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.

P1.10 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 peroxyl 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; *witekkor@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)

P1.11 BelaÃ¯di, JP; Denat, L; Perez, P; Soeur, J; Zobiri, O; Marrot, L*; L'OREAL R&I; *lmarrot@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"ঌ 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; ; *bhattachary4@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