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Journées scientifiques de la Société Française de Photobiologie

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Paris - Campus Pierre & Marie Curie

ABSTRACTS BOOK

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27-27 nov. 2024

Soleil et peau: doit-on se protéger au-delà des UV ?

Dr. Christelle COMTE

Dermatologue Paris, Présidente de la Société Française de Photodermatologie.

Impact toxicologique à long terme de pigments de tatouage

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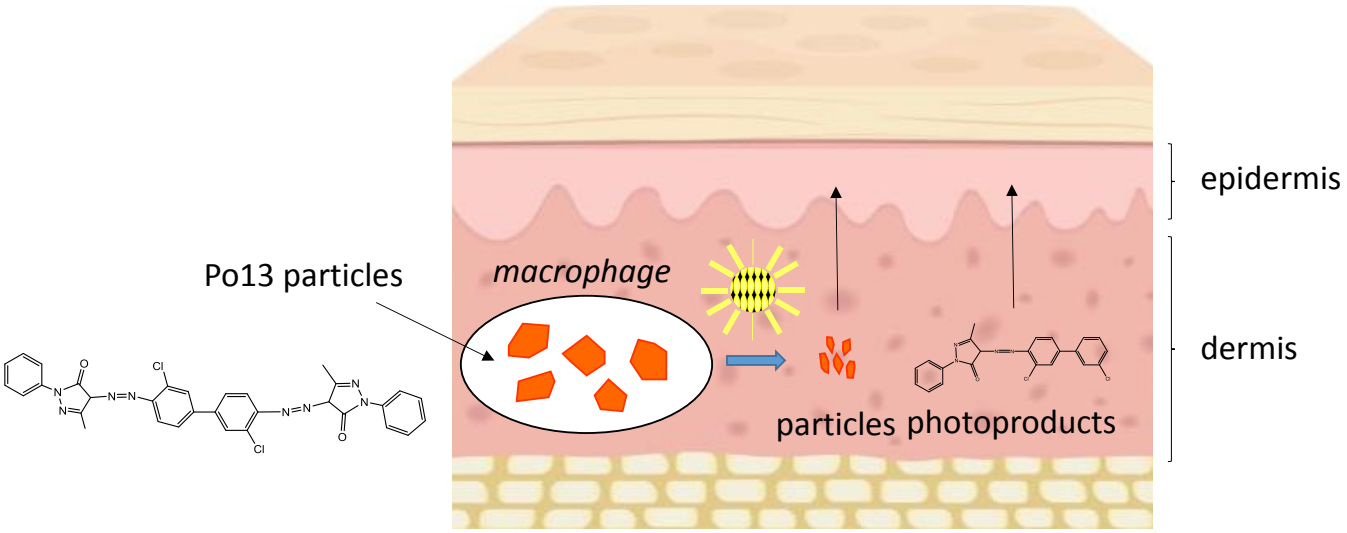
Mots-clés : tatouage ; pigments ; vieillissement ; photodégradation

Résumé :

Les pigments colorés utilisés dans le tatouage sont aujourd'hui essentiellement de nature organique. Maintenus dans le derme par les macrophages, ils sont susceptibles de subir différents processus de dégradation, dont l'impact de l'exposition à la lumière solaire. Nous explorons l'hypothèse que ces phénomènes de dégradation pourraient générer des produits solubles ou des petites particules capables de migrer vers l'épiderme où ils participeraient aux effets toxiques des tatouages. En effet, certains travaux proposent un lien entre le tatouage et des cancers cutanés. De plus, des allergies et des phénomènes de photosensibilisation ont été observés. Dans ce travail, nous nous sommes intéressés à une série de pigments, parmi lesquels le pigment diazo organique orange (Po13) s'est révélé particulièrement toxique. Des suspensions aqueuses ont été vieillies par exposition à la lumière solaire simulée à 40° C. La morphologie et la charge superficielle des particules de Po13 ont été peu modifiées par le vieillissement, mais leur taille a été réduite. Des photoproduits solubles ont été détectés dans les fractions liquides. Le photoproduit majoritaire (DCBP) a été produit en grande quantité en suspension dans l'isopropanol et purifié. Les profils toxicologiques des suspensions vieillies, de leurs fractions solubles et du DCBP ont ensuite été déterminés sur la lignée cellulaire de kératinocytes HaCaT. L'impact des suspensions de Po13 sur la viabilité n'a guère été affecté par le vieillissement. En revanche, les fractions solubles étaient plus toxiques après photovieillissement. Les suspensions et les filtrats n'ont induit ni la libération d'espèces réactives de l'oxygène ni la formation de cassures de brins d'ADN. Les échantillons n'ont montré que des effets limités sur le protéome des cellules HaCaT. À l'inverse, le DCBP était cytotoxique et induisait la production de ROS, mais n'était pas génotoxique. Le DCBP s'est avéré activer les monooxygénases CYP450 connues pour être impliquées dans le métabolisme des xénobiotiques. Au total, nos résultats montrent que le vieillissement de Po13 entraîne la libération de composés toxiques solubles. Des études en cours montrent des phénomènes de photodégradation similaires pour deux autres pigments rouges très utilisés dans les tatouages, Pr 254 et surtout Pr122.



Photovieillissement du pigment orange 13 (Po13) dans les tatouages conduisant à la production de particules et de photoproduits pouvant diffuser vers l'épiderme.

Low-energy DNA photoionization studied by time-resolved spectroscopy

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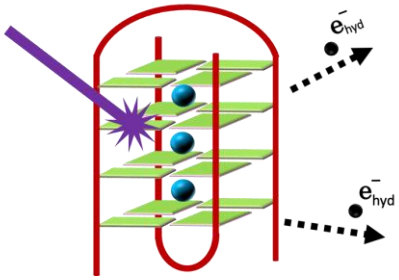
Mots-clés : Photoionization; DNA damage; G-quadruplexes; Guanine radicals; time-resolved spectroscopy;

Résumé :

Photoionization of DNA in aqueous environments has been studied since the 1990s, initially using 193 nm excitation. Over the past two decades, it has been found that this process can also occurs at lower excitation energies—energies at least 3 eV lower than the vertical ionization potential of DNA components. This unexpected behavior was investigated using nanosecond transient absorption spectroscopy with 266 nm excitation, allowing for the precise quantification of ejected electrons and base radicals.
At higher excitation energies, the quantum yields (ϕ) of one-photon ionization are largely unaffected by base-stacking or base-pairing. However, at lower energies, the quantum yields are strongly influenced by the DNA's secondary structure. Photoionization is not detected in monomers or unstacked oligomers, but in duplex structures, including genomic DNA, the yields range from 1 to 2×10^{-3}.
G-quadruplex structures, in particular, show a much greater tendency for low-energy photoionization, with ϕ values reaching up to 2×10^{-2}. By studying various structural parameters of the latter—such as metal cations in their central cavity, number of stacked guanine tetrads, and directionality—we propose an indirect mechanism. It involves excited state relaxation, where a small fraction of the excited population shifts to charge-transfer states involving neighboring bases. After charge separation, electron ejection occurs from the negatively charged region, leaving an electron hole in the DNA.
These findings are important for understanding UV-induced DNA damage, as low-energy photoionization contributes to oxidative lesions caused by UVB and UVA exposure. Furthermore, the photo-generation of charge carriers in DNA presents potential applications in developing biosensors that utilize photoconductivity.



Schematic representation of low-energy photoionization in a G-quadruplex followed by electron ejection.

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Non-Adiabatic Dynamics study on microhydrated DNA probe

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Mots-clés : Photochemistry, DNA probe,Non-adiabatic dynamics

Résumé :

The study of the native conformation of nucleic acids and its eventual perturbation induced by the coupling with external perturbation factors is fundamental to assess better the molecular mechanisms underlying their biological role.[1] Luminescent DNA probes [2] are tools of choice to evidence the presence of important structural modification of the nucleic acid structure finely and rapidly. I will present a non-adiabatic dynamics study, performed in the surface hopping formalism, on 2-thienyl-3-hydroxychromone, an environment-dependent luminescent organic DNA probe.[3] I will show that the first shell solvent water molecules undergo a rather complex reorganization upon light excitation.[4] This also involves the triggering of a water-mediated proton transfer process which leads to the formation of the tautomeric structure (Figure 1). The presence of this solvent-mediated transfer mechanism globally diminishes the intersystem crossing efficiency, and hence the population of the triplet state manifold, as compared to the non-solvated systems. The results also point out the non-innocent role of solvent networks in tuning complex photophysical processes, while opening competitive relaxation channels.

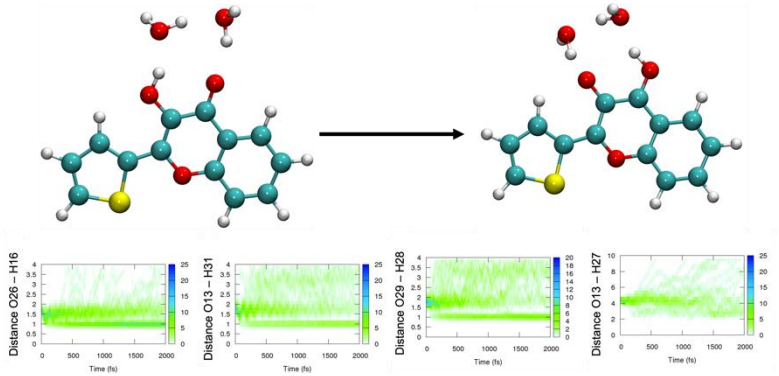

Figure 1. Tautomerization process

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Nouvelles drogues anti-cancéreuses ciblant les G-quadruplexes photoactivables dans un domaine spectral biocompatible par une stratégie inspirée de la photothérapie par rayons X

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Mots-clés : photothérapie dynamique activée par rayons X (PDTX), photoréaction, photosensibilisateur

Résumé :
Récemment, l'équipe du Dr L. Guy a découvert une réaction photochimique intramoléculaire prometteuse pour le développement d'un traitement anti-cancer à la croisée entre le photocageage de ligands des G-quadruplexes de l'ADN (G4) et la photothérapie dynamique (PDT). Cette photoréaction permet la conversion d'une bis-quinoléine **1** (qui n'interagit pas avec l'ADN, ne génère pas d'oxygène singulet $^1O_2$, non fluorescente) en une molécule diazonia bis-cationique **2** qui est quant à elle hautement toxique (ligand spécifique des G4, générant du $^1O_2$, fluorescente) (figure 1.A).[1,2] Cependant, ce système possède encore des limitations concernant en particulier la longueur d'onde d'activation de la réaction photochimique qui se situe dans l'UV.
Deux stratégies ont alors été explorées pour activer la réaction photochimique dans une gamme de longueur d'onde biocompatible : la première consiste à introduire des groupements auxochromes sur le chromophore de **1** conduisant à un décalage hypsochrome de ses propriétés d'absorption. Une irradiation biphotonique dans l'infra-rouge pourrait alors permettre le déclenchement de la réaction photochimique, mais à la fois les difficultés synthétiques et les modifications des propriétés biologiques du diazonia photo-généré sont des limitations majeures.
Nous avons commencé à investiguer une deuxième stratégie, plus prometteuse, qui consiste à utiliser des rayons X pour générer la photoréaction en s'inspirant des principes de la PDTX. De nouveaux dérivés de prodrogues **1** sont greffés à la surface de nanoparticules scintillatrices. Sous irradiation X, ces nanoscintillateurs sont donc capables d'émettre une lumière dans l'UV-visible pouvant alors, par transfert énergétique, conduire à la photoréaction des dérivés de **1** greffés sur les nanoscintillateurs par des fonctions d'ancrage telles que des phosphonates (en rouge) (figure 1.B).

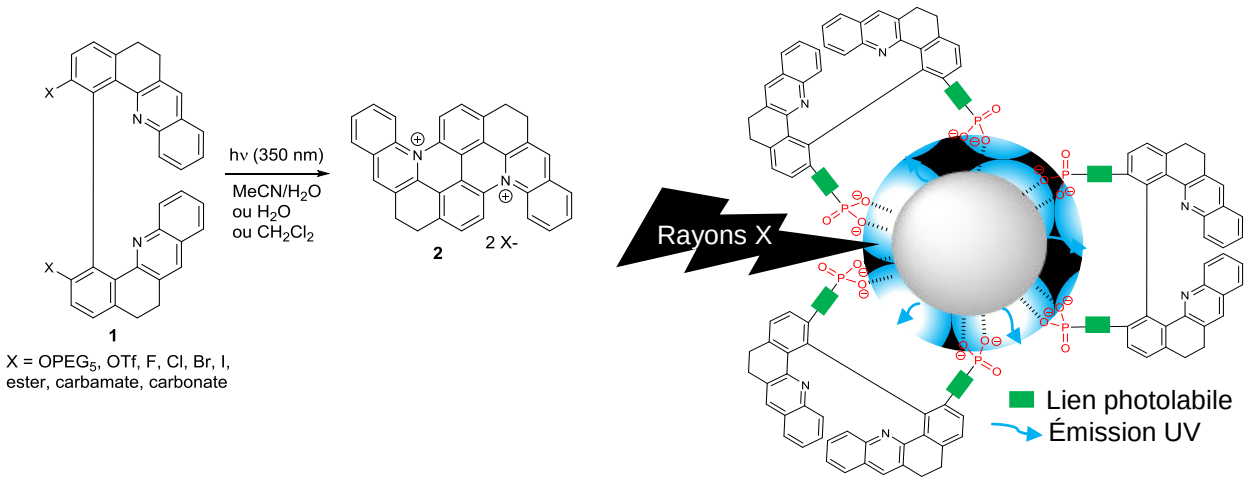

*Figure 1. A. Photoréaction de la bis-quinoléine **1** en diazonia **2**. B. Principe du nanoscintillateur de lanthanides décorés de **1**.*

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28-28 nov. 2024

Optogenetic manipulation of plant physiology via the LOV domain

Phototropins (phots) are plasma membrane-associated blue-light receptor kinases that control a variety of responses in plants that serve to optimise photosynthetic productivity. These include phototropism, leaf positioning, chloroplast accumulation movement and stomatal opening. The activity and regulatory action of their C-terminal kinase domain is controlled by a flavin-binding module known as the Light, Oxygen or Voltage sensing 2 (LOV2) domain located within the N-terminal region of the protein. This light-sensory module is proposed to cage and repress phot kinase activity in darkness, with this repression being alleviated following photo-activation through light-induced structural changes associated with LOV2. The LOV2 photo-switch has therefore been used to artificially regulate protein function through this caging/un-caging mechanism. One example is Blue Light-Induced K+ Channel 1 (BLINK1), a synthetic ion channel created by fusing the algal virus K^+ channel Kcv to the LOV2 photo-switch from oat phot1. We have used this optogenetic tool to modulate blue light-induced stomatal opening in *Arabidopsis thaliana*. Plants expressing BLINK1, specifically within the guard cells, show improved stomatal responses, carbon assimilation and water use efficiency in fluctuating light environments. We have also shown that the sensitivity of phot blue-light receptors in Arabidopsis can be modulated through targeted engineering of the LOV2 photo-switch and this approach can be used to increase photoreceptor function under low light conditions to improve photosynthetic productivity and biomass production.

Photoperiodism in cosmopolitan Phytoplankton

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Photoperiodism is the adaptation of living organisms to variations in day length and temperature, which vary over the course of the year and also according to latitude. Flowering is one of the most striking examples of photoperiodism in plants. Studies carried out on both model organisms such as *Arabidopsis thaliana* and cultivated plants adapted to different latitudes have identified the molecular components of photoperiodism belonging to the main timekeeping mechanisms (circadian clock), photoreceptors and downstream effectors (CONSTANS family).

Marine phytoplankton contributes as much as terrestrial plants to primary production, occupying a range of latitudinal niches between polar and temperate regions, with temperature and light being the main drivers of the spatial and seasonal patterns of phytoplankton abundances. However, photoperiodism has been poorly documented in marine microalgae, and the underlying molecular mechanisms are virtually unknown. To answer these questions, we are studying the Mamiellophyceae, a class of green phytoplankton, by combining laboratory studies on the well-established model organism Ostreococcus tauri and by exploiting the natural diversity of cosmopolitan species such as Bathycoccus prasinos. Latitudinal variations were observed in photoreceptors and CCT domain containing proteins of the circadian clock. Remarkably tropical strains lacks the canonical circadian oscillator described in *O. tauri*. In addition, pan-genomic analysis and physiological analysis of natural variants and metagenomics have highlighted the key role of temperature in the latitudinal and seasonal distribution of Bathycoccus in polar and temperate regions.

Higher-order nuclear reprogramming during plant adaptation to light

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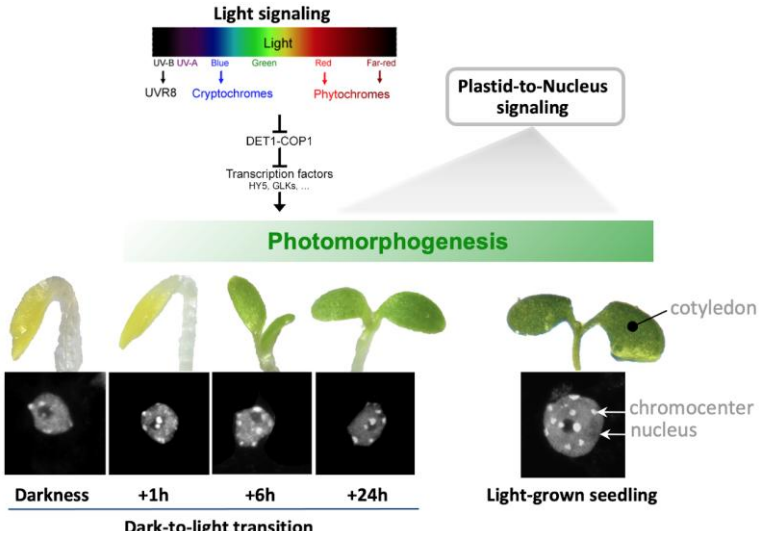
Mots-clés : Plant, Photomorphogenesis, Transcription, Nucleus organisation, Epigenome

Résumé : The reshuffling of nuclear architecture and the chromatin landscape is a recurring theme orchestrated in most developmental transitions of eukaryotic organisms. Plants display extraordinary capacities at deploying such mechanisms, a property that plausibly contributes to their high plasticity and fitness under changing environments. In addition to being used as a source of energy for photosynthesis, light sensing by specific receptors serves as critical information on the plant environment to adjust plant physiology and development continuously. This effect is particularly spectacular during seedling de-etiolation, a photomorphogenic transition induced in germinating seedlings by the initial light perception. Triggering chloroplast biogenesis and photosynthesis establishment, photomorphogenesis combines rapid disruptions in growth and cell specification linked to a massive reprogramming of genome expression. We identified that photomorphogenesis also involves dramatic changes of nucleus architecture and chromatin states at thousands of genes, which are controlled by DET1 and other light signaling components. In most cotyledon cells, this switch further involves a global increase of RNA Polymerase II activity, suggesting that light sensing triggers the release from a relatively quiescent to a more active transcriptional status. Accordingly, a spike-in-based RNA-seq approach unveiled that transcriptome size doubles during cotyledon photomorphogenesis due to a quasi-exclusive trend in gene upregulation. Our latest studies on light-regulated chromatin mechanisms and their links to the spatial organization of genes and transcription will be presented.



Work from the team showed that light perception and chloroplast activity drive multilevel nuclear and epigenome dynamics linked to a general increase of the transcriptional regime during Arabidopsis cotyledon photomorphogenesis. Here, the light-dependent formation of heterochromatic foci is shown.

Deciphering the Role of RITMO1 in *Phaeodactylum tricornutum*: Insights into the Diatom Circadian Clock

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Mots-clés : Diatoms, circadian clocks, photosynthesis, gene expression, transcriptomics

Résumé :

The synchronization of biological processes with daily light/dark cycles is essential for most organisms. These predictable environmental rhythms have driven the evolution of internal timekeepers known as circadian clocks. Those clocks have been demonstrated to play a major role in the regulation of physiological, developmental, and reproductive processes thereby increasing the fitness of the organisms. Circadian activity has been observed in various microalgae under laboratory and natural conditions [1]. However, the mechanisms underlying these rhythms, particularly their relationship to photosynthesis, remain unexplored in major phytoplanktonic organisms such as diatoms. Interestingly, despite endogenous rhythmicity, diatoms lack clear homologs of the canonical circadian components found in bacteria, algae, plants, or animals, suggesting a unique clock system [2].

To investigate circadian regulation in diatoms, we first defined physiological conditions for studying clock activity and highlighted the circadian regulation of various key processes in the model species *Phaeodactylum tricornutum*. To this aim, we generated different transgenic lines showing genetic alterations of the bHLH-PAS RITMO1 protein, previously proposed as a regulator of diel rhythms [3]. We showed that the obliteration of the single *RITMO1* gene and deregulation of its expression in ectopic over-expressing lines is sufficient to strongly affect circadian rhythms of cell fluorescence, gene expression and various photosynthetic parameters. Additionally, *RITMO1* knockout cells transformed with *RITMO1:Venus* construct under its native promoter rescued phenotypes only in lines with expression levels close to those of endogenous RITMO1, supporting its role in a tightly regulated feedback loop. By studying the expression of selected genes, we also showed that RITMO1 plays a key role in transcriptional control of circadian rhythms. To extend our understanding of diatom circadian mechanisms, we generated a comprehensive transcriptomic dataset under diel and free-running conditions (the absence of environmental cues, which isolates endogenous clock contributions). Our results confirmed that over 50% of genes retain rhythmic expression under free-running conditions, highlighting the critical importance of circadian regulation. Furthermore, we are assessing RITMO1's large scale role in gene expression regulation by examining genes deregulated in *RITMO1* knockout mutants.

By defining RITMO1 as a key endogenous clock component of diatoms and providing information about the circadian regulation of the entire P. tricornutum genome, these results represent a key step in the molecular characterisation of the phytoplankton circadian clock system, as well as its function and evolution and relation with photosynthesis. They also establish marine diatoms as novel powerful model system for chronobiology research.

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Biosynthesis of phytochrome chromophore in diatoms: characterization of heme oxygenase genes in *Phaeodactylum tricornutum*

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Mots-clés : Phytochrome, heme oxygenase, chromophore, biliverdin, diatoms

Résumé :

Phytochromes are photoreceptor proteins initially found in plants where they play critical roles in their growth and development[1]. They have been subsequently found in photosynthetic and non-photosynthetic bacteria, fungi and different alga, including diatoms[2], prominent marine microalgae deriving from secondary endosymbiosis events. Phytochromes perceive specifically red and far-red light colors thanks to a chromophore, a linear tetrapyrrole deriving from the cleavage of heme into biliverdin[2] through an enzyme, the heme oxygenase (HO). In plants, chromophore biosynthesis enzymes are located in the chloroplast, while in fungi HO is located at the outer mitochondrial membrane[3]. While diatom phytochromes are closely related to bacterial and fungal ones[4], nothing is known about the synthesis of their chromophore. Genome of the model diatom *Phaeodactylum tricornutum* (Pt) possesses four genes homologous to *HO*. By co-expressing recombinantly phytochrome with Pt*HO2*, a production of functional biliverdin could be highlighted while it was not the case with Pt*HO4 nor PtHO3*. Plastid localization was confirmed for two of them, Pt*HO2* and Pt*HO4*, by expression of Venus fusion HO proteins. Finally, to assess the functional involvement of these HO in the biosynthesis of phytochrome chromophore in *P. tricornutum*, we aim to generate knockout mutants of the different *HO* genes by CRISPR/Cas9. To do so, we have developed different plasmids compatible with the modular cloning technique to allow the expression of the Cas9, two distinct single guide RNA, and an antibiotic resistance gene on a single episomal vector. Phytochrome-dependent light sensing will be tested in the knocked-out cells by analysis of the expression of phytochrome regulated genes (and in the wild-type cells by RNA analysis). This work will bring knowledge about the functioning of phytochrome in diatoms and its complex evolutive history.

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L'optique adaptative en microscopie de fluorescence pour l'imagerie biologique in vivo à haute résolution

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Laboratoire ISMO, Orsay.

Near-infrared co-illumination of fluorescent proteins reduces photobleaching and phototoxicity

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Mots-clés : fluorescence imaging, fluorescent protein, photobleaching, phototoxicity

Résumé :

Probe photobleaching and phototoxicity are among the main limitations of fluorescence imaging. They impose severe constraints on the dose of excitation light, to the detriment of image contrast, spatio-temporal resolution and experiment duration. We will present a new method to reduce the photobleaching of fluorescent proteins and the associated phototoxicity. Our method exploits a photophysical process known as reverse intersystem crossing (RISC) to depopulate the triplet excited state of fluorophores, a precursor to photobleaching and a source of reactive oxygen species. We achieve RISC by co-illuminating the fluorophores at a near-infrared wavelength absorbed by their triplet excited state, during their excitation with visible light. We will show that this dual illumination method can be successfully applied to wide-field fluorescence imaging and that it works in live eukaryotic and prokaryotic cells with a wide range of fluorescent proteins, leading to a typical 4-fold reduction in photobleaching. Our method can, for example, substantially improve the monitoring of fluorescently-tagged replisomes in *E. coli*. A direct comparison with classical antifading media shows that dual illumination is more efficient. It also has the advantage that it does not require any specific preparation of the samples before imaging. We will further show that dual illumination reduces the phototoxicity caused by fluorescent protein excitation in bacteria and primary mouse neutrophils, and that near-infrared light does not significantly perturb the cells. This makes our method particularly well suited for in vivo dynamical studies.

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Influence of the chromophore configuration on photophysical properties of the gaf domain of bacterial phytochrome

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The chromophore-binding domain of phytochromes, GAF domain binds the chromophore biliverdin. Experimental absorption spectra of the chromophore inside the protein are available. Phytochromes, acting as photoreceptors, toggle between states that absorb red and far-red light, with absorption maxima varying by 50 to 70 nm depending on chromophore configuration.[1,2] We investigate the chromophore characteristics in a small cluster system within the surrounding residues by DFT level of theory. Moreover, Molecular Dynamics simulations for chromophore protein complex are carried out to elucidate the dynamic effect of protein on absorption spectra of the chromophore by QM/MM methodology.

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Terahertz ATR sheds light on real-time exchange kinetics occurring through plasma membrane during photodynamic therapy

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Mots-clés : Terahertz sensing, Photodynamique therapy, cell membrane

Résumé :
THz ATR spectroscopy provides, in a single measurement, the relative number of defects per membrane surface created by oxidative stress generated during photodynamic therapy (PDT), offering early, sensitive real-time information. THz spectroscopy is therefore a complementary technique to established (biological) assays and can be applied to any topic requiring the real-time examination of short-term plasma membrane permeabilization.

Methods to follow in real time complex processes occurring along living cell membranes such as cell permeabilization are rare. Here, we show that terahertz spectroscopy reveals early events in plasma membrane alteration generated during a photodynamic therapy protocol, events which are not observable in any other conventional biological techniques performed in parallel as comparison [1].

Standard biological laboratory experiments and physical measurements by THz spectroscopy were carried out in parallel to ensure the complementarity of the data collected, following the treatment of cancer cells by photodynamic therapy [1]. The results obtained indicate a real added value of THz spectroscopy compared to biological reference approaches. In particular THz measurements exhibit much better sensitivity. For instance, dose effects for concentrations below 0.33µM could not be detected using standard biological methods. Indeed, this new methodology provides both a quantitative estimate of the number of membrane surface defects created by oxidative stress during photodynamic therapy, but also more efficient measurements than biological approaches conducted in parallel. It also allows to obtain early information on the development of cellular responses, i.e. from the moment of light irradiation, as well as a real-time monitoring of the intracellular water content, on time scales of several hours. THz spectroscopy is thus a promising technique complementary to established classical biological studies and techniques, which can be applied to any subject requiring real-time examination of the modulation of cell water content.

This study validates the original and innovative application of THz spectroscopy, a physical technique that is applicable to biological and medical issues. Eventually, the technological development of terahertz radiation sources will allow to determine with precision the nature of the chemical elements involved in the modulations of intracellular water, following external stimuli such as PDT.

This work has proved that THz-ATR is a powerful promising and complementary technique to other biological approaches to monitor changes in cell permeability upon a photochemical stress, especially thanks to its high sensitivity and capacities to enable the early detection of membrane permeabilization processes in real-time on living cells. The THz-ATR has also proven to be a very powerful tool for studying the effectiveness of copolymer-based nanovectors as an agent for drug delivery, including photosensitizers.

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Modeling the effects of photo-activated drugs on biological membranes through molecular dynamics simulations

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Mots-clés: molecular dynamics, TIM-3, phosphatidylserine, photoisomerization, lipid membranes

Résumé :
This study presents a comprehensive analysis of the interactions between photo-induced molecular switches and model lipid bilayers, alongside the molecular dynamics of the IgV domain of TIM-3 protein with lipid membranes, through advanced molecular modeling and simulation techniques. We explore the effects of a cyclocurcumin derivative, a potential agent for light-activated chemotherapy, on lipid bilayers mimicking cell membranes[1]. Utilizing classical molecular dynamics and enhanced sampling simulations via the coupling of ABF and Metadynamics (meta-eABF) to determining free energy profiles for the penetration of the switch in the membranes, we investigate the chromophore's interaction and penetration into membranes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) and a complex mixture of DPPC, DOPC lipids and cholesterol. Our findings reveal concentration-dependent interactions with DPPC membranes and modulation of structural parameters through E/Z photoisomerization of cyclocurcumin derivative, offering insights into non-oxygen dependent therapies for hypoxic tumors[2]. In the more complex membrane, we showed that the cyclocurcumin derivative interacted differently, causing less profound damages and changes in the lipid bilayer[3]. However, for both membrane models we confirmed that the structural parameters of the bilayer are differently affected by two isomers, and hence can be modulated through photoswitching, offering interesting perspectives for future applications (Fig.1).

Furthermore, we characterize the interaction of the IgV domain of TIM-3 protein with a model lipid membrane, demonstrating stable yet dynamic complexes facilitated by phosphatidylserine-containing POPS lipids and Ca^{2+} ion. Enhanced sampling MD simulations highlight the thermodynamically favorable insertion of phosphatidylserine into the IgV binding pocket, suggesting mechanisms for modulating TIM-3 activity. This study not only elucidates the molecular basis of lipid-protein interactions but also provides a foundation for future immunotherapy strategies targeting the TIM-3 pathway in cancer treatment.

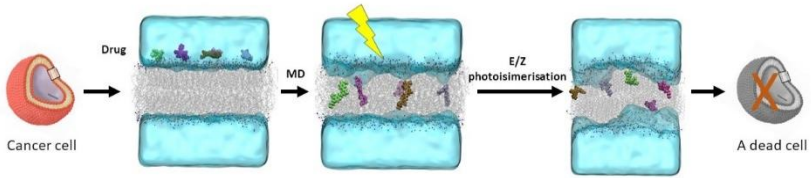

Figure 1. Schematic representation of the cell destruction process as a result of a cyclocurcumin derivative photoisomerization

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Efficient Delivering of a Photodynamic Therapy Drug into Cellular Membranes Rationalized by Molecular Dynamics

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Mots-clés : Photosensitizer ; Photodynamic Therapy; Drug Delivering; Membrane Internalization; Molecular Dynamics

Résumé :
Photodynamic therapy (PDT) is a light induced chemotherapy method, differentiate from the conventional chemotherapy due to the non-invasive nature, high tumour suppression ability as well as higher safety and success of the treatment. [1] In PDT, a photosensitizer (PS) agent is introduced to the living body, and it is irradiated with a light having longer wavelength to produce cytotoxic singlet oxygen and conceptually, an ideal PS is delivered to only the targeted cells, while healthy cells remain untouched and therefore the minimal side effect occurs while treatment proceeds. However, the success of PDT is limited because commercially available PDT drugs suffer from sufficient lipophilicity to penetrate the cell membrane and solubility in the physiological environment. To overcome this issue, drug delivery systems are used to improve the biocompatibility of the PS molecule and ensure controlled release into target cells. [2]

Here we have modeled the behavior of a tetrapyrrole derivatives as a drug, complexed by two β-cyclodextrin units in presence of a lipid bilayer. Our findings reveal insights into the internalization of the complex and its spontaneous dissociation inside the membrane, offering valuable information for targeted photodynamic therapy.

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29-29 nov. 2024

Wireless Nanolamps

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Investigating the $OCP^O \rightarrow OCP^R$ photoconversion mechanism in Orange Carotenoid Protein

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Mots-clés : photoprotection, orange carotenoid protein, transient absorption spectroscopy, two photon photoconversion

Résumé :

Orange carotenoid protein (OCP) is a photoactive protein that controls photoprotection in cyanobacteria. In the dark, OCP stays in an inactive orange form known as OCP^O. After blue or green illumination, a red active form is populated, known as OCP^R. OCP^R can interact with the phycobilisome, a cyanobacterial light-harvesting complex, inducing nonphotochemical quenching and leading to dissipation of harvested sunlight energy.[1-4] The details of $OCP^O \rightarrow OCP^R$ photoconversion are largely unknown. Various kinetic schemes of OCP^R formation upon photon absorption have been proposed, however, no coherent picture of the whole process has been developed so far. A cascade of intermediate forms is generated after photon absorption, however, due to the low quantum yield of these reactions, investigations of these processes are very challenging. It has been proposed that echinenone-functionalized OCP does not photoconvert to OCP^R upon single photon absorption and instead, OCP^R is formed only upon absorption of a second photon arriving roughly one second after the first one.[4] A custom experiment performed with two light pulses confirmed this hypothesis. The usage of two excitation laser pulses spaced by variable delay (Figure 1B) allowed to probe the existence of the intermediate state dubbed OCP^{1hv}, which needs to be re-excited in order to populate OCP^R (Figure 1A). The existence of a two-photon photoconversion mechanism allows cyanobacteria to better adapt to various irradiation conditions, allowing for the more adequate modulation of the dissipative processes that prevent the generation of the toxic reactive oxygen species in cyanobacteria.

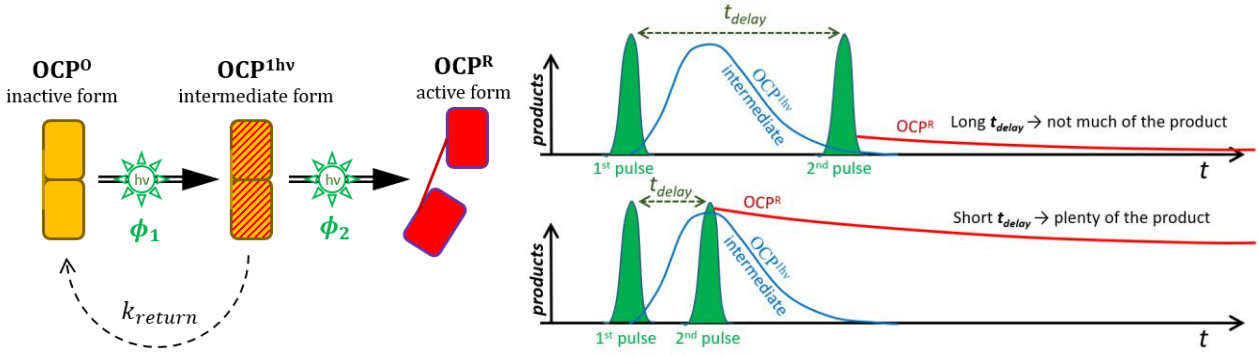

Figure 1.A) Two photon photoconversion scheme, B) Concept of the two-pulse irradiation experiment.

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Photoréactions ultrarapides dans les flavo-enzymes

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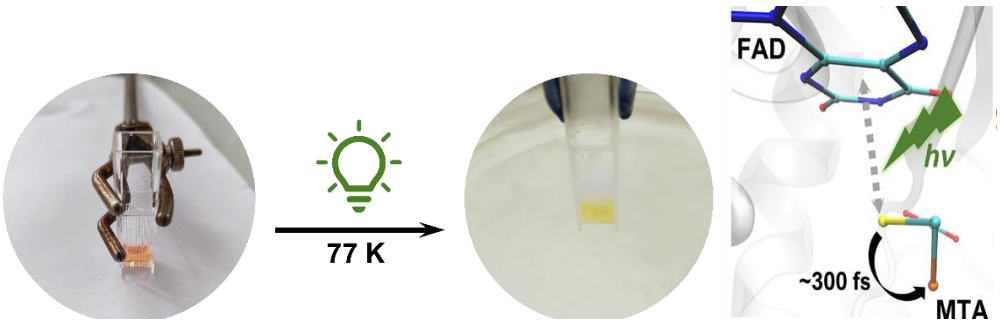
Mots-clés : flavine, protéines, spectroscopie ultrapide, dynamique moléculaire, photoswitch

Résumé :
Dans des cas exceptionnels, les enzymes à flavines utilisent la lumière de façon fonctionnel pour la catalyse, comme dans la fatty acid photodecarboxylase (FAP)[1]. Cependant, la plupart des flavo-enzymes ont des fonctions indépendantes de la lumière, bien que des processus photophysiques se produisent également dans ces cas. Ces processus peuvent avoir des fonctions photoprotectrices et peuvent également être exploités pour des applications photocatalytiques ou de photocommutation[2]. Ici nous présentons des études récentes utilisant la spectroscopie d'absorption et de fluorescence transitoire ultrarapide, sur des photoproduits à courte durée de vie dans des flavoprotéines 'non-photoactives', en explorant divers états d'oxydoréduction et de ligandation. Ils comprennent la découverte de deux photoréactions précédemment inconnues qui se produisent sur une échelle de temps de quelques centaines de femtosecondes ou moins.
D'abord, nous avons étudié des flavoprotéines oxydases, qui contiennent des acides aminés cationiques proches et nous avons observé une photo-oxydation quasi-instantanée de radicaux de flavine anioniques ($Fl^{\bullet -}$) et une ré-séparation de charges subséquente en quelques dizaines de picosecondes[3]. Nous montrerons qu'une telle photoréaction non-fonctionnelle se produit également dans le FAP.
Deuxièmement, nous avons étudié le complexe de transfert de charge (CT) formé par la flavine et un inhibiteur analogue au substrat, le méthylthioacétate (MTA), dans la sarcosine oxydase monomérique (MSOX). Suite à la population de l'état CT photo-excité, un état spectroscopiquement identique à l'enzyme non complexée est formé en ~300 fs dans un processus sans barrière et avec un rendement quantique proche de l'unité[4]. Cela implique l'anéantissement de toutes les interactions CT à cette échelle de temps. Le complexe CT initial est ensuite ré-formé de manière fortement activée à l'échelle nanoseconde. Ce sont les propriétés d'un système photochromique très efficace et absorbant dans le rouge (voire figure).
Ces deux nouveaux photoprocessus présentent des possibilités d'adaptation pour des applications en bio-ingénierie.



Photochromisme dans le système de flavoprotéine MSOX-MTA et mécanisme structurelle proposée

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A strategy to access to pure absorption spectra and switching quantum yields for reversible switchable fluorescent phytochromes

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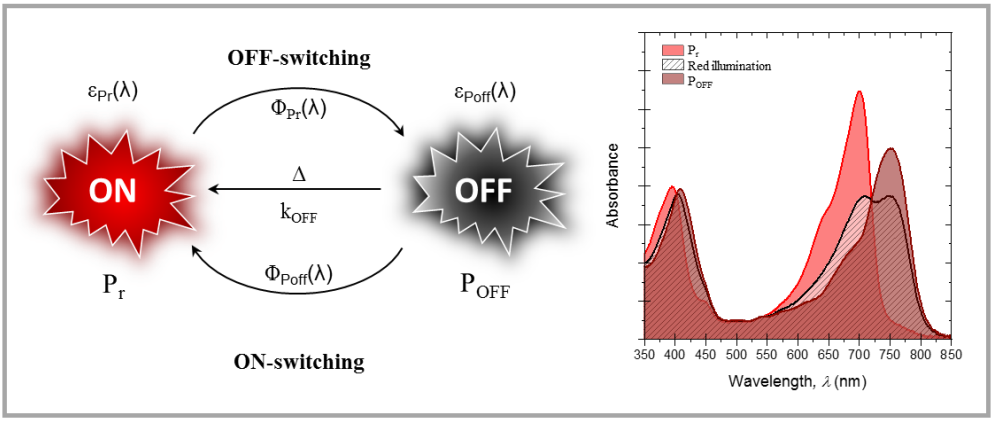
Key-words : Fluorescent protein, bacteriophytochrome, quantum yields, nanoscopy

Abstract :

Super-resolution fluorescence microscopy faces the recent challenge of developing new Reversibly Switchable Fluorescent Proteins (RSFPs) that operate in the red/NIR domain. We developed a new NIR-RSFP based on *Deinococcus radiodurans* bacteriophytochrome, which can switch from a red-absorbing ON form (P_r) to a far-red absorbing OFF form (P_{OFF}). The photoactivation of bacteriophytochromes involves several processes and intermediates, from femtoseconds to milliseconds, including *cis-trans* isomerization of the chromophore, deprotonation/protonation steps and structural changes in the protein. The use and optimization of our new NIR-RSFP require an understanding of its photoswitching mechanism, specifically to identify the controlling species (fluorescence and switching quantum yields).

We investigate the photophysics and photochemistry of the NIR-RSFP using UV-Visible steady-state spectroscopy (absorption and emission) and Resonance Raman spectroscopy. A particular challenge in characterizing bacteriophytochromes is the significant molar absorption coefficient of P_{OFF} in the red region, which results in a mixture of P_r and P_{OFF} forms after red illumination. We propose a strategy to obtain pure P_r and P_{OFF} spectra and accurately determine their fluorescence and switching yields, to address parameters that directly control these properties.



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Tracking exciton transport dynamics in Light-Harvesting Organic Nanoparticles with Ultrafast Fluorescence Spectroscopy.

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Keywords : Exciton diffusion, Fluorescence, annihilation, FRET, organic nanoparticles.

Abstract

Photosynthesis in natural photo-organisms relies on a highly organized network of chromophores embedded within proteins, known as Light-Harvesting Complexes (LHCs) **[1,2]**. These pigment-protein complexes enable the efficient absorption, transfer, and conversion of light energy. The high efficiency of these processes arises from exciton transport mechanisms, where electronic excitation energy is transferred through a combination of coherent and incoherent processes among chromophores **[3]**. Understanding these mechanisms is essential, as they are widely applied in synthetic light-harvesting systems and optoelectronic devices designed to replicate the performance of natural LHCs **[4]**.

In this study, we investigate exciton energy transfer dynamics within polymeric organic nanoparticles (ONPs) densely loaded with fluorophores, which are randomly distributed throughout the polymer matrix. These light-harvesting ONPs, developed for biophotonic applications, have demonstrated a giant antenna effect **[5]**, enhancing their performance in bioimaging and biosensing **[6]**, indicative of an efficient exciton diffusion.

To elucidate the mechanisms driving exciton transport within these ONPs, we employed a combination of time-resolved fluorescence spectroscopy techniques. First, anisotropy decay measurements were conducted using polarization-resolved up-conversion fluorescence spectroscopy with a time resolution of ~200 femtoseconds. The results reveal ultrafast sub-picosecond depolarization times, indicative of significant orientational disorder and rapid exciton hopping between excited donor fluorophores and their nearest ground-state neighbors via homo-FRET (Förster Resonance Energy Transfer).

Next, we assessed exciton diffusion properties by exploiting exciton-exciton annihilation (EEA) to extract the diffusion constant and diffusion length within the ONPs. Using streak camera-based photoluminescence spectroscopy (time resolution of 10 picoseconds) in a confocal microscope detection scheme, we determined the EEA rate from fluence-dependent isotropic exciton population decay kinetics **[7]**. We inferred a diffusion constant as high as ~0.5 nm²/ps for ONPs with a dye concentration of 0.36 M, corresponding to a diffusion length of ~ 70 nm **[7]**.

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