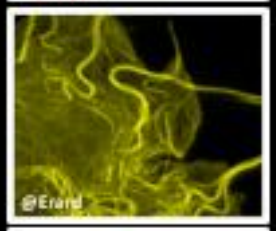

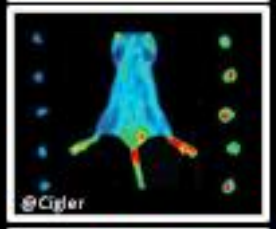

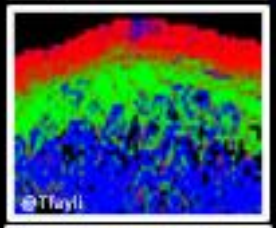

17th-19th October 2022

École normale supérieure Paris-Saclay
Université Paris-Saclay
Gif sur Yvette

PhysChemCell2022

Innovative strategies to probe living matter: quantitative analysis and imaging

Plenary Speakers

Pedro Gois (Portugal)
Petr Cigler (Czech Republic)
Thomas Laurell (Sweden)
Orly Reiner (Israel)

BIOPROBE | université PARIS-SACLAY

Axe 1

New Chemical Strategies

New Chemistries for Stimuli-Responsive Targeting Drug Conjugates

Pedro M. P. Gois

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Targeting drug conjugates, emerged as a powerful class of chemotherapeutic agents that are capable of sparing healthy tissues by liberating the cytotoxic payload upon specific antigen recognition. A considerable body of work in this field highlighted that targeting drug conjugates therapeutic efficacy, correlates well with the conjugate homogeneity and activation of the drug at the diseased site. Therefore, the linker technology used to connect both functions contributes decisively to the therapeutic usefulness of these constructs. In this communication will be presented our most recent finding on the design of functional likers for targeting drug conjugates, based on boron complexes (B-complexes)[1] that can be modulated to exhibit fluorescence and to respond to glutathione, pH or reactive oxygen species stimulus.[2-4]

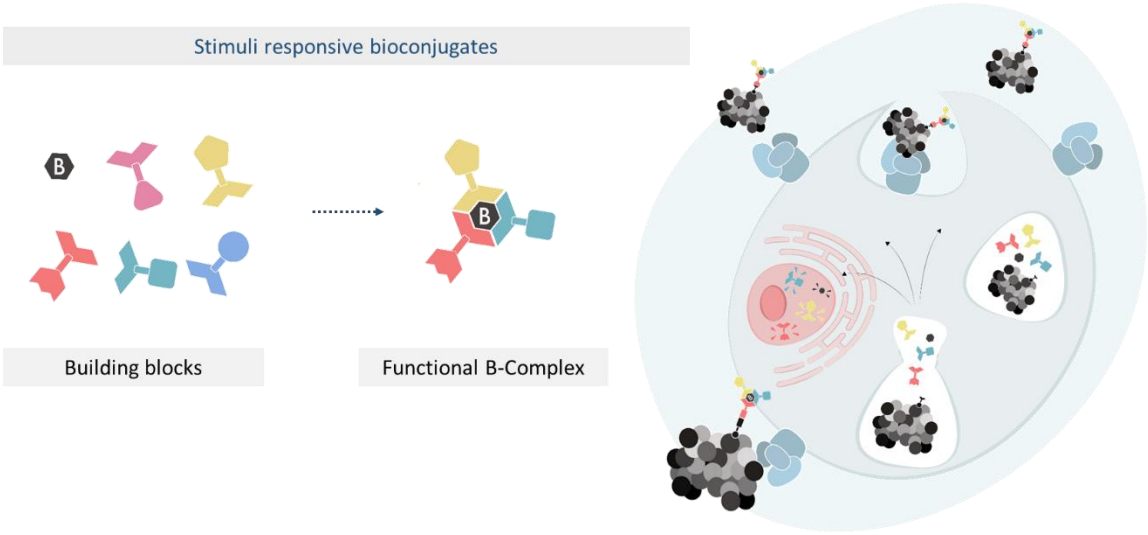

Figure 1: Modular, stimuli-responsive Targeting Drug Conjugates.

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Borinic acids as new, fast hydrogen peroxide–responsive triggers

Dominique Urban

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Hydrogen peroxide plays an important role in the fine balance between physiological and pathological processes. To detect this diffusible small molecule, we expanded the scope of organic triggers in developing borinic acids as an alternative and more sensitive trigger than the most conventional boronate-based sensors. We discovered that borinic acid is 10,000-fold more reactive than its boronic counterpart toward H_2O_2-mediated oxidation[1]. This improvement also proved effective for in-cell detection of exogenously as well as endogenously produced H_2O_2. We believe borinic acids represent a new and efficient tool allowing for the development of new devices for a better understanding of H_2O_2-mediated signaling processes.

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Photopharmacology: towards light-controlled TAM kinase activity

Sandrine Piguel

Biomolécules : Conception, Isolement, Synthèse (BioCIS), *Faculté de Pharmacie, Université Paris-Saclay : UMR8076*

Various strategies have been developed for triggering drug release by external stimuli, including light which is a particularly attractive source of stimulation. Indeed, light can be readily available and focused, allowing the precise temporal and spatial definition of the stimulus. Photopharmacology is an emerging field that utilizes photo-responsive molecules to enable control over the activity of a drug, using light. Photopharmacology aims at solving problems of off-target activity, selectivity and side effects of therapeutic treatments. Although kinase inhibitors have been successful as therapeutic drugs in cancer treatment, the lack of selectivity involving side-effects is still a major drawback. In order to circumvent this issue, the development of photo-controlled small molecules kinase inhibitors for dynamically controlling drug activity would be of significant benefit for the patients.
In this context, we developed unprecedented photo-controlled small molecules targeting the TAM receptors that play major parts in cancer development, autoimmune reactions and viral infections. Our approach is based on the photoactivatable protecting groups strategy. The blockade of the pharmacophore moiety with a photoremovable protecting group (PPG) renders the drug temporarily inactive. Then, irradiation with light will induce a photolytical reaction leading to the release of the active compounds. Starting from a small library of imidazo[4,5-b]pyridines recently identified as potent inhibitors of the TAM family in our team, we report the first photoactivatable caged inhibitors of TAM kinases. The design, the synthesis, the photocleavage properties, and the inhibitory activity under light of new photoactivatable small molecules will be discussed.

Fast and Bioorthogonal Release of Isocyanates in Living Cells from IminoSydnones and Cycloalkynes.

Maxime Riberaud, Frédéric Taran

Université Paris Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé, SCBM

Bioorthogonal click and release reactions are powerful tools for chemical biology allowing, for example, the selective release of drugs in biological media, including inside animals. Here we developed two new families of iminosydnones mesoionic reactants that allow a bioorthogonal release of electrophilic species under physiological conditions. Their synthesis and reactivities as dipoles in cycloaddition reactions with strained alkynes have been studied in detail. These mesoionic compounds react smoothly with cycloalkynes under physiological, copper-free reaction conditions to form a click pyrazole product together with a released alkyl- or aryl-isocyanate. With kinetic constants up to 1000 M^{-1} s^{-1}, this click and release reaction is among the fastest described to date, and represents the first bioorthogonal process allowing the release of isocyanate electrophiles inside living cells offering interesting perspectives in chemical biology.

CinNapht as Easily Tunable Naphthalimide/Cinnoline Fused-Hybrid Dyes for Fluorescence Imaging in Living Cells

Arnaud Chevalier[1*], Minh-Duc Hoang[1], Jean-Baptiste Bodin[2], Farah Savina[2], Jérôme Bignon[1], Philippe Durand[1], Rachel Méallet-Renault[2], Gilles Clavier[3]

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The development of new fluorescent structures that can be easily adapted is an important issue in the development of tools dedicated to fluorescence cell imaging. In this context, combining a stokes shift enhancement with a redshift of the absorption and emission wavelengths is one of the most addressed topics. The 4-amino-1,8-naphthalimide fluorophore (ANI) is a good example of tunable dye exhibiting a large stokes shift that's can be used for many applications.[1] The easy functionalization of their imide nitrogen atom enables fine tuning of the fluorophore properties such as solubility or organelle targeting.[2] However, their uses in imaging experiments are limited to the green region of the spectrum. Seeking to overcome this drawback, six-membered diaza ring of Cinnoline has been fused on Naphthalimide dye to give a donor-acceptor system called CinNapht.[3] These red shifted fluorophores were found to exhibit a large Stoke Shift and quantum yield up to 0.52. The photophysical and/or organelle targeting properties of CinNapht fluorophores can be easily modulated with our proposed synthetic pathway.[4] Here we present the synthesis and photophysical characterization of a panel of flurophores derived from a hybrid Naphthalimide/Cinnoline fused backbone called "CinNapht". The modularity of the CinNapht scaffold provides the access to a broad diversity of fluorophores in both photophysical behavior and in cell imaging purposes. They are therefore relevant alternatives to existing tools.

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New molecular tools for profiling active metalloproteases

Monika Kaminska[1], Pierrick Bruyat[2], Perrine Da Silva[3], Annabelle Bonino[4], Melissa De Souza[4], Robert Thai[4], Fabrice Beau[4], Carole Malgorn[4], Sarah Bregant[4*], Laurent Devel[4*]

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[2] CEA post-doctoral fellow program
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[4] Institut Joliot/DRF *CEA*
* : Auteur correspondant

Conventional proteomics approaches aiming at establishing the relation of proteases to disease states are limited by the fact that they take the total protein amount into account, whereas the protease functional status is often the sole relevant parameter. Within the arsenal of chemical probes developed to tackle the proteases activation status, activity-based probes (ABPs) have a prominent place[1]. A typical ABP is composed of i) a reactive "warhead", ii) a targeting motif, and iii) an analytical handle for subsequent characterization of the resulting covalent adduct. Since metalloproteases (MPs) use zinc-activated water molecule rather than a protein-bound nucleophile for catalysis, the ABPs targeting these proteases are systematically composed of a reversible inhibitor to which a photolabile group is attached[1]. Although capable of efficiently detecting endogenous MPs in biological fluids, the use of such photoaffinity probes is restricted to ex vivo applications and in vivo profiling of MPs remains unsolved to date.
To address this issue and by exploiting a ligand-directed chemistry, we recently developed original chemical probes capable of covalently modifying matrix metalloproteases (MMPs) active site without any external trigger[2,3]. In the frame of this presentation, we will present these approaches and will discuss how they can be exploited to profile metalloprotease active forms in biological fluids or in vivo.

1. Matrix Metalloproteinases: From Molecular Mechanisms to Physiology, Pathophysiology, and Pharmacology. Luiz G.N. de Almeida, Hayley Thode, Yekta Eslambolchi, Sameeksha Chopra, Daniel Young, Sean Gill, Laurent Devel, and Antoine Dufour. Pharmacol Rev. July **2022** 74:712–768.
2. Ligand-Directed Modification of Active Matrix Metalloproteases: Activity-based Probes with no Photolabile Group. Kaminska M, Bruyat P, Malgorn C, Doladilhe M, Cassar-Lajeunesse E, Fruchart Gaillard C, De Souza M, Beau F, Thai R, Correia I, Galat A, Georgiadis D, Lequin O, Dive V, Bregant S, Devel L. Angew Chem Int Ed Engl. **2021** Aug 9;60(33):18272-18279.
3. unpublished results - undergraduate internship (P. Da Silva LDD2 SCV **2022**, Université Paris-Saclay)

A new immunofluorescence methodology to follow G4 ligand distribution in cells

Daniela Verga

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Repetitive guanine-rich DNA and RNA sequences can form secondary structures known as G-quadruplexes (G4s). Bioinformatics analysis and biophysical, biochemical and cell-based assays unequivocally suggests that small synthetic molecules selective for G4 structures represent valuable chemical tools necessary to unveil G4 biological functions.[1] This is why strong effort has been focused on the synthesis of selective G4 ligands that can be used as G4 probes. The development of imaging techniques combined with G4 selective fluorescent tools are required to support these studies. Therefore, several imaging methods have been devised for tracking G4 ligand distribution *in cells*.[2] However, several G4 ligands are not fluorescent or are characterized by a very low intrinsic fluorescence. Thus, several labelling approaches based on both *ex situ* and *in situ* synthesis of fluorescent-tagged G4 ligands have been proposed.[3] In spite important developments, fluorescent tag functionalization did not overcome the issues associated to the spatial resolution of fluorescent microscopy; hence, signal amplification is required to allow G4 imaging with higher precision.

In this study, we proposed the development of a new visualization strategy called G4 ligand Guided Immunofluorescence Staining (G4-GIS) based both on specific recognition by small molecule and antibody signal amplification properties. By using PDC core as selective G4 ligand, we synthesized a series of immuno-tag (5-BrdU) modified PDC derivatives from PDC CuAAC precursors which selectively bind G4s with high affinity as confirmed by biophysical assays. *In situ* functionalization efficiency was tested *in vitro* and antibody recognition of the ligands bound to G4 structures was validated using a modified ELISA. Afterwards, both *ex situ* and *in situ* functionalized G4 ligands were incubated *in cells* and the new immunostaining method established. The latter provides a new useful high sensitivity approach for G4 ligand target detection.[4]

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Axe 2

Nanoprobes and Biosensors

Designing the interface of nanoprobes operating in biological environments

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The use of nanoparticles in diagnostics, therapeutics and imaging has revolutionized these fields with new properties not available with small molecules. Nanoparticle interface provide possibilities for polyvalent and independent attachment of different molecules serving as recognition/targeting structures, optical probes, spin probes or catalysts. However, nanoprobes with a solid interface require precise control of multiple factors related to surface chemistry. To avoid for example aggregation, off-target interactions, and protein corona formation, appropriate interface design is essential. This talk will present general nanoparticle design strategies and specific examples including nanodiamonds, plasmonic systems, and virus-like particles.

Biocompatible and photostable photoacoustic nanoparticular contrast agents based on bodipy-scaffold and polylactide polymers: synthesis, formulation and in vivo evaluation

Jean-Baptiste Bodin[1, 2], Clément Linger[2, 3], Théotim Lucas[3], Jérôme Gateau[3, 4], Gilles Clavier[5, 6], Nicolas Tsapis[2], Rachel Méallet[7]

[1] Institut des Sciences Moléculaires d'Orsay, Université Paris-Saclay, CNRS : UMR8214
[2] Institut Galien Paris-Saclay, Université Paris-Saclay, CNRS : UMR8612
[3] Laboratoire d'Imagerie Biomédicale, INSERM : U1146, Sorbonne Université : UM117, CNRS : UMR7371
[4] Institut Langevin - Ondes et Images - ESPCI ParisTech, CNRS UMR 7587, INSERM U979, ESPCI ParisTech, 1 rue Jussieu, 75238 Paris Cedex 05, France - France
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[6] Photophysique et Photochimie Supramoléculaires et Macromoléculaires, Université Paris-Saclay, CNRS : UMR8531, Ecole Normale Supérieure Paris-Saclay
[7] Institut des Sciences Moléculaires d'Orsay, Université Paris-Saclay, CNRS : UMR8214

We have synthesized a new Bodipy scaffold for photoacoustic (PA) imaging with the following properties: 1) absorption in the NIR region, 700-900 nm, where biological tissues absorb and scatter the less favoring the penetration depth for PA imaging, 2) a high extinction coefficient, 3) a low fluorescence quantum yield to increase the photoacoustic generation efficiency and 4) an alcohol function to initiate lactide polymerization. After Bodipy-initiated ring opening polymerization of lactide, the polylactide-Bodipy (PLA-Bodipy) was formulated into PEGylated nanoparticles (NPs) by mixing with PLA-PEG at different mass ratios. Formulated NPs of 100 nm exhibit excellent PA properties with absorption around 760 nm. Their PA efficiency allows to position them in between molecular PA absorbers and gold NPs. Bodipy-labelled NPs also present a much better photostability than cyanine-labelled PLA NPs. Bodipy-labelled NPs innocuity was verified in cultured macrophages. Finally, NPs were injected in healthy animals where they were easily detected using a commercial PA imaging system.

Focal adhesion nanosensors to study proteins assembly in synthetic cell model

Marcelina Cardoso Dos Santos

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The formation of focal adhesions (FAs) involves the recruitment of various types of proteins and it is extremely challenging to reconstruct dynamics of this process. Although several studies have partially revealed the architecture of FAs 1,2, the cartography and chronology of protein interactions leading to the assembly of FAs, and actomyosin cytoskeleton are poorly understood. Indeed, there is a lack of tools to monitor several proteins simultaneously at the nanoscale and the time resolution to address this fundamental issue.
In this work we are deciphering spatio-temporal dynamics of adhesion assembly in an artificial cell model using Quantum dot-to-dye FRET (Förster Resonance Energy Transfer) nanosensors. These versatile probes allow multiplexing and their potential has already been demonstrated in live cells and in vivo (zebrafish) samples 3. There are several advantages of using QDs as FRET donors: they are bright, photostable and have broad excitation and narrow emission spectra. However, the main advantage for this study is extended donor-acceptor distance allowing the measurement of protein-protein interaction up to 20 nm. Artificial cell model is build using Giant Unilamellar Vesicles (GUVs) containing transmembrane integrin, mimicking cell membrane, in which we microinject adhesion machinery, labeled with nanosensors. The microinjection of proteins one by one allow to measure the impact of each of them while avoiding undesired interactions with various other signaling pathways, a recurrent problem when working with living cells. To date, there appears to be no model that can accurately reconstruct cell adhesion while specifically controlling its composition from inside as well as outside.

A new YFP for imaging from FRET-FLIM to STED nanoscopy

Hélène Pasquier

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Yellow fluorescent proteins (YFP) are common optical reporters in FRET-based biosensors. We recently characterized the yellow fluorescent protein, tdLanYFP, derived from the tetrameric protein LanYFP. With a quantum yield of 0.92 and an extinction coefficient of 133 000 $mol^{-1}.L.cm^{-1}$, it is, to our knowledge, the brightest dimeric fluorescent protein available. Contrasting with EYFP and its derivatives, tdLanYFP has a very high photostability in vitro and in live cells. As a consequence, tdLanYFP allows imaging of cellular structures with sub-diffraction resolution using STED nanoscopy and is compatible with the use of spectro-microscopies in single molecule regimes. Its very low pK1/2 of 3.9 makes tdLanYFP an excellent tag even at acidic pHs. Finally, we show that tdLanYFP is a valuable FRET partner either as donor or acceptor in different biosensing modalities. Altogether, these assets make tdLanYFP a very attractive YFP for long-term or single-molecule live cell imaging including FRET experiments at acidic pH.

Digging for Glutathione function in Endoplasmic Reticulum physiology using roGFP-based redox probes

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Glutathione (GSH) is a small ubiquitous antioxidant, central to cell metabolism and involved in the etiology and progression of numerous diseases. Despite its millimolar concentration in the Endoplasmic Reticulum (ER), its role in ER physiology remains largely unknown, in part due to scarcity of tools to detect or fine-tune its metabolism. Ratiometric GSH-responsive probes have been developed to monitor and follow the dynamics of GSH subcellular levels and redox state. These genetic biosensors which can be organelle targeted, are used to evaluate GSH steady states but also to tackle its subcellular trafficking in cells. In an effort to elucidate GSH function in ER physiology (basal and ER stress conditions), we have developed experimental settings to characterize and modulate its ER transport. Using HEK293 cells, we evidenced that GSH can enter ER along a concentration-gradient, triggered by an increase of GSH cytosolic synthesis. This quickly resulted in a mild decrease of the ER redox potential, which promoted the reduction of select ER proteins without impacting the overall ER redox homeostasis. Contrary to yeast, we could not obtain clear-cut results for a role of the Sec61 translocon in ER GSH transport in mammals. We instead obtained initial evidence that different mechanisms are at play in mammalian cells: one passive dependent on GSH cytosolic concentration, and one active, stimulated upon depletion of ER Calcium stores. Screenings are underway to search for the GSH ER transporters involved in these two mechanisms.

Etude d'un système non canonique de réparation de l'ADN chez les Actinobactéries

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La molécule d'ADN est la cible de différents acteurs internes ou externes qui peuvent endommager le génome et concourir à l'introduction d'erreurs. Ces dernières peuvent conduire jusqu'à la mort cellulaire, ou encore, dans le cas des bactéries, à la formation d'organismes résistants aux antibiotiques. En réponse à la détection de cette dégradation, parmi un panel de mécanismes de réparation de l'ADN, certaines voies vont être activées et dans le meilleur de cas mener à la réparation de l'ADN.
Les *Actinobactéries* ont attiré l'attention de certaines équipes de cherche par l'absence dans leur génome du système MutS/MutL décrit pour son intervention dans la réparation des mésappariements (MMR) des bases de l'ADN. Indépendamment, des travaux récents ont mis en avant la présence chez ces organismes d'une protéine : NucS, qui présente une activité de reconnaissance des mésappariements et clivage double brin de l'ADN. Cependant, de nombreuses zones d'ombre demeurent : la validation *in vivo* de ses fonctions dans le MMR ou encore selon les suspicions de plusieurs équipes ; son intervention dans d'autres voies de réparation de l'ADN. Dans le cadre de ma thèse je m'intéresse ainsi à NucS, son rôle dans la cellule, ses fonctions, sa localisation.
Pour ce faire j'ai développé un système de marquage des cassures double brin chez *Corynebacterium glutamicum*, à partir d'une fusion entre la protéine Gam phagique et un marqueur fluorescent : mVenus. Cela me permet d'une part d'observer depuis leur formation les variations du nombre de cassure double brin dans le génome des cellules mais également de les observer sur plusieurs générations. Aujourd'hui mes efforts sont orientés vers le design d'un système de micro fluidique pour poursuivre mes analyses sur des échelles de temps plus importantes et avec plus de robustesse. Ce système de détection est donc tout à fait adapté au vivant et son expression peut être régulée au moyen de l'activation du promoteur sous lequel ce gène chimérique est sous le control.
J'utilise également d'autres fusions à partir de protéines telles que NucS qui sont impliquées dans des systèmes de réparation de l'ADN pour étudier la formation de mutation et la dynamique temporelle de leur réparation.

High Content Screening Confocal Laser Scanning Microscopy (HCS-CLSM) to decipher the mechanisms of bacterial pathogens exclusion by positive biofilms

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The increase in human consumption of animal proteins combined with public awareness towards sustainability and animal welfare implies changes in the management of meat production. Aligned with the One Health concept, alternative microbial solutions such as beneficial bacteria able to form positive biofilms and guide surface microbial ecology to limit microbial pathogen settlement in surfaces are promising tools that could complement existing biosecurity practices to maintain the hygiene of livestock buildings. Although the benefits of these positive biofilms have already been documented at farm level, the associated fundamental mechanisms and the rationale of the microbial composition of these new products are still sparse. We present a pipeline for rational strains selection for such applications based on genome and phenotype analysis using High Content Screening-Confocal Laser Scanning Microscopy (HCS-CLSM) combined with genetically engineered fluorescent strains and image analysis. This pipeline was applied to screen a collection of *Bacillus* spp. for their effect on the growth of several bacterial pathogens in different mixed biofilms models and to decipher the mechanisms of bacterial pathogens exclusion. The ability of *Bacillus* spp. to form stable multi-species biofilms will be studied in order to assemble positive consortia with enhanced ability to colonize surfaces and exclude pathogens.

Axe 3

Label-free chemical analysis and imaging

Acoustofluidics– A work horse in cell manipulation and isolation of extracellular vesicles

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The use of acoustic forces to manipulate cells and microparticles in microfluidic systems has shown a dramatic increase over the past years. The key to success relies on two factors: 1) the well-controlled conditions offered in the microfluidic domain in terms of laminar flow and hence, deterministic fluid behavior and 2) the possibility to build efficient acoustic resonators in microscale that generate well defined acoustic fields of sufficient energy density to move cells and microparticles. Over the past 20 years a global research community has developed around this theme and is governed under the title: Acoustofluidics.
Our early work focused on manipulating cells and develop acoustofluidic technology for precise cell separation where isolation of blood cells and subtypes of leukocytes as well as bacteria were demonstrated. We also saw the opportunity to target the clinical need for isolation and enumeration of circulating tumor cells which took us on a research trajectory where we now are performing clinical benchmarking studies versus CellSearch, the only clinically approved method for CTC enumeration. This required addressing both the need for high performance separation and high throughput, which many times is not compatible with microfluidics. The key motivation for this effort was that we saw an opportunity to address the CTC-question from a label free perspective, being independent of the EpCAM surface marker for selection but rather rely on the different biophysical properties of tumor cells versus leukocytes. Along with these developments the avenue to bring acoustic trapping (acoustic tweezer technology) into a microfluidic format for cell handling will be reviewed and the more recent developments to address the needs for new methods to isolate and manipulate nanoparticles will be discussed. Specific attention will be given to strategies that targets isolation, enrichment and purification of extracellular vesicles in biofluids for biomarker identification. Recent development includes upscaling the volumetric throughput for trapping of extracellular vesicles in dilute fluids such as urine and enable functional assays using isolated vesicles will be discussed.

Label-free nanoscale spectromicroscopy of drug nanocarriers

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The current sanitary crisis has outlined the need for new therapeutic strategies to cross biological barriers and increase drug bioavailability. Nanocarriers are engineered to protect the encapsulated drug and target diseased cells or organs. Among them, metal-organic framework nanoparticles (nanoMOFs) display one of the highest drug loading efficiencies, reaching almost 100%.[1,2] These organic-inorganic nanoparticles are built by the self-assembly of metal clusters and organic linkers leading to porous structures. Their versatile composition offers tunable properties according to the medical need.

For more than 15 years, nanoMOFs have been studied to treat cancer or infections.[3] Nevertheless, their behaviour remains to be unravelled: the drug distribution, interactions with biological environment and cellular fate are still not fully understood.[2,4,5] Therefore, a comprehensive characterization at the nanoscale is needed.

Here we used electron spectromicroscopy as a label-free technique for multimodal analysis of individual nanoMOFs. We have simultaneously imaged and analysed the composition of the nanoMOFs by coupling Scanning Transmission Electron Microscopy with Electron Energy Loss Spectroscopy (STEM-EELS), with a spatial resolution below 10 nm. Equipped with a monochromated gun, EELS provided access to a wide energy window, from infrared through UV-vis to soft X-rays, with a spectral resolution of less than 10 meV. Given the radiation sensitivity of nanoMOFs, special care was employed by using a cryo-holder and controlled electron doses for the analysis. First, we depict the beam-induced degradation with a careful assignment of the EELS features. Then, we show the intact spectral fingerprints collected at low doses (10 $\bar{e}/A^2$). Finally, we reveal chemical maps acquired in the three spectral ranges.

Our systematic study opens new possibilities to characterise sensitive specimens, from organic molecules to biomaterials. We further aim to quantify the drug loaded and decipher the cellular fate of nanoMOFs.

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Synchrotron multimodal imaging in a whole cell reveals lipid droplet core organization

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A lipid droplet (LD) core of a cell consists mainly of neutral lipids, triacylglycerols and/or steryl esters (SEs). The structuration of these lipids inside the core is still under debate. Lipid segregation inside LDs has been observed but is sometimes suggested to be an artefact of LD isolation and chemical fixation. LD imaging in their native state and in unaltered cellular environments appears essential to overcome these possible technical pitfalls. Here, imaging techniques for ultrastructural study of native LDs in cellulo are provided and it is shown that LDs are organized structures. Cryo soft X-ray tomography and deep-ultraviolet (DUV) transmittance imaging are showing a partitioning of SEs at the periphery of the LD core. Furthermore, DUV transmittance and tryptophan/tyrosine auto-fluorescence imaging on living cells are combined to obtain complementary information on cell chemical contents. This multimodal approach paves the way for a new label-free organelle imaging technique in living cells.

Single-molecule nanopore sensing of glycosaminoglycans

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Glycosaminoglycans are highly anionic linear polysaccharides expressed on the cell surface and in the extracellular matrix, which have prominent roles in a variety of physiological and pathological processes. They exhibit complex sulfation patterns and domain organization, thus making these carbohydrates as one of the most challenging biopolymer as regards structural analysis and functional assessment. In this context, we have investigated the label-free detection and analysis of glycosaminoglycan molecules at the single molecule level using sensing by a wild-type aerolysin nanopore. Heparin, chondroitin sulfate, dermatan sulfate, heparosan and hyaluronic acid saccharides were analyzed and could be distinguished, showing that aerolysin nanopore can detect and characterize glycosaminoglycans with various sulfate patterns, osidic bonds and epimers of uronic acid residues. Afterwards, this ability of the protein nanopore to probe all these structural determinants of glycosaminoglycans has been exploited to follow the regioselective desulfation of heparin by a sulfatase enzyme. Although, at this stage, discrimination of each of the constituent units of GAGs is not yet achieved at the single-molecule level, the resolution reached in this study is an essential step toward this ultimate goal.

Characterization of the chemical diversity of Sextonia rubra fruits by MALDI-CASI-FT-ICR mass spectrometry imaging and molecular networks

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Sextonia rubra (Mez.) Van der Werff (Lauraceae) is a tropical ligneous tree endemic to the Guiana Shield and the Brazilian Amazon[1]. It is known for its durability and in extenso for its use as a building material. The heartwood of this angiosperm consists of numerous lactone derivatives of which the two main molecules are rubrynolide and rubrenolide molecules. These two molecules have termicidal and anti-fungal activity, respectively. Therefore, they could be involved on a larger scale in the chemical defense mechanisms of this species[2].

However, its fruits, although described botanically, have never been studied further. Many recent studies show that the dispersal of an angiosperm species is influenced by the nature and quantity of specialized metabolites present in its fruits[3]. Meanwhile, the exploration of the chemodiversity of Amazonian fruits has led to the discovery of new compounds with pharmacological properties or applications in the food industry[4].

First, we developed imaging methods by MALDI-ToF and MALDI-CASI-FT-ICR mass spectrometry, either under native conditions or by the addition of cationizing agents. Then, we extracted fruits and each tissue with AcOEt and then analyzed the crude extracts by RPLC-ESI-HRMS/MS with data-dependent acquisition and positive mode. The resulting data were processed using mzMine 2 and MetGem software to construct molecular networks[5]. Finally, we explored the chemical diversity of volatile organic compounds by Head Space-Solide Phase MicroExtraction-GC-EI-MS.

Thus, the study of S. rubra fruits allows the development of analytical methods applicable to other plant species that could be taken up by the scientific community while understanding the chemical complexity of a fruit using modern analytical methods. These results constitute the first steps in the identification of specialized metabolites present in a larger system, the tree, whose ecological functions remain to be determined.

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A 3-Photon / THG Platform for Deep-Tissue Microscopy

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The reference technique for high-resolution 3D fluorescence imaging of biological tissues is two-photon (2P) microscopy with near infrared pulsed excitation (~950 nm, 80 MHz, 100 fs), now widely used in the neurosciences. However, the useful imaging depth of 2P microscopy is limited by scattering to ~500 µm in the live mouse brain cortex. Recently, three-photon (3P) microscopy with excitation in the 1300 or 1700 nm range has been introduced at Cornell University as a promising strategy to achieve deeper imaging of biological tissues (up to 1.5 mm in the mouse brain) [1, 2]. This ability is due to reduced scattering and stronger excitation confinement in this illumination regime. Although this approach is promising for tissue studies, several experimental aspects require further developments and better understanding to bring 3P microscopy to its full potential.

One first aspect is that efficient 3P imaging requires optimized laser sources which deliver ~100 nJ pulses at rates of 0.4 to 4 MHz. Thresholds for linear and nonlinear phototodamage must be characterized in order to determine the best excitation regime for a given application. In practice, for a given tolerable illumination level, a compromise needs to be done between imaging speed and depth.

Another important aspect is the number of observable parameters. The 3P excitation properties of common fluorophores such as fluorescent proteins are far from being fully characterized.

We present the development of a 3P microscopy setup optimized for multi-contrast in-depth microscopy. This work includes the validation of new generations of OPA sources optimized for this purpose (~1 MHz, ~50-80 fs) allowing to work at different wavelengths (1700, 1300 and 1030 nm) [3]. We then introduce a label-free imaging strategy which is compatible with 3P microscopy and provides different information. Our approach is based on the simultaneous detection of third harmonic generation (THG) and third order sum frequency generation (TSFG) signals and provides hemoglobin-specific contrast [4]. We illustrate its potential for selective imaging of red blood cells in live zebrafish embryos and adult zebrafish brain.

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Hybridization-chain-reaction is a relevant method for in situ detection of M2b-like macrophages during bone regeneration

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To better understand the mechanisms of tissue regeneration, it is necessary to characterise macrophages in vivo, as these cells play a fundamental role in this process. Macrophages are a heterogeneous cell population and, based on in vitro experiments, they have been divided into five different subtypes, referred to as M1, M2a, M2b, M2c and M2d, depending on the way they are activated and the cell surface markers they display. Whether all subtypes can be detected in vivo is still unclear. Our work aimed to identify in vivo macrophages during bone regeneration in a rat model. Classical immunolabelling techniques are limited to distinguish between different M2 macrophages. In this context, we have shown that in situ hybridization coupled with hybridization-chain-reaction detection (HCR) is an excellent alternative method for in situ detection of macrophages. Our work identified M2b-like macrophages in cryosections of regenerating bone. This work revealed that the HCR technique can provide valuable information for the characterization of macrophages in vivo and can be used to monitor therapeutic strategies for tissue repair.

Raman imaging of THP-1 cells: impact of eicosapentaenoic acid on the hydrolysis of cholesterol esters in lipid droplets

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Atherosclerosis – a cardiovascular disease and the major cause of morbidity and mortality in industrialized countries – is linked to the existence of atherosclerotic plaques characterized by cholesterol-laden macrophages called foam cells. In these cells, cholesterol esters (CE) are associated with triglycerides (TG) and form lipid droplets (LD). The only way to remove this excess of cholesterol is to promote the efflux of free cholesterol (FC) from macrophages to specific acceptors. Fournier, *et al.*, recently showed that eicosapentaenoic acid (EPA) reduces FC efflux on overloaded THP-1 macrophages *in vitro*, due to decreased EC hydrolysis. These *in vitro* observations could translate the difficulty of EPA to facilitate *in vivo* the antiatherogenic process of cholesterol efflux within advanced atherosclerotic plaques. The aim of this work is to study *in vitro* the impact of EPA on CE hydrolysis in the LD of human THP-1 macrophages by vibrational Raman microspectroscopy. For this, we used deuterated EPA and we recorded spectral images at cellular scale after different hydrolysis times. Results showed that EPA is involved in the formation of TG and phospholipids of lipid droplets. A slowdown in hydrolysis was observed after 24 hours with an increase in TG and a decrease in the intensity of the characteristic bands linked to deuteration. The size of LD without hydrolysis (t0) is larger than that after 24h (t1) or 48h (t2) of hydrolysis. The dimensions' decrease is more important when going from t0 to t1 than from t1 to t2. Principal component analysis made it possible to project the data according to the cellular compartment, the hydrolysis time and the supplementation of the medium.

Axe 4

Bridging the gap from nanoscale to *in vivo* imaging

Brain Folds and the Extracellular Matrix: Lessons from Brain

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Contribution of tissue clearing to 3D visualization of large biological samples

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Tissue clearing includes all the techniques that aim to render biological samples transparent. It enables the deep imaging of large tissues, organs or organisms by reducing the light absorption and the light scattering. The innovation relies on the possibility to image and visualize biological tissues in 3D in their native state without sectioning. Our facility is equipped with an imaging platform that develop in vivo imaging and tissue clearing techniques for the study of infectiology and immunity.

We harnessed these technologies to trace host-pathogen interactions through intravital imaging of recombinant pathogens (genetically encoded fluorescent or bioluminescent reporters) using either confocal microscopy or an In Vivo Imaging System (IVIS®). We also investigated viral and bacterial infections in model organisms (zebrafish and mouse) and farmed animals.

To image infectious processes at cellular resolution on complex biological samples (mouse organs and fish bodies), we recently set up in toto immuno-histochemistry, tissue clearing and 3D imaging approaches. This allowed us to provide a detailed description of the tropism of the respiratory syncytial virus (RSV) in the nasal cavity and the lungs of mice infected by nasal instillation. 3D visualization of the whole tissues enabled the identification of the infected cell subtypes, based on both morphological traits, position within the cellular network and the detection of the cytoplasmic viral factories, which are hallmarks of RSV infection.

When applied to fish species, clearing and 3D deep imaging represents novel tools to assess the pathophysiology of fish diseases and improve the 2D histology analyses. During the presentation, we will illustrate the contribution of these techniques in infectiology and anatomopathology.

In-vivo fast non-linear microscopy reveals intraneuronal transport impairment induced by slight molecular motor imbalances in the brain of zebrafish larvae

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Intracellular transport is a vital process, especially in neurons. Axonal transport deficit is found in neurodegenerative diseases. Conventional methods used to measure intraneuronal transport are limited by moderate spatiotemporal resolutions. We developed a method using photostable optically active nanocrystals (NC) tracers spontaneously internalized in endosomes1,2.

Here we demonstrate its application to zebrafish (Zf) larvae. We used NC exhibiting large second-order non-linear optical properties, injected in Zf brain. We harnessed these properties combined with fast raster scanning of the infrared laser beam to achieve 20 frames/s rate, allowing us to detect short pausing duration underpinning complex molecular mechanisms otherwise smeared out by low temporal resolution.

Using this method in axons of neurons with known polarization, we were able to separate the retrograde from the anterograde phases of motion. Our pipeline of video dataset analysis extracts statistical distributions of transport metrics in normal and perturbed situations. To test the sensitivity of our assay, we modulated active motors concentration, either by applying dynapyrazole, a retrograde motor dynein inhibitor, or by using transgenic Zf engineered with loss-of-function alleles of the anterograde motor Kif5aa. Dynapyrazole induces a 32% reduction of mobile NC, with a 37% reduction of their retrograde run length. In kif5aa mutants the retrograde run length is increased by 46% compared to wildtype.

The high sensitivity of our assay opens prospects in investigating in vivo endosomal transport molecular mechanism in depth.

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Adaptive optics fluorescence microscopy for bioimaging

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Both light-sheet fluorescence microscopy and two-photon excited fluorescence microscopy have opened doors to the deciphering of brain functions, thanks to their combined low phototoxicity, sectioning capability, and good spatio-temporal resolution. Nevertheless, when targeting in-depth imaging with high-resolution using high numerical aperture objectives, optical aberrations induced by the sample result in a significant loss of contrast and resolution. To compensate for these aberrations and increase image quality, we proposed an original implementation of Adaptive Optics (AO) on these microscopy setups, based on direct wavefront sensing from an extended scene Shack-Hartmann wavefront sensor [1]. This original approach leads to an efficient and fast correction [2] since it doesn't require iterative algorithms used in sensorless AO setups, and to an easier implementation without the complex and/or expensive use of either fluorescent beads or of a pulsed laser to generate a guide star.

We will show how this strategy of adaptive optics combined to light-sheet microscopy or two-photon excited fluorescence microscopy allows to enhance sensitivity and resolution for imaging in depth in the Drosophila, the Zebrafish or the mouse brain.

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An opto-microfluidic assay, to probe signaling and function in glomeruli-on-chip

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The identification of signaling pathways is the basis of understanding physio-pathological processes. This is particularly true for inflammation-related conditions such as Rapidly Progressive GlomeurloNephritis (RPGN). This pathology, for which ROS signaling is assumed to play a central role [1-2], causes terminal kidney failure. Identifying in situ the loci, the timing and the quantity of such oxidative molecules could provide a new readout of RPGN pathological transition. We propose an opto-microfluidic biomimetic assay, combining a thorough glomerulus reconstitution on-chip to decipher complex migration processes and advanced quantitative imaging methods using lanthanide-based luminescent nanoparticles to achieve fast ROS detection [3] in cell microsystems.

Our microfluidic framework is composed of two cellular compartments (capillary and urinary chambers of the glomerulus) separated by a membrane. In these systems, we co-culture a 3 cell type/layer system mimicking the glomerular organization and retaining filtration properties. Cell morphologies and differentiation were thoroughly characterized and maintained in culture on-chip for weeks.

These systems are built directly on an optically accessible surface, an “optical window”, allowing monitoring of the cells from the individual molecule to the tissue response, with virtually any high numerical aperture microscope. Based on this approach, we investigate ROS signaling in parietal epithelial cells through single lanthanide nanoparticle imaging to quantitatively assay ROS production levels.

Our microfluidic biomimetic platform tends to bridge the gap between in-vitro and in-vivo studies by bringing a relevant complexity (-e.g. 3D microenvironment and controlled co-cultures) while answering a biological question at the “meso-scale” of a tissue construct confined in an environment allowing the quantitative study from molecular to tissue responses.

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[2] Lazareth et al. Nat. Comm. 10, 3303 (2019)
[3] Casanova et al. Nat. Nanotech. 4, 581 (2009)

Timing a single ribosome in action: from in vitro to in cellulo observations

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Protein synthesis is a key process for all living organisms. It is orchestrated by the ribosome, a macromolecule capable of reading the genetic code carried by the messenger RNA (mRNA), to translate it into a chain of amino acids. As this mechanism involves multiple asynchronous steps, the observation of translation at the scale of a single ribosome can give a complete view of the process without averaging due to ensemble measurements.

We have developed an in vitro single-molecule fluorescence microscopy experiment based on a reporter system that functions as a eukaryotic ribosome "stopwatch" [1] and that allows both initiation and elongation rates to be measured on different mRNA coding sequences. Using this assay, we were able to quantify the kinetics of ribosome initiation on specific sequences called IRES, used by viruses to hijack eukaryotic ribosomes.

Recently, some teams in the world have developed experiments allowing similar measurements in cellulo [2]. They combine a system to detect the mRNA of interest, and a modification of the mRNA in question to detect the nascent protein. These systems open the way to, among other things, identify the localized translation of a given mRNA in a cell, and quantify the expression of the mRNA and its translation rate. We are currently developing similar strategies to observe in cellulo single translation events.

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Time shifting interferences for improved localization precision in Single Molecule Localization Microsocopy

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In Single Molecule Localization Microscopy, the positions of the fluorophores are obtained from a fitted Point Spread Function (PSF). This spatially based localization precision will then strongly depends on the PSF shape, which can be degraded by defocusing and aberrations. We proposed a new localization method where the uniform excitation is replaced by a time varying structured illumination over the entire field of view. The illuminated fluorophores will then have a modulated emission where the phase shift encodes their position.

As emitters can exhibit fast ON-time (typically shorter than 20 ms in dSTORM), the modulation frequency must be carefully chosen to allow demodulation. Even for a reduced field of view, shortest acquisition time of sCMOS cameras are still too slow for a sequential demodulation, this bottleneck has been overcome first by introducing an active optical element (Pockles cells, mirrors, ...) in front of the camera. The kHz modulation induced by the pattern shift, is sampled on four different subarrays acquired simultaneously by the camera, this permits to achieve fast demodulation of all emitters without losing events.

This technique, called ModLoc, can be implemented in different directions and provides a 2.4 improvement along the lateral direction. When it is applied in the z direction, it provides a unique uniform axial precision not only within the objective capture range but also up to several microns in depth, allowing a cell to be imaged with a uniform precision. We will discuss the various implementations of ModLoc and describe the recently developed detection strategies to improve furthermore its performances in terms of precision as sub 5 nm can now be obtained but also increased imaging depth as organoids can be imaged up to 40 µm along with a multi-proteins detection capability.

Dynamics of the calcium signal elicited by mechanical stimulation of the root in the model plant Arabidopsis

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Plant roots experience a variety of mechanical stresses during their progression in the soil. The pressure exerted by the soil and physical obstacles that roots have to overcome generate forces that are sensed by the tissues and transduced into cytosolic calcium concentration increases. The duration, amplitude, frequency and spatial distribution of the calcium elevation determine the calcium signature allowing specific responses to diverse cues. In order to address the question of the relationship between root deformation and calcium signaling we have developed a system combining fluorescent microscopy on plant expressing R-GECO1-mTurquoise Ca2+ nanosensor with a microfluidic device allowing to apply a controlled mechanical pressure on the root. Our experimental design proved to be suitable for investigating root deformation together with calcium variation induced by a mechanical stimulation. Our experiments revealed that (1) strain rather than stress of tissues is relevant to trigger the calcium signals, (2) calcium signal is elicited at the onset of a pulse of pressure and a second one is elicited when the pressure is released, (3) signals are localized to the tissue under pressure and do not propagate, (4) signals exhibit a strong attenuation upon repetitive stimulations.

The system we set up, thanks to its versatility, will open new perspectives, such as the identification of the molecular mechanisms underlying the calcium increase and the discovery of the adaptive outcome of local calcium signaling.

Poster session

P1	Antunez Dominguez Jesus Manuel	Miniaturized genotoxicity evaluation system for fast biomaterial-related risk assessment
P2	Assi Ali	Solar radiations alter stratum corneum water homeostasis : protective role and limitation of skin surface lipids
P3	Beguin Theo	Molecular origins of photobleaching of a yellow-emitting fluorescent protein
P4	Berdous Cecile	Emergent properties of a multi-species positive biofilm: structural and chemical analysis of the extracellular matrix
P5	Besse Laetitia	High content fluorescence microscopy and image analysis with an in-house Fiji plugin
P6	Bourge Mickael	Development of intracellular pH measurements to characterize the physiology of bacteroids in the legume-rhizobium symbiosis by combining cytometry and imaging.
P7	Bregant Sarah	Probes for covalent modification of Metalloproteinases
P8	David Marie-Odile	Development of Fluorescent Nanodiamonds for Biomedical Applications
P9	Devineau Stéphanie	Molecular imaging of single bacteria by Raman microscopy
P10	Dubois Camille	Mesure de force sur des cellules vivantes adhÃ©rentes par microscopie de fluorescence FRET
P11	Ibijbijen Amal	DEVELOPMENT OF BACTERIAL BIOSENSORS BASED ON NEW CARBOHYDRATE BIORECEPTORS
P12	Jalaber Hadrien	Plateforme SpICy - ICP, Orsay
P13	Lafargue Clément	Microscopie Electro-Optique de membranes
P14	Le Laurent	Fluorescence Lifetime Imaging Microscopy for the investigation of membrane tension
P15	Leveque-Fort Sandrine	Quantitative 3D single molecule imaging
P16	Martin Delphine	Ultrabright two-photon excitable fluorogenic probes for fast bioorthogonal wash-free labeling in live cells
P17	Mezaache Halla	Protein labelling with Fluorescent Nanodiamonds RÃ©sumÃ© poster based on affinity chromatography principles
P18	Michael-Jubeli Rime	HPLC/HR-MS(n) analyses and Raman imaging for the molecular characterization of complex lipid mixtures at the level of keratinocytes and reconstructed epidermis
P19	Michel Laurane	Visualisation of mitochondrial reductases with fluorogenic probes
P20	Monika Kaminska	Intravital imaging of endo-lysosomal pH gradients in the kidney proximal tubule.
P21	Pincet Lancelot	Adaptable and uniform illumination field as a tool for dye photophysics analysis in direct Stochastic Optical Reconstruction Microscopy
P22	Pontisso Ilaria	Combining experimental and modeling approaches to explore the interplay between moderate ER Ca2+ depletion and activation of Unfolded Protein Response
P23	Pucher Mathilde	Fluorogenic borinic acid-based probes for efficient hydrogen peroxide detection
P24	Tacke Eléonore	Late-stage functionalization of a fluorescent scaffold to afford a new generation of large Stokes shift red-emitting dyes with promising properties for biological imaging
P25	Zamiati Dounia	Quantitative FRET FLIM imaging to characterize lateral interactions between proteins anchored in the Endoplasmic Reticulum membrane

Miniaturized genotoxicity evaluation system for fast biomaterial-related risk assessment

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In the search for new synthetic biomaterials, it is important not only to assess their desired properties, but also, their unwanted effects. These pose a threat to the patients and can compromise the overall use of such biomaterial in biomedical applications. One of such effects is genotoxicity, as the ability of a chemical substance to damage the genetic material of organisms and potentially induce mutations. This effect correlates with cancer development and it is a crucial parameter to ensure biomaterials safety. Therefore, there are established tests, like the Ames test, that evaluate the genotoxicity of materials. However, these tests are complex, time consuming and rely heavily on manual work. As a result, they are not easily accessible to biomaterials laboratories and they are underrepresented in the literature. To address this issue, a simplified version of the Ames genotoxicity test was developed.

The proposed design implements fluorescence microscopy as read-out and allows side-to-side comparison and quantification of the sample of interest along with negative and positive controls in the same slide. The design is tailored to largely reduce reagent use, decrease testing time and simplify manual tasks. Of note, when coupled with a microfluidic pressure control system, the inoculation and staining steps can be automated to reduce manipulation, increase throughput and enhance repeatability. Nevertheless, the present miniaturized genotoxicity assay can be fully carried out manually using regular lab equipment, thus accessible for researchers that do not implement flow control instrumentation. Ultimately, the proposed design intends to provide a powerful tool along with a reliable method for the testing of genotoxicity of biomaterials, either on a routine basis or for punctual needs.

Solar radiations alter stratum corneum water homeostasis : protective role and limitation of skin surface lipids

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Skin is the largest organ of the body and plays several physiological functions such as a protective barrier against water loss and environmental stresses including solar radiations. Skin surface lipids (SSLs) film is a mixture of sebum and epidermal lipids. It covers the surface of the stratum corneum (SC) and thus participates to its barrier function. SSLs are composed of a complex of free fatty acids (FFAs) and other lipids that come from sebaceous secretion such as triglycerides, wax esters, and squalene, or from desquamation such as free and esterified cholesterols with FFAs. The aim of this study was to firstly evaluate the effect of solar radiations on the SC hydration and to explore the role of skin surface lipids in the protection of water homeostasis in the SC against solar radiations. Then, we evaluate the limits of the protection capacity of skin surface lipids, and we explore the protective action of sun filters on both SSLs composition and the water homeostasis in the SC.

In this study, we proposed a novel ex vivo approach to evaluate the impact of solar radiations on the SC. It is based on the hydration and the dehydration kinetics using Raman spectroscopy by calculating the vOH/vCH ratio to monitor the relative water content in the whole SC during the drying process. The composition of SSLs was analyzed using HT-GC/MS.

Our results showed an alteration in the water caption and homeostasis within the SC after solar radiations. In addition, we showed that SSLs confer a protection of the water uptake mechanism and water loss process in the SC and suggested that the protective role of SSLs against solar radiations works by absorbing a part of the energy of the solar radiations, either directly on the surface of the SC or on a slide (not in contact with the SC). This was confirmed by the decrease of Squalene, polyunsaturated fatty acids, and Cholesterol after irradiating the SSLs and analyzing it in HT-GC/MS. Meanwhile, this protective function may be limited and is dependent to the distribution of SSLs over the body surface. Thus, in the second part of this study, we showed the complementary protection that can be offered by the solar filters with the natural protection provided by skin surface lipids. This significant protection highlights the importance of using sunscreens to preserve the skin barrier integrity and the SSLs equilibrium.

Molecular origins of photobleaching of a yellow-emitting fluorescent protein

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Fluorescents Proteins are genetically encoded fluorescent probes that have become invaluable in bioscience/biophotonics as they allow the understanding of biological processes at the molecular level to get wonderful insights into living machines at work. The accurate monitoring of the biochemical event of interest deeply depends on their long term photostability, a poorly studied parameter until now. The improvement of the fluorescent protein photostability is a major challenge that requires a thorough understanding of the mechanisms involved in photobleaching. Here, we report the first results of a study aimed at elucidating the mechanisms involved in the photobleaching of Citrine, a yellow-emitting fluorescent protein. Two experimental approaches have been used so far. On the one hand, the analysis of the evolution of the absorption spectra over the irradiation time allowed to propose a first simple kinetic model for photobleaching. On the other hand, the first results of mass spectroscopy provided initial information on the chemical nature of the generated photoproducts. A cleavage of the protein leading to the degradation of the chromophore was identified. In addition, the mass spectra showed mass additions, but also mass removals. The first results of the influence of the presence of oxygen are presented and discussed.

Emergent properties of a multi-species positive biofilm: structural and chemical analysis of the extracellular matrix

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Bacterial cocktails are nowadays sprayed on the surfaces of livestock buildings to prevent the establishment of pathogenic microorganisms by the formation of a positive biofilms. These bacterial consortia are mostly composed of *Bacillus* spp. and lactic acid bacteria. We have recently observed in the laboratory a stimulation of the synthesis of extracellular polymeric substances (EPS) of *Bacillus* strains in contact with certain lactic bacteria. This induced massive production of EPS could confer new emerging properties to these mixed communities such as improved pathogen exclusion. In order to decipher the nature of these interactions and their effects, the structural dynamics of these mixed biofilms at the single cell scale was studied by confocal laser scanning microscopy coupled with high throughput image analysis. The possible modulation of the chemical composition of the extracellular matrix in mixed biofilms was analyzed by Fourier Transform Infrared Spectroscopy (FTIR). The EPS of *Bacillus* spp. biofilms are notably composed of amyloid fibers and polysaccharides presenting a specific infrared signature compatible with these tools. The knowledge of these properties will allow to improve the selection of strains for these applications.

High content fluorescence microscopy and image analysis with an in-house Fiji plugin

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Immunofluorescence microscopy analysis is extensively used to follow various cellular processes and in particular the DNA Damage Response. Here, we are presenting an in-house developed Fiji plugin, called MIC-MAQ for Microscopy Images of Cells-Multi Analysis and Quantifications, to analyze high content fluorescence microscopy images. This Fiji plugin is applied in the example of a multivariate Quantitative Image-Based Cytometry (QIBC) approach. This experiment determines the cell-cycle stage for each cell in a large asynchronously growing population. In parallel, the nuclear signal intensity and the counting of nuclear protein foci of a protein of interest are measured. The QIBC protocol was developed on U-2 OS cells. The cells were immunolabelled for nuclear proteins, de novo DNA synthesis in S-phase was detected by EdU labbeling and DNA staining with DAPI was performed. The images were acquired automatically using a widefield microscope. The MIC-MAQ plugin is then used to extract measurements from nuclei, based on a deep-learning Cellpose segmentation tool and examples of multiparametric data visualization will be presented.

Development of intracellular pH measurements to characterize the physiology of bacteroids in the legume-rhizobium symbiosis by combining cytometry and imaging.

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The legume-rhizobium symbiosis is characterized by the formation of symbiotic root nodules, in which the bacteria differentiate into nitrogen-fixing bacteroids. In some hosts, bacteroids are in state of terminal differentiation, presenting an elongated shape, an important polyploidy, and increased membrane permeability causing loss of viability. Higher symbiotic efficiency (a benefit to the host plant) was associated with this terminal bacteroid differentiation (TBD).
In order to identify novel features of TBD, we tested on free-living bacteria and bacteroids undergoing TBD (*Sinorhizobium meliloti* in *Medicago* nodules) a novel genetically encoded ratiometric fluorescent biosensor (pHP, derived from GFP) to estimate intracellular pH. A complementary approach in flow cytometry and imaging makes it possible to calibrate the biosensor, and to combine the statistical power of cytometry and the resolving power at the tissue level of imaging.

Probes for covalent modification of Metalloproteinases

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The detection of actors regulating normal or pathological biological processes still require highly finely-tuned molecular probes for improved selectivity and detection sensitivity. Activity-based probes field delivers selective tagging of a target protein of interest by probes derived from a affinity binder whose structure is extended with a tag and a chemical reactive function. The specific case of proteases tooks direct benefits from synthetic inhibitor structures to access activity-based probes, specifically for the cystein and serine family as their catalytic residues behave as “specifically hyperactive nucleophiles” to form their respective covalent adduct. For metalloproteinases whose catalytic mechanism does not cross any covalent bound transition state, alternative strategies are necessary to deliver a covalent adduct to be detected. Besides photoaffinity routes, we focus our efforts to produce selectively-labelled metalloproteases using the recently emerged proximity driven reactions. 1,2

One type of these approaches, using a matrix metalloprotease (MMP) inhibitor bearing a smooth labile linker cleaved upon nucleophilic addition, is expected to only tag the MMP of interest leaving unchanged their physiological function.

The second one uses a metalloprotease inhibitor including an additive function capable of SuFEX reaction planned to produce a covalent adduct, securing sustained MMP inhibition.

Both probes design was based on phosphinic scaffold included in amino-acids sequence leading to phosphinic pseudopeptide able to efficiently bind several MMP with tens to hundreds nanomolar affinities. The covalent labelling efficiency depending on the labile linker nature3 or the “SuFEX moeity” positionning will be presented and discuss in this poster as well the labelling specificity.

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Development of Fluorescent Nanodiamonds for Biomedical Applications

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Fluorescent nanodiamonds (fNDs) have unique properties: intense fluorescence, quantifiable and unalterable over time, ideal for labelling, non-toxic and with a chemically modifiable surface. Consequently, the use of fNDs as a marker in biology and medicine appears extremely interesting because it makes it possible to solve the photobleaching problem which is observed with most fluorophores. The nanoscale size of fNDs gives the advantage of an enormous trading surface that can also serve as a platform for drug delivery while carrying the functionality necessary for targeted addressing. Whatever the applications, the challenge is to functionalize wisely the nanodiamonds surface to control the nature of the chemical groups present on the surface that play a key role in : (i) the stability of their dispersion in biological fluids ; (ii) their toxicity, including cytotoxicity, which is one of the key characteristics for the use of fNDs in biodistribution studies in animals or, in the case of cells labelling ; (iii) the interactions and/or the reactions with the biomolecules to be labeled in such a way as to obtain stable ND-biomolecule assemblies while retaining the biological activity of biomolecules attached to nanodiamonds.

For that purpose, we developed different strategies based on the chemical grafting of a polymer corona at the surface of fNDs to label various species: from nanoparticles to biomolecules. We succeeded then to track alumina nanoparticles and followed their biodistribution in mice for durations as long as 9 months, proving thus their translocation from muscle to the liver and even to the brain. Furthermore, we designed the labeling of antibody and proteins while keeping their recognition specificity paving the way to further uses of fNDs in the field of immunology or to track the fate of these biomolecules in cells and even in body and for extended period.

Molecular imaging of single bacteria by Raman microscopy

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The detection and identification of bacteria involves the analysis of large populations following bacterial growth in standard laboratory conditions. This methodology does not allow the detection of viable but uncultivable bacteria nor can it be applied in situ. Moreover, the analysis of bacterial phenotypes at the population level masks cell-to-cell variations, a feature that may also play a role within a population or in the host response. While whole-genome sequencing has proven to be a powerful tool to overcome the limitations of the conventional approach to identify pathogenic isolates and genetic markers of drug resistance, less has been done on the imaging side.

Here, we exploited confocal Raman microscopy to investigate bacterial phenotypes at a single cell level. We developed a label-free Raman imaging technique for living bacteria that allowed us to map the molecular distributions of specific biomolecules at a cellular level. We applied this method to the analysis of pathogenic Klebsiella pneumoniae at a single cell level and to the formation of biofilms by environmental cyanobacteria. We suggest that the molecular imaging of single bacteria by Raman microscopy could be further applied to monitor bacterial growth or contamination in situ.

Mesure de force sur des cellules vivantes adhérentes par microscopie de fluorescence FRET

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La protéine vinculine intervient au niveau de la migration cellulaire, en créant un lien dynamique entre les filaments d'actine de la cellule et les intégrines qui s'accrochent au substrat sur lequel la cellule se déplace, via des zones d'adhésion focale. Nous mesurons le changement de conformation de cette protéine dans des cellules vivantes, en mesurant le transfert d'excitation dipolaire FRET entre deux fluorophores fixés aux deux extrémités de la protéine par un lien élastique. Nous avons mesuré de façon quantitative l'efficacité de ce transfert par des mesures d'intensité et par des mesures de durée de vie et mis en évidence l'effet de la force exercée en comparant un senseur de force VinTS et un contrôle VinTL. Le but à terme est de pouvoir exercer une force contrôlée et connue en utilisant une pince optique via une bille fonctionnalisée avec la protéine de notre choix.
METHODE : Nous avons mis en place un montage multimodal combinant microscopie de fluorescence FRET ratiométrique et pince optique. L'originalité de notre montage de FRET réside dans la détection simultanée de la fluorescence du donneur et de l'accepteur sans déplacement mécanique de filtre, sur deux caméras. Le montage dispose également d'un dispositif de pince optique en réflexion permettant d'accéder rapidement et précisément à la force exercée par la pince optique.
Des cellules CHO-K1 sont cultivées et transfectées sur la plateforme de biologie cellulaire de l'ISMO.
RESULTATS : Nous avons d'abord vérifié à partir de mesures de durée de vie pour 4 constructions de référence sur le montage de l'ICP que nos transfections donnaient bien les mêmes valeurs d'efficacité FRET que la littérature. Nous avons alors mesuré l'indice de FRET avec les mêmes constructions sur notre montage pour la calibration. Nous avons ensuite fait des mesures comparatives entre une transfection avec VinTS et une construction témoin VinTL non sensible à la force.
Nous avons effectué ces mêmes mesures sur le montage de mesure de FRET de l'Université de Rutgers avec différent traitement de surface des lamelles. On a bien mis en évidence une efficacité de FRET plus faible au niveau des adhésions focales des cellules plus accrochées aux lamelles.

Development of Bacterial Biosensors Based on New Carbohydrate Bioreceptors

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Food packaging plays an important role in extending the shelf life of packaged products by protecting them from physical, chemical, and environmental hazards and preventing cross-contamination. However, new functionalities can be attributed to packaging like ergonomic, informative, active, and intelligent properties.
As such, various packaging-related practices are being implemented effectively. However, there is an important issue that needs to be addressed, it is one of the biggest burdens on public health and a key barrier to socioeconomic development worldwide, it's the use of unsafe food products that are contaminated with bacteria, viruses, parasites, or chemicals that cause foodborne diseases. More than 200 diseases, ranging from cancer to diarrhea, are caused, in part, according to the World Health Organization (WHO), by contaminated foods that contain dangerous bacteria, viruses, parasites, or toxic chemicals. Biosensors, analytical devices capable of detecting specific analytes using biological molecules and converting them into detectable signals via optical, electrochemical, thermal, or mass-based transduction mechanisms, are one of the emerging research fields that address this issue. Biosensors are gaining tremendous research progress in various applications such as biomedical, agriculture, food, and the environment. The goal of our research is to develop a bioreceptor built primarily of biosourced materials for bacterial detection using electrochemical biosensors. In this poster, recent advances in the development of bioreceptors are displayed, as well as some preliminary results of our current study.

Keywords: Biosourced and Biobased materials, Synthesis, Toxins, Bacteria, Quality, Sensors, Biopolymers.

Plateforme SpICy - ICP, Orsay

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La plateforme SpICy réunit un ensemble de microscopes et un cytomètre multi laser permettant diverses études de spectro-analyses et spectro-imageries cellulaires, en fluorescence UV-visible. Certains de ces appareils fonctionnent en mode service, les autres sont en accès collaboratif ou à la demande. Le cytomètre en flux permet l'analyse à haut débit des propriétés spectrales et morphologiques de cellules individuelles de 0,5 à 100 µm environ. Il permet une caractérisation multiparamétrique des propriétés de fluorescence de populations cellulaires (4 lasers, 8 paramètres).

Le BIFLUOR est un microscope confocal à balayage équipé pour l'imagerie des déclins de fluorescence (FLIM-TCSPC), combinée à des images d'intensité par caméra et à un spectromètre fibré pour l'analyse des spectres d'émission. Il est utilisé notamment pour des applications d'imagerie FRET en cellule vivante. La plateforme SpICy intègre également deux microscopes plein champ à fluorescence permettant des analyses multicouleurs (Flora) et des analyses des spectres d'excitation (Mona). Ces microscopes sont équipés pour la vidéo microscopie à température ambiante ou à 37°C. Le laboratoire dispose de l'ensemble des infrastructures nécessaires à la manipulation de cellules procaryotes et de mammifères (laboratoire de culture de niveau L2 et autoclave notamment). Le personnel de l'Institut de Chimie Physique aide à la mise en place de protocoles et d'analyses sur ces instruments.

Microscopie Electro-Optique de membranes

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La microscopie Electro-Optique (MEO) est une méthode prototypale, mesurant les coefficients EO d'un échantillon fin. Je présenterai des images de cellules et de systèmes modèles (micropipettes en verre) ainsi qu'un modèle théorique impliquant le potentiel membranaire et l'orientation statistique des molécules d'eau voisines.
La MEO permet la mesure quantitative de l'effet electro-optique localement avec une résolution latérale optique de l'ordre de 500 nm. Nous avons montré que les cellules vivantes présentent un effet EO localement au niveau de leur membrane, ce qui est surprenant en considérant que les éléments chimiques constituant principalement la membrane (eau et lipides) ne sont pas intrinsèquement des matériaux electro-optiquement actifs. Le modèle théorique développé pour décrire ces images, s'appuyant sur des travaux concernant génération de seconde harmonique, met en jeu le potentiel membranaire et l'orientation statistique des molécules d'eau dans la double-couche électrique jouxtant la membrane polarisée. La variation d'indice optique sous les effets combinés d'un champ statique lié au potentiel membranaire, et d'un champ électrique oscillant extérieur est detectée par interférométrie.
Ceci ouvre la voie à une détection tout-optique et sans marqueurs du potentiel membranaire, applicable pour plusieurs problématiques de recherche en biophysique puisque le potentiel membranaire joue un rôle considérable dans de multiples phénomènes biologiques.
Pour confirmer la validité de ce modèle physique, je présenterai également des expériences de MEO sur un système modèle constitué d'une micropipette en verre plongée dans une solution de NaCl. Ces micropipettes utilisées habituellement par les biologistes pour l'électrophysiologie ont la particularité d'avoir, proche de la pointe effilée, des parois de verre extrêmement fines (<µm) qui constituent un bon système physique modème car elles sont polarisées sous des niveaux très intenses de champ électrique statique.

Fluorescence Lifetime Imaging Microscopy for the investigation of membrane tension

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Fluorescence lifetime is a relevant tool to measure local change of the physico-chimical environment inside a cell. Development of fluorescence probe has enabled biological investigation of specific parameters such as pH, viscosity, or ion concentration [1] by measuring the fluorescence lifetime variations. Meanwhile, ultra-fast detection offers the possibility to measure nanoseconds fluorescence lifetime with tens of picosecond precision. Recently, a new probe Flipper-TR has been developed to quantify the membrane tension [2]. Flipper-TR is a fluorescent live cell probe that specifically targets the plasma membrane of cells and reports membrane tension changes through its fluorescence lifetime changes.
On our homemade setup, a time-gated detection with a high-rate imager is used to record the fluorescence lifetime in a wide-field configuration. To achieve axial sectioning near the coverslip, we have implemented a total internal reflection fluorescence (TIRF) configuration for the illumination. Thus, time-resolved fluorescence imaging can be performed with approximately 100 nm axial sectioning. This configuration is convenient for the observation of ventral membrane [3].
We will present the use of Flipper-TR to understand how membrane tension influences the spatial regulation of lysosomal exocytosis. Spatial statistics shows that lysosomal exocytosis is not random at the adhesive part of plasma membrane of RPE1 but clustered at different scales. To investigate the link between exocytosis and membrane tension, fluorescence lifetime of Flipper-TR has been measured on cells cultured on adhesive ring-shaped micropatterns [4]. Different conditions influencing the membrane tension have been also experimented in our work.
To make the acquisition faster, we are implementing rapid-FLIM technique [5] processed with a fit free analysis achieving 1 Hz image rate. This approach can provide a video rate FLIM imaging and is more compatible with living cells experiment or FRET analyses.

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Quantitative 3D single molecule imaging

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Recent developments in fluorescence microscopy have made it possible to image beyond the diffraction limit and thus reach previously inaccessible observation scales. Single molecule localization techniques (PALM, STORM, PAINT, ...), thus allow to reach lateral localization precision of a few nanometers. However, as with all super-resolution techniques, improvement in the axial direction remains a major challenge in order to move towards a nanoscope with isotropic resolution, which furthermore would also be capable of imaging at depth (several tens of microns).

We proposed various alternatives to enhanced the axial resolution which take advantage of intrinsic information emitted by the fluorophore, and offers major assets compare to usual strategies based on point spread function engineering. By taking advantage of supercritical angle fluorescence intrinsically emits when the fluorophore is place close to the coverslip-sample interface, absolute axial position of the molecule can be retrieved. This approach being based on an evanescent emission, the capture range is limited but well suited to study adhesion processes. In order to quantitatively extract molecule localization information, we also propose a new excitation strategy to ensure a uniform illumination of the sample, but also the possibility to simultaneously distinguish spectrally very close fluorescent molecules such as CF647/660/680. This in particular allows us to image tracks deposited by migrating fibroblasts, which serves as guidance cue for cancer cell migration, and to (co)localize in 3D both tracks and clathrin-coated structures which are required to adhere to tracks.

For in depth imaging (up to 50 µm), we use a complementary strategy based on a time modulated excitation induced by a structured excitation. This permit to retrieve the fluorophore's position thanks to the phase of its modulated fluorescence, which offers a unique uniform precision below 7 nm.

Current developments and application will be presented as well as remaining challenges

Ultrabright two-photon excitable fluorogenic probes for fast bioorthogonal wash-free labeling in live cells

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Despite the growing interest on the development of fluorophores for live-cell imaging, challenges to be tackled still remain [1–2]. An ideal fluorescent probe for bioimaging should be water-soluble, cell-permeant, photostable and should absorb and emit at wavelengths compatible with live-cell imaging, with a high fluorescence quantum yield. Two-photon excitation (2 PE) offers a great opportunity as it allows excitation wavelengths in the NIR window where the absorption and diffusion of light by biological samples are minimized. This primordial advantage provides the capability to image deep inside a tissue and avoids autofluorescence as endogeneous fluorophores are not excited by two-photon excitation or only following very specific protocols [3].
Recently, we have developed very bright biocompatible probes for labelling mitochondria in living cells[4]. We then derivatized them to develop fluorogenic probes that could constitute interesting chemical biology tools, in particular for localizing drugs in the cell environment[5]. These probes are poorly or non-fluorescent and restore their fluorescence after reaction. This turn-on property allows an unambiguous labeling in cells and an excellent signal to background ratio. We have thus obtained the brightest two-photon excitable fluorogenic probes described to date (absorption cross-section >3000 GM).

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Protein labelling with Fluorescent Nanodiamonds based on affinity chromatography principles

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The goal here is to use Fluorescent Nanodiamonds for permanent labeling of proteins, because NDfs have characteristics allowing this labeling, such as: permanent and stable fluorescence, high biocompatibility as well as surface chemistry allowing high functionalization. For this, we developed a strategy of grafting based on the principle of affinity chromatography which has the benefit of offering an easy way to label many synthetic proteins. The strep-tag method was used for that purpose.

HPLC/HR-MS(n) analyses and Raman imaging for the molecular characterization of complex lipid mixtures at the level of keratinocytes and reconstructed epidermis

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The keratinocyte's differentiation is accompanied by profound modifications of the lipid composition to form the intercorneocyte lipid cement responsible for the skin barrier function. The aim of our work is to develop analytical approaches to study the biosynthesis of these lipids during keratinocytes differentiation.
Two analytical techniques were used:
1) Normal Phase Liquid Chromatography coupled with High-Resolution Mass Spectrometry. to separate the lipid classes of different polarities in one single run and to characterize the fine structures of lipids
2) Raman microspectral imaging; to follow the lipid evolution during differentiation and to provide information on their lateral packing within the Stratum Corneum.
Three models were used:
1) 2D cell model: to monitor the differentiation from the early stages
2) Epidermis reconstructed human model: to monitor the differentiation from Stratum Granulosum (SG) up to the Stratum Corneum (SC)
3) Native human SC: to study ceramide profiles In vitro, Ex vivo, and In vivo.
Results are as follows:
1) development of relevant analytical tools in the elucidation of complex lipid mixtures composition and their evolution at the cellular or tissue level
2) innovative application of Raman Microspectroscopy: several molecular modifications within the cell were observed indicating the transition between SG and SC
3) identification of two new subclasses of ceramides (1-OE (EO) Cer and A-1-O (EO) Cer) in the human reconstructed epidermis and in the SC.
Our multimodal approach has been able to provide a better understanding of lipid metabolism during keratinocyte maturation, in very detail at the molecular level, which would allow a more reasoned understanding of the physiological or pathological disturbances of the skin barrier function.

Visualisation of mitochondrial reductases with fluorogenic probes

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Mitochondria is the center of energy metabolism in the cell. Dysfunction of this organelle have been related to many diseases, such as cancer, diabetes, cardiovascular or neurodegenerative diseases, and more [1]. It is thus a promising therapeutic target which have been extensively studied in the last decades. A recent approach aims to design prodrugs exclusively activatable inside mitochondria and structurally designed to carry them into it using mitochondriotropic targeting moieties such as triphenylphosphonium or pyridinium. Recently, promising results have been obtained using the mitochondrial nitroreductase (NTR) as prodrug activating enzyme [2]. Unlike cytosolic NTRs that are exclusively active in a hypoxic environment, mitochondrial NTRs are active in a normoxic context thus making them serious candidates as intramitochondrial activators of prodrugs. The example of NTR shows the interest of the use of reductases, so it is relevant to look at other enzymatic activities in order to extend the panel of endogenous biocatalysts which can be used for in situ activation of mito-targeted pro-drugs.
Here we describe our work on the design of fluorogenic probes that allow the visualization of mitochondrial reductase activities, especially Azoreductase (AzoR). Starting from 4-amino-1,8-Naphthalimides, we have designed and synthetized both fluorophores and corresponding azo-bazed profluorescent probes. These sensors have been studied in vitro were found to be not only highly sensitive but also selective to AzoR. Confocal microscopy experiments conducted on different cell lines confirmed the presence of a mitochondrial AzoR that is expressed at different levels depending on the cell line. This interdisciplinary work involving organic chemistry, photophysics and cell biology has provided convincing results making AzoR a plausible and promising alternatives to NTR for specific drug delivery into mitochondria.

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Intravital imaging of endo-lysosomal pH gradients in the kidney proximal tubule

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As part of the imaging platform, we are developing new methods to visualize tubular cell function in vivo with intravital multiphoton microscopy. The endo-lysosomal system (ELS) in the proximal tubule (PT) plays a crucial role in kidney physiology by retrieving and degrading filtered plasma proteins to conserve important nutrients and cargo. Proteins are reabsorbed by receptor-mediated endocytosis (megalin/cubilin system), and defects in this process result in proteinuria, a widely used biomarker (1). Acidification of endo-lysosomal vesicles by V-ATPase pumps is critical for their function, however, the nature of normal pH gradients in the PT ELS was previously unknown. To address this knowledge gap, we are developing a new imaging-based methodology to track pH changes in real time as proteins traffick through the ELS. We have labelled lysozyme with 2 different fluorophores that can be excited simultaneously, but have different emission wavelengths. Since one fluorophore is pH sensitive, and the other insensitive, the emitted signals provide a ratiometric readout. Following intravenous injection and endocytosis of labelled lysozyme, we observed fast acidification of early endosomes in PTs, where receptor-ligand dissociation occurs, with a further decrease in pH in lysosomes responsible for protein degradation. In summary, we present a new technique to investigate pH changes in different ELS components in PT cells in vivo, and relate these to specialized functions. We are currently preparing other pH-dependent sensors with dual-labelled custom-designed peptides, which will allow us to make even more precise measurements.

Adaptable and uniform illumination field as a tool for dye photophysics analysis in direct Stochastic Optical Reconstruction Microscopy

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In fluorescence microscopy, wide-field illumination method is obtained by focusing an enlarged collimated laser beam in the back focal plane of the objective. The resulting irradiance field is then gaussian-shaped and induces a non-uniform excitation of the biological sample over the field of view thus impacting quantification. A method, called Aster [1] (Adaptable Scanning for Tunable Emission Regions) has been recently proposed to generate an effective uniform excitation by introducing a fast scanning (kHz) of the gaussian beam which generate dynamically a top hat excitation. ASTER thus offers a high flexibility, as the irradiance and energy can be varied by adjusting the speed and the scanned region, allowing to trigger single molecule emission over a large field of view (200 µm x 200 µm) while keeping a low laser power (300mW). Furthermore, another advantage of this strategy is the compatibility with optical sectioning techniques like TIRF or HILO, and the intrinsic speckle reduction associated to the fast scanning.

We will present the use of this technology to analyze fluorophores photophysics properties. Indeed, in Single Molecule Localization Microscopy (SMLM), the quality of the super-resolved image highly depends on the capacity to generate single molecule imaging conditions. STORM in particular strongly relies on the control of the photophysics of the dye [2,3,4]. Aster illumination offers a smart way to trigger a uniform photophysical response in the field, while being able to control the energy input by adjusting the scanning parameters. We will show the impact of these parameters on localization density, fluorophore blinking rates and photon counts, and how they can be easily tuned to optimize SMLM imaging.

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Combining experimental and modeling approaches to explore the interplay between moderate ER Ca^{2+} depletion and activation of Unfolded Protein Response

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The Endoplasmic Reticulum (ER) is the primary site of folding and quality control of one third of cellular proteins and is the major intracellular Ca^{2+} store. Depletion of the luminal [Ca^{2+}] disrupts the correct folding environment leading to an alteration of ER homeostasis and accumulation of misfolded proteins inside the lumen. In order to restore ER proteostasis and normal cellular functions, cells have developed an adaptive mechanism consisting in 3 specific signalling pathways. This response is commonly referred to as the Unfolded Protein Response (UPR) and leads to an increase of the protein folding capacity of the ER and to homeostasis restoration. Although long-term and strong UPR activation is much studied, the consequences of small amplitude, more physiological, luminal Ca depletions on the early activation of UPR has been largely unexplored. In this study, we investigate how moderate Ca^{2+} depletion impacts on the activation of the signaling pathways of the UPR. Ca^{2+} imaging experiments using genetically encoded Ca^{2+} of a data-driven computational model allows us to decipher, formalize, and quantify these complex signaling pathways. Given that luminal Ca^{2+} depletion and alteration of correct ER proteostasis are involved in a variety of pathologies such as diabetes, neurodegenerative diseases or cancer, a better understanding of the reciprocal crosstalk between Ca^{2+} and UPR will provide insight into the mechanisms of progression of these diseases.

Fluorogenic borinic acid-based probes for efficient hydrogen peroxide detection

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Reactive Oxygen Species (ROS) are involved in many physiological processes. Hydrogen peroxide (H_2O_2), which is the most stable and the most generated ROS, plays a major role as signaling molecule in several biological mechanisms.[1] However, its overproduction or accumulation (oxidative stress conditions) can be responsible for cellular lesions associated with aging, cancers or neurodegenerative diseases such as Alzheimer's or Parkinson's.[2] Thus, its detection could help for a better understanding of its role in these processes.
Many fluorescent molecular probes, based on various triggers, have been developed to allow the detection of H_2O_2.[3] The laboratory has shown that H_2O_2-mediated oxidation of arylborinic acids is dramatically faster than the commonly used boronic acid trigger.[4] We recently designed a new H_2O_2-selective fluorogenic probe, possessing a borinic acid as trigger and a 4-methylcoumarin as pro-fluorescent moiety.[5] However, this probe must be optimized for the detection of endogenously produced hydrogen peroxide for biological application.
Therefore, we present herein the synthesis of new optimized probes in terms of photophysical properties and the preliminary results for *in vitro* H_2O_2 detection.

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Late-stage functionalization of a fluorescent scaffold to afford a new generation of large Stokes shift red-emitting dyes with promising properties for biological imaging

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With the increasing interest of optical molecular imaging in medicine, fluorescence microscopy has seen constant development contributing to the emergence of new technologies and probes without discontinuity for the past decades. Fluorogenic probes are now considered as critical tools for the study of biological environments.[1] Therefore, there is definite interest in creating a new easily tunable chemical scaffold exhibiting fluorescent behavior that could later be used for the design of chemical fluorogenic probes. In this context, our group has investigated the synthesis of a fused ring cinnoline/naphthalimide hybrid here called “CinNapht” dyes.[2] The first generation of these new fluorophores exhibits original and promising properties in conventional fluorescence: a red emission, a large Stoke Shift, a strong solvatochromism, high chemo- and photostability and biocompatibility. Here we present a new synthesis pathway enabling an easy access to numerous analogues of CinNapht dyes by late-stage functionalization and on the study of their photophysical properties. We have re-designed the synthesis in order to obtain a fluorinated CinNapht-F that can react with a wide variety of amines in a SNAr type reaction. The SNAr reaction conditions as well as the scope of suitable amines partners have been investigated and showed that the SNAr reaction is suitable for a wide variety of amines. By using thought amine partner, we could have an easy access to new fluorophores with interesting photophysical properties associated with a true utility for cell imaging applications such as organelle imaging.

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Quantitative FRET FLIM imaging to characterize lateral interactions between proteins anchored in the Endoplasmic Reticulum membrane

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The Endoplasmic Reticulum (ER) is a highly dynamic organelle that continuously forms contacts with other cellular compartments at Membrane Contact Sites (MCS). Several proteins are involved in their formation and in the signaling processes located at MCSs. The study of these MCSs is a relatively recent undertaking, and much about them is still unknown. Numerous protein interactions are still unidentified, and their molecular organization is inaccessible to date.

We developed a FRET-FLIM imaging strategy to study MCSs. The fluorescent proteins (FPs)' FRET pair was chosen for their brightness, photostability, and their ability to be monomeric. We used them to tag members of membrane-bound proteins known to bridge the ER and the Plasma Membrane. We focused on three protein families: The E-SYTs, VAPs, and ORPs. An important notion to discover is whether our proteins of interest form complexes. That's why we chose to focus our work on characterizing lateral interactions between these proteins. From our FLIM data, we can characterize molecular interactions and quantify their relative intensities within the complexes.

Our preliminary results showed (1) that E-SYTs, VAPs, and ORPs form oligomers within their families, (2) the coiled coil domain of ORPs is essential for oligomerization within the ORP family, (3) E-SYT2 interact with both ORPs, and (4) ORP8 forms clusters with VAPB.

Moreover, our study demonstrated many advantages of using FRET FLIM. One of the main benefits of the method is that we can obtain quantitative measurements describing the geometry and affinity of the interaction studied. We also established that it is possible to observe intra-molecular conformation changes after a modification in the cell environment, and that we can study modifications in oligomerization levels after mutations. Another strong point of FRET FLIM is that we can distinguish between probable interactions and those only possible due to overexpression. Additionally, FLIM has been democratized over the last decade and is more easily accessible than other photonic imaging methods able to circumvent the diffraction limit.

Our future goal is to adapt our FRET-FLIM strategy for the space and time tracking of MCSs at the subcellular level.