French Group of BioInorganic Chemistry

Book of Abstracts

Anglet 2014

Sept 28th-Oct 1st

FrenchBIC

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Anglet 2014

	SUNDAY Septembre 28th 2014	MONDAY Septembre 29th 2014	TUESDAY Septembre 30th 2014	WEDNESDAY Octobre 1st 2014
8h30-8h50		Plenary Maria João Romão	Plenary Mercè Capdevila	K. Brillet
8h50-9h10				N. Ségaud
9h10-9h30				C. Buron
9h30-9h50		B. Guigliarelli	S. Gil-Moreno	J.-M. Latour
9h50-10h10		J. Moura	O. Palacios	A. Trehoux
10h10-10h30		J. Rendon	G. Lespes	Coffee
10h30-10h50		Coffee	Coffee	Break
10h50-11h10		Break	Break	N. Delsuc
11h10-11h30		C. Duboc	M. Bourrez	C. Bonnet
11h30-11h50		M. Swart	I. Pereira	I. Nazarenko
11h50-12h10		B. Garcia-Cirera	E. Lojou	S. Hostachy
12h10-12h30		I. López	S. Dementin	M. Isaac
12h30-13h50		Lunch	Lunch	Lunch
13h50-14h20		Presentation of the 3 Groups	Free Afternoon	
14h20-14h40		B. Boff		
14h40-15h00		P. Gamez		
15h00-15h20		A. Conte-Daban		
15h20-15h40		T. Santos-Silva		
15h40-16h00		Coffee		
16h00-16h20		Break		
16h20-16h40		M. Salmain		
16h40-17h00		E. Baudrin	Coffee	
17h00-17h20	Registration	H. Jamet	Break	
17h20-17h40		A. Company	J. Dominguez-Vera	
17h40-18h00		Poster Session	E. Artells	
18h00-18h20			E. Atrián-Blasco	
18h20-18h40			M. Beyler	
18h40-19h00			S. Eliseeva	
19h00-19h20			M. Beccia	
19h30-20h00	Diner	Diner	Diner	
20h00-20h30				
20h30-20h50	Plenary Olivia Reinaud	Assemblée Générale FrenchBIC		
20h50-21h10				
21h10-21h30				

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Sunday 28th September

Chair : Yves Le Mest

20h30 – 21h30 : Olivia Reinaud
Metal ions in biomimetic cavities

Monday 29th September

Chair: Vincent Nivière

8h30-9h30 : Maria J. Romão
Molybdenum enzymes in health and disease

9h30-9h50 : Bruno Guigliarelli
Structure and reactivity of the Molybdenum cofactor in periplasmic nitrate reductase as viewed by EPR spectroscopy

9h50-10h10 : José J. G. Moura
Novel orange proteins - Characterization of heterometallic Mo-Cu clusters

10h10-10h30 : Julia Rendon
Identification of a new semiquinone species stabilized in *E. coli* nitrate reductase A

10h30-11h10 : Coffee Break/Posters

Chair : Belen Albela

11h10-11h30 : Carole Duboc
O_2 activation by Mn thiolate complexes

11h30-11h50 : Marcel Swart
Catalase activity of Mn^{III} complexes

11h50-12h10 : Beltzane Garcia-Cirera
Insertion in a mesoporous silica of dinuclear Mn(III) compounds models of catalase

12h10-12h30 : Isidoro López
Single-site functional mimics of the oxygen evolving complex: is one site really enough ?

13h50-14h20 : Presentation of the 3 Groups

Chair: Peter Faller

14h20-14h40 : Bastien Boff
Rational design of intracellular Cu(I) chelators for treating copper overload in Wilson's disease

14h40-15h00 : Patrick Gamez
Fluorescent metal-binding molecules as potential anti-Alzheimer's agents

15h00-15h20 : Amandine Conte-Daban
Cu(II) chelator to inhibit the toxicity of Amyloid-β-Cu(II) against the Alzheimer's disease

15h20-15h40 : Teresa Santos-Silva
Metal complexes with biological activity – structural details of protein adducts

15h40-16h20 : Coffee Break/Posters

Chair: Olivia Reinaud

16h20-16h40 : Michèle Salmain
Artificial metalloenzymes derived from bovine betalactoglobulin for the asymmetric transfer hydrogenation of an aryl ketone

16h40-17h00 : Emmanuel Baudrin
Iron(III) chelators as a tool for the development of new antibacterial agents

17h00-17h20 : Hélène Jamet
Theoretical tools to study metalloprotein/ligand interactions: illustrations through studies of inhibitors of Tyrosinases

17h20-17h40 : Anna Company
Selective ortho-hydroxylation-defluorination of 2-fluorophenolates with bis(μ-oxo)dicopper(III) species

17h40-19h30 : Posters

Tuesday 30th September

Chair: Clotilde Policar

8h30-9h30 : Mercè Capdevila
Is there still something to learn from Metallothioneins?

9h30-9h50 : Selena Gil-Moreno
Revisiting *Neurospora crassa* metallothionein

9h50-10h10 : Òscar Palacios
Following the interaction of metallic compounds with biomolecules by mass spectrometry

10h10-10h30 : Gaëtane Lespes
Analytical strategies for bioinorganic chemical investigations

10h30-11h10 : Coffee Break/Posters

Chair : Christophe Léger

11h10-11h30 : Marc Bourrez
Proton-Coupled Electron Transfer on metal hydride complexes

11h30-11h50 : Inès C. Pereira
Structural insights into oxygen inactivation of *Desulfovibrio vulgaris* Hildenborough [NiFeSe] hydrogenase

11h50-12h10 : Elisabeth Lojou
Role of enzyme orientation at electrochemical interfaces for biotechnological processes

12h10-12h30 : Sébastien Dementin
Maturation and mechanism of the Carbon Monoxide Dehydrogenase (CODH) from *Desulfovibrio vulgaris*

Chair: Manuel Basallote

17h20-17h40 : Jose M. Dominguez-Vera
Bioinspired Magnetic Materials

17h40-18h00 : Ester Artells
Interactions and toxicology of silver nanoparticles in aquatic ecosystems

18h00-18h20 : Elena Atrián-Blasco
Gold(I) thiolates with optimum hydrophilic/lipophilic balance as anticancer drugs

18h20-18h40 : Maryline Beyler
Cyclen-based ligands for the complexation of Pb(II) and Bi(III) for alpha radioimmunotherapy

18h40-19h00 : Svetlana V. Eliseeva
Near-infrared emitting Zn^{II}-Ln^{III} “encapsulated sandwich” metallacrowns

19h00-19h20 : Maria R. Beccia
Engineering proteins to bind heavy metals efficiently and selectively

Wednesday 1st October

Chair: Katell Sénéchal-David

8h30-8h50 : Karl Brillet
An ABC transporter with two periplamic binding proteins involved in iron acquisition in *Pseudomonas aeruginosa*

8h30-9h10 : Nathalie Ségaud
New artificial metalloenzyme containing an iron coordinating active site

9h10-9h30 : Charlotte Buron
An artificial metalloenzyme made by covalent grafting of an iron(II) complex in β-lactoglobulin

9h30-9h50 : Jean-Marc Latour
Binuclear hydrolases: desperate search for a nucleophile

9h50-10h10 : Alexandre Trehoux
Diiron complexes and peroxo intermediates : influence of the second coordination sphere and oxidation catalysis

10h10-10h50 : Coffee Break/Posters

Chair : Olga Iranzo

10h50-11h10 : Nicolas Delsuc
A correlative approach for the imaging of a cell-penetrating peptide in skin slices

11h10-11h30 : Celia S. Bonnet
Zinc responsive contrast agents for MRI

11h30-11h50 : Iuliia Nazarenko
Polynuclear lanthanide-based dendrimers for biological optical imaging

11h50-12h10 : Sarah Hostachy
Development of rhenium carbonyl complexes for the labelling and bimodal imaging of biomolecules

12h10-12h30 : Manon Isaac
Lanthanide-based luminescent probe for time-gated detection of copper(I) : modulation of the antenna effect by cation/π interaction

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Plenary Lectures

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Metal ions in biomimetic cavities

Olivia Reinaud

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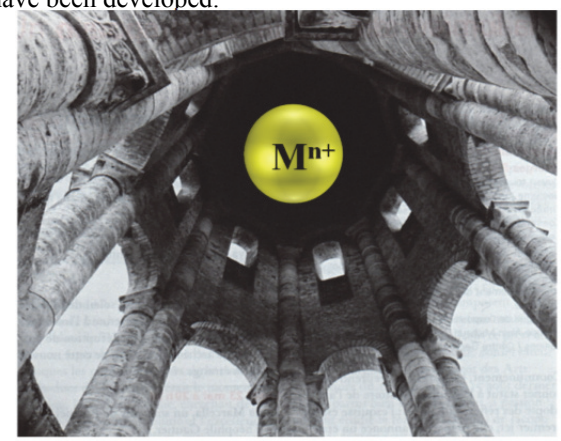
The aim of our work is to design supramolecular systems that will mimic both the coordination core and the hydrophobic pocket of a metallo-enzyme active site. Our strategy relies on the synthesis of cavity-based ligands that allow the control of the coordination spheres of the metal ion (1^{rst}, 2nd and 3rd), together with the approach and the binding of an exogenous molecule. Since many years, we have been developing systems based on the calix[6]arene scaffold, giving rise to the so-called "funnel complexes".[1] Various aspects such as dioxygen activation at a mononuclear Cu(I) center,[2] supramolecular control of hetero-multinuclear binding of metal ions,[3] guest covalent capture by a host,[4] and water-soluble receptors have been developed.[5]



Quite recently, we started to explore metal complexes based on the resorcin[4]arene scaffold, which provides a supramolecular environment different in shape, rigidity and binding properties, so-called "Bowl-complexes".[6]

Hence, various aspects of these cavity-complexes will be presented and the *Bowl* vs. *Funnel* supramolecular concepts for biomimetic metal complexes will be discussed.

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6. a) J. Gout, S. Rat, O. Bistri, O. Reinaud, *Eur. J. I. C.*, **2014**, 2819-2828 ; b) J. Gout, A. Višnjevac, S. Rat, O. Bistri, N. Le Poul, Y. Le Mest, O. Reinaud, *Eur. J. I. C.* **2013**, 5171–5180 ; c) J. Gout, A. Višnjevac, S. Rat, A. Parrot, A. Hessani, O. Bistri, N. Le Poul, Y. Le Mest, O. Reinaud, *Inorg Chem* **2014,** *53*, 6224–6234.

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Molybdenum Enzymes in Health and Disease

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Molybdenum-dependent (Moco) enzymes exist in all domains of life and their importance is exemplified by their ubiquity, their roles in metabolic diversity and in global geochemical cycles [1]. Crystallography has had a major impact in the field of Moco-enzymes and it has allowed identifying unexpected co-factors and to discover new metal ligands that challenged earlier proposed functions of the individual active sites. In humans, Mo occurs in four enzymes: sulfite oxidase, aldehyde oxidase, xanthine dehydrogenase and mitochondrial amidoxime reductase. These enzymes are involved in processes as diverse as nutrition, drug metabolism, detoxification and ROS-production and a mutation in Mo-metabolism leads to the failure of all molybdoenzymes being lethal for the organism.

Among mammalian Mo enzymes, one of the least studied is Aldehyde Oxidase (AOX). Mammalian AOXs are complex proteins characterized by a broad range substrate specificity, although their true physiological function is still to be unravelled. Very recently, it was recognized the emerging importance of the role of AOX in the metabolism of drugs and xenobiotics. We have solved the crystal structures of mouse and human AOX, native and complexed with the substrate and an inhibitor [2,3].
The combination of crystallographic data with kinetic, mutagenesis and molecular docking studies have made a decisive contribution to understand the molecular basis of the broad substrate specificity of AOXs. The ensemble of the crystal structures now available will allow to define in silico models for AO binding affinities of drugs under development, information of paramount importance in drug discovery.

References:
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3. Coelho, C, Foti, A., Santos-Silva T, Hartmann T, Leimkühler S and Romão MJ. Human Aldehyde Oxidase crystal structures in native and substrate and inhibitor-bound forms provide insight into the inhibition mode of xenobiotics. submitted

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Is there still something to learn from Metallothioneins?

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Metallothioneins (MTs) were discovered in the 50s when looking for a Cd^{2+}-binding protein in mammalian (horse) kidney. Since then, this ubiquitous and very big family of proteins that are characterized by their high capability for heavy metal ion coordination (metallo-) and elevated sulfur, *i.e.*Cys, content (-thioneins) has attracted much attention, being the protagonist of thousands of scientific papers[1]. Initially, MTs were exclusively considered as detoxifying agents against toxic metal ions. Afterwards, they have been related to many physiological events that include from the homeostatic control on Zn(II) and Cu(I) essential elements, until the control of redox processes and oxidative stress, among others. Nowadays, their functionality in living organisms is still a matter of debate. Our group has been involved in the study of MTs since the beginning of the 90s. During this almost 25 years of research we have analyzed the properties, and more specifically the metal-binding abilities, of a considerable number of MTs belonging to the most diverse organisms scattered through the Tree of Life[2]. The rigorous application of the same methodology for MT synthesis and analysis[3], has allowed the comparison of all the gathered results in a wide frame and rendered a more comprehensive picture of the properties of the whole family. This has allowed us not only to propose a new classification of their members following a gradation between genuine Zn-thioneins and clear Cu-thioneins, but it has also revealed many different peculiarities of the constituents of this family of metalloproteins that entitle us to state that much remains still to be studied and discovered about MTs. Some particular examples will be provided that will enlighten these affirmations as well as a spotlight in our latest and more interesting findings.

The financial support received from MINECO-FEDER (Projects BIO2012-39682-C02-01 to S.A. and BIO2012-39682-C02-02to M.C.) is acknowledged.

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Oral Communications

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Structure and reactivity of the Molybdenum cofactor in periplasmic nitrate reductase as viewed by EPR spectroscopy

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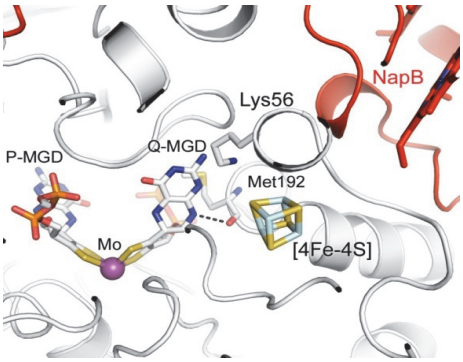
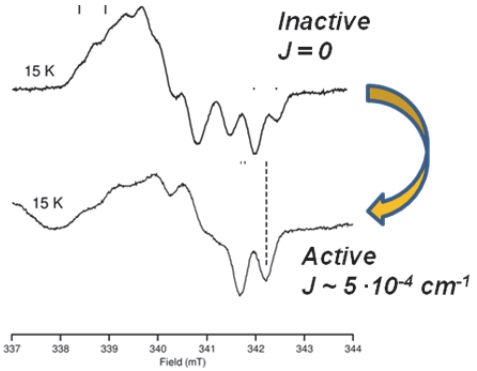
Molybdenum enzymes constitute a wide enzyme family found in nearly all organisms. In prokaryotes, these enzymes contain a mononuclear Mo-cofactor in which the Mo ion is coordinated by two pyranopterin guanosine dinucleotide (PGD) moieties and by an additional protein ligand. Despite the similarity of their Mo-bisPGD cofactor, these enzymes are very diverse in terms of structure and subunit composition and are able to use a broad diversity of substrates, being involved in the major biogeochemical cycle of carbon, nitrogen, sulfur and metalloids [1]. However, in spite of numerous crystallographic and spectroscopic studies, the molecular factors which trigger their reactivity remains largely debated [2].

During catalysis, the molybdenum ion cycles between the +IV and +VI redox states; the intermediate Mo(V) state is EPR-active (S=1/2) and EPR spectroscopy has proved essential for investigating the molybdo-enzymes reactivity. In periplasmic nitrate reductase, several Mo(V) species can be detected depending on the preparation conditions. By combining EPR spectroscopy, electrochemistry and DFT calculations on this model Mo-enzyme, we brought new insights on the activation process of these enzymes and on the role of the various spectroscopically detected Mo species in catalysis. We have shown that the so-called ''high *g* resting'' Mo(V) species is an inactive form that becomes catalytically competent the first time it is reduced [3]. Furthermore, studies of spin-spin interactions between the Mo(V) and the neighbour [4Fe-4S]$^{1+}$, before and after activation, gives insights into the Mo cofactor reactivity. The overall observations led us to conclude that the activation process occurs beyond the first coordination sphere of the Mo ion and entails the pterin moiety chemistry [4].





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Novel Orange Proteins - Characterization of heterometallic Mo-Cu clusters

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The ORange Protein (ORP) (11.8 kDa), isolated from Sulphate Reducing Bacteria, contains a mixed-metal sulphide cluster of the type $[S_2MoS_2CuS_2MoS_2]^{3-}$, non-covalently bound to the polypeptide chain[1,2]. The ORP was produced for NMR studies by heterologous expression in E.coli as the apo-form[3]. The holo-form was reconstituted by the *in situ* synthesis of the metal cluster upon the addition of copper sulphate and thiomolybdate (or thiotungstate). ^{13}C detection experiments enabled the extension and confirmation of the sequential assignment for both apo and reconstituted-forms of ORP[3]. The over-all solution structures of the apo and reconstituted ORP are similar and the mapping of the chemical shift differences between them was used to elucidate which region of the polypeptide chain is involved in the binding of the metal cluster. Since this cluster is diamagnetic, without any ^{13}C, ^{15}N or ^{1}H NMR observable nuclei, the exact location of this metal cluster is unclear. Moreover, efforts to obtain the X-ray structure of the holo-ORP resulted in the crystallization of the apo-protein. A strategy has been designed to obtain clusters that could aid in localizing its binding site using NMR methodologies[4]. Therefore, we have synthesized some M´–M (M´= MoS42–; M= Cu and Cd) clusters, which display spectral signals and could be used to probe the cluster-binding site. Since Mo and Cu are both spectroscopically silent in ORP, the copper atom can easily be replaced by cadmium to produce spin-spy active analogue compounds. Another strategy is based on the substitution reaction in which small organic ligands bind directly to the copper centre in molybdenum–copper hetero-dinuclear clusters, resulting in MoS_4–Cu–thiol. ^{113}Cd-Substitution and MoS_4–Cu–thiol (some with ^{19}F) constitute structural synthetic analogue compounds of ORP clusters that can be used to identify the cluster binding site of the ORP protein using conventional NMR spectroscopic techniques[4].

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Identification of a new semiquinone species stabilized in *E. coli* nitrate reductase A

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Isoprenoid quinones are liposoluble components of bioenergetic membranes shuttling electrons and protons between respiratory complexes in most living organisms. Interaction of quinones with membrane-embedded complexes at specific quinone processing sites allows transient generation of paramagnetic semiquinone intermediates.

The gut bacterium *Escherichia coli* naturally synthesizes three different quinones, namely the benzoquinone ubiquinone (UQ) and the naphthoquinones menaquinones (MK) and demethylmenaquinone (DMK). They differ in their midpoint redox potentials which range from +113 mV for the couple UQ/UQH2, to -74 mV for MK/MKH2. The midpoint potential of DMK is intermediate (+36 mV, -9 mV). To decipher the influence of the protein environment in tuning enzyme reactivity towards quinones, we use *E. coli* nitrate reductase A (NarGHI), a membrane-bound quinol oxidizing enzyme, as a model system [1]. As such, we have been able to report that NarGHI can stabilize menasemiquinones [3] or ubisemiquinones [4] within its quinol oxidation site. Intriguingly, Wissenbach *et al.* reported that DMK is not a substrate for NarGHI albeit this quinone predominates under these growth conditions [2]. We provide here experimental evidences for the efficient use of DMK as substrate and for the stabilization of demethylmenasemiquinones within NarGHI. Moreover, our EPR studies reveal unusual hyperfine characteristics for these radicals that are contrasted with corresponding existing experimental and theoretical data on NarGHI- and other protein-bound semiquinones. Overall, the nitrate reductase complex constitutes a valuable tool to address the relationships between reactivity, binding mode and stability level of semiquinones.

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O_2 activation by Mn thiolate complexes

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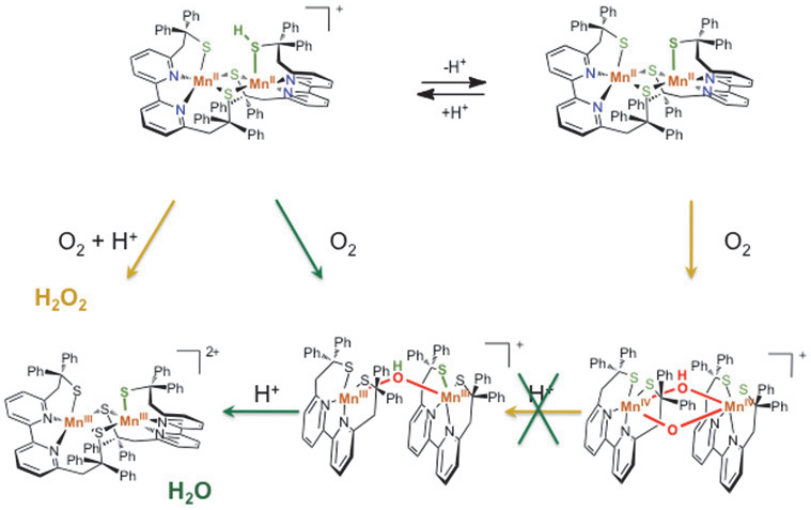
How dioxygen can be activated by molecular transition metal complexes remains a central question in bio-inorganic chemistry. Manganese is interesting in this sense because it is involved in many catalytic oxidation processes and also as a constituent of the active site of the evolving oxygen center of the photosystem II: the understanding of how the O-O bond can be broken can give insight on how it is formed. In this context, we have isolated a new thiolate dinuclear Mn(II) complex, which reacts which dioxygen. Interestingly, depending on its initial protonation state, its reactivity is different as well as the structure of the intermediate species involved. In this presentation, the characterization of the initial dinuclear Mn(II) complex, in which one the monodentate thiolate is protonated, will be presented including structural and magnetic data as well as its redox properties. Its reactivity towards O_2 will be discussed. Finally, its acid-base properties have been investigated and the reactivity of the deprotonated form with O_2 will also be discussed as well as the characterization of the intermediate species involved during the dioxygen reduction process. DFT calaculations have been performed to rationnalize the reactivity of this system.



Catalase activity of Mn^{III} complexes

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The study of oxidative stress and the close relationship this has with ageing means there is much interest in the effects of antioxidant complexes, which often contain manganese. Although experimental data in biological studies have shown very positive effects in prolonging the lifespan of mice,[1] there is much uncertainty about what is the origin of these effects and how they could be improved. Interestingly, very few chemical investigations have been performed on this subject,[2,3] and even less using computational chemistry. Therefore, we studied the mechanism of a catalase reaction of a manganese-salen complex that proved beneficial at the biological level, using advanced methods of computational chemistry.[4] The catalase mechanism contains two phases, a first one in which a Mn^{III}-salen complex captures an oxygen from a hydrogen-peroxide to form a high-valent Mn^{V}-oxo and water; and a second phase where the Mn^{V}-oxo complex converts a second hydrogen-peroxide into water and oxygen:

(i) Mn^{III}-salen + HOOH → Mn^{V}=O-salen + H_2O

(ii) Mn^{V}=O-salen + HOOH → Mn^{III}-salen + H_2O + O_2

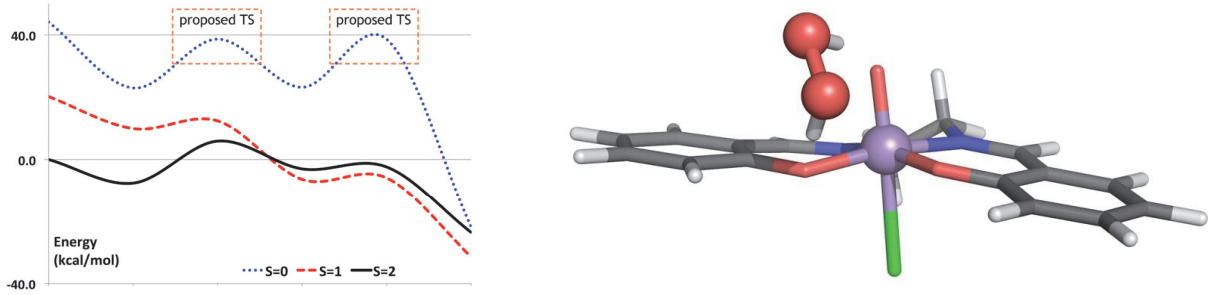

Figure 1. Energy profile for first phase, leading to Mn^{V}-oxo complex (left) ; binding of HOOH to Mn^{V}-oxo complex (right)

Besides a detailed description of all the different reaction pathways present in the mechanism and the important role of the spin state,[5] we have found new results and concepts that open the possibility of improving the efficiency and feasibility of the antioxidant complex. This is especially relevant for the initial part of the mechanism, where the manganese complex has to capture the hydrogen-peroxide in order to activate it. Two important aspects for the description of the reaction mechanism is the ability of the computational method to correctly describe the spin-state *and* the weak interactions, for which S12g performs excellently.[4]

Part of this work was supported by the COST Action CM1305 ECOSTBio (Explicit Control Over Spin-states in Technology and Biochemistry).

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Insertion in a mesoporous silica of dinuclear Mn(III) compounds models of catalase

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Aerobic organisms, as a by-product of the metabolism, generate some incomplete reduced oxygen species like $O_2^{\cdot -}$, H_2O_2 or the radical ·OH. This kind of species are called Reactive Oxygen Spices (ROS) and are much reactive than oxygen. Increased levels of ROS can cause oxidative damage destroying tissues, biomolecules and causing some degenerative diseases like Alzheimer, sclerosis or cancer.[1] Nevertheless, biological organisms have some defence mechanisms to fight against ROS. One of them is catalase that protects biological systems against oxidative damage caused by peroxides. These antioxidant enzymes are responsible for the decomposition of hydrogen peroxide into molecular oxygen and water.

In our group, Mn(III) dinuclear compounds, mimics of the catalase active site, were synthesized with the general formula $[\{Mn(L)(NN)\}_2(\mu\text{-}O)(\mu\text{-}n\text{-}RC_6H_4COO)_2](X)_2$, being NN = phen, bpy; *n* = *2-, 4-,* R = CH_3, Br, L = H_2O, ClO_4^-, NO_3^- or EtOH and X = ClO_4^-, NO_3^-. The catalytic activity is determined measuring the produced O_2 during the reaction.

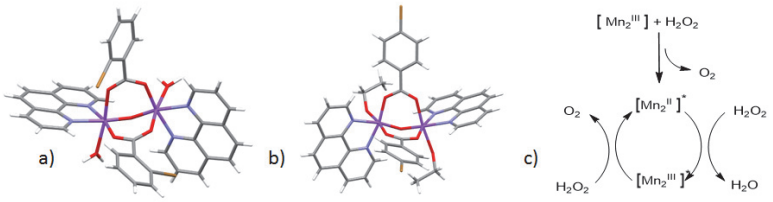

Figure 1. Scheme of dinuclears Mn(III) complexes: a) $[\{Mn(H_2O)(phen)\}_2(\mu\text{-}O)(\mu\text{-}2\text{-}BrC_6H_4COO)_2]^{2-}$ b) $[\{Mn(EtOH)(phen)\}_2(\mu\text{-}O)(\mu\text{-}4\text{-}BrC_6H_4COO)_2]^{2-}$ and c) scheme of the catalytic cycle of the disproportion reaction of the H_2O_2 using a Mn(III) compound.

Unfortunately, most of these compounds are not soluble in water or lose activity in aqueous media. With the aim to design efficient models stables in aqueous media and use them as drugs, we introduced them into mesoporous silica MCM-41. This silica can be functionalized to try to improve its activity as catalyst and also mimic the protein confinement.

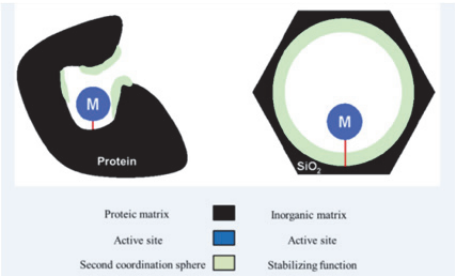

Figure 2. Comparison between protein (left) and silica pore (right). [2]

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Single-site Functional Mimics of the Oxygen Evolving Complex: Is One Site Really Enough?

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The developing of efficient and robust water oxidation catalysts (WOC) is presented as a fundamental step[1] in order to build a commercially viable photoelectrochemical cell in which energy contained in sunlight is converted into promising fuels like H_2 or MeOH. The natural ocurring WOC is called Oxygen Evolving Complex (OEC) and consists in a Mn_4O_5Ca cluster arranged in a cubane-like structure in which three manganese and one calcium atoms are linked by oxo bridge ligands. Several dinuclear and polynuclear metallic funtional mimics of OEC have been synthesized, nonetheless the recent discovery[2] of single-site Ru complexes working as WOCs has awakened a strong interest in this kind of catalysts. Thus, different electrochemical, spectrocopical (particularly rRAMAN spectroscopy) and computational techniques have been employed[3] in order to achieve a deeper description of the processes occurring at the molecular level during catalytic water oxidation mediated by the prototypical single-site complex $[Ru^{II}(trpy)(bpy)(H_2O)]^{2+}$ (**1**$^{2+}$) and a structurally related fluorinated compound. Our studies evidenced the evolution of **1**$^{2+}$ to the new rugged dinuclear Ru catalyst $[(trpy)(bpy)Ru^{IV}(\mu\text{-}O)Ru^{IV}(trpy)(O)(H_2O)]^{4+}$ (**1-dn**$^{4+}$) and other oxo-bridge complexes when the reaction proceeds.

1-dn^{4+} exhibits an unprecedented molecular structure in the field of homogenous water oxidation catalysis and was synthesized independently. The ruggedness of **1-dn**$^{4+}$ is due to the stability conferred by the trans di-oxo [Ru-O-Ru=O] scaffold indicating that this moiety can be key in the design of new long-term stable WOCs.

The mononuclear to dinuclear transformation here presented expands the current accepted mechanistic proposal[4] envisaged by Meyer et. al. showing numerous pathways that have remained unnoticed until now.

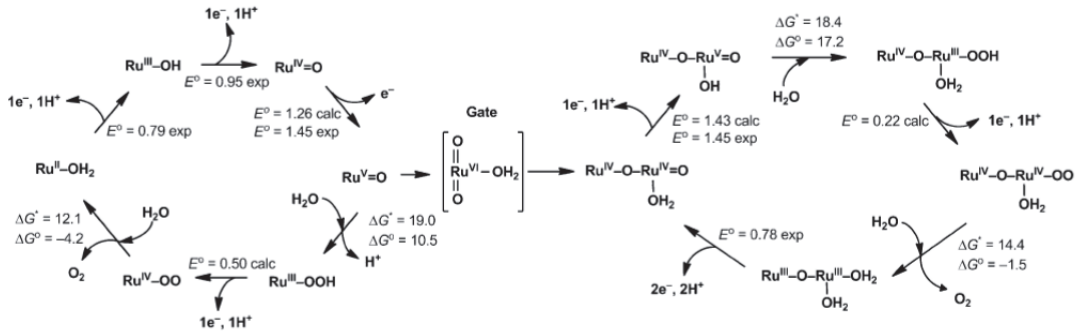

Figure. Proposed molecular scenario underlying in the catalytic water oxidation mediated by the single-site catalyst $[Ru^{II}(trpy)(bpy)(H_2O)]^{2+}$.

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Rational design of intracellular Cu(I) chelators for treating copper overload in Wilson's disease

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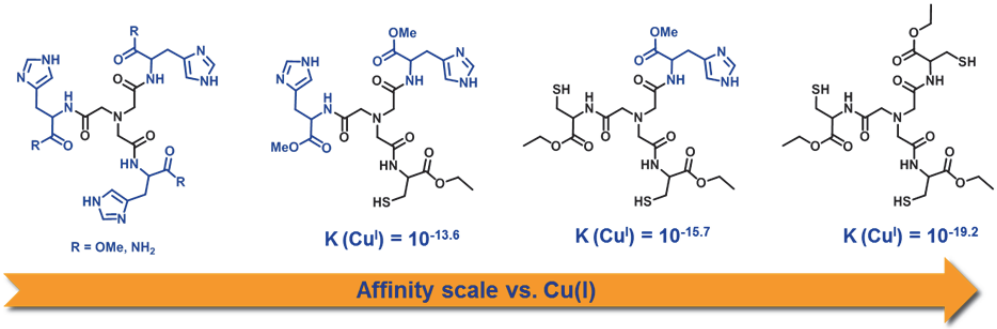
Metal overload plays an important role in several diseases or intoxications, like in Wilson's disease, a major genetic disorder of copper metabolism in humans. Indeed free Cu can promote Fenton-like reactions and be very toxic even at low concentration. Therefore, intracellular Cu concentration needs to be rigorously controlled so that it is only provided to the essential enzymes but does not accumulate to toxic levels.[1,2]

In Wilson's disease, impairment of the copper transport in hepatocytes, results in cytosolic Cu accumulation with associated cellular injury that is lethal if not diagnosed.[3] The current treatments are poorly selective for copper and induce major side effects. To design more efficient and selective treatment we design intracellular copper chelators targeted at the liver, the main organ of copper regulation. Excess intracellular copper is expected to be Cu(I), therefore we are designing ligands chelating this reduced copper oxidation state.[4]

In this communication, we will present the design of tripodal Cu(I) chelating agents inspired from proteins involved in Cu homeostasis which are reliable source of inspiration. They are based on a nitrilotriacetic acid scaffold with three coordinating amino acid such as cysteine (Cys) derivatives, which demonstrated very high affinity for the soft Cu(I) cation, with the formation of trigonal CuS_3 structure.[5] These thiolate-based ligands share many similarities with cysteine-rich metallothioneins (MTs) and particularly reproduce the high stabilities of Cu(I)-MTs complexes with dissociation constants of 10^{-19}.[6,7]



Moreover, it has also been recently demonstrated that histidine (His) residues from copper transporters Ctr1 have significant affinity for copper in both Cu(I) and Cu(II) oxidation state.[8] Hence, these tripodal architectures are currently functionalized with the latter to evaluate their impact on essential features, such as the affinity of the molecule for copper or the type of complexes which may be formed. Such fundamental information about copper chelation will afford crucial clues to design efficient drugs for Wilson's disease.

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Fluorescent metal-binding molecules as potential anti-Alzheimer's agents

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Alzheimer's disease (AD) is the most common lethal neurodegenerative disease featuring progressive impairments in memory, cognition and behaviour. AD principally affects the middle- to old-aged individuals (approximately one in four individuals over the age of 85 is affected by AD), and the number of patients is expected to triplicate within the next 35 years, from 35.6 million nowadays, to about 115 million in 2050, owing to the growing global demographics of the elderly. Consequently, an innovative approach for the development of effective anti-AD drugs needs to be established urgently, to prevent the public health disaster that awaits us.

Hence, we are currently designing novel metal-binding drugs based on amino acids that can also detect copper ions in AD brains by fluorescence.
Accumulating evidence suggests that brain tissues in AD patients are exposed to oxidative stress during the development of the disease, and redox metals like copper are clearly involved in this process. Therefore, small molecules containing 3-5 amino acids have been prepared in our group that include histidine residues as metal-binding groups and one or two natural or non-natural fluorescent amino acid(s) or/and fluorophores(s) as metal detectors (and for FRET purposes).

In this exposition, the first results achieved with these biocompatible metal protein attenuating compounds (MPACs) will be presented.

The authors thank the Ministerio de Economía y Competitividad of Spain (Project CTQ2011-27929-C02-01) and the Generalitat de Catalunya (2014SGR85).

Cu(II) chelator to inhibit the toxicity of Amyloid-β-Cu(II) against the Alzheimer's disease

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Alzheimer's disease is the most common neurodegenerative disease, with no known cure. Brain of Alzheimer's patients exhibits intracellular neurofibrillary tangles as well as extracellular senile plaques, consisting of insoluble fibrillar aggregates of the amyloid-β peptide (Aβ). The amyloid cascade hypothesis describes this aggregation; where monomeric Aβ aggregates first into oligomers, then into fibrils. In the presence of metal ions, this aggregation is modified[1]. Coordination of Aβ with Cu(II) generally favours the formation of oligomeric species, whereas Zn(II) generally induces fibrillisation of the peptide. Aβ-Cu(II) oligomers are considered to be more toxic than Aβ-Zn(II) fibrils[2], because of their capability to produce Reactive Oxygen Species (ROS).

One therapeutic strategy in tackling Alzheimer's disease is to reduce the formation of these toxic oligomeric species, by retrieval of Cu(II) from Aβ, through the use of a Cu(II) chelator. The ideal chelator must be able to (i) effectively retrieve Cu(II) from Aβ, (ii) stop or reduce the production of ROS, (iii) inhibit the formation of toxic oligomeric Aβ-Cu(II) species. The chelator used in this study, N,N'-Bis[(5-sulfonato-2-hydroxy)benzyl]-N,N'-dimethyl-ethane-1,2-diamine (L2), has previously shown promising results[3]. However, it is important to investigate the role of Zn(II), which is also present in high concentrations in the synaptic cleft[4] and can thus interfere in the Cu(II) removal process. We have investigated the metal exchange between Aβ and L2 and the detection of ROS production by UV-vis and EPR, and followed the aggregation of the peptide by fluorescence, verified by AFM.

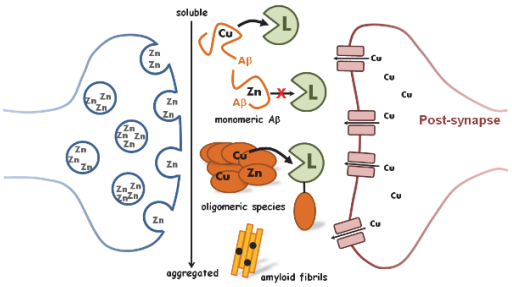

Figure1. Scheme of the amyloid cascade hypothesis.

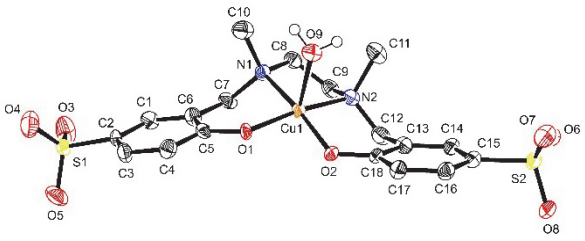

Figure2. X-ray determined structure of the L2-Cu complex.

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Metal Complexes With Biological Activity – Structural Details Of Protein Adducts

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Metal complexes have gained attention from the scientific community for their putative application in pharma as good drug candidates (Fig 1). For that, their pharmacokinetic properties need to be thoroughly determined. Our goal is to use X-ray crystallography to characterize the interaction of metal complexes with target and/or transport proteins.

Metal based carbonyl complexes have been successfully used in the last decade as prodrugs, releasing CO with impressive beneficial properties. They have been recognized as anti-apoptotic, anti-proliferative and anti-inflammatory species with good effects in different animal models of disease. The crystal structures of different experimental CORMs have been determined revealing their interaction with the model protein Hen Egg White Lysozyme (HEWL). Atomic resolution was attained and the protein-CORM adducts show how these small molecules can be transported in vivo, contributing to the elucidation of their mode of action.

Vanadium complexes are well known insulin enhancers with an increasingly role in fighting diabetes. Either in the form of VO^{2+} or coordinated to different organic ligands, also known as carriers, the effect of V has been observed in diabetic mice, effectively reducing glucose levels. The plasma protein transferrin has been proposed to be the major transporter of V in vivo, which putatively binds at the iron binding site. Using protein crystallography and Small Angle X-ray Scattering (BioSAXS), the interaction of different V complexes with transferrin and the model protein HEWL has been attended. The obtained structure show V in the +4 oxidation state, coordinated to one of the proteins side chains.

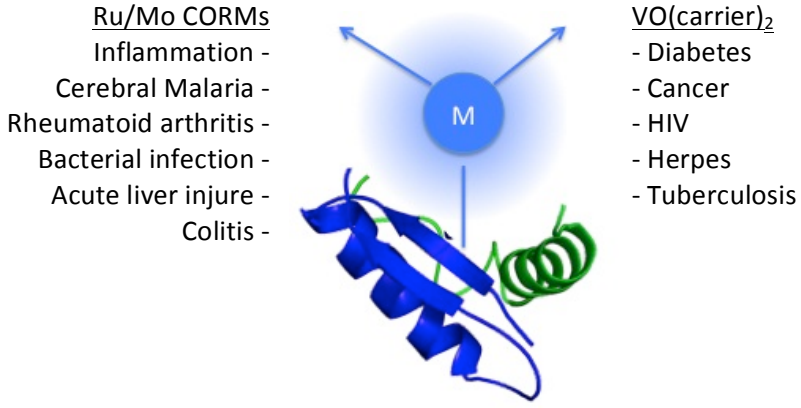

Figure 1. Accepted effects of metal containing CORMs and Vanadium complexes.

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Artificial metalloenzymes derived from bovine beta-lactoglobulin for the asymmetric transfer hydrogenation of an aryl ketone

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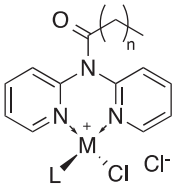
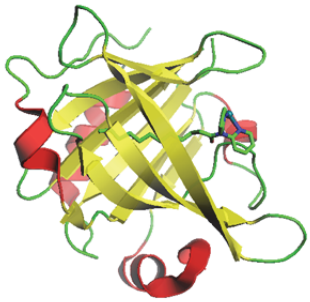
Our current research project aims at the design of artificial metalloenzymes for the asymmetric catalysis of organic reactions. These hybrid species result from the controlled assembling of synthetic metal cofactors with known catalytic activity to protein scaffolds. Their development is motivated by the complementary properties of metal catalysts and enzymes and the ability of these hybrid catalysts to act in environmentally friendly conditions. Incorporation of nonnative metal cofactors into proteins has been rationalized into three main strategies: covalent, supramolecular and dative anchoring.[1] Each of these strategies has its pros and cons but the supramolecular assembling approach has provided the most efficient catalysts in terms on both activity and selectivity so far.[2]

We focused our researches on the design of artificial transfer hydrogenases for the enantioselective reduction of ketones. To prepare artificial metalloenzymes by the supramolecular anchoring approach, we selected bovine beta-lactoglobulin (bLG) as protein host. bLG is a transport protein found in whey that displays a 8-stranded, antiparallel beta-barrel folding, forming a calyx lined with hydrophobic residues (see figure below). Most importantly, bLG binds various fatty acids with submicromolar dissociation constants and 1:1 stoichiometry.

We designed and synthesized a large series of bidentate diimine ligands derived from various fatty acids by appending a 2,2'-dipyridylamine moiety to their carboxylic acid function. Mononuclear, monocationic, half-sandwich Ru(II) and Rh(III) complexes (see structures below) were then obtained in excellent to quantitative yields from the ligands.[3]



M = Ru, L = bz or p-cym
M = Rh, L = Cp*
n = 8-16; n = 16, *trans*-Δ^{9} or *trans*-$\Delta^{9,12}$



Effective association of most of the ligands / complexes to bLG was assessed by various spectroscopic methods and confirmed by protein X-ray crystallography.[4] Eventually, the catalytic activity of the hybrids was tested on a benchmark transfer hydrogenation reaction in water using formate as hydrogen donor and the enantiomeric excess reached 32% thanks to the chiral environment brought by the protein.

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Iron(III) chelators as a tool for the development of new antibacterial agents

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Antibiotic resistance phenomena, which are notably due to the abusive use of these molecules and are the result of the selection of resistant strains, imply to find new therapeutic agents which can be active through new mechanisms. This is particularly urgent since the spreading of multi-drug resistant (MDR) bacteria has been accelerating during the last years to the point that we can presently observe the revival of infectious diseases which were previously contained. Tuberculosis is a typical example, with the appearance of half a million new cases per year linked to multi-drug resistant strains according to the world health organisation. Facing such problems, the presidents of the sciences academies of 14 countries signed, in May 2013, a common statement to attract attention on the problem and encourage the scientific and medical communities to develop studies aiming to find new tools for fighting these infections[1].

For several years now, our team has been working on the development of new antibacterial agents targeting the bacterial iron(III) acquisition system. Indeed, for their growth, bacteria need to scavenge iron(III) from their environment/host. They have thus developed a complex acquisition system through the release of siderophores, which are integrated through TON-B dependent membrane receptors and recycled (or not) after use[2]. On this basis, we are working on two types of systems. The first one consists in depriving the bacteria environment in iron(III) to limit its growth. This can be achieved through the use of iron(III) chelators. Another way is to use this acquisition pathway to favour the entry of antibiotics into the bacteria. This approach is known as the Trojan horse strategy[3]

In this presentation, we will illustrate these approaches though our latest results on 1-4 substituted piperazines molecules (Figure 1) and iron chelator-ciprofloxacine conjugates[4]. The biological properties of these systems will be discussed in relation with their iron(III) chelation properties.

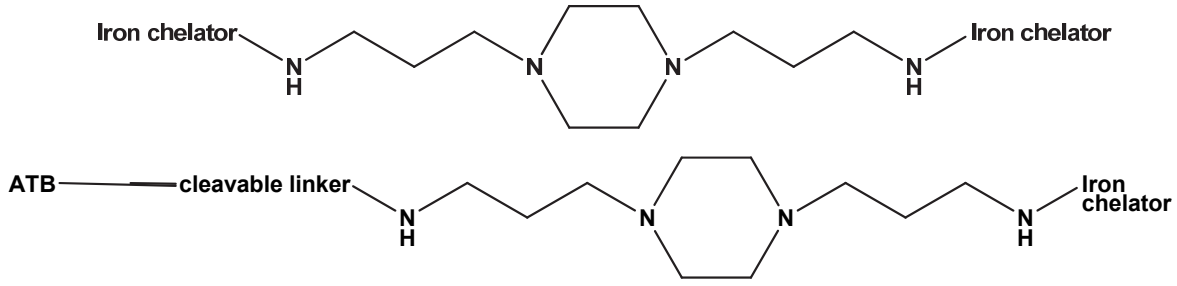

Figure 1: examples of new iron chelator-based systems targeting MDR bacteria

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Theoretical tools to study metalloprotein/ligand interactions: illustrations through studies of inhibitors of Tyrosinases

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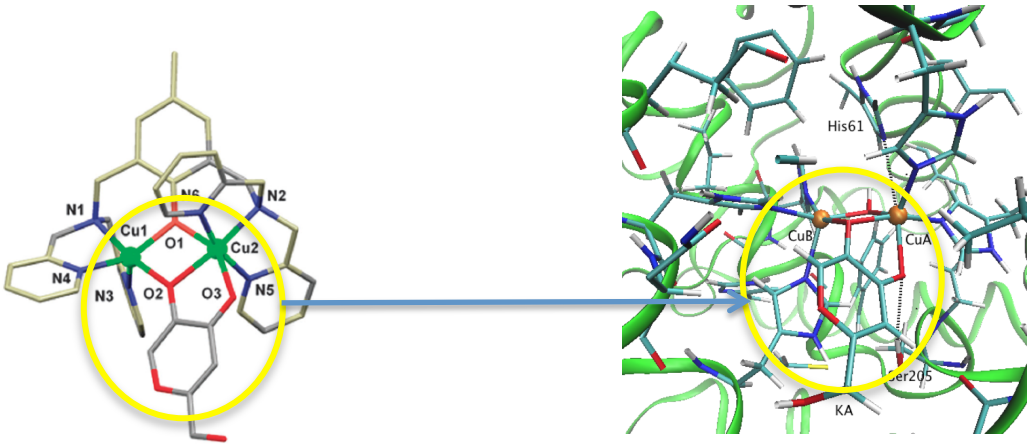
Modeling ligand/metalloprotein interactions is a challenge for theoretical chemistry. Size of systems is important, nature of interactions is complex, … Flexibility of the metalloprotein is also crucial. One solution is to use multi level approaches to take into account these different parameters. In our case, quantum studies on biomimetic complexes are done and used as starting point to explore interactions in the enzyme. Then short QM/MM dynamics simulations were performed to see the viability of the binding mode in the enzyme.



In this work, we present this approach to describe inhibitors of Tyrosinase. This enzyme contains in the active site two copper(II) atoms coupled magnetically in the *met* form. In mammals, it is involved in the biosynthesis of melanin-like pigments. Different inhibitors, tested in the enzyme and studied (X-Ray, NMR, EPR) on biomimetic complexes, will be described. Our approach allows us to compare the binding mode in the enzyme and in biomimetic complexes (1,2,3), to identify hot spot residues and for some inhibitors to rationalize their activities (4,5).

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Selective *ortho*-hydroxylation-defluorination of 2-fluorophenolates with bis(μ-oxo)dicopper(III) species

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Fluorinated organic compounds are renowned for their unique properties such as thermal stability, enhanced lipophilicity and ability to suppress metabolic detoxification, thus increasing the *in vivo* residence time.[1] Owing to these properties, fluorinated chemicals represent 20% of all pharmaceuticals and up to 30% of all agrochemicals[2] but they have increasingly become subjects of debate due to their toxicity, global warming potential, ozone depletion, environmental persistence, and bioaccumulation.[3,4] For this reason, degradation of fluorinated organic compounds or activation and transformation of C-F bonds into more reactive functional groups is of current interest. In nature, several microbial enzymes can break C-F bonds, and a wide range of fluorinated substrates including aliphatics (fluoroacetate, fluoropyruvate…) and aromatics (fluorobenzoates, fluorophenols…) can be defluorinated.

Tyrosinase is a ubiquitous dicopper enzyme that catalyzes the hydroxylation of phenols to catechols and the subsequent oxidation to quinones using dioxygen as oxidant.[5-7] Tyrosinase operates via a (η^2:η^2-peroxo)dicopper(II) species (**P**), that undergoes electrophilic attack over the arene. However, tyrosinase is incapable of hydroxylating 2-fluorophenols, which indeed are inhibitors of this enzyme.[8] Studies with model compounds have shown that **P** species are usually in equilibrium with a highly electrophilic bis(μ-oxo)dicopper(III) species (**O**), which may open the door to novel oxidative reactivity hitherto not attained by the peroxide isomer. Herein, we indeed show that **O** species $[Cu^{III}_2(\mu\text{-}O)_2(m\text{-}XYL^{MeAN})]^{2+}$ carries out an electrophilic *ortho*-hydroxylation-defluorination of 2-fluorophenolates to give the corresponding catechols (Figure 1). Isotopic labelling studies show that the incoming oxygen atom originates from the bis(μ-oxo) unit. *Ortho*-hydroxylation-defluorination occurs selectively in intramolecular competition with other *ortho*-substituents such as chlorine or bromine.[9] This reaction is also interesting from the perspective of environmental science, as it enables the transformation of the inert C-F bonds of common persistent pollutants into a more reactive C-OH unit.

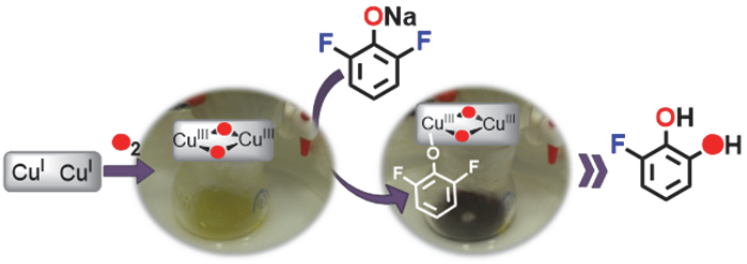

Figure 1. *Ortho*-hydroxylation-defluorination of 2-fluorophenolates carried out by a bis(μ-oxo)dicopper(III) species.

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Revisiting *Neurospora crassa* metallothionein

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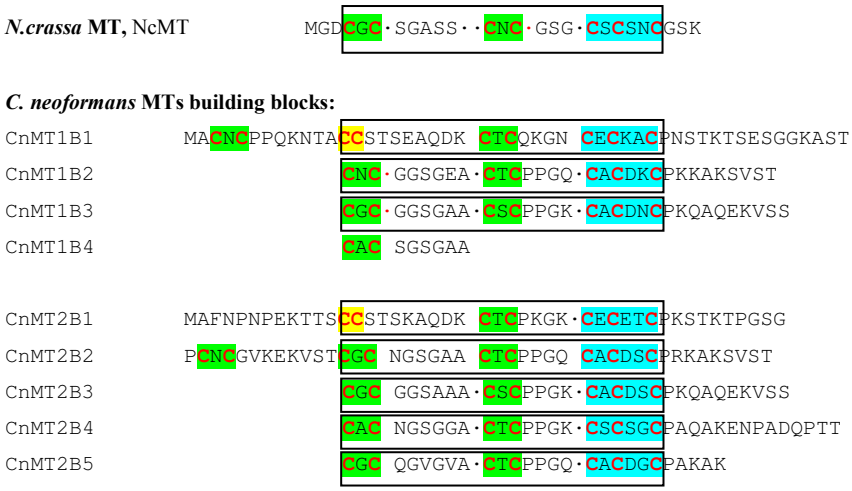
The metallothionein (MT) from the fungus *N. crassa* is a small MT made up of 25 amino acid residues, 7 of which are cysteines [1]. According to the bibliography, the seven cysteine residues are able to coordinate six Cu(I) ions[2]. This MT is only induced *in vivo* by copper, and not by any other transition metal, but *in vitro* it is also able to bind Zn(II), Cd(II), Co(II) and Ni(II)[3,4].

Because its Cys-x-Cys sequence, *N. crassa* MT (NcMT) shares a striking similarity with the ß domain of vertebrate MTs[5]. Particularly, the characteristic motif -CxCxxxxxCxC- from NcMT has been found in a variety of fungal MTs[6]. This high sequence similarity, mainly in the parallelism of the position of cysteine residues has suggested *NcMT* to be the representative of a primordial gene from which the vertebrate and fungal MTs have evolved[1].

Another fungus, *Cryptoccoccus neoformans*, has two different MT isoforms, CnMT1 and CnMT2, which are directly related with its virulence and pathogenicity, through their Cu-detoxification role[7]. For these two MTs, recently studied by our group, a higher capacity for Cu(I)-binding than other fungal MTs has been shown. The sequence of CnMT1 and CnMT2 appears to be built through evolution by the multiplication of blocks containing 7-Cys residues separated by short, non-coordinating stretches[8]. These building blocks exhibit a clear homology with the NcMT sequence.



To better understand the metal-binding behaviour of CnMT1 and CnMT2, and that of their constitutive building blocks, the metal-coordination abilities of NcMT have been revisited. For this purpose, it has been synthesized by recombinant techniques in Cu(II), Zn(II) and Cd(II) enriched media. Its Cd(II)/Zn(II), Cu(I)/Zn(II) metal replacement reactions have also been analyzed *in vitro* by spectroscopic (UV-Vis and CD) and spectrometric techniques (ESI-MS).

The financial support received from MINECO-FEDER (Projects BIO2012-39682-C02-01to S.A. and BIO2012-39682-C02-02 to M.C.) is acknowledged.

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Following the interaction of metallic compounds with biomolecules by mass spectrometry

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Due to the high number of existing metalloproteins and their involvement in several natural processes and diseases, the analysis of the interaction between proteins and metals became crucial for understanding the role of the metal ions in living organisms. Also, the increasing use of metal-based drugs for the treatment of different kinds of diseases imposes the need of developing new tools to study these interactions. In recent years, electrospray ionization mass spectrometry (ESI-MS) has emerged as an extremely valuable and powerful method to monitor the interactions between metal ions and different types of biomolecules. In spite of the relatively common use of nano-ESI sources for these purposes, this special methodology requires very specific conditions to be applied, while showing some limitations in reproducibility. The optimization of conventional ESI sources to the study of these interactions has become one of the current goals. Hence, we adapted the conditions of a standard ESI source instrument, equipped with a TOF high resolution analyzer, to study the interactions between metallic complexes (Pt(II), Ru(II), Re(I) and Tc(I)) and biomolecules (proteins and DNA)[1], as well as the interaction of several metal ions (Zn(II), Cd(II), Cu(I), Pb(II)) with Cys-rich proteins, namely metallothioneins[2]. When necessary, the MS results have been complemented with spectroscopic data (DC, UV-vis, fluorimetry) to better understand the nature of the metal-biomolecules interactions.

The financial support received from MINECO-FEDER (Project BIO2012-39682-C02-02) is acknowledged.

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Analytical strategies for bioinorganic chemical investigations

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Knowledge on the physico-chemical distribution of chemical elements in biological and/ or environmental media is a prerequisite to understand the origin, transport and fate of these elements, including their metabolism in organisms. Especially the bioavailability of elements, their chemical species or physical forms and/ or location are essential data to be determined which depend as well on the media. However, the acquisition of this information represents often an analytical challenge that requires a multi-technique approach. Among the different possible strategies, passive samplers (e.g. based on polymer hydrogel synthesized by droplet-flow millifluidic processes) and/or hyphenated techniques involving mass spectrometry (MS) (e.g. NanoLC-MS, FFF-ICPMS) for physico-chemical speciation analysis, and their possible combination with imaging techniques (e.g. TEM, NanoSIMS), have proven to be very powerful approaches.
In this presentation, we propose to address these different and complementary methods for bioinorganic chemical investigations. Application examples of elemental monitoring as well as evaluation of element migration, bioavailability, uptake, translocation, and internalisation will illustrate these multi-technique analytical strategies.

Proton-Coupled Electron Transfer on metal hydride complexes

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Proton-coupled electron transfer (PCET) is an ubiquitous type of elemental reaction, of fundamental importance, which is widespread in many biological or catalytic processes. The mechanism of PCET is mostly studied in organic compounds, where a O-H bond or a N-H bond is involved in the reaction. Less studies are done with metal complexes and very few in which a metal-hydrogen bond is directly involved. Indeed hydrogen atom transfer, when the proton and the electron acceptors are the same compound, have been studied but, to the best of our knowledge, no experimental work has been done involving a metal hydrogen bond, a proton acceptor (or donor) and an electron acceptor (or donor).

Due to the particular redox behaviors of metals, what we know about PCET in organic compounds might not be directly and simply transposed to metal hydride complexes. So, despite the challenging component caused by intrinsic instability and side-reactions of this kind of compounds, getting insights and deeper understanding of PCET with metal hydrides complexes is of great interest.

On the one hand, using a model complex of the active site of [FeFe]-hydrogenase bearing a pendant base, we studied the mechanism of the activation of proton reduction through the formation of a metal-hydride bond, as a function of the force of the acid. We were able to get some insights about the intrinsic properties of the catalyst.
On the other hand, using a well-known, well-described and sufficiently stable complex, as well as a combination of different oxidants and bases, we studied the mechanism of the oxidation of a metal hydride bond. We were able to get rid of most side-components and side-reactions in order to directly measure the kinetics of the PCET as a function of the different driving forces and, thus, to get direct information about the mechanism..

Structural insights into oxygen inactivation of *Desulfovibrio vulgaris* Hildenborough [NiFeSe] hydrogenase

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The genome of the sulfate reducing bacterium *Desulfovibrio vulgaris* Hildenborough encodes seven hydrogenases, four of which are periplasmic uptake hydrogenases, including two [NiFe], one [FeFe] and one [NiFeSe] hydrogenase. When selenium is available the main hydrogenase expressed in *D. vulgaris* is the [NiFeSe] enzyme, which has a high catalytic activity, particularly of H_2 production (1). The heterodimeric [NiFeSe] hydrogenase is an unusual lipoprotein, associated with the membrane by a lipidic group bound to the N-terminus of the large subunit, even though this protein lacks a typical lipoprotein signal peptide (2). It is the first lipoprotein described to be translocated by the Tat instead of the Sec pathway (2). Structurally, it is very similar to the [NiFe] hydrogenases, the major differences being that it has a selenocysteine as one of the ligands to the Ni in the active-site, and that the small subunit has a medial $[4Fe4S]^{2+/+}$ rather than a $[3Fe4S]^{+/0}$ cluster. The [NiFeSe] hydrogenase has some remarkable catalytic properties: it has a very high activity of H_2-production and does not form inactive Ni(III) species (Ni-A/Ni-B) upon contact with O_2, becoming active immediately upon reduction (3). This enzyme also shows promising properties in electrocatalytic studies (4).

The three-dimensional structure of the [NiFeSe] hydrogenase from *D. vulgaris* Hildenborough has been determined in its oxidised "as-isolated" form (5). Remarkably, this is the first structure of an oxidised hydrogenase of the [NiFe] family that does not contain a bridging oxide ligand at the active site. More recently, crystal structures of the reduced and reoxidized states were also obtained (6). This structural data together with other studies into the oxygen inactivation of this interesting hydrogenase, indicate that different hydrogenases of the [NiFe] family present different inactivation profiles, providing important insights for the design and improvement of biocatalysts suitable for H_2 production.

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Role of enzyme orientation at electrochemical interfaces for biotechnological processes

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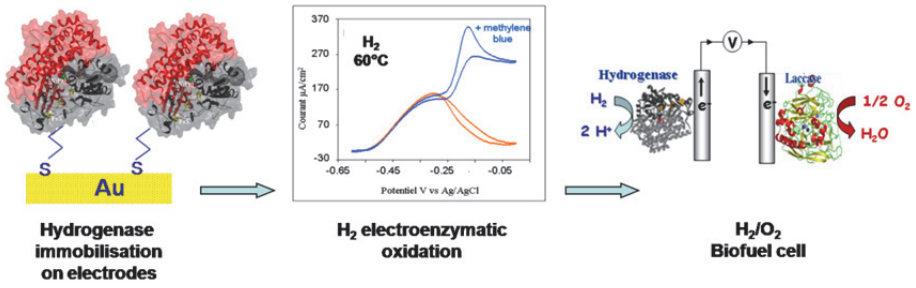
The [NiFe] membrane-bound hydrogenase from the hyperthermophilic bacterium *Aquifex aeolicus* is a good candidate for a new generation of H_2/O_2 biofuel cells, since it exhibits O_2, CO and temperature tolerances combined with high turnover for H_2 oxidation[1]. Coupled to a thermostable bilurubin oxidase, a H_2/O_2 biofuel cell was recently designed able to deliver power densities more than 1 mA/cm^2 in a wide range of temperature[2]. From this device, it was demonstrated that one of the limitation originates from the low electrical connection of the enzyme in the 3D-carbon nanofiber network necessary for the catalytic current enhancement[3]. Fundamental understanding, then control, of the enzyme interaction within these matrixes would help in the increase of both the stability of the biohybrid and the catalytic currents. We will especially report in this work the elucidation of enzyme orientation at electrodes using an original method coupling electrochemistry, AFM, PM-IRRAS and molecular dynamics[4,5].



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Maturation and mechanism of the Carbon Monoxide Dehydrogenase (CODH) from *Desulfovibrio vulgaris*

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Some microorganisms use Carbon Monoxide Dehydrogenase (CODH) to catalyse the reversible oxidation of CO into CO_2 according to the reaction: $CO_2 + 2H^+ + 2e^- \leftrightarrow CO + H_2O$. The Nickel-Iron (Ni-Fe) CODHs are found in some anaerobic organisms such as *Carboxydothermus hydrogenoformans* or the photosynthetic bacterium *Rhodospirullum rubrum* which use CO as sole energy source. This is probably due to the involvement of CODHs in the generation of a proton motive force in association with hydrogenases. In some cases, CODHs are involved in CO_2 fixation, in association with an Acetyl-CoA synthase. The precise role of the CODH from our model organism, *Desulfovibrio vulgaris* (Dv), is still unclear.

The biosynthesis of the active site of Ni-Fe CODHs requires at least one accessory protein in *R. rubrum* and *C. hydrogenoformans*. In particular, it is believed that the CooC protein favors the insertion of Ni in the active site (1,2). We have developed a system allowing the production of the Dv CODH in the presence or absence of its accessory protein CooC to determine the biochemical, kinetic and structural properties of biosynthetic intermediates of the Ni-Fe active site.

Furthermore, there is some controversy in literature about the active site's structure (3-6). On the basis of crystallographic investigations, several research groups have determined different active site structures, some of which would not be involved in catalytic cycle. Protein film voltammetry studies in our laboratory have revealed unprecedented kinetic properties of the Dv enzyme that shed new light on the structural heterogeneity observed by crystallography and may question the catalytic mechanisms proposed on the basis of these structures.

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Bioinspired Magnetic Materials

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Biogenic magnetite is a fascinating example of how nature can generate complex nanostructures for specific purposes. This biogenic magnetite exhibits fascinating magnetic properties very suitable for biotechnological applications, however they are recalcitrant to large-scale production.

Many research efforts have been devoted to the development of strategies for structurally mimicking these biogenic magnetic assemblies as a way to create bioinspired magnetic materials. Here I report a different approach: attempt to mimic the functionality of magnetic bacteria rather than their structure.

We have decorated non-magnetic probiotic bacteria with several thousands of magnetic nanoparticles on their external surfaces that induce the probiotic bacteria to behave as magnets at room temperature. The so called artificial magnetic bacteria[1] become directionally arranged following the magnetic field lines when submitted to an external magnetic field, as it occurs with native magnetic bacteria. Therefore artificial magnetic bacteria are capable of mimicking the functionality of natural magnetic bacteria despite their very different structure.

The methodology to make our artificial magnetic bacteria is based on probiotics, which are bacteria that confer health benefits for men, and involves mild chemical conditions and may be easily adapted to large-scale production.[2]

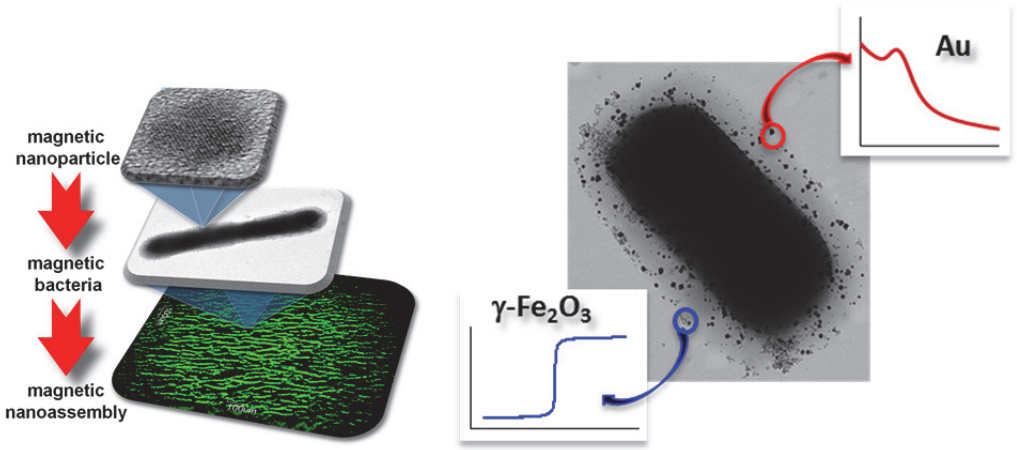

Figure 1: From single magnetic nanoparticles to artificial magnetic bacteria (left). TEM image of the first living magneto-optical material.

Likewise, "two-in-one" magneto-optical bacteria have been produced for the first time. We took advantage of two features of bacteria to synthesize this novel and bifunctional nanostructure: their metal-reducing properties, to produce gold nanoparticles, and their capacity to incorporate maghemite nanoparticles at their external surface.

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Interactions and toxicology of silver nanoparticles in aquatic ecosystems

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In the past 20 years, recent advances in nanotechnology have resulted in the generation of various nanostructured materials, which have unique physical and chemical characteristics. The metal oxide nanoparticles (MONPs) production is increasing rapidly and products containing MONPs have been introduced in our daily lives. Conservative market estimates for metal oxide nanoparticles in 2012 are 270 041 tons, rising to 1 663 168 tons by 2020[1]. However, the environmental release of NPs, the accumulation of these new pollutants in hydrographic basins and their effect on aquatic organisms are currently unclear. Understanding the toxic effects of these emerging xenobiotics is therefore crucial in order to anticipate the consequences of the potential degradation of ecosystems and their potential impact on health[2,3].

Silver nanoparticles (AgNPs) are the most extensively used nanoparticles in consumer products. More than 400 silver nanomaterials have been incorporated in day life products principally because of their antibacterial properties (textiles, cosmetics, air sanitizers or medical instruments) [4]. During manufacture, use or end of life of these products, a significant amount of NPs can be released into the environment; consequently, knowing the environmental transformations of AgNPs and their impacts on the organisms is important to anticipate their potential toxicity. In order to use "safer by design" NPs, this project propose a multiapproach annalysis regarding the potential modifications of the AgNPs in the environment by physico-chemical analyses (DLS, ICP, NMR, TEM…), and biological and toxicological analyses (survival studies, histology, gene expression, protein interactions…).

This work is part of the SERENADE (Safe(r) Ecodesign Research and Education applied to Nanomaterial Development) project whose goal is to create a dynamic network of academic research laboratories and industry to design tomorrow's nanomaterials that are safer for both humans and the environment.

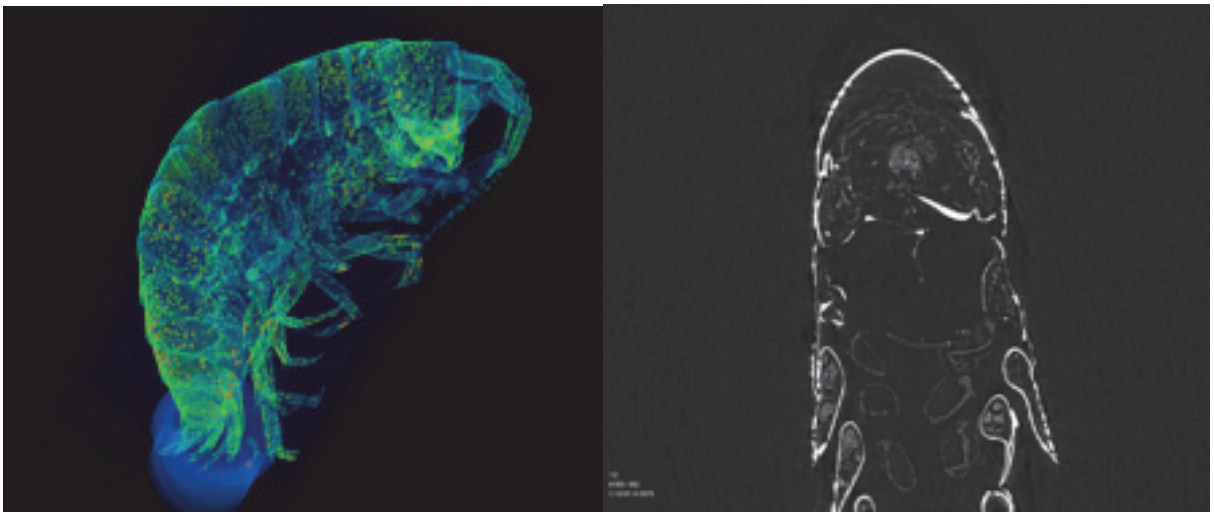

Figure 1. Gammarus fossarum after 96 h in 500 ug/L of SiO2 NPs observed using X-ray micro-tomography

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Gold(I) thiolates with optimum hydrophilic/lipophilic balance as anticancer drugs

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Cisplatin is one of the most employed metallic complexes for cancer treatment. However, its effectiveness is hindered by toxic side effects and the occurrence of tumour resistance. Since its discovery, the interest in the research of new metallic coordination and organometallic complexes with anticancer applications has significantly increased. Specially for those compounds that would show a great efficacy, but also a good selectivity for cancerous cells and reduced side effects. Among the new non-platinum as potential anticancer drugs, gold derivatives have gained increasing attention due to their generally strong tumour cell growth inhibiting effects and the observation that many of the compounds inhibit the enzyme thioredoxin reductase (TrxR) [1].

The side effects of most of the new anticancerous complexes could be given due to their lipophilicity. [2] For this reason, in the design of new metallic drugs, a balance between hydrophilicity and lipophilicity is required to be water soluble and at the same time be able to pass through the phospholipid cell membrane [3]. Accordingly, water solubility of the drugs could provide such balanced relationship. The use of water-soluble phosphanes can lead to the synthesis of water soluble or partially soluble complexes.

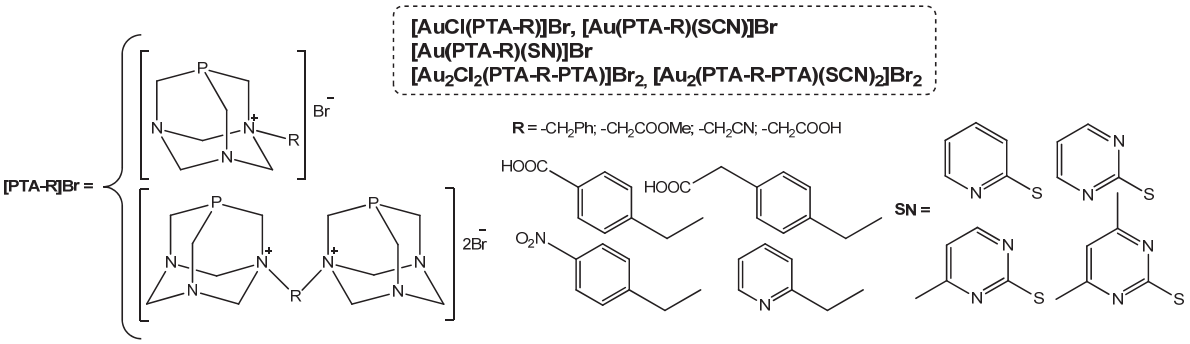

Fig. 1: Scheme of synthetized complexes

Within this frame, here we include the synthesis of new water-soluble thiolate gold(I) derivatives with different phosphanes derived from PTA (1,3,5-triaza-7-phosphaadamantane) (figure 1). Different experiments have been done to test the stability of these complexes and their lipophilicity/hydrophilicity balance. And some of them have been screened for their antitumor activity against human colon cancer cell lines, as well as their apoptotic activity evaluation.

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Cyclen-based ligands for the complexation of Pb(II) and Bi(III) for alpha radioimmunotherapy

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Alpha-radioimmunotherapy (α-RIT) represents an emerging therapeutic modality for tumor treatment which is currently under active investigation for curative applications (Figure 1).[1] In contrast with β-emitting radionuclides and their typical path length around 1 cm, the energy emissions of α-particle decays are directly deposited over a very short distance (40–100 μm), resulting in high linear energy transfer. The shorter path may also have the advantage of limiting the toxicity for normal tissues adjacent to the tumor.

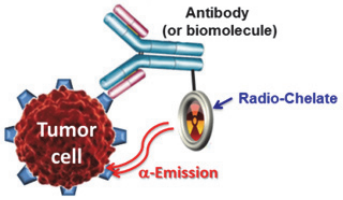

Figure 1. Principle of alpha-radioimmunotherapy.

^{212}Bi ($t_{1/2}$ = 60.6 min) and ^{213}Bi ($t_{1/2}$ = 45.6 h) are among the most studied radionuclides for α-RIT applications. However, ^{212}Pb ($t_{1/2}$ = 10.6 h) constitutes a promising α-particle emitting source. It is the longer-lived parent nuclide of ^{212}Bi, and serves as an *in situ* generator of ^{212}Bi.[2]

Radio-metals cannot be used in their free ionic form due to their high toxicity. The toxicity can be hidden by sequestering the ions in macrocyclic cavities. Our aim is to synthesize ligands for α-RIT that can trap hot bismuth and also validate the concept of *in situ* generator. This requires to design chelates for the radioactive isotopes of bismuth (^{213}Bi and ^{212}Bi) and for ^{212}Pb, the isotopic parent of ^{212}Bi.
Tetraazacycloalkanes, such as cyclen and cyclam derivatives, proved to be attractive ligands for RIT applications due to their high affinity for ionic metals. However, none of the already described ligands is able to complex both ions while filling the entire specifications for biomedical purposes. Therefore, cyclen-based ligands bearing pyridine-2-carboxylic acid pendant arms were synthesized and used as chelates for lead (II) and/or bismuth (III) (Figure 2). The "cold" complexes, especially those of bismuth (III), form quickly and are highly stable and inert, as proved by several thermodynamic and kinetic studies in solution.[3]

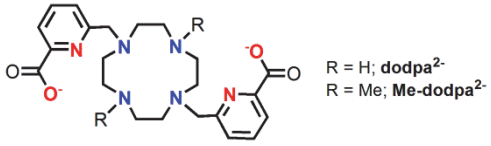

Figure 2. Cyclen-based ligands for Bi(III)-Pb(II) complexation.

An efficient ^{213}Bi labelling of the ligands was thus performed, resulting in a stable radio-complex in human plasma. The next step will be to take advantage of the ligand design to introduce bioconjugation functions by replacing one of the *N*-methyl groups to obtain a radiopharmaceutic.

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Near-infrared emitting Zn^{II}-Ln^{III} "encapsulated sandwich" metallacrowns

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A number of modern advanced technologies rely on unique spectroscopic properties of lanthanide(III) ions. In particular, their ability to generate characteristic sharp emission bands in the near-infrared (NIR) range has a growing interest in view of numerous exciting applications in biosciences, telecommunications and solar energy conversion.[1] The main fundamental challenges for the design of NIR-emitting lanthanide(III) compounds are: (1) efficient sensitization through appropriate chromophores, and (2) good protection from non-radiative desactivations through O-H, N-H and C-H vibrations.

Here, we present a novel strategy to overcome low quantum yields of NIR-emitting lanthanide(III) compounds. The approach is taking advantage of the unique structure of the"encapsulated sandwich" metallacrown (MC) complexes of the form $Ln^{III}[12\text{-}MC_{Zn(II),quinHA}\text{-}4]_2[24\text{-}MC_{Zn(II),quinHA}\text{-}8]$ (Ln^{III} = Y, Nd, Eu, Gd, Tb, Dy, Er, Yb) in which the MC framework is obtained by the self-assembly of Zn^{II} ions and tetradentate chromophoric ligands, quinaldichydroxamic acid (quinHA). Such structure allows locating lanthanide ions at a predetermined and relatively well shielded position to achieve high quantum yields and long luminescence lifetimes. Quantum yields of Nd^{III} and Er^{III} MCs in the solid state and in deuterated solvents are the highest values reported today among NIR-emitting lanthanide complexes containing C-H bonds.[2]

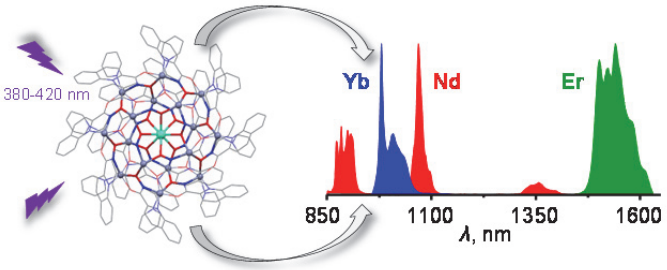

Figure 1. Crystal structure of $Dy^{III}[12\text{-}MC_{Zn(II),quinHA}\text{-}4]_2[24\text{-}MC_{Zn(II),quinHA}\text{-}8]$ and emission spectra of Yb^{III}, Nd^{III} and Er^{III} analogues in solid state under ligand excitation.

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Engineering proteins to bind heavy metals efficiently and selectively

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Using proteins to sequester toxic heavy metals can be a valid alternative to the existing decontamination approaches, because of the low production costs and the low environmental impact of these biomolecules.[1,2] In this frame, our aim is to optimise a protein scaffold for selective encapsulation of uranyl by combining recombinant protein production, site directed mutagenesis and/or post traductional modification, modelization by molecular dynamics and thermodynamic analysis of the metal-protein interaction.[3]

Using the regulatory calcium-binding protein Calmodulin (CaM) from A. thaliana as a structured template, we have designed various mutants by site-directed mutagenesis. We have analysed thermodynamics of uranyl ion binding to both sites I and II of CaM N-terminal domain and we have identified structural factors governing this interaction. Selectivity for uranyl ion has been tested by studying reactions of the investigated peptides with Ca^{2+}.[4] The allosteric effect depending on metal binding has been investigated by comparing thermodynamic parameters obtained for mutants having both sites I and II able to chelate metal ions with those of mutants consisting of just one active site. Our spectrofluorimetric and ITC analyses have shown that, by coupling the allosteric effect and the phosphorylation of a threonine in site I, CaM affinity for uranyl increases by a factor 100 with K_d values in the picomolar range.

This work improves the understanding of the molecular factors and mechanisms governing uranyl binding to proteins and hence its toxicity and speciation in cells. On top of that, this line of research will help in developing new tools for heavy metals bioremediation and biodetection purposes.

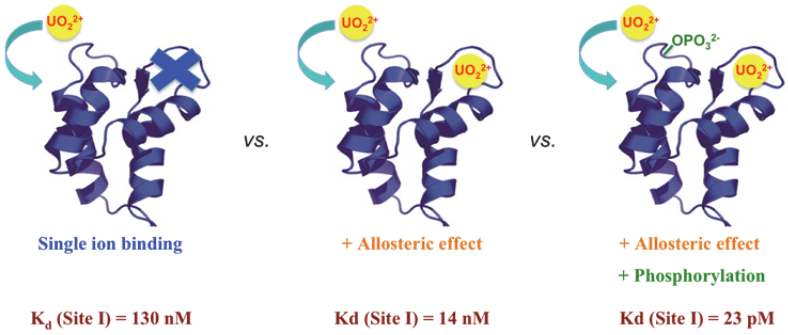

Figure 1. The effect of allostery and phosphorylation on Calmodulin N-terminal domain affinity (K_d) for uranyl ion.

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An ABC transporter with two periplamic binding proteins involved in iron acquisition in *Pseudomonas aeruginosa*

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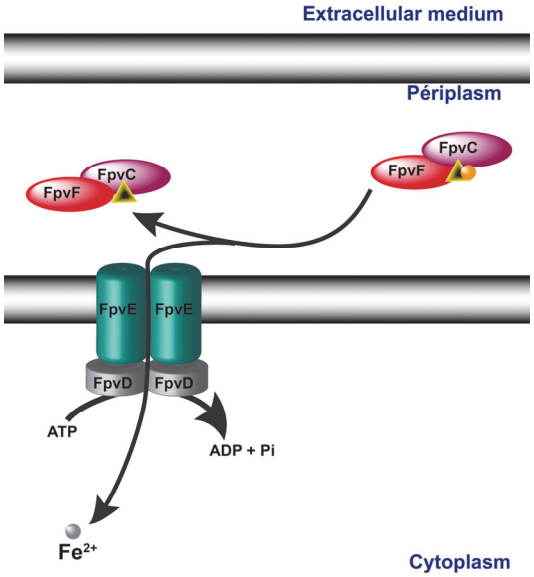
Pyoverdine (Pvd) is the main siderophore secreted by *Pseudomonas aeruginosa* PAO1 to obtain access to iron[1]. After extra-cellular iron chelation, pyoverdine-Fe uptake into the bacteria involves a specific outer-membrane transporter, FpvA[2]. Iron is then released in the periplasm by a mechanism involving no siderophore modification but probably iron reduction[3,4]. The proteins involved in this dissociation step are currently unknown. The pyoverdine locus contains the *fpvCDEF* operon, which contains four genes. These genes encode an ABC transporter of unknown function with the distinguishing characteristic of encompassing two periplasmic binding proteins, FpvC and FpvF, associated with the ATPase, FpvD, and the permease, FpvE. Deletion of these four genes partially inhibited cytoplasmic uptake of ^{55}Fe in the presence of pyoverdine and markedly slowed down the in vivo kinetics of iron release from the siderophore. This transporter is therefore involved in iron acquisition by pyoverdine in *P. aeruginosa*. Sequence alignments clearly showed that FpvC and FpvF belong to two different subgroups of periplasmic binding proteins. FpvC appears to be a metal-binding protein, whereas FpvF has homology with ferrisiderophore binding proteins. In vivo cross-linking assays and incubation of purified FpvC and FpvF proteins showed formation of complexes between both proteins. These complexes were able to bind in vitro Pvd-Fe or apo-Pvd. This is the first example of an ABC transporter involved in iron acquisition via siderophores, with two periplasmic binding proteins interacting with the ferrisiderophore.



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New artificial metalloenzyme containing an iron coordinating active site

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One of the major current challenges is to develop sustainable approaches to chemical synthesis. The power of chemical and biological synthesis can be merged by integrating non-natural synthetic chemistry into bio-synthetic pathways. By developing novel artificial metalloenzymes and incorporating them into cells, sustainable synthesis of molecules with novel structural features that are difficult to achieve in any other way could be achieved. This will result in novel building blocks that can be used as pharmaceutical intermediates, or complex molecules with novel or enhanced biological activity, such as antibiotics.

A new approach to artificial metalloenzymes was reported recently by our group ,[1] which comprises of a new active site in the hydrophobic pocket of the dimeric protein LmrR. This active site is composed of covalently anchored phenanthroline or bipyridine ligands at a specific position (figure 1).

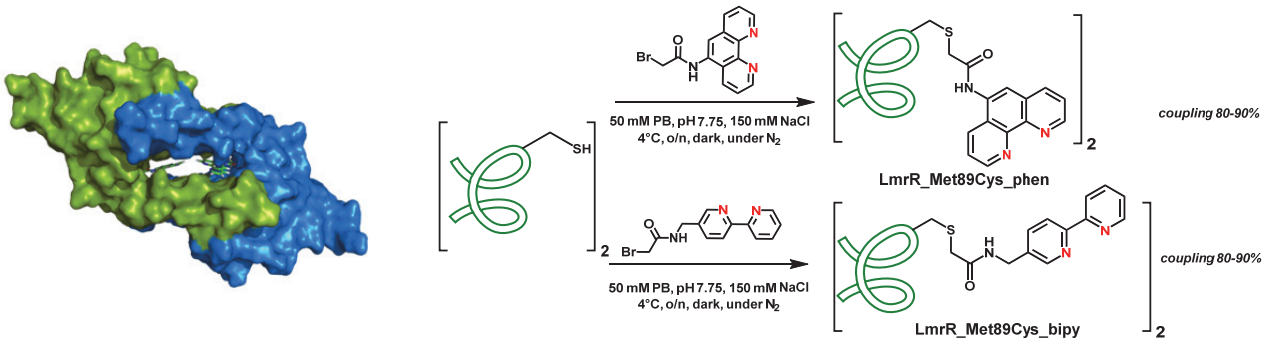

Figure 1. Left, Space filling structural representation of LmrR with phenanthroline ligands anchored ; right, anchoring of phenanthroline and bipyridine ligands to LmrR.

In the context of oxidation catalysis that mimicks peroxidases, we report here a study of the iron complexes formed with this new artificial protein.

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An artificial metalloenzyme made by covalent grafting of an iron(II) complex in β-lactoglobulin

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Various synthetic non heme monooxygenase systems are reported for more than 20 years. These systems have been shown to be precursors of intermediates such as high valent Fe(IV)-oxo, Fe(III)-peroxo and Fe(III)-hydroperoxo (figure 1).[1] These intermediates allow numerous organic molecules oxidation. Using iron(II) complexes with amine/pyridine ligands, we were able to prepare Fe(IV)-oxo using chemical oxidants (mCPBA, PhIO), or Fe(III)-hydroperoxo (by addition of H_2O_2). These intermediates are useful respectively for olefin epoxidation, and aromatic hydroxylation

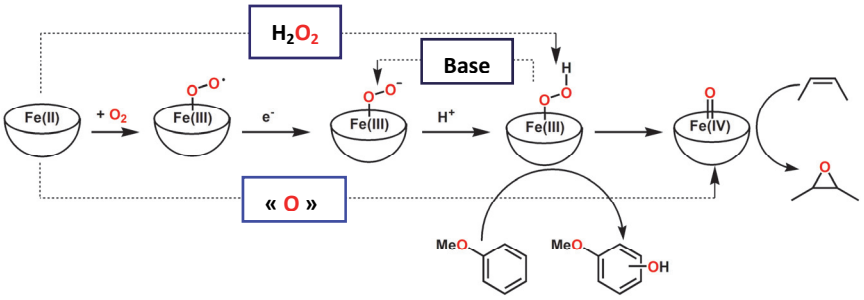

Figure 1: Cytochromes P450 oxygen activation simplify mechanism. Non hemic Iron(II) complex evolution after addition of various oxidative agents (dotted arrows).

(figure 1).[2]

One of the main problems related with this type of catalysts is that they are poorly selective (stereoselectivity, chemoselectivity and regioselectivity) unlike enzymatic systems.[3] In order to tackle this issue, we have modified one of our complexes in order to covalently attach it to the β-lactoglobulin protein (figure 2), thereby supplying a second coordination sphere to the catalyst as it is the case in enzymatic pocket.[4] Characterization and reactivity of the biohybrid system will be detailed in this presentation.

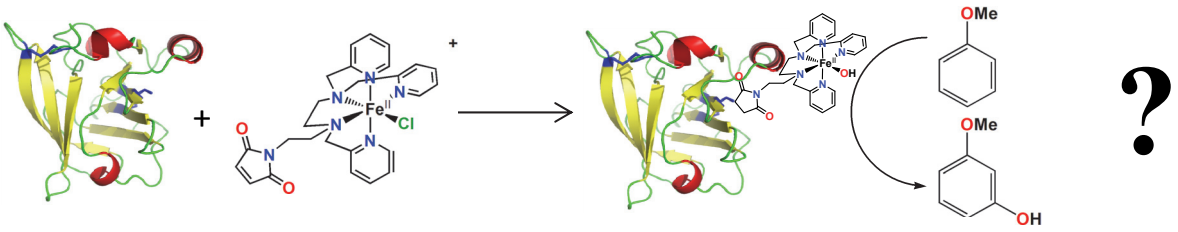

Figure 2: Principle drawing complex and β-lactoglobulin grafting and expected reactivity.

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Binuclear hydrolases: desperate search for a nucleophile

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Many biological hydrolytic reactions are performed by binuclear metal hydrolases with a wide variety of metal ions: Mn, Fe, Ni, Zn. The most widely studied of these enzymes are the Purple Acid Phosphatases which hydrolysize mono- or diphosphate esters.[1]Depending on the different isozymes, their active sites consist of a pair $Fe^{III}M^{II}$ with M^{II} = Fe^{II}, Mn^{II}, Zn^{II}.[2] In spite of a huge number of experimental and theoretical studies of the enzymes and model complexes, the nature of the active nucleophile involved in these hydrolytic reactions is still a matter of intense debate.

To address this question, we have performed spectroscopic and reactivity experiments using the following binuclear Fe^{III} Fe^{II} complex (Figure 1).[3]

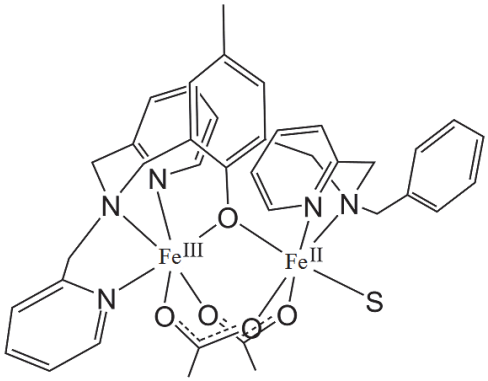

Figure 1: Structure of $Fe^{III}Fe^{II}$ complex (S = solvent)

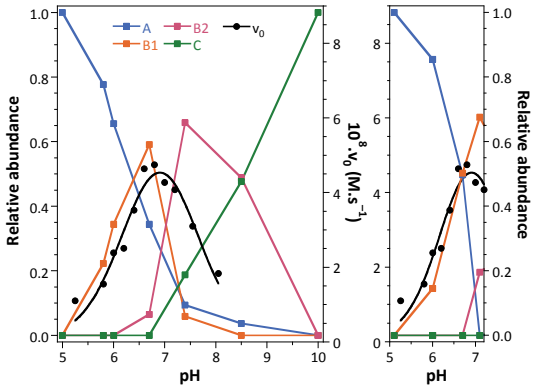

Figure 2: pH dependent speciation and activity

The very rich spectroscopic properties of these Fe^{III} Fe^{II} complexes enable us to use for the first time ^{1}H-NMR and Mössbauer spectroscopies to determine the species present in solution over the pH range 5 to 10. Comparison of the speciation curves with the reactivity profile (Figure 2) reveals a major role of the bridging hydroxide in the hydrolysis process.

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Diiron complexes and peroxo intermediates : influence of the second coordination sphere and oxidation catalysis

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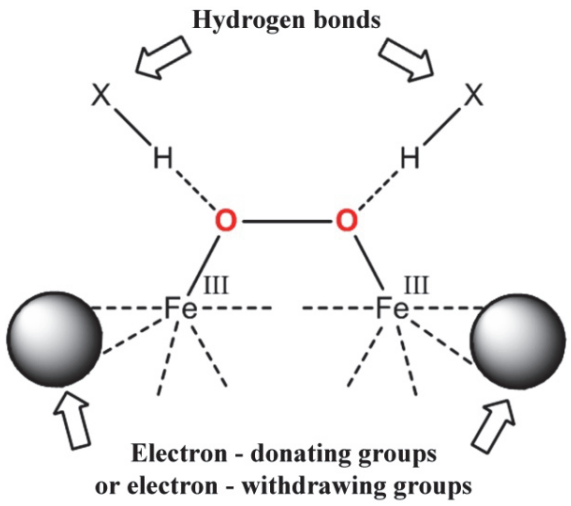
Iron monooxygenases are enzymes capable of activating dioxygen from the air in order to catalyze the selective oxidation of alkanes into alcohols.[1-3] Soluble methane monooxygenase for instance, is capable of oxidizing methane into methanol, thanks to its dinuclear iron active site.[2] Hence, numerous diiron complexes have been synthesized in order to mimic this catalytic activity, with the aim to develop new eco-friendly industrial processes.[4] During its catalytic oxidation cycle, a key diiron-μ-1,2-peroxo intermediate is formed, which is the focal point of our project.

Our project aims at investigating the influence of the second coordination sphere of diiron complexes on the stability/reactivity of peroxo intermediates. More precisely, it aims at studying how the microenvironment of the metal center may influence the formation and the stability/reactivity of peroxo species, which play an important role for the reductive activation of dioxygen. Only a few studies have been conducted regarding the influence of electron donating or electron withdrawing groups in the second coordination sphere of the metal ion on the stability and reactivity of diiron peroxo or diiron dioxo intermediates.

Therefore, we are focusing on diiron complexes bearing pyridine ligands substituted with electron donating groups, electron withdrawing groups or potential hydrogen bond donating groups. For example, we synthesized and characterized a series of symmetrical or non-symmetrical diiron(III) complexes, that bear electron donating groups in their second coordination sphere and show an interesting destabilization effect on diiron peroxo species.



We are now investigating the catalytic properties of these complexes in highly valuable oxidation reactions such as epoxidation, hydrogen atom abstraction or sulphide oxidation.

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A correlative approach for the imaging of a cell-penetrating peptide in skin slices

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Fluorescence microscopy is a well-spread and well-established technique to image labeled molecules in cells. This technique often allows reaching high resolution (< 100 nm) and good signal-to-noise ratio. However, it also possesses drawbacks, such as the involvement of probes' excited states and thus an associated deleterious reactivity, photo-bleaching and low tissue penetration. On the contrary, infrared (IR) imaging techniques only implies vibrational states, consequently, no photo damages are anticipated. Also, IR radiations can deeply penetrate tissues but the resolution is limited (> µm in the mid-IR range) due to the Rayleigh criterion. Thus, developing probes detectable by both techniques open up the opportunity for cross-correlative studies at different scales, from the sub-cellular to the tissues scale. We have recently shown that metal-carbonyl compounds ($M(CO)_n$) can be mapped inside cells using their IR-signature.[1,2] Interestingly $(L)M(CO)_n$ bearing specific ligands (L) are luminescent,[3] leading to a probe with two modalities called SCoMPI (Single Core Mutimodal Probe for Imaging).[4] We have demonstrated that such probes allowed performing correlative imaging at the cellular level, and now we aim at implementing this approach at the tissues scale. In this goal we have labelled a cell penetrating peptide (CPP) with a $(L)M(CO)_n$ probe. CPP are widely used to deliver biological active cargoes into cells since they are able to cross cell-membranes.[5] Recently, some researches have been devoted to the use of CPP in the topical and transdermal delivery of bioactive molecules, in particular in cosmeceutical and pharmaceutical fields.[6]

After a 6-hour skin-permeation experiment, the labelled CPP was found mainly in the *stratum corneum* and was not detected in the deeper layers of the epidermis. After a 24-hour exposure, it was found in the whole epidermis and not detected in the dermis, deeper than the dermo-epidermal junction. Images recorded by fluorescence microscopy and by synchrotron-based infrared microscopy were consistent, showing the labelled CPP at the same location (Figure 1). These proof-of-principle experiments lead to promising results and highlighted the fact that SCoMPI are useful tools for tissues imaging by IR and fluorescence microscopies.

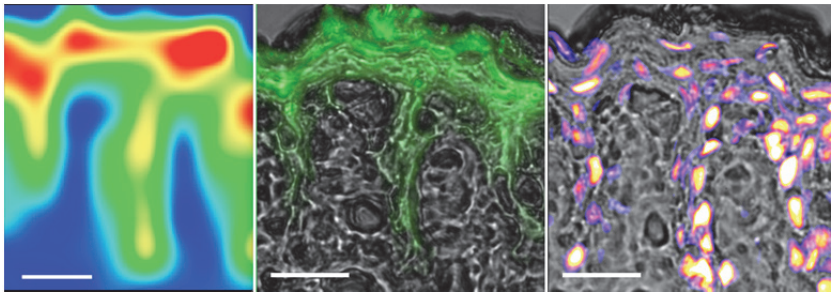

Figure 1. Skin slice after a 24-h exposure to a 2 10^{-2} M solution of the labelled CPP in water. ***Left***: mapping of the integral of the absorbance of the A_1-band (2040-2000 cm^{-1}). ***Middle***: bright field image merged with the luminescence signal of **1**. ***Right***: bright field image merged with the staining of nuclei by DAPI that shows the limits of the epidermis. Scale bar 20 µm.

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Anglet 2014

Zinc responsive contrast agents for MRI

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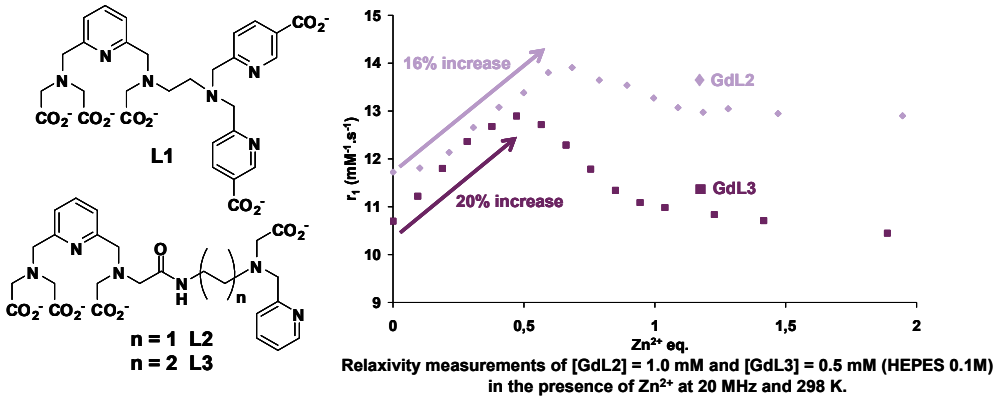
Magnetic Resonance Imaging has been devoted for a long time to obtaining anatomical and functional images. Recently emerging applications in molecular imaging seek information at the molecular level, looking at the biochemical or physiological abnormalities underlying the disease. This allows for a better understanding and earlier diagnosis. Unlike anatomic imaging, molecular imaging always requires an imaging probe that is selectively responsive to the parameter to detect. Gd^{3+}-based contrast agents are particularly well-adapted for this purpose and most often the changes on the efficacy (relaxivity) are based on changes of the hydration number and/or rotational dynamics of the complexes; these two parameters being the easiest to be tailored by the chemist[1].

Endogenous cations are known to play vital role in many fundamental biological processes. Their concentration is tightly regulated by the cell, and misregulation of these ions is connected to different pathologies. Zinc is the second most abundant transition metal ion in humans, and it plays a central role in controlling gene transcription and metalloenzyme function. Exposure to high zinc concentration can lead to neuronal death. It is also an important signalling ion in the brain, which is implicated in neurodegenerative diseases like Alzheimer's Disease.

We have recently developed zinc responsive contrast agents based on a pyridine unit already used for Gd^{3+} complexation [2], to which a zinc complexing unit has been added through a linker (cf Figure). Potentiometric studies have shown that the presence of the amide is necessary for the stability of Gd^{3+} complexes in the presence of zinc. GdL2 and GdL3 show a relaxivity response to zinc, and analyses of the ^{17}O NMR, and the NMRD profiles prove that changes of the rotational correlation time of the complexes are responsible for this behaviour. Finally the selectivity of the zinc complexing unit has been studied by relaxometry.



Relaxivity measurements of [GdL2] = 1.0 mM and [GdL3] = 0.5 mM (HEPES 0.1M) in the presence of Zn^{2+} at 20 MHz and 298 K.

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Polynuclear lanthanide-based dendrimers for biological optical imaging

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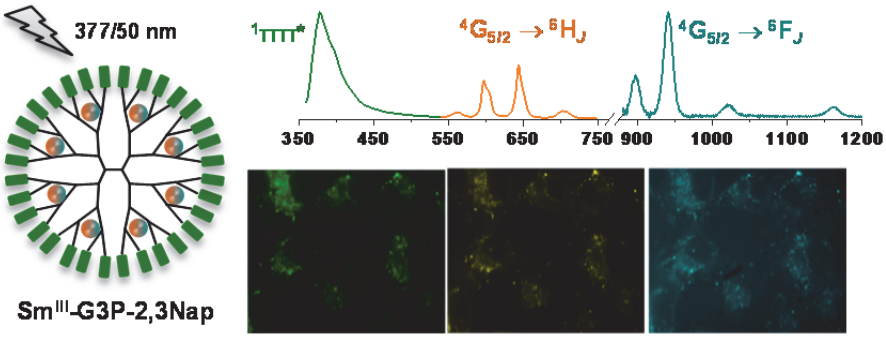
Luminescent lanthanide(III)-based compounds are very attractive for biological imaging as well as for other advanced technological applications. In comparison with organic fluorophores, they have long luminescence lifetime (in µs-ms range) and characteristic sharp emission bands, positions of which are not affected by different conditions and environments (chemical, biological, pH, temperature etc.). Several lanthanide ions emitting in the near-infrared (NIR) have a strong interest for biological applications due to the possibility to enhance signal-to-noise ratio as the background emission arising from biological tissues is reduced in this range of wavelengths.

Some challenges have to be considered when designing luminescent lanthanide-based compounds. The first one is to overcome low molar absorption coefficients of free lanthanide ions which can be achieved by surrounding them by appropriate chromophoric ligands able to act as "antennae" and efficiently sensitize lanthanide luminescence. The second challenge concerns low quantum yields and is of particular importance for NIR-emitting lanthanides. In this report we propose generation-3 poly(amidoamine) (PAMAM) dendrimers as a versatile platform. Such macromolecules can incorporate eight lanthanide ions inside their branches forming kinetically stable species while the periphery thirty-two amino groups can be substituted by suitable chromophores. [1] A combination of a large number of lanthanide cations and antennae in one macromolecule improves absorption/emission efficiency and allows to gain sensitivity of detection. Moreover, by using chromophoric groups possessing functional moieties the periphery of the PAMAM dendrimers can be further modified by covalent substitution with specific targeting molecules and/or drugs.

As a proof-of-concept demonstration generation-3 PAMAM dendrimers functionalized with thirty-two 2,3-naphthalimide chromophores and incorporating eight samarium(III) cations have been proved to exhibit characteristic emission in the visible and NIR domains upon single excitation wavelength, be non-cytotoxic (up to 2.5 µM), able to be uptaken by cells and act as a bioprobe in a fluorescence microscopy imaging experiments.[2]



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Development of rhenium carbonyl complexes for the labeling and bimodal imaging of biomolecules

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Fluorescence microscopy has been widely used over the past decades to study the function, localization and interactions of proteins in their native environment. Small-size probes have therefore been developed for the imaging of proteins not only by fluorescence microscopy but also by interesting alternative methods of imaging[1,2].

For instance, IR imaging, although less sensitive than fluorescence methods, induce no photo-bleaching or photo-damaging of cells due to the lower energies involved. The most widely used IR-probes are probably those based on metal-carbonyl moiety, as they show attractive properties for bio-imaging[3,4] (e.g. stability in biological environment and strong C=O absorption at 1800-2200 cm^{-1} in the transparency window of the cell). Interestingly, when bound to specific ancillary ligand with low π^* orbital's, metal-carbonyl complexes may also be luminescent[5].

Recently, our group has thus developed bimodal probes (IR and luminescent) based on rhenium-carbonyl complexes and used them to label various biomolecules and image them by both IR and fluorescence microscopies[6]. We aim now at labeling proteins, using the so-called traceless affinity labeling strategy developed in particular by Hamachi et al[2,7]. Here we present the synthesis of a traceless affinity label for human carbonic anhydrase IX, a protein overexpressed in some cancers. The label consists in an inhibitor of the protein linked to the desired probe by a moderately reactive linker (here an alkyloxyacyl imidazole). The ligand allows the selective binding of the label by the protein, while the acyl transfer from the ligand to one of the amino acids of the protein surface is enhanced by the proximity effect (Figure 1). The labeling studies will also be discussed.

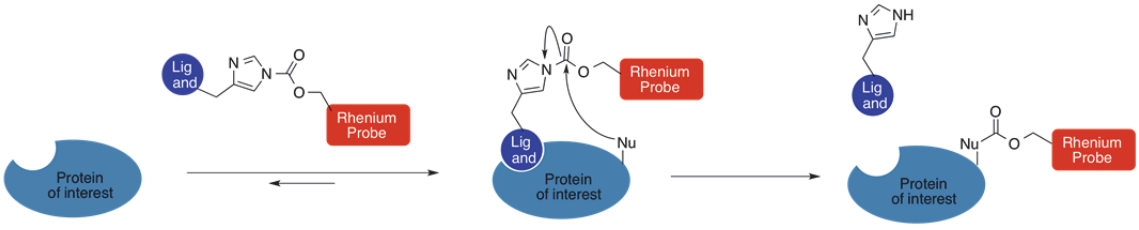

Figure 1 Principle of the ligand directed acyl imidazole chemistry

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Lanthanide-based luminescent probe for time-gated detection of copper(I) : modulation of the antenna effect by cation/π interaction

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Copper is an essential metal for life but it can also be toxic. Therefore, its homeostasis is tightly regulated by living systems. Serious diseases like Menkes and Wilson's disease are linked with a lack or an excess of copper. Hence it is important to design quantitative copper probes for a better understanding of its regulation and be able to diagnose diseases.

In cells, copper is at the oxidation state +I. We have developed a luminescent probe for Cu^+, based on the binding site of the copper chaperone CusF, a periplasmic protein in Gram negative bacteria. This protein binds copper with the side chains of two methionines (thioether), one histidine (imidazole) and one tryptophan (indole). The tryptophan is a very unusual ligand that is bound to copper through a cation/π interaction.[1,2] We have designed a peptide that mimics the binding site of CusF for selective Cu^+ binding and we have grafted on this peptide a DOTA-terbium(III) for light emission. In this peptide, a tryptophan serves both as a Cu^+ ligand and as an antenna for terbium sensitization. This luminescent probe is able to selectively detect Cu^+ among physiological cations.

In this communication, we will present the synthesis, the complexation properties and the photophysical properties of this probe. We will show how the cation/π interaction modulates the tryptophan antenna properties and changes the terbium emission.

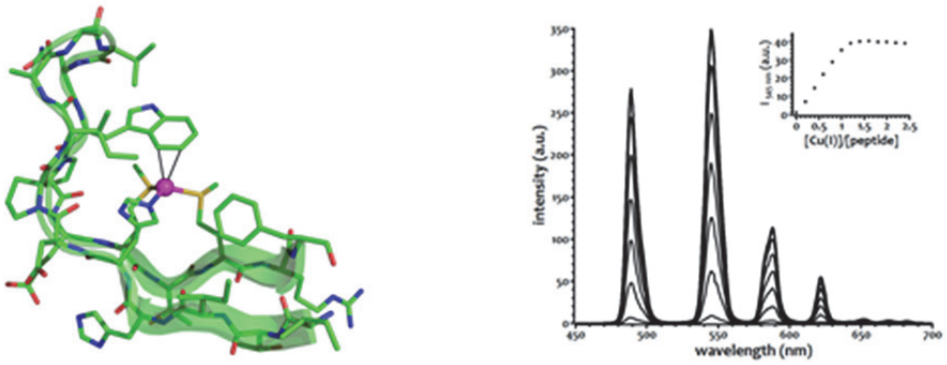

Figure : Copper(I) binding site of the protein CusF (left) and Tb^{3+} emission of the probe when Cu^+ is added (right).

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Poster Communications

FrenchBIC

Anglet 2014

Cu(I) removal as a new therapeutic approach against Alzheimer's disease

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Metal ions such as Zn, Cu and Fe have played an important role in the development of Alzheimer's disease by bonding to Aβ peptides, which can lead to aggregation of peptides to form *amyloid extracellular plaques*, one of the main physiological characteristics found in the brains of patients with AD [1]. On top of this, Cu ions coordinated to Aβ peptides can catalyse the production of Reactive Oxygen Species (ROS) that have an important role in oxidative stress of neuronal cells [2].

Several investigations are carried out in order to obtain a suitable ligand for these metals ions, and specially one able to stabilize one of the oxidation states of Cu or Fe to avoid the production of ROS. The ligands currently in research are used for the coordination of Cu(II). This work is focused on the application of a Cu(I) ligand.

The ability of the phosphane 1,3,5-triaza-7-phosphadaamante (PTA) to reduce Cu(II) and stabilize Cu(I) as a tetrahedral coordinated compound has been reported by Pombeiro et al. [3]. Also this phosphane has shown to be stable, highly soluble in water and most organic solvents, and can be modified by different positions, which makes of it an interesting ligand for coordination chemistry and applications in medicinal chemistry. [4]

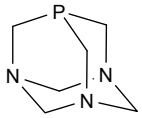

Fig. 1: Chemical structure of 1,3,5-triaza-7-phosphadamantane

Bearing this property in mind, several experiments using techniques such as UV-Vis spectroscopy, fluorescence studies, Nuclear Magnetic Resonance of ^{1}H and ^{31}P and EPR spectroscopy have been carried out in which the ability of PTA to reduce Cu(II) to Cu(I) and stabilize the low oxidation state in physiological conditions, and its ability to remove copper ions from Aβ which also leads to a stop in the ROS production. Also, preliminary experiments on Aβ aggregation have been performed.

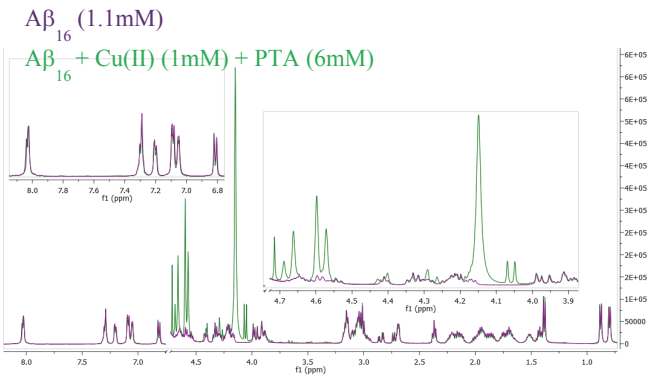

Fig. 2: ^{1}H-NMR of Aβ$_{16}$ and Aβ$_{16}$ in presence of Cu and PTA

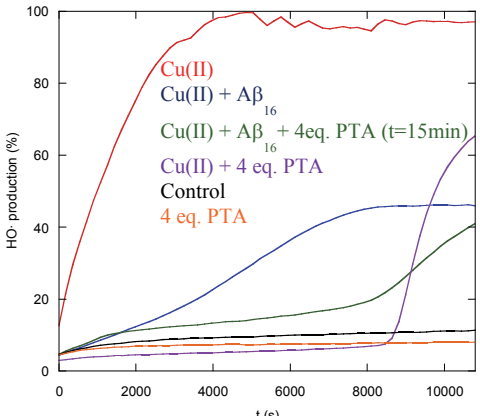

Fig. 3: CCA experiment showing stop of ROS production

The ligand PTA has demonstrated its efficiency reducing Cu(II) to Cu(I), which leads to the formation of a stable coordination complex, and removing Cu ions from Aβ, which leads to the arrest of ROS production.

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FrenchBIC

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Low potential inactivation of FeFe hydrogenases

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Hydrogenases, the enzymes that oxidize and produce H_2, can be covalently attached to electrodes, forming a stable protein films while maintaining direct electron transfer [1].

FeFe hydrogenases are inactivated under various conditions: presence of CO or O2, extreme potentials [2-3]. On one hand, using an original approach, which combines accurate electrochemical measurements and theoretical calculations, we elucidate the mechanism by which, under certain conditions, CO binding can cause permanent damage to the active site (H-cluster)[3]. One another hand, thanks to covalent immobilization of the enzymes, we show that FeFe hydrogenases inactivate at low potential, in a complex process that is mostly reversible. A form of the enzyme that is produced slowly and reversibly under reductive conditions has no proton activity under reductive conditions, although it can still oxidize H_2 under oxidative conditions [4].

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The kinetics of structural reorganization in macrocyclic complexes

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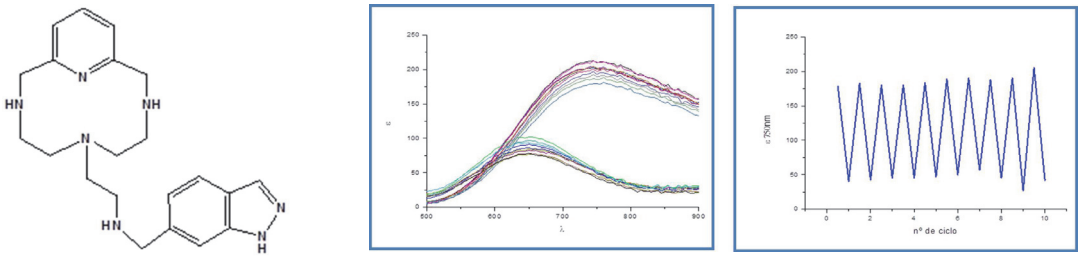
The interaction of metal ions with biomolecules, or their mimetics, cannot be simply considered from the thermodynamic point of view, kinetic aspects being fundamental for understanding the processes that are actually taking place. Of especial relevance are reorganization processes in which the initially formed adduct evolves to other species with a different interaction. From a kinetic point of view, the rate of those reorganization processes can be determined by the nature of the metal, the receptor or a combination of both. The classical interpretation or reaction mechanisms of inorganic metal complexes anticipates a dominant role of the metal ion, but the few references available on structural reorganizations in macrocyclic metal complexes with pendant arms suggest that this is not necessarily true for the case of some macrocyclic metal complexes with pendant arm[1]. In previous woks we have found that studying the kinetics of the acid-promoted decomposition of metal complexes can be used to detect the existence of molecular reorganizations and to study the kinetics of those movements [2] . In this communications some recent results using metal complexes of scorpiand-like ligands that show promising biological activity will be presented. The kinetics of the process are studied using stopped-flow with multiwavelength detection and global analysis of the data, and complemented with single-wavelength experiments using fluorescence detection.

The figure illustrates the reversibility of the spectral changes signalling the reorganization process in the Cu^{2+} complexes with a scorpiand-type ligand. In this case, the structural reorganization does not only involve the coordination-dissociation of the aliphatic NH group in the pendant arm commonly observed for related molecules. The results will be discussed and compared with those obtained for related systems.



Financial support by the Spanish MICINN and FEDER (Grant CTQ2012-37821-C02-02), Consolider- Ingenio 2010 Program (Grant CSD2010-00065) and Generalitat Valenciana PROMETEO GVA 2011-008 is gratefully acknowledged.

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Anglet 2014

Luminescence modulations of Rhenium tris-carbonyl complexes induced by structural variations

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Octahedral d^6 low-spin Re(I) tris-carbonyl complexes are of considerable interest as non-invasive imaging probes and have been deeply studied owing to their biological stability, low toxicity, large Stokes shifts and long luminescence lifetimes.[1] We reported recently the bimodal IR and luminescence imaging of a Re(I) tris-carbonyl complex with a Pyta ligand (4-(2-pyridyl)-1,2,3-triazole) in cells and labelled such metal-carbonyl complexes SCoMPIs for single core multimodal probes for imaging.[2] Re(I) tris-carbonyl complexes have unique photophysical properties allowing for their unequivocal detection in cells but present also some weaknesses such as a very low luminescence quantum yield in aqueous medium. Further optimizations would thus be desirable.

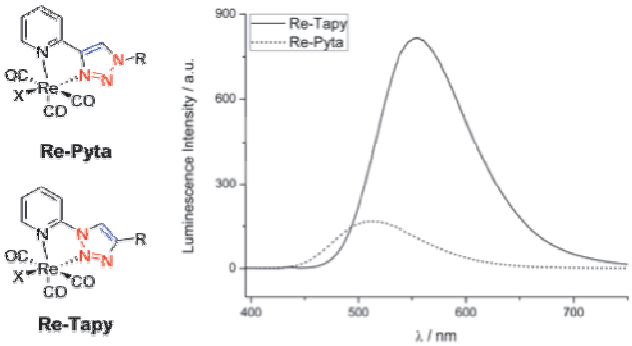

Figure 1. Re-Tapy *vs* Re-Pyta

We developed new Re(I) tris-carbonyl complexes prepared from different ancillary ligands. Complexes with benzothiadiazole-triazole ligands show interesting luminescent quantum yields in acetonitrile and may constitute valuable luminescent metal complexes in organic media. A series of complexes with bidentate 1-(2-quinolinyl)-1,2,3-triazole (Taquin) and 1-(2-pyridyl)-1,2,3-triazole (Tapy) ligands bearing various 4-substituted alkyl side chains has been designed and synthesized with efficient procedures. Their photophysical properties have been characterized in acetonitrile and in H_2O/DMSO (98:2) mixture and compared with the parent Quinta and Pyta-based complexes. Tapy complexes bearing long alkyl chain show impressive enhancement of their luminescent properties relative to the parent Pyta complex. Theoretical calculations have been performed to further characterize this new class of rhenium tris-carbonyl complexes. Preliminary cellular imaging studies in MDA-MB231 breast cancer cells reveal a strong increase in the luminescence signal in cells incubated with the Tapy complex substituted with a C12 alkyl chain. This study points out the interesting potential of the Tapy ligand in coordination chemistry, which has been so far under exploited.[3]

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DNA recognition by lanthanide-binding hexapeptides

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The expression of genetic information involves many recognition processes by interactions between a specific DNA sequence and a biomolecule. The development of molecules which can not only interact with DNA but highlight this interaction is essential for the comprehension of biological events and for medical diagnostic.

Lanthanide ions, with their unique spectroscopic properties prove to be powerful tools to detect supramolecular interactions between molecules and DNA. Thanks to their long-lived excited states, these metal ions allow the design of very sensitive probes and a time-resolved detection to overcome the natural luminescence of the biological material.[1]

In this context, we are designing efficient lanthanide-peptide complexes to detect the interaction with DNA through the metal-centered luminescence. The design of such probes requires the development of ligands which can effectively coordinate the metal ions, sensitize these metal ions by antenna effect and induce a change in the luminescence of the lanthanide in the presence of DNA.

It has been demonstrated that hexapeptides containing two unnatural amino acids bearing aminodiacetate side chains and a sequence Pro-Gly provide Ln^{III}-peptide complexes with high-enough stability to avoid dissociation in water at physiological pH. In fact, the Pro-Gly sequence promotes a β-turn conformation of the peptide, enhancing the coordination of the cation thanks to a spatial arrangement between the two chelating groups of the unnatural amino acids.[2-5]

Furthermore, organic fluorophores coupled to the N-terminus of these peptides act as intercalating agents and sensitizers of europium. Upon excitation of the sensitizer unit without DNA, the energy transfer from this unit to the lanthanide ion by antenna effect highlights the specific luminescence of the metal. In the presence of DNA, the sensitizer unit intercalates into this biomolecule and the energy transfer is no more possible, which significantly affects the metal-centered luminescence (cf. figure 1).[6]

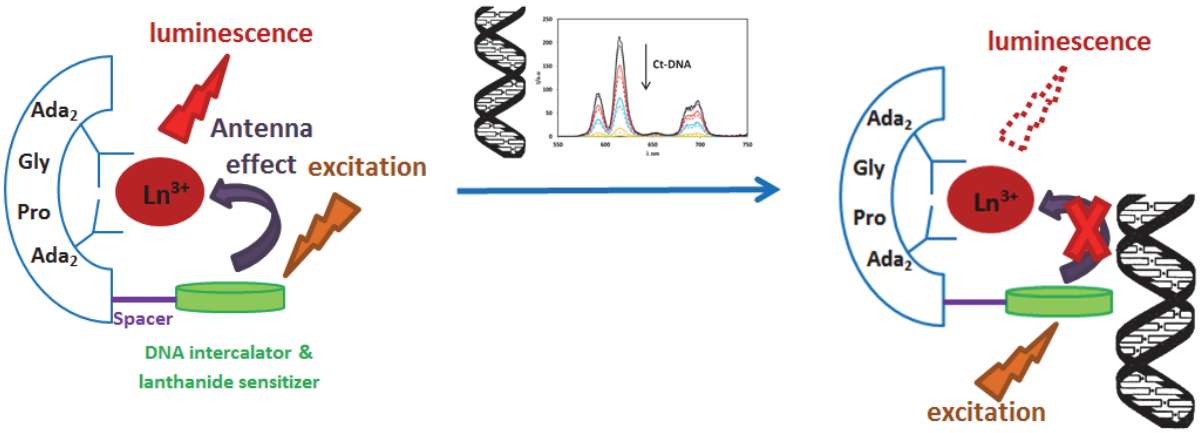

Figure 1 : detection of Ct-DNA by a lanthanide-binding peptide

We are currently designing new peptides inducing an increase in the lanthanide luminescence during interaction with DNA. This strategy consists of separating the DNA intercalator from the lanthanide sensitizer. The synthesis and preliminary properties of these novel peptide derivatives will be outlined in this communication.

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Reductive activation of *E. coli* Nitrate Reductase

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Prokaryotic nitrate reductases (NR) are molybdoenzymes that contain a Mo-bisPGD cofactor and catalyze the reduction of nitrate to nitrite. Several research groups have studied these enzymes, notably the spectroscopic signatures of the molybdenum cofactor (MoCo) [1-3], and the electron transfer chain [4]. For more than a decade, direct electrochemistry has been applied to probe the physical and chemical properties of the active site during catalysis [5-7]. Field and coworkers revealed that two prokaryotic NRs from *Paracoccus pantotrophus* (*Pp*) and *Synechococcus elongatus* (*Se*) activate upon reduction [7]. Elliott and coworkers have studied the respiratory NR from *E. coli* (*Ec*NR) and proposed a catalytic cycle [6], but the activation of this enzyme had not been reported. Our electrochemical investigation reveals that that *Ec*NR activates upon reduction (Figure 1), as observed with *Pp* and *Se* NRs [7-8]. We showed that 30 % of the sample is initially an inactive state, and that kinetics of activation depends on redox conditions in a complex manner. We propose a model that accounts for the kiknetics of reductive activation of *Ec*NR; we discuss the link between our observations and the properties of other Mo-bisPGD enzymes, such as DMSO reductase [9] or periplasmic NR [10].

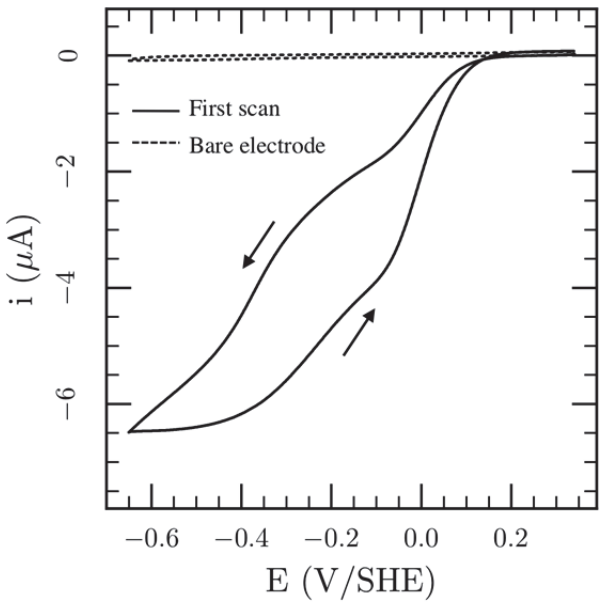

Figure 1. First cyclic voltammogram of a fresh film of *E. coli* NR starting at high potential. The upward scan (*cf.* arrows) shows more reduction current than the downard scan, revealing a reductive activation process.

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Manganese complexes for catechol oxidation: from synthesis to incorporation on a mesoporous support

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Intensive use of pesticides led to an excess of polluting molecules in water and soil. To replace the already existing expensive treatments, cheaper alternative solutions are of interest. In the case of catechol derivatives, catechol dioxygenase enzymes perform an oxidative cleavage of the aromatic ring using O_2 as oxidant. [1, 2] The mild conditions and the large availability of the oxidant make these enzymes a clever solution in such depollution issues.

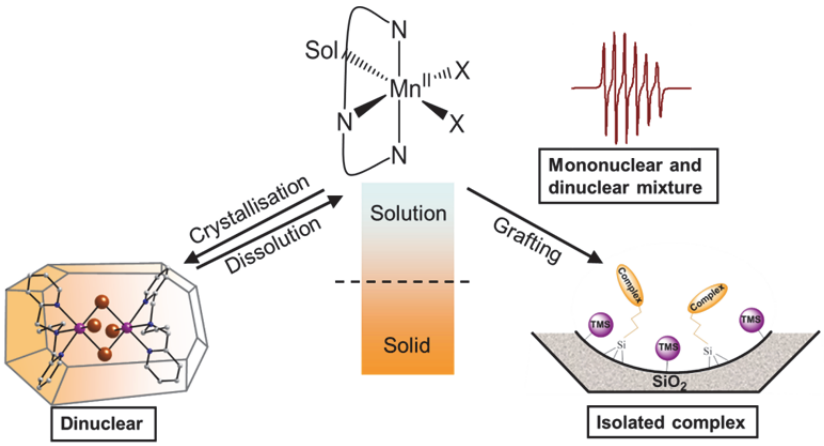

Figure 1: Crystallization and incorporation of manganese complexes into a modified mesoporous silica

This work present the synthesis, characterization and incorporation of manganese(II) complexes into a mesoporous silica to mimic both the enzymatic active site and the environment of the metalloprotein (Figure 1). [3, 4] By using a molecular stencil patterning strategy, we were able to double-functionalize the mesoporous support with both our manganese complexes and a second function to tune the chemical environment whereas the pores provide the confinement existing in the natural enzyme.

First catalysis tests will also be reported using both free and supported complexes. Catechol derivatives appear to be oxidized in the presence of oxygen to provide the quinone form along with other subproducts.

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Oxidation of the Beta-Amyloid peptide catalyzed by copper

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Almost 36 million people in the world are affected by dementia, most suffering from Alzheimer's disease (AD). One of the feature of AD is the formation of senile plaques in brain, mainly composed of the 40/42-residue Beta-Amyloid peptide (Aβ). It is known that the Aβ peptide is present in soluble form in healthy brain and found aggregated in Alzheimer's brain. In addition, some metals such as copper are present in high levels of concentration in senile plaques, and form complexes with Aβ. In the presence of a reducing agent (such as ascorbate), Cu-Aβ complexe can catalyze reactive oxygen species (ROS) production, including the hydroxyl radical (HO•) [1,2]. This latter is highly reactive and can cause oxidative damages on surrounding neuronal biomolecules and on the Aβ peptide itself.

Aβ peptide oxidation particularly targets the amino acid residues involved in copper coordination, since ROS are produced at the metal center of Cu-Aβ. EPR and NMR studies have shown that copper is bound to the N-terminal part of the peptide (Asp1, His6, 13 and 14) [3]. By using the tools of proteomic analyses by mass spectrometry (MS), we were able to characterize the oxidized amino acid residues and specify the nature of chemical modifications, on a truncated peptide (Aβ1-28) [4], and on the full-length peptide (Aβ1-40). We also have studied the time dependency of amino acid residues oxidation using high resolution mass spectrometry (HRMS). The results show that oxidation strongly damages the N-terminal part of the peptide, and would be thus responsible for a change in copper coordination. Furthermore, oxidation could also modify the way the peptide aggregates. Both processes are of interest for understanding the mechanisms involved in AD etiology.

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High-field pulse EPR nanometre-scale distance measurements of paramagnetic centres

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Nanometre-scale distance measurement between paramagnetic centres using pulse electron paramagnetic resonance (EPR) techniques is a versatile biophysical tool for probing the structures and functions of complex biological systems. The most common approach is to label the system with two nitroxide (S=1/2) spin labels (NSL) and measure their magnetic dipolar interaction using Pulse Electron Double Resonance (PELDOR), also known as DEER (Double Electron-Electron Resonance). The vast majority of the measurements have been made at 9 GHz (0.3 T). The use of higher magnetic fields and higher frequencies can greatly improve the sensitivity of the measurements. However, the large g-anisotropy of NSL makes the use of higher fields less effective. Gd^{3+} (S =7/2) complexes spin labels have been successfully used as an alternative for high-field PELDOR measurements, as their g-anisotropies are small and their EPR spectra are in fact narrower with increasing magnetic field. The same hold true for Mn^{2+} (S = 5/2) and we have been studying the use of Mn^{2+} complexes as spin labels. They are less redox sensitive than NSL and are less cytotoxic than Gd^{3+}, opening up the possibility of making *in vivo* measurements in cells. Our investigations on the use of a variety of spin labels for PELDOR and other pulse EPR techniques at high magnetic field (95 GHz) for nanometre-scale distance measurements will be discussed.

Rational design of peptide scaffolds coordinating uranyl

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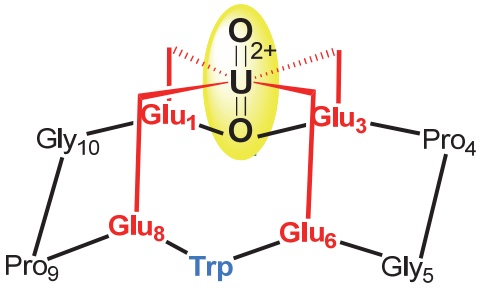
Actinides are very toxic metals, naturally present in environment at various concentrations. They are mainly used in nuclear industry and for military applications. Since they represent a potential threat both for environment and humans, their toxicity and detoxification are crucial societal and scientific issues.[1] This is why the understanding of their interactions with biomolecules is necessary to predict their toxicity and also to develop efficient detoxification agents.

Peptides are promising ligands of actinides, which present many advantages either for fundamental studies or the design of detoxifying molecules. First, peptides are mimics of protein binding sites since they offer the same chemical functions and structural properties. Therefore, they are good models to study the interactions of actinides with proteins and to predict high affinity uranyl binding sites. Moreover they are attractive water-soluble ligands to design non-toxic chelating agents as proven for other metal detoxification processes.[2]

Here, we will present peptide scaffolds, with a backbone conformation constrained in an antiparallel β-sheet. This structure induces the presentation of four amino acid side-chains bearing potential coordinating functions in the same direction and therefore preoriented to complex metal ions.[3] We demonstrated that this scaffold was an efficient ligand of uranyl ion, which promote the coordination of four carboxylate groups in the equatorial plane. Physico-chemical studies evidenced that a unique monometallic complex is formed with uranyl in water and that the complex structure is a well-defined β-sheet. A series of peptides was synthesized with various combinations of the four coordinating amino acids, in order to establish correlations between the peptide sequences and the ability to coordinate the uranyl cation. Uranyl complexes with nanomolar stabilities were obtained using acidic residues. A second generation of peptides with higher affinity amino acids is currently investigated in order to improve the stability of the uranyl complexes.



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Synthesis of platforms with constrained distance between pyridine-based Mn(II) complexes for EPR studies

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Measuring nanometer-scale distances has become an invaluable method to provide a microscopic picture about the overall structure of a biomacromolecule. This can be accomplished using EPR PELDOR (Electron Paramagnetic Resonance – Pulse Electron Double Resonance) which allows the accurate determination of distances ranging from 2 to 8 nm between two paramagnetic species.[1] This promising tool is of particular interest when structures are difficult to characterize by NMR or X-ray crystallography.[2]

The PELDOR method with stable radicals such as nitroxides has received a lot of attention, and can be performed nearly routinely. Nevertheless, nitroxides suffer from limitations, and are not appropriate for high-field measurements (W-band, 95 GHz) that would enhance sensitivity.[3] Mn(II) complexes are a relevant alternative because of their narrow high-field EPR spectrum which is essential to ease methodological development.

To calibrate the PELDOR method with Mn(II) complexes, we designed a family of modules with well-determined inter-paramagnetic centers distances (Fig.1). These platforms incorporate two Mn(II) derivatives[4] as paramagnetic moieties linked to a central rigid molecular rod.[5] Convergent synthesis relying on Pd-catalyzed chemistry (Sonogashira and Hartwig-Buchwald couplings) was used to assemble the linker, whereas different strategies were assessed to graft the pyridine-based Mn(II) ligand on the spacer. The Mn-Mn distances have been predicted using DFT calculations. The synthesis of various modules will be presented and the EPR spectra of these platforms will be discussed.

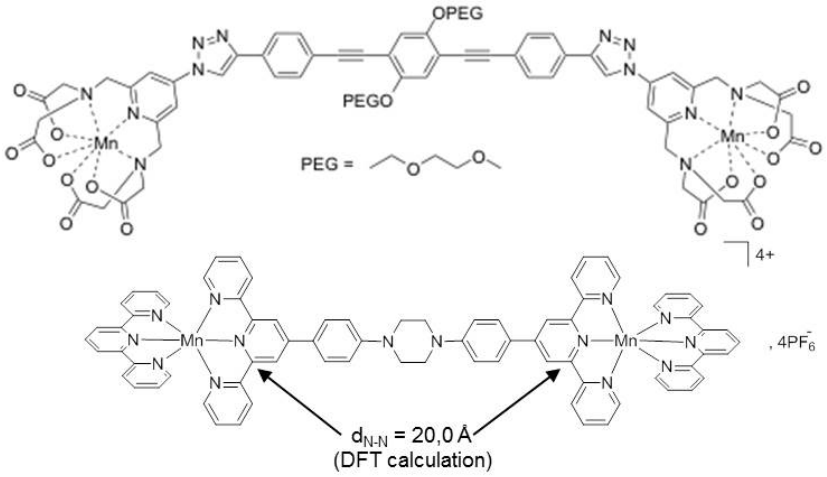

Fig.1: Structures of typical platforms

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Functional heterogeneity of the Carbon Monoxide Dehydrogenase (CODH) from *Desulfovibrio vulgaris*

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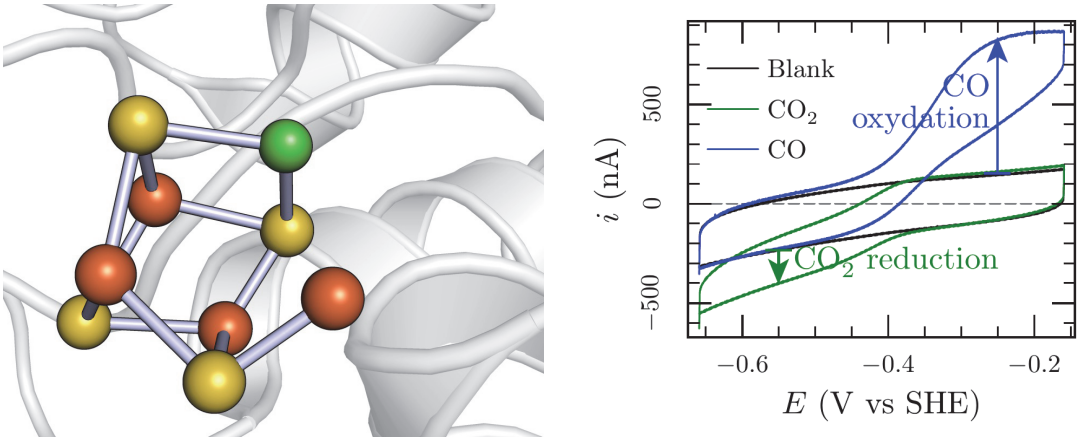
Some microorganisms use Carbon Monoxide Dehydrogenase (CODH) to catalyse the reversible oxidation of CO into CO_2 according to the reaction: $CO + H_2O \leftrightarrow CO_2 + 2H^+ + 2e^-$. The Nickel-Iron (Ni-Fe) CODHs are found in anaerobic organisms such as *Carboxydothermus hydrogenoformans* or the photosynthetic bacterium *Rhodospirullum rubrum* which use CO as energy source. This is probably due to the involvement of CODHs in the generation of a proton motive force in association with hydrogenases. In some cases, CODHs are involved in CO_2 fixation, in association with an Acetyl-CoA synthase. The precise role of the CODH from our model organism, *Desulfovibrio vulgaris* (*Dv*), is still unclear.



Left: structure of the active site of CODH. **Right:** cyclic voltammograms of a film of adsorbed CODH on a graphite electrode in the presence of CO (blue) or CO_2 (green).

We have developed a system for the almost homologous production of *Dv* CODH in the closely related bacterium *Desulfovibrio fructosovorans*. The enzyme forms stable active films on graphite electrodes that catalyze both the oxidation of CO or the reduction of CO_2 at high rates (figure). Electrochemical data suggest that the enzyme may be present under different forms of comparable activity but very different catalytic properties. This unprecedented behaviour may shine light on some of the properties of the CO dehydrogenases studied in the literature that have remained controversial in the past decades, such as the structure of its active site or its behavior with inhibitors (1-4).

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Receptor-based multipotent artzymes

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The importance of catalysis for the development of sustainable chemistry and the resulting societal impact is unambiguously recognized. We aim at preparing novel eco-friendly biocatalysts, namely artificial metalloenzymes or "artzymes", by combining the adenosine A_{2A} receptor[2] ($A_{2A}AR$) with catalytically active organometallic complexes, using the "Trojan horse strategy"[1].
For this, an agonist or an antagonist of $A_{2A}AR$, used as a "Trojan Horse", will be linked to a rhodium or a copper complex and subsequently combined with the receptor. The catalytic activity of the artzymes will be evaluated in the selective hydrogenation of double bonds and in Diels-Alder cyclizations.
$A_{2A}AR$ is expressed on the surface of CHO cells and many of its agonist and antagonist with different affinities are available. Thus, this receptor-based approach possesses all the features for creating multipotent artificial enzymes. Such artzymes will be capable of catalyzing cascades of different reactions following the concept of mutlicatalysis in a single-pot wherein a substrate is converted into desired products by a cascade of different reactions (**Figure 1**).

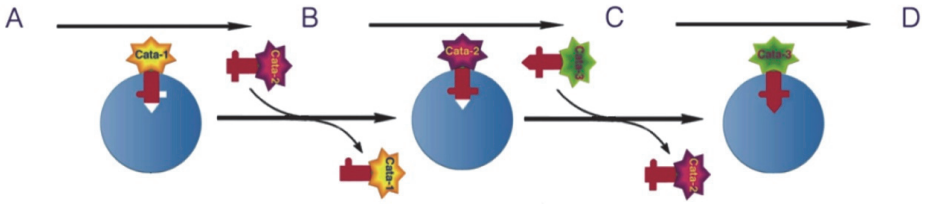

Figure 1. Multipotent artzyme catalyzing a three step reaction from substrate A to product D using the principle of displacement of organometallic counterparts by order of increasing affinities.

A diphenylphosphine rhodium(I) complex was linked to CGS-21680, an agonist of $A_{2A}AR$ (K_D = 27 nM), as a first step in the preparation of a receptor-based artzyme. In order to evaluate the catalytic activity of the receptor-based artzyme in the selective hydrogenation of double bonds, it will be compared with the activity of a soluble protein-based artzyme, also prepared following the "Trojan horse strategy" using the soluble protein Neocarzinostatin variant, NCS-3.24[3], which has affinity for testosterone derivatives ($K_{D\ testosterone}$ = 13 µM).
The NCS 3.24-based hydrogenase was prepared by incubation with the same rhodium complex linked to testosterone and was used to catalyse the hydrogenation of unsaturated amino acid derivatives. The results show a two-fold increase in the TON of the catalysis for the NCS 3.24-based hydrogenase *vs* the presence of the rhodium complex without protein. However, a low enantiomeric excess was observed in both cases (**Figure 2**). These results will be compared to those that will be obtained using the receptor-based hydrogenase.

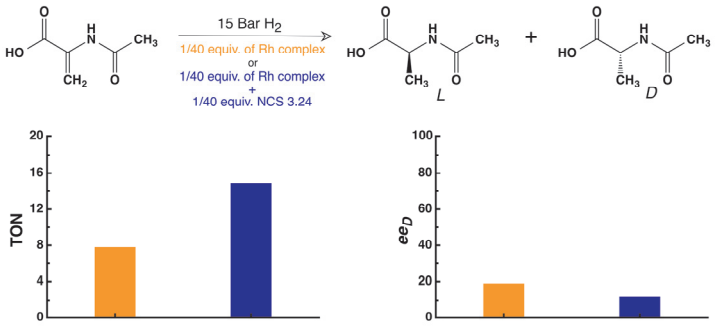

Figure 2. Results of the hydrogenation of catalyzed by NCS 3.24-based hydrogenase *vs* by rhodium complex without protein.

Subsequently, the same evaluation method will be used to compare the potential of the receptor-based artzyme in catalysing Diels-Alder cyclizations. Finally multicatalysis will be attempted.

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Reactivity of the molybdenum cofactor in Nitrate Reductase as probed by hyperfine spectroscopy

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The respiratory nitrate reductase (nNarGHI) from the gut bacterium *Escherichia coli* belongs to the so-called dimethylsulfoxide reductase family characterized by the presence of a Mo/W-bis(pyranopterin guanosine dinucleotide) cofactor at the active site [1]. Most importantly, it belongs to the subclass of Mo-enzymes using an aspartate as direct ligand of the Mo ion at the active site [3,4]. Early EPR spectroscopic studies performed on *E. coli* nNar have reported two characteristic pH-dependent Mo^V EPR signals, the low-pH and high-pH signals [2], both showing hyperfine coupling to a solvent exchangeable proton. At this stage, several open questions remain to be addressed. At first, the structure associated to these forms remains controversial and in particular how they relate to the two distinct Mo coordination modes inferred from crystallographic studies [3,4]. Secondly, their catalytic relevance is still discussed [5]. Finally, the reaction mechanism is still very much debated. For instance, binding of the substrate only weakly affects the Mo^V EPR signature raising the question of its mode of interaction to the Mo center [6].

The aims of the present work are to resolve the local structure of the high-pH and low-pH forms as well as the structural changes induced upon substrate binding using ESEEM/HYSCORE spectroscopy. As such, we have combined isotope enrichment strategies to simplify spectral analysis and assigned the detected nuclei to a specific Mo^V spectral form. These strategies have also been performed on the enzyme incubated with its substrate. Finally, DFT calculations based on available structural data have been used to understand the origin of the detected hyperfine couplings. These results lay the foundation for in-depth understanding of the structure of the Mo^V catalytic intermediates.

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Study of the successive one-electron photochemical events in a chromophore-catalyst molecular assembly

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We design, synthesize and characterize molecules that mimic the reactions observed in Photosystem II.[1] Akin to natural systems, these molecules are able to undergo light-induced charge separation, electron transfer, proton transfer and accumulation of oxidizing/reducing equivalents at a catalytic site.[2] These supramolecular systems contain a photoactive component covalently linked through a spacer to a catalytic cavity where a metal ion or cluster is located. The photosensitizer used is a $[Ru(bipy)_3]^{2+}$ (bipy = 2,2'-bipyridine) analogue, a counterpart to P680, which absorbs light in the visible region and triggers an electron transfer process. The resulting RuIII species has a reversible oxidation potential of 1.30 V vs. SCE, similar to that of P680, and is capable in principle of oxidizing a catalytic cluster. The catalyst used is a non-heme iron complex supposed to undergo a chemically-driven 2-electron, 2-proton removal process in order to form an oxo-iron species capable of performing oxidation reactions on organic substrates.[3]

Within the framework of the field of artificial photosynthesis we aim at substituting the chemical oxidants used to carry out these oxidation reactions with the energy provided by visible light.

Using flash photolysis and EPR techniques we have studied the two-electron light-driven oxidation of the Fe-catalyst unit by the ruthenium chromophore. The identification of the intermediates in this novel high valence ruthenium-iron complex, as well as the factors governing the energy and electron transfer processes towards the formation of reactive Fe-oxo species for their use as light driven catalysts within these molecular constructs are presented.

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Hybrid catalysts based on amyloid fibrils: a proof of concept(??)

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Metalloproteins are currently considered as the most powerful catalysts, with their very high speed and exquisite selectivity. However, their total specificity in reactivity and substrates makes their use exceptive in organic synthesis[1]. For a few decades, chemists have associated with biologists to create artificial metalloenzymes, made of a proteic environment and of a synthetic cofactor. These scaffolds are hoped to combine the unequalled speed and selectivity of enzymes with the large scope of reactivity of organometallic catalysts[2].

In this context, we focused on developing catalysts from amyloid fibrils. These protein scaffolds have unique physical properties, and were successfully used as bio nanomaterials[3]. Because of their typical β-sheet structures, they would ideally bind aromatic metal complexes. Upon addition of metals, we hope to form catalytically active species, which could confer regio-, stereo- or enantioselectivity to the substrates.

To do so, a series of 4' substituted terpyridine ligands was developed, and the properties of the corresponding Cu and Fe complexes were investigated. In parallel, the amyloid-b peptide was recombinantly produced, and its interactions with the different organometallic complexes were studied.

In a next step, directed evolution will be applied on the scaffold, to determine the mutations which would improve its catalytic potential.

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Zn in Alzheimer's disease, what's new ?

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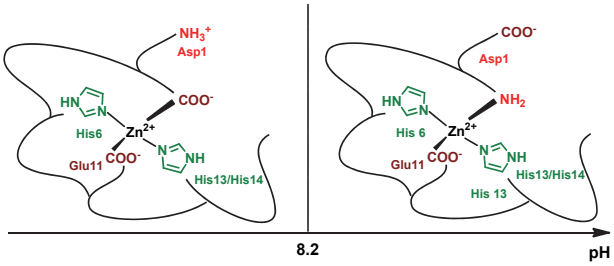
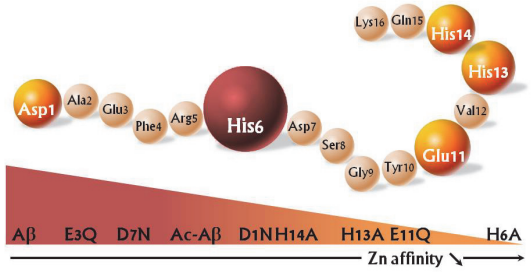
Alzheimer's disease (AD) is one of the most serious diseases mankind is now facing as its social and economical impacts are increasing rapidly. AD is very complex and the amyloid-β (Aβ) peptide as well as metallic ions (mainly copper and zinc) have been linked to its aetiology. While the deleterious impact of Cu is widely acknowledged, intervention of Zn is certain but still needs to be figured out.

In the present communication, we will first show results regarding Zn coordination to Aβ, both from structural and from affinity points of view. Structural insights were obtained mainly by X-ray Absorption Spectroscopy and NMR while Zn to Aβ affinity was determined by UV-Vis competition with a home-made competitor.

In a second part, we will also show some data regarding the importance of Zn in the Aβ aggregation process, one key event in the development of AD.

Last but not least, impact of Zn interference in Cu(II) chelation, regarded as one potential therapeutic approach against AD, will be illustrated.





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Day A, Conte-Daban A, Faller P, Hureau C. Manuscript in preparation.

Copper(II) Complexes of Phenanthroline Amino Acid Derivatives: Synthesis, Characterization and Evaluation of their DNA Cleavage and Cytotoxic Activity

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Copper(II) complexes have been intensely investigated in a variety of diseases and pathological conditions due to their therapeutic and imaging potential [1,2]. The development of these complexes requires a good knowledge of metal coordination chemistry and ligand design to control species distribution in solution and tailor the copper(II) centers in the right environment for the targeted functionality.

In this communication we present the synthesis and characterization of amino acid derivatives containing a phenanthroline unit. Their copper(II) coordination properties were studied using potentiometric and spectroscopic methods (UV-Vis and EPR). The data showed the formation of only one species at physiological pH, which is also the major species in the pH range 4.0 to 10.0. The structures of these species have been solved by X-ray crystallography and their redox potential determined by cyclic voltammetry. Their cytotoxic activity against different cancer cell lines (human ovarian (A2780), its cisplatin-resistant variant (A2780cisR) and human breast (MCF7) cancer cell lines) as well as their DNA cleavage properties have been evaluated. Despite having similar structures these copper(II) complexes present different biological activities. These preliminary results will be discussed based on their structures and redox potentials.

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Identification of the elusive sites of Tyr radicals in cytochrome c peroxidase by mutifrequency (9-285 GHz) EPR spectroscopy: Implications for oxidation of substrates bound to a site remote from the heme.

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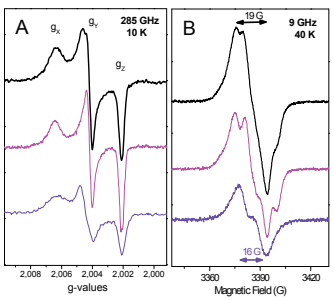
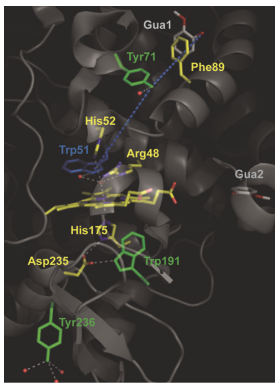
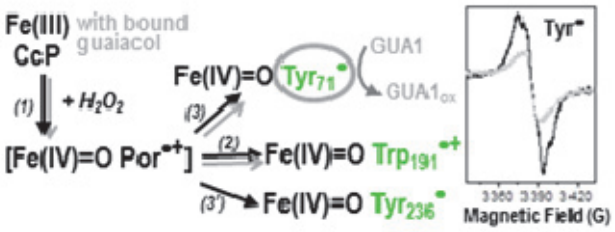
Trp- and Tyr-based radicals have been shown to play crucial roles when reacting in a concerted way with metal active site to accomplish catalytic functions in metalloproteins [1]. In heme enzymes, we and others have identified and characterized specific cases of Trp and Tyr radicals having an exquisite role as true catalytic intermediates and in long-range electron transfer, both related to the enzymatic reaction [2]. High-field/frequency EPR spectroscopy has been crucial for discriminating between Trp and Tyr radicals. Cytochrome c peroxidase (CcP) is the first heme peroxidase reported to utilize a Trp radical as catalytic intermediate. The enzyme is used as a functional and structural model for heme enzymes. The [Fe(IV)=O Trp191$^{\bullet+}$] intermediate is well-characterized [3], but the unambiguous identification of the site(s) for the formation of alternative Tyr radical(s) remained elusive. Previous characterizations of single Tyr mutations naturally led to the consensus that once a putative Tyr$^{\bullet}$ site is suppressed (by site-directed mutagenesis) the radical could be formed on other Tyr(s), with the underlying interpretation that formation of Tyr radicals in CcP would be a random event with no specific electron transfer pathway and/or catalytic function involved. Hence, it was important to clarify the situation in order to better understand other heme enzymes [3]. A systematic investigation of the location and reactivity of the Tyr radical(s) using multifrequency (9-285 GHz) EPR spectroscopy and multiple-site Trp/Tyr mutations in CcP allowed us to identified an unprecedented Tyr site (Tyr71) and its catalytic role in substrate oxidation, which also explained the recent report of an unexpected substrate binding-site in CcP by X-Ray crystallography [5]. Our findings reinforce the view that CcP is the mono-functional peroxidase which most closely resembles its ancestor enzymes, the catalase-peroxidases, in terms of the higher complexity of the peroxidase reaction [6]. The strategy used for identifying the elusive Tyr radical sites in CcP may be applied to other heme enzymes containing a high number of Tyr and Trp residues, and for which Tyr (or Trp) radicals have been proposed to be involved in their peroxidase or peroxidase-like reaction.







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Quantum chemical analysis of binuclear iron complexes highly active in H abstraction and nitrogen insertion reactions

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Among well documented series of binuclear iron complexes bridged by a μ-phenolato ligand and exhibiting mixed (N, O) coordination sphere, one complex has attracted our attention recently due to its very high activity towards H abstraction and NR insertion reactions.[1] Thanks to sophisticated spectroscopic and analytical methods, some insights have been gained on its molecular structure and hypotheses about the activation mechanism have been proposed. Most importantly a mixed-valent $Fe_2^{III,\ IV}$ = NTs (Ts = tosylate) species seems to be a key intermediate. Such Fe^{IV} = NR species attracts much interest nowadays, because of its analogy with long-time studied Fe^{IV} = O systems, and because this moiety seems to be critical for reactivity mechanism involving a NR group.[2]

The high reactivity of this intermediate precludes its isolation for further characterizations, and we thus turned to theoretical calculations to get more insight into its structural and electronic properties. We will thus present here DFT approaches used to firstly describe both initial $Fe_2^{II,III}$ and final $Fe_2^{III,III}$ – NHTs binuclear complexes, and secondly to characterize the $Fe_2^{III,\ IV}$ = N-tosylate intermediate. In order to be as reliable as possible, calibrations on homolog complexes have first been performed, completed by the estimation of Mössbauer parameters. A special focus will be made on the proposed $Fe_2^{III,\ IV}$ = NTs, with a careful exploration of the various possible spin states and Fe^{IV} = NTs bonding. Indeed due to a particular coordination environment, both Fe^{IV} high spin state (S = 2) and intermediate spin state (S = 1) were found at very close energies[3]. Some rationale gathering molecular electronic features (Figure 1) and reactivity properties will be proposed in the light of these calculations.

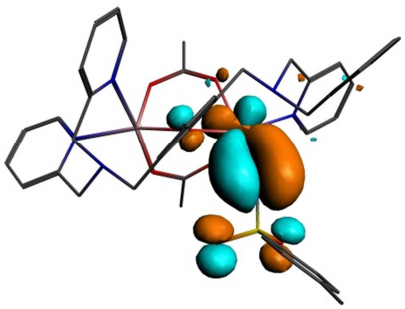

Figure 1. LUMO of electrophilic species $Fe_2^{III,IV}$ – NTs.

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Anchimeric Assistance in the Hydration of Two Nitrile Groups into Carboxamides in Mild Conditions by Temporary Interaction with a Ferrous Center: a Textbook Case.

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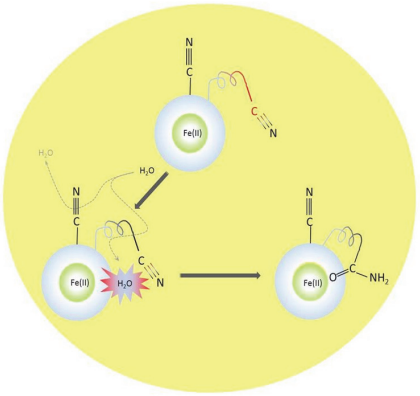

On this poster, we describe the hydration of two nitrile functions into carboxamides by a ferrous [Fe(II)] center in particularly mild conditions and very efficiently, and demonstrate that these unusual conditions result of anchimer assistance at the reaction site. Two bis(cyano-substituted) (tris 2-pyridyl methyl amine) ligands have been prepared and the structures of the corresponding $FeCl_2$ complexes are reported. These two ligands only differ by the position of the nitrile group on the tripod, respectively in α- and β-position with respect to the pyridine nitrogen. In any case, intramolecular coordination is impossible. Upon action of water, the nitrile groups are sequentially hydrated, however only if they are located in the α- position. The fact that the β-substituted β-$(NC)_2$TPA$FeCl_2$ complex is not water-sensitive confirms that the reaction proceeds in an intramolecular way at the vicinity of the metal center. Nitriles are responsible for the electron-deficiency of the ligand, which leads to activation of a coordinated water at the ferrous site. Because of its proximity with the coordination center, a nitrile is in turn activated and ready to undergo hydration in unexpected conditions, i.e. by an Fe(II) center at room temperature, with no need for its coordination. Additionally, in the bis α-substituted α-$(NC)_2$TPA$FeCl_2$ complex, both functions are converted in a very clean fashion, pointing out that this complex exhibits ligand flexibility, and is not deactivated after the first hydration. At a preparative scale, this reaction allows the one-pot conversion of the bis(cyano-substituted) tripod into a bis(amido-substituted) one in particularly mild conditions with a very good yield.

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Recent advances in oxidation catalysis.

Part 1 : Photoactivated artificial metalloenzymes

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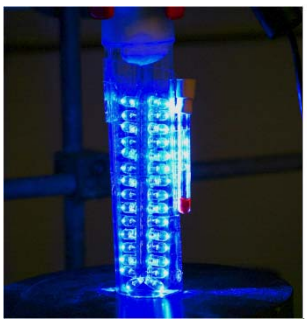
The need for sustainable chemical processes has been demonstrated many times over the last years. In this context the BioCE team of the LCBM is working on the development of new systems able to catalyzed oxidation reaction in an environmentally friendly way. We so decide to work with dioxygen as the oxidant and artificial metalloenzymes as catalysts in order to follow most of the rules of « green chemistry ». Recently, during our studies on the oxidation mechanism of aromatic substrates using the cristallo-kinetic methodology, we designed an artificial metalloenzyme by anchoring an inorganic iron complex into the NikA protein, a Ni transport protein in *E. coli*.[1] The X-rays studies revealed that the artificial metalloenzyme was able to hydroxylate two times the aromatic intramolecular substrate using only dioxygen and DTT. After the two catalysis cycles, the catalysts was inactivated by the coordination of the just created hydroxyl moiety to the iron disabling the oxidation of an exogenous substrate.

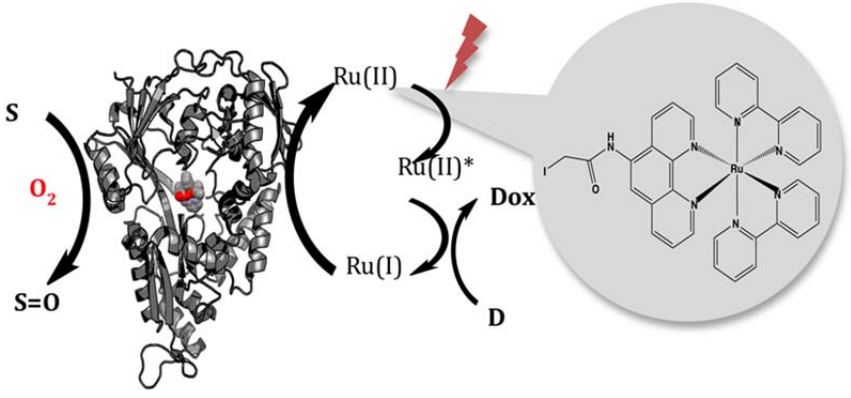
These promising results lead us to modify the inorganic complex by synthesizing new ligands free of oxidation moieties. After having verified that the modified catalysts were still able to activate dioxygen, we then had to demonstrate their ability to transfer the activated oxygen atom to a substrate of interest.

Special attention has been paid on the selection of the reducing system needed to activate the iron catalyst leading us to perform experiments under photocatalytic conditions.

In cristallo kinetic studies have been conducted on crystals of the new artificial metalloenzymes showing a single oxidation reaction on the ligand backbone when placed in a DTT/O_2 medium in the absence of an exogenous substrate.

The poster will present our latest results in this field.





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Novel Near-Infrared Emitting Lanthanide Nanoparticles as Imaging Agent for Living Cell Microscopy

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Near-infrared (NIR) luminescent lanthanides (Ln(III)) are key elements for numerous advanced materials and their corresponding applications, such as biomedical analysis and bioimaging. Since the luminescence intensity of free Ln(III) cations is strongly limited because of their low extinction coefficients, it is important to explore new approaches for controlling the photophysical properties of Ln(III) compounds. In addition, their special optical properties i.e. sharp emission bands, large energy difference between excitation and emission wavelengths and high resistance to photobleaching overcome their existing limitations. In order to obtain a sufficient number of photons per unit volume, Ln(III) ions have to be sensitized by appropriate chromophoric groups which function as "antenna" [1].

The use of nanoparticles allows to locate a large number of antennae and Ln(III) ions within one entity in order to increase detection sensitivity. Moreover, the presence of functional groups on the surface of nanoparticles gives an opportunity to achieve selectivity through attachment of specific targeting biomolecules.

Major challenges in cancer control and treatment are focused on early detection and diagnosis. Since the potassium channels play an important role in controlling physiological and pathological cell proliferation, important progresses have been achieved in their investigation. Particularly, Kv 10.1 potassium channels are expressed in over 70% of human tumors. In addition, these channels due to their extracellular accessibility are considered as effective therapeutic targets[2]. Currently used immunofluorescence techniques for molecular imaging of live tumor cells rely on organic fluorophores and have significant drawbacks: interference with biological autofluorescence, low photostability and inefficient light penetration. We propose NIR-emitting Ln(III)-based nanoparticles as a very promising novel agents for detection and identification of potassium channels.

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Interferences between metallic nanoparticles and metal homeostasis

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Metallic nanoparticles (NP) production has massively increased during the last decade as well as their use in commercial products. A major concern is currently to address the potential toxicity of the NP both on human health and on the environment. As the literature pointed out the involvement of an oxidative stress in the cytotoxicity of some metallic NP, we postulated that metallic NP may interfere with metal homeostasis, which is closely related to oxidative stress response. We thus investigated the molecular mechanisms of the interferences between metallic NP and metal homeostasis using eukaryotic and prokaryotic models, respectively. The interference of CuO nanoparticles with metal homeostasis in hepatocytes under sub-toxic conditions has been analyzed. In that case our results (1) showed how CuO-NP interfere with metal overload, metal exchange on metallothioneins, protein regulation and more generally, copper and zinc homeostasis. The interference between titanium from TiO_2 nanoparticles and iron homeostasis in *E. coli* as prokaryote model was also studied (2) and we obtained original data that will be also presented here.

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Copper chelators targeted to the liver to overcome the copper accumulation related to the Wilson's disease.

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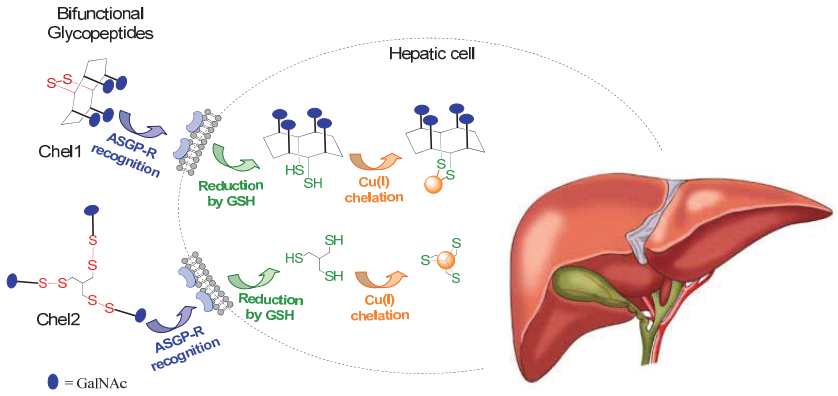
Wilson's disease is due to a disruption of copper homeostasis. In this genetic disorder, the ATP7B protein involved in Cu excretion out of the liver cell is malfunctioning due to a mutation. It results in a Cu overload in the liver, which is the key organ for Cu delivery and excretion in the body. This accumulation leads to the destruction of the tissues by oxidative stress. Current therapies mainly aim at limiting Cu absorption and enhancing its excretion but there are many side effects due to their lack of specificity.

In this context, we propose an innovative strategy that would selectively detoxify copper in liver cells. Since excess intracellular Cu is in the +I oxidation state, we figured that a chelator that would enter the hepatic cells and be specific for Cu(I) could represent an efficient strategy. Two bifunctional molecules Chel1 and Chel2 that contain a Cu(I) chelating unit associated to liver targeting functions have been obtained. They have been demonstrated to enter hepatocytes and to induce intracellular Cu chelation.[1]

The chelating units are cysteine-rich compounds inspired from proteins involved in Cu homeostasis. Their thiol functions coordinate Cu(I) with very large affinities and selectivities toward zinc. Importantly, the bifunctional compounds Chel1 and Chel2 can be considered as prodrugs since the coordinating thiol groups are hidden in disulfide bridges, which are reduced only after entering the targeted cells. The targeting units are directed to an abundantly and exclusively expressed lectin located at the surface of the hepatocytes: the asialoglycoprotein receptor (ASGP-R). This receptor is known to specifically recognize galactose and even more efficiently N-acetyl-galactosamine (GalNAc). The internalization of Chel1 and Chel2 in hepatic cells by endocytosis is allowed by their functionalization with several GalNAc residues in an appropriate geometry.[2,3]

These two molecules have different Cu chelating efficiency in vitro but demonstrate the same efficiency in hepatic cells. This effect may come from the ability of the two compounds to enter the targeted cells. Therefore, we compared the internalization efficiency of Chel1 and Chel2 in human hepatocytes by flow cytometry to compare the efficiency of the two targeting systems.



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Superoxide reductase as a model for oxygen activation

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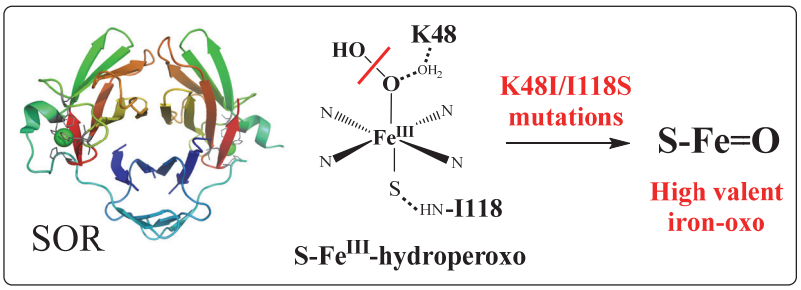
Superoxide reductase (SOR) is a non-heme iron enzyme involved in superoxide radical detoxification in microorganisms. Its active site consists into an atypical [FeN_4S_1] square pyramidal pentacoordinated iron center, where the N ligands are provided by four histidines and the S ligand by a cysteine in the axial position.[1,2]

Interestingly, SOR presents at least two striking similarities with cytochrome P450 oxygenase: a Fe^{3+}-OOH intermediate in its catalytic cycle and a [FeN_4S_1] coordination. However, unlike cytochrome P450, SOR does not cleave the O-O bond of the Fe^{3+}-OOH unit to generate a high-valent iron-oxo species, but rather cleaves the Fe-O bond to form its reaction product H_2O_2.[2,3]



Here we show that in SOR, the mutation of two second coordination sphere residues leads to the formation of a high-valent iron-oxo species in its active site, when the protein was reacted with H_2O_2.[4]

These data demonstrate that the evolution of the Fe^{III}-OOH intermediate and the cleavage of the Fe-O bond instead the O-O one is tightly controlled by different second coordination sphere residues.

Studies on the reactivity of the different SOR mutants as catalysts in oxidation reactions will be presented.[5]

This work illustrates the fact that SOR can be used as an unprecedented model to study the mechanisms of oxygen activation in metalloenzymes.

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Studying the reactivity of different Cu(II) complexes towards biomolecules

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Metal complexes have acquired in the last decades increasing interest to be used as chemotherapeutic or diagnostic agents. The serendipitous discovery of cisplatin in the 20th century led the medicinal inorganic chemistry to the front of the fight against cancer. Despite of its high efficiency against several kinds of cancer, the use of cisplatin is limited because side effects such as neurotoxicity, emetogenesis and nephrotoxicity[1]. Moreover, inherent or acquired resistance is a common problem in several Pt-based anticancer compounds. Therefore, non-platinum metal complexes have been increasingly tested in order to find more efficient anticancer drugs, with less toxicity and high affinity and specificity towards DNA. Ru, Au, Ti, Co, Fe or Cu have recently appeared as alternative and promising strategies[2].

Copper is a well-known essential element in living beings. It is involved in many crucial biological reactions and takes part in oxidation and reduction processes, growth and development. Nevertheless, high concentrations of copper in living organisms are also toxic for cell processes. Copper toxicity comes from the fact that it is able to generate reactive oxygen species (ROS) due to the interesting redox couple Cu(I)/Cu(II), displace other metals from their binding sites, and directly cleave nucleic acids such as DNA[3]. There is no doubt that copper and its related complexes appear as promising compounds of interest. Being essential for humans, it might be less toxic than other non-essential elements such as platinum and the resulting platinum-based compounds. Therefore, copper complexes may act with apparently less side effects[4].

According to some studies, N-donor ligands enable copper complexes to interact with biomolecules. Moreover, bidentate planar chelating ligands have been reported to intercalate DNA[4]. Four already known copper(II) complexes, which are depicted in figure **1**, have been synthesised. Three of them were firstly reported for their potential applications in the treatment of Alzheimer's disease[5] and the other one has been studied for its applications in catalysis[6]. Now, their reactivity as possible anticancer compounds has been tested. This work studies the interaction of these four copper complexes with different proteins and DNA by UV-VIS, CD and ESI-MS experiments.

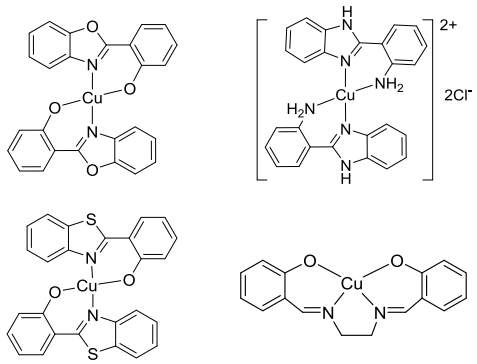

Figure **1**. The four studied copper(II) complexes.

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Near-infrared emitting lanthanide metal-organic frameworks for novel modalities of biological imaging

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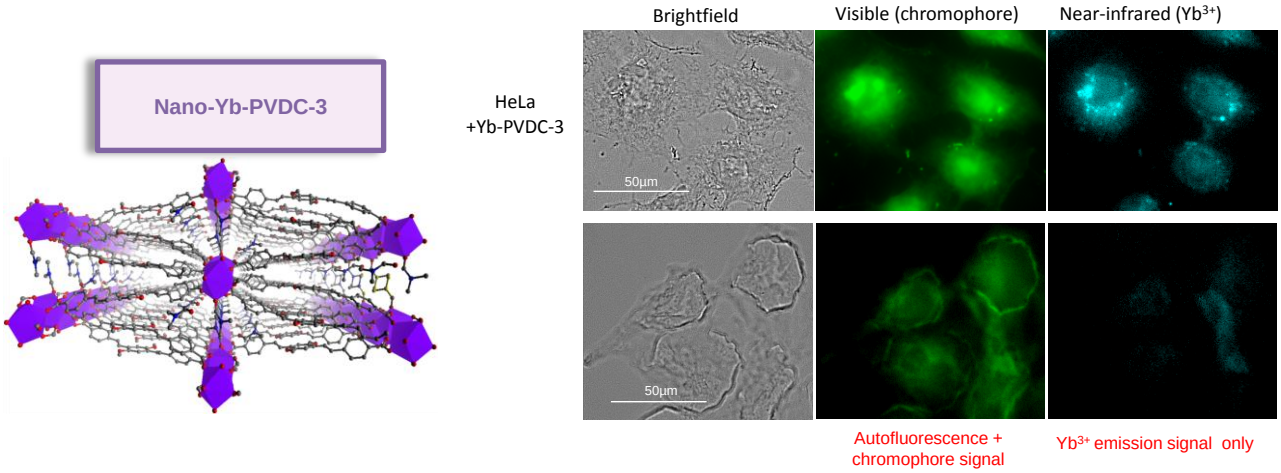
Fluorescence and luminescence are detection techniques that possess important advantages for bioanalytical applications and biologic imaging: high sensitivity, versatility and low costs of instrumentation. A common characteristic of biologic analytes is their presence in small quantities among complex matrices such as blood, cells, tissue and organs. These matrices emit significant background fluorescence (autofluorescence), limiting detection sensitivity.

The luminescence of lanthanide cations has several complementary advantages over the fluorescence of organic fluorophores and semiconductor nanocrystals, such as sharp emission bands for spectral discrimination from background emission, long luminescence lifetimes for temporal discrimination and strong resistance to photobleaching. In addition, several lanthanides emit near-infrared (NIR) photons that can cross deeply into tissues for non-invasive investigations and that result in improved detection sensitivity due to the absence of native NIR luminescence from tissues and cells (autofluorescence).

The main requirement to generate lanthanide emission is to sensitize them with an appropriate chromophore ("antenna effect").

An innovative concept for such sensitization of NIR-emitting lanthanides is proposed herein; the current limitation of low quantum yields experienced by most mononuclear lanthanide complexes is compensated for by using a large number of lanthanide cations and by maximizing the absorption of each discrete molecule, thereby increasing the number of emitted photons per unit of volume and the overall sensitivity of the measurement.[1,2] To apply this concept, we have created several metal-organic frameworks and succeeded in generating highly emissive NIR MOF reporters. We will discuss their structures, photophysical properties. We have optimized their sizes in order to make them compatible for cellular optical imagery. With this work, we report for the first time the use of NIR photons arising from lanthanide cations for cellular imaging.



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Luminescent dendrimers lanthanides complexes for NIR bio-imaging

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Lanthanide ions have unique photophysical properties which allow them to be used in several application domains from materials science, communication technology to biological imaging. For imaging purposes Ln(III) ions are good candidates especially due to their sharp emission bands spanning from the UV-vis to the NIR region, long excited states lifetimes and resistance to photobleaching. These properties allow better discrimination of the emission signal from the autofluorescence background. However, to obtain strong lanthanide luminescence, Ln(III) ions need to be sensitized by an appropriate chromophore and surrounded by a protective environment to reduce deactivation processes due to high-energy vibrations (water molecules).

For this purpose, we have developed Ln(III) complexes based on derivatives of polyamidoamine dendrimers. The presence of functional groups at the periphery and the multivalent character of the branches of these dendrimers allow their functionalization with a large number of chromophores and insertion of several Ln(III) ions into the same macromolecule. This approach permits to overcome the classical low luminescence quantum yield in the NIR range by enhancing the number of photons per unit volume.

Here we report generation 3 polyamidoamine dendrimers functionalized with thirty-two organic chromophores acting as antennae which absorb the excited light and transfer it to eight Ln(III) ions incorporated into the dendrimer branches. The photophysical properties of theses chromophores can be optimized for NIR imaging by modifying the backbone and the substituents. Moreover, we have been able to further modify the periphery of the dendrimer by introducing targeting moieties in order to enhance water solubility and biocompatibility. Complexation ability of the polyamidoamine dendrimers towards Ln(III) ions and photophysical properties of the resulted Ln(III)-dendrimer assemblies in the visible and the NIR ranges have been investigated. Moreover, luminescence microscopy experiments confirmed the potential of designed Ln(III)-dendrimers for bio-imaging applications.

Photoswitchable metal complexes: design and DNA binding properties

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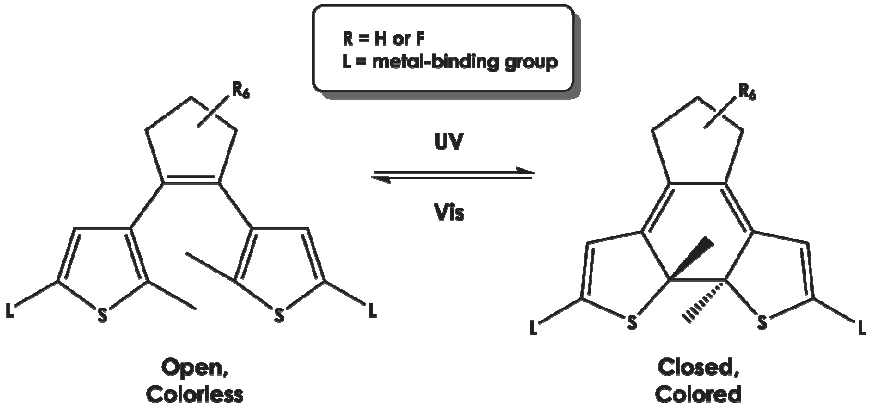
Dithienylethene-based compounds of the type illustrated in the figure below are well known for their potential use as molecular switches, as they undergo reversible ring closure upon irradiation with UV or visible light[1-3], giving rise to their contraction or expansion, respectively. Surprisingly, their use for biological applications has not yet been extensively studied.

Here, we present a new series of photoswitchable coordination compounds obtained from symmetrically-functionalized dithienylcyclopentene ligands. The open and closed forms of these metal complexes not only exhibit interesting optical properties, but also show clearly distinct DNA-interacting behaviours.



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Tidbits for the preparation of polyaminocarboxylate chelates used to design LnIII based near-infrared luminescent zinc finger probes

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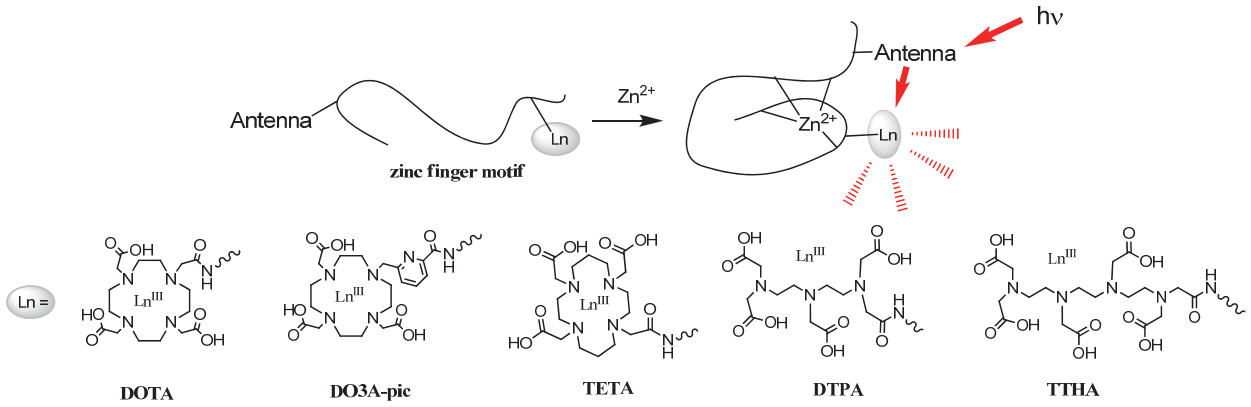

Metal ions are essential for life. In particular Zn^{2+} is widely required in cellular functions, and its dysregulation is implicated in neurodegenerative diseases or cancer[1]. A major comprehension of the physiological role of zinc required new tools to image zinc and its flux in living organisms. Interestingly, a class of proteins named zinc fingers binds specifically Zn^{2+} in which the metal plays a structural role contributing to the stability of the domain. Advantageously, we can exploited this property to design new smart zinc probes operating on the basis of Ln^{3+} ions emitting in the near-infrared and of zinc finger peptides for the selective binding of Zn^{2+} to ensure specific and sensitive response of the probe.

The design of these luminescent probes involves the grafting of Ln^{3+} complex on a zinc finger peptide. In this communication, we will report on the efficient synthesis of macrocyclic derivates based on cyclen, cyclam or triethylene (tri or tetra) amine. Preliminary models of zinc probes were developed using DOTA or DTPA as ligand for Eu^{3+} or Tb^{3+} lanthanides. Nevertheless, in the case of near- infrared lanthanide (Yb^{3+}, Nd^{3+}) their luminescence can be quenched by the surrounding water molecules in the coordination sphere and so required other ligands (TETA, DO3A-pic, TTHA)[2,3] giving an efficient shielding of the metal ions.

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Recent advances in oxidation catalysis.

Part 2 : New artificial metalloenzymes with a ruthenium core

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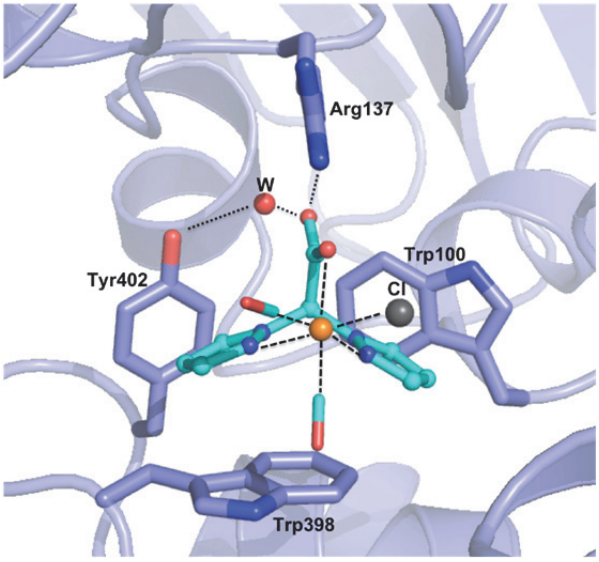
Enantiopurity of drugs is still a need, nevertheless, nowadays, lots of processes are still leading to a mixture of the both enantiomers. In this context, the BioCE team of the LCBM is working on the development of new systems able to selectively oxidize substrates : artificial metalloenzymes (ArMs). In fact, an ArM is composed of two subunits: an inorganic catalyst and a globular protein having a hydrophobic pocket.[1, 2] The former, anchored within the protein, regulates the reactivity *via* an active metal complex. Regarding the host protein, it can modulate the reaction selectivity due to its polypeptide scaffold.

Parallel to the work currently under investigation in the laboratory upon the design of artificial oxygenases (see poster Part 1), we have decided to elaborate new artificial oxygenases with a ruthenium core. Through a collaboration with Prof. Burzlaff, Erlangen, Germany, we performed the insertion in NikA, a Ni transport protein in *E. coli*, of two ruthenium (II) complexes where the ruthenium (II) atom is coordinated to a bis-(pyrazol-1-yl)acetate (bpza) or a (3,5-dimethylpyrazol-1- yl)acetate (bdmpza). These two ruthenium complexes have been selected because they model the active site of oxygenases showing a similarity with the 2-His-1-carboxylate facial triad motif. Moreover, similar scorpionate complexes were capable of epoxidation catalysis, in organic media, in the presence of iodobenzene diacetate.[3]

First, we have demonstrated the possibility to anchor successfully the complexes [Ru(bpza)$(CO)_2$Cl] or [Ru(bdmpza)$(CO)_2$Cl] into the binding site of NikA. Then, the reactivity in the oxidation catalysis of alkenes by the corresponding hybrids was undertaken.
The poster will present our latest results in this field.



X-rays structural analysis of the [Ru(bpza)$(CO)_2$Cl] complex anchored in NikA.

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Development of Bio-inspired Macromolecular Systems for Catalytic oxidation.

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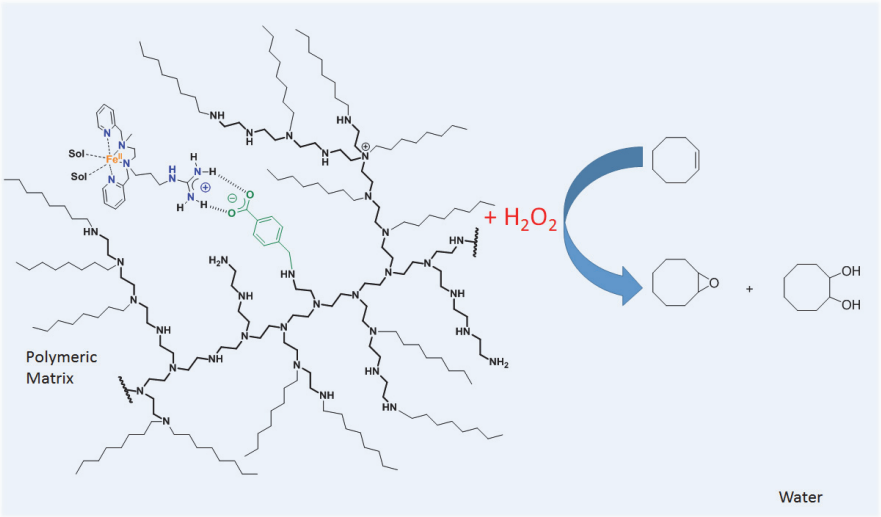
The development of catalytic systems that are able to directly use molecular dioxygen as oxidant is a major challenge in order to bring selective oxidation processes to cleaner spheres. Only a few examples of iron complexes have been demonstrated to activate molecular dioxygen and transfer an oxygen atom into the C-H bond of a substrate.[1,2] However, none of them can work as proper multiple-turn-over catalyst, since they all lack an efficient source of electrons and protons. Furthermore, most of these systems have been developed in organic solvents, which constitutes a major problem for the chemical industry in terms of waste treatment. The development of new processes using water as solvent is therefore another important detail to take into consideration. It is in this context that our project is aiming at developing water soluble artificial catalytic systems taking into consideration the three major characteristics of these enzymes:

(i) Activation of molecular dioxygen,
(ii) Specific input of electrons,
(iii) Availability of protons during catalysis.

In order to reach our goal, we plan to take lessons from Mother Nature and design macromolecular catalytic systems composed of both a catalytic center to activate O_2 and a redox cofactor to deliver electrons accurately. These two cofactors will be kept in proximity by a water soluble polymeric matrix, that will also provide a local hydrophobic environment and a potential source of protons located on the amines of the polymer backbone. However, this work has to be undertaken step by step in order to validate the different points. In this aim, the work has been started by comparative studies of the catalytic activity of the metal complex catalysts such as mononuclear or binuclear iron complexes and iron porphyrin in acetonitrile and water with H_2O_2 as oxygen atom source. Then, influence of polymeric matrix will be studied in water by incorporating the catalysts into the polymeric matrix. So far, the polymeric matrix and mononuclear iron(II) complexes have been synthesized and characterized respectively by NMR and ESI-MS studies coupled with X-Ray diffraction analysis. These complexes have been tested for catalytic oxidation with H_2O_2 as oxygen atom source in acetonitrile and water. Moreover, the influence of polymeric matrix on the catalytic yield was studied in water.



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New strategy in Pt-complexes design

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Platinum complexes, such as cisplatin and carboplatin, are widely used in chemotherapy since some time ago, due to its anticancer activity,. DNA is generally accepted to be the target for platinum-based drugs[1], which induce structural modifications on the helix, thus promoting apoptosis. However, in their uptake and biodistribution processes some interactions of platinum-complexes with proteins occur, causing side effects. Moreover, before the drug arrives to the target, part of the administrated dose is lost, producing a decrease in its efficiency. This leads to drug resistance in some tumours. For that reason, it is necessary the development of nobel platinum-drugs with lower protein interaction and higher DNA affinity. The study of these interactions can be achieve by using different techniques, but in recent years, electrospray ionization mass spectrometry (ESI-MS) has emerged as an extreme valuable and powerful method to monitor the interaction of metal-based drugs and proteins. In our group, the interaction of dmba (N,N-dimethylbenzylamine)-based Pt complexes, which showed sub-micromolar activity in several human tumour cell lines, with some proteins has been studied by ESI-MS. The interaction of the platinum complexes with a designed oligonucleotide, containing the GG Pt binding site motive, and its complementary strand has been also monitored by mass spectrometry[2,3].

On the other hand, the use of light sensitive complexes has also been established as a good strategy in order to avoid the mentioned side-effect problems. Azobenzenes have the particularity of switching from *cis* (Z) to *trans* (E) configuration and viceversa using UV light, which allows the control of this isomerization by irradiation.

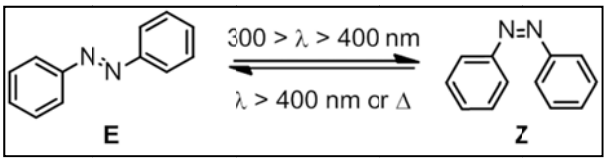

Figure 1. Azobenzene isomerization.

We have designed a new putative antitumoral agent, which consists in a mononuclear platinum complex with an azobenzene ligand functionalized with S- and N-donor atoms, as well as the dmba ligand to stabilize the complex. The azobenzene ligand, in its *cis* form, can act as a chelate ligand. The radiation-controlled switch from the initial *cis* to the *trans* configuration of the complex would presumably increase the activity of the drug in the irradiation site, by rendering an available position in the platinum environment. Hence, the activity of the complex could be controlled by irradiating the site where the drug-action is required. In this work we present the synthesis the platinum complex and the earlier studies of its behaviour.

The financial support received from MINECO-FEDER (Project BIO2012-39682-C02-02) is acknowledged.

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Reinforced ligands for implementation in oxidation catalysis

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Numerous synthetic models of iron monooxygenases have been reported for decades. These complexes have been very useful for the generation and spectroscopic characterization of reaction intermediates such as high valent Fe-Oxo, Fe(III)-peroxo and even Fe(III)-superoxo.[1] In contrast, rare are the complexes able to catalyze the oxidation of small organic molecules with efficiency and large turnover numbers. One major drawback to these systems is the oxidative degradation of the ligand during the catalytic reaction. Collins et al. devised an iterative method to identify and then replace the oxidation sensitive groups in their ligands leading to the TAML family.[2] These latter are efficient peroxide activators that can be used for water treatment for example.

In previous studies we have shown that (TPEN)Fe^{IV}-oxo is a good epoxidizing agent. However, a competitive bimolecular oxidative cleavage of the ligand was identified, probably leading to a lowered activity of the catalyst (cf. Figure).[3] The use of the ligand L624E instead of TPEN modulated the bimolecular cleavage but did not inhibit it.[4]

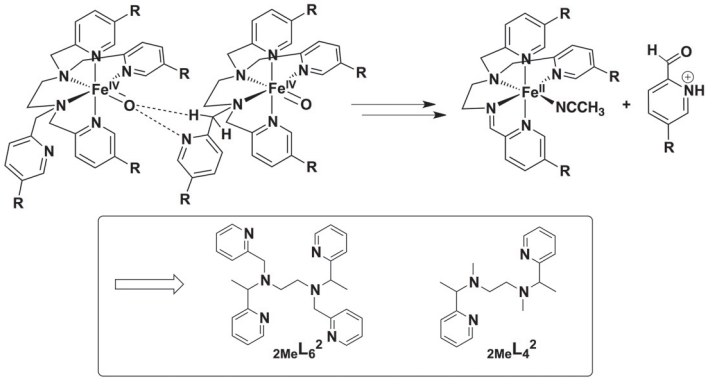

Figure. (top) Bimolecular oxidative cleavage of the ligands identified for Fe(IV)O complexes (R=H : TPEN; R=Et $L_6^2$4E); (bottom) new ligands used in this study.

In order to prevent this undesirable reaction, we have tackled chemical modifications of the ligands. Then, the synthesis of the hexadentate and tetradentate ligands 2MeL62 et 2MeL42 (cf. Figure) has been performed and their coordination chemistry with Fe(II) has been studied. These results will be presented as well as new the reactivity of the Fe(II) complexes in the presence of chemical oxidants and several organic substrates.

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Enhanced and selective DNA cleavage activity of redox-active metallopeptides based on PyTACN and BPBP ligands

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The development of artificial nucleases for the cleavage of phosphodiester bonds in DNA is a research area with great interest in multiple fields such as gene regulation, cancer therapy or protein mapping.[1] However, despite significant advances, the improvement of DNA cleavage while achieving specific DNA-recognition still remains as a challenge in chemical biology. In this context, peptides have been demonstrated to recognize specific sites in DNA. In particular, the recognition specificity of positively charged short peptides, such as SPKK or KWKK, to the minor groove of the DNA has been reported.[2] Within this regard, we focused our attention on the conjugation of complexes of non-toxic metals, such as Fe, Cu or Mn, containing the tetradentate ^{Me2}PyTACN and BPBP ligands to the cationic LKKL tetrapeptide sequence. Metal complexes containing the former tetradentate ligands have been described by our group as excellent catalysts for several oxidation reactions on a wide substrate scope with high efficiency and remarkable selectivity under mild experimental conditions, environmentally non aggressive.[3] Hence, the conjugation of these redox-active complexes to the tetrapeptide could be an attractive model for the design of novel nucleases.

Herein, we report the solid-phase synthesis, characterization and DNA cleavage studies of redox-active metallotetrapeptides incorporating ^{Me2}PyTACN and BPBP ligands. The preparation of the metal binding peptides started from the attachment of the 2-pyridylmethylene moiety to the tetrapeptidyl resin. Subsequent derivatization with the corresponding secondary amine on the solid support provides flexible incorporation of the corresponding metal-binding ligand to the LKKL peptide backbone. After acidolytic cleavage and further basification, metal complexation in solution affords the final metallotetrapeptides. Metal binding to ^{Me2}PyTACN and BPBP ligands was studied by HRMS (ESI), NMR and UV-VIS. Next, the resulting metallopeptides were screened with pUC 18 DNA in order to study the nuclease activity as well as their selectivity towards DNA. Interestingly, DNA cleavage studies of the redox-active metallopeptides showed that attachment of the ^{Me2}PyTACN and BPBP-based complexes to the LKKL peptide sequence resulted in an enhancement of the nuclease activity. Furthermore, the presence of the LKKL induced an improved binding affinity to the DNA minor-groove. This study puts forward the opportunity to obtain minor-groove selective nucleases.

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Ligand-centered redox activity in bis(phenolate)–Dipyrrin Complexes

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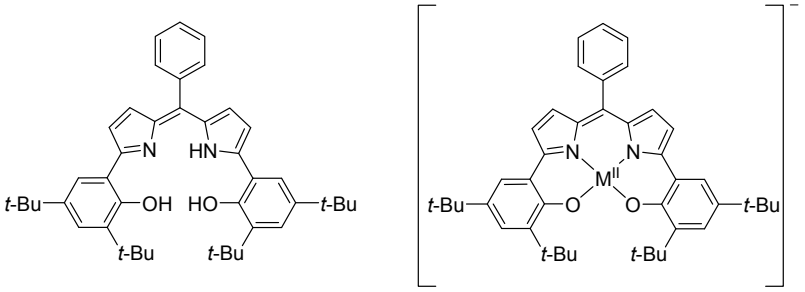
Metal-coordinated ligand radicals are found naturally in the active site of a number of metalloenzymes, some of them being essential for life. The most ubiquitous bio-radicals are porphyrinyls (as in cytochrome P450)[1] and phenoxyls (as in galactose oxidase).[2] These two classes of radicals are formed by oxidation of either coordinated cofactors or amino-acids and exhibit distinct properties, stabilities, and biogeneration pathways. In principle, one-electron oxidation of a M^{n+}–L species involving a redox-active ligand leads to either $M^{(n+1)+}$–L or the species M^{n+}–$L^{\bullet}$. Investigations on prototypical M^{n+}–phenolate and M^{n+}–porphyrin systems reveal that several interdependent factors such as the solvent, temperature, nature of the metal ion, and substituents may influence the site of oxidation. Bloom and Garcia in the early 1970s introduced a class of ligands that combine a "half-porphyrin" (dipyrrin) and two phenol moieties in a single and highly electron-rich trianionic ligand.[3] We will show in this communication that this ligand system supports a ligand centered redox activity. Full delocalization of the singly occupied molecular orbital (SOMO) gives the ligand an unprecedented hybrid porphyrinyl–phenoxyl radical character. It is highly stable and could be isolated as single crystals for structural characterizations.[4]



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Metal-based DNA-interacting agents from photoswitching ligands

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Photoactivated Chemotherapy (PACT) drugs, often based on transition-metal complexes, allow an enhanced control of their biological activity, thus reducing undesired side effects.[1] Dithienylcyclopentene (DTEs) molecules, also called bisthienylcyclopentenes, diarylethenes or dithienylethenes, undergo thermally irreversible cyclization reactions between open and closed forms when stimulated with radiation: UV light forces the "closing" of the molecule, whereas the open form is obtained under visible-light exposure[2] (see Fig. 1). This opening/closing process generates two molecules with distinct properties; for instance, an expansion (open form) or a contraction (closed form) of the molecule is observed. Furthermore, a modification of the optical/electronic properties takes placewhen the molecule is converted into one another.

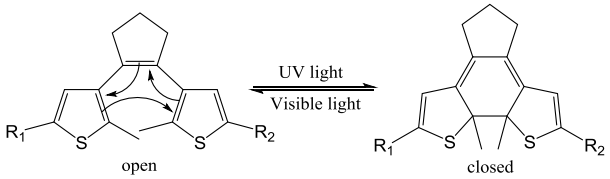

Fig. 1. Light-driven, thermally irreversible cyclization reaction between open and closed forms in DTE molecules.

DTEs are typically prepared *via* Suzuki-Miyaura cross-coupling reactions,[3] between a boronic ester derivative (that can be generated *in situ*) and an aryl bromide, in the presence of $[Pd(PPh_3)_4]$ under basic conditions (Fig. 1). Hence, photoswitchable arylated ligands may be produced through the formation of such a C-C bond. In the present work, several photoswitching Pt(II) and Ru(II) complexes obtained from DTE-based ligands are presented together with their DNA-interacting properties, which differ depending on their open or closed form.

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Tetranuclear Self-Assembled Clusters: Toward Luminescent Biomarkers

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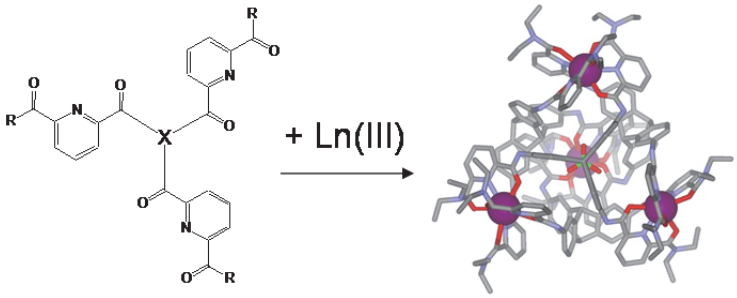
Lanthanides complexes have already been used for applications in several domains such as lightening devices, medicine and imaging agents for biological research and medicinal diagnostic (i.e., MRI and SPECT). Due to their unique luminescents properties (long luminescence lifetimes, sharp emission bands, large energy difference between the absorption and emission bands), lanthanide-containing systems can also be advantageously used for optical imaging in biology, especially by using near-infrared light, which is relatively transparent in biological tissues. Moreover, biological tissues do not emit significant near-infrared autofluorescence. This specific application requires a good sensitisation of metal-centred emission in aqueous media. A suitable chemical system must be thus carefully designed in order to fulfil several criteria.

In our laboratory, we focus on exploiting different types of multimetallic systems, where organic antennas are combined with several metallic cations in order to collect a high visible/near infrared light intensity for optical imaging. In this contribution, we present a supramolecular approach to achieve this purpose. The strategy consists in the tridimensional incorporation of four lanthanide cations in a well-defined discrete self-assembly.[1] The required tripodal ligands were synthetised by coupling an aromatic amine with modified tridentate binding moieties in order to increase the solubility. The resulting lanthanide complexes were characterised with different structural and physico-chemical methods. The luminescent properties of the corresponding europium and ytterbium complexes were studied. Through this detailed analysis, we show that the proposed system is promising for applications in cellular imaging.



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Molecular mechanism of nitric-oxide synthases

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Nitric oxide (NO) is a highly diffusive and reactive gas that was long thought to be toxic. However it has been found to be essential for the proper functioning of the body, as it is involved in neurotransmission, blood pressure regulation and activation of the immune system. In mammals, NO is synthetized by enzymes called NO-synthases (NOS). Three NOS isoforms – neuronal (nNOS), endothelial (eNOS) and inducible (iNOS) – have been identified, that differ in their primary sequence, function and localization.

NOS are flavohemoproteins that catalyze the stepwise oxidation of L-arginine to L-citrulline and NO, with N^{ω}-hydroxy-L-arginine as a stable enzyme-bound intermediate. This reaction consumes 1.5 equivalents of NADPH and 2 equivalents of O_2. NOS are multidomain proteins, comprised of a reductase domain, that binds FMN, FAD and NADPH, and an oxygenase domain, that binds heme, the substrate L-arginine and the cofactor (6R)-5,6,7,8-tetrahydro-L-biopterin (H_4B), linked by a calmodulin-binding domain. This redox active H_4B cofactor is essential to couple oxygen activation and substrate oxidation[1]. Uncoupled reactions yield reactive nitrogen and oxygen species (RNOS) that induce oxidative stress which can lead to several pathologies, such as cardiovascular diseases, diabetes or neurodegenerative diseases.

More recently, massive genome sequencing lead to the discovery of hundreds of NOS-like proteins (NOS-LPs) throughout the entire living kingdom. In the last decade, several bacterial NOS-LPs (bacNOSs) have been characterized and shown to resemble their mammalian counterpart[2]. They have thus been extensively used as models to study the mammalian enzymes. However bacNOSs exhibit significant differences from mammalian NOSs- the most striking one being the lack of a reductase domain. Moreover a comparative study conducted in our lab between a bacterial NOS-LP from *Bacillus subtilis* (bsNOS) and iNOS showed differences in behavior during the 2nd step of catalysis[3].

Here we focus on three bacterial NOS-LPs – from *Bacillus subtilis* (bsNOS), *Deinococcus radiodurans* (deiNOS) and *Geobacillus stearothermophilus* (gsNOS) – as well as on the I224V bsNOS mutant and its mammalian counterpart V346I iNOS. We aim at further characterizing these enzymes and their catalytic mechanism(s). To do so, we use rapid kinetic technics (stopped-flow, rapid-freeze quench) to trap and identify intermediates and to calculate their rate of formation and disappearance. Most NOS-LP-containing bacteria being unable to synthesize H_4B – including *Deinococcus radiodurans* – we also test the influence of tetrahydrofolate, which could be the native cofactor of these enzymes[4]. Finally the use of substrate and cofactor analogues will allow us to further probe the molecular mechanism of NO-synthases.

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Toward the imaging of micro-objects with electrogenerated chemiluminescence

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Electrogenerated chemiluminescence (ECL) is an analytical technique where chemiluminescence is obtained through the electrochemical generation of very reactive species[1, 2]. Specifically, it involves the generation of species at electrode surfaces that then undergo electron-transfer reactions to form excited states that emit light when returning to ground state. ECL is a technique of interest because no light source is required for the formation of excited states, preventing background signal coming from light source or autofluorescence. The most studied system so far is the ECL of $Ru(bpy)_3^{2+}$ (where bpy = 2,2'-bipyridine)[3-5]. For example, application of a highly oxidizing voltage to an electrode in the presence of $Ru(bpy)_3^{2+}$ can result in light emission through the complex reaction with a coreactant in aqueous solution. Most widely used coreactants are aliphatic amines like TPrA (tripropylamine) or other amine containing products like DBAE (2-(dibutylamino)ethanol).

The synthesis of ECL compounds for new applications is still needed. For example, the synthesis of a new family of ligand such PYTA in our group to produce Ruthenium complex for IR imagery of cells open a versatile route for the design of new Ruthenium complexes that can be used for ECL measurement[6]. These ligands are produced by simple and efficient click chemistry. New Ruthenium compounds were synthesized and their luminescence and electrogenerated chemiluminescence were characterized.

Then we decided to use ECL for the imaging of micro-objects such as polystyrene microbeads, when the beads are modified with Ruthenium complexes. Our goal is to understand how the ECL emission can occur and develop at the microbeads surface. Because of the complicated ECL mechanism, so first we focused at the understanding of the electrochemical signal from the modified beads. Several beads modifications were performed either with Ruthenium or Osmium complexes and their electrochemical behaviours were investigated.

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P02	Baffert Carole	Low potential inactivation of FeFe hydrogenases
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P04	Bertrand Hélène	Luminescence modulations of Rhenium tris-carbonyl complexes induced by structural variations
P05	Botz Alexandra	DNA recognition by lanthanide-binding hexapeptides
P06	Ceccaldi Pierre	Reductive activation of *E. coli* Nitrate Reductase
P07	Chaignon Jérémy	Manganese complexes for catechol oxidation: from synthesis to incorporation on a mesoporous support
P08	Cheignon Clémence	Oxidation of the Beta-Amyloid peptide catalyzed by copper
P09	Ching Vincent	High-field pulse EPR nanometre-scale distance measurements of paramagnetic centres
P10	Delangle Pascale	Rational design of peptide scaffolds coordinating uranyl
P11	Demay-Drouhard Paul	Synthesis of platforms with constrained distance between pyridine-based Mn(II) complexes for EPR studies
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P13	Ghattas Wadih	Receptor-based multipotent artzymes
P14	Grimaldi Stéphane	Reactivity of the molybdenum cofactor in Nitrate Reductase as probed by hyperfine spectroscopy
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P41	Zhu Jing	Toward the imaging of micro-objects with electrogenerated chemiluminescence

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