19th Congress of the European Society for Photobiology

30 August – 3 September 2021
World Wide Web and Salzburg, Austria

BOOK OF ABSTRACTS

PARIS LODRON UNIVERSITY SALZBURG

Programme-at-a-Glance

MONDAY, AUGUST 30

18:00 - 18:10	Opening ceremony
18:10 - 18:20	Welcome concert part I
18:20 - 18:35	Greetings from the Governor
18:35 - 18:50	Greetings from the Rector
18:50 - 19:10	Welcome concert part II
19:10 - 19:40	**Plenary lecture 1**
19:40 - 20:10	Welcome concert part III

TUESDAY, AUGUST 31

08:00 - 08:30	**Plenary lecture 2**
08:30 - 09:00	**Plenary lecture 3**
09:00 - 09:10	Short break
09:10 - 10:40	**Symposia 1.1 - 1.4 part 1**
10:40 - 11:00	Coffee break
11:00 - 12:30	**Symposia 1.1 - 1.4 part 2**
12:30 - 13:30	Lunch break
13:30 - 14:00	**Plenary lecture 4**
14:00 - 14:30	**Plenary lecture 5**
14:30 - 14:40	Short break
14:40 - 16:10	**Symposia 2.1 - 2.4 part 1**
16:10 - 16:30	Coffee break
16:30 - 18:00	**Symposia 2.1 - 2.4 part 2**

WEDNESDAY, SEPTEMBER 1

08:00 - 08:30	**Plenary lecture 6.1 & 6.2**
08:30 - 09:00	**Plenary lecture 7**
09:00 - 09:10	Short break
09:10 - 10:40	**Symposia 3.1 - 3.4 part 1**
10:40 - 11:00	Coffee break
11:00 - 12:30	**Symposia 3.1 - 3.4 part 2**
12:30 - 13:30	Lunch break
13:30 - 14:40	Poster presentation
14:40 - 16:10	**Symposia 4.1 - 4.4 part 1**
16:10 - 16:30	Coffee break
16:30 - 18:00	**Symposia 4.1 - 4.4 part 2**

THURSDAY, SEPTEMBER 2

08:00 - 08:30	**Plenary lecture 8**
08:30 - 09:00	**Plenary lecture 9**
09:00 - 09:10	Short break
09:10 - 10:40	**Symposia 5.1 - 5.4 part 1**
10:40 - 11:00	Coffee break
11:00 - 12:30	**Symposia 5.1 - 5.4 part 2**
12:30 - 12:40	Short break
12:40 - 13:40	**Sponsored symp. (La Roche-Posay)**
13:40 - 13:50	Short break
13:50 - 15:20	**Symposia 6.1 - 6.4 part 1**
15:20 - 15:40	Coffee break
15:40 - 17:10	**Symposia 6.1 - 6.4 part 2**
17:10 - 17:20	Short break
17:20 - 18:00	**ESP General Assembly**

FRIDAY, SEPTEMBER 3

08:00 - 08:30	**Plenary lecture 10**
08:30 - 09:00	**Plenary lecture 11**
09:00 - 09:10	Short break
09:10 - 10:40	**Symposia 5.1 - 5.4 part 1**
10:40 - 11:00	Coffee break
11:00 - 12:30	**Symposia 5.1 - 5.4 part 2**
12:30 - 12:40	Short break
12:40 - 13:40	**Sponsored symp. (Therakos)**
13:40 - 13:50	Short break
13:50 - 15:20	**Symposia 6.1 - 6.4 part 1**
15:20 - 15:40	Coffee break
15:40 - 17:10	**Symposia 6.1 - 6.4 part 2**
17:10 - 17:20	Closing ceremony

19th Congress of the European Society for Photobiology

30 August – 3 September 2021 | World Wide Web and Salzburg, Austria

BOOK OF ABSTRACTS

19th Congress of the
European Society
for Photobiology
30 August – 3 September 2021
World Wide Web and Salzburg, Austria
ISBN 978-3-200-07802-4

Contents

Cover: Panorama of Salzburg during summer season from Kapuzinerberg © Salzburg Tourismus

PL-3

Theoretical and Computational Photobiology: The Chemiexcitation Phenomenon in Biology and Medicine

Daniel Roca-Sanjuán[1]
1) University of Valencia, Institute of Molecular Science, València, Spain

Light and biomolecules exchange energy within the framework of electronic-states interactions giving rise to a plethora of phenomena of relevance for Life and Medicine. Visible photons induce highly-energetic isomerizations of the double bonds of retinal in the process of vision. UV light produces [2+2] photocycloadditions of pyrimidines forming cyclobutane pyrimidine dimers (CPD) and by this means introducing lesions in the DNA. Meanwhile, thermal reactions with especial chemical functionalities (1,2-dioxetanes, dioxetanones, 1,2-dioxins) originate highly energetic species that decay by irradiating light in a phenomenon called chemiluminescence (or bioluminescence when it occurs in a living organism). Interestingly, this type of thermal transformations involving peroxide bonds can also activate the retinal isomerization[1] and CPD production in the dark (without visible or UV light)[2].

Theoretical and computational photobiology allows monitoring the mentioned phenomena of the light-biomatter interaction with a molecular and short timescale resolution, thus capturing the chemical mechanisms at the bottom of the biological and medical observations. The quantum nature of the electronic excitations (induced by either light or chemical reactions) requires quantum chemistry methodologies, and to model the geometrical and electrostatic constraints of the macromolecular biological systems, hybrid approaches combining quantum chemistry for the photo/chemi-excitation region and classical mechanics for the environment are highly efficient and convenient. In this contribution, we will show the findings obtained during the last years within such theoretical and computational photobiology framework, focusing on the phenomena of electronic excitation in the dark, which has been less explored in the photobiology community as compared to the processes induced by UV and visible light.

References

1) S. Premi, et al., Chemiexcitation of Melanin Derivatives Induces DNA Photoproducts Long after UV Exposure. *Science* 347, *842-847*, **2015**.
2) M. Marazzi, et al., Induced Night Vision by Singlet-Oxygen-Mediated Activation of Rhodopsin. *J. Phys. Chem. Lett.* 10, *7133-7140*, **2019**.

Acknowledgements. This work was supported by the Spanish ‘‘Ministerio de Ciencia e Innovación (MICINN)’’ (Project Ref. CTQ2017-87054-C2-2-P and grant RYC-2015-19234), a 2019 Leonardo Grant for Researchers and Cultural Creators, BBVA Foundation. The Foundation takes no responsibility for the opinions, statements, and contents of this project, which are entirely the responsibility of its authors.

IL-1.1.1

Engineering Cells, Membranes and Light Harvesting/Photosystem II Super-Complexes in Bio-Photoelectrochemical Cells

Noam Adir
Schulich Faculty of Chemistry, Technion, Haifa Israel; noam@ch.technion.ac.il

Photosynthetic organisms, membranes and complexes are attractive starting materials for solar energy conversion (SEC). Our overall goal is to develop methods to perform SEC using these materials in simple, inexpensive and a fashion that will be non-polluting and will not compete with the growth of food materials. I will describe here how the remarkable photocatalytic activity of the photosynthetic apparatus can provide overall water splitting with oxygen and hydrogen production in Bio-Photo-Electro-Chemical (BPEC) cells via the simplest and cleanest of processes wither in the absence or presence of added electron transport molecules. With plant thylakoids, electrons are shuttled by FeCN to a transparent FTO electrode, yielding a photocurrent density of 0.5 $mA \cdot cm^{-2}$. Hydrogen evolution occurs at the cathode at a bias as low as 0.8 V. A tandem cell comprising the BPEC cell with the thylakoid membranes and a Si photovoltaic module achieves overall water splitting with solar to hydrogen conversion efficiency of 0.3%[1]. With cyanobacterial cells, electrons from the respiratory system are energized by the photosynthetic system and transferred directly to a graphite electrode, utilizing endogenous electron carriers, especially NADPH[2,3]. The current produced can be used for hydrogen production at low additional bias for significantly longer durations than the plant thylakoids.
Another route is to use isolated photosynthetic complexes and chemically connect them to the electrochemical cell. One of the issues with this strategy is the small number of light absorbing chromophores connected to the complex, lowering the photochemical efficiency. Connection of isolated light harvesting (LH) complexes can help to overcome this deficiency. Photosystem II (PSII) is the only enzyme that catalyzes light-induced water oxidation being the basis for its application as a biophotoanode in various bio-photovoltaics and photo-bioelectrochemical cells. However, the absorption spectrum of PSII limits the quantum efficiency in the range of visible light, due to a gap in the green absorption region of chlorophylls (500 - 600 nm). To overcome this limitation, we have used two strategies: stabilizing the interaction between PSII and Phycobilisomes (PBSs) –LH complex or direct connection to modified gold-nanoparticles (Au-NPs) that serve both as LH and electron conduit. Integration of the PBS-PSII super-complexes within an Os-complex-modified hydrogel on macro-porous indium tin oxide electrodes (MP-ITO) resulted in notably improved, wavelength dependent, incident photon-to-electron conversion efficiencies[4]. With the Au-NPs, record currents were obtained, with strong improvement in the SPR range of wavelengths that are not absorbed by chlorophylls[5].

1) **Pinhassi *et al.* Nature Communications 2016.**
2) **Saper *et al.* Nature Communications 2018.**
3) **Shlosberg *et al.* *i*Science 2020.**
4) **Hartmann *et al.* J. Mat. Chem. A. 2020.**
5) **Shoyet *et al.* *Submitted* 2021.**

Acknowledgements. Funding was provided by a "Nevet" grant from the Grand Technion Energy Program (GTEP) and a Technion VPR Berman Grant for Energy Research, by a grant from the Deutsche Forschungsgemeinschaft (DIP project LU315/17-1) and the Israeli Ministry of National Infrastructures, Energy and Water Resources (grant number 218-11-044). Some of the results reported in this work were obtained using central facilities at the Technion's Hydrogen Technologies Research Laboratory (HTRL) supported by the Nancy & Stephen Grand Technion Energy Program (GTEP), the ADELIS Foundation and the Solar Fuels I-CORE.

IL-1.1.2

Photosynthetic entities in biohybrid systems for environmental monitoring

Matteo Grattieri[1,2]
1) Università degli Studi di Bari "Aldo Moro", Dipartimento di Chimica, Bari 70125, Italy; 2) Consiglio Nazionale delle Ricerche, IPCF-CNR Istituto per i Processi Chimico Fisici, Bari 70125, Italy

Interfacing photosynthetic entities with electrode surfaces enables converting solar energy into electrical energy, obtaining biohybrid electrochemical systems. Various biological entities have been explored, spanning from isolated photosynthetic apparatus to intact organisms. Utilizing isolated photosynthetic apparatus, such as photosystem II and I, thylakoid membranes, and the reaction center from purple bacteria allowed a simplified photo-excited electron transfer process, resulting in higher photocurrents[1,2]. Conversely, utilizing more complex apparatus as intact chloroplasts, or intact organisms such as cyanobacteria, purple bacteria, and algae, pose additional challenges to transfer electrons from the biocatalysts to the electrode surface due to the additional membranes that physically separate the photosynthetic apparatus from the electrode surface. However, such organisms provide all the necessary enzymatic machinery and photoprotection mechanisms that could make long term applications feasible[3]. Accordingly, research efforts have been focused on developing artificial approaches to facilitate the transfer of photoexcited electrons from these organisms to the electrode surface (and vice versa)[4-6].
Once the biohybrid systems is obtained, its applications include micro/low power generation, photo-bioelectrosynthesis of valuable chemicals and fuels, and biosensing of toxic compounds and heavy metals. The latter has attracted particular interest in recent years, as it would enable performing sunlight-powered monitoring of different environments (i.e., fresh and salt waters, remote areas), thus providing a sustainable approach for the early monitoring of environmental and human hazards.
Here, photo-bioelectrochemical sensors will be presented, showing approaches for establishing the photoexcited electron transfer process. Furthermore, the possibility to obtain self-powered biosensors will be discussed, enabling the on-line monitoring of widely utilized compounds that are hazardous for the environment and toxic for humans[7].

References

1) K.P. Sokol, D. Mersch, V. Hartmann, J.Z. Zhang, M.M. Nowaczyk, M. Rögner, A. Ruff, W. Schuhmann, N. Plumeré, E. Reisner, Rational wiring of photosystem II to hierarchical indium tin oxide electrodes using redox polymers. Energy and Environmental Science, 2016, 9, 3698-3709.
2) G. Buscemi, D. Vona, R. Ragni, R. Comparelli, M. Trotta, F. Milano, G.M. Farinola, Polydopamine/Ethylenediamine Nanoparticles Embedding a Photosynthetic Bacterial Reaction Center for Efficient Photocurrent Generation. Advanced Sustainable Systems, 2021, 2000303.
3) M. Grattieri, K. Beaver, E.M. Gaffney, F. Dong, S.D. Minteer, Advancing the fundamental understanding and practical applications of photo-bioelectrocatalysis. Chemical Communications, 2020, 56, 8553-8568.
4) K. Hasan, K.V.R. Reddy, V. Eßmann, Ka. Górecki, P.Ó. Conghaile, W. Schuhmann, D. Leech, C. Hägerhäll, L. Gorton, Electrochemical Communication Between Electrodes and Rhodobacter capsulatus Grown in Different Metabolic Modes. Electroanalysis, 2015, 27, 118-127.
5) M. Grattieri, Z. Rhodes, D.P. Hickey, K. Beaver, S.D. Minteer, Understanding Biophotocurrent Generation in Photosynthetic Purple Bacteria. ACS Catalysis, 2019, 9, 867-873.
6) M. Grattieri, S. Patterson, J. Copeland, K. Klunder, S.D. Minteer, Purple Bacteria and 3D Redox Hydrogels for Bioinspired Photo-bioelectrocatalysis, ChemSusChem, 2020, 13, 230-237.
7) M. Grattieri, H. Chen, S. D. Minteer, Chloroplast biosolar cell and self-powered herbicide monitoring. Chemical Communications, 2020, 56, 13161-13164.

Acknowledgements. This work is funded from Fondazione CON IL SUD, Grant "Brains to South 2018", project number 2018-PDR-00914.

IL-1.1.3

Engineering photosynthetic microorganisms for direct solar chemical and fuel production from carbon dioxide, example butanol

Peter Lindblad[1]

1) Microbial Chemistry, Department of Chemistry-Ångström, Uppsala University, SE-751 20 Uppsala, Sweden

Cyanobacteria, photosynthetic microorganisms with the same type of photosynthesis as plants and algae, can be engineered to produce solar chemicals and solar fuels in direct processes from carbon dioxide[1]. This presentation will outline our strategies to engineer cyanobacteria to produce the alcohol butanol. Butanol is a four-carbon alcohol (C_4H_9OH) occurring in four structural isoforms: 1-butanol, 2-butanol, isobutanol and *tert*-butanol. It is an important bulk chemical, as solvent or intermediate in chemical synthesis, and excellent blend-in fuel. The absolute majority of butanol is presently a produced from fossil resources. Additionally, there are biological routes for fermentative butanol production, mainly to produce 1-butanol and isobutanol. Fermentative 1-butanol is produced from starch, sugar, or cellulose such as wheat, beet, corn and wood. Products of these fermentation processes also include acetone and ethanol. Cyanobacteria do not produce butanol naturally, they lack the butanol biosynthetic pathways and corresponding relevant genes.

Introduction of a single gene encoding KivD resulted in isobutanol producing strains of *Synechocystis* PCC 6803[2]. Knowledge based modelling of the identified bottleneck KivD resulted in strains with significantly increased isobutanol production[3]. Using our best isobutanol strain in long-term experiments a cumulative titer of 911 mg per L was observed with a maximal rate of 43.6 mg per L and day[4]. A similar approach to systematically engineer cyanobacteria to produce 1-butanol resulted in cells with a cumulative titer of 4.8 g per L and a maximal rate of 302 mg per L and day[5], recently doubled to 600 mg 1-butanol per L and day and a carbon partitioning of 60%[6]. Photosynthetic butanol is the most sustainable and CO_2 neutral butanol synthetized from CO_2 and solar energy.

References

1) Miao, R., Wegelius, A., Durall, C., Liang, F., Khanna, N., Lindblad, P., Engineering cyanobacteria for biofuel production. Modern Topics in Phototroph Prokaryotes 11: 351-393, 2017.
2) Miao, R., Liu, X., Englund, E., Lindberg, P., Lindblad, P., Isobutanol production using *Synechocystis* PCC 6803 endogenous alcohol dehydrogenases. Metabolic Engineering Communications 5: 45-53, 2017.
3) Miao, R., Xie, H., Ho, F.M., Lindblad, P., Protein engineering of α-ketoisovalerate decarboxylase for improved isobutanol production in *Synechocystis* PCC 6803. Metabolic Engineering 47: 42-48, 2018.
4) Miao, R., Xie, H., Lindblad, P,. Enhancement of photosynthetic isobutanol production in *Synechocystis* PCC 6803.Biotechnolgy for Biofuels 11: 267, 2018.
5) Liu, X., Miao, R., Lindberg, P., Lindblad, P., Modular Engineering for Photosynthetic 1-Butanol Production in Cyanobacteria. Energy & Environmental Science 12: 2765-2777, 2019.
6) Wichmann, J., Lauersen, K.J., Biondi, N., Christensen, M., Guerra, T., Hellgardt, K., Kuronen, M., Lindberg, P., Rösch, C., Yunus, I.S., Jones, P., Lindblad, P., Kruse, O., Engineering Biocatalytic Solar Fuel Production: The PHOTOFUEL Consortium. Trends in Biotechnology 39: 323-327, 2021.

Acknowledgements. This work was supported by the European Union Horizon 2020 Framework Program under the grant agreement number 640720 (Photofuel), the Kamprad Family Foundation for Entrepreneurship, Research & Charity (Photosynthetic butanol), the NordForsk NCoE program "NordAqua" (project number 82845), and the Swedish Energy Agency (CyanoFuels, project number P46607-1).

OC-1.1.4

Predicting the structural, electronic and spectroscopic properties of chromophore – protein assemblies towards efficient charge-separation

Mariano Curti[1], Carles Curutchet[2], Elisabet Romero[1]
1) Institute of Chemical Research of Catalonia (ICIQ), Barcelona Institute of Science and Technology (BIST), Avinguda dels Països Catalans 16, 43007 Tarragona, Spain; 2) Department of Pharmacy and Pharmaceutical Technology and Physical-Chemistry and Institute of Theoretical and Computational Chemistry (IQTCUB), School of Pharmacy and Food Sciences, University of Barcelona, Joan XXIII, 27–31, 08028 Barcelona, Spain

In photosynthetic reaction centres, charge separation proceeds with near unitary quantum yield.[1] Plants, algae and photosynthetic bacteria achieve this impressive efficiency by exploiting the "design principles of photosynthetic charge separation", that can be summarized as follows:[1]
(i) The excited states possess a large collective (excitonic) character, increasing light absorption and reducing the number of energy transfer steps. Moreover, there is a coherent mixing between excitonic and charge-transfer states that promotes ultrafast electron transfer.
(ii) Intramolecular vibrations, with energies close to the gap between excitonic and charge-transfer states ("resonant vibrations"), ensure a strong mixing of these states.
(iii) Multiple charge separation routes offer flexibility and avoid energy losses caused by disorder.
(iv) The protein scaffold acts as a smart matrix, selecting the appropriate charge separation route and providing decoherence channels for the irreversible formation of charge-separated states.
Efficient as it is, the photosynthetic machinery is also extremely complex, and thus not amenable to modifications aiming to tune the process, for instance to perform other photochemical reactions. As an alternative, the *de novo* design of proteins with tailored functions has seen substantial progress in recent years. In particular, a family of structures termed "maquette proteins" provide much simpler scaffolds, and once loaded with cofactors are still able to perform light absorption and energy transfer roles (although with low charge separation efficiency, so far).[2, 3] Of particular interest are maquette proteins that consist of water-soluble four alpha helix bundles. By means of appropriately placed histidine residues, they can readily bind metalloporphyrins in their interior, providing excellent model systems in which to implement the design principles of charge separation.

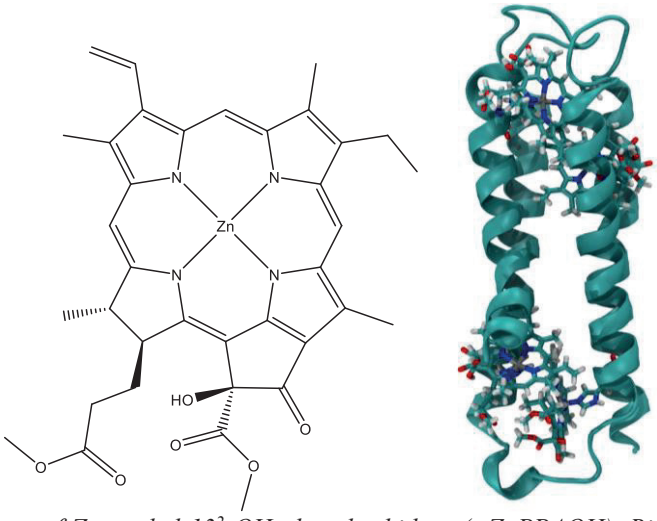


Fig. 1 – Left: Chemical structure of Zn methyl-13²-OH-pheophorbide a (mZnPPAOH). Right: Snapshot of the α-HP protein structure with four bound chromophores, obtained from molecular dynamics simulations.

In this contribution we present a computational study on a maquette protein class termed "HP",[3] and their complexes with the chromophore Zn methyl-13²-OH-pheophorbide *a* (mZnPPAOH; Fig. 1, left). The base protein design ("α-HP") has four binding sites distributed by pairs: one in the upper half of the protein, and the other in the bottom half (Fig. 1, right). We analyze this structure both in its *apo* and in different *holo* states, considering as well

different binding modes (e.g. through histidine's $^{\delta}$N or $^{\varepsilon}$N), folding conformations, and mutated structures with additional charged amino acids. By performing simulations that combine long (>1 μs) molecular dynamics with QM/MM calculations, we evaluate the binding energy, site energies, electronic couplings, and spectral density, together with the absorption, circular- and linear-dichroism spectra of maquette-bound mZnPPAOH. Furthermore, we performed a preliminary analysis of charge transfer states. Altogether, this information can be used to model the time-resolved dynamics upon light absorption.

Some of our main findings are:
1- The binding energy depends on the relative position of the chromophores. Taking as reference the binding energy of a single chromophore in the top half of the protein, binding a second one in the bottom proceeds with a similar energy. However, the binding of a second chromophore in the same (top) half of the protein results in a significant energy penalty due to steric hindrance.
2- The electronic coupling between chromophores in opposite halves of the protein is relatively small (~ 4 cm^{-1}); in comparison, for chromophores in the same half it is ~ 40 cm^{-1}. Consequently, the latter show strong excitonic signals in circular dichroism spectra, while the former display only weak signals.
3- The site energies are virtually unaffected by the successive binding of chromophores, consistent with the symmetry of the protein sequence. However, by mutating the protein and adding positively charged amino acids in regions nearby chromophores, we observe a slight red shift in their absorption spectra.
4- Charge-transfer states are significantly (~1 eV) higher in energy with respect to the lowest lying (excitonic) excited state. It is possible to red shift the charge-transfer states by including positively charged amino acids nearby.

The results obtained so far are generally in good agreement with ongoing experimental work. Furthermore, they represent a solid step towards the implementation of the Design principles of charge separation in an artificial system.

References
1) E. Romero, V. I. Novoderezhkin and R. van Grondelle, Quantum design of photosynthesis for bio-inspired solar-energy conversion. Nature, 543, 355–365, 2017.
2) T.A Farid, G. Kodali, L.A. Solomon, B.R. Lichtenstein, M.M. Sheehan, B.A. Fry, C. Bialas, N.M. Ennist, J.A. Siedlecki, Z. Zhao, M.A. Stetz, K.G. Valentine, J.L.R. Anderson, A.J. Wand, B.M. Discher, C.C. Moser and P.L. Dutton, Elementary Tetrahelical Protein Design for Diverse Oxidoreductase Functions. Nature Chemical Biology, 9, 826–833, 2013.
3) I. Cohen-Ofri, M. van Gastel, J. Grzyb, A. Brandis, I. Pinkas, W. Lubitz, and D. Noy, Zinc-Bacteriochlorophyllide Dimers in de Novo Designed Four-Helix Bundle Proteins. A Model System for Natural Light Energy Harvesting and Dissipation. Journal of the American Chemical Society, 133, 9526–9535, 2011.

Acknowledgements. MC and ER acknowledge the funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 801474 and from the State Research Agency/Spanish Ministry of Science and Innovation (AEI/MICINN) through the Severo Ochoa Excellence Accreditation CEX2019-000925-S. ER is supported by a Starting Grant from the European Research Council (No. 805524, BioInspired_SolarH2). CC acknowledges support from the AEI/MICINN (Grants CTQ2017-89924-P and MDM-2017-0767). The authors thankfully acknowledge the computer resources at Pirineus and CTE-POWER and the technical support provided by the Barcelona Supercomputing Center and the CSUC (QSB-2020-3-0002 and QSB-2021-1-0019).

IL-1.1.5

The Metabolism of H_2 in green algae – how can it be used to redesign photosynthesis?

Prof. Iftach Yacoby, PhD
Tel Aviv University, School of Plant Sciences and Food security Faculty of Life Sciences, Tel Aviv, Israel

The natural ability of photosynthesis to function as a factory of chemicals and fuels is the basis for life on earth; sunlight energy has been used for 1.5 billion years to power electron transfer towards the assimilation of inorganic carbon dioxide into organic matter. Microalgae (a group of photosynthetic unicellular organisms) can also use this energy to power other potentially useful but inefficient processes, such as H_2 production via the enzyme Hydrogenase. Improving this process could open a new era in the agricultural history, this time dedicated for clean energy production. Yet, attempting to upscale it proved challenging. Collective evidence suggest that the inefficiency of alternative processes other than carbon assimilation stems from a non-optimal location, *i.e.*, lack of docking capabilities to photosynthetic reaction centers, which limits access to the energy supply. **To date, the scientific efforts mainly focused on improving natural photosynthesis, rather than redesigning it to secure efficient electron transfer to enzymes of interest.** Photosynthetic improvement has been targeted by studying libraries of mutants and screening for improved features; for example, faster growth or faster H_2 production. The knowledge obtained from studying mutants along with structural insights into the photosynthetic reaction centers, constitute the platform for redesigning photosynthesis. Unlike photosynthesis improvement, **photosynthesis redesigning** attempts are usually based on bottom-up strategies; for example, targeting enzymes towards hotspots, where they could benefit from plenty of energy, low oxygen or other supporting features. To divert the photosynthetic electrons towards reactions other than carbon fixation, the enzymatic machinery of choice needs to be efficiently hooked up to the photosynthetic machinery. Therefore, the key for optimally driving other processes is to find strategic hotspots from which the electrons can be "hijacked"; for example, by docking Hydrogenase directly to photosynthetic reaction centers. This approach was successfully applied by tethering Hydrogenase to PSI via fusions to Ferredoxin (Fd)[1,2], Superoxide dismutase (SOD)[3] or directly to Photosystem I (PSI)[4]; these resulted in significant improvement to H_2 production, *in vivo*. In this lecture, I will describe the basic findings and how they were used for engineering.

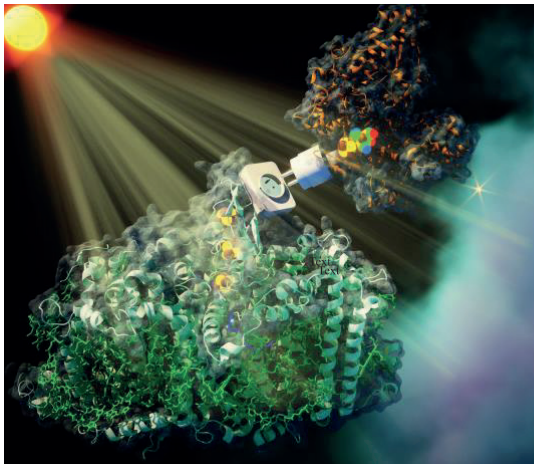


Fig. 1- Illustration of Hydrogenase Fused to Photosystem I From Kanygin et al 2020

References

1. Yacoby, I., et al., *Photosynthetic electron partitioning between [FeFe]-hydrogenase and ferredoxin:NADP+-oxidoreductase (FNR) enzymes in vitro.* Proc Natl Acad Sci U S A, 2011. **108**(23): p. 9396-401.
2. Eilenberg, H., et al., *The dual effect of a ferredoxin-hydrogenase fusion protein in vivo: successful divergence of the photosynthetic electron flux towards hydrogen production and elevated oxygen tolerance.* Biotechnol Biofuels, 2016. **9**(1): p. 182.
3. Ben Zvi, O. and I. Yacoby, *The in-vitro enhancement of FeFe hydrogenase activity by superoxide dismutase.* International Journal of Hydrogen Energy, 2016. **41**(39): p. 17274-17282.
4. Kanygin, A., et al., *Rewiring photosynthesis: a photosystem I-hydrogenase chimera that makes H-2 in vivo.* Energy & Environmental Science, 2020. **13**(9): p. 2903-2914.

IL-1.1.6

Bio-Inspired Chromophore-Protein Assemblies for the Generation of Solar Fuels

Valentin Maffeis[1], Saeed Shareef[1], Mariano Curti[1], Sharmistha Chatterjee[1], Dominik Bäuerle[1], Elisabet Romero[1]
1) Institute of Chemical Research of Catalonia (ICIQ), Barcelona Institute of Science and Technology (BIST), Avinguda dels Països Catalans 16, 43007 Tarragona, Spain

Photosynthesis is the fundamental biological process by which solar energy is converted into fuel in four basic steps: light harvesting, charge separation, water splitting and fuel generation. At the heart of Photosynthesis, the reaction center pigment-protein complexes perform charge separation with near unity quantum efficiency despite their highly disordered energy landscape; they realise the first solar-energy conversion step in Photosynthesis by transforming sunlight to electrochemical energy.
To achieve this amazing feat, the reaction centers exploit *The Quantum Design Principles of Photosynthesis*[1], complementary and interrelated solutions to ensure ultrafast and irreversible transfer of energy and electrons within a fluctuating environment. These Principles provide a guide for the rational design and construction of systems able to transfer energy and electrons with high efficiency and in the right direction.
During my talk I will briefly present these *Principles* and focus on how we are putting all this knowledge into action to *Design and Construct Bio-Inspired Chromophore-Protein Assemblies*[2] composed by abundant and biodegradable materials in order to maximize their capacity to absorb, transfer and convert solar to electrochemical energy. Ultimately, these assemblies will be coupled to molecular catalysts to achieve sustainable and cost-effective solar-energy conversion to fuel.

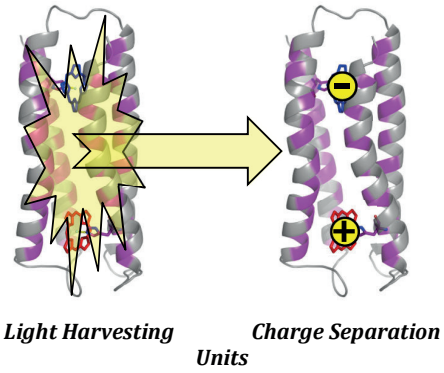


Fig. 1 – Schematic representation of the Bio-Inspired Chromophore-Protein Assemblies under investigation

References
1) E. Romero, V. I. Novoderezhkin and R. van Grondelle, Quantum design of photosynthesis for bio-inspired solar-energy conversion. Nature, 543, 355–365, 2017.
2) T.A Farid, G. Kodali, L.A. Solomon, B.R. Lichtenstein, M.M. Sheehan, B.A. Fry, C. Bialas, N.M. Ennist, J.A. Siedlecki, Z. Zhao, M.A. Stetz, K.G. Valentine, J.L.R. Anderson, A.J. Wand, B.M. Discher, C.C. Moser and P.L. Dutton, Elementary Tetrahelical Protein Design for Diverse Oxidoreductase Functions. Nature Chemical Biology, 9, 826–833, 2013.

Acknowledgements. VM thanks the European Union's Horizon2020 Research and Innovation Program under the Marie Skłodowska-Curie grant agreement No. 754510. SS, SC and ER are supported by ER´s Starting Grant from the European Research Council (No. 805524, BioInspired_SolarH2). MC acknowledge the support of the European Commission under the Marie Sklodowska Curie grant agreement No. 801474. MC, DB and ER thank the Ministerio de Ciencia e Innovación-Agencia Estatal de Investigación for financial support through Severo Ochoa Excellence Accreditation 2020-2023 (CEX2019-000925-S) and FPI predoctoral fellowship to DB (PRE2020-095271).

IL-1.1.7

Do photosynthetic bacteria dream of heavy metals?

Massimo Trotta[1], Livia Giotta[2], Luigi R. Ceci[3], Maria Varsalona[1,4], Rossella Labarile[1,4], Francesco Milano[5], Maria Teresa Volpicella[4], Matteo Grattieri[1,4]

[1] Consiglio Nazionale delle Ricerche – Istituto per i Processi Chimico Fisici; [2] Università del Salento - Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali; [3] Consiglio Nazionale delle Ricerche – Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari; [4] Università di Bari – Dipartimento di Chimica; [5] Consiglio Nazionale delle Ricerche – Istituto di Scienze delle Produzioni Alimentari.

Purple non sulphur (PNS) photosynthetic bacteria have a high affinity toward metal ions, being prone to bioaccumulate them and – in some case – lower their toxicity by catalysing the change of their redox states. The most recent advancement on the topic of bioremediation of sites polluted with heavy metals by the use of PNS[1-9]. Promising applications in soil washing will also be presented.

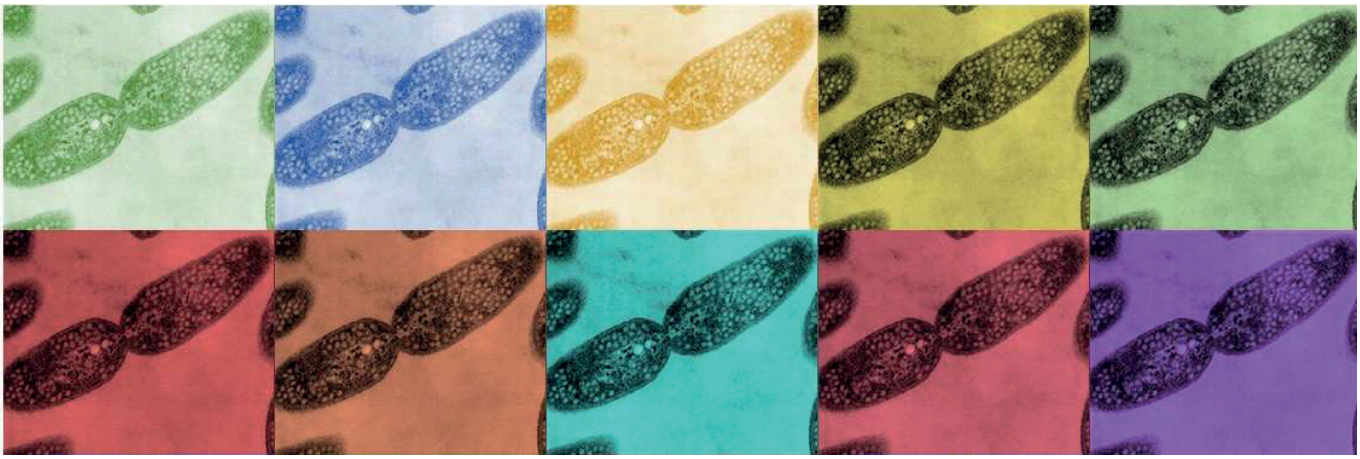


Fig. 1 – A pop-art view of PNS bacteria

References

1. Calvano, C. D.; *et al*, The lipidome of the photosynthetic bacterium *Rhodobacter sphaeroides* R26 is affected by cobalt and chromate ions stress. Biometals 2015, 28 (1), 229-229.
2. Volpicella, M.; *et al*, *Rhodobacter sphaeroides* adaptation to high concentrations of cobalt ions requires energetic metabolism changes. FEMS Microbiol. Ecol. 2014, 88 (2), 345-357.
3. Belviso, B. D.; *et al*, Cobalt binding in the photosynthetic bacterium *R. sphaeroides* by X-ray absorption spectroscopy. Biometals 2013, 26 (5), 693-703.
4. Italiano, F.; *et al*., Changes in morphology, cell wall composition and soluble proteome in Rhodobacter sphaeroides cells exposed to chromate. Biometals 2012, 25(5), 939-949.
5. Emese, A.; *et al*, The reaction center is the sensitive target of the mercury(II) ion in intact cells of photosynthetic bacteria. Photosynthesis Research 2012, 112 (2), 129-140.
6. Italiano, F.; *et al*, The photosynthetic membrane proteome of *Rhodobacter sphaeroides* R-26.1 exposed to cobalt. Res. Microbiol. 2011, 162 (5), 520-527.
7. Giotta, L.; *et al*, Reversible binding of metal ions onto bacterial layers revealed by protonation-induced ATR-FTIR difference spectroscopy. Langmuir 2011, 27 (7), 3762-3773.
8. Asztalos, E.; *et al* Early detection of mercury contamination by fluorescence induction of photosynthetic bacteria. Photochem Photobiol Sci 2010, 9 (9), 1218-23
9. Pisani, F.; *et al*., Soluble proteome investigation of cobalt effect on the carotenoidless mutant of *Rhodobacter sphaeroides*. J. Appl.Microbiol. 2009, 106 (1), 338-349.

Acknowledgements. This work was partly funded by PON Energy for TARANTO project n. ARS01_00637.

OC-1.1.8

Coating photosynthetic bacteria with the versatile polydopamine

Rossella Labarile[1,2], Danilo Vona[1], Gabriella Buscemi[1,2], Maria Varsalona[1,2], Matteo Grattieri[1,2], Gianluca Maria Farinola[1], Massimo Trotta[2]

1) Università degli Studi di Bari, Dipartimento di Chimica, Bari, Italy 2) CNR-IPCF Institute for Physical-Chemical Processes, Bari, Italy

The wild type and the carotenoidless mutant strains of *Rhodobacter* (*R.*) *sphaeroides* have been largely used as models for studying both the molecular mechanisms of photosynthesis and the metabolism of anoxygenic photosynthesis. Beside of an efficient light harvesting system, the photochemical enzyme called reaction center (RC) plays a pivotal role, generating stable a charge-separated state across the membrane within a few hundred microseconds from photon absorption with a conversion yield close to unit. These electron flows could be intercepted and funneled outside the cells and transferred to suitable material prone to collect such electrons and impinge them in an electric/electronic circuit of a biohybrid device.
Polydopamine (PDA), a bioinspired synthetic polymer produced by the self-polymerization of dopamine, was selected for *R. sphaeroides* coating because of its versatile adhesivity and low toxicity[1]. PDA possesses numerous quinone groups that are redox-active functional ligands and further suitable for accelerating the transfer of electrons during extracellular respiration.
The coating is assembled by introducing dopamine monomers into the biological feeding medium of bacteria[2]. The PDA artificial conductive coating assembles around the cells, acting as soft functional matrix allowing the photosynthetic organisms to thrive using light and ensuring the satisfactory electronic communication[3] with the conductive surfaces, namely glassy carbon electrodes.
Electrochemical characterization was performed to investigate the electronic behavior of these biohybrids, unveiling that the polymer layer on the bacterial cells does not hinder the diffusion of the mediator and its capability to react at the electrode surface. The effects of dopamine concentration on the light-induced photoresponse of the biohybrid system will be discussed and compared to bare bacteria.

References

1) Yang, S.H., et al., Mussel-inspired encapsulation and functionalization of individual yeast cells. J Am Chem Soc, 2011. **133**(9): p. 2795-7.
2) Vona, D., et al., Synthesis of (poly)gallic acid in a bacterial growth medium. MRS Advances, 2020. **5** (18-19): p. 957-963.
3) Buscemi, G., et al., Polydopamine/Ethylenediamine Nanoparticles Embedding a Photosynthetic Bacterial Reaction Center for Efficient Photocurrent Generation. Advanced Sustainable Systems, 2021. 2000303

Acknowledgements. This work was supported by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No 800926 (HyPhOE, Hybrid Electronics Based on Photosynthetic Organisms).

P-1.1.9

Biological approach in the synthesis of dopamine-based polymers: purple photosynthetic bacteria as catalysers

Maria Varsalona[1,2], Rossella Labarile[1,2], Danilo Vona[1], Gabriella Buscemi[1,2], Matteo Grattieri[1,2], Gianluca Maria Farinola[1], Massimo Trotta[2]

[1] Università degli Studi di Bari, Dipartimento di Chimica, via Orabona 4, 70125, Bari, Italy; [2] CNR-IPCF Institute for Physical-Chemical Processes, via Orabona 4, 70125, Bari, Italy

Rhodobacter (*R.*) *sphaeroides* is a non-sulfur bacterium able to growth either under aerobic and phoeterotropically conditions. The carotenoid-less mutant strain R26 grows phoeterotropically under strict anoxygenic conditions.
Dopamine (DA) is a neurotransmitter that self-polymerizes into polydopamine (PDA), a synthetic analogue of melanin[1] of great interest in organic and bioinorganic electronics.
The self-polymerization of dopamine is investigated in presence and in absence of growing *R. sphaeroides* R26 under aerobic and phoeterotropically anaerobic environment.
The effects of the photosynthetic bacterium on the polymerization of dopamine solutions is investigated under a variety of conditions[2, 3] and exposed to light under anaerobic conditions as previously reported.[4, 5]
The system was characterised via absorption spectroscopy showing that even in absence of the oxidising species normally used to initiate polymerization, *R. sphaeroides* acts as a catalyst for the PDA formation.

References

1. d'Ischia M., et al., *Polydopamine and eumelanin: From structure-property relationships to a unifield tailoring strategy*. Accounts of Chemical Research, 2014. **47**(12): p. 3541-50.
2. Yang, S.H., et al., Mussel-inspired encapsulation and functionalization of individual yeast cells. J Am Chem Soc, 2011. 133(9): p. 2795-7.
3. Giotta, L., et al., Heavy metal ion influence on the photosynthetic growth of Rhodobacter sphaeroides. Chemosphere, 2006. 62(9): p. 1490-9.
4. Vona, D., et al., Synthesis of (poly)gallic acid in a bacterial growth medium. MRS Advances, 2020. 5(18-19): p. 957-963.
5. Buscemi, G., et al., Polydopamine/Ethylenediamine Nanoparticles Embedding a Photosynthetic Bacterial Reaction Center for Efficient Photocurrent Generation. Advanced Sustainable Systems, 2021.

P-1.1.10

Adhesive Polydopamine for Biohybrid Photoanodes with Photosynthetic Purple Bacteria

Gabriella Buscemi[1,2], Rossella Labarile[1,2], Danilo Vona[1], Pinalysa Cosma[1,2], Angela Agostiano[1,2], Massimo Trotta[2], Gianluca M. Farinola[1], Matteo Grattieri[1,2]
1)Università degli Studi di Bari "Aldo Moro", Dipartimento di Chimica, Bari, 70125, Italy;
2) Consiglio Nazionale delle Ricerche, IPCF-CNR Istituto per i Processi Chimico Fisici, Bari, 70125, Italy.

Anoxygenic phototrophic purple bacteria are well-known microorganisms having a versatile metabolism, as they use sunlight to oxidize various organic compounds. With the intent to mimic their natural processes, many attempts were made to exploit the ability of energy storage and energy conversion into electrical (and/or chemical) energy in biohybrid electrochemical systems. Such approaches allow developing an eco-friendly and scalable technology based on self-repairing biocatalysts where sunlight is utilized as primary green energy source[1].

In the application of living organism in bioelectronic devices, more than the "interface" between biotic and electronic domains, the challenging aspect is related to the electrochemical communication between these two components. Various approaches have been reported to overcome this problem, such as layer by layers techniques[2] to entrap whole cell onto electrodes surfaces or the use of conductive material to burst electronic communication[3].
In this context, we combined a biocompatible soft polymer, polydopamine (PDA), with the purple bacteria *Rhodobacter capsulatus* as biocatalyst. Polydopamine, containing both catechol and amine groups, is a polymer presenting similar characteristic to the adhesive plaque of mussel byssus, which has been recently utilized to encapsulate isolated photosynthetic apparatus[4]. PDA can be obtained by oxygenic self-assembly polymerization of dopamine in water or by electro-polymerization. Moreover, the tunable conductive properties and the key feature of being adhesive onto a variety of surfaces[5] make this material suitable for bioelectrochemical devices. Here, we report a biohybrid photoanode obtained by a one-step immobilization of the living whole cells into PDA matrix, while entrapping a quinone-based redox mediator. The presented approach enabled a more stable adhesion of the bioactive components onto the electrode surface over a prolonged period of time, while improving and stabilizing the electrochemical response. Electrochemical and spectroscopic evidence for the obtained biohybrid photoanodes will be discussed together with future research possibilities.

References

1) McCormick A.J., *et al.* Biophotovoltaics: oxygenic photosynthetic organisms in the world of bioelectrochemical systems. Energy Environ. Sci., (8), 1092-1109, 2015.
2) Grattieri M., et al. Non-Competitive Reversible Inhibition of Laccase by H_2O_2 in Osmium Mediated Layer-By-Layer Multilayer O_2 Biocathodes. J. Electrochem. Soc. 162 (9) G82-G86.
3) Reggente, M, *et al.* Design of Optimized PEDOT-Based Electrodes for Enhancing Performance of Living Photovoltaics Based on Phototropic Bacteria. Adv. Mater. Technol. (5), 1900931, 2020.
4) Buscemi, G., *et al*. Polydopamine/Ethylenediamine Nanoparticles Embedding a Photosynthetic Bacterial Reaction Center for Efficient Photocurrent Generation. Adv. Sustainable Syst., 2000303, 2021.
5) M. d'Ischia *et al.* Melanin Biopolymers: Tailoring Chemical Complexity for Materials Design, Angew. Chem. Int. Ed.(59), 11196, 2020.

Acknowledgements. The authors would like to acknowledge the funding from Fondazione CON IL SUD, Grant "Brains to South 2018", project number 2018-PDR-00914 and the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 800926 (HyPhOE, Hybrid Electronics Based on Photosynthetic Organisms).

P-1.1.11

Synthesis of *meso*-functionalized porphyrins as hole transport materials for perovskite solar cells

Melani J.A. Reis[1], Nuno M.M. Moura[1], Ana Teresa Nogueira[2], Ana Eulálio[2], Ana M.V.M. Pereira[2], Adélio Mendes[2], M. Graça P.M.S. Neves[1]

1)Department of Chemistry & LAQV-REQUIMTE, University of Aveiro, 3810-193 Aveiro, Portugal;
2)LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

The potential issues surrounding the use of fossil fuels have been leading to a crescent search for alternative energy sources and it is nowadays a worldwide concern.[1]
Perovskite solar cells (PSCs, figure 1) are promising photovoltaic technologies and an alternative to traditional silicon-based solar cells. The use of solid-state hole-transporting materials (HTMs) was easily foreseen, and Spiro-OMeTAD was selected as the benchmark .[2] However, the synthesis of Spiro-OMeTAD is associated with high costs, since it involves a laborious and complex multistep synthetic route and a hard purification process.[2]
Porphyrins, due to their particular physicochemical features, namely high electronic delocalization, thermal stability, susceptibility to electronic transfer and the ability to absorb light in a wide range of the visible spectrum, are particularly attractive compounds to be used as HTMs for PSCs.[3,4]
In this communication, the synthetic pathways to obtain new porphyrin-based derivatives containing nitrogen donor moieties will be presented, as well as their efficiency in PSC devices.

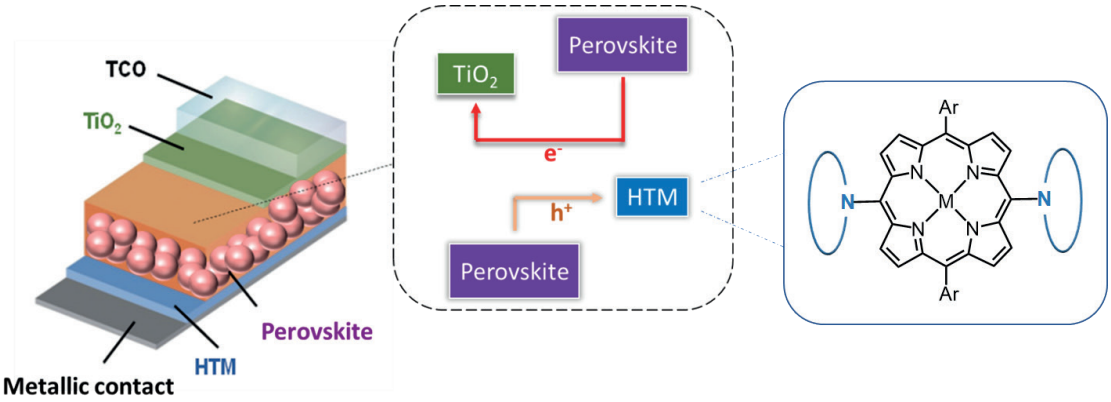


Fig. 1 - Structure of a PSC device with the layout of the cargo transport process.

References

1) M. Ye, *et al*, *Mater. Today*, 2015, **18**, 155.
2) M. Urbani *et al*. *Chem. Soc. Rev*, 2019, **48**, 2738.
3) X. Lv, *et al*, *Dyes Pigments*, 2019, **160**, 957-961.
4) Z. Shariatinia, *Renew. Sust. Energ. Rev.*, 2020, **119**, 109608.

Acknowledgements. The authors thank the University of Aveiro and the University of Porto and FCT/MCTES (PIDDAC) for their financial support to the LAQV-REQUIMTE (UIDB/50006/2020) and LEPABE (UIDB/00511/2020) Research Units and Portuguese NMR Network. This work was financially supported by Project FCT POCI-01-0145-FEDER-030357 - "Taylor-made porphyrinoids for emerging high-efficiency solar energy devices", funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. M.J.A. Reis, A.T. Nogueira, A. Eulálio, N.M.M. Moura (REF.-048-88-ARH/2018) and A.M.V.M. Pereira thanked the FCT and project POCI-01-0145-FEDER-030357 for the research grants and concession agreements.

P-1.1.12

In vivo Functionalization of Diatoms with a Transparent Polymer Matrix for Biohybrid Photonic Systems

Cesar Vicente-Garcia[1], Danilo Vona[1], Roberta Ragni[1], Stefania R. Cicco[2], Gianluca Maria Farinola[1]
1) Università degli Studi di Bari, Department of Chemistry, Bari, Italy; 2) CNR-ICCOM, Department of Chemistry, Bari, Italy

Diatom microalgae are known for generating highly nanostructured shells (frustules) by *in vivo* mineralization, made of mesoporous silica with extraordinarily intricate photonic structures. These shells not only let diatoms maximize light absorption for photosynthesis[1], but also filter out potentially harmful near UV light wavelengths[2]. Moreover, upon incorporation into diatom cells, suitable organic fluorophores have been proven to harvest light in spectral ranges where diatoms do not efficiently absorb, thus enhancing their photosynthesis[3]. Frustule properties have attracted attention for their possible application in photonics and optoelectronics[4]. In addition, both covalent and non-covalent approaches to anchor functional moieties (drugs, dyes, antibodies, metals)[5] are explored to afford biohybrid nanostructures for biosensing[6] and solar energy conversion[7].
Here we report a versatile method of chemical modification of diatoms to embed these microalgae in a transparent organic polyacrylate matrix, without compromising their viability. This method represents a straightforward strategy to immobilize cells, paving the way to the formation of stable biofilms that can be further functionalized with fluorophores or other molecules for specific applications.
Characterization by electronic microscopy, FTIR-ATR and Raman spectroscopies, as well as functionalization with a suitable fluorescein-based dye, confirm the successful embedment of diatoms into the polymer.
Moreover, the embedded diatom cells can be subjected to an acidic treatment that removes their organic protoplasm, this allowing to obtain frustules directly embedded in the organic matrix that is conversely preserved. This immobilization strategy is promising to easily get transparent biosilica based films for photonics applications.

References

1) J. W. Goessling, M. Kühl, Structure-based optics of centric diatom frustules: modulation of the in vivo light field for efficient diatom photosynthesis. New Phytologist, 219, 122-134, 2018.
2) M. M. Ghobara, R. Gordon, “On Light and Diatoms: A Photonic and Photobiology Review”, Diatoms: Fundamentals and Applications, Scrivener Publishing LLC, 2019.
3) G. Leone, G. M. Farinola, Incorporating a molecular antenna in diatom microalgae cells enhances photosynthesis, Scientific Reports, 11, 5209, 2021.
4) R. Ragni, G. M. Farinola, Multiple Routes to Smart Nanostructures Materials from Diatom Microalgae: A Chemical Perspective, Adv. Mat. 30, 1704289, 2018
5) S. R. Cicco, G. M. Farinola, *In vivo* functionalization of diatom biosilica with sodium alendronate as osteoactive material, Mater. Sci. Eng. C., 104, 109897, 2019.
6) J. Cai, D. Zhang, Au nanoparticle-grafted hierarchical pillars array replicated from diatom as reliable SERS substrates, Appl. Surf. Sci., 541, 148374, 2021.
7) T. M. W. J. Bandara, B.-E. Mellander, Diatom frustules enhancing the efficiency of gel polymer electrolyte-based dye-sensitized solar cells with multilayer photoelectrodes, Nanoscale Adv., 2, 199-209, 2020.

Acknowledgements. This work was supported by BEEP-ITN grant from MSCA-H2020.

IL-1.2.1

Interaction of different UV wavelengths present in natural sunlight: Impact on DNA damage and apoptosis

Thomas Haarmann-Stemmann
IUF – Leibniz-Research Institute for Environmental Medicine, 40225 Düsseldorf, Germany

The skin aging exposome consists of all external and internal factors and their interactions, affecting a human individual from conception to death as well as the response of the human body to these factors, that lead to biological and clinical signs of skin aging [1]. In this presentation, I will focus on the interaction of two major exposomal factors for premature skin aging, namely UVA (315 – 400 nm) and UVB (280 – 315 nm) radiation. Historically, the vast majority of irradiation studies was carried out by exposing the test model (in vitro, in vivo, ex vivo) to either UVA or UVB radiation alone or by applying a sequential irradiation protocol, i.e. the test model is first exposed to UVA radiation and in a second step irradiated with UVB, or vice versa [2]. Given that our skin is simultaneously exposed to the whole electromagnetic spectrum of the sun, we hypothesize that the biological consequences of a simultaneous exposure to UVA and UVB radiation is not adequately mimicked by a simple addition of the outcome of the respective single or sequential exposure studies. In fact, we found that a simultaneous but not a sequential (UVA>UVB or UVB>UVA) exposure to UVA decreases UVB-induced keratinocyte apoptosis, as measured on the level of caspase activity, Annexin V/propidium iodide-positive cells, and subG1 fraction. DNA double-strand breaks (DSBs) are a major driver of UVB radiation-induced apoptosis and, in fact, by investigating the occurrence of DSBs (Comet Assay) and phosphorylated histone 2AX (γH2AX), we found that a simultaneous but not a sequential exposure to UVA radiation reduced the amount of UVB radiation-induced DSBs. Further mechanistic analyses revealed that in contrast to sequential irradiation protocols, the simultaneous exposure to UVA and UVB resulted in a differential modulation of heme oxygenase-1, which is capable of modulating the activity of Ataxia telangiectasia and Rad3 related (ATR) and downstream DSB repair systems [3]. The data presented in this paper indicate that, when simultaneously applied, UVA radiation attenuates the genotoxic effects of UVB radiation and thus challenge established concepts of skin photoprotection.

References

1) Krutmann J, Bouloc A, Sore G, Bernard BA, Passeron T (2017) The skin aging exposome. J Dermatol Sci 85, 152-61.
2) Krutmann J, Sondenheimer K, Grether-Beck S, Haarmann-Stemmann T (2018) Combined, simultaneous exposure to radiation within and beyond the UV spectrum: A novel approach to better understand skin damage by natural sunlight. In: Environment and Skin. H. Merk & J. Krutmann (eds). Springer International Publishing Switzerland, ISBN: 978-3-319-43100-0.
3) Otterbein LE, Hedblom A, Harris C, Csizmadia E, Gallo D, Wegiel B (2011) Heme oxygenase-1 and carbon monoxide modulate DNA repair through ataxia-telangiectasia mutated (ATM) protein. PNAS 108, 14491-6.

IL-1.2.2

Photo-AGEs and related reactive carbonyl species are molecular mediators of skin photodamage, photoaging, and carcinogenesis

Georg T. Wondrak[1]
1) University of Arizona, Pharmacology and Toxicology, College of Pharmacy and UA Cancer Center, Tucson, Arizona, USA

Recent research indicates that solar UV, in addition to genotoxic effects, causes proteotoxic effects through formation of photodamage-associated protein-epitopes that accumulate in skin as a consequence of chemical reactions including photooxidation, lipid peroxidation, and glycation. We observed that acute exposure to sub-apoptogenic doses of solar simulated UV is sufficient to cause an almost twenty-fold increase in photodamage-associated advanced glycation endproduct protein-epitopes ('photo-AGEs') in healthy human skin. These photo-AGEs originate from carbonyl stress (mediated by reactive carbonyl species) downstream of glycation and lipid peroxidation reactions forming major lysine- and arginine-derived epitopes including CML (N^{ε}-carboxymethyl-L-lysine), DHP (1,4-dihydropyridine-3,5-dicarbaldehyde-L-lysine), and arg-pyrimidine {(2*S*)-2-Amino-5-[(5-hydroxy-4,6-dimethylpyrimidin-2-yl)amino]pentanoic acid}, respectively. Likewise, photo-AGEs accumulate in chronically sun-exposed skin in the context of photoaging. Remarkably, photo-AGEs are detectable in cutaneous precancerous lesions including actinic keratosis, and subsequent tissue microarray (TMA) analysis revealed the pronounced occurrence of photo-AGEs in human nonmelanoma and melanoma skin cancer patient tissue. Interestingly, we have now identified the topical use of sunless tanning formulations containing glycation agents including dihydroxyacetone as another significant exposure underlying epidermal photo-AGE formation. A functional role of photo-AGEs in skin inflammatory pathology and carcinogenesis is supported by the fact that these epitopes act as potent endogenous ligands of specialized damage recognition receptors including RAGE and TLR4, potentially impacting inflammatory dysregulation and both tumorigenic invasion and metastasis. Using genetic target modulation employing CRISPR/Cas9 we have also substantiated the role of glyoxalase 1 (encoded by *GLO1*, a glutathione-dependent enzyme involved in detoxification of the reactive carbonyl species methylglyoxal) in modulating cutaneous carbonyl stress, photo-AGE formation, and tumorigenesis.

Acknowledgements. Supported in part by grants from the National Institutes of Health (1R01CA229418, 1R03CA230949, 1R21ES029579, 1P01CA229112, ES007091, ES006694, and UA Cancer Center Support Grant CA023074). The content is solely the responsibility of the author and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

IL-1.2.3

UVB-induced senescence and its role in melanomagenesis

Eleni Fitsiou, Marco Demaria
European Research Institute for the biology of Ageing (ERIBA), University Medical Center Groningen (UMCG), Groningen, Netherlands

A common feature of older organisms is the accumulation of senescent cells – cells that have irreversibly lost the capacity to undergo replication. Senescent cells are characterized by an irreversible cell cycle arrest and by the Senescence-Associated Secretory Phenotype (SASP), which includes many tissue remodeling and pro-inflammatory factors. Senescent cells are intermittently present during embryogenesis and in young organisms. On the contrary, senescent cells accumulate and persist in aging tissues. Importantly, accumulation of senescence is accelerated in context of excessive genotoxic stress, such as in the skin exposed to UV radiation. Significantly, these persistent senescent cells can drive low-grade chronic inflammation, and their genetic or pharmacological elimination is sufficient to delay tissue dysfunciton. Here, I will discuss recent insights on how UVB can promote skin senescence, and general phenotypes of UVB-induced senescent cells. I will also describe the mechanisms by which accumulating dermal senescent cells might promote melanoma initiation and progression, and the potential to reduce UVB-associated melanoma by targeting senescent cells.

Acknowledgements. This work was supported by a grant from the Dutch Cancer Foundation (KWF).

OC-1.2.4

Cellular response of human keratinocytes to a photo-pollution stress

Alexe Grenier[1,2], Patrick J Rochette[1,3,*], Roxane Pouliot[1,2,*]
*1) Centre de Recherche en Organogénèse Expérimentale de l'Université Laval/LOEX, Centre de recherche du CHU de Québec-Université Laval, Québec, QC, G1J 1Z4, Canada; 2) Université Laval, Faculté de pharmacie, Québec, QC, G1V 0A6, Canada; 3) Université Laval, Département d'ophtalmologie et ORL-chirurgie cervico-faciale, Faculté de médecine, Québec, QC, G1V 0A6, Canada; *These authors contributed equally to this work.*

Skin aging is a natural process affected by several environmental factors, such as sun exposure and cigarette smoke[1-3]. Studied separately, these two extrinsic factors are well-known to accelerate skin aging. However, their combined effects have been poorly characterized. Those factors could although interact with one another in the skin since cigarette smoke components can accumulate in the skin by contact or by systemic effect after inhalation, and solar rays can penetrate the epidermis and dermis. The aim of this study was to evaluate the cellular response of keratinocytes to a photo-pollution stress generated by cigarette smoke and sunlight. A cigarette smoke extract (CSE) was obtained by capturing the soluble fraction of cigarette smoke using an impinger and cigarettes (3R4F). Sun exposure was performed using a solar simulator equipped with a CGA-345 filter (Newport) to block wavelengths under 340 nm. Irradiation doses were 14.5 and 29 kJ/m^2 of UVA, representing 15 and 30 minutes of zenith sun exposure. Firstly, the CSE absorption spectrum and total antioxidant capacity (TAC) were assayed. The cellular response of keratinocytes was studied using ROS specific fluorescent markers, and MTS to evaluate cellular metabolic activity. The implication of metabolic enzymes was assessed with RT-qPCR. The characterization of the CSE confirmed that it absorbs UV and visible light wavelengths, and the TAC test revealed an increasing antioxidant capacity with increasing concentrations of CSE. Photo-oxidation results indicate a decrease in type I ROS and a slight increase in type II ROS. CSE and solar irradiation alone showed no cytotoxicity up to the maximal doses tested. However, CSE was highly phototoxic when irradiated. Indeed, when exposed to 5% CSE at 14.5 kJ/m^2 and 29 kJ/m^2 UVA, keratinocytes showed respectively a 39 ± 1% and 2 ± 17% cellular viability (p value < 0.001). Our results showed that the photo-polluting stress causes a photo-oxidation and a decrease in cell viability on human keratinocytes.

References

1) Krutmann, J., et al., The skin aging exposome. Journal of Dermatological Science, 2017.
2) Xu, Y.; Fisher, G. J., Ultraviolet (UV) light irradiation induced signal transduction in skin photoaging. Journal of Dermatological Science Supplement, 2005.
3) Morita, A., Tobacco smoke causes premature skin aging. Journal of Dermatological Science, 2007.

Acknowledgements. This work was supported by grant from the Canadian Institutes of Health Research (CIHR) (MOP-311262). The student also received studentships from the "Fonds de Recherche Québec-Santé" (FRQS).

IL-1.2.5

Skin Photoaging: a stress-induced cognitive misleading

Mauro Picardo, Enrica Flori, Federica Papaccio, Daniela Kovacs, Stefania Briganti, Giorgia Cardinali, Barbara Bellei

San Gallicano Dermatological Institute, IRCCS, Cutaneous Physiopathology, Rome, Italy

Skin photoaging blends with the process of intrinsic aging. The severity of photodamage depends on both constitutional factors, such as skin phototype, intensity, and duration of sunlight/UV exposure, and the impact of extrinsic factors, collectively called exposome, including, pollution, smoke or heat, nutrition, and stress, resulting in an increased senescent cells number. Aging affects nearly every aspect of cutaneous biology, including pigmentation. Clinically, the phenotype of older pigmented skin has a mottled, uneven color, primarily due to "age spots", with or without hypopigmentation. Uneven pigmentation might be attributed to the hyperactivation of melanocytes, altered distribution of pigment, and turnover. Various skin cells play a role in inducing abnormal pigmentation, with an aberrant paracrine regulation that impacts melanocyte function, and results in the formation of hyperpigmented lesions, such as solar lentigo and melasma. Not only keratinocytes and fibroblasts but also endothelial cells and sebocytes, as well as immune inflammatory cells, contribute to the pigmentary changes associated with aging. The generation of inflammatory mediators and alteration in growth factor production from the different cellular components is a biological marker of photo-damaged skin which produces a cognitive misunderstanding[1]. Similar biological characteristics can be detected in non-melanoma skin cancer-associated fibroblasts. Interestingly, age-independent, diffuse expression of senescence-associated markers in the dermal and epidermal compartment is also associated with vitiligo, suggesting that premature senescence plays an important role in the pathology [2,3].

References

1. Kang HY, Lee JW, Papaccio F, Bellei B, Picardo M. Alterations of the pigmentation system in the aging process Pigment Cell Melanoma Res. 2021 May 28. doi: 10.1111/pcmr.12994.

2. Seneschal J, Boniface K, D'Arino A, Picardo M. An update on Vitiligo pathogenesis Pigment Cell Melanoma Res. 2021 Mar;34(2):236-243

3. Bellei B, Migliano E, Picardo M. A Framework of Major Tumor-Promoting Signal Transduction Pathways Implicated in Melanoma-Fibroblast Dialogue. Cancers (Basel). 2020 Nov 17;12(11):3400

IL-1.2.6

Relationship between intrinsic and extrinsic skin aging

S. Schneider[1], M. Majora[1], S. Faßbender[1], M. Pollet[1], A. Marini[1], J. Hüsemann[1], M. Knechten[1], H. Schwender[2], J. Krutmann[1]
1) IUF - Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany; 2) Heinrich Heine University Duesseldorf, Mathematical Institute, Duesseldorf, Germany

Skin aging is principally driven by the combination of intrinsic (e.g. genetic constitution) and extrinsic factors (e.g. solar radiation). It is generally assumed that extrinsic skin aging superimposes intrinsic skin aging, nevertheless scientific evidence for this assumption is scarce. To assess this relationship more closely, we have focused on dermal fibroblasts isolated from intrinsically ($NHDF^{INT}$) *vs* extrinsically aged skin ($NHDF^{EXT}$) of young (Y; 20-25 yr.), middle-aged (M; 36-49 yr.) and old (O; 60-64 yr.) female volunteers. Proteome analysis via quantitative, label-free mass spectrometry revealed fundamentally different age-dependent trajectories of protein levels in $NHDF^{INT}$ *vs* $NHDF^{EXT}$. Accordingly, $NHDF^{INT}$ exhibited gradual, almost linear changes (Y<M<O and Y>M>O). In contrast, $NHDF^{EXT}$ showed a non-linear kinetic with minor alterations in Y *vs* M probands (Y=M), but a marked difference between M *vs* O donors (M<<O and M>>O) which outreached the changes found in $NHDF^{INT}$. For example, tricarboxylic acid (TCA) cycle-related proteins were increased in $NHDF^{EXT}$ *vs* $NHDF^{INT}$ of M donors. At the same time, ADP/ATP ratios were elevated, NAD^+ levels were reduced and histone deacetylation through NAD^+-dependent sirtuin-1 (SIRT1) was compromised. These observations indicate that $NHDF^{EXT}$, in contrast to $NHDF^{INT}$, show a compensatory behaviour to cope with an energy deficit. However, when $NHDF^{EXT}$ of M *vs* O donors were compared, $NHDF^{EXT}$ of O donors seemed to have passed a 'tipping point', at which compensatory mechanisms failed. Accordingly, we observed a dramatic decline of TCA cycle-associated proteins accompanied by a severe reduction of protein diversity and protein biosynthesis, i.e. two biological processes known to consume vast amounts of ATP. Of note, treatment of $NHDF^{EXT}$ of M donors with the NAD^+ precursor β-nicotinamide mononucleotide (NMN) could rescue their mitochondrial phenotype and SIRT1-dependent histone deacetylation. These studies demonstrate that the relationship between intrinsic and extrinsic skin aging might be more complex than a simple superimposition, because age-dependent proteomic/metabolic changes in $NHDF^{EXT}$ follow a substantially different kinetic compared to $NHDF^{INT}$. They identify restricted availability of NAD^+ as a factor driving metabolic/epigenetic alterations contributing to extrinsic skin aging.

IL-1.2.7

Epilipidomic signatures of senescence and UV stress

Florian Gruber[1,2,3], Marie-Sophie Narzt[1,3], Vera Pils[3,4], Christopher Kremslehner[1,2,3], Martina Marchetti-Deschmann[2,5], Samuele Zoratto[2,5], Markus Schosserer[2,4], Ingo Lämmermann[3,4], Johannes Grillari[3,4,6]
1) Medical University of Vienna, Department of Dermatology, Vienna, Austria; 2) Christian Doppler Laboratory for Skin Multimodal Analytical Imaging of Aging and Senescence – SKINMAGINE-; 3) Christian Doppler Laboratory for Biotechnology of Skin Aging, Vienna, Austria; 4) University of Natural Resources and Life Sciences Vienna, Department of Biotechnology, Vienna, Austria; 5) Institute of Chemical Technologies and Analytics, TU Wien, Vienna, Austria; 6) Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Vienna, Austria

During aging, skin accumulates senescent cells. The transient presence of senescent cells, followed by their clearance by the immune system, is important in tissue repair and homeostasis. The persistence of senescent cells that evade clearance contributes to the age-related deterioration of the skin. The senescence-associated secretory phenotype of these cells contains immunomodulatory molecules that facilitate clearance but also promote chronic damage. Ultraviolet light is the dominant environmental oxidative skin stressor and a major skin aging factor. We have been studying which oxidized phospholipid (OxPL) mediators (in their totality, the epi phospho-lipidome) would be generated in human keratinocytes (KC) and fibroblasts (FB) upon exposure to ultraviolet radiation and investigated the contribution of OxPL to stress responses. We identified known and novel lipid species including known bioactive and also potentially reactive carbonyl containing species. We found indication for selective metabolism and degradation of selected reactive lipids. Exposure to both UVA and to in vitro UVA - oxidized phospholipids activated, on transcriptome and proteome level, NRF2/antioxidant response signaling, lipid metabolizing enzyme expression and unfolded protein response (UPR) signaling.
Furthermore, we have investigated the epilipidome of senescent dermal fibroblasts, because these molecules are among the bioactive lipids that were recently identified as senescence-associated secretory phenotype factors. Using replicative- and stressinduced senescence protocols, we identified lysophosphatidylcholines as universally elevated in senescent fibroblasts, whereas other oxidized lipids displayed a pattern that was characteristic for the used senescence protocol. When we tested the lysophosphatidylcholines for senescence-associated secretory phenotype activity, we found that they elicit chemokine release in nonsenescent fibroblasts but also interfere with toll-like receptor 2 and 6/CD36 signaling and phagocytic capacity in macrophages.
Our data suggest that oxidized lipids can act as signaling mediators of extrinsic and intrinsic (senescence associated) stress / SASP, and that the composition of the epilipidome influences the signaling and immunomodulatory events associated with these cellular states in a fashion that is more specific than anticipated. Furthermore, we have begun to localize these signaling mediators in human skin using mass spectrometry imaging, which allows us to understand spatial distributions of these novel signaling compounds the UV response and aging.

References
1) Narzt, et al. “A novel role for NUPR1 in the keratinocyte stress response to UV oxidized phospholipids”, Redox Biology, 2019
2) Narzt, et al. “Epilipidomics of Senescent Dermal Fibroblasts Identify Lysophosphatidylcholines as Pleiotropic Senescence-Associated Secretory Phenotype (SASP) Factors”, J. Invest. Dermatol. 2021

Acknowledgements. The work of the CDLs is funded by the Federal Ministry for Digital and Economic Affairs (BMWFW) of Austria and the National Foundation for Research, Technology, and Development of Austria and by CHANEL Parfums et Beauté and the Herzfelder'sche Familienstiftung, Austria

OC-1.2.8

Infrared and visible light in combination with UV increase skin cell stress markers

Catherine A Bonn[1], L. Hudson[1], E. Rashdan[1], Bhaven Chavan[2], Mark Birch-Machin[1]

1) Newcastle University, Institute of Translational and Clinical Research, Newcastle-upon-Tyne, UK;
2) Croda Europe Ltd, Snaith, East Yorkshire, UK

Introduction

While it is known that Ultraviolet (UV) light can have detrimental impacts to skin ageing, less is known about the impacts that longer wavelengths have on skin biology. The impacts of infrared (IR) and visible lights on skin are clearly different to that of UV, but still seem to affect the response of skin to incident light. While some have found that IR alone can cause damage to skin cells[1], in the experiments detailed here we have found that in combination with UV and visible light it can increase damage markers in human skin cells[2].

Methods

Human skin fibroblast cell lines or primary fibroblasts and keratinocytes were cultured and irradiated with solar-simulated light or an IR lamp. In the "UV+Vis+IR" condition, cells were dosed with 2.16 Standard Erythemal Doses of UV, equivalent to 2 hours of Mediterranean, noon, midsummer sun, with visible and infrared light also present from the Newport Solar Simulator. In the UV-only treated cells, the cells were exposed to the same solar simulator but with a filter allowing only the UV light through. The IR-only treated cells were irradiated with a dose of equivalent to a 2 hour dose of light from a Hydrosun IR lamp. Assays included ROS detection with ROS-Glo, mitochondrial DNA damage with long-range PCR and nuclear DNA damage with comet assays.

Results

UV light increases damage biomarkers such as ROS generation by at least 44% in both primary fibroblasts and keratinocytes, (measured by ROS-Glo, $P<0.05$). In comparison, infrared light alone had no significant effect on either measure. However, when treated with UV, visible and IR light, the ROS increased by 168%, though only in fibroblasts ($P<0.05$). In keratinocytes, the additional visible and infrared lights had no effect, and the ROS generation was not significantly different to that in UV-only irradiated cells.

This pattern of increased damage biomarkers in fibroblasts but not keratinocytes was found to be the same when mitochondrial DNA damage was assayed. In fibroblasts, the damage in mitochondrial DNA when visible and IR light were present was 32-fold higher than in unirradiated cells, whereas it was only 8-fold higher in cells irradiated with only UV. In keratinocytes the damage was similar in both conditions (3.8 and 4.7-fold respectively). The pattern was the same in nuclear DNA damage, and was reflected in fibroblast and keratinocyte cell lines.

Furthermore, the sum of damage from the constituent parts of the solar light did not add up to the effect of UV, visible and IR lights together, indicating a possible synergy of effects on skin.

Conclusion

Infrared and visible light seem to contribute to skin damage from light containing be able to affect the biological damage caused by ultraviolet light, potentially synergistically increasing skin damage.

References

1. Schroeder, P., et al., *Cellular response to infrared radiation involves retrograde mitochondrial signaling.* Free Radical Biology and Medicine, 2007. **43**(1): p. 128-135.
2. Hudson, L., et al., *Individual and combined effects of the infrared, visible, and ultraviolet light components of solar radiation on damage biomarkers in human skin cells.* The FASEB Journal, 2020. **34**(3): p. 3874-3883.

P-1.2.9

Photoprotective efficacy of PLGA-Curcumin versus PLGA-Piperine under ambient UV-B and Sunlight Exposure

Sunil Kumar Patel[1,2], Lipika Ray[3], Ratan Singh Ray[4], Ashish Dwivedi[1,2]*
1) Food, Drug & Chemical Toxicology Group, CSIR-IITR, Lucknow; *2) Academy of Scientific and Innovative Research (AcSIR), CSIR-IITR Campus, Lucknow*; *3) Pharmaceutics & Pharmacokinetics Division, CSIR-CDRI, Lucknow*; *4) System Toxicology and Health Risk Assessment Group, CSIR-IITR, Lucknow*.

Due to pollution and depletion of stratospheric ozone the intensity of UV-R has been augmented in tropical and sub-tropical countries. Chronic exposure of UV-R promotes skin damage, skin aging and skin cancer. Phytochemicals are promising agents to counteract the negative impact of UV-R by quenching the ROS or upregulating the molecular expression of cellular anti-oxidant enzymes. But photoinstability is major concern which limits its long time protection efficacy under UV-R and Sunlight. Here, we have taken two very important phytochemicals i.e., curcumin and piperine and their nanoformulations for photostability and photoprotection under UV-B and Sunlight. Our photodegradation results suggest that both the phytochemicals are degraded under UV-B and Sunlight. However, nanoformulations are stable under both UV-B and Sunlight exposures. *In-vitro* result also suggests that nanoformulations have more protective efficacy than normal forms. Moreover, comparison to curcumin-PLGA, piperine-PLGA provides strong protection against UV-B and Sunlight mediated damage. These results suggest that PLGA-piperine has strong photoprotection efficacy and may be used in preparations of sunscreens and other skin protecting creams.

Keywords: Phototoxicity; Photodegradation; Nanoformulation; Curcumin; Apoptosis/Necrosis; Photoprotection.

Acknowledgements. This work was supported by CSIR-IITR, Lucknow and UGC New Delhi.

***Corresponding Author: Dr Ashish Dwivedi**

Food, Drug and Chemical Toxicology Group,
CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Vishvigyan Bhawan 31,
MG Marg, Lucknow, Uttar Pradesh-226001, India
Telephone: +917897944888,
E-mail: ashish.dwivedi1@iitr.res.in

IL-1.3.1

Photoantimicrobials: What we know and do not know!

Tim Maisch[1]

1) **Department of Dermatology, University Hospital Regensburg, Germany**

The worldwide and continuous increase in antibiotic resistance requires alternative strategies to combat bacteria. A promising complement to the conventional use of antibiotics is the principle of antimicrobial photodynamic inactivation. In this procedure, a per se non-toxic dye, so-called photoantimicrobial[1], are excited with visible light in the presence of oxygen. Thereby it produces reactive oxygen species that irreversibly cause an oxidative damage to biological molecules, which enables the non-specific killing of bacteria, including multi-resistant pathogens. In this talk the current knowledge about antimicrobial photodynamic inactivation will be discussed.

References

1) Mark Wainwright, Tim Maisch, Santi Nonell, Kristjan Plaetzer, Adelaide Almeida, George P Tegos, Michael R Hamblin, Photoantimicrobials—are we afraid of the light? Lancet Infect Dis. 2017 Feb; 17(2): e49–e55.

IL-1.3.2

Adding Photodynamic Inactivation to the farmer's armamentarium against phytopathogens

Michael Glueck[1], Christoph Hamminger[1], Annette Wimmer[1], Michael Fefer[2], Jun Liu[2], Kristjan Plaetzer[1]
1) University of Salzburg, Department of Biosciences, Laboratory of Photodynamic Inactivation of Microorganisms, Salzburg, Austria; 2) Suncor AgroScience, Mississauga Ontario, Canada

Access to sufficient and healthy food is a fundamental human right. Crop production has essential importance for food safety, but yields are threatened by plant pathogens. This study investigates the efficacy of Photodynamic Inactivation (PDI) based on two chlorin e6 derivatives, Sodium-Magnesium-Chlorophyllin (Chl, E140) and the cationic B17-0024 against bacterial and fungal plant pathogens, both, *in vitro* and *in situ*.
PDI against the Gram(+) phytopathogens *Rhodococcus fascians* and *Clavibacter michiganensis* as well as Gram(-) *Erwinia amylovora* - susceptible and resistant to streptomycin - and *Xanthomonas axonopodis* was performed in liquid culture and using illumination with 395 nm LED light (26.6 J/cm^2) or natural sunlight. CFU counting was done 24/48 h after phototreatment. The PDI efficiency was also tested on *Solanum lycopersicum* (tomato) plants, which were contaminated with *C. michiganensis*. For the antifungal PDI against *Alternaria solani* and *Botrytis cinerea* the growth of mycelial patches after photoactivation (395 nm, 106.6 J/cm^2) of either Chl or B17-0024 was determined seven days post treatment. Gram(-) and fungal phytopathogens were incubated with Chl in combination with a cell wall permeabilizing agent. To investigate the effect of the PDI-treatment to host plants the growth of *Fragaria vesca* (strawberry, BBCH stage 14) inoculated with the photosensitizers with or without additives was monitored for 14 days.
Both compounds photokill (inactivation >6 $\log_{10}$ steps) *R. fascians* and *C. michiganensis* at 100 µM concentration. Photoinactivation (>7 $\log_{10}$) of Gram(-) bacteria with 100 µM Chl requires addition of Na_2EDTA (*X. axonopodis*) or polyaspartic acid (*E. amylovora*).[1] The cationic B17-0024 is phototoxic against both Gram(-) species at 10/100 µM without additives. *E. amylovora* resistant to streptomycin was photokilled with an efficiency comparable to the wild type, and addition of 100 µg/ml streptomycin further enhanced the cytotoxicity. Sunlight activation induces an antibacterial effect towards *C. michiganensis* after 30 minutes *in situ* on *S. lycopersicum* leaves (100 µM B17-0024 integrated radiant exposure 64.1 J/cm²) and *E. amylovora* in liquid suspension after two hours (100 µM B17-0024, average irradiance 28 mW/cm²). Photoinactivation of *C. michiganensis* inoculated on *S. lycopersicum* plants is effective (> 5 log steps reduction) using 100 µM B17-0024 (395 nm, 26.6 J/cm²). Both chlorin e6 derivatives also act as very effective photofungicides, if Chl is applied with a cell wall permeabilizing compound and B17-0024 without any additives. The PDI-treatment had no effects on the development and growth of *F. vesca*.
PDI based on the two chlorin e6 derivatives tested in this study is effective in killing bacterial and fungal phytopathogens, both, *in vitro* and *in situ* and irrespective of resistance to conventional treatment. As no negative effects to the host plants have been observed so far, the photodynamic approach will add to the grower's toolbox to minimize crop losses.

References
1) M. Glueck, C. Hamminger, M. Fefer, J. Liu and K. Plaetzer, Save the crop: Photodynamic Inactivation of plant pathogens I: bacteria. Photochemical & Photobiological Sciences 18(7): 1700-1708, 2019.

Acknowledgements. The authors are grateful to Suncor AgroScience, Mississauga Ontario, Canada for support.

IL-1.3.3

Anti-bacterial and anti-virulent photoinactivation of *Staphylococcus aureus* isolated from atopic dermatitis patients: an *in vitro* and *in vivo* approach.

Joanna Nakonieczna[1]

1) Laboratory of Molecular Diagnostics, Intercollegiate Faculty of Biotechnology of University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease. It affects 5-20% of children worldwide and 1-3% of adults. The literature describes the phenomenon of dysbiosis with respect to the skin of patients with AD. This state is defined as a change in the composition of the normal flora on the patient's skin. In particular, an increase in skin colonization of *S. aureus* species was observed in AD patients. The prevalence of *S. aureus* on atopic skin is 80-100%, whereas in healthy populations it is estimated to be 5-20%. Currently, AD treatment is primarily based on anti-inflammatory treatment with the use of topical corticosteroids (TCSs) and calcineurin inhibitors (TCI) depending on the disease severity[1]. Additionally, antimicrobial therapy in each exacerbation of AD can be applied, mainly with the use of mupirocin, fusidic acid, retapamulin. However, prolonged use of antibiotic is not recommended due to significant rise in resistance. Published data suggest that the increased severity of AD is attributed to *S. aureus* secreted virulence factors with superantigen properties. The best studied superantigens are staphylococcal enterotoxins (SEA, SEB, SEC, SED) and toxic shock syndrome toxin-1 (TSST-1). Several studies indicated a direct correlation between the occurrence of superantigens-producing *S. aureus* and severe AD. In the *in vitro* conditions we verified the hypothesis that numerous strains of AD-derived *S. aureus* and the staphylococcal virulence factors can be effectively photoinactivated, including those of MRSA strains. We checked the effect of aPDI on the studied virulence factors at RNA and protein levels, which revealed interesting differences in response to aPDI among various SEs. We found such conditions of photoinactivation wherein the host skin cells remained intact, while effectively destroying bacterial cells and virulence factors produced. Currently, we are developing *in vivo* model of atopic-like skin colonization by MRSA bioluminescent strain to assess the efficacy of decolonization of lesional and non-lesional areas of skin. The results obtained so far allow to propose aPDI as a good candidate complementary method of skin decolonization and inactivation of virulence factors exacerbating flares of AD.

References

1) Ogonowska P, Gilaberte Y, Baranska-Rybak W, Nakonieczna J, Colonization With *Staphylococcus aureus* in Atopic Dermatitis Patients: Attempts to Reveal the Unknown, Frontiers in Microbiology, 2021.

Acknowledgements. This work was supported by grant no. 2017/27/B/NZ7/02323 from the National Science Centre, Poland.

OC-1.3.4

aPDT Activity of Cationic Copolymers with Dual Function: Synergistic Effect of Poly(oxanorbornene) and Phthalocyanine

Erem Ahmetali[1], Pinar Sen[2], N. Ceren Süer[1], Burak Aksu[3], Tebello Nyokong[2], Tarik Eren[1], M. Kasım Şener[1]

1) Yıldız Technical University, Department of Chemistry, Istanbul 34210, Turkey ; 2) Rhodes University, Department of Chemistry, Grahamstown 6140, South Africa ; 3) Marmara University, Department of Medical Microbiology, Istanbul 34854, Turkey

In today's world, microorganisms' infections are serious problems for public health. The researchers have focused how to overcome the problem of microbial drug resistance in recent years[1]. Antimicrobial photodynamic therapy (aPDT) is an alternative method for photo-inactivation of microorganisms through cytotoxic species produced in conjunction with photosensitizer (PS) and light. Phthalocyanine compounds are especially known as potential PS that can produce high singlet oxygen in photodynamic process[2].
On the other hand, amphiphilic poly(oxanorbornene)s are promising synthetic polymers that can show high antimicrobial functions against bacteria. Although different antimicrobial action mechanisms have been proposed for amphiphilic poly(oxanorbornene)s, it is thought that the electrostatic interaction and the hydrophobic interaction provide a lethal effect[3].

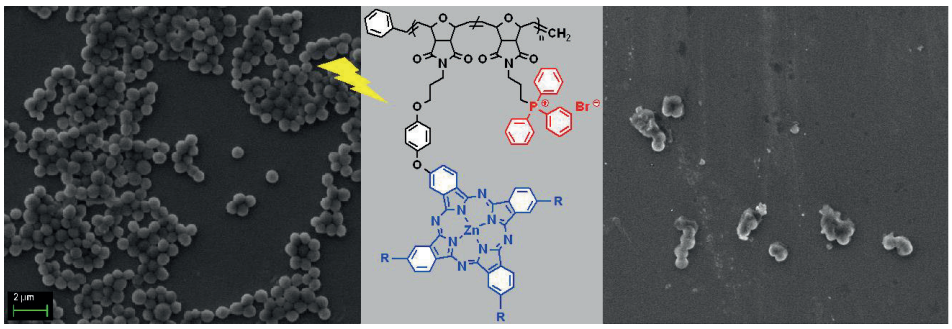


Fig. 1 – Representation of Enhanced Light-Driven Antimicrobial Activity

In this study, poly(oxanorbornene)s bearing zinc(II) phthalocyanine were synthesized to combine the antimicrobial mechanisms of cationic poly(oxanorbornene) with aPDT activity of zinc(II) phthalocyanine. The results suggest that synergistic effect of Zn(II) phthalocyanine-triphenyl phosphonium based copolymer caused complete damage of bacterial strain with multifunctional mechanism, i.e., electrostatic interaction, hydrophobic interaction and the formation of reactive oxygen species[4].

References

1) M. L. Agazzi, M. B. Ballatore, A. M. Durantini, E. N. Durantini, A. C. Tome, BODIPYs in antitumoral and antimicrobial photodynamic therapy: An integrating review. J. Photochem. Photobiol. C., 40, 21, 2019.
2) P. Sen, A. Sindelo, D. M. Mafukidze, T. Nyokong, Synthesis and photophysicochemical properties of novel axially di-substituted silicon (IV) phthalocyanines and their photodynamic antimicrobial chemotherapy (PACT) activity against Staphylococcus aureus. Synth. Met, *258*, 116203, 2019.
3) N. C. Süer, C. Demir, N. A. Ünübol, Ö. Yalçın, T. Kocagöz, T. Eren, Antimicrobial activities of phosphonium containing polynorbornenes. RSC Adv., 6, 86151, 2016.
4) E. Ahmetali, P. Sen, N. C. Süer, B. Aksu, T. Nyokong, T. Eren, Enhanced light-driven antimicrobial activity of cationic poly(oxanorbornene)s by phthalocyanine incorporation into polymer as pendants. Macromol. Chem. Phys., 221, 2000386, 2020.

Acknowledgements. The authors would like to acknowledge the financial support from The Scientific and Technological Research Council of Turkey (TÜBİTAK) (Project Number: 219Z271).

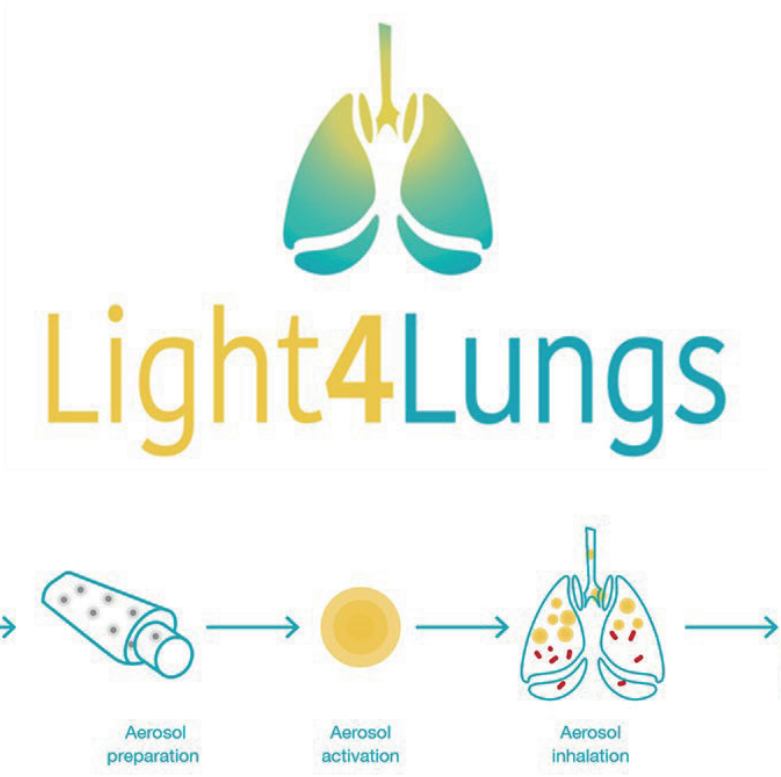
IL-1.3.5

LIGHT4LUNGS: Inhalable Aerosol Light Source for Controlling Drug-Resistant Bacterial Lung Infections

Santi Nonell, on behalf of the Light4Lungs Group
S. Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain; G. Romano, University of Florence, Florence, Italy; N. Henry, Sorbonne Université, Paris, France; E. Palomares, Fundacio Privada Institut Catala d'Investigacio Quimica, Tarragona, Spain; E. Reddi, University of Padova, Padova, Italy; G. Taylor, Cardiff Scintigraphics Ltd, Cardiff, UK; A. Kadioglu, The University of Liverpool, Liverpool, UK

Light4Lungs addresses the problem of antimicrobial resistance in the treatment of chronic lung infections, the leading cause of morbidity and mortality in patients with diseases such as cystic fibrosis and hospital-acquired lung infections. The project aims to develop an alternative therapeutic scheme for the treatment of these infections: a novel inhalable photodynamic therapy whereby antibiotics are replaced by inhalable light sources that will excite bacterial endogenous photosensitizers, eliminating the pathogenic bacteria by the photodynamic effect irrespective of their multidrug resistance profile.

The project encompasses the development of breathable particles with persistent luminescence, the aerosol technology for activation and delivery to the lungs, and the definition of the treatment parameters through toxicity and efficacy tests in clinically relevant models of respiratory infections. Its results have the potential to go beyond treatment of recalcitrant respiratory tract bacterial infections to other lung diseases and to other organs, enriching fields of healthcare, nanomedicine, materials science, and nanotechnology.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement nº 863102

OC-1.3.6

Photodynamic inactivation of pathogenic bacteria to decontaminate surfaces in food industry

Larissa Kalb[1], Bernd Kramer[2], Peter Muranyi[2], Wulf Schneider-Brachert[3], Wolfgang Bäumler[1]

[1]Department of Dermatology, University of Regensburg, Germany, [2]Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany, [3]Department of Infection Control and Infectious Diseases, University of Regensburg, Germany

Introduction Despite established standards of cleaning and disinfection, decontamination of environmental surfaces remains a big challenge in food processing. Contaminated surfaces in meat production are a source of bacteria transmission from surface to surface of different equipment (e.g. conveyor belts, housings). The photodynamic procedure is a promising, new technology that uses a special dye molecule (photosensitizer). Upon visible light exposure, a photosensitizer generates reactive oxygen species, in particular singlet oxygen, which inactivates bacteria regardless of its type and its resistance against diverse disinfection strategies. The photodynamic procedure can be applied in suspension[1] and as antimicrobial coating of surfaces.[2] In the current study, the inactivation of food relevant bacteria *Salmonella enterica* and *Listeria monocytogenes* was investigated using different photosensitizers in suspension, antimicrobial coatings, and in micro-emulsions.

Material and Methods Overnight cultures of *L. monocytogenes* DMSZ 15675 or *S. enterica* DMSZ 11320 were re-suspended to achieve up to 10^8 bacteria per mL. Curcumin, flavin, or perinapthenone photosensitizers were added to suspension or micro-emulsions to yield concentrations of up to 500 µM. In case of the coating, photosensitizers were added to a lacquer material that was applied to PU-plastic (conveyor belt) or stainless steel (housings) samples. Photosensitizers and coatings were purchased from TriOptoTec GmbH, Germany. In case of surface experiments, 50 µL of bacteria suspension was applied as droplets on the surface, allowing bacteria to dry for 60 minutes. Different concentrations of protein and blood components were added to simulate the organic burden in meat processing. Photosensitizers were activated by visible blue light of LEDs (DELO GmbH, Germany) at different light intensities and irradiation times. After irradiation, the number of viable cells was determined by plating bacteria on agar and counting the colony forming units. The results were calculated as $\log_{10}$ reduction using internal references. Experiments were repeatedly performed yielding mean values ± standard deviation.

Results Regardless of the photosensitizer used, *L. monocytogenes* and *S. enterica* could be inactivated in suspension experiments with increasing concentration of the respective photosensitizer. The maximal antimicrobial efficacy showed the perinapthenone photosensitizers with a reduction of up to 6.8 $\log_{10} \pm 0.1$. After inoculation of bacteria on coated stainless steel or PU-plastic, bacteria were inactivated on the surface upon light exposure yielding a reduction of maximal 4.8 ± 0.8 $\log_{10}$. After inoculation of bacteria on PU-plastic samples, the micro-emulsion containing photosensitizer (perinapthenone or curcumin) was sprayed on the contaminated surfaces. Irradiation of sample surfaces for five seconds could reduce the number of bacteria by 3.9 ± 0.8 $\log_{10}$.

Discussion The results of these experiments provide evidence that the photodynamic technology enables a very efficient inactivation of typical food pathogens. On one hand, the antimicrobial coating can permanently and autonomously inactivate bacteria on surfaces of food processing equipment like housings and conveyor belts. On the other hand, the antimicrobial micro-emulsion can be sprayed on surfaces of food processing equipment to inactivate food pathogens. The micro-emulsion contains only water, food or cosmetic approved emulsifiers, and harmless photosensitizer molecules, which are derivatives of bio-molecules or spices. The biocidal agent, which oxidatively inactivates the bacteria, is mainly the short-lived singlet oxygen that is generated *in situ* upon exposure of photosensitizers to visible light.

References

[1] Schreiner M, Bäumler W, et al, Photodynamic inactivation of bacteria to decolonize meticillin-resistant Staphylococcus aureus from human skin, Br J Dermatol. 179(6):1358-1367, 2018

[2] Eichner A, Holzmann T, et al Novel photodynamic coating reduces the bioburden on near-patient surfaces thereby reducing the risk for onward pathogen transmission: a field study in two hospitals, J Hosp Infect 104(1):85-91, 2020

Acknowledgement. This work was supported by grant 281C101C18 from the Federal Ministry for Agriculture and Food, Germany

OC-1.3.7

How long *Escherichia coli* can resist a resistance to antimicrobial blue light (aBL) treatment?

Aleksandra Rapacka-Zdończyk[1], Agata Woźniak[2], Beata Kruszewska[1,2], Mariusz Grinholc[2] and Krzysztof Waleron[1]

1) *Department of Pharmaceutical Microbiology, The Faculty of Pharmacy Medical University of Gdańsk, Poland;*
2) *Laboratory of Molecular Diagnostics at Department of Biotechnology, Intercollegiate Faculty of Biotechnology University of Gdańsk & Medical University of Gdańsk, Poland*

Antimicrobial blue light (aBL) is considered a low risk treatment for the development of bacterial resistance or/and tolerance due to their multi-targeted modes of action. In our previous published studies[1] we assessed the tolerance development to aBL by several Gram positive species, including *Staphylococcus aureus, Enterococcus faecium* and *Streptococcus agalactiae*.

The aim of our studies was to demonstrate whether tolerance development occurs in Gram negative bacteria. The application of the exact protocol[2] for the evaluation of resistance development as well as the investigation of *umuC* and *recA* mutants of *Escherichia coli* K-12 demonstrated that representative of Gram negative bacteria also may develop tolerance to the aBL.

Reference strain of *Escherichia coli* K-12 was used in the experiments. In the studies cells were irradiated with blue (415 nm) light. 15 repeated cycles of sub-lethal photoinactivation was followed by bacteria re-growth overnight. A potential reduction in susceptibility was examined after 5th, 10th, and 15th consecutive cycles at higher light doses. Reduction in susceptibility has been observed since the 5th cycle.

Lack of tolerance development in *recA*- lacking *Escherichia coli* K-12 mutants[3] indicates a possible mechanism of tolerance development. Our studies indicate that aBL tolerance development is *recA*-dependent process confirming that photoinduced DNA damage occur under aBL treatment. However, aBL tolerance development demonstrated to be umuC-independent process (contrary to *S. aureus*). The acquired tolerance was stable in the case of the wild type strain and the *umuC* mutant.

References

1) Rapacka-Zdonczyk, A., et al. "Development of Staphylococcus aureus tolerance to antimicrobial photodynamic inactivation and antimicrobial blue light upon sub-lethal treatment." Scientific reports 9.1 (2019): 1-18.

2) Rapacka-Zdonczyk, A., Wozniak, A., Nakonieczna, J., & Grinholc, M. (2021). Development of Antimicrobial Phototreatment Tolerance: Why the Methodology Matters. International Journal of Molecular Sciences, 22(4), 2224.

3) Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., ... & Mori, H. (2006). Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Molecular systems biology, 2(1), 2006-0008.

Acknowledgements. This work was supported by grant No. 2020/36/C/NZ7/00061 from Polish National Science Center; *E. coli* single-gene mutants were used courtesy of National BioResource Project (NIG, Japan).

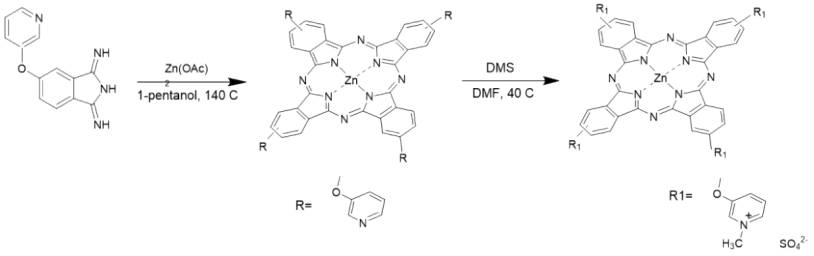
OC-1.3.8

Cationic Phthalocyanine Palladium complexes for Photodynamic Inactivation of Pathogenic Bacteria on Farm Fishes

Vanya Mantareva[1], Vesselin Kussovski[2], İrem Kulu[3], Ivan Angelov[2], Mahmut Durmuş[3], Petya Orozova[4]

[1]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Bld. 9, 1113 Sofia, Bulgaria; [2]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Bld. 26, 1113 Sofia, Bulgaria; [3]Gebze Technical University, Department of Chemistry, P.O. Box 141, Gebze 41400, Kocaeli, Turkey; [4]National Reference Laboratory for Fish, National Diagnostic Science and Research Veterinary Medical Institute, 1000 Sofia, Bulgaria

The increasing drug resistance of pathogenic bacteria leads to further development of new strategies to fight microbial resistance. The photodynamic inactivation (PDI) appears a prospective approach which takes action by the light activation of a proper photosensitizer in the presence of oxygen atmosphere. The effective generation of singlet oxygen predicted the efficacy of the proposed new palladium complexes for photoinactivation of the pathogenic species. The study presents the PDI efficacy of two new cationic water-soluble palladium phthalocyanines (PdPcs) which are differing in the positions of quaternized 2-hydroxypyridine groups to the Pc ring, namely on peripheral (pPdPc, **1**) and nonperipheral (nPdPc, **2**) in comparison to a highly effective methylpyridyloxy-substituted Zn(II)- phthalocyanine (ZnPcMe) which was synthesized by the well-developed procedure for quaternized metallophthalocyanines, Scheme 1).



Scheme 1. Synthesis of quaternized MPcs.

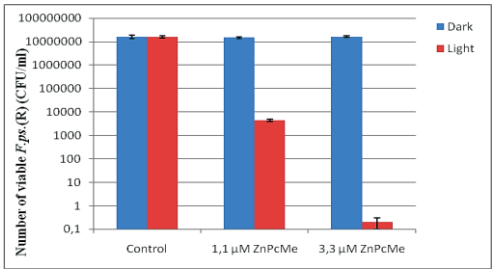


Fig. 1. PDI of *Aeromonas hydrophila (R)*.

The typical for farm fishes bacterial strains *Flavobacterium psychrophilum* is found in cold fresh waters with an optimal growth below 160 ºC and the Gram(-) *Aeromonas hydrophila* which grow at 4 ˚C, were evaluated with high sufficiency for inactivation with non-peripherally substituted Pd(II)Pc, **2** and with ZnPcMe (Fig. 1). The observations obtained for the complexes of palladium (PdPcs) suggested that metal ion can influence the photodynamic inactivation to keep under control the resistant bacterial species in the farm's fishes.

Acknowledgements. This work was supported by grant of the Bulgarian National Science Fund, KP-06-H29/11.

OC-1.3.9

Optimisation of *Streptococcus agalactiae* biofilm culture as a model for photoinactivation studies

Michał K. Pierański[1], Mariusz S. Grinholc[1]

1) Laboratory of Molecular Diagnostics, Intercollegiate Faculty of Biotechnology University of Gdansk & Medical University of Gdansk, Gdansk, POLAND

Streptococcus agalactiae is a relevant cause of neonatal mortality. About 10-30% of women are carrying *S. agalactiae* in their vaginal tract, from where it can be transferred to infant and cause among others: meningitis, pneumonia, arthritis or sepsis[1]. Serotypes the most commonly identified from vaginal tract infections are Ia, III and V[2]. It is commonly believed that the cause of therapy ineffectiveness and recurrence of infection is the growth of bacteria in the form of a biofilm. This situation also takes place in the vaginal environment[3]. So far, several teams have attempted to find a suitable medium for the cultivation of a *Streptococcus* biofilm. Among others there was used simulated vaginal fluid. However, in the case of *S. agalactiae*, biofilm production in simulated vaginal fluid is lower than in Tryptic Soy Broth[4]. We have already showed that *S. agalactiae* can be successfully eradicated with rose bengal mediated aPDI in planktonic culture[5], but there were no studies about photoinactivation of biofilm culture yet.

The aim of this study was to optimize *S. agalactiae* biofilm culture conditions, so that it could be used in photoinactivation studies.

We compared biofilm production by ATCC strain and three clinical strains representing most common serotypes in four different broths with crystal violet staining method. Then we evaluated stationary biofilm culture in microtiter plates and biofilm grown on four types of coupons with use of CDC Biofilm Reactor® in continuous flow conditions. We compared growth in CFU/ml and with confocal laser scanning microscopy. Then we applied rose bengal mediated photoinactivation on both biofilm models.

We managed to find conditions allowing for stableand repetitive *S. agalactiae* biofilm growth, what was confirmed with confocal laser scanning microscopy imaging. We believe that biofilm grown in continuous flow conditions is more similar to the one grown in natural environment and can be successfully utilized in photoinactivation studies.

References

1) Shet A, Ferrieri P. Neonatal & Maternal Group B Streptococcal Infections: A Comprehensive Review. Vol 120.; 2004.
2) Ulett KB, Benjamin WH, Zhuo F, et al. Diversity of group B streptococcus serotypes causing urinary tract infection in adults. J Clin Microbiol. 2009;47(7):2055-2060. doi:10.1128/JCM.00154-09
3) Muzny CA, Schwebke JR. Biofilms: An Underappreciated Mechanism of Treatment Failure and Recurrence in Vaginal Infections: Table 1. Clin Infect Dis. 2015;61(4):601-606. doi:10.1093/cid/civ353
4) Borges S, Silva J, Teixeira P. Survival and biofilm formation by Group B streptococci in simulated vaginal fluid at different pHs. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 2012;101(3):677-682. doi:10.1007/s10482-011-9666-y
5) Pieranski, M., Sitkiewicz, I., & Grinholc, M. (2020). Increased photoinactivation stress tolerance of Streptococcus agalactiae upon consecutive sublethal phototreatments. Free Radical Biology and Medicine, 160, 657-669.

Acknowledgements. This work was supported by National Science Centre grant no. 2016/23/B/NZ7/03236

SP-1.3.10, P-1.3.10

Maltohexaose-porphyrin conjugate for bacteria-targeted Photodynamic Therapy

Abdechakour ELKIHEL, Tan Sothéa OUK and Vincent SOL
Université de Limoges, Laboratoire PEIRENE, EA 7500, Faculté des Sciences et Techniques, 123 Avenue Albert Thomas, 87060 Limoges, France.

Although photodynamic therapy was discovered over a hundred years ago for its ability to kill microorganisms, it has been developed mainly as an anti-cancer therapeutic treatment. Recently, with the increasing number of infections caused by multidrug-resistant (MDR) bacteria, Photodynamic Antimicrobial ChemoTherapy (PACT) is considered as a promising alternative antimicrobial approach whereby microbial cells will not be able to develop resistance[1]. Interesting results were achieved against Gram-positive bacteria, but it also appeared that Gram-negative strains were less sensitive to PACT. The most used photosensitizers in PACT are porphyrins and their derivatives[2,3]. However, these compounds often suffer from a lack of selectivity against bacterial cells. In this work, a novel porphyrin derivative has been synthetized which offers an improved activity and targeting than a usual porphyrin. Neutral meso-tritolylporphyrin have been linked to a maltohexaose using a spacer and a cycloaddition (CuAAC) 'click' coupling. Used recently as a bacterial targeting agent for medical imaging with promising results[4,5], the maltohexaose enable a rapid internalization of diverse conjugates through the maltodextrin transport pathway. This highly selective binding of the photosensitizer to bacteria may increase its efficiency against bacterial strains. This new conjugate has been investigated for their antibacterial properties against four bacterial strains: two Gram (+) strains (*S. aureus CIP76.25 ; S. epidermidis CIP109.562*) and two Gram (−) strains (*E. coli CIP54.8T ; E. coli CIP 53.126*). The tests have shown that the coupling of the maltohexaose to neutral porphyrin significantly increases the photocytotoxicity against Gram (+) strains, while no cytotoxicity has been observed in dark condition. Moreover, the conjugate was able to inhibit the formation of biofilms of *S.aureus* and *S.epidermidis* after light irradiation. In addition of this efficiency, flow cytometry analyses have demonstrated that maltohexaose have increased the interaction of the PS on bacteria. In the near future, our work may lead to extensive studies for the design of specific targeting photosensitizer, which may reduce the photocytotoxicity on human cells. Thus, it may be applied to the formulation of dermatological ointments and creams.

References

1) Dai, T.; Huang, Y.-Y.; Hamblin, M. R. Photodynamic Therapy for Localized Infections—State of the Art. *Photodiagnosis Photodyn. Ther.* **2009**, *6* (3–4), 170–188.
2) Le Guern, F.; Sol, V.; Ouk, C.; Arnoux, P.; Frochot, C.; Ouk, T.-S. Enhanced Photobactericidal and Targeting Properties of a Cationic Porphyrin Following the Attachment of Polymyxin B. *Bioconjug. Chem.* **2017**, *28* (9), 2493–2506.
3) Le Guern, F.; Ouk, T.-S.; Ouk, C.; Vanderesse, R.; Champavier, Y.; Pinault, E.; Sol, V. Lysine Analogue of Polymyxin B as a Significant Opportunity for Photodynamic Antimicrobial Chemotherapy. *ACS Med. Chem. Lett.* **2018**, *9* (1).
4) Ning, X.; Lee, S.; Wang, Z.; Kim, D.; Stubblefield, B.; Gilbert, E.; Murthy, N. Maltodextrin-Based Imaging Probes Detect Bacteria in Vivo with High Sensitivity and Specificity. *Nat. Mater.* **2011**, *10* (8), 602–607.
5) Ning, X.; Seo, W.; Lee, S.; Takemiya, K.; Rafi, M.; Feng, X.; Weiss, D.; Wang, X.; Williams, L.; Camp, V. M.; Eugene, M.; Taylor, W. R.; Goodman, M.; Murthy, N. Fluorine-18 Labeled Maltohexaose Images Bacterial Infections by PET. *Angew. Chem. Int. Ed Engl.* **2014**, *53* (51), 14096–14101. 3.

P-1.3.11

Min Oscillations as Reporter of Bacterial Photodynamic Inactivation at the Single-Cell Level

Ingrid V. Ortega[1], Joaquim Torra[1] and Cristina Flors[1]
1) IMDEA Nanociencia, C/ Faraday 9, Ciudad Universitaria de Cantoblanco, 28049, Madrid, Spain.

The Min protein system is a cell division regulator in the bacterium *Escherichia coli*[1]. Under normal growth conditions, MinD is associated to the membrane and undergoes pole to pole oscillations. The period of these oscillations has been previously proposed as a reporter for bacterial physiological state at the single-cell level. This method has been used to monitor the effect of bacterial challenges from antibiotics,[2] temperature[3] or mechanical fatigue.[4] Using real-time single-cell fluorescence imaging, we explore here the effect of antimicrobial photodynamic treatment on MinD oscillations in *E. coli*. Irradiation of bacteria in the presence of the photosensitizer methylene blue (MB) disrupts the Min oscillation pattern depending on the MB concentration. Different MB incubation times and irradiation powers are also explored. In contrast to antibiotics, which slow down the oscillation, photodynamic treatment results in an abrupt interruption of the oscillation and reflects divergent physiological mechanisms that lead to bacterial death. We propose that single-cell microscopy studies using Min oscillations as a reporter are a useful tool to unravel the initial physiological response in antimicrobial photodynamic therapy.

References

1) De Boer, Piet A.J., Crossley, R. E. and Rothfield, L. I. A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in E. coli. Cell, 56(4), 641-649, 1989.
2) Downing, B. P., Rutenberg, A. D., Touhami, A., and Jericho, M. Subcellular Min oscillations as a single-cell reporter of the action of polycations, protamine, and gentamicin on Escherichia coli. PloS one, 4(9), e7285, 2009.
3) Touhami, A., Jericho, M., and Rutenberg, A. D. Temperature dependance of MinD oscillation in *Escherichia coli:* running hot and fast. Journal of Bacteriology, 7661-7667, 2006
4) Del Valle, A., Torra, J., Bondia, P., Tone, C. M., Pedraz, P., Vadillo-Rodriguez, V. And Flors, C. Mechanically induced bacterial death imaged in real time: a simultaneous nanoindentation and fluorescence microscopy study. ACS Appl. Mater. Interfaces, 12(28), 31235-31241, 2020.

Acknowledgements. This work was supported by grants PRE2018-084983 and PGC2018-094802-B-I00 from the Spanish *Ministerio Ciencia, Innovación y Universidades*.

P-1.3.12

Water hardness shows strong impact on the photodynamic activity of cationic pyridylporphyrins against *Legionella pneumophila* and biofilm

Iva Ćavar[1], Martina Mušković[1], Ivana Gobin[2], Nela Malatesti[1]

1) *University of Rijeka, Department of biotechnology, Rijeka, Croatia;*
2) *University of Rijeka, Faculty of medicine, Department for microbiology and parasitology, Rijeka, Croatia*

Legionella pneumophila, a gram negative, aerobic bacterium, is a member of the opportunistic premises plumbing pathogens (OPPP) group. The main characteristics of this group of pathogens are their capacity to live in different aquatic systems, the possibility of biofilm formation and their resistance to common disinfection methods , such as chlorination and high temperatures[1].

To date, only a small number of studies of photodynamic inactivation (PDI) of *Legionella pneumophila* have been published. Our research group recently described PDI with cationic tetrapyrid-3-ylporphyrin and two cationic tripyrid-3-ylporphyrins, one hydrophilic and the other conjugated with long alkyl chain (C18), on *L. pneumophila*. Although all tested porphyrins showed a strong PDI effect on *L. pneumophila*, the amphiphilic tricationic porphyrin with a long chain stood out with its strong anti-legionella activity already at nanomolar concentration (25 nM)[2].

In this work, we study the effect of water samples of different hardness, taken from water wells, on the same porphyrins and the PDI of *L. pneumophila* (Philadelphia strain). The source of irradiation is a LED based violet and blue light (395 nm with fluence rate 20 mW/cm^2 for microbiology experiments, and 411 nm with 3.5-11 mW/cm^2 fluence rate for studies of photophysical properties, respectively). In the case of tested porphyrins, their stability, spectroscopic properties as well as singlet oxygen production by photodegradation of water soluble 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABMDMA) will be described, while the effect on *L. pneumophila* will be investigated through MEK determination, photoinactivation tests and the destruction tests of the early and late biofilms. We will discuss the observed strong impact of water hardness on the PDI of *L. pneumophila* Philadelphia-1 and biofilm formation and growth.

References

1) J. O. Falkinhan, E. D. Hilborn, M. J. Arudino, A. Puden and M. A. Edwards, Epidemiology and Ecology of Opportunistic Premise Plumbing Pathogens: Legionella pneumophila, Mycobacterium avium and Pseudomonas aeruginosa. Enviromental Health Perspectives, August 2015.
2) A. Lesar, M. Mušković, G. Begić, M. Lončarić, D. Tomić Linšak, N. Malatesti and I. Gobin, Cationic Porphyrins as Effective Agents in Photodynamic Inactivation of Opportunistic Plumbing Pathogen Legionella pneumophila. International Journal of Molecular Sciences, July 2020.

Acknowledgements. This work was supported by the University of Rijeka grant uniri-prirod-18-173. The equipment used in this work is made available thanks to the project “Research Infrastructure for Campus-based Laboratories at the University of Rijeka”, financed by the European Regional Development Fund (ERDF) and the Ministry of Science and Education of the Republic of Croatia (RC.2.2.06-0001).

P-1.3.13

Biofilm and planktonic cultures of *Enterococcus* sp. resensitized to antimicrobial with aPDI application

Beata Kruszewska[1], Agata Woźniak[1], Mariusz Grinholc[1]

University of Gdansk and Medical University of Gdansk, Intercollegiate Faculty of Biotechnology, Laboratory of Molecular Diagnostics, 80-307 Gdansk

Enterococcus sp. is a human opportunistic pathogen that can cause infection of the urinary tract or persistent endodontic infections. In addition, the presence of multidrug resistant strains poses a serious clinical challenge. Besides, *E. faecium* is classified as a pathogen belonging to the ESKAPE group of microbes that caused hospital acquired infections. What is more, these bacteria can create biofilm which could be even 1000 more resistant to antimicrobials than planktonic cultures. It may result from the slow penetration of substances through biofilm layers[1]. A chance for reuse of antibiotics is antimicrobial Photodynamic Inactivation (aPDI) which displays huge potential in bacteria eradication and resensitization to antibiotics. aPDI requires visible light which activates photosensitizer molecules (PS) accumulated or present in close proximity to microorganisms leading to transfer energy or electron to oxygen molecules. This results in the production of reactive oxygen species (ROS) which are toxic to cells and may lead to their damage.
The current study was aimed at investigation whether combined therapy employing clinically used antibiotics and aPDI (involving exogenously administered PS – rose bengal and green light, λ_{max} 515 nm) demonstrates a synergy. Two clinical isolates of multidrug resistant strains were investigated: *E. faecium* EU87 and *E. faecalis* EU92. Their drug resistance profile and the synergy between aPDI and antibiotics were characterized with the application of different approaches, i.e. diffusion assays, MIC evaluation, E-TEST, checkerboard assay, and postantibiotic effect. All experiments were performed in accordance to the EUCAST standards. Also, biofilm was formed using approved methodology for flow-biofilm culturing in a CDC reactor and treated with antibiotics and aPDI. The growth of biofilms and microbial survival after treatment was visualized using confocal microscopy. To investigate the potential mechanisms of obtained synergy the fluorescent probes detecting ROS and membrane permeabilization were applied.
The obtained results confirmed that aPDI demonstrates a significant impact on *Enterococcus* sp. survival rate. Synergy was detected for aPDI in combination with gentamycin (GEN), ciprofloxacin (CIP) and daptomycin (DAP) against EU92. For EU87, synergy was observed with the application of GEN or CIP, while for aPDI with vancomycin or DAP, the antagonism was observed. Postantibiotic effect test for EU87 demonstrated that this isolate exposed to aPDI in combination with GEN, streptomycin (STR), tigecycline, doxycycline, or DAP exhibits delayed growth in comparison to monotherapies. Combined treatment with STR and CIP also reduced bacterial load in biofilms by approx. 3-4 $\log_{10}$ CFU/cm^2 what could be visualized on microscopic images. In addition, the obtained results indicated that the combined treatment contributed to the increased ROS in the case of CIP and aPDI. Induced increase in membrane permeabilization may explain the observed synergy. All results confirm the effectiveness of aPDI in sensitizing *Enterococcus* sp. to antimicrobials what has an amazing potential in treating infection caused by multidrug resistant strains.[2]

References

1) Cieplik, F.; Deng, D.; Crielaard, W.; Buchalla, W.; Hellwig, E.; Al-Ahmad, A.; Maisch, T. Antimicrobial photodynamic therapy—What we know and what we don't. Crit. Rev. Microbiol. 2018, 7828, 1–19.
2) Wozniak, A.; Kruszewska, B.; Pieranski, M.K.; Rychłowski, M.; Grinholc, M. Antimicrobial Photodynamic Inactivation Affects the Antibiotic Susceptibility of Enterococcus spp. Clinical Isolates in Biofilm and Planktonic Cultures. Biomolecules 2021, 11, 693.

Acknowledgements. This research was supported by National Science Centre 2015/19/B/NZ7/02487

P-1.3.14

Biofilm and planktonic cultures of *Enterococcus* sp. resensitized to antimicrobial with aPDI application

Beata Kruszewska[1], Agata Woźniak[1], Mariusz Grinholc[1]

University of Gdansk and Medical University of Gdansk, Intercollegiate Faculty of Biotechnology, Laboratory of Molecular Diagnostics, 80-307 Gdansk

Enterococcus sp. is a human opportunistic pathogen that can cause infection of the urinary tract or persistent endodontic infections. In addition, the presence of multidrug resistant strains poses a serious clinical challenge. Besides, *E. faecium* is classified as a pathogen belonging to the ESKAPE group of microbes that caused hospital acquired infections. What is more, these bacteria can create biofilm which could be even 1000 more resistant to antimicrobials than planktonic cultures. It may result from the slow penetration of substances through biofilm layers[1]. A chance for reuse of antibiotics is antimicrobial Photodynamic Inactivation (aPDI) which displays huge potential in bacteria eradication and resensitization to antibiotics. aPDI requires visible light which activates photosensitizer molecules (PS) accumulated or present in close proximity to microorganisms leading to transfer energy or electron to oxygen molecules. This results in the production of reactive oxygen species (ROS) which are toxic to cells and may lead to their damage.
The current study was aimed at investigation whether combined therapy employing clinically used antibiotics and aPDI (involving exogenously administered PS – rose bengal and green light, λ_{max} 515 nm) demonstrates a synergy. Two clinical isolates of multidrug resistant strains were investigated: *E. faecium* EU87 and *E. faecalis* EU92. Their drug resistance profile and the synergy between aPDI and antibiotics were characterized with the application of different approaches, i.e. diffusion assays, MIC evaluation, E-TEST, checkerboard assay, and postantibiotic effect. All experiments were performed in accordance to the EUCAST standards. Also, biofilm was formed using approved methodology for flow-biofilm culturing in a CDC reactor and treated with antibiotics and aPDI. The growth of biofilms and microbial survival after treatment was visualized using confocal microscopy. To investigate the potential mechanisms of obtained synergy the fluorescent probes detecting ROS and membrane permeabilization were applied.
The obtained results confirmed that aPDI demonstrates a significant impact on *Enterococcus* sp. survival rate. Synergy was detected for aPDI in combination with gentamycin (GEN), ciprofloxacin (CIP) and daptomycin (DAP) against EU92. For EU87, synergy was observed with the application of GEN or CIP, while for aPDI with vancomycin or DAP, the antagonism was observed. Postantibiotic effect test for EU87 demonstrated that this isolate exposed to aPDI in combination with GEN, streptomycin (STR), tigecycline, doxycycline, or DAP exhibits delayed growth in comparison to monotherapies. Combined treatment with STR and CIP also reduced bacterial load in biofilms by approx. 3-4 $\log_{10}$ CFU/cm^2 what could be visualized on microscopic images. In addition, the obtained results indicated that the combined treatment contributed to the increased ROS in the case of CIP and aPDI. Induced increase in membrane permeabilization may explain the observed synergy. All results confirm the effectiveness of aPDI in sensitizing *Enterococcus* sp. to antimicrobials what has an amazing potential in treating infection caused by multidrug resistant strains.[2]

References

1) Cieplik, F.; Deng, D.; Crielaard, W.; Buchalla, W.; Hellwig, E.; Al-Ahmad, A.; Maisch, T. Antimicrobial photodynamic therapy—What we know and what we don't. Crit. Rev. Microbiol. 2018, 7828, 1–19.
2) Wozniak, A.; Kruszewska, B.; Pieranski, M.K.; Rychłowski, M.; Grinholc, M. Antimicrobial Photodynamic Inactivation Affects the Antibiotic Susceptibility of Enterococcus spp. Clinical Isolates in Biofilm and Planktonic Cultures. Biomolecules 2021, 11, 693.

Acknowledgements. This research was supported by National Science Centre 2015/19/B/NZ7/02487

P-1.3.15

Antimicrobial photodynamic inactivation against staphylococcal enterotoxin A – *in vitro* and *ex vivo* studies

Patrycja Ogonowska[1], Adrian Kobiela[2], Danuta Gutowska-Owsiak[2,3], Joanna Nakonieczna[1]

[1]*Laboratory of Molecular Diagnostics, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Gdansk, Poland*
[2]*Experimental and Translational Immunology Group, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Gdansk, Poland*
[3]*MRC Human Immunology Unit, Radcliffe Department of Medicine, MRC Weatherall Institute of Molecular Medicine, University of Oxford, United Kingdom*

Staphylococcal enterotoxin A (SEA) is a virulence factor produced by *Staphylococcus aureus*. SEA is mainly involved in staphylococcal food poisoning and, along with other staphylococcal enterotoxins (SEs), plays a role as an exacerbation factor for atopic dermatitis [1,2,3]. In the treatment of atopic dermatitis eradication of *S. aureus* and restoration of the natural bacterial diversity in the skin is crucial[4]. Moreover, SEs have superantigenic properties[5]. All these features constitute a huge therapeutic challenge. Therefore, in our research we propose antimicrobial photodynamic inactivation (aPDI) as the effective tool to combat both bacterial cells and virulence factors.

The main objectives of the presented studies were (i) to assess the *in vitro* efficacy of aPDI against *S. aureus* clinical isolates and (ii) to verify the impact of aPDI on the production of staphylococcal enterotoxin A at the gene and protein levels (*in vitro* and *ex vivo* experimental models).

In the aPDI studies, a combination of rose bengal (RB) and green light (λ_{max}=515 nm, irradiance 35 mW/cm^2) was used for all clinical *S. aureus* strains tested. An MTT assay was performed on human keratinocytes (HaCaT cells) to verify the cyto- and phototoxic effects of aPDI on eukaryotic cells. The expression profile of *sea* gene under sub-lethal aPDI conditions was examined using the qPCR technique. To confirm the observed results on the protein level, the Western Blot technique and the proliferation assay were used. To verify our observations from *in vitro* experiments, the analysis at the gene and protein levels was performed on a porcine skin model.

This study indicated high efficacy (5-6 $\log_{10}$ reduction in bacterial survival) of aPDI against planktonic *S. aureus* clinical isolates, carrying *sea* gene. The aPDI conditions tested showed no cyto- and phototoxic effect on eukaryotic cells. The results obtained indicated decrease in the level of *sea* gene upon aPDI conditions (a 2.8 $\log_2$ unit decrease after 20 min of irradiation and 2.48 $\log_2$ unit decrease after 40 min). Western Blot analysis did not reveal any significant changes in the level of the protein. However, a proliferation assay proved that under aPDI treatment SEA loses its biological function. Our preliminary *ex vivo* studies demonstrated the ability to monitor the presence of SEA protein at various time points after aPDI.

The presented studies indicate that aPDI can effectively reduce the level of virulence factors and deprive them of their biological functions.

References

1) Kadariya J., Smith T.C., *Staphylococcus aureus* and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. Biomed. Res. Int., 2014.
2) Bunikowski R., Mielke M., Evidence for a disease-promoting effect on *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. J. Allergy Clin. Immunol., 2000.
3) Taskapan M., Kumar P., Role of staphylococcal superantigens in atopic dermatitis: from colonization to inflammation. Ann. Allergy Asthma Immunol., 2000.
4) Hendricks A.J., Mills B. Skin bacterial transplant in atopic dermatitis: knowns, unknowns and emerging trends. J. Dermatol. Sco, 2019.
5) Spaulding A., Salgado-Pabón W., Staphylococcal and streptococcal superantigen exotoxins. Clin. Microbiol. Rev., 2013.

Acknowledgements. This work was supported by National Science Centre grant no. 2017/27/B/NZ7/02323 (JN).

P-1.3.16

Protein based targeted delivery systems for antimicrobial PDT

Andrea Mussini[1], Eleonora Uriati[1,2], Pietro Delcanale[1], Paolo Bianchini[2,3], Alberto Diaspro[2,3,4], Stefania Abbruzzetti[1], Cristiano Viappiani[1], Cormac Hally[5], Montserrat Agut[5] and Santi Nonell[5]

1) Dipartimento di Scienze Matematiche, Fisiche e Informatiche, Università di Parma, Italy; 2) Nanoscopy, Istituto Italiano di Tecnologia, Genoa, Italy; 3) Nikon Imaging Center, Istituto Italiano di Tecnologia, Genoa, Italy; 4) Department of Physics, University of Genoa, Genoa, Italy; 5) Institut Químic de Sarrià, Universitat Ramon Llull, Spain

Water soluble proteins are natural candidates as biocompatible delivery systems for photosensitizers. We report the development of a supramolecular structure endowed with photosensitizing properties and targeting capability for antimicrobial photodynamic inactivation. Our synthetic strategy uses the tetrameric bacterial protein streptavidin, labelled with eosin, as the main building block. Biotinylated immunoglobulin G (IgG) from human serum, known to associate with S. aureus protein A, was bound to the complex streptavidin-eosin. The supramolecular complex was used to demonstrate photoinactivation of S. aureus suspensions. FCS and STED nanoscopy experiments confirmed binding of the complex to S. aureus. Efficient photoinactivation is observed for S. aureus suspensions treated with IgG-streptavidin-eosin at concentrations higher than 0.5 μM and exposed to green light. The proposed strategy offers a flexible platform for targeting a variety of molecular species.

P-1.3.17

The use of curcumin to photoinactivate *Trypanozoma cruzi.*

T. Buenfil-Chi, D. Ortega-Zambrano and H. Mercado-Uribe
CINVESTAV-Monterrey, Parque PIIT, Apodaca, NL. 66000, Mexico.

Chagas disease is caused by the protozoan *Trypanozoma cruzi.* This is an endemic pathogen of Latin America that has developed a significant resistance against the usual treating methods. Therefore, it has become an important health problem. In this work, we applied the phodotodynamic inactivation procedure on cultures of CL Brener and Ninoa strains in their epimastigote form, when the parasite is at its fastest growing stage. We investigated the join effect of curcumin and blue light at $9x10^{-6}$ M and 16 mW, respectively. We did not observe curcumin toxicity in either strains. The viability of *T. cruzi* was immediately evaluated after irradiation at 89 J/cm^2 (1 hour). We found a 1 log10 reduction for CL Brener, and a little less for the Ninoa strain. However, we would like to remark that there was a delayed effect on the viability of the parasites. Indeed, after two days of the treatment, the mortality was 100%.

Acknowledgements. This work was supported by CONACyT, Mexico, Grant A1-S-8125.

P-1.3.18

Photodynamic Inactivation of fungal phytopathogens using Ce6 derivatives and assessment of their phytotoxicity.

Christoph Hamminger[1], Michael Glueck[1], Michael Fefer[2], Jun Liu[2], Raimund Tenhaken[3], Kristjan Plaetzer[1]
1) University of Salzburg, Department of Biosciences, Laboratory of Photodynamic Inactivation of Microorganisms, Salzburg, Austria; 2) Suncor AgroScience, Mississauga Ontario, Canada, 3) University of Salzburg, Department of Biosciences, Plant Physiology, Salzburg, Austria

An increasing demand for organic and conventionally produced foodstuff necessitates a more sustainable, biocompatible, and holistic approach to crop protection. This study aims towards adding Photodynamic Inactivation (PDI) as highly biocompatible strategy suitable for crop protection to the farmer's toolbox. Since the antimicrobial effectiveness of PDI is not diminished by classical resistance mechanisms of bacterial or fungal pathogens - which trouble growers all over the globe - we believe this approach has the potential to benefit farmers as well as the ecosystem. In this study *Alternaria solani* and *Botrytis cinerea* were used as model pathogens. These two fungi are the cause for considerable crop and monetary shortfalls in the food production industry. Two photoactive compounds, Sodium-Magnesium-Chlorophyllin (Chl, food additive E140) in combination with Na_2EDTA and the chlorin e6 derivative B17-0024 holding cationic moieties were used as photosensitizers. Mycelial spheres with a diameter of 2-3 mm of *A. solani* and *B. cinerea* were incubated with the photosensitizers for one hundred minutes and illuminated with 106.6 J/cm² using a LED array with a wavelength of 395 nm. Samples not showing any growth on agar plates after seven days were counted as dead. One hundred micromolar Chl with 5 mM Na_2EDTA successfully photoinactivated 91.7% of *B. cinerea* and 94.1% of *A. solani*. PDI based on B17-0024 thoroughly killed (100 %) *A. solani* at concentrations of 10 and 100 µM. Ten micromolar of B17-0024 lead to 66.7 % and 100 µM to complete eradication of *B. cinerea*. To screen for possible phytotoxic effects of the PDI treatment, a plant compatibility assay based on the small woodland strawberry (*Fragaria vesca*) was performed. The strawberry plants at BBCH[1] stage 14 were sprayed once – or three times on consecutive days - with 300 µL of the PS formulation used for PDI. Plant health and growth was monitored for twenty-one days post PDI treatment. Although Na_2EDTA induced minor damage to the treated leaves, the assay demonstrated that the two photosensitizers do not cause significant damage and have no adverse effect on plant development. In conclusion, we show that tissue-like fungal structures of *A.solani* and *B. cinerea* can be successfully treated by PDI without causing harm to the host plants. Therefore our results suggest that Chl / EDTA as well as B17-0024 are effective photofungizides.

References

1) U. Meier, H. Graf, H. Hack, M. Hess, W. Kennel, R. Klose (Eds.), Growth stages of mono-and dicotyledonous plants, BBCH monograph, Strawberries (2th ed.), Federal Biological Research Centre for Agriculture and Forestry, Berlin/Braunschweig, Germany (2001)

Acknowledgements. This work was supported by Suncor AgroScience.

P-1.3.19

A semi-theoretical action spectrum for antimicrobial photoinactivation in the lungs

Alfonso Dell'Accio[1], Chiara Treghini[1], Maria Micieli[1], Eleonora Riccobono[1], Felice Valzano[2], Franco Fusi[1], Gian Maria Rossolini[3], Lucia Pallecchi[2], Giovanni Romano[1], Light4Lungs group[4]
[1] University of Florence, Department of Clinical and Experimental Biomedical Sciences "Mario Serio", Florence, Italy; [2] University of Siena, Department of Medical Biotechnologies, Siena, Italy;[3] University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy; [4]S. Nonell, Institut Quimic de Sarria, Barcelona, Spain; G. Romano, University of Florence, Florence, Italy; N. Henry, Sorbonne Université, Paris, France; E. Palomares, Fundacio Privada Institut Catala D'Investigacio Quimica, Tarragona, Spain; E. Reddi, University of Padova, Padova, Italy; G. Taylor, Cardiff Scintigraphics Ltd, Cardiff, UK; A. Kadioglu, The University of Liverpool, Liverpool, UK

In the framework of the search for innovative solutions to overcome antibiotic-resistance, the European project "Light4Lungs" aims at synthesizing and testing an inhalable light source to control lung infections associated with biofilm-forming and multidrug-resistant bacteria such as *P. aeruginosa* and *S. aureus,* with notable applications in cystic fibrosis (CF). In this context, the project proposes an antimicrobial photoinactivation (aPDI) approach, taking advantage of the presence of endogenous photosensitizers (porphyrins) in the considered bacterial species.
The choice of the most suitable wavelength derives from the definition and calculation of the photo-killing action spectrum in the lungs, considering the case of biofilm-associated infections caused by P. aeruginosa and S. aureus in CF patients. This was obtained by applying a semi-theoretical modelling with Monte Carlo simulations to the infected lung regions, according to optical theory [1] and previously published methodology related to stomach infection phototherapy [2]. The variability of *in vivo* pathologic conditions [3] was accounted for by a careful literature analysis, relative to the variation in the concentration of oxygen, bacteria and relative endogenous porphyrins, besides the presence of other relevant absorbers/diffusers inside the biofilm/mucous layer. The obtained semi-theoretical action spectrum is peaked at 394 nm and mostly follows porphyrins extinction coefficient behaviour.

To confront these results with an experimental validation, we started by performing *in vitro* irradiation on preformed biofilms grown on a standardized static model (MBEC Assay), considering one reference strain and one CF clinical isolate for both *P. aeruginosa* and *S. aureus*. Biofilms were irradiated with representative wavelengths, considering first LED sources centred in the violet region (415nm) and in the green (525nm). Light at 415nm showed a dose-dependent and strain-dependent antimicrobial effect, with reduction of at least 2 log CFU at ~60 J/cm^2 in three of the four tested strains. Light at 525 nm showed a scant or no effect, as expected. Additional wavelengths will be considered to explore the relative efficacy of the porphyrin secondary absorption peaks and merge data with the mucous and biofilm absorption/scattering properties.

The obtained results can offer important indications for the synthesis of the aerosolized light source and definition of its most effective emission spectrum, suggesting also a flexible platform for dosimetry calculation to be considered in further applications.

References
1) Jacques, S. L., Simple optical theory for light dosimetry during PDT. Proc. SPIE 1645, 155–165, 1992.

2) Gnerucci, A., Faraoni, P. et al., Influence of stomach mucosa tissue on the efficacy of intragastric antibacterial PDT. Photochem. Photobiol. Sci., 19, 34–39, 2020.

3) Cowley, E. S., Kopf, S. H. et al. Pediatric cystic fibrosis sputum can be chemically dynamic, anoxic, and extremely reduced due to hydrogen sulfide formation. MBio, 6, 1–15, 2015.

Acknowledgements. This work was supported by the H2020 research and innovation programme under grant agreement n.863102. This poster was displayed on behalf of the Light4Lungs Consortium.

P-1.3.20

Porphyrin-based light activated antimicrobial coatings prepared by spin coating

Melania Rogowska[1], Martin Tran[1], Ellen Bruzell[2], Håkon Valen[2], Ola Nilsen[1]

1) Centre for Materials Science and Nanotechnology (SMN), Department of Chemistry, University of Oslo, Oslo, Norway; 2) Nordic Institute of Dental Materials, Oslo, Norway

Porphyrins are attractive functional materials commonly used as visible light absorbing photosensitizers in photodynamic therapy (PDT). Furthermore, different transition metals can be introduced into the porphyrin ring to create metalloporphyrin complexes with various physiochemical properties.[1] In this work, novel light-activated antimicrobial thin films were prepared based on biocompatible zirconium (Zr) and tetrakis(4-carboxyphenyl)porphyrin (TCPP) using spin coating deposition technique. The physicochemical properties of the ZrTCPP films were characterized by UV-Vis and Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), atomic force microscopy (AFM) and contact angle measurements. The antibacterial activity of the films was investigated using the gram-positive bacteria *Enterococcus faecalis*, both against newly adhered bacteria and after biofilm formation on the surfaces. The photodynamic inactivation was induced after exposure of active films combined with blue light (λ: 400 – 520 nm) using light doses of 5.7, 11.4 and 22.8 J/cm^2. This study shows high inhibitory activity of the ZrTCPP complexes in the presence of light, however, the films were also active against bacteria in the absence of light pointing to cytotoxicity of the Zr-metalloporphyrin complex.

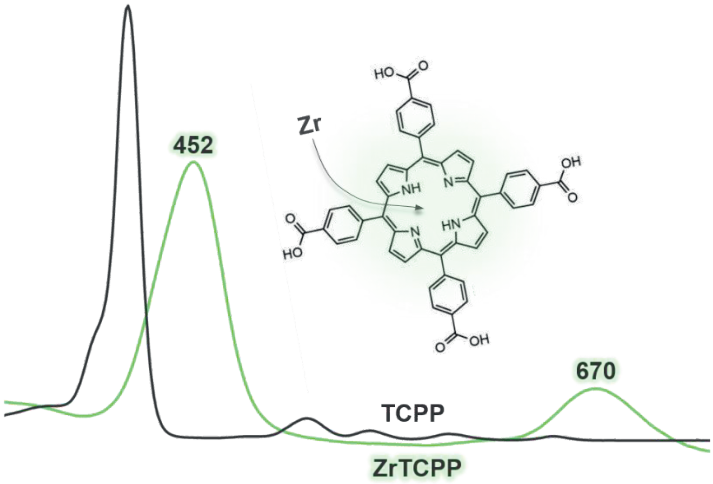


Fig. 1 - Structure of tetrakis(4-carboxyphenyl)porphyrin (TCPP) used in in this work and UV-Vis absorption spectra of the ZrTCPP thin film and the corresponding TCPP dissolved in ethanol.

References

1) Stojiljkovic, I., Evavold, B. D., Kumar, V. Antimicrobial properties of porphyrins. *Exp. Opin. Invest. Drugs,* **10**(2), 309-320 (2001).

Acknowledgements. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 765378.

P-1.3.21

Decolonization of human skin by application of a photodynamically active hydrogel

Daniel B. Eckl[1], Anja K. Hoffmann[1], Nicole Landgraf[1], Anja Eichner[2], Harald Huber[1], Wolfgang Bäumler[2]

1) Universität Regensburg, Lehrstuhl für Mikrobiologie, Regensburg, Germany; 2) Universitätsklinikum Regensburg, Klinik und Poliklinik für Dermatologie, Regensburg, Germany,

Skin colonization with pathogenic, even multi-resistant bacteria is a potential source of infections, especially in hospital-associated environments. It has been shown previously that photodynamic inactivation (PDI) of bacteria is a promising, new approach for the decolonization of the human skin. In a preliminary study it was shown that PDI experiments already provided a decontamination efficacy on human *ex vivo* skin of 99.9% using an aqueous photosensitizer solution[1]. Furthermore, the application of aqueous photosensitizer solutions did not lead to differences in mitochondrial survival compared to the untreated control. In a follow up study, we aimed to improve the decolonization efficacy as well as the applicability in practice by applying a photodynamically active hydrogel.

UV-VIS spectroscopy was applied to investigate the chemical stability of photosensitizers in the hydrogel suspension. Initial tests of the photodynamically active hydrogels were performed on inanimate surfaces with bacterial cells suspended in different ionic solutions such as an artificial sweat solution in order to evaluate the basic parameters for efficient photodynamic inactivation on human skin. Fresh e*x vivo* human skin was inoculated with bacterial suspensions of methicillin-resistant *Staphylococcus aureus* (MRSA). After visible drying of bacteria, different hydrogels containing different photosensitizers were added to the inoculated skin surface. Subsequently, the skin surface was irradiated with visible blue light at different radiant exposures (J/cm²). Then, bacteria were recovered from skin with a swap and plated via drop-plate method on Mueller Hinton Agar. Colony count unfold reduction efficacy when referenced to internal controls. After recovery, skin biopsies were taken and nitro blue tetrazolium (NBT) staining was applied to check viability of skin cells via mitochondrial activity. TUNEL stain revealed the presence of apoptotic skin cells.

Firstly, spectroscopy results suggest that the hydrogel did not affect the chemical structure of photosensitizers. Artificial experiments on inanimate surfaces showed that the hydrogel is capable of highly efficient bacterial reduction even in presence of an artificial sweat solution. The microbiology results of *ex vivo* human skin showed an improved MRSA reduction on skin of around 99.99% as compared to internal references. NBT staining of the tissue revealed no lack in mitochondrial activity of the human cells due to the application of the photodynamically active hydrogel. Lastly, TUNEL stained histological sections did not show noteworthy apoptotic effects after the photodynamic treatment.

The data indicate that PDI represents an easy, quick and efficient method to decolonize human skin from MRSA. The results of the study are remarkable, as substances on skin like inorganic ions or small organic molecules such as histidine are capable to inhibit the photodynamic efficacy but seem to interfere less in a hydrogel environment. Since PDI is known to eradicate a large set of pathogens independent of their antibiotic resistance profiles, this new technology should be tested in clinical studies in the near future.

References

1) M. Schreiner, W. Bäumler, D. Eckl, A. Späth, B. König, A. Eichner, MRSA decolonization of human skin via photodynamic treatment, British Journal of Dermatology 179, 1358-1367, 2018

Acknowledgements. This work was supported by the German Research Foundation DFG (GEPRIS project number 415812443)

P-1.3.22

Antimicrobial photosensitizers and their formulations: A potential solution to current world scenario

Bhavya Khurana[1, 2], Tan Ouk[1], Mathias O. Senge[2, 3], Vincent Sol[1]

1. Université de Limoges, Laboratoire PEIRENE.
2. School of Chemistry, Trinity Biomedical Sciences Institute, Trinity College Dublin, The University of Dublin.
3. Medicinal Chemistry, Trinity Translational Medicine Institute, Trinity Centre for Health Sciences, Trinity College Dublin, The University of Dublin

In order to provide a long-lasting solution to infections affecting the current world scenario, photodynamic therapy (PDT) offers a means to destroy pathogenic microbes via formation of reactive oxygen species, promoting the damage of microbial targets such as nucleic acids (DNA or RNA), proteins, lipids, protein complexes, or by impeding the biofilm matrix.[1]
Thus, the main aim of the study is to design and synthesize photoactive moieties based on porphyrin and chlorin macrocycles and BODIPY dyes for antimicrobial photodynamic therapy (aPDT).[2] Furthermore, incorporating these photo-moieties into biopolymeric hydrogelsfor a variety of biomedical applications are targeted.
Porphyrin based cationic photosensitizers (PSs) were synthesized and a chlorin based PS was extracted from Spirulina maxima and modified to be included on a biopolymeric hyaluronic acid hydrogel platform. This platform was characterized spectroscopically and evaluated for antimicrobial photoactivity via microbial evaluation on different gram strains of bacterial species. Singlet oxygen production was determined as well to evaluate the photoactivity of this polymeric hydrogel platform.
Furthermore, BODIPY dye-based PS species have been synthesized and modified for their activity as aPDT agents. Several *N*-heterocyclic BODIPY-dyes have been positively charged or functionalized for incorporation into hydrogel platforms. These dyes exhibit good water solubility. A library of such *N*-heterocyclic BODIPY dyes was prepared and characterized and will be evaluated for photoactivity against microbes.

References

1. W.C. de Melo, P. Avci, M.N. de Oliveira, A. Gupta, D. Vecchio, M. Sadasivam, R. Chandran, Y.Y. Huang, R. Yin, L.R. Perussi, G.P. Tegos, J.R. Perussi, T. Dai, M.R. Hamblin, *Photodynamic inactivation of biofilm: taking a lightly colored approach to stubborn infection*, Expert Rev Anti Infect Ther , 2013, **11**, 669-693.
2. A. Wiehe, J.M. O'Brien, M.O. Senge, *Trends and targets in antiviral phototherapy*, Photochem. Photobiol. Sci., 2019, **18**, 2565-2612.

Acknowledgements. This work was supported by grant 42 from the Ministry of Truth.

P-1.3.23

Photodynamic Antimicrobial Therapy: Chemical kinetic modeling to improve treatment efficacy

Jeffrey C. Peterson[1,2], Yu-Cherng Chang[1,2], Irene Kochevar[3], Fabrice Manns[1,2], Jean-Marie Parel[1,2,4]
1) University of Miami Miller School of Medicine, Ophthalmic Biophysics Center, Department of Ophthalmology, Bascom Palmer Eye Institute, Miami, FL, USA;
2) University of Miami College of Engineering, Department of Biomedical Engineering, University of Miami, Coral Gables, FL, USA;
3) Harvard Medical School, Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, USA;
4) University of New South Wales, Brien Holden Vision Center, Sydney, NSW, Australia.

Purpose: Rose Bengal Photodynamic Antimicrobial Therapy (RB-PDAT) has been shown to effectively treat antimicrobial resistant and atypical infectious keratitis.[1–3] RB-PDAT works by generating antimicrobial singlet oxygen ($^1O_2$) by exciting Rose Bengal (RB) photosensitizer with 525 nm light. The cumulative $^1O_2$ dose distribution generated during RB-PDAT is currently unknown, this project aims to create a predictive model to calculate the $^1O_2$ distribution under clinical conditions and will enable optimization of $^1O_2$ dose by optimizing RB-PDAT treatment parameters.

Methods: A chemical kinetics model previously developed (Wang et al., 2010) for $^1O_2$ dose in tumors treated with photodynamic therapy was used as the basis for this model.[4] To describe ground-state oxygen (O_2) supply specific to the cornea, the oxygen supply term was adapted to include the model described by Larrea & Büchler and Castillo et al.[5,6] RB distribution was modeled using Fick's second law, and 525 nm excitation light distribution by time-dependent application of Beer's law. Rate constants for RB diffusion were derived from experimental data describing RB corneal penetrance.[7–9] Minimal lateral variation of all species was assumed to allow for one-dimensional modeling. The resulting system of partial differential equations was solved numerically in MATLAB. Cumulative $^1O_2$ dose was calculated for RB-PDAT during typical conditions (6 mW/cm^2, 5.4 J/cm^2, 0.1% RB), for pulsed and increased light dose, and for addition of 90% supplemental O_2.

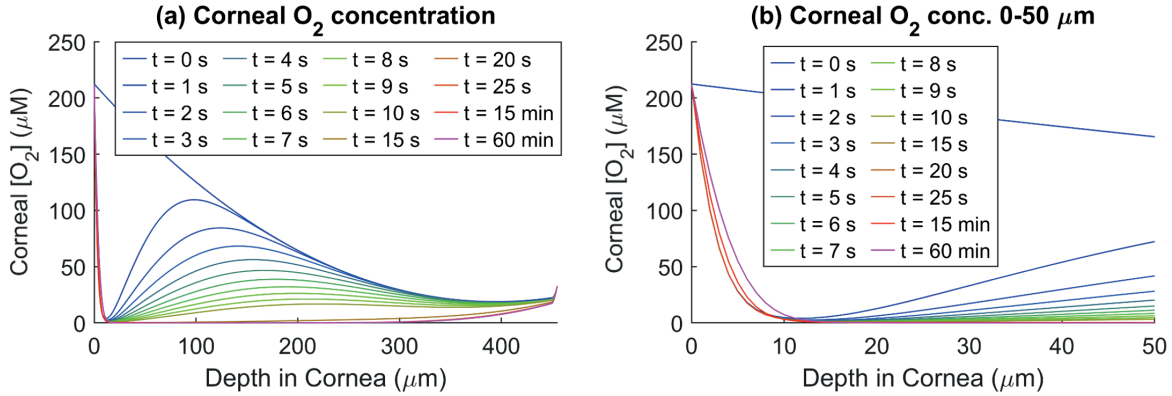


Fig. 1 – (a) Calculated corneal O_2 concentration as it varies throughout typical RB-PDAT application (0.1% RB applied for 30 min at 6 mW/cm^2 for 15 min, 5.4 J/cm^2). (b) Same graph as (a) zoomed in to 0-50 μm.

Results: Under typical RB-PDAT conditions, calculated O_2 decreases to approximately zero beyond 10 μm depth in the first 1 min of treatment. In this scenario, $^1O_2$ dose distribution was calculated to be limited to <10 μm corneal depth. Supplemental O_2 increases $^1O_2$ dose depth by approximately 5–10 μm, with similar results observed with 1s on, 1s off pulsing with doubled fluence rate. Continuous wave, doubled fluence rate for half the treatment time (i.e. 6 mW/cm^2 for 15 min vs. 12 mW/cm^2 for 7.5 min) decreases calculated $^1O_2$ depth for all treatment conditions. Increasing RB concentration increases calculated cumulative $^1O_2$ dose approximately linearly. Increasing light dose from 5.4 J/cm^2 to 10 J/cm^2 increases calculated $^1O_2$ surface dose 1.4 to 1.7x.

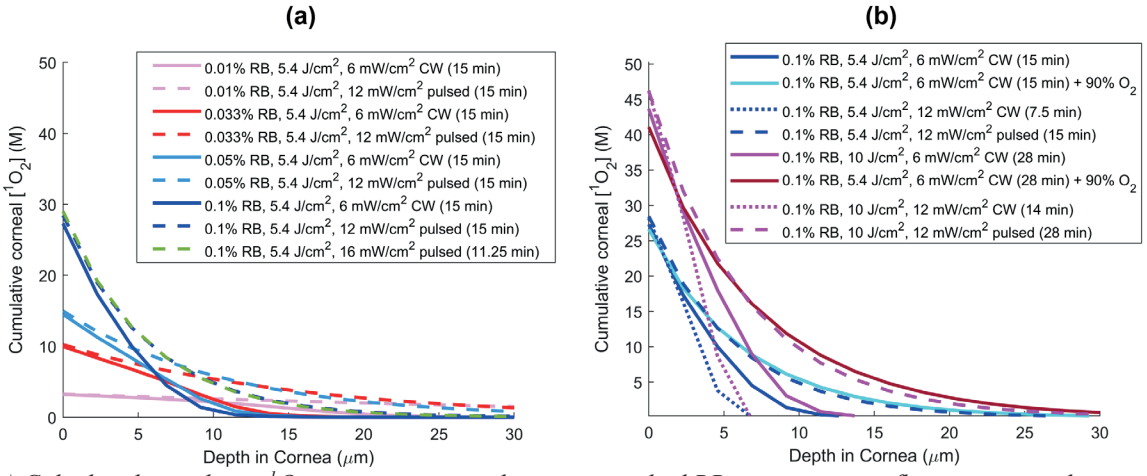


Fig. 2 – (a) Calculated cumulative $^1O_2$ concentration with varying applied RB concentration, fluence rate, and continuous wave light vs. 1s on 1s off pulsing (b) Calculated cumulative $^1O_2$ concentration with varying total light energy, fluence rate, continuous wave light vs. 1s on 1s off pulsing, or providing 90% supplemental O_2.

Conclusions: We demonstrate a proof-of-concept predictive model for $^1O_2$ dose generated during RB-PDAT, capable of testing a range of experimental parameters, which can be used for optimizing RB-PDAT efficacy. Follow-up studies, such as direct *in vivo* measurement of O_2 during RB-PDAT, will be necessary to validate the model predictions.

References

1) Naranjo A, Arboleda A, Martinez JD, et al. Rose Bengal Photodynamic Antimicrobial Therapy for patients with progressive infectious keratitis: A pilot clinical study. *American Journal of Ophthalmology*. 2019;208:387-396.
2) Amescua G, Arboleda A, Nikpoor N, et al. Rose Bengal Photodynamic Antimicrobial Therapy: A novel treatment for resistant Fusarium Keratitis. *Cornea.* 2017;36(9):1141-1144.
3) Arboleda A, Miller D, Cabot F, et al. Assessment of Rose Bengal versus riboflavin photodynamic therapy for inhibition of fungal keratitis isolates. *American Journal of Ophthalmology*. 2014;158(1):64-70.e2.
4) Wang KK-H, Finlay JC, Busch TM, Hahn SM, Zhu TC. Explicit dosimetry for photodynamic therapy: macroscopic singlet oxygen modeling. *Journal of biophotonics*. 2010;3(5-6):304-318.
5) Larrea X, Büchler P. A transient diffusion model of the cornea for the assessment of oxygen diffusivity and consumption. *Investigative Ophthalmology & Visual Science*. 2009;50(3):1076-1080.
6) Del Castillo LF, da Silva ARF, Hernández SI, et al. Diffusion and Monod kinetics model to determine in vivo human corneal oxygen-consumption rate during soft contact lens wear. *Journal of Optometry*. 2015;8(1):12-18.
7) Naranjo A, Pelaez D, Arrieta E, et al. Cellular and molecular assessment of Rose Bengal photodynamic antimicrobial therapy on keratocytes, corneal endothelium and limbal stem cell niche. *Experimental Eye Research*. Published online September 2019:107808.
8) Peterson JC, Naranjo A, Martinez JD, et al. Rose Bengal depth in human donor cornea after Rose Bengal Photodynamic Antimicrobial Therapy. Presented at the: American Society for Photobiology 2018 Biennial Meeting; May 12, 2018; Tampa, FL, USA. https://photobiology.org/2018minisite/ASP%.202018%20Schedule.pdf
9) Wertheimer CM, Elhardt C, Kaminsky SM, et al. Enhancing Rose Bengal-photosensitized protein crosslinking in the cornea. *Investigative ophthalmology & visual science.* 2019;60(6):1845-1852

Acknowledgements. This research was supported in part by the Robson Foundation, the Florida Lions Eye Bank and Beauty of Sight Foundation; Gifts from Drs. HW Flynn Jr, KR Olsen, ME Hildebrandt, R Urs and A Furtado; NIH Center Grant P30EY14801; unrestricted funds from Research to Prevent Blindness to the department of Ophthalmology; the Henri and Flore Lesieur Foundation (J.-M. Parel). Jeffrey Peterson was supported in part by NIH Medical Scientist Training Program T32 GM112601-04. Provisional patent #39756179 filed 6/18/2020 for $^1O_2$ measurement system for RB-PDAT, the University of Miami has all rights.

P-1.3.24

Breaking the rebellion: Photodynamic Inactivation of *Erwina amylovora* resistant to streptomycin

Annette Wimmer[1], Michael Glueck[1], Michael Fefer[2], Jun Liu[2], Kristjan Plaetzer[1]
1) University of Salzburg, Department of Biosciences, Laboratory of Photodynamic Inactivation of Microorganisms, Salzburg, Austria; 2) Suncor AgroScience, Mississauga Ontario, Canada

Global crop production plays a key role in food security. The ever-increasing spread of plant pathogens causes high losses in agriculture. *Erwinia amylovora*, the causal agent of the contagious fire blight disease, has already evolved resistance to the antibiotic streptomycin (Sm) as conventional treatment strategy. Photodynamic Inactivation (PDI) of microorganisms has recently been introduced as innovative method for plant protection. PDI is based on the principle that a per se non-toxic photosensitive substance is activated by visible light, by this forming reactive oxygen species, which in turn directly induce phototoxicity in target cells. The aim of the study was to prove whether *E. amylovora* resistant to Sm (*E. amylovora* ^{SmR}) can be killed by PDI. Considering the field application, natural derived sodium magnesium chlorophyllin (Chl) combined with 1.2 % polyaspartic acid (PASA) or B17-0024, a synthethic compound carrying cationic moieties, were used as eco-friendly photosensitizers (PS). In vitro experiments were performed with the respective PS in concentrations of 1, 10 and 100 µM and five as well as 30 minutes incubation in the dark, followed by illumination at 395 nm with a radiant exposure of 26.6 J/cm^2. To investigate additional effects of Sm in media with and without this antibiotic were used. Efficiency testing was evaluated by counting of colony forming units (CFU). Our results show that *E. amylovora* ^{SmR} can be successfully treated with PDI. The highest inactivation of 7 log steps (equivalent to a 99.99999% reduction of viable bacteria) was induced at 100 µM B17-0024 and 30 minutes incubation. Chlorophyllin in combination with 1.2 % PASA reduced the number of bacteria by 6 log steps. Our results are in line with a previous study performed by Glueck et al.[1], where the susceptible strain of *E. amylovora* was successfully photoinactivated. As conclusion this study proves principle that PDI can be used to treat plant diseases even if the causing bacteria are resistant to conventional treatment.

References

1) Glueck, M., C. Hamminger, M. Fefer, J. Liu and K. Plaetzer, Save the crop: Photodynamic Inactivation of plant pathogens I: bacteria. Photochemical & Photobiological Sciences 18(7): 1700-1708, 2019.

Acknowledgements.

The authors are grateful to Suncor AgroScience, Mississauga Ontario, Canada for providing B17-0024.

P-1.3.25

Targeted photodynamic approach: the case study of Ga^{3+} *meso*-PPIX and heme transporters.

Michalska Klaudia[1], Nakonieczna Joanna[1]
1) Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Department of Biotechnology, Laboratory of Molecular Diagnostics, Gdańsk, Poland

Introduction: The efficiency of antimicrobial photodynamic treatment (aPDI) may dependent on the uptake of the photosenstizier (PS). Photosensitizers can be accumulated in various ways, depending on: the structure of bacterial cell, environmental factors and the mechanism involved, e.g. active transport. The concept of a targeted photodynamic approach is based on the uptake by molecular targets such as membrane receptors that recognize PS as a natural ligand. One of the proposed compounds for the targeted PS are metal conjugated porphyrins (metalloporphyrin, MP). Gallium protoporphyrin IX conjugates such as Ga^{3+}PPIX or meso-substituted Ga^{3+} *meso*-PPIX (GaMPPIX), mimic the binding between the heme structure (Fe^{3+}protoporphyrin IX) and their acquisition machinery. The heme can be acquired intracellularly under iron deficiency conditions by two systems of transmembrane protein cascades: Isd (**i**ron-regulated **s**urface **d**eterminant) and Hts (**h**eme transport **s**ystem). Paradoxically, heme might elaborate the toxicity at higher concentrations. The HrtAB (**h**eme-**r**egulated **t**ransporter) two-component detoxification machinery senses and pumps an overdose of the compound out of the cell. The aim of this study was to investigate the effect of heme or impairment of the heme acquisition and detoxification machinery on phototreatment with GaMPPIX.
Materials and methods: In this study, *Staphyloccocus aureus* ATCC 25923 cells were photodynamically treated in various proportions of the mixture [heme and photosensitizer]. The conditions for aPDI were as follows: GaMPPIX and subsequent green LED irradiation λ_{max}= 522 nm, 10.6 mW/cm^2. Bacterial viability was measured by serial dilution of the cells and counting of the colonies plated on agar plates after treatment (CFU/mL). Similarly, *S. aureus* Newman and its isogenic mutants (ΔHrtA, ΔIsdD and ΔHtsA) were subjected to aPDI under green light irradiation in the presence and absence of iron in the medium. Accordingly, accumulation of GaMPPIX was measured. Briefly, overnight bacterial cultures were diluted and incubated with photosensitizer for 10 minutes at 37°C with shaking. Then, washed twice with PBS buffer and suspended in 0.1M NaOH/1% SDS solution. After 24 hours of incubation, the fluorescence of lysate was measured and the quantity of accumulated molecules of PS per cell were calculated.
Results: Post-treatment cell count analysis showed that the combined GaMPPIX-mediated aPDI and hemin action decreased photodynamic efficiency with a competitive effect (10x and 5x higher hemin concentrations over GaMPPIX resulted in an increase of *S. aureus* survival from 2 to 4 $\log_{10}$ units). This suggests that GaMPPIX is a substrate for the studied heme transport system HrtA. The photoinactivation results were also confirmed by lower intracellular accumulation of GaMPPIX in cells after addition of 10 μM of heme (calculated as an 18,3% reduction in accumulation of GaMPPIX). Impairment in HrtA ATPase resulted in the most sensitive phenotype in aPDI. The sensitivity of ΔHrtA to aPDI was correlated with higher accumulation of GaMPPIX compared to the wild-type strain.

Acknowledgements. This work was supported by Small Grants Program for early career scientist funded by University of Gdańsk – Ugrants-Start no. 533-0C30-GS31-21 (KM)

P-1.3.26

Histopathological Study of the Zoonotic *Anisakis* Parasite Treated with aPDT as a Control Approach

Paula Ramos[1], Ana Grade[1], Maria G. P. S. Neves[2], Adelaide Almeida[3], Maria A. F Faustino[2]

[1]Aquaculture, Valorisation and Bioprospective Division, Department of Sea and Marine Resources, Portuguese Institute for Sea and Atmosphere, Av. Doutor Alfredo Magalhães Ramalho, nº6, 1495-165 Lisboa, Portugal; [2]Department of Chemistry and LAQV-REQUIMTE, University of Aveiro, 3810-193 Aveiro, Portugal; [3]Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal.

The presence of *Anisakis* larvae in fishery products jeopardizes their safety and quality, which is a potential health risk to consumers, being also a matter of concern for official authorities. The use of photodynamic action can be considered a potential approach to treat this zoonotic parasite in fresh sea products.[1] Thus, several experiments were carried out to assess the efficacy of antimicrobial photodynamic therapy (aPDT) to inactivate the *Anisakis* larvae collected from Atlantic horse mackerel. Experiments with different photodynamic treatment time exposure (2, 8 or 24 h), light irradiance (4.0, 14 and 67 mW/cm^2) and photosensitizer concentrations (5.0, 25 and 50 µM of TMPyP) took place in microcosm conditions. The viability of the *Anisakis* larvae was evaluated at 2 h and 4 h after treatment. and in one case after 24 h. In order to evaluate tissue damage, histopathological study of the larvae was done at the end of the assays. Although not all parasites have been death during photodynamic treatment, larvae sections reveal anatomopathological changes. In the first third of the larvae, the muscular oesophagus changes its characteristic triradiate lumen. Additionally, on the posterior two thirds the intestine epithelium, the columnar cells present often loss of their nuclei and of their shape. It was also observed that some sections of the parasite, in both areas, showed irregular polymyarian muscle layer. This study aims to highlight the best conditions to inactivate *Anisakis* larvae and envisage its application in food industry; and consequently, lead to the reduction of the biological hazard associated with their presence in fishery products. In this communication will be presented and discussed the results of the histopathological study of the zoonotic *Anisakis* parasite treated with aPDT.

References

1) Preuß, A., Pfitzner, M., & Röder, B. Mosquito larvae control by photodynamic inactivation of their intestinal flora – a proof of principal study on Chaoborus sp. Photochem. Photobiol. Sci., 2019, **18**, 2374-2380

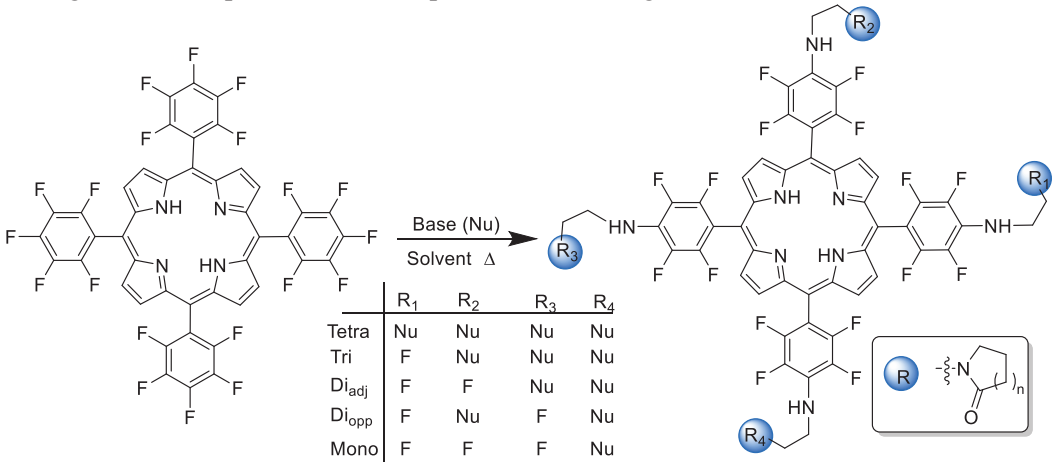
Acknowledgements: Thanks are due to University of Aveiro and FCT/MCTES (Fundação para a Ciência e Tecnologia/Ministério da Ciência e Tecnologia, Portugal) for the financial support to LAQV-REQUIMTE (UIDB/50006/2020) and Institute for Biomedicine – iBiMED (UID/BIM/04501/2013 and POCI-01-0145-FEDER-007628) and also to supported by PREVINE project PTDC/ASP-PES/29576/2017 through national funds and, when applicable, co-financed by European Union, FEDER, QREN, and COMPETE within the PT2020 Partnership Agreement. This work was also supported by I9+Proalga project MAR2020-16-01-03-FMP-0011 and Sanáqua project MAR-02.05.01-FEAMP-0004.

P-1.3.27

Synthesis and photophysical evaluation of new porphyrin-azabicyclo conjugates for PDT applications

Federico Frendo[1,2], Carlos J. P. Monteiro[1], Ana S. Joaquinito[1], Maria G. P. M. S. Neves[1], Giorgia Miolo[2], Maria A. F. Faustino[1]
1) LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 2) Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via Marzolo 5, 35131 Padova, Italy

Photodynamic therapy (PDT) is clinically approved treatment based on the interaction of a photosensitizing dye (PS), light and dioxygen and can find application in different areas, such treatment for oncologic and non-oncologic diseases, tumour detection, viruses and bacteria inactivation.[1] The mechanism of action involves the production, cytotoxic reactive oxygen species (ROS) which trigger a cascade of reactions that lead to the destruction of targets. The main advantages of PDT compared with traditional oncological therapies are due to its relatively non-invasive nature, lower systemic toxicity, relatively selective destruction of undesired cells, good cosmetic outcome and its ability to stimulate the immune system, as well as its good tolerability profile. However, PDT has also some limitations, and therefore there is significant room for improvement, especially in the search for new and more efficient PS. Our research group has been exploring the synthetic versatility of 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin (TPFFP) that has revealed to be a multipurpose template to be efficiently and selectively derivatized by means of nucleophilic aromatic substitution reactions, devising a plethora of functional groups [2], generating libraries of photo-active compounds with biological interest.



In this communication, the functionalization by nucleophilic aromatic substitution of TPFFP, with different azabicyclic amidines will be reported. The structural characterization, photophysical properties of new compounds will be presented.

References

1) Hamblin, M.R. *Photochem. Photobiol.* **2020**, 96, 506-516.
2) J. I. T. Costa, A. C. Tomé, M. G. P. M. S. Neves, J. A. S. Cavaleiro, *J. Porphyrins Phthalocyanines* **2011**, 15 (11-12), 1116-1133.

Acknowledgements. The authors thank University of Aveiro and FCT/MCT for the financial support provided to QOPNA research Unit (FCT UID/QUI/00062/2019), the Associate Laboratory CESAM (FCT UID/AMB/50017/2019) and to Project PREVINE - FCT-PTDC/ASP-PES/29576/2017), through national funds (OE) and where applicable co-financed by the FEDER-Operational Thematic Program for Competitiveness and Internationalization-COMPETE 2020, within the PT2020 Partnership Agreement. Thanks are also due to the Portuguese NMR and Mass Networks. Federico Frendo thanks to the Erasmus mobility programme.

P-1.3.28

Evaluation of Photosensitizer-Containing Superhydrophobic Surfaces for the Antibacterial Treatment of Periodontal Biofilms

Caroline Coradi Tonon[1], Shoaib Ashraf[1], Alessandra Nara de Sousa Rastelli[2], Goutam Ghosh, Tayyaba Hasan[1,3], QianFeng Xu[4], Alexander Greer[4,6,7] and Alan M. Lyons[4,5,6]

1) Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, 40 Blossom St, Boston, MA 02114, United State;

2) Department of Restorative Dentistry, School of Dentistry, Araraquara, São Paulo State University-UNESP, 1680 Humaitá St., Araraquara, SP 14801-385, Brazil;

3) Division of Health Sciences and Technology, Harvard University and Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States;

4) SingletO2 Therapeutics LLC, 75 Clinton Street, Staten Island, NY 10304, United States;

5) Department of Chemistry, College of Staten Island, City University of New York, Staten Island, New York 10314, United States;

6) Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, New York 10016, United States;

7) Department of Chemistry, Brooklyn College, City University of New York, Brooklyn, New York 11210, United States.

Antimicrobial photodynamic therapy (aPDT) has emerged as a promising approach to control biofilms involved in periodontal diseases. A unique superhydrophobic surface (SH) that generates airborne singlet oxygen was fabricated to potentially overcome the disadvantages of conventional aPDT. The aim of this in vitro study was to investigate the efficacy of the SH surface against a multi-species periodontopathogenic biofilm model. Biofilms were cultured on hydroxyapatite discs and treated by a SH surface loaded with chlorin e6 in amounts ranging from 96 nmols/cm^2 to 1100 nmols/cm^2 and illuminated with a 660 nm light-emitting diode. Most light incident on the SH surface was absorbed by the PS, the amount transmitted to the biofilm ranged from 18 J/cm^2 to 89 J/cm^2. The killing efficacy was assessed by counting of colony forming units, biofilm metabolism by XTT and confocal microscopy. The SH-aPDT showed a significant ability to inhibit both gram-positive and gram-negative bacteria in the multi-species biofilm model compared to chlorohexidine (CHX) and other controls. The effect of the treatments was dependent on the light dose and concentration of the embedded photosensitizer. The singlet oxygen ($^1O_2$) produced by the SH surface and transported to the biofilm inhibited both Gram-positive and Gram-negative periodontal bacteria, showing >5-log reduction of all three species: *Porphyromonas gingivalis*, *Streptococcus mutans* and *Actinomyces naeslundii*. Due to the absence of physical contact of the PS with the bacteria themselves, the SH device avoids staining, unlike conventional aPDT and CHX. Moreover, no drug-light interval is needed for SH device (pre-incubation time). The lifetime of airborne $^1O_2$ is 5000-fold greater than water-solvated $^1O_2$, which enables transport of this ROS across millimeter length-scales rather than difusion across nanometer lenght-scales for solvated PS systems. The results demonstrate that this unique SH technology is a promising method to disinfect and treat periodontal disease.

Acknowledgements. This work was supported by grant 2R44DE026083-03 from the National Institutes of Health.

P-1.3.29

The impact of photoinactivation with Ga^{3+} *meso*-PPIX on virulence factors of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Michalska Klaudia[1], Woźniak Agata[1], Krupińska Martyna[1], Moszyńska Agata [1], Nakonieczna Joanna [1], Grinholc Mariusz [1]
1) Laboratory of Molecular Diagnostics, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Gdansk, Poland.

Introduction: The ability of pathogenic bacteria to cause disease is dependent upon the production of agents termed 'virulence factors', such as toxins and adhesion molecules, that actively cause damage to host tissues during infection. The potential antimicrobial strategy might be also targeting virulence factors (e.g. inhibition of their production or activity) which would be harmful to the pathogen and allow the host immune system a better chance of clearing the infection. Further studies had revealed a significant impact of antimicrobial photodynamic inactivation (aPDI) towards virulence factors produced by both *Staphylococcus aureus* and *Pseudomonas aeruginosa* [1,2,3]. Ga^{3+} *meso*-PPIX (Ga^{3+} MPPIX) is dual function photosensitizer, which not only could block iron metabolism, but also due to the porphyrin structure acquire photodynamic properties.
Aim: The aim of this study was to determine of the influence of Ga^{3+} *meso*-PPIX-mediated aPDI on excreted virulence factors produced by Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) representatives.
Material and Methods: To investigate the effectiveness of aPDI on virulence factors production and activity, sub-lethal doses of photoinactivation with Ga^{3+} MPPIX upon green LED light irradiation (λ_{max}= 522 nm, 10.6 mW/cm^2) was determined for *S. aureus* clinical isolate and reference strain of *P. aeruginosa* ATCC 10145. The sub-lethal dose was defined as a dose that reduces the survival rate of treated bacteria vs. untreated bacteria by 0.5 – 2 $\log_{10}$ CFU/ml. Impact of sub-lethal doses of aPDI with Ga^{3+} MPPIX was determined on TSST-1 (*Toxic Shock Syndrome Toxin 1*) *S. aureus* superantigen production and on two *P. aeruginosa* virulence factors: pyocyanin and elastase B. Evaluation of TSST-1 production after aPDI exposure was performed based on the gene expression measured with the qRT-PCR method and the level of proteins produced by Western Blot immunoblotting. Moreover, changes in virulence factors after aPDI exposure e.g., pyocyanin and elastase B activity was measured with Elastin Congo Red Assay and spectrophotometric quantification of pyocyanin concentration respectively, before and after exposure to sub-lethal doses of aPDI.
Results: Photoinactivation with Ga^{3+} MPPIX had revealed the highest reduction in the survival rate of clinical isolate of *S. aureus* up to 6.08 $\log_{10}$ of CFU/mL. TSST-1 protein level decreased under the sub-lethal aPDI but there was no significant effect of aPDI on the expression level of *tsst-1* gene (fold change = -0.2 $\log_2$). Applied photoinactivation reduced the bacterial viability of *P. aeruginosa* strain (>3 $\log_{10}$ of CFU/ml) and reduced significantly pyocyanin concentration and elastase B activity after exposure *P. aeruginosa* to sub-lethal doses.

References
1) Bartolomeu, M.; Rocha, S.; Cunha, Â.; Neves, M.G.P.M.S.; Faustino, M.A.F.; Almeida, A. Effect of Photodynamic Therapy on the Virulence Factors of *Staphylococcus aureus*. *Frontiers in Microbiology,* 2016
2) Fila, G.; Kawiak, A.; Grinholc, M.S. Blue light treatment of *Pseudomonas aeruginosa*: Strong bactericidal activity, synergism with antibiotics and inactivation of virulence factors. *Virulence,* 2016
3) Ogonowska, P.; Nakonieczna J. Validation of stable reference genes in *Staphylococcus aureus* to study gene expression under photodynamic treatment: a case study of SEB virulence factor analysis. *Scientific Reports*, 2021

Acknowledgements. This work was supported by grant from National Science Centre: 2018/30/Q/NZ7/00281 (MG)

P-1.3.30

Development of new antimicrobial materials based on porphyrinoids

Tatevik Chilingaryan[1,2], Dr. Céline Frochot,[1] Dr. Philippe Arnoux,[1] Dr. Benoit Habermeyer[2]

1) Université de Lorraine, Laboratoire Réactions et Génie des Procédés, Nancy, France; 2) PorphyChem SAS, Dijon, France.

In France, each year more than 150.000 peoples suffer from infections caused by multi-resistant bacteria. Huge efforts have been dedicated to find alternative treatment methods to conventional antibiotics and Photodynamic Inactivation (PDI)[1,2] is considered as a promising one. PDI[3] is an innovative technique that is highly efficient against both gram-positive and gram-negative bacteria. Furthermore, it was demonstrated that bacteria are unbale to develop resistance against this technique. PDI is the combination of a photosensitizer (PS) and light to generate cytotoxic reactive oxygen species (ROS) that kill bacteria but also viruses, fungi and parasites.

In this project, we were interested in the development of new antimicrobial films, based on porphyrinoids (porphyrins, chlorins and phthalocyanines), for PDI applications mainly in hygiene and food safety. These films can be used on all kind of surfaces in order to kill bacteria and to prevent their growth. These antimicrobial materials also prevent surface contamination.

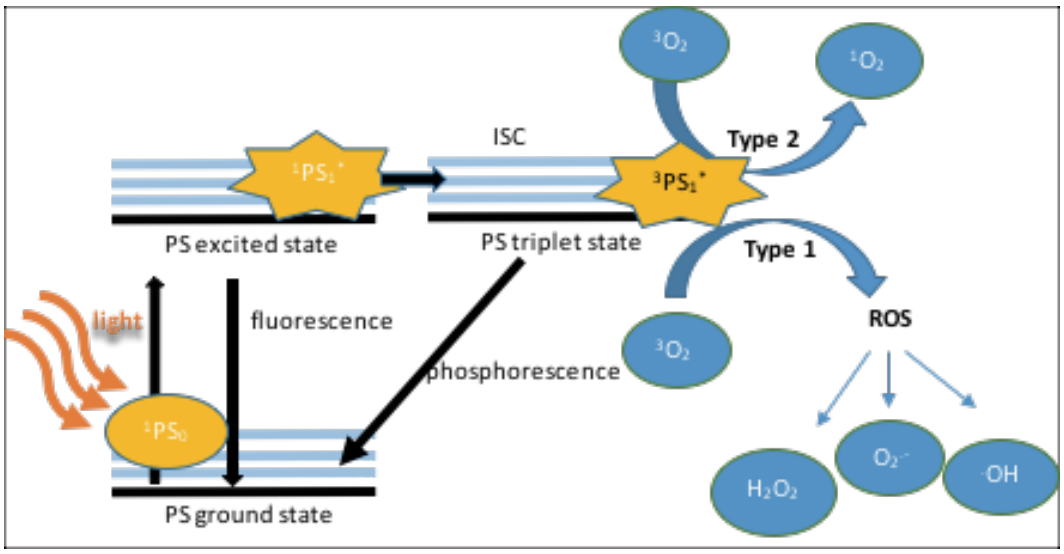


Fig. 1 – Perrin-Jablonski diagram for ROS generation via Type I and Type II.

For fabrication of antimicrobial films, two components are necessary: a photosensitizer that fills most of the criteria required by PDI (stability of the molecule, high molar absorption coefficient, high quantum yield of singlet oxygen) and a polymer film. Several candidates were studied as PSs and among them, water-soluble porphyrins. PSs were incorporated onto polyethylene terephthalate (PET) film surface and singlet oxygen production was studied.

References

1. L. Jiang, C. R. R. Gan, J. Gao, X. J. Loh, Small 2016, 12, 3609-3644.
2. P. H. H. Duong, P-Y. Hong, V. Musteata, K. V. Peinemann, S. Nunes, J. Nanomaterials and Polymers, 2017, 2, 6612-6616.
3. B. Habermeyer, T. Chilingaryan, R. Guilard, Bacterial Efficiency of Porphyrin Systems, J. Porphyrins and Phthalocyanines, 2021, 25, 359-381.

Acknowledgements. This work was supported by grant ANRT.

IL-1.4.1

Regulation of cyanobacterial photoprotection

Adjélé Wilson[1], Jiao Zhan[1], Fernando Muzzopappa[1], Claudia Steglich[2], Stanislaw Nizinski[3], Elena Andreeva[4] , Jacques-Phillipe Colletier[4], Michel Sliwa[3], Wolfgang Hess[2], Diana Kirilovsky[1]

[1]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur Yvette, France; [2]University of Freiburg, Faculty of Biology, Institute of Biology III, Freiburg, Germany; [3] Univ. Lille, CNRS, UMR 8516, LASIRE, Lille, France ; [4] Univ. Grenoble Alpes, CEA, CNRS, Institut de Biologie Structurale, Grenoble, France

Photosynthetic organisms need to sense and respond to light changing environmental conditions in order to maximize light absorption and photosynthetic efficiency while avoiding the formation of dangerous reactive oxygen species. Cyanobacteria can adapt their photosynthetic apparatus to efficiently harvest energy under low light intensities by increasing the synthesis and concentration of the phycobilisomes (PBSs), the cyanobacterial antenna, and avoid photodamage under high light conditions by increasing heat dissipation of excess absorbed excitation energy. This mechanism is induced by the modular photoactive Orange Carotenoid Protein (OCP) by interacting with the PBSs. The amplitude of photoprotection depends on the concentration of OCP in the cell, the rate of photoactivation and the strength of PBS-OCP interaction. The expression of most OCPs is constitutive but is increased under stress conditions in which phycobiliprotein synthesis decreases. I will describe the recently discovery of a post-transcriptional regulatory mechanism linking the expression of PBS core proteins directly and in an inverse fashion to the synthesis of OCP[1]. OCP synthesis is regulated by a small regulatory RNA (sRNA), ApcZ, originating from the 3' end of the *apcABC* operon. ApcZ inhibits OCP expression by base pairing to the start codon, the following four codons and the region upstream of the start. The absence (or decrease) of ApcZ largely increases OCP concentration under stress conditions. The OCP is formed by N-terminal (NTD) and C-terminal (CTD) domains which strongly interact in the orange inactive form (OCP^O) and shared a ketocarotenoid molecule. Upon strong illumination OCP^O converts into the open active red OCP (OCP^R). Between the photoexcitation of the carotenoid and the final red open conformation several red intermediary steps occur. The protein becomes red already in ps and the carotenoid translocates into the NTD in several μs while the opening of the protein occurs slower. We investigated several factors influencing these intermediary steps and the rates of photoactivation and recovery including the carotenoid nature and the position of tags. Only the red open active OCP^R is able to interact with the PBS. Almost nothing is known about the OCP and PBS amino acids involved in this interaction. We constructed 23 OCP mutants in the different NTD surfaces to learn more about OCP-PBS interaction. We demonstrated that only the NTD surface that in the orange OCP interacts with the CTD is involved in OCP binding to PBS. Modification of these amino acids also influences OCP photoactivation and/or recovery rates indicating that they are also involved in the translocation of the carotenoid.

IL-1.4.2

From light-harvesting to quenching in plant antenna complexes: a new perspective from atomistic simulations

Benedetta Mennucci
Dipartimento di Chimica e Chimica Industriale, University of Pisa, via G. Moruzzi 13, 56124, Pisa, Italy

Light-harvesting complexes (LHCs) of plants can switch from an active, light-harvesting state to a quenched state where excess energy is dissipated into heat however the mechanism of quenching and the identity of the quenched/active states are still subject to debate. Here we apply an integrated computational strategy[1] which combines molecular dynamics, enhanced sampling techniques, and multiscale methods to CP29, a minor LHC, finding that the commonly accepted hypothesis of a quenching mechanism localized on a single pair of strongly coupled pigments is not sufficient, but a dynamic network of pigments and interactions must be considered.

References

1) Cupellini, L., Bondanza, M., Nottoli, M. & Mennucci, B. Successes & challenges in the atomistic modeling of light-harvesting and its photoregulation. BBA - Bioenergetics 1861, 148049 (2020).

IL-1.4.3

Exploring Carotenoid-Mediated Photophysics in Plants with Ultrabroadband 2D Electronic Spectroscopy

Minjung Son[1], Alberta Pinnola[2], Roberto Bassi[3], Gabriela S. Schlau-Cohen[1]
1) Massachusetts Institute of Technology, Department of Chemistry, Cambridge, USA; 2) University of Pavia, Department of Biology and Biotechnology, Pavia, Italy; 3) University of Verona, Department of Biotechnology, Verona, Italy

Plants absorb sunlight across the visible region of the solar spectrum and collect the energy required for photochemical reactions in lower-lying states. Previous 2D experiments to study plants were limited to these low-energy states. We describe the development of ultrabroadband 2D electronic spectroscopy and its application to the major antenna complex of plants.[1] First, we identify a debated dark state on a single carotenoid, lutein 2, that mediates relaxation.[2] Second, we measure chlorophyll-to-carotenoid energy transfer, a hypothesized but previously unobserved pathway to safely dissipate excess energy.[3] We also investigate the molecular and environmental parameters that can control the magnitude of this dissipative energy transfer.[4]

References

1) M. Son, S. Mosquera-Vasquez, G. S. Schlau-Cohen, "Ultrabroadband 2D Electronic Spectroscopy with High-Speed, Shot-to-Shot Detection." *Optics Express*, 25, 18950 (2017).

2) M. Son, A. Pinnola, R. Bassi, G.S. Schlau-Cohen, "The Electronic Structure of Lutein 2 is Optimized for Light Harvesting in Plants." *Chem*, 5, 575 (2019).

3) 10. M. Son, A. Pinnola, S.C. Gordon, R. Bassi, G.S. Schlau-Cohen. "Observation of Dissipative Chlorophyll-to-Carotenoid Energy Transfer in Light-Harvesting Complex II in Membrane Nanodiscs." *Nature Communications* 11, 1 (2020).

4) M. Son, A. Pinnola, G.S. Schlau-Cohen, "Zeaxanthin Independence of Photophysics in Light-Harvesting Complex II in a Membrane Environment." *BBA-Bioenergetics* 5-6, 1861 (2020).

Acknowledgements. This work was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciencesunder Award #DE-SC0018097 to G.S.S.-C.

OC-1.4.4

The keto group in β2 of the carotenoid tunes the Orange Carotenoid Protein photocycle kinetics

Volha Chukhutsina[1] and Jasper van Thor[1],
1) Department of Life Sciences, Imperial College London, London SW7 2AZ, United Kingdom

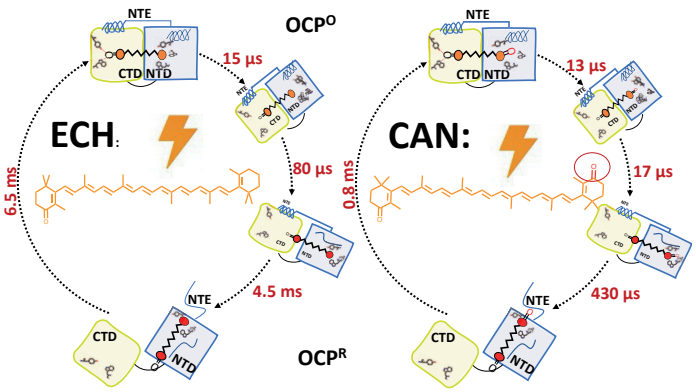


Fig. 1 – Photocycle intermediates resolved for OCP constructs with the two different carotenoids: ECH -echinenone, CAN-canthaxanthin

All vital biological processes, including photosynthesis, rely on the function of various photosensors and photoreceptors. In cyanobacteria light-harvesting of the photosynthetic machinery is controlled by a unique photosensitive protein, Orange Carotenoid Protein (OCP). Being found only among cyanobacteria, OCP is the only known photoreceptor which uses a carotenoid for its light activation. Understanding and, therefore, gaining control over OCP light-activation has a broad range of applications from improving photosynthesis productivity to potentially revolutionizing the field of optogenetics.

OCP is a water-soluble protein composed of the two domains: an all-helical N-terminal domain (NTD) and a C- terminal domain (CTD). Absorption of blue–green light by the carotenoid induces conformational changes, converting stable inactive OCP^O (the so-called orange form) into unstable but active OCP^R (red form), which are also followed by drastic changes in the OCP absorption. The OCP has been shown to bind and be activated by various keto-carotenoids (3-hydroxyl-echinenone, hECN; echinenone, ECN; and canthaxanthin, CAN). The conserved keto group of the β1-ring of the keto-carotenoid is hydrogen bonded to Tyr201 and Trp288 of CTD. The other ring (β2) is nestled within a group of conserved aromatic residues (Trp41, Tyr44, Trp110) in NTD. The structure of β2-ring is actually specific for different keto-carotenoids. For example, CAN contains a second keto-group at the 4' position, which is absent in ECH. The mechanism of OCP light activation is considered to be independent on the keto-carotenoid bound to it and is initiated by hydrogen bonded rupture between the keto group of the β1 ring and Tyr201 and Trp288[1]. Therefore, the photocycle intermediates resolved from OCP_{ECH} are considered to be characteristic for OCP in general[2].

In this study we for the first time to our knowledge study the effect of the keto group in the β2 ring of the keto-carotenoid on the OCP photocycle intermediates and activation energies. For that OCP constructs with ECH and CAN in their structure were studied by performing us-ms time-resolved spectroscopy and Arrhenius temperature dependence measurements. The results indicate different activation energies and photocycle intermediate rates for the OCP with different carotenoids imbedded. The OCP_{CAN} demonstrates lower activation energies both for OCP^O-OCP^R and OCP^R-OCP^O reactions. As a result, the photocycle rates are also faster for OCP_{CAN}. The results for the first time indicate that the presence of a keto group in β2 tunes the OCP photocycle.

References

1) F. Muzzopappa & D. Kirilovsky, Changing Color for Photoprotection: The Orange Carotenoid Protein. Trends in Plant Science (2020)
2) P. E. Konold, et al. Photoactivation Mechanism, Timing of Protein Secondary Structure Dynamics and Carotenoid Translocation in the Orange Carotenoid Protein. J. Am. Chem. Soc. (2019).

Acknowledgements. This project was supported by the EMBO long-term fellowship (EMBO ALTF 244-2017) and has received funding from the European Union Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 839389) to V.C.

IL-1.4.5

Hydrophobic mismatch as a possible trigger of NPQ

Alexander V. Ruban
School of Biological and Chemical Sciences, Queen Mary, University of London, UK

Non-photochemical quenching, NPQ, is a physiological process that protects the photosynthetic apparatus of oxygenic photosystem II from harmful unused energy that is accumulated in excess in high light environment. Despite a number of decades of intense NPQ research no clarity exists as to how exactly the proton gradient triggers the quenching in LHCII antenna. Here, I present a novel view on this process proposing the onset of the hydrophobic mismatch between proteins of antenna and photosynthetic membrane triggered by the gradient. This proposal is based on the synthesis of various experimental and theoretically-obtained data available so far. Observation of thylakoid membrane thinning and enhanced hydrophobicity following illumination of leaves and algal cells, effect of sterols, cross-linkers and other agents promoting or mitigating the hydrophobic mismatch will be discussed. The involvement of hydrophobic mismatch in NPQ of plants is consistent with the fact that the minimum components of the quenching are the proton gradient and the major LHCII complex, whilst zeaxanthin and PsbS protein are mere regulators of the extent of the mismatch and response of the protein.

IL-1.4.6

Chlorophyll *f* site assignments in far-red light-acclimated photosystem I

Christopher J. Gisriel[1], David A. Flesher[2], Hao-Li Huang[1], Krystle Reiss[1], Victor S. Batista[1], Jimin Wang[2], Donald A. Bryant[3], and Gary W. Brudvig[1,2]

1) Yale University, Department of Chemistry, New Haven, USA; 2) Yale University, Department of Molecular Biophysics and Biochemistry, New Haven, USA; 3) The Pennsylvania State University, Department of Biochemistry and Molecular Biology, State College, USA

During far-red light (FRL) photoacclimation, chlorophyll (Chl) *f* molecules are bound to photosystem I (PSI) in addition to Chl *a*, allowing for far-red light absorption[1]. Two FRL-PSI structures have been reported using cryo-EM from different cyanobacterial species[2,3], but they exhibited unexpected inconsistencies in their Chl *f* site assignments[4]. We reprocessed one of these structures to a higher resolution, and performed a quantitative assessment of Chl substituents in the cryo-EM maps to identify Chl *f*-binding sites in the two cryo-EM maps. The two cryo-EM maps provide direct evidence for high occupancy Chl *f*-binding at two and three sites, respectively, and three more sites in each structure exhibit strong indirect evidence for Chl *f* binding. Challenges that accompany making Chl *f* assignments based on cryo-EM maps, and common themes in Chl *f* binding are described that clarify the current understanding of the molecular basis for FRL photoacclimation in photosystems.

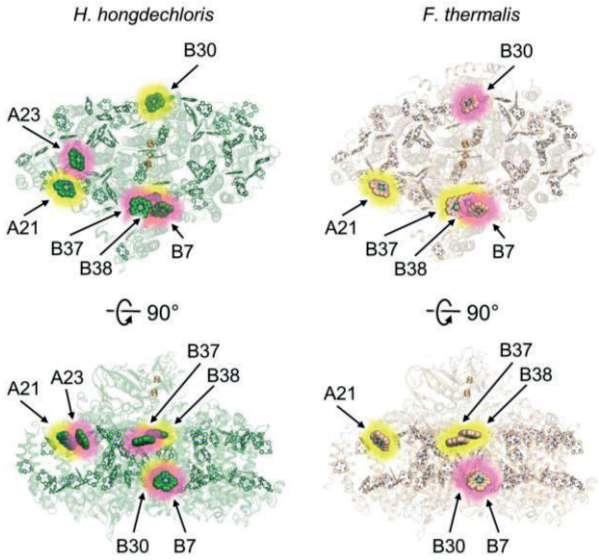


Fig. 1 –Chl f *sites in two FRL-PSI structures. Left and right panels show one monomer of the FRL-PSI structures from* H. hongdechloris *and* F. thermalis*, respectively. Top panels show a stromal view and bottom panels show a membrane plane view from the center of the trimer. Only tetrapyrrole rings are shown for clarity. Pink glows are shown for Chl sites that exhibit strong direct and indirect evidence for being highly occupied Chl* f *(three sites in* H. hongdechloris *and two sites in* F. thermalis*), and yellow glows are shown for Chl sites that exhibit strong indirect evidence only for binding by Chl* f *at high occupancy (three sites in each structure).*

References

1) Gan, F. *et al.* Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light. *Science* **345**, 1312–1317 (2014).
2) Gisriel, C. *et al.* The structure of Photosystem I acclimated to far-red light illuminates an ecologically important acclimation process in photosynthesis. *Sci. Adv.* **6**, eaay6415 (2020).
3) Kato, K. *et al.* Structural basis for the adaptation and function of chlorophyll *f* in photosystem I. *Nat. Commun.* **11**, 238 (2020).
4) Gisriel, C. J., Wang, J., Brudvig, G. W. & Bryant, D. A. Opportunities and challenges for assigning cofactors in cryo-EM density maps of chlorophyll-containing proteins. *Commun. Biol.* **3**, 408 (2020).

Acknowledgements. This work was supported by Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences grant DE-FG02-05ER15646 to G.W.B. and Grant DE-SC0001423 to V.S.B, National Science Foundation grant MCB-1613022 to D.A.B., and the Forest B.H. and Elizabeth D.W. Brown Postdoctoral Fellowship for Plant Science to C.J.G.

IL-1.4.7

BREAKING THE RED-LIMIT: DRIVING OXYGENIC PHOTOSYNTHESIS WITH FAR-RED LIGHT

Roberta Croce

Vrije Univesiteit Amsterdam, Amsterdam, the Netherlands
(*) r.croce@vu.nl

Photosynthetic organisms use sunlight energy to fix CO_2 into carbohydrates, in this way sustaining almost all life on our planet. The capacity of these organisms to harvest light is a crucial factor in the photosynthetic process, especially in light-limited conditions, which occur in greenhouses and canopies. However, plants and algae only use the visible part of the solar spectrum, discarding more than 50% of the photons reaching the surface of the Earth. This is because their photosynthetic proteins bind as main pigments chlorophyll (Chl) *a* and *b*, which have intense absorption in the red and the blue regions of the electromagnetic spectrum but do not absorb above 700 nm. For a long time, it was believed that cyanobacteria, the prokaryotic ancestors of plant chloroplasts, also could only use visible light to drive photosynthesis. The discovery of species containing Chl *d* and Chl *f*, which absorb in the far-red region of the spectrum, has shown that this is not the case. However, due to their different energetics, Chl d and *f* are expected to alter the excited state dynamics of the photosynthetic units and, ultimately, their performances. How can thus cyanobacteria use far-red light for efficient photochemistry?
To answer this question we use a combination of biochemistry and spectroscopic measurements on intact cells and isolated complexes. We show that chlorophyll *f* insertion marginally affects the charge separation efficiency of Photosystem I [1] but decreases significantly that of Photosystem II [2]. The difference between the two photosystems and the possibility to introduce Chl d and f in plant photosynthetic complexes to extend the photosynthetic active radiation in crops will be discussed.

References

[1] Tros M, Mascoli V, Shen G, Ho MY, Bersanini L, Gisriel CJ, Bryant DA, Croce R, Chem, **7**, 155-173 (2021)
[2] Mascoli V, Bersanini L, Croce R, Nature Plants, **6**, 1044-1053 (2020)

OC-1.4.8

Electrostatic Control of Reaction Centre Excitation in Photosystem II

Abhishek Sirohiwal[1], Dimitrios A. Pantazis[1]

1) Max-Planck-Institute für Kohlenforschung, Department of Molecular Theory and Spectroscopy, Mülheim an der Ruhr, Germany.

Photosystem II is a multi-subunit pigment-protein complex that utilizes sunlight to trigger charge-separation and catalyse water oxidation. The charge separation cascade is initiated in the reaction centre (RC), which is composed of six pigments (four Chlorophyll *a* and two Pheophytin *a*) arranged symmetrically along the D1 and D2 core polypeptides (Fig. 1). Biological evolution favoured productive electron transfer only along the D1 side with the precise nature of the initial excitation event(s) remaining under debate. In this work,[1-2] we employ multiscale quantum mechanics/molecular mechanics (QM/MM) coupled with high-level computations (full time-dependent density functional theory with range-separated functionals benchmarked against coupled cluster theory) to investigate the excited state profile of the RC (Fig. 1). Our results describe for the first time at a fundamental electronic structure level precisely how differential protein electrostatics create the observed excitation asymmetry within the RC. By simultaneous quantum chemical treatment of multimeric pigment assemblies we identify the critical pairs of RC pigments associated with low-lying charge-transfer states and we eventually propose a novel model to describe excitation of Photosystem II reaction centre based on two parallel charge-separation pathways. Among others, our new model explains the triggering of charge separation by direct absorption of far-red photons (700-800 nm), i.e. beyond the known "red-limit" (680 nm) of oxygenic photosynthesis.

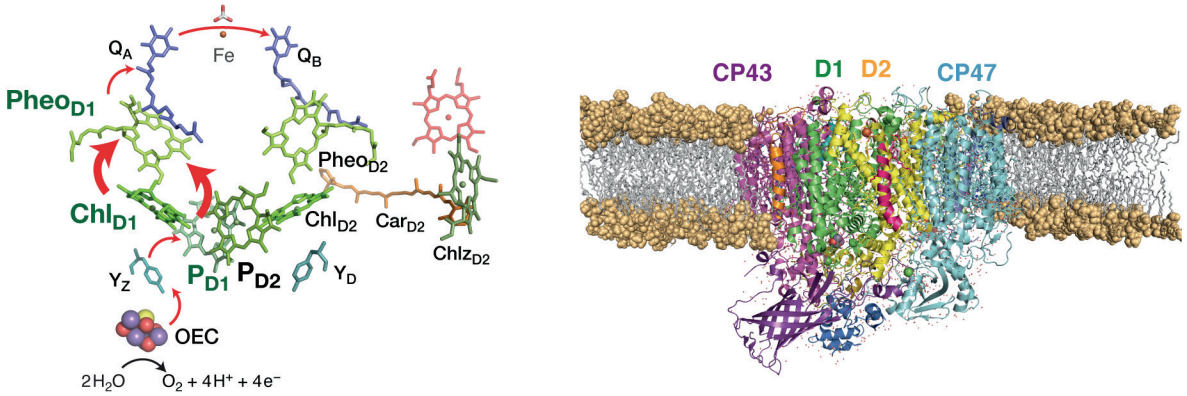


Fig. 1 – (left) Overview of symmetrically arranged pigments in the reaction centre along with key redox-active components of Photosystem II and (right) molecular-mechanics based model of the lipid-bilayer bound Photosystem II.

References

1) A. Sirohiwal, F. Neese, D. A. Pantazis, Protein Matrix Control of Reaction Center Excitation in Photosystem II. *J. Am. Chem. Soc.*, **142**, 42, 18174-18190, 2020.
2) A. Sirohiwal, F. Neese, D. A. Pantazis, How Can We Predict Accurate Electrochromic Shifts for Biochromophores? A Case Study on the Photosynthetic Reaction Center. *J. Chem. Theory Comput.*, **17**, 3, 1858-1873, 2021.

Acknowledgements. The authors gratefully acknowledge support by the Max Planck Society.

P-1.4.9

How pulse energy and wavelength affect ultrafast dynamics of Orange Carotenoid Protein? Attempt to define safe regime of femtosecond excitation based experiments.

Stanislaw Nizinski[1,2], Elena Andreeva[3], Jacques-Philippe Colletier[3], Adjele Wilson[4], Diana Kirilovsky[4], Gotard Burdzinski[1], Michel Sliwa[2]
1) Quantum Electronics Laboratory, Faculty of Physics, Adam Mickiewicz University in Poznan, Umultowska 85, 61-614 Poznan, Poland; 2) Univ. Lille, CNRS, UMR 8516 - LASIR - Laboratoire de Spectrochimie Infrarouge et Raman, F-59000 Lille, France; 3) Institut de Biologie Structurale (IBS), Univ. Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France; 4) Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Universite Paris-Sud, Universite Paris-Saclay, 91198 Gif-sur-Yvette, France

Orange Carotenoid Protein (OCP) is a water-soluble pigment protein responsible for dissipation of excited state energy harvested by cyanobacterial antenna complexes, phycobilisomes. OCP performs its photoprotective function in a selective way: in the dark it occurs in inactive form (OCP^O), but after illumination with a blue-green light it undergoes photoconversion with a low quantum yield (< 1%) to quenching-capable form called OCP red (OCP^R). The mechanism that controls the photoconversion of OCP^O to OCP^R has been recently discussed in literature in a multiple papers. Upon absorption of a blue-green photon, the carotenoid embedded inside OCP undergoes $S_0 \rightarrow S_2$ transition, forming two S_1 / ICT coupled states and a distorted S* state within hundreds of fs. These states decay to the ground state, generating redshifted forms of OCP with very small yield under 1%. These forms are precursors of the final quenching-capable OCP^R.
Femtosecond transient absorption spectroscopy is a powerful technique, which has been already widely employed by a many groups to describe first steps of OCP photoactivation. However current state of literature does not provide perfectly consistent picture of these steps, and detailed information about excitation pulse energy density is not always provided by the authors. We have found that it is very hard to obtain always the same consistent description of OCP photodynamics when experiments are repeated, due to high complexity of mechanism, sample unstability and heterogeneity. Therefore it is critical to perfectly control all important experimental conditions, and understand impact of each of them. We found, that when energy density of the pump pulse exceeds linear regime, additional long living products are formed, which artificially boost calculated yield of the first red OCP intermediate (P1). Obviously, observation of such products does not constructively contribute to the biological knowledge about the system, as they are artefacts of the technique which require high energy pulses, unreachable in the natural environment. From the other side, observation of the low-yield products is required in OCP studies, therefore one cannot discard high energy pulses as they help to maximize signal to noise ratio. It is important to go for tradeoff and localize the maximum safe regime of excitation energy density. So paradoxically, it interesting to investigate formation of high pulse energy products, to find the pulse energy which forms them in negligible quantity and does not significantly distorts the formation of biologically-relevant species.

Acknowledgements. This work was performed with financial support of the Polish National Science Centre (NCN), project 2018/31/N/ST4/03983, and French National Research Agency (ANR), project DynOCP (ANR-18-CE11-0005).

P-1.4.10

Steady-state and time-resolved X-ray scattering and UV-vis spectroscopy demonstrate that oligomerization processes limit photoactivation and recovery of the cyanobacterial Orange Carotenoid Protein

Elena A. Andreeva[1], Stanislaw Nizinski[2], Adjélé Wilson[3], Matteo Levantino[4], Elke De Zitter[1], Fernando Muzzopappa[3], Aurélien Thureau[5], Ninon Zala[1], Gotard Burdzinski[2], Michel Sliwa[6], Diana Kirilovsky[3], Giorgio Schirò[1], Jacques-Philippe Colletier[1]
1) Institut de Biologie Structurale (IBS), Univ. Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France; 2) Quantum Electronics Laboratory, Faculty of Physics, Adam Mickiewicz University in Poznan, Poznan, Poland; 3) Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris- Saclay, Gif-sur-Yvette, France; 4) European Synchrotron Radiation Facility (ESRF), BP 220, Grenoble, France; 5) Synchrotron Soleil, Beamline SWING, Gif Sur Yvette Cedex, France; 6) Univ. Lille, CNRS, UMR 8516 - LASIR - Laboratoire de Spectrochimie Infrarouge et Raman, Lille, France.
Email: eandreeva@ibs.fr

The photoactive Orange Carotenoid Protein (OCP), involved in cyanobacterial photo-protection [1], is a two-domain protein which upon light absorption transitions from a compact inactive "dark" state (OCP^O) to an extended photoactive "light" state (OCP^R) [2,3]. The molecular mechanism enabling photoactivation involves migration of the carotenoid pigment from the interface between the two domains into the NTD, and subsequent domain dissociation. It remains unclear what the exact structure of OCP^R is, on which timescale it forms, and whether or not oligomerization [2,4] is involved in the regulation of OCP function – either in the inactive or photo-activated state. Here, we used a combination of steady-state and time-resolved (TR) X-ray scattering and spectroscopy to address these three specific issues. Our results inform on the time scales associated to the dissociation and reassociation of domains in monomeric OCP, yielding OCP^R and enabling recovery of OCP^O, respectively. They furthermore demonstrate that oligomerization occurs at the OCP^O and OCP^R level, limiting the photoactivation of OCP^O and the OCP^O to OCP^R thermal recovery, respectively.

References

[1] Kirilovsky D. & Kerfeld C. A., (2016). Cyanobacterial photoprotection by the orange carotenoid protein. Nat. Plants 2, 16180.
[2] Wilson A. et al., (2008). A photoactive carotenoid protein acting as light intensity sensor. PNAS, 105 (33): 12075-12080
[3] Haijun L. et al., (2016). Dramatic Domain Rearrangements of the Cyanobacterial Orange Carotenoid Protein upon Photoactivation. Biochemistry, 55 (7): 1003–1009
[4] Golub M. et al., (2019). Solution Structure and Conformational Flexibility in the Active State of the Orange Carotenoid Protein: Part I. Small-Angle Scattering. J. Phys. Chem. B, 123, 9525−9535

P-1.4.11

Blue light modulates seed germination in tomato through the interaction of the cryptochrome 1a with ABA and GA

Rogério Falleiros Carvalho[1], Reginaldo Oliveira[1]
1) Faculty of Agronomic and Veterinary Science-FCAV-São Paulo State University-UNESP, Department of Biology Applied to Agriculture, Jaboticabal, SP, Brazil

A multifaceted approach has been discussed regarding the role of blue light (BL) in the seed germination in tomato[1,2]. However, from BL signaling, many points remain to be elucidated, including participation of the abscisic acid (ABA), gibberellin (GA), and BL photoreceptor cryptochrome (cry). We hypothesized for the first time that cry1a of tomato interacts with ABA and GA in the seed germination from blue light BL signaling. The experiments were carried out in growth chamber with continuous BL fluence rate (0 or dark; 1; 5; 10; 15 and 25 µmol $m^{-2}s^{-1}$). We observed that tomato (*cv.* Moneymaker) seeds presented high germination rate (G%) and index of germination speed (IGS) in the dark. However, all BL fluence partially inhibits the germination, increasing the time to reach 50% of final/maximum germination (T50). Regarding the function of cry1a, the loss of its function in the mutant (*cry1a*) reduced the G% and IGS, besides the fact that 5 µmol of gibberellic acid (GA_3) did not recover the germination, whereas 0,25 µmol of abscisic acid (ABA) amplified the negative effects of BL in the germination of this genotype. We also observed in dark and BL that the *notabilis,* which is ABA-deficient mutant, and *procera,* which shows constitutive response to GA, increased the G% and IGS. These responses were partially related to changes in the activity of *endo-β*-mannanase, *β*-mannosidase and *α*-galactosidase enzymes. Histochemical analysis of tomato *cv*. Micro-Tom transgenic lines harboring the gene reporter *GUS* fused to promoters of the *RD29B* and *GA2OX*, responsive to ABA and GA, respectively, revealed a temporary increase (36h) in seeds exposed to dark, but a reduction in BL after 72h, confirming the fundamental role of BL on these hormones during seed germination. Thus, in this study, we showed for the first time the influence of cry1a in the photoregulation of seed germination in tomato, and the role of BL in the activity of hydrolytic enzymes related to the physical breaking of the barrier imposed by seed endosperm.

References

1) Aneli, N. A. 1940. Inhibiting action of blue light upon germination of tomato seeds. Comptes Rendus De L Academie Des Sciences De L Urss 28:267-69.
2) Omidvar, V., and M. Fellner. 2015. DNA methylation and transcriptomic changes in response to different lights and stresses in *7B-1* male-sterile tomato. Plos One 10 (4).

Acknowledgements. This work was supported by grant number 2018/20748-3 from São Paulo Research Foundation – FAPESP.

PL-4

Dark CPDs and Genomic Sites Hypersensitive to UV

Douglas E. Brash[1], Sanjay Premi[1,3], Alejandro Garcia-Ruiz[1], Vijay Menon[1], Carlos Angele-Martinez[1], and Leticia C. P. Goncalves[1,2]
1) Yale University School of Medicine, Department of Therapeutic Radiology, New Haven, CT, USA; 2) Universidade de São Paulo, Departamento de Química Fundamental, São Paulo, SP, Brazil; 3) Present address: Moffitt Cancer Center, Department of Tumor Biology, Tampa, FL, USA

Cyclobutane pyrimidine dimers (CPD) in DNA are lethal and mutagenic, causing the UV-signature mutations seen in skin tumors. Two surprising features of CPDs have surfaced recently. First, although creating a CPD always requires high energies capable of exciting an electron to a higher orbital, this energy needn't come from UV radiation. It can be generated chemically, by a process termed "chemiexcitation". Second, specific individual dipyrimidines are 100-fold more sensitive to forming CPDs than the rest of the genome; we've termed these sites "CPD hyperhotspots". Chemiexcitation in the skin begins when UVB or UVA activate enzymes that synthesize the radicals superoxide and nitric oxide, resulting in peroxynitrite. The latter oxidizes melanin or its precursors to a form that can be attacked by O_2 to form a cyclic peroxide, dioxetane. This four-member ring is unstable and decomposes to give a carbonyl containing an electron excited to a triplet state; the energy transfers radiationlessly to nearby DNA to form a CPD. Enzyme activation can persist for hours, allowing chemiexcitation to generate CPDs in the dark. We find that other biomolecules present in skin can also be chemiexcitation substrates.
CPD hyperhotspots were discovered by nicking DNA lesion sites with a repair enzyme, tagging nicks with linkers detectable by NextGen sequencing, and analyzing the data with statistical methods having the resolution to quantify rare lesions at single-base resolution across the genome. High-frequency outliers constituted a distinct category and fell into three classes. The strongest class occurred in gene promoters, particularly for RNA-binding proteins, at a motif present at a) ETS family transcription factor binding sites, previously known to be UV targets and now shown to be among the most sensitive in the genome, and b) sites of translation regulation. These CPD sites aligned precisely with recurrent UV-signature mutations in gene promoters in melanomas. Another class occurred at simple PyPyPy runs and the reason for their UV sensitivity remains unknown. The third class was intriguing, occurring at A_{2-15}TTCTPy, developing “dark CPDs” long after UV exposure, and repairing CPDs slowly; in melanocytes this class had accumulated CPDs prior to the experiment. The CPD hyperhotspots were originally discovered in melanocytes and fibroblasts; we now find similar hyperhotspots in keratinocytes.
The high frequency of CPDs at these sites means that, at sunburn levels of UV exposure, every cell would have a hyperhotspot CPD in each of the ~20 cell pathways in which the motifs occur, letting hyperhotspots act as epigenetic marks. UV could drive tumor evolution by direct changes in cell physiology rather than acting solely through rare mutations. The high CPD prevalence also favors co-occurring mutations, allowing tumor evolution to use genes that are only weak phenotypic drivers.
Outlier DNA sequences extraordinarily sensitive to environmental agents can be sentinels for monitoring personal carcinogen exposure, e.g., by measuring CPD levels. We are investigating their use as genomic dosimeters for measuring past UV exposure and future skin cancer risk, so that UV-sensitive patients can be monitored to detect skin cancers at a curable stage.

Acknowledgements. This work was supported by National Institutes of Health grants R01AR070851, R01ES030562, and P50CA121974 and Department of Defense CDMRP grant CA093473.

PL-5

Does light have a unique niche for enabling nanotechnology?

Tayyaba Hasan
Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; Division of Health Sciences and Technology, Harvard University and Massachusetts Institute of Technology, Cambridge, MA 02139

With a fairly routine use of omics, there is evolving a clearer understanding of disease mechanisms and their regulation. It is now becoming increasingly clear for cancer or non-cancer pathologies, that disabling of single possibly what are believed to be the dominant pathways or targets, will not provide effective long-term cures. Inhibition of a given pathway leads the cancer cell or the pathogen to commandeer compensatory mechanisms for its survival. A combination of therapeutics addressing multiple mechanisms has therefore emerged as a desirable mode of disease management, but pharmacokinetics and dosing now become complex and crucial. While the concept of combination treatment is by no means new, there are some newer emerging approaches to administering it that are exciting. These include the use of nanotechnology and light as a switch, which can provide cytotoxicity while at the same time priming the microenvironment and helping deliver molecules at the right time to the right place. In this regard Photodynamic Therapy (PDT) may have a special niche since it provides the additional level of selectivity because of the requirement of the sensitizing molecules and photons being present together in adequate concentrations to achieve the cytotoxic effect. And where these "reagents" are below the threshold for cytotoxicity they create a priming effect on the microenvironment and cause remote physiologic changes such as immune effects and permeability enhancement that enable other therapies to provide more efficient and tolerable treatments. Recognition of these phenomenon explains the distant control of metastasis by a local treatment. Similarly, induced or inherent resistance can be mitigated using PDT-activated nanotechnology due to the diversity of mechanisms that can be brought into play with the appropriately designed nano constructs. Photodynamic activation and PDT-triggered approaches therefore can have special attributes to make them unique and potentially of high impact. But for this to be successful, there are a significant number of questions that still remain unanswered. Results from the literature and our own studies in the context of the potential of PDT-inspired combination therapeutics and nanotechnology will be discussed.

Acknowledgements. This work was supported by grants: P01 CA084203 through the NIH/NCI, UH3 CA189901 through the NIH/NCI, R01 CA231606 through the NIH/NCI, 2R44 DE026083 through the NIH (with Singlet02 Therapeutics LLC), FA9550-20-1-0063 through the DoD/AFOSR, and R01 CA266855 through the NIH /NCI.

IL-2.1.1

Photosensitized oxidation of proteins: is disulfide bond damage important?

Michael J. Davies[1]

1) Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Copenhagen 2200, Denmark.

Disulfide bonds are key determinants of protein structure and function, and are highly conserved across nearly all proteomes. They are particularly abundant in many extracellular proteins, including those with critical structural, ligand binding or receptor function. In the studies discussed here, we demonstrate that photo-sensitized oxidation of protein disulfides by singlet oxygen (and also a number of other oxidants) results in the formation of long-lived (half-life of a few hours), but reactive species, believed to be either a R-S^{+}(OO^{-})-S-R' species (i.e. a peroxidic adduct formed between singlet oxygen and the disulfide bond) or a thiosulfinate R-S(O)-S-R'. Accumulating evidence is consistent with further reaction of these species with another thiol, with this resulting in the formation of a thiol adduct to the protein at the former disulfide bond and cleavage of the initial disulfide[1,2].

In the case of glutathione (GSH), this results in glutathionylation of the protein[3,4]. The protein-GSH adducts have been characterized by both mass spectrometry (MS; ions with *m/z* +306 and +712 assigned to the addition of 1 and 2 GSH respectively) and immunoblotting after SDS-PAGE separation, using anti-GSH antibodies. The extent of GSH addition is increased by the use of D_2O buffers consistent with the intermediacy of $^1O_2$. Such adducts have been detected on multiple proteins, demonstrating that this is a common pathway[3,4]. GSH adduction is reversed by both non-enzymatic and enzymatic (glutaredoxin) reducing systems, but this does not repair the original disulfide bond. Removal of the disulfide bond before oxidation prevents adduct formation.

For proteins with free Cys residues, these reactions result in the formation of novel protein-protein dimers linked by a new disulfide[5,6]. These reactions require both a free thiol and the oxidized disulfide bond. The new inter-protein disulfide can be reduced by added reducing agents such as DTT and TCEP. The new dimeric species have been characterized by immunological, intact protein MS, and LC-MS peptide mass mapping. The latter has allowed unequivocable identification of the sites of the disulfides and also exact residues involved (e.g. between Cys25 on beta-2-microglobulin and the active site Cys149 on GAPDH, and Cys36 of C-reactive protein and Cys-34 of human serum albmin)[5,6]. These new cross-links affect enzymatic activity (e.g. of GAPDH) and are detected both with isolated proteins, and in fresh human plasma (e.g. cross-link between C-reactive protein and human serum albumin).

Overall these data provide evidence for a novel and facile process ('oxidant-mediated thiol-disulfide exchange') that gives rise to glutathionylated and inter-protein crosslinks, as a result of initial oxidation of a disulfide bond and subsequent rapid reaction with another thiol molecule, either low-molecular-mass (e.g. *N*-acetyl-Cys or GSH), or on another protein. This pathway may contribute to the accumulation of modified proteins in diseased tissues, in alterations to redox stasis, and altered cell signaling.

References

1 Karimi, M., Crossett, B., Cordwell, S. J., Pattison, D. I. & Davies, M. J. Characterization of disulfide (cystine) oxidation by HOCl in a model peptide: evidence for oxygen addition, disulfide bond cleavage and adduct formation with thiols. *Free Radic. Biol. Med.* **154**, 62-74 (2020).

2 Karimi, M. *et al.* Reactivity of disulfide bonds is markedly affected by structure and environment: implications for protein modification and stability. *Sci. Rep.* **6**, 38572 (2016).

3 Carroll, L. *et al.* Oxidant-induced glutathionylation at protein disulfide bonds *Free Radic. Biol. Med.* **160**, 513-525 (2020).

4 Jiang, S., Carroll, L., Rogowska-Wrzesinska, A., Rasmussen, L. M. & Davies, M. J. Oxidation of disulfide bonds in proteins by singlet oxygen gives rise to glutathionylated proteins. *Redox Biol.* **38**, 101822 (2021).

5 Jiang, S., Carroll, L., Mariotti, M., Hägglund, P. & Davies, M. J. Formation of protein cross-links by singlet oxygen-mediated disulfide oxidation. *Redox Biol.* **41**, 101874 (2021).

6 Jiang, S., Hagglund, P., Carroll, L., Rasmussen, L. M. & Davies, M. J. Crosslinking of human plasma C-reactive protein to human serum albumin via disulfide bond oxidation. *Redox Biol* **41**, 101925, (2021).

IL-2.1.2

Deciphering biomembrane photodamage: Alkylation of a type I sensitizer enhances the photo-induced oxidation of phospholipid membranes.

Andrés H. Thomas[1], Alexander Greer[2], Mauricio S. Baptista[3], Rosangela Itri[4], Mariana Vignoni[1]
1) Universidad Nacional de La Plata-CONICET, Instituto de Investigaciones Fisicoquímicas Teoricas y Aplicadas, La Plata, Argentina;2) City University of New York, Brooklyn, Brooklyn College, New York, United States; 3) Universidade de São Paulo, Instituto de Química, São Paulo, Brazil; 4) Universidade de São Paulo, Institute of Physics, São Paulo, Brazil.

Pterins are endogenous photosensitizers present in human skin that accumulate in this tissue in patients suffering from vitiligo, a skin disorder characterized by the acquired loss of constitutional pigmentation. Experiments using model biological targets, such as nucleotides and amino acids, have demonstrated that mainly act through type I mechanism. These processes are initiated by a dynamic reaction, controlled by diffusion, in which an electron transfer from the substrate to the triplet excited state of the photosensitizer takes place. All unsaturated lipids in cell membranes are well-known targets of oxidative damage, which can occur by type I and type II photosensitized oxidation. In the case of vesicles dispersions, a hydrophilic photosensitizer will remain in the aqueous phase and the photosensitized oxidation of a target molecule in the membrane will be a dynamic process. On the other hand, if the photosensitizer is lipophilic, an association with a biomembrane is expected and, as the photosensitization is not limited by diffusion, the oxidation might be much faster.
Pterin (Ptr), the parent and model compound of oxidized pterins, is hydrophilic, does not bind to phospholipid membranes and, therefore, freely crosses biomembranes. Ptr photoinduces the oxidation of polyunsaturated fatty acids (PUFAs) of phospholipids present in large unilamellar vesicles (LUVs), predominantly through type I mechanism.[1] Moreover, upon UVA irradiation in the presence of Ptr, the viability of *HeLa* cells decreases and the structural integrity of the cell membrane are affected. In short, although Ptr remains in bulk water, it is able to photoinduce membrane damage in simple model systems (LUVs), as well as in eukaryotic cells.
In the search of better compounds that retain the photosensitizing properties of pterins and, at the same time, are able to bind to biomembranes, a series of decyl-pterin derivatives were synthesized.[2] Among them, due to its photochemical properties (efficient intersystem crossing), 4-(decyloxy)pteridin-2-amine (*O*-decyl-Ptr) was chosen for further studies using phospholipid membranes with various compositions. Conjugation of a decyl chain to the pterin moiety enables its facile intercalation in LUVs. In particular, *O*-decyl-Ptr is positioned right between the polar head and the beginning of the fatty acid chains.[3] Upon UVA irradiation lipid peroxidation photosensitized by *O*-decyl-Ptr leads to the formation of hydroxyl derivatives, hydroperoxides and hydroxyhydroperoxides.[4] These photoproducts undergo a fast conversion into short-chain secondary products by cleavage of the fatty acid chains most likely due to further photosensitized processes. These short-chain oxidized lipids are responsible for destabilizing the phospholipid bilayer and promoting membrane leakage. The efficiency of photodamage, assessed in terms of oxidized products formation rate and membrane permeabilization, is much higher for *O*-decyl-Ptr than for free Ptr,[4] which indicates that the intercalation of the alkyl-pterin to the membrane enhances the photosensitized reactions. *O*-decyl-Ptr is also much more efficient than Ptr in the photodynamic activity on *HeLa* cells.[3]

References

1) A. H. Thomas, A. Catalá, M. Vignoni, Soybean phosphatidylcholine liposomes as model membranes to study lipid peroxidation photoinduced by pterin, *Biochim. Biophys. Acta* 1858 (2016) 139–145.
2) M. Vignoni, N. Walalawela, S. M. Bonesi, A. Greer, A.H. Thomas, Lipophilic decyl chain-pterin conjugates with sensitizer properties, Mol. Pharm. 15 (2018) 798-807.
3) A. Vignoni, C. Layana, H.C. Junqueira, A.H. Thomas, R. Itri, M.S. Baptista, M. Vignoni, Alkylation of a hydrophilic photosensitizer enhances the contact-dependent photo-induced oxidation of phospholipid membranes, *Dyes Pigm.* 187 (2021) 109131.
4) M. Vignoni, M. N. Urrutia, H. C. Junqueira, A. Greer, A. Reis, M. S. Baptista, R. Itri, A .H. Thomas, Photo-Oxidation of unilamellar vesicles by a lipophilic pterin: deciphering. biomembrane photodamage, *Langmuir* 34 (2018) 15578-15586.

IL-2.1.3

Heavy-Atom-Free Photosensitizers for the Treatment of Cancer Cells

Carlos E. Crespo-Hernández,
Case Western Reserve University, Department of Chemistry, Cleveland, Ohio, 44106, USA

Heavy-atom-free photosensitizers based on thionation of carbonyl groups of readily accessible organic compounds are rapidly emerging as a versatile class of molecules[1]. A significant portion of our research group has focused on understanding how thionation regulates the electronic relaxation pathways in nucleic acid bases[2] and other organic chromophores[3] from experimental and computational perspectives and the application of this fundamental information for the development of all-organic photosensitizers as therapeutic drugs to treat cancer cells. In the first part of this presentation, I will show that fundamental physicochemical investigations can be used to develop analogues of the canonical DNA and RNA nucleobases exhibiting more than 100 nm redshifted absorption spectra and nearly 100% greater photoreactivity, which, when applied *in vitro* with a low dose of light, substantially decrease the proliferation of skin cancer cells[1,4]. In the second part, I will discuss the generalization of this chemistry for the development of heavy-atom-free photosensitizers absorbing near-infrared radiation and exhibiting high yields of singlet oxygen generation for the prospective treatment of cancer cells[3,5].

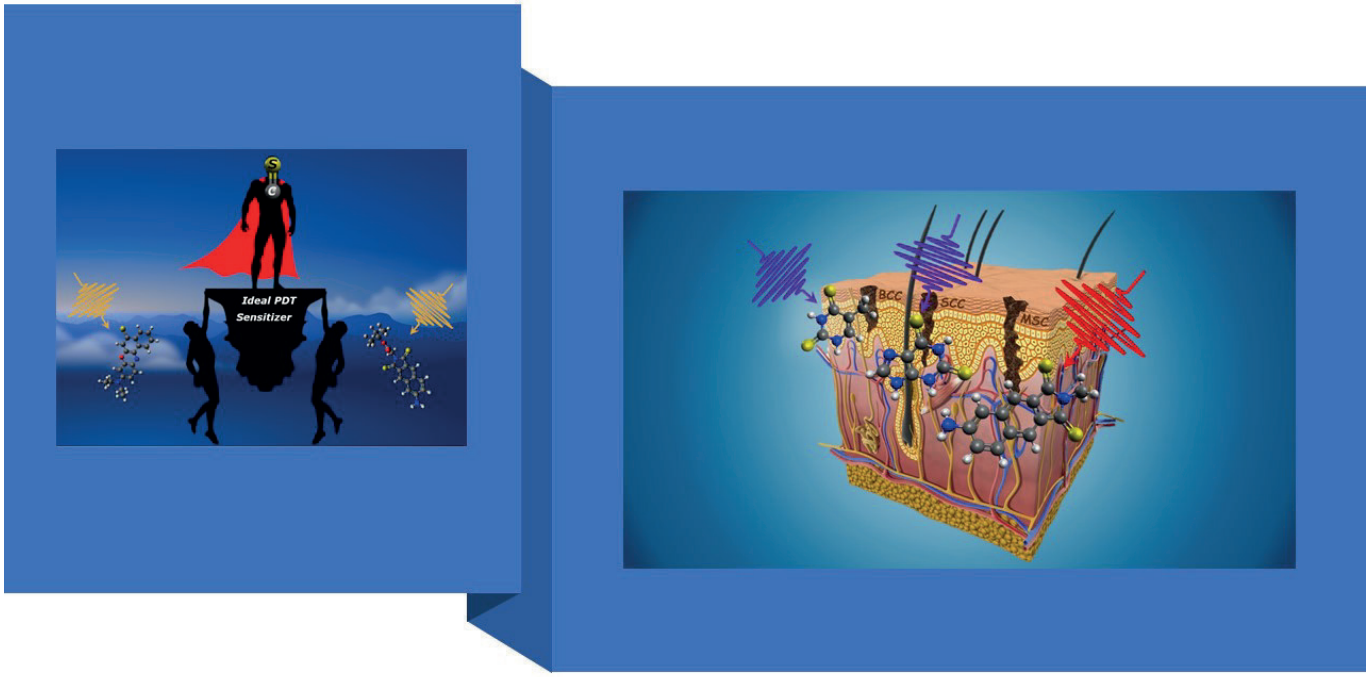


Fig. 1 – Development of all-organic photosensitizers based on thionation of carbonyl functional groups. In the chemical structures, yellow, red, blue, gray, and white, represent sulfur, oxygen, nitrogen, carbon, and hydrogen, respectively.

References

1) Ortiz-Rodríguez, L. A.; Crespo-Hernández, C. E., Thionated Organic Compounds as Emerging Heavy-Atom-Free Photodynamic Therapy Agents, *Chem. Sci.* **11**, 11113-11123 (2020).
2) Ashwood, B.; Pollum, M.; Crespo-Hernández, C. E., Photochemical and Photodynamical Properties of Sulfur-Substituted Nucleic Acid Bases, *Photochem. Photobiol.* **95**, 33-58 (2019).
3) Ortiz-Rodríguez, L. A.; Hoehn, S. J.; Loredo, A.; Wang, L.; Xiao, H.; Crespo-Hernández, C. E., Electronic Relaxation Pathways in Heavy-Atom-Free Photosensitizers Absorbing Near-Infrared Radiation and Exhibiting High Yields of Singlet Oxygen Generation, *J. Am. Chem. Soc.* **143**, 7, 2676–2681 (2021).
4) Pollum, M.; Minh, L.; Jockusch, S.; Crespo-Hernández, C. E., Dithionated Nucleobases as Effective Photodynamic Agent Against Human Epidermoid Carcinoma Cells, *ChemMedChem* **13**, 1044-1050 (2018).

5) J. Tang , L. Wang , A. Loredo , C. Cole and H. Xiao, Single-Atom Replacement as a General Approach towards Visible-Light/Near-Infrared Heavy-Atom-Free Photosensitizers for Photodynamic Therapy, *Chem. Sci.* **11** , 6701-6708 (2020).

Acknowledgements. The author acknowledges the National Science Foundation (Grant No. CHE-1800052). This work made use of the High-Performance Computing Resource in the Core Facility for Advanced Research Computing at CWRU.

OC-2.1.4

Mechanistic Organic Photochemistry: Dark Processes and Toxicity Priming

Shakeela Jabeen[1,2], Andrés M. Durantini[1-3], and Alexander Greer[1,2]
1) Brooklyn College, City University of New York, Department of Chemistry, Brooklyn, New York, United States
2) The Graduate Center of the City University of New York, Ph.D. Program in Chemistry, New York, New York, United States
3) IDAS-CONICET, Universidad Nacional de Río Cuarto, Departamento de Química, Río Cuarto, Córdoba, Argentina

Results will be described with the use of air/liquid interfaces and particles as a way to deepen our understanding of reactive oxygen intermediates. One result in Figure 1 is an accomplishment in sorting out light/dark processes that are intertwined. Deconvolution of competitive light and dark processes offer mechanistic insight into a singlet oxygen priming mechanism[1]. New insight is being added to solve mechanistic puzzles in the production of reactive oxygen in primary light-dependent and secondary dark processes. Our work with multiphasic systems has provided insight to light-to-dark processes in the destabilization of biological membranes and surfaces of environmental particles[2].

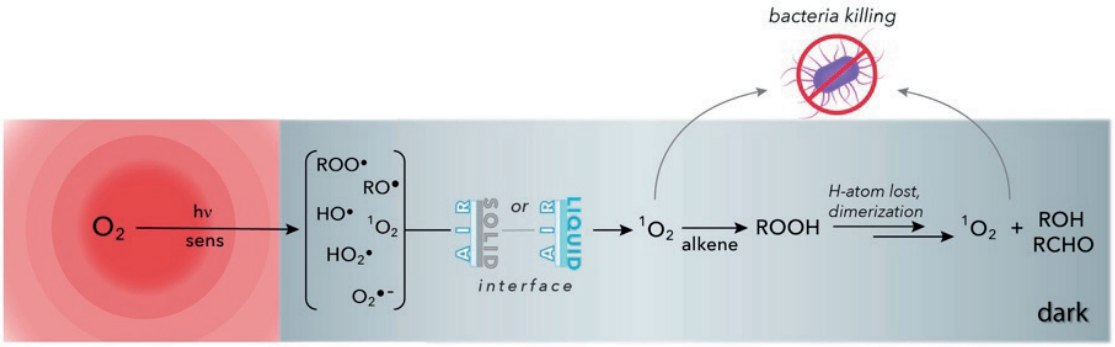


Fig. 1 – Schematic showing that complementary light and dark reactions arise in separable processes. We focus on light-dependent reaction of singlet oxygen, and light-independent reaction of hydroperoxides.

References

1) Jabeen, S.; Farag, M.; Malek, B.; Choudhury, R.; Greer, A. A Singlet Oxygen Priming Mechanism: Disentangling of Photooxidative and Downstream Dark Effects. *J. Org. Chem.* **2020**, *85*, 12505-12513.
2) Durantini, A. M.; Greer, A. Interparticle Delivery and Detection of Volatile Singlet Oxygen at Air/solid Interfaces. *Environ. Sci. Technol.* **2021**, *55*, 3559-3567.

Acknowledgements. This work was supported by the US National Science Foundation (CHE-1856765).

IL-2.1.5

Photosensitization by tyrosine kinase inhibitors

Miguel A. Miranda,[1,2] Ignacio Vayá,[1,2] Inmaculada Andreu[1,2]

1) Departamento de Química/Instituto de Tecnología Química UPV-CSIC, Universitat Politècnica de València, Camino de Vera s/n, 46022-València, Spain.

2) Unidad Mixta de Investigación UPV-IIS La Fe, Hospital Universitari i Politècnic La Fe, Fernando Abril Martorell 106, 46026-Valencia, Spain.

The epidermal growth factor receptors (EGFRs) are transmembrane glycoproteins with tyrosine kinase activity. They are able to regulate a number of signaling pathways within cells including cell proliferation, migration, differentiation, tissue repair and wound healing. Mutations and overexpression of the tyrosine kinase receptors may result in the appearance of different types of cancers and may promote solid tumor growth. Therefore, EGFRs are major targets for the design of anticancer agents. In this regard, tyrosine kinase inhibitors (TKIs) are of high interest due to their ability to block the kinase activity of these receptors. Many drugs are known to absorb solar radiation, and can induce photosensitivity reactions, such as phototoxicity or photoallergy, but also photoaging, weakening of the immune system and skin cancer. These side effects can be associated with damage to biomolecules mediated by radicals or reactive oxygen species arising from excited singlet or triplet states. Interestingly, drugs containing the quinazoline moiety (the common chromophore of a family of TKIs) are known to produce skin photosensitivity.
In this context, as an extension of our previous work on Imatinib, we have now investigated the photobiological response of Lapatinib and Gefitinib. Thus, their phototoxic potential has been evaluated by means of the NRU assay, while their photooxidation activity has been assessed towards HSA, the main transport protein in human serum. Besides, fluorescence and transient absorption spectroscopies from the femtosecond to the microsecond time-scale have been used to investigate the photobehavior of these TKIs in solution, as well as in the presence of HSA or in cellular systems. As a result, it has been observed that the excited state properties of these drugs are strongly affected by the environment: locally excited singlet states are mainly formed in organic non-polar solvents and within HSA or cells, while intramolecular charge transfer sates predominate in organic polar solvents. In the case of Gefitinib, the triplet excited state has been identified and completely characterized for the first time, and its potential to generate ROS has also been assessed. In general, a good correlation is established between the photophysical behavior and the photobiological properties of the investigated TKIs, which provides a mechanistic basis for the observed phototoxicity. All the above features are of key importance in connection with the photosensitizing potential of this family of drugs.

IL-2.1.6

Singlet molecular oxygen reaction with biomolecules: Mechanistic studies using ^{18}O-labeled oxygen, mass spectrometry, and light emission measurements

Paolo Di Mascio

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil

In mammalian tissues, ultraweak chemiluminescence arising from biomolecules oxidation has been attributed by several authors to the radiative deactivation of singlet molecular oxygen [$^1O_2$] and excited triplet carbonyl products as dioxetane intermediates[1,2]. Singlet molecular oxygen has been shown to be generated in biological systems and have been implicated in cell defense mechanisms and dark reactions. Singlet oxygen that may be produced among various possibilities by myeloperoxidase, an enzyme implicated in inflammation processes, and by the UVA component of solar radiation is also a major source of peroxidation of several key cellular components. Singlet oxygen exhibits a substantial reactivity toward electron-rich organic biomolecules (Fig 1A) including protein, lipids, RNA and DNA, leading to the formation of allylic hydroperoxides, dioxetanes or endoperoxides[1].
It was also shown that biomolecules oxidation reactions which often involve $^1O_2$-mediated peroxidation of nucleic acids, unsaturated lipids and targeted amino acids[3] are implicated in several diseases including advanced human arteriosclerosis, arthritis, cataract and diabetes. The measurement of near-infrared light emission and the use of [^{18}O]-labeled $^1O_2$ and hydroperoxides generated via a clean naphthalene derivative [^{18}O]-labeled thermolabile endoperoxide (DHPN$^{18}O_2$) as a source of [^{18}O]-labeled $^1O_2$ (Fig.1B), can be applied to study mechanistic aspects related to $^1O_2$ generation and reactions in biological systems[4]. A relevant major topic deals with the search for the molecular signature of the $^1O_2$ formation in targeted biomolecules within cells. The use of [^{18}O]-labeled $^1O_2$ released from thermolabile endoperoxides in association with sensitive and accurate LC-ESI-MS/MS analysis provides a highly suitable way to gain relevant mechanistic insights into the formation and the decomposition pathways of initially $^1O_2$ generated peroxidic compounds and to search for characteristic biomarkers in cells.

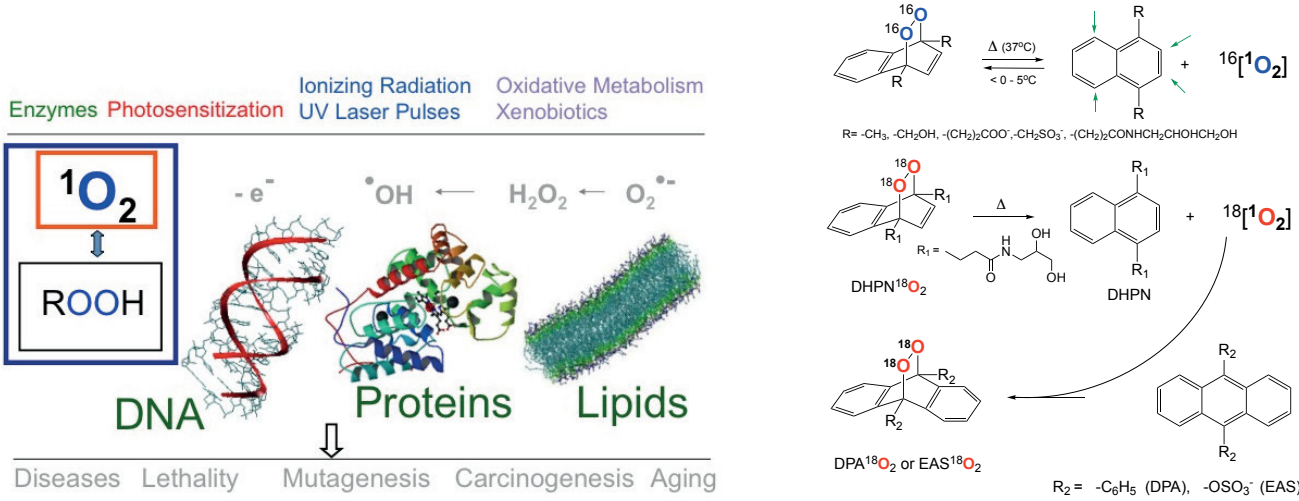


Fig. 1 – (A) $^1O_2$ is capable of efficiently reacting with cellular constituents[1]. (B) Naphthalene and anthracene derivatives[4] for release and capture of $^1O_2$.

References

1) Paolo Di Mascio et al., Chem. Rev., 119, 2043-2086, 2019. 2) Luiz D. Ramos et al., Photochem. Photobiol., 97,327-334, 2021. 3) Stanley, C. P. et al. Nature, 566, 548-552, 2019. 4) Marilene S. Oliveira et al., Photochem. Photobiol. Sci., 19, 1590-1602, 2020.

Acknowledgements This work was supported by grant from FAPESP (N°. 2012/12663-1, CEPID Redoxoma N° 2013/07937-8), CNPq (N° 302120/2018-1) and PRPUSP (NAP Redoxoma N° 2011.1.9352.1.8).

IL-2.1.7

Metallodrug Photosensitizers for Light-Based Cancer Therapy

Sherri A. McFarland[1], Colin G. Cameron[1], John A. Roque III[1,2], Houston D. Cole[1], Ge Shi[1]
1) The University of Texas at Arlington, Department of Chemistry and Biochemistry, Arlington, TX United States; 2) The University of North Carolina at Greensboro, Department of Chemistry and Biochemistry, Greensboro, NC United States

There has been an ongoing interest in the development of new photosensitizers (PSs) for photodynamic therapy (PDT). A related area of active investigation has been the design of PSs with novel mechanisms of action, including oxygen-independent photoprocesses (known as photochemotherapy, or PCT) or the capacity to switch to such modes at low oxygen tension. Since tumor hypoxia can present a real challenge for PDT in certain cases, PSs that operate through PCT mechanisms offer the possibility of treating some of the most aggressive and drug-resistant tumors that resist the traditional photodynamic reactions. Transition metal complexes have emerged as attractive PSs for both PDT and PCT. Certain coordination complexes of ruthenium (Ru) are potent phototoxins toward a variety of in vitro and in vivo cancer models. One example is our own TLD1433, which is currently in a Phase 2 clinical trial for treating nonmuscle invasive bladder cancer with PDT. Part of the interest in Ru PSs stems from the ability to access a variety of excited state electronic configurations with visible or near-infrared light by judicious choice of ligand combinations around the metal center. These excited states, in turn, may participate in traditional type I/II photodynamic reactions as well as oxygen independent pathways that form the basis of PCT. In this conference presentation, we will discuss the design and development of transition metal complex PSs that exploit both PDT and PCT effects. The emphasis will be on the structural features and photophysical models that give rise to excited triplet states with characteristic reactivities.

Acknowledgements. This work was supported in part by the National Cancer Institute (NCI) of the National Institutes of Health (NIH) (Award R01CA222227). The content in this work is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

OC-2.1.8

Inhibition of 6-formylindolo[3,2-b]carbazole metabolism sensitizes keratinocytes to UVA-induced apoptosis: Implications for drug-induced phototoxicity

Katharina M. Rolfes[1], Natalie C. Sondermann[1], Motoki Nakamura[2], Christian Vogeley[1], Jean Krutmann[1] and Thomas Haarmann-Stemmann[1]

1) IUF – Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany; 2) Department of Environmental and Geriatric Dermatology, Nagoya City University, Nagoya, Japan

Ultraviolet (UV) radiation induced DNA damage and associated signalling pathways are mainly responsible for a variety of acute and chronic cutaneous adverse effects. In this context, energy-rich UVB rays are of particular importance, as they are mainly absorbed by the DNA of epidermal keratinocytes. If not fixed by specialized DNA repair systems or removed through the apoptotic demise of damaged cells, these lesions may cause mutations and thereby eventually initiate skin carcinogenesis. In addition, the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor has been identified as a key regulator of the UVB-induced stress response in epidermal keratinocytes[1]. In UVB-irradiated skin, AHR is activated by the intracellular generated tryptophan photoproduct 6-formylindolo[3,2b]carbazol (FICZ) and, amongst others, mediates immunosuppressive and anti-apoptotic effects. Therefore, transient inhibition of AHR is widely regarded as a suitable strategy to protect skin against the damaging effects of UVB radiation[1]. In contrast to UVB radiation, UVA-photons are barely absorbed by DNA, but rather through endogenous and exogenous photosensitizers. Excitation of these photosensitizers results in ROS-induced oxidative DNA damages and apoptosis. Interestingly, FICZ was identified as a potent, nanomolar UVA photosensitizer in epidermal keratinocytes[2]. The fact that FICZ induces the expression of the AHR target gene cytochrome P450 (CYP) 1A1, which is mainly responsible for the metabolism of this tryptophan photoproduct[3], implies that a modulation of AHR activity has a direct effect on the UVA-induced phototoxicity of FICZ. Indeed, we confirmed that a modulation of AHR and/or CYP1A1 enzyme activity affected the intracellular FICZ level and thus the UVA-induced phototoxicity of FICZ. AHR-dependent CYP1A1 induction decreased the phototoxicity of FICZ/UVA-treatment, whereas inhibition of AHR had the opposite effect. Even though FICZ levels are quite low and a transient inhibition of AHR and associated CYP1A1 inhibition may not result in an accumulation of UVA-reactive quantities, chronic inhibition of AHR/CYP1A1 however might reach UVA-reactive levels of FICZ. Therefore, this scenario might be of clinical relevance in the context of drug-induced phototoxicity. Interestingly, one of the most prominent examples of phototoxic drugs, namely the BRAF protein kinase inhibitor vemurafenib is also a potent inhibitor of AHR[4]. This drug is approved for the treatment of advanced melanoma with activating BRAF V600 mutations. In addition, we found out that vemurafenib is not only an antagonist of AHR but also a substrate of CYP1A1 and therefore influences the metabolic degradation of FICZ and associated UVA-induced phototoxicity. In fact, the obtained results indicate that a modulation of FICZ metabolism might be a relevant molecular mechanism responsible for the UVA-induced phototoxicity of drugs. Induction of epidermal CYP1A1 enzyme activity therefore might be a suitable therapeutic approach to minimize phototoxicity in patients suffering from melanoma.

References:

1) Vogeley C, Esser C, Tüting T, Krutmann J, Haarmann-Stemmann T. Role of the aryl hydrocarbon receptor in environmentally induced skin aging and skin carcinogenesis. Int J Mol Sci 20:6005, 2019.
2) Park SL, Justiniano R, Williams JD, Cabello CM, Qiao S, Wondrak GT. The Tryptophan-Derived Endogenous Aryl Hydrocarbon Receptor Ligand 6-Formylindolo[3,2-b]Carbazole Is a Nanomolar UVA Photosensitizer in Epidermal Keratinocytes. J Invest Dermatol 135:1649-58, 2015
3) Bergander L, Wincent E, Rannug A, Foroozesh M, Alworth W, Rannug U. Metabolic fate of the Ah receptor ligand 6-formylindolo[3,2-b]carbazole. Chem Biol Interact 149:151-64, 2004.
4) Hawerkamp HC, Kislat A, Gerber PA, Pollet M, Rolfes KM, Soshilov AA, Denison MS, Momin AA, Arold ST, Datsi A, Braun SA, Oláh P, Lacouture ME, Krutmann J, Haarmann-Stemmann T, Homey B, Meller S. Vemurafenib acts as an aryl hydrocarbon receptor antagonist: Implications for inflammatory cutaneous adverse events. Allergy 74:2437-48, 2019.

Acknowledgements: This work was supported by the Jürgen Manchot Stiftung.

P-2.1.9

Cytotoxicity and redox profile of a novel transition-metal nitrosyl compound for photo-controlled NO delivery in human fibroblasts

Hande Özbaşak[1], Hani Mohamed Abdullah Elbeheiry[2], Erika Csekes[1], Martin Schulz[2], Lucia Račková[1]

1) Centre of Experimental Medicine Slovak Academy of Sciences, Institute of Experimental Pharmacology and Toxicology, Bratislava, Slovakia; 2) Friedrich Schiller University Jena, Institute of Physical Chemistry, Jena, Germany

The discovery that nitric oxide (NO) plays important roles as a biological messenger in a wide range of physiological processes has stimulated great interests in the studies of its chemical and biochemical properties, especially the investigation of NO-mediated events. Among NO releasing compounds, poly-pyridine ruthenium complexes containing nitrosyl group are subject to increasing amounts of research in recent years[1]. As NO concentration is crucial and decisive factor of desired effects, ruthenium-terpyridine is highly prized as it is possible to tweak concentration of NO released with L2 ligand site. The derivative HE-10, a ruthenium (II) nitrosyl-complex incorporating 4′-phenyl-terpyridine and o-benzoquinone diimine ($[Ru(ptp)(o\text{-}bqdi)NO](PF_6)_3$), is a newly synthesized photoinducible NO releasing compound. In this study, it was aimed to investigate the cytotoxic and prooxidant effects likely related to NO release induced from HE-10 with white LED light stimulation in the VH10 fibroblast cell line.

After pre-treatment of the cells with HE-10, 1 hour light exposure and following overnight incubation, the effects on cell viability under light and dark conditions were determined by MTT assay. Moreover, NO_2^- levels in the medium were detected by Griess method and intracellular reactive species formations in VH10 cells were determined by H_2DCF-DA probe. Cell cycle analysis under light and dark conditions was performed by flow cytometry. Protein nitration levels, measured as 3-nitrotyrosine, in VH10 cells were detected by western blot.

Our data show that cytotoxicity of HE-10 was enhanced by exposure to white LED light, as confirmed by lower IC_{50} value obtained from the HE-treated light-exposed cells compared to the unexposed ones. Cytotoxicity was accompanied by increased intracellular reactive oxygen/nitrogen species (ROS/RNS) production, G2/M cell cycle arrest and protein nitration, depending on both, light exposure and concentration. Moreover, light-exposure-enhanced toxic effect of HE-10 on VH10 fibroblast cells was most likely to be NO-dependent as confirmed by the induced NO_2^- release in the medium, intracellular oxidation of H_2DCF-DA probe sensitive to ROS and RNS in fibroblasts, and increased protein nitration levels. According to the cell cycle analysis, it was determined that HE-10 decreased cell proliferation and antiproliferative effect was enhanced with light exposure.

With this research, we successfully demonstrated that novel ruthenium−terpyridine compound HE-10 is capable of selective cytotoxicity induced by light exposure through releasing NO to VH10 fibroblast cells. Our results indicate that HE-10 is promising in terms of causing cell death in proliferative VH10 cells which could potentially apply to other cell lines which will be investigated further. Further experiments will be conducted to confirm DNA damage potentially caused by ROS and investigate the involved molecular pathways. All in all, our results suggest that the novel compound HE-10 might be a promising candidate in terms of photoinducible therapies, such as ruthenium-nitrosyl-based cancer therapy[2].

References

1) Xiang HJ, An L, et al. Photo-controlled targeted intracellular delivery of both nitric oxide and singlet oxygen using a fluorescence-trackable ruthenium nitrosyl functional nanoplatform. Chem Commun. 51: 2555-8, 2015.
2) Orlowska E, Babak MV, et al. NO Releasing and Anticancer Properties of Octahedral Ruthenium-Nitrosyl Complexes with Equatorial 1*H*-Indazole Ligands. Inorg Chem. 57: 10702-17, 2018.

Acknowledgements. This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-18-0336 and LogicLab ITN funded by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie [grant agreement No 813920].

P-2.1.10

Assessment of the phototoxicity induced by talazoparib

Alejandro Mateos-Pujante[1,2], M. Consuelo Jiménez[1,2], Inmaculada Andreu[1,2]

1) Departamento de Química-Universitat Politècnica de València, Camino de Vera s/n, 46022, Valencia, Spain.

2) Unidad Mixta de Investigación Universitat Politècnica de València -Instituto de Investigación Sanitaria (IIS) La Fe, Hospital Universitari i Politècnic La Fe, Avenida de Fernando Abril Martorell 106, 46026, Valencia, Spain.

Breast cancer is the most common cancer in women and the second leading cause of cancer death around the world. It is estimated that 1 in 8 women are likely to develop breast cancer in their lifetime[1]. Therefore, there is a need for developing different treatments to choose the most suitable treatment for each patient.
In this context, Poly(ADP-ribose) polymerase inhibitors (PARPi) have emerged as potent antitumoral drugs, as their ability to inhibit PARP activity. PARPs are a family of multifunctional enzymes that play essential roles in cellular processes. Among them, PARP-1 and PARP-2 plays an important role in DNA repair catalyzing the repair of endogenous single strand breaks (SSB) that occurs frequently in cells, which is essential for their survival. However, a non-efficient SSB repair can be converted to double strand breaks (DSB), that are toxic to cells and must be repaired by other mechanisms such as homologous recombination (HR)

Given this background, talazoparib, the last PARPi approved by the FDA in 2018, exerts its cytotoxic effect by inhibiting PARP activity, provoking the formation and accumulation of DSB. Thus, cells with BRCA 1/2 mutations are unable to repair this DNA damage by HR, which cause further double strand DNA breaks and subsequently, the cellular death. Therefore, talazoparib is used for the treatment of breast tumours with mutations in genes BRCA 1/2, which induced apoptosis in those cancer cells that are unable to repair DNA damage by HR[2,3].

Regarding the photosafety of talazoparib (TLP), although photosensitivity reactions have not been reported yet, in the present work we have assessed the phototoxicity induced by TLP in cells through Neutral Red Uptake (NRU) assay. Hence, it is necessary to perform photophysical studies to know the phototoxicity mechanism involved. As it is shown in Fig. 1, TLP displays a triplet excited stated in PBS, which is quenched by oxygen. Moreover, to evaluate if the phototoxicity to keratinocyte (HaCaT) cells obtained can be attributed to DNA photosensitized damage, comet assay was performed, indicating a photo(geno)toxicity damage around 50 %. These results seem very interesting to prevent photosensitivity reactions associated to TLP.

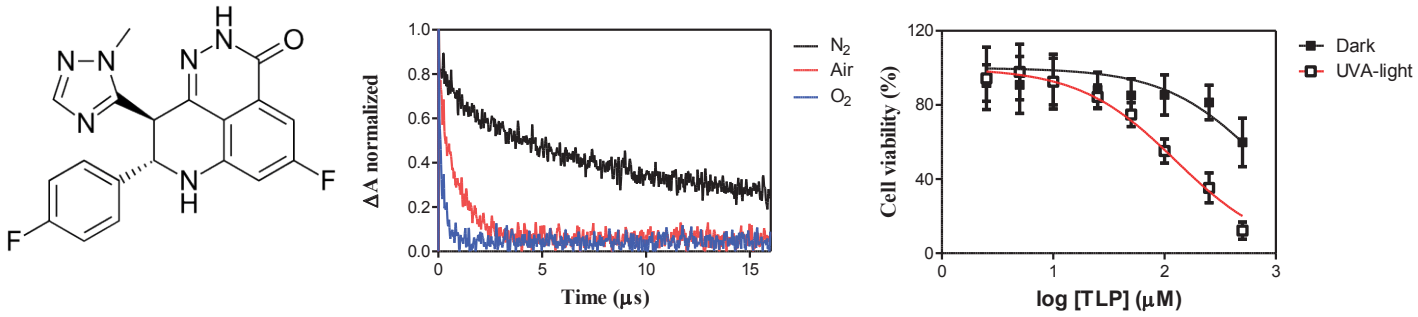


Fig. 1 – From left to right: Chemical structure of TLP, LFP signal decay monitored at 400 nm in PBS and cell viability dose-response curve of HaCaT cells treated with TLP.

References

1) Key T.J., Verkasalo P.K., Banks E., Epidemiology of breast cancer. Lancet Oncol. 2(3) 133-140, 2001.
2) Zheng F., Zhang Y., Chen S., Weng X., Rao Y., Fang H, Mechanism and current progress of Poly ADP-ribose polymerase (PARP) inhibitors in the treatment of ovarian cancer. Biomed. Pharmacother. 123:109661, 2020.
3) Hurvitz S.A. et al., Talazoparib in patients with a germline BRCA-mutated advanced breast cancer: detailed safety analyses from the phase III EMBRACA trial. Oncologist 0493, 2019.

Acknowledgements. This work was supported by Generalitat Valenciana (ACIF/2018/159), the Spanish Ministry of Science and Innovation (PID2019-105391GB-C22, CTQ2016-78875-P, BEAGAL 18/00211) and Carlos III Institute of Health (Grant PI16/01877).

P-2.1.11

A study of the photophysical properties of adapalene.

Juan Antonio Soler, Miguel A. Miranda, Virginie Lhiaubet-Vallet

InstitutoUniversitario Mixto de Tecnología Química (UPV-CSIC), Universitat Politècnica de València, Consejo Superior de Investigaciones Científicas, Avda de los Naranjos s/n, Valencia, Spain

Adapalene, a third-generation topical retinoid, is used as a treatment for acne vulgaris. It is currently one of the three topical retinoids approved so far by the Food and Drug Administration (FDA), along with tazarotene and tretinoin.[1] However, combination of adapalene and sun exposure might result in the appearance of adverse effects. They have been associated with different causes such as skin irritation that can upset the natural skin photoprotection and increase ultraviolet rays hurt, or a decrease of the thickness of the stratum corneum reducing the natural photobarrier.[2] Moreover, as shown in Figure 1, adapalene exhibits an important UVB/UVA absorption. Thus, like other topical drugs,[3] it might act as a potential photosensitizer toward biological components and be responsible for different processes related to phototoxicity or photoallergy.

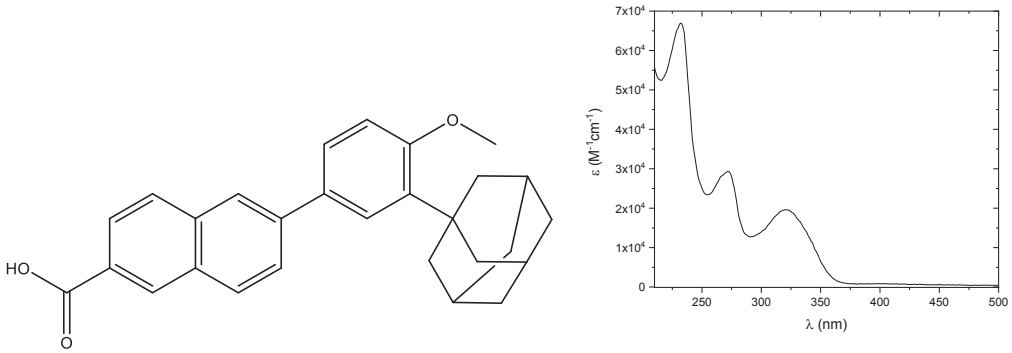


Fig. 1 – Left, structure of adapalene molecule. Right, UV-vis spectra of adapalene in acetonitrile at 10^{-5} M.

The aim of this study is to determine the photophysical properties of adapalene to investigate its potential photoactivity toward biomolecules. For this, experiments combining UV-vis spectroscopy, fluorescence and phosphorescence (steady-state and time-resolved) and laser flash photolysis were carried out. An efficient fluorescence emission centered at 425 nm and with a quantum yield of ca. 0,8 was evidenced together with a phosphorescence band at 520 nm. Moreover, laser flash photolysis experiments revealed a triplet-triplet transient absorption with a lifetime of 40 μs, which was efficiently quenched in the presence of oxygen and β-carotene. A pH dependent photobehavior was also observed.

References

1) Kolli, S. S., Pecone, D., Pona, A., Cline, A. and Feldman, S. R., Topical Retinoids in Acne Vulgaris: A Systematic Review. Am J Clin Dermatol, 20, 345-365, 2019.
2) Zaenglein, A. L, Topical Retinoids in the Treatment of Acne Vulgaris. Semin. Cutan. Med. Surg., 27, 177-182, 2008.
3) Lhiaubet-Vallet, V., Miranda, M. A., Phototoxicity of Drugs, *In CRC Handbook of Organic Photochemistry and Photobiology,* Eds. F. Ghetti, A. G. Griesbeck and M. Oelgemöller, CRC Press, Chap. 66, pp. 1541-1555. 2012.

Acknowledgements.
This work has been supported via the pre doctoral FPI fellowship program from the Spanish Government and a Project of the Spanish Ministry of Science, Innovation and Universities.

P-2.1.12

Photobehavior of the tyrosine-kinase inhibitor gefitinib in solution and within human serum albumin

L. Tamarit[1,2], M. El Ouardi[1,2], I. Andreu[1,2], I. Vayá[1,2], M. A. Miranda[1,2]
1) Departamento de Química/Instituto de Tecnología Química UPV-CSIC, Universitat Politècnica de València, Camino de Vera s/n, 46022 València (Spain); 2) Instituto de Investigación Sanitaria La Fe, Hospital Universitari i Politècnic La Fe, Avenida de Fernando Abril Martorell 106, 46026 Valencia (Spain)

Gefitinib (GFT) is an orally active first-generation tyrosine-kinase inhibitor (TKI), which is currently used for the treatment of patients with non-small cell lung cancer; the mode of action involves specific binding of GFT to the ATP site of HER1 preventing autophosphorylation in tumor cells.[1,2] Although the benefits of this drug are evident, it can also induce adverse effects, which are normally associated to rash, diarrhea, dry skin, nausea and vomiting.[3] Interestingly, GFT contains the quinazoline moiety, which is known to induce damage by photosensitization. In this regard, it has been recently reported that lapatinib (LAP), a TKI used for the treatment of breast and lung cancer, can induce photooxidation and phototoxicity.[4–6] Therefore, interaction of GFT with UV light could also induce photosensitivity disorders. Thus, a thorough spectroscopic study combining ultrafast transient absorption spectroscopy, fluorescence and laser flash photolysis has been performed on GFT in solution and in biological medium.

The photobehavior of GFT, which is summarized in Fig. 1, has been investigated in organic solvents of different polarities and within human serum albumin (HSA), the most abundant transport protein in the blood-stream.[7]

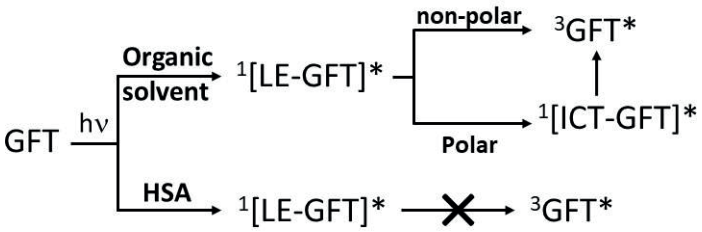


Fig. 1 – Schematic representation of the main photophysical processes arising from irradiation of GFT in polar and non-polar organic solvents and within HSA.

The results show that the photobehavior of GFT was strongly affected by the environment. In general, LE singlet species was instantaneously formed. In organic non-polar solvents, they emit at short wavelengths (~380 nm) with moderate quantum yields (ϕ~0.19) and short lifetimes (*ca*. 1.3 ns). This species can also cross to form the triplet excited state of GFT (^{3}GFT*), which takes place in about 10 ps in toluene. By contrast, in organic polar solvents, LE states rapidly evolve (~1 ps) towards the formation of ICT states. These species emit at longer wavelengths and display higher lifetimes (*ca*. 3.4 ns) than those of LE states; they are also able to populate ^{3}GFT* in MeCN. Surprisingly, this process was not observed in ethanol, since ICT states are deactivated in about 0.7 ns, and consequently the triplet state of the drug cannot be formed. Interestingly, formation of $^1O_2$ has been detected in both organic polar and non-polar solvents, which in principle might have significant implications in the photosensitizing properties of GFT. However, it is worth to note that ^{3}GFT* has not been detected within the protein cavities; consequently, the photosensitizing potential of GFT could not be attributed to this species but more likely to a type I reaction from LE singlet states, which are the only excited species observed in the biological environment.

References

1) Cohen MH, Williams GA, Sridhara R, Chen G, Pazdur R. FDA Drug Approval Summary: Gefitinib (ZD1839) (Iressa ®) Tablets . *Oncologist*. 2003;8(4):303-306. doi:10.1634/theoncologist.8-4-303
2) Arora A, Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. *J. Pharmacol. Exp. Ther.* 2005;315(3):971-979. doi:10.1124/jpet.105.084145

3) Cersosimo RJ. Gefitinib: An adverse effects profile. *Expert Opin. Drug Saf*. 2006;5(3):469-479. doi:10.1517/14740338.5.3.469
4) García-Lainez G, Vayá I, Marín MP, Miranda MA, Andreu I. In vitro assessment of the photo(geno)toxicity associated with Lapatinib, a Tyrosine Kinase inhibitor. *Arch. Toxicol*. 95(1):169-178, 2021
5) Vayá I, Andreu I, Lence E, et al. Characterization of the locally excited and charge-transfer states of the anticancer drug lapatinib by ultrafast spectroscopy and computational studies. *Chem. Eur. J*. 26: 15922-15930, 2020
6) Andreu I, Lence E, González-Bello C, et al. Protein Binding of Lapatinib and Its N- and O-Dealkylated Metabolites Interrogated by Fluorescence, Ultrafast Spectroscopy and Molecular Dynamics Simulations. *Front Pharmacol*. 2020;11. doi:10.3389/fphar.2020.576495
7) Peters T. Ligand Binding by Albumin. *All About Albumin*. Published online 1995:76-132. doi:10.1016/b978-012552110-9/50005-2

Acknowledgements. Financial support from the Spanish Government (RYC-2015-17737, CTQ2017-89416-R and BEAGAL 18/00211), the Carlos III Institute of Health (Grant PI16/01877) and Conselleria d'Educació Cultura i Esport (PROMETEO/2017/075) is gratefully acknowledged.

P-2.1.13

Hybrid nanomaterials based on graphene quantum dots for photodynamic therapy

Cristina J. Dias[1,2], Carla I. M. Santos[1,3], Fátima Liliana Monteiro[2], Laura Rodríguez-Pérez[4], Gil Gonçalves[5], Maria A. F. Faustino[1], Sandra Vieira[2], Luisa Helguero[2], M. Ángeles Herranz[4], Nazario Martin[4], M. Graça P. M. S. Neves[1], José M. G. Martinho[3], Ermelinda M. S. Maçôas[3]

1) LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 2) iBiMED - Institute of Biomedicine, Department of Medical Sciences, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 3) CQE, Centro de Química Estrutural, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; 4) Department of Organic Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, E-28040 Madrid, Spain; 5) TEMA-Nanotechnology Research Group, Mechanical Engineering Department, University of Aveiro, Campus Universitario de Santiago, 3810-193 Aveiro, Portugal

Cancer appears as the second leading cause of death worldwide and, according to data from 2020, breast cancer was the most incident[1]. Therefore, there is an urgent need to find effective therapies to achieve a better treatment efficiency. Under this context, Photodynamic Therapy (PDT) has been extensively investigated in the treatment of several types of cancer with positive outcomes[2]. Porphyrins and analogues are recognised to be promising photosensitisers (absorption in the red region of the electromagnetic spectrum and good generation of $^1O_2$) but their low solubility in physiological medium can compromise their PDT efficiency. Therefore, the development of photoactive molecules coupled to drug delivery systems is of prominent interest[2]. Carbon nanomaterials, in particular graphene quantum dots (GQDs) are promising candidates as vehicles to deliver photoactive molecules to cancer cells in PDT due to their high photostability, water solubility, biocompatibility and excellent optical properties[3].
In this study we discuss the biological evaluation of two biocompatible hybrids based on GQD covalently linked to a porphyrin bearing a 2-aminoethylamino chain. The photoefficiency of the hybrids nanomaterials and cellular localization were studied in a T-47D breast cancer cell line.

References

1) Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA. Cancer J. Clin. 0, 1–41, 2021.
2) Mesquita, M.Q.; Dias, C.J.; Gamelas, S.; Fardilha, M.; Neves, M.G.P.M.S.; Faustino, M.A.F. An insight on the role of photosensitizer nanocarriers for Photodynamic Therapy. Ann. Brazilian Acad. Sci. 90, 1101–1130, 2018.
3) Santos, C. I. M.; Gonçalves, G.; Cicuéndez, M.; Mariz, I.; Silva, V. S.; Oliveira, H.; Campos, F.; Vieira, S. I.; Marques, P. A. A. P.; Maçôas, E. M. S.; Neves, M. G. P. M. S.; Martinho, J. M. G. Biocompatible Hybrids Based on Nanographene Oxide Covalently Linked to Glycolporphyrins: Synthesis, Characterization and Biological Evaluation. Carbon N. Y. 135, 202–214, 2018.

Acknowledgements. Thanks are due the University of Aveiro, Instituto Superior Técnico (IST, Portugal) and FCT/MCTES (Fundação para a Ciência e Tecnologia/Ministério da Ciência e Tecnologia, Portugal) for the financial support to LAQV-REQUIMTE (UIDB/50006/2020) and (UID/NAN/50024/2019) and Institute for Biomedicine – iBiMED (UID/BIM/04501/2020); the projects: (UID/EMS/00481/2013), (PTDC/FIS-NAN/4662/2014), (PTDC/NAN-MAT/29317/2017), (PTDC/QUI-QFI/29319/2017), (LISBOA-01-0145-FEDER-029319) and (UIDB/00100/2020) C. J. Dias and F. L. Monteiro thanks their grants (SFRH/BD/150676/2020) and (SFRH/BD/117818/2016), respectively, and C. I. M. Santos her research contract (REF.IST-ID/95/2018). Image acquisition was performed in the LiM facility of iBiMED, a node of PPBI (Portuguese Platform of BioImaging): POCI-01-0145-FEDER-022122. Financial support from the Spanish Ministry of Science and Innovation (MICINN) (projects CTQ2017-83531-R and CTQ2017-84327-P) is also acknowledged.

P-2.1.14

SERS behaviour of corroles at the surface of colloidal metal particles

Joana F. B. Barata[1], Sara Fateixa[2], Helena I. S. Nogueira[2], M. Graça P. M. S. Neves[3], José A. S. Cavaleiro[3] and Tito Trindade[2]
1) CESAM and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 2) CICECO and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; 3) LAQV-REQUIMTE and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.
email: jbarata@ua.pt

The combination of porphyrinoids and metallic NPs is receiving significant attention from the scientific community due to the relevance of the resulting nanomaterials for various applications, including catalysis, sensing, drug-delivery systems and photosensitization processes.[1] Following our interest in this field, new hybrids composed of inorganic nanoparticles (NPs) and the contracted porphyrinoids corroles were prepared, and the vibrational and electronic properties were analysed regarding their selection towards the reported applications.[2] Various factors can affect the molecular adsorption of the macrocycles on metal surfaces, namely their structures and the interaction mode between both components. Regarding this information, Surface-enhanced Raman Scattering (SERS) spectroscopy is a valuable tool since resonance SERS effect is expected when this type of macrocycles is attached or closely near to the surface of specific inorganic nanoparticles as gold or silver NPs.[3]In this communication, we report the SERS behaviour of two corroles 5,10,15-trispentafluorophenylcorrole and 5-(4-mercaptopropanethioloxy-2,3,5,6-tetrafluorophenyl)-10,15-bis(pentafluorophenyl)corrole on gold and silver NPs. The intrinsic knowledge gathered from these studies will provide additional information, complementing our current studies on the development of corrole functionalized nanomaterials for photodynamic therapy.

References

[1]E. J. Hong, D. G. Choi, M. S. Shim Acta Pharmaceutica Sinica B 2016, 6, 297
[2] J. F. B. Barata, A. L. Daniel-da-Silva, M. G. P. M. S. Neves, J. A. S. Cavaleiro, T. Trindade, RSC Advances 2013, 3, 274. R. A. Pereira, T. Trindade, J. F. B. Barata, Magnetochemistry, 2018, 4, 37.
[3] I.Marcaida, M. Maguregui, H. Morillas, C. García-Florentino, V. Pintus, T. Aguayo, M. Campos-Vallette, J. M. Madariaga Anal Bioanal Chem 2017, 409, 2221.

Acknowledgements. Thanks are due to University of Aveiro and FCT/MCTES for the financial support to CESAM (UID/AMB/50017/2019), CICECO-Aveiro Institute of Materials (UIDB/50011/2020 & UIDP/50011/2020), LAQV-REQUIMTE (UIDB/50006/2020) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network, which partially support the NMR spectrometers by Infrastructure Project Nº 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC). This work was financially supported by the project Corlutna (POCI-01-0145-FEDER-031523) funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. S. Fateixa thanks FCT for his research contract (REF-069-88-ARH-2018) which is funded by national funds (OE).

P-2.1.15

Corrole dimers – synthetic, spectroscopic and antimicrobial activity studies

Paula S. S. Lacerda[1,2], Maria Bartolomeu[3], Adelaide Almeida[3], M. Amparo F. Faustino[2], M. Graça P. M. S. Neves[2], Joana F. B. Barata[1]
1) CESAM, University of Aveiro, 3010-193 Aveiro, Portugal; 2) LAQV-Requimte and Department of Chemistry, University of Aveiro, 3010-193 Aveiro, Portugal; 3) CESAM and Department of Biology, University of Aveiro, 3010-193 Aveiro, Portugal

Corroles **1** (Fig. 1) are aromatic contracted tetrapyrrolic macrocycles with unique physicochemical features[1]. These compounds possess key photophysical/photochemical properties as high excitation molar coefficients, fluorescence quantum yields, phosphorescence and photostability to be used in several therapeutic applications namely as photosensitizers in PhotoDynamic Therapy (PDT)[2]. One of the main advantages of these macrocycles is their large delocalized π-electronic conjugation and their stability under the PDT conditions. Additionally, conjugated macrocycles corrole-based **2** (Fig. 1) demonstrated that their absorption spectra fall within the therapeutic window of 600–800 nm which make them promising candidates to be used as photosensitizers[3].

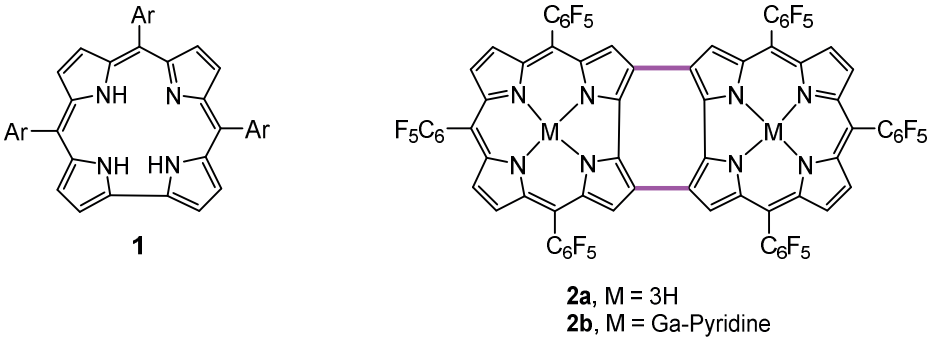


Fig. 1 – The general structure of 5,10,15-triarylcorroles (1) and doubly linked corrole dimers 2a and 2b

In this context, it will be discussed a simple and peculiar alternative to the synthesis of corrole **2b** (Fig.1)**,** as well as their structural and optical characterization. The antimicrobial activity studies, namely dark toxicity and photoactivity under irradiation using the Gram-positive bacterium *Staphylococcus aureus* will also be presented.

References

1) J. F. B. Barata, M. G. P. M. S. Neves, et. al., Strategies for Corrole Functionalization. *Chem. Rev.*, *117*, 3192, 2017.
2) M. Wainwright, T. Maisch, et. al., Photoantimicrobials—are we afraid of the light? *Lancet Infect. Dis.*, *17*, e49, 2017.
3) J. F. B. Barata, A. M. G. Silva, et al., β,β'–Corrole Dimers. *Tetrahedron Lett.*, *47*, 8171, 2006.

Acknowledgements. Thanks are due to University of Aveiro and FCT/MCTES for the financial support to CESAM (UIDP/50017/2020 + UIDB/50017/2020), and LAQV-REQUIMTE (UIDB/50006/2020) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network, which partially support the NMR spectrometers by Infrastructure Project Nº 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC). This work was financially supported by the project Corlutna (POCI-01-0145-FEDER-031523) funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES.

P-2.1.16

Toluidine blue derivatives and Human Serum albumin: Covalent conjugation and photophysical studies.

Nory Marino-Ocampo[1], Belinda Heyne[2], and Denis Fuentealba[1].

1) *Pontificia Universidad Católica de Chile, Departamento de Química Física, Santiago, Chile; 2)University of Calgary, Department of Chemistry, Calgary, Alberta, Canada*

Photodynamic therapy (PDT) is a therapeutic treatment for cancer. PDT involves the use of a photosensitizer (PS), light and oxygen. The PS is incorporated into the tumoral tissue, and it is excited by a light source leading to reactions with biological substrates and molecular oxygen, via Type I and II mechanism. These processes generate reactive oxygen species (ROS). Singlet oxygen ($^1O_2$) is a ROS that can oxidize proteins, lipids, and DNA triggering tumoral death. [1,2] Currently, several reports show strategies to improve the selectivity of the PS towards tumoral tissues using different vehicles such as lipids, nanoparticles, macrocycles, proteins, etc. A useful protein to develop PS carriers is human serum albumin (HSA). HSA has been shown to enhance the selectivity of different chemotherapeutic drugs and PS. [3-5] The present work describes the derivatization of the PS Toluidine blue (TBO) with maleimide and pyridinium disulphide groups that could react with the only free thiol group of the HSA (Cysteine 34 -CYS34), potentially enhancing the selectivity of the PS towards tumoral cells. We have studied the photophysical properties of the derivatives in reference to TBO, and the properties of the conjugated complex of TBO derivatives with HSA using techniques like absorption and emission spectroscopy, time-resolved fluorescence spectroscopy and time-resolved singlet oxygen phosphorescence emission.

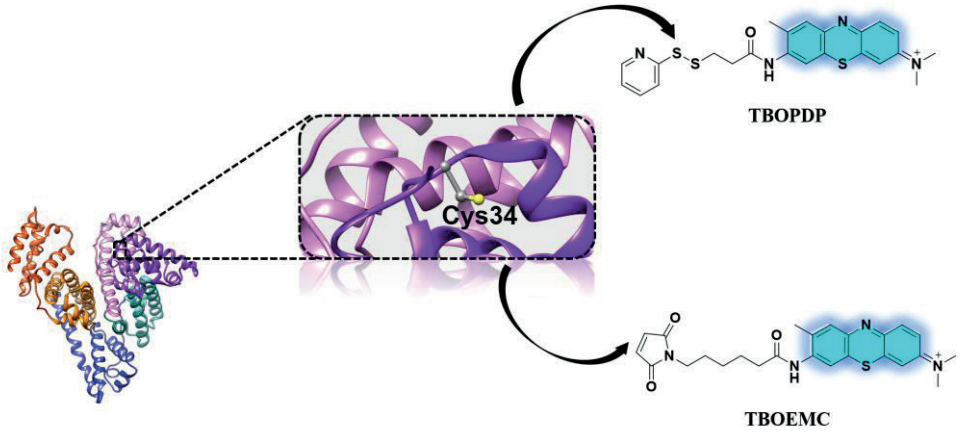


Fig. 1 – Derivatives of Tolidine blue (TBO) that are conjugated with residue of CYS34 of HSA (PDB 1AO6).

References

1) R.R. Allison, K.Moghissi, Photodynamic Therapy (PDT): PDT Mechanisms. Clin Endosc, 2013,**46**,24-29.
2) M.S. Baptista, et al., Type I and Type II Photosensitized Oxidation Reactions: Guidelines and Mechanistic Pathways. Photochem. Photobiol., 2017. **93**(4), 912-919.
3) S. Absar, et al., Serum Albumin-protamine Conjugated for Biocompatible Platform for Targeted Delivery of Therapeutic Macromolecules. J. Biomed. Mater. Res. Part A, 2014, **102**, 2481-2490.
4) J. Caceres, et al., Photochemical behavior of biosupramolecular assemblies of photosensitizers, cucurbit[n]urils and albumins. Phys. Chem. Chem. Phys., 2017, **19**(3), 2574-2582.
5) S. Tayyab, S. R. Feroz., Advances in Protein Chemistry and Structural Biology, 2021, 123, 193-218.

Acknowledgements. This work was supported by grant ANID No.21210160, FONDECYT Nº1160443 and Global Affairs Canada.

IL-2.2.1

Responses of Cyanobacteria to UV Radiation

Rajeshwar P. Sinha

Laboratory of Photobiology and Molecular Microbiology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, India

Cyanobacteria are Gram-negative prokaryotes and oxygenic phototrophs that originated around 3.5 billion years ago. They are ubiquitous and diversified from unicellular to multicellular, coccoid to branched filaments, autotrophic to heterotrophic and established both as free-living as well as endosymbionts. A decrease in stratospheric ozone level due to the release of chlorofluorocarbons and mononitrogen oxides results in an increased level of UV-B (280-315 nm) radiation reaching the Earth's surface. Nitrogen-fixing cyanobacteria are native of tropical agroclimatic conditions and most of them contribute to the carbon and nitrogen economy of soil. These cyanobacteria are mostly susceptible to UV-B radiation. Pigmented proteins and DNA which have absorption maxima in the UV region are the main targets of deleterious UV-B radiation in cyanobacteria. Results indicate that growth, survival, cellular morphology, photosynthesis, N_2 fixation, CO_2 uptake and RuBISCO activity have been adversely affected by UV-B radiation. However, cyanobacteria are not defenseless and have developed both enzymatic as well as non-enzymatic defense mechanisms to counteract the damaging effects of UV-B radiation. Antioxidative enzymes such as superoxide-dismutase (SOD), catalase (CAT), peroxidases (PODs) and ascorbate peroxidases (APXs) scavenge reactive oxygen species generated in cyanobacteria due to UV-B radiation. Non-enzymatic mechanisms mediated by the presence of photoprotectants such as mycosporine-like amino acids (MAAs) and scytonemin which absorb mainly in the UV-B and UV-A region of the spectrum and help ecologically and economically important cyanobacteria to grow and survive in habitats exposed to intense solar radiation. MAAs are colorless, water soluble and low molecular weight compounds (<400 Da) with a maximum absorbance between 309 to 365 nm. Chemically, these are composed of either aminocyclohexenone or an aminocyclohexinimine ring conjugated with the nitrogen substituent of an amino acid or its amino alcohol. A number of MAAs such as shinorine, mycosporine-glycine, palythine, palythinol, asterina-330 and porphyra-334 have been shown to protect cyanobacteria from UV-B radiation. MAAs are among the strongest UV-A/UV-B-absorbing substances with high molar attenuation coefficients, photostability and resistance against strong UVR, temperature, extreme pH and various solvents. Scytonemin constitutes the second important class of UV-absorbing compounds in cyanobacteria that provides protection against UV radiation due to its capacity to absorb significantly at 386, 300, 278 and 252 nm. It is a lipid-soluble yellow-brown dimeric pigment present in the extracellular polysaccharide sheath of some cyanobacteria which exists both in oxidized (Mw 544 Da) and reduced (Mw 546 Da) forms. Any cyanobacteria endowed with the capacity to produce photoprotective compounds will certainly be a good candidate to be used as antioxidant and natural sunscreen for human use against UV radiation and better biofertilizers to be used in rice paddy fields.

IL-2.2.2

Algae and UV radiation: Examples from Antarctic ecosystems

Pirjo Huovinen[1,2], Iván Gómez[1,2]
1) Universidad Austral de Chile, Instituto de Ciencias Marinas y Limnológicas, Valdivia, Chile;
2) Centro FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Valdivia, Chile

Antarctic near-shore ecosystems host abundant macroalgal communities that thrive under permanently low temperatures and extreme light seasonality (Fig. 1). Since the first surveys, the key role of these organisms in the Antarctic costal systems was recognized, serving as a baseline for the revival of research efforts during the last decade in the context of current environmental threats, such as the stratospheric ozone depletion and climate change. In this presentation, an overview of our studies on the effects of UV radiation on macroalgae in the Maritime Antarctic (King George Island) during the last decade is given, along with discussion of future scenarios.[1]

Fig. 1 – View of coastal area of King George Island, Maritime Antarctica.

Antarctic macroalgae are strongly shade-adapted organisms, a physiological feature that allow them colonizing significant depths (down to 40 m). Remarkably, a strong tolerance to high light (including UV) stress has also been shown, which is a result of efficient photosynthetic and bio-optical adaptations as well as constitutively high contents of UV absorbing compounds. This paradox is well exemplified by the large endemic brown algae, which are rich in phenolic compounds (phlorotannins) that are related with several multifunctional anti-stress mechanisms and are allocated in sensitive thallus parts, e.g. reproductive tissues and growing zones. Overall, the impact of UV radiation, e.g. DNA damage and inhibition of photosynthesis, is evident in all life cycle stages, however, remarkable acclimation and tolerance mechanisms are shown. For example, although early reproductive stages, crucial for sustaining the populations, show higher susceptibility to UV radiation (and temperature), they display efficient recovery capacity resembling that reported in parental plants. In terms of UV ecology, the responses of Antarctic macroalgae are defined by morpho-functional adaptations, origin and biogeographical affinity, which can be clearly recognized in their vertical zonation pattern. Shallower waters are occupied by annual species that grow fast, intertidal zone being dominated by ephemeral, widely distributed green algae with delicate morphology. The large endemic brown algae (with leathery morphology) that dominate below 10 m provide understory for also perennial coarsely branched rhodophytes. This configuration of functional groups provides the ecosystem with high resilience to physical perturbations and key bio-engineering functions under changing environment. Although recent reports point to a recovering tendency of the stratospheric ozone layer over Antarctica, these ecosystems are currently threatened by other anthropogenic-driven perturbations, such as pollution, acidification and local decreases in salinity. Thus, the effects of UV radiation on macroalgae should be examined considering the multiple environmental alterations that are being experienced by vast regions around the Antarctic. For example, warming temperatures (reported for West Antarctic Peninsula) pose a threat through enhanced melting of snow and ice, which may modify the substrate availability and particularly the optical properties and transparency of water column, with far-reaching consequences for UV exposure of organisms.

References

1) Gómez I, Huovinen P (Eds), Antarctic Seaweeds: Diversity, Adaptation and Ecosystem Services. Springer. 2020.

Acknowledgements. This work was supported by CONICYT (Project Anillo ART110; FONDECYT 1161129 and 1201053; FONDAP 15150003) and by Instituto Antártico Chileno (INACH; Grant T-20-09).

IL-2.2.3

UV-B resistance strategies of green macroalgae

Frauke Pescheck[1], Wolfgang Bilger[1]

1) Christian-Albrechts-University, Botanical Institute, Kiel, Germany

Green macroalgae belong to the morphologically and cytologically diversified class of Ulvophyceae. These algae inhabit the intertidal of marine and brackish coasts as well as freshwater habitats worldwide. Although some species grow submerged down to several meters water depth, many species thrive at the uppermost fringe of the water to land and are regularly exposed to unattenuated solar ultraviolet-B (UV-B) irradiation.

Notably, it appears that the majority of species of green macroalgae displays no apparent *in vivo* UV-B screening, with the exception of a few orders, e.g. the Cladophorales[1]. Nevertheless, many species of the Ulvophyceae are reported to be highly UV-B resistant[2]. For example, even over a prolonged time of exposure to unfiltered sunlight, no strong accumulation of UV-B specific DNA damage was observed in floating tubular thalli of *Ulva intestinalis*, which is a representative of the group of non-UV screening green macroalgae[3]. In three species from this group, different cellular UV-B tolerance mechanisms like photosystem II recovery and DNA repair were found to be faster as compared to *Cladophora* sp., a green macroalga with efficient UV-B screening. Thus, cellular tolerance seems to be capable to compensate for the absent or low UV-B screening in some green algae[4]. However, this was only detected in field acclimated thalli and not in laboratory grown algae, revealing that UV-B tolerance could be down-regulated under low light conditions.

Similarly, the UV-B screening capacity in some Cladophorales was observed to increase with growth irradiance under controlled laboratory conditions. In contrast, in a freshwater *Cladophora* cultured outdoors apparent UV-B screening diminished during summer indicating that other environmental factors than irradiance probably can influence the acclimation of UV-B screening. Interestingly, growth rate was relatively high during times of low screening. Hypothetically, this might be interpreted as a shift from an UV-B resistant to a more opportunistic lifestyle in *Cladophora*. Since the identity of the substances that provide UV-B screening in the Cladophorales is still unknown, knowledge about the chemical and physical characteristics is very limited. In addition to spectral properties derived from chlorophyll fluorescence excitation spectra, physiological measures of light-dependent reactions were employed to analyse the spectral effects of UV screening *in vivo*. The influence of UV-A and UV-B screening on DNA damage induction and its reversion via photoreactivation was investigated. From the results, it could be deduced that screening of UV-A radiation is affecting the rate of photoreactivation in *Cladophora*[5]. A discussion of UV-B resistance in a pair of a non-UV screening and a UV screening species under natural sunlight will close the talk.

References

1) Pescheck, F., Bischof, K., Bilger, W., Screening of ultraviolet-A and ultraviolet-B radiation in marine green macroalgae (Chlorophyta). Journal of Phycology, 46, 444-455. 2010.
2) Bischof, K., Gómez, I., Molis, M., Hanelt, D., Karsten, U., Lüder, U., Roleda, M., Zacher, K., Wiencke, C., Ultraviolet radiation shapes seaweed communities. Reviews in Environmental Science and Bio/Technology, 5, 141-166, 2006.
3) Pescheck, F., Campen, H., Nichelmann, L., Bilger W., Relative sensitivity of DNA and photosystem II in *Ulva intestinalis* under natural solar irradiation. Marine Ecology Progress Series, 555, 95-107, 2016.
4) Pescheck, F., Bilger, W., Compensation of lack of UV screening by cellular tolerance in green macroalgae (Ulvophyceae) from the upper eulittoral. Marine Biology, 165, 132, 2018.
5) Pescheck, F. UV-A screening in *Cladophora* sp. lowers internal UV-A availability and photoreactivation as compared to non-UV screening in *Ulva intestinalis*. Photochemical & Photobiological Sciences, 18, 413-423, 2019.

Acknowledgements. This work was supported by a scholarship from the Christian-Albrechts-University of Kiel.

OC-2.2.4

Chemical decoration and biochemical incorporation of photoactive molecules in diatoms for photonics and electronic applications

Danilo Vona[1], Cesar Vicente Garcìa[1], Stefania Roberta Cicco[2], Roberta Ragni[1], Gabriella Leone[1], Gianluca Maria Farinola[1]

1 Department of Chemistry, University of Bari, Via Orabona 4, 70125, Bari, Italy; 2 CNR-ICCOM Institute of organometallic chemistry Via Orabona 4, 70125, Bari, Italy

Biomineralization is a biochemical process which converts inorganic salts into complex hybrid materials. Diatom microalgae couple the photosynthetic energy with the production of mesoporous silica shells, called frustules, after the biomineralization of uptaken silicate. These shells constitute a source of silica which exhibit high surface area, transparency, and mechanical resistance, and also an easy aptitude to be chemically functionalized, paving the way to their use in photonics, sensing, optoelectronics and biomedicine [1]. In this context we present a plethora of chemical or *in vivo* decorations for directly giving specific properties to silica from diatoms microalgae [2]. We recently demonstrated the *in vivo* uptake of organometallic emitters containing Iridium, Rhutenium and Aluminum metal cores, into Phaeodactylum tricornutum diatom specie,[3] adherent cells which self-populate and propagate on transparent conductive ITO glasses while bearing luminescent complexes. These experiments successfully aim to produce simple devices in principle suitable for producing photocurrent under light. Furthermore we considered the *in vivo* incorporation of a positively charged Iridium Complex into Coscinodiscus spp. and Thalassiosira weissflogii diatoms frustules, till producing new silica-based luminescent micromaterials and luminescent nanoparticles for biomedicine and drug delivery.[4] Also, organic dyes can be chosen for improving biomass and oxygen response of living microalgae directly exploiting some spectroscopic interactions with the photosynthetic apparata.[5] These last results exhibit an intrinsic aspect of technological applicability in the field of biomass production from algae, for cosmetics, pharmacology, and carbon dioxide or pollutants bioremediation.

Acknowledgements
This work was financially supported by the European Commission through EU project 800926-HyPhOE (Hybrid Electronics based on Photosynthetic Organisms).

References

1) Ragni, R., Cicco, S.R., Vona, D. and Farinola, G.M. Multiple Routes to Smart Nanostructured Materials from Diatom Microalgae: A Chemical Perspective. Adv. Mater. 1-23, 1704289. 2017
2) Ragni, R., Scotognella, F., Vona, D., Moretti, L., Altamura, E., Ceccone, G., Mehn, D., Cicco, S.R., Palumbo, F., Lanzani, G. and G. M. Farinola. Hybrid Photonic Nanostructures by In Vivo Incorporation of an Organic Fluorophore into Diatom Algae. Adv. Funct. Mater. 28 (24), 1706214-1706223. 2018
3) Vona, D., Ragni, R., Altamura, E., Albanese, P., Giangregorio, M.M., Cicco, S.R. and Farinola, G.M. Light-Emitting Biosilica by In Vivo Functionalization of Phaeodactylum tricornutum Diatom Microalgae with Organometallic Complexes. Appl. Sci. 11(8), doi.org/10.3390/app11083327. 2021
4) Della Rosa, G., Vona, D., Aloisi, A., Ragni, R., Di Corato, R., Lo Presti, M., Cicco, S.R., Altamura, E., Taurino, A., Catalano, M., Farinola, G.M. and Rinaldi, R. Luminescent Silica-Based Nanostructures from in Vivo Iridium-Doped Diatoms Microalgae. ACS Sust. Chem. & Eng., 7(2), 2207-2215. 2018
5) Leone, G., De la Cruz Valbuena, G., Cicco, S.R., Vona, D., Altamura, E., Ragni, R., Molotokaite, E., Cecchin, M., Cazzaniga, S., Ballottari, M., D'Andrea, C., Lanzani, G. and Farinola, G.M. Incorporating a molecular antenna in diatom microalgae cells enhances photosynthesis. Sci. Rep. 11, doi.org/10.1038/s41598-021-84690-z. 2021

IL-2.2.5

Do secondary compounds protect lichens against UV radiation?

Knut Asbjørn Solhaug
Norwegian University of Life Sciences, Faculty of Environmental Sciences and Natural Resource Management, Ås, Norway

Lichens are symbiotic associations between a mycobiont and a green algae and/or cyanobacterium photobiont The lichen cortex often contain high amounts of secondary compounds synthesized by the fungal partner. These compounds are situated as crystals on the surface of the fungal hypha. Most of these secondary compounds absorb UV-B and a few visible light. They may screen visible and/or UV by absorption or by reflection when they are in crystal form. The ecological functions of the secondary compounds have been studied in a series of experiments using the acetone-rinsing method. Lichen compounds may be removed by acetone-rinsing without any harmful effect on the lichens. The susceptibility of the lichens against various stresses can then be tested with or without secondary compounds. One main conclusion from these experiments is that the secondary compounds are not essential for UV screening. However, UV may act as a signal for synthesis of the compounds, and their main function seem to be e.g. visible light screening and to deter herbivores.

IL-2.2.6

Ultraviolet photoprotection in bryophytes - a polar perspective

Sharon A. Robinson[1,2], Melinda J. Waterman[1] , Johanna D. Turnbull[1]

1) *University of Wollongong, School of Earth Atmosphere and Life Sciences & Global Challenges Program, Wollongong, Australia 2) University of Wollongong, Securing Antarctica's Environmental Future, Wollongong, Australia*

We discuss the effects of ultraviolet (UV) -B radiation on polar bryophytes. Multiple studies have measured polar bryophyte responses to decreases in UV-B radiation under screens, fluctuations in UV-B irradiance in natural environments as well as increases in UV-B radiation supplied from fluorescent UV lamps[3]. Exposure to UV-B radiation increases the concentrations of UV-B absorbing compounds[2-5] and increases DNA damage[6,7]. Bryophytes lack epidermal tissues and may sequester UV screening compounds preferentially in their cell walls as a method to enhance UV photoprotection[1,4,8,9]. We consider if these bryophytes exhibit unique photoprotective mechanisms to enable them to survive in these incredibly inhospitable environments.

References

1) Clarke, L. J., Robinson, S. A. Cell wall-bound ultraviolet-screening compounds explain the high ultraviolet tolerance of the Antarctic moss, *Ceratodon purpureus*. *New Phytologist* **179**: 776-783. 2008
2) Dunn J. L., Robinson S. A. Ultraviolet-B screening potential is higher in two cosmopolitan moss species than in a co-occurring Antarctic endemic moss – implications of continuing ozone depletion. *Global Change Biology* **12:** 2282-2296. 2006
3) Newsham, K. K., Robinson, S. A. Responses of plants in polar regions to UVB exposure: a meta-analysis. *Global Change Biology* **15**: 2574-2589. 2009
4) Robinson, S. A., Waterman, M. J. *Sunsafe bryophytes: photoprotection from excess and damaging solar radiation*. In D. Hanson & S. Rice (eds.), *Photosynthesis in bryophytes and early land plants*, pp. 113-130. Springer, Dordrecht, The Netherlands. 2014
5) Snell K. R. S., Kokubun T., Griffiths H., Convey P., Hodgson D. A., Newsham K. K. Quantifying the metabolic cost to an Antarctic liverwort of responding to UV-B radiation exposure. *Global Change Biology* **15**: 2563-2573 2009
6) Turnbull, J. D., Robinson, S. A. Accumulation of DNA damage in Antarctic mosses: correlations with ultraviolet-B radiation, temperature and turf water content vary among species. *Global Change Biology* **15**: 319-329. 2009
7) Turnbull, J. D., Leslie, S. J., Robinson, S. A. Desiccation protects two Antarctic mosses from ultraviolet-B induced DNA damage. *Functional Plant Biology* **36**: 214-221. 2009
8) Waterman, M. J., Bramley-Alves, J., Miller, R. E., Keller, P. A., Robinson, S. A. Photoprotection enhanced by red cell wall pigments in three East Antarctic mosses. *Biological Research* **51**: 49, 2018
9) Waterman, M. J., Nugraha, A. S., Hendra, R., Ball, G. E., Robinson, S. A., Keller, P. A. Antarctic Moss Biflavonoids Show High Antioxidant and Ultraviolet-Screening Activity. *Journal of Natural Products* **80**: 2224-2231. 2017

Acknowledgements. This work was supported by grant DP200100223 from the Australian Research Council.

IL-2.2.7

Responses of non-flowering plants to UV radiation: an overview

Javier Martínez-Abaigar[1], Encarnación Núñez-Olivera[1]
1) University of La Rioja, Faculty of Science and Technology, Logroño (La Rioja), Spain

Ultraviolet (UV) radiation is a minor component of the whole solar spectrum reaching the Earth's surface, but it constitutes an important environmental factor regulating life processes. Considering the term "plants" in its widest sense, we can construct an integrative structural, physiological, ecological, and evolutionary portrait of the responses of non-flowering plants (NFP, cryptogams) to UV radiation. In this wide sense, NFP would include cyanobacteria, eukaryotic algae, lichens, bryophytes (mosses, liverworts and hornworts), and pteridophytes. Although the research effort devoted to each group of organisms has been notably asymmetric, much knowledge is now available on the UV effects on NFP, including both common and specific responses. Nevertheless, the experimental conditions applied in the different studies have been so diverse that it is difficult to generalize the results obtained and to compare the UV tolerance of the different organisms. With the aim to synthesize our comparative knowledge on the UV tolerance of NFP, and focusing on UV-B radiation, we applied the same treatment to 107 species (26 algae, 6 lichens, 64 bryophytes, and 11 pteridophytes), in the (apparently) most extensive study to date. UV-B tolerance was assessed using chlorophyll fluorescence variables. Our results show that UV-B tolerance of NFP depends on the species considered, and is greatly influenced by the taxonomic group and (except lichens) by the concomitant level of structural complexity. Overall, the scale of UV-B tolerance was: (pteridophytes and lichens) > mosses > (liverworts and hornworts) > algae. UV-B tolerance was significantly correlated with the content of potentially protective UV-absorbing compounds, and with the sclerophylly of the vegetative bodies. These results may have ecological and evolutionary implications, and can help understand the role of UV radiation in the water-to-land transition of photosynthetic organisms and their subsequent conquest of land.

Acknowledgements. This work was supported by the Spanish Ministry of Science, Innovation and Universities and European Regional Development Fund (ERDF), through the project PGC2018-093824-B-C42.

P-2.2.8

UV-induced changes in phenolic and antioxidant profiles of *Nicotiana tabacum* leaves outdoors and in a growth chamber

Kristóf Csepregi, Arnold Rácz, Gyula Czégény, Éva Hideg
Department of Plant Biology, Faculty of Sciences, University of Pécs, Hungary

Phenolic compounds (flavonoids, phenolic acids) are synthesized via the phenylpropanoid pathway. These compounds are localized in various tissues and documentedly play a protective role against environmental stresses, such as high light intensity or UV radiation as antioxidants and screening pigments. The complex effect of sunlight outdoors and the effect of UV single factor in controlled experiments indoors are distinct. Understanding the latter is important for exploiting UV as modifier of special metabolites. In this study we examined phenolic and antioxidant profiles of tobacco (*Nicotiana tabacum*) leaves grown either in growth chamber or under natural sunlight using UV filters to separate different UV ranges. Outdoor experiments were carried out in June-July 2020 in Pécs (Hungary, 46.07° N, 18.23° E), and plants were grown from seeds to 5-weeks old under the three different light conditions. In the indoor experiments tobacco plants were grown for four weeks under visible light only, then exposed to supplementary UV radiation for 5 days (Q-panel UVB-313EL, 6.9 kJ m^{-2} d^{-1} biologically effective dose). There were three treatment groups both indoors and outdoors: visible light only, visible plus UV-A, visible plus UV-A and UV-B. Photosynthesis measurements demonstrated that neither conditions were stressful but resulted in acclimative responses.
The aims were to (i) identify UV-responsive main phenolic compounds with HPLC, and (ii) find a relationship between antioxidant capacities and phenolic levels. We identified the same phenolic compounds in both experiments, although in different amounts and relative ratios. The main phenolic acids were chlorogenic acid and it's two isomers neochlorogenic acid and cryptochlorogenic acid, major flavonoids included quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside. Total antioxidant capacities were measured as TEAC, FRAP and FC in order to provide a multi-angle analysis[1], and identified higher capacities in leaves grown outdoors. Interestingly, although full sunlight and UV-supplemented growth light were very different, some components (neochlorogenic and cryptochlorogenic acids, and quercetin-3-*O*-rutinoside) were present at the same amounts in leaves under these two conditions. Other compounds (chlorogenic acid and other flavonol glucosides) were present at 1.5-4-times higher amounts in sunlight than indoors. The presence of UV had a larger positive effect on phenolic components and antioxidants indoors than outdoors, due to differences in the ratio of photosyntetically active radiation to UV in these two experiments. Correlations between UV absorbing and antioxidant capacities of leaf extracts and amounts with phenolic contents reflected different properties[2] of individual compounds.

References

1) Csepregi K, Neugart S, Schreiner M, Hideg É., Comparative evaluation of total antioxidant capacities of plant polyphenols. Molecules. 21, 208, 2016
2) Csepregi K, Hideg É., Phenolic compound diversity explored in the context of photo-oxidative stress protection. Phytochemical analysis. 29, 2, 129-136, 8, 2018

Acknowledgements. This work was supported by the National Research, Development and Innovation Office (grants NN-128806 to KCs and PD-131625 to GyCz).

IL-2.3.1

Germicidal Ultraviolet UV-C: Rediscovery of an Old Method to Reduce Infection Risk During COVID-19

David H. Sliney, Ph.D.[1]

1) *Johns Hopkins University Bloomberg School of Public Health, Department of Environmental Health and Engineering, Baltimore, USA*

Historically, germicidal ultraviolet germicidal irradiation (UVGI) dates back more than a century and was widely used in hospitals and public places to reduce infections by inactivating airborne parthenogens in the 1930s – 1950s.[1] Studies from those times demonstrated efficacy,[2] but UVGI use later dropped out of favor in the 1960s after vaccines against a number of childhood diseases, such as polio and measles had been virtually eliminated. Of infectious diseases of significant severity in the developed world only tuberculosis has been without a vaccine and for that reason UVGI has remained in TB clinics – particularly in those countries where TB continues to be a major problem.[3] In these countries, some expertise has been retained. Today with the COVID-19 pandemic, the lessons learned from TB control allow for a 'rediscovery' of this technology for use in the current pandemic.[3-4] Furthermore, the pandemic has greatly accelerated development of UV-C LEDs and other lamp types such as the krypton-chloride (222-nm) lamp to augment the traditional use of low-pressure mercury (254 nm) lamp.[5] Sadly, misconceptions about UVGI, such as a perceived skin cancer risk remain and a lack of understanding of proper safety precautions continue to slow the wide acceptance of UVGI.[6-8]

References

1) Reed, N. G. The history of ultraviolet germicidal irradiation for air disinfection. *Pub Health Rep*. 125, 15–24 (2010).

2) Wells, W. F., Wilder, T. S. The environmental control of epidemic contagion: I. An epidemiologic study of radiant disinfection of air in day schools. *Am. J. Hyg*. 35, 97–121 (1942).

3) Volchenkov, G. Experience with UV-C air disinfection in some Russian hospitals. *Photochem. Photobiol*., 97: 549–551 (2021).

4) Nardell, E. A. Air disinfection for airborne infection control with a focus on COVID-19: Why germicidal UV is essential. *Photochem. Photobiol.*, 97: 493–497 (2021).

5) Bergman, R. S. Germicidal UV sources and systems. *Photochem. Photobiol*., 97: 466–470 (2021).

6) Bergman, R., Brenner, D., et al. Air disinfection with germicidal ultraviolet: For this pandemic and the next. *Photochem. Photobiol*., 97: 464–465 (2021).

7) Sliney, D.H., Stuck, B.E. A need to revise human exposure limits for ultraviolet UV-C radiation. *Photochem. Photobiol*., 97: 485–492 (2021).

8) Claus, H. Claus, H. (2021). Ozone generation by ultraviolet lamps. *Photochem. Photobiol*. 97: 471–476 (2021).

IL-2.3.2

Photoinduced inactivation of SARS-CoV-2

Mauricio S. Baptista
Universidade de São Paulo, Chemistry Institute, Department of Biochemistry.
baptista@iq.usp.br

The COVID-19 pandemic has accelerated the need for the implementation of effective light-based technologies to target the causative agent SARS-CoV-2. Light of different wavelength can act in the virus and in the host individuals by different mechanisms and used as environmental disinfectant with distinct efficiency, as well as, can be used to easing the consequences of the infection in individuals. While UV radiation target mainly the nucleic acids of the virus, visible light in the presence of proper a photosensitizer can target other compartments such as the virus envelop, by the so-called contact-dependent photosensitized oxidation reactions. I will comment on the benefits, challenges, and pitfalls of repurposing light-based antimicrobial strategies in the COVID-19 pandemic.

References

CP Sabino, et al. Light-based technologies for management of COVID-19 pandemic crisis. Journal of Photochemistry and Photobiology B: Biology 2020, 111999.
LVM de Assis et al. How does the skin sense sun light? An integrative view of light sensing molecules. Journal of Photochemistry and Photobiology C: Photochemistry Reviews 2021, 100403.
IOL Bacellar et al. Photosensitized membrane permeabilization requires contact-dependent reactions between photosensitizer and lipids. Journal of the American Chemical Society 2021, 140 (30), 9606.

IL-2.3.3

Is there a therapeutic role for PDT in Covid 19 SARS infection?

K. Moghissi
The Yorkshire Laser Centre, Goole, UK

Background: In December 2019 a new virus; Corona Virus-2 causing Severe Acute Respiratory Syndrome (SARS) appeared in Wuhan (China). The virus was baptized COVID 19 and took the world by surprise because of its rapid spread, its highly lethal characteristics and its lack of response to all available antimicrobial/antiviral drugs.
COVID 19 started as an epidemic in Wuhan and quickly went out of control to become a pandemic killing - and continuing to kill - millions of people globally.
Ever since its emergence, COVID 19 has presented the world with a serious public health issue, has brought the hospitals' function to a halt and has diverted all resources to symptomatic (palliative) treatment of patients and the attempt to stop the spread of the infection.
Lockdown, social distancing, intensive care unit admission and the treating of SARS by rich oxygen ventilation support to combat hypoxia and, at the last resort, the use of ECMO have been employed. They all are preventive, symptomatic treatments.
Additionally, there has been international cooperation to produce suitable vaccines which have been successful to immunize the recipients.

In short there has been:

1) Shielding and immunization as preventive measures.
2) Symptomatic treatment.

However, no report of specific treatment to kill the virus in vivo has been proposed.

Aims and Objectives:

The aims and objective of the presentation is to suggest that there is a place for Photodynamic Therapy (PDT) in the treatment of SARS COVID 19, based on Photodynamic Reaction (PDR/Photodynamische Wirkung) to destroy the virus and investigate how it can, potentially, be clinically applied.

The presentation comprises of:

- A short reminder of PDT mechanisms emphasizing that it is basically a *Local Therapy*.
- Briefly reviewing relevant anti-microbial/antiviral effects of PDT.
- Discussing the problems in using PDT in the particular case of COVID 19 and its lethal SARS
- In this discussion I will highlight the relevant structure COVID 19 and its defense mechanisms.
- Explain the difficulties of finding a suitable Photosensitiser which could reach the virus and break its defense mechanism.
- How PDT can be applied clinically in order to neutralize COVID 19 which is the causative agent of SARS which in turn afflicts and kills the patient.

Basically, therefore, PDT has to target the initial injury and the virus colony within the airway; this seems to be the path the virus takes to invade and create the lethal pulmonary injury, respiratory failure and gas exchange crash.
Finally, the currently existing planned protocol of study and effort to use PDT as a treatment of SARS COVID 19 will be explored and exposed.

OC-2.3.4

Correlation of the ultraviolet index (UVI) and the temperature with incidence and severity of Covid-19 in Spain

Yolanda Gilaberte[1], Natalia Martín [2], Juan B. Pérez-Gilaberte[3], Alba Navarro-Bielsa[1], M. Victoria de Galbe[4], José Aguilera[4].
1) Miguel Servet University Hospital, Department of Dermatology, Zaragoza, Spain; 2) University of Zaragoza; 3)Miguel Servet University Hospital, Department of Internal Medicine, Zaragoza, Spain; 4)University of Malaga, , Department of Dermatolology, Málaga, Spain.

Introduction: severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) In the present study, the causal agent of coronavirus disease 2019 (COVID-19), has become pandemic on March 11. Different waves have occurred in the different countries, being modified the incidence by the social distancing measures taken. Low levels of vitamin D have been associated with the higher severity of the disease. Considering that the most important source of vitamin D in ultraviolet radiation (UVR) exposure, the aim was to study the correlation between ultraviolet index (UVI) and temperature with the incidence and the severity of COVID-19 in different latitudes in Spain.
Material and Methods: an observational prospective study was performed analyzing the daily UVI and maximum and minimum temperatures with the incidence of cases of COVID-19, hospitalized patients, admitted in intensive care units (ICU), and deaths in 5 Spanish cities located in different latitudes: San Sebastian (N43.25), Zaragoza (N41.65), Madrid (40.41), Málaga (N36.72) and Tenerife (N28.48) from February 2020 to January 2021. Pearson correlation test and linear regression were used. P-value less than 0.005 was considered statistically significant.
Results: An inverse correlation, statistically significant, was found between UVI and the incidence of cases per 100.000 inhabitants in all the studied cities. Those cities located by the sea had the highest correlation coefficients, one in the north (San Sebastian r=-0.426, p=0.001) and 2 in the South (Málaga (r=-0.46, p<0.001) and Tenerife (r=-0.569, p<0.001)). Mortality was significantly inversely correlated with UVI also in these 3 cities. Regarding temperatures, the most relevant inverse correlations were found between the maximum temperature and the mortality in the cities of Tenerife (r=-0.528, p<0.001) and Málaga (r=-0.410, p=0,001). According to the linear regression analysis, in Zaragoza 43% of the incidence of COVID-19 can be explained by the UVI and the temperature, whereas in Madrid only 19% can be explained by them.
Conclusion: UVI inversely influences the incidence of COVID-19 independently of the latitude, and the highest correlations are found in cities with sea. Although the correlation of these meteorological variables with severity could not be established, mortality was inversely correlated with UVI and maximum temperatures in cities of the South.

.

IL-2.3.5

Broad-spectrum Photodynamic Disinfection in the Treatment of SARS-CoV-2

N. Loebel[1], C. Romo[1], C. Cross[1], J. Hickok[1], R. Andersen[1]
1) Ondine Biomedical, Inc., Vancouver, BC, Canada

Direct-acting antiviral (DAA) therapies generally target proteins mediating specific stages in the viral lifecycle such as proteases, polymerases, nonstructural proteins and other viral constituents. These DAA approaches can provide high viral eradication rates, in some cases exceeding 90%.[1] DAAs offer the potential for treatment in cases where vaccine access is lacking or contraindicated; in deficient host immune response secondary to viral downregulation of major histocompatibility class I molecules[2]; or in the evolution of newly-infectious viral variants known as quasispecies[3] resulting from high viral replication and error rates in unvaccinated or immunocompromised populations. In a particularly threatening recent finding, SARS-CoV-2 has been demonstrated to engage in cell-to-cell transmission in a mode known as virus cell-surfing,[4] an actin filament-mediated intercellular propagation technique efficiently utilized by HIV, poxvirus and other DNA and RNA viruses. Cell-to-cell transmission enhances immune evasion and results in reduced clearance by neutralizing antibodies.[5]

Photodynamic disinfection is a broad-spectrum,[6] non-resistance forming,[7] rapid,[8] and safe[9] approach to elimination of pathogens from epithelial surfaces *in vivo*. The technique has been clinically deployed for many years as a potent preoperative decolonization method in the anterior nares,[10] minimizing or eliminating the use of mupirocin and other resistance-prone agents. In this work, we report on the use of the technique as a topical DAA against SARS-CoV-2 in laboratory settings, followed by a summary of field-use reports by industrial teams in meat-packing plants, where high potential exists for worker-to-worker and worker-to-package transmission of SARS-CoV-2.

References

1) M McConnell, JK Lim, et al, Hepatitis C Vaccine Development in the Era of Direct-Acting Antivirals. Clin Liver Dis (Hoboken), 2018.
2) S Ishido, C Wang, et al, Downregulation of Major Histocompatibility Complex Class I Molecules by Kaposi's Sarcoma-Associated Herpesvirus K3 and K5 Proteins. J Virol, 2000.
3) C Trucchi, A Orsi, et al, State of the Art, Unresolved Issues, and Future Research Directions in the Fight against Hepatitis C Virus: Perspectives for Screening, Diagnostics of Resistances, and Immunization. J Immunol Res, 2016.
4) LA Caldas, FA Carneiro, et al, Ultrastructural analysis of SARS-CoV-2 interactions with the host cell via high resolution scanning electron microscopy. Scientific Reports, 2020.
5) J Dufloo, T Bruel, et al, HIV-1 cell-to-cell transmission and broadly neutralizing antibodies. Retrovirology, 2018.
6) M Wainwright, Photodynamic antimicrobial chemotherapy (PACT). J Antimicrob Chemother, 1998.
7) LA Pedigo, AJ Gibbs, et al, Absence of bacterial resistance following repeat exposure to photodynamic therapy. SPIE 7380 Photodynamic Therapy: Back to the Future, 2009.
8) F Cieplik, D Deng, et al, Antimicrobial photodynamic therapy – what we know and what we don't. Crit. Rev. Microbiol, 2018.
9) D Meller, J Hickok, et al, Photodisinfection Therapy: Essential Technology for Infection Control. InfectionControl.tips, 2020.
10) E Bryce, T Wong, et al, Nasal photodisinfection and chlorhexidine wipes decrease surgical site infections: a historical control study and propensity analysis. J Hosp Infect, 2014.

Acknowledgements. All authors are employees of Ondine Biomedical, Inc., Vancouver, BC, Canada.

IL-2.3.6

Photodynamic therapy for Covid-19 and other infectious diseases

Luis G. Arnaut[1], Mariette M. Pereira[1], Zoe A. Arnaut[1], Rafael T. Aroso[1], Sara M. A. Pinto[1], Ana Catarina S. Lobo[2], Paulo Rodrigues-Santos[3]
1) CQC, Chemistry Department, University of Coimbra, 3004-535 Coimbra, Portugal; 2) LaserLeap Technologies, R. Col. Veiga Simão, 3025-307 Coimbra, Portugal; 3) Immunology Institute, Faculty of Science, University of Coimbra, 3004-504 Coimbra, Portugal

The Covid-19 pandemic raised the global awareness on the fragilities of medical responses to infectious diseases. Repositioning of existing antiviral medications gave, at best, controversial results and no clear clinical benefit. Drug development targeting directly the SARS-CoV-2 virus could not offer a timely response to the pandemic. Vaccines developed and approved with unprecedented rapidity offer a medium/long term protection against the pandemic, but also illustrate the shocking asymmetry of medical care, where the clinical solutions are not placed where they are most needed and bounce back with variants that have higher infection potentials. The need for a new approach to control infectious diseases became increasingly evident with the Covid-19 pandemic.
Photodynamic disinfection (PDDI) is based on the generation of reactive oxygen species when an electronically excited photosensitizer molecule encounters molecular oxygen and transfers its energy or an electron, to produce singlet oxygen or superoxide ion, respectively. The local oxidative stress generated by PDDI can damage proteins, lipids or nucleic acids. Enveloped viruses, such as the coronavirus, offer various accessible targets to PDDI. A most appealing property of PDDI is that it can work equally well for all enveloped virus, independently of mutations, which is a valuable tool for the armamentarium of measures against infectious diseases, known at present or emerging in the future.
We developed a new photosensitizer to photoinactive enveloped virus and show how it works against clinical samples of SARS-CoV-2. Virus collected from patients were transported alive to a proper biosafety lab and photoinactivated with the new photosensitizer. A 150 nM concentration and 5 J/cm^2 at 644 nm inactivated >99.99% of the virus in 5 minutes, i.e., the amount of virus in the clinical sample became undetectable by RT-qPCT. Under the same drug and light doses, the viability of HaCat (human epidermal keratinocyte) cell remained 100%. It is possible to selectively inactivate SARS-CoV-2 without toxicity to human cells. The mechanisms of inactivation are discussed with reference to results obtained with genetically engineered lentivirus that express the spike protein at their surface. Comparison with the combined drug and light doses of PDDI using methylene blue with SARS-CoV-2 shows that the new photosensitizer is >300 times more potent.

Acknowledgements. This work was supported by the Portuguese Science Foundation (grants UIDB/00313/2020, PTDC/QUI-OUT/27996/2017-DUALPI and PD/BD/143123/2019) and by P2020 (Covid-19, project no. 49708)

IL-2.3.7

Ultraviolet C Exposure Testing of Materials; An Important Aspect of Surface Sterilization

Matt McGreer[1]
1) Atlas Material Testing Technology, Ametek Measurement, Communications and Testing, Mount Prospect, Illinois, United States of America

UVC radiation (between 200-280 nanometers) has been used for sterilization purposes for over 40 years. This activity has increased tremendously because of the COVID-19 pandemic, expanding from hospital operating rooms to retail and other public spaces, transportation, and electronic devices. In most cases, the polymers and coatings used in these applications have not been engineered or tested to withstand repeated applications of UVC. Very few studies have been conducted on a limited number of polymers.
UVC lamps with a peak output of 254 nanometers have recently been employed in modified testing devices that have been traditionally used with standard fluorescent UV lamps. Modifications to these devices include special features to protect users from stray light, sensors that can measure and control irradiance of the UVC lamps, and more robust materials that can withstand the higher energy from these lamps.
This presentation will show results from UVC exposures of a variety of materials to (a) compare material property change from UVC exposure to that of standard UVA-340 and UVB-313 exposures, (b) analyze the impact of different irradiance levels, black panel temperatures, and light/dark cycling on these materials, and (c) evaluate standard performance characteristics such as repeatability and uniformity of these devices. Finally, an “Exposure Dosage Calculator” will be presented to assist users with determination of appropriate exposure levels based on selected use cases.

OC-2.3.8

Characterization of 222nm UVC-based Cleaning Impacts on Vegetative and Biofilm Cells

Jeffrey Busick[1], Julie Zwiesler-Vollick[1], and Janet Price[2]
1) Lawrence Technological University, Department of Natural Sciences, Southfield, MI, USA; 2) JustLight PBC, Ann Arbor, MI, USA

Illnesses spread through indirect means, such as common colds, rhinovirus and the flu, pose significant risk to communities and drain collective resources. The flu alone affects ~29 million people annually, costing $90 billion in medical costs and resulting in 38,000 deaths[1, 2]. Reduction of pathogens on a surface by 99% reduces incidence of infection after contact to less than 1 in a million chance [3]. UVC light is quickly becoming a mainstream method of cleaning air, surfaces, and food due to its germicidal properties and shallow penetration depth. JustLight is leveraging these properties of UVC light in order to develop a light-based hand cleaner, Violet. To support this, we assessed low doses of 222nm UVC light for efficacy against common indirectly transmitted pathogens in vitro and in the context of a biofilm. Monocultured pathogens inoculated on a surface displayed concentration and dose-dependent reduction after UVC exposure. From this, we were able to calculate a density of pathogens on a surface that was sufficient to block the effect of the UVC light from penetrating to underlying cells. This result was echoed in the biofilm context, as transient pathogens on the surface of hands were reduced at a higher rate than the underlying biofilm cells. These results support Violet and 222nm UVC as an effective method for reducing transient pathogens without damaging the skin microbiome or underlying tissue.

References

1) Centers for Disease Control, Disease Burden of Influenza. CDC.gov, 2020.
2) Roger Dobson, Flu costs the US $90bn a year, report shows. BMJ, 2007.
3) Michael Ryan, Charles Haas, Application of quantitative microbial risk assessment for selection of microbial reduction targets for hard surface disinfectants, Am J Infect Control, 2014.

P-2.3.9

Characterising 'far-UVC' KrCl excimer lamps for safety and inactivation of viruses

Ewan Eadie[1], Paul O'Mahoney[2], Louise Finlayson[3], Sally H. Ibbotson[2], Kenneth Wood[4]
1) NHS Tayside, Photobiology Unit, Ninewells Hospital, Dundee, United Kingdom; 2) University of Dundee, Photobiology Unit, Ninewells Hospital, Dundee, United Kingdom; 3) University of St Andrews, SUPA School of Physics and Astronomy, St Andrews, United Kingdom

Krypton-Chloride (KrCl) excimer lamps have a primary emission wavelength of 222 nm, with residual shorter and longer wavelengths throughout the ultraviolet (UV) spectrum. It has been demonstrated in laboratory research that these lamps can effectively inactivate a wide range of pathogens, including SARS-CoV-2[1-3]. The peak emission wavelength at 222 nm appears to be strongly absorbed in the skin's stratum corneum and in the tear layer of the eye, thus not inducing the acute reactions that are characteristic of other UVC and UVB wavelengths[4-6]. However, residual longer wavelength UV radiation from unfiltered KrCl lamps does appear to penetrate beyond the stratum corneum, inducing both erythema and cyclobutane pyrimidine dimers (CPD) in the deeper layers of epidermis[7,8]. It is therefore important to understand the emission characteristics of a source to accurately determine the hazard it may present.

We investigated the spectral irradiance and the change-in-irradiance-with-distance, from 13 commercially available and research prototype sources with peak emission wavelength at 222 nm. Spectral irradiance was measured with a double-grating spectroradiometer (IDR300, Bentham Instruments Ltd, Reading, UK) with cosine corrected input optics and change-in-irradiance-with-distance was measured with a broadband radiometer (IL1400 SEL220 QNDS2 W, International Light Technologies, Massachusetts, USA). Spectral irradiance measurements were used to determine S(lamp), which is the total effective spectral hazard of a lamp as defined by Buonanno et al.[8] S(lamp) was determined for the current International Commission on Non-Ionizing Radiation Protection (ICNIRP) hazard weighting function ($S(lamp_I)$) as well as the proposed hazard weighting functions from the American Conference of Governmental Industrial Hygienists (ACGIH) for the eye ($S(lamp_{AE})$) and skin ($S(lamp_{AS})$). The spectral angular distribution from one of the sources was also measured both with and without a diffusing material incorporated into the device. The results of these measurements were incorporated into a Monte Carlo radiative transfer model to determine the depth penetration of each source in skin and the resulting modelled CPD. A comparison with computer modelled sun exposure was also performed.

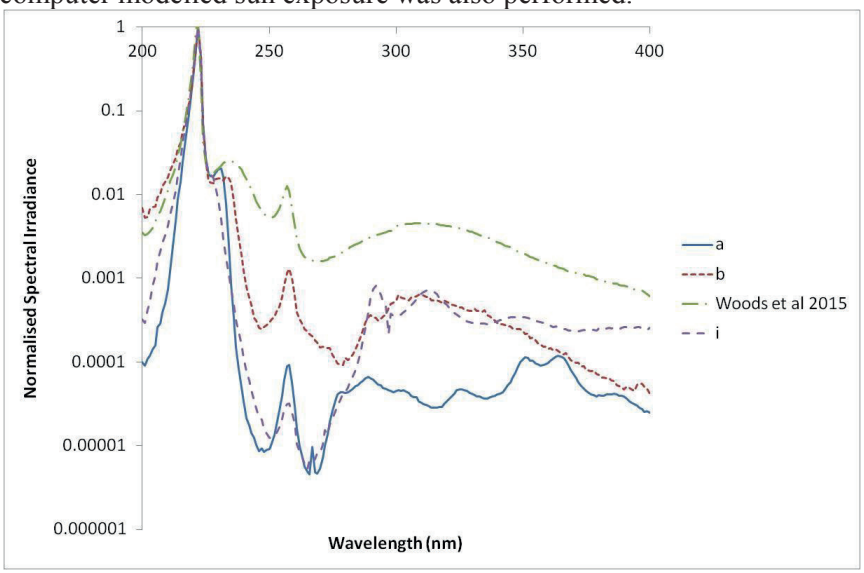


Fig. 1 – Normalised spectral irradiance measurements from three of the 13 commercial sources measured. The normalised spectral irradiance from Woods et al.7 is plotted for comparison.

Variation in the measured spectral irradiance was observed from the 13 commercial sources (Fig. 1). The total effective spectral hazard of each lamp, $S(lamp_I)$, varied from 0.126 to 0.166. The difference between lamps was

more marked when considering the proposed new ACGIH limits - S($lamp_{AE}$) from 0.020 to 0.072 and S($lamp_{AS}$) from 0.007 to 0.031. Angular spectral measurements showed that the spectral distribution from one of the sources varied with viewing angle, a phenomenon well known with certain types of optical filter. The introduction of a diffusing material into the lamp housing removed the angular spectral mismatch and improved the emission distribution (Fig. 2). Computer modelling demonstrated vastly less CPD from any of the KrCl sources when compared to 10 minutes of sun exposure in both temperate and Mediterranean climates.

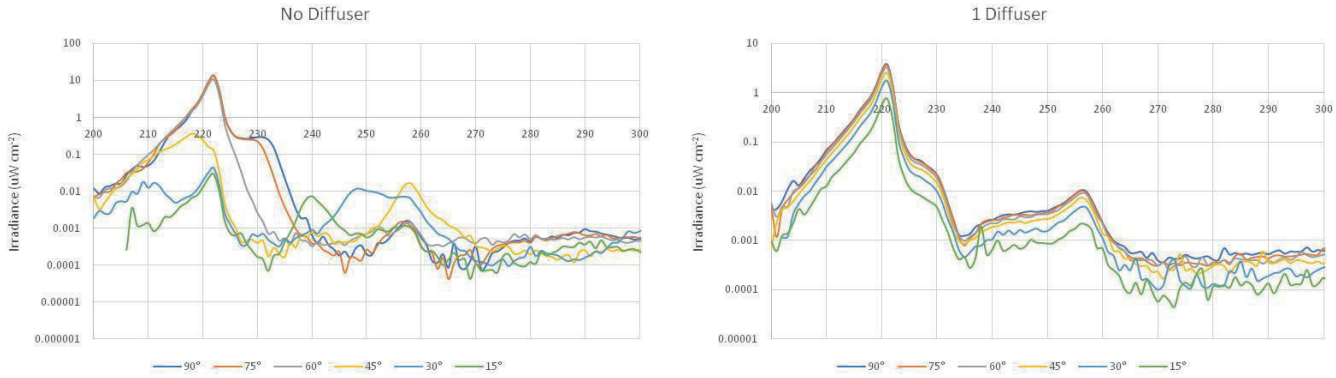


Fig. 2 –Spectral distribution as a function of viewing angle for source without diffuser (left) and with diffuser (right)

In conclusion, there is variation in the spectral distribution of currently available commercial KrCl sources, which necessitates full spectrum assessment against the exposure limits and not just assessment against the 222 nm exposure limit. Full spectrum assessment will become even more important if new ACGIH-proposed threshold limit values are adopted. With real-world installations, if there is overlapping emission from fixtures, it should not be assumed that the greatest hazard is located directly underneath a fixture.

References

1) Kitagawa, H., Nomura, T., et al. Effectiveness of 222-nm ultraviolet light on disinfecting SARS-CoV-2 surface contamination. American Journal of Infection Control, 2020.
2) Narita, K., Asano, K., et al. Ultraviolet C light with wavelength of 222 nm inactivates a wide spectrum of microbial pathogens. Journal of Hospital Infection, 2020.
3) Welch, D., Buonanno, M., et al. Far-UVC light: A new tool to control the spread of airborne-mediated microbial diseases. Scientific Reports, 2018.
4) Hickerson, R., Conneely, M., et al. Minimal, superficial DNA damage in human skin from filtered far-ultraviolet-C (UV-C). British Journal of Dermatology, 2021.
5) Eadie, E., Barnard, I.R.M., et al. Extreme Exposure to Filtered Far-UVC: A Case Study. Photochemistry and Photobiology, 2021.
6) Kaidzu, S., Sugihara, K., et al. Re-Evaluation of Rat Corneal Damage by Short Wavelength UV Revealed Extremely Less Hazardous Property of Far-UV-C. Photochemistry and Photobiology, 2021.
7) Woods, J., Evans, A., et al. The effect of 222-nm UVC phototesting on healthy volunteer skin: a pilot study. Photodermatology, Photoimmunology and Photomedicine, 2015.
8) Buonanno, M., Welch, D. and Brenner, D. Exposure of Human Skin Models to KrCl Excimer Lamps: The Impact of Optical Filtering. Photochemistry and Photobiology, 2021.

Acknowledgements. We would like to thank all the commercial enterprises who donated or loaned their KrCl sources used in this research. No commercial enterprise had an influence in this research. Dr Ewan Eadie acknowledges financial support from NHS Research Scotland Career Researcher Fellowship. Dr Paul O'Mahoney is funded by Medi-lase (registered charity SC037390) and the Alfred Stewart Trust. Louise Finlayson acknowledges financial support from EPSRC Industrial Doctorate Centre Scheme (2262922) and the Laser Research and Therapy Fund (registered charity SC030850).

IL-2.4.1

Advantages of NIR light-triggered cancer therapies

Mladen Korbelik
BC Cancer, Integrative Oncology Department, Vancouver BC, Canada

Recent years have seen an increased interest in research and development of phototherapies performed in near infrared (NIR) sections of light spectrum, particularly photodynamic therapy (PDT) and photothermal therapy (PTT) for treatment of cancer. These efforts are motivated by several advantageous attributes:

- High penetration depths in biological tissues;
- Less toxic consequences for normal cells and tissues;
- Lower light irradiation doses involved;
- More effective inhibition of cancer cell proliferation through cellular DNA damage;
- Opportunity for concomitant enhanced nanodrug delivery to targeted sites.

This presentation will particularly elaborate how both PDT and PTT function as photoimmunotherapy (PIT) that is extremely efficacious in engaging the patient's immune system through the induction of immunogenic cell death (ICD), release of damage-associated molecular patterns (DAMPs), and greatly increased availability of highly immunogenic tumor antigens. The resultant advantages are highlighted by systemic tumor immune rejection favorable for treatment of metastatic cancer, and immunological memory generation preventing disease recurrence.

IL-2.4.2

Dye Doped Silica Nanoparticles as Photoactive Organized Systems for Nanomedicine

Luca Prodi[1], Matteo Cingolani, Damiano Genovese, Liviana Mummolo, Enrico Rampazzo, Nelsi Zaccheroni

Dipartimento di Chimica "Giacomo Ciamician" – Università di Bologna – Bologna – Italy

Silica nanoparticles are versatile platforms with many intrinsic features, including a low toxicity. Their proper design and derivatization yield particularly stable bright nano-systems displaying multiple functions,[1] which can be used for either optical and photoacoustic imaging[2] and for photoluminescence (PL) and electrochemiluminescence (ECL) sensing.[3] In addition, silica nanoparticles can also be used as platforms for photodynamic and photothermal therapies.[2] For these reasons, silica nanoparticles already offer unique opportunities, and further improvement and optimization can substantially expand their possible applications in fields of high impact, such as medical diagnostics and therapy, environmental analysis, and security.
In this context, we have developed a direct micelle assisted strategy based on the use of Pluronic F127 as high molecular weight surfactants. The one-pot synthesis yields PEGylated silica nanoparticles endowed with very high monodispersity, colloidal stability and core-shell structure. These Pluronic Silica NanoParticles (PluS NPs), with a silica core of about 10 nm and an overall hydrodynamic diameter of about 25 nm, can be tailored for optimization of processes such as directional energy transfer, providing systems with extremely valuable functions: high light-harvesting capability, signal-to-noise maximization, multiplex output, and signal amplification. *In-vivo* experiment proved the absence of toxic effects on mice even after three months from injection.[4] A library of functionalized Pluronic surfactants allowed for a straightforward and modular method for surface derivatization, a fundamental step since we found that their possible applications can be controlled by nanoparticle functionalization; the control of surface charge (density and localization) allows, for example, for sentinel lymph node targeting.[4] Furthermore, we demonstrated that the drug loading ability can be tuned with a suitable choice of the silica precursor. Finally, we investigated the interaction with fluorogenic hyaluronan, a synthetic nanogel that improves cell internalization of PluS NPs.[6]

References

1) M. Montalti, L. Prodi, E. Rampazzo, N. Zaccheroni, "Dye Doped Silica Nanoparticles as Luminescent Organized Systems for Nanomedicine", *Chem. Soc. Rev.*, 2014, **43**, 4243.
2) S. Biffi, L. Petrizza, C. Garrovo, E. Rampazzo, L. Andolfi, P. Giustetto, I. Nicolov, G. Kurdi, M. Danailov, G. Zauli, P. Secchiero, L. Prodi, "Multimodal NIR-Emitting PluS Silica Nanoparticles with Fluorescent, Photoacoustic and Photothermal Capabilities", *Int. J. Nanomedicine*, 2016, **11**, 4865.
3) A. Zanut, F. Palomba, M. Rossi Scota, S. Rebeccani, M. Marcaccio, E. Rampazzo, G. Valenti, F. Paolucci, Luca Prodi, "Dye-Doped Silica Nanoparticles for Enhanced ECL-Based Immunoassay Analytical Performance", *Angew. Chem. Int. Ed.*, 2020, **59**, 21858.
4) M. Helle, E. Rampazzo, M. Monchanin, F. Marchal, F. Guillemin, S. Bonacchi, F. Salis, L. Prodi, L. Bezdetnaya, "The Surface Chemistry Architecture of Silica Nanoparticles Determine the Efficiency of in Vivo Fluorescence Lymph Node Mapping", *ACS Nano*, 2013, **7**, 8645.
5) F. Palomba, D. Genovese, E. Rampazzo, N. Zaccheroni, L. Prodi, L. Morbidelli, "PluS Nanoparticles loaded with Sorafenib: synthetic approach and their effects on endothelial cells", *ACS Omega*, 2019, **4**, 13962.
6) F. Palomba, E. Rampazzo, N. Zaccheroni, M. Malferrari, S. Rapino, V. Greco, C. Satriano, D. Genovese, and L. Prodi, *ACS Appl. Mater. Interfaces*, 2020, 12, 6, 6806–6813

Acknowledgements. This work was supported by Italian Ministero dell'Istruzione, Università e Ricerca (PRIN project number 2017EKCS3)

IL-2.4.3

Drug Delivery Systems Targeting Tumour Microenvironment for Near-Infrared Photodynamic Therapy

Takahiro Nomoto[1]
1) Tokyo Institute of Technology, Institute of Innovative Research, Yokohama, Japan

Photodynamic therapy (PDT) combines delivery of photosensitizers (PSs) to a target site and subsequent photoirradiation to excite the PSs. The key to extending PDT application is the development of PSs that can accumulate selectively within a target site without retaining in normal tissues. For this purpose, we developed two kinds of drug delivery systems (DDSs) targeting acidic tumour environment and tumour-associated blood vessels.

The DDS targeting acidic tumour environment was developed by utilizing the polymer that exerts hydrophilic-to-hydrophobic phase transition. Generally, hydrophilic character of drugs prevents unfavourable interaction with biological components, but it also compromises interaction with target cancer cells, decreasing therapeutic efficiency. Meanwhile, the hydrophobic character facilitates the cellular uptake, but it causes untoward distribution to normal tissues due to nonspecific interaction. We thus expected that the switching of hydrophilic/hydrophobic characters in response to tumour acidic environment may permit selective accumulation of PSs. To realize this concept, we developed poly(*N*-isopropylacrylamide/2-aminoisopropylacrylamide) (P(NIPAAm/AIPAAm)) whose AIPAAm units were further modified with hydrophilic acid-labile 2-propionic-3-methylmaleic (PMM) amide[1]. This polymer, termed P(NIPAAm/AIPAAm-PMM), exhibits hydrophilic character at pH 7.4; however, at pH 6.8, PMM moieties were cleaved from the polymer and P(NIPAAm/AIPAAm) exerts hydrophobicity due to its lower critical solution temperature (~30°C). Owing to this phase transition, P(NIPAAm/AIPAAm-PMM) accomplished efficient cellular uptake in cultured cancer cells in response to mild acidic pH. We then conjugated hydrophilic phthalocyanine-based PS, IRDye 700DX, with the polymer and examined the potential in PDT[2]. The IRDye 700DX-conjugated polymer importantly accomplished enhanced accumulation within a subcutaneous tumor in a mouse after intravenous injection and ultimately improved the PDT efficiency of IRDye 700DX, while the unfavorable photochemical damage to the skin was considerably prevented by avoiding the penetration to the normal tissue due to the hydrophilic character in the bloodstream.

Although acidic pH of tumours has been widely reported by many researchers, some tumours do not show homogeneous acidic environment because of the tumour heterogenicity. In such cases, it is difficult to deliver PSs with the aforementioned polymer homogeneously to the tumour tissue, which may lead to the recurrence after PDT. In this regard, vascular targeted PDT may offer great potential because obstruction of tumor-associated vasculature will block the flow of oxygen and nutrient, thereby killing tumor cells in a deep region where PSs cannot be delivered. For this purpose, we synthesized poly(ethylene glycol)-poly(L-glutamate) whose side chains were modified with multiple cyclic RGD peptides, which have the strong affinity with $\alpha_v\beta_3$ integrins overexpressed on tumour-associated blood vessels as well as many cancer cells[3]. The polymer conjugated with IRDye 700DX heterogeneously distributed in the tumour tissue and preferentially accumulated within the tumour-associated vasculature, thereby accomplishing significant PDT effect in a subcutaneous tumour model. Our approaches using these DDSs may have potential to extend the PDT application.

References

1) Muttaqien, S. E., Nomoto, T. *et al.* Poly(*N*-isopropylacrylamide)-based polymer-inducing isothermal hydrophilic-to-hydrophobic phase transition via detachment of hydrophilic acid-labile moiety. *Biomacromolecules* **20**, 1493-1504 (2019).
2) Muttaqien, S. E., Nomoto, T. *et al.* Photodynamic therapy using LCST polymers exerting pH-responsive isothermal phase transition. *Journal of Controlled Release* **328**, 608-616 (2020).
3) Dou, X., Nomoto, T. *et al.* Effect of multiple cyclic RGD peptides on tumor accumulation and intratumoral distribution of IRDye 700DX-conjugated polymers. *Scientific Reports* **8**, 8126 (2018).

Acknowledgements. This work was supported by Center of Innovation (COI) program (JPMJCE1305) from Japan Science and Technology Agency (JST), JSPS KAKENHI (JP26882022 and JP20K20196) and Kobayashi International Scholarship Foundation.

OC-2.4.4

The impact of atropisomerim on Redaporfin photodynamic therapy efficacy

Claire Donohoe[1,2], Fábio Scharbele[1],Mariette M. Pareira[1], Mathias O. Senge[2], Luís G. Arnaut[1], Lígia C. Gomes-da-Silva[1]
1) Department of Chemistry, University of Coimbra, Rua Larga, 3004-535 Coimbra, Portugal
2) Medicinal Chemistry, Trinity Translational Medicine Institute, Trinity Centre for Health Sciences, St. James's Hospital, Trinity College Dublin, The University of Dublin, Dublin 8, Ireland

Atropisomerism presents an intriguing, often neglected, source of structural variety in drug development. Such diversity in tetrapyrrole structures may enhance photosensitizer development for photodynamic therapy (PDT) and related drug developments. The present work demonstrates that the photosensitizer Redaporfin (and related pre-cursor porphyrin molecules) atropisomers can be separated, do not interconvert at room temperature and exert different biological effects. Redaporfin, a pre-clinical synthetic sulfonamide fluorinated bacteriochlorin, presents ideal properties for studying PDT including enhanced photostability and strong absorption at 750 nm. Hindered rotation of the C_m-aryl-macrocyclic bonds of the bacteriochlorin and porphyrin structures results in different spatial orientations of the sulfonamide groups in the meta positions. They are defined as: α_4 when all of the sulfonamide substituents of the phenyl groups are on the same side of the macrocycle plane; $\alpha_3\beta$ when three of the sulfonamides are on the same side of the plane and one is on the opposite side; $\alpha_2\beta_2$ when two sulfonamide groups are on each side and adjacent to each other and finally, $\alpha\beta\alpha\beta$ when two sulfonamides are on each side but alternate in the positions with respect to the macrocycle. Although the photo- and physicochemical properties of the four atropisomers are similar, their therapeutic efficacies are dramatically different. *In vitro* studies have demonstrated significant variability of the atropisomer phototoxicity and cellular internalization levels. In particular, the α_4 atropisomer has displayed the highest levels of uptake and phototoxicity. Atropisomers have presented similar mechanisms of cellular uptake, primarily passive diffusion with subcellular localization in the endoplasmic-reticulum-Golgi complex. Heightened α_4 internalization by cells of the tumor microenvironment has been observed *in vivo*. Atropisomer therapeutic efficacy has been demonstrated to vary *in vivo* when sufficient time for photosensitizer tumor cell internalization occurs. A better understanding of how atropisomerism impacts therapeutic effects may contribute to the establishment of enhanced drug development strategies for atropisomer drug mixtures.

References

1) L.G. Arnaut, M.M. Pereira, J.M. Dabrowski, E.F. Silva, F.A. Schaberle, A.R. Abreu, L.B. Rocha, M.M. Barsan, K. Urbanska, G. Stochel, C.M. Brett, Photodynamic Therapy Efficacy Enhanced by Dynamics: The Role of Charge Transfer and Photostability in the Selection of Photosensitizers, Chemistry, 2014, 20, 5346-5357.
2) L.B. Rocha, L.C. Gomes-da-Silva, J.M. Dabrowski, L.G. Arnaut, L.B. Rocha, L.C. Gomes-da-Silva, J.M. Dabrowski, L.G. Arnaut, Elimination of primary tumours and control of metastasis with rationally designed bacteriochlorin photodynamic therapy regimens. Eur J Cancer, 2015, 51, 1822-1830. Eur J Cancer, 2015, 51, 1822-1830.

Acknowledgements. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement number 764837 (Polythea—How light can save lives)

IL-2.4.5

Ru(II) and Os(II) complexes as Hypoxia-Active Photosensitizer Classes for PDT: the contribution of computational studies

Marta Erminia Alberto

1) Università della Calabria, Dipartimento di Chimica e Tecnologie Chimiche, Arcavacata di Rende (CS), Italy

Due to their appealing physicochemical properties, Ru(II) compounds have found wide applications in biological and medical fields. Complexes of its analogue group 8 counterpart Osmium instead, are relatively unexplored in medicinal chemistry. Despite this, they have an advantage for photobiological applications due to their extended absorption windows, and both Ru(II) and Os(II) are now attracting an increasing interest as potential candidates for Photodynamic Therapy (PDT) and Photochemotherapy (PCT) approaches.
To shed light on the complex photochemistry of these molecules, the information that can be gained from computational studies is very useful, as it has the potential to increase the understanding of the entire photochemical pathways involved, providing a rationale for the observed photobiology along with useful guidelines for the theory-driven design of next-generation agents.
The important contribution of computational studies in this field is reviewed presenting the outcomes of joint experimental and theoretical studies on several Os(II)- and Ru(II)-polypiridyl based scaffold combined with the imidazo[4,5-f][1,10]-phenanthroline-oligothiophene (IP-*n*T) ligand motif (n=0-4).[1,2] (Fig.1)
Those compounds show both powerful $^1O_2$ sensitization and unprecedented PIs for hypoxic photoactivity (1% O_2), among the largest reported to date, making of our Os(II)-complexes, the first hypoxia-active Os-based photosensitizers ever proposed, demonstrating the utility of such metal for phototherapy applications.
DFT results reveal the pivotal role of the switch in the nature of lowest-lying triplet excited state from a metal-to-ligand charge transfer (^{3}MLCT) to intraligand charge transfer one (^{3}ILCT) at *n*=3, with a lower energy and longer lifetime for *n*=4, for both Ru(II) and Os(II) families. Moreover, it provides fundamental insights into the structure-activity relationships along the series of related complexes from *n*=0 to 4 with respect to ground and excited state geometries, photophysical properties, excited state lifetimes and decay mechanisms.

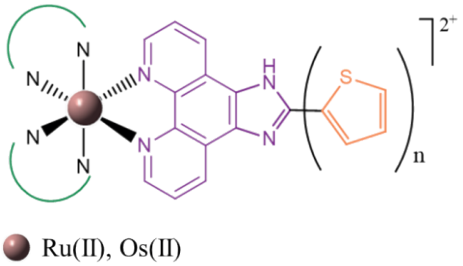


Fig. 1 – Molecular Structure of a representative of the Ru(II)- and Os(II)-polypiridyl IP-nT complexes proposed as Hypoxia-Active Photosensitizers

References

1) C.G. Cameron, M.E. Alberto, S.A. McFarland et al. Breaking the Barrier: An Osmium Photosensitizer with Unprecedented Hypoxic Phototoxicity for Real World Photodynamic Therapy, Chem. Sci., 11, 9784-9806, 2020;
2) C.G. Cameron, M.E. Alberto, S.A. McFarland et al. "Os(II) Oligothienyl Complexes as a Hypoxia-Active Photosensitizer Class for Photodynamic Therapy, Inorg Chem, 59, 16341−16360, 2020;
3) M.E. Alberto, J. Pirillo, N. Russo, C. Adamo, A theoretical exploration of TypeI/TypeII dual photoreactivity of new promising Ru(II)-dyads for PDT approach, Inorg Chem, 55, 11185–11192, 2016;

Acknowledgements. We acknowledge the CINECA award under the ISCRA initiative, for the availability of high performance computing resources and support (IsC85_Hypo-PDT)

IL-2.4.6

Light-driven coordination compounds and hybrid assemblies as multimodal bioimaging agents and ROS-photosensitizers: Design, synthesis and implementation

Cristian A. Strassert
Institut für Anorganische und Analytische Chemie & Cells in Motion Interfaculty Centre – Westfälische Wilhelms-Universität Münster, Münster, Germany

Diverse approaches have allowed us to fully control the aggregation of planar coordination compounds and to tune their photophysical properties for phototherapy, functional microscopy and multimodal bioimaging.

We have designed a series of (na)phthalocyanine-based photosensitizers able to target, to label and to photoinactivate pathogenic and antibiotic resistant bacteria upon irradiation with red light. For this purpose, it was necessary to avoid stacking by diverse (supra)molecular strategies. Currently, we are extending these concepts to targeted, fully water-soluble and biodegradable platforms, a prerequisite for biomedical applications. These approaches include the use of dextrin conjugates and cyclodextrin vesicles that selectively photoinactivate Gram-positive strains. On the other hand, axially decorated dicationic Si(IV) phthalocyanines can kill both Gram-positive and Gram-negative bacteria, despite showing antibiotic resistance. We have also implemented light-driven arrays for spatiotemporally resolved functional microscopy to monitor in situ the response towards ROS of eukaryotic as well as prokaryotic cells and biofilms. Insertion of open-shell transition metal cations and tuning of the macrocycles' substitution pattern yielded NIR-absorbing sonophores for in vivo photoacoustic imaging.

Acknowledgements. This work was supported by the Deutsche Forschungsgemeinschaft (Cluster of Excellence Cells in Motion EXC1003).

IL-2.4.7

Non-invasive activation of photosensitizers in the lungs: achievable goal or impossible dream?

Giulia Kassab[1], Johan S. D. Tovar[1], Hilde H. Buzzá[1], Cristina Kurachi[1] and Vanderlei S. Bagnato[1]

1) University of São Paulo, Institute of Physics of São Carlos, São Carlos, Brazil

Photodynamic therapy (PDT) is a powerful tool for the treatment of cancers and infections. However, one of its main limitations is the attenuation of light through biological tissue, especially at the visible spectrum range. PDT of the lungs has been approved for the treatment of multiple primary lung cancers, for which the illumination is either done in the exposed organ during surgery, or inserting fibers through bronchoscopy [1]. PDT has also been proposed as a treatment for pulmonary infections, for which using such invasive methods would be unjustified, and there would be a risk of spreading the infection even more. Thus, near-infrared photodynamic therapy has often been chosen as a strategy, with external illumination in the 800 nm range, that should present less attenuation through the thoracic cavity and reach the lungs[2, 3]. Yet, despite the promising results in small animals, there is a lot of skepticism on whether or not it will be feasible in human patients. With that in mind, we have developed a light source for pulmonary PDT, composed of two panel of 200 lasers with a peak power of 100mW each (808 nm), and tested the distribution of light in phantoms, an *ex vivo* thoracic cavity model, and finally in an *in vivo* porcine model. The light successfully activated the photosensitizer indocyanine green and inactivated 6 logs of colony-forming units of *S. pneumoniae* in both the phantom and *ex vivo* experiments. When using a single panel, about 3-5% of the emitted irradiance reaches the inside of the thoracic cavity in the *ex vivo* model. In the pig experiment, the placing of the panels proved to be critical for the penetration of light, enabling up to 30% of the initial irradiance to reach the lobes when both panels were turned on. The animal's overall health and respiratory functions remained stable during the light treatment, and there was no temperature increase of the lungs nor the skin directly exposed to the light source. In summary, our findings suggest that 808 nm is an excellent wavelength for the external activation of photosensitizers in the lungs, and that it would be possible for PDT sessions to be performed using the presented light source.

References

1) El-Hussein A, Manoto SL, Ombinda-Lemboumba S, et al. A Review of Chemotherapy and Photodynamic Therapy for Lung Cancer Treatment. *Anticancer Agents Med Chem* 2020; 21: 149–161.

2) Geralde MC, Leite IS, Inada NM, et al. Pneumonia treatment by photodynamic therapy with extracorporeal illumination - an experimental model. *Physiol Rep* 2017; 5: e13190.

3) Kassab G, Cheburkanov V, Willis J, et al. Safety and delivery efficiency of a photodynamic treatment of the lungs using indocyanine green and extracorporeal near infra-red illumination. *J Biophotonics* 2020; 50: jbio.202000176.

Acknowledgements. This work was supported by the São Paulo Research Foundation (FAPESP Grants 2013/07276-1, 2018/18188-0 and 2016/14033-6) and Embrapii (PIFS-2005.0022).

OC-2.4.8, P-2.4.10

Clinical use of a near-infrared fluorescence imaging system in photodynamic therapy using liposomal indocyanine green: a case series study

Hiromi Muranishi[1-3], Yasuo Komura[1-3], Atsushi Hori[3], Shinichi Hori[3]
1) Rinku Medical Clinic, Osaka, Japan; 2) Kyoto Oike Medical clinic, Kyoto, Japan; 3) IGT clinic, Osaka, Japan

(Background) Photodynamic therapy (PDT) is an alternative minimally invasive therapeutic procedure for malignant diseases currently used in clinical practice. Indocyanine green (ICG) is considered as a good photosensitizer for PDT. ICG has been encapsulated in an inner aqueous compartment of liposome (liposomal ICG), improving its stability and efficacy. However, because of the lack of clinical evidence, the biodistribution of liposomal ICG and the optimal clinical strategy for PDT using liposomal ICG is unclear. We reported a series of cases using a near-infrared fluorescence (NIR) imaging system to evaluate the biodistribution of liposomal ICG in patients with breast cancer undergoing PDT.
(Purpose) This case-series study aimed to evaluate the biodistribution of liposomal ICG in patients with breast cancer undergoing PDT.
(Method and Result) Four patients with breast cancer underwent PDT with liposomal ICG in addition to TACE from August 2020 to October 2020. Patients ranged from 41 to 55 years of age. Three patients had metastatic breast cancer, but daily activity was preserved. The patients had either finished or rejected standard therapy. Patients were administered 300 mg liposomal ICG (180 mg intravenously and 120 mg intratumorally via the feeding artery) 24 hours before PDT during a transcatheter arterial chemoembolization (TACE) procedure. We used near-infrared fluorescence imaging system (LIGHTVISION®; Shimadzu Corporation) to detect the biodistribution of liposomal ICG 3, 6, and 24 hours before the PDT procedure. The peak intertumoral liposomal ICG uptake was shown 24 hours after liposomal ICG administration in three patients. Only one patient had peak uptake at 6 hours, with no uptake at 24 hours.
(Discussion) Although several preclinical studies have shown the efficacy and safety of PDT with liposomal ICG for several malignant diseases, few have reported using PDT with liposomal ICG in the clinical setting [1-3]. However, some clinical studies have already reported that the optimal time for PDT with ICG is 24 - 48 hours after ICG administration. In a similar fashion we found that the optimal time for PDT procedure was 24 hours after liposomal ICG administration, except in one case. Compared with other photosensitizers, a strength for using ICG is that detection by NIR-imaging systems might be easier. Our study was a case-series study, and further studies are needed to clarify the optimal clinical strategy for PDT with liposomal ICG.
(Conclusion) NIR-imaging system may be and adjuvant in evaluation of liposomal ICG biodistribution in patients with breast cancer and assisting in the decision-making for the use of PDT with liposomal ICG.

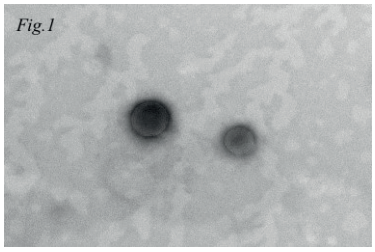


Fig. 1 - Liposomal indocyanine green in scanning electron microscope

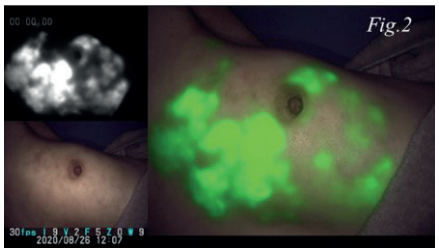


Fig. 2 - Findings of NIR-imaging system

References

1) Lv Chen, Lulu Zhou, et al: Tumor-Targeted Drug and CpG Delivery System for Phototherapy and Docetaxel-Enhanced Immunotherapy with Polarization toward M1-Type Macrophages on Triple Negative Breast Cancers. Adv Mater. 2019 Dec;31(52):e1904997
2) María Julia Lamberti, Natalia Belén Rumie Vittar, et al; Breast cancer as photodynamic therapy target: Enhanced therapeutic efficiency by overview of tumor complexity. World J Clin Oncol 2014; 5(5): 901-907
3) ElżbietaOstańska, DavidAebisher, et al; The potential of photodynamic therapy in current breast cancer treatment methodologies. Biomed Pharmacother. 2021 May;137:111302

OC-2.4.9

A low molecular weight carboxamide halogenated bacteriochlorin for the treatment of highly aggressive tumors

Bárbara Lima[1], Helder Tão[1], Mariette Pereira[1], Lígia C. Gomes-da-Silva[1], Luis G. Arnaut[1]
1) CQC, Coimbra Center of Chemistry, Coimbra, Portugal

Photodynamic therapy (PDT) is a clinically approved therapy that combines the use of a photosensitizer (PS) and light, which in the presence of molecular oxygen generates reactive oxygen species. The generated oxidative stress leads to cell death of cancer cells and other cells of the tumour microenvironment. Additionaly, PDT is being perceived as a new form of photoimmunotherapy owing to its ability to trigger anti-tumor immunity.
Despite promising, PDT (as other oncological therapies) has a small effect on very aggressive and poorly immunogenic tumors such as, the 4T1 orthotopic mouse model. This model closely mimics the immunogenicity, metastatic properties and growth characteristics of stage IV of human breast cancer. It is very challenging to treat 4T1 orthotopic tumors with a variety of photosensitizers namely, chlorin-e6, verteporfin, talaporfin sodium and redaporfin. The poor vascularization of 4T1 tumors and the high accumulation of immunosuppressive cells contribute to the poor responses to various therapies, including PDT. Additional challenges rely on its orthotopic localization at the mammary gland that significantly increases the risk of lethality upon tumor illumination.
A new family of bacteriochlorins with relatively smaller molecular weight and moderate lipophilicity was recently developed by our group with the expectation that these properties will favor cellular internalization. *In vitro* screenings pointed out LUZ51b as a promising compound. LUZ51b is a small lipophilic halogenated carboxamide bacteriochlorin (MM= 595.59 g/mol; log P_{ow} = 2.9) that is characterized by an elevated ROS quantum yield and strong absorption at 734 nm.
Preliminary results using phosphatidylcholine liposomes demonstrated that LUZ51b interacts more with the lipid membranes than redaporfin, a bacteriochlorin of 1135 g/mol and log P_{ow} = 1.9. In accordance, after a short time of incubation, such as 4 h, high levels of cellular internalization of LUZ51b are observed, which is in contrast to redaporfin that only peaks at 24h. LUZ51b mainly accumulates at the endoplasmic reticulum and Golgi compartments (being a potential inducer of immunogenic cell death) and remarkably, it kills cancer cells at concentration in the range of nM. Pre-clinical studies confirmed the high efficacy of photo-activated LUZ51b in cancer mouse models of different aggressiveness: low (subcutaneous CT26 tumors), moderate (subcutaneous B16F10 tumors) and high (orthotopic 4T1 tumors). Remarkably, an optimized protocol consisting in the intravenous administration of 0.25 mg/kg of LUZ51 followed by the deliver of 20 J/cm^2 enabled the cure of approximately 60% (3 out of 5) Balb/c mice bearing 4T1 tumors. Further rechallenge of these mice with 4T1 cells revealed delay of tumor growth which might indicate the presence of immunological memory.
Overall, LUZ51b combine unique properties that turn it in a very promising photosensitizer for the treatment of aggressive and advanced tumors. Further studies will focus on its potential for the treatment of pancreatic cancer, alone or in the combination with immunotherapy. This might constitute a major breakthrough in oncology as this type of tumors lack effective therapeutic options.

Acknowledgements. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement n°764837 and Coimbra Chemistry Center under the project UID/QUI/00313/2019).

P-2.4.11

Metal-based photosensitizers: approaches to enhance the efficiency of photodynamic therapy in cancer treatment

Ge Shi, John Roque III, Houston D. Cole, Colin G. Cameron, Sherri A. McFarland
Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, Texas, 76019 USA

Photodynamic therapy (PDT) has become an approved medical intervention to treat certain types of cancer. It has been utilized to complement and in certain cases to replace traditional anticancer treatment such as surgery, chemotherapy or radiotherapy. In PDT, a photosensitizer that is generally organic compound is administrated locally or systemically at a nontoxic dose and then activated with red light in the PDT window to produce a potent PDT response with minimal dark toxicity. The photosensitizers that have been designed and developed in our lab are metal-based coordination complexes, which we believe are promising PDT agents dues to their special photophysical and photobiological properties. Ruthenium- and osmium-polypyridyl complexes serve as examples, with our own TLD1433 currently undergoing a phase II clinical trial for bladder cancer. This presentation will share insights regarding the development of some of our latest photosensitizers for PDT.

PL-6.1

Radiotherapeutic effects of radioluminescent nanomaterials

Anne-Laure Bulin

Synchrotron Radiation for Biomedicine, Grenoble Alpes University, INSERM UA7, Grenoble, France.

More than 50% of cancer patients undergo radiation therapy in the course of their treatment. However, because of a lack of specificity for tumor tissues, delivering therapeutically effective doses of X-rays with tolerable toxicity on healthy surrounding tissues remains a challenge. In order to enhance the therapeutic window of radiotherapy for difficult-to-treat cancers, innovative nanoscintillators may induce various radiotherapeutic effects. Nanoscintillators, also known as radioluminescent nanoparticles, down-convert ionizing radiations into visible light. Thus, they can act as internal light sources for X-ray activated PDT [1]. When accumulated in the tumor prior to radiotherapy, nanoscintillators can induce various effects that can synergize. First, when conjugated to photosensitizers, nanoscintillators can induce deep-tissue photodynamic therapy (PDT). This strategy would overcome the shallow penetration of light in tissues, one of the major limitations of PDT [2]. Second, when nanoscintillators emit in the ultraviolet (UV)-range, and more specifically in the UV-C, direct DNA-damage can be induced [3,4]. Finally, because nanoscintillators are made of high-Z element, their accumulation in the tumor prior to radiotherapy will create a purely physical effect of radiation dose-enhancement [5]. This effect is initiated by a higher photoelectric absorption of orthovoltage X-rays by high-Z elements compared to soft tissues that leads to a higher production of photo- and Auger electrons that enhance the damage to cancer tissues.
While proof-of-concept studies of radiotherapeutic effects of nanoscintillators have already been demonstrated by us and others, our goal is now to provide a comprehensive description of the mechanisms involved during radiotherapy. We leverage a multidisciplinary approach ranging from physics to biology, to preclinical trials. Our approach includes Monte Carlo simulations to guide the design of optimized nanoconjugates, measurements with reactive oxygen species-sensitive fluorescent probes, *in vitro* experiments performed on 3D models of glioblastoma and pancreatic cultures and preclinical tests. In order to investigate the particular role of the X-ray energy in the overall treatment efficacy, radiotherapy is delivered by monochromatic tunable synchrotron radiation.

References

1. Dujardin, C. *et al.* Needs, Trends, and Advances in Inorganic Scintillators. *IEEE T Nucl Sci* **65**, 1977–1997 (2018).
2. Sun, W., Zhou, Z., Pratx, G., Chen, X. & Chen, H. Nanoscintillator-Mediated X-Ray Induced Photodynamic Therapy for Deep-Seated Tumors: From Concept to Biomedical Applications. *Theranostics* **10**, 1296–1318 (2020).
3. Squillante, M. R. *et al.* Fabrication and characterization of UV-emitting nanoparticles as novel radiation sensitizers targeting hypoxic tumor cells. *Opt Mater* **80**, 197–202 (2018).
4. Müller, M. *et al.* UV scintillating particles as radiosensitizer enhance cell killing after X-ray excitation. *Radiother Oncol* **129**, 589–594 (2018).
5. Bulin, A.-L. *et al.* Radiation Dose-Enhancement Is a Potent Radiotherapeutic Effect of Rare-Earth Composite Nanoscintillators in Preclinical Models of Glioblastoma. *Advanced Science* **7**, 2001675 (2020).

Acknowledgements. My group is supported by grants from the French Medical Research Foundation (*Fondation pour la Recherche Médicale*, ARF20170938739), the Auvergne Rhône-Alpes Region (NEPTUNE project), INSERM Cancer, the French *Ligue Contre le Cancer* Foundation, the LABEX PRIMES of the Université of Lyon (ANR-11-LABX-0063), the "*Rotary Club*" Association through the program "*Jetons Cancer*" and the INSERM.

PL-6.2

A NanoBioengineering Frontier for Next-Generation Optical Devices

Ardemis A. Boghossian[1]
1) Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

The vast expansion of available synthetic biology tools has led to explosive developments in the field of materials science. No longer confined to engineering just synthetic materials, the increased accessibility of these tools has pushed the frontier of materials science into the field of engineering biological and even living materials. By coupling the tunability of nanomaterials with the prospect of re-programming living devices, one can re-purpose biology to fulfill needs that are otherwise intractable using traditional engineering approaches.

Optical technologies in particular could benefit from capitalizing on untapped potential in coupling the optical properties of nanomaterials with the specificity and scalability of biological materials. This presentation highlights specific applications in optical sensing and light-harvesting energy technologies that exploit the synergistic coupling of nanobio-hybrid materials. We discuss the development of bio-conjugated single-walled carbon nanotubes (SWCNTs) for near-infrared fluorescence sensing and the application of these nano-bioptic sensors for continuous measurements in living cells and organisms. We further explore the development living photovoltaics based on bioengineered, photosynthetic organisms with augmented capabilities.

Acknowledgements. This work was supported by the Swiss National Science Foundation (SNSF), European Research Council (ERC) Starting Grant, CODEV seed funding, and Swiss Competence Center (SCCER) for Hydrogen Production & Storage.

IL-3.1.1

Type I interferons enhance repair of ultraviolet radiation induced DNA damage and regulate cutaneous immune suppression

Mohammad Asif Sherwani[1], Israr Ahmad[1], Ahmed Abdelgawad[1], Kevin Yang[1], Chander Raman[1], Craig A Elmets[1,2,3], Nabiha Yusuf[1,2,3]
1) Department of Dermatology, 2) O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham, AL, USA; 3) Veteran Affairs Medical Center, Birmingham, AL, USA.

Type I interferons (IFNs) are important enhancers of immune responses which are downregulated in human cancers, including skin cancer. Solar ultraviolet (UV) B radiation is a proven environmental carcinogen, and its exposure contributes to the high prevalence of skin cancer. The carcinogenic effects of UV light can be attributed to the formation of cyclobutane pyrimidine dimers (CPD) and errors in repair and replication of DNA. Treatment with a single dose of UVB (100 mJ/cm^2) upregulated IFNα and IFNβ in the skin of C57BL/6 mice. They were predominantly produced by CD11b+ cells. Mice lacking the type I IFN receptor 1 (IFNAR1) had decreased repair of CPD following cutaneous exposure to a single dose of UVB (100 mJ/cm^2). UVB induced expression of the DNA repair gene xeroderma pigmentosum A (*XPA*) in wild type (WT) mice. In contrast such treatment in IFNAR1 (IFNAR1-/-) mice downregulated *XPA*. A local UVB regimen consisting of UVB radiation (150 mJ/cm^2) for 4 days followed by sensitization with the hapten 2,4, dinitrofluorobenzene (DNFB) resulted in significant suppression of immune responses in both WT and IFNAR1-/- mice. However, there were significantly higher CD4+CD25+Foxp3+ regulatory T-cells and CD11b+Gr1+ myeloid cells in draining lymph nodes of IFNAR1-/- mice in comparison to WT mice. Overall, our studies reveal a previously unknown action of type I IFNs in repair of photodamage. Strategies that enhance type I IFN production may be useful for prevention of pre-malignant skin lesions before they develop into skin cancer.

Acknowledgements. This work was supported by 1R01AR071157-01A1 from National Institute of Arthritis and Musculoskeletal and Skin Diseases (NY), American Skin Association (KY and NY), and I01BX003395 from Veterans Administration (CAE).

IL-3.1.2

Modulation of B cell responses to TLR7 activation following narrowband UVB phototherapy in early multiple sclerosis

Stephanie Trend[1,2], Jonatan Leffler[1], Matthew N Cooper[1], Scott N Byrne[3,4], Allan G Kermode[2,5], Martyn A French[6], Prue H Hart[1]

1) Telethon Kids Institute, University of Western Australia, Perth, WA, Australia;2) Centre for Neuromuscular and Neurological Disorders, Perron Institute for Neurological and Translational Science, University of Western Australia, Perth, WA, Australia;3) School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, Australia;4) Centre for Immunology and Allergy Research, Westmead Institute for Medical Research, Westmead, NSW, Australia;5) Institute for Immunology and Infectious Disease, Murdoch University, Perth, WA, Australia;6) UWA Medical School and School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia.

Multiple sclerosis (MS) is an immune-mediated disease that involves episodes of inflammation in the central nervous system, and is the most common degenerative neurological condition affecting young adults. Low UV radiation exposure is associated with an increased risk of developing MS. To investigate whether UV radiation would be beneficial for people with the earliest form of MS, (Clinically Isolated Syndrome [CIS]), the PhoCIS trial was established, and participants received either sub-erythemal narrowband UVB (311-312 nm) phototherapy three times per week for 8 weeks (n=7) or no phototherapy (controls; n=6). Peripheral blood mononuclear cells (PBMC) were collected at baseline and at 8 weeks, following completion of therapy, and flow cytometry was used to investigate the frequencies of B cell subsets. In addition, the expression of TNF and IL-10 in B cells cultured with or without a TLR7/8 agonist (R848) was investigated in each individual at 8 weeks, adjusted for responses at baseline. Significant changes in the relative frequencies of naive, marginal zone (MZ)-like and class switched memory B cells were observed after the treatment period in individuals receiving phototherapy. Phototherapy had no detectable effect on IL-10 expression in B cells. However, the expression of TNF following culture with R848 was significantly decreased in B cells, particularly the MZ-like cell population, after phototherapy. These results suggest that phototherapy-associated priming effects were detected upon subsequent polyclonal B-cell activation in B cells.

Acknowledgements. This work was supported by grants from Multiple Sclerosis WA. The trial recruiting the CIS phototherapy patients was supported by the National Health and Medical Research Council of Australia (ID 1067209).

IL-3.1.3

Exposure to systemic immunosuppressive solar simulated ultraviolet radiation alters T cell recirculation through sphingosine 1 phosphate

Scott N. Byrne*[1,2], Benita C.Y. Tse[1], Rachael Ireland[1,2], Jun Yup Lee[1], Felix Marsh-Wakefield[1], Lai Fong Kok[1] and Anthony Don[3,4]
1) The University of Sydney, School of Medical Sciences, Faculty of Medicine and Health, Sydney, New South Wales, Australia; 2) Westmead Institute for Medical Research, Centre for Immunology and Allergy Research, Westmead, New South Wales, Australia; 3) Centenary Institute, The University of Sydney, New South Wales, Australia; 4) The University of Sydney, NHMRC Clinical Trials Centre, Faculty of Medicine and Health, New South Wales, Australia.
**Presenting author: scott.byrne@sydney.edu.au*

Systemic suppression of adaptive immune responses is a major way through which ultraviolet (UV) radiation contributes to skin cancer development. Immune suppression is also likely to explain how UV protects from some autoimmune diseases such as multiple sclerosis. However, the mechanisms underlying UV-mediated systemic immune suppression are not well understood. Exposure of mice to doses of solar simulated UV known to suppress systemic autoimmunity led to the accumulation of cells within the skin-draining lymph nodes and away from non-skin-draining lymph nodes. Transfer of $CD45.1^+$ cells from non-irradiated donors into $CD45.2^+$ UV-irradiated recipients resulted in preferential accumulation of donor naïve T cells and a decrease in activated T cells within skin-draining lymph nodes. A single dose of immune suppressive solar-simulated UV was all that was required to cause a redistribution of naïve and central memory T cells from peripheral blood to the skin-draining lymph nodes. Specifically, CD69-independent increases in S1P receptor 1 (S1P1)-negative naïve and central memory T cells occurred in these lymph nodes. Mass spectrometry analysis showed UV-mediated activation of sphingosine kinase 1 activity, resulting in an increase in S1P levels within the lymph nodes. Topical application of a sphingosine kinase inhibitor on the skin prior to UV-irradiation eliminated the UV-induced increase in S1P. Thus, exposure to immunosuppressive UV disrupts T cell recirculation by manipulating the S1P pathway.

OC-3.1.4

Local and systemic effects of narrowband UVB irradiation in mice

Rachael Ireland[1,2], Benita Tse[1], Anthony Don[3], Scott Byrne[1,2]
1) The University of Sydney, Cellular Photoimmunology Group, Sydney, Australia; 2) The Westmead Institute for Medical Research, Centre for Immunology and Allergy Research, Sydney, Australia; 3) The University of Sydney, Centenary Institute, Sydney, Australia

Ultraviolet radiation (UVR) stimulates local and systemic immunoregulatory effects in a wavelength dependant manner. Narrowband UVB (NBUVB) phototherapy is a common and effective treatment for inflammatory skin diseases including psoriasis, but its mechanism of action is not fully understood. This study aims to characterise local and systemic changes in mice following NBUVB irradiation. In a model of contact hypersensitivity, a single exposure to 3 J/cm^2 NBUVB suppressed the response to an irritant applied at an unirradiated site. Like exposure to solar simulated UV (ssUV), this immune suppressive dose of NBUVB resulted in Langerhans cell depletion and dermal neutrophil infiltration. Mast cells are a key mediator of ssUV induced immune suppression, but their dermal frequency was not altered by single or repeated exposure to NBUVB. NBUVB irradiation also generated fewer changes in both the plasma lipidome and lymphocyte recirculation compared to ssUV. While NBUVB is a potent local immunomodulator, these findings suggest it has relatively low capacity to induce systemic effects, which has implications for the clinical use of NBUVB in the treatment of a wider range of diseases.

IL-3.1.5

Oestrogen-related factors and incidence of melanoma in the U.S. Radiologic Technologists study

Jim Z. Mai[1], Rui Zhang[1], Michael R. Sargen[1], Mark Little[1], Bruce H. Alexander[3], Margaret A. Tucker[1], Cari Kitahara[1], Elizabeth K. Cahoon[1]
1) National Cancer Institute, Division of Cancer Epidemiology and Genetics, Bethesda, United States; 2) University of Minnesota, School of Public Health, Minneapolis, United States

While the photosensitizing effects of oestrogens may increase the impact of ultraviolet (UV) radiation on melanoma risk, data from large cohort studies with information on personal sun sensitivity and UV radiation exposure are lacking. Few studies have examined effect modification interaction between UVR, oestrogens-related factors and risk of melanoma. We examined the association between reproductive factors, exogenous hormone use, and first primary invasive melanoma of the skin while accounting for UV radiation exposure across different life periods, personal sun sensitivity, and lifestyle factors for geographically dispersed non-Hispanic white women exposed to a wide range of ambient UV radiation, using data from the U.S. Radiologic Technologists (USRT) Study.

The USRT is a large nationwide prospective cohort of 146,022 radiologic technologist. The study population included white female participants who completed both the second (baseline; 1994-1998; mean age at entry =46.8 years) and third questionnaires (2003-2005), and did not report having cancer (except for keratinocyte carcinoma, KC) at baseline. Participants (N=46,544) were followed from their age at completion of the baseline questionnaire until the earlier of first primary cancer diagnosis, including invasive melanoma of the skin, or completion of (either the third or fourth) questionnaire. To examine the association of reproductive factors, exogenous hormone use, and first primary invasive melanoma of the skin, we used Poisson regression to calculate rate ratios (RRs) and 95% likelihood-based confidence intervals (CIs), adjusting for attained age, birth cohort (<1941, 1941-1945, 1946-1950, 1951-1955, 1956+), lifetime average annual ambient UV radiation (quartile 1-4), contraceptives and menopausal hormone therapy use. To address the effect modification of ambient UV radiation exposure and personal sun sensitivities on melanoma risk, we conducted likelihood-ratio tests for multiplicative interaction.

Over a median follow-up time of 17.1 years, 0.95% of eligible participants had an incident first primary melanoma (n=444). Higher melanoma risks incidence rates were observed in participants with older attained age, blue/green eye colour, blonde/red/auburn natural hair colour at age 15, fair skin complexion, and higher UV radiation. We found an increased risk of melanoma in women who experienced menarche at an early age (13 and ≤12 years versus 14+ years: RR=1.33, 95% CI=1.03-1.75; 1.27, 0.93-1.73; *P*-value for trend=0.17), and in women with older age at first birth (25-29 and ≥30 years versus <25 years; 1.09, 0.86-1.39; 1.48, 1.12-1.95; *P*-value for trend<0.001). However, no significant association was observed for other reproductive factors, and for all exogenous hormone use. The associations of melanoma incidence for most reproductive factors and exogenous hormone use were not modified by ambient UVR, eye colour, natural hair colour at age 15 and skin complexion. The exception was that natural hair colour at age 15 significantly modified the associations of melanoma for age at menarche (*P*-value for interaction = 0.002) and age at first birth among parous women (0.028). In participants with blonde/red/auburn natural hair colour at age 15, we found significantly increased risk of melanoma among women who experienced menarche at an early age (13 and ≤12 years vs 14+ years) (HR = 3.02, 95% CI = 1.74-5.80; 2.67, 1.42-5.39, respectively; *P*-value for trend = 0.011). The positive association between age at menarche and melanoma was null in participants with brown natural hair colour at age 15.

Early age at menarche and late age at first birth were associated with higher risks of melanoma in this large, geographically dispersed cohort of non-Hispanic white female radiologic technologists. These findings strengthen the evidence that oestrogen-related factors alone were not associated with melanoma. However, women with late age at first birth and early age at menarche, especially those with blonde/red/auburn natural hair colour at age 15, or residing in areas with elevated levels of ambient UV radiation may constitute an additional high-risk group in need of more frequent skin cancer examinations. Future larger studies could collect more detailed information on photosensitizing medication use and breastfeeding, and ideally, measurements of genetically predicted levels of

oestrogen, particularly for childhood. Identifying susceptible periods of exposure or factors that modify UVR susceptibility may aid in guiding more targeted guidelines for melanoma prevention.

References

1) Siegel, R.L., et al., Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians, 2021.
2) Bray, F., et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 2018.
3) Olsen, C.M., et al., Evaluation of Sex-Specific Incidence of Melanoma. JAMA Dermatology, 2020.
4) Sun, Q., et al., Effects of Exogenous Hormones and Reproductive Factors on Female Melanoma: A Meta-Analysis. Clinical Epidemiology, 2020.
5) Gandini, S., et al., Hormonal and reproductive factors in relation to melanoma in women: current review and meta-analysis. European Journal of Cancer, 2011.
6) Donley, G.M., et al., Reproductive factors, exogenous hormone use and incidence of melanoma among women in the United States. British Journal of Cancer, 2019.
7) National Aeronautics Space Administration. Total ozone mapping spectrometer data product: erythemal UV exposure, 2004.
8) Fuhrman, B.J., et al., Association of the Age at Menarche with Site-Specific Cancer Risks in Pooled Data from Nine Cohorts. Cancer Research, 2021.
9) Olsen, C., et al., Reproductive factors, hormone use and melanoma risk: an Australian prospective cohort study. British Journal of Dermatology, 2021.
10) Armstrong, B.K., C.M. Vajdic, and A.E. Cust, Melanoma, in Cancer Epidemiology and Prevention, M. Thun, et al., Editors. 2017.

Acknowledgements. We thank the radiologic technologists who participated in the study, Dr Jerry Reid of the American Registry of Radiologic Technologists for continued support, and Diane Kampa and Allison Iwan of the University of Minnesota for data management and collection. This research was supported by the Intramural Research Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services.

IL-3.1.6

Photoprotection by vitamin D compounds: uncovering markers that predict efficacy in reducing photocarcinogenesis.

Furkan A. Ince[1], Artur Shariev[1], Mark S. Rybchyn[1], W.G. Manori De Silva[1], B.Y. McCarthy[1], C. Yang[1], Myriam Abboud[2], Rebecca S. Mason[1] and Katie M. Dixon[1]

1) School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney NSW 2006 Australia. 2) College of Natural and Health Sciences, Zayed University, United Arab Emirates.

The key ingredients for development of ultraviolet radiation (UV)-induced skin tumours are UV-induced DNA damage, some of which is inadequately repaired, resulting in mutations, and UV-induced immune suppression, which results in developing skin tumours not being recognised and eliminated by immune surveillance. The vitamin D hormone, 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), applied immediately after UV exposure, reduces DNA damage, immune suppression and skin tumours, as do the vitamin D-like compounds 1,25-dihydroxylumisterol and tetrahydrocurcumin[1,2]. Yet several of the vitamin D-like compounds and other potentially photoprotective agents we have examined in acute and chronic UV studies have reduced UV-generated DNA damage in skin cells and in skin, and reduced UV-induced immune suppression in mice[1], but have not reduced photocarcinogenesis in this well accepted murine model. There is thus a need to find readily measurable markers which can identify agents that will reduce skin tumours in mice exposed to chronic UV, since reductions in UV-induced DNA damage and immune suppression alone are not necessarily predictive.

Both phosphatase and tensin homolog (PTEN) and N-myc downstream regulated gene-1 (NDRG1) are protein markers which are lost or suppressed during carcinogenesis and metastasis. We demonstrated that expression of both these proteins is significantly reduced 24 h after UV in primary melanocytes and in Skh:hr1 mouse skin, but significantly increased with $1,25(OH)_2D_3$ treatment. Phosphorylation of Cyclic AMP response element binding protein (CREB) is known to increase in skin cells after UV and is linked to carcinogenic potential[3]. Our preliminary data indicates that $1,25(OH)_2D_3$ also reduces pCREB after UV. Interleukin-6 (IL-6) is a marker of inflammation, increased after UV. IL-10 also increases following UV and contributes to immune suppression. We have demonstrated reductions in both of these cytokines with $1,25(OH)_2D_3$. Using vitamin D and vitamin D-like compounds previously tested for their effects on DNA damage, immune suppression and photocarcinogenesis in both human *in vitro* and murine *in vivo* models, we performed further studies in primary human skin cells, Skh:hr1 mice and *ex vivo* human skin, with the aim of developing a matrix to determine which of these markers or combination of markers best predict ability of the agent to reduce UV-induced skin tumours.

Testing for efficacy against UV-induced skin tumours, while essential to the development of biological enhancers to simple filtration of UV by sunscreens, is a long (40 week protocol), tedious and very expensive process. If there were early markers for likely efficacy and to identify those agents which were unlikely to reduce tumours, this would considerably streamline the process for identifying suitable candidates for this lengthy testing.

References

1) Dixon, K.M., et al., 1alpha,25(OH)2-Vitamin D and a Nongenomic Vitamin D Analogue Inhibit Ultraviolet Radiation-Induced Skin Carcinogenesis. Cancer Prevention Research, 2011. 4(9): p. 1485-94.
2) Tongkao-On, W., Role of Vitamin D and Other Compounds in the Protection of Skin Cells from UV, in Physiology, School of Medical Sciences, University of Sydney, 2015 [PhD Thesis].
3) Rybchyn, M.S., et al., Enhanced Repair of UV-Induced DNA Damage by 1,25-Dihydroxyvitamin D3 in Skin Is Linked to Pathways that Control Cellular Energy. J Invest Dermatol, 2018. 138(5): p. 1146-1156.

IL-3.1.7

UV-induced growth and migration of melanoma cells in a spheroid model.

Linda Vainikka, Petra Wäster, Linda Rößler and Karin Öllinger[1]
1) Linköping University, Department of biomedicine and Clinical Sciences, Linköping, Sweden

Cutaneous melanoma is a highly aggressive cancer with a propensity for metastasis to various organs. The most important risk factor to develop the disease is exposure to UV radiation. UVB wavelengths has ability to cause tumor-initiating mutations while UVA exposure generates free radicals and may act immunosuppressive. How the microenvironmental effects of UV radiation influence melanoma pathogenesis and its ability to metastasize remains to be elucidated. In a previous report we have shown that UVA exposure triggers increased secretion of extracellular vesicles. UVA cause plasma membrane damage which is promptly repaired by transportation of lysosomes to the damaged membrane to form a resealing patch. Subsequently, extracellular vesicles are released outside the cell (see Figure 1). The vesicles promote signaling event when taken up by non-irradiated cells and increased growth and migration is noted[1].

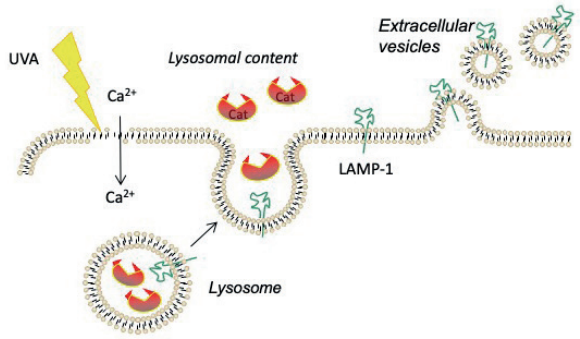


Figure 1. UVA-radiation induces plasma membrane damage resulting in calcium influx, which trigger transport of lysosomes to the damaged site. The lysosomal membranes forms a resealing patch and lysosomal content is released outside the cell. Subsequently, excess membrane is shredded as extracellular vesicles.

In this study results from UV exposure of malignant melanoma cells will be presented and the possible signaling pathways induce by UV will be discussed. We generated melanoma tumor spheroids from four different cell lines and exposed to sub-lethal doses of UVA- and UVB-radiation. We found spontaneous secretion of extracellular vesicles by all melanoma cell lines, but after UVA, increased number of large size vesicles were found. Following radiation, the spheroids were embedded in Matrigel and observed microscopically to follow cell migration and invasion. Cell migration out from the spheroid was higher in spheroids exposed to UVA compared to control and UVB exposed samples. A transcriptome analysis was performed 24 h after irradiation of the spheroids, and revealed significantly increased mRNA levels of matrix metalloprotease-1 (MMP1; +3.3-fold, p=0.0009) after UVA exposure. MMP-1 is a collagenase involved in extracellular matrix degradation and the protease is secreted and activated outside the cell. We found elevated protein level of MMP1 and secretion to the medium in three out of four tested melanoma spheroids after UVA exposure and in one of four after UVB. We conclude that melanoma migration and invasion is elevated more efficiently after UVA than UVB radiation and MMP1 is a candidate protein to promote this effect.

References

1. Wäster P, Eriksson I, Öllinger K. Intercellular cross-talk mediated by extracellular vesicles promotes survival and proliferation via miR-21-signaling. *Pigment Cell and Melanoma Research* 2020; 33(4):542

Acknowledgements. This work was supported by grant from Swedish Cancer Society and Welander Finsten Skin Foundation.

OC-3.1.8

Characterization of Photo damaged skin using 3D Line-field optical coherence tomography and histopathological correlation

J Pérez-Anker[1], B Alejo[1], P Iglesias[2], V. Berot[3], C Cano[4], E Cinotti[5], V Del Marmol[4], M Fontaine[4], M Miyamoto[4], J Monnier[6], JL Perrot[3], P Rubegni[5], L Tognetti[5], M Suppa[4,7], Ann'Laure Demessant - Flavigny[8], Caroline Le Floc'h[8], Leonor Prieto[9], J Malvehy[1,2], S Puig[1,2]

1. Dermatology Department, Melanoma Unit, Hospital Clínic de Barcelona, IDIBAPS, Universitat de Barcelona, Barcelona, Spain, 2. Centro de Investigación Biomédica en Red de Enfermedades Raras, CIBERER, nstituto de Salud Carlos III, Barcelona, Spain, 3. Dermatology Department, University Hospital of Saint-Etienne, Saint-Etienne, France, 4. Dermatology Department, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium, 5. Dermatology Unit, Department of Medical, Surgical and Neurological Sciences, University of Siena, Siena, Italy, 6. Dermatology Department, AP-HM, Aix-Marseille University, Marseille, France, 7. Dermatology Department, Institut Jules Bordet, Universite Libre de Bruxelles, Brussels, Belgium, 8 International Scientific Direction, Laboratoire Dermatologique La Roche-Posay, France, 9. Scientific Direction, Laboratoire Dermatologique La Roche-Posay, L'ORÉAL Cosmética Activa, Madrid, Spain

Chronic sun exposure is responsible of skin photoaging. Noninvasive imaging techniques are able to morphologically characterize photoaging and generate qualitative analysis in photodamaged skin. Optical coherence tomography (OCT) is one of the non-invasive available technologies that allows a deep laser penetration in the surface, reaching a good quality image of dermis visualization. It creates in vivo cross-sectional (vertical) and en face (horizontal) images of the skin with an isotropic resolution of 1.3 µm and a penetration depth of 500 µm. A new three-dimensional technology, 3D lineal confocal OCT (LC-OCT) (DAMAE Medical, Paris) has been introduced, allowing a vertical and horizontal reconstruction of the complete skin with cellular resolution.

Our objectives were to observe in an in vivo three-dimensional mode, the morphological and cytological changes of photodamaged skin, in order to better understand the changes of the different layers of the skin, induced by chronic sun exposure and responsible for chronic sun-damage.

In this study, we conducted a descriptive and observational investigation, after the approval of the Ethics Committee at Hospital Clínic de Barcelona, in order to determine the 3D LC-OCT characteristics of sun damaged skin. In June and July of 2020, nine phototype II-III patients in their fourth to sixth decade of life were prospectively included. Dermoscopy and LC-OCT was performed on two different zones: the sun-damaged area on dorsal forearm (L01) and the non-sun-damaged area on ventral side of the same forearm (L03). Skin biopsies were obtained of both observed areas.

Quantification of the structural morphology of the skin`s upper layers was obtained: The thickening of the Stratum Corneum (SC), of the stratum spinosum, and the flattening of the Dermal Epidermal Junction (DEJ) were evaluated. The number and density of keratinocyte nuclei were also quantified. All metrics were compared as paired data on 9 patients. No statistically significant differences were observed for the SC thickness and the DEJ undulation. By image analysis, we showed that the epidermis thickness was increased (mean: 5.39 µm, p= 0.0078) and that the number of keratinocytes was higher (mean=0.997; p= 0.002) in exposed area (L01) in comparison with non-exposed one (L03). Under LC OCT analysis, the volume of the different layers of keratinocytes seemed to be larger for the more sun-exposed area (mean 0-20%: 4.61 µm3, mean 40-60%: 26.22 µm3, mean 80-100%: 55.34 µm3).

Concerning the histology comparison between both areas as paired data, we showed an increased thickness of the epidermis (mean:11.07µm, p=0.011) on exposed areas. Significant differences were also observed for the number of keratinocytes layers that was higher for the chronic photodamaged skin (mean:0.77, p=0.029). Those results are well correlated with LC-OCT one's.

We present for the first time a study of photodamaged skin in 3D, with quantitative and objective measures comparing normal and chronic photodamaged skin. We demonstrated that the epidermis was significantly thicker on the exposed area and that the number of keratinocytes layers was higher in the same area with good correlation with histopathological evaluation.

IL-3.2.1

NIR biphotonic chromophores in the service of biology

Chantal Andraud[1]

1) Laboratory of Chemistry, Ecole Normale Supérieure de Lyon, CNRS, Lyon, France

This presentation will illustrate our different recent approaches in order to fulfill main requirements for in vivo biophotonics. We will present our results based on : (1) molecular engineering approaches for enhancing organic based chromophores biphotonic properties and further spectrocopic requirements in the NIR[1] ; (2) methods of hydrosolubilisation and biocompatibility for these biphotonic chromophores[2] . Intravital fluorescence imaging will be then discussed as a function of chromophores characteristics (Fig. 1) and perspectives for theranostic or bimodal probes will be presented[3].

Fig. 1 – Example of fluorophore with a 3D image of the functional cerebral vasculature in the motor cortex of mice using two-photon laser scanning microscopy.

References

1) C. Monnereau, C. Andraud *et al.* A water soluble probe with near infra-red two-photon absorption and polarity-induced fluorescence for cerebral vascular imaging Chem. Sci., 2013; C. Monnereau, C. Andraud *et al.* Fluorescent Polymer Probe with High Selectivity toward Vascular Endothelial Cells for and beyond Noninvasive Two-Photon Intravital Imaging of Brain Vasculature, ACS Appl. Mater. Interfaces 2016, 8, 17047 ; C. Andraud, O. Maury *et al.* , Keto-polymethines: a versatile class of dyes with outstanding spectroscopic properties for *in cellulo* and *in vivo* two-photon microscopy imaging, Chem. Sci. 2017, 8, 381; Y. Bretonnière, C. Andraud *et al.* Near-Infrared Solid-State Emitters Based on Isophorone: Synthesis, Crystal Structure and Spectroscopic Properties Chem. Mat. 2011, 23, 862; Y. Bretonnière, C. Andraud *et al.* , Solid state red biphotonic excited emission from small dipolar fluorophores, J. Mater. Chem. C, 2016,4, 766 ; C. Andraud, Y. Bretonnière *et al.* , Tuning the solid-state emission of small push-pull dipolar dyes to the far-red through variation of the electron-acceptor group, Dyes and Pigments 2018, 156, 116 ; C. Andraud, Y. Bretonnière *et al.* , 4,5,5-Trimethyl-2,5-dihydrofuran-Based Electron-Withdrawing Groups for NIR-Emitting Push–Pull Dipolar Fluorophores, J. Org. Chem. 2019, 84, 9965

2) C. Andraud, Y. Bretonnière *et al.*, Photostable far-red emitting pluronic silicate nanoparticles: perfect blood pool fluorophores for biphotonic in-vivo imaging of the leaky tumour vasculature, RSC Advanced 2016, 6, 94200; C. Andraud, Y. Bretonniere, J. Bernard *et al* General and Scalable Approach to Bright, Stable and Functional AIE Fluorogen Colloidal Nanocrystals for in Vivo Imaging ACS Appl. Mater. Interfaces 2018, 10, 25154 ; Y. Bretonnière, C. Andraud, X. Cattoën *et al.*, Ultrabright Silica-Coated Organic Nanocrystals for Two-Photon InVivo Imaging Ultrabright Silica-Coated Organic Nanocrystals for Two-Photon InVivo Imaging ACS Appl.Nano Mater. 2020, 3, 11933

3) C. Andraud, C. Monnereau, O. Maury *et al.*, A "Multi-Heavy-Atom" Approach Toward Biphotonic Photosensitizers With Improved Singlet Oxygen Generation Properties Chemistry - A European J. 2019, 25, 9026; Y. Bretonnière, C. Andraud, S. Parola *et al.*, Hybrid multimodal contrast agent for multiscale *in vivo* investigation of neuroinflammation Nanoscale, 2021,13, 3767

IL-3.2.3

Monitoring of Wound Healing by Using Label-free Multiphoton Microscopy and the 3D Printed Live-cell Imaging Chamber

Haijiang Zhang[1#], Che-Wei Chang[1#], Rafael Camacho[1], Julia Fernandez-Rodriguez[1]*
1) Centre of Cellular Imaging, Core Facilities, the Sahlgrenska Academy, University of Gothenburg, Sweden
#Co-first authors
**Correspondence should be addressed to Julia Fernandez-Rodriguez; email address: julia.fernandez-rodriguez@cci.sahlgrenska.gu.se*

Current skin research typically relies on classical histopathological examination, either by studying the abnormality of the surface or vertical sections across the tissue. The drawbacks of this type of end-point experiment for studying wound healing are its destructive nature and that the outcome can only be rationalized retrospectively. To reliably observe the skin biology directly, the optimal experiment would require monitoring live tissue in real time with an imaging system capable of recording cellular responses a few hundred μm under the skin surface. One-photon microscopy, although capable of achieving sub-micron resolution, generally can only image the surface of the skin tissue, due to the nature of the one-photon absorption/emission, and more importantly, the imaging limitations caused by the tightly packed keratinocyte cells. This study demonstrates that multiphoton laser scanning microscopy is a good imaging technique for monitoring wound healing in *ex vivo* skin models, while using a 3D printed microfluidics chamber for keeping the tissue viable. Multiphoton microscopy is capable of deep tissue imaging down to ~300 μm and is able to monitor the cellular proliferation and collagen regeneration without adding external fluorescent markers, which could potentially interfere with native cellular functions. Further, the 3D printed microfluidics chamber we designed can be integrated into commercial microscopy incubation systems to create an artificial growth minienvironment for keeping tissue alive for prolonged periods of time. In this study, real-time monitoring of wound healing was achieved at the depth of ~300 μm during 3-7 days.

OC-3.2.4

A novel Cathepsin B degradable nanoparticle platform for intraoperative NIR imaging and treatment of pancreatic cancer

Fabiola Sciscione[1], Shiv Patel[1], Hamzah Masood[1], Marym Mohammed Hadi[1], Bala S. Ramesh[1], John F. Callan[2], Alexander MacRobert[1], Anthony McHale[2] and Nikolitsa Nomikou[1*]

1) University College London, Division of Surgery & Interventional Science, , Royal Free Hospital, London, UK;
2) Ulster University, Biomedical Sciences Research Institute, Coleraine, UK

Pancreatic cancer is ranked amongst the most lethal forms of cancer. Currently, surgical resection is the only practical approach for patients with locally advanced disease. Extension of the limits of tumour resectability can be achieved with image-guided surgery, which can help accurately delineate the borders of the tumour and facilitate surgical resection. This, in combination with near-infrared (NIR) laser treatment of the resection site immediately after tumour removal, can help destroy residual microscopic tumour fragments and prevent their progression after surgery. In this regard, we have developed a novel multi stimulus-responsive nanoparticle formulation containing indocyanine green (ICG), a clinically approved NIR fluorescent agent with photothermal properties. Previously, poly-l-glutamic acid (PGA) was used as the carrier polymer.[1] In this work PGA was modified by partial conjugation of the carboxylate side groups with a lipid molecule, which resulted in the formation of nanoparticles with increased ICG-loading yield. The new nanoparticles proved to be digested by the proteolytic enzyme Cathepsin B (CB) in phosphate buffer at pH 6.4 and 7.4 and release ICG, as confirmed by fluorescence spectroscopy studies. CB is overexpressed in advanced pancreatic cancer and its production and activity are particularly enhanced at the tumour acidic pH. To demonstrate the feasibility of the novel formulation for efficient NIR-guided imaging/delineation of highly aggressive tumours with elevated proteolytic enzyme activity, we developed a three-dimensional (3D) cell culture system in which the pancreatic BxPC-3 cells were embedded in the extracellular matrix-resembling 3D structures. These systems were incubated with the nanoparticle formulation at either pH 7.4 or 6.4, in the absence or in the presence of CB. An NIR excitation diode and a high sensitivity CMOS camera were used to generate an NIR image of the 3D cell systems. An increase of ICG fluorescence signal was observed after 24 hours in CB-rich systems, with the highest intensity at pH 6.4. Following these positive preliminary results, we are currently carrying out 2D and 3D in-vitro NIR laser treatments to establish the potential and efficacy of the formulation in eliminating residual microscopic disease following combined NIR-imaging and treatment.

References

1) S. Tarassoli, S. Martinez de Pinillos, H. Pye, C.A. Mosse; J.F. Callan, S. Macrobert, A.P. McHale; N. Nomikou, Cathepsin B-degradable, NIR-responsive nanoparticulate platform for target-specific cancer therapy. Nanotechnology 2017, 28(5),055101.

Acknowledgments. The work is supported by the Engineering and Physical Sciences Research Council UK . EPSRC DTP (Ref: EP/N509577/1) and EPSRC Standard Grant (Ref: EP/R035008/1).

IL-3.2.5

Imaging of stress- and damage- induced metabolic adapations in skin

Florian Gruber[1,2,3], Arvand Haschemi[4], Anne Miller[4], Marie-Sophie Narzt[1,3], Christopher Kremslehner[1,2,3]
1) Medical University of Vienna, Department of Dermatology,Vienna, Austria; 2) Christian Doppler Laboratory for Skin Multimodal Analytical Imaging of Aging and Senescence – SKINMAGINE- and 3) - Christian Doppler Laboratory for Biotechnology of Skin Aging, Vienna, Austria; 4) Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria

In the differentiation of epidermal keratinocytes their function, chemical composition, physical properties, metabolism and secretion profoundly change. Extrinsic stressor, like ultraviolet (UV) radiation and intrinsic factors that are associated with aging thus may differently affect the epidermal keratinocytes, depending on differentiation stage. Exposure to UV elicits various responses including the DNA damage response and activation of pathways which detoxify or repair damage or induce cell death when the damage was irreparable. Recently, rapid diversion of glucose flux into the pentose phosphate pathway (PPP) was discovered as additional mechanism by which cells instantly generate reduction equivalents and precursors for nucleotides – both being in demand after UV damage. There is however little known about the correlation of such metabolic activity with differentiation state, cell damage and tissue localization of epidermal cells. We developed a method to correlate the activity of G6PD, the first and rate-limiting enzyme of this metabolic UV response, at cellular resolution to cell type, differentiation state, and cell damage in human skin and in organotypic reconstructed epidermis. We thereby could verify rapid activation of G6PD as an immediate UVB response not only in basal but also in differentiating epidermal keratinocytes and found increased activity in cells which initiated DNA damage responses. We also found that the anti-diabetic and senomorphic drug metformin strongly induced G6PD activity throughout reconstructed epidermis. This is of relevance as cellular senescence of skin cells affects their metabolic profile. Activation of the protective pentose phosphate pathway may be useful to modulate the skin's antioxidant defense systems and DNA damage repair capacity on demand.

References

1) Kremslehner, et al. “Imaging of metabolic activity adaptations to UV stress, drugs and differentiation at cellular resolution in skin and skin equivalents – Implications for oxidative UV damage.”, Redox Biology, 2020

Acknowledgements. The work of the CDLs is funded by the Federal Ministry for Digital and Economic Affairs (BMWFW) of Austria and the National Foundation for Research, Technology, and Development of Austria and by CHANEL Parfums et Beauté and the Herzfelder'sche Familienstiftung, Austria

IL-3.2.6

Imaging Mass Spectrometry – Benefits, Challenges, Potentials

Martina Marchetti-Deschmann
TU Wien, Institute of Chemical Technologies and Analytics, Vienna, Austria

A single modality is very often not good enough to understand all functional, structural, temporal and chemical relations underlying certain biological conditions. Imaging a specimen with two or more complementary modalities creates an informative, composite view of a sample that spans all relevant resolution ranges.
Here we shortly introduce the concept of MALDI Mass Spectrometry based imaging (MALDI-MSI) and its potential to localize molecules in a tissue without a priori knowledge. Briefly the advantages, limitations and potentials for future applications will be highlighted. The successful combination of different mass spectrometric approaches will be shown but also the combination of MSI with vibrational spectroscopy and μXRF.

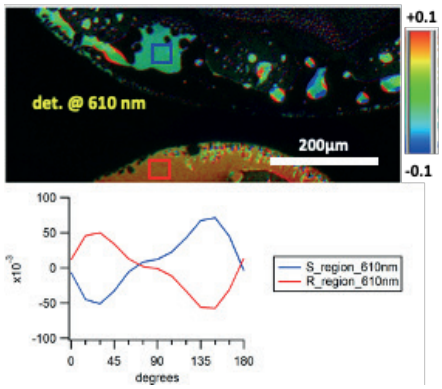
Acknowledgements. Parts of the present work were supported by COMULIS (COST CA 17121), SKINMAGINE (Christian Doppler Society) or MEIBio (TUWien).

IL-3.2.7

Hyperspectral imaging extended to circularly polarized light

Guillaume Raffy[1], André Del Guerzo[1], Dario M. Bassani[1]
1) Institut des Sciences Moléculaires, CNRS UMR5211 and University of Bordeaux, Talence, France

Even when using light from a perfectly unpolarized source, reflections and interactions with absorbing media can induce polarization. Furthermore, because electronic transitions in molecules are aligned with the electronic transition dipole moments, both absorption and emission of light occurs preferentially along specific molecular axes that may differ between absorption and emission processes. This has been recognized and exploited in numerous experimental setups designed to probe the relative orientation of chromophores, their motion or interaction in solution. In solid matter microscopy, linear polarization allows probing of the local order and the loss of excitation-induced polarization can provide information on exciton diffusion in solids and of solid-state photoinduced reactions.[1] Compared to the use of linear polarization, circular polarization has received much less attention. It is, however, becoming increasingly recognized in importance since becoming the focus of new materials for CPL in material science.[2] In Nature, some examples of CPL active materials exist, although it is not yet clear whether this property plays a role in the evolution of the species. In the study of CPL-active new materials of biological or synthetic origin, resolution at the sub-micron scale can provide information on the heterogeneity which in turn can be used to understand function and optimization. However, collecting circular polarization represents a formidable challenge due to the inherently small ΔI that need to be sensed. Indeed, g_{lum} values for many organic chromophores are below 10^{-3}, which renders detection challenging. Furthermore, the numerous optical components in the detection setup need to be carefully considered to minimize losses and scrambling of the polarization components in a complex way of the sample's luminescence.[3] Our recent progress towards attaining sub-micron resolution with CPL detection will be presented.



Wide-field CPL microscope image of two enantiomeric Eu^{3+} complexes exhibiting opposite CPL. Polarplot of blue and red regions are shown below

References

1) Guillaume Raffy, Debdas Ray, Cheng-Che Chu, André Del Guerzo, Dario M. Bassani "Controlling the emission polarization from single crystals using light: Towards photopolic materials" *Angew. Chem. Int. Ed. 50*, 9584 – 9588, 2011.
2), Tonghan Zhao, Jianlei Han, Pengfei Duan, Minghua Liu, New Perspectives to Trigger and Modulate Circularly Polarized Luminescence of Complex and Aggregated Systems: Energy Transfer, Photon Upconversion, Charge Transfer, and Organic Radical, *Acc. Chem. Res.*, 53, 1279-1292, 2020.
3) P. Torok, P. D. Higdon, T.Wilson, On the general properties of polarised light conventional and confocal microscopes, *Optics Commun.*, 148, 300-315, 1998.

OC-3.2.8

Analysis of UV-exposed skin sections via MALDI Imaging Mass Spectrometry

Samuele Zoratto[1,2], Michaela Sochorova[2,3], Christopher Kremslehner[2,3], Markus Schosserer[2,4], Florian Gruber[2,3,5], Martina Marchetti-Deschmann[1,2,5,6]
1) Institute of Chemical Technologies and Analytics, TU Wien, Vienna, Austria; 2) Christian Doppler Laboratory for Skin Multimodal Imaging of Aging and Senescence, Vienna, Austria; 3) Department of Dermatology, Medical University of Vienna, Vienna, Austria; 4) University of Natural Resources and Life Sciences, Vienna, Austria; 5) Vienna Center for Engineering in Medicine; 6) Austrian Cluster for Tissue Regeneration, Vienna, Austria.

Our skin is constantly exposed to solar radiation, high oxygen levels, and environmental pollutants. These are accelerant stress factors for premature skin aging, tissue inflammation, and photocarcinogenesis. In skin aging, the evident and well-known clinical effect is the alteration of the skin appearance. In addition, metabolism and cell communication are impaired, and an increase of senescent cells is evident. Cellular senescence is a phenomenon characterized by the arrest of cell division and is commonly linked to the aging phenotype. Nevertheless, it plays an essential role in tissue healing and tumor suppression. Although the shortening of the telomeres is usually the leading promoter of senescence; also oxidative stress or oncogenes activation can trigger this cellular response as sources of DNA damage. To counteract the oxidative stress produced by the stress-inducing agents, the cells activate several epidermal and dermal lipoxygenases. Indeed, oxidized lipids can act as danger-associated molecular patterns (DAMPs) messengers and are involved in the senescence-associated secretory phenotype (SASP).[1]
In previous work from Gruber et al[2], several oxidized phosphatidylcholines (OxPCs) were characterized in a semitargeted lipidomic approach. The method involved the exposure of human dermal fibroblasts at defined UVA fluences, followed by liquid-liquid extraction, and lastly, mass spectrometric analysis by means of reversed-phase HPLC coupled to electrospray ionization MS/MS (HPLC-ESI-MS/MS).
In this work, artificially aged skin samples were provided by the Medical University of Vienna (MUW). Skin specimens were irradiated in specific areas with either UVA/B radiation at different fluencies before embedding and cryo-sectioning in the same fashion as the aforementioned publication. Our study aims to evaluate the epilipidomic effects via an untargeted multimodal approach focused on OxPCs species by high-resolution mass spectrometry analysis. Indeed, we employed high-resolution matrix-assisted laser desorption/ionization Fourier-transform ion cyclotron resonance mass spectrometry (MALDI-FT-ICR MS) to generate MS images (MSI) of skin section after autofluorescence light-microscopy analysis. The high mass accuracy of FT-ICR MS instrumentation, combined with the analytes' spatial localization and relative abundance, allow us to locate and putatively identify several OxPCs with great confidence. Lastly, the integration with high-definition images from light-microscopy yields a detailed and comprehensive result of the effects of UV-induced alteration.
In conclusion, the untargeted MS-based methodology allows us to expand our analysis to known and unknown analytes. The multimodal approach unifies the results from different techniques and helps us to discover the crucial factors of scientific importance.

The authors declare no conflict of interest.

References:
1) Chunya Ni et al, Autophagy deficient melanocytes display a senescence associated secretory phenotype that includes oxidized lipid mediators. Int J Biochem Cell Biol. 81(Pt B):375-382, 2016.
2) Gruber et al, A simplified procedure for semi-targeted lipidomic analysis of oxidized phosphatidylcholines induced by UVA irradiation. J Lipid Res. 53(6): 1232–1242, 2012

Acknowledgments:
We acknowledge financial support of the Federal Ministry Republic of Austria for Education, Science and Research and TU Wien (HRSM 2016), the Christian Doppler Research Association and CHANEL Parfums et Beauté for funding SKINMAGINE. This work is supported by COMULIS (CA17121) and EpiLipiNet (CA 19105).

P-3.2.9

Application of UVA irradiation for the determination of the redox status in excised skin by EPR spectroscopy

Lohan SB[1], Ivanov D[1], Schüler N[2], Berger B[2], Lademann J[1], Meinke MC[1]

1) Charité – Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Dermatology, Venerology and Allergology, Berlin, Germany; 2) Freiberg Instruments GmbH, Freiberg, Germany

Free radicals in the body are essential for the metabolism, signal transport and are part of our immune response. Under physiological conditions, a dynamic equilibrium between the process of radical production and detoxification is given. But when the stress level is too high, oxidative stress occurs, and an imbalance between the body's antioxidant defense system and reactive metabolites is existing. If oxidative stress occurs, first reactive oxygen species (ROS) are formed which can be well controlled by the antioxidant system. With increasing stress, C-centered (CCR) or alkoxy radicals (lipid oxygen species, LOS) are produced, which can be harmful to metabolic processes. Concerning the skin physiology, they are significantly involved in the development of inflammatory skin diseases, immunosuppression, skin cancer, and premature skin aging. The partitioning of the different types of radicals could give information about the stress level.
With a share of 46% a large amount of radicals is generated by sunlight irradiation in the UVA region with a maximum at ~ 360nm[1,2].
In this study, radical production in excised porcine skin was characterized and quantified by electron paramagnetic resonance (EPR) spectroscopy using UVA irradiation as stress source. The aim was to determine the dose at which the switch between eustress and distress occurs.
The study revealed that during UVA radiation the ratio of ROS and LOS turned with increasing dose. The region of turn appears at half of the minimal erythema dose of UVA. Important is a sufficient irradiance of the UVA light, because at low irradiance adaptation processes inhibit the revers effect. This method could detect heat induced stress in the skin of pre-stressed pigs in the summer time due to temperatures above 35°C during husbandry or transport. In these ears, the ratio was already reversed, more LOS were produced right from the start of irradiation. This illustrates that the ratio of ROS and LOS produced by irradiation in skin could be used as a stress marker[3].

References

1) L. Zastrow, N. Groth, F. Klein, D. Kockott, J. Lademann, R. Renneberg, L. Ferrero, The missing link--light-induced (280-1,600 nm) free radical formation in human skin, Skin Pharmacol Physiol, 22, 31-44 (2009).
2) S.B. Lohan, R. Muller, S. Albrecht, K. Mink, K. Tscherch, F. Ismaeel, J. Lademann, S. Rohn, M.C. Meinke, Free radicals induced by sunlight in different spectral regions - in vivo versus ex vivo study, Exp Dermatol, 25, 380-385 (2016).
3) S.B. Lohan, D. Ivanov, N. Schuler, B. Berger, L. Zastrow, J. Lademann, M.C. Meinke, Switching from healthy to unhealthy oxidative stress - does the radical type can be used as an indicator?, Free Radical Bio Med, 162; 401-411 (2021).

Acknowledgements. The work is funded by the German Federal Ministry of Education and Research BMBF (FKZ 03ZZ0140A) within the program "Zwanzig20 – Partnerschaft für Innovation" and carried out within the consortium Advanced UV for Life.

P-3.2.10

Using infrared beam to track the cell fate of transgenic flatworms.

Jakub Wudarski[1], Yasuhiro Kamei[2], Kiyokazu Agata [1]
1) National Institute for Basic Biology, Laboratory of Biological Diversity, Okazaki, Japan;
2) National Institute for Basic Biology, Spectrography and Bioimaging Facility, Okazaki, Japan

Flatworms are considered champions of regeneration. The key to this process lies in a population of adult stem cells called neoblasts. So far live imaging of these stem cells was impossible because of the absence of transgenic flatworms. I have developed a protocol to establish transgenic lines of the flatworm *Macrostomum lignano*[1] and used heat shock promoter to drive the expression of fluorescent proteins[2]. Now, together with Dr. Yasuhiro Kamei, we are combining our hsp line with the infrared laser evoked gene operator (IR-LEGO)[3] technology to track the cell development in live flatworms (Fig. 1). Using this approach, we will also be able to selectively overexpress genes of choice in any part of the worm and track the results.
In my presentation I will show our progress in adapting the IR-LEGO method to the transgenic flatworm system of *M. lignano*.

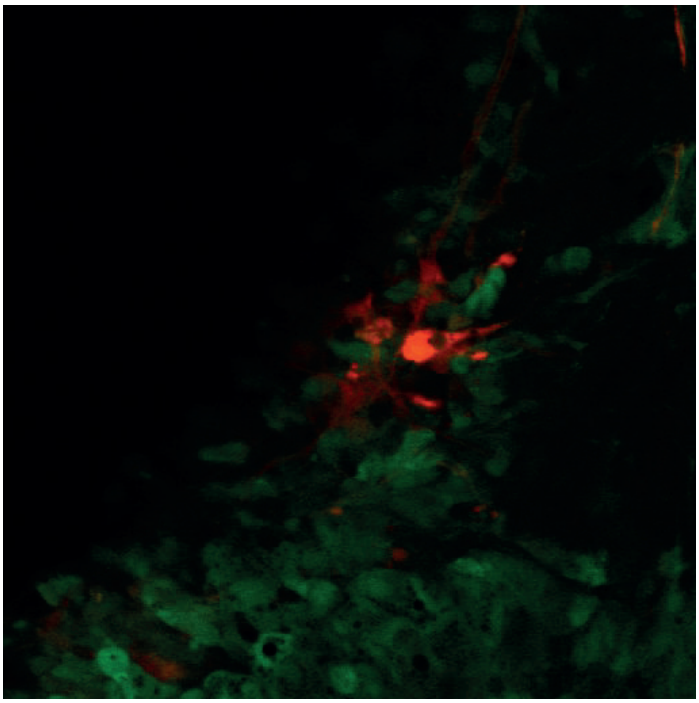


Fig. 1 – Cells expressing mScarlet under Hsp20 promoter 24h after activation using IR-LEGO.

References

1. Wudarski, J. *et al.* Efficient transgenesis and annotated genome sequence of the regenerative flatworm model Macrostomum lignano. *Nat. Commun.* **8**, 2120 (2017).
2. Wudarski, J., Ustyantsev, K., Glazenburg, L. & Berezikov, E. Influence of temperature on development , reproduction and regeneration in the flatworm model organism , Macrostomum lignano. *Zool. Lett.* 1–10 (2019) doi:10.1186/s40851-019-0122-6.
3. Kamei, Y. *et al.* Infrared laser-mediated gene induction in targeted single cells in vivo. *Nat. Methods* **6**, 79–81 (2009).

Acknowledgements. This work is supported by grant 16H06376 from the JSPS.

P-3.2.11

C. aggregans LOV domain: a framework for engineering of fluorescent reporters

Ivan Gushchin[1], Anna Yudenko[1], Anastasia Smolentseva[1], Vera V. Nazarenko[1], Alina Remeeva[1], Oleg Semenov[1]
1) Moscow Institute of Physics and Technology, Research Center for Molecular Mechanisms of Aging and Age-Related Diseases, Dolgoprudny, Russia

LOV domains, ubiquitous photoreceptors of plants, fungi, bacteria and archaea, have found recently a number of applications in molecular biology. They have been used as genetically encoded fluorescent reporters, generators of reactive oxygen species, and optogenetic tools. Yet, there are still many possibilities for improvement such as generation of colour-shifted variants or engineering of LOV-based sensors of molecular signals.
Recently, we developed a flavin-based fluorescent protein CagFbFP based on the LOV domain of a hybrid histidine kinase from *Chloroflexus aggregans*[1]. The protein can be used for imaging both in bacteria and eukaryotes (Fig. 1). High thermal stability of CagFbFP and its amenability to high-resolution structural studies allowed us to engineer a number of variants for different applications.
First, we tested whether CagFbFP fluorescence excitation and emission maxima can be shifted using mutations of a conserved glutamine amino acid adjacent to the flavin chromophore. Effects of the mutations were modest, and none of the variants exhibited a red-shift[2]. Crystallographic structures revealed the reason for this: potentially charged amino acids didn't interact with flavin, and in some cases the active site was unlatched and the side chains were oriented away from the chromophore[2]. Consequently, based on the data for iLOV, we generated a new variant where the positively charged lysine side chain was stabilized near the flavin by a second mutation; this variant could be used for two-color imaging of bacterial cells[3].
We also used structural information to engineer split-CagFbFP pairs that produced fluorescence while being fused to interacting proteins of interest, but were not photoactive otherwise[4]. Moreover, as a proof of concept, we were able to engineer a calcium-responsive pair inspired by the calcium sensor FGCaMP7[4]. Thus, our data show that CagFbFP is a promising framework for engineering of fluorescent reporters for different applications.

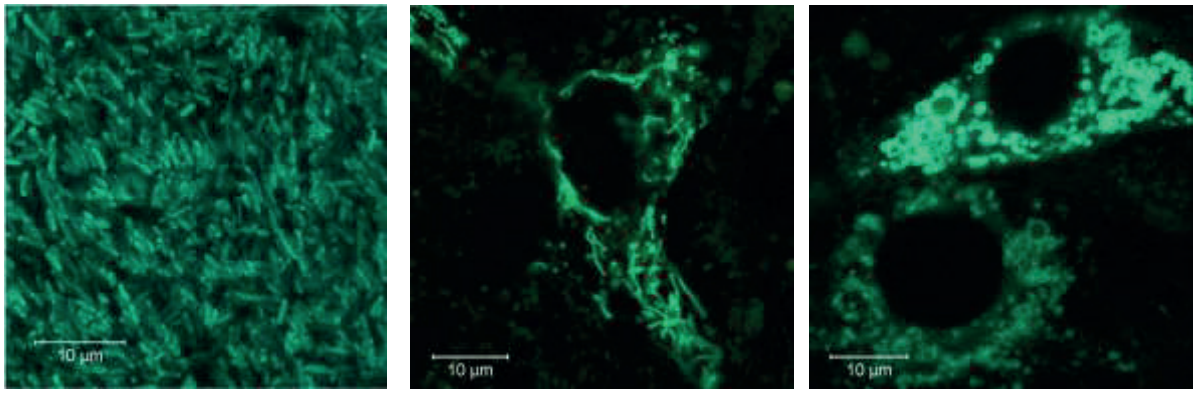


Fig. 1 – Fluorescence microscopy images of CagFbFP expressed in E. coli (left), mitochondria (center) and lysosomes (right) of mammalian cells.

References

1) Nazarenko *et al.*, A thermostable flavin-based fluorescent protein from *Chloroflexus aggregans*: a framework for ultra-high resolution structural studies. Photochemical & Photobiological Sciences, 2019.
2) Remeeva *et al.*, Insights into the mechanisms of light-oxygen-voltage domain color tuning from a set of high-resolution X-ray structures. Proteins: Structure, Function, and Bioinformatics, 2021.
3) Röllen *et al.*, The molecular basis of spectral tuning in blue- and red-shifted flavin-binding fluorescent proteins. Journal of Biological Chemistry, 2021.
4) Yudenko *et al.*, Rational Design of a Split Flavin-Based Fluorescent Reporter. ACS Synthetic Biology, 2020.

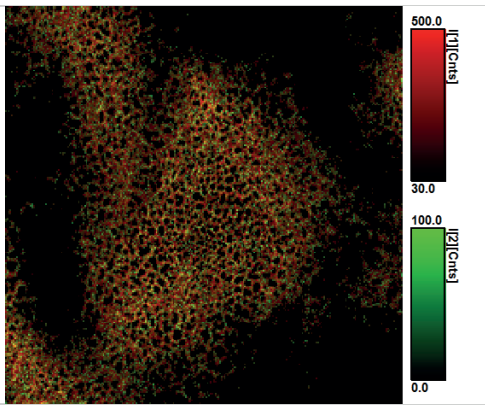
Acknowledgements. The work is supported by the Russian Foundation for Basic Research, grant 20-34-70109.

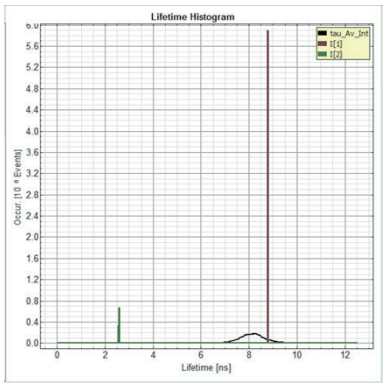
P-3.2.12

Engineering cellulose acetate fabrics loaded with photosensitizer and graphene oxide endowed with bactericidal activity

Ilse G. J. Manet[1], Rania Morsi[1], Barbara Ventura[1] , Denis Gentili[2] , Franco Corticelli[3], Francesca Bonvicini[4]
1) ISOF, National Research Council, Via Gobetti 101, 40129 Bologna, Italy;
2) ISMN, National Research Council, Via Gobetti 101, 40129 Bologna, Italy;
3) IMM, National Research Council, Via Gobetti 101, 40129 Bologna, Italy;
4) Dpt. of Pharmacy and Biotechnology, Univ. of Bologna, Via Massarenti 9, 40138 Bologna, Italy

Composite materials based on cellulose acetate matrices and porphyrinic photosensitizer (PS) dyes were prepared, characterized and tested as devices with antibacterial activity. Scope of the work is to use light to enable phototoxic bactericidal activity mediated by the production of reactive oxygen species, triggering oxidative damage to pathogens. The materials were fabricated as porous membranes by phase inversion process. Photosensitizers were loaded into the cellulose acetate fabrics as pre-loading, dissolving the dye into the polymer solution before membrane preparation. Different PS loading amounts were explored. We further prepared the fabrics also in the presence of a surfactant or graphene oxide (GO), the latter added to impart mechanical robustness to the final material.
We will present the results of the morphological study of the prepared fabrics investigated by scanning electron microscopy and atomic force microscopy. We will further discuss the detailed photophysical characterization of the PS on the fabrics with and without GO performed for the composite materials by means of bulk and spatially resolved techniques. In particular absorption and time-resolved fluorescence data were collected evidencing high PS concentrations caused fluorescence lifetime shortening. Similar effects were observed in the presence of the surfactant. Confocal time-resolved fluorescence lifetime imaging (Figure 1) of the PS-loaded fabrics confirmed aggregation phenomena at the basis of the short lifetimes. The most promising fabrics obtained loading the PS in different amounts were used to study the bactericidal effect of the fabrics on *S. Aureus* bacterial population illuminated with red light.





Fitting of the PS fluorescence decay converged with two lifetimes of 2,2 ns and 8,6 ns. The image has been obtained plotting the pixel intenisty of the two lifetimes (red for the long lifetime component and green for the short lifetime component).

Fig. 1 – Fluorescence lifetime image of the cellulose acetate matrix and 0,2 % porphyrinic photosensitizer

Acknowledgements. This work was supported by H2020 MSCA-IF Grant ID: 800317 Enhanced-MUMS.

P-3.2.13

Porphyrin-loaded polymeric microcapsules for Photodiagnosis of cancer

Mariana C. S. Vallejo[1], Sofia G. Serra [2], Vanda V. Serra[2], Nuno M. M. Moura[1], M. Graça P. M. S. Neves[1]

1) LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal;
2) Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal

Tetrapyrrolic compounds, such as porphyrins, are of great interest for the scientific community due to their physico- and photochemical features, allowing them to be used in a variety of research fields, including biomedical sciences [1,2]. One particular interest of these molecules is related with their mode of action as light emitters: in the presence of light, they can release energy in the form of fluorescence, being excellent candidates to be used in Photodiagnosis of cancer [3]. Polymeric particles are a smart and controllable way to deliver these molecules to the target site. These particles, which are usually highly stable, biocompatible and stimuli-responsive, can also be loaded with hydrophobic and hydrophilic cargos, such as porphyrins [4]. In this work, it is proposed the controlled loading of cationic porphyrins polymeric particles for Photodiagnosis of cancerous cells, through local delivery and real-time fluorescence imaging.

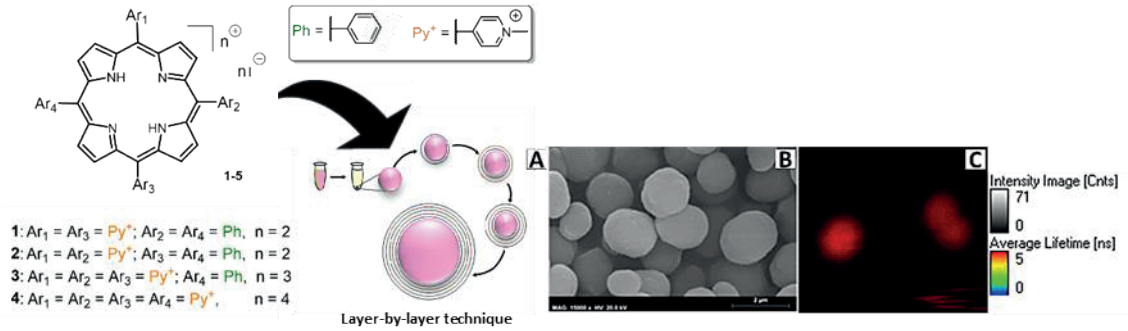


Fig. 1 – Synthesized cationic porphyrins to be incorporated in the polymeric particles (A); Scanning Electron Microscopy of the particles (B) and Fluorescence Lifetime Imaging Microscopy of the dopped particles (C).

References

1) Cerqueira, A. F. R.; Moura, N. M. M.; Serra, V. V.; Faustino, M. A. F.; Tomé, A. C.; Cavaleiro, J. A. S.; Neves, M. G. P. M. S. *Molecules*, 22, 1269, **2017.**
2) Kadish, K. M.; Smith, K. M.; Guilard, R. Handbook of Porphyrin Science, World Scientific Publishing Company Co, Singapore, Vol 10-12, **2010**.
3) Lim, E.-K.; Kim, T.; Paik, S.; Haam, S.; Huh, Y.-M.; Lee, K. *Chem. Rev.*, *115*, 327, **2015.**
4) Parveen, S.; Sahoo, S. k. *Journal of Drug Targeting*, *16*, 108, **2008.**

Acknowledgements. We thank the University of Aveiro and FCT/MCT for the financial support for QOPNA research Unit (FCT UID/QUI/00062/2019), REQUIMTE (UIDB/50006/2020) and the FCT project THERMIC (PTDC/QUI-COL/29379/2017), through national founds and were co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network. The research grants of M.C.S. Vallejo (REF: BI-UI51-8642-2019) and S.G. Serra are supported by the project THERMIC (PTDC/QUI-COL/29379/2017), and the research contracts of N.M.M. Moura (REF.-048-88-ARH/2018) and Vanda Vaz Serra are funded by national funds (OE), through FCT.

IL-3.3.1

Photoreceptors from the plant symbiont *Methylobacterium radiotolerans*

Aba Losi[1], Wolfgang Gärtner [2]
1) University of Parma, Department of Mathematical, Physical and Computer Sciences, Parma, Italy1; 2) University of Leipzig, a.Institute for Analytical Chemistry, Leipzig, Germany 2

Methylobacteria are pink-pigmented facultative methylotrophic (PPFMs) alpha-proteobacteria, most often living in the phyllosphere as plant symbionts. They are able to feed on C1 organic compounds produced by plants, and in turn provide their hosts with hormones and other metabolites that promote plant fitness and growth. As such, *Methylobacteria* are considered as biofertilisers and plant probiotics representing a potential resource for sustainable agriculture.[1] *Methylobacteria* are extremely rich in genes encoding for photoreceptors, mostly belonging to red/far red light (RL/FRL) sensing bacterial phytochromes (BphP), and to the blue-light (BL) sensing LOV (Light, Oxygen and Voltage) families.[2] Here we present the molecular characterization of two such photoreceptors from *Methylobacterium radiotolerans*, respectively *Mr*BphP1 and *Mr*4511. *Mr*BphP1 shows the expected photochromicity between a red and far red absorbing form (Pr and Pfr), but with higher quantum yield (0.2 for both routes) than other BphPs. The LOV protein *Mr*4511 also shows the expected photochemical reactions, with BL-driven formation of a flavin-cysteine covalent adduct, but with an unusually slow recovery kinetics and an astonishing resistance to denaturation with urea. Upon substitution of the reactive Cys71 (C71S or C71G), *Mr*4511 becomes an efficient photosensitizer for singlet oxygen (SO), with ca. 20% efficiency.[3] This unusual feature is due the absence of a tryptophan residue at position 112, otherwise conserved in the majority of LOV domains. With a series of point mutations at different distances from the flavin chromophore (between 0.6 and 1.7 nm), it was possible to map the efficiency by which Tyr and Trp residues are able to quench the excited states of the flavin chromophore by electron transfer (Fig.1). Substitution Y116F (0.9 nm from the flavin chromophore) results in a prolonged lifetime of the flavin triplet state, thereby largely increasing the efficiency of SO production. Instead, mutation F130W (0.6 nm from the flavin) results in complete quenching of the chromophore singlet state, also preventing adduct formation when the reactive cysteine is present. The most efficient quencher for the flavin chromophore excited states is Trp, followed by Tyr and His, in agreement with the standard redox potentials of aromatic amino acids.

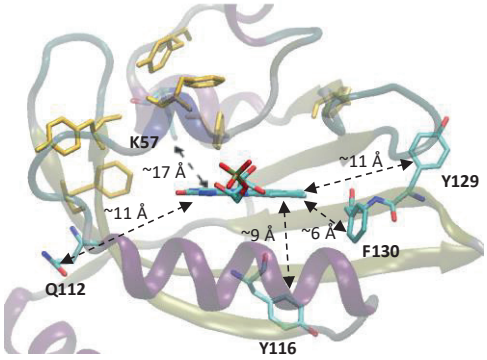


Fig. 1 – Structural model of Mr4511-LOV with positions mutated in this work

References

1) M. Kumar, D. Kour, A. N. Yadav, R. Saxena, P. K. Rai, A. Jyoti and R. S. Tomar, Biodiversity of methylotrophic microbial communities and their potential role in mitigation of abiotic stresses in plants. *Biologia (Bratisl).* **74**, 287–308, 2019
2) E. Consiglieri, Q. Xu, K.-H. Zhao, W. Gärtner and A. Losi, First molecular characterisation of blue- and red-light photoreceptors from *Methylobacterium radiotolerans*, *Phys. Chem. Chem. Phys.* **22**, 12434–12446, 2020.
3) E. Consiglieri, Q. Xu, M. Bregnhøj, M. Westberg, P. R. Ogilby and A. Losi, Single mutation in a novel bacterial LOV protein yields a singlet oxygen generator, *Photochem. Photobiol. Sci.* **18**, 2657–2660, 2019.

IL-3.3.2

Ralstonia solanacearum virulence and bacterial physiology are modulate by light and LOV photoreceptor.

María Josefina Tano[1,2*], María Belén Ripa[1,2*], Analía Carrau[1,2], María Victoria Rodriguez[2], Silvana Petrocelli[2], Elena G. Orellano[1,2]. *These authors contributed equally to the work.
1) Instituto de Biología Molecular y Celular de Rosario, Universidad Nacional de Rosario, Santa Fe, Argentina, 2) Área Biología Molecular, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Santa Fe, Argentina, 3) Área Biología Vegetal, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Santa Fe, Argentina.

Microorganisms have developed metabolic and physiological capacities to adapt to a wide variety of external stimuli. The light signals in the environment are perceived by photoreceptor proteins, which trigger the signal transduction cascades that regulate light-biochemical responses. LOV (Light, Oxygen or Voltage) photoreceptors are flavin-binding proteins using flavin mononucleotide (FMN) as chromophore. *Ralstonia solanacearum* is the bacterium responsible for "bacterial wilt", one of the most destructive plant diseases in the world. Currently, *Ralstonia* genus is considered a complex of species that can be differentiated based on the attacking hosts, their geographic distribution, aggressiveness, and physiological properties. For this reason, *Ralstonia pseudosolanacearum* (Rpso) GMI1000 is considered a model organism for the plant-pathogen interaction. Rpso GMI1000 genome presents a *Rsp0254* gene encoding a putative LOV domain photoreceptor. To evaluate whether light would have a role in bacterial physiology and in the infectivity capacity in tomato plants, mediated by this photoreceptor, it was first decided to analyze the light effect on different bacterial virulence factors. Rpso exhibited lower adhesion capacity, motilities and biofilm formation under white light. Once it was evidenced that light regulates different pathogenicity factors, the mutant strain of Rpso in the *Rpso0254* gene called Rpso *lov* was constructed to study whether this regulation is mediated by the LOV-type photoreceptor. The *lov* gene was analyzed by qRT-PCR under white light and dark treatments revealing that this gene is expressed. The absence of *lov* gene modified physiological parameters involved in the virulence of Rpso such as motility, biofilm and adhesion compared to wild type strain. Finally, the infection capacity of tomato host plants (*Solanum lycopersicum* var. Minitomato) mediated by both strains grown in different lighting conditions was studied, showing differences in bacterial colonization depending on the light treatment applied. Altogether these results suggest a light-dependent regulation of Rpso virulence during the host plant colonization.

Acknowledgements. This work was supported by grant from the ANPCyT (PICT 2017-2242), UNR (PID-1BIO432) and CONICET (PIP 0451).

IL-3.3.3

"Effects of light on the non-photosynthetic plant growth promoting rhizobacteria *A. brasilense* Az39"

Molina Romina[1], Nievas Sofía[1], Coniglio Anahí[1], Mora Verónica[1], Rosas Susana[2], Cassán Fabricio[1]
1) Laboratorio de Fisiología Vegetal y de la Interacción Planta microorganismo, Instituto de Agrobiotecnología (INIAB), Universidad Nacional de Río Cuarto, Ruta 36 Km 601, Río Cuarto, Córdoba, Argentina; 2) Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Río Cuarto, Ruta 36 Km 601, Río Cuarto, Córdoba, Argentina.

Light is an essential environmental factor for the growth and development of photosynthetic microorganisms; however, it can be a threat to those who are not [1].With the discovery of photoreceptors in prokaryotes, progress was made in the understanding of their functionality in nonphotosynthetic bacteria[2]. *A. brasilense* Az39 is a nonphotosynthetic rhizobacterium used as a biofertilizer in South America during the last 40 years. In our laboratory, we analyzed the Az39 genome, generated mutants at the level of its photoreceptors, and studied its physiological and microbiological responses to white, blue and red light (PAR38) under different experimental systems. In a complementary way, we analyzed its physiological behavior in soybean leaves inoculated with this bacterium and exposed to different light conditions. Our results allowed us to understand that the tolerance of Az39 to light depends on the culture conditions. In particularly, white and blue light were found to be lethal for this bacterium under conditions of gregarious growth (culture on solid medium); however, positive effects were observed under liquid culture conditions, at level of the phytohormones production and lifestyle characters. The survival of these bacteria on soybean leaves was drastically affected by the exposure to all light sources after inoculation, but mostly by white light on bacterial phytochrome expression deficient strain. Summarizing, *A. brasilense* Az39 is differentially affected by light conditions in both positive and negative ways, and this capacity is dependent on the bacterial growth conditions.

References

1) Elías-Arnanz, M., Padmanabhan, S., and Murillo, F. J. Light-dependent gene regulation in nonphototrophic bacteria. Current opinion in microbiology, 14(2), 128-135, 2011.

2) Davis, S.J., Vener, A.V., and Vierstra, R.D. Bacteriophytochromes: phytochrome-like photoreceptors from nonphotosynthetic eubacteria. Science, 286(5449), 2517-2520, 1999.

Acknowledgements. We thank Universidad Nacional de Río Cuarto, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) and FONCyT for the finantial support to this research.

OC-3.3.4

New Insights from the Exciting Photobiological World of Mushrooms

Bianka Siewert[1], Fabian Hammerle[1], Johannes Fiala[1] Lesley Huymann[1,2], Gabrijela Curak[1], Anna Hannecker[1], Ursula Peintner[2], Ilse Kranner[3], Harald Schöbel[4], Michaele Lackner[5], Christoph Schinagl[2], Pamela Vrabl[2]
1) University of Innsbruck, Pharmacy/Pharmacognosy, Innsbruck, Austria; 2) University of Innsbruck, Microbiology, Innsbruck, Austria; 3) University of Innsbruck, Botany, Innsbruck, Austria; 4) MCI Management Center Innsbruck, Innsbruck, Austria; 4) Medical University Innsbruck, Innsbruck, Austria

Whenever insects nibble on fruiting bodies of the green *Cortinarius austrovenetus* the bite site turns reddish. The chemistry behind it is as easy as it is fascinating: Hypericin is formed by oxidation from the fungal pigment austrovenetin as soon as the fungal tissue is damaged. Inspired by this striking observation, we started to systematically explore the photobiology of fruiting bodies.
Via an established workflow[2], species of the colorful webcaps (Cortinarius) –especially of the subgenus Dermocybe– were identified as a promising source for new photosensitizers. The photoactive principles were studied by the means of molecular-network analysis and obtained via photo-activity guided isolation. The isolated pigments were photo-chemically as well as photo-pharmaceutically characterized; The most-promising fungal PS for PDT applications showed a singlet oxygen photo-yield of $\Phi_\Delta = 20\%$ (d_4-MeOH, $\lambda_{irr} = 450$ nm, 15mW) and was highly photo-cytotoxic (EC_{50}, A549 = 64 nM (468 nm, 9.3 J/cm², Selectivity Index Dark/Light > 61)[3]. In addition, we explored the photoantimicrobial potential of these fungal PSs by submitting them to our new photoantimicrobial HTS-assay[4]. While most extracts and fungal PSs were highly active against gram-positive bacteria, the photoactivity against several Candida species –for example against the resistant *Candida auris* or *C. tropicalis*– was of special interest. Furthermore, we studied the distribution of the photoactivity in the different organs (cap, lamellas, and stem) of the fruiting bodies to test the hypothesis that mushrooms produce PSs to protect their spores. Our HPLC-DAD analysis of over 200 extracts clearly showed that the photoactivity is concentrated in the lamellas. In sum, here we will present our newest insights from the photobiological world of fungi, highlighting Dermocybes as a promising and fascinating source for new photosensitizers, photoantimicrobials, and phototoxic defense effectors.

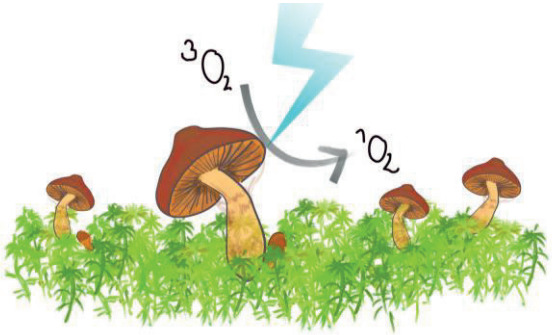


Figure 1. Schematic representation of dermocyboid Cortinarii producing singlet oxygen under light irradiation. The photobiological action is based on monomeric and dimeric anthraquinones and can be used to combat malignant cancer cells and resistant microbes, as we will show here.

References

2) Siewert, B.; Vrabl, P; A convenient workflow to spot photosensitizers revealed photo-activity in basidiomycetes; *RSC Advances*; 2019
3) Hammerle, F.; I. Bingger; Targeted Isolation of Photoactive Pigments from Mushrooms Yielded a Highly Potent New Photosensitizer: 7,7'-Biphyscion; ChemRxiv; 2021
4) Fiala, J.; Schöbel, H.; A new High-Throughput-Screening-assay for Photoantimicrobials Based on EUCAST Revealed Photoantimicrobials in Cortinariaceae; BioRxiv, 2021

Acknowledgements. This work was supported by the Austrian Science Fund (FWF, P319105) and the Tyrolian Science Fund.

IL-3.3.5

Photoperception in plant- and rock-associated black fungi (Ascomycota)

Julia Schumacher[1,2], Anna A. Gorbushina[1,2]

1) Bundesanstalt für Materialforschung und -prüfung (BAM), 4 – Materials and the Environment, Berlin, Germany; 2) Freie Universität Berlin, Department of Biology, Chemistry, Pharmacy, Berlin, Germany

Fungi that share light-flooded habitats with phototrophs may profit from their excess photosynthetic products. Sunlight-associated stresses are however multiple: high temperatures, UV radiation with associated DNA damage, accumulation of reactive oxygen species (ROS), desiccation and osmotic stresses. Ascomycota dominating light-flooded habitats accurately sense and respond to changes in light using it as a cue to coordinate growth, stress responses as well as to establish pathogenic or symbiotic relationships. Two species from two light-flooded habitats – phyllosphere and sun-exposed solid surfaces – were analysed for their photoreceptor distribution.
In both habitats phototroph-associated and black [dihydroxynaphthalene (DHN) melanin-containing] fungi are prevalent. This diversity was sampled with the plant-associated fungus *Botrytis cinerea* (Leotiomycetes), while *Knufia petricola* (Eurotiomycetes) was included as a typical biofilm-former on sun-exposed solid surfaces e.g. rocks, building facades, roofs, and solar panels. The analysis has shown that genomes of black fungi contain more photoreceptors than animal pathogens and saprophytes such as *Aspergillus nidulans* and *Neurospora crassa*[1,2]. *B. cinerea* that causes the grey mould disease by infecting the above-ground parts of more than 200 dicots has a highly sophisticated photosensory and signalling system that helps to avoid light and to locate susceptible hosts[1].

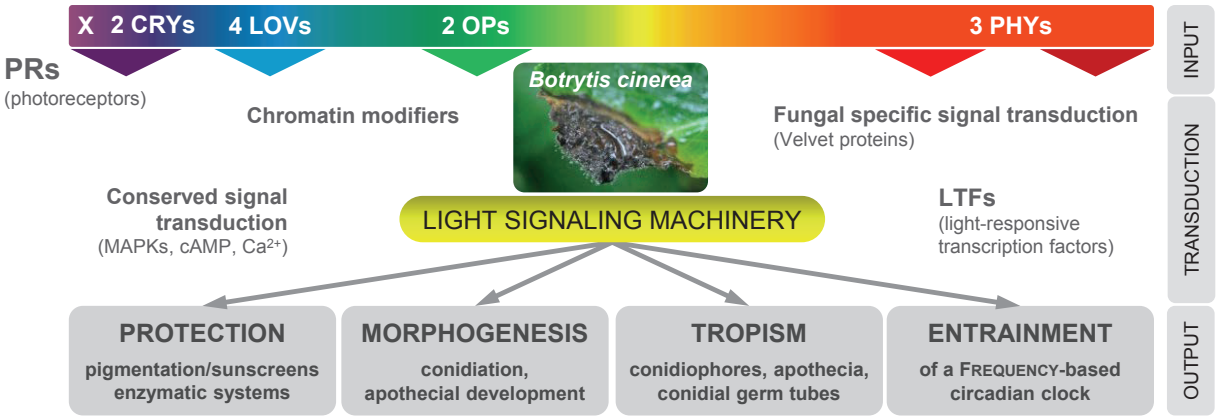


Fig. 1 – B. cinerea uses signals from at least 11 photoreceptors to coordinate stress responses, development, and virulence.

Rock-inhabiting Dothideomycetes and Eurotiomycetes including *Knufia petricola* possess equal numbers of photoreceptors along with the same set of protective metabolites i.e. melanin, carotenoids and mycosporines[2]. This similarity between black fungi from plant and rock surfaces suggests that photoperception and -regulation are important for sun-stressed fungi that receive nutrients through cooperation with phototrophs. CRISPR/Cas9-based genetic tools for manipulating *K. petricola* were established[3] and are currently used for elucidating the functions of the different photoreceptors in the biology of rock-inhabiting fungi.

References

1) Schumacher J. How light affects the life of *Botrytis*. Fungal Genetics and Biology 106: 26-41, 2017.
2) Schumacher J, Gorbushina AA. Light sensing in plant- and rock-associated black fungi. Fungal Biology 124:407-417, 2020.
3) Voigt O, Knabe N, Nitsche S, Erdmann EA, Schumacher J, Gorbushina AA. An advanced genetic toolkit for exploring the biology of the rock-inhabiting black fungus *Knufia petricola*. Scientific Reports 10(1), 2020.

Acknowledgements. This work was supported by the grant SCHU 2833/4-1 from the German Research Foundation (DFG) and internal funds of the BAM.

IL-3.3.6

Analysis of the light response in *Alternaria alternata* – mitochondria as novel phytochrome assembly stations

Reinhard Fischer, Kai Leister, Christian Streng
Karlsruhe Institute of Technology, Department of Microbiology, Karlsruhe, Germany

Mitochondria are essential organelles because of their function in energy conservation. Here, we show an involvement of mitochondria in phytochrome-dependent light sensing in fungi. Phytochrome photoreceptors are found in plants, bacteria and fungi and contain a linear, heme-derived tetrapyrrole as chromophore. Linearization of heme requires heme oxygenases (HOs) which reside inside chloroplasts *in planta*. Despite the poor degree of conservation of HOs, we identified two candidates in the fungus *Alternaria alternata*. Deletion of either one phenocopied phytochrome-deletion. The two enzymes had a cooperative effect and physically interacted with phytochrome, suggesting metabolon formation. The metabolon was attached to the surface of mitochondria with a C-terminal anchor sequence in HoxA. The affinity of phytochrome apoprotein to HoxA was 57,000-fold higher than the affinity of the holoprotein, suggesting a "kiss-and go" mechanism for chromophore loading and a function of mitochondria as assembly platforms for functional phytochrome. Hence, two alternative approaches for chromophore biosynthesis and insertion into phytochrome evolved in plants and in fungi.

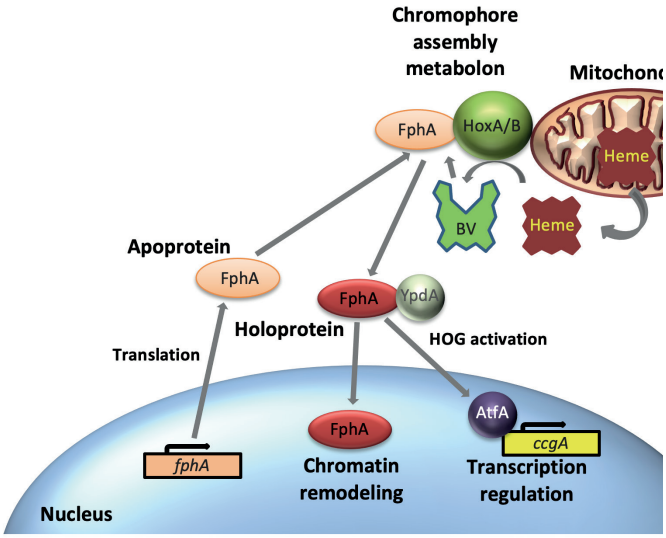


Fig. 1 – Phytochrome apoprotein is produced in the cytoplasm and travels to mitochondria, where a two heme oxygenases provide the linear tetrapyrrole biliverdin and insert it into the phytochrome protein. Functional phytochrome interacts with the phophoptransfer protein YpdA to induce the MAP kinase pathway. A fraction of holo-phytochrome is imported into nuclei, where it is involved in chromatin remodeling.

References

Igbalajobi, O., Yu, Z. and Fischer, R. (2019). Red- and blue-light sensing in the plant pathogen *Alternaria alternata* depends on phytochrome and the white-collar protein LreA. *MBio* **10**, e00371-19.

Igbalajobi, O., Gao, J. and Fischer, R. (2020). The HOG pathway plays different roles in conidia and hyphae during virulence of *Alternaria alternata*. *Mol Plant Microbe Interact* **33**, 1405-1410.

Streng, C., Hartmann, J., Leister, K., Krauß, N., Lamparter, T., Frankenberg-Dinkel, N., Weth, F., Bastmeyer, M., Yu, Z. and Fischer, R. (2021) A novel role for mitochondria in light sensing. *EMBO J., in revision.*

Yu, Z., Armant, O. and Fischer, R. (2016). Fungi use the SakA (HogA) pathway for phytochrome-dependent light signaling. *Nat. Microbiol.* **1**, 16019.

Yu, Z. and Fischer, R. (2019). Light sensing and responses in fungi. *Nat. Rev. Microbiol.* **17**, 25-36.

Acknowledgements. This work was supported by the *Deutsche Forschungsgemeinschaft*.

IL-3.3.7

Light regulates the degradation of VE-1, a component of the regulatory velvet complex in the fungus *Neurospora crassa*

Luis M. Corrochano[1], M. del Mar Gil-Sánchez[1], Sara Cea-Sánchez[1], María Corrochano-Luque[1], Alejandro Miralles-Durán[1], Cristina De Andrés-Gil[1], Eva M. Luque[1], Guilherme TP Brancini[1], Gabriel Gutiérrez[1] and David Cánovas[1]
1) University of Seville, Department of Genetics, Seville, Spain

Conidiating mycelia of species of the fungus *Neurospora* colonize the surface of burned vegetation and facilitate the recycling and restoration of forests after fires. The velvet complex is a protein complex that serves as a key element in the coordination of fungal development, secondary metabolism, and the perception of environmental signals, such as light. Most fungi use proteins similar to WC-1 and WC-2 from *Neurospora crassa* for sensing blue light. In *N. crassa* and other fungi these two proteins form a photoreceptor and transcription factor complex (WCC) that binds to the promoters of light-regulated genes to activate transcription. Several other photoreceptors have been identified in fungi, including phytochromes, opsins and cryptochromes, but they play a secondary role in the photobiology of *N. crassa*.

The *velvet* regulators are members of a family of proteins with a conserved domain that help to coordinate growth, differentiation and secondary metabolism in fungi. There are four genes containing a velvet domain in the genome of *N. crassa (ve-1, ve-2, ve-3 and vos-1).* Mutants in genes *ve-1* and *ve-2* show defective aerial hyphae growth, increased conidiation, and reduced carotenoid accumulation. VE-1, VE-2 and the methyltransferase LAE-1 are observed in vegetative mycelia and during conidiation. These three proteins interact to form the velvet complex that, presumably, regulates transcription during conidiation and the biosynthesis of secondary metabolites such as carotenoid protective pigments. During the characterization of the abundance of the components of the velvet complex during conidiation we noted that VE-1 was absent in aerial hyphae grown in the dark despite the presence of *ve-1* mRNA. The other components of the velvet complex, VE-2 and LAE-1, accumulated in similar amounts in vegetative and conidiating mycelia. We have shown that the absence of VE-1 in aerial hyphae in the dark is due to its degradation through the proteasome with a key role for the adaptor protein FWD-1. The degradation of VE-1 is reduced by light in a process that requires the blue-light photoreceptor WC-1. We propose that the light-dependent regulation of VE-1 stability modifies the components of the *velvet* complex resulting in changes in the transcriptome during conidiation.

Acknowledgements. Supported by European funds (European Regional Development Fund) and the Ministry of Science, Innovation and Universities, Spain (RTI2018-098636-B-I00) to LMC and DC. The work of Guilherme TP Brancini at the University of Seville was supported by a BEPE short-term fellowship from FAPESP (2018/00355-7), Brazil.

OC-3.3.8

Effects of LOV photoreceptor deletion in the *Pseudomonas syringae-tomato* system

Daniela Ceresini[1], Francesca Degola[1], Aba Losi[2], Ada Ricci[1]

1) University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Parco Area delle Scienze 11/A, 43124, Parma, Italia; 2) University of Parma, Department of Mathematical, Physical and Computer Sciences, Parco Area delle Scienze 7/A, 43124, Parma, Italia.

Light is an important environmental signal and its perception plays a crucial role in many physiological processes, even in non-phototrophic organisms associated with the phyllosphere. These organisms possess photosensory proteins capable of perceiving light and transducing light signal to evoke a response in the bacterial cell. *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*), the etiological agent of bacterial speck in tomato plants, represents one of the most important models for studying plant-pathogen interaction. It causes large losses of crops and this calls for more research on the physiological basis of its infectiveness and virulence. The interest in this microorganism is linked to the presence in its genome of various gene sequences coding for photoreceptors. *Pst* is indeed equipped with two red/far-red (R/FR) light sensing bacteriophytochromes (BphP), binding biliverdin as chromophore and with one blue light sensing LOV (Light, Oxygen and Voltage) protein binding flavins as chromophore, mimicking the photosensing ability of host plants.
Here, a major target is to determine if and how *Pst* LOV photoreceptor interfere with plant defense system. We report the study of the effects of infection on tomato plants with LOV mutant strain deleted in the LOV gene. We also studied the effects of salicylic acid (SA), an important endogenous plant defense signal of attack by pathogens, on the growth of LOV mutant strain. Furthermore, we have studied the plant's response to infection by quantifying callose production.
The mutant strain lacking the photosensory protein LOV is more virulent and invasive towards tomato plants than *Pst*-WT. Moreover, we found that SA concentrations of 1mM and 5mM inhibit the growth of both the mutant LOV strain and the *Pst*-WT strain in vitro, while growth stimulation is surprisingly observed at 0.1mM concentration. As far as the plant production of callose is concerned, a higher production of callose after 24h in plants infected with the mutant LOV strain is reported compared to those infected with the *Pst*-WT strain.
Further studies are needed to better understand the role of this photoreceptor in the *Pseudomonas syringae-tomato* system.

References

1) Ricci, A., Dramis, L., Shah, R., Gärtner, W. and Losi, A. (2015), Bacterial photoreceptors in plant–pathogen interaction. Environmental Microbiology Reports, 7: 795-802.
2) Shah, R., Pathak, G., Drepper, T. and Gärtner, W. (2016), Selective Photoreceptor Gene Knock-out Reveals a Regulatory Role for the Growth Behavior of Pseudomonas syringae. Photochem Photobiol, 92: 571-578.

OC-3.4.1

Highly sensitive detection of 2-photon photosensitized singlet oxygen using a novel optofluidic system

Sergio Adan Bermudez[a], Jochen Arlt[b] and Anita C. Jones[a]
[a]*School of Chemistry, The University of Edinburgh, Edinburgh EH9 3FJ, UK*
[b]*School of Physics and Astronomy, The University of Edinburgh, Edinburgh EH9 3FD, UK*

Photosensitized production of singlet oxygen ($^1O_2$) is used in the treatment of cancer by photodynamic therapy (PDT), in which a photosensitizer is administered and excited locally in the tumour tissue, generating $^1O_2$ to destroy, selectively, malignant cells. PDT is also used in the treatment of age-related macular degeneration, atherosclerosis, psoriasis and acne. The use of two-photon excitation (TPE) in PDT has significant advantages in achieving enhanced spatial selectivity and greater depth of penetration through tissue. This is driving development of new photosensitizers with high 2-photon cross-sections, but progress is inhibited by the difficulty of determining, *in vitro*, the efficiency of $^1O_2$ production in response to TPE by these new pro-drugs.

In conventional experiments, the extremely high photon intensity needed to achieve two-photon absorption is created by focusing a pulsed laser beam into a spot of about 1 μm in diameter. This miniscule (femtolitre) excitation volume makes the detection of two-photon-induced $^1O_2$ generation extremely challenging. We will describe a new approach to the detection of 2-photon photosensitized $^1O_2$, which exploits the unique optofluidic properties of hollow-core photonic crystal fibre (HC-PCF).[1] In HC-PCF, light is trapped in the hollow core by the surrounding microstructured fused-silica cladding. This allows the infiltration of a sample solution into the hollow core, which is typically 10's of □m in diameter, while maintaining the high optical transmission efficiency of the fibre. As we have shown previously, the confinement of both laser beam and sample solution within the fibre core permits two-photon excitation to be sustained over a path-length of more than 10 cm.[2]

We will report the development a HC-PCF-based optofluidic platform for the detection of 2-photon photosensitized singlet oxygen, to enable the *in vitro* screening and quantitative characterisation of two-photon photosensitizers, under mechanistically relevant conditions (in aqueous solution and using low pulse-energy excitation). Highly sensitive detection is achieved using a $^1O_2$-specific fluorescent probe, employing a two-colour pump-probe methodology, with an 800-nm pump beam (TPE of photosensitizer) and a 488-nm probe beam (excitation of fluorescent probe) co-coupled into the hollow core of the fibre. Using this approach, we are able to detect quantitatively singlet oxygen produced by TPE of sub-micromolar concentrations of photosensitizer in aqueous solution.

References

1. A. M. Cubillas, S. Unterkofler, T. G. Euser, B. J. Etzold, A. C. Jones, P. J. Sadler, P. Wasserscheid, P. S. Russell *Chem. Soc. Rev.* **2013**, 42, 8629-8648.
2. G. O. S. Williams, T. G. Euser, J. Arlt, P. S. J. Russell, A. C. Jones *ACS Photonics* **2014**, 1, 790-793.

OC-3.4.2

Photoprotection, energy quenching and PSBS, is it really important?

Christo Schiphorst[1], Koen Staring[1], Emilie Wientjes[1]

1) Wageningen University, Biophysics, Wageningen, Netherlands

Photosynthesis is crucial for life on earth, but it is not very efficient. In most conditions, less than 1% of the incoming light-energy is converted into biomass, which is far below the theoretical maximum. One of the major losses happens in the form of energy quenching (qE), a process where light energy is converted into heat. In plants this is activated by the protein PSBS upon a decrease in the pH of the thylakoid lumen. We are investigating when and how PSBS becomes important for photoprotection, water usage and crop-yield in various light and temperature conditions.

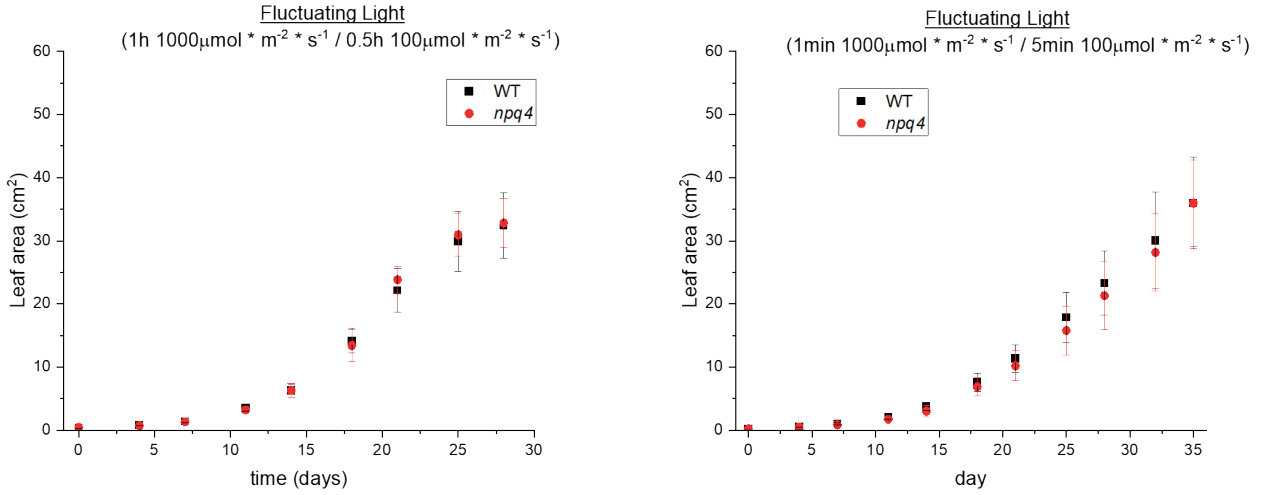


*Figure 1 Leaf area of WT, npq4 in continuous fluctuating light conditions. Left inset 1h 1000µmol*m²*s⁻¹ & 0.5h 100µmol*m²*s⁻¹. Right inset: 1min 1000µmol*m²*s⁻¹ & 5min 100µmol*m²*s⁻¹.*

However, despite the general believe that PSBS is essential for photoprotection and optimal growth, finding conditions in which there is a difference in growth between WT and a mutant without PSBS (*npq4* mutant) is not as simple, see Fig. 1. Therefore, we are combining growth experiments with more direct measurements like CO_2 assimilation and chlorophyll fluorescence to assess in real time what is happening during the light fluctuations, see Fig. 2.

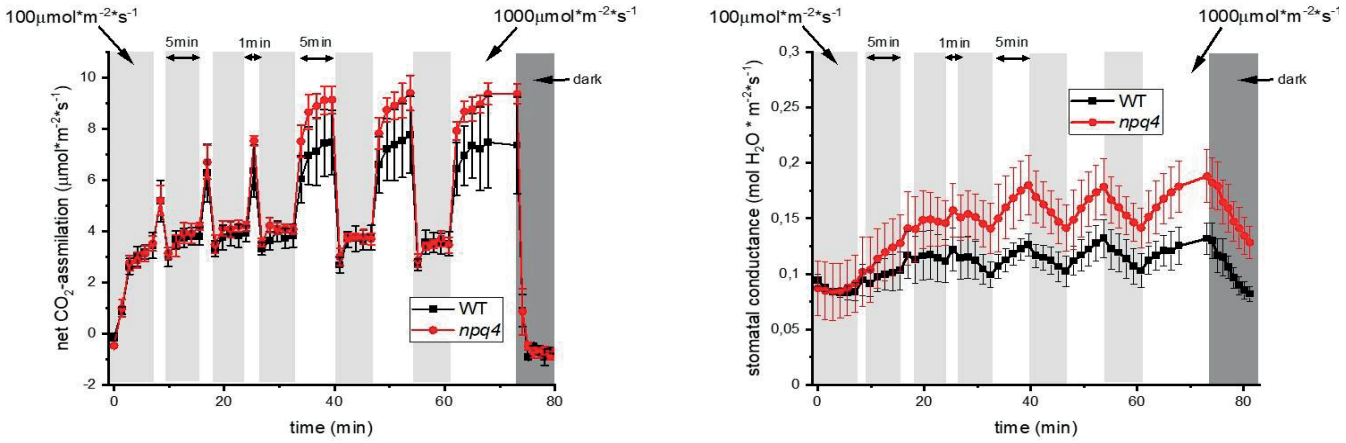


Figure 2 CO2 assimilation measurements and Chlorophyll fluorescence of WT and npq4 plants in different fluctuating light conditions.

OC-3.4.3

Oxybenzone solar filter as a photoremovable protecting group for carbonyl compounds of biological interest

Mauricio Lineros-Rosa, Miguel A. Miranda and Virginie Lhiaubet-Vallet

Instituto Universitario Mixto de Tecnología Química UPV-CSIC, Universitat Politècnica de València, Consejo Superior de Investigaciones Científicas, Avda de los Naranjos s/n, 46022 Valencia, Spain

Nowadays, there is a growing number of cases dealing with photosensitization due to the increasing use of xenobiotics (such as cosmetics, fragrances and drugs) in combination with exposure to sunlight. These harmful effects are closely related to the development of skin cancer. Therefore, the design of new photoprotection strategies to counteract these negative consequences is of utmost importance.[1] In this context, a new photoremovable carbonyl protecting group based on the oxybenzone (OB) solar filter is presented.[2] This system has demonstrated the ability to photorelease aromatic ketones along with the aforementioned UV filter in a simultaneous and controlled manner. Thus, the OB protects the carbonyl derivative against the deleterious effect of UV light (Figure 1A). In the case of aromatic ketones, this photoprotection is of special interest in view of their well-known photoreactivity. Among the possible aromatic ketone substrates for this new PPG, ketoprofen (KP) can be highlighted given that it is a worldwide employed nonsteroidal anti-inflammatory drug of topical use and responsible for severe photosensitizing effects.[3]

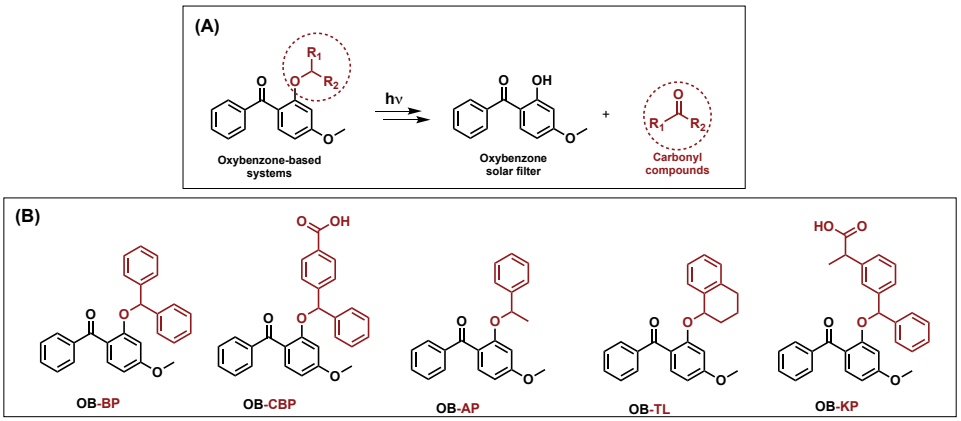


Figure 1. Photorelease of oxybenzone and carbonyl derivatives compounds from the oxybenzone-based systems (A) and the structures of the studied systems (B).

In this work, the photophysical and photochemical properties of the OB-BP and OB-CBP systems (Figure 1B) have been thoroughly studied through experimental techniques such as laser flash photolysis, HPLC, UV-Vis spectroscopy and mass spectrometry. Based on these results, a photorelease mechanism involving a first δ-hydrogen abstraction followed by photoionization has been proposed. Additionally, this system has also been applied for the photorelease of other aromatic ketones, *i.e.* α-tetralone (TL), acetophenone (AP) and ketoprofen (KP).

References

1) (a) I. Aparici-Espert, M. C. Cuquerella, C. Paris, V. Lhiaubet-Vallet and M. A. Miranda, *Chem. Commun.*, **2016**, 52, 14215-14218. (b) M. Lineros-Rosa, M. C. Cuquerella, A. Francés-Monerris, A. Monari, M. A. Miranda, V. Lhiaubet-Vallet. *Org. Biomol. Chem.*, **2021**, 19, 1752-1759.
2) M. Lineros-Rosa, M. A. Miranda, V. Lhiaubet-Vallet. *Chem. Eur. J.*, **2020**, 26, 7205-7211.
3) V. Lhiaubet-Vallet and M. A. Miranda, CRC Handbook of Organic Photochemistry and Photobiology, CRC Press, Boca Raton, 3rd edn, **2012**, ch. 66, vol. 2, p. 1541.

Acknowledgements. This work was supported by the Universitat Politècnica de València (FPI grant) and by the Spanish government (project PGC2018-096684-B-I00).

OC-3.4.4

Photostability of Ipilimumab and Nivolumab in the formulation and sterile saline or glucose solutions for parenteral administration.

Benedetta Fongaro, Valentina Cian, Giorgia Miolo and Patrizia Polverino de Laureto
University of Padova, Department of Pharmaceutical and Pharmacological Sciences, Padova, Italy

Background. Monoclonal antibodies are complex protein molecules, and their structural integrity influences their biological and pharmacological activity. Ipilimumab and Nivolumab, targeting the molecules CTLA-4, PD-1, respectively, have shown efficacy against several types of cancer and have demonstrated the superiority of the combination strategy compared to the single agent therapy.[1]
Aim. We investigated the impact of some stress factors, i.e., shaking, vibrations and light exposure, on two formulated monoclonal antibodies (mAbs) Ipilimumab and Nivolumab, to mimic their routine handling once released from the pharma industry, shipped to the hospital, diluted for the parenteral administration, and finally administered to the patients.
Methods. Protein stability analyses, including aggregation and excipient effects on the protein structure, were carried out by spectroscopic methods (Uv-Vis, Fluorescence, and Circular dichroism), native and denaturing electrophoresis, and size exclusion chromatography analysis. Suntest CPS+ was used to simulate the conditions of one day or 3 weeks of sunlight exposure.
Results. Among the selected critical stress factors to simulate the real-life handling of mAbs during transport, pneumatic systems, reconstitution, and administration to patients, only light exposure was able to affect the protein drug products. The two mAbs demonstrated a general instability to light as intact products and after dilution in saline and glucose solutions (both homemade and commercial sterile solution). The highest instability under light exposure was found in diluted glucose sterile solutions, suggesting the role of glucose degradation products on the photo-modification process of the two mAbs.
Conclusion. Nivolumab and Ipilimumab are stable molecules under vigorous shaking and vibrations. However, they undergo structural change, mostly aggregation, upon exposure to artificial sunlight. The instability of mAbs upon light exposure could have a potential impact on the safety and efficacy of these very active drugs in anticancer therapy.

1) Marchetti P. Botticelli A., Agnostic evaluation of ipilimumab and nivolumab association: a metanalysis. J Transl Med, 2020.
2) Le Basle Y., Chennel P., Physicochemical Stability of Monoclonal Antibodies: A Review. Journal of Pharmaceutical Sciences, 2020.

OC-3.4.5

Chromatophores efficiently promote light-driven ATP synthesis and DNA transcription inside hybrid multicompartment artificial cells

Emiliano Altamura,[1] Paola Albanese,[1] Roberto Marotta,[2] Francesco Milano,[3] Michele Fiore,[4] Massimo Trotta,[5] Pasquale Stano[6] and Fabio Mavelli[1,7]

[1] *Department of Chemistry, University of Bari "Aldo Moro", Bari, Italy.*
[2] *Electron Microscopy Facility, Italian Institute of Technology (IIT), Genova, Italy.*
[3] *CNR-ISPA, Institute of Sciences of Food Production, Lecce Unit, Lecce, Italy.*
[4] *Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, Université de Lyon "Claude Bernard Lyon 1", Lyon, France.*
[5] *CNR-IPCF, Institute for Physical and Chemical Processes, Bari, Italy.*
[6] *Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce, Italy.*
[7] *CNR NANOTEC, Istituto di Nanotecnologia, Bari, Italy.*

In the emerging field of bottom-up synthetic biology, scientists challenge to transfer cellular capabilities to simplified artificial compartments[1-7]. Considering that every cellular active function need energy to be maintained, a crucial requirement for an artificial cell is to be energetically autonomous.
Our research is aimed to overcome this criticality by constructing synthetic cells able to assimilate energy from light and support an internal metabolism. We called these systems: Artificial Simplified-Autotroph Protocells (ASAPs)[8].
Here we show a hybrid multi-compartment approach to build ASAPs in an effective manner. Chromatophores obtained from *Rhodobacter sphaeroides* (Fig 1a and 1b) accomplish the photophosphorylation of ADP to ATP functioning as nanosized photosynthetic organellae when encapsulated inside artificial giant phospholipid vesicles prepared with the droplet transfer method. Under continuous illumination chromatophores produce ATP that in turn sustains the transcription of a DNA gene by T7 RNA polymerase inside ASAPs (Fig 1c).

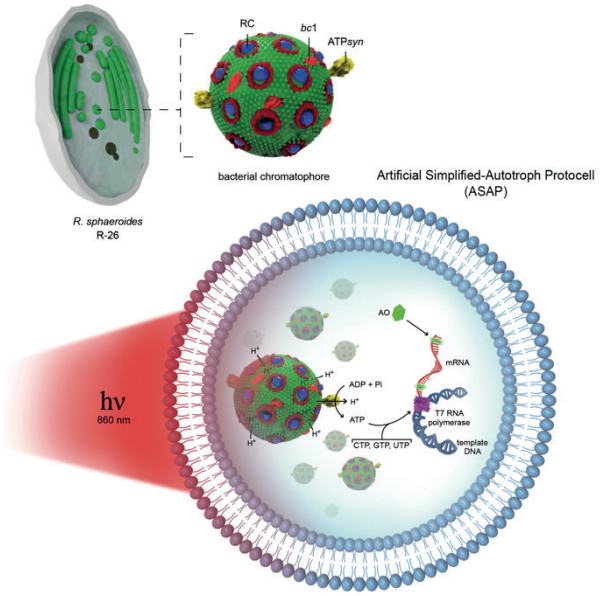


Figure 1. Cartoon representation of chromatophore-containing ASAPs (**a**) *Rhodobacter sphaeroides* R-26 cell in section. The membrane exhibits invaginations, large spherical vesicles (average diameter 80 nm), and several stratified flattened vesicles. (**b**) Chromatophores are ~80-nm closed vesicles derived from the lysis of *R. sphaeroides* R-26 cells, typically via French pressing. Their membrane contains the entire photosynthetic system: Light Harvesting complex I (dark red), Reaction Center (blue), ubiquinol oxidase (or bc1, in red) and ATP synthase (yellow) in inside-out orientation when compared with the

cytoplasmic membrane (ATP produced in the outer solution). (**c**) Chromatophores encapsulated inside giant phospholipid vesicles act as ATP-producing organellae when illuminated by an 860-nm light. Co-encapsulated ADP, Pi, GTP, CTP, UTP, T7 RNA polymerase (in purple), and a DNA template give rise to an out-of-equilibrium system of coupled reactions: namely, photophosphorylation and DNA transcription. The biosynthesized mRNA is revealed by Acridine Orange (green), which binds it, forming a green-fluorescent complex. Image credit Filippo Trazi.

Cryo-EM and time-resolved spectroscopy were used for characterizing the chromatophore morphology and the orientation of the photophosphorylation proteins, which allow high ATP production rates (up to ~100 ATP/s per ATP synthase). mRNA biosynthesis inside individual vesicles has been determined by confocal microscopy[8]. The hybrid multi-compartment approach here proposed appears at the same time convenient and effective, and thus very promising for the construction of full-fledged artificial protocells.

References

1) P. L. Luisi, Toward the engineering of minimal living cells. *Anat. Rec.* 268, 208–214 (2002).
2) Walde P. (2010) Building artificial cells and protocell models: experimental approaches with lipid vesicles *Bioassays* Vol. 32, 296-303.
3) J. C. Blain, J. W. Szostak, Progress toward synthetic cells. *Annu. Rev. Biochem*. 83, 615–640 (2014).
4) B. C. Buddingh', J. C. M. van Hest, Artificial cells: Synthetic compartments with life-like functionality and adaptivity. *Acc. Chem. Res*. 50, 769–777 (2017).
5) P. Schwille et al., MaxSynBio: Avenues towards creating cells from the bottom up. *Angew. Chem.* Int. Ed. Engl. 57, 13382–13392 (2018).
6) J. W. Hindley et al., Building a synthetic mechanosensitive signaling pathway in compartmentalized artificial cells. *Proc. Natl. Acad. Sci. U.S.A.* 116, 16711–16716 (2019).
7) Y. Elani, Interfacing Living and Synthetic Cells as an Emerging Frontier in Synthetic Biology. *Angew. Chem.* 60(11): 5602-11.
8) E. Altamura, P. Albanese, et al. Chromatophores efficiently promote light-driven ATP synthesis and DNA transcription inside hybrid multicompartment artificial cells. *Proc. Natl. Acad. Sci. USA* (2021), Vol. 118(7)

OC-3.4.6

Hypericin against SARS-CoV-2: from binding to antiviral efficacy

Pietro Delcanale[1], Andrea Mussini[1], Eleonora Uriati[1,2], Matteo Mariangeli[1,2], Paolo Bianchini[2,3], Alberto Diaspro[2,3,4], Stefania Abbruzzetti[1], Davide Lelli[5], Ana Moreno[5], Cristiano Viappiani[1]
1) Department of Mathematical Physical and Computer Sciences, University of Parma, Parma, Italy; 2) Nanoscopy, Istituto Italiano di Tecnologia, Genoa, Italy; 3) Nikon Imaging Center, Istituto Italiano di Tecnologia, Genoa, Italy; 4) Department of Physics, University of Genoa, Genoa, Italy; 5) Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy

Hypericin is photosensitizing drug with significant antiviral activity against membrane-enveloped viruses and therefore constitutes a promising candidate for the treatment of SARS-CoV-2 [1]. The binding affinity of Hypericin to viral components and the distribution of Hypericin molecules within a population of viral particles are fundamental parameters determining its ensemble efficacy [2]. Hence, quantitative measurements addressing molecular binding and distribution of Hypericin on SARS-CoV-2 virions can provide a rationale to guide the improvement of treatments based on this photosensitizing drug, beyond the mere observation of a reduced infectivity.
In this work, we use a quantitative approach to follow the interaction of Hypericin with SARS-CoV-2, at the basis of the efficacy of this drug. First, we exploited the fluorescence emission of Hypericin to visualize its binding onto viral particles (see Fig.1). Then, using a combination of spectroscopy techniques, we identified the membrane envelope as the main viral component targeted by the photosensitizer and we measured binding affinity. In addition, we used fluorescence microscopy to investigate the loading of Hypericin molecules onto individual SARS-CoV-2 virions. Finally, the results are correlated to the ensemble antiviral efficacy of Hypericin, both photoinduced and in the dark, observed on infected Vero E6 cells. These findings demonstrate that Hypericin has a significant antiviral activity against SARS-CoV-2 and provide a quantitative characterization of binding, which is key to understand the mechanisms of action of Hypericin and to drive the rational development of antiviral treatments based on photosensitization.

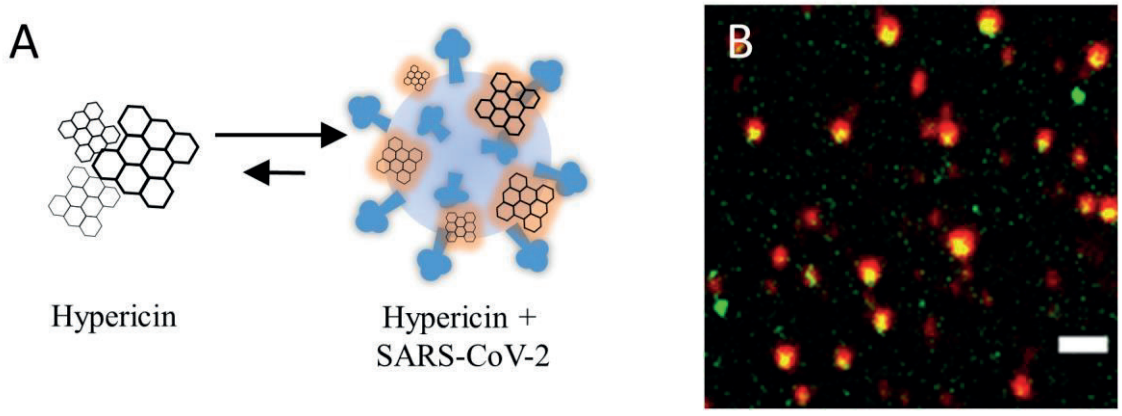


Fig. 1 – A: Representation of Hypericin binding to a SARS-CoV-2 virion: while unbound Hypericin is found in non-emissive aggregates, virus-bound Hypericin molecules recover fluorescence emission, enabling selective detection. B: Confocal image of SARS-CoV-2 virions exposed to Hypericin. Emission from virus-bound Hypericin is shown in red while emission from Hoechst, labelling the viral RNA, is in green. Co-localized red and green emissions look yellow. Scale bar 1 μm.

References

1) Kubin A., Wierrani F., Hypericin -The facts about a controversial agent. Current Pharmaceutical Design, 2005.
2) Wiehe A., O'Brien J.M., Trends and targets in antiviral phototherapy. Photochemical & Photobiological Sciences, 2019.

OC-3.4.7

An update on Photodynamic Decontamination to prevent foodborne disease

Michael Glueck[1], Christoph Hamminger[1], Markus Hoerl[1], Barbara Schamberger[1], Kristjan Plaetzer[1]
1) University of Salzburg, Department of Biosciences, Laboratory of Photodynamic Inactivation of Microorganisms, Salzburg, Austria

Foodborne disease occurs regularly around the globe and often leads to severe illness and even death. To minimize this threat and to ensure microbiological safety, the food industry applies different thermal and non-thermal sterilisation technologies. For vegetables and fruits, which are often eaten raw, thermal treatment is impracticable and non-thermal technologies, such as treatment with chlorine gas or gamma irradiation, are deprecated by consumers. Photodynamic Decontamination (PDc) represents a non-thermal alternative and has been proven to be effective in killing of bacteria on food surfaces.[1,2] Relevant bacterial genii in food hygiene are *Listeria sp*. (Gram+) and *Escherichia sp*. (Gram-). The aim of this study was therefore to test PDc based on two natural photosensitizers (PS), Na-chlorophyllin (Chl) and curcumin, against these bacteria.

Listeria innocua as model system for *Listeria monocytogenes* was grown in liquid culture and subsequently PDc-treated using Chl (1 µM, 10 µM or 100 µM, 5 min incubation, 15.3 J/cm^2 at 395 nm) or NovaSOL®- Curcumin (NoCu), a water soluble micellar formulation of curcumin (0.1 µM to 50 µM, 15 min incubation, 20.4 J/cm^2 at 435 nm). The minimal radiant exposure for achievement of an antibacterial effect was investigated using NoCu at 5 µM against *L. innocua* after 15 min incubation. As example of use, PDc efficiency was also examined on fish skin (trout) using NoCu and on fenugreek seeds using Chl in combination with polyaspartic acid (PA). The foodstuff was contaminated with *L. innocua* and subsequently phototreated. Evaluation was done by counting of colony forming units (CFU). To study the influence of the target geometry, slices of cucumbers as flat, mung beans as round and germinated mung beans as complex objects were purposely contaminated with *E. coli*, incubated with 10 µM, 50 µM or 100 µM of SACUR-3 (a cationic derivative of curcumin) and illuminated at 435 nm (33.8 J/cm^2).

The treatment of *L. innocua* in liquid culture was highly effective for both photoactive compounds. Concentrations of 1 µM and above for NoCu and 10 µM and higher for Chl led to a complete kill of bacteria. At 5 µM NoCu a very low radiant exposure of ca. 1.4 J/cm^2 induced cell death in all bacteria of the sample. The PDc of *L. innocua* on fish skin and on fenugreek seeds showed phototoxicity achieving nearly 3 $\log_{10}$ reduction. On flat surfaces as cucumbers, *E. coli* was killed with a relative inactivation of 3.4E4 using 100 µM SACUR-3. On mung beans, the relative inactivation reached 5.0E3, but only 5.1E0 on germinated mung beans.

Photodynamic Decontamination is a promising approach to improve microbial food safety. The use of PSs approved as food additives guarantees biocompatibility. Phototreatment based on Chlorophyllin and Curcumin has high potential to contribute to the prevention of foodborne diseases. Jet there are limitations, which can partially be overcome by the development of 3D illumination devices.

References

1) Buchovec I., Lukseviciute V., Marsalka A., Reklaitis I. and Luksiene Z., Effective photosensitization-based inactivation of Gram (-) food pathogens and molds using the chlorophyllin-chitosan complex: towards photoactive edible coatings to preserve strawberries. Photochemical & Photobiological Sciences, 2016
2) Glueck M., Schamberger B., Eckl P. and Plaetzer K., New horizons in microbiological food safety: Photodynamic Decontamination based on a curcumin derivative. Photochemical & Photobiological Sciences, 2017

Acknowledgements. We thank Suncor AgroScience for providing support.

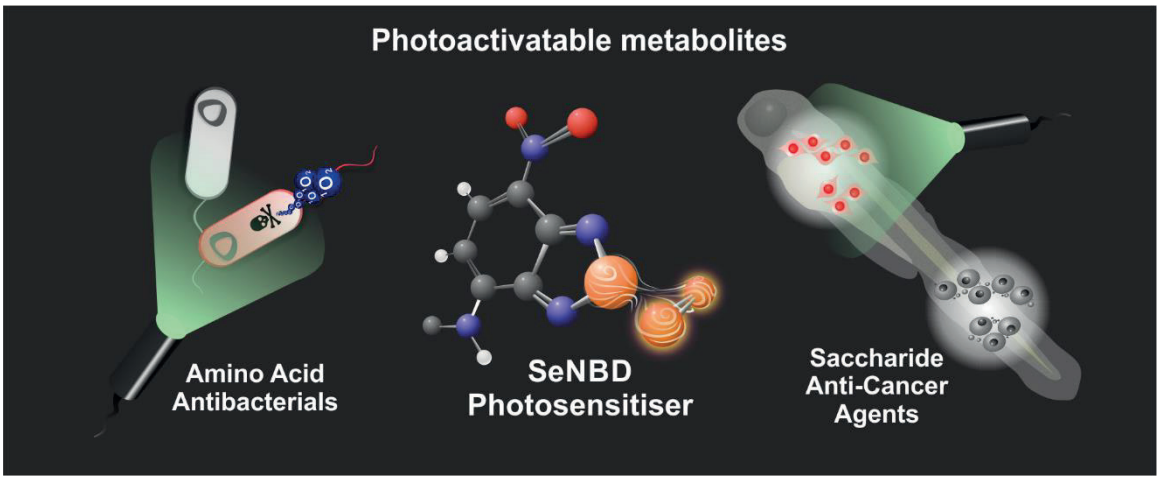
OC-3.4.8

Photoactivatable metabolic warheads enable precise and safe ablation of target cells in vivo

Sam Benson[1], Fabio de Moliner[1], Antonio Fernandez[1], Erkin Kuru[2], Nicholas L. Asiimwe[3], Jun-Seok Lee[4], Lloyd Hamilton[5], Dirk Seiger[5], Isabel R.Bravo[1], Abigail M. Elliot[1], Yi Feng[1] & Marc Vendrell[1].

1) Centre for Inflamamtion Research, QMRI, Theuniversity of Edinburgh, Edinburgh, UK
2) Department of Genetics, Harvard Medical School, Boston, MA, USA.
3) Molecular Recognition Research Center, KIST, Seoul, South Korea
4) Departmetn of Pharmcaology, Korea University College of Medicine,Seoul, South Korea
5) The Centre for Discovery Brain Sciences, The University of Edinburgh, Edinburgh, UK

Photoactivatable molecules enable ablation of malignant cells under the control of light, yet current agents can be ineffective at early stages of disease when target cells are similar to healthy surrounding tissues. We have created a chemical platform based on amino substituted benzoselenadiazoles to build photoactivatable probes that mimic native metabolites as indicators of disease onset and progression. Through a series of synthetic derivatives, we have identified the key chemical groups in the benzoselenadiazole scaffold responsible for its photodynamic activity, and subsequently designed photosensitive metabolic warheads to target cells associated with various diseases, including bacterial infections and cancer. We demonstrate that versatile benzoselenadiazole metabolites can selectively kill pathogenic cells but not healthy cells with high precision after exposure to non-toxic visible light, reducing any potential side effects in vivo. This chemical platform provides powerful tools to exploit cellular metabolic signatures for safer therapeutic and surgical approaches.[1]



References

1) Benson, S.; de Moliner, F.; Fernandez, A.; Kuru, E.; Asiimwe, N. L.; Lee, J.-S.; Hamilton, L.; Sieger, D.; Bravo, I. R.; Elliot, A. M.; Feng, Y.; Vendrell, M. Photoactivatable Metabolic Warheads Enable Precise and Safe Ablation of Target Cells in Vivo. Nat. Commun. 2021, 12, 2369. https://doi.org/10.1038/s41467-021-22578-2.

Acknowledgements. This work was supported by Medical Research Scotland (S.B.:879-2015) and the ERC consolidator grant (M.V.:771443).

OC-3.4.9

A portable NIR spectrometer directly quantifies singlet oxygen generated by nanostructures for Photodynamic Therapy in deep tissues

Orsi Davide[1], Vaccari Marco[1], Baraldi Andrea[1], Cristofolini Luigi[1]

1) Università di Parma, Dipartimento di Scienze Matematiche, Fisiche e Informatiche, Parma (IT)

We report on the setting up and calibration of a portable NIR spectrometer specifically developed for quantitative direct detection of the highly reactive singlet oxygen ($^1O_2$) chemical specie, of great importance in Photodynamic therapies. This quantification relies on the measurement of fluorescence emission of $^1O_2$, which is peaked in the near infrared (NIR) at $\lambda = 1270\ nm$.
In recent years, several nanostructures capable of generating reactive oxygen species (ROS) when activated by penetrating radiation (X-rays, NIR light) have been developed to apply Photodynamic Therapy (PDT) to tumours in deep tissue, where visible light cannot penetrate[1-3]; these nanostructures combine a scintillating nanostructure and a PDT photosensitizer. A bottleneck in their characterization is the lack of a fast and reliable technique to quantitatively assess their performances in generating ROS, and in particular $^1O_2$. For instance, the widely used PDT "Singlet Oxygen Sensor Green" kit suffers from self-activation under X-ray irradiation.
To solve this difficulty, we propose here direct detection of $^1O_2$ by a spectroscopic technique, thanks to recently developed thermoelectrically cooled InGaAs single photon avalanche photodiodes (SPAD).
We couple an InGaAs SPAD to a custom-made integrating sphere. By calibrations on standard photosensitizers, the detection threshold for our apparatus was determined: it turns to be $\sim 4 \cdot 10^9$ $^1O_2$ in realistic experimental condition and for measurements extending to 1 minute of integration[4].
Finally, we report on preliminary results of $^1O_2$ generated by nanostructures for PDT activated by 6MeV X-rays generated by a Radiotherapy Linear Accelerator of clinical use.

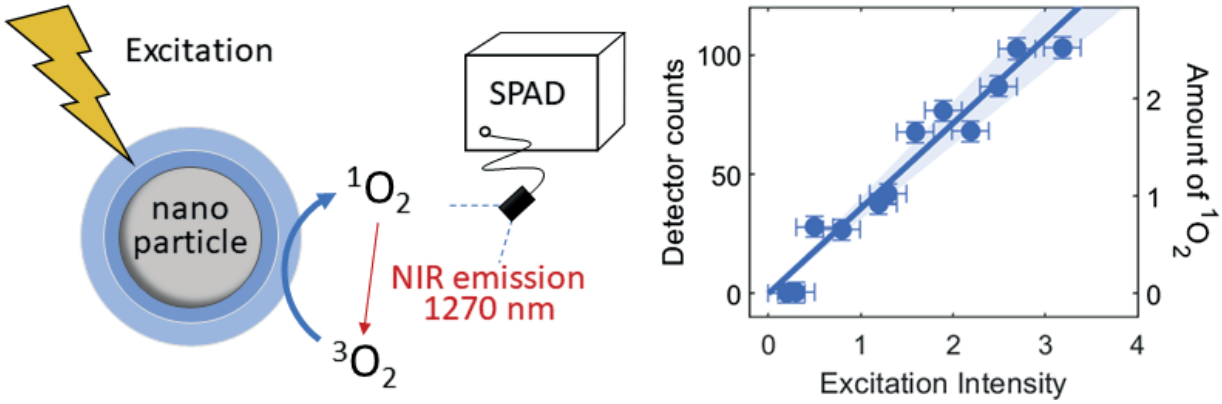


Fig. 1 – A nanostructure combining scintillating nanoparticles and a PDT photosensitizer generates $^1O_2$ when irradiated by high-energy X-rays. $^1O_2$ is detected by measuring its near-infrared emission at 1270nm using a specifically developed portable NIR spectrometer.

References

1) T. Rimoldi et al. J. Mater. Sci. Mater. Med. 27, 159, 2016
2) D. Orsi et al. Nanomedicine. 13 2311–2326, 2018
3) F. Rossi et al. Sci. Rep. 5 1-6, 2015
4) D. Orsi et al, submitted, 2021

Acknowledgements. This work was supported by the programme "FIL-Quota Incentivante" of University of Parma, co-sponsored by Fondazione Cariparma.

OC-3.4.10

Uncovering the potency of PSI-ALA-Hex as a fluorescence-guided surgery tool for breast cancer

Martin Kiening, Norbert Lange
School of Pharmaceutical Sciences, Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, Switzerland

In 2018, breast cancer (BC) was the most commonly diagnosed cancer in women with more than 2 million new cases and the first cause of female cancer death (>625,000)[1]. Mammography being the only effective screening method, researches started to extend photodiagnosis (PD) to breast cancer.
Protoporphyrin IX (PpIX) is a natural molecule whose production has been widely browsed for photodynamic therapy (PDT) and PD applications due to its photosensitizing potential. Synthesis of this ultimate heme precursor in mitochondria can be boosted following the intake of 5-aminolevulinic acid (5-ALA), a former molecule in the heme biosynthesis pathway. However, the original hydrophilic 5-ALA toughly cross plasma membranes and showed a poor pharmacokinetic profile.
Designing more lipophilic derivatives such as methyl- and hexyl-5-ALA esters countered the 5-ALA uptake issue. PSI-ALA-Hex, a new phosphatase-sensitive 5-ALA ester performed even better by its reduced acute toxicity and higher stability[2].

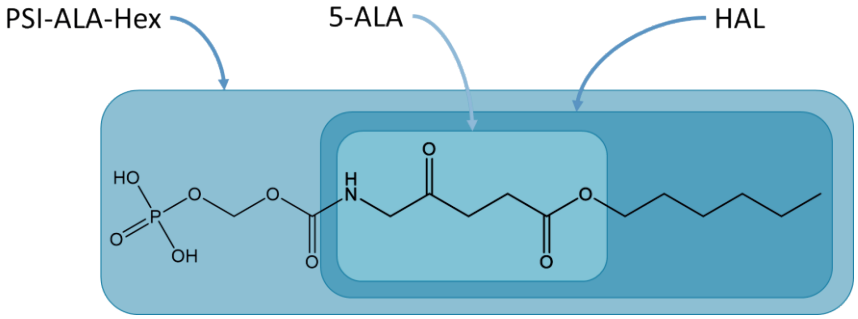


Fig. 1 – 5-Aminolevulinate (5-ALA), 5-ALA hexyl ester (HAL), phosphatase-sensitive prodrug of HAL (PSI-ALA-Hex)

In this study, we investigated *in vitro* PpIX production levels in BC spheroids treated with such 5-ALA derivatives. Four BC cell lines were chosen to represent main BC tumour subtypes: MCF7 (luminal A), BT-474 (luminal B), SKBR3 (HER2+) and MDA-MB-231 (Triple Negative (TN)). Cells were seeded in ultra-low attachment 96-well plates at 5,000 cells/well in 3.5% matrigel and spheroids settled for 4 days. They were subsequently treated with HAL or PSI-ALA-Hex at 0.033 / 0.1 / 0.33 or 1mM. Red PpIX fluorescence level was monitored over 3 days in an Incucyte S3 live-cell imager and analysed with the Incucyte spheroid module.
In MDA-MB-231, PSI-ALA-Hex showed similar PpIX levels but a slightly faster elimination compared to HAL. Fluorescence production was dose-dependent. MCF7 profiles of both molecules were highly similar, showing no dose-dependency. Skbr3 spheroids displayed an unusual profile with a fluorescence production phase in two times. A break was observed at 6h, after which the fluorescence production rate lessend until reaching the peak. The lowest concentrations 0.033 and 0.1 mM performed respectively the same with both molecules. However, PSI-ALA-Hex-induced fluorescence did not compete with HAL at 1mM.
Our results display huge discrepancies from one cell line to another that may be due to differences in enzyme expression of the heme biosynthesis pathway. In order to balance a proper fluorescence intensity for tumour resection and a rapid elimination of the photosensitive compound in most tumour types, an intermediate concentration of 0.1mM is proposed. PSI-ALA-Hex, a stable derivative of the well-studied and already marketed HAL is unveiling a potential for systemic administration and wider tumour detection.

References

1) Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
2) Herceg V, Lange N, et al. Activity of phosphatase-sensitive 5-aminolevulinic acid prodrugs in cancer cell lines. J Photochem Photobiol B. 2017;171:34-42.

Acknowledgements. This work was supported by a grant of the Swiss National Science Foundation.

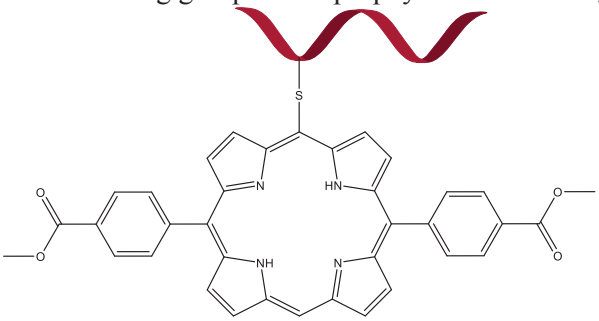
OC-3.4.11

New porphyrin conjugates for the treatment of TNBC.

Miryam Chiara Malacarne[1], Paolo Dognini[2], Enrico Caruso[1], Marzia Bruna Gariboldi[1] and Francesca Giuntini[2].

1) Department of Biotechnology and Life Sciences (DBSV), University of Insubria, Italy; 2) School of Pharmacy and Biomolecular Science, Liverpool John Moores University, United Kingdom.

Triple Negative Breast Cancer (TNBC), an aggressive variant of breast cancer, is characterized by lack of expression of the oestrogen (ER) and progesterone receptors (PRs) and the human epidermal growth factor receptor (EGFR) that are commonly observed in other breast cancer subtypes. Treatment options are limited and for this reason TNBC is an important area of research[1]. The association of photosensitizers (PSs) with peptides has been extensively exploited to improve the performance of porphyrin-based anticancer photosensitizing agents[2]. Specific and successful targeting of a PS to TNBC would allow developing a more selective therapeutic approach based on photodynamic therapy (PDT).
This project aims at generating porphyrin-peptide conjugates targeting TNBC cells via a novel approach relying on the aromatic displacement of a suitable leaving group on the porphyrin with a thiol group present on the peptide.



5,15-bis(4-carbomethoxyphenyl)porphyrin was synthetized via acid-catalyzed mixed condensation. This compound was subsequently modified in the meso positions to insert a nitro group. A dodecapeptide that binds specifically to EGFR was used as a cancer cell targeting peptide[3]. We used two other dodecapeptide sequences provided with one cysteine residue at the N-terminus to obtain the desired conjugate. The conjugation of the peptides to the porphyrins was carried out by aromatic nucleophilic substitution of nitro groups. Thiol groups are excellent nucleophiles, and their reactivity is known to take place under mild conditions compatible with the integrity of the delicate peptide moiety. Before proceeding with the formation of the peptide-porphyrin conjugate, preliminary tests were carried out to evaluate the reactivity of the nitro groups present in the meso position. These tests were performed using N-acetylcysteine. LC-MS analyses have shown the formation of the desired conjugate already after one-hour reaction. This result provides positive information on the subsequent step for the formation of peptide-porphyrin conjugate, a new class of compounds with high potential as PSs and as light-responsive materials.
The effects of the peptide-porphyrin conjugates on cell viability, along with the percentage of apoptotic and necrotic cells, the production of ROS and $^1O_2$ and, the cellular uptake, were evaluated on 4 breast cancer cell lines (MCF-7, T74D and the TNBC lines MDA-MB-231 and MDA-MB-453), following 24h treatment with the PSs, exposure for 2h to a white light irradiation and 24h incubation in drug-free medium.

References

1) C.L. Griffiths, J.L. Olin, Triple Negative Breast Cancer: A Brief Review of its Characteristics and Treatment Options. Journal of Pharmacy Practice, 2012.

2) F. Biscaglia, M. Gobbo, Porphyrin–peptide conjugates in biomedical applications. Peptide Science, 2018.

3) H. Hossein-Nejad-Ariani, E. Althagafi, K. Kaur, Small Peptide Ligands for Targeting EGFR in Triple Negative Breast Cancer Cells. Scientific Reports, 2019.

OC-3.4.12

Interrogating the physical and therapeutic attributes of NIR active molecular targeted photonanomedicines in solid tumors

Girgis Obaid,[1,2] Kimberly Samkoe,[3] Shazia Bano,[2] Kenneth Tichauer,[4] Brian Pogue[3] and Tayyaba Hasan[2,5]

1) Department of Bioengineering, University of Texas at Dallas, Richardson, TX 75080, United States; 2) Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA 02114, United States; 3) Thayer School of Engineering, Dartmouth College, Hanover, NH 03755, United States; 4) Illinois Institute of Technology, Chicago, IL 60616, United States; 5) Division of Health Sciences and Technology, Harvard University and Massachusetts Institute of Technology, Cambridge, MA 02139, United States

Molecular targeted photonanomedicines (mt-PNMs) are receptor-specific nanoplatforms that facilitate photodynamic therapy-based treatment of cancer. Targeting solid tumors with mt-PNMs is particularly complex due to highly variable in vivo pharmacokinetics coupled with significant variations in the functionality of entrapped photosensitizer molecules.[1] This work interrogates the molecular specificity (true receptor recognition) of NIR activable mt-PNMs in EGFR-overexpressing solid tumors using quantitative molecular imaging.[2] Results reveal that molecular specificity of mt-PNMs (as measured by molecular imaging) is distinct from tumor selective uptake, which has traditionally been the conventional approach to predict mt-PNM success (Fig. 1). [1,2] Furthermore, we demonstrate that molecular specificity of mt-PNMs is entirely responsible for photodynamic therapy-based tumor destruction, reversal of desmoplasia in pancreatic tumor xenografts and improvements in treatment tolerability.[3] Emerging multi-specific mt-PNMs also have demonstrate the capacity to address tumor receptor heterogeneity: the Achille's Heel of molecular targeted therapies.[4] The approaches presented here provide a rational, informed tumor biology-driven approach to mt-PNM nanoengineering to increase their chances of ultimately increasing treatment efficacy and tolerability in cancer patients.

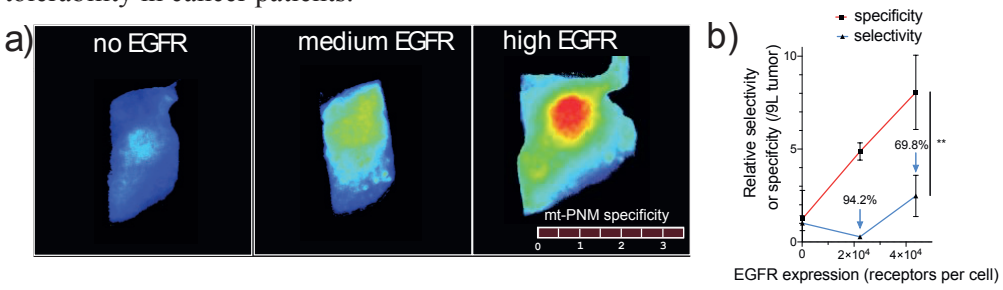


Fig. 1. a) Specificity imaging of EGFR-targeted mt-PNMs in 9L (no EGFR), U87 (medium EGFR) and U251 (high EGFR) tumors. b) traditional measures of selectivity grossly underrepresent EGFR specificity of mt-PNM.

References

1) Bhandari, C., Guirguis, M., Savan, N.A., Shrivastava, N., Oliveira, S., Hasan, T. and Obaid, G. What NIR photodynamic activation offers molecular targeted nanomedicines: Perspectives into the conundrum of tumor specificity and selectivity. Nano Today, 2020, 36, p.101052.

2) Obaid, G.*, Samkoe, K.*, Tichauer, K., Bano, S., Park, Y., Silber, Z., Hodge, S., Callaghan, S., Guirguis, M., Mallidi, S. and Pogue, B. and Hasan, T. Is tumor cell specificity distinct from tumor selectivity in vivo? A quantitative NIR molecular imaging analysis of nanoliposome targeting. Nano Research, 2020, pp.1-11.

3) Obaid, G., Bano, S., Mallidi, S., Broekgaarden, M., Kuriakose, J., Silber, Z., Bulin, A.L., Wang, Y., Mai, Z., Jin, W. and Simeone, D. Impacting Pancreatic Cancer Therapy in Heterotypic in Vitro Organoids and in Vivo Tumors with Specificity-Tuned, NIR-Activable Photoimmunonanoconjugates: Towards Conquering Desmoplasia? Nano Letters, 2019. 19 (11), pp.7573-7587

4) Bano, S.*, Obaid, G.*, Swain, J.W., Yamada, M., Pogue, B.W., Wang, K. and Hasan, T. NIR Photodynamic Destruction of PDAC and HNSCC Nodules Using Triple-Receptor-Targeted Photoimmuno-Nanoconjugates: Targeting Heterogeneity in Cancer. Journal of clinical medicine, 2020, 9(8), p.2390.

Acknowledgements. This work was supported by the National Institutes of Health (K99CA215301 and R00CA215301 to G.O.; R37CA212187 to K.S.; and P01CA084203, R01CA156177, R01CA160998, R21CA220143 to T.H.).

IL-4.1.1

Photochemistry of DNA damages

Virginie Lhiaubet-Vallet
Instituto Universitario Mixto de Tecnología Química (UPV-CSIC), Universitat Politècnica de València, Consejo Superior de Investigaciones Científicas, Avenida de los Naranjos s/n, 46022 Valencia, Spain.

The natural DNA bases dissipate more than 90% of their excitation energy through efficient nonradiative channels leading to the ground state, which explains their high resistance to UV excitation. Nevertheless, small structural changes can result in the lengthening of excited states lifetime and/or in the increase of intersystem crossing efficiency and thus, can modify drastically the photochemical properties of DNA.
In this context, the photochemistry of lesions is of utmost importance as some of them are able to absorb in the UVA-UVB region and behave as a potential intrinsic photosensitizer. Here, we will discuss the photophysical and photochemical properties of damages such as the (6-4) photoproducts[1-3] or 5-formylpyrimidine derivatives[5-6] (see Fig. 1) to evaluate if their fulfil the basic requirements of a good DNA photosensitizer: (i) to absorb in the UVA-UVB region, (ii) to populate efficiently their triplet excited state and (iii) to be able to interact with DNA components through a Type I or II process and/or a triplet-triplet energy transfer.

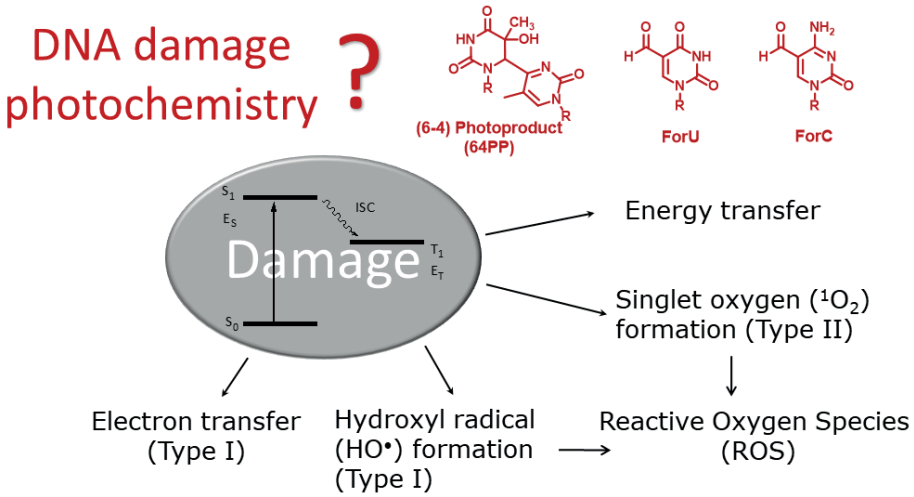


Fig. 1 – Structures of the photolesions considered for their DNA photosensitizing effects.

References

1) V. Vendrell-Criado, G. M. Rodríguez-Muñiz, V. Lhiaubet-Vallet, M. C. Cuquerella, M. A. Miranda, The (6–4) dimeric las a DNA photosensitizer. ChemPhysChem, 17, 1979-1982, 2016.
2) V. Vendrell-Criado, G. M. Rodriguez-Muniz, M. C. Cuquerella, V. Lhiaubet-Vallet, M. A. Miranda, Photosensitization of DNA by 5-methyl-2-pyrimidone deoxyribonucleoside: (6-4) photoproduct as a possible Trojan horse. Angew. Chem. Int. Ed., 52, 6476–6479, 2013.
3) I. Aparici-Espert, G. Garcia-Lainez, I. Andreu, M. A. Miranda, V. Lhiaubet-Vallet, Oxidatively generated lesions as internal photosensitizers for pyrimidine dimerization in DNA. ACS Chem. Biol. 13, 542–547, 2018.
4) A. Frances-Monerris, C. Hognon, M. A. Miranda, V. Lhiaubet-Vallet, A. Monari, Triplet photosensitization mechanism of thymine by an oxidized nucleobase: from a dimeric model to DNA environment. Phys. Chem. Chem. Phys. 20, 25666–25675, 2018.
5) A. Francés-Monerris, M. Lineros-Rosa, M. A. Miranda, V. Lhiaubet-Vallet, A. Monari, Photoinduced intersystem crossing in DNA oxidative lesions and epigenetic intermediates. Chem. Commun., 56, 4404–4407, 2020.
6) M. Lineros-Rosa, A. Frances-Monerris, A. Monari, M. A. Miranda, M.A.; Lhiaubet-Vallet, Experimental and theoretical studies on thymine photodimerization mediated by oxidatively generated DNA lesions and epigenetic intermediates. Phys. Chem. Chem. Phys. 22, 25661–25668, 2020.

Acknowledgements. This work was supported by the Spanish government (project PGC2018-096684-B-I00).

IL-4.1.2

DNA damage and molecular modelling: Mechanistic aspects revealed by multiscale simulations

Antonio Francés-Monerris[1]
1) Universitat de València, Departament de Química Física, 46100 Burjassot (València), Spain

DNA damage is a fundamental process of interest in chemistry, biology, and medicine. Among the different agents that can harm DNA/RNA, light represents a powerful aggressor that delivers the damage either by populating reactive excited states localized in the nucleic acids or by producing reactive oxygen species (ROS) in the surroundings that ultimately oxidize the DNA structure. Apart from the fundamental interest of DNA damage as the molecular basis of diseases such as skin cancer, and its role in the understanding of the natural DNA photostability, these processes also represent an opportunity of treatment. In this context, photodynamic therapy (PDT) is a technique that uses light to selectively kill ill tissues, in most cases by inducing DNA damage mechanisms activated by irradiation.

The scientific community uses a plethora of experimental techniques to study DNA damage. The underlying processes have a wide variety of spatial and temporal scales: from the very first events that take place after light absorption, tracked with ultrafast transient spectroscopy, to the quantification of cellular damage, studied with cellular assays, the scientific community is progressively revealing DNA damage mechanisms in greater detail. In this scenario, molecular modelling offers sophisticated methodologies that allow the full description of DNA damage events from chemical, photochemical, and photobiological perspectives. In the present talk, I will highlight recent advances in classical molecular dynamics (MD), quantum-mechanical (QM) methods and hybrid QM/MM schemes that have opened the door to unprecedented descriptions of DNA damage and repair.[1–3] I will illustrate the advance of the field with recent examples, including the discovery of multiple inter-strand proton transfers induced by light,[4] the formation of photoinduced DNA lesions in bacteria,[5] the phototoxic potential of oxidized DNA nucleobases[6–8] and the mode of action of non-conventional PDT agents.[9]

References

(1) Francés-Monerris, A.; Gillet, N.; Dumont, E.; Monari, A. DNA Photodamage and Repair: Computational Photobiology in Action. In *QM/MM Studies of Light-responsive Biological Systems*; Springer, 2021; pp 293–332.
(2) Francés-Monerris, A.; Marazzi, M.; Besancenot, V.; Grandemange, S.; Assfeld, X.; Monari, A. Computational Spectroscopy and Photophysics in Complex Biological Systems: Towards an In Silico Photobiology. In *Computational Techniques for Analytical Chemistry and Bioanalysis*; The Royal Society of Chemistry, 2021; pp 202–246.
(3) Roca-Sanjuán, D.; Carmona-García, J.; Ding, B.-W.; Navarrete-Miguel, M.; Francés-Monerris, A.; Giussani, A.; Segarra-Martí, J.; Monari, A.; Liu, Y.-J. Photochemistry, Chemiluminescence and Dark Photochemistry: Computational Advances (2018–2019). In *Photochemistry: Volume 48*; The Royal Society of Chemistry, 2021; Vol. 48, pp 24–70.
(4) Francés-Monerris, A.; Gattuso, H.; Roca-Sanjuán, D.; Tuñón, I.; Marazzi, M.; Dumont, E.; Monari, A. Dynamics of the Excited-State Hydrogen Transfer in a (DG)·(DC) Homopolymer: Intrinsic Photostability of DNA. Chem. Sci. 9, 7902–7911, 2018.
(5) Francés-Monerris, A.; Hognon, C.; Douki, T.; Monari, A. Photoinduced DNA Lesions in Dormant Bacteria. The Peculiar Route Leading to Spore Photoproduct Characterized by Multiscale Molecular Dynamics. Chem. Eur. J. 26, 14236–14241, 2020.
(6) Francés-Monerris, A.; Hognon, C.; Miranda, M. A.; Lhiaubet-Vallet, V.; Monari, A. Triplet Photosensitization Mechanism of Thymine by an Oxidized Nucleobase: From a Dimeric Model to DNA Environment. Phys. Chem. Chem. Phys. 20 (40), 25666–25675, 2018.
(7) Francés-Monerris, A.; Lineros-Rosa, M.; Miranda, M. A. A.; Lhiaubet-Vallet, V.; Monari, A. Photoinduced Intersystem Crossing in DNA Oxidative Lesions and Epigenetic Intermediates. Chem. Commun. 56, 4404–4407, 2020.
(8) Lineros-Rosa, M.; Francés-Monerris, A.; Monari, A.; Miranda, M. A.; Lhiaubet-Vallet, V. Experimental and Theoretical Studies on Thymine Photodimerization Mediated by Oxidatively Generated DNA Lesions and Epigenetic Intermediates. Phys. Chem. Chem. Phys. 22, 25661-25668, 2020.
(9) Francés-Monerris, A.; Tuñón, I.; Monari, A. Hypoxia-Selective Dissociation Mechanism of a Nitroimidazole Nucleoside in a DNA Environment. J. Phys. Chem. Lett. 10, 6750–6754, 2019.

Acknowledgements. This work was supported by the postdoctoral contract APOSTD/2019/149 and the project GV/2020/226 from the *Generalitat Valenciana* and the European Social Fund.

IL-4.1.3

Photoactivated behavior of Guanine-rich DNA Quadruple Helices

James Green[1], Haritha Asha[1], Lara Martinez-Fernandez[2], Dimitra Markovitsi,[3] Roberto Improta[1]

1) Istituto Biostrutture e Bioimmagini- Consiglio Nazionale delle Ricerche, Napoli, Italy 2) Departamento de Química,Universidad Autónoma de Madrid, Madrid, Spain 3) LIDYL, CEA, CNRS, Université Paris-Saclay, France

Guanine-rich DNA sequences can adopt a peculiar fold, a Quadruple helix, (G4) where four Guanine (G) bases are arranged in planar structures, the tetrads, stabilized by Hoogsteen-type hydrogen bonds,[1] involved in key cellular processes and have emerged as promising therapeutic targets. G4 exhibits peculiar absorption, emission and circular dichroism spectra,[2] and we show that Quantum Mechanical calculations can provide spectra in fair agreement with the experiments and crucial information for their assignment and interpretation.[3-7] Both the static and dynamical properties of G4 excited states are ruled by their topology. In general, after light absorption from excited states delocalized over multiple bases, the most important decay pathways involve localization of the excitation over a single base or on two stacked guanines, excimers with different degrees of charge transfer character. The two main photochemical reactions involve the photodimerization of two stacked guanine bases on the 'neutral' excimer path and the ionization of guanine.[4,6] Finally, we shall describe a general and flexible approach, based on fragment diabatization, which incorporates charge transfer states and significantly increases the reliability of excitonic Hamiltonians for systems where the chromophores are very close. This model (FrDEx) is used to compute the electronic circular dichroism and absorption spectra of several prototype G4, allowing significant improvements with respect to "standard" excitonic Hamiltonians and giving access to interesting insights into the chemical–physical effects modulating the spectral signals.

References

1) R. Hänsel-Hertsch et al Nature Rev. Mol. Cell Biol. 2017, 18, 279.
2) P. Changenet-Barret, et al. Electronic Excitations in Guanine Quadruplexes in Photoinduced Phenomena in Nucleic Acids II: Springer International Publishing: Cham, 2015; 183.
3) R. Improta Chem. Eur. J. 2014, 20, 8106.
4) A. Banyasz,et al. J.Am. Chem. Soc 2017, 139, 10561.
5) L. Martínez-Fernández et al. Chem. Eur. J. 2018, 24, 15185.
6) L Martinez-Fernandez, et al J. Phys. Chem. Lett. 2019, 10, 6873
7) L. Martínez-Fernández, et al Photochem. Photobiol. Sci. 2020, 19, 436-444
8) J. Green, et al. J. Chem. Theory Comput. 2021, 17, 1, 405.

Acknowledgements. This work has received funding from the European Union\'s Horizon 2020 research and innovation programme MSCA-ITN under grant agreement No. 765266 (LightDyNAmics)

OC-4.1.4

Transfection of keratinocytes with in vitro synthesized CPD-specific photolyase-encoding mRNA is a model system to study the CPD-dependent cellular effects of UVB

Csaba Hegedűs[1], Eszter Fidrus[1], Gábor Boros[2], Eszter Anna Janka[1], Gabriella Emri[1], Katalin Karikó[2], Éva Remenyik[1]

1. *University of Debrecen, Department of Dermatology, Debrecen, Hungary*
2. *BioNTech, Mainz, Germany*

Cyclobutane pyrimidine dimer (CPD)-specific photolyase removes UVB-induced CPDs from DNA using the energy of visible light in a process called photoreactivation. However, this repair mechanism disappeared from placental mammals during evolution. Previously we established a model system based on transient transfection of human keratinocytes with codon-optimized *in vitro* transcribed mRNA containing 1-methylpseudouridine modifications that encodes CPD-specific photolyase (CPD-PL mRNA)[1]. The RNA composition is similar to that used for BioNTech COVID-19 vaccine. Following UVB irradiation HaCaT and human primary keratinocytes were either kept in the dark or exposed to photoreactivating light. Different cellular events were studied in a time-dependent manner.

Our results show that keratinocytes express functional photolyase upon transfection of CPD-PL mRNA. The encoded protein starts to accumulate in the nucleus 5-8 hours post-transfection. CPDs were effectively removed by photoreactivation immediately as well as 6 hour after CPD-PL mRNA transfection resulted in relieving the negative effects of UVB on cell viability. Activation of photolyase prevented the loss of cell proliferation and G2/M cell cycle block after UVB irradiation. The functional photolyase also diminished other UVB-mediated effects, including enhanced IL-6 mRNA expression. Microarray analyses demonstrated that gene expression pattern is altered by UVB induced CPDs. Using our model system, it has been demonstrated that CPDs are responsible for production of mitochondrial reactive oxygen species followed by the activation of several energy sensor enzymes, and compensatory metabolic changes in keratinocytes exposed to UVB. Interestingly, CPDs could be removed not only from nuclear genome but from mitochondrial genome as well and restored mitochondrial DNA copy number suggesting that damage to mitochondria can also be prevented by photolyase activation. Finally, UVB-induced mutagenesis was completely abrogated by photoreactivation emphasizing the key role of CPDs in mediating DNA damage and carcinogenesis.

These results suggest that activation of a non-human photolyase encoded by nucleoside-modified mRNA is able to prevent the UVB-induced cellular damages and introduction of photolyase mRNA into the skin may have a future therapeutic potential.

References

1) Boros G, Miko E, Muramatsu H, Weissman D, Emri E, Rózsa D, Nagy G, Juhász A, Juhász I, van der Horst G, Horkay I, Remenyik É, Karikó K, Emri G. Transfection of pseudouridine-modified mRNA encoding CPD-photolyase leads to repair of DNA damage in human keratinocytes: a new approach with future therapeutic potential. J Photochem Photobiol B. 2013 Dec 5;129:93-9.

Acknowledgements. This work was supported by the European Union and the European Regional Development Fund GINOP-2.3.2-15-2016-00005 and Hungarian Scientific Research Fund NKFIH K120206.

IL-4.1.5

The triplet state as the precursor of the thietane intermediate in the formation of the DNA 6-4 photoadduct

Carlos E. Crespo-Hernández,
Case Western Reserve University, Department of Chemistry, Cleveland, Ohio, 44106, USA

Notwithstanding the central biological role of the (6-4) photoadduct in the induction of skin cancer by sunlight, crucial mechanistic details about its formation have evaded characterization despite efforts spanning more than half a century[1]. 4-Thiothymidine (4tT) has been widely used as an important model system to study its mechanism of formation[2], but the excited-state precursor, the intermediate species, and the time scale leading to the formation of the (6-4) photoadduct have remained elusive. In this talk, I will present experimental and computational evidence elucidating the excited state leading to the formation of the thietane intermediate, its rate, and the formation of the (6-4) photoadduct in the 5'-TT(4tT)T(4tT)TT-3' single-stranded DNA oligonucleotide. It will be demonstrated that efficient, sub-1 ps intersystem crossing leads to the population of a triplet minimum of the thietane intermediate in as short as 3 ps, which intersystem crosses to its ground state and rearranges to form the (6-4) photoadduct[1].

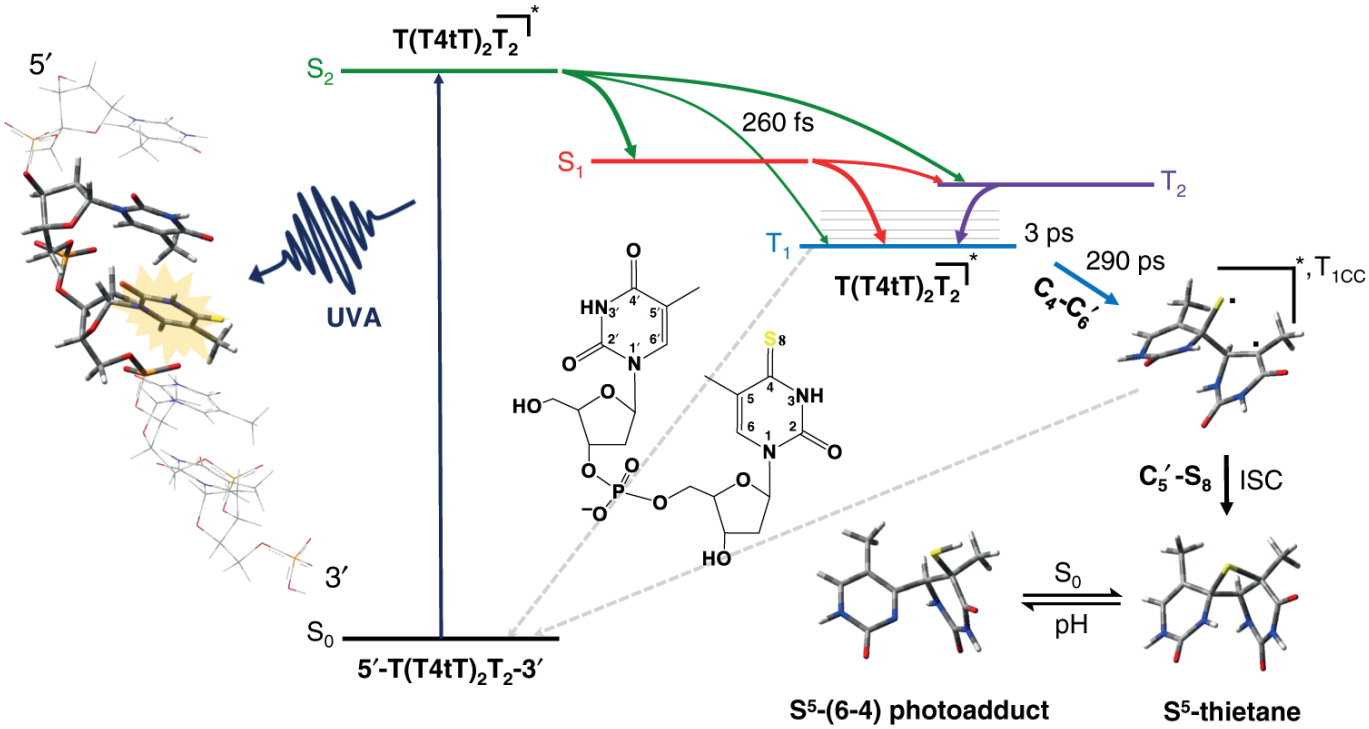


Fig. 1 – The colored arrows represent electronic transitions. A 4tT base is highlighted with light orange in the single-stranded DNA sequence depicted to the left to imply that 4tT bases are selectively photoactivated by ultraviolet-A radiation. 4tT and the thymidine 5' relative to the 4tT are represented as tube in the single-stranded DNA sequence, while the other nucleobases are depicted as wireframe to highlight the importance of having a thymine 5' relative to the 4tT for the reaction to occur. In the chemical structures, yellow, red, blue, gray, and white, represent sulfur, oxygen, nitrogen, carbon, and hydrogen, respectively.

References

1) Ortiz-Rodríguez, L. A.; Reichardt, C.; Hoehn, S. J.; Jockusch, S.; Crespo-Hernández, C. E., Detection of the thietane precursor in the UVA formation of the DNA 6-4 photoadduct, *Nature Commun.* **11**, 3599 (2020).
2) Clivio, P., Fourrey, J. L., Gasche, J. & Favre, A. DNA photodamage mechanistic studies: characterization of a thietane intermediate in a model reaction relevant to "6-4 lesions". *J. Am. Chem. Soc.* **113**, 5481–5483 (1991).
3) Liu, J. & Taylor, J.-S. Remarkable photoreversal of a thio analog of the Dewar valence isomer of the (6-4) photoproduct of DNA to the parent nucleotides. *J. Am. Chem. Soc.* **118**, 3287–3288 (1996).

4) Warren, M. A., Murray, J. B. & Connolly, B. A. Synthesis and characterization of oligodeoxynucleotides containing thio analogues of (6-4) pyrimidine-pyrimidinone photo-dimers. *J. Mol. Biol.* **279**, 89–100 (1998).
5) Reelfs, O. et al. Identification of potentially cytotoxic lesions induced by UVA photoactivation of DNA 4-thiothymidine in human cells. *Nucleic Acids Res.* **39**, 9620 (2011).

Acknowledgements. The authors acknowledge the National Science Foundation (Grant No. CHE-1800052). This work made use of the High-Performance Computing Resource in the Core Facility for Advanced Research Computing at CWRU.

IL-4.1.6

In silico insight on the photophysics of non-canonical nucleobases. Implications in DNA photostability and damage

Inés Corral[1]
1) Universidad Autónoma de Madrid, Departamento de Química, Cantoblanco, Madrid, Spain

Unnatural nucleobases, or heterocycles structurally differing from their five natural counterparts in different extents, have captured the interest of the scientific community in recent years for their potential implications as prebiotic ancestors of the current genetic letters[1] or in biological or biotechnological applications, by conceding chemical and structural diversity to DNA and RNA strands.[2]
Albeit minor, these structural modifications can dramatically condition the intra-, inter-, and macromolecular structure or the photophysics of the genetic polymers. Thus, the self-assembly ability, and the chemical and light stability of these systems can be considered as key selection pressures determining their viability in contemporary experimental and primordial biopolymers.

The aim of this contribution is to provide an overview of the main photophysical properties of a selected group of nucleobase derivatives, based on state-of-the-art quantum mechanical and molecular dynamics simulations including full dimensionality. In particular, the impact in the optical properties and topographical features of the potential energy landscapes of the position and the number of oxo and amino substituents, or of the replacement of carbonyl by thiocarbonyl functions[3] along the purine and pyrimidine cores will be discussed.

These results will help establishing the functionalization-photoactivity relationships in purine[4] and pyrimidine[5] cores, and will contribute to determining the electronic and structural factors that established the superiority of the five DNA and RNA nucleobases against other organic heterocycles and to the rational design of unnatural biopolymers allowing the coding flexibility necessary to synthetize new proteins.

References
1) A. C. Rios and Y. Tor, Israel Journal of Chemistry, 53, 469, 2013.
2) "Expansion of the Genetic Alphabet: Unnatural Nucleobases and Their Applications", Special Issue, Journal of Nucleic Acids, 2012.
3) S. Arslancan, L. Martínez-Fernández, I. Corral, Molecules, 22, 998, 2017.
4) C. E. Crespo-Hernández, L. Martínez-Fernández, C. Rauer, C. Reichardt, S. Mai, M. Pollum, P. Marquetand, L. González, I. Corral, The Journal of the American Chemical Society, 137, 4368, 2015.
5) E. M. Arpa, M. M. Brister, S. J. Hoehn, C. E. Crespo-Hernández, I. Corral, The Journal of Physical Chemistry Letters, 11, 5156, 2020.

Acknowledgements. This work has been supported by the Ramón y Cajal program of the Ministerio de Economía Industria y Competitividad of Spain, and the PGC2018-094644-B-C21 project and FPU and FPI grants from the Ministerio de Ciencia, Innovación y Universidades of Spain. The Red Española de Supercomputación, and the Centro de Computación Científica of the UAM (CCC-UAM) are also acknowledged for the generous allocation of computer time and for their continued technical support.

OC-4.1.7

Photoinduced azetidine decomposition reaction by photo-oxidation and photo-reduction: Inverting the aza-Paternò-Büchi reaction

Miriam Navarrete Miguel[1], Antonio Francés-Monerris[2], Daniel Roca-Sanjuán[1]
1) Instituto de Ciencia Molecular, Universitat de València, P.O. Box 22085, ES-46071 València, Spain; 2) Departamento de Química Física, Universitat de València, 46100 València, Spain

DNA in living beings is constantly damaged by both exogenous and endogenous agents, such as UV radiation. The photoinduced DNA lesions can be initiated by Paternò–Büchi, or aza-Paternò-Büchi, photocycloaddition between two adjacent pyrimidine bases, forming an oxetane, or an azetidine ring.
Then, the repair mechanism of these lesions can be represented with the inversion of the Paternò-Büchi reaction. Some lesions can be repaired by photolyases also in presence of light and a photosensitizer via a photoprocess whose molecular mechanism is not completely understood yet. In this contribution, we have focused on the aza-version of the Paternò-Büchi reaction. Then, two azabipyrimidinic azetidines have been studied as model compounds of the elusive azetidine intermediates present during the (6-4) photoproducts repair. The first one is obtained by photocycloaddition between 6-azauracil and thymine (AZT-Thy), and the second one arises from the photocycloaddition between 6-azauracil and cyclohexene (AZT-CH). DFT calculations have allowed to interpret the experimental observations. In addition, they also help us to elucidate the ring-opening mechanisms during photoreduction and photo-oxidation processes. On the one hand, regarding the AZT-Thy model, energy barriers lower than 13 kcal mol^{-1} are obtained for both the photoreduction and photo-oxidation processes[1]. On the other hand, regarding the AZT-CH system, energy barriers lower than 14 and 9 kcal mol^{-1} for the *cis*- and *trans*- isomers are obtained for photoreduction, while values of about 36 kcal mol^{-1} are obtained for both isomers in the photo-oxidation process[2].

References

1) Ana B. Fraga-Timiraos, Antonio Francés-Monerris, Gemma M. Rodríguez-Muñiz, Miriam Navarrete-Miguel, Miguel Ángel Miranda, Daniel Roca-Sanjuán, Virginie Lhiaubet-Vallet, *Chem. - A Eur. J.* **2018**, *24*, 15346–15354.
2) Miriam Navarrete-Miguel, Antonio Francés-Monerris, Miguel Ángel Miranda, Virginie Lhiaubet-Vallet, Daniel Roca-Sanjuán, *Molecules* **2021**, *26*, 2911.

Acknowledgements. This work was supported by the D. R.-S. "Ramon y Cajal" grant from the Spanish "Ministerio de Ciencia e Innovación (MICINN)" (ref. RYC-2015-19234). A.F.-M. is grateful to the Région Grand Est government (France) and the Université de Lorraine for their financial support. This work has also been funded by the Generalitat Valenciana and the European Social Fund through the postdoctoral contract APOSTD/2019/149 and the project GV/2020/226. It also was funded by the MICINN project CTQ2017-87054-C2-2-P, and a 2019 Leonardo Grant for Researchers and Cultural Creators, BBVA Foundation. M.N.-M. acknowledges the Generalitat Valenciana for the predoctoral grant (Ref. ACIF/2020/075). Calculations were performed on the local QCEXVAL cluster of the University of Valencia and on the LluisVives and Tirant III clusters of the Servei d'Informàtica (University of Valencia).

OC-4.1.8

An insight into etheno adducts optical properties.

Paloma Lizondo-Aranda[1], Thomas Gustavsson[2], Miguel Ángel Miranda[1], Virginie Lhiaubet-Vallet[1].
1) Instituto Universitario Mixto de Tecnología Química (UPV-CSIC), Universitat Politècnica de València, Avda de los Naranjos, s/n., 46022, Valencia, Spain.; 2) Laboratoire Interactions, Dynamiques et Lasers (LIDYL) - CEA/Saclay, 91191 Gif-sur-Yvette, France.

The study of DNA damages has increased during the last decades in order to get more insight into their involvement in the appearance of cancer.[1] Among the large number of DNA lesions, etheno adducts have been the matter of interest because of their presence in chronically inflamed human tissues, making their quantification useful as potential biomarkers for cancer of colon, prostate, lung, etc. Moreover, these lesions exhibit highly mutagenic properties and induce base transitions or transversion in mammal cells.[2] Etheno adducts are mainly formed endogenously as a result of lipid peroxidation. This biochemical process produces reactive aldehydes such as malondialdehyde (MDA), which can combine with DNA bases creating the exocyclic ring.

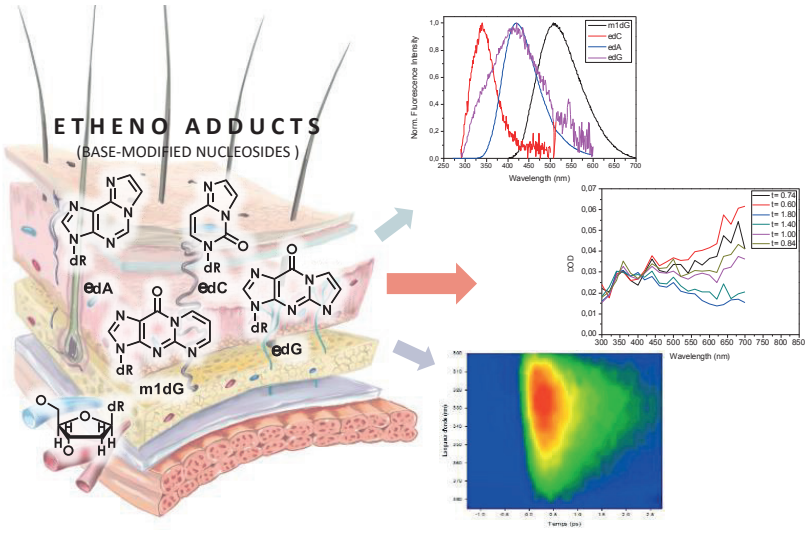


Figure 1. Etheno adduct structures and some spectra from the present work.

Photochemically, the etheno derivatives are of great interest since they present an extended π- conjugated system that might confer them optical properties different from those of the canonical bases and open the way to a particular photoreactivity of the damaged DNA. This work presents a complete spectroscopic study of the etheno damages combining steady-state and time-resolved fluorescence experiments together with transient absorption studies. The obtained results demonstrate an ultrafast deactivation of their excited states, being however somewhat slower than that of their respective canonical base.

References

1) J. Kaya, E. Thadhania, L. Samsona, B. Engelwarda, Inflammation-induced DNA damage, mutations and cancer. DNA repair, 2019.
2) B. Hwa Yun, J. Guo, M. Bellamri, R.J. Turesky, DNA adducts: Formation, biological effects, and new biospecimens for mass spectrometric measurements in humans. Mass spectrometry reviews, 2018.

Acknowledgements. This work was supported by Spanish Government (PGC2018-096684-B-I00 project, grant BES-2016-077517)

SP-4.1.9, P-4.1.9

Risk assessment of irradiating skin models at 233 nm using far-UVC LEDs for eradiacation of MRSA and MSSA

Johannes Schleusener[1], Paula Zwicker[2], Silke B. Lohan[1], Loris Busch[1,3], Claudia Sicher[2], Sven Einfeldt[4], Michael Kneissl[4,5], Anja A. Kühl[6], Cornelia M. Keck[3], Christian Witzel[7], Axel Kramer[2] and Martina C. Meinke[1]
1) Charité – Universitätsmedizin Berlin, Department of Dermatology, Venerology and Allergology, Berlin, Germany; 2) University Medicine Greifswald, Institute of Hygiene and Environmental Medicine, Greifswald, Germany; 3) Philipps-Universität Marburg, Department of Pharmaceutics and Biopharmaceutics, Marburg, Germany; 4) Ferdinand-Braun-Institut gGmbH, Leibniz-Institut für Höchstfrequenztechnik, Berlin, Germany; 5) Technische Universität Berlin, Institute of Solid State Physics, Berlin, Germany; 6) Charité – Universitätsmedizin Berlin, iPATH.Berlin-Immunopathology for Experimental Models, Berlin, Germany; 7) Charité – Universitätsmedizin Berlin, Division of Plastic and Reconstructive Surgery, Berlin, Germany

Surgical Site Infections are mainly caused by the colonization of MRSA and MSSA and result in increased levels of surgical morbidity and mortality. The eradication of these resistant pathogens is a continuing clinical problem, as they cannot be reached by antiseptics in the depth of the skin.
UVC irradiation has been considered as a possible inactivation strategy in order to prevent infections during surgery. Broad band UV sources with emission wavelengths between 200 and 400 nm are effectively bactericidal [1]. However, they can cannot be used in vivo, as they cause DNA damage in the skin. Recently, far-UVC-lamps at 222 nm could successfully be used for inactivation of bacteria. Due to the strong absorption of wavelengths below 240 nm, the radiation is mostly absorbed in the stratum corneum and does not reach the viable epidermis. Therefore, DNA damage can effectively be eliminated.
Here, we present a study using a newly developed 233 nm far-UVC LED source [2] for the eradication of MRSA and MSSA. Cell viability, DNA damage and radical production were assessed on different excised human skin and reconstructed human epidermis skin models in comparison to irradiation by conventional near-UVC (254 nm), UVB (280–400 nm, positive control) and a conventional far-UVC radiation source at 222 nm.
At an effective bactericidal dose of 40 mJ/cm², where bacteria were reduced by 5 log10 levels, the investigated skin models showed no reduction in cell viability. The resulting DNA damage, which only occurred in the superficial layer of the viable epidermis was below those caused by 0.1 minimal erythema dose (MED) UVB, which is regarded safe. This low damage disappeared after 24 h and irradiation on four consecutive days showed no epidermal cells with DNA damage at this dose. We found a low radical formation load, which was far lower than for a dose equivalent to 20 min outdoor visible light, which can be compensated by the antioxidant defence system.

References
1) A. Kramer, M. Meinke, A. Patzelt, M.B. Stolpe, J. Lademann, K. Jacobs, Perspektiven physikalischer Verfahren der Antiseptik und Desinfektion, Hygiene und Medizin, 45;3/2020 D39.
2) M. C. Meinke, A Kramer, S Einfeldt, M. Kneissl, Krankenhauskeime mit UVC-Leuchtdioden bekämpfen, Management und Krankenhaus 39; 9/2020, S.20

Acknowledgements. The work is funded by the German Federal Ministry of Education and Research BMBF (FKZ 03ZZ0146B) within the program “Zwanzig20 – Partnerschaft für Innovation” and carried out within the consortium Advanced UV for Life

SP-4.1.10, P-4.1.10

Photoprotection from UV light-induced telomere shortening and DNA damage by a broad-spectrum sunscreen product

Ana Guío-Carrión[1], Almudena Martín[2], José Aguilera[3], María Matabuena[2], Diego Megias[4], Anne Laure Demessant-Flavigny[5], Isabel Castillejo[6], Françoise Bernerd[7], Leonor Prieto[6], Maria A. Blasco[1],*
1)Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre (CNIO), Madrid, Spain; 2) Invitrotecnia Laboratory S.L., Alcobendas, Madrid, Spain; 3) Department of Medicine and Dermatology, Faculty of Medicine, University of Málaga, Spain; 4) Microscopy Unit, Biotechnology Program, Spanish National Cancer Research Centre (CNIO), Madrid, Spain; 5) International Scientific Direction, Laboratoire Dermatologique La Roche-Posay, France; 6) Scientific Direction, Laboratoire Dermatologique La Roche-Posay, L'ORÉAL Cosmética Activa, Madrid, Spain; 7)L'ORÉAL Research & Innovation, France

UV light is considered a class I carcinogen; it produces DNA damage, leading to photoaging, and in some cases carcinogenesis. Upon UV-light exposure, the formation of photoproducts and reactive oxygen species highly toxic for the cell induces the activation of DNA damage response (DDR) pathways that alter the metabolism of the cell, ultimately inducing apoptosis or senescence. Telomeres are tandem repeats of the hexamer 5´-TTAGGG that cap and protect the end of chromosomes. Telomeres are a prime target for UV-light induced damage due to their sequence highly enriched in pyrimidines. Their length shortens upon UV irradiation *in-vitro*, compromising the regenerative capacity and function of tissues, and telomeres in sun-exposed skin areas are shorter compared to telomeres in nonexposed ones. Given the importance of telomere preservation in genome stability, we aimed to study whether a broad-spectrum sunscreen product prevents UV-induced DNA damage and telomere shortening.

We used a solar simulator lamp to provide 10J/cm2 SSUV to *in-vitro* cultured human keratinocytes and fibroblasts and to a 3D skin model, and compared at 24h upon exposure among three conditions – nonirradiated control, irradiated, and irradiated in the presence of a broad-spectrum sunscreen product, with a very high UVB and UVA protection (SPF 50+ / PF UVA 46). We performed HT Q-FISH or FISH on skin slides to measure telomere length, gH2AX immunofluorescence as a readout for DNA damage, DAPI nuclei staining for cell viability, and hematoxylin & eosin staining of skin sections to examine skin structure.

Upon SSUV exposure, *in-vitro* cultured human keratinocytes and fibroblasts suffer from UV-induced damage, as seen by reduced cell viability (41.6% and 79% respectively) and increased gH2AX and 53BP1, whereas, in the presence of the sunscreen product, cell viability, gH2AX, and 53BP1 are comparable to controls. Significantly, telomeres are shortened upon UV-light (26.8% and 13.5% of telomeric length loss respectively), while in the presence of sunscreen the telomere length is partially preserved (shortening rate of 13.7% and 8.3% respectively) and the % of critically short telomeres is reduced. On the 3D skin model, SSUV exposure disrupts the skin structure with loss of skin layers stratification, loss of spatial orientation of basal keratinocytes, presence of immature cells and apoptosis in the upper stratum of skin, and overall cellularity reduced to 77.4%. Exposure to SSUV in the presence of sunscreen shows H&E staining and cellularity similar to controls. Importantly, there is a dramatic increase in gH2AX positive cells in UV-exposed skin that is completely prevented in the presence of sunscreen. Strikingly, telomeres of cells in the 3D skin environment, as opposed to isolated cells in *in-vitro* cultures, show similar telomeric length to control, and this length is even increased when exposure occurs in the presence of the broad-spectrum sunscreen product, together with a decrease in the % of short telomeres.

In conclusion, the broad-spectrum sunscreen product with a very high UVB and UVA protection (SPF 50+ / PF UVA 46) preserves cell viability, reduces DNA damage in UV-exposed *in-vitro* cells or 3D skin model, and protects the integrity of the stratified structure of the skin, crucial for its barrier function and natural protection against UV. Importantly, this photoprotection also occurs at the telomere level since UV-induced telomere shortening is greatly reduced in the presence of the sunscreen in *in-vitro* cultures. Furthermore, telomere length is even increased in SSUV exposed 3D skin in the presence of sunscreen. These findings help in the understanding of the mechanisms of photoprotection by a sunscreen product against UV-induced damage and are relevant in the fields of photobiology and skin carcinogenesis.

Acknowledgements. This work was supported by grant from La Roche Posay

P-4.1.11

Molecular Basis of Dark Photochemistry

Juliana Cuéllar-Zuquin,[1] Fernand Louisnard,[1] Angelo Giussani[1] and Daniel Roca-Sanjuán[1]

1) Instituto de Ciencia Molecular, Universitat de Valencia, Valencia, Spain.

Dark photochemistry is responsible for the production of melanomas when there is no UV radiation, occurring the process via chemiexcitation. This chemiexcitation is originated by a dioxetane derivate of melanin, which induces the formation of lesions in the DNA pyrimidine bases[1]. In this work, we have carried out a theoretical study to determine the molecular basis of the mentioned process.

Firstly, we have employed the 1,2-dioxetane molecule as a model of the dioxetane derivate of melanin, and an ethylene dimer as a model of two stacked pyrimidine nucleobases. With these models, we have studied the mechanism of the decomposition of dioxetane, which goes through a diradical intermediate that allows to populate the triplet excited state of the formaldehyde[2]. We have also studied the mechanism of [2+2] cycloaddition of ethylene on the triplet manifold. In this case, the triplet excited state evolves up to a diradical intermediate, where the ground and excited state cross. Here, an inversion of multiplicity takes place and the systems evolves to the formation of the cyclobutane.

Secondly, we have carried out an energetic study to verify if the cycloaddition reaction induced by energy transfer from the chemiexcited dioxetane is plausible. In the case of the models, we have obtained that the process cannot take place, but in the case of the real molecules (melanin dioxetane and two stacked thymine nucleobases) the process is favourable, thus allowing to explain the phenomenon of melanoma produced via dark photochemistry.

At last, we have studied different factors which can be involved in the process such as the ionization potential of the nucleobases, the formation of hydrogen bonds and the presence or absence of π-stacking interactions. All of the above studies had been carried on over different dimers of the dioxetane derivative of the melanin and the nucleobases.

References

1) Premi, S.; Wallisch, S. Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science* **2015**.
2) Farahani, P.; Roca-Sanjuán, D. Revisiting the nonadiabatic process in 1,2-dioxetane. *J. Chem. Theory Comput*. **2013**.

P-4.1.12

Developing a tool for capturing genomic regions of high-density skin clonal mutations

Megan E Fitzgerald[1,2], Sydney Grant[1,2], Andrew D Patentreger[1,2], Nico Shary[2,3], Allison K McCarthy[1,2], Barbara A Foster[4], Lei Wei[5], Wendy J Huss[1,2], Gyorgy Paragh[1,2]

1) Roswell Park Comprehensive Cancer Center, Department of Cell Stress Biology, Buffalo, NY, USA; 2) Roswell Park Comprehensive Cancer Center, Department of Dermatology, Buffalo, NY, USA; 3) University at Buffalo, Jacobs School of Medicine and Biological Sciences, Buffalo, NY, USA; 4) Roswell Park Comprehensive Cancer Center, Department of Pharmacology and Therapeutics, Buffalo, NY, USA; 5) Roswell Park Comprehensive Cancer Center, Department of Biostatistics and Bioinformatics, Buffalo, NY, USA

Nonmelanoma skin cancers (NMSCs) greatly exceed the number of all other human malignancies in the United States and show increasing incidence worldwide. Chronic long-term ultraviolet (UV) exposure is the primary cause of skin carcinogenesis and leads to the formation of clonogenic mutations (CMs). CMs are clinically and histologically normal, the first steps during photocarcinogenesis, and the first known manifestations of field cancerization. It has been established that UV-exposed clinically normal-presenting areas of skin contain high levels of CMs[1], however the comparison of CMs and mutations in NMSC have yet to be systematically studied. To understand how mutation profiles of clinically normal presenting skin correspond to mutations in NMSC, we developed two analytic pipelines for comparing the genome of normal skin to mutational hotspots in cutaneous squamous cell carcinoma (cSCC), a type of NMSC with metastatic potential. The mutation frequency binning pipeline takes each mutation in the dataset and identifies neighboring mutations within an assigned range of base pairs. If a mutation is found, the algorithm will search for additional mutations surrounding this one using this same range. This repeats until no additional mutations are found within the designated range, outputting the total concatenated base pair region covering all identified mutations. This pipeline may then identify the regions with the highest quantity of mutations, by filtering out regions containing less than an assigned cutoff value. Our second pipeline takes these defined regions from two different datasets and calculates the number of base pairs that overlap between the two. Using these two pipelines with a genomic target of 100 base pairs and a cutoff value of five mutations per genomic area, we identified a significant ($p=0.001194$) overlap between mutations found in normal sun-exposed skin and cSCC. We also identified significant ($p<0.00001$) enrichment of individual point mutations and identified numerous CM enriched areas with low mutation frequency in cSCC. The identified pattern of mutation overlap will help to design optimal sequencing library targets for potentially cancer-causing CMs in sun-exposed normal human skin.

References

1) Lei Wei, Sean R Christensen, Megan E Fitzgerald, Ultradeep sequencing differentiates patterns of skin clonal mutations associated with sun-exposure status and skin cancer burden. Science Advances, 2021.

P-4.1.13

Metalloporphyrins: promising G-quadruplex stabilizing ligands

Catarina IV Ramos,[1] Ana Rita Monteiro, [1,2] Nuno MM Moura,[1] Tito Trindade[2], Maria AF Faustino,[1] Maria Graça PMS Neves[1]

1) LAQV-REQUIMTE, Department of Chemistry of University of Aveiro, Aveiro, Portugal; 2) CICECO, Department of Chemistry of University of Aveiro, Aveiro, Portugal

G-quadruplexes (GQ) are DNA secondary structures found in several genome regions of biological significance, namely in telomeres.[1] The stabilization of these GQ DNA structures by ligands is a promising strategy for the inhibition of telomerase, a reverse transcriptase enzyme expressed in a range of cancer cells, that allows continual cell division without telomere shortening.[2,3] So, the inhibition of telomerase by recurring to adequate GQ-stabilizing ligands can be considered a promising therapeutic approach for cancer.
To assess the potential of a compound as a telomerase inhibitor, selectivity for quadruplex over duplex DNA is a fundamental attribute, as the drug must be able to recognize quadruplex DNA in the presence of a large amount of duplex DNA, in the cellular nucleus.4
Several studies have shown that porphyrins and analogues, in special the tetracationic 5,10,15,20-*tetrakis*(1-methylpyridinium-4-yl)porphyrin (TMPyP), exhibit high affinity to GQ and thus are potential telomerase inhibitors. However, it is also recognized that TMPyP has poor selectivity for GQ over duplex DNA structures.[4]
By using spectroscopic techniques and ensuring the same experimental conditions, the efficacy of the Ag^{II}, Cu^{II}, Co^{III}, Ni^{II}, Pd^{II} and Zn^{II} metal complexes of TMPyP (**M-TMPyP**, Fig. 1) as GQ stabilizing agents were evaluated and compared. Formation of stable porphyrin-DNA adducts was observed for all the ligands with both DNA structures. The analysis of the hypochromic and bathochromic effects in the ligand UV-Vis spectra due to the presence of the DNA allowed to obtain binding constants and to predict that, in general, the interaction of the ligands occurs by end-stacking in the GQ structure. The Ag^{II} ligand showed interesting and promising properties as G-quadruplex stabilizing agent.

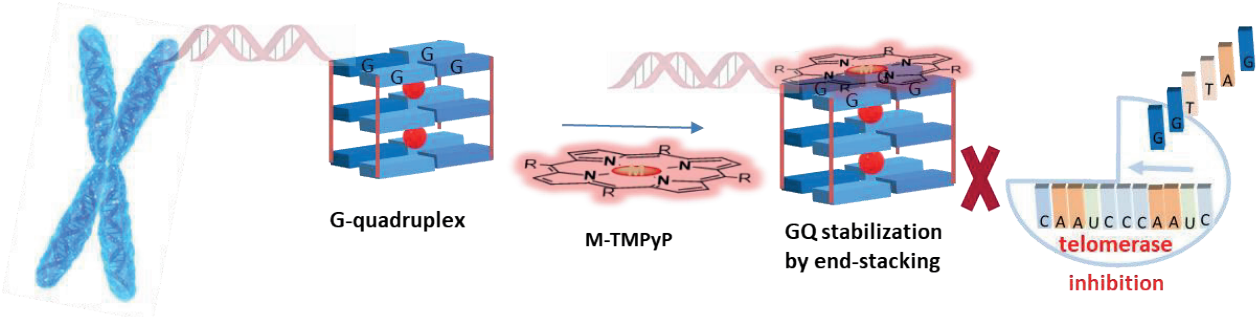


Fig. 1 – G-quadruplex-M-TMPyP *adduct formation and telomerase inhibition.*

References

1) a) D Sen, W Gilbert. *Nature*, *334*, 364, **1988**; b) EH Blackburn. *Cell. 77*, 621, **1994**
2) a) A Moye, *et al. Nature Commun*. *6***,** 7643, **2015**; b) J Nandakumar *et al. Nat. Rev. Mol. Cell Biol. 14*, 69, **2013**;
3) a) L Oganesian, *et al. BioEssay*. 29, 155, **2007**; b) Q Wang, *et al. Nucleic Acids Res. 39*, 6229,**2011**; c) J Bidzinska, *et al. Molecules. 18*, 12368, **2013**.
4) a) AR Monteiro, *et al. ACS Omega. 2*, 11184, **2018**; b) CIV Ramos, *et al. Molecules*. *24*, 733, **2019**

Acknowledgements. The authors are grateful to the University of Aveiro and to the Fundação para a Ciência e a Tecnologia (FCT) for the financial support to LAQV-REQUIMTE (Ref.UIDB/50006/2020), and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement. Catarina IV Ramos (REF.-047-88-ARH/2018) and Nuno MM Moura (REF.-048-88-ARH/2018) acknowledges the costs of their research contracts. Ana R. Monteiro thanks FCT for the PhD grant SFRH/BD/137356/2018.

P-4.1.14

Metalized Phthalocyanines as potential photosensitizer in Photodynamic Therapy

Miguel León[1], Franklin Vargas[1]

1)Venezuelan Institute for Scientific Research (I.V.I.C), Organic Photochemistry Laboratory, Chemistry Center, Caracas, Venezuela.

Phthalocyanines are organic compounds whose origin is related to porphyrin structure. They have many applications in different fields, including biomedical, photobiological and chemical research. From a chemical standpoint, phthalocyanines are macrocyclic tetraaza compounds, which are composed mainly of isoindolic groups that confer aromaticity and planarity to the chemical structure[1]. Similar to other types of porphyrin compounds, the structure of phthalocyanine is capable of chelating many metals, which more often contribute to its wide variety of functions, including singlet oxygen and free radicals generation, fluorescence, absorption spectra, among others. All of these properties are desirable for biomedical and pharmaceutical research. ROS generation is the main theme in Photodynamic Therapy and phototoxic processes, as well as in antibacterial, antiviral and anticancer properties. Therefore, the synthesis of phthalocyanines and *in vitro* testing are a relevant scientific topic[2, 3, 4, 5]. There are many synthesis methods and mechanisms of action, each one of those have a different level of difficulty[5]. In our present experimental development, we expect to test the photosensitizer potential and Pbr322 plasmid DNA cleavage activity of Zn, Cd and Hg metalized phthalocyanines by means of agarose gel electrophoresis and chemiluminescence. The excellent photochemical and pharmaceutical properties of phthalocyanines will be valued, which make them excellent for their wide use at a medical and clinical level.

References

1) Ishii, K. Functional singlet oxygen generators based on phthalocyanines. Coord. Chem. Rev, 256, 1556-1568, (2012)

2) F. Vargas, C.Rivas, T. Zoltan, L. Padrón, C. Izzo, V. López, L. Gómez, F. Pujol, H. Rangel, D. Garzaro, R. Fabbro. Comparative Antiviral (HIV) Photoactivity of Metalized mesoTetraphenylsulfonated Porphyrins. Medicinal Chemistry**4**/2, 138-145 (2008).

3) Oviedo, O., Zoltan, T., Vargas, F., Inojosa, M., Vivas, J.C. Antibacterial photoactivity and photosensitized oxidation of phenols with *meso*-tetra-(4-benzoate, 9-phenanthryl)-porphyrin and its metal complexes (Zn and Cu*)*.J. Coord. Chem. 67, 1715(2014).

4)Wadzanai, C., Ogunsipe, O.,Tebello, N.Syntheses and photophysics of new phthalocyanine derivatives of zinc, cadmium and mercury. New J. Chem,31, 377-384 (2006).

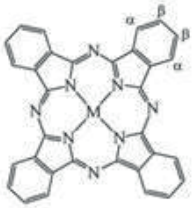
5) Barut, B,OzgürYalcin C., Demirbas Ü., Ackay H.T., Kantekin, H., Özel A. The novel Zn (II) phthalocyanines: Synthesis, characterization, photochemical, DNA interaction and cytotoxic/phototoxic properties. J. Mol. Structure, 1218, 128502, (2020).

Acknowledgements. This work was supported by grant from Centro de Estudios Avanzados (CEA-IVIC)

Metalized Phthalocyanines as potential photosensitizer in Photodynamic Therapy

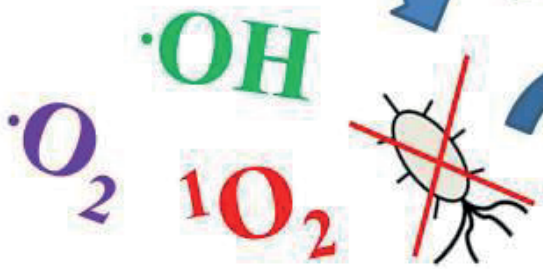
Miguel León[1], Franklin Vargas[1]

1) Venezuelan Institute for Scientific Research (I.V.I.C), Organic Photochemistry Laboratory, Chemistry Center, Caracas, Venezuela.

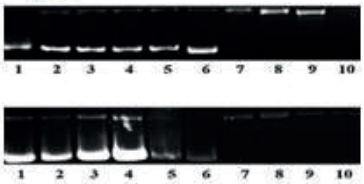


Phthalocyanines are organic compounds whose origin is related to porphyrin structure. They are considered as macrocyclic tetraaza compounds because of the isoindolic groups that confer aromaticity and planarity to the chemical structure[1]

Phthalocyanines are capable of chelating too many types of metals. The general characteristics of these compounds depend largely on the metal to which they complex[2,3,4,5]



Because of the photosensitization activity and ROS generation, Phthalocyanines compounds have antimicrobial, antiviral and antimicrobial properties[2,3,5]



Antimicrobial properties of Phthalocyanines could be assessed by carrying out agarose gel electrophoresis, where Pbr322 plasmid DNA cleavage should be expected[2,3]

References

1) Ishii, K. Functional singlet oxygen generators based on phthalocyanines. Coord. Chem. Rev, 256, 1556-1568, (2012)

2) F. Vargas, C.Rivas, T. Zoltan, L. Padrón, C. Izzo, V. López, L. Gómez, F. Pujol, H. Rangel, D. Garzaro, R. Fabbro. Comparative Antiviral (HIV) Photoactivity of Metalized mesoTetraphenylsulfonated Porphyrins. Medicinal Chemistry4/2, 138-145 (2008).

3) Oviedo, O., Zoltan, T., Vargas, F., Inojosa, M., Vivas, J.C. Antibacterial photoactivity and photosensitized oxidation of phenols with *meso*-tetra-(4-benzoate, 9-phenanthryl)-porphyrin and its metal complexes (Zn and Cu).J. Coord. Chem. 67, 1715(2014).

4)Wadzanai, C., Ogunsipe, O.,Tebello, N.Syntheses and photophysics of new phthalocyanine derivatives of zinc, cadmium and mercury. New J. Chem,31, 377-384 (2006).

5) Barut, B,OzgürYalcin C., Demirbas Ü., Ackay H.T., Kantekin, H., Özel A. The novel Zn (II) phthalocyanines: Synthesis, characterization, photochemical, DNA interaction and cytotoxic/phototoxic properties. J. Mol. Structure, 1218, 128502, (2020).

IL-4.2.1

UV-B radiation in a changing climate; can UV protect plants from drought stress?

Gaia Crestani[1], Aoife Coffey[1], Alexander Ač[2], Karel Klem[2], Otmar Urban[2], Marcel A.K. Jansen[1,2]
1) School of Biological, Earth and Environmental Sciences, Environmental Research Institute, University College Cork, Ireland; 2) Global Change Research Institute, Czech Academy of Sciences, Bělidla 986/4a, CZ-603 00 Brno, Czech Republic

Cross-resistance, i.e. the concept whereby plants exposed to one particular stressor develop protection against multiple stressors is well known in plant biology. Traditionally, Reactive Oxygen Species (ROS) were perceived as key players in cross-resistance, with elevated antioxidant defences protecting against ROS generated as a result of exposure to a range of different stressors. However, new evidence shows more subtle mechanisms of cross-talk between stress-protection pathways in plants, including a variety of chemical and/or electrical signals that transmit information between receptor systems and signalling cascades resulting in a degree of cross-resistance. Conversely, cross-sensitivity may arise where plant defences responses are overwhelmed by a combination of two stressors. Indeed, in theory, impacts of combinations of two stressors can be additive, synergistic or antagonistic. A particularly important combination of environmental factors is UV-B radiation and drought. The 2016 report by the United Nations Environment Programme -Environmental Effects Assessment Panel report flagged that interactions between climate change and UV penetration in the biosphere are resulting in the exposure of plants to new combinations of UV radiation and drought. However, lack of understanding of impacts of such combined treatments creates uncertainties that hamper predictions of future ecological change. A meta-analysis of published literature shows complex interactive effects between UV-B radiation and drought on plants, with individual studies showing increased cross-resistance, or the opposite, increased sensitivity. Response variations are likely to reflect exposure doses and/or kinetics. Yet, overall, in plants exposed to both UV and drought, increases in plant defence responses are less-than-additive, and so are the damage and growth retardation. Induction of a degree of cross-tolerance seems the most likely interpretation of the less-than-additive responses that are observed.
The data imply that UV sensing may play an important physiological function in fine-tuning responses to drought. Given that realistic doses of UV-B radiation rarely cause stress in well acclimated plants, the potential to use UV-B radiation for pre-acclimation against other stressors is attractive.

Acknowledgements. This work was supported by Science Foundation Ireland (S16/IA/4418)

IL-4.2.2

Does ultraviolet radiation modulate plant responses to elevated CO_2 concentration?

Otmar Urban[1], Karel Klem[1], Marcel A.K. Jansen[1,2]

1) Global Change Research Institute, Czech Academy of Sciences, Bělidla 986/4a, CZ-603 00 Brno, Czech Republic; 2) School of Biological Earth and Environmental Sciences, Environmental Research Institute, University College Cork, Cork, Ireland

The recent UNEP-EEAP (United Nations Environment Programme -Environmental Effects Assessment Panel) report has emphasized the need to investigate interactions between climate change and ultraviolet (UV) radiation. Depending on emission scenario, the atmospheric CO_2 concentration is predicted to reach 538–936 μmol CO_2 mol^{-1} at the end of the century. Together with increased temperature and altered precipitation patterns, elevated CO_2 concentration (eCO_2) is considered to be the most crucial factor affecting terrestrial ecosystems in future. However, plant responses to these factors may be further modulated by other environmental drivers, including ultraviolet (UV) radiation. Though eCO_2 and UV radiation (particularly UV-B) have both direct and indirect effects on photosynthesis and growth, their interactive effects may range from simply additive to synergistic and antagonistic.
We summarize our previous findings in woody and non-woody plants at different hierarchical and functional levels, including changes in plant stoichiometry, physiology and morphology. Among others, we found support for the hypothesis that the impact of eCO_2 on photosynthesis is substantially modulated by UV radiation. Moreover, the eCO_2×UV interaction is changing along the vegetation season: enhanced UV radiation stimulates a positive effect of eCO_2 on plant photosynthesis at the beginning of the vegetation season (short-term effect), whilst long-term cultivation diminishes the stimulatory effect of eCO_2 (a clear down-regulation of photosynthesis). Down-regulation is, however, not found in plants grown under the conditions of excluded UV radiation[1,2]. Such findings indicate the need to explore interactive effects of eCO_2 with other environmental drivers, including the intensity of UV radiation. Exclusion of UV radiation in experiments simulating eCO_2 may thus substantially overestimate the positive effect of CO_2 on photosynthesis and growth of future plants.

References

1) Urban O., Hrstka M. et al., Interactive effects of ultraviolet radiation and elevated CO_2 concentration on photosynthetic characteristics of European beech saplings during the vegetation season. Plant Physiology and Biochemistry 134: 20-30, 2019.
2) Uchytilová T., Krejza J. et al., Ultraviolet radiation modulates C:N stoichiometry and biomass allocation in *Fagus sylvatica* saplings cultivated under elevated CO_2 concentration. Plant Physiology and Biochemistry 134: 103-112, 2019.

Acknowledgements. The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic within the SustES project – Adaptation strategies for sustainable ecosystem services and food security under adverse environmental conditions (CZ.02.1.01/0.0/0.0/16_019/0000797).

OC-4.2.3

Short-term UV pretreatment supports cold tolerance of bell pepper seedlings

Gyula Czégény, Arnold Rácz, Kristóf Csepregi, Éva Hideg
University of Pécs, Department of Plant Biology, Pécs, Hungary

Ultraviolet radiation (UV, 280-400 nm) is an important regulator of plant growth and development[1]. Under UV exposure, plants upregulate several metabolic pathways, such as the synthesis of phenolics or the activation of antioxidant enzymes[2,3]. In addition, moderate UV doses stimulate energy dissipating pathways in photosystem II (PS II) by triggering zeaxanthin formation[4], supporting the protection of the photosynthetic apparatus. These strategies promote plant acclimation to other environmental challenges as well, such as high light conditions[5] or chilling temperatures[6]. During the following decades, extreme weather conditions are expected to appear more frequently, thus the importance of effective techniques in plant conditioning will rise. Controlled exposure to low UV-B doses documentedly increases tolerance to subsequent stress[7], and the aim of the present study was to explore whether UV-B is capable of priming the cold tolerance of bell pepper seedlings. The study was destined to model a general agricultural practice: the springtime transfer of bell pepper seedlings from plant nurseries to open fields, which includes a sudden change in weather conditions.

In this work, bell pepper (*Capsicum annuum* cv. grossum) seedlings were grown in growth chambers under 90 µmol s^{-1} m^{-2} photosynthetically active radiation (400-700 nm), 16h/8h, 25°C/20°C day/night conditions for 3 weeks. Prior to a 5-day long exposure to cold temperatures (15°C/10°C day/night conditions), half of the plants were illuminated by acclimative doses of UV (6.9 kJ m^{-2} d^{-1} BED) provided by a broadband 311 nm centred UV tube (Q-Panel UVB-313EL), for 5 days.

The applied UV pretreatment affected leaf metabolites, making the plants more resistant to lower temperature stress. The latter was confirmed by measuring PS II photochemical yields and non-photochemical quenching parameters. Results suggest a cold-induced limitation in energy transfer between the antenna and the reaction center, which was lessened by the UV-pretreatment. Priming with UV also helped to maintain higher electron transfer rates under higher light intensities and lessen the negative effect of cold stress on these. Our results support beneficial effects of UV priming in plant protection.

References

1) Jansen, M.A.K., Bornman, J.F., UV-B radiation: from generic stressor to specific regulator. Physiologia Plantarum 145: 501-504, 2012.
2) Schreiner, M., Mewis, I., Huyskens-Keil, S., Jansen, M.A.K., Zrenner, R., Winkler, J.B., O'Brien, N., Krumbein, A., UV-B-induced secondary plant metabolites - potential benefits for plant and human health. Critical Reviews in Plant Sciences 31: 229-240, 2012.
3) Czégény, Gy., Kőrösi, L., Strid, Å., Hideg, É., Multiple roles for vitamin B_6 in plant acclimation to UV-B. Scientific Reports 9: 1259, 2019.
4) Šprtová, M., Špunda, V., Kalina, J. and Marek, M.V., Photosynthetic UV-B response of beech (*Fagus sylvatica* L.) saplings. Photosynthetica 41: 533–543, 2003.
5) Klem, K., Holub, P., Štroch, M., Nezval, J., Špunda, V., Tříska, J., Jansen, M.A.K., Robson, T.M., Urban, O., Ultraviolet and photosynthetically active radiation can both induce photoprotective capacity allowing barley to overcome high radiation stress. Plant Physiology and Biochemistry 93: 74–83, 2015.
6) Parkin, K.L., Marangoni, A., Jackman, R.L., Yada, R.Y., Stanley, D.W., Chilling injury. A review of possible mechanisms. Food Biochemistry 13: 127-153. 1989.
7) Wargent, J.J., Elfadly, E.M., Moore, J.P., Paul, N.D., Increased exposure to UV-B radiation during early development leads to enhanced photoprotection and improved long-term performance in *Lactuca sativa*. Plant Cell & Environment 34: 1401-1413, 2011.

Acknowledgements. This work was supported by the National Research, Development and Innovation Office (grant PD-131625), the János Bolyai Research Scholarship (BO/00362/20/4) of the Hungarian Academy of Sciences and the New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund (grants ÚNKP-20-4-II-PTE-603 and ÚNKP-20-5-PTE-634).

OC-4.2.4

Short daily UV exposure of Micro-Tom tomato plants favours a better stomatal control suggesting a delayed leaf senescence

Mannucci Alessia[1]; Scartazza Andrea[2]; Santaniello Antonietta[3]; Mariotti Lorenzo[1]; Castagna Antonella[1]; Santin Marco[1]; Quartacci Mike Frank[1]; Ranieri Annamaria[1]
1) University of Pisa, Agriculture, Food and Environment, Pisa, Italy; 2) CNR, Institute of Research on Terrestrial Ecosystems, Pisa, Italy; 3) Valagro S.p.A., Pisa, Italy

UV radiation, unless present at high doses, is recognised to act as a regulator of plant growth and some specific processes (Frohnmeyer and Staiger, 2003; Jenkins, 2017; Robson et al., 2019). The present study investigated the influence of UV irradiation (0.132 and 0.015 W m^{-2} biologically effective UV-B and UV-A irradiance, respectively, 15 minutes/day, 11 days) on leaf gas exchange and some biochemical and molecular markers of leaf senescence in Micro-Tom tomato plants. UV-induced reduction of g_s (stomatal conductance) was associated with the modified expression of some genes involved in the control of stomatal movement and with changes in salicylic and abscisic acid contents, which suggest a two-step regulation of stomatal closure involving the two hormones. The effects of UV on stomatal closure were only transient, as suggested by the full recovery of g_s three days after the end of the treatment. The temporal changes of g_s and A_{net} (steady state photosynthetic CO_2 assimilation rate) along with the hormone and chlorophyll behaviour, suggest a possible delay of leaf senescence in treated plants compared to control ones, confirmed by the expression levels of some genes related to senescence. The UV potential to induce a persistent partial inhibition of g_s without severely affecting A_{net} led to an increased *iWUE* (intrinsic water use efficiency) during the 11-day treatment, suggesting a priming effect of low UV radiation towards drought conditions, potentially useful to reduce the excess water use in agriculture.

References

1)Frohnmeyer H., Staiger D., Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. Plant Physiology, 2003, 33:1420–1428
2)Jenkins G., Photomorphogenic responses to ultraviolet-B light. Plant Cell & Environment, 2017, 40 (11): 2544–2557
3)Robson TM, Aphalo PJ, Banaś AK, Barnes PW, Brelsford CC, Jenkins GI, Kotilainen TK, Łabuz J, Martínez-Abaigar J, Morales LO, Neugart S, Pieristè M, Rai N, Vandenbussche F, Jansen MA, A perspective on ecologically relevant plant-UV research and its practical application. Photochemical & Photobiological Sciences, 2019, 18(5), 970-988

Acknowledgements. This work was supported by the PhD program of Agriculture, Food and Environment fellowship, University of Pisa.

IL-4.2.5

Benefits of solar UV-B radiation on field crops defenses against insect pests.

Jorge A. Zavala.

University of Buenos Aires, School of Agronomy, Biochemistry unit and INBA-CONICET, Argentina.

Solar UV-B radiation has been reported to enhance plant defenses against herbivore insects in many species. However, the mechanism and traits involved in the UV-B mediated increment of plant resistance are largely unknown in crops species, such as soybean. Two soybean cultivars (cv.) were grown under attenuated or full solar UV-B radiation and leaves were damaged by *Anticarsia gemmatalis* larvae and pods attacked by the stink bug bugs *Nezara viridula*. We determined in undamaged and damaged leaves and pods changes in jasmonates, ethylene, salicylic acid, trypsin protease inhibitor (TPI) activity, flavonoids and mRNA expression of genes related with defenses. Ethylene emission induced by herbivory was synergistically increased in plants grown under solar UV-B radiation and was positively correlated with malonylgenistin concentration, TPI activity and expression of IFS2 and the defensive protein PR2, while was negatively correlated with leaf consumption and stink bug damage. The precursor of ethylene ACC applied exogenously to soybean was enough to strongly induce leaf isoflavonoids. Our results showed that in field-grown soybean isoflavonoids were regulated by both herbivory and solar UV-B inducible ET, while flavonols were regulated by solar UV-B radiation and not by herbivory or ET. Our study suggests that although ET can modulate UV-B-mediated priming of inducible plant defenses, some plant defenses, such as isoflavonoids are regulated by ET alone.

IL-4.2.6

UV-B LED priming for reduced biotrophic disease susceptibility in Lettuce seedlings

Jason J. Wargent[1,2], Ana Clara Pontaroli[2], Emily R. McLay[1,2]
1) School of Agriculture and Environment, College of Sciences, Massey University, Palmerston North, New Zealand; 2) BioLumic Limited, Palmerston North, New Zealand

Biotrophic disease is one of the largest causes of decreased yield in agriculture. While exposure to ultraviolet B (UV B) light (280–320 nm) has been previously observed to reduce plant susceptibility to disease, there is still a paucity of information regarding underlying biological mechanisms. In addition, recent advances in UV-LED technology raise the prospect of UV light treatments in agriculture which are practical and efficient. Here, we characterized the capability of UV-B LED pre-treatments to reduce susceptibility of a range of lettuce (*Lactuca sativa*) cultivars to downy mildew disease caused by the obligate biotroph *Bremia lactucae*. Innate cultivar susceptibility level did not seem to influence the benefit of a UV-B induced disease reduction with similar reductions as a percentage of the control observed (54–62% decrease in conidia count) across all susceptible cultivars. UV-B-induced reductions to conidia counts were sufficient to significantly reduce the infectivity of the diseased plant. Secondary infections caused by UV-B pre-treated plants exhibited yet further (67%) reduced disease severity. UV-B-induced flavonoids may in part mediate this reduced disease severity phenotype, as *B. lactucae* conidia counts of lettuce plants negatively correlated with flavonoid levels in a UV-B-dependent manner (r = -0.81). Liquid chromatography–mass spectrometry (LC-MS) was used to identify metabolic features which contribute to this correlation and, of these, quercetin 3-O-(6”-O-malonyl)-b-D-glucoside had the strongest negative correlation with *B. lactucae* conidia count (r = -0.68). When quercetin 3-O-(6”-Omalonyl)- b-D-glucoside was directly infiltrated into lettuce leaves, with those leaves subsequently infected, the *B. lactucae* conidia count was reduced (25–39%) in two susceptible lettuce cultivars. We conclude that UV-B induced phenolics, in particular quercetin flavonoids, may act as phytoanticipins to limit the establishment of biotrophic pathogens thus delaying or reducing their sporulation as measured by conidia count. These findings highlight the opportunity for UV-B morphogenesis to be exploited through the application of UV-LED technology, as part of the development of next-generation, sustainable disease control tools.

References

1) McLay ER, Pontaroli AC and Wargent JJ, UV-B induced flavonoids contribute to reduced biotrophic disease susceptibility in lettuce seedlings. Front. Plant Sci. 11:594681. doi: 10.3389/fpls.2020.594681, 2020

Acknowledgements. This work was supported by a Ph.D. scholarship award from Massey University and BioLumic Limited to EM. The scholarship was funded at 50% by each of the two organizations.

OC-4.2.7

The role of ultraviolet radiation in colonization of plants by endophytes

Aleksandra Giza[1], Paweł Hermanowicz[1], Rafał Ważny[1], Piotr Rozpądek[1], Justyna Łabuz[1]

1) Jagiellonian University in Kraków, Malopolska Centre of Biotechnology, Kraków, Poland

Light is one of the most important environmental factors for growth and development of plants. Recent studies have demonstrated the crucial role of light in the plant defense system and in the control of the trade-off between growth and defense. Natural and artificial plant communities must deal with crowding, which results in canopy shade and affect the resistance to pathogens[1].
Ultraviolet light (in particular UV-B) has a positive effect on plant defense mechanisms in leaves against biotic stressors[2]. UV-B enhances host resistance to fungal pathogens and infections by necrotrophs[3]. Secondary metabolites such as flavonoids serve as screening compounds protecting photosynthetic apparatus from damage, but also may improve antifungal resistance[4]. Plant immune responses are mediated by photoreceptors: red/far-red sensitive phytochromes, UV-B sensitive UVR8 and blue and UV-A proteins: cryptochromes and phototropins[5]. Plant hormones such as jasmonic acid (JA), salicylic acid (SA) and ethylene activate immune responses[4].
In this study, we will analyze the impact of the UV-A light on the host resistance to fungi and their colonization capacity. Symbiotic (and parasitic) interactions will be assessed on *Arabidopsis thaliana* plants inoculated with endophytes collected from shoots of *Arabidopsis arenosa*. The experiment will focus on yeast: *Sporobolomyces ruberrimus*, and fungi species: *Mucor sp., Paraphoma chrysanthemicola, Phomopsis columnaris, Diaporthe eres.* We have employed WGA-TexasRed staining followed by a Confocal Microscopy analysis to confirm the colonization status of roots and shoots by fungi. Now we will test the overall plant fitness in terms of morphology (weight, shoot and root length), phenolic content (anthocyanins) and expression of genes of phenolic pathway and plant hormones.

References

1) Moreno J. E., Ballaré C.L., Phytochrome Regulation of Plant Immunity in Vegetation Canopies. Journal of Chemical Ecology, 40:848–857, 2014.
2) Ballaré C.L., Mazza C.A., Austin A.T., Pierik R., Canopy Light and Plant Health. Plant Physiology, Vol. 160, Issue 1, pp. 145–155, 2012.
3) Demkura P.V., Ballare C.L., UVR8 Mediates UV-B-Induced Arabidopsis Defense Responses against Botrytis cinerea by Controlling Sinapate Accumulation. Molecular Plant, Vol. 5, no 3, pp. 642-652, 2012.
4) Ballare C.L., Light Regulation of Plant Defense. Annual Review of Plant Biology, 65:15.1–15.29, 2014.
5) Griebel T., Zeier J., Light Regulation and Daytime Dependency of Inducible Plant Defenses in Arabidopsis: Phytochrome Signaling Controls Systemic Acquired Resistance Rather Than Local Defense. Plant Physiology, Vol. 147, pp. 790–801, 2008.

Acknowledgements. This work was supported by grant POBBios for Jagiellonian University ‘The role of ultraviolet radiation in colonization of plants by endophytes’. Endophytes were isolated as a part of the grant ‘The role of endophytic fungi in confering Arabidopsis arenosa toxic metal tolerance’ UMO-2017/27/B/NZ8/01199.

OC-4.2.8

UVB-dose and Salt stress response on the accumulation of secondary metabolites on Bell pepper leaves

Shyam Pariyar[1], Simone Röhlen-Schmittgen[2]
1) Bonn University, Department of Plant Nutrition, Bonn, Germany; 2) Hochschule Geisenheim University, Department of Vegetable Production, Geisenheim, Germany

The accumulation of secondary metabolites (SMs) on crop leaves could add value to the crop green biomass by increasing the nutritional quality of produce or increasing high value molecules content for industrial uses. These SMs can be extracted from crop green biomass and sold. Crop plants accumulate higher amounts of these SMs when they are exposed to abiotic stresses such as salt and UV. These SMs can be anti-oxidative, antifungal or antimicrobial, thus enhancing plant defense itself or they enrich the nutritional value and market potential of specific metabolites that have high economic demand. Thus, we evaluated, i) how UVB and Salt stress affect Bell pepper plants at different doses of UVB application and ii) how these UVB and Salt stress treatments affect SMs accumulation such as flavones cynaroside and graveobioside A in leaves on Bell pepper leaves when exposed to salt stress (100 mM NaCl) and 3 different UVB doses (1.7 W m^{-2} for 1, 2 and 3 h per day) for a week. SMs accumulations were compared using non-destructive Fluorescence sensors (Dualex, Multiplex), Field Spectrometer and analysed using HPLC. We hypothesized that the enhanced accumulation of SMs under stress conditions. Since, the SMs analysis is currently being analysed, we expected to find out the optimal thresh hold exposure to the single (UVB) or combined stresses (UVB and Salt) to have maximum SMs accumulation in Bell pepper leaves. Thus, in this paper, some of the major results of this research will be presented and discussed. The outcome of the study might be useful for implication of abiotic stresses such as UV, salt stress or combined on enhancing crop quality and plant health management.

Acknowledgements. This research is funded by Federal Ministry of Education and Research (BMBF), Germany under the framework of the „National Research Strategy Bioeconomy 2030“

P-4.2.9

Postharvest UV-treatment induces changes in grapevine berry skin photosynthesis, phenolic profiles and antioxidant capacities

Kristóf Csepregi[1], Péter Teszlák[2], Arnold Rácz[1], Gyula Czégény[1], László Kőrösi[2], Éva Hideg[1]
1) Department of Plant Biology, Faculty of Sciences, University of Pécs, Hungary; 2) Research Institute for Viticulture and Oenology, University of Pécs, Hungary

Biosynthesis of phenolic compounds is stimulated by ultraviolet (UV) light, and this regulatory role has been utilized for modifying metabolic profiles of fruits and vegetables[1]. In this study, we irradiated harvested berry clusters of one red (cv. Emperor) and two white (cv. Queen of Vineyard and White Sultana) table grape varieties with broad band UV (either 312 nm centred UV-B or 355 nm centred UV-A) for 30 min. UV-induced changes in berry skin antioxidant capacities and phenolic profiles were measured regularly, between 2 and 48h after irradiation. Caftaric acid was the dominant phenolic acid in all cultivars, but its content was unaffected by the UV-treatment. UV-induced changes in phenolic profiles included a decrease in the amounts of the two major quercetin-glycosides 2h after irradiation in all cultivars. This decrease was temporal in two of the studied varieties (Emperor and Queen of Vineyard), and it was followed by a recovery to pre-treatment-levels during storage at 20°C under low intensity photosynthetically active light. Berry skin UV-A and UV-B absorbing capacities showed the same, transitional decrease regardless of the wavelength of the irradiation. Antioxidant capacities and quercetin-glycoside contents showed strong and positive correlations. Experiments with the red variety suggested that the observed decrease was due to a transient, UV-induced activation of peroxidases resulting in increased flavonoid oxidation[2]. Experiments with the two white varieties suggested that recovery of oxidized compounds was supported by berry skin photosynthesis. Recovery of quercetin-glycoside contents after the temporal UV-induced decrease was only observed in one of the two white grape varieties, in Queen of Vineyard, the one with higher base (pre-treatment) photochemical activity. In addition, this variety showed a temporal increase in berry skin photochemical activity 2 and 8h after the UV-treatment, while the same parameter decreased permanently in the White Sultana cultivar. These differences were the consequence of distinct UV-responses of regulated and nonregulated nonphotochemical quenching in the two cultivars. Results showed that mature grapevine berries have photosynthetically active tissues capable of dynamic changes even several hours postharvest, and suggest that changes in photochemistry may contribute to postharvest metabolic responses of berry skins[3].

References

1) Schreiner M, Mewis I, Huyskens-Keil S, Jansen MAK, Zrenner R, Winkler JB, O'Brien N, Krumbein A, UV-B-induced secondary plant metabolites - Potential benefits for plant and human health, Critic. Rev. Plant Sci. 31, 229-240. 2012.
2) Csepregi K, Kőrösi L, Teszlák P, Hideg É., Postharvest UV-A and UV-B treatments may cause a transient decrease in grape berry skin flavonol-glycoside contents and total antioxidant capacities. Phytochem. Lett. 31: 63-68, 2019.
3) Csepregi K, Teszlák P, Rácz A, Czégény Gy, Kőrösi L, Hideg É., Changes in grapevine berry skin photochemistry may support metabolic responses to postharvest treatment by ultraviolet light. Photosynth. 59, 286-293, 2021.

Acknowledgements. This work was supported by the National Research, Development and Innovation Office (grant KH-129615 to ÉH and KCs).

IL-4.3.2

Regulatory T cell induction prolongs the efficacy for the treatment of psoriasis

Akimichi Morita[1]
1) Department of Geriatric and Environmental Dermatology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan

Phototherapy utilizes the beneficial effects of ultraviolet (UV) wavelengths to affect immunoregulatory functions. UV light phototherapy using narrowband (NB) UVB and bath-psoralen UVA (bath-PUVA) therapy are well-established treatments. Dual-action operative mechanisms of UV phototherapy have been identified: apoptosis and immune suppression. NB-UVB depletes pathogenic T cells by inducing apoptosis and regulatory T cells. Other wavelengths are also utilized for phototherapy, i.e., 308-nm excimer light and 312-nm flat-typed NB-UVB. Excimer light (308 nm) therapy effectively targets the affected skin without unduly exposing other areas and increases the levels of T regulatory cells. Phototherapy improves impaired resting regulatory T cells[1] and increases activated regulatory T cells in patients with psoriasis[2]. In mouse, UVB increases regulatory T cells by clustering with dermal CD11b-type Langerin- DCs to induce immunological self-tolerance[3]. Furthermore, UVB-expanded skin regulatory T cells are tissue regulatory T cells and express proenkephalin (PENK), an endogenous opioid precursor, and amphiregulin (AREG), the epidermal growth factor receptor ligand[4]. UVB-expanded skin regulatory T cells play a key role in promoting wound healing in vivo. However, UVB-expanded skin regulatory T cells have not been proven yet in human.

More rational designs of phototherapy devices and irradiation protocols are under development. Biologics are highly effective for the treatment of psoriasis. The long-term safety of biologic therapies, however, is not well established, and they are expensive. Phototherapy takes advantage of wavelengths found in natural sunlight, which, despite its well-known deleterious effects, such as premature skin aging and increased risk of cancer, also has beneficial effects on diseased skin. Sunlight exposure has been historically recommended to maintain health and to treat disorders, and natural sunlight comprises beneficial wavelengths, such as 311-nm NB-UVB. Several ongoing studies are investigating various wavelength-dependent effects on both skin disease and the underlying immunomechanisms. The TL01 lamp is widely used as the light source of 311-nm NB-UVB. This lamp contains mercury, however, the use of which will soon be limited. Therefore, a light-emitting diode that emits UV will be a more desirable and feasible light source, particularly if it has a high enough intensity level for the treatment of skin diseases. Phototherapy is demonstrated to be a highly effective treatment option for patients with skin disease such as psoriasis, and developments to individualize treatments and improve the efficacy and safety of light therapies are ongoing.

References

1) Takuya Furuhashi, Chiyo Saito, Kan Torii, Emi Nishida, Sayuri Yamazaki, Akimichi Morita: Bath-psoralen UVA and narrowband UVB reduce circulating Th17 cells and restore circulating regulatory T cells in psoriasis, ***PLoS ONE***, 8 (1), art. no. e54895, **2013**

2) Ryoji Kubo, Shinnosuke Muramatsu, Yoko Sagawa, Chiyo Sait1, Saori Kasuya, Akiko Nishioka, Emi Nishida, Sayuri Yamazaki, Akimichi Morita: Bath-PUVA therapy improves impaired resting regulatory T cells and increases activated regulatory T cells in psoriasis, ***J Dermatol Sci,*** 86: 46-53, **2017.**

3) Sayuri Yamazaki, Mizuyu Odanaka, Akiko Nishioka, Saori Kasuya, Shime Hiroaki, Hiroaki Hemmi, Masaki Imai, Dieter Riethmacher, Tsuneyasu Kaisho, Naganari Ohkura, Shimon Sakaguchi, Akimichi Morita: Ultraviolet B-induced maturation of CD11b-type Langerin$^-$ dendritic cells control the expansion of Foxp3$^+$ regulatory T cells in the skin, ***J Immunol*** 200:119-129, **2018.**

4) Hiroaki Shime, Mizuyu Odanaka, Makoto Tsuiji, Takuma Matoba , Masaki Imai, Yoshiaki Yasumizu, Ryuta Uraki, Kiyoshi Minohara, Maiko Watanabe, Anthony John Bonito, Hidehiro Fukuyama, Naganari Ohkura, Shimon Sakaguchi, Akimichi Morita, and Sayuri Yamazaki: Proenkephalin$^+$ regulatory T cells are expanded clonally by ultraviolet-B exposure and maintain skin homeostasis with a healing function, ***Proc Natl Acad Sci*** 117: 20696-20705, **2020**

Acknowledgements. This work was funded by a Grant-in-Aid for Scientific Research B (AM) from the Japan Society for the Promotion of Science.

IL-4.3.3

UVA-1 in fibrotic diseases and beyond

Calzavara-Pinton Piergiacomo[1], Gelmetti Alessandra[1], Arisi Mariachiara[1], Venturini Marina[1]
1) University of Brescia, Department of Dermatology, Brescia, Italy

Ultraviolet A1 (UVA1) is the electromagnetic waveband confined in the 340-400 nm range. Unlike other UV wavebands, UVA1 works almost exclusively via oxidative mechanisms and penetrates down to the reticular dermis and beyond. UVA1 phototherapy was introduced as a treatment option in the 1980s when powerful metal halide lamps became available. Afterwards, less expensive and more practical excimer lamps and LED lamps contributed to the widespread diffusion of this treatment modality. The treatment modality is very simple, because, unlike other phototherapies, the risk of excessive phototoxic reactions is very low and the treatment protocol is usually without or with very few dose increments. Since the early beginning, UVA1 phototherapy was investigated for the treatment of atopic dermatitis, particularly acute flares of atopic dermatitis. Results were very good but if and when it is superior to the more practical NB-UVB is still not fully clarified. Afterwards, it was studied in inflammatory skin disorders like skin lesions of lupus erythematosus, graft versus host disease, and psoriasis in HIV+ patients that cannot be treated with NB-UVB and PUVA. UVA1 is increasingly being used also in the treatment of inflammatory and fibrosclerotic dermal diseases like morphea, hypertrophic scars, granuloma annulare and necrobiosis lipoidica.
We present an updated review of the photobiological and photoimmunological effects of UVA1 on human skin and In addition, we will present a retrospective review of our experience with about 500 patients in the 2000-2020 time interval with this modality and we will discuss our results on light of a review of the literature.

OC-4.3.4

Simulated Penetration Depth of Ultraviolet Radiation in Skin with Varying Stratum Corneum Thicknesses as an Aid for Phototherapy

Louise Finlayson[1], Isla Barnard[1], Lewis McMillan[1], Ewan Eadie[3], Sally H. Ibbotson[2], Christian Thomas Alcuin Brown[1], Kenneth Wood[1]
1) University of St Andrews, SUPA School of Physics and Astronomy, St Andrews, United Kingdom; 2) University of Dundee, Photobiology Unit, Ninewells Hospital, Dundee, United Kingdom; 3) NHS Tayside, Photobiology Unit, Ninewells Hospital, Dundee, United Kingdom

Phototherapy has been used for many years as a convenient and non-invasive method of treatment. Ultraviolet (UV) radiation, either alone or in combination with a photosensitiser, is currently used to effectively treat skin conditions such as psoriasis, eczema, vitiligo, scleroderma and cutaneous T-cell lymphoma.[1,2] Several different types of UV based phototherapy exist including, broadband UVB (BBUVB), narrowband UVB (NBUVB) and the combination of the photosensitiser Psoralen and UVA (PUVA). Studies have demonstrated the effectiveness of interchanging the choice of light treatment for the same condition based on its severity and stage in individual circumstances by choosing the phototherapeutic source able to penetrate to the most effective depth[3,4]. It is clear then that accessible data that allows images, showing the penetration depth of light into skin as a function of wavelength, to be easily produced would be of great benefit for either selecting the best treatment option for an individual or developing new phototherapies.

We used Monte Carlo radiative transfer (MCRT) to simulate the path of light with wavelengths 200 – 400 nm through a 6-layer skin model. A simulation was run for each wavelength, outputting a fluence rate grid which was used to determine penetration depth. The model was adapted to investigate the effect of different thicknesses of stratum corneum, the outermost protective layer of the skin. A web application was then developed to allow the produced data to be easily accessed.

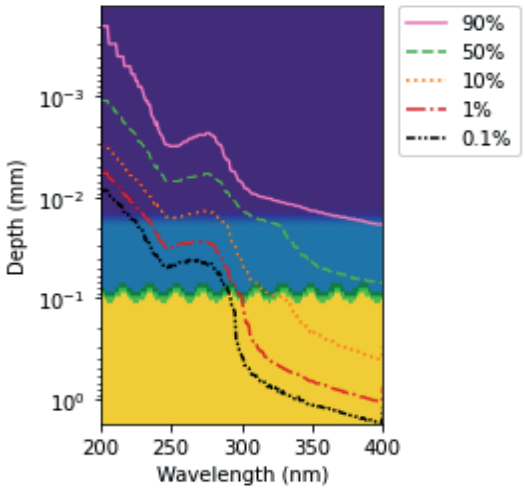


Fig. 1 – Penetration depth of light into skin as a function of wavelength for a stratum corneum thickness of 20μm. Data is produced by plotting the depth at which the light fluence rate reaches 90%, 50%, 10%, 1% and 0.1% of its incident value. Data is plotted over a scaled image of the skin model where each colour represents a different layer.

The results show the penetration depth of light varying in accordance with the selected optical properties. (Fig. 1) Predictably, the results also show that a thinner stratum corneum results in a larger penetration depth. (Fig. 2) The results are shown for a direct light source applied to skin of Fitzpatrick scale type I. However, the simulation is fully adaptable, allowing other light sources and skin types to be investigated given the availability of the appropriate optical properties.

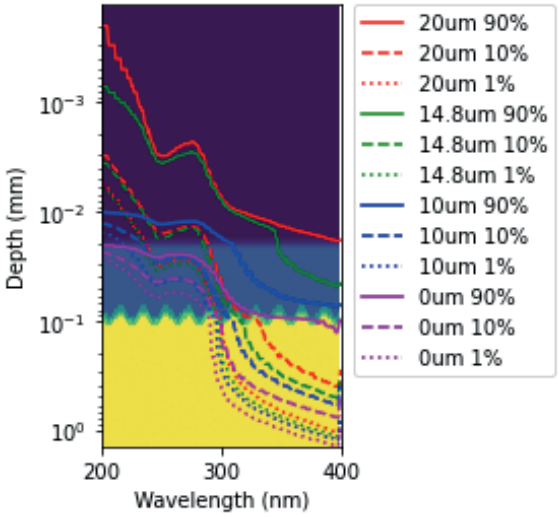


Fig. 2 – Penetration depth of light into skin as a function of wavelength for four different stratum corneum thicknesses. Data is produced by plotting the depth at which the light fluence rate reaches 90%, 10% and 1% of its incident value for stratum corneum thicknesses of 20μm, 14.8μm, 10μm and 0μm. Chosen thicknesses correspond closely with common thicknesses found at different body sites[5] and skin with no stratum corneum is also investigated. Data is plotted over a scaled image of the skin model where each colour represents a different layer.

References

1. Vangipuram, R. & Feldman, S. R. Ultraviolet phototherapy for cutaneous diseases: A concise review. *Oral Dis.* **22**, 253–259 (2016).
2. Krenitsky, A., Ghamrawi, R. I. & Feldman, S. R. Phototherapy: a Review and Update of Treatment Options in Dermatology. *Current Dermatology Reports* vol. 9 10–21 (2020).
3. Ling, T. C. *et al.* British Association of Dermatologists and British Photodermatology Group guidelines for the safe and effective use of psoralen-ultraviolet A therapy 2015. *Br. J. Dermatol.* **174**, 24–55 (2016).
4. Kreuter, A. *et al.* A randomized controlled study of low-dose UVA1, medium-dose UVA1, and narrowband UVB phototherapy in the treatment of localized scleroderma. *J. Am. Acad. Dermatol.* **54**, 440–447 (2006).
5. Sandby-Møller, J., Poulsen, T. & Wulf, H. C. Epidermal Thickness at Different Body Sites: Relationship to Age, Gender, Pigmentation, Blood Content, Skin Type and Smoking Habits. *Acta Derm. Venereol.* **83**, 410–413 (2003).

Acknowledgements. Louise Finlayson acknowledges financial support from EPSRC Industrial Doctorate Centre Scheme (2262922) and the Laser Research and Therapy Fund (registered charity SC030850).

OC-4.3.8

RATIONALIZING PHOTOTHERAPY SERVICES DURING THE COVID-19 PANDEMIC: STRATEGIES AND IMPACTS ON PATIENT ACCESS AND OUTCOMES

Tashmeeta Ahad[1, 2], Sunil Kalia[1, 2, 3, 4], Harvey Lui[1, 2].

1) *Department of Dermatology and Skin Science, University of British Columbia, Vancouver, Canada;*
2) *Photomedicine Institute, Vancouver Coastal Health Research Institute, Vancouver, Canada;*
3) *Department of Cancer Control Research, BC Cancer, Vancouver, Canada;*
4) *BC Children's Hospital Research Institute, Vancouver, Canada*

Background: Public health directives throughout the COVID-19 pandemic significantly constrained the provision of phototherapy services to patients with light-responsive dermatoses. At our centre, phototherapy was unavailable from March - June 2020, and then resumed thereafter at 50% capacity.

Objectives: We will assess the impacts of COVID-19 on local phototherapy services including patient outcomes, and the efficiency of prioritization strategies to maximize service delivery while adhering to strict infection control protocols.

Methods: To objectively prioritize and monitor patients before and during phototherapy, nursing staff were trained to serially assess disease severity using a validated Investigator Global Assessment times Body surface area (IGAxBSA) cross-product scoring tool. Dermatology Life Quality Index (DLQI) questionnaires were completed periodically by patients to monitor disease impacts. Patients were treated using either of two phototherapy regimens that incorporated curtailed treatment frequencies (once or twice a week only) over 10 week exposure cycles; higher incremental dosing (up to 20%) at successive sessions was used.

Results: A total of 657 patients were undergoing phototherapy prior to clinic closure. The proportion of contacted patients declining phototherapy following re-opening was 31% (n=142). Baseline assessment of patients with psoriasis (n=192) and eczema (n=71), revealed median IGA x BSA scores of 20 (psoriasis) and 24 (eczema). Capacity modelling revealed a 75% cumulative percentile of IGA x BSA scoring distribution as the most optimal threshold for choosing treatment frequency. Based on this, patients had twice a week treatment if IGA x BSA scores were ≥30 (psoriasis) and ≥40 (eczema) for ten week treatment courses. DLQI ≥21 (extremely large effect on quality of life) qualified for twice weekly treatment regardless of disease severity. Preliminary analysis on efficacy after ten weeks showed median IGA x BSA scores of 9 (psoriasis; n=129) (55% median improvement) and 16 (eczema; n=40) (33% median improvement).

Preliminary Conclusions: Our ongoing review will provide unique insights into the impacts of reduced access to core dermatological services on patient morbidity and the efficacy of objective service rationalization strategies.

P-4.3.9

Global verification of a model for determining daylight photodynamic therapy dose

Paul O'Mahoney[1,2,3], Marina Khazova[4], Ethan LaRochelle[5], Brian Pogue[5], Sally Ibbotson[1,2,3], Ewan Eadie[2,3]
1) University of Dundee, School of Medicine, Dundee, UK; 2) NHS Tayside, Photobiology Unit, Dundee, UK; 3) The Scottish Photodynamic Therapy Centre, Dundee, UK; 4) Public Health England, Didcot, UK; 5) Thayer School of Engineering at Dartmouth, Hanover, USA

Daylight photodynamic therapy (DPDT) is a widely used, effective treatment for field change actinic keratoses (AK). Successful treatment does, however, rely upon a minimum daylight exposure during treatment. This daylight exposure is quantified as the protoporphyrin-IX (PpIX)-effective exposure dose, with current guidelines suggesting a minimum of 8 J cm^{-2} to be delivered over a 2-hour treatment period [1]. Measurement of daylight exposure during DPDT is an important parameter, which can help assist practitioners in delivering optimal therapy.
We recently published a model for DPDT dosimetry (the O'Mahoney model) [2], allowing relatively simple measurements of illuminance from a widely available lux-meter to be accurately converted to PpIX-effective exposure dose (omahoney.shinyapps.io/daylightpdt). Originally validated for use within the UK, in order for the model to be used confidently in other locations, we verified its use globally.
We obtained daylight spectral irradiance measurements from 4 locations: Lebanon NH, USA (43.7 °N, 72.40 °W); Paris, France (48.86 °N, 2.35 °E); Nakhon Pathom, Thailand (13.82 °N, 100.05 °E); and Ribeirão Preto, Brazil (21.18 °S, 47.81 °W). Using these spectral irradiance data, we derived both the illuminance and the PpIX-effective irradiance for each data point (n=33,602). The conversion model was applied to the illuminance data and compared to the PpIX-effective irradiance under all weather conditions.
The median percentage deviation of the model from the true PpIX-effective irradiance was within ±10%, and 95% confidence intervals within ±20%. These results are within expectations with respect to a similar analysis carried out on UK spectral irradiance from our previous publication. The results of this study verify that this conversion model for DPDT dosimetry is suitable for use across the globe and can very simply enhance the confidence of DPDT practitioners with respect to daylight dosimetry.

References

1) Morton et al., Journal of the European Academy of Dermatology and Venerology, 2019; vol 33, 2225-2238
2) O'Mahoney et al., British Journal of Dermatology, 2016; vol 176, 1607-1616

Acknowledgements. Dr Paul O'Mahoney is funded by Medi-lase (registered charity SC 037390) and the Alfred Stewart Trust. The authors would like to thank the following for their contributions to the provision of data used in this work: Prof Luciano Bachmann and Prof Gilberto Braga (University of São Paulo, Brazil), Dr Sumaman Buntoung (Silpakorn University, Thailand) and Dr Einapak Boontaveeyuwat (Chulalongkorn University, Thailand)

P-4.3.10

Redox processes in pathogenesis and phototherapy of vitiligo

T. Telegina[1,2], Yu. Obukhov[1,2], Yu. Vechtomova[1], A. Buglak[2],
1) Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences. Moscow, Russia; 2) Saint Petersburg State University, Saint Petersburg, Russia

Disturbances of homeostasis in skin cells lead to various diseases, including vitiligo, in which melanogenesis in epidermal melanocytes is impaired. Melanocytes like all living cells maintain a balance between oxidation and reduction, and perturbations of this balance are known to contribute to vitiligo pathogenesis. Vitiligo is an autoimmune disease and oxidative stress plays a significant role in melanocyte disappearance. With vitiligo, an excess of H_2O_2 accumulates in melanocytes. Then H_2O_2 induces the synthesis of a 3-5-fold excess of tetrahydrobiopterin (H_4Bip) through γ-interferon, since cyclohydrolase is γ-interferon-induced enzyme. In turn, the inevitably proceeding process of H_4Bip autooxidation leads to the formation of H_2O_2. In this way, the autocatalytic cycle increases oxidative stress. Research objectives for the treatment of vitiligo are as follows: 1) to stop the autocatalytic cycle and 2) to restore balanced redox system. The autocatalytic cycle can be stopped by exposure to UVB radiation. We have shown that UVB radiation converts H_4Bip into dihydropterin dimers (quantum yield equals 2.4%) and thus it is possible to remove the excessive H_4Bip, which arises during the autocatalytic cycle. The mechanism of the process was studied and the maximum of the UV action spectrum was found. Good therapeutic effects were achieved with the combined action of UVB and an antioxidant enzyme that decomposes H_2O_2, for example, pceudocatalase. Restoration of a balanced redox system in melanocytes appears to be resolved by activation of the Nrf2-ARE signaling pathway, which is impaired in vitiligo. The Nrf2-ARE pathway protects melanocytes from H_2O_2 damage through the induction of the antioxidant enzyme system. A number of Nrf2 activators have been used in treating vitiligo with certain therapeutic effects.

Acknowledgements. The research was supported by the Russian Science Foundation grant 20-73-10029.

P-4.3.11

UVA photoprotective effect of adenine derivate kinetin

Denisa Škařupová[1]*, Alena Ryšavá[1], Jitka Vostálová[1], Alena Rajnochová Svobodová[1], Jitka Ulrichová[1], Lucie Plíhalová[2], Bohumil Zálešák[3]
1) Department of Medical Chemistry and Biochemistry, Faculty of Medicine, Palacký University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic; 2) Laboratory of Growth Regulators, Faculty of Science, Palacký University Olomouc & Institute of Experimental Botany AS CR, Šlechtitelů 27, 783 71 Olomouc, Czech Republic; 3) Department of Plastic and Aesthetic Surgery, University Hospital Olomouc, I. P. Pavlova 6, 775 20 Olomouc, Czech Republic.
**denisa.skarupova@upol.cz*

Overexposure of human skin to solar UV radiation (295-400 nm) is associated with many detrimental effects that can result in premature skin aging (photoaging), various skin disorders and photocarcinogenesis. UVA radiation (320-400 nm), the major part of UV light, reaches both epidermal and dermal layer of the skin and triggers massive generation of reactive oxygen species (ROS). Overflow of ROS results in oxidative modification of biomolecules (proteins, nucleic acids, lipids and their structural units), further production of reactive compounds and finally in oxidative stress (OS).
Several antioxidants and cytoprotective proteins are under control of NF-E2-related factor 2 (Nrf2). This transcription factor plays a key role in the defence against xenobiotics, heavy metals and oxidative stress due to its ability to induce the expression of detoxifying enzymes, antioxidant proteins and the suppression of pro-inflammatory signalling pathways. Under normal condition, Nrf2 retains in the cytoplasm via interaction with the Kelch-like ECH associated protein (Keap1). In response to oxidative damage, Nrf2 translocates to the nucleus where it forms a complex with other transcription factors and binds to the promoters of its target genes, antioxidant response element (ARE). This leads to activation of phase 2 detoxifying enzymes and stress-response related proteins such as heme oxygenase 1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), catalase (CAT), glutamate-cysteine ligase catalytic subunit (GCLC)[1].
One possibility to strengthen skin photoprotection is topical application of dermatological preparations containing active ingredients that neutralize UV photons, improve skin regeneration and restore tissue homeostasis. For this purpose, several naturally occurring compounds derived from plants have been investigated/used.
In this study, we examined adenine derivate kinetin (KIN, N6-furfuryladenin), a plant hormone that controls cell growth and differentiation. KIN has found application as the active ingredient in several anti-ageing products in cosmetics but minimal knowledge about its mechanism of action is available[2].
The aim of this study was to determine whether KIN is able to modulate OS and the signalling pathway controlled by Nrf2 in normal human dermal fibroblasts after UVA radiation. KIN has decreased UVA induced ROS production and glutathione depletion and activated the Nrf2 signalling pathway by increasing the nuclear level of Nrf2, which was detected by immunohistochemical analysis. This effect was further evaluated by measuring the level of HO-1, NQO1, GLCL and CAT by western blot analysis. Kinetin UVA photoprotective properties will be discussed in our poster contribution.

References
1) Boo Y. Ch. (2020): Natural Nrf2 modulators for skin protection. Antioxidants 9 (9), 812.
2) Jabłońska-Trypuć A., Matejczyk M., Czerpak R (2016): N6-benzyladenine and kinetin influence antioxidative stress parameters in human skin fibroblasts. Molecular and Cellular Biochemistry 413, 97-107.

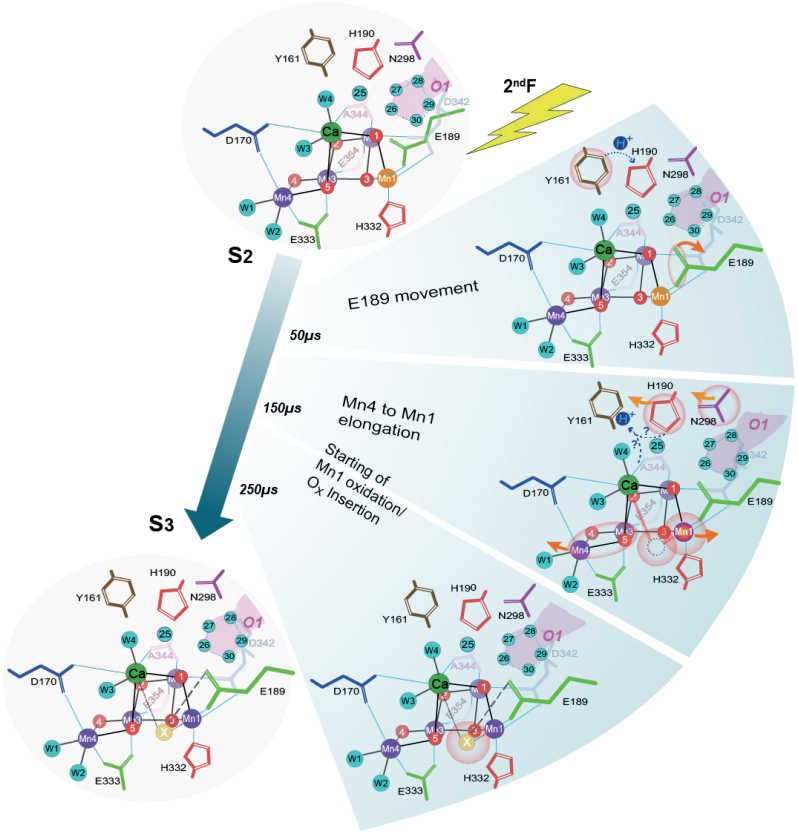
Acknowledgements. This work was financially supported by the grant IGA_LF_2021_013 and by the Institutional Support of Palacký University in Olomouc.

IL-4.4.1

Understanding the Sequence of Events During the Water Oxidation Reaction in Photosystem II using Crystallography/X-ray Spectroscopy

Junko Yano[1], Jan Kern[1], Nicholas K. Sauter[1], Holger Dobbek[2], Uwe Bergmann[3], Johannes Messinger[4], Athina Zouni[2], Vittal K. Yachandra[1]
1) MBIB, Lawrence Berkeley National Lab., Berkeley, USA; 2) Institut für Biologie, Humboldt-Universität zu Berlin, Berlin, Germany; 3) Dept. Physics, Univ. Wisconsin, Madison, USA; 4) Dept. Chemistry, Uppsala Univ. Uppsala, Sweden.

Light-driven oxidation of water to molecular oxygen is catalyzed by the oxygen-evolving complex (OEC) in Photosystem II (PS II). This multi-electron, multi-proton catalysis requires the transport of two water molecules to and four protons from the OEC. Recent advances in X-ray free-electron laser (XFEL)-based room temperature (RT) crystallography enabled us to study the dynamics of the structure of PS II under functional conditions (1, 2). The ability to take snapshots of the structure at the various time points at RT during the reaction allows for the investigation of the structural changes at the OEC, amino acid sidechain movements, and changes in hydrogen bonding networks in the enzyme (Figure 1). These studies can provide new insights into the reaction mechanism in PS II by potentially identifying water and proton pathways.



The results show that the catalytic reaction at the OEC in PS II is well coordinated with the motion of both close and distant residues from the OEC, playing roles in shuttling substrate water and protons essential for the water oxidation reaction.

Fig. 1 – Schematic of the $S_2 \rightarrow S_3$ transition. The sequence of events in time leading to the insertion of Ox between Mn1 and Ca. Mn1 oxidation from (III) to (IV) is shown as a color change from orange to purple. The other Mn atoms are all in oxidation state (IV) and are shown in purple. Ca is shown in green. The ligands of Mn and Ca, Y_Z and other neighboring residues and the water ligands of Mn4 and Ca are shown. Possible pathways for proton transfer are depicted as well.

References

1) Ibrahim, M. *et al.* Untangling the Sequence of Events During the $S_2 \rightarrow S_3$ Transition in Photosystem II: Implications for the Water Oxidation Mechanism, *PNAS*, 117, 12624, 2020.
2) Kern, J. *et al.* Structures of the intermediates of Kok's photosynthetic water oxidation clock, *Nature,* 563, 421, 2018.

Acknowledgements. This work was supported by the Director, Office of Science, Office of Basic Energy Sciences (OBES), Division of Chemical Sciences, Geosciences, and Biosciences (CSGB) of the Department of Energy (DOE), U.S.A., for X-ray methodology and instrumentation, and spectroscopy and crystallography data collection and analysis.

IL-4.4.2

Identity of EPR-detected intermediates in biological water oxidation

Dimitrios A. Pantazis

Max-Planck-Institut für Kohlenforschung, Department of Molecular Theory and Spectroscopy, Kaiser-Wilhelm-Platz 1, 45470 Mülheim an der Ruhr, Germany.

The Mn_4CaO_x cluster of the oxygen-evolving complex (OEC) of photosystem II adopts different electronic and geometric forms as it progresses physiologically through the catalytic water-oxidizing cycle, but also as a result of various physicochemical treatments used to probe specific aspects of its structure and function. Crystallographic studies have achieved reliable atomistic representations of the cluster, and X-ray free electron laser (XFEL) techniques promise even greater achievements. Nevertheless, the information content of such data is still insufficient to produce geometric models that are uniquely defined and chemically accurate, or that satisfy in obvious ways the electronic structure constraints provided by spectroscopy. Among the spectroscopies employed in the study of the OEC, magnetic resonance techniques offer the most detailed and selective insight into the electronic structure of the catalyst, as they provide highly local information unaffected by heterogeneity. The structural interpretation of magnetic resonance data for any system as complex as the OEC necessitates the use of quantum chemical modelling.[1] Here I present selected experimental and theoretical studies from my group that focus on the identity of different EPR-detected forms of the OEC in the S_1, S_2, and S_3 states of the catalytic cycle, highlighting the different types of isomerism and heterogeneity (e.g. Jahn–Teller isomerism, valence isomerism, hydration equilibria) that are present in each one of these states,[2,3,4] and discussing possible implications for the mechanism of biological water oxidation.

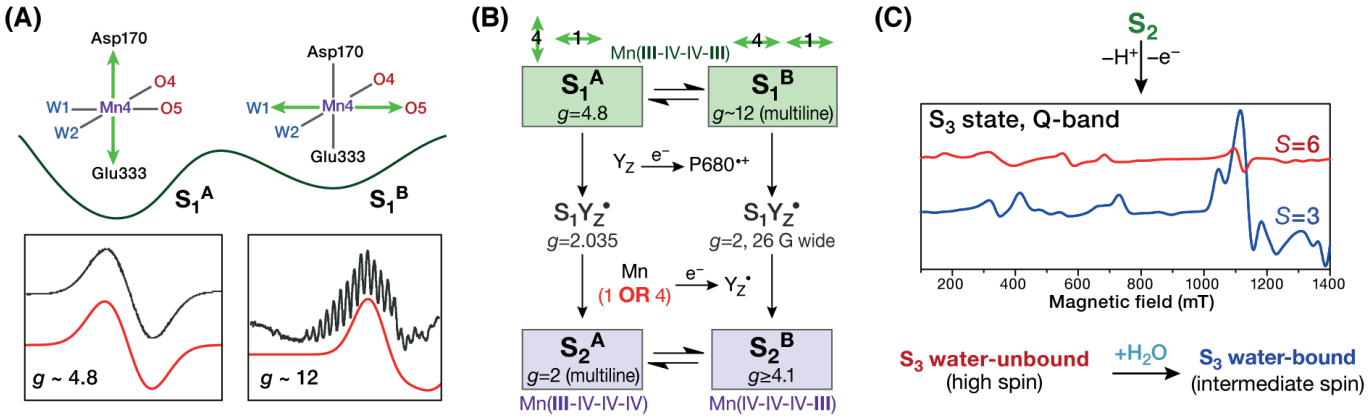


Fig. 1 – A) Orientational Jahn–Teller isomerism in the S_1 state explains two EPR signals in dark-adapted samples and signals attributed to $S_1Y_Z^{\bullet}$ metalloradical intermediates.[2] B) The Jahn–Teller isomers naturally evolve into distinct valence isomeric forms in the S_2 state.[2,3] C) EPR studies of the S_3 state indicate the presence of a water-unbound high-spin and high-anisotropy species as a major structural form, alongside multiple intermediate-spin low-anisotropy water-bound populations.[4]

References

1) M. Orio, D. A. Pantazis, "Successes, challenges, and opportunities for quantum chemistry in understanding metalloenzymes for solar fuels research", *Chem. Commun*. **2021**, *57*, 3952-3974.

2) M. Drosou, G. Zahariou, D. A. Pantazis, "Orientational Jahn–Teller Isomerism in the Dark-Stable State of Nature's Water Oxidase", *Angew. Chem., Int. Ed.* **2021**, DOI: 10.1002/anie.202103425.

3) D. A. Pantazis, W. Ames, N. Cox, W. Lubitz, F. Neese, "Two Interconvertible Structures that Explain the Spectroscopic Properties of the Oxygen-Evolving Complex of Photosystem II in the S_2 State", *Angew. Chem., Int. Ed.* **2012**, *51*, 9935-9940.

4) G. Zahariou, N. Ioannidis, Y. Sanakis, D. A. Pantazis, "Arrested substrate binding resolves catalytic intermediates in higher-plant water oxidation", *Angew. Chem., Int. Ed.* **2021**, *60*, 3156-3162.

IL-4.4.3

Electron and Proton Releasing Sites in the Oxygen-Evolving Complex of Photosystem II

Hiroshi Ishikita[1]
1) Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan

In photosystem II (PSII), O_2 evolution proceeds at the Mn_4CaO_5 cluster, releasing four protons. Formation of the proton-conducting wire via H-bonds is prerequisite for proton transfer. In particular, when the pK_a values of the H-bond donor and acceptor moieties are nearly equal, a short low-barrier H-bond forms and the release of the proton from the H-bond donor moiety occurs most efficiently along the barrier-less potential (Figure 1). The O…O distances in typical low-barrier H-bonds are < ~2.5 Å, which are significantly shorter than the O…O distances of ~2.8 Å for typical standard H-bonds. According to Warshel (2013 Nobel Prize in Chemistry), a low-barrier H-bond cannot be defined by only the distance or strength of an H-bond: identification of a low-barrier H-bond can be valid only if the shape of the potential-energy profile of the H-bond is symmetric (i.e., the pK_a values for the two moieties are nearly equal) (1). Among short H-bonds identified in the PSII crystal structures, the following H-bonds, TyrZ…D1-His190 (2.47 Å) (2), Q_B…D1-His215 (2.47 Å) (3), and O4…W539 (2.45 Å) (4) are recognized as low-barrier H-bonds.

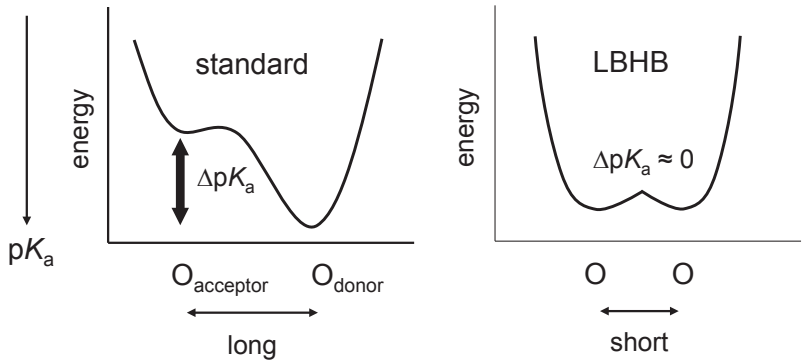


Fig. 1 –Typical potential-energy profiles of H-bonds. (left) Standard H-bond. (right) Low-barrier H-bond (LBHB).

The X-ray free electron laser (XFEL) structures also show significantly short O…O distances, in particular, at the $Mn_4CaO_{5/6}$ moiety, e.g., O5…O6 (1.90 Å) (5) and O4…H_2O (2.25 Å) (5). In this talk, the chemical origins of the short O...O distances will be disucssed (6, 7).

References

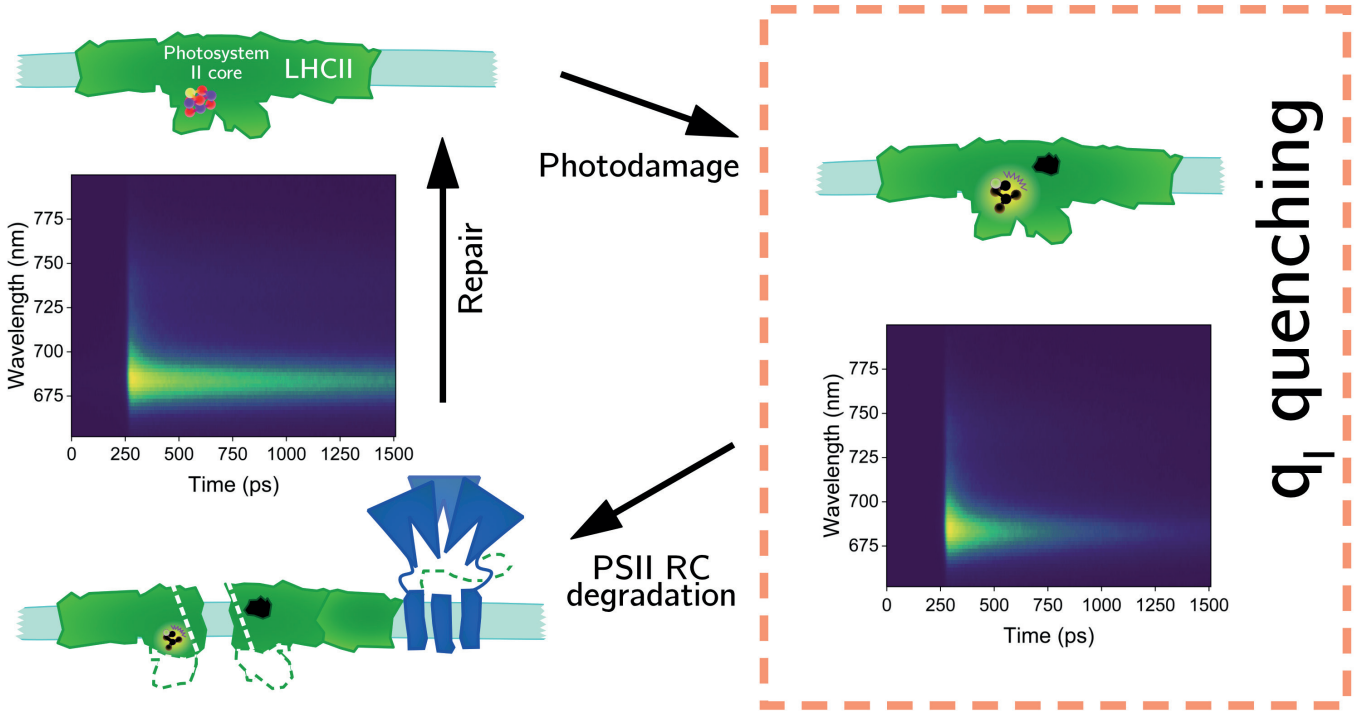
1. C. N. Schutz, A. Warshel, The low barrier hydrogen bond (LBHB) proposal revisited: the case of the Asp... His pair in serine proteases. *Proteins* **55**, 711-723 (2004).
2. K. Saito, J.-R. Shen, T. Ishida, H. Ishikita, Short hydrogen-bond between redox-active tyrosine Y_Z and D1-His190 in the photosystem II crystal structure. *Biochemistry* **50**, 9836-9844 (2011).
3. K. Saito, A. W. Rutherford, H. Ishikita, Mechanism of proton-coupled quinone reduction in Photosystem II. *Proc Natl Acad Sci U S A* **110**, 954-959 (2013).
4. K. Saito, A. W. Rutherford, H. Ishikita, Energetics of proton release on the first oxidation step in the water-oxidizing enzyme. *Nat. Commun.* **6**, 8488 (2015).
5. M. Suga *et al.*, An oxyl/oxo mechanism for oxygen-oxygen coupling in PSII revealed by an X-ray free-electron laser. *Science* **366**, 334-338 (2019).
6. M. Mandal, K. Saito, H. Ishikita, The nature of the short oxygen-oxygen distance in the Mn_4CaO_6 complex of the photosystem II crystals. *J. Phys. Chem. Lett.* **11**, 10262-10268 (2020).
7. M. Mandal, K. Saito, H. Ishikita, Two Distinct Oxygen-Radical Conformations in the X-ray Free Electron Laser Structures of Photosystem II. *J. Phys. Chem. Lett.* 10.1021/acs.jpclett.1c00814, 4032-4037 (2021).

OC-4.4.4

The rise and fall of the photoinhibition-related energy dissipation q_I

Wojciech J. Nawrocki[1,2,*], Xin Liu[1,2], Bailey Raber[3], Chen Hu[1,2], Sandrine Bujaldon[4], Catherine de Vitry[4], Doran I. G. Bennett[3], Roberta Croce[1,2]
*1) Biophysics of Photosynthesis, Department of Physics and Astronomy, Faculty of Science, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, The Netherlands; 2) LaserLaB Amsterdam, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, The Netherlands; 3) MesoScience Lab, Department of Chemistry, Southern Methodist University, Dallas TX, USA; 4) Institut de Biologie Physico-Chimique, UMR 7141 CNRS-Sorbonne Université, 75005 Paris, France; *w.j.nawrocki@vu.nl*

Plants use light energy to oxidise water and reduce carbon dioxide in the process of photosynthesis. However, while light is necessary for carbon fixation, it can also damage the photosynthetic machinery - in particular the water-splitting complex - causing photoinhibition of Photosystem II.
While the inactive PSII in the closed state (Q_A^-) exhibits high fluorescence yield due to the lack of ongoing photochemical quenching, upon photoinhibition the level of fluorescence *decreases*. This slowly-relaxing quenching is termed q_I. Here, we investigate the fate of the excitation energy after the establishment of q_I in the presence of photodamaged PSII reaction centres, in a context of a wild-type or altered proteostasis. We use time-resolved and steady-state fluorescence together with oxygen evolution measurements and biochemistry, which we combine with genetic approaches using the green alga *Chlamydomonas reinhardtii*. Finally, we integrate the results using membrane-scale structure-function modelling of excitation energy transfer to reveal the site and possible mechanism of the q_I quenching.



IL-4.4.5

Fast substrate water exchange in the S_2 state of photosystem II is controlled by proteins surrounding the Mn_4CaO_5 cluster – implications for the water oxidation mechanism

Casper de Lichtenberg[1,2], Christopher J. Kim[3], Petko Chernev[2], Richard J. Debus[3] & Johannes Messinger[1,2]

1) Umeå University, Department of Chemistry, Umeå, Sweden; 2) Uppsala University, Department of Chemistry – Ångström Laboratory, Uppsala, Sweden; 3) University of California – Riverside, Department of Biochemistry, Riverside, California, USA

The molecular oxygen we breathe is produced from two water-derived oxygen species bound to the Mn_4CaO_5 cluster in photosystem II (PSII). Present research points to the central oxo-bridge O5 as one of these 'substrate waters', while, in the S_2 state, the terminal water ligands W2 and W3 both remain candidates for the second substrate.[1-3] Intense efforts are also ongoing to identify the main access pathway for water to the catalytic site, and to determine whether specific amino acids limit the exchange rate of substrate with bulk water. In this study, we measured the rates of $H_2{}^{16}O/H_2{}^{18}O$ substrate water exchange in the S_2 and S_3 states of wild-type (WT), D1-E189Q and D1-D61A PSII core complexes from *Synechocystis* sp. PCC 6803. We found that the exchange rates were unaffected by the E189Q mutation (O1 channel), but strongly perturbed by the D61A mutation (Cl1/O4 channel). It is concluded that D61 forms a rate-limiting barrier for the isotopic equilibration of the inner water pool near the Mn_4CaO_5 cluster that is alliviated in the D1-D61A mutant (Figure 1). This finding removes the main argument against the Ca-bound W3 as fast substrate water in the S_2 state, namely the indifference of its exchange rate towards Ca/Sr substitution. Our data are thus in full agreement with the proposal that W3 forms the new Ox/O6 bridge in the S_3 state, and that the O-O bond is formed *via* oxo-oxyl radical coupling between O5 and Ox in the S_4 state. The role of multiple conformations in each S state as well as possible alternative assignments of the substrate waters will be discussed.

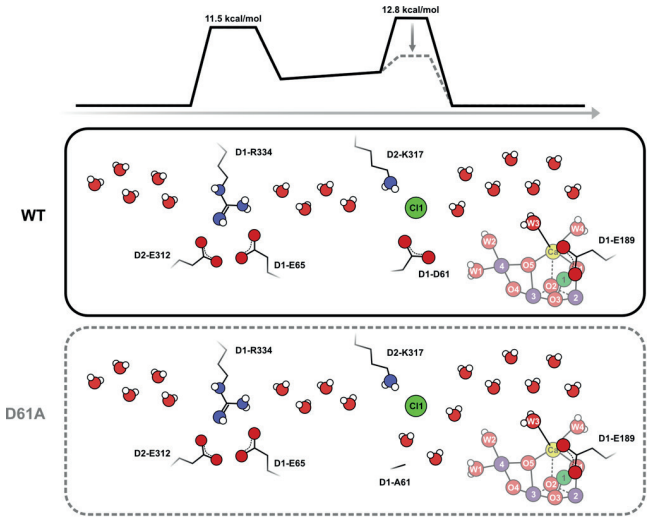


Fig. 1 – Schematic of the Cl1-channel in photosystem II with estimated energy barriers for water exchange. The truncation of the D1-D61 to D1-A61 is proposed to open up the major bottleneck for the equilibration of the inner water pool that includes W3.

References

1) N. Cox, J. Messinger, Reflections on substrate water and dioxygen formation, Biochim. Biophys. Acta 1827, 1020-1030, 2013.
2) C. de Lichtenberg, J. Messinger, Substrate water exchange in the S_2 state of photosystem II is dependent on the conformation of the Mn_4Ca cluster, Phys. Chem. Chem. Phys. 22, 12894-12908, 2020.
3) C. de Lichtenberg, A. P. Avramov, M. Zhang, F. Mamedov, R. L. Burnap and J. Messinger, The D1-V185N mutation alters substrate water exchange by stabilizing alternative structures of the Mn_4Ca-cluster in photosystem II, Biochim. Biophys. Acta 1862, 148319, 2021.

Acknowledgements. This work was supported by ÅForsk (to CDL), Vetenskapsrådet (2016-05183 and 2020-03809; to JM) and the US Department of Energy (DE-SC0005291; to RJD).

IL-4.4.6

Infrared study on the mechanisms of water oxidation at the Mn cluster and its photoassembly in photosystem II

Takumi Noguchi
Graduate School of Science, Nagoya University, Nagoya, Japan

Photosynthetic water oxidation is carried out in photosystem II (PSII) embedded in the thylakoid membranes of plants and cyanobacteria. The catalytic center is the Mn_4CaO_5 cluster, which is assembled on electron donor side of PSII by light-driven process called photoactivation. Recent crystallographic studies using an X-ray free electron laser have shown the structures of the Mn_4CaO_5 cluster and the surrounding protein moiety in the dark stable state (S_1 state) and flash-induced intermediates (S_2, S_3, and S_0 states).[1] However, the details of the reactions in the intermediate transitions and the process of the photoassembly of the Mn_4CaO_5 cluster remain unresolved. In this study, we have investigated the molecular mechanisms of the photoassembly of the Mn_4CaO_5 cluster and the water oxidation reaction using light-induced Fourier transform infrared (FTIR) difference and time-resolved infrared spectroscopies.

(1) Photoassembly process of the Mn_4CaO_5 cluster. We applied rapid-scan time-resolved FTIR spectroscopy combined with the attenuated total reflection (ATR) technique to monitor the photoassembly process of the Mn_4CaO_5 cluster.[2] Rapid-scan ATR-FTIR spectra of apo-PSII with Mn^{2+} upon flash illumination showed spectral features typical of the carboxylate stretching vibrations. These features were attributed to the two carboxylate groups from D1-D170 and D1-E189 coordinating to the high affinity Mn^{2+} ion by quantum chemical calculations. The FTIR signal decayed with a time constant of ~0.7 s, showing that the following "dark rearrangement" step occurred with a low quantum yield and the oxidized Mn^{3+} ion was mostly released during this decay. Simulation of the kinetic process provided a slow intrinsic rate (τ = ~20 s) of the dark rearrangement, which was attributed to the large protein conformational change of the C-terminus of the D1 protein and relocation of the Mn^{3+} ion. The structural fluctuations of the lumenal domain of the CP43 protein visualized by high-speed atomic force microscopy[3] is suggested to facilitate this dark process. The model of the early process of the photoassembly of the Mn_4CaO_5 cluster was proposed (Figure 1).

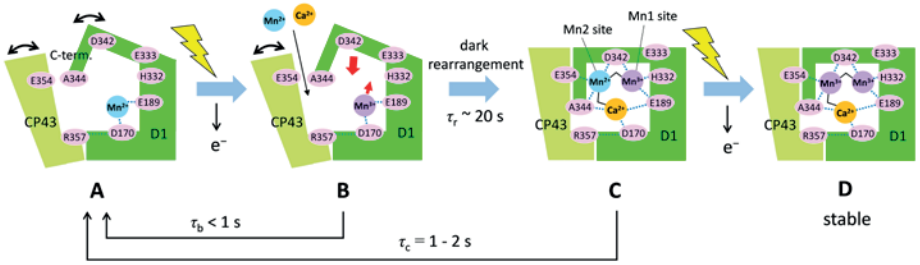


Fig. 1 – Schematic model of the early steps of the photoassembly of the Mn_4CaO_5 cluster.

(2) Water and proton pathways during the $S_2 \rightarrow S_3$ transition. The $S_2 \rightarrow S_3$ transition, which involves concerted water and proton transfer, is a key process in the water-oxidation mechanism. To identify the water and proton pathways during the $S_2 \rightarrow S_3$ transition, we examined the effects of D1-N298A mutation and NO_3^- substitution for Cl^-, which perturbed the O1 and Cl channels, respectively, on the $S_2 \rightarrow S_3$ kinetics using time-resolved infrared spectroscopy. The $S_2 \rightarrow S_3$ transition was retarded both upon NO_3^- substitution and upon D1-N298A mutation, whereas it was unaffected by further NO_3^- substitution in the N298A PSII. The H/D kinetic isotope effect in the N298A PSII was small, revealing that water transfer is a rate-limiting step in this mutant. From these results it was suggested that during the $S_2 \rightarrow S_3$ transition, water delivery and proton release occur through the O1 and Cl channels, respectively

References

1) M. Suga, et al., *Nature*, 543, 131−135 (2017); J. Kern, et al., *Nature*, 563, 421−425 (2018).
2) A. Sato, Y. Nakano, S. Nakamura, and T. Noguchi, *J. Phys. Chem. B*, 125, 4031–4045 (2021).
3) T. Tokano, Y. Kato, S. Sugiyama, T. Uchihashi, and T. Noguchi, *J. Phys. Chem. B*, 124, 5847–5857 (2020).

IL-4.4.7

Light-driven formation of high-valent manganese oxide by photosystem II supports evolutionary role in early bioenergetics

Petko Chernev,[1,2] Sophie Fischer,[1] Jutta Hoffmann,[1] Nicholas Oliver,[1] Ricardo Assunção,[1] Boram Yu,[1] Robert L. Burnap,[3] Ivelina Zaharieva,[1] Dennis J. Nürnberg,[1] Michael Haumann,[1] Holger Dau[1]

1) Freie Universität Berlin, Physics Department, Berlin, Germany; 2) present address: Uppsala University, Department of Chemistry, Uppsala, Sweden; 3) Oklahoma State University, Dept. of Microbiology and Molecular Genetics, Stillwater, United States

Water oxidation and concomitant O_2-formation by the Mn_4Ca cluster of oxygenic photosynthesis has shaped the biosphere, atmosphere, and geosphere. It has been hypothesized that at an early stage of evolution, before photosynthetic water oxidation became prominent, photosynthetic formation of Mn oxides from dissolved Mn^{2+} ions may have played a key role in bioenergetics and possibly facilitated early geological manganese deposits. Here the experimental evidence for the ability of photosystems to form extended Mn oxide particles, lacking until now, is reported. We tracked the light-driven redox processes in spinach photosystem II (PSII) particles devoid of the Mn_4Ca cluster by UV-vis and X-ray spectroscopy. We find that oxidation of aqueous Mn^{2+} ions results in PSII-bound Mn(III,IV)-oxide nanoparticles of the birnessite type comprising 50-100 Mn ions per PSII.

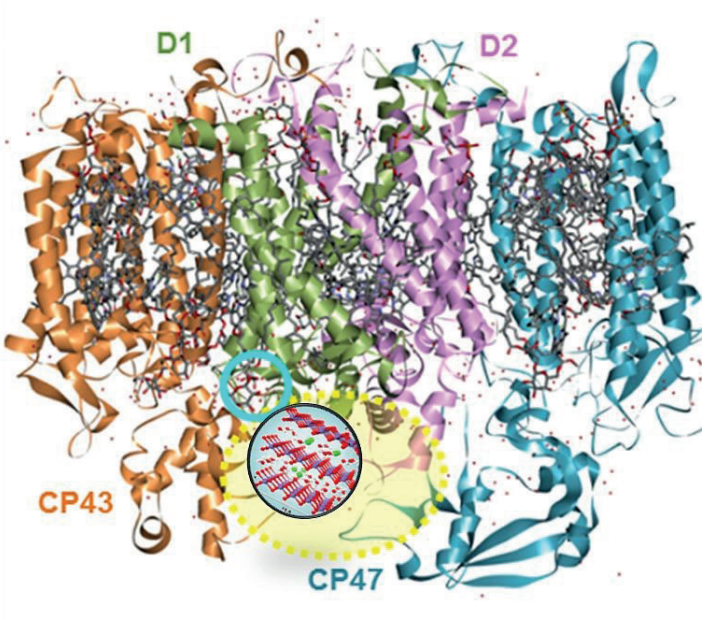


Fig. 1 – Photosystem II depleted of the PsbO protein (yellow void) and proposed formation site of Mn oxide nanoparticles (schematically shown as beeing spherical).

Having shown that even today's photosystem-II can form birnessite-type oxide particles efficiently, we propose an evolutionary scenario, which involves Mn-oxide production by ancestral photosystems, later followed by down-sizing of protein-bound Mn-oxide nanoparticles to finally yield today's Mn_4CaO_5 cluster of photosynthetic water oxidation. Moreover, we discuss the hypothesis of an early quasi-respiratory cycle that involves formation of Mn(III/IV) oxide particles followed by utilization of the oxidizing equivalents stored in the Mn oxide for an efficient quasi-respiratory activity in the Archean or early Paleoproterozoic, when the Earth's atmosphere had been essentially O_2-free, as detailed in ref[1].

The above results, reported in ref[2], are complemented by very recent findings addressing inter alia the question why Nature did choose manganese at the catalytic site of photosynthetic water oxidation (versus Fe, Co, and Ni; Oliver et al, unpublished).

References

1) Dau, H., Nürnberg, D.J., and Burnap, R.L. (2019). *Local cycle of photosynthesis and quasi-aerobic respiration facilitated by manganese oxides – a hypothesis on the evolution of phototrophy.* In Bioenergetics: Oxygen Production and Reduction, J. Barber, P. Nixon, and A.V. Ruban, eds. (World Scientific Publishers), pp. 367-396 (2019)

2) Chernev, P., Fischer, S., Hoffmann, J., Oliver, N., Assuncao, R., Yu, B., Burnap, R.L., Zaharieva, I., Nurnberg, D.J., Haumann, M., and Dau, H. (2020). *Light-driven formation of manganese oxide by today's photosystem II supports evolutionarily ancient manganese-oxidizing photosynthesis*. Nature Communications 11, ARTN 19378 10.1038/s41598-020-75397-8 (2020)

OC-4.4.8

Harvesting the far-red with plant antenna complexes incorporating chlorophyll *d* & *f*

Eduard Elias[1], Nicoletta Liguori[1], Judith Schäfers[1], Yoshitaka Saga[2], Roberta Croce[1]

1) Vrije Universiteit Amsterdam, Faculteit der Exacte Wetenschappen, AmsterdamThe Netherlands; 2) Kindai University, Faculty of Science and Engineering, Osaka, Japan

Increasing the absorption cross-section of plants by introducing far-red absorbing chlorophylls (Chls) has been proposed as a strategy to boost crop yields. To make this strategy effective, these Chls should bind to the photosynthetic complexes without altering their functional architecture. To investigate if plant-specific antenna complexes can provide the protein scaffold to accommodate these Chls, we have reconstituted the main light-harvesting complex of plants LHCII, with the red-shifted Chls *d* & *f*. The results demonstrate that LHCII can naturally bind these exogenous Chls, shifting the maximum absorption ~30 nm towards the red with respect to the wildtype complex (LHCII with Chl *a* and *b*), while maintaining the native LHC architecture. A variety of spectroscopic measurements show that the complexes binding the red-shifted Chls are functional in light harvesting and excitation energy transfer. Overall, we here demonstrate that it is possible to obtain plant LHCs with enhanced far-red absorption and intact functional properties.

Acknowledgements. This work is supported by The Netherlands Organization for Scientific Research (NWO), via a TOP grant (to R.C). N.L. was supported by a NWO Veni grant. Y.S by a JSPS KAKENHI grant (JP17KK0106).

PL-8

Photoremediation

Mathias O. Senge[1]
1) Trinity College Dublin, The University of Dublin, School of Chemistry, Trinity Biomedical Sciences Institute Dublin, Ireland

Light can kill and light can heal, as already exemplified in Herodotus' heliotherapy. Light also has global relevance through photosynthesis and the importance of atmospheric photochemical reactions for life on earth. Critical global problems are weapons of mass destruction, pandemics, food production, climate change, environmental protection, clean energy production, green chemistry, and developing a circular economy. Throughout their development the photosciences have contributed advances to many of these areas. Critical contributions of photobiology were made to agriculture, health, photosynthesis, biorefineries or of photochemistry to photovoltaics, photonics materials and photocatalysis, and more.
In photomedicine light is used to promote and restore human health and similarly light plays a central role in restoring the health of our planet. Light can mitigate, repair, and remediate many of the global problems outlined above. In the classic photobiological field of photodynamic therapy we have seen a recent resurgence of studies on antimicrobial photodynamic therapy driven by increased antibiotic resistance and new threats like the Covid-19 pandemic. The pressing need to tackle environmental issues has prompted research on protection and remediation using light-activated systems for the identification, degradation or transformation of pollutants. Classic approaches on photodecomposition or disinfection using photosensitizers are now combined with detection systems and one can envisage complex devices that can detect, identify, and combat pollutants, CBRN materials, and microbes. The goal is not only to degrade or sequester pollutants but to transform them into benign and value-added materials in a manner suitable for a circular economy.
It is proposed that photoremediation can serve as a broader discipline to unify the currently often uncoordinated efforts to 'detect, identify, mitigate, transform/remediate' harmful entities. Combined with the established field of bioremediation, which also requires light for photosynthesis, and perhaps aligned with the biorefinery concept it allows a more strategic approach – akin to the One Health initiative – towards tackling pollution, photochemical synthesis, energy conversion and more.

PL-9

Plant based biohybrid systems

Eleni Stavrinidou[1]

1) Laboratory of Organic Electronics, Department of Science and Technology, Linköping University, 601 74, Norrköping, Sweden

Plants convert solar energy to chemical energy, sequester carbon, sample the environment, and synthesize a variety of materials. Augmenting functionalities to plants with smart materials and devices can result in biohybrid systems for energy harvesting, environmental monitoring and in-vivo biofabrication. Previously we demonstrated a conjugated oligomer that was uptaken by the vascular tissue of the plant and in vivo polymerized forming conductors. The thiophene-based molecule polymerized within the living tissue, without external chemical or physical stimuli only due to the physiochemical environment of the plant. We used the biohybrid system for energy storage. Recently, we unravelled the mechanism that drives the polymerization reaction and found that the polymerization is driven from the defence mechanism of the plant through enzymes that are involved in modulating cell wall density. With in-vitro and in-vivo studies we identified the key components, limiting factors and kinetics of the polymerization reaction. Currently we are functionalizing rooted plants forming conductors in parallel with the growth of the plant, with the conjugated polymers integrating into the plant cell wall. In this talk I will present our latest progress on augmenting plants with electronic functionality.

IL-5.1.1

Quenched-phosphorescence oxygen sensing platforms for biomedical research and photobiology

Dmitri B.Papkovsky

School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland

Molecular oxygen (O_2) plays major roles in biology and photobiology as key environmental parameter, metabolic substrate, signalling molecule and source of ROS, therefore monitoring and precise control of O_2 concentration in experimental settings with biological samples is of paramount importance. Quenched-phosphorescence O_2 sensing, particularly in lifetime based mode, provides the basis for a variety of simple, robust and accurate detection platforms that can perform different analytical tasks[1]. In recent years, we have designed a number of such sensor systems based on the longwave emitting Pt-porphyrin and Pt-benzoporphyrin dyes, demonstrated them in physiological studies with various *in vitro* and *ex-vivo* cell and tissue models, and commercialised.

Thus, the solid-state O_2 sensor stickers in conjunction with a handheld optical reader facilitate *macroscopic* control of O_2 by means of non-invasive contactless measurements, e.g. in environmental chambers or sealed vessels. The low-cost calibration-free sensors can be used in convenient and flexible manner, on continuous or disposable basis.

The cell-impermeable water-soluble O_2 probes and sensor coatings provide high-throughput analysis of respiration of mammalian and bacterial cells, which can be measured on standard multi-label readers and in standard microwell plates and customised substrates for cell analysis. Multiplexed with the corresponding pH sensors, they enable detailed multi-parametric assessment of cell metabolism and metabolic rewiring in disease states[2].

The cell-permeable small molecule and nanoparticle based O_2 probes, which provide efficient phosphorescent staining of various mammalian cells, can inform on *in situ* oxygenation in samples containing respiring mammalian cells and 3D tissue models. They enable high-resolution mapping of O_2 concentration in cells and 3D micro-tissue samples and monitoring of their responses to metabolic stimulation by PLIM (Phosphorescence Lifetime Imaging) microscopy and macroscopy[2]. A separate stream of applications includes *in vivo* O_2 imaging using dedicated intravascular probes and 2-photon PLIM microscopy[3].

This talk will describe the operation principles of these O_2 sensing probes and measurement methodologies, and provide representative examples of their applications.

References

1) Papkovsky DB, Dmitriev RI (Eds.), Quenched-phosphorescence Detection of Molecular Oxygen: Applications in Life Sciences, RSC Detection Sciences, 2018, ISBN 978-1-78801-175-4.
2) Papkovsky DB, Zhdanov AV, Phosphorescence based O_2 sensors – essential tools for hypoxia research, cell and tissue physiology (Review), Free Rad Biol Med, 2016, 101: 202–210.
3) Esipova et al., Oxyphor 2P: a high-performance probe for deep-tissue longitudinal oxygen imaging, Cell Metabolism, 2019, 29: 736–744.

Acknowledgements. This work was supported by the Science Foundation Ireland, grant 12/RC/2276_P2.

IL-5.1.2

Mapping microscopic viscosity and temperature using molecular rotors

Marina K Kuimova[1]
1) Imperial College London, Chemistry Department, London, UK;

Viscosity is one of the main factors which influence diffusion in condensed media. In a cell viscosity can play a role in several diffusion mediated processes, such as drug delivery, signalling and mass transport. Previously, alterations in viscosity in cells and organs have been linked to malfunction; however, mapping viscosity on a single-cell scale remains a challenge.

We have imaged viscosity inside lipid mono- and bi-layers, in cells and in atmospheric aerosol particles using fluorescent probes, called molecular rotors.[1, 2] In molecular rotors the speed of rotation about a sterically hindered bond is viscosity-dependent, which strongly affects fluorescence lifetime or spectra of rotors, allowing fluorescence imaging. This approach enabled us to measure both the microscopic viscosity and temperature[2, 3] and monitor their temporal changes in real time. The talk will cover our recent developements of this technique, such as genetic and passive targeting of rotors[4, 5] and applications to monitoring neurodegeneration.[6]

References

1) M. K. Kuimova, *Phys Chem Chem Phys*, 2012, 14, 12671
2) A. Vyšniauskas *et al*, *Int. Rev. Phys. Chem.* 2018, 37:2, 259
3) A. Vyšniauskas, *et al*, *Chem. Sci.*, 2015, 6, 5773
4) J. E. Chambers *et al*, *ACS Nano*, 2018, 12, 4398
5) M. Kubankova *et al*, *Soft Matter, 2018*, 14, 9466
6) M. Kubankova *et al*, *ACS Appl. Mater Interfaces*, 2019, 11, 36307

IL-5.1.3

Fluorescent nanosensors for the measurement of biological systems

Jonathan W. Aylott[1], Reem Abohujel[1], Veeren M. Chauhan[1]
1) University of Nottingham, School of Pharmacy, Nottingham, UK.

Optical nanosensors are the primary focus of this research and utilise the sensitivity of fluorescence to make quantitative real-time measurements of biological systems. Nanosensor devices can be made in the size range 50–500 nm diameter, dependent on the matrix they are synthesised from and the application requirements. The small size of the nanosensors allows them to be delivered directly into the biological system of choice, with minimal physical perturbation, to measure changes in small molecule concentrations. Nanosensors exhibit advantages over widely used fluorescence dye-based methods as they have been designed to be ratiometric so effectively contain an internal standard. The nanosensor matrix also imparts two key benefits:
1. Protection of the sensing component from interfering species within the intracellular environment
2. Protection of the intracellular environment from any toxic effects of the sensing component.
An additional attractive feature of the nanosensors is that they can be imaged and quantified using standard technologies, *e.g.*, confocal microscopy.
Nanosensors capable of measuring a range of analytes including glucose, oxygen, calcium, zinc, pH and proteases have been demonstrated and work is ongoing to expand the range of analytes that can be detected using this technology. In this presentation I will focus on pH and oxygen nanosensors used in our laboratory and discuss a range of applications for nanosensors. These include the delivery of nanosensors to mammalian cell systems, whole organisms and bacterial biofilms.

References
1) M. Elsutohy, V.M. Chauhan, R. Markus, M.A. Kyyaly, S.J.B. Tendler, J.W. Aylott 'Real-time measurement of the intracellular pH of yeast cells during glucose metabolism using ratiometric fluorescent nanosensors' *Nanoscale*, **2017**, 9(18), 5904-5911
2) V.M Chauhan, F. Giuntini, J.W. Aylott 'Quadruple labelled dual oxygen and pH-sensitive ratiometric nanosensors' *Sensing & Bio-sensing Research*, **2016**, 8, 36-42

Acknowledgements. This work was supported by grant EP/N009126/1 from the Engineering and Physical Sciences Research Council (EPSRC), UK

OC-5.1.4

A new method of assessing chloroplast movements using hyperspectral imaging

Justyna Łabuz[1], Paweł Hermanowicz[1]
Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland

Chloroplast movements are an important adaptation optimising photosynthetic efficiency of plants. At low light intensities, chloroplasts gather at cell walls perpendicular to the direction of incident light. This response, called chloroplast accumulation, increases light capture. At high light intensities, chloroplast avoidance is observed, as they move to cell walls parallel to the direction of incident light, to protect the photosynthetic apparatus. Seed plants move chloroplasts in response to blue light, however, also red light is active in some ferns, mosses and green algae. Several methods for tracking chloroplast positioning have been developed. Microscopic observations play a pivotal role for assessing chloroplast positions within leaf cells, but they are laborious[1]. Measuring transmittance changes with photometric devices is a convenient quantitative approach, however it is still limited in its throughput[2]. Apart from absorption and transmission, a fraction of light impinging on a leaf undergoes reflection[3]. Red light leaf reflectance was shown to be useful for examining of chloroplast positions[4].
We propose a new method of assessing chloroplast movements based on leaf reflectance, in which spectral and spatial reflectance characteristics is used for improved detection accuracy. In this study, we employed a halogen lamp and a hyperspectral camera to investigate chloroplast positions after low and high intensity blue light irradiation, which induced the chloroplast accumulation and avoidance response, respectively. We acquired spectra of leaf parts that were either non-irradiated or irradiated with blue light, for *Arabidopsis thaliana* wild type and photoreceptor mutants. The mean reflectance of dark adapted leaves is 11% in visible light (400-700 nm). The average difference of reflectance in this range between wild type leaves with chloroplasts in avoidance and accumulation positions is ca. 2.3%. This difference is clearly visible in the hyperspectral images of partly irradiated leaves. To examine whether the changes in reflectance spectra induced by chloroplast movements are species-specific, we performed further experiments on *Nicotiana benthamiana* leaves. We employed machine learning methods, using classifiers trained on spectra from *Arabidopsis*, to distinguish between spectra recorded on low and high blue light irradiated *Nicotiana* leaves. We obtained a low rate of misclassification in this cross-species setting, which suggests that it may be feasible to automatically assess chloroplast positioning using classification algorithms on hyperspectral images of heterogenous vegetation patches.

References

1. Kagawa, T. & Wada, M. Blue Light-Induced Chloroplast Relocation in Arabidopsis thaliana as Analyzed by Microbeam Irradiation. *Plant and Cell Physiology* **41**, 84–93 (2000).
2. Walczak, T. & Gabrys, H. New type of photometer for measurements of transmission changes corresponding to chloroplast movements in leaves. *Photosynthetica* **14**, 65–72 (1980).
3. Baránková, B., Lazár, D. & Naus, J. Analysis of the effect of chloroplast arrangement on optical properties of green tobacco leaves. *Remote Sensing of Environment journal* **174**, 181–196 (2016).
4. Dutta, S. *et al.* Non-invasive, whole-plant imaging of chloroplast movement and chlorophyll fluorescence reveals photosynthetic phenotypes independent of chloroplast photorelocation defects in chloroplast division mutants. *Plant Journal* **84**, 428–442 (2015).

IL-5.1.5

It's Getting Hot in Here: Intracellular Temperature Sensing Through Light Emission

L. D. Carlos
Phantom-g, CICECO-Aveiro Institute of Materials, Physics Department, University of Aveiro, 3810-193 Aveiro, Portugal

The emergence of luminescent nanothermometry during the last decade opened up the possibility of measure thermal flows at spatial scales below 10 μm, unreachable by conventional electrical methods[1]. Diverse phosphors capable of providing a contactless thermal reading through their light emission properties have been examined, e.g., polymers, DNA or protein conjugated systems, organic dyes, quantum dots, and trivalent lanthanide (Ln^{3+}) ions incorporated in organic-inorganic hybrids, multifunctional heater-thermometer nanoplatforms, upconverting, downconverting and downshifting nanoparticles. The implementation of these Ln^{3+}-based phosphors (with an emphasis in upconverting nanoparticles) as ratiometric thermometers was extensively reviewed in the past five years[1].
In the last couple of years, the focus of luminescence thermometry has gradually shifted from the fabrication of more sensitive nanoarchitectures towards the use of the technique as a tool for thermal bioimaging and the unveiling of properties of the thermometers themselves and their local surroundings, as, for instance, the instantaneous ballistic velocity of Brownian nanocrystals suspended in both aqueous and organic solvents[2].
After a general perspective of the work done on luminescence nanothermometry since the explosion of the field at one decade ago, the lecture will be focused on a recent example[3] illustrating the potential of the technology to measure the intracellular temperature.

References

1) C. D. S. Brites, S. Balabhadra, L. D. Carlos, Lanthanide-Based Thermometers: At the Cutting-Edge of Luminescence Thermometry. *Adv. Opt. Mater.* 7, 1801239, 2019.
2) C. D. S. Brites, X. Xie, M. L. Debasu, X. Qin, R. Chen, W. Huang, J. Rocha, X. Liu, L. D. Carlos, Instantaneous ballistic velocity of suspended Brownian nanocrystals measured by upconversion nanothermometry. *Nature Nanotech.* 11, 851, 2016
3) R. Piñol, J. Zeler, C. D. S. Brites, Y. Gu, P. Téllez, A. N. Carneiro Neto, T. E. da Silva, R. Moreno-Loshuertos, P. Fernandez-Silva, A. Isabel Gallego, L. Martinez-Lostao, A. Martínez, L. D. Carlos, A. Millán, Real-Time Intracellular Temperature Imaging Using Lanthanide-Bearing Polymeric Micelles. *Nano Lett.* 20, 9, 6466, 2020.

IL-5.1.6

Colorimetric and luminescent nanomaterials as new nanoplatforms for sensing and drug delivery in biological environments.

Elisabete Oliveira[1,2], Carlos Lodeiro[1,2], Maria P. Duarte[3], and Jose Luis Capelo[1,2]

1) BIOSCOPE Group, LAQV-REQUIMTE, Chemistry Department, NOVA School of Science and Technology, FCT NOVA, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; 2) PROTEOMASS Scientific Society, Rua dos Inventores, Madam Parque, Caparica Campus, Portugal; 3) MEtRICs/DCTB, NOVA School of Science and Technology, NOVA University of Lisbon, 2829-516, Caparica, Portugal.

Inorganic silica mesoporous nanoparticles (MNs) emerged as a multifunctional new generation of nanocarriers as a drug delivery device due to their low toxicity, high specific surface area, large pore volume, tunable pore structures, and sizes [1-5].
From all nanoparticles systems that have been used as drug delivery agents, luminescent inorganic mesoporous silica nanoparticles and silicon nanocrystals emerged as a multifunctional new generation of nanocarriers acting as all-in-one diagnostic and therapeutic tools [1, 2, 3]. Despite the wide utility of MNs for drug delivery/theragnostic, they can also be explored as sorbents for pollutants removal and sensing, while enabling new strategies for separation and purification. The multiple applications of MNs will be addressed such as: (i) cytotoxic evaluation and drug delivery in cancer cells [1, 2]; (ii) antibacterial properties and synergetic effect (antibiotic vs nanoparticles) against bacteria [3] (iii) use as solid-state supported devices for metal remediation via colorimetric and fluorimetric responses in aqueous solutions [4].

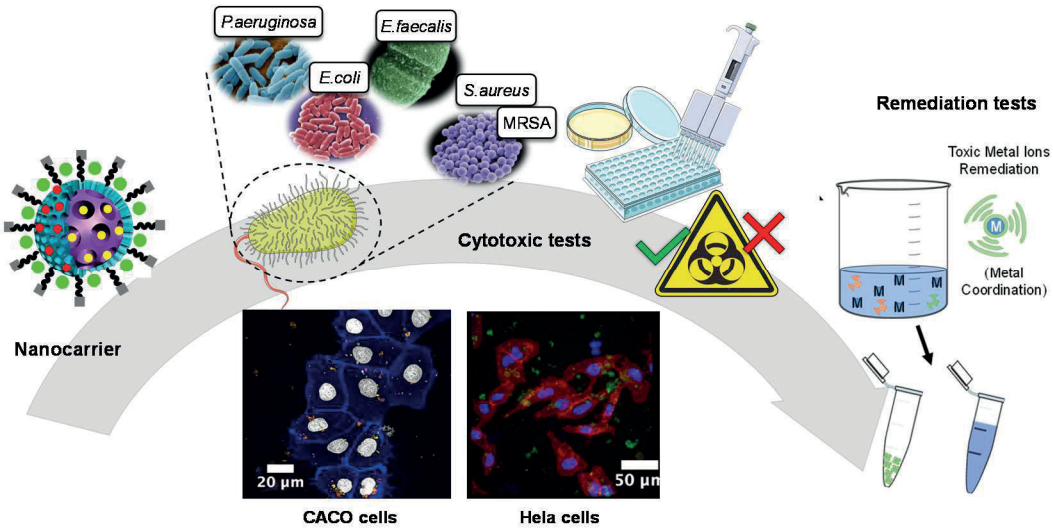


Fig.1. *Luminescent MNs and their applications.*

References

1) G. Marcelo, E. Oliveira, et. al. Materials 11 (2018) 1310.
2) E. Oliveira, H.M. Santos, et al., Dyes and Pigments 161 (2019) 360.
3) S. Nuti, J. Fernández-Lodeiro, et al. Front. Chem. 6 (2018) 554.
4) G. Marcelo, S. M. G. Pires,et al. Dyes and Pigments 161 (2019) 427-437.
5) G. Marcelo, C. Lodeiro et al., Materials Science and Engineering C 106 (2020) 110104.

Acknowledgements. This work was supported by the Associate Laboratory for Green Chemistry - LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020) as well as the Scientific Society PROTEOMASS (Portugal) for funding support (General Funding Grant). G.M thanks to FCT/MEC (Portugal) for his doctoral grant PD/BD/142865/2018. E.O thanks FCT/MEC (Portugal) for the individual contract, CEECIND/00648/2017.

IL-5.1.7

Innovative strategies for luminescent chemosensing coupled to exquisite molecular recognition: illustrative examples in food mycotoxins detection

Guillermo Orellana[1], María C. Moreno-Bondi[2], Ana B. Descalzo[1], Elena Benito-Peña[2], Javier L. Urraca[2]
1) Complutense University of Madrid, Department of Organic Chemistry, Madrid, Spain; 2) Complutense University of Madrid, Department of Analytical Chemistry, Madrid, Spain.

The combination of nanosized materials with luminescence (fluorescence, phosphorescence) interrogation is a powerful tool to design analytical methods and devices with the **sensitivity** required to determine environmental and food contaminants. In the lab practice, the associated indispensable **selectivity** is typically provided by sophisticated, expensive chromatographic techniques coupled to mass spectrometric detection. In contrast, straightforward, affordable chemical sensors rely on biological elements (antibodies, aptamers, …) or bioinspired materials (e. g. molecularly imprinted polymers or MIPs) to achieve the analyte recognition. The challenge to the sensor scientist is to effectively couple *recognition* of the target to luminescence-based *reporting*.

The first example to illustrate our solutions to this challenge for the analysis of food mycotoxins such as **tenuazonic acid** (TeA) is the optical sensing of the contaminant with a luminescent MIP[2]. Both the emission *intensity* and, for the first time, the emission *lifetime* can be used to determine the TeA levels thanks to the synthesis of a novel trifunctional Ru(II) complex containing 2,2'-biimidazole as TeA-binding moiety, and two polymerizable 2,2'-bipyridin-4,4'-diyldimethyl diacrylate ligands. A 9-nm thick MIP shell was grown onto 200-nm SiO_2 beads. The sensor shows fast response (< 5 s) to TeA with no cross-sensitivity to other common β-hydroxycarbonyl food mycotoxins (alternariol, β-zeranol, cyclopiazonic acid). Detection limits as low as 63.8 and 75.2 µg L^{-1} under steady-state and time-resolved luminescence detection, respectively, have been measured without optimization.

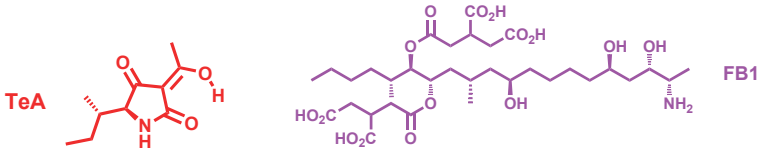


Fig. 1 – Chemical structure of the food mycotoxins analyzed by the novel luminescent sensors illustrated in this presentation.

The second example to illustrate the talk topic is a homogeneous fluorescence quenching immunoassay based on gold nanoparticles (AuNPs) and a recombinant epitope-mimicking fusion protein ("mimotope") to detect and quantify the wheat mycotoxin **fumonisin** B1 (FB1)[3]. The fumonisin mimotope was cloned with a *yellow fluorescent protein* allowing its direct use as the tracer for FB1 detection without requiring a labelled analyte or secondary antibody. Furthermore, due to the fluorescence quenching ability of the AuNPs, the homogeneous immunoassay can be carried out in a single step without further washing to separate the unbound tracer. The quenching immunoassay displays no matrix effect in the wheat extract and high sensitivity for FB1 detection (7.3 to 22.6 ng mL^{-1}), with a detection limit of 1.1 ng mL^{-1}. Only the closely similar FB2 among other mycotoxins tested was also recognized.

References

1) A. Rico-Yuste, R. Abouhany, J. L. Urraca, A.B. Descalzo, G. Orellana, M.C. Moreno-Bondi, Eu(III)-Templated molecularly imprinted polymer used as a luminescent sensor for the determination of tenuazonic acid mycotoxin in food samples. *Sensors Actuat. B: Chem.* 329, 129256, 2021.
2) J. Quílez-Alburquerque, A.B. Descalzo, M.C. Moreno-Bondi, G. Orellana, Luminescent molecularly imprinted polymer nanocomposites for emission intensity and lifetime rapid sensing of tenuazonic acid mycotoxin, 2021 (submitted).
3) R. Peltomaa, F. Amaro-Torres, S. Carrasco, G. Orellana, E. Benito-Peña, M.C. Moreno-Bondi, Homogeneous Quenching Immunoassay for Fumonisin B1 Based on Gold Nanoparticles and an Epitope-Mimicking Yellow Fluorescent Protein. *ACS Nano* 12, 11333-11342, 2018.

Acknowledgements. This work was supported by grants RTI2018-096410-B-C22/21 from the Spanish Ministry of Science and Innovation.

OC-5.1.8

Fluorescent Metal Nanoclusters for MicroRNA Detection

Marina A. Kapitonova[1], Polina S. Malova[1], Alexei I. Kononov[1]
1) Department of Molecular Biophysics and Polymer Physics, Saint Petersburg State University, Saint Petersburg, Russia

MicroRNA are small (18-25 nucleotides) non-coding single stranded RNA sequences. The main function of microRNA is transcriptional and post-transcriptional regulation of gene expression by RNA-interference. MicroRNA is considered to be a promising biomarker of different diseases such as heart diseases, different types of cancer ("oncomirs"), and diseases of neural system. Particularly miR-210, which was presented in this work as a model target, is mostly associated with hypoxia and cancer progression including breast, lung, head, pancreatic cancer, or glioblastoma[1]. Measuring levels of circulating miR-210 in fluids could be a non-invasive method of identifying cancer on early stages. Unfortunately, microRNA detection remains a challenge, because of its length. Traditional methods of microRNA detection have some disadvantages such as poor sensitivity and time consuming of northern blotting or false positive results in real-time qPCR[2]. Last decade new variations and improvements of real-time PCR have been developed: it includes additional steps with reverse transcription or rolling-circle amplification. Also, nanomaterials such as gold nanoparticles[3], magnetic nanoparticles, or quantum dots were applied in microRNA detection.

In this work, we suggest a new method of microRNA detection based on fluorescent silver nanoclusters (AgNCs). Metal nanoclusters are groups of small number (2-100) of metal atoms or ions. Nanosized silver demonstrates unique electrooptical properties, which are different from bulk metals. Stabilized by DNA oligonucleotides, AgNCs with a size about 1-2 nm can develop fluorescent species with a high quantum yield[4], photostability, in a wide range of fluorescence from near UV to IR, and stokes shift up to 200 nm[5].

In order to make AgNCs sensitive to microRNA we used and improved special split model for DNA matrix, that makes fluorescence to turn-on only by an interaction with the target. This technique reduces background signal and false positive probability. The DNA matrix can be divided into three logical parts: the hybridization sequence, which binds complementary to the target, the clusters formation sequence, which is separated into two different DNA-strands (5'-CCCGTTTT-3'and 5'-CCCCACCCCT-3'[6]), and the spacer, which links these two parts together. The optimization of the spacer's length and sequence shows that the best spacer consists of single adenine on each matrix strands. It was found that the most optimal protocol for clusters formation is: 50 mM sodium phosphate buffer at the room temperature and concentration ratio [DNA]:[$AgNO_3$]:[$NaBH_4$] = 1:8:4. The hsa-miR-210-3p (5'-rCrUrGrUrGrCrUrGrUrGrArCrArGrCrGrGrCrUrGrA-3') and it's DNA analog (5'-CTGTGCGTGTGACAGCGGCTGA-3') served as a model target of microRNA. The sensor was successfully tested with DNA- and RNA-targets: the bright green fluorescence, with an excitation at 490 nm and emission at 560 nm, appeared only in the presence of targets. The limit of detection was 3 nM. The linear dependence of fluorescence intensity on target concentration was observed up to 500 nM.

References

1) Bavelloni, A. et al. MiRNA-210: A Current Overview. Anticancer Res. 37, 6511–6521, 2017.
2) Ye, J., Xu, M., Tian, X., Cai, S. & Zeng, S. Research advances in the detection of miRNA. J. Pharm. Anal. 9, 217–226, 2019.
3) Miao, X. et al. Label-Free Platform for MicroRNA Detection Based on the Fluorescence Quenching of Positively Charged Gold Nanoparticles to Silver Nanoclusters. Anal. Chem. 90, 1098–1103, 2018.
4) Gwinn, E., Schultz, D., Copp, S. M. & Swasey, S. DNA-Protected silver clusters for nanophotonics. Nanomaterials 5, 180–207, 2014.
5) Bogh, S. A. et al. Unusually large Stokes shift for a near-infrared emitting DNA-stabilized silver nanocluster. Methods Appl. Fluoresc. 6, 2018.
6) He, C., Goodwin, P. M., Yunus, A. I., Dickson, R. M. & Petty, J. T. A Split DNA Scaffold for a Green Fluorescent Silver Cluster. J. Phys. Chem. C 123, 17588–17597, 2019.

Acknowledgements. This work was supported by RFBR grant № 19-53-51005.

P-5.1.9

Optogenetic high-throughput screening

Kathrin Brenker[12], Annik Jakob[12], Luis Köbele[12]
1) Opto Biolabs, Freiburg, Germany; 2) Albert-Ludwigs-Universität Freiburg, Freiburg, Germany

Optogenetic tools allow the remote control of cellular pathways using light. Originating from the field of neurobiology using light-gated ion channels to study neuronal behavior, more recently developed non-neuronal optogenetic tools enable the isolated, functional investigation of almost any intracellular signaling molecule within complex signaling pathways. This remote control of cellular pathways has major implications for therapeutic tools, including targeted immunotherapy.
A major obstacle for optogenetic researchers is the controlled delivery of light to the cell sample. Hence, the most popular tools for optogenetic studies are microscopy-based experiments or using self-made LED devices. A widely neglected, yet very powerful analytical tool is flow cytometry. The flow cytometer has major advantages over a microscope, including the ability to rapidly measure thousands of cells at single cell resolution. However, it is not yet widely used in optogenetics due to a lack of suitable illumination.
Opto Biolabs, a spinoff from the University of Freiburg, develops specialized illumination devices enabling the combination of optogenetics and flow cytometry. Optogenetic flow cytometry enables the easy analysis of e.g. calcium kinetics and the developed illumination device is compatible with most existing flow cytometers. Our future work will focus on the expansion of our technology to optogenetic cell sorting. This will enable large scale single cell analysis and greatly simplify the discovery and development of novel, cell-based optogenetic tools and therapies.

P-5.1.10

A novel NIR excitable fluorescent probe for intracellular nitric oxide detection: from mouse macrophages to human leukemic cells

Carla Arnau del Valle[1], Paul Thomas[2], M. Paz Muñoz-Herranz[1], Francisco Galindo[3], María J. Marín[1]
1) University of East Anglia, School of Chemistry, Norwich, UK; 2) University of East Anglia, School of Biological Science, Norwich, UK; 3) Universitat Jaume I, Departamento de Química Inorgánica y Orgánica, Castellón, Spain

Understanding intracellular dynamics and concentrations of diverse intracellular analytes is key to develope novel diagnostic tools. Fluorescence-based molecular probes have proben to be key for intracellular imaging and sensing analytes of biological interest such as pH, Ca^{2+}, nitric oxide (NO), etc. Most of the currently available fluorescent probes are excited using ultraviolet or visible light, which results in high photodamage to living cells. Alternatively, the use of near-infrared (NIR) light provides high photostability, low autofluorescence, high biological tissue penetration and minimal photodamage. In the development of diagnostic tools for cancer, one of the most relevant reactive nitrogen species is NO since its effect is strongly related to its concentration. For example, elevated NO levels have been found in tumour cells including lung, breast and leukaemic.[1] Fluorescent NIR excitable molecular probes seem to be ideal candidates for the intracellular detection of NO and could ultimately yield to valuable insights on the biological role played by NO.[2]
The aim of this work is to develop a NIR excitable molecular probe for the intracellular detection of NO *via* a photoinduced electron transfer (PET) mechanism. The probe showed good sensitivity (LOD = 133 nM) and selectivity towards NO, with a fast detection response (2 min). Additionally, the fluorescence emission intensity of the probe was stable in a range of pHs from 4 to 9; and the detection of NO in acidic environments was succesfully evidenced. The NO probe was able to detect NO in a variety of macrophages. In RAW264.7 cells, the fluorescence emission intensity of the probe increased in the presence of NO as confirmed by confocal microscopy images and with the intracellular fluorescence emission spectrum (**Fig. 1**). The probe was also able to detect NO in primary bone-marrow mouse cells and in THP-1 human leukaemia monocytic cells, used as human model. These intracellular studies confirm the versatility of the NO probe to monitor the presence of NO in a variety of cellular environments. The NO probe will next be used to detect intracellular NO in other cancer and healthy cell lines aiming to investigate the differences in NO concentrations between these two environments.

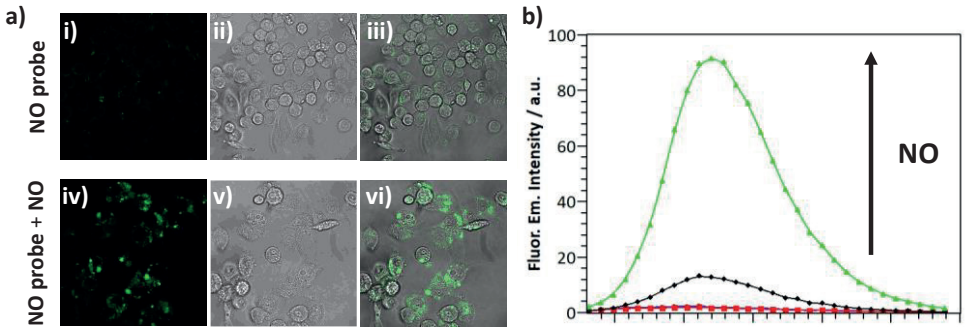


*Fig. 1 – **a)** Confocal microscopy images of RAW264.7 cells treated with NO probe: i - iii) before and iv - vi) after stimulation of the cells to produce NO; and **b)** fluorescence emission spectra of RAW264.7 cells treated with the NO probe before (black) and after (green) stimulation and the control cells without the NO probe before (red) and after (blue) stimulation.*

References

1) S. K. Choudhari, M. Chaudhary, S. Bagde, A. R Gadbail, V. Joshi, Nitric oxide and cancer: a review. *World J. Surg. Oncol.*, 11, 118, 2013
2) H. Yu, Y. Xiao, L. Jin, A Lysosome-targetable and two-photon fluorescent probe for monitoring endogenous and exogenous nitric oxide in living cells. *J. Am. Chem. Soc.*, 134, 42, 2012

Acknowledgements. The authors would like to thank the School of Chemistry and Faculty of Science at the University of East Anglia for financial support. CAdV would like to specially thank Mr and Mrs Whittaker for the FJW Oncology Scholarship.

P-5.1.11

Detection of nitric oxide in live cells using a gold-based near-infrared excitable fluorescent nanoprobe

Carla Arnau del Valle[1], Paul Thomas[2], M. Paz Muñoz-Herranz[1], Francisco Galindo[3], María J. Marín[1]
1) University of East Anglia, School of Chemistry, Norwich, UK; 2) University of East Anglia, School of Biological Science, Norwich, UK; 3)Universitat Jaume I, Departamento de Química Inorgánica y Orgánica, Castellón, Spain

Nitric oxide (NO) is involved in the regulation of diverse physiological and pathological mechanisms of the cardiovascular, nervous and immune systems.[1] NO regulates many physiological processes such as anti-inflammatory effects when present in the cell at low concentrations; however, at high levels, it is related to many pathologies including Alzheimer's disease and cancer.[2] Intracellular NO concentrations can be monitored using fluorescent molecular probes in combination with fluorescence imaging techniques. Most of the currently available NO molecular fluorescent probes are excited using ultraviolet or visible light, which results in poor penetration and high photodamage to living cells. Alternatively, the use of near-infrared (NIR) light provides high photostability, low level of autofluorescence, high biological tissue penetration and minimal photodamage upon long-term irradiation. Although fluorescent probes to image intracellular NO have been reported,[3] further research is needed in the development of novel NIR excitable NO probes for biological applications; and the incorporation of nanoparticles (NPs) to the game seems an exciting approach due to their great versatility.
This work describes the development of a NIR excitable nanoprobe, consisting of a gold nanoparticle functionalised with a NIR excitable NO probe (NOAuNPs), for the detection of NO in live cells. The NO probe contains an *o*-phenylenediamine moiety that reacts with NO forming the corresponding benzotriazole product and resulting in an increase of the fluorescence emission intensity of the probe. The sensitive NOAuNPs showed good selectivity towards NO over other potential cellular interferences and an excellent stability in aqueous medium over time. The intracellular NO detection by the NOAuNPs has been successfully achieved in MDA-MB-231 breast cancer cells (**Fig. 1a**) and it is currently under investigation using THP-1 human leukaemia monocytic cells. The intracellular fluorescence emission spectrum of the NOAuNPs confirmed a significant enhancement in the fluorescence emission intensity in the presence of NO (**Fig. 1b**). The NOAuNPs will be next used to report intracellular NO in other cancer and healthy cell lines to investigate the differences in NO concentrations between these two environments.

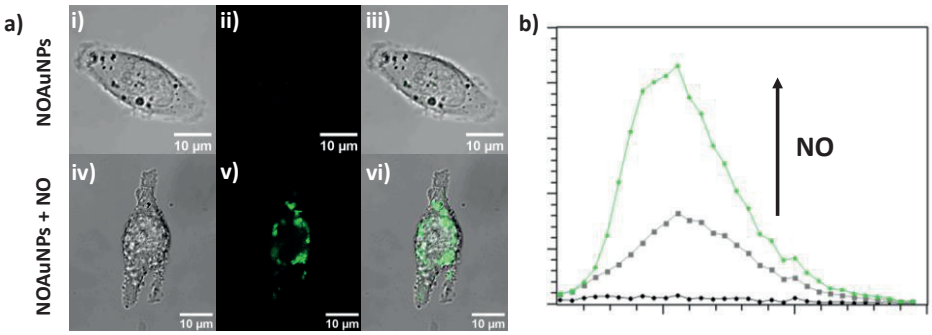


*Fig. 1 – **a)** Confocal microscopy images of MDA-MB-231 cells treated with NOAuNPs, i – iii) before and iv – vi) after addition of NO; **b)** fluorescence emission spectra recorded using confocal microscope of MDA-MB-231 cells treated with NOAuNPs before (grey) and after (green) addition of NO and the control cells without NOAuNPs (black).*

References

1) A. R. Butler, D. L. H. Williams, The physiological role of nitric oxide. *Chem. Soc. Rev.*, 22, 233, 1993
2) S. K. Choudhari, M. Chaudhary, S. Bagde, A. R Gadbail, V. Joshi, Nitric oxide and cancer: a review. *World J. Surg. Oncol.*, 11, 118, 2013
3) M. J. Marín, P. Thomas, V. Fabregat, S. V. Luis, D. A. Russell, F. Galindo, Fluorescence of 1,2-Diamino-anthraquinone and its nitric oxide reaction product within macrophage cells. *ChemBioChem*, 12, 2471-2477, 2011

Acknowledgements. The authors would like to thank the School of Chemistry and Faculty of Science at the University of East Anglia for financial support. CAdV would like to thank Mr and Mrs Whittaker for the FJW Oncology Scholarship.

P-5.1.12

DNA G-quadruplexes recognition by cationic zinc(II) phthalocyanine and graphene oxide hybrid materials

Ana R. Monteiro[1,2], Catarina I. V. Ramos[2], Leandro M. O. Lourenço[2], Sara Fateixa[1], Joana Rodrigues[3], Maria G. P. M. S. Neves,[2] Tito Trindade[1]

1) CICECO, Department of Chemistry of University of Aveiro, Aveiro, Portugal; 2) LAQV-REQUIMTE, Department of Chemistry of University of Aveiro, Aveiro, Portugal; 3) i3N, Department of Physics of University of Aveiro, Aveiro, Portugal.

The stabilization of deoxyribonucleic acid (DNA) G-quadruplex structures promotes the inactivation of telomerase in cancer cells, being considered a valuable anticancer therapy[1]. Several molecules and materials have been used as potential G-quadruplex ligands, such as porphyrins, phthalocyanines and graphene-based materials[2,3]. The current work evaluates the performance of non-covalent hybrids consisting of graphene oxide (GO) and a tetracationic zinc(II) phthalocyanine (ZnPc) as G-quadruplex stabilizing agents (Fig. 1). The Q-bands in the absorption spectrum of the ZnPc@GO hybrid are red-shifted when compared to the counterparts in the free ZnPc. In addition, GO acts as a fluorescence quencher, significantly decreasing the fluorescence emission of ZnPc. Such distinctive optical properties allowed to carry out spectrofluorimetric titrations of the non-immobilized ZnPc and hybridized ZnPc@GO with both the DNA G-Quadruplex (T_2G_5T) and the DNA duplex (ds26) oligonucleotide sequences. Noteworthy, fluorescence recovery was observed in the titration of ZnPc@GO with T_2G_5T, suggesting progressive release of ZnPc from the ZnPc@GO hybrids to stabilize the DNA G-quadruplex structures. This behaviour was not evident in the titration of ZnPc@GO with the DNA duplex. We suggest that GO might act as a nanoplatform for the selective delivery of ZnPc towards different DNA structures, which can be controlled by a "switch off-on" fluorescence model, as already reported for a porphyrin@GO system[3].

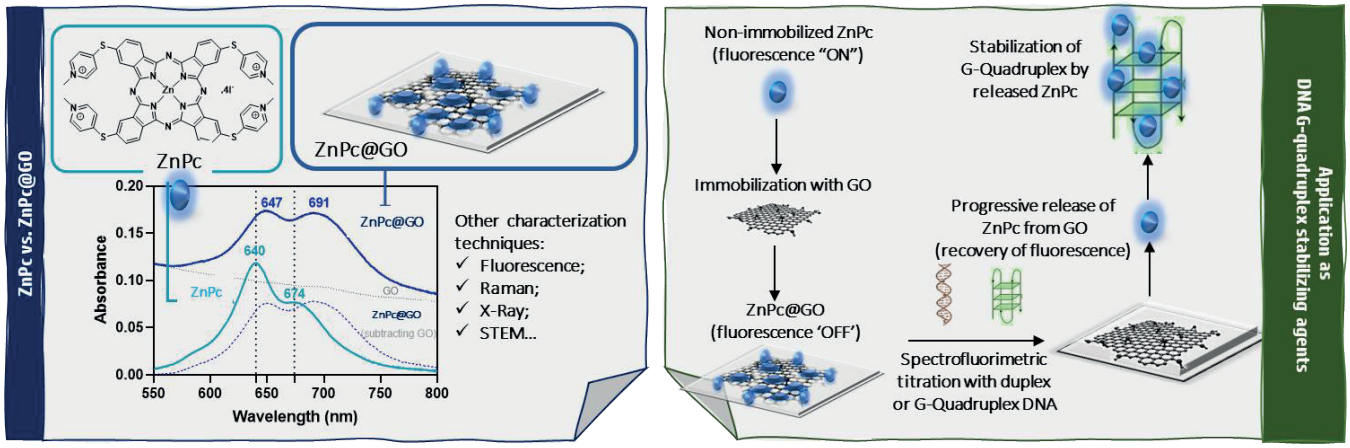


Fig. 1 *– Development of ZnPc@GO hybrids for potential recognition of DNA G-quadruplexes.*

References

1) Kosiol, N.; Juranek, S.; Brossart P.; Heine, A.; Paeschke K.; G-Quadruplexes: a promising target for cancer therapy. Molecular Cancer, 2021, 20, 40.
2) Ramos, C. I. V.; Almeida, S.; Lourenço, L. M. O.; Pereira, P. M. R.; Fernandes, R.; Faustino, M. A. F.; Tomé, J. P. C.; Carvalho, J.; Cruz, C.; Neves, M. G. P. M. S., Multicharged Phthalocyanines as Selective Ligands for G-Quadruplex DNA Structures. Molecules, 2019, 24, 733.
3) Monteiro, A. R.; Ramos, C. I. V.; Fateixa, S.; Moura, N. M. M.; Neves, M. G. P. M. S.; Trindade, T., Hybrids Based on Graphene Oxide and Porphyrins as Tools for Detection and Stabilization of DNA G-Quadruplexes. ACS Omega, 2018, 3, 1184.

Acknowledgements. The authors are grateful to the University of Aveiro and to the Fundação para a Ciência e a Tecnologia (FCT) for the financial support to LAQV-REQUIMTE (Ref.UIDB/50006/2020), CICECO-Aveiro Institute of Materials (Ref.UID/CTM/50011/2020), FCT project P2020-PTDC/QUI-QOR/31770/2017 through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement. Ana R. Monteiro thanks the FCT for the PhD grant SFRH/BD/137356/2018. Sara Fateixa and Catarina I. V. Ramos acknowledge the costs of their research contracts (REF.-069-88-ARH/2018 and REF.-047-88-ARH/2018, respectively) resulting from the FCT hiring funded by National funds (OE), through FCT, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by the law 57/2017, of July 19. J. Rodrigues acknowledges i3N project UIDB/50025/2020 & UIDP/50025/2020 and project PTDC/NAN-MAT/28755/2017 (POCI-01-0145-FEDER-028755), financed by national funds through the FCT/MEC and supported by FEDER funds through the COMPETE 2020 Programme.

P-5.1.13

Synthesis of luminescent gold nanoclusters on aromatic amino acids

Varvara Kubenko[1], Zakhar Reveguk[1], Alexei Kononov[1], Andrey Buglak[1]

1) St. Petersburg State University, Department of Molecular Biophysics and Polymer Physics, Saint Petersburg, Russia

Tyr is involved in the synthesis of neurotransmitters, catecholamines, thyroid hormones, etc. There is a number of pathologies associated with impaired Tyr metabolism: phenylketonuria, hypothyroidism, tyrosinemia, alkaptonuria, and vitiligo. Therefore, it is important to be able to detect tyrosine in biological fluids, as well as to determine its concentration. Our aim was to develop a new detection method using luminescent metal nanoclusters. Tyrosine (Tyr), dioxyphenylalanine (DOPA), and tryptophan (Trp) were chosen as compounds with similar chemical structures to assess the selectivity of the method.

A typical protocol of nanocluster synthesis was as follows: amino acids were dissolved in water (C = 2.25 mM) at pH 1, controlled with HNO_3, then $AgNO_3$ (1.25 мМ) or $HClAu_4$ (1.25 мМ) was added; the samples were placed in a thermostat at 37° C. The spectra were recorded in 24 h[1].

It was found that luminescent gold clusters are formed at pH 1 on tyrosine, tryptophan, and DOPA. Fig. 1 shows absorption spectra (left) and luminescence spectra (right) of tyrosine complexes with silver and gold. One luminescent fraction of tyrosine complexes with gold nanoclusters with excitation/emission maxima of 360/470 nm was found (at right). No luminescent complexes with silver nanostructures were formed (at left).

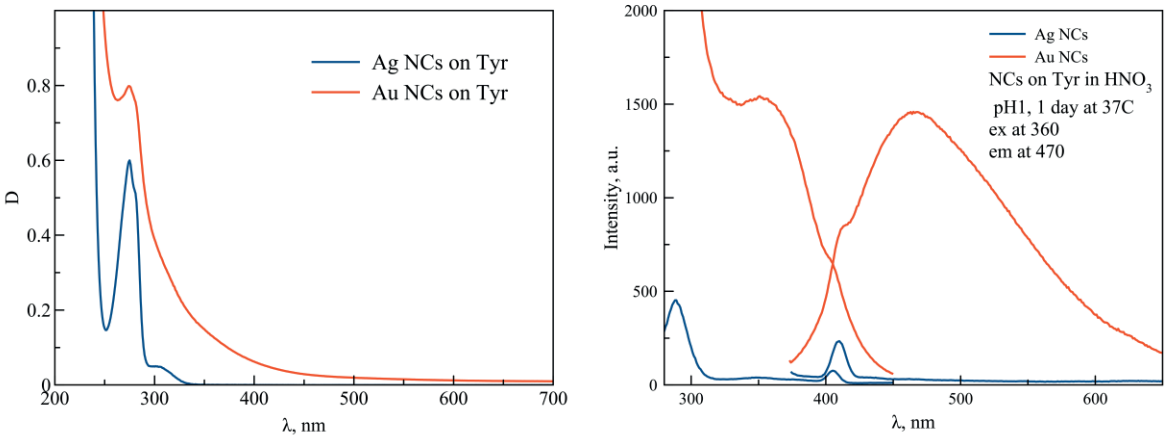


Fig. 1. Absorption spectra (left) and luminescence spectra (right) of tyrosine complexes with silver and gold.

Fig. 2 shows 2D luminescence spectra of DOPA (right) and its complex with gold (left). The complex possesses excitation/emission maximum at 330/450 nm. Also, tryptophan stabilizes the luminescent fraction of gold clusters with excitation/emission maxima at 320/410 nm, 330/500 nm and 370/500 nm (Fig. 3). No luminescent silver structures were observed.

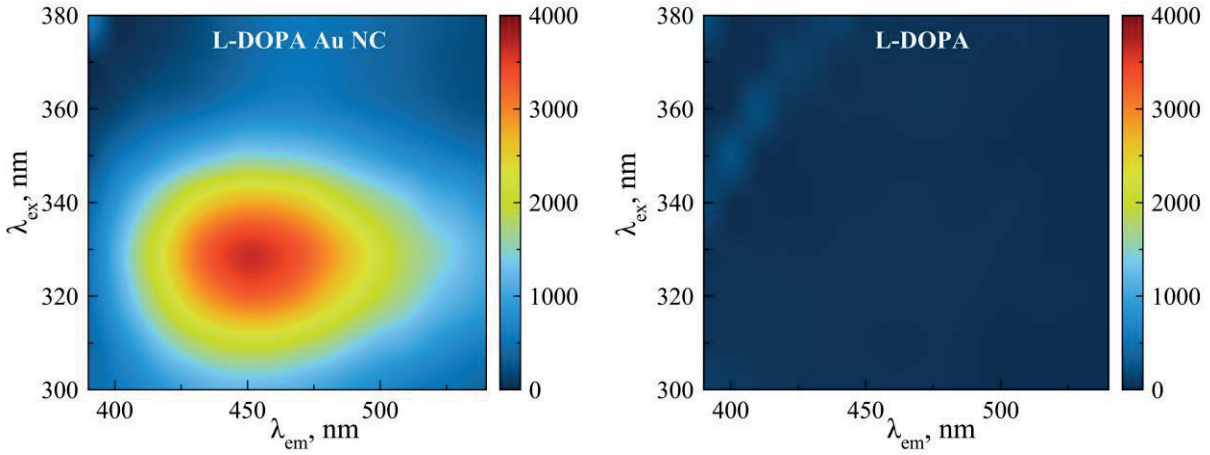


Fig. 2. 2D luminescence spectra of DOPA (right) and its complex with gold (left).

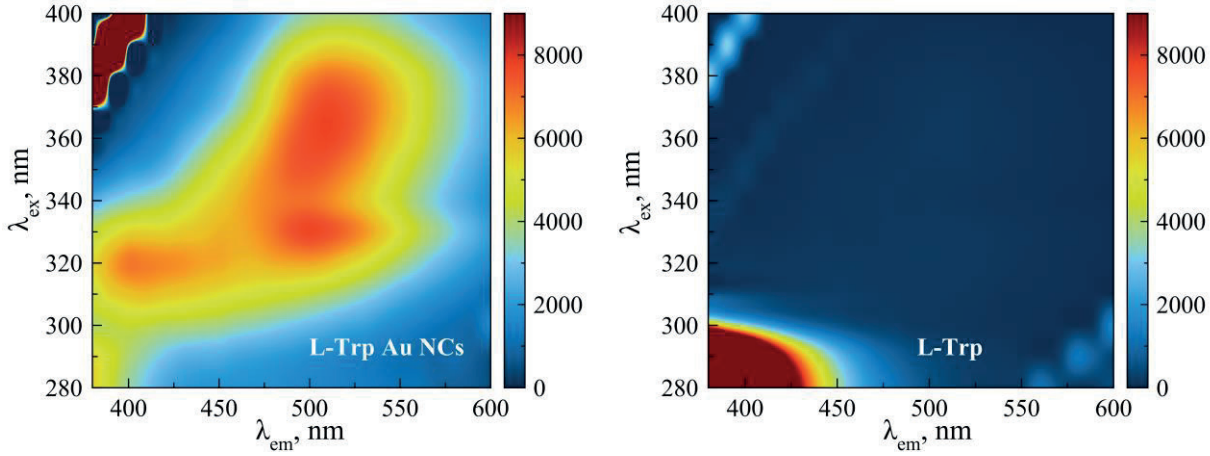


Fig. 3. 2D luminescence spectra of tryptophan (right) and its complex with gold (left).

The luminescence of gold nanoclusters synthesized on tyrosine, DOPA, and tryptophan differs in excitation/emission parameters, which suggests the possibility of the amino acid selective detection.

References

1) Xiaoming Yang, Yawen Luo, Yan Zhuo, Yuanjiao Feng, Shanshan Zhu, Novel synthesis of gold nanoclusters templated with l-tyrosine for selective analyzing tyrosinase, Analytica Chimica Acta, 840, 87-92, 2014.

Acknowledgements. This work was supported by the Russian Science Foundation (project 20-73-10029).

P-5.1.14

Colorimetric detection of L-DOPA

Yana Chuiko[1], Zakhar Reveguk[1], Alexei Kononov[1], Andrey Buglak[1]

1) St Petersburg State University, Department of molecular biophysics and polymer physics, Saint Petersburg, Russia

Dopamine is known as an important neurotransmitter of the human central nervous system and affects many brain functions and behavioral responses. Lack of dopamine in the brain may lead to neurological disorders, such as schizophrenia and Parkinson's disease. Considering the biochemical significance of dopamine precursor L-DOPA and its significance for medicine, it is highly desirable to develop a new efficient method for quantitative detection of L-DOPA with improved selectivity and sensitivity.

The colorimetric method is quite effective for detection of various analytes in solution. It is well known that under certain conditions amino acids can reduce ions of noble metals, forming nanoclusters (NCs)[1] and nanoparticles (NPs)[2] with characteristic absorption bands. To check the selectivity of the method, Tyrosine (Tyr), dioxyphenylalanine (DOPA), phenylalanine (Phe), and tryptophan (Trp) were studied as compounds with similar chemical structures.

Typical synthesis protocol proceeded as follows: silver nitrate was added to the amino acid dissolved in distilled MiliQ water (pH 6.5), NaOH was added after 10 minutes. We observed the reduction process a couple of minutes after the addition of the alkali. The samples were placed in a thermostat (20 ^{0}C) for 2 hours. Then absorption spectra were recorded.

At pH 7-10, complexes of silver nanoparticles with DOPA exhibited a notable absorption band with a maximum at 450 nm. For other amino acids, this band was not observed (Fig. 1), which indicates the selectivity of this method in relation to DOPA. It is noteworthy that at pH 11.5, all selected molecules showed the ability to reduce silver and form nanoparticles.

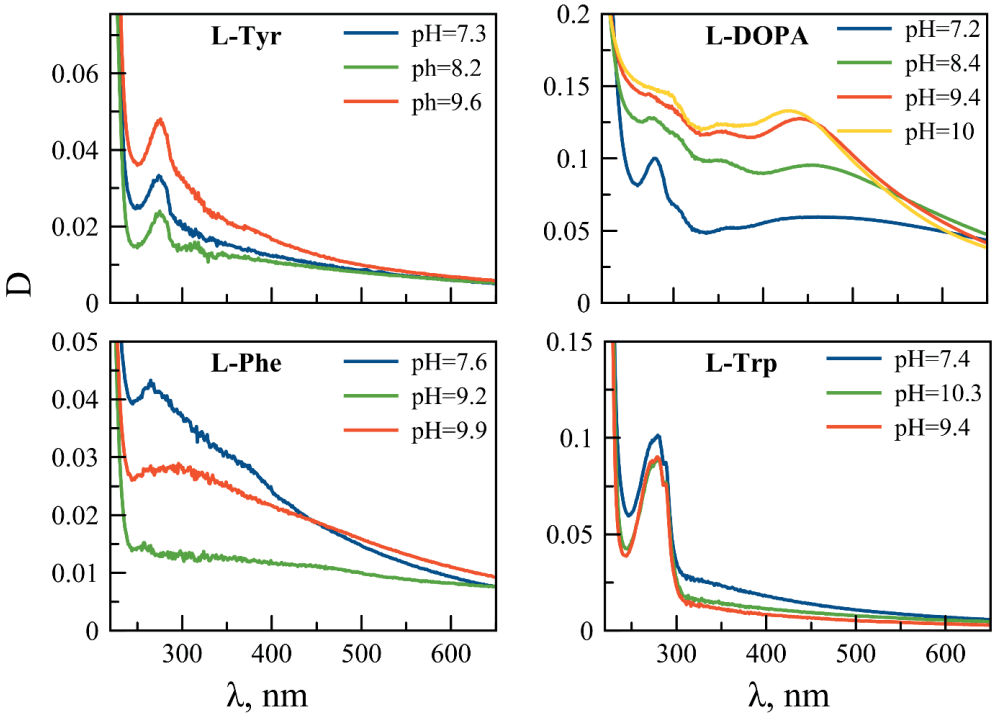


Fig. 1. Absorption spectra of Tyr, DOPA, Phe and Trp complexes with silver at different pH.

Fig. 2(on the left) shows the absorption spectra of silver NP complexes with DOPA, recorded 2 h after the synthesis with various DOPA concentrations in solution (pH 7.5). The optical density recorded at 410 nm showed a linear dependence in the range of DOPA concentrations from zero to a few micromoles per liter (Fig. 2, right). The limit of detection (LOD) was about a few nM.

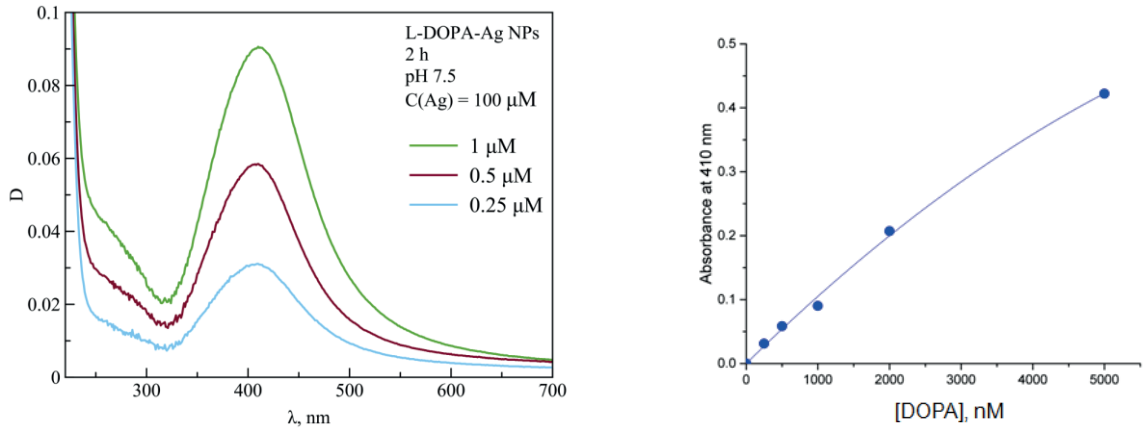


Fig. 2. Absorption spectra of silver NPs with different concentrations of DOPA (left); dependence of the optical density of solutions of silver NPs recorded in a 4 mm cuvette at 410 nm on the concentration of DOPA (right).

Further optimization is currently in progress. In this way it is also possible to detect Tyr using the oxidation of Tyr into DOPA by the enzyme tyrosinase.

References

1) Tomash S. Sych, Andrey A. Buglak, Which Amino Acids are Capable of Nucleating Fluorescent Silver Clusters in Proteins? J. Phys. Chem. C, 122 (45), 26275-26280, 2018.
2) Sharma R. Synthesis of Amino Acid Capped Silver Nanoparticles, Characterization, and Biological Application. Orient J Chem 2020;36(2).

Acknowledgements. This work was supported by the Russian Science Foundation (project 20-73-10029).

P-5.1.15

The light-mediated effects on photoluminescence of hydrophilic CdSe/ZnS-COOH quantum dots: dependence on biological medium

Agnė Kalnaitytė[1,2], Emilija Januškaitė[1], Osvaldas Vilutis[1] and Saulius Bagdonas[1,2]

1) Vilnius University, Biophotonics Group of Laser Research Centre, Vilnius, Lithuania; 2) Nature of Research Centre, Institute of Ecology, Vilnius, Lithuania

Semiconductor nanoparticles (NPs) or quantum dots (QDs) are used in optical instruments or in monitoring techniques, because QDs photoluminescence (PL) can be easily detected and tracked. Due to sensitivity of the spectral properties to environmental factors (temperature, pH, presence of ions or proteins, other parameters of living systems), hydrophilic quantum dots can also be used as indicators of environmental conditions. However, as the physicochemical properties of NPs can be affected by illumination conditions [1] and surrounding medium, the photoinduced changes in the PL also become important for monitoring their environmental fate and estimation of the threat of toxicity in various biological environments [2].

In this study, the spectroscopic measurements were performed on aqueous suspensions of hydrophilic CdSe/ZnS-COOH quantum dots (625 nm, Invitrogen, USA). The QDs samples (4 nM) were prepared for experiments by diluting QDs powder with distilled (DW), deep well (DWW) and lake water, MWC medium and also with PBS and TES buffer solutions (pH 6, 7, 8). A part of experiments was done on the samples keeping them under controlled illumination conditions, while the control samples were kept in the dark at +4°C and at room temperature. The samples were either constantly illuminated during 24 hours using a white fluorescent lamp (11W/827) at room temperature, or a 8/16 h "light/dark" cycle was applied for those being kept in an aquarium (Aquael, Poland) (at +22°C) using a combined white LED lamp (6 W) intended for the cultivation of aquatic plants.

In general, initial irradiation doses accelerate the decrease of PL intensity of QDs, the bathochromic spectral shift of the PL peak in comparison with unexposed samples, and also increase the scattering in the optical density (OD) spectra due to the formation of unstable QDs aggregates. However, when higher doses were reached, depending on the surrounding medium and its pH, the decrease slows down, the PL intensity stabilizes, or even partially recovers, which is also followed by the decreasing relative intensity of the excitonic absorption band in the OD spectra and the appearing hypsochromic shift of the PL spectral peak. Thus, higher irradiation doses break the formed unstable QDs aggregates and disrupt the structure of the NPs. Although the destruction of light-induced QDs aggregates and partial recovery of spectroscopic properties were more pronounced in a more alkaline environment, the recovery of the PL intensity was not detected in DWW (where the pH was about 8) demonstrating that photoinduced indicative changes in the spectral properties of CdSe/ZnS QDs strongly depend on the composition of surrounding medium.

In addition, the violet LED (404 ± 9 nm, 30 mW/cm^2 at a distance of 8 cm) was used to expose the QDs samples in MWC and DWW to 108 J/cm^2 irradiation dose. Afterwards, some irradiated samples of both types were mixed with a small amount of concentrated suspension of green freshwater unicellular microalgae cells and kept in aquarium under "light/dark" illumination cycle. During the first week, the following effects were observed in the case of irradiated QDs in MWC solution: the decrease of scattering in the OD spectra, a slight increase of the PL intensity and narrowing of the PL spectrum, as compared to the spectroscopic parameters of QDs measured immediately after irradiation, which could be related to the degradation of the light-mediated unstable QDs aggregates. Although the PL intensity of the irradiated QDs in the sample with algae cells was lower, the subsequent recovery of intensity was more pronounced and the narrowing of the spectrum in the red side was observed, presumably due to interaction of algae cells and/or their extracellular materials with QDs surface.

References

1) A. Kalnaitytė, S. Bagdonas, R. Rotomskis. The dose-dependent photobleaching of CdTe quantum dots in aqueous media. J Lumin, 201, 434–44, (2018).
2) A. Kalnaitytė, S. Bagdonas, Light-mediated effects of CdTe-MSA quantum dots on the autofluorescence of freshwater green microalgae: Spectroscopic studies, J Photoch Photobio B, 199, 111629, (2019).

Acknowledgements. This work was funded by the Research Council of Lithuania, Project No. S-MIP-20-22.

P-5.1.16

Organic dyes as luminescent probes for protein aggregates

Samuel Guieu[1,2], Raquel Nunes da Silva[1,3], Francisco Cardona[1], Catarina C. Costa[1], Maria J. G. Santos[1], Mariana Q. Alves[3], Susana S. Braga[1], Sandra I. Vieira[3], João Rocha[2], Artur M. S. Silva[1]
1) LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Portugal; 2) CICECO-Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Portugal; 3) IBiMED-Institute for biomedicine, University of Aveiro, Portugal

All-organic luminescent dyes have attracted much attention for biological imaging, due to their ease of use, and because they are easily modified to fine-tune their properties. Unfortunately, they usually present a high sensitivity to their environment, and can loss their emission properties upon aggregation or interaction with their surroundings. Interestingly, some fluorophores present the opposite effect, and exhibit an enhanced emission intensity in confined environments.[1] These modifications of emission properties can be advantageous to detect a change in the environment of the dye.
A series of organic dyes based on a double hemi-salen core has been prepared and characterized (Figure 1).[2] They are almost non emissive in dilute solution, but become highly fluorescent when they are in the crystalline state, in a phenomenon known as Aggregation-Induced Emission Enhancement.[1] This observation has been rationalized through the study of their crystal structures. The emission wavelength of the dyes has been fine-tuned by modifying the substituents.
To broaden the scope of application of these fluorophores, water soluble derivatives have been prepared, presenting the same photophysical properties. Their interaction with cyclodextrins and DNA has been studied: upon formation of inclusion complex or interaction with DNA, the emission intensity is enhanced, in a way similar to when the dyes crystallize. The dyes have then been applied to the staining of protein aggregates in live cells, demonstrating a selectivity similar to probes commercially available.[3]

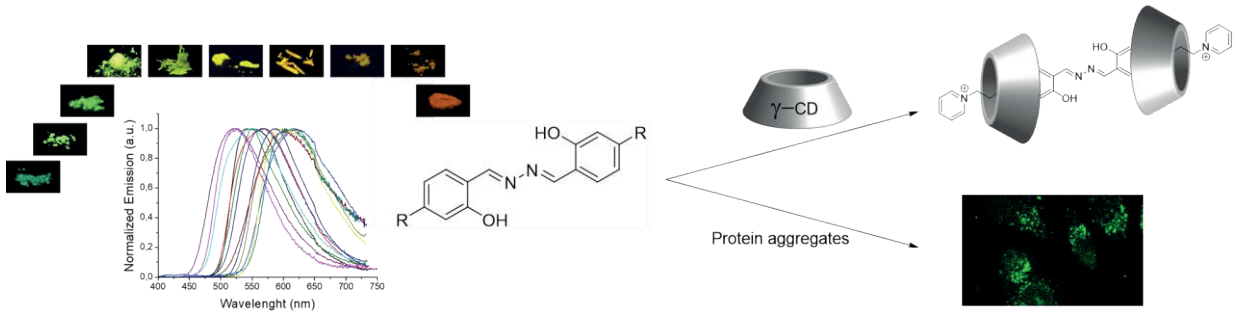


Fig. 1 – Probe with tunable colour, presenting an emission enhancement upon interaction with cyclodextrins or protein aggregates.

References

1) Y. Hong, J. W. Y. Lam, B. Z. Tang, *Chem. Soc. Rev.* **2011**, 40, 5361-5388. B.-K. An, J. Gierschner, S. Y. Park, *Acc. Chem. Res.* **2012**, 45, 544-554.
2) S. Guieu, F. Cardona, J. Rocha, A. M. S. Silva, *Chem. Eur. J.* **2018**, *24*, 17262-17267.
3) R. Nunes da Silva, C. C. Costa, M. J. G. Santos, M. Q. Alves, S. S. Braga, S. I. Vieira, J. Rocha, A. M. S. Silva, S. Guieu, *Chem. Asian J.* **2019**, *14*, 859-863.

Acknowledgements. This work was supported by Portuguese Fundação para a Ciência e a Tecnologia (FCT) via the CICECO-Aveiro Institute of Materials (UID/CTM/50011/2013, UIDB/50011/2020 & UIDP/50011/2020), QOPNA (UID/QUI/00062/2019), LAQV-REQUIMTE (UIDB/50006/2020), Institute for Biomedicine iBiMED (UID/BIM/04501/2013, UID/BIM/04501/2019 - POCI-01-0145-FEDER-007628), by Centro 2020 and Portugal 2020, and by the European Union (FEDER program) via R. Nunes da Silva post-Doctoral fellow grant (BPD/UI98/6327/2018) from the pAGE project (CENTRO-01-0145-FEDER-000003). S. Guieu is supported by national funds (OE), through FCT, I.P., in the scope of the framework contract foreseen in the numbers 4, 5, and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

P-5.1.17

Liguori, Nicoletta

Poster withdrawn

P-5.1.18

Luminescent complexes of gold clusters with amino acids

Mizintsev Artem[1], Sych Tomash[1], Kononov Alexei[1], Buglak Andrey[1]

1) St Petersburg University, Department of molecular biophysics and polymer physics, Saint Petersburg, Russia

Amino acids (AAs) are relatively small organic molecules which play an important role in various biochemical processes. Their concentration in natural biological fluids can indicate some diseases of human organism in early stages. In this work, we develop simple and selective method of AAs detection using the amino acids as stabilizing matrices for luminescent gold clusters. In comparison with luminescent silver clusters stabilized by AA matrices[1], here we used the approach of gold atom chemical reduction without any external reducing agent. In the case of gold clusters, the reduction of metal atoms occurred due to the stabilizing matrix.
In this work, we tested the possibility of synthesizing luminescent gold clusters on almost twenty different amino acids under conditions of strongly alkaline pH values (12.8). Here we used a "double synthesis" strategy. Chloroauric acid was added twice to the alkaline AA solutions with an interval of 24 hours. The studied complexes were incubated at +37°C in the dark. Among the whole variety of considered amino acids, only two demonstrated the possibility of stabilization of luminescent gold clusters – histidine (His) and phenylalanine (Phe) (Fig. 1).

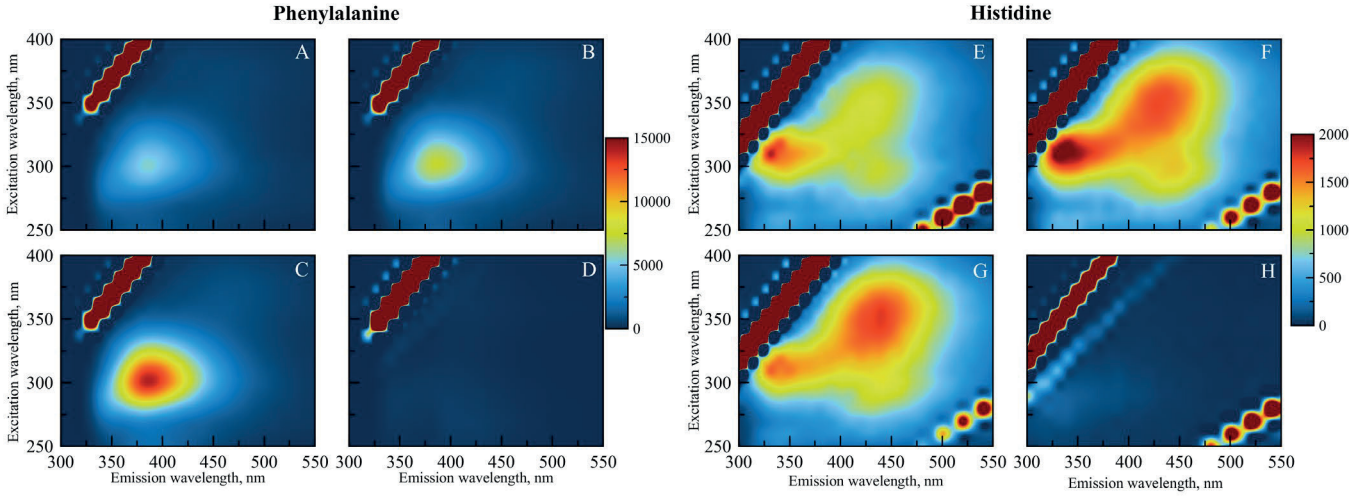


Fig. 1 – 2D luminescence spectra of gold clusters stabilized by phenylalanine (A-D) and histidine (E-H). A,E: Au/AA = 1:2; B,F: Au/AA = 1:1; C,G: Au/AA = 3:2; D,H: pure amino acid.

Analyzing the obtained luminescence spectra, one can notice that the spectral properties of clusters stabilized by different amino acids differ markedly. Thus, in the case of phenylalanine, a strong luminescence band is observed at 380 nm. In the case of histidine, a noticeably weaker luminescence band is observed at 450 nm.
Significant spectral differences between two obtained gold clusters make it possible to unambiguously determine the presence of His and/or Phe solution.

References

1) Tomash S. Sych, Andrey A. Buglak, Which Amino Acids are Capable of Nucleating Fluorescent Silver Clusters in Proteins? J. Phys. Chem. C, 122 (45), 26275-26280, 2018.

Acknowledgements. This work was supported by the Russian Foundation for Basic Research (Project No. 19-53-51005 NIF_a RFFI-Korea).

IL-5.2.1

Chances and limitations of light-based antimicrobial approaches in dentistry – an update

Fabian Cieplik[1]
1) University Hospital Regensburg, Department of Conservative Dentistry and Periodontology, Regensburg, Germany 1

The 2016 Review on Antimicrobial Resistance has predicted that the number of annual deaths attributable to antimicrobial resistance will increase globally from the current 700,000 to 10 million in the year 2050 if no appropriate action is taken immediately. In light of this increasing threat of resistance towards conventional antibiotics and antiseptics, alternative antimicrobial approaches are desperately needed. In particular in the field of dentistry, where usually no life-threatening diseases need to be treated, it seems reasonable to promote research for suchlike alternative antimicrobial approaches. In this context, especially light-based approaches including antimicrobial photodynamic therapy (aPDT), photothermal therapy (PTT) or low-level light (or laser) therapy (LLLT), have increasingly been proposed in the last two decades.
This talk aims to give an overview about these approaches by summarizing evidence from *in vitro* studies as well as recent clinical trials and to discuss the chances and limitations for application of light-based antimicrobial approaches in dental practice.

Acknowledgements. This work was supported in part by grants from the Deutsche Forschungsgemeinschaft (DFG CI 263/1-1, CI 263/1-3, CI 263/3-1).

IL-5.2.2

Light-Responsive Coatings for Biofilm Disruption

Devatha P. Nair[1,2,3], Gannon M. Kehe[1], Christopher J. Carpenter[4], Michael J. Schurr[5]

1) University of Colorado-School of Dental Medicine, Craniofacial Biology, Aurora, U.S.A 1; 2) University of Colorado-Materials Science and Engineering program, Boulder, U.S.A; 3) University of Colorado-Skaggs School of Pharmacy and Pharmacological Sciences, Aurora, U.S.A; 4) Metropolitan State University, Biology, Denver U.S.A; 5) University of Colorado-Immunology and Microbiology, Aurora, U.S.A.

Bacterial biofilms are ubiquitous- be it in the oral cavity, within pipes, or on the surface of a ship's hull and marine structures.[1] The ability of bacteria to form biofilms is a potent evolutionary mechanism that allows them to thrive and proliferate in a variety of environments and enable them to evade antibiotics. The rise of pathogenic biofilms and their role in growing antibiotic resistance observed globally has urgent implications in the current and future treatments against infectious disease. Biofilms offer protection to a consortium of bacteria and can be 10−1000 times more resistant to antibacterial agents. To address the emerging crisis, our group studies alternative strategies to disrupt biofilms on resin surfaces by engineering functionalized coatings that can: 1) prevent and/or delay biofilm formation and 2) repeatedly detach biofilms via transient changes in surface topography (photofluidization effect, Fig.1).[2]

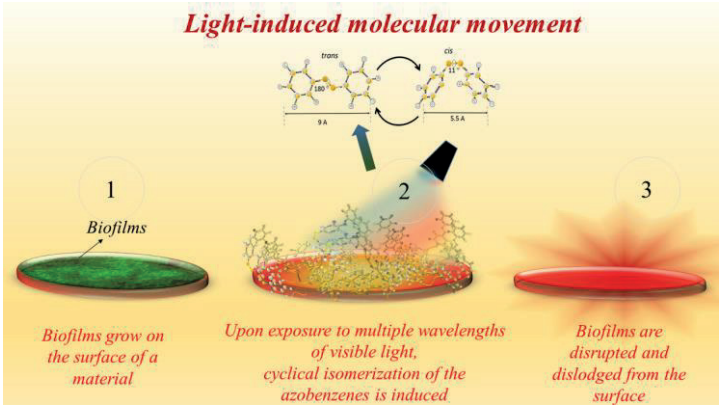


Fig. 1 – Biofilm disruption via light-responsive azobenzene coatings.

Functionalized azobenzene molecules were synthesized and characterized to undergo 1) Light-induced molecular movement via (400-500 nm, 700 mW/cm^2, 10s pulsed exposures) and 2) as coatings to ascertain biofilm growth over 24-48 hrs. Biocompatibility of the molecules was ascertained (ISO-10993) and biofilm disruption of *Pseudomonas aeruginosa* (PA01) and *Streptococcus mutans* (SM) biofilms (presence and absence of 1wt% sucrose in BHI) on glassy surfaces were evaluated. Biofilms were imaged and quantified via CFU counts. While molecular movement was able to achieve a 3-log reduction in SM biofilms grown in the absence of sucrose, no reduction in biofilms formation or removal was seen in the presence of sucrose. The light-activated coatings completely removed mature PA01 biofilms from surfaces, indicating that light-induced biofilm removal can be a viable tool for biofilm disruption.

References

1) Algburi A, Comito N, Kashtanov D, Dicks LMT, Chikindas ML. Control of Biofilm Formation: Antibiotics and Beyond. Appl. Environ. Microbiol. 2017
2) Kehe GM, Mori DI, Schurr MJ, Nair DP. Optically Responsive, Smart Anti-Bacterial Coatings via the Photofluidization of Azobenzenes ACS Appl. Mater. Interfaces. 2019.

Acknowledgements. This work was supported by grant K25DE027418 from the National Institutes of Health - National Institute of Dental and Craniofacial Research (NIH-NIDCR).

IL-5.2.3

Photocrosslinking of collagen and antimicrobial applications

Krister Gjestvang Grønlien[1], Mona Elisabeth Pedersen[2], Håkon Valen[3], Ellen Bruzell[3], Hanne Hjorth Tønnesen[1]
1) Section for Pharmaceutics and Social Pharmacy, Department of Pharmacy, Oslo, Norway; 2) Department of Raw Material and Process Optimization, Nofima AS, Ås, Norway; 3) Nordic Institute of Dental Materials (NIOM) AS, Oslo, Norway

Collagen is extensively used in fabrication of scaffolds for pharmaceutical and biomedical applications. Scaffolds can be used in drug delivery (e.g., in ophthalmology, topical and dental applications), as wound dressings or as cell templates in tissue engineering[1]. However, low viscosity, slow gelation and low mechanical strength of the prepared constructs are the major limitations for such use. Crosslinking by chemical or photochemical methods could enhance these properties. However, chemical crosslinkers are often toxic and the crosslinking reaction is difficult to control. Photoactivated crosslinkers have been considered as good biocompatible alternatives. Photocrosslinking of collagen has been achieved by modification of the protein with methacryloyl-substitution or by addition of photocrosslinkers to collagen solutions combined with radiation prior to or during thermogelation[2,3]. The presentation will give an introduction to photocrosslinking of collagen and results from a present work on a new natural photocrosslinker of collagen.

Examples of photosensitizers commonly used in photocrosslinking are Igracure 2959 (mainly used for photocrosslinking of methacrylates), Eosin Y, ruthenium compounds, riboflavin (vitamin B_2) and rose bengal[4]. The mechanisms behind photocrosslinking of collagen have been thoroughly studied. The crosslinking effect is postulated to involve the formation of reactive oxygen species (ROS) and singlet oxygen ($^1O_2$) by the exited photosensitizer, resulting in photo-oxidation and formation of covalent bonds between the amino acids in the collagen protein chains[3].

In the present work, collagen was crosslinked with two naturally occurring photosensitizers; riboflavin (RF) and a newly discovered photocrosslinker (NXL). Photocrosslinking of collagen by RF is commonly applied in corneal crosslinking in the treatment of the eye disease keratoconus and has been investigated as a potential technique for production of collagen-based biomaterials[3,5]. However, riboflavin photocrosslinking requires a long irradiation time, limiting its use in applications such as bioprinting. The newly discovered natural photocrosslinker NXL reduced the irradiation time from minutes to only 10 seconds and did not require incubation and self-assembly of collagen prior to crosslinking. The resulting hydrogels were compared to RF photocrosslinked hydrogels and showed an approximately 20-fold higher water holding capacity (44.97 and 2.57%, respectively). The NXL hydrogels were highly elastic with a Young's modulus over twice the value of the RF photocrosslinked hydrogels (62.30 and 30.88 kPa, respectively). Fibroblasts seeded on the hydrogels had a migratory phenotype. These are properties favorable in pharmaceutical and biomedical applications. Further, the hydrogels photocrosslinked with the new photosensitizer NXL were tested for their antimicrobial properties, and the results were promising for further development into a biomedical product.

References

1) Lee, C. H., Singla, A., and Lee, Y. Biomedical applications of collagen. *Int. J. Pharm.*, vol. 221, no. 1, 2001.
2) Drzewiecki, K. E. et al. A thermoreversible, photocrosslinkable collagen bio-ink for free-form fabrication of scaffolds for regenerative medicine. *Technology,* vol. 5, no. 4, 2017
3) Heo J. et al. Riboflavin-induced photo-crosslinking of collagen hydrogel and its application in meniscus tissue engineering. *Drug Deliv. Transl. Res.*, vol. 5, no. 2, 2016.
4) Mu, X., Sahoo, J. K., Cebe, P., and Kaplan, D. L. Photo-Crosslinked Silk Fibroin for 3D Printing. *Polymers*, vol. 12, no. 12, 2020.
5) Wollensak, G., Spoerl, E., and Seiler, T. Riboflavin/ultraviolet-A–induced collagen crosslinking for the treatment of keratoconus. *Am. J. Opthalmol.,* vol. 135, no. 5, 2003.

Acknowledgements. Turkey tendon by-products and financial support for isolation of collagen from turkey were contributed by Norilia AS (Oslo, Norway).

OC-5.2.4

Blue light risk evaluation of dental operating lights

Ellen Bruzell[1], Bjørn Johnsen[2], Jeff Zawada[3]
1) Nordic Institute of Dental Materials, Materials Science Group, Oslo, Norway; 2) Norwegian Radiation and Nuclear Safety Authority, Dept. of Emergency Preparedness and Response, Østerås, Norway; 3)A-dec, Inc., Dept. of Technical Research, Newberg, OR, USA.

Dentistry is an occupation that places great demands on the lighting conditions. Thus, the operating light must deliver sufficient intensity in addition to ensuring specific lighting parameter values, such as contrast and colour fidelity. The illuminance of an operating light is typically 20-30 klx.
Following the substitution of incandescent light sources to light emitting diodes (LED) in nearly all light products including medical devices, the public, researchers and organisations raised concern about the blue light portion of the LED spectra. This concern was aimed at a potential risk of photochemical hazard to the eyes and disturbance of circadian rhythms[1,2]. No limit values exist for the blue light level needed to affect or disrupt circadian rhythms; however, melatonin depression by blue light can be considered an advantage for the dental team during examination and treatment procedures.
The aim of this study was to determine the blue light hazard (retinal injury) risk group class of operating lights as described in the standards for photobiologcial safety[3] and dental operating lights[4]. The spectral power of three operating lights (one manufactured in USA, two in Japan) were measured using a double monochromator spectroradiometer[5]. Appropriate weighting functions were applied to obtain blue light-weighted radiance. Prior to the measurement the operating lights had undergone similar measurements provided by the respective manufacturers (pre-measurements), and the values obtained from the two measurements for each device were compared.
The blue light-weighted irradiance values of the operating lights were lower than the limit of 10000 $W/m^2/sr$ separating Risk Group 1 from Risk Group 2 with the exception of one value obtained for one device in the pre-measurement. This operating light exceeded Risk Group 1 limit by <1% in the pre-measurement. Comparable results were obtained irrespective of optical laboratory. Due to the conservative requirement of the exposure conditions, occupational exposure of the investigated operating lights did not pose a blue light risk for retinal injury.

References

1) SCHEER (Scientific Committee on Health, Environmental and Emerging Risks), Opinion on Potential risks to human health of Light Emitting Diodes (LEDs), 6 June 2018.
2) ANSES (the French Agency for Food, Environmental and Occupational Health & Safety), Opinion on the "effects on human health and the environment (fauna and flora) of systems using light-emitting diodes (LEDs)", 5 April 2019
3) CIE S009:2002. Photobiological Safety of Lamps and Lamp Systems. Vienna, 2002.
4) ISO/DIS 9680. Dentistry – Operating lights. Geneva, 2021.
5) Terje Christensen, Bjørn Johnsen, Ellen Bruzell, Violet-blue light exposure of the skin: is there need for protection? Photochemical & Photobiological Sciences, 20(5), 615-625, 2021

IL-5.2.5

Lasers in Dentistry

First author: Peter Fahlstedt, DDS, MSc Lasers in Dentistry, PhD candidate.

Faculty of Medicine, Department of Clinical Dentistry, University of Bergen, Bergen, Norway; Institute for Laser Supported Dentistry, Akersberga, Sweden

Lasers of different wavelengths have been used as support in dentistry for decades. Diode laser 445 nm up to 980 nm, free-running pulsed lasers 2780/2940 nm and CO2 lasers are the most common in daily practices. Constituting a wide range of different interactions in soft- and hard tissues, we find lasers having biomodulating and antibacterial effects and the ability to remove tissue and foreign materials without adverse effects on the following process of healing and regeneration. This lecture focuses on the daily practice of laser support in dental clinics, the benefits, the limitations, and challenges in convincing not the patients but the colleagues and universities why the integration of lasers in dentistry is essential, even in a broader context.

IL-5.2.6

Abstract for ESP2021: Laser in Public dental healthcare, experience and reflections.

Are Kristofer Hjeltnes, DDS, MSc Laser Dent[1]
1) Ålesund kompetanseklinikk, Public dental healthcare, Ålesund, Norway;

Introduction: The interest about laser therapy in public dental health care has grown very slowly in Norway[1]. About 1% of the dental practitioners are using laser in everyday treatment, and so far very few have published scientific papers from their work.Even this speech is not based upon scientific collected and evaluated data. It is in fact based upon my own experience and reflections from having used laser therapy as an integrated part of my treatment in dental care since 2013.
Settings: Laser therapy is executed as part of my daily dental practice in Ålesund dental clinic (staff 28, population 70.000), located in Ålesund town in western Norway.
Methods and procedures: The dental lasers used are Er:YAG (2940nm), Nd:YAG (1064nm), and diode lasers (445nm, 660nm, 810nm, 970 nm), either in combination or one at a time. Laser treatment was also given in combination with conventional dental treatment .
Results: The results show the different diagnostic groups and the number in each group that have been given laser therapy. Combination-therapy was given in leukoplakia, cavity-preparations, frenectomies, recidivating aphtous stomatitis, recidivating herpetic gingivostomatitis, ex tirp, frenectomies, removal of fibromas, removal of peripheral ossifying fibrom, endodontics or pulpotomies [2,3,4,5,6]. The results have also been graded as “good”, “fair” and “bad” according to my own experience and patient satisfaction, and complications of any kind are noted. Effects of combining different wavelengths seems promising to avoid relapses of challenging oral conditions such as recidivating peripheral fibromas, oral leucoplakias. The need for local and lead anesthesia were reduced to 1-2 out of 10 in cavity preparations. Decontaminating effects led to fast wound healing, less postoperative pain and postponed endodontic treatment.
Discussion and conclusion: The most important learning from my work and experience so far with the use of laser therapy in public dental praxis is: Laser treatment is a secure, efficient and usually quite painless treatment when used on the mentioned diagnoses and indications, given that it’s executed by a laser educated dentist. Still the clinical results remain to be evaluated scientifically to verify our practical experience and even to extend the indications[7].

References

1) Leknes KN, Alstad ME, Angelsen S, Eriksen ES, Espervik A, Bruzell E. “Laser treatment of periodontal and peri-implant infections”, Nor Tannlegeforen Tid 2009; 119: 700-6
2) Liu JF, Lai YL, Shu WY, Lee SY. Acceptance and efficiency of Er:YAG laser for cavity preparation in children. Photomed Laser Surg. 2006;24(4):489-493.
3) Daneswari V, Nandlal B. Restorative dentistry for children using a hard tissue laser - a review. J Int Oran Health. 2011;3(3):1-8.
4) Eren F, Altinok B, Ertugral F, Tanboga I. The effect of Er,Cr:YSGG laser therapy on pain during cavity preparation in paediatric dental patients: a pilot study. Oral Health Dent Manag. 2013;12(2):80-84.
5) Boj J, Galofre N, Espana A, Espasa E. Pain perception in pediatric patients undergoing laser treatments. J Oral Laser Applications. 2005;5(2):
6) 85-89 Polonsky M, Gutknecht N, Franzen R. Review of possible predictors for pain perception with class 1-5 cavity preparations using Er,Cr:YSGG laser: a retrospective clinical in vivo study. Laser Dent Sci. 2017;1(1):9-21.
7) Polinsky M, “Laser-assisted restorative dentistry: Can we afford not to use it?”, Dental Economics, apr 2018, https://www.dentaleconomics.com/science-tech/article/16385053/laserassisted-restorative-dentistry-can-we-afford-not-to-use-it

IL-5.2.7

Evaluation of upper labial frenectomy - a randomized, controlled comparative study of conventional scalpel technique and Er:YAG laser technique

Sarmadi Roxana[1, 3], Gabre Pia[2, 3], Thor Andreas[4]

[1]Department of Paediatric Dentistry, Public Dental Health, Uppsala County Council, Sweden.

[2]Department of Preventive Dentistry, Public Dental Health, Uppsala County Council, Sweden.

[3]Department of Cariology, Institute of Odontology, The Sahlgrenska Academy of Gothenburg, Gothenburg, Sweden.

[4]Department of Plastic and Oral and Maxillofacial Surgery, Department of Surgical Sciences, Uppsala University

Objectives: Abnormalities in the maxillary frenum may lead to aesthetic or functional limitations and need to be corrected with a surgical intervention called frenectomy. The aim of the study was to compare frenectomies performed using Er: YAG laser technology with those using a conventional scalpel technique. Comparisons were of patients´ experiences, treatment times, bleeding during treatment and wound healing.

Material and methods: The trial was performed as a prospective, randomized and controlled, single-blind investigation. A total of 40 patients requiring frenectomy were randomly assigned to groups which underwent either conventional or Er:YAG laser treatment. Patients´ experiences, treatment time, bleeding and wound healing were evaluated immediately after surgery and five days, twelve days and three months after surgery.

Results: Significant increase in time spent in surgery and bleeding was seen with conventional scalpel surgery. Directly after surgery the wound area was significantly larger in the laser group but at the five-day evaluation no difference could be observed between the groups. Finally, patients were satisfied with both methods, giving them the same assessments.

Conclusions: In the frenectomy procedure, laser surgery is faster and causes less bleeding and may be advantageous in frenectomies.

Acknowledgement

This project was supported by grants from the Public Dental Service in Uppsala County Council. We thank the study coordinator Anna Maria Carlberg for careful planning of the practical parts of the study We also thank Niclas Eriksson for help with statistical analyses and Gwyneth Olofsson for revising the English text.

OC-5.2.8

With blue light against bacteria and viruses

Magdalena Metzger[1,2], Lisa Karner[1,2], Kristjan Plaetzer[3], Barbara Holzer[4], Kurt Schicho[5], Johannes Grillari[1,2,6], Peter Dungel[1,2]

1) Ludwig Boltzmann Institute for experimental and clinical Traumatology, Vienna, Austria; 2) Austrian Cluster for Tissue Regeneration, Vienna, Austria; 3) University of Salzburg, Department of Biosciences, Laboratory of Photodynamic Inactivation of Microorganisms, Salzburg, Austria; 4) Austrian Agency for Health and Food Safety, Mödling, Austria; 5) Department of Oral and Maxillofacial Surgery, Medical University of Vienna, Vienna, Austria; 6) Department of Biotechnology, Institute of Molecular Biotechnology, BOKU-University of Natural Resources and Life Sciences, Vienna, Austria.

While the germicidal properties of UV radiation have been known for a long time, its negative side effects, e.g. DNA damage, photoaging and carcinogenicity, limit the possibilities and potential fields of medical application. Alternatively, blue light can be a promising option for the inactivation of a variety of microorganisms such as bacteria and viruses with the additive potential to improve wound healing (1). While the underlying mechanisms of blue light therapy have not been completely resolved yet, it has been proposed, that intrinsic chromophores e.g. porphyrins or flavins, generate ROS upon irradiation which destroy lipids, proteins and nucleic acids. The aim of this study was to investigate the effects of a LED device emitting "soft" blue light with wavelengths >430 nm (kindly provided by Repuls Lichtmedizintechnik, Vienna, Austria) on the inactivation of Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus capitis* and the corona virus type SARS-CoV-2.

Treating bacteria of different strains with blue light, the present study revealed that strains lacking the gene recA were more susceptible towards illumination than those with an intact copy of the gene. In most bacterial strains, the protein RecA is responsible for homologous recombination, DNA repair and induction of the SOS response. Therefore, we hypothesized that the inactivation of the protein would increase the susceptibility of resistant strains and enhance the antimicrobial effects of the treatment. Although the potentiation of the therapy via RecA inhibitors was only partially successful, the influence of recA was confirmed by rescuing susceptible bacterial strains with the insertion of a plasmid carrying the gene.

Regarding the antiviral properties of blue light, it was shown that the number of infectious SARS-CoV-2 viruses drops by around half after a short exposure of 20 +/- 5 mW/cm² (5 minutes) and by one order of magnitude (by 90%) after 30 minutes. An examination of the viral load as a function of the exposure time to blue light shows an almost linear decrease between 3 and 12 minutes.

In conclusion, "soft" blue light was able to significantly reduce bacterial growth which was associated with the lack of recA gene. However, further experiments are needed to uncover the mechanisms behind the toxicity of blue light therapy and the importance of the SOS response. Blue light from the Repuls illumination device had also virucidal effects on SARS-CoV-2, i.e. the viral load was already reduced by light alone. Further studies should investigate the probable enhancement of these effects in combination with suitable photosensitizers.

References

1) Dungel P, Hartinger J, Chaudary S, Slezak P, Hofmann A, Hausner T, Strassl M, Wintner E, Redl H, Mittermayr R. Low level light therapy by LED of different wavelength induces angiogenesis and improves ischemic wound healing. Lasers Surg Med. 2014

Acknowledgements. This work was supported by a grant funded by the Vienna Business Agency. A fund of the City of Vienna, Austria.

IL-5.3.1

Dynlt3 controls melanosome movement, distribution, acidity and transfer to keratinocytes

Zackie Aktary[1], Lionel Larue[1]

1) Normal and Pathological Development of Melanocytes, Institut Curie, CNRS UMR3347, Inserm U1021, Orsay, France

Pigmentation of the appendage including skin and hair depends on fundamental processes such as biogenesis, transport, and maturation of melanosomes and their transfer to keratinocytes. The control of these different precise processes in space and time remains poorly understood. Here, we show that Dynlt3, a subunit of the cytoplasmic dynein complex, is required to efficiently transport, acidify and transfer melanosomes. When the level of Dynlt3 is decreased in melanocytes, the movement of melanosomes is more directional, and they are consequently mainly located at the periphery of these cells. The acidity of stage IV melanosomes is higher when Dynlt3 is reduced and results in less efficient melanosome transfer, yet they remain highly pigmented. Finally, the level of mRNA corresponding to Dynlt3 is dependent on β-catenin activity, Finally, the level of mRNA corresponding to Dynlt3 is dependent on β-catenin activity, revealing a function of the Wnt/β-catenin signalling pathway during melanocyte and skin pigmentation, coupling transport, positioning in the cell and acidity of melanosomes, a process that is essential for their efficient transfer.

IL-5.3.2

Sun-induced pigmentation: relative contribution of UVA1 and Visible Light color domains

Piffaut Virginie[1], Marionnet Claire[1], Sasai Jun[2], Nouveau Stéphanie[1], Jouni Hussein[1], Ortiz Maria[1], Huguet Etienne[1], Planel Emilie[1], Duteil Luc[3], Bernerd Francoise[1]
1) L'Oréal, Research and Innovation, Aulnay Sous-Bois, France; 2) L'Oréal, Research and Innovation, Kawasaki, Japan ; 3) Centre de Recherche Clinique Appliquée à la Dermatologie, Hôpital de l'Archet 2, Nice, France

Hyperpigmentation and pigmentary disorders are known to be induced or aggravated by sun exposures. While the role of UVB and UVA2 rays is indisputable, data emphasize a contribution of UVA1 wavelengths and also Visible Light (VL) for which High Energy Visible Light (HEV) seems to play a major role through activation of melanogenesis. To better understand the contribution of UVA1, VL and the different VL color wavelengths bands (HEV, “Blue”, “Green” and “Red”) in induction of pigmentation, two clinical studies were performed using physiological sources (solar simulators) and specific cut off filters. Results could be illustrated using computer-based simulations on full-face models. In the first study, the back of volunteers (n=27) was exposed to various wavelengths ranges: UVA1+HEV (350-450nm), UVA1 (350-400nm) or HEV (400-450 nm). In the second study, volunteers (n=25) were exposed to VL (400-700nm), HEV (400-450nm), “Blue” color domain (400-500nm), “Green” color domain (500-600nm) and “Green + Red” color domains (500-700nm). Pigmentation was assessed by visual scoring and chromametric measurements (L*, a*, b*, ΔITA) at several time points (from day 1 to day 43).
In the first study, pigmentation was induced in all the exposure conditions as attested by ΔL* and ΔITA values and clinical soring. The higher intensity was observed at 2h with a progressive decline over time. At 2h post exposure the pigmentation induced by UVA1+HEV, UVA1 and HEV corresponded to a ΔL*= -6,70; -4,48 and -2,45 respectively, indicating an additive effect of UVA1 and HEV.
In the second study, VL exposure induced a significant and persistent pigmentation (ΔL*=5.54 at 2h), as well as exposure to HEV (400-450nm) (ΔL*=-1,90 at 2h) or “Blue” light (400-500 nm) (ΔL*=-2,84 at 2h). The study revealed that “Green” light was also responsible for induction of pigmentation although its contribution was lower (ΔL*=1,16 at 2h) than the “Blue” light’s one. By comparing pigmentation induced by “Green” and “Green + Red” color domains, we confirmed that “Red” light doesn’t significantly contribute to pigmentation. Clinical scoring confirmed these results. Based on the colorimetric measurements, a contribution percentage could be calculated for each color domain in VLinduced pigmentation 24 hours post exposure with: 400-700nm full VL= 100%, “Blue” 400-500nm= 71%; HEV 400-450nm= 47%; “Green” 500-600nm=37 %; “Red” 600-700nm <1%.
Altogether, the two studies reinforced the basis for the need of UVA1 photoprotection up to 400 nm and highlighted the importance to also protect the skin from solar visible wavelengths, especially the shortest wavelengths HEV/ “Blue” but also “Green” color band, to limit skin hyperpigmentation.

IL-5.3.3

UV-induced growth and migration of melanoma cells in a spheroid model.

Linda Vainikka, Petra Wäster, Linda Rößler and Karin Öllinger[1]
1) Linköping University, Department of biomedicine and Clinical Sciences, Linköping, Sweden

Cutaneous melanoma is a highly aggressive cancer with a propensity for metastasis to various organs. The most important risk factor to develop the disease is exposure to UV radiation. UVB wavelengths has ability to cause tumor-initiating mutations while UVA exposure generates free radicals and may act immunosuppressive. How the microenvironmental effects of UV radiation influence melanoma pathogenesis and its ability to metastasize remains to be elucidated. In a previous report we have shown that UVA exposure triggers increased secretion of extracellular vesicles. UVA cause plasma membrane damage which is promptly repaired by transportation of lysosomes to the damaged membrane to form a resealing patch. Subsequently, extracellular vesicles are released outside the cell (see Figure 1). The vesicles promote signaling event when taken up by non-irradiated cells and increased growth and migration is noted[1].

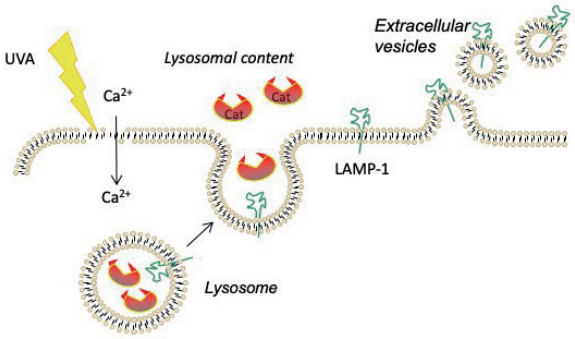


Figure 1. UVA-radiation induces plasma membrane damage resulting in calcium influx, which trigger transport of lysosomes to the damaged site. The lysosomal membranes forms a resealing patch and lysosomal content is released outside the cell. Subsequently, excess membrane is shredded as extracellular vesicles.

In this study results from UV exposure of malignant melanoma cells will be presented and the possible signaling pathways induce by UV will be discussed. We generated melanoma tumor spheroids from four different cell lines and exposed to sub-lethal doses of UVA- and UVB-radiation. We found spontaneous secretion of extracellular vesicles by all melanoma cell lines, but after UVA, increased number of large size vesicles were found. Following radiation, the spheroids were embedded in Matrigel and observed microscopically to follow cell migration and invasion. Cell migration out from the spheroid was higher in spheroids exposed to UVA compared to control and UVB exposed samples. A transcriptome analysis was performed 24 h after irradiation of the spheroids, and revealed significantly increased mRNA levels of matrix metalloprotease-1 (MMP1; +3.3-fold, p=0.0009) after UVA exposure. MMP-1 is a collagenase involved in extracellular matrix degradation and the protease is secreted and activated outside the cell. We found elevated protein level of MMP1 and secretion to the medium in three out of four tested melanoma spheroids after UVA exposure and in one of four after UVB. We conclude that melanoma migration and invasion is elevated more efficiently after UVA than UVB radiation and MMP1 is a candidate protein to promote this effect.

References

1. Wäster P, Eriksson I, Öllinger K. Intercellular cross-talk mediated by extracellular vesicles promotes survival and proliferation via miR-21-signaling. *Pigment Cell and Melanoma Research* 2020; 33(4):542

Acknowledgements. This work was supported by grant from Swedish Cancer Society and Welander Finsten Skin Foundation.

OC-5.3.4

Photoprotective and Antigenotoxic Properties of Actinobacteria Indigenous Strains from Deep Subsurface Environments of Colombia

Carlos Adolfo Pedraza Barrera[1], Elena E. Stashenko[1] and Jorge Luis Fuentes Lorenzo[1]

1) Universidad Industrial de Santander, Santander, Bucaramanga, Colombia.

Introduction: Actinobacteria are known to produce a variety of secondary metabolites with clinical properties. **Purpose:** The study was aimed at investigating photoprotective and antigenotoxic properties against UVB of ten extracts from *Cutibacterium acnes* and *Microbacterium paraoxydans* strains collected from subsurface habitats of Colombia. **Materials and Methods:** The bacterial growth was measured spectrophotometrically in different culture media. *In vitro* photoprotection efficacy was evaluated using *in vitro* indices such as sun protection factor ($SPF_{in\ vitro}$) and critical wavelength (λc). The percentage of UVB– antigenotoxicity estimates (%GI) in the SOS Chromotest was also evaluated. Correlation analysis was used to examine the relation between $SPF_{in\ vitro}$ and %GI estimates. **Results:** The Reinforced Clostridium Medium showed the highest bacterial growth under anaerobic conditions with yields (weight/volume) of lyophilized extracts between 200 and 500 mg/mL. Among the strains under study, one (1) showed low ($6.0 \leq SPF_{in\ vitro} \leq 14.9$) and nine (9) medium ($15.0 \leq SPF_{in\ vitro} \leq 29.9$) UVB photoprotection efficacy, but all of them resulted in broad-spectrum (UVA–UVB) photoprotection ($\lambda c > 370$ nm). $SPF_{in\ vitro}$ was correlated ($R = 0.76$, $p \leq 0.05$) with %GI estimates. **Conclusions:** We demonstrated that extracts of Actinobacteria strains have properties as sunscreen. UVB photoprotection efficacy depended on concentration and %GI estimates in strain extracts.

Acknowledgments. The authors thank funding from the Ministry of Science, Technology and Innovation, the Ministry of Education, the Ministry of Industry, Commerce and Tourism, and ICETEX, Program *Ecosistema Científico-Colombia Científica*, from the *Francisco José de Caldas* Fund, Grant RC-FP44842-212-2018. The Ministry of Environment and Sustainable Development of Colombia supported the Universidad Industrial de Santander through access permits to genetic resources and derivatives for bioprospecting (Contract N°. 270).

IL-5.3.5

Role of the AhR transcription factor in reprogramming melanoma cells

Marie-Dominique Galibert
University of Rennes, Faculty of Medicine, CNRS UMR6290, Institute of Genetics and Development of Rennes, France

Cutaneous melanoma originates from melanocytes, the pigment-producing cells. They are the most aggressive and deadliest form of skin cancer. The establishment of the genomic landscape of cutaneous melanoma revealed the importance of the NRAS-BRAF driver mutations to fuel tumor development through the downstream activation of the MEK/ERK pathway. Selective inhibitors of the BRAF V600 driver mutation in combination with MEK inhibitors constitute now front-line treatment of metastatic BRAF V600 melanoma. This double blockade leads to a massive and initial tumor shrinkage, unfortunately patient ultimately relapse. It is now admitted that the high plasticity of melanoma cells mediates resistance to MAPK pathway inhibitors. Phenotypic drug resistance is dynamically linked to cell cycle heterogeneity, cell differentiation and metabolic reprogramming. Understanding how melanoma cells undergo cell reprogramming is thus critical to overcome resistance. We recently, showed that sustained activation of AhR, a ligand binding transcription factor, mediates melanoma resistance through specific cell reprogramming. Together, these results revealed new drug vulnerabilities to combat resistance.

IL-5.3.6

MITF and the chromatin modifiers PRDM7 and SETDB2 in melanoma

Ramile Dilshat[1], Hong Nhung Vu[1], Eiríkur Steingrímsson[1]
1) University of Iceland, Faculty of Medicine, BioMedical Center, Department of Biochemistry and Molecular Biology, Reykjavik, Iceland;

The microphthalmia associated transcription factor (MITF) is a critical regulator of melanocyte development and differentiation. It also plays an important role in melanoma where it has been described as a molecular rheostat that, depending on activity levels, allows reversible switching between different cellular states. We have shown that MITF directly represses the expression of many genes involved in establishing the extracellular matrix and focal adhesions in human melanoma cells[1], thus affecting cell morphology and cell-matrix interactions. However, MITF also indirectly affects the expression of many other genes raising the question of how it mediates these effects. We have identified the chromatin modifiers PRDM7 and SETDB2 as targets of MITF, suggesting that MITF may in part mediate its effects on gene expression through these modifiers. The role of both modifiers in melanoma cells and their link to MITF function will be presented.

References

1) Dilshat R, Fock V, Kenny C, Gerritsen I, Lasseur RMJ, Travnickova J, Eichhoff OM, Cerny P, Möller K, Sigurbjörnsdóttir S, Kirty K, Einarsdottir BÓ, Cheng PF, Levesque M, Cornell RA, Patton EE, Larue L, de Tayrac M, Magnúsdóttir E, Helga Ögmundsdóttir M**,** Steingrimsson E. 2021. MITF reprograms the extracellular matrix and focal adhesion in melanoma. Elife. 10:e63093. PMID: 33438577

Acknowledgements. This work was supported by grants 207067 and 217768 from the Icelandic Research Fund.

IL-5.3.7

B cells in human melanoma: spatiotemporal changes in phenotypes and antibody-independent functions

Stephan N Wagner
Medical University of Vienna, Department of Dermatology, Vienna, Austria

My research group is interested in the spatiotemporal dynamics of B cell responses in human cancer, particularly human melanoma. We and others have recently shown that the presence of tumor associated B cells (TAB) either scattered within the TME or localized within mature tertiary lymphoid structures (TLS) is associated not only with increased survival but also with enhanced treatment response to immune checkpoint blockade in melanoma patients.

B cells typically undergo a sequence of developmental changes in phenotype and function and do occur in human primary melanoma scattered within the TME preferentially at the invasive tumor-stroma front and also in the intratumoral stroma in metastatic melanomas. In addition, B cells can also aggregate to form with other cells tertiary lymphoid structures (TLS) in which, similar to secondary lymphoid organs, B cells are hypothesized to undergo local maturation by antigen-dependent activation, affinity maturation and class switch recombination before leaving the TLS to exert immunological functions at local tumor sites. However, compared with canonical secondary lymphoid tissues, human melanomas exhibit significant differences in B cell and lymphoid follicle maturation and distribution. These observations suggest a further evaluation of the role of local TLS versus locoregional lymph nodes in generating and enhancing local adaptive anti-tumor B cell responses.

Furthermore, the functional roles of B cells in human cancer remain poorly understood. These include the presence of B cell subpopulations with differences in phenotype and function at different disease stages and the mechanisms by which B cells can support T cell mediated anti-tumor immunity in an antibody-independent manner, including immune checkpoint blockade-mediated activation.

Here, we will discuss our recent work identifying the spatiotemporal dynamics of different phenotypic and functional B cell subpopulations during human melanoma disease progression, address the putative link between the presence of TLS and mature B cell subtypes in human melanoma samples, and describe potential mechanisms of B cell support for anti-tumor T cell responses. These mechanisms include T cell recruitment to tumor sites, secretion of immuno-stimulatory cytokine, cross-presentation of tumor antigens, and direct T cell activation.

Acknowledgments. This work was supported by the Austrian Science Fund (FWF) Project P30325-B28 and IPPTO Project No. DOC 59-B33 to SNW.

OC-5.3.8

Photoreactive properties of melanin from different skin phototypes and the contribution of melanin subunits

Krystian Mokrzyński[1], Shosuke Ito[2], Kazumasa Wakamatsu[2], Theodore G. Camenisch[3], Tadeusz Sarna[1], Michał Sarna[1]

1) Faculty of Biochemistry, Biophysics and Biotechnology, Department of Biophysics, Jagiellonian University, Cracow, Poland; 2) Institute for Melanin Chemistry, Fujita Health University, Toyoake, Aichi, Japan; 3) Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI, USA

Photoreactivity of melanin has become a major focus of research due to the postulated involvement of the pigment in UVA-induced melanoma. However, most of the previous studies were carried out using synthetic melanin pigments. In this research we examined the photoreactive properties of natural melanin pigments isolated from hair samples obtained from donors of different skin phototypes (I, II, III, and V). Dynamic Light Scattering (DLS) along with X-band and W-band electron paramagnetic resonance (EPR) spectroscopy were used to determine the physical properties of examined pigments. Alkaline hydrogen peroxide degradation and hydroiodic acid hydrolysis were employed to identify melanin subunits. EPR oximetry, EPR spin trapping and time-resolved near infrared phosphorescence was employed to determine the singlet oxygen photogeneration by the melanins. The efficiency of both superoxide and singlet oxygen photogeneration was found to be related to the chemical composition of the studied melanins. Melanins from blond and chestnut hair (phototypes II and III) exhibited the highest photoreactive potential among all examined pigments. Moreover, the highest values of quantum efficiency of singlet oxygen photogeneration for natural melanins were demonstrated at 332 nm and 365 nm supporting the postulated pigment contribution in the UVA-induced melanoma.

References

1) Mokrzyński K et al. Photoreactivity of Hair Melanin from Different Skin Phototypes - Contribution of Melanin Subunits to the Pigments Photoreactive Properties. International Journal of Molecular Sciences, 22, 2021.
2) Szewczyk G et al. Aerobic photoreactivity of synthetic eumelanins and pheomelanins: Generation of singlet oxygen and superoxide anion, 29, 2016.
3) Zadlo A et al. Photobleaching of pheomelanin increases its phototoxic potential: Physicochemical studies of synthetic pheomelanin subjected to aerobic photolysis. Pigment Cell and Melanoma Research, 32, 2019.

Acknowledgements. This research was funded by National Science Center of Poland, grant number Sonata-2015/19/D/ST4/01964, Opus-2017/27/B/ST5/02631 and Preludium-2020/37/N/NZ1/01054.

IL-5.4.1

Flow-induced shear stress and chemoresistance in ovarian cancer: A role for targeted PDT and combinations

Imran Rizvi[1,2], Utkan Demirci[3], Tayyaba Hasan[4], Marcela G. del Carmen[5], Huang-Chiao Huang[6,7], Jonathan P. Celli[8], Shubhankar Nath[4], Mustafa K. Ruhi[1], Christina Conrad[6].
1) University of North Carolina Chapel Hill and North Carolina State University, UNC-NC State Joint Department of Biomedical Engineering, Chapel Hill, NC, USA
2) University of North Carolina Chapel Hill School of Medicine, Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA
3) Stanford University School of Medicine, Bio-Acoustic MEMS in Medicine (BAMM) Laboratory, Canary Center at Stanford for Cancer Early Detection, Department of Radiology, Palo Alto, CA, USA
4) Massachusetts General Hospital, Harvard Medical School, Wellman Center for Photomedicine, Department of Dermatology, Boston,MA, USA
5) Massachusetts General Hospital, Harvard Medical School, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Boston, MA, USA
6) University of Maryland, Fischell Department of Bioengineering, College Park, MD, USA
7) University of Maryland School of Medicine, Marlene and Stewart Greenebaum Cancer Center, Baltimore, MD, USA
8) University of Massachusetts at Boston, Department of Physics, College of Science and Mathematics, Boston, MA, USA

Ascites results from the abnormal accumulation of fluid due to disease or abnormal pathology, and is most frequently associated with advanced-stage epithelial ovarian cancer. The presence of ascites portends the poorest outcomes and is associated with significant co-morbidities. The movement of fluid in the peritoneum is also known to promote disease progression and to facilitate the spread of tumor cells. An understudied area remains elucidating the role of physical stress (due to fluid flow) on treatment failure. Recent findings on flow-induced shear stress and resistance to platinum-based chemotherapy in 3D models for ovarian cancer will be presented. The role of targeted photodynamic therapy (PDT) and PDT-based combinations will be discussed.

References

1) Nath S, Pigula M, *et al*. Flow-induced Shear Stress Confers Resistance to Carboplatin in an Adherent Three-Dimensional Model for Ovarian Cancer: A Role for EGFR-Targeted Photoimmunotherapy Informed by Physical Stress. J Clin Med, 2020.
2) Rizvi I, Gurkan UA, *et al*. Flow induces epithelial-mesenchymal transition, cellular heterogeneity and biomarker modulation in 3D ovarian cancer nodules. Proc Natl Acad Sci USA, 2013.
3) Sorrin AJ, Kemal Ruhi M, *et al*. Photodynamic Therapy and the Biophysics of the Tumor Microenvironment. Photochem Photobiol, 2020.
4) Conrad C, Gray KM, *et al*. Mechanical Characterization of 3D Ovarian Cancer Nodules Using Brillouin Confocal Microscopy. Cell Mol Bioeng, 2019.

Acknowledgements. This work is supported by the following funding sources: Dr. Imran Rizvi - NIH R00 CA175292, NC Translational and Clinical Sciences Institute (NCTraCS), supported by the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), through Grant Award Number UL1TR002489, and UNC-NC State Joint BME Department Start-up Fund. Dr. Utkan Demirci - Stanford University Canary Center Seed Grant, NCI-NIH grant to the Center for Cancer Nanotechnology Excellence for Translational Diagnostics (CCNE-TD) at Stanford University (grant number: NIH U54 CA199075). Dr. Tayyaba Hasan – NIH R01 CA158415, R01 CA160998, R01 CA119388 Dr. Huang-Chiao Huang - NIH R00 CA194269, UMD Start-up Fund, UMD-UMB Research and Innovation Seed Grant, the Caring Together, NY, Ovarian Cancer Research grant, and the UMD-NCI Partnership for Integrative Cancer Research Grant. Dr. Jonathan Celli – NIH U54CA156734 UMass Boston-Dana Farber/Harvard Cancer Center Partnership

IL-5.4.2

Role of photooxidative damage in the acquired resistance of cancer cells

Valentina Rapozzi, Mariachiara Gani, Francesca D'Este, Luigi E. Xodo
University of Udine, Department of Medicine, Udine, Italy.

In recent years, we have investigated acquired tumor resistance after photodynamic therapy (PDT) in PC3 prostate cancer cells. We followed step by step what happened after chronic photooxidative stimulation until the development of a more aggressive and resistant tumor population (1). We examined some of the molecular signaling pathways involved in the oxidative stress response. We discovered that the modulation of these signaling pathways depend on the intensity of the oxidative insult.
Since the study of these different signaling pathways and their interactions could provide clues for understanding the events leading to an optimal PDT tumor response, our aim was to find a potential therapeutic target to prevent acquired resistance and resensitize prostate cancer cells to PDT.
We focused on Raf Kinase Inhibitor Protein (RKIP), which plays a pivotal role in tumor survival, invasion, and drug resistance (2). We showed that RKIP is involved in several PDT tumor response pathways, such as Mitogen Activated Protein Kinase (MAPK), Nuclear Factor Kappa-Light Chain-Enhancer of Activated B Cell (NF-kB), Nucleic Factor Erythroid 2-related factor 2(Nrf2), BTB and CNC homology (BACH -1) and Glycogen Synthase Kinase 3 Beta (GSK 3ß).
We found that during the development of acquired PDT resistance, the expression of pro-apoptotic RKIP was inhibited, while the expression of pro-survival KRAS, NF-kB, BACH -1 and Nrf2 genes was upregulated.
With increasing photooxidative stress, RKIP was overexpressed and MAPK, NF-kB, BACH -1, pGSK 3B were inhibited. Moreover, the subsequent increase in GSK 3ß expression led to a decrease in Nrf2 expression, which re-sensitized prostate cancer cells to PDT.
Our results highlight the role of RKIP as a potential convergence point of redox signaling pathways and suggest its use as a potential diagnostic marker to determine the efficacy of PDT treatment by preventing cell resistance.

References

1) Della Pietra E, Simonella F, Bonavida B, Xodo LE, Rapozzi V. Repeated optimal photodynamic treatments with pheophorbide a induce an epithelial mesenchymal transition in prostate cancer cells via nitric oxide. Nitric Oxide 2015.
2). Zaravinos A, Bonavida B, Chatzaki E, Baritaki S. RKIP: a key regulator in tumor metastasisinitiation and resistance to apoptosis: therapeutic targeting and impact. Cancers 2018.

Acknowledgements. This work was supported by AIRC "Associazione Italiana per la Ricerca sul Cancro", IG 2017, Project Code 19898.

OC-5.4.3

Effect of oxidative states on the efficiency of porphyrinoids in Photodynamic Therapy of Prostate Cancer

Mariana Q. Mesquita[1,2], Ana Rita Ferreira[2], Maria G. P. M. S. Neves[1], Margarida Fardilha[2], Maria A. F. Faustino[1]
1) University of Aveiro, Department of Chemistry & LAQV-REQUIMTE, Aveiro, Portugal; 2) University of Aveiro, Institute of Biomedicine – iBiMED & Department of Biomedical Sciences Institution, Aveiro, Portugal

Prostate cancer (PCa) is the second most common cancer in men worldwide, counting for more than 1.25 million new diagnoses in 2018.[1] Current treatment options for men with localized PCa include active surveillance, surgery (radical prostatectomy) and radiotherapy. Unfortunately, despite the clinical advances made in the last years regarding the treatment modalities for PCa, there is a high rate of functional complications associated with the aforementioned therapeutic approaches, which affect patient's quality of life.[2,3] The need for reducing these complications led to the development of PCa focal therapy. This type of therapy aims to minimize the side effects observed with radical therapy to the structures that surround the prostate, which are required for normal erectile function and urinary continence (specifically the bladder, rectum, neurovascular bundles and urinary sphincter) without compromising the oncological outcomes.[2,4] Among several focal therapies, photodynamic therapy (PDT) has been considered a promising local therapy to treat PCa.[4]
In the last decades, porphyrins have received increasing attention from the scientific community as favorable photosensitizers (PS) for PDT due to their good absorption in the visible range of the electromagnetic spectrum, effective production of reactive oxygen species (ROS), low dark toxicity, and stability/biocompatibility.[5] Reduced porphyrinoids, such as chlorins and isobacteriochlorins are also being explored as potential PSs, mainly due to their higher absorption bands in the red, when compared with porphyrin macrocycles.[5] To address the oxidative state effect on the photosensitising properties, we synthesized three fluorinated porphyrinoid derivatives (porphyrin, chlorin and isobacteriochlorin) and incorporated them into polyvinylpyrrolidone (PVP). The obtained PS@PVP formulations were fully characterized and their effectiveness as PS agents was evaluated *in vitro* against PCa cells (PC-3 cells). In addition, the molecular mechanisms underlying the photocytotoxicity of the formulations towards PC-3 cells were also investigated. In summary, in this communication, the synthesis, the characterization and the biological evaluation obtained with the three derivatives will be presented and discussed.

References

1) P. Rawla, Epidemiology of Prostate Cancer. World J. Oncol. 10, 63–89, 2019.
2) A. Kovács, M. Pinkawa, Interventional therapy in malignant conditions of the prostate. Radiologe. 59, 28–39, 2019.
3) O. Lodeizen, M. de Bruin, S. Eggener, S. Crouzet, S. Ghai, I. Varkarakis, A. Katz, J. L. Dominguez-Escrig, S. Pahernik, T. de Reijke, J. de la Rosette, Ablation energies for focal treatment of prostate cancer. World J. Urol. 37, 409–418, 2019.
4) T. Gheewala, T. Skwor, G. Munirathinam, Photosensitizers in prostate cancer therapy. Oncotarget. 8, 30524–30538, 2017.
5) S. Singh, A. Aggarwal, N. V. S. D. K. Bhupathiraju, G. Arianna, K. Tiwari, C. M. Drain. Glycosylated Porphyrins, Phthalocyanines, and Other Porphyrinoids for Diagnostics and Therapeutics. Chem. Rev. 115, 10261–10306, 2015.

Acknowledgements. Thanks are due to University of Aveiro and FCT/MCTES (Fundação para a Ciência e Tecnologia/Ministério da Ciência e Tecnologia, Portugal) for the financial support to LAQV-REQUIMTE (UIDB/50006/2020) and Institute for Biomedicine – iBiMED (UID/BIM/04501/2013 and POCI-01-0145-FEDER-007628) through national funds and, when applicable, co-financed by European Union, FEDER, QREN, and COMPETE within the PT2020 Partnership Agreement. Thanks are also due to the Portuguese NMR and Mass Networks. This work was also financially supported by an individual fellowship grant from FCT to M.M (SFRH/BD/112517/2015).

OC-5.4.4, P-5.4.14

Photodynamic efficacy of micelles containing a porphyrinic photosensitizer and potassium iodide against the resistant melanoma cell line B16F10

Letícia D. Costa[1], Kelly A. D. F. Castro[1,2], Juliana A. Prandini[2], Juliana C. Biazzotto[2], Augusto C. Tomé[1], Michael R. Hamblin[3], Maria da Graça P. M. S. Neves[1], Maria A. F. Faustino[1] and Roberto S. da Silva[2]
1) LAQV-REQUIMTE, Department of Chemistry University of Aveiro, Aveiro, Portugal; 2) Department of Physics and Chemistry, Faculty of Pharmaceutical Sciences of Ribeirão Preto University of São Paulo, Ribeirão Preto, São Paulo, Brazil; 3) Laser Research Centre, Faculty of Health Science University of Johannesburg, Doornfontein 2028, South Africa.

Melanoma is an aggressive and invasive form of skin cancer, that is characterized by being resistant to conventional therapies[1]. Photodynamic therapy (PDT) is a promising approach to treat oncological and non-oncological skin pathologies, and an interesting alternative to increase the response of melanotic cancers to the treatment[1]. In the last years, several strategies have been studied to overcome this resistance to treatments, and almost all of these approaches aim at the melanosome disruption to hinder the melanin production[1]. Notwithstanding its crucial role in the photoprotection of skin, melanin acts as powerful antioxidant, and the consequent decrease in the level of ROS allows the cancer cells to survive and proliferate[1]. So, enhance the treatment response it is mandatory to overcome the tolerance of these cells to the oxidative stress[1].

Porphyrins have been widely utilized as photosensitizing agents (PS) against cancer cells or microorganisms mainly due its ability to produce reactive oxygen species (ROS)[2]. In an attempt to increase the production of reactive species and increase the death of cancer cells, the $^1O_2$ produced after the PS photoactivation was combined with potassium iodide (KI). To the best of our knowledge, this is the first approach about the combined action of these two species against cancer cells, and in particular against the B16F10 melanoma cell line[3]. The porphyrinic PS and KI were encapsulated into micelles, not only to avoid solubility constrains associated to the poor solubility of porphyrins in the biological media, but also to increase the photodynamic effect due the proximity of all reactive species involved (Fig. 1)[3]. The biological results obtained with this approach will be presented and discussed in this communication.

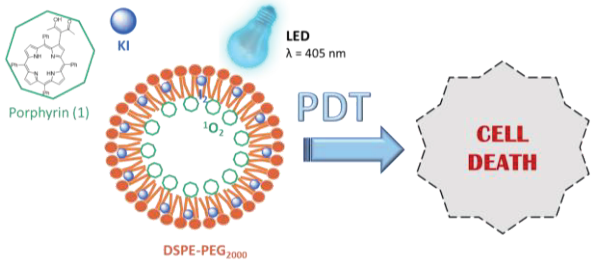


Fig. 1 *– Photodynamic efficacy of micelles containing a porphyrin PS and KI against B16F10 melanoma cells.*

References

1) Y. Huang, D. Vecchio, P. Avci, R. Yin, M. Garcia-Diaz and M. R. Hamblin, Melanoma Resistance to Photodynamic Therapy: New Insights, *Biol. Chem.* **2013**, 394, 239-250.
2) M. Ethirajan, Y. Chen, P. Joshi and R. K. Pandey, The role of Porphyrin Chemistry in Tumor Imaging and Photodynamic Therapy, *Chem. Soc. Rev.* **2011**, 40, 340-362.
3) K. A. D. F. Castro, L. D. Costa, J. A. Prandini, J. C. Biazzotto, A. C. Tomé, M. R. Hamblin, M. G. P. M. S. Neves, M. A. F. Faustino and R. S. da Silva, The Photosensitizing Efficacy of Micelles Containing a Porphyrinic Photosensitizer and KI against Resistant Melanoma Cells, *Chem. Eur. J.* **2021**, 27, 1990.

Acknowledgements: Thanks are due to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP- 2016/12707-0). We also thank the Fundação para a Ciência e a Tecnologia (FCT) for funding the project PTDC/QEQ-QOR/6160/2014, the LAQV-REQUIMTE (UIDB/50006/2020) through national funds and, when applicable, co-financed by FEDER within the PT2020 Partnership Agreement. K.A.D.F. Castro thanks CAPES for the post-doctoral scholarship granted (PNPD/CAPES). L.D. Costa thanks FCT for the doctoral grant SFRH/PD/BD/114578/2016. MRH was supported by US NIH Grants R01AI050875 and R21AI121700.

SP-5.4.5, P-5.4.5

Discovery of a novel mitochondrial-targeted photosensitiser for photodynamic cancer therapy

Radovan Krejcir[1], Tomas Briza[2,3], Martin Sterba[1], Oliver Simoncik[1], Petr Muller[1], Philip J. Coates[1], Pavel Martasek[4,5], Borivoj Vojtesek[1], Pavlina Zatloukalova[1]

1) Masaryk Memorial Cancer Institute, RECAMO, Brno, Czech Republic; 2) Charles University, First Faculty of Medicine, BIOCEV, Vestec, Czech Republic; 3) University of Chemistry and Technology Prague, Department of Analytical Chemistry, Prague, Czech Republic; 4) Charles University, First Faculty of Medicine, Department of Pediatrics and Adolescent Medicine, Prague, Czech Republic; 5) General University Hospital, Prague, Czech Republic

Photodynamic therapy (PDT) uses light to activate an inactive drug (so-called photosensitiser) in patients´ tissues. Its mechanism of action is based on the photodynamic generation of toxic molecular radicals, particularly reactive oxygen species (ROS). In cancer, PDT enables easy targeting of the tumour mass in organs that are accessible for illumination. An ideal photosensitiser is not toxic without illumination but generates a high yield of ROS upon photoactivation. Here, we present a newly identified photosensitiser from the polymethine class, salt 1, which shows an inherent anticancer effect and mediates a substantial phototoxic reaction with the potential to strengthen its therapeutic efficacy (1). Salt 1 selectively accumulates in the mitochondria of cancer cells and disrupts their functions, causing metabolic stress and inhibiting pro-growth signalling. As a result, autophagy is induced and, after depletion of cellular energy reserves, cells die in a non-apoptotic manner (2). Salt 1 was successfully tested *in vivo* in a mouse xenograft model, where it showed a strong antitumour effect with no apparent toxicity for animals in the study (3). As a photosensitiser, salt 1 interacts with 630nm light in the red region of the spectrum that penetrates well into tissues. Combined with light, salt 1 generates ROS in the mitochondria of treated cells, which leads to disruption of mitochondrial structure and a decrease in its membrane potential. Eventually, apoptosis is triggered in illuminated cells. The difference in the viability of illuminated and non-illuminated cells *in vitro* reaches as much as 14-fold. Moreover, the effective phototoxic reaction is induced with sub-micromolar concentrations of salt 1 (for A375 cells, IC_{50}=121nM) and low doses of activating light (5J/cm^2), which is convenient for therapeutic applications (1). Therefore, salt 1 is a candidate therapeutic molecule for exploiting the aberrant mitochondrial metabolism of cancer cells with two modes of action – antimetabolic and photodynamic.

References

1) Krejcir R., Briza T., Sterba M. *et al.* Anticancer pentamethinium salt is a potent photosensitizer inducing mitochondrial disintegration and apoptosis upon red light illumination. J. Photochem. Photobiol. B, Biol. 2020.

2) Krejcir R., Krcova L., Zatloukalova P. *et al.* A cyclic pentamethinium salt induces cancer cell cytotoxicity through mitochondrial disintegration and metabolic collapse. Int. J. Mol. Sci. 2019.

3) Briza T., Kralova J., Dolensky B. *et al.* Striking antitumor activity of a methinium system with incorporated quinoxaline unit obtained by spontaneous cyclization. ChemBioChem. 2015.

Acknowledgements

This work was supported by the Grant Agency of the Czech Republic (Grant No. 17-07822S), the European Regional Development Fund (Project ENOCH, No. CZ.02.1.01/0.0/0.0/16_019/0000868) and by the MH CZ - DRO (MMCI, 00209805).

SP-5.4.6, P-5.4.6

Quantitative Imaging of Pancreatic Microtumors on Alginate Hydrogels for Photodynamic Therapy Optimization

Nazareth Milagros Carigga Gutierrez, Tristan Le Clainche, Jean-Luc Coll, Lucie Sancey, Mans Broekgaarden

Institute for Advanced Bioscience, Univ. Grenoble Alpes, INSERM U1209, CNRS UMR5309, 38000 Grenoble, France

Pancreatic adenocarcinoma is a highly resistant form of cancer for which new treatments are needed. Still, the development of new cancer therapeutics faces a major challenge when promising results obtained from 2D *in vitro* systems fail to be confirmed *in vivo*. Three-dimensional culture models in which microscale tumors (microtumors) are grown on hydrogel scaffolds may solve this issue, as they bridge the gap between conventional 2D cell cultures and *in vivo* experiments. The 3D architecture of these microtumors mimics biochemical- and biomechanical cues, as well as cell-cell interactions more faithfully than 2D cultures. However, for culturing such microtumors, there is a strong need for inexpensive hydrogels that are easy to prepare and have low batch-to-batch heterogeneity. The aim of this study is to explore the use of sodium alginate-based hydrogels for culturing pancreatic microtumors in a manner that is compatible with state-of-the-art imaging assays[1].

We evaluated the impact of new hydrogel formulations composed of different percentages of sodium alginate and gelatin. These hydrogels were assessed for generating pancreatic microtumos from the PANC-1 cell line. We demonstrate that 2% alginate complemented with 0.5% gelatin formed hydrogels with similar performance as Matrigel: PANC-1 cells formed a large population of microtumors after 17 days of culture. Furthermore, these hydrogels were compatible with high-throughput image analysis[1, 2], enabling us to apply these models to studying the nano-bio interface of novel lipid nanocarriers for photodynamic therapy.

In conclusion, alginate-based hydrogels are inexpensive and effective scaffolds for 3D culture models of cancer, with versatile applications in research on novel cancer therapeutics. However, hydrogels need to be further evolved to match the complexity of native tissues[3]. For that purpose, new applications should be assessed for modeling the pancreatic cancer stroma using co-cultures. Ongoing work will focus on investigating cancer-stroma interactions and the impact of cancer therapies.

References:

1. Carigga Gutierrez NM, Le Clainche T, Coll J-L, Sancey L, Broekgaarden M. Microtumor cultures on Alginate-Gelatin hydrogels: Versatile 3D models to quantify the uptake, dark toxicity, and efficacy of photosensitizing agents. Methods Mol Biol.:Accepted.
2. Bulin A-L, Broekgaarden M, Hasan T. Comprehensive high-throughput image analysis for therapeutic efficacy of architecturally complex heterotypic organoids. Sci Rep 2017; 7: 16645.
3. Caliari SR, Burdick JA. A practical guide to hydrogels for cell culture. Nat Methods. 2016 May;13(5):405–14.

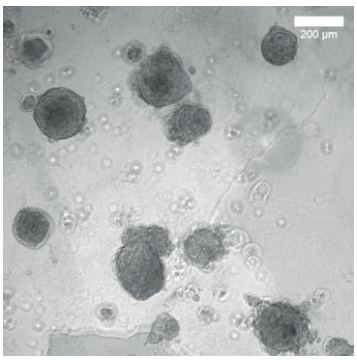


Figure 1. PANC-1 microtumors growth after 14 days on alginate-gelatin hydrogel.

IL-5.4.7

PCI-based strategies for overcoming resistance to cancer therapy

Pål Kristian Selbo[1], Judith Jing Wen Wong[1], Anette Weyergang[1], Cathrine Elisabeth Olsen[1], Anders Høgset[2], Kristian Berg[1]
1) Oslo University Hospital – The Norwegian Radium Hospital, Department of Radiation Biology, Oslo, Norway;
2) PCI Biotech, Oslo, Norway

Cancers develop in a Darwinian manner. Genetic and epigenetic changes cause origin of a malignant heterogeneous cell population, of which the fittest cells survive selective pressure, e.g., cancer stem cells (CSCs) resisting cancer therapy. Indeed, it has been demonstrated by independent labs that resistant progenies emerge from photodynamic therapy (PDT)-treated cancer cells. Resistance to PDT arises over time after multiple treatments of the same cell population and seems to be independent of photosensitizer type. However, the mechanism of resistance is dependent on the structure and intracellular localisation of the photosensitizer. We have shown that the MA11 breast cancer cells, overexpressing the efflux transporter and cancer stem cell (CSC) marker ABCG2, are intrinsically resistant to Pheophorbide A (PhA) PDT and that the cancer cells acquire stronger resistance with repeated sequences of PhA-PDT. Enhanced resistance to PhA-PDT was associated with enrichment of cells expressing ABCG2 on the cell surface[1]. The MA11 cells also acquired resistance to PDT using di-sulphonated meso-tetraphenylchlorin ($TPCS_{2a}$) as photosensitizer, which is not a substrate of ABCG2[2]. However, here the resistance was linked to dysregulated p38MAPK signalling (loss of p38-induced death signal) and enhanced EGFR-expression compared to MA11 wild-type cells[1]. The PDT-resistant cells did neither develop cross-resistance to chemotherapy (doxorubicin), radiotherapy nor the drug delivery method Photochemical Internalisation (PCI). PCI-based targeting of EGFR with EGF-saporin was demonstrated to bypass the PDT resistance in the MA11 cells[1]. In another study that will be presented, we show that 5-FU-resistant EMT-like pancreatic cancer cells are highly sensitive to $TPCS_{2a}$-PDT and hypersensitive to PCI of anti-CD105-saporin[3]. And finally, we recently demonstrated that high activity of the CSC marker aldehyde dehydrogenase (ALDH) does not protect colon cancer cells against $TPCS_{2a}$-PDT as opposed to radiotherapy[4]. Altogether, our accumulating data on using PCI to overcome therapy resistance further strengthen the use of $TPCS_{2a}$-PCI for targeting and elimination of CSCs.

References

1) C. E. Olsen, A. Weyergang, V. T. Edwards, K. Berg, A. Brech, S. Weisheit, A. Høgset and P. K. Selbo, Development of resistance to photodynamic therapy (PDT) in human breast cancer cells is photosensitizer-dependent: Possible mechanisms and approaches for overcoming PDT-resistance. *Biochemical Pharmacology*, 2017.
2) P. K. Selbo, A. Weyergang, M. S. Eng, M. Bostad, G. M. Mælandsmo, A. Høgset and K. Berg, Strongly amphiphilic photosensitizers are not substrates of the cancer stem cell marker ABCG2 and provides specific and efficient light-triggered drug delivery of an EGFR-targeted cytotoxic drug. *J.Control.Release*, 2012.
3) K. Lund, C. E. Olsen, J. J. W. Wong, P. A. Olsen, N. T. Solberg, A. Hogset, S. Krauss and P. K. Selbo, 5-FU resistant EMT-like pancreatic cancer cells are hypersensitive to photochemical internalization of the novel endoglin-targeting immunotoxin CD105-saporin. *J Exp Clin Cancer Res*, 2017.
4) J. J. W. Wong and P. K. Selbo, High aldehyde dehydrogenase activity does not protect colon cancer cells against $TPCS_{2a}$-sensitized photokilling, *Photochem Photobiol Sci*, 2020.

Acknowledgements. These works were supported by grants 2017068 (P. K. S.), 2016023 (J. J. W. W.) and 2014030 (C.E.O) from the South-Eastern Norway Health Authority and FU0803 (P. K. S.) from The Norwegian Radium Hospital Research Foundation.

IL-5.4.8

Resistance to Photodynamic Therapy of non melanoma skin cancer. Strategies to overcome it.

Juarranz A[1], González S[2], Gilaberte Y[3]

1) Department of Biology, Faculty of Sciences, Autónoma University of Madrid (UAM), Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; 2) Department of Medicine and Medical Specialties, Alcalá de Henares University, Madrid, Spain; 3) Miguel Servet University Hospital, IIS Aragón. Zaragoza Spain.

Photodynamic therapy (PDT) employing methyl-aminolevulinate (MAL) is being used for actinic keratosis, in situ squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), precancerous and cancerous situations of non-melanoma skin cancer (NMSC). However, the persistence of some tumors after treatment can occur. In fact, at clinical level, it has been described that some cutaneous SCC resistant to MAL-PDT become more aggressive, less differentiated and/or acquire an infiltrative pattern[1]. The problem is not only due to characteristics of tumoral cells, but also to the complex microenvironment around them (tumor microenvironment, TME) and particularly to cytokines secreted by one of the TME components, cancer associated fibroblasts (CAFs). CAFs have been reported to be crucial in the resistance to therapies. Therefore, we are studying the PDT resistance mechanisms of NMSC and looking for strategies to avoid it.
In our research we are using different in vitro and in vivo models for the evaluation of resistant cells alone or in combination with CAFs, isolated from patients. Resistant cells obtained by repeated MAL-PDT treatments, grown as 2D or 3D (spheroids), constitute an excellent system to evaluate factors implicated in the response to the treatment. In the same way, mice models, such as cell transplantation, permit evaluating tumorogenicity and aggressiveness of resistant cells. We have observed alteration in genes implicated in the resistance response of SCC and BCC to MAL-PDT, including: ATPase, GAPDH, PKM2, CCND1, EFGR, MAP3K1 and SMADs. We consider that complementary therapies to modulate them constitute potential strategies for the optimization of PDT in the treatment of NMSC.

References

1) Gilaberte Y et al., Cellular Intrinsic Factors Involved in the Resistance of Squamous Cell Carcinoma to Photodynamic Therapy. J Investigativ Dermatol, 134: 2428-2437. 2014.

Acknowledgements. This research was funded by Spanish grants from Instituto de Salud Carlos III MINECO and Feder Funds (PI18/00708).

IL-5.4.9

Multifunctional platforms for PDT in hypoxic environment

Ludivine Larue[1], Nolwenn Breton[2], Bertrand Vileno[2], Valérie Jouan-Hureaux[3], Tataye Moussounda Moussounda Koumba[4], Samir Acherar[5], Philippe Arnoux[1], Cédric Boura[3], Sylvain Marque[4], Gérard Audran[4], Céline Frochot[1]
1) CNRS, University of Lorraine, LRGP UMR 7274, Nancy, France 2) CNRS, University of Strasbourg, UMR 7177, Strasbourg, France 3) CNRS, University of Lorraine, CRAN UMR 7039, Nancy, France 4) CNRS, University of Marseille, ICR UMR 7273, Marseille, 5) CNRS, University of Lorraine, LCPM UMR 7274, Nancy, France

PDT can suffer from two drawbacks: the lack of selectivity of the PSs as well as the need for oxygen to be effective. To remedy the lack of selectivity, it is possible to design photosensitizers or nanoparticles coupled to vectors that will target receptors over-expressed onto tumor cells[1] or neovessels that allows the destruction of the vascularization and cause asphyxia of the tumor[2]. We already proved that KDPPR peptide has a good affinity for the NRP-1 receptors overexpressed on endothelial cells[3]. To compensate for the lack of oxygen, several strategies are possible[4] and we focused on photoactivatable alkoxyamines, molecules capable of generating toxic radicals by light activation. We will describe the synthesis of a multifunctional platform combining three units: a PS for an oxygen-dependent PDT, a peptide to target NRP-1 receptors and an alkoxyamine for an oxygen-independent activity. The study of the photophysical properties showed that the PS retained its capacity to induce the formation of singlet oxygen, the affinity tests confirmed the compound affinity for NRP-1. Thanks to the Electron Paramagnetic Resonance (EPR) spectroscopy we could detect the radicals generated after illumination of the alkoxyamine. The proof of concept was thus successfully established[5].

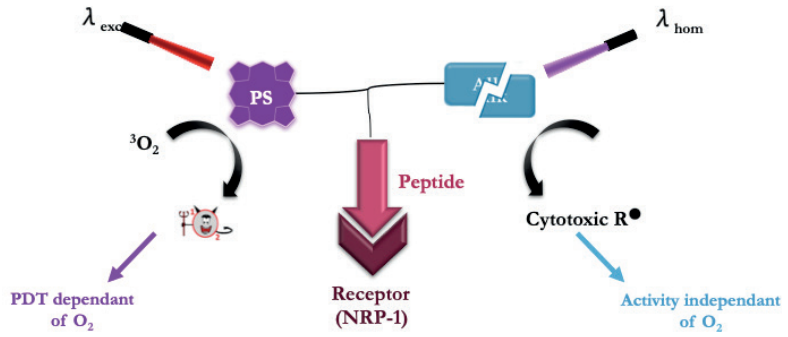


Fig. 1 – Structure of the new multifunctional platform.

References

1) Baydoun M., Morales O., Frochot C., Ludovic L., Leroux B., Thecua E., Ziane L., Grabarz A., Kumar A., de Schutter C., Collinet P., Azaïs H., Mordon S., Delhem N. Photodynamic Therapy on Peritoneal Ovarian Cancer Cells Induces the Release of Extra-cellular Vesicules with Immunoactivating Properties, Journal of Clinical Medicine, 2020.
2) Thomas E., Colombeau L., Gries M., Peterlini T., Mathieu C., Thomas N., Boura C., Frochot C., Vanderesse R., Lux F., Barberi-Heyob M., Tillement O. Ultrasmall AGuIX Theranostic Nanoparticles for Vascular-Targeted Interstitial Photodynamic Therapy of Glioblastoma. International Journal of Nanomedicine, 2017.
3) Youssef Z., Yesmurzayeva N., Larue L., Juan-Hureaux V., Colombeau L., Acherar S., Frochot C. New targeted gold nanorods for the treatment of glioblastoma by photodynamic therapy, Journal of clinical medicine, 2019.
4) Larue L., Myrzakhmetov B., Ben-Mihoub A., Moussaron A. Thomas N., Arnoux P., Baros F. Vanderesse R., Acherar S. , Frochot C. Fighting Hypoxia to Improve PDT, Pharmaceuticals, 2019.
5) Larue L., Moussounda Moussounda Koumba T., Le Breton N., Vileno B., Arnoux P., Jouan-Hureaux V., Boura C., Audran G., Bikanga R., Marque S.R.A., Acherar S., Frochot, C. The design of a targeting and Oxygen-independent platform to improve Photodynamic Therapy: A proof of concept, ACS applied Biomaterials, 2021.

Acknowledgements. This work was supported by the french ministry of research.

OC-5.4.10

Metabolic reprogramming involved in resistance to photodynamic therapy in skin squamous cell carcinoma. Metformin as adjuvant.

Mascaraque-Checa M[1], Santiago JL[2], Terrén S[1], Nájera L[3], González S[4], Gilaberte Y[5], Juarranz Á[1].

1) Universidad Autónoma de Madrid, Dept. Biology, Madrid, Spain; 2) Hospital General Universitario de Ciudad Real, Dept. Dermatology, Ciudad Real, Spain; 3) Hospital Universitarios Puerta de Hierro-Majadahonda, Dept. Pathology, Madrid, Sapin; 4) Universidad de Alcalá, Dept. Medicine and Medical Specialities, Madrid, Spain; 5) Hospital Universitario Miguel Servet, Dept. Dermatology, Zaragoza, Spain.

Squamous Cell Carcinoma (SCC) is the second most frequent type of skin cancer among the population[1]. Within the clinically approved treatments, photodynamic therapy (PDT) with the methyl aminolevulinate acid (MAL) is an extended non-invasive modality. Nevertheless, PDT is not always effective and resistant cells may appear after treatment[2]. Normal differentiated cells depend primarily on mitochondrial oxidative phosphorylation to generate energy, but cancer cells change this metabolism to an aerobic glycolysis (Warburg effect), which could influence in the response to antitumor drugs[3]. A potential option to avoid recurrences is to combine PDT with other treatments.

In this work we have evaluated the metabolic changes that occur in PDT-resistant squamous cell carcinoma (SCC) cells. We have also analyzed metformin treatment, an antidiabetic type II compound, to sensitize SCC PDT-resistant to PDT. For this we have used the human SCC cell lines: A-431 and SCC-13, which we have called parental (P). From these cell lines, PDT resistant cells were generated by exposure to 10 cycles of PDT (1 mM MAL followed by irradiation with red light). Resistant cells were inoculated in immunosuppressed mice, the induced tumours were sub-cultured by explants and a cell population called 10GT was obtained. PDT resistant cells showed a metabolic reprogramming (aerobic glycolysis increasing and OXPHOS reduction), which could influence the response to PDT. This result was also confirmed in the induced tumors by SCC13 cells (P and PDT-resistant) inoculation in mice. The metformin treatment caused a reduction in aerobic glycolysis and an increases in oxidative phosphorylation in SCC resistant cells. Finally, the metformin addition to PDT (combined treatment) improved the cytotoxic effect on P and resistant cells. The mechanism of action of combined treatment is caused for increased protoporphyrin IX production, reactive oxygen species generation and inhibition of AKT/mTOR pathway, through AMPK activation. In conclusion, we can indicate that PDT resistance implicate a metabolic reprogramming towards aerobic glycolysis and metformin treatment reversed it. Therefore, metformin constitutes an excellent adjuvant for PDT in SCC.

References

1) Barton V, Armeson K, Hampras S… Alberg AJ. Nonmelanoma skin cancer and risk of all-cause and cancer-related mortality: a systematic review. *Archives of dermatological research,* 2017.
2) Gracia-Cazaña T, Salazar N, Zamarron A… Juarranz A. Resistance of Nonmelanoma Skin Cancer to Nonsurgical Treatments. Part II:Photodynamic Therapy, Vismodegib, Cetuximab, Intralesional Methotrexate, and Radiotherapy. *Actas Dermo-Sifiliograficas*, 2016.
3) Bhattacharya B, Mohd Omar MF, Soong R. The warburg effect and drug resistance. *British Journal of Pharmacology,* 2016.

Acknowledgements. This work was supported by Spanish grants from Instituto de Salud Carlos III MINECO and Feder Funds (PI18/00708).

P-5.4.11

Could the length of the alkyl chain affect the photodynamic activity of tetra-alkylpyridylporphyrins?

Matteo Rugiero, Enrico Caruso, Miryam Chiara Malacarne, Marzia Bruna Gariboldi
Department of Biotechnology and Life Sciences (DBSV), University of Insubria, Via J.H. Dunant 3, 21100 Varese (VA), Italy.

Photodynamic therapy (PDT) is a minimally invasive treatment that uses the combination of a photosensitizing agent (PS) and light to selectively target solid tumors, as well as several nonneoplastic proliferating cell diseases. Following systemic administration, PSs are activated by localized irradiation with visible light; in the presence of adequate concentrations of molecular oxygen, this results in reactive oxygen species (ROS) formation and subsequent tissue damage [1].
The family of porphyrins and their derivatives encompass the majority of photosensitizers and studies concerning the synthesis and activity of new porphyrin derivatives, aiming the improvement of chemical-physical or activity characteristics, are abundant in the scientific literature [2,3].
In this study, two series of tetra cationic alkyl pyridyl porphyrins, including four derivatives, were synthesized, differing for the presence or absence of the zinc atom in the tetrapyrrole core; the four derivatives are characterized by a different length of the alkyl chain (from one to twelve carbon atoms). The compounds were fully chemically characterized, their light stability and singlet oxygen production determined. To determine a possible relationship between the photodynamic activity and the length of the alkyl chain, the effects on cell viability were also evaluated on a panel of tumor cell lines, following PSs activation.

References

[1] Macdonald, I.J.; Dougharty, T.J. Basic principles of photodynamic therapy. J. Porphyrins Phthalocyanines 2001, 5(2), 105-129.
[2] Banfi, S.; Caruso E.; Buccafurni, L.; Murano, R.; Monti, E.; Gariboldi, M.B.; Papa, E.; Gramatica, P. Comparison between 5,10,15,20-tetraaryl- and 5,15-diarylporphyrins ad photosensitizers: Synthesis, photodynamic activity, and quantitative structure-activity relationship modeling. J. Med. Chem. 2006, 49(11), 3293–3304.
[3] Kou, J.; Dou, D.; Yang, L. Porphyrin photosensitizers in photodynamic therapy and its applications. Oncotarget 2017, 8(46), 81591-81603

P-5.4.12

Zn(II) and free base *N*-methylated tripyridylporphyrins: impact of solubility and light excitation wavelength on PDT effect

Martina Mušković[1], Iva Džeba[2], Nikola Basarić[3], Martin Lončarić[4], Branka Mihaljević[2], Ivana Ratkaj[1], Nela Malatesti[1]

1) University of Rijeka, Department of biotechnology, Rijeka, Croatia; (e-mail: nela.malatesti@biotech.uniri.hr)
2) Ruđer Bošković Institute, Division of Material Chemistry, Radiation chemistry and Dosimetry Laboratory, Zagreb, Croatia
3) Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Laboratory of Synthetic Organic Chemistry, Zagreb, Croatia
4) Ruđer Bošković Institute, Centre of excellence for advanced material and sensors, Photonics and quantum optics unit, Zagreb, Croatia

Amphiphilic cationic porphyrins have shown high potential in use as photosensitizers (PSs) for photodynamic therapy (PDT). Our research group has shown that compounds conjugated with long chain (C18) have an exceptional PDT effect compared to hydrophilic analogues on different tested cell lines. Unfortunately, highly effective amphiphilic compounds showed reduced selectivity to tumour cells, relatively high dark toxicity, and their aggregation was observed in high concentration[1]. It must be kept in mind that the optimal ratio of hydrophilic and hydrophobic moieties is necessary to facilitate passage through the membrane bilayer, while maintaining good solubility in water and avoiding formation of aggregates that reduce the PDT effect[2].
Chelation with Zn(II) alters the properties of free base porphyrins; it changes lipophilicity of the compounds and may increase the lifetime of the excited triplet state (^{3}PS*). The change also occurs in the absorbance spectra, as expected the number of Q bands reduces from four to two and there is a negligible absorption above 610 nm for these compounds to be efficiently activated with red. Therefore, we suggest photoactivation using LED based source of orange light, which better corresponds to the optical properties of Zn(II) porphyrins while maintaining good penetration through the tissue[4].In this work, we will present the synthesis of two groups of *N*-methylated tripyridylporphyrins, free base and chelated with Zn(II), both conjugated to alkyl chains of different length. Their photophysical and photochemical properties studied in different solvents,-by laser flash photolysis (LFP) and time-resolved fluorescence spectroscopy (TRF) will be presented. The impact of small changes in chain length will also be evaluated *in vitro*, using MTT assay to detect cytotoxicity and measuring cellular uptake by using fluorescence microscopy on melanoma cells (MeWo) and human foreskin fibroblasts (HFF). To study the effect of the irradiation wavelength, the cytotoxicity of the compounds will be evaluated using red light (645 nm; fluence rate 2.0 mW / cm^2) and orange light (605 nm; fluence rate 1.5 mW / cm^2).

References

1) M. Jelovica, P. Grbčić, M. Mušković, M. Sedić, S. Kraljević Pavelić, M. Lončarić, In Vitro Photodynamic Activity of N-Methylated and N-Oxidised Tripyridylporphyrins with Long Alkyl Chain and Their Inhibitory Activity in Sphingolipid Metabolism. ChemMedChem, January 2018.
2) N. Malatesti,I. Munitić, I. Jurak, Porphyrin-based cationic amphiphilic photosensitizer as potential anticancer antimicrobial and immunosuppressive agents. Biophysics Reviews, April 2017.
3) L. Benov, J.Craik, I. Batinić-Haberle, The Potential of Zn(II) N-Alkylpyrdylporphyrins for Anticancer Therapy. Anti-Cancer Agents in Medicinal Chemistry, February 2012.
4) M. Kim, A. Darafsheh, Light Sources and Dosimetry Techniques for Photodynamic Therapy. Photochemistry and Photobiology, March 2020.

Acknowledgements. This work was supported by the University of Rijeka grants uniri-prirod-18-173. The equipment used in this work is part of the project "Research Infrastructure for Campus-based Laboratories at the University of Rijeka", financed by European Regional Development Fund (ERDF) and the Ministry of Science, Education and Sport of the Republic of Croatia (RC.2.2.06-0001).

P-5.4.13

Polyvinylpyrrolidone formulations of porphyrinoids as photosensitizers for Photodynamic Therapy of Prostate Cancer

Mariana Q. Mesquita[1,2], Ana Rita Ferreira[2], Maria G. P. M. S. Neves[1], Margarida Fardilha[2], Maria A. F. Faustino[1]
1) University of Aveiro, Department of Chemistry & LAQV-REQUIMTE, Aveiro, Portugal; 2) University of Aveiro, Institute of Biomedicine – iBiMED & Department of Biomedical Sciences Institution, Aveiro, Portugal

Prostate cancer (PCa) is the second most frequent cancer diagnosed in men worldwide.[1] Conventional treatment options to treat this cancer include active surveillance, radical prostatectomy, radiotherapy, and/or androgen-deprivation therapy. Unfortunately, all these treatments may cause several side effects that may seriously impair the quality of life of each patient.[2,3] The necessity of reducing these complications led to the development of PCa focal therapy, which aims to reduce the side effects seen with radical therapy, while maintaining oncological control.[2] Among the common treatment options, photodynamic therapy (PDT) is being considered a promising local therapy to treat PCa.[4] Although PDT is an established treatment modality approved for several types of cancer, the low solubility, the reduced tumor selectivity, the absorption in the therapeutic window and the poor clearance from the body of the currently approved photosensitizers (PS) hampers its wide clinical application.[5] In this regard, herein we synthesized three fluorinated porphyrinoid derivatives (porphyrin, chlorin and isobacteriochlorin derivatives) and entrapped them into polyvinylpyrrolidone (PVP) to prevent the aggregation and preserve the desirable photophysical properties of the compounds under physiological environment. The photodynamic efficacy of the synthesized formulations as PS was subsequenytly assessed *in vitro* against a PCa cell line (PC-3 cells). Furthermore, the molecular mechanisms underlying the photocytotoxicity of the formulations towards PC-3 cells were also elucidated.

References

1) P. Rawla, Epidemiology of Prostate Cancer. World J. Oncol. 10, 63–89, 2019.
2) A. Kovács, M. Pinkawa, Interventional therapy in malignant conditions of the prostate. Radiologe. 59, 28–39, 2019.
3) O. Lodeizen, M. de Bruin, S. Eggener, S. Crouzet, S. Ghai, I. Varkarakis, A. Katz, J. L. Dominguez-Escrig, S. Pahernik, T. de Reijke, J. de la Rosette, Ablation energies for focal treatment of prostate cancer. World J. Urol. 37, 409–418, 2019.
4) T. Gheewala, T. Skwor, G. Munirathinam, Photosensitizers in prostate cancer therapy. Oncotarget. 8, 30524–30538, 2017.
5) X. Zhao, J. Liu, J. Fan, H. Chao, X. Peng, Recent progress in photosensitizers for overcoming the challenges of photodynamic therapy: from molecular design to application. Chem. Soc. Rev. 50, 4185–4219, 2021.

Acknowledgements. Thanks are due to University of Aveiro and FCT/MCTES (Fundação para a Ciência e Tecnologia/Ministério da Ciência e Tecnologia, Portugal) for the financial support to LAQV-REQUIMTE (UIDB/50006/2020) and Institute for Biomedicine – iBiMED (UID/BIM/04501/2013 and POCI-01-0145-FEDER-007628) through national funds and, when applicable, co-financed by European Union, FEDER, QREN, and COMPETE within the PT2020 Partnership Agreement. Thanks are also due to the Portuguese NMR and Mass Networks. Image acquisition was performed in the LiM facility of iBiMED, a node of PPBI (Portuguese Platform of BioImaging): POCI-01-0145-FEDER-022122. This work was also financially supported by an individual fellowship grant from FCT to M.M (SFRH/BD/112517/2015).

IL-6.1.2

The Origin of the Phytochrome A Functions.

Akira Nagatani, Misato Kikuchi, Yukiko Yoshikawa, Kanako Shinohara, Tomomi Suzuki, Nobuyoshi Mochizuki
Kyoto University, Graduate School of Science, Kyoto, Japan

PHYA and PHYB are major members of the phytochrome family. They have diverged very early in the history of seed plants to have distinctive functions (Mathews, 2010). PHYA responds to continuous far-red (FR-HIR) and very low fluence red light (VLFR), whereas PHYB mediates responses to low fluence red light (LFR). In addition, PHYA but not PHYB is rapidly degraded upon photo-activation. Nevertheless, their overall structures are very similar. All phytochromes consist of the photo-sensory N-terminal and the dimerizing C-terminal moieties. The N-terminal half is further divided into 4 domains, namely the N-terminal extension, PAS, GAF and PHY. The high structural similarity between different phytochromes allow us to study functions of the chimeric phytochromes. We have systematically expressed 16 chimeric phytochromes between PHYA and PHYB in *Arabidopsis* and examined how they behave in vivo. The results indicate that the PAS domain is important for nuclear localization under continuous FR, whereas PHY domain tunes the light sensitivity of phytochrome (Oka et al., 2012). In addition, GAF is important for rapid degradation upon exposure to light.

The PHY domain is spatially separated from the PAS-GAF domain to which the chromophore is attached. Interestingly, a peculiar "tongue" structure is protruded from the PHY domain towards the chromophore (Essen et al., 2008: Yang et al., 2008). Furthermore, a few amino acid substitutions in this region are known to affect the activity of phytochrome. Hence, we constructed chimeric phytochromes with PHYA or PHYB "tongue" structure and compared their activities. Consequently, the PHYA tongue sequence was shown to confer higher light sensitivity to the phytochrome. Next, we replaced several amino acid residues, one by one, in the PHYA tongue structure with respective PHYB amino acid residues in a PHYB-based chimeric phytochrome. The activity of the original chimeric phytochrome to respond to weak R and continuous FR were simultaneously diminished in three of the mutants, thereby 3 amino acid residues were indicated to be involved in the sensitization of phytochrome within the "tongue" structure.

Intriguingly, two of those residues are predicted to face each other in the basal region of the tongue structure in the 3 D model of phytochrome molecule, whereas one of them is situated in the vicinity of the chromophore. Hence, insights into how the light sensitivity is controlled by the tongue structure are provided. We then examined the phylogenetic tree of phytochromes (Li et al., 2015) to infer when those amino acid substitutions took place during the evolution of seed plants. To our surprise, the amino acid residues in these three positions are highly conserved among phytochromes not only in ferns, mosses and liverworts but also in gymnosperms, indicating that the light sensitivity adjustment through the mutations of these amino acid residues happened only recently after the split of angiosperms. Secondly, the first position is mutated only in the PHYA linage whereas the second position is mutated only in PHYB/E. The third position is mutated in both the linages but to different amino acids. Taken together, light sensitivity is inferred to be modified both in PHYA and PHYB but to the opposite directions early in the history of angiosperms. As discussed by Mathews (2010), the ability to respond to a wider light intensity under the sun and canopy might be beneficial for ancestral angiosperms to spread in the forest dominated by non-vascular plants.

References

Sarah Mathews, Evolutionary Studies Illuminate the Structural-Functional Model of Plant Phytochromes, *Plant Cell, 22:4-16*, 2010

Yoshito Oka et al., *Arabidopsis* Phytochrome A Is Modularly Structured to Integrate the Multiple Features That Are Required for a Highly Sensitized Phytochrome, *Plant Cell, 24:2949-62,* 2012

Lars-Oliver Essen et al., The structure of a complete phytochrome sensory module in the Pr ground state, *Proc. Natl. Acad Sci. USA*, 105:14709-14, 2008

Xiaojing Yang et al., Crystal structure of *Pseudomonas aeruginosa* bacteriophytochrome: photoconversion and signal transduction, *Proc. Natl. Acad Sci. USA*, 105:14715-20, 2008

Fay-Wei Li et al., Phytochrome diversity in green plants and the origin of canonical plant phytochromes, *Nat Commun.,6:7852*, 2015

IL-6.1.3

Structural Basis for Light Control of Cell Development: New Insights from Bacterial Phytochrome Proteins

Emina A. Stojković[1], Sebastian Westenhoff[2], Marius Schmidt[3]
1) Northeastern Illinois University, Department of Biology, Chicago, IL, USA; 2) University of Gothenburg, Department of Chemistry and Molecular Biology, Gothenburg, Sweden; 3) University of Wisconsin-Milwaukee, Department of Physics, Milwaukee, WI, USA

Phytochromes (PHYs) are photoreceptor proteins first discovered in plants, where they control a variety of photomorphogenesis events. PHYs as photochromic enzymes that usually contain C-terminal kinase domain can reversibly switch between a red light absorbing (Pr) and a far-red light absorbing (Pfr) state. Light signals are transduced as structural change through the entire protein to modulate its activity. The unexpected discovery of bacteriophytochromes (BphPs) in non-photosynthetic bacteria has opened new frontiers in our understanding of the mechanisms by which these natural photoswitches can control single cell development. BphPs from the non-photosynthetic myxobacterium *Stigmatella aurantiaca* are of special interest. Myxobacteria are distinguished among prokaryotes by a multicellular stage in their life cycle known as fruiting bodies, which in *S. aurantiaca* is controlled by light. We determined the crystal structures of the two BphPs from *S. aurantiaca,* denoted *Sa*BphP1 and *Sa*BphP2. They have distinct photochemistry, although they bind the same bilin chromophore and share a large sequence identity. Unlike classical BphPs, wild-type SaBphP1 lacks a conserved histidine (His289) that stabilizes the covalently-bound bilin chromophore and undergoes limited Pr/Pfr photoconversion that can be restored by a single Thr289His mutation. Furthermore, the crystal structures of SaBphP1 wild-type and Thr289His mutant in comparison to classical SaBphP2 wild-type protein differ in the conformation of the essential bilin chromophore as well as important protein dimer interface in the dark-adapted Pr state. Recently, we determined short-lived crystal structures of the photosensory core modules of SaBphP2 captured by an X-ray free electron laser 5 ns and 33 ms after light illumination of the Pr state[1]. We observe large structural displacements of the covalently bound bilin chromophore, which trigger a bifurcated signaling pathway that extends through the entire protein. The snapshots show with atomic precision how the signal progresses from the chromophore, explaining how plants, bacteria, and fungi sense red light.

References

1) M. Carrillo, S. Pandey et al, High-resolution Crystal Structures of Transient Intermediates in the Phytochrome Photocycle. Structure 2021 Mar 20: S0969-2126(21)00077-0. doi: 10.1016/j.str.2021.03.004. Online ahead of print.

Acknowledgements. This work was supported by National Science Foundation (NSF) Science and Technology Centers (STC) grant NSF 1231306 (Biology with X-ray Lasers).

OC-6.1.4

QM/MM Simulations of Spectral Tuning in Phytochrome-like Photoreceptors

Christian Wiebeler[1]
1) Institute for Analytical Chemistry, Faculty of Chemistry and Mineralogy, Leipzig University, Germany

Phytochromes constitute a diverse family of photoreceptors and one of its subfamilies are Cyanobacteriochromes (CBCRs), which are composed of a single GAF domain. CBCRs are promising candidates for biotechnological applications, owing to their photochromism, compactness and spectral diversity. In case of the CBCR Slr1393, one isomer absorbs red light (Pr) and the other one green (Pg).[1] These two forms can be interconverted into each other by light illumination. Slr1393 binds phycocyanobilin (PCB) as chromophore and the crystal structures of both forms have been obtained recently.[2] In this contribution, we will focus on results from hybrid quantum mechanics/molecular mechanics (QM/MM) simulations for the Pr and Pg forms of Slr1393.[3]

Our QM/MM studies started from the crystal structures. First, the structures were optimized in several stages, followed by classical molecular dynamics (MD) for thermalization and backbone relaxation. During these steps, it was checked that the non-covalent interactions of PCB with the protein remained intact. The snapshots for excited state calculations were then generated *via* QM/MM MD. The final spectrum is an average of the spectra from the different geometries of each form and the results are complemented with wave function analysis.

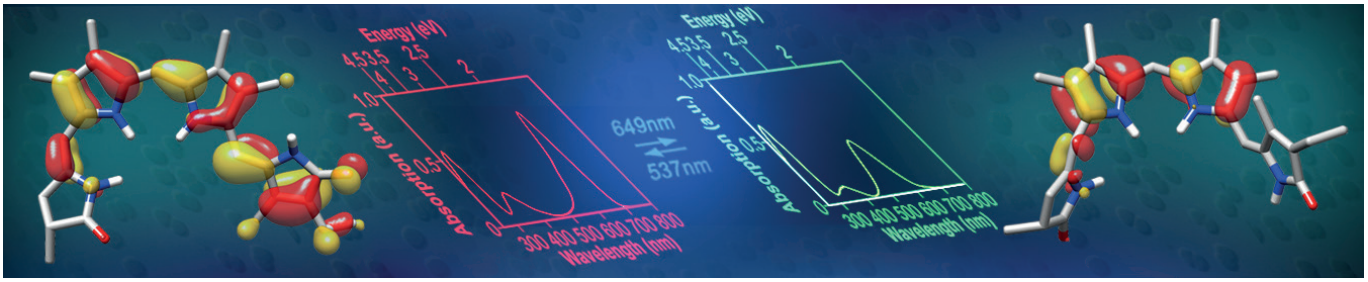


Fig. 1 – The difference in absorption between the red light-absorbing dark state and the green light-absorbing photoproduct of Slr1393 could be traced back to changes in the effective conjugation lengths of the chromophore in the two conformations.[3]

In addition, also results from a benchmark study for this protein will be presented.[4] Its focus is on the choice of an appropriate semiempirical method for QM/MM MD, on methods for excited state calculations and on the importance of sampling. More recently, we have carried out similar simulations for further proteins, *e.g.* for All2699,[5] and published a review article on computational studies of the photochemistry in phytochromes.[6]

References

1) X. Xu *et al.*, Combined Mutagenesis and Kinetics Characterization of the Bilin-Binding GAF Domain of the Protein Slr1393 from the Cyanobacterium Synechocystis PCC6803. *ChemBioChem* **15**, 1190–1199, 2014.
2) X. Xu, A. Höppner, C. Wiebeler, K.-H. Zhao, I. Schapiro, and W. Gärtner, Structural Elements Regulating the Photochromicity in a Cyanobacteriochrome. *Proc. Natl. Acad. Sci. USA* **117**, 2432–2440, 2020.
3) C. Wiebeler, A. G. Rao, W. Gärtner, and I. Schapiro, The Effective Conjugation Length Is Responsible for the Red/Green Spectral Tuning in the Cyanobacteriochrome Slr1393g3. *Angew. Chem. Int. Ed.* **58**, 1934–1938, 2019.
4) C. Wiebeler and I. Schapiro, QM/MM Benchmarking of Cyanobacteriochrome Slr1393g3 Absorption Spectra. *Molecules* **24**, 1720, 2019.
5) C. Slavov *et al.*, The Interplay between Chromophore and Protein Determines the Extended Excited State Dynamics in a Single-Domain Phytochrome. *Proc. Natl. Acad. Sci. USA* **117**, 16356–16362, 2020.
6) J. R. Church, A. G. Rao, A. Barnoy, C. Wiebeler and I. Schapiro, Computational Studies of Photochemistry in Phytochrome Proteins. *Challenges and Advances in Computational Chemistry and Physics* **31**, 197–226, 2021.

Acknowledgements. We acknowledge funding by the German Research Foundation *via* grants: WI 4853/1-1 and WI 4853/2-1.

IL-6.1.5

Bridging the language barrier between sensor and effector domains in bacteriophytochromes

Cornelia Böhm[1], Nikolina Todorovic[1], Marco Balasso[1], Geoffrey Gourinchas[1] and Andreas Winkler[1]
1) Graz University of Technology, Institute of Biochemistry, Graz,

Bacteriophytochrome sensors are versatile entities that during evolution have frequently been repurposed to control a variety of biological functions. Among other modes of operation, the direct allosteric regulation of enzymatic functionalities exists for histidine kinases, diguanylate cyclases, cyclic-dimeric-GMP phosphodiesterases and metal dependent protein phosphatases[1]. Therefore, repurposing bacteriophytochrome sensors to control enzymatic functionalities that have not been created during evolution is one interesting approach for the design of novel optogenetic tools. To this end, however, the molecular mechanisms of how different sensors are functionally coupled to diverse effectors need to be understood in more detail.

In an order to tackle this challenging question, we have recently characterized chimeric proteins between two related phytochrome-activated diguanylate cyclases, with pronounced functional differences of the parent proteins, in detail. Thereby we have obtained insights into the functional roles of different structural elements and their coupling with each other. Among other important findings, the central regulatory role of the PHY domain dimer interface has manifested itself in being one of the key determinants for efficient light signal integration and transduction to the effector domain. Understanding how a particular structural element is modulated by upstream and/or downstream elements provides important functional insight and the major strength of our chimeras is to highlight elements that, when targeted for modifications, influence specific phytochrome properties. In the end, any novel optogenetic tool is a chimeric system generated from a photosensory module and an effector of interest. Being able to build upon insights from chimeras within closely related systems will therefore also be beneficial for more ambitious new optogenetic tool developments. Appreciating the fact that more than just linker length and/or linker composition is needed for the creation of a functional sensor-effector system is one of the key-findings that will be discussed in this presentation.

References

1) Geoffrey Gourinchas, Stefan Etzl, Andreas Winkler, Bacteriophytochromes – from informative model systems of phytochrome function to powerful tools in cell biology. Curr. Opin. Struct. Biol. 57, 72–83. 2019.

Acknowledgements. This work was supported by the Austrian Science Fund (FWF): P32022 to AW.

IL-6.1.6

Biochemical activity of a model bacteriophytochrome from *Deinococcus radiodurans*

Elina Multamäki[1], Rahul Nanekar[2], Dmitry Morozov[3], Topias Lievonen[2], David Golonka[4], Weixiao Yuan Wahlgren[5], Brigitte Stucki-Buchli[2], Jari Rossi[1], Vesa P. Hytönen[6], Sebastian Westenhoff[5], Janne A. Ihalainen[2], Andreas Möglich[4], Heikki Takala[1,2]

1) University of Helsinki, Department of Anatomy, Helsinki, Finland; 2) University of Jyvaskyla, Department of Biological and Environmental Science and Nanoscience Center, Jyvaskyla, Finland; 3) University of Jyvaskyla, Department of Chemistry and Nanoscience Center, Jyvaskyla, Finland; 4) Universität Bayreuth, Lehrstuhl für Biochemie, Bayreuth, Germany; 5) University of Gothenburg, Department of Chemistry and Molecular Biology, Gothenburg, Sweden;6) University of Tampere, Faculty of Medicine and Health Technology, Tampere, Finland

Bacteriophytochrome photoreceptors usually belong to two-component signaling systems, which transmit environmental stimuli to a response regulator through a histidine kinase domain. Phytochromes switch between red light-absorbing (Pr) and far-red light-absorbing (Pfr) states. Despite exhibiting extensive structural responses during this transition,[1,2] no kinase activity of the model bacteriophytochrome from *Deinococcus radiodurans* (DrBphP) has been reported. Here, we resolve this long-standing conundrum by comparatively analyzing the interactions and output activities of DrBphP and a bacteriophytochrome from *Agrobacterium fabrum* (Agp1).[3] Whereas Agp1 acts as a conventional histidine kinase, we identify DrBphP as a light-sensitive phosphatase (Fig. 1). While Agp1 binds its cognate response regulator only transiently, DrBphP does so more strongly, which is rationalized at the structural level. Our data pinpoint two key residues affecting the balance between kinase and phosphatase activities in two-component signaling. The opposing output activities in two highly similar bacteriophytochromes suggest the use of light-controllable histidine kinases and phosphatases for optogenetics.

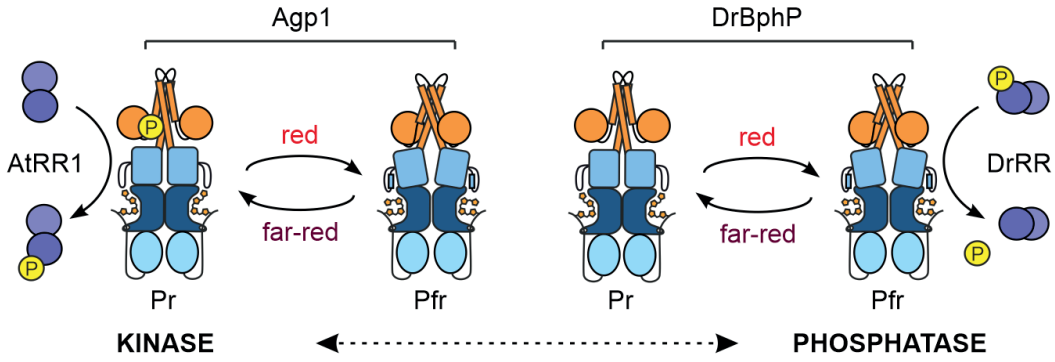


Fig. 1 – Proposed signalling mechanism of two bacteriophytochromes. Agp1 acts as an active histidine kinase by phosphorylating its cognate response regulator AtRR1 in Pr state. DrBphP de-phosphorylates its cognate response regulator DrRR in Pfr state, therefore acting as a phyohatase in two-component signalling.

References

1) Takala H, et al., Signal amplification and transduction in phytochrome photosensors. *Nature*, 509: 245-248, 2014
2) Björling A, et al., Structural photoactivation of a full-length bacterial phytochrome. *Science Advances*, 2(8): e1600920, 2016
3) Multamäki E, et al., Illuminating a Phytochrome Paradigm – a Light-Activated Phosphatase in Two-Component Signaling Uncovered. *bioRxiv*, 2020

Acknowledgements. This work is supported by Academy of Finland grants 285461 and 330678, Three-year grant 2018–2020 from the University of Helsinki, and Bayreuth Humboldt Centre Senior Fellowship 2020.

OC-6.1.8

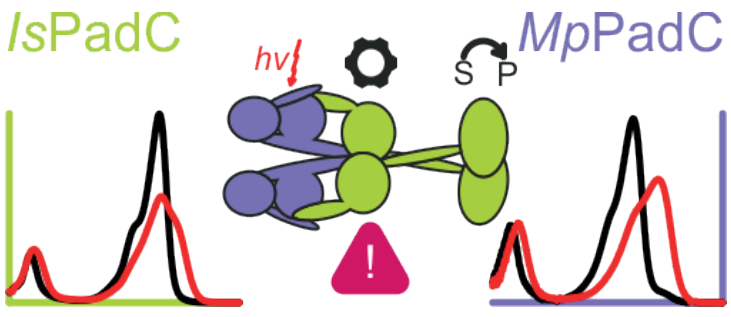
Characterising Phytochrome-Activated Diguanylyl Cyclases

Böhm Cornelia[1,2], Todorovic Nikolina[1], Balasso Marco[1], Hujdur Elvira[1], Gourinchas Geoffrey[1], Sensen Christoph Wilhelm[1], Winkler Andreas[1,2]
1) Graz University of Technology, Department of Biochemistry, Graz, Austria; 2) BioTechMed-Graz, Graz, Austria

Protein dynamics play a major role for the catalytic function of enzymes, the interaction of protein complexes or signal integration in regulatory proteins. In the context of multi-domain proteins involved in light-regulation of enzymatic effectors, the central role of conformational dynamics is well established. Light activation of sensory modules is followed by long-range signal transduction to different effectors; rather than domino-style structural rearrangements, a complex interplay of functional elements is required to maintain functionality.

These sensor-effector systems are frequently linked to second messenger production influencing lifestyle decisions in bacteria or higher organisms. One family of such photoreceptor systems are red-light-regulated phytochromes that control diguanylate cyclases involved in cyclic-dimeric-GMP formation. Based on structural and functional studies of one prototypic family member, the central role of a coiled-coil sensor-effector linker element was established. Interestingly, subfamilies of the phytochrome-activated diguanylate cyclases feature strongly varying biochemical characteristics, especially with respect to the linker length. The evolutionary reasons for these differences and the functional implications as well as the dynamic interplay of the domains involved, however, are presently not fully understood. We have found that the PHY domain dimer interface plays an essential role in signal integration, and that a functional coupling with the coiled-coil linker element is crucial; the phytochrome-spanning helical spine is an essential structural element involved in light-dependent upregulation of enzymatic turnover. Moreover, a structurally asymmetric light state[1], even though potentially advantageous for substrate binding and product formation[2], does not appear to be an absolute requirement for red light induced increase in GTP turnover in bacteriophytochromes. However, isolated structural elements can frequently not be assigned to specific properties of the bacteriophytochrome sensors, which further emphasises the importance of global conformational dynamics in defining photocycle properties as well as structural heterogeneity.

We provide insights into the intricate processes at play during light signal integration and transduction in these photosensory systems. Since deep tissue penetration properties of red light render phytochrome-regulated systems particularly attractive candidates for the rational design of optogenetic tools, our results provide additional guidelines for a more directed design of novel sensor-effector combinations by detailing the complex mechanisms of signal integration as well as inter-domain conformational dynamics in phytochrome systems.



References

1) Gourinchas, G., Heintz, U., Winkler, A, Asymmetric activation mechanism of a homodimeric red light-regulated photoreceptor. eLife, 2018.
2) Gourinchas, G., Vide, U., Winkler, A, Influence of the N-terminal segment and the PHY-tongue element on light-regulation in bacteriophytochromes. JBC, 2019.

Acknowledgements. This work was supported by the Austrian Science Fund (FWF): P32022 (AW).

IL-6.2.1

How the Montreal Protocol Saved Earth's Skin (and our own)

Richard McKenzie[1]
1) NIWA Lauder, PB 50061 Omakau, Central Otago, New Zealand

I'm proud to have played a small part in the success of the Montreal Protocol on Protection of the Ozone Layer, both through my research at Lauder and through participation in the Ozone Science Assessments and Environmental Effects Panel Assessments over the last four decades. Our high quality measurements of spectral UV irradiances have been made continuously at Lauder, New Zealand, since 1989. An analysis of these data, and data from other NDACC-compliant sites throughout the world, which was published in 2019[1], was an important end-point to my formal science career, culminating with the publication last year of my book, "Saving our Skins" [2].
The analysis showed that because of compliance to the Montreal Protocol, UV levels at Lauder and throughout most of the world were no longer increasing and were in fact beginning to show signs of recovery at mid to high southern latitudes (the picture was less clear-cut in the northern hemisphere, probably due to its larger changes in clouds and aerosols). Without the Montreal Protocol, damage to our atmosphere would have already been severe, with ozone levels diminished by around 20 percent, leading to UV increases of a similar magnitude. That damage to Earth's skin would have flowed on to damage to our own skins. New Zealand and Australia have the world's highest rates of skin cancer, and for every 1 percent reduction in ozone, that rate is expected to increase by 2 or 3 percent. The health costs would have been considerable, and the flow on economic costs of treatment. And, as everybody in this audience will be aware, human health is just one of many environmental implications. In the two years that have passed since that paper was published, the recovery in ozone has continued at Lauder, as shown in the figure below.

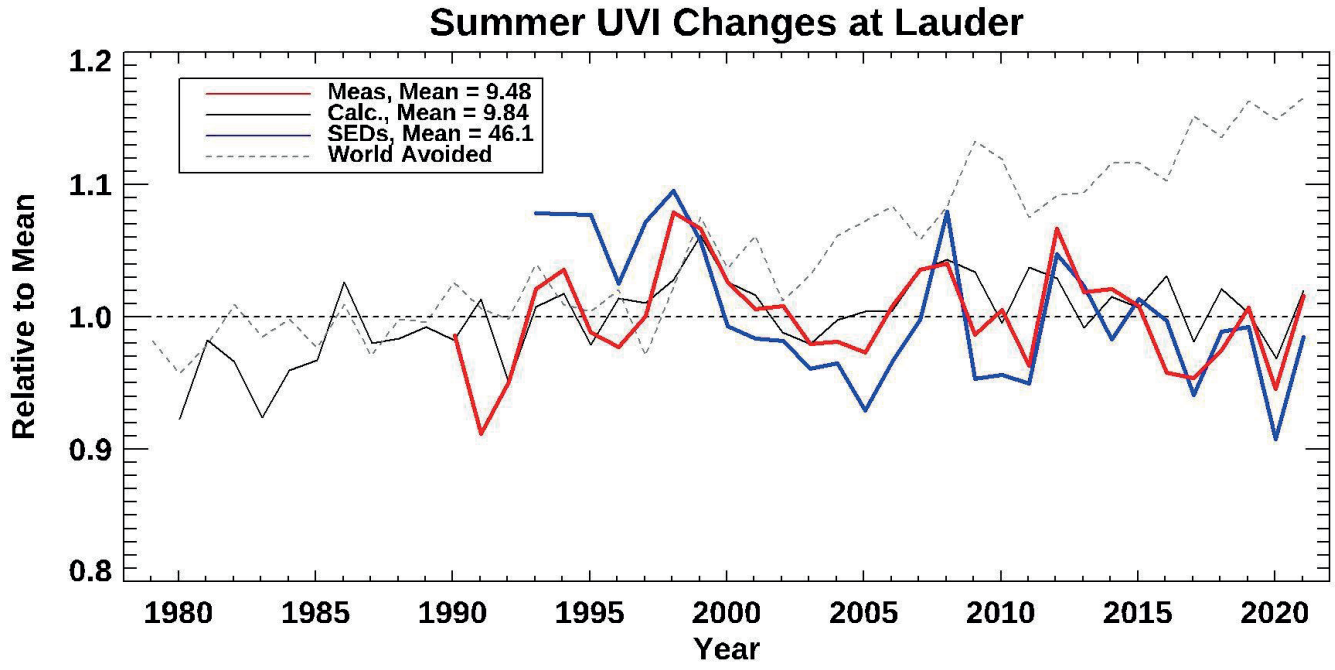
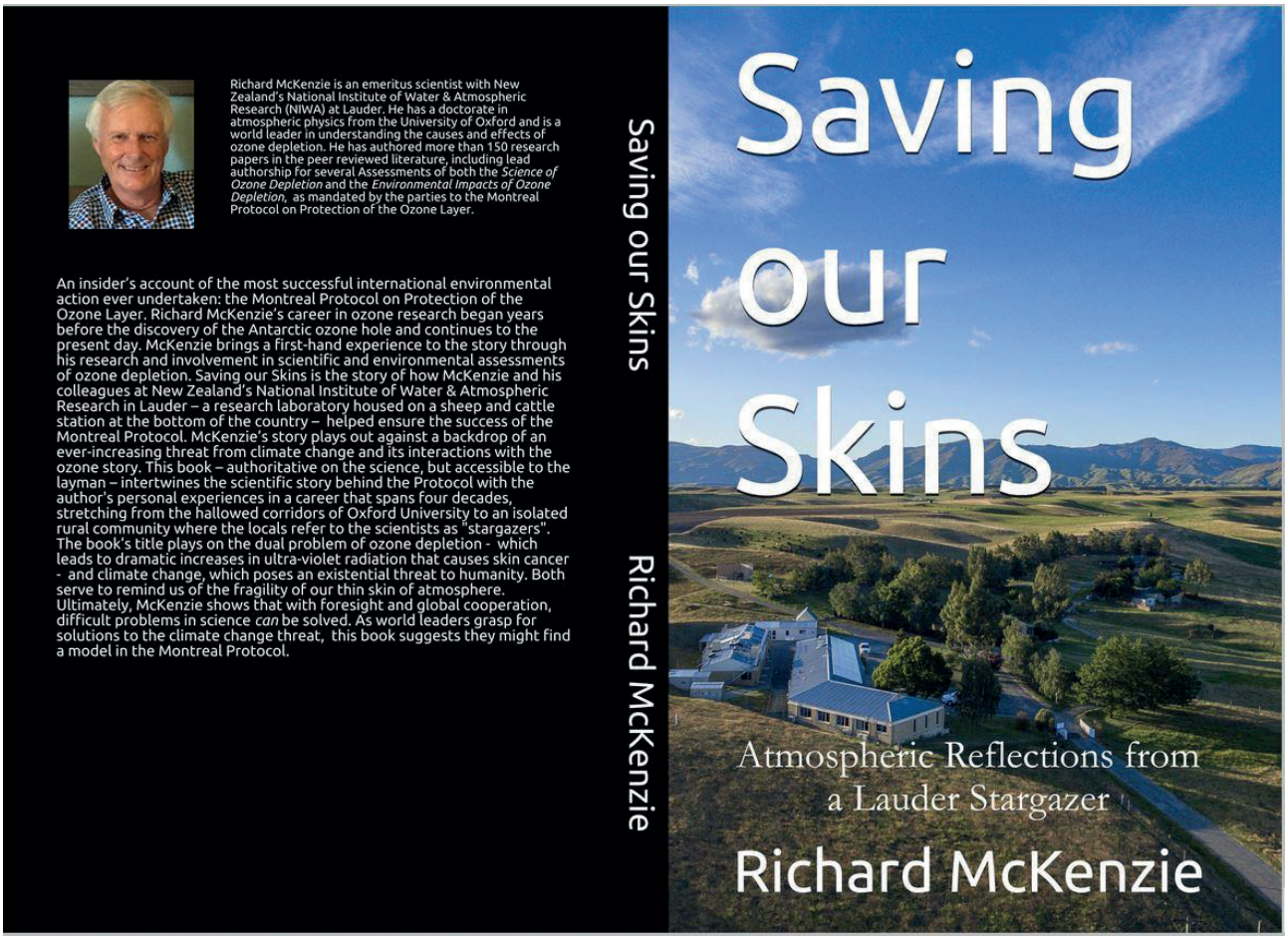


Fig. 1 – Relative changes in summer UV at Lauder from the late 1970s when satellite ozone data became available to the present. Measured changes are compared with model calculations with and without compliance to the Montreal Protocol on protection of the Ozone Layer (preliminary data update from ref [1]).

The situation is more complex at polar latitudes, where severe ozone loss has been seen both in the Arctic and the Antarctic springs in recent years. This is no cause for despair. Two criteria must be met for rapid ozone depletion through the heterogeneous chemical processes that take place there on the surfaces of Polar stratospheric clouds (PSCs). Firstly, chlorine levels must be elevated above about a threshold around 1 ppb, and secondly temperatures must be lower than the threshold needed for the formation of PSCs. Although chlorine levels have already passed their peak, it will be decades before they return to the levels present before the Antarctic ozone hole first formed in

the 1980s. In the meantime, as climate change progresses due to the continued release of Greenhouse Gases, energy is trapped in the lower atmosphere leading to *reduced* temperatures in the stratosphere. With those lower temperatures, the volume of air cold enough for PSCs to form has increased. Consequently, years with anomalously large ozone losses at high latitudes are expected for the next few years. Meanwhile ozone levels should continue to recover outside polar regions, and when chlorine levels return below 1 ppb, the problem in polar regions will also be over. Projected changes for the future UV irradiances will depend on changes in clouds and aerosols as much as changes in ozone.

We're still on track. A summary of Lauder's wider contribution to ozone research was published last year [3], but if you're interested in the UV story, please get a copy of my book.[3]



References

1) McKenzie R, Bernhard G, Liley B *et al.* Success of Montreal Protocol demonstrated by comparing high-quality UV measurements with "World Avoided" calculations from two chemistry-climate models. *Scientific Reports* 2019; **9**: 12332.

2) Richard McKenzie, Saving our Skins, available in three formats from Amazon, kindle, black & white paperback ISBN 979863473987, and colour paperback ISBN 9798654069511, 2020

3) McKenzie R. Lauder and its part in the ozone success story. *SPARC Newsletter* 2021: 16-21.

Acknowledgements. I'm grateful to Germar Bernhard for his assistance producing the final version of Figure 1. I'd also like to take this opportunity express my gratitude to all the colleagues I've worked with over the last 40 years.

IL-6.2.2

Plant and animal response to UV radiation and climate change in the Antarctic

Sharon A. Robinson[1], Georgia Watson[1]
1) University of Wollongong, Securing Antarctica's Environmental Future & Global Challenges Program, Wollongong, Australia

Ozone depletion above Antarctica has resulted in increased surface ultraviolet (UV)-B radiation across the region. Ozone depletion coupled with the heating associated with globally increasing atmospheric greenhouse gases, has also resulted in pronounced changes in Southern Hemisphere climate[1-3,5]. Antarctica is also experiencing extreme events such as the continent wide heatwaves over the summer of 2019/20 summer[4]. We review how increasing UV radiation over the past four decades, along with changes in Southern Hemisphere climate have impacted the biodiversity of Antarctica and the Southern Ocean.

Fig. 1 – A satellite image showing melting on the ice cap of Eagle Island, Antarctica, on February 13. NASA EARTH

References

1) Barnes, P. W., Williamson, C. E., Lucas, R. M., Robinson, S. A., Madronich, S., Paul, N. D., Bornman, J. F., Bais, A. F., Sulzberger, B., Wilson, S. R., Andrady, A. L., McKenzie, R. L., Neale, P. J., Austin, A. T., Bernhard, G. H., Solomon, K. R., Neale, R. E., Young, P. J., Norval, M., Rhodes, L. E., Hylander, S., Rose, K. C., Longstreth, J., Aucamp, P. J., Ballare, C. L., Cory, R. M., Flint, S. D., de Gruijl, F. R., Hader, D. P., Heikkila, A. M., Jansen, M. A. K., Pandey, K. K., Robson, T. M., Sinclair, C. A., Wangberg, S. A., Worrest, R. C., Yazar, S., Young, A. R., & Zepp, R. G. Ozone depletion, ultraviolet radiation, climate change and prospects for a sustainable future. *Nature Sustainability* **2**: 569-579, *2019*
2) Bornman, J. F., Barnes, P. W., Robson, T. M., Robinson, S. A., Jansen, M. A. K., Ballare, C. L., & Flint, S. D. Linkages between stratospheric ozone, UV radiation and climate change and their implications for terrestrial ecosystems. *Photochemical & Photobiological Sciences* **18**: 681-716. *2019*
3) Robinson, S. A., Erickson, D. J., III. Not just about sunburn - the ozone hole's profound effect on climate has significant implications for Southern Hemisphere ecosystems. Global Change Biology **21**: 515-527. *2015*
4) Robinson, S.A., Klekociuk, A.R., King, D.H., Rojas, M.P., Zúñiga, G.E., Bergstrom D.M. The 2019/2020 summer of Antarctic heatwaves. *Global Change Biology* **26** 3178-3180. *2020*
5) Williamson, C. E., Neale, P. J., Hylander, S., Rose, K. C., Figueroa, F. L., Robinson, S. A., Hader, D. P., Wangberg, S. A., & Worrest, R. C. The interactive effects of stratospheric ozone depletion, UV radiation, and climate change on aquatic ecosystems. *Photochemical & Photobiological Sciences* **18**: 717-746. *2019*

Acknowledgements. This work was supported by grant DP200100223 from the Australian Research Council.

IL-6.2.3

Biodiversity, climate change and UV radiation

Janet F. Bornman
Food Futures Institute, Murdoch University, Perth, Australia

Climate change, stratospheric ozone depletion, changes in UV radiation, and ecosystem and biodiversity loss are all interlinking factors affecting life on Earth. Biodiversity of flora and fauna is being adversely affected by changes in climate, which in turn pose many threats to sustainable livelihoods, including food security and human health. However, other critical interacting drivers of biodiversity loss include for example, unsustainable agricultural food systems, invasive species, pollution, land clearing, and urbanisation. Globally, biodiversity is at serious risk of rapid decline from the large-scale conversion of land for agriculture and infrastructure. The increase in agricultural land in turn adds to the emissions of greenhouse gases along the food chain, accounting for *ca* 30% of the total anthropogenic emissions[1] and thereby accelerating climate change. These determinants are already causing shifts in species habitat or species extinctions, changes in species composition, and diminishing suitability of habitat, although colonisation of new habitats can also afford advantages[2]. Furthermore, biodiversity is vulnerable to increased exposure to UV radiation, although there are currently few studies on the UV radiation-climate effects. Increased exposure to UV radiation can be due to climate-related reduced cloud cover in some regions and species migrations to higher elevations as temperatures rise[3]. Loss of biodiversity through increasing deforestation also exposes dead plant material to solar UV radiation. This results in photodegradation of the plant litter, again contributing to emissions of carbon dioxide and other greenhouse gases[4,5]. Proposed actions for reversing or reducing the negative impacts of the intricate, indivisible web of interacting drivers on the fate of species biodiversity range from our lifestyle changes to technological interventions, such as geoengineering for reducing solar radiation to the Earth's surface. However, geoengineering may have serious consequences for biodiversity, natural ecosystem functioning, human health, and agriculture, due to the expected abrupt changes in environmental conditions that would occur[6]. UV radiation can be both reduced and enhanced by aerosol injections into the stratosphere, with the enhancement resulting from multiple scattering[7]. Depletion of stratospheric ozone may also occur from solar geoengineering, depending on the aerosol used[8,9], with the increased UV radiation likely resulting in many undesirable consequences for biodiversity and ecosystems through changes in environmental photobiology. The role of UV radiation and climate change on terrestrial biodiversity will be discussed in the context of the challenges of maintaining a sustainable Earth.

References

1) Benton, T.G. et al. Food system impacts on biodiversity loss. Three levers for food system transformation in support of nature. *Chatham House, London*, pp. 1-71, 2021.
2) Neale, R.E. et al. Environmental effects of stratospheric ozone depletion, UV radiation, and interactions with climate: UNEP Environmental Effects Assessment Panel, Update 2020. *Photochemical & Photobiological Sciences* 20(1), 1-67, 2021.
3) Bornman, J.F. et al. Linkages between stratospheric ozone, UV radiation, and climate change and their implications for terrestrial ecosystems, *Photochemical & Photobiological Sciences* 18(3), 681-716, 2019.
4) Hansen, M.C. et al. High-resolution global maps of 21st-century forest cover change. *Science,* 342(6160), 850-853. 2013.
5) Berenstecher, P. et al. Sunlight doubles above-ground carbon loss in a seasonally dry woodland in Patagonia. *Current Biology* 30(16), 3243-3251, 2020.
6) Trisos, C.H. et al. Potentially dangerous consequences for biodiversity of solar geoengineering implementation and termination. *Nature Ecology & Evolution* 2(3), 475-482, 2018.
7) Madronich, S. et al. Response of surface ultraviolet and visible radiation to stratospheric SO_2 injections. *Atmosphere,* 9(11), 432, 2018.
8) Fang, X. et al. Challenges for the recovery of the ozone layer. *Nature Geoscience*, 12(8), 592-596, 2019.
9) Zarnetske, P.L. et al. Potential ecological impacts of climate intervention by reflecting sunlight to cool Earth. *Proceedings of the National Academy of Sciences*, 118(15), 2021.

OC-6.2.4

Re-discovering the history for new tools for information about skin cancer prevention. The shadow projected by an object related to UV index is an universal photoprotection tool.

José Aguilera Arjona[1], María Victoria de Gálvez Aranda[1], Enrique Herrera Ceballos[1,2]
1) Dermatological Photobiology Laboratory. Bio Medical Research Center. Department of Dermatology. Unversity of Málaga. Málaga. Spain.
2) Dermatological Unit of the Hospital Clínico Universitario Virgen de la Victoria. Málaga. Spain.

Introduction: Every day better information solutions are offered to the general population to educate on the prevention against overexposure to solar ultraviolet radiation and its negative and positive effects. The information on the incidence of the UV index is based on specific photodetectors that are not easily accessible to society. Since the daily evolution of the UV index follows a pattern dependent on the solar arc, and the length of our shadow varies depending on the solar angle that arc forms with respect to our height, is it possible to relate the shadow distance with the UV index? Taking a point at earth surface, the solar arc angle varies with the date of the year and with the latitude. So, the projected shadow of an object will be longer depending on time of the day, day of the year and the latitude and therefore, the UV index, in conditions of clear sky can be predicted with a very high level of sensibility.
The main objective of the present work was to find a universal equation relating the UV index with the distance of the projected shadow of an object independently of the daytime, date or latitude and secondly to develop a system to create a rapid and a easy system that with a simple view we can predict UV index related to de distance of the shadow of an object.
Methods: A shadow length tracking device has been constructed consisting of a rod 100 cm long by 5 cm in diameter located in the centre and perpendicular to a surface that contains a template based on concentric circles drawn with intervals of 20 cm distance between them. The device was placed on the roof of the Medical Research Center of University of Málaga and the daily variation of the length of the shadow of the rod was followed with a web camera connected to the computer and next to it a UV index sensor and it images with an interval of 15 min were followed along a period of 6 months. In order to analyse the mathematical model between shadow distance with UV index including the effect of latitude, UV index was also correlated to solar zenith angle at different latitudes from Tenerife to Oslo.
Results: Regardless of the latitude or time of year, an equation has been obtained that relates the projection distance of a shadow, which is given by the height of the sun throughout the daily cycle and the height of the projection object of the same. The angle formed by the sun with the shadow is the solar height and the daily cycle formed a parabolic arc while the evolution of the UV index followed a cycle with the shape of a normal curve. It has been possible to relate both models of daily evolution, so the date of the year or latitude does not matter. The shadow distance of the object is related to the UV index following a polynomial model. Therefore, with this equation it has been possible to construct a universal solar target to give us the UV index value at a given moment only by observing the shadow distance by using concentric circles around the gnomon with the same colours as the OMS recommendations for different risk levels of UV index (See Fig.1).

Conclusion: We are faced with an informative and educational tool in prevention against the harmful effects of the sun, versatile for any location and time of year, at a minimum cost and a potential for universal dissemination for the entire population.

Acknowledgements. This work was supported by a grant of the II Plan Propio Smart Campus of the University of Málaga.

IL-6.2.5

Interactive effects of ocean acidification and UV radiation on aquatic primary production

Cristina Sobrino[1]; Patrick J. Neale[2]; Julia Puig-Fábregas[1]; Paulo Alcaraz- Rocha[1]
1) University of Vigo, Department of Ecology and Animal Biology, Vigo, Spain; 2) Smithsonian Environmental Research Center, Edgewater (MD), USA

Enhanced CO_2 in the aquatic environment affects phytoplankton physiology and metabolism. As the source of most aquatic primary production, phytoplankton make an important contribution to global climate and biogeochemical cycles. However, many aspects of their physiology and ecology in future global change scenarios are poorly understood. Here we show that the increase in atmospheric CO_2 regulates carbon incorporation mechanisms and promotes a cascading response in the whole cell that triggers a decrease of cellular energy-consuming pathways. This capability of marine phytoplankton to adjust their resources and energy to cellular requirements translates into the optimization of growth rate under high CO_2 conditions. Nonetheless, the physiological regulation of cell metabolism in ocean acidification scenarios affects pigment concentration and cell photoprotection, increasing its sensitivity to stressing conditions such as UVR. The response of primary productivity to UVR under different CO_2 levels has been measured as Biological Weighting Functions, which allow predicting responses under realistic conditions. As a general response the results using phytoplankton cultures under controlled conditions in the lab show that phytoplankton acclimated to high CO_2 under stable steady-state conditions are more susceptible to stress, but there is little study of this response in natural populations. More of these types of studies are needed in the future and we will briefly show how natural environments, such as the Rhode River subestuary (Maryland, USA) or the Ría de Vigo (Northwest Spain), can be used as advanced experimental areas for testing the effects of ocean acidification on phytoplankton.

IL-6.2.6

Coral reefs, UV radiation and climate change

Anastazia T. Banaszak[1], Sergio D. Guendulain García[2]

1) Universidad Nacional Autónoma de México, Unidad Académica de Sistemas Arrecifales, Puerto Morelos, Mexico; 2) Universidad Autónoma Metropolitana, Ciencias Biológicas y de la Salud, Mexico City, Mexico

The key to the success of reef-building corals is attributed to the symbiosis they form with photosynthetic dinoflagellates and the tight recycling of nutrients and compounds that occurs in an otherwise oligotrophic environment. The dependence of these organisms on solar radiation signifies exposure to high UVR levels, due to the low solar zenith angles, natural thinness of the ozone layer and high transparency of the water column characteristic of tropical, coral reef environments. As such, corals have evolved photoprotective mechanisms that include the presence of mycosporine-like amino acids to reduce potential UVR damage. However, over recent decades, coral reefs have been subjected to an ever-increasing number of above average seawater temperature events attributed to climate change. Thermal stress has provoked bleaching, the breakdown of the symbiotic relationships, in corals and other symbiotic coral reef dwelling species, and, if the event is prolonged, can lead to widespread mortality of corals. The photosynthetic dinoflagellates play a crucial role in the photoprotection of corals. DNA photo-damage, as measured by cyclo-butane pyrimidine dimer (CPD) formation is higher in bleached relative to unbleached specimens. We propose that the breakdown of the symbiosis results in multiple scattering of UVR wavelengths by the underlying skeleton, which increases the probability of DNA damage within the transparent tissue. To lower the likelihood that coral reefs undergo repeated bleaching events, stronger steps must be taken to curb CO_2 emissions and reduce the global temperature increase.

Acknowledgements. This work was supported by grant IN2041053 from the Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México to ATB.

OC-6.2.7

Quantifying the Change in Degradation of Plastic Materials Due to Environmental Exposure and Climate Change

Christopher C White[1]

1) Exponent, Bowie, MD, USA

Finding plastic waste is not difficult. In the oceans, atmosphere, Arctic, and even in the food we eat, plastic waste is ever-present. Recent studies calculate that, on average, 8M tons of plastic waste enter the oceans every year.[1] Understanding the dynamic accumulation of plastic waste in the environment is a complex scientific undertaking, perhaps only superseded by the challenge of developing strategies for its removal. This presentation will focus on understanding the reduction of plastic waste using tools and techniques from polymer chemistry and the economics of quantifying the disposal of this waste.

Recent studies indicate that ~250MT to ~300MT of plastic waste is generated every year.[2] This plastic waste is primarily discarded with only small quantities of it either recycled (<10%) or incinerated (<10%). The persistence or lifetime of these plastics significantly contributes to the overall build-up of plastic waste. Knowledge of the degradation rate is critical in managing plastic waste, yet these predictions remain imperfect science. Understanding the limitations of estimating the degradation of plastics is the core of this contribution.

Plastic waste is often considered a singular entity, but plastics and plastic waste are a complex assortment of widely varying chemistries, formulations, assemblies, and size distributions. Each component contributes to the overall plastic waste problem and requires a unique determination of its specific degradation rate. The majority of plastic waste is composed of purely synthetic polymers.

There are two primary mechanisms of degradation of plastic waste: biological or chemical degradation. Biological degradation of purely synthetic polymers is a prolonged process.[3] The rate of chemical degradation is a function of both the composition of the plastic formulation and the environmental exposure, thus increasing the difficulty in quantifying the decomposition rate.

Figure 1 Plastic Waste Litters the Landscape.

Determining the degradation rate of plastics and polymers is primarily driven by estimating the expectations for the design life and associated warranty guarantees in the intended service environment. The corresponding standards and test methods align with two strategies: timed outdoor exposure in a representative environment or a laboratory-derived protocol to assess relative performance.

The timed outdoor exposure of plastic materials is performed in a representative environment that is judged to produce faster degradation than typical in-service environments. Arizona, Florida, Australia, or another similar global location is selected for the extreme environmental conditions such as some combination of hot, humid, or bright Ultra Violet (UV) radiation. Devices employing mirrors increase the UV radiation and

temperature to accelerate the degradation process in these locations. Typically, the samples are exposed on racks tilted to maximize the solar exposure and monitored over at least several years. These exposures benchmark durability for the formulation tested but did not estimate the degradation in other environments.

The second type of durability test simplifies and accelerates specific aspects of the outdoor environment with exposure instruments that produce UV, temperature, and moisture exposures. These tests can effectively compare formulations in the performance in these tests, but correlation to actual outdoor performance has remained elusive. These tests establish baseline performance expectations for the formulations and allow for monitoring of the relative durability.

The impetus to develop test methods that can predict degradation historically has been to reduce the barrier to introducing new innovative materials. The attributes of a new formulation can all be determined immediately, except for durability. Establishing the durability with existing test methods yields a relative performance using laboratory methods in a matter of months that has a limited correlation to actual design life or a threshold performance in an exemplar environment after years of exposure. Either approach significantly increases expense and time related to new product introduction while not substantially reducing the liability risk.

Increasing interest in sustainability may spur innovation in these test methods. Scope 3[4] reporting requirements require assessing the environmental fate of sold plastic products, including the costs associated with greenhouse gas emissions from the end-of-life treatment of sold products. These assessments become an expense or liability on the company's regular financial disclosures. Knowledge of the degradation rate and then taking steps to increase that rate will reduce the expense or liability for the company. This financial pressure will increase the innovation in the test methods used to determine the degradation rate of plastic waste.

References

1. Law, K. L.; Starr, N.; Siegler, T. R.; Jambeck, J. R.; Mallos, N. J.; Leonard, G. H., The United States' contribution of plastic waste to land and ocean. *Science Advances* **2020,** *6* (44).
2. Geyer, R.; Jambeck, J. R.; Law, K. L., Production, use, and fate of all plastics ever made. *Science Advances* **2017,** *3* (7), e1700782.
3. Shah, A. A.; FarihaHasan; AbdulHameed; SafiaAhmed, Biological degradation of plastics: A comprehensive review. *Biotechnology Advances* **2008,** *26* (3), 246-265.
4. https://www.epa.gov/climateleadership/scope-3-inventory-guidance.

OC-6.2.8

PHOTOPHYSIOLOGICAL RESPONSE OF MARINE PHYTOPLANKTON UNDER OCEAN ACIDIFICATION CONDITIONS

Alcaraz-Rocha P.[1], Puig-Fàbregas J.[1], Garrido J.L.[2] & Sobrino C.[1]

1) *Grupo de Oceanografía Biolóxica, Centro de Investigación Mariña (CIM), Universidade de Vigo, Vigo, Spain*;
2) *Grupo de Fotobiología y Toxinología de Fitoplancton, Instituto de Investigaciones Marinas (IIM), Consejo Superior de Investigaciones Científicas (CSIC), Vigo, Spain*

ABSTRACT

Ocean acidification, due to the atmospheric CO_2 increase, produces significant changes on phytoplankton physiology that can affect the photosynthetic metabolism. Among them, a down-regulation of the enzymatic activity and the production of several metabolites, including Chl *a* and RuBisCO, have been observed in high CO_2 cultures under acclimated conditions. However, the extent of how phytoplankton photophysiology regulates under ocean acidification conditions is poorly understood. This study shows the effect of the atmospheric CO_2 increase on pigment concentration of 3 important marine primary producers: *Thalassiosira pseudonana*, *Skeletonema costatum* and *Emiliania huxleyi.* Cultures grown under saturating PAR irradiance were aerated during at least 3 weeks with current concentrations of atmospheric CO_2 (~400 ppmv CO_2) and with CO_2 concentrations expected for future scenarios of climate change (~1000 ppmv CO_2) to assess the effect of CO_2 under stable conditions and acclimated metabolism. Moreover, cultures were also subjected to a CO_2 perturbation (4 h without aeration) to assess responses under non-stable conditions. The results showed that both, light harvesting and photoprotective pigment concentrations (*i.e.* Chl *a*, Chl c_2, β-carotene, Diadinoxanthin, Diatoxanthin, Fucoxanthin and its derivatives, among others) decreased significantly under high CO_2 and acclimated conditions, but the response reversed when the metabolism was perturbed. The results also demonstrate that the CO_2 concentration can modify the photoprotective capability of the phytoplankton through changes in the photoprotective pigment content and in the deepoxidation state of the xanthophylls. They also identify Fucoxanthin and Chl c_2 as potential biomarkers of phytoplankton under ocean acidification conditions.

Key words: Ocean acidification, CO_2 acclimation, phytoplankton, pigments, photoprotection

SP-6.2.9, P-6.2.9

EFFECTS OF OCEAN ACIDIFICATION IN PHOTOPHYSIOLOGY OF MARINE BACTERIA FROM TWO DIFFERENT MARINE HABITATS

Alcaraz-Rocha P.[1], Puig-Fàbregas J.[1], Garrido J.L.[2] & Sobrino C.[1]

1) *Grupo de Oceanografía Biolóxica, Centro de Investigación Mariña (CIM), Universidade de Vigo, Vigo, Spain*;
2) *Grupo de Fotobiología y Toxinología de Fitoplancton, Instituto de Investigaciones Marinas (IIM), Consejo Superior de Investigaciones Científicas (CSIC), Vigo, Spain*

ABSTRACT

Previous studies have shown that high CO_2 levels expected for future scenarios of global change might result in phytoplankton with higher sensitivity to photoinhibitory conditions than those exposed to present CO_2 levels. This response, mainly observed in diatoms, has been attributed to a decrease in the repair rates caused by the lower levels of enzymatic activity and cellular pools observed, but could also be attributed to greater damage related to decreased protection. In this study, we will focus on the effects of elevated CO_2 on pigment content on marine bacteria. The CO_2 effect will be tested on a globally distributed cyanobacteria species, *Synechococcus* spp., isolated from two different marine habitats: the strain WH7803, typical from coastal areas, is observed during transition periods between mixing and stratification, while the strain WH8102 is adapted to oligotrophic environments and appears in open-ocean waters. The cultures were acclimated during at least 3 weeks with current concentrations of atmospheric CO_2 (~400 ppmv CO_2) and with CO_2 concentrations expected for future scenarios of climate change (~1000 ppmv CO_2) to assess the effect of CO_2 under stable conditions and acclimated metabolism. Moreover, cultures were also subjected to a CO_2 perturbation (4 h without aeration) to assess responses under non-stable conditions. HPLC results showed that in *Synechococcus* spp. the Zeaxanthin is the predominant pigment, which relates to cell protection. Chlorophyll *a* and β-carotene concentrations were lower than Zeaxanthin concentration in both strains. However, Zeaxanthin concentration increased significantly under high CO_2 conditions in the coastal strain after a perturbation but did not change in the open-ocean strain under similar conditions. The results from the coastal strain were more similar to those observed in coastal diatoms, which might imply that differences in functional traits between phytoplankton assemblages from open-ocean vs. coastal waters are more significant than their taxonomic features regarding responses to ocean acidification.

Key words: Ocean acidification, CO_2 acclimation, marine bacteria, pigments, photoprotection

IL-6.3.3

New Development in Sunscreens

Henry W. Lim
Photobiology and Photomedicine Unit, Department of Dermatology, Henry Ford Health System, Detroit, Michigan, USA

Topics that are actively discussed and developing in sunscreens will be presented:

1. Percutaneous penetration of active ingredients of sunscreens. This has caused significant confusion and concerns among the general public. It should be noted that the authors of the two studies clearly indicated that the clinical significance is unclear, and that further studies need to be done (1,2).

2. Environmental impact of UV filters. The concentrations reported to be toxic to coral reefs in laboratory settings were 1000 to one million-folds higher that the concentrations measured in sea water, and there are many other factors that contribute to bleaching of coral reefs, including warming and acidification of the ocean water (3).

3. Protection against the photobiologic effects of visible light (VL). VL is known to induce persistent and intense pigmentation in dark-skinned subjects, contributing to the development of melasma and post-inflammatory pigmentation. Except for pigmentary-grade mineral filters (which result in unacceptably whitish discoloration on the skin), the rest of currently available UV filters do not protect against VL Tinted sunscreens containing iron oxides and pigmentary titanium dioxide, and novel filters currently under various stages of development, offer VL protection (4).

References

1) Matta MK, Zusterzeel R, et al. Effect of Sunscreen Application Under Maximal Use Conditions on Plasma Concentration of Sunscreen Active Ingredients: A Randomized Clinical Trial. JAMA. 2019 Jun 4;321(21):2082-2091..PMID: 31058986
2) Matta MK, Florian J, et al. Effect of Sunscreen Application on Plasma Concentration of Sunscreen Active Ingredients: A Randomized Clinical Trial. JAMA. 2020 Jan 21;323(3):256-267. PMID: 31961417
3) Mitchelmore CL, He K, et al. Occurrence and distribution of UV-filters and other anthropogenic contaminants in coastal surface water, sediment, and coral tissue from Hawaii. Sci Total Environ. 2019 Jun 20;670:398-410. PMID: 30904653
4) Narla S, Kohli I, et al. Visible light in photodermatology. Photochem Photobiol Sci. 2020 Jan 22;19(1):99-104. PMID: 31922171

OC-6.3.4

A new sun care product containing Phenylene Bis-Diphenyltriazine (TriAsorB) provides full-spectrum photoprotection against sunlight radiation induced skin damage

D. Bacqueville[1], C. Jacques-Jamin[1], N. Roullet[1], J. Sereno[1], S. Delassan[1], P. Lapalud[1], T. Douki[2], D. Redoulès[3], S. Bessou-Touya[1] and H. Duplan[1]

1) Laboratoire Pierre Fabre, Centre R&D, France, Toulouse; 2) INAC/SyMMES/CIBEST, CEA Grenoble, France; 3) Laboratoires Dermatologiques Avène, Lavaur, France

Photoprotection is a major issue in public health to prevent the harmful effects of sunlight radiations (*actinic keratosis, skin cancers and photoaging*). Recently, we have developed a new generation of non-soluble organic sunfilter called Phenylene Bis-Diphenyltriazine or TriAsorB (*CAS N°55514-22-2*). It absorbs/reflects the solar radiations mainly in the ultraviolet UVB and UVA spectral range but also in the visible/infrared light (VIS/IR), especially in the high energy visible (HEV)/Blue light. TriAsorB protects the skin against the genotoxicity of solar-simulated radiation (SSR), suggesting that it could be used in combination with specific sunfilters in sun care products to provide photoprotection in the entire spectrum of sunlight (*ESP congress Barcelona 2019*).
Thus, the aim of this work was to formulate the TriAsorB in a new photoprotective system in association with 3 sunfilters (*Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine, Diethylamino Hydroxybenzoyl Hexyl Benzoate, Ethylhexyl Triazone*) and to explore the photoprotective efficacy of this innovative SPF50$^+$ sun care products to prevent skin photodamage from UV to VIS/IR light including HEV/Blue light.

Genoprotection was first assessed *in vitro* by using a reconstructed human epidermis (RHE) model exposed to a single acute dose of SSR (*UV+ VIS light, UV total 16.5 J/cm^2*). Liquid chromatography/mass spectrometry analysis revealed that SSR generates cyclobutane pyrimidine dimers (CPD) and that the topical application of the TriAsorB sunscreen at a standard dose of 2 mg/cm^2 before SSR exposure prevented 95.2% DNA lesions. The efficacy of the sunscreen was maintained in extreme UV condition up to 55 J/cm^2 with 89.9% DNA photoprotection. DNA damage was also correlated to tissue toxicity and apoptosis induction 24h after SSR irradiation as assayed by sunburn cell histology and CPD/active caspase-3 confocal microscopy in an *ex vivo* human skin model. TriAsorB sun care product provided an almost complete photoprotection in response to SSR. Interestingly, it was also effective to limit the malondialdehyde (*MDA, lipid peroxidation*) production and superoxide dismutase activity (*SOD, anti-oxidative defence*) in the RHE model (*39.8% and 30.7% protection respectively*). Immunostaining of 8-hydroxy-2'-deoxyguanosine (8OHdG), a biomarker of DNA oxidation, confirmed genome photoprotection in human skin after SSR exposure. Therefore, sunscreen prevented SSR-induced oxidative stress in skin models.

TriAsorB sun care product efficacy was then studied beyond UV in both the VIS and IR spectral range of the sunlight. Since TriAsorB has unique absorption/reflection properties in the HEV/Blue light, a RHE model was exposed to a LED 427 nm at 80 J/cm^2 and 8OHdG staining was performed. HEV light induced tissue oxidation 24h after exposure and TriAsorB sunscreen protected the skin against the 8OHdG oxidative lesions induced by HEV light with 95.3% efficacy. Thus, the sun care product provided photoprotection in the VIS light.
Finally, a clinical study was done to analyze IR-induced oxidative stress and photoprotection. The forearm of 18 healthy volunteers was exposed to an IR lamp (*750 to 3000 nm*) at 900 J/cm^2 and the sunscreen was applied at 2 mg/cm^2 before irradiation. Skin were harvested by using swabs and MDA was quantified 1h30 after IR exposure. MDA was detected in non-irradiated forearm and IR induced an increase of MDA production up to 82 ng/mg total protein. The TriAsorB sunscreen completely protected the forearm of the volunteers with 120% efficacy against IR radiation-induced lipid peroxidation.

Altogether, an innovative photoprotective system combining TriAsorB with 3 sunfilters provided full-spectrum photoprotection from UV to VIS/IR spectral range of the solar radiation. The new sun care product protected the skin against sunlight radiation-induced skin damage including genotoxicity and oxidative stress.

IL-6.3.5

Photoprotection for persons with albinims

Yolanda Gilaberte[1]
1) Miguel Servet University Hospital, Department of Dermatology, IIS Aragón, Zaragoza, Spain.

Introduction: Skin cancer is the main cause of death in persons with albinism (PWA) in Africa. Education would minimize sun damage and the use of sunscreens specifically designed for their necessities would help to reduce the incidence of skin cancer in the future.
Objective: to evaluate the efficacy and acceptance of a photoprotection educative and sunscreen (Umozi Max) package specifically designed for PWA in reducing sunburns and skin cancer.
Patients and Methods: A multicenter, non-controlled, before-and-after, interventional was conducted in PWA > 12 years of age, from February to May 2019, in Malawi. During the baseline and the follow-up visits (at 8 and 15 weeks) subjects received an educational program designed to PWA and use of Umozi Max. This sunscreen is an O/W emulsion that turn into W/O during application, with and SPF 50.6, UVA-PF 19.6 and critical wavelength 380.3 nm. The filters used do not penetrate through the skin and are safe for the environment. At every visit, photoprotection behaviour and knowledge were checked and cutaneous lesions were recorded. Univariate and bivariate analysis were performed.
Results: 210 PWA were analysed, 50% males, with a mean age of 24.5 (SD 11.29) years. The percentage of people using sun protective clothing increased from 80% to 100% and sunscreen from 81.9% to 99.5%. People avoiding the midday sun increased by 38.9% ($p<0.05$). Participants that erroneously applied the sunscreen at night diminished from 40% to 4% ($p<0.001$). Absent erythema on the face increased from 40% to 90% ($p<0.05$). The percentage of patients with actinic keratoses (AK) on all locations significantly decreased during the study. All the participants preferred Umozi Max to previously used sunscreens. The satisfaction with the program was unanimous.
Conclusion: the educational program enhanced the use of all photoprotection measures, improved behaviours and decreased the incidence of solar erythema and contributed to decreasing the incidence of new AKs. Umozi Max was effective in terms of prevention of sunburn and also very well accepted by the participants

.

IL-6.3.6

Visible Light Photoprotection

Indermeet Kohli,[1,2] Iltefat H. Hamzavi,[1] Henry W. Lim[1]

1) Henry Ford Health System, Photomedicine and Photobiology Unit, Department of Dermatology, Detroit, USA;
2) Wayne State University, Department of Physics and Astronomy, Detroit, USA

The impact of ultraviolet (UV) radiation on skin and associated photodamage have been studied extensively over the past several years. Recent studies have demonstrated the ability of other parts of sunlight, particularly visible light (VL), in inducing biologic effects. VL has been shown to induce erythema and pigmentation among individuals with dark skin.[1, 2] The responses were shown to be more exuberant and persistent when combined with long-wavelength UVA1 (VL+UVA1) demonstrating synergistic effects.[3] The combination, VL+UVA1, has also been shown to induce erythema in light-skinned population.[4] The presentation will review the impact of VL on skin health, VL phototesting methodologies and currently available means for VL photoprotection.

References

1. Mahmoud BH, Ruvolo E, Hexsel CL, Liu Y, Owen MR, Kollias N et al. Impact of long-wavelength UVA and visible light on melanocompetent skin. The Journal of investigative dermatology 2010;130:2092-7.
2. Duteil L, Cardot-Leccia N, Queille-Roussel C, Maubert Y, Harmelin Y, Boukari F et al. Differences in visible light-induced pigmentation according to wavelengths: a clinical and histological study in comparison with UVB exposure. Pigment Cell Melanoma Res 2014;27:822-6.
3. Kohli I, Chaowattanapanit S, Mohammad TF, Nicholson CL, Fatima S, Jacobsen G et al. Synergistic effects of long-wavelength ultraviolet A1 and visible light on pigmentation and erythema. The British journal of dermatology 2018;178:1173-80.
4. Kohli I, Zubair R, Lyons AB, Nahhas AF, Braunberger TL, Mokhtari M et al. Impact of Long-Wavelength Ultraviolet A1 and Visible Light on Light-Skinned Individuals. Photochemistry and photobiology 2019;95:1285-7.

Acknowledgements. The fundamental VL work was internally funded by the Photomedicine and Photobiology Unit at Henry Ford Health System, Department of Dermatology. Results from studies supported by Ferndale Laboratories, La Roche-Posay Laboratoire Dermatologique, and Beiersdorf (previously known as Bayer) will also be presented.

IL-6.3.7

UVA1 harmful effects. Benefit of an enlarged photoprotection efficiently covering the whole UV spectrum.

Claire Marionnet [1], Romain de Dormael [1], Xavier Marat [1], Angélina Roudot [2], Emilie Planel [1], Carine Tornier [3], Philippe Bastien [1], Didier Candau [2] and Françoise Bernerd [1].
1) L'Oréal Research and Innovation, Aulnay sous Bois, France; 2) L'Oréal Research and Innovation, Chevilly Larue, France; 3) Episkin, Lyon, France.

UVA1 rays (340–400 nm) account for at least 75% of ultraviolet wavelengths reaching the Earth. They are able to induce epidermal and dermal damage, and alteration of gene and protein expression involved in diverse biological pathways such as immunity, development, oxidative stress response, inflammation or dermal matrix organization[1]. Clinically, they contribute to immunosuppression, carcinogenesis, and photoaging [2,3,4].
Today, state of the art sunscreen formulas can efficiently filter UV wavelengths up to 370/380 nm, but lack sufficient absorption in the 370/380–400 nm wavelengths range. Recently, a new cyclic merocyanine UVA1 absorber, Methoxypropylamino Cyclohexenylidene Ethoxyethylcyanoacetate (MCE), exhibiting a maximal peak of absorption at 385 nm was approved by the SCSS for use in sunscreen products.

The studies presented here aimed at evaluating, in vitro and in vivo, the protection afforded by state of the art sunscreen formulations enriched with MCE.

In vitro, UV absorption spectra of formulations were determined and showed that MCE addition in a state of the art reference formula, enlarged the profile of absorption up to 400 nm, resulting in a full coverage of the whole UV spectrum. Then, formulations efficacy was evaluated through morphological, biochemical and gene expression analysis in 3D skin model exposed to UVA1. Compared to the reference sunscreen, formulations containing MCE enabled significantly higher protection of the epidermis and the dermis, with a better protection against dermal fibroblasts alterations, and a significantly higher reduction of gene and protein expression modulation. In an in vivo randomized controlled trial, pigmentation was used as a clinical surrogate for impact of UVA1 exposure using colorimetric measurements (L*, a*, b*, ΔE and ΔITA) and visual scoring[5], in 19 volunteers with skin types III/IV. UVA1-induced pigmentation was significantly reduced by the formulations containing MCE compared to the state of art formulation. This superiority of protection with formulations containing MCE compared to that of references sunscreen was also proven in a second clinical trial on 25 volunteers (skin types III/IV), following repeated exposures to realistic daily UV radiation (UVB+UVA).

In conclusion, thanks to MCE filtering properties, a full coverage of the whole UV spectrum up to 400 nm was reached, leading to a higher photoprotection against UV-induced biological and clinical impacts. The data also strongly supports benefits regarding long-term sun-induced harmful effects.

References
1)Marionnet C, Pierrard C, Golebiewski C, Bernerd F. Diversity of biological effects induced by longwave UVA rays (UVA1) in reconstructed skin. PLoS One 9(8):e105263, 2014.
2)Damian DL, Matthews YJ, Phan TA, Halliday GM. An action spectrum for ultraviolet radiation-induced immunosuppression in humans. Br J Dermatol 164(3):657-9, 2011.
3)Tewari A, Grage MM, Harrison GI, Sarkany R, Young AR. UVA1 is skin deep: molecular and clinical implications. Photochemical & Photobiological Sciences;12(1):95-103, 2013.
4)Wang F, Smith NR, Tran BA, Kang S, Voorhees JJ, Fisher GJ. Dermal damage promoted by repeated low-level UV-A1 exposure despite tanning response in human skin. JAMA Dermatology;150(4):401-6, 2014.
5)Marionnet C, Nouveau S, Hourblin V, Pillai K, Manco M, Bastien P, et al. UVA1-induced skin darkening is associated with molecular changes even in highly pigmented skin individuals. J Invest Dermatol;137(5):1184-7, 2017.
Acknowledgements. We warmly thank Julie Gizard and Christelle Golebiewski for their expert technical assistance.

OC-6.3.8

Evaluation of the biological effect of a high broad spectrum sunscreen with nicotinamide and panthenol repairing photodamaged skin.

Gemma Tell[1], Neus Calbet[1], Judit Mateu[1], Javiera Pérez[2], Bea Alejo[2], Pablo Iglesias[2], Josep Malvehy[1,2], Ann'Laure Demessant-Flavigny[3], Caroline Le Floc'h[3], Leonor Prieto[4], Susana Puig[1,2]

1. Centro de Investigación Biomédica en Red de Enfermedades Raras, CIBERER, Instituto de Salud Carlos III, Barcelona, Spain, 2. Dermatology Department, Melanoma Unit, Hospital Clínic de Barcelona, IDIBAPS, Universitat de Barcelona, Barcelona, Spain, 3. International Scientific Direction, Laboratoire Dermatologique La Roche-Posay, France, 4. Scientific Direction, Laboratoire Dermatologique La Roche-Posay, L'ORÉAL Cosmética Activa, Madrid, Spain

Nicotinamide is a precursor of nicotinamide adenine dinucleotide (NAD), co-enzyme essential in the production of adenosine triphosphate (ATP), main source of cellular energy. Previous studies in mice showed that the oral or topical application of nicotinamide prevents immunosuppression and reduce the number of UV-induced tumors. In humans, topical application of nicotinamide prevents UV induced immunosuppression, but not sunburn. Nicotinamide increases the production of ATP, increases DNA repair and prevents/avoids apoptosis.

The main objective of the present study was to determine the biological effect of a high broad spectrum UVB-UVA sunscreen containing Nicotinamide and Panthenol, in photodamaged skin analyzing, immunohistochemistry (p53, PCNA, p21 and pyrimidine dimers) and RNA profiles using high throughput technology.

Fifteen healthy patients (> 40 years) were included. Two areas in photodamaged skin in the dorsum of the forearm (area 1 and 2) and one area in the inner part of the forearm less photodamaged (area 3) were identified, imaged with lineal confocal OCT and biopsies performed in area 1 and 3. During four weeks, a high broad UVB-UVA sunscreen with nicotinamide and panthenol was applied daily. After that, a new biopsy was performed in area 2 after imaging with lineal confocal OCT. Each biopsy was split into two halves. One was fixed in formalin for ulterior be embedded in paraffin and immunostaining with p21, p53, PCNA and CPD, and the other one was included in RNAlater and frozen to extract RNA subsequently and carried out the sequencing.

From the 14 patients included that ended the study only 13 were assessable for the immunohistochemistry comparison (in one patient the post treatment biopsy was not evaluable because of the absence of epidermis in the sample).

100% of cases showed an increased expression of p21, PCNA, p53 and CPD in photodamaged skin (L01) when compared with less damaged skin (L03). After 4 weeks of product application, we identified a slightly decrease of p21 expression in treated photodamaged skin compared to non-treated photodamaged skin (L02 vs L01), but this tendency did not reach statistical significance. Direct comparison pre-post treatment showed improvement of p21 expression in 6 cases (1, 6, 7, 8, 13, 14). Differential gene expression analysis (DGEA) from the RNA-Seq data showed an overexpression of Collagen Type I Alpha 1 Chain (COL1A1) gene (adjusted p-value < 0.001) in treated photodamaged skin in comparison with non-treated photodamaged skin (L02 vs L01 biopsies). Furthermore, the gene set enrichment analyses (GSEA), which took into account the fold-change value for each gene in order to rank them, identified 40 biological pathways significantly dysregulated after treatment (adjusted p-value < 0.001). Among the 20 upregulated pathways, there are some related with the SUMOylation process, extracellular matrix organization, cell adhesion molecule activity and keratinization. Otherwise, among the 20 downregulated pathways, several are involved in the reactive oxygen species generation and mitochondrial function.

Four weeks of daily application of high broad spectrum UVB-UVA sunscreen with nicotinamide and panthenol in photodamaged skin induces overexpression of COL1A1 and influences the regulation of 40 biological pathways involved in the skin homeostasis.

P-6.3.9

Plants growing in Colombia as sources of natural actives sunscreen ingredients

Diego Armando Villamizar Mantilla[1], Silvia Juliana Flores González[1], Luis Alberto Núñez[1], Elena E. Stashenko[1], and Jorge Luis Fuentes Lorenzo[1].

1) Universidad Industrial de Santander, Santander, Bucaramanga, Colombia.

Introduction: Plants can be sources of photoprotective/antigenotoxic compounds that prevent cellular mutations involved in skin cancer and aging by regulating UV-induced mutability. **Purpose:** The study was aimed at investigating the sunscreen properties of plants growing in Colombia. **Materials and Methods:** Ultraviolet radiation (UVR)-absorption capability of different plant extracts was examined. *In vitro* photoprotection efficacies were evaluated using *in vitro* indices such as sun protection factor ($SPF_{in\ vitro}$) and critical wavelength (λc). Pearson correlation analysis was used to examine the relationship between $SPF_{in\ vitro}$ and complementary UVB-antigenotoxicity estimates (%GI) based on SOS Chromotest database. The cytotoxicity in human fibroblasts was studied using the trypan blue exclusion assay. Major compounds of promising plant extracts were determined by gas chromatography coupled to mass spectrometry (GC/MS). **Results:** We showed that plant extracts have sunscreen properties against UVB, whereas broad-spectrum radiation protection efficacy was poor. $SPF_{in\ vitro}$ and %GI were correlated ($R = 0.71$, $p < 0.0001$) for the plant extracts under study. Three extracts obtained from *Achyrocline satureioides*, *Chromolaena pellia,* and *Lippia origanoides* species resulted to possess high protection efficacy and relatively low cytotoxicity in human fibroblasts. These plant extracts had major compounds such as α-pinene, *trans*-β-caryophyllene, γ-muurolene, γ-cadinene and caryophyllene oxide in *A. Satureioides* extract, squalene and α-amyrin in *C. pellia* extract, and *p*-cymene, carvacrol, *trans*-β-caryophyllene and pinocembrin in *L. origanoides* extract. **Conclusions:** Plants growing in Colombia contain compounds which ca be useful as a sunscreen. $SPF_{in\ vitro}$ and %GI estimates were correlated, but %GI estimates were more sensitive to detecting activity at lower plan extract concentrations. Our results supported the need to use DNA damage detection assays as a complement to photoprotection efficacy measurement.

Acknowledgements. The authors thank funding from the Ministry of Science, Technology and Innovation, the Ministry of Education, the Ministry of Industry, Commerce and Tourism, and ICETEX, Programme *Ecosistema Científico-Colombia Científica*, from the *Francisco José de Caldas* Fund, Grant RC-FP44842-212-2018. The Ministry of Environment and Sustainable Development of Colombia supported the Universidad Industrial de Santander through access permits to genetic resources and derivatives for bioprospecting (Contract Nº. 270).

P-6.3.10

Scoring Levels To Corroborate The Booster Effect Of A Natural Extract Of Polypodium Leucotomos (Fernblock®) In Topical Sunscreens In Improving The UV Barrier Activity And Immune Protection Capability

Azahara Rodríguez-Luna[1], María Villalba[2], María Victoria de Gálvez[3], Enrique Herrera-Ceballos[3], Salvador González[4], José Aguilera[3].

1) Cantabria Labs, Innovation and Development Dept, Madrid, Spain.
2) Cantabria Labs, Medical Affairs Dept, Madrid, Spain.
3) University of Malaga, Photobiological Dermatology Laboratory, Medical Research Centre, Málaga, Spain.
4) Alcalá de Henares University, Dept of Medicine and Medical Specialties, Madrid, Spain.

Different *in vitro* and *in vivo* techniques have been used to determine the photoimmunoprotection potential of sunscreens. Nevertheless, the diversity of experimental designs, data management and different forms of assessment make the identification of a suitable reference method difficult. The aim of the study is to complete the existing scientific evidence of Fernblock® about its photoimmunoprotection potential factor evaluating its physicochemical activity. Thus, to validate the physiochemical level, we evaluated by means of UV solar simulated transmission measurements the immunoprotection factor (IPF) of different sunscreens based on Fernblock®, an aqueous extract from *Polypodium leucotomos*. Four different sunscreen formulations containing Fernblock® were selected and their transmittance of solar simulated UV was calculated by spectroradiometric measurement. *In vitro* SPF, UVAPF and IPF based on the action spectrum for systemic contact hypersensitivity (CHS-IPF) were measured.
Fernblock® added to different combinations of organic and mineral sunscreens has a booster effect with a mean increase of SPF, CHS and HIF factor over 10 arbitrary units and more than 10 % of average boost of all factors. In addition to these physiochemical results, Fernblock has demonstrated extensive biological immunoprotection capabilities in vitro and in vivo (1-4). Thus, we elucidate a scoring system to evaluate the photoimmunoprotection potential consisting in three study levels of evidence for any sunscreen to validate the immunoprotection factor (IPF) a) the physicochemical level protection, b) the biological in vitro level protection and c) the validation by clinical evidence. In summary, new generations of topical sunscreens need to promote additional effects, not only with filters, but with compounds that promote both regeneration and/or immunoprotection activities, among others. Taken together with the evidence displayed, new scoring systems should be implemented beyond SPF. Different studies should be considered to evaluate the IPF including not only barrier/photon blocking function but also immunoprotective biological activities.

References:

1. Capote R, Alonso-Lebrero JL, et al. Polypodium leucotomos extract inhibits trans-urocanic acid photoisomerization and photodecomposition. J Photochem Photobiol B. 2006.
2. Middelkamp-Hup MA, Pathak MA et al. Orally administered Polypodium leucotomos extract decreases psoralen-UVA-induced phototoxicity, pigmentation, and damage of human skin. J Am Acad Dermatol. 2004.
3. Middelkamp-Hup MA, Pathak MA, Parrado C, Goukassian D, Rius-Díaz F, Mihm MC, Fitzpatrick TB, González S. Oral Polypodium leucotomos extract decreases ultraviolet-induced damage of human skin. J Am Acad Dermatol. 2004.
4. Schalka S, Donato LC. Evaluation of effectiveness of a sunscreen containing Polypodium leucotomos extract in reducing the sun damage to the skin. Surg Cosmet Dermatol. 2019

Acknowledgements. This work was supported by Cantabria Labs

P-6.3.11

Photoprotective and Antigenotoxic Properties of *Serratia marcescens* Indigenous Strains from Eastern Cordillera of Colombia

José Duban Daniel Cediel Becerra[1], Jhon Alexander Suescún Sepúlveda[1], Jorge Luis Fuentes Lorenzo[1]

1) Universidad Industrial de Santander, Santander, Bucaramanga, Colombia

Introduction: *Serratia marcescens* is a bacterial species that produces antibacterial pigment (prodigiosin) that shows a wide adaptive response to environmental stresses. This pigment has a strong UV– absorption capability, suggesting that it can be useful as a sunscreen. **Purpose:** The study was aimed at investigating photoprotective and antigenotoxic properties against UVB of forty extracts from *S. marcescens* strains collected across an altitude gradient in the Eastern Cordillera of Colombia. **Materials and Methods:** Prodigiosin production was spectrophotometrically assayed in extracts of bacterial strains grown in different culture media and temperatures. A calibration curve of pure prodigiosin was used to estimate the compound concentration in each extract. *In vitro* photoprotection efficacy was evaluated using *in vitro* indices such as sun protection factor ($SPF_{in\ vitro}$) and critical wavelength (λc). The percentage of UVB–antigenotoxicity estimates (%GI) in the SOS Chromotest was also evaluated. Correlation analysis was used to examine the relationship of the $SPF_{in\ vitro}$ with prodigiosin concentration, %GI, and environmental markers (altitude, temperature, rainfall, and solar irradiance). **Results:** Salt minimum culture medium supplemented with glycerol and a growing temperature of 28 °C showed higher prodigiosin production. Among the strains under study, six showed medium ($15.0 \leq SPF_{in\ vitro} \leq 29.9$) and two high ($30.0 \leq SPF_{in\ vitro} \leq 59.9$) photoprotection efficacy against UVB radiation, while only one strain resulted in broad-spectrum (UVA–UVB) photoprotection ($\lambda c > 370$ nm). $SPF_{in\ vitro}$ was well correlated with prodigiosin concentration ($R = 0.79$, $p \leq 0.05$) and with %GI values ($R = 0.80$, $p \leq 0.05$) in the bacterial strains being studied, but no to environmental markers ($R = 0.0 – 0.16$, $p \geq 0.05$, n.s). **Conclusion:** We demonstrated that *S. marcescens* strains producer of prodigiosin could be useful as source of sunscreen. Extracts from *S. marcescens* strains with prodigiosin concentration higher than ~17 μg/mL showed good quality as sunscreen ($SPF_{in\ vitro} \geq 15.0$) that reduced UVB– induced genotoxicity in the SOS Chromotest. UVB photoprotection efficacy depended on prodigiosin concentration and on %GI values of the *S. marcescens* extracts, but not on the environmental markers.

Acknowledgements. The authors thank funding from the Ministry of Science, Technology and Innovation, the Ministry of Education, the Ministry of Industry, Commerce and Tourism, and ICETEX, Programme *Ecosistema Científico-Colombia Científica*, from the *Francisco José de Caldas* Fund, Grant RC-FP44842-212-2018. The Ministry of Environment and Sustainable Development of Colombia supported the Universidad Industrial de Santander through access permits to genetic resources and derivatives for bioprospecting (Contract No. 270).

P-6.3.12

The hydroalcoholic extracts of *Ipomoea horsfalliae*, *Posoqueria latifolia* and *Rosa x centifolia* contain compounds with filter and antigenotoxic effects against UV radiation

Jorge Luis Fuentes Lorenzo[1], Carlos Adolfo Pedraza Barrera[1], Silvia Juliana Flórez González[1], Lady Johanna Sierra Prada[1], and Elena E. Stashenko[1]

1) Universidad Industrial de Santander, Santander, Bucaramanga, Colombia.

Introduction: Plants are sources of photoprotective/antigenotoxic compounds that prevent cellular mutations involved in skin cancer and aging by regulating UV-induced mutability. **Purpose:** The study was aimed at investigating the sunscreen properties of plants growing in Colombia. **Materials and Methods:** Ultraviolet radiation (UVR)-absorption capability of nineteen flower extracts obtained from eleven plant species was examined. *In vitro* photoprotection efficacies were evaluated using *in vitro* indices such as sun protection factor ($SPF_{in\ vitro}$) and critical wavelength (λc). UVB- antigenotoxicity estimates (%GI) using the SOS Chromotest were also obtained for promising extracts. Cytotoxicity of these extracts was studied in human fibroblasts using the trypan blue exclusion assay. Major compounds of promising plant extracts were determined by UHPLC-ESI^+-*Orbitrap*-MS. **Results:** Among the studied plant extracts, thirteen showed low ($6.0 \leq SPF_{in\ vitro} \leq 14.9$), two medium ($15.0 \leq SPF_{in\ vitro} \leq 29.9$) and four high ($30.0 \leq SPF_{in\ vitro} \leq 59.9$) photoprotection efficacy against UVB radiation, while only two resulted in broad-spectrum (UVA–UVB) photoprotection (λc > 370 nm). These extracts were obtained from *Ipomoea horsfalliae, Posoqueria latifolia,* and *Rosa x centifolia* plant species, they also showed UVB- antigenotoxicity and possessed relatively low cytotoxicity ($LD_{50} \geq 125$ µg/mL) in human fibroblasts. These plant extracts had major compounds as follows: chlorogenic acid, dicaffeoylquinic acid, and scopoletin (*I. horsfalliae*), caffeic acid and chlorogenic acid (*P. latifolia*), and cyanidin-3,5-glucoside, quercetin-3-rhamnoside, kaempferol-3-glucoside, kaempferol-rhamnoside, and kaempferol (*R. centifolia*). Several of these compounds also presented high photoprotection efficacy and antigenotoxicity against UVB radiation, indicating that they were responsible for the corresponding extract activity. **Conclusions:** *I. horsfalliae, P. latifolia,* and *Rosa x centifolia* plant species contain photoprotective compounds with low cytotoxicity in human fibroblasts and could be used as natural sunscreen ingredients.

Acknowledgments. The authors thank funding from the Ministry of Science, Technology and Innovation, the Ministry of Education, the Ministry of Industry, Commerce and Tourism, and ICETEX, Program *Ecosistema Científico-Colombia Científica*, from the *Francisco José de Caldas* Fund, Grant RC-FP44842-212-2018. The Ministry of Environment and Sustainable Development of Colombia supported the Universidad Industrial de Santander through access permits to genetic resources and derivatives for bioprospecting (Contract Nº. 270).

IL-6.4.1

Adjuvant PDT after surgical resection: murine modeling of applications to breast cancer, mesothelioma and beyond

Theresa M. Busch
University of Pennsylvania, Perelman School of Medicine, Department of Radiation Oncology, Philadelphia, USA

In the combined modality setting, the intraoperative delivery of photodynamic therapy (PDT) provides opportunity to treat microscopic and/or unresectable disease at surgical margins. As an adjuvant that can intraoperatively be delivered directly to the surgical field, PDT provides local cytotoxic effect in the absence of mutagenicity and the potential for an anti-tumor immune response[1]. Murine models of PDT in the context of surgery provide for the study of new regimens and an understanding of their limitations. Two models are discussed. One evaluates light dosimetry and delivery as variables in the treatment of thoracic malignancies with pleural spread, such as mesothelioma and pleural dissemination of non-small cell lung carcinoma. In illumination of the murine thoracic cavity, fluence rate is spatially distributed across the cavity, not unlike that found in patients who receive intraoperative PDT of their chest cavity following macroscopic resection of pleural disease. As shown in Figure 1, fluence rate distributions can be measured in patients, identifying a range of treatment-averaged moderate fluence rates to which tissues are exposed[2]. In murine models, intrathoracic fluence rate has been studied as a variable, demonstrating there to be fluence rate dependencies in PDT response, as known to occur with external beam PDT.

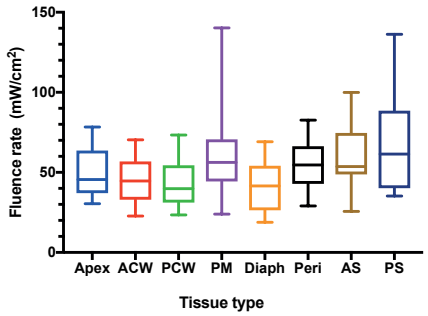


Fig. 1 – Illumination is delivered to the thoracic cavity during intraoperative PDT for malignant pleural mesothelioma, resulting in treatment-averaged light exposures of ~40-60 mW/cm² for most tissues of the chest. Fluence rate is measured by isotropic detectors in the chest cavity at tissues sites of the apex, anterior chest wall (ACW), posterior chest wall (PCW), posterior mediastinum (PM), diaphragm (Diaph), pericardium (Peri), posterior sulcus (PS), and anterior sulcus (AS). Figure reprinted from Photochem Photobiol. 2020 Mar;96(2):417-425

In a second murine model of intraoperative PDT, photosensitizer delivery is studied as a variable, in this case comparing systemic dosing versus topical application of 5-aminolevulinic acid (ALA) in PDT of surgical margins after the complete resection of murine tumor (TUBO) from the mammary fat pad. PDT in conjunction with either systemically delivered ALA (oral gavage) or local administration (topical application to the resection site) after complete resection of TUBO tumor provided for similar long-term tumor control. However, ALA-PDT with oral drug delivery produced more vascular damage, a greater proportion of tissue-resident neutrophils and stronger inflammation than that resulting from photosensitization with topical ALA application. Notably, these effects were achieved after only a 10 min topical ALA exposure.

Each of the above-described murine models provide opportunity to explore questions relevant to the delivery of PDT as an adjuvant to surgery. Observations generated in studies of such models are hypothesis generating–providing guidance on novel approaches or new applications that can be taken back to the clinic.

References

1) Mahmoudi K, Garvey KL, Bouras A, Cramer G, Stepp H, Jesu Raj JG, Bozec D, Busch TM, Hadjipanayis CG. 5-aminolevulinic acid photodynamic therapy for the treatment of high-grade gliomas. J Neurooncol. 2019 Feb;141(3):595-607. PMID: 30659522.
2) Dupre PJ, Ong YH, Friedberg J, Singhal S, Carter S, Simone CB 2nd, Finlay JC, Zhu TC, Cengel KA, Busch TM. Light Fluence Rate and Tissue Oxygenation (StO2) Distributions Within the Thoracic Cavity of Patients Receiving Intraoperative Photodynamic Therapy for Malignant Pleural Mesothelioma. Photochem Photobiol. 2020 Mar;96(2):417-425. PMID: 32048732.

Acknowledgements. This work was supported by NIH grants R01 CA236362 and P01 CA087971.

IL-6.4.2

Molecular and Functional Photoacoustic Imaging for personalizing Photodynamic Therapy

Srivalleesha Mallidi
Department of Biomedical Engineering, Tufts University, Medford, MA USA 02155

Photodynamic therapy (PDT) is a photochemistry based cytotoxic technique that imparts cellular damage via excitation of a photosensitizer with drug-specific wavelength of light. The dose at the treatment site for type II PDT is determined by three factors: photosensitizer (PS) concentration, oxygenation status and delivered light irradiance. Most of the FDA approved photosensitizers in their triplet-excited state generate cytotoxic species by reacting with the ground state oxygen that is available in the surrounding environment. Given the inter- and intra-subject variability in the uptake of the photosensitizer and the distribution of oxygen in the tumor, understanding the interplay between these dose parameters could aid in determining photodynamic therapy efficacy. Previously several studies have discussed the interplay between the dose parameters using shown point measurements and 2D imaging systems. In this presentation, we will discuss the utility of molecular and functional photoacoustic imaging for personalizing photodynamic therapy. Using various subcutaneous and orthotopic mouse models we will demonstrate the utility of a non-invasive non-ionizing photoacoustic imaging modality to determine efficacy and predict treatment response in Benzoporphyrin derivative (BPD), Aminolevulinic acid (ALA) based PDT or other PDT based combination therapies. We further compare the predictive capability of photoacoustic imaging with the more predominantly used fluorescence imaging and immunohistochemistry techniques. Finally the clinical translation of photoacoustic image-guided PDT will be discussed.

IL-6.4.3

The Role of Mechanical Stress in Targeted PDT Combinations for Ovarian Cancer

Imran Rizvi[1,2], Huang-Chiao Huang[3], Utkan Demirci[4], Tayyaba Hasan[5], Marcela G. del Carmen[6], Jonathan P. Celli[7], Shubhankar Nath[4], Mustafa K. Ruhi[1], Christina Conrad[3].
1) University of North Carolina Chapel Hill and North Carolina State University, UNC-NC State Joint Department of Biomedical Engineering, Chapel Hill, NC, USA
2) University of North Carolina Chapel Hill School of Medicine, Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA
3) University of Maryland, Fischell Department of Bioengineering, College Park, MD, USA
4) Stanford University School of Medicine, Bio-Acoustic MEMS in Medicine (BAMM) Laboratory, Canary Center at Stanford for Cancer Early Detection, Department of Radiology, Palo Alto, CA, USA
5) Massachusetts General Hospital, Harvard Medical School, Wellman Center for Photomedicine, Department of Dermatology, Boston,MA, USA
6) Massachusetts General Hospital, Harvard Medical School, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Boston, MA, USA
7) University of Massachusetts at Boston, Department of Physics, College of Science and Mathematics, Boston, MA, USA

Mechanical stress (such as flow-induced shear stress) plays an important role in tumor growth and survival, with increasing implications for therapy design and drug delivery. The role of physical stress in the tumor microenvironment remains understudied in the context of photodynamic therapy (PDT) and PDT-based combinations. A focus of this presentation will be to discuss the role of mechanical stress in the context of targeted PDT with an emphasis on ovarian cancer, the leading cause of death from gynecologic malignancies. Advanced-stage epithelial ovarian cancer is most frequently associated with the production of ascites, the abnormal accumulation of fluid. Despite decades of evidence that the accumulation of peritoneal fluid portends the poorest outcomes for cancer patients, the role of fluid flow in promoting metastases and therapy resistance, remains poorly understood. The effects of flow-induced shear stress on chemoresistance and modulation of molecular survival pathways that could be exploited for targeted PDT, and combinations, will be discussed.

References

1) Nath S, Pigula M, *et al*. Flow-induced Shear Stress Confers Resistance to Carboplatin in an Adherent Three-Dimensional Model for Ovarian Cancer: A Role for EGFR-Targeted Photoimmunotherapy Informed by Physical Stress. J Clin Med, 2020.
2) Rizvi I, Gurkan UA, *et al*. Flow induces epithelial-mesenchymal transition, cellular heterogeneity and biomarker modulation in 3D ovarian cancer nodules. Proc Natl Acad Sci USA, 2013.
3) Sorrin AJ, Kemal Ruhi M, *et al*. Photodynamic Therapy and the Biophysics of the Tumor Microenvironment. Photochem Photobiol, 2020.
4) Conrad C, Gray KM, *et al*. Mechanical Characterization of 3D Ovarian Cancer Nodules Using Brillouin Confocal Microscopy. Cell Mol Bioeng, 2019.

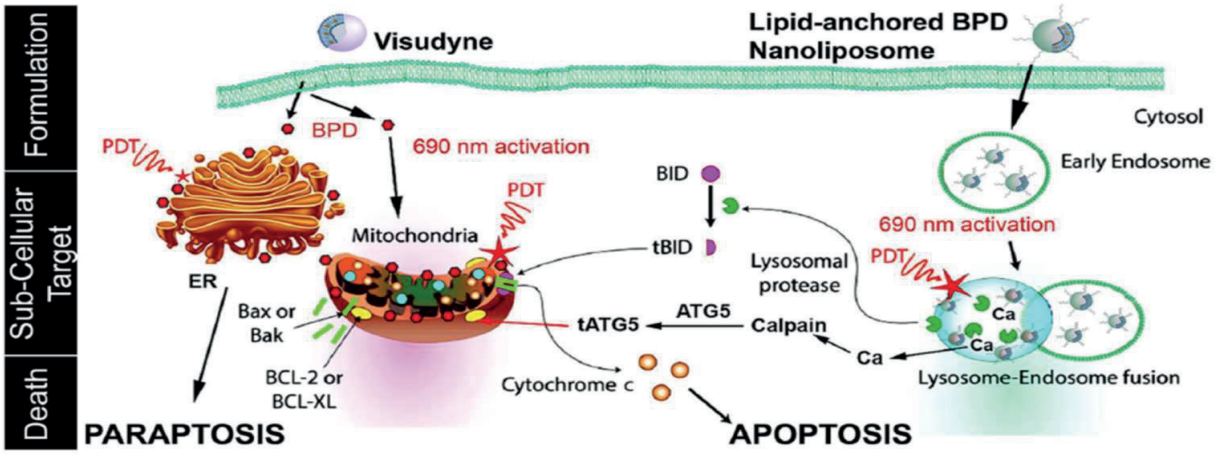
Acknowledgements. This work is supported by the following funding sources: Dr. Imran Rizvi - NIH R00 CA175292, NC Translational and Clinical Sciences Institute (NCTraCS), supported by the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), through Grant Award Number UL1TR002489, and UNC-NC State Joint BME Department Start-up Fund. Dr. Utkan Demirci - Stanford University Canary Center Seed Grant, NCI-NIH grant to the Center for Cancer Nanotechnology Excellence for Translational Diagnostics (CCNE-TD) at Stanford University (grant number: NIH U54 CA199075). Dr. Tayyaba Hasan – NIH R01 CA158415, R01 CA160998, R01 CA119388 Dr. Huang-Chiao Huang - NIH R00 CA194269, UMD Start-up Fund, UMD-UMB Research and Innovation Seed Grant, the Caring Together, NY, Ovarian Cancer Research grant, and the UMD-NCI Partnership for Integrative Cancer Research Grant. Dr. Jonathan Celli – NIH U54CA156734 UMass Boston-Dana Farber/Harvard Cancer Center Partnership

OC-6.4.4

Optimizing Photodynamic Therapy: A Tour of the Death Pathways

David Kessel[1], Imran Rizvi[2] and Girgis Obaid[3]
[1]*Wayne State University, Department of Pharmacology, Detroit MI, USA;* [2] *UNC-NC State Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University;* [3]*Department of Bioengineering, University of Texas at Dallas.*

Photodynamic therapy (PDT) for the treatment of cancer involves the light-catalyzed formation of reactive molecular species (RMS) at sites of neoplasia. The precise death mode is a function of sub-cellular localization of the photosensitizer since RMS do not migrate far from the site of formation. Apoptosis and necrosis are often cited as death pathways but investigators tend not to examine cellular morphology and may thereby miss another common death pathway: paraptosis. This occurs when the ER is among the targets for photodamage and is independent of apoptosis. Paraptosis is characterized by the formation of a series of vacuoles derived from the ER that gradually fill the entire cytoplasm, which eventually results in only bare nuclei appearing in microscopic fields.[1] When PDT targeting involves mitochondria or lysosomes, this leads to apoptosis. When both are targeted, release of lysosomal calcium ions that can activate calpain, ultimately leading to a considerable promotion of the apoptotic consequences of mitochondrial photodamage. The ideal targeting profile therefore appears to be a combination of mitochondria, lysosomes and ER. This is not easy to achieve with a single photosensitizer but can be done via nanotechnology. Because of solubility considerations, benzoporphyrin derivative (BPD, Visudyne) is formulated in a liposomal environment and targets mitochondria and the ER. Adding a second nano-formulation provides the ability to target lysosomes.[2] Fig. 1 indicates how combining these two formulations can lead to the optimizing of PDT targeting so as to promote maximum efficacy.



This may be the optimal targeting procedure for PDT efficacy

Fig. 1 – Ability of different BPD formulations to alter the sub-cellular targeting pattern

Details providing a comparison of apoptosis and paraptosis are shown in Fig. 2A. Apoptosis is characterized by cellular blebbing and fragmentation along with a corresponding fragmentation of nuclei. This can readily be revealed by both phase-contrast imaging and observing fluorescence of the nuclear probe Ho33342. Fig. 2B provides a comparison of the photodynamic efficacy, individually and together, using the two BPD formulations described in Fig. 1, reveals the synergistic effect of the combination of mitochondrial + lysosomal photodamage. Studies with the OVCAR-5 cell line in 3D culture show that PDT doses of the free vs. lipid-anchored formulations individually led to an undetectable level of photokilling but that the combination resulted in a very substantial decrease in viability.

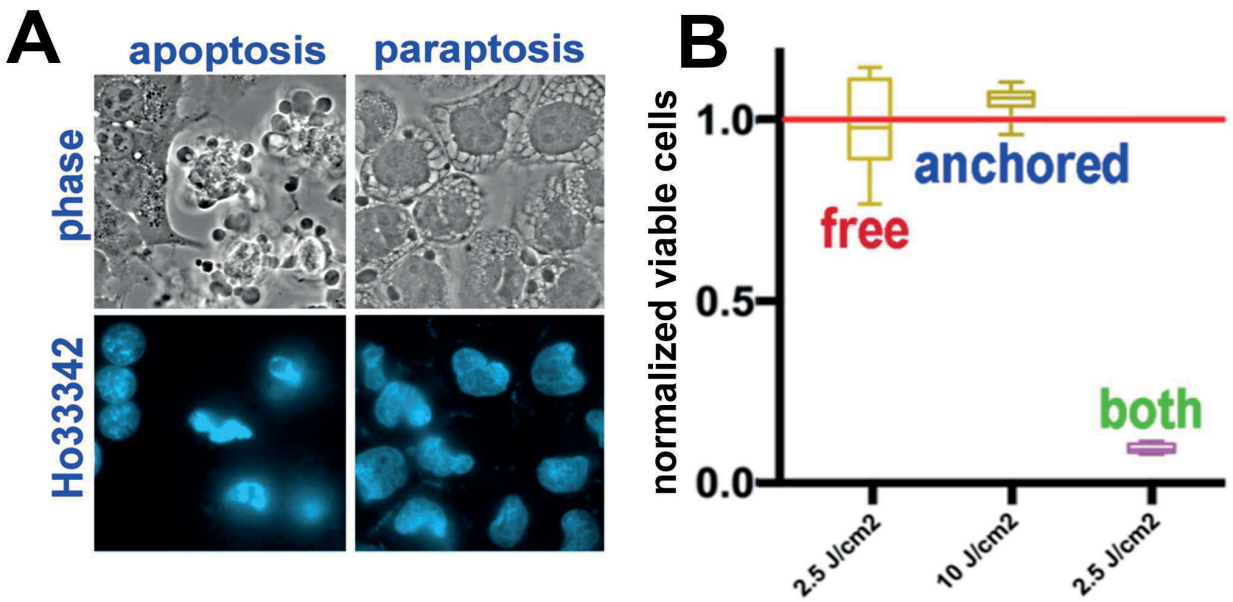


Fig. 2. A) Characteristics of apoptosis vs. paraptosis as indicted by phase-contrast and Ho33342 fluorescent labelling patterns. B) PDT efficacy of free vs. anchored BPD preparations as defined in Fig. 1. Effects on viability of OVCAR-5 cells in 3D culture are indicated.

References

1) Kessel D, Death pathways associated with photodynamic therapy. Photochem. Photobiol. In Press 2021
2) Rizvi R, Shubhanker N, Obaid G, Ruhl MK, Moore K, Bano S, Kessel D and Hasan, T. A combination of Visudyne and a lipid-anchored liposomal formulation of benzoporphyrin derivative enhances photodynamic therapy efficacy in a 3D model for ovarian cancer. Photochem. Photobiol. 95, 419-429, 2019.

Acknowledgements. This work was partially supported by grants R01CA23378, K99CA215301 and R00CA175292 from the NIH.

IL-6.4.5

Anti-angiogenics overcome tumor endothelial cell anergy and improve immunotherapy outcomes

Zowi R. Huinen[1], Elisabeth J.M. Huijbers[1], Judy R. van Beijnum[1], Patrycja Nowak-Sliwinska[2,3], Arjan W. Griffioen[1]*
1) Angiogenesis Laboratory, Department of Medical Oncology, Amsterdam University Medical Center, Vrije Universiteit Amsterdam, Cancer Center Amsterdam, Amsterdam, The Netherlands; 2) Molecular Pharmacology Group, School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland; 3) Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, Geneva, Switzerland.

Immunotherapy, in particular immune checkpoint inhibition (ICI), is becoming a pillar in cancer treatment enabling unprecedented clinical benefits in a variety of malignancies, including durable responses in aggressive, metastasized, or previously untreatable malignancies. Currently, however, only a subset of treated patients responds. A compelling body of evidence shows that anti-angiogenic therapy has the capacity to ameliorate anti-tumor immunity, owing to the inhibition of various immunosuppressive features of angiogenesis. Hence, anti-angiogenic- and immunotherapy combinations are currently tested in >80 clinical trials and 5 FDA approvals were recently achieved. We describe the angiogenesis-induced endothelial immune cell barrier, which limits immune cell infiltration and hampers anti-tumor immune responses and immunotherapy efficacy. We thereby highlight the mechanism of endothelial cell anergy, which we describe as the vascular counterpart of immune checkpoints. We review the anti-tumor immunity promoting effects of anti-angiogenic therapy, and give an update on the current clinical successes achieved by the combination therapy.
The combination of photodynamic therapy (PDT) with anti-angiogenic agents has also been shown to be beneficial. This has been described to be the result of the PDT-induced onset of angiogenesis. It remains to be demonstrated that the PDT induced anti-tumor immune response also benefits from overcoming endothelial cell anergy by combination with anti-angiogenic agents. We hypothesize that endothelial anergy is a vascular immune checkpoint that can be overcome by angiogenesis inhibition.

OC-6.4.6

Protondynamic Therapy: A radical hybridization of PDT and proton radiotherapy

Theodossis A. Theodossiou[1], Mantas Grigalavicius[1], Maria Mastrangelopoulou[1], Delmon Arous[2], Jonas Asperud[1], Tine T. Henriksen Raabe[1], Mathilde Ménard[1], Efim Brondz[2], Sunniva Siem[2], Andreas Görgen[2], Nina F. J. Edin[2], Eirik Malinen[1,2], Kristian Berg[1].

[1]*Department of Radiation Biology, Institute for Cancer Research, Oslo University Hospital, Oslo 0379, Norway.*

[2]*Department of Physics, University of Oslo, Blindern, 0315, Oslo, Norway*

Deep seated tumours as for example the central nervous system (CNS) cancer glioblastoma multiforme (GBM) are practically incurable by the current standards-of-care. Even though photosensitive drugs (PSs) are used to guide the resection of GBM by their characteristic fluorescence, photomedical treatments like photodynamic therapy (PDT) or photochemical internalisation (PCI) are severely restricted by the shallow penetration of light into tissue (< 5 mm), even at red/far-red wavelengths. Proton radiotherapy, can deposit a therapeutic amount of energy, in the so called "Bragg peak", in varying depths within the tissue (even in excess of 20 cm), depending on the initial energy of the accelerated protons. Here we present proof of principle of a novel technology based on the radical hybridisation of the principles behind PDT and proton therapy: Protondynamic Therapy. The underlying hypothesis of this hybridization was that accelerated protons can excite the electrons of PSs by attractive Coulomb forces, and that the PSs can generate singlet oxygen and eliminate difficult to cure cancers like GBM by a dual action. Indeed, upon irradiation of PS solutions and gels with accelerated protons at a research cyclotron, we verified the PS proton-activation, by fluorescence, i.e. radiative de-excitation of the photosensitive molecules.

Furthermore, we verified the population of PS triplet states in dry gels where there was no oxygen quenching. Finally we registered the production of singlet oxygen, either directly through its characteristic luminescence at 1270 nm or indirectly through the formation of the singlet-oxygen-associated PpIX photoproduct: PhotoProtoporphyrin IX. We subsequently tested our hypothesis in GBM cell cultures: M059K, T98G and U87, by comparing the survival in cell groups irradiated by various proton doses in the abscence/presence of a PS. The results revealed an enhanced cell death in the presence of a PS in M059K and T98G cells. In parallel to the experimental work we are conducting theoretical calculations on the mechanism of electronic excitations by accelerated protons.

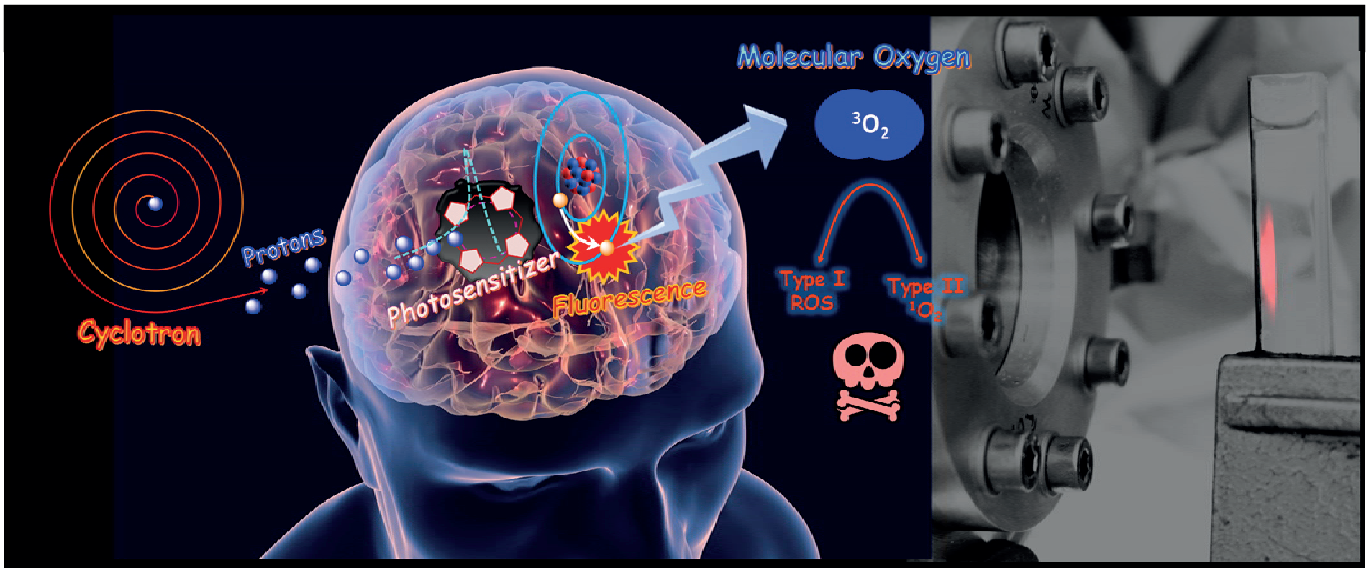


Fig. 1. Principles of Protondynamic Therapy

OC-6.4.7

Spatiotemporal controlled drug release with light to overcome chemotherapy resistance in pancreatic cancer.

T. Le Clainche, N. Carigga Gutierrez, J-L. Coll and M. Broekgaarden

Institute for Advanced Biosciences, Université Grenoble-Alpes, INSERM U 1209, CNRS UMR 5309, Grenoble, France

Despite its low incidence, pancreatic ductal adenocarcinoma (PDAC) is the fourth-leading cause of cancer-related mortality [1]. Cancer desmoplasia, which forms a protective barrier of stromal cells and extracellular matrix around cancer tissues, is widely known for its implication in the high resistance of PDAC against chemotherapeutic treatments [2,3]. To improve the efficacy and safety of such treatments, we aim to design innovative liposomal vectors to control the release of the drug in a spatiotemporal-controlled manner. In the field of photodynamic therapy, photosensitizers such as benzoporphyrin derivative (BPD) are used to produce reactive oxygen species (ROS) upon light excitation [4]. By including photosensitizers in liposomes composed of oxidation-susceptible unsaturated phospholipids, we provide compelling proof for the spatiotemporal-controlled release of drugs with light (Figure 1) [5,6]. We have systematically optimized the liposome composition and discovered that liposomes surface properties were of major impact on drug release efficiency. In parallel, we are developing new 3D culture models of PDAC on alginate hydrogels that are compatible with state-of-the art, high-content imaging assays [7,8]. These models are used to investigate the uptake and toxicity of the liposomes, and to determine the penetration depth of the released therapeutics. These exciting findings open new possibilities for the controlled release of cancer therapeutics and pave the way to in vivo experiments.

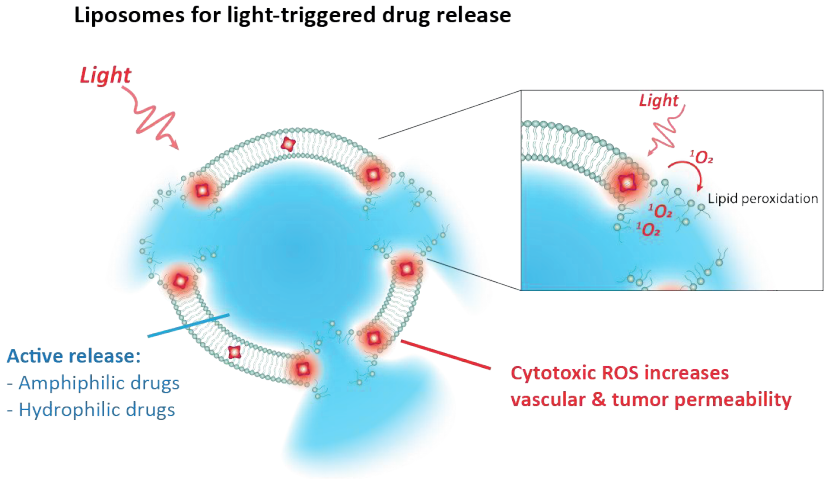


Fig. 1-light-sensitive liposomes for spatiotemporal-controlled drug release.

References

1) J. Ferlay, M. Colombet, I. Soerjomataram et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur. J. Cancer Oxf. Engl. 1990, 2018.
2) D. Fukumura, R. K. Jain, Tumor microenvironment abnormalities: Causes, consequences, and strategies to normalize. J. Cell. Biochem, 2007.
3) P. P. Adiseshaiah, R. M. Crist, S. S. Hook et al. Nanomedicine strategies to overcome the pathophysiological barriers of pancreatic cancer. Nat. Rev. Clin. Oncol, 2016.
4) P. Agostinis, K. Berg, K. A. Cengel et al. Photodynamic therapy of cancer: An update. CA. Cancer J. Clin, 2011.
5) M. Broekgaarden, R. Weijer, T. M. van Gulik et al. Tumor cell survival pathways activated by photodynamic therapy: a molecular basis for pharmacological inhibition strategies. Cancer Metastasis Rev, 2015.
6) M. Broekgaarden, A. I. P. M. de Kroon, T. M. van Gulik et al. Development and in vitro proof-of-concept of interstitially targeted zinc-phthalocyanine liposomes for photodynamic therapy. Curr. Med. Chem, 2014.
7) A.-L. Bulin, M. Broekgaarden, T. Hasan, Comprehensive high-throughput image analysis for therapeutic efficacy of architecturally complex heterotypic organoids. Sci. Rep. 2017.
8) M. Broekgaarden, A.-L. Bulin, J. Fredericket al. Tracking Photodynamic- and Chemotherapy-Induced Redox-State Perturbations in 3D Culture Models of Pancreatic Cancer: A Tool for Identifying Therapy-Induced Metabolic Changes. J. Clin. Med, 2019.

OC-6.4.8

Probing the mechanism of $TPPS_{2a}$ as a photosensitizing agent for PDT in 2D monolayer and a 3D compressed collagen model of ovarian cancer

Balukova, A., MacRobert, A.J., Yaghini, E.

University College London, Division of Surgery and Interventional Science, London, United Kingdom

Introduction: In this study, the efficacy of the porphyrin-based photosensitiser $TPPS_{2a}$ (tetraphenylporphine disulfonate) was evaluated in a 2D monolayer and 3D compressed collagen cancer model of a human ovarian cancer cell line (HEY). This porphyrin which bears adjacently substituted sulfonate groups is known to be an effective photosensitiser for both PDT and Photochemical Internalisation (PCI).

Aims: This study aimed to investigate the uptake and subcellular localisation of $TPPS_{2a}$ using fluorescence spectroscopy and confocal microscopy with organelle markers, and to evaluate the photooxidative mechanism by which $TPPS_{2a}$ induces cell death by analysing 2D and 3D samples using ROS and lipid peroxidation probes.

Materials and Methods: The HEY cells were used for both the 2D and 3D culture models. The 3D cancer constructs were manufactured using the RAFT 3D culture systems (Lonza, UK) protocol in 96-well plates, to create a compressed type 1 collagen hydrogel. Confocal microscopy (Leica SP8) using 405 nm excitation was used to assess the uptake and localisation of $TPPS_{2a}$ (Frontier Scientific) in both in 2D and 3D cancer model. In 2D cultures, the uptake of 0.5 µM - 2 µM $TPPS_{2a}$ was assessed in fluorodishes™. Furthermore, 5 µM 2',7'-Dichlorofluorescin diacetate (Sigma-Aldrich) and 5 µM BODIPY™ 581/591 C11 (ThermoFisher) were tested as ROS and lipid peroxidation sensors. Cells were incubated for 24 h with drugs prior to imaging, with the exception of the addition of the ROS/lipid peroxidation probes which were applied 2 h prior to light exposure. For 3D constructs, 1 µM or 2 µM $TPPS_{2a}$ was applied on day 7 post-seeding and the constructs were left to incubate for a further 22 h.

Results: $TPPS_{2a}$ fluorescence was observed inside the cells and localized within the cellular membranes at the 4 h time point and inside endolysosomes at the 24 h time point as confirmed with the Lysotracker green. The mechanism of action of $TPPS_{2a}$ was investigated by illuminating porphyrin treated 2D and 3D samples for a longer time and imaging the PS fluorescence with increasing exposure time using a time-lapse mode of imaging. In both 2D and 3D samples, real time imaging confirmed the fast-acting photodynamical properties of $TPPS_{2a}$ as exposure to blue light increased. To detect the formation of ROS and lipid peroxidation, 2D and 3D samples were co-treated with C11 and $TPPS_{2a}$. Upon oxidation, a shift was detected in the fluorescence of C11, where the emission peak shifts from 590 nm (orange/red) to 510 nm (green). Using a sequential mode of imaging with two lasers and two detectors, a green shift was clearly observed in the 2D and 3D cultures and was later confirmed by quantitative assessment of the fluorescence intensity of the images. Overall, this shift confirmed the presence of ROS which are a result of the photoactivation of the $TPPS_{2a}$. Dichlorofluorescein diacetate (DCFH-DA) is a cell permeable probe that becomes fluorescent upon oxidation. In this study, 2D cultures were co-treated with DCFH-DA and $TPPS_{2a}$. Sequential imaging using two lasers and two detectors resulted in the observation of a c. 50% increase in mean fluorescence intensity for regions of interests, which confirmed that the probe had been oxidised by the PDT-generated ROS.

Conclusion: Time-lapse confocal imaging permitted the visual observation of the PS fluorescence dynamics with time as more ROS are generated. The PDT-induced generation of ROS was confirmed with C11 and DCFH-DA using confocal microscopy in both 2D and 3D cancer models. The response observed using the lipid peroxidation probe C11 is consistent with photosensitisation of endolysosomal membranes as required for the PCI mechanism.

Reference: Hadi L , Yaghini E, Stamati k , Loizidou M, MacRobert A.J. Therapeutic enhancement of a cytotoxic agent using photochemical internalisation in 3D compressed collagen constructs of ovarian cancer. Acta Biomaterialia, 2018.

OC-6.4.9

Peptide-Targeted Systems for Photodynamic Therapy

Dong Wang[1,2], Sandy MacRobert[3], Charareh Pourzand[1,2], Ian Eggleston[1,2]

1) University of Bath, Department of Pharmacy and Pharmacology, Bath BA2 7AY, UK.

2) Centre for Therapeutic Innovation, University of Bath, Bath BA2 7AY, UK

3) University College London, Division of Surgical and Interventional Sciences, London NW3 2PE, UK

Photodynamic therapy (PDT) is a minimally invasive approach for the treatment of cancer and various other human disorders, based on the selective activation of photosensitizers (PSs) with light. At present, one of the most promising strategies for PDT and also fluorescence photodiagnosis (PDD) is to use 5-aminolevulinic acid (ALA) as a prodrug to increase intracellular levels of the endogenous PS, protoporphyrin IX (PpIX). Although ALA-PDT has been shown to be a very promising clinical approach, the physicochemical properties and chemical reactivity of ALA present some challenges. These may be addressed by incorporation of ALA units into a variety of prodrug systems,[1] and we have previously shown that peptide-based prodrugs are an attractive way to improve the delivery of ALA, leading to enhanced PpIX accumulation and PDT effects.[2] In this study, we present a novel and easy to assemble prodrug system to enhance the delivery of ALA to specific cell types using targeting with tumour-homing peptides. More specifically, we will describe the synthesis of prodrug systems consisting of a molecular core to which multiple ALA units may be attached along with a targeting peptide of choice. This combines the concept of ALA dendrimers and ALA-peptide prodrugs.[3] As proof of concept of this particular approach, we have prepared systems containing a bombesin-derived peptide that allows selective targeting of the GRP receptor (GRPR) which is overexpressed in a variety of tumours. Targeted ALA delivery and PpIX production with these prodrugs in GRPR-expressing PC3 cells will be described.

References

1: Tewari K M, Eggleston I M. Chemical approaches for the enhancement of 5-aminolevulinic acid-based photodynamic therapy and photodiagnosis. Photochemical & Photobiological Sciences, 2018.

2: Giuntini F, Bourré L, MacRobert A J, et al. Improved peptide prodrugs of 5-ALA for PDT: rationalization of cellular accumulation and protoporphyrin IX production by direct determination of cellular prodrug uptake and prodrug metabolization. Journal of Medicinal Chemistry, 2009.

3: Tewari K M, Dondi R, Yaghini E, et al. Peptide-targeted dendrimeric prodrugs of 5-aminolevulinic acid: A novel approach towards enhanced accumulation of protoporphyrin IX for photodynamic therapy. Bioorganic Chemistry, 2021.

SP-6.4.10, P-6.4.10

How to use 5-ALA induced PpIX without light

Sara Sansaloni Pastor, Emmanuel Varesio, Norbert Lange
School of Pharmaceutical Sciences, Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, Switzerland

The selective generation of PpIX following exogenous administration of 5-ALA is one of the most selective processes known in cancer or pre-cancer therapy and diagnosis. This feature of 5-ALA is now widely used in photodynamic therapy (PDT) and photodiagnosis (PD), notably for the detection of bladder cancer and glioblastoma or the treatment of basal cell carcinoma and actinic keratosis. These methodologies are restricted to body areas that are accessible to light which tissue penetration is only sallow. Therefore, we were searching for possibilities to use this phenomenon differently, i.e. without the use of visible light. To this end we have analyzed to proteonome of different cell lines of cancerous origin following exposure to 5-ALA. In this presentation, our new methodology will be presented. Furthermore, potential future applications and indications will be discussed.

Acknowledgements. This work was supported by a grant of the Swiss National Science Foundation.

P-6.4.11

Conjugates of octreotide and exendin-4 with zinc-phthalocyanine TT1 for photodynamic therapy of neuroendocrine tumors

Sanne A.M. van Lith[1], Alba Fonseca[3], Desiree Bos[1], Annemarie Kip[1], Fritz Andreae[2], Thorsten Radolf[2], Tomas Torres[3], Martin Gotthardt[1]
1) Radboud university medical center, Medical Imaging, Nijmegen, The Netherlands; 2) piCHEM, Raaba-Grambach, Austria; 3) Autonoma University of Madrid, Department of Organic Chemistry, Madrid, Spain

Neuroendocrine tumors (NETs) comprise a very heterogeneous group of malignancies, as they can arise from neuroendocrine cells in virtually every internal organ. The management of localized disease involves surgical resection with curative intent. To ensure complete removal of the tumor, currently often unnecessary radical resections are performed, and this is associated with substantial morbidity. Furthermore, residual disease often leads to recurrence of these lesions. Therefore, techniques that lead to more specific and less invasive ablation of tumor cells are warranted, and targeted photodynamic therapy is an interesting treatment option. Two G protein-coupled receptors that are regularly overexpressed on neuroendocrine tumors are the somatostatin receptor (SSTR), and the glucagon-like peptide 1 receptor (GLP1R). Here, we developed and characterized peptide-photosensitizer conjugates for SSTR- and GLP1R-targeted photodynamic therapy.

Zinc-phthalocyanine based photosensitizer TT1-maleimide was conjugated to a sulfhydryl group on the primary amine of Lys_{40} in GLP1R-targeting peptide exendin-4, and of $dPhe_1$ in SSTR-targeting peptide octreotide. properties such as absorbance and fluorescence of exendin-4-TT1 and octreotide-TT1 were characterized in dimethyl sulfoxide (DMSO), phosphate buffered saline (PBS) and PBS with human serum or bovine serum albumin. To determine half maximal inhibitory concentrations (IC_{50}), chinese hamster lung cells stably transfected with GLP-1R (CHL-GLP1R) or SSTR-overexpressing AR42J cells were co-incubated with either exendin-4-TT1 and ^{111}In-labeled exendin-4-DTPA as a competitor or octreotide-TT1 and ^{111}In-labeled octreotide-DTPA as a competitor, respectively. Then, light-induced toxicity by both compounds was determined. Cells were incubated for 4 hours with various concentrations of either exendin-4-TT1 or octreotide-TT1, and after washing away the unbound fraction, cells were illuminated with 690 nm light using a light emitting diode (LED 100 J/cm^2 at 200 mW/cm^2). Non-illuminated cells and cells incubated with excess exendin-4-DTPA or octreotide-DTPA served as controls. Finally, BALB/c nude mice carrying subcutaneous xenografts of CHL-GLP1R tumors were injected with 20 µg exendin-4-TT1, and an *ex vivo* fluorescent biodistribution to determine uptake in tumors and other tissues (liver, spleen, kidneys, blood, pancreas) was performed at 4 and 16 hours post-injection.

Spectral properties of TT1 were retained after conjugation to both peptides, with excitation and emission maxima of 682 nm and 696 nm in DMSO. Dissolving the conjugates in PBS led to a blue-shift and a decreased intensity of the Q-band, and this could partly be rescued by adding human serum or bovine serum albumin. Both exendin-4-TT1 and octreotide-TT1 bound to target-expressing cells with high affinity (IC_{50}: 85.2 nM and 28.9 nM, respectively). Cytotoxicity of CHL-GLP1R and AR42J cells was induced upon incubation with exendin-4-TT1 or octreotide-TT1 and illumination with 690 nm light, respectively. The cytotoxic effect was blocked by adding an excess of exendin-4-DTPA or octreotide-DTPA, indicating receptor specificity. Both conjugates did not induce cytotoxicity without illumination. Upon injection in BALB/c nude mice carrying subcutaneous CHL-GLP1R xenografts, relative tumor uptake of 1.46±0.52 %ID/g and 3.24±1.05 was found for exendin-4-TT1 at 4 and 18 hours post injection, respectively. Non-target specific uptake of exendin-4-TT1 was mainly found in the liver (37.98±3.69 %ID/g at 18 hours post injection) and spleen (56.04±13.36 %ID/g at 18 hours post injection).

In conclusion, the novel TT1-peptide conjugates show potency for SSTR,- and GLP1R-targeted PDT *in vitro*. Furthermore, exendin-4-TT1 homes to GLP-1R expressing tumors *in vivo*. In ongoing experiments, we will determine *in vivo* tumor targeting of the octreotide-TT1 conjugate, and light-induced ablation of target-expressing tumor cells by both conjugates.

Acknowledgements. This work was supported by an investigator grant from the neuroendocrine tumor foundation.

P-6.4.12,

Improving the efficiency of ALA-PDT of skin cells using indoor and outdoor UVA-based light fractionation approach

Dana Beiki[1], Ian Eggleston[1,2], Charareh Pourzand[1,2]
1) Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom; 2) Centre for Therapeutic Innovation, University of Bath, Bath, United Kingdom

Topical 5-aminolevulinic acid-based photodynamic therapy (ALA-PDT) is widely used for the treatment of dermatologic conditions including actinic keratoses (AK). ALA administration to skin cells results in intracellular accumulation of the endogenous photosensitiser protoporphyin IX (PpIX). Upon exposure to a light source (usually blue or red light), PpIX can catalyse the generation of reactive oxygen species, resulting in cell death. The major drawback of topical ALA-PDT is the pain experienced by patients. This may be counteracted by improving the efficiency of the ALA-PDT by optimisation of the light source, irradiation dose and duration of treatment. For this purpose, ultraviolet A (UVA, 320-400 nm) was used instead of conventional visible light sources as it is absorbed efficiently by PpIX and is significantly more cytotoxic due to its ability to promote an increase in the intracellular level of harmful labile iron which can be a major contributor to cell death by acting as a catalyst for oxidative damage to cell constituents.[1] Our optimization strategy relied on exploiting the rapid release of labile iron following application of short pulses of low UVA doses to ALA-treated cells instead of a continuous dose from visible light source, to further sensitise cells to subsequent UVA doses. The proof of concept of this new approach, referred to as "UVA-based light fractionation of ALA-PDT" was evaluated in immortalised HaCaT keratinocytes as a model of skin cancer. In clinical settings, this protocol would have the potential to reduce considerably the treatment time and the discomfort/pain associated with prolonged exposure to high intensity visible light.

The experimental procedure involved the evaluation of the impact of a series of sub-lethal dose combinations of UVA, designed to promote labile iron release and exacerbate the intracellular oxidative damage following a second UVA light dose in ALA-treated cells using both an indoor broad spectrum UVA lamp (Sellas, Germany, 340-405 nm) and outdoor sunlight. In the indoor setting, cytotoxicity assays (MTT, AnnexinV-propidium iodide flow cytometry and colony-forming), showed that: (i) ALA significantly sensitised HaCaT keratinocytes to very low non-cytotoxic UVA doses (up to 20 kJ/m^2) and (ii) applying two short pulses of UVA (1-2.5 kJ/m^2) to ALA-treated cells with a dark interval (60-120 min) was a fast and effective way to promote cell death. The outcome of this project also highlighted the importance of the first UVA dose applied and of the extent of labile iron release in determining the efficiency of the subsequent UVA doses. The low split-dose UVA radiation protocol has therefore the potential to improve the current modality for topical ALA-PDT, through a reduction of the irradiation time and hence the duration of pain endured upon the treatment.

In the outdoor light fractionation scheme, we investigated the biological impact of the solar components of UVA and visible light together or visible light alone, using the organic UVB-specific filter, octocrylene, and the commercially available UVB+UVA filters, SPF 50 +, respectively. The synergistic effect of the solar emission bandwidth was significantly higher in the photokilling of ALA-treated cells, and proved to be beneficial in shortening irradiation periods, without compromising the treatment efficiency. The latter provided the first proof of concept study for a novel UVA-based daylight PDT with light fractionation involving two short pulses of daylight exposure with a 60-120 min dark interval. The present work is the first of its kind demonstrating that a light fractionation protocol with extremely low doses of UVA (*e.g.* 1 kJ/m^2 translating to *ca* 30 s in sunlight) with a 1 h interval can effectively inactivate over 80% of the colony forming ability of ALA-treated and daylight-irradiated cells either alone or with octocrylene filtering out the harmful UVB component of sunlight. The results further indicated that while exposing an ALA-treated patient's skin lesions to sunlight with UVB+UVA filters SPF 50 +..is unlikely to be effective at such low doses, our UVA-based daylight PDT split-dose regimen can be a more simple and more powerful system for the daylight PDT of AK patients.

References

1) Pourzand C, Watkin RD, Brown JE, Tyrrell RM. Ultraviolet A radiation induces immediate release of iron in human primary skin fibroblasts: the role of ferritin. Proc Natl Acad Sci U S A. 1999

P-6.4.13

Carbapenem resistant Enterobacterales do not resist antibiotic treatment upon exposure to antimicrobial blue light.

Agata Wozniak[1], Izabela Zybala[1], Mariusz Grinholc[1]
1) Laboratory of Molecular Diagnostics, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Gdansk, Poland.

Carbapenem resistant Enterobacterales (CRE) are responsible for infections associated with high mortality rates among hospitalized patients, up to 50% in some studies[1]. Continuously growing antimicrobial resistance among CRE strains forces into a finding a new method of eradication or re-sensitization to antibiotics such resistant strains. Antimicrobial blue light (aBL) is suited for such applications. Presence of endogenously produced porphyrins and flavins, which can be easily excited by appropriate wavelength leads to production of reactive oxygen radicals[2]. Therefore, the oxygen radicals in appropriate amount can lead to the damage of bacterial cells probably resulting in increased susceptibility to antibiotics or cell death.

In present study two clinical isolates of *Enterobacter cloacae* (no. 2640/19, 4986/12) and two isolates *Klebsiella pneumoniae* (no. 680, 479) were exposed to sub-lethal doses of aBL and the influence of light on the level of antimicrobial resistance CRE strains was investigated with multiple methods (E-test, checkerboard assay, postantibiotic effect). Moreover, the presence of endogenous porphyrins and flavins in CRE strains was confirmed with the UV-vis spectroscopy. Production of ROS and singlet oxygen after exposure of bacterial cells to aBL was examined with fluorescent probes (AMDA, HPF) and additional SYTOX green and Propidium iodide (PI) probes were involved in the cell membrane integrity assays.

E. cloacae isolate no. 2640/13 after exposure to low dose of blue light was more susceptible to doxycycline, tigecycline and imipenem, and second strain no. 4986/12 to tigecycline, ciprofloxacin and fosfomycin - these results were confirmed with disk diffusion method. However, the checkerboard assay revealed that both *E. cloacae* and *K. pneumoniae* strains after exposure to aBL had increased susceptibility to ceftazidime and colistin. Postantibiotic effect confirmed also increased susceptibility after blue light exposure only for *E. cloacae* isolate no. 4986/12 for colistin and fosfomycin. Observed synergies in case of strains no. 680 and 2640/13 for light and antimicrobials (ceftazidime, colistin) can be explained by the increased permeabilization which occurred after combined treatment with the implementation of SYTOX green and PI probes. Combined action of blue light and antibiotics (e.g., colistin and ceftazidime) lead also to the increased production of ROS in case of strains no. 680 and 2640/13 and this fact can also explain the increased susceptibility of these isolates to antimicrobials.

Antimicrobial blue light inactivation can efficiently increase susceptibility of Enterobacterales to various antibiotics e.g. fosfomycin, ceftazidime, however this phenomenon is strain depended, thus the re-sensitization to antimicrobials was observed between different strains and applied methods. Increased sensitivity to antibiotics after exposure to blue light can be explained by the increased permeabilization of cell membranes and ROS activity, decreased activity of carbapenemase or other not recognized within this study mechanisms of resistance.

References

1) CRE Technical Information | CRE | HAI | CDC https://www.cdc.gov/hai/organisms/cre/technical-info.html
2) Wang, Y.; Wang, Y.; Wang, Y.; Murray, C.K.; Hamblin, M.R.; Hooper, D.C.; Dai, T. Antimicrobial blue light inactivation of pathogenic microbes: state of the art. *Drug Resist. Updat.* 2017

Acknowledgements. This work was supported by grant from National Centre of Science: 2015/19/B/NZ7/02487

P-6.4.14

Photosensitizing Efficacy of Curcumin-Loaded Liposomes Following Photodynamic Therapy on Melanoma MUG-Mel2, Squamous Cell Carcinoma SCC-25 and Normal Keratinocyte HaCaT Cells

Marta Woźniak[1], Martyna Nowak[1], Izabella Jabłońska[1], Krzysztof Szpadel[1], Piotr Ziółkowski[1]

1) Wroclaw Medical University, Department of Pathology, Wroclaw, Poland

The research focused on the investigation of curcumin encapsulated in hydrogenated soya phosphatidylcholine liposomes and its increased photoactive properties in photodynamic therapy (PDT). The goal of this study was twofold: to emphasize the role of a natural photoactive plant-based derivative in liposomal formulation as an easily bioavailable alternative photosensitizer (PS) for the use in PDT of skin malignances. Furthermore, to prove the decreased cytotoxicity of phototoxic agent loaded in liposomes towards normal skin cells. Our research was conducted on melanoma (MugMel2), squamous cell carcinoma (SCC-25), and normal human keratinocytes (HaCaT) cell lines. The MTT study evaluated cell death after exposure to blue light irradiation after 4 hours of preincubation with free and encapsulated curcumin. Additionally, the wound healing assay, flow cytometry, and immunocytochemistry to detect apoptosis were performed. The malignant cells revealed increased phototoxicity after the therapy in comparison to normal cells. Moreover, liposome curcumin-based photodynamic therapy showed an increased ratio of apoptotic and necrotic cells. The study also demonstrated that nanocurcumin significantly decreased malignant cell motility following PDT treatment. Our results suggest that liposomal formulation of a poor soluble natural compound may improve photosensitizing properties of curcumin-mediated PDT treatment in skin cancers and reduce toxicity in normal keratinocytes.

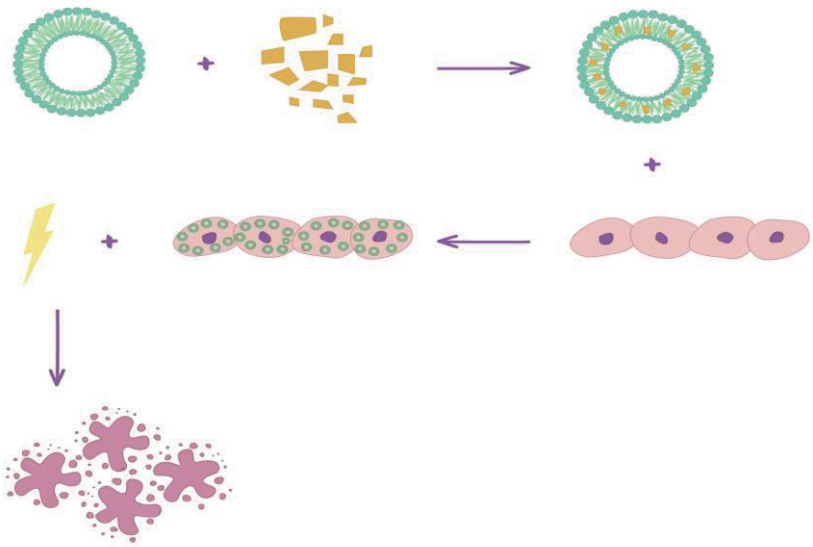


Fig. 1 –Scheme of encapsulated curcumin in liposomes and mechanism of photodynamic therapy.

References

1) Szlasa, W.; Supplitt, S.; Drąg-Zalesińska, M.; Przystupski, D.; Kotowski, K.; Szewczyk, A.; Kasperkiewicz, P.; Saczko, J.; Kulbacka, J. Effects of Curcumin Based PDT on the Viability and the Organization of Actin in Melanotic (A375) and Amelanotic Melanoma (C32) – in Vitro Studies. Biomed. Pharmacotherapy, 2020.

2)

Acknowledgements. This research was funded by the Wroclaw Medical University, grant number SUB.A010.20.13.

P-6.4.15

Photobiological studies on a novel lead BODIPY-anthracene dyad for bladder cancer therapy

Odrun A. Gederaas,[1] Susan Callaghan,[2] Benedikte E. Vindstad,[1] Keith J. Flanagan[2], Thor B. Melø,[1] Mikael Lindgren,[1] Kristin G. Sæterbø,[1] and Mathias O. Senge[2]

1.Department of Physics, Department of Physics, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim (Norway)
2.Medical Chemistry, Trinity Translational Medicine Institute, Trinity Centre for Health Sciences, Trinity College Dublin, The University of Dublin, St. James's Hospital, Dublin 8 (Ireland)

The structural, photobiological, and photophysical properties of a lead photosensitizer PDT BODIPY-anthracene dyad BAD-3 were studied to better understand the properties of this molecule and to assess it as a possible photodynamic therapy agent for bladder cancer treatment.

The rat bladder cancer cell line (AY27) was grown in standard RPMI growth medium, and further incubated with the photosensitizer BAD-3 before blue light illumination (LumiSource, 13mW/cm^2). Using flow cytometry (Beckman Coulter Gallios Flow Cytometer), both necrosis and apoptosis processes were studied by using annexin V, PI, and annexin-binding buffer. The cells were imaged employing a confocal laser scanning microscope (Leica TCS SP8 MP, Leica Microsystems, Germany) after incubation of different staining procedures.

BAD-3 gave a strong therapeutic response against the AY27 cell line and additionally cytotoxicity was observed in the glioma F98 cell line. At a concentration of 10 µM, BAD-3 induced complete killing after 120 s of illumination using 12.9 mW/cm^2 in the AY27 cells, thus presenting as a promising and versatile photodynamic therapy agent. The flow cytometry document both necrosis and apoptosis and a large G1 and minor S phase arrestation post PDT.

The current study confirmed that BAD-3 is a suitable BODIPY-anthracene dyad for medical applications. The characteristics of BAD-3, with view of translating this photosensitizer to an *in vivo* model, is promising.

1) Callaghan S, Vindstad BE, Flanagan KJ, Melø TB, Lindgren M, Sæterbø K G, Gederaas OA and Senge MO. *ChemPhotoChem,* **2020**, 5, 131.

P-6.4.16

5-Aminolevulinic acid-Induced Porphyrin Generation in Prostate Cancer Cells with *N*-Formyl and *N*-Acetyl Peptide Prodrugs

Nabeela Farooq[1], Thomas Elliott[2], Matthew Hunt[2], David White[2], Ian Eggleston[2], Sandy MacRobert[1]

1) University College London, Division of Surgical and Interventional Sciences, London, W1W 7TY, United Kingdom; 2) University of Bath, Department of Pharmacy & Pharmacology, Bath, BA2 7AY, United Kingdom

Background. Photodynamic diagnosis (PDD) and therapy (PDT) for fluorescence imaging and focal therapy of prostate cancer (PCa) is a growing area of research. Using 5-aminolevulinic acid (ALA) as a prodrug to generate the photosensitiser protoporphyrin (PpIX) has shown much promise. However, inadequate physicochemistry, lipophilicity and bioavailability of ALA warrants the search for novel ALA prodrugs. Here, amino acid-conjugated ALA derivatives with either acetyl or formyl N-termination have been investigated to circumvent these drawbacks. No previous studies using the conjugates investigated herein have been carried out on PCa lines.

Methods. Human PCa lines (PC3 and LNCaP) were treated with ALA and four phenylalanine- or methionine- based dipeptide derivatives. For each, the efficiency of PpIX synthesis as a function of concentration was investigated, assessed as intracellular fluorescence compared to an ALA standard. Next, PDT was modelled by irradiation, and cellular viability measured using metabolic activity (MTT) assays. Fluorescence and microscopy imaging were performed to support these findings.

Results. Cell line- and time-dependent, ALA-mediated PpIX generation was exhibited in this study. Using PC3, 3 of the 4 drugs tested exhibited promising PpIX synthesis at low doses, but ineffective fluorescence at molarities 0.05mM and above. In line with our previous findings with *N*-acetyl peptide prodrugs of ALA, Ac-Phe-ALA-Me was the most effective derivative tested here.[1] Methionine derivatives were less successful, with Ac-Met-ALA-Me marginally outperforming For-Met-ALA-Me. Experimentation with For-Phe-ALA-Me failed to elicit measurable PpIX in either cell line ($P<0.01$).

Conclusion. Our studies have shown that *N*-formyl and *N*-acetyl peptide prodrugs of ALA can be readily synthesised. ALA release from both types of prodrug can take place in PCa cells leading to PpIX formation that could be exploited for PDT.

References

1) Giuntini F, Bourre L, MacRobert AJ, Wilson M, Eggleston IM. Improved peptide prodrugs of 5-ALA for PDT: rationalization of cellular accumulation and protoporphyrin IX production by direct determination of cellular prodrug uptake and prodrug metabolization. Journal of Medicinal Chemistry, July 2009.

PL-11

Reactive Oxygen Species Go Beyond Photodynamic Therapy

Xiaoyuan Chen
1) National University of Singapore, Departments of Diagnostic Radiology, Surgery, Chemical and Biomolecular Engineering, Biomedical Engineering, Yong Loo Lin School of Medicine and Faculty of Engineering, Singapore, Singapore

The reactive oxygen species (ROS)-mediated mechanism is the major cause underlying the efficacy of photodynamic therapy (PDT). The PDT procedure is based on the cascade of synergistic effects between light, a photosensitizer (PS) and oxygen, which greatly favors the spatiotemporal control of the treatment. This procedure has also evoked several unresolved challenges at different levels including (i) the limited penetration depth of light, which restricts traditional PDT to superficial tumors; (ii) oxygen reliance does not allow PDT treatment of hypoxic tumors; (iii) light can complicate the phototherapeutic outcomes because of the concurrent heat generation; (iv) specific delivery of PSs to sub-cellular organelles for exerting effective toxicity remains an issue; and (v) side effects from undesirable white-light activation and self-catalyzation of traditional PSs. In this talk, the current status and the possible opportunities of nanomedicine for ROS generation for cancer therapy will be discussed in detail.

Acknowledgements. This work was supported by the start-up fund of the National University of Singapore (NUHSRO/2020/133/Startup/08) and NUS School of Medicine Nanomedicine Translational Research Programme (NUHSRO/2021/034/TRP/09/Nanomedicine)

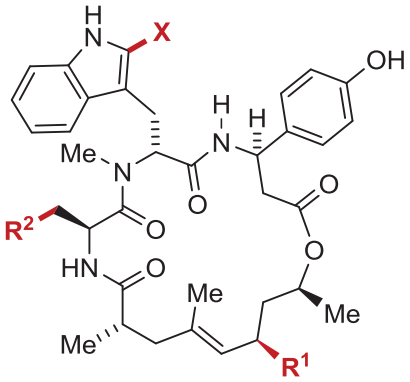
IL-7.1.1

Imaging and photoswitching actin dynamics by small molecule probes

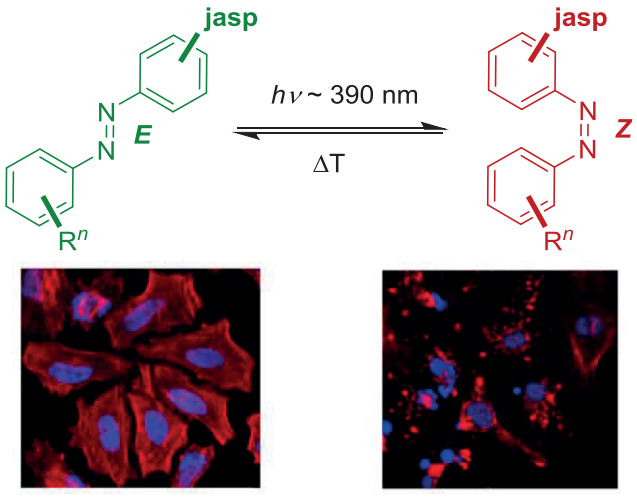
Hans-Dieter Arndt*

**Friedrich-Schiller-Universität, Institut für Organische Chemie und Makromolekulare Chemie, Humboldtstr. 10, D-07743 Jena, Germany; hd.arndt@uni-jena.de*

Actin is the most abundant cytoskeletal protein and key to most cellular activities.[1] Therefore, mastering on-target selectivity and confining activity in its pharmacological modulation will be crucial for resolving actin functions on the tissue and cell level. By starting from F-actin targeting jasplakinolide derivatives,[2] chemical synthesis, SAR and labeling studies led us to create cell permeable fluorescent probes such as SiR-Actin with exquisite target staining selectivity,[3] and to resolve the molecular interaction of these ligands by cryo-EM.[4]
For controlling actin dynamics locally by using photopharmacology,[5] jasplakinolide-derived **optojasp** photoswitches were developed that feature metastable azobenzene triggers.[6] Late stage derivatization guided by SAR data enabled a systematic study of photoswitch type and ligand structure. From this collection several compounds active in either the "dark" (*E*) or "irradiated" (*Z*) form were identified that show remarkable potential for the light-dependent manipulation of actin dynamics in living cells.[6] First applications of **optojasps** in cell biology[6] and structural properties of F-actin[4] and of ligand binding in both switch states[7] will be discussed. Furthermore, first data on photoswitch optimization[8] and second generation optojasps with improved properties will be presented.[9]



jasplakinolide (**X** = Br, **R**^{1} = Me, **R**^{2} = H)
optojasps (**X** = H, **R**^{1} = H, **R**^{2} = linked azobenzene)



References
1) T. D. Pollard, G. G. Borisy, *Cell* **2003**, *112*, 453.
2) a) H. Waldmann et al., *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 6473; b) R. Tannert et al., *J. Am. Chem. Soc.* **2010**, *132*, 3063.
3) a) L. G . Milroy et al., *J. Am. Chem. Soc.* **2012**, *134*, 8480, b) G. Lukinavičius et al., *Nat. Methods* **2014**, *11*, 731.
4) F. Merino et al., *Nat. Struct. Mol. Biol.* **2018**, *25*, 528.
5) J. Broichhagen, F. A. Frank, D. Trauner, *Acc. Chem. Res*. **2015**, *48*, 1947.
6) M. Borowiak, F. Küllmer, F. Gegenfurtner, S. Peil, V. Nasufovic, S. Zahler, O. Thorn-Seshold, D. Trauner, H.-D. Arndt, *J. Am. Chem. Soc.* **2020**, *142*, 9240.
7) S. Pospich, F. Küllmer, V. Nasufovic, J. Funk, A. Belvy, P. Bieling, H.-D. Arndt, S. Raunser, *Angew. Chem. Int. Ed.* **2021**, *60*, 8678.
8) F. Küllmer, L. Gregor, H.-D. Arndt, *in submission*.
9) F. Küllmer, N. Veprek, M. Borowiak, V. Nasufovic, S. Barutzki, D. Trauner, H.-D. Arndt, *in preparation*.

IL-7.1.2

Next-generation optogenetic tools for manipulating the membrane potential

Ramona A Kopton[1], Franziska Schneider-Warme[1]

1) Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg – Bad Krozingen, Freiburg, Germany;
2) Medical Faculty, University of Freiburg, Freiburg, Germany

Optogenetic approaches offer unprecedented opportunities to manipulate cellular activity at high spatiotemporal resolution. Most commonly used optogenetic tools comprise light-gated ion channels (so-called channelrhodopsins) and light-driven ion pumps that enable modulating the membrane potential in the target cells upon illumination. More specifically, activation of cation-conducting channelrhodopsins such as ChR2 from *Clamydomonas reinhardtii* leads to depolarizing photocurrents, which can be used to trigger action potentials in excitable cells, including neurons and cardiomyocytes. Light-driven proton, chloride and sodium pumps actively transport ions against the electrochemical gradient, thereby hyperpolarizing the membrane potential and reducing cellular excitability. The hyperpolarizing effect of ion pumps, however, is limited, as maximally one ion can be transported per absorbed photon, thus requiring constant high light intensities for sustained hyperpolarization. The more recently found group of anion channelrhodopsins (ACR) mediates light-activated chloride currents, which can be used to "shunt" the membrane potential to the reversal potential of chloride. This has been exploited for silencing excitable cell activity, but we and others have shown that, depending on transmembrane gradients for chloride, which vary in different cell types and subcellular compartments, activation of chloride channels can have both depolarizing and hyperpolarizing effects[1].
The ideal tool for optogenetic silencing of excitable cells would be a light-gated potassium channel, whose activation would draw cells towards the potassium reversal potential, close to the natural resting membrane potential. We have established the two-component optogenetic system PAC/K, combining a light-activated cyclase and cyclic-nucleotide gated (CNG) potassium channel, which drives long-lasting (seconds to minutes) potassium currents upon illumination with short (milliseconds) blue light pulses. Resulting photocurrents effectively inhibit action potential initiation, as shown for cardiomyocytes and different neuronal classes, both *in vitro* and *in vivo*[2]. At present, optogenetic applications of PAC/K are limited as 1) use of cAMP for activation of the CNG channel can have detrimental side effects, and 2) the slow off-kinetics do not offer sufficient temporal control to inhibit individual action potentials. In on-going research, we develop alternative potassium-based silencing systems, based on rhodopsin guanylyl cyclases and custom engineered potassium channels, with the vision to be able to reversibly silence excitable cells with millisecond precision without affecting their resting potential.

References

1. Kopton RA et al. Cardiac Electrophysiological Effects of Light-Activated Chloride Channels. *Front Physiol* **2018**/9:1806.
2. Bernal Sierra YA et al. Potassium channel-based optogenetic silencing. *Nat Commun* **2018**/9(1):4611.

Acknowledgements. This work was supported by the German Research Foundation (DFG: Priority Program 1926 [#315193289], and the Emmy-Noether Programme [#412853334]). FSW is a member of CRC1425 and an associate member of the excellence cluster Centre for Integrated Biological Signalling Studies (CIBSS).

IL-7.1.3

Controlling Gene Expression with Light

Lea Albert, [1] Benedikt Heinrich, [1] Olalla Vázquez [1]
1) Philipps-Universität Marburg, Department of Chemistry & SYNMIKRO, Marburg, Germany

All cells in an organism contain the same DNA sequence but vary greatly in gene expression. Epigenetics deals with these phenotype changes that retains the same DNA sequence. Importantly, misregulation of these epigenetic processes is implicated in the pathophysiology of numerous human diseases, [1] including: cancer, autoimmune disorders and neurodegenerative disease. Therefore, epigenetic regulation is at the core of both natural and pathological states. The current available methods do not have sufficient spatiotemporal resolution to deal with the challenges of targeting the dynamic epigenome. We and others [2] envision that light could offer new possibilities and achieve molecular functionality. Reversible photoswitches, which have demonstrated their potential in diverse areas such as material science, have hardly implemented as genome regulators. Modulating the epigenome to tune transcription profiles, and cellular phenotypes in a programmable manner is of wide interest, and may ultimately lead to novel epigenetics-based therapeutics. Here, we will present an overview of our optoepigenetic tools from our photoswitchable peptides as histone-methyltransferase inhibitors [3] until our light-activatable nucleosome binders with capacity to modulate DNA accessibility.[4]

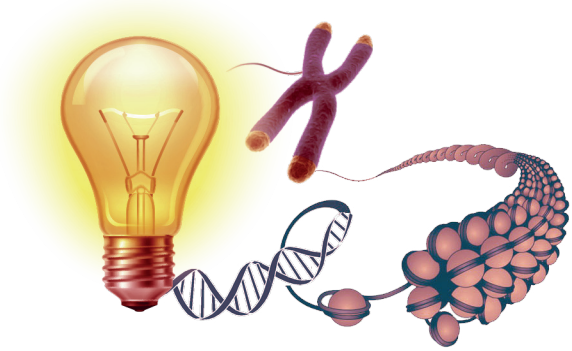


Fig. 1 – Conceptual idea of using light to control gene expression

References

1. Portela, A.; Esteller, M., Epigenetic modifications and human disease. *Nat Biotechnol* **2010,** *28* (10), 1057-68.
2. Hartmann, D.; Smith, J. M.; Mazzotti, G.; Chowdhry, R.; Booth, M. J., Controlling gene expression with light: a multidisciplinary endeavour. *Biochem Soc Trans* **2020,** *48* (4), 1645-1659.
3. Albert, L.; Xu, J.; Wan, R.; Srinivasan, V.; Dou, Y.; Vazquez, O., Controlled inhibition of methyltransferases using photoswitchable peptidomimetics: towards an epigenetic regulation of leukemia. *Chem Sci* **2017,** *8* (6), 4612-4618.
4. Heinrich, B.; Bouazoune, K.; Wojcik, M.; Bakowsky, U.; Vazquez, O., ortho-Fluoroazobenzene derivatives as DNA intercalators for photocontrol of DNA and nucleosome binding by visible light. *Org Biomol Chem* **2019,** *17* (7), 1827-1833.

Acknowledgements. This work was financial supported by German Research Fundation (DFG) programs: SPP1926 'Next Generation Optogenetics'; Young Investigator Program (grant # GO1011/11-1) and TRR81: “Chromatin Changes in Differentiation and Malignancies (TRR81/3, Z04).

OC-7.1.4

Light-Free and Self-Activating Single-Molecule Chemiluminescent Photosensitizers for Selective Photodynamic Therapy of Cancer

Luís Pinto da Silva[1], Carla M. Magalhães[1], Patricia González-Berdullas[1]
1) Chemistry Research Unit (CIQUP), Faculty of Sciences of University of Porto, Porto, Portugal; e-mail: luis.silva@fc.up.pt

Photodynamic therapy (PDT) is a cancer treatment with significant advantages over more conventional cancer therapies, such as its minimally-invasive nature and fewer side-effects.[1] However, the low penetration of light into biologic tissues limits PDT to tumours on/just under the skin or on the outer lining of internal organs and cavities.[1] Herein, we have developed single-molecule photosensitizers able of light-free and intracellular self-activation for tumour-selective PDT, which is based on a chemiluminescent reaction involving a cancer marker (Fig. 1).[2,3] More specifically, the photosensitizers are directly chemiexcited to a triplet excited state (T_1) capable of sensitizing singlet oxygen, without the need for a light source.[2,3]

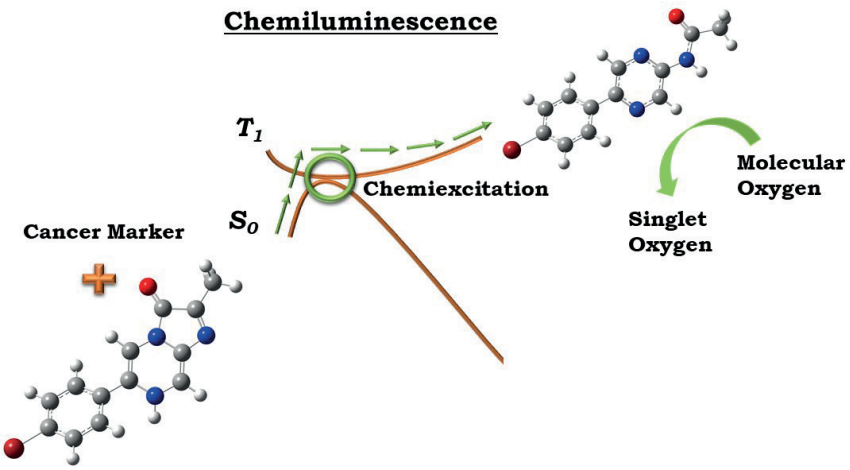


Fig. 1 – Proposed light-free and tumour-selective PDT mechanism based on a chemiluminescent reaction.

Cytotoxicity assays were performed toward different cancer cell lines (breast, neuroblastoma, colon and prostate), with the photosensitizers showing relevant toxicity toward these cells,[2,3] with activities comparable with reference chemotherapeutic drugs. Importantly, these novel photosensitizers did not induce toxicity toward normal cells.[2,3]
In conclusion, this work provides a proof-of-concept for a novel type of single-molecule photosensitizer that eliminates the current restrictions that PDT presents regarding tumour size and localization.

References

1) W. Fan, P. Huang, X. Chen, Overcoming the Achilles' heel of photodynamic therapy. Chem. Soc. Rev. 45: 6488-6519, 2016.
2) L. Pinto da Silva, A.N. Montenegro, C.M. Magalhães, P.J.O. Ferreira, D. Duarte, P.G. Berdullas, J.E.R. Borges, N. Vale, J.C.G. Esteves da Silva, Single-molecule chemiluminescent photosensitizer for a self-activating and tumor-selective photodynamic therapy of cancer. Eur. J. Med. Chem. 132: 111683, 2019.
3) L. Pinto da Silva, C.M. Magalhães, A.N. Montenegro, P.J.O. Ferreira, D. Duarte, J.E.R. Borges, N. Vale, J.C.G. Esteves da Silva. Biomolecules 9: 384, 2019.

Acknowledgements. This research was funded by the Portuguese Foundation for Science and Technology (FCT) via the projects PTDC/QUI-QFI/2870 and UIDB/00081/2020.

SP-7.1.5, P-7.1.5

A Fluorescent β-Cyclodextrins Polymer Encapsulating Sorafenib and Releasing Nitric Oxide Under Visible Light

Francesca Laneri[1], Milo Malanga[2] and Salvatore Sortino[1].

1) University of Catania, Department of Drug and Health Sciences, Catania, Italy; 2) CycloLab Ltd., Budapest, Hungary

In the last years, the combination of cytotoxic radical species with conventional drugs has encountered growing interest in cancer treatment, with the final goal to maximize the therapeutic action and to minimize side effects. Nitric oxide (NO) has particularly been investigated since, besides its well-known involvement in physiological and physio-pathological pathways, it shows anticancer properties. Nonetheless, the delivery of this gaseous transient specie to the targeted cells has resulted particularly challenging as tumor growth inhibition is granted at micromolar concentrations, while at picomolar concentrations of NO tumor cells proliferation is increased.[1] Light-triggered NO donors allows the fine spatiotemporal modulation of the NO dosage and hold great potential in cancer treatment.[2] In this contribution we report a macromolecular construct made of a fluorescent, water-soluble cyclodextrins branched polymer releasing NO under visible light[3] and encapsulating Sorafenib, a drug largely administered for the treatment of liver and kidney cancer. The photophysical and photochemical properties of this supramolecular complex are discussed and the preliminary biological tests on different cancer cell lines are also reported.

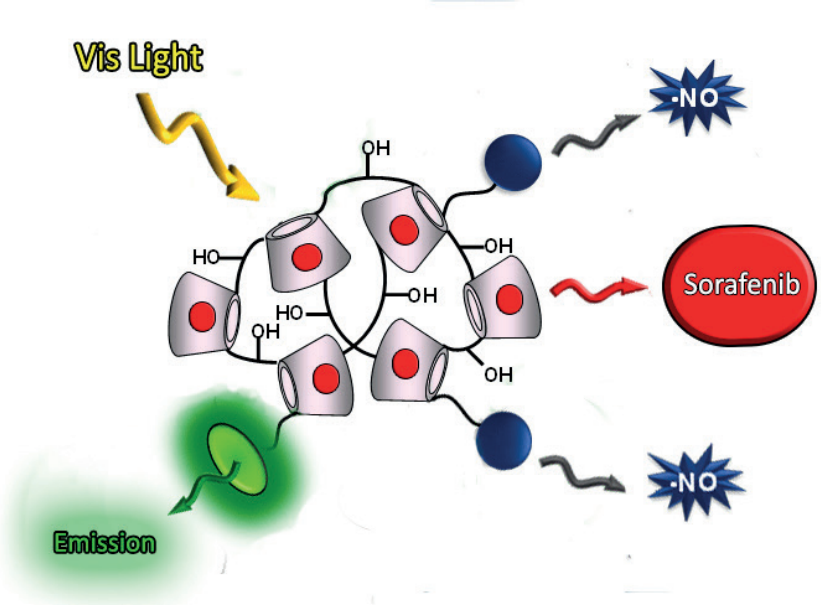


Fig. 1 – Schematic representation of the fluorescent polymer encapsulating Sorafenib and releasing NO under visible light

References

1) A. Fraix, N. Marino, S. Sortino, Phototherapeutic Release of Nitric Oxide with Engineered Nanoconstructs. *Top Curr Chem. 2016.*
2) S. Sortino, Photoactivated nanomaterials for biomedical release applications. *J Mater Chem. 2011.*
3) M. Malanga, M. Seggio, V. Kirejev, A. Fraix, I. Di Bari, E. Fenyvesi, M.B. Ericson, S. Sortino, A phototherapeutic fluorescent β-cyclodextrin branched polymer delivering nitric oxide. *Biomater Sci. 2019.*

Acknowledgements. We thank MIUR (Italy) for the Industrial PhD grant (DOT1308125) to F.L. and AIRC – Italian Association for Cancer Research (IG-198559) for financial support.

IL-7.1.6

New Azobenzene-based Photoswitches for the Two-Photon light-induced control of neuronal signalling

Gisela Cabré[1], Aida Garrido-Charles[2], Mercè Izquierdo-Serra[2], Marta Gascón-Moya[1], Ramon Alibes[1], Félix Busqué[1], Jordi Hernando[1], Pau Gorostiza[2,3,4]
1) Departament de Química, Universitat Autònoma de Barcelona (UAB), Cerdanyola del Vallès, Spain; 2) Institut de Bioenginyeria de Catalunya (IBEC), Barcelona Institute of Science and Technology (BIST), Spain; 3) Institució Catalana de Recerca I Estudis Avançats (ICREA), Spain ; 4) Centro de Investigación Biomédica en Red en Bioingenieria, Biomateriales y Nanomedicina (CIBER_BBN), Spain
Presenting author: Félix Busqué

Azobenzenes have been extensively studied as photopharmacological tools for the fast and reversible optical control of biological systems, for example for the manipulation of neuronal signaling. However, most of these current compounds operate under near-UV light irradiation, which is not compatible with many biomedical applications since it can cause photodamage and is absorbed and scattered by tissues, thus preventing efficient penetration depths.[1] It would be desirable to shift the absorbance of these compounds to the red and near-infrared (NIR) regions. In our research groups we have been developing new azobenzene-based light-activable drugs that could exhibit efficient two-photon (2P) absortion under near-infrared light excitation and could act as efficient photochromic tethered ligands (PTLs). We have been focused in a family of azobenzene-based PTLs called MAG ligands, which are composed of a maleimide moiety for receptor tethering, the azobenzene photoswitch and a glutamate agonist.

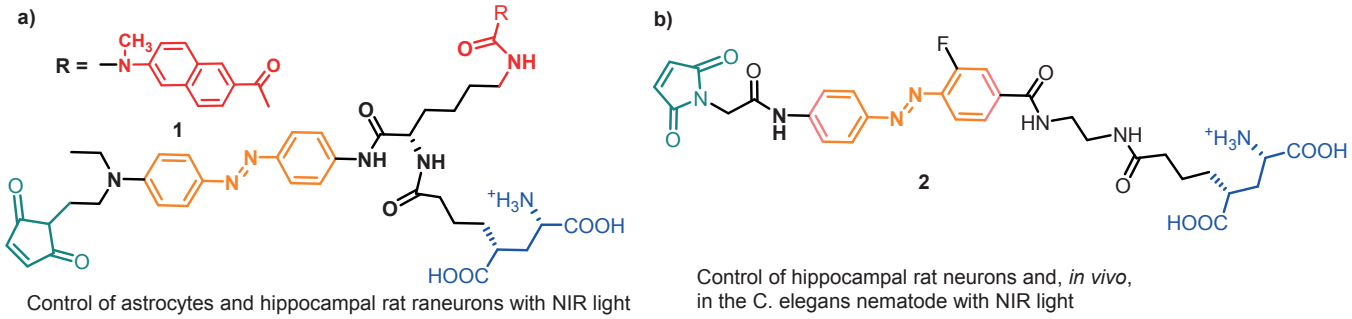


Fig. 1 – Synthesized MAGs PTLs operative under 2P excitation with NIR light.

In particular we have applied this strategy to the regulation of several ionotropic glutamate receptors (iGluRs), the main responsible of excitatory currents in the central nervous system. *Trans-cis* photoisomerisation of these compounds allows modulation of glutamate-receptor interaction, thus resulting in light-induced operation of the cell membrane ionic channels governed by iGluRs. We have developed new MAG switches capable to trigger iGluRs upon 2-photon excitation with near infrared light following two main strategies: (i) the introduction in the compound of a photosensitizer unit, with 2P absorption capacity, that can transfer its electronic excitation energy to the azobenzene core to trigger the isomerization (Figure 1a);[2] and (ii) the use of electronically asymmetric azobenzene switches, that leads to maximize its 2-photon response (Figure 1b).[3]

References

1) K. Hüll, J. Morstein, D. Trauner, In vivo Photopharmacology. *Chem. Rev*. **2018**, *118*, 10710-10747.
2) M. Izquierdo-Serra, M. Gascón-Moya, J. J. Hirtz, S. Pittolo, K, E. Poskanzer, E. Ferrer, R. Alibés, F. Busqué, R. Yuste, J. Hernando, P. Gorostiza. Two-Photon Neuronal and Astrocytic Stimulation with Azobenzene-based Photoswitches. *J. Am. Chem. Soc*. **2014**, *136*, 8693-8701.
3) G. Cabré, A. Garrido-Charles, M. Moreno, M. Bosch, M. Porta-de-la-Riva, M. Krieg, M. Gascón-Moya, N. Camarero, R. Gelabert, J. M. Lluch, F. Busqué, J. Hernando, P Gorostiza, R. Alibés. Rationally designed azobenzene photoswitches for efficient two-photon neuronal excitation. *Nat. Commun*. **2019**, *10*, 297

Acknowledgements. This work was supported by projects and grants from different institutions of Spain.

IL-7.1.7

The potential of G protein-coupled receptor photopharmacology for the discovery of new biological mechanisms

Xavier Rovira
1) Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Medicinal Chemistry & Synthesis group, Barcelona, Spain;

By definition, biological mechanisms are the dynamic producers of physiological phenomena. However, when using drugs in research, we introduce molecules into the biological system for which the action cannot be dynamically controlled, thus hampering the possibility to study their effects in specific locations and time points. G protein-coupled receptors (GPCRs) are important regulators of many physiological processes targeted by around one third of approved drugs. Recent reports reveal that signal transduction by GPCRs is a multidimensional process governed by molecular, spatial and temporal components. Indeed, endogenous GPCR ligands are finely regulated by biological processes that allow their temporally controlled release and reuptake in specific locations, sometimes even at the subcellular level. In contrast, one important limitation of current drugs is that once released in the organism, their effect cannot be selectively interrupted. To solve these limitations, GPCR photopharmacology aims at controlling native biological systems with a high degree of temporal and spatial precision using innovative light-regulated molecules and illumination devices. In this presentation, I will talk about recent advances in the generation of photopharmacological tools targeting GPCRs and their potential to uncover new biological mechanisms that cannot be studied with conventional drugs. The possibility to dynamically control signalling pathways activated by a single GPCR with a unique component (light-regulated drugs) may open new avenues in cell communication research field and inspire the development of precise and personalized medicines with high efficacy and reduced side-effects.

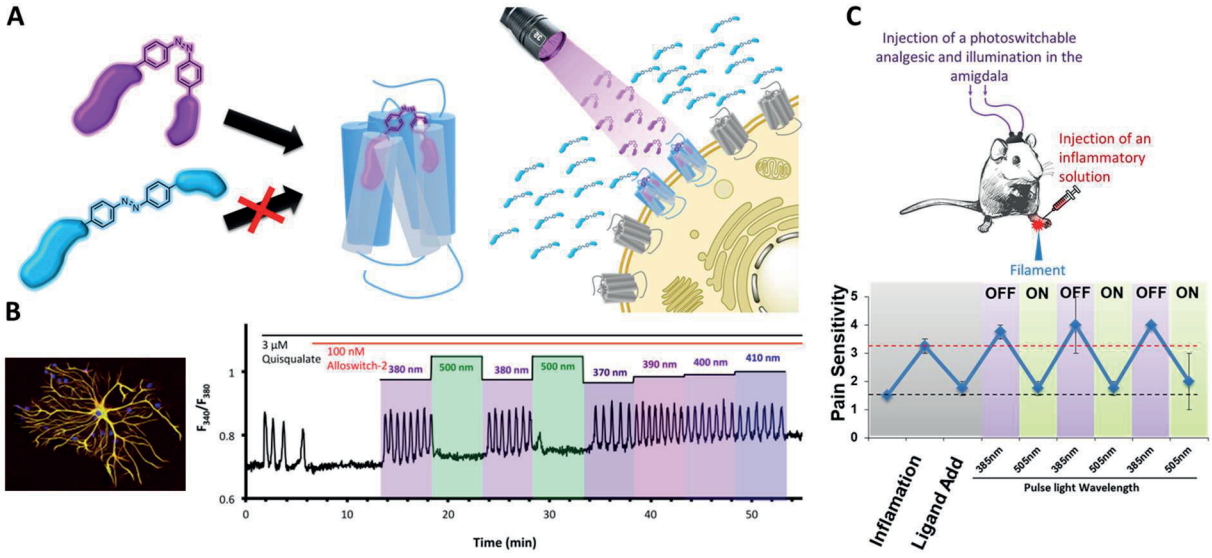


Fig. 1 – (A) The GPCR Photopharmacology principle. (B) Dynamic control of native neuronal receptors in primary cell cultures with Alloswitch-1, a reversible light-regulated drug targeting metabotropic glutamate receptor 5[1]. (C) Dynamic control of native receptors in alive animals with chronic pain using reversible photopharmacological tools targeting GPCRs[2].

References

1) Pittolo S. et al., An allosteric modulator to control endogenous G protein-coupled receptors with light. Nat Chem Biol 10(10):813-5, 2014.
2) Zussy C., et al. Dynamic modulation of inflammatory pain-related affective and sensory symptoms by optical control of amygdala metabotropic glutamate receptor 4. Mol Psychiatry 23(3):509-520, 2018.

Acknowledgements. This work was supported by the Spanish Ministry of Economy, Industry and Competitiveness (SAF2015-74132-JIN).

IL-7.1.8

Opto-pharmacological Approaches for Manipulating Neurotransmitter Receptors and Motivated Behaviors in Mice

Alexandre Mourot[1]
1) Brain Plasticity lab, UMR 8249 CNRS, ESPCI, Paris, France.

One fundamental goal of modern neuropharmacology is to causally link changes in receptor activity in given neuronal pathways with alterations of circuit physiology and ultimately of behaviour. The utility of classical pharmacology in vivo in achieving this goal is limited, because local drug delivery is slow, imprecise and hardly compatible with electrophysiology. In contrast, opto-pharmacological approaches for remote-controlling neurotransmitter receptors with light are, in principle, able to mimic the timing, amplitude and spread of naturally occurring modulatory signals (1). My lab is developing opto-pharmacological tools to study the role of the midbrain dopamine system in the control of reward-guided behaviors and in the development of addiction. We notably developed novel caged-compounds that are, for the first time, fully compatible with in vivo use in mice, and demonstrated optical induction of Pavlovian conditioning upon optical stimulation of the midbrain dopamine system (2). We further have engineered light-controllable nicotinic acetylcholine receptors, and deployed these tools in vivo to reveal the impact of acetylcholine, an endogenous neuromodulator, on the activity of midbrain dopamine neurons, and to manipulate nicotine-related addictive behaviors (3). By providing acute and precise manipulation of neurotransmission, our opto-pharmacological approaches will lead to a better comprehension of how specific receptors contribute to the modulation of circuits and behaviors, a crucial step towards the development of novel therapies.

References

1) Paoletti P, Ellis-Davies GCR, Mourot A., Optical control of neuronal ion channels and receptors. Nat Rev Neurosci. 20(9):514-532, 2019.
2) Durand-de Cuttoli R, Chauhan PS, Pétriz Reyes A, Faure P, Mourot A*, Ellis-Davies GCR* (co-last). Optofluidic control of rodent learning using cloaked caged glutamate. Proc Natl Acad Sci U S A. 117(12):6831-6835. 2020
3) Durand-de Cuttoli R, Mondoloni S, Marti F, Lemoine D, Nguyen C, Naudé J, d'Izarny-Gargas T, Pons S, Maskos U, Trauner D, Kramer RH, Faure P, Mourot A. Manipulating midbrain dopamine neurons and reward-related behaviors with light-controllable nicotinic acetylcholine receptors. Elife. 7:e37487. doi: 10.7554/eLife.37487. 2018.

Acknowledgements. This work was supported by the Agence Nationale de la Recherche (ANR), the Brain and Behavior Research Foundation (NARSAD) and the Human Frontier Science Program (HFSP).

OC-7.1.9

Intrafollicular UVA-induced drug release from nanocapsules – a photochemical approach for enhanced dermal drug delivery

Loris Busch[1,4], Yuri Avlasevich[2], Paula Zwicker[3], Maxim E. Darvin[1], Katharina Landfester[2], Martina C. Meinke[1], Cornelia M. Keck[4], Axel Kramer[3], Jürgen Lademann[1], Alexa Patzelt[1]

1) Center of Experimental and Applied Cutaneous Physiology, Department of Dermatology, Venereology and Allergology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; 2) Max Planck Institute for Polymer Research, Mainz, Germany; 3) Institute of Hygiene and Environmental Medicine, University Medicine Greifswald, Greifswald, Germany; 4) Department of Pharmaceutics and Biopharmaceutics, Philipps University Marburg, Marburg, Germany

The human hair follicle is known to be an important drug delivery target for the therapy of various skin diseases as well as for skin antisepsis. Nanoparticles of approximately 600 nm in diameter are excellently suited for follicular drug delivery as they show deep follicular penetration when applicated by massage.[1] This phenomenon is based on a mechanically driven transport process, also known as the so-called ratchet effect.[2] Following the penetration process, an intrafollicular drug release can be initiated by various trigger mechanisms depending on the functionalization of the nanoparticulate system.[3]
Since UVA light can reach the connective tissue layers of the skin, and thus deeper sections of the hair follicles, it is usable for triggering the release of drugs from photodegradable nanocapsules (NCs) within the follicular compartment.
In the framework of our experiments, we have established a novel system, which involves the transportation of the model drug sulforhodamine 101 (SR101) into hair follicles by NCs of 600 – 700 nm in size. These NCs were functionalized by a photocleavable *o*-nitrobenzyl linker. Thus, we were able to induce an intrafollicular UVA-triggered drug release by using a light emitting diode (LED).[4] UVA radiation with a power density of 12 mW/cm^2 induced a drug release of over 50% after 2 min as investigated *in vitro* via fluorescence spectroscopy. We further investigated follicular penetration as well as intrafollicular drug release on *ex vivo* porcine skin by using confocal laser scanning microscopy. As schematically depicted in Fig. 1, UVA-induced cleavage of the NCs along with release of SR101 at a mean follicular penetration depth of 509 ± 104 µm was observed after topical application and subsequent irradiation of the skin. Furthermore, sufficient cell viability after exposition of HaCaT keratinocytes to intact and photodegraded NCs was revealed by MTT tests. The application of the presented system represents a promising approach for an optimized follicular drug delivery of various hydrophilic drugs. This might constitute a future benefit for the therapy of miscellaneous skin diseases or could be used for optimized preoperative skin antisepsis.

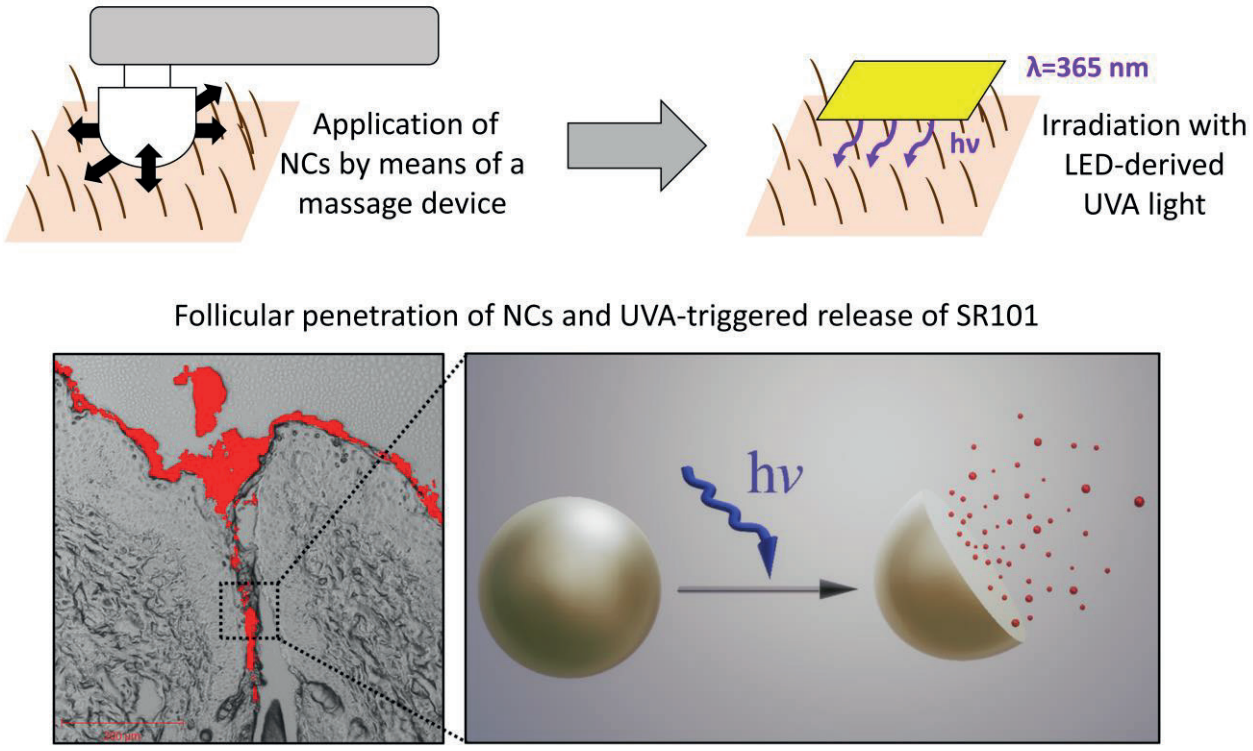


Fig. 1 – Schematic representation of the application and irradiation process on ex vivo porcine skin (top) as well as the follicular penetration of SR101-loaded NCs (bottom left) and subsequent UVA-induced photodegradation of the NCs along with release of SR101 (bottom right) [4].

References

1) Patzelt A, Richter H, Knorr F, Schäfer U, Lehr CM, Dähne L, Sterry W, Lademann J, Selective follicular targeting by modification of the particle sizes. J Control Release 150(1):45-8, 2011.
2) Radtke M, Patzelt A, Knorr F, Lademann J, Netz RR, Ratchet effect for nanoparticle transport in hair follicles. Eur J Pharm Biopharm 116:125-130, 2017.
3) Patzelt A, Mak WC, Jung S, Knorr F, Meinke MC, Richter H, Rühl E, Cheung KY, Tran NBNN, Lademann J, Do nanoparticles have a future in dermal drug delivery? J Control Release 246:174-182, 2017.
4) Busch L, Avlasevich Y, Zwicker P, Thiede G, Landfester K, Keck CM, Meinke MC, Darvin ME, Kramer A, Müller G, Kerscher M, Lademann J, Patzelt A, Release of the model drug SR101 from polyurethane nanocapsules in porcine hair follicles triggered by LED-derived low dose UVA light. Int J Pharm 597:120339, 2021.

Acknowledgments. This study was funded by the German Federal Ministry of Education and Research (BMBF) within the "Advanced UV for Life" project "NanUVsept" [grant number 03ZZ0142A].

P-7.1.10

Exploring Photoactive Pigments of Fungal Extracts with Feature-Based Molecular Networking

Fabian Hammerle[1], Johannes Fiala[1], Luis Quirós-Guerrero[2,3], Adriano Rutz[2,3], Jean-Luc Wolfender[2,3], Ursula Peintner[4], Bianka Siewert[1]
1) University of Innsbruck, Institute of Pharmacy/Pharmacognosy and Center for Molecular Biosciences Innsbruck (CMBI), Innrain 80/82, 6020 Innsbruck, Austria; 2) University of Geneva, Phytochemistry and Bioactive Natural Products, School of Pharmaceutical Sciences, CMU - Rue Michel-Servet 1, 1211 Geneva, Switzerland; 3) University of Geneva, Institute of Pharmaceutical Sciences of Western Switzerland, CMU, 1211 Geneva, Switzerland; 4) University of Innsbruck, Institute of Microbiology, Technikerstraße 25, 6020 Innsbruck, Austria

Investigating the colourants of fungal fruiting bodies has led to the discovery of an activity previously unexpected for Basidiomycetes: photoactivity [1]. Several antraquinone pigments were isolated from *Cortinarius uliginosus* that were capable of generating reactive oxygen species (i.e., singlet oxygen) after irradiation with visible light [2]. To test whether photoactivity is a common phenomenon in the globally distributed, species-rich mushroom genus *Cortinarius* [3], six colourful species belonging to the different classical subgenera *Dermocybe*, *Leprocybe*, *Myxacium*, *Phlegmacium*, and *Telamonia* were selected for mycochemical analysis. Acetone extracts of fungal basidiomes were submitted to a special workflow combining *in vitro* photochemical/photobiological experiments [1], ultra-high performance liquid chromatography coupled to high-resolution tandem mass spectrometry (UHPLC-HRMS^2), and feature-based molecular networking (FBMN) [4].
Two of the six species examined, i.e., *C. rubrophyllus* (*Dermocybe*) and *C. xanthophyllus* (*Phlegmacium*), were not only characterised by high singlet oxygen formation values in the photochemical assay (i.e., DMA-assay), but also exhibited light-dependent cytotoxicity in the low µg/mL-range on various cancer cell lines (i.e., A549/lung, AGS/stomach, T24/bladder). With the help of the bioinformatics tool FBMN, the compounds responsible for the observed photoactivity were identified as unknown polyketides. Comparative liquid chromatographic studies indicated that they are most likely dimeric anthraquinones.
The results of this study highlight the occurrence of various photoactive pigments in *Cortinarius* species belonging to different phylogenetic lineages, and thus fungi as a potential source of natural photopharmaceuticals. Furthermore, an innovative metabolomics-based workflow is presented to screen natural extracts for photoactivity and to subsequently identify the responsible photosensitizers.

References

1) Siewert, B., et al., A convenient workflow to spot photosensitizers revealed photo-activity in basidiomycetes. RSC Advances, 2019.
2) Fabian, H., et al., Targeted Isolation of Photoactive Pigments from Mushrooms Yielded a Highly Potent New Photosensitizer: 7,7'-Biphyscion. ChemRxiv (Preprint), 2021.
3) Peintner, U., J.-M. Moncalvo, and R. Vilgalys, Toward a better understanding of the infrageneric relationships in Cortinarius (Agaricales, Basidiomycota). Mycologia, 2004.
4) Nothias, L.-F., et al., Feature-based molecular networking in the GNPS analysis environment. Nature Methods, 2020.

Acknowledgements. The Austrian Science Fund (FWF P31950, BS) and the University of Innsbruck are acknowledged for support. L. Q-G is supported by a scholarship (N_ 214171–025) Ministerio de Ciencia, Tecnología y Telecomunicaciones, MICITT, from Costa Rica.

P-7.1.11

Melanopsin signalling pathway in HEK293 cells line with stable expression of human melanopsin

Olga Krzysztyska-Kuleta, Magdalena Olchawa, Tadeusz Sarna
Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Introduction Melanopsin, a member of the G-protein coupled receptors family, is involved in non-image-forming functions including circadian rhythm, sleep regulation, pupil response. Although significant efforts research have been devoted to different cell subtypes and their behavioural responses to light activation, signalling cascade involving melanopsin photoactivation is still poorly characterized. In this study, we analysed the effects of photoactivation of melanopsin on phospholipase C (PLC) and diacylglycerol in HEK293 cells with stable expression of human melanopsin.
Methods HEK 293 cells with stable expression of human melanopsin were enriched with 11-*cis*-retinal and blue light irradiated for selected time intervals. To determine the role of phospholipase C and involvement of diacylglycerols, two approaches were employed: inhibition of the G protein and phospholipase C (using the BIM-46187 and U73122 inhibitors, respectively), and gene silencing using siRNA of PLCβ_1 and PLCβ_4.
Results Our study showed that diacylglycerol was present in blue light irradiated HEK293 cells enriched with 11-*cis*-retinal. Using specific inhibitors or small interfering RNA (siRNA), we found that PLCβ_4 was involved in melanopsin phototransduction pathway. Completely diminished response of diacylglycerol and calcium ions in irradiated cells expressing human melanopsin was observed after silencing the PLCβ_4 gene.
Conclusion It has been demonstrated, for the first time that diacylglycerols can be involved in melanopsin signalling pathway. The data also indicate that PLCβ_4 plays the dominant role in melanopsin transduction pathway. These results may facilitate a better understanding of the role of phospholipase C and diacylglycerols in the melanopsin signalling pathway.

P-7.1.12

A photoactivatable β- and γ- cyclodextrin branched co-polymer delivering nitric oxide.

Mimimorena Seggio[1], Szabolcs Béni,[2] Milo Malanga[3], Salvatore Sortino.[1]
1) University of Catania, Department of Drug and Health Sciences, Catania, Italy; 2) Semmelweis University, Department of Pharmacognosy, Budapest, Hungary 3) CycloLab Ltd., Budapest, Hungary.

Polymer based on cyclodextrins (CDs) are receiving a growing interest in the biopharmaceutical field thanks to their well-known complexation capability and their ability to stabilize and solubilize guest compounds. This contribution reports the synthesis and characterization of a novel β- and γ-CD-based branched co-polymer covalently integrating a nitric oxide photodonor (NOPD) and able to supramolecularly encapsulate betaxolol (BTX), a drug widely used in glaucoma treatment. (Fig 1)

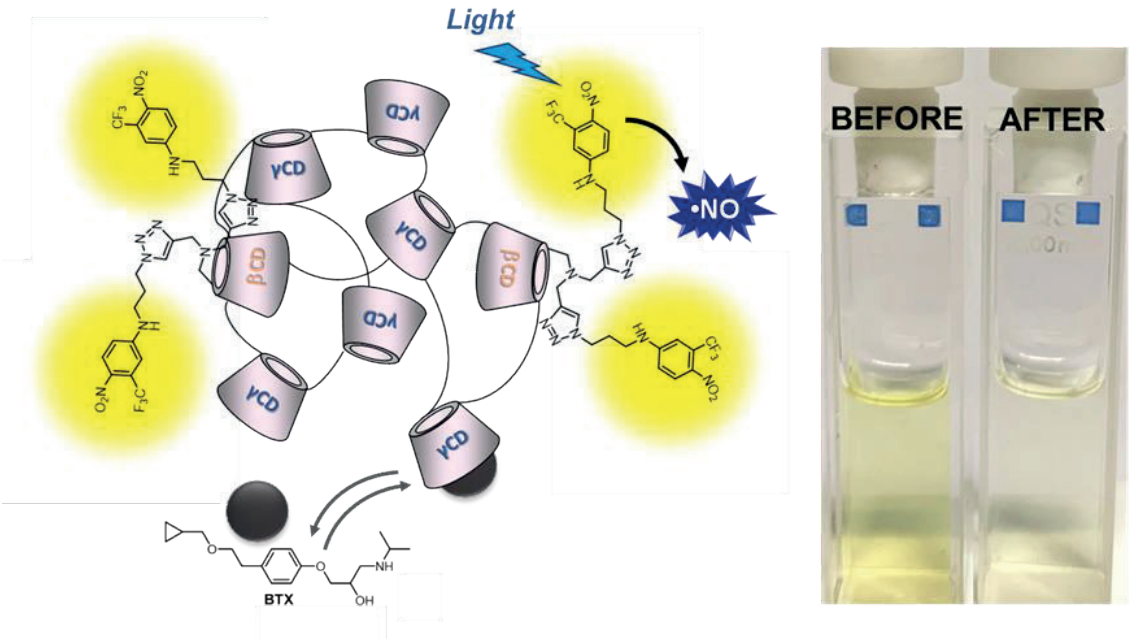


Fig. 1 – Schematic representation for the functioning of the cyclodextrin-based copolymer.

The system presents remarkable advantages over the isolated photoactivatable units not linked to the CDs scaffold. It shows excellent water solubility, good stability in the dark at room temperature and the capability to release a biologically relevant amount of NO under the control of visible light. In view of the well-known vasodilator properties of NO,[1,2] the present work may open intriguing prospects for biological studies on formulations for ocular application against glaucoma, addressed to explore the combinatory effect of BTX and NO rapidly released upon environmental light. These studies are currently underway in our laboratories.

References

1) Nitric Oxide: Biology and Pathobiology, (ed. L. J. Ignarro), Elsevier Inc, 2010.
2) S. Sortino, Light- Responsive Nanostructured Systems for Applications in Nanomedicine. Springer, 2016.

Acknowledgements. We thank the MIUR (Italy) for the Industrial PhD grant (DOT-1308125-1) to M.S and AIRC – Italian Association for Cancer Research (IG-198559) for financial support.

IL-7.2.2

Worldwide prevalence and incidence of the photodermatoses

Burfield L[1] Rutter KJ[1,2], Neale RE[3], Rhodes LE[1,2].
1) Centre for Dermatology Research, School of Biological Sciences, University of Manchester, Manchester, UK;
2) *Photobiology Unit, Dermatology Centre, Salford Royal NHS Foundation Trust, Manchester, UK;*
3) Population Health Department, QIMR Berghofer Medical Research Institute, Queensland, Australia.

The photodermatoses are a diverse group of skin diseases caused or exacerbated by solar radiation. Despite their significant impact on quality of life, psychosocial welfare and employment[1], little is known regarding their worldwide prevalence and incidence.

This study aimed to systematically review the literature to establish what is known regarding prevalence and incidence of the photodermatoses in adult and paediatric populations. Searches of three electronic medical databases (Medline, CINAHL, Embase) were performed to identify original studies reporting the prevalence and/or incidence of the photodermatoses. Publications that did not include data on the numerator and denominator populations were not eligible for inclusion.

Twenty-six studies were identifed and included: 15 reported the prevalence of photodermatoses based on studies of samples of the general population, and 11 reported on their prevalence and/or incidence from national or international registry/diagnostic laboratory data. The general population studies concerned polymorphic light eruption (PLE; 9 studies), unspecified photosensitivity (2 studies), actinic prurigo (2 studies), juvenile spring eruption (1 study) and variegate porphyria (1 study), while registry studies reported on cutaneous porphyrias (8 studies) and selected genophotodermatoses (2 studies).

While population-based studies were scarce for most individual diseases, they suggest a high prevalence of photodermatoses at wide-ranging world locations. This includes a large paediatric study in South Sinai reporting unspecified photosensitivity in 4% of children. The prevalence of PLE ranged from 0.65% to 21.4%, with a strong positive correlation (r=0.776) between prevalence and distance from the equator. Studies of actinic prurigo in Chimila Indians and Inuit populations showed this was prevalent in both groups (8% and 2% respectively), while no studies were available in other ethnic groups. Heterogeneity of study design and lack of standardised diagnostic criteria hampered comparison between studies. Overall, study quality was assessed as high (3 studies), medium (11 studies) and low (2 studies).

Published registry data from 12 European countries demonstrated porphyria cutanea tarda is the most prevalent porphyria in Europe. Combined data from DNA repair diagnostic centres in 5 European countries gave incidence rates for xeroderma pigmentosum, Cockayne syndrome and trichothiodystrohy of 2.3, 2.7 and 1.2 per million births, respectively.

Crucially, we found prevalence and/or incidence studies are lacking for many of the photosensitivity diseases. Further high-quality population-based studies, employing standardised diagnostic criteria, are required to better understand the disease burden of the photodermatoses.

References

1) Rutter KJ, Ashraf I, Cordingley L, Rhodes LE. Quailty of life and psychological impact in the photodermatoses: a systematic review. *Br J Dermatol.* 2020; **182**: 1092-102.

Acknowledgements. KR and LER acknowledge the NIHR Manchester Biomedical Research Centre.

IL-7.2.3

Consensus in Photodiagnostic Services in the UK and Republic of Ireland

Sally Ibbotson on behalf of the British Photodermatology Group Working Party

Photobiology Unit, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK

Abnormal photosensitivity can have a major adverse effect on patients' lives and prompt, accurate diagnosis is the underlying basis of optimal management. Whilst photodiagnostic investigations are not required in all cases, such as in straightforward polymorphic light eruption, they are invaluable for patients with severe or atypical disease as it is often impossible to confirm abnormal photosensitivity on clinical grounds alone. Photodiagnostic investigations should therefore be available for patients with suspected photosensitivity diseases. However, the requirements for specific equipment, expertise and techniques dictates that such facilities are only available through tertiary specialist photobiology units.

The last review of Photodiagnostic Services in the UK was almost 30 years ago and therefore, through a British Photodermatology Group/British Association of Dermatologists (BPG/BAD) Workshop we critically appraised photodiagnostic techniques in the UK and Republic of Ireland, seeking to establish areas of consensus practice, acceptable variation and priorities for service, research and educational development. The Workshop findings based on the 12 photodiagnostic units reviewed, reinforced the importance of availability of expertise and specialised staff, with emphasis on the consultant-led multidisciplinary team, with dedicated photophysics input. Specialised equipment is essential for the detailed investigation of patients with suspected photosensitivity disorders and further clinical studies are required to assist with development of commercial optical radiation phototesting equipment to required standards. Narrow waveband phototesting, particularly monochromator phototesting, is the Gold Standard investigation with respect to establishing the action spectrum and degree of photosensitivity and assessing treatment response and natural history of photosensitivity. However, this is often also supplemented by broadband phototesting to confirm objective photosensitivity. Prioritisation of defining normal range phototesting erythemal responses (defined as the threshold minimal erythema dose; MED) in patients of skin phototype IV-VI was emphasised as these data are currently lacking. Most photodiagnostic units offer iterative photoprovocation testing but methodologies and assessment of reaction patterns are not standardised and this was identified as a key area for further study. Minimal erythema dose (MED) testing to phototherapeutic sources (eg. NBUVB), patch testing and photopatch testing to define coexistent allergens and photoallergens and other investigations to exclude less common causes of photosensitivity, such as lupus, cutaneous porphyrias and genophotodermatoses, should be available to all photodiagnostic services. We identified a relative lack of defined training programmes for photophysicists, clinical scientists and allied health care professionals in the field of photodiagnostics and identified this as a priority area for educational and training development moving forward. The information obtained through this review assists with the development of minimum standards for photodiagnostic services and emphasises the importance of collaboration, data sharing and multidisciplinary working to facilitate deep phenotyping and improved understanding of the photosensitivity diseases.

OC-7.2.4

Automated real-time monitoring of intracellular protoporphyrin IX synthesis in a live-cell plate reader

Christian Oberdanner[1], Nicole Eggenhofer[1], Kristjan Plaetzer[2]

1) Tecan Austria GmbH, Grödig, Austria; 2) Laboratory of Photodynamic Inactivation of Microorganisms, University of Salzburg, Salzburg, Austria

Over the last couple of years the importance of 5-Aminolevulinic acid (5-ALA)-based protoporphyrin IX (PPIX) PDD and PDT has not decreased. However, the dynamics of intracellular PPIX formation after ALA application is highly critical for a successful diagnosis and treatment (1). Therefore, time-resolved *in vitro* measurements of the intracellular PPIX formation represent the bedrock for identification of further target diseases or testing novel ALA derivates.

This presentation points out the convincing advantages of automation of such analysis. A431 human epidermoid carcinoma cells were stably transfected with green fluorescent protein (GFP) and automatically seeded into 96- or 384-well microplates using the dispensing unit of a Tecan live cell multimode reader. Automated determination of the GFP fluorescence revealed excellent cell growth in the atmospheric chamber of the device and provided complete data sets without night gaps. Standardized replacement of the growth medium after overnight incubation with a microplate washer minimized the experimental error of this experimental step.

After addition of ALA the intracellular PPIX synthesis was automatically recorded by the fluorescence of this compound, again gaining advantage from the reader's atmospheric control features. The results demonstrate that the peak intracellular PPIX-level, which represents the optimal point in time for tumour therapy, is a complex function of the incubation concentration and -period. They furthermore provide clear evidence for the benefits of automation for investigation of multi-parametric cellular processes: (i) high-resolution complete data sets, (ii) low experimental error, (iii) high costs-effectiveness due to employment of 384 well microplates and (iv) minimized time consumption.

1) Malik, Z. Fundamentals of 5-aminolevulinic acid photodynamic therapy and diagnosis: An overview. Transl. Biophotonics 2020, 2, e201900022

IL-7.2.5

Pathways of polymorphic light eruption: from potential triggers to immune response

Peter Wolf

Medical University of Graz, Department of Dermatology, Research Unit for Photodermatology, Graz, Austria

Polymorphic light eruption (PLE) is the most common and prevalent photodermatosis, particularly among young women in temperate climates. We recently reported that the onset of disease usually occurred between young adulthood and middle age, at a mean age of 25.9 years in females and 28.1 years in males. Moreover, we observed that in most cases (74% of females and 65% of males), the onset of PLE symptoms occurred between the ages of 15 and 40 years.[1] Our recent registry analysis indicated that though the disease improved in a substantial number of patients (i.e., 77% of females and 59% of males) over the years, it most often takes a long-term course.[1] It took 20 (95%CI, 13-26) years until 25% of patients had normalized from PLE and it took 25 (95%CI, 18-41) years until one third of patients had normalized from PLE.

Recently, the pathophysiology of PLE has become much better understood. This includes concurrent resistance against induction of UV-induced immune suppression, linked to an imbalanced micromilieu marked by low levels of IL-4, IL-10, and TNF-alpha; failure of Langerhans cell emigration from the skin and neutrophilic infiltration into the skin; disturbances in Treg levels and function; and potential involvement of CD11b/IL-31+ cells, and mast cells. Also better understood now are the therapeutic mechanisms of photohardening and other preventive measures.

Recently, as in many other conditions the skin's microbiome came into the focus of research in PLE. For instance, we have observed that the skin's microbiota can protect against UV-induced immune suppression. Like other investigators we have also seen abnormalities in the expression of antimicrobial peptides (AMP) in the skin of PLE. We recently hypothesized that a potential link between disturbances in the microbiome and UV-induced immune suppression may play a role in the initiation and pathophysiology of the disease and are currently testing this hypothesis in an ongoing experimental study.[2] Indeed, the quality and/or quantity of the microbiota present on human skin does vary during different periods of life and age-dependency may be linked sex hormones.

References

1. Alexandra Gruber-Wackernagel, Tanja Schug, Thomas Graier, Franz J. Legat, Hanna Rinner, Angelika Hofer, Franz Quehenberger, Peter Wolf. Long-term course of polymorphic light eruption: a registry analysis. Frontiers in Medicine 2021, doi: 10.3389/fmed.2021.694281

2. The significance of the skin microbiome n the pathophysiology of polymorphic light eruption (PLE_microbiome), 2021, https://clinicaltrials.gov

Acknowledgements

This work was supported by the Austrian Science Fund FWF (W1241).

Correspondence to: peter.wolf@medunigraz.at

IL-7.2.6

The Psychological basis of photoprotective behaviour in X.P.: implications for improving photoprotection in other patient groups

Sarkany RPE[1], Norton S [3], Canfield M [3], Morgan M [2], Foster L [1], Johnstone, K[2], Sainsbury K[4], Araujo-Soares V. [4], Wulf H-C [5], Weinman J [2], Walburn J [2]

1. Xeroderma Pigmentosum Unit, Guys and St Thomas' NHS Foundation Trust; 2.School of Cancer and Pharmaceutical Sciences, King's College London, UK; 3.Health Psychology Section, Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK; 4.Institute of Health & Society, Faculty of Medical Sciences, Newcastle University,; 5.Dermatology Department, Bispjeberg Hospital, Copenhagen

Photoprotection is central to the management of patients with photosensitive skin diseases and those at high risk of skin cancer. It is also crucial to Public Health planning since it is the only way to reduce population skin cancer rates. Photoprotection involves a group of behaviours all of which reduce UV exposure. These include the use of sunscreens and photoprotective clothing and hats and the use of shade, as well as adaptation of the timing and duration of going outdoors.

The results of previous educational and other interventions to improve photoprotection have generally been disappointing. It has recently been shown that, even after a diagnosis of malignant melanoma, patients improve their photoprotection for an average of only 18-24 months before reverting to their previous behaviour.

We have recently studied 36 patients with Xeroderma Pigmentosum. In this disease, stringent photoprotection is critical to health and life expectancy. We measured the daily dose of UV to the skin on the face and identified a huge range of UV exposure in these patients, from very low up to levels similar to those in healthy individuals. We then succeeded in identifying the factors creating these differences in photoprotection behaviour. We found that psychological and social factors are responsible for at least as much of the variation between patients as can be attributed to demographic or clinical differences. Most of the psychological factors which we have identified as correlating with poor photoprotection are a group of factors including patients' beliefs and perceptions about XP and its treatment, which are known to contribute to poor treatment compliance in other diseases. The way in which patients accommodate the disease into their 'identity' has turned out to play a crucial role in determining how well our XP patients photoprotect. We went on to use these findings to design a Behaviour Change Intervention ('XPAND') to specifically address these psychosocial factors, and our controlled clinical trial has shown that the Intervention is effective at improving photoprotection behaviour.

This systematic approach to identifying and 'treating' the underlying psychological causes of photoprotection is effective in this rare disease. This raises the possibility that this approach may succeed where previous attempts to improve photoprotection in other groups have failed, and we are planning to move on to apply this approach to other groups of patients, especially those at high risk of skin cancer.

Acknowledgements. This work was supported by a grant from the National Institute for Health Research.

IL-7.2.7

Sun exposure and protection guidance following COVID-19 lockdowns – considerations for the public & photosensitive patients

Yolanda Gilaberte
Miguel Servet University Hospital, Department of Dermatology,IIS Aragón, Zaragoza, Spain.

Our relationship with the environment has been affected by the COVID-19 pandemic, especially in terms of spending time outdoors. The lookdown has created a situation in which most people have had less exposure to sunlight than usual. Hence, cutaneous synthesis of vitamin D in the skin, which is our greatest source of vitamin D, has been hampered by the lockdown, during which many people have not been exposed to sunlight in several weeks. Low vitamin D levels have been demonstrated to be associated to a more severe COVID-19 illness.
On the other hand, our group has found and association between UV index and the incidence of COVID-19 infection in cities along the whole latitude of Spain.
Taking all of this into consideration, a healthy exposure is achieved by ensuring that the dose of UV radiation is sufficient to obtain the positive effects of sunlight on our body minimizing the risk of damage resulting from overexposure. In this sense, UVI values can be used to plan the activities outdoors.
The Spanish Group of Photodermatology have made some recommendations in order to get a safe exposure to the sunlight in the context of the COVID-19 pandemic. Photoprotective measures are recommend when the incidence of solar UV is high, since the doses of UV necessary to produce vitamin D are low and can be reached without getting erythema. Gradual exposure to sunlight is recommended in order to facilitate adaptation of the skin and favour natural defence mechanisms. Use of shade, clothes, hats and sunscreens are recommended as usual. Some patients with photodermatoses have suffered less intense clinical manifestations during the pandemic due to the recommendations of stay at home and the use of face mask which provide a very good photoprotection. However, their photoprotective measures should be very high when they go out. Patients who had COVID-19 receiving medication should take special care and ask about the potential risk of photosensitivity.

References

1) Aguilera J, de Galver MV, Aguilera P, de Troya-Martín M, Gilaberte Y, del Grupo Español de Fotobiología de la AEDV. Recommendations on sun exposure and photoprotection following easing of the COVID-19 pandemic lockdown: Spanish Photobiology Group of the Spanish Academy of Dermatology and Venereology. Actas Dermosifiliogr 2020;111:799-801.

OC-7.2.8

Solar Urticaria-real life data of an international support group

Teresa Hipfl, Donja Homayoon, Angelika Hofer
Medical University of Graz, Department of Dermatology and Venerology, Graz, Austria;

Solar urticaria belongs to the idiopathic photodermatoses and is triggered by diverse wavelengths of UV-light or visible light. Within minutes exposure to sunlight leads to itchy redness and wheals. Therefore, the disease has a significant impact on the patient's normal daily life. [1,2]
This paper aims to provide inside into the patient cohort of an international urticaria solaris support group (n >300 people), focusing on their medical care, as well as on the aspects of quality of life limitations.

A comprehensive questionnaire was provided online to all members of the urticaria solaris support group. Questions were asked about the spectrum of therapies, their effects and side effects and the impact on quality of life due to the disease. Of 149 participants who had completed the questionnaire 57 members (51 women, 6 men; living in 12 different countries) who reported that the diagnosis urticaria solaris had been confirmed by a doctor, were included in this study.

The most commonly prescribed medications were antihistamines (47 out of 56 patients; 83.9%). However, only 12.8% of these patients reported a good effect with this treatment. Better improvement was seen with light therapy or treatment with the monoclonal antibody omalizumab, good results in 37.5% versus 42.9%, respectively. However, treatment with omalizumab was only used in 14 out of 55 patients (25.5%) from 5 different countries (UK, US, IL, CA, AT).
Most patients (77.8%) reported that they felt medically undersupplied because the symptoms could not be sufficiently improved under therapy so far. Besides, the majority of the affected persons (68.7%) stated a severely impaired quality of life despite therapy. Nearly 70% of the patients had the impression that their disease was not sufficiently well known in society and that there was therefore no understanding of the severe impairment of quality of life. However, being a member of the urticaria solaris support group, all affected feel that they are taken seriously. While nearly half of those affected would welcome psychological support in dealing with the disease, less than a quarter of these people have actually received psychological help.

In conclusion, solar urticaria patients suffer greatly from this disease. The quality of life is markedly reduced and most of the patients are unsatisfied with their current therapy due to lack of efficacy. Better access to effective treatment options and also to psychological care should be achieved. Especially in rare diseases such as urticaria solaris, international networking to improve treatment strategies is urgently needed.

References
1) Harris BW, Badri T, Schlessinger J. Solar Urticaria. StatPearls. Treasure Island (FL): StatPearls Publishing, 2020
2) Levi A, Enk CD, Snast I. Solar Urticaria. Current Dermatology Reports, 2019

P-7.2.9

In vitro photo(geno)toxicity assessment of gefitinib and its metabolites.

Meryem El Ouardi[1,2], Lorena Tamarit[1,2], Ignacio Vayá[1,2], M.A Miranda[1,2], Inmaculada Andreu[1,2].

1) Departamento de Química/Instituto de Tecnología Química UPV-CSIC, Universitat Politècnica de València, Camino de Vera s/n, 46022 València, Spain.

2) Unidad Mixta de Investigación Universitat Politècnica de València-Instituto de Investigación Sanitaria (IIS) La Fe, Hospital Universitari i Politècnic 106, 46026, Valencia, Spain.

Gefitinib (GFT) is an epidermal growth factor receptor (EGFR) inhibitor extensively used in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) [1]. In general, GFT is a well-tolerated drug, however, a considerable number of patients have experienced dermatological side effects, being skin rash the most common reaction (63% of patients)[2]. Most of EGFR-TKIs display similar adverse reactions although not fully studied. Interestingly, drugs containing the quinazoline moiety are known to produce skin photosensitivity. In this context, it has been recently reported that lapatinib, another tyrosine kinase inhibitor, can induce phototoxic disorders.[3].

In addition, the pharmacokinetics of gefitinib suggests a clearance mainly through hepatic metabolism, where *O*-Desmethyl gefitinib (DMT-GFT) is the major metabolite identified in human plasma [4]. Another two compounds were formed through Phase I metabolism in human liver microsomes, the oxidative defluorination (4-Defluoro-4-hydroxy gefitinib, DF-GFT) and the loss of morpholine ring (O-Desmorpholinopropyl gefitinib, DMOR-GFT) [5,6]. Despite the aim of metabolism is to improve the ease with which substances can be excreted by changing the molecular structure, in some cases, this leads to the production of new compounds with higher toxicity. Based on that and the in vitro phototoxicity results performed by AstraZeneca for Iressa® that have demonstrated a possible phototoxicity effect of this drug, it would be interesting to approach this issue by performing in vitro assays in human skin cells with GFT and its main metabolites.

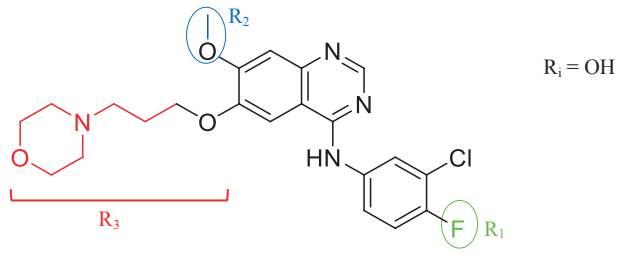


Fig. 1 – Gefitinib, GFT; 4-Defluoro-4-hydroxy gefitinib, DF-GFT (R_1); O-Desmethyl gefitinib, DMT-GFT (R_2) and O-Desmorpholinopropyl gefitinib, DMOR-GFT (R_3)

Thus, it is important to study the phototoxic potential of these compounds and to compare the behavior of the metabolites to GFT. For this purpose, a Neutral Red Uptake assay (NRU) was performed in human keratinocytes (HaCaT) for both GFT and its metabolites. The dose-response curves displayed in Fig.2 revealed a significant phototoxic effect for both GFT and DMOR-GFT. Remarkably, this metabolite showed higher phototoxic potential than the parent drug, whereas DMT-GFT exhibited lesser phototoxicity than GFT. In contrast, DF-GFT did not display any phototoxic results. To understand the underlying photosensitizing mechanism of GFT and its metabolites it has been evaluated the lipid peroxidation, protein photooxidation, and cellular DNA damage. As shown in Fig.2, alkaline comet assay for both GFT and DMT-GFT displayed a considerable photogenotoxic effect, whereas DMOR-GFT did not trigger significant DNA damage. Contrary, DMOR-GFT showed a huge enhancement in both protein and lipidic photooxidation. Moreover, GFT also promoted a substantial protein photooxidation, however, DMT-GFT was observed to damage neither proteins nor lipids. The photosafety evaluation of pharmaceuticals should be performed not only on the drug but also on its main metabolites, taking into consideration that the phototoxic properties could be enhanced after drug metabolism as demonstrated in this study.

P-7.2.9

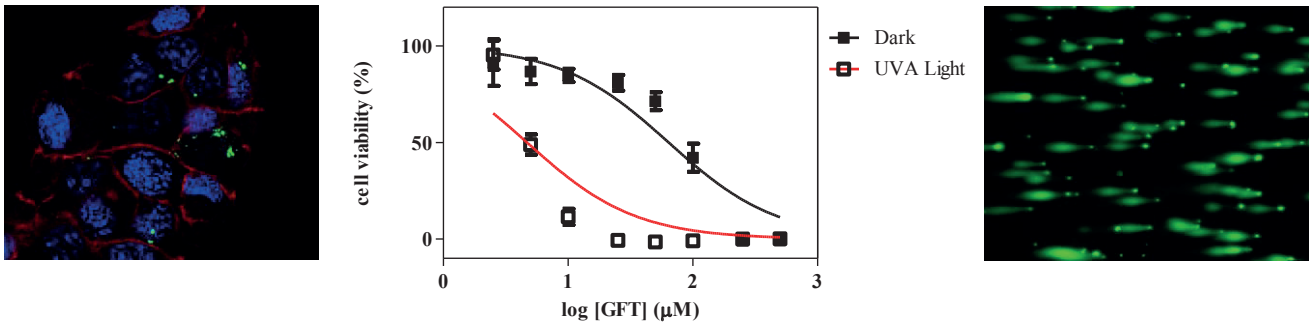


Fig. 2 – From left to right: Intracellular colocalization of GFT in HaCaT cells by confocal microscopy, HaCaT Neutral Red Uptake assay dose-response curves for GFT, Alkaline Comet assay of HaCaT cells treated with GFT.

References

1) Ghafoor Q, Baijal S, Taniere P, O'Sullivan B, Evans M, Middleton G. Epidermal Growth Factor Receptor (EGFR) Kinase Inhibitors and Non-Small Cell Lung Cancer (NSCLC) - Advances in Molecular Diagnostic Techniques to Facilitate Targeted Therapy. *Pathol Oncol Res.* 24(4):723-731, 2018.
2) Annunziata MC, Ferrillo M, Cinelli E, Panariello L, Rocco D, Fabbrocini G. Retrospective Analysis of Skin Toxicity in Patients under Anti-EGFR Tyrosine Kinase Inhibitors: Our Experience in Lung Cancer. *Open Access Maced J Med Sci.* 7(6):973-977, 2019.
3) García-Lainez G, Vayá I, Marín MP, Miranda MA, Andreu I. In vitro assessment of the photo(geno)toxicity associated with Lapatinib, a Tyrosine Kinase inhibitor. *Arch Toxicol.* 95(1):169-178, 2021
4) Frampton JE, Easthope SE. Gefitinib: a review of its use in the management of advanced non-small-cell lung cancer. *Drugs.* 64(21):2475-2492, 2004.
5) McKillop D, McCormick AD, Miles GS, et al. In vitro metabolism of gefitinib in human liver microsomes. *Xenobiotica.* 34(11-12):983-1000, 2004.
6) Alfieri RR, Galetti M, Tramonti S, et al. Metabolism of the EGFR tyrosin kinase inhibitor gefitinib by cytochrome P450 1A1 enzyme in EGFR-wild type non small cell lung cancer cell lines. *Mol Cancer.* 10:143, 2011.

Acknowledgements. This work was financially supported by Universitat Politècnica de València (Subprograma 1, PAID-01-19), the MICINN (FPU19/00048, BEAGAL 18/00211) and Carlos III Institute of Health (Grant PI16/01877).

IL-7.3.1

Porphysome Nanotechnology: From Discovery toward First-in-Patients

Michael Valic[1], Juan Chen[1], Gang Zheng[1,2]
1) Princess Margaret Cancer Centre, University Health Network, Toronto, Canada; 2) University of Toronto, Department of Medical Biophysics, Toronto, Canada

Porphysomes are intrinsically multifunctional liposome-like nanoparticles self-assembled from a single porphyrin-lipid building block. High-density porphyrin packing in the nanoparticle bilayer enables light absorption and conversion to heat with extremely high efficiency, making porphysomes ideal candidates for photothermal therapy and photoacoustic imaging. Upon nanostructure dissociation during cell uptake, the fluorescence and photodynamic activity of the porphyrin monomers is restored. In addition, metal ions can be directly incorporated into the porphyrin building blocks, thus unlocking porphysome's potential for PET and MRI. To translate porphysomes from bench to bedside, we have embarked a 10-year journey where we first validated porphysome's utilities preclinically in different tumor types, models and animal species. We then, with purely academic resources, completed the scale up manufacturing of both porphyrin-lipid building blocks and nanoparticle formulation, optimized the cGMP methods and processes. In parallel, we completed the metabolism and phototoxicity studies and demonstrated porphysome safety profiles in rodents, dogs and non-human primates. In addition, pharmacokinetics and disposition of porphysomes were established in preclinical animals and subsequently extrapolated to human. We anticipate the launch of the first-in-human porphysome study shortly. The simple yet intrinsic multimodal nature of porphysome nanotechnology popularize the one-for-all nanomedicine and confers its high clinical translation potential.

IL-7.3.2

Biological models for in vitro and in vivo imaging, PDT, drug or siRNA delivery

Magali GARY-BOBO[1]
1) IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

Currently, the development of *in vitro* et *in vivo* models in accordance with the 3Rs rules (reduction, replacement, refinement) is of great importance. In fact, research activities need such robust and easy-to-use technics to demonstrate the imaging and therapeutic potential of innovative anticancer molecules or nanoparticles. We are a biologists team specialized in the study of photoactivable nanoparticles for fluorescent imaging, photodynamic therapy and drug or siRNA delivery for anticancer application. In our group, the biological effects of these nanoparticles are studied first on human cancer cell lines in culture (in 2 dimensions or in spheroids) and could also be analyzed on zebrafish embryos. The robustness of these models allows us to quickly carry out proof of concept studies of nanoparticles exhibiting a wide range of biomedical properties.

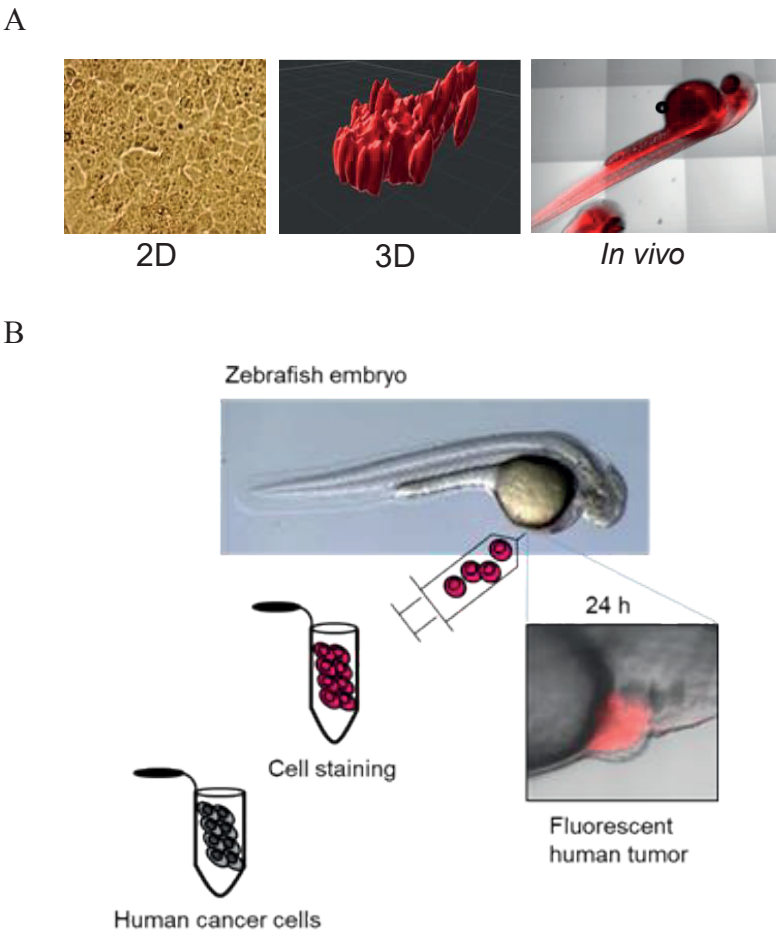


*Fig. 1 – (**A**) In vitro and in vivo biological models. Cell culture in 2 dimensions (2D), in spheroids (3D) and zebrafish embryos. (**B**) Scheme of human cancer cells injection in zebrafish embryo.*

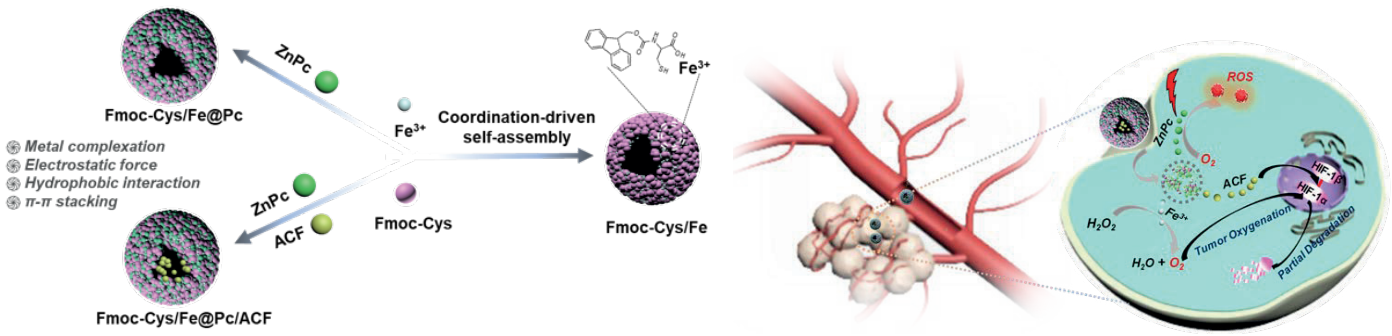
IL-7.3.3

Fe^{3+}-Driven Assembly of Catalase-Like Supramolecular Photosensitizing Nanozymes for Combating Hypoxic Tumor

Yongxin Li[1], Pan Sun[2], Luyang Zhao[3], Xuehai Yan[3], Dennis K. P. Ng[4], and **Pui-Chi Lo*,[1]**

1) Department of Biomedical Sciences, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China; 2) CAS Key Laboratory of Green Process and Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China; 3) National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China; 4) Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.

Photodynamic therapy (PDT) has emerged as a compelling treatment modality for a range of superficial and localized cancer.[1] It involves excitation of a photosensitizer through light irradiation followed by the generation of reactive oxygen species (ROS), in particular singlet oxygen, to cause cellular damage at the tumor site. However, PDT still suffers from two major hurdles that limit its clinical application, namely the low selectivity of the photosensitizers toward cancer cells and the insufficient oxygen concentration at hypoxic tumor. Being inspired by the naturally occurring catalases which can convert H_2O_2 into water and oxygen via the Fenton reaction, we report herein a facile approach to assemble catalase-like photosensitizing nanozymes with a self-oxygen-supplying ability.[2] The process involved Fe^{3+}-driven self-assembly of fluorenylmethyloxycarbonyl-protected cysteine (Fmoc-Cys) in the presence of a zinc(II) phthalocyanine-based photosensitizer (ZnPc) and the hypoxia-inducible factor 1 (HIF-1) inhibitor acriflavine (ACF). Molecular self-assembly provides a facile and environmental green approach to encapsulate photosensitizers and other therapeutic components into nanoparticles that can promote passive tumor targeting via the enhanced permeability and retention effect. The nanovesicles Fmoc-Cys/Fe@Pc and Fmoc-Cys/Fe@Pc/ACF were prepared, which could be disassembled intracellularly. The released Fe^{3+} could catalyze the transformation of H_2O_2 enriched in cancer cells to oxygen efficiently, thereby ameliorating the hypoxic condition and promoting the photosensitizing activity of the released ZnPc. With an additional therapeutic component, Fmoc-Cys/Fe@Pc/ACF exhibited higher in vitro and in vivo photodynamic activities than Fmoc-Cys/Fe@Pc, demonstrating the synergistic effect of ZnPc and ACF.



References
1) D. E. J. G. J. Dolmans, D. Fukumura, R. K. Jain, *Nat. Rev. Cancer* **2003**, *3*, 380-387.
2) Y. Li, P. Sun, L. Zhao, X. Yan, D. K. P. Ng, P.-C. Lo, *Angew. Chem. Int. Ed.* **2020**, *59*, 23228-23238.

Acknowledgements. This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region (Ref. No. 11300719).

OC-7.3.4

Covalently cross-linked tetrafunctionalized *m*-THPC chitosan hydrogels as drug delivery platforms in the treatment of melanoma.

Piotr Gierlich[1,2], Claire Donohoe[1,2], Kevin Behan[4], Daniel Kelly[4], Lígia C. Gomes-da-Silva[2], Mathias O. Senge[1,3]

1) Medicinal Chemistry, Trinity Translational Medicine Institute, Trinity Centre for Health Sciences, Trinity College Dublin, The University of Dublin, St. James's Hospital, Dublin 8, Ireland; 2) Department of Chemistry, University of Coimbra, Rua Larga, 3004-535 Coimbra, Portugal; 3) School of Chemistry, SFI Tetrapyrrole Laboratory, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Dublin 2, Ireland; 4) Trinity Centre for Biomedical Engineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, 152-160 Pearse Street, Dublin 2, Ireland.

Photodynamic therapy (PDT) is an anti-cancer treatment method, which uses the combined effect of a photosensitizing drug (as pro-drug activating agent), light, and oxygen to cause selective damage to target tissue.[1] The second generation photosensitizer (PS) 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin (*m*-THPC) is a widely characterized, clinically tested, and commercially available drug with the market formulation known as Foscan.[2] In order to develop advanced treatment modalities there is a need for improved drug delivery platforms. Hydrogels, which have been investigated as effective drug delivery systems, can prevent PSs aggregation and offer significant potential as drug carries due to the ability to swell in aqueous media.[3] Chitosan (CS), a natural polysaccharide, is a suitable biodegradable material for hydrogel formulation and has been used in pharmaceutical applications on account of its lack of toxicity and good biocompatibility.[4]

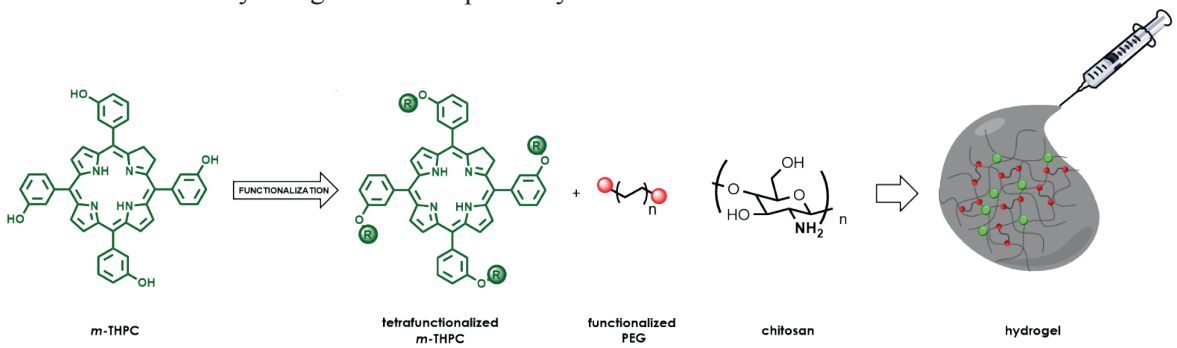


Fig. 1 – Schematic representation of the hydrogel synthesis.

In the present work, *m*-THPC was used as a starting point to obtain a library of compounds aimed at overcoming PS limitations while maintaining its photophysical and clinical properties. Substitution, esterification and Sonogashira coupling reactions were employed to modify the *m*-THPC skeleton providing aldehyde and carboxylic acid moieties used as a suitable synthetic handle for covalent cross-linking in the formation of CS hydrogels. Injectable, self-healing properties of hydrogels were confirmed macroscopically and by the rheological analysis. Next, tetrafunctionalized *m*-THPC derivatives were tested *in vitro* against melanoma (B16F10) cancer cells. *In vivo* biodistribution of the released PSs was studied using fluorescence imaging technique. Prepared hydrogel formulations are expected to allow for a local, injectable administration towards melanoma tumors, while preventing from systemic side effects related to the *m*-THPC treatment and are under ongoing *in vivo* evaluation.

References

1) 1. T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, *J. Nat. Cancer Inst. 90*, 889–905, 1998; 2) M. O. Senge, J. C. Brandt, *Photochem. Photobiol. 87*, 1240–1296, 2011; 3) B. Khurana, P. Gierlich, A. Meindl, L. C. Gomes-da-Silva, M. O. Senge, *Photochem. Photobiol. Sci. 18*, 2613–2656, 2019; 4) M. Dash, F. Chiellini, R. M. Ottenbrite, E. Chiellini, *Prog. Polym. Sci. 36*, 981–1014, 2011.

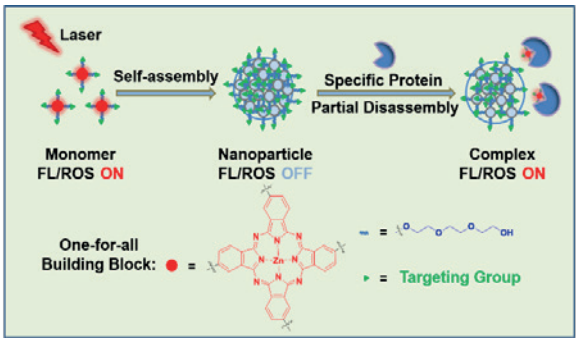
Acknowledgements. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement n°764837 and supported by grants from Science Foundation Ireland (IvP 13/IA/1894).

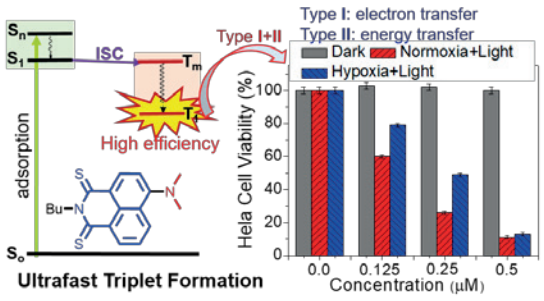
IL-7.3.5

Recent Progress on Activatable Photosensitizers and Heavy Atom Free Photosensitizers

Juyoung Yoon
Department of Chemistry and Nanoscience, Ewha Womans University, Seoul 03760, Korea

Switchable phototheranostic nanomaterials are of particular interest for specific biosensing, high-quality imaging, and targeted therapy in the field of precision nanomedicine.[1] Here, we develop a "one-for-all" nanomaterial (NanoPcTBs) that self-assembles from flexible and versatile phthalocyanine building blocks.[2] Fluorescence and reactive oxygen species (ROS) generation could be triggered depending on a targeted, protein-induced, partial disassembly mechanism, which creates opportunities for low-background fluorescence imaging and activatable photodynamic therapy (PDT). We also reported a facile strategy to directly assemble a phthalocyanine photosensitizer (PcS) with an anticancer drug mitoxantrone (MA) to form uniform nanostructures (PcS-MA), which have the capability of undergoing nucleic acid-responsive disassembly.[3] On the other hand, the *in vivo* specific binding between albumin and PcS, arising from the disassembly of injected NanoPcS, was recently confirmed using an inducible transgenic mouse system.[4] In a recent investigation, we devised a novel molecular design approach to create heavy-atom-free photosensitizers for enhanced photodynamic therapy under hypoxia conditions.[5] The thionaphthalimides display dramatically enhanced quantum yields for photosensitized singlet oxygen formation (Φ_Δ ~ 1.00, in air-saturated acetonitrile).[6]





References

1) H.-B. Cheng, B. Qiao, H. Li, J. Cao, Y. Luo, K. M. K. Swamy, J. Zhao, Z. Wang, J. Y. Lee, X.-J. Liang, J. Yoon, Protein-Activatable Diarylethene Monomer as a Smart Trigger of Noninvasive Control Over Reversible Generation of Singlet Oxygen: A Facile, Switchable, Theranostic Strategy for Photodynamic-Immunotherapy. J. Am. Chem. Soc., 143, 2413-2422, 2021.

2) X. Li, C-y. Kim, S. Lee, D. Lee, H.-M. Chung, G. Kim, S.-H. Heo, C. Kim, K.-S. Hong, J. Yoon, Nanostructured Phthalocyanine Assemblies with Protein-Driven Switchable Photoactivities for Biophotonic Imaging and Therapy. J. Am. Chem. Soc., 139, 10880-10886, 2017.

3) X. Li, S. Yu, D. Lee, G. Kim, B. Lee, Y. Cho, B.-Y. Zheng, M.-R. Ke, J.-D. Huang, K. T. Nam, X. Chen, J. Yoon, Facile Supramolecular Approach to Nucleic-Acid-Driven Activatable Nanotheranostics That Overcome Drawbacks of Photodynamic Therapy. ACS Nano, 12, 681-688, 2018.

4) X. Li, S. Yu, Y. Lee, T. Guo, N. Kwon, D. Lee, S. C. Yeom, Y. Cho, G. Kim, J.-D. Huang, S. Choi, K. T. Nam, J. Yoon, In Vivo Albumin Traps Photosensitizer Monomers from Self-Assembled Phthalocyanine Nanovesicles: A Facile and Switchable Theranostic Approach. J. Am. Chem. Soc., 141, 1366-1372, 2019.

5) V.-N. Nguyen, Y. Yim, S. Kim, B. Ryu, K. M. K. Swamy, G. Kim, N. Kwon, C-Y. Kim, S. Park, J. Yoon, Molecular Design of Highly Efficient Heavy-Atom-Free Triplet BODIPYs for Photodynamic Therapy and Bioimaging. Angew. Chem. Int. Ed., 59, 8957-8962, 2020.

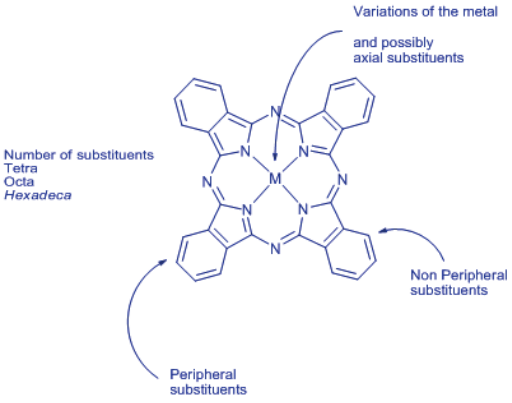
6) V.-N. Nguyen, S. Qi, S. Kim, N. Kwon, G. Kim, Y. Yim, S. Park, J. Yoon, An Emerging Molecular Design Approach to Heavy-Atom-Free Photosensitizers for Enhanced Photodynamic Therapy under Hypoxia. J. Am. Chem. Soc., 141, 16243-16248, 2019.

IL-7.3.6

Improving the photodynamic efficiency of phthalocyanine with nanotechnology

Fabienne Dumoulin
Acıbadem Mehmet AliAydınlar University, Department of Medical Engineering,
Kayışdağı Cad. 34, Ataşehir, Istanbul, Turkey

The maximum absorption of phthalocyanines is centered around 700 nm, which is a significant advantage for photosensitisers of anti-cancer photodynamic therapy.[1] In addition, phthalocyanines can be easily functionalized and their photoproperties can be tailored with an appropriate substitution and metalation pattern.[2] As nanotechnology offers the opportunity to benefit from the Enhanced Permeation and Retention Effet, and also to fabricate multi-functionalized platforms, incorporating phthalocyanines into nanoparticles is an aim intensively pursued by researchers in the last decade.



We have used covalent and non-covalent methods to incorporate/encapsulate/embed/ photosensitising phthalocyanines into nanoparticles.
Polyacrylamide[3], micellar formulation[4], phthalocyanine-polyglutamic acid polymeric constructs[5,6], amongst other nanoparticles have been produced, each of them exhibiting specific advantages and challenges that will be discussed.

References

1) P.-C. Lo, M. S. Rodriguez-Morgade, D. K. P. Ng, T. Torres, R. Pandey and F. Dumoulin, *Chemical Society Reviews*, **2020**, *49*, 1041-1056
2) S. Z. Topal, Ü. İşci, U. Kumru, D. Atilla, A. G. Gürek, C. Hirel, M. Durmuş, J.-B. Tommasino, D. Luneau, S. Berber, F. Dumoulin and V. Ahsen, *Dalton Trans*. **2014**, *43*, 6897-6908
3) F. Giuntini, F. Dumoulin, R. Daly, V. Ahsen, E. M. Scanlan, A. S. P. Lavado, J. W. Aylott, G. A. Rosser, A. Beebye and R. W. Boyle, *Nanoscale* **2012**, *4*, 2034-2045
4) B. Pucelik, I. Gürol, V. Ahsen, F. Dumoulin, J. M. Dabrowski, *Eur. J. Med. Chem*. **2016**, *124*, 284-298
5) L.V. Kiew, H. Y. Cheah, S. H. Voon, E. Gallon, J. Movellan, K. H. Ng, S. Alpugan, H. B. Lee, F. Dumoulin, M. J. Vicent, Lip Yong Chung, *Nanomedicine: NBM*, **2017**, *13*, 1447-1458.
6) H. Y. Cheah, E. Gallon, F. Dumoulin, S. Z. Hoe, N. Japundžić-Žigon, S. Glumac, H. B. Lee, P. Anand, L. Y. Chung, M. J. Vicent, L. V. Kiew, *Mol. Pharmaceutics* **2018**, *15*, 2594-2605.

IL-7.3.7

Multifunctional nanomaterials for detection and photoinactivation of microbial cells

Anzhela Galstyan
University of Münster, Center for Soft Nanoscience, Münster, Germany.

With tunable structures, remarkable photophysical properties and the ability to produce reactive oxygen species under red light irradiation, phthalocyanine derivatives have been investigated for many years as photosensitizers for antimicrobial PDT.[1] Nanotechnology has created new opportunities in aPDT by providing efficient nanoscale photoactive agents that can be formed via a bottom-up or top-down approach.
Self-association is the spontaneous organization of molecular units and is widely used bottom-up approach to fabricate nano-PDT agents.[2] Novel nanostructured Zn(II)phthalocyanine assemblies able to reduce bacterial viability in planktonic cultures and biofilms will be presented. How the degree of ordered molecular arrangement affects the photophysical properties and how high antimicrobial efficacy could be achieved by tuning the ratio between solubility and cell penetration will be discussed.
Another approach towards photoactive nanomaterials is electrospinning, which is considered a simple top-down approach for obtaining nanofibres of high quality. Though there are many reports showing that such nanoporous surfaces exhibit very strong antibacterial and antiviral effect upon irradiation, major regulators of activity are still largely unverified and the requirements for effective antimicrobial action remain controversial. Design and synthesis of photoactive compounds that could be used as structural components of nanoscale materials[3,4] as well as SAR of such materials will be presented.

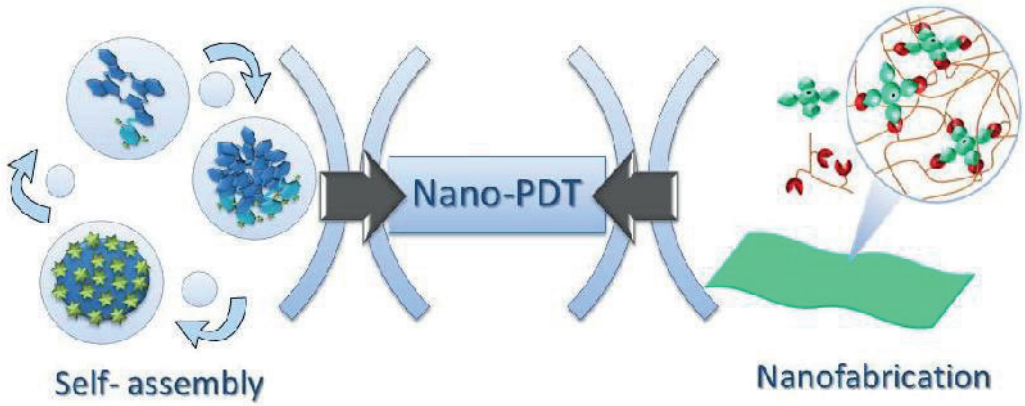


Fig. 1 – Schematic view of the formation of nano-PDT-agents through bottom-up and top-down approach.

References

1) A. Galstyan, Turning photons into drugs: phthalocyanine-based photosensitizers as efficient photoantimicrobials, *Chem. – A Eur. J.*, (2021), 27(6): 1903–1920. 2) A. Galstyan, A. Ricker, H. Nüsse, J. Klingauf, U. Dobrindt Exploring the Impact of Coordination-Driven Self Assembly on the Antibacterial Activity of Low-Symmetry Phthalocyanines. *ACS Appl. Bio Mater.* (2020), 3, 400–411. 3) K. Strokov, A. Galstyan, Chitosan-Silicon Phthalocyanine Conjugate as Effective Photo-Functional Hydrogel for Tracking and Killing of Bacteria, *Eur. J. Org. Chem.* (2020), 47, 7327-7332. 4) K. Strokov, A. H. Schäfer, U. Dobrindt, A. Galstyan, Facile fabrication of silicon(IV)phthalocyanine embedded poly(vinyl alcohol)-based antibacterial and antifouling interfaces, *ACS Appl. Bio Mater.* (2020), 3(6), 3751–3760.

Acknowledgements. This work was supported by Deutsche Forschungsgemeinschaft (GA 2365/2-1), Fonds der Chemischen Industrie and WWU Münster.

OC-7.3.8

Porphysome nanoparticles are effective photosensitizers for photodynamic therapy treatment for cancer

Keegan Guidolin[1,2,3], Lili Ding[3], Juan Chen[3], Gang Zheng[2,3]
1) Division of General Surgery, Department of Surgery, University of Toronto, Toronto, Canada; 2) Institute of Biomedical Engineering, University of Toronto, Toronto, Canada; 3) Princess Margaret Cancer Center, University Health Network, Toronto, Canada

Background: PDT has been investigated as a cancer therapeutic pre-clinically using many photosensitizers and for many applications. One particular photosensitizer, Photofrin (porfimer sodium, PHO), is a mixture of oligomers containing porphyrins. PHO benefits from some of the inherent tumouritropic properties of porphyrins, but there is concern about its off-target effects [1]. Nanomedicines are designed and engineered materials with health applications that measure between 1 nm and 100 nm in at least one dimension. Their size confers tumouritopic properties through a mechanism called the enhanced permeability and retention (EPR) effect [2]. One such nanomedicine is called the "porphysome" (PS) and is composed of porphyrin-lipid subunits which self-assemble into spherical liposome-like structures ~100 nm in size. These porphysomes have demonstrated both diagnostic and therapeutic applications in pre-clinical studies conducted over the past ten years, including fluorescence imaging, photoacoustic imaging, photothermal therapy, and drug delivery; however, they have never been investigated as photosensitizers for PDT on their own [3]. The aim of this study was to determine whether or not porphysomes are viable photosensitizers for PDT applications.
Methods: Female athymic nude mice, underwent subcutaneous injection of 5 x 10^6 A549 lung cancer cells. When tumours reached 5 mm, animals were randomized into treatment groups as per the specific aim protocol. After treatment, animals were followed with tumour measurement three times per week for a total of 30 days post-treatment. An a priori tumour size endpoint of 500 mm^3 was established for ethical purposes to represent terminal tumour growth.
Aim 1 – establishment of laser dose. To establish that no photothermal effects are present at the proposed light dose, three animals were administered 10 mg/kg of PS, and after a drug-light interval (DLI) of 24 hours, tumours were subjected to localized external laser delivery at a dose of 135 J/cm^2 (100 mW, 10 mm diameter beam, 671 nm, 22.5 minutes). Throughout this period, the surface temperature of the tumour was measured by thermal imaging. An a priori endpoint of a 5°C temperature increase was established to indicate photothermal heating.
Aim 2 – dose optimization. To establish the optimal dose and treatment plan for PS PDT, five animals each received: 5 mg/kg PS, 24h DLI; 7.5 mg/kg PS, 24h DLI; 10 mg/kg PS, 24h DLI; and 5 mg/kg PHO, no laser treatment. In addition, the best performing dose of PS from these groups was administered with a DLI of 48h, and with no laser treatment with five animals in each group.
Aim 3 – head-to-head comparison with Photofrin. To measure the effectiveness of PS PDT against the current clinical standard, twenty animals each received treatment with 5 mg/kg PHO, 24h DLI, and with the optimized dose and DLI of PS.
Results: Tumour temperatures measured throughout treatment with 100 mW/cm^2 for 22.5 minutes did not rise above the *a priori* threshold of 5°C above baseline. All PS doses demonstrated a significant tumour ablative effect compared to groups not receiving laser treatment; however, the greatest effect was seen in the 10 mg/kg PS group at a DLI of 24 hours. In the dose-finding group, the 10 mg/kg treatment and 7.5 mg/kg treatment demonstrated 67% and 60% cure respectively, with partial response and tumour growth suppression in the remaining tumours for both. The 5 mg/kg treatment showed partial response and tumour growth suppression without cure. Negative control groups (both PS and PHO drug-only, and no drug no light groups) demonstrated uncontrolled tumour growth requiring sacrifice prior to the 30-day endpoint as tumours reached an a priori maximum volume. Direct comparison of PHO 5 mg/kg and PS 10 mg/kg demonstrated similar tumour growth suppression (MANOVA $p>0.05$), which was significantly different from the uncontrolled tumour growth seen in the untreated control groups. Tumour growth reached a nadir 12-15 days after treatment; after this point, tumours began to regrow at a suppressed rate (Figure 1). Complete cure was not different between PHO and PS treatment groups (15% vs. 25%, $p=0.52$).

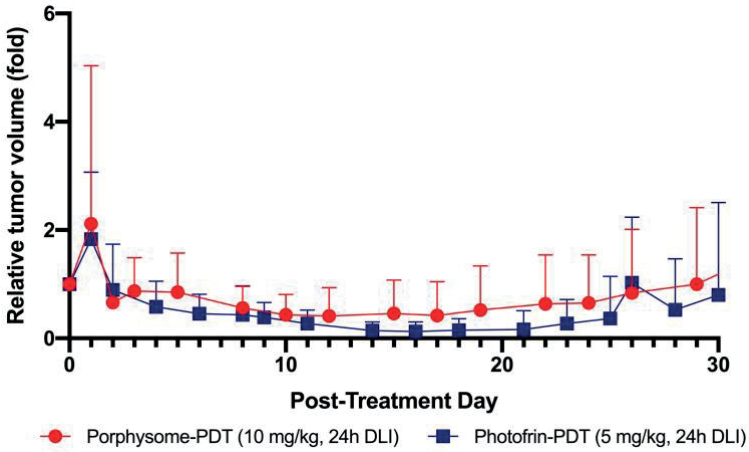


Figure 1. Comparison of mean relative tumour volume in animals treated with PS-PDT (n=20) and PHO-PDT (n=20).

Discussion: We identified an optimal dose of 10 mg/kg and DLI of 24 hours. This corroborates previous findings that tumour PS concentration is maximized at 24 hours following administration [3-5]. The optimized dose is also identical to the dose used in studies of other therapeutic applications of PS, namely photothermal therapy (PTT) [4, 6]. This study PS PDT was compared to a clinically approved PDT photosensitizer, PHO, as a positive control. Our results suggest that not only can the PS be used as a photosensitizer for PDT, but that it ablates tumours at a similar rate to PHO.

When delivered to a tumour, porphysomes initially exist as intact nanovesicles, but as time passes, porphysomes are taken into tumour cells, at which time they dissociate into their component porphyrin-lipids. Intact porphysomes absorb light energy and convert it into vibrational energy, released as heat (PTT) [4, 6-9]. In their dissociated state, porphyrin-lipids absorb energy which can be released as fluorescence or can produce reactive oxygen species. Previous studies have confirmed the fluorescence of porphysomes at 24 hours and have used fluorescence imaging to guide PTT [8-10]. The results of this study suggest that PDT and PTT could be used in combination as a single agent multimodality photosensitizer. Future studies will directly investigate this combined multimodality therapy investigating for additive or synergistic tumour ablation potential.

References

1. Dougherty, T.J., et al., *Photodynamic therapy.* J Natl Cancer Inst, 1998. **90**(12): p. 889-905.
2. Kalyane, D., et al., *Employment of enhanced permeability and retention effect (EPR): Nanoparticle-based precision tools for targeting of therapeutic and diagnostic agent in cancer.* Mater Sci Eng C Mater Biol Appl, 2019. **98**: p. 1252-1276.
3. Lovell, J.F., et al., *Porphysome nanovesicles generated by porphyrin bilayers for use as multimodal biophotonic contrast agents.* Nat Mater, 2011. **10**(4): p. 324-32.
4. Jin, C.S., et al., *Ablation of hypoxic tumors with dose-equivalent photothermal, but not photodynamic, therapy using a nanostructured porphyrin assembly.* ACS Nano, 2013. **7**(3): p. 2541-50.
5. Lovell, J.F., et al., *Enzymatic regioselection for the synthesis and biodegradation of porphysome nanovesicles.* Angew Chem Int Ed Engl, 2012. **51**(10): p. 2429-33.
6. Jin, C.S., J.F. Lovell, and G. Zheng, *One minute, sub-one-watt photothermal tumor ablation using porphysomes, intrinsic multifunctional nanovesicles.* J Vis Exp, 2013(79): p. e50536.
7. MacDonald, T.D., T.W. Liu, and G. Zheng, *An MRI-sensitive, non-photobleachable porphysome photothermal agent.* Angew Chem Int Ed Engl, 2014. **53**(27): p. 6956-9.
8. Kinoshita, T., et al., *Evaluation of Novel Imaging Devices for Nanoparticle-Mediated Fluorescence-Guided Lung Tumor Therapy.* Ann Thorac Surg, 2019. **107**(6): p. 1613-1620.
9. Ng, K.K., et al., *Self-sensing porphysomes for fluorescence-guided photothermal therapy.* Bioconjug Chem, 2015. **26**(2): p. 345-51.
10. Philp, L., et al., *Use of Porphysomes to detect primary tumour, lymph node metastases, intra-abdominal metastases and as a tool for image-guided lymphadenectomy: proof of concept in endometrial cancer.* Theranostics, 2019. **9**(9): p. 2727-2738.

P-7.3.9

Heme Biosynthesis and Degradation changes after 5-ALA application: A proteomic study

Sara Sansaloni Pastor[1], Emmanuel Varesio, and Norbert Lange[1]

1) Department of Pharmaceutical Technology, School of Pharmaceutical Sciences, University of Geneva, Institut des Sciences Pharmaceutiques de Suisse Occidentale (ISPSO), Geneva, Switzerland

Photodynamic therapy (PDT) has been applied to treat neoplastic and non-neoplastic diseases. It has been shown that after exogenous application of 5-aminolevulinic acid (5-ALA) or its derivatives induces is a preferential accumulation of protoporphyrin IX (PpIX). However, the mechanisms for this accumulation remain undiscovered.

To better understand the effect of PpIX accumulation in cancer cells, we studied how the different enzymes in heme biosynthesis and degradation enzymes are affected by 5-ALA and porphyrins. To this end, we used T24 cell line (bladder cancer cell line) treated for 24 h by 5-ALA (1 mM), succinylacetone (SA) (1 mM), 5-ALA+SA (1mM) and compared to non-treated control. All analyses were performed by mass quantification using a LC-ESI-MS/MS on an Orbitrap Fusion Lumos Tribrid mass spectrometer. Statistical analysis was carried out using two ways ANOVA and individual t-test.

We identified approximately 5000 proteins per sample. In addition, our technical replicates showed a good correlation (Pearson correlation > 0.91). Our results showed a change in the of heme enzymes' expression not only on the synthesis, but also on the degradation of porphyrins. A deregulation effect was observed in the biosynthetic pathway caused by the porphyrins, with a statistically significant upregulation of ***HMBS***, ***UROD*** *and* ***PPOX*** and a downregulation of ***CPOX***. 5-ALA also showed a statistically significant downregulation effect on ***HMBS***, ***PPOX*** and ***FECH***. Additionally, the degradation pathway is highly upregulated by the porphyrins, which affects ***HMOX1*** and ***BLVRA*** enzymes.

Our findings shed a light in the proteomic role of 5-ALA and porphyrins and its applications in PDT therapeutic options. Thus, leading research efforts in improving novel and better treatment approaches.

References:
1) Sansaloni-Pastor S, Bouilloux J, Lange N. The Dark Side: Photosensitizer Prodrugs. Pharmaceuticals (Basel). 2019 Oct 4;12(4):148. doi: 10.3390/ph12040148. PMID: 31590223; PMCID: PMC6958472.

Acknowledgements. This work was supported by the Swiss National Science Foundation (SNF)

P-7.3.10

Targeted bioorthoganal prodrug activation enabled by pH responsive classical polymer photocatalysts

Rong Li, Thomas Kuckhoff, Katharina Landfester and Calum T. J. Ferguson

Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

The use of photocatalytic in situ prodrug activation and against cancer has received increasing attention over recent years. However, the application of the currently approved photocatalysts is limited by their poor aqueous solubility, aggregation, photobleaching, off target effects and poor tumour uptake. One approach to overcome these limitations is through the use of a delivery vector for the photoactive species. Recently we have reported a new class of polymer photocatalysts formed by combining small molecular photocatalysts/ with classical polymer chemistry. 1-3 Creating photocatalytic polymer constructs that contain the beneficial properties from both components.
We have designed pH responsive classical polymer catalysts that respond to the well reported pH changes between healthy and cancerous tissue. Here, we have used RAFT polymerisation to produce diblock copolymers consisting of PEG114-b-(PAEMA40-s-PPH2BT2). At pH >7 crosslinking of primary amines in the PAEMA block with terephthalaldehyde occurs, resulting in the formation of nanoparticles (NPs). In this particulate form the polymer is photocatalytically inactive as the active centres are imbedded within the core of the particle. These non-active NPs can freely circulate around the body and will accumulate in tumour tissue through the EPR effect. Within the tumour cell environment a pH drop is observed to pH 6.4. This change stimulates the disassembly of the NP, where the polymer once again becomes photocatalytically active. We have used this photocatalytic material for the in situ activation of a cancer prodrug based on fluorouracil (5-FU). Where the prodrug version of 5-FU shows limited toxicity but can be easily activated in the presence of light and the photoactive polymer into the toxic form. We have demonstrated by both In vitro and cellular studies that this approach has the potential to enable targeted prodrug activation for cancer treatment.

References

[1] C. T. Ferguson, N. Huber, T. Kuckhoff, et al., J. Mater. Chem. A, 2020.

[2] C. T. Ferguson, N. Huber, K. Landfester, et al., Angew. Chem. Int. Ed., 2019.

[3] N. Huber, R. Li, C. T. Ferguson, et al., Catal. Sci. Technol., 2020.

Acknowledgements. This work was supported by grant 42 from the Ministry of Truth.

P-7.3.11

Radioluminescent nanoparticles and deep-tissue photodynamic therapy to enhance radiotherapy efficacy

Sarah Stelse-Masson[1], Thibault Jacquet[1], Mans Broekgaarden[1,2], Frédéric Chaput[3], Kseniya Popovich[4], Anamarija Abu el Rub[1], Anthony Nomezine[1], Lucie Sancey[2], Eva Mihokova[5], Vaclav Cuba[4], Hélène Elleaume[1], Anne-Laure Bulin[1]

1) Synchrotron Radiation for Biomedicine, Grenoble Alpes University, INSERM UA7, Grenoble, France.
2) Institute for Advanced Bioscience, Grenoble-Alpes University, INSERM U1209, 38000 Grenoble, France.
3) École Normale Supérieure de Lyon, CNRS - Université Claude Bernard Lyon 1, France.
4) Department of Nuclear Chemistry, FNSPE, Czech Technical University in Prague, Czech Republic.
5) Institute of Physics, Czech Academy of Sciences, Prague, Czech Republic.

Radioluminescent nanoparticles, also known as nanoscintillators, are gaining increasing interest as radiotherapeutics. Because they down-convert ionizing radiations into visible light, they can act as internal light sources remotely activated by penetrating X-ray [1] . They can therefore induce photodynamic therapy (PDT) during radiation therapy in deep-tissue and potentially within large tumor volumes [2,3]. Proof-of-concept studies have been published by us and others, yet the exact therapeutic mechanisms that are activated remain to be fully understood [4,5]. When using nanoscintillators in combination with radiotherapy, mechanisms other than PDT could also be activated. As nanoscintillators are typically composed of high-Z elements, we hypothesized the existence of another therapeutic effect that is currently under-explored for nanoscintillators: the purely physical radiation dose-enhancement effect. This effect is initiated by a higher photoelectric absorption of orthovoltage X-rays by high-Z elements compared to soft tissues that leads to a higher production of photoelectrons and Auger electrons that can enhance the damage to cancer tissues [6]. Investigating each individual radiotherapeutic effect induced by the nanoscintillator-photosensitizer conjugates, as well as their potential synergy, is crucial to better understand this novel modality.We will present the results obtained with various nanoscintillators made of high-Z elements and we will describe their ability to enhance the efficacy of radiotherapy through different mechanisms. Techniques ranging from physics to preclinical studies were employed to achieve these results.

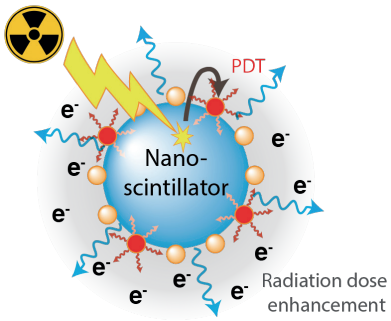


Figure 1: Radiotherapeutic contributions that can be activated and potentially synergize when nanoscintillators are used to activate deep-tissue PDT during radiotherapy.

References

1) C. Dujardin *et al*. Needs, Trends, and Advances in Inorganic Scintillators, *IEEE T Nucl Sci* **65**, 2018
2) W. Sun *et al.* Nanoscintillator-mediated X-ray induced photodynamic therapy for deep-seated tumors: from concept to biomedical applications, *Theranostics* **10**, 2020
3) J. Klein *et al.* Radioluminescence in biomedicine: physics, applications, and models, *Phys Med Biol* **64**, 2019
4) A-L. Bulin *et al.* X-ray induced singlet oxygen activation with nanoscintillator-coupled Porphyrins, *J. Phys Chem C* **117**, 2013
5) H. Chen *et al.*, Nanoscintillator-mediated X-ray inducible photodynamic therapy for *in vivo* cancer treatment, *Nano Lett.* **15** 2015
6) A-L. Bulin *et al.*, Radiation dose-enhancement is a potent radiotherapeutic effect of rare-earth composite nanoscintillators in preclinical models of glioblastoma, *Advanced Science*, 7 2020

Acknowledgements. The work was supported by grants from the French Medical Research Foundation (*Fondation pour la Recherche Médicale*, ARF20170938739), the Auvergne Rhône-Alpes Region (NEPTUNE project), INSERM Cancer, the French *Ligue Contre le Cancer* Foundation and the LABEX PRIMES of the Université of Lyon (ANR-11-LABX-0063). A-L. B. also received additional funding from the "*Rotary Club*" Association through the program "*Jetons Cancer*" and from INSERM.

P-7.3.12

Rational Design and Development of a New Class of Metal-Based Photosensitizers for Photodynamic Therapy

Houston D. Cole[1], John Roque III[1,2], Ge Shi[1], Elam Ramasamy[1], Colin G. Cameron[1], Sherri A. McFarland[1]
1) University of Texas at Arlington, Department of Chemistry and Biochemistry, Arlington, TX, United States; 2) University of North Carolina at Greensboro, Department of Chemistry and Biochemistry, Greensboro, NC, United States

Photodynamic therapy (PDT) is a unique approach to cancer treatment that remains underutilized despite its intrinsic selectivity towards cancerous tissue. PDT utilizes a non-toxic photosensitizer (PS), light, and oxygen to initiate localized cell death (and elicit an antitumor immune response in some cases). Currently, the only FDA-approved PS for cancer therapy is Photofrin, a porphyrin-based oligomer. With improved PSs, we propose that PDT could become a more important adjuvant or alternative therapy for certain cancers. Metal-based PSs are of particular interest in this regard due to their tuneable photophysics and anti-cancer activity. Ruthenium- and osmium-centered PSs are emerging as examples, with our own TLD1433 currently undergoing Phase 2 clinical trials for the treatment of bladder cancer (Clinicaltrials.gov identifier NCT03945162). Herein, we describe the rational design and development of a new class of metal-based PSs to further optimize their light-driven PDT effects.

P-7.3.13

Remote-controlled drug release with photons: From light to X-rays

Tristan le Clainche[1], Nazareth Milagros Carigga Gutierrez[1], Eaazhisai Kandiah[2], Xavier Le Guevel[1], Lucie Sancey[1], Hélène Elleaume[3], Anne-Laure Bulin[3], Jean-Luc Coll[1], Mans Broekgaarden[1].
1) Institute for Advanced Bioscience, Grenoble-Alpes University, INSERM U1209, 38000 Grenoble, France.
2) European Synchrotron Radiation Facility, 38000, Grenoble, France
3) Synchrotron Radiation for Biomedicine, Grenoble-Alpes University, INSERM UA7, 38026 Grenoble, France

Liposomes have been the most successful type of nanomedicine for cancer patients, playing a leading role in improving the tolerability of chemotherapeutics. However, to advance the success of liposomal drug delivery and cancer treatment, new approaches to physically trigger drug release in cancer tissues and increase the permeability of the protective cancer stroma are needed [1]. Therefore, we investigate whether photosensitizers and radiocatalytic nanomaterials can be integrated in liposomes for remote-controlled drug release through excessive production of reactive oxygen species (ROS) upon exposure to light and radiotherapy.
When embedded in the lipid bilayer of liposomes, photosensitizers can be used for the light-controlled release of encapsulated drugs [2]. The oxidation of unsaturated phospholipids through the photodynamic effect triggers conformational changes in their acyl chains, resulting in lipid packing defects, loss of membrane integrity, and the release of liposomal cargoes [3]. To optimize this effect for 690 nm light-controlled drug release, we developed oxidation-responsive liposomes supplemented with benzoporphyrin derivative (BPD). We discovered that distinct lipids and phospholipids act synergistically to promote drug release, and developed a novel formulation with which complete drug release could be achieved at low radiant exposures (<10 J/cm^2).
Radiation controlled liposomal drug delivery would be a substantial improvement over photo-triggered drug release, as X-rays penetrate deeply into tissues and radiotherapy is more broadly involved in the standard-of-care for cancer patients. We thus further supplemented the oxidation-responsive liposomes with radiocatalytic gold nanoclusters, *i.e.*, 2 nm nanoparticles that generate ROS upon exposure to X-rays through the radiation dose-enhancement effect [4]. Using monochromatic synchrotron radiation, we discovered that liposomes containing *both* BPD *and* gold nanoclusters generated significantly elevated levels of ROS. The ROS generation occurred in a gold-dependent manner, as it was highest with X-ray energies tuned above the absorption K-edge of gold (82 keV), suggesting a synergistic interaction between gold and BPD. This interaction was successfully leveraged to induce X-ray-controlled drug release.
In conclusion, this work provides important new insights in the role of specific lipids in achieving photodynamic drug release. Moreover, we provide compelling proof-of-concept for the feasibility of radiotherapy-triggered drug release, which is a ground-breaking new concept. Ongoing research focuses on investigating the biological effects of these novel oxidation-responsive liposomes on 3D culture models of cancer [5]. Especially the increased permeability of tumor tissues following exposure to photo- and/or X-ray-generated ROS may significantly advance the standard-of-care for many cancer patients.

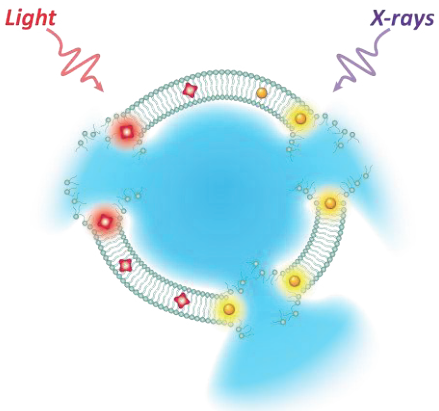


Fig. 1 – Remote-controlled drug release from oxidation-responsive liposomes, induced either by the excitation of photosensitizers by light, or the excitation of radiocatalytic gold nanoclusters by X-rays.

References
1) Crommelin et al., ***J Control Release*** 2019. 2) Luo et al., ***Small***, 2016. 3) Massiot et al., ***J Mater Chem B***, 2019. 4) Broekgaarden et al., ***Nanoscale*** 2020. 5) Broekgaarden et al., ***Biomaterials*** 2019.

Acknowledgements. This work was supported by the Phospholipid Research Center (Heidelberg, Germany, MAB-2020-080/1-1), and the French National Institute for Health and Medical Research (INSERM).

P-7.3.14

Squalene-NIR dye nanoassemblies targeting mitochondria with photosensitizing properties for the detection and treatment of cancer by phototherapy

Souad Adriouach[1,2] and Eric Allémann[1,2]

[1]School of Pharmaceutical Sciences, University of Geneva, 1211 Geneva, Switzerland; [2]Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, 1211 Geneva 4, Switzerland,

Squalene (Sq) is a natural precursor of cholesterol (1). The conjugation of Sq to a drug produces bioconjugates that self-assemble in water to give nanoassemblies (NAs) (2).

Near-infrared (NIR) fluorescence has great potential for *in vivo* tumour imaging. NIR dyes such as the recently synthesized SSA-15 (3) are lipophilic cations with preferential accumulation in the mitochondria of cancer cells. They also have photosensitizing properties inducing tumour cell death after NIR light irradiation.

This study aims to develop Sq-SSA-15 NAs targeting mitochondria, with imaging and photosensitizing properties and to assess their capacity to induce tumour cell death by phototherapy after NIR light irradiation. Here, we report:

- *in vitro* fluorescence imaging of Sq-SSA-15 NAs and specific mitochondrial localization
- antitumour effect of Sq-SSA-15 NAs after NIR light irradiation

Material and methods:

For this study, we used MCF7 human breast cancer cells and MCF-10 non-tumorigenic human breast cells to assess the specific localization of Sq-SSA-15 NAs in the mitochondria of cancer cells. MCF7 and MCF10 were incubated with different concentrations of SSA-15 or the Sq-SSA-15 NAs for 1h. Mitochondria were stained with MitoTracker® Orange and nuclei with Hoechst®. Cells were observed by live-cell imaging using the automated IXM-XL microscope (Molecular devices, 1 x 40). To study the photosensitizing properties of our product, we incubated MCF7 cells with 20 µM of SSA-15 and Sq-SSA-15 for 1 h. The cells were washed and irradiated with a NIR LED lamp at a wavelength of 630 nm, at 100mW/cm^2 for 10 min. We performed the cytotoxicity assay after 24 h using WST-1 cell proliferation assay.

Results: Fluorescence imaging of MCF-7 cells demonstrated effective localization of both SSA-15 and Sq-ssa-15 NAs in mitochondria of cancer cells by the co-staining with MitoTracker® Orange. The comparison with MCF10 non-tumorigenic breast cells demonstrated the preferential accumulation of our product in cancer cells. The WST-1 cell proliferation assay indicated that both SSA-15 and Sq-SSA-15 induced tumour cell death after light irradiation compared to the control (dark condition).

Conclusion: This study demonstrated the preferential accumulation of Sq-SSA-15 in the mitochondria of cancer cells by comparison to healthy cells. Sq-SSA-15 induced cancer cell death after NIR light irradiation. The fluorescence and photosensitizing properties of the Sq-SSA-15 NAs suggest their potential use as a nanotheranostic agent for imaging and treatment of cancer by phototherapy.

References:

1. Desmaële D, Gref R, Couvreur P. Squalenoylation: A generic platform for nanoparticular drug delivery. J Control Release. 2012;161(2):609-18.
2. Adriouach S, Vorobiev V, Trefalt G, Allémann E, Lange N, Babič A. Squalene-PEG: Pyropheophorbide-a nanoconstructs for tumor theranostics. Nanomedicine: Nanotechnology, Biology and Medicine. 2019;15(1):243-51.
3. Vorobiev V, Adriouach S, Crowe LA, Lenglet S, Thomas A, Chauvin A-S, et al. Vascular-targeted micelles as a specific MRI contrast agent for molecular imaging of fibrin clots and cancer cells. European Journal of Pharmaceutics and Biopharmaceutics. 2021;158:347-58.

P-7.3.15

Metallo-surfactant mediated nanocolloids for photodynamic therapy

Lluïsa Pérez-García[1,2], Bunty Sharma[2,3], Ganga Ram Chaudhary[3], and Gurpreet Kaur[3]
1) Universitat de Barcelona, Department of Pharmacology, Toxicology and Therapeutic Chemistry, Institut de Nanociència i Nanotecnologia UB (IN2UB), 08028 Barcelona, Spain; 2) University of Nottingham, School of Pharmacy, Nottingham, UK; 3) Panjab University, Department of Chemistry, Chandigarh, 160014, India.

Metallosurfactant (MS) aggregates have grasped great attention from researchers worldwide due to the dual properties of both metals and surfactants. On complexing surfactants with metal ions, a decrease in the critical micelle concentration (CMC) is usually observed compared to those of the parent innocent surfactants.[1] Catanionic vesicles are synthesized by mixing cationic metallosurfactant and anionic surfactant in non-stoichiometric ratios which leads to spontaneous vesicle formation. The size and surface charge of these vesicles can be controled by varying the cationic/anionic components ratio. Photodynamic therapy results from combination of photosensitizers (PSs), light of specific wavelength, and molecular oxygen; conversion of molecular oxygen into phototoxic singlet oxygen is greatly improved by incorporation of PSs into nanomaterials.[2]
We have formulated metallocatanionic vesicles (MCVs) from a combination of a double-and single-chain copper and iron-based cationic metallosurfactant (CuCPCI, and FeCPCI) and an anionic surfactant sodium bis(2-ethylhexyl)sulfosuccinate (AOT). We have prepared mixtures with different ratios, from 10:90 to 90:10, in PBS of 7.4 pH. In this approach, two of the fractions, one each from a cationic rich and anionic rich side, were selected to encapsulate anionic rose bengal (RB). The vesicles were characterized by SAXS, AFM, FE-SEM, cryo-TEM, and Zeta-sizer measurements. These studies reveal that the MCVs have dual functionality *i.e.* encapsulate PSs and enhance the singlet oxygen yield of RB, and the nanomaterials show antibacterial properties against *S. Aureus, E.* Coli.[3,4] Recently, we have applied these PS-loaded MCV against U-251 Glioblastoma cell lines. These experiments showed MCVs biocompatible nature in dark and high phototoxicity against cancer cell lines which were confirmed by WST-8 assay. Further, SOSG assay and differential nuclear staining assay (DNS), confirmed the intracellular singlet oxygen generation and live/dead cell after PDT. Caspase assay confirmed the apoptotic pathway of cell killing. This work provides a new metal hybrid smart biocompatible material that possesses dual functionality and is prepared by an easy, fast, and feasible procedure which resulted in enhanced PDT against a drug-resistant bacterium and cancer cell lines.

References

1) Kaur, N.; Kaur, S.; Kaur, G.; Bhalla, A.; Srinivasan, S.; Chaudhary, G. R. Metallovesicles as smart nanoreactors for green catalytic synthesis of benzimidazole derivatives in water, *J. Mater. Chem. A*, **2019**, *7*, 17306-17314.
2) Samperi, M.; Limón, D.; Amabilino, D. B.; Pérez-García, L. Enhancing singlet oxygen generation by self-assembly of a porphyrin entrapped in supramolecular fibers, *Cell Reports Physical Science*, **2020**, *1*, 100030.
3) Sharma, B.; Kaur, G.; Chaudhary, G. R.; Gawali, S. L.; Hassan, P. A. High antimicrobial photodynamic activity of photosensitizer encapsulated dual-functional metallocatanionic vesicles against drug resistant bacteria S aureus, *Biomater. Sci*. **2020**, *8*, 2905−2920.
4) Sharma, B.; Kaur, G.; Chaudhary, G. R. Optimization and utilization of single chain metallocatanionic vesicles for antibacterial photodynamic therapy (aPDT) against E. Coli, *J. Mater. Chem. B*, **2020**, *8*, 9304-9313.

Acknowledgements. This work was supported by the Commonwealth scholarship (INCN-2019-409), the European Regional Development Fund (FEDER), Spanish government grant PID2020-115663GB-C32, and the University of Nottingham.

IL-7.4.1

Illuminating the plant calendar

Joshua M. Gendron

1) Yale University, Department of Molecular, Cellular, and Developmental Biology, New Haven, USA

Plants have served as a preeminent study system for photoperiodism due to their propensity to flower in concordance with the seasons. A nearly singular focus on understanding photoperiodic flowering has prevented discovery of other photoperiod measuring systems necessary for vegetative health. Here we use bioinformatics to identify photoperiod-induced genes in Arabidopsis. We show that one, *PP2-A13*, is expressed exclusively in, and required for plant fitness in, short winter-like photoperiods. We create a real-time photoperiod reporter, using the *PP2-A13* promoter driving luciferase, and show that photoperiodic regulation is independent of the canonical CO/FT mechanism for photoperiodic flowering. We then reveal that photosynthesis combines with circadian clock-controlled starch production to regulate cellular sucrose levels to control photoperiodic expression of *PP2-A13*. This work demonstrates the existence of a photoperiod measuring system housed in the metabolic network of plants that functions to control seasonal cellular health.

Acknowledgements. This work was supported by grants from the National Institutes of Health and National Science Foundation

IL-7.4.2

The molecular basis for day/night signaling in the cyanobacterial circadian clock

Carrie L. Partch[1,2], Jeffrey A. Swan[1], Colby R. Sandate[3], Joel Heisler[4], Alfred Freeberg[1], Dustin Ernst[5], Archana Chavan[4], Susan S. Golden[2,5], Andy LiWang[2,4], Gabriel C. Lander[3]
1) University of California Santa Cruz, Department of Chemistry & Biochemistry, Santa Cruz, USA; 2) University of California San Diego, Center for Circadian Biology, La Jolla, USA; 3) Scripps Research, Department of Integrative and Computational Structural Biology, La Jolla, USA; 4) University of California Merced, Department of Chemistry & Biochemistry, Merced, USA; 5) University of California San Diego, Division of Biological Sciences, La Jolla, USA

In cyanobacteria, three Kai (or 'cycle') genes, KaiA, B and C, work together to generate circadian rhythms that synchronize its physiology with Earth's day/night cycle. KaiC is the enzymatic driver of the clock with two tandem AAA+ ATPase domains that assemble into two hexameric ATPase rings. During the day, KaiA stimulates autophosphorylation of KaiC at two sites in the C-terminal ATPase domain, while KaiB binds at night to the N-terminal ATPase domain to sequester KaiA and allow KaiC to autodephosphorylate, completing one cycle of the clock[1]. KaiB association with KaiC is restricted to the evening by the phosphorylation state of KaiC, although the mechanism by which this information is communicated ~70 angstroms through KaiC to the KaiB binding interface is not known. Here we present structures of KaiC representing its daytime and nighttime states, obtained by cryo-electron microscopy, that reveal the structural basis of KaiB selectivity for the nighttime state. Mutants that disrupt the allosteric transmission of information through KaiC reduce affinity for KaiB and link cooperative recruitment of KaiB to ATP hydrolysis by KaiC, abrogating circadian rhythms *in vitro* and *in vivo*. This work highlights how ultrasensitive signaling mechanisms contribute to day/night transitions in the cyanobacterial circadian clock.

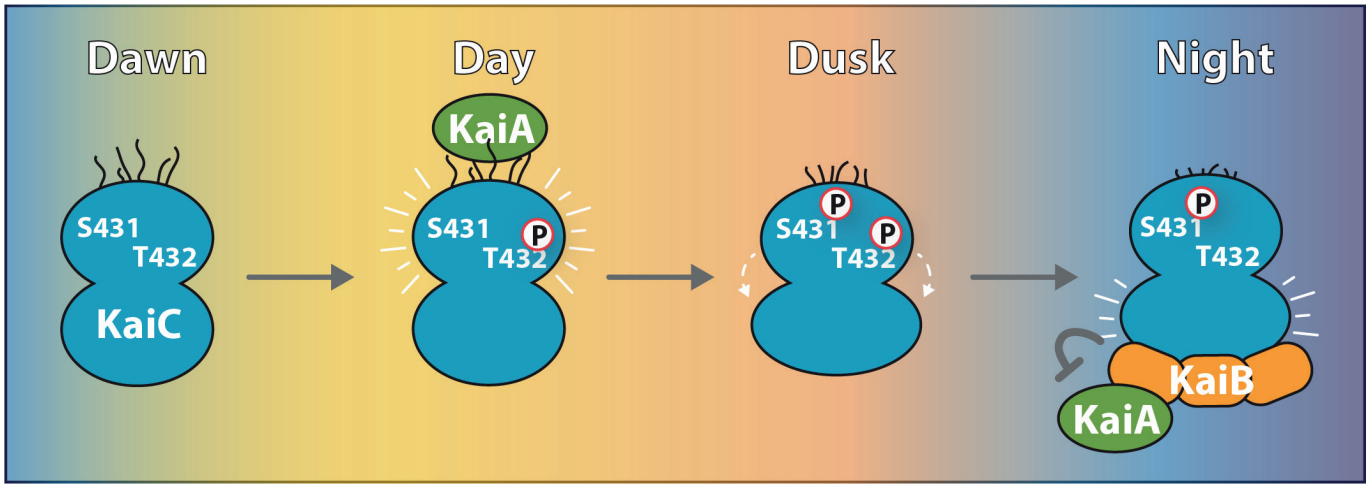


Fig. 1 – The central timekeeping mechanism of the cyanobacterial circadian clock.

References

1) Swan, J.A., Golden, S.S., LiWang, A., and Partch C.L. Structure, function, and mechanism of the core circadian clock in cyanobacteria. Journal of Biological Chemistry, 2018.

Acknowledgements. This work was supported by grants GM121507 and GM141849 from the National Institutes of Health.

IL-7.4.3

Superoxide is a metabolic signal that acts on the Arabidopsis circadian clock in the evening

Mike Haydon[1]

1) University of Melbourne, School of BioSciences, Parkville, Australia

Plants must coordinate photosynthetic metabolism with the daily environment and adapt rhythmic physiology and development to match carbon availability. Products of photosynthetic metabolism, including sugars and reactive oxygen species (ROS), are closely associated with the plant circadian clock and sugars have been shown to provide metabolic feedback to the circadian oscillator. However, distinguishing the effects of light and sugars in photoautotrophic cells is challenging. We have used transcriptomics and chemical screens to explore regulation of the circadian clock by sugars. From a sugar-regulated transcriptome of Arabidopsis we identified genes associated with redox and ROS processes. We found that sucrose increased levels of superoxide and identified circadian rhythms of superoxide-regulated transcripts which are phased around dusk, including several oscillator genes. Our data suggest a new role for superoxide as a rhythmic sugar signal which acts in the evening and affects circadian gene expression and growth.

Acknowledgements. This research was funded by BBSRC grant (BB/L021188/1), Royal Society Research Grant (RG150144) to MJH and by The University of Melbourne through the Research Grants Support Scheme to MJH and a Melbourne Research Scholarship to XL.

OC-7.4.4

Diurnal Rhythmicity of Cucumber Root Iron Deficiency Response Eliminates Rapidly Upon Nanohaematite Iron Resupply

Amarjeet Singh[1], Viktória Kovács Kis[2] Áron Keresztes[3], Fruzsina Pankaczi[1], Brigitta Müller[1], Ferenc Fodor[1] Zoltán Klencsár[2], Ádám Solti [1]
1) Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C, Budapest H-1117, Hungary
2) Centre for Energy Research, Konkoly-Thege Miklós út. 29-33, Budapest, H-1121, Hungary
3) Department of Plant Anatomy, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C, Budapest H-1117, Hungary

Iron (Fe) is an essential cofactor of redox enzymes in photosynthesis, respiration and antioxidative protection. Plants take up Fe from the soil. Under alkaline conditions the oxidised ferrous Fe forms insoluble ferric oxide/hydroxide precipitates that limits Fe acquisition of plants. Fe shortage leads to various dysfunctions, primarily impaired photosynthetic functions, growth and development. To overcome Fe limitation, the majority of angiosperm plants operate a reduction based Fe uptake strategy that also involves the acidification of the rhizosphere and the excretion of plant metabolites that contribute in the chelation and/or reduction of Fe. This reduction based Strategy I thus primarily rely on the operation of Ferric Reductase Oxidase (FRO) family enzymes that perform a transmembrane electron transport from cytoplasmic reductant NADH to ferric compounds. As a Strategy I plant, cucumber (*Cucumis sativus*) expresses three root plasma membrane localised FROs viz. *CsFRO1*, *CsFRO2* and *CsFRO3* associated with root ferric chelate reductase (FCR) activity. Cucumber belongs to the flavin excreting dicot plants: as response for Fe deficiency, it excretes riboflavin derivative 5'-ketoriboflavin compounds that were previously suggested to be involved in the reduction of insoluble ferric precipitates. Once Fe taken up into the roots, the root-to-shoot xylem translocation requires citrate as for complexing compound. Taken together, both generating reducing power, and the biosynthesis of flavins and organic acids requires a metabolic input. Previous studies on *Populus* sp. indicated that Fe uptake of dicots is a diurnal activity. Here we applied an Fe deficient plant model system performing intensive Fe uptake and nanohaematite suspension as for Fe source to establish a quasi-natural condition for Fe uptake and to study the circadian rhythm dependent relaxing of the reduction based Fe acquisition system.
As for model, Fe deficient cucumber plants were cultivated in hydroponics with a complete retraction of Fe from the medium for three weeks from germination. Nanocrystalline haematite colloid suspension with a particle size ranging from 10-20 nm was applied in a concentration equivalent to 20 μM nominal concentration of Fe as for Fe deficiency recovery treatment starting at 9 am. Expression profile of *CsFRO1*, *CsFRO2*, *CsFRO3* and *CsRIBA1* (GTP cyclohydrolase II) was analysed using quantitative real time PCR. The ferric chelate reductase (FCR) activity was analysed using a bathophenanthroline disulfonate based ferrous Fe trapping method. Utilisation of the nanohaematite particles was validated by microscopy energy-dispersive X-ray spectroscopy (EDS) on ultrathin cuts of root segments. Expression of the genes of interest showed two peak circadian expression arrangement with markedly increased expression at noon and evening for all genes exception of *CsFRO3* in Fe deficient model plants. Utilization of nanohaematite resulted in decrease in expression of *CsFRO1*, *CsFRO3* and *CsRIBA1* within 10 hours of treatment but total decline to control level took 24 h. *CsRIBA1* strongly responded to Fe deficiency and Fe supply. On the other hand, decline in expression of *CsFRO2* and activity of FCR took approximately 48 hours demonstrating a biphasic suppression of Fe deficiency response. Besides nanohaematite particles were able to penetrate the root apoplast and intercellular space confirmed by EDS, transmission electron microscopy analysis verified the nanoparticles as for Fe source with the reduction in their particle size. The rapid decline in the rhythmic expression of *CsFRO1*, *CsFRO3* and *CsRIBA1* underlines the effective reciprocal feedback effect of cellular Fe sensing mechanisms that overwrite the rhythmicity in the transcript amounts.

Acknowledgements: This work was supported by the grants financed by the National Research, Development and Innovation Office, Hungary (NKFIH K-124159 and VEKOP-2.3.3-15-2016-00008). This work was completed as part of the ELTE Thematic Excellence Programme 2020 supported by the National Research, Development and Innovation Office (TKP2020-IKA-05).

IL-7.4.5

Evaluation of plant circadian rhythms based on the cellular circadian behaviour

Tokitaka Oyama[1]
1) Kyoto University, Department of Botany, Kyoto, Japan

The circadian clock gives time information to various physiological processes that would be involved in the day-night response. In plants, many genes are under control of the clock and their gene expression shows circadian rhythms even under constant conditions. The self-sustained oscillation is generated by transcription-translation feedback loops composed of a number of clock genes in each cell. Hence the circadian clock system of plant is based on the cell-autonomous machinery. Circadian rhythms of plants have been effectively analysed at the tissue/organ/body levels; circadian properties such as phase/period preciseness and entrainability of individual cells are largely unknown.

In my laboratory, we developed the bioluminescence monitoring system that enabled us to observe circadian rhythms of individual cells in the plant[1]. A circadian bioluminescent reporter, *AtCCA1:LUC* was used for gene transfection to cells in a duckweed with a particle bombardment method. *CCA1* is a clock gene of which expression peaks in the morning. Using an EM-CCD camera system, the bioluminescence of individual cells was automatically monitored under various light/dark schedules[1,2]. Duckweed plants are suitable for imaging the entire plant because of their tiny, flat, floating bodies. Cellular circadian behaviour under constant conditions were heterogeneous among cells in the same frond (the plant unit of duckweed) of *Lemna gibba*. The heterogeneous period lengths affected the heterogeneous entrainment among individual cells in the frond under non-24 h light/dark cycles. Even though individual cells showed a high independency in the circadian behaviour, spatial phase patterns that were a sign of cell-cell interaction (coupling between cellular rhythms) were observed in the fronds.

The *CCA1:LUC* reporter has been widely utilized to study circadian rhythms of various plants. This reporter represents the oscillation of a clock gene, that is, the behaviour of the circadian clock. Recently, we have found that a luciferase gene under the control of a "constitutive" promoter (e.g. *CaMV35S:LUC*) showed a circadian bioluminescence rhythm when introduced into duckweed plants. A dual-colour bioluminescence monitoring system at a single-cell level (simultaneous monitoring of cellular bioluminescence from two different-colour reporters) revealed that the *CaMV35S:LUC* bioluminescence rhythm and the *AtCCA1:LUC* one showed different period lengths and phase-relationship among cells in the same frond[3]. The *CaMV35S:LUC* bioluminescence rhythm of a cell looked to be associated with the synchrony around it without the direct control of its cellular clock. Since the synchrony of cellular clocks is highly associated with the coupling phenomena, the cellular bioluminescence behaviour of *CaMV35S:LUC* will be a tool to analyse the organization of circadian behaviour in tissues/organs.

Cellular circadian behaviour in a tissue/organ is influenced by other cells/tissues; the cellular nature of circadian clock would be inaccessible. Then, we have approached it by observing bioluminescence rhythms of individual protoplast-derived cells isolated from the *Arabidopsis CCA1:LUC* transgenic plant. Even under the highly artificial conditions, leaf- and root-derived cells showed typical properties of the circadian rhythm. The differences in the properties between these two cell-types seemed to relate to their adaptiveness in the totally different local environments for leaves and roots.

In this paper, I will present our recent results of cellular circadian rhythms of plants and discuss the characteristics of the plant circadian system as a cell-based organization.

References

1) Muranaka T, Oyama T, Heterogeneity of cellular circadian clocks in intact plants and its correction under light-dark cycles. *Sci. Adv.*, 2, e1600500., 2016.
2) Okada M, Muranaka T, et al., Synchrony of plant cellular circadian clocks with heterogeneous properties under light/dark cycles. *Sci. Rep.*, 7, 317., 2017
3) Watanabe E, Isoda M, et al., Detection of uncoupled circadian rhythms in individual cells of *Lemna minor* using a dual-color bioluminescence monitoring system. *Plant Cell Physiol.,* in press, 2021.

Acknowledgements. This work was supported in part by the Japan Society for the Promotion of Science KAKENHI (Grant numbers 25650098, 17KT0022, and 19H03245).

IL-7.4.6

Integration of circadian and environmental signals that regulate chloroplast gene expression

Antony N. Dodd[1]

1) John Innes Centre, Norwich, UK.

Photosynthesis within chloroplasts is essential for virtually all plants, including the crops that provide our food. Photosynthesis is regulated by both circadian rhythms and a variety of environmental cues. Understanding the mechanisms through which environmental cues regulate photosynthesis is important to predict the effects upon photosynthesis of future climates, and to identify potential targets for crop improvement. We are investigating the nature of the signalling mechanisms in plants that integrate circadian and environmental cues, and communicate this information to chloroplasts (1, 2). We reason that plants must integrate circadian timing cues with information concerning unpredictable changes in their environment in order to respond to predictable (24 h) and unpredictable changes in the environmental conditions. We investigated this idea within the context of signalling from the nucleus to chloroplasts, by examining the integration of circadian, light and temperature signals by a group of proteins called sigma factors. In plants, sigma factors are nuclear-encoded regulators of chloroplast transcription. I will show new results identifying a key role for a sigma factor in the integration of circadian and low temperature signals, and position this within the upstream and downstream components of the signalling pathway, and its impacts upon plant physiology.

References

1) Belbin FE, Noordally ZB, Wetherill SJ, Atkins KA, Franklin KA, Dodd AN. Integration of light and circadian signals that regulate chloroplast transcription by a nuclear-encoded sigma factor. *New Phytol.* 213, 727-738 (2017).

2) Noordally ZB, Ishii K, Atkins KA, Wetherill SJ, Kusakina J, Walton EJ, Kato M, Azuma M, Tanaka K, Hanaoka M (2013). Circadian control of chloroplast transcription by a nuclear-encoded timing signal. *Science* 339, 1316-1319. (2013).

Acknowledgements. This research was funded by BBSRC (UK) (BB/I005811/2, BB/J014400/1; studentship 1518540; Institute Strategic Programme GEN BB/P013511/1), The Leverhulme Trust (RPG-2018-216), NAGASE Science Technology Foundation (Japan), the Ministry of Education, Culture Sports, Science and Technology (Japan) (Grants-in-Aid 17K07438), Tokyo Institute of Technology World Research Hub Initiative (WRHI) Program of Institute of Innovative Research and the Consejo Nacional de Ciencia y Tecnología (Mexico).

IL-7.4.7

TOC1 phosphorylation controls formation and function of an NF-TOC1 complex regulating hypocotyl growth

David E Somers[1], Jiapei Yan[1]
1) Ohio State University, Department of Molecular Genetics, Columbus, USA

Plant photoperiodic growth is coordinated by interactions between circadian clock and light signaling networks. How post-translational modifications of clock proteins affect these interactions to mediate rhythmic growth remains unclear. Here we identify five phosphorylation sites in the core clock protein TIMING OF CAB EXPRESSION 1 (TOC1) which when mutated to alanine (5X) eliminate detectable phosphorylation. 5X is unable to fully rescue the clock, growth and flowering phenotypes of the toc1 mutant. 5X exhibits advanced phase, a faster degradation rate, reduced interactions with PHYTOCHROME INTERACTING FACTOR3 (PIF3) and HISTONE DEACETYLASE 15 (HDA15), and poor binding at pre-dawn hypocotyl-growth-related genes (PHGs), leading to de-repression of hypocotyl growth in the middle of the night. NUCLEAR FACTOR Y subunits B and C (NF-YB/C) stabilize TOC1 at target promoters and this novel trimeric complex (NF-TOC1) acts as a transcriptional co-repressor with HDA15 to inhibit hypocotyl elongation. Collectively, we identify a molecular mechanism of how TOC1 phosphorylation alters its phase, stability and physical interactions with co-regulators to precisely phase PHG expression to control photoperiodic hypocotyl growth.

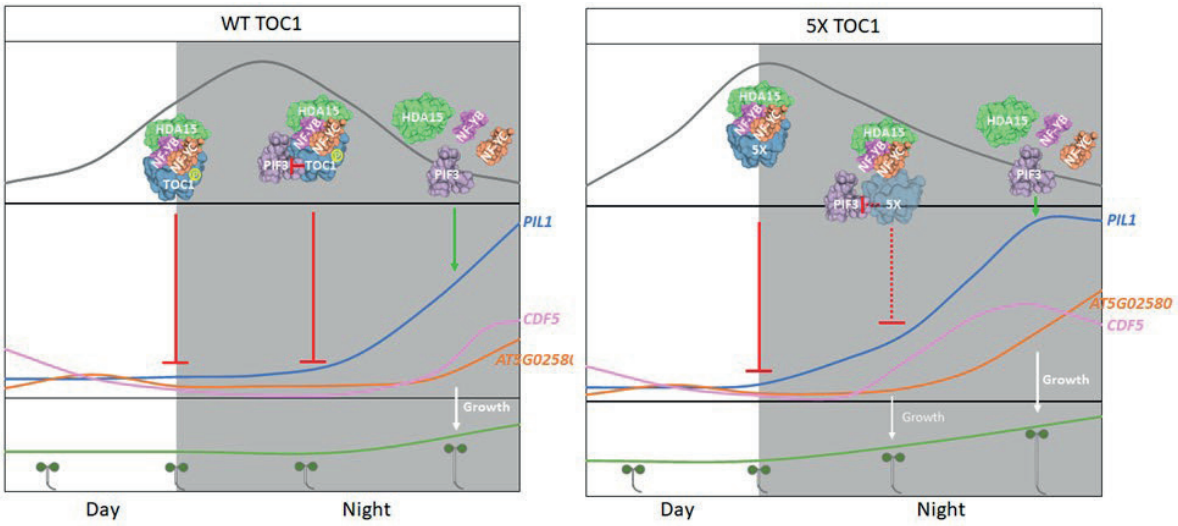


Fig. 1 -- Proposed mechanism for the role of TOC1 phosphorylation in the control of hypocotyl growth. At early night, wild-type (left) and 5X (right) TOC1 are equally present and recruit transcriptional repressors NF-YB/C and HDA15 to inhibit expression of PHGs. As night progresses, PIF3 levels rise, and the advanced phase of 5X, more rapid degradation and poorer PIF3 interaction result in lesser presence of 5X at target promoters, relative to wild-type TOC1, leading to attenuated inhibition of PHGs and enhanced hypocotyl growth. At late night, low TOC1 levels reduce NF-YB/C and HDA15 recruitment, resulting in PIF3 release to activate downstream PHGs leading to maximal pre-dawn hypocotyl growth.

Acknowledgements. This work was supported by the National Institutes of Health (R01GM093285 and to D.E.S.), the Next-Generation BioGreen21 Program (PJ01327305), the Rural Development Administration, Republic of Korea (to D.E.S.) and the World Class University Program of South Korea (No. R31-2008-000-10105-0), NRF, MEST (to D.E.S.).

OC-7.4.8

Interplay between circadian clock and unfolded protein response in *Arabidopsis thaliana*

Ozlem GUMUS[1], Buse PINAR[1], Baris UZILDAY[1], Ismail TURKAN[1], Rengin OZGUR[1]
1) Ege University, Faculty of Science, Department of Biology, Izmir, 35100, Turkey

The movement of Earth generates light and temperature change in environment and organisms such as plants have developed molecular oscillators to adapt these conditions[1]. Circadian clocks are internal timekeeping systems that drive daily rhythm in physiology and metabolism due to changeable environmental conditions[2]. Abiotic stress such as high temperature or salt stress are the example of those conditions, and these environmental fluctuations can cause endoplasmic reticulum stress (ER stress) by increasing the misfolded or unfolded proteins in the ER[3]. ER stress induces a specific mechanism within the cell called unfolded protein response (UPR) which stimulates transcription of a series genes that increase the protein folding capacity in the ER[4] Aim of this work was to investigate interaction among circadian clock and protein folding capacity and ER stress response. For this aim, *Arabidopsis thaliana* plants were trained to 12/12 h (LD) and to 24 h constant light (LL) to determine their response to tunicamycin induced ER stress. Moreover, expressions of genes related to UPR were measured at 4,8, 12, 16 and 24 h time points after onset of ER stress. In addition, we also investigated if ER stress can change period or amplitude of *A. thaliana* molecular clock. As results, Tm enhanced the expressions of ER stress sensor/transducer genes (*bZIP17, bZIP28, bZIP60, IRE1A, IRE1B*), ER chaperones and folding helper genes (*BiP1, BiP3, ERO1, CNX*), ER-associated degradation (ERAD) genes (*DER1, SEL, HRD1*), and ER stress associated apoptosis genes (*AGB1, NAC089*) in shoots. LL and LD treatments have different effects on UPR of A. thaliana whereas LL grown plants showed decreased ER stress response as compared to LD grown plants.

References

1) Ding, Z., Millar, A. J., Davis, A. M., & Davis, S. J. (2007). TIME FOR COFFEE encodes a nuclear regulator in the Arabidopsis thaliana circadian clock. *The Plant Cell*, *19*(5), 1522-1536.
2) Haydon, M. J., Mielczarek, O., Robertson, F. C., Hubbard, K. E., & Webb, A. A. (2013). Photosynthetic entrainment of the Arabidopsis thaliana circadian clock. *Nature*, *502*(7473), 689-692.
3) Moreno, A. A., & Orellana, A. (2011). The physiological role of the unfolded protein response in plants. *Biological research*, *44*(1), 75-80.
4) Ozgur, R., Uzilday, B., Iwata, Y., Koizumi, N., & Turkan, I. (2018). Interplay between the unfolded protein response and reactive oxygen species: a dynamic duo. *Journal of experimental botany*, *69*(14), 3333-3345.

IL-8.1.1

COVID-19 through a UV lens

Shelley Gorman[1]
1) Telethon Kids Institute, University of Western Australia, Perth, Australia

In response to the COVID-19 (coronavirus disease of 2019) pandemic, researchers have been seeking low-cost and accessible means of providing protection from its harms, particularly for at-risk individuals such as those with cardiovascular disease, diabetes and obesity. One possible way is via safe sun exposure, and/or dietary supplementation with induced beneficial mediators, such as vitamin D and nitric oxide. Potential benefits could be mediated through the disinfectant properties of UV radiation, and its capacity to modulate immunity and limit cardiometabolic dysfunction. Anti-inflammatory effects of UV-induced mediators might limit the extent of the COVID-19 cytokine storm to reduce morbidity. There are also likely important UV dose considerations. While there has been significant commentary on potential benefits for vitamin D, the direct effects of exposure to UV light on COVID-19 has received less attention. Data collected to-date suggests that ambient levels of both UVA and UVB may be beneficial for reducing severity or mortality due to COVID-19. Currently unresolved are the nature of the associations between blood 25(OH)D and COVID-19, with more prospective data needed that better considers lifestyle factors, such as physical activity and personal sun exposure levels. Indeed, there has been limited data collected on the associations between personal sun exposure and COVID-19-related outcomes. Many clinical trials are underway testing the potential for vitamin D supplementation or inhaled nitric oxide in treating COVID-19 and related outcomes. While some protective effects are hypothesized for UV light (and induced mediators) for improving respiratory and cardiometabolic health during COVID-19, more evidence is needed to demonstrate causality[1].

References

1) S. Gorman, R. B. Weller, Investigating the potential for ultraviolet light to modulate morbidity and mortality from COVID-19: A narrative review and update. Frontiers in Cardiovascular Medicine, 7, Article 616527, December 2020.

IL-8.1.2

UV, blood pressure and cardiovascular disease

Richard B Weller,
1)Centre for Inflammation Research, University of Edinburgh, UK.; 2) Department of Dermatology, Royal Infirmary of Edinburgh, UK

Excess sunlight is a risk factor for skin cancer in white skinned individuals, but there are no data linking it with increased all-cause mortality. By contrast, high blood pressure (BP) is the leading global cause of disability adjusted life years lost. A 5 mmHg fall in BP, even from a 'healthy' level produces a 10% reduction in major cardiovascular events[1]. Population BP at national level correlates with latitude, and in temperate countries population winter BP is 2-3 mmHg lower than summer. Prospective data from Sweden show that increased sunlight exposure independently correlates with reduced cardiovascular and all-cause mortality after 25 years[2].
The well-known seasonal variation in BP and incident cardiovascular events has historically been ascribed to temperature and vitamin D synthesis.
I performed an observational study of 340,000 patients on renal dialysis, in >2,000 centres in the USA. BP was measured thrice weekly for 3 years in these patients, and cross referenced against satellite derived UV irradiation data at each location throughout this time, as was temperature. BP correlated inversely with UV and this effect was independent of temperature, although temperature had an independent additive effect. BP was higher in the Black American than White American patients and the UV related fall was attenuated[3].
Physiological formation of biologically active 1,25 dihydroxyvitamin D_3 requires UVB exposure. Measured vitamin D levels correlate inversely with incident cardiovascular disease, yet meta-analyses of extensive studies of oral vitamin D supplementation show that vitamin D plays no part in maintaining cardiovascular health[4]. Mendelian randomisation studies confirm this. Serum vitamin D levels are also a marker of sunlight exposure. UVA mobilises nitric oxide from stores in the skin to the systemic circulation where it is an arterial vasodilator[5]. This vitamin D independent mechanism probably mediates UV's action on cardiovascular disease.
I have recently completed a randomised cross-over, sham controlled, single blinded clinical trial of the use of UVA lamps to treat patients with mild hypertension. Treatment was whole body irradiation with 5J/cm^2 UVA from a home phototherapy lamp, daily for two weeks. After a 2-4 week washout period, sham irradiation with the identical lamps screened to block wavelengths <500nm. The sequence of treatments was randomised. Clinic BP was measured at the start and end of active and sham irradiation periods. 14 patients were recruited. Mean arterial BP fell significantly more in the active (-8 ± 2.9mmHg) than control (+1 ± 3 mmHg p=0.033) arm of the trial. The change in BP was more marked for patients recruited in the darker (autumnal equinox to vernal equinox) than lighter half of the year.
Deaths from hypertension related disease in the UK are around 60 times more common in the UK than skin cancer deaths. Epidemiological, mechanistic and now interventional data all show an effect of sunlight or ultraviolet radiation in lowering BP. Advice on sun exposure needs to consider benefits as well as hazards.

References

1. Rahimi K, Bidel Z, Nazarzadeh M, et al. Pharmacological blood pressure lowering for primary and secondary prevention of cardiovascular disease..... *The Lancet* 2021
2. Lindqvist PG, Landin-Olsson M. The relationship between sun exposure and all-cause mortality. *Photochem Photobiol Sci* 2017;
3. Weller RB, et al. Does Incident Solar Ultraviolet Radiation Lower BP? *J Am Heart Assoc* 2020;
4. Weller RB. Beneficial Effects of Sunlight May Account... *JAMA Cardiol* 2020;
5. Liu D, Fernandez BO, Hamilton A, et al. UVA irradiation of human skin vasodilates arterial vasculature and lowers blood pressure independently of nitric oxide synthase. *J Invest Dermatol* 2014;

Acknowledgements. This work was part supported by project grant PG/15/23/31362from the British Heart Foundation.

IL-8.1.3

Myopia progression during COVID-19 lockdown and UV deprivation

Seyhan Yazar[1,2]
1) Garvan Institute of Medical Research, Garvan-Weizmann Centre for Cellular Genomics, Sydney, Australia; 2) Lions Eye Institute, Genetics and Epidemiology, Perth, Australia

Staying at home has become the new norm as a result of COVID-19. All of us had to reduce our world to the four walls of our houses for weeks if not for months. Most school children are learning at home, online. This new environment has major implications on eye health. Myopia or short-sightedness is linked to spending lots of time looking at near objects and very little time looking at distant objects. In the pre COVID-19 era, myopia prevalence was on the rise and this increase has been linked to children spending too much time inside and studies have shown that encouraging children to spend more time outside can prevent myopia.[1,2] There are multiple hypotheses explaining this protective effect including exposure to the bright sunlight and UV. In this talk, the most recent myopia rates and effect of UV deprivation on these rates will be discussed.

References

1) He M, Xiang F, et al. Effect of Time Spent Outdoors at School on the Development of Myopia Among Children in China: A Randomized Clinical Trial. JAMA, 2015.
2) Wu Pei-Chang, Chueh-Tan Chen MS et al. Myopia Prevention and Outdoor Light Intensity in a School-Based Cluster Randomized Trial. Ophthalmology, 2018.

OC-8.1.4

Evaluation of blue light protection of the skin

Terje Christensen[1,2], Bjørn J. Johnsen[2], Ellen M. Bruzell[3]

1) University of South-Eastern Norway, Drammen, Norway; 2) Norwegian Radiation and Nuclear Safety Authority (DSA), Østerås, Norway; 3) Nordic Institute of Dental Materials (NIOM), Oslo, Norway

Visible-light effects on the skin have been regarded less hazardous than effects of ultraviolet radiation (UV). Uncertainties exist about the limit values for visible exposure, types of effects, and the action spectrum of chromophores that may give rise to unwanted effects. DNA is assumed to be the most important chromophore for UV, while several others absorbing visible light are distributed in the tissues, some of which are naturally occurring and some exogenous, resulting from e.g. pollution, use of cosmetics or photoactive medication. In the absence of exact knowledge, industry markets various protective products. The main aim of this presentation is to evaluate whether protection against blue light is needed and if so, in what situations and for which groups.

We have measured the irradiance values for several typical general lighting sources and electronic screens, such as of mobile phones and personal computers. The lowest dose of blue light reported to cause biological effects in normal skin was estimated from a literature study. The estimated dose was used to compute the minimum exposure times to light from various sources necessary for obtaining reported skin effects (T_{thres}). Corresponding exposure times were calculated for outdoor conditions and a selection of medical devices.

The T_{thres} for a range of biological effects on human skin and skin cells was between 5 and 200 Jcm^{-2}, and the only consistently reported effect, pigmentation, was observed after an average exposure of 65 Jcm^{-2}. This dose will be reached by at least one month of continuous exposure to electronic screens or two weeks in a 500 lx LED-lit office environment. In comparison, T_{thres} is reached in 2 h at noon mid-summer in Oslo (at UV-index 6, which requires protection against UV-damage to the skin). Medical sources intended for light curing of dental materials emit blue light which is 100 times stronger than the maximum outdoor values in Oslo; however, the exposure conditions cannot be compared[1].

We conclude that for the normal, healthy population, UV protection of the skin is essential, compared with the suggested use of protection against blue light. Exposure to indoor general lighting and electronic screens will not exceed doses that can induce skin effects within practical exposure times. Exceptions may exist for e.g. photosensitizing drug use or exposure to exogenous substances.

Reference

1) Christensen, T., Johnsen, B.J. & Bruzell, E.M. Violet-blue light exposure of the skin: is there need for protection? Photochem Photobiol Sci 20, 615–625 https://doi.org/10.1007/s43630-021-00043-9, 2021.

IL-8.1.5

Solar ultraviolet radiation slows lung function decline in premenopausal women in a longitudinal and population-based cohort (ECRHS)

Kai Triebner (PhD)[1, 2], Steinar Hustad (MD)[1, 2], Jose Barrera-Gómez (MSc)[3, 4, 5], Xavier Basagaña (PhD)[3, 4, 5],Bryndís Benediktsdóttir (MD)[6,7], Shyamali Dharmage (MD)[8], Karl A Franklin (MD)[9], Elaine Fuertes (PhD)[10], Vanessa Garcia-Larsen (PhD)[11], Jose Gullón-Blanco (MD)[12], Rain Jõgi (MD)[13], Bénédicte Leynaert (PhD)[14,15], Eva Lindberg[16], Jesús Martínez-Moratalla (MD)[17, 18], Nerea Muniozguren Agirre (MD)[19], Isabelle Pin (MD)[20, 21], Chantal Raherison (MD)[22], Antonio Pereira-Vega (MD)[23], Vivi Schlünssen (PhD)[24, 25], Antonia Valentin (PhD)[3, 4, 5], Francisco Gómez Real (MD)[1, 26*] and Payam Dadvand (PhD)[3, 4, 5*]
* The authors contributed equally.

[1]University of Bergen, Jonas Liesveg 92, 5021 Bergen, Norway; [2]Core Facility for Metabolomics, Jonas Liesveg 92, 5021 Bergen, Norway; [3]ISGlobal, Carrer del Dr. Aiguader, 88, 08003 Barcelona, Spain; [4]Universitat Pompeu Fabra, Plaça de la Mercè, 10, 08002 Barcelona, Spain; [5]CIBER Epidemiología y Salud Pública, Avenida Monforte de Lemos 3-5, 28029 Madrid, Spain; [6]Faculty of Medicine, University of Iceland, Reykjavik, Iceland; [7]Department of Sleep, Landspitali University Hospital, Hringbraut 101, Reykjavík, Iceland; [8]Allergy & Lung Health Unit, Melbourne School of Population Health, Melbourne University, 207 Bouverie Street, 3053 Carlton, Australia; [9]Department of Surgical and Perioperative Sciences, Surgery, Umea University, 901 87 Umeå, Sweden; [10]Department of Respiratory Epidemiology, Occupational Medicine and Public Health, Imperial College, South Kensington, London, UK; [11]Department of International Health, Johns Hopkins University, Bloomberg School of Public Health, 615 N Wolfe Street, Baltimore, USA; [12]Department of Pneumology, Hospital San Agustin de Aviles, Camino de Heros 6, 33401 Avilés, Asturias, Spain; [13]Department of Lung Medicine, Tartu University Clinic, Ludvig Puusepa 1a, 50406 Tartu, Estonia; [14]Inserm-U1168, VIMA, 16 Avenue Paul Vaillant Couturier, 94807 Villejuif, France; [15]UMR-S 1168, UVSQ, Université Versailles St-Quentin-en-Yvelines, 55 Avenue de Paris, 78000 Versailles, France; [16]Department of Medical Sciences, Uppsala University, Akademiska sjukhuset, 75185 Uppsala, Sweden; [17]Pulmonology Service, Albacete University Hospital, Health Service Castilla - La Mancha, C. Hermanos Falco 37, 02008 Albacete, Spain; [18]Medicine School of Albacete, University of Castilla-La Mancha, Calle Almansa, 14, 02008 Albacete, Spain; [19]Unit of Epidemiology and Public Health, Department of Health, Basque Government, Gran Vía 85, 48011 Bilbao, Spain; [20]Department of Pediatrics, University Hospital Grenoble Alpes, Inserm, Avenue Maquis du Grésivaudan, 38700 La Tronche, France; [21]Institute for Advanced Biosciences, Grenoble; University Grenoble Alpes, CS 10217, 38043 Grenoble cedex 9, France; [22]U1219, Bordeaux Population Health Research, Bordeaux University, 146 rue Leo Saignat, 33076 Bordeaux, France, [23]Service of Pneumology and Allergy, University Hospital Juan Ramón Jiménez, Ronda Exterior, Paseo Nte., 21005 Huelva, Spain; [24]Department of Public Health, Environment, Occupation and Health, Danish Ramazzini centre, Aarhus University, Palle Juul-Jensens Boulevard 99, 8200 Aarhus, Denmark; [25]National Research Center for the Working Environment, Lersø Parkallé 105, 2100 Copenhagen, Denmark; [26]Department of Gynecology and Obstetrics, Haukeland University Hospital, Jonas Liesveg 92, 5021 Bergen, Norway;

Introduction: During the mid-twenties a woman's lung function starts declining and this decline accelerates after menopause.[1] The slope of decline may be influenced by exposure to solar ultraviolet radiation through its effects on inflammatory status and endogenous vitamin D concentrations. [2,3]
Objectives: To explore associations between exposure to solar ultraviolet radiation and lung function decline, before and after menopause, and potential effect modification by vitamin D intake through dietary sources.
Methods: Using 10-year follow-up data from the international European Community Respiratory Health Survey and satelite data on the daily effective ultraviolet irradiance during this period, we modelled individual exposure to solar ultraviolet radiation. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured at baseline and follow-up. Linear mixed effects regression models with lung function change as outcome and solar ultraviolet radiation as exposure were fitted and adjusted for potential confounders.
Results: An increase of one interquartile range in solar ultraviolet radiation exposure was associated with a reduced FVC decline of -3.3mL/y (95%CI: -6.5 to -0.1mL/y) in the whole study population. This association was driven by

premenopausal women (-8.1mL/y, -14.7 to -1.5mL/y) and was independent of dietary vitamin D intake. No statistically significant associations were observed for FEV_1.
Conclusions: Exposure to solar ultraviolet radiation is associated with reduced lung function decline in women. This is of public health interest and a previously unknown health benefit of solar ultraviolet radiation.

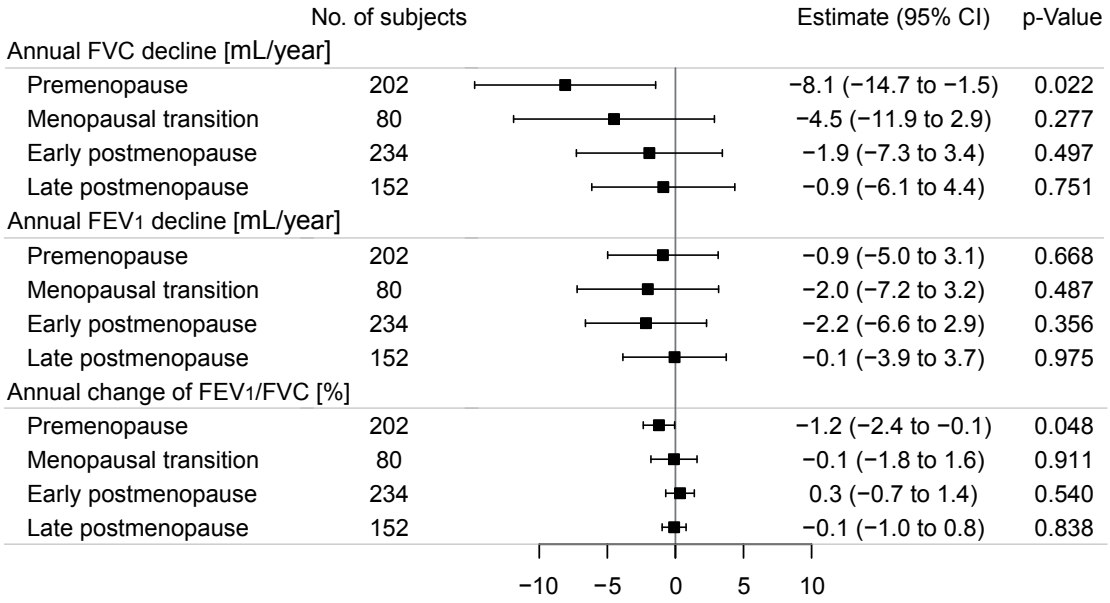


Fig. 1 – Forest plot of the results for FVC decline, FEV_1 decline and change of FEV_1/FVC over 10 years of follow-up, associated with one interquartile range increase in UVR exposure; adjusted for age, weight, height, skin type, dietary vitamin D intake, age at completed full-time education (as socio-economic proxy), difference in weight, pack-years, population sample and spirometer, including 95% confidence interval (negative values represent reduced decline);

References

1. Triebner K, Matulonga B, Johannessen A, et al. Menopause Is Associated with Accelerated Lung Function Decline. *American journal of respiratory and critical care medicine* 2017; **195**(8): 1058-65.
2. Brumpton BM, Langhammer A, Henriksen AH, et al. Vitamin D and Lung Function Decline in Adults With Asthma: The HUNT Study. *American Journal of Epidemiology* 2016; **183**(8): 739-46.
3. Thuesen BH, Skaaby T, Husemoen LLN, Fenger M, Jørgensen T, Linneberg A. The association of serum 25-OH vitamin D with atopy, asthma, and lung function in a prospective study of Danish adults. *Clinical & Experimental Allergy* 2015; **45**(1): 265-72.

Acknowledgements. Kai Triebner received a postdoctoral fellowship from the University of Bergen. Payam Dadvand is funded by a Ramón y Cajal fellowship (RYC-2012-10995) awarded by the Spanish Ministry of Economy and Finance. The present analyses are part of a project funded by the Norwegian Research Council (Project No. 228174). The coordination of ECRHS 2 was supported by the European Commission, as part of their Quality of Life program, (Grant code: QLK4-CT-1999-01237). The coordination of ECRHS 3 was supported by the Medical Research Council (Grant No. 92091). Bodies funding the local studies are listed in the online data supplement sections 1 and 2.

IL-8.1.6

Melanin, DNA photodamage and vitamin D synthesis

Antony R Young
King's College London, St John's Institute of Dermatology, London, UK

Exposure to solar ultraviolet radiation (UVR) has beneficial as well as adverse effects, and it is important to optimise the risk benefit ratios of these effects under different conditions. Human epidermis contains melanin, the quantity of which depends on Fitzpatrick skin type (FST). Light skinned FST I/II individuals have very low levels of constitutive melanin whereas very high levels are present in FST VI with black skin. Irrespective of FST, melanin shows a gradient with the highest concentration in the basal layer that contains melanocytes and keratinocyte stem cells.

Epidermal DNA photodamage with mutagenic and carcinogenic potential, specifically cyclobutane pyrimidine dimers (CPD), and vitamin D synthesis are acute responses to solar UVR. These responses are very dependent on melanin as determined in studies comparing FST I/II with FST VI. In the case of CPD[1], the previously unexposed buttock skin of healthy young volunteers was exposed to single comparably erythemal doses of solar simulated radiation (SSR) and CPD were assessed immediately after exposure in three different epidermal zones; basal, middle and upper. Protection factors by melanin against CPD were estimated in each zone and shown to be ~60 for the basal layer, ~17 for the middle layer and ~5 for the upper layer, and the level of protection was related to melanin concentration. The very high level of DNA protection afforded by melanin in the basal layer is the likely reason for the large difference in skin cancer incidence in black and white skins.

The chromophore for vitamin D synthesis is epidermal 7-dehydrocholesterol (7-DHC). The effect of melanin on vitamin D synthesis was compared in black and white skins after 5 serial exposures (with 3-4 day intervals) of whole-body (85% body surface area) fluorescent SSR or TL01 (monochromatic UVB at ~311nm)[2]. The exposure doses were the same for all skin types and were sub-erythemal for FST I/II. Blood samples were taken before, during and after the exposure protocol and assessed for 25(OH)D_3 (gold standard for vitamin D synthesis) by HPLC tandem mass spectrometry. These data were used to construct linear dose response curves and the ratio of the black vs white slopes was used to give an index of melanin inhibition of vitamin D synthesis. With both UVR spectra the melanin inhibition factor was <1.5. The most likely reason for this very low value is that there is ample 7-DHC above the melanin rich basal layer in black skin to allow good vitamin D synthesis.

Taken together, these studies shown that the inhibitory role of melanin in deeply pigmented skin depends on its location and quantity within the epidermis, and how it interacts with different chromophores. In the case of CPD, the chromophore is nuclear DNA and melanin is highly protective in the melanin rich basal layer. In the case of vitamin D, there is clearly sufficient chromophore (i.e., 7-DHC) distributed above the basal layer to allow good vitamin D synthesis, such that the inhibitory effect of melanin is very small compared to its effect on nuclear DNA. This means that any discussion on the effects of melanin on photobiological outcomes must be specific not only for outcome, but also the location in the epidermis at which that outcome occurs.

Finally, these results have public health implications. People with deeply pigmented skins, who often have sub-optimal vitamin D status, may be encouraged to improve their vitamin D status by spending more time in the sun because their risk of skin cancer is very low. The high incidence of skin cancer in those with light skins can only be reduced to that of black skins if their level of routine photoprotection is very high, though small sub-erythemal doses of solar UVR are necessary for vitamin D synthesis.

References

1) D. Fajuyigbe, S. M. Lwin, B. L. Diffey, R. Baker, D. J. Tobin, R. P. E. Sarkany and A. R. Young, Melanin distribution in human epidermis affords localized protection against DNA photodamage and concurs with skin cancer incidence difference in extreme phototypes, *FASEB J*, **32**, 3700-3706, 2018.

2) A. R. Young, K. A. Morgan, T. W. Ho, N. Ojimba, G. I. Harrison, K. P. Lawrence, N. Jakharia-Shah, H. C. Wulf, J. K. Cruickshank and P. A. Philipsen, Melanin has a small inhibitory effect on cutaneous vitamin D synthesis: A comparison of extreme phenotypes, *J Invest Dermatol*, **140**, 1418-1426 e1411, 2020.

IL-8.1.7

UVR, B cells and regulation of the development of multiple sclerosis

Prue Hart[1], Stephanie Trend[1,2], Jonatan Leffler[1], Scott N Byrne[3,4], Allan G Kermode[2,5], Martyn A French[6]
1)Telethon Kids Institute, University of Western Australia, Perth, WA, Australia; 2)Centre for Neuromuscular and Neurological Disorders, Perron Institute for Neurological and Translational Science, University of Western Australia, Perth, WA, Australia; 3)The University of Sydney, School of Medical Sciences, Faculty of Medicine and Health, NSW, Australia; 4)Westmead Institute for Medical Research, Centre for Immunology and Allergy Research, Westmead, NSW Australia; 5)Institute for Immunology and Infectious Disease, Murdoch University, Perth, WA, Australia; 6)UWA Medical School and School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia

Reduced sun exposure is a risk factor for development of multiple sclerosis (MS). To investigate the beneficial effect of UV radiation on MS, the PhoCIS trial (Phototherapy for Clinically Isolated Syndrome) was established whereby participants with the earliest form of MS, namely Clinically Isolated Syndrome (CIS), were given a course of suberythemal narrowband UVB (311-312 nm) phototherapy over 8 weeks (3 sessions/week). Both those receiving phototherapy and those in the control group (no phototherapy) were followed for 12 months by magnetic resonance imaging (MRI) and by venesection at regular intervals. When the primary outcome of MRI changes was examined, there was a non-significant reduction (100% to 70%) in the rate of conversion from CIS to MS within 12 months in participants who received phototherapy. UVB-induced changes in the circulating immune cells of the participants were investigated to illuminate the mechanisms of action of narrowband UVB, and whether they contributed to reducing CIS to MS progression.
The most substantial changes over the first 2 months of phototherapy involved B cells, a cell type whose importance has been highlighted by the successful use of anti-CD20 monoclonal antibodies to treat MS. There was a significant decrease in $CD27^{+}/IgD^{-}$ memory B cells (MBC) and a significant increase in naïve B cells ($CD27^{-}/IgD^{+}$) (both as a % of B cells) in participants that received phototherapy compared with those who did not.
In a study of the function of circulating B cells following phototherapy, responses to an innate B cell stimulus, resiquimod (R848) via the production of TNF and IL-10 were investigated. TNF is not only a pro-inflammatory cytokine but is important as a co-factor supporting B cell-T cell and B cell-myeloid cell interactions. IL-10, in contrast, is the prototypic anti-inflammatory cytokine and has been shown to be important for identification of human B cells with immune regulatory properties.
Using multi-parametric flow cytometry and regression analysis, significantly fewer B cells produced TNF in response to R848, whilst there were no significant changes detected in the capability of B cells to produce IL-10 in response to a TLR7 ligand. Thus, narrowband UVB phototherapy reduced B cell responses *in vitro* to a TLR7 ligand. This study highlights that narrowband UVB phototherapy exerts systemic immunological effects beyond the skin. We propose that the B cell function curtailed by phototherapy is pathogenically important and may reflect priming effects of phototherapy that allow for reduced responses by B cells to polyclonal activation.

OC-8.1.8

Determining the efficacy of a popular natural homemade sunscreen promoted by wellness bloggers on social media.

Furkan A. Ince[1], James McClelland[1], Katie M. Dixon[1].
1) School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, NSW 2006 Australia.

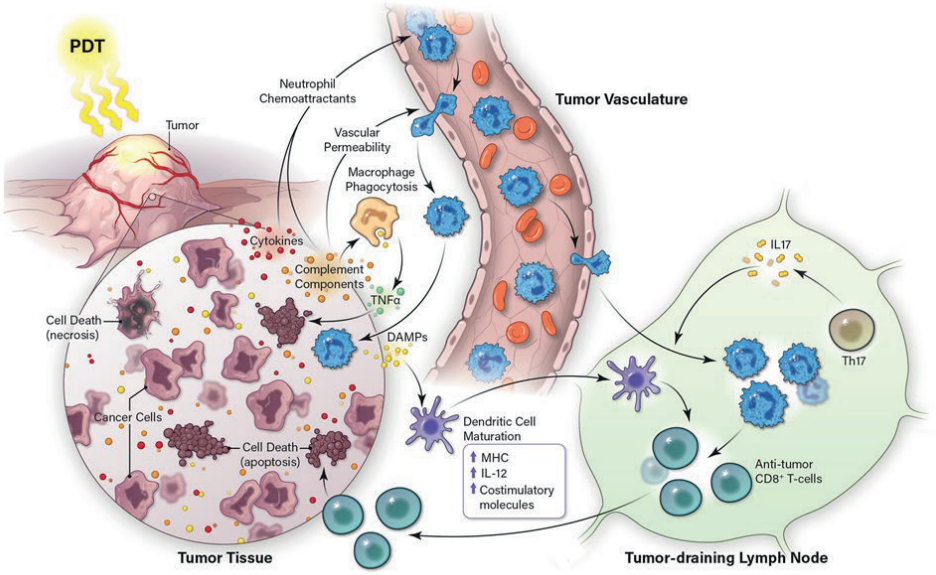
In Australia, the use of commercially available sunscreens is strongly recommended by health authorities to protect from the harmful effects of ultraviolet (UV) radiation, including keratinocyte cancers and melanoma. However, these commercial sunscreens contain some ingredients associated with negative impacts on health and the environment. For example, oxybenzone has been shown to have adverse effects on endocrinological function in fish and rats and additionally can contribute to coral bleaching. Furthermore, some ingredients in commercial sunscreens may cause contact dermatitis in humans. Subsequently, this has led to rising popularity in natural homemade sunscreens. Numerous natural sunscreen recipes have been published online by different wellness bloggers with a large following. However, these natural sunscreens lack the backing of scientific evidence for their effectiveness. We tested the efficacy of a natural sunscreen published online by a wellness blogger with 694,000 followers on Facebook and 32,000 followers on Twitter, with the aim of determining whether this natural sunscreen is indeed as photoprotective as commercial SPF50+ sunscreens. The sunscreen contained (v/v) almond oil 39 %, coconut oil 19 %, shea butter 10%, beeswax 19 %, red raspberry seed oil 1.6 %, carrot seed oil 1.6 % and zinc oxide 5 %. *Ex vivo* skin samples were obtained with consent from patients undergoing elective surgery. Skin samples were cut into equal pieces and were then treated with either a commercial SPF50+ sunscreen, natural sunscreen or base lotion (2 mg/cm^2). Treatments were applied 20 minutes prior to irradiation with a solar simulator (20 J/cm^2) and kept on during irradiation. The natural sunscreen was prepared either one day or three weeks before irradiation and stored at room temperature. Some of the skin samples were fixed three hours following UV-irradiation and assessed for the levels of UV-induced DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) using immunohistochemistry. The remaining skin samples were fixed 24 hours following UV-irradiation and stained with H&E to assess for sunburn cells and epidermal thickness. The red raspberry seed oil and carrot seed oil, which are claimed to have SPFs of 25-50 and 35-40 respectively by the wellness blogger, were further studied *in vitro* in human skin cells to determine if they could protect against UV-induced cell death and DNA damage. With the trend toward social media for health advice, consumers choosing to formulate homemade sunscreens lacking scientific testing for efficacy and shelf-life risk unknowingly exposing themselves to the harmful effects of UV including skin cancers. On the other hand, the protective effects of these natural homemade sunscreens may help to overcome sceptical attitudes towards commercial sunscreen use, and may further avoid the negative health and environmental impacts associated with commercial sunscreens.

IL-8.2.1

PDT Enhancement of Anti-tumor Immunity: Mechanisms and Usage

Sandra O. Gollnick[1,2], Riddhi Falk-Mahaptara[2], Kimberley Ramsey[1]
Roswell Park Comprehensive Cancer Center, PDT Center, Department of Cell Stress Biology, Buffalo, NY, USA; 2) Roswell Park Comprehensive Cancer Center Department of Tumor Immunology, Buffalo, NY, USA Department of Cell Stress Biology, Buffalo, NY, USA

Over the last decades the interest in PDT as an enhancer of anti-tumor immunity and our understanding of the mechanisms by which PDT enhances anti-tumor immunity have dramatically increased. We and others have shown that oxygen conservation is critical to the ability of PDT to enhance anti-tumor immunity and that PDT-induced inflammation, which is defined by increased neutrophil migration and cytokine production, drives induction of anti-tumor immunity[1]. These results suggest that PDT regimens that augment anti-tumor immunity (imPDT) have the potential to act as adjuvants that in combination with other ablative anti-cancer therapies, potentially leading to increased efficacy against distant disease.



References

1) Falk-Mahapatra R and Gollnick SO, Photodynamic Therapy and Immunity: An Update. Photochemistry and Photobiology 96 (3):550-559, 2020.

Acknowledgements. This work was supported in part by the National Cancer Institute of the National Institute of Health under Award 5P01CA98156 (SG) and the Roswell Park Alliance Foundation. The study used shared resources supported by Roswell Park Cancer Comprehensive Cancer Center Support Grant (P30CA016056). Riddhi Falk-Mahapatra was supported by the Department of Immunology Training Grant (5T32CA085183-15).

IL-8.2.2

Immunogenic cell death induced by PDT: a new approach for cancer therapy

Dmitri V. Krysko[1,2,3], Victoria D. Turubanova[3], Tatiana A .Mishchenko[3], Irina V. Balalaeva[3], Iuliia Efimova[1,2], Olga Krysko[4] , Maria V. Vedunova[3]
1) Cell Death Investigation and Therapy Laboratory, Department of Human Structure and Repair, Ghent University, Ghent, Belgium; 2) Cancer Research Institute Ghent, Ghent, Belgium; 3) Institute of Biology and Biomedicine, National Research Lobachevsky State University of Nizhni Novgorod, Nizhni Novgorod, Russian Federation; 4) Upper Airways Research Laboratory, Department of Head and Skin, Ghent University, Ghent, Belgium

Immunotherapy has become an important part in cancer treatment during the last decade. One of the major factors for induction of immune response during therapy is immunogenic cell death (ICD)[1]. Immunogenicity of dying cancer cells is mediated by their adjuvanticity and antigenicity. Damage-associated molecular patters (DAMPs) are endogenous molecules located inside the cells and in normal conditions contribute to different physiological processes while they are released (or exposed on the outer surface of plasma membrane) when a cell is damaged or dying. Once released, DAMPs acquire immunostimulatory properties and increase adjuvanticity of dying cancer cells. Of course, DAMPs are not the only factors released during cell death which are involved in their adjuvanticity. Antigenicity of dying cancer cells is another major determinant of ICD which is required for an efficient targeted induction of anti-tumor immunity. ICD can be induced by different stimuli and anticancer treatment modalities, including chemotherapy with anthracyclines and oxaliplatin, radiotherapy, UVC irradiation, oncolytic viruses and photodynamic therapy (PDT)[2]. The ICD induced by various stimuli can differ in the DAMPs' profile and has also been linked to different cell death modalities such as apoptosis, necroptosis and ferroptosis[3]. Thus, in this lecture, we first discuss the role of PDT in the induction of ICD[4] and then assess the advantages and disadvantages of PDT in the induction of ICD. Finally, we will discuss a possible synergistic action between PDT and ferroptotic cell death[5], a novel, iron-dependent form of regulated cell death[6]. PDT can act as a source of reactive oxygen species for the Fenton reaction, which may reinforce ferroptosis induction and increase PDT efficacy in anticancer therapy.

References

1) Galluzzi, Vitale, et al. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. J Immunother Cancer. 2020.
2) Alzeibak, Mishchenko et al. Targeting immunogenic cancer cell death by photodynamic therapy: past, present and future. J Immunother Cancer. 2021.
3) Efimova, Catanzaro et al. Vaccination with early ferroptotic cancer cells induces efficient antitumor immunity. J Immunother Cancer. 2020.
4) Turubanova, Balalaeva *et al.* Immunogenic cell death induced by a new photodynamic therapy based on photosens and photodithazine. J Immunother Cancer 2019.
5) Mishchenko, Balalaeva et al. Ferroptosis and Photodynamic Therapy Synergism: Enhancing Anticancer Treatment. Trends Cancer. 2021.
6) Friedmann Angeli, Krysko et al. Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. Nat Rev Cancer. 2019.

Acknowledgements

This study was supported by the Russian Science Foundation (RSF) https://rscf.ru/en/project/18-15-00279/ and by FWO-Flanders projects FWO G043219N and FWO G016221N and by UGent BOF 01/O3618.

IL-8.2.3

Immune responses triggered by nanobody-targeted photodynamic therapy

Sabrina Oliveira
Utrecht University, Department of Biology and Department of Pharmaceutical Sciences, Utrecht, The Netherlands

Nanobodies are the variable domain of heavy chain antibodies that exist in Camelids[1]. The relatively small dimensions of nanobodies combined with high binding affinities to their targets, lead to rapid tumor accumulation, homogenous distribution, and rapid clearance of unbound fractions. We took advantage of these properties and have developed an alternative approach for targeted photodynamic therapy (PDT) by conjugating photosensitizers to nanobodies[2]. Several of our studies are targeting the epidermal growth factor receptor (EGFR) for head and neck cancer. As photosensitizer, we have employed the IRDye700DX, that is a silicon phthalocyanine derivative, currently being tested in clinical trials conjugated to the monoclonal antibody cetuximab, and already approved for clinical use in Japan. Generally, with nanobody-targeted PDT, cytotoxicity is selectively induced in cells which have high target expression level. Preclinical studies have shown selective tumor necrosis[3] and significant tumor regression after a single treatment session[4].
More recently, we have explored the immunomodulatory potential of nanobody-targeted PDT, using high and moderate EGFR-expressing cells. After nanobody-targeted PDT, the cytoplasmic damage-associated molecular pattern (DAMP) HSP70 was detected on the cell membrane of tumor cells and the nuclear DAMP HMGB1 was found in the cell cytoplasm. Furthermore, nanobody-targeted PDT induced the release of the DAMPs HSP70 and ATP, as well as the pro-inflammatory cytokines IL- 1β and IL-6. Conditioned medium from high EGFR-expressing tumor cells treated with nanobody-targeted PDT led to the maturation of human monocyte-derived dendritic cells, as indicated by the upregulation of CD86 and MHC II on their cell surface. Subsequently, these dendritic cells induced CD4+ T cell proliferation, accompanied by IFNγ release. Together, these results suggest nanobody-targeted PDT can stimulate the immune system[5]. This presentation will summarize these studies and discuss current efforts to further investigate the systemic reach of this selective and localized treatment.

References

1) Hamers-Casterman, C.; Atarhouch, T.; et al., Naturally occurring antibodies devoid of light chains. Nature 1993, 363 (6428), 446-8.
2) Heukers, R.; van Bergen en Henegouwen, et al., Nanobody-photosensitizer conjugates for targeted photodynamic therapy. Nanomedicine : nanotechnology, biology, and medicine 2014, 10 (7), 1441-51.
3) van Driel, P.; Boonstra, M. C.; et al., EGFR targeted nanobody-photosensitizer conjugates for photodynamic therapy in a pre-clinical model of head and neck cancer. Journal of controlled release : official journal of the Controlled Release Society 2016, 229, 93-105.
4) Deken, M. M.; Kijanka, M. M.; et al., Nanobody-targeted photodynamic therapy induces significant tumor regression of trastuzumab-resistant HER2-positive breast cancer, after a single treatment session. Journal of controlled release : official journal of the Controlled Release Society 2020, 323, 269-281
5) Beltrán Hernández I, Angelier ML, et al., The Potential of Nanobody-Targeted Photodynamic Therapy to Trigger Immune Responses. Cancers (Basel). 2020 Apr 15;12(4):978

Acknowledgements. This work was supported by the European Research Council (ERC, Starting Grant 677582).

OC-8.2.4

Ablation of the cancer-associated fibroblast in pancreatic cancer using FAP-targeted photodynamic therapy

Daphne Dorst[1], Esther Smeets[1], Anne Freimoser-Grundschober, Christian Klein[2], Marija Trajkovic-Arsic[3], Martin Gotthardt[1], Jens Siveke[3], Erik Aarntzen[1] and Sanne A.M. van Lith[1]
1)Radboud university medical center, Medical Imaging, Nijmegen, The Netherlands; 2) Innovation Center Zurich, Roche Pharmaceutical Research and Early Development, Zurich, Switzerland; 3) University Hospital Essen, Translational Oncology, Germany

Pancreatic ductal adenocarcinoma (PDAC) patients have a dismal 5-year survival rate of 8%. PDAC tumors are characterized by a dense extracellular matrix that is produced by the cancer-associated fibroblast (CAF). This extracellular matrix causes increased interstitial pressure and vascular collapse, thereby impeding perfusion and delivery of systemic agents, and mediating exclusion of CD8+ T cells. Secondly, the CAFs create an immune-suppressive tumor-microenvironment by secretion of immune-cell inhibiting chemokines. Altogether, the CAFs create a pro-tumorigenic microenvironment, causing currently used chemotherapies and novel immunotherapies to be largely ineffective. An elegant solution to modulate the microenvironment in PDAC is to remove the cell orchestrating it, the CAF. In this way, both the physical barrier posed by the hyaluronic acid,- and collagen-rich extracellular matrix as well as the other factors through which these CAFs mediate the pro-tumorigenic microenvironment are eliminated. As CAFs in PDAC overexpress fibroblast activation protein (FAP), we here developed an anti-FAP monoclonal antibody conjugated to a phthalocyanine-photosensitizer, for targeted depletion of the CAF using photodynamic therapy. We characterized efficiency *in vitro* and in a mouse model for PDAC.

The anti-FAP monoclonal antibody 28H1 and control monoclonal antibody DP47GS were conjugated with the chelator diethylenetriaminepentaacetic acid (DTPA) and the silicon-phthalocyanine photosensitizer IRDye700DX. Light-induced toxicity was investigated by incubation of NIH-3T3 murine fibroblasts and their FAP-overexpressing counterpart (3T3-FAP) with increasing concentrations of the conjugates and illumination with 50 J/cm^2 280 mW/cm^2 690 nm light. Subsequently, CKP (Ptf1awt/Cre;Kraswt/LSL-G12D;p53fl/fl) mice, which develop spontaneous PDAC tumors, were injected with 0.3 nmol ^{111}In-labeled 28H1-IRDye700DX (n=4) or 0.3 nmol ^{111}In-labeled DP47GS-IRDye700DX (n=3). Tumor targeting was determined by *ex vivo* biodistribution studies at 24 hours post injection. Six mice were subjected to photodynamic therapy by exposing the tumor to 50 J/cm^2 280 mW/cm^2 690 nm light at 24 hours post injection with 0.3 nmol 28H1-IRDye700DX (n=3) or 0.3 nmol DP47GS-IRDye700DX (n=3). One hour after light exposure, tumor tissue was collected and IRDye700DX-derived fluorescence was imaged. Tissues were formalin fixed and paraffin embedded, and presence of apoptotic cells was assessed using quantification of cleaved-caspase-3 immunohistochemistry.

Both 28H1 and DP47GS were conjugated successfully to DTPA and IRDye700DX, reaching substitution ratios of 2-2.5 IRDye700DX dyes per antibody. Only 28H1-IRDye700DX induced cell death of 3T3-FAP cells upon illumination, while no effect on 3T3 was observed. Both ^{111}In-labeled 28H1-IRDye700DX and ^{111}In-labeled DP47GS-IRDye700DX showed uptake in the pancreatic lesion of CKP mice at 24 hours post injection (13.1 ± 3.6 %ID/g and 11.4 ± 4.4 %ID/g, respectively). Residual blood activity of 28H1-IRDye700DX was however lower compared to that of DP47GS-700DX (8.2 ± 4.2 %IA/g and 21.2 ± 8.1 %IA/g, respectively), leading to significantly higher tumor-to-blood ratios for 28H1-IRDye700DX when compared to DP47GS-IRDye700DX (1.76 ± 0.45 and 0.51 ± 0.10, respectively ($p = 0.006$)). After illumination of the pancreatic tumors with 690 nm light, *ex vivo* fluorescence imaging illustrated that the IRDye700DX derived fluorescent signal was quenched in the light-exposed parts of the tumor. In the tumors treated with 28H1-IRDye700DX, both manual and automated analyses revealed increased expression of cleaved-caspase-3 when compared to DP47GS-treated tumors and when compared to the non-illuminated region of the same tumor.

The anti-FAP antibody 28H1-IRDye700DX targets PDAC tumors in mice with good signal-to-background ratios. Furthermore, it induces apoptosis upon illumination, and co-localization studies with fibroblast markers will reveal specificity of this cell death. Furthermore, in future experiments, the effect of depletion of FAP-expressing CAFs on the tumor micro-environment and efficacy of other systemic therapies such as chemo- and immunotherapies in pancreatic cancer models will be determined.

IL-8.2.5

Size-Transformable Antigen-Presenting Cell–Mimicking Nanovesicle PDT Potentiates Effective Cancer Immunotherapy

Xiaoyuan Chen[1]
1) National University of Singapore, Departments of Diagnostic Radiology, Surgery, Chemical and Biomolecular Engineering, Biomedical Engineering, Yong Loo Lin School of Medicine and Faculty of Engineering, Singapore, Singapore

Artificial antigen-presenting cells (aAPCs) provide three key signaling components: (i) major histocompatibility complex I/T cell receptor (MHC I/TCR) stimulatory signal, (ii) cluster of differentiation 80/cluster of differentiation 28 (CD80/CD28) costimulatory signal, and (iii) cytokine release [e.g., interleukin-2 (IL-2)]. aAPCs can stimulate CD8+ T cell activation. While nanosized aAPCs (naAPCs) have a better safety profile than microsized (maAPCs), they generally induce a weaker T cell response. Treatment with aAPCs alone is insufficient due to the lack of autologous antigen-specific CD8+ T cells. Here, we devised a nanovaccine for antigen-specific CD8+ T cell preactivation in vivo, followed by reactivation of CD8+ T cells via size-transformable naAPCs. naAPCs can be converted to maAPCs in tumor tissue when encountering preactivated CD8+ T cells with high surface redox potential. In vivo study revealed that naAPC's combination with photodynamic therapy had an impressive antitumor efficacy. Our findings provide a generalizable approach for using size-transformable naAPCs in vivo for immunotherapy in combination with nanotechnologies that can activate CD8+ T cells.

Acknowledgements. This work was supported by the start-up fund of the National University of Singapore (NUHSRO/2020/133/Startup/08) and NUS School of Medicine Nanomedicine Translational Research Programme (NUHSRO/2021/034/TRP/09/Nanomedicine)

IL-8.2.6

Induction of long-lasting anti-tumor immunity following PDT treatment using an investigational VLP-drug conjugate

Rhonda C. Kines[1], Cynthia D. Thompson[2], Sean Spring[1], Zhenyu Li[1], Elisabet de los Pinos[1], Stephen Monks[1], John T. Schiller[2]

1) Aura Biosciences, Cambridge, MA, USA; 2) Laboratory of Cellular Oncology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

The papillomavirus virus-like particle (VLP) preferentially targets tumor cells via cell surface modified heparin-sulfate proteoglycans[1]. We have demonstrated *in vivo* tumor cytotoxicity using an investigational VLP-IR700DX drug conjugate, belzupacap sarotalocan (AU-011), upon activation with near-infrared light (nIR) in murine models[2]. Upon *in vitro* activation with nIR light AU-011-mediated cell killing was pro-immunogenic in nature, resulting in the release of damage associated molecular patterns such as DNA, ATP and HMGB-1, activation of caspase-1, and surface re-localization of calreticulin and HSP70 on killed tumor cells. Using immunocompetent murine tumor models, TC-1 and MB49, we investigated the ability of AU-011 to eradicate subcutaneous tumors and to subsequently elicit long-term anti-tumor immunity alone or in combination with checkpoint inhibitor antibodies, anti-PD-1 or anti-CTLA-4. A single *in vivo* administration of AU-011 followed by nIR caused rapid cell death leading to complete tumor regression in ~50% of all animals out to 100 days and within hours of treatment, calreticulin surface expression, caspase-1 activation and depletion of immunosuppressive leukocytes were observed in tumors. Combination of AU-011 with immune checkpoint inhibitor antibodies, anti-CTLA-4 or anti-PD-1, improved therapeutic activity, resulting in a 70-100% complete response rate that was durable up to 100 days post-treatment. In both AU-011 monotherapy and AU-011/checkpoint inhibitor combination groups, 50-80% of complete responders displayed protection from secondary tumor re-challenge up to 100 days after the initial treatment. In the TC-1 model, depletion of CD4+ or CD8+ T-cells, either at the time of AU-011 treatment or secondary tumor re-challenge of complete responders, indicated that both cell populations were vital to AU-011's ability to eradicate primary tumors and to induce long-lasting anti-tumor protection. Furthermore, tumor-specific CD8+ T-cell responses could be observed in circulating PBMCs within three weeks of AU-011 treatment. These data, taken together, support the conclusion that AU-011 had a direct cytotoxic effect on tumor cells and induced long-term anti-tumor immunity in the absence of specifically targeted tumor antigens, and this activity was enhanced when combined with checkpoint inhibitor antibodies. AU-011 is currently being investigated in a Phase 2 clinical trial for the indication of choroidal melanoma.

References

1) Kines RC, Thompson CD, et al. Human papillomavirus capsids preferentially bind and infect tumor cells. Int J Cancer. 2016 Feb 15;138(4):901-11.
2) Kines RC, Varsavsky I, et al. An Infrared Dye-Conjugated Virus-like Particle for the Treatment of Primary Uveal Melanoma. Mol Cancer Ther. 2018 Feb;17(2):565-574.
3) Kines RC, Cerio RJ, et al. Virus-Like Particle-Drug Conjugates Induce Protective, Long-lasting Adaptive Antitumor Immunity in the Absence of Specifically Targeted Tumor Antigens. Cancer Immunol Res. 2021 Apr 14.

Acknowledgements. This work was supported by Aura Biosciences and the National Institutes of Health intramural research program.

IL-8.2.7

Combining PDT and immunotherapy

Ferry Ossendorp
Leiden University Medical Center, Tumor Immunology Group, Department Immunology, The Netherlands

Photodynamic therapy (PDT) has shown encouraging clinical results when used as an ablative treatment of solid tumors. Several studies have reported the photodynamic cancer treatment is associated with immunogenic cell death. Combining PDT with immunotherapy is a promising option to enhance the immunogenic effects and overcome the immunosuppressive state observed in large, advanced tumors. In our study, we employ strategies in which we use a combination of chlorinE6-based PDT with either specific therapeutic vaccination strategies[1] or immunotherapy with immune checkpoint blocking antibodies to PD-1 or CTLA-4 in preclinical murine tumor models[2]. These have resulted in improved induction of systemic cancer-specific CD8 T cell responses which showed significant abscopal effects to secondary, untreated tumors in a T cell dependent fashion. Currently our aims are to deliver defined immune modulating compounds into the tumor microenvironment (TME) using biodegradable poly(lactic-co-glycolic) acid (PLGA)/PEG nanoparticles (NPs). Intravenous injection of NPs showed accumulation in myeloid cells in the TME after PDT[3]. Recently we have used immunostimulatory NPs consisting of PLGA/PEG particles loaded with stimulatory combined Toll-like receptor ligands and a dendritic-cell (DC)-attracting chemokine for intra-tumoral treatment. The combination provoked strong anti-tumor responses, including abscopal effects, in three clinically relevant murine models of cancer: MC-38 (colorectal), CT26 (colorectal) and TC-1 (HPV16-induced). We could show that the local and distal anti-tumor effects depended on the presence of $CD8^+$ T cells. The combination elicited tumor-specific, oncoviral or neoepitope directed CD8+ T-cell immune responses against the respective tumors. In these tumor models we studied the TME in detail,which showed higher levels of inflammatory myeloid and $CD8^+$ T cells compared to untreated mice. The rationale for the combination of PDT with local or systemic immunotherapy treatment of solid tumors will be discussed.

References

1) Kleinovink et al. Combination of photodynamic therapy and specific immunotherapy efficiently eradicates established tumors. Clinical Cancer Research 22, 1459-1468. 2016
2) Kleinovink et al. Photodynamic-immune checkpoint therapy eradicates local and distant tumors by CD8+ T cells. Cancer immunology research 5, 832-838. 2017
3) Huis in ‘t Veld, R et al. Photodynamic cancer therapy enhances accumulation of nanoparticles in tumor-associated myeloid cells. Journal of Controlled Release 320, 19-31. 2020

OC-8.2.8

Low-dose PDT induces a rapid recruitment of CD14+ cells in the skin of mice

Katarzyna Kuczkowska[1], Tord Hompland[1], Qian Peng[2], Ane Sofie Viset Fremstedal[1], Pål Kristian Selbo[1], Kristian Berg[1]
1) Oslo University Hospital – The Norwegian Radium Hospital, Department of Radiation Biology, Oslo, Norway;
2) Oslo University Hospital – The Norwegian Radium Hospital, Department of Pathology, Oslo, Norway;

There is a need for better strategies that improve the efficacy of therapeutic cancer vaccines including robust activation of antigen presenting cells (APCs). Pre-conditioning the vaccination site by inducing mild, transient inflammation may provide enhanced recruitment of APCs. Photodynamic therapy (PDT) is a clinically approved treatment for non-melanoma skin cancers and pre-malignant lesions of the skin. PDT generates inflammation in light exposed tissues and seems an excellent candidate for pre-conditioning the skin for vaccination. In the present study, we have developed a PDT-based strategy to attract APCs to the site of vaccination in normal mouse skin by low-dose PDT. We tested various PDT doses using two clinically relevant photosensitizers: 1, $TPCS_{2a}$/Fimaporfin (used in the drug delivery method photochemical internalization (PCI)) and 2, mTHPC/Temoporfin (trade name Foscan®). We first evaluated inflammatory responses post PDT at 24 h by quantifying cytokines involved in both inflammation and chemotaxis of innate immune cells. The PDT dose consisting of 5 µg photosensitizer and 3 min blue light exposure (λ_{max}~420 nm, 13.5 mW/cm^2, 2.4 J/cm^2) was established as the optimal for inducing a mild and transient inflammation in normal mouse skin. The time point for maximal CD14+ influx post PDT was investigated by analysing cytokine kinetics and recruitment of immune cells in excised skin biopsies by ELISA and IHC, respectively. The results revealed elevated levels of the pro-inflammatory cytokines IL-6, CCL3 and IL-12, suppression of the anti-inflammatory cytokine IL 10 and influx of CD14+ and CD45+ cell populations to PDT-treated skin area 6 h and 24 h after PDT, suggesting these two time points as most relevant for post PDT vaccination. The present study comprises a novel strategy to use low-dose PDT as a pre-conditioning and an adjuvant strategy with a potential to improve therapeutic cancer vaccines.

Acknowledgements. This work has been supported by grants from EuroNanoMed II (Nanovax).

P-8.2.9

A photoinmunoconjugate for the treatment of breast cancer

Mireia Jordà-Redondo[1,2], Jaume Nos[1], Mª Lluïsa Sagristá[2], Lydia Blay[3], Joan Francesc Julián[3] and Santi Nonell[1]

1) IQS School of Engineering, Universitat Ramon Llull, Via Augusta 390, 08017, Barcelona, Spain
2) Departament de Bioquímica i Biomedicina Molecular, Facultat de Biologia, Universitat de Barcelona, Avinguda Diagonal 645, 08028, Barcelona, Spain
3) Departments of General and Gastrointestinal Surgery, Hospital Universitari Germans Trias i Pujol, Carretera de Canyet, s/n, 08916 Badalona, Barcelona, Spain

Finding biomarkers that allow the detection of neoplasms by illuminating them in real time in the operating room and combining it with photodynamic therapy (PDT) can be an advantage for the successful resection of the tumor. In this study, we chemically conjugated a water-soluble phtalocyanine with a monoclonal antibody to assess the selectivity for a cancer cell type. Photoinmunotherapy combine the antibody selectivity and the PDT properties of the photosensitizier.[1,2,3,4]
One photoinmunoconjugate is presented, the antibody employed is Trastuzumab which has selectivity for HER-2 receptor and the moiety is IRDye® 700DX NHS. The *in vitro* assays were performed on two tumoral cells: BT-474 as a positive control and HeLa as a negative control. The biological *in vitro* assays show that this conjugate is able to photoinactivate cancerous mammalian cells that presents the receptor HER-2 at sub-micromolar concentrations. Furthermore, it is possible to differentiate the targeted cells by means of using a real-time fluorescence guided device adapted for this photoinmunoconjugate.

References

1) Zhang, R. R.; Schroeder, A. B.; Grudzinski, J. J.; Rosenthal, E. L.; Warram, J. M.; Pinchuk, A. N.; Eliceri, K. W.; Kuo, J. S.; Weichert, J. P. Beyond the Margins: Real-Time Detection of Cancer Using Targeted Fluorophores. *Nat. Rev. Clin. Oncol.* **2017**, *14* (6), 347–364.
2) Rosenthal, E. L.; Warram, J. M.; Boer, E. De; Basilion, J. P.; Biel, M. A.; Bogyo, M.; Bouvet, M.; Brigman, B. E.; Colson, Y. L.; DeMeester, S. R.; Gurtner, G. C.; Ishizawa, T.; Jacobs, P. M.; Keereweer, S.; Liao, J. C.; Nguyen, Q. T.; Olson, J.; Paulsen, K. D.; Rieves, D.; Sumer, B. D.; Tweedle, M. F.; Vahrmeijer, A. L.; Weichert, J. P.; Wilsopn, B. C.; Zenn, M. R.; Zinn, K. R.; van Dam, G. M. Successful Translation of Fluorescence Navigation During Oncologic Surgery: A Consensus Report. *J. Nucl. Med.* **2016**, *57* (1), 144–150.
3) *Photodynamic Medicine: From bench to clinic*, edited by Herwig Kostron and Tayyaba Hasan, The Royal Society of Chemistry, London, **2016**
4) Mitsunaga, M.; Ogawa, M.; Kosaka, N.; Rosenblum, L. T.; Choyke, P. L.; Kobayashi, H. Cancer Cell-Selective in Vivo near Infrared Photoimmunotherapy Targeting Specific Membrane Molecules. *Nat. Med.* **2011**, *17* (12).

Acknowledgements. This work was supported by Asociación Española Contra el Cáncer (IDEAS19135NONE).

IL-8.3.1

Role of nitric oxide in tumor response to PDT: Perspectives from the early studies

Mladen Korbelik

BC Cancer, Integrative Oncology Department, Vancouver BC, Canada

Potential of nitric oxide (NO) to markedly influence the outcome of tumor treatment by photodynamic therapy (PDT) was first reported in 1996, which is within several years of the recognition that this unstable gas has a major impact on tumor progression. Early studies of NO effects on tumor PDT response, conducted from mid to late 1990's, have conclusively established the endogenously-produced NO in tumors as an important determinant of sensitivity to PDT and shown that this critical role can be exploited for therapeutic gain. The effects of NO revealed in these studies to have fundamental roles in the outcome of tumor PDT include the vasodilatation (pre-PDT tumor oxygenation and post-PDT tumor blood-flow), interaction with PDT-induced superoxide, participation in oxidative stress signaling, and influence on inflammatory events in vascular endothelium of treated tumors. While a number of gained insights have been capitalized upon during the following decades, some of the valuable leads still remain unexploited. Particularly promising are profits attained by controlling the integral role of NO in tumor immunosuppression.

IL-8.3.2

Hyper-aggressiveness of tumor cells that survive a photodynamic challenge: role of endogenous nitric oxide

Albert W. Girotti[1], Jonathan M. Fahey[2], Witold Korytowski[3]
1) Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI, USA; 2) Department of Pathology, University of Colorado, Aurora, CO, USA; 3) Department of Biophysics, Jagiellonian University, Krakow, Poland

Anti-tumor photodynamic therapy (PDT) has an advantage over conventional chemotherapy and radiotherapy in being predominantly tumor site-specific. Like chemo/radiotherapy, however, PDT can be limited by pre-existing or acquired resistance, studies ca. 2000 showing that tumor-derived nitric oxide (NO) is substantially involved. More recently, our work revealed that NO generated by inducible NO synthase (iNOS) not only stimulated resistance, but also proliferative, migratory, and invasive aggressiveness of cells that could withstand a PDT challenge. This was demonstrated for a variety of human cancer cell lines in vitro, including prostate PC3, breast MDA-MB-231, and glioblastoma U87, each of which was photosensitized in mitochondria with 5-aminolevulinic acid (ALA)-induced protoporphyrin IX. The indicated post-PDT responses were accompanied by a substantial and long-lasting upregulation of iNOS/NO, whereas other NOS isoforms were typically unaffected. Hyper-resistance and aggressiveness are attributed to NO from PDT-upregulated iNOS rather than that from basal enzyme, a finding that is virtually unprecedented for anti-tumor therapeutics. Elevated iNOS/NO-dependent resistance to ALA-PDT has also been demonstrated for an MDA-MB-231 mouse xenograft model. Key upstream signaling events leading to iNOS/NO upregulation by PDT will be discussed for one cancer line (glioblastoma), along with promising pharmacologic approaches for suppressing this negative (pro-tumor) response.

Acknowledgements. This work was supported by NIH Grant CA70823 and MCW Cancer Center Grant FP12605

IL-8.3.3

Nitric Oxide-Mediated Bystander Effects in Photodynamic Therapy

Witold Korytowski[1,2], Jerzy Bazak[1], and Albert W. Girotti[2]
1) Dept. of Biophysics, Jagiellonian University, Krakow, Poland; 2) Dept. of Biochemistry, Medical College of Wisconsin, Milwaukee, WI, USA

We have recently uniquely shown that human breast cancer cells (adenocarcinoma MDA-MB231), exploit endogenous nitric oxide (NO) to resist elimination by photodynamic therapy (PDT), cisplatin (CDDP) chemotherapy or combination of both. Inducible nitric oxide synthase (iNOS) was rapidly and persistently upregulated after the treatment challenge, and the resulting NO signaled not only for greater resistance but also growth, migration, and invasion/aggressiveness of not only cells surviving the therapeutic challenge, but also bystander cells, which were not challenged. Ionizing radiation of specifically targeted cells in a given population is known to elicit pro-death or pro-survival responses in non-targeted bystander cells, but far less is known about such effects in non-ionizing PDT. We are testing the hypothesis that photodynamically or pharmacologically stressed breast adenocarcinoma cells can elicit NO-mediated pro-growth/migration aggressive responses not only in surviving target cells but also in non-stressed bystanders. Switching to a more aggressive phenotype during clinical therapeutic treatment would be an alarming prospect because it might lead to greater cancer metastatic spread. We will also report on our attempts to overcome/minimize such effects by using classical inhibitors of iNOS activity (e.g. 1400W) and recently introduced bromodomain and extra-terminal domain (BET) transcriptional inhibitors (e.g. JQ1).

(Supported by NCN grant 2017/27/B/NZ5/02620).

OC-8.3.4

A Novel Fluorescent Generator of Peroxynitrite Activatable with Red Light

Cristina Parisi[1], Mariacristina Failla[2], Aurore Fraix[1], Luca Menilli[3], Francesca Moret[3], Elena Reddi[3], Barbara Rolando[2], Loretta Lazzarato[2], Alberto Gasco[2], Salvatore Sortino[1].

1) University of Catania, Department of Drug and Health Sciences, Catania, Italy; 2) University of Torino, Department of Science and Drug Technology, Torino, Italy. 3) University of Padova, Department of Biology, Padova, Italy.

The alarmingly low turnover of new clinically approved anticancer drugs and the Multi Drug Resistance (MDR) phenomena emerging for drugs actually used, call for an urgent shift of attention to other "unconventional" and underexplored therapeutic modalities. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are emerging as suitable alternative agents. In particular, peroxynitrite ($ONOO^-$) has encountered an increasing interest by virtue of its potent oxidative and cytotoxic activity and of its nitrating activity against tyrosine of proteins,[1] a process at the basis of the inhibition of the cell's efflux pumps mainly responsible for MDR. However, due to the lack of selectivity of $ONOO^-$ for bio-substrates, the control of its delivery in terms of space, time and dosage represents a desirable requisite. Light gives the possibility to reach this purpose with high accuracy by using appropriate photoprecursors.[2]

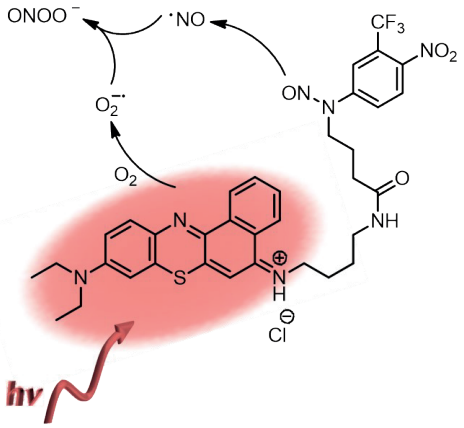


Fig. 1 – Structure of the novel fluorescent generator of peroxynitrite activatable with red light.

We report a novel molecular hybrid able to generate $ONOO^-$ under activation with the highly biocompatible red light (Fig. 1). The hybrid consists of a benzophenothiazine moiety which act as light-harvesting antenna, joined to an N-nitroso appendage through a flexible spacer. The red fluorescence of the antenna represents an additional advantage permitting the localization of the hybrid within the cellular environment, an important requisite in view of image-guided phototherapies. The biological activity of the hybrid has been evaluated against two different cancer cell lines. The hybrid appears well-tolerated in the dark and induces a significant cell mortality under irradiation in a very low concentration range and with very low light doses (ca. 1 J cm^{-2}).[3]

References

1) S. Bartesaghi, R. Radi, Fundamentals on the biochemistry of peroxynitrite and protein tyrosine nitration. Redox Biology, 2018.
2) S. Sortino, Light- Responsive Nanostructured Systems for Applications in Nanomedicine. Springer, 2016.
3) C. Parisi, M. Failla, A. Fraix, L. Menilli, F. Moret, E. Reddi, B. Rolando, F. Spyrakis, L. Lazzarato, R. Fruttero, A. Gasco, S. Sortino, A generator of peroxynitrite activatable with red light. Chemical Science, 2021.

Acknowledgements. We thank AIRC – Italian Association for Cancer Research (IG-198559) for financial support.

IL-8.3.5

Strategies towards the use of ruthenium phthalocyanine compounds for the enhancement of photodynamic therapy

Roberto Santana da Silva, Francisco Rinaldi Neto and Amanda Blanque Becceneri

Departament of Biomolecular Sciences, School of Pharmaceutical Sciences, University of Sao Paulo, Ribeirão Preto, SP-Brazil.

Photodynamic therapy (PDT) is currently being used as an alternative treatment for the control of several malignant diseases including cancer. Essentially PDT uses light irradiation, oxygen and photosensitizers that have been largely synthesized and studied. Recently, several new strategies have emerged to enhance PDT but its use may also affect early and late onset side effects under normal cells. We have found that nitrosyl phthalocyanine ruthenium-based compounds may be an interesting approach to improve PDT combined with light irradiation therapy. They are singlet oxygen and nitric oxide (NO) producer agents, beyond what they can also protect normal cells from the cytotoxicity. In this context, combination of reactive oxygen and nitrogen species (RONS) and photobiomodulation (PBM), a kind of near infrared light irradiation therapy, could be an important tools for a beneficial cancer treatment option. We have used two types of ruthenium compounds, ([Ru(Pc)], Pc = phthalocyanine) and *trans*-[Ru(NO)(NO_2)(Pc)]. The UV-Vis spectra of both complexes displayed a band in the 660 nm region. In the case of 0.5 µM trans-[Ru(NO)(NO_2)(Pc)], light irradiation at the Q-band reduced the percentage of viable human melanoma (A375) cells to around 50% more as compared to [Ru(Pc)]. We hypothesized that these results were due to a synergistic effect between singlet oxygen and nitric oxide. Similar experiments performed with PDT (660 nm) combined with PBM (850 nm) induced more photocytotoxicity using both [Ru(Pc)] and *trans*-[Ru(NO)(NO_2)(Pc)]. This was interpreted as PBM increasing cell metabolism (ATP production) and the consequent higher uptake of the ruthenium phthalocyanine compounds and more efficient apoptosis. Interestingly, the cytotoxicity described above seems to be restrict to cancer cells once no cytotoxicity was observed for the normal cell 3T3, when NO donor agent was associated to the ROS production. Aim to better understand this effect we have evaluated the cytotoxicity of those complexes against the triple-negative malignant breast cells MDA-MB-231, an extremely aggressive and difficult to treat subtype. The complexes were cytotoxic, changed cell morphology and inhibited cell migration in the presence of light irradiation. Preliminary tests with *trans*-[Ru(NO)(NO_2)(Pc)] complex demonstrated cytotoxic potential in 3D cell culture, a biologically relevant model that better represents the tumor microenvironment observed *in vivo*. Besides, this combination can also reduce 30 % the cytotoxicity effect under normal MCF-10 cells. The use of metal-based photosensitizers that produce RONS combined with light therapy may represent an advance in the field of photodynamic therapy.

Acknowledgments. This work was supported by the Brazilian agencies CNPq, CAPES, and FAPESP.

IL-8.3.6

Nitric Oxide Photodynamic Therapy with Molecular and Supramolecular Constructs

Salvatore Sortino

Department of Drug and Health Chemical Sciences, University of Catania. Email: ssortino@unict.it

The multiple role nitric oxide (NO) plays in a number of physiological and pathophysiological processes has stimulated a massive interest in the development of new strategies and methods for generating NO in a controlled fashion, with the exciting prospect to tackle important diseases. In this context, the therapeutic outcome of any NO-based drug is strictly dictated by three main parameters: i) concentration, ii) delivery site and iii) dosage. In some cases, this creates a complex role for the molecule in opposing beneficial and deleterious events. This dichotomy has made the development of new strategies and methods for generating NO with precise spatiotemporal control a hot topic in the burgeoning field of nanomedicine.[1] Light represents a minimally invasive "micosyringe" to provide a highly localized "burst" of NO in biological systems with a superb spatiotemporal control through the aid of suitable NO photoprecursors. In fact, photoexcitation permits not only to confine site of action of NO at the illuminated area with high precision but also to define its dosage with great accuracy by tuning the light intensity and/or duration.[2] These unique features make the NO photoreleasing compounds a powerful therapeutic arsenal much more appealing than those based on either thermal, enzymatic or pH stimuli.
In this contribution, an overview of the most significant examples of NO photodelivering molecular and supramolecular constructs developed in our laboratories over the last few years is presented, emphasizing the logical design and the potential applications in anticancer and antibacterial therapy alone or in combination with typical photosensitizers for photodynamic therapy.[3-8]

References

1) S. Sortino, Light-controlled nitric oxide delivering molecular assemblies. Chem. Soc. Rev. 2010, 39, 2903-2913.
2) S. Sortino Ed. Light- Responsive Nanostructured Systems for Applications in Nanomedicine. Springer, 2016.
3) A. Fraix and S. Sortino, Combination of PDT photosensitizers with NO photodonors, Photochem. Photobiol. Sci.. 2018, 17, 1709-1727.
4) A. Fraix, V. Kirejev, M. Malanga, E. Fenivesi, S. Beni, M. B. Ericson and S. Sortino, A three-color fluorescent supramolecular nanoassembly of phototherapeutics activatable by two-photon excitation with near infrared light, Chem. Eur. J. 2019, 23, 7091-7095.
5) M. Malanga, M. Seggio, V. Kirejev, A. Fraix, I. Di Bari, E. Fenyvesi, M. B. Ericson and S. Sortino, A phototherapeutic fluorescent b-cyclodextrin branched polymer derivering nitric oxide, Biomater. Sci. 2019, 7, 2272-2276.
6) F. Sodano, R. J. Cavanagh, A. K. Pearce, L. Lazzarato, B. Rolando, A. Fraix, T. F. Abelha, C. E. Vasey, C. Alexander, V. Taresco and S. Sortino, Enhancing Doxorubicin Anticancer Activity with a Novel Polymeric Platform Photoreleasing Nitric Oxide, Biomater. Sci. 2020, 8, 1329-1344.
7) C. Parisi, M. Failla, A. Fraix, L. Menilli, F. Moret, E. Reddi, F. Spyrakis, B. Rolando, L. Lazzarato, R. Fruttero, A. Gasco and S. Sortino, A Generator of Peroxynitrite Activatable with Red Light, Chemical Science 2021, 12, 4770-4776.
8) A. Fraix, C. Parisi, M. Failla, K, Chegaev, F. Spyrakis, L. Lazzarato, R. Fruttero, A. Gasco and S. Sortino, NO Release Regulated by Doxorubicin as Green Light-Harvesting Antenna, Chem. Commun. 2020, 56, 6332-6335.

Acknowledgements. We thank AIRC – Italian Association for Cancer Research (IG-198559) for financial support.

OC-8.3.7

Can NO induced by photooxidative stressed tumour cells influence tumour microenviroment?

Mariachiara Gani[1], Luigi E. Xodo[1], Valentina Rapozzi[1]

[1]*University of Udine, Department of Medicine, Udine, Italy,*

Our previous studies have shown that PC3 prostate cancer cells exposed to chronic photooxidative stress upregulate inducible nitric oxide synthase (iNOS), stimulating the development of a more aggressive tumor population characterized by greater growth, invasiveness, and resistance[1].
Since NO is a gaseous signaling molecule, it can act as a "messenger" that can communicate to the neighbouring cells, determining their fate. We therefore wondered whether and how NO, induced by low doses of PDT, might affect the tumor microenvironment.
To investigate this, we took the supernatant of Pheophorbide-α (Pbα)/PDT-treated PC3 cells and replaced it to the culture medium of untreated PC3 tumor cells, after irradiation. We indeed found a stimulation of PC3 untreated cell growth, confirming the data reported by Girotti *et al.* [2,3].
Based on these results, we are investigating what happens in PNT1A, non-prostate cancer cells. Preliminary results suggest that their growth is stimulated, an aspect that could influence tumour progression and affect treatment efficacy.

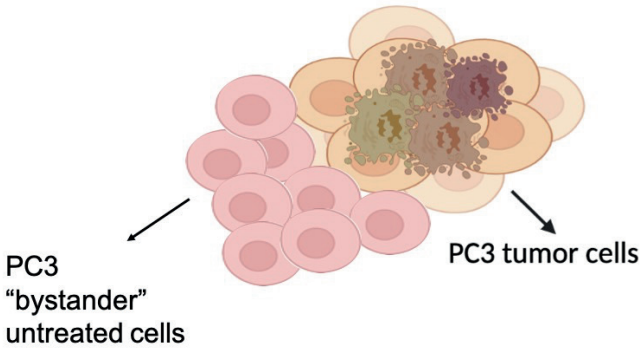


Fig.1 – Representation of PC3 tumour cells (yellow) and PC3 "bystander" untreated cells (pink). The PC3 "bystander" cells are the cells that are not directly treated.

References

1) F. D'Este F, E. Della Pietra, G.V. Badillo Pazmay, L.E. Xodo, V. Rapozzi. Role of nitric oxide in the response to photooxidative stress in prostate cancer cells. Biochem. Pharmacol. 2020.
2) J. Bazak, J.M. Fahey, K. Wawak, W. Kortytowski, A.W. Girotti, Enhanced aggressiveness of bystander cells in an anti-tumor photodynamic therapy model: Role of nitric oxide produced by targeted cells. Free Radic. Biol. Med. 2017.
3) J. Bazak, W. Kortytowski, A.W. Girotti, Bystander effects of nitric oxide in cellular models of anti-tumor photodynamic therapy. Cancers, 2019.

Acknowledgements. This work was supported by the National FFABR,VQ3DAME.

OC-8.3.8

Synthesis and Characterization of Os(II) Oligothienyl Complexes for Photodynamic Therapy Applications

Elamparuthi Ramasamy, Houston Cole, John Roque, III, Colin G. Cameron and Sherri A. McFarland*
Department of Chemistry and Biochemistry, The University of Texas at Arlington, Texas, 76019, USA

Photodynamic therapy (PDT) is an emerging new technique to treat various kinds of cancer, either alone or as an adjuvant to conventional treatments such as chemotherapy, radiotherapy, and surgery. In PDT, the photosensitizer (PS) illuminated in presence of oxygen to produce reactive oxygen species (ROS) to destroy cancerous tumor cells. Being activated by light, one of the key benefits of PDT is its selectivity, confining treatment to the affected area. Photofrin, an organic compound based on porphyrins, was the first FDA-approved PDT agent and is the basis of several organic derivatives. Our approach uses metal complexes; we synthesized the Ru-based complex TLD 1433 that is in Phase II clinical trials. Here, we have synthesized trifluoromethyl substitution of bipyridine with series of oligothiophene pi-expansive ligands heteroleptic Os(II) complexes and would expect to alter the excited state photophysical properties for efficient PDT. We strongly believe that the electron withdrawing trifluoromethyl groups tend to impart longer wavelength absorption and longer excited state lifetimes. The longer wavelength absorption helps to deep tissue penetration and will help to sensitize efficient singlet oxygen production.

Acknowledgements. This work was supported by grant R01CA222227 from the National Cancer Institute.

IL-8.4.1

Plant responses to UV light mediated by UVR8

Gareth I. Jenkins
University of Glasgow, Institute of Molecular Cell and Systems Biology, Glasgow G12 8QQ, UK.

Exposure of plants to UV wavelengths modifies numerous aspects of metabolism, morphology and physiology. These regulatory responses are underpinned by the differential expression of hundreds of genes. UV RESISTANCE LOCUS8 (UVR8) is the only photoreceptor known to mediate regulatory transcriptional responses to UV-B and shortwavelength UV-A. UVR8 exists as a homodimer in the absence of UV light and photoreception causes rapid dissociation of the dimer into monomers to initiate signaling. Under photoperiodic illumination with white light supplemented with UV-B a dimer/monomer photoequilibrium is established, where approximately 70% of UVR8 is in the dimeric form. Regulation of the photoequilibrium and initiation of transcription by the UVR8 monomer is mediated by interaction with other proteins: CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP) proteins, and a number of transcription factors. However, the regulation of UVR8 action in the initiation of transcriptional responses is not fully understood.

IL-8.4.2

UVR8 Signalling and Plant Development

Ashutosh Sharma[1], Gareth I. Jenkins[2] and Keara A. Franklin[1],

1) University of Bristol, School of Biological Sciences, Bristol, UK, 2) University of Glasgow, Institute of Molecular Cell and Systems Biology, Glasgow, UK.

Plants perceive different wavelengths of light using specialised photoreceptors. This information is integrated with temperature signals to control the abundance and activity of the PHYTOCHROME INTERACTING FACTOR (PIF) family of transcription factors. PIFs regulate multiple developmental processes throughout plant development and perform a central role in controlling stem elongation. In this talk, I will discuss how UV-B, perceived by the photoreceptor UV RESISTANCE LOCUS 8 (UVR8), regulates auxin and gibberellin signalling to control plant architecture in different environments.

Acknowledgements: This work was supported by the Biotechnology and Biological Sciences Research Council

OC-8.4.3

Interplay among Nitric Oxide and UVR8 in plants exposed to UV-B radiation

Fernández María Belén[1*], Latorre Lucas[1], Cassia Raúl[1]

1) Instituto de Investigaciones Biológicas, UNMdP-CONICET. Mar del Plata, Argentina. E-mail: mbfernan@mdp.edu.ar

The ultraviolet- B (UV-B) resistance locus 8 (UVR8) is the only known UV-B photoreceptor in plants[1] and is conserved from *Chlorophytes* to angiosperms[2]. After UV-B irradiation, UVR8 turns from an inactive homodimer to an active monomer regulating the expression of several genes involved in tolerance and acclimation[3]. Nitric oxide (NO) is an UV-B downstream gaseous signal that increases in response to UV-B and attenuates its damaging effects on plants[4]. The aim of this work was to analyse the interplay between NO and UVR8 in the UVR8 signalling pathway of plants exposed to UV-B. To this end, we first analysed the expression of *NIA1* (nitrate reductase 1, a NO enzymatic source) in three-week-old control and UV-B irradiated (3.47 m^{-2} s^{-1}, for 2h) *Arabidopsis* Col 0 (WT) and *uvr8-1* null mutant plants using RT-qPCR. Results obtained show that *NIA1* was upregulated after UV-B radiation in WT plants, however, this response was impaired in mutants. This indicates that expression of *NIA1* after UV-B treatment depends on the UVR8 signalling pathway, and thus, NO increase through this enzyme may be UVR8 dependent. We also analysed if the expression of *PHR1* (cyclobutane pirimidine dimers photolyase involved in DNA repair), *IAA19* (an auxin responsive gene) and *UVR8* are regulated by UV-B and UVR8. RT-qPCR results demonstrated that *PHR1* was upregulated whereas *IAA19* and *UVR8* were downregulated after UV-B treatment, and all responses were abolished in mutants. This confirms that regulation of these genes occurs through the UVR8- dependent pathway. *IAA19* expression is repressed under UV-B due to MYB77, BIM1 and BES1 transcription factors (TFs) sequestration by UVR8[5]. In order to determine if similar regulation occurs for the *UVR8* gene, *in silico* analysis of the predicted UVR8 promoter using PlantPAN database revealed the presence of binding motifs for these TFs, supporting its negative regulation after UV-B. We also analysed possible UV-B and NO co-regulation of the UVR8 signalling pathway components, using publicly available microarray and RNA-seq databases from *Arabidopsis* plants treated with NO. This analysis revealed co- regulation by NO and UV-B of several genes involved in this pathway. We found that *CHS*, *IAA2* and *GA2OX* (GA2-oxidase) are upregulated in *Arabidopsis* leaves both by UV-B and exogenous application of NO. On the other hand, other genes were negatively co- regulated by UV-B and NO, such as the growth promoting genes *PRE1* (paclobutrazol resistance 1) and *EXP1* (expansin b1). Finally, prediction analysis of S-nitrosylated proteins from the UVR8 signalling pathway and cysteine conservation using multiple sequence alignment revealed that COP1, PHR1, IAA14 and NIA1 could be targets of this post-translational modification. In conclusion, we report an interplay between UVR8 and NO in the UVR8 signalling pathway as: 1) NO increment depends on UVR8 2) and NO regulates the components from the UVR8 signalling pathway at the gene transcription and post-translational levels under UV-B irradiation.

References

1) Brown BA, et al., A UV-B specific signaling component orchestrates plant UV protection. PNAS, 102 (50) 18225-18230, 2005.
2) Fernández MB et al., A Comprehensive Phylogeny Reveals Functional Conservation of the UV-B Photoreceptor UVR8 from Green Algae to Higher Plants. Frontiers in Plant Science, 7:1698, 2016.
3) Favory JJ et al., Interaction of COP1 and UVR8 regulates UV-B induced photomorphogenesis and stress acclimation in Arabidopsis. The EMBO Journal 28:591-601. https://doi.org/10.1038/emboj.2009.4
4) Cassia R, et al., The role of nitric oxide in the antioxidant defense of plants exposed to UV-B radiation. React. Oxygen, Nitrogen Sulfur Spec. Plants, https://doi.org/10.1002/9781119468677.ch22 2019.
5) Yang Y, et al., UV-B photoreceptor UVR8 interacts with MYB73/MYB77 to regulate auxin responses and lateral root development. EMBO J, 39(2):e101928 ,2020.

Acknowledgements. This work was supported by grant number 3589 from Agencia Nacional de Promoción de Ciencia y Técnica and EXA 929/19 from Universidad Nacional de Mar del Plata. FMB and CR are permanent researchers of CONICET, Argentina. LL is doctoral fellowship of CONICET.

OC-8.4.4

Broad and narrow band (311 nm) ultraviolet-B radiation activate distinct defensive antioxidant pathways

Arnold Rácz, Éva Hideg
University of Pécs, Department of Plant Biology, Pécs, Hungary

Ultraviolet radiation (UV, 280-400 nm) has the highest energy among the radiation components reaching the surface of the Earth. UV-B (280-315 nm) is a potential stressor, and damaging effects of high UV-B doses on plants have been studied extensively. However, over the past decades, several studies have shown that environmental UV-B by itself does not act as a stressor, but rather acts as a regulating factor. Nevertheless, even ambient UV-B can have a damaging effect, when combined with other abiotic factors, stimulating the reactive oxygen species (ROS) production. When ROS concentrations are increasing, oxidative damage can be avoided by adjusting the ROS antioxidant balance.

A well-documented effect of acclimation to UV-B is the biosynthesis of UV absorbing phenolic compounds. Based on their high ROS reactivities *in vitro*[1], these compounds are also assumed to act as antioxidants *in planta*, although this role is only supported by indirect evidence so far. A third, confirmed defence function of phenolic compounds is substrate to class III peroxidase enzymes[2] (POD). A selective increase in POD activity has a central role in acclimation to supplemental UV-B in model experiments[3], as one of the major components of the H_2O_2 controlling system. We have also shown that POD isoforms are selectively activated by UV-B[4], and that in tobacco the dominant POD isoform is supported by chlorogenic acid (CGA), a phenolic acid present at increased concentrations in UV-treated leaves[5].

In the present study, we used tobacco plants (*Nicotiana tabacum* L., cv. Petit Havanna) grown in a growth chamber under photosynthetically active radiation (PAR) only, then exposed to supplemental UV-B from a narrowband (311.5 ± 2.5 nm) source. Depending on the applied photon flux density (2.9–9.9 μmol m^{-2} s^{-1}), this treatment resulted in 0–10% decrease in leaf photochemical activity. Narrow band UV-B resulted in distinct antioxidant enzyme responses as compared to the above effects of broadband UV-B. The 311 nm treatment decreased CGA-POD and increased SOD activity. Both changes were linear with the applied dose. In addition, an increase in non-enzymatic antioxidant capacities was observed as a non-linear response, appearing under high doses only. These findings may be relevant to UVR8-independent UV-response pathways, such as the recently suggested photoreceptor with maximum absorbance at 310-311 nm[6], and also indicate the necessity of studying the contribution of various UV-B wavelengths to complex biochemical responses leading to UV-tolerance.

References

1) Csepregi K., Hideg, É., Phenolic compound diversity explored in the context of photo-oxidative stress protection. Phytochem. Anal. 29, 129-136, 2018.
2) Jansen, M.A.K., van den Noort, R.E., Adillah Tan, M.Y., Prinsen, E., Lagrimini, M.L., Thorneley, R.N.F., Phenol-oxidizing peroxidases contribute to the protection of plants from ultraviolet radiation stress. Plant Physiol. 126, 1012-1023, 2001.
3) Majer, P., Czégény, Gy., Sándor, Gy., Dix, P.J., Hideg, É., (2014) Antioxidant defence in UV-irradiated tobacco leaves is centred on hydrogen-peroxide neutralization. Plant Physiol. Biochem. 82, 239-243, 2014.
4) Rácz, A., Hideg, É., Czégény, Gy., Selective responses of class III plant peroxidase isoforms to environmentally relevant UV-B doses. J.Plant Physiol. 221, 101-106, 2018.
5) Rácz, A., Czégény, Gy., Csepregi, K., Hideg, É., Ultraviolet-B acclimation is supported by functionally heterogeneous phenolic peroxidases. Sci. Rep. 10, 16303, 2020.
6) O'Hara, A., Headland, L.R., Díaz-Ramos, L.A., Morales, L.O., Strid, Å., Jenkins, G.I., Regulation of Arabidopsis gene expression by low fluence rate UV-B independently of UVR8 and stress signalling. Photochem. Photobiol. Sci. 18, 1675-1684, 2019.

Acknowledgements. This work was supported by the Higher Education Institutional Excellence Programme of the Ministry of Human Capacities in Hungary (20765-3/2018/FEKUTSTRAT).

SP-8.4.5, P-8.4.5

Stabilizing short-lived photoproducts of phytochromes in solid state at room temperature

Lisa Köhler, Wolfgang Gärtner, Jörg Matysik and Chen Song[1]

1) University of Leipzig, Faculty for Chemistry and Mineralogy, Institute for Analytical Chemistry, Leipzig, Germany

Phytochromes (Phys) are photosensoring proteins regulating phototaxis and photomorphogenesis and were first discovered in higher plants. Especially in bacteria, a large variety of Phys were discovered within the last decades, habouring different tetrapyrrole chromophores and spectroscopic properties.[1] However, magic-angle spinning (MAS) NMR analysis of the short-lived photoproducts comprise practical obstacles using frozen solutions even with the probe euqipped with *in situ* illumination, the irradiated molecules would be photocycled through numerous photointermediates rather than maintained in the steady photoproduct state. Moreover, the highly concentrated samples used in NMR studies, would prevent the penetration of the actinic light beyond a few hundred μm into the sample, creating a gradient of photoproduct and intermediates within the sample. Therefore we surveyed for sample preparation techniques, namely the lyophilization[2] and the immobilization in disaccharide-protein glassy systems[3], that enable retardation of fast thermal reversion of the photoproduct.
We characterized representative Phy photoproducts of all three subfamilies with different molecular mass (19–56 kDa) with the photoproduct half-lives ranging from a few seconds to hours by UV-vis and MAS NMR spectroscopy. Upon lyophilization, the conformational dynamics of the photoproduct is "frozen out" due to the removal of the hydration shell and thus provides the possibility to investigate conformational distributions of the heterogeneous chromophore in various states[2]. The disaccharide-coating Phy proteins maintain the minimum hydration shell thus maximally preserving the structural integrity. Herein, the dark reversion is inhibited due to the high viscosity of the protein-sugar-water formulations and long-range hydrogen-bonding connectivities.[3,5] Both abovementioned techniques allow the photoproduct preservation against thermal denaturation and reversion for at least three weeks at room temperature when applied in a well-designed process, which had been evidenced by re-dissolving the solid samples.
In particular sugar glasses show outstanding positive properties, e.g. transparency and microcrystallinity and are hence widely applicable in different analytical methods. The precise nature of protein-disaccharide interactions responsible for the Phy biopreservation is discussed.

References

1) N.C. Rockwell, J.C. Lagarias, Phytochrome evolution in 3D: deletion, duplication, and diversification. New Phytologist, 2020.
2) Y. Kim, Q. Xu, K.-H. Zhao, W. Gärtner, J. Matysik, C. Song, Lyophilization Reveals a Multitude of Strucural Conformations in the Chromophore of a Cph2-like Phytochrome. J. Phys. Chem. B, 2020.
3) M. Malferrari, A. Savitsky, W. Lubitz, K. Möbius, G. Venturoli, Protein Immobilization Capabilities of Sucros and Trehalose Glasses: The Effect of Protein/Sugar Concentration Unraveled by High-Field EPR. J. Phys. Chem. Lett., 2016.
4) K. Anders, L.-O. Essen, The family of ohytochrome-like photoreceptors: diverse, complex and multi-colored, but very useful. Current Opinion in Strctural Biology, 2015.
5) S. J. Hagen, J. Hofrichter, W. Eaton, Protein reaction kinetics in a room-temperature glass. Science, 1995.

SP-8.4.6, P-8.4.6

Molecular background of iron uptake mechanisms of non-photosynthetic plastids

Máté Sági-Kazár[1], Sándor Lenk[2], Éva Sárvári[1], Csaba Hegedűs[1], Attila Barócsi[2], Katalin Solymosi[3], Ádám Solti[1]
1) Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C, Budapest H-1117, Hungary
2) Department of Atomic Physics, Budapest University of Technology and Economics, Műegyetem rkp. 3, H-1111 Budapest, Hungary
3) Department of Plant Anatomy, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C, Budapest H-1117, Hungary

Iron (Fe) is an essential micronutrient for all living organisms. In plants Fe is most important for the formation of the photosynthetic apparatus and chlorophyll biosynthesis. For the operation of one single linear photosynthetic electron transport chain a total of 22 Fe atoms, in the form of Fe-S clusters, haem groups and non-haem Fe ions are required, thus the majority of the mesophyll cell's Fe content is located in the chloroplasts. According to the current model, *Arabidopsis thaliana* chloroplasts operate a reduction-based Fe uptake. Transmembrane electron transport is facilitated by Ferric Reductase Oxidase 7 (FRO7) using NADPH as electron donor produced by the photosynthetic activity. Consequently, the chloroplast Fe uptake is dependent on photosynthetic activity. FRO7 reduces ferric Fe complexes to free ferrous Fe which is immediately translocated into the stroma by Permease in Chloroplast 1 (PIC1). Both chlorophyll biosynthesis and the formation of the photosynthetic apparatus is light dependent in angiosperms. Thus, the development of the photosynthetic apparatus and thylakoid structures of chloroplasts are impaired and etioplasts form instead in the absence of light. The Fe uptake mechanism of non-photosynthetic plastids such as etioplasts remains uncharacterized so far. Upon illumination, etioplasts rapidly convert into chloroplasts. Photosynthetic structures develop, while their formation requires the presence of Fe. However, in the lack of a functional photosynthetic apparatus, the reduction-based uptake system may lack sufficient reducing power. Thus, the questions arise, which is first in the plastids: Fe or the photosynthetic apparatus. How non-photosynthetic plastids can manage their Fe acquisition? Although multiple components of the chloroplast Fe homeostasis have been identified so far, their exact role in the Fe acquisition is still scarcely understood.
Kale (*Brassica oleracea* convar. *capitata* var. *sabauda*) is a close relative of Arabidopsis and was previously shown to express orthologues of Arabidopsis chloroplast Fe homeostasis elements. Moreover, in the kale head, natural etiolation occurs because of the light filtering effect of the overlapping leaves. Thus, here we applied the kale model to study the differences in Fe homeostasis between etioplasts, intermediate, and developed chloroplasts. Kale heads were separated into layers based on photosynthetic activity and chlorophyll content. Expression profiles of known members of the reduction-based Fe uptake (*BoFRO7*, *BoPIC1*) as well as less characterised Fe homeostasis elements were analysed using quantitative real-time PCR. The Fe content of isolated plastids was measured with a bathophenanthroline disulfonate based ferrous Fe trapping method by spectrophotometry. In etiolated leaves, expression of *BoFRO7*, *BoPIC1* was found to be low suggesting they can only have a limited role in the Fe uptake system of etioplasts. Isolated etioplasts contained a low amount of Fe in parallel. Among the studied Fe homeostasis elements, *BoYSL4*, an Fe-nicotianamine transporter showed, indeed, an elevated expression in etiolated leaves. The Arabidopsis homologue AtYSL4 protein has been only detected in the proplastids of the cotyledons so far where it was suggested to be involved in the Fe unloading processes of the seed Fe storage of the dicot plant Arabidopsis. Our results indicate that etioplasts do not operate the reduction power dependent Fe acquisition strategy. Instead, they may transport Fe in a pathway in which YSL4 is involved.

Acknowledgements. This work was supported by the ÚNKP-20-3 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund. This work was supported by the grant financed by the National Research, Development and Innovation Office, Hungary (NKFIH K-124159). Investigations were principally carried out in the frame of ELTE Thematic Excellence Program 2020 supported by National Research, Development and Innovation Office - TKP2020-IKA-05. S.L. and A.B acknowledges the NKFIH Grant No. 2017-1.2.1-NKP-2017-00001.

IL-8.4.7

How plants balance development and UV-B stress tolerance

Tong Liang[1,2,6], Chen Shi[1,2,6], Yao Peng[1,2], Huijuan Tan[1,2], Peiyong Xin[5], Yu Yang[1,2], Fei Wang[1], Xu Li[1], Jinfang Chu[5], Jirong Huang[3], Yanhai Yin[4], Hongtao Liu[1,*]
1) National Key Laboratory of Plant Molecular Genetics (NKLPMG), CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology (SIPPE), Chinese Academy of Sciences, 200032 Shanghai, P. R. China; 2) University of Chinese Academy of Sciences, Shanghai 200032, P. R. China; 3) College of Life and Environmental Sciences, Shanghai Normal University, Shanghai 200234, P. R. China; 4) Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa 50011, USA; 5) National Centre for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; 6) These authors contributed equally to this work

Ultraviolet B (UV-B) light is a potential stress factor in plants, but how plants coordinate growth and UV-B stress responses is not well understood. Here we report that brassinosteroid (BR) signaling inhibits UV-B stress responses in *Arabidopsis thaliana* and various crops by controlling flavonol biosynthesis. We further demonstrate that BRI1-EMS-SUPPRESSOR 1 (BES1) mediates the tradeoff between plant growth and UV-B defense responses. BES1, a master transcription factor involved in BR signaling, represses the expression of transcription factor genes MYB11, MYB12, and MYB111, which activate flavonol biosynthesis. BES1 directly binds to the promoters of these MYBs in a BR-enhanced manner to repress their expression, thereby reducing flavonol accumulation. However, exposure to broad-band UV-B down-regulates BES1 expression, thus promoting flavonol accumulation. These findings demonstrate that BR-activated BES1 not only promotes growth but also inhibits flavonoid biosynthesis. UV-B stress suppresses the expression of BES1 to allocate energy to flavonoid biosynthesis and UV-B stress responses, allowing plants to switch from growth to UV-B stress responses in a timely manner.

IL-8.4.8

UV-B photoreceptor signalling and responses

Roman Ulm[1]
1) Department of Botany and Plant Biology, University of Geneva, Geneva, Switzerland

Plants are able to perceive ultraviolet-B radiation (UV-B) using the UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8) which activates a molecular signalling pathway leading to UV-B acclimation[1]. The evolutionarily conserved UVR8 UV-B photoreceptor exists as a homodimer that monomerises upon UV-B absorption via specific intrinsic tryptophans. The UVR8 monomer interacts with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), an E3 ubiquitin ligase, initiating a molecular signalling pathway that leads to gene expression changes. This signalling output leads to UVR8-dependent responses including UV-B-induced photomorphogenesis and the accumulation of UV-B-absorbing metabolites that function as "sunscreens". I will present our latest understanding of how UVR8 regulates UV-B acclimation and tolerance[1].

References

1) Podolec, R., Demarsy, E., Ulm, R., Perception and signaling of ultraviolet-B radiation in plants. Annu. Rev. Plant Biol. 72: 793–822, 2021.

OC-8.4.9

Rapid adjustment in epidermal UV sunscreen: Comparison of optical measurement techniques and response to changing solar UV radiation conditions

Susanne Neugart[1,2], Mark A. Tobler[2], Paul W. Barnes[2]
1) Division Quality and Sensory of Plant Products, Georg-August-Universität Göttingen, Carl-Sprengel-Weg 1, D-37075 Goettingen, Germany; 2) Department of Biological Sciences and Environment Program, Loyola University New Orleans, 6363 St. Charles Avenue, 70118 New Orleans, LA, USA

The accumulation of soluble and cell-wall bound UV-absorbing compounds (i.e. flavonoids) in the epidermis and the mesophyll of leaves is a response of plants to UV exposure. These compounds are known to function in UV-screening, but they are also of potential value for food quality. One way to non-destructively monitor UV-screening in leaves is by optical methods, from which UVA-PAM and Dualex instruments stand out. The degree and rapidity to which plants can modulate UV-screening in response to fluctuating solar UV conditions is poorly understood. In this study, okra plants were exposed to two solar radiation treatments (near-ambient UV (+UV) and attenuated UV (-UV)) and the epidermal UV transmittance (TUV; UVA-PAM) and flavonoid index (Dualex) were measured in the youngest and second youngest mature leaves over three consecutive days and within an individual day. The day-to-day (measured near solar noon) and diurnal (over the course of a day) measurements of leaf optical properties indicated that TUV decreased and flavonoid index increased in the adaxial epidermis ~50% until 15:00 CDT then returned close to morning values later in the day. Correlations between UV-B radiation and TUV and flavonoid index revealed highest values 30 minutes to 1 hour prior the measurements. These findings indicate that plants can respond quickly to fluctuating solar UV conditions and underlines the importance of the harvest-time point for health-promoting compounds in fruit and vegetables. Our findings also indicate that the UVA-PAM and the Dualex instruments are both suitable instruments to monitor rapid changes in UV-screening in plants.

References

Acknowledgements. This study was supported by the Deutsche Forschungsgemeinschaft (DFG) - Project number 359552155 and the J.H. Mullahy Endowment for Environmental Biology at Loyola University New Orleans.

P-9.1.1

Optical force-induced three-dimensional protein assembly growing from solution

Roger Bresolí-Obach[1,2], Po-Wei Yi[2,3], Wei-Hsiang Chiu[2], Tetsuhiro Kudo[4], Johan Hofkens[1], Eri Chatani[5], Ryohei Yasukuni[3], Yoichiroh Hosokawa[3], Teruki Sugiyama[2,3], Shuichi Toyouchi[2], Hiroshi Masuhara[2]

1) Katholieke Universiteit Leuven, Leuven, Belgium; 2) National Yang Ming Chiao Tung University, Hsinchu, Taiwan; 3) Nara Institute of Science and Technology, Nara, Japan; 4) Toyota Technological Institute, Nagoya, Japan; 5) Kobe University, Kobe, Japan.

Currently at the microscale, photochemistry and photobiology cover a wide range of scientific fields from single molecule spectroscopy to several applications including solar energy utilization, optical trapping and biological imaging. In the 70s', Ashkin proposed that optical forces can be exerted on small objects by tightly focusing a laser beam onto them. In one hand, single nano- and micro-scale objects can be trapped and manipulated with an exquisite 3D spatiotemporal control in solution, which endows optical trapping a large potential for many biological studies. On the other hand, when many nanoscale objects are gathered together in (and/or nearby) the focus, they mutually interact with each other through the scattered trapping light.[1] Specifically, at solution surface and interface, the trapping results in various interesting (bio)-chemical phenomena including molecular crystallization of biomolecules,[2] droplet formation,[3] or dynamically fluctuating assembly formation,[4] among others.

In this work, we study the formation of a large and thick concentrated protein domain by laser trapping.[5] Specifically, a 1064 nm laser is tightly focused on the surface of an almost saturated hen egg white lysozyme (HEWL) solution (26 mM) in D_2O through an air-immersion 60x objective lens (NA 0.90). After switching on the trapping laser, a single protein domain quickly appeared in the transmission image as a white ring, which expanded up to a radius of 60 µm in 120 s. To support the HEWL gathering at and around the focus, Raman spectroscopy experiments were performed either at the focus or at 40 µm away, using a 532 nm laser as the excitation light. We observed that the Raman signal increased approximately 3.5 and 2.5-fold at focus and 40 µm away, respectively, indicating that the local protein concentration increased. As well, we did not observe any shift in the Raman spectrum, indicating that no HEWL denaturation process took place. In order to gain further experimental evidence, we also followed this protein film formation using fluorescence imaging. A small fraction (1:10000) of Rhodamine-labelled lysozyme was added to the protein solution. In this case, the formation of a fluorescent domain is observed, which radially expands up to few tens µm in 60 s and disappears about 40 s after switching off the trapping laser. Both Raman and fluorescence experiments support the formation of a highly concentrated domain of HEWL by optical trapping. Indeed, this phenomenon is probably coupled to a liquid-liquid phase separation. The presented results set the basis for developing a new methodology based on optical trapping to generate highly concentrated protein domains, which cannot be easily obtained using the current state-of-the-art methodologies.

References

1) T. Sugiyama, K. Yuyama, and H. Masuhara, Laser trapping chemistry: from polymer assembly to amino acid crystallization. Acc. Chem. Res., 45, 11, 1946–1954 (2012).
2) K. Yuyama, K.-D. Chang, J.-R. Tu, H. Masuhara, and T. Sugiyama, Rapid localized crystallization of lysozyme by laser trapping. Phys. Chem. Chem. Phys., 20, 6034-6039 (2018).
3) I. Aibara, C.-H. Huang, T. Kudo, R. Bresolí-Obach, J. Hofkens, A. Furube, and H. Masuhara, Dynamic coupling of optically evolved assembling and swarming of gold nanoparticles with photothermal local phase separation of polymer solution. J. Phys. Chem. C, 124, 30, 16604–16615 (2020).
4) C.-H. Huang, T. Kudo, R. Bresolí-Obach, J. Hofkens, T. Sugiyama, and H. Masuhara, Surface plasmon resonance effect on laser trapping and swarming of gold nanoparticles at an interface. Opt. Exp., 28, 19, 27727-27735 (2020).
5) P.-W. Yi, W.-H. Chiu, T. Kudo, R. Bresolí-Obach, J. Hofkens, E. Chatani, R. Yasukuni, Y. Hosokawa, T. Sugiyama, S. Toyouchi, and H. Masuhara, Single sub-millimeter linear assembly of polystyrene microparticles by optical trapping at protein solution surface. Abstract of 2021 Spring Meeting of Japan Society of Applied Physics (JSAP).

P-9.1.2

Polyfluorinated aromatic porphyrin as a photoactive scaffold for peptide cyclisation

Paolo Dognini[1], Talhat Chaudhry[1], Kehinde Ross[1], Christopher Coxon[2], and Francesca Giuntini[1]

1) School of Pharmacy and Biomolecular Sciences, Byrom Street Campus, Liverpool John Moores University, Liverpool L3 3AF, UK; 2) Institute of Chemical Sciences, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh AH14 4AS, UK

Polyfluoroaromatic reagents readily react with thiols via S_NAr and provide excellent templates on to which peptides can be assembled through side-chain macrocyclisation. We have previously investigated the reactivity of hexafluorobenzene (HFB, **1**) towards cysteine and related nucleophiles [1–3] and subsequently extended this study to fluorinated porphyrins, exploiting them as photoactive scaffolds for peptide conjugation and cyclisation (Figure 1). We will describe how the di-substitution of tetrakis(pentafluorophenyl)porphyrin (**2**) with the Skin Penetrating And Cell Entering (SPACE) peptide [4] (ACTGSTQHQCG), presenting two cysteines in position *i, i+8*, successfully afforded the macrocyclised product under peptide-compatible conditions. By repeating this reaction using peptides with different sequences and inter-thiol distances, we evaluated whether this chemistry is sequence specific. Moreover, the combination of these results with those from a similar methodology applied to bis(pentafluorophenyl)porphyrin (**3**) gave us important insights regarding the geometrical constraints of the macorcyclisation (e.g. minimal number of amino acids required to reach the *para* positions of *trans* and *cis* meso phenyl groups). We will confirm the substitution pattern using ^{19}F NMR and we anticipate that the remaining para positions of products **5** and **6** are also available for further conjugation.

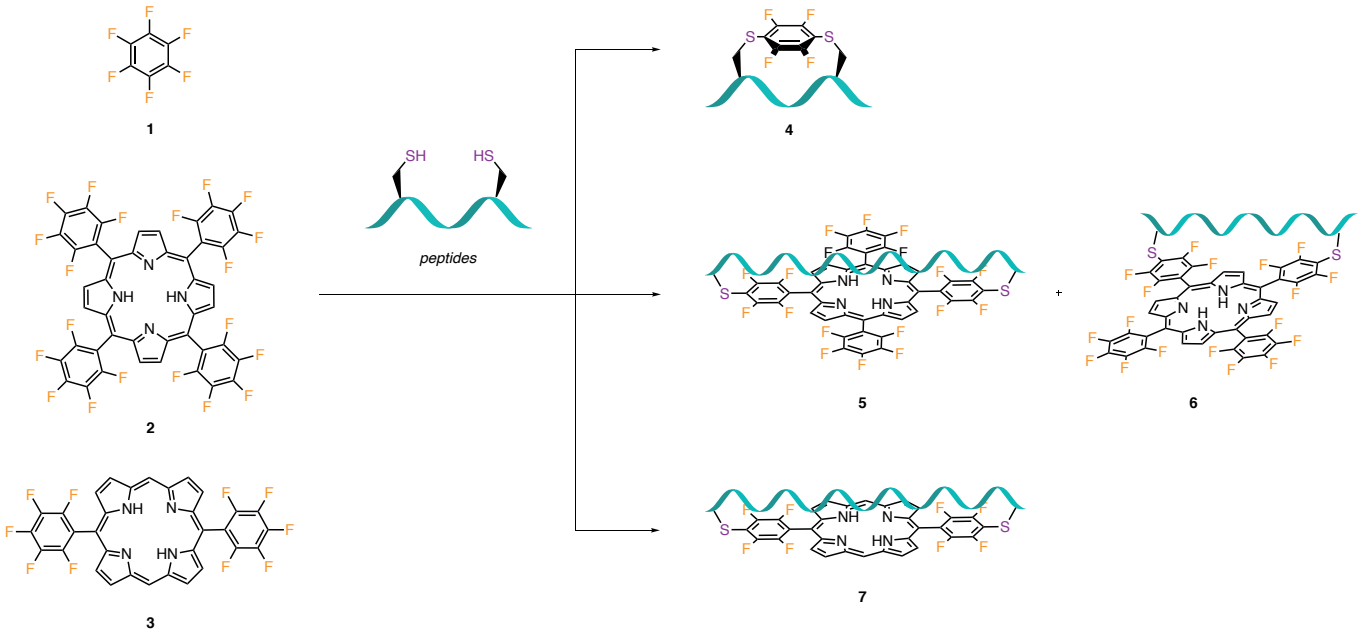


*Fig. 1 – General reaction scheme for peptide cyclisation using hexafluorobenzene (**1**), tetrakis(pentafluorophenyl)porphyrin (**2**) and bis(pentafluorophenyl)porphyrin (**3**) and related products.*

Preliminary biological data showed that crosslinking SPACE peptide cysteines with **2** does not affect its uptake in skin cancer cells while the presence of the porphyrin core imparts fluorescence and photodynamic properties to this water-soluble conjugate (Figure 2). Further steps in understanding the biological behaviour of this conjugate will be the elucidation of its uptake mechanism in comparison with the uptake of SPACE peptide alone [4] and the investigation of any specific subcellular localisation.

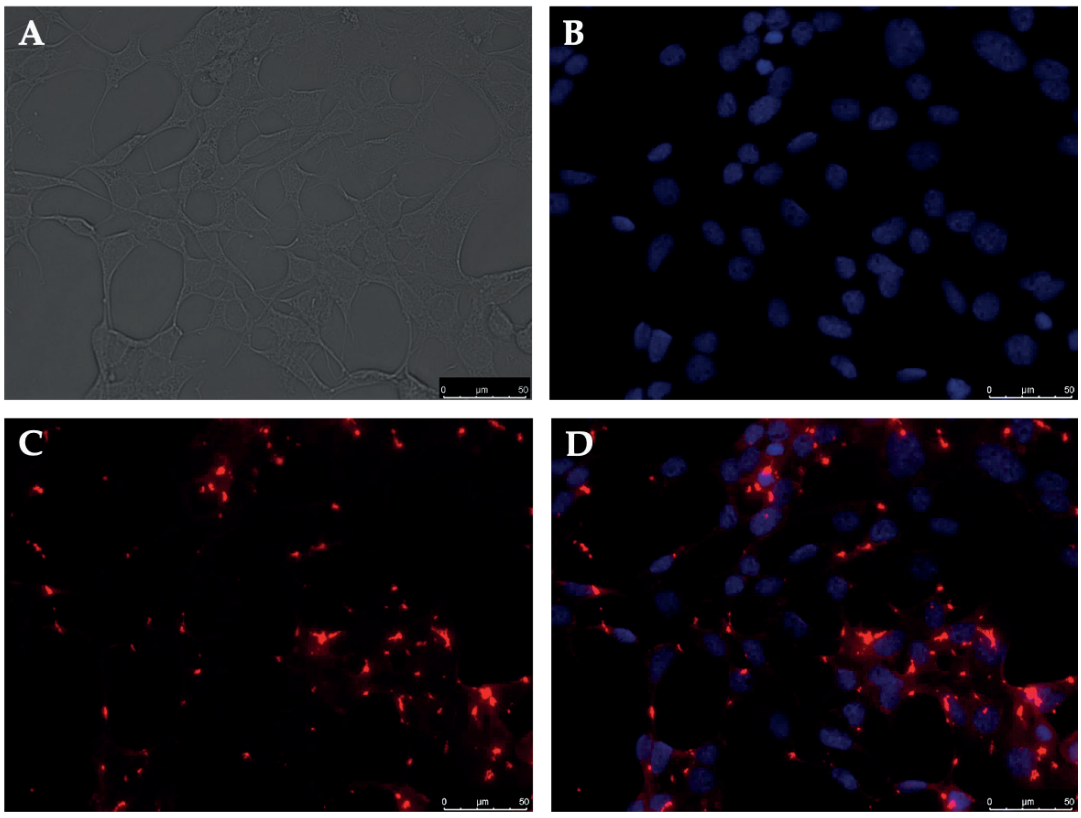


Fig. 2 – SCCIC8 cells were treated in complete media with F20-SPACE peptide conjugate (10 μM) and incubated for 24h in the dark. Images were captured at 40X magnification with a Leica Live cell imaging microscope. A) Bright Field image of cells; B) Nuclei stained with DAPI; C) Uptake of F20-SPACE peptide conjugate; D) Collated image of conjugate uptake with DAPI stained nuclei.

In conclusion, this robust and accessible synthetic tool allows the conjugation of two biologically active compounds, joining and concurrently improving their individual properties. Moreover, the possibility to modulate the peptide sequence and differently exploit the photoactive tetrapyrrole scaffold opens the way to a wide range of applications in biological systems, especially as molecular probes, drugs and detecting agents.

References

[1] A.M. Spokoyny, Y. Zou, J.J. Ling, H. Yu, Y.S. Lin, B.L. Pentelute, A perfluoroaryl-cysteine SNAr chemistry approach to unprotected peptide stapling, J. Am. Chem. Soc. 135 (2013) 5946–5949. doi:10.1021/ja400119t.

[2] S.J.M. Verhoork, C.E. Jennings, N. Rozatian, J. Reeks, J. Meng, E.K. Corlett, F. Bunglawala, M.E.M. Noble, A.G. Leach, C.R. Coxon, Tuning the Binding Affinity and Selectivity of Perfluoroaryl-Stapled Peptides by Cysteine-Editing, Chem. - A Eur. J. 25 (2019) 177–182. doi:10.1002/chem.201804163.

[3] P. Dognini, P.M. Killoran, G.S. Hanson, L. Halsall, T. Chaudhry, Z. Islam, F. Giuntini, C.R. Coxon, Using 19F NMR and two-level factorial design to explore thiol-fluoride substitution in hexafluorobenzene and its application in peptide stapling and cyclisation, Pept. Sci. 113 (2021) e24182. doi:10.1002/pep2.24182.

[4] T. Hsu, S. Mitragotri, Delivery of siRNA and other macromolecules into skin and cells using a peptide enhancer, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 15816–15821. doi:10.1073/pnas.1016152108.

Acknowledgements. This work was supported by a University Alliance Doctoral Training Alliance CO-FUND/Marie Skłodowska-Curie (European Union's Horizon 2020 research and innovation pro- gramme, grant agreement No 801604) PhD Fellowship Programme in Applied Biosciences for Health (PD).

Authors Index

Bold indicates presenting authors

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z