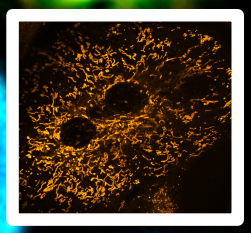
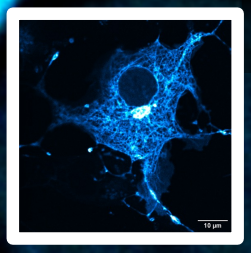
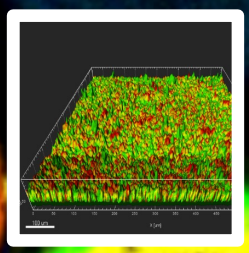
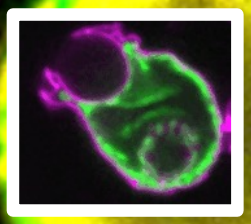
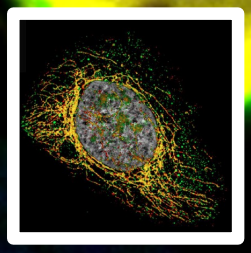
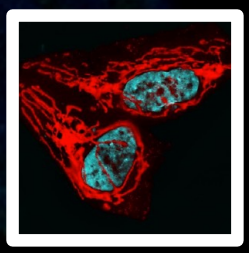
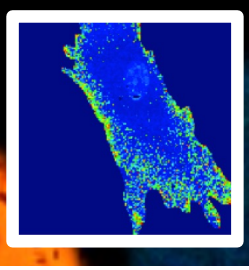








PhysChemCell2024

From Molecules to Organisms: Advancing Labelling, Imaging and Analysis of Biological Samples

Oct 14-16

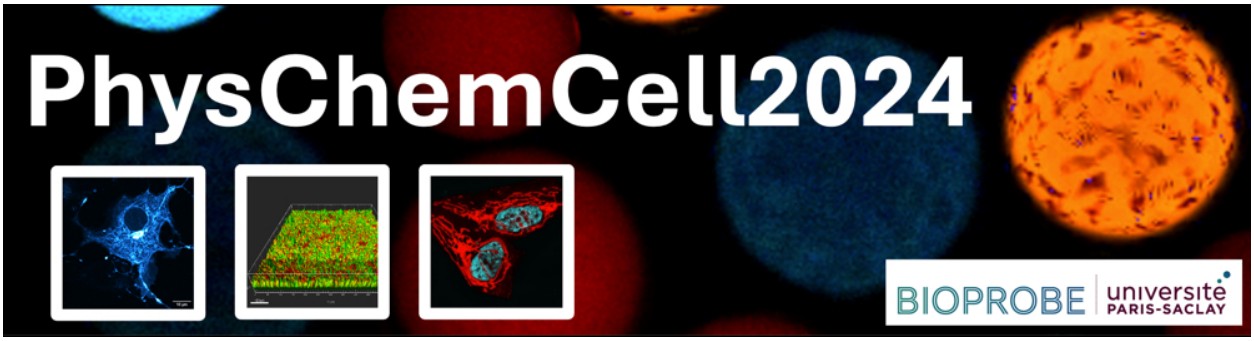
Site Henri Moissan Université Paris-Saclay Orsay

Plenary Speakers

- Peter Jönsson, Lund
- Senada Koljenovic, Antwerp
- Francesca Pennacchietti, Stockholm
- Marc Vendrell, Edinburgh

BIOPROBE | université PARIS-SACLAY

https://physchemcell2024.sciencesconf.org/



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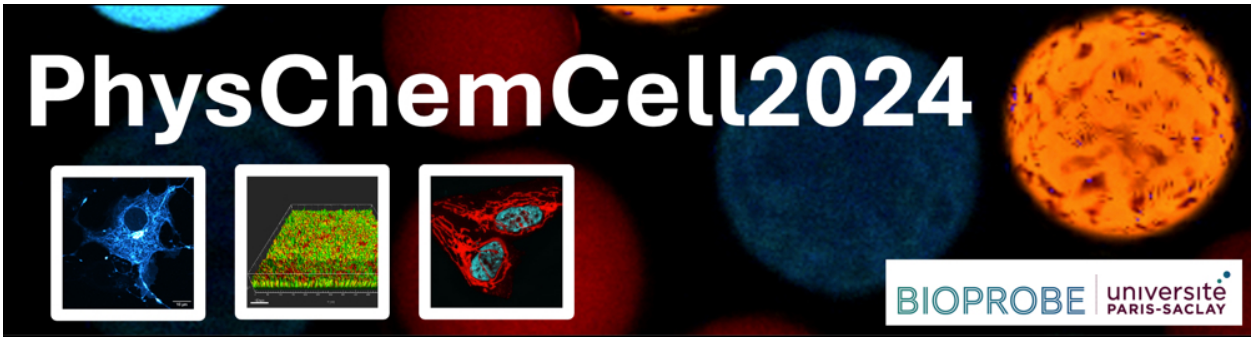
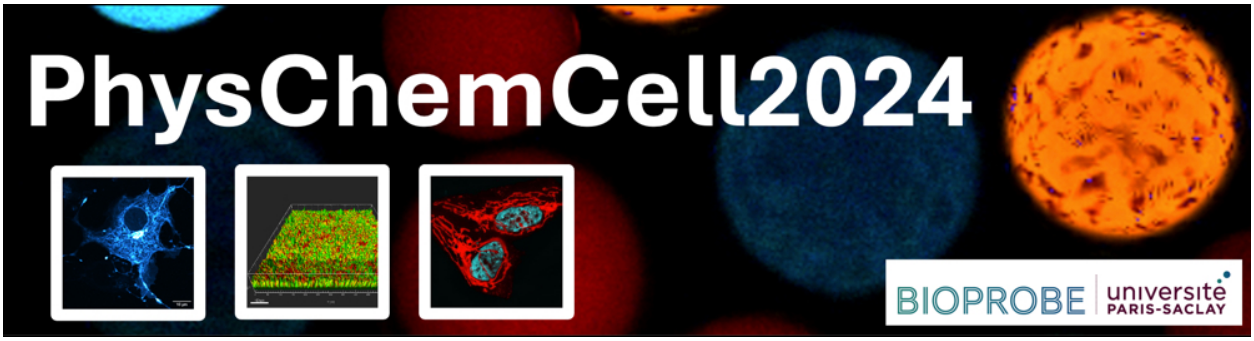


Program



Monday, October 14

Session 1 : New chemical probes, labeling and activation strategies for a living environment

Chairs : **Florence Mahuteau-Betzer** (Curie) and **Arnaud Chevalier (**ICSN)

Hervé Daniel Auditorium – Henri Moissan (Bat 670)

14:00-14:15 Conference Opening
Boris Vauzeilles, ICSN and BioProbe Co-Chair

14:15-15:15 Translating fluorescent probes for imaging immune cell function in humans
Marc Vendrell, Centre for Inflammation Research and IRR Chemistry Hub, The University of Edinburgh, Edinburgh, UK

15:15-15:35 An azo-based fluorogenic smart probe to visualize a mitochondrial azoreductase activity in live cells
Laurane Michel, ICSN

15:35-15:55 Two-photon excitable fluorogenic probes for Click-to-Release bioorthogonal reactions
Aurélie Rodriguez, Institut Curie

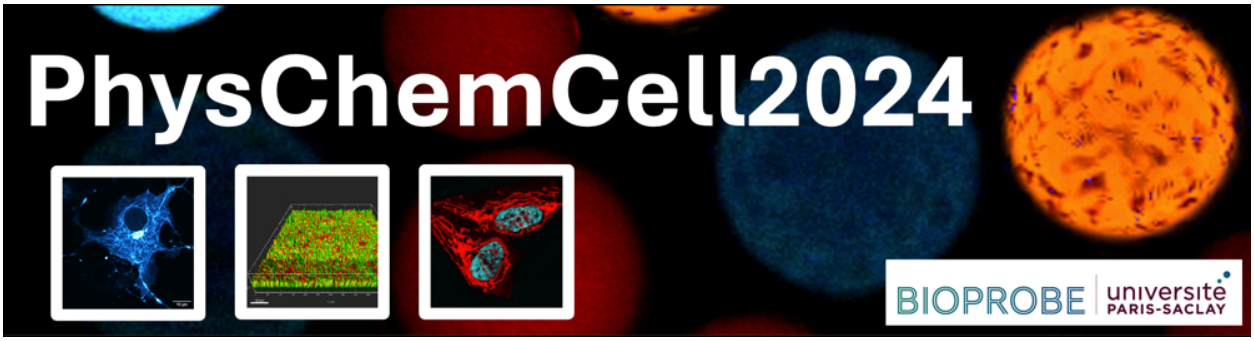
15:55-16:15 Coffee Break

16:15-16:45 L'interdisciplinarité au CNRS
Maguy Jaber

16:45-17:05 Decoding day-long maturation of the PCFPs of the EosFP family and engineering fast-maturing variants
Virgile Adam, Institut de Biologie Structurale, Grenoble

17:05-17:25 A tunable and versatile chemogenetic near infrared fluorescent reporter
Lina El Hajji, Laboratoire des Biomolécules

17:25-18:30 BioProbe General Assembly including a presentation from SATT
Marie Erard, ICP and BioProbe Co-Chair



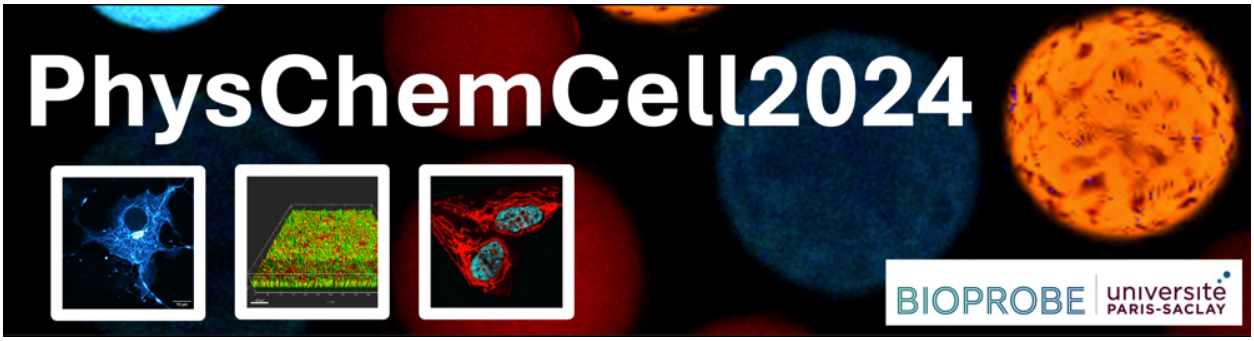
Tuesday, October 15

Session 2 : Biosensors and Nanoprobes from design to application in imaging

Chairs : **Rima Haddad** (LSHL) and **Oliver Nüsse** (ICP)

Hervé Daniel Auditorium – Henri Moissan (Bat 670)

9:00-10:00	Protein binding kinetics in immune-cell contacts and its influence on T-cell activation **Peter Jönsson**, Division of Physical Chemistry, Lund University, Sweden
10:00-10:20	Bioengineered condensates to interact and manipulate membrane-bound organelles in cells **Emma Pasquier**, ENS
10:20-10:40	Coffee Break
10:40-11:00	In vitro evaluation of a strategy of treatment associating Platinum nanoparticles and FLASH Radiotherapy **Pauline Maury**, IGR
11:00-11:20	Quantum Dot-Based FRET Nanosensors for Talin-Membrane Assembly and Mechanosensing. **Marcelina Cardoso Dos Santos**, I2BC
11:20-11:40	Optical Biosensors for the Detection of Bacteria **Mariah Harris**, ISMO
11:40-12:00	CAP-PHOTOAC: Control of the optical absorption properties of nanovectors for photoacoustic imaging **Riccardo Ossanna**, ISMO & PPSM
12:00-14:00	Lunch



Tuesday, October 15

Session 3 : Label-free chemical analysis and imaging

Chairs : **Hafsa Korri-Youssoufi** (ICMMO) and **Ali Tfayli** (Lip(Sys)^2)

Hervé Daniel Auditorium – Henri Moissan (Bat 670)

14:00-15:00 Improving cancer surgery: need for objective photonic techniques.
Senada Koljenovic, UZA Antwerp, Belgium

15:00-15:20 Multicontrast three-photon microscopy for tissue applications
Stella Dees, LOB

15:20-15:40 Using Infrared Nanospectroscopy (AFM-IR) to study protein assemblies: the case of Prion assemblies dynamic.
Jérémie Mathurin, ICP

15:40-16:00 Development of a handheld multispectral imager prototype for drug analysis.
Erwin Winkler Martinez, Lip(Sys)^2

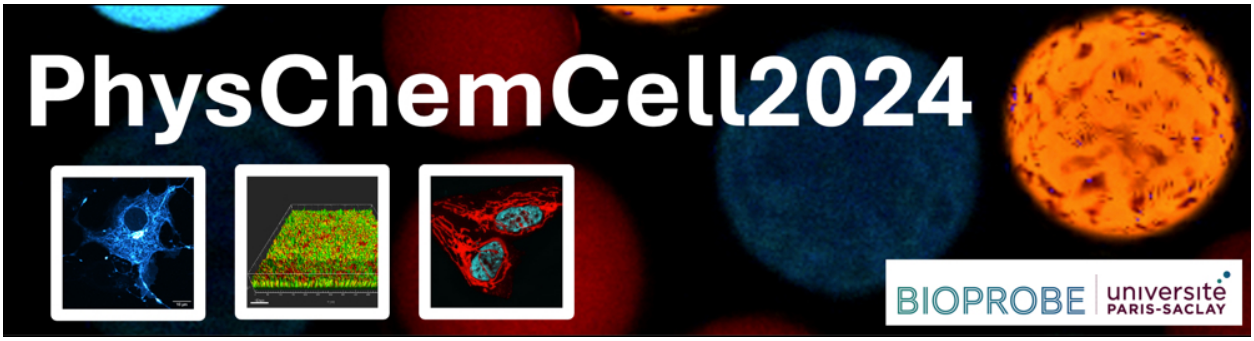
16:00-16:20 Coffee Break

16:20-17:20 SOLEIL is the French synchrotron, both a large-scale facility and a research laboratory
Frederic Jamme, Christophe Chevalier and Pierre Montaville, Soleil

17:20-17:40 Single cell analysis using synchrotron radiation µFTIR spectroscopy.
Christophe Sandt, Soleil

17:40-18:00 Noninvasive evaluation of the skin barrier in reconstructed human epidermis: impact of blue light exposure/
Lea Habib, Lip(Sys)^2

18:00-20:00 Cocktail and Poster Session



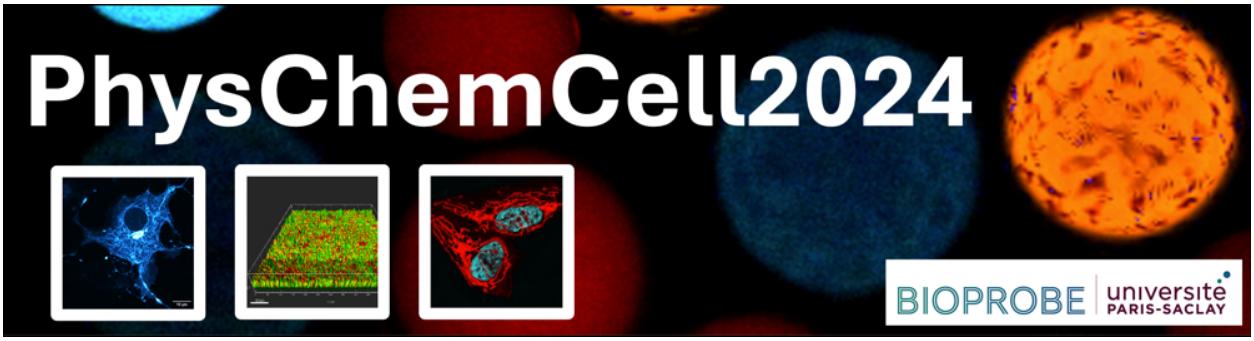
Wednesday, October 16

Session 4 : Bridging the gap from nanoscale to in vivo imaging

Chairs : **Sandrine Lévêque-Fort** (ISMO) and **Larbi Amazit** (IBVB)

Hervé Daniel Auditorium – Henri Moissan (Bat 670)

9:00-10:00	Red photocontrollable fluorescent proteins for live-cell nanoscopy **Francesca Pennacchietti**, KTH Royal Institute of Technology, Sweden
10:00-10:20	Measuring axonal transport in models of Alzheimer's disease using neurotropic fluorescent nanodiamonds **Baptiste Grimaud**, LuMIn & LAMBE
10:20-10:40	Agile two-photon microscope for fast 3D single-particle translation and rotation tracking **François Marquier**, LuMIn
10:40-11:00	Coffee Break
11:00-11:20	Flux : a new contrast for simultaneous multi-targets imaging in 3D single molecule localization microscopy **Laurent Le**, ISMO
11:20-11:40	Visualizing the self-assembly mechanism of viruses through fluorescence microscopy and interferometric scattering at the single molecule level **Karen Perronet**, LuMIn
11:40-12:00	Defining and enhancing single molecule regime with dynamic excitation control **Lancelot Pincet**, ISMO
12:00-12:20	Periodic light modulations for low cost wide-field imaging of luminescence kinetics. **Ian Coghill**, ENS
12:20	Pick up lunch



Keynote Speakers

Translating fluorescent probes for imaging immune cell function in humans

Marc Vendrell[1]

[1] Centre for Inflammation Research and IRR Chemistry Hub, The University of Edinburgh, Edinburgh, UK.

Fluorescent activatable probes are valuable tools for live-cell imaging because of their tunability and target specificity.[1] Our group has designed fluorogenic amino acids and peptides for high-resolution biological imaging and translational medicine. Our team have demonstrated that this approach can generate probes to visualize infectious pathogens (e.g., fungal pathogens in *ex vivo* human lung tissue[2]) and subsets of immune cells in live cells and *in vivo*[3] and in *ex vivo* human biopsies.[4] We have designed our fluorescent amino acids to: 1) be compatible with conventional solid-phase peptide synthesis, 2) maintain the biomolecular recognition features of the native peptides and 3) emit fluorescence preferentially after target binding, improving signal-to-noise ratios for imaging. Furthermore, we have reported fluorogenic analogues with emission >600 nm to prepare of cyclic peptides for imaging tumor cells using multiphoton imaging *in vivo*.[5] Recently, we have extended the toolbox with smaller amino acids, which include the first phenylalanine-based fluorogenic building blocks for detection of urinary tract *Candida* infections,[6] the smallest turn-on fluorescent amino acids for peptide-PAINT imaging and super-resolution microscopy,[7] and to fluorogenic tags for small proteins associated with immune cell function like interleukins and cyclophillins.[8,9]

[1] Nat. Rev. Chem. **2020**, 4, 275.
[2] Nat. Commun. **2016**, 7, 10940.
[3] Nat. Commun. **2020**, 11, 4027.
[4] Nat. Commun. **2022**, 13, 2366.
[5] a) Chem. Sci. **2020**, 11, 1368; b) Angew. Chem. Int. Ed. **2022**, 61, e20211302.
[6] Angew. Chem. Int. Ed. **2022**, 61, e202117218.
[7] Angew. Chem. Int. Ed. **2023**, 62, e202216231.
[8] ACS Cent. Sci. **2024**, 10, 143.
[8] ACS Cent. Sci. **2024**, 10, 969.

Protein binding kinetics in immune-cell contacts and its influence on T-cell activation

Peter Jönsson *

[1] Division of Physical Chemistry, Department of Chemistry, Lund University, Lund, Sweden

Binding between ligands and receptors across contacting cells is vital for several biological processes. This includes activation of T cells, where T-cell receptors (TCRs) bind to antigen presented by MHC complexes (pMHC). How strong this interaction needs to be to start an immune response is, however, unclear. I will here highlight some of our recent results on the affinity and lifetime between TCRs and pMHC molecules displaying different peptides, from cognate to self, and new methods devised by us to quantify these parameters. For this purpose, we use a combination of single molecule and total internal reflection fluorescence microscopy to study the distribution and movement of differently labelled protein molecules in the contact between a living cell and a cell membrane mimic called a supported lipid bilayer. I will also discuss how the weak interaction between the co-receptor CD4 and pMHC can influence T-cell signaling and how the binding can be influenced by auxiliary binding molecules in the cell contact. This demonstrates that it is not only the protein-protein interaction per see that determines the binding magnitude in cell contacts and thereby if a T cell should be activated or not.

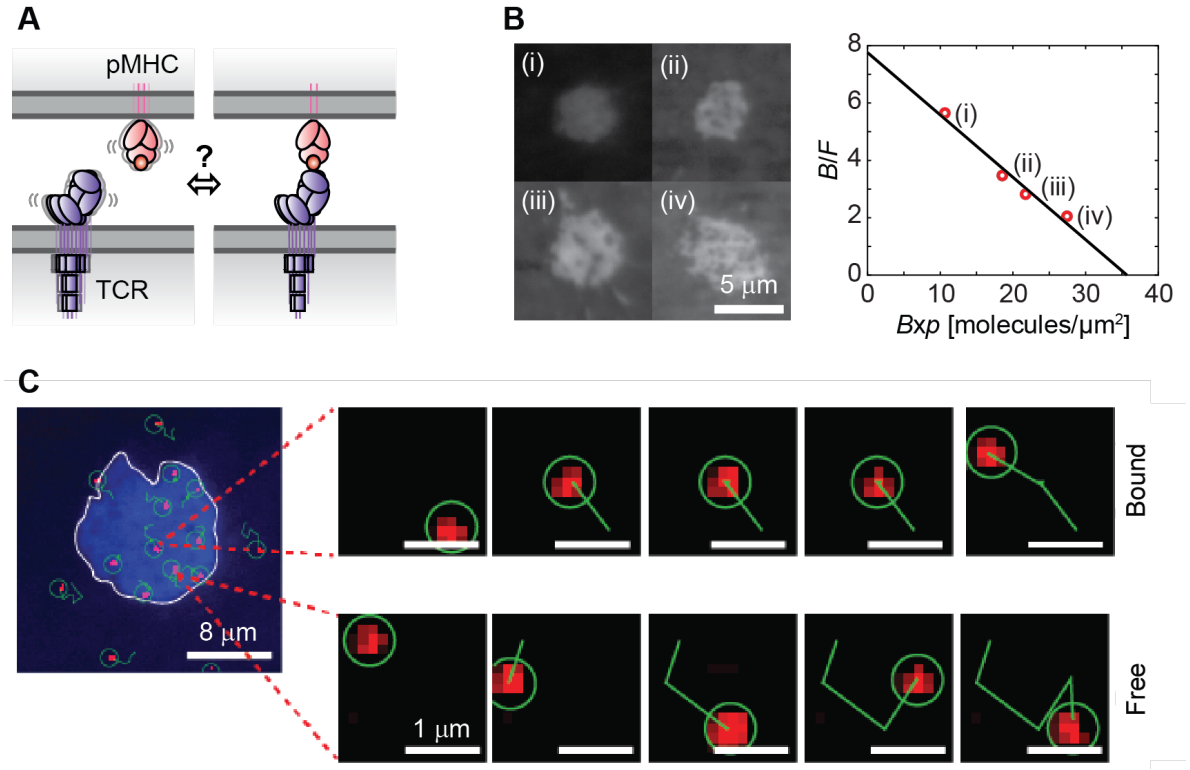


Figure 1. (A) Binding between TCR and pMHC is the first step in T-cell activation, however, how strong this interaction needs to be to start an immune response is unclear. **(B-C)** We study this using a combination of total internal reflection fluorescence microscopy and single molecule imaging. Image in B is reproduced from Dam et al. Front. Physiol. 11, 613367 (2021).

Improving cancer surgery: need for objective photonic techniques

Senada Koljenovic[1]

[1] UZA Antwerp, Belgium

Patients with solid cancer are often treated with surgery. The goal is to remove the tumor with a margin of surrounding healthy tissue. Unfortunately, this is often not achieved. For example, in oral cancer surgery adequate margin rate is only about 15% to 26%.

Intraoperative assessment of tumor resection margins can dramatically improve surgical results. It enables the surgical-pathological team to directly perform additional tissue resection, if necessary, to achieve a so-called “first time right results”. However, current methods are laborious, subjective, and logistically demanding. This hinders broad adoption of Intraoperative assessment of tumor resection margins, to the detriment of patients. Inadequate resection margins result in the need for a 2nd, sometimes 3rd operation, combined or not with chemotherapy or radiotherapy.

Therefore, an objective easy-to-use technique is needed, to accurately assess all resection margins intraoperatively. The challenges in pathology and the opportunities of photonic techniques in general will be discussed. The development of a high-wavenumber Raman spectroscopic technology, for quick and objective intraoperative measurement of resection margins will be presented.

Red photocontrollable fluorescent proteins for live-cell nanoscopy

Francesca Pennacchietti[1]

[1] KTH Royal Institute of Technology; francesca.pennacchietti@scilifelab.se

Cells orchestrate their biological function through basic mechanisms, such as diffusion-mediated compartmentalization and coordination macromolecular interaction. Observing their interplay directly inside living cells and tissues provide the maximum level of information but requires minimally invasive imaging strategies. In this context, photocontrollable fluorescent proteins (FPs) play a crucial role as tags in optical super-resolution microscopy and functional live-cell imaging. They exhibit a range of switching behaviours compatible with various super-resolution techniques and require only modest light doses to activate the optical switch.

To this end we have previously shown that reversibly switchable FPs enable fast (1 Hz for a 50 x 50 μm^2) and gentler (< 1 kW/cm^2 illuminations) nanoscopy [1]. Additionally, irreversibly photoconvertible FPs can achieve photolabeling with high spatiotemporal precision. Nevertheless, their photophysical complexity poses challenges in expanding such techniques toward multiplexing and in vivo imaging. Here, we explore novel photoswitching mechanisms for fluorescent proteins in the red and near-infrared region of the spectra and assess their compatibility with live cell imaging at the nanoscale [2]. Finally, we present strategies to combine the spectral and photophysical fingerprint of distinct photocontrollable FPs to achieve multiplexing in live cell imaging at the nanoscale and photolabeling studies [3].

[1] L. A. Masullo et al., Enhanced photon collection enables four-dimensional fluorescence nanoscopy of living systems *Nature Communications,* **2018**, 9 (1), 3281, https://doi.org/10.1038/s41467-018-05799-w.

[2] F. Pennacchietti et al. Fast reversibly photoswitching red fluorescent proteins for live-cell RESOLFT nanoscopy *Nature Methods,* **2018,** 15 (8), 601-604, https://doi.org/10.1038/s41592-018-0052-9.

[3] F. Pennacchietti Blue-shift photoconversion of near-infrared fluorescent proteins for labeling and tracking in living cells and organisms, *Nature Communications* **2023**, 14:8402, https://doi.org/10.1038/s41467-023-44054-9.

SOLEIL is the French synchrotron, both a large-scale facility and a research laboratory

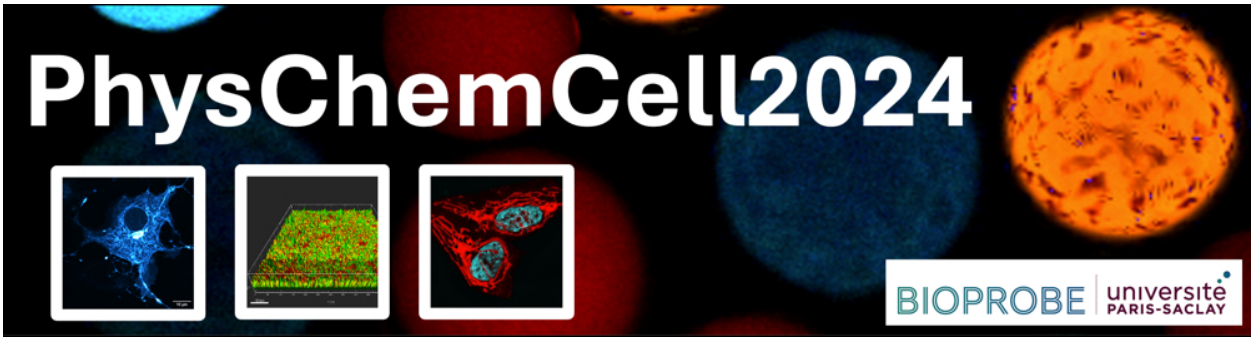
Frederic Jamme[1], Pierre Montaville[1] and Christophe Chevalier[2]

[1] Synchrotron SOLEIL, L'Orme des Merisiers, 91190 Saint-Aubin, France
[2] INRAE, VIM, Équipe Influenza, 78350 Jouy-en-Josas, France

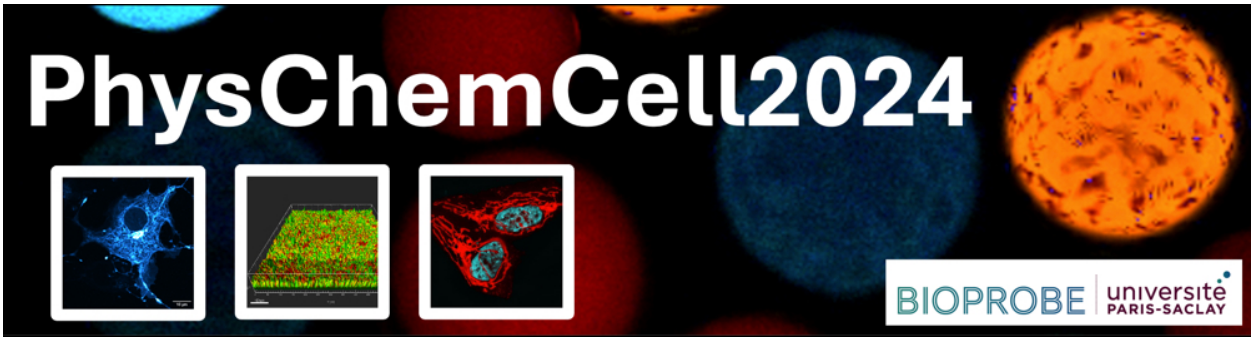
The SOLEIL synchrotron is both a service platform open to all scientific communities and a research laboratory. Synchrotron radiation enables measurements across a wide variety of length and time scales whilst exploiting its wide photon energy range, from hard X-ray to infrared, to provide chemical and morphological characterization with high selectivity. Life science research at synchrotron radiation sources has been remarkably productive since their inception over 40 years ago. In particular, X-ray crystallography beamlines have had a major impact for structural biology. Recent revolutions in both single-particle cryo-electron microscopy (cryo EM) and prediction of protein folds have changed the way we look at integrative approaches. The integration of cryo EM with synchrotron-based techniques will greatly facilitate the correlation of molecular and structural information from the atomic to cell levels. The combined expertise of the life science beamline teams at SOLEIL is the basis for the development of a post-upgrade approach to multimodal and integrative biology. Today, new synchrotron based bioimaging techniques, enlarge the scope of cell and tissue imaging with a huge potential for biological research and medical diagnosis. The ongoing upgrade of the SOLEIL synchrotron will contribute to the emergence of methods aimed to improve the imaging capabilities of cells, tissues, and organs at high spatial and temporal resolution. The Health and Well-Being Scientific Section at SOLEIL is an active group consisting of 30 experts in various fields.

A model system studied at SOLEIL as an in-house project allows us to highlight the contribution of synchrotron radiation for studying interfaces in biology. XPA is a naturally fluorescent protein that spontaneously crystallizes in-vivo. The understanding of the transition of XPA from the intracellular liquid dilute phase to the crystalline solid dense phase required to combine live fluorescence imaging and cryo soft X-ray microscopy and X-ray diffraction analysis. This multiscale approach revealed that XPA forms liquid condensates that are closely associated to biological membranes of intracytoplasmic organelles. It also provided a description of the dynamics of these XPA liquid condensates, a spatial description at the cellular and meso scale of the phase transition from the liquid to the crystalline state in the cellular environment, and the autophagic cellular response to the liquid liquid phase separation of XPA.

Protein crystallization processes are also associated with some kinds of virus-host interactions, such as it happens with the crystallization of NS1 protein of the influenza A virus during host infection. NS1 is involved in the pathogenicity of influenza A viruses (IAVs), amongst the most dangerous viruses for both human and animal health. Many types of structures have been described in IAV-infected cells, avian viruses led to a particular signature in IAV-infected (human and avian) cells characterized by numerous NS1 associated polygonal-shaped inclusions (size 1 to 3 microns) within the cytoplasm of the majority of the infected cells since the early stage of infection. Our preliminary results obtained with electron microscopy on ultrathin sections revealed crystalline organization of the NS1 inclusions It is worth investigating how the formation of these crystalline structures occurs, from the mechanisms initiating the LLPS process to the partners and/or components necessary for their formation, and what is the impact on the surrounding cell environment. These relevant questions will require the use of live imaging and cryo-imaging techniques to describe the dynamics, morphological and chemical changes linked to the NS1 protein and to the surrounding cell environment.



Oral Communications



Session 1

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New chemical probes, labeling and activation strategies for a living environment

An azo-based fluorogenic smart probe to visualize a mitochondrial azoreductase activity in live cells

MICHEL L.,[1] AUVRAY M.,[2] BADET-DENISOT M.-A. ,[1] DURAND P.,[1] MAHUTEAU-BETZER F., [2] CHEVALIER A[1]

[1] *Institut de Chimie des Substances Naturelles, CNRS UPR2301, 1 Avenue de la Terrasse, 91198 Gif sur Yvette, France*
[2] *Institut Curie, CNRS UMR9187 - Inserm U1196, Bâtiment 110 Rue Henri Becquerel, 91401 Orsay, France*

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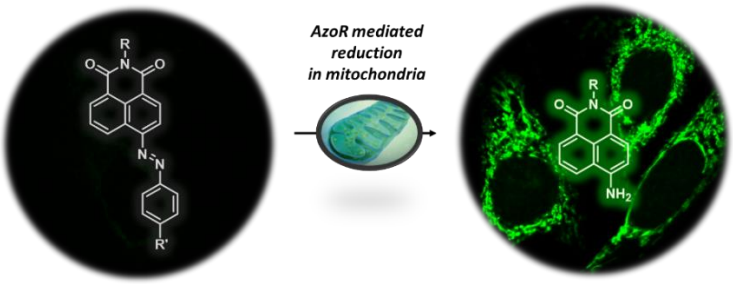
Mitochondria is the center of energy metabolism in the cell. Dysfunctions of this organelle have been related to many diseases, such as cancer, diabetes, cardiovascular or neurodegenerative diseases, and more.[1] In this context, the study of biological phenomena at the intramitochondrial level is particularly relevant. Fluorescence imaging, and more specifically fluorogenic smart probes, are potent tools to observe chemical transformations at the subcellular level. This approach has allowed the identification of an intramitochondrial nitroreductase activity.[2] Recently, this enzymatic activity has been exploited for the activation of prodrugs selectively inside mitochondria.[3]

Here, we describe our works on the design and utilization of fluorogenic probes thought for the observation of an intramitochondrial azoreductase activity in live cells. We have designed and synthesized azo-based probes derivated from 4-amino-1,8-Naphthalimides fluorophores. The particularity of these probes lies in their non-emissive structure, making them interesting OFF-ON probes. Their azo N=N double bond can be reduced by an AzoR activity, restoring a brightly emitting naphtalimide fluorophore. These sensors have been studied in vitro with multiple enzymes and were found to be stable under biological conditions, as well as highly sensitive and selective to AzoR. Confocal microscopy experiments conducted on different living cell lines showed the presence of a mitochondrial AzoR, 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 alternative to NTR for specific drug delivery into mitochondria.



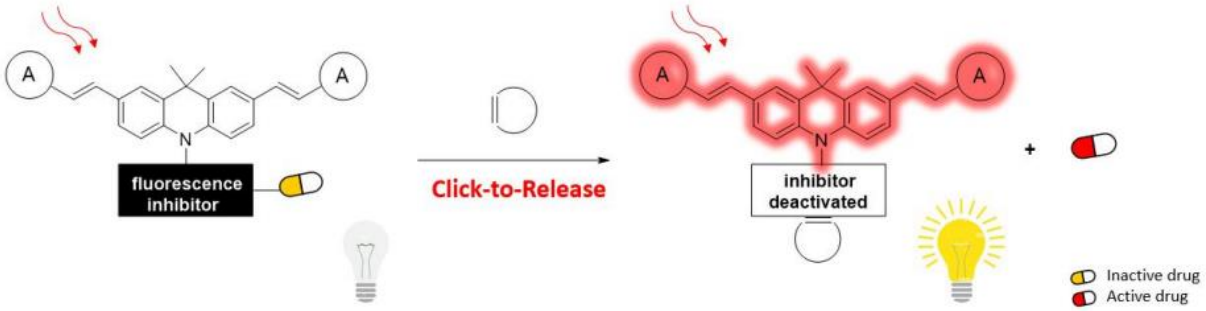
[1] Murphy, M. P.; Hartley, R. C., Nat. Rev. Drug Discov. 2018, 17, 865.
[2] (a) Chevalier, A.; Khdour, O.; Zhang, Y.; Hecht, S. Prodrug and profluorescent compounds for selective mitochondrial imaging and therapeutic targeting. WO2017218537A1, 2017; (b) Chevalier, A.; Zhang, Y.; Khdour, O. M.; Kaye, J. B.; Hecht, S. M., J. Am. Chem. Soc. 2016, 138 (37), 12009-12012.
[3] Rivera-Fuentes, P.; Tirla A.; Nguyen J., Org. Biomol. Chem., 2021, 19, 2681-2687

Two-photon excitable fluorogenic probes for Click-to-Release bioorthogonal reactions

A.Rodriguez, D. Naud-Martin, G. Fontaine, K. Renault and F. Mahuteau-Betzer*

CMBC, UMR9187-UI1196, Institut Curie, rue Henri Becquerel, 91401 ORSAY

Fluorogenic bioorthogonal reactions are interesting tools for tracking small molecules or biomolecules in living organisms.[1] In this context, our team developed two-photon excitable fluorogenic probes activatable by bioorthogonal reaction.[2] Two-photon excitation, by shifting absorption towards the red, significantly increases the signal-to-noise ratio and decreases photodamage, while allowing imaging about 10 times deeper than conventional fluorescence imagery. Indeed, two-photon excitation probes are particularly powerful to imaging precisely in complex biological media.



In this work, we will present our ongoing research concerning the development of new two-photon excitable fluorogenic probes. The design of these probes is inspired by probes developed within the team, which have shown satisfactory spectral properties and a high turn-on. 2 These probes are therefore very interesting for designing new theranostic tools through Click-to-Release reactions.[3] Click-to-Release reactions will allow both the release of the drug and the turn-on of the fluorescence allowing the localisation of the released drug.

[1] Shieh, P.; Bertozzi, C.R. Design Strategies for Bioorthogonal Smart Probes. *Org. Biomol. Chem.* **2014**, 12(46), 9307-9320. DOI: 10.1039/c4ob01632g.

[2] Auvray, M.; Naud-Martin, D.; Fontaine, G.; Bolze, F.; Clavier, G.; Mahuteau-Betzer, F. Ultrabright two-photon excitable red-emissive fluorogenic probes for fast and wash-free bioorthogonal labelling in live cells. *Chem. Sci.* **2023.** DOI: 10.1039/d3sc01754k

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Decoding day-long maturation of the PCFPs of the EosFP family and engineering fast-maturing variants

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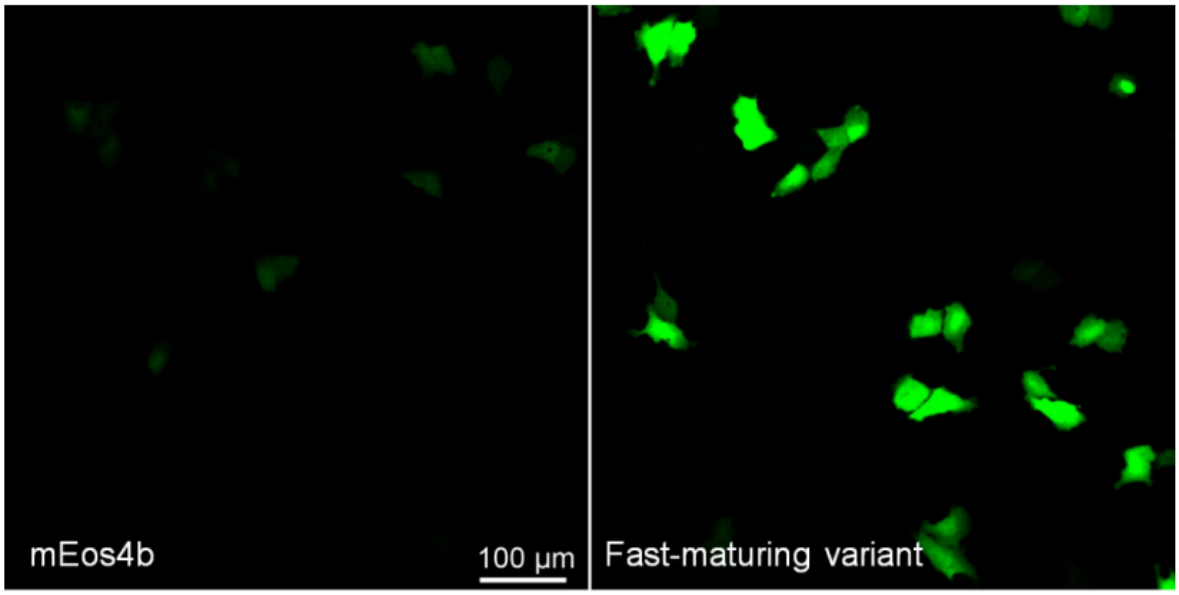
Green-to-red photoconvertible fluorescent proteins (PCFPs) are widely used in super-resolution techniques such as photoactivated localization microscopy (PALM) or minimal emission fluxes (MINFLUX). Our recent NMR study of a PCFP of the EosFP family revealed extremely slow chromophore maturation, which can impact cellular brightness and labeling efficiency, especially in live cells and when short-lived proteins of interest are labeled. Despite reports of faster maturing fluorescent protein variants, the underlying mechanisms remain poorly understood, and various measurement techniques complicate comparisons. We developed a method to accurately measure fluorescent proteins maturation rates and we systematically compared multiple PCFPs of the Eos family. To our surprise, we found that many of them showed apparent maturation time of more than a day. Using X-ray crystallography, dynamics simulations, and targeted mutations, we engineered faster-maturing PCFPs without compromising other photophysical properties such as photostability or brightness. These engineered variants demonstrated improved imaging performance in both prokaryotic and eukaryotic cells.



U2OS cells, 13 hours post-transfection

A tunable and versatile chemogenetic near infrared fluorescent reporter

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Recent developments in protein engineering allowed the generation of near-infrared emitting fluorescent proteins (NIR-FP), which open interesting prospects of multiplexing and deep-tissue imaging. Such proteins fluoresce upon the covalent binding of biliverdin, and thus their use is limited to contexts where this ligand is readily available. Here we present the engineering of a near-infrared fluorescent chemogenetic reporter named nirFAST (near-infrared fluorescence-activating and absorption shifting tag), a small protein tag of 14 kDa able to bind and stabilize the fluorescent state of HPAR-3,5DOM fluorogenic chromophore, which is otherwise dark when free in cells [1]. nirFAST was engineered by red-shifting the absorption and emission properties of frFAST [2], a far-red emitting chemogenetic reporter. Molecular engineering of the fluorogenic chromophore (fluorogen) combined with directed evolution allowed the development of nirFAST, a variant forming a tighter and brighter assembly with the new fluorogen with 636/715 nm absorption/emission peaks. nirFAST was successfully shown to fluoresce in mammalian cells, with higher cellular brightness compared to emiRFP670 and miRFP713, two NIR-FP with comparable absorption and emission properties to nirFAST. nirFAST also bears the advantage of being smaller in size than NIR□FP, and thus having a reduced genetic footprint. Additionally, nirFAST was successfully used in more complex organisms such as chicken embryo tissues and zebrafish larvae. We showed that nirFAST is orthogonal to pFAST, a promiscuous chemogenetic tag able to bind and activate the green fluorescence of HMBR [3], allowing two color imaging in different biological contexts, as well as the generation of fluorescent cell-cycle indicator. Finally, splitting nirFAST into two fragments allowed the generation of a near-infrared emitting protein-proximity inducing tool. The promising spectral properties and initial characterization of nirFAST in multicellular organisms allow us to envision its use not only as a fluorescent reporter of cellular activity at the level of single cells and in more complex organisms, but also in its split form as a tool to control and visualize protein proximity in cells.

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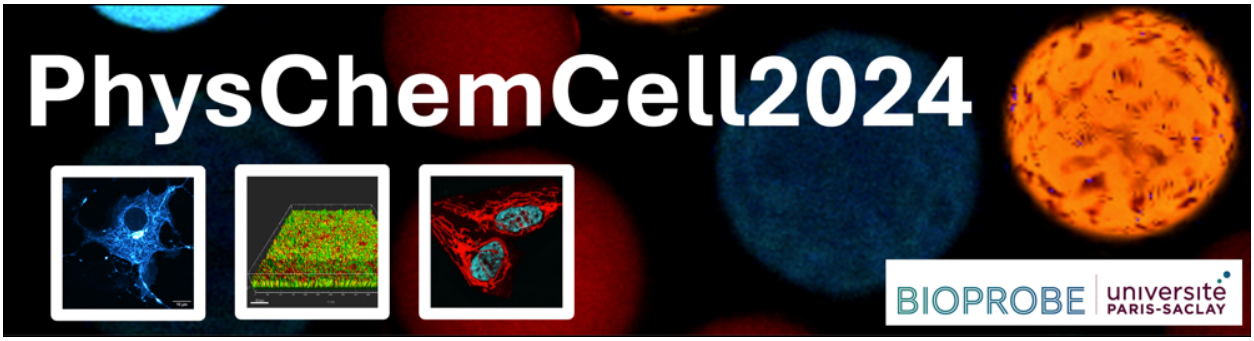

Session 2

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Biosensors and Nanoprobes from design to application in imaging

Bioengineered condensates to interact and manipulate membranebound organelles in cells

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The dynamic interactions between the location and timing of biochemical processes within organelles is crucial for determining cell fate and function. Advanced biochemical, genetic, imaging, and "omics" approaches have revealed many important features of the biogenesis and functions of cellular organelles. In particular, recent studies suggest that communication between membrane organelles, through the establishment of membrane contact sites (MCS), could play crucial roles for their functions[1]. However much less is known about putative contact sites between membrane-bound and membrane-less organelles (also known as biomolecular condensates)[2]. One key limitation comes from the biochemical complexity of condensates that make them difficult to study and manipulate in a cell context.

To further advance in that direction, we are developing a novel methodology allowing the controlled assembly/disassembly in cells of bioengineered membrane-less organelles and that recapitulate some biophysical features of liquid-like condensates[3]. Our engineered condensates are based on protein scaffolds designed to undergo phase separation in cells in a reversible manner[3,4]. We demonstrated that it is possible to specifically target the assembly of engineered condensates on the surface of lysosomes, making them suitable for addressing fundamental questions about the interplay between condensates and lysosome interactions: How condensate nucleation and growth are modulated when interacting with the lysosomes? Conversely, how lysosome spatiotemporal dynamics is impacted when interacting with condensates? This method is versatile and could, in principle, be applied for studing other cellular organelles.

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In vitro evaluation of a strategy of treatment associating Platinum nanoparticles and FLASH radiotherapy

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Recent advancements in radiotherapy have focused on enhancing local tumor control while minimizing side effects. Two promising approaches have emerged: (i) the use of metallic nanoparticles (NPs) that selectively accumulate in tumors to increase localized dose deposition, and (ii) the FLASH effect, characterized by ultra-high dose rates (UHDR > 100 Gy/s) that spare normal tissues while maintaining tumor control. This study investigates the *in vitro* potential of a combined strategy utilizing both modalities. We developed and characterized reproducible 4T1 cell spheroids (700 µm diameter), assessing tumor heterogeneity through the quantification of necrotic/apoptotic cells and hypoxia via flow cytometry. Spheroids were incubated with platinum nanoparticles (Pt-NPs) at a concentration of 0.5 mmol/L for 14 hours. Pt-NP penetration within the 3D models was visualized using confocal microscopy and quantified through ICP-MS analysis.

Both Pt-NP-treated and untreated spheroids were irradiated in 0.2 ml Eppendorf tubes submerged in a water-filled plexiglass phantom. Radiation doses ranging from 0 to 12 Gy were administered using: (i) a conventional electron beam (9 MeV, 600 UM/min, Clinac 2300C/D, Varian) and (ii) a UHDR electron beam (10 MeV, mean dose rate: 200 Gy/s, instantaneous dose rate: $1x10^6$ Gy/s, FLASHKNiFE, THERYQ). Dosimetric verification was performed with EBT3 films. Post-irradiation, spheroids were disaggregated, and clonogenic assays were conducted to determine survival fractions (SF) and quantify the NPs' radio-enhancement effects. Results indicated that Pt-NPs effectively penetrated the spheroids, as confirmed by confocal imaging. The Pt-NPs significantly enhanced the radiation effects, as described by the linear quadratic model. Notably, at 2 Gy, a radio-enhancement effect of 13.2% and 12.4% was observed for conventional and UHDR irradiations, respectively. The enhancement effect of Pt-NPs was preserved under UHDR conditions. Ongoing analyses, including flow cytometry and gammaH2AX foci formation, aim to further characterize cell death mechanisms under both irradiation modalities with or without NPs.

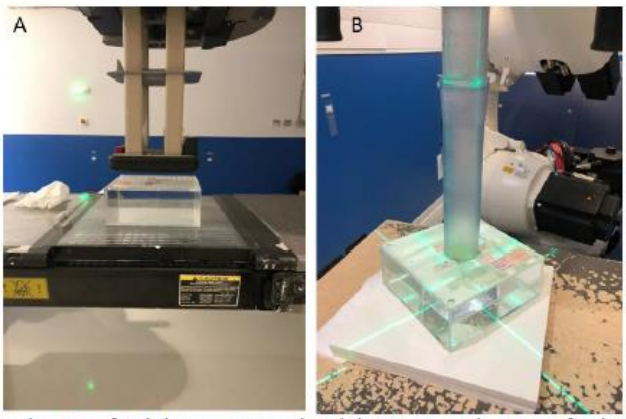

Figure 1. Experimental setups for (A) conventional or (B) UHDR irradiations of spheroids placed in 0.2 ml Eppendorf tubes.

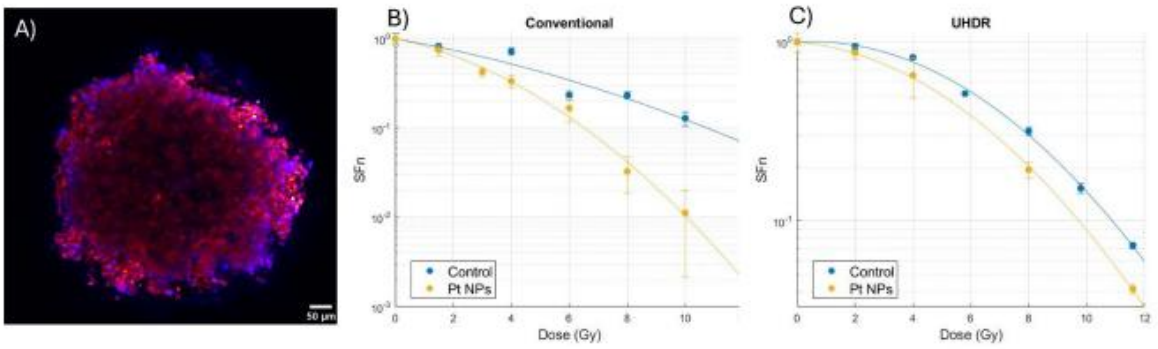

Figure 2. (A) Confocal microscopy of 4T1 spheroid exposed to Pt-NPs. Nuclei and NPs are labeled in blue and red respectively. Survival fractions of 4T1 spheroids with and without Pt NPs when irradiated in (B) conventional dose rate or (C) UHDR

Quantum Dot-Based FRET Nanosensors for Talin-Membrane Assembly and Mechanosensing

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Understanding the mechanisms of assembly and disassembly of macromolecular structures in cells relies on solving biomolecular interactions. However, those interactions often remain unclear because tools to track molecular dynamics are not sufficiently resolved in time or space. In this study, we present a straightforward method for resolving inter- and intra-molecular interactions in cell adhesive machinery, using quantum dot (QD) based Förster resonance energy transfer (FRET) nanosensors [1]. Using a mechanosensitive protein, talin, one of the major components of focal adhesions, we are investigating the mechanosensing ability of proteins to sense and respond to mechanical stimuli. First, we quantified the distances separating talin and a giant unilamellar vesicle (GUV) membrane for three talin variants. These variants differ in molecular length. Second, we investigated the mechanosensing capabilities of talin, i.e., its conformational changes due to mechanical stretching initiated by cytoskeleton contraction. Our results suggest that in early focal adhesion, talin undergoes stretching, corresponding to a decrease in the talin-membrane distance of 2.5 nm. We demonstrate that QD-FRET nanosensors can be applied for the sensitive quantification of mechanosensing with a sub-nanometer accuracy.

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Optical Biosensors for the Detection of Bacteria

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Throughout human history, bacterial infections have posed significant challenges to public health.[1] With the discovery of antimicrobial agents and antibiotics to combat bacterial infections came the ability of bacteria to become resistant to these drugs due to genetic mutations and overuse.[1,2] As current antimicrobial medicines are becoming ineffective, there is an urgent need for new medicines and an urgent push to improve early detection of bacterial infections. Currently, effective bacterial detection methods require the ability to identify pathogenic bacteria with sensitivity and specificity but such methods can be expensive and time consuming. [3,4] To overcome the current limitations of existing detection techniques, biosensors utilizing nanoparticles (NPs) and fluorescence have emerged as alternatives by offering detection in faster and more efficient ways. In the present work, we developed two optical biosensors (in suspension and on a surface) using fluorescent organic NPs as the sensing element, a pH sensor, and fluorescence as the reader device. Fluorescent organic NPs allow for great photostability, easy cellular uptake, and tunable within the visible range making them valuable for biosensing applications.[5] In suspension, we observed that the photophysical properties of the individual compounds in varying pH buffers remained consistent when they become bound together in solution by copper-free SPAAC click chemistry, with the NPs unresponsive to the pH change while the sensor displayed increased fluorescence intensity in acidic media. Preliminary bacteria sensing test showed that the fluorescence intensity of the combined solution (NPs + pH sensor) increased over time, correlating with the acid production by the tested bacteria (which lowered the media's pH). When immobilized onto a glass surface, the photophysical properties and pH sensing capabilities were retained. By plotting the intensity ratio of the pH sensor over the intensity of the NPs, a pKa of the luminescent surface was determined to be 5.7 $\pm$ 0.15, which is within the physiological range for bacteria detection. Further tests to stabilize the pH sensor on the surface are needed before beginning bacteria growth with the surface. In conclusion, there is potential in our optical biosensors for detecting bacteria by sensing pH change by fluorescence detection.

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CAP-PHOTOAC: Control of the optical absorption properties of nanovectors for photoacoustic imaging

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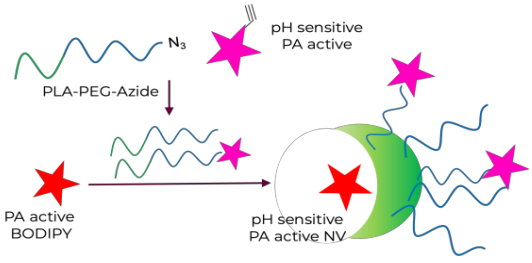
Optically absorbing structures can generate sound waves via the photoacoustic (PA) effect, which enables high-resolution imaging of molecules or nanoparticles in biological tissues. BODIPY dyes are commonly used as molecular PA agents due to their high absorption coefficient, tunable absorption maxima, and photostability. Past work from our research group[1] has produced a novel BODIPY-based chromophore that, when conjugated to a lactide chain and grafted onto a PEGylated nanoparticle (NP), shows remarkable absorption properties and biocompatibility, making it an excellent platform for PA imaging and drug delivery.

In this study, we aim to develop a stimulus-responsive PA agent by synthetizing and characterizing a pH-sensitive molecule using the BODIPY scaffold, which is a dye structure already used in the creation of pH-sensitive fluorophores[2]. Our work produced two BODIPY molecules of novel synthesis, equipped with a clickable handle and a 3-nitro-4-phenol group. Its phenolphenolate interconversion provides the capacity of responding to pH changes in the cellular environment[3]. The pH-sensitive BODIPYs are going to be conjugated to PLA-PEG and formulated into pH sensitive PLA-PEG nanoparticles. Eventually, the most promising pH sensitive molecule could be added to a previously developed design for BODIPY-labeled PLA-PEG NPs to create multifunctional NPs that provide control over the optical absorption without adding toxicity. These nanoparticles will be biocompatible and pH-sensitive PA agents that we intend to use to evaluate their intratissue distribution and intracellular uptake kinetics in a murine model of rheumatoid arthritis. Overall, this work aims to showcase the potential of these NVs as a clinically relevant, biocompatible, and photostable platform for personalized medicine and nanomedicine, enabling real-time visualization of drug distribution and patient response.



Acknowledgements

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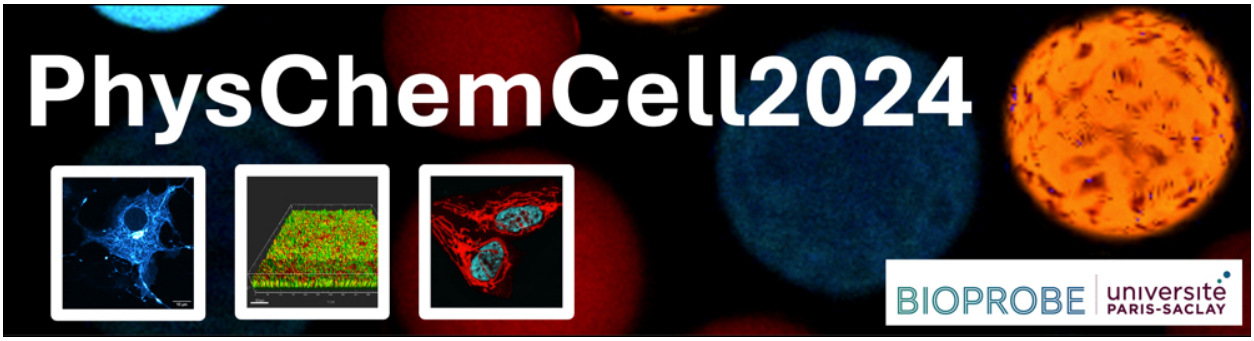

Session 3

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Label-free chemical analysis and imaging

Multicontrast three-photon microscopy for tissue applications

Stella Dees[1], Payvand Arjmand[1], Pierre Mahou[1], Júlia Ferrer Ortas[1], Frédéric Druon[2], Chiara Stringari[1], Nicolas Olivier[1], Willy Supatto[1], Emmanuel Beaurepaire[1*]

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Multiphoton microscopy is widely used for high-resolution 3D imaging of intact biological tissues. Recent work has shown that 3-photon (3P) excitation offers a significant advantage over 2-photon excitation for in-depth imaging of scattering specimens. The optimal excitation wavelengths for in-depth 3P microscopy are 1300 nm and 1700 nm, where scattering and absorption are minimal. The implementation of 3P imaging therefore requires efficient femtosecond lasers operating at these wavelengths with repetition rates in the MHz range [1]. In addition to 3P-excited fluorescence, such laser sources also allow efficient imaging with complementary contrasts such third-harmonic generation (THG) and third-order sum frequency generation (TSFG) [2]. All these processes can be obtained simultaneously by spatially and temporally synchronizing pulses at different wavelengths. We have implemented a platform for dual-beam 3P imaging, and have shown that TSFG with femtosecond pulses can detect the presence of absorbers due to 3-photon resonance. In particular, red blood cells can be detected in a label-free manner due to the Soret absorption band of hemoglobin at 400-440 nm [2]. We are currently investigating the potential of TSFG for tissue imaging.

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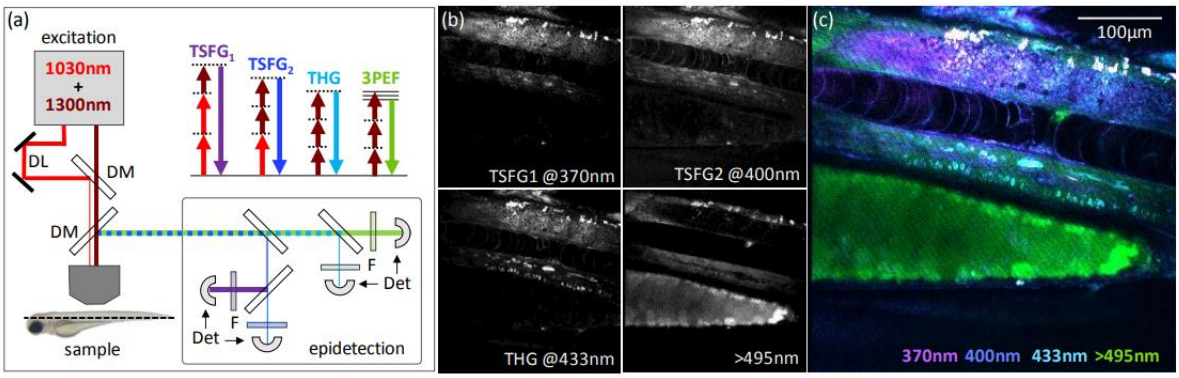

Figure: Multicontrast 3-photon microscopy. (a) Experimental setup for 3P-THG-TSFG imaging. (b, c) Label-free color TSFG imaging of a live 2 dpf zebrafish embryo obtained by synchronizing 1030nm and 1300nm pulses.

Using Infrared Nanospectroscopy (AFM-IR) to study protein assemblies: the case of Prion assemblies dynamic

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Infrared spectroscopy is a powerful tool to study the secondary structure of protein assemblies. However, due to the diffraction limit, classic IR microscopy spatial resolution is limited to few microns and cannot be used to analyze unique nanoscale objects such as protein assemblies. This physical limitation can be circumvented by using an IR nanospectroscopy technique called AFM-IR. This well-established technique combines an AFM and a tunable IR laser source to perform sub-micrometric IR analysis [1]. In this present work we used AFM-IR to explore the structural heterogeneity of infectious synthetic prion assemblies and their intrinsic dynamicity.

The prion pathology is based on autonomous structural information propagation towards single or multiple protein conformational changes. Since this last decade, the prion concept referring to the transmission of structural information has been extended to several regulation systems and pathologies including Alzheimer and Parkinson's diseases. The unified theory in Prion replication implies structural information transference from the prion to a non-prion conformer through a mechanism also called improperly, with regards to biophysical considerations, "seeding" phenomenon. Recently, MAP2 team reported that prion replication is intrinsically source of structural diversification [2], [3]. The coexistence of multiple prion assemblies with different structural and replication propensity questions i) how prion assemblies self-organized, ii) how this diversity is maintained within the same media and iii) how different PrP population escape to best replicator selection process during prion replication. Together with classic liquid AFM, it is possible to use AFM-IR to answer some of these points as it highlights, at the single assembly's scale, an intra-assembly's heterogeneity. This revealed that prion assemblies, rather than a canonical amyloid assembly, constitute a complex dynamic system far from the equilibrium where at least two different subpopulations coexist through catalytical material exchange.

While this work demonstrates the analytical capabilities of AFM-IR to study unique nanoscale protein assemblies, it also highlights it principal limitation which is the requirement to work in dry air, far from the relevant physico-chemical conditions. As a perspective, we will present a new project called NanEAU-IR which try to address this issue by developing AFM-IR analysis in water environment.

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Development of a handheld multispectral imager prototype for drug analysis.

Erwin Winkler Martinez[1], Ali Tfayli[1], Thomas Nappez[2], Jean-Philippe Michel[3], Alexandre Michelet[4], Douglas N. Rutledge[1], Pierre Chaminade[1], Sana Tfaili[1*]

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The prevalence of substandard and falsified (SF) drugs requires the development of effective identification and differentiation methods to distinguish genuine from SF medications. SF drugs have harmful consequences, including increased morbidity, and mortality. Near-Infrared (NIR) spectroscopy provides a rapid, specific, and non-destructive method for the identification of SF. This well-established technique has demonstrated its effectiveness in detecting poor-quality medicines. NIR spectroscopy can be coupled with methods like; hyperspectral imaging, multispectral imaging, or point spectra collection. These methods alongside advanced data treatment and chemometrics, have proven to be essential in drug analysis. The objective of this project was to develop a low-cost, portable handheld device, named SnapCup. The use of low‐cost handheld spectrophotometers in low-income countries offers a cost-effective and quicker alternative to traditional laboratory methods (Mass Spectrometry).

The study monitored the homogeneity of paracetamol tablets with varying concentrations of the active pharmaceutical ingredient (API:100%, 80%, 50%, 30%, and 0%) and assessed the functionality of the portable device in detecting SF drugs. The study employed spectrophotometers coupled with chemometric techniques such as NCLS, k-means, PCA, and ICA. Two benchtop devices in the NIR range (Spotlight 400 and spectrum Two N) successfully identified API within the tablet formulations at different concentrations. Two handheld visible‐NIR spectrometers, the Specim IQ and SnapCup (prototype), were evaluated for their efficiency in the detection of SF drugs. However, it was noted that the Specim IQ device currently does not offer a practical implementation for drug analysis, and further developments are anticipated. Our portable device, SnapCup, showed promising results in differentiating API concentrations after applying data treatment and chemometric analysis. This project was funded by BIOPROBE (Appel à Projets 2023 - Preuve de concept).

Keywords: Near-infrared spectroscopy, Substandard and falsified drugs, Hyperspectral and multispectral imaging, handheld devices

Single cell analysis using synchrotron radiation µFTIR spectroscopy

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Fourier Transform InfraRed microspectroscopy (µFTIR) is a powerful technique for characterizing the biochemical composition of cells and tissues at the micron scale without the need for dyes or markers. The FTIR spectrum of a cell reflects its lineage, metabolism, cell cycle stage, differentiation level, and physiological or pathological state. Utilizing synchrotron radiation (SR) as an infrared source allows measuring the spectrum from individual cells in 2D or 3D culture, or from single cells within tissues. This presentation will showcase several biomedical applications of SR-µFTIR.

SR-µFTIR could detect metabolic differences between cells genetically modified to carry the wild-type (WT) bcr-abl gene responsible for the Chronic Myeloid Leukemia, and cells carrying the T315I mutation which confers resistance to most antileukemic drugs. The spectral differences between these cells revealed various biochemical changes including variations in RNA expression which were corroborated in transcriptome analysis. This study showed that even a single point mutation can significantly impact the overall metabolism of a cell(1).

The IR spectra of glioblastoma cells grown in hypoxic conditions exhibited distinct changes in their lipid and carbohydrate compositions compared to cells grown under normoxic conditions. These alterations could be related to the accumulation of lipid droplets in the cytoplasm and a shift towards glycolytic metabolism. In addition, acute hypoxic signature could be replicated using hypoxia-mimicking drugs such as DMOG while chronic hypoxia was different. This study showed that µFTIR can be used to monitor the effects of the cellular microenvironment and chemical treatments(2).

The µFTIR spectra of human and murine Mesenchymal Stem Cells (MSC), Embryonic Stem Cells (ESC) and induced Pluripotent Stem Cells (iPSC) were measured in 2D cultures. By employing an innovative experimental design, isogenic cells at various pluripotency stages were obtained. Bona-fide ESCs derived from embryos were first differentiated into multipotent MSCs which were then reprogrammed into iPSCs via transfection with embryonic transcription factors (OSKM for murine, OSLN for human). This process yielded isogenic ESC, MSC and iPSC from the same lineage. We used these cells to demonstrate that iPSCs retrieve a chemical composition very similar to that of their parent ESC, distinct from that of their MSC progenitors. This shows µFTIR utility in studying pluripotent stem cell differentiation(3).

Finally, µFTIR can also be applied to analyze cells within tissues. For example, µFTIR provides insights into the differentiation process of hair progenitor cells as they mature into the hair shaft in human hair follicles(4).

In conclusion, µFTIR demonstrates significant potential in various biomedical applications by providing chemical insights into cellular metabolism and differentiation, the impact of genetic

mutations, environmental conditions, and chemical treatments on cells, all without requiring any markers.

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Noninvasive evaluation of the skin barrier in reconstructed human epidermis: impact of blue light exposure

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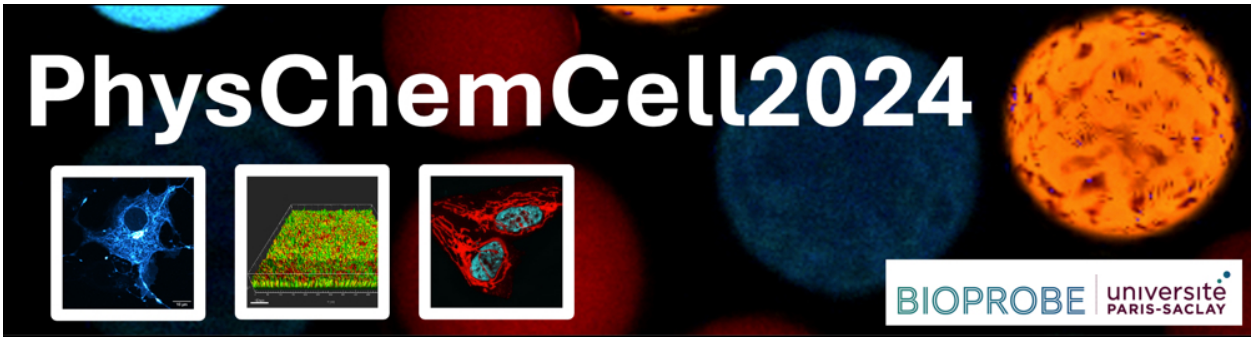
Reconstructed human epidermis (RHE) models, derived from 3D cultures of keratinocytes, are increasingly used as models for safety and efficacy testing in skin research. To accurately evaluate these models, it is crucial to perform both molecular and functional characterizations. A noninvasive method is particularly desirable for assessing the skin barrier in these models. Therefore, the first part of this study aimed to apply the speckle technique to evaluate the skin barrier in RHE.
While the harmful effects of UV radiation on the skin barrier are well-documented, the impact of blue light—an abundant component of both sunlight and artificial light sources—remains less understood. The second part of this study explored the effects of blue light exposure on RHE

To monitor differentiation, speckle analyses and Raman microspectroscopy were performed on RHE samples at two stages of maturation: Day 17 (D17) and Day 20 (D20). To assess the impact of blue light exposure, RHE samples were exposed to blue light at wavelengths of 415 nm and 455 nm during various maturation stages. Raman spectra, speckle images, and normal-phase liquid chromatography coupled with high-resolution mass spectrometry (NPLC/HR-MS^n) were used to analyze both irradiated and non-irradiated (control) samples.

Between D17 and D20, there was an observed increase in several Raman parameters, including the percentage of the stratum corneum, lateral lipid packing, lipid-to-protein ratio, and changes in protein secondary structure. Additionally, both the degree of light polarization (DOP) and the speckle grain size (dx) increased during this period, indicating changes in skin barrier properties.
Blue light exposure resulted in alterations in the structural organization of lipids and proteins in RHE, modifications in lipid composition, and changes in DOP and dx. The extent of these changes varied depending on the specific wavelength and dose of blue light exposure.

In conclusion, speckle proved to be a valuable noninvasive method for assessing the skin barrier in reconstructed human epidermis (RHE) models. Comparisons with Raman microspectroscopy validated this approach, offering comprehensive molecular and functional characterization of these skin models. The findings also suggest that exposure to blue light can disrupt the integrity of the skin's protective barrier, potentially increasing sensitivity to environmental stressors and contributing to skin damage.



Session 4

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Bridging the gap from nanoscale to *in vivo* imaging

Measuring axonal transport in models of Alzheimer's disease using neurotropic fluorescent nanodiamonds

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Defects in axonal transport at the molecular level are observed in neurodegenerative diseases, but their roles in the development of the pathology are unknown. We developed a method to measure axonal transport based on the endocytosis of fluorescent nanocrystal (nanodiamonds, FNDs) into neurons, followed by recording their movement using fast videomicroscopy[1]. The goal of this project is to extend this method to human cerebral organoids (hCO), which present a diversity of cell types closer to that of the brain.

To facilitate internalization into hCOs, which are denser than two-dimensional cultures and less mature than primary mouse neurons, our strategy is to graft a neurotropic peptide (RVG29) onto the FNDs. We have generated and characterized a first batch of FNDRVG29 nanoconjugates, starting from commercial FND-PEGs (nominal size ≈40 nm) prefunctionalized by maleimide groups, and from custom-synthesized RVG29-C. Direct characterization of the complex by MALDI-TOF mass spectrometry (MS) is challenging due to the considerable difference in molecular mass between FNDs and RVG29. We therefore opted for a chemical cleavage of the peptide, which should only release its N-terminal fragment if it is covalently bound to the FND and which is measurable by MS. To overcome the quantitative limitations of MS, we recently obtained an isotopically labelled peptide with an identical structure to RVG29 but 10 Da heavier. We thus hope to quantify the amount of peptide that reacted with FNDs.

In addition to the physicochemical characterization of the conjugates, and prior to studies on hCOs, we developed neuronal cultures derived from human induced pluripotent stem cells (iPSCs). Specifically, we differentiate neural progenitors with and without mutations of the BIN1 gene (a risk factor for Alzheimer's disease). Differentiation into neurons and axonal growth (in microfluidic channels) was validated by immunofluorescence. Neurons are also functional, as evidenced by calcium influx (visualised by a genetically encoded probe) and spontaneous electrophysiological activity (detected using a high-density microelectrode array on which the culture was grown for 2 weeks).

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Agile two-photon microscope for fast 3D single-particle translation and rotation tracking

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We present a two-photon microscopy setup to measure intraneuronal transport parameters in a 3D sample in a super localisation regime thanks to the non-linear optical response from nanoparticles (second harmonic (SH) generation). We take advantage of a Digital Micromirror Device (DMD) to perform digital holography and change the focus position of the excitation laser. We create a pattern of excitation in the vicinity of the nanoparticle, which allows us to super-localise the particle in real time (millisecond regime), with a localization precision of less than 5 nm by maximum likelihood approach [1]. The DMD is fast enough to track the nanoparticle during its motion. We also use the holograms to correct the wavefront and obtain thus a diffraction-limited spot at the laser focus. The tracking method has been tested on nanoparticules ($BaTiO_3$ nanospheres, ~100 nm diameter) internalized in living cells displaying directional trajectories and typical go and stop phases.

We aim at completing the intraneuronal transport parameters, inferred from the x(t),y(t),z(t) positions, with the measurement of the rotational movement of vesicles. This additional parameter is useful to understand how the molecular motors are driven along the microtubules [2,3]. The nanoparticles SH signal depends on their crystalline axis and polarization of the excitation laser. By rotating the incident polarization and detecting along two orthogonal polarizations, we are able to track the translation motion as well as the rotation of the nanocrystal [4]. Our first measurements display standard deviations around 1-2° for azimuthal and polar angles θ and φ.

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Flux: a new contrast for simultaneous multi-targets imaging in 3D single molecule localization microscopy

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Single-molecule localization microscopy has emerged as a widely adopted technique for achieving super-resolution imaging in biological samples. Given the intricate nature of biological phenomena, there is a growing demand for techniques that enable the simultaneous localization of various biomolecules, allowing for a quantitative understanding of their interactions. Initially derived from conventional imaging methods, multiplexing has been accomplished by selecting fluorophores with distinct absorption and/or emission spectra. However, this approach has limitations and imposes constraints on the choice of fluorophores and the level of multiplexing attainable.

An alternative strategy involves observing dyes with closely spaced spectral profiles that are excited by a single laser, typically favoring red dyes. This spectral demixing approach relies on incorporating a carefully chosen dichroic filter in the emission path, splitting it into two spectrally complementary channels. While this approach either confines the field of view when using a single camera or necessitates the use of a second camera, it also requires a robust association of events between the channels which typically discards up to 50% of events.

To address these constraints, we will demonstrate that alternative intrinsic properties can serve as effective means to distinguish fluorophores, even in cases where their absorption and emission spectra strongly overlap thus minimizing chromatic aberrations. Specifically, we will illustrate that the photon flux of a fluorophore, linked to its brightness, can be utilized as a parameter to identify simultaneously multiple dyes revealing different proteins in a single acquisition. This offers the significant advantage of direct compatibility with a single-camera setup. We will elucidate the advantages and limitations of this approach in the context of DNA-PAINT imaging, comparing its performance with traditional spectral demixing strategies for multi-proteins imaging. Additionally, we will present an extension to accommodate imaging with three simultaneous colors.

Moreover, since the photon flux technique doesn't necessitate any alterations to the optical setup, integrating it with 3D single molecule localization microscopy is seamless. Specifically, we will showcase the direct integration of photon flux demixing with astigmatism, as well as with supercritical angle fluorescence, enabling axial super-resolution—commonly referred to as DONALD or SALM

Finally, we will show that the intrinsic contrast offered by the flux, can also be used in association with spectral demixing. First flux information can be used to recover unpaired localization in spectral demixing events due to a low signal-to-noise ratio in one channel, both in 2D and 3D configuration. Secondly, we will also discuss how flux and spectral properties could be smartly intertwined to further increase the number of proteins imaged simultaneously.

Visualizing the self-assembly mechanism of viruses through fluorescence microscopy and interferometric scattering at the single molecule level

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The diversity of viruses we are facing dictated us to learn how to live with them. To facilitate this coexistence and, above all, combat their proliferation, it has been necessary to understand how they work. This need has led to numerous studies on viruses of various sizes and compositions. Up to now, the results obtained through ensemble measurements contribute to improving our knowledge upon assembly pathways of viral capsids and their dependence on salt and pH. Indeed, most of the techniques used give average results for an entire population, without being able to access to the inherent heterogeneity of the assembly mechanism. Total internal reflection fluorescence microscopy (TIRFM) allows us to work not only at the level of the individual capsid, but also at the level of the individual subunit. This experimental setup has been used on the Cowpea Chlorotic Mottle Virus (CCMV), whose dimers and single-stranded RNA were fluorescently labelled.

We followed the self-assembly dynamics of CCMV at equilibrium, and accessed to information such as the mean number of capsid subunits per RNA and the time between two consecutive binding events. These initial experiments showed, as expected, an increase in particle size and a decrease in binding rate as the subunit concentration increased. These results, combined with those obtained by interferometric scattering (mass photometry), indicated that the self-assembly mechanism followed through fluorescence microscopy could be close to give fully formed capsids. Finally, non-equilibrium experiments seemed to suggest that the early steps of assembly were faster when the salt concentration was closer to physiological conditions.

Defining and enhancing single molecule regime with dynamic excitation control in SMLM imaging

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A significant limitation of SMLM super-resolved imaging is the challenge of generating a high-quality single-molecule regime to minimize reconstruction artifacts. In particular, dSTORM imaging is characterized by the complexity of controlling the photophysical behavior of its fluorophores. We propose using localization density as a universal quantifiable experimental indicator of this blinking regime quality. Through simulations, we will define how localization density is governed by the characteristic duty cycle of photoswitching and fluorophore density, allowing us to derive a quantitative criterion for single-molecule regime quality, dependent on the localization algorithm used. Applying this criterion to challenging dense samples via localization density maps enables us to highlight the local decline in singlemolecule regime quality within the observed region.

To standardize the single-molecule regime quality across the dSTORM field of view, we propose a dynamic excitation system, IASTER (Intelligent Adaptative Scanning for Tunable Excitation Regions), capable of generating tailored excitation for different regions. Following a photophysical study of Alexa Fluor 647, we can define an irradiance map to be applied, which will be subjected to a feedback loop in parallel with the acquisition. This approach aims to enhance the uniformity and quality of the single-molecule regime, ultimately improving dSTORM images quality. Experimental results on cytoskeleton proteins will be presented to evidence the benefit of irradiance adaptation during the acquisition. We will also discuss alternative output of this smart microscopy implementation.

PERIODIC LIGHT MODULATIONS FOR LOW COST WIDEFIELD IMAGING OF LUMINESCENCE KINETICS

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Luminescence lifetime imaging (LLI) is a valuable technique in many domains, such as in bioimaging (e.g., for improving contrast[1]) and image-based sensing (e.g., for mapping oxygenation at a surgical site[2]). Given that the majority of the photoactive species used in LLI have lifetimes in the µs-ns range, where standard cameras are not fast enough to capture the signals involved, sophisticated and costly camera setups, such as those with modulable sensors[3] or fast gating[4], are needed. In this work, we introduce two protocols, that we coined RIOM and HIOM, which give access to LLI in the ms-µs range, but with standard cameras. We performed initial validation of these techniques using a well characterized photoactive system (a reversibly photo-switchable fluorescent protein called Dronpa-2), before demonstrating their performance in recovering phosphorescence lifetimes for a number of optical sensors. We extended this work with the optical sensors by imaging oxygen concentrations. Finally, we also explored the potential usefulness of the protocols for acquiring kinetic fingerprints of the physiological state of plants. In this, we were able to follow the uptake of an inhibitor of photosynthesis (DCMU) into the leaves of an Arabidopsis plant after application at its roots.

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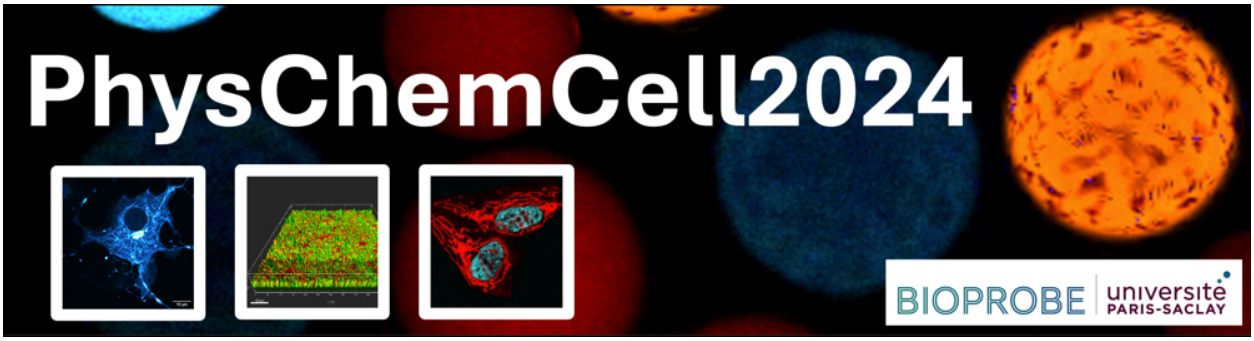

Posters

Clickable fluorescent probes for biological application

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Fluorescent probes are a powerful tool in bioimaging and diagnostics due to their ability to emit fluorescence upon interaction with specific biomolecules or environmental changes.

Recently, sydnones, a class of mesoionic compounds, have emerged as promising scaffolds for click chemistry. Sydnones, five-membered ring with a mesoionic structure, characterized by a unique electronic distribution that allows for diverse chemical reactivity linear and strained alkynes[1]. These characteristics make sydnones ideal candidates for the development of clickable fluorescent molecules.

In this context, we have developed clickable and fluorescent molecules based on high-performance two-photon absorption fluorophores to which sydnones will be attached. These fluorophores, developed within the laboratory, called *Acri-Py*, exhibit exceptional effective cross-section values, and emit light in the NIR (Near-Infrared) region[2]. Thus, in addition to benefiting from the inherent advantages of two-photon absorption (precise imaging, reduced photodamage, excitation in the NIR), these molecules can be easily coupled for various applications thanks to the sydnone core.

Here, we will present the first *Acri-Py-Syd (p) and Acri-vi-Syd (o)* obtained in the laboratory, as well as their spectral properties.

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Investigating nonlinear photodamage in multiphoton light-sheet microscopy: from numerical simulation to live imaging

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Scaling up the imaging speed in multiphoton microscopy to capture fast biological processes, such as a beating heart *in vivo*, is an active and promising field of research [1]. However, it requires to balance image quality, signal level, induced photodamage and optical setup complexity. For example, we have shown that fast cardiac imaging with multiphoton light-sheet microscopy requires the tunability of laser parameters, such as wavelength or pulse repetition rate, to optimize two-photon excitation with low photodamage [2]. Here, we propose to further investigate the origin of photodamage in multiphoton light-sheet microscopy and to devise a strategy to mitigate them. By combining numerical simulation and experiments on live zebrafish embryos, we investigate the beam propagation through biological, quantify photodamage, identify their origin and propose a new strategy to improve signal-to-photodamage ratio.

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Synthesis of novel probes based on borinate ester trigger for the detection of hydrogen peroxide

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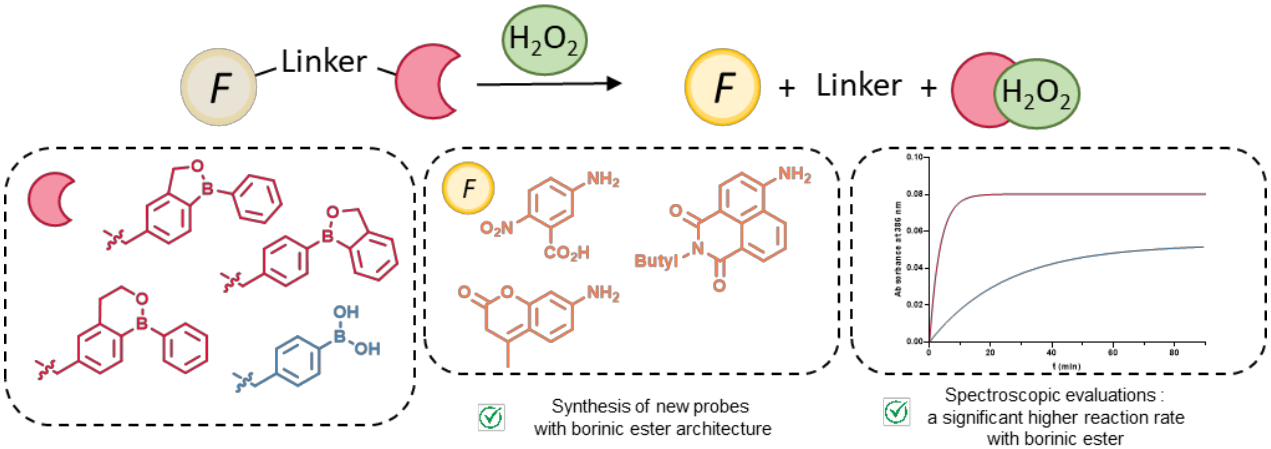
Abstract:

Hydrogen peroxide is well known in the biological environment as part of the ROS (reactive oxygen species) family. Present in cells in low concentration, a higher concentration (called oxidative stress) induces toxicity, possibly leading to several pathologies: neurodegenerative and cardiovascular diseases, cancers and diabetes.[1] A plethora of scientist groups were interested in studying the formation, propagation and impact of hydrogen peroxide by using mostly fluorescent probes. These probes were generally composed of two parts: the fluorescent part and the reactive part (trigger). The latter was well investigated and the design, based on boronic acid/boronate ester, is well explored, and explained by an easy synthesis.[2] Unfortunately, the kinetics were not fast enough to induce a real-time estimation of the concentration of hydrogen peroxide and lower the image resolution. In recent studies, borinic acid – a boron atom connected to two carbons instead of one for boronic acid – showed a better kinetic reaction than boronic acid.[3] However, borinic acids are difficult to obtain due to synthesis or purification issues. To counter these drawbacks, our project is to develop several original probes with borinate ester as a trigger included in a benzoxaborole-type backbone, supposed to be easier to access. The second part of the probe is composed of a chromophore group (5-ANBA, 4-ANI, 7-AMC) connected to the borinate ester with a carbamate as a self-immolating linker. Characterization and application in the presence of hydrogen peroxide were conducted by UV-vis spectrometry and HPLC as soon as the probes were obtained. In the presence of hydrogen peroxide, the borinate ester probes showed a faster reaction rate compared to their homologous boronic acid, confirming our proof of concept.



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Keywords: probes, borinate ester, hydrogen peroxide.

Label-free metabolic imaging and energy costs in algae

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We recently described a new non-invasive and non-destructive label free microscopic method that quantifies the dynamic metabolic activity of a cell (Mazlin et al, 2022; Bey et al., 2023). The method is label free as the contrast is due to interference between the illuminating beam (light-emitting diodtomographic image either using a static mode to show the morphology of the biological sample or dynamic mode, which highlights the metabolic contrast within a cell on a movie.

We successfully applied this method to diatoms under environmental stresses (iron or phosphate deficiency) (Bey et al 2023). The cells were immobilized in agar and a film was taken for a few seconds. We then computed the standard deviation of each pixel of the movie. We were able to show in diatoms that the detected dynamic signal was a metabolic signal as it was dependant on photosynthetic activity (signal values dependant of the LED wavelength and of the use of PSII inhibitors) (Bey et al 2023).

To correlate the metabolic signal within a cell with the energy consumption (expressed in ATPeq) we used an autotrophic model organism Chlamydomonas. We studied respectively a wild type strain and a mutant deleted of the chloroplastic gene encoding the large subunit of the Rubisco, ΔrbcL, this mutant is unable to fix atmospheric CO_2 and is devoid of pyrenoid (Johnson et al. 2010). We correlated, in the ΔrbcL mutant, the dynamic signal to the cost in ATPeq consumption for building starch. We also studied the dynamic signal of the polar diatom *Fragilaripsis cylindrum* experiencing the polar night and evaluated the cost in ATPeq of this quiescent stage for two months (Joli et al 2023)

The method that we will describe in detail is easy to implement and could be very valuable for various biological materials and conditions.

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Impact of exogenous neuroglobin on ionizing radiation-induced ferroptosis cell death in human breast cancer cells

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Our study investigates the role of neuroglobin (NGB), a hemoprotein found in neurons and certain tumor types, including breast cancer. NGB is believed to promote cell survival under oxidative stress or in response to treatments like paclitaxel [1]. Notably, a secreted form of NGB has been detected in the tumor microenvironment of breast cancer tissue, which may protect against cancer cell death under stress conditions. This study aims to explore the impact of exogenous NGB on ionizing radiation (IR)-induced ferroptosis, a type of iron-dependent cell death characterized by lipid membrane peroxidation. We have first shown that exogenously supplied NGB coupled to Alexa-488 is internalized by MCF-7 breast cancer cells using flow cytometry and spinning disk confocal microscopy. Further confirmation of NGB internalization is underway using western blot analysis of total protein extracts from MCF-7 cells. We are currently in the process of determining the optimal treatment conditions to induce ferroptosis-dependent lipid peroxidation in MCF-7 cells using C11-Bodipy [2]. Cells have been first incubated with C11-Bodipy then with hydrogen peroxide (used here as a positive control) to induce oxidation. Preliminary results show using fluorescence microscopy and spectroscopy that C11-Bodipy oxidation depends a lot on experimental conditions (medium composition, hydrogen peroxide oxidation, phenol red,…). We have also been investigating the level of glutathione peroxidase 4 (Gpx4) which is essential in ferroptosis by detoxifying lipid peroxides. Since chemical ferroptosis inducers are known to synergize with IR to promote iron-dependent lipid peroxidation [3], MCF-7 cells were exposed to IR followed by incubation with suboptimal concentration of erastin or RSL3 for 24 hours and the level of Gpx4 were analyzed by western blot. The results showed a slight decrease in Gpx4 protein levels when MCF-7 cells were treated with erastin or IR alone, and a significant twofold reduction in Gpx4 levels when both treatments were combined. Future experiments will involve the use of the iron chelator deferoxamine and the ferroptosis inhibitor ferrostatin-1 to confirm that the observed lipid peroxidation and reduction in Gpx4 levels induced by the combination of IR and erastin are specific events leading to cell death via ferroptosis. Once these parameters are established, the effect of NGB pretreatment on its potential to prevent lipid peroxidation and protect GPX4 from degradation in response to IR and erastin will be tested.

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Monitoring the ribosome dynamics at the single molecule level

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Protein synthesis is a complex multi-step process involving many factors that need to interact in a coordinated manner to properly translate the messenger RNA. As translating ribosomes cannot be synchronized over many elongation cycles, single molecule studies, mainly using total-internal-reflexion fluorescence microscopy, have been introduced to bring a deeper understanding of translation dynamics. In order to perturb as little as possible the translation machinery and to use cell extracts, we decided to monitor the passage of individual, unmodified mammalian ribosomes at specific fluorescent primers hybridized along a mRNA. Because of the ribosome helicase activity, the double strand formed by the oligonucleotide and the mRNA is opened while the ribosome translates this region of the mRNA. Two different oligonucleotides are hybridized at two different places on the mRNA. Thus, the consecutive loss of the fluorescence signal of both oligonucleotides allows us to measure the translation speed distribution of single ribosomes. We use this system to study IRES- and cap-dependent initiation, show that the IRES initiation strongly limits the rate of the first elongation step [1], and compare 3 IRES, CrPV, HCV and EMCV, in different cell extracts. We are currently developing a magnetic tweezers assay to get complementary information on the elongation kinetics during frameshifting. Thanks to its versatility, this method is a valuable tool to investigate the role of translation machinery modifications in human diseases.

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Biocapteurs Électrochimiques à Base de Fructose-6-Phosphate pour la Quantification de l'AMF dans les Fluides Humains

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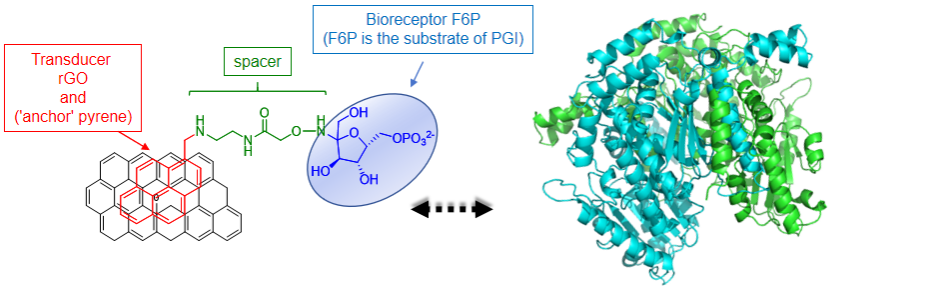
L'enzyme glycolytique phosphoglucose isomérase (PGI) est une protéine multifonctionnelle ayant plusieurs activités extracellulaires. Une fois sécrétée à l'extérieur de la cellule, la PGI est connue comme une cytokine nommée "facteur de motilité autocrine" (AMF) détectée dans le sérum ou l'urine des patients atteints de cancer avec un pronostic défavorable, et donc considérée comme un biomarqueur métastatique suspecté. Récemment, une activité enzymatique sérique élevée de PGI a également été détectée chez les patients atteints de stéatose hépatique non alcoolique (NAFLD). Ainsi, la PGI sécrétée dans le sérum humain apparaît comme un nouveau biomarqueur pour un diagnostic précoce et non invasif du cancer et/ou de la NAFLD.

La technologie des biocapteurs a le potentiel de fournir une détection rapide et précise de plusieurs biomarqueurs pathologiques. Les objectifs du projet sont le développement de nouveaux biocapteurs électrochimiques d'intérêt clinique pour le diagnostic de ce biomarqueur du cancer dans des fluides humains réels, en combinant l'expertise d'une équipe de recherche en chimie (ICMMO, Orsay) et d'autres équipes spécialisées en oncologie. Le projet repose sur la preuve de concept initiale démontrée pour la détection de l'AMF dans le plasma humain supplémenté en AMF [1, 2] et vise à concevoir des biocapteurs électrochimiques basés sur des nanomatériaux en utilisant des "électrodes à sérigraphie" (SPE) abordables et pratiques. Les biocapteurs doivent inclure un espaceur avec des propriétés "anti-fouling" et un motif sucre spécifiquement conçu pour reconnaître le biomarqueur. Le biocapteur est censé avoir la spécificité et la sensibilité nécessaires pour la quantification du biomarqueur PGI sécrété dans les fluides humains des échantillons réels de patients atteints de cancer ou de NAFLD et de patients sains.



Biocapteur de Type F6P

Biomarqueur Pathologique Ciblé (Cancer, Diabète) :
Phosphoglucose Isomérase (PGI)

Ce projet promet de fournir des outils diagnostiques rapides, précis et non invasifs, améliorant ainsi le diagnostic précoce et le pronostic des patients.

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Investigating the Role of Unconventional Myosins in Membrane Deformations and Organelle Dynamics

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Unconventional myosins, specifically Myo1C and Myo7A, are actin-dependent motor proteins that play crucial roles in endomembrane dynamics and the attachment of organelles to the actin cytoskeleton. However, the precise molecular mechanisms governing these processes remain largely unexplored. Our aim is to elucidate the specific functions of Myo1C and Myo7A in membrane deformations and dynamics of lysosomes. These acidic organelles are critical for recycling of macromolecules and act as a cellular hub for metabolism and signaling. We hypothesize that unconventional myosins regulate lysosomal membrane tension.

In our project, we develop a new bottom-up approach that combines biochemical reconstitution assays with purified membranes from intracellular organelles. We started to investigate the contribution of Myo1C and the actin cytoskeleton in membrane deformation using *in vitro* reconstitution assays based on Giant Unilamellar Vesicles (GUVs). First results indicated that Myo1C does not deform GUV membranes. Next, we employed membrane tension probes (Flipper or lyso-Flipper) and Fluorescence Lifetime Microscopy (FLIM) to measure membrane tension of GUVs. Preliminary results indicated an increase in fluorescence lifetime of GUVs that have been covered with Myo1C and actin suggesting a role of the actomyosin network in membrane rigidification. In addition, we aim at replacing GUVs by purified lysosomes in order to verify the observed membrane rigidifying effect of the actomyosin cage. For this, we have set up a protocol to purify lysosomes from various cell lines including non-transformed cells. We have confirmed the presence of Myo1C and actin in the purified lysosome fraction but did not detect Myo7A so far. Next, we will monitor membrane tension of purified lysosomes using lyso-Flipper in the presence and absence of purified myosins and actin, and the findings will be compared with those for GUVs.

This research will advance our understanding of how unconventional myosins, through linking membranes to the actin cytoskeleton, regulate lysosomal dynamics. It will contribute to the understanding of complex diseases such as cancer that often show deregulation in the actomyosin cytoskeleton.

Development of fluorescent nanomaterials for of bacteria sensing

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Bacteria detection is an important activity in relevant sectors such as health, food industry and quality control. The proposed project involves the development of biosensors for real-time detection of bacteria, with a detection system featuring rapid fluorescence readout based on a "mix and detect" approach. The transducers to be developed will be based on fluorescent organic macromolecules (porphyrins combined with fluorescent Graphene dots nanomaterials that enable off/on luminescence following the FRET (Förster Resonant Energy Transfer) phenomenon. FRET (Förster Resonance Energy Transfer) occurs through the detection of energy transfer between a donor and an acceptor interacting at distances between 1 to 10 nm. FRET.

This study investigates the formation of nanocomposites and the occurrence of FRET with nanocomposites formed various graphene quantum dots (GQDs) acting as donors (D) and different porphyrins acting as receptor. We expect that the optical properties beside the ability of the two nanomaterials to interacts through pi-stacking and ionic interaction favor the conditions to obtain the FRET. For this purpose, the synthesis of various porphyrins bearing hydroxyl groups and pyridinium groups as negatively and positively charged are investigated as acceptors and two graphene dots non-doped and N-doped are synthetized and investigated as donor (Figure 1A). The composites were synthetized by optimization of the conditions such as solvent and the ratio. Photophysical properties are studied by UV-Visible and fluorescence of the donor and acceptor as well as the composites.

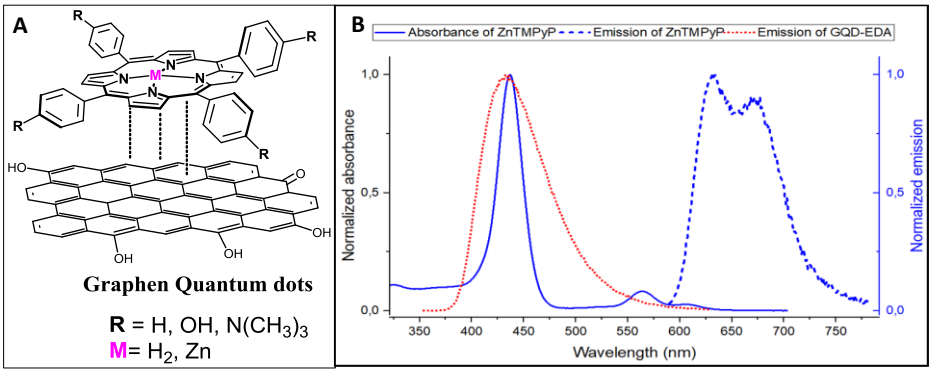

Figure 1: (A) Scheme of the porphyrines/GQDs complexe, (B) Emission and fluorescence spectra of the donor and acceptor

We demonstrate that the condition to obtain FRET is favored following the optical properties of the two materials (Figure 1B). In the case of the complex three of the proposed D-A couples presented quenching of the donor fluorescence after addition of acceptor. Evidence of FRET is not as straightforward as envisioned. However, studies of assemblies of composite formed with between ZnTMPyP and non-doped GQDs suggest the presence of photoelectron transfer, explaining the GQDs band quenching after addition of porphyrin, and the absence of sensitization from donor to acceptor.

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The mesoSPIM initiative Paris-Saclay An open-source light-sheet microscope for 3D imaging of large cleared tissues and organisms.

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Recent imaging workflows have rendered possible the examination of whole-organ and organism in three-dimensions (3D) at high-resolution. In parallel with imaging technology development, the emergence of tissue clearing techniques which aim to render large tissues transparent by reducing light scattering and absorption is a significant step-forward allowing optical inspection of over cm biological organism. Given the centimeter-size of cleared samples, a dedicated imaging technology is necessary and light-sheet microscopy has been a game-changer improving considerably the 3D fluorescence imaging throughput of large specimens. Combination of tissue clearing and light-sheet imaging enables the contribution of the IERP phenotyping platform to several studies[1-7] from model organisms to livestock to finely describe the anatomy of whole organs/organisms in the fields of comparative anatomy, developmental biology and infectiology (pathophysiology) in label-free chemical approaches. Currently, the restricted and time-limited access to commercial systems are bottlenecks that IERP will overcome by building, together with physicists specialized in optics at ENS Paris-Saclay, the first mesoscale Selective Plane Illumination Microscope1 in France. This microscope design will diversify the equipment pool and services available for large-scale imaging in Ile-de-France providing open-access state-of-the-art 3D imaging of whole organism within the IERP phenotyping platform.

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Time controlled excitation for Fluorescence Lifetime Imaging Microscopy without time-resolved detection

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Fluorescence Lifetime Imaging Microscopy (FLIM) stands as a valuable and well-established tool for enriching the functional insights derived from biological observations. Fluorescence Lifetime enables the exploration of local physico-chemical properties (such as pH, ion concentration, viscosity, membrane tension, etc.) within cells. However, all FLIM techniques currently rely on time-resolved detection, facing challenges such as a limited number of detected photons (TCSCPC) and compromised contrast (Time-gated intensifier and frequency domain). Additionally, these detectors are costly and not easily accessible to biologists. These constraints also hinder the integration of fluorescence lifetime with single-molecule microscopy without trade-off.

In the context of this project, we are removing the time-resolved detection for thereby increasing the overall count of detected photons. To ensure that our measurement continues to be contingent on fluorescence lifetime, we are considering the excitation of the fluorophore with two pulses separated by a known delay. In proximity to excitation saturation, the number of excited molecules is restricted. If the second pulse exhibits a delay at the nanosecond timescale, only a limited population in the ground state can be stimulated. Consequently, the emitted fluorescence light becomes dependent on the delay of the second pulse. By precisely controlling the delay between the two pulses, the fluorescence light becomes reliant on the fluorescence lifetime and can be accurately retrieved.

Our initial exploration involves investigating DPFLIM (Double Pulse Fluorescence Lifetime Imaging) within a confocal microscope, providing a focused beam for saturating the fluorophore. Results obtained with a colorant solution are presented, and we will also demonstrate its adaptability to wide-field imaging.

To enhance the sensitivity of the lifetime measurement, we are also experimenting with exciting the fluorophore at a high repetition rate using a GHz laser. Saturation is expected to occur when the excitation repetition rate aligns with the relaxation time scale, typically in the nanosecond range for organic dyes. By transitioning the laser repetition rate from MHz to GHz, emitted fluorescence becomes saturated at different frequencies based on the fluorophore lifetime.

Specific targeting of new borinic acids as hydrogen peroxide-responsive probes to proteins using HALO-Tag Strategy.

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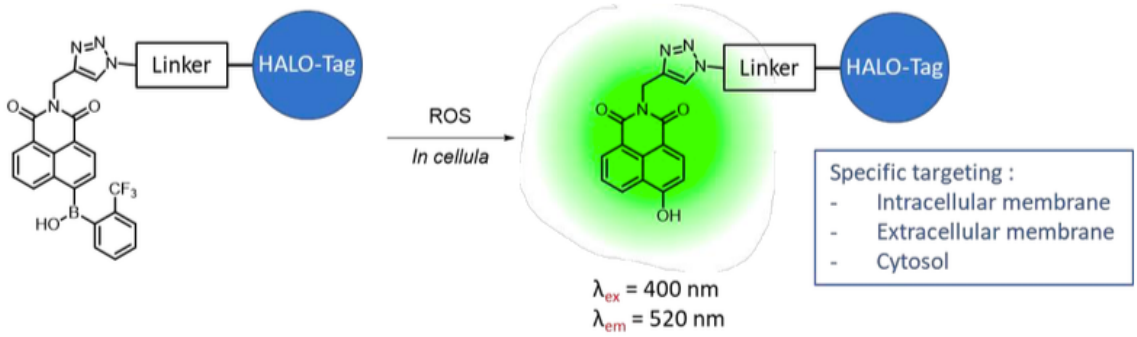
Reactive Oxygen Species (ROS) are involved in numerous physiological processes. In particular, hydrogen peroxide (H_2O_2) at low concentration plays a critical role in the regulation of numerous biological activities as a signaling molecule. However, aberrant production or accumulation of highly diffusive H_2O_2 leads to oxidative stress conditions, which can cause biomolecules lesions associated with aging, cancer and several neurodegenerative diseases such as Alzheimer's or Parkinson's.[1]

In order to detect hydrogen peroxide, many fluorogenic organic probes have been developed, relying on a boronate trigger. However, this design suffers from slow kinetics, preventing a special resolution of hydrogen peroxide imaging. To address this issue, our team has successfully developed borinic acid-based fluorogenic probes, which display kinetic constants 10,000-fold faster than their boronic acid analogs toward H_2O_2.[2]

In this work, we wish to improve the design of the *"Off-on"* fluorogenic borinic acid-based sensors by adding a protein-specific targeting moiety, i.e. an HALO-Tag[3], to a naphthalic scaffold. The specific targeting of this new sensor will allow analysis of redox events at different subcellular levels.



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High content analysis reveals *in vitro* genotoxic and cytotoxic effects of microcystins (-LR, -RR and -LF) in human HepaRG cells

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Microcystins (MCs) are cyclic hepatotoxins produced by various species of cyanobacteria. Their structure includes two variable amino acids leading to more than 100 variants. MC-LR is a potent tumor promoter which inhibits protein phosphatase activities, but increasing evidences suggest that variant MC-LF might be even more toxic. The aim of the current study was to characterize the cytotoxicity and the genotoxicity of microcystins congeners MC-LR, -RR and -LF using high content analysis (HCA) in a human hepatoma cell line HepaRG. Undifferentiated HepaRG cells were processed for high content analysis after 24 hours of treatment with dose ranging from 0.39 to 50 µM MC-LR and MC-RR and from 0.05 to 6.25 µM MC-LF. A total of 6 cellular markers were evaluated for viability, apoptosis and DNA damage investigation (cell count, active Caspase-3, γH2AX, phospho H3, p53 phospho S15 and ATM phospho S1981). A significant increase in each of these markers was observed which was correlated with the onset of cytotoxicity. The results also suggest that MC-LF elicited cytotoxic effects at lower concentrations compared to MC-LR and MC-RR. These findings provide key information in the mechanism of action of microcystin congeners.

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Chemical and genetical screening for identification of ER glutathione transporter during ER Ca^{2+} depletion

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The Endoplasmic Reticulum (ER) is a major site of protein folding and quality control but it is also fundamental for Ca2+ storage. Protein maturation frequently involves the formation of disulfide bonds by a dedicated machinery that regulates oxidative folding. Therefore, the redox poise of the oxidative environment of the ER is tightly regulated. Importantly, high levels of luminal Ca2+ concentration are maintained by redox regulation of calcium transporters while ER Ca2+ depletion itself induces a decrease of the ER redox potential, at least in part due to the ER import of the reducing molecule glutathione (GSH). This questions a possible role of GSH ER transport in the control of ER calcium levels. In mammalian cells, GSH is synthesized in the cytosol and its entry in the ER has not been extensively explored. To gain insight in this cellular process, we have created a cell line stably expressing an improved version of the genetically encoded Grx1-roGFPiE GSH biosensor to monitor luminal EGSH as a proxy of GSH transport in the organelle. We observed that different drugs triggering the depletion of luminal Ca2+ could induce an ER reductive shift that was abolished during GSH depletion, confirming the likely GSH import in the ER in conditions of low luminal Ca2+. In addition, we employed a second biosensor, an ER-targeted Glutaredoxin 1 (Grx1) and we monitored its glutathionylation status both by differential dPEG alkylation and by means of clickable GSH. Results obtained so far indicate that a decrease of luminal Ca2+ concentration induces increase in ER GSH, suggesting the trigger of GSH import.

In order to investigate further the mechanism concerned, we set up two high-throughput screenings (chemical and genetical) to identify specific molecules that could inhibit or stimulate GSH luminal entry as well as determine specific genes participating in this mechanism. Chemical screening allowed us to test more than 1600 molecules from which a small set of promising hits has been selected for further characterization.

Serial multiphoton microscopy : towards large-scale mapping of label-free nonlinear optical signals in fixed mouse brains.

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Understanding the role of myelin in the development and function of the brain is crucial for unraveling the mechanisms underlying neurological disorders. However, mapping opaque brain tissue with subcellular precision during different developmental stages has remained challenging. We present an update of the platform, ChroMS [1], that combines label-free third harmonic generation [2] signals outlying tissue heterogeneity with specific fluorescent signals to target myelinated areas in mouse brains. ChroMS ensures perfect channel registration across the whole field of view, which would allow for quantitative correlation pixel by pixel between THG and fluorescent signals, thus determining the specificity of the THG signals for the myelinated area of the brain. We hope that this work will allow the quantification of the distribution of myelin, a vital component essential for efficient neural communication, utilizing the label-free third-harmonic generation (THG) signal [3].

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Conformational exploration of HSULF-2, an Intrinsically Disordered Protein (IDP): benchmarking of coarse-grained models

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In 2002, a new category of 2 human sulfatases (HSULF1 and HSULF2) that execute enzymatic activity at interior of substrate, termed endosulfatases, compared to known sulfatases at extremity of substrate, is discovered [1]. Human endosulfatase 2 (HSULF2) is found overexpressed in numerous diseases, especially breast cancer and inflammation, and is thus a promising therapeutic target. However, no experimentally resolved structure has been yet uploaded in the Protein Data Bank (PDB), which can be attributed to the fact that its hydrophilic domain (HD) is (1) of low homology to all other sulfatases; (2) an intrinsically disordered region (IDR). Therefore, in order to comprehend HSULF2 activities, it remains critical but always challenging to obtain HSULF2 structure either experimentally or computationally, and study protein flexibility and dynamics.

In this context, we are exploring the conformational space of HSULF2 using 4 coarse-grained models: MARTINI3 [2], SIRAH [3] , UNRES [4] , and CALVADOS2 [5] with AlphaFold2 (AF2) structure. Coarse-grained models are adapted for this study, as the simplification enables phase space to be explored more rapidly. What is more, this modeling research is also connected and fed by experimental data provided through structural and functional characterization carried out at the Laboratory of Analysis, Modelling, Materials for Biology and Environment (LAMBE) using biochemical and analytical chemistry tools, particularly mass spectrometry [6-8] , and biophysical tools.

One important measure of global compacity is the radius of gyration R(g), which was previously experimentally determined [9] . We thus compare the R(g) from molecular dynamics simulations with this experimental value to benchmark the different CG models.

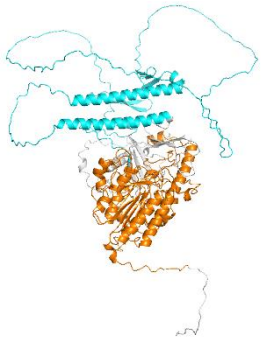

Figure 1 3D structure of HSULF2 predicted by AlphaFold2. Hydrophile Domain is colored in cyan, where high order is observed.

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Probing viral infection induced axonal transport defects in live zebrafish larvae, *via* non-linear nanocrystals high-speed tracing

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Axonal transport is essential to maintain the proper functioning of neurons, as it ensures the traffic of cargoes such as vesicles towed by molecular motors along the cytoskeleton. Indeed, axonal transport deficits constitute early pathological features of neurodegenerative diseases [1], originating from various possible causes including genetic risk factors, proteinopathies or pathogens infections.

We recently described an optically active nanoparticle-based tracing assay to measure *in vivo*, in zebrafish (Zf) larvae central nervous system (CNS), axonal transport parameters in healthy and pathological conditions [2]. Intracerebral injection of potassium titanyl phosphate ($KTiOPO_4$, KTP) (size ≈120 nm, nanoKTP) nanocrystals induces their spontaneous endocytosis in brain cells thus labelled detected at the level of the endosomal compartments. The combination of femtosecond pulsed excitation laser at 1040 nm wavelength with the optical translucency of Zf larvae enable the detection of a high signal to background ratio. Adding to our assay the automatized extraction of nanoKTP trajectories from the fast raster scans, we are able to quantify axonal transport impairment in various transport parameters (velocity, pausing frequency, pausing time…).

In the present study we aim to characterize the biodistribution of nanoKTP after intracerebral injection in the optic tectum of Zf larvae and to investigate the impact of human neurotropic viral infection on axonal transport in these larvae. NanoKTP were injected in three distinct Zf transgenic lines expressing fluorescent reporters either in endothelial cells, in microglia, and in post-mitotic neuronal cells. In a second time, wild type zebrafish larvae were coinjected with nanoKTP and Sindbis viral suspension. For this assay, we used human recombinant Sindbis-mCherry virus in order to detect mCherry fluorescent protein positive neuronal cells in the brain of the infected larvae. Two-photon observations of fluorescent Zf transgenic lines showed that nanoKTP injected in the brain parenchyma appear as isolated or aggregated forms. Rare isolated particles have been detected in the endothelial cells, thus showing the lack of diffusion in the blood vessels. On the contrary, numerous nanoKTP are phagocytosed by microglia. Ongoing experiments will allow us to determine their endocytosis in postmitotic neurons.

While we previously showed that neurotropic viruses harness axonal transport to spread within the Zf CNS [3], little is known about the pathophysiological mechanisms of the disease during the early infection phase (consequences of infection on endosomal transport). Injection of Sindbis-mCherry led, 24 h post injection to the detection of neuronal cells bodies and axonal compartment having the form of periventricular neurons (PVN), a cellular type by which nanoKTP are up-taken in endosome that further display axonal transport. [2] However, in preliminary experiment, we could not find any nanoKTP in Sindbis infected neuron 24 h post infection and concomitantly failed to observe any directed motion, while in control nanoKTP-injected naïve larvae transport was easily detected.

We will now further investigate the reasons why nanoKTP are static, tackling a few questions: 1) does Sindbis virus infection impact PVN endocytosis capacity or does it stop endolysosomal transport; 2) is the phenotype visible from the earliest stages of infection; 3) does it correspond to early signs of neuronal excitotoxicity, which can be rescued by NMDA receptor blockade?

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Microfluidic platform for real-time investigation of intestinal epithelial interaction with bacteria

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Campylobacter jejuni is the most common cause of foodborne gastroenteritis in humans with about 550 million annual infections worldwide. The extracellular vesicles (EVs) of *C. jejuni* have an important impact during pathogenicity but their role in invasion of the host intestinal epithelial cells remains largely unknown [1]. *In vitro* models lack the complexity of tissue and fail to replicate the dynamic interactions between EVs and human intestinal epithelial cells accurately, while animal infection models bring ethical concerns. To bridge this gap, we propose a microfluidic platform integrated with an impedimetric sensor for real-time monitoring of *C. jejuni* EVs interaction with human intestinal epithelial cells. When cultured in this microfluidic device, Caco-2 epithelial cells underwent 3D morphogenesis and spatially organized in spheroid-like structures. Functional assays revealed that *C. jejuni* secretome and EVs have a significant cytotoxic effect on Caco-2 cultured on plates. However, 3D Caco-2 spheroids showed increased resistance to the toxicity of secreted virulence factors of *C. jejuni*. By combining the impedance spectroscopy and live cell imaging, the platform allowed real-time monitoring of cellular spatial growth and sensitive detection of the EVs ability to reach and damage intestinal epithelial cells organized in 3D morphologies. Thus, the developed microfluidic device offers a promising platform for investigating host-microbe interactions, and may have a broad impact on biomedical research of gastroenteritis [2].

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Speeding up live imaging with multiphoton light-sheet microscopy by optimizing both laser wavelength and repetition rate

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Scaling up the imaging speed in multiphoton microscopy is an active field of research [1]. However, its application to live imaging requires a challenging balance between image quality, signal level, induced photodamage, and optical setup complexity. Among promising strategies, multiphoton light-sheet microscopy takes advantage of an orthogonal geometry to parallelize the excitation and the detection and to increase the imaging rate. However, it requires the tunability of laser parameters, such as wavelength and pulse repetition rate, to optimize two-photon fluorescence excitation with low photodamage [2]. Wavelength adjustment is required to both maximize the fluorophore excitation and minimize water absorption. Adjusting the laser repetition rate modulates the pulse peak intensity to balance signal, heating and nonlinear photodamage. However, both parameters cannot be tuned simultaneously in most available femtosecond laser sources used in multiphoton microscopy. It is now generally recognized that a tunable laser repetition rate is essential for the application of advanced multiphoton microscopy in biology [1]. Here, we introduce a novel approach to tune the laser repetition rate in multiphoton light-sheet microscopy based on passive pulse splitting. Our approach is simple to align, light efficient, and can be used with a commercial wavelength-tunable source. We illustrate its potential by performing very high-speed multiphoton light-sheet imaging of live specimens with optimized laser wavelength and pulse repetition rate.

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