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Abstracts

18.1 Ablonczy, Z*; Herrmann, J; Pallitto, P; Drake, RR; Koutalos, Y; Crouch, RK; Donello, J; Department of Ophthalmology, Medical University of South Carolina, Charleston, SC, USA, Allergan, Inc., Irvine, CA, USA, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ, USA, Department of Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, USA; ablonczy@musc.edu

The accumulation of lipofuscin and A2E depend on retinal anatomy

Both lipofuscin and its major component, A2E, has been implicated in retinal pigment epithelium (RPE) phototoxicity in vitro. In mice, lipofuscin and A2E exhibit a high spatial correlation increasing with the amount of A2E; however in humans, they do not co-localize. Therefore, we set out to determine the relationship of lipofuscin and A2E distributions in species with distinct retinal anatomies. RPEs from rabbits and macaques were mounted flat onto conductive slides and autofluorescence (AF) was imaged with a Maestro 2 hyperspectral imaging system. After coating with matrix (sinnapinic acid in 80% ethanol), the tissues were analyzed in a Bruker Autoflex II TOF-TOF mass spectrometer at a 25-250 μm spatial raster in the range of m/z 250-2500. The comparison of AF and mass spectrometric images revealed remarkable differences in lipofuscin, A2E, and lipid distributions between the species. Rabbits have a merangiotic retina. In rabbits, lipofuscin accumulated most in the middle of the visual streak, while A2E was highest in the far periphery and decreased toward the visual streak in a linear manner. Macaques have a fovea. In macaques, lipofuscin was markedly highest in the perimacula, while A2E was most abundant in the far periphery and decreased toward the perimacular area in a concentric fashion. In both species, lipofuscin broadly correlated with literature reports on vascular and photoreceptor density and A2E exhibited a distinctly inverse relationship. Taken together with our previous data on mice and humans, these results support the idea that lipofuscin accumulates in the RPE adjacent to intense retina metabolism while elevated levels of A2E represent retinal areas with a reduced metabolic demand. However, it is still unclear whether bisretinoids accumulate in the RPE in consequence of higher rates of formation or lower rates of breakdown in the periphery.

15.2 Adar, S*; Hu, J; Lieb, JD; Sancar, A; University of North Carolina; sheera@email.unc.edu

DNA repair genomics: Mapping DNA damage and DNA repair at single-nucleotide resolution across the human genome

Ultraviolet radiation induces pyrimidine photodimers in DNA that present a barrier to transcription and replication, and compromise the ability of a cell to function. Nucleotide excision repair is the sole mechanism for removing these damages from the human genome. During human excision repair, dual incision of the damaged strand results in removal of a ~27 nucleotide-long single stranded oligomer. We have recently developed two genomic methods for mapping DNA damages and DNA repair at single nucleotide resolution across the human genome. Damages-seq relies on the replication-blocking properties of the damages to precisely map their location. In eXcision Repair-seq (XR-seq) we capture the excised oligonucleotide released during repair in vivo, and subject it to high-throughput

sequencing. We used Damage-seq and XR-seq to produce genome-wide maps of two UV-induced DNA damages and follow the kinetics of their repair: cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts ((6-4)PPs). Our results show preferential repair of actively transcribed and open chromatin regions. Conversely, repair at heterochromatic and repressed regions is relatively low and continues even two days following UV irradiation. Comparing repair kinetics with existing somatic mutation data from melanoma cancer cells shows late-repaired regions are associated with a higher level of cancer-linked somatic mutations. The new genomic assays we have developed will be a powerful tool in identifying key components of genome stability, and understanding the genetic and epigenetic changes resulting from genotoxic stress.

**6.5 Adar, S*; Hu, J; Lieb, JD; Sancar, A; University of North Carolina; sheera@email.unc.edu
*DNA repair genomics: Mapping DNA damage and DNA repair at single-nucleotide resolution across the human genome***

Ultraviolet radiation induces pyrimidine photodimers in DNA that present a barrier to transcription and replication, and compromise the ability of a cell to function. Nucleotide excision repair is the sole mechanism for removing these damages from the human genome. During human excision repair, dual incision of the damaged strand results in removal of a ~27 nucleotide-long single stranded oligomer. We have recently developed two genomic methods for mapping DNA damages and DNA repair at single nucleotide resolution across the human genome. Damages-seq relies on the replication-blocking properties of the damages to precisely map their location. In eXcision Repair-seq (XR-seq) we capture the excised oligonucleotide released during repair in vivo, and subject it to high-throughput sequencing. We used Damage-seq and XR-seq to produce genome-wide maps of two UV-induced DNA damages and follow the kinetics of their repair: cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts ((6-4)PPs). Our results show preferential repair of actively transcribed and open chromatin regions. Conversely, repair at heterochromatic and repressed regions is relatively low and continues even two days following UV irradiation. Comparing repair kinetics with existing somatic mutation data from melanoma cancer cells shows late-repaired regions are associated with a higher level of cancer-linked somatic mutations. The new genomic assays we have developed will be a powerful tool in identifying key components of genome stability, and understanding the genetic and epigenetic changes resulting from genotoxic stress.

P2.12 Alabugin, IV*; Roy, S; Breiner, B; Yang, WY; Mohamed, R; De Faria, T; Florida State University; alabugin@chem.fsu.edu

Alkyne photochemistry for double DNA-cleavage and aldehyde release

We present two C1-C5 cycloaromatization reactions enabled by the high energy of alkyne functionality. The C1-C5 photocyclization of enediyne leads to four H-atom transfers from the environment and, thus, can be utilized for the design of efficient reagents for double-strand DNA photocleavage. The ratio of double- (ds) to single-strand (ss) DNA damage by hybrid molecules that combine enediynes with lysine and related basic peptides and amino acids exceeds the ds:ss ratios provided by the natural enediynes, the most potent family of anticancer agents in Nature. Furthermore, the reactivity towards DNA and the ds:ss ratio are increased dramatically at the lower pH of hypoxic cancer tissues. This increase is based on the new photophysical approach to selective DNA photodamage based on pH-gated photoinduced electron transfer between an appropriately positioned amino group and the DNA-damaging "warhead". [1] On the other hand, the newly discovered "self-terminating" C1-C5 photocyclization of enynes leads to photorelease of formaldehyde and related carbonyl compounds. [2] 1. Breiner, B.; Kaya, K.; Roy, S.; Yang, W.-Y.; Alabugin, I. V. Hybrids of Amino Acids and Acetylenic DNA-Photocleavers: Optimizing Efficiency and Selectivity for Cancer Phototherapy. Invited "Perspective". *Org. Biomol. Chem.* 2012, 10, 3974-3987. 2. The Missing C1-C5 Cycloaromatization Reaction: Triplet State Antiaromaticity Relief and Self-Terminating Photorelease of Formaldehyde for Synthesis of Fulvenes from Enynes. Mohamed, R. K.; Mondal, S.; Jorner, K.; Faria Delgado, T.; Ottosson, H. *J. Am. Chem. Soc.*, 2015, 137, 15441-15450.

12.2 Alex, A; Li, A; Men, J; Jerwick, J; Tanzi, RE; Zhou, C*; Lehigh University, Massachusetts General Hospital; chaozhou@lehigh.edu

An Integrated Optical Coherence Microscopy Imaging and Optical Stimulation System for Optogenetic Pacing in Drosophila melanogaster

Electrical stimulation is the clinical standard for cardiac pacing. Although highly effective in controlling cardiac rhythm, the invasive nature, non-specificity to cardiac tissues and possible tissue damage limits its applications. Optogenetic pacing of the heart is a promising alternative, which is non-invasive and more specific, has high spatial and temporal precision, and avoids the shortcomings in electrical stimulation. *Drosophila melanogaster*, which is a powerful model organism with orthologs of nearly 75% of human disease genes, has not been studied for optogenetic pacing in the heart. Here, we developed a non-invasive integrated optical pacing and optical coherence microscopy (OCM) imaging system to control the heart rhythm of *Drosophila* at different developmental stages using light. The OCM system is capable of providing high imaging speed (130 frames/s) and ultrahigh imaging resolutions (1.5 μm and 3.9 μm for axial and transverse resolutions, respectively). A light-sensitive pacemaker was developed in *Drosophila* by specifically expressing the light-gated cation channel, channelrhodopsin-2 (ChR2) in transgenic *Drosophila* heart. We achieved non-invasive and specific optical control of the *Drosophila* heart rhythm throughout the fly's life cycle (larva, pupa, and adult) by stimulating the heart with 475 nm pulsed laser light. Heart response to stimulation pulses was monitored non-invasively with OCM. This integrated non-invasive optogenetic control and in vivo imaging technique provides a novel platform for performing research studies in developmental cardiology.

PI.21 Anand, S*; Thomas, E; Hasan, T; Maytin, EV; Cleveland Clinic, Harvard Medical School; anands@ccf.org

Oral Vitamin D3 As A Neoadjuvant For Combination Photodynamic Therapy (cPDT): Preclinical Studies In A Murine Model To Predict The Therapeutic Response In Vitamin D3 Deficient Human Populations

Combination PDT (cPDT), in which the tumor is preconditioned with a differentiation-promoting agent prior to ALA-PDT, is a promising and evolving approach for the treatment of non-melanoma skin cancers. We have shown that a cPDT approach using the active hormonal form of vitamin D3 (calcitriol) given systemically prior to PDT, resulted in elevated protoporphyrin (PpIX) levels and subsequent PDT-induced cell death in A431 SCC tumors. However, since calcitriol may pose a risk for hypercalcemia in humans, we replaced calcitriol with natural dietary vitamin D3 (D3; 10-fold) fed over a 10-day period. In this study, we asked whether vitamin D3 deficiency might alter the PpIX-elevating response. Nude mice were fed with a D3 deficient diet for 4, while control mice were fed the normal diet. Human A431 SCC cells were implanted subcutaneously, and the mice were then either switched to a 10K diet or given a HED of 50,000 IU D3 by oral gavage. Tumors and tissues including skin and serum (to measure vitamin D3 levels) were collected for analyses. Following D3 supplementation, a tumor-selective increase in the levels of PpIX and in expression of markers of differentiation (E-Cadherin) was observed in both the D3-deficient and the normal control mice. As compared to other methods of VD delivery, a pretreatment regimen involving 10 days of a 10K diet, the response was similar in VD-deficient and normal mice with respect to the PpIX levels in the tumors, whereas 3 days of a 10K diet was insufficient to bring PpIX levels to the same level as in normal mice. Serum levels of D3 and its metabolic intermediates, and expression levels of heme- and vitamin D3-metabolic enzymes in tumors, skin, and other tissues were analyzed and were supportive of the PpIX induction results. These data suggest that a clinical study using oral vitamin D3 as a neoadjuvant is a new promising approach that could be effective for the treatment of human skin cancer in both normal and VD-deficient populations.

17.4 Anand, S*; Maytin, EV; Cleveland Clinic; anands@ccf.org

Lack Of Tumor Development In C/EBP β Knockout Mice After UVB Exposure Involves Increased Apoptosis And Decreased EGFR And ERK1/2 Expression And Phosphorylation

The CCAAT/Enhancer Binding Proteins (C/EBPs) are a family of six (C/EBP α , β , γ , δ , ϵ , and ζ) leucine zipper transcription factors that control normal functions such as regulation of cell cycle, metabolism and differentiation. Recently, the involvement of these factors in cancer, inflammation, apoptosis and ER stress has been established. In current study we investigated the role of C/EBP β in carcinogenesis induced by sun exposure. Although, C/EBP β has been involved in the regulation of skin development and differentiation, its role in skin carcinogenesis is not clear. To investigate the role of C/EBP β in UVB-carcinogenesis, C/EBP β knockout mice (C/EBP β KO), along with their heterozygote (Het) and wild type (WT) littermates, were exposed to UVB (progressively increasing doses up to 175 mJ/cm² UVB, 3 times a week) for 20 weeks. At week 25, each WT mice had developed many tumors, whereas C/EBP β KO mice completely lacked tumor development. As anticipated, Het mice had an intermediate response. To characterize the short-term events following UVB exposure that would eventually contribute to the tumor phenotype, mice were exposed to UVB (100 mJ/cm²) and skin was harvested at different times for analyses by immunohistochemistry and western blot. An enhanced apoptosis response (TUNEL and Caspase-3 cleavage) and elevated expression levels of TNF α were observed in C/EBP β KO epidermis. Additionally, the expression and phosphorylation of EGFR and ERK1/2 were down regulated in C/EBP β KO epidermis, as compared with their WT littermates. The observation of enhanced apoptotic response via the extrinsic pathway involving TNF α upregulation and compromised EGFR and ERK1/2 signaling were postulated as the mechanism underlying the lack of tumor response in C/EBP β KO mice. Our results suggest that in addition to its well-established functions in skin physiology, C/EBP β may also play an important role in skin carcinogenesis involving the regulation of apoptotic and survival pathways.

4.3 Anbil, S*; Rizvi, I; Baglo, Y; Broekgaarden, M; Maytin, E; Hasan, T; Howard Hughes Medical Institute, Harvard Medical School, Harvard Medical School, Massachusetts General Hospital, Wellman Center for Photomedicine, Cleveland Clinic Lerner Research Institute; anbil.sriram@mgh.harvard.edu

Biomodulation of metabolic and signaling pathways to enhance photodynamic therapy efficacy for pancreatic adenocarcinoma and oral squamous cell carcinoma

Vitamin based biomodulation of metabolic and paracrine-signaling pathways represents a promising approach to overcome tumor heterogeneity and poor treatment selectivity. Among these approaches includes biomodulation of the heme pathway with calcitriol to increase accumulation of protoporphyrin IX (PpIX), the photosensitizer responsible for the cytotoxic effect of aminolevulinic acid (ALA)-based photodynamic therapy (PDT). Because cancer associated desmoplasia has been implicated in treatment resistance in several contexts, an opportunity exists to increase the susceptibility of these tumors to PDT through stromal rehabilitation while also improving the therapeutic index via heme pathway biomodulation. We investigate the ability of the vitamin hormones calcipotriol or retinoic acid to serve as dual-purpose biomodulatory and stromal reprogramming agents to enhance PDT efficacy in context of oral squamous cell carcinoma (OCA) and pancreatic ductal adenocarcinoma (PDA). OCA is highly prevalent in low resource settings where few feasible modalities exist for early detection and treatment. In contrast, PDA is frequently detected at late stage and characterized by a unique milieu comprising activated mesenchymal cells that facilitate tumor progression. Our approach has significance in both contexts: it may improve the theranostic potential of low-cost image guided PDT for OCA, and may enable synergy between PDT and other chemo/biologic therapies for PDA via modulation of tumorigenic signaling pathways. Using in-vitro and in-vivo approaches, we assess the ability of vitamin hormones to improve the selectivity and homogeneity of PpIX accumulation in OCA and PDA. In 2D and 3D cultures, we assess the ability of calcipotriol and retinoic-acid to induce quiescence in activated fibroblast lines, and assess the impacts of this "reprogramming" in PDA lines. A combined biomodulatory and reprogramming approach to improve PDT efficacy in PDA lines is tested.

4.1 Arnaut, LG*; Pereira, MM; Dabrowski, JM; Schaberle, FA; Rocha, LB; Gomes-da-Silva, L; Calvete, MJF; Soares, HT; Silva, AD; Lobo, ACS; University of Coimbra, Portugal, Jagiellonian University, Poland, Luzitin SA, Portugal; lgarnaut@ci.uc.pt

Photodiagnosis and Photodynamic Therapy: Can They Ever Be Combined In The Same Molecule?

The new frontiers of cancer detection and treatment demand the visualization of diseased tissue with millimetric precision. Screening requires that diagnostic techniques are also affordable, portable and minimally invasive. Fluorescence imaging fulfills these requisites but requires fluorophores that target tumors with high NIR absorbance, intense fluorescence, large Stokes shifts, photostability and low toxicity. We disclose a deformed silicon phthalocyanine absorbing at 743 nm and emitting at 759 nm, and show that it accumulates in 4T1 cells implanted in a mammary gland of BALB/c mice. Fluorescence from 1 mm tumors was observed 30 min post-injection. PDT also benefits from sensitizers that target tumors, have high NIR absorbance and low photodecomposition, but they must also efficiently generate ROS. We have shown that a halogenated bacteriochlorin (named redaporfin) offered 86% cure rates of BALB/c mice bearing CT26 tumors. The phthalocyanine and redaporfin differ mostly in phototoxicity. Switching phototoxicity ON and OFF is discussed.

10.2 BÃ©rubÃ©, R*; Drigeard Desgarnier, MC; Rochette, PJ; University Laval and Centre Hospitalier Universitaire de Quebec Research Center; roxanne.berube@hotmail.com

Persistence Of DNA Damage Induced By Chronic UVB Irradiation In The Human Genome

Exposure to UVB rays is a major risk factor in skin cancer initiation. In fact, UVB wavelengths are responsible for the formation of cyclobutane pyrimidine dimers (CPD), a pre-mutagenic damage, that lead to the C ? T and CC ? TT mutations found in non-melanocytic skin cancer. Recently, we have shown that a chronic irradiation with low dose of UVB (CLUV) leads to the formation of CPD that remains unrepaired (residual CPD). We then aim to determine the distribution, the localization and the impact of those residual CPD on the human genome. Four different cultures of human diploid fibroblasts were irradiated using a precise CLUV. Metaphase spreads were prepared from the irradiated cells and CPD were revealed. Chromosomes were counted and classified according to the number of sister chromatids per chromosome containing CPD (0, 1 or 2). We observed that residual CPD are tolerated in the genome and are diluted through cellular division. To localize residual CPD in the genome, we have optimized a chromatin immunoprecipitation (ChIP) protocol. Two fractions were obtained using an anti-histone 3 acetyl lysine 9 for euchromatin and anti-histone 3 tri methyl lysine 9 for heterochromatin. The amount of residual CPD induced by the CLUV according to the chromatin compaction status will be quantified using an anti-CPD ELISA. Using BrdU incorporation, we have quantified the occurrence of sister chromatids exchange (SCE) in CLUV and unirradiated cells. We have shown that the CLUV treatment catalyzes SCE as we observe 10% more SCE in irradiated when compared to unirradiated cells. This clearly indicates that residual CPD lead to genomic instability. Taken together, our results have demonstrated that exposure to chronic irradiation of UVB wavelengths leads to genomic instability through the formation of residual CPD in the genome that are not repaired but rather diluted with cell division.

PI.10 Baptista, MS; Cadet, J; Di Mascio, P; Ghogare, AA*; Greer, A; Hamblin, MR; Lorente, C; Ribeiro, MS; Thomas, A; Vignoni, M; Institute of Chemistry, University of Sao Paulo, University de Sherbrooke, Brooklyn College and Graduate Center of the City University of New York, Massachusetts General Hospital and Harvard Medical School, INIFTA, Universidad Nacional de La Plata (UNLP) and CONICET, Institute of Energy and Nuclear Research; aghogare@brooklyn.cuny.edu

Guidelines for Defining Type I and II Photosensitized Oxidation

Here, ten tips are presented for a standardized definition of type I and II photosensitized oxidation reactions. Because of varied notions of photosensitized oxidation reactions, a checklist of

recommendations is provided for their definitions. Type I and type II reactions are oxygen-dependent and involve unstable species such as peroxy radical and singlet oxygen. This exercise was an outgrowth of a mini-symposium on singlet oxygen chemistry in Cambury, Brazil in 2014.

8.4 Bazak, J; Korytowski, W*; Fahey, JM; Girotti, AW; Jagiellonian University, Medical College of Wisconsin and Jagiellonian University, Medical College of Wisconsin; witekko@mcw.edu

Nitric Oxide-mediated Bystander Cell Responses in an Anti-tumor Photodynamic Therapy Model

Non-ionizing photodynamic therapy (PDT) can induce a bystander effect, but far less is known about this than the ionizing radiation-induced counterpart. In the present study, we tested the hypothesis that photodynamically-stressed prostate cancer PC3 cells can elicit nitric oxide (NO)-mediated pro-growth/migration responses in non-stressed bystander cells. A novel approach was used whereby both cell populations existed on a culture dish, but made no physical contact with one other. Visible light irradiation of photosensitized (targeted) cells resulted in a large and prolonged upregulation of inducible NO synthase (iNOS) along with a slower, less pronounced upregulation in bystander cells. This was accompanied by post-irradiation appearance of NO-derived DAF-FM fluorescence, the level of which increased gradually in both cell compartments. Like targeted cells, bystanders exhibited a significant increase in growth and migration rate, both responses being strongly attenuated by an iNOS inhibitor (1400W) or NO scavenger (cPTIO). Incubating bystander cells with conditioned medium from targeted cells failed to stimulate growth/migration, ruling out involvement of relatively long-lived effectors. The pro-survival/pro-growth kinases Akt and ERK-1/2 exhibited progressive post-irradiation activation in bystander cells, NO again playing a key role. This is the first reported evidence for NO-enhanced bystander aggressiveness in the context of PDT and illustrates the need for pharmacologic iNOS inhibitors as PDT adjuvants. (Supported by NIH/NCI Grant CA70823)

PI.11 Bela[~]di, JP; Denat, L; Perez, P; Soeur, J; Zobiri, O; Marrot, L*; L'OREAL R&I; lmarr@rd.loreal.com

TRACES OF POLLUTANTS FROM PARTICULATE MATTER INDUCE A STRONG PHOTOTOXIC STRESS IN HUMAN KERATINOCYTES EXPOSED TO UVA1: REQUIREMENT OF AN APPROPRIATE PHOTOPROTECTION STRATEGY

Dermatological impact of pollution is not yet fully characterized, however skin is probably exposed to very low concentrations of pollutants. In fact, literature suggests that Polycyclic Aromatic Hydrocarbons (PAH) could be provided either by topical penetration of ultrafine particles or by systemic distribution from lungs through blood circulation. Phototoxic impacts of particulate matter PM, PM extract and various PAH on normal human keratinocytes exposed to daily UV (d-UV from 300-400 nm) or to UVA1 (340-400 nm) were compared. Surprisingly, UVA1 was often as potent as d-UV (and sometimes more) in impairing cell survival. Moreover, benzo[a]pyrene (BaP) and indeno[1,2,3-cd]pyrene (IcdP) were phototoxic at very low concentrations (few nanomoles per litre), consistent with concentrations reported in blood of smokers or people exposed to strong pollution. Reactive oxygen species were generated within cells by co-exposure to BaP or IcdP and UVA1, suggesting that photo-oxidative stress contributed to cell death. Finally, comparison of the photoprotection provided to keratinocytes by two formulations differing in their UVA absorption ability confirmed the impact of wavelengths longer than 340 nm in such a "photo-polluting" stress. Our results emphasized the need of an appropriate daily photoprotection for people living in polluted area.

26.3 Bhattacharya, S*; Lehtivuori, H; Forest, KT; ; bhattacharya4@wisc.edu

Structural insights into phytochrome fluorescence

Use of fluorescent proteins in studying in-vivo processes in mammalian systems requires development of near-infrared biomarkers due to clear signals unimpeded by absorption or autofluorescence of biomolecules. Bacteriophytochromes (BphPs) that use biliverdin as their chromophore have been

engineered to form monomeric near-infrared biomarkers. The original design template for a fluorescent phytochrome was the chromophore binding domain of *Deinococcus radiodurans* (DrCBD), with a D207H substitution. This variant proved to be the hallmark for the next generation of phytochrome biomarkers including IFP1.4 (D207H +11substitutions), Wi-Phy (D207HY263F) and the iRFP family based on *Rhodospseudomonas palustris*. We solved the x-ray crystal structures of IFP1.4 and several monomeric DrCBD variants, to explain the origins of fluorescence in derived BphPs. A comparative study revealed two important themes. First, hydrophobic packing around the D-ring increases fluorescence by limiting D-ring motion. Second, while the 207th residue is critical to photochemistry it certainly does not need to be His in order to maximize fluorescence. In fact, the highest fluorescence quantum yield to date in this family belongs to a D207L variant in which waters are excluded from the chromophore vicinity (WiPhy2). Higher quantum yield and longer excited state lifetimes than in the parent suggest the loss of a de-excitation pathway via proton transfer. Continuing our structural analysis, we have turned to the iRFP family of phytofluors and uncovered a surprisingly stable interaction between phytochrome and the heme oxygenase partner that provides biliverdin. We have also engineered a smaller phytochrome variant without the characteristic figure of eight knot topology. Our engineered knotless chromophore binding domain folds and binds bilin. Combining structural insights with protein engineering will help us understand how holo-phytochrome is assembled and design better near-infrared fluorophores.

PI.2 Binder, D*; Bier, C; Hilgers, F; Graberger, A; Loeschcke, A; Kohlheyer, D; Pietruszka, J; Jaeger, KE; Drepper, T; University Dusseldorf, FZ Juelich, IBG-1, FZ Juelich; *d.binder@fz-juelich.de*
Photocaged Carbohydrates As Versatile Tools For Synthetic Bio(techno)logy And Single Cell Applications

Optogenetic tools are light-responsive components that allow for a simple triggering of cellular functions with unprecedented spatiotemporal resolution and in a non-invasive fashion. In particular, light-regulated gene expression exhibits an enormous potential for various biotechnological and synthetic biology applications. Here, we report on the development and evaluation of light-responsive microbial expression systems based on caged compounds such as photocaged IPTG or arabinose. These photocaged carbohydrates are highly feasible to accurately control target gene expression in different biotechnologically relevant production hosts in a rapid and gradual fashion. Microfluidic single cell analysis further revealed that native expression heterogeneity, observed for conventional inducer molecules, can be abrogated by using photocaged carbohydrates as inducers. Apparently, their increased membrane-permeability superseded specific inducer uptake systems. Finally, the biotechnological applicability of light-responsive inducers was demonstrated by distinct improvements of production yields for terpenoids and antibiotics produced in different industrially relevant Gram-positive and Gram-negative expression hosts. In vivo expression analyses revealed that photocaged carbohydrates together with their corresponding transcriptional regulator/promoter system can be employed as optogenetic plug-and-play modules for synthetic biology approaches. These expression modules can be applied in novel photomicrobioreactors and single cell cultivation platforms to precisely control expression of target genes and thereby fully automatize the optimization of microbial production processes. Especially for closed (e.g. anaerobic) systems and increasing numbers of parallelized expression cultures, non-invasive and spatiotemporal light induction will provide a higher-order control.

16.1 Blankenship, RE; Washington Univ.; *blankenship@wustl.edu*

Molecular Mechanisms of Photosynthetic Antenna Regulation

All photosynthetic organisms contain a light-harvesting antenna system. Photosynthetic antenna systems are extremely diverse in terms of structural organization and type of pigment utilized. In addition to the light absorption function of the antenna, it is essential for all photosynthetic organisms to have regulatory mechanisms that serve to protect them against excess light. Several of these regulatory mechanisms involve excited state quenching processes. These mechanisms are referred to as

Non-Photochemical Quenching (NPQ) to distinguish them from the normal excited state quenching by photochemistry that leads to productive energy storage. Remarkably, there are several different types of NPQ that are distinct mechanistically and are almost certainly independent evolutionary inventions. This talk will center on two of the mechanisms of NPQ. These are the Orange Carotenoid Protein (OCP) that is found in photosynthetic cyanobacteria and the redox-induced quenching that has recently been discovered in the Fenna-Matthews-Olson (FMO) protein found in Green Sulfur Bacteria. Cyanobacterial OCP serves to regulate energy collection in the phycobilisome antenna complex, and contains a photoactivated 3'-hydroxyechinenone carotenoid molecule as the pigment, which is photoconverted from an orange form to a red form. While in the red form, the OCP binds to the phycobilisome and quenches excitations. The regulation in the FMO protein takes place via a pair of redox-active cysteine residues, which are near to two of the bacteriochlorophyll pigments. Under oxidizing conditions, these residues oxidize to form thiyl radicals, which directly quench the excited states of the nearby bacteriochlorophylls by electron transfer processes. These regulatory systems have been investigated using an interdisciplinary approach involving ultrafast spectroscopy, mass spectrometry, mutational analysis, molecular modeling, X-ray and neutron crystallography, EPR spectroscopy and electrochemistry.

6.7 Blankenship, RE; Washington Univ.; blankenship@wustl.edu

Molecular Mechanisms of Photosynthetic Antenna Regulation

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20.5 Boissout, F; Perez, P; Soeur, J; Marrot, L*; L'Oreal R&I; Imarrot@rd.loreal.com

Antioxidant baicalin displays a higher toxic impact on p53-mutated keratinocytes (HaCat or A431 carcinoma cells) versus normal human keratinocytes.

Baicalin is a flavonoid present in *Scutellaria baicalensis* and its anti-inflammatory and antioxidant properties have been well described. Moreover, anti-tumorigenic activity of baicalin has also been reported in leukemia or pancreatic cancer. In the dermatological field, some studies have shown that baicalin could protect skin against UV stress, but data dealing with skin cancer targeting are scarce. In this study, biological effects of baicalin were investigated on precancerous HaCaT keratinocytes and on human skin carcinoma cell line A431 (both cell types harboring UV-induced p53 mutations) compared to primary normal keratinocytes (NHEK). In a defined range of concentrations (between 0.1

and 1 mM) baicalin selectively killed HaCaT and A431 whereas it displayed almost no toxic effect on NHEK. To mimic the situation of patches involving p53-mutated cells often found in photo-aged skin, a co-culture model (HaCaT + NHEK) was used. This approach further demonstrated the preferential toxicity of baicalin towards HaCaT. Investigation of mechanisms involved in baicalin-induced cell death was performed comparing monoculture HaCaT versus NHEK. We observed that apoptosis was the main pathway as shown by flow cytometry using annexin-V labelling and caspase activity on a fluorescent peptide. These results suggest that baicalin could reduce clonogenic expansion of p-53 mutated epidermal cells in addition to providing an anti-oxidant protection. Such a flavonoid, and also natural extracts containing baicalin, might be interesting compounds in skin photocancer prevention.

3.3 Broome, A-M*; Dixit, S; Zhu, Y; Moore, A; Medical University of South Carolina;
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Targeted Gold Nanoparticles for PDT Drug Delivery in Brain Tumors

Therapeutic drug delivery across the blood-brain-barrier is not only inefficient but also nonspecific, thereby posing a major shortcoming in effective treatment of brain cancer. Widespread use of photodynamic therapy (PDT) as a localized treatment modality in brain tumor therapy has been partially hampered by non-targeted phototoxicity towards healthy tissue. Development of nanoparticles selectively targeted to cell surface receptors that can act as drug delivery vehicles is critical for improving the therapeutic responsiveness in inaccessible tumors, such as glioblastomas. Gold nanoparticles (Au NPs) provide an excellent platform with a surface that can be tailored to attach biomolecules for targeted drug delivery and biocompatible coatings that can efficiently encapsulate the hydrophobic photosensitizer drug, Pc 4, thereby reducing off-site cytotoxicity. Our research demonstrate a novel double targeted, noncovalent Au NP drug delivery agent, which selectively delivers drugs to brain tumors for PDT. Double-targeted Au NPs loaded with the drug silicon phthalocyanine (Pc 4) have been compared with previously studied single targeted Au NPs. More specific and efficient uptake is observed upon dual targeting. Upon activation of Pc 4 by PDT after delivery by the double-targeted Au NPs increased cell death is observed as compared to systemically delivered Pc 4.

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Informing and Implementing Combinational Approaches in Photodynamic Therapy

Multimodality combinations of surgery, chemotherapy, molecular therapy, and radiotherapy play a major role in the treatment of cancer. Photodynamic therapy (PDT) can also uniquely contribute to cancer treatment in the combinational setting. The integration of PDT in a multimodality approach can be guided by pre-existing or therapy-altered characteristics of a tumor, such as its microenvironment or molecular signature. In this way, treatment can be rationally designed to synergize the anti-tumor effects of the separate modalities. However, in other circumstances, combined modality therapy is not designed. Rather, it is implicit to PDT delivery. For example, there is necessarily the combination of surgery with PDT when PDT is delivered intraoperatively to the site of tumor resection. Irrespective of whether combined therapy is designed or dictated, both the positive and negative interactions of the multiple therapies must be considered to guide clinical application. We discuss several combined modality approaches to PDT that are studied preclinically and clinically, considering the potential effects of each on the subsequent modality. In the case of intraoperative PDT, we've observed an effect of preceding surgery on the therapeutic potential of PDT that immediately follows. In combinations of PDT with molecular targeting drugs, we describe our recent data on the activation of epidermal growth factor receptor (EGFR) after high fluence rate PDT and consider fluence rate for its potential influence on the design of this multimodality approach. Ultimately, the elucidation and exploitation of interactions between PDT and other therapies will guide the design of new multi-modality treatments, as well as inform approaches to improve delivery of combinations that are already used clinically.

6.12 Busch, TM; University of Pennsylvania; buschtm@mail.med.upenn.edu

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14.5 Cannistraro, VJ; Pondugula, S; Song, Q; Taylor, JS*; Washington University; taylor@wustl.edu

Rapid deamination of cyclobutane pyrimidine dimers at TCG sites in the FOS nucleosome in vivo

C to T UV signature mutation hotspots in the p53 tumor suppressor gene of skin cancers occur primarily at methylated PyCpG sites that have been correlated with UV-induced cyclobutane pyrimidine dimer (CPD) formation. These mutations can be explained by the rapid deamination of the C or 5-methyl-C in the CPD's to U or T followed by insertion of A by the DNA damage bypass polymerase \hat{I} . As a consequence, the relative mutagenicity of C-containing CPDs is expected to depend on the relative deamination rates. In vivo, DNA is largely bound to nucleosomes which we have shown in vitro modulate the deamination rates of TmCG CPD's by 12-fold over a complete turn of the DNA helix. To determine the extent to which this occurs in vivo, we determined the deamination rates of CPD's at TCG sites in a stably positioned nucleosome within the FOS promoter in HeLa cells by LMPCR. A procedure for in vivo hydroxyl radical footprinting with Fe-EDTA was developed, and together with results from a cytosine methylation protection assay, we determined the translational and rotational positions of the TCG sites. Consistent with the in vitro observations, deamination was slower for one CPD located at an intermediate rotational position compared to two other sites located at outside positions. Photoproduct formation was also highly suppressed at one site, possibly due to its interaction with a histone tail. Most importantly, deamination of CPDs at TCG sites were faster than at all other C-containing dipyrimidine sites.

6.3 Cannistraro, VJ; Pondugula, S; Song, Q; Taylor, JS*; Washington University; taylor@wustl.edu

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28.1 Carell, T; LMU Munich; *thomas.carell@lmu.de*

DNA Bases beyond Watson and Crick

Epigenetic information is stored in the form of modified cytosine bases in the genome. Setting and erasing of epigenetic imprints controls the complete development process. I will discuss the latest results related to the function and distribution of the epigenetic marker bases 5-hydroxymethylcytosine (hmC), 5-formylcytosine (fC), 5-carboxycytosine (caC) and 5-hydroxymethyluracil. These nucleobases control epigenetic programming of stem cells and some of these bases are also detected at relatively high levels in brain tissues. I will particularly cover mass spectroscopic approaches to decipher the biological functions of the new bases of which some were described in the past as pure DNA lesions. In particular, results from quantitative mass spectrometry, new covalent-capture proteomics mass spectrometry, and isotope tracing techniques will be reported. The data allow us to unravel the chemistry in stem cells and the protein networks that are controlled by the epigenetic base modifications.

P2.14 Carter, KA*; Wang, S; Geng, J; Luo, D; Shao, S; Lovell, JF; University at Buffalo, State University of New York; *kcarter@buffalo.edu*

Metal Chelation Modulates Phototherapeutic Properties of Mitoxantrone-Loaded Porphyrin-Phospholipid Liposomes

An ongoing goal of drug delivery systems is to deliver therapeutic payloads while reducing the total exposure of the drug to healthy tissues and organs. To this end, nanoparticles such as liposomes have shown promise as delivery vehicles and have achieved several examples of clinical translation. Liposomes incorporating porphyrin-phospholipid (PoP) can be formulated to release entrapped contents in response to nearinfrared (NIR) laser irradiation. Here, we examine effects of chelating copper or zinc into the PoP. Cu(II) and Zn(II) PoP liposomes, containing 10 molar % HPPH-lipid, exhibited unique photophysical properties and released entrapped cargo in response to NIR light. Cu-PoP liposomes exhibited minimal fluorescence and reduced production of reactive oxygen species upon irradiation. Zn-PoP liposomes retained fluorescence and singlet oxygen generation properties; however, they rapidly self-bleached under laser irradiation. Compared to the free base form, both Cu- and Zn-PoP liposomes exhibited reduced phototoxicity in mice. When loaded with mitoxantrone and administered intravenously at 5 mg/kg to mice bearing human pancreatic cancer xenografts, synergistic effects between the drug and the light treatment (for this particular dose and formulation) were realized with metallo-PoP liposomes. The drug-light-interval affected chemophototherapy efficacy and safety.

31.4 Chiou, YY*; Yang, Y; Rashid, N; Ye, R; Selby, CP; Sancar, A; UNC Chapel Hill; *yychiou@email.unc.edu*

Mammalian Period Performs Both Repressor and Activator Functions by Displacing the CLOCK-BMAL1 Activator Complex in a Cryptochrome-Dependent Manner

The mammalian circadian clock is based on a transcription-translation feedback loop (TTFL) consolidated by secondary loops. In the primary TTFL, the CLOCK-BMAL1 heterodimer acts as the transcriptional activator, and Cryptochrome (CRY) and Period (PER) proteins function as repressors. PER represses by displacing CLOCK-BMAL1 from promoters in a CRY-dependent manner. Interestingly, genes with complex promoters may either be repressed or activated by PER, depending

on the particular promoter regulatory elements. Here, using mouse cell lines with defined mutations in clock genes, and RNA and ChIP-seq analyses, we elucidate the dual functions of PER as activator and repressor in a context-dependent manner.

12.1 Choe, R; University of Rochester; *Regine_Choe@urmc.rochester.edu*

Monitoring hemodynamic responses to treatments for bone graft and breast cancer with diffuse optical and correlation tomography

Diffuse optical and correlation tomography quantifies 3D distribution of microvascular oxyhemoglobin, deoxyhemoglobin, water and lipid concentrations, scattering and blood flow in deep tissue using photons in the near-infrared spectral window. These physiological parameters have great potential to assess therapeutic efficacy of breast cancer treatments and tissue-engineering based treatments for bone grafts. In addition, the use of light which is non-ionizing and inexpensive instrumentation makes diffuse optical and correlation tomography attractive for translational research. Here, we introduce clinical and preclinical research tools and approaches to test the capabilities of diffuse optics in early prediction of therapeutic efficacy. For monitoring vascularization of bone graft, we developed a non-contact scanning scheme to acquire large spatial dataset necessary for reliable 3D image reconstruction. Different temporal and spatial blood flow responses between autograft and allograft were observed, demonstrating the potential to utilize blood flow for prediction of treatment efficacy. In a murine breast tumor model, we found a close correlation between the tumor volume and the blood flow changes. Clinical data showing feasibility to predict treatment efficacy at early time point will be presented.

15.3 Choi, JH*; Kemp, MG; Sancar, A; Korea Research Institute of Standards and Science, Daejeon, Korea, University of North Carolina School of Medicine; *junchoi@kriss.re.kr*

Development of an in vivo assay to detect DNA excision repair events in mammalian cells

Nucleotide excision repair is the sole DNA repair system for removing UV-induced DNA lesions, cyclobutane pyrimidine dimers (CPDs) and the (6-4) photoproducts, as well as bulky base adducts induced by numerous chemical carcinogens and chemotherapeutic agents from the human genome. Studies in vitro and in vivo have shown that the UV damage is removed from the genome in the form of an oligonucleotide approximately 24- to 32-nt in length. Because there are limitations to many of the currently available methods for investigating UV photoproduct repair in vivo, we developed a convenient non-radioisotopic method to directly detect DNA excision repair events in human cells. The methodology was shown to be highly sensitive to directly detect the generation of excision products even within minutes following UV irradiation. Moreover, our techniques allowed us to examine repair events in vivo following exposure of cells to different types of DNA damaging agents causing bulky base adducts, DNA crosslinks or DNA-protein crosslinks. We suggest that the new techniques presented here will be a useful and powerful approach for studying the mechanism of human nucleotide excision repair in vivo.

27.6 Christensen, T*; Danielsen, T; Jaworska, A; Brunborg, G; Bruzell, EM; Norwegian Radiation Protection Authority and Centre for Environmental Radioactivity (CERAD CoE), Norwegian Radiation Protection Authority, Norwegian Institute of Public Health and Centre for Environmental Radioactivity (CERAD CoE), Norwegian Institute of Public Health, Nordic Institute of Dental Materials; *terje.christensen@nrpa.no*

Wavelength Dependent Increase In Cell Sensitivity After Glutathione Inhibition By Methacrylate Monomers

Reduction in the amount of glutathione (GSH) in cells can lead to increased sensitivity to physical and chemical agents. Methacrylate monomers (MM) are precursors of polymethacrylates which are used in dental and medical biomaterials and in a wide variety of other products. MM can increase cell sensitivity to long wavelength ultraviolet and visible radiation. The aim of this study is to elucidate if

MM can induce sensitivity also to shorter wavelength radiation. A concentration of 3 mM 2-hydroxyethyl methacrylate (HEMA) in a serum free medium was added to ZF4 zebrafish embryo fibroblasts during logarithmic growth and kept on the cells for up to 4 h. Cell death was assayed by the Alamar blue assay 3 days after irradiation. The level of GSH was quantified by a commercial glutathione assay kit. Buthionine sulfoximine (BSO) was used as positive control for depletion of GSH. HEMA reduced the level of GSH relative to control. The cells were irradiated with either broadband UVA (Osram 9W/78), UVB (Philips 9 W PL 12) or 225 keV X-rays. HEMA was non-toxic to the cells under these conditions and did not absorb optical radiation with wavelength longer than 250 nm. Following cell pretreatment with HEMA, the survival relative to controls was reduced from 72.3 % to 23.0 % after exposure to 100 kJ/m² of UVA, from 75.3 % to 65.5 % after exposure to 0.25 kJ/m² UVB and from 70.0 % to 67.9 % after 16 Gy X-rays. The change in cellular sensitivity due to HEMA was more pronounced with UVA- than with UVB- and X-ray irradiation. UVA-induced cellular damage is characterized by formation of reactive oxygen species, most notably hydrogen peroxide. UVB damage, on the other hand, includes direct interaction with DNA, whereas ionizing radiation induces breaks in the DNA strands, by direct hits or via the formation of hydroxyl radicals. These different mechanisms of action are likely to be associated with the observed sensitivity modifications due to HEMA treatment.

14.4 Coelho, SG*; Miller, SA; Michele, TM; Center for Drug Evaluation and Research, FDA, Center for Devices and Radiological Health, FDA; sergio.coelho@fda.hhs.gov

Long-term Skin Pigmentation after a Single Sunburn Reveals Altered Hemidesmosome Plasticity

Despite educational efforts to reduce the risk for skin cancers from preventable UV overexposure, sunburns occur quite frequently, especially in vulnerable populations such as children. Severe sunburn events can occur in the absence of using protective measures, but may also occur during careless use of sunscreens and/or other protective measures to prolong exposure times in the sun. Until now, no long-term evaluation of the consequences from a single sunburn had ever been done. Based on our work, approximately 32-61% of individuals with moderate sunburn from a single exposure (2-4x their minimal erythema dose) developed a long-lasting pigmentation (LLP) effect that persisted for greater than 9 months. By studying 6 individuals (3 LLP+ and 3 LLP-) in detail, the goal was to investigate whether this long-term effect was signaling overt changes to melanin production/distribution, cellular proliferation and/or skin morphology. Based on immunohistochemistry, the increased visual pigmentation was corroborated with increased melanin staining in the basal layer of the epidermis in LLP+ individuals. This increase in pigmentation was not due to any increased melanogenesis, but rather retention of melanin in basal keratinocytes. There was a measureable increase in basement membrane interdigitation at the epidermal-dermal junction. This basement membrane plasticity was characterized by decreased hemidesmosome density by electron microscopy, attenuation of hemidesmosomal partners (integrin alpha 6 beta 4 and plectin) by immunofluorescence and spatial regulation of SoxF family transcription factor (Sox7) by proximity ligation assays. The detected histopathological features resemble characteristics of solar lentigos, which are known risk factors for precancerous lesions. Our results further underscore advice to encourage individuals to use various sun protective measures.

30.1 Crespo-Hernandez, CE; Case Western University; carlos.crespo@case.edu

DNA + Light: From Nucleic Acid Bases to Modifications that Sensitize Damage in Cells

DNA is the carrier of genetic information for almost every organism on Earth. The genome's vulnerability to damage through the absorption of solar ultraviolet radiation has sustained interest in the light-induced chemistry of the nucleic acid bases for decades. Emphasis in this area by our research group has recently shifted toward understanding the electronic and structural elements that regulate the relaxation pathways in the nucleic acid bases and how this fundamental information may be used to advance nucleic acid derivatives for applications in topical phototherapy. Our most recent and exciting results will be presented in this Symposium. NSF funding is acknowledged (CHE-1255084).

16.6 DA, Myles; MJ, Cuneo*; Oak Ridge National Laboratory; mylesda@ornl.gov

Towards a full atomic resolution model of a photosynthetic antennae complex

An understanding of the molecular details that underlie solar energy capture in photosynthetic organisms will lead to a better understanding of energy transfer in natural photosynthetic systems, and, in addition, will aid in the development of biohybrid photosynthetic systems. Current x-ray crystal structures of photosynthetic machinery reveal details of the pigment-protein architecture and interactions that regulate and control energy transfer but do not resolve hydrogen atoms. This is significant because, in many cases, hydrogen bonding interactions are likely to be important in the stabilization of antenna pigments and in fine-tuning and control of their site-energies. Important questions remain on how the individual site energies of protein bound chlorophyll molecules are modulated and tuned by local hydrogen bonding and electrostatic interactions with the protein scaffold. The extra level of detail provided by neutron diffraction experiments can contribute to better understanding of the spatio-energetic landscape and exquisitely tuned properties of the photosynthetic apparatus.

14.1 Dabrowski, JM*; Pucelik, B; Stochel, G; Arnaut, LG; Faculty of Chemistry, Jagiellonian University, Krakow, Poland, Chemistry Department, University of Coimbra, Coimbra, Portugal; jdabrows@chemia.uj.edu.pl

Molecular Engineering of Photosensitizers for Enhanced Photodynamic Therapy Against Pigmented Melanoma

Photodynamic therapy (PDT) has emerged as a promising strategy in oncology. Its multiple approaches to optimize therapeutic regimens and the targeting of tumor vasculature may be especially useful in the treatment of melanoma. However, PDT of pigmented melanoma has generally been unsuccessful because insufficient light penetration through tissue and high concentration of melanin that acts as an optical shield and as antioxidants. PDT photosensitizers and protocols need to be carefully optimized in order to overcome these challenges. The treatment of melanoma requires highly active photosensitizers that absorb at long wavelengths (700-800 nm) where melanin does not absorb and the energy is still sufficient to generate reactive oxygen species (ROS). In this work the relevant photosensitizer properties (electronic absorption, photostability, nature of generated ROS, polarity and delivery) and treatment parameters (drug and light doses, drug-to-light intervals, radiant exposure and tumor margin) to optimize PDT for melanoma are discussed. Comprehensive in vitro studies against B16F10 melanoma cells with photostable NIR absorbing bacteriochlorin (redaporfin) showed that redaporfin-P123 micelles led to higher cellular uptake and increased oxidative stress compared with photosensitizer alone after short incubation times. Neither redaporfin encapsulated in Pluronic nor P123 micelles alone exhibited cytotoxicity in a broad concentration range. On the other hand, they cause strong light dose depended apoptosis and necrosis. Vascular-targeted PDT using redaporfin in P123 against B16F10 tumors in C57BL/6J mice with light doses of 74 J/cm² led to 100% complete cures (no tumor regrowth ca. one year post-treatment). This remarkable result reveals that redaporfin has nearly ideal properties and its modification with Pluronic block copolymers helps to overcome the resistance of melanoma cells via increased tumor selectivity and enhanced ROS generation in Pluronic micelles.

PI.4 Datta, P; Philipsen, PA*; Olsen, P; Petersen, B; Johansen, P; Morling, N; Wulf, HC; Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark, Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark; peter.alshede.philipsen@regionh.dk

Major inter-individual variation in the UVB induced increase and maximal level of 25-hydroxy vitamin D

Vitamin D influences skeletal health as well as other aspects of human health. Even when the most obvious sources of variation such as solar UVB exposure, latitude, season, clothing habits, skin

pigmentation and ethnicity are selected for, variation in the serum 25-hydroxy vitamin D (25(OH)D) response to UVB remains extensive and unexplained. Our study assessed the inter-individual variation in 25(OH)D response to UVR and the maximal obtainable 25(OH)D level in 22 healthy participants (220 samples) with similar skin pigmentation during autumn/winter from October through December with negligible ambient UVB. During nine weeks the participants received identical UVB doses on identical body areas until a maximal level of 25(OH)D was reached. To examine if the maximal 25(OH)D level had been reached the participants were subsequently sent on a one-week sun holiday in Hurghada, Egypt. Major inter-personal variation in both the maximal obtainable UVB-induced 25(OH)D level (range 85-216 nmol/l, mean 134 nmol/l) and the total increase in 25(OH)D (range 3-139 nmol/l, mean 48 nmol/l) was found. Linear modelling including measured 25(OH)D baselines as personal intercepts explained 54.9% of the variation. By further including individual personal slopes in the model, as much as 90.8% of the observed variation could be explained. The explained variation constituted by personal differences in slopes thus represented 35.9%. Age, vitamin D receptor gene polymorphisms, height and constitutive skin pigmentation (a skin area not exposed to UVB) explained 15.1% of this variation. In total this linear model explained 70% of the observed variation. Despite elimination of most known external sources of variation, our study demonstrated inter-individual variation corresponding to an observed maximal difference of 136 nmol/l in the total increase of 25(OH)D and 131 nmol/l in the maximal level of 25(OH)D.

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A Non-Invasive Modality for In Vivo Detection of Neutrophil Influx in Preclinical Models of Mesothelioma

Photodynamic therapy (PDT) frequently leads to a rapid influx of innate immune cells, most especially neutrophils, into the site of treatment. Signals released by these neutrophils help to steer the adaptive immune response, and it is therefore no surprise that their depletion is associated with poorer outcomes in preclinical studies of tumor models. Conversely, treatment resistance can develop due to instigation of survival factors that include vascular endothelial growth factor (VEGF) and endothelial growth factor receptor (EGFR), or if the adaptive immune response is driven toward a more regulatory subset. These relationships are further complicated when PDT is performed in the context another inflammation-inducing treatment, such as surgery. In order to better understand the spatial and temporal aspects of neutrophil influx during and after intraoperative PDT, we have used a non-invasive, chemiluminescent modality for the in vivo detection of neutrophils in pre-clinical models of mesothelioma. Mice bearing AB12 (murine mesothelioma) tumors were injected intraperitoneally with luminol, which releases light in the presence of peroxide radicals produced by the neutrophil enzyme myeloperoxidase. Studies were performed to longitudinally evaluate the effects of surgery, followed by PDT, in contrast to PDT as an individual entity. Using this technique, neutrophil influx was readily detected after even minor injury. Moreover, this modality was able to differentially distinguish the spatial localization of neutrophils to the incision and PDT-treated sites. Ongoing studies will seek to correlate this influx with the expression of resistance factors and tumor response in mesothelioma models, towards the goal of understanding the induction of innate immunity in combinations of PDT with surgery.

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Challenges of and Solution for Interstitial Photodynamic Therapy

Abstract not available.

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Investigating the Chemical Composition of Human Retinal Lipofuscin in Association with Age Related Macular Degeneration (AMD)

Age related macular degeneration (AMD) is a common retinal disorder that affects the elderly and is the leading cause of blindness in the Western world. The excess buildup of lipofuscin in the retinal pigment epithelium (RPE) is considered to be a major risk factor for AMD. RPE lipofuscin, thought to be derived from the ingestion of photoreceptor cells' outer segments, has the capability of causing photooxidative damage from excessive visible light exposure. A2E is a commonly studied fluorophore known to be a component of RPE lipofuscin. This study aims to look at the photoreactivity of A2E and its possible reactions with amino acids under blue light exposure. Human retinal lipofuscin is isolated from human donor eyes diagnosed with AMD according to the method previously described by Feeney-Burns. The organic soluble fraction of lipofuscin is collected, dried, and reconstituted using methanol for use in high performance liquid chromatography tandem mass spectrometry (LC/MS) coupled with a photo diode array and fluorescence detectors. Separately, A2E is synthesized and reacted with various amino acids under blue light conditions. The products are then analyzed using the same methods as RPE lipofuscin and results are compared to that found in the human donor tissue. Results show possible reactions with particular amino acids and A2E, particularly that of lysine. Post-translational modifications of lysine have commonly been associated with inflammation and disease. These data suggests the vulnerability of amino acids and protein in vivo to photooxidative damage elicited by A2E. Further analysis can assist in determining the pathogenesis of AMD and the role A2E plays in disease progression.

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Impact of human skin dermis on the repair efficiency of UV-induced DNA damage in the epidermis
Skin, constituted of the epidermis and the dermis, is exposed to solar ultraviolet radiations (UVR). Exposure to UVR leads to the generation of cyclobutane pyrimidine dimers (CPD), a highly mutagenic DNA damage responsible for skin cancer driver mutations. An efficient CPD repair is important to avoid mutation induction and skin cancer. A previous study brought evidence that the interactions between dermal fibroblasts and epidermal keratinocytes play a role in promoting epidermal CPD repair after UVB irradiation. However, very little is known about the mechanisms involved and the factors responsible for this dermal-epidermal crosstalk modulating UV-induced damage repair in keratinocytes. We used a tissue-engineered skin model to investigate the impact of dermis and its components on epidermal CPD repair. This skin model is produced exclusively from human fibroblasts and keratinocytes. Fibroblasts were cultured for 35 days in the presence of ascorbic acid to have them secrete and assemble their own extracellular matrix and thus form a thick fibrous dermal sheet, on top of which keratinocytes were seeded and grew during 10 days. We compared CPD repair, after 400 J/m² UVB, in keratinocytes seeded either on dermal sheet or on culture dish plastic. We observe a faster repair of epidermal CPD in the presence of a dermal sheet. We are now investigating which element of the dermis is influencing CPD repair efficiency in epidermal cells, i.e. extracellular matrix components and/or fibroblasts secreted factors. This project aim to shed light on the influence of dermal components on epidermal CPD repair efficiency and will help to determine the nature of these constituents. Understanding how the fibroblast"keratinocyte crosstalk influences response to UVB-induced genotoxicity represents an important step toward skin cancer prevention.

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Combined actions of UVB and UVA on DNA damage and repair

The major role played by solar UV radiation in the onset of skin cancer is mostly explained by its DNA damaging properties. An important aspect of this genotoxicity is the respective role of UVB and UVA since the proportion between the two wavelength ranges depends on the irradiation conditions and the photoprotection strategies applied. Induction of pyrimidine dimers (cyclobutane pyrimidine dimers, pyrimidine (6-4) pyrimidone photoproducts) and oxidative lesions (oxidized bases, single strand breaks) are the most frequent DNA lesions in UV exposed cells and skin. Importantly, the nature of the photoproducts is less strictly different between UVB and UVA than previously thought as

illustrated by the formation of cyclobutane dimers as the major DNA lesion in the UVA range. Data accumulated in the recent years allow us to draw a picture of the frequency of DNA damage not only after exposure to pure UVB or UVA, but also to the full complexity of sunlight. In addition, the combination of UVB and UVA has to be considered for some kind of damage (e.g. Dewar valence isomers). Modulation of the genotoxicity of UVB by UVA can also result from the alteration of the DNA repair capacities. This has been observed in both skin explants and culture keratinocytes. The latter phenomenon can be triggered by protein oxidation but may also result from difference in activated signaling pathways by either UVB or UVA. The bulk of these data has to be considered to design efficient photoprotection.

15.8 Drigeard Desgarnier, MC*; Rochette, PJ; University Laval and Centre Hospitalier Universitaire de Quebec Research Center, Canada; *mc.desgarnier@gmail.com*

Improvement Of UV-induced DNA Damage Repair By Chronic Low Dose Of UV

Ultraviolet B (UVB) is a carcinogen responsible for the induction of non-melanocytic skin cancer. UVB wavelengths exhibit their mutagenic potential by inducing two main types of DNA damage: cyclobutane pyrimidine dimer (CPD) and 6-4 photoproducts (6-4 PP). In humans, those pre-mutagenic bulky adducts are repaired by the nucleotide excision repair (NER) pathway. Since decade, the protective role of NER against skin cancer is well established; nonetheless, most of studies are based with acute dose. Few evidences in the literature are suggesting that chronic irradiation with a low sub-lethal doses of mutagen could enhance the NER capacity. However, it has never been tested whether conditioning cells with chronic low UV irradiations (CLUV) would impact NER efficiency, and the mechanisms involved in the DNA repair response after this treatment are not completely understood. Thus, we have irradiated normal human dermal fibroblasts (NHDF) with a CLUV regime, which consists of 75 J/m² UVB every 12 hours for 7.5 days (total 1125 J/m²). Twelve hours following the last irradiation, cells were irradiated or not with an acute UVB dose (400 J/m²). Our results show that CPD induced by a single acute UVB dose are 50% repaired 24h post-irradiation whereas CLUV pre-stimulated cells repaired significantly faster (70% of CPD are repaired 24h post-irradiation). The repair of 6-4PP was not improved by the CLUV pre-stimulation treatment. Our data indicate that CLUV pre-stimulation enhance the NER of CPD but not 6-4PP. Since DDB2, a DNA damage recognition protein is known to influence CPD repair efficiency but not 6-4PP, we investigate its level after the CLUV pre-stimulation. We found a 2-fold increase of DDB2 protein and mRNA levels in CLUV pre-treated cells. As DDB2 is known to be down-regulated in skin cancer, our results indicate that the CLUV treatment might have a protective effect against this neoplasia

21.4 Drouin, R*; Lapointe, G; Genereux, M; Bouffard, C; Laval University, Quebec City, QC, University of Sherbrooke, Sherbrooke, QC; *Regen.Drouin@USherbrooke.ca*

Characterization of UV and Sun Exposure Behaviors and Skin Cancer in the Estrie Region of Quebec Province

Since the Estrie is the region of Quebec Province with the highest incidence of skin cancer (SC), we conducted a large-scale survey on the Estrie population to characterize UV and sun exposure behaviors of people. A representative sample of 8,737 adults [18 years old, 4462 women (W) and 4275 men (M)] was surveyed. There were 12 questions related to the type of skin, UV exposure in tanning salon, sunbathing, risk of tanning, use of UV protection, meaning of sun tanning, number of sunburns and development of SC. Data were collected through a random digit dial telephone survey. On the Fitzpatrick scale, 17.7% of the respondents had skin types 1 and 2; 67.7% had types 3 and 4 and 14.6% had types 5 and 6. Out of 6781 adults aged between 18 and 64 years, 31% (W 44 vs. M 18%) have been to a tanning salon and 48.2% (W 60.6 vs. M 35.9%) have been sunbathing with the sole purpose of tanning. During the last 10 years, 50.7% of the respondents spent vacations in the South to catch the sun. To reduce the sun exposure, the respondent used sunscreen (79% of the respondents), clothes (72.4%), staying in the shade (74.5%) and avoiding the midday sun (63.1%). Sun tanning can mean SC (65.2%) and accelerated skin aging (62.6%) as side effects, but most respondents were there because

they wanted to improve their health (63.1%), vitamin D synthesis (57.8%), relaxation (40.3%), well-being (39.6%), beauty enhancement (33.9%), treatment of skin problems such as acne or psoriasis (20.7%), seduction (16.6%), improve self-confidence (11.4%), improve chances of success (4.6%), and power (2.4%). A majority of the surveyed people consider sunburns and burns (62.8%), sun spots, changes in skin colour (53.6%), higher risks of developing SC (88.5%) and accelerated skin aging (72.9%) as risks associated with tanning. Only 4.4% of the respondents did not suffer sunburns, 48.3% (W 53.1% vs. M 43.2%) of the people had had between 1 and 9 sunburns and 47.3% (W 41.7% vs. M 53.2%) at least 10 sunburns.

31.1 Duffy, JF*; Chinoy, ED; Zitting, KM; Harvard Medical School and Brigham and Women's Hospital; jduffy@hms.harvard.edu

Light Effects on the Human Circadian Timing System

Nearly all organisms possess a biological timing system that produces rhythms in physiology and behavior with a ~24-hour cycle length. These near-24-h circadian rhythms are synchronized to the 24-h day by signals from the environment, and in humans this is primarily achieved by exposure to light and darkness. As a diurnal species, adult humans whose circadian system is synchronized with their environment will be alert during the day and able to sleep for an extended time during the night. The consequences of not being entrained are experienced by those such as shift workers who must remain awake at night to work and then attempt to sleep during the day, and by individuals who have recently traveled across multiple time zones. Light exposure has phase-dependent effects on circadian rhythms, with the magnitude and direction of alterations in rhythm timing dependent on the biological time at which the light exposure occurred. Other features including wavelength, illuminance, duration, and pattern of exposure also impact the circadian response to light. Thus, light exposure at some times of day will produce shifts in circadian rhythm timing to earlier hours, light at other times of day will produce shifts to later hours, and there are times at which the same duration, wavelength, and intensity of light will produce almost no change in circadian rhythm timing. In addition to the entraining and phase-shifting effects that light produces on human circadian rhythms, there are also direct effects, including suppression of the hormone melatonin and increases in alertness. With understanding of the direct and indirect light effects on the human circadian system, researchers have used that information to design therapies to improve on-shift alertness and off-shift sleep in night workers, to more quickly adjust the biological clocks of travelers, and to shift sleep timing in individuals with circadian rhythm sleep disorders.

6.13 Duffy, JF*; Chinoy, ED; Zitting, KM; Harvard Medical School and Brigham and Women's Hospital; jduffy@hms.harvard.edu

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11.2 Essen, LO; Philipps University; essen@chemie.uni-marburg.de

Microbial members of the photolyase/cryptochrome family: Common structures, common mechanisms ?

Members of the photolyase-cryptochrome family (pc-family) are manifold involved in light-driven DNA repair and blue-light dependent signaling. All members utilize in their C-terminal, alpha-helical domain a U-shaped FAD chromophore for catalysis or light absorption [1,2]. However, unlike other, well studied "canonical" representatives of the pc-family, e. g. plant [3] and animal cryptochromes or class I and (6-4) photolyases, several subfamilies (class II photolyases, proteobacterial cryptochromes) developed novel electron transfer pathways for its photoreduction as well as hitherto unknown antenna cofactors. The advent of bifunctional members of the pc-family, e.g. DASH-type and algal cryptochromes, blurred the distinction between cryptochromes and photolyases. For example we found (6-4) photolyase activity for the animal-like cryptochrome of the green algae *Chlamydomonas reinhardtii* (CraCRY) that otherwise shows blue- and red-light activity in vivo. Its 1.9Å structure reveals the common bilobal architecture, where the N-terminal Rossma-like fold harbors 8-hydroxydeazaflavin as antenna. The co-crystal structure with (6-4) lesion comprising duplex DNA proves that CraCry is indeed a fully-fledged (6-4) photolyase. To investigate photoactivation of CraCRY, the conserved tryptophan triad was mutated and analyzed by UV/Vis spectroscopy. CraCRY forms FADH* upon blue light illumination without any reductive agents. Our data show that Y373 is the endogenous electron donor to the tryptophan triade by forming a long-lived radical state. Other examples of the microbial pc-family will be presented, which indicate a repeated re-usage of DNA-photolyases as signaling proteins.

27.4 Evans, CL; Wellman Center for Photomedicine, Harvard Medical School, Massachusetts General Hospital; evans.conor@mgh.harvard.edu

Quantitative Imaging of Oxygen in Tissues with Bright Porphyrin Phosphors and Bandages

Proper tissue oxygenation is crucial in situations ranging from treating chronic wounds to therapeutic intervention in cancer. Our research has been focused on developing a platform for real-time, quantitative imaging of oxygen tension within tissue. First, we have developed a set of click-chemistry compatible, bright planar porphyrin molecular oxygen sensors based on near-infrared phosphorescence quenching. These meso-unsubstituted molecules have considerably higher phosphorescence quantum yield than existing commercial probes, enabling rapid oxygen tension sensing and image acquisition. Second, we have developed a simple, but extensible, click-chemistry based scheme that allows for the rapid growth of custom dendrimer layers surrounding these new porphyrin sensors that not only provide an extended oxygen sensing dynamic range, but are also designed to enable cellular uptake even in highly acidic tumor compartments. These new sensors have been tested in a three-dimensional in vitro model of ovarian cancer where they readily penetrate throughout large nodules and report oxygenation changes. Third, to translate these oxygen sensors into the clinic, we have developed oxygen sensing bandages that can be applied to skin or wounds for transcutaneous oxygen imaging. The formulation of the bandage enables the simple readout of tissue oxygenation with commercial cameras and smartphones for rapid quantification of oxygen by untrained operators.

8.3 Fahey, Johathan M.; Girotti, Albert W.*; Medical College of Wisconsin; agirotti@mcw.edu
ROLE OF ENDOGENOUS NITRIC OXIDE IN HYPER-AGGRESSIVENESS OF CANCER CELLS SURVIVING A PDT-LIKE OXIDATIVE CHALLENGE

Nitric oxide (NO) is a pleiotropic free radical molecule generated naturally by enzymes of the nitric oxide synthase (NOS) family. Many tumors exploit low levels of endogenous NO for survival, growth, and invasive signaling and also to resist various anti-tumor therapies, including photodynamic therapy (PDT). Studies carried out in the laboratory ca. 6 years ago were the first to demonstrate that inducible NOS (iNOS) is rapidly and persistently elevated in several types of cancer cell subjected to a photodynamic challenge mimicking 5-aminolevulinic acid (ALA)-based PDT. In recent studies, we discovered that two human prostate cancer lines (PC3 and DU145) and a breast cancer line (MDA-MB-231) exploit iNOS/NO to not only resist photokilling, but also to promote aggressive expansion of surviving cells. For example, 24 h after an ALA/light challenge (fluence ~ 1 J/cm²), PC3 survivors proliferated (MTT assay), migrated (gap-closure assay), and invaded (trans-well assay) much more rapidly than dark controls. Each of these responses was suppressed by an iNOS inhibitor or NO scavenger, consistent with NO involvement. In agreement with accelerated invasion, surviving cells exhibited a marked NO-dependent activation of matrix metalloproteinase-9 (MMP-9, shown by in-gel zymography), down-regulation of MMP-9 inhibitor TIMP-1, and upregulation of alpha-6 and beta-1 integrins. Photostress-surviving MDA-MB-231 cells were also found to proliferate and migrate/invade more rapidly than dark controls in vitro. The significance of these findings was recently confirmed at the in vivo level. Using SCID mice bearing MDA-MB-231 tumor xenografts, ALA as a pro-sensitizer, and a 633 nm LED light source, we showed that ALA-PDT significantly reduced tumor expansion rate relative to light-only controls. Importantly, administered iNOS inhibitors further reduced this rate, implying that tumor iNOS/NO was reducing PDT efficacy, presumably by acting cytoprotectively as observed in vitro. (Supported by NIH/NCI grant CA70823)

21.5 Fajuyigbe, D*; Damato, E; McMahon, S; Penney, G; Coleman, A; Sarkany, R; Young, AR; ;
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Objective Assessment Of UVR-induced Erythema And Pain Sensitivity In Different Skin Types

Constitutive skin pigmentation is thought to be responsible for variations in response to UVR but the standard measure of sensitivity, i.e. visual assessment of the minimal erythema dose (MED) may be obstructed by high levels of melanin (dark skin). Our aim was to explore confocal microscopy using its 830nm wavelength (minimally absorbed by melanin) to analyse erythema irrespective of skin colour. Volar forearms of healthy African (type VI) and fair-skinned volunteers (types I/II) were exposed to a geometric dose series of solar simulated radiation. Exposures were based on standard erythema doses (SED). 24h post-exposure, blood vessels videos were captured within a 0.5x0.5mm² frame and the blood vessel area and blood cell counts per second (flow) were automatically extracted. We found a significant linear correlation between vessel area and SED for black ($p=0.0005$, slope=0.0023) and white skin ($p=0.0001$, slope=0.0943) thus suggesting that white skin is roughly 40 times more sensitive to UVR. Further analysis showed significant correlation between flow and area for white ($R^2=0.903$, $p=0.001$) and black skin ($R^2=0.562$, $p=0.03$). It is clear that confocal microscopy offers a novel approach for the objective determination of sensitivity to UVR irrespective of skin colour. Furthermore, on the same skin sites, we assessed skin type differences in the relationship between visible erythema and pain sensitivity using thermal and mechanical stimuli. The baseline thermal sensitivity threshold was $47.80^{\circ}\text{C} \pm 0.42$ with no significance difference between black and white skin ($p=0.32$). When SSR exposure was expressed as MED, there was no skin type difference ($p=0.0001$) in increased sensitivity to thermal pain. However, black skin unlike white skin showed resistance to mechanical stimuli regardless of dose ($p=0.78$). These results extend the previous findings of erythema and pain sensitivity correlations in white skin, it remains unclear why black skin was resistant to mechanical stimuli.

11.3 Faraji, S*; Dreuw, A; University of Southern California, USA, Ruprecht-Karls University of Heidelberg, Germany; *shirin.faraji@uni-heidelberg.de*

Utilizing Light for Repair of Light-induced DNA Damages: The Clever Mode of Action of DNA Photolyases

UV radiation triggers various chemical reactions in DNA such as intra-strand cross-linking between adjacent pyrimidines causing genetic mutations. The pyrimidine dimers are supposed to be the major players in the formation of skin cancer. DNA photolyases are enzymes initiating cleavage of mutagenic photolesions by a photo-induced electron transfer from flavin adenine dinucleotide to the lesion. Using state-of-the-art hybrid quantum mechanical/molecular mechanical dynamics, we have carried out a series of simulations to completely map out the entire evolution of functional processes involved in molecular mechanism of this important biological function. We have demonstrated that the electron catalyzing the repair is generated via an intermolecular Coulombic decay (ICD) process [1]. In fact, this is the first example for ICD in a real biological system. We have presented the most energetically feasible electron-induced splitting mechanism in which the initial step is electron transfer coupled to proton transfer from the protonated Histidine to the lesion, which proceeds simultaneously with intramolecular OH transfer along an oxetane-like transition state [2]. In agreement with recent experimental time resolved findings [3], the photolesion can be split and original bases restored. The experimental spectroscopic signature of the detected 6-4PP intermediate is assigned theoretically to a specific molecular structure determining the operating molecular mechanism of the electron-induced restoration of (6-4) photolesions. Thereby, all pieces of the electron-induced (6-4) photolesion repair puzzle are finally put together [4]. 1. P. Harbach, M. Schneider, S. Faraji, A. Dreuw, *J. Phys. Chem. Lett.* 4, 943 (2013). 2. S. Faraji and A. Dreuw, *J. Phys. Chem. Lett.* 3, 227 (2012), S. Faraji, G. Groenhof and A. Dreuw, *J. Phys. Chem. B.* 117, 10071 (2013) 3. J. Li et al, *Nature* 466, 887 (2010). S. Faraji and A. Dreuw, *Ann. Rev. Phys. Chem.* 64, (2014). 4. S. Faraji, D. Zhong, and A. Dreuw, *Angew. Chem. Int. Ed. Engl.*, accepted for publication (2016).

31.2 Gaddameedhi, S; Washington State University; *shobhan.gaddameedhi@wsu.edu*

Circadian Clock, UV-DNA Repair and Skin: Implications in Skin Carcinogenesis and Sunburn Erythema

Epidemiological studies of humans and experimental studies with mouse models suggest that sunburn as a result of exposure to excessive UV light and damage to DNA confer an increased risk for melanoma and non-melanoma skin cancer. Previous reports have shown that both nucleotide excision repair, which is the sole pathway for removing UV-induced DNA photoproducts, and DNA replication, are regulated by the circadian clock in mouse skin. Furthermore, the timing of UV exposure during the circadian cycle has been shown to affect skin carcinogenesis in mice, with up to a 5-fold difference in invasive carcinoma. Because sunburn and skin cancer are causally related, we investigated UV-induced sunburn apoptosis and erythema in mouse skin as a function of circadian time. Interestingly, we observed that sunburn apoptosis, inflammatory cytokine induction, and erythema peaked at 3-fold following an acute early morning exposure to UV when compared to following an afternoon/evening exposure. Furthermore, the circadian rhythmicity of these responses was found to be correlated with activation of ATR-mediated DNA damage checkpoint signaling and p53 activity, which is known to control the process of sunburn apoptosis. These data provide the first evidence that the circadian clock plays an important role in skin carcinogenesis and the erythema response in UV-irradiated mouse skin. Since mice are nocturnal and humans are diurnal, we expect the circadian clock outputs of the two organisms to exhibit opposite phases. On this basis it would be expected that humans may be less prone to UV-induced skin toxicity in the morning and more prone in the evening. While presenting the above findings on mouse models, this presentation will highlight the potential application of the circadian clock that modulate the skin responses to DNA damaging mediated therapeutics that are commonly used in the field of dermatology.

26.5 Gaertner, W.; MPI Chemical Energy Conversion; *gaertner030151@gmail.com*

The GAF-3 domain from Slr 1393: Photochemistry and Insight into Structural Changes

Cyanobacteriochromes are photochromic photoreceptors carrying bilins as chromophores. Their light-driven reactions are similar to those of canonical photochromes. They have attracted strong attention of scientists as CBCRs show a much broader absorbance range and, in addition, these proteins collate the

entire photochemistry and chromophore binding capability in one single GAF domain. GAF3 of Slr1393 from *Synechocystis* PCC6803 generates a red light-absorbing parental state and a green light-absorbing photoproduct state ($\lambda_{\text{max}} = 650\text{nm}, 535\text{ nm}$, respectively). Both forms can be fully photo-converted into each other. Time-resolved absorption spectroscopy in the us-to-ms time range identified a single intermediate for each conversion direction. Three dimensional structure of both parental and photoproduct state could be solved (with 1.6 and 1.8 Å resolution), and also an intermediate state was trapped with a chromophore isomerized into the red-absorbing state and the protein surrounding still fixed in the green-absorbing state. The observable fluorescence, the small size, and the efficient photochemistry make CBCR-GAF promising tools for optogenetic applications.

18.5 Gaillard, ER*; Tournear, JC; Northern Illinois University; gaillard@niu.edu

Investigating the Cell Death Mechanisms of ARPE-19 Cells using Modified ARPE-Derived ECM to Model Aging and Disease

Purpose: RPE cell death is a symptom of age related macular degeneration (AMD), but it is unclear what mechanisms of cell death are involved in this process. This study aims to modify ARPE-derived extra cellular matrix (ECM) in order to investigate the ARPE-19 cell death mechanism associated with various stresses modeling both age-related modifications and inflammation. Methods: A series of modifications were performed on ECM derived from ARPE-19 cells to model aging and inflammation. These modifications included non-enzymatic glycation, A2E, blue light irradiated A2E and nonenzymatic nitration. These modifications were done on ECM coating 24-well plates. Post modification, healthy ARPE19 cells were seeded onto the ECM and allowed to attach for 30 min. Unattached cells are removed and fixed for further study. Attached cells were allowed to grow prior to viability analysis. After 24 hours of growth, both unattached and attached cells were stained with Annexin-V and PI then subjected to analysis using flow cytometry to investigate apoptosis versus necrosis as mechanisms of cell death. Results: Cell viability is observed to decrease under all stresses when compared to unmodified ECM. Cells grown on blue light irradiated A2E modified ECM showed not only a loss in viability, but also a change in proliferation and cell morphology. There was evidence of both apoptosis and necrosis as mechanisms of cell death. When analyzing both the unattached and attached simultaneously, a further decrease in cell viability was observed suggesting that attachment to modified ECM is impaired. Conclusions: These data suggest that more than one mechanism of cell death is involved under aging and disease conditions. Both necrotic and apoptotic cells were observed supporting the idea that cells have difficulty in both cell attachment and proliferation. This study provides insight into the mechanisms of RPE cell death in AMD and can help to further understand the pathogenesis of the disease.

26.2 Gallagher, KD; Mihalas-Sanchez, E; Mapara, A; Duong, P; Nugent, A; Bizhga, D; Patel, H; Woitowich, NC; Stojkovic, EA*; Northeastern Illinois University; e-stojkovic@neiu.edu

Phytochromes in Myxobacteria: Implications for Light-Controlled Morphogenesis

Bacteriophytochromes (BphPs) are red-light photoreceptors that require biliverdin (BV), open-chain tetrapyrrole as a cofactor for photoactivity. BphPs belong to a large phytochrome family of photoreceptors found in various plants and microorganisms but their role in non-photosynthetic organisms remains largely unknown. Here we present the first structural and functional characterization of two BphPs from the non-photosynthetic myxobacterium *Stigmatella aurantiaca*. Among prokaryotes, myxobacteria are distinguished by a unique multicellular stage in their life-cycle in which fruiting bodies are formed. In contrast to closely related *Myxococcus xanthus*, which forms fruiting bodies in the dark, *S. aurantiaca* produces orange-pigmented fruiting bodies only in the presence of light. Besides BphPs, the *S. aurantiaca* genome annotation indicates the presence of a putative heme oxygenase, which is essential in BV synthesis. However, the genome of *M. xanthus* completely lacks BphPs and heme oxygenase genes. Our hypothesis is that BphPs may play a role in the fruiting body formation of *S. aurantiaca*. Like classical BphPs, SaBphP1 and SaBphP2 are composed of a photosensory module covalently linked to a histidine kinase. They share 41% sequence

identity, both bind BV and undergo red to far-red light (Pr/Pfr) photoconversion. Unlike classical BphPs, including SaBphP2, wild-type SaBphP1 lacks a highly conserved His that stabilizes BV, a feature also common to other myxobacterial BphPs. Interestingly, SaBphP1 undergoes limited Pr/Pfr photoconversion that can be restored by a single Thr (Thr289) to His mutation in the BV-binding photosensory module. Currently, we are investigating the role of BphPs in fruiting body formation by inactivating/mutating genes coding for BphPs and screening for expected phenotypes. Our goal is to determine what mechanistic changes accompany light-induced morphogenesis in myxobacteria and the novel role of photoreceptors in these non-photosynthetic microorganisms.

PI.22 Garc a Calavia, P*; Mar n, MJ; Chambrier, I; Cook, MJ; Russell, DA; University of East Anglia; P.Garcia-Calavia@uea.ac.uk

Zinc Phthalocyanine functionalised gold nanoparticles for photodynamic cancer therapy

Phthalocyanines (Pcs) are widely used photosensitisers for photodynamic therapy (PDT). Such molecules predominantly produce singlet oxygen after light excitation. Hydrophobic Pcs have been shown to be ideal photosensitisers for PDT.(1) The main disadvantage of such Pcs is that their hydrophobicity presents problems for in vivo and in vitro delivery.(2) For this reason, numerous studies have focused on the use of nanoparticles as delivery vehicles.(3) Nanoparticles present further advantages as they can be additionally functionalised with targeting ligands that increase selectivity towards cancerous tissue.(2) In this study, gold nanoparticles (AuNPs; ca. 4 nm) were synthesised and functionalised with a mixed monolayer of polyethylene glycol and a zinc phthalocyanine (Pc). Two zinc phthalocyanines were explored. The difference between the two molecules was the length of the carbon chain that connects the Pc to the gold core. The chain was composed of either three (C3Pc) or eleven (C11Pc) carbon atoms. Fluorescence emission intensity was found to be higher for free C11Pc. Conversely, when the Pc was conjugated to AuNPs, higher fluorescence emission intensity was observed for C3Pc. These results open the possibility for an increased production of singlet oxygen and better cytotoxic effects. A comparison between C3Pc and C11Pc based AuNPs was studied in vitro using SK-BR-3 human breast adenocarcinoma cells, with and without the presence of a breast cancer specific targeting agent. Results on cell viability show a significant difference between C3Pc and C11Pc when they are not functionalised with the targeting ligand. Conversely, the functionalisation of the AuNPs with the specific anti-HER2 antibody leads to similar photodamage by both Pc systems. (1) Josefsen, L. B. et al., *Theranostics* 2012, 2, 916. (2) Obaid, G. et al., *Angew. Chem., Int. Ed.* 2012, 51, 6158. (3) Chatterjee, D. K. et al., *Adv. Drug Delivery Rev.* 2008, 60, 1627.

PI.6 Gasparro, FP; Hamden Hall Country Day School; fgasparro@hamdenhall.org

What is Photobiology? An Introduction for High School Students.

This year a group of very talented Tampa area high school students will be attending our meeting for a day. They will be the Tampa Area Science Fair Finalists and they will be setting up their posters for our expert scientists to view. In addition, they have been invited to attend a special symposium in which several ASP members will be asked to present their work at a high school level. In this introductory talk, I will give an overview of the basics of photochemistry and photobiology so that the students will be familiar with some of the terminology and elementary principles. The primary purpose of this symposium is to introduce these talented students to the idea of conducting photobiology research and thus to consider choosing photobiology as a field of study and career

6.1 Gasparro, FP; Hamden Hall Country Day School; fgasparro@hamdenhall.org

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PI.19 Ghogare, AG*; Miller, JM; Mondal, B; Lyons, AM; Cengel, KA; Busch, TM; Greer, A; Brooklyn College and The Graduate Center of the City University of New York, University of Pennsylvania, College of Staten Island and The Graduate Center of the City University of New York; aghogare@brooklyn.cuny.edu

"All-in-One' Photodynamic Device and its Performance for In-vivo Sensitizer Release

This poster describes progress in the development of an all-in-one PDT device for local delivery of sensitizer, oxygen and illumination. Questions we sought to address included: Does the silica device tip biofoul? Do cells adhere to the tip and impede sensitizer photorelease? Does fluorination of the tip increase biofouling resistance in the pointsource PDT technique? In this regard, we have used biomaterial (e.g., proteins, cells, etc.) from SQ20B head and neck tumors and whole blood for an assessment of fouling of the silica tips by adsorption. It was shown that by exchanging the native silica tip for a fluorinated tip, a better non-stick property led to an increased sensitizer output by ~10%. The fluorinated tip gave a sigmoidal photorelease where singlet oxygen is stabilized to physical quenching, whereas the native silica tip with unprotected SiO²H groups gave a slower (pseudolinear) photorelease. A further benefit from fluorinated silica is that 15% less biomaterial adheres to its surface compared to native silica based on a bicinchoninic acid assay (BCA) and X-ray photoelectron spectroscopy (XPS) measurements. We discuss how the fluorination of the device tip increases biofouling resistance and can contribute to a new pointsource PDT tool.

2.6 Ghogare, AA*; Debaz, C; Abramova, I; Greer, E; Oliveira, MS; Prado, FM; Di Mascio, P; Greer, A; Brooklyn College and Graduate Center of the City University of New York, Brooklyn College, Baruch College, University of Sao Paulo; aghogare@brooklyn.cuny.edu

An Unusual Photooxygen-Atom Exchange Reaction of Nitrosamines with Molecular Oxygen: Dependence on Nitrosamine Substituents

Despite the decades-long interest in N-nitrosamine organic chemistry and toxicity, no photochemical oxygen atom exchange process with molecular oxygen has been reported. Little is known of the peroxy intermediates involved in the direct photolysis of nitrosamines in the presence of molecular oxygen. This presentation describes results on scrambling of oxygen atoms in the photolysis of two of four nitrosamines in the presence of 18-O labeled oxygen gas. HPLC/MS and HPLC-MS/MS data show that 18-O labeled nitrosamines were generated for N-nitrosodiphenylamine and N-nitroso-N-methylaniline. In contrast, nitrosamines N-butyl-N-(4-hydroxybutyl)nitrosamine and N-nitrosodiethylamine do not exchange the 18-O label and instead decomposed to amines and/or imines under the conditions. Our mechanistic proposal is the formation of nitrooxide, hexaoxadiazocane and trioxazetidine intermediates followed by an oxygen extrusion process to account for exchange of the oxygen atom label.

PI.20 Ghogare, AA*; Malek, B; Fang, W; Walalawela, N; Choudhury, R; Liu, Y; Zhao, Y; Xu, Q; Lyons, AM; Greer, A; Brooklyn College and Graduate Center of the City University of New York, Brooklyn College, College of Staten Island and Graduate Center of the City University of New York; aghogare@brooklyn.cuny.edu

Phase Separation of Reactive Oxygen Species: Singlet Oxygen Chemistry at Interfaces

Highlights are described from our studies of interfacial singlet oxygen. The talk will provide examples of surfactant traps for airborne singlet oxygen at the air-water interface (with the sensitizer "in absentia"), and the regioselective formation and characterization of surfactant hydroperoxides. The preparation of a 3-D printed superhydrophobic surface and first example of a triphasic photosensitizer

with regions that are controllably dry, partly wetted, and/or fully wetted will also be discussed. Singlet oxygen was directly detected by its NIR luminescence at 1270 nm at or above the air-liquid surface, in the plastron of the superhydrophobic surface and within water. Singlet oxygen is fascinating, not in a singular way, but from the multiplicity of reactions it undergoes. The above reactions are of utility in synthesis or are biologically relevant models of singlet oxygen at membrane or marine aerosol surfaces.

17.2 Girardi, M*; Deng, Y; Ediriwickrema, A; Lewis, J; Suh, HW; Fong, Linda; Saltzman, WM; Yale University, Yale University; michael.girardi@yale.edu

Engineering Bioadhesive Biodegradable Nanoparticle Encapsulation of Organic Sunscreen Agents to Enhance Their Performance and Safety

Skin cancer is the most common malignancy in the USA, and exposure to the sun's ultraviolet radiation (UVR) is the primary risk factor. Therefore, strategies designed to protect the skin from UVR exposure, including topically applied sunscreens, may markedly decreased the incidence of and costs associated with skin cancer. The ideal sunscreen formulation might be expected to provide all-day, broad UVA/UVB, waterproof/sweatproof protection from a single application, and as well appease any safety concerns by preventing penetration of the organic active ingredients into the skin cells and blood stream. Towards these goals, we have developed and are assessing the efficacy of encapsulation of organic sunscreen agents into biodegradable, bioadhesive nanoparticles (BNPs). BNPs were designed with a polylactic acid core linked to a hyperbranched polyglycerols corona that was terminated with aldehydes after exposure to sodium periodate. Upon application the skin, the BNPs form covalent bonding to the stratum corneum with uniform coverage. In pre-clinical testing, relative to commercially available sunscreen, BNP-encapsulated sunscreen showed superior substantivity, the capacity to prevent any detectable skin absorption of the active organic agent, and protection against UVR-induced cyclobutane pyrimidine dimer formation. Importantly, reactive oxygen species (ROS)-induced DNA damage following UVR exposure was clearly evident with commercial sunscreen, but owing to the prevention of penetration, absent with the BNP formulation. Thus, formulations utilizing BNP-encapsulation may enhance the performance and safety of the currently approved sunscreen agents.

6.14 Girardi, M*; Deng, Y; Ediriwickrema, A; Lewis, J; Suh, HW; Fong, Linda; Saltzman, WM; Yale University, Yale University; michael.girardi@yale.edu

Engineering Bioadhesive Biodegradable Nanoparticle Encapsulation of Organic Sunscreen Agents to Enhance Their Performance and Safety

Skin cancer is the most common malignancy in the USA, and exposure to the sun's ultraviolet radiation (UVR) is the primary risk factor. Therefore, strategies designed to protect the skin from UVR exposure, including topically applied sunscreens, may markedly decreased the incidence of and costs associated with skin cancer. The ideal sunscreen formulation might be expected to provide all-day, broad UVA/UVB, waterproof/sweatproof protection from a single application, and as well appease any safety concerns by preventing penetration of the organic active ingredients into the skin cells and blood stream. Towards these goals, we have developed and are assessing the efficacy of encapsulation of organic sunscreen agents into biodegradable, bioadhesive nanoparticles (BNPs). BNPs were designed with a polylactic acid core linked to a hyperbranched polyglycerols corona that was terminated with aldehydes after exposure to sodium periodate. Upon application the skin, the BNPs form covalent bonding to the stratum corneum with uniform coverage. In pre-clinical testing, relative to commercially available sunscreen, BNP-encapsulated sunscreen showed superior substantivity, the capacity to prevent any detectable skin absorption of the active organic agent, and protection against UVR-induced cyclobutane pyrimidine dimer formation. Importantly, reactive oxygen species (ROS)-induced DNA damage following UVR exposure was clearly evident with commercial sunscreen, but owing to the prevention of penetration, absent with the BNP formulation. Thus, formulations utilizing BNP-encapsulation may enhance the performance and safety of the currently approved sunscreen

agents.

5.3 Givens, Richard S.*; Elles, Christopher G.; Houk, Amanda L.; University of Kansas; givensr@ku.edu

Expanding The Range Of Caged Bioinitiators: 2-PE (500 - 700 nm) Excitation Of p-Hydroxyphenacyl Caged ATP

The photoremovable protecting group, p-HydroxyPhenacyl (pHP), is demonstrably effective at delivering biological ligands for investigations such as triggering nucleotide release of ATP and GTP that activate ATPase and GTPase signal protein action associated with GAP protein which controls cell growth. Likewise, release of GABA was used to reveal the effect of GABA spillover during axon development (plasticity) during activation of the Lateral Superior Olive (LSO) to GABAergic receptors on the Medial Nucleus of the Trapezoid Body (MNTB) in the brainstem of newborn auditory systems. Developing methods to explore the intricate mechanisms, including the physical parameters of kinetic rate constants and activation barriers, requires tools that control the temporal, spatial and quantity of initiator released. The pHP chromophore has been used to release an impressive array of bioactive substrates ranging from phenols and carboxylates to phosphates and sulfonates. The release occurs within a nsec in near quantitative yield with 10 to 100% efficiency. Whereas all previous applications depended on one-photon excitation at 280 - 330 nm, we now demonstrate the same photochemistry using a Ti:Sapphire pulsed laser for broadband two-photon absorption (2-PA). The 2-PA measurements reveal a strong transition at 4.5 eV (550 nm), the lowest-energy bands of p-hydroxyacetophenone and pHP diethyl phosphate with cross sections of $\sigma_{2PA} = 23 \pm 11$ and 11 ± 6 GM, respectively. These 2-PA absorptions are superimposable on the 1-PA spectra of pHP at ~310 nm. This has been extended to pHP ATP, a classic caged initiator. The 2-PA absorption spectra are consistent with excitation to the same S3 ($1\ddot{I}\ddot{I}^*$) excited state for both 1- and 2-PA activation. Two-photon uncaging is now possible using the visible light range 500-720 nm even at basic pH ~8.0. The fundamental spectroscopy, mechanism and representative applications will be discussed. Houk, Givens, Elles, J. Phys. Chem., 2016, 107, 0000.

9.2 Glaser, AK; Chen, Y; Liu, JTC*; UW Seattle; jonliu@uw.edu

Design and optimization of optical-sectioning microscopes via realistic simulations of diffraction, refraction, and scattering in tissues

There has been great interest in developing optical-sectioning microscopes, including light-sheet microscopes, for sub-cellular-resolution imaging of tissues both for biological investigations and clinical applications. Unfortunately, conventional Monte-Carlo tissue scattering simulations do not account for certain effects that are often significant for high-resolution tissue microscopy, such as diffraction and refractive beam-steering/aberrations. Therefore, our group has been developing various improved simulation methods to more-realistically investigate and optimize the performance of microscopes for disease detection and surgical guidance. We will discuss the advantages of these techniques, including an enhanced Monte-Carlo technique and a fractal model of refractive-index turbulence, in the context of confocal and light-sheet microscopes being developed within our group.

2.1 Glazer, EG*; Heidary, DK; Wachter, E; Sun, Y; U Kentucky; ec.glazer@uky.edu

Phototherapy and Photoreporters " Efforts Towards Inorganic Therapeutics and Research Tools

The discovery and development of new therapeutic agents is slowed by complications from off-target effects and difficulties in correctly identifying mechanism of action, along with mechanisms for undesired toxicity. Many cytotoxic metal complexes interact with or damage DNA. While this provides for effective cell killing, the non-selective mechanism leads to general toxicity in terms of impacts on healthy tissues, and off-mechanism effects provide another danger. A promising approach is to develop compounds capable of targeting and damaging key biological molecules of particular importance in a specific disease, such as specific nucleic acid structures, sequences, or proteins.

Agents that can be induced to form covalent bonds to their targets using light as an external trigger will be discussed, as such systems would permanently damage or inactivate essential biological molecules in a spatially limited treatment area. We are developing a research platform to aid in the advancement of a family of coordination complexes that can form covalent bonds only upon irradiation with visible to near-IR light. These compounds can be designed to target different nucleic acids or proteins within the cell, depending on the compounds' structure and charge. We are also developing reporter assays that provide functional information on various essential cellular processes to elucidate both the mechanism of action and potential off-target, off-mechanism toxicity.

25.4 Gollnick, SO; Roswell Park Cancer Institute; sandra.gollnick@roswellpark.org

PDT Enhanced Anti-Tumor Immunity: Mechanisms and Exploitation

Photodynamic therapy (PDT) enhancement of anti-tumor immunity has been demonstrated in both clinical and pre-clinical settings. The ability of PDT to enhance anti-tumor immunity is thought to be due to several factors including stimulation of immunogenic cell death, release of tumor-specific antigens, induction of acute inflammation and over-whelming of the host's clearance system. Interestingly PDT-enhancement of anti-tumor immunity appears to be regimen dependent suggesting that regimens that optimally enhance anti-tumor immunity can be developed. These findings suggest that PDT may provide both local and systemic control of disease and that PDT has the potential to be used in combination with other anti-cancer modalities that lack immune enhancing abilities. However, several recent studies have shown that enhancement of anti-tumor immunity can also result in immune escape via simultaneous enhancement of immune regulatory molecules. These studies suggest that combination of immune-enhancing PDT regimens with blockade of immune regulatory molecules may increase control of local and distant disease. Data supporting each of these concepts will be presented in this overview seminar.

26.6 Gomelsky, M; GOMELSKY, MARK; University of Wyoming; gomelsky@uwyo.edu

Synthetic Photobiology in Near-infrared

Several branches of medicine, including cancer immunotherapy, neurology and regenerative medicine, are rapidly adapting the use of engineered cells. A major concern in using engineered cells lies in our poor ability to control them. Synthetic photoregulated systems responsive to light in the near-infrared optical window (NIRW; ~670-900 nm) can help overcoming this problem. NIRW light is harmless and penetrates deep into mammalian tissues. Microbial photoreceptors, bacteriophytochromes, naturally respond to NIRW light. We will present several types of engineered bacteriophytochrome-based optogenetic (synthetic photobiology) tools. One toolset involves NIRW light-activated nucleotide cyclases that can be used to control cAMP and cGMP levels with high spatial and temporal precision. These cyclases have been engineered by fusing bacteriophytochrome photosensory modules with homodimeric nucleotide cyclases. We have engineered NIRW light-activated adenylate cyclases with various levels of enzymatic activities and different lit form stabilities. Another set of engineered bacteriophytochrome-based tools involves NIRW light-dependent systems to control gene expression in mammalian cells. Two prototype systems will be discussed. One system is based on the NIRW light-activated cyclic dinucleotide-mediated expression, while another one is based on the NIRW light-dependent bacterial antirepressor-repressor system. Performance of the newly developed bacteriophytochrome tools will be discussed.

P2.15 Gomes, MC*; Cunha, A; Tome, JPC; Trindade, T; G, Zheng; University of Aveiro, University of Lisbon, University Health Network Toronto, University of Toronto ; clara.gomes@ua.pt

Carbohydrate Coated Gold Nanoparticles as SERS probes for Confocal Raman Microscopy of Cancer Cells

Amongst the wide variety of imaging techniques used in Biology, Confocal Raman Microscopy (CRM) has been used as one of the most relevant techniques considering its high sensitivity and non-

destructive nature. This technique combines the Raman spectroscopy features, allowing the characterization of specimens in terms of their chemical composition, with the Confocal Microscopy. As a result, a mapping image can be acquired, assessing the distribution of a specific specimen inside a cell without the need for staining. Here, Gold Nanoparticles (AuNP) has earned an outstanding role being used as transducers for Surface-Enhanced Raman Scattering (SERS). Due to their easy surface functionalization, AuNP can be coated with a Raman reporter molecule and at the same time functionalized with several biomolecules, for increased selectivity. During the last years, AuNP-based SERS probes have been used to monitor several cellular responses to outside stimulus or even to detect cancer distribution in tissues. Intending to develop novel AuNP-based SERS probes with specificity to cancer cells, we synthesized a carbohydrate (glucose and galactose) coated 60 nm AuNP (AuNP@Glu and AuNP@Gal) and tested their ability as SERS probes using CRM. For this purpose, we used Rat 9Lluc glioma as cancer cells model and evaluated the AuNP surface nature in terms of uptake efficiency and as SERS probes for Bioimaging. The experimental uptake extent, unveiled that AuNP@Gal presented higher uptake over the glucose ones. This can be explained by the higher levels of galectin-1 observed by Western blot, on the 9Lluc glioma cells membrane, when compared with the GLUT-1 levels. However, due to the high sensibility of CRM and the surface enhancement signal provided by the gold, we were able to observe both AuNP SERS signal and mapping it inside the 9Lluc glioma cells. Moreover, it was possible to observe the AuNP distribution inside the cells, being notorious their absence in the nucleus. This work showed that carbohydrates coated AuNP can be used as SERS probes for cancer cells, allowing us to take advantages from a new type of microscopy, and opening new prospects for the use of these SERS probes for in vivo tumour identification. M. C. Gomes thanks FCT for the grant SFRH/BD/88334/2012. Thanks are due to FCT and FEDER, for funding the project PTDC/CTM/101538/2008, QOPNA (Pest-C/QUI/UI0062/2011), CICECO (Pest-C/CTM-LA0011/2011) and CESAM (PEst-C/MAR/LA0017/2011) research units. Thanks are also due to Terry Fox Research Institute, the Princess Margaret Cancer Foundation, the Canadian Institutes of Health Research, the Ontario Institute for Cancer Research, the Natural Sciences and Engineering Research Council of Canada, the Canada Foundation for Innovation.

29.5 Gottlieb, R; College of Syntonic Optometry; raygottlieb@me.com

Syntonic Phototherapy - an Introduction

Syntonic phototherapy uses low-level, non-coherent, non-polarized, broad-band filtered light delivered through the eyes. The eyes permit nearly direct, non-invasive application of light to the reservoir of blood circulating through the retina as well as to non-visual, retinal photoreceptor systems that signal circadian and other brain centers. This input is thought to rebalance and resynchronize poorly functioning homeostatic, autonomic and circadian causes of visual weakness. Syntonics is especially effective in treating brain injury, headache, strabismus, eyestrain, eye pathology, and attention and learning dysfunctions. Patients typically look at prescribed colors for 20-minute periods, several times a week for twenty sessions. Special visual field, pupil, and binocular vision tests, along with patient symptoms and medical history determine the syntonic prescription, progress and final outcome measures. Presentation will include discussion of kinetic and color visual fields, pupil fatigue, theoretical and historical background, and will review cases showing initial and post-treatment data and case resolution. Optometrists, ophthalmologists and psychologists in many countries currently practice this 85 year-old therapy.

25.1 Griffioen, AW*; Huijbers, EJ; Nowak-Sliwinska, P; van Beijnum, JR; VU Medical Center; aw.griffioen@vumc.nl

Targeting the tumor vasculature; extracellular vimentin as an ideal target for the treatment of cancer

The identification of specific markers of the tumor vasculature is of key importance. We have identified the intermediate filament protein vimentin as being overexpressed in colorectal carcinoma endothelial cells, as compared to normal colon- and angiogenic placenta endothelial cells. Although

vimentin is known to be a cytoskeletal protein, evidence accumulates that it is not exclusively an intracellular protein. Here, we demonstrate that vimentin is excreted by, and expressed at the surface of, angiogenically activated endothelial cells, and that this externalization of vimentin is dynamic. Surface expressed vimentin is involved in migration and sprouting and can be targeted using antibodies to inhibit these processes. Furthermore, targeting tumor endothelial vimentin in a preclinical tumor model using a monoclonal antibody impairs tumor angiogenesis and tumor growth. The role of a splice variant of vimentin will be discussed, as well as the translational development towards a clinically used treatment regimen against cancer. Both combination of such treatment with phototherapy and the use of extracellular vimentin as marker to target photosensitizers will be discussed in this presentation.

P2.4 Gupta, S*; Chakraborty, S; Clark, RJ; Saltiel, J; Florida State University; sgupta2@fsu.edu

Photochemistry of Cholestatrienol - a Fluorescent Analogue of Cholesterol

Cholestatrien-3 β -ol (CTL) is a natural product(ref.1) that has found considerable use as a fluorescent probe for cholesterol intracellular trafficking(ref.2). Although known since 1930, the photochemical reactivity of dehydroergosterol(ref.3), an analogue of CTL, has been ignored. We encountered CTL as a trace impurity in 7-dehydrocholesterol, the precursor in the synthesis of vitamin D3. As it is highly fluorescent, it interfered with our initial emission studies in the vitamin D3 field. Its use in biochemical applications prompted us to investigate its photoproducts. Irradiation of CTL (313 nm) in ethanol indicated the formation of a photoproduct, whose UV spectrum is consistent with an s-trans-diene moiety that could form by photoaddition of ethanol to one of CTL's double bonds. Subsequent irradiation of CTL in tetrahydrofuran gave the same product as in ethanol proving that photoaddition is not the major photoreaction. 1D 1H, 2D COSY NMR and UV-Vis. and MALDI spectra of this major photoproduct are consistent with CTL photorearrangement to pentacyclic: (3aR,9aS)-3-(6-hydroxy-6-methylheptan-2-yl)-3a,9a-dimethyl-1,2,3a,4,6,7,8,9,9a,9b,10b-dodecahydrobenzo[2,3]cyclopropa[1,2-b]-asindacen-7-ol. The structure assignment of this pentacyclic product was definitively established by X-ray crystallography. References: 1.Wassif, CA; Brownson, KE; Sterner, AL; Forlino, A; Zervas, PM; Wilson, WK; Starost, MF; Porter, FD (2007) Hum. Mol. Genet. 16, 1176-1187. 2.Scheidt, HA; Muller, P; Herrmann, A; Huster, D (2003) J. Biol. Chem. 278, 45563-45569. 3.Morton, RA; Heilbron, IM; Spring, FS (1930) Biochem. J. 24, 136-140. (Moved from P1.14)

25.6 Hasan, T; Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School; thasan@mgh.harvard.edu

Combination treatments in cancer therapeutics: role of spatiotemporal synchronization

This talk will introduce platforms for cancer combination therapy that utilizes near infrared light activation not only for photodynamic damage but also as an extrinsic mechanism to initiate release of complimentary drugs to suppress dynamic bursts in molecular signaling networks that promote tumor cell survival and treatment escape. The targets are determined not only by the intrinsic biology of cancer cells but also by responses to external stimuli that change the microenvironment and induce stress. The goal is to achieve optimal therapeutic response by delivery/co-delivery with concomitant activity of photodynamic, molecular inhibitor and chemotherapeutic agents, selectively within the tumor in response to the microenvironment changes. This approach overcomes challenges in achieving synergistic interactions using sequential drug delivery, which is compromised by the differential pharmacokinetics of individual agents.

16.7 Hashimoto, H*; Yukihiro, H; Sugai, Y; Fujiwara, M; Iha, M; Sakaguchi, K; Katsumura, S; Gardiner, AT; Cogdell, RJ; Kwasnei Gakuin University, South Product, Osaka City University, University of Glasgow; hideki-hassy@kwansei.ac.jp

Incorporation of Algal Carotenoids into the Light-Harvesting System from a Purple Photosynthetic

Bacterium

Fucoxanthin (Fx) is a carotenoid that is mainly bound to the light-harvesting complexes (LHCs) from algae. It produces intra-molecular charge transfer (ICT) excited-state under polar environment following photoexcitation up to the excited state. This ICT state plays a key role for the highly efficient energy-transfer from Fx to chlorophylls in the LHC from algae. In this study we were successful, for the first time, to incorporate Fx into the LHCs from a purple photosynthetic bacterium, *Rhodospirillum rubrum* G9+ (a carotenoidless strain). Femtosecond pump-probe spectroscopy was applied thus fabricated reconstituted LH1 complex in order to discuss the Fx to bacteriochlorophyll energy-transfer.

17.5 He, Yu-Ying; ; yyhe@medicine.bsd.uchicago.edu

Autophagy regulates UV-induced DNA damage repair and controls photocarcinogenesis

Autophagy is a cellular catabolic process that is essential for maintaining tissue homeostasis and regulating various normal and pathologic processes in human diseases including cancer. One cancer-driving process is accumulation of genetic mutations due to impaired DNA damage repair, including nucleotide excision repair that removes DNA damage caused by UV radiation and other chemicals. Here we show that autophagy positively regulates nucleotide excision repair through enhancing DNA damage recognition by the DNA damage sensor proteins XPC and DDB2 via two pathways. First, autophagy deficiency down-regulates the transcription of XPC through TWIST1-dependent activation of the transcription repressor complex E2F4/RBL2. Second, autophagy deficiency impairs the recruitment of DDB2 to UV-induced DNA damage sites through TWIST1-mediated inhibition of EP300. In mice, the pharmacological autophagy inhibitor Spautin1 promotes UVB-induced tumorigenesis, whereas the autophagy inducer rapamycin reduces UVB-induced tumorigenesis. These findings demonstrate the crucial role of autophagy in maintaining proper nucleotide excision repair in mammalian cells and suggest a previously unrecognized tumor-suppressive mechanism of autophagy in cancer.

20.3 He, Yu-Ying; University of Chicago; yyhe@medicine.bsd.uchicago.edu

UV-induced inflammation at the molecular, cellular, and organismal levels

Skin cancer is the most common cancer in the US. The incidence of skin cancer continues to rise at an alarming rate annually. Exposure to ultraviolet B radiation (UVB, 280-315 nm) from the sun is the major environmental risk factor for skin cancer. UV radiation causes DNA damage and lead to accumulation of oncogenic mutations. In addition, UV also damages self noncoding RNA to mediate acute the inflammatory response, collectively known as sunburn. In humans, sunburn sensitivity is positively associated with increased risk of skin cancer including both non-melanoma skin cancer and melanoma. However, the mechanism in regulating UV-induced inflammation is not well understood. Here we show the molecular basis for regulation of UV-induced inflammation at the molecular, cellular, and organismal levels.

P2.16 Hill, HZ; Rutgers NJ Medical School; hill@njms.rutgers.edu

Keeping Tabs on Your Lab: Recognition and Detection of Data Manipulation

Over the last 10 years, there has been a marked increase in retractions of articles in the scientific literature. Even more unsettling is the fact that well over half of these retractions are due to scientific misconduct. Scientists are increasingly becoming aware that not all data generated in their laboratories is reflective of the truth. In order to make certain that results have not been influenced by the experimenter(s), the wise chief can employ certain tests to ascertain that no tampering of results has occurred. Data alteration can fall into at least 3 categories: plagiarism, image manipulation and numerical fabrication. Commercial products are available to test for plagiarism and image alteration. An excel spreadsheet has been developed that allows for the easy testing of three types of number manipulation that might be encountered when counting cells in suspension or colonies. Examples will

be shown to demonstrate image rearrangements and suspicious numbers. Ways to avoid these pitfalls will be discussed.

4.2 Huang, HC*; Rizvi, I; Liu, J; Hasan, T; Massachusetts General Hospital and Harvard Medical School; hhuang11@partners.org

Repurposing Tetracyclines To Potentiate A Bi-Directionally Interactive Combination Of Photodynamic Therapy And Irinotecan For Cancer

It is becoming clear that the most effective treatments for complicated cancer will involve interactive regimens that target several non-overlapping pathways, preferably such that each component enhances the others to improve outcomes while minimizing side effects. Towards this goal, we developed a combination of photodynamic therapy (PDT) and irinotecan, where each component reinforces the others beyond their individual tumor destruction pathways, to synergistically reduce tumor burden and prolong animal survival. Furthermore, we repurposed FDA-approved tetracycline antibiotics, which have been shown to affect a range of targets in cancer, to overcome resistance that may render irinotecan treatment ineffective. This repurposing of FDA approved, non-cancer drugs presents an opportunity to design rapidly-translatable, mechanism-based therapies for cancer. The results will highlight insights into the cooperative effect of combination PDT and irinotecan, as well as the repurposed tetracyclines on the treatment response and molecular markers, in challenging mouse models of pancreatic and ovarian cancers.

20.1 Imokawa, G*; Terazawa, S; Mori, S; Yasuda, M; Nakajima, H; Chubu University, Tokyo University of Technology; imokawag@dream.ocn.ne.jp

New Evidence for an Essential Role of the p38/MSK1/NFkappaBp65Ser276 Axis during Intracellular Signaling Pathways Leading to UVB-induced Cutaneous Inflammation and Hyperkeratinization

UVB exposure is the most harmful stimulus that deteriorates healthy skin. These effects mainly include cutaneous inflammation and abnormal keratinization where prostaglandin E2, cyclooxygenase (COX)2, macrophage granulocyte colony stimulatory factor (GM-CSF), interleukin (IL)-8 and transglutaminase 1 (TGase1), play pivotal roles. The stimulated expression of those factors has been implicated to be highly associated with the signaling axis leading to the facilitated translocation of NFkB from the cytosol to nuclei during UVB-activated signaling cascades. However, the possible therapeutic interruption of those signaling cascades has a definite risk because inhibiting many of the signaling factors, such as p38, ERK and JNK is required for complete interruption but may also abolish constitutive anti-UVB carcinogenetic cascades, such as apoptotic signaling. Fortunately, we have discovered an interesting phenomenon where the specific inhibition of only mitogen-stress activated protein kinase (MSK)-1, is attainable by astaxanthin (AX) or by MSK1 inhibitor H89 and results in a remarkable abrogation of the increased expression of those inflammatory and keratinization factors. We found that post-irradiation treatment with AX or H89 significantly abrogates the increased expression of COX2, PGE2, GM-CSF, IL-8 and TGase1, which is accompanied by a specific abrogation of the phosphorylation of MSK1 and NFkappaBp65Ser276 without any suppressive effects on the activation of p38, ERK, JNK and casein kinase or on the stimulated translocation of NFkB from the cytosol to nuclei in UVB-exposed human keratinocytes. We have also confirmed an association between the specific inhibition of MSK1 activation and the attenuated expression of TGase1 by silencing MSK1 in UVB-exposed human keratinocytes. This suggests an essential role of the p38/MSK1/NFkappaBp65Ser276 axis during intracellular signaling pathways leading to UVB-induced cutaneous inflammation and hyperkeratinization.

PI.15 Ito, H*; Tamura, M; Matsui, H; Indo, HP; Majima, MJ; University of Tsukuba, Kagoshima University; toi.homer@gmail.com

Mitochondrial Reactive Oxygen Species Accelerated Cellular Uptake of 5-Aminolevulinic Acid in

Gastric Cancer Cells

5-aminolevulinic acid (ALA), which is a precursor of heme, has been clinically used for photodynamic therapy (PDT) and photodynamic diagnosis (PDD). Cancer cell specific porphyrin accumulation used to be involved by ALA treatment, whereas a mechanism had not been elucidated. There could be following three mechanisms for porphyrin accumulation in cancer: Inhibition of ALA metabolism, Promotion of ALA uptake, and Inhibition of porphyrins. We recently reported that over-generation of endogenous nitric oxide (NO) by gene transfection of inducible NO synthase (iNOS) inactivated ferrochelatase, which is an enzyme to chelate iron into porphyrin structure, to enhance porphyrin accumulation. Thus, this mechanism is likely to contribute cancer specific porphyrin accumulation. However, the uptake level and the pathway of ALA were not unclear. It is known that a membrane transporter, PEPT1 is a major transporter of ALA and is expressed in gastric cancer. We have been confirmed that a gastric cancer cell line over-generate reactive oxygen species from mitochondria (mitROS). We hypothesized that mitROS affected upregulation of PEPT1 and accelerated cellular uptake of ALA. In this study, we clarified that mitROS induced PEPT1 expression and cancer cell specific uptake of ALA using following cell lines: rat gastric mucosal cells RGM1, its cancer-like mutated cells RGK1, and manganese superoxide dismutase (MnSOD) overexpressed RGK cells RGK-MnSOD. Since MnSOD is expressed in mitochondria, mitROS can be specifically scavenged. We demonstrated cancer specific upregulation of PEPT1 and observed downregulation in RGK-MnSOD cells. Additionally, radio-labelled ALA was incorporated in RGK1 rather than RGK-MnSOD. In conclusion, cancer cellular mitROS induced PEPT1 upregulation and acceleration of ALA uptake. In addition, we are now investigating about porphyrin excretion from cells.

P2.17 Ito, H; Tamura, M; Nagano, Y; Matsui, H*; Indo, HP; Majima, HJ; University of Tsukuba, Kagoshima University; hmatsui@md.tsukuba.ac.jp

Indomethacin-Derived Mitochondrial Reactive Oxygen Species Accelerated Cancer Specific Porphyrin Accumulation to Enhance Photodynamic Therapeutic Effect in Gastric Epithelial Cells

Photodynamic therapy is useful for the treatment of cancer because it is minimally invasive for patients. Certain porphyrin compounds and their derivatives have been used as the photosensitizer because they accumulate specifically in cancerous tissues. However, the detailed mechanism of this phenomenon has not been clarified. We previously reported that a proton-coupled folate transporter, HCP1, transported porphyrins and that regulation of the protein was associated with cancer-specific reactive oxygen species from mitochondria (mitROS) (Hiyama K et al. J Porph Phtal. 2013; 17: 36-43). Therefore, over-generation of mitROS could increase HCP1 expression and the effect of photodynamic therapy. We investigated whether pretreatment with indomethacin influenced photodynamic therapy by using a rat normal gastric mucosal cell line, RGM1, its cancer-like mutated cell line, RGK1, and a manganese superoxide dismutase (MnSOD)-overexpressed RGK cell line, RGK-MnSOD. Indomethacin promotes the generation of cellular mitROS by inhibiting the electron transport chain, and MnSOD scavenges the mitROS. We elucidated that indomethacin enhanced cancer-specific mitROS generation and increased HCP1 expression. Furthermore, RGK1 cells showed higher cellular incorporation of hemato-porphyrin and better therapeutic effect with indomethacin treatment whereas RGK-MnSOD cells did not show a difference. Thus, we concluded that indomethacin improved the effect of photodynamic therapy by inducing increased mitROS generation in cancer cells.

13.4 Jarrett, SG; Wolf Horrell, EM; D'Orazio, JA*; Univ. KY; jdorazio@uky.edu

Hormonal regulation of the repair of UV photodamage in melanocytes by the MSH-MC1R signaling axis

UV radiation represents a major causative risk factor for melanoma by promoting the formation of UV signature mutations. Nucleotide excision repair (NER), the genomic maintenance pathway responsible for clearing UV photodamage to prevent mutagenesis and malignant degeneration, is regulated

hormonally by melanocyte stimulating hormone (MSH) and the melanocortin 1 receptor (MC1R). Loss-of-signaling MC1R polymorphisms that lead to defective cAMP second messenger generation are major inherited risk factors for melanoma development in part because of suboptimal NER and higher rates of UV mutagenesis. We have determined that the MC1R-cAMP signaling axis enhances NER through activation of cAMP-dependent protein kinase (PKA) and phosphorylation of ataxia telangiectasia and Rad3-related protein (ATR) on the S435 residue. This post-translational event recruits the key NER factor XPA to ATR and together, XPA and p-S435 ATR efficiently localize to sites of UV photodamage in chromatin to facilitate NER. The MC1R agonists melanocyte stimulating hormone (MSH) or adrenocorticotrophic hormone (ACTH) efficiently promote generation of p-S435 ATR, accelerate repair of UV photoproducts and reduce UV mutagenesis. In contrast, MC1R antagonists agouti signaling protein (ASIP) or beta-defensin 3 (BD3) inhibit PKA-mediated ATR phosphorylation, impair NER and increase UV mutagenesis. Our data suggest that melanocytic NER is directly influenced by MC1R signaling ability and the presence of MC1R agonists and antagonists in the melanocytic milieu.

6.4 Jarrett, SG; Wolf Horrell, EM; D'Orazio, JA*; Univ. KY; jdorazio@uky.edu

Hormonal regulation of the repair of UV photodamage in melanocytes by the MSH-MC1R signaling axis

UV radiation represents a major causative risk factor for melanoma by promoting the formation of UV signature mutations. Nucleotide excision repair (NER), the genomic maintenance pathway responsible for clearing UV photodamage to prevent mutagenesis and malignant degeneration, is regulated hormonally by melanocyte stimulating hormone (MSH) and the melanocortin 1 receptor (MC1R). Loss-of-signaling MC1R polymorphisms that lead to defective cAMP second messenger generation are major inherited risk factors for melanoma development in part because of suboptimal NER and higher rates of UV mutagenesis. We have determined that the MC1R-cAMP signaling axis enhances NER through activation of cAMP-dependent protein kinase (PKA) and phosphorylation of ataxia telangiectasia and Rad3-related protein (ATR) on the S435 residue. This post-translational event recruits the key NER factor XPA to ATR and together, XPA and p-S435 ATR efficiently localize to sites of UV photodamage in chromatin to facilitate NER. The MC1R agonists melanocyte stimulating hormone (MSH) or adrenocorticotrophic hormone (ACTH) efficiently promote generation of p-S435 ATR, accelerate repair of UV photoproducts and reduce UV mutagenesis. In contrast, MC1R antagonists agouti signaling protein (ASIP) or beta-defensin 3 (BD3) inhibit PKA-mediated ATR phosphorylation, impair NER and increase UV mutagenesis. Our data suggest that melanocytic NER is directly influenced by MC1R signaling ability and the presence of MC1R agonists and antagonists in the melanocytic milieu.

P2.18 Jones, RN*; Erhard, S; Gen, A; Malham, M; Olsen, KW; Dale, R; Loyola University Chicago; RJONES10@LUC.EDU

Selective Cell Targeting In Zebrafish Embryos

To attack cancer cells in a specific manner, we have developed a folate-targeted, photodynamic therapy (PDT) agent. The folate (FA) and the chlorin e6 (Ce6) photosensitizer were attached to bovine serum albumin (BSA) using carbodiimide chemistry. Since over 50% of cancers overexpress folate receptors, FA-BSA-Ce6 can be taken up by them and is an effective PDT agent. Cancer cells are not alone in expressing many folate receptors. Embryos need folate and have more folate receptors than adult cells. This suggested that FA-BSA-Ce6 could be used to study development in zebrafish. Zebrafish are good candidates for these studies because they share a high degree of sequence and functional homology with humans and their embryos and larvae are transparent making it easy to see the impact of treatment without invasive techniques. The folate receptor has ~50% amino acid identity between zebrafish and humans. To map the expression of the zebrafish folate receptor, we used reverse transcription-polymerase chain reaction (RT-PCR) on total RNA prepared from 9 embryonic stages. Expression was seen before the zebrafish maternal-zygotic transition, suggesting that folate receptor

mRNA is maternally loaded. Whole-mount in situ hybridization was performed and all of the stages tested with the antisense probe demonstrated the presence of folate receptor mRNA. Stages through 1 day post fertilization (dpf) express the folate receptor globally. By 2 dpf, expression is seen in the head and heart of the embryo. To confirm the presence of the folate receptor protein, 3 dpf embryos were treated with fluorescent-tagged folate. Fluorescent-tagged folate embryos demonstrated fluorescence in the head and cloaca, which is where the mRNA was observed. Embryos at 4 dpf were exposed to 1, 2.5, 5 and 10 μ M FA-BSA-Ce6 and then treated with 1 to 6 min of 660 nm light. The lower concentrations and light times allowed the embryos to recover while the higher ones resulted in non-viable embryos.

P2.19 Justiniano, R*; Park, SL; Williams, JD; Wieland, DR; Wondrak, GT; University of Arizona, UA Cancer Center and College of Pharmacy; wondrak@pharmacy.arizona.edu

Photodynamic Elimination of Melanoma and Nonmelanoma Skin Cancer Cells using the Endogenous Tryptophan Photoproduct 6-Formylindolo[3,2-b]carbazole (FICZ)

Recently, we have demonstrated that 6-formylindolo[3,2-b]carbazole (FICZ), a tryptophan-derived photoproduct and endogenous high affinity aryl hydrocarbon receptor (AhR) agonist generated in skin as a result of UV exposure, displays activity as a nanomolar UVA-photosensitizer. Here, we report that the endogenous photosensitizer FICZ can be used for the photodynamic elimination of human melanoma and nonmelanoma skin cancer (NMSC) cells. Employing a panel of cultured human squamous cell carcinoma (SCC-25) and melanoma skin cancer cells (A375, G361, LOX), photodynamic induction of apoptosis was achieved by the combined action of solar simulated UVA (6.6 J/cm²) and FICZ (10 nM), whereas exposure to the isolated action of UVA or FICZ did not impair cell viability. Comet analysis revealed the introduction of Fpg-sensitive oxidative base lesions following combined FICZ/UVA exposure, a genotoxic photodynamic effect observed also upon FICZ photoexcitation using a blue light source (LED 460 nm). Photooxidative effects were substantiated by detection of glutathione depletion, MitoSOX Red fluorescence microscopy, and upregulation of stress response gene expression (HMOX1, HSPA1A, HSPA6), all of which were antagonized by antioxidant cotreatment (NAC, sodium azide). In SKH1 mouse skin, an experimental photodynamic regimen (involving topical application of FICZ combined with UVA exposure) blocked the tumorigenic progression of UVB-induced mouse papillomas suggesting the preclinical utility of the endogenous tryptophan-derived photosensitizer FICZ for the photodynamic elimination of cutaneous malignant cells.

P1.16 Justiniano, R*; Park, SL; Williams, JD; Perer, J; Lesson, JL; Wondrak, GT; UA Cancer Center and College of Pharmacy, University of Arizona; wondrak@pharmacy.arizona.edu

B6-Vitamins are Endogenous Photosensitizers of Photo-oxidative and Genotoxic Stress in Cultured Human Keratinocytes and Reconstructed Epidermis

UVA-driven cutaneous photo-oxidative stress may originate from specific endogenous chromophores acting as photosensitizers. Recently, we have documented the photosensitization of cultured human skin keratinocytes and fibroblasts exposed to micromolar concentrations of B6-vitamins (pyridoxine/pyridoxamine/pyridoxal) causing UVA-driven inhibition of proliferation, cell cycle arrest, and apoptosis. Here, we demonstrate that B6-vitamins are micromolar sensitizers of photo-oxidative and genotoxic stress in cultured human keratinocytes and reconstructed epidermis. First, induction of apoptosis was substantiated by flow cytometric analysis of annexinV-FITC/PI-stained primary keratinocytes exposed to the combined action of UVA (6.6 J/cm²) and B6-vitamin (order of potency: pyridoxal > pyridoxine = pyridoxamine). Flow cytometric assessment of DCF fluorescence and chemiluminescent detection of glutathione depletion confirmed the occurrence of photo-oxidative stress elicited by combined exposure to UVA and B6-vitamins. Comparative gene expression array analysis revealed that combined exposure to pyridoxal and UVA induced pronounced ER (DDIT3, HSPA6) and redox (EGR1, GSTM3, SOD1) stress responses in primary keratinocytes. Comet analysis indicated the introduction of Fpg-sensitive oxidative DNA base lesions observable only in response to

combined B6/UVA exposure. In human reconstructed epidermis (EpidermTM; MatTek), pyridoxal pre-incubation followed by UVA exposure caused genomic 8-oxo-dG and TUNEL positivity, substantiating the occurrence of UVA-driven photodynamic effects that may be relevant to human skin exposed to high concentrations of B6-vitamins.

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Light-induced difference FTIR spectroscopy of photolyase

Photolyases (PHRs) are DNA repair enzymes by light, which specifically revert UV-induced photoproducts, cyclobutane pyrimidine dimers (CPD) or (6-4) photoproduct, into normal bases. Flavin adenine dinucleotide (FAD) is the chromophore of PHRs, and enzymatically active fully reduced state (FADH⁻) is formed by light-induced electron/proton transfers from oxidized and neutral radical states (activation process). Upon illumination of the FADH⁻ form, the repair of the photoproduct is initiated by the electron transfer from FADH⁻ to the photoproduct. Molecular mechanism of activation and repair processes of PHRs is intriguing, and light-induced difference Fourier-transform infrared (FTIR) spectroscopy is a powerful tool for this aim. Hydrated film samples have been used for photoreceptive proteins such as rhodopsins, which can monitor even a single water molecule [1]. Nevertheless, for FTIR study of PHRs, we wanted a sample with higher water content to facilitate enzyme turnover. We thus established two different methods [2], re-dissolved samples and concentrated solutions, for the study of *Xenopus* (6-4) PHR [3] and *E. coli* CPD PHR [4], respectively. Our FTIR results will be presented including the trial of functional conversion between CPD and (6-4) PHRs. References [1] H. Kandori, *Biochim. Biophys. Acta* 1460, 177-191 (2000). [2] D. Yamada and H. Kandori, *Methods Mol. Biol.* 1148, 361-376 (2014). [3] Y. Zhang Y. et al. *Biochemistry* 50, 3591-3598 (2011); Y. Zhang Y. et al. *J. Phys. Chem. Lett.* 2, 2774-2777 (2011); D. Yamada et al. *Biochemistry* 51, 5774-5783 (2012); D. Yamada *Biophys. Physicobiol.* 12, 139-144 (2015); D. Yamada et al. *Biochemistry* 55, 715-723 (2016). [4] I M. M. Wijaya et al. *Biochemistry* 52, 1019-1027 (2013); I M. M. Wijaya et al. *Biochemistry* 53, 5864-5875 (2014); I M. M. Wijaya et al. *BIOPHYSICS* 11, 39-45 (2015); I M. M. Wijaya et al. *J. Am. Chem. Soc.* in press (2016).

9.3 Kanick, SC; Dartmouth College; *stephen.c.kanick@dartmouth.edu*

Sub-diffusive imaging of tissue microstructure in a wide field of view

This study uses structured light imaging to provide quantitative wide-field maps of biomarkers of tissue microstructure. Reflectance images sampled over multiple spatial frequencies allow independent separation of absorption and scattering properties that independently define density and fractal dimension. This multi-scale scatter imaging approach is used to differentiate tissue types in a preclinical murine tumor model. The approach is also translated in a pilot clinical investigation to differentiate the microstructure of tissue types from samples obtained during breast conserving surgeries.

PI.17 Karumanchi, DK*; Pantoja, YV; Street, AM; Freeman, DA; Roth, FA; Gaillard, ER; Northern Illinois University; *kalyank04@gmail.com*

Sustained Release Liposomal Drug Delivery for Treating Ocular Angiogenesis

Diabetic Retinopathy (DR) and Age Related Macular Degeneration (AMD) are the most common ocular diseases and a leading cause of blindness in American adults. Angiogenesis observed in these two diseases is characterized by the growth of new blood vessels into the retina damaging its surface in the process. The new blood vessels are fragile, "leaky" and pool blood into the retinal space, further damaging the retina. Laser treatments and drugs like Lucentis and Avastin are available for controlling the diseases. These drugs are anti-VEGF antibodies that inhibit the growth of new blood vessels. The intravitreal injections, administered every month are inconvenient, painful as well as very expensive. Our interest in this research project is to encapsulate the protein drug in nanostructures, prolong the time of drug release into the eye, thereby, decreasing the frequency as well as the cost factor for these

treatments. Liposomes are artificial vesicles composed of phospholipids and cholesterol which form lipid bilayers and a hollow hydrophilic core where the drugs can be encapsulated. Abrishami et al have been able to obtain a sustained release of the anti-VEGF drugs up to a period of 42 days. We have been successful in encapsulating a model protein into our stable liposomal formulations and attain a controlled release over a period of 6 months in vitro. Currently, we are studying the in vivo drug release kinetics and distribution using Dutch belted rabbits as animal models to test the efficacy of the nanocarrier. With this study, our efforts would be to decrease the frequency of intravitreal injections from 12 to 2 per year, thereby effectively making the treatment more economically feasible.

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Early Diagnosis of Diabetes Through The Eye

Diabetes mellitus is a metabolic disorder characterized by high blood sugar levels which give rise to complications in the eye, kidneys and the brain. Diabetes triggers the development of ocular diseases like diabetic retinopathy, glaucoma and cataracts which are the leading cause of blindness around the world. The most common method for the diagnosis of diabetes involves measuring the blood sugar levels in the body. One major disadvantage of this method is the fluctuating blood sugar levels which contribute to false negative results. This leads to delay in treatment, eventually causing permanent damage to the organs. Therefore, diagnosis of diabetes at an early stage is very crucial. One biomarker for diabetes related diseases is the formation of Advanced Glycation Endproducts (AGEs) that result from the Maillard reaction of proteins with glucose. α -crystallin in the ocular lens is a small heat shock protein with no protein turnover and hence acts as a record for post-translational modifications especially glycation which forms fluorescent AGEs. We have used steady state and time resolved fluorescence measurements to study the spectroscopic changes in alpha crystallin with increase in time of glycation and the intact lenses from diabetic and non-diabetic donors. Overall, this study was focused on developing a non-invasive diagnostic tool for early detection of diabetes mellitus.

15.5 Kemp, MK*; Lindsey-Boltz, LA; Sancar, A; Wright State University, University of North Carolina; kemp.2@wright.edu

Multiple Roles for the ATR Kinase in the Cellular Response to UV-induced DNA Damage

The ATR (ataxia telangiectasia and rad3-related) protein kinase regulates numerous cellular responses to ultraviolet (UV) light-induced DNA damage, including cell cycle progression, DNA replication, and apoptosis. How ATR is activated following UV irradiation to mediate specific cellular outcomes is unclear and is an important issue given that ATR has become an attractive target for cancer prevention and treatment strategies. Four main mechanisms have been proposed for ATR activation by UV-damaged DNA, including: (1) direct recognition of the damage by the kinase or associated regulatory factors; (2) the generation of single-stranded DNA gaps by nucleotide excision repair-dependent removal of UV photoproducts; (3) replicative polymerase stalling and uncoupling from DNA helicase activity during chromosomal DNA replication; and (4) interference with RNA polymerase progression during gene transcription. Our laboratory has therefore utilized a combination of biochemical and cell biological approaches to better define the activation modes and downstream functions of ATR in response to UV-induced DNA damage. For example, we have shown that ATR kinase activity is directly stimulated by the binding of ATR and its co-activator protein TopBP1 to bulky DNA adduct-containing DNA in the absence of other DNA metabolic processes. Furthermore, we recently defined a minimal set of proteins that couple the removal of DNA adducts by nucleotide excision repair and the generation of gaps in DNA to the activation of ATR. We have also characterized a variety of protein-protein and protein-DNA interactions that influence the kinetics and amplitude of ATR signaling in response to replication stress induced by DNA polymerase stalling at UV photoproducts. More recently, we have uncovered a novel pro-apoptotic function for ATR in response to UV-induced transcription stress in non-replicating cells. Our findings therefore provide critical insights into the mechanisms of ATR activation and the cellular functions of ATR in human cells exposed to UV

radiation.

PI.18 Kim, MA*; Jung, YC; Kim, EJ; Lee, HK; Amorepacific R&D Center; alsdk@amorepacific.com
Biological Effects of Heat Generated by Infrared Exposure on Human Skin in vivo

Human skin is constantly exposed to sunlight, which comprises 6.8% of ultraviolet (290-400nm), 38.9% of visible light (400-760nm), and 54.3% of infrared radiation (760nm-1mm). Recently, not only the ultraviolet (UV) responses, but the effect of infrared (IR) radiation on human skin has been studied. IR spectral region has been known to cause premature skin aging, and the heat generated by IR might be involved in the skin aging as well. Since the heat is known to regulate the expression of MMP-1, MMP-3, MMP-12 and tropoelastin, fibrillin-1. To prevent this premature aging, the effective IR or heat blockers are being developed widely. However, the appropriate clinical evaluation method for this efficacy has not been studied heretofore. In this study, we investigated the biological impact of heat generated by IR on human skin in vivo, in order to set up the evaluation method. Thirteen healthy subjects participated in a study of IR radiation. Subjects exposed their back side, which is the region between scapula line and waist, to the IR emitter. Measurements were conducted on baseline, and after IR irradiance of 0.24W/cm² in a room with controlled temperature and relative humidity (24±2°C and 40±2%). Skin surface temperature, erythema, and blood flow of IR exposed region were measured. As a result, the skin surface temperature and the erythema significantly increased after IR exposure, compared to the baseline. Skin blood flow also significantly increased as well. The molecular vibrations and rotations might lead to the increase of skin surface temperature, and the temporarily appeared erythema is thought to be affected by increased blood flow and vasodilation. From the results, biophysical properties of skin surface temperature, erythema, and blood flow could be evaluating indices for the heat protection efficacy measurement. Further studies regarding materials which are effective for the heat protection and the standardized evaluating method should be investigated.

13.5 Kolbe, L; Beiersdorf AG; ludger.kolbe@beiersdorf.com

Photoprotective Properties Of Licochalcone A From Glycyrrhiza Inflata On Human Skin In Vitro And In Vivo

Licorice extract is frequently used in the western world as for candies and as a sweetener in the food and tobacco industry. In the Far East, however, licorice is well known as a basic compound for several traditional medicines for a broad range of diseases. Pharmacological activities have been attributed to several phenolic ingredients and terpene saponins found in different Glycyrrhiza species. Licochalcone A from Glycyrrhiza inflata has previously been shown to possess anti-bacterial and anti-parasitic anti-inflammatory activity. We focused on the anti-oxidative and anti-inflammatory properties of licochalcone A. The production of reactive oxygen species (ROS), induced by UV irradiation and environmental stress leads to oxidative tissue damage and is one of the major causes of premature skin aging. We examined the in vitro inhibitory effects of licochalcone A on various pro-oxidative/ pro-inflammatory reaction cascades including: fMLP and zymosan induced oxidative burst of neutrophils, UV-induced lipid peroxidation and interference with signal transduction cascade in fibroblasts and keratinocytes. Licochalcone A inhibits the activation of NF- κ B after UV-irradiation and activates protective Nrf2 pathways. In vivo, licochalcone A reduces skin redness after irradiation of human skin with solar simulated radiation. A study with a licochalcone-containing formulation confirmed the anti-oxidative efficacy on UVA-induced ultra weak photon emission. Taken together these data demonstrate the potent inhibitory capability of topically applied licochalcone A against oxidative skin damage caused by ROS. The anti-oxidative activity of Licochalcone A at submicromolar concentrations and the broad action profile makes it a promising candidate for dermatological and cosmetic topical products in photoprotection.

19.4 Kovar, JL*; Craddock, M; Volcheck, B; Xing, K; Draney, D; Padhye, N; Urlacher, T; LI-COR

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IRDye® 700DX: A demonstration of structural stability and photodynamic principles with small molecule conjugates

IRDye® 700DX (aka IR700) is a near-infrared phthalocyanine dye with structural attributes designed for photodynamic therapy (PDT) applications. Photolytic cleavage of Si-O-Si linkages of IRDye 700DX has been suggested as a factor in cell-killing. This effect was examined for the dye carboxylate at pH 4-8 using HPLC. IRDye 700DX fluorescence intensity was stable at pH levels >6 with no significant increase in degradation products such as structural loss of either axial arm or decrease in the dye concentration in the solutions. We demonstrate the principles of PDT apply to IRDye 700DX-labeled targeting agents such as small molecules. RGD, EGF, chlorotoxin (CLTX), and anti-EGFR Affibody® molecules were labeled and cells examined morphologically before and after irradiation with 690 nm light (32 J/cm²). Reduced cell viability, a loss of mitochondrial potential, and an increase in Caspase 3/7 activity for RGD, EGF, and CLTX conjugates were noted at 20 h post-irradiation. IRDye 700DX exhibited a 4-fold increase in singlet oxygen production over non-dye controls with a significant reduction when sodium azide was added. IRDye 700DX production of reactive oxygen species, superoxide and hydroxyl radicals, was assessed using fluorogenic probes with and without specific scavengers for each radical. These data suggest cytotoxic effects, necrosis or apoptosis, may be dependent on the location of the probe at the time of light exposure, cell membrane versus intracellular. Taken together, these data support IRDye 700DX as a Type I and Type II photosensitizer that exhibits a high degree of photostability when undergoing PDT.

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Combination of verteporfin-PDT and PI3K pathway inhibitor BEZ235 synergistically enhances endothelial cell growth inhibition and apoptosis

Photodynamic therapy (PDT) induces cell injury and death through generation of reactive oxygen species (ROS) after light activation. Verteporfin is a photosensitizer that has been approved for the treatment of age-related macular degeneration and is under investigation for vascular-targeted cancer therapy. Verteporfin-mediated PDT induces rapid apoptotic cell death in SVEC mouse endothelial cells by activating mitochondria-initiated cell death pathways. However, we found that PDT activated pro-survival phosphatidylinositol 3-kinase (PI3K) signaling pathway, which was associated with cell regrowth after treatment. Thus, the goal of this study is to test the hypothesis that the therapeutic outcome of verteporfin-PDT can be enhanced by targeting PDT-induced pro-survival PI3K/mTORC signaling pathway. In this study, we combined verteporfin-PDT and dual PI3K/mTORC pathway inhibitor, BEZ235 to enhance treatment response in SVEC cells. We found that compensatory upregulation of PI3K/mTORC signaling post PDT was significantly inhibited by BEZ235 as indicated by a dramatic reduction in the phosphorylation of AKT and s6 protein. Our results demonstrate that combining PDT with BEZ235 not only induced more cell apoptosis but also resulted in more durable inhibition in cell proliferation. Enhanced treatment outcome of this combination therapy was shown by both short-term cell proliferation and long-term clonogenic assay. These results provide the basis that targeting pro-survival PI3K signaling pathway is an effective approach for enhancing therapeutic response to verteporfin-PDT.

P2.1 Kumagai, M*; Yamada, D; Iwata, T; Yamamoto, J; Getzoff, ED; Iwai, S; Kandori, H; Nagoya Institute of Technology, Osaka University, The Scripps Research Institute; mai519kuma@gmail.com

Comparison of photoactivation and photorepair of T(6-4)T and T(6-4)C photoproducts by Xenopus (6-4) photolyase on FTIR study

Photolyases (PHRs) are DNA repair proteins that revert UV-induced photoproducts. Two types of PHRs have been reported: CPD PHR repairs cyclobutane pyrimidine dimers (CPDs), while (6-4) PHR repairs pyrimidine-pyrimidine (6-4) photoproducts ((6-4) PPs). Flavin adenine dinucleotide (FAD) is the chromophore of PHRs. The oxidized and neutral radical forms are resting states, and fully reduced (FADH⁻) form is enzymatically active. Upon the illumination to the FADH⁻ form, the repair of

photoproduct is achieved as a trigger for the electron transfer from FADH⁻ to the photoproduct. We have measured the photoactivation and photorepair processes of Xenopus (6-4) PHR by light-induced difference Fourier-transform infrared (FTIR) spectroscopy [1-5]. When the photoactivation process was compared between substrate-bound and unbound (6-4) PHR, different structural changes were observed in the α -helices and neutral His residue(s). In addition, hydrogen-bonding environment of C=O groups from (6-4) PP was influenced upon photoactivation. Thus, FTIR spectroscopy can detect faint changes which cannot be detected by X-ray crystallography. (6-4) PHR can repair both T(6-4)T and T(6-4)C, where the OH and NH₂ groups are bound at C5 position of 5' side, respectively. Here, to investigate structural perturbation of the enzyme by (6-4) PP binding, photoactivation and photorepair process of T(6-4)T and T(6-4)C were compared by FTIR spectroscopy. We observed the spectral difference between presence of T(6-4)T and T(6-4)C PP in the photoactivation and photorepair processes. Identification of vibrational bands by use of isotope labeling and mutants is now in progress. Molecular mechanisms of substrate recognition, repair by (6-4) PHR will be discussed based on the observation. [1] Zhang Y. et al. *Biochemistry* 50, 3591-3598 (2011) [2] Zhang Y. et al. *J. Phys. Chem. Lett.* 2, 2774-2777 (2011) [3] Yamada D. et al. *Biochemistry* 51, 5774-5783 (2012). [4] Yamada D. et al. *Biophys. Physicobiol.* 12, 139-144 (2015). [5] Yamada D. et al. *Biochemistry* 55, 715-723 (2016).

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Chemometric Analysis of Chromoprotein Photocycles

The archetypical microbial retinal protein, bacteriorhodopsin, undergoes a photocycle triggered by light absorption by the retinal chromophore, that is followed by a sequence of dark reactions resulting in the pumping of a proton across the cell membrane. The rigorous chemometric analysis of the spectrotemporal matrices obtained in our time resolved multichannel absorption spectroscopic experiments was initiated by Jack Saltiel in the nineties. Our work together yielded a more accurate description of the intermediate spectra and kinetics and, with the combination of singular value decomposition with exponential fit-assisted self-modeling (SVD-EFASM), we found a previously undetected intermediate. More recently, we applied similar chemometric methods to elucidate the photocycle of the soluble chromoprotein, photoactive yellow protein (PYP). PYP is a blue-light sensor from the bacterium *Halorhodospira halophila* with a covalently bound p-coumaric acid chromophore. SVD, exponential fit and target testing yielded intermediate spectra serving as input in the following global spectrotemporal model fit, using a sufficiently complex photocycle scheme with reversible transitions. The obtained dependence of the rate coefficients on the presence of chaotropic or kosmotropic Hofmeister co-solutes suggested that the conformational change of PYP leading to the most unfolded signaling state takes place gradually, and starts already in microseconds with the relaxation after the chromophore isomerization. At present we are studying the Hofmeister effect on the kinetics of the bacteriorhodopsin photocycle. These effects, observed almost exclusively in the millisecond range, will be discussed based on available spectroscopic and X-ray structural data, and on a recent model of the Hofmeister effect on the structural stability of proteins [Dar et al., *J. Phys. Chem. B* 111 (2007) 5344-5350].

14.2 Lawrence, KP*; Sarkany, RPE; Acker, S; Herzog, B; Young, AR; King's College London, BASF Grenzach GmbH; *karl.lawrence@kcl.ac.uk*

Wavelengths from 385 " 405nm cause photodamage to skin cells

The adverse effects of solar UVR (~295 " 400nm) on the skin are well documented, especially in the UVB region (~295-315/320nm), and sunscreens have been shown to be beneficial in inhibiting a wide range of photodamage. The effects of long-wave UVA (>380nm) and visible radiation on the skin are much less well known, but increasingly studied. Most sunscreen formulations provide very little protection in the long wave UVA region (380-400nm) and almost none from shortwave visible wavelengths (400-420nm). We demonstrate photodamage in this region using high irradiance,

narrowband LED arrays at 385nm and 405nm, using environmentally relevant doses. The endpoints include cell viability, DNA damage (cyclobutane pyrimidine dimers " CPD), differential gene expression and oxidative stress in vitro in HaCat keratinocytes, and pigmentation and erythema in vivo in human volunteers. For most endpoints we found a clear dose-response relationship for both sources. There was a highly significant reduction in cell viability (385nm $p < 0.0001$; 405nm $p < 0.0001$), increase in reactive oxygen species (385nm $p < 0.0001$; 405nm $p = 0.0001$), and several genes associated with adverse effects were significantly upregulated including HMOX-1, MMP-1, IL-1a, IL-6 and IL-8 (385nm $p < 0.02$; 405nm $p < 0.003$). PON-2 was upregulated by 385nm but not 405nm (385nm $p = 0.0002$; 405nm $p = 0.1122$). At high doses of 385nm radiation there was an increase in CPD production, but no effect at 405nm. In addition we demonstrate that these sources can induce skin-type dependent changes in erythema, IPD and delayed tanning in vivo. This work provides new insight into photodamage and may lead to new strategies to provide improved photoprotection in this poorly protected region.

6.10 Lawrence, KP*; Sarkany, RPE; Acker, S; Herzog, B; Young, AR; King's College London, BASF Grenzach GmbH; *karl.lawrence@kcl.ac.uk*

Wavelengths from 385 " 405nm cause photodamage to skin cells

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17.1 Lewis, J; Turban, J; Girardi, M*; Yale University; *michael.girardi@yale.edu*

Immune mechanisms of p53 mutant keratinocyte clonal expansion and cutaneous carcinogenesis

We have recently demonstrated that keratinocyte (KC)-derived malignancy induced by chronic UVB exposure is facilitated by Langerhans cells (LC), and that LC promotion of clonal expansion of p53-mutant KC is correlated to epidermal growth factor IL-22 expression, in a T-cell independent manner. Thus, to better understand the mechanisms of innate immunity in LC-facilitated UV-carcinogenesis, we characterized innate lymphoid cells (ILC) in the presence and absence of LC, before and after chronic UVB exposure. Flow cytometric analysis of ILC transcription factors and cytokine expression revealed that among skin-isolated ILC (CD45+ Thy1+ CD11b- CD11c- B220- Gr1- Fc-epsilon-R1-Ter119-) from untreated LC-intact mice, the predominant subsets identified were (T-bet+) ILC1 and (Gata-3+) ILC2, while (Eomes+) cNK and (Rorc+) ILC3 were relatively infrequent. However, this distribution was dramatically altered by chronic UVB exposure that resulted in reduced T-bet+, Eomes+ and Gata-3+ populations and a marked increase in Rorc+ ILC3. Examination of cytokine expression following chronic UVB exposure revealed markedly increased levels of IL-22+ ILC, the

majority of which co-expressed IL-17A+, while levels of IFN γ + and IL-13+ ILC remained relatively low. IFN γ -producing cells were CD2+ CCR6- and primarily Sca1- whereas IL-13, IL-22 and IL-17A were produced by Sca1-bright CCR6-dim cells. The majority of IL-22+ and IL-17A+ cells also expressed CD2. Notably, LC-deficiency was associated with a marked reduction in the frequency of IL-13+ ILC2 and IL-22+ ILC3. These results help elucidate cutaneous ILC phenotypic populations under chronic UV exposure and under LC influences, and suggest that LC and IL-22-producing ILC3 cooperate to make fundamental contributions to skin cancer development by facilitating clonal expansion of p53-mutant keratinocytes.

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Tracking Photoinduced Charge Separation in DNA

Recent studies of photoinduced charge separation in DNA have progressed rapidly as a consequence of collaborations with groups of laser spectroscopists and theoreticians. Recent studies of photoinduced charge separation in DNA have progressed rapidly as a consequence of collaborations with groups of laser spectroscopists and theoreticians. Old theories for the transport of positive charge (holes) and negative charge (electrons) founded on singlet step superexchange and multi-step incoherent hole hopping have been superseded by hole injection followed by formation of a conduction channel or trapped polaron. Direct evidence for formation of the trapped polaron in poly(adenine) sequences (A-tracks) of variable length has now been obtained by transient spectroscopy, as has the formation of the guanine hole trap. The efficiency of hole transport across an A-track is determined by the competition between charge recombination and charge transport in the polaron, both of which are subject to energy gap laws. Beyond five A-T base pairs the efficiency of charge separation becomes very small except when charge recombination is inhibited. Upon reaching a deep hole trap, the polaron is irreversibly trapped and decays slowly by charge recombination with the chromophore anion radical produced during the charge injection process. Electron transport is less efficient than is hole transport in DNA, because of faster charge recombination in the initially formed contact radical ion pair.

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Tracking Photoinduced Charge Separation in DNA

Recent studies of photoinduced charge separation in DNA have progressed rapidly as a consequence of collaborations with groups of laser spectroscopists and theoreticians. Recent studies of photoinduced charge separation in DNA have progressed rapidly as a consequence of collaborations with groups of laser spectroscopists and theoreticians. Old theories for the transport of positive charge (holes) and negative charge (electrons) founded on singlet step superexchange and multi-step incoherent hole hopping have been superseded by hole injection followed by formation of a conduction channel or trapped polaron. Direct evidence for formation of the trapped polaron in poly(adenine) sequences (A-tracks) of variable length has now been obtained by transient spectroscopy, as has the formation of the guanine hole trap. The efficiency of hole transport across an A-track is determined by the competition between charge recombination and charge transport in the polaron, both of which are subject to energy gap laws. Beyond five A-T base pairs the efficiency of charge separation becomes very small except when charge recombination is inhibited. Upon reaching a deep hole trap, the polaron is irreversibly trapped and decays slowly by charge recombination with the chromophore anion radical produced during the charge injection process. Electron transport is less efficient than is hole transport in DNA, because of faster charge recombination in the initially formed contact radical ion pair.

4.6 Liebmann, J*; Born, M; Pfaff, S; von Felbert, V; Philips GmbH, Innovative Technologies, Aachen, Germany, University Hospital, RWTH Aachen University, Aachen, Germany;

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Randomized Clinical Trial On The Efficacy And Safety Of UV-Free Blue Light For Treating Mild Psoriasis Vulgaris

UV is used for phototherapy of different skin conditions like psoriasis. Though effective, it is carcinogenic making it a hazardous tool to treat skin diseases over longer periods of time. LED technology made it possible to investigate the effect of distinct wavelengths on human skin including the visible range. Here, UV-free blue light at 453nm reduces proliferation while inducing differentiation without causing toxicity in keratinocytes up to high fluences. Therefore, blue light could be a new treatment option for psoriasis - which is characterised by increased proliferation and disturbed differentiation of keratinocytes. The small size of LEDs enables the integration into compact and even wearable devices for localized treatment. We here present data from a randomized clinical trial using blue light for treating localized psoriasis plaques. We developed a wearable light device (453nm), delivering a fluence of 90J/cm² during 30 minutes of treatment for home use. We chose patients with two mirror plaques and randomized which plaque would be treated and which would be left untreated as a control. Patients were asked to treat the target plaque daily (at least 5 times / week) at home for an initiation treatment period of 4 weeks followed by a period of 8 weeks with 3 applications per week with at least one treatment free day in between treatments (maintenance treatment). After the last treatment (week 12) patients were followed up for 4 weeks without irradiation. The investigator assessed the local psoriasis severity index (LPSI) to evaluate treatment efficacy on study visits. The change from baseline (CfB) in LPSI, as primary endpoint, showed a significant improvement of the target plaques compared to the control plaques. So far no toxic or carcinogenic effects of blue light have been found. The patient compliance was high (97.87%) and no adverse events related to the blue light treatment or the use of the device were found. Satisfaction and usability of the blue light therapy was rated excellent on an average System Usability Scale (SUS) score by the patients.

11.4 Lin, C.; UCLA; clin@mcdb.ucla.edu

The blue light-dependent phosphorylation of Arabidopsis CRY2

Arabidopsis cryptochrome 2 (CRY2) undergoes blue light-dependent phosphorylation, which regulate the function and protein turnover of CRY2. A study of blue light-dependent CRY2 phosphorylation by mass spectrometry and mutagenesis analyses demonstrate that CRY2 contains two types of phosphorylation in the CCE domains. One type of CRY2 phosphorylation occurs in a serine-cluster of the CCE domain that causes electrophoretic mobility upshift, whereas the other type of phosphorylation occurs outside of the serine cluster that do not cause mobility upshift. We identified four closely related protein kinases, referred to as CIK1 to CIK4 (CRY-Interacting Kinase 1 to 4), and investigated the substrate specificity, phosphorylation sites, and physiological functions of those kinases. Results of our biochemical analyses demonstrate that CIKs phosphorylate the serine-cluster of CRY2 in the substrate-specific and blue light-specific manner. Genetics study demonstrates that CIKs are major protein kinases that phosphorylate CRY2, resulting in blue light-dependent ubiquitination and degradation of the photoreceptor.

12.3 Liu, JTC; UW Seattle; jonliu@uw.edu

Miniature MEMS-scanned dual-axis confocal microscopes for point-of-care pathology

Recent advances in the design and miniaturization of optomechanical components have enabled the development of handheld and endoscopic confocal microscopes for point-of-care analyses of tissue microanatomy and molecular biomarkers of disease. We are currently developing a handheld line-scanned dual-axis confocal microscope, with MEMS-based scanning, for high-speed (>15 Hz) microscopic imaging of superficial (<250-microns deep) tissues. The line-scanned DAC architecture enables fast frame rates to mitigate motion artifacts during handheld clinical use. Validation studies, performed with reflectance targets and fluorescently stained fresh tissues, show that this device has the potential to enable early detection and surgical guidance. Future clinical applications include the examination of suspicious lesions in the oral cavity as well as for guiding the resection of brain tumors.

12.5 Lovell, JF; SUNY Buffalo; jflovel@buffalo.edu

Porphyrin-phospholipid Liposomes : Emerging Applications in Chemophototherapy

Near infrared (NIR) light penetrates human tissues with limited depth, thereby providing a method to deliver non-ionizing radiation within the bounds of well-defined target tissue volumes. Light-based therapies including photodynamic therapy (PDT) and laser-induced thermal therapy (LITT) have been validated clinically for curative and palliative treatment of solid tumors. However, these monotherapies can suffer from incomplete tumor killing and have not displaced existing ablative modalities. The combination of phototherapy and chemotherapy (chemophototherapy, CPT), when carefully planned, can be an effective cancer treatment option that is relatively unexplored. Here, we will discuss our lab's efforts to develop light responsive nanovesicles based on porphyrin-phospholipid (PoP) liposomes to enable enhanced drug deposition at tumor sites. PoP liposomes also enable unique imaging approaches and facile ligand-targeting of the nanovesicles to target tissues.

31.3 Lucas, RJ; University of Manchester; robert.lucas@manchester.ac.uk

Using light to tell time

A master circadian clock in the hypothalamic suprachiasmatic nuclei (SCN) orchestrates twenty-four hour rhythms in mammalian physiology and behaviour. The SCN clock is reset to local time primarily by a projection from the retina that conveys information about the diurnal light dark cycle. The conventional view of this sensory pathway is that it efficiently extracts information about background light intensity (irradiance) as the most reliable indicator of time of day. We have been studying the visual information reaching the SCN in the mouse and show that neurones in the mouse SCN also respond to changes in colour and according to spatial patterns. The former provides an additional time of day cue that is used to set the phase of circadian clocks. The appearance of information about spatial patterns in the SCN has less obvious relevance for telling time of day, but may rather relate to the SCN's other function as a control centre behavioural and physiological state.

3.4 Luo, D*; Carter, KA; Razi, A; Geng, J; Shao, S; Giraldo, D; Sunar, U; Ortega, J; Lovell, JF; University at Buffalo, State University of New York, McMaster University, Wright State University; dandanlu@buffalo.edu

Doxorubicin Encapsulated In Long Circulating Stealth Liposomes Conferred With Light-triggered Drug Release

Delivery of drugs at sites of target at therapeutically effective levels is a central challenge for drug delivery in cancer therapy. There is an increasing interest in designing liposomes and other type of nanoparticles that can release encapsulated drugs in response to external (e.g. light, heat, or ultrasound) or environmental triggers (e.g. pH, enzymes). Stealth liposomes can be used to extend the blood circulation times of encapsulated therapeutics. Inclusion of small amount (i. e. 2 mol. %) of porphyrin-phospholipid (PoP) imparted near infrared light-triggered release of doxorubicin (Dox) from stealth liposomes. The type and amount of PoP affected drug loading, serum stability and drug release induced by NIR light. Cholesterol and PEGylation were required for Dox loading, but slowed light-triggered release. By studying these factors we have developed Dox-loaded stealth liposomes which exhibit a long circulation time (half-life in mice: 21.9 h) and were stable in storage for months. Following intravenous injection and NIR irradiation, Dox deposition increased 7 fold in treated subcutaneous human pancreatic xenografts. Photo treatment induced mild tumor heating and complex tumor hemodynamics. A single chemo-phototherapy treatment with Dox-loaded stealth PoP liposomes (5-7mg/kg Dox) eradicated tumors while corresponding chemo- or photodynamic therapies were ineffective. A low dose 3 mg/kg Dox photo-treatment with stealth PoP liposomes was more effective than a maximum tolerated dose of free (7 mg/kg) or conventional long-circulating liposomal Dox (21 mg/kg). To our knowledge, Dox-loaded stealth PoP liposomes represent the first reported long-circulating nanoparticle capable of light-triggered drug release.

P2.13 M, Bayda*; C, Redwood; S, Gupta; O, Dmitrenko; J, Saltiel; Florida State University, Tallahassee, FL; Present address: Faculty of Chemistry, Adam Mickiewicz University, Poznan, PL, Florida State University, Tallahassee, FL, University of Delaware, Florida State University; mbayda@amu.edu.pl

Mechanisms of Photoisomerization of the Vitamin D3 isomers Pre-, Provitamin-D3 and Lumisterol in EPA Glass at 77 K

We recently measured fluorescence spectra in the course of the photoisomerizations of previtamin and provitamin D3 (Pre and Pro) to tachysterol (Tachy) at 77 K in EPA glass. Our analysis using singular value decomposition with self-modeling led to the conclusion that Pre exists as the s-cis,s-cis-conformer (cZc-Pre) which gives, exclusively, the unstable s-cis,s-cis-conformer of Tachy (cEc-Tachy) and Pro gives tEc-Tachy, the stable s-trans,s-cis-conformer, as the major photoproduct. The major Pre photoproduct from Pro is tZc-Pre and not the expected cZc-Pre. Accordingly, the Pre to Tachy cis-trans photoisomerization proceeds via a conformer specific one bond twist process as proposed by Havinga. We extended these studies to lumisterol (Lumi), whose structure differs substantially from that of its stereoisomer, Pro. Initially, the light induced ring openings of Pro and Lumi are expected to give (-)cZc(-)-Pre and (+)cZc(+)-Pre, respectively. In the case of Pro, most of the initially formed cZc-Pre proceeds to tZc-Pre, the precursor of tEc-Tachy. In contrast, our new results show that under the same conditions most cZc-Pre formed from Lumi retains its conformation and isomerizes to cEc-Tachy. We detected no (+)cZc(+)-Pre fluorescence. Its formation as an intermediate from the ring-opening of Lumi was established by UV absorption measurements.

27.1 Macia, N; Planas, O; Nonell, S; Heyne, B*; University of Calgary, University Ramon Llull; bjmheyne@ucalgary.ca

Singlet Oxygen at the Nanoscale.

Singlet oxygen remains at the forefront of research, whether it is in bacterial photo-inactivation, in cancer treatment, or in nanoscience. Contrary to other reactive oxygen species, singlet oxygen is emissive, and by consequent its demeanor could be monitored in vivo. Due to its convenient mode of generation by photosensitization, singlet oxygen is the holly grail of imaging techniques, as it can offer both temporal and spatial resolution. However, there is a catch. Only one singlet oxygen molecule in 0.1 million is emissive! Therefore, monitoring this species in vivo poses a great challenge. Challenge, which can only be overcome by developing novel monitoring tools that largely outperform the current ones. In this study, we have engineered a series of novel core-shell nanoparticle where the plasmonic core of the nanoparticles plays a dual role of enhancing both the production of singlet oxygen and its radiative decay. By consequence, our plasmonic hybrid nanoparticles generate a signal of singlet oxygen phosphorescence significantly more intense than expected from the amount of singlet oxygen generated in solution. We have taken advantage of this dual amplification to detect singlet oxygen emission signal in biological media, such as Gram-negative and Gram-positive bacteria. We demonstrate the plasmonic hybrid nanoparticles are endowed with strong light-induced antimicrobial activity even towards the more resilient Gram-negative bacteria.

6.11 Mallidi, S*; Ichikawa, M; Alkhateeb, A; Khan, AP; Mai, Z; Hasan, T; Harvard Medical School; Mallidi.Srivalleesha@mgh.harvard.edu

Personalizing Photodynamic therapy based treatment strategies with Photoacoustic imaging

To achieve effective outcome in photodynamic therapy (PDT), it is paramount to understand the dynamic changes in the tumor microenvironment (oxygenation), photosensitizer consumption and adjust light dose accordingly. Towards this goal, I will present the utility of non-invasive 3D ultrasound guided photoacoustic imaging (PAI) to understand the heterogeneous changes in blood oxygen saturation during treatment and post treatment. Photoacoustic imaging, as the nomenclature suggests, involves acoustic signal generation by irradiating tissue with nanosecond laser pulses that satisfy the thermal stress confinement conditions. PAI provides tissue optical absorption information at deeper penetration depths with sensitivity similar to optical imaging and resolution on par with

ultrasound imaging. Given that PAI and ultrasound imaging share the same receiver electronics, the images are inherently co-registered to provide both anatomical and tissue optical information. Leveraging the 3D imaging and real-time imaging capabilities of ultrasound guided PAI, we identify regions not responding to PDT and have the potential to recur using various subcutaneous and orthotopic mouse models. We further compare the predictive capability of photoacoustic imaging with the more predominantly used fluorescence imaging and immunohistochemistry techniques for both Benzoporphyrin derivative and aminolevulinic acid based PDT. Finally the strategies to push the envelop for ultrasound-guided PAI as an important aid in tumor diagnosis, customizing patient-specific treatment, and monitoring the therapeutic progression and outcome in vivo not only for PDT but other therapies will be discussed.

12.4 Mallidi, S*; Ichikawa, M; Alkhateeb, A; Khan, AP; Mai, Z; Hasan, T; Harvard Medical School; Mallidi.Srivalleesha@mgh.harvard.edu

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P2.5 MARIONNET, C*; PIERRARD, C; GOLEBIEWSKI, C; CANDAU, D; BERNERD, F; L'Oreal Research and Innovation; cmarionnet@rd.loreal.com

A broader filtration of UVA1 wavelengths improves skin photoprotection, in vitro

Sun exposure can lead to photoaging and photocarcinogenesis. It involves UVB (290-320 nm) and UVA (UVA2, 320-340 nm and UVA1, 340-400 nm). UVA1 rays represent majority of UV that reach the Earth surface. They generate reactive oxygen species and contribute to harmful effects including immunosuppression and carcinogenesis in vivo. At the molecular and cellular level we previously showed in a model of reconstructed skin, that UVA1 exposure led to epidermal and dermal damage, such as fibroblast apoptosis and a strong alteration of gene and protein expression involving essential biological pathways. However, sunscreen formulas that efficiently filter UV wavelengths up to 370 nm lack, up to now, a sufficient absorption in the range of 370-400 nm UVA1 wavelengths. The present study aimed at investigating if an enlargement of spectral absorption could improve the protection against UVA1-induced biological damage. Three filtrating formulations were used: a state of the art formulation (SAF) (absorbing 280-370 nm UV rays), and two other formulations including prototype UVA1 filters, allowing a gain of absorption in the UVA1 range (280-385nm, 280-395 nm, respectively). The efficiency of these three formulations was tested after topical application onto reconstructed skin prior UVA1 exposure. At different time points post UVA1 exposure, cell and tissue morphology, as well as gene and soluble protein expression were assessed. Results show that, as

compared to an unprotected human reconstructed skin, SAF could prevent UVA1-induced cell and tissue damage as well as the modulation of gene and protein expression, although the protection was not complete. The use of formulations including UVA1 filters provided a superior protection than the SAF. On most of the tested endpoints, the broadening of UVA1 absorption spectrum led to a better protection. These results show that a broader filtration of UVA1 wavelengths can afford a significant better biological protection against UVA1-induced damage.

5.6 Markovitsi, D.; LIDYL, CEA, CNRS; dimitra.markovitsi@cea.fr

High Energy Long-Lived Excited States in Model Duplexes and Natural DNA

According to the picture emerged during the past decade, the energy of a UV photon absorbed directly by DNA duplexes may be redistributed among their bases. An important part of the excitations evolves toward charge transfer (CT) states which emit around 420 nm, at lower energy than the fluorescence of mono-nucleotides (330 nm). In the case duplexes with alternating guanine-cytosine or adenine-thymine sequence, a counterintuitive relaxation pathway, involving High-energy Long-lived Emitting Mixed (HELM) states, was identified. HELM states emit at 305 nm, decay on the ns time-scale and are very sensitive to structural disorder. Quantum chemistry calculations showed that they result from mixing between Frenkel excitons and purine-to-pyrimidine CT states and extend over at least four bases on both strands. The properties of HELM states were also detected in the fluorescence of calf thymus DNA. Conformational motions break the coherence of HELM states repopulating pp* states and giving rise to delayed fluorescence, which was indeed observed for this natural system. (Vaya

13.3 MARROT, L; ; lmarrot@rd.loreal.com

Deleterious synergy between pollution and sunlight: pollutants from particulate matter aggravate oxidative impact of UVA1 in skin models.

Atmospheric pollution is becoming a serious health concern in industrial countries and particulate matter (PM) from combustion is considered as particularly deleterious. In fact, ultrafine particles carry toxic compounds such as poly aromatic hydrocarbons (PAH). Moreover, they can translocate from lung capillaries to blood circulation and be distributed in the whole body. Up to now, no precise estimation of pollutants concentration in living skin is available. However, pollutants might reach dermis and epidermis either by penetration from skin surface or by systemic exposure. Some PAH are photo-reactive and phototoxic: sunlight and pollution might thus synergistically compromise skin health. After summing up current knowledge about dermatological damage induced by pollution, experimental data obtained in vitro using normal human keratinocytes or reconstructed epidermis will be presented. At very low concentrations (in the nanomolar range), some PAH such as benzopyrene or indenopyrene displayed a strong phototoxicity under UVA1 irradiation (340-400 nm). Even when cytotoxicity was low, PAH-induced photo-oxidative stress could impair mitochondrial function (membrane polarization and ATP production) and impact endogenous glutathione (GSH) homeostasis. Interestingly, among genes controlling GSH metabolism, SLC7A11 was particularly overexpressed (at gene and protein levels). This protein is an antiporter in charge of cystine supply. SLC7A11 upregulation suggests that regeneration of GSH might be of huge importance to ensure protection against "photo-pollution" stress. As proof, pretreatment of cells by buthionine sulfoximine BSO, an inhibitor of GSH biosynthesis, significantly increased PAH-induced phototoxicity. Our results highlight that pollutants could aggravate skin photodamage: specific photoprotection strategies for skin care in polluted area will be discussed.

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Metal Coordination Complexes as Photosensitizers for Photodynamic Therapy

Photodynamic therapy (PDT), a method that combines a photosensitizer (PS) and light in the presence of oxygen to destroy tumors, was first used in the field of oncology more than 40 years ago. Despite its vast potential and some isolated success, PDT remains underutilized as a cancer treatment. The number of variables that must be optimized with respect to the light component and the absence of large, comparative randomized clinical trials have been implicated as well as drawbacks associated with the organic PSs that have been traditionally employed for PDT. Metal coordination complexes for light-mediated cancer therapy are an attractive alternative to their organic counterparts. These systems possess a variety of excited state configurations that can be accessed through rational and systematic changes to their very modular, yet structurally diverse, architectures. These excited states can be tuned to participate in oxygen and oxygen-independent photoprocesses through catalytic and stoichiometric reactions, and offer many additional advantages over purely organic PSs. Our group has developed ruthenium (Ru)- and osmium (Os)-based coordination complexes for photoactivated cancer therapy, with a lead compound entering human clinical trials for treating bladder cancer this year. Herein we will discuss the attributes of Ru and Os PSs for PDT, the development process, and future directions.

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Cyanobacteriochrome Regulation of Second Messenger-Dependent Processes in Cyanobacteria

Microorganisms adapt to changes in their external environment by sensing and amplifying external first messenger signals using second messengers to alter their metabolism and behaviors. Levels of the second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) are regulated by a variety of environmental stimuli and play critical roles in controlling cellular processes such as biofilm formation, motility, central metabolism, and cell differentiation in a range of bacteria. C-di-GMP is a ubiquitous second messenger synthesized and degraded by proteins that contain GGDEF, EAL or HD-GYP domains. GGDEF domain proteins exhibit diguanylate cyclase (DGC) activity, resulting in the synthesis of c-di-GMP from two GTP molecules. EAL and HD-GYP domains have phosphodiesterase (PDE) activity, resulting in c-di-GMP degradation. C-di-GMP signaling systems have been largely characterized in pathogenic gamma proteobacteria; however, DGC or PDE proteins that are prominent in cyanobacterial species remain largely underexplored. Many putative c-di-GMP synthesis or degradation output domains are found in genes that also harbor light-responsive signal input domains, suggesting that light is an important signal for altering c-di-GMP homeostasis. Indeed, such output

domains are often the second most common output domain in photoreceptors"only outnumbered by a histidine kinase output domain. Cyanobacteria differ from other bacteria as they have a higher percentage of c-di-GMP signaling enzymes associated with photoreceptors and different photoreceptors are utilized. These results suggest that c-di-GMP plays key roles in adaptation of cyanobacteria to the photoenvironment. Our recent studies demonstrate that light regulates c-di-GMP levels in cyanobacteria and this photoregulation is associated with distinct c-di-GMP-dependent physiological responses. The range of identified cyanobacterial responses under control of light-dependent regulation of c-di-GMP will be discussed.

13.2 Narzt, MS; Nagelreiter, IM; Bochkov, VN; Latreille, J; Grillari, J; Tschachler, E; Gruber, F*; Medical University of Vienna, CDL Skin AUT, Medical University of Vienna, AUT, University of Graz, AUT, Chanel PB, Pantin, FRA, VBIT-BOKU, CDL Skin, Vienna, AUT, Medical University of Vienna, CDL Skin, Vienna, AUT; florian.gruber@meduniwien.ac.at

Phospholipid oxidation in UV stress and cellular senescence in the skin.

Oxidation of lipids and proteins is a hallmark of photodamaged and aged skin, and also potentially causative for age-related aesthetic decline and pathologic damage. To study which oxidation products are generated by long wavelength UV light, by age promoting stressors and in cellular senescence of various skin cell types, we developed and applied lipidomic analysis of oxidized and non-oxidized phospholipids (PL) using a HPLC-MS/MS method. With that approach we could quantify >400 oxidized lipid species in keratinocytes and fibroblasts and could determine UV fluence dependent changes. In parallel, we also performed analysis of global mRNA expression and of selected cyto/chemokines and stress response enzymes on protein level. We will present an overview of lipid species regulation patterns in UV stress and in recovery in keratinocytes and fibroblasts, and how UV-generated oxidized lipids can act as bioactive mediators in cellular stress and senescence.

25.5 Nowak-Sliwinska, P; University of Geneva; patrycja.nowak-sliwinska@unige.ch

Design of optimal combination therapy for the treatment of disease

The concept of photodynamic therapy (PDT), i.e. the treatment of disease via the administration of a photosensitizer and the localized application of light, is nowadays successfully established in the clinic for some ocular disorders and to a limited extent in oncology. In oncological applications it is mostly applied for superficially growing tumors in the skin, or in hollow organs. It is now being realized that combining PDT with other treatment strategies may give opportunities to overcome some of the limitations that have precluded efficient PDT-based anti-cancer strategies. However, new combination treatments will only be efficient when designed in an optimal way. In order to do this many aspects should be considered, such a choice of drugs, doses applied, level of oxygenation, sequencing, synergistic/antagonistic activities and resistance issues. The biggest challenge in the design of combination therapies is the immense number of possible drug mixtures. We have developed a algorithm-based technology to rapidly identify optimal combination therapies, by testing only a limited number of drug mixtures. This talk will highlight the specifics of this technology and our effort to use this methodology for personalized cancer treatment.

P2.20 Olsen, KW*; Jones, RN; Hira, S; Kiernan, K; Khaleeluddin, H; Kooistra, R; Sullivan, K; Kanzok, S; Loyola University Chicago; kolsen@luc.edu

BSA Based, Folate Directed Photodynamic Therapy Agent's Effectiveness In HeLa Cells

We have developed a novel photodynamic therapy (PDT) agent that specifically targets cancer cells. We used carbodiimide chemistry to attach folate (FA) and chlorin e6 (Ce6) to bovine serum albumin (BSA). A typical synthesis had 1 FA and 10 Ce6 per BSA. More than 50% of cancers overexpress the folate receptor. The folate containing conjugate can be taken up by the cell into the cytoplasm via receptor mediated endocytosis. Therefore, FA-BSA-Ce6 has double selectivity due folate-targeting plus limited-area light exposure. Ce6 has a large extinction coefficient of 59,000 M⁻¹cm⁻¹ at 660 nm

(Lee et al., 2012). Both FA and Ce6 are hydrophobic. Covalently attaching them to BSA increased their solubility, which should allow more efficient delivery to tumors. A singlet oxygen sensor (p-nitroso-N,N'-dimethylaniline) was used to demonstrate reactive oxygen species production when illuminated at 660 nm. FA-BSA-Ce6 was specifically taken up into the cells via receptor mediated endocytosis. Hela cells exposed to FA-BSA-Ce6 in the dark showed no cytotoxicity. Upon exposure to light, the conjugate was dose (time and concentration) dependent. Concentrations less than or equal to 2 μM were not effective at killing Hela cells but concentrations of at 5 μM and above lead to 95% cell death with a 4 min exposure to 660 nm light.

14.6 Osseiran, S*; Wang, H; Suita, Y; Roider, E; Fisher, DE; Evans, CL; Massachusetts General Hospital; evans.conor@mgh.harvard.edu

Towards Label-Free Evaluation of Oxidative Stress in Human Skin Exposed to Sun Filters

Skin cancer is the most common form of cancer in North America, and its incidence remains on the rise despite growing use of sunscreen. Given the regulatory history of cosmetics in the US, there is a concern regarding the safety of sun filters that remains to be tested. Indeed, previous experiments have shown that they induce oxidative stress in in vitro cultures, warranting the study of these compounds in intact human skin. Label-free optical methods for evaluating oxidative stress rely on fluorescence of NADH and FAD, two key metabolic co-enzymes. However, sun filters exhibit fluorescence that interferes with that of NADH. Here, we present a method based on fluorescence lifetime imaging microscopy to decompose the fluorescence of NADH and sun filters to (1) specifically localize the compounds spatiotemporally as they penetrate through skin, and (2) to exclude the sun filter signal from that of NADH to measure oxidative stress.

PI.8 Palasuberniam, P*; Chen, B; University of the Sciences in Philadelphia; ppalasuberniam@mail.usciences.edu

Therapeutic Enhancement of Aminolevulinic Acid-based Tumor Imaging and Therapy

Photodynamic therapy (PDT) involves the combination of a photosensitizer and light of a specific wavelength. Upon light activation in the presence of oxygen, photosensitizer molecules generate reactive oxygen species that cause cytotoxicity by inducing oxidative stress. Aminolevulinic acid (ALA) is a pro-drug used for the diagnosis and PDT treatment of various solid tumors based on endogenous production of heme precursor protoporphyrin IX (PpIX). Although nearly all types of human cells express heme biosynthesis enzymes and produce PpIX, tumor cells are found to have more PpIX production and accumulation than normal cells, allowing for the detection and treatment of solid tumors. The objective of my research is to explore therapeutic approaches to enhance ALA-based tumor detection and therapy. We have found that high ABCG2 transporter activity in triple negative breast cancer cells (TNBC) contributed to reduced PpIX levels in cells, causing them to be more resistant towards ALA-PDT. The administration of an ABCG2 inhibitor, Ko143, was able to reverse cell resistance to ALA-PDT by enhancing PpIX mitochondrial accumulation and sensitizing cancer cells to ALA-PDT. Ko143 treatment had little effect on PpIX production and ALA-PDT in normal and ER- or HER2-positive cells. Furthermore, since some tyrosine kinase inhibitors (TKI) are known to block ABCG2 transporter activity, we screened a panel of tyrosine kinase inhibitors to examine its effect on enhancing PpIX fluorescence and ALA-PDT efficacy. Several TKIs including lapatinib and gefitinib showed effectiveness in increasing ALA-PpIX fluorescence in TNBC leading to increased cell death after PDT administration. These results indicate that inhibiting ABCG2 transporter using TKIs is a promising approach for targeting TNBC with ALA-based modality.

10.1 Pandey, RP; none; renu.lasers@gmail.com

Low Level Laser Therapy and Memory Enhancement: Effect of Different Energy Doses

The amount of information that can be stored in short-term memory can vary. An often cited figure is 7 ± 2 items, based on the results of a George A Millers experiment on short-term memory, published in

1956. This clinical study aimed to see the effect of different energy doses of Low Level Laser Therapy (LLLT) on memorizing capacity of the young population aged 20 years to 30 years. 106 subjects with minimum of a 10th grade education with similar cultural background volunteered in the study. The subjects were divided into Group A and Group B according to the laser set they used. The Group A consisted of 56 (Mean±SD 23.7±4.8) subjects and Group B consisted of 50 subjects (Mean±SD 24.89±3.61). Two sets of different multi-diode lasers (Q10 & Q1000) with different energy output but same wavelengths (650, 808) and energy density (38.89mJscm²) were applied for 3 minutes on the frontal and occipital region of the head of the participants simultaneously. For the purpose two equivalent tests of non-associated meaningful words were prepared. One test was used as pre test to determine the baseline working memory at start of the study. The lasers were applied twice a week for 4 weeks. Q10 with energy output of 14mJ/s was applied by group A and Q1000 with energy output of 42mJ/s was applied by group B. Depending on the type of laser applied the participants were divided into two groups. At the end on the 8th application on day 30 the participants took another equivalent "non-associated memory test" and the results were analyzed. For Group A the mean ± SD value score of pre-test was 1.7±1.7 and post test was 1.7±1.7, (p-value0.6090). For Group B the mean value score of pre-test was 2.14±1.5, and the post test score was 2.22±1.04 (p-value0.0553). Using the score of 7 as 100% memory retention, the average percentage of the data was calculated. For group A pre test scored 23.9% and post test scored 53.6% while for group B the pretest scored 30.6% and post test scored 32.6%.

10.1 Pandey, RP; none; *renu.lasers@gmail.com*

Low Level Laser Therapy and Memory Enhancement: Effect of Different Energy Doses

The amount of information that can be stored in short-term memory can vary. An often cited figure is 7±2 items, based on the results of a George A Millers experiment on short-term memory, published in 1956. This clinical study aimed to see the effect of different energy doses of Low Level Laser Therapy (LLLT) on memorizing capacity of the young population aged 20 years to 30 years. 106 subjects with minimum of a 10th grade education with similar cultural background volunteered in the study. The subjects were divided into Group A and Group B according to the laser set they used. The Group A consisted of 56 (Mean±SD 23.7±4.8) subjects and Group B consisted of 50 subjects (Mean±SD 24.89±3.61). Two sets of different multi-diode lasers (Q10 & Q1000) with different energy output but same wavelengths (650, 808) and energy density (38.89mJscm²) were applied for 3 minutes on the frontal and occipital region of the head of the participants simultaneously. For the purpose two equivalent tests of non-associated meaningful words were prepared. One test was used as pre test to determine the baseline working memory at start of the study. The lasers were applied twice a week for 4 weeks. Q10 with energy output of 14mJ/s was applied by group A and Q1000 with energy output of 42mJ/s was applied by group B. Depending on the type of laser applied the participants were divided into two groups. At the end on the 8th application on day 30 the participants took another equivalent "non-associated memory test" and the results were analyzed. For Group A the mean ± SD value score of pre-test was 1.7±1.7 and post test was 1.7±1.7, (p-value0.6090). For Group B the mean value score of pre-test was 2.14±1.5, and the post test score was 2.22±1.04 (p-value0.0553). Using the score of 7 as 100% memory retention, the average percentage of the data was calculated. For group A pre test scored 23.9% and post test scored 53.6% while for group B the pretest scored 30.6% and post test scored 32.6%.

P2.21 Petroff, JT; Baumann, SM; Solomon, E; Jez, JM; McCulla, RD*; Saint Louis University, Washington University in St. Louis; *rmccull2@slu.edu*

Selective Oxidations In Biological Samples By Atomic Oxygen

Early investigations into the reactivity between atomic oxygen [O(3P)] and biomolecules revealed that O(3P) was more selective than other reactive oxygen species (ROS). The oxidation of biomolecules by endogenous or environmental ROS generates a myriad of different oxidation products that may have important roles in disease pathology and redox signaling in cells. The photodeoxygenation of various

aromatic heterocyclic oxides, such as dibenzothiphene S-oxide (DBTO), has been suggested to generate ground state atomic oxygen [O(3P)] as an oxidant. Using DBTO derivatives as a source of O(3P), initial investigation revealed O(3P) can selectively oxidize biomolecules in complex environments. For example, O(3P) has been shown to selectively oxidize plasmalogens in low-density lipoprotein, quantitatively oxidize cysteine residues in proteins, and induce strand-scission in DNA. To achieve selective oxidations in complex mixtures of biomolecules, current work has focused on preparing DBTO derivatives whose properties allow the targeting of specific biomolecules for oxidation in a biological milieu. For example, the ability of lipophilic DBTO derivatives to affect the redox state of the RubisCO large subunit in Arabidopsis leaf extract was explored.

27.3 Philipsen, PA*; Morgan, KA; Harrison, G; Petersen, B; Wulf, HC; Young, AR; Bispebjerg Hospital, Denmark, King's College London, United Kingdom; *Peter.Alshede.Philipsen@regionh.dk*
The CIE action spectrum for pre-vitamin D requires adjustment to be valid in vivo for 25(OH)D in humans

The validity of the CIE action spectrum for the conversion of 7-dehydrocholesterol (7-DHC) to pre-vitamin D₃ has been questioned (Norval et al., PPS, 2010). We designed experiments to test this spectrum and compare it with 2 others (Olds, PhD thesis, 2009 and Bolsee et al. Appl. Optics, 2010) that are blue-shifted compared with the CIE spectrum. This was done with full body (FB - 86%) or partial body (PB - ~5%) exposures, each with 3 very different UVR emission spectra (FB: Arimed B, UV6, Waldmann F85/PUVA; PB: UV6, two solar simulated spectra with different amounts of UVB (2% and 8%)). Each participant had 5 serial exposures of 2 SED with intervals of 2-3 days. Blood was drawn before each exposure and 2 days after the last exposure and analyzed for 25(OH)D by LCMS. 75 participants (skin type I/II, 29M/46F, mean age 26.3±4.9y) completed the study, done in winter-spring 2011-2014 to minimize ambient UVR influence. Individual increases in 25(OH)D vs SED were linear with accumulated dose and dependent on baseline 25(OH)D value. The slopes of the curves were significantly different ($p < 0.0001$) for FB and PB. We weighed the UVR dose (J/m^2) response curves with the three action spectra. Our hypothesis was that the correct action spectrum would result in dose response slopes that were independent of emission spectra. The CIE spectrum resulted in significant differences in dose response slopes for PB: $p < 0.0001$ and FB: $p < 0.00001$. This was also the case for the Olds spectrum, PB: $p = 0.004$ and FB: $p < 0.00001$. However, weighting with the Bolsee spectrum showed non-significant differences for PB: $p = 0.841$ ($r^2 = 0.83$) and just significant differences for FB: $p = 0.049$ ($r^2 = 0.84$). We also tested the effect of a 1-9nm blue-shift on the CIE spectrum in 1 nm steps. For the PB there is no significant difference in slope between UVR sources for 3 - 6 nm shifts ($p > 0.12$) and for the FB there is no significant differences for 4 - 6 nm shifts ($p > 0.18$). A 5 nm blue-shift gave the best fits for both PB ($r^2 = 0.83$) and FB ($r^2 = 0.85$) studies, where the slopes for the different UVR sources were equal. We conclude that the CIE action spectrum needs a 5nm blue-shift, which is a better match with the 292 nm 7-DHC absorption peak. This supports a suggested blue-shift (McKenzie et al., PPS, 2012:11:1174). Furthermore, our data suggest that UVA has no significant effect on vitamin D production.

21.2 Philipsen, PA*; Bodek, M; Petersen, B; Grage, M; Heydenreich, J; Thieden, E; Eriksen, P; Wulf, HC; Bispebjerg Hospital, Copenhagen, Denmark, Danish Meteorological Institute, Copenhagen, Denmark; *Peter.Alshede.Philipsen@regionh.dk*
Population UVR exposure doses are highly depended on environmental observation - based on personal UVR measurements

Personal UVR exposure is much depended on the weather, so we investigated the relation between personal UVR exposure and several meteorological observations. Personal UVR exposure was measured in Standard Erythema Doses (SED) using a wrist worn electronic dosimeter. Behaviour like work/off-work and sunbathing was recorded in a diary on a daily basis. In May through September 2009, 44 Danish farmer families (in all 152 persons) participated in the study. Each family had 1 to 3 participating children. The mean ages for adults and children were 44 years (range 32-67 years) and 11

years (range 5-19 years) respectively. 148 people completed the study and 17303 out of the 19995 collected days were analysed. Meteorological data were collected on a daily basis for the entire period and grouped as follows: maximum temperature grouped into 5-degree intervals (<15, 15-20, 20-25, 25-30, >30 Celsius), rain grouped into two groups (above or below 1mm), sunshine-hours as high or low, cloud cover as: Clear sky, partly clouded, mostly clouded, total overcast. These meteorological parameters were used as "model input variables" to predict personal UVR exposure for farmers, spouses and children separately. The measured average UVR doses (SED) were 206.1, 143.7 and 187.6 for the farmers, spouses and children respectively. The highest personal UVR dose was on days with no rain and much sunshine (2.0 SED, ambient UVR 26.2 SED) and lowest on rainy days with less sunshine (0.7 SED, ambient UVR 15.3 SED). The percentage of ambient UVR received on days with no rain and much sunshine was 10%, significantly higher than the 5% received on less sunny and rainy days. The personal UVR was higher on days with higher temperature, but did not influence the percentage of ambient UVR received. On sunny clear sky days where ambient UVR was highest, people received not only the highest personal UVR dose but also the highest percentage of ambient UVR. (Funded by EC-project ICEPURE (227020) www.icepure.eu)

18.2 Pilat, AK*; Zadlo, AC; Sarna, TJ; Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland; ania.pilat@uj.edu.pl

The effect of iron enrichment on expression of heme oxygenase-1 and ferritin in retinal pigment epithelium cells containing phagocytized melanosomes

ARPE-19 cells containing phagocytized photobleached melanosomes or untreated melanosomes were previously shown to exhibit differential ability to bind metal ions by the containing, which could affect the cells susceptibility to oxidative stress. Here we asked whether the protective mechanism involves differential expression of heme oxygenase-1 (HO-1) and ferritin. ARPE-19 cells on day 7-post plating were loaded by phagocytosis with untreated or photobleached melanosomes isolated from bovine RPE or black latex beads used as control particles. Heme oxygenase-1 and ferritin were quantified by western blot analysis after selected time post-phagocytosis. The stress was confirmed as sub-lethal by cell survival analysis using real-time quantification of propidium iodide fluorescence. Particle phagocytosis induced transient increased in HO-1 protein level and the increase was greater for melanosomes than for black latex beads. By the day 7 post-phagocytosis, HO-1 level were equivalent in cells containing melanosomes versus black latex beads. Iron enrichment increased HO-1 and ferritin in a dose-dependent manner. We did not observe any cytotoxic effect of iron enrichment in cells containing phagocytized untreated or photobleached melanosomes. Phagocytosis of photobleached melanosomes induced an increase in HO-1 and GPx protein level. The results indicate possible role for HO-1 and ferritin in RPE iron homeostasis. Our observation raises the possibility that cells containing pigment granules may differ in expression levels of other iron sensitive proteins aside from ferritin. Supported by grant for young researchers (the department statutory activity) K/DSC/003274 (AP).

10.6 Pollum, M*; Guan, L; Ahsanuddin, S; Baron, E; Lam, M; Crespo-Hernandez, CE; Case Western Reserve University; mmp71@case.edu

DNA and RNA Analogs for Topical Photodynamic Therapy

Photodynamic therapy (PDT) is an effective treatment option for a variety of skin cancers and diseases. Recently, the DNA analog 4-thiothymidine (4tT) has demonstrated efficacy as a PDT agent against rapidly-dividing malignant cells while leaving normal cells unharmed. Its phototoxic activity and low off-target effects make this thio base a highly promising PDT candidate. However, the depth of tissues able to be treated with 4tT is limited by its absorption cutoff at ~365 nm. A thio base photosensitizer able to absorb longer wavelengths of light is needed because longer wavelengths can reach more invasive skin cancers and diseases. Recently, we found that doubling the sulfur substitution of the nucleobase increases its photoreactivity and simultaneously shifts its absorption spectrum into the near-visible region (~395 nm) where light can penetrate more than 100% deeper into tissues. In vitro screening experiments performed in our laboratory using epidermoid carcinoma cells (A431)

have revealed several doubly-substituted thiobase derivatives that are effective photosensitizers. Cell proliferation assays were used to determine the optimal thiobase doses, which do not significantly impact cell survival in the absence of UVA light (360-400 nm). Exposure of the thiobase-treated cells to a low dose of UVA light (<5 J/cm²) results in up to 60% cell death, while the same UVA exposure has no effect on untreated cells. The demonstrated photodynamic efficacy of these novel thiobase derivatives against skin cancer cells, coupled with their expected phototoxicity in deeper human tissues than the recently reported 4tT photosensitizer, fuels the prospect of using these sulfur-substituted DNA and RNA analogs for topical PDT. [The authors acknowledge the CAREER program of the National Science Foundation (Grant No. CHE-1255084) for financial support.]

P2.6 Pucelik, B*; Paczynski, R; Vinagreiro, CS; Gonzalves, NPF; Arnaut, LG; Pereira, MM; Dabrowski, JM; Jagiellonian University, Krakow, Poland, Jagiellonian University and Malopolska Centre of Biotechnology, Krakow, Poland, University of Coimbra, Coimbra, Portugal, Luzitin SA Ed. Bluepharma, Coimbra, Portugal; pucelik@chemia.uj.edu.pl

Tuning the photophysical properties of fluorinated porphyrin derivatives: effect on biological activity and its application in design of efficient anticancer and antimicrobial PDT agents

Tetrapyrrolic macrocycles have been recently explored as scaffolds for the development of more efficient photosensitizers for photodynamic therapy of cancer (PDT) as well as photodynamic inactivation (PDI) of microorganisms. Herein, we perform the comparison of physicochemical and photophysical properties of the series of structurally related fluorinated sulphonamide porphyrin derivatives designed for PDT and PDI application. Our studies report that the chemical modification of the macrocycle by tunable substituents provides an access to the desired biological properties needed for efficient treatment. Thus, we evaluate the broad-spectrum photodynamic activity of investigated photosensitizers against a variety of cancer cell lines and a panel of pathogenic microbes consisting of the Gram-positive and Gram-negative bacteria. All of tested photosensitizers showed favorable characteristics due to strong absorption in visible or near infrared region of spectrum and high singlet oxygen quantum yields. Moreover, selected compounds was found to be a highly effective antimicrobial agents against Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative (*E. coli*, *P. aeruginosa*, *P. putida*) bacteria which are most resistant to antimicrobial PDT. The death of prevalent pathogens was dependent on the PS concentration and illumination time. Significantly, completed eradication of the initial population of microorganisms was observed after photodynamic inactivation mediated porphyrin-based photosensitizer. On the other hand, in the following studies investigated porphyrins and bacteriochlorins indicated the lack of dark cytotoxicity and strong photodynamic effect towards cancer cells (A549, CT26, 2H11, B16F10). The improved photodynamic activity of selected compounds reveal that fluorinated porphyrins and their tetrahydro- derivatives are worth further in vivo investigations not only on animal tumor models but also might prove beneficial to treat the localized wound infections.

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Photochemistry in Confined Spaces: Medium is the Message

It is well known that chemical reactions, activated by heat or light, occurring in biological assemblies differ from that in conventional media such as organic solvents including water. Uniqueness of these reactions has led to continued search for new supramolecular structures (media) that would mimic biological systems. The hosts that have attracted attention includes micelles, liquid crystals, LB films, organic and inorganic hosts, clays, silica, zeolites, etc. This type of investigation under the broader title of "'supramolecular chemistry' that emphasizes the importance of weak interactions has witnessed a renaissance since the award of Nobel Prize to Cram, Lehn and Pederson. The early investigation (pre-1900) of supramolecular photochemistry dealt with crystals while the recent one's deal with host-guest assemblies involving synthetic hosts in water. Reactions carried out in crystals as well as in host-guest assemblies in water share features and they both could be understood based on the topochemical principles established to understand reactions in crystals. This lecture will highlight a few observations

from our laboratory on photoreactions in various media. Studies from our and several other laboratories have established that molecules like humans behave differently when confined. Photochemistry within a water-soluble organic capsule, V. Ramamurthy, *Acc. Chem. Res.*, 48, 2904, 2015. Supramolecular photochemistry: From molecular crystals to water-soluble capsules, V. Ramamurthy and S. Gupta, *Chem. Soc. Rev.*, 44, 119, 2015. Supramolecular Photochemistry as a Synthetic Tool, V. Ramamurthy and J. Sivaguru, *Chem. Rev.*, 2016, ASAP. (Special issue on Photochemistry in Organic Synthesis)

14.3 Ramazanov, RR*; Maksimov, DA; Kononov, AI; St.Petersburg State University;
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Potential Hot Spots for Solar-Induced DNA Lesions

UV-induced DNA lesions are not randomly distributed among the bases. Local DNA structure can affect both photophysical and photochemical aspects of DNA photo-damage. We report on the electronic spectra of DNA stacked bases calculated for a wide range of base stacking conformations taken from PDB data bank and molecular dynamics simulations. Quantum mechanical calculations on stacked thymine, cytosine, adenine (R. R. Ramazanov, D. A. Maksimov, A. I. Kononov, *J. Am. Chem. Soc.* 2015, 137, 11656), and guanine dimers reveal low-lying excited excitonic states in the stacked bases with reduced inter-base distance. Absorption of terrestrial solar radiation at 300 nm by such structures increases dramatically with respect to canonical forms of stacking. The low-lying excited states at about 300 nm in the dimers may also serve as a trap for the excitation energy transfer from the neighboring bases in the helix. We thus conclude that such sites with non-canonical stacked geometries can be hot spots for sunlight-induced DNA damage.

P1.9 Rege, K*; Urie, RR; Flake, TJ; Mushaben, M; Heys, JJ; Jaffe, M; Arizona State University (ASU), ASU, Montana State University (MSU), MSU, Midwestern University;
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Plasmonic Nanocomposites for Rapid Tissue Sealing and Repair

Laser tissue welding is a potential alternative to sutures or staples that are conventionally used for tissue approximation in soft tissue surgeries. Laser tissue welding is a technique where a chromophore absorbs photons and converts them into heat to seal apposed tissue edges. Significant barriers to laser tissue welding including insufficient closure strength and extensive thermal damage have hindered the clinical application of this technique. In this work, we overcome these barriers in ex vivo tissue welding and spatiotemporal modeling. In this presentation we discuss results of ex vivo laser tissue welding using gold nanorod-elastin-like polypeptide crosslinked matrices, gold nanorod-collagen hydrogels, and gold nanorod-silk thin films. These plasmonic biopolymer nanocomposites were shown to return up to 68% of native tissue tensile strength and 54% of native tissue bursting point pressure. Additionally, we discuss the predictions of a spatiotemporal model developed to optimize the parameter space in laser tissue welding.

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Electronic excitation energy transfer in DNA and DNA-Ag cluster complexes.

We report on the electronic excitation transfer in single-stranded and double-stranded cytosine oligonucleotides and also in the complexes of DNA duplexes with Ag nanoclusters studied by steady-state and time-resolved femtosecond spectroscopy. Fluorescence up-conversion spectra of the cytosine oligonucleotides are discussed in the frame of electronic excitation transfer to the low-lying exciton states of stacked cytosines with reduced inter-base distance that have been predicted by QM calculations (R. R. Ramazanov, D. A. Maksimov, A. I. Kononov, *J. Am. Chem. Soc.* 2015, 137, 11656). Steady-state fluorescence excitation spectra of the DNA-Ag cluster complexes show that the energy is transferred from 10-30 DNA bases to Ag cluster. Fluorescence decay curves for the cytosine

oligonucleotides and the DNA-Ag cluster complexes show that the processes of energy transfer occur within <100 fs. The obtained results are explained by coherent excitonic mechanism of the transfer. The observed efficient energy transport along DNA strand can affect the distribution of UV-induced lesions in DNA.

27.5 Rizvi, I*; Briars, E; Bulin, A-L; Anbil, S; Vecchio, D; Broekgaarden, M; Hanna, W; Celli, JP; Hasan, T; Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Wellman Center for Photomedicine, Massachusetts General Hospital, Wellman Center for Photomedicine, Massachusetts General Hospital and Howard Hughes Medical Institute, University of Massachusetts Boston; rizvi.imran@mgh.harvard.edu

Photodynamic therapy-based combinations overcome tumor endothelial cell-induced heterogeneity and chemoresistance in 3D ovarian cancer cultures

Identifying the molecular, cellular, and microenvironmental cues that lead to heterogeneity and treatment resistance is critical to developing strategies to target unresponsive regions of stubborn disease. Photodynamic therapy (PDT) has been shown to synergize with conventional agents and to overcome the evasion pathways that cause resistance. Developing PDT-based combinations that target resistant tumor populations and cooperate mechanistically with conventional agents is an increasingly promising approach to improve therapeutic efficacy while minimizing toxicity, particularly in complex disease sites. Here, 3D in vitro models that restore the architectural features, physical stress and heterocellular signaling experienced by tumors in vivo are described in the context of metastatic ovarian cancer, the leading cause of death among gynecologic malignancies. The potential value, and challenges, associated with developing complex cell-based models that include communication with stromal partners (e.g. tumor endothelial cells, which are emerging as dynamic regulators of cell cycle and treatment resistance), will be presented, with a particular focus on addressing resistance to conventional agents with PDT-based combination regimens. We show that priming hetero-cellular ovarian cancer nodules with verteporfin-based PDT prior to treatment with chemotherapy (carboplatin and paclitaxel) significantly decreases the size and viability of complex 3D tumors and decreases heterogeneity in response. The potential molecular and phenotypic basis of this enhanced efficacy and predictability in response will be presented.

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Quantitative Spectroscopy for Optical Monitoring of PDT

The use of reflectance and fluorescence spectroscopy for monitoring (pre-) clinical photodynamic therapy in has been under development for over 20 years. A large body of work has developed around the use of these types of optical measurements for monitoring patient individualised pharmacokinetics and in the application of PDT dosimetry. Metrics based on the measurement of photosensitiser content of tissue, the light fluence (rate), the presence of reactive oxygen species and the physiological response during and following PDT are being studied in pre-clinical models and some stage I/II clinical trials optimizing PDT. The application of quantitative spectroscopy is illustrated in 3 ongoing clinical studies utilizing topical PDT in the skin, intra-luminal PDT in the GI tract and interstitial PDT in the head and neck, and in a recent pre-clinical application of targeted-PDT for head and neck cancer. In these studies light fluence rate measurements are combined with reflectance and fluorescence spectroscopy to quantitatively determine changes in tissue physiology and photosensitiser content. Our results show that the photosensitiser under investigation has a critical influence on which parameters are most appropriately used to perform dosimetry. Changes in fluence rate during PDT were strongly influenced by changes in tissue optical properties caused by changes in light scattering coefficient, oxygenation and blood volume. These effects were fluence rate dependent and not always predictable. Monitoring fluorescence photobleaching during therapy and using this as a PDT dose metric is very challenging in an environment of changing tissue optical properties. In all cases the volume of interrogation of optical measurements is critical parameter particularly for interstitial PDT. We have found that truly quantitative measurements of photosensitiser fluorescence before PDT can predict the

clinical response in some applications. The clinical implications of these types of measurements and future applications of imaging will be discussed.

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Resistance of oral cancer cells to 5-aminolevulinic acid-mediated photodynamic therapy

Oncologic photodynamic therapy (PDT) has been used as an adjuvant treatment for oral squamous cells carcinoma (OSCC). The aim of this study was to investigate whether OSCC (SCC9 cells) develop resistance after several cycles of 5-aminolevulinic acid-mediated PDT (5-ALA-PDT), and to verify whether the expression of markers associated with cell survival (NF- κ B, Bcl-2, iNOS, mTOR, and Akt) is altered during this process. SCC9 cell line was subjected to one cycle of PDT (630 nm, 5.86 J/cm², 150 mW, 150 s). Survival cells after this first PDT cycle were cultured again and submitted to more five cycles, increasing the radiation dose. Four resistant cell populations were obtained. The resistant populations showed significant increase on pNF- κ B, iNOS, pmTOR, and pAkt. In conclusion, OSCC cells exhibited increased viability after five cycles of 5-ALA-PDT and overexpression of proteins associated with resistance. This fact must be carefully considered when initiating PDT for OSCC.

5.8 Schanze, KS; University of Florida; kschanze@chem.ufl.edu

Triplet States in Organometallic Conjugated Materials

Triplet excited states (excitons) play an important role in the application of organic materials. For example, in organic light emitting diodes, harvesting of triplet excitons affords a substantial enhancement in external quantum efficiency and luminous efficiency. In organic solar cells, recombination to produce triplet states can reduce the efficiency of photocurrent generation. Our research program has explored the properties of triplet excitons in π -conjugated polymers and model oligomer systems. This work has taken advantage of the effect of spin-orbit coupling enhancement by heavy metal centers that are strongly coupled to the π -conjugated electronic system. In particular, platinum(II) acetylides and orthometalated platinum(II) complexes give rise to enhanced intersystem crossing efficiency and in some cases room temperature phosphorescence. This allows facile investigation of the energetics and dynamics of triplet exciton states. Topics of interest include triplet excited state structure and delocalization in conjugated molecules, the effect of electronic structure on intersystem crossing efficiency, triplet exciton diffusion length, triplet states in organic solar cells and in near-infrared light emitting diodes.

16.2 Schlau-Cohen, G.S.; MIT; gssc@mit.edu

Elucidation of the Photoprotective Mechanisms in Algal Light Harvesting

In photosynthetic light harvesting, absorbed energy migrates through a protein network to reach a dedicated location for conversion to chemical energy. In green algae, this energy flow is efficient, directional, and regulated. The regulatory response involves complex and complicated multi-timescale processes that safely dissipate excess energy, thus protecting the system against deleterious photoproducts. We explore the mechanisms behind this photoprotective process in a light-harvesting complex implicated in dissipation, light-harvesting complex stress response (LHCSR). By characterizing the conformational states and dynamics of individual proteins, we identify the extent of energy dissipation in single LHCSR proteins and how the extent of dissipation changes in response to pH and carotenoid composition, two components known to play a role in photoprotection. From this information, we explore how individual complexes contribute to the balance between efficiency and adaptability in photosynthetic light harvesting.

6.8 Schlau-Cohen, G.S.; MIT; gssc@mit.edu

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Recent advances in 3d modelling of the UV body exposure

In this talk an overview in the progress of 3d modelling will be given as well as on recent developments and on future challenges. UV exposure of a human can be calculated by using 3d body models. Nowadays body models are flexible to a high degree. Models for each gender and for different ages are available. Each body model consists of tens of thousands of polygons, which deliver a realistic and detailed proxy of a body even for small parts like the ears. The posture can be changed, as well as clothing and hairstyle. Additionally the models can perform movements such as walking, running or cycling. The UV irradiance falling on a certain part of the body may result from measurements or from radiative transfer model calculations. The later enable modelling at any time and location as well as the weighting with any action spectrum. The exposure pattern differs for different action spectra. The evaluation of 3d-modelled body exposure was done in the past. Nonsurprisingly the agreement of modelled and measured values is best for clear sky. The agreement for cloudy sky depends on the time scale: as longer the period the better is the agreement. An important input parameter to the model must be delivered by studies on behaviour. Behaviour includes location, activity (postures), clothing and duration, may change fast and may have typical diurnal patterns. Recently we analysed several behaviour studies to build up a typical week at a beach resort. This includes the behaviour at the beach (e.g. walking, lying, swimming), at the hotel (e.g. balcony) and at other nearby locations (e.g. side walk cafes) in the urban environment and sports activities. It includes also the clothes worn in dependence of location, activity and time. For this a variety of postures, clothing and radiation environments were combined to calculate the UV exposure pattern of the body for a week of vacation. With that, body modelling becomes a very helpful tool for sun protection and health care.

P2.7 Schmalwieser, A.W.*; Blumthaler, M.; Klotz, B.; Schwarzmair, M.; Baumgartner, D.; Schauburger, G.; University of Veterinary Medicine, Institute of Physiology and Biophysics, Div. of Biomedical Physics, Medical University Innsbruck, University of Graz, Kanzelhöhe Observatory; alois.schmalwieser@vetmeduni.ac.at

The Austrian UVA-Network

In 2012 the Austrian UVB-Network (www.uv-index.at) was enlarged by UVA-broadband instruments. Three out of 12 stations were equipped with detectors type J033 (CMS-Schreder, Austria) which responds to 310 nm to 400 nm ($r_{50\%}=330$ " 375 nm) and possess a cosine-like response. At two stations both global and diffuse (using a shadow-ring) UVA radiation is measured. The stations are located in Vienna (16.4°E, 48.3°N, 153 m), Innsbruck (11.4°E, 47.3°N, 577m) and Mt. Gerlitz (13.9°E, 46.7°N, 1526 m). The devices are cared according international recommendations for UV-Index measurements. Measurements are corrected by a calibration matrix in respect to solar elevation and total ozone column. Here we present the global and diffuse UVA measurements during a period of up to 4 years as well as a comparison of global and diffuse and a comparison to erythemally effective

UV and to total global radiation. At Vienna (153 m asl) the daily global UVA dose reaches 12 kJ/m² in January and 70 kJ/m² in June and July while the corresponding values of the diffuse component are 9 kJ/m² and 40 kJ/m² respectively. The ratio between diffuse and global shows a clear annual cycle whereas in winter it is between 0.75 and 0.9 while in summer it can be within 0.4 and 0.8. The ratio between UV-Ery and UVA is highest in August reaching a value of 0.12 and lowest in January with 0.04. The ratio between UVA global and total global is relative constant during the year varying around 0.055 with a higher variability in winter which may be partly driven by the higher uncertainties due to low irradiances. At the alpine station Mt. Gerlitz (1526 m) the daily global UVA dose reaches higher values than at Vienna. The ratio of daily global UVA doses from these two stations is not constant over the year but depends on season.

2.2 Schnermann, MJ*; Nani, RN; Gorke, AP; National Cancer Institute; *martin.schnermann@nih.gov*
Heptamethine Cyanine Near-IR Uncaging Chemistry: Discovery and Applications

Many key fundamental and applied questions in biology require unraveling issues relating to the spatial and temporal organization of multi-cellular systems. While the combination of photocaged small molecule probes and the spatially controlled application of light could in principle provide key insights, existing photoremovable caging groups are often not suitable, particularly for organismal applications. This is due to the general requirement of UV or blue light, which suffers from associated toxicity and poor tissue penetration. By contrast, light between 650 and 900 nm, often referred to as the near-IR window, is cytocompatible and exhibits much improved tissue penetration. We have developed a single photon uncaging reaction initiated by 690 nm light using readily synthesized C4'-dialkylamine-substituted heptamethine cyanines. Release occurs through a reaction sequence comprising regioselective photooxidative C-C cleavage and then hydrolysis of the C4'-amine. The oxidative cleavage step was known to be the basis of cyanine photobleaching and our efforts are the first to deliberately apply this remarkable reactivity for small molecule delivery. The precisely controlled introduction of biological stimuli will enable studies within complex model organisms that address cell-cell interactions or control/monitor cell fate. In this area, we are developing advanced tools to spatially pattern gene expression. From a medical perspective, the ability to release therapeutic molecules using the targeted application of near-IR light will facilitate the creation of innovative drug delivery strategies. In this area, we are developing antibody-based theranostic tools with the goal of treating otherwise unresectable tumors and micrometastases. Details regarding the development of this approach, our mechanistic studies, and long-term goals will be discussed.

26.4 Shin, H; Zeng, X; Ren, Z; Yang, X*; University of Illinois at Chicago; *xiaojing@uic.edu*
Structural Basis of Color Perception and Signal Integration in Cyanobacteriochromes

Phytochromes are bilin-binding photoreceptors that were first characterized as red-light photoreceptors in plants. Thanks to large-scale genome sequencing efforts and recent discovery of cyanobacteriochromes, the phytochrome superfamily has been significantly expanded. Bilin-binding photoreceptors collectively exhibit extraordinary versatility in action spectra, domain architecture and signaling properties. We will present our ongoing crystallographic and biochemical studies that aim to understand how color perception, signal integration and long-range signaling are achieved in multi-sensor bilin-based photoreceptors at the molecular level.

P2.8 Shirsath, NP*; Ober, J; Frei, R; Mayer, G; Heinemann, A; Wolf, P; Research Unit of Photodermatology, Department of Dermatology and Venereology, Medical University of Graz, Graz, Austria, Center for Medical Research, Medical University of Graz, Graz, Austria, Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria;
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PVA pretreatment down-modulates key inflammatory cytokines and leads to reduced activation of the signal transducer and activator of transcription (STAT1/3) pathway in the imiquimod model of

psoriasis

Psoriasis is a chronic disease for which PUVA (psoralen+ultraviolet-A) is one of the most effective treatment options. Here by using the IMQ (Imiquimod) model we dissect the early cellular events and signals promoting psoriasis. We left mouse skin untreated (group1) or pretreated it with 0.25J/cm² PUVA followed by IMQ at 48 and 72h (group2). In group3, IMQ was applied after a gap of 8 days post PUVA. PUVA pretreatment significantly reduced the macroscopic and microscopic psoriasiform skin changes and decreased influx of innate immune cells. Bone marrow neutrophils showed less responsiveness towards CXCL1 in a migration assay. Moreover, significant downmodulation of key inflammatory cytokines was achieved with reduced expression of phospho STAT1/3 levels in skin sections (in group2) after PUVA treatment. However, this effect of PUVA was lost when a gap of 8 days was kept to apply IMQ, pointing to an indirect effect of PUVA modulating psoriatic manifestation.

P2.25 Silber, ZS*; Obaid, G; Kuriakose, J; Broekgaarden, M; Wang, Y; Hui, J; Tsourkas, A; Hasan, T; Northeastern University, Massachusetts General Hospital, University of Pennsylvania; zack.silber8@gmail.com

Tumor-selective photodynamic therapy using copper-free click-conjugated liposomes

Photodynamic therapy (PDT) utilizes the photoirradiation of a photosensitizer (PS) to convert available oxygen into a cytotoxic reactive molecular species. Liposomal formulations of PSs retain their photoactivity and promote PS uptake in tumors through the enhanced permeability and retention effect. By conjugating tumor-targeting moieties at the surface of liposomal PSs, tumor cell selectivity is increased and off-target phototoxicity is reduced, allowing higher PDT doses to be tolerated. We have developed a liposomal platform that utilizes copper-free click chemistry to surface-conjugate antibodies modified with an azide moiety through two distinct methods. This reaction occurs bioorthogonally, without the need for toxic copper catalysts and yields no side products. The first method utilizes a Protein Z molecule that is site-specifically photocrosslinked to the Fc region of an anti-EGFR antibody, Cetuximab. The second method utilizes a stochastically conjugated azide linker that has no control over antibody orientation at the liposome surface. The site-specific formulation allows complete control over the antibody's positioning at the liposome surface without interfering with cellular antigen binding. The site-specific antibody-liposome formulation also has higher conjugation efficiency than the stochastic method. However, in vitro binding assays show that the stochastic antibody liposomes have a higher rate of binding to high-EGFR expressing cells. We have characterized the size and zeta potential of the antibody liposomes using dynamic light scattering following conjugation. We are currently examining the selective phototoxicity and pharmacokinetic behavior of the two conjugation methods in vitro and in vivo. The developed clicked antibody liposomal construct is a flexible and reproducible platform for targeted drug delivery, allowing a variety of targeting ligands to be attached and multiple hydrophobic and hydrophilic PDT agents to be used in combination.

4.4 Simone, C; University of Pennsylvania; Charles.Simone@uphs.upenn.edu

Advances in Photodynamic Therapy for Thoracic Malignancies: Frontiers for Mesothelioma and Lung Cancer

Abstract not available.

29.1 Sliney, David H.; Sliney, David; Johns Hopkins Bloomberg School of Public Health; david.sliney@att.net

What are the Spectral Limits of Visual Radiation?

Historically, the concept of the nature of light has endured some controversies - from the ancient Greek concept that light originated in the eye, to Newton's corpuscular theory, on to wave-particle duality. However, the true extent of the visible spectrum has not been possible to standardize. The CIE defines

infrared and ultraviolet photobiological spectral bands (e.g., UV-A and IR-B), but the CIE recognizes the variable spectral limits of vision. The limits of visibility really extend from about 310 nm in the ultraviolet (in youth) to about 1100 nm in the near-infrared, but depend very much on the radiance, that is, "brightness" of the light source. Recent suggestions that infrared vision occurs from two-photon isomerization are based only on non-linear effects from ultra-short pulses.

15.6 Sobol, R; University of South Alabama Mitchell Cancer Institute; lstrong@burkinc.com

Cellular mechanisms of repair and response to oxidized nucleotides

Abstract not available.

3.1 Sokolov, KV; The UT M.D. Anderson Cancer Center; ksokolov@mdanderson.org

Nanoparticle clusters for cancer imaging and therapy

Synthesis of heterogeneous hybrid nanoparticles can be advantageous in a variety of biomedical applications. However, integration of multiple components in a single nanostructure is a challenging task. Most of the existing approaches to synthesis of hybrid nanoparticles require cumbersome multi-step protocols and result in nanostructures with limited tunability of physical and chemical properties. Here, we present a general idea of using controlled self-assembly of individual very small ca. 5 nm diameter nanoparticles into well-defined nanoclusters with desirable properties for in vivo applications. Two specific examples of this approach will be discussed: (1) synthesis of biodegradable plasmonic nanoparticles, and (2) magneto-plasmonic nanoclusters (MPNs) with a strong near-infrared (NIR) absorbance and a high magnetic moment in an external magnetic field. We demonstrated that self-assembled nanoclusters greatly improve sensitivity of MRI and photoacoustic imaging, magnetic cell separation and cell manipulation in an external magnetic field. Furthermore, biodegradable nanoclusters can dissociate into ca. 5 nm constituent particles under physiological condition that facilitates accelerated excretion from the body.

29.3 Solntsev, KM*; Boxrud, P; Lorenz, JA; DeSa, RJ; OLIS, Inc.; kyril@olisweb.com

Photochemical/spectroscopic studies in turbid and scattering media

The talk is dedicated to the scientific research in business environment. The research at OLIS involves the development and utilization of various state of the art optical spectrometers. This privately-held American company continues to serve the biophysical, biochemical, and bioenergetics fields with absorbance, fluorescence, and circular dichroism spectroscopy for sophisticated research and routine measurements. Our newest product line "the CLARiTY series" has eliminated the previously insurmountable barrier to accurate absorbance spectroscopy of live, whole, particulate, aggregated, turbid samples. Few sample cases will be discussed. They involve steady-state and time-resolved measurements of redox reaction in cell suspension, photoswitching of MOFs, monitoring vitamin A delivery & uptake in intact frog eye retina, and many more. Finally, an active CLARiTY spectrophotometer will be demonstrated and the audience will have to chance to take the absorbance spectra of their own samples. Bring your own turbid sample! OLIS website: <http://olisweb.com/> Dr. Solntsev's website at GaTech: <http://ww2.chemistry.gatech.edu/solntsev/>

2.5 Sova, SL; University of Maryland Baltimore County; ssoval@umbc.edu

Photooxidative Crosslinking and Affinity Labeling of Proteins Using Naphthalene Imides

Determining protein structures and interactions are crucial to understanding their biological function. Photoaffinity labeling and oxidative crosslinking have been demonstrated by us using naphthalimide and naphthalimide derivatives as a new structural probes of proteins. N-(4-Hydroxyphenyl propionic acid)-1,8-naphthalene imide (NI-Tyr) and N-(3,4-dihydroxyphenyl propionic acid)-1,8-naphthalene imide (NI-Dopa) actively target the binding site of mushroom tyrosinase. Enzymatic oxidation was accompanied by the recovery of fluorescence intensity in both cases. Competitive kinetic assays

showed mixed inhibition of the monophenolase activity and activation of the diphenolase activity, indicating these naphthalimides were oxidized within the active site; however no permanent covalent linkage was observed. Despite failing to label mushroom tyrosinase, these compounds do oxidatively crosslink proteins such as lysozyme. Though the location of the crosslink has not been determined, the compounds are viable for probing transient protein interactions. In separate work, N,N'-bis[2-(ethanoic acid)]-1,4,5,8-naphthalene diimide does nonspecifically label bovine serum albumin. To make this diimide more site specific, asymmetric naphthalaldiimides containing a site-specific substrate or inhibitor are being synthesized as new photoaffinity labels with molar extinction coefficients nearly 1000-fold higher than traditional benzophenone-based compounds.

19.1 Spring, BQ*; Sears, RB; Zheng, LZ; Mai, Z; Watanabe, R; Sherwood, ME; Pogue, BW; Pereira, SP; Villa, E; Hasan, T; Northeastern University, Emmanuel College, Massachusetts General Hospital, University of California San Diego, Dartmouth College, University College London;
b.spring@neu.edu

Optical Molecular Image-Guided Treatment of Residual Microscopic Tumors and Suppression of Multiple Treatment Escape Pathways

This talk will highlight two recent strategies guided by fluorescence molecular imaging for preventing tumor recurrence. The first enables selective photodestruction of residual deposits of microscopic tumors missed by conventional treatments that frequently seed local and distal recurrence using a unique antibody-conjugate that enables a combination of molecular targeting and cancer cell activation. This approach also facilitates visualization and monitoring of residual tumor deposits and their molecular expression profiles using fluorescence endomicroscopy. Second, we will present a newly developed nanoparticle drug delivery system motivated by molecular imaging of dynamic molecular signaling pathways associated with tumor regrowth and invasion. The nanoparticle supports photo-initiated release of multikinase inhibitors "at the right time and the right place" to suppress multiple modes of treatment escape. These new approaches support an expanded role for the use of image-guided photomedicine to suppress disease recurrence in the surgical bed and to reduce toxicities of molecular inhibitors and chemotherapy.

8.1 Suschek, C. V.; Department of Trauma and Hand Surgery, Medical Faculty of the Heinrich-Heine-University Dusseldorf; *suschek@hhu.de*

Nitric Oxide Derivatives and Skin Environmental Exposure to light: From Molecular Pathways to Therapeutic Opportunities

The physiological as well as pathophysiological role of cutaneous nitric oxide (NO) in human skin has been under investigation since first reports of nitric oxide synthase (NOS)-expression in human skin tissue in 1992. Already in the first years on NO research it became obvious that NO plays a pivotal role in the dermal response to environmental stimuli like ultraviolet radiation, heat, and cold. Additionally to enzymatically produced NO a range of non-enzymatic pathways for NO generation has been identified. Substantial quantities of NO radicals are continuously generated in the human skin, and formation of NO can be enhanced 3-5 fold by illumination with UVA light, especially in the outer skin. This phenomenon is attributed to the photolysis of photolabile nitroso compounds, which play an outstanding role in mammalian physiology. Previous work has shown that human skin is quite rich in these compounds, which attain local concentrations far higher than found in the blood circulation under normal conditions. Both are known to deliver free NO radicals upon UVA photolysis even at the modest UVA fluxes as found in sunlight at sea level. Accordingly, the degree of exposure of skin to ambient light will significantly affect the NO status of human skin, which plays an outstanding role in the regulation of local hemodynamic parameters, inflammation, infection, wound healing, and protection from the injurious effects of UV radiation.

27.2 Sutherland, JC; Augusta University; *jsutherland@augusta.edu*

Cellular Killing and Transformation by Ultraviolet and Ionizing Radiation: a Fundamental Difference

The clonogenic survival curves of cells growing in vitro as a function of dose are generally similar for ultraviolet (UV) and ionizing radiations, and are often represented by similar mathematical expressions. But the validity of some widely used expressions for survival curves are problematic because the underlying theory ignores repair. Cellular transformation is frequently described by a linear-quadratic (LQ) function, because they are often non-linear. However, this description is essentially ad hoc as it is not based on underlying assumptions about the transformation process. In addition, this fails to account for the plateau in the plot of transformants per survivor at doses yielding very low ($< 10^{-3}$) survival. The repair-dependent theory of cell survival and transformation uses a single set of assumptions to characterize both survival and transformation, and can describe the shapes of both, including the high-dose plateau in survival frequency.* But there is a fundamental difference between the results for mammalian cells exposed to UV and ionizing radiations. In the case of x-rays and neutrons, the survival curves can be fit by the expressions generated by the repair-dependent model only if different parameters are used for the two processes. In contrast, in the case of UV, the dose-response curves for survival and transformation can be fit using the same values of the shared parameters. This difference is due to the fact that the theory underlying the repair-dependent model is based on the assumption that a single radiation event never results in more than one potentially lethal damage. This is true for the absorption of a UV photon, but generally not true for a radiation process, such as the absorption of an x-ray that may result in an energetic electron, or the impact of a neutron, that can result in an energetic proton. Both processes can produce multiple damages per event. * Sutherland, J. (2014). *Physics in Medicine and Biology* 59: 5073-5090.

P2.9 Taniguchi, M*; Du, H; Lindsey, JS; North Carolina State University; mtanigu@ncsu.edu

PhotochemCAD 3. Diverse Modules for Photophysical Calculations with Accompanying Spectral Databases

PhotochemCAD is a software program and accompanying spectral database for use in photochemistry and photobiology. The development of "PhotochemCAD" was motivated by the desire to have at one's fingertips spectral data (absorption spectra with molar absorption coefficients, emission spectra with quantum yields, references to the original photochemical literature) for a wide variety of representative compounds and the ability to perform photochemically relevant calculations using such spectral data. The program enables calculations (Förster energy transfer, oscillator strength, fluorescence lifetime, multicomponent analysis, blackbody radiator, transmission) and simulations (energy transfer, artificial spectrum creation based on Lorentzian and Gaussian distributions). PhotochemCAD 1 and 2 were released in 1998 and 2005, respectively. PhotochemCAD 3 contains the following revisions: (1) expanded spectral database, (2) multiple database handling capability, and (3) user interface enhancements. In addition to the original spectral database (~150 compounds), over 1000 new spectral data have been incorporated, which includes (i) ~400 commercial fluorophores (cyanine dyes, BODIPYs, Q-dot, Alexa Fluor, fluorescence proteins, etc.) and (ii) ~650 tetrapyrrole macrocycles (porphyrins, phthalocyanines, chlorins, bacteriochlorins). The spectral data in the PhotochemCAD database can be exported to other programs, and spectral data recorded by the user can be easily imported to the PhotochemCAD database. The availability of this program and database was anticipated to facilitate the design and analysis of a wide variety of photochemical systems (e.g., energy-transfer dyads, artificial light-harvesting architectures, molecular photonic devices, etc.). PhotochemCAD 3 will be available in due course for free downloading at <http://www.photochemcad.com>

6.9 Timlin, JA; Sandia National Laboratories; jatimli@sandia.gov

Quantitative Imaging of Photosynthetic Pigments and Proteins in Live Cells

In order to facilitate efficient energy harvesting and transfer, the endogenous pigments in photosynthetic organisms such as chlorophylls and carotenoids are collocated in pigment-protein

complexes and have an inherently high degree of spectral overlap. Photosynthetic pigments and proteins vary within and between organisms and are dynamically regulated in response to changing environmental conditions. The identity, abundance, and localization of photosynthetic pigments are critical to understanding light harvesting and the reactions that produce energy within the cell. Spectral imaging methods coupled with multivariate analysis are uniquely suited to untangle the highly overlapped spectral signatures from photosynthetic pigments and reveal global pigment localization and dynamics in intact, living cells. In this talk, I will introduce the state-of-the-art in spectral imaging and multivariate analysis methods with an emphasis on preprocessing techniques that we have developed for robust analysis. Examples will highlight applications of hyperspectral confocal fluorescence microscopy and hyperspectral confocal Raman microscopy to identify and map multiple photosynthetic pigments in cyanobacteria, green algae, and land plants in response to changing conditions (environmental, genetic differences, etc.). These methods provide increased fundamental understanding of global pigment dynamics and function within and across photosynthetic organisms. The results have important implications for synthetic biology, genetic engineering, and development of biohybrid or bio-inspired devices.

16.3 Timlin, JA; Sandia National Laboratories; jatimli@sandia.gov

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20.4 Tong, L*; Wu, S; Ohio University; tongll@ohio.edu

Carnosol as a therapeutic compound in preventing and treating UVB-induced skin cancer through its anti-inflammatory and pro-apoptosis characteristics.

Repeated overexposure of ultraviolet B (UVB) radiation is known to be carcinogenic, although the detailed mechanisms remain ambiguous. Carnosol is a natural compound extracted mainly from rosemary and sage leaves, which is edible and thus environmental friendly. It has been shown to have anti-inflammatory and anti-cancer effects. However, very little is known on the effect of carnosol on UVB-induced skin cancer. In this report, we studied the effect of carnosol on UVB-induced non-melanoma skin cancer formation and progression. By repeated exposing to UVB radiation, normal human skin cells can be transformed to cancerous skin cells, while treating the cells with carnosol could significantly reduce the transformation rate. The mechanisms possibly relies on the fact that carnosol can partially reduce UVB-induced reactive oxygen species (ROS) elevation and thus protect UV-induced DNA damage in normal skin cells. Our data also demonstrated that carnosol can impede the UVB-induced NF-kappaB activation, by protecting IkappaB degradation and thus reduce NF-kappaB phosphorylation level at active site and reduces its DNA binding activity. The manipulation of

NF-kappaB activity in turn affects a series of cellular responses including inflammation and cell survival signaling pathways. In addition, carnosol can also prohibit skin cancer cell progression with or without UV radiation. Based on our data, carnosol could be a potential therapeutic for chemoprevention and treatment of UVB-induced skin cancers.

17.3 Tong, X; Mirzoeva, S; Bridgeman, B; Veliceasa, D; Crawford, SE; Cornwell, M; Volpert, OV*; Northwestern University, Chicago, IL, USA, Northwestern University, Stanley Manne Children's Research Institute, North Shore University Research Institute; olgavolp@northwestern.edu

Regulation of UVB-induced carcinogenesis by Apigenin and Thrombospondin-1

Ultraviolet B (UVB) radiation is the main cause of non-melanoma skin cancer, which is increasingly prevalent of late despite the use of sunblock. Thus new agents and mechanisms useful for prevention or treatment of UVB-induced cancer are highly important. Endogenous angiogenesis inhibitor thrombospondin-1 (TSP1) is expressed by the epidermal keratinocytes and its critical role in cutaneous angiostasis is well known. Importantly, TSP1 expression is potently inhibited by UVB. Plant-derived bioflavonoid apigenin is a nutraceutical chemopreventive agent with anti-proliferative and anti-angiogenic effects, which inhibits UVB-induced carcinogenesis in skin through multiple mechanisms. We discovered that apigenin restores TSP1 expression in UVB-irradiated keratinocytes and skin. Using TSP1-null hairless mice we have shown that endogenous TSP1 has protective role in UVB-induced carcinogenesis and is critical for chemopreventive effect of apigenin in skin. We showed dramatic increases in UVB-induced cutaneous angiogenesis and inflammation in the absence of TSP1. Moreover, restoring TSP1 activity in UVB-irradiated skin with bioactive peptide mimetic was sufficient to recapitulate wide variety of apigenin's effects in UVB-exposed skin including its anti-angiogenic, anti-inflammatory and anti-proliferative actions. Seeking mechanisms underlying TSP1 regulation by the UVB and apigenin, we identified miRNA-dependent downregulation of TSP1 mRNA. Apigenin restored TSP1 expression through increased cytoplasmic presence of the RNA-binding protein HuR, where it bound TSP1 mRNA and resulted de novo synthesis of TSP1 protein. Together, our data provide new mechanism by which apigenin restores cutaneous homeostasis and prevents UVB-induced carcinogenesis.

20.2 Tong, X; Mirzoeva, S; Bridgeman, B; Plebanek, MP; Cornwell, M; Crawford, SE; Volpert, OV*; Northwestern University, Chicago, IL, USA, North Shore University Research Group, Evanston, IL, USA; olgavolp@northwestern.edu

Regulation of cutaneous inflammation by Thrombospondin-1

Ultraviolet B (UVB) radiation is the main cause of non-melanoma skin cancer, whose incidence steadily increases despite the use of sunblock agents. Thus new agents and mechanisms useful for prevention or treatment of UVB-induced cancer are highly important. The contribution of UVB-induced inflammation and immunosuppression, respectively, are critical for UVB-induced skin carcinogenesis. While critical roles of endogenous angiogenesis inhibitor thrombospondin-1 (TSP1) in cutaneous angiostasis is well established its immunomodulatory effects in UVB-irradiated skin are not well understood. Importantly, TSP1 expression is potently inhibited by UVB. Using TSP-1 null hairless mice we have demonstrated that the lack of endogenous TSP1 causes dramatic increases in UVB-induced cutaneous angiogenesis and acute inflammation as well as baseline inflammatory activity. Seeking immune cell populations affected TSP1 we found profound differences between the myeloid cell populations in the bone marrow of the wild type and TSP-1 null mice exposed to UVB radiation and the recruitment of the circulating myeloid cells. These differences translated in significantly increased macrophage infiltration and neutrophil presence in the UVB-exposed skin. In addition, the lack of endogenous TSP1 strongly altered cytokine profile in skin keratinocytes and in circulation in a way that favored inflammatory and proliferative activities. Importantly, restoring TSP1 function in UVB-irradiated skin and in TSP1-null mice with small bioactive peptide mimetics significantly decreased UVB-induced skin edema, inflammatory infiltrates and cytokine production. Our analyses point to the critical role of TSP1 in cutaneous inflammation and potential utility of TSP1

peptide mimetic to mitigate the UVB inflammatory effects in skin.

P2.10 Tournear, JC*; Denius, K; Gaillard, ER; NIU; jentournear@me.com

Investigating the Effects of Blue Light Irradiation on Retinal Pigment Epithelial (RPE) Cell Death in Relation to Age Related Macular Degeneration (AMD)

Age related macular degeneration (AMD) is a common retinal disorder found in the elderly and is the leading cause of blindness in the Western world. Cell death is a symptom of age related macular degeneration (AMD), but it is unclear the mechanism in which RPE cells undergo for this symptom to occur. A2E is a widely studied fluorophore that has long been correlated with AMD and when exposed to blue light acts as a photosensitive generator of singlet oxygen. This study aims to modify ARPE-derived extra cellular matrix (ECM) using A2E and blue light irradiated A2E in order to investigate the ARPE-19 cell death mechanism. Post modification, healthy ARPE-19 cells were seeded onto the ECM and allowed to attach for 30 min. Unattached cells are removed and fixed for further study. Attached cells were allowed to grow prior to viability analysis. After 24 hours of growth, both unattached and attached cells were stained with Annexin-V and PI then subjected to analysis using flow cytometry to investigate apoptosis versus necrosis as mechanisms of cell death. Blue light irradiated A2E modified ECM showed not only a loss in viability, but also a change in proliferation and cell morphology. When analyzing both the unattached and attached simultaneously, a further decrease in cell viability was observed suggesting difficulty attaching to modified ECM. Both necrotic and apoptotic cells were observed supporting the idea that cells have difficulty in both cell attachment and proliferation. This study showed implications for the symptom of AMD of cell death and can help to further understand the pathogenesis of the disease.

10.5 Trehan, A; Wang, C; Crockett, M; Buhimschi, A; Jing, H; Buhimschi, I; Gooden, D; Gasparro, F*; Hamden Hall Country Day School, Yale University, Nationwide Childrens Hospital, Duke University; fgasparro@hamdenhall.org

The Effect of Psoralen Ring Substituents and DNA Base Composition on Photoreactivity

From a library of 76 new psoralen derivatives, 9 compounds were selected based on their potential photoreactivity with DNA. Among the substituents were halogens, hydrophobic alkyl chains and aminomethyl alkyl chains. These new compounds were compared to two commonly available psoralen derivatives: 8-methoxypsoralen (8-MOP) and 4'-aminomethyl-trimethylpsoralen (AMT). The psoralens were irradiated with either UVA or UVB radiation in solutions with a series of synthetic oligonucleotides. Initial studies were performed in a self-complementary ten base alternating oligonucleotide (AT-10: A-T-A-T-A-T-A-T-A-T, T_m=20C). To test the effect of oligonucleotide stability on psoralen photochemistry, two additional self-complementary oligonucleotides were also studied (ATGC1: G-T-A-T-A-T-A-T-A-C, T_m=24C, and ATGC2: G-C-A-T-A-T-A-T-G-C, T_m=28C). After UV exposure (at 4 C) the samples were analyzed by Matrix-Assisted Laser-Desorption-Ionization-Time-of-Flight-Mass-Spectrometry (MALDI-TOF-MS). In the 8-MOP/AMT solutions with AT-10, the original oligonucleotide was detected at 3023 Da. The 8-MOP/AMT adducted AT-10 oligos were detected at 3239 and 3280 Da respectively. These studies confirmed the well-characterized photoreactivity of AMT as compared to 8-MOP (38.5% vs 6.37%). Among the new compounds, halogen-substituted compounds were virtually unreactive in terms of adduct formation. When a 3-aminopropoxy substituent was added at the 8-position of psoralen, the level of adduct formation reached 57.5%. When a more bulky 4-methylpiperazino group was substituted, reactivity fell to 18.6%. Adduct formation was comparable when either UVA or UVB was employed. In ATGC1 and ATGC2 there were dramatic increases in AMT photoaddition (37% and 19% compared to 15% AMT in AT-10 after just a 5 min UVA exposure). All of the new psoralen compounds had been shown to be highly active in a clonogenic assay suggesting that routes other DNA modification may play a role in their efficacy. Synthesis of new psoralen derivatives with superior photoreactivity is an important goal in cancer therapeutics.

21.6 Turner, J*; Parisi, AV; Igoe, D; Amar, A; University of Southern Queensland;
joanna.turner@usq.edu.au

Using Smartphone Hardware To Measure Ultraviolet Radiation.

Smartphones are increasingly being used to provide people with the Ultraviolet Index (UVI) values and help them control their ultraviolet (UV) exposure. Various approaches have been applied to provide this service, some of which include external sensors and algorithm based prediction systems (Apps). Whilst these are excellent methods to improve education and understanding about UV exposure, one of the areas where the current products commercially available fail, (when no external sensor is available either due to availability or financially) is to account for areas where connectivity is intermittent or non-existent for an app that relies on network based information. In regional and remote areas of Australia, connectivity can still be an issue. In most UVI algorithm applications, the calculation of UVI is achieved from data collected in the nearest largest city that has the appropriate weather data collection systems. For some areas this could be an inappropriate estimation and provide incorrect UVI values. Additionally it fails to account for factors such as variation in cloud compared to location, and proximity of structures in the landscape. In order to determine UVI correctly, measurement of UVB radiation as well as UVA radiation is required. Previous work has shown it is possible to detect UVA radiation with existing hardware already contained within a smartphone (Igoe, Parisi & Carter, 2013). To measure UVI using existing smartphone hardware, UVB radiation must also be detectable within the same system. The feasibility of the use of smartphone hardware will be discussed and the smartphone's sensitivity to UVB radiation will be presented by considering data collected on the dark response, temperature response, irradiance response and spectral response for three different smartphone models. Discussion will also be included on the feasibility of the use of the smartphone as a UV measurement tool. Igoe, D., A. Parisi and B. Carter (2013)Photochem. Photobiol., vol. 89, pp. 215-218.

6.16 Turner, J*; Parisi, AV; Igoe, D; Amar, A; University of Southern Queensland;
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Using Smartphone Hardware To Measure Ultraviolet Radiation.

Smartphones are increasingly being used to provide people with the Ultraviolet Index (UVI) values and help them control their ultraviolet (UV) exposure. Various approaches have been applied to provide this service, some of which include external sensors and algorithm based prediction systems (Apps). Whilst these are excellent methods to improve education and understanding about UV exposure, one of the areas where the current products commercially available fail, (when no external sensor is available either due to availability or financially) is to account for areas where connectivity is intermittent or non-existent for an app that relies on network based information. In regional and remote areas of Australia, connectivity can still be an issue. In most UVI algorithm applications, the calculation of UVI is achieved from data collected in the nearest largest city that has the appropriate weather data collection systems. For some areas this could be an inappropriate estimation and provide incorrect UVI values. Additionally it fails to account for factors such as variation in cloud compared to location, and proximity of structures in the landscape. In order to determine UVI correctly, measurement of UVB radiation as well as UVA radiation is required. Previous work has shown it is possible to detect UVA radiation with existing hardware already contained within a smartphone (Igoe, Parisi & Carter, 2013). To measure UVI using existing smartphone hardware, UVB radiation must also be detectable within the same system. The feasibility of the use of smartphone hardware will be discussed and the smartphone's sensitivity to UVB radiation will be presented by considering data collected on the dark response, temperature response, irradiance response and spectral response for three different smartphone models. Discussion will also be included on the feasibility of the use of the smartphone as a UV measurement tool. Igoe, D., A. Parisi and B. Carter (2013)Photochem. Photobiol., vol. 89, pp. 215-218.

13.1 Tyrrell, RM; University of Bath; *prsrmt@bath.ac.uk*

UVA and oxidative stress: Will antioxidants enhance endogenous protection?

UVA radiation leads to a sustained and diffusible oxidative stress in skin. This has led to a belief that the damage caused may be prevented by exogenous antioxidants. Among the reactive oxygen species generated by UVA radiation are singlet oxygen and hydrogen peroxide and various radical species including superoxide which probably results from tissue specific UVA activation of NADPH oxidases, including 1 and 4. UVA radiation also directly leads to a rapid increase in the labile iron pool as a result of ferritin degradation and cyclooxygenase-dependent release of free heme and activation of quite distinct and cell-type dependent stress pathways including heme oxygenase 1 and metalloproteinases. UVA-mediated oxidative stress and disruption of heme and iron homeostasis is modulated by the activities of constitutive endogenous antioxidants, as well as constitutive and inducible antioxidant enzymes. These factors contribute to the restoration of cellular homeostasis following an oxidant stress and must be understood when considering the potential of supplemented topical and systemic antioxidants to enhance protection.

16.4 Ueda, Nozomi*; Ono, Yukiko; Iwata, Tatsuya; Iwaki, Masayo; Kandori, Hideki; Nagoya Institute of Technology; *cin11019@nitech.jp*

Artificial photosynthesis with a single redox centre based on the engineered flavoprotein LOV

One of the key concepts of artificial photosynthesis is a light-induced charge separation, of which the lifetime is long enough for the redox center to react with external chemical species or solid electrodes. To achieve this, a molecular unit that is made of a photosensitizable electron donor-spacer-acceptor (D-S-A) triad has been regarded as a principle molecular design after inspired by the molecular structure of natural photosynthetic reaction centres. In this presentation, we propose a different type of molecular design of artificial photosynthesis, in which a single photosensitizable redox center can function as both electron donor and acceptor. A plant photosensor, LOV (light-oxygen-voltage) protein, which contains a neutral oxidized form of FMN (flavin mononucleotide) molecule embedded in a 10-kDa polypeptide, was used as a template model compound to demonstrate the possibility of artificial photosynthesis with a single redox center. In the wild type LOV, light irradiation generates the excited state of FMN, followed by the adduct formation between FMN and the thiol group of the nearby Cys residue. When the Cys was mutated to the Ala (denoted as LOV C/A), the excited state of FMN oxidized the external electron donor ferrocyanide to ferricyanide and the semiquinone form of FMN (FMNH) was accumulated in the anaerobic condition. In the presence of methylene blue (MB) as an electron acceptor in addition to ferrocyanide, the reduction of MB was observed, which was concomitant with the formation of ferricyanide and less accumulation of FMNH. In the aerobic condition or in the absence of LOV protein, the redox reduction of MB was abolished. The results suggest that the single photosensitizer FMN molecule in LOV C/A functions as the electron acceptor first, and then the FMNH as the electron donor. Compared to the D-S-A triad, the single redox catalyst may have the advantage of easy manufacturing and broad application.

16.5 Urban, VS*; Stingaciu, LR; O'Neill, H; Liberton, M; Pakrasi, HB; Ohl, M; Oak Ridge National Laboratory, Julich Centre for Neutron Science, Washington University, St. Louis; *urbanvs@ornl.gov*

Thylakoid Membrane Dynamics in Cyanobacteria

Cyanobacteria are photosynthetic prokaryotes that make major contributions to the production of the oxygen in the Earth atmosphere. The photosynthetic machinery in cyanobacterial cells is housed in flattened membrane structures called thylakoids. The structural organization of cyanobacterial cells and the arrangement of the thylakoid membranes in response to environmental conditions have been widely investigated. However, there is limited knowledge about the internal dynamics of these membranes in terms of their flexibility and motion during the photosynthetic process. We present a direct observation of thylakoid membrane undulatory motion in vivo and show a connection between membrane mobility and photosynthetic activity. High-resolution inelastic neutron scattering experiments on the cyanobacterium *Synechocystis* sp. PCC 6803 assessed the flexibility of

cyanobacterial thylakoid membrane sheets and the dependence of the membranes on illumination conditions. We observed softer thylakoid membranes in the dark that have three to four fold excess mobility compared to membranes under high light conditions. Our analysis indicates that electron transfer between photosynthetic reaction centers and the associated electrochemical proton gradient across the thylakoid membrane result in a significant driving force for excess membrane dynamics. These observations contribute to a deeper understanding of the relationship between photosynthesis and cellular architecture.

19.3 Valic, MS*; Ye, T; Zhang, C; Jiang, W; Chen, J; Bernardini, MQ; Zheng, G; University of Toronto and Princess Margaret Cancer Centre, Princess Margaret Cancer Centre; michael.valic@mail.utoronto.ca

All-Organic Nanovesicles for Multimodal PET/CT and Optical Fluorescence Assessment of Lymphatic Disseminations

Cancer mortality is linked increasingly to early metastatic disease occult at the time of primary diagnosis. The detection of metastatic disease in the lymph nodes—a common event in breast, colon, and cervical cancer—has substantial clinical impact for prognosis and planning therapeutic strategies. However, identification of lymph node metastasis is problematic owing to varying sensitivity and specificity of non-invasive imaging techniques such as CT, MR, and PET. Recent advances in the development of "lympho-tropic" imaging agents to assess nodal disease have shown tremendous preclinical promise for non-invasive nodal characterization. One such agent is an all-organic unilamellar nanovesicle (Porphysome) with intrinsically activatable biophotonic properties. In addition to being biodegradable and biocompatible, Porphysomes can stably chelate radioactive copper-64 (Cu-64) to serve as a highly accurate PET imaging tool. Herein we describe the clinical development of radio-pharmaceutical Porphysome kits manufactured in accordance with current Good Manufacturing Practice (cGMP) and complying with United States Pharmacopeial (USP) standards for non-sterile pharmaceutical compounding. One-pot radio-labelling of single use Porphysome kits with copper-64 (II) chloride prepared parenteral injections of dose 5 mCi (185 MBq) with high radiochemical purity (> 95%). Intending to strengthen radio-labelled Porphysome signal accumulation and signal activation in lymph nodes in vivo, the physiochemical (size, surface charge and chemistry) and photobiological (fluorescence activation) properties of the Porphysome nanovesicles were optimized to promote measurable multi-modal signal enhancement in lymph nodes following parenteral administration. We anticipate these optimized "lympho-tropic" Porphysome kits will provide the desired signal selectivity and enhancement in lymph nodes burdened with metastatic disease in preclinical models with lymphatic dissemination.

15.4 Van Houten, B; UPCI; Nixonw@upmc.edu

Single molecule analysis of DNA repair enzymes reveals a novel "recognition at a distance" mechanism.

Nucleotide excision repair (NER) is a highly conserved DNA repair mechanism that processes a variety of helix-distorting lesions in DNA, such as UV-induced photoproducts. To shed light on the dynamic protein-DNA interactions during the damage recognition stage of eukaryotic NER, we employed single-molecule fluorescence microscopy, as well as atomic force microscopy (AFM). We found that quantum dot-tagged *Saccharomyces cerevisiae* Rad4-Rad23, once bound to UV-irradiated DNA, either forms non-motile complexes or conducts one-dimensional search in two distinct diffusive modes: random linear diffusion and constrained motion. Deletion of β -hairpin 3 in Rad4 resulted in increased constrained and random motion on DNA. A six amino acid deletion of the tip of β -hairpin 3 increased constrained motion and was fully able to complement UV resistance of a β mutant. Taken together these data indicate that Rad4 uses a novel "dynamic recognition at a distance" to identify UV photoproducts. PARP1 has recently been shown to play a role in NER and have used single molecule approaches to probe its interaction on DNA. AFM experiments indicate that PARP1 binds at nicks, AP sites, and DNA ends primarily as a monomer. Fluorescence microscopy revealed

that: 1) APE1 can co-localize with PARP1; 2) APE1 diffuses more rapidly than PARP1 on DNA; 3) surprisingly, addition of NAD does not increase PARP1 dissociation from DNA; 4) PARylated PARP1 shows higher motility and less dissociation than non-PARylated PARP1; and 5) interestingly, PARP inhibitor olaparib increases PARP1 motility on AP-DNA damage arrays. Supported by NIH R01ES019566 to B.V.H.

29.2 Vega, MC*; Gaillard, ER; Northern Illinois University; *mvegal@niu.edu*

Identifying Novel Fluorophores in Human RPE Melanolipofuscin

Age related macular degeneration (AMD) is a debilitating and poorly understood retinal degenerative disease that causes progressive loss of central vision. The retinal pigment epithelium (RPE) cells are critical for maintaining the photoreceptors and retinal tissue. Auto-fluorescent pigment granules, known as melanolipofuscin, accumulate in RPE cells with age. The current study aims to identify novel fluorophores in human RPE melanolipofuscin which may lead to the development of new diagnostic techniques for the early detection of age related macular degeneration. Human RPE melanolipofuscin is extracted from human donor eyes as previously described by Feeney-Burns and subjected to a Folch extraction. The organic soluble portion of melanolipofuscin is collected, dried under argon gas, and reconstituted in HPLC grade methanol for analysis using high performance liquid chromatography tandem mass spectrometry (LC/MS/MS) coupled to a fluorescence detector (Surveyor LC with PDA, Thermo Finnigan LCQ Advantage MS, Surveyor FL). Fluorescence detection and tandem mass spectrometry data are analyzed for the identification and structure elucidation of the fluorescent components of human RPE melanolipofuscin. The fluorophore, A2E, has been observed as a component of human RPE melanolipofuscin. The presence of additional fluorophores has been confirmed. Tandem mass spectrometry analysis of these fluorophores has provided structural information for these vitamin A derivatives. Human RPE melanolipofuscin is observed to accumulate in the RPE with age and this accumulation has been suggested to closely correlate with the onset of AMD. Fluorescent components of melanolipofuscin have been identified in human melanolipofuscin extracts. These fluorophores may lead to the development of a new fluorescence based diagnostic technique for the early detection of AMD.

P2.11 Vignoni, M; Walalawela, N; Ghogare, AA*; Bonesi, SM; Thomas, AH; Greer, A; Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA) and UNLP-CONICET, Brooklyn College and Graduate Center of the City University of New York, Centro de Investigaciones de Hidratos de Carbono (CIHIDECAR) and FCEN-CONICET; *aghogare@brooklyn.cuny.edu*

Synthesis and characterization of long chain pterin derivatives: O vs N substitution

We describe a synthetic process for the N- and O-alkylation of 2-aminopteridin-4-(3H)-one (pterin) by nucleophilic substitution (SN2). A decane chain was introduced either at N or O of the amide leading to two new alkylated pterins. Dialkylation of both N and O amide pterin sites was not observed. However, two adducts from N-amine condensation of dimethylformamide (DMF) were obtained as by-products since DMF was used as solvent. The new pterin derivatives were characterized by NMR and mass spectrometry, and also absorption and emission spectra, fluorescence lifetime and singlet oxygen production were measured. The use of pH changes and comparison to the N-methylated pterin (2-amino-3-methylpteridin-4-(3H)-one), which exists in the keto form lacking the acidic NH, was key to assessing the alkylation at N and O positions in the new pterins. Unlike typical pterins, our new long chain pterins show lipophilic solubility, making them potential fluorescent probes in cell membranes.

25.3 Vikdal, M; Selbo, PK; Hompland, T; Sellevold, S; Fremstedal, AS; Rofstad, EK; Weyergang, A; Peng, Q; Berg, K*; Oslo University Hospital -Radium Hospital, Oslo, Norway; *kristian.berg@rr-research.no*

Tumor vascular shutdown after photochemical internalization (PCI). Impact on treatment outcome

Photochemical internalization (PCI) is a novel technology for release of endocytosed macromolecules

into the cytosol. The technology is based on the use of photosensitizers located in endocytic vesicles that upon activation by light induce rupture of the endocytic vesicles and thereby release of the macromolecules into the cytosol. PCI has been shown to enhance the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane. Although PCI was developed for targeting the parenchyma cells of solid tumors recent studies have shown that the vasculature may also be damaged by this treatment as shown by e.g. in vitro studies of endothelial cells, CE-MRI studies of tumor perfusion and in vivo-ex vivo survival studies. The tumor rim seems to be less sensitive than the tumor center to PDT and also to some extent to PCI. CE-MRI studies indicate that the resistance in the tumor rim to PCI may be related to persistent vasculature. Accordingly, adjuvant bevacizumab treatment resulted in a strong curative effect in orthotopically located HT1080 fibrosarcomas not seen after PCI of bleomycin alone. However, in the highly different subcutaneously growing WiDr colon carcinoma bevacizumab showed no beneficial effect and even antagonistic effects in some treatment setups. In order to further understand the parameters regulating the response of the tumor vasculature to PCI we are currently performing more in-depth MRI studies that will be presented.

15.7 Wang, K; Taylor, JS*; Washington University; taylor@wustl.edu

Effect of nucleosome formation and bending on cyclobutane dimer formation in T11-tracts

It is well established that DNA photoproducts produced by sunlight are responsible for the majority of the mutations associated with skin cancers. What is not so well established is the physical or mechanistic origin of the variation in mutation type and frequency within a gene, which must result from a complex interplay between the frequency of photoproduct formation, chemical transformation, repair, and translesion synthesis. To begin to dissect the various contributions of these factors, we have been examining the role of chromatin structure on DNA photoproduct formation and chemical transformation. It was demonstrated a number of decades ago that nucleosomes can greatly modulate cyclobutane pyrimidine dimer (CPD) formation with a 10 bp periodicity that was attributed to the effect of bending on the conformation and dynamics of DNA. Dipyrimidine sites for which the phosphodiester backbone faced out showed the greatest photoproduct yield, whereas sites facing in showed the least. The original studies of CPD modulation by nucleosomes were carried out with heterogenous nucleosomal DNA from degradation of chromatin. A number of subsequent studies with defined nucleosomal sequences did not, however, appear to show the same effect. To reinvestigate the modulation of CPD formation by nucleosomes we have studied CPD formation in T11-tracts at 7 different translational positions in a rotationally phased defined DNA sequence using a circular permutation synthesis strategy. We find that CPD formation in the T11-tracts is strongly modulated by a nucleosome, with maxima that are shifted to the 5'-side compared to heterogeneous sequences and in accord with what was previously observed for a T9-tract. The position of the maxima, however, also depended on the translational position. CPD formation was also shown to be strongly modulated in rotationally phased nucleosome-free circular DNA in the same way as found in the nucleosome, indicating that bending is the major factor in controlling CPD formation frequency.

10.4 Ward, WW*; Turner, C; Rutgers University; crebb@rci.rutgers.edu

Purifying GFP By Modified Three-Phase Partitioning

Three-phase partitioning (TPP) has been used by our group and others to purify green-fluorescent protein. TPP has reduced processing time from several months to one or two days. In purifying recombinant GFP from transformed E. coli cells, we have made three very useful modifications. We have switched from using t-butanol to the much less expensive isopropanol and we apply TPP directly to freshly harvested, un-lysed cells. Under the influence of 1.6 M ammonium sulfate, the alcohol behaves as a low polarity solvent, dissolving the cell membrane. The mixed solvent enters through the large cell wall pores and precipitates all DNA and most protein. The aggregated macromolecules become entombed within the cells while the more soluble GFP easily exits--usually 40% pure. We employ three precipitation stages rather than one or two. Then, one round of hydrophobic interaction

chromatography increases purity to 90% or higher.

6.17 Ward, WW*; Turner, C; Rutgers University; crebb@rci.rutgers.edu

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5.1 Whitten, DG*; Pappas, HC; Donabedian, PL; Hill, EH; Chi, EY; Neumann, AK; University of New Mexico, University of New Mexico; whitten@unm.edu

Synthetic Oligo- and Poly-(phenylene ethynylene)-Electrolyte/Biological Interfaces

Our presentation will introduce a class of materials and compounds based on charged phenylene ethynylene polymers (CPE) and oligomers (OPE) that we have developed as fluorescence-based biosensors and antimicrobials. The focus of the presentation will be on recent findings and new applications we have developed. The first example is our discovery that certain OPE can induce or enhance bacillus spore germination in a dark process. This provides a new mode of antibacterial activity in that the resulting vegetative cell can be killed by either dark or light-activated biocidal activity of the same OPE. Another topic we will discuss is the selective binding of certain OPE with fibrils formed from aggregation of mis-folded proteins associated with Alzheimer's disease and sensing and therapeutic possibilities that might be developed from these interactions. Finally we compare the different paths used by an OPE and PPE to deactivate pathogenic Candida cells.

29.6 Wigle, JC*; Cone, MT; Mason, JD; Figueroa, E; Hokr, BH; Bixler, JN; Gonzales, CC; Rockwell, BA; Yakovlev, VV; Fry, ES; Air Force Research Laboratory, Texas A&M University; jeffrey.wigle@us.af.mil

Measuring Absorption Coefficients in Human Retinal Pigmented Epithelium Cells and Subcellular Fractions

Retinal pigmented epithelium (RPE) is the tissue where most of the light entering the eye is absorbed and, therefore, the site of laser injury for wavelengths of 400-1200nm. An in vitro model of this interaction, using an immortalized human RPE cell line, was developed to study light-tissue interactions at these wavelengths. We have previously shown that an induced resistance to a lethal pulse of 2- μ m laser radiation occurs in these cells when exposed to low levels of red light (photobiomodulation, PBM) 24h prior to the laser challenge (adaptive response). While "downstream" physiological effects have been extensively studied, little is known about the initiation of the cascade which is hypothesized to start with light absorption by the mitochondria. But true light absorption by the mitochondria has never been measured because cells and organelles in suspension have very large scattering cross sections compared to absorption cross sections. Because of this, "linear" absorption measurements (intensity of light before and after it passes through a sample) overestimate true absorption because these values include losses due to scattering, for which there is no correction. However, photon absorption is essential for PBM and, since light-tissue interaction models also use absorption coefficients to predict laser bioeffects, we measured absorption coefficients of human RPE cells and subcellular components. The technique we used is based on cavity ring-down spectroscopy (CRDS), but the traditional mirrored cavity is replaced with a high-reflectivity

integrating cavity which prevents scattered light from escaping. So integrating cavity ring-down spectroscopy (ICRDS) provides a true, direct measurement of absorption. At wavelengths of 400-700 nm absorption coefficients are 7 to 85-fold lower than corresponding attenuation coefficients, as a function of increasing wavelength, and the mitochondria absorption coefficients are dependent upon their oxidation state.

13.6 Wondrak, GT; UA Cancer Center and College of Pharmacy, University of Arizona;
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Redox-directed Interventions Targeting Skin Photodamage: An 'Inside-Out' Approach Harnessing NRF2

Exposure to solar ultraviolet (UV) radiation is a causative factor in skin photodamage and carcinogenesis. Recent studies suggest that pharmacological modulation of cellular stress response pathways may protect skin against environmental insult. The protective role of cutaneous gene expression orchestrated by the transcription factor NRF2 (nuclear factor erythroid 2-related factor 2) in solar photoprotection, wound healing, and antioxidant defense against environmental carcinogenic insult has now been substantiated. We have explored the molecular mechanism underlying skin photoprotection by natural product-based NRF2-inducers (including tanshinone I and dihydrotanshinone) that protect human skin cells and reconstructed skin against solar UV. In addition, in an attempt to test feasibility of NRF2-dependent systemic photoprotection by dietary constituents, we focused our photoprotection studies on the apocarotenoid bixin, an FDA-approved food colorant from the seeds of the achiote tree (*Bixa orellana*) native to tropical America. Consumed by humans since pre-Columbian times, this apocarotenoid is now used worldwide as a dietary additive and cosmetic ingredient (referred to as 'annato'; E160b) with established safety record and systemic bioavailability/pharmacokinetic profile upon oral administration. Our research indicates that bixin activates NRF2 through the critical Cys-151 sensor residue in KEAP1, orchestrating a broad cytoprotective response in cultured human keratinocytes as revealed by antioxidant gene expression array analysis (AKR1C2, GCLC, NQO1, SLC7A11, FTH1, TXNRD1, NCF2, SRXN). Systemic administration of bixin suppressed acute skin photodamage, attenuating epidermal oxidative DNA damage and inflammatory responses in *Nrf2*^{+/+} (but not in *Nrf2*^{-/-}) SKH-1 mice, confirming the NRF2-dependence of bixin-based systemic photoprotection. Taken together, these data demonstrate feasibility of achieving NRF2-dependent 'Inside-Out' skin photoprotection by systemic administration of a natural food additive consumed worldwide.

21.3 Wright, CY*; Albers, PN; South African Medical Research Council and University of Pretoria,
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Solar UV radiation: measurement, monitoring and public awareness in sub-Saharan Africa

Skin cancer is an under-researched, yet important public health problem in sub-Saharan Africa where it mainly affects individuals with fair skin, individuals with oculocutaneous albinism and more recently, individuals with HIV/AIDS. A key modifiable skin cancer risk factor is exposure to solar ultraviolet (UV) radiation. Reduction of excess personal sun exposure can be achieved by altering the timing of outdoor activities and using clothing, hats, shade, and sunscreen. In the past ten years, several studies have been carried out to understand the continental status quo including systematic reviews on the health impacts of excess sun exposure, cross-sectional epidemiological studies on sunbed prevalence, adult and child knowledge and understanding of the UV Index and individual understanding of skin phototype, as well as analysis of skin cancer registry data. Together, these data provide information that can be used to inform the development of skin cancer prevention and sun awareness campaigns tailored to at-risk groups in sub-Saharan Africa.

8.2 Wu, S*; Tong, L; Ohio University; *wus1@ohio.edu*

Constitutive nitric oxide synthases as a chemopreventive target for UVB-induced skin

carcinogenesis

Ultraviolet B light (UVB) is a hazardous environmental carcinogen that affects multiple oncogenic signaling pathways. Two of these signaling circuits are the reactive oxygen species/reactive nitrogen species (ROS/RNS)-mediated DNA damage and NF-kappa B-mediated anti-apoptotic pathways. In this presentation, we will provide evidences that constitutive nitric oxide synthases (cNOS), including both NOS1 (neuronal) and NOS3 (endothelial), mediates UVB-induced DNA damage (partially) and NF-kappa B activation (totally) via unique mechanisms. The activation and uncoupling of cNOS increases ROS/RNS production immediately after UVB irradiation and inhibition of cNOS can significantly reduce UVB-induced DNA damage. Meanwhile, the elevation of ROS/RNS also induces the phosphorylation of the alpha-subunit of eukaryotic initiation factor 2 (eIF2-alpha) and translational inhibition of I kappa B synthesis, which leads to the activation of NF-kappa B. The UVB-induced NF-kappa B activation is totally diminished in cNOS null HEK293T, and the inducibility of NF-kappa B can be restored by overexpressing cNOS in the cells. Finally we will demonstrate that inhibition of cNOS can completely suppress the transformation of keratinocytes induced by UVB-irradiation. Our results suggest that cNOS inhibitor could be a potential chemopreventive agent for UVB-induced skin cancer formation by reducing DNA damage-caused cell mutagenesis and suppressing NF-kappa B-mediated cell survival pathway.

3.2 Xie, J; University of Georgia; jinxie@uga.edu

Ferritin-facilitated photodynamic therapy against cancer

Photodynamic therapy is an emerging cancer treatment modality. Despite of the great promise, there is a lack of a reliable delivery vehicle for tumor targeted delivery of photosensitizers. Previous efforts have been focused on polymer- or liposome-based nanocarriers, which are often associated with a suboptimal drug loading rate and a large particle size. Our recent studies found that ferritin, a compact protein cage (~12 nm), can load photosensitizers such as zinc hexadecafluorophthalocyanine (ZnF16Pc) at high efficiency (~60wt%). Meanwhile, ferritins can be modified by either chemical or genetic methods to introduce targeting ligands to the particle surface. In particular, we've successfully introduced folic acid, RGD4C, and a FAP targeting scFv, to the surface of ferritins. By using these surface modified ferritins as carriers, we are able to selectively deliver ZnF16Pc to cancer cells, tumor endothelial cells, and tumor associated fibroblasts. With appropriate photoirradiation, the treatment leads to efficient tumor growth control, with minimal toxicity to the skin and other normal tissues. Compared with artificial carrier based delivery, the technology affords advantages such as low toxicity, low immunogenicity, and biodegradability and holds great promise in clinical translation.

9.1 Xu, Chris; Cornell University; cx10@cornell.edu

In vivo Multiphoton Imaging of Mouse Brain

Over the last two decades, multiphoton microscopy has created a renaissance in the brain imaging community. It has changed how we visualize neurons by providing high-resolution, non-invasive imaging capability deep within intact brain tissue. Multiphoton imaging will likely play an essential role in understanding how the brain works at the level of neural circuits, which will provide a bridge between microscopic interactions at the neuronal level and the macroscopic structures that perform complex computations. In this paper, the fundamental challenges of deep tissue, high-resolution optical imaging are discussed. New technologies for in vivo structural and functional imaging of mouse brain using long wavelength excitation and three-photon microscopy (3PM) will be presented. We will discuss the requirements for imaging the dynamic neuronal activity at the cellular level over a large area and depth in awake and behaving animals. Finally, we will speculate on the possible future directions to further improve the imaging depth and speed in biological tissues.

29.4 Yacout, SM*; Elsawa, SF; Gaillard, ER; Northern Illinois University; sallymyacout@gmail.com
Evaluating the Influence of Melanin in Cytokine Secretion in Photo-stressed Retinal Pigment

Epithelial Cells

The retinal pigment epithelium (RPE) is a monolayer of post-mitotic cells that maintain retinal health and preserve vision. It is well known that these cells regulate ocular immune response by expressing and secreting cytokines and by presenting antigens. A factor that may influence the role of RPE cells in immune response is pigmentation. While the function of RPE melanin is not fully understood, experimental evidence suggests the pigment behaves as a photo-protectant and antioxidant. Given the beneficial nature of RPE melanin, it is possible that this pigment also impacts retina immune response. Of particular interest is the photo-stress elicited immune response in the aged human retina. In this study tissue culturing was utilized and UV exposure to the retina with time was modeled by exposing human cell line ARPE-19 to UV-C radiation. Cells were pigmented by phagocytizing bovine melanin and unpigmented cells were used as a control. Secretion of the cytokine interleukin-6 (IL-6) was monitored using an enzyme-linked immunosorbent assay (ELISA) and gene expression was detected using PCR. Elevated IL-6 secretion was observed in pigmented cells under dark and photo-stress conditions compared to unpigmented dark control cells. Additionally, UV-C irradiation resulted in an increase in IL-6 secretion in pigmented cells compared to irradiated unpigmented cells. It is suggested that inflammation contributes to age-related atrophy of the retina and degradation of melanin with time may result in ocular disease such as age-related macular degeneration.

28.2 Yamamoto, J; Brettel, K*; Osaka University, I2BC/CEA Saclay; *klaus.brettel@cea.fr*

On The Time Scale Of The Repair Of (6-4) Photoproducts By Their DNA Photolyase

UV irradiation induces two major types of harmful crosslinks between adjacent pyrimidines in DNA: cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts (6-4PPs). In many organisms, these lesions are repaired by photolyases, flavoproteins that require light for their catalytic action (see comprehensive review by A. Sancar, Chem Rev. 2003, 103, 2203-2237). For CPDs, the repair mechanism involves electron transfer (ET) to the lesion from the photoexcited state of the fully reduced flavin cofactor FADH⁻, splitting of the intra-dimer bonds and return of the excess electron to the semi-reduced flavin FADH⁰. These reactions take place within ~1 ns and are highly efficient (repair quantum yield of 50-100%). Repair of 6-4PPs by (6-4) photolyase has a much lower quantum yield (about 5-10%), and the repair mechanism is less understood. Li, Liu, Tan, Guo, Wang, Sancar and Zhong (Nature 2010, 466, 887-890) provided evidence for ET from excited FADH⁻ to the 6-4PP and proton transfer from a histidine residue within 500 ps, but electron return did not take place in the accessible time window of 3 ns, indicating that repair of 6-4PPs takes much longer than that of CPDs. From flash sequence experiments on a seconds time scale, we have provided evidence that two successive photoreactions are required for the repair of the 6-4PP, each of them presumably involving ET from excited FADH⁻ to the lesion and electron return to FADH⁰ (Yamamoto et al., Angew. Chem. Int. Ed. 2013, 52, 7432-7436). Here we will present results of recent transient absorption experiments (using a dedicated setup that provides a time resolution of up to 300 ps without excessive signal averaging; Byrdin et al., Rev. Sci. Instrum. 2009, 80, 043102) that aimed at establishing the time scale of 6-4PP repair by monitoring electron return for each of the two photoreactions in the nano- and microsecond range.

28.4 Yang, Linlin; Jian, Yajun; Setlow, Peter; Li, Lei*; Indiana University Purdue University Indianapolis (IUPUI), UConn Health; *lilei@iupui.edu*

Spore photoproduct within DNA is a surprisingly poor substrate for its designated repair enzyme "the spore photoproduct lyase"

UV radiation of DNA generates three pyrimidine dimers, cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) and 5-thymine-5,6-dihydrothymine, i.e. the spore photoproduct (SP). All these dimers can be repaired by direct reversal enzymes where CPDs and 6-4PPs are repaired by the respective photolyase and SP is repaired by spore photoproduct lyase (SPL). All these dimers can also be repaired by the general but relatively inefficient nucleotide excision repair (NER) pathway. Prof. Sancar's earlier seminal work demonstrated that the CPD

photolyase exhibits roughly 105-fold higher affinity toward CPD than undamaged DNA, explaining the highly efficient CPD repair in duplex DNA by the enzyme. It was widely expected that SPL may exhibit a similarly high affinity toward SP in duplex DNA leading to an efficient SP repair. Surprisingly, our latest in vitro studies using SP-containing short oligonucleotides, pUC 18 plasmid DNA, and E. coli genomic DNA found that they are all poor substrates for SPL in general, exhibiting turnover numbers of 0.01-0.2 min⁻¹. The faster turnover numbers are reached under single turnover conditions, and SPL activity is low with oligonucleotide substrates at higher concentrations. Moreover, SP-containing oligonucleotides do not go past one turnover. In contrast, the dinucleotide SP TpT exhibits a turnover number of 0.3 ~ 0.4 min⁻¹, and the reaction may reach up to 10 turnovers. These observations distinguish SPL from DNA photolyases. To the best of our knowledge, SPL represents an unprecedented example of a major DNA repair enzyme that cannot effectively repair its substrate lesion within the normal DNA conformation adopted in growing cells. Factors such as other DNA binding proteins may have to cooperate with SPL to enable the efficient SP repair in germinating spores. Moreover, SP can be produced in non-spore-forming microorganisms; our results indicate that the SP repair in these species may be slow and potentially problematic.

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Multifunctional Prodrugs for the Spatiotemporal Combination of Photodynamic Therapy and Site-Specific Chemotherapy

A non-invasive or minimally invasive tumor ablation method is an attractive tool for controlling local and regional tumors. This approach can be complementary to primary treatment options without causing systemic side effects or severe physical burdens from the treatments. Photodynamic therapy (PDT) is one such a regimen, which has been used in the clinic for various tumors. However, the therapeutic efficacy of PDT is limited by various factors leading to incomplete tumor ablation for certain cases. The spatial and temporal limits of the major effector of PDT, singlet oxygen, have been suggested as potential causes of incomplete ablation in highly heterogeneous tumor and PDT. We recently developed a prodrug strategy that could overcome these limits by using a unique combination of PDT and site-specific chemotherapy. The prodrug is composed of a photosensitizer and anticancer drugs via a singlet oxygen-cleavable linker. In particular, we designed the prodrugs using fluorescent photosensitizers. Thus, the prodrugs can be imaged using optical imaging both in vitro and in vivo. Upon illumination, the prodrugs cause immediate PDT damage and sustained damage by locally released anticancer drugs, which are released during the illumination at the target. Recent progress in our research will be presented including mechanistic proofs of the dual damage and targeted multifunctional prodrugs.

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The Effect of In Vitro Photoaging of Bovine RPE Melanosomes on Morphology, Reactivity and Photoprotective Efficiency of the Partially Photobleached Pigment Granules

Melanosomes in the human retinal pigment epithelium (RPE) undergo distinct age-related changes that could affect their biological functions. To analyze physicochemical nature of such changes, purified bovine RPE melanosomes were subjected to irradiation with intense visible light " an in vitro model for melanin photoaging. Melanin content, size and morphology of untreated and partially photobleached melanosomes were examined by electron paramagnetic resonance (EPR) spectroscopy and atomic force microscopy (AFM). The efficiency of control and photobleached melanosomes to inhibit photosensitized oxidation of lipids mediated by methylene blue (MB) was compared. The topography of melanin functional groups and their reactivity in the studied pigment granules were determined by monitoring their interaction with the nitroxide TEMPO choline, dysprosium (III) ions, and singlet oxygen, employed as molecular probes. Although the results show that the more extensively photobleached melanosomes, compared to untreated melanosomes, inhibited the photosensitized oxidation of lipids less efficiently, weakly photobleached melanosomes were actually a

better protective agent. The latter effect could be due to an increased exposure of melanin functional groups, responsible for the interaction with the tested reagents, while the reduced efficiency of more extensively photobleached melanosomes to inhibit photosensitized oxidation of lipids could be explained by partial degradation of the melanin active sites. AFM analysis of control and photobleached melanosomes confirmed this by showing that weakly photobleached melanosomes were mostly stripped of outer layer structures and exhibited exposed melanin nanoaggregates. An increased exposure of melanin functional groups dramatically affected the efficacy of photobleached melanosomes to interact with reagents as demonstrated by saturation recovery EPR employing dysprosium (III) as a powerful relaxing agent

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Dynamics and mechanisms of UV-damaged DNA repair by photolyases

UV radiation can damage DNA to mainly form cyclobutane pyrimidine dimer or (6-4) photoproduct. Such lesion may eventually lead to skin cancer. Photolyase, a flavin photo enzyme, can revert such damage with high repair efficiency. Here, we combine femtosecond spectroscopy and molecular biology and have completely mapped out the entire repair evolution at the most fundamental level by following the dynamics from the initial reactants, to the fleeting intermediates and to the final repaired products. By resolving more than six elementary steps in the complex enzymatic reaction, we captured three electron-transfer reactions and bond-breaking and -forming processes. These dynamics are in synergy to achieve a maximum repair efficiency. Various mutations were also carried out to identify the critical residues in the active site for function. We carefully examined the Class I, Class II, Class III and ssDNA photolyases and observed a unified electron transfer mechanism and determine the critical role of the unique folded structure of cofactor.

14.7 Zinflou, C; Rochette, PJ; University Laval and Centre Hospitalier Universitaire de Quebec Research Center, Canada; corinne.zinflou.1@ulaval.ca*

Ultraviolet A - Induced Oxidation in Cornea : Characterization Of The Early Oxidation-Related Events

Molecular and epidemiological data show that chronic exposure to sunlight ultraviolet (UV) rays, especially to UVA wavelengths (315 - 400 nm), is toxic for eyes and might be linked to many ocular diseases. UVA are the main component of solar UV reaching the eyes. Their absorption by cellular photosensitizers efficiently enhances oxygen reactive species production, leading to an oxidative stress induction. It has been described that UVA-induced oxidative stress plays a role in UVA toxicity. However, events triggered by oxidation and involved in the toxic effects of UVA radiations remain unknown. We have thus investigated the early cellular and molecular changes linked to oxidation in the cornea, the most UV-exposed ocular structure, following an ocular exposure to UVA rays. Three markers were used to assess changes throughout the three corneal layers (epithelium, stroma and endothelium) of UVA-irradiated (6000 kJ/m²) rabbit eyes: (1) oxidized mitochondrial flavins/reduced NAD(P) (ox-Fvm/NAD(P)H) ratio as a sensor of mitochondrial activity and redox state; (2) 8-oxo-7,8-dihydroguanine (8-oxodG) formation in nuclei and mitochondria as a marker of photo-oxidative DNA damage and (3) levels of S-glutathionylated proteins (SG-Prot) as a marker of transiently impaired redox signaling. In response to UVA, we found significantly higher ox-Fvm/NAD(P)H ratio, reflective of a disrupted mitochondrial redox balance, in the most posterior parts of the cornea. Besides, UVA-induced 8-oxodG are concentrated in nuclear DNA of the epithelium, while they are found in both nuclear and mitochondrial DNA in stromal and endothelial layers. Finally, altered patterns of SG-Prot appear in the three layers immediately after UVA exposure and seem to correlate with UVA penetrance. Our data indicate that mitochondrial anomalies and prolonged disruption of proteins normal functions, under stress favored by a chronic UVA exposure, are events potentially critical for the development of UVA toxicity.

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