2023 FrenchBIC meeting

Centre Moulin Mer, Logonna-Daoulas

16th-19th April 2023

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Organizing committee

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Program

Day	Time	Code	Speaker / Event	Session
Sunday 16 April	16:00 - 20:00		Registration	
	20:00 - 21:00		Dinner	
	21:15 - 22:15	PL1	Y. LE MEST/O. REINAUD/J.-P. MAHY	Session S1
				Chairman: M. Réglier
Monday 17 April	09:00 - 09:45	PL2	Y. MEKMOUCHE	Session S2
	09:45 - 10:00	OC1	S. YELISETTY	Chairman: B. Colasson
	10:00 - 10:15	OC2	J. ARNONE	
	10:15 - 10:30	OC3	U. CONTALDO	
	10:30 - 11:15		Coffee Break	
	11:15 - 11:30	OC4	Y. WANG	Session S3
	11:30 - 11:45	OC5	E. REMADNA	Chairwoman: M. Seemann
	11:45 - 12:00	OC6	L. SHAMSEDDINE	
	12:00 - 12:15	OC7	E. BODIO	
	12:30 - 14:00		Lunch	
	14:00 - 14:45	PL3	S. FLOQUET	Session S4
	14:45 - 15:00	OC8	Y. NICOLET	Chairman: J.-P. Mahy
	15:00 - 15:15	OC9	A. SALAME	
	15:15 - 15:30	OC10	T. PICHON	
	15:30 - 16:15		Coffee Break	
	16:15 - 16:30	OC11	C. ORAIN	Session S5
	16:30 - 16:45	OC12	K. CARIOU	Chairwoman: O. Reinaud
	16:45 - 17:00	OC13	M. LEFEVRE	
	17:00 - 17:05		U. ALCI - *Origalys*	
	17:30 - 19:30		Poster Session 1	
	19:30 - 20:45		Dinner	
	20:45 - 22:15		General Assembly	
Tuesday 18 April	08:45 - 09:30	PL4	M. SEEMANN	Session S6
	09:30 - 09:45	OC14	N. HAMON	Chairman: S. Floquet
	09:45 - 10:00	OC15	I. ELCHENNAWI	
	10:00 - 10:15	OC16	A. FASANO	
	10:15 - 11:00		Coffee Break	
	11:00 - 11:15	OC17	I. MICHAUT-SORET	Session S7
	11:15 - 11:30	OC18	J. DE FREITAS	Chairman: C. Léger
	11:30 - 11:45	OC19	A. NHARI	
	11:45 - 12:00	OC20	S. AHAMMAD	
	12:00 - 12:05		M. PATOU - *Jasco*	
	12:15 - 13:30		Lunch	
	13:30 - 17:30		Social Activity	
	17:30 - 19:30		Session Poster 2	
	19:45 - 21:30		Gala Dinner	
Wednesday 19 April	09:00 - 09:45	PL5	B. COLASSON *(sponsored by Wiley)*	Session S8
	09:45 - 10:00	OC21	O. MANGEL	Chairwoman: Y. Mekmouche
	10:00 - 10:15	OC22	Q. SHEN	
	10:15 - 10:45		Coffee Break	
	10:45 - 11:00	OC23	A. ALDINIO-COLBACHINI	Session S9
	11:00 - 11:15	OC24	K. MAJEE	Chairwoman: C. Hureau
	11:15 - 11:30	OC25	E. DE LA MORA	
	11:30 - 11:50		Closing Ceremony	
	11:50 - 12:00		Lunch Box Distribution and Departure	

Plenary lecture

Towards eco-compatible chemistry: metalloenzyme mimics and artificial metalloenzymes

Jean-Pierre Mahy[a,*] Frédéric Avenier[a], Wadih Ghattas[a], Rémy Ricoux[a]

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In view of the current economic and ecological contexts, the questions of setting up clean and eco-efficient processes as well as saving our energy resources appear to be fundamental. There is thus a need to develop reactions that would solve the problem of the selective transformation of chemicals under mild conditions. This urges researchers and industrials to develop ''green chemistry'' processes which must not only include catalytic processes to limit waste, but also to use harmless solvents, the ideal of which would be water, with low temperatures and pressures to limit energy consumption. Nature has already solved this problem using biocatalysts that fulfill all the conditions mentioned above: enzymes and, in particular, metalloenzymes.

To meet this challenge, our group has long chosen to develop two main strategies of research (Figure 1) that are based respectively on:

- The synthesis of bio-inspired catalysts based on metal complexes in which a metal cation is coordinated to ligands that mimic the first coordination sphere of this metal by active site amino-acid side chains inside the active site of the protein [1]
- The generation of new biocatalysts: artificial metalloenzymes or "Artzymes" (AMEs)[2] that combine the robustness and wide range of reactions of chemical catalysts with the ability of enzymes to work under mild conditions in an aqueous medium and with high selectivity. these new hybrid biocatalysts can be obtained by combining a metal complex with a protein, so that they could be able to catalyze selective reactions under eco-compatible conditions.[3,4]

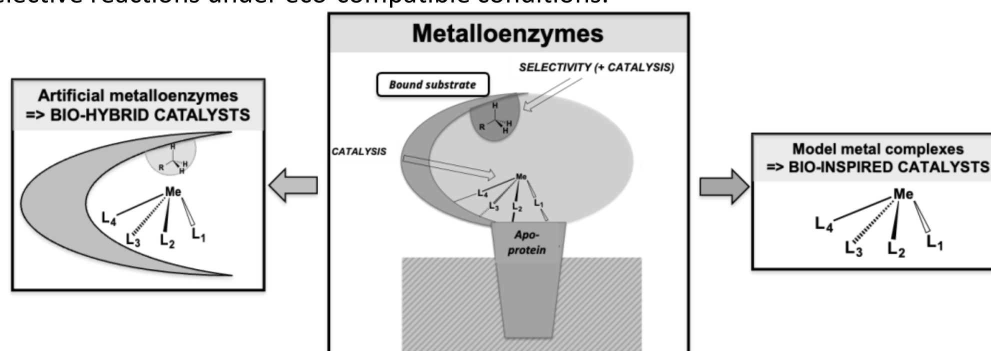

Figure 1. Two strategies to mimic metalloenzymes: elaboration of biohybrid and bioinspired catalysts based on metal complexes

The most recent developments of our research along these two axes will be presented here:

- The transfer of unprotected amine catalyzed by bio-inspired non-heme iron(II) complexes,[5]

- The design of new artificial metalloenzymes which perform the photocatalytic production of hydrogen and the reduction of carbon dioxide,[6] or have a metal-adaptive coordination site and display a Ni-dependent quercetinase activity.[7]

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2. Schwizer, F. et al. *Chem. Rev.* **2018,** *118*, 142.
3. Mahy, J.P. et al., *Chem. Commun.* **2015**, *51*, 2476.
4. Mahy, J.P. et al., in: *Modern Biocatalysis: Advances towards Synthetic Biological Systems*, Eds. Williams G.; and Hall, M.; RSC (UK) **2018**, 3, pp. 53-87
5. Boullé, A. et al., *Chem. Commun.* **2023,** *59*, 79-81
6. Oliveira Udry, G. A. et al., *Int. J. Mol. Sci.* **2022**, *23*, 14640
7. Beaumet, M. et al. *J. Inorg. Biochem.* **2022,** 235, 111914

Une histoire d'O

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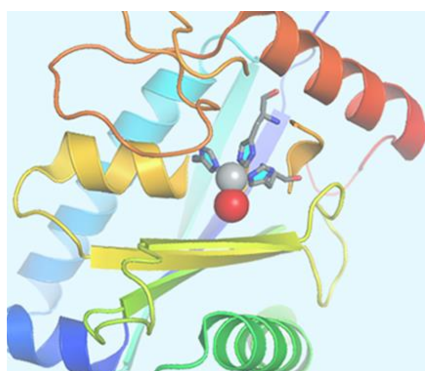
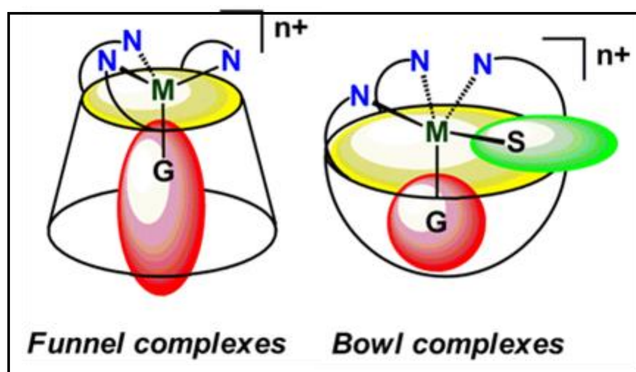
Molecular recognition in a confined space is a classical event in biology, at the basis of essential processes such as signaling or catalysis. A metal ion is often associated with it, active as a Lewis acid or redox center. The third element inevitably present is water, either as a solvent or as a molecular actor involved in recognition or catalysis phenomena. Having developed biomimetic cavity systems capable of binding and thus controlling the reactivity of a metal ion,[1-3] we are interested in the particular role that water can play as a solvent but also as a molecule interacting with the cavity complexes. A set of surprising results obtained with calixarenes1,4 and resorcinarenes[5-8] ("funnel" and "bowl" complexes) will be presented.





Proteic metallo-site **Supramolecular modeling**

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2. J.-N. Rebilly, B. Colasson, O. Bistri, D. Over, O. Reinaud, *Chem. Soc. Rev.* **2015**, *44*, 467-489. DOI: 10.1039/c4cs00211c
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4. N. Le Poul, B. Colasson, G. Thiabaud, D. Jeanne Dit Fouque, C. Iacobucci, A. Memboeuf, B. Douziech, J. Řezáč, T. Prangé, A. de la Lande, O. Reinaud, Y. Le Mest, *Chem. Sci.* **2018**, *9*, 8282–8290. DOI: 10.1039/C8SC03124J
5. E. Brunetti, L. Marcelis, F. E. Zhurkin, M. Luhmer, I. Jabin, O. Reinaud, K. Bartik, *Chem. Eur. J.* **2021**, *27*, 13730–13738. DOI: 10.1002/chem.202102184.
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Hybrid catalysts based on surface functionalized laccases

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Active heterogeneous chemoenzymatic catalysts that combine enzymes and metal complexes are increasingly attractive for selective transformations, especially the oxidation of organic substrates. These combinations lead to a huge diversity of properties, and synergies appear between the catalysts leading for example to increased turnover numbers.[1,2,3] It is crucial to control every level of the catalytic system design, ranging from the choice of enzymes, catalysts and material, and their functionalization, to their orientation, loading and organization. We recently achieved a site-directed immobilization of a fungal laccase into glutaraldehyde activated Si(HIPE) monoliths (High internal Pickering Emulsion).[4] Laccase (EC 1.10.3.2) is a robust biocatalyst, whose active sites are composed of two copper-containing centers: a surface located T1 Cu^{II} center responsible for the oxidation of substrate and a trinuclear copper T2/T3 center (TNC) responsible for the reduction of O_2 into H_2O.[5] Selectively decorating the inner surface of the foam with variants containing a unique solvent-exposed lysine located either near (K_{157}) or opposite (K_{71}) to the T1 substrate oxidation site named as $UNIK_{157}$ and $UNIK_{71}$ respectively, leads to tune the catalytic efficiency of the immobilized laccases. We will present our latest architectures based on the oriented grafting of the enzyme surface with a Pd based complex. Alcohol oxidation and laccase functionalized silica-based materials will be discussed.

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5. Solomon, E.I.; Heppner D. E.; Johnston, E.M.; Ginsbach, J. W.; Cirera, J.; Qayyum, M.; Kieber-Emmons, M. T.; Kjaergaard, C. H.; Hadt, R.G.; Tian, L. *Chem Rev.* **2014**, *114*, 3659-3853

Use of Molybdenum complexes for the safeguard of the honeybees

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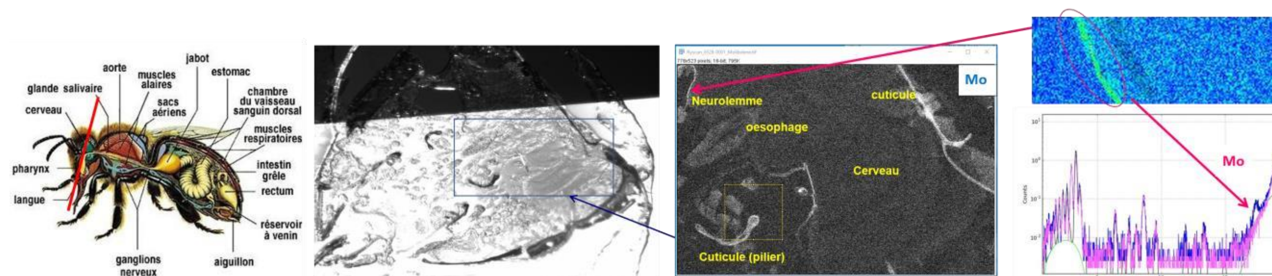
Bees are experiencing a worrying decline around the world. Beyond this decline, 20,000 species of plants are also affected by their disappearance and nearly 40% of our diet. Molybdenum is an essential trace element for life. Found in over fifty enzymes, Mo plays an important role in plants and in animals. For several years we are developing molecular complexes based on Mo for applications in biology.[1,2] In particular, very simple coordination complexes have proved to be very active in beekeeping. Non-toxic, these molecules seem to stimulate the immune system of bees, which become more resistant to temperature variations, parasites and bee diseases and therefore provoke a significant decrease of the colonies mortality. A first part of this talk will be focus on the choice of the complexes and their effects in beehives in different conditions and different countries.[3] A second part will be dedicated to understanding of the role played by these complexes within the bees' organism, thanks to many techniques such as ICP-MS, X-Ray Fluorescence experiments performed on Synchrotron SOLEIL or XPS studies.



Localization of molybdenum within the head of a bee fed with a Mo-complex

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2. A. Fuior, D. Cebotari, M. Haouas, J. Marrot, G. Minguez Espallargas, V. Guérineau, D. Touboul, R. Rusnac, A. Gulea and S. Floquet, *ACS Omega*, **2022**, https://doi.org/10.1021/acsomega.2c00705
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FrenchBic 2023
Groupe français de chimie bioinorganique

Understanding enzymes through bioinorganic chemistry explorations: IspG and IspH, two targets for the development of new antimicrobials.

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Antibiotics save millions of lives each year. However, antibiotic resistance is one of the biggest threats to public health as some bacterial infections impossible to treat with the existing therapeutic repertoire are emerging everywhere.[1] As a consequence, there is increasing urgency to discover new drugs with innovative modes of action, acting on new bacterial targets, to avoid entering into a 'post-antibiotic era' where common infections or insignificant injuries lead to fatalities.

Targets that could be explored to combat antibacterial resistance are unstable and difficult-to-isolate essential bacterial enzymes, including certain metalloenzymes, that were not previously discovered due to lack of knowledge and technology to characterise them.

IspG and IspH are such enzymes. These oxygen-sensitive enzymes are essential for the survival of numerous disease-causing microbes, including those posing a major challenge for new drug development. They are involved in the last two steps of the methylerythritol phosphate pathway and provide Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP, Figure 1), key compounds for the synthesis of terpenoids. Importantly, these enzymes do not exist in humans and are therefore valuable targets for the development of new specific antibacterial drugs. They contain a [4Fe-4S] centre and catalyse unprecedented reactions that are sources of inspiration for new antibacterial strategies.[2,3,4]

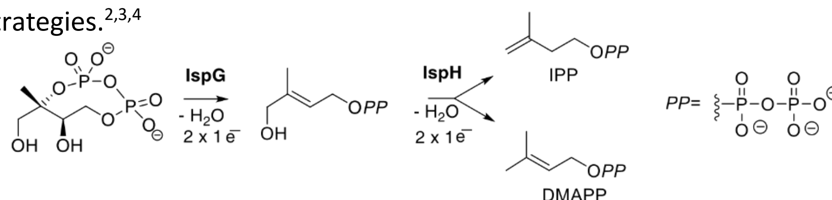

Figure 1. Reactions catalysed by IspG and IspH

In this talk, we will present the discovery, characterisation and breakthroughs made in the elucidation of the catalytic mechanism of these metalloenzymes as well as the exploitation of the first results to discover new highly efficient inhibitors.[2,3,5,6,7]

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2. H. Jobelius, G. I. Bianchino, F. Borel, P. Chaignon, M. Seemann, *Molecules*, **2022**, *27*, 708.
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Supramolecular design for biomimetic model complexes

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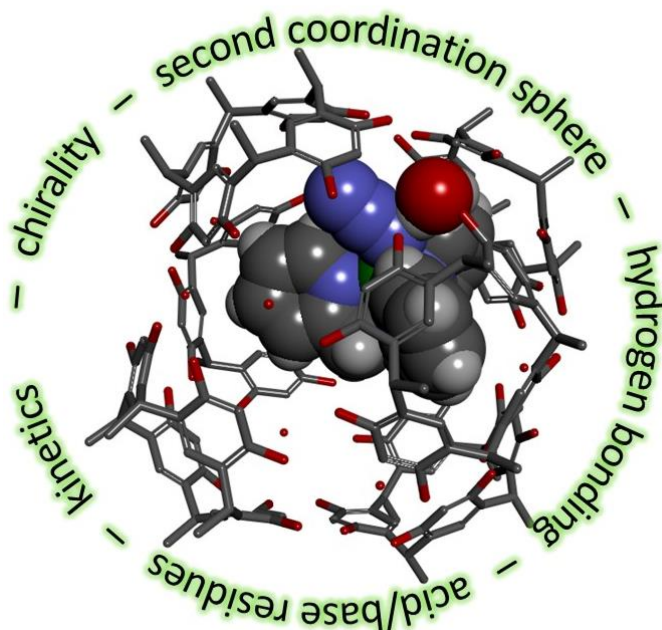
The design of biomimetic models of metalloenzymes needs to take into account many factors and is therefore a challenging task. While most of the works rely on the introduction of functional groups on the ligands in the periphery of the metal centres, supramolecular chemistry appears to be a fruitful alternative for controlling the properties of metal complexes. In this presentation, we discuss an original strategy to control the second coordination sphere of a metal centre and its distal environment.[1] A biomimetic model is assembled with a tris(2-pyridylmethyl)amine (TMPA) ligand-based complex encapsulated in a supramolecular hydrogen-bonded capsule. The characterization of some of the properties resulting from this design will be presented. This simple and broad scope strategy is unprecedented in biomimetic studies and appears as a very promising method for the stabilization of reactive species and for the study of their reactivity.



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Oral communications

FrenchBic 2023
Groupe français de chimie bioinorganique

Fluorescent amyloid-beta peptide for real time probing of reactive oxygen species generated by bound Cu

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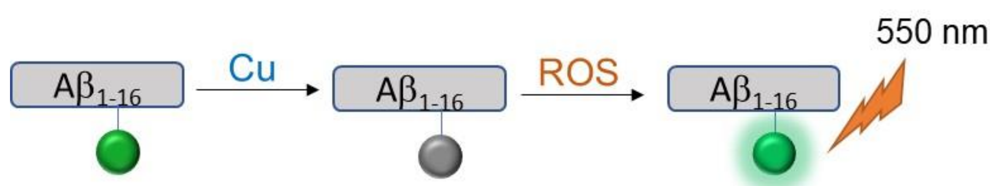
Amyloid-β (Aβ) peptide binds with Cu and produces ROS (superoxide, H_2O_2, and HO•) which has been well established to contribute to the oxidative stress in Alzheimer's disease. However, the direct generation of ROS and its contribution to the toxicity of Aβ(Cu) complex in vivo is not clear. Among ROS, OH radicals are short-lived and diffuse less which makes them relevant species to directly monitor their production by bound Cu at Aβ site. Herein we identify naphthalene monoimide (NMI) as a fluorescent tool that is capable of probing OH radical species selectively. We synthesized $A\beta_{1\text{-}16}$ peptide conjugated with the fluorophore NMI to obtain $A\beta_{1\text{-}16}^{NMI}$ and studied the ROS production catalyzed by Cu bound to $A\beta_{1\text{-}16}^{NMI}$ in presence of ascorbate and O2. NMI present close to the catalytic site of Cu bound to $A\beta_{1\text{-}16}^{NMI}$ showed several-fold turn-on increase in fluorescence response towards ROS production. Further screening and experiments with radical scavengers like catalase enzyme established the selectivity of NMI to HO• among other oxidant species (H_2O_2, superoxide, HOCl and singlet oxygen). We also studied the proximal effect of NMI to understand its ability to capture HO• generated using catalytically active Cu and Fe-EDTA systems as a proximal and bulk source of generation respectively. Aβ1-16NMI when subjected to both systems and presence of DMSO (scavenger for bulk HO•), NMI clearly responded only to HO• produced by Cu bound to $A\beta_{1\text{-}16}^{NMI}$, but not to the HO• produced in the bulk by Fe-EDTA, revealing the NMI reactivity to the proximal generation of HO•. We further delineated the mechanism of NMI turn-on response as chemical modification and characterized it as HO• induced oxidative demethylation of NMI (φ_{NMI} = 0.007) resulting in demethylated NMI (NMIDM) with high quantum efficiency (φ_{NMIDM} = 0.21). The ability of NMI on $A\beta_{1\text{-}16}^{NMI}$ to react with HO• has also been validated *in cellulo* conditions. In conclusion, we identify NMI as a potential probing tool to measure the ROS catalyzed by Cu bound to $A\beta_{1\text{-}16}$ and further being investigated to measure in presence of full-length Aβ peptide.



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2. E. Falcone, M. Okafor, N. Vitale, L. Raibaut, A. Sour, P. Faller, Coord. Chem. Rev. 2021, 433, 213727.

FrenchBic 2023
Groupe français de chimie bioinorganique

Design of a cascade reaction by heterogeneous artificial metalloenzymes

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Oxidation reactions are important processes in organic synthesis. In industry, making these processes less toxic and more respectful of the environment and health is a daily challenge. Enzyme catalysis is so far the best solution to combine selectivity, toxicity, energy costs, and safety. Nevertheless, the range of reactions is often restricted to natural substrates or analogues, whereas man made catalysis is more adapted for new to nature reaction. Then, our interdisciplinary project aims to propose more sustainable alternatives, through a novel heterogeneous catalytic methodology based on the design of bio-inspired artificial metallo-enzymes (ArMs).[1] These catalysts allow thus to work in a wide range of operating conditions (low pH, high temperatures, organic or aqueous solvents…) and to be reusable.[2] To perform catalysis under heterogeneous conditions, cross-linked enzyme crystals (CLEC) are created *via* the insertion of complexes into crystals of NikA, a Ni importer from *E. coli*. To go further, the project aims at a cascade of reactions, including a hydrothiolation followed by a sulfoxidation in order to add a sulfoxide function on an alkene (see scheme).

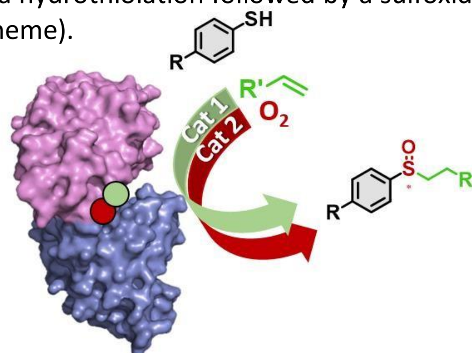

Figure 1. Tandem reaction by CLEC ArMs

Up to now, several complexes, known to carry out these reactions, have been synthesized. The $Fe(III)N_2Py_2$ complex is used for hydrothiolation and Schiff base V(IV) ones for sulfoxidation.[3] In the case of the Bolm inspired ligands, modifications to anchor them in the NikA cavity have been developed. Indeed, a carboxylate moiety on the Schiff base ligand has been introduced to form hydrogen bonds with arginine residues present in the cavity.[4] During this communication, I will present the synthesis and characterization of a series of vanadium complexes with these modified ligands. The characterization of ArMs with those V complexes will be discussed, using diverse methods with a special attention to X-ray diffraction. Homogeneous catalysis (complex alone) will then be compared to heterogeneous catalysis (CLECS of ArMs) on either thioether substrates for sulfoxidation or alkenes for hydrothiolation. Only the sulfoxidation part has been studied for the moment. Among the four complexes synthesized, two of them display catalytic activity for sulfoxidation reactions and are inserted into NikA-based CLEC. A yield of 90% is achieved on the methyl p-tolyl sulfide oxidation with a Schiff base V(IV) complex using H_2O_2 as the oxidant, and about 1500 TON with a CHP as the oxidant. We are currently testing the hydrothiolation reaction, and hope to share these results in the meantime. Finally, the cascade reaction will be undertaken either in solution or in NikA-based CLEC.

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Copper as metal-cofactor, active site and substrate? The case of blue oxidases involved in bacterial copper resistance

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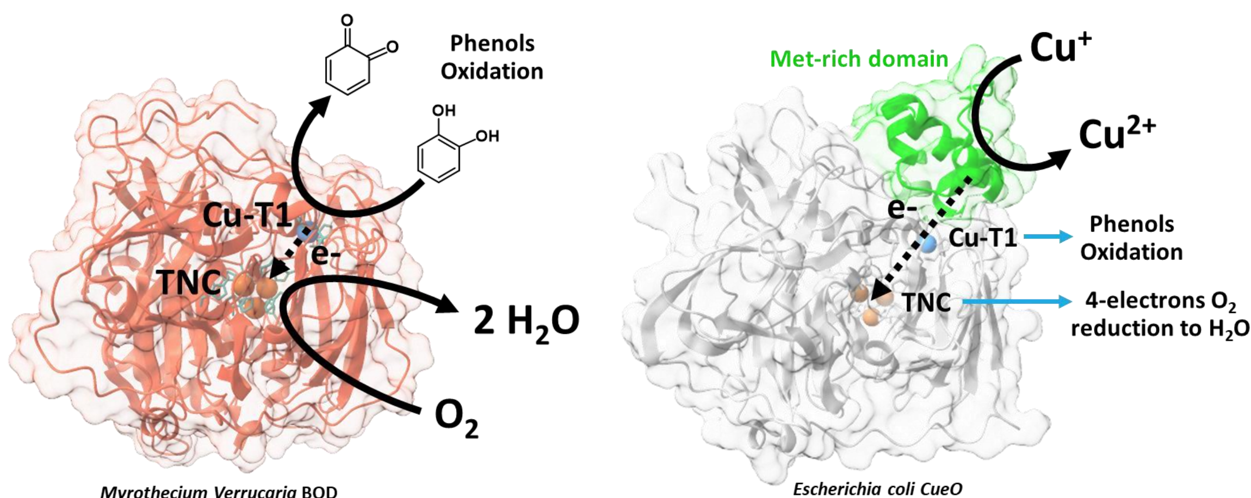
Blue oxidases or multicopper oxidases (MCOs) are enzymes that contain four Cu atoms spatially and functionally organised into two active sites. The mononuclear Cu-T1, which gives them their blue coloration, first carries out the oxidation of phenols and then the electrons are transferred to the trinuclear copper center, which finally catalyses the reduction of oxygen to water. Copper efflux oxidases (CueOs) are a subgroup of the MCO family. Unlike MCOs, the phenolic substrate binding site of CueOs is deeply buried under a methionine-rich (Met-rich) domain. This results in less phenoloxidase activity than in MCOs,[1] but allows CueO to catalyse the oxidation of cuprous to cupric ions. Depending on the microorganism, the Met-rich domain exhibits a variety of structural organisations and a number of committed Met residues.[2-5] How this Met-rich domain affects the cuprous oxidase activity of CueOs, and further the bacterial resistance to copper, is thus a relevant biological question. Here we address this question by studying the *in vitro* activities of the widely described CueO from *Escherichia coli* and a novel CueO enzyme from *Hafnia alveii*.



Myrothecium Verrucaria BOD

Escherichia coli CueO

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Bioinspired mono-copper complexes: synthesis, activity and mechanism

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In the context of challenging substrate oxidations by copper-containing enzymes, Lytic Polysaccharide Mono-Oxygenase (LPMO) is an important biological example of a catalyst capable of activating strong C−H bonds of polysaccharides using dioxygen (and electrons) or hydrogen peroxide as co-substrate.[1, 2] Among possible Cu/O_2 species, **high-valent copper oxygen adducts (e.g. $[CuO]^+$ or $[CuOH]^{2+}$)[3] have been proposed as key oxidizing intermediates. (Figure 1)**

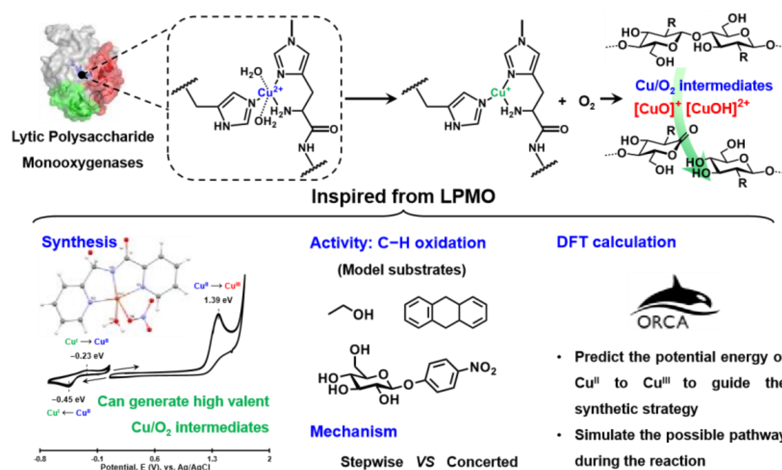

Figure 1. Proposed active site of lytic polysaccharide monooxygenase (LPMO) for strong C−H bond oxidation (up); Research strategy of mono-copper complexes that inspired from LPMO (down).

Bioinspired from LPMO, we have prepared and characterized new mono-copper complexes that can stabilize high valent Cu/O_2 species. Their properties (e.g. XRD, EPR, UV-Vis, electrochemistry) and reactivity towards various substrates have been investigated. Our experimental data, combined with DFT calculations will be presented.

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Compounds [(CC)Au(NHC-R)X] a new family: Syntheses and biological studies (SAR)

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Metal-based cancer treatments appeared in the late 1970s with the appearance of platinum-based compounds.[1] Although these treatments are used in therapy today, they have many inconveniences. This results in the development of significant side effects (neurotoxicity, nephrotoxicity)[2] or the development of resistance.[3] For this reason, studies have been conducted by replacing platinum(II) with isoelectronic gold(III). However, the low stability of Au(III) ion in physiological environment prompted the development of ligand systems improving the redox stability of Au(III) complexes. Within this frame, many gold (III) complexes with ligands (C^N) or (C^N^C) have been synthesized and have been shown to be active with a micromolar range of activity.[4] In particular, Au(III) complexes with (C^N^C) ligands appeared stable even in the presence of reducing agents such as glutathione.[5] However, out of the four available coordination sites, three are blocked with these ligands, leaving little possibility of functionalization or coordination of gold(III) with biomolecules. The objective here is to synthesize a new family of gold(III) complexes, replacing the ligand (C^N^C). After a first study of compounds with a biphenyl ligand (C^C) and a dinitrogen ligand (N^N) which appeared too labile for further drug development,[6] we focused on the synthesis of a new family of complexes composed of a biphenyl ligand (C^C), a chlorido ligands and pyridinyl-NHC based on imidazole and benzimidazole scaffolds. These new organogold complexes were tested on different human cancer cell lines. Further biological studies have been performed (toxicity,uptake, excretion)[7,8] to determine structure-activity relationship.

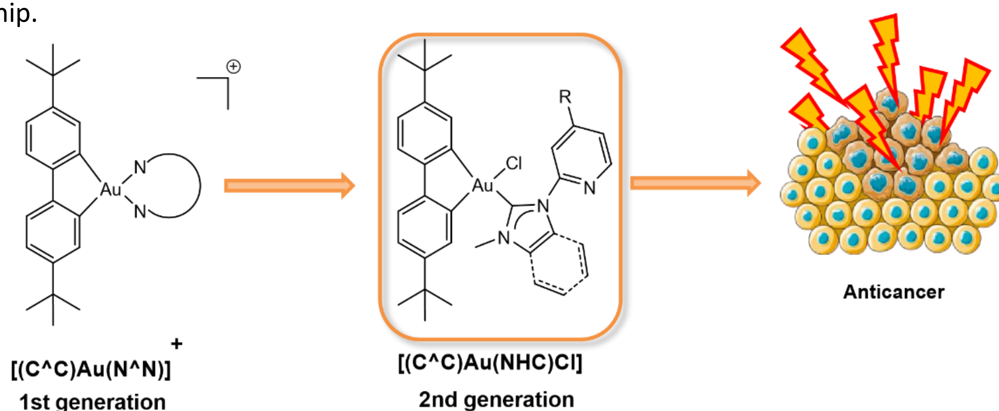

Fig. 1 Optimization of gold(III) complexes with pyridinyl-NHC ligands and assays for anticancer activity

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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 posttranslationally modified peptides) produced by bacteria. *Ruminococcus gnavus E1*, 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 partner amino 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 used for priority pathogens including *Clostridium difficile, Enterococcus faecalis* and *Streptococcus pneumoniae*.[5,6] The presentation will address the biochemical and functional characterizations of the other four RumC peptides and will give insights into the mode of action of these sactipeptides.

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AzaMDIPY, a new class of NIR-emitting fluorophores

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In recent years, aza-boron-dipyrromethenes (aza-BODIPYs) have gone from being a scientific curiosity to a class of fluorophores of major interest just like rhodamines or cyanines. These nitrogenous analogues of BODIPYs have built their reputation thanks to their ease and speed of synthesis, their high chemical and photochemical stability, their good quantum yields, and especially thanks to the fact that they absorb and emit light in the NIR-I (700-900 nm). This corresponds to wavelengths sought after for *ex vivo* and *in vivo* optical fluorescence imaging or even for surgical assistance.

Some aza-BODIPYs, that we have developed,[1] can even emit in the NIR-II (1000-1700 nm), which allows to further improve the resolution of the images. This area is much more difficult to access and many works have been undertaken to try to reach this area by modifying the substituents of the aza-BODIPYs, by rigidifying them, by extending their conjugated system, but very few groups have focused their attention on the boron atom of the aza-BODIPY core. Indeed, this fluorophore can be considered as an aza-dipyrromethane ligand chelating a boron atom, which rigidifies this structure (figure 1). We can therefore wonder what would happen if we replaced this boron atom by a metal. The few previous studies have often resulted in unstable and low emissive compounds. This is why we have been inspired by salen chemistry to stabilize our complexes (figure 1).

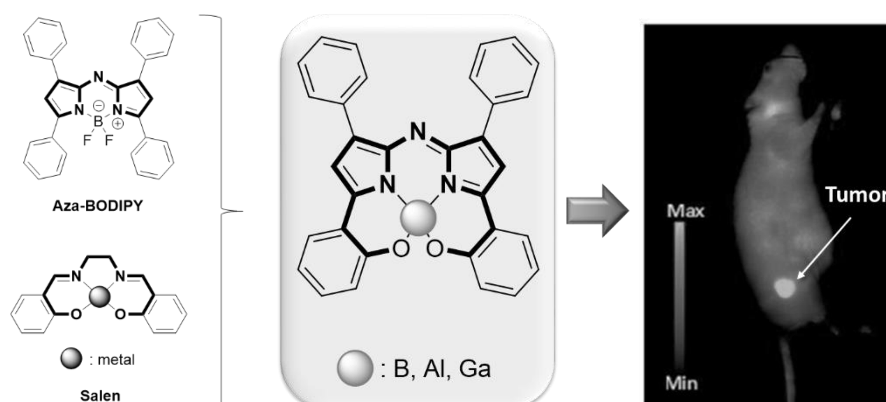

Figure 1: Structure of **azaMDIPY** and observation at 800 nm of mouse bearing U87 tumor at 24h after intravenous injection of an **azaGaDIPY** compound (0.25g/L).

In this presentation we will describe the synthesis and characterization of different aza-Metal-DIPYs, where the metal is a group 13 element.[2] Their photophysical properties will be determined, investigations that will be completed by theoretical studies that will give elements of rationalization of the observed phenomena. Finally, the "biocompatibilisation" of the gallium complex and its biological evaluation as a contrast agent will be presented.

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FeFe-hydrogenase active site assembly: toward the function of the radical SAM maturase HydE

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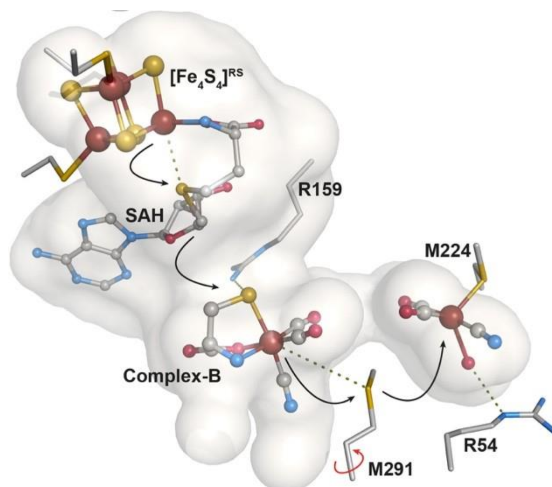
Transition metals are essential to all living organisms because they open chemistries that would not be possible when using only the 22 amino acids that constitute enzymes. Therefore, transition metals are often found at the heart of important reactions, notably in energy metabolism. For instance, transition metals play a key role in the metabolism of gases (H_2, CO_2, N_2) by microorganisms.[1] The organometallic cofactors, which constitute the active sites of the corresponding metalloenzymes (hydrogenase, CODH or nitrogenase) are nowadays inserted into the apo-enzymes by dedicated multiprotein machineries.

In this presentation, we will focus on the assembly of the $[2Fe]_H$ center of the FeFe-hydrogenase. This center contains two irons, both bound to cyanide and carbon monoxide ligands and an azadithiolate bridging molecule.[2] Notably we will discuss in details the role of the two radical *S*-adenosyl-L-methionine (SAM) enzymes HydG and HydE in this process.[3,4]



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Spectroelectrochemical evidences of Fe-CO intermediates in the CO_2 catalytic reduction by Fe porphyrins

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The study of the electrochemical reduction of carbon dioxide (CO_2) to carbon monoxide (CO) and other C_1 products offers us increasingly efficient ways to control CO_2 reduction reaction (CO_2RR) and to further develop the production of fuels and chemical feedstock. To achieve these processes, molecular catalysts made of earthabundant metals are the core of our attention at the Laboratoire d'Electrochimie Moléculaire (LEM). Decades of electrochemical studies demonstrated that iron porphyrins are excellent homogenous catalysts for CO_2 electroreduction to CO.[1,2] Among them, [Fe(pTMA)Cl]Cl_4 is one of the most efficient catalyst.[3,4]

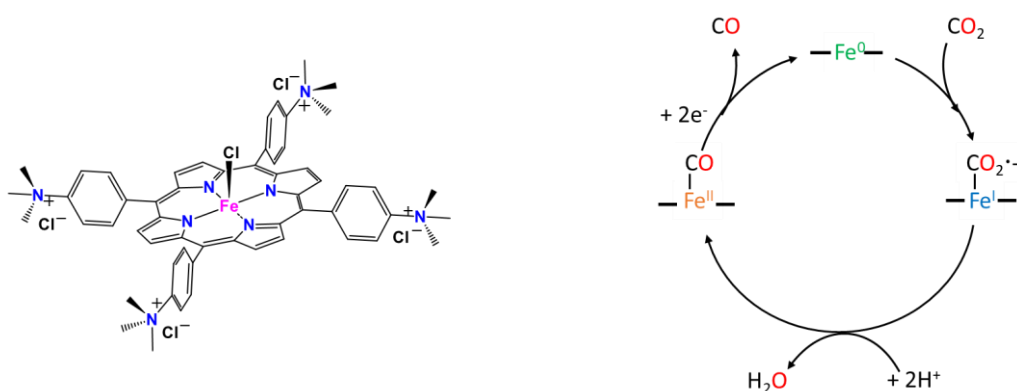

Figure 1. Left. Structure of [Fe(pTMA)Cl]Cl_4. **Right.** Plausible catalytic cycle for CO_2 electroreduction to CO by an iron porphyrin in the presence of a proton source.

Even though electrochemistry has been used for decades to understand the mechanistic aspects of this catalytic reduction of CO_2 by Fe porphyrins, the lack of spectroscopic signatures prevents us to define the structures of intermediates along the catalytic cycle. The coupling of spectroscopies and electrochemistry (spectroelectrochemistry, SEC) is a powerful tool to elucidate this mechanism.[5,6] Our most recent results obtained in closed collaboration with Uppsala University will be presented here. It allowed us to combine infrared and UV-visible SEC that proved to be invaluable for catching Fe^{X}-CO intermediates (X = III, II, I).

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Coupling a carbon monoxide dehydrogenase with hydrogenase mimics through carbon nanotubes for room temperature Water Gas Shift Reaction

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The Water Gas Shift Reaction (WGSR, $CO + H_2O = CO_2 + H_2$) is a transformation used on industrial scales to control the H_2 to CO ratio in syngas, a mixture of H_2, CO and CO_2. This is a key process to condition syngas for subsequent conversion into energy carriers (synthetic natural gas, liquid fuels) or a variety of commodity chemicals (methanol, formaldehyde, ethylene…). Currently, this reaction is carried out at high temperatures and pressure using metal oxides as catalysts (Fe, Cr, Cu, Zn, Al). This process comes with a high energy cost, and other drawbacks such as selectivity issues and sensitivity to impurities.[1] Meanwhile, carboxydotrophic microorganisms also carry out this reaction using enzymes: a [NiFe] Carbon Monoxide Dehydrogenase (CODH) oxidizes CO to CO_2, giving two protons and two electrons ($CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$). These electrons are carried over to a [NiFe] Hydrogenase (H_2ase) through a ferredoxin, allowing the reduction of two protons to molecular hydrogen ($2H^+ + 2e^- \rightarrow H_2$). This process in its entirety constitutes the biological WGSR, and takes place in mild conditions. My PhD project consists in trying to harness this bio-WGSR by immobilizing a CODH and either a hydrogenase or a hydrogenase mimic onto Multi-Walled Carbon Nanotubes (MWCNT) to allow for the exchange of electrons between both catalysts.

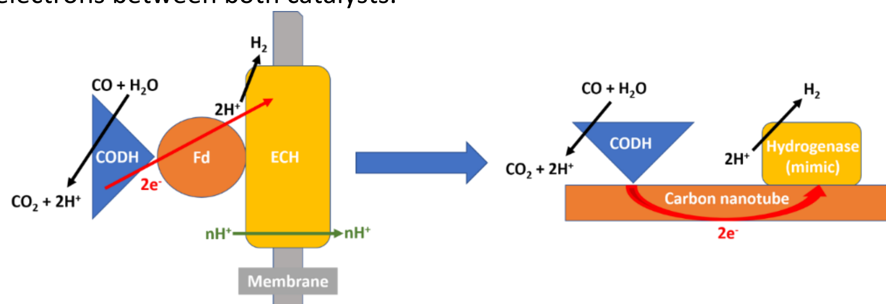

Figure 1: Schematic representation of the biological WGSR, followed by a representation of an *in vitro* bio-WGSR

Our team is specialized in the heterologous production in *E. coli* and one day purification of [NiFe]CODH from *Rhodospirillum rubrum* (RecRrCODH). This enzyme has been extensively studied by protein film voltammetry, with optimization of its immobilization on MWCNT through their functionalization, achieving high corresponding current for both CO oxidation and CO_2 reduction with low overpotential.[2,3] Using similar immobilization techniques, the [NiFeSe]H_2ase from *Desulfomicrobium bacculatum* (DbH_2ase) has also been studied by protein film voltammetry, showing high activity for the hydrogen evolution reaction (HER).[4] In a similar fashion, a hydrogenase mimic was studied: the $[Ni^{II}(P^{Cy}{}_2N^{Arg}{}_2)_2]^{8+}$ complex, inspired by the hydrogenase's active site, is a highly active catalyst towards HER and hydrogen oxidation reaction (HOR).[5] The complex was immobilized on MWCNT and electrochemically tested for activity towards the HER in aqueous buffers at various pH. These studies lead to WGSR trials using CODH and the $[Ni^{II}(P^{Cy}{}_2N^{Arg}{}_2)_2]^{8+}$ complex, which will be presented.

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Electrodes modified with Rhodium or Cobalt organometallic complexes for hydrogen production in aqueous electrolyte at neutral pH

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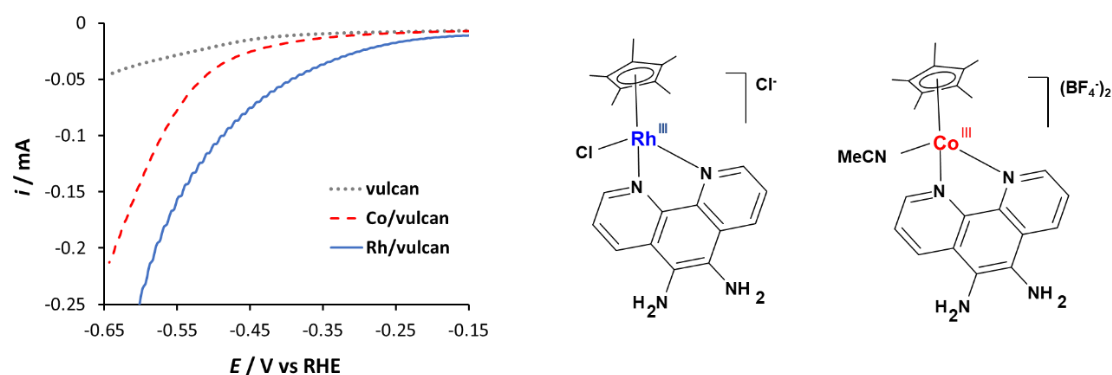
We are currently engaged in a project[1] aimed at demonstrating the fixation of dinitrogen (N_2, a very stable molecule) to ammonium (NH_4^+, an essential fertilizer) by a cathode colonized by a mixed biofilm accepting electrons directly from the electrode or in the form of dihydrogen (H_2) electrogenerated at the electrode.

In this context, our primary objective is to elaborate electrodes that are biocompatible and can catalyse H_2 production under biological conditions, i.e. in aqueous electrolyte at neutral pH. Our strategy to modify electrodes relied on organometallic complexes with phenanthroline ligand disubstituted with amine groups. The diamine moiety allows the grafting of these complexes on oxidized carbon surfaces through covalent and conjugated bonds.[2]

Here we report results on electrodes modified by $[Cp^*RhCl(phendiamine)]^+$ or $[Cp^*Co(CH_3CN)(phendiamine)]^{2+}$ (Scheme 1). The rhodium complex grafted on oxidized carbon black powder (Vulcan) has already shown its efficiency and stability under strongly acidic conditions.[3] Herein, we scrutinize its electrochemical behaviour in less acidic electrolytes (phosphate buffer) and compare them to those of the cobalt related complex, which could represent more abundant transition metal alternative. The hydrogen production efficiency is also evaluate for both type of modified electrodes.



Scheme 1. Voltammetry of modified electrodes in phosphate buffer (20 mM) and NaCl (0.1 M), pH 6.8, 5 mV s^{-1}, 2000 rpm. Structures of $[Cp^*RhCl(phendiamine)]^+$ and $[Cp^*Co(CH_3CN)(phendiamine)]^{2+}$.

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Amino-Metallocenyls Moieties for Therapeutic Applications

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Metal-based drugs constitute an ever-growing class of therapeutic agents, with a wide array of modes of actions,[1] that can allow to overcome therapeutic dead ends. A sub-category of these drugs consists of organometallic compounds,[2] which are defined as metal complexes containing at least one metal-carbon bond. They have many applications in the fields of chemical biology and medicinal chemistry. A very successful example is the ferrocene-containing compound ferroquine,[3] which is a close derivative of the antimalarial drug chloroquine and which is currently in clinical phase II trials as an antimalarial drug candidate.

In this context we pursue two lines of research: 1. The incorporation of metallocenyl-moities into drug-frameworks to improve their therapeutic profile and 2. the development of new methods to acces original metallocenyl-containing synthons.

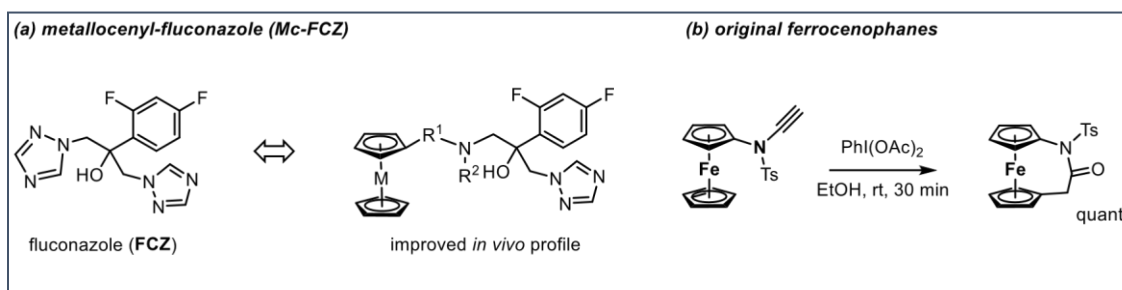

Figure 1. (a) New antimycotic metallocenyl analogues of fluconazole and **(b)** synthesis of novel ferrocenophanes

During this talk we will first present our latest results on the development of promising metal-based antimycotic[4] compounds based on fluconazole (FCZ) analogues incorporating a metallocenyl moiety (Figure 1a).[5] We will then describe a new synthetic methodology allowing access to unprecedented amino-ferrocenophanes from ynamides (Figure 1b).[6]

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ATCUN peptides in Alzheimer's disease context

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by amyloid-β peptide (Aβ) deposits leading to the senile plaques which are enriched with metal ions such as copper (Cu). Bound to Aβ peptide, Cu ions can cycle between its two redox states +I and +II, fuelled with reductants (e.g., ascorbate) and O_2. Therefore, they play an important role in the production of reactive oxygen species (ROS) contributing in the overall increase oxidative stress linked to the disease growth.[1] Consequently, there is a wide field of research that aim to prevent this deleterious peptide-metal interaction through the removal of Cu ions from Aβ. In this context, Cu chelators have thus emerged in the literature and AminoTerminal Copper and Nickel motif (ATCUN) H_2N-Xxx-Zzz-His (XZH) are of interest. They are biologically relevant motif found in the blood and serum, they bind Cu^{II} with a high affinity and form Cu^{II} complexes resistant to reduction by ascorbate.[2] Hence they have the ability to retrieve Cu^{II} ions from Cu(Aβ) complexes and prevent Cu(Aβ)-induced ROS formation.[3] Here, we report the design, synthesis and study of ATCUN motif-containing peptides whose sequences were rationally modified to probe how the nature of the first two amino-acid residues affect the rate of Cu^{II} extraction from Aβ and how it is link to the Cu(Aβ)-induced ROS production. The Cu^{II} binding rate was followed by fluorescence experiments thanks to the addition of a tryptophan residue (Trp) in the peptide sequence. Ascorbate consumption kinetic assays were used to study the ability of the new peptides to retrieve Cu^{II} from Cu(Aβ) and to redox silence it. All the Cu^{II} complexes were characterized by UV-Visible and EPR spectroscopies while their redox ability was probed by electrochemistry. We highlighted in this research that all the ATCUN motifs studied formed the same thermodynamic complex but that the addition of a second histidine in position 1 or 2 allowed for an improvement in the Cu^{II} uptake kinetics (Figure 1).[4]

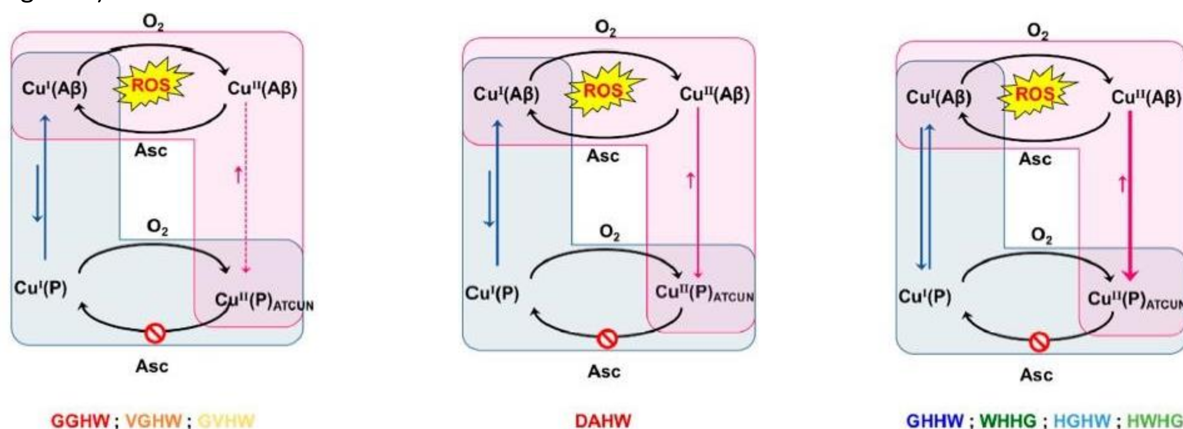

Figure 1. The three possible routes of arrest of Asc consumption as a function of the peptides in play.

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FrenchBic 2023
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Designing Ln^{3+} luminescent pyclen complexes for optical imaging: from proof of concept to biological application

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Lanthanide Luminescent Bioprobes (LLBs) exploit the particular properties of lanthanides such as sharp and intense emission bands spread from the visible to the Near Infra-Red (NIR) and long luminescent lifetimes (up to ms for Eu and Tb), which make them useful tools for time-resolved optical imaging.[1] The conception of an efficient LLB must meet several criteria:[2] (i) the coordinating sphere of the Ln^{3+} ion must be saturated to avoid interactions with OH or NH surrounding vibrators and a consequent luminescent quenching, (ii) the complexes should be kinetically inert toward the biological media, (iii) LLBs must present a high brightness to obtain images of high quality. Finally, the introduction of an additional coupling function for conjugation with targeting biomolecules is the essential asset to foresee a biological application. Polyazacycloalcanes are well known to form highly stable metal complexes. Among them, thanks to the rigidity brought by its pyridine unit, pyclen has emerged as an interesting platform for the design of lanthanide chelates. Our group previously developed a specific *N*-regiofunctionalization of pyclen with two picolinate and one acetate pendant arms which afforded non-symmetric Ln^{3+} chelates with great photophysical properties under one-photon excitation (Figure 1).[3] However, UV-excitation limited the potential of this bioprobes for *in vivo* imaging. We will present here the conception of two new generations of LLBs exploiting the advantages of two-photon excitation (biological transparency windows, high resolution) by functionalization of pyclen with π-conjugated antennas.[4] Cell imaging was successful thanks to the high brightness of the bioprobes, even with both excitation and detection in the NIR channel. Imaging and toxicity studies performed on Zebrafishes also demonstrated the potential of these LLBs for *in vivo* imaging. We then confirmed that the introduction of a coupling function on the pyclen skeleton for further targeting preserves the photophysical properties of the bioprobes.

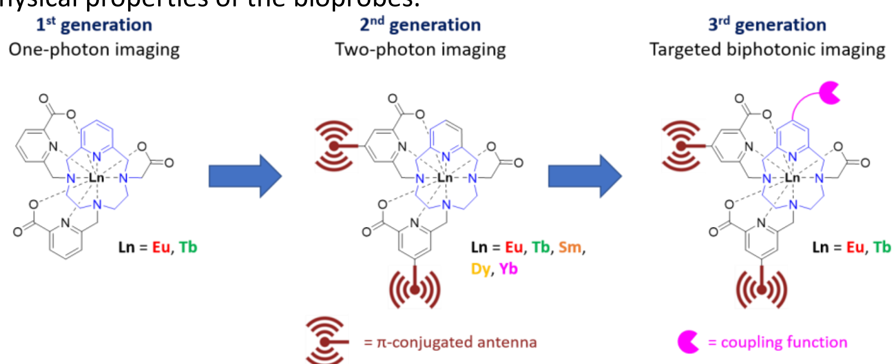

Figure 1: General structures of the Lanthanide Luminescent Bioprobes (LLBs) developed.

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Structural and functional characterization of proteins involved in Iron-Sulfur cluster biogenesis in *Mycobacterium tuberculosis*: towards the discovery of antibacterial drugs?

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Iron-sulfur (Fe-S) clusters are inorganic cofactors that are essential in various biological processes (e.g., DNA repair, respiration, photosynthesis, cofactor biosynthesis).[1] They exist in different forms and oxidation states, the most common types being [4Fe-4S] and [2Fe-2S] clusters. In bacteria, two major pathways for Fe-S cluster biogenesis and delivery have been identified: the Isc and the Suf systems. In *Escherichia coli*, the Isc system is the housekeeping pathway for Fe-S biogenesis, whereas the Suf system is active under environmental stress and iron limitation.[2] Interestingly, Mycobacterium *tuberculosis* (*Mtb*), the causative agent of Tuberculosis (TB), contains only the Suf system as Fe-S assembly machinery.[3] Suf system is essential for *in vitro* growth of *Mtb* under normal conditions[4,5] and recently proved to be a point of vulnerability in *Mtb.*[6] Moreover, the Suf components of *Mtb* are induced during iron starvation, a process experienced by the pathogen in host tissues, indicating that Fe-S assembly and therefore Fe-S metabolism may be important in the establishment of latent infection.[7] Similarly, the *Mtb* Suf system is up-regulated under nitrosative and oxidative conditions, stressors of the innate immune response.[8] Therefore, there is mounting evidence that Suf sytem is essential for *Mtb* pathogenicity and targeting it might open novel avenues for the development of novel anti-TB drugs through disturbing the pathogen's Fe-S metabolism. A requisite of that consists in characterizing *Mtb* Suf proteins.

Structural and functional characterization of the Suf proteins from the *Mtb* suf operon is mostly unknown and constitutes the main goal of our research. We will present the first characterization of two proteins of the Suf system from *Mtb*.

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Chimeric hydrogenase complexes: a new strategy to study structure function relationships

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NiFe hydrogenases are enzymes that catalyse hydrogen oxidation and production. They are composed of two subunits, the large one harboring the dinuclear NiFe active site, and the small subunit containing three FeS clusters that mediate long range electron transfer.

Here we focus on two enzymes from *E. coli*, Hyd1 and Hyd2, representative of the "O_2 tolerant" and standard (or "O_2 sensitive") NiFe hydrogenases, respectively. The catalytic bias (or catalytic directionality), the overpotential requirement for H_2 oxidation and the inhibition by CO are also very different in Hyd1 and Hyd 2.[1]

Whether these properties are related to the large or the small subunit has been debated.[2,3,4]

To gain further understanding with a new approach, we have designed and produced a chimeric NiFe hydrogenase complex, composed of the large subunit of Hyd1 and the small subunit of Hyd2. Forcing the "non-natural" interaction between these two subunits required the fine tuning of the dimer interface. Protein Film Electrochemistry allows an accurate investigation of the previously mentioned properties (small molecules inhibition and catalytic bias).[5] The comparison between the WT dimeric enzymes and the chimeric complex gives a precise picture of which subunit(s) define catalytic bias and reactions with CO and O_2.

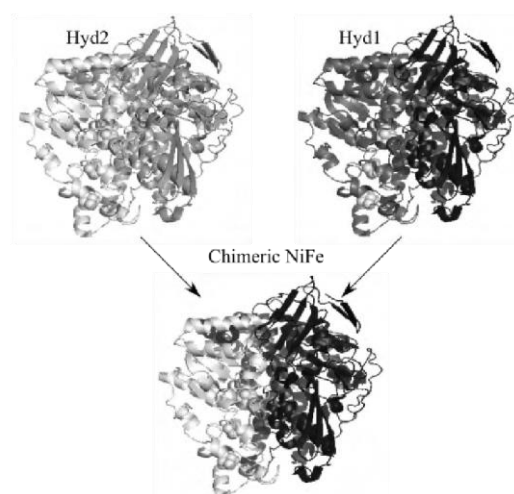

Figure 1. On top, the 2 WT NiFe hydrogenase from *E. coli*, Hyd2 in light gray (the small subunit is the lighter), Hyd1 in dark gray (the small subunit is the lighter). At the bottom, the chimeric NiFe hydrogenase, composed of the large subunit of Hyd1 and the small subunit of Hyd2.

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An additional layer of regulation of the peroxide FurC (PerR) sensor in cyanobacteria

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Metal and redox homeostasis in cyanobacteria are tightly controlled to preserve the photosynthetic machinery of vegetative cells and the nitrogen fixation of heterocyst from mismetallation and oxidative cell damage. The FUR (ferric uptake regulation) family of transcription factor proteins is the main actor of this control.

FurC, a FUR family member, works as the PerR (peroxide response) paralog in *Anabaena sp. PCC7120*. Despite its importance, this regulator remained poorly characterized. Although FurC lacks the typical CXXC motifs present in FUR proteins, it contains a tightly bound zinc per subunit. FurC:Zn stoichiometrically binds zinc and manganese in a second site, being manganese more efficient in the binding of FurC:Zn to its DNA target *PprxA*. In presence of ferrous iron, an irreversible inactivation of the regulator occurs by metal catalyzed oxidation as in other described PerR.

However, oligomerization analyses of FurC by SEC-MALLS-RI (size exclusion chromatography coupled to light scattering measurement and refractometry) evidence an additional level of regulation: the occurrence of different aggregates ranging from dimers to octamers. These oligomers, that appeared upon oxidation of thiols by H_2O_2 or diamide were bound by intermolecular disulfide bonds. Nevertheless, in the absence of metal ions this oligomerization be fully reversed by dithiothreitol reductant. Comparison of models for FurC:Zn dimers and tetramers obtained using AlphaFold Colab and SWISS-MODEL allowed to infer the residues forming both metal-binding sites and to propose the involvement of Cys86 in reversible tetramer formation. SEC-MALLS-RI analyses in anaerobiosis or/and in presence of metal ions were performed to follow the DNA binding and the oligomerization properties.

Our results decipher the existence of two levels of inactivation of FurC:Zn of Anabaena sp. PCC7120, a reversible one through disulfide-formed FurC:Zn tetramers and the irreversible metal catalyzed oxidation. This additional reversible regulation may be specific of cyanobacteria.[1]

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Molecular electrochemical reduction of N_2-to-NH_3 with a Mn catalyst

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A current chemistry challenge is the (photo)-electrochemical production of ammonia (NH_3) from atmospheric N_2 without using H_2 or emitting CO_2, as an alternative to the current Haber-Bosch process which consumes 1% of the world energy and emits nearly 1.5% of the CO_2. The nitrogen reduction reaction ($N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$) implies a cascade of elementary steps that require a delicate balance of reactivity at the metal active site M (cleavage of the N-N triple bond without creating a too strong, poisoning M-N interaction; avoiding competitive reduction of protons to H_2 …).[1,2] To date, examples of molecular electrocatalysts including abundant metals remain scarce, although recent spectacular progress has been made, notably with Fe and Mo based catalysts.[3,4] In this presentation, we will present and discuss reactivity and mechanistic aspects of a Mn catalyst able to reduce dinitrogen to ammonia in homogeneous as well as in electrochemical conditions.[5]

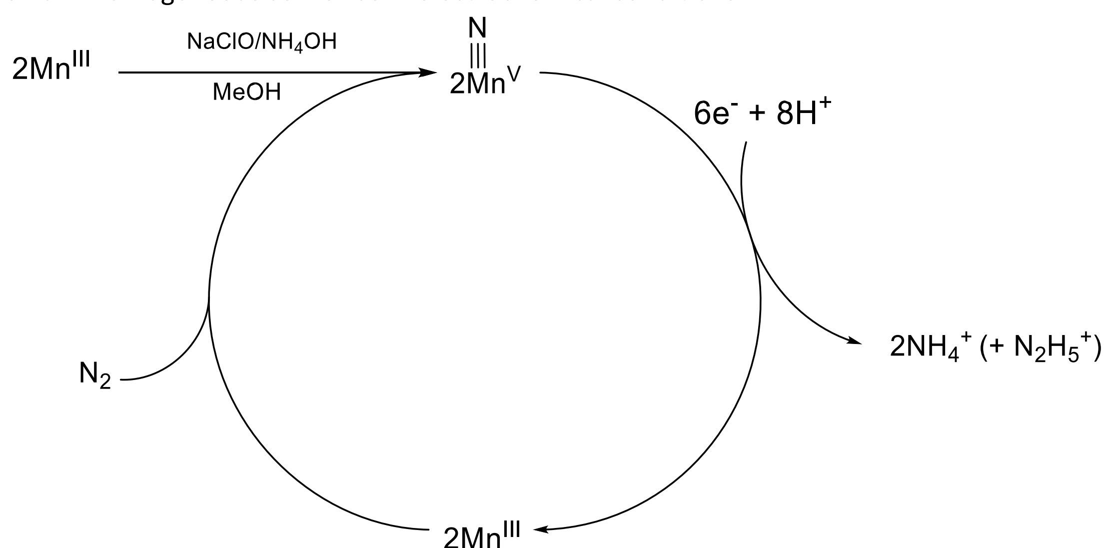

Figure 1: electrocatalytical cycle scheme for N_2 reduction to ammonia and possible side products.

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Cu^{+} is able to displace Zn^{2+} from Hsp33 zinc finger in Cu^{+} stress conditions: formation of a tetranuclear cluster

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Copper has well-known antimicrobial, antiviral and fungicidal properties.[1] Its toxicity has been attributed to the formation of reactive oxygen species (ROS) and the accompanying oxidative stress.[2] However, another mechanism observed under anaerobic conditions, inducing protein misfolding and aggregation, could explain this toxicity, as recently evidenced by the group of M. Ilbert.[3,4,5] Molecular chaperones such as DnaK or HSP33 play a major role in the bacterial defense against Cu^{+}-induced aggregate formation[5]. Interestingly, these chaperones are zinc finger (ZF) proteins, with a Zn^{2+} ion bound to four amino acids in a tetrahedral environment. ZF sites play a structural role in giving proteins their tertiary structure, but they can also be reactive sites to trigger protein activity under oxidative stress. This has been demonstrated for Hsp33, whose oxidation of the ZF cysteine triggers a conformational change that makes the protein active for its biological function: activated Hsp33 is a holdase that prevents other proteins from unfolding and aggregating. Under anaerobic Cu^{+} stress conditions, it was demonstrated by the group of M. Ilbert that the zinc atom at the core of the zinc finger can be trans-metallated by copper, activating Hsp33. The exact nature of the Hsp33-Cu complex has not been determined. Using a model peptide of the zinc finger site of Hsp33,[6] we have studied the reaction of this zinc finger with Cu^{+} in biologically relevant conditions by various spectroscopic techniques (including UV-vis absorption, fluorescence and EXAFS).

In this communication, we will present our results and show that Zn^{2+} can be replaced by a tetranuclear Cu^{+} cluster within the zinc finger site, even in the presence of glutathione in the mM range. Our results suggest activation of Hsp33 by formation of such a cluster when natural Cu^{+}-binding proteins are saturated with Cu^{+}.

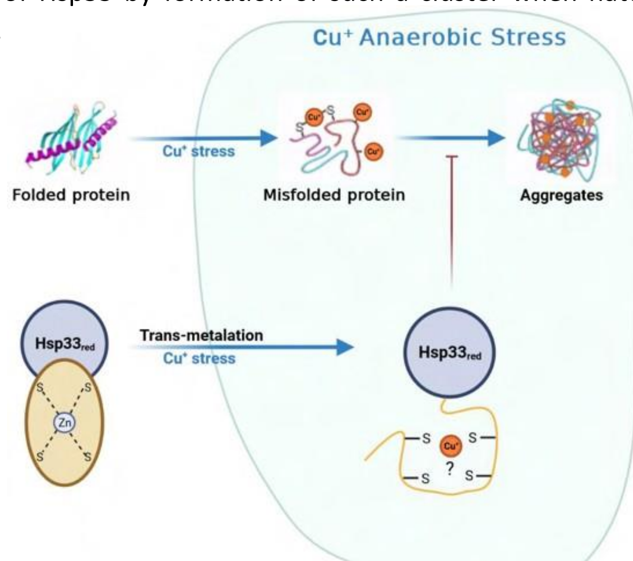

Figure 1. Inhibition of the toxicity of copper under anaerobic condition

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Synthesis and Characterisation of Molybdenum Complexes with Dithiolene containing Peptides

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The industrial revolution has increased the atmospheric concentration of CO_2, which makes it a major concern for the environment. The abundance of this polluting gas can be tackled in a useful manner by CO_2 valorization, that is its conversion to high value-added chemicals and fuels. This valorization can be the basis of a greener and more sustainable chemical industry. However, the thermodynamic and kinetic stability of CO_2 makes its activation a challenging task. Formate dehydrogenases are enzymes present in Nature which have a Mo or W metal center in their active site bound to two pyranopterin guanosine dinucleotide units, known as the molybdenum cofactor (Moco). These enzymes can reversibly convert formate to CO_2 and thus, they have attracted a lot of interest in recent years.[1] Many research groups have tried to develop biomimetic and bioinspired models of these enzymes, but only a few are hardly functional.[2] Our aim is to develop a new generation of bioinspired Mo complexes by incorporating dithiolene units in peptides and studying their structures, properties and reactivities using both experimental and theoretical approaches.

The commercially available tripeptide glutathione was chosen as starting peptide and its Cys residue was modified to introduce dithiolene units. The Mo complexes were prepared either in DMF/water and DMSO/water solvent mixtures and were characterized by UV-Vis and, EPR spectroscopies, and by theoretical calculations based on Density Functional Theory (DFT). Finally, the electrochemical properties were studied via Cyclic Voltammetry (CV) and confronted with DFT computations to get insight into the nature of the species formed during the redox processes. Interestingly, our data indicate the formation of different Mo complexes, namely a Mo(V)-oxo and a Mo(VI)-dioxo complex in DMF/water and DMSO/water mixtures, respectively. The electrochemical studies show that these Mo complexes are capable to cycle between the (+VI), (+V) and (+IV) redox states, all being relevant for CO_2 valorization reactions.

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Impact of a base on the reaction mechanism of the C-H bond activation by a di-copper complex: a DFT approach

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While hard to obtain in a an environmentally benign and efficient way, the activation of C-H bonds is a key reaction in synthesis. It is however done in nature by several enzymes including the particulate methane monooxygenase (pMMO), which makes it a topic of great interest for the design of bio-inspired complexes.[1] Several complexes have thus been synthesized to obtain a Cu^{II}-Cu^{III} center, thought to be a key intermediate in the pMMO reaction mechanism.[2] Recently, a bis-(μ-hydroxo) dicopper (II-II) complex coordinated to dipyridylethane naphthyridine (DPEN) ligand was shown to be able to form the Cu^{II}-Cu^{III} species upon electrochemical oxidation.[3] With this complex, the activation of a toluene C-H bond was observed at room temperature. The mechanism was demonstrated to be stoichiometric and to become catalytic in the presence of a base.[4]

In our study, we were able to reproduce the reaction in both cases by using DFT and Intrinsic Bond Orbitals (IBO) methods. When no base was present in the system, the reaction was happening according to an oxidative asynchronous concerted Proton Coupled Electron Transfer (cPCET) mechanism with an energy barrier of around 17kcal/mol. In the presence of lutidine however, the species formed upon oxidation no longer was a Cu^{II}-Cu^{III} species but a Cu^{II}-$O^{\bullet}$-Cu^{II} one. Because of this, the reaction was occurring following a HAtom Transfer (HAT) mechanism and the energy barrier had been decreased.

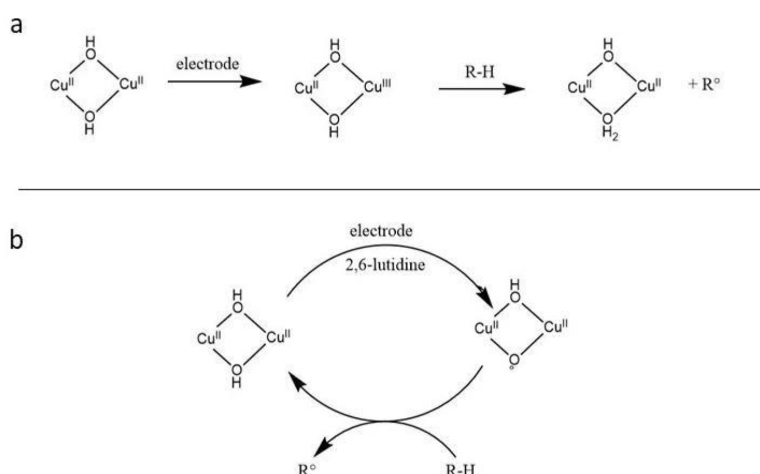

Figure 1. Reaction mechanism of the DPEN complex, a. in the absence of base, b. in the presence of base

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Using laccase/electrode interactions to understand electron transfer pathways

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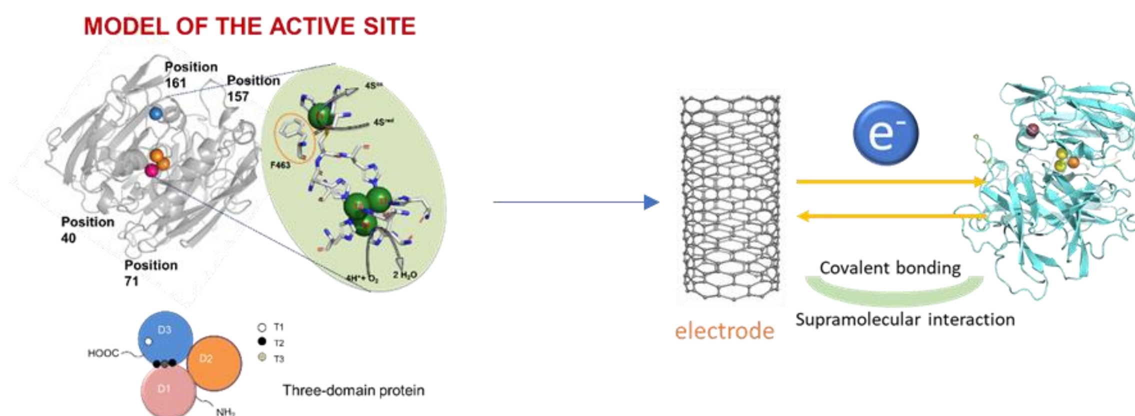
Laccases are particularly robust copper-containing oxidases with distinctive spectroscopic features. In aerobic conditions, they couple the mono-electronic oxidation of phenolic substrates (at a T1 surface located Cu center) to the 4e- reduction of dioxygen into water (at an embedded tri-nuclear Cu center). The search for efficient enzymatic fuel cells has facilitated the design of low potential/high current bioelectrodes for oxidizing or reducing substrates. Direct electron transfer (DET) between the electrode and the biocatalyst is influenced by protein orientation. Oriented immobilization of laccase onto an electroactive surface is a strategy to maximize the direct electron transfer between redox enzyme and electrodes. Our research group has previously shown synergistic combinations of laccase and multi-walled-carbon-nanotube (MWCNT) modified electrodes. We also reported an efficient electrocatalytic reduction of molecular oxygen by a rationally oriented fungal laccase covalently bound to MWCNTs as biocathode.



In our work, we use several laccase variants to further characterize the DET between enzyme active sites and the electrode materials. We aim to get more insights into the structure of hybrid materials characterization, into different electron pathways, and to better understand factors that influence the electrochemical behavior of the system.

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Mass transport limited adsorption experiments show that *E. coli* Hydrogenase-1 is much more active than solution assays suggest

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Hydrogenases are a group of metallo-enzymes that catalyze the reversible conversion of dihydrogen into protons and electrons ($H_2 \rightleftharpoons 2H^+ + 2e^-$). These proteins are widespread in nature, occurring in bacteria, archaea, and some eukarya with various mechanisms, activities (reversible/irreversible H_2 uptake or evolution) and efficiencies.[1] Nowadays, the worldwide interest in H_2 as a clean energy carrier and basic chemical agent drives researchers to study hydrogenases.
A measure of the enzyme efficiency is its absolute turnover frequency (k_{cat}, the rate at which it converts its substrate). However, its value is often challenging to determine for redox catalysts. Solution assays are largely employed to this aim, but the need to utilize redox mediators, often bias the k_{cat} estimation.[2] Protein Film Electrochemistry (PFE) can be used as an alternative to overcome the mediators' limits. The technique requires the immobilization of the redox enzyme on an electrode which acts as a substitute for its natural redox partner, in a configuration allowing direct electron transfer. In presence of the protein substrate, the catalytic reaction generates a current proportional both to k_{cat} and the amount of enzyme electrically connected to the electrode (G), providing a mean to k_{cat} estimation as long as G is known.[3] In practice, the electroactive coverage is often difficult to determine.
Hydrogenase-1 from *E. coli* (*Ec* Hyd-1) is a NiFe hydrogenase which catalyzes the irreversible oxidation of dihydrogen at a bimetallic NiFe active site.[4] We observed that it spontaneously adsorbs on freshly polished graphite electrodes, when they are rotating, even from sub-nanomolar solutions of enzyme. Following the process over time by cyclic voltammetry (PFE method in which the current is recorded as a function of time; see **Figure**), we could demonstrate that it is mass transport-limited. This property helped provide a finer estimation for the upper bound of G, thus a lower boundary for k_{cat}, which showed that *Ec* Hyd-1 is significantly more active than what solution assays suggest.[5,6]

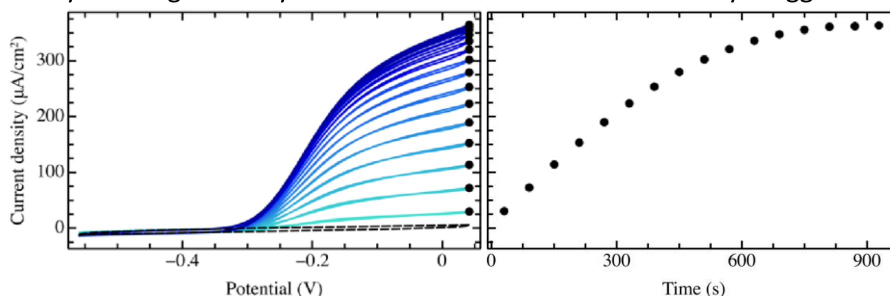

Figure. *Ec Hyd-1 adsorption experiment.* Left panel: successive voltammograms (normalized for the electrode surface; recorded from the bottom to the top as a function of time) of a rotating graphite electrode at 3000 rpm, immersed in a solution containing 0.7 nM *Ec* Hyd-1 and equilibrated with a flux of 1 bar H2. Right panel: plot of the maximum current density for each voltammogram as a function of time.

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Selective CO_2 Electroreduction to CO in Aqueous Medium by a Cobalt Quaterpyridine type Complex upon Immobilization on Carbon Nanotube

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Due to continuous use of nonrenewable fossil fuels, there is a remarkable increase of atmospheric carbon dioxide (CO_2) which is the main greenhouse gas. Use of alternative energy could solve the environmental issues as well as increasing energy demand to some extent.[1] In this regard, CO_2 could be converted into value added C1 or C2 feedstock and would be helpful to mitigate the atmospheric CO_2. CO_2 electroreduction by metal–polypyridine complexes with first row transition metals are showing great attention.[2] Co bipyridine and terpyridine based catalysts towards electrochemical CO_2 reduction are suffering from low CO production Faradaic yields, low selectivity producing H_2 as a by-product. Whereas, Co complexes based on quaterpyridine or its derivatives have been hardly investigated for the study of electrochemical CO_2 reduction.[3,4] Furthermore, important limitation of homogeneous catalysts in terms of low solubility and catalytic activity in either organic or aqueous media may be overcome by using heterogeneous system such as by immobilizing the molecular catalyst onto a solid support material.

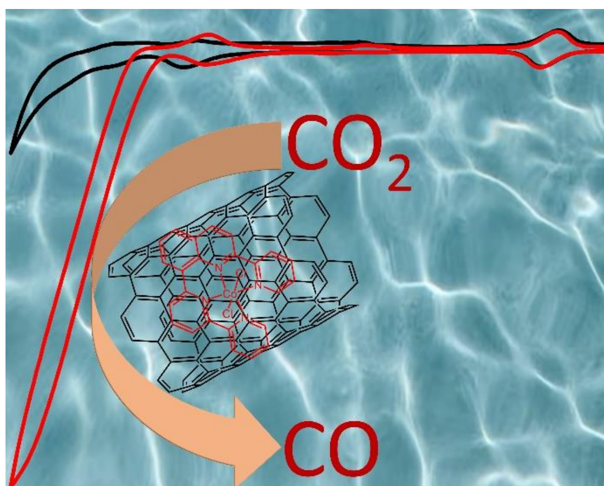

Figure 1. CO_2 electroreduction by immobilised $Co(bpq)Cl_2$ catalyst in aqueous media.

Herein, we have studied electrochemical CO_2 reduction under homogeneous as well as heterogenous condition (**Figure 1**) by $[Co(bpq)]^{2+}$ complex[5] where, bpq= 8-([2,2'-bipyridin]-6-yl)-2-(pyridin-2-yl)quinoline, that have quaterpyridine backbone but greater conjugation and might be able to stabilize the low-valent cobalt center during the reduction process. Under homogeneous condition (organic solvent such as DMF or aqueous medium), the $Co(bpq)Cl_2$ catalyst shows lack of stability and undergoes electrodeposition on the electrode surface during the controlled potential electrolysis (CPE) process. Further, the unmodified $Co(bpq)Cl_2$ complex is directly immobilized onto CNT via adsorption. This immobilized catalyst has been used for the study for electroreduction in aqueous media. It has been observed that catalytic activity can be improved for the catalyst on moving to heterogeneous CO_2 reduction process from the homogeneous one. The catalyst shows high selectivity towards the CO production during heterogeneous CO_2 reduction process in aqueous media.

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Cyclophane formation and β-hydroxylation catalysed by fused radical SAM and HExxH domain proteins with a unique structural fold

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Cyclic peptides are important drug molecules, including antibiotics and tumor suppressors, that are used clinically. One of the challenges in synthetic chemistry is to expand the available macrocyclization methods, either enzymatic or chemical, to generate new and diverse cyclic peptide scaffolds that will enable the modular synthesis of peptide libraries for drug discovery platforms. We identified a group of post-translational modifying bacterial enzymes that establish single and multiple cyclophane macrocycles and β-hydroxylations over diverse short peptide sequences.[1] The cross-links consist of C-C bonds, occurring on three-residue motifs that include tryptophan, phenylalanine, tyrosine, or histidine residues to form indole-, phenyl- or imidazole-bridged cyclophanes. The restricted rotation of the aromatic ring and the induced planar chirality in the asymmetric indole bridge confer higher stability and restricted conformations to the modified peptides. Cross-links and β-hydroxylations are catalyzed in separated domains, while the first are installed by a radical SAM enzyme domain, the later are catalyzed by a new family of non-heme iron oxoglutarate-dependent oxygenase domain characterized by two conserved motifs: a HExxH motif previously described in Zn-dependent metalloproteases and a unique PWRxxxRP motif involved in oxoglutarate binding. Crystallographic structures of the oxygenase domain revealed a novel fold and unveiled how it has been adapted to coordinate an iron ion, to bind an oxoglutarate molecule and cyclized peptidic substrates to carry out oxoglutarate-dependent β-hydroxylations over specific sites of the macrocycle.

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Poster communications

Synthesis of pyclen-based zinc-responsive MRI contrast agents

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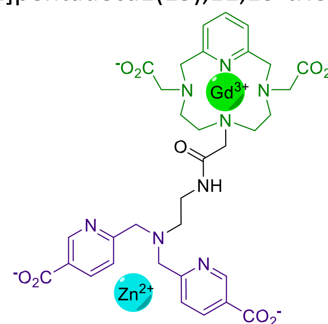
Magnetic resonance imaging (MRI) is a non-ionising, non-invasive diagnostic technique that provides highresolution visualisation of soft tissue and vascular structures. More than 70 million examinations are performed each year worldwide. Today, nearly 50% of clinical MRI procedures use paramagnetic contrast agents to enhance signal resolution and sensitivity. Gd^{3+} based contrast agents are particularly well-adapted for this purpose. Moreover, they can be rendered responsive to a biomarker, such pH, enzymatic activity, the presence of cations… Indeed, changes in their efficacy (relaxivity) are often based on changes in the hydration number and/or rotational dynamics of the contrast agents, which are the easiest parameters to be tailored by the chemist.[1]

Zinc, the second most abundant metal ion in humans, plays a major role in the functioning of the body. However, its quantitative distribution and exact role are not well understood. Its concentration is highly regulated and any disturbance of its homeostasis is implicated in various pathologies such as cancers (prostate, pancreas and breast), neurodegenerative diseases, or diabetes.[2] Therefore, monitoring Zn^{2+} in vivo by non-invasive technique such as MRI is important in biomedical research to understand its biological role, and to provide earlier diagnosis for specific pathologies.[3]

Gd-based Zn-responsive contrast agents are typically composed of three parts: (1) the Gd^{3+} complex, the MRI active part; (2) the Zn^{2+}-bonding unit, and (3) a linker between the two. In the team, we have previously developed a first generation of Zn-responsive systems based on a pyridinic unit for Gd^{3+} complexation.[4] We have further modified the Zn-binding unit to allow for Zn quantification and the systems respond to Zn^{2+} in the presence of Human Serum Albumin (HSA).[5,6] We now aim at further modifying the Gd^{3+}-complex using pyclen (3,6,9,15tetraazabicyclo[9.3.1]pentadeca1(15),11,13-triene) derivatives. Pyclen is a macrocyclic unit suitable for Gd^{3+} complexation. The resulting complexes display high thermodynamic stability and kinetic inertness together with fast complexation properties. We will describe here the synthesis of a family of ligands based on pyclen (green) to which a DPA (2,2'-Dipicolylamine) derivative (purple), the zinc binding part, has been appended via a linker (black) (Figure).



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Structural insights into a NifEN-independent nitrogenase

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NifDK is the nitrogenase catalytic component, where nitrogen gas (N_2) is reductively cleaved into ammonia (NH_3). This crucial process is known as Biological Nitrogen Fixation and is performed only by diazotrophic prokaryotes. NifDK is organized as a $\alpha_2\beta_2$ heterotetramer that contains a permanent [Fe_8S_7] cluster termed P-cluster and located between each NifD and NifK subunits, and one of the most complex sulfoferric cofactors found in nature known as FeMo-co or [Fe_8S_9CMo-*R*- homocitrate] at the active-site and located within each NifD subunit. To date, all well characterized nitrogenase FeMo-co biosynthesis process occurs in an external scaffold protein known as NifEN. However, recently we have discovered the first nitrogenase in nature demonstrated to act both as scaffold for the assembly of its own metallic cofactor and as nitrogenase *per se,* establishing a new paradigm in metalloprotein biosynthesis.

In this project, we aim at studying the tridimensional structure of a NifEN-independent nitrogenase using cryo-electron microscopy technique (cryo-EM) in order to understand what makes it unique as a scaffold and as a nitrogenase.

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Electrocatalytic CO_2-to-CO conversion by carbon-nanotube-supported O_2-sensitive carbon monoxide dehydrogenases operating in Deep Eutectic solvents

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A major challenge for our society is to tackle global warming by minimizing the concentration of greenhouse gases in the atmosphere. While the first solution seems to be to reduce their emission, a complementary solution is to recycle CO_2 (the most abundant greenhouse gas) by converting it into building blocks such as CO.

A promising approach is to take advantage of highly efficient and specific natural catalysts such as CO Dehydrogenase (CODH)[1] enzymes, able to reduce CO_2 to CO by lowering the activation energy of the reaction. They rely on active sites comprised of abundant metals, iron and nickel, buried inside a protein scaffold. This structure is responsible for their high reversible catalytic activity with minimal overpotential. However, CODH is highly-oxygen sensitive and must be handled under strictly anaerobic conditions.

Key to achieving efficient bio-electrocatalysis is the design of tailored electrodes with a minimized enzyme electrode distance in order to avoid electron transfer rate limitations and to design innovative and sustainable enzymatic devices. For this purpose, carbon nanotube (CNT)-based electrodes have proven ideally suited. Once modified with adapted anchoring groups,[2] they can accommodate a high enzyme loading on their surface and provide high film conductivity.

This study shows a new method for enhancing the catalytic activity under aerobic conditions through the development of CODH-modified electrodes in particular organic media. Deep Eutectic Solvents (DESs),[3] a subclass of ionic liquids with low toxicity and low manufacturing cost, have diffusion and oxygen solubility limiting properties.[4,5] We have studied the electrocatalytic CO_2 reduction at CNT-supported CODH in DES with the aim of enabling an efficient CO_2-to-CO conversion in a non-natural environment as a means of increasing their oxygen tolerance.

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Does simplifying the design of macrocyclic lanthanide chelates lead to convincing results in luminescence?

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Polyazacycloalkanes have shown their interest as macrocyclic platforms for the complexation of cations with fine thermodynamic and kinetic properties, with and/or without *N*-functionalization.[1] Pyclen derivatives (pyclen = 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene) are specific azacycloalkanes with an aromatic pyridine unit included in the macrocyclic ring. This provides an additional rigidity to the structure that can impose coordination environments leading to an improvement of the chelating properties. Functionalized pyclen ligands with three pendant acetate arms, such as **PCTA** (***Figure 1***) form rather stable complexes with metal ions such as trivalent lanthanides (Ln^{3+}), both in terms of thermodynamic stability and dissociation kinetics.[2,3]
Ln^{3+} complexes are of particular interest due to the numerous applications related to their optical properties when containing π-conjugated coordinating pendants. Thanks to the modularity of the platform, pyclen derivatives *N*-functionalized by a combination of picolinate and acetate pendants, have been largely studied in our group, in particular for Ln^{3+} complexation for their photophysical properties.[4,5] It has been possible to obtain various regioisomers distinguished with properties close to or even better than the famous **DOTA** in terms of stability and inertness. Two of them (**PC1A2PA-disym**, **PC1A2PA-sym**, Figure 1), presented the adequate number of coordinating atoms (CN=9 and no water molecule to quench the photo-emission) to form luminescent Ln-complexes and were then studied for their photophysical properties with Tb^{3+} and Eu^{3+}.

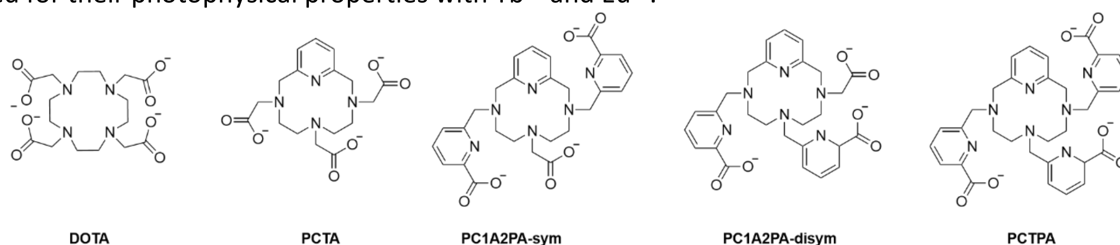

Figure 1: Pyclen-based ligands for Ln(III)-complexation.

However, the synthesis of the ligands (**PC1A2PA-disym** and**PC1A2PA-sym**) involves tricky methods using different successive protection and deprotection steps.[3,4] An alternative ligand was considered and relies on the “simple and easier” introduction of three coordinating picolinate arms. The so obtained **PCTPA** would possess 10 coordinating atoms that can potentially participate in the encapsulation of trivalent lanthanides cations while preserving similar properties that its **PC1A2PA**derivatives. The question of the coordination scheme is however important and has attracted our attention. The efficient and low-step synthesis of **PCTPA** will be presented as well as its Eu^{3+}, Tb^{3+}, Lu^{3+} complexes prior to their structural and photophysical properties, to finally answer the question: does simplifying the design of our macrocyclic lanthanide chelates lead to convincing results in luminescence?

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Development of theranostic tools: biomimetic metal complexes of β-lactamases

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The development of novel theranostic tools, for example for Parkinson's disease (PD), would be possible using artificial metalloenzymes with β-lactamase activity.

Firstly, biomimetic complexes of zinc containing dinuclear β-lactamases will be synthesized using ligands that closely mimic those of the active site in natural β-lactamases. Secondly, A_{2A} adenosine receptor antagonists that are drugs commonly used to treat PD, will be covalently attached to the biomimetic metal complexes of β-lactamases to from conjugates (Figure 1A).[1,2] A_{2A} is membrane receptor naturally expressed in the brain. Thus, the conjugates are vectored into the brain wherein they can act as a first drug that bonds A_{2A}. The conjugate bound into the membrane receptor also forms an artificial metallo-β-lactamase at the surface of the cell.[3] The hydrolytic activity of this artificial enzyme will then be used to selectively release a MonoAmine Oxydase (MAO) inhibitor masked into a pro-drug β-lactam. It is noteworthy that such inhibitors are also known drugs for PD. In a similar manner, a β-lactam pro-imaging prob could be selectively unmasked by the artificial enzyme thus providing on the disease progression (Figure 1B).

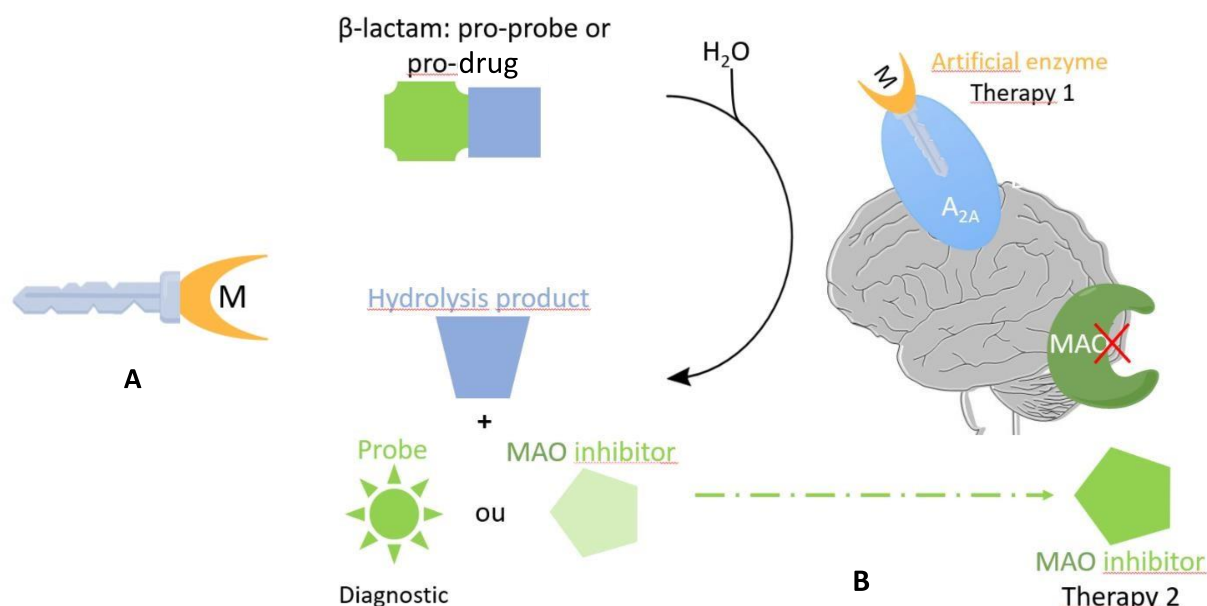

Figure 1: **A**) Conjugate of an A_{2A} antagonist and biomimetic metal complexes of β-lactamases. **B**) Strategy for the development of a theranostic tool for PD.

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Influence of Molecular Crowding on Enzymatic Activity and Self-Assembly Processes

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Mimicking cellular or extracellular environment with a simplified and well-defined set of macromolecules may help in understanding real biological phenomena. This approach was initially applied to study enzymatic activity and interactions between biomolecules in concentrated solutions of polymers. Recently, the progress in this field is also oriented toward controlled self-assembly of bio-related materials.

In this contribution, we present the effect of crowding on the activity of selected enzymes. In such a complex system, we measure reaction kinetics and evaluate intermolecular interactions. On the other hand, we investigate the influence of crowding on the formation of supramolecular metal-containing complexes. We evaluate related kinetic and thermodynamic parameters to rationalize self-assembly process in such conditions.

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Revealing the Mechanism of the Metalloenzyme IspH to Develop Novel Antibacterial Agents

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The methylerythritol phosphate (MEP) pathway is of great interest for the identification of novel antimicrobial agents.[1] It is found in chloroplasts and many pathogenic bacteria such as *M. tuberculosis*, which is responsible for tuberculosis. Also parasites such as *P. falciparum*, responsible for malaria, employ the MEP pathway. The last enzyme of this pathway, which is called IspH or LytB, catalyzes the reductive dehydroxylation of HMBPP into the two products IPP and DMAPP (Figure 1a).[2] These products are precursors for terpenoids, essential molecules for the survival of all living organisms. In humans, IPP and DMAPP are produced by the so-called mevalonate pathway which in turn is independent of IspH. In the context of rising antimicrobial resistance of many pathogens, IspH is thus a suitable target for the development of novel antimicrobial agents.

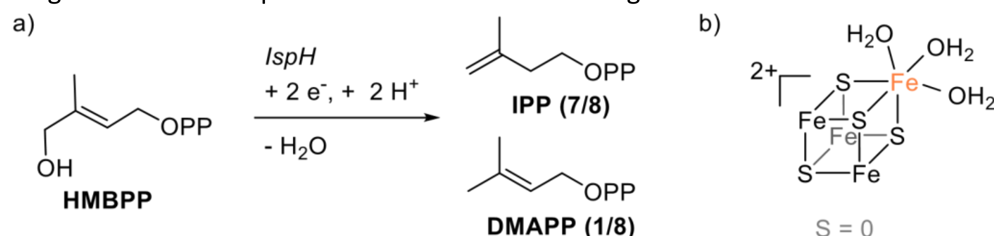

Figure 1. a) The enzymatic reaction catalyzed by IspH. b) Structure of the $[4Fe4S]^{2+}$ cluster of IspH.

IspH is an oxygen sensitive enzyme harboring a $[4Fe\text{-}4S]^{2+}$ cluster with a unique fourth Fe atom coordinated to three water molecules (Figure 1b). The $[4Fe\text{-}4S]^{2+}$ center plays an important role in the conversion of the substrate and several of the mechanistic steps have already been elucidated by the application of spectroscopic techniques such as Mössbauer or EPR spectroscopy.[3,4] However, the final step is not yet well understood as the thermodynamically favored product DMAPP is produced in lower quantities than its configurational isomer IPP. In the case of *E. coli* IspH, IPP and DMAPP are formed in a ratio of 7:1 (Figure 1a).

In order to understand this step, several *E. coli* IspH mutants have been purified under anaerobic conditions. It has been shown that they are involved in the product formation as they change the IPP : DMAPP ratio. They are currently being analyzed by spectroscopic means as well as X-ray crystallography in order to understand their role in the catalysis. Revealing the complete mechanism of IspH will help to develop novel antimicrobials that are urgently needed in the fight against bacterial and parasitic infections.

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In vitro chemical Fe-S reconstitution experiments in glycine/cysteine-rich proteins from *Megavirinae* giant viruses

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In 2003, mimivirus, initially mistaken for an intracellular parasitic bacterium, was identified as the first giant virus with a large particle size of around 700 nm-diameter and a complex DNA-genome (1.2Mb) of about 1000 proteins.[1] Mimivirus is the first representative and prototype member of the *Mimiviridae* family. In *Megavirinae*, a sub-family of *Mimiviridae*,[2] we recently identified a new family of small proteins with a high glycine, cysteine, and aromatic residues content, and endowed with very unusual Fe-S cluster binding properties.[3] This family was coined **GciS** for **G**lycine/**C**ysteine-rich **I**ron-**S**ulfur proteins[3]. This protein is conserved in all *Megavirinae* members. The GciS protein, of unknown function, is very abundant in the viral particles and likely essential for the virus/host interaction.

Our studies revealed that the air-purified recombinant protein overproduced *in E. coli* incorporated sub-stoichiometric amounts of (S=0) $[2Fe\text{-}2S]^{2+}$ and linear (S=5/2) $[3Fe\text{-}4S]^{1+}$ clusters, the latter geometry being scarce in proteins.[3-4]

Here, we present the *in vitro* chemical Fe-S reconstitutions performed on both recombinant (partly metalated) and demetalated GciS proteins from all clades of *Megavirinae.* The anaerobically reconstituted GciS protein displayed UV/vis and EPR spectra consistent with the presence of above 0.7 – 0.9 $[4Fe\text{-}4S]^{2+}$ cluster per protein. Upon exposure to air, the $[4Fe\text{-}4S]^{2+}$ cluster was converted into a mixture of linear $[3Fe\text{-}4S]^{1+}$ and $[2Fe\text{-}2S]^{2+}$ clusters. This unusual Fe-S binding behavior was shared between all clades, suggesting that it is an intrinsic property of the family and a valuable clue to identify the function of these Fe-S viral proteins.

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A new family of viral glycine/cysteine-rich proteins with remarkable Fe-S cluster binding properties

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In 2003, the discovery of giant viruses has been highly unexpected and has changed the perception of *viral size and complexity*.[1-3] These giant viruses are characterized by large particle size (reaching up to 2.5 µm) and a complex genome (up to 1.5 Mb), encoding thousands of proteins.[4] A small protein of 6kDa featuring an amino acid composition peculiarly rich in cysteine and glycine residues was identified in the *Mimiviridae* family. This protein is very abundant in the viral particles and it has no predicted function but it is probably essential for the structure of the virion or for infection. This protein is conserved in all members of the *Mimiviridae* family. Recently we reported the ability of the recombinant purified protein to accommodate several types of Fe-S clusters including a diamagnetic (S=0) $[2Fe\text{-}2S]^{2+}$ and the unusual (S=5/2) linear $[3Fe\text{-}4S]^{1+}$ cluster.[5] This protein was named GciS for Glycine/Cysteine-rich Iron-Sulfur protein.

In the present study, we highlight an unusual feature of GciS protein to perform Fe-S cluster conversions from the linear [3Fe-4S] to the [2Fe-2S] and [4Fe-4S] clusters under reductive conditions. The kinetics of this cluster conversion was monitored in the timescale between minutes and hours by combining in-depth EPR, Mossbauer and UV-visible experiments. We hypothesize that the Fe-S cluster conversion property is related to the high conformational flexibility and dynamic changes of the protein thus providing a unique capacity to interconvert between the Fe-S clusters using exchangeable cysteine ligands.

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Mechanistic insights into thiols oxidation and ROS production by catalytic Cu(II) complexes with anticancer thiosemicarbazones

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Cancer cells show increased Cu levels, which are required to sustain cell proliferation, angiogenesis, and metastasis.[1] Therefore, α-Pyridyl thiosemicarbazones (TSCs) able to coordinate copper are gaining increasing interest because they have the potential to enhance the selectivity towards cancer vs healthy cells. Copper can catalyze the oxidation of thiols to disulfides in aerobic conditions with the production of oxygen reactive species (ROS).[2] When TSC ligands bind Cu, they can reduce the ratio between reduced and oxidized glutathione (GSH/GSSG) in the cell and lead to cellular apoptosis or necrosis.[2] Between Cu chelators with anticancer activity, Triapine (3-aminopyridine-2,2-carboxaldehyde thiosemicarbazone, 3AP) and Dp44mT (di2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone) have entered clinical trials and they belong to two different cytotoxicity classes. Dp44mT is active in nM range, whereas 3AP works in µM range which was linked to a different ROS production upon thiols oxidation by the corresponding Cu^{II}-TCS complexes.[3]

In this regard, we wanted to understand the differences between Cu^{II}-Dp44mT and Cu^{II}-3AP in the redox mechanism that involves GSH oxidation. It is shown that Cu^{II}-Dp44mT is a better catalyst than Cu^{II}-3AP.[4] GSH reduces Cu^{II}-3AP faster than Cu^{II}-Dp44mT, but the latter produces ROS faster because, unlike 3AP, it withstands the reductive dissociation by GSH (Figure 1)[4]. The difference in the reactivity with GSH could be due to a different hard/soft character of the complexes, as suggested by the performed density functional theory (DFT) calculations.[4] Following, we tried to understand the reactivity and selectivity of Cu^{II}-Dp44mT towards different cellular thiol-containing molecules such as cysteine. For instance, it could be shown that cysteine oxidation is kinetically faster, but upon its addition to GSH in physiological concentrations cysteine does not affect the rate of GSH oxidation. This indicates that Cu^{II}-Dp44mT has a selectivity for GSH over cysteine.

Figure 1. Different fate of CuII-TSCs in the presence of GSH and impact on the generation of ROS.

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Assembly of the [FeFe]-hydrogenase active site

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[FeFe]-hydrogenases use a unique organometallic complex, termed the H-cluster, to reversibly convert H_2 into protons and low potential electrons. It can be best described as a [Fe_4S_4] cluster coupled to a unique 2Fe center ([$2Fe]_H$), where the reaction actually takes place. The latter corresponds to two iron atoms, each bound to one CN− and one CO ligands. A unique azadithiolate and an additional bridging CO connect the two iron atoms.

The [$2Fe]_H$ center is built stepwise thanks to the well-orchestrated action of dedicated enzymes that belong to the Hyd machinery. HydE, HydF, and HydG – termed the Hyd maturases – are the three [FeS] cluster-containing accessory proteins that directly participate in this assembly. HydE and HydG both belong to the radical SAM (*S*-adenosyl-L-methionine) enzyme superfamily. They use the one-electron reduction of a [Fe_4S_4] cluster to cleave SAM into methionine and a highly reactive 5´-deoxyadenosyl radical species (5´-dA•), that in turn will trigger radical-based reaction to the substrate. Over the last decade, it has been shown that HydG uses L-tyrosine as a substrate to produce the CN and CO ligands of the [$2Fe]_H$ subcluster. In addition to the radical SAM cluster, HydG harbors an auxiliary [Fe_4S_4] cluster that actually corresponds to a regular [Fe_4S_4] cluster connected to a dangling iron, bound to a L-cysteine. Upon reaction, this species is stepwise converted into a new one termed complex-B that corresponds to a (κ^3-L-cysteinate)$Fe^{II}(CN)(CO)_2$.

More recently, it has been shown that HydE uses this complex-B as substrate. Using syn-B, a synthetic mimic of complex-B, two intermediates were identified and characterized by resonance paramagnetic resonance spectroscopy, demonstrating that HydE catalyzes the radical addition of the 5´-dA• onto the sulfur atom of complex-B. Yet, the exact product of HydE is unknown, After HydG and HydE, HydF is the third maturase enzyme. It is a GTPase with a FeS cluster-binding domain, a dimerization domain, and a [Fe_4S_4] cluster. HydF is responsible for hosting and delivering a [$2Fe]_H$ precursor to the apo-hydrogenase, hence leading to an active enzyme.

Here we will present our recent data on HydE to decipher its molecular mechanism.

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Syntheses, structures and CO release properties of neutral Mn(I) tricarbonyl complexes with 8-hydroxyquinoline and imidazole ligands

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Carbon monoxide (CO) is known in the popular culture as an odourless lethal gas, earning his name of "silent killer". Since the past decade, CO has emerged as a promising therapeutic agent for anticancer treatment when administrated in optimal concentrations.[1] However, due to its gaseous form, CO remains difficult to handle. To overcome this issue, Carbon Monoxide Releasing Molecules (CORMs) have emerged as a suitable mode of administration of CO.[2] These molecules are mostly organometallic complexes, enabling the releasing of CO upon a given external stimulus, such as visible light These complexes, named photoCORMs, remain the most widely used so far.[3] Despite recent advances, efforts are still ongoing to synthesize new carbonyl complexes absorbing light in the visible region with high potency of vectorization for the controlled delivery of CO.[4.] In this context, we present a new series of photo-activable [Mn(8-hydroxyquinoline)$(CO)_3$X] with X = imidazole derivatives. We developed a short steps synthesis from a dimeric structure $Mn_2(8\text{-HQ})_2(CO)_6$ and applied a series of imidazole ligands (Figure 1). The use of various imidazole-based ancillary ligands allows the synthesis of a wide range of complexes with a high degree of modularity due to the presence of allyl, hydroxyl, or amine groups on the imidazole ring that could be used as anchoring points for the introduction of specific vectors.

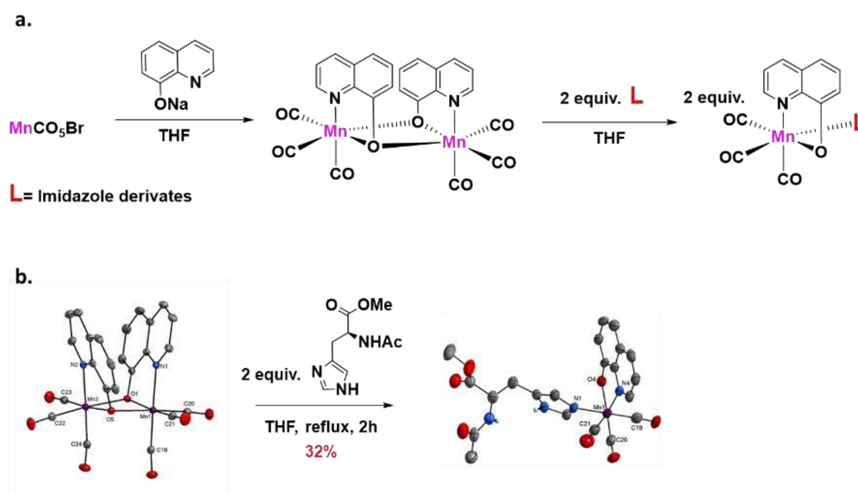

Fig.1 *Synthesis pathway of these new photoCORMs. a) General synthesis pathway b) Synthesis of [Mn(8HQ)(CO)₃NAc-His-OMe] with corresponding X-ray structures.*

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Evaluation of Pt(IV) conjugates of oxaliplatin and SODmimics to reduce oxaliplatin induced peripheral neuropathy

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The anti-cancer agent oxaliplatin (Ox)[1] is an efficient chemotherapeutic agent widely used in clinics with a main indication in metastatic colorectal cancer. However, a major limiting factor in its administration is its toxicity and in particular the peripheral neuropathy it induces,[2] which pathophysiology has been linked to the generation of oxidative burst. The development of drugs with better therapeutic indices is therefore of critical importance. Several modulators of the redox balance have demonstrated interesting results in counteracting Ox-induced neurotoxicity. Manganese Superoxyde Dismutase (SOD)-mimics are low molecular weight Mn complexes able to reproduce *in vivo* the activity of the MnSOD, a metalloenzyme involved in the cellular protection against oxidative stress, in catalyzing the dismutation of the anion superoxide into H_2O_2 and O_2. In our group, we develop MnSOD mimics bioinspired from the active site of MnSOD, specific of superoxide,[3] and that were shown to exert an anti-inflammatory activity in epithelial cells (HT29-MDM2) linked to an antioxidant effect.[4] We have recently described that the combined treatment of oxaliplatin with MnSOD mimics prevented the appearance of sensitive axonal neuropathy and neuromuscular disorders induced by Ox, in mice.[5]

From these results, the advantages of a molecule bearing oxaliplatin and an antioxidant are clear: it would facilitate the administration, as well as, potentially, reduce the low selectivity problems of Pt(II) drugs. This option can be envisioned by employing Pt(IV) complexes, intrinsically more inert than their Pt(II) analogs and that would just be reduced at the tumor microenvironment. We present here the development of stable Pt(IV) conjugates and their in vitro and in vivo evaluation.

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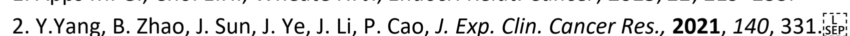
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Diphosphine-containing organogold(III) complexes: a new class of structural anticancer drugs

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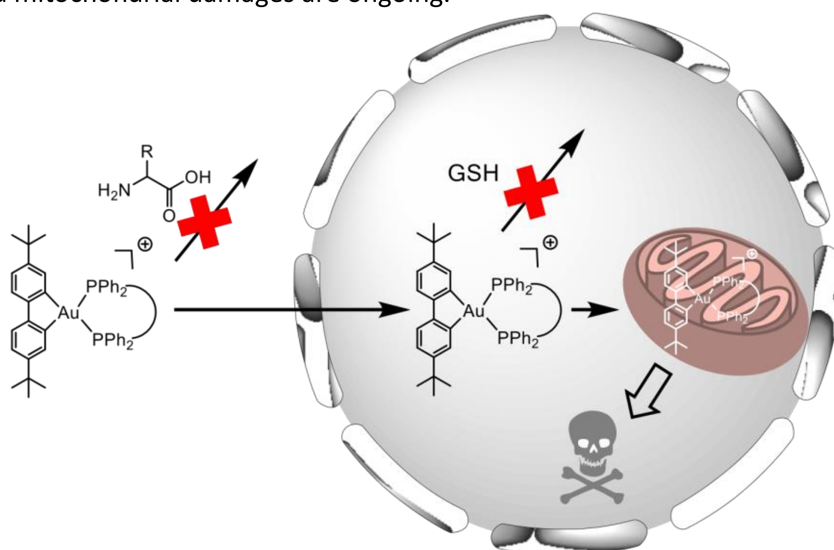
Metal-based complexes have been reported to trigger anticancer effect *via* either the direct coordination of the metal center to biomolecules like cisplatin derivatives on DNA[1] or through molecular recognition like ruthenium-based kinases inhibitors developed by Meggers.[2] Thus the knowledge of the reactivity toward biomolecules is of major relevance when developing new metal-based anticancer drug candidates. Moreover, in the case of gold-based complexes, the question of the redox stability has also to be considered. We previously reported the anticancer activity of a family of organogold(III) complexes with a (C^C) biphenyl ligand and a (N^N) ligand. We demonstrated the redox stability of the $[(C^\wedge C)Au]^+$ moiety in the presence of glutathione and the high lability of the (N^N) ligand in the presence of cysteine and histidine residues.[3] Based on these results, we replaced the (N^N) chelate by a diphosphine ligand (P^P). These $[(C^\wedge C)Au(P^\wedge P)]^+$ complexes appeared particularly active on lung cancer cells. Using mass spectrometry techniques, we demonstrated the higher stability of this new family of complexes with respect to the (N^N) analogs in the presences of various biomolecules including GSH. The maintainance of the structure could be demonstrated by cryo-X-Ray Fluorescence cell mapping. Upon correlation with optical fluorescence organelle labelling, we could establish the intracellular accumulation of the complex inside mitochondria. Biochemical demonstration of complex-induced mitochondrial damages are ongoing.



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Bacterial copper resistance: Cu sites and role of the periplasmic protein CopI

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CopI is a periplasmic protein of 15kDa which is induced by high copper concentration and is directly involved in the copper resistance of the purple bacterium *Rubrivivax gelatinosus*.[1,2] Homolog exists in other environmental bacteria but also pathogens such as *Vibrio cholerae* or *Pseudomonas aeruginosa*. CopI is highly induced by Cu and possess a scarce green type cupredoxin site. It is the first single domain cupredoxin with at least a second Cu site. The cupredoxin center and a highly conserved His/Met-rich region are required for Cu resistance while the His-rich N-terminal region is not required.

In order to get insights into the different Cu binding sites and possible electron transfer routes between them, we have performed biophysical studies on the wild-type protein as well as specific mutants targeting the Cu binding modules from the aminoacid sequence.

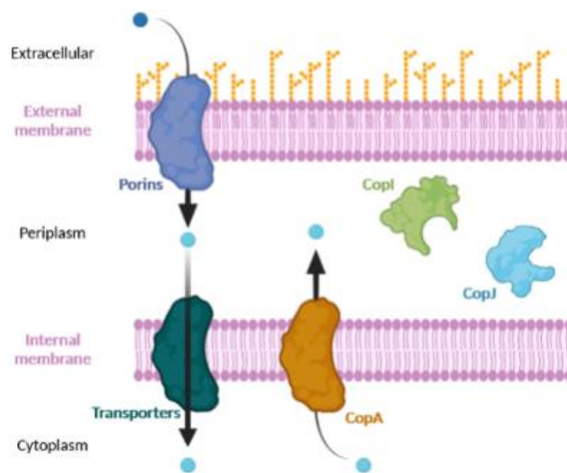

Figure 1. Schematic representation of the proteins involved in Cu homeostasis in *Rubrivivax gelatinosus*

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IspH as an interesting target for the discovery of novel anti-infectives

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One of the most concerning global health threats is the increasing incidence of anti-infective resistance to pathogens which will be the major cause of death in the next 20 years, according to the WHO. One way to tackle the emerging resistance against existing antibiotics is to focus on an innovative and underexploited series of target enzymes. Isoprenoids are vitally important in all organisms and their precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), are produced via two different pathways. In mammalians, fungi, archaea, and some bacteria, the isoprenoid precursors are synthesized via the mevalonate pathway,[1] in contrast with most bacteria and some parasitic protozoa of the Apicomplexa phylum that use the 2*C*-methyl-D-erythritol 4-phosphate (MEP) pathway.[2] This makes the MEP pathway a very interesting drug target against relevant pathogens such as *Pseudomonas aeruginosa* (responsible for nosocomial infections), *Mycobacterium tuberculosis* (causative agent of tuberculosis), and *Plasmodium falciparum* (causative agent of malaria).

IspH is an oxidoreductase enzyme containing an oxygen-sensitive [4Fe-4S] cluster that catalyses the last step of the MEP pathway converting HMBPP into IPP and DMAPP (Fig. 1). Several studies in the last decades contributed to better understand the mechanism of this enzyme, opening an interesting scenario for the discovery of new antibiotics. All the anti-infectives that have been investigated so far against IspH can be divided into four categories: substrate analogues,[3] alkyne and pyridine diphosphate,[4] and nondiphosphate compounds.[5] However, none of these inhibitors were reported to be active against the parasites responsible for malaria and the bacterium causing tuberculosis. From here, a multidisciplinary approach has been applied for the discovery of new classes of inhibitors against IspH from *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis,* and *Plasmodium falciparum.*

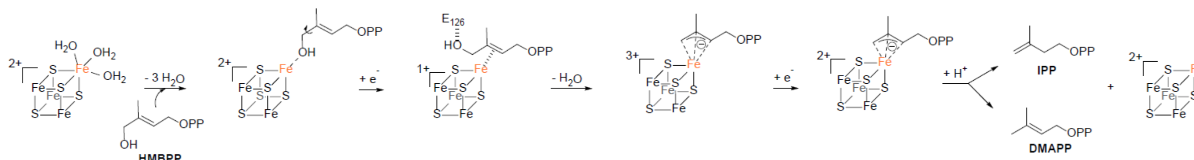

Figure 1 : Proposed mechanisms of IspH

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EPR and Mössbauer spectroscopic characterizations of OrpR in whole cells

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In bacteria, Enhancer Binding Proteins (EBPs) are key players of σ^{54}-regulation that control transcription of genes expression in response to environmental signals. OrpR is one key EBP that has been recently identified in the sulfato-reducing bacterium *Desulfovibrio vulgaris* Hildenborough (*Dv*H).[1] It presents the three typical domains found in bEBPs, namely a N-terminal sensory domain, a central AAA^+ domain, and a C-terminal DNA binding domain. One peculiarity of OrpR is the presence of a FeS cluster in the N-terminal sensory domain.[2]

Two different forms of OrpR have been identified. The active form is observed in reducing medium, for instance in physiological conditions when *Dv*H is producing H_2S. On the opposite, the inactive form is observed when the redox potential of the medium increases, due to an oxidative stress for example.

In previous communications, we have demonstrated that purified OrpR[2] and OrpR overproduced in *E. coli* accommodate a $[4Fe\text{-}4S]^{2+}$ cluster. We will present here recent EPR and Mössbauer studies performed on whole *Dv*H cells that aimed to identify and discriminate the active and inactive forms of OrpR.

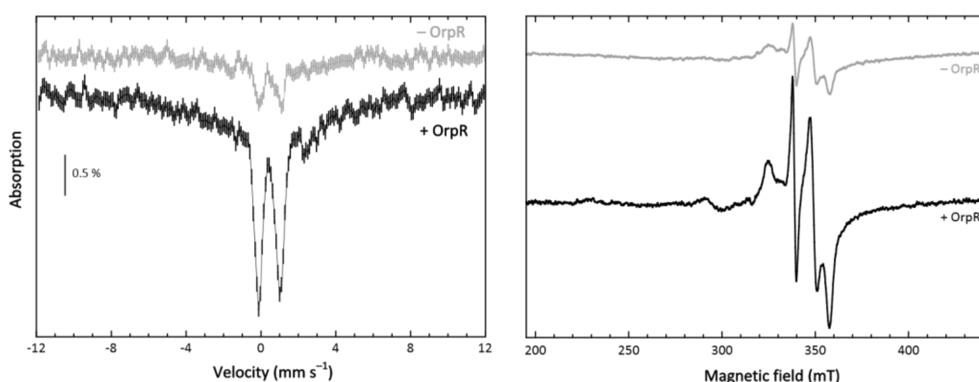

Figure 1. Left: 6 K Mössbauer spectra recorded on *Desulfovibrio vulgaris* Hildenborough cells with a 0.06 T external magnetic field applied parallel to the γ-beam. Right: EPR spectra recorded at X-band (9.48 GHz) and 15 K with a 10 mW microwave power and a 10 G modulation amplitude.
Cells were grown in anaerobia on minimal media containing a source of ^{57}Fe. Top and grey traces: Control cells with inactivated *orpR* gene. Bottom and black traces: Cells overproducing the active form of OrpR.

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Study of the supramolecular interaction between the anion $[SiW_{11}O_{39}]^{8-}$ and the Amyloid-β1-16 peptide by NMR and ITC

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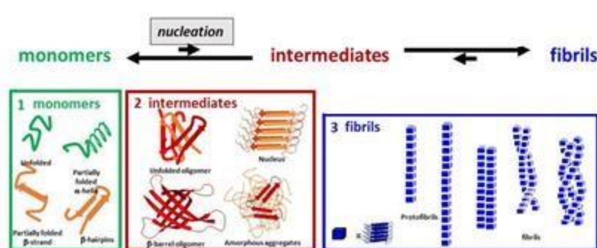
The formation of Amyloid β peptide aggregates has clearly been identified as a key feature of Alzheimer's disease, although its exact role in the development of this pathology has not been clearly deciphered so far. This aggregation results from a very complex interaction process involving monomers, soluble oligomers and solid fibrils. The intermediates species (soluble oligomers) are proposed to be the most toxic forms for the brains.[1]

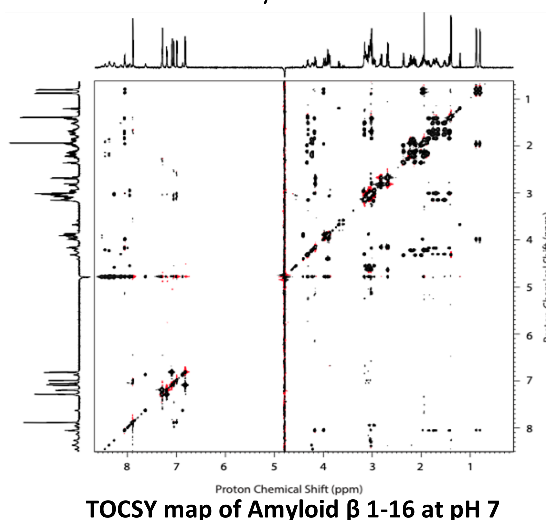


Aggregation mechanism of Amyloid β peptide in the context of Alzheimer disease

To better understand this mechanism, we are studying Polyoxometalates (POMs) as aggregation modulator agents. POMs or molecular oxides are stable inorganic, highly soluble and tuneables nanomolecules. They can have different geometries and sizes ranging from tenths of nanometers to a few nanometers for nano wheels.[2] POMs have recently started to be studied for their influence on peptide aggregation.[3]

In this presentation, I will describe our recent results concerning the interaction of the $[SiW_{11}O_{39}]^{8-}$ anion with the $A\beta_{1-16}$ fragment of the amyloid-β peptide.[4] This interaction has been studied by 1D and 2D NMR, which allowed us to determine an affinity constant and identify the interaction sites as well as the impact of the interaction process on the conformational space probed by this intrinsically disordered peptide. The thermodynamic data were further confirmed by ITC measurements.

monolacunary Keggin POM $[SiW_{11}O_{39}]^{8-}$



TOCSY map of Amyloid β 1-16 at pH 7

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Structural insight into the substrate recognition by the radical SAM oxygenase DarE in darobactin biosynthesis

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Darobactin A is a remarkable bicyclic heptapeptide that belongs to the ribosomally synthetized and post translationally modified peptide (RiPP) family. It contains a unique ether crosslink between two tryptophan residues alongside a carbon-carbon crosslink between one tryptophan and one lysine.[1]

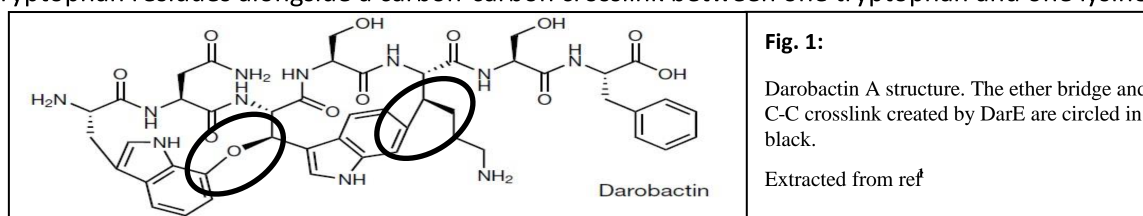

Fig. 1:

Darobactin A structure. The ether bridge and C-C crosslink created by DarE are circled in black.

Extracted from ref[1]

These two cyclisations give darobactin A a rigid β-strand conformation that confers to the molecule specific antibiotic properties against gram-negative pathogens through inhibition of BamA, a protein of the β-barrel assembly machinery (BAM) complex involved in the insertion of outer membrane proteins.[2]

Darobactin A is originated from a propetide, DarA, that is maturated by a radical SAM enzyme, DarE, responsible for the formation of the two distinct crosslinks. DarA shows the classical features of a RiPP peptide, namely a N-terminal sequence called the leader, rather long comprising 49 amino acids over the 58 total in the peptide, the core peptide of 7 amino acids where the modifications occur and that constitute the darobactin, and a follower containing 2 amino acids. The leader and follower will be excised to release the antibiotic. The darobactin ether bond is unique as it is not trivial to chemically make this type of cross link. In Nature, radical-based chemistry is involved and the oxygen atom is provided by molecular oxygen (O_2). [3] As radical SAM enzymes contain iron sulfur clusters and are known to be sensitive to O_2, the precise mechanism by which DarE manage to use oxygen is of significant interest.

Several variations of the darobactin have also been identified from homologs with a very low conservation on the leader sequences of their pro-peptide. However, those variants are characterized by single-point mutations on the core region in residues not involved in any crosslink.[1] While looking at sequence analysis, we noticed a variation in the N-terminal sequence of DarE correlating with the leader changes on DarA. Combining sequence analyses and structural models, we identified a motif composed of two α-helices and conserved amino acids that could be involved in the recognition of DarA by DarE.

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Towards Deciphering the Cytotoxicity of Ferrocifens

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In 2020, 2.2 million new cases of breast cancers were estimated worldwide. It is the first female cancer and led to about 685 000 deaths for the same year. Depending on the tumor subtype and the presence of metastases, several options are available to treat them: hormonotherapy, chemotherapy, surgical resection (until a possible mastectomy) and/or radiation. The endocrine therapy consists in a dose of Tamoxifen (TAM), a selective estrogen receptor modulator (SERM). Even if it is widespread, TAM is inefficient on hormone-independent breast cancers, since these tumors do not express the estrogen receptor.[1,2]

To overcome this issue, Jaouen's group had the idea to include a ferrocenyl moiety to the TAM structure as it is considered to be a bioisotere of a phenyl group. This new family was named Ferrocifens and surprisingly showed a high cytotoxicity on both non-hormone (MDA-MB-231, or triple-negative) and hormone-dependent (MCF-7) breast cancer cell lines. Since then, efforts were carried out to find the mechanism of action (MoA) of this new family to offer new therapeutics to women suffering of breast cancer. [3-5]

To decipher the MoA involved in the antiproliferative activity of ferrocifens, a chemical proteomic approach called Activity-Based Protein Profiling (or ABPP) can be employed to identify the protein targets (see workflow in Figure 1). With this chemoproteomic strategy, two directions will be undertaken: protein targets fishing and identification and cellular imaging studies. For these purposes, an alkyne derivative of a highly toxic compound, patented by the start-up Feroscan, will be employed (see structure in Figure 1). With all the information collected, we hope to better understand the mechanism of action of these molecules, to select among the library of existing molecules those that are the most efficient and the most selective towards cancer cells. In conclusion, the results obtained can be the starting point of the adaptation of ferrocifens to offer more efficient and selective molecules targeting cancer cells.[6-9]

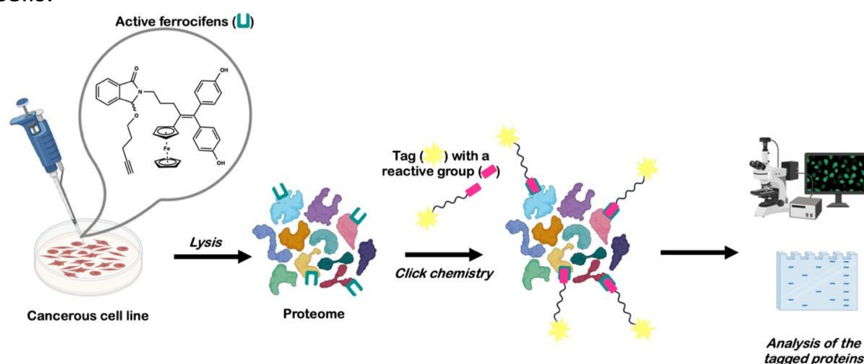

Figure 1. Workflow of the strategy to decipher the cytotoxicity of Ferrocifens

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New artificial binuclear metalloenzyme for binding and stabilization of a semiquinone radical

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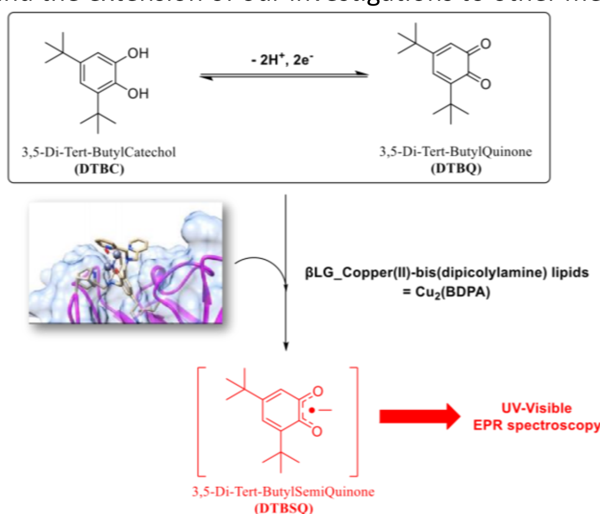
The elaboration of artificial metalloenzymes (AMEs) has emerged as a promising strategy for obtaining new biocatalysts both for natural and non-natural reactions, as well as for studying the role of the second coordination sphere of metal cations in enzymatic catalysis. Tyrosinases (Ty, EC 1.14.18.1) are coppercontaining metalloenzymes that are widely distributed in microorganisms, plants and animals, wherein they catalyze the oxidation of phenolic compounds to catechols (phenolase activity) and of catechols to *o*quinones (catecholase activity). Many dinuclear copper (II) complexes that possess a good catecholase activity have been reported in the literature,[1] without however constituting good mimics of the metalloprotein. Indeed, they lack the interactions with the second coordination sphere amino acids that can allow, in metalloproteins, the stabilization of highly reactive species that lead to an increase in their lifespan. Accordingly, metal-semiquinone intermediate complexes have recently been identified in AMEs by the groups of *DeGrado*[2] and *Roelfes*[3] who reported the stabilization of the 3,5-di-tert-butylsemiquinone (DTBSQ) radical resulting from the one-electron oxidation of 3,5-di-tert-butylcatechol (DTB-C), respectively inside a de novo engineered metalloprotein [DFsc-ZnII2] [2] and inside an artificial metalloprotein where 2,2'bipyridyl alanine (bpyA) metal complexes were incorporated into the lactococcal multidrug resistance regulator (LmrR).[3] *Salmain et al.*[4] exploited the affinity of beta-lactoglobulin (βLG) for fatty acids to design AMEs that catalyze the reduction of trifluoroacetophenone by hydrogenation transfer with up to 25% enantiomeric excesses. Based on these preliminary observations, we decided to associate this protein with a binuclear copper complex (Cu2-bis-dipicolylamine, Cu2(BDPA)) functionalized with a lauric acid (LA) tail, in order to get a new artificial metalloenzyme capable of oxidizing catechol derivatives into quinones. The obtained -LG-LA-Cu2(BDPA) AME showed no catecholase activity, but, to our satisfaction, showed a definite ability to stabilize the DTB-SQ radical intermediate. Here we present the UV-vis. and EPR characterizations of the complexes obtained and the extension of our investigations to other metals such as Zn^{II}.



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Nickel complexes for hydrogen oxidation and production without overvoltage

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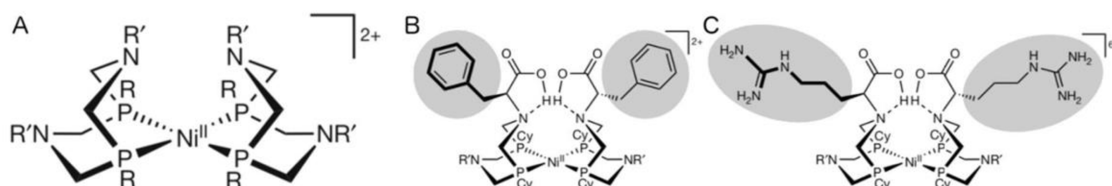
The design of efficient transition metal (platinum-free) catalysts for H^+/H_2 conversion is a prerequisite for the large-scale deployment of fuel cells and electrolysers.[1] Many inorganic catalysts are available for the electrochemical production of hydrogen,[2] but they work in a unidirectional way and with a high "overvoltage", i.e. under conditions that are much more reducing than what is allowed by thermodynamics. This overvoltage implies that a device that produces hydrogen by electrolysis of water dissipates as heat a fraction of the electrical energy that makes it work. This is an obstacle for the construction of devices to temporarily and efficiently store (without energy loss) the intermittently produced energy by wind or solar power. A small number of bioinspired mononuclear nickel complexes developed over the last five years can catalyse both the oxidation and the production of hydrogen at high rates and under very energy efficient conditions. These complexes belong to the large family of molecular catalysts known as "DuBois".[3] They are Ni complexes in a phosphorous environment, bearing bioinspired amine groups that promote proton transfer and heterolytic dihydrogen splitting. The ligands are functionalised with substituents (R and R' in Figure 1A) that significantly modify their catalytic properties. Two examples of complexes, based on the generic structure, are represented in Figure 1 (panels B and C).



The goal of my work is to understand how structural details determine the kinetics of the catalytic cycle steps and the catalyst properties in this family of mononuclear Ni complexes. My studies rely on recently proposed kinetic models in the group, that allow the use of electrochemical methods to decipher catalytic mechanisms of bidirectional redox catalysts in an effort to understand what makes them work in an (ir)reversible way, with or without overpotential.[4,5] New designs of these complexes are also envisioned to allow grafting on electrodes to facilitate the study of their mechanisms.

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Bioinspired complexes for recalcitrant biomass valorization

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Lignocellulosic biomass constitutes one of the largest global sinks of photosynthetically fixed carbon and is increasingly considered as a renewable resource for biofuels, biochemicals and biomaterials production.[1] Nevertheless, biomass valorization is still a challenge because of its "recalcitrance", responsible of its high cost.

In Nature, many organisms including fungi and bacteria are able to degrade lignocellulosic biomass by producing various enzymes (oxidative and hydrolytic) which act in a synergistic manner.[2] Among oxidative components, Lytic polysaccharide monooxygenases (LPMOs) are recently discovered copper-containing enzymes that play a major role in polysaccharides deconstruction.[3,4,5] LPMOs catalyze the glycosidic bond cleavage by hydroxylation of a strong carbonhydrogen bond, in presence of O_2 and electrons or H_2O_2; further leading to structural disruption and making the polysaccharides more accessible to hydrolytic enzymes.[6] The active site of LPMO is composed of a surface-exposed Cu(II) ligated by a histidine-brace motif. The conception of bioinspired catalysts with LPMOs activity while using biologically relevant transition metal ions appears as a relevant approach.

A few years ago, it has been reported the first low molecular-weight model of LPMOs promoting oxidative cleavage in aqueous medium and at room temperature.[7] Since this first report, several copper complexes were described in the literature, but the catalytic activities still lack proof-of-concept on realistic models closer to the biomass ultrastructure.[8,9,10] On this basis, we aim at developing new copper-based complexes and mimics of LPMO and evaluating their oxidative activity, either on model substrates or complex biomass under mild conditions (water, ambient temperature, O_2 or H_2O_2).

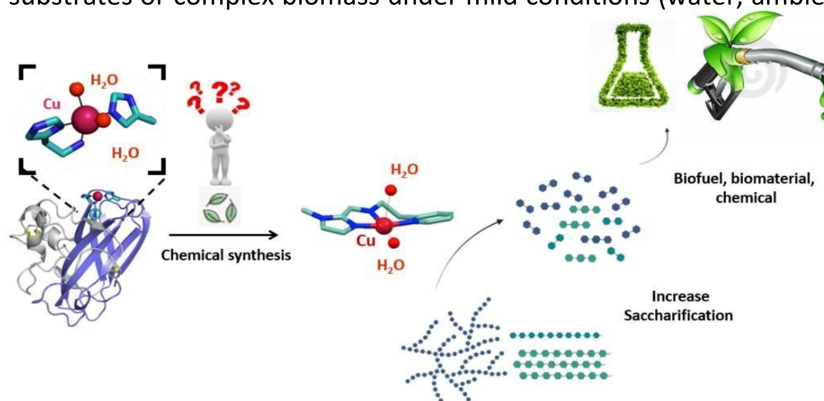

Figure: Scheme of the project strategy in two steps, first the chemical synthesis of bioinspired complexes, and then, the activity screening on substrates in order to modify them, for instance increase the saccharification of biomass polysaccharides to produce biofuel. Other modification could lead to biomaterial or chemicals.

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Installing a LPMO-inspired copper binding site onto amyloidlike fibrils, for radical reactions

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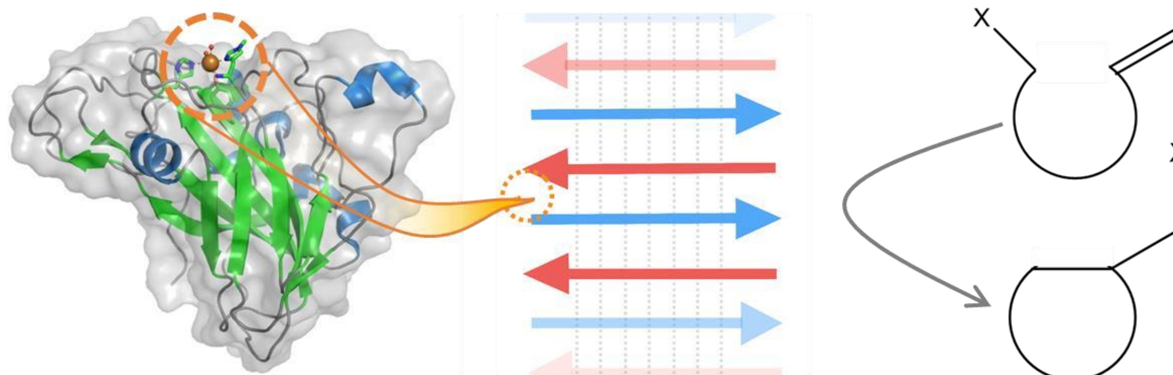
Flexibility of short peptides often impairs their use as ligands for the development of catalytic metal complexes. A way to lock a peptide's conformation is to involve it in a network of intermolecular interactions, such as in amyloid fibrils.[1] The group of Korendovych has demonstrated the potential of such method to develop catalytically active peptide-metal complexes.[2-4]
Lytic Polysaccharide MonoOxygenases (LPMO) are copper enzymes able to oxidatively cleave polysaccharide (eg. cellulose, chitin) that are receiving chemist's attention because of their potential applications.[5]
Here, we describe a "minimalist de novo protein design" approach aiming at anchoring a LPMO-inspired copper site onto amyloid-like fibrils. As the copper in LPMO is chelated by two non-equivalent histidines, the design was made in order to preform the metal site via the use of two different peptides, each bringing one histidine. Their sequence was built on those of the "Amyloid Inspired Peptides" developed by the group of Guler.[6]



We show that even though this system reveals poor activity towards oxidation reactions, it does show interesting catalytic properties towards Atom Transfer Radical Cyclization (ATRC).

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Bioelectrochemistry as a tool to decipher the role of Met-rich domains in copper detoxification by multicopper oxidases

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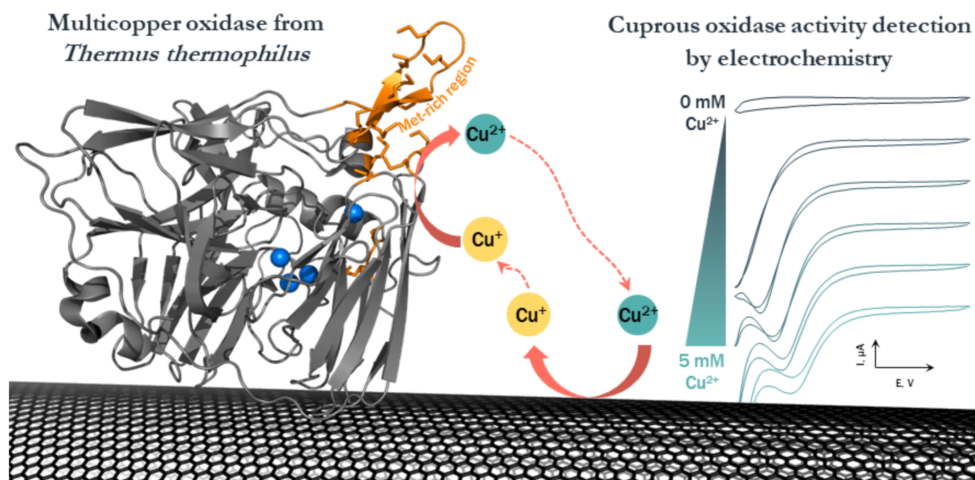
Bioelectrochemistry is a powerful tool allowing to study a multitude of biological redox processes in the electrochemical cell. It is particularly useful for the investigation of redox enzyme mechanisms as it allows an instantaneous condition change and requires only picomolar quantities of protein adsorbed on the electrode. Interfacing enzymes with electrodes also allows considering multiple biotechnological applications, from enzymatic fuel cells to biosensors.

Multicopper oxidases (MCOs) are redox enzymes present in all domains of life that contain a couple of Cu-centres: mononuclear T1 and trinuclear T2/T3.[1] The coordination of these copper centres is highly conserved, but these MCOs can play various physiological roles.

Earlier, we showed that the MCO from *T.thermophilus* gives rise to a new catalytic wave upon Cu^{2+}-addition.[2] We rationalised and ascribed this wave to a cuprous oxidase activity displayed by this enzyme thus allowing us to propose its physiological role in the copper detoxification.[3] We observed a similar wave for another MCO with confirmed cuprous oxidase activity, CueO from *E.coli*, which has a large methionine-rich domain presumably involved in the copper binding.[4] Here we present the utility of bioelectrochemistry and other methods in study of the cuprous oxidase activity of MCOs related notably to the Met-rich domain.



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Siderophore-linked Ruthenium Catalysts for Targeted Allyl Ester Prodrug Activation within Bacterial Cells

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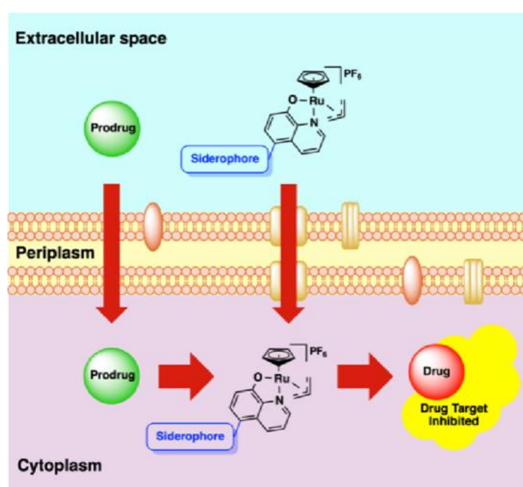

Due to rising resistance, new antibacterial strategies are needed, including methods for targeted antibiotic release. In this work, siderophore-linked ruthenium catalysts were investigated for the activation of an antibacterial prodrug within cells. A fluoroquinolone prodrug, moxifloxacin, was developed and shown to be compatible with the catalysts under micro-aerobic, biologically-relevant conditions. The co-addition of the catalysts and prodrug to *E. coli* showed a combinative effect, with the dihydroxybenzoic acid- and azotochelin-linked catalysts showing most promise for cellular uptake and thus intracellular prodrug activation.

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Bidirectional and reversible catalysis: from redox enzymes to molecular machines

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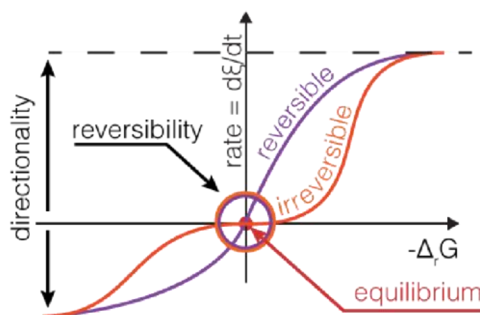

Studies of catalysts usually focus on measuring the *rate* of the catalyzed reaction, and on identifying their *mechanism*.

Of equal importance are the following distinct questions: what makes them uni- or bidirectional, and how much thermodynamic driving force is required to make them work at a significant rate.

We define as 'reversible' a bidirectional catalyst that allows a reaction to proceed at a significant rate in response to even a small departure from equilibrium, resulting in fast and energy-efficient chemical transformation.

Of course, the typical design of voltammetric experiments, where the electrode potential is changed and the current measured, makes it easy to force a redox catalyst to work forward or backward and to examine how the rate of a catalyzed reaction depends on the applied thermodynamic driving force. But biological motors (such as ATP synthase or kinesin) can also be driven by applying a chemical or mechanical force, to look at the relation between kinetics and thermodynamics. The same questions regarding the 'directionality' and 'reversibiliy' of these catalysts can then be asked, and the rate equations are remarkably similar.

New electrochemical data for the catalytic oxidation and production of H_2 under various configurations (by molecular catalysts or enzymes undergoing direct or mediated electron transfer) as well as literature data describing the function of molecular motors illustrate the concepts, questions and challenges that we face when we aim for understanding what makes redox catalysts reversible.

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Understanding the assembly machinery of the nitrogenase active site

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Reduced nitrogen in the form of ammonia (NH_3) is fundamental to all life and many industrial processes. The HaberBosch 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 throughout the production.[1] Interestingly, nature has always been a huge player in nitrogen fixation, yet in a more sustainable 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 – 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[2]. Using computational modelling approach, we were able to predict some 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 and its unique chemistry. This in turn may help inspire the development of more efficient catalysts for the production of ammonia.

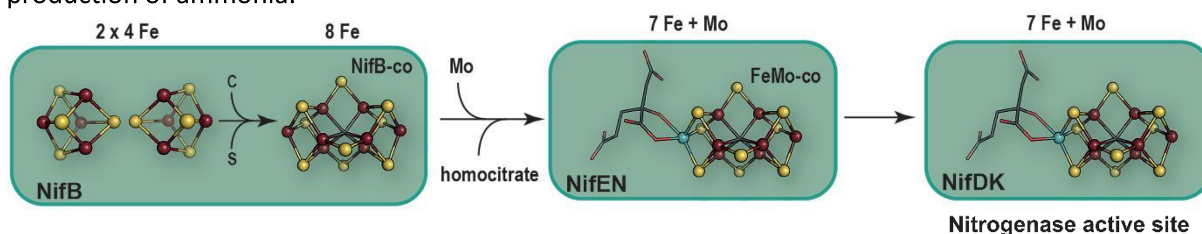

Figure 1. Schematic illustration of the biosynthesis of the FeMo cofactor of nitrogenase

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