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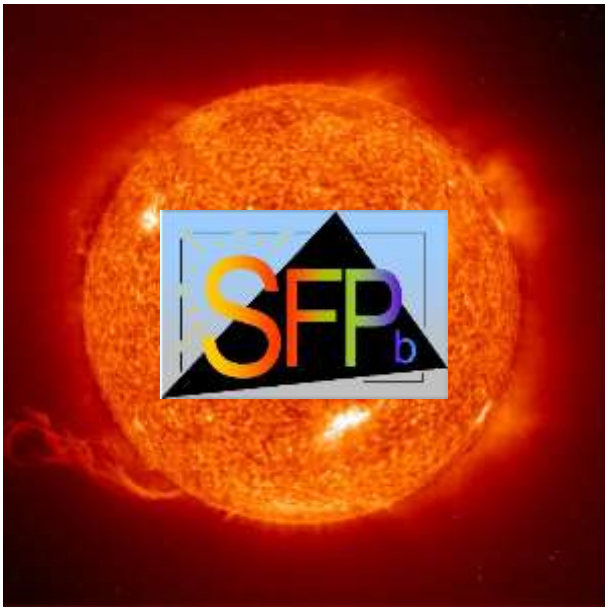
« Photobiologie sous le soleil »



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Sorbonne Université - Campus de Jussieu, Paris

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Journées réalisées avec le soutien de Sorbonne-Université et de L'Oréal

Résumés des Présentations

Session 1

Photochimie des biomolécules : théorie et spectroscopie

Conférences Invitées

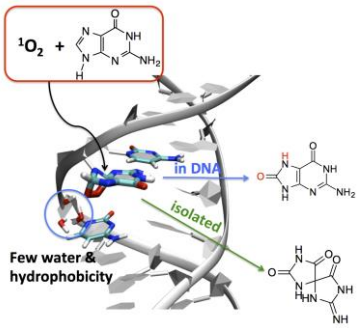
Structural insights into DNA photolesions in and out the nucleosome

Elise Dumont

Formation and repair of DNA lesions embraces a rich and combinatorial chemistry, where atomic-scale simulations are most helpful to complement and expand experimental evidences. I will present our recent efforts to achieve a robust, computationally-driven description of DNA photolesions, amongst which 64-PP and cyclobutane pyrimidines dimers.

Intensive multiscale molecular dynamics, coupling a description for an electronically-active subsystem and the rest of the double-stranded helix, allow to trace back triplet-triplet energy mechanisms between DNA and type II photosensitizers [1], singlet oxygen attack onto guanine [2], and delineate rule-of-thumbs for the non-covalent forces that drive sequence recognition [3].

I will also illustrate the power of all-atom, classical molecular dynamics to investigate the structure and repair of clustered DNA lesions.



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Theoretical insights to understand when Bioluminescence and Fluorescence spectra should be alike or not.

Isabelle Navizet
Université Paris-Est, Laboratoire Modélisation et Simulation Multi Echelle, MSME, UMR 8208 CNRS, UPEM, 5 bd Descartes, 77454 Marne-la-Vallée, France
isabelle.navizet@u-pem.fr, http://msme.u-pem.fr/

The emitting light in fireflies or other bioluminescent species arises from the electronic relaxation of oxyluciferin, an organic compound resulting from the oxidation of the D-luciferin substrate inside an enzyme called luciferase.

As the bioluminescent systems are already used as a marker in biology, man needs to understand what are the chemical and physical important factors responsible for the emitted light's color. In order to have insight of the mechanism of the light emission, both experimental and theoretical joint studies have been performed. Fluorescence experiments (in the lab or numerical) on the products of the bioluminescence are usually performed to better understand the system. Are these experiments always relevant?

In order to theoretically study bioluminescent and fluorencence of biological photoemitters' systems, the use of quantum mechanical/molecular mechanical (QM/MM) methods is required. Accurate QM level is needed for dealing with electronic transition and charge transfer phenomena. Taking into account the surrounding protein at the MM level is essential in order to understand the color modulation and influence of the enzyme.
The presentation will present briefly the methods used and will discuss examples of how theoretical studies can give complementary insights to the experimental results for the understanding of such complex phenomena. Fluorescence and bioluminescence phenomena will be compared. Influence of the surrounding environment will be discussed.

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Chimiluminescence du dioxétane : simulations de dynamique moléculaire ab initio et analyse par machine learning

Morgane Vacher
Université de Nantes et CNRS, Laboratoire CEISAM

La chimiluminescence est l'émission de lumière à la suite d'une réaction chimique non adiabatique. L'une des molécules les plus simples ayant des propriétés chimiluminescentes est le 1,2-dioxétane. Il a été observé que le rendement du procédé chimiluminescent était faible dans le 1,2-dioxétane (0,3%), mais il augmente à 35% en substituant les atomes d'hydrogène par des groupes méthyle. La raison de cette augmentation impressionnante est longtemps restée incomprise. Tout d'abord, nous
l'adressons en simulant la dynamique de la réaction de décomposition. D'autre part, bien que les simulations soient essentielles à la compréhension de réactions chimiques, un défi actuel est l'analyse approfondie de la grande quantité de données produites. Ici, nous présentons des modèles d'apprentissage automatique capables de faire des prédictions précises et de mettre en évidence des règles empiriques qui font aujourd'hui parti des connaissances chimiques.

Photodynamics of fluorescent proteins applied in RESOLFT nanoscopy

Lucas M. Uriarte, Olivier Devos, Cyril Ruckebusch, Steve Meech, Dominique Bourgeois, J. P. Colletier, Martin Weik and Michel Sliwa
Univ. Lille, CNRS, UMR 8516, LASIR, Lille, France
School of Chemistry, University of East Anglia, Norwich, UK
Institut de Biologie Structurale, Univ. Grenoble Alpes, CEA, CNRS, Grenoble, France
Michel.Sliwa@univ-lille.fr

Recently, reversibly photoswitchable fluorescent proteins (RSFPs) have been widely applied in super-resolved fluorescence microscopy, such as reversible saturable optical fluorescence transition (RESOLFT), a super-resolved microscopy technique that allows for a significant reduction in the illumination intensities and in photobleaching. Even though photo-physical parameters (switching, fluorescence quantum yields…) are linked to the resolution and image acquisition speed, the switching mechanism that controls these parameters is still a matter of debate. Using electronic and vibrational time-resolved transient absorption spectroscopy from the femtosecond to the millisecond time scale we studied the mechanism of off-to-on and on-to-off photoswitching in WT and different mutants of rsEGFP2 which is the most common protein used in RESOLFT super-resolution microscopy. We will discuss here the existence of different isomerization mechanims between different off and on states. We will show then for rsEGFP2 how the protein cage controls meaningful parameters for RESOLFT nanoscopy: fluroescent quantum yield, photo-dynamics and switching yield.

Session 1

Photochimie des biomolécules : théorie et spectroscopie

Communications Orales

Computational Investigation on Bioluminescence of NanoLuc-Furimamide Complex: Effect of Protonation

Mehdi Sahihi et Isabelle Navizet

Université Paris-Est, Laboratoire Modélisation et Simulation Multi Echelle, MSME UMR 8208 CNRS, 5 bd Descartes, 77454, Marne-la-Vallée, France.

E-mail: Mehdi.sahihi@gmail.com

Here, the fluorescence and bioluminescence of furimamide and its different protonated and deprotonated states are studied. Also, the energetics of different protonation states are investigated, as well. Furthermore, we clarify the interaction of furimazine-NanoLuc and the stability of their complex using molecular docking and molecular dynamics (MD) simulation methods, respectively. The spectroscopic properties of furimamide are studied by density functional theory (DFT) and time dependent TD-DFT calculations on eight possible light emitters: three neutral forms, amide anion, three pyrazine cations and pyrazine dication. We modelled furimamide and its protonated and deprotonated analogues, using theoretical approaches (TD-DFT calculations at QM and QM/MM levels) in different environments (in gas phase, in PCM model and in NanoLuc)[1]. We have compared the obtained theoretical emission spectra to clarify the effect of protonation and various media on fluorescence of furimamide.[2–4]

Keywords: Bioluminescence, NanoLuc, Furimamide, QM/MM, Molecular Docking, MD simulation.
Programs: Gaussian G09, Gromacs 2019, AutoDock 4.2.

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Fluorescence emission of the oxyluciferin and some of its analogues by QM and QM/MM methods

Madjid Zemmouche, Cristina García-Iriepa et Isabelle Navizet

Université Paris-Est, Laboratoire Modélisation et Simulation Multi Echelle, MSME UMR 8208 CNRS, 5 bd Descartes, 77454, Marne-la-Vallée, France.

e-mail: madjid.zemmouche@u-pem.fr

In this work we have performed theoretical study on the firefly oxyluciferin, the resulting reactant of bioluminescence reaction responsible for the light emission. It has been already experimentally proposed different analogues of natural oxyluciferin, showing different emission colours. In particular, the structure of natural oxyluciferin has been modified by atom or groups substitutions. The aim of this study is to determine the influence of substitution and modification within oxyluciferin structure on the colour modulation in the fluorescence emission. To do this, we have modelled the oxyluciferin and its synthesized analogues, whose experimental bioluminescent spectra are red and blue-shifted regarding the natural one, using theoretical approaches (TD-DFT calculations at QM and QM/MM levels) in different environments (in gas phase, in PCM model and in protein)[1]. We have compared the obtained theoretical emission spectra with the available experimental spectra.[2–4]

Keywords: Bioluminescence, oxyluciferin, MD, QM/MM.
Programs: Amber14, Gaussian G09 and G09/Tinker coupling.

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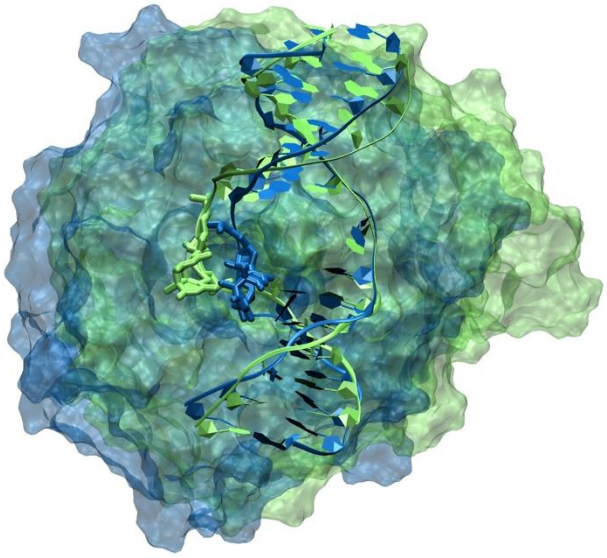
Elucidating the different repair rates of cyclobutane pyrimidine dimers in DNA oligomers and their recognition by repair enzymes

Cécilia Hognon[1], Chen-Hui Chan[2], Christophe Chipot[1,3], François Dehez[1,3], Jean-Luc Ravanat[4], Thierry Douki[4], Elise Dumont[2,*] and Antonio Monari[1,*]

[1]Université de Lorraine and CNRS, LPCT UMR 7019, F54000 Nancy, France; [2]Université de Lyon, ENS de Lyon, CNRS, Université Lyon 1, Laboratoire de Chimie UMR 5182, F69342 Lyon, France; [3]Laboratoire International Associé Centre National de la Recherche Scientifique et University of Illinois at Urbana Champaign; [4] Univ. Grenoble Alpes, CEA, CNRS, INAC, SyMMES/CIBEST, F- 38000 Grenoble

E.mail : cecilia.hognon@univ-lorraine.fr

The repair of photo-induced DNA lesions through nucleotide excision repair (NER) machinery is still the source of important questions. In particular, it has been observed that the repair rate of the different cyclobutane pyrimidine dimers, i.e. the photochemical induced dimerization of two π-stacked pyrimidines (T<>T, T<>C, C<>T, C<>C), depends on the nucleobases involved in the lesion[1]. TT derivatives (T<>T) are removed more slowly than those containing cytosine. Using all-atom molecular dynamics simulations and free-energy calculations, we demonstrate that variation of the repair rate observed in human skin and in cultured cutaneous cell is associated to the recognition of the four lesions by the DDB2 protein moiety. Indeed, while C<>C and C<>T experience excellent interaction with the repair protein interface, T<>T and T<>C are slightly displaced from the recognition region, hence hampering optimal repair. The observed DNA deformation and the free-energy profile underlying CPD extrusion correlate with the experimental repair rate, and provide a structural rationale for the repair rates of CPD by the NER machinery.



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Formation of inclusion complexes between temoporfin and β-cyclodextrins

Yakavets I[1,2,3], Lassalle HP[1,2], Yankovsky I[3], Ingrosso F[4], Monari A[4], Bezdetnaya L[1,2], Zorin V[3]

1. *Institut de Cancérologie de Lorraine, Vandoeuvre-lès-Nancy, France*
2. *Centre de Recherche en Automatique de Nancy, CNRS, Université de Lorraine, Nancy, France*
3. *Department of Biophysics, Belarussian State University, Minsk, Belarus*
4. *Université de Lorraine, CNRS, LPCT, F-54000 Nancy, France*

The interaction between the potent photosensitizer meta-tetrakis(3-hydroxyphenyl)chlorin (temoporfin, mTHPC) and a series of β-cyclodextrins (β-CDs) was investigated using spectroscopic analysis and molecular dynamics simulations. The possibility of improving its poor aqueous solubility with β-CDs was estimated by measuring both equilibrium solubility and association constants. Parameters of binding isotherms revealed that mTHPC strongly interacts with β-CD derivatives, forming 1:2 inclusion complexes in aqueous solution. We demonstrated that apparent binding constants strongly depend on mTHPC concentration due to the porphyrin self-aggregation. The estimated "correct" binding constants demonstrated that completely methylated β-CD exhibits the highest affinity (K = 1.1×10^7 M^{-1}) as compared to randomly methylated β-CD (K = 7.1×10^5 M^{-1}) and 2-hydroxypropyl substituted β-CD (K = 1.7×10^5 M^{-1}). As a corollary of our results concerning the high binding affinity and efficient solubilization of mTHPC by β-CD, one can assume the application of β-CDs as mTHPC nanovectors [2]. Thus, the knowledge of correct values of binding constants could be helpful for the selection of optimal composition of β-CD-based delivery system for mTHPC-mediated PDT.

This work was supported by the Institut de Cancérologie de Lorraine; French "Ligue Nationale Contre le Cancer (CCIR-GE)"; Belarusian Republican Foundation for Fundamental Research (BRFFR) [grant number M18MB-002]; the Ministry of Education of the Republic. The authors thank biolitec research GmbH (Jena, Germany) for providing with mTHPC.

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Dynamics of pH-induced conformational changes in the Cyan Fluorescent Protein (CFP): towards a better understanding of the structure-photophysics-dynamics relationship.

Hélène Pasquier

Laboratoire de Chimie Physique, UMR CNRS 8000, 91405 Orsay

Recent development of cyan fluorescent proteins (CFPs) follows a step by step evaluation of the spectroscopic effects of crucial mutations within a family of spectrally identical and evolutionary close variants. Through this process, it was revealed that an adequate and well-chosen single mutation at position 148 improves the CFPs photophysics and reduces its pH sensitivity [1]. More generally, CFP and its mutants are model compounds allowing us to decipher the pH-induced conformational changes occurring in fluorescent proteins and their time scale.

Here we report a study aiming at following the fluorescence decay kinetics of CFP and its variant CFP-H148G under out-of-equilibrium initial conditions. At fixed pH (5 or 7.4), both proteins display complex fluorescence decay kinetics [2]. We perform pH-jump experiments targeting the determination of the time scale over which the fluorescence decay kinetics of both proteins evolve upon sudden acidification. This in turn reveals the time scale for the protein structural relaxation upon pH-jump.

To this end, with a previously validated approach [3], we use droplet microfluidics to produce out-of-equilibrium initial conditions by rapid mixing (≤ 1 ms) within the very small (few 100 pL) volume of water-in-oil droplets. In CFP the results reveal a population exchange on a time scale of a few hundred ms between the two dominating long-lived fluorescence components. These results will be discussed in the light of the crystallographic structure analysis obtained for both proteins at neutral and acid pH.

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Transfert d'électron ultrarapide dans les flavoenzymes : états produits radicaux et photocatalyse

Lipsa Nag[1], Andras Lukacs[2], Hannu Myllykallio[1], Ursula Liebl[1], Marten Vos[1]

1. *LOB, Ecole Polytechnique, Institut polytechnique de Paris, CNRS, INSERM, 91128 Palaiseau*
2. *Department of Biophysics, Medical School, University of Pecs, Hongrie,*

Les flavoprotéines sont omniprésentes et impliquées dans de nombreuses fonctions biologiques qui exploitent la capacité des flavines à agir en tant qu'intermédiaires de transfert d'électrons et/ou de protons. La plupart des flavoprotéines ne sont pas naturellement photoactives, mais certaines sont impliquées dans des processus photobiologiques. La photoréduction des flavines peut être obtenue principalement par transfert d'électrons à partir d'acides aminés tryptophane (TrpH) ou tyrosine (TyrOH) proches, ou par des substrats dans le site actif, vers la flavine excité. Parmi les photoproduits possibles, la forme radicalaire $TyrOH^{\circ+}$, probablement très instable (pK ~2), n'avait jamais été observé. En utilisant la spectroscopie de fluorescence et d'absorption ultra-rapide de variants de l'ARN méthyltransférase bactérien TrmFO[1] et la glucose oxydase, une flavoprotéine modèle bien étudiée[2], nous avons récemment proposé que cet état puisse être formé avec une durée de vie de quelques picosecondes et nous avons déterminé sa signature spectrale. Cette observation implique que l'oxydation de TyrOH n'induit pas nécessairement sa déprotonation concertée.

Enfin, dans les photoenzymes, la catalyse peut être initiée par des interactions de transfert de charge entre chromophore et substrat. Nous avons commencé à étudier la photoréduction picoseconde de la flavine dans la photodécarboxylase d'acides gras[3], où tous les acides aminés aromatiques susceptibles de concurrencer cette réaction sont situés loin de la flavine.

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Session 2

Lumière-Perception et collection chez les organismes photosynthétiques

Conférences Invitées

Structural bases of light utilization in diatoms

Clarisse Uwizeye[1], Serena Flori[1,2], Guillaume Allorent[1], Florence Courtois[1], Davide dal Bo[1], Johan Decelle[1], Denis Falconet[1], Giovanni Finazzi Giovanni Finazzi[1], Benoit Gallet[3] Cécile Giustini[1], Pierre Henri Jouneau[1], Claire Seydoux[1], Guy Schoehn[1],

[1]Institut de Recherche Interdisciplinaire, Université Grenoble Alpes, CEA Grenoble

[2]Marine Biology Association of the UK, Plymouth, United Kingdom

Oxygenic photosynthesis is almost equally distributed in the land and the ocean. However, the latter contains much less photosynthetic biomass than the former, suggesting that the efficiency of CO_2 assimilation is very high in ocean phototrophs (phytoplankton).

What are the regulation mechanisms that allow improving phytoplankton photosynthesis? One of the 'master regulators' of photosynthesis is the Proton Motive Force (PMF), i.e. a gradient of proton and ions that is generated by photosynthesis. The PMF controls several steps of this process: light absorption (via a negative feedback: Non Photochemical Quenching), electron flow and ATP synthesis.

Using molecular engineering and in vivo spectroscopy, we have pinpointed molecular actors (photoreceptors[1], ion channels[2], redox transporters[2]) that regulate the PMF and its consequences on cell physiology in the diatom *Phaeodactylum tricornutum*. These elements actively modify the light acclimation responses of this organism in laboratory conditions. Using single cell tomography we have also identified structural elements of this regulation, showing the existence of subcellular interactions between the energy producing organelles, the chloroplast and mitochondria[3-5].

A survey of several phytoplankton members suggests a conservation of these interactions and of their role in driving acclimation to the environment.

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Photoprotection and electron transport mechanisms in green algae

Xenie JOHNSON

Photosynthesis and Environment Team, Aix Marseille Univ., CEA, CNRS, BIAM, Cadarache.

In plants, algae and cyanobacteria, photosynthetic electron transport is tightly linked to CO_2 assimilation. Depending on CO_2 availability, either the light reactions or carbon metabolism can be limiting for photosynthesis [1], therefore modulating the redox poise of the terminal electron acceptors downstream of photosystem I (PSI) as well as the inter-photosystem electron carriers. Environmental variations such as changes in light intensity or temperature modify the energetic balance between metabolism and light reactions. Electron transport regulation tunes this balance in order to avoid over-reduction of the photosynthetic apparatus and photodamage [2]. The process of light acclimation includes three major mechanisms conserved in all oxygenic phototrophics: (i) regulation of light capture and PSII turnover, (ii) photosynthetic control, (iii) regulation of CO_2 assimilation and primary carbon metabolism [3]. Using the model green algae, *Chlamydomonas reinhardtii,* these three processes can be studied in isolation and together using combinations of single and double mutants with biophysical and biochemical analysis. To understand photosynthetic regulation in response to light, mutants without CO_2 assimilation (*rbcL*), without photosynthetic control (*pgr5*) or lacking mechanisms in PSII photoprotection (*ape1*) are informative to understand the interplay between photoprotection and energetics [4-6]. The major aim of this work is to understand what limits photosynthetic productivity.

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Bases moléculaires et importance écologique de l'acclimatation chromatique chez les cyanobactéries marines du genre *Synechococcus*

Théophile Grébert[1], Hugo Doré[1], Jo Sanfilippo[2], Adam A. Nguyen[3,4], Suman Pokhrel[3,4], Bo Chen[2], Morgane Ratin[1], Allissa M. Haney[2], Jonathan A. Karty[5], Laurence Garczarek[1], Wendy M. Schluchter[3,4], David M. Kehoe[2] & Frédéric Partensky[1]

[1]Station Biologique, UMR 7144 CNRS & Sorbonne Univ., Equipe 'Ecologie du Plancton Marin' (ECOMAP), 29680 Roscoff, France; [2]Dept of Biology, Indiana Univ., Bloomington, IN 47405, USA; [3]Dept of Biological Sciences, Univ. New Orleans, LA 70148, USA; [4]Dept of Chemistry, Univ. New Orleans, LA 70148, USA; [5]METACyt Biochemical Analysis Center, Dept of Chemistry, Indiana Univ., Bloomington, IN 47405, USA.

Les cyanobactéries marines du genre *Synechococcus* se caractérisent par la grande diversité pigmentaire de leurs complexes antennaires ou phycobilisomes (PBS). Dans les zones océaniques, ces cellules possèdent toutes des PBS sophistiqués, composés de phycocyanine et de deux types de phycoérythrines (PE-I et PE-II). Certaines cellules sont spécialisées dans la collecte de lumière verte ou bleue, tandis que d'autres peuvent modifier dynamiquement leur spectre d'absorption de la lumière afin de capturer la couleur dominante : vert ou bleu. Ce processus appelé 'acclimatation chromatique de type IV' (CA4) est lié à la présence d'un îlot génomique existant sous deux configurations (CA4-A et -B ; [1]). Les populations possédant ces deux types d'îlots occupent des niches très complémentaires dans l'environnement marin [2]. D'un point de vue moléculaire, les deux types d'acclimatation chromatique impliquent deux régulateurs transcriptionnels, FciA et FciB [3], et deux couples phycobiline lyase/lyase-isomérase: MpeZ et MpeY chez les CA4-A [4, 5], MpeW et MpeQ chez les CA4-B [Grébert et al., in prep.], que nous avons caractérisés par diverses approches (mutagénèse, expression hétérologue, spectrométrie de masse LC/MS/MS pour identifier le type de chromophores et leur site de fixation, etc.). Nos données montrent que les processus CA4-A et -B agissent 'en miroir' l'un de l'autre et supportent un scénario évolutif selon lequel l'acquisition de l'îlot CA4-B aurait permis à des spécialistes de la lumière bleue de devenir des acclimateurs chromatiques, tandis que des spécialistes de la lumière verte auraient acquis cette capacité en acquérant un îlot CA4-A.

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Chronobiologie et perception de la lumière à l'environnement

François-Yves Bouget

Laboratoire d'Océanographie Microbienne, Banyuls sur Mer

Session 2

Lumière-Perception et collection chez les organismes photosynthétiques

Communications Orales

Production of reactive oxygen species during leaf senescence

Ginga Shimakawa[1], Karin Krupinska[2], Anja Krieger-Liszkay[1]

[1]Institute for Integrative Biology of the Cell, CEA, CNRS, Université Paris-Saclay, 91198 Gif-sur-Yvette, France
[2]Institute of Botany, University of Kiel, Olshausenstraße 40, 24098 Kiel, Germany

Generation of reactive oxygen species (ROS) in chloroplasts may play a crucial role in triggering the initiation of leaf senescence[1]. We studied the generation of ROS and changes in the photosynthetic electron transport chain in two barley varieties. During senescence chlorophyll content decreased and photosynthetic electron transport was inhibited as shown for flag leaves collected from barley varieties Lomerit and Carina grown in the field and in controlled conditions. Spin trapping electron paramagnetic resonance (EPR) was used to investigate the production of reactive oxygen species in thylakoid membranes during senescence[2]. EPR measurements were performed with specific spin traps to discriminate between singlet oxygen on one hand and reactive oxygen intermediates on the other hand. The results show that the generation of reactive oxygen intermediates increases in both varieties during senescence. Singlet oxygen increased only in the variety cv. Lomerit while it remained constant at a low level in the variety cv. Carina. In Lomerit photosystem II activity decayed much earlier than in Carina in field grown material, while no difference was observed in material grown under controlled conditions. Measurements in the presence of inhibitors of photosystem II and of the cytochrome b6f complex revealed that in senescing leaves reduction of oxygen at the acceptor side of photosystem I was the major, but not the only source of superoxide anions. This study shows that during senescence the production of individual reactive oxygen species varies in different barley varieties and different growth conditions. Abiotic stresses like UV, fluctuating light, extreme temperatures or temporary drought may affect photosystem II activity and singlet oxygen production in field-grown Lomerit, thereby inducing a different senescence scenario than under controlled conditions where only superoxide is generated in both, Lomerit and Carina.

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Phytochromes in diatom microalgae: red and far-red light perception in the Ocean

Carole Duchêne[1], Jean-Pierre Bouly[1], Juan Pierella Karlusich[2], Chris Bowler[2], Angela Falciatore[1], Marianne Jaubert[1]

1. Laboratoire de Biologie du chloroplaste et perception de la lumière chez les microalgues UMR7141, CNRS, Sorbonne Université, Institut de Biologie Physico-Chimique , Paris, France
2. Ecole Normale Supérieure, Institut de Biologie de l'ENS (IBENS), Paris, France

Light is an essential source of energy and information for photosynthetic organisms. In the marine environment, the light field is structured by depth, as red and far-red light are quickly attenuated while blue and green penetrate deeper in the water column. Diatoms are important marine microalgae, which are responsible for 40% of the marine primary production. They possess a wide array of blue and green photodetectors (cryptochromes, aureochromes, rhodopsins). More surprisingly, diatoms also possess red/far-red phytochrome photoreceptors (DPHY), which regulate gene expression in response to far-red light (750nm) in the model diatom *Phaeodactylum tricornutum.* This finding brings up questions about the function and the significance of phytochrome-mediated far-red light signaling in the oceans. To address these questions, we have generated *P. tricornutum* transgenic lines, expressing YFP under the control of a DPHY-regulated promoter. By measuring the YFP signal of cells grown in different conditions (light colors, intensities, red and far-red ratios, cell concentrations, etc.), we have been able to follow the DPHY activity in vivo, and to start characterizing its in vivo photochemical properties (sensitivity, reversibility), giving hints at the conditions in which it can be active in the marine environment. In parallel, we opened the questions of far-red sensing to other species by studying the occurrence of DPHY genes in the meta-omics *Tara Oceans* data. We showed that although not all diatoms possess phytochromes, phytochrome-containing diatoms are present in the seas all around the world.

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ORANGE CAROTENOID PROTEIN: INSIGHTS ON ITS MOLECULAR MECHANISM AND POSSIBLE BIOMEDICAL APPLICATIONS

Silvia Leccese[1], Claude Jolivalt[1], Thomas Onfroy[1], Adjélé Wilson[2], Diana Kirilovsky[2], Alberto Mezzetti[1]

[1]Laboratoire de Réactivité de Surfaces UMR CNRS 7197, Sorbonne Université
[2] I2BC, UMR CNRS 9198, CEA-Saclay, Université Paris-Saclay

Orange Carotenoid Protein (OCP) is a water-soluble photoactive protein involved in photoprotection of the photosynthetic apparatus of cyanobacteria. The chromophore is a carotenoid; under strong light, OCP changes its conformation (and its colour) from a so called orange form OCP^O to a red form OCP^R; the latter is able to bind the phycobilisome (the antenna system of cyanobacteria) and to quench the excess of light energy, which could lead to photodamage. We have characterized the light-induced conformational changes of OCP, using FTIR difference spectroscopy, and focusing on the conformational changes of carotenoid during the photo-activation. In addition, we have investigated OCP inside mesoporous silica SBA-15 nanoparticles. OCP remains photoactive under these conditions. On the one hand this makes it possible to better study and characterize the effect of the external environment on the photocycle, trying to understand the influence of the constraint of the matrix. On the other hand, it represents a first step towards the use of the OCP@SBA-15 system, as possible optical photochromic devices and as sensors of other parameters, such as pH and temperature. Different kinds of SBA-15 nanoparticles have been used, with different porous sizes and different internal surfaces. The most interesting results (i.e. complete photoactivation) were obtained using nanoparticles whose surfaces where chemically modified by the insertion NH_2 groups. Possible applications in fluorescence imaging using OCP@SiO_2 as switch on/off for energy transfer and applications in antioxidant drug delivery are also presented.

Light-harvesting and photoprotection in the eukaryotic phytoplankton of the global ocean

Juan J. Pierella Karlusich, Zoé Fournout, Etienne Dvorak, Federico ibarbalz, Chris Bowler

Institut de Biologie de l'École Normale Supérieure

Marine eukaryotic phytoplankton accounts for around 30% of Earth´s net primary production. Their light antenna contain the light-harvesting complex (LHC) proteins, which are not found in prokaryotic phytoplankton (cyanobacteria). This family of pigment-binding proteins are considered the most abundant membrane proteins on Earth. They collect and transfer energy from the sunlight to the photosystems, but they also include stress-induced variants involved in photoprotection under excess light. Here, we have explored the data generated by *Tara* Oceans through metagenomics and metatranscriptomics to characterize LHC diversity and distributions in phytoplankton communities from both surface and deep chlorophyll maximum layers (0-200 m) across the major ocean basins. Our results provide a comprehensive picture of the main eukaryotic contributors to marine photosynthesis and in the different patters of adaptation and acclimation (changes in gene copy number and expression) driven by environmental variables, as well as latitudinal and depth gradients among the LHC subfamilies involved in photoprotection *vs* light-harvesting.

REGULATION OF PHOTOSYNTHESIS BY UV-B PHOTOPERCEPTION IN MICROALGAE

Guillaume Allorent[$], Florence Courtois[$], Linnka Lefebvre-Legendre[#], Richard Chappuis[#], Denis Falconnet[$], Kuntz Marcel[$], Thuy B. Truong[£], Krishna K. Niyogi[£], Marie Dannay[$], Chloé Bertin[$], Roman Ulm[#], Michel Goldschmidt-Clermont[#], Giovanni Finazzi[$]

[$]Univ. Grenoble-Alpes, CNRS, INRA, CEA, IRIG-LPCV, Grenoble, France; [#]Department of Botany and Plant Biology, University of Geneva, Geneva, Switzerland and [£]Howard Hughes Medical Institute, Department of Plant and Microbial Biology, University of California, Berkeley, CA. (guillaume.allorent@cea.fr)

Sunlight is essential for life on earth. Photosynthetic organisms transform light into chemical energy, through a series of redox reactions that produce NADPH and ATP. These compounds are consumed by numerous metabolic pathways including the Calvin-Benson cycle, which assimilates CO_2 and produces the biomass used by other organisms in the food chain. Besides this function, light carries signaling information that influences the physiology of both animals and plants. Because light quantity and quality vary depending on environmental conditions, photosynthetic organisms must cope with these changes.

Under natural conditions, these organisms are exposed both to the Photosynthetic Active Radiation (PAR, which ranges from 400 to 700 nm) and UV (< 400 nm)[1], which constitutes an intrinsic component of the sunlight. Originally seen as an actor of damage and stress, the UV-B part of UV radiations is now considered as an important regulator of biological processes [2]. UV-B affects crop productivity but also aquatic ecosystems and the question of how levels of UV-B affect life-processes is becoming more relevant, especially in photosynthesis.

Here we report that in the green microalgae *Chlamydomonas reinhardtii*, UV-B shapes the photosynthetic apparatus by optimizing its light absorption capacity and utilization. These modifications are under the control of UVR8, a UV-B photoreceptor [3, 4], which mainly induces UV-B specific proteins involved in the protection of the photosynthetic apparatus against high light stress [5].

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Fatty Acid Photodecarboxylase - a New Photoenzyme Converting Fatty Acids to Alkanes using Visible Light

Pavel Müller[1], Klaus Brettel[1], Damien Sorigué[2], Poutoum-Palakiyem Samire[2], and Frédéric Beisson[2]

[1]*Institute for Integrative Biology of the Cell (I2BC), CEA Saclay, CNRS, University Paris-Sud, University Paris-Saclay, 91198 Gif-sur-Yvette, FRANCE*

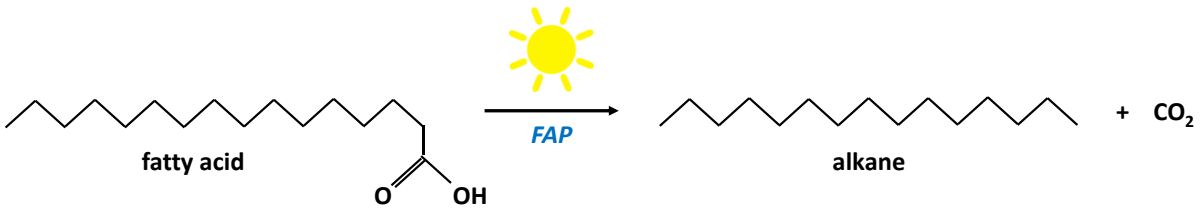
[2]*Biosciences and Biotechnologies Institute of Aix-Marseille (BIAM), CEA Cadarache, CNRS, Aix-Marseille University, 13108 Saint-Paul-lez-Durance, FRANCE*

E-mail: pavel.muller@i2bc.paris-saclay.fr

Green algae *Chlamydomonas reinhardtii* and *Chlorella variabilis* have recently been found to produce long-chain n-alkanes and n-alkenes in a light-dependent manner.[1] It turns out that the hydrocarbons are yielded by decarboxylation of the corresponding fatty acids and this reaction is catalyzed by a novel type of photoenzyme belonging to the protein family of glucose-methanol-choline oxidoreductases. We have named the photoenzyme 'Fatty Acid Photodecarboxylase' – 'FAP'.

We have studied the mechanism of FAP function by time-resolved fluorescence and transient absorption spectroscopy.[2] FAP contains a non-covalently bound and fully oxidized flavin adenine dinucleotide cofactor (FAD), which absorbs UV & visible light up to ~530 nm. Situated < 4 Å from the carboxylate of the substrate (i.e., the fatty acid: $R\text{-}COO^-$), the photoexcited FAD abstracts an electron from $R\text{-}COO^-$ within ~300 ps, generating a pair of radicals: $FAD^{\bullet -}$ and $R\text{-}COO^{\bullet}$. $R\text{-}COO^{\bullet}$ spontaneously decarboxylates, giving rise to an alkyl radical $R^{\bullet}$ and CO_2. The electron is then transferred back from $FAD^{\bullet -}$ to $R^{\bullet}$ within ~100 ns. This process is likely coupled to a transfer of a proton from a donor (XH), which is yet to be identified. The resulting alkane/alkene and CO_2 are replaced by a new substrate within a few tens of milliseconds.

The discovery of FAP opens a new avenue for a "green" production of fuel-like hydrocarbons from non-fossil sources[3] and sets the stage for design and development of new flavin-based catalysts.



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Session 3

Photobiologie humaine en environnement naturel

Conférences Invitées

Rayonnement solaire UV en France métropolitaine et en outremer

Applications : érythème et synthèse de la vitamine D

C. Brogniez[1], F. Auriol[1], C. Deroo[1], M. Catalfamo[1], F. Minvielle[1], J.-M. Metzger[2], P. Da Conceicao[3], Y. Pignon[3]

[1]Laboratoire d'Optique Atmosphérique, Université de Lille, Sciences et Technologies, Villeneuve d'Ascq, France
[2]UMS 3365 - OSU Réunion, Université de La Réunion, St-Denis de La Réunion, France
[3]UMS 3470 - OSU Pytheas, Observatoire de Haute Provence, St-Michel l'Observatoire, France

Depuis plusieurs années, des mesures spectrales quotidiennes de rayonnement solaire UV sont effectuées sur 3 sites français : dans les Hauts-de-France, dans les Alpes et sur l'Île de La Réunion (1). Les spectres mesurés permettent de calculer les éclairements effectifs (i) pour l'érythème et (ii) pour la synthèse de la vitamine D. L'indice UV, qui est un indicateur simple du risque sanitaire de l'exposition au rayonnement UV, est déduit de l'éclairement érythémateux. La durée d'exposition conduisant à un érythème dépend du phototype et a été déterminée à partir de l'indice UV en utilisant la classification des types de peaux selon leur facilité à rougir (2). De même, la durée d'exposition nécessaire à une production suffisante de vitamine D dépend du phototype mais également de la surface de peau exposée. Elle a été déterminée grâce à une relation proposée dans la littérature (3). La comparaison des 2 durées indique si une quantité suffisante de vitamine D peut être produite avant l'apparition d'un érythème. Les séries temporelles des éclairements effectifs et des durées d'exposition ont permis d'établir des climatologies sur les 3 sites. Toutes ces mesures ne fournissent qu'une valeur approchée de l'éclairement susceptible d'être reçu à une date ultérieure par un individu. En conséquence, afin de permettre au public de prendre des mesures de protection solaire adaptées, des prévisions d'indice UV sont mises en place. Les prévisions d'indice UV distribuées par Météo-France dépendent de plusieurs facteurs dont la nébulosité, prédite elle-même par un modèle de prévision. La couverture nuageuse pouvant varier rapidement au cours d'une journée, son influence sur les éclairements effectifs et donc sur les durées d'exposition a été étudiée au moyen d'un code de transfert radiatif (1).

Les résultats précédents ne peuvent être utilisés directement, en effet les éclairements effectifs mesurés correspondent à une surface plane et horizontale, qui n'est pas représentative du corps humain (4). Par ailleurs l'âge des individus influe leurs capacités à développer un érythème et à synthétiser la vitamine D (5). Les durées doivent ainsi être reconsidérées.

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Vivre avec le Soleil, l'éducation solaire à l'école

Pierre Cesarini

Association Sécurité Solaire, centre collaborateur de l'OMS

Les expositions solaires excessives, en particulier celles de l'enfance, sont la cause d'une augmentation forte du nombre de cas de cancers de la peau et de cataractes. Dans ce contexte, *La Sécurité Solaire* a lancé en 2006 le programme *Vivre avec le soleil* qui repose sur 4 piliers : un guide de l'enseignant diffusé gratuitement, un site web, des formations et des évaluations récurrentes.

Conçu par des formateurs issus de l'Education nationale et de l'équipe de *La main à la pâte* (Académie des sciences) ainsi que par des experts de l'association, le guide propose des activités, articulées avec les programmes et les objectifs que poursuivent les enseignants. La pédagogie qui y est proposée, la démarche d'investigation, aide les élèves à construire par eux même et à mémoriser les messages de prévention (1). L'acquisition de compétences psycho-sociales est observée, en particulier dans les milieux socialement défavorisés (2). La nature du guide comme les modalités opérationnelles du programme permettent une mobilisation massive des enseignants rendus autonomes par un outil simple à utiliser. La formation et l'accompagnement facilitent la mise en œuvre des activités mais aussi la collaboration entre les enseignants et les acteurs locaux (infirmières, animateurs…) ainsi que la sensibilisation des familles. Différentes évaluations sont régulièrement conduites depuis son lancement en 2006. Outre qu'elles ont montré un impact positif sur les connaissances, attitudes, et comportements des élèves (1), elles permettent de mesurer chaque année la participation des enseignants et des élèves. Avec un coût à l'élève bénéficiaire limité à quelques dizaines de centimes, l'efficience est optimale. Le succès rencontré en France (plus de 50.000 enseignants et 1 M. d'élèves bénéficiaires), grâce à des financements publics, s'étend actuellement à l'international (Québec, Allemagne, Portugal, Espagne, Brésil…).

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EFFETS CUTANES CONSECUTIFS A L'ASSOCIATION ENTRE SOLEIL ET POLLUANTS ATMOSPHERIQUES.

Laurent MARROT PhD
Senior Expert L'OREAL, Domaine Exposome, Recherche Avancée
1 av E Schueller
93600 Aulnay sous Bois

Du fait de l'urbanisation croissante, des nombreuses personnes sont soumises à une exposition élevée à certains polluants tels que l'ozone ou les particules fines. L'effet de la pollution sur la santé est bien connu, mais ce n'est que récemment que son impact dermatologique a été décrit dans la littérature. Il apparait que vivre dans un environnement pollué fragilise la peau et accélère le vieillissement cutané (déstabilisation de la fonction barrière, rides, taches pigmentées). De plus, il est hautement probable, qu'en plus de l'effet délétère de surface, certains polluants tels que les HAP (hydrocarbures aromatiques polycycliques) soient véhiculés vers la peau profonde par la voie sanguine, à des faibles teneurs inférieures au micro-molaire. Toxiques à l'obscurité, certains HAP tel que le benzopyrène, sont aussi phototoxique sous irradiation UV et l'on peut penser que cette photo-réactivité va contribuer au stress cutané final en milieu urbain. Certaines données vivo chez les fumeurs soumis à ces HAP suggèrent une aggravation des effets UVA, alors que d'autres interrogent la possible atténuation des effets UVB par filtration ou diffusion par les particules. Dans cette présentation, en plus d'une synthèse bibliographique sur le sujet, des données expérimentales issues de modèles vitro (cellules cutanées et peaux reconstruites) illustreront le concept de stress photopolluant associé à la toxicité de certains HAP sous irradiation UVA.

Session 3

Photobiologie humaine en environnement naturel

Communications Orales

Modulation by sunlight of the genotoxic effects of polycyclic aromatic hydrocarbons in human skin

Thierry Douki[1], Anne von Koschembahr[1], Antonia Youssef[1], David Béal[1], Marie-Thérèse Leccia[2], Jean-Phillipe Giot[2] & Anne Maitre[2]

1) Univ. Grenoble Alpes, CEA, CNRS, SyMMES (UMR 5819)
2) Grenoble University Hospital Center

Co-exposure of skin to carcinogenic polycyclic aromatic hydrocarbons (PAH) and solar UV radiation is of particular interest in occupational safety. Published in vitro studies on cultured cells have suggested that UVB enhances the genotoxicity of benzo[a]pyrene (B[a]P) by activating the AhR pathway and overexpressing the cytochrome P450 enzymes responsible for the conversion of B[a]P into DNA damaging metabolites. Our present work involves more realistic conditions, namely ex-vivo treatment of human skin explants and simulated sunlight (SSL) as a UV source. We first found that SSL, applied either before or after topical application of B[a]P, led to a lower expression of cytochrome P450 genes than with B[a]P only. Accordingly, the level of DNA adducts to the diol-epoxide metabolite of B[a]P (BPDE) was lower when skin was exposed to both B[a]P and SSL than to B[a]P alone. We extended our work to a more realistic PAH exposure by using organic extracts of coal tar pitch. We used both a raw organic extract and a synthetic mixture mimicking the PAH fraction. The same observations than with B[a]P were made. These results indicate that UV significantly impairs B[a]P and PAH metabolism, and decreases rather than increases immediate toxicity. The time-course observations made with B[a]P yet suggest that this phenomenon might be a delay rather than a complete reduction. It thus remains to clearly establish whether UV-induced decrease in metabolism efficiency may not change an acute exposure into a more chronic one as the result of an increased residence time of parent PAH in skin.

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Session 4

Photomédecine

Conférences Invitées

Thérapie photo-dynamique solaire

Christophe Bedane

Service de Dermatologie, CHU Limoges

Phytophotodermatoses

Martine Avenel-Audran

Service de Dermatologie et Vénéréologie, CHU Angers

On entend par phytophotodermatoses les dermatoses qui sont dues à l'action combinée du contact avec une plante et de l'exposition au soleil.

Les plantes en cause contiennent des agents chimiques photosensibilisants, les furocoumarines, psoralènes et isopsoralènes dont le nom dérive de Psoralea, une plante de la famille des Fabacées utilisée autrefois en Inde pour traiter le vitiligo (une dermatose dépigmentante). Les psoralènes se retrouvent la plupart du temps dans toutes les parties des plantes mais leur taux peut varier en fonction des saisons.

Le spectre d'action du rayonnement solaire se situe dans les UVA (320-400 nm) avec un pic maximal d'absorption des psoralènes à 335 nm.

Ce contact combiné plantes plus soleil provoque une réaction phototoxique souvent favorisée par un milieu humide qui augmente la diffusion sur la peau des psoralènes. La lésion clinique qui en résulte est un coup de soleil localisé à la zone de contact avec la plante, avec ses signes habituels, érythème, bulles puis pigmentation ou parfois pigmentation seule.

La dermite des prés d'Oppenheim en est le tableau clinique le plus classique, reconnaissable par l'aspect linéaire, strié, des lésions reproduisant parfois le dessin d'une feuille. Les circonstances de survenue sont beaucoup plus souvent la tonte de la pelouse qui projette des débris d'herbe sur le torse et les jambes nus du jardinier que le classique bain de rivière de la description princeps de 1932. Ces lésions, légèrement douloureuses, n'apparaissent que 24 heures après le contact et évoluent vers une pigmentation qui peut durer plusieurs semaines. Les plantes en cause ici sont surtout des Apiacées (anciennes Ombellifères) comme la redoutable grande Berce, le céleri, le panais, la carotte, le cerfeuil sauvages et bien d'autres… et aussi les Moracées comme le figuier.

La « lime disease », dermatose érythémateuse et bulleuse aux aspects cliniques trompeurs, en particulier chez l'enfant, est due au contact avec le jus de citron vert. On la voit aussi chez les barmen.

Chez les Rutacées de l'espèce Citrus, les psoralènes sont situés dans l'écorce des fruits, comme la fameuse essence de bergamote très utilisée dans les parfums, en particulier l'eau de Cologne, et responsable de la dermite en breloque avec ses « coulées » pigmentées. C'est la raison pour laquelle il est déconseillé d'appliquer des parfums avant de s'exposer au soleil.

La rue de jardin, autre Rutacée est responsable de brûlures sévères. A cette famille appartient aussi la fraxinelle dont les fleurs fanées laissent exsuder une huile spontanément inflammable et qui pourrait être le buisson ardent de Moïse décrit dans la bible.

Session 4

Photomédecine

Communications Orales

Photo-protection des kératinocytes contre les effets des UVA par un extrait de fruit de Sechium edule (chayotte)

E. Metral[1,2,3], W. Rachidi[2], O. Damour[3], F. Demarne[1], N. Bechetoille[1]

[1]Gattefossé, R&D, Saint-Priest, France ; [2]CEA/INAC/SyMMES/CIBEST, Université de Grenoble Alpes, Grenoble, France ; [3]BTC/LSC HCL de Lyon, Lyon, France

La photoprotection est cruciale pour prévenir les effets délétères à long terme des UVs sur la peau, tels que les cancers et le photo-vieillissement. Les plantes sont de plus en plus utilisées, aujourd'hui, pour développer des agents photo-protecteurs naturels encore plus efficaces. Cependant, les mécanismes moléculaires responsables de ces activités de photoprotection sont souvent mal compris. L'objectif de cette étude a été d'étudier les effets photo-protecteurs d'un extrait de fruit de chayotte (*Sechium edule extract,* SEE) contre les méfaits des UVA sur les kératinocytes humains normaux. Nous avons montré que SEE protégeait les kératinocytes contre la cytotoxicité induite par les UVA, diminuait l'accumulation intracellulaire d'espèces réactives de l'oxygène et réduisait les lésions de l'ADN induites par l'oxydation après exposition aux UVA. SEE diminuait également la formation de dimères de pyrimidine (CPD) dans les kératinocytes irradiés par les UVA et augmentait la réparation de ces photo-produits 24h après l'exposition. En utilisant les biopuces de réparation de l'ADN (LX Repair), nous avons démontré que les kératinocytes traités par SEE avaient des activités de réparation enzymatiques de l'ADN plus efficaces pour les sites abasiques, les CPD et les thymine glycols. Par conséquent, les activités photo-protectrices de SEE contre les UVA sont portées par une combinaison d'activités antioxydante, de réduction des dommages à l'ADN et d'amélioration des capacités de réparation de l'ADN.

Proteomic analysis to identify candidate biomarkers associated with skin co-exposure to ultraviolet radiations and Benzo[a]pyrene

Fayyad-Kazan M[1], Bourgoin-Voillard S[1], Fitoussi R[2], Beauchef G[2], Seve M[1] & Rachidi W[3]

[1] *Université de Grenoble Alpes, LBFA et BEeSy, INSERM U1055, PROMETHEE Proteomic Platform, 2280 rue de la Piscine, BP 53, 38041, Grenoble, France*

[2] *Laboratoires Clarins, 5 rue Ampère, 95300, Pontoise, France*

[3] *Université Grenoble Alpes, SyMMES/CIBEST UMR 5819 UGA-CNRS-CEA, IRIG/CEA-Grenoble, 38000 Grenoble, France.*

Human skin, and mainly the outer epidermis, is continuously exposed to environmental stressors, mainly air pollutants (such as ultraviolet radiations (UVR) and polycyclic aromatic hydrocarbons (PAHs); such as benzo[a]pyrene (BaP)). Exposure to UVA and UVB has been associated with skin aging and sunburn, respectively. Moreover, combined exposure to UVA and PAHs could significantly worsen the skin damage. On the other hand, exposure to BaP has been strongly associated with cancer development in different organs. Intriguingly, skin tumor incidence has been shown to be significantly increased in response to BaP + UVA treatment, compared to BaP- or UVA-treatment alone. Till date, a detailed analysis of the biological processes that are altered following co-exposure of human skin to BaP + UVR has not been performed. In this study, proteins were extracted from the cytosol, membrane or nuclear fraction of human epidermal skin cells being exposed to either UVR alone, or UVR + BaP and compared to non-exposed cells. Mass-spectrometry (MS)-based proteomic analysis was carried out to identify differentially expressed protein in UVR + BaP conditions. MS-followed by bioinformatics analysis led to the identification of 147 skin biology-related proteins following skin exposure to UV + BaP. These deregulated proteins appeared to be involved in different biological processes including epidermis development, keratinocyte differentiation, autophagy, cell proliferation and collagen biosynthetic processes. Further validation of these deregulated proteins could enable identification of certain markers of skins exposed to UVR + BaP. Moreover, dissection of the signalling pathways in which these deregulated proteins are involved could account for better understanding of the molecular events taking place following skin exposure to UV + BaP.

Screening of photoactive nanoparticles in stroma-rich multicellular tumor spheroids

Yakavets I[1,2,3], Francois A[1,2], Maklygina Y[1,2,4], Loschenov V[4,5], Lassalle HP[1,2], Zorin V[1], Bezdetnaya L[1,2]

1. *Institut de Cancérologie de Lorraine, Vandoeuvre-lès-Nancy, France*
2. *Centre de Recherche en Automatique de Nancy, CNRS, Université de Lorraine, Nancy, France*
3. *Department of Biophysics, Belarussian State University, Minsk, Belarus*
4. *Prokhorov General Physics Institute of the Russian Academy of Sciences, Moscow, Russia*
5. *National Research Nuclear University MEPhI, Moscow, Russia*

Conventional 3D multicellular tumor spheroids of head and neck squamous cell carcinoma (HNSCC) consisting exclusively of cancer cells have some limitations. In order to better mimic *in vivo* HNSCC tumor microenvironment, we have constructed 3D stroma-rich *in vitro* model of HNSCC, using cancer-associated MeWo skin fibroblasts and FaDu pharynx squamous carcinoma cells [1]. The developed spheroids were optimized, characterized by fluorescence microscopy and immunohistochemical analysis of spheroid cryo-section and appeared to reproduce sufficiently a stroma-rich HNSCC tumors. The expression of stromal components in heterospheroids was confirmed by immunochemical staining. The generated co-culture FaDu/MeWo spheroids were applied to study the behavior of Temoporfin, clinically approved second-generation photosensitizer, and its liposome-based nanoformulations [2]. Overall, the developed stroma-rich spheroids enlarge the arsenal of *in vitro* pre-clinical models for high-throughput screening of anti-cancer nanomedicines.

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