POSTERS

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Three dimensional diffusion of singlet oxygen in lipid suspensions using Monte Carlo simulations. Juergen Baier[1,*], Thomas Fuss[1,*], Christopher Wiesmann[1,*], Johannes Schwanzl[1,*], Max Maier[1,*] and Wolfgang Baeumler[6,*]. [1]University of Regensburg / Physics, Regensburg, Germany, [6]University of Regensburg / Dermatology, Regensburg, Germany.

Among others, singlet oxygen can be generated by an energy transfer of a light absorbing photosensitizer. The nonradiative deactivation of singlet oxygen is accompanied by radiative deactivation leading to infrared luminescence at 1270 nm, which is widely employed for singlet oxygen detection. The lifetime of single oxygen depends on the environment in which the singlet oxygen decays, which can be described by a theoretical system of differential equations. But usually no diffusion of singlet oxygen during different environments is considered. When adding phosphatidylcholine to water, droplets of the fatty acid were formed. It has been shown that ATMPn is exclusively localised in lipids when added to aqueous suspensions of fatty acids. By exciting the suspension singlet oxygen will be generated only in lipid. However, singlet oxygen can escape into the aqueous environment. In that way the lifetime of singlet oxygen depends on the exchange of singlet oxygen between water and lipid. This could explain the measured intermediate lifetime of 10 μs of singlet oxygen in the luminescence experiments of the suspension of ATMPn, which is shorter than the value for pure phosphatidylcholine (14 μs) and larger than the value of water (3.5 μs). To understand this process of diffusion of singlet oxygen Monte Carlo simulations were made for lipid suspension, which consists of two different environments for singlet oxygen. It could be shown that the diffusion length of singlet oxygen must be approximately equal to the dimension of the lipid areas. Moreover the droplets of lipid must be composed on bilayer of fatty acids which were surrounded by water. Simulations with solid droplets of lipid could not produce the experimental data in acceptable conditions.

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Examininationation of the fluorescence yield in mutant glucokinase by use of computer modeling. Jennifer L Greene*, Bogumil Zelent*, Jane M Vanderkooi* and Franz Matschinsky*. Department of Biochemistry and Biophysics, Philadelphia, PA, USA.

Fluorescence of genetically engineered glucokinase that is conjugated with glutathione reductase were compared with the native enzyme. Tryptophan fluorescence yield from human glucokinase exhibits a 70% increase in yield upon saturation binding of D-glucose. The large change in fluorescence allows one to use Trp fluorescence to monitor binding in genetically engineered GST conjugated glucokinase in which particular residues are replaced. Binding affinity selectivity followed this sequence for nearly all variants: D-glucose > D-mannose > D-mannoheptulose > 2-deoxy-D-glucose >> L-glucose. Replacing W99, W167 and W257 caused wide variation in sugar binding constant and fluorescence change. Computer modeling of the mutant enzymes using CHARMM molecular mechanics force field was carried out to see whether resonance energy transfer between Trp residues can account for the variations in intensity, specifically for W167F and W257F. The conclusion is that an increase in fluorescence when sugar binds is consistent with a change in conformation that results in higher quantum yield, but energy transfer is unlikely to be the sole reason for this. All Trp residues contribute to the fluorescence intensity, and small local changes in the environment contribute to the quantum yield; however, mutating a single Trp residue still leaves ambiguity as to the cause of the increasing fluorescence since the glucokinase undergoes conformational changes.

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Analysis of UV-induced pigmentation from repeated exposures and evaluation of melanin redistribution. Sergio G Coelho[1,2], Sharon A Miller[1], Barbara Z Zmudzka[1], Janusz Z Beer and Vincent J Hearing[2,*]. [1]Center for Devices and Radiological Health, Food and Drug Administration, Rockville, MD, USA, [2]Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.

Constitutive skin color, i.e. pigmentation, serves as a natural sunscreen to protect us from harmful effects of ultraviolet (UV) exposure (including skin cancer induction). The exposure to natural or artificial UV (e.g., sunlamps) increases the production of melanin leading to development of pigmentation several days after the exposure. We previously studied the mechanisms of skin tanning and found that a single UV exposure induces redistribution of melanin to the upper layers of the skin. We believe that this phenomenon increases photoprotection of the skin. Now, we report observations on the effects of repeated UV exposures on melanin distribution in the epidermis. Ten healthy volunteers of skin phototypes 2-3 had three (3x3 cm) sites on their back exposed to a typical sunlamp using 3 tanning schedules with doses of 4.3 kJ/m2, 2.9 kJ/m2 or 1.9 kJ/m2, accumulated during 14 visits over an 8-week period. UV-induced color changes were followed using diffuse reflectance spectroscopy, visual assessment and computer-assisted digital image

* Denotes non-member.

evaluation (CADIE). Epidermal biopsies were analyzed using digital images of Fontana-Masson staining and examined for distribution of melanin in the three-epidermal layers (basal, granulosum, and spinous). In all cases, repeated UV exposures resulted in increases of the total epidermal melanin content. However, preliminary analyses show considerable variation of responses. While in some cases the highest total melanin content was observed after the highest cumulative dose, in other cases the intermediate cumulative doses were most effective in increasing the melanin content. Also, in some cases redistribution of melanin towards the skin surface seemed to be stronger after intermediate rather than the highest cumulative doses. These observations confirm the importance of localization of melanin not only with respect to skin surface appearance, but also in potentially playing a role in the photoprotective function of the pigment.

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Tocopherol related radioprotective compounds. Anil K Singh* and Gopu K*. Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai-400076, India, Mumbai, Maharastra, India.

Tocopherol (vitamin E) is known to play important role in modulating intracellular signaling pathways that rely on reactive oxygen intermediates. It can reduce the early damages produced in cells and tissues through inonizing radiations, and thus it acts as radioprotective / anti-oxidant agent. However, the radioprotective activity of vitamin E gets limited due to its fat-soluble nature. Therefore, development of water-soluble vitamin E compounds is highly desirable. In this context, we have synthesized some bromo-, acetyl, bromo-, acetyl, formyl-, acetyl, acetate- and phospho- derivatives of a- tocopherol and examined their relative radioprotective / antioxidant properties employing glutathione peroxidase method. Enhancement of radioprotective / antioxidant activity for water-soluble derivatives is observed. It is further noted that phospho- and acetyl, acetate- derivatives show relatively higher radioprotective / antioxidant activity as compared to vitamin E itself.

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Development of antioxidant/radioprotective molecules based on compounds in vitamin A series. Anil K Singh* and Solomon Libsu*. Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai-400076, India, Mumbai, Maharastra, India.

Compounds in vitamin A series have been known to possess, among others, antioxidant properties. Work over the last decade has revealed that these compounds act synergistically with vitamin E (a-tocopherol), a well-known lipid-soluble antioxidant. A major drawback of these compounds has been their fat-soluble nature. It is thus thought desirable to develop aqua-soluble derivatives of these compounds. To this end, several ionyl compounds bearing additional hydrophilic moieties like a carbohydrate, amine, amino acid, and phosphate have been synthesized. Further, their antioxidant / radioprotective properties are being examined by glutathione peroxidase method.

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The influence of glycerol concentration on the hydrogen bonding network in glycerol/water mixtures. Jennifer L Dashnau, Kim A Sharp* and Jane M Vanderkooi*. Department of Biochemistry and Biophysics, Philadelphia, PA, USA.

General solvent effects are known to induce a Stokes shift in the fluorescence emission of fluorophores that is commonly described to first-order approximation by the Lippert equation. The spectral shift is related to the energy difference between fluorophore ground and excited states that is caused by the rearrangement of solvent molecules around the excited state dipole moment of the probe. Since fluorescence emission is exquisitely linked to the nature of the surrounding solvent, it is important to understand the dynamics of the solvent system employed in analyzing spectral shifts. Glycerol/water is a type of solvent system commonly used in temperature-dependent studies–mainly for its ability to form glassy matrices at low temperatures. However, a detailed, molecular-level description of the dynamics of this system is yet to be obtained. In the present work, explicit solvent molecular dynamics simulations and infrared spectroscopy were used to determine the hydrogen bond patterns of glycerol/water mixtures as a function of glycerol concentration. The ability of glycerol/water mixtures to inhibit ice crystallization and form a glass is directly linked to the concentration of glycerol in solution and the ability to disrupt the hydrogen bond network of water. Increasing glycerol concentration depleted the amount of water with bulk-like character available for ice formation. Water molecules in the first hydration shell concentrated around the hydroxyl groups of glycerol as the alkyl groups of glycerol self-associated. Glycerol-glycerol intermolecular hydrogen bonds became the dominant interaction in the first hydration shell and the percolation nature of the water network was disturbed. At glycerol concentrations beyond that required for disruption of the water network, glycerol/water mixtures remained glassy at low temperature and the glycerol-water hydrogen bond favored a more linear arrangement. High glycerol concentration mixtures mimicked the strong hydrogen bonding pattern seen in ice, yet crystallization did not occur. These results further explain those obtained from our previous study (Biophys. Chem. (2005) 114: 71-83).

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Photosensitized type I oxygenation of cholesterol in model dyads. ISABEL M MORERA[1,*], INMACULADA ANDREU[1,*], FRANCISCO BOSCA[2,*] and MIGUEL A MIRANDA[2,*]. [1]Departamento de Química. Universidad Politécnica de Valencia. Camino de Vera s/n, Valencia, Spain, [2]Instituto de Tecnología Química. UPV-CSIC. Universidad Politécnica de Valencia, Camino de Vera s/n, Valencia, Spain.

Among the structural components of cell membranes, Cholesterol (Ch) accounts for a substantial part of the total lipids. As an unsaturated lipid, Ch is susceptible to oxidative degradation, which can result in potentially pathologic consequences. This process can be promoted by UVA-irradia-

tion in combination with photosensitizing agents. Thus, drugs containing the benzophenone (Bz) chromophore (such as ketoprofen, Kp) can photosensitize chemical modifications of biomolecules, including lipid peroxidation. However, Bz-photosensitized Ch oxidation has not been previously reported, in spite of its potential mechanistic interest. In the present work, this issue has been addressed by means of Kp-Ch dyads, prepared through conjugation of beta- and alpha-Ch with (S)- and (R)-Kp. Following the methodology applied to related lipid-drug models, the Kp-Ch dyads have been submitted to time-resolved and steady-state photolysis studies. By comparing the triplet lifetimes of the beta-and alpha-Ch derivatives in CH_2Cl_2, it becomes clear that only the latter are reactive, with a rate constant for intramolecular H-abstraction from the C-7 allylic position of ca. 10^8 M^{-1} s^{-1}. Although the triplet states of the alpha-dyads are very short-lived (ca. 10 ns), the resulting biradicals are much longer-lived ((R)-Kp-Ch: 240 ns and (S)-Kp-Ch: 350 nm). Under anaerobic conditions, the biradicals undergo intramolecular C-C radical coupling. However, oxygen trapping of the C-7 radical gives rise to the corresponding hydroperoxide and ketone oxidation products. This supports a Type I photooxygenation mechanism; blocking the type II pathway is achieved in the dyads through the dramatic shortening of their Kp triplet lifetimes, which do not allow for an efficient diffusion-controlled oxygen quenching.

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Interaction of A2E with radicals generated in photosensitized reaction. Anna Pawlak[1,*], Agnieszka Broniec[1,*], Albert Wielgus[2,*], Joan E Roberts[3,*], T. George Truscott[4,*], Ruth Edge[5,*], Suppiah Navaratnam[5,*] and Tadeusz Sarna[1,*]. [1]Jagiellonian University, Krakow, Poland, [2]Laboratory of Pharmacology and Chemistry, Research Triangle Park, NC, USA, [3]Department of Natural Sciences, New York, NY, USA, [4]School of Chemistry and Physics, Keele, Staffordshire, UK, [5]Free Radical Research Facility, Warrington, Cheshire, UK.

A pyridinium bisretinoid (A2E) is the only identified human ocular lipofuscin (Lf) component, accumulation of which in the retinal pigment epithelium (RPE) is linked to etiology of age-related maculopathy. Although photoreactivity of A2E is very low, it has been postulated that this blue absorbing chromofore may be involved in phototoxicity to the outer retina. Using pulse radiolysis and sensitized energy transfer approach, we have determined that energy of the lowest excited triplet state of A2E lies in the range 110-123kJ/mol. Formation of the radical forms of A2E was studied by laser flash photolysis employing rose bengal and all-trans retinal as photosensitizers, and NADH as electron donor. Our data indicate that A2E, under the experimental conditions used, can undergo one-electron reduction via interaction with NAD radical formed in a primary photosensitized electron transfer reaction. We have previously shown that the anion radical form of A2E exhibits relatively long lifetime and high reactivity with molecular oxygen. Therefore it can be speculated that oxidative stress conditions may result from generation of A2E anion radical in this highly oxygenated retinal tissue.

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Investigation of photodynamic inactivation of bacteria using the detection of singlet oxygen luminescence. Juergen Baier[1,*], Tim Maisch[2,*], Barbara Franz[2,*], Max Maier[1,*], M. Kabdthalers[2,*], Rolf Markus Szeimies[2,*] and Wolfgang Bauemler[2,*]. [1]University of Regensburg / Physics, Regensburg, Germany, [2]University of Regensburg / Dermatology, Regensburg, Germany.

In view of the increasing resistance of bacteria to antibiotics, photodynamic inactivation of bacteria is a promising new technique. It is known, that Gram(+) and Gram(-) bacteria can be killed by antibacterial photodynamic inactivation depending on the used photosensitizer. The objective was to evaluate localisation of the photosensitizer Photofrin in Gram(+) S. aureus and Gram(-) E. coli by detection of singlet oxygen time-resolved by its luminescence at 1270 nm directly. Singlet oxygen was generated by energy transfer from the photoexcited Photofrin, dissolved in aqua dest. After incubation of S. aureus or E. coli with Photofrin and subsequent irradiation, the viability of S. aureus decreased yielding 99.9% dead bacteria, whereas the viability of E. coli was hardly affected. Sodium azide, quencher of singlet oxygen, inhibited the killing of S. aureus. Fluorescence microscopy showed an uptake of Photofrin by S. aureus but not by E. coli. Due to the limited resolution of the microscope, the subcellular localization of Photofrin in bacteria failed and therefore a detailed insight into the mechanisms of action was not possible. However, the localization of Photofrin is correlated to the localization of singlet oxygen, which is correlated to luminescence decay time of singlet oxygen measured. The resolution of this method is given by the diffusion length of singlet oxygen, which is very short in a biological environment. When incubating E. coli with 300 μg/ml Photofrin for 90 min no singlet oxygen luminescence was detected confirming the results of cell viability experiment. When incubating S. aureus with Photofrin, a singlet oxygen luminescence decay time of 6 μs ± 2 μs was measured. Adding the quencher Sodium azide the luminescence decay time was shortened (3 μs ± 1 μs). Obviously, the decay time of luminescence is an intermediate time of singlet oxygen decaying in phospholipids (14 μs ± 2 μs) of membranes and in the surrounding water (3.5μs ± 0.5 μs). Thus, singlet oxygen seems to decay in outer cell wall areas of S. aureus, which is then the subcellular localization of Photofrin. The luminescence decay time in large agglomerates of bacteria was much longer (40 μs ± 16 μs) than in the suspension with single bacteria. This is the first time that singlet oxygen was measured directly by its luminescence inside living bacteria.

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Development of analytical tool for evaluation of photoreactivity of pharmaceutical substances. Satomi Onoue* and Yoshiko Tsuda*. Analytical R&D, Pfizer Global Research and Development, Pfizer Japan Inc., Aichi, Japan.

Phototoxic responses after administration of photosensitive pharmaceutics have been recognized as undesirable side effects. Thus, screening for phototoxicity is necessary in the

early phases of the drug discovery process, even before introducing drugs and chemicals into clinical therapy, as this may help prevent unwanted drug reactions in humans. This investigation is aimed at designing a model system for the assessment of photosensitive/phototoxic potential through analytical and biochemical methods. Here, we evaluated the generation of singlet oxygen and superoxide, by the colorimetric determination, upon exposure of some representative phototoxic/non-phototoxic compounds to light. Most photosensitive/phototoxic drugs tested, even weak UV absorbers, at the concentration of 200 μM showed significant production of ROS in less than 18 hours of light exposure (30,000 lux). On the other hand, ROS generated from weak/non-phototoxic compounds, including the strong UV absorber benzocaine, were low or negligible. Exposure of quinine to light resulted in significant degradation (half-life time, $t_{0.5}$; 6.4 hr), however it was dramatically attenuated by the addition of ROS scavengers, especially sodium azide ($t_{0.5}$; 122.6 hr). Photodynamic peroxidation of linoleic acid as a model of phototoxic injury and the photodegradation of quinine, a typical photosensitizer, were also investigated to further clarify the role of ROS in the photochemical/phototoxic response. Concomitant exposure of photosensitive/phototoxic compounds (200 μM) and linoleic acid (1 mM) for 18 hours led to the marked formation of lipoperoxide. The results suggested that the tested compounds, which are known to be photosensitive/phototoxic, have the ability to generate ROS under light exposure, and that this photochemical reaction could be associated with their photoinstability and/or phototoxic responses. Upon these findings, determination of ROS, generated from photo-irradiated compounds, may be an effective predictive model in recognizing their photosensitive/phototoxic potential.

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Approach to potential risks of phototoxicity. Asako Ishibashi*. Nagoya Laboratories Pfizer Japan Inc, Aichi, Japan.

This study was initiated to address photosensitivity, which has emerged as a major issue in a drug discovery. The purpose of the study was to identify descriptors that can be applied to new drug designs, and to avoid potential photoreactivity risks at the design stage. Our approach to risk prediction was molecular orbital calculation using launched drugs and our own compounds. The study showed that the HOMO-LUMO gap can potentially be used as a guide to avoid risk.

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Ethenyl indoles as neutral and hydrophobic fluorescence probes. Anil K Singh* and Prasanta Hota*. Department of Chemistry, Indian Institute of Technology, Bombay, Powai, Mumbai - 400 076, India, Mumbai.

Extrinsic fluorescence probes have numerous applications in biology and chemistry. For example these can be used for characterizing specific biomolecular interaction and for probing the microenvironment of organized assemblies. Several fluorescence probes are known but most of these charged in nature. Availability of neutral fluorescence probes is severely limited. The charged fluorescence probes undergo secondary Columbic interactions with the host system. Therefore, the results of probing studies using these probes are difficult to analyze and understand. Development of neutral fluorescence probes is, therefore, highly desirable. In this context, we have synthesized and examined fluorescence probe properties of ethenyl indoles, namely 3-(4-nitrophenyl ethenyl-E)- NH-indole, 3-(4-nitrophenyl ethenyl-E)-N-acetyl indole, and 3-(4-nitrophenyl ethenyl-E)-N-benzenesulfonyl indole. It is found that these compounds can satisfactorily be used for studying the ligand-protein interaction, and also as reporters of the microenvironment of organized assemblies of various micelles. It is further found that ethenyl indoles bearing electron withdrawing group at the indole nitrogen atom are generally less fluorescent but show linear enhancement of their fluorescence intensity upon increasing protein concentration. Hence, these are useful as probe for protein quantification.

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A Stark spectroscopic study of semiquinone FAD in DNA photolyase. Goutham Kodali* and Robert J Stanley*. 201 Beury Hall, Philadelphia, PA, USA.

DNA photolyase is a light-driven flavoprotein that repairs cyclobutylpyrimidine dimers (CPD) in UV-damaged DNA via an ultrafast photoinduced electron transfer reaction from the fully-reduced anionic flavin adenine dinucleotide (FADH-) cofactor to the CPD. The basic electronic properties of oxidized FAD previously obtained from our laboratory suggest that the electric dipole moment of the CPD induces an electrochromic shift in the electronic transition energies of the oxidized FAD in DNA photolyase. These results would indicate that the substrate electric field plays a critical role in the electron transfer process. A similar electrochromic shift has been reported for the energy levels of the semiquinone FAD in DNA photolyase upon CPD binding3. In an effort to provide further insight into this result, we have explored the change in the electronic structure of the electronic states of the semiquinone FAD in DNA photolyase using Stark spectroscopy. Our results support the electrochromic shift model. Spectral shifts produced by this model are in good agreement with the previous experiments. References: (1) Stanley, R. J., and Siddiqui, M. S. (2001). A Stark Spectroscopic Study of N(3)-Methyl, N(10)-Isobutyl-7,8-Dimetylisoalloxazine in Non-Polar Low Temperature Glasses: Experiment and Comparison with Calculations J. Phys. Chem. A 105 (49), 11001. (2) MacFarlane IV, A.W. and Stanley, R.J. (2001). Evidence of Powerful Substrate Electric Fields in DNA Photolyase: Implications for Thymidine Dimer Repair. Biochemistry 40 (50), 15203. (3) Schelvis, J. P. M., Ramsey, M., Sokolova, O., Tavares, C., Cecala, C., Connell, K., Wagner, S., and Gindt, Y. M. (2003). Resonance Raman and UV-Vis Spectroscopic Characterization of FADH. in the Complex of Photolyase with UV-damaged DNA. J. Phys. Chem. B 107 (44), 12352.

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Laser flash studies of 10, 11- carbamazepine. Efrain Betancourt[1,*], Carmelo Garcia[2,*] and Rafael Arce[1,*]. [1]University of Puerto Rico, Rio Piedras Campus, San Juan, PR, [2]University of Puerto Rico, Humacao Campus, Humacao, PR.

Although 10, 11- carbamazepine is a well known and widely used antiepileptic drug (AED); it produces a range of side effects, including photoallergic reactions. In order to optimize the process of designing new AEDs, there is a need to understand the mechanism of the phototoxicity induced by these and similar drugs. In this study, the laser flash technique is used to identify and study the reactions of the possible species responsible for their phototoxicity. Laser flash transient spectra of 10, 11-carbamazepine were obtained in PBS 7.4, methanol and acetonitrile. In all solvents, the 266-nm UV laser irradiation leads to the production of the cation radical and the corresponding electron. The radical has an absorption maximum at 500 nm. This assignment was done on the basis of an equal decrease of absorption in presence of both O_2 and N_2O. The solvated electron has a broad band peaking at 700 nm in PBS and at 600 nm in methanol. Besides, there is also a transient absorbing at 400 nm, which was tentatively assigned to a triplet, because the decay of the intensity of ΔOD is faster in presence of O_2. The kinetic curves at 425 nm show a weak component of the ΔOD signal that could be attributed to a triplet because the initial decrease in intensity is faster in presence of O_2 than in presence of N_2. The long lived component of these curves is assigned to the radical cation since the absorption is larger for the solvents in which the solvated electron is more stable: PBS 7.4 $>$ CH_3OH $>$ CH_3CN. The financial support by the Chemistry Department is gratefully acknowledged.

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Photochemical degradation of 1,8-dinitropyrene adsorbed on silica gel 60 Å as model of atmospheric particulate matter. Maria C Morel*, Ileana Alers* and Rafael Arce*. University of Puerto Rico, Río Piedras Campus, San Juan, Puerto Rcio.

Dinitropyrenes, well known mutagenic and carcinogenic compounds, are found in the atmospheric particle matter. The transformations of these ubiquitous pollutants in the atmosphere are not well understood. Thus, it is relevant to be able to understand their environmental fate because they absorb in the solar region (400 nm), and possible photoreactions in the atmospheric particulate matter can occur. In this study, 1,8-DNP is used as a model compound to study its photochemical and photophysical properties on the surface of silica gel 60 Å as a model of atmospheric particulate matter. The absorption spectra of unirradiated 1,8-DNP adsorbed on silica gel 60 Å present maxima at 292 nm and 397 nm, similar to those observed in acetonitrile, except for the absence of the band at 245 nm. Broadening of the absorption bands was also noted due to interactions between the surface and the 1,8-DNP molecules. Fluorescence emission spectra of unirradiated samples reveal a broad emission band at 460 nm as observed in solution, while a new band at 339 nm is also observed. Continuous photolysis of 1,8-DNP adsorbed on silica gel 60 Å results in a decrease of its characteristic absorption band at 397 nm. Simultaneously, a new band with maxima at 450 nm grows with irradiation time indicating its photodegradation and further transformation into products. A broad fluorescence emission band at 518 nm (excitation at 397 nm) further indicates the phototransformation of the adsorbed 1,8-DNP. Similar bands observed in irradiated solutions of 1,8-DNP in acetonitrile are due to the presence of products such as hydroxy-nitropyrenes and pyrenediones. Separation and identification of the stable adsorbed photoproducts by HPLC is in progress. The incorporation of these functionalities is in agreement with the proposed nitro-nitrite rearrangement for the phototransformation of other nitroarenes. The financial support by NIH SCoRE (grant 55066M08102), RISE (grant 5R25GM061151) and the Chemistry Department is gratefully acknowledged.

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Development of physical and functional standards for biological assays. Adolfas K Gaigalas*, Kenneth D Cole*, Lili Wang* and Srikant Bykadi*. National Institute of Standards and Technology, Gaithersburg, MD.

Work is underway at NIST to characterizing solutions of ricin and other proteins with the objective of providing reference solutions of these proteins. A calibrated spectrophotometer is used to measure the absorbance, and amino acid analysis is used to obtain concentrations of proteins to develop reference solutions of known concentration and molar extinction coefficient. These reference solutions are intended for users who wish to check their measurement system. Measuring the absorption of photons at 266 nm and 280 nm have been traditional methods for determining the concentrations of DNA and proteins respectively. To obtain greater sensitivity, fluorescence measurements have been adapted to biological assays. We have developed fluorescence based reference materials to assist in the quantification of antigens on the surface of lymphocytes. The application of these reference materials is in clinical flow cytometry. In addition to probing the properties of protein solutions, light can also influence the functioning of biological systems. Examples are photoinduced tissue damage and many applications of photodynamic therapy. We invite suggestions regarding standards for improving measurements in the area of photoinduced effects in biological systems.

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Studies of transient intermediates of 1-Nitropyrene in different environments. Eduardo F Pino* and Rafael Arce*.

1-Nitropyrene is a widely distributed environmental pollutant which is highly mutagenic and carcinogenic. Also, is the most abundant nitroaromatic compound in the environment. In order to understand its photochemical and photophysical properties phosphorescence and laser flash photolysis techniques in different environments have been used to identify transient intermediates that could be involved in the phototransformation processes. Phosphorescence spectra were recorded in polar and non-polar glasses such as EPA

(mixture of diethyl-ether, isopentane and ethanol, in 5:5:2 proportion), diethyl ether and 3-methylpentane at 77 K. These present two maxima at 640 nm and 700 nm. Furthermore, in 3-methylpentane the spectra present a shoulder at 540 nm. The phosphorescence obtained in all glasses suggest a lowest excited triplet state (π, π^*) with a lifetime of 0.054 s. Under steady state irradiations at 77 K a small band at 450 nm is observed that could be assigned to a pyrenyloxyl radical. The transient absorption spectra of laser irradiated 1NP at 355 nm in solvents of different polarities and viscosity (benzene, cyclohexane, acetonitrile and methanol), and in the presence of N_2 or O_2, present two maxima, one at 450 nm (pyrenyloxyl) and the other to 530-550 nm and a shoulder at 570 nm (triplet state). Increasing the solvent polarity shifts the triplet maximum to longer wavelengths (from 530 to 570 nm) characteristic of a π, π^* triplet absorption. The ratio of the transient absorption intensities changes with observation times in the presence of O_2 and in solvent with different polarities, suggesting the presence of different species. The financial support by NIH SCoRE (grant 55066M08102) is gratefully acknowledged.

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Photophysical and photochemical properties of 1-Nitropyrene in different solvents. Zulma I Garcia-Berrios* and Rafael Arce*. University of Puerto Rico, San Juan, PR, Puerto Rico.

1-Nitropyrene (1NP), a well known mutagenic and highly carcinogenic polycyclic aromatic hydrocarbon (PAH), is the most abundant nitro-PAH emitted to the environment through different combustion processes. Its transformation in the atmosphere is still debated, and a possible environmental fate for 1NP is through its photodecomposition in the organic liquid-like region of the atmospheric aerosols. To follow its photodegradation and to study the effects of the nature and polarity of the solvent and the presence of O_2 and N_2 on the photodegradation rates and products distribution, an HPLC system coupled with a UV-Vis spectrophotometer was used. The photodegradation rate of 1NP was higher in methanol (10^{-3} s^{-1}) in N_2 and O_2 saturated solutions in comparison with other solvents (hexane, benzene, 3-methylpentane, and EPA = diethylether-isopentane-ethanol (5:5:2)) in which a rate constant of 10^{-4} s^{-1} was determined. The higher rate in methanol can be explained considering the low bond dissociation energy of the C-H in methanol facilitating the hydrogen abstraction as part of the mechanism. Quantum yields have been determined for hexane and methanol of the order of 10^{-3}. The relative yields for the identified photoproducts at ≈30% photodegradation, in N_2 or O_2 saturated solutions are respectively: for hexane, 1-hydroxypyrene (31.9%, 55.8%), 1,6-pyrenedione (10%, 12%), 1,8-pyrenedione (0.5%, 1.4%), and 1-nitro-2-hydroxypyrene (9.7%, 11.8%); for methanol, 1-hydroxypyrene (40%, 26%), 1,6-pyrenedione (0.8%, 4%), 1,8-pyrenedione (0.6%, 4%), and 1-nitro-2-hydroxypyrene (0.2%, 2%); for benzene, 1-nitro-2-hydroxypyrene (1%, 51%); for 3-methylpentane, 1-hydroxypyrene (4%, 4%), and 1-nitro-2-hydroxypyrene (n.o., 26%); and for EPA, 1-hydroxypyrene (44%, 33%), and 1-nitro-2-hydroxypyrene (n.o., 28%). Other decomposition products are formed. The formation of the principal photoproduct, 1-hydroxypyrene, can be interpreted as resulting from a hydrogen abstraction from the solvent by the pyrenyloxyl radical formed through the nitro-nitrite rearrangement. The financial support by NIH SCoRE (grant 55066M08102) and RISE (grant 5R25GM061151) is gratefully acknowledged.

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Spatial distribution of protein damage in keratinocytes produced by singlet oxygen. Colin F Chignell*, Yu-Ying He*, Sarah Council* and Li Feng*. Laboratory of Pharmacology and Chemistry, Research Triangle Pk., NC.

Singlet oxygen may be generated in cells by either endogenous or exogenous photosensitizers as a result of exposure to UV or visible irradiation. We have used immunospin trapping (*Free Rad. Biol. Med.* 36, 1214, 2004) to identify the subcellular targets of singlet oxygen generated by rose bengal (RB). Confocal fluorescence microscopy of HaCaT keratinocytes incubated with RB clearly showed that the dye entered the cells and was located in the perinuclear region probably associated with the Golgi apparatus. Previous studies by Davies and coworkers (*Photochem. Photobiol. Sci.* 3, 17, 17) have shown that long lived protein hydroperoxides (POOH) are present in cells exposed to singlet oxygen generating dyes. The addition of reducing metal ions (M^+) to POOH results in the generation of protein derived radicals which react with the spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) to give a stable adduct: PH + $^1O_2$ → POOH + M^+→ M^{++} + POO• → PO• + DMPO → DMPO/•OP. We incubated the keratinocytes with RB and exposed them to visible light followed by the addition of Fe^{++} and DMPO. The cell extract was separated by SDS gel electrophoresis and proteins containing DMPO adducts were detected by Western blotting using an anti-DMPO specific antibody. While extensive formation of protein-DMPO adducts was observed it was not possible to identify the labeled bands. In order to determine the subcellular localization of the protein-DMPO adducts, we therefore repeated the RB/light exposure and then incubated the cells with Cu^+ and DMPO. After staining with antibody against DMPO followed by a secondary Alexa Fluor 488 goat anti-rabbit IgG, the intracellular distribution of protein-DMPO adducts was determined by confocal microscopy. The subcellular localization of the protein hydroperoxides was coincident with that of Rose Bengal. This approach may provide information on the spatial distribution of singlet oxygen generated in cells.

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Ultrafast kinetics of HbI and HbI mutants from *Lucina pectinata*. Cacimar A Ramos Alvarez[1,*], Lourdes Lopez Caban[1,*], Michel Negrieri[2,*], Jean-Louis Martin[2,*] and Juan Lopez-Garriga[1,*]. [1]University of Puerto Rico, Mayaguez, PR, Puerto Rico, [2]Laboratoire d'Optique et Biosciences, Paris, France.

Hemoglobin, since its discovery, has been the model system for numerous studies that involves the relation between

structure and function. Hemoglobin I (HbI) from Lucina pectinata contains 142 aminoacids residues and presents and unusual heme pocket among the globin families, which is believe to play a key role in the selectivity and particular affinity with certain ligands [1,2]. The role of those residues surrounding the active site can be investigated by the photoinduce dynamics of the various complexes using an ultrafast laser system to resolve the evolution of the transient species [3-5]. Another radical approach widely used involved the expression of mutant variations of the hemeprotein, where some residues are changed in order to elucidate the effect that this mutations has on the kinetics signal of the protein. Wild type HbI (Wt-HbI) from L. pectinata posses an unusual glutamine and a series of phenylalanine residues surrounding the heme center of the protein. A mutant of this position (B10) was expressed where the natural phenylalanine was substituted with a Valine (Val). Ultrafast kinetic measurement where made using NO as the ligand and they where compare with Mb and WtHbI. The ultrafast setup uses and ultrafast laser source coupled to a CCD detector where a wide wavelength region can be scan simultaneously. On the initial portion of the plot it was observed that ≈80% of the signal was recovered for the HbI PheB10Val mutant complex against only ≈54 and 48 % for the Wt-HbI and Mb species respectively. This values present that HbI-PheB10Val is ≈26% faster than its Wt-HbI counterpart. On much longer time scales, ≈350 ps, it is observed that almost 97% of the signal is recovered for the Wt-HbI and the HbI-Mutant species. On the other side for the Mb case only ≈85% of the signal has been recovered at that time. Under the time window observed for this experiments no complete recovery of the signal of the Mb complex where achieved.≈

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Two-photon photodynamic therapy for the treatment of neovascular age-related macular degeneration. Mamta Khurana[1,*], Aliaksandr Karotki[1,*], Hazel Collins[2,*], Harry L Anderson[2,*] and Brian C Wilson[1,*]. [1]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, [2]Department of Chemistry, Oxford University, Oxford, UK.

Age related macular degeneration (AMD) is a major cause of severe vision loss in older population. It occurs due to ingrowth of new leaky blood vessels (neovasculature) from the choriocapillaris. The resulting blood leakage leads to destruction of photoreceptors in the fovea and loss of central vision. "Standard" one-photon (1-γ) photodynamic therapy (PDT) using Visudyne (QLT Inc, BC) is an approved method of AMD treatment. PDT is based on combined action of light and drug (photosensitizer) to produce a photocytotoxic effect. Unfortunately, 1-γ PDT also has the potential to damage healthy tissues lying above and below the neovasculature due to photosensitizer accumulation and its wide-beam 1-γ excitation. Highly-targeted two-photon (2-γ) excitation is a possible solution to this problem. Due to its non-linear nature, the probability of 2-γ excitation is greatest in the focal plane, which intrinsically avoids out-of-focus damage to healthy tissues. We investigated the efficiency of Visudyne as 2-γ PDT agent and compared it to the archetypal photosensitizer Photofrin. Since neovascular endothelium is targeted in AMD, an endothelial cell line (YPEN-1) has been selected as model. 2-γ PDT of Photofrin and Visudyne were investigated using a highly focused 300 femtosecond laser pulses from a Ti:sapphire laser operating at 850 nm with 90 MHz pulse repetition rate. An assay was developed for quantification of the cellular damage using cell permeant stain Hoechst33258 and cell viability stain SYTOX. We report that Visudyne (LD_{50} (Dose to kill 50% of cells) = 500 J/cm^2, 10 μM, 7.2 μg/ml) is about an order of magnitude better 2-γ photosensitizer than Photofrin (LD_{50} = 7500 J/cm^2, 25 μg/ml). We also demonstrate for the first time the quadratic dependence of the cellular response to 2-γ PDT. This *in vitro* work will lead to the design of optimized *in vivo* studies in animal models of AMD.

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Palmitate removal by hydroxylamine in bovine rhodopsin. Wesley C Jackson, Zsolt Ablonczy* and Rosalie K Crouch. Medical University of South Carolina, Department of Ophthalmology, Charleston, South Carolina, USA.

The intracellular C-terminal fragment of the photosensitive visual pigment rhodopsin is highly conserved in most all visual systems. Two cystein amino acids are acylated with fatty acids at position 322 and 323 on bovine rhodopsin. Normally the acyl groups consist of a palmitate (≥80%) bound via a thioester bond. To study pigment formation with the 11-cis retinal or other retinal analogues, hydroxylamine historically has been used remove native retinal chromophore from the opsin. As acylation has been proposed in other G-protein coupled receptors to be part of signaling processes, we have examined the effect of hydroxylamine on the stability of bovine (Bos turus) rhodopsin acylation. Our study demonstrates that increasing concentrations of hydroxylamine compromise the structural integrity of the thioester bond, cleaving palmitate groups as well as evacuating the retinal from the opsin binding pocket. The second objective of this study focuses on the primary site of deacylation, i.e., which palmitate is removed first during hydroxylamine exposure. The role of this deacylation on the structure, and function, and photochemical properties of the protein are being studied. The clinical relevance to these studies focuses on poorly understood genetic disorders of the retina such as macular degeneration, where the lack of 11-cis retinal due to the improper transport is known to be a major risk factor for blindness. Supported by NIH grants EY04939 and EY 14793, and an unrestricted grant from RPB.

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Function of transport and sensory rhodopsins in photosynthetic bacteria. Kwang-Hwan Jung*, Ah Reum Choi* and Sa Ryong Yoon*. Dept of Life Science and Interdisciplinary Program of Integrated Biotechnology, Seoul, South Korea.

Microbial rhodopsins are retinal-binding, seven transmembrane proteins which function as ion pumps and sensory receptors. A gene encoding a homologue of type I microbial rhodopsin found in the genome of cyanobacteria (www.kazusa.or.jp). The *Anabaena* sp. PCC7120 sensory

rhodopsin (ASR) shows a visible light-absorbing pigment (absorption max = 550 nm) in the presence of all-trans retinal and the mutation of retinal binding pocket (P206D & E) shifts the absorption maximum to the blue. ASR is interacted with a 14 kDa soluble transducer and it serves as a photoreceptor for chromatic adaptation by proteomics analysis. The proton acceptor of ASR is not D75 (D85 in BR) but D217 in the cytoplasmic portion of ASR. The 14 kDa transducer functions as a transcription factor to bind promoter region of phycobilisome biosynthesis genes and ASR operon. Gloeobacter violaceus PCC7421 is believed to be a primitive cyanobacterium because of lack of thylakoid. The *Gloeobacter* rhodopsin (GR) gene encoded a polypeptide of 298 amino acids, with a molecular weight of 33 kDa. This gene is localized alone in the genome unlike *Anabaena* opsin which is together with 14kDa transducer gene. The gene was functionally expressed in *Escherichia coli* and bound all-trans retinal to form a pigment and show a light-driven pumping activity like proteorhodopsin. The pigment did not exhibit proton transport activity when Asp121 (Asp85 in BR) is replaced with Asn and Glu132 (Asp96 in BR) to Gln in GR. The efficient proton pumping and rapid photocycle of this GR pigment are strongly suggested that *Gloeobacter* rhodopsin functions as a proton pump in its natural environment.

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Time-resolved spectroscopy of the photoreceptor system of the ciliate *Blepharisma japonicum*. Giovanni Checcucci[1,*], Francesco Lenci[1,*], Mathilde Mahet[2,*], Pascal Plaza[2,*] and Monique M Martin[2,*]. [1]Istituto BioFisica CNR, Pisa, Italy, [2]Department de Chimie, UMR CNRS-ENS 8640, Ecole Normale Superieure, Paris, France.

Oxyblepharismin (OxyBP), a phenanthroperylene quinone derivative closely related to hypericin, is the photoreceptor chromophore for the step-up photophobic response of the light-adapted (blue-colored) form of the ciliate *Blepharisma japonicum*. A readily extractable and very stable, non-covalently bound oxyblepharismin-protein complex (OBIP, 200 kDa,), has been proposed to mediate the photophobic response of the blue form of *B. japonicum*. We studied the primary photochemical processes taking place in OBIP, by means of optical subpicosecond transient absorption spectroscopy. We showed that OBIP exhibits distinctive spectro-temporal behaviors, different from those of the free chromophore in organic solution. Up to 50% of the excited-state decay of OBIP occurs in the picosecond regime, the remaining part being a nanosecond component similar to that of the free OxyBP chromophore. The results are explained in terms of heterogeneity of the OBIP sample. Two independent classes of chromoprotein are proposed: a "reactive" species, which presents a specific 685 nm band decaying in 4 and 56 ps and a "non-reactive" one, which behaves like the free chromophore in solution. A bimolecular photooxidation of OxyBP in the presence of 1,4 benzoquinone was performed to record the absorption spectrum of the OxyBP radical cation. Comparison with reactive OBIP suggests that electron transfer could be involved in the primary photoprocesses of OBIP and possibly trigger the sensory transduction chain of *B. japonicum*. In addition, the specificity of the chromophore-protein interaction was probed through the study of the artificial complex that OxyBP forms with Human Serum Albumin (HSA). OxyBP-HSA happens to be spectroscopically much closer to free OxyBP than to OBIP. This highlights the specific nature of the interaction between OxyBP and its native protein partner and further supports the proposal that OBIP is the actual photoreceptor for the photophobic response of *B. japonicum*.

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Characterization of the gene encoding a blue fluorescent protein from *Vibrio fischeri* strain Y1. Hajime Karatani*, Takashi Osaki*, Masashi Yasui* and Shogo Ohta*. Department of Biomolecular Engineering, Graduate School of Science and Technology, Kyoto Institute of Technology, Sakyo-ku, Kyoto, Japan.

Yellow bioluminescent *Vibrio fischeri* Y1 cells produce a blue fluorescent protein (Y1-BFP, $\lambda_{max} \approx 461$ nm) as well as a yellow fluorescent protein (YFP, $\lambda_{max} \approx 538$ nm). Unlike YFP, playing as a secondary emitter, the functional role of Y1-BFP in bioluminescence (BL) has been unknown. In this study, the gene encoding Y1-BFP (Y1-BFP gene) was cloned and compared with the gene encoding YFP. The cloning of the Y1-BFP gene was attained by two kinds of PCR methods; one was a degenerate PCR with two mixed primers designed based on the partial amino acid sequences of Y1-BFP and the other was an inverse PCR with two primers designed based on the partially identified nucleotide sequences. The Y1-BFP gene was found to be 600 bp in length. The gene encoding YFP was amplified by PCR using *V. fischeri* Y1 genomic DNA as a template with reference to the reported sequence of the YFP gene, called *lux*Y. The sequence of the YFP gene cloned was confirmed to be exactly identical with the reported sequence (582 bp). The deduced amino acid sequence of Y1-BFP showed approximately 40 % homology to that of YFP. A recombinant Y1-BFP polypeptide with a histidine-tag was successfully produced in *E. coli* strain BL 21 by expression of the cloned gene inserted in a pETBlue-2 plasmid vector. The recombinant Y1-BFP was constructed by simply mixing the recombinant polypeptide and the Y1-BFP chromophore isolated from the wild Y1-BFP.

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A new evaluation method for plant defense activators based on potentiation of elicitor-responsive photon emissions (ERPE) in rice cells. Hiroyuki Iyozumi*, Hidehiro Inagaki* and Kimihiko Kato*. 678-1, Iwata, Shizuoka, Japan.

Living organisms are generating ultraweak photon emissions(10^0-10^4photons/sec/cm^2) as a by-product in their metabolic processes, so-called biophotons. Plants, including cultured cells, generate relatively high level of ultraweak photon emissions during a defense response to pathogen attack or elicitor treatment. ERPEs are controlled in plant defense signaling. For example chitin oligomer induced ERPE is generated through the phosphatidic acid (PA) signaling path-

way in similar to other elicitor responses of rice. We found that when rice cells are primed for defense by pretreatment with a plant defense activator, ERPE in rice cells become faster and stronger. Such priming effect on ERPEs was found among a variety of plant defense activators. Based on this fact, we developed a new evaluation method for plant defense activator as follows; I) dispense rice suspension culture into dishes, II) add each candidate or solvent control into cells, III) incubate treated cells for 2-4 h and check if each chemical has any disturbance on cell metabolism by photon counting, IV) add elicitor solution, e.g. chitin oligomer, V) measure ERPE from rice cells, VI) estimate the potentiation rate of ERPE from chemical-pretreated cells against that from solvent-pretreated cells. VII) Select chemicals, which show enough potentiation of ERPE. Now we are developing similar evaluation methods for other crops.

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The hydroxypyridinone iron chelator CP94 is superior to desferrioxamine at increasing PpIX fluorescence in cultured human cells for photodynamic therapy. Andrew Pye*, Leo Salter* and Alison Curnow*. Cornwall Dermatology Research, Truro, Cornwall, UK.

Photodynamic therapy (PDT) is a cancer therapy that combines the selective accumulation of a photosensitizer in tumor tissue with visible light (and tissue oxygen) to produce reactive oxygen species. This results in cellular damage and ablation of tumor tissue. The administration of the pro-drug aminolevulinic acid (ALA) or its methyl-ester (MAL) to cells results in the production of the photosensitizer protoporphyrin IX (PpIX) through the enzymes of the heme biosynthesis pathway. This temporarily accumulated PpIX can then be activated by visible light resulting in a photodynamic effect that is related to photosensitizer concentration. One proposed way of increasing the concentration of photosensitizer in cells is by the use of iron chelators. This has the potential to increase the accumulation of PpIX by reducing its subsequent bioconversion to heme. This research compares directly for the first time the effects of the novel hydroxypyridinone iron chelating agent CP94 and the more clinically established iron chelator desferrioxamine (DFO) on the enhancement of both ALA and MAL induced PpIX accumulation in cultured human cells. Cultured human cells were incubated with a combination of various ALA, MAL, CP94 and DFO concentrations and the resulting PpIX accumulations in the cells were quantified fluorometrically. The use of iron chelators in combination with ALA was shown to significantly increase the amount of PpIX accumulating within the cells. The use of iron chelators with MAL also resulted in a significantly increased accumulation of PpIX in the cells. Whether incubated with ALA or MAL, CP94 was shown to be significantly superior to DFO in the enhancement of PpIX accumulation in both normal and tumor cell types.

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Differential susceptibility of Candida albicans and Candida glabrata to hydrophobic and cationic photosensitizers. Yeissa M Chabrier-Roselló[1], Thomas H Foster[2], Soumya Mitra[2] and Constantine G Haidaris[1]. [1]University of Rochester School of Medicine and Dentistry, Rochester, NY, USA, [2]University of Rochester School of Medicine and Dentistry, Rochester, NY, USA.

Meeting the challenge of adaptive and inherent resistance of *Candida* species to currently used antifungal agents will require the development of novel strategies to control infection. Photodynamic treatment (PDT) represents a promising therapeutic approach for mucocutaneous and cutaneous candidiasis. Previous studies demonstrated that the clinically approved, hydrophobic photosensitizer Photofrin was capable of inducing photodynamic damage to *C. albicans* but not *C. glabrata* as measured by a metabolic assay. Successful photodynamic treatment of oral *C. albicans* infection using methylene blue in a mouse model by Teichert et al. (Oral Surg. Oral Path. Oral Rad. Endo. 93:155, 2002) prompted us to test an experimental cationic photosensitizer, meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP-1363; Frontier Scientific, Logan, UT) against *C. albicans* and *C. glabrata*. Organisms resuspended to a density of 10^8 cells/ml were exposed to increasing concentrations of TMP-1363 (0.1-10 micrograms/ml) for 5 minutes, and excess compound was removed by washing. Significant, photosensitizer dose-dependent inhibition of metabolic activity against both *C. albicans* and *C. glabrata* was observed using concentrations as low as 1 microgram/ml TMP-1363 at a fluence of 9 J/cm^2 compared to organisms treated identically but not irradiated. Furthermore, significant inhibition of metabolic activity was achieved using 3 micrograms/ml TMP-1363 against *C. albicans* biofilms at a fluence of 18 J/cm^2 compared to treated, unirradiated biofilms. The resistance of *C. glabrata* to PDT using Photofrin may be due to decreased cell association and/or increased efflux of Photofrin from the cell compared to TMP-1363. Demonstrating the feasibility of PDT with cationic photosensitizers against both *C. albicans* and *C. glabrata* is of clinical importance since successful treatment of *C. albicans* infection often results in emergence of a subsequent infection with a more resistant non-albicans *Candida* species such as *C. glabrata*. Supported by National Institutes of Health grant DE016537.

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Differential susceptibility of Candida albicans and Candida glabrata to hydrophobic and cationic photosensitizers. Yeissa Chabrier-Rosello. 124 Crittenden Way, #5, Rochester, NY, USA.

Meeting the challenge of adaptive and inherent resistance of *Candida* species to currently used antifungal agents will require the development of novel strategies to control infection. Photodynamic treatment (PDT) represents a promising therapeutic approach for mucocutaneous and cutaneous candidiasis. Previous studies demonstrated that the clinically approved, hydrophobic photosensitizer Photofrin was capable of inducing photodynamic damage to *C. albicans* but not *C.*

glabrata as measured by a metabolic assay. Successful photodynamic treatment of oral *C. albicans* infection using methylene blue in a mouse model by Teichert et al. (Oral Surg. Oral Path. Oral Rad. Endo. 93:155, 2002) prompted us to test an experimental cationic photosensitizer, meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP-1363; Frontier Scientific, Logan, UT) against *C. albicans* and *C. glabrata*. Organisms resuspended to a density of 10^8 cells/ml were exposed to increasing concentrations of TMP-1363 (0.1-10 micrograms/ml) for 5 minutes, and excess compound was removed by washing. Significant, photosensitizer dose-dependent inhibition of metabolic activity against both *C. albicans* and *C. glabrata* was observed using concentrations as low as 1 microgram/ml TMP-1363 at a fluence of 9 J/cm^2 compared to organisms treated identically but not irradiated. Furthermore, significant inhibition of metabolic activity was achieved using 3 micrograms/ml TMP-1363 against *C. albicans* biofilms at a fluence of 18 J/cm^2 compared to treated, unirradiated biofilms. The resistance of *C. glabrata* to PDT using Photofrin may be due to decreased cell association and/or increased efflux of Photofrin from the cell compared to TMP-1363. Demonstrating the feasibility of PDT with cationic photosensitizers against both *C. albicans* and *C. glabrata* is of clinical importance since successful treatment of *C. albicans* infection often results in emergence of a subsequent infection with a more resistant non-albicans *Candida* species such as *C. glabrata*. Supported by National Institutes of Health grant DE016537.

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Molecular determinants of photofrin -PDT and laserphyrin -PDT. Takeshi Hirata[1,*], Jitsuo Usuda[1,*], Shuji Ichinose[1,*], Keishi Ohtani[1,*], Kimito Yamada[1,*], Hidemitsu Tsutsui[1,*], Tetsuya Okunaka[1,2,*] and Harubumi Katou[1,*]. [1]First Department of Surgery, Tokyo Medical University, Tokyo, Japan, [2]Respiratory Disease Center Sanno Hospital, International University of Health and Welfare, Tokyo, Japan.

It is very important to elucidate the mechanism of action and identify molecular determinants, in order to increase the number of clinical applications and develop new photosensitizers. We have previously reported that photodynamic therapy (PDT) using some photosensitizers, such as phthalocyanine (Pc 4) damages anti-apoptotic protein Bcl-2, and that Bcl-2 is a molecular target of PDT. We examined the molecular targets of photofrin-PDT and laserphyrin-PDT, by evaluating the photodamage of Bcl-2. We found that Bcl-2 was a molecular determinant of photofrin-PDT but not laserphyrin-PDT. Our results show that laserphyrin-PDT does not damage Bcl-2 and Bcl-2 overexpressing cells are resistant to the sensitivity against PDT. Photofrin-PDT damages Bcl-2 and induces apoptosis earlier than laserphyrin-PDT. We conclude that photofrin-PDT damages different molecular targets from laserphyrin-PDT. Many advanced cancer cells have elevated amounts of Bcl-2 protein and we hypothesize that in most situations, Bcl-2 photodamage eliminates the normal protection against cell death. In this presentation, we evaluated the role of photodamage to Bcl-2 in regulating the fate of cancer cells after PDT using photofrin and laserphyrin, and we discuss the target molecules and new clinical applications. In addition, we could confirm depression of P 21 after PDT of laserphyrin in our own experiments example of DNA assay. P 21 works as controlling element in the down stream of P 53 which is apoptosis controlling factor for cell cycle in G1 term, and it is said to be relating to apoptosis. We suggest that laserphyrin-PDT strongly inhibits P 21 and activates Bax to induce apoptosis.

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Slug modulates hair growth in normal and UVR-exposed skin. Allison Parent*, Kimberly Newkirk* and Donna Kusewitt. Department of Veterinary Biosciences, Columbus, OH.

Slug is a member of the Snail family of zinc finger transcription factors involved in epithelial-mesenchymal transformation during development, carcinoma progression, and wound healing. Slug is expressed at low levels in many organs of the body, including the skin, where it is found primarily in hair follicles. Our studies suggest that Slug plays an important role in both normal and UVR-enhanced hair growth in the mouse. Onset of the first cycle of hair growth is delayed by at least 2 days in newborn Slug knockout mice compared to wild type mice. At birth, when anagen hair follicles are elongating, Slug expression is seen in differentiating keratinocytes located above the dermal papilla, in scattered cells within the dermal papilla, and in the elongating keratinocyte strand. By 3-5 days after birth, Slug expression is associated with the dermal papilla, adjacent hair bulb keratinocytes, and the outer root sheath of anagen follicles. There is little Slug expression in hair follicles at 12 d after birth, when most follicles are in catagen. In telogen hair follicles at 18 d after birth, Slug expression is largely restricted to cells of the dermal papilla. In response to shaving and ultraviolet radiation exposure, hair growth in mice is stimulated and the hair cycle is synchronized. Compared to wild type mice, Slug knockout mice exhibit a prolonged period of hair growth following shaving and UVR exposure. The first cycle of active hair growth in UVR-exposed Slug knockout lasts approximately 4 weeks longer than in wild type mice. The role of Slug in hair growth, both in newborn and UVR-exposed mice, is likely to be related to its known functions as a regulator of keratin expression, cell movement, and apoptosis.

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Comparative evaluation of fluorescence diagnosis in implanted human bladder cancer cells on chick chorioallantoic membrane using hypericin formulations with N-methyl pyrrolidone or albumin. Constance Lay Lay Saw[1,*], Paul Wan Sia Heng[1,*], William Wei Lim Chin[2,*], Khee Chee Soo[2,*] and Malini Olivo[2,*]. [1]Department of Pharmacy, National University of Singapore, Singapore, [2]Division of Medical Sciences, National Cancer Centre Singapore, Singapore.

The clinical application of hypericin (HY) in photodynamic diagnosis of bladder cancer had been demonstrated to perform better than white light endoscopy. However, further

studies are needed to make the use of HY better accepted clinically. Human plasma protein is currently the popular ÄeffectiveÄ HY transporter / carrier in use, but an alternative, an acceptable pharmaceutical solvent was proposed. We had reported that formulation of HY using a biocompatible solvent and penetration enhancer, N-methyl pyrrolidone (NMP) was effective for delivery of HY across the in vivo chick chorioallantoic membrane (CAM). This present study reports further investigation on the HY-NMP formulations in CAM implanted with human bladder cancer cells as a potential fluorescence diagnostic agent of cancer. The formulation of HY with human serum albumin (HY-HSA 0.5 %) used in clinics was included as a control group. The red to blue (I(R)/I(B)) intensity ratio of fluorescence images was used as a diagnostic algorithm, to differentiate the uptake of HY in the tumor and adjacent regions on CAM. Results indicated that HY-NMP 0.05 % was significantly better than HY-HSA 0.5 %. The findings of the I(R)/I(B) in tumor and adjacent tissues supported the potential usefulness of NMP as the alternative to human plasma protein, in clinical fluorescence diagnosis using HY. The NMP formulations investigated was able to produce a higher contrast between the tumor to adjacent tissues, at earlier time point intervals compared with HY-HSA at 0.5 %.

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Activation of the immune response against distant untreated lesions in recurrent angiosarcoma treated with photodynamic therapy. Patricia S Thong[1,*], Malini Olivo[1,*], Kiang-Wei Kho[1,*], Vanaja Manivasager[1,*], Ramaswamy Bhuvaneswari[1,*] and Khee-Chee Soo[1,2,*]. [1]Division of Medical Sciences, Singapore, Singapore, [2]Department of Surgery, Singapore, Singapore.

Background: The conventional treatment approach for angiosarcoma is radical surgery followed by post-operative radiotherapy. However recurrences are common, often at regional and distant sites and the five-year prognosis is poor. In this case report, we describe photodynamic therapy (PDT) using a novel chlorin-e6-based photosensitiser, as a promising alternative for treatment of recurrent angiosarcoma. Methods: A patient with recurrent multifocal angiosarcoma of the scalp and nape, and with distant recurrence on the arm, was recruited for PDT, which was carried out over several treatment sessions. Fotolon® (Haemato-science GmbH, Germany), a combination of chlorin-e6 and polyvinylpyrrolidone (PVP), was administered at a dose of 2.0-6.0 mg/kg body weight. 3 h later, the lesions were irradiated with 665 nm laser light for a dose of 65-200 J/cm2. Results: There were no observable side effects or complications arising during or after PDT. As early as four months after PDT, treated soft tissue is seen to heal well. Repeat PDT appeared to activate the patient's immune response against untreated neighbouring and distant lesions. Conclusions: Photodynamic therapy is a promising treatment option for recurrent angiosarcoma as it is safe and easy to carry out in an outpatient clinic and has no long-term side effects. It can be repeated as often as is needed to achieve local control of the disease. It also has the added advantage of being able to activate an immune response against distant untreated lesions.

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34 Regulation of tumor suppressor ING3 in response to UV radiation. Yemin Wang*, Marco Garate* and Gang Li*. Department of Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada.

The novel tumor suppressor ING3 has been demonstrated as a modulator of p53-mediated transcription, cell cycle control, and apoptosis. Our previous study showed that ING3 was DNA-damage inducible and promoted UV-induced apoptosis via caspase-8/Fas pathway in melanoma cells. However, the exact mechanism of ING3 upregulation by DNA damage agents remains unclear. In this study, we demonstrated that UV radiation not only upregulated ING3 protein in a dose-dependent manner, but also induced the re-distribution of ING3 between cytosol and nucleus. The accumulation of ING3 protein was dramatically increased in the nucleus after UV radiation. We also observed that only high dosage of UV radiation enhanced the mRNA level and promoter activity of ING3. On the other hand, the half-life of ING3 protein was increased three folds after DNA damage. In the presence of MG132, an inhibitor of proteasome, the ING3 level increased significantly, while no obvious induction of ING3 was observed after UV irradiation. Moreover, in the presence of staurosporine, an inhibitor of protein kinase C, UV-mediated induction of ING3 was blocked. This will be further confirmed by selective protein kinase C inhibitors or RNA interfering technology. In conclusion, our results showed that induction of ING3 by UV irradiation was regulated at both transcription and protein stability levels, which may involve the protein kinase C signaling pathway.

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35 Fluoroquinolone antibiotics having the potential to interfere with fluorescence-based diagnosis. L S Matchette*, Anant Agrawal* and T J Pfefer*. FDA Center for Devices and Radiological Health, Rockvilleq, Maryland.

Fluorescence, both intrinsic and exogenously induced, is being used for diagnosis of abnormal tissue. Excitation wavelengths used by these methods range from 320-450 nm. The presence of optically active (absorbing, fluorescing) drugs is rarely taken into account by practitioners of fluorescence diagnosis and has the potential to yield either false positive or false negative results. Therefore, we are measuring, in vitro, the relative quantum yields of fluoroquinolone antibiotics in an effort to identify those that require further characterization in vivo. Our aim is to quantify a potential to interfere by (1) comparing the relative quantum yield of fluoroquinolone antibiotics to those of known tissue fluorophores and (2) taking into account drug tissue concentrations during treatment. Quantum yields are determined relative to a working standard of Rhodamine 6G in ethanol. The working standard was calibrated against a fluorescein standard developed by the National Institute of Standards and Technology (NIST). We concentrated our initial efforts on (1) the fluoroquinolone antibiotics, ciprofloxacin, norfloxacin and ofloxacin and (2) the intrinsic tissue fluorophores, NADH, FAD and protoporphyrin IX. When ciprofloxacin, norfloxacin and ofloxacin were excited at wavelengths 310 to 390

nm, emission occurred from 350-650 nm with quantum yields ranging from 0.3- 0.03. Quantum yields for intrinsic fluorophores excited at their peak absorption wavelengths were 0.02 (NADH, 340 nm), 0.035 (FAD, 450 nm) and 0.087 (protoporphyrin IX, 408 nm). A review of the literature shows that these fluoroquinolones have a large volume of distribution and can be found in high concentrations in almost every organ during a treatment regime. The product of the drug tissue concentration and quantum yield, which we term the Fluorescence Effective Concentration, with certain assumptions, is such that it is likely these fluoroquinolones have the potential to interfere during fluorescence diagnosis techniques.

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36 Photoinactivation of clinical multi-drug resistant pathogens in Hong Kong. Christine M Yow[1,*], WM Lai[2,*], KC Wong[3,*], HM Tang[1,*], Ellie S Chu[1,*] and Ricky W Wu[1,*]. [1]Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong, HKSAR, [2]Department of Medical Microbiology, Prince of Wales Hospital, Shatin, Hong Kong, HKSAR, [3]Department of Microbiology, United Christian Hospital, Kwun Tong, Hong Kong, HKSAR.

Hypericin (HY) is one of the herbal therapeutic agents which has been extensively studied on tumor cells but nevertheless not on photobactericidal study. At present, methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* are three of the commonest multi-drug resistant (MDR) pathogens arouse worldwide concern. Here we explored the photodynamic inactivation (PDI) potential of HY on *S. aureus* (MSSA), wild type *Escherichia coli* and wild type *K. pneumoniae* and compared with their clinical MDR isolates: MRSA, ESBL *E. coli* and ESBL *K. pneumoniae*. All bacterial strains were sensitised with a range concentrations of HY (0.5 - 80 μM) for ½ hour and then exposed to light (10 - 30 Jcm^{-2}). Dark controls were included. After treatment, viable bacterial counts were enumerated for calculation of survival fraction. At 4 μM and 30 Jcm^{-2} light dose, HY showed complete killing of MSSA and MRSA. At 80 μM HY with 30 Jcm^{-2}, only 80% killing of *E.coli* (WT) and ESBL *E. coli*; whereas 50% killing of *K. pneumoniae* (WT) and ESBL *K. pneumoniae* were obtained. This suggested that Hypericin were effective to MRSA and MSSA but not the gram negative ESBL pathogens in this study. Acknowledgement: This project was supported by Internal Competitive Research Grant ICRG PolyU A-PF33, 2004.

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37 Reintroduction of a classic vitamin D UV source. R. M Sayre[1,2,*], J. C Dowdy[2,*] and J G Shepherd[3,*]. [1]Dept. of Medicine, Division of Dermatology, Memphis, TN, USA, [2]Rapid Precision Testing Laboratory, Cordova, TN, USA, [3]KBD, Inc, Cresent Springs, KY, USA.

Beginning around 1930 sunlamps claiming to provide ultraviolet (UV) exposure to make vitamin D were sold to the public in the US and Canada for home use. Demand for such lamps declined, following the advent of vitamin D fortification of foods, until they were generally discontinued about 1960. With the recent realization that even with dietary supplementation many people do not get enough solar UV exposure to maintain sufficient vitamin D levels, there has been growing interest in the availability of sunlamps for this purpose. The original Sperti Sunlamp consisted of a filtered intermediate pressure mercury lamp, approved by the American Medical Association in 1940 as a sunlamp, with a label claim noting that vitamin D would occur due to exposure. In an intermediate pressure mercury lamp's spectrum are Ultraviolet B emission lines at 297, 302, and 313 nm able to convert 7-dehydrocholesterol in the skin to previtamin-D3 initiating the natural process of vitamin D formation. Today's KBD vitamin D Lamp, an updated model of the earlier type source, still employs an intermediate pressure mercury source. However, in order to comply with modern safety guidance, the source is better filtered to remove unnecessary UVC radiation and is equipped with a timer to control the dose administered. The 5 minute timer provides an exposure, at 20 inches from the user's skin, of 1 Standard Erythemal Dose (SED). The SED, an internationally standardized measure of erythematogenic UV radiation equivalent to an erythemal effective exposure of 10 mJ/cm^2, represents a sub-erythemal dose for even the most sensitive skin type I individual. This lamp will be compared to other sources including sunlight in its ability to photo initiate vitamin D formation.

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38 Further characterisation of UV-induced regulatory T cells. Agatha G Schwarz*, Akira Maeda* and Thomas Schwarz*. Department of Dermatology, University Kiel, Schittenhelmstrasse 7, Kiel, Germany.

Painting of contact allergens onto UV-exposed skin results in tolerance which is mediated via regulatory T cells (Treg) which act in an antigen-specific fashion and express CD4 and CD25. In contrast to the classical CD4+CD25+ Treg which act in a contact-dependent manner, UV-induced Treg suppress mostly via the release of IL-10, indicating that they may represent a distinct subtype of Treg. This study was performed to further characterize these cells. Transfer of Treg generated by sensitization with dinitrofluorobencene (DNFB) through UV-exposed skin into naive mice inhibited the sensitization against DNFB. Upon depletion with antibodies directed against the glucocorticoid inducible TNF family-related receptor (GITR) or the surface molecule neuropilin, transfer of suppression was lost. Furthermore, UV-induced Treg express FoxP3. UV-induced Treg act antigen-specific since injection of DNFB-specific Treg into the ears of oxazolone (OXA)-sensitized mice does not affect the OXA challenge. However, the OXA challenge is suppressed when DNFB-specific Treg are activated before OXA challenge with DNFB. Hence, activation of Treg is antigen-specific, however, once activated their suppressive activity is non-specific (by-stander suppression). Thus speculations exist about the therapeutic potential of Treg generated in response to antigens that are not necessarily the precise antigen

driving the pathogenic process. Therefore, we asked whether multiple injections of DNFB-specific Treg into ears of naive mice followed by multiple DNFB challenges finally result in sensitization against DNFB. DNFB-specific Treg were injected once per week into the left ears of naive mice and DNFB challenge performed 24 h later. After 3 injections a challenging dose of DNFB was applied on the right ear. This resulted in a pronounced ear swelling, indicating that subsequent boosting of Treg had caused sensitization. These data demonstrate that UV-induced Treg express GITR, neuropilin as well as FoxP3 and that they act via by-stander suppression. However, constant boosting of Treg with antigen doses in the challenging range results in sensitization which may limit their therapeutic potential.

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39 The interaction of the photobactericides methylene blue and toluidine blue with a fluorophore in the *P. aeruginosa* cells. Marina N Usacheva*, Matthew C Teichert*, Chet E Sievert* and Merrill A Biel*. Advanced Photodynamic Technologies, Inc. 2520, University Ave SE, Suite 101, Minneapolis, Minnesota.

The involvement of the outer membrane proteins along with lipopolysaccharides (LPS) in the bacterial photodamage induced with methylene blue (MB) and toluidine blue (TB) and red light could clarify the reasons for the MB and TB different photobactericidal efficacy. One way to bind the dyes to outer membrane proteins is using siderophores to form a siderophore-receptor protein complex linking the protein. This study is aimed to determine the interaction between the dyes and the bacterial receptor protein-siderophore system taking advantage of the strong fluorescence of pyoverdin, a siderophore responsible for *P. aeruginosa* fluorescence in the visible spectral region. An investigation of the fluorescence of *P. aeruginosa* cells excited at 488 nm in the presence of increasing dye concentrations was carried on using confocal laser scanning microscopy. It was demonstrated that the intensity of cell fluorescence at 522 mn was progressively decreased with the dye increasing concentrations. Regardless of the dye concentration, no emission was observed at 680 nm (peak of dye emission), when fluorescence was excited at 488 nm. The Stern-Volmer constants of cell fluorescence quenching with the dyes were evaluated and compared to the association constants of the dye's complexes with LPS. Analysis of the results suggests that the quenching of the *P.aeruginosa* fluorescence was associated with the formation of ground-state complexes between the dyes and the pyoverdin-FpvA protein system, where MB was more effective than TB. In contrast, TB formed far firmer complex with LPS P.a characterized by an association constant, which is an order higher than that of the pyoverdin-FpvA protein system. The different affinity of the dyes for the pyoverdin-protein and LPS $_{P.a.}$ will affect the contribution of dye interactions with these biopolymers in the bacterial photodamage.

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40 Usefulness of photodynamic therapy with 5-aminolevulinic acid (ALA-PDT) for skin invasion of breast cancer. Kimito Yamada[1,2,*], Jitsuo Usuda[1,*], Takeshi Hirata[1,*], Keishi Ohtani[1,*], Mitsuhiro Kubota[1,*], Akihiko Ogata[1,2,*], Norio Kohno[2,*] and Harubumi Kato[1,*]. [1]1st. Dept. of Surgery, Tokyo Medical University Hospital, Tokyo, Japan, [2]Dept. of Breast Oncology, Tokyo Medical University Hospital, Tokyo, Japan.

We experienced a case of breast cancer with skin invasion. Photodynamic therapy with 5-aminolevulinic acid (ALA-PDT) was useful for quality of patient's life as one of multidisciplinary therapy. **Case** 76 year-old woman. divorced. Family history (-). She noticed a breast tumor in her left breast 3 years ago, but she left alone. And these 2 years her sister had disinfected every day. Because the tumor invaded to skin, wetty and often bled. In October 2003, she was referred to our department from her home doctor for more detailed examination and treatment. On her first visit to our department, the tumor directly invaded to skin, major pectoral muscle, intercostal muscle, with necrosis and infection. Chest CT revealed that main tumor was 15x12x4cm in diameter and there was metastasis into the left breast, 4x3x2cm in diameter with Axillar LN metastasis. Bone scintigram revealed the metastasis of Th10-L1,3-5. Result of biopsy was Invasive ductal carcinoma, ER3+, PgR3+, HER2/nue1+. The number of CA15-3 was raised to 56.0(>28) c-T4N3M1§ageIV. So systemic chemotherapy was performed. (DTX25mg+Epirubicin25mg/m2)x10 times. (2003,11-2004,3) Concurrently ALA-PDT, 20% ALA white vaserin 10g, ALA 2.5g, 4 Hr rapping 200J/cm2,630nm), irradiated by Excima dye laser.(2003.12/25)After that the skin lesion got scar and surface got dry. She could take a bath. Following Radiation Tx(Linac Xray 4mV) was performed of total 50 Gy. (2004,4-6) Pamidoronate 90mg was dripped per 4 weeks for bone metastasis. Anastrozole 1mg per day. Now she attends our hospital without recurrence.

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41 Evaluation of two-photon excitation of novel photosensitizer chlorin e6 C15 monomethyl ester. Ping Chen[1,*], Guoqing Tang[1,*], Lie Lin[1,*], Jianzhong Yao[2,*] and Zheng Huang[3,*]. [1]Institute of Modern Optics, Tianjing, P. R.China, [2]College of Pharmacy, Shanghai, P. R. China, [3]Radiation Oncology Department, Aurora, CO, USA.

Two-photon excitation (TPE) has received increasing attention due to its potential applications in two-photon fluorescent microscopy and imaging, three-dimensional optical data storage, fabrication of photonic crystal and photodynamic therapy (PDT). There are several advantages when using femtosecond laser as a light source for PDT because TPE offers a high peak power with a comparatively low average power. The linear absorption by biomaterials may be weak at the wavelength range where the two-photon absorption (TPA) occurs therefore lowering collateral damage to healthy tissue and more precise delivery of light energy to target tissue with a high degree of spatial specificity. A femtosecond laser two-photon excitation system has been used to study the photo-

dynamic effect of chlorin e6 C15 monomethyl ester. Our previous studies demonstrate that this novel photosensitizer has a high quantum yield of singlet oxygen compared to chlorine e6 and has a considerably strong frequency-up-converted fluorescence emission while being excited at 800 nm with TPE and a considerable large TPA cross-section. In this pilot study we tested its photodynamic effect on human liver carcinoma cells. Light pulses of 800 nm were generated by two femtosecond laser sources at a repetition rate of 100 MHz with a pulse width of 60 fs. Light was delivered to the cancer cells in the presence of a low concentration of chlorin e6 C15 monomethyl ester. Eosine stain was utilized to identify TPE PDT induced cell death. Microscopic examination showed that chlorin e6 C15 monomethyl ester mediated TPE PDT induced noticeable morphological changes, cell death and possible bystander effects - indicating a strong photodynamic effect. The intracellular site-specific TPE PDT and its effect will be further studied.

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42 The relation between cytokine expression induced by photodynamic therapy and anti-tumor effect. Keishi Ohtani*, Jitsuo Usuda*, Tatsuya Inoue*, Takeshi Hirata*, Syuji Ichinose*, Yukari Kuroiwa*, Tetsuya Okunaka* and Harubumi Kato*. Shinjuku-ku Nishi-shinjuku 6-7-1, Tokyo.

Photodynamic therapy (PDT) is an effective treatment for malignant tumor, which consists of exposing lesions to a tumor-specific photosensitizer and light. It is approved for use as a primary therapy for early stage of lung cancer locating at hilum of lung. A problem to be solved is to expand the adaptability of PDT for the advanced lung cancer, especially to bring about stenosis of the airway. We examined about immune response and anti-tumor effect after PDT by molecular biological approach. Lewis lung carcinoma (LLC) cells were treated by laserphyrin-PDT. After that the expression of mRNA of IL-2, IL-6, TNF-alpha and INF-gamma increased. Therefore, in order to make clear the relation between the expression of cytokines and anti-tumor effect, we examined the photosensitivity of cytokine-gene-transfected cells, namely LLC/IL-2, LLC/IL-6 and LLC/INF-gamma in vitro and in vivo. As a result of colony assay in vitro, LLC/IL-2 cells were not effective in comparison with LLC cells. And next, we examined the anti-tumor effect of laserphyrin-PDT to tumor that transplanted in C57BL/6J mouse. As a result of this, the recurrence rate of LLC/IL-2 was increase and the survival rate of LLC/IL-2 was decrease. Therefore, we conceived that IL-2 inhibited the anti-tumor effect of PDT. This result suggested that PDT could become more effective by using a molecule targeted therapeutic drug and chemotherapy together.

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43 Human Bruch's membranes include blue light absorbing products of lipid oxidation that act as potent photosensitizers. Malgorzata Rozanowska[1,*], Mike Boulton[1,*], Matthew Davies[1,*], Anna Pawlak[2,*] and Bartosz Rozanowski[3,*]. [1]School of Optometry and Vision Sciences, Cardiff, United Kingdom, [2]Department of Biophysics, Krakow, Poland, [3]Department of Genetics and Cell Biology, Krakow, Poland.

Bruch's membrane (BrM) is a 5 layer structure that separates the retina from the choroid. With age it accumulates lipids, products of lipid peroxidation, and protein crosslinks that result in a decrease of its permeability. Our aim was to determine the susceptibility of human BrM to photooxidation and its ability to photogenerate reactive oxygen species. BrMs were isolated from human eyes from donors above 60 years old. Mechanically homogenized BrMs were subjected to chloroform/methanol extraction and extracts were used either for preparation of liposomes or were solubilized in an organic solvent. Electron spin resonance (ESR) oximetry, iodometric assay of lipid hydroperoxides, TBA-assay for detection of secondary poducts of lipid peroxidation, ESR spin trapping, laser flash photolysis combined with absorption spectroscopy and time-resolved detection of singlet oxygen phosphorescence were used to investigate the susceptibility of BrM extract to light-induced oxidation and determination of its photosensitizing properties. Absorption spectrum of solubilized BrM extract exhibited a steep increase with decreasing wavelengths. The rates of photo-induced oxygen uptake in suspension of BrM liposomes exhibited an increasing efficiency with decreasing irradiation wavelengths within the range of 500 to 312 nm when normalized to equal number of incident photons. This action spectrum was similar to BrM absorption spectrum and to the action spectrum for oxidized docosahexaenoic acid. Light-induced photooxidation was accompanied by loss of unsaturated lipids, accumulation of lipid hydroperoxides, and formation of TBA-reactive substances. Irradiation of BrM in the presence of a spin trap, DMPO led to the formation of DMPO-OOH radical adducts. Photoexcitation of solubilized BrM extract with 355 nm or 420 nm laser light led to photosensitized generation of singlet oxygen with quantum yields up to 16%. In conclusion, human BrM from elderly donors is susceptible to light-induced oxidation and contains potent photosensitizers generating reactive oxygen species and lipid peroxidation when excited with ultraviolet or blue light. It can be speculated that irradiation with blue light of BrM may decrease its permeability and prove toxic to cells of neighbouring retinal pigment epithelium and/or choroid.

44

44 Sunscreens and UVA protection - In silico experiments to meet established and emerging standards for assessment of UVA protection. Uli Osterwalder[1,*], Stefan Mueller[2,*], Anna Gril[1,*] and Bernd Herzog[2,*]. [1]Ciba Specialty Chemicals Inc., Basel, Switzerland, [2]Ciba Specialty Chemicals Inc., Grenzach-Wyhlen, Germany.

The need for UVB and UVA protection during work and leisure is generally recognized. Over the last 5 years new UVA and broad-spectrum UV filters such as Bisoctrizole (Tinosorb® M) or Bemotrizinol (Tinosorb® S) became available. This allows the formulators to create sunscreens with superior broad-spectrum protection than ever. The open question in most countries still is how to communicate the degree of UVA protection to the consumer. We present a brief overview of the available methods for assessment of UVA protection. Besides established UVA methods and standards such as Persistent Pigment Darkening (Japan),

Australian UVA standard, UVA/UVB ratio (United Kingdom) and UVA balance (Germany), new methods, e.g. an improved UVA balance that takes into account photostability, are emerging. Besides the in vivo and the in vitro methods a third category - in silico - is now emerging. By simulation of the UV protection performance of sunscreens, new insight how to meet established and emerging standards can be gained. The latest simulation program is based on the absorption spectra of the individual UV filters and assumptions on the non-uniform sunscreen film on the skin. Photostability and synergy between UV filters are also taken into account. Simulation examples with conventional and new UV filter combinations are presented.

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45 Is the importance of UVB risk now being systematically underestimated? John C Dowdy[1,*], Robert M Sayre[1,2,*] and Julian M Menter[3,*]. [1]Rapid Precision Testing Laboratory, Cordova, TN, USA, [2]Divison of Dermatology Department of Medicine, Memphis, TN, USA, [3]Morehouse School of Medicine, Atlanta, GA, USA.

Examination of a series of human and animal UV action spectra in the photomedical and photobiological literature suggests that systematic errors may have significantly distorted risks presented in a number of studies. These systematic problems can be described as poor stray light rejection in monochromators, scatter around broadband filters, band width of monochromatic bands, and failure to specifically measure and account for low levels of effective radiation in polychromatic sources. Extrapolation and interpolations in generalized action spectra have tended to artificially worsen the effects of these systematic problems. Failure to account for these errors has resulted in overestimation of the effects of UVA while underestimating the relative effects of UVB. Such errors of increased UVA sensitivity in action spectra especially decrease the relative significance of the UVB in sources rich in UVA such as sunlight. Consequently, as changes in ozone level proceed, risk evaluations based on disproportionate spectral hazard weighting may significantly undervalue the effect of increasing UVB while over emphasizing the unchanging levels of UVA. This net underestimation of the impact of ozone depletion has serious implications upon assessment of deleterious long term biological consequences.

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46 The effects of topical application of creams containing glycolic acid or salicylic acid on the photocarcinogenicity of simulated solar light in male and female SKH-1 mice. Paul C Howard[1,*], Barbara J Miller[1,*], Brett T Thorn[2,*] and Paul W Mellick[3,*]. [1]Division of Biochemical Toxicology, Jefferson, AR, USA, [2]Z-Tech Incorporated, Jefferson, AR, USA, [3]Toxicologic Pathology Associates, Jefferson, AR, USA.

Topical creams containing alpha or beta hydroxy acids are used on solar exposed skin. In order to determine the effect of these keratolytic agents on sunlight induced skin cancers, a 52-week study photococarcinogenesis study was conducted on male and female Crl:SKH-1 (hr^-/hr^-) hairless mice treated topically with creams containing 4% or 10% glycolic acid (pH 3.5) or 2% or 4% salicylic acid (pH 4) administered simulated solar light (SSL) from filtered 6.5 kW xenon arc lights. Starting at 8 weeks of age, the mice were treated each morning (5 days/week) with the creams and each corresponding afternoon with 0, 6.85, or 13.7 mJ-CIE/cm^2 SSL. Untreated mice were exposed to the same doses of SSL including 20.55 mJ-CIE/cm^2. Mice were weighed weekly and the location, size and number of skin lesions were recorded. The treatment continued for 40 weeks and the mice observed for an additional 12 weeks prior to sacrifice. The mice were sacrificed at the 52 week completion of the study, or during the study if any lesion reached 10 mm. All mice were necropsied and skin lesions were examined by histopathology and classified. The tumor indices that were quantified were mean time to 1 mm tumor, tumor multiplicity, and histopathological diagnosis. SSL induced a dose-dependent increase in all tumor indices regardless of treatment. The control vehicle decreased the time-to-lesion in SSL-exposed mice when compared to control. The inclusion of glycolic acid in the cream did not affect time-to-lesion, or tumor incidence or multiplicity, with the exception of an increase at 10% at 6.85 mJ-CIE/cm^2 in male mice. The inclusion of salicylic acid in the creams had a photoprotective effect, increasing the time-to-lesion, and decreasing tumor incidence and multiplicity. (*The contents of this abstract should not be considered FDA policy*).

47

47 Photodegradation quantum yields of chlorinated phenothiazines. Luis E Piñero[1,*], Carmelo Garcia[1,*], Rafael Arce[2,*] and Rolando Oyola[1,*]. [1]University of Puerto Rico at Humacao, Humacao, PR, Puerto Rico, [2]University of Puerto Rico at Rio Piedras, San Juan, PR, Puerto Rico.

The mechanism that induce the phototoxic response of 2-chlorophenothiazine derivatives is still unknown. To better understand the relationship between the molecular structure of phenothiazines and their phototoxic activity, we studied the photophysics and photochemistry of 2-chlorophenothiazine derivatives in several solvents, determined their photodestruction quantum yields under anaerobic conditions using monochromatic light (313 nm) and performed theoretical calculations to explained our experimental results. Using absorption- and emission-spectroscopy, ^{1}H- and ^{13}C-NMR, GC-MS, and steady-state photolysis, we demonstrated that the photochemistry of 2-chlorophenothiazines under aerobic conditions in organic solvents does not induce the drug photodegradation. This in fact implies that, whatever amount of singlet oxygen is produced, it does not react with the drug ground state. Nevertheless, photodegradation is observed in air saturated aqueous solutions, indicating that the photochemistry of 2-chlorophenothiazine derivatives in water is very different. The photochemistry of these compounds in organic polar solvents (such as methanol and acetonitrile) under anaerobic conditions induces photodegradation, but not if the solvent is non-polar. Therefore, we postulate a "triplet state-polar solvent" complex, which is responsible for the neutral radical formation and consecutive photore-

actions. The photodestruction quantum yield of 2-chlorophenothiazine derivatives depends on the solvent, but not on the 10-substituent. The distribution of photoproducts, on the other hand, strongly depends on the 10-substituent, but not very much on the solvent. Finally, it was demonstrated that the phototoxic effect of chlorinated phenothiazines is not an intrinsic property of neither of their transients. This phototoxic response can not be attributed to singlet oxygen either, because all phenothiazine derivatives (chlorinated and non-chlorinated) have the same singlet oxygen quantum yield. We conclude that, whatever is inducing the phototoxic side-effect, must include - at least - one drug intermediate and one membrane component.

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48 Enhancement and reduction of aminolevulinic acid (ALA) mediated phototoxicity on EGFR positive cells by epidermal growth factor (EGF) and inhibitors of the EGF Receptor. Allison M Marrero[1,2,*], Weiguo Liu[1,2,*], Janet Morgan[1,*], Mary Jo Bowman[1,*] and Allan R Oseroff[1,*]. [1]Dermatology, Buffalo, NY, [2]Molecular Pharmacology & Cancer Therapeutics, Buffalo, NY.

Binding of epidermal growth factor (EGF) to its receptor (EGFR) normally stimulates cell proliferation, differentiation and survival. Tumor cells often express elevated levels of EGFR, which can influence tumor growth via survival pathways. FaDu cells (human head and neck squamous cell carcinoma cell line) express elevated levels of EGFR. EGFR inhibitors (EGFR-I), such as Iressa and AG1478, when combined with ALA-PDT for treatment of EGFR positive cells (FaDu) enhance the anti-tumor response, possibly by inhibiting EGF-stimulated signaling pathways, growth arrest and morphological changes. Further investigation found that EGFR-I administered prior to Photodynamic Therapy (PDT) caused increased phototoxicity, the greatest effects occurring after 24h exposure prior to ALA-PDT. Cells were arrested in G0/G1 with decreased cells in S-phase and G2/M, however the overall PpIX was increased by an unknown mechanism. EGFR-I also caused increased peripheral benzodiazepine receptor (PBR) expression, which correlates with increased PpIX synthesis. Conversely, if cells were stimulated with EGF for 24 h, the PpIX levels decreased and the cells were protected from phototoxicity. The effect of EGF alone on FaDu cell growth and survival is minimal over 4-24h. However the literature suggests that EGF can stimulate the induction of BCRP/ABCG2 in EGFR+ cells (such as head and neck cancers), if they are exposed to it for 24h. This has been demonstrated as an increase in the side population, the supposed cancer stem cells, which are defined by their expression of BCRP. We hypothesize that when EGF stimulation is combined with ALA-PDT, induction of the BCRP causes efflux of PpIX, which decreases phototoxicity and enables proliferation. In contrast, EGFR-I decreases the effects of EGF on side population maintenance and expansion, PpIX levels are maintained, and the side population decreases, demonstrated as decreased survival.

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49 Photodynamic gene therapy for lung cancers. Jitsuo Usuda[1,*], Shuji Ichinose[1,*], Takeshi Hirata[1,*], Keishi Ohtani[1,*], Kimito Yamada[1,*], Kinya Furukawa[2,*], Tetsuya Okunaka[3,*] and Harubumi Kato[1,*]. [1]6-7-1, Nishishinjuku, Shinjuku-ku, Tokyo, Japan, [2]3-20-1, Chuo, inashikkigun, amimachi, Ibaraki, Japan, [3]8-10-6, Akasaka, Mimatoku, Tokyo, Japan.

Photodynamic therapy (PDT), a treatment for cancer, utilizes a photosnesitizer and laser irradiation to produce reactive oxygen in cells. In Japan, the United States and other many countires, PDT is a standard treatment option for stage 0 (TisN0M0) and stage I (T1N0M0) centrally located early stage lung cancer. PDT can preserve lung function, can be repeated and be used by combined with chemotherapy. Recently, mono-L-aspartyl chlorine e6 (NPe6, Laserphyrin), which was a second generation photosensitize and low photosensitivity, was approved by the Japanese government and the phase II clinical study using the photosensitizer and a new diode laser demonstrated excellent anti-tumor effect and low skin photosensitivity. NPe6 has an absorption profile that closely matches the emission profile of chemiluminescence using Renilla luciferase/coelenterazine. In this study, we proposed that coelenterazine and NPe6 when taken up by Renilla luciferase-transfected cells, as a consequence of their intracellular proximity, produce sufficient light to release singlet oxygen by photoactivation and to trigger individual cell death. We examined the chemiluminescence could kill cancer cell. We transfected Renilla luciferase gene into A549, lung cancer cells. Eight h after the transfection, the cells were treated with NPe6 and coelenterazine. Twenty four h after the transfections, we observed typical morphologicaly apoptosis in A549 cells using fluorescent microscope. These results indicate that the intrafluorescence can produce a high quantum yield of singlet oxygen and kill cancer cells, and this new cancer treatment, photodynamic gene therapy, using trasfection of Renilla lucuferase and photosensitizer, can induce apoptosis. In the future, photodynamic gene therapy without laser irradiation can treat solid tumors through the production of intracellular light.

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50 Photodynamic therapy using Laserphyrin (NPe6) for centrally located early lung cancer. Yoshihiko Tsunoda*, Jitsuo Usuda*, Shuji Ichinose*, Keishi Ohtani*, Takeshi Hirata*, Kimito Yamada*, Kinya Furukawa* and Tetsuya Okunaka*. Dept. of Thoracic Surgery, Tokyo, JAPAN.

In central type early stage lung cancer, the tumor must be located only as far as the segmental bronchi and be carcinoma in situ or with only limited invasion into the bronchial wall. Sputum cytology examinations are the only means available for detection of centrally located early lung cancer, especially roentgen graphically occult lung cancers. From February 1980 to December 2005, a total number of 204 patients with 264 lesions of centrally located early stage lung cancer underwent photodynamic therapy (PDT) in the Department of Thoracic Surgery, Tokyo Medical University Hospital. The histological type was squamous cell carcinoma

in 258 lesions. There were 185 clinical stage 0 lesions and 79 stage I lesions. CRs and PRs were obtained in 224 lesions (84.8%) and 40 lesions (15.2%) out of 264 lesions. The 264 lesions were classified in four groups according to maximum longitudinal tumor extent. Of these, 56 lesions were less than 0.5 cm, 124 lesions were 0.5 less than 1.0 cm, 50 lesions 1.0 cm less than 2.0 cm, 34 lesions 2.0 cm in maximum diameter. The CR rates were 94.6%, 93.5%, 80%, 44.1% respectively. As a photosensitizer, we used Photofrin or Laserphyrin (mono-L-aspartyl chlorine e6, NPe6). After a phase II clinical study, Laserphyrin was approved by the Japanese government and has been on sale from June 2004. From July 2004 to December 2005, we performed Laserphyrin-PDT for 28 lesions of centrally located early stage lung cancer in Tokyo Medical University Hospital. Four h after the administration of Laserphyrin 40 mg/m2, we irradiated using diode laser (100 mJ/cm2). Before PDT, we evaluated the tumor lesions and tumor depth using autofluorescence bronchoscopy and endobronchial ultrasonography (EBUS), and we confirmed the area of laser irradiation. The rate of CR was 92.9% (26lesions) in 28 lesions. For Laserphyrin-PDT, Skin photosensitivity was very low and the clean-up bronchoscopies were not frequently needed, and the period of hospitalization was shorter compared to that for Photofrin-PDT. We conclude that PDT using Laserphyrin will be a standard option for stage 0 (TisN0M0) and stage I (T1N0M0) centrally located early stage lung cancer.

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51 Increased expression of p53 enhances transcription-coupled repair of UVC-induced DNA damage in human cells. Diana Dregoesc and Andrew J Rainbow. Department of Biology, Hamilton, Ontario, Canada.

The p53 tumour suppressor protein has many roles in cellular functions that protect the cell from uncontrolled proliferation, including DNA repair. Ultraviolet (UV) light-induced DNA damage is repaired by nucleotide excision repair (NER), which is divided into two subpathways: global genome repair (GGR) and transcription-coupled repair (TCR). While it is generally accepted that p53 contributes to GGR, the involvement of p53 in TCR remains controversial. In the present work, we investigated the role of p53 in both GGR and TCR of UVC-induced DNA damage in human fibroblasts. We employed a non-replicating recombinant human adenovirus, AdCA17lacZ that can efficiently infect human fibroblasts and express the beta-galactosidase (beta-gal) reporter gene under the control of the human cytomegalovirus promoter. We examined host cell reactivation (HCR) of beta-gal expression for the UVC-treated reporter construct in normal human fibroblasts and in xeroderma pigmentosum (XP) and Cockayne syndrome (CS) fibroblasts deficient in GGR, TCR, or both. HCR was examined in fibroblasts that had been pre-infected with Ad5p53wt (which results in increased expression of p53) or a control adenovirus, AdCA18 (which expresses the luciferase gene). HCR in pre-Ad5p53wt-infected cells was significantly enhanced in normal, CS-B (TCR-deficient), and XP-C (GGR-deficient), but not XP-A (TCR- and GGR-deficient) fibroblasts compared to that in pre-AdCA18-infected cells when beta-gal expression was examined at 12 hr after infection. The increased HCR in pre-Ad5p53wt-infected cells persisted in CS-B, but not in normal and XP-C cells, until at least 24 hr after infection. In addition, when cells were also pre-treated with UVC, pre-Ad5p53wt-infected XP-C cells showed increased HCR compared to pre-Ad5CA18-infected XP-C cells when beta-gal was examined at both 24 and 40 h after infection. These results indicate that increased expression of p53 results in an enhancement of both the TCR and GGR pathways of UVC-induced DNA damage in human cells.

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52 Quantitative assessment of genotoxicity induced by simulated solar UV on reconstructed skin Episkin. Laurent Marrot*, Jean Philippe Belaidi*, Christophe Jones*, Philippe Perez* and Jean Roch Meunier*. L'OREAL Phototoxicity 1 av. E. Schueller, Aulnay sous Bois, France.

Even if cultured normal human keratinocytes constitute a relevant and validated model for photogenotoxicity studies, it is important to also get information when these cells are in a conformation closer to this of human skin. In this regard, reconstructed skin models, such as Episkin, are very convenient. Numerous studies on photobiology have been reported with those models. However, in most cases, data were obtained thanks to immuno-histochemistry, a technique which is generally rather qualitative than quantitative. Here, the reconstructed skin Episkin (including dermis with fibroblasts embedded in collagen) was exposed to simulated solar UV radiation comparable to zenithal sunlight in terms of spectral power distribution. Biological endpoints related to genotoxicity were then quantitatively assessed. A dose dependant induction of DNA breaks and pyrimidine dimers was observed using an adapted protocol of the single cell gel electrophoresis (comet assay). Thanks to an ELISA approach, we observed that p53 accumulation, analyzed 24 hours post UV, reached a plateau for exposure times longer than 15 minutes. Finally, expressions of genes controlled by p53 were measured by quantitative RT-PCR: (i) p21 and GADD45 were highly induced; (ii) at UV doses where XPC expression was doubled, this of p48 was not significantly stimulated; (iii) a clear induction of MDM2 required relatively high UV doses. This study shows that reconstructed skin can be a precious tool as a part of in vitro strategies aiming at assessing photoprotection and photogenotoxicity.

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53 A novel polymer-based formulation is safer and more effective than acetone for the delivery of topical caffeine to mediate Ultraviolet B radiation-induced skin cancer in mice. Coimbatore s Sreevidya*, Mehmoosh Ghorbani*, Bakul Bhatt*, Gregg Siegel* and Stephen E Ullrich*.

Caffeine has been shown to decrease the incidence and progression of skin cancer in an ultraviolet (UV) radiation exposed mouse model. Most studies have used acetone or ethanol as a vehicle for topical application of caffeine, but used repeatedly, they can dehydrate the skin and cause further damage leading to poor delivery of caffeine. We assessed whether N-polymer, a novel combination of film-

forming polymers, could be an efficient and safe vehicle for topical caffeine application to inhibit skin cancer initiation and progression using high-risk hairless SKH-hr1 mice. Mice were exposed to 5 kJ/m ^{2} UVB radiation three times a week for 8 weeks and then treated for 32 weeks with 1.2% caffeine dissolved in N-polymer or acetone. We found that tumor incidence, multiplicity and size were significantly lower when N-polymer rather than acetone was used as the vehicle. These results indicate that caffeine inhibits UV radiation-induced skin cancer in mice and that N-polymer is a more effective vehicle than acetone for delivering topical caffeine. Mice skin treated with N-polymer were normal whereas acetone treated mice developed wrinkles, looked dry and pale.

54 UVA light and singlet oxygen quantum yield of endogenous photosensitizers determined directly by its luminescence. Juergen Baier[1,*], Claudia Poellmann[1,*], Tim Maisch[2,*], Max Maier[1,*] and Wolfgang Baeumler[2,*]. [1]University of Regensburg / Physics, Regensburg, Germany, [2]University of Regensburg / Dermatology, Regensburg, Germany.

The UVA component of solar radiation has been shown to produce deleterious biological effects in which singlet oxygen plays a major role. In tissue the UVA light is only weakly absorbed by a limited number of molecules, which may act then as photosensitizer. After UVA light absorption, the photosensitizer molecules cross over to its triplet state and transfers energy to generate singlet oxygen. To provide doubtless evidence for a correlation of UVA damage in tissue and singlet oxygen, it must be shown that these endogenous photosensitizers generate singlet oxygen to a sufficient extent. Comparable to exogenous photosensitizers, the efficacy of singlet oxygen generation (quantum yield) must be determined. In the present experiments flavins, NADH/NADPH, urocanic acid or different fatty acids where investigated. These endogenous photosensitizers were excited in the range of UVA light using a Nd:YAG laser at 355 nm. Singlet oxygen was detected directly by its time resolved luminescence at 1270 nm. The respective decay rates and rate constants of singlet oxygen were determined, in particular at different oxygen concentrations. The singlet oxygen quantum yield (Φ_Δ) could be calculated. For e.g. riboflavin in fully aerated solution of H_2O, a singlet oxygen quantum yield of $\Phi_\Delta = 0.54 \pm 0.07$ was determined. That value is comparable to exogenous photosensitizers used in photodynamic therapy (Photofrin $\Phi_\Delta = 0.33$). The singlet oxygen quantum yield depends critically on the oxygen concentration, i.e. the oxygen partial pressure (pO_2) in the respective experimental setup. That is important when comparing experiments of in vitro ($pO_2 \sim 150$ mmHg) and conditions in vivo such as the skin ($pO_2 < 20$ mmHg). The results show a decrease of Φ_Δ with decreasing oxygen concentration. Our investigations provide clear evidence that UVA light at 355 nm generates singlet oxygen in endogenous sensitizers such as flavins, urocanic acid or fatty acids.

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55 Polymorphism of the XPD DNA repair gene is associated with an early onset of basal cell carcinoma. Edu Suarez[1,2,*], Abigail Ruiz[1,*], Jocelyn Matias[2,*], Rafael Ortiz[2,*], Jaime R Villa[3,*] and Jaime L Matta[1,*]. [1]Department of Pharmacology and Toxicology, Ponce School of Medicine, Ponce, Puerto Rico, USA, [2]Department of Biology, University of Puerto Rico, Ponce, Puerto Rico, USA, [3]Parra Building, Damas Hospital, Ponce, Puerto Rico.

Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common human cancers collectively termed as non-melanoma skin cancer (NMSC). Inter-individual variation have been identified in DNA repair capacity (DRC) in relation to cancer risk. NMSC risk is clearly associated with chronic exposure to sunlight and a low DRC. Polymorphisms of DNA repair genes involved in the NER pathway are important as candidate genes that may contribute to variations between individuals DRC and cancer susceptibility. A candidate susceptibility gene is the XPD polymorphism in exon 23, position 751 that causes an amino acid substitution of lysine for glutamine. Individuals with NMSC who have the variant Gln allele are at increased risk of developing a secondary primary cancer. However, no studies have been undertaken to determine whether polymorphisms in the XPD gene are associated with the early onset of NMSC. As part of a population study aimed at testing the hypothesis that reduced DRC may explain differences in NMSC risk, we identified a group of eight individuals that developed NMSC at an early age ($\leq$35 years) to examine the association between XPD Lys751Gln genotype status and risk of early onset. Participants were residents of PR and $\geq$21 years old with confirmed BCC. DNA was extracted from peripheral lymphocytes. The XPD Lys751Gln genotype was assessed using EcoRI RFLP's. Controls were selected from age-matched population without NMSC. The presence of the C allele (A/C or C) in this younger subpopulation resulted in a statistical significant ($p=0.03$, Student's t-test) increase (O.R.$=7.0$) in NMSC risk when compared to wild type (A). The presence of the XPD Lys751 genotype is associated with an early onset of BCC in the population studied. This is the first study that demonstrates that the XPD Lys751 genotype is associated with a higher risk of BCC in younger persons.

56

56 Identifying biosensor genes that quantify human epidermal response to UV radiation. Ahmet Altiner[1,2,*], Christine Tock[1,*], Lexa Turner[1,*], Atsushi Terunuma[1,*], Sharon Miller[1,3,*], Carole Yee[1,*], Mark Udey[1,*] and Jonathan Vogel[1,*]. [1]Dermatology Branch, NCI, Bethesda, MD, [2]Howard Hughes Medical Institutes, Bethesda, MD, [3]Food and Drug Administration, Rockville, Maryland.

The cumulative dose of UV exposure the human epidermis sustains remains as the most important risk factor for developing a variety of skin cancers. Currently, the methods to predict an individual's life-time UV exposure dose depend heavily on the medical history and personal recall. An assay that utilizes patterns of gene expression as biomarkers to

quantify total exposure to UV light can serve as a reliable personal dosimeter, and may help stratify persons into risk categories for developing skin cancer. To identify predictive gene panels, 20 healthy volunteers with Fitzpatrick type 2 skin were exposed to various doses of ssUVR and UVA related to their individual erythema dose (0.1 MED, 1 MED) and a standard dose of 100 J/m2, with and without prior application of sunscreen (spf 15). We measured global RNA expression levels 24 hours after exposure using Affymetrix HG-U133A2 high-density microarray genechips. Data from 140 chips was subjected to the RMA algorithm, and analyzed according to their exposure dose and type (control, 0.1 MED ssUVR, 1 MED UVA etc). Two thirds of the samples were used as training sets, with the remaining samples used to verify the results. Using a paired t-test analysis, we initially compared ssUVR samples (100J/m2 and 1 MED) with controls, and identified 26 genes that were consistently up or down regulated in a dose dependent manner ($p<0.001$). These genes correctly predicted the ssUVR dose with 100% accuracy for 100J/m2 and 1 MED test genechips, and with 70% accuracy for 0.1 MED test genechips ($p<0.001$). Similar analyses revealed that the 26 genes were not able to predict the dose of UVA genechips, but continued to function as reliable biosensors for sunscreen-treated ssUVR samples with 100% accuracy ($p<0.001$). These findings suggest that these discreet set of genes function as biosensors to assess doses of UVB and not UVA. These 26 genes can also be used to accurately quantify the amount of protection spf 15 sunscreen offers. Further analyses that elucidate similar biosensor genes for UVA can assist in predicting exposure type in addition to amount.

57

57 A decline in DNA repair capacity is a risk factor for non-melanoma skin cancer independently of tumor site and history of UV exposure. Abigail Ruiz[1,*], Juan Ramos[2,*], Jaime Villa[3,*], Roy Armstrong[4,*] and Jaime Matta[1,*]. [1]Ponce School of Medicine, Ponce, Puerto Rico, USA, [2]Department of Radiology, Houston, TX, USA, [3]Parra Building, Ponce, Puerto Rico, USA, [4]Department of Marine Sciences, Mayaguez, Puerto Rico.

Exposure to UV radiation, DNA damage, DNA repair capacity (DRC), immunological responses are important factors interact in the tumorigenesis process of non-melanoma skin cancer (NMSC). Most NMSC tumors develop on sun-exposed areas of the body (SEA), although in some persons, NMSC tumors also develop sun-protected areas (SPA). The purpose of this study was to test the hypothesis that the anatomical site of the tumor and the history of UV exposure influence a decrease in DRC when participants are compared to participants without skin cancer. A six year case control study involving 822 participants was performed to compare DRC of persons with (n=474) or without (n=348) NMSC in Puerto Rico (PR). San Juan, PR has the second highest UV index in the USA. An epidemiological questionnaire was administered to each participant to solicit skin cancer risk factor and history of UV exposure. DRC was measured using a cell-host reactivation assay with a luciferase reporter gene. The controls without skin cancer had a 7.31% (± 0.24, SEM) DRC. The mean DRC of participants with tumors in SEA was 3.05 % (±0.18) and 3.36 % (±0.41) for participants with SPA tumors. These decreases of 33% and 27% respectively were statistically significantly from controls but not between SPA and SEA. The history of the UV exposure increases the number of participants with tumors in SEA but not the DRC. Epidemiological results on additional key risk factors are presented in the population studied. These findings support the hypothesis that the DRC is an important risk factor for NMSC. The anatomical sites of the tumor and the history of UV exposure do not significantly influence the strong effect of DRC in terms of risk of developing NMSC. However, these factors when examined individually are important risk factors. Supported by RCMI-NIH grant # 2G12 RR03050-17.

58 Plant alkaloid sanguinarine protects from ultraviolet B radiation-mediated cutaneous damages in SKH1 hairless mouse. Haseeb Ahsan*, Shannon Reagan-Shaw*, David Eggert*, Farrukh Afaq*, Hasan Mukhtar* and Nihal Ahmad*. University of Wisconsin - Madison, Madison, WI.

Ultraviolet B (UVB; 280-320 nm) light in the solar spectrum is believed to be the major cause of non-melanoma skin cancers, the most prevalent cancer in the USA. UVB functions as a complete carcinogen and known major factor in a variety of other cutaneous disorders. Therefore, there is an urgent need to develop novel approaches for prevention of UVB-mediated damages. Here, we have studied the chemopreventive effects of sanguinarine, a benzophenanthridine alkaloid, isolated from the roots of *Sanguinaria Canadensis*. Sanguinarine possesses antibacterial, antifungal, and anti-inflammatory activities and is used in toothpastes and mouthwashes. We have shown that sanguinarine inhibits UVB exposure-mediated damages in HaCaT keratinocytes via apoptotic elimination of UVB-damaged cells. This study was designed to investigate the photoprotective effects of sanguinarine *in vivo* in SKH-1 hairless mice. The mice were subjected to a single exposure of UVB (180 mJ/cm^2) with either a pre-treatment (30 min prior to UVB) or post-treatment (5 min after UVB) of sanguinarine (5 μmol/0.2 ml ethanol per mouse). The mice were euthanized 24 h following UVB and further studies were conducted. Our data demonstrated that sanguinarine (both pre- and post- treatments) resulted in an inhibition of UVB radiation-mediated increases in i) skin edema (assessed by bi-fold skin thickness), and ii) hyperplasia and infiltration of leukocytes (assessed by H&E staining). Further, sanguinarine treatments also resulted in a significant decrease in H_2O_2 production (determined by immunostaining). Furthermore, as assessed by immunohistochemical- and western blot- analyses, sanguinarine also inhibited UVB-mediated increases in the levels of i) ornithine decarboxylase (ODC), and ii) proliferating cell nuclear antigen (PCNA); markers of tumor promotion. Interestingly, the post-treatment of sanguinarine afforded equal protection compared to the pre-treatment; suggesting that sanguinarine-mediated responses are not sunscreen effects. Our data suggest that sanguinarine may be developed as an agent for the prevention of UVB-mediated damages.

59

59 Photodynamic therapy resistant human colon carcinoma HT29 cells show cross-resistance to UVA but not UVC. Natalie J Zacal* and Andrew J Rainbow*. Department of Biology, Hamilton, Ontario, Canada.

We have reported previously the isolation of photodynamic therapy (PDT) resistant human colon carcinoma HT29 cells. HT29/P14, HT29/A11 and HT29/N8 were isolated following repeated in vitro PDT treatment to the 1-10% survival level followed by regrowth of single surviving colonies using the photosensitizers Photofrin, Aluminium Phthalocyanine Tetrasulphonate (AlPcS4) and Nile Blue A respectively. These PDT resistant variants show increased expression of the Hsp27 and BNip3 protein and a decreased expression of mutant p53 protein compared to parental HT29 cells. Since mutant p53 and increased expression of Hsp27 have been associated with resistance to various chemotherapeutic agents, whereas BNip3 is a potent inducer of apoptosis, we were interested in determining whether these PDT resistant cells were cross resistant to other cytotoxic agents. In the present report we used a colony survival assay to examine the sensitivity of the PDT resistant HT29 variants and several other clonal variants of HT29 cells to UVA and UVC. The HT29 PDT resistant variants showed cross-resistance to UVA, but not UVC. Cell sensitivity to UVA or UVC was then correlated with Hsp27, BNip3 and p53 protein levels in the PDT resistant variants as well as in several clonal variants of HT29 cells that express different levels of Hsp27, BNip3 and p53. We show that decreased mutant p53 correlates with increased resistance to UVA but not UVC and increased BNip3 shows a weak correlation with increased sensitivity to UVC but not UVA. An HT29 cell line over expressing Hsp27 alone showed increased resistance to PDT compared to that of parental HT29 cells but no increase in resistance to UVA or UVC suggesting that although Hsp27 over expression alone results in increased PDT resistance it does not result in an increased resistance of HT29 cells to UV.

60

60 Effects of topical exposure to retinyl palmitate-containing cream on the photocarcinogenicity of simulated solar light (SSL) in SKH-1 mice. Qingsu Xia[1,*], Mary D Boudreau[1,*], Peggy Webb[1,*], Barbara J Miller[1,*], Greg R Olson[2,*], Alan R Warbritton[2,*], Paul C Howard[1,*] and Peter P Fu[1,*]. [1]Division of Biochemical Toxicology and National Toxicology Program Center for Phototoxicology, Jefferson, AR, USA, [2]Toxicologic Pathology Association, Jefferson, AR, USA.

A 52-week study was conducted to determine if the topical application of creams containing 0.1% and 0.5% (wt/wt) RP to the skin of SKH-1 mice would enhance the carcinogenicity of simulated solar light (SSL) emitted from 6.5 kW filtered xenon arc lamps. The levels of SSL used in this study were 0-, 6.85-, 13.7-, and 20.55-mJ•CIE/cm^2. SSL was administered to mice in the morning, and cream formulations were applied in the afternoon 5 days/week for a period of 40 weeks. The incidence of squamous cell papillomas were increased in the vehicle control compared with untreated mice that received either no SSL or were exposed to 6.8 mJ•CIE/cm^2. Topical treatment with 0.1% RP increased the incidence of squamous cell carcinomas in situ and carcinomas in male mice exposed to 6.8- and 13.7- mJ•CIE/cm^2 when compared with the vehicle control. Treatment with 0.5% RP did not show an upward trend in skin neoplasms compared with the 0.1% RP group. The multiplicity (number of tumors per mouse) of squamous cell papillomas was markedly increased in vehicle control mice compared with untreated mice at SSL doses of 0, 6.8- and 13.7- mJ•CIE/cm^2. The multiplicity of squamous cell carcinomas in situ and carcinomas was higher in vehicle than untreated mice at 6.8- but not at 13.7- mJ•CIE/cm^2. There was an increase in the multiplicity of squamous cell papillomas in mice treated with 0.1% and 0.5% RP at SSL doses of 6.8- and 13.7- mJ•CIE/cm^2. Treatment with 0.1% RP and exposure to SSL at 13.7 mJ•CIE/cm^2 increased the multiplicity of squamous cell carcinomas in situ and carcinomas; however, treatment with 0.5% RP did not increase these neoplasms. In general, the average number of neoplasms, primarily as papillomas, increased with 0.1% and 0.5% RP treatment compared with vehicle and untreated controls. (The contents of this abstract should not be considered FDA policy)

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61 Impact of UVR on vertical movement and photosynthetic efficiency of Euglena gracilis Klebs. Peter R Richter[1,*], Donat P Häder[1,*], Virginia E Villafañe[2,*] and E. Walter Helbling[2,*]. [1]Institut für Biologie, Friedrich-Alexander-Universität, Erlangen, Germany, [2]Estación de Fotobiología Playa Unión, & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rawson, Chubut, Argentina.

Movement behavior and photosynthetic efficiency of the unicellular flagellate *Euglena gracilis* Klebs was investigated in vertical Plexiglas columns (depth = 65 cm, diameter = 15 cm). The columns containing the organisms were irradiated from above with a solar simulator (HÖnle lamp) receiving irradiances of 162, 32.6 and 1.08 W m^{-2} for PAR (400-700 nm), UV-A (315-400 nm) and UV-B (280-315 nm). Two treatments were implemented: One column was covered with a 295 nm cut-off filter (thus receiving UVR-280-400 nm, and PAR), and the other with a 395 nm cut-off filter (thus receiving only PAR). Cell samples were drawn from different depths (ie., surface, 20, 40 and 65 cm) from which movement parameters were measured by image analysis, and photosynthetic efficiency determined with a pulse amplitude modulated fluorometer (Water PAM, Walz). UVR had significant effect on both photosynthesis and swimming behavior. Photosynthetic efficiency increased with depth and samples exposed to UVR had a significant decrease in photosynthetic yield as compared those exposed only to PAR. Cells at the surface showed a negative gravitactic behavior (i.e., swimming upward) whereas the fraction of cells swimming downward increased with depth below 20 cm. At 40 cm depth about 50 % of the cells swam upward and 50 % downward whereas at 65 cm depth $>$ 70 % of the cells showed a downward movement. This resulted in that the largest fraction of cells was at the bottom (i.e., 65 cm),

while the number of cells towards the surface decreased. This situation reversed under low irradiance or dim light conditions, as more cells were found near the surface. Our data indicate that gravitaxis and thus the depth distribution of *E. gracilis* clearly depends on the radiation exposure.

62

62 Do ambient levels of both UV-A and UV-B radiation affect leaf litter chemistry and decomposition, and performance of soil fauna? Titta Kotilainen[1,*], Jari Haimi[1,*], Riitta Tegelberg[2,*], Riitta Julkunen-Tiitto[2,*], Elina Vapaavuori[3,*] and Pedro J Aphalo[1,*]. [1]Dep. Biological and Environmental Science, Jyväskylä, Finland, [2]Natural Product Research Laboratory, Joensuu, Finland, [3]Suonenjoki Research Station, Suonenjoki, Finland.

Our experimental setup was based on the use of plastic films strongly attenuating different parts of the UV spectrum. The experimental setup consisted in three treatments: 1) UV-B+, UV-A+ (near ambient control, AB), 2) UV-B−, UV-A+ (Ab) and 3) UV-B−, UV-A&Minus; (ab). The experiment was done on eight grey alder (*Alnus incana*) and twelve white birch (*Betula pubescens*) trees growing in the field. All the treatments were on different branches of each tree. Senescent leaf samples collected in the fall were used in decomposition experiments. Laboratory experiments were conducted to study the effects of the leaf litter from different UV treatments on soil organisms and on the decomposition rate. In an experiment with woodlice, jars with alder litter had more woodlice feaces and litter remaining at the end of the experiment in Ab treatment than in AB control and the most in ab treatment. In another decomposition experiment with soil and litter the cumulative CO_2 production was lowest in ab treatment, in both tree species. In the same experiment, litter decomposition rate was lowest in AB control compared to Ab, in both species. Chemical analyses showed that there were more extractable tannins in birch and more residual tannins in alder, but no effect of UV. The C/N ratio analysed from the litter and from the woodlice feaces was higher in birch. Total lignin concentration was lowest in ab treatment compared to AB control, with intermediate values in Ab treatment, in both species. Both UV-A and UV-B radiation affect chemical properties of the litter and changes in litter properties cause some downstream effects on litter decomposition. The fact that UV-A radiation is effective is relevant to assessing the applicability of action spectra, indicating that spectra with no action in the UV-A band are not applicable to some of these responses.−

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63 Motility of freshwater zooplankton from a temperate lagoon from Patagonia (Chubut, Argentina) throughout a year cycle: Influence of solar radiation. Rodrigo J Gonçalves*, Elena S Barbieri*, Virginia E Villafañe* and E. Walter Helbling*. Estación de Fotobiología Playa Unión (EFPU) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Argentina, Rawson, Chubut, Argentina.

We investigated the impact of solar ultraviolet radiation (UVR, 280-400 nm) on motility (i.e., swimming speed) of dominating zooplankton species (cladocerans and copepods) from a freshwater lagoon of Patagonia, Argentina, during a yearly cycle (February 2005 to February 2006). Zooplankton samples were collected with a net (200 μm mesh size) from surface waters every 2-3 weeks; samples were put in UVR-transparent containers and exposed to solar radiation, inside temperature-controlled water bath under three radiation treatments: 1) PAB (280-700 nm), 2) PA (320-700 nm), and 3) P (400-700 nm), and a control (no radiation). Swimming speed of free motile individuals was measured every 2 h of exposure with an image-analysis system from video recordings. Optical characteristics of the water body, i.e., the attenuation coefficient of PAR (400-700 nm) (K_{dPAR}) and mean irradiance (Im) in the upper mixed layer, were estimated from the measured irradiance and published models taking into account the concentration of chlorophyll-a (Chl-a) and chromophoric dissolved organic matter (CDOM). Chl-a was variable between 4 and 800 μg l^{-1}, and it was found to be the most important variable controlling KdPAR (range: 2-17 m^{-1}). Carnivorous-cyclopoid copepods (*Metacyclops* sp) and herbivorous cladocerans (*Daphnia* sp.) dominated the zooplankton community at different times showing a negative relationship in their abundance. Additionally, the abundance of cladocerans was negatively correlated with Chl-a concentration, suggesting a top-down control of the phytoplankton population. Swimming speed of both zooplankton groups increased relatively when K_{dPAR} was low (autumn / winter) and it decreased when KdPAR was high. Solar ultraviolet radiation had relatively little impact on swimming speed and these changes in motility seem to be more related to factors such as the underwater radiation field / KdPAR and prey concentration, than to the incident solar radiation.

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64 Summer variability of UVR effects on phytoplankton primary production in a tropical coastal area of the Southern China Sea. Kunshan Gao[1,2,*], Gang Li[1,*], E. Walter Helbling[1,3,*] and Virginia Villafañe[1,3,*]. [1]Marine Biology Institute, Shantou University, Shantou, Guangdong, China, [2]Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, China, [3]Estación de Fotobiología Playa Unión & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rawson, Chubut, Argentina.

During summer (June to September) 2005 we carried out simulated *in situ* experiments to determine the effects of solar UV radiation (UVR, 280-400 nm) on primary productivity of natural phytoplankton assemblages in the coastal water (23° 24′,117° 07′) of the Southern China Sea. Surface sea water was collected and exposed to solar radiation for 2-3 hours under 3 radiation treatments (i.e. PAB, 280-700 nm; PA, 320-700 nm and P, 400-700 nm) at 7 levels of irradiance (using neutral density screens) to obtain photosynthesis versus irradiance (P-E) curves. Periods of high phytoplankton biomass were associated with the presence of tropical storms (i.e., heavy rain), during which stratification in the water column allowed for the development of a bloom (>20 μg chl-a L^{-1}) dominated by the diatom Skeletonema costatum;

the rest of the time, natural assemblages were dominated by monads / flagellates. During the study period UVR inhibition was variable, but it reached values as high as 50%. Carbon fixation during cloudy days was higher in samples receiving UVR than in those under PAR-only treatment, indicating the use of short wavelength energy for photosynthesis. On the other hand, primary production was significantly reduced by UVR during sunny days. We also found differences in responses depending on the size structure of the assemblages: Photosynthetic inhibition of piconanoplankton cells (<20 μm) was higher than that in microplankton (> 20 μm) suggesting that the smaller cells are more sensitive to solar UVR than larger ones.

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65 Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis and DNA of the economically important cyanobacterium Arthrospira (Spirulina) platensis. Kunshan Gao[1,2,*], Ping Li[1,*], Teruo Watanabe[3,*] and E. Walter Helbling[1,4,*]. [1]Marine Biology Institute, Shantou University, Shantou, Guangdong, China, [2]Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Huibei, China, [3]Hainan DIC Microalgae CO., LTD., Haikou International Commercial Centre 38, Haikou, Hainan, China, [4]Estación de Fotobiología Playa Unión & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rawson, Chubut, Argentina.

We conducted experiments to assess the combined effects of ultraviolet radiation (using a Sol 1200W mercury lamp, Honle, Germany) and temperature (i.e., 15 °C, 22 °C and 30 °C) on morphology, photosynthesis and DNA of the cyanobacterium Arthrospira (Spirulina) platensis (Strain 439) at three biomass concentrations: (1) low (100 mg (dry weight) L^{-1}); (2) medium (160 mg (dry weight)L^{-1}) and, (3) high (240 mg (dry weight)L^{-1}). The samples were exposed to two radiation treatments (UVR+PAR, and PAR) receiving an irradiance of 344, 76, and 2.6 W m^{-2} for PAR, UV-A (315-400 nm), and UV-B (280-315 nm), respectively. A significant UVR-induced breakage of the spiral filament was found at 15 and 22 °C, but not at 30 °C. High PAR level also produced in a significant breakage at 15 and 22 °C, but only at low biomass densities, although to much less extent as compared to the UV-R+PAR treatment. The impact of UVR on the photosynthetic quantum yield (Y) was highest at 15 °C and lowest at 30 °C; in addition, the effect of UVR on Y decreased with increasing biomass density. Following the pattern of change in morphology and decrease in Y, UVR-induced DNA damage was highest at 15 °C, and lowest at 30 °C. Increasing cell biomass density resulted in less damage to the spiral structure, photosynthetic efficiency and DNA. Our data indicate that temperature has a strong effect on the observed UVR impact on A. platensis, with a better performance to negligible impact with increasing temperature.

66

66 Patterns and modeling of solar radiation in South Central Chile(32° S) during 2003-2004. Klaudia Hernández[1,2,*], Beatriz Yanicelly [1,2,*], Aldo Montecinos[3,*], Giovanni Daneri[2,4,*] and E. Walter Helbling[5,*]. [1]Programa de Postgrados en Oceanografía, Concepcion, Chile, [2]Departmento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepci[:oacute:, Concepcion, Chile, [3]Departamento de Geofísica y ciencias de la tierra (DEGEO), Universidad de Concepción, Concepcion, Chile, [4]Centro de de Investigación en Ecosistemas de la Patagonia (CIEP), Coyaique, Chile, [5]Estación de Fotobiología Playa Unión y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rawson, Chubut, Argentina.

In Southern central Chile, Concepcion (36 °S), the annual and local variability of solar radiation and their relationship with cloudiness and ozone have been scarcely studied. Concepcion is under the occasional influence of low ozone air masses that increase solar UVB radiation (280-320 nm). We analyzed the daily variability of solar radiation (PAR and UVR) and its relationship with meteorological and atmospheric variables such as ozone, cloudiness and wind stress. In order to describe the ozone variations the energy ratio of 305/340 nm (GUV-511C) was calculated and compared with satellite data obtained from NASA. Here, a time series-model is presented to describe the PAR, UVA, UVB, as a function of the ozone concentration, time of the year and cloudiness. The results showed that the different bands considered in the annual cycle co-varied in phase with the maximum (spring-summer) and minimum (autumn-winter) values. The meridian pseudostress and cloudiness also showed an annual cycle, with dominance of south winds and low cloudiness during summer. The model also described a coupling between solar radiation and cloudiness and winds in intraseasonal and daily scales. A three months lag was observed between the incident radiation and the stratospheric ozone, which was maximum during spring time. The 305/340 ratio was a good ozone variation index and showed daily fluctuations with periods of 10 days. The high variability of the incident radiation in daily and annual cycles at Concepcion was modulated by zenital angle (59%), and the meteorological factors mainly cloud index (30%) and ozone (4%).

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67 Solar UVR induced inhibition / enhancement of photosynthesis in phytoplankton assemblages from coastal waters off SE China. Kunshan Gao[1,2,*], Yaping Wu[1,*] and E. Walter Helbling[1,3,*]. [1]Marine Biology Institute, Shantou University, Shantou, Guangdong, China, [2]Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, China, [3]Estación de Fotobiología Playa Unión & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rawson, Chubut, Argentina.

We carried out experiments to evaluate short-term impact of UVR on photosynthesis of phytoplankton assemblages from coastal waters off SE China, during March 2004 - Sep 2005. Sea surface samples were taken and exposed to different radiation treatments with the presence or absence of

PAR or UVR using sharp cut-off filters. Five different treatments were implemented: 1) PAB, 280-700 nm; 2) PA, 320-700 nm; 3) P, 400-700 nm; 4) UVR only, using a UG-11 filter, and 5) UV-A only, using both UG-11 and 320 nm Schott filters. Solar UVR significantly inhibited photosynthetic rates of phytoplankton assemblages during spring and winter, with UV-A accounting for most of the observed inhibition; very little or no UVR-induced inhibition was observed during summer. Assimilation numbers during summer were significantly higher than those in spring and winter, with mean values of samples exposed to PAR of 7.62 (summer), 2.83 (spring), and 4.35 μg C (μg chl a)$^{-1}$ h^{-1} (winter), respectively. Experiments conducted with samples receiving only UVR or UV-A radiation (i.e., no PAR) confirmed the previous findings that tropical phytoplankton assemblages can use UV-A as source of energy for photosynthesis. In fact, the amount of carbon fixed under UV-A-only treatment was as much as 0.48 μg C (μg chl a)$^{-1}$ h^{-1}. The seasonal variation on the impact of UVR on phytoplankton carbon fixation could be attributed to differences in vertical mixing and stratification and in species composition.

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68 Photochemical degradation of colored dissolved organic matter in a brackish tidal marsh-estuarine system of the Chesapeake Bay. Maria Tzortziou[1,*], Patrick J Neale[2,*], Christopher L Osburn[3,*], Charles L Gallegos[2,*], Patrick J Megonigal[2,*] and Jay R Herman[4,*]. [1]University of Maryland, College Park, MD, USA, [2]Smithsonian Environmental Research Center, Edgewater, MD, USA, [3]US Naval Research Laboratory, Washington, DC, USA, [4]NASA/Goddard Space Flight Center, Greenbelt, MD, USA.

Tidal marsh ecosystems in the Chesapeake Bay act as important local sources of dissolved organic carbon for adjacent estuarine waters. However, the quality and bioavailability of the dissolved organic compounds exported from these marsh systems remain largely unknown. Moreover, little is known about the photo-reactivity of this material and the effects of UV exposure on its optical quality and composition. Previous studies suggest that biological and chemical processes in the shallow waters of the Chesapeake Bay are affected by solar UV radiation despite strong attenuation with depth. Our measurements in tidal brackish marshes of the Rhode River along the western shore of the Bay suggest that UV exposure can lead to significant degradation of marsh-exported colored dissolved organic matter (CDOM) and the bleaching of both its absorption and fluorescence emission bands. Absorption loss occurred in different regions of the spectrum depending on the spectral characteristics of the light irradiating CDOM (i.e. different cut-off filters). This differential loss of absorbance changes the exponential slope of the absorbance spectra (S_{CDOM}). For a cut-off filter of 395 nm, most of the absorption loss was observed at wavelengths at and above 400 nm. This resulted in an increase in S_{CDOM} compared to the dark treatment. As the cut-off wavelength moved lower, absorption was also lost at the lower UV wavelengths with a subsequent decrease of S_{CDOM}. These results illustrate that solar exposure can cause either an increase or a decrease in S_{CDOM}, depending on the spectral quality of light. Our findings are consistent with previous laboratory experiments using monochromatic (laser) sources that show such exposures cause the most decrease in absorbance at the wavelength of irradiation, as is predicted by a charge-transfer theory of CDOM absorbance. We used measured changes in the CDOM optical characteristics to derive spectral weighting functions for UV photobleaching and determine the effects of solar exposure on CDOM quality in this environment.

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69 Interaction of ultraviolet radiation and vertical mixing effects on photosynthesis in the Ross Sea Polynya. Patrick J Neale[1,*], Cristina Sobrino[1,*], Ann E Gargett[2,*], Hae-Cheol Kim[1,*], Linda A Franklin[1,*] and Jesse Phillips-Kress[1,*]. [1]Smithsonian Env. Res. Ctr, Edgewater, MD, [2]CCPO, Norfolk, VA.

Extensive algal blooms occur in a polynya north of the Ross Ice Shelf, Antarctica but little is known about the effects of solar UVR despite importance of the bloom to Southern Ocean production and heightened exposure to UVB during seasonal ozone depletion. Several approaches were used to quantitate photosynthetic response as a function of UV spectral exposure. A xenon lamp based spectral incubator was used to describe the spectral response (biological weighting functions) of UV inhibition in the context of a photosynthesis-irradiance model (BWF/P-I). Model predictions are compared with results of deck incubations +/- UV and in situ profiles of daily production. The early bloom was dominated by the colonial alga, *Phaeocystis antarctica*, while the mid-to-late bloom assemblage was a mixture of diatoms and *P. antarctica*. Depth profiles with a fast repetition rate fluorometer (FRRF) showed high PSII quantum yield (Fv/Fm = 0.6) early, falling to low yield (Fv/Fm $<$ 0.35) as the bloom developed, consistent with previous findings of iron limitation in the region. Sensitivity to UV was the highest yet measured for Southern Ocean phytoplankton, with slow rates of recovery. The BWF/P-I model was applied to in situ irradiance and predicted strong inhibition by UV and PAR in the upper 3-5 m, closely matching the depth variation of daytime production as measured using an in situ array. However, inhibition as indicated by Fv/Fm extended to 15 m, suggesting that vertical transport and slow recovery enhance inhibition of water column production. Simultaneous, high-resolution (7s Δt) time-series measurements were made of Fv/Fm at 6 m and near-surface acoustic backscatter (tracking downward transport of microbubble clouds). The highest variability in Fv/Fm at 6 m occurred when the upper 3 m received high inhibiting irradiance. Fv/Fm variation was correlated in time with the direction of vertical transport, i.e. transport from the surface decreased Fv/Fm while upward transport increased Fv/Fm at 6 m. Both wind-driven Langmuir cells and internal waves contribute to vertical transport in the Ross Sea Polynya and are important in determining the overall effect of UV exposure in the surface layer.

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70 Dissimilar effects of increased CO^2 on growth,light and carbon fixation of two phytoplankton species under par and UVR exposure. Cristina Sobrino*, Patrick J Neale* and Mary L Ward*. Smithsonian Env Res Ctr, Edgewater, MD.

Understanding the repercussion of increased atmospheric CO_2 on aquatic ecosystems requires information on the effects on phytoplankton growth and photosynthesis which are in turn related to the CO_2 dependence of organic production (i.e. presence of different CO_2 concentrating mechanisms (CCMs)), nutrient fluxes and exposure to photoinhibitory UVR, among other factors. We studied the effect of increased CO_2 on growth, light absorption and carbon fixation under PAR and UVR exposures in two phytoplankton species with different CCMs. *Nannochloropsis oculata* has a HCO_3^- transport system and a C3 pathway in photosynthetic carbon fixation and *Thalassiosira pseudonana* uses a C4 pathway where a major fraction of net carbon fixation depends on the synthesis of a C4 intermediate. Nutrient replete cultures of both species were acclimated to present atmospheric levels (0.03% CO_2) and predicted future high CO_2 levels (0.1% CO_2) under PAR exposures. After acclimation, photosynthesis under PAR and photoinhibition by UVR+PAR were measured as ^{14}C assimilation during 1h incubations. Exposure to 80 UVR+PAR spectral treatments of varying light intensity and spectral composition allowed the calculation of the Biological Weighting Functions for inhibition of photosynthesis under different CO2 conditions. Increased CO2 enhanced growth (μ, day^{-1}) and photosynthetic rates (P^B_{max}, gC $gChl^{-1}$ h^{-1}) in *Thalassiosira* while the opposite was observed in *Nannochloropsis*. Both species adapted to increased CO_2 through changes in light harvesting, though by different mechanisms. *Thalassiosira* under high CO_2 reduced chlorophyll per cell increasing the resource use efficiency. In contrast, *Nannochloropsis* showed higher chlorophyll per cell and a decrease in the optical absorption cross section normalized by chlorophyll. Sensitivity to UVR was not different with or without the CO_2 supply in *Nannochloropsis* but increased in *Thalassiosira* under high CO_2. We hypothesize that high CO_2 conditions stressed *Nannochloropsis* enhancing the repair rates and predisposing the cells to counteract additional UVR damage. In contrast, high CO_2 conditions induced downregulation of the photosynthetic apparatus in *Thalassiosira* and increased sensitivity to UVR, the latter probably due to a downregulation of repair enzymes.

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71 UV Radiation and herbivory shape intertidal macroalgal assemblages in Antarctica. Michael Y Roleda*, K. Zacher*, M. Molis*, D. Hanelt* and C. Wiencke*. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany.

Marine macroalgae are very important primary producers in coastal ecosystems, serving as food for herbivores and as habitat for many organisms. While most research on ultraviolet radiation (UVR) is focused on the physiological aspects of single organisms, little is known on the impact of UVR in combination with herbivory on community assemblages. Field-experiments on macrobenthos are likewise scarce, especially in the Antarctic region. Therefore the effects of UVR (280-400 nm) and consumers on early successional stages of an intertidal hard bottom macroalgal community on King George Island, Antarctica, were studied. In a two-factorial design 32 experimental units (PAR + UVA + UVB = 280 to 700 nm; PAR + UVA = 320 to 700 nm; PAR = 400 to 700 nm vs. grazer, no grazer) were installed in Antarctic summer between December and March for 106 days. Species recruitment and dry mass of macroalgae on ceramic tiles were followed. Both UV radiation and herbivory exhibited a significant impact on macroalgal recruitment and succession in the Antarctic intertidal. Species composition and diversity were significantly higher in the UV-depleted treatment compared to the treatment exposed to the full solar spectrum. Additional laboratory experiments showed that spores from intertidal macroalgae were generally well adapted to UVR. After initial photoinhibition a quick recovery of the photosynthesis across all light treatments was found. Although UVR induced DNA damage, spore vitality was not affected. The obtained data on the physiological performance explains the ecological success of macroalgal species in this intertidal community. The effects of UVR and herbivory may change the community structure and alter trophic interactions in this system. Whether these effects are persistent requires further studies.

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An MRP2 transport model from the malpighian tubules of the cricket, *acheta domesticus*. Monica A Davis[1,2]. [1]College of Charleston, Charleston, SC, [2]Medical University of South Carolina, Charleston, SC.

Special excretory transporters have evolved in organisms to defend against harmful xenobiotics and molecules. The ability found in insects to excrete substrates of multidrug resistance associated protein, MRP2, has led us to study the Malpighian tubules of the cricket, *Acheta domesticus*. These tubules transport the fluorescent MRP2 substrate, Texas Red, as detected previously by confocal fluorescence microscopy and digital image analysis. These tubules have shown inhibition of several MRP2 transport inhibitors on this transport process and so a model system has been developed in order to collect the transported molecules. The role of MRP2 transport in the accumulation of carcinogens in breast cancer and the selective uptake of photosensitizers in cancer cells will be better studied with the establishment of this tubule model. In the cricket, there are 114 tubules that flow into an ampulla, from which a ureter flows to the hindgut. In order to collect the excretion from all tubules, a small section of the gut at this connection was removed and sutured at its proximal end, and PE10 tubing was inserted at the distal end. The tubules were placed in a small chamber of continually oxygenated Ringers solution with 5 μM of Texas Red, and the PE10 tubing attached to the gut of the cricket was inserted through the plastic wall of an inner chamber filled with oil. The excretion collected under oil was analyzed for Texas Red with a Perkin-Elmer spectrofluorimeter. In time

periods of 135-190 minutes, we observed 0.7 to 5.3 μl-volume fluid droplets, all of which contained Texas Red. Texas Red concentration was observed in the droplets of 0.2-13 μ moles (N=4). This data demonstrates that this model offers the possibility of further study of the metabolic pathways and transport.

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Investigation of the mechanism of resistance of A549 lung cancer cells to light-independent merocyanine 540 uptake. Joshua D Fuller[1], Linda R Jones[1] and Karl Karnaky[2,*]. [1]Department of Physics, College of Charleston, Charleston, SC, [2]Department of Cell Biology, Medical University of South Carolina, Charleston, SC.

Merocyanine 540 (MC540) is a photosensitizer that has been used experimentally to purge leukemia cells from bone marrow. MC540 is taken up in leukemia cells and is excluded from normal bone marrow cells. However the mechanism of selective MC540 uptake has not been established. Human A549 lung cancer cells are known to be resistant to MC540 photosensitization. A549 cells have membrane transport proteins including MRP1 and p-glycoprotein that exclude certain drugs associated with multidrug resistance (MDR). The goal of this study was to investigate the role of MDR in A549 resistance to MC540 in the absence of light. The effect of specific inhibitors of MRP1 and p-glycoprotein-mediated efflux was determined quantitatively with fluorescence spectroscopy of intact cells. Previous work in this lab with a cricket malphigian tubule model indicated that MC540 is a substrate of MRP1. However, results indicate that inhibition of any one membrane protein is insufficient to prevent the efflux of MC-540 from lung cancer cells. Three inhibitors, CDNB, Verapamil and Cyclosporin A, were used. There was no significant difference in fluorescence between cells incubated with the inhibitors and the controls except for Cyclosporin A. It actually reduced the fluorescence by about fifteen percent. Further work is necessary to investigate light-enhanced uptake.

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Photosensitizer quantification in the rat esophagus. George A Khouri, Linda R Jones, David Hall*, Herbert C Wolfsen*, Michael B Wallace* and Norris W Preyer *. Department of Undergraduate Biology, College of Charleston, Charleston, SC.

Barrett's esophagus is a common result of chronic acid reflux from the stomach. People with Barrett's esophagus are at increased risk of esophageal cancer. Ablation of Barrett's esophagus, particularly in advanced precancerous stages called high-grade-dysplasia, has been shown to reduce the risk of cancer development. The only FDA approved method of ablation is photodynamic therapy (PDT) with porfimer sodium (Photofrin; Axcan, Quebec, Canada). The optimal light and drug dose for Barrett's esophagus ablation is unknown. The aim of this project is to quantify mucosal concentrations of porfimer sodium in human esophagus with fiber optic reflectance measurements as a means to optimize dosing. We are currently using a rat model of normal esophagus to investigate the effect of porfimer sodium on reflectance spectra. Adult Wistar rats were fed purified rodent feed for two weeks in order to minimize the involvement of chlorophyll in the spectral measurements. Two mg/kg porfimer sodium was administered via tail vein injection. After 24 hours, rats were euthanized and the esophagus was removed. Reflectance spectra of the esophagus were measured with an Ocean Optics fiber optic spectrometer from 400 to 700 nm. The Photofrin was then extracted from the esophagus using Scintigest as a tissue solubilizer. After incubation for seven days in the dark at 37°C, the sample was centrifuged and the porfimer sodium content of the supernatant was determined. Fluorescence measurements with 410 nm excitation were compared to standard curves of porfimer sodium solubilized in Triton X-100. Absorption spectra of the supernatant were also compared to standard curves. We have found that absorption measurements of the supernatant are more reliable than fluorescence measurements owing to quenching of the fluorescence emission at higher porfimer sodium concentrations. Results indicate that porfimer sodium localizes in the normal rat esophagus at a concentration of up to 4 to 5 mg/kg. However, a portion of that porfimer sodium is expected to be in aggregated form in vivo and not photoactive. The main feature of the reflectance spectra of sensitized esophagus is an increase in the reflectance above 600 nm. In conclusion, fiber based reflectance spectroscopy is capable of quantifying in vivo esophageal porfimer sodium concentrations in an animal model. Further studies will compare spectroscopic measurements in human patients undergoing PDT.

ABSTRACTS

SUN-A

Free electron lasers: A primer for their use in photobiology and photomedicine*. H.F. Dylla*. Jefferson Lab, Newport News, VA.

Lasers, since their invention in the 1960's, have been applied to numerous basic research problems in the biological and medical sciences and have also become a practical tool for a variety of medical diagnostic measurements and clinical treatments. A number of research centers in the 1990's have employed free electron lasers to exploit the unique properties of these lasers for biomedical applications. These unique properties include higher powers than available from conventional lasers, broad tunability over infrared through ultraviolet wavelengths, and short-pulse (< picosecond) time structures that maximize the transfer of laser energy to the irradiated material and allow dynamical studies at timescales appropriate for chemical interactions. The world's most powerful and versatile free electron laser is operational at the DOE's Jefferson Lab in Newport News, VA. This laser provides up to multi-kilowatts of power in the infrared, and kilowatt class power in the far infrared, visible and ultraviolet. A versatile user facility is co-located with the FEL where experimental set-ups can be arranged to use this unique light source. The author will describe this light source facility and briefly review the existing and planned applications of this source in photobiology. * This work is supported by the Office of Naval Research, the Air Force Research Laboratory, the Commonwealth of Virginia and the Laser Processing Consortium

SUN-B,a

From structure-function to photobiotechnology of phytochromes. Pill-Soon Song[1,*], Yun-Jeong Han[3,*], Hyoyeon Lee[3,*] and Jeong-Il Kim[2,*]. [1]Faculty of Biotechnology, Jeju, Korea, [3]Environmental Biotechnology Research Center, Jinju, [2]Kumho Life & Environmental Science Laboratory/ Chonnam, Gwangju.

This talk describes a story of how the basic study of the structure-function relationship of phytochromes as the photomorphogenic receptors in plants has led to the development of photobiotechnology of turf grass and other plants through interdisciplinary research. Phytochromes are the phyto-photoreceptors mediating a variety of photomorphogenic responses to red/far-red wavelength lights. Phytochromes exist in two photochromically interconvertible isomers, the red-light absorbing Pr and far-red light absorbing Pfr forms. The ratio of red and far-red wavelength lights (R: FR ratio) determines the photoequilibrium of the Pr and Pfr forms to induce and/or modulate plants photoresponses. Thus, a high R:FR ratio induces photoequilibrium of phytochrome toward the functionally active form, Pfr. In contrast, a low R:FR ratio under shade conditions shifts the photoequilibrium to the inactive Pr form, resulting in shade avoidance responses as exhibited by multiple phenotypic changes including stem elongation, internodes extension, retardation of leaf growth, and early flowering. In crop plants, these shade-avoiding responses lead to significant losses of yields. We have developed bathochromic mutant phytochromes whose Pr-absorption spectra move to longer wavelengths so that they can be activated even at low R: FR ratios to suppress shade avoidance responses in plants. We have also characterized their in vivo functions in terms of shade tolerance. Changing highly conserved amino acid residues in the bilin lyase domain of oat phytochrome A, we obtained several absorption wavelength-shifted mutants including bathochromic mutants with up to 12 nm shift. In addition, we generated a hyperactive phytochrome mutant that enhances plants shade tolerance. We introduced these phytochromes into turf grasses in order to produce economically and environmentally beneficial shade-tolerant grass cultivars. (This research is supported by BioGreen 21 program/ RDA and NCRC grant #R15-2003-012-01003-0.)

SUN-B,b

Can we model the biological effects of polychromatic light? John Sutherland*. East Carolina Univ, Greenville, NC.

When trying to understand photochemical and photobiological processes in the laboratory, it is frequently helpful to be able to work with essentially monochromatic light. However, most natural and man made light sources consist of broad spectrum light, i.e., they are polychromatic. Sunlight is the most important example. Thus, understanding photobiology in the real world outside the laboratory requires that we understand how polychromatic spectra influence photobiology. If the response being studied is a linear function of the incident light then the magnitude of the effect scales as the product of the dose of light at each relevant wavelength times the cross section for the particular process integrated over all wavelengths where both parameters are non-zero, as shown in Equation 1. Equation 1 This familiar equation is adequate for photochemical reactions which are linear functions the dose of incident light, but usually inadequate for light induced biological reactions, which frequently not linear functions of dose. For example, if a certain dose at one wavelength can kill 50% of the cells in a population and a certain dose at another wavelength can kill 60% of the cells in a similar population, applying the same does of both wavelengths simultaneously will not kill 110% of the target cells. I will review some of the biological systems where accurate predictions of the biological effects of polychromatic light are required, some of the theoretical approaches that have been proposed to address the problem and recent

experimental advances in light sources that should make it possible to acquire complete experimental data sets that have not been practical in the past. I shall also discuss the experiments that should be performed to validate any theory developed to predict the biological consequences of polychromatic irradiations.

SAM-1,a

Expanding the fluorescent protein toolkit: Engineering new colors and improved FRET pairs. Robert E Campbell*. University of Alberta, Edmonton, Alberta, Canada.

A decade ago researchers reported that it was possible to blue shift the emission peak (504-509 nm) of the *Aequorea victoria* green fluorescent protein (GFP) into the cyan region (475-490 nm) of the visible spectrum by substituting the tyrosine-derived phenol(ate) moiety of the wild-type chromophore with a tryptophan-derived indole. To date, all of the commonly used monomeric cyan fluorescent proteins (CFPs), including enhanced CFP (ECFP) and Cerulean, retain this tryptophan-derived chromophore despite intrinsic and undesirable spectroscopic properties such as the broad and double-humped emission peak. Starting from the *Clavularia* coral-derived tetrameric cFP484, we have engineered a new monomeric teal fluorescent protein (mTFP1) with a tyrosine-derived chromophore that provides several advantages over cyan fluorescent alternatives currently available. We expect that this new protein will find immediate practical application as a bright label in biological fluorescence imaging and in fluorescence resonance energy transfer (FRET) type experiments in particular. In related and ongoing work, we have used rational engineering and directed evolution to create a variety of new proteins that fluoresce in the blue to cyan region of the visible spectrum. Directed evolution of proteins that harbor a variety of novel chromophore structures has produced bright and interesting new variants that may find practical application in biological imaging.

SAM-1,b

Engineering fluorescent proteins for wavelength shift and photostability. Nathan C Shaner*. University of California, San Diego, La Jolla, CA, USA.

Fluorescent proteins are genetically encodable tags that have become ubiquitous tools in molecular and cell biology. While wild-type fluorescent proteins sometimes possess sufficiently beneficial properties to be used unmodified, many applications require improvements in brightness or photostability, reduction of oligomerization, or other specific properties that require additional engineering of the wild-type protein. The previously engineered monomeric red fluorescent protein, mRFP1, has been engineered through a combination of rational design and directed evolution into a set of monomeric fluorescent proteins spanning from blue through far-red, providing a more diverse array of genetically encodable tags. The resulting far-red fluorescent proteins have been studied in further detail after the discovery that certain variants exhibited the novel property of reversible photoactivation. While fluorescent proteins typically bleach at a substantially slower rate than many small-molecule dyes, lack of photostability remains an important limiting factor for many experiments requiring large numbers of images of single cells. Screening methods focusing solely on brightness or wavelength are highly effective in optimizing both properties, but the absence of selective pressure for photostability in such screens leads to unpredictable photobleaching behavior in the resulting fluorescent proteins. We demonstrate that selection specifically for photostability is also feasible, resulting in a new generation of highly photostable variants.

SAM-1,c

Novel marker proteins from *Entacmaea quadricolor*. Joerg Wiedenmann*. University of Ulm, Dept. of General Zoology and Endocrinology, Ulm, Germany.

The color variability of the sea anemone *Entacmaea quadricolor* depends on the content of cyan, orange and red fluorescent proteins. Despite their intense fluorescence, the red shifted emitters show structural features commonly found among non-fluorescent chromoproteins. Engineered derivatives useful for live cell imaging include thermostable, pseudomonomeric and red shifted variants.

SAM-1,d

Excited state proton transfer pathways in wtGFP and GFP mutants. Deborah Stoner-Ma[1,*], Jerome Nappa[2,*], Peter J Tonge[1,*] and Stephen R Meech[2,*]. [1]Department of Chemistry, Stony Brook, NY, USA, [2]School of Chemical Sciences and Pharmacy, Norwich, UK.

Green fluorescent protein (wtGFP) and related proteins are commonly used tools in cellular and molecular biology. The intrinsic fluorescence of these proteins, due to their autocatalytically formed chromophore, has permitted their use as visual markers for protein trafficking, localization and interactions, as well as their use as sensors for intracellular or organelle conditions. In recent years, there has been a rapid expansion in the number and types of applications of these proteins and hence a continuous need for new, specifically tuned reporters. Improved understanding of the photophysical properties and behavior of these proteins is therefore of great importance. The research to be presented includes the results of an exploration into the photophysics of wtGFP using vibrational spectroscopy, a method which can provide insight into structural changes of photoactive proteins. The process of excited state proton transfer (ESPT) in wtGFP has been studied using time-resolved techniques and has allowed for the identification of Glu222 as the terminal proton acceptor. Several mutants have been examined as well which appear to utilize an alternate pathway for proton transfer. This new pathway, direct from the chromophore to an introduced aspartate residue, allows for the recovery of ESPT in mutants in which the standard proton transfer pathway has been disabled.

SAM-1,e

Chromogenic cross-link formation in green fluorescent protein. Rebekka M Wachter*, Liping Zhang*, Lauren J Pouwels* and Gabrielle D Malo*. Department of Chemistry and Biochemistry, Tempe, AZ, USA.

In recent years, the diversity of known protein prosthetic groups generated from genetically encoded amino acid residues has expanded tremendously. A number of post-translational modifications are oxidative in nature and utilize molecular oxygen as the final electron acceptor. An important class concerns the specific oxidation of a tyrosine residue to generate built-in redox cofactors in various enzyme systems. However, a distinct and entirely unprecedented tyrosine oxidation process is exemplified by a group of colored proteins, whose founding member is green fluorescent protein (GFP). This family of proteins requires neither metal nor enzyme systems to generate an internal chromogenic cross-link. A spontaneous main-chain cyclization reaction triggers the sensitivity towards molecular oxygen by priming the protein active site for the transfer of redox equivalents to oxygen. Thus, the brightly fluorescing chromophores that are the trade-mark of GFP-like proteins are the result of an oxidative modification mediated by peptide condensation rather than metal chemistry. To better understand why chromophore biogenesis occurs in GFPs but not more generally in all sorts of proteins, we have used X-ray crystallography to examine the structural parameters essential for this process. In addition, we have carried out extensive maturation kinetic studies on intact and slow-maturing GFP variants. The emerging picture of the GFP self-modification process includes factors such as conformational pre-organization, modulation of proton dissociation constants, general base catalysis, hydration-dehydration equilibria and slow proton abstraction steps.

SAM-2,a

Physics and optics of the human eye. David Sliney*. USA Center for Health Promotion and Preventive Medicine, Gunpowder, MD.

Studies of the effects of intense light and ultraviolet radiation often fail to provide sufficient details of the exposure geometry and measurement technique to quantify exposures to the cornea, lens or retina in animal models. The impact of source position or the use of inappropriate radiometric quantities can greatly weaken laboratory studies. Furthermore, environmental studies of sunlight generally ignore the importance of the geometry of the special geometry of sunlight exposures. Few epidemiological studies pay appropriate attention to the changing exposures of the crystalline lens 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 have seldom been 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.

SAM-2,b

Retinal healing by near-infrared light therapy. J T Eells[1,*], M M Henry[2,*], J N VerHoeve[1,*], T M Nork[1,*], M.T.T Wong-Riley[2,*], D K Kirk[3,*], K Valter[3,*] and J Stone[3,*]. [1]The University of Wisconsin-Milwaukee, Milwaukee, [2]The Medical College of Wisconsin, Milwaukee, [3]The Australian National University, Canberra, Australia.

Mitochondrial dysfunction and oxidative stress are central in the pathogenesis of retinal and optic nerve diseases ranging from age-related macular degeneration to glaucoma. The broad objective of our research program is to identify the molecular signaling pathways that regulate the process of retinal aging and degeneration with the long-term goal of learning how to protect cells from degeneration. Low-energy photon irradiation by light in the far-red to near-infrared (NIR) spectral range (630-1000 nm) using low energy lasers or light emitting diode arrays has been shown to accelerate wound healing and improve recovery from ischemic injury in the heart. The therapeutic effects of NIR light are believed to be triggered by the interaction of NIR light with the mitochondrial enzyme, cytochrome oxidase, and culminate in improved cellular mitochondrial energy metabolism, antioxidant production and cell survival. Research in our laboratories has demonstrated that NIR-LED photo-irradiation increases the production of cytochrome oxidase in cultured primary neurons and prevents apoptosis induced by metabolic inhibitors. In vivo studies have documented the therapeutic benefit of NIR-LED treatment in the survival and functional recovery of the retina following acute injury produced by histotoxic hypoxia or high-intensity laser injury. Recent studies have also provided evidence that NIR-LED treatment attenuates photoreceptor degeneration in a rodent model of retinitis pigmentosa. These findings provide a link between the actions of NIR light on mitochondrial oxidative metabolism in vitro and cell injury in vivo. They provide a mechanistic explanation for the therapeutic efficacy of NIR light and support the potential for the use of NIR-LED therapy in the treatment of retinal injury and degenerative retinal diseases.

SAM-2,c

Beneficial effects of visible light. Joan E Roberts. Fordham University, New York City, NY, USA.

Mammals, including humans, experience an increase and decrease in the production of most hormones and neurotransmitters over a 24 hour period. The circadian (Latin: circa dies- about a day) system refers to the coordination of these daily biological actions. Although the human circadian system is regulated by endogenous clocks, the most powerful external regulator of the circadian response in humans is blue visible light [460-490 nm] which is transmitted through the eye. When blue visible light impinges on the retina, it sends a signal to the suprachiasmatic nucleus (SCN) in the hypothalamus leading to a cascade of hormonal changes in the pituitary, pineal, adrenal and thyroid glands. There are

specific photoreceptor(s) and photopigments in the retina responsible for regulating the circadian response. This presentation will review the precise human circadian photoreceptor, its location in the eye, the putative photopigment which has been identified and confirmed with knockout mice; and the action spectrum responsible for circadian regulation (at least for neural melatonin modulation) and finally the biological consequences of disrupting the human circadian response.

SAM-2,d

Retinal damage from light and non-light initiated pathways. Rosalie Crouch * and Jie Fan*. Department of Ophthalmology, Charleston, SC.

Retinal damage can occur from light induced activation of the visual pigments which have a dysfunction in the deactivation mechanism and by constitutive activation of the visual pigment by non-light induced processes. These two processes are present in serious clinical disorders causing blindness. One of the main enzymes involved in the deactivation of visual pigment is rhodopsin kinase (RK), which phosphorylates the activated pigment. Studies on the rhodopsin kinase knockout mouse (RK-/-) have shown the retina has rapid degeneration under cyclic light. If the pigment lacks the native chromophore, 11-cis retinal, such as in the Rpe65-/- mouse, slow degeneration occurs, due to a basal activity of the apoprotein. This apoprotein is unexpected phosphorylated. Our studies on the RK-/-::Rpe65-/- show that RK is the kinase responsible for the removal of this phosphorylation and that, on removal of this phosphorylation, degeneration is extremely severe. These results imply that a separate mechanism not involving the light-induced activated form of rhodopsin is responsible for this degeneration. Supported by NIH grants EY04939 and EY 14793, and an unrestricted grant from RPB.

SAM-2,e

Blue light induces A2E oxidation in the rat eye. Albert R Wielgus[1,*], Fred B Lih[2,*], Ken B Tomer[2,*], Colin F Chignell[1] and Joan E Roberts[3]. [1]Laboratory of Pharmacology and Chemistry, Research Triangle Park, NC, USA, [2]Laboratory of Structural Biology, Research Triangle Park, NC, USA, [3]Department of Natural Sciences, New York, NY, USA.

Blue light induced retinal injury has been associated with age-related macular degeneration. A2E is a blue light absorbing retinal chromophore that increases with age. Our previous studies have determined that A2E has little phototoxicity; however the oxidation products of A2E can contribute to retinal photodamage. Thus, we examined the potential phototoxic effect of blue light exposure in the rat eye and its relationship to A2E and oxidized A2E. Albino Sprague-Dawley rats were dark adapted for 24 hours. Control rats (n=10) remained in the dark while the experimental animals (n=10) were exposed to blue light (220 ftc) for 6 hours. Rats were sacrificed immediately following light treatment. The eyes were enucleated under dim red light and frozen in liquid nitrogen. Isolated retinas were homogenized in the Folch extraction mixture and then they were subjected to an additional chloroform extraction. Dry extracts were reconstituted and divided for determination of organic soluble compounds. Fatty acids were analyzed as methyl esters via EI/GC/MS, A2E and other chromophores using HPLC, and A2E oxidation products by LC-MS. Blue light exposure of the rat eye does not significantly change the fatty acid composition of the retina. The A2E concentration (normalized to fatty acid content) in blue light exposed animals was found to be lower than the A2E concentration in rats housed under normal light conditions. The concentration of the isomer of A2E, *iso*-A2E, was also lower under blue light exposure than under normal light conditions. On the other hand, the amount of A2E oxidized forms was higher in the animals exposed to blue light. It appears that blue light exposure promotes the oxidation of A2E and *iso*-A2E to A2E epoxides in the rat eye. As these A2E oxides are toxic to retinal tissue, this may partially explain blue light induced retinal injury. These studies suggest that similar phototoxic effects may also take place in human eyes; however additional research in that area is required.

SAM-2,f

Blue light damage to the retina: Role of chronically photooxidized RPE melanin. Tadeusz J Sarna[1,*], Andrzej Zadlo[1,*], Grzegorz Szewczyk[1,*], Mariusz Zareba[1,*], Michele H Henry[2,*] and Janice M Burke[2,*]. [1]Department of Biophysics, Krakow, Poland, [2]Department of Ophthalmology, Milwaukee, WI, USA.

Melanin in the retinal pigment epithelium (RPE) is believed to act as a antioxidant that protects the outer retina against adverse photoreactions and oxidative stress. However, melanosomes of the human RPE are long-lived organelles with almost no metabolic turnover that are theoretically susceptible to photoinduced oxidation. We investigated effects of aerobic irradiation of purified RPE melanosomes, isolated from cow and porcine eyes, with intense visible and near UV radiation, on their antioxidant properties and ability to photogenerate superoxide anion. Antioxidant properties of control and irradiated melanosomes were analyzed by measuring the melanosome ability to inhibit iron/ascorbate-induced peroxidation of lipids extracted from of cow eye retinas, iron-catalyzed free radical decomposition of hydrogen peroxide, and to bind ferric ions. The ability of the melanosomes to photogenerate superoxide anion was determined by ESR-spin trapping using DMPO as a spin trap. Our data show that while control, non-irradiated RPE melanosomes, inhibit iron-induced peroxidation of lipids in a concentration dependent manner and lower the yield of free hydroxyl radicals generated by the Fenton reaction, melanosomes irradiated with high doses of light are less efficient in inhibiting the peroxidation of lipids and generation of free hydroxyl radicals, and even become prooxidant. The reduced efficiency of the irradiated partially bleached melanosomes to act as an antioxidant appears to be related to the melanosome photooxidation that lowers their ability to bind, hence sequester, iron ions. Although it is not clear whether similar light-induced oxidation/bleaching of the RPE melanosomes occurs *in situ*, the consequence of chronic photoinduced changes in RPE melanosomes may be a diminished capacity

of melanin to help protect aged cells from oxidative damage. Supported by National Eye Institute grant R01 EY013722 and by Polish Ministry of Science and Information Technology grant 3 PO4A 00925.

SAM-3,a

Regulatory concerns for the photosafety testing of human drugs. Robert E Osterberg*. ACLAIRO Pharmaceutical Development Group, Vienna, VA, USA.

In 1938, a retrospectively phototoxic antimicrobial drug product (Elixir of Sulfanilamide) was instrumental in creating the Federal Food, Drug and Cosmetic Act. This powerful Act placed the burden of safety testing for any drug intended for human and/or animal use squarely upon the pharmaceutical industry. However it wasn't until the late 1980's that the FDA's Center for Drug Evaluation and Research (CDER) seriously began to consider the phototoxic potential of drugs by instituting internal policies, procedures and instructions to its drug reviewers. These instructions suggested, on a case-by-case basis, that for a potentially phototoxic drug the drug sponsor should submit to the appropriate CDER division protocols for investigating that potential. During this period of time, the CDER was involved in the review of drugs that exhibited a strong phototoxic potential such as psoralens, fluoroquinolones and nonsteroidal anti-inflammatory drugs and drugs that influenced phototoxic reactions such as retinoic acid derivatives and immunosuppressive drugs. Following several public presentations on the subject of phototoxicity, the CDER drafted and finalized in May of 2003 a Guidance for Industry, Photosafety Testing. This guidance is intended to assist drug sponsors in deciding whether they should test for photoirritation and assess the potential of their drug product to enhance UV-associated skin carcinogenesis. While several groups of molecules are known to enhance photoirritation and perhaps skin photo cocarcinogenesis, many classes of drugs have never been tested. Such drugs may either be administered systemically or topically and may directly influence photoirritation or alter biological processes or optical or structural features of the skin that function as protective mechanisms. Proposed regulatory approaches to identifying photochemical irritants and testing for enhancement of UV-associated skin carcinogenesis are discussed within the document. The guidance is obtainable at http://www.fda.gov/cder/guidance/index.htm in the pharm/tox section.

SAM-3,b

EU regulatory issues and perspectives with photosafety assessment. Peter Kasper*. Federal Institute for Drugs and Medical Devices (BfArM), Bonn, Germany.

A European guidance on photosafety testing of pharmaceuticals has been implemented in 2002. According to this document, photosafety assessment is usually needed if a drug substance absorbs UVB, UVA, or visible radition and is either topically applied or reaches skin or eyes following systemic exposure. In such cases preclinical studies addressing the endpoints phototoxicity, photoallergy, and photogenotoxicity (as a screening approach for predicting photocarcinogenic hazard) are recommended. The guideline endorses the use of *in vitro* models when available, particularly for phototoxicity (3T3 NRU PT) and photogenotoxicity (-clastogenicity) testing. Accumulating data and experiences with regulatory photosafety testing over the past years suggest that some of the guideline recommendations and concepts may need a re-evaluation. Issues of current discussions include the following aspects: (1) the criteria for deciding whether photosafety testing is needed are too unspecific and need inclusion of quantitative aspects (level of molar absorbance and skin exposure); (2) the high rate of positive findings in *in vitro* models, particularly from photo-clastogenicity studies, and their impact on the assessment process (strategy of avoidance or *in vivo* follow-up testing); (3) the pros and cons of testing several endpoints in parallel versus a tiered approach starting with an initial phototoxicity assessment; (4) the timing of photosafety testing during drug development. A concerted effort in compiling data from (mainly unpublished) regulatory/industry photosafety testing is proposed. Such a database would facilitate an evaluation of different testing approaches and may provide the scientific evidence needed to justify any revisions of the current testing paradigm.

SAM-3,c

Relevance, issues and limitation on the use of the 3T3-NRU in vitro phototoxic assay. Nathalie Alépée*, Isabelle Domingo*, Samuel Blond*, Christine Biagini* and Stephan Chevalier*. Pfizer PRGD Safety Science, Amboise, France, France.

Many types of chemicals are reported to induce phototoxicity. They are distributed in sun-exposed tissues and absorb radiation in the wavelength range of 290-700 nm. The spectral irradiance distribution of UVB, UVA and visible lights is usually associated to the 290-320, 320-400 and >400nm regions, respectively. The in vitro 3T3 Neutral Red Uptake phototoxicity test (NRU-PT) is based on a comparison of the cytotoxicity of a compound when tested in the presence and absence of exposure to UVA light. This assay was adopted by the European Agency for the Evaluation of Medicinal Products and the Food and Drug Administration for guidance on photosafety testing. The 3T3-NRU phototoxicity test was shown to be predictive of acute effects in animals and in human for UVA/visible absorbers. Chemicals absorbing in the UVA or visible range and positive in this test are highly likely to be phototoxic in vivo following systemic or topical applications. There are some limitations to the 3T3-NRU assay related to the biological system itself, such as the lack of metabolic activation, the dissolution of water-insoluble substances or complex drug formulations. The concentrations inducing an acute phototoxic effect in the cells may also considerably exceed the concentrations inducing phototoxicity in humans. In addition, the phototoxic potential of chemicals designed for topical application can not be determined. In order to determine the relevance of this assay for chemicals absorbing only in the 290 to 320 nm UVB range, strict UVB absorbers were tested. 2/3 of these were reported to be photoirritants in vivo. This study demonstrated that the 3T3 NRU-PT assay was not suitable to discrim-

inate the phototoxicity potential of strict UVB absorbers after 5 joules/cm2 UVA light irradiation. The in vitro 3T3 NRU-PT was also tested with an exposure to UVB light only in order to evaluate the phototoxic potential of strict UVB absorbers. In these conditions, none of known classic UVA and UVB phototoxicants or strict UVB absorbers showed evidence of cytotoxicity or phototoxicity. The potential of two in vitro tests, the red blood cell phototoxicity test and the human 3-D skin model phototoxicity test to evaluate UVB absorbers and/or topical chemicals will be described.

SAM-3,d

The use of UV absorption measurements and photostability to understand photosafety risk. Brian Henry*. Pharmaceutical Sciences, Sandwich, Kent, UK.

Photoreactive drugs that are present in light exposed tissues need to be assessed for their potential to cause harmful reactions in patients. The European and US regulatory authorities have recently issued guidelines to help frame the photosafety assessment process and potential label descriptions. The key trigger for photosafety assessments are: drug present in the skin and absorption of light. The EU guidelines also suggest that photodegradation or photosafety structural alerts should also be considered. We have used standard protocols to measure UV absorption and photodegradation in aqueous solution of a wide range of commercially available drugs with known photosafety issues in the clinic. The Molar Extinction Coefficients (MEC) was calculated for all observed peaks of light absorption and at the 290nm intercept. The MECs compounds with known clinical photosafety issues was typically in the range of 10,000 to 30,000Mol/cm – none had MECs <1000Mol/cm. So whilst an UV absorption measurement does not fully describe the photoreactivity potency of a molecule in a biological matrix, it does allow a simple, standardised measurement that can be used as part of a risk assessment to trigger further photosafety evaluation for a new compound. The compounds demonstrated a wide range photodegradation in the photostability assay. No relationship could be established between the degradation rate and either clinical evidence of photosafety issues or activity in the 3T3 *in vitro* photo irritation assay. There can be a complex series of events that follow light activation of a molecule and subsequent biological damage – it may not be possible to assess these by just measuring photodegradation of aqueous drug solutions alone We have developed screening methodology for light absorption and photodegradation measurement as part of a photosafety risk assessment process. Whilst light absorption measurements appear useful as an initial trigger for further safety evaluation, solution based photostability assessments do not. Photoreactivity screens that better measure how a drug molecule uses absorbed light energy or are converted to species that could cause biological damage will be useful and should be further developed.

SPM-1,a

Interactions of changing climate and photobiogeochemical cycles in aquatic environments. Richard G Zepp[1,*], David J Erickson III[2,*], Nigel D Paul[3,*] and Barbara A Sulzberger[4,*]. [1]US Environmental Protection Agency, Athens, Georgia, USA, [2]Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA, [3]Lancaster Environment Centre, Lancaster, UK, [4]EAWAG, Dübendorf, Switzerland.

Global biogeochemistry plays a critical role in controlling life processes, climate and their interactions, including effects on atmospheric greenhouse gas concentrations. Recent evidence indicates that the light-driven part of aquatic biogeochemical cycles is being altered by interactive global changes in solar UV irradiance, warming, precipitation, clouds and atmospheric circulation. Four specific examples are reviewed here: 1) changes in runoff from land to water; 2) changes in stratification and mixing; 3) warming-induced changes in microbial cycling of UV-absorbing colored dissolved organic matter; 4) changes in water to atmosphere fluxes of trace gases. For example, warming and precipitation change can alter the transfer of organic matter from terrestrial to aquatic ecosystems and thereby influence UV penetration into water bodies, with major consequences for aquatic biogeochemical processes. Moreover, future changes in climate may enhance stratification, reduce vertical mixing and alter the mixed layer depth of lakes and the ocean, which will alter UV effects on biogeochemistry in the surface layer. These changes in biogeochemistry include modification of carbon cycling that is linked to effects on the cycling of metals and mineral nutrients such as nitrogen. Changes in metal cycling can also affect the light-induced production of reactive oxygen species in aquatic systems, and these changes in oxidative activity affect contaminant decomposition and microbial cycling of carbon and other elements. Interactions between changing solar UV and climate change in aquatic environments are changing water-to-atmosphere exchange of trace gases, such as methyl bromide and dimethylsulfide. Methyl bromide can catalyze depletion of the ozone layer and oceanic emissions of dimethylsulfide produce particulates (i.e., sulfate aerosols) that directly and indirectly (via clouds) have a cooling effect on the marine atmosphere. (Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy).

SPM-1,b

Phytoplankton photosynthesis in a scenario of global change: Interactive effects of UVR, mixing and CO2 in tropical environments. E. Walter Helbling[1,2,*], Kunshan Gao[1,*] and Virginia E Villafañe[1,2,*]. [1]Marine Biology Institute, Shantou University, Shantou, Guangdong, China, [2]Estación de Fotobiología Playa Unión, & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rawson, Chubut, Argentina.

Some of the potential outcomes of global warming are the increase of stratification in the water column, changes in both ocean circulation patterns and mixing rates. These changes could induce a higher stress in phytoplankton cells

by exposing them to higher UVR levels due to their circulation within a shallower upper mixed layer (UML). On the other hand, it has been shown that an increase of CO2 concentration favored phytoplankton enhancing their growth rates. Our experiments conducted in a freshwater lagoon of a tropical region of Southern China (23o 26N, 116o 42E) were designed to assess the impact of UVR on phytoplankton photosynthesis (i.e., evaluated through measurements of photosynthetic quantum yield, Y). Two mixing regimes (1 rotation in 10 minutes / 1 hour down to 2.1 optical depths) were imposed in cells acclimated to two CO_2 concentrations - ambient level and 1100 ppm. UVR was the most important variable affecting Y, followed by mixing and finally by CO_2 concentration. During the study period (March to May, 2005) the inhibition due to UVR was $>$ 50%, with UV-A accounting for most of the observed impact. The rate of decrease of photosynthetic quantum yield per hour of exposure was higher under low than under high mixing speed. Cells acclimated to ambient CO_2 levels were slightly (but significantly) more sensitive to UVR than those acclimated to high CO_2, when large dinoflagellates (i.e., *Ceratium* spp. and Peridinium spp.) accounted for most of biomass. On the other hand, when piconanoplankton cells ($<$ 2um) dominated, there were no differences between ambient and high CO_2 acclimated cells. Our data thus clearly suggest that the combined impact of UVR, mixing and CO_2 concentration is highly dependent on the species composition and / or size structure of the assemblages.

SPM-1,c

Influence of increased solar UV radiation and other climatic changes on terrestrial ecosystems. Donald T Krizek*. USDA/ARS, Sustainable Agricultural Systems Laboratory, Beltsville, MD.

Since the early 1970s',there has been growing concern about the possible biological effects of stratospheric ozone reduction and the attendant increase in solar ultraviolet-B (280-320 nm) radiation. The impact of global warming and other climatic changes on plant productivity that may result from increased carbon dioxide and other trace gases may be profound. Changes are expected in the frequency and intensity of precipitation and storms as a consequence of global warming. To develop a meaningful assessment of terrestrial ecosystem responses to global climate change, there will be need to conduct ecosystem studies across and within multiple trophic levels in order to examine the interactive effects of UV-B, carbon dioxide and tropospheric ozone levels along with other stress factors. In conjunction with these stress factors, increased UV-B radiation may alter many important ecosystem processes such as biomass production, plant-herbivore interactions, disease incidence, change in species abundance and composition, and mineral nutrient cycling. The response of plants to enhanced UV-B levels has been investigated using both UV supplementation and UV exclusion techniques. Based on these studies, one can draw some generalizations as to the likely response of terrestrial plants to projected changes in global climate. Higher plant species are expected to vary greatly in their response to increased UV-B radiation with the most typical effects being a reduction in height, leaf area, and shoot biomass, and an increase in phenolics and other secondary compounds. The overall effect of global warming on crop production may be either positive or negative, depending on the cropping system and the severity of market disruptions. Crop simulation models may be needed in reaching decisions as to how best to achieve food security and mitigate environmental change.

SPM-1,d

Impacts of ultraviolet radiation in tropical marine ecosystems. Yasmín Detrés*, Roy A Armstrong* and Juan L Torres*. Department of Marine Sciences, Mayagüez, PR, USA.

The coastal and neritic waters of the Caribbean Sea are dominated by highly productive interconnected ecosystems including mangrove forests, seagrass beds and coral reefs. Tropical latitudes are exposed to high year-round UV radiation due to the smaller solar zenith angles and the natural thinning of the ozone layer towards the equator. Furthermore, the low attenuation of these wavelengths in clear tropical waters allows the penetration of UV to significant depths reaching benthic organisms in the euphotic zone. For the last nine years (1996 to 2005) we have assessed the impact and responses of UVR in the climax marine macrophytes *Rhizophora mangle* and *Thalassia testudinum*, as well as in the reef building coral species *Montastrea faveolata*, *Acropora cervicornis*, and *Porites furcata* at La Parguera Marine Reserve, southwestern Puerto Rico. These assessments included UV exclusion and supplementation experiments in the field and in the laboratory. Studies with macrophytes considered the impact of UV in growth, photosynthetic and photoprotective pigments composition, photosynthetic efficiency, and changes in leaf optical properties. Research with hermatypic corals evaluated the changes in the content and distribution of Mycosporine like amino acids (MAA's), growth, photosynthetic pigments composition, and fecundity with exposure to UV. This study required accurate measurements of UV irradiance above and underwater and the optical characterization of the water column. A comprehensive summary of the impacts of UVR on these organisms, trends in UV fluxes, and the optical properties of these waters will be presented.

SPM-1,e

Impacts of changes in climate and UV-B radiation on Arctic terrestrial ecosystems. Terry Callaghan*. Royal Swedish Academy of Sciences, Abisko, Sweden.

In northern latitudes, temperature has increased, particularly during winter and spring, and snow cover has decreased. Projections for the next 100 years suggest continued warming, particularly during winter. Recent past and projected climate changes amplify global trends because of feedbacks from Arctic surfaces and ecosystems to the climate system. Measurements of UV-B radiation show localised recent increases that, combined with earlier melt of snow, are likely to affect ecosystems. Within the Arctic Climate Impact Assessment, ecologists have assessed the impacts of these changes on ecosystems. The dominant re-

sponse of Arctic species to climate change is likely to be relocation rather than adaptation. Some species are at risk, but productivity and number of species are very likely to increase overall. Crossing environmental thresholds, and extreme winter events will trigger changes in populations. Forest is projected to replace much of the tundra affecting biodiversity, ecosystem function and land surface-atmosphere processes. However, forest advance is slow and not possible everywhere due to environmental and sociological processes. Rapid climate change that exceeds the ability of species to re-locate will increase disturbances such as fires, disease and pest outbreaks. Displacement of tundra by forests and shrublands will decrease albedo, which increases the positive feedback to climate warming. Warming and drying of soils in parts of Alaska have already changed the carbon status of this tundra from sink to source. Elsewhere, discontinuous permafrost is thawing, soils have become waterlogged and methane emissions have increased. Future vegetation responses to warming are projected to lead to a small, long term carbon sink in the Arctic, although uncertainties are high. Enhanced CO2 and UV-B affect plant tissue chemistry and have subtle but long-term impacts on ecosystem processes that reduce nutrient cycling with the potential to affect productivity and herbivory.

SPM-2,a

Photogenotoxicity testing: A sensible screen for photocarcinogenicity? Elmar Gocke*. F. Hoffmann-La Roche AG, BASEL, Switzerland.

Prolonged exposure of unprotected human skin to short wavelength UV light (UV-B) is implicated in inducing adverse consequences, mainly due to the direct absorption of the high energy UV photons by DNA. Other chromophores present in the skin or the eyes can enhance the phototoxic action and extend the effective light spectrum to long wavelenghth UV (UV-A) or even visible light by absorbing the incident energy and generating e.g. reactive oxygen radicals or degradation products. In drug development phototoxic liabilities should be recognized in early development phases as they can have strong impact on the safety and acceptance - and consequently the commercial success - of a new drug. The EMEA photosafety guideline recommends parallel testing for phototoxicity/ photogenotoxicity/ photoallergenicity for compounds which have light-absorbing properties and are present in sun exposed organs. This recommendation can logically be contrasted with a tiered approach to photosafety testing. Photogenotoxicity tests employed in drug safety assessment are largely derived from standard in vitro genotoxicity tests adapted for concomitant light exposure. Photogenotoxicity data for a number of in house development candidates (fluoroquinolone, chloroquine analogue, Ro19-8022 and pyridone analogues) are presented. Tests for clastogenicity (i.e. chromosomal damage) are found to be more sensitive to photogenotoxic action than tests for gene mutations (i.e. Ames test). Analogously to general (dark) genotoxicity testing the result of the photogenotoxicty test is appraised as predictor of photocarcinogenic properties. Data accumulated over the last years show that established in vitro phototoxins induce in vitro photogenotoxicity, specifically photoclastogenicity, almost without exception. Thus the use of a photoclastogenicity assay as screen for photocarcinogenicity may well represent a redundant effort because the hazard identification might be satisfied by the in vitro phototoxicity test. Unless the quality of the photogenotoxicity result yields valuable information on the photocarcinogenic risk it can be questioned whether the performance of the photogenotoxicity assays will yield added value for compounds which have been shown to possess phototoxic properties.

SPM-2,b

Pre-clinical photoirritation and photoallergy testing. J F Nash*. The Procter & Gamble Company, Cincinnati, OH, USA.

Photosensitization is a general term used to describe two clinical events namely photoirritation(toxicity) and photoallergy. Photoirritation is produced by the interaction between UV or visible radiation and a chemical resulting in an acute, adverse skin reaction. Photoirritation is common to many drugs. The clinical manifestation of this interaction is an exaggerated sunburn with urticaria, erythema and tenderness. A photoirritation reaction can be evoked in all subjects provided the concentration of chemical and dose of light are appropriate. The 3T3-NRU Phototoxicity Test (OECD 432) is used to determine photoirritation potential of a compound. If the material cannot be tested in vitro or a NOEL needs to be established, photoirritation testing has been done in several species including mice, rabbits and Guinea pigs. The Guinea pig test is the preferred test for determination of the photoirritation potential of compounds. Photoallergy is a cell-mediated immunologic reaction to a chemical that has been made antigenic by the interaction with UV or visible light. This reaction is similar to allergic contact dermatitis, but differs in that chemicals require activation by light to elicit the response. Photoallergy is less frequent compared to photoirritation. Clinically, photoallergy skin responses may be distinguished from photoirritation by the increased severity with repeat exposure, time course for eliciting a response, spreading edema, vesiculation and itching. Presently, photoallergy can only be assessed using in vivo methods. The preferred animal species is the Guinea pig. However, mouse models for systemic administered compounds are often used since they tend to be less expensive and additional pharmacokinetic data may exist in this species. For routes of exposure other than topical, plasma and/or skin concentrations may be needed to optimize the interaction between drug and light exposure. Many of the known human photoallergens are also photoirritatnts. Therefore, testing for photoallergy is recommended after a phototoxicity assessment.

SPM-2,c

Preclinical phototoxicity testing of pharmaceuticals. Miklos Csato*. F. Hoffmann-La Roche Ltd., Dept. PRBN-T, Bldg 73/101A, Grenzacherstr. 124, Basel, Switzerland.

To address phototoxicity in preclinical development a strategy involving spectrophotometric evaluation of drugs followed by in vitro and in vivo characterization has been

established. In the in vitro assay growth and viability of drug-treated 3T3 murine cultured fibroblasts kept in dark vs. exposed to sub-lethal UV-irradiation was evaluated. The assay was set out to detect in vitro potential of a drug, i.e. used for hazard identification. Further characterization was done in vivo in the hairless rat. The rat was selected based on availability of pharmacokinetic information and the fact that UV light transmittance of the epidermis in the truncal skin is similar to that in humans. The animals were treated repeatedly, once daily for a week, through the appropriate route and after the last dosing, at the Tmax of a systemic drug in the blood, skin sites were exposed to different doses of sub-erythemal UV-A irradiation. Resulting erythema was indicative of a phototoxic reaction induced by a test compound. The assay has been evaluated with different classes of human phototoxicants. 466 compounds have been tested through the approach. Selection of the compounds for the testing was done by evaluating their UV and visible light absorption characteristics. Strong absorption particularly in the longer UV-A range was frequently associated with phototoxicity. Approximately 30% of the tested compounds proved to be positive in the in vitro test. 35 compounds could not be conclusively evaluated due to their poor water solubility. Among the compounds advanced to in vivo characterization all in vitro negatives remained so in vivo, while approx. 20% of the in vitro positive molecules did not induce phototoxicity in vivo. The test strategy is practicable and appropriate to identify and work up drug phototoxicity through preclinical development.

SPM-2,d

Ocular toxicity testing. Joan E Roberts*. Department of Natural Sciences, Fordham University, Lincoln Center Campus, 113 West 60th Street, New York City, New York, USA.

Although the human eye is constantly subjected to both artificial and sun light, damage rarely occurs from this exposure unless the eye is aged or the light is particularly intense. However, many drugs, dietary supplements, cosmetics and diagnostic dyes have the potential to induce damage to the eye leading to transient or permanent blinding disorders. The degree to which a particular exogenous photosensitizing substance is capable of producing phototoxic and toxic side effects in the eye depends on several parameters including: l) the chemical structure; 2) the absorption spectrum of the drug; 3) binding of the drug to ocular tissue (lens proteins, melanin, DNA); and 4) the ability to cross blood-ocular barriers (amphiphilic or lipophilic). For instance, compounds that have either a tricyclic, heterocyclic or porphyrin ring structure and are incorporated into ocular tissues are potentially phototoxic agents in the eye. The magnitude of the damage to the eye (photoefficiency) can be predicted first by screening with in vitro, in situ, and photophysical techniques. This relieves the burden of extensive in vivo analysis. An alternative way light can destructively interact with drugs in the eye is to disrupt the circadian oscillators (dopamine and melatonin) which control disc shedding of the retina. The daily removal of the upper portion of the rod outer segments (disc shedding) is a natural method of controlling light damage to the retina. With simple, inexpensive testing, compounds can be screened for their potential ocular toxicity and phototoxicity at their developmental stage. It may be that a portion of the molecule can be modified to reduce toxicity or phototoxicity while leaving the primary drug effect intact. Preclinical safety testing may prevent ocular side effects that can range from mild, reversible blurred vision to permanent blindness.

SPM-3,a

LHCII: From light-harvesting to photoprotection. Andy A Pascal*. SBFM / DBJC, Gif-sur-Yvette, France.

The light-harvesting antenna of photosynthetic organisms is designed to maximise the absorption cross-section of the system, and thus to increase photosynthetic yield in low light conditions. However, plants are subject to light intensities which can vary by several orders of magnitude and have thus evolved mechanisms to dissipate the excess energy when incident light is no longer limiting. These dissipative processes have in some cases to be induced and turned off again on timescales going from tens of seconds to several minutes. We have been making measurements on the fastest-reacting mechanism, which involves a conformational change in the major light-harvesting protein of photosystem II, called LHCII. We describe the changes observed in pigment properties associated with this conformational change in vitro, and also attempt to quantify the extent to which this change is observed in vivo.

SPM-3,b

Testing the biological system: Can a low energy chlorophyll carry out water splitting? Alison Telfer*. Imperial College London, London.

The marine organism *Acaryochloris marina* which lives underneath a marine colonial ascidian in extreme shade appears to have adapted to the near absence of photosynthetically useful light by using chlorophyll (Chl) d rather than Chl a as its major pigment. Chl d differs from Chl a, because it has a formyl group in place of the vinyl group on ring I of the porphyrin head group. Its QY absorption is shifted approx 35 nm to the red relative to Chl a. Consequently the excited state energy gap of the radical pair would be approx 100 mV less for photons of 715 nm as compared to the 680 nm light absorbed by the primary electron donor in Chl a PSII reaction centres. The question has therefore been posed whether there is sufficient energy for Chl d to carry out water splitting. We have been isolating PSII preparations from *A. marina* in order to discover whether the minor amount of Chl a, present in this organism, is an integral and important component of the PSII primary electron transfer reactions or is present for some other reason. Active PSII preparations have been elusive but recent low temperature transient absorption data using PSII-enriched preparations will be presented which attempts to untangle this conundrum.

SPM-3,c

Synthetic models duplicating early events in PSII. Ally Aukauloo[1,*], Annamaria Quaranta[2,*], Fabien Lachaud[1,*], Yuanjun Hou[2,*], Christian Herrero[2,*], Pierre Dorlet[1,*], Marie-France Charlot[1,*] and Sun Un[2,*]. [1]Laboratoire de Chimie Inorganique, Orsay, France, [2]Service de Bioénergétique, Gif-sur- Yvette CEDEX, France.

Splitting water to H_2 and O_2 using sunlight as the only source of energy has constantly relinquished chemists on the side of the bench. However, this scientific task in the actual worldwide energy context is regaining interest. This is because Hydrogen is called to play a crucial role as the future energetic vector. This problem can be divided into two parts, in one, the photooxidation of water to O_2 and H^+ and secondly the reduction of protons to hydrogen. Our approach to perform these reactions is biomimetic. The first reaction is carried at Photosystem II, which uses sunlight to oxidise water following a four electron process. This apparatus can be divided in two parts, a photoactive site and a catalytic part. We will discuss on the modelisation of the first photoinduced electron trade within Photosystem II.

SPM-3,d

Bioinspired energy conversion schemes. Ana L Moore*, Gary F Moore*, Michael Hambourger*, Miguel Gervaldo*, Paul A Liddell*, Devens Gust* and Thomas A Moore*. Arizona State University, Department of Chemistry & Biochemistry, Tempe, AZ, USA.

The long-term objective of our research is the design of bioinspired schemes that couples solar energy to the oxidation of water and the subsequent use of the reducing equivalents to synthesize energy rich/reduced compounds. In an initial approach, a photoelectrochemical cell that oxidizes carbohydrates, alcohols, or other organic compounds, uses light to boost the reduction potential of the resulting electrons, and reduces hydrogen ions to hydrogen at neutral pH is being developed. The photoanode consists of a Gratzel-type nanoparticulate TiO_2 electrode coated with a porphyrin sensitizer. Upon visible light excitation of the porphyrin sensitizer, electrons are injected from the S_1 state of the porphyrin into the TiO_2 conduction band. These electrons are then passed through the external circuit to a microporous platinum cathode where hydrogen evolution occurs. It was found that excitation of the photoanode with light absorbed only by the porphyrin results in evolution of hydrogen from the cathode with a quantum yield of ca. 5%. Glucose or other reduced carbon compounds in the anode solution are oxidized by the appropriate NAD-linked dehydrogenase enzyme reducing NAD^+ to NADH. NADH is oxidized by the porphyrin radical cation, regenerating the porphyrin ground state for subsequent rounds of photo-excitation. Key to the operation of the cell is the coupling of the anode photoreactions to the oxidation of biological fuels by the NADH/NAD^+ coenzyme and NAD-linked dehydrogenase enzyme and the facile and cyclical electron donation to the oxidized sensitizer by NADH, generating NAD^+, which is not reduced by charge recombination reactions at the photoanode. The design, synthesis, and electrochemical and photophysical characterization of a series of synthetic models of the donor side of PSII where a porphyrin chromophore plays the role of P680 will also be presented. In these biomimetic models, the porphyrin moiety is covalently attached to either tyrosine or to a hydrogen bonded phenol/imidazole pair. Initial experiments with these models adsorbed on the photoanode of the photoelectrochemical will be described.

MON-A,a

Following the photon: From T cells to tumors. Frances P Noonan*. Laboratory of Photobiology and Photoimmunology, Washington, DC.

Sunlight is fundamental to our environment but has the negative consequence of initiating skin cancer in susceptible populations. Of particular concern is the most lethal of the skin cancers, cutaneous malignant melanoma. This tumor of the melanocyte, the pigment cell of the skin, is currently one of the fastest increasing cancers and is frustratingly resistant to current therapies. The mechanisms by which sunlight causes melanoma are controversial and not well understood and have been difficult to access experimentally. This lack of understanding has been a major barrier to effective prevention and treatment. Outstanding fundamental questions include which wavelengths in sunlight are responsible for melanoma, the roles of pigment - protective and/or a prooxidant melanoma promoter, UV-induced DNA damage and repair and UV-induced immunosuppression. This talk will address some of these questions using data derived using a mouse model for UV-induced melanoma which recapitulates the etiology, genetics and histopathology of human disease.

MON-A,b

Thermal imaging and smart packaging: The photophysics way. Lisa Kelly*. Department of Chemistry and Biochemistry, Baltimore, MD, USA.

Fluorescent sensors have found broad-ranging applications in biological, chemical, and engineering applications. An enormous number of chemical and biological sensors is reported in the literature. In addition, there has been growing interest in the creation of "smart materials," capable of fast, reversible responses to environmental stimuli. These materials offer great prospects as "smart coatings" to map, in two dimensions, changes in environmental conditions across a surface or object. Photophysical processes in molecular assemblies provide a simple basis for designing "smart materials." Our laboratory has focused on incorporating these assemblies into temperature-sensitive materials. When formulated into a polymeric system, the material may be coated on the tip of a bifurcated optical fiber or spin- or spray-casted onto an object. When used with appropriate illumination and imaging devices, remote, full-field measurements of temperature, pressure, humidity, or other environmental properties become viable. This lecture will focus on how photophysics and polymer design can be combined to create fluorescent coatings for non-contact thermal imaging. Exciplex and excimer formation in polymer-encapsulated and polymer-bound perylene dyes serves as the basis of the functional coatings. The temperature-dependent equilibria offer

a dually (two color) emissive material. The relative intensities provide an absolute and real-time temperature of the coated surface. Insight into the mechanism of the thermal response is provided by steady-state and time-resolved fluoresence spectroscopies. In parallel, fluorescent polymers that are thermally and irreversibly modified can provide the basis of "smart packages." Polymer systems will be described where the fluorescent dye is quenched. When a thermal reaction causes the quencher to disappear, the material becomes fluorescent and indicates that the package has exceeded an undesirable thermal threshold. This chemistry will be discussed, along with ways in which the threshold can be modified via the polymer architecture.

MON-A,d

UVA-induced DNA damage in human skin cells. Thierry Douki*. Laboratoire "Lésions des Acides Nucléiques, Grenoble, France.

Exposure to solar ultraviolet radiation (UV) is known to be the major cause of the induction of skin cancers. DNA damages induced by the most energetic part of the UV portion of solar light (UVB: 280-320 nm) are now well identified. They mostly consist in dimerization products involving adjacent pyrimidine bases (thymine T and/or cytosine C). Several types of dimeric photoproducts can be formed, including cyclobutane dimers (CPDs), (6-4) photoproducts and their related Dewar valence isomers. The other part of the solar UV spectrum (UVA: 320-400 nm) is at least 10 to 20 times more abundant than UVB. UVA are known to be mutagenic but the DNA lesions involved in this deleterious process are not clearly identified. Using a sensitive and specific liquid chromatography / mass spectrometry assay, we showed that UVA irradiation induced the formation of CPDs in significant yield within both primary culture of human cutaneous cells and human skin biopsies. Interestingly, in both cultured cells and whole skin, the frequency of CPDs was higher than that of 8-oxo-7,8-dihydroguanine, the major UVA-induced oxidized DNA base. These observations rule out oxidative stress as a major process involved in the genotoxicity of UVA. Another interesting feature of the distribution of UVA-induced DNA lesions is the lack of formation of (6-4) photoproducts and Dewar isomers, strongly suggestive of the involvement of a photosensitized triplet energy transfer process in the formation of CPDs upon UVA irradiation. This was confirmed by the predominance of TT-CPD with respect to the related TC, CT and CC derivatives. Measurements within cultured cells and whole skin also showed that CPDs are removed less efficiently after UVA than UVB irradiation. Altogether, these observations show that CPDs are most likely to play a major role in the genotoxic properties of UVA radiation.

MAM-2,a

Sunlight, skin cancer protection campaigns and vitamin D deficiency. Susan L Walker*. Department Environmental Dermatology, London, UK.

Cutaneous synthesis of vitamin D (VitD) is one of the few benefits of exposure to solar ultraviolet radiation. The role of VitD in calcium homeostasis and bone health is relatively well understood but there is emerging evidence that poor VitD nutriture increases the risk of colon, breast and prostate cancer as well as autoimmune and cardiovascular diseases. VitD is synthesised by solar ultraviolet B radiation (UVB; 295-315nm) the waveband that is also the prime cause of sunburn and skin cancer. Exposure to sunlight is essential in order maintain adequate VitD status, as dietary sources of VitD alone are inadequate, but a balance needs to be struck between a reduction in UVB to prevent skin cancer and enough UVB exposure to meet the body's requirements for VitD. Dermatologists often state that 15 minutes exposure of the face, arms and hands to noonday summer sunlight two or three times a week is 'sufficient' for VitD synthesis, but these recommendations are based on very limited human studies and actual amount of UVB needed is uncertain. Given that VitD deficiency is widespread at temperate latitudes, with ≈25% of free-living adults becoming deficient during the winter months it is essential that the amount and frequency of sunlight exposure required to maintain VitD is accurately determined. This need is not just a concern for white-skinned subjects who have an elevated risk of skin cancer but is also relevant to individuals with pigmented skin - a group in which photobiological research is very limited. There is a clear need to establish recommendations for optimising VitD nutriture without increasing risk of skin cancer but it is essential to formulate appropriate public health messages about sun exposure for different ethnic groups. The aim of this review is to outline the risk/benefits associated with sun exposure and highlight the gaps in current research in this area.

MAM-2,b

Solar UV Doses of American youths and adults are inadequate for sufficient Vitamin D_3 production. Dianne E Godar[1,*], Stanely J Pope[2,*], John Streicher[3,*], Steven J Mackin[4,*], Sergio G Coehlo[1,*], William B Grant[5,*] and Michael F Holick[6,*]. [1]US Food and Drug Administration, Rockville, MD, [2]Sun Systems & Svc, Inc., Oak Park, MI, [3]National Oceanic and Atmospheric Administration, Research Triangle Park, NC, [4]Solartech Inc., Harrison Township, MI, [5]Sunlight, Nutrition and Health Research Center (SUNARC), San Francisco, CA, [6]Boston University School of Medicine, Boston, MA.

Solar UV radiation (290-400 nm) affects human health in both detrimental and beneficial ways. The detrimental health effects include skin cancers (melanoma and non-melanoma), while the beneficial health effects include vitamin D_3 production. Vitamin D_3 is not only important for bone and muscle health, but may also reduce the incidence of multiple sclerosis and type 1 diabetes mellitus in children and a variety of cancers such as prostate, breast, and colon in adults, along with tooth loss and hip fractures in elderly people. Evaluation of the vitamin D status (measured as 25-hydroxyvitamin D in serum) of many Americans show they have insufficient (<50 nmol/L) levels, indicating they are not getting adequate solar UV doses to make sufficient vitamin D_3, according to the current recommendations (200 IU/day for people under 50 yr; 400 IU/day for people over 50 and under

70 yr; 600 IU/day for people over 70 yr). We decided to investigate this situation using the solar UV doses of indoor-working Americans (about 10,000) to see if they are making sufficient vitamin D_3. We find many Americans are getting inadequate incidental solar UV exposures to make sufficient amounts of vitamin D_3: Fitzpatrick skin types I/II during the winter (and fall in the north); skin types III/IV all year, except summer (and spring in the south); skin types V/VI all year. People over 40 yr can only make about two thirds and people over 60 yr can only make about half the amount of vitamin D_3 that young adults (0-20 yr) can make. If people diligently wear SPF 15 sunscreen, they will dramatically reduce (>90%) their production of vitamin D_3. Furthermore, few people make ample vitamin D_3 by the current suggested level (1,000 IU/day), except some skin types I/II during the summer and some skin types I/II and III/IV that take a two-three week vacation during the summer. Thus, most Americans are not going outside enough to get solar UV doses that can make sufficient amounts of vitamin D_3 for about half the year or more, depending on age and skin type.

MAM-2,c

Sunscreens and Vitamin D: A review. J F Nash*. The Procter & Gamble Company, Cincinnati, OH, USA.

Reducing the damage to human skin from repeated exposure to solar UV radiation has been the goal of public health campaigns throughout the world. However, this safe sun strategy has been questioned based on the concern that reducing solar UV exposure may be responsible for vitamin D insufficiency, which is reportedly on the rise. Solar UV, primarily short wavelengths (290-320 nm), is responsible for the conversion of 7-dehydrocholesterol to form cholecalciferol or pre-vitamin D3 in human skin. Pre-vitamin D3 enters the circulation and is converted to 25-hydroxyvitamin D in the liver and then to the biologically active 1,25-dihydroxyvitamin D in the kidneys. As such, the skin benefits of reducing or, in theory, eliminating solar UV exposure by use of sunscreens could be offset by the vitamin D insufficiency and negative health effects. Experimentally, it has been reported that acute or repeated application of a sunscreen will reduce artificial UV-induced increases in mean serum 25-hydroxyvitamin D (25-OH Vit D) levels. However, these experimental findings are unlikely to manifest as vitamin D insufficiency under consumer use. For example, in prospective clinical trails of daily sunscreen use coupled with sun avoidance behavior, normal serum concentrations of 25-OH-Vit D have been reported. There are practical reasons for this including improper product application, sunscreens do not totally block UV transmission or maintain photoprotection for extended periods, and incidental sun exposure sufficient to allow adequate vitamin D production to take place. In any event, achieving vitamin D sufficiency is possible by dietary supplements, i.e., multi-vitamin with calcium. This approach would provide the health benefits without encouraging intention exposure to a known human carcinogen, solar UV.

MAM-2,d

The relationship between Vitamin D, UVB and the immune system. Margherita Cantorna*. Pennsylvania State University; Department of Veterinary and Biomedical Sciences, University Park, PA, United States.

A major source of vitamin D results from its manufacture via a photolysis reaction in the skin. Interestingly the wavelength of light required for vitamin D synthesis overlaps considerably with the documented immunosuppressive effects of UVB. The inverse relationship between sunlight exposure, vitamin D status and autoimmunity suggests that the availability of vitamin D through UVB may be a risk factor for autoimmune disease development. It is clear that alterations in vitamin D status impact immune function. In particular, vitamin D deficiency results in the exacerbation of many experimental models of autoimmunity. Conversely, supplementation with the hormonally active form of vitamin D (1,25(OH)2D3) completely blocks the development of experimental autoimmune diseases. Comparisons of the effects of vitamin D versus UVB on immune function highlight a number of similarities and a number of important differences. UVB has been shown to increase the susceptibility of mice and rats to infectious diseases while 1,25(OH)2D3 has not been shown to compromise the ability of infected mice to fight a fungal or viral infection. In addition, there is evidence that at least for tuberculosis vitamin D and 1,25(OH)2D3 may actually increase resistance to infection. In experimental autoimmunity the effects of UVB have been shown to suppress experimental multiple sclerosis before induction of disease but to exacerbate disease after induction. 1,25(OH)2D3 is effective at suppressing experimental multiple sclerosis both before and after disease induction. It seems likely that some of the UVB effects on the immune system might be a result of increased vitamin D production. However, the definitive experiments have not been done to clearly demonstrate the relative role of vitamin D versus UVB on the immune system.

MAM-2,e

Strength of evidence for vitamin D status and internal malignancy. Marianne Berwick*. University of New Mexico, Albuquerque, New Mexico, USA.

Numerous papers and talks have recently been published that suggest that serum vitamin D would help prevent some cancer incidence and in some cases mortality. The major mechanism for this preventive effect has been postulated to be control of cellular proliferation and apoptosis. Most of the studies have focused on breast, colorectal and prostate cancers. The data to support the inverse association between serum vitamin D levels and these cancers varies from somewhat persuasive to only interesting suggestions. A number of issues are critical to consider when evaluating the postulated effects of vitamin D. Among these are study design, the actual measurement of serum vitamin D, the validity and reproducibility of the measures, and the optimal level of vitamin D itself. Many of the studies have measured proxies for serum vitamin D, such as single nucleotide polymor-

phisms (SNPs)with varying results. This presentation will present the data available to date and summarize its strength.

MAM-3,a

Dosimetric and spectroradiometric investigations of glass filtered solar UV. Alfio V Parisi[1], David J Turnbull[1,*] and Michael G Kimlin[2,*]. [1]Centre for Rural and Remote Area Health, Faculty of Sciences, Toowoomba, Qld, Australia, [2]Institute of Health and Biomedical Innovation, Brisbane, Australia.

Exposure to UV radiation is known to be a causative factor in the induction of skin cancers and other sun-related disorders. Past research has shown that UVA (320 to 400 nm) plays a significant role in human skin carcinogenesis. Studies have also shown that UVA plays an important role in skin damage, immune suppression, DNA damage, photoageing and wrinkling. Exposure from filtered solar UV can contribute to the cumulative UV exposure of humans. The spectrum of the filtered UV transmitted through the material is substantially different from that of the unfiltered solar UV spectrum. For glass, this results in a change in the relative ratio of UVA to UVB irradiances and a consequent change in the biologically damaging UV exposures. For these environments where the UVB wavelengths have been removed and the UVA wavelengths are still present, it is necessary to consider the erythemal irradiances due to these UVA wavelengths. The transmission through glass of solar UV on a horizontal plane in the field was investigated for a range of solar zenith angles with a spectroradiometer. The short wavelength cut-off and the irradiances of the erythemal UV were investigated for different glass thicknesses of 2 mm to 6 mm for window glass and for automobile windscreen glass. A newly developed thin film dosimeter for the assessment of UVA exposures that is based on phenothiazine in thin film form and filtered by a thin film of mylar was also employed to measure the solar UVA exposures filtered through these different glasses.

MAM-3,b

UVR-induced picophytoplankton cell death: resolving the vertical extent of UVR-death layer in the ocean. Susana Agusti* and Moira Llabres*. IMEDEA, CSIC -UIB, Esporles, Mallorca, Islas Baleares, Spain.

For photosynthetically active radiation (PAR) scientist have defined the extent of the photic layer as the depth receiving the 1% of the PAR incident on the surface. For phytoplankton and other aquatic organisms there is not consensus as yet about a similar definition for the depth of the UVR-active layer. There are a variety of processes that could be used (e.g. inhibition of photosynthesis, DNA damage, etc) and there is also considerable uncertainty in the accuracy of present indices relating UVR penetration to damage because those indices are based in the sensitivity of organisms other than phytoplankton. Recent results demonstrated that ultraviolet radiation (UVR) could induce significant cell death in natural communities of picophytoplankton, with Prochlorococcus showing the highest sensitivity to UVR, and Synechococcus the lowest. The LD50 values (LD50= doses of UVR needed to decimate to half the picophytoplankton populations) described for picophytoplankton represent daily UVR doses easily attainable in natural conditions. By using measurements of underwater penetration of UVR and the values of picophytoplankton UVR LD50, we calculated the depth of the UVR-active layer of the ocean as that receiving UVR levels sufficient to cause severe cell death to piocophytoplankton. The UV death-active layer varied from 30 m for Synechococcus and eukaryotic picophytoplankton but extended more than 60 m for Prochlorococcus in the oligotrophic subtropical Atlantic Ocean.

MAM-3,c

Genotypic and phenotypic variation in excessive PAR and UV sensitivity in marine phytoplankton. Willem vd Poll * and Anita GJ Buma*. Dept. of Ocean Ecosystems, University of Groningen, Haren, The Netherlands.

Phytoplankton experiences strong and rapid fluctuations in irradiance level and quality. As a consequence, regulatory and acclimatory processes are constantly optimizing light harvesting and photoprotective performance. Furthermore, photoregulation and -acclimation potential is species specific. Near surface irradiance can exceed photosynthetic requirements and cause effects that vary from reduced photosynthetic efficiency (photoinhibition) to viability loss. These effects are enhanced by UV radiation exposure. In a series of experiments we assessed consequences of pre-experimental nutrient limitation and photoacclimation to static and dynamic irradiances for excessive irradiance stress. Excessive irradiance effects (PSII efficiency, xanthophyll cycling, viability loss) were determined during brief exposures to high irradiance that mimicked near surface irradiance. Experiments were performed with diatoms (Chaetoceros brevis, Thalassiosira antarctica, Thalassiosira weissflogii) and the prymnesiophyte Emiliania huxleyi to identify genotypic variation in excessive irradiance sensitivity. Our results show strong modulation of excessive irradiance sensitivity by pre-experimental nutrient-status and photoacclimation for all species. Furthermore, E. huxleyi was more sensitive than the diatoms, which exhibited small differences in sensitivity. A crucial protective role of pigment composition in general and the xanthophyll cycle in particular against excessive PAR and UV induced photoinhibition and viability loss is suggested.

MAM-3,d

Phytoplankton dynamics throughout a yearly cycle in a freshwater lagoon of patagonia: physiological responses to solar radiation. Elena S Barbieri*, Rodrigo J Gončalves *, Virginia E Villafañe* and E. Walter Helbling*. Estación de Fotobiología Playa Unión (EFPU) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Argentina, Rawson, Chubut, Argentina.

A time series study was carried out in the temperate Chiquichano lagoon in Patagonia, Argentina (43° 14S, 65° 18W) from February 2005 to February 2006. The aim of the study was to determine the dynamics of phytoplankton as well as the impact of solar radiation on photosynthetic efficiency

(which was determined using a pulse amplitude modulated Water-PAM fluorometer) and pigments contents. Samples for experimentation and other determinations (e.g., CDOM, nutrients, species composition) were collected every two weeks, the evening before experimentation, pre-filtered to remove zooplankton (<100 um), and kept in dark (18 °C) throughout the night until the following morning. Then, three radiation treatments (duplicates) were implemented: a) PAB (280-700 nm), uncovered quartz tubes; b) PA (320-700 nm), tubes covered with a cut-off filter at 320 nm, and c) P (395-700 nm), tubes covered with cut-off filter at 395 nm. The samples were exposed to solar radiation from 9 AM to 5 PM, inside a water-bath (for temperature control), and every hour photosynthetic parameters were measured; also samples for dark recovery were taken at noon and at the end of the experiment and measured every hour until 8 PM, being the last measurement the following morning. Phytoplankton dynamics and concentration thoroughout the year was strongly controlled by zooplankton (i.e., *Daphnia menucoensis*) grazing so that Chl-a concentration varied from > 800 ug L-1 during periods when its abundance was low, to about 4 ug L-1 when *D. menucoensis* dominated the zooplankton population. There was a significant impact of UVR on photosynthesis throughout the year, but samples collected during fall and winter (i.e., when KdPAR was low) were more sensitive than in other seasons. A significant recovery was observed in darkness for all radiation treatments and samples, but it was not complete even after 20 hours. Chronic damage varied between 2 and 85% of the original yield value, and this variation was a function of the previous exposure to solar radiation.

MAM-3,e

UV effects across multiple trophic levels in freshwater ecosystems: comparative spectroscopy of biological weighting functions. Patrick J Neale[1,*], Robert E Moeller[2,*], Gabriella Grad[3,*], Craig E Williamson[2,*], Wade H Jeffrey[4,*], Robert W Sanders[5,*] and Mark H Olson[6,*]. [1]Smithsonian Env. Res. Ctr, Edgewater, MD, [2]Dept. Zoology, Oxford, OH, [3]Dept. Earth Env. Sci., Bethlehem, PA, [4]Center Env. Diagnostics Bioremediation, Pensacola, FL, [5]Dept. Biology, Philadelphia, PA, [6]Dept. Biology, Lancaster, PA.

Quantitative models of UV response are fundamental to assessing the importance of changes in environmental UV due to ozone depletion and variation in UV absorbing dissolved organic matter (CDOM) in the aquatic environment. Essential features of these models include a description of spectral dependence (biological weighting function, BWF) and time-dependent response as a function of exposure. Environmentally relevant weighting functions are best defined from polychromatic exposures, the results of which can be used to extract the implicit spectral dependence. The technique of using simultaneous incubation of an array of samples to filtered xenon irradiance (the "photoinhibitron") was initially developed to characterize spectral weighting functions of UV inhibition of photosynthesis. As part of a multiple trophic level study of UV effects in freshwater lakes, modifications of this approach are being used to characterize weighting functions for UV effects on other aquatic organisms, including bacterioplankton, protists, zooplankton and fish larvae. A three-year study (2003-6) is being conducted with the biota of Lake Giles, which has relatively low CDOM and high UV transparency. The BWFs for phytoplankton and bacteria indicate a generally high level of sensitivity to UV. There were seasonal (April vs. July) variations in the sensitivity of photosynthesis in 2003-4, UVA sensitivity was lower in July. Compared to microbial rate processes, zooplankton and fish larvae (survivorship) have a similar sensitivity to UVB, but are less sensitive to UVA. Zooplankton typically have an exposure threshold beyond which survivorship rapidly drops. These results enable a direct comparison of UV effectiveness between and within trophic levels, and indicate the potential for indirect effects of UV through differential responses between trophic levels.

MAM-3,f

Sensitivity of the early life stages of macroalgae to ultraviolet readiation. Michael Y Roleda[1], Christian Wiencke[2,*], Dieter Hanelt[3,*] and Kai Bischof[4,*]. [1]Biologische Anstalt Helgoland, Alfred Wegener Institute for Polar and Marine Research, Marine Station, Helgoland, Germany, [2]Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, Bremerhaven, Germany, [3]Biozentrum Klein Flottbek, University of Hamburg, Ohnhorst-str. 18, Hamburg, Germany, [4]Institute for Polar Ecology, University of Kiel, Wischhofstr. 1-3, Kiel, Germany.

The reproductive cells of macroalgae are regarded as the algal life history stages most susceptible to various environmental stresses, including UV radiation (UVR). Furthermore, UVR is proposed to determine of the upper depth distribution limit of macroalgae on the shore. These hypotheses were tested by UV-exposure experiments, using spores and young thalli of the eulittoral Rhodophyceae *Mastocarpus stellatus* and *Chondrus crispus* and various sublittoral Laminariales (Phaeophyceae) with different depth distribution from Helgoland (German Bight) and Spitsbergen (Arctic). Our results clearly support the above hypotheses. UV-B-induced DNA damage in spores of *M. stellatus* and *C. crispus* was higher compared to the macrothalli. Similarly, photosynthesis in spores of *Laminaria digitata* was much stronger photoinhibited after exposure to the same dose of UV-B radiation as in the macrothalli of the same species. The degree of photoinhibition was lower in eulittoral species and higher in sublittoral species. After UV stress, recovery of photosynthetic capacity was also faster in eulittoral species compared to sublittoral species. DNA damage was lowest while repair of DNA damage was highest in eulittoral species compared to sublittoral species. When the negative impact of UVR prevails, spore germination is inhibited. This was observed in deep water Laminariales whereas the same UVR doses do not inhibit germination of shallow water species. A potential acclimation mechanism to increase UV tolerance of brown algal spores is the species-specific ability to increase the content of UV-absorbing phlorotannins in response to UV-exposure. The species-specific susceptibility of the early life stages of macroalgae UVR plays an important role for the determination of zonation patterns and probably also for shaping up community structure.

MAM-4,a

Introduction to the spectroscopy and dynamics of xanthophylls. Harry A Frank*. Department of Chemistry, Storrs, CT, USA.

Xanthophylls are a major class of photosynthetic pigments that play a number of important roles in many different biological systems. This talk will introduce the field and briefly touch on the topic of the adaptation mechanism by which higher plants protect themselves from high-light stress. An ultrafast time-resolved spectroscopic investigation of all the major xanthophyll pigments from spinach: zeaxanthin, lutein, violaxanthin and neoxanthin, has been performed. The experimental data and associated computational analyses reveal the inherent spectral properties and ultrafast dynamics of the allowed and forbidden excited states, the elucidation of which are important for understanding the manner in which the molecules function in nature.

MAM-4,b

On the function of lutein and zeaxanthin in higher plant chloroplast photoprotection. Roberto Bassi[1,2,*], Luca Dall'Osto[1,*], Luca Dall'Osto[1,*], Giulia Bonente[2,*] and Giovanni Giuliano[3,*]. [1]Dipartimento Scientifico e Tecnologico, Università di Verona, Verona, Italy, [2]Laboratoire de Génétique et Biophysique des Plantes, Université Aix-Marseille II, Marseilles, France, [3]Enea, Casaccia Research Center, Rome, Rome, Italy.

The xanthophyll composition of higher plants and green algae is one of their best conserved biochemical characteristics. This suggests that each xanthophyll species has a special function despite the highly similar characteristics of purified xanthophylls. In the dark, or in low light conditions, three xanthophyll species are found, namely lutein, violaxanthin and neoxanthin. Upon exposure to excess light, violaxanthin is transformed into zeaxanthin. In order to elucidate the function of xanthophyll species, we have undertook a systematic genetic analysis of the xanthophyll biosynthetic pathway in order to obtain, per each xanthophyll species, a mutant plant containing: (a) all xanthophylls but one and (b) a single xanthophyll species. These genotypes have been obtained for zeaxanthin and lutein while only partial success was reached for violaxanthin and neoxanthin. The results so far obtained support the view that each carotenoid has specialized function in light harvesting or different aspects of protection from photooxidation. Moreover, it appears that the function of carotenoid in photosynthesis can be understood in the framework of their binding to specific sites of antenna proteins belonging to the Lhc family, besides their intrinsic properties as lipid-free molecules. References Fiore A., Dall' Osto, L., Bassi R. and G. Giuliano (2006) Elucidation of the beta-carotene hydroxylation pathway in Arabidopsis thaliana reveals a fundamental role of epoxixanthophylls in photoprotection. Submitted Dall' Osto,L. Caffarri,S. Bassi, R. (2005) A mechanism of non-photochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26. The Plant Cell. 17(4):1217 Havaux M, Dall' Osto L, Cuine S, Giuliano G, Bassi R. (2004) The effect of zeaxanthin as the only xanthophyll on the structure and function of the photosynthetic apparatus in Arabidopsis thaliana. J. Biol. Chem. 279, 13878

MAM-4,c

Zeaxanthin photophysics in hydrated solvents and human retina. Tomas Polivka*. Institute of Physical Biology, Nove Hrady, Czech Republic.

The carotenoid zeaxanthin in hydrated ethanol forms two types of aggregates: J-zeaxanthin (head-to-tail aggregate, absorption band at 530 nm), and H-zeaxanthin (card-pack aggregate, absorption band at 400 nm). In this contribution we show that by choosing proper values of three parameters: (1) pH; (2) initial concentration of zeaxanthin; and (3) the ratio of ethanol/water; it is possible to control whether J- or H-zeaxanthin is formed. Time-resolved experiments showed that excitation of the 530 nm band of J-zeaxanthin produces a different relaxation pattern than excitation at 485 and 400 nm, showing that the 530 nm band is not a vibrational band of the S_2 state, but a separate excited state formed by J-type aggregation. The S_1 lifetimes of the aggregates yield 20 and 30 ps for H- and J-zeaxanthin, respectively, which are markedly longer than monomeric zeaxanthin (9 ps). In addition, zeaxanthin bound to a xanthophylls-binding protein (XBP) isolated from human retina was also studied. The results from the native XBP and the zeaxanthin-enriched XBP were then compared to those for zeaxanthin in ethanol, hydrated ethanol, and in detergent micelles. Both steady state and transient absorption spectra show that incorporation of xanthophylls into the protein causes a red shift of the spectra. We have observed an influence of the protein environment on the S_1 lifetime of zeaxanthin, which has a longer (12 ps) lifetime in XBP protein than in solution (9 ps). The most pronounced effect was found for vibrational relaxation in the S_1 state, which is significantly slower for xanthophylls in the XBP protein compared to micelles and solution. Comparison of results obtained for XBP enriched by zeaxanthin and its stereisomer meso-zeaxanthin showed that this effect is more pronounced for meso-zeaxanthin, suggesting a specific site for binding this carotenoid to XBP.

MAM-4,d

Xanthophyll accumulation and photoprotection in the human retina. John T Landrum[1,*] and Richard A Bone[2,*]. [1]Department of Chemistry and Biochemistry, Miami, Florida, [2]Department of Physics, Miami, Florida.

Introduction: The leaf xanthophylls, lutein and zeaxanthin, are accumulated at high concentrations in the macula of the human retina and lower levels in the lens. Evidence suggests that these carotenoids function in the retina to reduce the damage produced by incident blue light, lowering the risk for age-related macular degeneration (AMD). Comparison of retinal carotenoids in AMD diagnosed eyes to controls is consistent with the protection hypothesis. Similarly, risk of cataract extraction is lower for individuals consuming high levels of these carotenoids. With the increasing prevalence of age related eye disease considerable attention has been directed toward the question of reducing the risk for these

diseases in the general population. Methods: We have investigated the effects that differing doses of lutein taken by subjects will have on the serum level and macular level in a four month study period. Doses equivalent to 0, 5, 10, and 20 mg/d of free lutein (given as lutein diester) were examined. Macular pigment optical density measurements were evaluated during supplementation and compared to baseline. Results: Serum changes were insignificant for placebo subjects and increased significantly to 170% of baseline for 5 mg/d dose, 335% for a 10 mg/d dose, and 590% for a 20 mg/d dose. The average rate of increase in the MPOD values for each group was insignificantly different from zero except for the 20 mg dose. However, over the study period a clear positive trend was observed between rate MPOD increase with dose. Conclusion: Serum and MPOD increase in response to carotenoid intake for small groups in a dose dependent fashion. Individuals show a large variability in their response to supplemental carotenoid intake.

MAM-4,e

Photoprotective effects of lutein and zeaxanthin in the eye - A review of published information and recent data in monkeys. Wolfgang Schalch[1,*] and Felix M Barker[2,*]. [1]DSM Nutritional Products Ltd., Kaiseraugst, Switzerland, [2]Pennsylvania College of Optometry, Philadelphia, Pennsylvania.

Introduction: The substantial accumulation of lutein (L) and zeaxanthin (Z) in the macula and their ability to quench exited species provides the basis for the hypothesis that these yellow xanthophylls can be photoprotective in the eye and could contribute to risk reduction of age-related macular degeneration (AMD). The lecture reviews relevant data from the literature on the photoprotective abilities of L and Z. Furthermore, it will report results from a recent experiment in monkeys. In this experiment, the effect of supplementation with L and Z on photoprotection against blue light laser irradiation of the retina has been directly investigated. **Methods:** Eight rhesus monkeys, depleted of carotenoids and demonstrating no macular pigment, were subjected to low power 476 nm laser energy delivered either within or outside the foveal area of the retina. This was repeated after the animals had been supplemented with Z (OPTISHARP™) or L for 24 weeks. **Results:** Threshold energy levels for photochemical blue light damage of foveal and parafoveal areas of carotenoid-depleted monkeys were low and similar to parafoveal areas of control animals. Foveal areas of control animals demonstrated significant photo-protection in the form of substantially elevated thresholds. During supplementation of the carotenoid-depleted monkeys, the optical density of macular pigment increased steadily but, after supplementation, had not reached the levels observed in control animals. Consistent with this, photo-damage thresholds did not rise to the level of the control animals. However, significant photo-protection against the blue light hazard was observed by comparison of supra-threshold lesion sizes. **Conclusion:** Supplementation of carotenoid-depleted Rhesus monkeys with Z or L, leads to partial return toward normal foveal optical density and concomitant return of significant photo-protection against blue-light photochemical damage. This complements the reviewed data on the role of L and Z in photoprotection and supports the hypothesis that intake of these xanthophylls can contribute to risk reduction of AMD.

MAM-5,a

Photophysical and photochemical properties of bromine and iodine derivatives of rhodamine-123. Guilherme L Indig, Paulo F Moreira*, Sandra M Silva* and Bindu Abraham*. University of Wisconsin-Milwaukee, Milwaukee, WI, USA.

We have employed a combination of spectroscopic and analytical techniques to explore the photophysical and photochemical properties of five bromine and iodine derivatives of rhodamine-123 (Rh123). Namely, the mono-, di-, tri- and tetrabromo derivatives of the parent dye cation, along with the respective diiodo-Rh-123 analog. These dyes are currently under investigation as potential drug candidates for use in a variety of phototherapeutic applications, including the extracorporeal purging of residual tumor cells from bone marrow grafts used in autologous bone marrow transplantations. Our findings have indicated that the effects of heavy-atom substitution on the photophysical properties of these dyes are primarily in keeping with classical theoretical predictions. That is, the relative magnitudes of S1->T1 intersystem crossing (ISC) efficiency was found to linearly increase upon increasing the heavy-atom perturbation along this dye series. However, the relative magnitudes of reverse (T1->S0) ISC efficiency were found to follow a linear relationship with heavy-atom perturbation only when the bromine derivatives of Rh123 were taken into consideration. The transient decay of the diiodo-Rh123 derivative has shown unexpected kinetics, what suggests the presence of substantial populations of free radical intermediates within the timeframe of our kinetic measurements. The relative values of photobleaching efficiency along the series of halogen-derivatives of Rh123 considered here were found to follow the order: I2>Br4>Br3>Br2>Br1. The mechanism of photochemical decomposition of bromine derivatives of Rh123 appears to be highly conserved along this dye series. A major channel of photodecomposition of these dyes was found to lead to the formation of reaction products expected from the sequential debromination of the respective parent dye cation. On the other hand, a parallel channel of photodecomposition was found to lead to the formation of products that strongly absorb in the 380-400 nm region of the spectrum. These last products are presumably formed through singlet oxygen-mediated reactions.

MAM-5,b

Photodegradation pathways of a thiophene-containing drug substance. Patrick J Jansen*, William K Smith* and Steven W Baertschi*. Eli Lilly and Company, Indainapolia, IN.

Photostability testing of a granule formulation of a thiophene-containing drug using an artificial daylight lamp (D65, Toshiba, Japan) at an intensity of approximately 2000 lux (visible light intensity) revealed that the formulation was sensitive to light. When additional material was irradiated in

a different laboratory using a xenon arc lamp in order to generate larger quantities of the photoproducts for characterization, different photoproducts were observed. The photoproducts from xenon lamp exposure were determined to be the initially formed photoproducts that are converted to the secondary photoproducts over time or when heated. Solution studies designed to probe the mechanism of formation of the initial photoproducts have been conducted and indicate that the thiophene-containing drug is a singlet oxygen sensitizer. The singlet oxygen produced upon irradiation reacts with the thiophene moiety of the drug, presumably via a 4 +2 cycloaddition, to give an unstable endoperoxide intermediate that degrades via various pathways to produce the observed photodegradation products. The structures, mechanistic experiments, and proposed photodegradation pathways will be discussed.

MAM-5,c

A photostability mechanistic case study: Formation of a photo-induced dimer. Michael A Coutant*, Kim McClure*, Jon Bordner*, Andrew Jensen*, Jane Li* and Ivan J Samardjiev*. Pfizer Global Research and Development, Groton, CT.

During the evaluation of CP-944,629 drug substance 10X ICH photostability purposeful degradation sample, a large discrepancy in the mass balance of the sample was observed. Correspondingly, a significant amount of sample remained undissolved in the original preparation. This degradation product exhibited little to no solubility in a number of organic and aqueous dissolving solvents. The isolation, identification and characterization this photodegradation product is discussed. The dimer was determined to be a 4+4 addition of two molecules in a head-to-tail orientation, analogous to the formation of dianthracene. To facilitate the understanding of this reaction, the solid-state photoinduced dimerization of three structurally analogous 1,2,4-triazolo[4,3-a]pyridine compounds (CP-944,629, CP-863,187 and CP-930,139) were investigated. The structure of the dimer for CP-944,629 was determined by mass spectrometry, NMR spectroscopy and single crystal x-ray crystallography. Mass spectral information was used to confirm the formation of photodimer in the two other analogous compounds. The differences in the yield of solid-state photodimerization product were correlated to their crystal packing and small variations in subsistent groups.

MAM-5,d

Photochemistry and photosensitivity of fluoroquinolones. Suppiah Navaratnam[1,2,*], Fernando Lorenzo[1,3,*], Ruth Edge[1,4,*] and Norman Allen[3,*]. [1]Free Radical Resarch Facility, Warrington, UK, [2] Biosciences Research Institute, Salford, UK, [3]Deaprtment of Chemistry, Manchester, UK, [4]School of Physical and Geographical Sciences, Keele, UK.

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 photohapten. We have investigated photophysical properties of three such quinolones and determined the triplet energy levels and singlet oxygen yield for five of them. Steady- state fluorescence measurements were used to determine the pKa* 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 $Br2^{-\cdot}$. Other photophysical parameters such as triplet-triplet absorption spectra, intersystem crossing efficiency, triplet life-time, excited singlet oxygen formation etc. have also been determined. Reaction of the radicals of these drugs, produced by the action of light, with amino acids such as tyrosine and tryptophan as well as the redox potentials of these radicals have been determined. Mechanisms for primary photochemical reactions and the implication in photosensitivity of these compounds will be discussed in detail. Acknowledgements: Experiments were performed at CCLRC Free Radical Research Facility, which is supported by the EC Access to Large Scale facilities.F.L. thanks the BIO-MED network for studentship

MPM-1,a

DNA damage and repair in scleractinian corals: Optimization of DNA extraction. Anastazia T Banaszak. Unidad Academica Puerto Morelos, Puerto Morelos, Quintana Roo, Mexico.

UV-B is known to cause DNA damage, principally by the formation of thymine dimers, but little research has been conducted in coral reef environments where UV doses are high. The majority of tropical reef-dwelling corals form a mutualistic symbiosis with the dinoflagellate *Symbiodinium* yet the limited number of studies on DNA damage and repair in corals have focused on the whole coral rather than on the symbiotic components separately. The aim of this research is to quantify DNA damage and repair in both the coral host and the algal symbiont. The first step in this investigation was to optimize the extraction of DNA from the host, *Porites astreoide*s, as well as the symbiont. The optimization was divided into a series of steps: the preservation of the samples, separation of the coral tissue from the skeleton, separation of the host tissue from the algal cells to prevent contamination, extraction of DNA from both samples and purification of both samples of DNA. The best preservation method was freezing at low temperatures without a preservative such as ethanol. The best method to separate coral tissue from the skeleton without the induction of DNA damage used razor blade scrapes. The coral tissue was successfully divided into host and algal components and the DNA extracted using different protocols including commercially available kits as well as a number of published DNA extraction techniques. All of the kits resulted in clean but very low yields of DNA whereas several modifications of pub-

lished techniques yielded high quantities of clean DNA suitable for the quantification of thymine-dimer formation using antibodies.

MPM-1,b

Increased UV radiation effects on the photosynthetic and photoprotection pigments of the Caribbean shallow-water coral acropora cervicornis. Juan L Torres[1,*], Roy A Armstrong[1,*], Jorge Corredor[1,*] and Fernando Gilbes[2,*]. [1]University of Puerto Rico, Lajas, PR, [2]University of Puerto Rico, Mayaguez, PR.

Clear coral reefs waters are highly transparent to ultraviolet radiation (UVR). The effects of increased UVR on the production of photosynthetic and photoprotective pigments in the Caribbean shallow-water branching coral *Acropora cervicornis* Lamarck were studied during a transplant experiment in La Parguera, Puerto Rico. Colonies of *A. cervicornis* were transplanted from the shelf edge at 20m depth to San Cristobal Reef at 1m depth. On-site colonies normally living at those depths were used as controls. The downwelling irradiance (i.e. underwater downwelling irradiance without normalization by above-water Ed measurements) for the total UVR region (EdUVR) at the shelf edge and at San Cristobal Reef showed an exponential decay similar to that of the downwelling irradiance at the Photosynthetically Active Radiation (EdPAR). Nonetheless, incident EdUVR at San Cristobal Reef was up to six-fold that at the shelf edge. Pigments were quantified through High Performance Liquid Chromatography (HPLC) analysis. A positive correlation was found between photosynthetic pigments concentration and reduced UVR, while the concentration of photoprotective UV-absorbing compounds (mycosporine-like amino acids or MAAs) was negatively correlated with reduced UVR. Severe bleaching was experienced by those colonies of *A. cervicornis* transplanted from deep to shallow areas resulting in significantly decreased photosynthetic pigments concentration compared to controls, although no significant changes were observed in zooxanthellae densities. This suggests that a specific targeted effect of UVR on the photosynthetic capacity of the zooxanthellae caused the bleaching. Bleached colonies survived by significantly increasing the UVR protection with increased MAAs concentrations and a possible relocation of resources. While several physical factors may influence reef corals physiology, the results suggest that shallow-water corals could be significantly affected by increases in UVR resulting from the thinning of the Earth ozone layer.

MPM-1,c

Potential transfer of UVR protection from coral to fish. Frederique LM Kandel[1,2,*], Mindy Mizobe[2,*], Kenia Whitehead[3,*] and Robert A Kinzie[1,2,*]. [1]Department of Zoology UH Manoa, Honolulu, HI, USA, [2]Hawaii Institute of Marine Biology, Kaneohe, HI, USA, [3]The Institute for Systems Biology, Seattle, WA, USA.

To better understand the mechanisms by which coral reef organisms cope with the physical and physiological stresses associated with exposure to ultraviolet radiation (UVR), we have begun to characterize UVR blocking compounds in coral reef fish. Previous work has shown that the mucus covering the body of reef fish absorbs in a wavelength range characteristic of the class of UV absorbing compounds known as mycosporine-like amino acids (MAAs). Fishes are not thought to be capable of synthesizing these compounds de novo and are believed to obtain them from their diet. In order to explore the potential sources and transfer of these protective compounds between organisms that inhabit coral reef ecosystems, we have exploited the strong site attachment and restricted diet of the reef dwelling coralivorous butterflyfish *Chaetodon multicinctus*. The epidermal mucus of fish, and the corals that constituted the bulk of their diets, were sampled at three depths exhibiting measured differences in UVR exposure. The MAAs in these samples were analyzed and identified using LC-MS/MS and the profiles obtained for the fish mucus and corals compared. Our data reveal that *Chaetodon multicinctus* epidermal mucus contains a complement of MAAs found in the corals they feed upon, and resolves trends in the relative abundance of individual MAAs that reflects species level and depth differences in the corals sampled.

MPM-1,d

Characteristics of the UVB-induced biosynthesis of phenolic compounds in Glycine max (L). Richard H Grant*, Wilfred Vermerris*, Javier Campos*, Cheryl Bawhey*, Kent Apostol* and Thomas Housley*. Dept. of Agronomy, Purdue University, W. Lafayette, IN, USA.

Single leafets of Williams 82 ad Essex cultivars of soybean were exposed to a combined UVB-BE irradiance of 1.2 J m^{-2} $h(^{-1}$. Leaflets were excised, freeze dried and analyzed for changes in the expression of genes involved in the production of phenolic compounds after 1/2 h, 2 h, 4 h, and 6 h of exposure. In addition, after three days of 6h exposure each, leaflets were excised, freeze dried and assayed spectrophotometrically for UVB-absorbing compounds. Results show that the UVB exposure resulted in the production of detectable levels of UVB-absorbing compounds (after 18h) in Essex, whereas Williams 82 showed only a slight increase in UVB absorbing compounds. Reverse transcriptase PCR (RT-PCR) showed that the expression of the genes encoding phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (CA4H), and 4-coumarate-CoA ligase (4CL-1, -2 and -3) did not vary in response to the UVB exposure, but the expression of one of the eight chalcone synthase (CHS) genes showed greater up-regulation after 4 hours of exposure in Essex than Williams 82. We conclude that the UVB-enhanced production of UVB-absorbing compounds is at least partly controlled by at least one CHS gene in Essex. The lack of up-regulation of CHS in Williams 82, a cultivar that is less sensitive to UVB exposure than Essex, indicates that the cultivar must use means other than the production of UVB-absorbing compounds to mitigate the impacts of UVB exposure. These findings offer opportunities to further investigate the plant response to UVB exposure at the molecular level.

MPM-1,e

Do ambient levels of both UV-A and UV-B radiation affect leaf litter chemistry and decomposition, and performance of soil fauna? Titta Kotilainen[1,*], Jari Haimi[1,*], Riitta Tegelberg[2,*], Riitta Julkunen-Tiitto[2,*], Elina Vapaavuori[3,*] and Pedro J Aphalo[1,*]. [1]Dep. Biological and Environmental Science, Jyväskylä, Finland, [2]Natural Product Research Laboratory, Joensuu, Finland, [3]Suonenjoki Research Station, Suonenjoki, Finland.

Our experimental setup was based on the use of plastic films strongly attenuating different parts of the UV spectrum. The experimental setup consisted in three treatments: 1) UV-B+, UV-A+ (near ambient control, AB), 2) UV-B−, UV-A+ (Ab) and 3) UV-B−, UV-A&Minus; (ab). The experiment was done on eight grey alder (*Alnus incana*) and twelve white birch (*Betula pubescens*) trees growing in the field. All the treatments were on different branches of each tree. Senescent leaf samples collected in the fall were used in decomposition experiments. Laboratory experiments were conducted to study the effects of the leaf litter from different UV treatments on soil organisms and on the decomposition rate. In an experiment with woodlice, jars with alder litter had more woodlice feaces and litter remaining at the end of the experiment in Ab treatment than in AB control and the most in ab treatment. In another decomposition experiment with soil and litter the cumulative CO_2 production was lowest in ab treatment, in both tree species. In the same experiment, litter decomposition rate was lowest in AB control compared to Ab, in both species. Chemical analyses showed that there were more extractable tannins in birch and more residual tannins in alder, but no effect of UV. The C/N ratio analysed from the litter and from the woodlice feaces was higher in birch. Total lignin concentration was lowest in ab treatment compared to AB control, with intermediate values in Ab treatment, in both species. Both UV-A and UV-B radiation affect chemical properties of the litter and changes in litter properties cause some downstream effects on litter decomposition. The fact that UV-A radiation is effective is relevant to assessing the applicability of action spectra, indicating that spectra with no action in the UV-A band are not applicable to some of these responses.−

MPM-1,f

Coupling short-term variation in UV-B levels with induction of UV-screening compounds. Joe H Sullivan[1,*], Chenping Xu[1,*], James R Slusser[2,*] and Wei Gao[2,*]. [1]Department of Plant Science and Landscape Architecture, College Park, MD, USA, [2]USDA UV-B Monitoring and Research Program, Fort Collins, CO.

A substantial number of studies have been conducted over the last several decades in order to assess the potential impacts of long-term increases in UV-B radiation (UV-B between 280-320 nm) that might result from continued depletion of stratospheric ozone. However, in addition to stratospheric ozone levels and seasonal changes, tropospheric conditions such as cloudiness exert a much larger influence on short-term fluctuations in levels of ambient UV-B received a given location. The effects of short-term changes in UV-B radiation on plant growth, phytochemistry and physiological processes have received relatively little attention in the literature. The USDA UV-B Monitoring and Research Program provides an excellent opportunity to monitor long-term changes in solar UV-B radiation and the responses of plants to short-term variation in UV-B levels on a near real time basis. In these studies Barley (*Hordeum vulgare*) and soybean (*Glycine max*) were used as model systems. Emerging seedlings of these species were grown under either near ambient levels of UV-B or under reduced levels (ca 90% reduction) in the field. Periodic measurements on leaf phytochemistry, photosynthetic integrity (Fv/Fm) and levels of DNA damage were made following contrasting periods of ambient levels of UV-B in order to test whether development and subsequent sensitivity to UV-B radiation was altered by short-term variation in UV-B. Ambient levels of UV-B modulated the levels of flavonoids and other putative UV-screening compounds in both species. These in turn exerted some influence on photoprotection as assessed by reductions in Fv/Fm ratios and accumulation of DNA dimmers in Barley and to a lesser extent in soybean. These results suggest that short-term fluctuations in solar UV-B levels may modulate screening compound levels and subsequent protection from UV-B damage at higher UV-B levels. Species-specific differences may be related to the kinetics of the induction of the phenylpropanoid pathway.

MPM-2,a

Structural distortions in psoralen cross-linked DNA seen at high-resolution. Pui S Ho[1,*], Franklin A Hays[1,*], Yonggang He[2,*], Brandt Eichman[3,*], Andrea Vaiana[4,*], John Hearst[5,*] and Wei Kang[2,*]. [1]Department of Biochemistry & Biophysics, Corvallis, OR, [2]Department of Chemistry, Corvallis, OR, [3]Department of Biological Sciences, Nashville, TN, [4]Institute de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France, [5]Department of Chemistry, Berkeley, CA.

Psoralen is a tricyclic photochemotheraputic drug that acts by covalently cross-linking thymine bases across the two-strands of DNA double-helices. Our earlier 2.2 Å resolution crystal structures of deoxyoligonucleotides showed that psoralen diadducts induce formation of four-stranded Holliday junctions, a structural intermediate that is involved in mechanisms of genetic recombination and in repair of DNA adducts. We have now determined a higher-resolution (1.5 Å) single-crystal structure of the drug-induced junction in the sequence d(CCGCTAGCGG), which reveals more precisely the structure of the drug, the linkage between the drug and DNA bases, and the distortions in both that are associated specifically for the psoralen diadduct with DNA. *Ab initio* calculations on the drug and thymine-adducts from this crystal structure reveal that the greatest distortions relative to idealized structures are associated with the thymine cross-linked to the six-membered pyrone ring of the drug, resulting in significant destabilization of the cyclobutyl bonds and the stacking between the thymine base and the psoralen rings. The asymmetric distortions and energies between the pyrone and furan-sides of the drug-DNA complex can account for the difference in the photodissociation of psoralen diadducts

to double-stranded DNA as compared to those to free thymine bases.

MPM-2,b

A photochemically-inactivated Epstein-Barr virus vaccine. Cara Orr[1,*], M. Victor Lemas[1,*], Dexue Fu[1,*], Jeff Bitzan[1,*], Linda Post[1,*], Janice Davis[1,*], John Hearst[2,*], Richard Ambinder[1,*] and Weson Hsieh[1,*]. [1]Johns Hopkins School of Medicine, Baltimore, MD, United States, [2]University of California, Berkeley, Berkeley, CA.

Epstein-Barr virus (EBV) associated lymphoma is an important complication of a variety of immunodeficiency conditions. Evidence suggests that immune response to EBV antigens may protect against the development of these malignancies. In an effort to try to prevent the development of these malignancies we have developed individualized EBV-lymphoblastoid cell line vaccines that express the immunodominant latency antigens commonly expressed in post-transplant lymphoma and administered these to patients awaiting organ transplantation before immunosuppression. Patient B cells are transformed with GMP-produced laboratory strain B95.8 virus, expanded, inactivated with UV-S59 and then administered to patients as a subcutaneous vaccine. In preclinical studies, S59 (a psoralen cross-linker), inactivates EBV biologically (preventing lymphocyte transformation) while preserving immunogenicity as demonstrated by interferon-gamma secretion assays and FACS analysis. In clinical studies we have administered vaccine to 13 patients including 4 seronegative patients. There have been no vaccine related toxicities. In order to detect transmission of laboratory strain virus we have developed primers that selectively amplify wild type virus, lab strain virus or both. In mouthwash and peripheral blood of patients who were EBV seropositive prior to vaccination, we have been able to detect wild type virus but not laboratory strain virus. In seronegative patients who were vaccinated, no virus of any sort has been detected. Analysis of cellular immune responses to EBV antigens in 5 patients who were seropositive prior to vaccination demonstrate a negligible impact on T cell responses, whereas in a seronegative patient (only one analyzed to date) there was a dramatic impact on CD4(+) and CD8(+) responses. These results suggest that S59-UV cross-linking is effective at blocking transmission of laboratory strain virus from the vaccine and that the vaccine itself is effective at boosting EBV-specific T cell responses.

MPM-2,c

Vaccination with killed but metabolically active Listeria monocytogenes. John D Roback[1,*], Levan J Lezhava[1,*], William Luckett[2,*], Thomas W Dubensky[2,*], Christopher D Hillyer[1,*] and Martin Giedlin[2,*]. [1]Emory University School of Medicine, Atlanta, GA, USA, [2]Cerus Corporation, Inc., Concord, CA, USA.

Background: Vaccination following bone marrow transplantation (BMT) is challenging since vaccines must be highly safe and also effective at stimulating an immune response. We recently described a novel vaccine technology in which the bacterium *Listeria monocytogenes* (*Lm*), rendered incapable of nucleotide excision repair through site-directed mutagenesis (ΔactA/ΔuvrAB), is treated with the proprietary psoralen amotosalen and UVA light. The resulting killed but metabolically active (KBMA) *Lm* vaccine, which is non-replicating but viable, can safely deliver antigens for immunization. We tested the safety and efficacy of this vaccine, engineered to express an immunogenic protein from murine cytomegalovirus (KBMA-*Lm*-MCMV), in a mouse BMT model. **Methods:** C57BL/6 BMT recipients were transplanted with 5×10^6 BM cells, and a subset also received donor lymphocytes ($0\text{-}30 \times 10^6$) from mice that were either naive or immunized with 10^7 cfu *Lm*-MCMV on day −7. Selected BMT mice were vaccinated with 10^7 cfu KBMA *Lm*-MCMV on days 1-3 and 21-23. **Results:** KBMA-*Lm*-MCMV vaccination was very safe, with no lethality seen, and was also highly effective. 10-15% of CD8+ T-cells were MCMV-specific in mice that received immune donor lymphocytes and vaccination by 8 days after BMT. BMT recipients that did not receive vaccination did not show significant levels of antiviral T-cells. When lymphocyte doses between $0.5-30 \times 10^6$ were combined with KBMA vaccination, similar levels (up to 15%) of antiviral T-cells were identified at day 28. *In vivo* CTL assays were performed to quantify long-term MCMV-specific cytolytic activity. In the absence of vaccination, specific lytic activity was $< 10\%$. In contrast, BMT mice that received donor lymphocytes and vaccination displayed 100% specific lysis of antigen-labeled targets 200 days after treatment. **Conclusions:** Immunization using the safe KBMA *Lm*-MCMV vaccine elicited rapid (within 8 days) and extensive expansion of MCMV antigen-specific CD8+ T-cells in BMT recipients. This approach is effective using as few as 0.5×10^6 splenocytes, and leads to long-term (>200 days) persistence of virus-specific lytic activity *in vivo*. Given the resulting high levels of durable antigen-specific lytic activity, this may represent a broadly applicable approach to prevent viral disease after transplantation.

MPM-3,a

Effect of lipophilic/hydrophilic character on the mitochondrial localization of ester derivatives of rhonamine-123. Inessa Belostotsky*, Maria G Paez*, Sandra M Silva* and Guilherme L Indig. University of Wisconsin, Milwaukee, WI, USA.

The observation that enhanced mitochondrial transmembrane potential is a prevalent tumor cell phenotype has provided the conceptual basis for the development of mitochondrial targeting as a novel therapeutic strategy for (photo)chemotherapy of neoplastic diseases. Because the plasma transmembrane potential is negative on the inner side of the cell, and the mitochondrion transmembrane potential is negative on the inner (matrix) side of this organelle, extensively conjugated cationic molecules (dyes) displaying appropriate structural features are electrophoretically driven through these membranes and tend to accumulate inside energized mitochondria. As a result of the higher mitochondrial transmembrane potential typical of tumor cells, a number of cationic dyes preferentially accrue and are retained for longer periods in the mitochondria of these cells as compared to

normal cells, and this differential in both drug loading and retention brings about the opportunity of attacking and destroy tumor cells with a high degree of selectivity. It is reasonable to infer that, for desirable degrees of tumor cell selectivity to be achieved the mechanisms of cellular uptake and intracellular distribution of any putative mitochondrial drug candidate must be primarily controlled by transmembrane potentials, with just minor contributions arising from lipophilic partitioning or any other competitive phenomena. Here we describe how the lipophilic/hydrophilic character of five model mitochondrial drug candidates (i.e. the methyl, n-propyl, i-propyl, n-pentyl, and n-octyl ester derivatives of rhodamine-110) affect the degree of specificity with which these dyes localize in energized mitochondria, and the extent to which transmembrane potentials effect their mitochondrial localization and subcellular distribution. Our results have indicated that the range of lipophilic/hydrophilic character associated with a high degree of potential-driven mitochondrial specificity tends to decrease to negligible levels when the lipophilic character of the dye approaches values of octan-1-ol/water partition coefficient close to two orders of magnitude above that of the methyl ester derivative.

MPM-3,b

Sanguinarine inhibits ultraviolet radiation-mediated activation of mitogen activated protein kinase- and nuclear factor kappa B- pathways in SKH-1 hairless mouse skin. Haseeb Ahsan*, Shannon R Reagan-Shaw*, David Eggert*, Farrukh Afaq*, Hasan Mukhtar* and Nihal Ahmad*. University of Wisconsin, Madison, WI.

Studies from our laboratory have demonstrated that sanguinarine (13-methyl benzodioxolo[5,6-c]-1,3-dioxolo[4,5-i]phenanthridinium), a benzophenanthridine alkaloid derived from the root of Sanguinaria *canadensis* and other poppyfumaria species inhibits ultraviolet (UV) B-exposure-mediated damages in HaCaT human keratinocytes and in the skin of SKH1 hairless mouse. Mechanism of the photoprotective effects of sanguinarine is largely unexplored. In this study, employing SKH-1 hairless mouse model, we tested the hypothesis that the photoprotective effects of sanguinarine are mediated via modulations in mitogen activated protein kinase (MAPK)- and nuclear factor kappa B (NF-κB)- pathways. The SKH1 mice were subjected to a single exposure of UVB radiation (180 mJ/cm^2) and received either a pretreatment (30 min prior to UVB) or post-treatment (5 min after UVB) of sanguinarine (5 μmol/0.2 ml ethanol per mouse). The mice were euthanized 24 h following UVB exposure for further studies. Our data demonstrated that sanguinarine resulted in inhibition of UVB exposure-mediated (i) phosphorylation of extracellular-signal-regulated kinases (Erk1/2), (ii) c-Jun N-terminal kinases (JNK), and (iii) p38. Further, as evident from immunohistochemical- and immunoblot- analyses, we found that sanguinarine inhibited UVB exposure-mediated (i) activation of NF-κB (ii) phosphorylation and degradation of IκBα, and (iii) increase in inducible nitric oxide synthase and Cyclooxygenase-2. Our data we suggest that that sanguinarine protects against the adverse effects of UV radiation via modulations in MAPK- and NF-κB- pathways and provides molecular basis for the photoprotective effects of sanguinarine in an in vivo animal model system that possesses relevance to human situations. Because sanguinarine is used in toothpastes and mouth rinses as an anti-inflammatory agent, based on our work, it is conceivable to design sanguinarine containing emollient or patch as well as sunscreen and skin-care products for prevention of skin cancer and other conditions which are believed to be caused by UV radiation. More detailed studies should be conducted to assess the effectiveness of sanguinarine in prevention of UV exposure-mediated skin tumorigenesis.

MPM-3,c

Investigation of antitumor efficacy using chlorin e6-polyvinylpyrrolidone in multiple cancer models. William WL Chin[1,*], Paul WS Heng[2,*], Ramaswamy Bhuvaneswari[1,*], Othmar Dill[1,*] and Malini Olivo[1,*]. [1]Division of Medical Sciences, National Cancer Centre Singapore, 11 Hospital Drive, Singapore, Singapore, [2]Department of Pharmacy, National University of Singapore; No. 18 Science Drive 4, Block S4, Singapore, Singapore.

During the past decades much research has been focused on developing effective drug delivery systems for the preparation of chlorins as potential photosensitizers for PDT. This report describes the evaluation of a new water-soluble formulation of chlorin e6 (Ce6) consisting of a complex of trisodium salt Ce6 and polyvinylpyrrolidone (PVP) for the application of photodynamic therapy (PDT). We have investigated and compared the pharmacokinetics and photodynamic activity of Ce6 formulated in different molecular weights of PVP in several mouse xenograft models. In vivo and in vitro studies were conducted to determine production of singlet oxygen, cell phototoxicity, tumor selectivity and extent of distribution and clearance in the various organs and also antitumor effects of the various formulations. All in vitro PDT procedures resulted in cell phototoxicity in all the formulations tested. The effects of various drug and light doses of Ce6-PVP mediated photodynamic therapy (PDT) on tumor growth of MGH human bladder carcinoma xenografts were elucidated. Significant tumor necrosis was observed at 48 hr post PDT when irradiated at 1 hr drug-light interval (DLI) compared to 3 and 6 hr DLI in the MGH bladder model. Ce6-PVP has lesser toxicity to mice compared to its parent compound Ce6. This observation was attributed to the rapid systemic elimination of Ce6-PVP. Antitumor efficacy was further evaluated in mice bearing human small-cell lung cancer (SCLC and human non-small-cell lung cancer (NSCLC). Difference in sensitivity of the antitumor effect of Ce6-PVP was observed between these lung xenografts model. Significant antitumor effects was observed in the NSCLC and SCLC model at the appropriate drug concentrations. Taken together, these results indicate that Ce6-PVP has good tolerability in mice and is able to induce direct photodamage against a wide range of tumors.

MPM-3,d

Oral feeding of anthocyanidin- and hydrolyzable tannin-rich pomegranate fruit extract modulates early biomarkers of photocarcinogenesis in SKH-1 hairless mouse epidermis. Farrukh Afaq*, Bilal B Hafeez*, Deeba N Syed*, Mee-Hyang Kweon* and Hasan Mukhtar*. Department of Dermatology, Madison, WI, USA.

Exposure to solar ultraviolet (UV) radiation, particularly its UVB (280-320 nm) component, is the primary cause of skin cancers and other cutaneous pathologies in human population, more so in Caucasian individuals. There has been considerable interest in the identification of natural agents capable of affording protection to skin from the adverse effects of solar UVB radiation. Pomegranate from the plant *Punica granatum* possesses strong antioxidant, anti-inflammatory and anti-proliferative properties. Recently, we have demonstrated that treatment of normal human epidermal keratinocytes with pomegranate fruit extract (PFE) inhibits UVB-mediated activation of NF-κB and MAPK pathways (Afaq *et al.* Photochem. Photobiol. 81:38-45, 2005). Here, we evaluated the effect of PFE on early biomarkers of photocarcinogenesis employing SKH-1 hairless mice. PFE was provided in drinking water (0.2%, wt/vol) to SKH-1 hairless mice for 14 days before a single UVB (180 mJ/cm^2) irradiation. At 1, 8 and 24 hrs following UVB exposure, animals were sacrificed and skin biopsies were collected for biomarker assessment. We found that oral feeding of PFE inhibited UVB-induced: (i) skin edema, (ii) hyperplasia, (iii) infiltration of leukocytes, (iv) lipid peroxidation, and (v) hydrogen peroxide generation. We next assessed the effect of PFE on markers of UV-induced DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) and 8-hydroxy-2'-deoxyguanosine (8-OHdG). Oral feeding of PFE significantly inhibited UVB-induced formation of CPDs and 8-OHdG. Employing western blot and/or immunohistochemical analyses, we found that oral feeding of PFE inhibited UVB-induced: (i) PCNA, (ii) ODC, and (iii) COX-2. Importantly, PFE treatment further enhanced UVB-mediated increase in tumor suppressor p53 and cyclin kinase inhibitor p21. These data show that oral feeding of PFE to mice affords substantial protection from the adverse effects of UVB radiation via modulation in early biomarkers of photocarcinogenesis. PFE could be a useful photochemopreventive agent.

MPM-3,e

(-)-Epigallocatechin-3-gallate from green tea prevents photocarcinogenesis in mice through interleukin-12-dependent DNA repair mechanism. Santosh K Katiyar[1,2,*], Syed M Meeran[1,*] and Sudheer K Mantena[1,*]. [1]University of Alabama at Birminghm, Birmingham, AL, USA, [2]Birmingham VA Medical Center, Birmingham, AL, USA.

We have previously shown that topical application of (-)-epigallocatechin-3-gallate (EGCG) prevents photocarcinogenesis in mice. EGCG also prevents ultraviolet (UV) radiation-induced immunosuppression through the induction of interleukin (IL)-12. As IL-12 possesses anti-tumor activity and DNA repair ability, we determined whether prevention of photocarcinogenesis by EGCG is mediated through EGCG-induced IL-12-dependent DNA repair. For this purpose we used genetically modified mouse model [IL-12 knockout (KO) mice on C3H/HeN background] and DNA repair-deficient cells from patients suffering from xeroderma pigmentosum complementation group A (XPA). The effect of EGCG was determined on photocarcinogenesis and UVB-induced DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) in IL-12 KO mice and compared with their wild-types (WT), and in XPA-deficient and proficient cells using immunohistochemistry, immunocytostaining and dot-blot analysis. In photocarcinogenesis protocol, mice were exposed to UVB (180 mJ/cm2) with or without the pretreatment of EGCG (1 mg/cm2 skin) three times a week for 30 weeks. We found that treatment of EGCG prevented photocarcinogenesis in WT mice in terms of tumor incidence and tumor multiplicity but did not prevent it in IL-12 KO mice, suggesting the role of EGCG-induced IL-12 in prevention of photocarcinogenesis. EGCG reduced UVB-induced DNA damage in the form of CPDs and sunburn cells in the skin of WT mice more rapidly compared to non-EGCG treated mice. In contrast this effect of EGCG was not observed in IL-12 KO mice. Further, EGCG was able to repair UVB-induced CPDs in in vitro XPA-proficient cells obtained from healthy person but did not repair in XPA-deficient cells indicating that nucleotide excision repair mechanism is involved in DNA repair. These results identify a new mechanism of anti-photocarcinogenic effect of EGCG which indicates that prevention of photocarcinogenesis by EGCG is mediated through EGCG-induced IL-12-dependent DNA repair.

MPM-3,f

Phototoxicity of DIMET-PC on tetrahymena *thermophila* and human skin basal cell carcinoma. Emmanuel Ojadi* and Marlene DeAbreu*. University of Massachusetts Dartmouth, North Dartmouth, MA, USA.

DIMET-PC, an *amphiphilic* (soluble in both water and oil systems) phthalocyaninium ion was synthesized and tested as a photosensitizer for use in the photodynamic therapy of skin carcinomas. Hydrophility was achieved by quarternizing two of the aza N-sites which caused the compound to also retain its lipophilicity. The compound was tested and showed positive phototoxicity on the protozoan Tetrahymena *thermophila* cultures. Further test on human skin cancer cells also found that DIMET-PC is phototoxic to human skin basal cell carcinoma cell cultures.

MPM-4,a

Photocalorimetry: A tool for photostability testing. Meena Dhuna[1,*], Simon Gaisford[1,*], Michael A O'Neill[1,*], Anthony E Beezer[1,*], Joseph A Connor[2,*] and David Clapham[3,*]. [1]School of Pharmacy, University of London, London, UK, [2]Natural Resources Institute, Medway Sciences, University of Greenwich, Kent, UK, [3]GlaxoSmithKline, New Frontiers Science Park, Essex.

Recent work[1] has resulted in the development of a novel photocalorimeter, one which offers the potential to perform

photostability testing of pharmaceuticals. The instrument is designed to allow the direct study of photosensitive materials in liquid, solid or semi-solid states and, in combination with appropriate data analysis methodologies, the derivation of thermo-kinetic information to monitor photodegradative processes. The photocalorimeter uses an optical beam splitter and liquid-light guides to direct light from a 300 W Xe arc lamp into sample and reference ampoules (20 mL) of a Thermal Activity Monitor (TAM, Thermometric Ltd). The light entering both ampoules is adjustable using a number of focusing and shuttering assemblies; the amount of light entering the sample side is set to a predefined level while, the reference side is adjusted until a zero baseline signal is achieved. Thus, the TAM itself acts as a null-adjust balance to ensure equivalence between the two sides. It is possible to quantify the amount of light entering the sample side using an actinometer. The photodegradation of 2-nitrobenzaldehyde (2-NB)as a chemical actinometer was investigated. The reaction follows zero order kinetics[2]; thus a constant deflection from zero in the photocalorimeter should be observed. In order to relate the reaction to a calorimetric output, the rate constant (k) or the enthalpy (ΔH) is required. Since neither parameter is well established in literature an auxiliary method was required to determine a value for k. The reaction was followed by monitoring the changes in pH as the photodegradation product, 2-nitrosobenzoic acid, concentration increased during irradiation. The data showed that an additional oxidation process accompanied the photodegradation. Addition of EDTA resulted in zero-order kinetics. An oxidation event would clearly impact on the measured response in the photocalorimeter, which settled to give a zero order signal after approximately 5 hours of light exposure. This means that while the photodegradation of 2-NB offers potential as a chemical actinometer, careful consideration must be taken during its preparation and use. These topics will be discussed in this presentation. [1]Dhuna et al (2005) British Pharmaceutical Conference, MICC, UK. [2]Allen JM et al (2000) J Pharm Biomed Anal 24:167-178.

MPM-4,b

Wavelength-dependent photoisomerization of montelukast sodium. Lee J Klein*, Li Li*, Allen Templeton*, Andreas Abend*, Brian Hill*, Nathan Pixley*, William Farina* and Bob Reed*. Merck & Co., Inc., West Point, PA, USA.

Montelukast sodium, a leukotriene inhibitor indicated for allergic rhinitis and asthma, undergoes reversible photoisomerization about a central stilbene-like double bond. To determine the effect of wavelength on the position of this isomerization, a systematic study was carried out with the aid of an optical bench apparatus consisting of a 1000 Watt Xe(Hg) lamp, thermostatted IR filter, monochromator, and spectroradiometer (along with associated power supplies, accessories, and optics). Methanolic solutions of pure montelukast sodium as well as the pure isomeric product were circulated through quartz flow cells positioned in monochromatic beams of known intensity and were assayed with time via online UV-Vis absorbance as well as a standard validated HPLC method. Results indicate that the quantum yields for the cis-trans and trans-cis photoconversions are not equal, and hence the equilibrium position of this photoisomerization is wavelength-dependent. Data acquired with the optical bench apparatus correlate well with results obtained for solutions of montelukast exposed to controlled doses of ambient fluorescent light and the ICH visible photostress. This experimental approach, when expanded to multiple wavelengths, can be used to predict the formation and distribution of known photodegradates given spectroradiometric data for any light source. Other potential applications include the rapid identification of the minimum light protection measures necessary for drug substances or drug products.

MPM-4,c

Photostability testing and photostabilisation of transdermal delivery systems. Heiko Spilgies* and Dirk Schenk*. Novosis AG, Miesbach, Germany.

Transdermal Delivery Systems (TDS) are typically stored in light-proof pouches. Nevertheless, regulatory authorities often request information on photostability tests according to ICH guideline Q1B. A case study will be presented how a photostability test was set up. Information can be used to estimate the in-use photostability. A photosensitive drug combination was stabilised by using an overtape containing a photoabsorber in the adhesive matrix. The photoprotective effect could be optimised by chosing an absorber with an absorption spectrum matching that of the drug(s) to be stabilised.

MPM-4,d

Photoantimicrobial chemotherapy (PACT). Mark Wainwright*. School of Pharmacy & Chemistry, Liverpool, UK.

It is well established that light interacts with unsaturated molecules, causing electronic promotion and potentiating chemical reaction via electron transfer. With conventional drugs, this interaction with light can lead to phototoxicity as a side effect in the patient, via the production of reactive oxygen species (ROS), as seen with fluoroquinolones, sulfonamides, phenothiazines etc. Photoantimicrobial chemotherapy (PACT) utilises the energy provided by light to increase the activity of drug molecules specifically designed to absorb light and to produce therapeutically useful yields of ROS. The production of singlet oxygen by photoantimicrobials circumvents typical microbial defences, and explains the susceptibility of conventional drug-susceptible and drug-resistant strains of bacteria to this approach, e.g. in methicillin- and vancomycin-resistant Gram-positive pathogens. The reactivity of singlet oxygen allows photoantimicrobial activity across bacteria (Gram +/-), viruses, yeasts/moulds and protozoa. The limiting factor of light delivery means that the approach is, at present, suitable only for topical/local application, although this is not limited to skin infection: for example, PACT is currently in use in pulmonary tuberculosis. PACT thus offers effective local therapy of the diseased or colonised state and may be used both in the treatment of resistance and in the conservation of those systemic antibiotics which are still effective.

MPM-4,e

An examination of photostability testing as a function of clinical development phase. Allen C Templeton*. Merck Research Laboratories, West Point, PA.

The extent of photostability testing needed to guide product development activities such as formulation design, packaging systems development, and manufacturing process scaleup vary significantly as a function of clinical development phase. The present work attempts to develop a roadmap for photostability testing decisions as a function of clinical phase in view of the ICH guidance. The types of studies presented are divided up between regulatory filing requirements and fundamental stressing studies to guide decision-making. The studies discussed will encompass risk management principles in terms of providing the most appropriate data (while not stretching to overkill) for preformulation, Phase I, and Phase IIb-III development phases. Several miniature case studies will be presented to develop the concepts further and illustrate the principles.

MPM-5,a

Surface interaction of quantum dots with some aliphatic and aromatic amines. Raquel E. Galian and J. C. Scaiano. University of Ottawa; Faculty of Sciences; Deparment of Chemistry, Ottawa, ON, CANADA.

Quantum dots (q-dots) have been receiving much attention in bioimaging due to their unique properties, broad absorption spectra, narrow emission fluorescence and high intensity response. Nevertheless, studies of their photophysical and photochemical properties in the interaction with other compounds are not totally developed. An enhancement, and sometime a decrease, in the luminescence of q-dots with some specific amines have been reported. In this work, the effect of some aliphatic amines (triethylamine), cyclic amine (1,4-diazabicyclo[2.2.2]octane, DABCO) and aromatic amines (2-aminoantracene and 2-aminopyrene) on the luminescence of q-dots have been investigated. We have used home made CdSe q-dots with triocthylphosphine (TOPO) as a ligand and commercial capped CdSe(ZnS) q-dots of different size. The steady state and time resolved fluorescence techniques have been used to examine these interactions. The fluorescence of CdSe q-dots (μM) is quenched by the presence of triethylamine taking an hour to equilibrate the intensity at each addition of the quencher with a small blue-shift in the maximum of emission. The capped q-dots fluorescence showed less efficiency in the quenching process, and the intensity after one hour is partially restored, suggesting a possible reversible exchange of the amine and TOPO ligand. Similar behaviour has been observed with DABCO, but the maximum of emission at the first addition is red-shifted and when the fluorescence is re-stored the maximum return to the initial value (small shift are observed but the behaviour is reproducible). The fluorescence lifetime is also reduced with increasing concentration of the quencher due to dynamic quenching that could operate in combination with other specific interactions on the surface of q-dots. The quenching dependence with the nanoparticles size and the mechanism involved in the process will be discussed.

MPM-5,b

New fluorescent methods for studying peptide-membrane interactions. Matthew J Tucker*, Jia Tang* and Feng Gai*. Department of Chemistry, Philadelphia, PA.

Unnatural amino acids with novel spectroscopic properties can be used in a variety of applications in biochemistry and biophysics. Here, we show that p-cyano-phenylalanine (Phe_{CN}) offers rather interesting fluorescent and infrared properties which may be used in peptide and protein conformational studies. In particular, we demonstrate that Phe_{CN} can be used as a fluorescence resonance energy transfer (FRET) donor to tryptophan (Trp); and this amino-acid FRET pair can be applied to study in detail the mechanism of peptide-membrane interactions. In addition, we show that Phe_{CN} can be used alone as a sensitive fluorescent probe to investigate the kinetics of peptide dimerization in a membrane environment.

MPM-5,c

Computer aided design of small peptides for serpin depolymerization. Pramit K Chowdhury*, Wei Wang*, Michelle R Bunagan*, Jason W Klemke*, Jia Tang*, Jeffrey W Saven*, Barry S Cooperman* and Feng Gai*. University of Pennsylvania, Philadelphia, PA.

Polymerization of serpin protease inhibitors (serpins) is involved in a variety of diseases that include liver cirrhosis, dementia and emphysema. Several blocking peptides, mostly homologous to the reactive center loop (RCL) of the serpins have been used to inhibit the polymer formation process. Here we have used a computationally assisted design strategy, to facilitate the search of more efficient peptide sequences, in conjugation with fluorescence correlation spectroscopy (FCS), to study the mechanism of depolymerization. Our results show that one of the designed sequences is more efficient than the others in breaking up the polymers. In addition FCS, having single molecule sensitivity allows us to have a peek into the heterogeneity associated with the process. The distribution of diffusion times obtained by fitting the autocorrelation decays reveals the presence of a lag phase in depolymerization.

MPM-5,d

Investigation of photophysical and photochemical properties of 1-indoly-2-arylethenes. Anil K Singh* and Abera Asefa*. Department of Chemistry, Indian Institute of Technology, Bombay, Powai, Mumbai - 400 076, India, Mumbai, MH, India.

Electronically excited state properties of donor-acceptor diphenylpolyenes have been extensively been examined and reviewed. This is partly because these compounds serve as models for the photobiologically significant linear polyenes like retinoids and carotenoids. In this context, we have synthesized several 1-indolyl-2-arylethenes, and examined their absorption, fluorescence emission and photoismerization properties in organic solvents and micelles. The results indicate involvement of dipolar species in the excited state

dynamics of some of the compounds. The electronic effect of substituents and the solvent polarity on fluorescence and photoisomerization behaviors of these ethenes is described in detail. Role of charge transfer excited states in the photoprocesses of ethenes and linear polyenes in general is discussed.

MPM-6,a

UV-induced CD4+DX5+ are CD1d-restricted and regulate tumor immunosurveillance by suppressing CTL activity. Noor M Khaskhely*, Yasuko Miyahara*, Nasser Kazimi*, Scott Byrne* and Stephen E Ullrich*. Department of Immunology, University of Texas, MD Anderson Cancer Center, Houston, TX, YSA.

The ultraviolet radiation present in sunlight plays a critical role in the initiation and promotion of non-melanoma skin. Previously we showed that Natural Killer T (NKT) cells mediate UV-induced suppression of tumor immunity, in that we could transfer suppression to non-irradiated mice by transferring as little as 2 million NKT cells. We hypothesized that suppression was due to the ability of CD4+, DX5+ NKT cells to block the induction of cytotoxic T (CTL) cells. To confirm this, suppressor NKT cells were generated in C3H/HeN mice by chronic UV-irradiation. Splenic NKT cells (CD3+, CD4+, DX5+, CD44+, CD62L+, Vb8.1/8.2+) were purified from the spleens of the irradiated mice by magnetic bead separation. 2 x 106 cells were injected into normal non-irradiated mice, and these mice were then immunized with UV-2240 tumor cells. When spleen cells from these mice were isolated 14 days after immunization, their ability to generate a CTL reaction was significantly suppressed. Injecting CD4+, DX5- cells isolated from UV-irradiated mice did not block the induction of CTL activity. To find the signals needed to activate NKT cells in vitro, the NKT cells were first cultured for 3 days in 96 well plates with plate bound anti-CD3. The cells were then washed and added to a second culture containing immunized spleen cells and tumor cells. Activation with anti-CD3 was not sufficient to induce immune suppressive activity. However, when the cells were first activated with anti-CD3 and then incubated with ?-irradiated UV-2240 tumor cells, they were able to suppress the generation of anti-tumor CTLs. Anti-CD1 monoclonal antibody blocked the ability of the tumor cells to activate the NKT cells to suppress CTL function. FACS indicated that tumor cells express CD1 on their cell surface. Although analysis of the culture supernatants by ELISA demonstrated the activated NKT cells secreted IL-13, anti-IL-13 did not totally block the induction of immune suppression. These data indicate that UV-induced NKT cells suppress CTL generation both in vitro and in vivo. Moreover, they indicate that the activation of the suppressor NKT cells is CD1 restricted, and that the tumor cells that activate the NKT cells express CD1 on their cell surface. Although the cells secrete IL-13, it does not appear to be involved in the suppression of CTL generation in vitro.

MPM-6,b

***CIS*-urocanic acid initiates gene transcription in primary human keratinocytes.** Kazuyo Kaneko[1], Ulli Smetana-Just[1,*], Mary Matsui[2], Antony R Young[1], Mary Norval[3] and Susan L Walker[1,*]. [1]St John's Institute of Dermatology, London, UK, [2]Clinique Laboratories, Melville, NY, US, [3]Medical Microbiology, Edinburgh, UK.

Trans-urocanic acid (*trans*-UCA) is a major chromophore for UVR in the epidermis and isomerisation to *cis*-UCA takes place upon absorption of UVR. Many *in vivo* studies suggest that *cis*-UCA plays a role in UV-induced immunosuppression; however, its mechanism remains unclear. In this study, changes in the gene expression and protein synthesis of cytokines and prostaglandin E_2 (PGE_2) were assessed following UCA treatment of primary human keratinocytes. Firstly, gene arrays were used to compare the transcriptional profiles of keratinocytes treated with *trans*- or *cis*-UCA (10μg/ml) or solar simulated UVR (12J/cm^2 ~ 2-3 minimal erythema doses for fair skin) or untreated at 24hr. About 700 genes were regulated by UVR based on at least a 2-fold change, and 20 of these genes were also up-regulated by *cis*-UCA. *Trans*-UCA had no effect on gene expression. To confirm the array results, the expression of the major genes induced by *cis*-UCA and/or UVR was quantified independently, using RT-PCR. The mRNAs of cyclooxgenase-2 (COX-2), TNF-α, IL-6, IL-8, IL-18, thioredoxin reductase-1 and haemoxygenase-1 were up-regulated by both *cis*-UCA and UVR. Secondly, secretion of cytokines and PGE_2 into the culture supernatant was measured using ELISA 24hr after treatment with PBS, *trans*-UCA (100μg/ml), *cis*-UCA (10, 50 and 100μg/ml) or UVR (12J/cm^2). *Cis*-UCA increased the secretion of PGE_2, TNF-α, IL-1, IL-6, IL-8 and IL-18 significantly in a dose-dependent manner compared to PBS. UVR had the same effect as *cis*-UCA, but *trans*-UCA did not alter protein secretion. The results suggest that the induction of immunosuppression by *cis*-UCA may involve the initiation of gene transcription in primary human keratinocytes.

MPM-6,c

Natural UV filters and anti-oxidants in skin protection. Fritz Boehm[1,*], Ruth Edge[2,*], Ernesto Fernandez[3,*] and T. George Truscott[2,*]. [1]Photobiology Lab, Charite Hospital, Humboldt University, Berlin, Germany, [2]Keele University, School of Chemistry and Physics, Keele/Staff., UK, [3]University of Valparaiso, Chemistry and Pharmacy School, Valparaiso, Chile.

None of the current sunscreens are perfect for man 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 dietary 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, b-car-

otene, 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. $NO_2^{\cdot}$ and singlet oxygen). Protection factors varied from 5−20 with the most efficient being based tomato lycopene. Results on the lichen compounds, e.g. usnic acid and calycine 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. Reactions with vitamin C were more efficient for usnic acid than calycine with corresponding rate constants of $3x10^7$ $M^{-1}s^{-1}$ for calycine and $3.5x10^8$ $M^{-1}s^{-1}$ for usnic acid. Vitamin E quenches the radical cations with rate constants of 2.2 x 10^7 $M^{-1}s^{-1}$ and 6.8 x 10^7 $M^{-1}s^{-1}$ for calycine and usnic acid respectively. For Trolox the corresponding values are 2.0 x 10^8 $M^{-1}s^{-1}$ and 1.9 x 10^8 $M^{-1}s^{-1}$ suggesting there could be benefits in using vitamins E and C with these potential sunscreens since this would remove any radical cations produced. Interestingly, the values for Trolox (in the aqueous phase) are more efficient than for vitamin E (in the lipid phase). Future studies will show if lichen extracts are efficient singlet oxygen quenchers. I thank Drs S. Navaratnam, Wanda Quilhot and F. Rancan and Professor Kirsten Boehm for collaboration. Pulse radiolysis 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).

MPM-6,d

Contradictions in the safe sun message for vitamin D production and erythemic risk. Robert M Sayre[1,2,*] and John C Dowdy[2,*]. [1]Division of Dermatology, Memphis, TN, USA, [2]Rapid Precision Testing Laboratory, Cordova, TN, USA.

A primary benefit of sunlight exposure is the production of vitamin D and the primary acute risk of sunlight exposure is sunburn. Recent studies report that the safe sun public health messages are compatible with producing vitamin D by sunlight exposure. Several reports suggest that one is able to make sufficient vitamin D through casual non-midday exposure even while wearing a sunscreen. This is based on assuming a one to one relationship between vitamin D benefit and erythemic sunburn risk. This is untrue because the UVB vitamin D response spectrum and erythemal action spectrum, which extends through the UVA, are different and the UVB to UVA ratio of sunlight constantly changes with solar elevation throughout the day. We collected a series of solar spectra throughout one day and divided the data set into a series of 1 standard erythemal dose (SED) intervals. Vitamin D effective dose was then calculated for each SED increment. The results showed the solar effectiveness for vitamin D production varied with the changing solar elevation. When the sun was at or near its zenith, the vitamin D effectiveness per SED reached a maximum for the day. With decreasing solar elevations, vitamin D effectiveness declined more rapidly than erythemic risk. For lower solar elevations vitamin D effectiveness approaches erythemic risk such that erythema predominates over vitamin D production. We also examined effects of wearing a sunscreen on solar potential to produce vitamin D. Contrary to published reports we find that sunscreens are more effective in attenuating UV effectiveness for vitamin D production than in protecting against erythema. We demonstrate calculation of vitamin D blocking factors over two fold greater than corresponding sun protection factors. Our analyses demonstrate that prescribed sun safe behaviors can not lead to optimal UV exposure for vitamin D with minimal risk.

MPM-6,e

Sunscreens and UVA protection - Comparison of established and emerging methods for assessment of UVA protection. Uli Osterwalder* and Bernd Herzog*. Ciba Specialty Chemicals Inc., Basel.

The need for UVB and UVA protection during outdoor activities is generally recognized. Over the last 5 years new UVA and broad-spectrum UV filters such as Bisoctrizole (MBBT, Tinosorb® M) or Bemotrizinol (BEMT, Tinosorb® S) became available. This allows the formulators to create sunscreens with far superior broad-spectrum protection than ever. The open question in most countries still is how to communicate the degree of UVA protection to the consumer. Besides established UVA methods and standards such as Persistent Pigment Darkening (Japan), Australian UVA standard, UVA/UVB ratio (United Kingdom) and UVA balance (Germany), new methods are emerging, e.g. an improved UVA balance that takes into account photostability. The methods were rated by their ability to discriminate high and low UVA protection. The following ranking was found for the in vitro methods: UVA Balance > UVA/UVB ratio > Australian UVA standard (1996) > critical wavelength. The improved UVA balance is the only in vitro method that allows the necessary differentiation between high and low protection in the UVA range over the full period of time a sunscreen is expected to be effective. Long-lasting UVA protection can be achieved with the new UV filters, but also with the help of photo-stabilizing the classic UVA Filter Avobenzone.

MPM-6,f

Comparable photoreactivity of hair melanosomes, soluble eumelanin and phaeomelanin at low concentration: Low melanin a risk factor for UVA photo-damage via superoxide? Rachel Haywood* and Martin Lee*. RAFT Institute of Plastic Surgery, Middlesex, United Kingdom.

Data is lacking of the effects of UVA irradiation on human melanosomes. We have shown previously that melanosomes isolated from human oriental and black cat hair are photoreactive, producing superoxide at low concentrations when exposed to UVA irradiation comparable to UK levels of sunlight (irradiance 1.3 mW/cm2). We have now investigated the UVA-irradiation of human hair melanosomes isolated from different coloured hair samples using electron spin resonance spectroscopy and spin trapping. In addition, the comparable irradiation of synthetic phaeomelanins synthesised from L-dopa, L-cysteine and tyrosinase was studied. Oriental hair melanosomes isolated by three different meth-

ods and UVA-irradiated with 5,5-dimethyl-1-pyrolline-N-oxide (DMPO) were photoreactive at low concentrations, with an initial increase in superoxide radical-adduct production with melanin concentration comparable to that observed in soluble synthetic eumelanin. Data was obtained using 5 min NaOH (90oC) treatment which showed a peak ($\approx$ 0.3 mg/ml melanin) and then decline in superoxide radical-adduct formation with increasing melanin concentration; however, this method appeared unsuitable for similar studies of auburn and blonde hair. Two enzyme methods were also investigated: a method based on proteinase K and dithiothreitol and the second on papain and dithiothreitol. The latter resulted in good melanosome yields, separation from residual keratin with no visible melanosome disruption and was suitable for melanosome isolation from brown, blonde and auburn hair. Melanosomes isolated from a range of different coloured hair samples by this method, and synthetic phaeomelanins, showed similar photoreactivity at low concentration (maximal $\approx$ 0.3 mg/ml melanin concentration) and increasing photoprotection $> \approx$ 0.3 mg/ml melanin, which was independent of hair colour and broadly comparable to soluble synthetic eumelanin. These preliminary results suggest that all soluble melanins and melanosomes may be comparable in properties at constant fluence, and that melanosome concentration could be more significant with respect to UVA-photodamage via superoxide than pigment type.

MPM-6,g

Molecular responses of normal human caucasian melanocytes in culture exposed to simulated solar UV: Could melanin and its precursors behave as endogeneous photosensitizers ? Laurent Marrot*, Jean-Philippe Belaidi*, Christophe Jones*, Philippe Perez* and Jean Roch Meunier*. L'OREAL Phototoxicity 1 av E. Schueller, Aulnay sous Bois, France.

Melanocytes have a central role in the response of skin to sunlight exposure. They are directly involved in UV-induced pigmentation as a defence mechanism. However, their alteration can lead to melanoma, a process where the role of sun overexposure is highly probable. The aim of this work was to analyze the behaviour of melanocytes from fair skin under irradiation mimicking environmental sunlight in terms of spectral power distribution. To do this, normal human Caucasian melanocytes in culture were exposed to UV radiation from a solar simulator (SSUV: 300-400 nm). Even at relatively high doses (12 kJ/m2 UVB and 110 kJ/m2 UVA), cell death was limited. Moreover, p53 accumulation was three times lower in melanocytes than in unpigmented cells such as fibroblasts after SSUV exposure. However, an important fraction of melanocyte population was arrested in G2/M phase, and this correlated well with a high induction level of the gene GADD45, 4 hours post-exposure. Among the genes involved in DNA repair, gene XPC was the most inducible since its expression increased more than two fold 15 hours, whereas expression of P48, also involved in the nucleotide excision repair pathway, was only slightly increased. In addition, an early induction of Heme Oxygenase 1 (HO1) gene, a typical response to oxidative stress, was observed for the first time in melanocytes. Interestingly, this induction remained significant when melanocytes were exposed to UVA radiation only (320-400 nm) and stimulation of melanogenesis prior to irradiation further increased HO1 induction. Such a behavior confirmed previous results showing that photoinduced DNA breakage by UVA (detected using the comet assay) was also enhanced when melanogenesis was triggered. This work provides new data about the stress reponse of human melanocytes exposed to UV and underlines the probable involvement of sunlight in melanoma initiation.

TUE-A

A discussion of bio and bio-inspired constructs that would provide global scale sustainable energy for human use. Thomas Moore*. Center for the Study of Early Events in Photosynthesis, Tempe, AZ.

Faced with the reality of anthropogenic climate change - a problem facing humanity that is no less significant than war, famine, disease, overpopulation, the plight of refugees and the guarantee of human rights across the lands - humans must find sustainable ways to power their societies. Solar energy input to the biosphere is about 1024 joules/year, making human needs of even a projected 1021 joules/year a deceptively achievable goal. One key to global-scale use of solar energy is the sustainable synthesis of energy-rich fuel materials such as hydrogen and reduced carbon compounds. The latter have the almost inestimable advantage that the energy infrastructure for distribution and use is in place and they offer energy densities comparable to that of fossil fuels. To close the carbon/combustion loop, energy-rich reduced carbon compounds must be synthesized from CO2 and the electrons for doing this reduction must come from the oxidation of water. This process could be carried out sustainably by photosynthetic microorganisms and by human engineered hybrid systems using solar energy and natures catalysts to template electroreductive synthesis starting from CO2. Photosynthetic, biosynthetic and respiratory enzymes provide paradigms for catalysis of these energy converting processes. These catalysts function at room temperature, at near thermodynamic efficiency, and without the use of precious metals (opper, manganese, iron and nickel are typically used at their active sites). They have evolved reaction mechanisms that overcome key limitations of current human-engineered catalysts, e.g., carbon-carbon bond cleavage is facile and the O2/H2O half reaction operates with essentially no overpotential. In this school, I will present some of the research which is necessary to underpin progress and hope to initiate discussion and analysis of the challenges presented by the need to provide sustainable energy for human use.

TAM-1,a

What makes DNA so photo(un)stable? Bern Kohler*. Department of Chemistry, Columbus, OH.

DNA photodamage by UV light proceeds from excited electronic states associated with the nucleobases. Despite decades of study, there is poor understanding of how the initial excited states give rise to the various photolesions. Dramatic

progress has been made in recent years at directly observing the dynamics of these states using ultrafast laser spectroscopy. A remarkable feature of monomeric bases are the very high rates of nonradiative decay seen in the condensed phase, which result in fluorescence lifetimes of just hundreds of femtoseconds. It has been argued that these short lifetimes imbue the bases with a high degree of photostability, which protects the critical information encoded in the genome. Recently, however, we have shown that high yields of long-lived excited states are observed when oligonucleotides are excited by femtosecond UV laser pulses. These states have dark character, meaning they do not decay radiatively to a significant degree. They also depend strongly on sequence, consistent with nearest-neighbor interactions arising from vertical base stacking. The existence of these states shows that excitations in these complex, multichromophoric polymers are not predictable from the photophysical properties of their building blocks alone. It also raises questions about what interactions, if any, are ultimately responsible for the photostability of the double helix. In this presentation, the dynamics of how nucleic acids 'process' excess electronic energy will be reviewed with examples drawn from experiments on DNA model systems ranging from single bases to double-stranded oligonucleotides.

TAM-1,b

Cooperative effects in UV photon absorption by model DNA helices. Dimitra Markovitsi*, Delphine Onidas*, Francis Talbot*, Sylvie Marguet*, Thomas Gustavsson* and Elodie Lazzarotto*. Laboratoire Francis Perrin CEA-CNRS URA 2453, Gif-sur-Yvette, France.

The distribution of DNA lesions induced by UV radiation depends on the sequence around the hotspots suggesting cooperativity between bases. We have shown that such cooperativity may intervene at the very first step of a cascade of events and related to the electronic states reached directly by photon absorption at 267 nm (excited states delocalized over several bases and auto-ionizing states). Delocalization of excited states has been evidenced by probing intrinsic fluorescence of poly(dA).poly(dT) induced by femtosecond pulses (Markovitsi et al. J. Am. Chem. Soc., 2005, 127, 17130). Emission was probed by fluorescence upconversion and time-correlated single photon counting, over a large time domain (from 100 fs to 100 ns). The time-resolved fluorescence decays and fluorescence anisotropy decays were discussed in relation with the steady-state absorption and fluorescence spectra in the frame of exciton theory. We studied ionization of model helices induced by nanosecond pulses using time-resolved absorption spectroscopy (Marguet, S. et al. J. Phys. Chem. B in press). The variation of the hydrated electron concentration with the absorbed laser intensity has shown that one photon ionization takes place for $(dAdT)_{10}(dAdT)_{10}$, $(dA)_{20}(dT)_{20}$ and $(dA)_{20}$ but not for $(dT)_{20}$. The spectra of all adenine containing oligomers at the microsecond time-scale correspond to the adenine deprotonated radical formed in concentrations comparable to that of the hydrated electron. The quantum yield for one photon ionization of the oligomers (ca. 10^{-3}) is higher by at least one order of magnitude than that of dAMP showing clearly that organization of the bases in single and double helices leads to an important lowering of the ionization potential.

TAM-1,c

DNA photonics-probing light-Induced dynamics in DNA on the femtosecond time scale. Torsten Fiebig* and Eugene F Merkert*. Chemistry Center, Chestnut Hill.

The first generation of experiments on photoinduced electron transfer (ET) in DNA has spawned a basic mechanistic picture from which simple kinetic models were derived. In these models ET through the base stack has been reduced to a static donor-bridge-acceptor problem. Recent experimental and theoretical results have demonstrated that structural dynamics are critical for a comprehensive mechanistic understanding of the ET process. While the initial controversies regarding the long-range conductivity properties and wire-type behavior of DNA have been settled, a new field, DNA Photonics, has emerged around the photophysics of nucleic acids. The contributions that can be expected from future studies in DNA Photonics will likely answer the question whether and to what extend DNA can be used as a functional building block in molecular nanoscale devices. They will also be focused on the complex interactions between structural and electronic properties of DNA which are profound for biomedical applications such as DNA-targeted drug design. In this paper we report about our recent experimental efforts which are part of the second generation of studies to expand the new and highly exciting field of DNA Photonics. Experimental data from several different classes of functionalized DNA systems will be presented to illuminate the relationship between structural dynamics and charge injection/migration using state-of-the art femtosecond broadband spectroscopy. Our results present strong evidence for the involvement of hydrogen bond dynamics which must be considered as a specific mode of solvation dynamics inside the DNA helix. Finally, we emphasize the importance of the initial electronic excitation. Thus, ultrafast electronic energy migration, dissipation and (de)localization must be included into the theoretical description of light-induced dynamics in DNA.

TAM-1,d

Excited state dynamics of DNA constituents : The role of the solvent. Thomas Gustavsson[1,*], Roberto Improta[2,3,*], Nilmoni Sarkar[4,*], Elodie Lazzarotto [1,*] and Dimitra Markovitsi[1,*]. [1]Laboratoire Francis Perrin, Gif-sur-Yvette, France, [2]Dipartimento di Chimica, Universita Federico II, Napoli, Italy, [3]Bioimmagini /CNR, Napoli, Italy, [4]Department of Chemistry, Kharagpur, WB, India.

Since a few years, there is a renewed interest in characterizing the electronically excited states of DNA and its constituents. Various ultrafast spectroscopic techniques have been applied with success to nucleobases (adenine, thymine, cytosine, guanine) and the corresponding nucleosides and nucleotides, showing that the excited states decay on a subpicosecond time scale. The mechanism responsible for the ultrafast non-radiative deactivation (internal conversion) is

in general not well known, even if several theoretical studies point towards the existence of near barrier-less reaction paths (not necessarily the same for the different bases), implying important ring deformation, leading from the excited state through a conical intersection to the ground state. The prevailing picture is that the ultrafast decays observed for the various bases are due to purely intramolecular mechanisms, little or not affected by the solvent. However, gas phase studies have shown that much longer lived states, having nanosecond lifetimes, exist in vacuum, implying that the environment may play an important role. Up to this date though, nearly all ultrafast studies have been performed in aqueous solution while only a few investigations in other solvents have been reported. One may say that the role of the solvent in the excited state deactivation of nucleobases is neither clearly identified nor well understood. In order to gain additional information on this matter we have chosen to investigate several uracils (including uracil and thymine) in various non-aqueous solvents, such as acetonitrile and linear alcohols, and compare the findings with our recent results obtained in aqueous solution. By combining time-resolved fluorescence spectroscopy and TD-DFT calculations on the excited state we show that the relative ordering of the first excited $\pi\pi^*$ and $n\pi^*$ states depend strongly on the solvent. For example, contrary to the situation in aqueous solution, in acetonitrile these states become near-degenerate, which opens up an additional decay channel for the optically bright $\pi\pi^*$ state. This is in accordance with the observations of much faster fluorescence decays in acetonitrile than in other solvents.

TAM-2,a

Mode of action of extracorporeal photopheresis. T Schwarz*. Department of Dermatology, Kiel, Germany.

The basis of extracorporeal photopheresis (ECP) is the reinfusion of leukocytes, which have been exposed to 8-methoxypsoralen and UVA light. It has been initially developed for the treatment of CTCL. Despite its clinical use for many years its mode of action still remains elusive. ECP induces apoptosis of nearly all leukocytes. It was suggested that the infusion of these cells causes a kind of immune response against the malignant cells, a phenomenon called transimmunization. ECP, however, is also beneficial for the treatment of autoimmune diseases, solid organ transplant rejection and GvHD, diseases which usually require an immunosuppressive approach. There is unanimous agreement that ECP is not associated with any severe side effects, quite unusual if this therapy causes immunosuppression. Since UV radiation of the skin exhibits the capacity to induce tolerance via induction of regulatory T cells, we studied whether ECP might induce a similar state of tolerance following extracorporeal treatment of leukocytes. For this purpose we utilized a murine model of contact hypersensitivity (CHS). Splenocytes and lymph node cells of mice, which were sensitized with DNFB, were exposed to ECP in vitro. These cells were injected intravenously into naive mice, which were subsequently sensitized with DNFB. Animals, which had received ECP-treated cells, were significantly suppressed in their CHS response. Induction of suppression was lost when lymph node cells were depleted of CD11c+ cells before ECP treatment. Suppression was cell-mediated and antigen specific. Transfer of tolerance was lost when cells were depleted of CD4+ or CD25+ subpopulations, indicating that experimental ECP induces regulatory cells, possibly of the CD4/CD25 lineage. Together, these data suggest that infusion of experimental ECP induced apoptotic cells produces highly active antigen specific regulatory cells. Further studies are underway to better understand the nature of these regulatory cells and their mechanism of induction.

TAM-2,b

Photopheresis for the treatment of cutaneous T cell lymphoma. John A Zic*. Vanderbilt University Division of Dermatology, Nashville, TN, USA.

Photopheresis (ECP) is an immunomodulating procedure available for the treatment of cutaneous T cell lymphoma (CTCL) since 1987. A concentrated white blood cell (WBC) sample spiked with 8-methoxypsoralen (methoxsalen) is exposed to a UVA light source then all blood components are returned to the patient. Treatment of mycosis fungoides and Sezary syndrome, the most common variants of CTCL, with ECP has been reported in over 400 patients though no randomized clinical trials have been reported. The combined overall response rate for all stages of CTCL is 55.7% (244/438) with 17.6% (77/438) achieving a complete response. Efficacy in treating certain clinical stages (IB, IIA, III, IVA) and skin stages (T2, T4) of MF and Sezary syndrome is favorable though prospective controlled trials comparing ECP to other standard therapies are needed. The use of ECP to treat early stage patients remains controversial. A retrospective study of 50 patients with CTCL treated at our institution included a univariate and multivariate analysis of 49 clinical and laboratory variables to assess predictors of response and survival. Key variables associated with response to ECP ($p<0.05$): higher percent increase in leukopheresis bag WBC, lymphocytes, and monocytes at 6 to 10 months of ECP and decreased lactate dehydrogenase levels at 6 to 10 months of ECP. Key variables associated with improved survival ($p<0.05$): baseline eosinophil count $<$ 300#/cmm; higher percent increase in leukopheresis bag monocytes at 6 to 10 months of ECP; and baseline CD8 cell count $>$160#/cmm. Efforts to establish the effectiveness of combining ECP with other newer immunoadjuvant therapies and modifications of the procedure to enhance immunomodulation are exciting prospects for patients with CTCL.

TAM-2,c

Photopheresis in early stage mycosis fungoides. Elma D Baron[1,2,*]. [1]University Hospitals of Cleveland/Case Western Reserve Univ, Cleveland, OH, USA, [2]Louis Stokes Veterans Affairs Medical Center, Cleveland, OH, USA.

Background: Extracorporeal photopheresis (ECP) has been used for nearly 20 years for the treatment of cutaneous T-cell lymphoma (CTCL). A substantial body of literature reports that this form of photoimmunotherapy improves or stabilizes the course of disease in a subset of patients across all stages of disease.However, current clinical approach usu-

ally reserves ECP for patients who do not respond to other treatments or for patients with late-stage disease or Sezary syndrome (SS). Methods: A comprehensive Pubmed/Medline literature search was performed to identify studies reporting the use and efficacy of ECP in early stage CTCL. Information regarding prognostic factors and survival of early stage patients treated with ECP was also obtained and summarized. Results: The current literature contains reports of 119 early stage patients treated with ECP or ECP plus adjuvant therapy from 1987- 2006 in 17 different studies. Response rates of treatment for this patient population with ECP and ECP plus adjuvant therapy varied from 50%-100%. Conclusions: Given the very low side effect profile of ECP compared with other therapies and its demonstrated efficacy,this treatment modality appears to be beneficial even for patients with earlier stages of CTCL. Randomized prospective studies are needed to establish the role of ECP in this disease subset.

TAM-2,d

Extracorporeal photoimmunetherapy in immune mediated disease. Robert Knobler*. Department of Dermatology, Vienna, Vienna, Austria.

After receiving FDA approval for the use of Extracorporeal Photoimmunetherapy (ECP, photopheresis) in the palliative treatment of refractory cutaneous T-cell lymphoma(CTCL) its possible use in other indications where T-cells may play an important role has been extensively investigated. At present ECP is being used, with varying degrees of success in the treatment of selected autoimmune diseases including systemic sclerosis, pemphigus vulgaris, refractory systemic lupus erythematosus, refractory atopic dermatitis and inflamatory bowel disease. In addition ECP has increasingly been shown to be effective in supporting control of solid organ transplant rejection(Heart, Lung and Kidney). Multiple recent studies have documented that ECP may be of critical importance in the supportive treatment of both acute and chronic Graft versus Host Disease (GvHD)after allogeneic bone-marrow transplantation as well as in its prevention. FDA controlled multi-center prospective randomized studies in the various indication suggested should help document and better establish the value of ECP not only in the field of dermatology but other medical specialties as well. Research into and better understanding of the true mechanisms of action involved in ECP may help optimize its future use and exact indications.

TAM-3,a

Light dosimetry for photodynamic therapy: Introduction and clinical implementation. Jarod C Finlay[1,*], Timothy C Zhu[1,*], Andreea Dimofte[1,*], Xiaodong Zhou[1,*], Jun Li[1,*], Joseph S Friedberg[2,*], Douglas M Fraker[2,*] and Stephen M Hahn[1,*]. [1]Department of Radiation Oncology, Philadelphia, PA, [2]Department of Surgery, Philadelphia, PA.

The accurate measurement of light delivery in photodynamic therapy (PDT) is a complex problem involving the choice of light delivery and measurement techniques, tissue optical properties, and sensitizer localization. We summarize the basic concepts behind light delivery and measurement, and present the results of a series of studies on the factors influencing the measurement of light in PDT. Differences between isotropic and nonisotropic measurement devices can cause a difference in measured dose of up to 70% between systems. Changes in refractive index of tissue can cause similar differences in the dose measured by isotropic detectors, depending on their design. Both of these effects can be predicted on a theoretical basis. We will present the results of our clinical implementation of various dosimetry systems in clinical trials of PDT for the prostate and intraperitoneal and pleural cancers. Strategies for quantifying and accounting for the effects of inter- and intrapatient variations in tissue optical properties and their implications for PDT dosimetry have also been investigated. We have developed methods for characterizing the variations in absorption and scattering properties over the entire volume of the human prostate, and have demonstrated the ability of optical diffusion theory to predict the delivered light dose to tissue using this information. We will discuss the implications of these methods for clinical dosimetry and treatment planning.

TAM-3,b

PDT dosimetry - From pre-clinical models to clinical practice. Dominic J Robinson*. Center for Optical Diagnostics and Therapy, Rotterdam, The Netherlands.

The therapeutic effect following PDT depends on a combination of parameters that include drug dose, drug-light interval, and light fluence (rate). These parameters are in general investigated in pre-clinical models and some stage I/II clinical trials optimizing PDT. Since early in the 1980's and in parallel with the development of PDT, investigators have carried out research in the field of in-vivo light dosimetry where in-situ light measurement methods were developed. It was shown that the actual fluence rate in tissue does not only depend on the amount of light delivered, but also on the optical properties and geometry of the tissue, leading to a build up in fluence rate, especially in hollow organs. Traditionally, the approach to light delivery in PDT is to adjust the output power of the source to a value equal to the intended fluence rate multiplied by the surface area to be treated. This ignores not only the build up of fluence rate, but also the inter-patient variation in build up factor. Only a small number of clinical studies have taken this effect into account. This has lead in some cases to treatment failure and adverse side effects following PDT. More recently it has been recognised that state of the art light dosimetry alone is not sufficient for standardising the dose delivered during therapy. Various pre-clinical techniques have been developed that report the deposition of singlet oxygen and/or the vascular response during PDT. These methods are now being applied during PDT in the clinic. We present an overview of how these data can be incorporated into treatment protocols for clinical PDT and how dosimetric information will be acted upon in the heterogeneous clinical environment.

TAM-3,c

Light dosimetry for photodynamic therapy of Barrett's esophagus. Linda R Jones[1,*], Norris W Preyer[1,*], Herbert C Wolfsen[2,*] and Michael B Wallace[2,*]. [1]Department of Physics, Charleston, South Carolina, US, [2]Department of Gastroenterology and Hepatology, Jacksonville, Florida.

Introduction: Photodynamic therapy with porfimer sodium using 630nm red light is the only approved method for ablation of Barrett's esophagus in the setting of high grade dysplasia. However, the ideal dosimetry of photodynamic therapy has not been determined. The current methods of light and pharmacologic dosing may result in either excessive tissue damage, resulting in stricture formation, or inadequate tissue damage, resulting in persistent sub-squamous dysplasia. The objective of this study was to develop quantitative methods for optimization of light dosimetry and to compare our findings with published clinical results. Methods: A multilayer optical model of the esophagus was constructed using the optical properties obtained from ex vivo, snap frozen, pig esophagus. Pig esophagus was separated into mucosal and muscle layers by blunt dissection. Diffuse reflectance and transmittance was measured with a Perkin Elmer Lamda 45 UV/Vis spectrophotometer with an internal integrating sphere attachment. Absorption and scattering coefficients were derived with the inverse adding doubling method. A three-dimensional cylindrical Monte Carlo program that is capable of modeling heterogeneous layers was used to determine necrosis depth for various light doses and porfimer sodium content. The simulation retains and follows the photons scattered into the lumen. Results: The absorption coefficients for mucosa and muscle layers were found to be 0.068 and 0.109 mm-1 respectively. The scattering coefficients for mucosa and muscle layers were 1.27 and 0.722 mm-1, respectively. Predicted necrosis depth ranged from 0.2 mm for a light dose of 85 J/cm to 2.3 mm for a light dose of 300 J/cm. Conclusions: Predicted necrosis depth correlated well with the thickness of Barrett's dysplasia based on previous studies. Similar methods may be applied to the study of other components of the photodynamic reaction process (tissue photosensitizer concentrations and oxygen levels).

TAM-3,d

Metallic nanoparticles as molecular-specific contrast agents for in-vivo optical imaging of dysplasia. David J Javier*, Nitin Nitin* and Rebecca Richards-Kortum*. 6100 Main St., Keck Hall 116, Houston, TX.

Metallic nanoparticles can be used as an ideal platform for the development of contrast agents due to their strong surface plasmon resonance. The scattering property, stability and biocompatibility of metallic nanoparticles can be exploited to develop in-vivo optical imaging approaches. Furthermore, probes which bind cancer-related biomarkers can be immobilized on the surface of the nanoparticles to provide molecular specificity. In the presentation, the author will discuss the challenges and general considerations in the development of nanoparticles as molecular-specific contrast agents for cancer imaging. The synthesis of gold-based and silver-based metallic nanoparticles is based on reduction methods to produce nanospheres (diameter of 50 nm) and nanorods (aspect ratio of 50/10 nm). The conjugation of biomarkers is facilitated using either hydrophobic and electrostatic interactions (direct conjugation) or metal-sulfur lattice interactions (indirect conjugation). Biomarkers selected to target specific cell receptor include antibodies for EGFR binding, peptides for MMP cleavage, phage-display libraries designed for RGD binding, and aptamers for phospholipid detection. The specificity of the biomarker-conjugated nanoparticles is evaluated using confocal microscopy. Upon fabrication and conjugation process, the scattering properties of the contrast agents are analyzed using diffuse reflectance spectroscopy. In this set-up, the diffuse reflectance of the unconjugated and biomarker-conjugated nanoparticles is evaluated in different media and tissue-like phantoms. The importance of stability and scattering property will be emphasized in the overall design of the contrast agents.

TAM-3,e

Using fluorescence resonance energy transfer to design novel photodynamic therapy agents. Juan Chen[1,*], Klara Stefflova[2,*], Hui Li[1,*] and Gang Zheng[1,*]. [1]Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Chemistry, University of Pennsylvania, Philadelphia, PA.

Fluorescence resonance energy transfer (FRET) occurs when the electronic excitation energy of a donor fluorophore is transferred to a nearby acceptor molecule. In recent years, FRET has been successfully used in designing activatable fluorescence reporter probes (also known as molecular beacons) for *in vivo* cancer imaging. These FRET-based probes can yield particularly high tumor-to-background ratios since they are nonfluorescent in the native state, but become highly fluorescent upon target activation. Our group is the first to apply the FRET concept to design an activatable photodynamic therapy agent (PDT beacon). It comprises a disease-specific linker, a photosensitizer (PS) and a singlet oxygen ($^1O_2$) quencher/scavenger (Q), such that there is no photosensitization until the linker interacts with a specific target molecule, such as a tumor-specific enzyme or mRNA. For example, we synthesized a matrix metalloproteinase-7 (MMP7) triggered PDT beacon containing a PS and a Q conjugated to the opposite ends of a MMP7-specific peptide sequence. Proximity of PS and Q quenches fluorescence and photoreactivity of the PS by energy transfer. In the presence of cancer overexpressing MMP7, the substrate sequence is cleaved and Q is removed from the vicinity of PS. Thus, the PDT beacon lights up (by emitting fluorescence) and destroy (by producing cytotoxic $^1O_2$) the cancer cells, while leaving normal cells undetectable and unharmed. Furthermore, we also applied the FRET concept for assessing the PDT therapeutic outcome *in situ*. We synthesized a PDT agent with a built-in apoptosis sensor, comprising a caspase-3 peptide substrate, a PS and a suitable Q (completely quenching fluorescence and partially quenching $^1O_2$). We demonstrated both *in vitro* and *in vivo* that apoptosis-induced peptide cleavage results in detectable fluorescence restoration in cancer cells upon *in situ* PDT treatment. In summary, by intro-

ducing FRET to PDT, we have developed two novel PDT strategies and demonstrated their feasibilities.

TAM-4,a

Chronic UVA irradiation of human HaCaT keratinocytes induces malignant transformation associated with acquired apoptotic resistance. Yu-Ying He[1], Jingbo Pi[2,*], Jian-Li Huang[1,*], Bhalchandra A Diwan[3,*], Michael P Waalkes[2,*] and Colin F Chignell[1]. [1]NIEHS/NIH, Research Triangle Park, NC, [2]NCI/NIEHS, Research Triangle Park, NC, [3]NCI/NIEHS, Frederick, MD.

Ultraviolet A (UVA, 315-400 nm), constituting about 95% of ultraviolet irradiation in natural sunlight, represents a major environmental challenge to the skin and is clearly associated with human skin cancer. It has proven difficult to show direct actions of UVA as a carcinogen in human cells. Here we demonstrate that chronic UVA exposures at environmentally relevant doses in vitro can induce malignant transformation of human keratinocytes associated with acquired apoptotic resistance. As evidence of carcinogenic transformation, UVA-Long-Treated (24 J/cm^2 once/week for 18 weeks) HaCaT (ULTH) cells showed increased secretion of matrix metalloproteinase (MMP-9), overexpression of keratin 13, altered morphology and anchorage-independent growth. Malignant transformation was established by the production of aggressive squamous cell carcinomas after inoculation of ULTH cells into Nude mice. ULTH cells were resistant to apoptosis induced not only by UVA but also by UVB and arsenite, two other human skin carcinogens. ULTH cells also became resistant to apoptosis induced by etoposide, staurosporine and doxorubicin hydrochloride. Elevated phosphorylation of protein kinase B (PKB, also called AKT) and reduced expression of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) were detected in ULTH cells. The resistance of ULTH cells to UVA-induced apoptosis was reversed by either inhibition of phosphatidylinositol 3-kinase (PI-3K) or adenovirus expression of PTEN or dominant negative AKT. These data indicate that UVA has carcinogenic potential in human keratinocytes and that the increased AKT signaling and decreased PTEN expression may contribute to this malignant transformation. Further comparisons between the transformed ULTH and control cells should lead to a better understanding of the mechanism of UVA carcinogenesis and may help identify biomarkers for UVA-induced skin malignancies.

TAM-4,b

Modulation of nucleotide excision repair by the transcription factor E2F1. David Mitchell[1,*], Thomas Berton[1,*], Ruifeng Guo[1,*], Lakshmi Paniker[1,*], Joyce Reardon[2,*], Aziz Sancar[2,*] and David Johnson[1,*]. [1]The University of Texas MD Anderson Cancer Center, Smithville, Texas, [2]The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

E2F1 is an important transcription factor primarily targeted to genes involved in the S-phase of the cell cycle. It has been implicated in apoptosis and appears to play a role in numerous carcinogenesis pathways. We have recently used transgenic and knockout mice to show that E2F1 is involved in the efficiency of nucleotide excision repair (NER) capable or up-regulating the removal of UV damage (i.e., (6-4) photoproducts) in the skin in over-expressing mice and down-regulating NER in knockout mice. The mechanisms underlying these effects do not appear to involve gene regulation since early NER genes (e.g., XPA, XPC, DDB2) are not affected by E2F1 activity. Recently our attention has turned to analyzing the direct interaction between E2F1 and DNA damage sites including the proteins involved in damage recognition as well as the DNA damage directly. Micro-irradiation, immunocytochemistry, and immunoprecipitation data support the idea that E2F1 and perhaps other transcription factors may play an important role in signaling the presence of DNA damage as well as increasing accessibility and/or stabilization of the repair complex during the early stages of NER. In addition to apparent E2F1-protein interactions during the early stages of damage recognition we have also shown that E2F1 interacts directly with the DNA damage itself, similar to the "molecular hijacking" phenomena described for other transcription factors and damage types. The role of E2F1 in NER is far from clear and certainly intriguing; continuation of these studies will serve to expand our understanding of E2F1 as well as the comprehensive and complex function of NER in apoptosis and carcinogenesis.

TAM-4,c

Protein Kinase C epsilon sensitizes skin to the development of squamous cell carcinomas possibly by imparting resistance to apoptosis and promotion of survival of ultraviolet radiation-induced sunburn cells. Ajit K Verma* and Moammir H Aziz*. Department of Human Oncology, Madison, Wisconsin, USA.

Protein Kinase C ϵ (PKCϵ), a calcium-independent and phospholipid-dependent serine/threonine kinase, is among the PKC isoforms (α, δ, ϵ, μ and ζ) expressed in both human and mouse epidermis. We have reported that FVB/N PKCϵ transgenic mouse lines 224 and 215, which overexpress PKCϵ protein approximately 8- and 18-fold, respectively, over endogenous levels in the basal epidermal cells and cells of the hair follicle, are highly sensitive to UVR-induced cutaneous damage and development of squamous cell carcinoma (SCC) (Cancer Resaerch 64, 7756-7765, 2004). To find clues about the mechanisms by which PKCϵ sensitizes skin to UVR carcinogenesis, we found that UVR exposure of PKCϵ transgenic mice, as compared with their wild-type littermates, reduced the appearance of sunburn cells. Sunburn cells are the UVR-induced DNA-damaged keratinocytes undergoing apoptosis. To test the hypothesis that PKCϵ mediates resistance to apoptosis and promotion of survival of sunburn cells, we analyzed the expression of proteins linked to cell death (Fas/Fas-L, FADD and survivin) and survival (STAT-3, c-myc, cyclin D1, cyclin E and cdc25A) pathways. The level of expression of these proteins was determined after both acute (single 2kJ/m2) and chronic (4 doses, 2kJ/m2/dose, 3 x weekly) UVR exposure. PKCϵ overexpression upregulated UVR-induced expression of cytokines (TNFα, G-CSF, GM-CSF, IL-5, IL-6 and IL-10) and prostaglandin biosynthetic enzyme COX-2. The expression

of Fas/Fas-L and Fas associated death domain (FADD) adaptor proteins was inhibited while the level of expression of apoptotic inhibitor protein survivin was attenuated. PKCϵ overexpressing transgenic mice, when given either single or chronic UVR exposures, elicited constitutive activation of STAT-3 and accompanied upregulation of STAT-3 regulated genes (*c-myc*, *cyclin D1*, *cyclin E* and *cdc25A*). The results indicate that PKCϵ may impart sensitivity to UVR carcinogenesis by inhibiting apoptosis and promoting the proliferation of UVR-initiated preneoplastic cells.

TAM-4,d

A Specific codon 172 mutation in the p53 gene results in increased UV sensitivity. Cara L Benjamin[1], Chengming Zhu[1,*], David L Mitchell[2], Geng Liu[3,*], Guillermina Lozano[3,*] and Honnavara N Ananthaswamy[1]. [1]Department of Immunology, Houston, TX, USA, [2]Department of Carcinogenesis, Smithville, TX, USA, [3]Department of Molecular Genetics, Houston, TX, USA.

The tumor suppressor, p53, is responsible for many cellular activities including control of cell cycle progression and apoptosis. Clinical disorders such as Li-Fraumeni Syndrome involve mutations in the p53 gene and result in a predisposition to early onset tumor development and multiple tumor formation. A mouse model for the study of Li-Fraumeni Syndrome has been developed and reported previously involving a mutation at hotspot codon 172 changing an arginine to proline (R172P). This model is being studied for the response to UV-induced skin carcinogenesis. Early data shows an increase in sensitivity to UV-irradiation in terms of dermatological damage and increased skin-fold swelling following acute UV-irradiation. In response to DNA damage *in vitro*, mutant p53 MEFs display no sensitivity to IR by clonogenic assay. In contrast, UV irradiation resulted in a significant decrease in number of colonies formed in the $p53^{R172P}$ MEFs when exposed to FS20 sunlamps at increasing exposure, while other p53 mutants tested were not sensitive. To understand if the UV sensitivity of these cells is due to repair deficiency or cell cycle control disregulation, DNA from UV irradiated MEFs was examined by radioimmunoassay to detect (6-4) Photoproducts and Cyclobutane Pyrimidine Dimers (CPDs). Analysis showed that the R172P MEFs repaired the (6-4) PDs slower than either wild type control or cells harboring a different mutation at the same position in p53, but given time they were able to the eliminate the photodamage. These cells showed no deficiency in ability to repair CPDs. To investigate the regulation of cell cycle control in response to UV-induced DNA damage in these cells, cell cycle analysis and apoptosis assays are being performed. Therefore, these results indicate that the $p53^{R172P}$ mutation has an increased sensitivity to UV-irradiation both *in vivo* and *in vitro* and that the p53 defect does not interfere with the ability to repair DNA damage, implying that the sensitivity may be due to alteration in cell cycle control.

TAM-4,e

Repair dependent radiation survival: A stochastic model. John C Sutherland[1,2,*]. [1]Physics Department, Greenville, NC, USA, [2]Biology Department, Upton, NY, USA.

A stochastic model is derived that expresses the probability that an irradiated cell or virus survives exposure to a radiation dose as a function of the number of damages produced in a given target, the average probability that damages can be repaired, and a maximum repair capacity. The occurrence of damages is presumed to be random within a population. Survival is expressed as weighted sums of Poisson probabilities, with closed form solutions found in terms of Euler gamma functions. One limiting case provides a rationale for the observation that cells differing only in DNA repair capacity can exhibit single-exponential survival with greatly differing log-linear slopes. Another limiting case results in summations corresponding to those appearing in single-target-multi-hit target theory, extended to include the closed-form gamma function solution. The general case and limiting subsets span a range of observed survival data from single exponential to broad shoulders with no initial slope. Survival data analyzed using this model include both prokaryotic and eukaryotic cells. This stochastic model builds on concepts that have been developed over time to characterize cellular responses to radiation, and is consistent with established mechanisms of cellular damage and responses to radiation insults, i.e., the repair of DNA damage. It separates parameters into those that depend only on the radiation insult and those that are associated with repair. Analytical methods are demonstrated for obtaining these parameters from experimental survival data. Implications for applying survival functions the prediction of survival of cells exposed to polychromatic radiation such as sunlight and the influence of repair on the infectivity of viruses released into the environment and exposed to sunlight are discussed.

TAM-4,f

Alterations induced in mitochondrial DNA of human cells by UV and ionizing radiation. Helene Z Hill[1,*], Karen Hubbard[2,*], Mark Steinberg[3,*], James J Dermody[4,*], Wendy K Pogozelski[4,*], Douglas R Spitz[6,*], Edouard I Azzam[1,*] and Sonia M de Toledo[1,*]. [1]Department of Radiology, Newark, NJ, [2]Department of Biology, New York, NY, [3]Department of Chemistry, New York, NY, [4]Department of Microbiology and Molecular Genetics, Newark, NJ, [6]Department of Radiation Oncology, Iowa City, IA.

The 17 KBp mitochondrial DNA, like that of bacteria, is circular and replicates asynchronously. It lacks the protective cover of chromatin and is more easily mutated compared to genomic DNA. Repeat sequences are frequent and provide a platform for the development of deletions that range in size from a few bases to more than 10,000. One of these, the so-called Common Deletion, accumulates with age especially in slow turn-over tissues with high rates of respiration and is found in tissues of patients with degenerative neurological diseases, as well as in solar-exposed skin. Such deletions are believed to be caused by reactive oxygen-species that arise both endogenously and from exogenous sources. In our studies, DNA was extracted from cultured cells from a variety of sources that had been exposed to UV from an FS20 lamp in the presence and absence of glutathione and to γ-rays. Purified DNA was analyzed by real-time PCR using primers that encompass the Common Deletion region,

as well as primers designed to detect total mitochondrial DNA. Eight new deletions in the Common Deletion region have been characterized. Six of these must have arisen from the misannealing of direct repeats while two appear to be the result of inverted repeats requiring the twisting of mitochondrial DNA in such manner as to produce so-called Hoogsteen base-pairing. Very low doses of γ-rays of less than 10 cGray induce an increase in mitochondrial genomes seen after 24 hours. Linearizing the mitochondrial genome with XhoI, a restriction enzyme that makes a single cut, results in a 4-fold increase in mitochondrial copy number suggesting that the multiple genomes known to be present in each mitochondrion may be aggregated in the natural state. The results of these studies indicate that the mitochondrial genome is exquisitely sensitive to the *milieu intérieur* and responds in a variety of ways depending on the circumstances.

TAM-5,a

Control of DNA repair by photolyase. Dongping Zhong*.

Photolyase uses light energy to split ultraviolet-induced cyclobutane pyrimidine dimers in damaged DNA, but its molecular mechanism has never been directly revealed. Here, we report the direct mapping of catalytic processes through femtosecond synchronization of the enzymatic dynamics with its repair function. We observed direct electron transfer from the excited flavin cofactor to the dimer in 170 ps and back electron transfer from the repaired thymines in 560 ps. Both reactions are strongly controlled by active-site solvation to achieve maximum repair efficiency. These results show that the photocycle of DNA repair by photolyase is through a radical mechanism and completed on subnanosecond time scale at the dynamic active site with no net electron change in redox states of the flavin cofactor.

TAM-5,b

Probing the roughness of the folding energy landscape of beta-sheets. Feng Gai*. University of Pennsylvania, Philadelphia, PA.

Using a laser-induced temperature jump (T-jump) IR technique, we have shown that the turn formation is the rate limiting step in beta-hairpin folding. Therefore, it is possible to diminish the folding free energy barrier of beta-sheets by using rigid turns that are predisposed to fold in the unfolded state. In this talk, we will discuss the folding mechanism of a three-stranded beta-sheet that contains such rigid turns. Our results show that this beta-sheet folds on the nanosecond time scale, suggesting that the folding free energy barrier is indeed very small. Further Langevin dynamics simulations allowed us to explicitly determine the roughness of the folding energy landscape of this beta-sheet.

TAM-5,c

The role of structure in ultrafast dynamics of hemoglobins-ligand complex from *Lucina pectinata*. Juan Lopez-Garriga*. Chemistry Department, University of Puerto Rico, Mayaguez Campus, Mayaguez, PR, USA.

Hemeproteins play an essential role in a wide variety of physiological functions by their capacity to bind and release external ligands or by their redox properties. Thus, the study of invertebrate hemoglobins is very useful because of their structural similarity and functional diversity from hemoglobin and myoglobin. Hence the use of the hemoglobins from *Lucina pectinata* presents a novel system in terms of function-structure relationship. This mollusk is an intriguing invertebrate that lives in the sulfide-rich mangroves and is characterized by the presence of intracellular chemoautotrophic symbiotic bacteria that need to be supplied with both, hydrogen sulfide and oxygen through hemoglobin I (HbI) and hemoglobins II and III (HbII and HbIII), respectively. In hemeproteins, the nature of the distal heme environment and the heme iron electronic structure are essential factors in ligand binding dynamics. Regarding this, Pump and probe ultrafast time resolved absorption studies were performed in HbICO, HbIICO and HbI mutants; HbI(Phe/B10/Tyr)CO, HbI(Phe/B10/Val)CO and HbI(Gln/E7/Val)CO to evaluate the effect of distal heme environment and structure. The relaxation time constants of hemeCO derivatives were for HbI 9.19ps, HbII 2.87ps, HbI(Phe/B10/Tyr) 6.23ps, HbI(Phe/B10/Val) 4.29 ps, and HbI(Gln/E7/Val) 5.80 ps. The data indicated that the dynamics of the HbI mutants resembled that of HbI, suggesting that they both have similar structural conformational arrangement. The difference between HbII and the HbI(Phe/B10/Tyr) in signal relaxation suggests the direct influence of the monomeric and dimeric nature the chemical structure, respectively. Similarly, in HbI the docking site process may help to establish a barrier to the reverse rebinding process and thereby inhibits ultrafast geminate ligand rebinding in the closed conformation. These differences may account for the different ligand affinity of HbI and HbII for hydrogen sulfide and oxygen, respectively.

TAM-6,a

UV-Induced regulatory T cells. Thomas Schwarz*. Department of Dermatology, University Kiel, Kiel, Germany.

Topical application of contact allergens onto UV-exposed skin does not result in sensitization but causes hapten-specific suppression which is mediated via regulatory T cells (Tr). These cells express CD4, CD25 and CTLA-4, bind dectin-2 and release IL-10. Upon intravenous injection UV-induced Tr migrate into the lymph nodes but not into the skin. This explains why intravenously injected Tr prevent the sensitization but do not suppress the elicitation phase of contact hypersensitivity. However, when Tr are injected intracutaneously into the ears of sensitized mice the effector phase of contact hypersensitivity is efficiently suppressed. This migration behaviour is due to the expression of the lymph node homing receptor L-selectin but not of the ligands for the skin homing receptors E- and P-selectin. IL-12 exhibits the capacity to prevent UV-induced immunosuppression and even to break established UV-mediated tolerance by yet unknown mechanisms. IL-12 was recognized to be able to reduce UV-induced DNA damage presumably via the induction of nucleotide excision repair (NER). UV-induced DNA damage is an essential molecular trigger for UV-mediated immunosuppression, implying that the restoring effect of IL-12 may

be linked to its capacity to reduce DNA damage. Indeed, IL-12 was able to prevent UV-induced immunosuppresion in wild type mice (wt) but not in NER-deficient mice (Xpa-/-). In contrast, IL-12 exhibited the capacity to break already established UV-induced tolerance both in wt and Xpa-/-, indicating this effect to be independent of NER. Inhibition of sensitization by UV is due to the depletion of Langerhans cells (LC) which is triggered by UV-induced DNA damage. Accordingly, LC depletion by UV was prevented upon injection of IL-12 into wt but not in Xpa-/- mice. Immunofluorescence staining revealed DNA damage carrying cells in the regional lymph nodes upon UV exposure. The number of these cells was remarkably reduced when UV-exposed mice had received IL-12. In turn, IL-12 did not reduce the number of DNA damage carrying cells in Xpa-/- mice. Taken together, this indicates that the prevention of UV-induced inhibition of sensitization IL-12 is linked to its capacity to induce NER while breaking of tolerance is independent of NER and mediated via another yet to be determined mechanism.

TAM-6,b

Vaccination to induce tolerance using ultraviolet light. J P Dutz*, M. Ghoreishi* and H. Najar*. Departments of Medicine and Dermatology, Vancouver, Canada.

Transcutaneous immunization (TCI) is an effective method of inducing immune responses to protein and peptide Ag. Topical application of Toll Like Receptor (TLR) 9 family agonists (CpG adjuvant) promotes the generation of cytotoxic lymphocytes (CTL) specific for either co-applied or locally injected antigen. We explore the effect of UV irradiation on TCI. The generation of Ag-specific CTL to OVA protein, but not class I MHC-restricted OVA peptide, is inhibited by TCI through UV-irradiated skin. Application of protein or class II MHC-restricted OVA peptide to UV-irradiated skin induces transferable Ag-specific tolerance. This tolerance is mediated by CD4(+)CD25(+) T regulatory (T(reg)) cells. These Ag-specific T(reg) cells inhibit the priming of CTL following protein immunization in the presence of CpG adjuvant. We next demonstrate, using IL-10-deficient mice and adoptive T cell transfer, that IL-10 is required for the direct inhibition of CTL priming following immunization through UV-irradiated skin. However, IL-10 is not required for the induction of T(reg) cells through UV-irradiated skin as IL-10-deficient T(reg) cells are able to mediate tolerance. Rather, host-derived IL-10 is required for the function of UV-generated T(reg) cells. These experiments indicate that protein and peptide TCI through UV-irradiated skin may be used to induce robust Ag-specific tolerance to neo-Ags.

TAM-6,c

CD4+ T cells in lymph nodes of UVB-irradiated mice suppress immune responses to newly-introduced antigens both in vitro and in vivo. Prue H Hart*, Shelley Gorman* and John J Finlay-Jones*. Telethon Institute for Child Health Research, Perth, Western Australia, Australia.

The mechanisms by which erythemal UVB irradiation modulates systemic immune responses to antigens applied to non-irradiated sites are poorly understood. We have found no evidence for qualitative or quantitative changes to CD11c+ antigen presenting cells that handle sensitizing antigens applied to sites distant to those irradiated. Instead, regulatory CD4+ T cells were identified in the skin-draining lymph nodes of UVB-irradiated, but otherwise naive mice. A transgenic mouse strain (DO11.10) was utilised in which >85% of CD4+ T cells expressed the ovalbumin (OVA 323-339) T cell receptor. This mouse enabled in vitro and in vivo OVA-specific responses to be investigated without a direct requirement for antigen sensitisation (thus avoiding potential effects of UV on OVA-presenting APC in the sensitization phase). CD4+ T cells from the skin-draining lymph nodes of UVB-irradiated mice had significantly reduced capacity to respond to presentation of OVA protein following adoptive transfer into naive BALB/c in vivo, and the OVA323-339 peptide in vitro. Transfer of CD4+ T cells from skin-draining lymph nodes of UVB-irradiated antigen-naive mice significantly suppressed contact hypersensitivity responses in recipient mice to an experimental hapten. Depletion of CD4+CD25+ cells abrogated this UVB-suppressive effect in the in vitro proliferation assay. There was also a significant increase in the proportion of CD4+CD25+Foxp3+ cells in the skin-draining lymph nodes of UVB-irradiated mice. The potential of these regulatory cells to regulate responses to incoming antigens at distant non-irradiated sites broadens the biological impact of UVB irradiation of skin on immunity.

TAM-6,d

UV-induced activation of suppressor B cells is mediated through both RAF and serotinin receptors. Scott N Byrne[1], Yumi Matsumura[1,*], Dat X Nghiem[1,2,*], Yasuko Miyhara[1,*] and Stephen E Ullrich[1,2]. [1]Department of Immunology, Houston, TX, USA, [2]The Graduate School of Biomedical Sciences, Houston, TX, USA.

Inflammatory mediators such as PAF and cis-UCA are released after UV radiation and orchestrate UV-induced immune suppression. They also contribute to skin cancer formation. However, little is known about the cellular targets of these inflammatory mediators. Mice were exposed to immunosuppressive doses of UVB then hapten sensitized 4 days later. Draining lymph node cell suspensions were prepared and cells transferred into naive syngeneic hosts 18h later. As expected, transferring FITC+ lymph node cells from UV irradiated mice into normal recipient animals induced immune suppression. We confirmed that the FITC+ cell responsible for inducing immune suppression 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 PAFR knock out mice transfer of suppression was still observed. However, when cells were isolated from UV-irradiated WT mice treated with a dual PAF and 5HT2a receptor antagonist, or from PAFR KO mice that had been pretreated with a serotonin receptor antagonist, transfer of immune suppression was lost. Thus, both PAF and serotonin receptor signaling was required for the activation of suppressor B cells. These data identify a novel function for both PAF and serotonin/cis-UCA in modulating immune function,

activation of regulatory B cells that induce immune suppression.

TAM-6,e

Can modern sunscreens prevent immunosupression? Anny M Fourtanier*. L'Oreal Recherche 90 rue du Général Roguet, Clichy cedex, FRANCE.

Sunscreens were initially designed to protect against sunburn/erythema. Their efficacy against this acute reaction is labelled by the sun protection factor (SPF). As a lot of other biological damage, particularly those induced by the long UV wavelengths have been described these last 25 years, it became important to improve sunscreen formulation and efficacy. Today with the introduction of new UV filters and the knowledge of the best way to combine them to obtain good absorption spectra these goals have been achieved. However, some published studies have raised doubts about the sunscreen ability to prevent UVR induced immunosuppression or to offer comparable protection against erythema and immunosuppression. Five recent (2001-2004) published human studies, followed by an international expert meeting and a consensus paper (J. Invest. Dermatol. 2005) addressed this point. These studies have compared the capacity of sunscreens, with different levels of UVB and UVA protection, to inhibit UV induced suppression of either the induction arm or the elicitation arm of the contact hypersensitivity (CHS) or the delayed type hypersensitivity (DTH) responses. All demonstrated that, to offer a good protection against immunosuppression, at least equal to the erythema protection a sunscreen must provide a high UVA filtering capacity.

TAM-6,f

Deficient inflammatory response to UVB in neonatal mice. Agnieszka Wolnicka-Glubisz[1,2,*], Jesse Damsker [3,*], Stephanie Constant [3,*], Stephanie Corn[4,*], Edward De Fabo [2,*] and Frances Noonan[2,*]. [1]the Jagiellonian University, Krakow, Poland, [2]the George Washington University, SPHHS, Washington, DC, USA, [3]the George Washington University, SMHS, Washington, DC, USA, [4]Ohio State University, Department of Veterinary Biosciences, Columbus, OH, USA.

Childhood sunburn is a major risk factor for cutaneous malignant melanoma indicating critical differences between adults and children in the UV responsiveness of skin. Neonatal but not adult UV irradiation of hepatocyte growth factor/scatter factor (HGF/SF) transgenic mice initiates melanomas which resemble human disease. Irradiation of adult FVB or HGF/SF transgenic mice with UVB (280-320nm) but not with UVA (320-400nm) initiated a dose and time dependent influx of inflammatory $CD11b^+$ and $Ly6G^+$ cells and a decrease in MHC Class II^+ cells in the skin detected by FACS analysis and immunohistochemistry. In contrast, in neonatal wild-type or transgenic skin no infiltrating cells were detectable in response to any dose of radiation from any UV source although in both adult and neonatal skin DNA damage and edema were readily detected in response to UV. Topical trinitrochlorobenzene (TNCB) did not initiate an inflammatory infiltrate in neonatal skin and there was no inflammatory infiltrate in the peritoneal cavity in response to thioglycollate. Neonatal sensitization with TNCB was tolerogenic, resulting in a significantly lowered contact hypersensitivity response in adults on resensitization. Neonatal blood contained abundant neutrophils with an immature phenotype which exhibited enhanced chemotaxis to the bacterial product fMLP but impaired chemotaxis to the chemokine Gro-alpha and a decreased expression of its receptor CXCR2. We propose this deficient inflammatory response is a significant previously unrecognized factor in neonatal immune tolerance which, together with the absence of a cytocidal inflammatory infiltrate in UVB irradiated neonatal skin, facilitates survival of UV initiated melanocytic cells and thus subsequent melanoma development.

TAM-7,a

PDT enhances the immune cell response to basal cell carcinoma antigens. Sandra O Gollnick[1,*], Edith Kabingu[1,*] and Allan R Oseroff[2,*]. [1]PDT Center, Dept. Cell Stress Biology, Buffalo, NY, USA, [2]Dept of Dermatology, Buffalo, NY, USA.

Basal cell carcinoma (BCC) is the most common form of skin cancer, affecting 800,000 Americans each year. The sonic hedgehog proteins and their receptors play an important role in the development of skin cells. Mutations in the patch-1 (PTCH-1) gene, which encodes for a receptor for sonic hedgehog proteins, have been identified as the causative agent in BCC. BCC also overexpress a second receptor for the hedgehog proteins known as HIP-1 (hedgehog interacting protein-1), which is not mutated in BCC. Preclinical studies have shown that vaccination with dendritic cells loaded with HIP-1 can prevent development of BCC. We have identified a HIP peptide that binds to the human major compatibility complex molecule HLA-A2. Previous experiments by others and us have shown that PDT enhances antitumor immune responses in animal models. To determine whether PDT was able to enhance the anti-tumor immune responses in a clinical setting, the ability of PDT to enhance recognition of HIP-1 was examined in patients treated with PDT. Sera were collected from patients expressing HLA-A2 before and 7-14 days after PDT and analyzed for HIP specific IFN-gamma producing lymphocytes as an indication of increased immune response. Our results showed that following PDT, the number of IFN-g producing lymphocytes increased dramatically (50% to >130%) in the patients following PDT. Overall, these data demonstrates that there is an enhancement in the HIP responsive immune cells following PDT.

TAM-8,a

Upregulation of copper and vascular endothelial growth factor (VEGF) in nasopharyngeal carcinoma following hypericin mediated photodynamic therapy (PDT). Ramaswamy Bhuvaneswari[1,*], Gan Yap Yik-Yuen [2,*], Patricia S Thong[1,*], Mac M Ho[1,*], Khee-Chee Soo[1,*] and Malini Olivo[1,*]. [1]National Cancer Centre Singapore, Singapore, Singapore, Singapore, [2]National Institute of Education, Singapore, Singapore, Singapore.

Objectives: Recent studies have shown that the availabil-

ity of trace element copper is critical to the initiation and development of angiogenesis. Based on this principle the current study investigates the role of copper in the promotion of angiogenesis growth factor VEGF in nasopharyngeal carcinoma after treatment with Hypericin mediated photodynamic therapy (PDT). Methodology: Human nasopharyngeal carcinoma (NPC) cell line HK1 was xenografted on 6-8 weeks old male Balb/c nude mice. Hypericin, a potent photosensitizer was used for the treatment. The serum and tumors were collected at 24, 48 and 72hr post PDT. Enzyme-Linked Immunosorbent Assay (ELISA) kits were used to study the copper and VEGF levels in serum and tumor tissue. Both mouse and human VEGF were measured. Nuclear microscopy was also used to quantify the concentration of elemental copper in tumors. Results: An increase in copper was observed in serum and tumor tissue after treatment with hypericin-PDT compared to the non-PDT group. Analysis by nuclear microscopy also showed a significant increase in copper in tumor post PDT. Though the human VEGF level in the serum and tumor tissue decreased at 24 and 48 hr post PDT, it was upregulated at 72 hr post PDT. Mouse VEGF level in the serum was also found to display a similar trend as in the human VEGF. The increase in copper in serum and tumor after PDT and the increase in VEGF at 72hr post-PDT suggests that copper plays an important role in angiogenesis. Conclusion: The results show a significant increase in copper after Hypericin mediated PDT, and potentially a significant factor in the upregulation of VEGF, leading to tumor regrowth after PDT.

TAM-8,b

Peptide-induced inflammatory increase in vascular permeability improves photosensitizer delivery and inter-subject photodynamic treatment efficacy. Xiaodong Zhou[1,*], Bin Chen[2,*], P J Hoopes[3,*], Tayyaba Hasan[4] and Brian W Pogue[4,5]. [1]Division of Medical Physics, Radiation Oncology Department, Philadelphia, PA, [2]Department of Pharmaceutical Sciences, Philadelphia, PA, [3]Department of Surgery, Hanover, NH, [4]Wellman Center for Photomedicine, Boston, MA, [5]Thayer School of Engineering, Hanover, NH.

Photodynamic therapy treatment can exhibit high inter-subject variability due to the inherent differences in drug delivery throughout tissue to be treated. In this study, the concept of using a chemical modifier to increase perfusion was analyzed, using Substance P, a peptide known to increase vascular permeability in tissues. The effect of this peptide was quantified in terms of its impact as a model to promote drug delivery via artificially induced acute inflammation. The transvascular permeability coefficient was quantified and the mean value increased from 0.026 to 0.043 microns/sec with the addition of substance P. Correspondingly, there was a 40-50% increase in uptake of the photosensitizer in the tumor parenchyma, for substance P injected tumors, as compared to those without. This increased drug uptake also resulted in an increased tumor doubling time from 4 days with regular PDT to 6.2 days with the substance P and PDT. Most interestingly, there was also a significant reduction in the inter-individual variability in response to this latter PDT treatment from 64% to 13% coefficient of variation, indicating that increased permeability helped to make the treatment more repeatable between subjects. The resulting treatment was then more effective and more reliable in terms of dosing between subjects.

TAM-8,c

An in vitro investigation into the enhancement of the efficacy of amindevulinic photodynamic therapy using iron chelation. Andrew Pye*, Toni Wakeham*, Sara Horton*, Leo Salter* and Alison Curnow. Cornwall Dermatology Research, Truro, Cornwall, UK.

Aminolevulinic acid photodynamic therapy (ALA-PDT) combines the selective accumulation of a photosensitizer in tumor tissue with visible light irradiation (and tissue oxygen) to produce reactive oxygen species (ROS). This results in cellular damage and ablation of tumor tissue. The use of iron chelators in combination with ALA has the potential to increase accumulation of the photosensitizer protoporphyrin IX (PpIX) in the cell by reducing its bioconversion to heme. Chelation of iron should therefore increase the efficacy of ALA-PDT by increasing the amount of photosensitizer in the cell. In addition however iron has a role to play in ROS production (through Haber-Weiss and Fenton reactions) and its chelation could also result in a reduction in the efficacy of ALA-PDT. Therefore a study was conducted to determine the importance of iron and its chelation on ALA-PDT efficacy. ROS-induced DNA damage, cell viability, and PpIX accumulation were all determined in human fetal lung fibroblasts incubated with ALA with and without iron chelators (using single cell gel electrophoresis (comet assay), the XTT cell viability assay, and fluorometric detection of PpIX respectively). The iron chelators studied were desferrioxamine (DFO) and the novel hydroxypyridinone iron chelator CP94. It was observed that iron chelation increased the accumulation of PpIX in the cells incubated with ALA and that this correlated strongly to both increased DNA damage and cell cytotoxicity following red light irradiation. These results demonstrate that the effects of iron chelation on the concentration of photosensitizer are more significant in determining the efficacy of ALA-PDT than the effects of iron chelation on iron mediated ROS reactions. This is an important finding that adds more evidence to support the use of iron chelation in combination with ALA-PDT to improve treatment efficacy.

TAM-8,d

Photodynamic therapy with organically modified silica (ORMOSIL) nanoparticles using the hydrophobic photosensitizer HPPH (2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide a): comparison of the free and nanoparticle encapsulated forms in vitro and in vivo. Janet Morgan[1,*], Indrajit Roy[3,*], Dhruba J Bharali[3,*], Ravindra K Pandey[2,*], Allan R Oseroff[1,*], Paras N Prasad[3,*] and Earl J Bergey[3,*]. [1]Dept. of Dermatology, Buffalo, NY, USA, [3]Institute for Lasers, Photonics and Biophotonics, Buffalo, NY, USA, [2]Cell Stress Biology, Photodynamic Therapy Center, Buffalo, NY, USA.

Enhanced tumor selectivity and effectiveness of photosen-

sitizers (PS) are desirable goals in photodynamic therapy (PDT). Many PS have some degree of selectivity for tumors, but the ratio of tumor to normal (T/N) tissue retention is often low. Also, many PS are hydrophobic, tend to aggregate which makes them less photoactiveand difficult to formulate for *in vivo* administration. Recent developments suggest that some PS also are substrates for ATP-dependent multidrug resistant (MDR) phenotype proteins (in particular ABCG2/BCRP), which pump them out of cells, decreasing intracellular levels available for photochemical reactions. Delivery systems in which PS are encapsulated in a nanoparticle produce aqueous-dispersible formulations which have the potential to increase tumor to normal tissue ratios resulting in enhanced selectivity, and to overcome the MDR phenotype, by protecting the PS and inhibiting its access to the pump's binding site. We synthesized and characterized HPPH-ORMOSIL nanoparticles, and compared them to free HPPH *in vitro* and *in vivo*, using the Colon-26 mouse colon carcinoma, which expresses the ABCG2 pump *in vitro*, but not *in vivo*. Similar amounts of singlet oxygen were produced by the two formulations on an equimolar basis. At equal administered doses, the HPPH in nanoparticles was more effective at photosensitizing cells than in its free form, because it could not be pumped out by the ABCG2 protein. *In vivo*, the pharmacokinetics at similar doses differed somewhat, with an earlier and increased T/N tissue ratio with nanoparticles. Retention in the skin and in other organs was less with particulate HPPH than for free HPPH. Otherwise tumor accumulation did not differ substantially. *In vivo*, tumor response did not differ substantially at high fluence rates, but was enhanced with nanoparticles at lower fluence rates and doses. ORMOSIL-HPPH are an effective, selective and stable formulation choice for PS delivery to tumors.

TAM-8,e

Killing of antibiotic resistant bacteria by rose bengal. Craig Dees*, Rebecca Adams*, Rhiannon Carr*, Timothy C Scott* and Eric A Wachter*. Provectus Pharmaceuticals, Inc., Knoxville, TN.

Infection with methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant enterococci (VRE)are increasingly a problem in health care and other facilities. We demonstrate that using Rose Bengal and light provided by a simple flourescent bulb is highly efficient in killing high numbers of these two antibiotic resistant bacteria in vitro. Killing of bacteria was not dependent on the presence of oxygen. Therefore, we examined the effects of light on Rose Bengal. Rose Bengal after illumination was converted to tetrachloroerythosin. Therefore, the mechanism of bacterial killing is likely to be by the generation of free radical iodine. It may be possible to treat cutaneous infections caused by antibiotic resistant bacteria using very low concentrations of Rose Bengal and ambient light or a very simple light source.

WED-A

Management of phototherapy and choice of light source. Hans C Wulf*. Department of Dermatology, Copenhaven.

Ultraviolet radiation is a wellknown treatment modality for several skin diseases such as psoriasis and eczema. Light sources may emit ultraviolet radiation in the UVB and UVA range. Broad spectrum UVB may be used as well as narrow band UVB. There has been a tendency to go to the narrow band,since it is considered to be more efficacious. However, the skin diseases that are treated are chronic diseases and therefore the patients must be able to tolerate the treatment without long-term side effects. Therefore we have to consider if narrow band UVB is more carcinogenic than broad band UVB. UVA is much less carcinogenic than UVB and is considered as a treatment alternative for topic dermatitis. PUVA treatment consists of a combination of 8-methoxypsoralen and broad band UVA. It is used in severe cases of psoriasis and eczema, but it has been proven to be considerably more carcinogenic than UVB treatment and it is very important to limit the use of PUVA to very severe cases. UVA may also be used for the treatment of more rare diseases such as scleroderma and possibly lupus erythematosus. When the right light sources have been chosen the next step is to choose the right light dose. This is highly depending on the skin type of the patient or degree of pigmentation and can be guided by reflectance measurements. Systems for determining the ideal light dose will be presented and has been shown to reduce the accumulated UV dose by up to 70%, when treating psoriasis and atopic dermatitis, and thus diminish the risks associated with treatment.

WAM-1,a

Effects of UVC-and UVB-induced photooxidation of DNA on the intercalation of thiazole orange dye: A highly sensitive reporter for oxidative damage. Colleen C Trevithick-Sutton, Larisa Mikelsons*, Vasilisa Filippenko* and J. C Scaiano.

A novel method of identifying oxidative damage to DNA is reported. Thiazole orange, (TO), an intercalating cyanine dye, fluoresces strongly when intercalated in DNA, but not free in solution. Upon UVB- or UVC-induced photooxidation of DNA, the change in TO fluorescence is greater than the change in any of the other spectral or biochemical indicators (UV-visible absorbance, circular dichroism, and agarose gel electrophoresis). Fluorescence of ethidium bromide, the classical agarose gel electrophoresis stain, follows the same trend but to a lesser extent. This methodology can provide a fast screening method to identify damage to DNA including both pyrimidine dimer and 8-oxo-G formation, regardless of strand breakage, giving an early warning of the onset of damage.

WAM-1,b

Singlet oxygen oxidation of isolated and cellular DNA. Jean Cadet[1,*], Thierry Douki[1,*], Jean-Luc Ravanat[1,*], Glaucia R Martinez[2,*], Marisa HG Medeiros[2,*] and Paolo Di Mascio[2,*]. [1]Lésions des Acides Nucléiques, DRFMC/SCIB, CEA/Grenoble, Grenoble, France, [2]Departamento de Bioquimica, Universidade de Sao Paulo, Sao Paulo, Brazil.

Singlet oxygen oxidation reactions of guanine components, the specific nucleic acid targets of the latter reactive oxygen species, have received major attention during the last

decade. In this respect we should emphasize the outstanding contributions made by Chris Foote and his associates concerning the elucidation of mechanistic pathways and the structural assessment of endoperoxide and dioxetane intermediates. Thus [4+2] Diels-Alder addition of $^1O_2$ across the 4,8-bond of the guanine moiety leads to diastereomeric endoperoxides that may rearrange into a 8-hydroperoxide. Reduction of the latter intermediate, whose formation was inferred from low temperature ^{13}C NMR measurements of a suitable model compound, leads to the generation of 8-oxo-7,8-dihydroguanine (8-oxoGua) as a minor process for isolated nucleosides. In fact it was found that the main degradation products are the 4R and 4S diastereomers of spiroiminodihydantoin nucleosides which are likely to arise from dehydration of the 8-hydroperoxyguanine moiety leading to a quinonoid intermediate. This is followed as shown by labeling experiments by a nucleophilic addition of a water molecule at C5 and further rearrangement of the resulting unstable 5-hydroxy-7,8-dihydroguanine. However, there is no evidence for the occurrence of the latter degradation pathway within isolated DNA. In fact, studies involving a chemical source of singlet oxygen have showed that the reaction of $^1O_2$ with the guanine moiety of calf thymus DNA leads to the overwhelming 8-oxoGua formation. Similar specific oxidation reaction of the guanine moiety was shown to take place in the DNA of human monocytes. Interestingly 8-oxoGua was found to be generated in prokaryotic and eukaryotic cells upon exposure to UVA radiation as the likely predominant contribution of $^1O_2$ produced by still unknown endogenous type II photosensitizer(s). No information on the formation in isolated and cellular DNA of secondary $^1O_2$ oxidation products of 8-oxoGua including spiroiminodihydantoin and oxaluric acid is available so far.

WAM-1,c

The excited state dynamics of Ni(II) tetraphenyltetrabenzoporphyrin: Photophysical and theoretical investigations. Michael A Rodgers*, Andrey V Zamyatin* and Alexandra V Soldatova*.

Metallotetrapyrroles having first row transition metal centers have been shown to undergo extremely rapid radiationless deactivation from their photo-generated excited states. Earlier studies from this laboratory have focused on metallophthalocyanines (MPc) and metallonaphthalocyanines (MNc); here we report recent investigations of a metellotetraphenyl-tetrabenzoporphyrin (MTPTBP). The photodynamic properties of nickel (II) porphyrins having various kinds of peripheral substitution have been extensively investigated by the research groups of Holten, Kim and others; in this work we have investigated the excited state dynamics of NiTPTBP by ultrafast transient absorption spectrometry and have employed Time Dependent Density Functional Theory to characterize the nature and the energy of electronic states that lay below the lowest £k, £k* state. *f*n Calculations revealed that the energy of the Q-state, the lowest singlet of £k £k * character, lies at 1.941 eV, compared to the experimental value for the Q band absorption maximum of 1.928 eV. Below the Q-state and above the ground state six states were found, two pairs of almost degenerate singlet and triplet LMCT states, one 3 £k £k* *f*x*f*nstate (1.559 eV, 3E) and one 3(dz2,dx2-y2) (1.431 eV, 3B2). Upon photoexcitation with 400 nm light the 1 £k, £k * (S1) state is generated from the primary S2 state within the instrument response function (ca 200 fs). The difference absorption spectrum of a toluene solution of NiTPTBP (5 £gM) at 1 ps post excitation (the first observed transient, FOT) was positive and featureless over the 450 nm to 750 nm range except for a strong negative absorption signal around 645 nm indicating ground state bleaching. The FOT absorption decayed rapidly and was replaced by a positive band that grew up to the red side of the ground state bleaching signal. These rates were wavelength dependent with lifetimes ranging from 0.8 ps-1 to 2.0 ps-1. The new spectral band also shifted to the blue with time; both spectral and kinetic features indicating vibrational cooling. After ca 5 ps this spectral motion had stabilized and the resulting spectral shape was characteristic of a d,d state. This feature decayed to the ground state with a lifetime of ca 30 ps.

WAM-1,d

The triplet energy of thymine in DNA. Virginie Lhiaubet-Vallet[1,*], M. Consuelo Cuquerella[1,*], Jose Vicente Castell[2,*], Francisco Bosca[1,*] and Miguel Angel Miranda[1]. [1]Instituto de Tecnología Química UPV-CSIC, Avda de Los Naranjos s/n, Valencia, Spain, [2]Centro de Investigación, Hospital La Fe, Avda de Campanar 21, Valencia, Spain.

Photosensitization of thymine cyclobutane dimers (T<>T) occurs through triplet-triplet energy transfer. Although the feasibility of this process lies on the triplet energy of thymine in DNA, the precise value of this parameter has not yet been definitively established. Nevertheless, it appears to be markedly different from that of free thymine or thymidine. In DNA, this problem has been addressed by the use of photosensitizers of different triplet energies to produce T<>T lesions; this way, E_T has been estimated at 290 kJ/mol or even lower. The aim of the present study was to determine in a more accurate way the triplet energy of thymine in DNA or, more precisely, the minimum value of E_T for a photosensitizer to produce T<>T lesions in DNA. As fluoroquinolones seem to be near to this limit, it appeared reasonable to take them as starting point. So, two members of the family with E_T values close to each other, defining a very narrow range comprising the threshold energy capable of triggering T<>T formation, have been chosen. Accordingly, Norfloxacin (NFX) and its acetylated derivative ANFX were selected as possible candidates as a peripheral substitution appeared more convenient than variations at the basic skeleton. First, it has been evidenced from the observation of single strand breaks after T4 endonuclease V enzymatic treatment and subsequent gel electrophoresis that NFX photosensitizes formation of T<>T, while ANFX does not. Then, Laser Flash Photolysis experiments have been performed and the triplet energies of NFX and ANFX have been estimated at 273 and 268 kJ/mol, respectively, on the basis of triplet-triplet energy transfer quenching by a set of biphenyl and naphthalene derivatives. Hence, the triplet energy of thymine in DNA (i.e. the value for a photosensitizer to produce T<>T) has be estimated at 270 kJ/mol.

WAM-2,a

Photosensitized reduction and DNA covalent binding of alkylating quinones. Antonio E Alegria[1,*], Nadya Martinez[1,*], Sujit Kumar-Ghosh[1,*], Garcia Carmelo[1,*] and Rafael Arce[2,*]. [1]University of Puerto Rico, Department of Chemistry, Humacao, PR, USA, [2]University of Puerto Rico, Department of Chemistry, Rio Piedras, PR.

Photodynamic therapy (PDT) is a cancer treatment that uses a combination of red laser light, a photosensitizing agent and molecular oxygen to bring about a therapeutic effect. Porphyrins (POR), phthalocyanines (PC), chlorins (CHL) and others are currently being used in photodynamic treatment (PDT) of tumors due to their large absorption coefficients in the 500-800 nm range. In the presence of air these will photosensitize the production of singlet oxygen and superoxide. Singlet oxygen production, the so-called Type II pathway, is claimed as the most important process which kills tumor cells. However, Type I pathways, those involving photoreduction or photooxidation of substrates, have also been proposed as photocytotoxic events in PDT, especially in hypoxic environments. As example of Type I pathway, in this work we show that alkylating quinones binds covalently DNA under anoxia after being photoreduced by different red-light-absorbing dyes. Photolysis of anaerobic aqueous mixtures at wavelength maxima above 600 nm and at pH 7.4 containing either aluminum phthalocyanine tetrasulfonate (AlPcS4) chlorin e6 (CHLORIN), pheophorbide-a (PHEO) or a novel tetracationic phthalocyanine derivative (TETCHLORIN) in the presence of the quinones diaziquone (AZQ), carboquone (CARBOQ) or 2,5-dicloro-diaziridinyl-1,4-benzoquinone (AZDClQ) produce the corresponding semiquinones. Photolysis of these mixtures under the conditions stated above but in the presence of DNA and at pH 5.5 produces quinone-DNA covalent adducts. Evidence is obtained which suggests binding of these quinones to DNA through the open aziridine ring. In general, the quinone CARBOQ yielded the largest amounts of adducts photosensitized by the dyes studied here. No quinone-DNA adducts were detected if samples were at pH 7.4.Thus, both photoreduction of these quinones and an acidic environment are needed for these quinones to bind DNA. This work suggests a potential mode of therapy with special applications to hypoxic regions in solid tumors which are characterized by an acidic environment.

WAM-2,b

Survivin, a member of the inhibitor of apoptosis (IAP) family, is induced by PDT and is a target for improving treatment response. Angela J Ferrario[1,*], Natalie Rucker[1,*], Sam Wong[1,*], Marian Luna[1,*] and Charles J Gomer[1,2,*]. [1]Childrens Hospital Los Angeles, Los Angeles, CA, United States, [2]University of Southern California, Los Angeles, CA, United States.

A goal of our laboratory is to improve the effectiveness of Photodynamic Therapy (PDT) for the treatment of solid tumors. We recently discovered PDT induces the expression and activation of survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, in mouse and human cancer cells. Survivin is activated by phosphorylation at Thr-34. Activated survivin inhibits caspase 9, blocks apoptosis, and is associated with resistance to chemotherapy and radiation. Survivin is a client protein for the 90 kDa heat shock protein (hsp90) and the binding of survivin to hsp90 assists in the maturation, proper folding, assembly, and transport of this IAP protein. Additional hsp-90 client proteins include Akt, HIF-1 and Bcl-2. A derivative of geldanamycin, 17-allylamino-17-demethoxygeldanamycin (17-AAG), interferes with binding of client proteins to hsp90 and leads to misfolding of client proteins, ubiquination and proteasome degradation. We hypothesize that PDT efficacy is reduced by treatment-mediated activation of survivin and that targeting the survivin pathway can increase PDT responsiveness. To address this, we first examined cellular and molecular responses following PDT, 17-AAG, and the combination of PDT plus 17-AAG in human BT-474 breast cancer cells. Cells treated with both PDT and 17-AAG displayed decreased expression of phospho-survivin, phospho-Akt and Bcl-2 proteins and this was accompanied by increased apoptosis and cytotoxicity. We next used a human melanoma cell line, YUSAC2/T34A-C4, stably transfected with an inducible dominant negative (Thr34> Ala) survivin under the control of a tetracycline-regulated (tet-off) promoter. Withdrawal of tet from culture medium causes these cells to express a phosphorylation-defective dominant negative survivin mutant. We observed that PDT of melanoma cells expressing dominant negative survivin had increased cleavage of the caspase substrate poly ADP-ribose polymerase, apoptosis and cytotoxicity when compared to results following PDT of melanoma cells expressing wild type survivin. These results demonstrate that targeting survivin, and other hsp-90 client proteins, with 17-AAG improves in-vitro PDT responsiveness and suggest that manipulation of the antiapoptotic pathway maintained by survivin may enhance PDT-mediated cancer therapy.

WAM-2,c

Effects and mechanisms of vascular permeabilization induced by vascular-targeting PDT. Bin Chen[1,*], Brian Pogue[2,3,*], Jack Hoopes[2,*] and Tayyaba Hasan[3,*]. [1]Dept. Pharmaceutical Sciences, Univ Sciences in Philadelphia, Philadelphia, PA, [2]Thayer School of Engineering, Dartmouth College, Hanover, NH, [3]Wellman Center for Photomedicine, MGH, Boston, MA.

Tumor vasculature represents an attractive target for cancer therapy due to its accessibility to blood-borne therapeutic agents and the dependence of tumor cells on a functional blood supply for the survival and growth. The property that photosensitizers are exclusively localized within the vasculature shortly after systemic administration plus the ability to accurately deliver light by current laser fiber technology provides an effective and selective way to target tumor blood vessels through intravascular generation of reactive oxygen species (ROS). Vascular targeting PDT based on a short drug-ligt interval is currently under clinical trial for prostate cancer. Here we studied the effect of vascular-targeting PDT on vascular barrier function in prostate tumor models using intravital microscopy and whole-body fluorescence imaging

techniques. Immunofluorescence study was performed to examine photosensitization-induced endothelial cytoskeleton changes. Our results demonstrate that vascular-targeting PDT permeabilizes blood vessels through the formation of endothelial intercellular gaps, which are likely due to endothelial cell microtubule depolymerization after vascular photosensitization. PDT-induced vascular permeabilization greatly enhances the extravasation of circulating molecules. Loss of endothelial barrier function can ultimately lead to tumor vascular shutdown and the trapping of circulating molecules. Our results suggest that tumor vascular barrier dysfunction induced by vascular targeting PDT has significant implications in drug transport and tumor cell metastasis.

WAM-2,d

Heterogeneity in vascular response to photodynamic therapy. Theresa M Busch*, E. P Wileyto*, Arjun G Yodh*, Guoqiang Yu*, Shirron Carter*, Elizabeth Rickter* and Min Yuan*. University of Pennsylvania, Philadelphia.

One component of photodynamic therapy (PDT) action is its effect on tissue vasculature. Damage to tumor vasculature will contribute to therapeutic response. Conversely, acute changes in tumor blood flow during illumination for PDT can compromise tissue oxygen supply, potentially limiting the production of cytotoxic oxygen species. It is well known that one factor mediating vasoreactivity to PDT is the treatment regimen; we are now considering the interaction of treatment regimen with regional heterogeneity in vascular histology as an effector of vascular responses during PDT. Studies were performed in intradermal radiation induced fibrosarcoma (RIF) murine tumors. Using immunohistochemical methods, tissue microenvironment was characterized by EF3 binding to label hypoxia, CD31 binding to label vascular structure, and smooth muscle actin binding to label vascular maturity. Diffuse correlation spectroscopy was employed to monitor vasoreactivity during PDT, measured as changes in tumor blood flow. The data show Photofrin-PDT at 75 mW/cm^2 (135 J/cm^2) led to greater vasoreactivity and more hypoxia in tumor regions containing more mature vascular structure. These changes were accompanied by relative decreases in cytotoxicity in the hypoxic tumor region. During low fluence rate PDT (38 mW/cm^2, 135 J/cm^2) the differential in vasoreactivity between tumor areas of differing vascular maturation was abrogated. This was accompanied by less regional heterogeneity in tumor hypoxia during PDT and no regionally-dependent protection against cytotoxicity. Regional differences in vasoreactivity during PDT at low fluence could be created if PDT was performed in a tissue with higher overall vascular maturity. These data suggest the presence of an interactive relationship between PDT regimen and vascular maturity in determining vessel response during illumination for PDT. The consequences of this interaction are relevant to therapeutic outcome as measured by differences in clonogenic survival.

WAM-3,a

UV doses worldwide. Dianne E Godar*. US Food and Drug Administration, Rockville, MD, USA.

UV affects human health. Human exposure to UV causes beneficial health effects, like vitamin D_3 formation, while it also causes detrimental health effects: sunburn, ocular damage, photoaging, immune suppression, DNA damage and mutations, and skin cancers (melanoma and non-melanoma). In countries with fair-skinned populations, skin cancer is the most diagnosed of all cancers. In the U.S., there were over one million new cases of skin cancer last year. That means 1 out of every 285 people got skin cancer. Skin cancer of fair-skinned individuals is increasing at an alarming rate (4-6% per yr) around the world and has now reached so-called pandemic proportions. Thus, it is important to know what UV doses people around the world get throughout their lives. This review briefly covers how the outdoor UV doses are weighted for different biological effects (e.g., erythema and skin cancer), the most commonly used measuring devices for terrestrial (e.g., Brewer spectrophotometers and radiometers) and personal UV doses (e.g., badges and watches), the natural and other effects on terrestrial (e.g., pollution and clouds) and personal UV doses (e.g., trees and buildings), the time people spend outside during the daylight hours (an annual average of 10%), and their ambient exposures, but will concentrate on the terrestrial and especially the personal UV doses of adult outdoor and indoor workers as well as children and adolescents around the world. Overall, outdoor-working adults around the world get about 10%, while indoor-working adults and youths get about 3% (2-4%) of the total available annual UV (relative to a horizontal plane, but real human geometry UV doses will also be discussed). People′s UV doses increase with increasing altitude and decreasing latitude (and ozone); most indoor working adult Europeans (and Canadians) get 10,000-20,000, Americans get 20,000-30,000, and Australians are estimated to get 20,000-50,000 J/m^2 per yr (excluding vacation, which can increase the dose by 30% or more).

WAM-3,b

UV radiation exposure related to age, sex, occupation, and sun behavior - based on time-stamped personal dosimeter readings. Elisabeth Thieden*, Peter Philipsen*, Jakob Heydenreich* and Hans Christian Wulf*. Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark.

OBJECTIVE: To assess individual time-related (time-stamped) UV radiation (UVR) dose pattern and sun exposure behavior. DESIGN: Open prospective observational study. STUDY SUBJECTS: Two hundred eighty-five Danish volunteers with apparently healthy skin: children, adolescents, indoor workers, sun worshippers, golfers, and gardeners (age range, 4-68 years). MEASUREMENTS: We developed a personal electronic UVR dosimeter in a wristwatch (SunSaver) and measured continuously time-related UVR doses in standard erythema dose (SED) and corresponding sun exposure behavior from diaries, resulting in 346 sun-years (median, 119 days. The estimated yearly UVR doses were calculated based on personal and ambient measured doses. RESULTS: The median estimated yearly UVR dose was 173 SEDs (range, 132 SEDs [indoor workers]-224 SEDs [gardeners]), with no significant difference by age ($p = .25$) or sex ($p = .75$). The SED of girls (175 SEDs) was significantly

(p = .04) higher than that of boys (116 SEDs). Subjects younger than 20 years had an increase of 5 SEDs per year (p = .03). Sunbathing or exposing shoulders (risk behavior) outside the beach resulted in a median of 2.5 SEDs per day in northern Europe and 3.2 SEDs per day in southern Europe; however, at the beach, corresponding values were 4.6 and 6.9 SEDs per day. Children and adolescents received more than half their total UVR dose at the beach. Sunburning doses above 10 SEDs per day were connected with sunbathing or exposing shoulders. Of the UVR dose, 50% was received between noon and 3 PM. Only the gardeners received most of their UVR dose (55%) on working days. CONCLUSIONS: High UVR doses are connected with risk behavior, except for outdoor workers. There is no need to change sun exposure habits on days without risk behavior. Only 25% of the lifetime UV dose was received before the age of 20 and the annual UV dose was thus independent of age.

WAM-3,c

A: Youth solar UV radiation exposure: A review. B: Factors influencing UV exposure of New Zealand primary schoolchildren, based on personal dosimetry. Caradee Wright[1,*], Anthony Reeder[1,*], Greg Bodeker[2,*] and Andrew Gray[3,*]. [1]Social and Behavioural Research in Cancer Group, Dunedin, New Zealand, [2]National Institute of Water and Atmospheric Research, Central Otago, New Zealand, [3]Department of Preventive and Social Medicine, Dunedin, New Zealand.

A. This presentation will review the methods used to quantify youth solar ultraviolet radiation (UV) exposure, related outdoor activities and sun protective practices. A holistic approach is essential if research is to inform the development of appropriate messages for preventive strategies to reduce excess and harmful UV exposure. Two databases were searched and 29 studies retrieved which report measurement or assessment techniques documenting UV exposure patterns and related outdoor activities. Polysulphone film badges were the main measurement instrument used in 10 studies, with questionnaire, survey data, observation, a model, electronic dosimeters, biological dosimeters, colorimeter and UV colouring labels used in the remaining studies. Methods used to record activities included self-report, parental report and observation. Measurement duration and unit of UV exposure varied in most studies, but a method common to 15 studies was measured personal UV exposure as a percentage of ambient UV. Studies are required which document precise time-stamped UV exposure, concurrent activities, sun protection usage, sun-related knowledge, attitudes and behaviours for children and adolescents. B.This study examined hypothesized relationships between solar UV exposure, concurrent outdoor activities and sun protection; sun-related knowledge, attitudes and behaviors; skin type; and school and community sun protection / skin cancer prevention efforts. The aim was to create a comprehensive research database with the goal of helping to inform health promotion interventions targeted at preventing harmful UV radiation exposure in early life. The analysis was based on quantified UV exposure from personal UV monitors and self-reported data collected from a sample of primary schoolchildren from 27 schools in 5 geographically-different regions in New Zealand. Overall, mean total daily UV exposure was 0.9 SED (1 SED = 100 Jm-2). Structural equation modeling was used to examine hypothesized causal pathways between variables and these results are discussed. This project was initiated and undertaken by the Social and Behavioural Research in Cancer Group and funded by a project grant from the Cancer Society of NZ Inc.

WAM-3,d

Do we need biological dosimetry in the skin? Antony R Young*. King's College London, London, UK.

UVR dosimetry of the skin may be approached from biological and physical standpoints. The assessment of the minimal erythema dose (MED) is the most ubiquitous biological technique in which the MED is expressed in physical units such as J/m^2. In effect, the MED uses the skin as a delayed integrating dosimeter that incorporates the action spectrum for erythema and the emission spectrum of the UVR source. The relatively new concept of the standard erythema dose (SED) incorporates these spectra based on a reference erythema action spectrum and a given emission spectrum. However, the SED cannot be readily applied to solar UVR because its spectrum is in a state of flux. It is relatively easy/feasible to make accurate spectral measurements of ambient solar UVR at a fixed geographical site. It is more difficult to determine the amount, fraction and spectral distribution of ambient UVR that reaches the surface of the skin at a given anatomical location on a free moving body. However, it is very difficult to determine these factors at a given target zone within the skin, e.g. the basal layer. This is all the more complex because the skin (i) is not a neutral density filter, (ii) has endogenous photosensitizers, (iii) reflects/scatters radiation and (iv) is subject to the influence of exogenous chromophores such as photosensitising drugs and sunscreens. This presentation will review possible options and techniques for the development of non-erythema biological dosimetry within the skin and assess their significance in risk (e.g. skin cancer) and benefit (e.g. vitamin D synthesis) evaluation.

WAM-3,e

Can personal UV Dosimeter studies be used to estimate the need for protection? Hans Christian Wulf*. University of Copenhagen, Bispebjerg, Copenhagen NV., Denmark.

Can personal UV Dosimeter studies be used to estimate the need for protection? Hans Christian Wulf, Professor, University of Copenhagen, Bispebjerg, Denmark There is a considerable discrepancy between the amount of environmental biologically weighted ultraviolet radiation (measured in SED) and the dose received by persons. The average person receives 5 SED on a summer day in Denmark and in spring in southern Europe and 10 SED in southern Europe in summer. The average Dane can tolerate 5 SED before getting erythema of the skin. The average Dane getting the average dose in Denmark in summer and in spring in southern Europe do not seem to need any sunscreen to avoid

sunburn (5/5), while in the summertime there would be a need for a factor 2-protection to avoid sunburn (10/5). However, the sensitivity of the skin is very different from person to person and erythema appears after 2 SED in sensitive persons and after up to 10 SED in insentive persons of the Danish population. The maximum dose received in spring in southern Europe is up to 30 SED per day and during the summertime up to 54 SED per day measured by dosimetry. If a very sensitive person gets that kind of dose an SPF of 27 (54/2) would be needed to avoid sunburn. This illustrates the difficulty in making personal advice about sunscreens e.g. use of shadow and other ways of reducing the exposure in daily life and during vacation. Individual calculations will be given for both different groups of workers and for different behavior.

WAM-4,a

An optical bench apparatus for wavelength-dependent photochemical characterization. Lee J Klein*, Yao Zhou*, Li Li* and Allen Templeton*. Merck & Co., Inc., West Point, PA, USA.

An optical bench apparatus for the rapid wavelength-dependent characterization of photolabile drug substances and drug products is described in detail with examples. Key components of the apparatus include high output light sources (both continuous and line sources), filters, a monochromator, associated optics, and a spectroradiometer. Figures of merit and examples of applications for studying wavelength-dependent photochemistry will be discussed.

WAM-4,b

Design of experiment approach to development of a photosensitive pharmaceutical tablet film coating utilizing spectroscopy of surrogate thin films. David J Lavrich[1,*], Yun Mao[1,*], Mary Golden[2,*], Erica Bush[1,*], William E Bowen[1,*], Charles DeLuca[1,*], Aquiles Leyes[1,*] and Saurabh Palkar[3,*]. [1]Merck and Co., Inc., West Point, PA, USA, [2]Boston University, Boston, MA, USA, [3]Johnson and Johnson Pharmaceutical Research and Development, Spring House, PA, USA.

Photosensitive tablet formulations can be protected from light by a variety of means. In the simplest case the tablets can be placed in light protective packaging. The packaging, however, provides no protection once the tablets are removed. Another more versatile method is film coating the tablets with a light protective coating. Development of the coating can require several experimental attempts utilizing a wide range of light protective excipients, excipient mixtures, excipient concentrations, and film coating thicknesses that are evaluated for a range of formulation potencies. The desire is to develop the simplest formulation with minimal processing that provides adequate light protection across a wide range of potencies. A Design of Experiment (DOE) approach is utilized to enable the development of a film coated tablet while using minimal experimental resources without sacrificing scientific understanding of the several factors under investigation. Several film coat formulations are prepared on microscope slides and are studied with spectroscopic techniques to develop a model understanding of the light protective properties of the films. This information is employed to produce light protective film coated tablets that are evaluated for efficacy by exposure in light chambers.

WAM-4,c

A case study for in-use photostability of a photosensitive IV product. Andreas Abend*, Hui Xu* and Robert Reed*. Sumneytown Pike, West Point, PA.

The spectral power distribution and illuminance in emergency care units of four Philadelphia area hospitals was determined using the OL-754 from Optronics Lab Inc. We found that all emergency rooms were illuminated by cool white fluorescent light bulbs and the brightness ranged from 300 to 2000 Lux. In addition to the fluorescent lights, some rooms were also illuminated by window light, although the spectral power distribution near the windows was dominated by the spectrum of the fluorescent light present. In addition to these primary light sources, all emergency care units were equipped with halogen spot lights. These lights produce a focused light beam, which, when focused directly into the orifice of the instrument at close range (1 foot), reached brightness levels of up to 150 kLux. The impact of the fluorescent light on the stability of a light sensitive drug available as an IV formulation was determined. When stored according to the label instructions, this drug product is very stable. When removed from the package to prepare the drug for administration, the drug product is protected by amber glass, and no significant degradation is noticeable. However, in order to infuse the drug into the patient, the drug needs to be transferred from the amber vial into an IV suitable syringe. Also, the drug is expected to be infused into the patient over a defined period of time using an existing IV line. Since both the syringe and IV tubing materials that will be used to administer the drug are typically clear, photo degradation during the drug administration process can occur. Using a photostability chamber with white fluorescent light bulbs, the propensity of this drug to degrade in the various stages of drug administration was determined. This included measuring the stability inside the amber vial, inside clear glass syringes, and when injected through IV tubing. The same studies were conducted using a halogen light source. The results of this study provided further guidance for the administration process, which suggests protecting the drug product inside the syringe from light until the administration is complete.

WAM-4,d

Gelatin-induced photoisomerization of an active pharmaceutical ingredient in formulations. Allen C Templeton[1,*], Yao Zhou[1,*], Anthony Leone[1,*], Lee Klein[1,*], Randal Seburg[2,*] and Robert Reed[3,*]. [1]Merck Research Laboratories, West Point, PA, [2]Cima Labs, Minneapolis, Minnesota, [3]XenoPort, Inc, Santa Clara, California.

It is well known that conjugated polyenes may undergo isomerization when exposed to light. The triene-containing active pharmaceutical ingredient (API), such as vitamin D3 1, undergoes cis-trans isomerization when exposed to light

at wavelengths < 330 nm. However, it was observed that this isomerization also occurs in formulations exposed to light of wavelengths well outside the absorption spectrum of 1 (330-436 nm). The cause of this unexpected photoinstability of the vitamin D3 was explored as a function of wavelength in a series of monochromatic irradiation under accelerated light exposures. The results suggest that photoisomerization of 1 is mediated by gelatin which is a fundamental component of many pharmaceutical formulations. Luminescence spectroscopy was used to further understand the role the gelatin serves in mediating the photo-isomerization.

WAM-4,e

Unexpected photochemistry in pharmaceutical products: A review on the role of diluents, excipients, and product components in promoting pharmaceutical photochemistry. Robert A Reed[1,*], Allen C Templeton[2,*], William E Bowen[2,*], Lee Klein[2,*], Paul A Harmon[2,*] and Yu Lu[3,*]. [1]XenoPort Incorporated, Santa Clara, CA, [2]Merck & Company, Inc., West Point, PA, [3]Vertex Pharmacetuicals Incorporated, Cambridge, MA.

The impact of light exposure on drug substance and drug product quality has been known for almost as much time as the modern pharmaceutical industry has been in existence. The overall impact of photostability is evident from an examination of the USP 27 (2004) Reference Table 'Containers for Dispensing Capsules and Tablets'. Of the 743 pharmaceutical products listed in the table, 248 (33%) require light resistant packaging. Clearly, developing an improved understanding of photostability would improve the ability of pharmaceutical applicants to effectively control and respond to the specific requirements of each product. We have launched substantial investigations in understanding product systems where the drug itself does not seem to be absorbing incident light, yet the product is found to be photosensitive. While there is a large and diverse body of literature noting direct degradation of pharmaceutical substances upon exposure to light, there has been only scant attention paid to the photostability of drug products where the drug is involved in the photochemistry through non-obvious mechanisms. By our estimates, approximately 15% (or 37 products) of the compounds listed as requiring light resistant packaging in the USP do not have appreciable absorption beyond 300 nm and thus beg the question: What is promoting the photo-instability of the product? The 300 nm wavelength represents a key differentiation determined by a combination of considerations of emission profiles for typical light sources and transmission properties of glasses and typical primary packaging materials. In this presentation, we review both our work and the extant literature on unexpected photochemistry in pharmaceutical products as it pertains to 1) diluent mediated, 2) excipient mediated and 3) product component mediated photochemistry. We will show that photostability testing is necessary, even when the drug molecule itself does not absorb light at wavelengths > 300 nm.

WPM-5,a

Time-resolved crystallography and signal transduction. Keith Moffat*. Department of Biochemistry & Molecular Biology, Chicago, IL, USA.

Time-resolved X-ray crystallography can now be conducted with atomic structural resolution and nanosecond to picosecond time resolution, which enables the structures of short-lived intermediates generated after absorption of a photon to be determined. This will be illustrated by our studies of PAS/LOV domain and GAF domain photosensory proteins.

WPM-5,b

DNA photolyase: An unsolved mystery of substrate suicide. Robert J Stanley*.

DNA photolyase is a monomeric flavoprotein that repairs DNA damaged by ultraviolet radiation. The DNA lesion, a cyclobutylpyrimidine dimer (CPD), is bound in a base-flipped form, in which the CPD is extracted from the DNA duplex so that repair can be facilitated by ultrafast electron transfer from the photoexcited reduced flavin cofactor. There is evidence that this electron transfer reaction takes only in the presence of substrate. We provide a model of the protein: substrate system, including ultrafast and steady-state spectroscopic data, that suggests that the protein uses the electric field of the CPD substrate to gate the electron transfer step and thus the demise (repair) of the CPD. The significance of this model is considered in the general context of electron transfer-catalyzed enzymatic reactions.

WPM-5,c

LOV domain photoreceptors in plants and bacteria. Trevor E Swartz[1,*], Tong-Seung Tseng[2,*], Marcus Frederickson[1,*], Winslow R Briggs[2,*] and Roberto A Bogomolni[1,*]. [1]University of California, Santa Cruz, Santa Cruz, CA, USA, [2]Department of Plant Biology, Stanford, CA, USA.

A new member of the PAS domain super family is the LOV domain, which functions as a light-sensory module in plant, algae, and fungal blue-light receptors. The plant blue-light receptor phototropin contains two LOV domains and a serine/threonine kinase. In *Arabidopsis*, the phototropin receptors mediate phototropism, chloroplast relocation and stomotal opening. The LOV domain is activated by a unique mechanism in which light absorption results in formation of a cysteinyl adduct (a C-S bond between the sulfur of a cysteine and a carbon of the flavin chromophore) signaling state. Many bacteria, including *Brucella melitenesis*, *Erythrobacter litoralis*, *Pseudomanas syringae* and *Xanthamonas campestris*, contain genes that code for proteins in which a LOV domain is coupled to a histidine kinase. Sensor histidine kinases are essential in environmental sensing by bacterial two-component systems, which are generally involved in gene transcription regulation. We have cloned, expressed in *E. coli* and affinity purified four LOV-domain-histidine kinase proteins (LOV-HPKs). All four LOV-HPKs bind a flavin chromophore (provided by the *E. coli* host) and undergo light-induced absorption changes typical of LOV-domain receptor modules. In addition, we have demonstrated that all four proteins act as light-activated histidine kinases. Although this strongly suggests that these proteins all function as light receptors and belong to a new family of pho-

toreceptors, the light responses these proteins mediate is unknown.

WPM-5,d

Ultrafast studies on novel blue-light photoreceptors. Cosimo Bonetti[1,*], Tilo Mathes[2,*], Ivo H. M. van Stokkum[1,*], Marie-Louise Groot[1,*], Rienk van Grondelle[1,*], Peter Hegemann[2,*] and John T.M. Kennis[1,*]. [1]Section of Biophysics, Department of Physics and Astronomy, Faculty of Sciences, Vrije Universiteit, Amsterdam, The Netherlands, [2]Institut für Biologie / Experimentelle Biophysik,Humboldt Universität zu Berlin, Berlin, Germany.

Time resolved visible pump, mid-infrared probe and time resolved fluorescence experiments have been performed on a novel class of blue-light photoreceptors, the BLUF (Blue Light sensing Using Flavin) domain family. Time-resolved fluorescence experiments on the Slr1694 BLUF domain from *Synechocystis* revealed four components of flavin adenine dinucleotide (FAD) excited-state decay, with lifetimes of 6 ps, 26 ps, 90 ps, and 340 ps. No kinetic isotope effect on the excited-state lifetime was observed for BLUF domains dissolved in D_2O buffer, indicating that the fluorescence of flavin is quenched by photo-induced electron transfer by a conserved tyrosine residue. In ultrafast transient absorption experiments, excitation at 470 nm triggers the reaction that leads to signaling state formation; the structural changes between flavin molecule and side chain residues inside the protein are followed by monitoring in time the difference absorption over a mid-IR spectral window from 1800-1200 cm^{-1}. We find that the long-lived signaling state is formed on a time scale of 300 ps. In its spectra a down shift of the carbonyl bands appears, indicating a weakening of the C4=O and C2=O stretches that results from a formation of H-bonds to these groups. Beside the carbonyl bands, a strengthening of C=N stretch vibrations is recorded. Our results support a model where light-induced electron transfer causes a hydrogen-bond rearrangement, whereby the hydrogen bonds from the amino moiety of a highly conserved glutamine to tyrosine and the flavin N5 are broken, followed by a 180° glutamine flip and formation of new hydrogen bonds between the glutamine and an FAD carbonyl group.

WPM-6,a

An action spectrum for UVR-induced skin cancer. Paul D Forbes*. Charles River Laboratories, Inc, Horsham, PA.

Based on data from several dozen studies using a variety of light sources at two research institutes, the SCUP-m action spectrum for UVR-induced photocarcinogenesis was identified as the appropriate weighting function to describe the proportional contributions of wavelengths throughout the UVR (250-400nm region) to skin cancer induction in albino hairless mice. This action spectrum subsequently became the basis for risk based weighting function for human exposure to UVR sources (CIE Collection in Photobiology and Photochemistry; 2000 Non-melanoma Skin Cancer; 138/2 CIE TC 6-32). Several of the original studies utilized a xenon arc lamp which was carefully characterized, but from which a small amount of scattered radiation could only be estimated. The details of the stray radiation have been determined through the use of modern specdtroradiometric equipment. An evaluation of the source spectra with and without the stray radiation shows that there is no significant impact of this stray radiation on the original conclusions regarding the shape of the SCUP-m photocarcinogenesis action spectrum, and that a comprehensive analysis demonstrates the validity of the SCUP-m action spectrum for UVR-induced skin cancer.

WPM-6,b

Effects of topical exposure to Aloe vera plant extracts on the photocarcinogenicity of simulated solar light in SKH-1 mice. Mary D Boudreau[1,*], Paul W Mellick[2,*], Barbara J Miller[1,*], Paul C Howard[1,*] and Frederick A Beland[1,*]. [1]Division of Biochemical Toxicology and the National Toxicology Program Center for Phototoxicology, Jefferson, Arkansas, U.S.A., [2]Toxicologic Pathology Associates, Jefferson, Arkansas, U.S.A.

A 52-week study was conducted to determine if the topical application of creams containing 3% or 6% (wt/wt) Aloe vera plant components or 0.56 μg or 56.0 μg Aloe-emodin, an aglycone of Aloe vera, would enhance the carcinogenicity of simulated solar light (SSL) delivered by filtered 6.5 kW xenon arc lamps to SKH-1 hairless mice. The levels of SSL used were 0-, 6.85-, 13.7-, and 20.55-mJ•CIE/cm^2. The Aloe vera plant components included extracts of the inner leaf gel, the whole leaf, and charcoal-filtered whole leaf. Cream formulations were applied to the dorsal skin of mice in the morning, and SSL was administered in the afternoon 5 days/week for a period of 40 weeks. A 12-week post-exposure observation period was followed by euthanasia, a complete necropsy, and microscopic examination of all skin lesions for histopathology. The incidence (number of tumor-bearing animals) of squamous cell neoplasms of the skin of SKH-1 mice was highly dependent on exposure to SSL. Except for one squamous cell papilloma, no squamous cell neoplasms developed in groups that were not exposed to SSL. In male and female mice exposed to 13.7-mJ•CIE/cm^2, the incidence of squamous cell papillomas was higher in all groups treated with Aloe vera plant extracts compared to the vehicle control group. In male mice, but not in female mice, there was a trend toward increased incidence of squamous cell carcinomas associated with groups treated with Aloe vera plant extracts. In both male and female mice exposed to 13.7-mJ•CIE/cm^2•, the multiplicity (average number of neoplasms per animal) of squamous cell papillomas and carcinomas in situ was higher in groups treated with Aloe vera plant extracts compared with the vehicle control. There was no significant difference among the plant extracts in the multiplicity of these neoplasms. (Views expressed in this abstract do not necessarily reflect those of the FDA).

WPM-6,c

Skin cancer and photobiological studies in Puerto Rico. Jamie L Matta*. Ponce School of Medicine, Ponce, PR.

During the last six years, we have conducted in Puerto Rico (PR) a large case-control study in the molecular epi-

demiology of non-melanoma (NMSC) and melanoma skin cancer involving nearly 1,000 participants. The focus of this study has been to examine the hypothesis that reduced DNA repair capacity (DRC) increases the risk to melanoma and non-melanoma skin cancers (NMSC). Data from clinical case-controlled retrospective studies is stratified by DNA repair level and adjusted for age, skin type, familial history of skin cancer and severe sunburns in a lifetime in order to calculate odds ratios for key skin cancer risk factors. Persons with NMSC have a statistically significant reduction of 42% in the age-adjusted DRC. For every 1% reduction in DRC (in relation to controls), the risk of NMSC increases 21% and the risk developing melanoma increases 11%. We have also measured environmental UVA and UVB levels over a six year period. This large-scale population study provides evidence that varying doses of UV radiation have a profound influence on key characteristics of NMSC. We are also undertaking a study to examine by means of gene microarrays the differential expression of DNA repair and other genes that are involved directly or indirectly with tumorigenesis in melanoma. We are also using microarray technology to identify specific genes associated with the genetic progression of melanoma both in Florida and in Puerto Rico. Finally, polymorphisms in codon 751 of the XPD gene are being studied to explain the early onset of basal cell carcinoma in PR.

WPM-6,d

Role of coat color in UV-carcinogenesis of adult mice. F M Strickland[1,*], Glen Merlino[2,*], Lynn Lamoreux[3,*] and Frances Noonan[4,*]. [1]Department of Dermatology, Detroit, MI, [2]National Institute of Health, Bethesda, MD, [3]Texas A&M University, College Station, TX, [4]George Washington University, Washington, DC.

Chronic treatment of adult agouti C3H/HeN mice with a combination of UV radiation and ethanol has been shown to induce primary skin tumors, including squamous cell carcinomas, fibrosarcomas an melanomas. In the present study, we investigated the influence of genes controlling pigmentation in skin cancer development in our model. Congenic pigment mutants were generated on the C57BL/6 genetic background and intercrossed with the C57BL/6-[Tg]HGF and C57BL/6-[Tg]Dct:LacZ transgenic strains. The Dct:LacZ transgene is expressed in melanocytes and their precursors and enables us to identify these cells and follow their development during chronic UV irradiation. C57BL/6 animals with the following genotypes and coat colors were studied: A/A (agouti), -a/a (black), e/e (yellow), E/e (black) and parallel groups containing the HGF transgene. Beginning at 8 weeks of age the dorsal fur of the animals was shaved and the animals were exposed three times per week for 20 weeks to 10 kJ/m2 UVB from FS40 sunlamps (60% UVB 40% UVA, 0.5% UVC). The fur was removed weekly or as needed. Parallel groups were treated with a combination of 25% ethanol/water and UV radiation. The ethanol was applied to the UV-irradiated skin immediately after each UV exposure. A/A, a/a, and E/e mice developed skin tumors after approximately 12 weeks of UV treatment. In contrast mice that possessed the HGF transgene and darkly pigmented skin (a/a and E/e) failed to develop skin tumors, suggesting that the high levels of eumelanin were protective. Yellow mice (e/e) developed a few benign pigmented lesions but failed to develop either melanoma or non-melanoma skin cancers in this study. The results suggest that genes controlling pigmentation can influence UV carcinogenesis with greater complexity that previously appreciated.

WPM-6,e

Preliminary analyses on the efficacy of Celecoxib, a COX-2 inhibitor, in the development of cutaneous malignant melanoma in the HGF/SF transgenic melanoma mouse model. Ed De Fabo*, F P Noonan* and J Bahn*. Laboratory of Photobiology & Photoimmunology, Washington, DC.

Cyclooxygenase-2 inhibitors have shown efficacy in the treatment of some types of cancers including skin cancer. To study whether or not Celecoxib (Celebrex; a cyclooxygenase-2 inhibitor) can affect the development of cutaneous malignant melanoma in an experimental animal model (HGF/SF transgenic melanoma mouse model) celecoxib was administered at a dose of 750 mg/kg either 6 weeks after initiation by UV radiation at a UV dose known to induce melanoma (Early Celecoxib Group) or 270 days after UV exposure (Late Celecoxib Group). Identical control groups (no celecoxib but UV irradiated) were also included in the study. Celecoxib was mixed with and administered in powdered form (Purina mouse chow 5001) ad libitum. Animals were observed 7 days per week and shaved approximately every 2 weeks throughout the experiment and observed for lesion development. Any lesion arising was removed when the lesion reached 1 cm in any dimension and placed in formalin (10%). Any lesion found on an animal which expired before termination (14 months) was excised and placed in 10% formalin as well. At the conclusion of this 2 year study preliminary analyses of the first observable lesion, recorded as melanoma or not a melanoma based upon previous melanoma development in this mouse model, was carried out. Kaplan-Meier survival analysis was applied to the data. Presently, the data indicate no significant difference in lesion development between animals given celecoxib and UV, either early or late, versus control food (no celecoxib and UV). Although histopathology has not been completed at this time, it appears that, based on observable lesion development in this animal model, celecoxib was not efficacious in the blocking the development of UV-induced lesions in either early or late treatment groups.

WPM-7,a

Photodynamic therapy stimulates anti-tumor immunity in murine models. Michael R Hamblin[1,2,3,*], Ana P Castano[1,2,*] and Pawel Mroz[1,2,*]. [1]Wellman Center for Photomedicine, Boston, MA, USA, [2]Department of Dermatology, Boston, [3]Harvard-MIT Division of Health Science and Technology, Cambridge.

Cancer is a leading cause of death among modern peoples largely due to metastatic disease. The ideal cancer treatment should target both the primary tumor and the metastases with the minimal toxicity. This is best accomplished by educating

the body's immune system to recognize the tumor as foreign so that after the primary tumor is destroyed, distant metastases will also be eradicated. Photodynamic therapy (PDT) involves the IV administration of photosensitizers followed by illumination of the tumor with red light producing reactive oxygen species that cause vascular shutdown and tumor cell apoptosis. Anti-tumor immunity is stimulated after PDT due to the acute inflammatory response, priming of the immune system to recognize tumor-specific antigens, and induction of heat-shock proteins. Nevertheless the immune response after PDT is variable and sub-optimal, and the determinants that affect its efficiency are imperfectly understood. Mice are an ideal model to study this process due to the availability of genetically altered strains and a variety of tumor types. We will describe three strategies that can both potentiate immunity and provide valuable information on mechanisms involved. (I) Tumor cells can be transduced to express foreign proteins that act as model tumor associated antigens such as green fluorescent protein, ovalbumin, b-galactosidase and firefly luciferase. PDT can then cause dendritic cells to present these antigens to T-cells. (II) A class of immune suppressor cell known as CD4+CD25+ T-regulatory cells that can inhibit cytotoxic T-cells from destroying the tumor can be depleted using either low-dose cyclophosphamide or an anti-CD25 antibody in combination with PDT. (III) PDT can be combined with non-specific immune adjuvants that are frequently derived from microbial products and are generally agonists of toll-like receptors, to increase inflammation and immune response after PDT. Evidence of immune response after combination PDT treatments of mouse tumors is provided by: (I) a more effective local tumor response to PDT; (II) rejection of a subsequent tumor rechallenge in cured mice; (III) demonstration of lymphocyte recognition and destruction of tumor cells ex vivo; (IV) regression of distant untreated tumors in mice bearing multiple tumors only one of which received PDT.

WPM-7,b

Photodynamic therapy generates anti-tumor immunity against a beta-galactosidase expressing tumor but not its wild-type counterpart. Pawel A Mroz* and Michael R Hamblin*. Wellman Center for Photomedicine, Boston, MA, USA.

Photodynamic therapy can stimulate anti-tumor immunity against cancer by increasing expression of cytokines and promotion of immune recognition of the tumor cells. Identification of tumor associated antigens (TAA) recognized by CD8+ T cells and the corresponding major histocompatibility complex class I (MHC-I) restricted epitopes was a groundbreaking step in cancer immunology. TAA-specific CD8+ T cells represent an important component of the host's immune response against malignant diseases. The goal of this study was to investigate application of PDT to an established TAA model. Lacking a well defined murine TAA, we utilized a beta-galactosidase (beta-gal, a bacterial protein) transduced colon tumor cell line CT26.CL25 that stably expressed beta-gal as well as its class I MHC restriction element H-2Ld syngeneic to Balb/c mice. PDT with a regimen of 1mg/kg BPD IV, and 120 J/cm2 690-nm after 15 minutes successfully cured both tumor types with 100% effectiveness. After 90 days tumor free interval the mice were rechallenged with the identical tumor and CT26.CL25 cured mice rejected the rechallenge while naive and CT26 cured mice demonstrated tumor progression. The CT26.CL25 cured mice did not reject CT26 wild type tumor cells. Experiments with mice bearing two CT26.CL25 tumors (one in each leg) and only one tumor treated with PDT, showed that the immune response was strong enough to destroy an already established tumor. Cytotoxic T lymphocytes removed from lymph nodes from cured mice are able to recognize and destroy tumor cells in vitro demonstrating a long lasting tumor-specific immunity.

WPM-7,c

Intratumor induced inflammation generates maximally activated macrophages that can eradicate cancerous cells. Nobuto Yamamoto*. Socrates Institute For Therapeutic Immunology, Philadelphia, PA.

Photodynamic action on mammalian tissues immediately results in severe inflammation, leading to macrophage activation. Intratumor PDT-induced inflammation generates maximally activated macrophages that can eradicate local as well as metastasized cancerous cells. Inflammation in mammalian tissues activates phospholipase A2 to releases lysophospholipids that efficiently activate macrophages. Because cancerous tissues contain alkylphospholipids, PDT-induced inflammation of cancerous tissue produces alkyl-lysophospholipids and alkylglycerols that activate macrophages with approximately 400 times more efficiency than lysophospholipids. These results imply that highly activated macrophages can kill cancerous cells. Inflammation-primed macrophage activation process is the principal macrophage activation cascade that requires serum vitamin D3-binding protein (known as Gc protein) and participation of B and T lymphocytes. Stepwise hydrolysis of Gc protein with the inducible membranous beta-galactosidase of inflammation-primed B cells and the membranous Neu-1 sialidase of T cells yields a potent macrophage activating factor (MAF), the protein with N-acetylgalactosamine as the remaining sugar. Thus, Gc protein is the precursor for the principal MAF. Stepwise treatment of highly purified Gc protein with immobilized beta-galactosidase and sialidase generated the most potent macrophage activating factor (termed GcMAF) ever discovered which produces no side effect in humans. Administration of 100 ng GcMAF per human and 100 pg GcMAF per mouse results in the maximal activation of macrophages, which develop enormous variation of receptors. When human macrophages were treated in vitro with GcMAF (100 pg/ml) for 3 hrs, the macrophages were highly activated. The activated macrophages can recognize and kill a variety of cancerous cells indiscriminately. When prostate and breast, cancer patients were treated with less than 25 weekly administrations of 100 ng GcMAF, the majority of cancer patients, excluding anemic patients, exhibited healthy control levels of the serum prognostic index, alpha-N-acetylgalactosaminidase, indicating eradication of the tumors. GcMAF therapy also develops antibodies against the tumors.

WPM-7,d

Efferocytosis after tumor PDT and its impact. Mladen Korbelik*. British Columbia Cancer Agency, Vancouver, B.C., Canada.

Treatment of solid cancers by photodynamic therapy (PDT) elicits a strong host response prompted by rapidly-induced massive tumor tissue injury, which culminates in the development of adaptive immune response recognizing the treated lesion as its specific target. One of the key tasks of tumor PDT-elicited host response is efferocytosis (dead cell disposal) because the host is suddenly (within a short time after PDT treatment) faced with a threatening burden of a large number of dead cells in the targeted lesion. Efferocytosis has a direct influence on the resolution of PDT-associated inflammation, and it is increasingly evident that it has a critical impact on the establishment of the adaptive immunity against PDT-treated tumors. Efferocytes (primarily macrophages and dendritic cells) have a variety of receptors that can be engaged in phagocytosis of cellular corpses. Intracellular antigens of dying tumor cells (normally hidden to immune surveillance elements) are known to survive this phagocytic process, and are, particularly at high antigen loads, effectively cross-presented to T cells. This communication will discuss eat me signals displayed on necrotic and apoptotic cells in PDT-treated tumors, innate immune pattern recognition receptors (PRRs) that recognize them, and receptors on efferocytes that interact with PRRs and are associated with the ingestion of dead cell corpses. Elaborated will be the approach aimed at intervening in this process to alter the engagement of particular PRRs or even efferocyte populations involved and the potential impact this can have on tumor antigen recognition and eventual outcome of tumor PDT.

WPM-7,e

Neutrophils play a critical role in activation of tumor specific CD8+ T Cell responses. Sandra O Gollnick*, Philaretos C Kousis* and Barbara W Henderson*. PDT Center, Dept. Cell Stress Biology, Buffalo, NY, USA.

Tumor specific immune responses are frequently suppressed in tumor bearing hosts and a primary goal of immunotherapy is to overcome this suppression and stimulate effective anti-tumor immunity capable of controlling the growth of primary and disseminated disease. Simulation of acute local inflammation by genetic manipulation to create tumors that express pro-inflammatory cytokines and stimulate leukocyte infiltration can lead to enhanced anti-tumor immunity, but this method is time consuming and difficult to implement in the clinic. Photodynamic therapy (PDT) is an FDA approved therapy for the treatment of early disease and palliation of advanced disease that induces acute local inflammation at the tumor site in a regime dependent manner. High local levels of pro-inflammatory cytokines and rapid infiltration of the tumor by host leukocytes characterize acute local inflammation induced by PDT. We have used PDT to determine the mechanisms by which acute inflammation enhances anti-tumor specific immune responses. We show that induction of acute local inflammation by PDT treatment of murine tumors results in the generation of increased numbers of activated tumor specific CD8+ effector T cells. This increase in effector T cells is accompanied by an increased ability to control tumor growth and the formation of a strong anti-tumor immune memory response. We further show that the enhancement of tumor specific CD8+ effector T cell numbers and function is dependent upon the ability of neutrophils to access the treated tumor; mice depleted of neutrophils or that have defective neutrophil migration did not exhibit enhanced effector T cell responses after induction of acute local inflammation by PDT. These studies demonstrate that induction of acute local inflammation by PDT can result in enhanced numbers of activated CD8+ effector T cells and that enhancement of tumor specific immune responses by PDT is dependent upon neutrophil access to the tumor site. We propose that treatment regimes that induce local acute inflammatory responses can be devised to enhance anti-tumor immunity and to control disseminated disease.