Université Grenoble Alpes

ÉCOLE DOCTORALE
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EDCSV PhD Day 2022

23th of June 2022

Booklet

PLENARY CONFERENCE

Invited speaker: Dr. Nicolas BALDOVINI

Flavors, Perfumes, synthesis, andmodelization

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ORAL PRESENTATION: MORNING SESSION

Bio-orthogonal click chemistry as a selective tool towards *Streptococcus pneumoniae*

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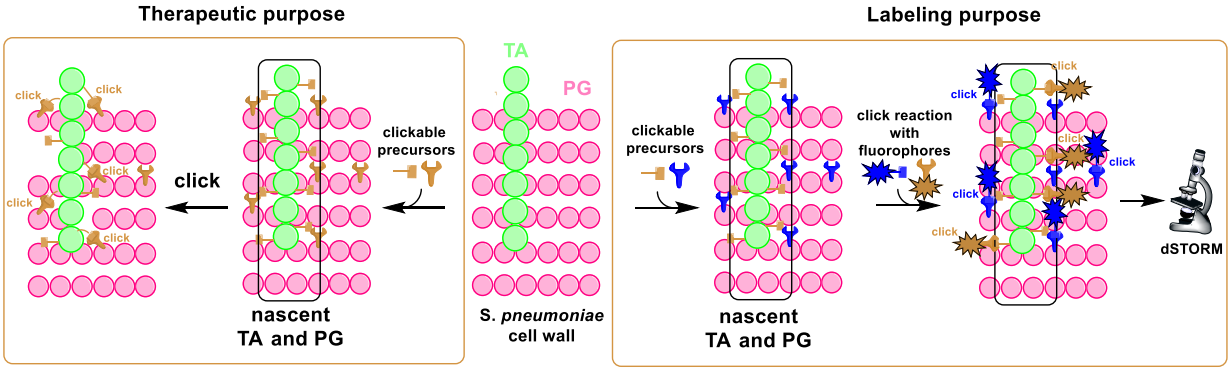
Bio-orthogonal click chemistry has been widely used in chemical biology for the past few years as they are fast, specific and biocompatible reactions. Indeed, these reactions are easily performed at physiological pH and temperature and the precursors are unreactive towards functional groups frequently present in biological systems. Our main focus is to use these types of reactions on the cell wall of the gram-positive bacterium *Streptococcus pneumoniae* both for therapeutic and labeling purposes.

The bacterial cell wall of *S. pneumoniae* is composed of two main biopolymers, the peptidoglycan (PG) and teichoic acids (TA). While PG synthesis is well known,[1] TA have only been recently labeled and their biosynthesis explored.[2] A comparison between the assembly of PG and TA suggests that it occurs simultaneously in a similar location on the cell surface.[3] Therefore, we are exploring whether it is possible to cross-link together PG and TA using incorporation of click precursors in both biopolymers followed by a click reaction between both moieties. The first preliminary results seem to suggest that the assembly of the bacterial wall could be disrupted after the two click precursors are incorporated into PG and TA.

On the other hand, a combined approach of metabolic labeling using copper-free click chemistry and high-resolution dSTORM (direct stochastic optical reconstruction microscopy) has recently revealed with unprecedented details the PG synthesis sites and their relative dynamics along the cell cycle.[4] Therefore, we are now labeling and observing PG and TA in two colors in the same cell in order to detect fine variations in the localization and architecture of their synthesis site(s).



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Architecture of SpoIIIA-SpoIIQ, a protein nanomachine involved in bacterial sporulation

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Bacterial sporulation is a morphological differentiation process that produces a dormant spore, resistant to most disinfection treatments, including antibiotics. We study sporulation in the non-pathogen model organism *Bacillus subtilis*, but this mechanism is also found in important pathogens such as *Clostridium difficile*, causing problems of dissemination and persistence. Spore maturation requires the SpoIIIA-SpoIIQ complex (AQ). It is proposed to be a new transport machinery allowing the mother cell to feed the spore with molecule(s) required for its development. This nanomachine displays structural similarities with endotoxin secretion systems[1]. In support of this hypothesis, we discovered that the AQ component SpoIIIAG assembles into oligomeric rings that form a conduit between the mother cell and developing spore[2]. However, the global architecture of the AQ complex, as well as its mode of transport and relationship with other secretion systems remain unknown. My PhD project aims at unraveling these aspects by determining the **3D structure of the AQ complex** at atomic resolution using **cellular cryo-electron tomography (ET)** on thin sections of sporulating cells obtained by **cryo-focused ion beam milling (FIB)** coupled to scanning electron microscopy (SEM).

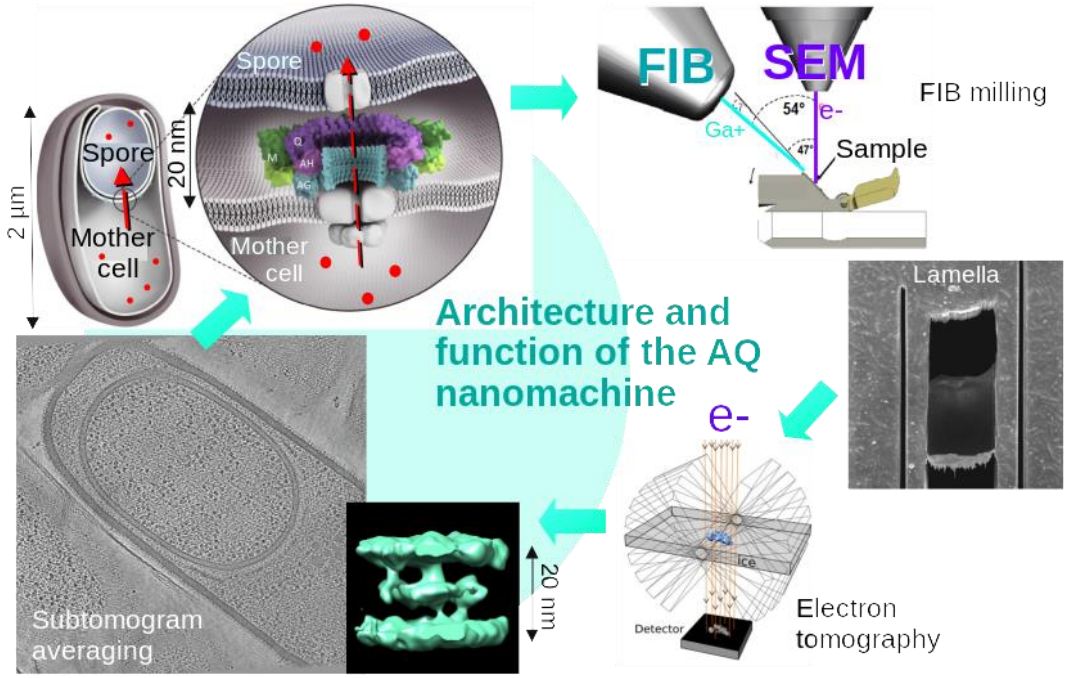

Figure 1. Unraveling the molecular architecture of the AQ complex in its cellular environment. To access and preserve deep cell regions, we section sporulating cells into nanometric lamellae in cryo condition using FIB milling monitored by SEM. Cryo-ET acquisitions allow to acquire images of the AQ complex in different orientations by rotating the sample under the electron beam[3]. The tilt-series are then processed to reconstruct the information in 3D and extract the volumes of interest containing the AQ complex.

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STUDY OF THE COMPETITION BETWEEN THE AROMATIC CYCLOPROPANTION AND BENZYLIC C-H INSERTION

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Polycyclic skeletons containing seven-membered rings are found in numerous natural products, having interesting biological activities.[1] Unlike many medium sizes (five or six membered rings), the synthesis of seven-membered ring-containing skeletons is more challenging and there are few reports on of the efficient strategies. Thus, building this kind of carbocycles has attracted the interest of researchers over the past decades. Different reactions have been used for this purpose such as cycloaddition, ionic cyclisation, or metathesis.[2] The Buchner reaction is a type of cyclopropanation which have ability to enlarge an aromatic ring to form cycloheptatriene by insertion of a carbene.[3] The intramolecular version is particularly suitable for the synthesis of polycyclic structures previously mentioned.

The development of organometallic catalysis has allowed major advances for the Buchner reaction. Metal complexes makes it possible to catalyze aromatic cyclopropanation in an efficient and selective way.[4,5] Moreover, asymmetric Intramolecular Buchner reaction was found as an efficient strategy to prepare enantioenriched seven-membered carbocycle-containing bicyclic compound.[6] Nevertheless, the carbene environment strongly influences the chemoselectivity of this transformation, and to date, this influence is incompletely described and explains some observations poorly.

Thus, we decided to study the chemoselectivity of the intramolecular carbene insertion catalzyed by dirhodium(II). This study reveals that α-diazo-β-amides can undergo the Buchner reaction and/or the C-H insertion, controlled by different elements. Herein, we reported our results on the effect of the carbene substituents as well as the length of the carbon chain on the chemoseclectivity. DFT calculations are performed to help to explain entirely the outcome of the transformation.

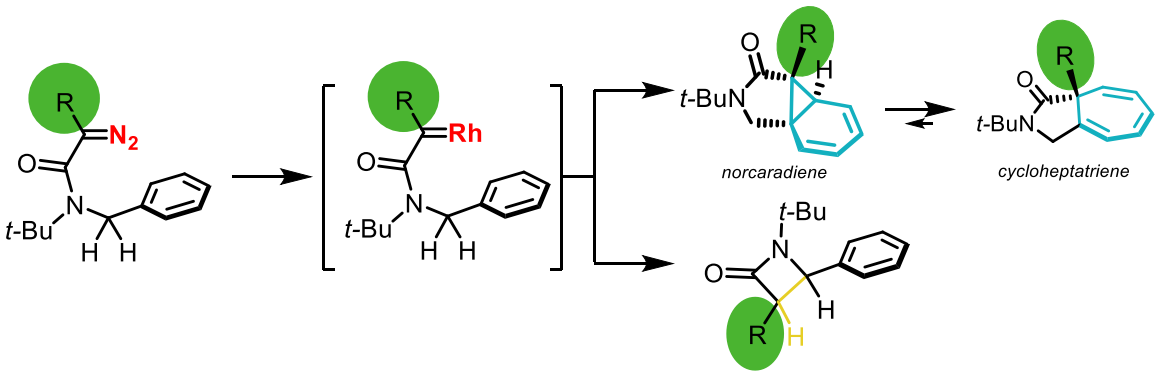

Figure. Metallo-carben insertion pathways

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Anaerobic UQ biosynthetic pathway in *Escherichia coli*

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Ubiquinone (UQ) is a redox active lipid which mediates the transfer of electrons in aerobic respiratory chains in eukaryotes and proteobacteria[1]. *Escherichia coli* synthetizes UQ *via* an O_2-dependent biosynthetic pathway that requires nine biochemical reactions carried out by Ubi-enzymes and accessory proteins **(Figure 1)**. Interestingly, *E. coli* was also reported to synthesize UQ in anaerobic conditions. We identified *ubiT, ubiU* and *ubiV* as essential for UQ biosynthesis under anaerobic conditions[2] and we showed that the oxic and anoxic UQ biosynthetic pathways differ only by the three hydroxylation reactions. Under aerobic conditions, these three reactions are carried out by the flavoprotein monooxygenases UbiI, UbiH and UbiF **(Figure 1)**, which use dioxygen as a source of hydroxyl group. Conversely, in anaerobic conditions, our new data show that UbiU and UbiV catalyse the hydroxylation steps in a 4Fe-4S clusters-dependent manner and use a metabolite of the shikimate pathway as a hydroxyl donor **(Figure 1)**. However, we proved also that dioxygen was not toxic for UbiU and UbiV activities. The role of the UbiT protein is still unclear but it possesses a sterol carrier protein 2 (SCP2) domain, similar to the UbiJ protein, which organizes a soluble Ubi complex in aerobic conditions and binds hydrophilic UQ biosynthetic intermediates[3]. Furthermore, we have explored the regulation of the *ubiT-V* genes as well as their conservation in proteobacteria. The preliminary data show that *ubiU* and *ubiV* are expressed under anoxic conditions in an FNR-depending manner whereas the expression of *ubiT* seems to be constitutive. The phylogenetic analyses demonstrated that the *ubiT-V* genes are conserved exclusively in proteobacteria containing UQ[2].

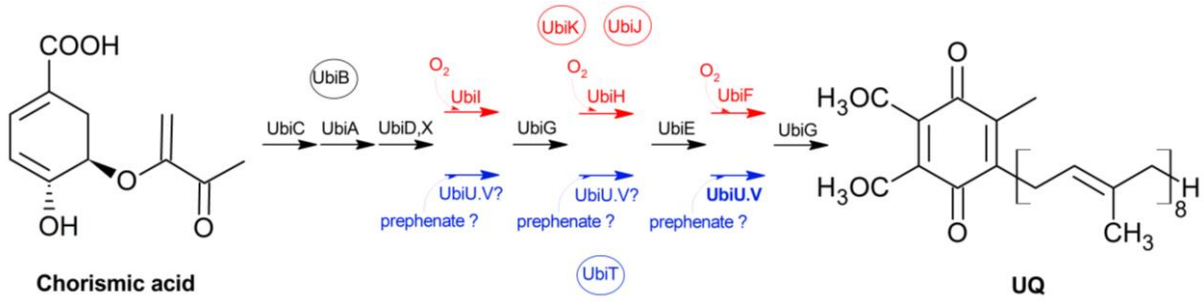

Figure 1 : UQ biosynthetic pathways. Ubi-enzymes specific to the O_2- dependent pathway (red), to the O_2-independent pathway (blue), or common to both pathways (black). The same color code applies to the accessory factors (circled). Adapted from Pelosi et al.[2]

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Genetic control of bacterial growth to improve bioproduction performance

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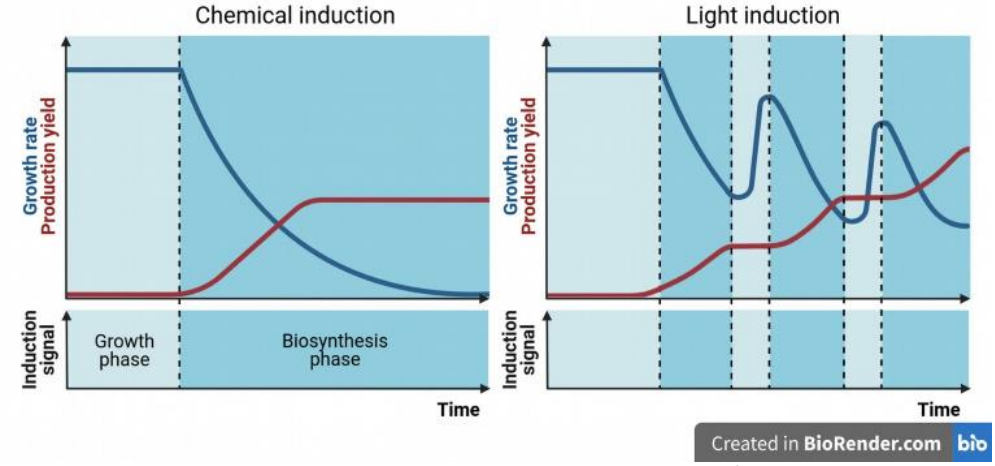
Bioproduction using microorganisms as microscopic factories is attractive and certainly a more sustainable alternative to production of useful compounds by the chemical industry. However, biotechnology still has to overcome certain challenges to be competitive[1]. Maximizing the production of a compound of interest imposes a burden for the cell that has evolved to optimally allocate resources rather to growth than to the synthesis of a particular metabolite. One way to overcome this challenge is to allow the cells to focus on one task at a time, dissociating biosynthesis from growth[2].

In order to achieve this dissociation, our group has developed a genetic growth switch that controls the growth rate of the bacterium *E. coli* by a chemical inducer. Since preventing growth for the benefit of biosynthesis generates a huge selection pressure, we have to ensure the stability of the control circuit. Our group has constructed two versions of the control system and I have assessed the stability of both. Furthermore, we have verified the reversibility of the growth control. In addition, I have measured the production yield of a model compound in these two systems in order to quantify the re-allocation of cellular resources.

For an optimal control, we would like to rapidly turn growth on and off. This is not possible with a chemical inducer that would have to be mechanically removed from the system. We therefore set out to develop an optogenetic control of growth. Using light as an induction signal presents several advantages and opens up a new world of possibilities in terms of growth/biosynthesis induction patterns. We expect to conceive a cybergenetic system with our approach, that allows for alternate phases of growth and biosynthesis, thereby maximizing the product yield, as illustrated in the figure below[3].

Maximization of the yield of the bioproduction of an interesting compound. (Left) In the classical approach, after



reaching a biomass threshold, the growth is inhibited, leading to the reallocation of resources towards biosynthesis. In our first strains, the control is achieved through chemical induction making this induction less flexible than with light (Right), which allows more sophisticated induction patterns. Using control theory or empirical optimization, we expect to identify optimal induction patterns that maximize the production yield. Figure based on Pouzet et al. [3]

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FLASH COMMUNICATION: MORNING SESSION

Rhenium-bipyridyl-carbonyl molecular catalyst heterogenization in LSH layered hydroxides; characterization, redox properties and effect of the catalyst anchoring on CO_2 electroreduction activity

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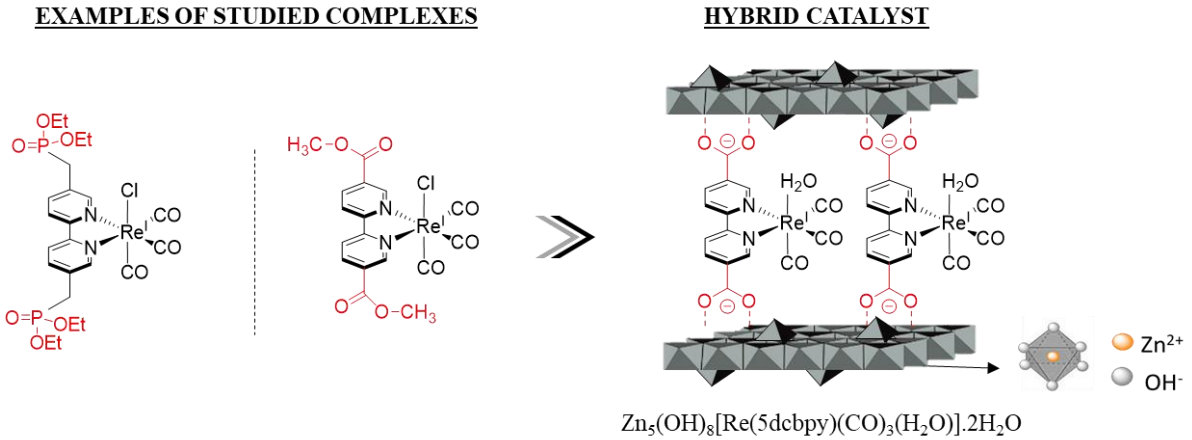
Heterogenization of molecular catalysts on (photo)electrode surfaces is required to design devices enabling to store renewable energy in chemical bonds. Among the various strategies to immobilize molecular catalysts, direct chemical bonding presents some advantages due to the robustness of the linkage [1]. When the catalyst is, as it is often the case, a transition metal complex, the anchoring group has to be connected to the complex through the ligands and an important question is thus raised on the influence of this function on the redox properties and on the catalytic properties of the complex. Although the role of substituents present on ligands of molecular catalysts has been studied in many cases [2], the question of the effect of anchoring groups has not been specifically reported. Herein we will illustrate this issue through the detailed investigation of rhenium tris-carbonyl bipyridine (bpy) complexes. We will show that the presence of methoxycarbonyl groups on bpy (cf. scheme), used as a mimic for covalent attachment of the Re complex on a material, such as LSH, dramatically decrease the potential of the formation of the catalytic active species for CO_2 electroreduction. A detailed study combining cyclic voltammetry and spectroscopies reveals the underlying reasons for such a drastic effect. Then, we will show that using different anchoring groups, such as diethylmethyl phosphonate ester not conjugated with the bpy ligand (cf. Scheme), allows preserving the catalytic activity toward CO_2 electroreduction at reasonable applied potential.



Scheme

This work was supported by the ANR: CALHYCO2 ANR-19-CEO5-0015 Project

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Deciphering the molecular mechanisms underlying the tolerance and accumulation of metals in a hypertolerant green microalga of the *Coelastrella* genus

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Pollution by heavy metals is a significant risk for ecosystems and human health. Bioremediation processes can be an eco-friendly solution for the treatment of contaminated environments. In this context, the identification and characterization of organisms that tolerate and accumulate metals are essential to develop such approaches.

The Plants, Stress & Metals team from the Cell & Plant Physiology Laboratory (LPCV) isolated a metal-hypertolerant green microalga from an environment contaminated by the toxic radionuclide uranium. My PhD project aims at analysing the tolerance and accumulation of uranium in this alga and at deciphering the molecular mechanisms involved.

To this aim, I used a combination of cell physiology and systems-based approaches.

The tolerance properties of the *Coelastrella sp*. alga towards uranyle nitrate was analyzed using growth curves in mixotrophic media containing different concentrations of the radionuclide. Comparisons with other algae (e.g. *Chlamydomonas reinhardtii* or *Chlorella vulgaris*) was done to describe the specific traits of *Coelastrella sp.*. *Coelastrella sp.* was able to grow and maintain high photosynthetic performance in the presence of 200 µM (50 ppm) uranyl nitrate, whereas *C. reinhardtii* and *C. vulgaris* cannot. The capacity of the alga to take up and accumulate uranium was analyzed by inductively coupled plasma mass spectrometry (ICPMS) and was found to be very important.

As a first step towards the identification of the molecular mechanisms supporting the tolerance and accumulation properties of *Coelastrella sp.* to metals, we sequenced and annotated the algal genome. Transcriptomic analyses will be the second step using *Coelastrella sp.* cells challenged with uranium. This approach should identify key genes involved in the tolerance and accumulation properties of the microalga to this radioelement.

Endothelial cell reprogramming in cancer environment towards the identification of new therapeutic targets

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The endothelial cells are known to be involved in the angiogenesis, vascular tone, hemostasis, immune system filtration and maintain the vascular integrity. To achieve this role, they have to be linked each other thanks to the Vascular Endothelial (VE) cadherin. In cancer context, it has been found that endothelial cells participate to the tumor progression by providing blood and nutrients thanks to new vascular networks. It has been found that the VE-cadherin is phosphorylated at site tyrosine 685. As a consequence, the extracellular domain is rapidly cleaved by proteases secreted by the tumor[1]. This domain is found in the bloodstream of the cancer patient whereas the intracellular domain is quickly degraded. These modifications of VE-cadherin are a hallmark of endothelial cells dysfunction. Herein, we study the role of the phosphorylation at Y685 in endothelial functions in Knock-in (KI) mice carrying the mutation at this site and therefore inhibiting the ability of phosphorylation at this site[2],[3]. So far, our study shows in highly vascularized organ such as lungs, the endothelial cells genes are highly deregulated especially in the cell adhesion and angiogenesis. The cell adhesion dysfunction was confirmed by an increase of cell filtration found in the bronchoalveolar lavage in the KI. The inhibition of the phosphorylation at Y685 has also an important role in the kidney, another highly vascularized organ where it has been found a decrease of filtration taking place in the glomeruli composed of mesangial cells, podocytes and endothelial cells. The podocytes and the endothelial cells have to interact each other indirectly through secreted proteins to maintain the glomeruli function[4]. Our study showed that the KI endothelial cells could induce podocyte dysfunction. Nephrin involved in podocyte junctions and WT1 in the differentiation of podocyte are downregulated in the KI whereas TRPC6, calcium channel impacting the Nephrin function is overactivated in the KI.

Therefore, the phosphorylation of Y685 in the endothelial cells have an impact on the podocyte in the glomeruli leading to the filtration dysfunction. This phosphorylation plays a key role in the cell adhesion and angiogenesis in the lungs. Thus, the Y685 phosphorylation could be a potential target in several diseases related with kidney and lungs.

3-3

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Bismuth based perovskite nanocrystals for photoelectrocatalytic CO_2 reduction

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Closing the carbon cycle is an important challenge in the context of global warming. Valorization of the CO_2 produced by the industry by its transformation into useful products or fuels using visible solar light as a driver is a promising, efficient and sustainable means to address this challenge. Bio-inspired photoelectrocatalysis can be used for the CO_2 conversion using only environment friendly compounds. The solar light not being efficient alone to break the C=O bond in CO_2, an external photosentisizer (PS) coupled to co-catalyst will be used in this process. The PSs used often are hard to synthesize, toxic and have low stability; in addition, their properties can be easily optimized. In this project, we propose to use halogenated perovskite NCs as PSs for the photoelectrocatalysis. The perovskites have been recently very successfully used for the photovoltaics; however, most of them are based on lead, a highly toxic element. We propose to synthesize alternative perovskites based on bismuth, $A_3Bi_2X_9$ (A= cation; X=Br, I).[1] These much less toxic NCs have optoelectronic properties well adapted for the CO_2 photoreduction.[2] Later, they will be coupled to a metal co-catalyst, which is efficient for the CO_2 reduction into green fuels and then deposited on NiO electrodes to fabricate efficient photoeletrocatalytic devices.

The Combination of Cs^+, Bi^{3+}, and halide anions (Br, I) can give rise to several different crystalline materials with varying structural and compositional modifications.[3] In our synthesis of layered 2D bismuth-based perovskites, we focus on two solution-based methods, hot-injection and LARP.[4] By optimizing synthesis protocols, we managed to obtain stable $Cs_3Bi_2I_9$ NCs with the size of 13±2 nm and band gaps in the range of 2.12-2.48 eV, as well as luminescent $Cs_3Bi_2Br_9$ nanoplatelets. Synthesis details, structural and optoelectronic properties will be discussed as well as their perspectives for the photoelectrocatalytic CO_2 reduction applications.

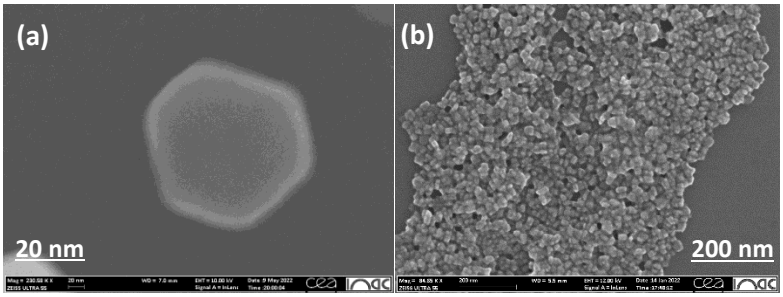

Figure (a) $Cs_3Bi_2Br_9$ nanoplatelets (b) $Cs_3Bi_2I_9$ NCs.

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Drug Discovery: Evaluation of an Innovative Anti-Cancer Compound

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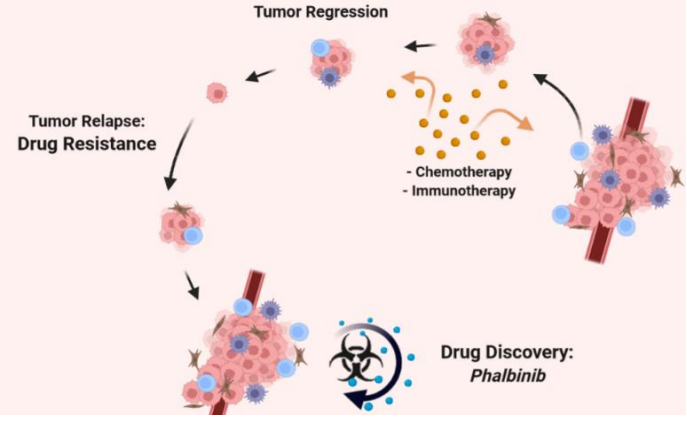
It is established that lung cancer is the biggest cancer killer worldwide[1], with 1.38 million deaths in 2008[2]. With an incidence of 470,000 new cases in one year, lung cancer is also the leading cause of cancer death in Europe (one-fifth of the total), with 388,000 estimated deaths in 2018[3]. Chemotherapy is the mainstay of treatment for lung cancers. However, drug resistance is a major cause of treatment failure. Consequently, the development of new therapeutic agents and innovative combination of treatments are promising strategies to overcome resistance in lung cancers[4]. In our laboratory, we explore the therapeutic effects of a new chemical anticancer agent (named ***Phalbinib***) in different lung cancer cell lines, alone or in combination with standard chemotherapies. We performed viability experiments and assessed the IC_{50} of ***Phalbinib*** and derivatives on various lung cancer cell lines (PC9, H322, H358, A549) and in various other cancer types. We found that the IC_{50} of ***Phalbinib*** is around 2µM for almost all cancer cell lines. Because of at low concentrations, we evaluated the effect of this drug on cell cycle and on different cell death pathways. We further evaluated the effects of this drug on the cell cycle with flow cytometry experiments, and its ability to induce apoptosis or non-apoptotic cell death. The promising anti-proliferative effects of ***Phalbinib*** prompted us to start *in vivo* experiments that are currently ongoing. Altogether, we are developing a very promising anticancer agent for the treatment of lung cancers.



Drug resistance in cancer: Phalbinib an Innovative Anti-Cancer Compound

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Potentialities of a mesoporous activated carbon as a virus detection probe in urban water environment

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Enteric viruses are widely spread in water environments, some being harmful for human communities, such as human Noroviruses Genogroup I (NoV GI) or Adenoviruses (AdV). Regular epidemics highlight the usefulness of analysing such viruses in wastewaters as a tool for epidemiologists to monitor the extent of their dissemination among populations. Despite an increasing number of studies dedicated to this topic, a lack of efficient and standardised techniques remains, especially in collecting viruses from complex media such as wastewater[1].

In this context, a mesoporous Powdered Activated Carbon (PAC) was chosen for its high porosity and high adsorption capacity to investigate sorbent ability to be used as part of virus detection probes. PAC-based probes were developed in our lab and used to prospect PAC efficacy in virus adsorption and above all, the feasibility of virus retrieval from them, allowing to further analysis such as molecular quantification[2].

Our results pointed out that PAC-based probes exhibited a high adsorption efficacy in virus suspensions (adsorbed viruses > 90%), with no material saturation within our experimental framework. Experiments on PAC probes reusability suggested that they should be used three times at the most for a maximum efficiency. Values of virus retrieval from PAC were low (recovered viruses = 11-14%), illustrating the need for the techniques to be improved. Finally, a preliminary field assay using PAC-based probe in wastewaters demonstrated that our catch-and-retrieve protocol yielded to the detection of autochthonous human NoV GI and AdV, suggesting its promising application as virus detection tool in such high loaded and complex waters.

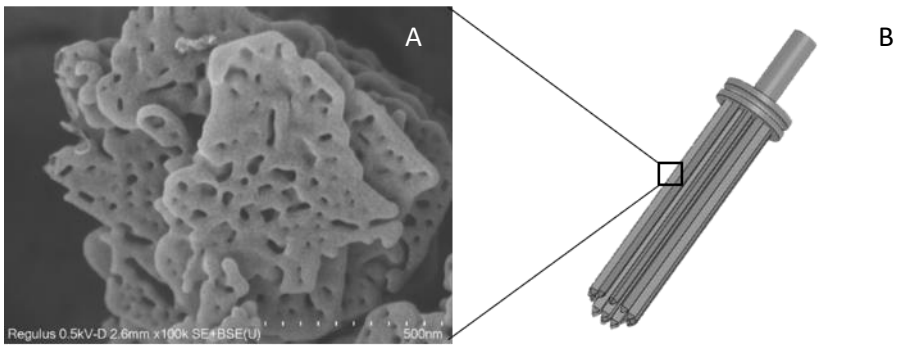

Figure 2. Scanning electron microscopy image of mesoporous activated carbon (A) and PAC probe design (B)

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Graphene aerogel for metal extraction

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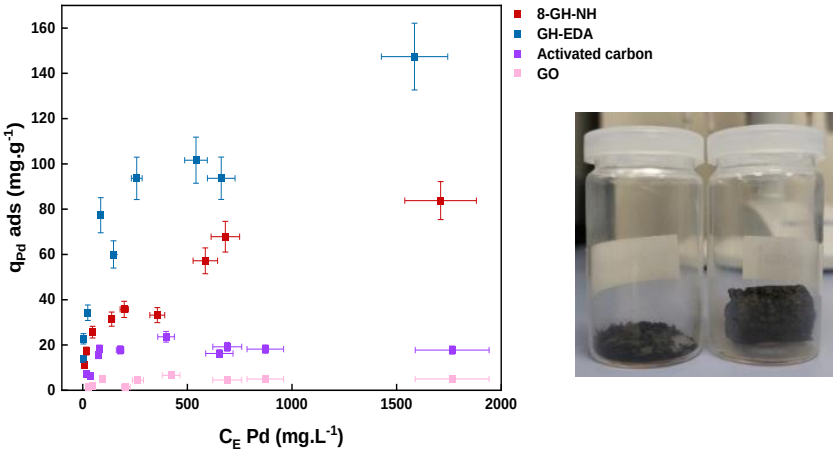
As the figure below shows, GH is a macroscopic and monolithic structure composed of carbon atoms. This monolith is obtained during a partial reduction of the graphene oxide (GO) by chemical or hydrothermal synthesis, leading to a self-assembly of 3D-graphene scaffolds. GHs present a large specific surface area (about 500 m^2/g) and a porous structure in the form of cavities. Due to their remarkable properties, such as high mechanical strength, thermal and chemical resistance, electrical conductivity and good adsorption capacity, GH have attracted a lot of interest currently, especially for applications as supercapacitor[1]. However, the use of graphene aerogels for the selective extraction of metallic species in aqueous solutions has been less explored.

During this study, the focus has been put on palladium (Pd), due to its supply risk and economic importance especially in the field of catalysis. Different organic and inorganic materials have been developed for the solid/liquid extraction of palladium, such as functionalized resins, silica, activated carbon or organic membranes[2]. The objectives of this PhD work are to synthesize various GH materials for the palladium extraction in nitric acid media, properly characterise and understand their physicochemical properties and extraction performance, correlate this to reference materials, and establish structure-property relationships.



Adsorption isotherms of functionalized GHs compared to reference adsorbents; Pictures of a graphene aerogel powder (left) and 3D-graphene aerogel monolith (right)

Two functionalization approaches have been studied to maximise the extraction performance and the stability of materials: a non-covalent approach allowing the attachment of lipophilic extractant molecules to the surface of the graphene aerogel by physisorption, and a covalent approach leading to a chemical link between the GO surface and the extractant motifs. The synthesis, functionalization, characterization and evaluation of various GH, particularly adapted for palladium extraction in nitric acid media, were successfully accomplished and show an enhanced affinity of functionalized GH towards Pd. The adsorption capacity of the materials has been determined in batch reactor upon varying the extraction parameters such as acidity, nitrate concentration, time and palladium concentration. The study puts in evidence the key role of surface functionalization of GHs on the Pd extraction efficiency compared to reference adsorbents. For further study, Pd extraction will be performed by a column in order to compare to the batch reactor results.

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ORAL PRESENTATION : AFTERNOON SESSION

Reconstruction of a genome-scale metabolic model of *Microchloropsis gaditana* with detailed lipid metabolism.

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Microchloropsis gaditana (formerly *Nannochloropsis gaditana*) is a promising microalga for biofuel applications due to its ability to accumulate a high level of lipids [1–3]. To optimize *M. gaditana* growth conditions and develop new strains with higher lipid yields, a broad level understanding of the organism's metabolism is required. Computational models such as genome-scale metabolic models can be used to unravel intracellular metabolic fluxes of a given microorganism and identify interesting strain design strategies [4,5].

In this work, we present iMgadit a new genome-scale metabolic model of *M. gaditana*, which encompasses 864 genes associated with 2289 reactions and 1956 metabolites distributed across eight compartments: extracellular, cytosol, chloroplasts stroma and lumen, endoplasmic reticulum, peroxisome and mitochondrial matrix and intermembrane space. Since *M. gaditana* is a promising strain for biofuels production, lipid biosynthesis and degradation pathways were exhaustively described in iMgadit and account for 44 % of model reactions: more than 1000 reactions in total. Our model includes two biomass objective functions representing biomass composition under two growth conditions: photoautotrophic growth with and without nitrogen starvation. Both biomass objective functions composition was defined based on previous published data [6] and experimental data particularly for lipid contents under nitrogen-rich and nitrogen-poor conditions. Model conformity to standards for formatting and consistency was assessed with Memote Test suite [7]. The model was then used to predict growth rates and flux distributions under several conditions: heterotrophy and photoautotrophy with different light intensities. Model predictions were validated with experimental data.

iMgadit constitutes a powerful tool to simulate and predict *M. gaditana* metabolism under diverse cultivation conditions. Based on iMgadit content, two-dimensional pathway maps were drawn. These maps provide a systems-level visualization of *M. gaditana* metabolism.

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Photonic and plasmonic properties of a nano-architecture assembled by DNA

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Quantum dots (QD) are small semiconductor nanoparticles, which exhibit photoluminescence (PL) properties thanks to electron-hole recombination. They are particularly robust to photobleaching, have high quantum yields (from few to several tens of percent) and have already found numerous applications in optoelectronic devices or in biological imaging as probes. However, QD suffer from a small extinction cross-section, leading to a small probability of interaction with an incident excitation light and to a limitation of their brightness.

On the contrary, other nanoparticles such as gold nanorods (GNRs) have high extinction cross-section and exhibit strong plasmonic effects, tunable from 515 nm to ~1400 nm depending on their dimensions.[1] This plasmonic effect is more intense at the GNR tips (hot-spots), where the incident electric field can be exalted from tens to hundreds of times depending on the shape and size of the particle.

In my PhD project, I synthesize and functionalize these nanoparticles with DNA strands[2] to realize a distance-controlled auto-assembly composed of a GNR linked with one or multiple QD at its hot-spots (Figure 1). This will allow to increase the QD excitation field and thus their PL intensity, and optimize the distance between these two particles through the modification of the length of the DNA strand. Once this study completed, we will explore the use of this auto-assembly for the detection of short DNA/RNA strands (miRNA), paving the way toward new biosensors and contrast agents based on nanoparticle assemblies.

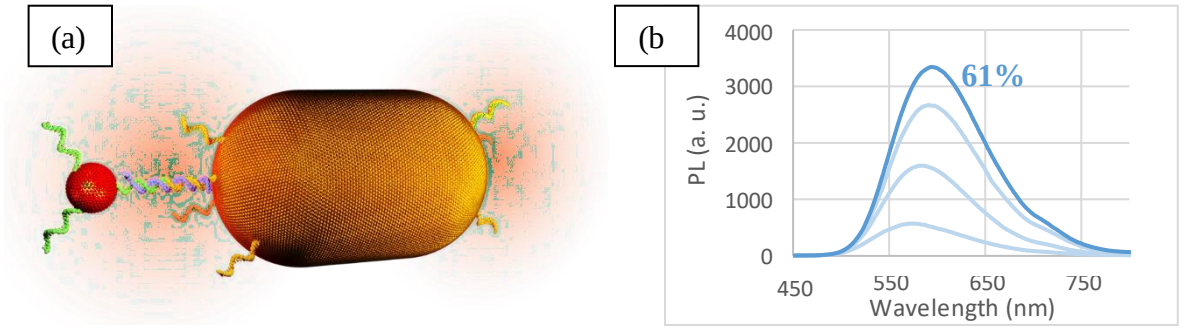

Figure 1. a) schematic representation of a QD placed at a GNR hot-spot using DNA, with the exalted electric field outlined in red light b) PL of the QD solution as synthesized (dark blue) with the corresponding quantum yield, and PL of successive aliquots taken during synthesis (light blue)

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Ionic Liquids 2.0: Designer Functional Soft Matter for Energy

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Ionic Liquids[1] (ILs) belong to a fascinating class of materials intensively studied and developed both for their fundamental interests and a hand full of technological applications due to their unique combination of *tuneable-by-design* properties such as low vapour pressure and flammability, high thermal and (electro)chemical stabilities, and ionic conductivity, to name a few. Encoding a liquid crystalline behaviour into the chemical structures of ILs allows for the dawn of stimuli-responsive (dynamically self-assembling/healing) functional materials, *i.e.* Thermotropic Ionic liquid Crystals (TILCs)[2]. Interestingly, the 'material marriage' of ILs with thermotropic liquid crystals (TLCs)[3] opens an exploratory research arena both for (i) in depth (fundamental) studies of the interplay linking the hierarchical self-assembly of functional soft matter into precise morphologies and (1D/2D/3D) dimensionality-controlled ionic transport properties and (ii) their technological uses as a key-enabling sub-component (*i.e.* the electrolyte) at the heart of new generations of *safer-by-design*[4] (electrochemical) energy storage and conversion devices such as batteries[5] and supercapacitors, and fuel cells, respectively.

In this communication, we will detail the molecular design strategy, syntheses, and multi-scale structure/ionic transport correlations of a series of anionic conductors (A-TILCs) based on a di-*n*-octadecylimidazolium ($C_{18}C_{18}Im^+$) cation with 4 anions, namely iodide [I^-], bromide [Br^-], bis(trifluoromethane)sulfonimide [$TFSI^-$], and dicyanamide [$N(CN)_2^-$]: See **Fig. 1**. Relying on cross-fertilizing DSC, POM, and SAXS/WAXS characterizations, we will show that this series of A-TILCs share a lamellar organization (smectic A (SmA) mesophase) with ionophobic and ionophilic slabs encoding *tuneable-by-design* and nanoconfined 2D ionic transport. As reflected in their tuneable transition temperatures and ionic conductivity values (EIS), we will discuss how anion metathesis is authorizing the fine-tuning of A-TILCs. Finally, we will elaborate on the role of selected stimuli (*e.g.* electric/magnetic fields, light, *etc.*) to master mosaicity *vs.* long range order in TILCs (through *on-demand* control of their dynamic self-assembly) and the (growing) interest for self-healing into next generation energy storage/conversion devices.

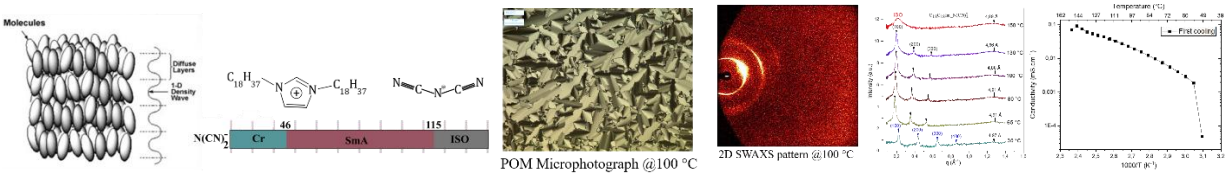

Fig. 1: A-TILC/$C_{18}C_{18}Im^+/N(CN)_2^-$. <u>Left Panel</u>: Schematic representation of the supramolecular organization of its SmA mesophase, its temperatures of phase transitions, and its POM microphotograph @100°C featuring a paramorphotic truncated focal-conic fan (homogeneously aligned) texture (The scale bar is 190 μm). <u>Right Panel</u>: SAXS/WAXS 2D pattern @100°C and 1D profiles @150, 130, 100, 80, 65, and 30°C and temperature-dependent ionic conductivity (EIS data).

<u>Acknowledgments</u>: We are indebted to the French National Agency of Research (ANR) for the funding of the collaborative research project **CITADEL** under the grant <u>ANR-19-CE05-0028</u>. HP, MM & PR express their gratitude to CNRS & UGA for the support of the related research activities within the (Joint Research Unit) UMR5819-SyMMES & UMR5279-LEPMI labs.

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Fighting oxidative stress thanks to Ni-SOD mimics

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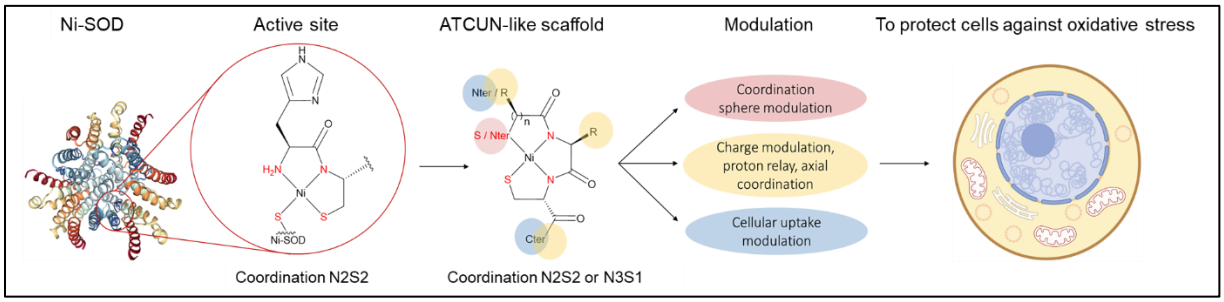
During the Great Oxidation event, 2.5 billion years ago, cyanobacteria started the production of huge amounts of dioxygen. Thus, living organisms of the time had to deal with the emergence of this species, at the origin of oxidative stress. Evolution endowed them with several strategies including protective enzymes in charge of the detoxification of Reactive Oxygen Species (ROS) from cells.[1] Among them, Ni-SOD is specialized in the capture and consumption of superoxide ($O_2^{\bullet-}$). Ni-SOD is a prokaryotic redox enzyme, which catalyses the dismutation of $O_2^{\bullet-}$ into H_2O_2 and O_2, thereby assisting cells to maintain optimal intracellular ROS concentrations.[2]

The remarkable catalytic activity of this enzyme prompted us to design small biomimetic Ni^{II} complexes with antioxidant properties as therapeutic agents for the numerous diseases causes by oxidative stress. These bio-inspired complexes also represent interesting tools to decipher the catalytic mechanism of this metalloenzyme.

The structure of Ni-SOD active site was mimicked by Ni^{II} complexes based on an ATCUN-like scaffold.[3] This motif enabled easy chemical modulation, formation of stable Ni^{II} complexes with square-planar geometry, and biocompatibility for future cellular assays. The complexes designed in this work show an intrinsic superoxide-dismutase activity, which can be tuned by modulating parameters such as coordination sphere, charge, presence of a proton relay, etc. The effects of these modulations on the catalytic activity will be discussed in the presentation.



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Looking for new plasma biomarkers of Non-Alcoholic Fatty Liver Disease (NAFLD) progression using discovery and targeted proteomics

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Liver diseases are a major global health problem, causing more than 2 million deaths per year worldwide[1]. Most liver diseases result from viral infections, alcoholism and NAFLD (Non-Alcoholic Fatty Liver Disease). NAFLD is highly prevalent (25% of the global population worldwide [2,3]) and closely associated to the rise of obesity in the world[1]. Patients with NAFLD may progressively develop NASH (Non Alcoholic Steato-Hepatitis) requiring liver transplantation in the most advanced stages[1]. Liver biopsy remains the reference examination to detect NAFLD, but it is an expensive, invasive and unusable examination for massive screening[3]. Although a large number of potential biomarkers has been discovered for NAFLD patient stratification, none of them is presently able to reach the performance of liver biopsy and histopathological examination. The development of non-invasive tests to diagnose NAFLD patients from the earliest stage is still a crucial need.

As a contribution to the field, we carried out a LC-MS/MS proteomic discovery study using plasma samples (n=160) from NAFLD patients diagnosed at Grenoble hospital hepatology department and classified into five groups of disease severity (fibrosis progression), according to liver biopsy and histopathological examination. This discovery study led to the identification of 114 plasma proteins with differential abundances between the five groups of NAFLD patients.

Among these 114 putative biomarkers for patient stratification, we selected 15 proteins for further evaluation based on two criteria: (1) a specific expression in the liver and (2) an absence of previous description as NAFLD biomarker in the literature. Then, these 15 proteins were precisely quantified in the 160 plasma samples obtained from NAFLD patients. For this purpose, a list of 33 signature peptides was established and a scheduled SRM assay was optimized using AQUA peptides as quantification standards. These quantitative analyses confirmed two plasma proteins as potential biomarkers to discriminate the severity stages of NAFLD.

In order to verify the relevance of our results, a validation study targeting these two plasma proteins will be launched by SRM and ELISA (enzyme-linked immunosorbent assay) in an independent cohort of NAFLD patients.

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FLASH COMMUNICATION: AFTERNOON SESSION

Integrated Bioelectrodes/Biopolymer-Microneedle Devices for Transdermal Electrochemical Sensing

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Abstract: Transdermal detection devices have attracted considerable interest owing to their tremendous promise for wearable and rapid monitoring of personal health. Selective and sensitive amperometric biosensors exploiting enzymes such as glucose oxidase (GOx) with needle devices have already revolutionized the treatment of diabetes[5]. Technical challenges for in/on-vivo devices generally include cost, accuracy, biocompatibility, invasivity and patient comfort, as well as stability limitations inherent to enzymatic electrodes[2]. Herein, we describe an original 2nd generation glucose biosensor employing the emerging FAD-dependent glucose dehydrogenase[3] (FAD-GDH) and a physically adsorbed electron transfer mediator on a carbon nanotubes electrode integrated with photochemically cross-linked dextran-methacrylate (Dex-MA). A series of Dex-MA with different degrees of substitution have been synthesized and their performance after cross-linking revealed attractive mechanical properties e.g. ability to pierce materials mimicking the human skin, and good swelling properties. Moreover, the beneficial effects on bioelectrocatalytic and biosensing performances clearly revealed that dextran hydrogels provide a favourable microenvironment for biosensing, keeping the high selectivity of the sensor. Finally, we will briefly present our first steps towards the development of polymer microneedle devices for transdermal sensing.

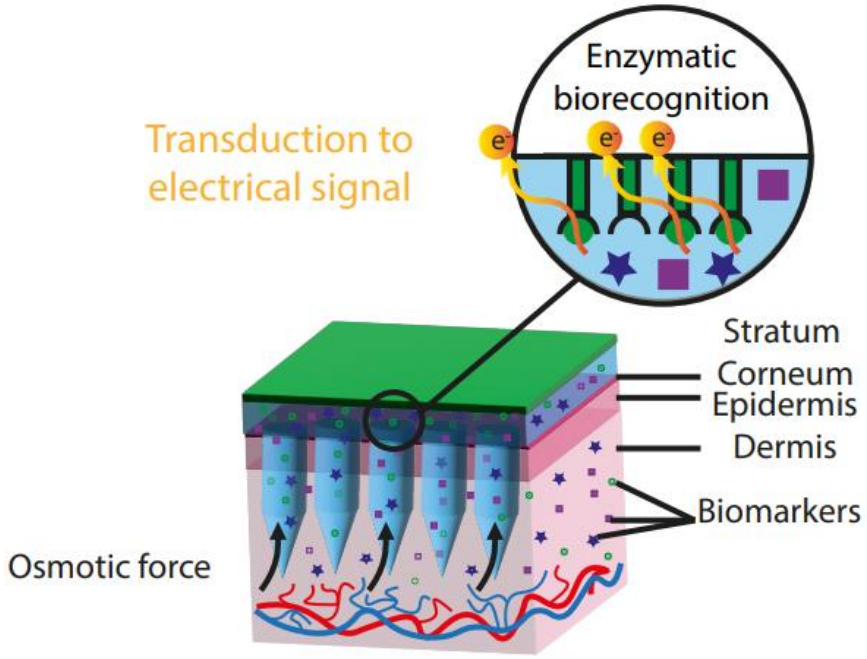

Figure 1: Integrated amperometric biosensor to cross-linked Dex-MA MNs patch.

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Inducing osteogenesis of mesenchymal stem cells through the use of mechano-bactericidal nanostructured titanium

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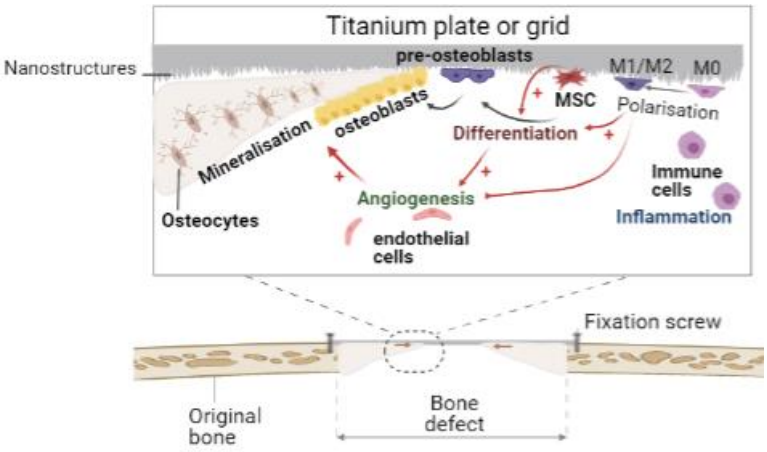
In recent years, much progress has been made in the development of bone substitutes to meet clinical needs for the reconstruction of large bone fragments. Despite these advances, most failures in bone graft surgery are related to nosocomial infections. Preventing the occurrence of such infections on the surface of implantable biomaterials while providing better osteoinductive properties is a real challenge. In this context, our collaborator at RMIT University has developed two different types of nanotopographies on the surface of titanium, which provide titanium an intrinsic bactericidal effect[1]. In collaboration with this laboratory, our team has shown that these mechano-bactericidal nanostructured biomaterials not only allow adhesion and proliferation of mesenchymal stem cells (MSCs) but also exhibit osteoinductive properties (i.e. the ability of a material to induce the differentiation of a cell into a bone cell)[2]. Therefore, our mechano-bactericidal titanium surfaces with both bactericidal and osteoinductive properties have emerged as promising alternatives for improving the success rate of bone reconstructive surgery. MSCs are highly responsive to mechanical stimuli and their fate depends on the topography of the support. Indeed, our results suggest that nanostructures on the surface of a scaffold can control mechanotransduction pathways, ultimately leading to the differentiation of MSCs into bone-forming osteoblasts. We are currently studying the signaling pathways induced by our different nanostructures. This would help us to optimize the nanotopography of our titanium supports to improve their osteoinductive properties. We will also test the efficacy of our titanium in aiding tissue repair in an animal model of induced critical bone defect.



Schematic view of the expected synergistic effect for bone regeneration of our nanostructured titanium in physiological condition

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Understanding the assembly of the nitrogenase active site: toward a greener approach to nitrogen fixation

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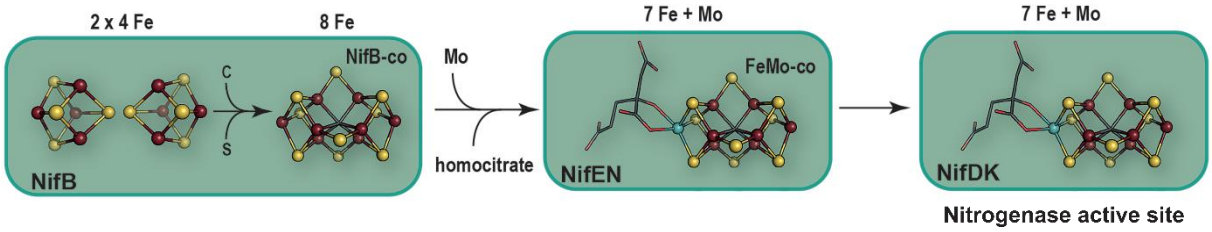
Reduced nitrogen in the form of ammonia (NH_3) is fundamental to all life and many industrial processes. The Haber-Bosch process, which converts hydrogen and nitrogen to ammonia, made ammonia fertilizer widely available and significantly increased crop yield in a short time. However, it is an exceedingly energy-demanding process that requires high temperature and pressure, largely driven by fossil-fuel and leaves a massive carbon footprint embedded in all the different products that come through this process[1].

Interestingly, nature have always been a huge player in nitrogen fixation, yet in a more sustainable and seemingly effortless way. Indeed, about half of the nitrogen intake of the human body comes from an enzyme called nitrogenase – the only enzyme known to be capable of reducing N_2 to NH_3 at ambient temperature and pressure. The FeMo-co active site of nitrogenase is a [$MoFe_7S_9C$-*R*-homocitrate] center – perhaps one of the most sophisticated metalloclusters that exist in nature. It is synthesized by different accessory proteins that constitute the NIF (NItrogen Fixation) assembly machinery[2]. NifB is considered as the key enzyme in this mechanism because it is responsible for the fusion of two [Fe_4S_4] centers, combined with a carbide ion insertion and the addition of a sulfide ion to produce a [Fe_8S_9C] precursor termed NifB-co[3]. By combining X-ray crystallography, spectroscopy and *in vitro* analyses, we have identified the presence of a unique 8-Fe intermediate prior to the formation of the NifB-co[4]. In addition, the scaffolding protein NifEN also plays an important role in the machinery as it receives NifB-co from NifB protein and tailors the cluster into the final nitrogenase cofactor FeMo-co[3]. Using computational modelling approach, we were able to predict some interesting interactions between NifB and NifEN. This result can be combined with practical structural study and functional analysis of the NifEN-NifB complex to elucidate the intermolecular interactions between these components, as well as define the order of mechanisms involved in components recruitment and cluster transfer.

This study provides a deeper understanding of the biosynthesis of the nitrogenase active site, leading us one step closer to developing more efficient catalysts to produce ammonia through inspiration given by the unique properties of this enzyme.



Schematic illustration of the biosynthesis of the FeMo cofactor of nitrogenase

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STUDYING THE PHOTOACTIVE PROTPERTIES OF THE ORANGE CAROTENOID PROTEIN USING SERIAL X-RAY CRYSTALLOGRAPHY AND NMR

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In many species of cyanobacteria, large pigment-protein complexes called Phycobillisomes (PBS) employ a variety of pigments to harvest blue-green light, convert it into excitation energy, and funnel into the Photosystem II (PSII) reaction centers to initiate the first steps of photosynthesis. However, under strong light conditions, the pigments of the PSII and PBS can remain excited for too long and transition to their triplet excited states. These triplet states cause the pigments to fluoresce, and interact with molecular oxygen to produce $^1O_2$, a potent oxidizer responsible for mutation and cell death.

The water-soluble Orange Carotenoid Protein (OCP) is present in a wide variety of cyanobacterial species where it mitigates PBS fluorescence under high-irradiance conditions through a process called non-photochemical quenching. Structurally, the 35kDa protein features an all alpha-helical N-Terminal Domain (NTD) (residues 1-165), an alpha-helix/beta-sheet C-Terminal Domain (CTD) (residues 190- 317), ~25 unstructured linker residues which join the domains together, and a carotenoid as it's chromophore[1]. In dark conditions, OCP is orange and inactive, known as OCP^O, but in the presence of blue-green light, the protein photoactivates into its active red state, OCP^R. Once active, the NTD and CTD separate, connected only by the linker region, and the NTD acts as the effector domain, binding to the PBS such that the carotenoid can then quench excess excitation energy which is dissipated as heat [2], before recovering back to OCP^O. However, in order to ensure that excitation energy is not quenched in normal light conditions, the OCP has a noticeably small quantum yield of under 0.3% as many structural steps must be followed from the transition of OCP^O to OCP^R. This has presented many difficulties in studying not only the photocycle of the OCP following photoactivation, but also the structural characterization of the active OCP^R state. The OCP has found itself subject to much interest as of recent, as it's light-driven domain dissociation marks it as a desirable candidate for potential use in the field of optogenetics, as well as future applications in the optimization of photosynthetic pathways.

My thesis project aims to study the photocycle and active state dynamics of the OCP through several biophysical and biochemical means. Currently, we are designing mutants of OCP with altered photophysical properties to identify the roles and relationships of critical residues in the structure. We are using macromolecular crystallography to obtain static structures of these mutants, through which we establish ties between structure and function. We are also employing kinetic and serial crystallography, at synchrotron sources and eventually XFEL facilities, to trap intermediate states which follow photon absorption, allowing us to structurally characterize the much-debated photocycle. OCP presents itself as a difficult candidate to study by NMR, but we nevertheless hope that this technique will provide structural information on the elusive active OCP^R state. Understanding both the photocycle and active state dynamics are crucial for designing an OCP variant capable of reaching its potential in the applied biotechnological sphere.

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Role of contractility in the chiral swirling of endothelial cells

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Chirality, defined as the property of an object not being superimposable to its mirror image, is a biologically conserved feature, with critical implications in diverse physiological processes, such as tissue morphogenesis and embryonic development. More recently, it has been shown that individual cells and cell collectives exhibit spontaneous chiral symmetry break when migrating or when constrained on confined circular surfaces[1–3]. Despite the lack of a clear mechanism explaining the manifestation of this intrinsic property, several studies point at a key role of the actomyosin cytoskeleton in this phenomenon[2–4]. In particular, it has been demonstrated that network contraction and connectivity are key determinants of chiral cell alignment and cell rotation headedness[2,5,6]. However, the exact contribution of contractile forces to the emergence and maintenance of cellular chirality remains to be elucidated.

Our project thus focuses on the role of contractility in establishing chirality among endothelial cells. By confining cells on disk-shaped micropatterns of different sizes, we first show that both single and double cells undergo persistent chiral swirling. Focusing on cell doublets, we additionally demonstrate that both the rotation and the expression of this chiral phenotype are strongly dependent on the contractile forces produced by the actin cytoskeleton. In particular, our results suggest that an optimal amount of forces is required to maintain the stable chiral bias observed within the population. Any deviation from this optimum impairs both the rotation of the cells and their bias, resulting in, either its enhancement or complete reversion. Finally, our preliminary traction force measurements reveal specificities in the force profiles of clockwise and counter-clockwise rotating cells.

Taken together, these results suggest that the balance of forces exerted within the cell doublet and their total magnitude could represent important factors governing the emergence and maintenance of cellular chirality.

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Subject: Mutated calcium channel in Exertional Heat Stroke

Exertional Heat Stroke (EHS) is a life-threatening disease that occurs in young individuals who engage in prolonged and strenuous activity. It is characterized by an elevation of the core body temperature with a dysfunctions of the central nervous system (from confusion to coma) and is a leading cause of sudden death in athletes. EHS shares several pathophysiological characteristics with malignant hyperthermia (MH), a pathology triggered by volatile halogenated anesthetics, among which an abnormal in vitro contracture test of the skeletal muscle[1]. The underlying causes of MH are dysregulated calcium metabolism in skeletal muscle due to mutations in genes coding for proteins involved in the ECC (Excitation Contraction Coupling) process. Despite strong similarity with MH [2], EHS is still poorly characterized and its genetic causes are still largely unknown.

Genetic predisposition to EHS was investigated in our lab by whole exome sequencing on a cohort of soldiers. Four unrelated patients were found to harbor genetic variants of the same gene, *CACNA2D4*, a voltage-gated L-type channel regulatory subunit, which is an isoform of one of the gene involved in the ECC complex. In the first part of my thesis work, I have explored whether *CACNA2D4* is expressed in skeletal muscle and if the genetic variants found related to EHS have an impact on the skeletal muscle L-type Calcium channel.

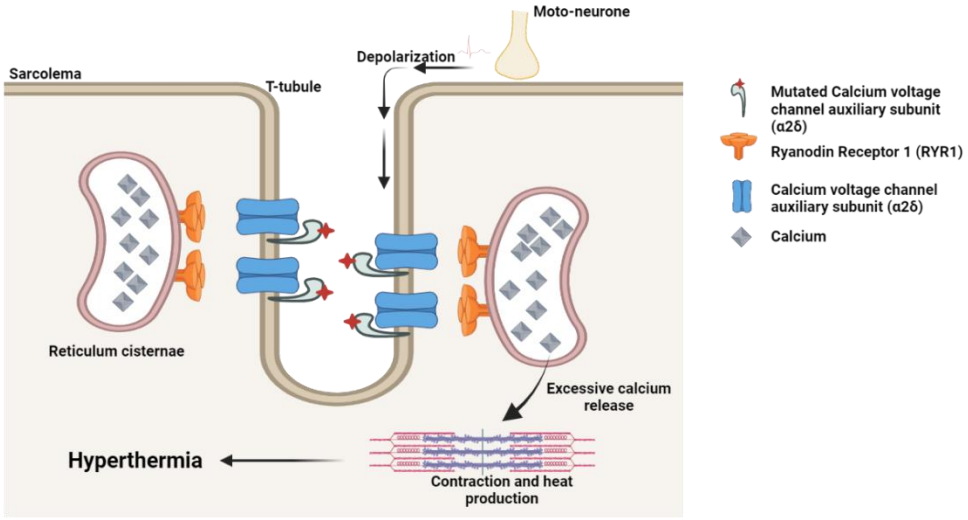

Figure : Excitation Contraction Coupling (ECC) in skeletal muscle.

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Thermotropic Liquid Crystals meet Electrochemical Energy Storage for enabling Self-healing Lithium Metal Batteries

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Fulfilling the United Nation Sustainable Development Goal N°7 (UN SDG 7) and aiming at a carbon-neutral European continent by 2050 (EU Green deal) are calling for higher energy density and *safer-by-design* batteries. Substituting graphite by Li metal (Li°) for the negative electrode in Li-Metal Batteries (LMBs) is opening doors to an effective way to increase (*by more than one order of magnitude*) the energy density, but it is not enough. Indeed, the seminal carbonate-based liquid electrolytes which enabled of the 45 year-long technological and commercial success story of LiBs (Nobel Prize in Chemistry 2019) are not suitable for LMBs, which are plagued by the growth of Li° dendrites[1], causing deleterious consequences shortening the lifespan of this type of energy storage device (see **Fig.1**). Liquid electrolytes present indeed a series of drawbacks decreasing the coulombic efficiency of batteries till posing serious safety concerns (leakage, fire, etc.) that need to be solved. Thermotropic Ionic Liquid Crystals (TILCs), i.e. the fusion of dynamically self-assembling thermotropic liquid crystals (TLCs) with ionic transport properties of ionic liquids (ILs) are promising surrogates of their liquid analogs for LMBs. TILCs are indeed emerging as designer soft-matter electrolytes for electrochemical devices[2] due to their unparallel abilities to encode, by molecular engineering, 1D, 2D & 3D ionic transport of cat/an-ions through stimuli-responsive nanostructures, paving the way toward self-healing LMBs. Strikingly, TILCs are currently surging as 2.0 solutions to the long-standing Li dendrite challenge through both theoretical[3] and experimental[4] *proof-of-concept* demonstrations under a LMB configuration.

In this communication, we will unfold how one can synthesize and formulate TILCs by the simple mixing of a TLC matrix[5] with a series of Li salts, leading to advanced electrolytes featuring a smectic A mesophase. Through detailed structure/property correlations (DSC, POM & SAXS/WAXS characterizations) we will discuss how Li salts influence both the dynamic self-assembly and thermal transitions of TILCs (see **Fig.1**). Advanced electrochemical characterizations (CV & EIS) will be leveraged to report performances resulting in an electrochemical stability window reaching 3.8-3.9 V vs. Li^+/Li, ionic conductivity of 0.2 $mS.cm^{-1}$ @ 120°C, and Lithium transference numbers $t^+_{Li} > 0.50$.

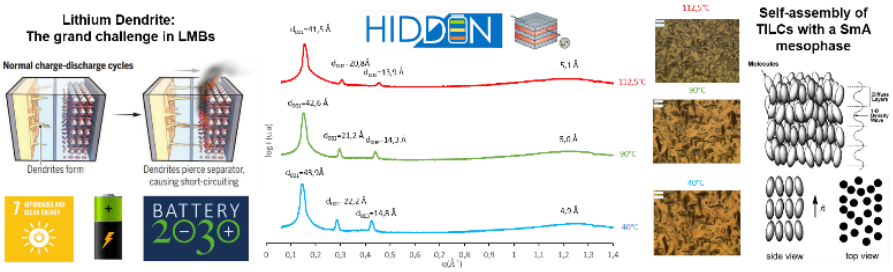

Fig.1: left panel: growth of Li dendrites plaguing LMBs when cycled, ultimately resulting in piercing of the separator, short-circuits and thermal runaway. Central & right panels: SAXS/WAXS 1D patterns and POM microphotograph (focal conic fan textures) recorded at 112.5°C, 90°C & 40°C and schematic representation of a smectic A (SmA) mesophase

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Photo-switchable electrodes modified with dithienylethene-based coordination polymer

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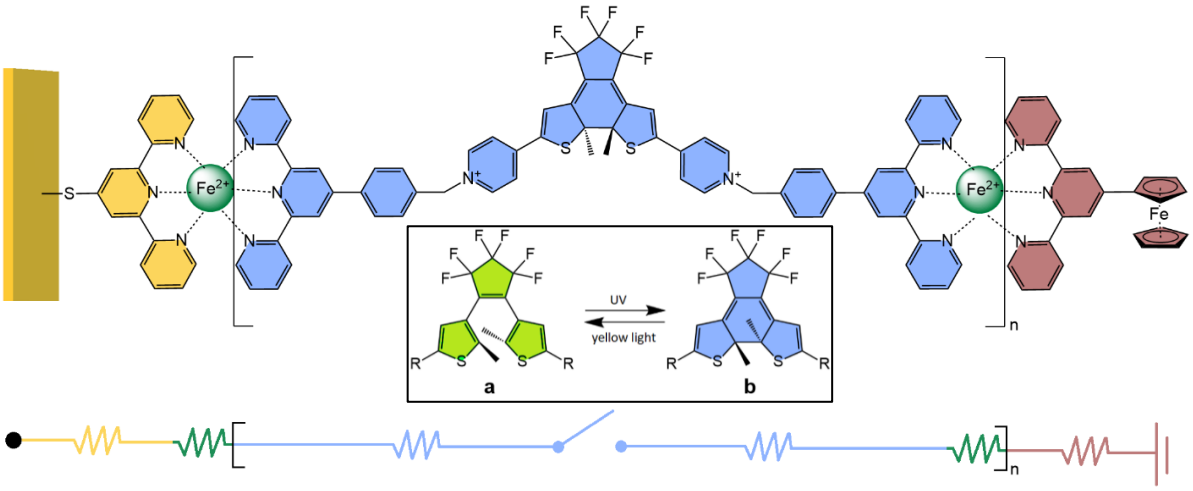
Switchable films consist of molecules whose properties may be reversibly tuned by external stimuli such as electromagnetic irradiation, electric field, pH, or thermal driving[1]. Dithienylethene (DTE) is a good candidate for the design of photochromic devices. Irradiation with UV light allows the interconversion between the non-conjugated open form to the fully conjugated closed form, while the irradiation with visible light (yellow or red) converts it back to the open form. The change in the conjugation causes a characteristic change in the UV-VIS absorption spectra[2]. In this experiment we functionalised a gold electrode layer-by-layer with anchoring ligand, iron(II), bis-terpyridine DTE ligand (repeated several times to obtain layers with different thicknesses) and ferrocene-terpyridine terminal group, as shown in the Figure.



A schematic representation of a molecular circuit with a substrate functionalised with anchoring terpyridine group (yellow), DTE bitopical ligand (blue) and a ferrocene probe (brown). Inset: open (a) and closed (b) state of the DTE unit. The colours on the graph represent the actual colour of the solutions of the compounds.

In the experiment the functionalised substrate served as the working electrode. The transfer coefficient and the oxidation and reduction rate of the ferrocene probe were determined using the Laviron's theory which relates the variations of the anodic and cathodic peak voltage during linear potential sweep voltammogram with the sweep rate.[3] Two important features were observed. Firstly, with the increase of the number of layers, and thus, the thickness, the electron transfer ratio exponentially decreases with a constant beta attenuation factor. Finally, the electron transfer ratio was reversibly photo-modulated by exposure to UV (opening of the DTE) and yellow light (closure of the DTE).

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POSTER ABSTRACTS

Adsorption of therapeutic proteins to material surfaces encountered during manufacturing, storage, and administration

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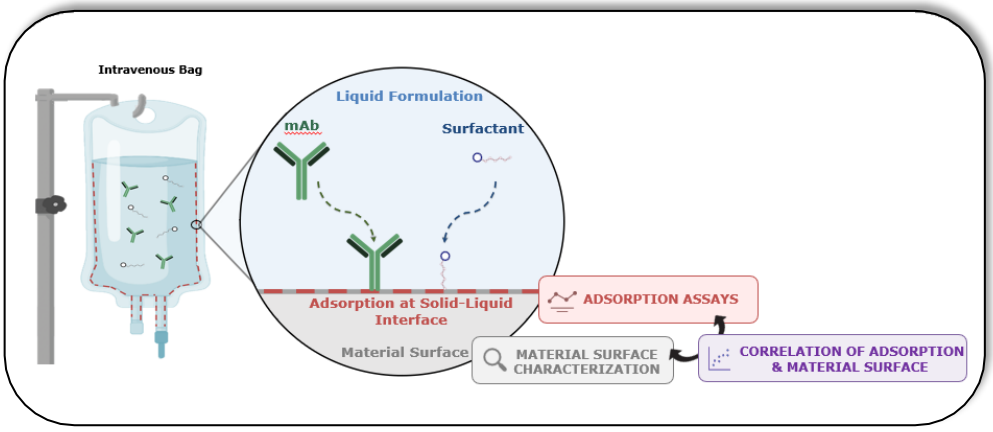
Monoclonal antibodies (mAbs) are therapeutic proteins exposed to different solid-liquid interfaces during production, purification, storage, and administration. The risk of surface interaction can lead to protein concentration loss and protein aggregation, with potential immunogenic effects on patients[1]. The behaviour of protein adsorption at solid-liquid interfaces will depend on the whole system, including the surface in contact, the solution excipients, and the protein[2]. Surfactants are used to prevent mAb adsorption on surfaces, polysorbate 80 (PS80) being a commonly used surfactant in therapeutic protein formulations[3].

It was recently demonstrated, using plastic model surfaces (96 well-plates), that surfactant protection efficacy to prevent mAb adsorption depends on the surface material[4]. However, are model surfaces representative of in-use medical plastic surfaces, (e.g. intravenous plastic bags)? How to measure mAb adsorption directly on medical plastic surfaces? Which are the correlations between material surface, surfactant protective efficacy and mAb adsorption?

The aim of this project is to develop fundamental knowledge in protein-material interactions, studying the adsorption of mAbs and surfactants at the solid-liquid interface, considering the complexity of materials, formulation excipients and the reversibility of adsorption. The novelty of the project is to investigate the adsorption directly on medical plastic surfaces, which, combined with the detailed surface characterization, will contribute to the development of a screening protocol for protein adsorption on plastic materials, beneficial to study protein stability at early stages during drug development.

Figure 1: Intravenous plastic bag showing the adsorption of monoclonal antibodies (mAbs) and surfactants at the solid-liquid interface.



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Influence of wind-induced snow transport on soil temperature and vegetation in alpine meadows

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Soil thermal regimes in the root zone have a considerable influence on plant life in alpine grasslands and thus are often use to derive useful bioclimatic variables . However, snowcover variability at small and meso-scale in mountainous environment has a huge influence on soil temperature and plant spatial distribution, and is strongly driven by snow transport by wind. Taking advantage of growing computational resources, operational systems simulating snowpack evolution at mountain range scale currently tend to move toward hectometric resolution in order to enhance the representation of this local variability, and to represent processes causing it. It may also allow to enhance simulations of soil thermal regimes performed with those models. Soil thermal regimes computed with the ISBA soil scheme coupled to Crocus, in it's current operational use in the Alps, are evaluated against a large dataset of measurements in open areas in the 2100-2800m altitude range. A strong cold bias is observed in summer is observed, whereas simulated winter temperature show no correlation with observations. We present then a model predicting snow transport by wind coupled to the state-of-the-art snow model Crocus and designed to allow the simulation of snowpack evolution at a resolution of 250 m on the entire french Alps during several years. The intermediate resolution which is used and the need for computational efficiency leaded to the choice of a simple scheme decoupled from the atmosphere. The effect of this model on simulated soil thermal regimes is evaluated.

Impedimetric aptasensors: fiding the optimal immobilization system for anti-adenosine aptamer

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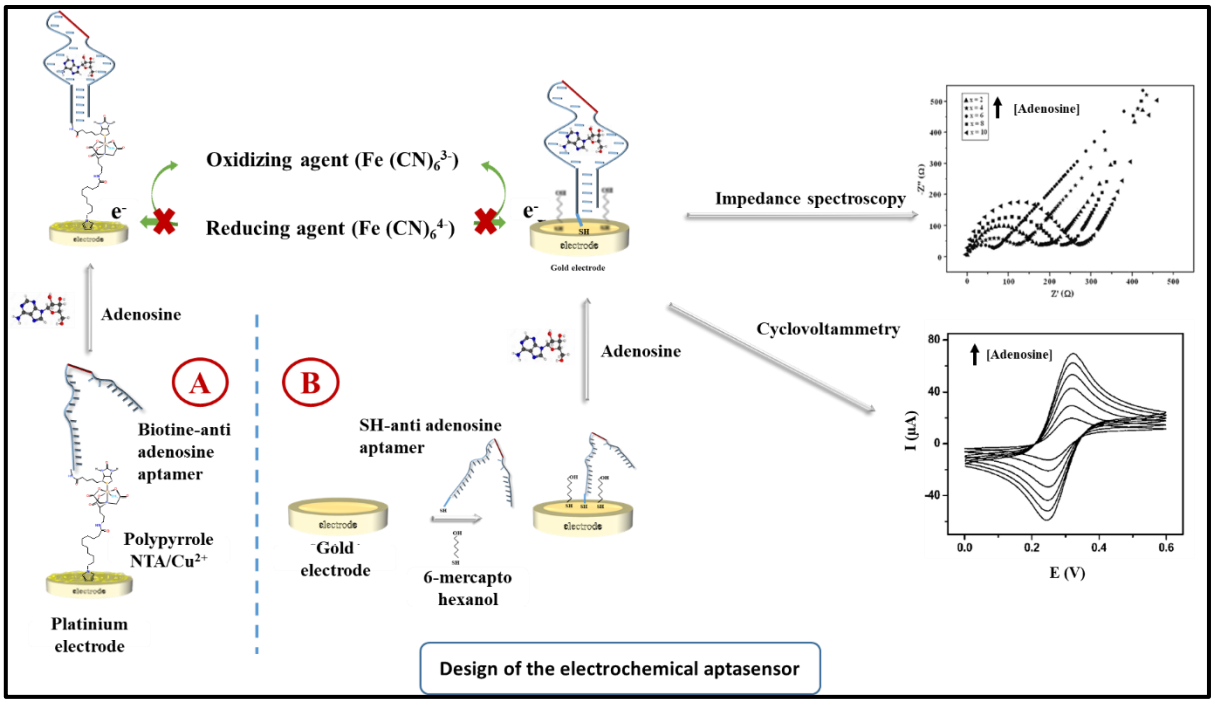
Aptamers are single-stranded DNA or RNA sequence capable of binding a large spectrum of molecules. They have gained widespread attention as biorecognition elements and are an attractive alternative to antibodies, especially for small molecules [1]. On the other hand, electrochemical biosensors became essential tools for biomarkers detection due to their high sensitivity (detection limits to the femtomolar level [2]) and the possibility to be miniaturized into portable devices. Despite these advantages of both the recognition element and the transduction method, the crucial step for the design of this type of biosensor remains the immobilization of the aptamer. Here we compare polymer-based (A) and gold electrode-based (B) methods of immobilization of the anti-adenosine aptamer for the elaboration of a femtomolar and selective adenosine aptasensor



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Aptamer Switches Regulated by Post-Transition/Transition Metal Ions

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Aptamers are synthetic single-stranded oligonucleotides that exhibit high binding affinity and specificity towards their target. They are exquisite recognition elements for elaborating molecular biosensing platforms, based in most cases on the structural switching principle. However, the existing aptaswitches have some limitations that can include aptamer structural pre-requisites, tedious engineering and slow response kinetics.

We exploited the properties of transition/post-transition metal ions to induce, through their coordination to nucleobases, significant disrupting effects on nucleic acid architectures. We conceived a small-molecule sensor simple to design and easy to use, based on the DNA switching activity from an inactive, metal ion-complexed state to an active, target-bound conformer. The sensing approach relied on the single labeling fluorescence anisotropy readout mode.

Using Pb^{2+} and Cd^{2+} to induce switching, we were able to demonstrate the feasibility of such a sensor with several small molecules and with several aptamers of different structures. This method is fast and inexpensive. We were also able to demonstrate the potential applicability of this method in complex media (urine matrix) for L-Tyrosinamide and cocaine.

We envision that our scheme could be easily adapted to a variety of readout methods, especially electrochemical ones.

Impact of intermittent hypoxia on ischemic cardiomyopathy progression:role of insulin and adrenergic cross-talk

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Background Patients with obstructive syndrome apnea (OSA) exhibit poor prognosis after myocardial infarction (MI). Intermittent hypoxia (IH), the hallmark feature of OSA, promotes sympathetic hyperactivity, hyperinsulinemia and insulin resistance, and has been identified as a major contributing factor of post-MI cardiac remodeling and contractile dysfunction. Here, we hypothesize that activation of sympathetic nervous system and insulin resistance by IH induces desensitization of cardiac adrenergic and insulin signaling pathways that contribute tosubsequent ischemic cardiomyopathy aggravation.

Methods MI is induced in C57bl6 mice by permanent ligation of the left coronary artery. Mice were then randomized to IH (21–5% FiO2, 60 s cycle, 8 h/day) or normoxia for up to 6 weeks. Longitudinal follow-up of mice is performed through evaluation of systemic insulin sensitivity (dynamic insulin tolerance test), evaluation of cardiac sympathetic activity (spectral analysis of heart rate variability (HRV) and determination of cardiac function/remodeling (echocardiography, RT-qPCR, histology). At the completion of IH protocol, cardiac interstitial fibrosis and hypertrophy are evaluated by RT-qPCR and histology (Sirius Red and WGA staining, respectively). Assessment of adrenergic and insulin signaling pathways and there cross-talk are performed by Western blot.

Results IH induces an aggravation of MI-induced cardiac contractile dysfunction in miceassociated with significant decrease in ejection fraction (19,4±2,1% vs 33,3±3,9% in IH vs. N respectively, $p<0,05$). In both N and IH groups, MI induces significant cardiac remodeling characterized by upregulation of several markers of cardiac fibrosis (Col1a1 and Col3a1) and hypertrophy (Acta1 and MyH7), which tend to be higher in IH compared to N. In our MI model,IH does not induce significant systemic insulin resistance, tend to increase LF power with no significant modification of LF/HF ratio compared to N mice. Histological experiments will aimat confirming IH-induced aggravation of post-MI cardiac remodeling and ongoing Western blot will determine whether IH specifically affects insulin and adrenergic signaling.

Conclusion These results demonstrate that IH is responsible for aggravation of contractile function in our mice model of ischemic cardiomyopathy. On-going experiments will determinewhether alteration of adrenergic and insulin signalling could be involved as a contributing mechanisms.

Key Words: Obstructive sleep apnea, intermittent hypoxia, ischemic cardiomyopathy, sympathetic activity, insulin resistance.

Brain connectivity in Huntington's disease

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Huntington's disease (HD) is a neurological disorder characterized by the dysfunction and loss of neurons predominantly in the striatum and the cortex. It is caused by an abnormal CAG expansion in the first exon of the *huntingtin* gene, which results in a polyglutamine expansion in the N-terminal part of the huntingtin protein (HTT)[1]. HD manifestations appear in adults, but growing evidence suggests a developmental contribution to HD[2]. Neuronal tracts are affected in early-stage HD patients such as the corticospinal tract or the fornix, but no evidence has been found yet in mouse models of HD[3].

Using brain clearing approaches, we are investigating if neuronal tract defects are established during development in the CAG140 mouse model of HD. Our preliminary observations suggest that the development of tracts such as that of the anterior commissure and the post-commissural fornix may be altered during postnatal development. We also found a striking difference in brain volume of 3 months old mice as the brain of females CAG140 mice is smaller than their wild-type counterparts. This difference is left to investigate to see if it is due to a global reduction of the brain or if some structures are more affected than others.

As the establishment of neuronal connectivity relies on growth cone dynamics, we are studying this process in cortical neurons *in vitro*. The lab has already proven that neuronal growth is limited in another mouse model of HD as microtubular structuration is defective in the growth cone[4]. Using microfluidic devices developed in the lab, we are showing that growth cone dynamics are also disrupted in CAG140 cortical neurons, as the growth cones are less stable than the control ones.

Overall, our project will describe the mechanisms underlying early alterations in circuit connectivity in the HD brain.

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« 3D electron microscopy as a tool to assess the metabolism of symbiotic microalgae »

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Symbiotic associations between a host cell and intracellular photosynthetic cells (microalgae) are widespread in aquatic ecosystems and were key in the evolution of eukaryotes. In this work, the study of the cellular architecture the free-living and symbiotic microalgae (Chlorella) was addressed using a FIB-SEM-based 3D reconstruction to assess the physiology and metabolism of the algal cells. Live cells were cryofixed with high pressure freezing, freeze substituted and resin embedded. Quantitative morphometrics analysis showed an increase in the total cell volume by 3-fold in the symbiont respect to the free-living Chlorella. Morphological differences of important energetic-organelles also were observed: the chloroplast, pyrenoid and mitochondria showed an increase in volume of 4.3, 3.4 and 2.1 – fold respectively in symbiotic microalgae. Additionally, storage compounds such as starch increased in volume. Moreover, the network-like shape of the mitochondria and several thylakoid membranes crossing the pyrenoid matrix also differentiate symbiont and free-living Chlorella. These results suggest that the physiological capacities (photosynthetic and respiratory activity) are enhanced in symbiosis (perhaps increasing the benefits for the host), and need to be confirmed with more cells in the future, so enhancing image processing (e.g. deep learning). Additionally, more studies on the physiological changes (photosynthesis, carbon fixation) in both free-living and symbiont "Chlorella" should be combined to further understand the physiological activity of the symbiotic microalga, and the influence of the host.

Exploring integrin/BMPRI coupling in breast epithelium integrity

Breast cancer is the most common cancer affecting women worldwide, accounting for about 30% of new cancer cases diagnosed each year and for 15% of cancer deaths in women. Breast cancer is not a single disease but rather represents a collection of tumor subtypes with diverse pathological features, molecular signatures and clinical outcomes. Intra-tumoral heterogeneity reflects distinct breast epithelial cells that serve as the cells of origin for malignant transformation. **Understand how an aberrant microenvironment impinges on the phenotypic diversity of breast cells or affects tumor heterogeneity is not well understood.**
Bone Morphogenic Protein 2 (BMP2), which controls extracellular matrix composition and tissue biomechanics is abnormally elevated in luminal breast tumors. Moreover, abnormal BMP-Receptor signaling has been reported in breast cancers. Our lab has previously shown the cooperation between BMPR and integrin to drive cell adhesion processes and Smad signaling as effector downstream BMP receptor in 2D. We are repositioning the contribution of the cooperation between integrin and BMPR in spatially organized multicellular contexts by considering the geometry (2D versus 3D), the polarity and the biomechanics of the breast epithelium. Our hypothesis is that signaling resulting from BMPR/ integrin cross-talk may impact on the organization of epithelial cells by affecting cell sorting, cell competition and the direction of cell extrusion within epithelial tissue. We are investigating whether and how BMP2 targets a subpopulation of MCF10A breast cells to promote intra-tumoral heterogeneity. Our results show that BMP2 induces a partial EMT which is associated with a change in the expression pattern of integrin and extracellular matrix in 2D environment. MCF-10A cells adopt an elongated shape when treated with BMP2. PIV analysis of time-lapse data obtained on MCF-10A monolayers indicates that BMP2 stimulation induces a fluidization of the monolayer by driving collective cell migration in 2D monolayer. In 3D environment, BMP2 treatment reveals a transition from acini to spheroid which correlate with a redistribution of integrin. Based on organoids, micropatterns and adaptative microscopy, we anticipate an integration of integrin dynamics as a molecular basis for tissue fluidization within the context of intra-tumoral heterogeneity.

Design, synthesis and characterization of monoclonal antibody mimics against the CD-20 antigen.

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In the late 90's, the monoclonal antibodies (mAbs) became a major tool for therapeutic purposes. However, some restrictions provided strong arguments for the development of alternative agents like aptamers, that integrate the benefits of mAbs while circumventing these limits. As a proof of concept, we propose to design small synthetic antibody mimics of the Rituximab, uses to treat some lymphomas by targeting the CD20 antigen. In order to prepare efficient and selective mimics, a selection of recognition elements must be achieved. Aptamers were chosen because of their very low immunogenicity, their high affinity generally for their target, their structural and chemical stability and the fact that their production is carried out by organic synthesis. Few DNA sequences were thus selected from a random ssDNA library using the systematic evolution of ligands by exponential enrichment (SELEX) method, coupled to capillary electrophoresis (CE). Among the modified SELEX methods, CE-SELEX was preferred due to its multiple benefits such as less consumption of samples, a natural binding environment, and a higher screening efficiency. The affinity and specificity of the best candidates for their target, were then characterized in solution by CE and Isothermal Titration Calorimetry (ITC). Later on, these recognition elements will be combined on macromolecular scaffolds using chemical ligations such as oxime, CuAAC (copper(I)-catalyzed azide alkyne cycloaddition) and thioether to assess the multivalent effects.

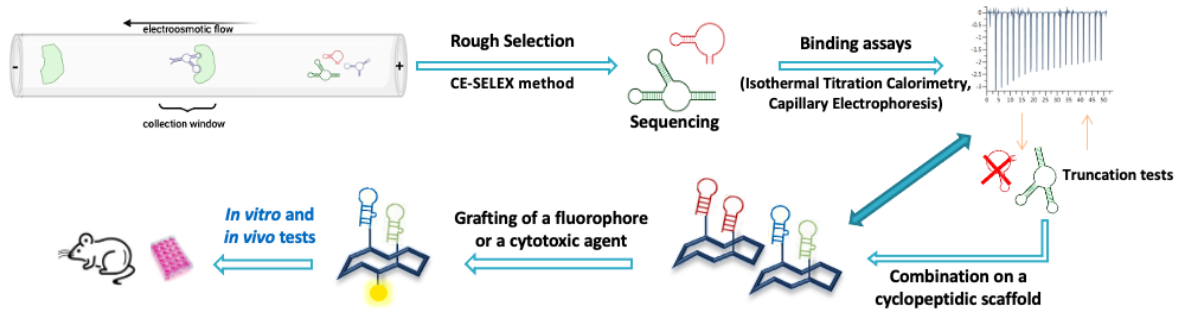

Figure 1 : Project overview from the selection process to the multivalent system characterization.

Nano-electro-mechanical sensor mass spectrometry for viralparticles characterization

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Nano-Electro-Mechanical Sensor Mass Spectrometry (NEMS-MS) is an emerging technology,able to analyse species in the MDa to the GDa mass range, beyond the reach of conventional MS instruments.[1] It allows single particle measurements, independently of their charge state, and with a mass-independent resolution. These properties make NEMS-MS an increasingly valuable and adaptedtool for the characterization of viruses, Virus-Like Particles (VLPs), but also of synthetic Nanoparticles.

The NEMS (Nano-Electro-Mechanical Sensors) used are doubly pinned silicon beams, oscillating in plane, in two specific vibrational modes, combined in an array of 20 devices. (Fig 1, Leftpanel) This method works by quantifying the changes in each of the modes of the NEMS device's resonance frequencies, for every individual particle landing, and from there deducing the particle massand landing position on the beam. Our VLPs of choice were the empty (genome-free) capsids of *E.coli*bacteriophage T5.

Several nano-ESI (Electrospray Ionisation) source parameters were identified as influencing the mass measurement of viral particles using NEMS-MS. These include the solvent composition, solutionfeeding flow rate, nano-ESI voltage and inlet capillary temperature. For this purpose, we designed a controlled experiment, in which, the nano-ESI flow rate was varied over the course of a NEMS-MS acquisition. This allowed us to isolate the effect of desolvation on the resulting mass measurement (Fig1, Right panel). In addition, viral particle integrity and aggregation status was characterised by NTA (Nano Tracking Analysis), prior to NEMS-MS analysis. The observed mass differences were evaluatedin light of known measurement uncertainties of NEMS, and our results may provide insights on the nebulization and desorption mechanism of large particles with sizes as large as the droplets formed bynano-ESI.[2]

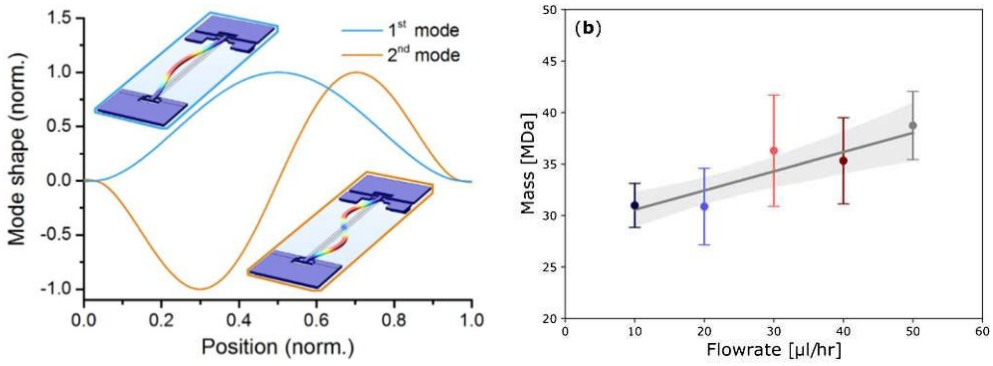

***Figure 1:** NEMS device's vibration modes (Left panel). Mass measurements as a function of ESI flow rate (right panel).*[2]

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Pseudomonas Aeruginosa IspH crystallographic structure: a starting point for rational design of novel antinfectives

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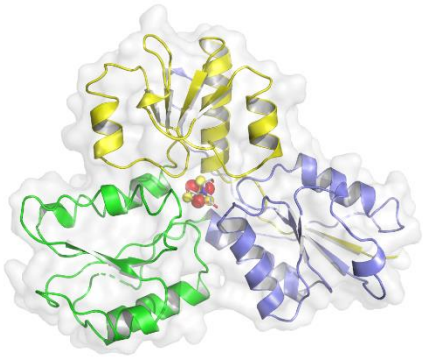
The aim of this project is to solve the crystallographic structures of *Pseudomonas aeruginosa*, *Mycobacterium Tubercolosis* and *Plasmodium Falciparum* IspH in order to identify and design new inhibitors by combining crystallographic fragment screening and *in silico* docking analysis. IspH is the last enzyme of the methylerythritol phosphate (MEP) pathway which occurs in Gram-negative bacteria like *Pseudomonas aeruginosa* (classified as a pathogen of critical priority by the WHO in 2017[1]), in parasites, like *P. falciparum* and in plant chloroplasts. Since this pathway does not exist in humans it is therefore a valuable target for the development of new specific antibacterial/antiparasitic drugs. IspH catalyzes a $2H^+/2e^-$ reduction and dehydroxylation thanks to the presence of a 4Fe-4S cluster highly oxygen sensitive which requires to work in anaerobic conditions. The purification and the activity assay of the enzyme were carried out under oxygen exclusion using a gloves-box and the protocol described for the *E.coli* IspH[2]. So far the crystallization conditions for *Pa*IspH were not known, good diffracting crystals were obtained after performing a screening thanks to the crystallization robot of the IBS Metallo group. Data sets were collected on BM07-FIP2 beamlines at ESRF (Grenoble, France). The structure was solved by molecular replacement using the existing Alphafold[3] model to determine the phases. The *Pa*IspH crystallographic structure will finally help us in the identification of new inhibitors which will be tested *in vitro* by our collaborators in Strasbourg. The newly identified inhibitors will be cocrystallized with *Pa*IspH; this work would represent a good starting point for the development of innovative drugs with unprecedented modes of action that are urgently needed to fight against multidrug-resistant infectious diseases.



Structure of *P.Aeruginosa* IspH. Surface representation of the monomeric IspH: the three α/β domains (domain A: yellow; domain B: green; domain C: blue). The central cluster is fixed by cysteines from all three domains.

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Targeting BET bromodomains of *Candida auris* with small molecule inhibitors as a potential new antifungal strategy

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Candida auris is an emerging, multidrug-resistant yeast pathogen capable of nosocomial transmission that has caused outbreaks in hospitals and long-term care facilities around the world. Strains of *C. auris* resistant to all three major classes of antifungal drugs (azoles, polyenes and echinocandins) have been identified in the clinic, raising an urgent need for new antifungal therapeutic strategies.

BET (Bromodomain and Extra-Terminal) proteins are chromatin-associated proteins that regulate gene transcription and chromatin organization. BET proteins bind chromatin via two small helical domains called bromodomains, epigenetic reader modules that recognize acetylated lysine residues in the N-terminal tails of histones. Human BET bromodomains have been intensely studied as potential drug targets against diverse diseases, including cancer, inflammatory disease and cardiovascular disease. BET proteins are also expressed in *Candida* species and we have previously shown that inactivation of the fungal BET protein Bdf1 results in the decreased viability and virulence of *C. albicans*[1].

More recently, we developed a FRET-based assay to measure the acetylpeptide binding activity of the two *C. auris* Bdf1 bromodomains. Using high-throughput and focused chemical screening we identified small molecule inhibitors of these domains, including one compound that exhibits a sub-micromolar IC50 value and several that show selectivity towards the *C. auris* bromodomains relative to their human counterparts. High-resolution crystal structures of *C. auris* bromodomains in the unbound and inhibitor-bound states revealed the detailed inhibitor binding modes, providing insights into how to optimize the potency and selectivity of these compounds towards Bdf1 bromodomains. Overall, these findings provide encouraging support for BET inhibition as a potential new antifungal strategy against *Candida auris*.

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SPECTROELECTROCHEMICAL AND MECHANISTIC STUDIES OF THE REDUCTION OF NITROUS OXIDE BY RHENIUM BIPYRIDINE CATALYSTS

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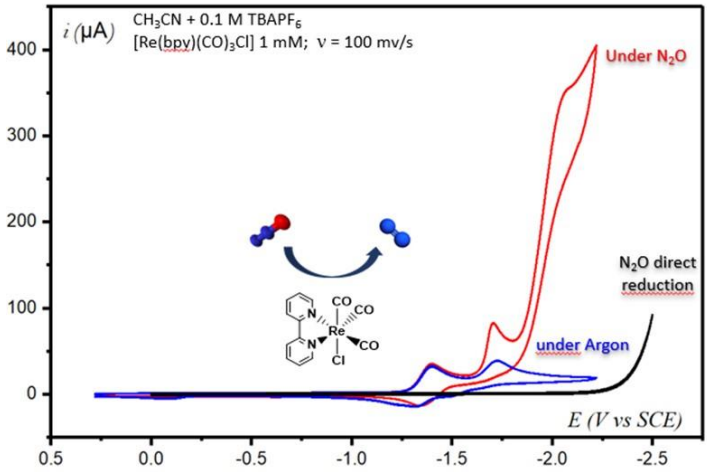
Abstract : Due to increasing concerns regarding global warming an interest in N_2O(g) reduction is gradually increasing in the recent days. N_2O(g) has been categorized as the greatest contributor to the stratospheric ozone depletion and is regarded as the third most significant anthropogenic greenhouse gas. Reduction of N_2O to N_2 gas is therefore of interest. N_2O being an inert molecule, its electrochemical reduction (deoxygenation) requires the implementation of catalytic processes. In this presentation we will show that electrogenerated reduced forms of rhenium bipyridyl carbonyl complexes are selective catalysts for deoxygenation of nitrous oxide in organic medium in the presence of water (proton source). [1] Then comparing their mechanism of catalytic action for N_2O electroreduction with the one of different aromatic organic catalysts, having reversible redox systems, we will emphasize differences between innersphere vs. outersphere catalysis respectively. [2] Cyclic voltammetry analysis indicates that Re-carbonyl bipyridine complexes are first reduced to produce the catalytic active species {Re^0}, then N_2O binds to the reduced metal center and the resulting adduct is further electro-reduced to trigger N-O bond breaking leading to N_2. This proposed mechanism is further assessed by forming the catalytically active rhenium complexes followed by addition of N_2O, and monitoring the kinetics reaction by IR and UV-VIS spectroelectrochemical measurements.



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Impact of C-type lectin receptors (CLRs) in the infectious process of coronavirus

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The first step of any immune strategy relies on the ability to identify, to sense, the presence of threats whatever their nature; being potentially pathogenic fungi, bacteria, parasites or viruses. C-type lectins receptors (CLRs) are one class of pathogen recognition receptors (PRRs) and are involved at the surface of our dendritic cells in pathogen detection. They are specifically dedicated to the recognition of carbohydrate-based molecular patterns associated to pathogens. Many innate immune cells express a large variety of CLRs, which shape immune response as a consequence of their pattern recognition capacity. CLRs expressed can also differ from one cell type to another, allowing a specific tuning of the response following recognition. Thus, CLRs such as MGL, Langerin, and DC-SIGN are major players in recognition of pathogenic fungi, bacteria, parasites and also viruses.[1]

In these battle for infection, some pathogens have found strategies to circumvent the initial role of CLRs in immunity activation and even hijack CLRs for their benefit in the context of their infection process. Indeed, the subversion of several C-type lectin receptors has been reported, among them L-SECtin, L-SIGN, and most importantly DC-SIGN which is one of the most widely reported as used in viral infection processes. Indeed, these three receptors have been reported to promote *cis* or *trans* infection of several viruses. One or several of these CLRS are used notably by HIV, Dengue, Ebola virus as well as Zika virus.[2]

Finally, in the context of the actual world-scale global coronavirus outbreak, COVID-19, focus is brought now on the SARS coV-2 virus. In the last two decades, it is the third alert, outbreak caused by a coronavirus, after the first severe acute respiratory syndrome (SARS) in 2002-2003, due to the SARS coV-1, and later the MERS-coV in 2012. Thus, coronaviruses represent clearly major threats for the future. SARS-Cov-2 uses ACE2 as primary receptor as does SARS-Cov-1[3]. Moreover, in the case of the SARS-Cov-1 infection, a role for L-SECtin, L-SIGN and/or DC-SIGN has been suggested as alternate receptors or enhancer factors of ACE2-mediated virus infection. Indeed, the surface spike protein of coronavirus is largely glycosylated providing many potential anchor points for the CLRs. In addition, L-SIGN is expressed in human lung tissue on type II alveolar cells, which are target for the SARS coV-2. To date, the potential role of CLRs toward the new SARS cov-2 have not been investigated.

Our group is developing antagonist targeting CLRs for a decade now, and more particularly toward DC-SIGN. Many of these tools are already available and their capacity to compete with the SARS coV-2 binding to the corresponding CLRs will be investigated. The best compounds identified will be candidate for competition experiment within a cellular model of infection with one of our collaborators (Ebola and/or Coronavirus competition system).

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Bunyavirales is a large order of segmented negative sense single stranded RNA viruses (sNSV) that comprises more than 450 viruses divided into 12 families. Arthropods and rodents are their natural reservoirs and man is occasionally infected leading to meningitis, encephalitis or hemorrhagic fevers. The recent increase in the number of outbreaks, while no treatment has yet been developed, prompts us to study these viruses. We focus our analysis on the RNA dependent RNA polymerase (known as L) which is an essential enzyme that catalyzes two critical steps of the viral cycle: replication, that generates copies of the viral RNA (vRNA), and transcription that produces a viral messenger RNA using a cap-snatching mechanism. Here, we present a partial cryo-electron microscopy (cryo-EM) structure of Hantaan virus polymerase (HTNV-L) that displays the protein CORE. It reveals interactions between the polymerase and viral RNA (vRNA), which are necessary for activity. The C-terminal region of HTNV-L is too flexible to be determined in the cryo-EM structure and we therefore present its structural prediction done with AlphaFold. Modifications of the vRNA ends and usage of capped RNA displaying variable length lead to the visualization of *in vitro* transcription activity. Cryo-EM and activity assays will serve as a basis for further structural studies to understand the mechanisms of HTNV transcription.

Aptamer-Target Crosslinks Formation

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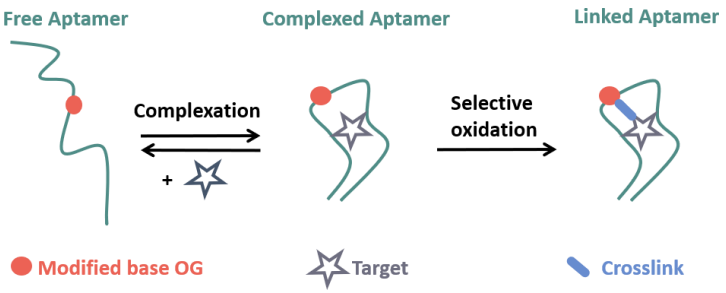
Aptamers are short single-stranded oligonucleotides in the series RNA or DNA that have been selected to recognize with high affinity and specifically a target via low energy interactions. With the aim of increasing the selectivity and specificity of aptamers, attempts have been made to chemically crosslink an aptamer to its target. This could have an application for decontamination purposes.

To generate the chemical crosslink, we proposed to use the well-known reactivity of 8-oxo-7,8-dihydroguanine (OG) oxidized base that following oxidation is known to react efficiently with nucleophilic residues.[1] To demonstrate the feasibility of this concept, we used an anti-L-argininamide aptamer. Three guanine bases of this aptamer, located in the recognition site, were replaced one at a timeby OG. Using fluorescence polarization assays, we first demonstrated that such a replacement has a limited impact on the affinity of the aptamer for its target (the affinity is either increased or decreased depending on the localization of the modification.

Following oxidation of the OG-containing oligonucleotide complexed with its target, using 2 NH_4^+, $IrCl_6^{2-}$ as an oxidant, an efficient crosslink formation between the oligonucleotide and its targetwas observed, as demonstrated by capillary electrophoresis and HPLC-coupled to mass spectrometry. In absence of OG in the oligonucleotide, no crosslink was generated. When the OG-containing oligonucleotide was oxidized in absence of its target, specific oxidation of OG was observed, generating spiro and guanidino derivatives in agreement with the literature. However, at low target concentration where free aptamer remains in solution, the formation of crosslinks was still almost quantitative, suggesting that crosslinks are also generated via non-specific interactions between the positively charged L-argininamide and the polyanionic aptamer. Work is in progress through the optimization of the reaction parameters and the use of competitors to reduce these unspecific interactions. Another strategy, using another aptamer that recognizes a non-positively charged target will be investigated.



General principle of crosslink formation by selective oxidation of the modified base OG

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Mild Green Reduction Method of Graphene Oxide using Ascorbic Acid: From Physico-Chemical Analysis to Electrochemical Performance as Supercapacitors

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Since the discovery of graphene in 2004[1], it has been considered a "Wonder" material with lots of potential due to its attractive properties. However, due to the high cost and difficulty of producing and handling single sheets of graphene, the presence of graphene in the market has been hindered. Luckily, various methods have been devised to synthesize graphene-like materials that would be easier and cheaper to produce. Among such methods, the chemical exfoliation of graphite, yielding reduced Graphene Oxide (rGO), is one of the commonly used synthesis methods nowadays. Unfortunately, such a method, although efficient and low cost, is not a green one as it involves the use of hydrazine hydrate (Hz) as a reducing agent. Since hydrazine is a very toxic chemical, research has been conducted to find alternatives to its use. Many reducing agents have been experimented, yet Ascorbic Acid (AA) proved itself to be an efficient reducing agent, offering a mild green reduction process[2]. While many diverse protocols involving AA have been used, a rationalization of the correlation between important reaction parameters and rGO properties is lacking.

In this psoter, a full study on the reduction of graphene oxide using AA has been done including varying the reaction parameters such as the amount of AA, the reaction temperature and the reaction time. Various characterization techniques have been employed to analyze the structural and morphological differences of the rGO reduced with AA and Hz, including XPS, XRD, TGA, SEM, Raman spectroscopy, Elemental Analysis (EA) and conductivity measurements. The relationship between experimental conditions and graphene intrinsic properties will hence be presented. Furthermore, being a material of high interest for supercapacitor applications, the electrochemical storage performances of the rGO samples reduced with those 2 reducing agents have been assessed. It will also be shown that the obtained results pave the road for the employment of AA as a mild green reduction method for, not only rGO, but also different rGO based materials, including pillared graphene materials that have been showing promising performances as supercapacitors[3].

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A molecular approach to graphitic carbon nitride: towards new photosensitizers and catalysts

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In the context of climate change, utilizing CO_2 instead of simply releasing it could be of the utmost importance. Drawing inspiration from natural photosynthesis, artificial photosynthesis could provide a way to store solar energy in the chemical bonds of molecules of interest using the sun and CO_2.

This process is actually made of three steps: absorb a photon to generate a hole/electron pair, retain the electron long enough for the desired reaction to take place and actually do the reaction using the proper catalyst. While a wide array of system may achieve this, one particular material has been receiving a lot of attention recently[1] : graphitic carbon nitride. Indeed, this inexpensive organic material can perform all three steps on its own. However while it can do them all it is efficient at doing so in none of them. Worse: controlling its synthesis is difficult and its characterization even more so. This leads to a very limited understanding of its properties. Particularly problematic are the catalysis step which is performed generally by using a co-catalyst such as platinum[2] and its absorption band located in the UV band of the spectrum which makes it poorly adapted to solar use.

However, it has been shown that oligomers of g-CN monomer's heptazine, which can be synthesized in a controlled manner, show very similar properties to the polymeric material[3]. This provides exploration pathways. Working with heptazine and then with the oligomers allows a reasoned functionalization and easy characterization which can be applied to g-CN, provided an understanding of the heptazin's challenging chemistry[4]. This work presents new methods for heptazine functionalization; apply them to enhance heptazine's absorption in the visible light and using it as a photosensitizing a photocathode (fig.1).

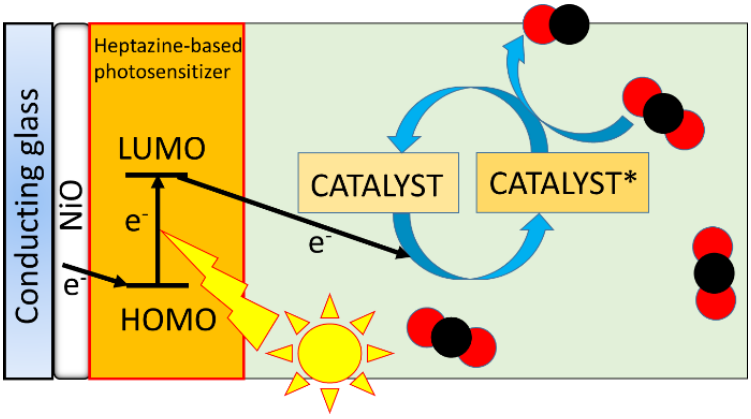

Figure 1: Photocathode design using an heptazine-based photosensitizer

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DEVELOPMENT OF MOLECULAR CATALYSTS USING D^{6}METALS FOR CO_2 REDUCTION

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In a world facing an increase of anthropogenic greenhouse gas emissions (mainly CO_2) in parallel with the need of finding means of producing sustainable energy and storing it at a low cost, an approach towards the development of scientific solutions applicable in industries is of necessity. Photoelectrochemical (PEC) carbon dioxide reduction (CO2R) is process designed to tackle both ofthe challenges, by utilizing solar energy as the driving force for the electrochemical conversion of CO_2 into more valuable small carbon-based product.

In that context, molecular catalysts based on Earth-abundant transition-metal able to reduce CO_2 areextensively investigated to replace the prototypical [Re(bpy)$(CO)_3$Cl] (bpy = 2,2'-bipyridine) catalyst.[1] Group 6 molecular compounds remain underexplored relative to other transition metals.[2] In this project, we are working towards the development of polypyridinyl complexes, characterization of their electrocatalytic performance in the dissolved state (homogeneous system)[3] and eventually proceeding to their immobilization on the photocathode surface (heterogeneous system)[4] for further investigation.

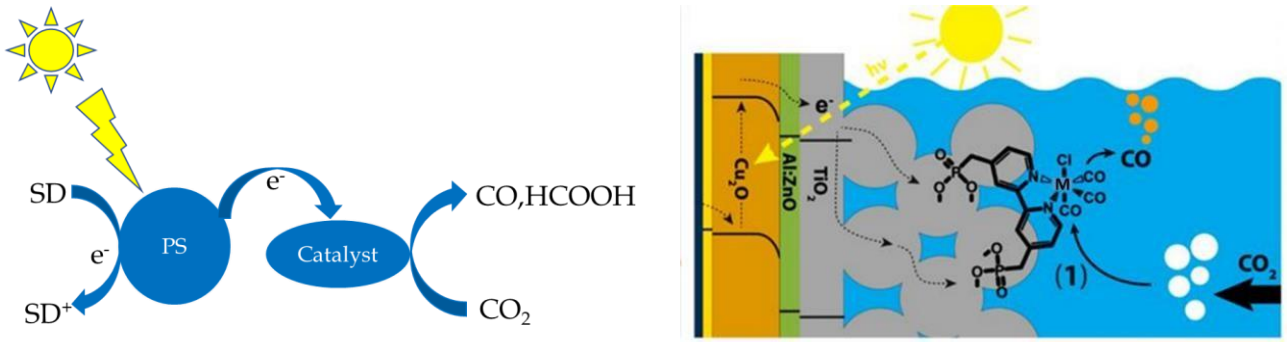

Figure 1. Association of molecular photosensitizer and catalyst (left), A photoelectrode comprisinga modified Cu_2O-based photocathode and an immobilized molecular catalyst (right).

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Human and climatic impact on the demographic history of four emblematic species of butterflies from peat bogs of Franche Comté, and implications for conservation management

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The increasing loss of biodiversity over the last decades requires the scientific community to precisely identify the drivers of population declines to brake the ongoing sixth extinction[1]. Notably, land-use and climate changes are sources of fragmentation and quality degradation of species habitats. Then, specialist and/or low-dispersing species, such as butterflies, are the most challenged in maintaining their populations[3].

In France, the Franche-Comté region is home to a large network of peatlands, and, as such, has an important responsibility in conserving the peat bogs biodiversity. Notably, a program titled "Des Ailes pour les Tourbières[2]" aims at protecting four patrimonial species of butterflies: The Violet Copper (*Lycaena helle*), the Scarce Heath (*Coenonympha hero*), the Large heath (*Coenonympha tullia*) and the Cranberry Fritillary (*Boloria aquilonaris*). These species differ in their ecologies, specifically in their host-plant specialization and their habitat structure. This Ph.D. project is a part of this program and uses population genetics, landscape ecology and landscape genetics frameworks to understand the influence of climate and human activities on these species, at the scale of the departments of Jura and Doubs.

Described as glacial relicts in the literature, the four species show the same pattern of demographic history, with an ancient climatic decline after the last glaciation and a recent one, probably induced by human land development. Besides, species distribution model projections in future climate scenarios indicate that local conditions may be unsuitable for these butterflies if the temperature rise is too important and/or if actions are not conducted to limit its consequences on habitats. Population genetic structure and diversity analyses show that the four species have a metapopulation functioning and, for three of them a source-sink dynamic. However, the source populations of these species are not located in the same area of the landscape, suggesting that features influencing dispersion may be species-specific.

The next landscape connectivity study, based on graph theory[4], will precise the influence of habitats configuration and landscape matrix on the genetic differentiation of the four species populations. It aims at identifying features impeding or enhancing gene flow and will be the basis of management and conservation recommendations, adapted to each species and determined in collaboration with natural areas management experts and naturalists.

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Building a Huntington disease cortico-cortical neuronal network-on-chip for drug investigation

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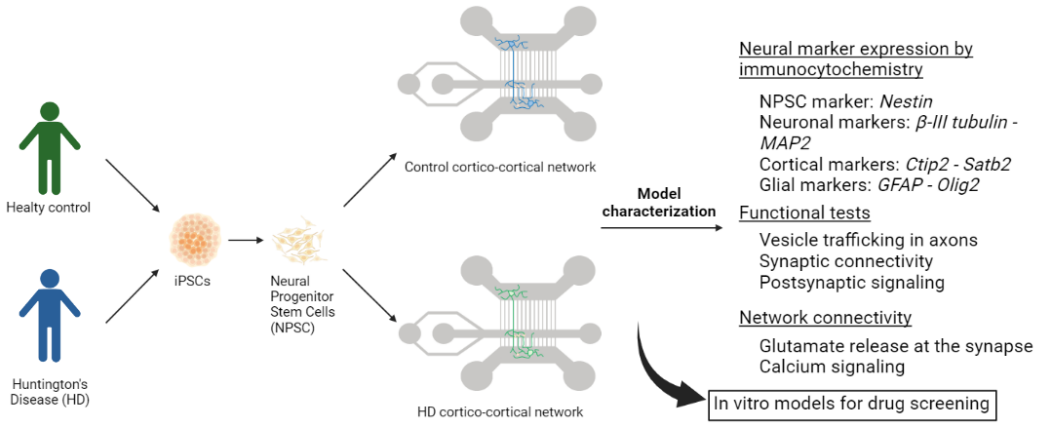
Huntington Disease is a genetic neurodegenerative disorder caused by the abnormal expansion of a glutamine-encoding CAG repeat in the huntingtin (HTT) gene[1]. The pathology is characterized by the dysfunction and death of neurons in the brain. HTT, a scaffolding protein, is involved in the axonal transport of vesicles containing Brain-Derived Neurotrophic Factor (BDNF). In HD, mutated HTT impairs BDNF trafficking and leads to a reduction in the amounts of BDNF provided to the striatum, which in turn leads to death of both striatal and cortical neurons[1,2].

The laboratory has developed microfluidic devices to reconstitute a mature neuronal network, in which presynaptic, synaptic and postsynaptic events are compartmentalized[2,3]. Culturing primary neurons from HD mouse model already provided a “disease-on-a-chip” platform ideal to fully decipher presynaptic dynamics, synaptic morphology and transmission, postsynaptic trafficking and signaling, as well as global network dynamics in healthy and pathogenic conditions.

Here, we developed a human HD brain-on-a-chip by using human cortical neurons derived from induced pluripotent stem cells (iPSCs) to reconstruct a HD functional cortico-cortical network. We use neurons derived from HD patient-specific induced pluripotent stem cells that contain both wild type and mutated HTT gene. We are currently characterizing and validating this model of human HD neuronal network, with the objective of providing a predictive model for drug testing and for the investigation of disease mechanisms.



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Deciphering the pathophysiological role of ATAD2, a bromodomain-containing testis-specific factor

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ATAD2 is an evolutionarily conserved, nuclear protein that contains a bromodomain that has been found to interact specifically with the acetylation mark of Lysine 5 of Histone 4 [1]. ATAD2 expression has been detected in testis, embryonic stem cells (ESCs) [2] as well as in various tumors [1]. Concerning its function in physiological setting, we have previously shown that ATAD2 is a facilitator of histone turnover in ESCs [2] and recently demonstrated that ATAD2 regulates chromatin binding of histone chaperones FACT and HIRA around transcriptional start sites (TSS) by recycling nucleosome-bound chaperones [3]. Through these functions ATAD2 can increase the dynamics of chromatin and could therefore aid in large- scale chromatin rearrangements. Given these facts the current work on ATAD2 in our lab is oriented towards developing mouse models of cancer including Hepatocellular Carcinoma (HCC) and Non-Small Cell Lung Cancer (NSCLC), in which we aim to examine ATAD2 involvement. Our hypothesis is that as a chromatin factor that increases the accessibility to chromatin, ATAD2, enhances the recruitment and action of oncogenic factors and its absence in the genetic background of these cancer models would impair cancer development and progression. Concurrently, we are aiming to further understand the function of ATAD2 in spermatogenesis, during which it is physiologically expressed. ATAD2 is suspected as a mediator of histone eviction during the transition from nucleosomal-based chromatin of immature sperm cells to the protamine-based chromatin of mature spermatozoa. While mice that have the deletion of the Atad2 gene exhibit no significant decrease in fertility under normal conditions, they do appear to exhibit mild defects in histone replacement during spermatogenesis. In summary, my thesis aims at defining the functional impact of ATAD2's role in mediating histone chaperone-based chromatin dynamics, in different physiopathological model systems.

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Adaptive immunity plays a crucial role in the body's defense against non-self and altered-self components involved in infectious and tumoral diseases. Especially, CD8+ T cells have the ability to recognize and lyse cells presenting specific peptides through class I HLA molecules and cytotoxic molecules secretion. Hence, enhancing the number of peptide-specific T cells could accelerate and facilitate disease resolution. We propose here a systematic method for designing a therapeutic vaccine able to induce and boost a number of selected antigen-specific CD8+ T cells using a patented cell line of plasmacytoid dendritic cells (PDC line). Furthermore, this strategy could also avoid immune escape mechanism through reduced antigen presentation and restore functional T cells in case of altered activity.

Robust biodiversity measures for metabarcoding

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Biodiversity indicators are tools for better management of ecosystems, for example to monitor changes in biodiversity or to compare areas between them. They are based on the estimation of the relative abundance of each species present. The metabarcoding used to decode the information contained in environmental DNA leads to biased estimates of these abundances. This work investigates two biases: differences in amplification efficiency during the PCR (Polymerase Chain Reaction) phase and in the proportion of DNA targeted by the used marker among the total DNA of each species.

A mathematical model has realistically modeled the PCR amplification. Together with a specific experimental protocol, it aims at correcting the amplification biases. The results on simulated data are satisfactory.

A study on a community of alpine plants has also shown, thanks to a digital droplet PCR treatment, large variations in the proportion of chloroplastic DNA, targeted here by the marker, compared to the total DNA concentration usually used to assay samples.

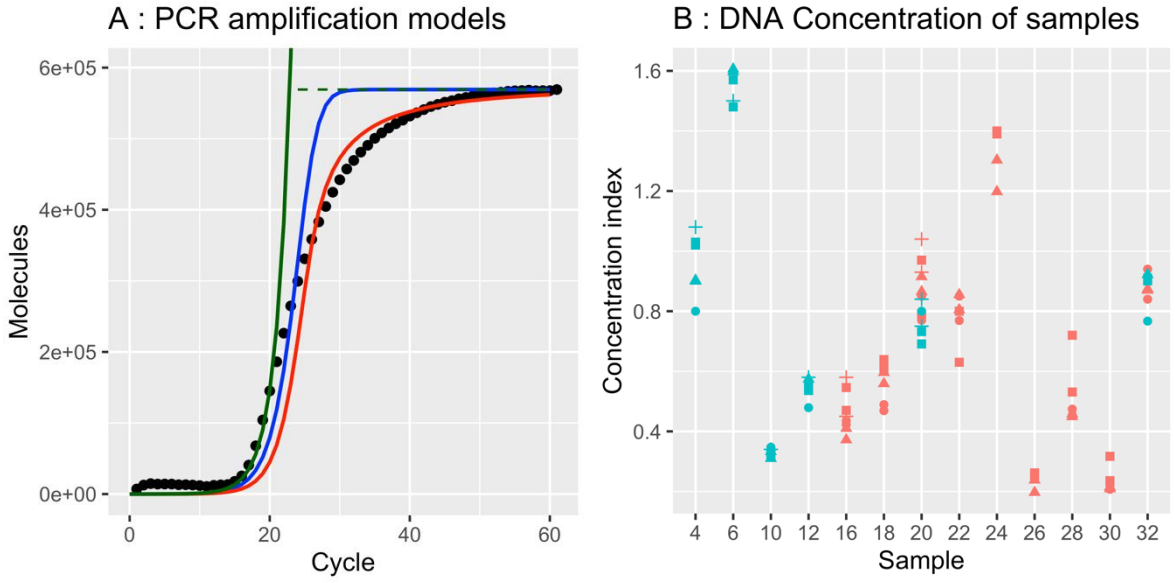

Figure: Consideration of two biases impacting metabarcoding analysis. Panel A: Modeling of PCR kinetics. Black dots: real data observed by qPCR. Green curve: common exponential model in the literature. Red and blue curves: two different models developed in this work. Panel B: Concentration index of target DNA measured by ddPCR under different experimental conditions with identical total DNA concentration for 13 alpine plants.

Redox signalling involved in the biogenesis of chloroplast

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The biogenesis of chloroplast and the assembly of the photosynthetic apparatus are regulated by transcription of both plastid and nuclear genes, Photosynthesis associated plastid genes (*PhAPGs*) and Photosynthesis associated nuclear genes (*PhANGs*). The *PhAPGs* are transcribed by Plastid-encoded RNA Polymerase (PEP). The active PEP in chloroplast has an unknown 3D structure that has four catalytic subunits (α2, β, β′, β″) and 12 PEP-associated proteins (PAPs) encoded by the nucleus[1]. Mutation of most *pap* genes in plants lead to albino phenotypes and makes the PEP non-functional.

During the primary photosynthetic reactions, there is formation of reactive oxygen species. The photosynthetic apparatus is protected by superoxide dismutase. It catalyses the dismutation of superoxide to hydrogen peroxide. $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. In plants, superoxide dismutases with Fe^{3+}, Cu^{2+} or Mn^{3+} at their catalytic site have been identified. Among the 12 PAPs bound to active PEP, PAP9 (FSD3) and PAP4 (FSD2) are Fe superoxide dismutases that are observed only in the chloroplast. Thus, PAP9 and PAP4 could protect the PEP from oxidative stresses. Previous studies have shown that double mutant *pap9-pap4* leads to albino phenotype, that indicates that the photosynthetic apparatus has not been formed because the PEP was not functional[2]. We are interested in studying the catalytic activity of these SODs *in vitro* and *in planta* (*Arabidopsis thaliana*) since they are essential for the PEP function.

Another redox protein that interacts with PEP is PRIN2 (Plastid Redox Insensitive 2). It has been reported that the deletion of *prin2* gene leads to albino phenotype; the plants are unable to perform photosynthesis strongly suggesting that PRIN2 is also essential for the PEP activity. PRIN2 exists in dimeric form in the plastids. It undergoes reduction of disulphide bridge and becomes monomeric. In this form, it interacts with PEP complex and activate it.[4] PRIN2 has several redox partners such as PAP10 (thioredoxin that could reduce its disulfide bridge) and CSP41b (Chloroplast Stem-loop binding Protein) that has DNA binding regions[3].

During my thesis, we are interested in identifying the interactions of PRIN2 with CSP41b *in vitro* and its interaction with PEP *in planta*. We also want to characterise the role of PAP4 and PAP9 in the PEP *in planta:* whether it is structural or catalytic.

The objectives are:

- to analyse the role of PAP4 and PAP9 in the PEP complex both *in vitro*[5] and *in planta*.
- to biophysically characterize the redox partners PRIN2 and CSP41b *in vitro* and to solve its 3D structure by Cryo-EM.
- to investigate the interacting redox partners of PRIN2 by proximity labelling and BiFC.
- to isolate chloroplasts from *Sinapis alba* for the purification of PEP complex.

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Structural Basis of Human Melanogenic Enzymes

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The production of the pigment melanin in human requires the activity of at least three melanogenic enzymes, tyrosinase (TYR) and tyrosinase-related proteins 1 (TYRP1) and 2 (TYRP2), which regulate the type and amount of melanin produced. Despite their essential role, the catalytic mechanism and specificity are still under strong debate. The lack of structural data hampers the understanding of their molecular bases and the design of specific inhibitors to treat pigmentation disorders or melanoma[1]. Recently, the solved crystal structure of human TYRP1 challenged previous assumptions claiming it is a redox enzyme[2,3]. In view of the existence of pathological mutations for all three melanogenic enzymes, it is thus very likely that each enzyme plays a unique function in human melanogenesis[4]. Therefore, it is crucial to obtain 3D structures of all three melanogenic enzymes at atomic level as well as to identify their respective metabolites in order to improve our current knowledge on the human melanin biosynthesis pathway. With all these information, compounds of great efficiency and specificity can then be designed to treat melanogenesis disorders.

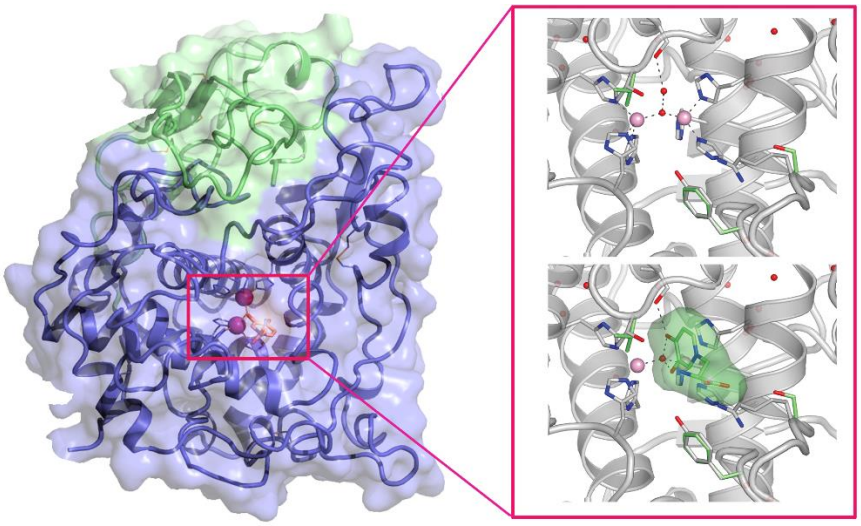

Figure. Cartoon representation of TYRP1 protein with close-up view of the active pocket. *On left, the tyrosinase-like domain and cysteine-rich domain of TYRP1 protein are coloured in blue and green respectively. The two metal ions at the active pocket are represented as magenta spheres. Close-up of the active site structure, as displayed in the inset, reveals metal-coordinating histidine residues (shown as stick models), metal bridging water molecules (red spheres) and substrate mimosine (stick model with green surface). Hydrogen-bonding interactions are also shown as black dotted lines.*

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Electrospun Azo-Cellulose Fabric: A Smart Polysaccharidic Photo-Actuator

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Key words: Cellulose, Azobenzene, Electrospinning, Nanofiber, Photo-actuator

Abstract

Electrospinning is a versatile and simple method of continuous fiber production that uses strong electric fields to draw polymer solutions or melts with nano/micro scale diameters. Electrospun fabrics made of polysaccharides and their functionalized derivatives have captivated ever-increasing attention for their potential biomedical applications.[1] Recently, our team has been working on electrospinning of functionalized polysaccharide derivatives for diverse applications [2] including photo-responsive actuators, *i.e.* photo-actuators.[3] So far, a new type of photo-responsive fabric was produced *via* electrospinning of cellulose tris(4-phenyl azobenzoate) (Azo-Cel). In this poster presentation, we present (i) the detailed electrospinning process of Azo-Cel in various organic solvent systems as well as the acquired morphology of fibers in the self-standing fabric, and (ii) the photo-responsive variations of the surface free energy and shape of the textiles that are triggered by *cis/trans* isomerization of the azobenzene moiety in Azo-Cel by means of Water Contact Angle and *in-situ* actuation behavior analyses under UV/Vis light irradiations.[4]

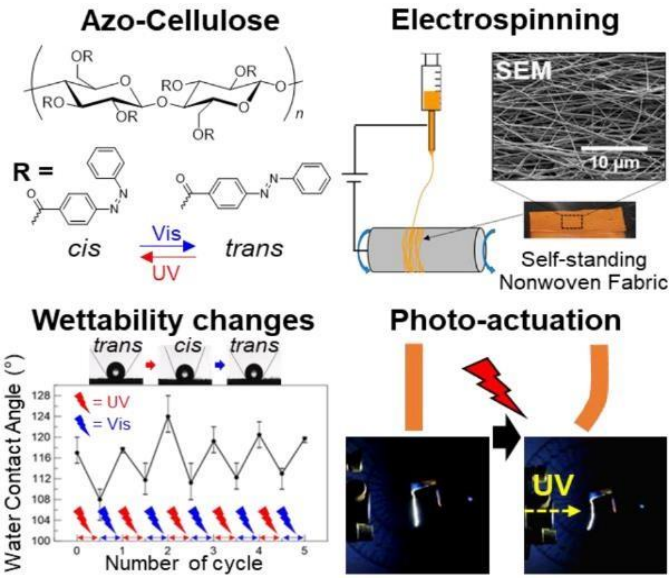

Figure. Graphical Abstract: Overall scheme of the work.

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Electrospun Polysaccharidic Textiles: Toward Highly Efficient Chiral Resolutions

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Mots-clés: Polysaccharide, membrane filtration, Electrospinning, Nanofiber, enantioselectivity

Polysaccharides have attracted attention as sustainable and renewable sources of bioactive materials. Modifications of native polysaccharides with functional group(s) are one of the promising approaches for the creation of bio-based smart materials such as photo-actuators for artificial muscles[1] and chiral selectors in chiral HPLC columns[2]. Electrospinning is a highly versatile method to process solutions or melts of polymers, including polysaccharides and their derivatives, into continuous fibers with diameters ranging from tens of nanometres to a few micrometers.[3] It is the method of choice to form nanofibers with a high specific surface area (SSA) and good mechanical properties that can be used as nonwoven textiles for membrane separation technology. Membrane separation processes are particularly advantageous for molecular purifications due to cost-effectiveness, high efficiency, low energy usage, simplicity, convenience for up-and/or downscaling, continuous operability, and, environmental friendliness. Therefore, electrospun enantioselective membranes made of polysaccharidic chiral selectors with large SSA, tunable porosity, high permeability, and good mechanical properties are promising chiral separation materials. We hereby present the first successful electrospinning of a well-established polysaccharidic chiral selector, tris(3,5-dimethyl phenyl carbamates) (CDMPC), to fabricate self-standing nonwoven membranes and the chiral resolutions of a racemic compound, (*R*,*S*)-1-(1-naphthyl)ethanol, via membrane filtrations to yield the *S*-enantiomer-rich mixture.[4]

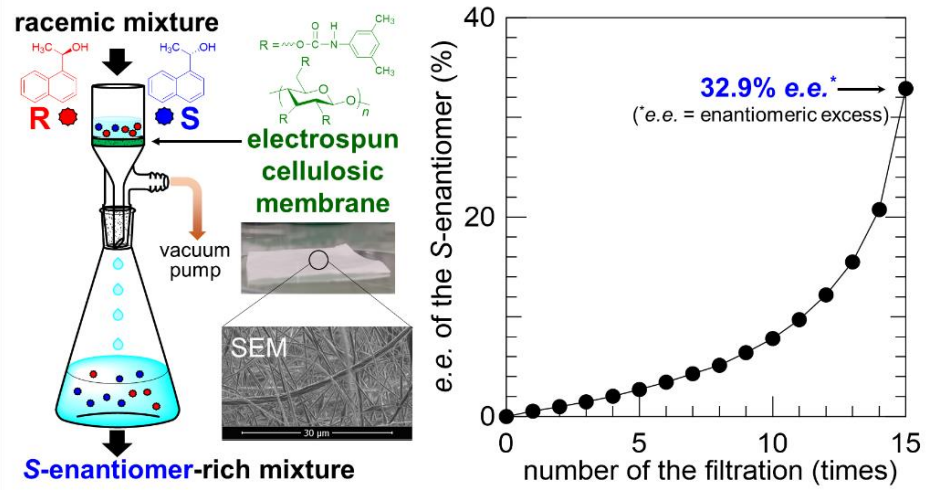

Figure. Chiral resolution of (*R*,*S*)-1-(1-naphthyl)ethanol using electrospun CDMPC membranes via repeated vacuum filtrations.[3]

Electrospinning of a series of CDMPCs having various degrees of polymerizations (DPs) and amylose tris(3,5-dimethyl phenyl carbamates) (ADMPC) is also studied based on the effect of DP on their fiber diameters and thermal properties.

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Otsuka, I.; Pandey, K.; Ahmadi-Nohadani, H.; Nono-

Study of Excitatory/Inhibitory balance in the hippocampus of a transgenic mouse model of Alzheimer disease

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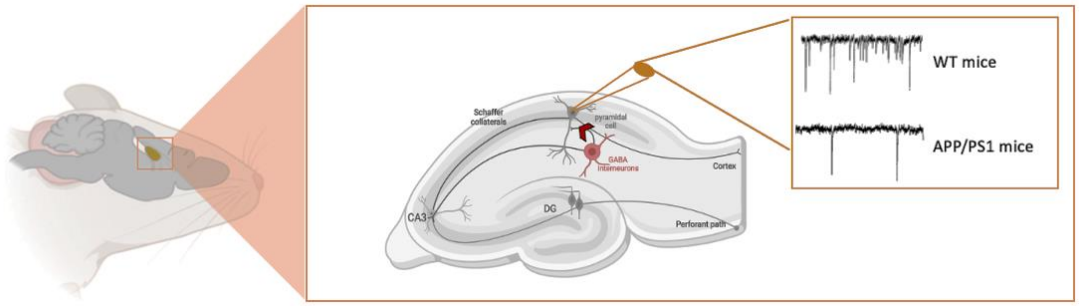
Alzheimer's disease (AD) is a progressive neurodegenerative disease and the first cause of dementia. At early stage, soluble Aβ oligomers (Aβo) have been showed to impaired synaptic plasticity and enhance network hyperexcitability in the hippocampus, the structure involved in learning and memory processes[1] [2].

To explore the pathogenic outcome occurring during this prodromal period, our team use one-month-old APP/PS1 mice which overproduce Aßo, starting at P14 and simulated a key aspect of early AD. Using electrophysiological approach on those mice, our team showed a neuronal hyperactivity[3].

Hyperactive state reported in other AD mice models is dependent on a significant reduction of hippocampal GABAergic interneuron activity, highlighting an impairment of the balance between excitatory and inhibitory components[4].

Using a multimodal approach combining electrophysiology and molecular biology technique, my PhD project aims at further understanding whether an alteration of the inhibitory control of CA1 pyramidal neurons by GABAergic interneurons is responsible for the modifications characterized in one-month-old APP/PS1 mice.

The result obtained during the first year of PhD confirmed our hypothesis showing a disruption of the basal inhibitory synaptic transmission and an alteration in the long-term potentiation of inhibitory transmission. During the second year of her thesis, I will investigate the cellular mechanisms at the origin of the disturbances of the inhibitory transmission. I will focus on a membrane protein responsible for chloride ion transport, KCC2[5]. A defect in the expression of this protein could be at the origin of the imbalance in the excitation/inhibition balance highlighted in our model.



Hippocampal inhibitory transmission patch-clamp recordings in WT and APP/PS1 mice

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Exploring Two-Partner Secretion systems in the virulence of Gram-negative bacteria

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Pseudomonas aeruginosa (*Pa*) is a gram-negative opportunistic human pathogen that can lead to both acute and chronic infections. It is responsible for about 10% of nosocomial infection and it is now a huge threat to human health. Indeed, it belongs to the WHO critical priority list for finding new drugs to fight against it as more and more clinical strains are resistant to the most used antibiotics. To do so, anti-virulence approaches are studied which aims to target essential functions for infection such as virulence factor. *Pa* has a panoply of surface-attached and exported proteins involved in virulence that can be secreted by several ways, including Two Partner Secretion (TPS) systems, a type of secretion system widespread in pathogens and environmental bacteria. It is composed of one transporter, called TpsB inserted in the outer membrane that enables the translocation of the effector protein, TpsA. TpsA proteins can be classified in different categories: adhesins, proteases, hemolysins and contact-dependent growth inhibition. *Pa* encodes for at least five TPS: LepA protease, CdiA (contact-depedent growth inhibition), CdrA adhesin, Exolysin (ExlA) a pore-forming toxin present in a minority of clinical strains and PdtA a filamentous hemagglutinin (FHA)-like adhesion recently identified[1]. In a first project, ExlA and homologous proteins called ExlA-like in other *Pseudomonas* species were studied and in particular the structure-function relationship in those proteins. The second project aims to study PdtA : it belongs to the HMW adhesion family of proteins with HMW1 of *Haemophilus influenzae*, an adhesin known to be immunogenic[2]. The implication of PdtA in *P. aeruginosa* virulence is still unclear, as it depends on the infection model used. On *C. elegans* the mutant without *pdtA* was less virulent that the wild type[3] but in zebrafish embryos the mutant has the same phenotype as the wild type strain[4]. We know that PdtA is expressed during infection as antibodies raised agaisnt PdtA were found in patients' sera[4] and the protein is well conserved in clinical strains that is why it is a potential candidate for a vaccine or for monoclonal antibodies based therapy. However, the studies on PdtA were impeded due to the lack of laboratory strains expressing the protein. To work on this protein we collected thanks to the CHU of Grenoble 46 clinical strains isolated from patients with bacteremia and some patients' sera from patients suffering from cystic fibrosis.

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Visualizing the Transiently Populated Closed-State of Human HSP90 ATP Binding Domain

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Molecular chaperones are involved in many different mechanisms. Due to their importance, alteration of one member of this family can cause numerous diseases such as cancer. For this reason, they are good targets for therapeutic ligands.1

In this study, we investigated an homo-dimer of two times 90 kDa: HSP90α.

HSP90 is composed of 3 domains: C-terminal, middle and N-terminal domain (NTD). In the NTD, there is an unusual ATP-binding site named the Bergerat fold. Indeed, the cycle activity of HSP90 is coupled with ATPase activity and a set of complex conformational rearrangements. This domain is also the target of most therapeutic ATP-competitive inhibitors developed. Because of its importance, the HSP90-NTD structure was highly studied mainly by X-ray cristallography. More than 300 structures are available on the PDB but static models cannot represent the dynamic of HSP90-NTD. Therefore, we decided to study the different conformations of the N-terminal domain of human HSP90α using liquid state NMR and molecular dynamics.

We demonstrated that HSP90 ATP-lid exchanges between two conformations in millisecond time scale and elucidating the structures of both the major and minor states. The major state corresponds to previously determined X-ray structures. The ATP-lid samples a closed conformation distant by up to 30 Å from the major state. This is the first time that this important structural change is observed. Combining NMR relaxation experiments and molecular dynamics, we have investigated the stability of minor state. Further simulations will be performed to characterize the exchange between both states. These important results will be particularly useful for the drug design of new therapeutic ligands targeting this domain.[3]

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Exploiting Serial Crystallography in Structure-based Drug Design

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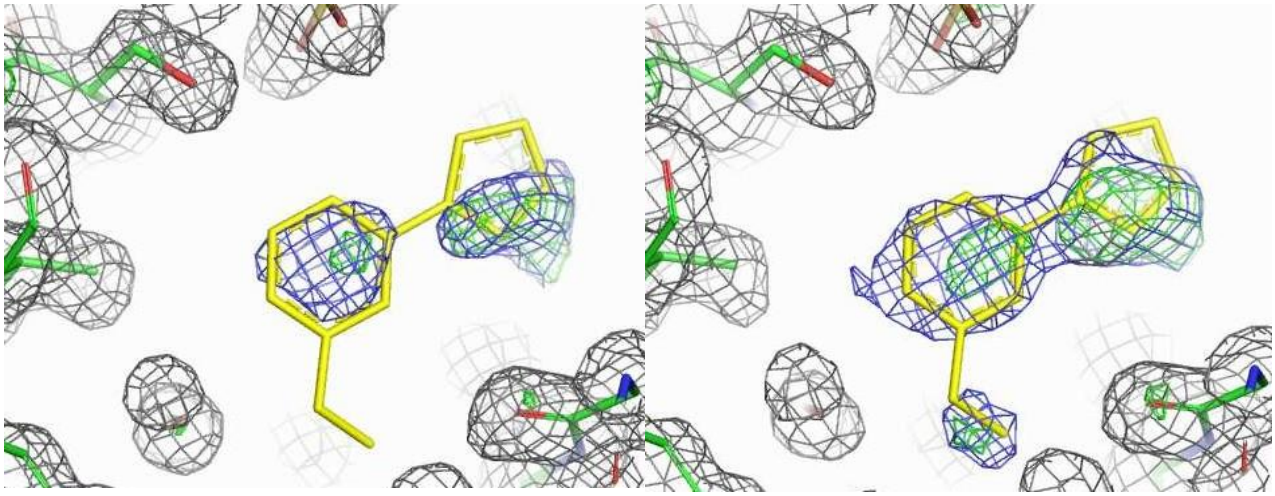
In Structure-based Drug Design, determining the structure of ligands in complex with target proteins is critical to the drug design process. X-ray Crystallography is a modern tool in structural biology used to investigate these molecular structures at high resolution. [1]

The aim of this project is to improve the electron density maps around these ligands, thereby improving the atomic models. Diffraction data from these complexes are collected using serial X-ray crystallography from many micro crystals. We are developing algorithms such as Genetic algorithms and Hierarchical cluster analysis that can identify isomorphic groups from these large pools of data to improve ligand electron density. [2-3]



Electron density map (gray-around model and blue-around ligand: 2fo-fc 1 sigma, green: fo-fc 3 sigma) of all datasets merged (left image) compared to that of selected datasets by Genetic algorithm (right image).

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Role of the tyrosine kinase Pyk2 in synaptic function and in the pathophysiology of Alzheimer's disease

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Aims: A genome wild association study has identified PTK2B, a gene which encodes for Pyk2, a neuronal Ca^{2+}-activated non-receptor tyrosine kinase implicated in synaptic function, as a major risk factor for Alzheimer's disease (AD)[1]. Several studies have validated the implication of Pyk2 in the pathophysiology of AD but contradictory findings have been reported[2–4]. The objective of this project is to further understand the role of Pyk2 in physiological and pathophysiological synaptic functions in the context of AD.

Methods: Murine primary neuronal culture (DIV 14) were transfected with Life-actin GFP and Pyk2 plasmids with altered kinase activity to assess the influence of PyK2 kinase activity on dendritic spines density and morphology. Furthermore, we measure Pyk2 expression and phosphorylation profile in the hippocampus and cortex of APPxPS1-21 transgenic mice, a model of AD.

Results: Pyk2 overexpression leads to a decrease in synaptic density when it is overexpressed in neurons. This effect does not appear to be related to its kinase activity since this decrease is also observed with the inactive mutant Y402F of Pyk2. In APPxPS1 mice, we observed a strong decrease of Pyk2 phosphorylation in the cortex at 6 and 9 months.

Conclusions: Together these results highlight the physiological role of PyK2 in the structural features of excitatory synapses. Because PyK2 phosphorylation displays a long lasting reduction in APPxPS1-21 mouse model of AD, we will study further the consequences of an altered PyK2 activity on excitatory neurotransmission in the context of AD.

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Deciphering the interaction between VASH-SVBP detyrosination enzymes and microtubules using single molecule TIRF microscopy

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Microtubules are key eukaryotic cytoskeletal elements that are regulated by a mechanism called the 'tubulin code,' which involves the differential expression of α- and β- tubulin isotypes and the post-translational modifications (PTMs) of tubulin[1]. A prominent tubulin PTM is the cyclic removal and re-addition of the C-terminal tyrosine residue of α-tubulin. This cycle of detyrosination/tyrosination is a tubulin PTM, where it is imperative to maintain the equilibrium as any change can lead to undesired pathological conditions. Vasohibins (VASH1 and VASH2), which are ubiquitous proteins, were identified as the first tubulin-detyrosinating enzymes[2]. Small vasohibin-binding protein (SVBP), whose loss has been associated with impaired neuronal development, acts as a chaperone and co-factor for vasohibin activity.

Even though the crystal structure of VASHs in complex with SVBP was resolved recently[3], mechanisms underlying the interaction of this enzyme with microtubules remain poorly explored. During my thesis, I am examining the nature of interaction between VASH-SVBP complexes and taxol-stabilised microtubules in-vitro, using single-molecule total internal reflection fluorescence (TIRF) microscopy experiments, and correlating to their detyrosinating activity using immunofluorescence assays. I started by comparing the active complex to a catalytically dead mutant, where the catalytic cysteine residue of VASH was mutated to alanine. I compared their binding on microtubules enriched in tyrosinated or detyrosinated tubulin. Unlike the detyrosinated microtubules, tyrosinated microtubules are the usual substrate of these enzymes. After getting an idea that in case of the active complex, there is a question of substrate evolution over time (owing to its active nature on tyrosinated microtubules), I am continuing the rest of my project with the mutated full length and truncated versions of the complexes. Since the VASH structure is typically composed of three main domains namely, the N-terminal, the core domain, and the C-terminal, with this study I would like to further pinpoint the role of each of the domains. This study will not only help in gaining an insight into the detailed functional and behavioural aspects of these enzymes that regulate such a crucial physiological cycle but also in designing vasohibin inhibitors that might be useful in preventing abnormally increased tubulin detyrosination levels associated with cancer, heart diseases, and neurodegeneration.

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Ruminococcus gnavus' Sactipeptide RumC1; a Possible Alternative to Conventional Antibiotics?

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Antibiotic resistance is considered as one of the main health challenges around the world. The WHO is alerting that the death toll caused by antimicrobial resistance might reach 10 million cases in 2050 (1). One of the possible alternatives to conventional antibiotics is a class of antimicrobial peptides called RiPPs (Ribosomally synthesized and post-translationally modified peptides) produced by bacteria. *Ruminococcus gnavus*, a strictly anaerobic commensal bacterium residing in the human colonic microbial community (2), possesses a regulon encoding for five peptide isoforms called RuminococcinC (RumC1-C5) (3). These peptides belong to the "Sulfur-to- Alpha Carbon Thioether Peptides" class, also known as "Sactipeptides". In other words, they carry intramolecular thioether bonds between the sulfur atom of a cysteine residue and the Cα of a partneramino acid. These thioether bonds are inserted by Radical-SAM enzymes, called sactisynthases. Two genes encoding for such enzymes are present in the regulon (3).

We recently demonstrated that RumC1 presents a double hairpin structure held by four thioether bonds inserted onto the precursor peptide during the maturation step by a radical-SAM enzyme (4). We also reported that RumC1 possesses a strong antimicrobial efficacy with minimal inhibitory concentrations (MIC) that are similar to or less than those of the reference antibiotics usedfor priority pathogens including *Clostridium difficile*, *Enterococcus faecalis* and *Streptococcuspneumoniae* (5,6) . Here we will present the biochemical characterizations of the maturation enzymeand the RumC1 peptide, as well as the biological activity of the mature peptide.

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SPHINX31, a spliceosome inhibitor targeting the kinase SRPK1, induces replicative stress and cell death in lung cancer cells with acquired resistance to platinum salts

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Lung cancer, including the Non-Small Cell Lung Carcinoma (NSCLC) histological subtype, is a leading cause of cancer-related death worldwide. Acquisition of resistance to therapies such as platinum salts, the gold standard chemotherapy in NSCLC, is one of the major trick supporting patient's poor prognosis. Deregulation of splicing factors involved in pre-mRNA alternative splicing has been associated with lung tumor progression. However, less is known regarding the contribution of RNA splicing defects to lung tumor escape from therapies. Recently, pharmacological inhibitors targeting different components/regulators of the spliceosome machinery have emerged as potential anti-cancer drugs, such as SPHINX31 that inhibits SRPK1 a kinase implicated in splicing regulation through the phosphorylation of various serine/arginine (SR)-rich proteins. In order to investigate whether RNA splicing defects contribute to acquired resistance to platinum salts in NSCLC, we generated cellular models of resistance derived from two NSCLC cell lines (namely H358, H460) by sub-culturing these cells (denoted hereafter H358S and H460S) with increasing concentrations of cisplatin during 4-6 months in order to obtain resistant cells (as a bulk, denoted hereafter H358R and H460R). In this study, we showed that H358R and H460R cells are more susceptible to SPHINX31-induced cell death as compared to isogenic parental cells. We found that this enhanced vulnerability correlates with higher genomic instability, as detected by increased expression of P-H2AX(Ser139) a marker of DNA double-strand breaks. This increase was also observed upon SRPK1 knock-down using siRNA. By performing flow cytometry, we further demonstrated that a fraction of resistant cells incorporates less BrdU upon SPHINX31 treatment while the inverse was observed in parental cells. In addition, using SIRF analyses, we showed that SPHINX31 strongly decreases PCNA protein recruitment at nascent replication forks in resistant cells but not in sensitive ones. These results suggested that SPHINX31 induces replication fork stalling/slowing-down thus replicative stress in resistant cells. In order to unravel the molecular mechanisms involved, RNA-seq experiments were performed. As compared to untreated cells, we found that SPHINX31 differentially regulates the splicing of different genes in all cellular models, with exon skipping representing the most frequent events. When Gene Ontology analyses were done to identify gene sets enrichment upon SPHINX31 treatment, we found enrichment of genes involved in DNA replication in resistant cells only, with five genes of interest, namely CDCA3, DBF4B, RFC5, WIZ and TERF-1. Moreover, we showed that resistant cells display higher levels of P-ATR(Thr1989) auto-phosphorylation and of its downstream target P-CHK1(Ser345), as compared to parental cells. In these cells, SPHINX31 or SRPK1 knock-down reversed activation of the ATR/CHK1 checkpoint and SPHINX31 also prevented the recruitment of TOPBP1 and ATRIP proteins into chromatin-enriched fraction. Altogether, our results identified a role of SRPK1 in the management of DNA replicative stress and the control of genomic stability in NSCLC cellular models with acquired resistance to platinum salts and highly suggested that the use of SRPK1 inhibitors could counteract platinum-salts resistance in lung cancer.

Two-component systems of *Pseudomonas aeruginosa*: From a large-scale study to targeted approaches.

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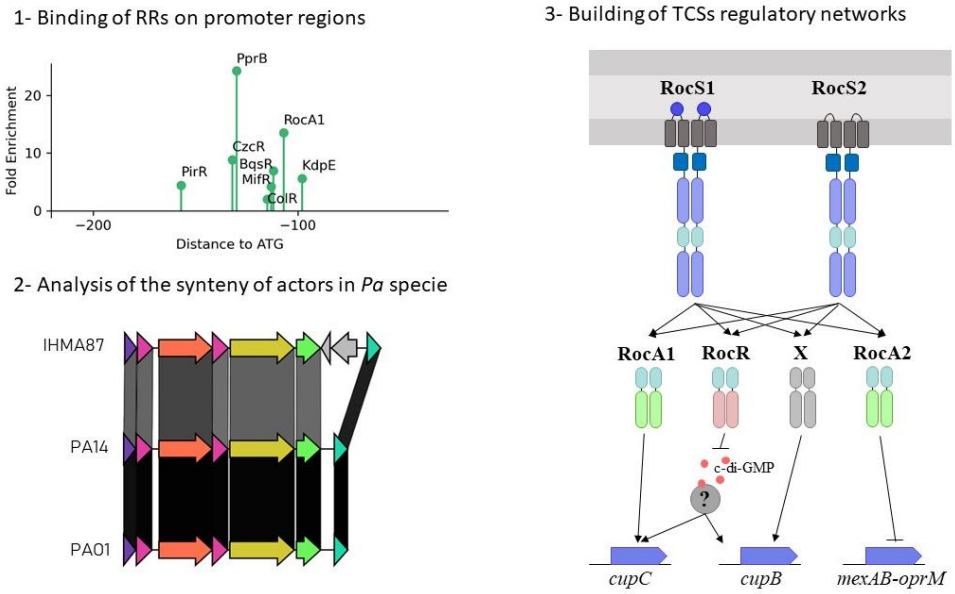
Pseudomonas aeruginosa (*Pa*) has one of the most complex bacterial regulatory networks, which is largely responsible for the high adaptability of this pathogen. Two-component systems (TCSs) play a major role in these networks: they allow bacteria to perceive and adapt to physicochemical changes in their environment. TCSs generally consist of two partners communicating by phosphate transfer: a histidine kinase (HK) which detects the signal and transmits it to the response regulator (RR) which is often a transcription factor. Particularly numerous in *Pa*, TCSs are highly studied because of their importance in antibiotic resistance and virulence. However, our knowledge of the signals they detect and the genes they regulate is largely incomplete. A recent study conducted by our team used the DAP-seq (DNA Affinity Purification-sequencing) technique to determine *in vitro* the set of binding sites for 55 Pa RRs on the genomes of 3 strains belonging to the 3 main phylogenetic groups[1]. This study provides the first complete determination of the TCS regulatory network in *Pa*, highlighting the great complexity of this network and the specificities between strains. Thanks to the exploitation of the DAP-seq results and the analysis of the synteny of the genes coding for the different actors of this network (RRs, HKs and targeted genes), our work aims to decipher the functioning of some *Pa* TCSs involved in virulence and antibiotic resistance. We show that the Roc (Regulation of cup) system, known to be particularly interconnected (2 HKs and 3 RRs), includes an additional RR that has not been characterized so far. This fourth RR activates *cupB* operon (*cupB1-6*) that code for a fimbriae involved in biofilm formation. Our results suggest that in addition to its complexity, the Roc system is diverse among species of *Pa*. We also demonstrated that the strain IHMA87 possesses a specific TCS carried by an integrative and conjugative element involved in copper resistance.



Methodology for DAP-seq investigation

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Interactions surface/cellules via la fonctionnalisation de surface : protection bactéricide et réalisation de biocomposants

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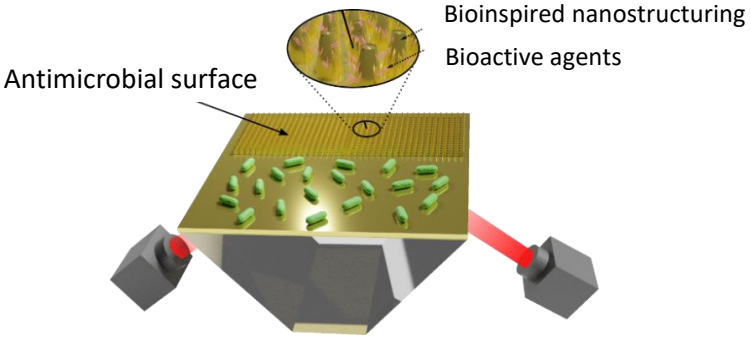
Microbial proliferation in human environments has been one major public health concern for years. Nosocomial and implants infections are particularly prevalent each year (3,500 to 9,000 deaths per year in France[1]) especially due to pathogens antibiotic resistance. To effectively fight against these micro-organisms, it is crucial to develop surfaces that can limit adhesion and proliferation in its early stages. Numerous methods providing antimicrobial properties to materials already exist or are under development. They are often divided into two categories: chemical functionalization using bioactive or anti-adhesive agents[2] and physical micro- and nanostructuring, often inspired by natural surfaces such as cicada wings.[3]

The main objective of this PhD is to study the synergy of these two approaches, as it has been little considered. By doing so, it could be possible to get rid of some drawbacks that single approaches may have, such as chemical degradation, cytotoxicity and biological waste accumulation onto the surfaces. The antimicrobial activities of single and combined approaches are quantified and compared using microbiological characterization as well as Surface Plasmon Resonance imaging (SPRi) to better understand interactions between the micro-organisms and the engineered surfaces.



Chemical functionalization and nanostructring to tackle micro-organisms proliferation – Convert L. et Costella M.

This project is a result of a cotutelle between the University of Sherbrooke (Québec) and the University of Grenoble-Alpes (France). First, the use of antimicrobial peptides (AMP) is investigated, as it is a promising candidate for tackling efficiently a wide range of micro-organisms.[4] AMP activity can be greatly modified by several immobilization parameters thus peptide orientation as well as linker length are investigated. In a second step, different bioinspired nanostructurations known to have antifouling and antimicrobial properties[5] are developed with various morphological parameters such as the aspect ratio, sharpness, density, order, etc. Finally, the two approaches are combined and characterized and multiple parameters such as solvent type, AMP concentration and regioselective functionalization are investigated to create a panel of efficient and durable antibacterial surfaces.

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Synaptic activity promotes amyloidogenic cleavage of APP and production of Aβ

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Accumulation of Aβ peptides is a key player in the development of Alzheimer's disease (AD) physiopathology. Aβ peptides are produced by the sequential cleavage of transmembrane protein APP by proteases BACE-1 and γ-secretase, respectively. Recent findings showed increased production of Aβ is driven by synaptic activity[1]. Thus, this study aims at identifying the conditions by which synaptic activity affects APP processing and Aβ peptides production.

For this purpose, we design a plasmid containing human APP695 with and without the Swedish mutation (swe) tagged with the fluorescent moiety mCherry at the c-terminus. This mutation promotes an important increase in the BACE-1 cleavage of APP leading to an early onset of AD in Human. After, the transfection of this plasmid in murine primary cortical neurons (DIV14), we monitored APP processing by measuring the fluorescence in live neurons exposed to increased synaptic activity by exposing for 15 minutes the neurons to chemical long-term potentiation (cLTP) protocols (bicuculline [50 µM] and 4-aminopyridine [2.5 mM]) in the presence of γ and/or β-secretase inhibitors [5µM, 1µM].

We showed that exposure to cLTP induced a reduction of APP fluorescence in both APP695 and APPswe. The presence of the mutation swedish on APP induces 2 folds increased in the reduction of APP fluorescence driven by cLTP when compare to APP. Pharmacological inhibition of γ and/or β secretases by DAPT and B-IV, respectively, is blocking the reduction in APP fluorescence driven by synaptic activity in neurons.

Our findings suggest that APP processing by amyloidogenic pathway can be monitored in live neurons by measuring the fluorescence of APP-tagged proteins. We showed that synaptic activity promotes BACE cleavage of APP leading to Aβ production. These finding highlight the role of synaptic activity in the physiopathological pathway leading to AD.

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BCC7 protein contributes to the *Toxoplasma* basal pole by interfacing between the MyoC motor and the IMC membrane network

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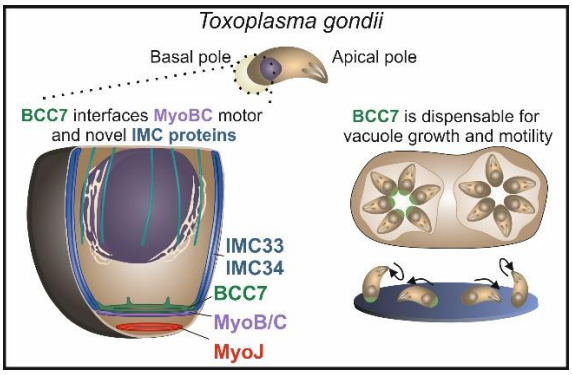
Toxoplasma gondii is an obligate intracellular protozoan parasite of a wide range of homeotherm animals worldwide. It has evolved a stage called tachyzoite which multiplies in host cells by producing internally two daughter cells. These nascent tachyzoites bud off their mother and repeat the division process until the expanding progenies escape to settle and multiply in other host cells. Over these intra- and extra-cellular phases, the tachyzoite maintains an essential apicobasal polarity that emerges through a unique bidirectional budding process of the elongating cells [1]. This process requires the assembly of several molecular complexes that, at the nascent pole, encompass structural and myosin motor elements. To characterize a basal pole marker recently identified in the lab named BCC7 with respect to the posterior acto-myosin J and myosin C motors [2], we used conventional biochemistry, advanced proteomic and in silico analysis in conjunction with live and super resolution microscopy of transgenic fluorescent tachyzoites. We documented that BCC7 forms a ribbed ring below which myosin C motor entities distribute regularly. In addition, we identified among thirteen BCC7 putative partners two novel and five known members of the Inner Membrane Complex (IMC) family which ends at the apical side of the ring. Therefore, BCC7 could assist the stabilization of the IMC plaques and contribute to the parasite biomechanical properties.



BCC7 basal pole localization in a close proximity with MyoB/C, MyoJ and the new discovered IMC33 and IMC34; BCC7 was also found to be dispensable for the tachyzoite lytic cycle (in particular for motile and growing skills). .

The work presented in this abstract corresponds to a manuscript currently under revision (Vigetti *et al*, 2022).

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The photoconversion efficiency of mEos4b depends on laser illumination conditions used in PALM

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Green-to-red photoconvertible fluorescent proteins (PCFPs) have played a key role in the development of photoactivated localization microscopy (PALM), one of the super resolution techniques that enables visualization of biological structures at the nanoscale. Efficient green-to-red photoconversion (PC) is crucial for high data quality. Yet, it remains unclear how the illumination conditions used in PALM influence the PC efficiency (PCE) of commonly used PCFPs[1,2]. Here[3], we investigated the effects of 405-nm and 488-nm light intensities on the PCE of mEos4b, a popular PCFP because of its brightness and high monomeric quality. We show that when PC is driven by 405-nm illumination, 488-nm illumination reduces the PCE of mEos4b due to increased bleaching of the green state. Furthermore, we show that while low intensity 405-nm light is crucial for efficient green-to-red conversion, intense 405-nm illumination reduces the PCE, which we attribute to nonlinear bleaching of the green state by 405-nm light. We observed similar relationships between the 405-nm light intensity and the PCE of other commonly used PCFPs, such as Dendra2 and PCstar. Our results contribute to improved understanding of the complex photophysics of PCFPs, which may guide the design of optimized illumination schemes and may inform the development of new PCFPs with desired characteristics.

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