Conférences

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Groupe français de chimie bioinorganique
Murol 2016

Perspectives nouvelles en biocatalyse : des enzymes artificielles pour la biologie de synthèse.

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La biologie de synthèse a fait récemment son entrée dans les laboratoires de recherche avec l'objectif de récréer ab initio une cellule vivante pour construire de nouveaux organismes capables d'accomplir des fonctions d'intérêt dans divers domaines. Dans le domaine de la biocatalyse, des gènes codants pour des enzymes bien identifiées sont introduits dans des cascades métaboliques pour produire des molécules d'intérêt thérapeutique. Pour réaliser cet objectif, il est fait appel à la panoplie des transformations chimiques présentes dans les organismes vivants. La récente synthèse biocatalysée de l'artémisinine en est une brillante illustration.

De son côté, pendant plus d'un demi-siècle, la chimie synthétique a quant à elle développé des réactifs de plus en plus sophistiqués pour réaliser des transformations non catalysées par le vivant. Dans cette approche, la panoplie de ces réactions est bien plus riche et d'une plus grande diversité. En ce début de 21e siècle, la chimie se trouve à la croisée des chemins où la manipulation du vivant rend possible le développement d'enzymes artificielles capables de réaliser des transformations chimiques non classiques et enrichir ainsi la panoplie du vivant. Ce sont ces nouveaux développements que nous tenterons de décrire en les mettant en perspectives avec la biologie de synthèse.

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Nouveaux chélates azamacrocycliques pour le diagnostic et la thérapie des cancers : du concept à l'image

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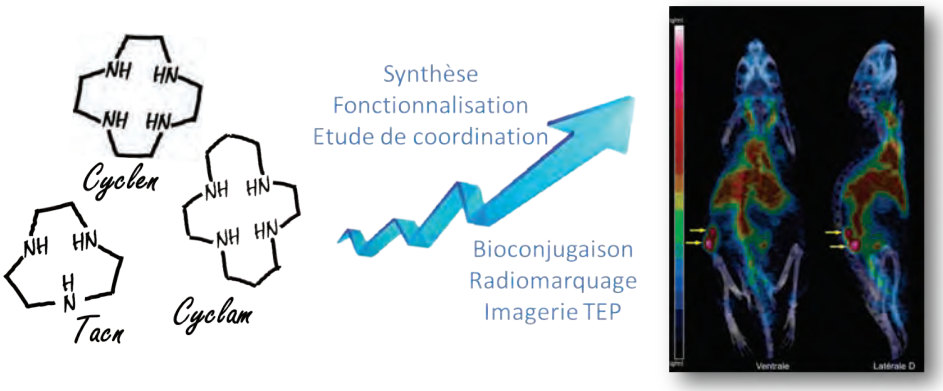
La Médecine Nucléaire ouvre actuellement de nouvelles perspectives pour le diagnostic et l'imagerie fonctionnels des tumeurs, pour leur caractérisation, celle de leur environnement et pour la thérapie. Les agents d'imagerie et de thérapie traditionnels ont été complètement remplacés ces dernières années par les agents d'immunoscintigraphie d'imagerie par Tomographie par Emission de Positons (TEP) ou la RadioImmunoThérapie (RIT). Ces techniques sont des outils précieux pour le développement et la validation de nouvelles molécules thérapeutiques.
Devant la réussite récente de telles techniques, une envolée spectaculaire a été remarquée en matière de vectorisation de radionucléides tant pour l'imagerie TEP que pour la RIT en développant de nouveaux radiopharmaceutiques, véritables « taxis moléculaires » basés sur des isotopes radioactifs chélatés par des molécules organiques. Le principe repose sur l'injection au patient du complexe métallique par l'intermédiaire d'une biomolécule vecteur, spécifique des antigènes exprimés à la surface des cellules tumorales ou de zones anatomiques particulières. De la nature du radioisotope dépendra l'application : imagerie TEP (émetteur β+ : 64Cu, 68Ga…) et radioimmunothérapie (émetteur α ou β- : 212Pb, 212, 213Bi, 67Cu, 90Y…).
Les dérivés bifonctionnels des polyazacycloalcanes font partie des agents chélatants les plus prisés en médecine nucléaire. Ils présentent le double rôle de complexer le radioélément et de se lier facilement au vecteur immunospécifique. Comparés à d'autres ligands, ces macrocycles, souvent basés sur des plateformes tacn, cyclen ou cyclam, ont l'avantage d'être fonctionnalisables « sur mesure » selon le métal ciblé. Ils répondent ainsi complètement au cahier des charges de l'application offrant une cinétique de métallation rapide, une stabilité thermodynamique et une grande inertie en milieu compétiteur ou bioréducteur.
Les dernières avancées de notre équipe dans le domaine seront présentées notamment au travers de travaux sur le développement de nouveaux radiopharmaceutiques du 64Cu pour l'imagerie TEP.



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Des bactéries qui ne perdent pas le nord

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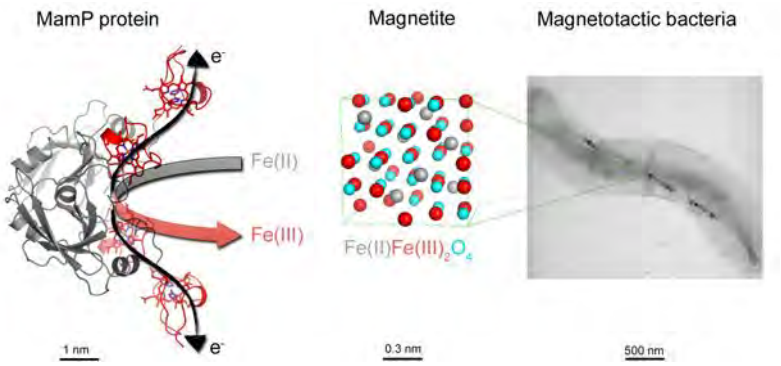
Les bactéries magnétotactiques (MTB) sont des procaryotes aquatiques d'horizons phylogéniques divers, capables de nager le long des lignes du champ géomagnétique. Cette capacité singulière est rendue possible grâce à l'alignement d'organelles magnétiques spécifiques (les magnétosomes) formant une véritable aiguille de boussole intracytoplasmique. Ces magnétosomes sont des vésicules protéo-lipidiques qui encapsulent un cristal magnétique dont la taille est comprise entre 25 et 100 nm (monodomaine, aimantation permanente), composé de magnétite (Fe_3O_4) ou de greigite (Fe_3S_4). Contrairement aux nanoparticules magnétiques chimiquement synthétisées, les nanocristaux biominéralisés par les MTB ont des morphologies bien définies avec une gamme de taille uniforme étroite. Ces caractéristiques sont typiques d'une minéralisation biologiquement contrôlée : la synthèse des magnétosomes est contrôlée génétiquement par un groupe de gènes, les gènes mam, qui codent pour des protéines associées aux magnétosomes, retrouvées chez toutes les MTBs, et uniquement chez elles.

Un premier axe de nos recherches couple des approches de biodiversité, de génomique comparative et de génétique. Ces études nous ont permis ainsi de retracer l'évolution de la magnétotaxie chez ces micro-organismes[1] mais également d'isoler en culture pure et de caractériser le génome de la première bactérie magnétotactique capable de biominéraliser de la magnétite ou de la greigite selon les conditions environnementales[2]. Un deuxième axe de recherche s'intéresse plus particulièrement aux fonctions des protéines codées par les gènes mam. Ces approches moléculaires qui associent génétique, biologie structurale, biophysique et enzymologie visent à caractériser in vitro la fonction des protéines Mam. Nous nous intéressons notamment aux protéines redox contenant un domaine cytochrome unique au MTBs (baptisé domaine magnétochrome) [3]. Nos travaux ont permis de démontrer le rôle de ce domaine dans le contrôle de l'état d'oxydation du fer lors de sa biominéralisation[4]. L'ensemble de ces approches fondamentales nourrissent des études appliquées exploitant les potentialités biotechnologiques des magnétosomes, en biorémédiation et imagerie médicale[5].

Cette conférence présentera l'ensemble de nos approches en se focalisant plus particulièrement sur la caractérisation des métalloprotéines originales impliquées dans les processus de biominéralisation.



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Communications

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Etude de la dynamique fonctionnelle de l'ACCO par marquage de spin couplé à la Résonance Paramagnétique Electronique

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L'oxydase de l'acide carboxylique 1-aminocyclopropane (ACCO), qui appartient à la famille des cupines, est une enzyme non-hémique à Fe(II) qui catalyse l'oxydation à deux électrons de l'acide aminé ACC, en présence de O_2 et d'ascorbate, ce qui constitue la dernière étape de synthèse de l'éthylène.[1] Il a été suggéré que la partie C_{term} de l'ACCO soit flexible et que sa mobilité joue un rôle dans la catalyse.[2, 3] L'étude de la flexibilité structurale de l'ACCO en conditions catalytiques représente une première étape dans la compréhension de la dynamique fonctionnelle de l'ACCO, et plus largement des cupines. Le marquage de spin combiné à la Résonance Paramagnétique Electronique RPE est une technique puissante et adaptée pour obtenir des informations structurales sur les systèmes biologiques. Cette technique est basée sur l'insertion de sondes paramagnétiques exogènes (marqueurs de spin), et sur l'analyse de leurs propriétés dynamiques par spectroscopie RPE.[4-7] Ces sondes sont des radicaux stables, de type nitroxyde, qui vont réagir avec une ou plusieurs cystéines (naturelle ou introduite par mutagénèse dirigée) d'une protéine. Le spectre RPE reflète la mobilité de la sonde et fournit par conséquent des informations structurales locales. Le marquage de spin couplé à la RPE permet également des mesures de distances nitroxyde-nitroxyde par RPE pulsée dans le cas des protéines, ainsi que dans les métalloprotéines, où les centres métalliques peuvent être utilisés comme des sondes intrinsèques pour les mesures métal-NO. Cette communication présentera les résultats obtenus lors du marquage de l'ACCO, qui contient 4 cystéines naturelles, et des études à température ambiante de l'ACCO en présence des co-facteurs. Les résultats de mesures de distances NO-NO et métal-NO par RPE pulsée (DEER) seront également présentés.

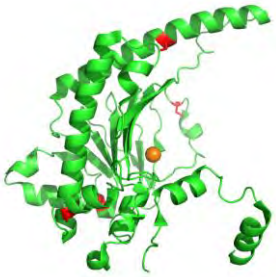

Figure 1. Structure cristallographique de ACCO de *Petunia hybrida***.** Les résidus cystéines sont présentés en rouge, le Fe(II) est indiqué en orange.[8]

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Des métalloenzymes artificielles pour une chimie tandem.

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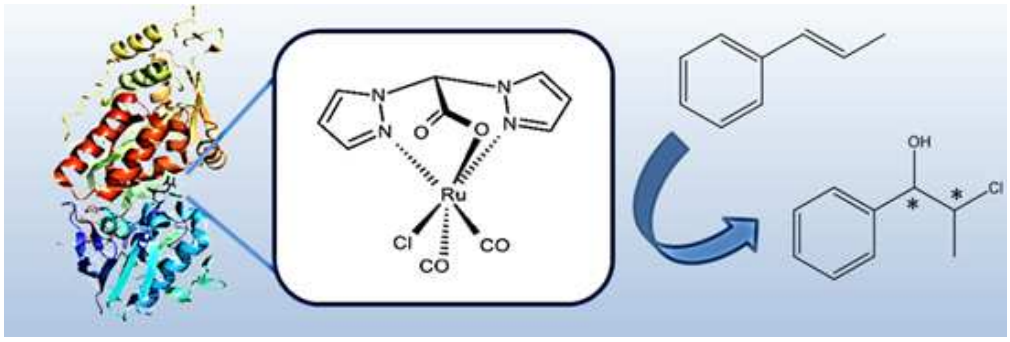
La prise de conscience de l'impact des activités anthropiques sur l'environnement et la santé humaine a conduit l'équipe BioCE, en collaboration avec l'équipe BioCAT, toutes deux du Laboratoire de Chimie et Biologie des Métaux de Grenoble, à mettre au point de nouveaux catalyseurs d'oxydations respectant les principes de chimie verte :[1] les métalloenzymes artificielles.[2] En combinant les avantages de la biocatalyse et de la catalyse homogène, de nouvelles alternatives aux solvants polluants et aux produits chimiques dangereux ont été trouvées.[3] Dans ce contexte, nous avons décidé d'incorporer un complexe de ruthénium (II), mimant le site actif des oxygénases et capable de réaliser des époxydations en milieu organique,[4] au sein de la protéine NikA. Ce nouvel hybride présente une réactivité différente du complexe parent, offrant des conditions d'oxydation douces pour la production de chlorhydrines, en milieu aqueux. L'utilisation de ce nouveau catalyseur permet d'obtenir ces synthons chiraux de façon chimio- et régio-sélective. Ces résultats illustrent l'avantage d'employer les métalloenzymes artificielles comme catalyseur : la cavité protéique active et stabilise le complexe inorganique tout en améliorant la sélectivité de la réaction. Afin de développer ces systèmes, une stratégie d'évolution dirigée du site actif de la protéine sera mise en place afin d'améliorer l'énantiosélectivité de la réaction.



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Impact of copper on amyloid-β aggregation and ROS production linked to Alzheimer's disease

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Alzheimer's disease (AD) is the leading cause of dementia in the elderly affecting nearly 44 million people worldwide. The underlying mechanisms of this complex disorder are not clear, but there is evidence and a relatively wide agreement that the so-called amyloid cascade is a key and early event in the development of AD. This hypothesis proposes an increased extracellular accumulation of the peptide amyloid-β (Aβ), which leads to its aggregation, first into oligomers and then into fibrils found in amyloid plaques, a post-mortem hallmark of the disease [1].

In vitro and *in vivo* studies indicate that metal ions and in particular Cu ions have a relevant role in AD. Due to its redox ability, Cu can participate in the formation of Reactive Oxygen Species (ROS), such as $O_2^{\bullet-}$, $^{\bullet}OH$ and H_2O_2, responsible for oxidative stress implicated in AD. It has been reported that Cu binds to Aβ and hence Aβ-Cu complexes can be directly involved in ROS production [2]. Moreover, Cu modulates the important aggregation of Aβ, leading to toxic oligomeric species. As a consequence, Aβ-Cu complexes might be the cross point of ROS production and Aβ aggregation linked to Aβ-induced toxicity in AD.

We are studying the ROS production of Aβ peptide at different ratios Aβ/Cu in the presence of a physiological reductant (ascorbate) in order to demonstrate the particular contribution of the complexes Aβ-Cu. These studies will also allow us to evaluate the binding affinity of Aβ-Cu complexes. The generation of ROS is monitored by the detection of the ascorbate consumption by absorbance measurements (at 265 nm). Figure 1 shows the initial ascorbate consumption rate, which reflect the initial rate of ROS production, obtained for two different Cu concentrations. The following conclusions can be drawn from the results obtained: 1) for ratios Aβ/Cu < 1, ROS production is very high and free Cu seems to importantly contribute to the initial ascorbate consumption rate; 2) the ROS observed at around stoichiometric concentrations (ratio Aβ/Cu = 1) would not exclusively correspond to the ROS production of the complexes Aβ-Cu and small changes in Aβ concentration around the stoichiometric concentration lead to an noteworthy change in ROS production; 3) the initial ascorbate consumption rate observed for ratios > 2 would correspond to the ROS production of the complex Aβ-Cu; thus the ROS production ascribed to the complexes Aβ-Cu could be determined. This shows that, when one wants to measure the ROS produced by Aβ-Cu only (and be able to neglect the contribution of free Cu), at least a two-fold excess of Aβ has to be used. The results will be compared with those obtained for other peptides, such as the H13A mutant Aβ and the acetylated Aβ, which show a lower and higher affinity constant, respectively, than that of Aβ.

Lastly, since Cu can both catalyze the generation of ROS and influence the aggregation of Aβ, as mentioned before, we intend to study the relation between Cu-induced aggregation of Aβ and their consequences on ROS production.

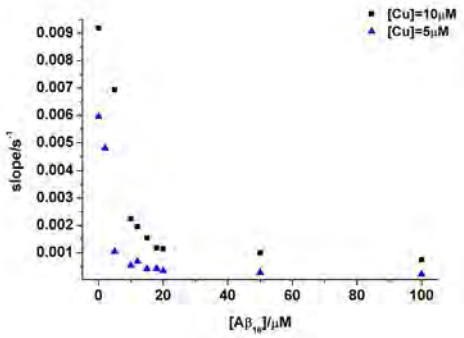

Figure 1. ROS production (ascorbate consumption rate) for different [Aβ] and [Cu]=5, 10 μM

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Complexes dinucléaires du cuivre bis-µ hydroxo pour l'oxydation d'alcanes

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Le méthane est le constituant principal du gaz naturel et constitue une importante source d'énergie. Actuellement, il est transformé en méthanol par des procédés industriels impliquant des températures et des pressions élevées. Les procédés alternatifs permettant de travailler dans des conditions douces pour l'oxydation directe du méthane sont donc d'un intérêt considérable. Dans la nature, l'oxydation aérobie du méthane en méthanol est réalisée par des enzymes, les méthanes mono-oxygénases (MMO), qui peuvent être de type soluble (sMMO) ou membranaire (pMMO). Cette dernière est caractérisée par un site dinucléaire à cuivre qui permet l'activation du dioxygène. Sur la base de calculs théoriques récents, la présence d'une espèce dinucléaire cuivre-oxygène à valence mixte semble déterminante dans le mécanisme d'oxydation catalytique.[1]
Notre objectif est de développer et de caractériser des complexes du cuivre susceptibles de former des intermédiaires à valence mixte de type Cu(II)-O_2-Cu(III) par oxydation d'espèces bis-µ-hydroxo. Nous nous sommes intéressés à deux types de complexes dinucléaires porteurs de bras polypyridine, la différence résidant essentiellement au niveau de l'espaceur (phénolate ou naphthyridine). Concernant la série avec le pont phénolate, des études spectroscopiques et électrochimiques, couplées à des calculs théoriques, ont été réalisées sur divers complexes, afin de rationaliser l'effet de dissymétrisation du complexe ainsi que l'influence du substituant en position para sur le phénolate, sur les propriétés redox.[2,3] L'électrochimie démontre une oxydation centrée sur le ligand, conduisant à un radical phénoxyle dont le potentiel redox est fortement dépendant du groupement substituant et de la symétrie du complexe. La modulation des propriétés redox permet ainsi d'envisager l'oxydation électrocatalytique de substrats simples. Concernant le complexe porteur d'un espaceur naphthyridine (Figure 1),[4] l'oxydation électrochimique couplée à la spectroscopie RPE et aux calculs théoriques révèle la formation d'une espèce à valence mixte Cu(II)-Cu(III) à basses températures.

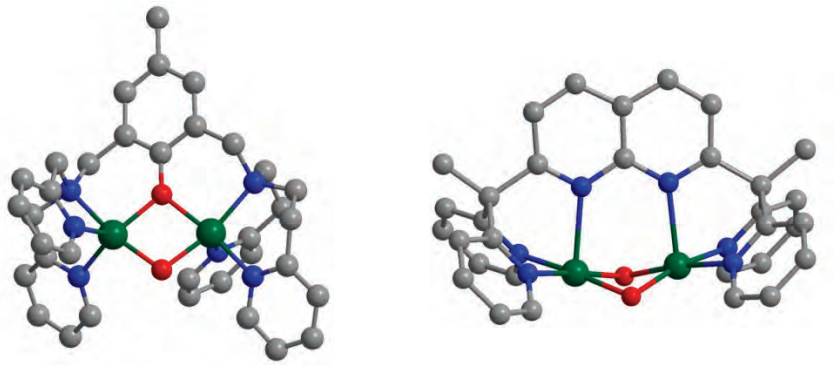

Figure 1. Structures RX des complexe bis-cuivre µ-hydroxo à pont phenoxo (gauche) et naphtyridine (droite)

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High-Field pulse EPR Nanometric Distance Measurements between MnDOTA centers

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Nanometric distance measurements by pulse electron paramagnetic resonance (EPR) techniques are versatile biophysical tools for probing the structures and functions of complex biological systems. A well-established approach is to introduce nitroxide (S=1/2) spin labels onto the biomacromolecules via site-directed spin labelling. The distances between labels are determined by measuring the magnetic dipolar interactions between the nitroxyl unpaired electrons using pulse electron double resonance (PELDOR also known as DEER).[1] The measurements are commonly performed at 9.5 GHz on frozen solutions. The use of higher magnetic fields and higher frequencies inherently improves the sensitivity of the measurements. However, at high field the EPR spectrum of radical labels broaden due to their g-anisotropy. This poses technical challenges and complicates the analysis due to orientation selection effect. Furthermore, typical radicals are readily reduced under normal cellular environment, limiting their utility in the cellular context. Stable high spin metal centres with small g-anisotropies such as Gd(III) (S=7/2) and Mn(II) (S=5/2) have been introduced as alternatives for high field PELDOR distance measurements. At high fields their EPR spectra is dominated by their central $|-1/2>|1/2>$ transition, which narrows proportionally to $\sim D^2/\nu_0$, where D is a zero-field splitting parameter and ν_0 is the spectrometer frequency. Gd(III)–Gd(III) PELDOR have been performed on various biological systems with good accuracy and high sensitivity.[2] However, analogous experiments with Mn(II) remain much less explored. Mn(II) based pulse-EPR distance measurements are very attractive for biological applications because unlike Gd(III), it is endogenous in biological environments and is found in numerous enzymes and proteins. Mn(II) is also interesting because it can often replace Mg(II) in many enzymes, such as kinases, nucleic acid constructs and nucleotide binding domains due to their similarity in size and charge.

Here we present Mn(II)-Mn(II) PELDOR distance measurements at 95 GHz on polyproline II helices[3] and also an oligo(phenylene-ethynylene) spacer doubly spin labelled with Mn(II)DOTA, which are distinguished by their small zero-field interaction. The measured Mn-Mn distances and distribution profiles were in good agreement with the expected values from molecular models. Additional features in the frequency-domain spectra became apparent at certain experimental conditions, which likely arose from contributions from the pseudo-secular component of the dipolar interaction that were found to be non-negligible for Mn(II)DOTA. These results and the potentials and limitations of Mn(II)DOTA-based spin-labels for measuring nanometric distances in proteins and other biological systems will be discussed.

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Complexes NHC-métaux monétaires : concepts et exemples autour de leurs application biologiques potentielles

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Il est maintenant bien reconnu que, de par les propriétés intrinsèques des éléments métalliques, le domaine des « métallodrogues » est porteur de bénéfices potentiels en thérapie par rapport aux médicaments organiques classiques.[1] Malgré de grands succès dans certains domaines (ex : complexes ammine de platine(II) en tant qu'anticancéreux, complexes aminopolycarboxylate des gadolinium(III) comme agents de contraste…), une difficulté fréquente à la conception de métallodrogues est que les équilibres de coordination pouvant résulter en une liaison à des sites biologiques indésirables et/ou à une décoordination non souhaitée du métal.

Pour certaines de ces applications potentielles, la famille des ligands carbène *N*-hétérocyclique (NHC) pourrait permettre de grandes avancées. En effet, ces ligands sont aujourd'hui utilisés largement en chimie organométallique, principalement pour des applications en catalyse. Leurs complexes sont aisément préparés et, dans le cas de nombreux élément métalliques (dont les métaux du groupe 11), la liaison carbone–métal présente une stabilité vis-à-vis de l'hydrolyse permettant de considérer des applications biologiques. D'ailleurs, l'origine principale de leur succès en catalyse, est, justement, l'absence de décoordination du métal.

Au sein de notre laboratoire, nous menons, depuis plusieurs années des recherches dans ce domaine. D'une part, après avoir montré que des complexes de ligands NHC simples (et pour la plupart, déjà connus pour des applications en catalyse) pouvaient présenter une cytotoxicité marquée vis-à-vis des cellules cancéreuses,[2] nous nous sommes intéressés à la fonctionnalisation des complexes pour leur conférer des propriétés intéressantes pour une utilisation en milieu biologique (hydrosolubilité, luminescence, ciblage potentiel etc…). Ces méthodes de synthèses, basées sur des stratégies de type « *click chemistry* » ont porté leur fruits et ont permis l'obtention d'une famille de composés diversifiés dont nous commençons à déterminer les propriétés biologiques.[3]

Par ailleurs, dans ce type de recherche de *chimie bioinorganique médicinale*,[1] il est souhaitable, voir nécessaire, de disposer d'outils permettant un criblage rapide de bibliothèques de complexes métalliques. Dans le domaine des propriétés antibactériennes, pour lesquels les complexes Ag-NHC sont de bons candidats, nous avons utilisé le Biofilm Ring Test®, pour cribler rapidement une bibliothèque de complexes métal–NHC et mettre en évidence certains composés actifs contre des souches bactériennes formant des biofilms.[4]

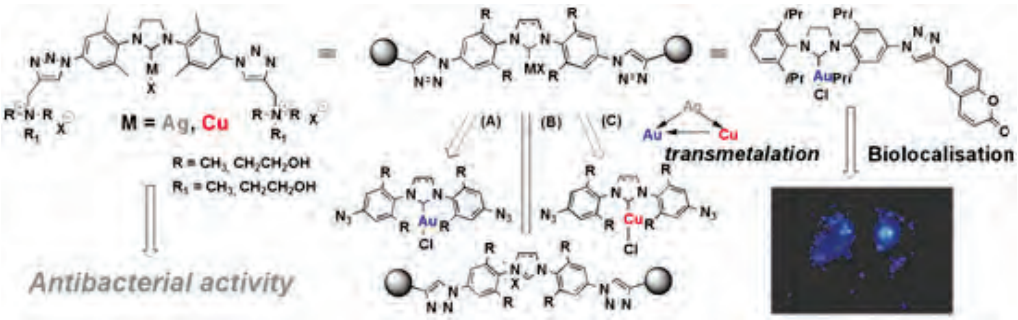

Figure 1. La famille de composés considérés

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Hybrid *β*-lactoglobulin/prochiral palladium(II) pincer complexes systems: a new outlook on artificial metalloenzymes design

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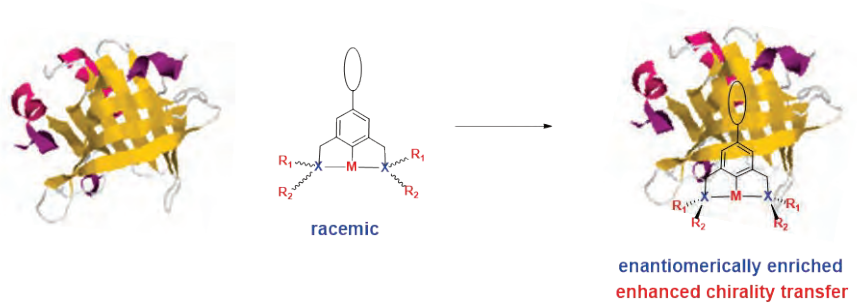
Artificial metalloenzymes are one of the modern and attractive approaches to perform stereoselective catalytic transformations.[1] These are hybrid species which contain a catalytically active transition metal complex incorporated within a host biomacromolecule, typically a protein, peptide or DNA.[2,3] Such a concept allows to combine typical advantages of both enzymatic and organometallic catalysts, such as high catalytic selectivity and efficiency of enzymatic systems and wide substrate scope of transition metals catalysts, and, at the same time, to overcome some of the limitations of both systems. Our approach consists in the utilization of transition metal complexes based on prochiral hemi-labile ligands, since the host protein could force them to confer a specific stereoconfiguration. This makes possible to bring the chirality closer to the catalytic metal center and, therefore, to increase the enantioselectivity of catalyzed reactions.

In this contribution, we report the synthesis of new NCN pincer palladium(II) complexes with prochiral hemi-labile ligands and the study of their structural properties. Furthermore, the supramolecular anchoring of these complexes to bovine *β*-lactoglobulin (β-LG) was studied both experimentally and theorically by computational calculation and preliminary catalytic activity was examined on aldol condensation reactions.



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Smart theranostics: a useful tool for tracking metal-based complexes

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Since the pioneer discovery of cisplatin for biological applications by Rosenberg, metal complexes have become the most currently investigated and used class of compounds in cancer chemotherapy.[1] Gold and ruthenium-based derivatives gave very promising results as anticancer agents,[2] although it is still challenging to understand their mechanism of action in order to improve the efficiency and to limit the side effects of such compounds.

To deal with this issue, we have drawn our inspiration from theranostics: we attached a fluorophore on metal-based complexes to be able to track them *in vitro*. In previous works, we proved the efficiency of the use of an amino-phosphine linker to bind BODIPYs, and metallated-DOTA moities to Ru(II) and Au(I) complexes to visualize them by optical imaging or SPECT *in vitro* and *in vivo*, respectively.[3]

In this study,[4] we present several metal complexes involving Au(I), Ru(II), and Os(II) metal ions, and among them two novel gold complexes, which are compared to auranofin and to their phosphonium analogue. A challenging question is whether the gold phosphine complex is a prodrug that is administered in an inactive precursor form, or rather that the gold atom remains attached to the phosphine ligand during treatment. To tackle this issue we choose a phosphine-based smart probe as a ligand, whose strong fluorescence depends on the presence of the gold atom. The *in vitro* biological action of the gold complexes and the phosphonium derivative were investigated and a preliminary *in vivo study* in healthy zebrafish larvae allowed us to evaluate gold complex biodistribution and toxicity. The different analyses carried out showed that these gold complexes behaved differently from phosphonium and auranofin, both *in vitro* and *in vivo*.

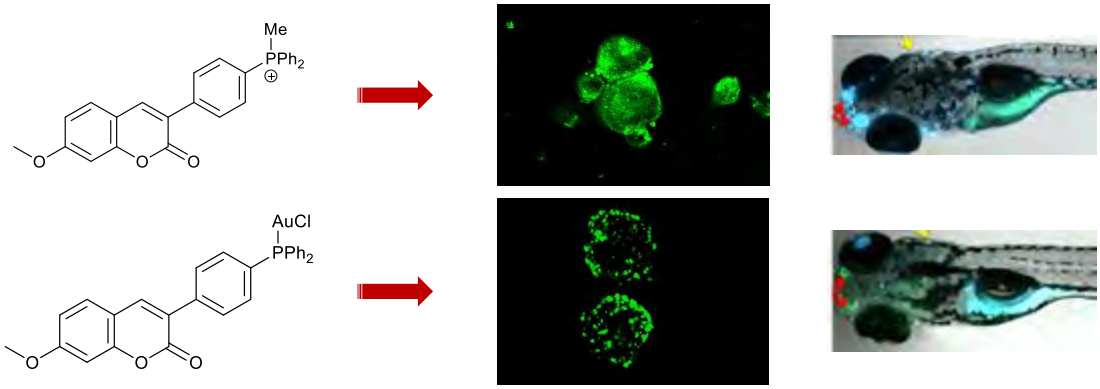

Figure 1: investigated compounds & two-photon microscopy images (MDA, 50 µM, λ_{ex} = 750 nm).

Two-photon microscopy experiments demonstrated that the cellular targets of these gold complexes are not the same as those of the phosphonium analogue. Moreover, despite similar IC50 values in some cancer cell lines, gold complexes displayed a low toxicity *in vivo*, in contrast to the phosphonium salt. They are therefore suitable for future *in vivo* investigations (Figure 1).

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Peptides to mimic uranyl binding sites in phosphoproteins

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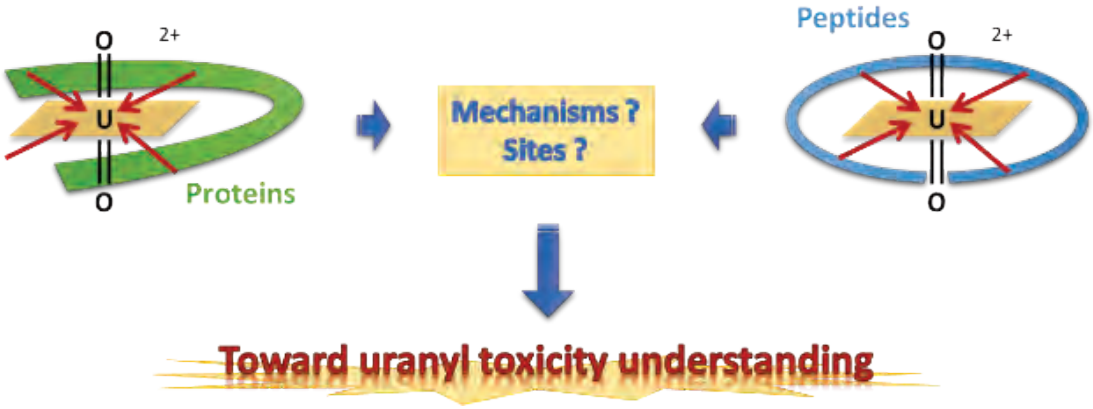
Uranium, mostly known for its industrial exploitation as nuclear fuel, is the heaviest natural element found on earth. The aqueous and physiological relevant form of this actinide is the linear dioxo-cation uranyl. This metal has no biological function and is toxic. In case of contamination, uranyl is absorbed into the blood and very quickly redistributed in the body. Many studies show that it mainly targets the kidneys but also the bones where it can stay for a very long time.

Over the past decades, some target proteins were found to interact strongly with uranyl *in vivo*. Among these are some blood proteins, such as fetuin or transferrin, and osteopontin, a phosphorylated protein that is involved in bone growth mechanisms. These biomolecules are responsible for the retention, transport and distribution of uranyl species *in vivo*. However the interaction mechanisms are not well known and there is still today a lack of knowledge about it.



In order to elucidate these interactions, we designed simplified and optimized peptide models that are able to chelate uranyl in aqueous media. More recently, we introduced amino acids containing phosphate groups that are known to efficiently interact with this cation. Here, we will present the synthesis of such phosphopeptides as well as their uranyl binding properties. We will more specifically discuss the influence of the nature, position and number of phosphorylated amino acids that were introduced in the peptides sequences.

These peptides are relevant models to mimic uranyl binding sites found in phosphorylated proteins such as osteopontin, and thus are promising tools to elucidate contamination mechanisms.

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Electrochemical oxidation of aminoindane substrates mediated by copper complexes

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Water splitting reaction by means of robust and biocompatible molecular systems has attracted much interest because it is the key challenge in the conversion of sunlight into chemical fuels[1]. We still need to understand and control several key reactions and trap their intermediates. One of these key reactions is the water oxidation to dioxygen. This reaction is performed in Nature by the Oxygen Evolving Centre of Photosystem II composed of 4 manganese ions and 1 calcium ion.[2] However, there are examples in the literature which report that this reaction can be catalyzed by other metals such as copper ions.[3] The inspiration for using copper-based catalysts for water oxidation came by copper's low cost, high abundance and his well-known biomimetic chemistry with dioxygen.

Moreover, in view of the significance of catalyzed oxidations for industrial applications, a key goal in current research is the photochemical oxygen atom transfer to different substrates through water activation. To achieve this goal we need to understand mechanisms of oxidation catalysis by studying copper-oxygen intermediates.

In a previous work of our lab using Cu^{I} precursors, Réglier et al. have described the stereoselective hydroxylation of a substrate after exposure in O_2.[3] The substrate is bound covalently to the tertiary amino group of the ligand so that an intramolecular oxygen atom transfer from copper to the ligand is favored, obtaining the cis-hydroxylated product (Figure 1).

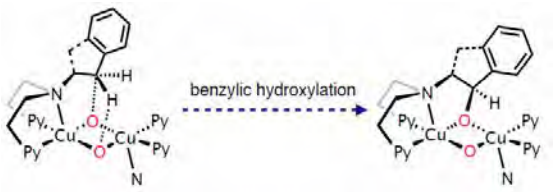

Figure 1: Intramolecular oxygen atom transfer to the substrate

Following that work, we investigate the oxygen atom transfer on 2-aminoindane group via H_2O activation and Cu^{II} complexes using electrochemical methods. Also, we analyze the products formed after the bulk electrolysis of our copper complex (cis-hydroxylated product and O_2) and we try to characterize the copper-oxygen species involved (Figure 2).

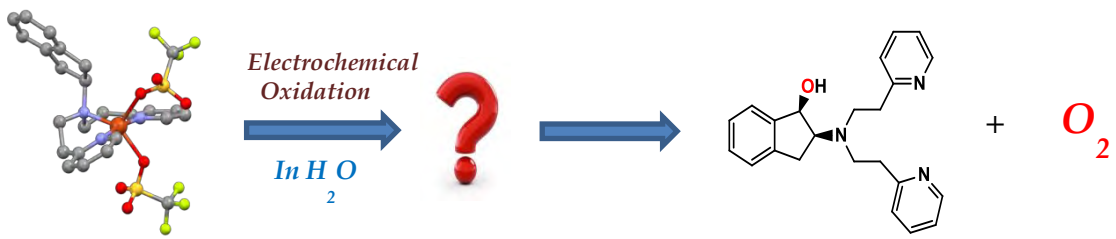

Figure 2: Approach followed for water oxidation and water activation

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Un complexe de type nickel pincer dans le site actif de la lactate racemase

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La racémisation du lactate est une activité enzymatique permettant à de nombreux microorganismes de produire ou d'utiliser les deux isomères (L- et D-) du lactate indifféremment. Nous avons montré que la lactate racémase (LarA) est une enzyme à nickel nécessitant l'activation par trois protéines accessoires (LarB, LarC, et LarE) (1).
En combinant des données de spectrométrie de masse et de crystallographie par rayons X, nous avons pu déterminer la structure du cofacteur présent dans LarA. Ce cofacteur est un dérivé de l'acide nicotinique lié à une lysine et qui forme un complexe de type pincer avec un atome de carbone, deux atomes de soufre et une histidine comme ligands (Figure 1) (2).

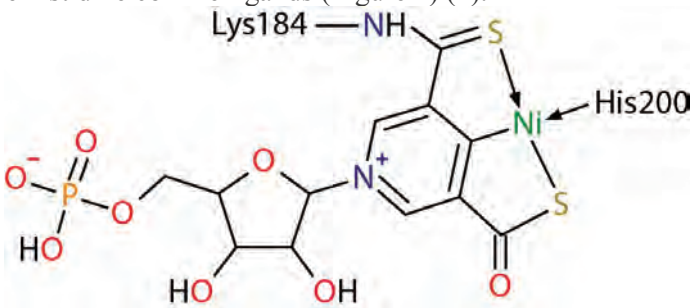

Figure 1. Structure du cofacteur de la lactate racémase, le Ni pincer pyridinium-3-thioamide-5-thioacide carboxylique mononucléotide.

Bien que des complexes similaires aient été synthétisés auparavant, c'est le premier exemple d'un complexe de ce type dans une enzyme. Le rôle de ce cofacteur serait de capturer un hydrure afin de permettre la formation de pyruvate comme intermédiaire de réaction.
La présence dans de nombreux génomes procaryotiques des gènes encodant les protéines accessoires en absence de gène codant pour la lactate racémase suggère que ce cofacteur puisse être utilisé par d'autres enzymes que LarA (1).
Récemment, nous avons déterminé le rôle des protéines accessoires : LarB catalyse la carboxylation du pyridinium, LarE insére deux atomes de soufre dans le produit de LarB et LarC fournit le nickel pour le complexe pincer (3). La possibilité de synthétiser le cofacteur de la lactate racémase *in vitro* ouvre désormais la voie à l'étude des propriétés physico-chimiques de ce premier complexe pincer d'origine enzymatique.

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Rational immobilization of multicopper oxidases on functionalized carbon nanomaterials for the design of oxygen-reducing biocathodes

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Carbon nanotubes (CNTs) have many advantages for the direct wiring of metalloenzymes: excellent conductivity, high specific surface and porosity, versatile functionalization processes and intimate interactions with enzymes [1]. We have especially investigated the covalent and non-covalent functionalization of CNTs to achieve rational MCO attachment and orientation for the realization of an efficient direct electron transfer between the electrode and the enzyme active site. MCOs, such as laccases and bilirubin oxidases, possess a set of two copper-containing centers: a surface-located T1 mononuclear copper centre, responsible for the oxidation of phenols and the successive electron transfers to a buried trinuclear T2/T3 centre (TNC), responsible for the activation of O_2 and its subsequent four-electron reduction into water. CNT modification by functional groups represents a versatile technique to achieve MCO direct wiring on electrodes via the T1 copper centre and efficient bioelectrocatalytic oxygen reduction at low overpotential [2-4]. These nanostructured bioelectrodes were combined with different types of anodes for the design of enzymatic fuel cells [5].

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Mn-SiO_2 hybrid materials with catalase-like activity: towards the stabilization of Mn_2 complexes in water solution

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Reactive oxygen species such H_2O_2, $O_2^{\bullet-}$ and $HO^{\bullet}$ (ROS) are endogenously produced during the metabolism and, if their concentration is not under control, they may perform undesirable oxidations and become cytotoxic. Hence, cells are loaded with high amounts of very efficient ROS scavengers.[1,2] Mn-catalase (Mn-CAT) is one of the enzymes that protects cells from damage caused by H_2O_2, catalyzing its decomposition into H_2O and O_2.[3,4]

In our group, we have synthesized a series of binuclear Mn(III) compounds (Figure 1) that are models of the catalytic center of the Mn-CAT. These models are able to effectively decompose H_2O_2 but their low stability in water does not allow to use them as antioxidant agents.[3] In order to overcome this limitation, we inserted the aforementioned compounds in mesoporous silica (MCM-41). The hybrid material obtained was active in water solution, but the mesostructure was not preserved during the catalysis.[5]

Currently, we present the synthesis and characterization of new materials synthesized from mesoporous silica previously functionalized with pyridine function. The activity of this material is as good as our previous one. Furthermore, it is more stable and both the mesostructure and the activity are preserved after several additions of H_2O_2. The study of the nature of the Mn complex-silica interaction is under progress.

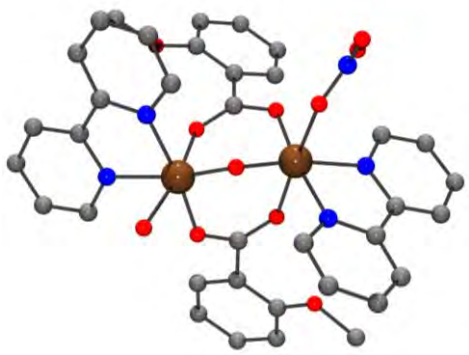

Figure 1. *Binuclear manganese complex.*

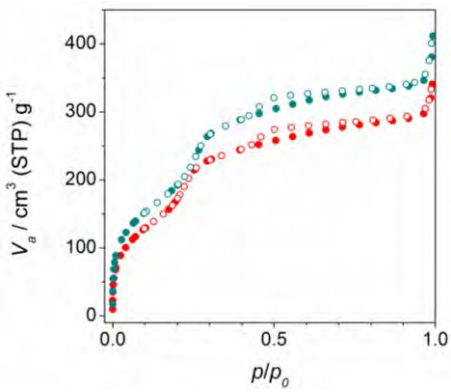

Figure 2. *N_2 sorption isotherms for material Py@SiO2-Mn2 (red) and for the same material after two additions of H_2O_2 (dark cyan).*

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Local structures of the molybdenum cofactor in Nitrate Reductase probed by advanced EPR spectroscopy

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Molybdenum enzymes having a single Mo atom at the active site are widespread in living organisms. They mainly catalyse two-electron redox reactions and are often associated with oxygen transfer reactions. Many of them are involved in bioenergetic processes [1]. An example is provided by Nitrate reductase A (NarGHI) from *Escherichia coli,* a respiratory enzyme that catalyzes the two electron reduction of nitrate into nitrite. Its molybdenum-containing active site exhibits two main paramagnetic Mo^V EPR signals from the so-called high-pH (hpH) and low-pH species [2] according to the pH conditions in which they are predominant. The catalytic relevance of both species has been previously discussed [3,4]. In contrast to the hpH species, the lpH Mo^V EPR signature has been shown to be sensitive to the presence of the substrate (NO_3^-), raising the question of its mode of interaction to the Mo center [5]. In this work, we aim at resolving the local structure of the hpH and lpH species as well as the structural changes induced upon substrate binding through the detection of ^{14}N and ^{15}N magnetic interactions to the Mo(V) ions using advanced EPR (HYSCORE) spectroscopy. DFT calculations have been performed to understand the origin of the detected hyperfine couplings and to propose structural models for the Mo(V) intermediates which account for available spectroscopic data. Our results lay the foundation for in-depth understanding of the structure of the Mo^V catalytic intermediates in NarGHI.

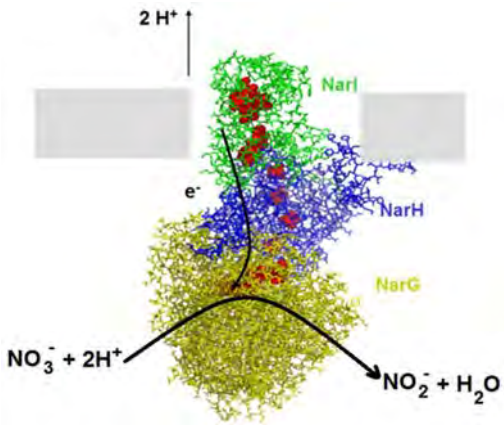

Figure 1 - Crystallographic structure of NarGH obtained by X-ray diffraction

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Propriétés de complexation d'un nouveau chélatant bifonctionnel pour l'imagerie TEP

Complexe Cu(II) – cyclam *C*-alkylé

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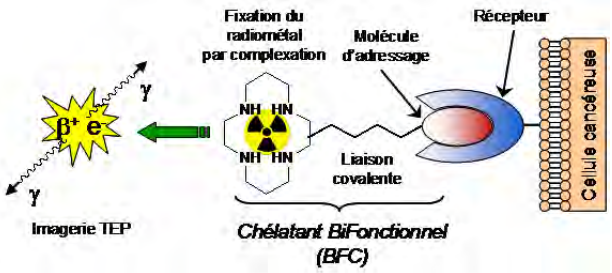
Technique incontournable en médecine nucléaire, la Tomographie par Emission de Positons (TEP), fournit des images particulièrement informatives pour les cancérologues. Cependant, l'amélioration du diagnostic demande la mise au point de nouveaux traceurs radioactifs. A ce jour plusieurs radioisotopes sont en usage médical, tel que ^{11}C, ^{18}F, ^{123}I, ^{99m}Tc, ^{188}Re etc. Le cuivre-64 acquiert un intérêt croissant en tant que radioisotope émetteur de positons puisqu'il possède de bonnes caractéristiques pour une application en imagerie: temps de vie et énergie de la particule émise adéquats, chimie bien connue en milieu aqueux. Le ^{64}Cu doit être incorporé par un chélatant bifonctionnel (BFC), permettant la coordination du métal et la création d'une liaison covalente avec une molécule d'adressage.

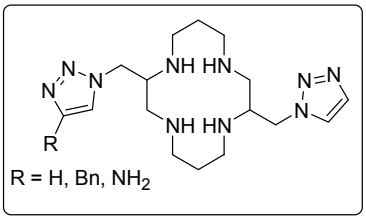


Pour être évalué et validé en tant que BFC utilisable en TEP, une molécule doit répondre aux critères suivants:

- grande affinité et sélectivité vis-à-vis du cuivre(II) dans des conditions physiologiques;
- grande inertie cinétique vis-à-vis des réactions de dissociation: démétallation, transchélation, protonation, hydrolyse;
- résistance vis-à-vis des réactions réductrices ou oxydantes;
- conditions de complexation appropriées (faible concentration, température ambiante, formation rapide).

De nombreux chélatants évalués sont construits sur la base des polyamines cycliques *N*-fonctionnalisées, notamment des dérivés de DOTA et TETA. Or, la faible stabilité *in vivo* de complexes métalliques de ces systèmes justifie le développement des séquestrants moléculaires plus performants.

Notre équipe développe actuellement une nouvelle stratégie de synthèse de tétraazamacrocycles *C*-fonctionnalisés permettant la libération des azotes secondaires du cycle et ainsi l'augmentation du pouvoir complexant des chélateurs. La synthèse et l'évaluation des propriétés de complexation de nouveaux dérivés de type dioxocyclam et cyclam *C*-alkylés seront présentées.



A.-S. Felten, N. Petry, B. Henry, N. Pellegrini-Moïse and K. Selmeczi, *New J. Chem.* **2015**, DOI: 10.1039/C5NJ01927C

Oxidation of the Amyloid-Beta peptide and consequences on copper coordination and ROS production

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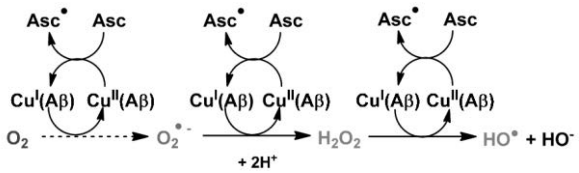
Alzheimer's disease (AD) is the most frequent form of dementia in the elderly. One of the features of AD is the formation of senile plaques in brain, mainly composed of the 40/42-residue Amyloid-Beta peptide (Aβ). The Aβ peptide is present in soluble form in healthy brain and found aggregated in AD brain. Furthermore, 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, Cu-Aβ complexes can catalyze Reactive Oxygen Species (ROS) production, including the hydroxyl radical ($HO^{\bullet}$)[1, 2]. The latter is highly reactive and can cause oxidative damages to surrounding neuronal biomolecules and to 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. Electron Paramagnetic Resonance (EPR) and Nuclear Magnetic Resonance (NMR) studies have revealed the coordination of both Cu(I) and Cu(II) to the peptide[3, 4] and mass spectrometry allowed us to better characterize the coordination sphere of Cu during electron transfer, along with the resulting oxidized residues of Aβ[5]. With this in mind, we studied the impact of metal-catalyzed oxidation of Aβ regarding the ROS production, by using fluorescence spectroscopy with 3-CCA oxidation as a probe, and the coordination of Cu(I) and Cu(II) to the oxidized Aβ species, by using X-Ray Absorption Near Edge Spectroscopy (XANES) and EPR. The results showed an increase of fluorescence as the peptide get oxidized, due to an increase amount of ROS exiting Cu-AB and being scavenged by 3-CCA. This latter phenomenom was linked to a change in Cu-Aβ coordination, leading to loosely bound copper. Thus, ROS production and Aβ oxidation could appear as playing together, becoming an important mechanism involved in the etiology of Alzheimer's disease, leading to neurodegeneration.



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Direct electrochemistry and photochemistry of FeFe-hydrogenases

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FeFe-hydrogenases are enzymes that catalyse the reversible oxidation of dihydrogen. The active site, called H-cluster, consists of an iron-sulfur cluster bound to a diiron site. The Fe atoms of the diiron site are coordinated by three intrinsic CO ligands; the binding of a fourth CO ligand (Hox-CO state) reversibly inhibits the enzyme by preventing H_2 binding. FeFe-hydrogenase could be used to produce H_2 from water and light, but an obstacle is the fact that the H-cluster is sensitive to light in the UV-Vis range, since the irradiation at room temperature could induce the photo-dissociation of one intrinsic CO and the degradation of the active site[1]. The photodissociation of extrinsic CO has been studied using spectroscopic and computational methods[1-5]. The effect of white light on the binding of extrinsic CO was also shown with electrochemistry[6]. However, there are no kinetic data related to the CO photo-dissociation process and moreover the photo-damage during catalysis has not been characterized so far.

To investigate further and more quantitatively the effect of light on the active site of FeFe-hydrogenases, we used protein film voltammetry (PFV) to study the enzymes from *Chlamydomonas reinhardtii* and *Clostridium acetobutylicum* at room temperature, under turnover conditions, irradiating the enzyme film on the electrode with laser diodes at different wavelengths. The fit of a mathematical model to the experimental data allowed the determination of the kinetic constants of the inhibition process, in the dark and under irradiation. We also carried out TD-DFT calculations, on models of the active site, to study the photochemistry of the diiron site in the CO-inhibited state, to characterize the electronic structure of excited states and the electronic transitions induced by irradiation.

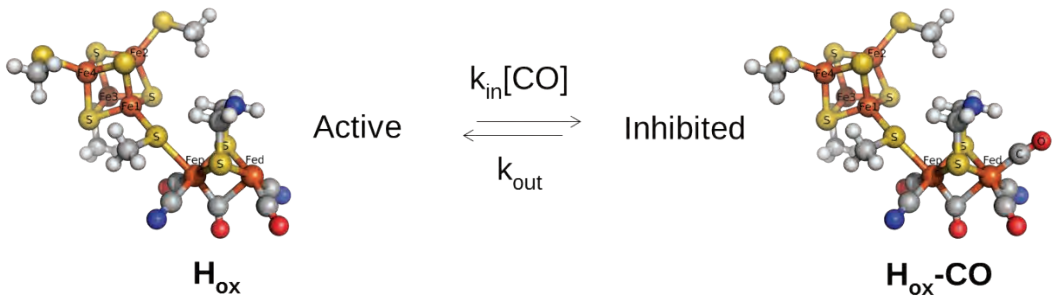

Figure 1 Structure of the H-cluster in oxidizing conditions (H_{ox}) and inhibited by extrinsic CO (H_{ox}-CO). The bond of extrinsic CO in the apical coordination position of the distal iron (Fe_d), inhibits the H-cluster (H_{ox}-CO); k_{in} and k_{out} are the kinetic constants of CO binding and release respectively.

We show that the kinetics of CO release from the H-cluster are dependent on the wavelength of the excitation, and that light affects the turnover rate of hydrogenase. We describe the excited states and the electronic transitions involved in the photodissociation of CO.

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CooT and CooJ: towards a better understanding of Carbon monoxide dehydrogenase maturation in *Rhodospirillum rubrum*

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CooC, CooT and CooJ from *Rhodospirillum rubrum* are accessory proteins, essential for the maturation of carbon monoxide dehydrogenase (CODH). CODH is a key enzyme in hydrogenogenic carboxydotrophs (e.g. *Rhodospirillum rubrum* or *Carboxydothermus hydrogenoformans*), able to use CO as a sole carbon and energy source. In this case, the oxidation of CO into CO_2, catalyzed by CODH, is coupled to the production of dihydrogen, catalyzed by a [NiFe]-hydrogenase, according to the reaction: $CO + H_2O \rightarrow CO_2 + H_2$. In 2001, crystal structures of CODH from *C. hydrogenoformans* (*Ch*CODH) [1] and *R. rubrum* (*Rr*CODH) [2] were solved (Fig. 1), revealing the unique structure of the active site (C-cluster), constituted of a $[NiFe_4S_4]$ cluster (Fig. 1). Additional Fe_4S_4 clusters are present to mediate electron transfer from C-cluster to a ferredoxin, CooF, which transfers electrons to hydrogenase. Although the mechanism is well studied [3], not much is known about the biosynthesis of the C-cluster. While the FeS part of the cluster is thought to be assembled by the bacterial Isc or Suf systems, the insertion of the nickel ion *in vivo* depends on the accessory proteins CooC, CooT and CooJ.

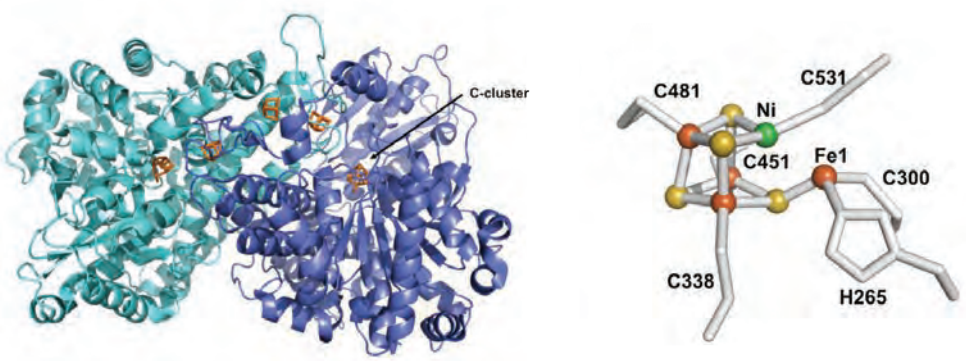

Figure 1: Structure of *R. rubrum* CODH at 2.8 Å (PDB code: 1jqk) and its active site (modified from [2]).

CooC in *R. rubrum* is a membrane associated ATPase proposed to carry out the ATP-dependent insertion of nickel into the C-cluster. The structures of *C. hydrogenoformans* CooC in complex with zinc and ADP were solved [4], but the mechanism of nickel insertion remains elusive. The roles of CooT and CooJ are even less well understood. While data for CooJ are very limited, there are no data published for CooT.
Here we present the first structural and biophysical characterization of the accessory proteins CooT and CooJ, which will provide the basis for further studies investigating the biosynthesis of CODH's C-cluster.

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Macrocyclic Gd(III) Complexes with Pendant Crown Ethers Designed for Binding Zwitterionic Neurotransmitters

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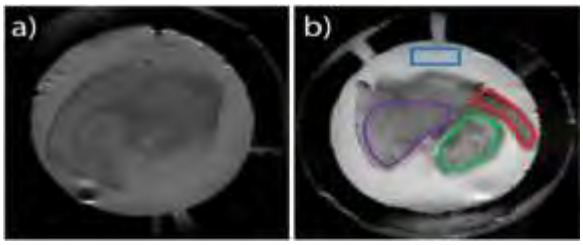
Magnetic resonance imaging (MRI) has been increasingly used as a promising technique to non-invasively visualize physiological events with excellent temporal and spatial resolution. In particular, smart MRI contrast agents that are able to report on the physico-chemical status of the tissues, start to have a strong impact in neuroscience.[1] In spite of the key role of neurotransmitters in signal transduction and cognitive functions, their non-invasive *in vivo* monitoring remains an important challenge.

In this work, we report the design and *in vitro* characterization of a series of lanthanide-based probes responsive to zwitterionic neurotransmitters with the aim to track in vivo concentration changes of neurotransmitters using MR. The design of these imaging probes relies on a ditopic binding approach of zwitterionic neurotransmitters to the Ln^{3+} complexes, involving interactions (i) between a coordinatively unsaturated and positively charged Ln^{3+} chelate and the carboxylate function of the neurotransmitters and (ii) between an azacrown ether appended on the chelate and the amine group of the neurotransmitters.[2]

The contrast agents were found to exhibit high relaxivities and the neurotransmitter binding leads to a remarkable relaxivity change, related to a decrease in hydration number of the Ln^{3+} complexes. Gadolinium complex, **GdL**, discriminates zwitterionic neurotransmitters from monoamines, though little selectivity against bicarbonate was observed. Ultimately, **GdL** was successfully used to monitor neural activity in *ex vivo* mouse brain slices by MRI.[2b]



MR images of a mouse brain slice in the absence (a) and in the presence (b) of 500 µM **GdL**.

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Ru(II) complexes and associated nanoparticles as photosensitizers in photodynamic therapy (PDT)

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Metalloproteins evidence the importance of transition metal in living systems for the transport, structuration and catalytic activities (enzymes). Now, it is also well known that coordination complexes and organometallic compounds can be used as drugs in medicine for imaging (optical or magnetic imaging, Gd^{3+} complexes in MRI for example) or therapy such as *cis*-Pt for the treatment of cancers. Photodynamic therapy (PDT) is a method of medical treatment for which light excitation of a photosensitizer (PS), and an energy transfer (ET) to surrounding oxygen ($^3O_2$), allow the generation of toxic reactive oxygen species, and especially singlet oxygen, leading to the cell death. This technique, applied by humans for 3000 years and already known by the Egyptians, proves to be very efficient and promising in several pathologies [1]. Octupolar coordination Ru(II)-polypyridyl complexes were already investigated for their stability, inertness, biological activity such as DNA interaction [2] and optical properties such as ^{3}MLCT excited-state properties [3], leading to a wide range of applications in therapy [4]. One can exploit the triplet character of the excited-state of such Ru(II) complexes to use them as sensitizers [5]. In this domain, we will present our strategy and results using hydrosoluble Ru(II) complex as PS (see Figure 1) in linear, and two-photon excited PDT [6]. The use of grafted PS in hybrid multifunctional nano-platforms for potential application in theranostic [7] or encapsulation and confinement of metallic complexes within biocompatible polymeric nanoparticles [8] will also be discussed.

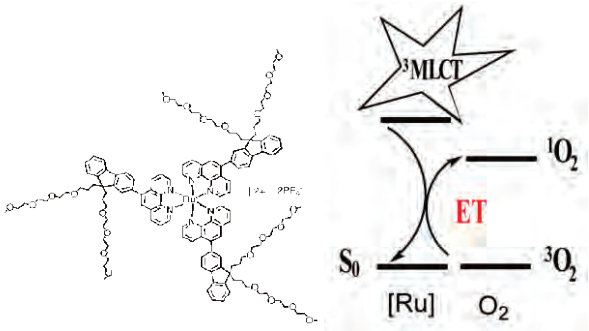

Figure 1. Example of a [Ru(II)] complex-based photosensitizer (PS) used in the singlet oxygen production ($^1O_2$) for photoynamic therapy (PDT) application.

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BODIPY: a highly versatile platform for the design of Monomolecular Multimodal Imaging Probes

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Molecular imaging is a highly promising field of research and innovation with potential in a wide range of applications, including prognostic, diagnostic, drug discovery and development of theranostics.[1] The combination of two molecular imaging techniques can provide synergistic advantages and compensate the drawbacks of each imaging system while taking advantage of their individual strengths. In particular, combining optical and nuclear imaging may find important preclinical and clinical applications. For example, the radionuclide component can provide early quantitative data in a target tissue, and subsequent events can be monitored longitudinally by an optical method. In clinic, such bimodal imaging can be used for surgery assistance. Indeed, preoperative PET/SPECT scan will allow the localization of the tumor, while intraoperative optical imaging will help for a better delineation of tumor margins and resection of the tumor tissues.

In this context, we are developing MOnomolecular Multimodal Imaging Probes (MOMIP) based of one promising fluorophore, BODIPY.[2] Versatile multimodal platforms were thus designed by tuning the luminescent properties of the dye, the coordination properties of the macrocyclic chelator and the nature of the functional group for bioconjugation. Some of these MOMIP have been attached to biomolecules such as antibodies, peptides or lipopolysaccharides, and investigated *in vivo*.[3] The synthesis, photophysical studies and the *in vivo* validation of these bimodal systems will be presented.

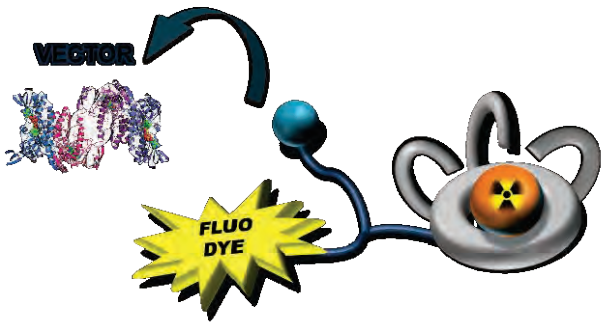

Figure 1: General structure of the MOMIP

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Influence of a conserved Val to Ile substitution in bacterial NO-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.

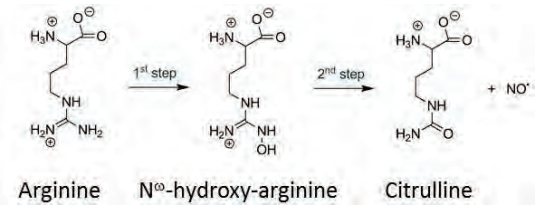

Figure 2- Reaction catalyzed by NO-Synthases

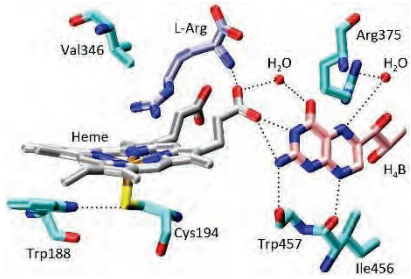

Figure 1-iNOS active site with substrate arginine and cofactor H4B

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 (mNOSs) - 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 another difference between mNOSs and bacNOSs: the replacement of a valine residue (V346 in murine iNOS) by a slightly bulkier isoleucine (I224 in bsNOS). This residue is thought to be on the way out of nitric oxide and this Val to Ile substitution has been shown to be at least partially responsible for the slow rates of NO˙ release measured in bacNOSs[4]. Here we present results on the I224V bsNOS mutant and its mammalian counterpart V346I iNOS. We characterized the ferric form of these enzymes, as well as their ferrous-nitrosyl complexes using UV-visible, resonance Raman and EPR spectroscopies. The latter allows us to see subtle differences between those NOS and small changes due to the presence of substrate or cofactor. We also conducted a kinetic study (stopped-flow and freeze quench) to better understand how this Val/Ile influences the catalytic mechanism.

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Manganese SOD-mimics showing an anti-inflammatory activity on a cellular model of Inflammatory Bowel Diseases

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In the search for a better understanding of diseases at the molecular scale, oxidative stress has been identified as an important event implicated in several diseases ranging from inflammatory diseases to neurodegeneration or cancer. During the oxidative burst, an overproduction of reactive oxyen species (ROS) takes place, thus endogenous defenses are overwhelmed. The superoxide dismutases (SODs) are a family of metallo-enzymes implicated in the reduction of oxidative stress and inflammation process by tightly controlling the concentration of superoxide in cells. In the case of Inflammatory Bowel Diseases (IBD), a chronic inflammation of the gut takes place and it was shown that SODs are either overproduced in an inactive or overwhelmed.[1] The use of antioxidant compounds that mimic the activity of endogenous enzymes such as SODs is thus of interest as it can help to control and resolve the inflammation process. Numerous SOD mimics are reported in the literature, that present different type of ligand or metal center, as well as different anti-oxidant activity.[2-5]

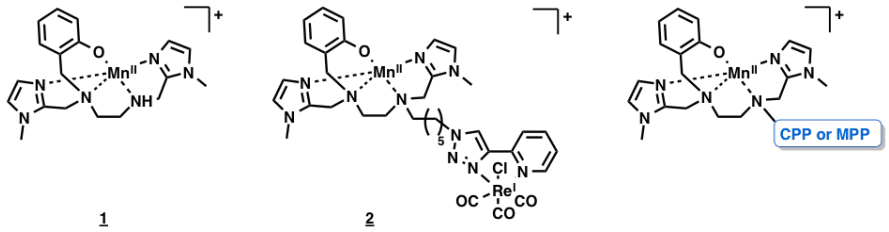

Figure 1. Parent complex **1**, a Re-probe derivative **2** and cell penetrating peptides (CPP) or mitochondria penetrating peptides (MPP) derivatives

The Mn^{II} complex $[Mn\mathbf{L}]^+$ (**1**; **HL**=*N*-(2-hydroxybenzyl)-*N,N'*-bis[2-(N-methylimidazolyl)methyl]ethane-1,2-diamine) is a SuperOxide Dismutase mimics (SODm) with a catalytic constant *in vitro* of 5.0 10^6 $M^{-1}s^{-1}$ corresponding to 1% of the one of the enzyme, and a redox potential of 0.22 V *vs.* SCE.[3-5] These features makes it as a promising SODm and derivatives bearing either a multimodal probe (**2**), or cell penetrating peptides (CPP) or mitochondria penetrating peptides (MPP) have been synthesized.

In this work, the anti-inflammatory activity of **1** and **2** on a cellular model of inflammation and oxidative stress is investigated. **1** and **2** exerts an intracellular anti-inflammatory activity, which is dose-dependent as demonstrated by looking at different markers of inflammation (IL8 and COX2) using biochemistry techniques. The quantification by EPR of **1** and **2** in cell lysates, and the determination of their location using spatially-resolved X-ray fluorescence, are correlated with their activity. Interestingly, these complexes seem to complement mitochondrial MnSOD in activated cells, which is promising for future applications. The effect of the functionalization of **1** by cell penetrating peptides or mitochondria penetrating peptides on its activity is also investigated.

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Spectroscopie Mössbauer *in Cellulo* : Etude des Clusters Fe/S au Sein des Protéines ISCA

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L'assemblage des clusters Fer-Soufre (Iron-Sulfur Clusters, ISC) est un processus essentiel chez tous les organismes vivants.[1,2] Les clusters Fe/S sont tout d'abord assemblés au sein de protéines d'échafaudage avant d'être transférés à des protéines cibles. Les protéines dites de type A sont impliquées dans ces étapes de transfert aux cibles. Il a été démontré que les deux protéines de type A rencontrées chez les eucaryotes, notées ISCA1 et ISCA2, sont des protéines clés pour la formation des clusters Fe_4S_4 présents au sein des protéines mitochondriales chez les mammifères.[3] Cependant, la nature des clusters Fe/S au sein des protéines ISCA (Fe_4S_4, Fe_2S_2 ou Fe) *in vivo* reste inconnue.

Pour comprendre la machinerie, très complexe, d'assemblage des clusters Fe/S chez les mammifères, nous avons entrepris des études par spectroscopie Mössbauer, un outil de choix pour sonder le Fer. Cette technique est spécifique à l'isotope ^{57}Fe et tous les noyaux ^{57}Fe présents au sein d'un échantillon sont détectés. Les signatures dépendent des états d'oxydation et de spin ainsi que de l'environnement des ions Fer, ce qui permet l'identification et la quantification des différentes formes de Fer présentes au sein d'un échantillon.

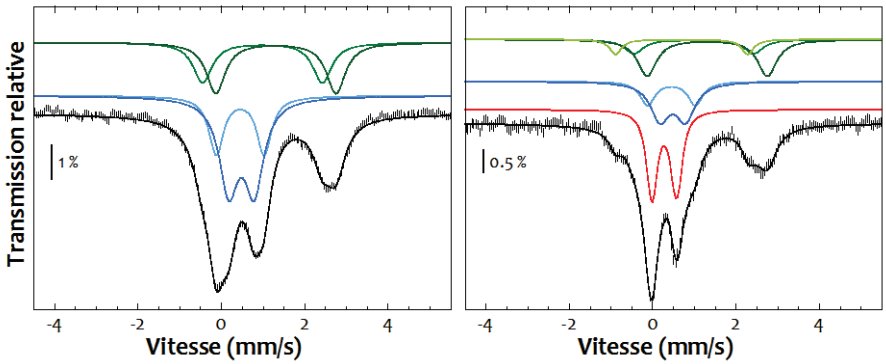

Figure 1. Spectres Mössbauer expérimentaux (hachures verticales) enregistrés à 5.5 K sous un faible champ magnétique externe sur des cellules d'*E. coli* surexprimant (à droite) ou non (à gauche) la protéine ISCA2 de souris. Le spectre théorique (trait plein noir) est superposé. Les différentes contributions sont indiquées en couleur. Celle des clusters Fe/S est en rouge.

Cette présentation s'attachera aux études menées par spectroscopie Mössbauer sur des cellules bactériennes entières surexprimant ou non les protéines de souris ISCA1 et/ou ISCA2. L'abondance et la nature des clusters Fe/S qui sont détectés seront discutées. Ces résultats seront mis en perspective par rapport aux publications récentes portant sur des études RMN *in vitro* des protéines ISCA1 et ISCA2 humaines.[4,5]

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Receptor-based artificial metalloenzymes on living cells

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To produce end-molecules, synthetic chemistry exploits an array of diverse efficient catalysts, which often require harsh and toxic conditions. In contrast, biocatalysis exploits enzymes. These can function under environmentally friendly conditions but posses a relatively limited scope. To overcome these limitations and diversify the reactions that enzymes can catalyze, chemists created artificial metalloenzymes as hybrids incorporating an organometallic catalyst into a biomolecular host.[1] Despite the many advantages provided by artificial metalloenzymes their usefulness is still limited by the necessity of new batches of protein for every catalytic essay.

In this context, we incorporated a copper(II)-1,10-phenathroline complex (**1**) known to efficiently catalyze Diels-Alder cyclization reactions into the adenosine A_{2A} receptor (A_{2A} AR) expressed on the surface of HEK cells.[3] This was achieved by covalently binding a strong antagonist of the receptor (**2**, K_D = 2 nM) with the copper catalyst (**1**). The resulting catalytic entity (**3**) maintained a strong affinity for the receptor (K_D = 4.7 nM) as observed in competition assays with radio-ligand binging (Fig 1, left). Subsequently, catalysis of the Diels-Alder cyclization reaction of cyclopentadiene (**4**) with azachalcone (**5**) was performed using HEK cells expressing the A_{2A} AR in absence and in presence of catalytic entity (**3**) at 4 °C for 73 h (Fig 1, right).

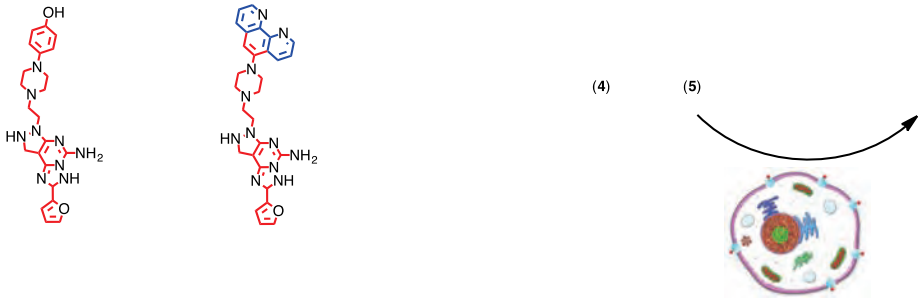

Fig 1: Left, catalytic entity (**3**) made by the bonding of complex (**1**) on antagonist (**2**). Right, schematic representation of HEK cells expressing the A_{2A} AR in presence of catalytic entity (**3**) catalyzing the Diels-Alder cyclization reaction of cyclopentadiene (**4**) with azachalcone (**5**).

In absence of (**3**), the reaction yield was 18 % and there was no selectivity toward any of the four possible products (0 % *ee*). In presence of (**3**), the reaction yield was 78 %, which can be credited to the presence of the copper catalyst. Additionally, the *ee* was 15 % emphasizing the role of the receptor in inducting selectivity in the catalysis.

After the reaction, 70 % of the cells remained alive and could proliferate again, which brings a major advantage in the field of artificial enzymes as it eliminates the need for preparing fresh protein. Additionally, because the catalysis was performed on the surface of living human cells, new perspectives for artificial enzymes can now be foreseen in the field of medicinal chemistry.

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Reactivity of CO Dehydrogenases with O2.

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Reducing CO_2 to high value products may be a possible solution to CO_2 accumulation in the atmosphere and depletion of fossil fuels resources. However CO_2 reduction at low activation energy is very challenging. Indeed man made chemical catalysts have very poor performance so far. But nature present catalysts more efficient: enzymes.

Carbon monoxide dehydrogenases (CODH) catalyze the reversible reduction of CO_2 to CO

$$CO_2 + 2e^- + 2H^+ \leftrightarrow CO + H_2O$$

The active site of CODH isolated from anaerobic bacteria is a [Ni-4Fe-4S] cluster[1,2] (Fig 1B). In the group, we study various aspects of CODH from different organisms, including physiological function, maturation of the active site, mechanism and the reactivity of O_2.

CODHs are presented as extremely oxygen-sensitive, which prevents their use as catalyst for CO_2 reduction. However the mechanism of their inactivation by oxygen is not known. Here we have investigated the effect of oxygen on two CODHs: CODH II from *Carboxydothermus hydrogenoformans* (*Ch*) and the CODH from *Desulfovibrio vulgaris* Hildenborough (*Dv*)[4], using protein film voltammetry[3]. In this technique, the CODH is adsorbed onto an electrode so that electron transfer to or from the protein is direct (Fig 1A). In the presence of carbon monoxide (CO) we measure a CO-oxidation current which is proportional to the enzymatic activity. We analyzed the effect of short exposures to different concentrations of O_2.

We showed that O_2 reacts quickly with the active site of CODHs[5], but the inactivation can be partially reverted by a reductive poise and the extent of reactivation varies from one CODH to the other. We showed that *Dv* CODH is more resistant to O_2 than *Ch* CODH.

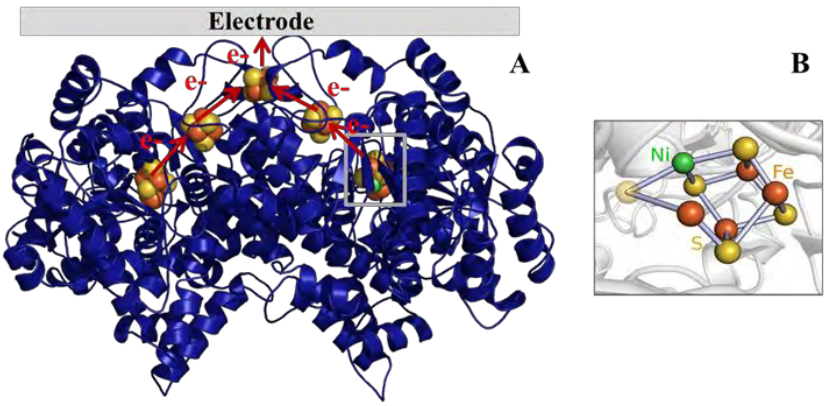

Figure 1: (A) Representation of CODH II from *Carboxydothermus hydrogenoformans* adsorbed onto an electrode. (B) Structure of the active site of CODH II from *Carboxydothermus hydrogenoformans*.

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Uranium binding by microbial iron chelators: from solution to soil studies

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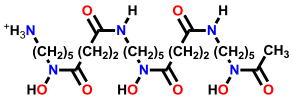
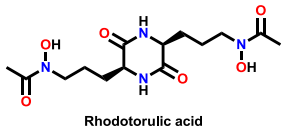
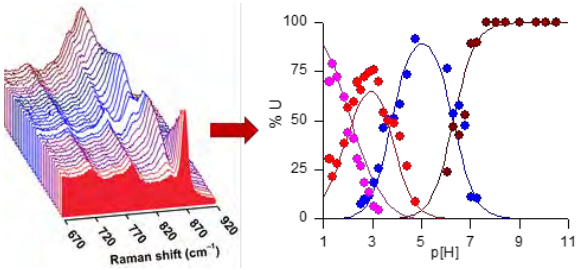
Siderophores are ubiquitous, high-affinity iron(III) chelators excreted by virtually all bacteria and yeasts under iron-stress conditions. Their primary biological role is to supply the microorganisms with iron, an essential nutrient and growth factor. As a mean to circumvent the extremely low bioavailability of this element at physiological pH, siderophores react with ferric (oxo)hydroxides and form thereby water-soluble complexes which are transported across the cell membranes according to an energy-driven mechanism involving specific outer-membrane uptake receptors. Hydroxamates are common bidentate chelating groups found in many siderophores, desferrioxamine B and rhodotorulic acid being emblematic representatives.





As their concentration in soils is typically in the µg/kg range, these compounds might significantly increase the solubility, migration rate, and bioavailability of highly-toxic actinides in case of environmental contamination. Indeed, desferrioxamines B and E for example are not only able to dissolve $Pu(OH)_4$ [1], but moreover both ligands mediate efficiently plutonium(IV) uptake by several bacterial strains [2]. Thus, in relation to the management and remediation of contaminated fields or the disposal of nuclear wastes in geological repositories, it is of outmost importance to gain a deeper understanding of the coordination chemistry of f-element by siderophores and related chelators. However, predicting and modeling the metal speciation in water and soils requires an accurate knowledge of the thermodynamic and kinetic parameters related to the complex formation and dissociation equilibria. Because such data are scarce and often unreliable in the case of the transuranium cations [3], considerable research efforts are still required.

The conformational issues and actinide binding properties of linear and cyclic monohydroxamic acids will be addressed before discussing the desferrioxamine B/uranium(VI) system [4,5]. Speciation was inferred from potentiometric, spectrophotometric, Raman and calorimetric titrations combined with NMR and capillary zone electrophoretic measurements. X-ray absorption and Raman spectroscopies enabled to probe the chemical environment around the uranium center in the different species prevailing in solution. The proton-assisted step-by-step dissociation mechanism of the $[UO_2(DFB)H_2]^+$ complex investigated by stopped-flow spectrophotometry will be discussed too. Finally, uranium dissolution tests performed on UO_2-rich soils (pitchblende) will be presented.



This work is supported by the program Défi NEEDS Environnement (project "Actisol") and the Conseil Régional de Bourgogne (contract PARI II "CDEA").

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Nucleolipids, self-assembly and physico-chemistry

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Nucleolipids are amphiphile molecules with very interesting properties. Indeed, they are able to self-organize into various supramolecular objects (vesicules, micelles, fibers...). These assemblies could be used for a large number of applications from drug delivery[1] to stem cell culture[2] including medical imaging[3].

Nevertheless, how external parameters, such as pH, metal ions[4], and the type of nucleolipides (nucleobase, lipidic chains...) influence the supramolecular formation is still unclear. Using different techniques (TEM, UV-vis, DLS...), we investigated the influence of metal ions on the self-organized structure formed.

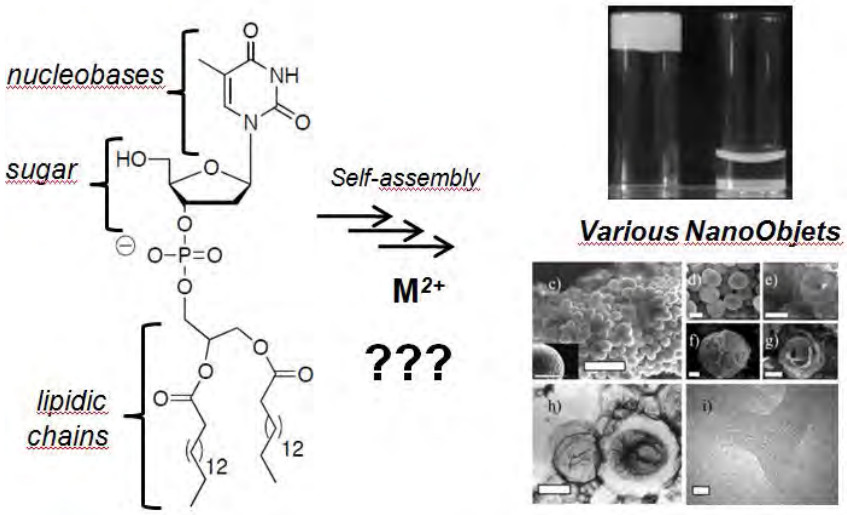

Figure. Left side, the detailed chemical structure of nucleolipids; Right side, macroscopic (top) & microscopic (bottom) images obtained by TEM & SEM of objects formed.

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Oxidation of veratril alcool. Bioinspired model of galactose oxidase.

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Galactose oxidase (GOase)[1] is a mononuclear fungal copper metalloenzyme, which belongs to the class of copper type-2 enzymes.[2] Under aerobic conditions it catalyses the two-electron oxidation of D-galactose and a broad range of primary alcohols to the corresponding aldehydes under simultaneous reduction of molecular oxygen to hydrogen peroxide.[3]

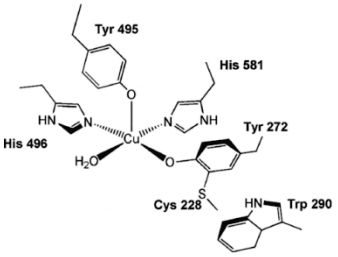

Figure 1. The active site of GOase, determined by X-ray crystallography. [4]

With the aim to synthetize a bioinspired model of GOase was realized the synthesis of Bis[2-(2H-benzotriazol-2-yl)-4,6-ditert-pentylphenolato]copper(II) as a green polycrystalline compound which in DMF is forming a brown monocrystalline pentacoordinated copper-centered complex compound. The structure of both compounds was determined by X-ray crystallography. Elemental Analysis, IR and Mass spectroscopies are in accordance with the determined crystallographic structure. The compound was characterized by electrochemical and spectroelectrochemical methods.

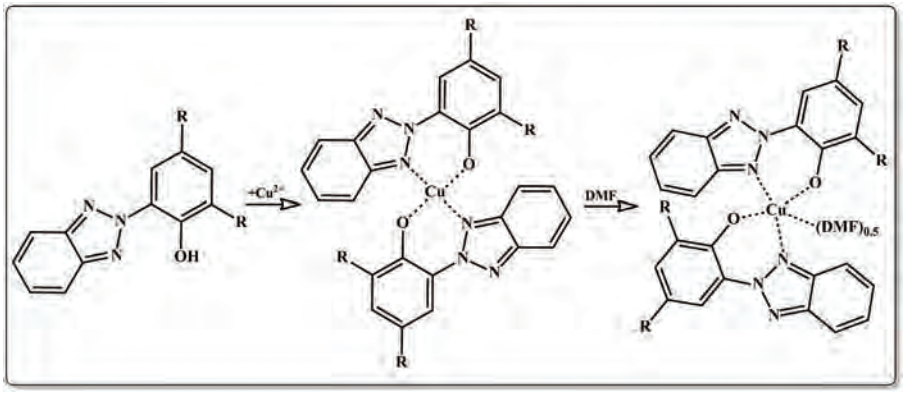

Figure 2. Reaction scheme of Cu-complexes synthesis. R = *tert-pentyl*.

The oxidation of veratryl alcohol was performed in DMF under different conditions. From Cyclic Voltammetry data the oxidation of alcohol into aldehyde occurs with TOF > 6.6 s^{-1}. The formation of aldehyde was identified as well by HPLC.

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Ni-Fe complexes mimicking the active site of Ni-Fe Hydrogenase.

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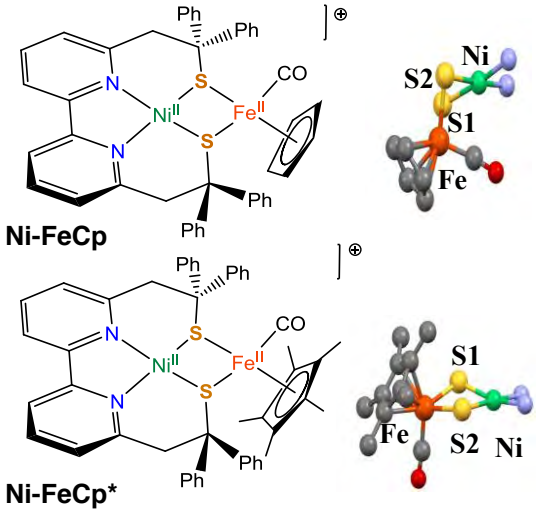
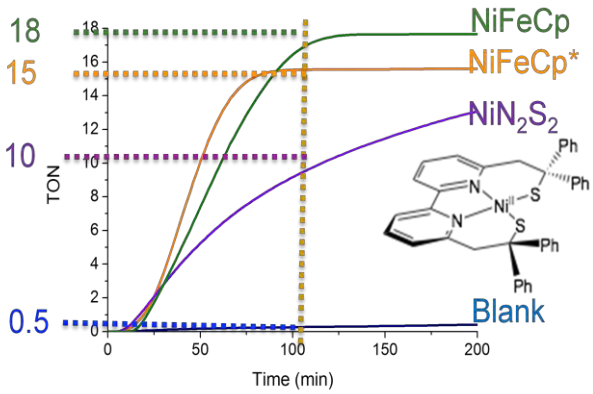
Hydrogenases, one of the rare families of organometallic biomolecules, are unique catalysts for both the production of hydrogen from water and its oxidation back to water. The Ni-Fe Hydrogenase contains two metal ions, Ni and Fe, bridged by two cysteinyl thiolates. Another two cysteine residues are bound to the Ni ion whereas three diatomic ligands (one CO and two CN^-) are coordinated to the Fe ion.
Our objectives are to design and develop new models that mimic not only the main structural properties of the active site but also its function.

Two new catalysts have been synthesized and characterized. The Ni-FeCp complex, where structural properties displays the best analogy with the active site of the Ni-Fe hydrogenase, shows the best reactivity towards proton reduction in MeCN compared to mononuclear Ni complex. We were thus able to correlate the structure and the reactivity of the catalysts.





Catalytic study (50 equiv. of Et_3NHBF_4 [C]= $1.84.10^{-4}$ M, Applied potential: -2.1 V).

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EPR Study of Copper(II) Complexes with Polyaza-macrocycles of Interest for ^{64}Cu-Based PET Imaging

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Molecular imaging plays a steadily growing role in medical diagnosis and evaluation of therapeutic effects of new drugs. Among the widely used techniques, positron emission tomography (PET) is a non-invasive imaging modality providing insights into the physiological processes that occur within a living organism. ^{64}Cu is a particularly promising radionuclide for use in PET imaging. However, the use of ^{64}Cu-based radiotracers requires the development of high affinity chelators forming stable, inert, and non-reducible complexes under physiological conditions in order to prevent the release of the radioelement.

Among the variety of linear and cyclic chelators considered so far as potential ^{64}Cu-based radiotracers [1-3], researches evidenced high in-vivo performances of cyclic polyaminocarboxylates (Figure 1) [4,5]. In the present study, cyclam and cyclen functionalized with four carboxylate (TETA^{4-}, DOTA^{4-}) or carboxamide (TETAM) pendant arms, as well as mixed derivatives bearing three acetate and one acetamide groups (TE3AAM^{3-}) were considered. The cross-bridged cyclam ligand CB-TE2A^{2-} bearing two carboxylate arms was also studied.

Copper(II) binding has been monitored by electron paramagnetic resonance (EPR) spectroscopy, allowing us to unravel the speciation as a function of the solution composition and pH. EPR spectroscopy is certainly one of the most informative methods since characteristic signatures of the paramagnetic species are generally obtained in solid state or in frozen solution. The determination of the *g* factors as well as the hyperfin coupling constants provide valuable information about the coordination sphere around the metal center. In this study, we have established correlations between crystallographic and EPR data (H_2O, 100 K) for the $^{63/65}$Cu(II) complexes as a function of pH, which provide insights into the coordination geometry both in solution and in the solid state.

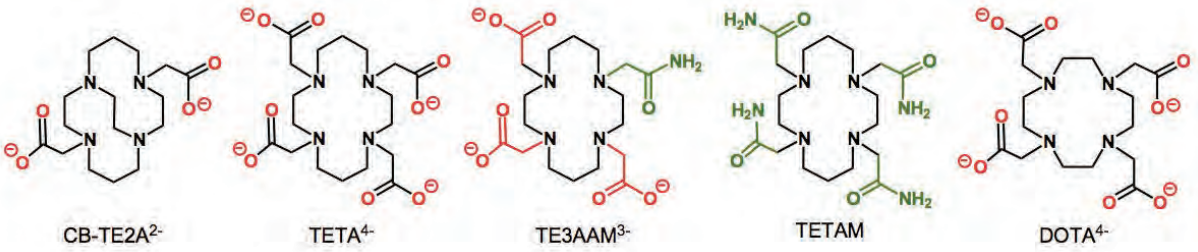

Figure 1. Ligands considered for EPR analysis of Cu(II) complexes

Support was provided by the CNRS, the Université de Bourgogne, and the Conseil Régional de Bourgogne through the program PARI II "Pharmaco-imagerie et agents théragnostiques".

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The use of metals bound to peptides as genetically encodable spin-labels for nanometric distance determination in proteins

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Distance measurements are of importance in structural biology, an arguably equally important biological problem is the understanding the quaternary structures of proteins, the arrangement of protein complexes and how proteins interact with each other, especially in the cellular milieu which can significantly differ from test-tube conditions. Pulse electron-electron double resonance (PELDOR or DEER) has become proven method for studying structures of biological systems. The technique can measure dipolar interaction between paramagnetic centers and has been used measure distances as long as 10 nm. One of its great advantages is that such measurements can be done on systems in a wide variety of environments, even within the complex interior of cells. However, the conventional nitroxide-based DEER approaches are limited by the instability of radicals in the reducing environment of the cytosol. Because of this limitation metal-based spin-labels are more appealing. To date such labels have involved a redox stable paramagnetic metal ion encapsulated inside a well-define ligand sphere that has a high binding efficiency for the metal. As we have recently showed Mn(II)-DOTA can be used as an effective spin label for high-field, high-frequency DEER meassurements.[1] However nearly all ligand systems have been synthetic and, like nitroxide labelling, requiring a linking group. Rather than labelling proteins by chemical reactions, a genetically encoded spin-label would greatly simplify sample preparation, and also facilitate *in situ* and ultimately *in vivo* DEER measurements. To this end we have explore the use of short peptide sequences as anchoring points for metals ions, in this way the spin label could be directly ensemble *in situ* by adding the metal ion to the peptide-tagged protein. Two approaches were tested: binding of Mn(II) to hexa-histidines (His-Tag) and binding of Gd(III) to lanthine binding loops (LBT). His-tags are commonly used as a way of purifying overexpressed proteins while LBT have been extensively used in MRI. Both systems were study on synthetic peptides and overexpressed triple-helix bundles carrying a peptide tag at each end. In these peptides the two metal ion anchoring position were separated by ~4nm. His-tags affinity for Mn(II) was low but this did not significantly impair the use of Mn(II) bound to His-tags as spin-labels for DEER measurements which were in agreement with the estimated distances. LBT affinity for Gd(III) is much higher which resulted the absence of free Gd(III). The advantages and disadvantages of these to systems as well as the perspective of they use for *in situ* and *in vivo* DEER measurements will be discussed.

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Role of a highly conserved amino in the intramolecular electron transfer acid within the molybdoenzymes from the DMSO reductase family

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Molydboenzymes are ubiquitous in living organisms and catalyze, for most of them, oxidation-reduction reactions of a large range of substrates. These enzymes are involved in the biogeochemical cycles of essential elements (C, S, N) and their studies may find application in the field of energy, environment or in health (1).

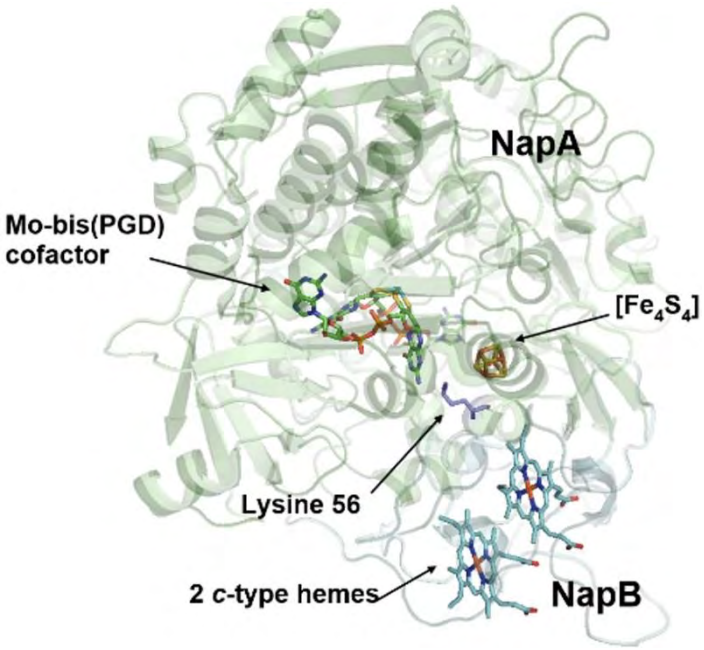

Figure 1. Crystallographic structure NapAB from *Rhodobacter sphaeroides* at 3.2 Å resolution (2)

Periplasmic nitrate reductase (NapAB) from *Rhodobacter sphaeroides* catalyzes the 2-electron reduction of nitrate in nitrite. Its active site, named the molybdenum cofactor, is a Mo bis-(pyranopterin guanine dinucleotide) found in all molybdoenzymes from the bacterial DMSO reductase family. A [4Fe4S] cluster and 2 *c*-type hemes form the intramolecular electron transfer chain (3). Lysine 56 (K56) is a highly conserved amino acid within enzymes from the DMSO reductase family. This residue connects through hydrogen-bond network the [4Fe-4S] center to the end of one pyranopterin ligand of the Mo and is expected to be involved in the electron transfer. In this work, we investigated the role of the K56 amino acid by site-directed mutagenesis, activity assays measurements, redox titrations and EPR and HYSCORE spectroscopies of the purified K56M and K56H mutants. Our data show that mutation of K56 strongly affects enzyme's activity while integrity and structural properties of the metallic centers of the K56M and K56H mutants are conserved. Surprisingly, we found that kinetics of electron transfer between the two hemes and the [4Fe-4S] was dramatically impaired in the mutants. These results are discussed in light of the redox properties changes of the centers.

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Nouveaux catalyseurs moléculaires pour l'activation de l'eau

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Splitting water is a good way to cleanly provide protons and electrons for the synthesis of alternative energy carriers like H_2 or MeOH. Developing efficient and cheap catalysts are prerequisite to the achievement of such technologies. Ideally, these catalysts should operate at low overpotentials and be coupled to a photoactive unit to achieve light-driven processes. The interest of molecular chemists in this area has been mostly focusing on mimicking enzymatic systems, performing a plethora of oxidative transformations under mild conditions and sometimes with the help of solar energy.[1,2]

$$2\,H_2O \longrightarrow 1/2\,O_2 + 4\,H^+ + 4\,e^- \qquad (1)$$

$$H_2O + S \longrightarrow SO + 2\,H^+ + 2\,e^- \qquad (2)$$

In this context, our efforts have recently been directed at the development of new ligands platforms that could favor the formation of highly oxidized metal-oxo species by activation of a water bound molecule in order to perform clean oxidative transformations as an alternative to most pollutant processes currently used in industry.[3,4]

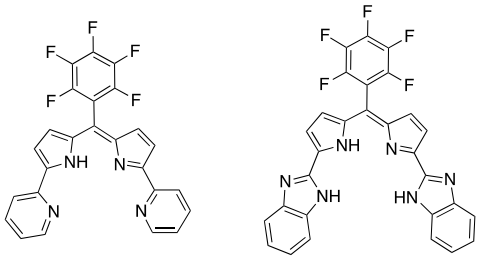

Figure 1 Examples of ligand platforms presented in this study

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Interaction Frataxine-Metal: cas du CuI et CuII

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L'ataxie de Friedreich est la plus fréquente des ataxies héréditaires humaines. C'est une maladie neurodégénérative autosomique récessive touchant environ une personne sur 50 000. Dans la majorité des cas, la maladie est causée par l'expansion du triplet (GAA) dans l'intron 1 du gène FXN, qui code pour une protéine mitochondriale, la frataxine. Dans les cellules malades, l'expression de la frataxine est réduite, ce qui conduit à des dysfonctionnements dans le métabolisme du fer comme la diminution de la biosynthèse des centres fer-soufre et des hèmes, l'accumulation du fer dans la mitochondrie, et l'hypersensibilité des cellules au stress oxydant [1,2]. Néanmoins, le rôle physiologique de la frataxine reste encore un sujet à controverse.
La structure de la frataxine est conservée de la bactérie à l'homme. Elle présente à sa surface de nombreux acides aminés chargés négativement qui pourraient interagir avec des cations métalliques [3]. Le but de ce travail est de savoir si la frataxine est impliquée dans le métabolisme d'autres métaux que celui du fer.

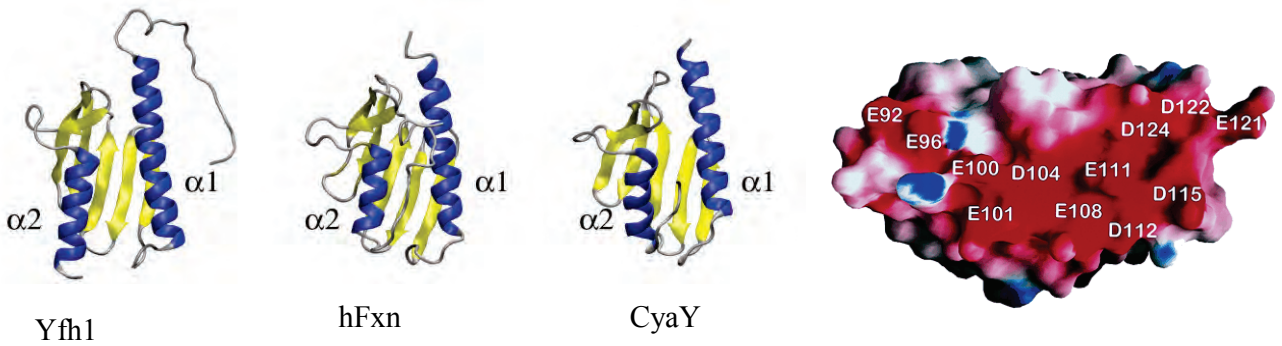

Figure : Structure des frataxines de levure (Yfh1), humaine (hFxn) et bactérienne (CyaY). Surface négative de la frataxine

Dans cette étude, après avoir extrait et purifié yfh1 la frataxine de levure, nous avons étudié in vitro l'interaction de cette protéine avec les métaux mitochondriaux (Fe, Cu, Zn, Mn) par spectrophotométrie de fluorescence, chromatographie d'exclusion stérique et microcalorimétrie. Nous avons montré que tous les métaux étudiés interagissent avec yfh1, avec une meilleure affinité que celle du fer.
Ensuite, par la technique de l'écoulement interrompu « Stopped-flow », nous proposons les mécanismes de complexation de CuII et de CuI par la frataxine. Ces résultats préliminaires pourraient permettre une meilleure compréhension de la fonction de cette protéine dans la synthèse des centres [Fe-S] et de son rôle dans le stress oxydant.

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Control d'une réaction radicalaire par la tryptophane lyase NosL

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Le nosiheptide est un antibiotique de la famille des thiopeptides. Il a longtemps été utilisé comme complément alimentaire pour l'élevage des poulets et des porcs. Ces dernières années, le nosiheptide a suscité un regain d'intérêt, suite à la mise en évidence de son efficacité contre un certain nombre de souches multi résistantes de pathogènes gram positifs. Le nosihpetide correspond à un peptide riche en résidus cystéine hautement modifié (Figure 1), contenant cinq cycles thiazole et un cycle pyridine tetra-susbtitué. Il comporte aussi un cyclé dérivé de l'acide 3-méthyl-2-indolique (MIA) synthétisé indépendamment à partir du tryptophane et subséquemment inséré dans le peptide.

La tryptophane lyase NosL, responsable de la synthèse du MIA, appartient à la superfamille des protéines à radical *S*-adénosyl-L-méthionine (SAM). Ces enzymes utilisent la réduction d'un centre Fe_4S_4 pour produire, à partir de la SAM, une espèce 5´-déoxyadénosyl radicalaire hautement réactive. Cette dernière va alors servir d'activateur pour déclencher une réaction radicalaire permettant la conversion du L-tryptophane en MIA.

Au cours des dernières années, nous avons réalisé une étude structurale (1) et fonctionnelle (2), couplée à une analyse par spectroscopie de résonance paramagnétique électronique (3) qui nous a conduit à réviser totalement le mécanisme réactionnel de l'enzyme. Nos résultats illustrent la plasticité de la matrice protéique pour la sélection et le contrôle d'une réaction radicalaire sans précédent.

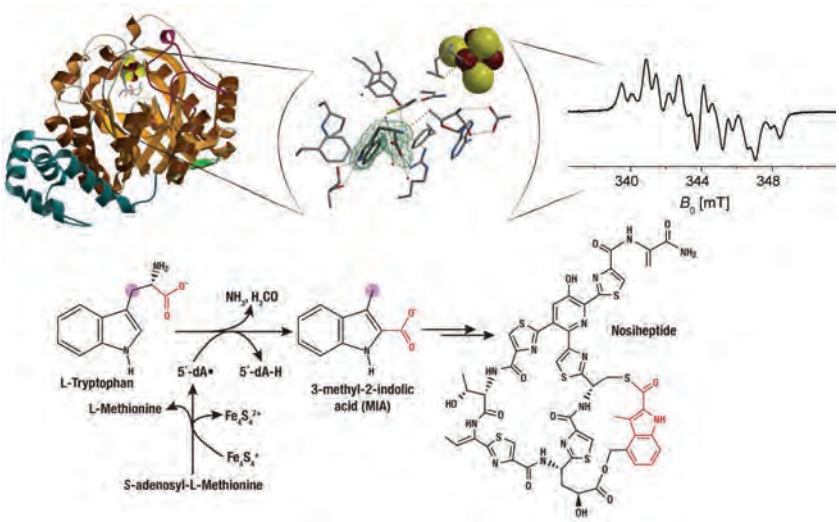

Figure 1 : Structure de NosL, zoom de son site actif présentant le tryptophane lié, spectre de résonance paramagnétique électronique correspondant à un intermédiaire radicalaire et réaction catalysée par NosL pour la synthèse du MIA avant son insertion dans le nosiheptide.

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Le superoxide est un intermédiaire dans la production de H_2O_2 par le complexe Cu(I)/Aβ et l'O_2.

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Les plaques amuloïdes, un de marqueurs de la maladie d'Alzheimer, contiennent une concentration élevée en peptide amyloïde β (Aβ) qui, lors du développement de la maladie, passe d'une forme monomérique soluble à une forme agrégée insoluble.

Les espèces les plus toxiques vis-à-vis des neurones sont les espèces oligomériques intermédiaires du processus d'agrégation. En effet, ces espèces peuvent produire, suite à leur complexation par les ions métalliques Cu et en présence d'agent réducteur, des espèces réactives oxygénées (ROS) tels que le peroxyde d'hydrogène (H_2O_2) et le radical hydroxyle ($OH^{\bullet}$).

Les mécanismes exacts de la production des ROS par le complexe Cu/Aβ restent peu clairs. Generalement, la litterature suggère que la réduction d'O_2 en H_2O_2 par le complexe Cu/Aβ est directe sans liberation de superoxide ($O_2^{\bullet-}$) (Figure1 : mécanisme 2). En 2010 Zhou et. al. ont proposé que cette réduction passe par la formation de $O_2^{\bullet-}$. Cependant il n'existait aucune donnée expérimentale démontrant la présence de $O_2^{\bullet-}$ dans le cycle catalytique des ROS.

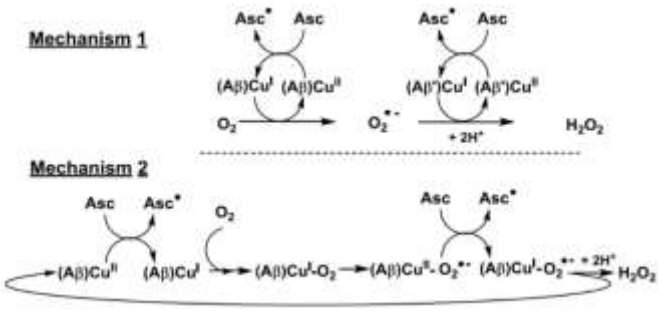

Figure1. Mécanismes potienciels pour la production de H_2O_2 pas le complexe Cu/Aβ à partir de O_2.

A l'aide une étude d'électrochimie et l'utilisation de la superoxyde dismutase-1 (SOD-1), nous avons démontré pour la première fois que la production de H_2O_2 par le complexe Cu/Aβ passe principalement par un $O_2^{\bullet-}$ intermédiaire (Figure 1 : mécanisme 1). Cette découverte change le regard porté sur le mécanisme de production de H_2O_2 par le complexe Cu/Aβ. Le $O_2^{\bullet-}$ pourrait donc participer aux dommages oxydatifs de la maladie d'Alzheimer, ce qui est d'intérêt dans la recherche de l'inhibition de la toxicité du complexe Cu/Aβ.

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Etude cinétique de l'inhibition des hydrogénases [FeFe] par le dioxygène

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Les hydrogénases sont des enzymes qui catalysent l'oxydation de l'hydrogéne en protons de manière réversible. Elles sont classées en deux catégories selon métaux contenus dans leur site actif: les hydrogénases [FeFe] ou [NiFe].

Il faut comprendre l'inactivation de ces enzymes dans des conditions oxydantes (aérobies et anaérobies) pour pouvoir espérer les utiliser dans des biopiles ou pour la photoproduction d'hydrogène.

Récemment, en étudiant par électrochimie directe les hydrogénases [FeFe] de *Chlamydomonas reinhardtii* (*Cr*) et *Clostridium acetobutylicum* (*Ca*), nous avons démontré l'existence de deux formes inactives oxydées de l'enzyme en équilibre avec la forme active[1]. Ces enzymes sont aussi inhibées par le dioxygène,[2] mais l'expérience de la figure 1 montre qu'elles réagissent de façon très différentes.

Afin de comprendre l'origine de cette différence, nous avons interprété des expériences de chronoampérométrie comme celles de la fig 1 en utilisant un modèle cinétique qui prend en compte l'équilibre avec les deux formes inactives oxydées. Cela nous a permis de déterminer précisément les différentes constantes cinétiques qui décrivent l'inhibition par le dioxygène, de comparer différentes enzymes, et de caractériser le mécanisme d'inhibition.

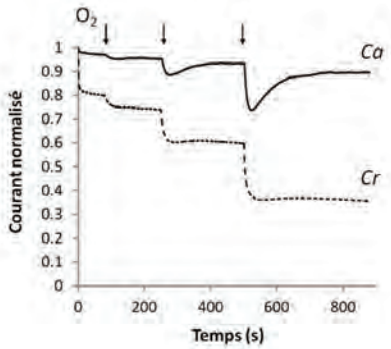

Figure 1. Courant catalytique d'oxydation du dihydrogène par les hydrogénases [FeFe] de *Ca* (—) et *Cr* (- - -) greffées sur électrode avec lesquelles elles échangent les électrons directement (0.04V vs. SHE, 1 atm. H_2, pH= 7, T= 12°C), les flèches marquent les injections de 1.25, 6.25 et 25 µM d'O_2.

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Influence of iron(III) on the cyclization of catecholamines through Pictet-Spengler reactions

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Catecholamines represent a widespread class of neurotransmitter in the central nervous system. Dopamine and adrenaline are naturally secreted *in vivo* but are also commonly used as medicines. Under specific circumstances, catecholamines reactivity/metabolisms can be modified and lead to the formation of neurotoxic tetrahydroisoquinoline derivatives[1]. It is known that this class of molecules could decompose giving formaldehyde which can in a second step lead to a secondary reaction called the Pictet-Spengler Reaction (PSR)[2]. Similar reaction was reported *in vivo* notably for L-dopa treated parkinsonian patients with the formation of Tetrahydropapaveroline[3] or 1,2,3,4-tetrahydroisoquinoline alkaloids (for instance salsolinol) for chronic alcoholism[4].

The PSR consists in the condensation of formaldehyde with an arylalkylamine type compound leading to one or two bicyclic products depending on the substituents of such molecules. In the case of catecholamines, two regioisomers of tetrahydroisoquinolines (THIQ) can be obtained[5].

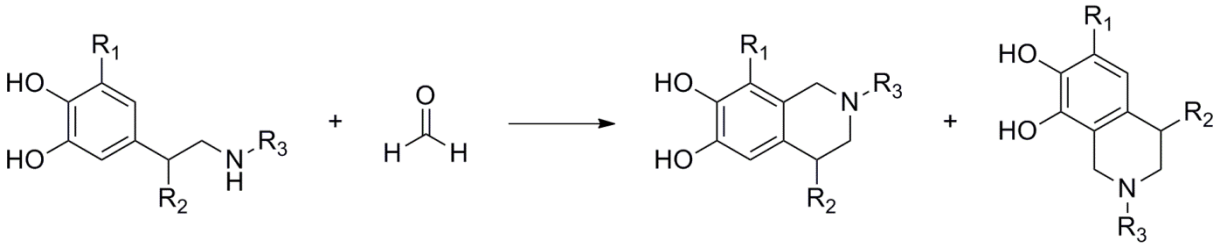

Figure 1 Formation of two regioisomers of tetrahydroisoquinolines through a Pictet-Spengler reaction

In a previous work[6], it was shown that the kinetic and stereioselectivity of the PSR reaction was affected by the presence of transition metals, namely Cu^{2+} and Fe^{3+}, two ions typically encountered *in vivo*. In the present work, we will discuss our results on the influence of the presence of ferric ions on the reactivity of catecholamines with formaldehyde notably on the regioselectivity of the cyclization through PSR.

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Synthesis and solution chemistry of new substituted piperazines iron(III) chelators

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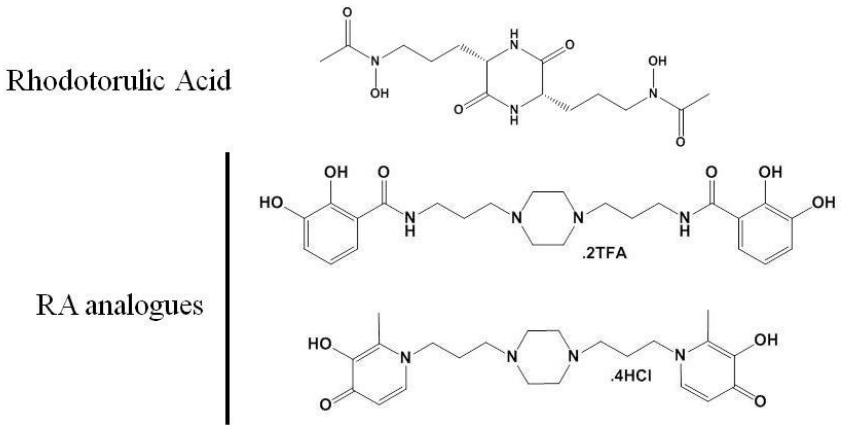
Antibiotic resistance phenomena towards infectious agents (parasites, virus or bacteria) are becoming extremely threatening for public health. Indeed, these resistance problems lead to some difficulties to treat some diseases that we expected to have eradicated during the last decades, such as turberculosis[1] or plague[2], to quote only two of them. The appearance more and more frequent of new multidrug-resistant (MDR) strains of bacteria implies the exploration of new therapeutic routes to tackle this threat.

For a few years, our group has been working on the preparation of siderophores analogues-antibiotic conjugates to specifically target bacteria and increase their influx through the siderophore pathway[3,4]. To control the vectorization of the conjugates inside the bacteria, it is important to understand the coordination chemistry of the siderophores analogues towards iron(III). Among the large siderophore family, Rhodotorulic Acid (RA), produced by *Rhodotorula Pilimanae*[5], is a peculiar system as it is a tetradentate piperazine-based ligand that possesses two hydroxamate ligands to chelate ferric iron. It thus leads to a complex with a 2:3 Fe:L stoichiometry under physiological conditions. Substituted piperazines are found in a large variety of drugs such as antidepressants, anxiolytics, anthelmintics agents, antibiotics and antihistamines and possess a very low acute toxicity in mamal[6]. In this communication, we will present the synthesis and complexation behaviour of RA analogues based on substituted piperazine core and catechol or hydroxypyridinone coordinating groups.



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A modular approach for the synthesis of ditopic ligands of easily-incrementable length

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We report here the design and efficient synthesis of homoditopic and heteroditopic ligands that consist of a central rigid oligo(phenylene-ethynylene) (OPE)[1] spacer of incrementable length and terminal chelating units of various nature: terpyridine, picolinic acid, DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate). A convergent synthesis of OPE spacers bearing alternate phenyl rings with and without small PEG chains (diethyleneglycol methyl ether), which were introduced to improve water solubility, was first devised using Sonogashira couplings between ethynylbenzene derivatives and a iodinated building block equipped with the PEG chains. Using this strategy, spacers of increasing length – up to seven phenyl-acetylene units – and terminated with a wide range of chemical groups (amine, ethynyl, aldehyde, carboxylic acid, azide) suitable for further modifications were easily obtained. Subsequent functionalizations with various chelating units successfully afforded symmetrical and dissymmetrical platforms (Figure).

This approach allows for the easy access to platforms incorporating two metal complexes separated by a controlled and predictable distance.[2] The accessible ditopic ligands could find interesting applications in diverse fields such as model compounds for pulse EPR spectroscopy,[3] metal-organic frameworks (MOFs),[4] supramolecular chemistry or architectures with multimodal properties.

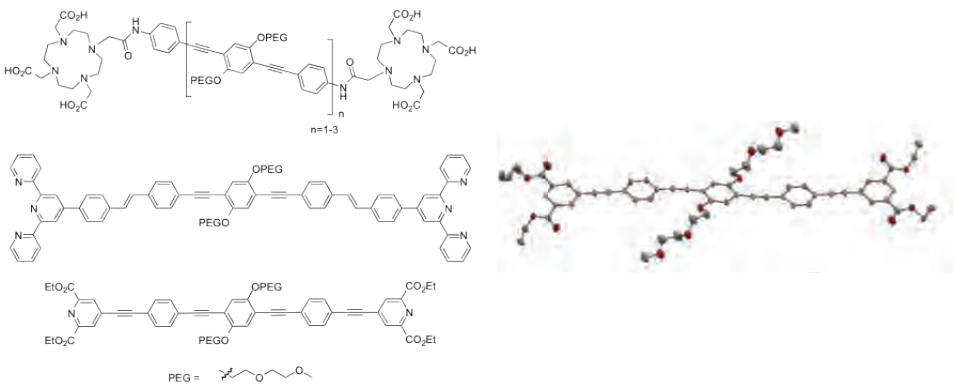

Figure 1. Examples of ditopic ligands and ORTEP drawing of a dipicolinic diethyl ester derivative

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Coordination of Zn(II) with human and murine Amyloid-β

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Alzheimer's disease (AD) is the most common neurodegenerative disease, with no known cure. In the brain of Alzheimer's patients are present intracellular neurofibrillary tangles and 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 and later 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).

A better understanding of the AD mechanism requires investigations on mouse and rat models. However, the murine Aβ peptide, which differs from the human Aβ peptide by three point mutations, do not show amyloid deposition[3]. Consequently, studies are performed on transgenic mice or rats that produce the human Aβ (hAβ) peptide in addition to their own peptide (mAβ). The Cu(II) coordination to murine and human peptides differ and this has impact on redox process.[3]

Here we want to determine the coordination of Zn(II) on human and murine peptides and their differences. We also want to see whether they have an impact on aggregation. The aggregation of the peptide is evaluated by fluorescence and verified by AFM, while the different coordination is evaluated XANES, ^{1}H-NMR, affinity constant.

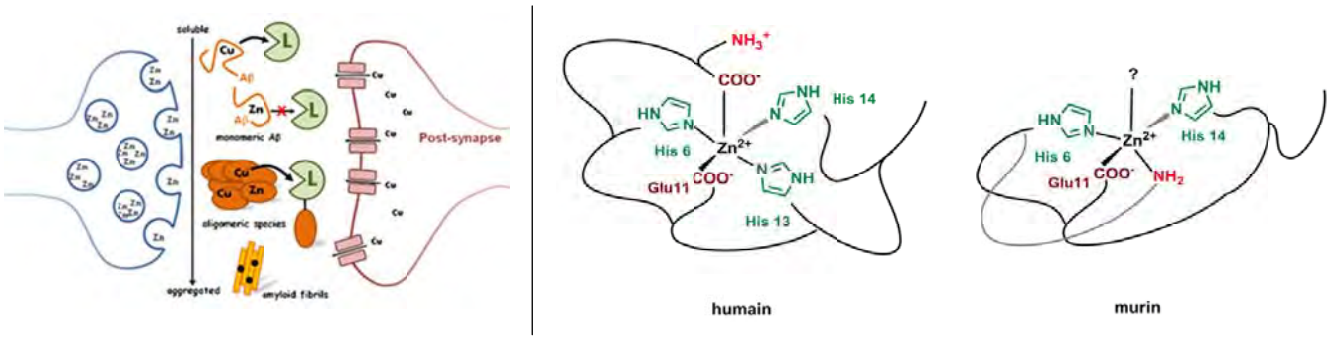

Figure 1. Proposed binding modes of Zn(II) to hAβ and mAβ.

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EPR Study of Copper(II) Complexes with Polyaza-macrocycles of Interest for ^{64}Cu-Based PET Imaging

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Molecular imaging plays a steadily growing role in medical diagnosis and evaluation of therapeutic effects of new drugs. Among the widely used techniques, positron emission tomography (PET) is a non-invasive imaging modality providing insights into the physiological processes that occur within a living organism. ^{64}Cu is a particularly promising radionuclide for use in PET imaging. However, the use of ^{64}Cu-based radiotracers requires the development of high affinity chelators forming stable, inert, and non-reducible complexes under physiological conditions in order to prevent the release of the radioelement.

Among the variety of linear and cyclic chelators considered so far as potential ^{64}Cu-based radiotracers [1-3], researches evidenced high in-vivo performances of cyclic polyaminocarboxylates (Figure 1) [4,5]. In the present study, cyclam and cyclen functionalized with four carboxylate (TETA^{4-}, DOTA^{4-}) or carboxamide (TETAM) pendant arms, as well as mixed derivatives bearing three acetate and one acetamide groups (TE3AAM^{3-}) were considered. The cross-bridged cyclam ligand CB-TE2A^{2-} bearing two carboxylate arms was also studied.

Copper(II) binding has been monitored by electron paramagnetic resonance (EPR) spectroscopy, allowing us to unravel the speciation as a function of the solution composition and pH. EPR spectroscopy is certainly one of the most informative methods since characteristic signatures of the paramagnetic species are generally obtained in solid state or in frozen solution. The determination of the *g* factors as well as the hyperfin coupling constants provide valuable information about the coordination sphere around the metal center. In this study, we have established correlations between crystallographic and EPR data (H_2O, 100 K) for the $^{63/65}$Cu(II) complexes as a function of pH, which provide insights into the coordination geometry both in solution and in the solid state.

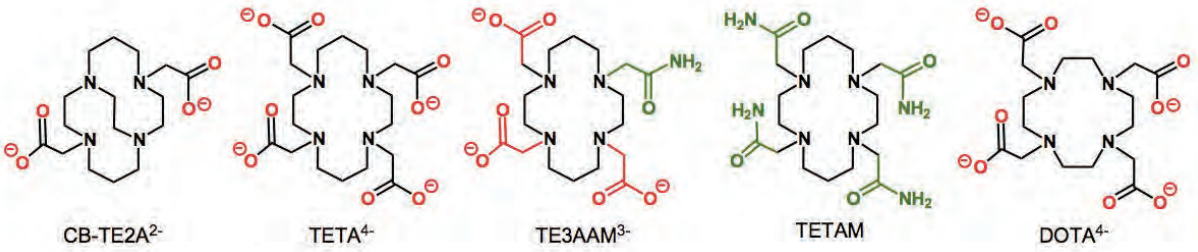

Figure 1. Ligands considered for EPR analysis of Cu(II) complexes

Support was provided by the CNRS, the Université de Bourgogne, and the Conseil Régional de Bourgogne through the program PARI II "Pharmaco-imagerie et agents théragnostiques".

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Into the giant viruses' world: characterization of a new iron-sulfur protein

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Giant viruses were discovered about more than a decade ago. They display unexpected features for viruses, namely sizes of the viral particle higher than 0.5 µm and complex genomes containing from 500 to 2500 genes, depending on the virus. Most of their genes encode proteins never encountered before in a virus or without homology in the cellular world. Four families are identified to date, Mimiviridae, Pandoraviridae, Pithoviridae and Molliviridae[1-4], and it is believed that these viruses are widespread and abundant in the environment.

Mimivirus, the first giant virus identified as such, is the fourth largest known virus in Mimiviridae family in particle size (750nm) and genome complexity [5,6]. Its 1.2 Mbp genome was predicted to encode more than 1 000 proteins, less than 50% of which having assigned functions. Transcriptional analysis of Mimivirus infecting *Acanthamoeba* hosts revealed transcripts corresponding to unpredicted genes[7]. Among them, the most transcripts (R633b gene) encode a protein which displays a brownish color when overexpressed in *Escherichia coli*. This small protein of 5.6 kDa is conserved in the Mimiviridae family, features an atypical sequence (about 50% of glycine, high cysteine content) and seems essential in the viral infection process while its function is unknown.

We have undertaken a detailed characterization of this peculiar protein from Mimivirus and Megavirus chilensis by combining UV-Visible absorption spectroscopy, EPR spectroscopy (amibs, viruses, *E. coli* cells overexpressing this protein, recombinant purified proteins) to elementary analysis (Fe, acid-labile sulfide) and *in vitro* Fe-S reconstitutions. Our first results show that this viral protein contains an Fe-S cluster indeed different from the typical Fe-S clusters found in cellular proteins (rubredoxin-like cluster, [2Fe-2S], [3Fe-4S] or [4Fe-4S]), and whose structure remains to be elucidated.

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Zn impact on the Cu(II) chelation in the context of 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. In this study, differents [N,O] chelators are tested, like the L2 and NBPMG ligands.[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 Cu(II) removal from Aβ by chelators (in presence or absence of Zn(II)) and the detection of ROS production by EPR and UV-vis, and monitored the aggregation of the peptide by Thioflavine T fluorescence, verified by AFM.

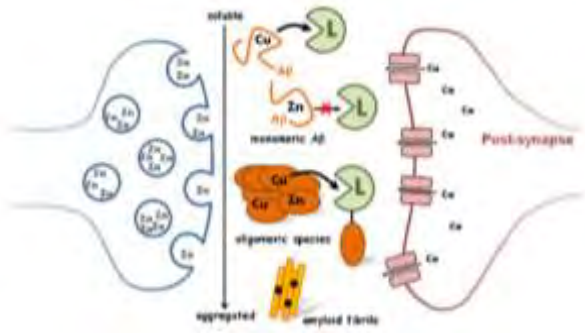

Figure 1. Scheme of the amyloid cascade hypothesis.

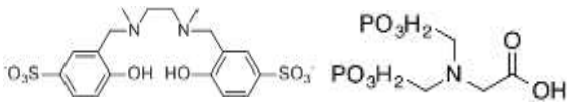

Figure 2. Structures of Cu(II) chelators : L2 and NBPMG.

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Complexes peptides-lanthanide : vers de nouvelles sondes luminescentes de l'ADN

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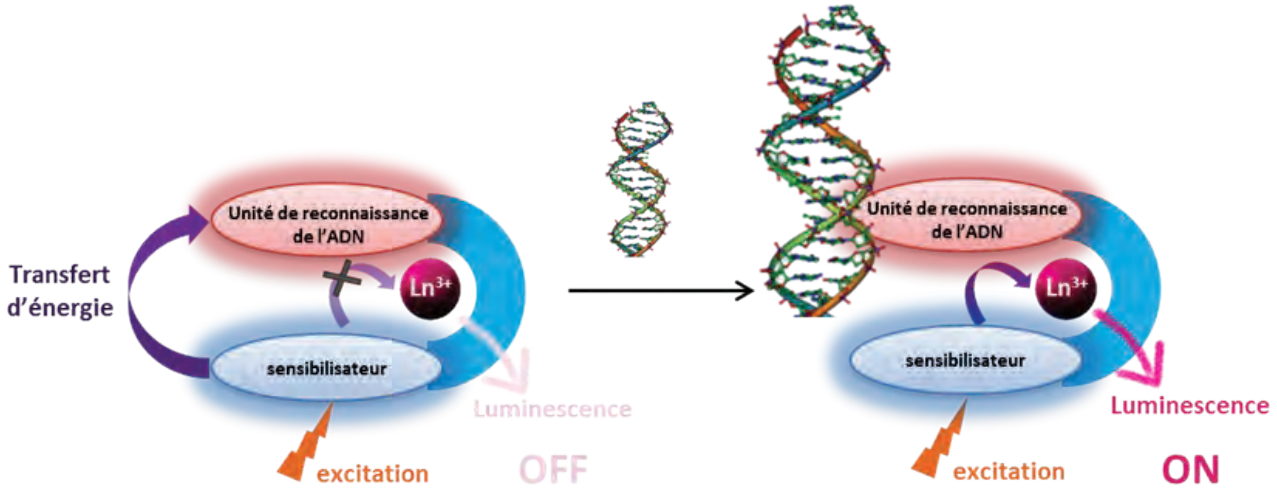
L'expression de l'information génétique dépend de nombreux processus de reconnaissance impliquant l'ADN. L'élaboration d'outils moléculaires capables d'interagir avec l'ADN et de mettre en évidence cette interaction est donc un domaine de recherche de fort impact scientifique pour la compréhension d'événements biologiques essentiels et pour le diagnostic médical. Les ions lanthanides (Ln^{3+}) s'avèrent être des candidats très prometteurs dans l'élaboration de telles sondes, grâce à leurs propriétés spectroscopiques particulières. En effet, la luminescence des lanthanides est très intense avec des bandes d'émission fines et caractéristiques, peu sensible au photoblanchiment et permet une détection résolue en temps grâce à de longs temps de vie de luminescence.

Dans ce contexte, nous avons développé des complexes peptidiques de lanthanides efficaces pour détecter de telles interactions avec l'ADN à travers une augmentation de la luminescence du lanthanide.

Les ligands choisis au laboratoire sont des hexapeptides structurés, largement solubles dans l'eau et présentant l'avantage d'être aisément fonctionnalisables. Afin d'obtenir des complexes de lanthanides de stabilité suffisante à pH physiologique, deux acides aminés non naturels Ada2 porteurs de chaînes latérales aminodiacétates ont été introduits dans la séquence peptidique. L'enchaînement Ada2-Pro-Gly-Ada2 favorise une conformation en coude β, qui permet une coordination simultanée du cation par les deux acides aminés chélateurs, renforçant ainsi la stabilité du complexe. Ce peptide a été fonctionnalisé en position N-ter par une unité de reconnaissance de l'ADN5 et en C-ter par un sensibilisateur du lanthanide.



Principe de détection de l'ADN à travers la luminescence centrée sur le métal

La synthèse et les propriétés de ces nouvelles sondes luminescentes de l'ADN seront décrites dans cette communication.

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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.

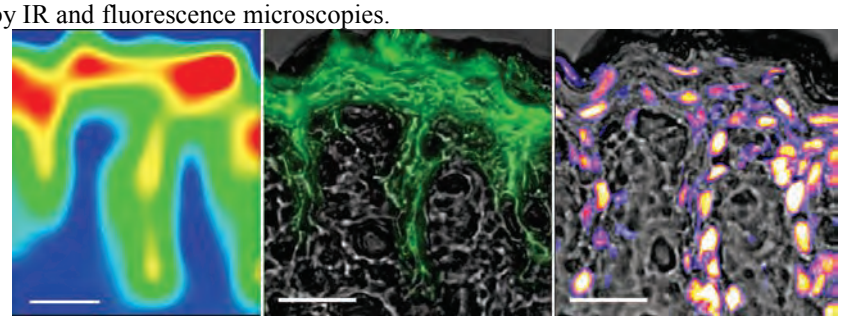

Figure 1. Skin slice after a 24-h exposure to a 2 10^{-2} M solution of the labelled CPP in water. ***Left***: mapping of the integral of the absorbance of the A_1-band (2040-2000 cm^{-1}). ***Middle***: bright field image merged with the luminescence signal of **1**. ***Right***: bright field image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

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Carbon monoxide dehydrogenase (CODH): maturation of the active site

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Ni-containing Carbon Monoxide Dehydrogenases (CODHs) catalyze the reversible conversion between CO and CO_2 in some anaerobic bacteria and archaea. They play a key role in energy conservation and/or CO_2 fixation.
The active site of CODHs, called C-cluster, is a distorted Ni–3Fe–4S cubane coordinated to a unique fourth iron (1,2, see figure). The function of the accessory proteins, CooC, CooF, CooJ and CooT, in the maturation of the complex C-cluster is unclear because the corresponding genes are not always present in the CODH operons. However, CooC is undoubtedly the most important of the accessory proteins of CODH because it is present in all the Ni-CODH producing organisms; its putative role is to favor the insertion of nickel into the protein.

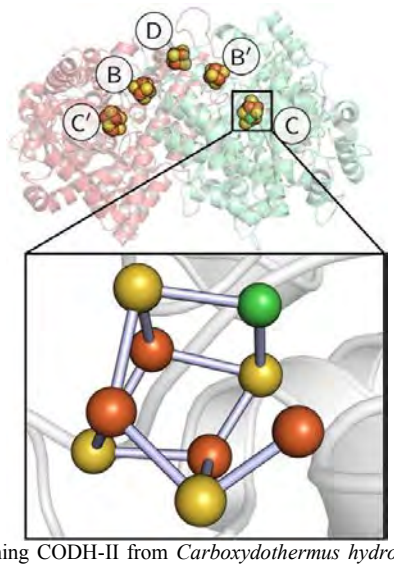

Figure 1: Structure of the homodimeric Ni-containing CODH-II from *Carboxydothermus hydrogenoformans* (pdb accession number 1SU8). Each subunit (in red and cyan) contains a C-cluster (C and C′) and a [4Fe–4S] cluster (B and B′). The interfacial [4Fe–4S] D-cluster (D) is ligated by two cysteines of each monomer. The monomers are intertwined so that the B-cluster of the one monomer receives or gives electrons to the C-cluster of the other monomer. Inset: Structure of the Ni–4Fe–4S C-cluster. The iron atoms are in orange, the sulfur atoms in yellow and the nickel atoms in green.

Desulfovibrio vulgaris (*Dv*) contains an annotated CODH operon containing two genes that encode for the CODH enzyme and only the maturase CooC but these enzymes have not been characterized so far (3). To understand the precise role of CooC, we have designed a recombinant system allowing for the explicit study of the *Dv* CODH produced both in the absence and presence of CooC in *D. fructosovorans*. We thus have compared the kinetic, spectroscopic and structural properties of the two forms of the *Dv* CODH.

Our results support the idea that CooC folds the active site so that it can bind exogenous nickel. Moreover, we have observed an unprecedented nickel-dependent activation process of the already Ni-loaded CODH. Interestingly, this activation mechanism is apparently not related to structural changes of the active site (4).

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Caracterization of an orphan human cytochrome P450: Spectroscopic and Modeling studies

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Cytochrome P450 2U1 (CYP2U1) has been identified from the human genome and is highly conserved in the living kingdom [1]. In humans, it has been found to be predominantly expressed in the thymus and in the brain [2]. However, CYP2U1 is considered as an "orphan" enzyme as few data are presently available on its physiological function(s) and active site topology. Its only substrates reported so far were unsaturated fatty acids such as arachidonic acid [2] and, much more recently, *N*-arachidonoyl-serotonin [3]. CYP2U1 is up-regulated in a variety of cancer tissues such as colorectal or breast cancer tissues [4, 5]. Very recently, mutations in the CYP2U1 gene has been found to be related in appearance of hereditary spastic paraplegia (HSP), a neurological disease [6]. In order to characterise this protein we expressed CYP2U1 in *E. Coli* and tested some imidazole and pyridine derivatives. Docking experiments in our 3D-model [7] allowed us to explain the mode of interaction of these compounds and the regioselectivity observed for two known substrates arachidonic acid [2] and *N*-arachidonoyl serotonin [3]. We also localized mutations of CYP2U1 implicated in HSP affecting conserved amino acids on our 3D model and constructed by virtual mutagenesis models of these mutants. These models permitted to better understand the consequences of these mutations on the structure and activity of CYP2U1. This study can help to identify endogenous and/or exogenous molecules as substrates of CYP2U1 in order to provide new insights on its implication either in physiological processes or in human diseases.

Collaborations: D[rs] Ph. Beaune and M.-A. Loriot (INSERM U775); D[rs] G. Stevanin and A. Brice (INSERM S975); C. Goizet (EA 4576).

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EPR studies of NO-synthases

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In mammals, the NO-synthases (NOSs) are responsible for the production of nitric oxide (NO) involved in a number of biological activities ranging from signalling to cytotoxic processes. Their mechanism is based on that of CYP450, the active site of the oxygenase domain being composed by a heme-thiolate moiety. In addition to CYP450, NOSs possess a tetrahydro-biopterin cofactor responsible for the reduction of the oxyferrous compound, and the heme thiolate ligand is hydrogen-bonded to a nearby tryptophan residue. In the last 10 years, the improvement of gene sequencing techniques has led to the discovery of numerous proteins homologous to mammalian NOSs throughout the living kingdom (NOS-like proteins, NOS-LPs) in particular in bacteria. Despite homology to mammalian NOS, bacterial NOS-LPs bear some differences: essentially, they lack the reductase domain, bacteria do not generally have the machinery to synthesize the biopterin cofactor, and the NO production of NOS-LPs is smaller compared to NOSs.

We have performed comparative studies on mammalian and bacterial NOS enzymes.[1-3] EPR is a technique particularly suited to study the heme active site of NOS as well as the radicals involved in the electron transfer processes. Results obtained for *Deinococcus radiodurans* NOS will be presented.

Another aspect of interest is the interaction between the oxygenase and reductase domains of mammalian NOS and how the electron transfer between them is regulated. We are starting a new project using EPR and PELDOR (pulse electron-electron double resonance) to study this and will present preliminary data.

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Designing a new generation of electrochemical cells for the study of enzymes that reduce CO2

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Carbon monoxide dehydrogenases (CODH) catalyzes the reversible oxidation of CO to CO_2 using at $NiFe_4S_4$ active site[1,2].

$$CO + H_2O \leftrightarrow CO_2 + 2e^- + 2H^+$$

We study the mechanism of CODH using protein film voltammetry (PFV)[3]. In this technique (**figure**), a redox enzyme is immobilized on an electrode in a configuration, in which the electrons are transferred directly from the electrode to the enzyme. In this setup, the current is proportional to the enzymatic activity, which makes it easy to follow its variations as a function of the experimental conditions. When the enzyme turns over, it consumes the substrate in the vicinity of the electrode[3]. Traditionally, to prevent substrate depletion at the electrode, electrochemists use rotating disk electrodes (RDE). However, this is not enough in the case of CODH. Because CODH is very active, the RDE does not prevent depletion, which complicates the electrochemical response and makes it impossible to study the CODH mechanism. To overcome this problem, we are designing a new electrochemical cell to study the highly active redox enzymes. First, *in silico*, using mechanical fluid simulations, we are looking for suitable geometries to enhance the rate of substrate transport toward the electrode. After that, we will build and test prototypes of electrochemical cell, which will be used to study the mechanism of CODH enzyme.

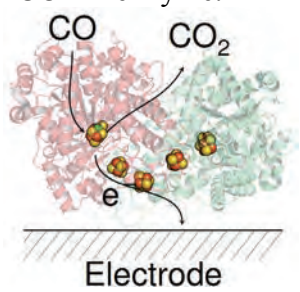

Figure: Schematic presentation of the protein film voltammetry experiment, the colored part corresponds to the structure of CODH that is absorbed at the electrode surface where is direct electron transfer

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QSoas, a powerful y=f(x) data analysis program

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In our team, we use protein film voltammetry[1] to study the mechanisms of redox enzymes. In this technique, an enzyme is immobilized onto an electrode in a configuration in which electron transfer is direct, and the current that flows through the electrode provides a direct reading of the activity. This techniques lends itself very well to quantitative interpretation, but no data analysis software on the market provided enough flexibility to perform the kind of analysis we needed. This is why we wrote our own software for data analysis.

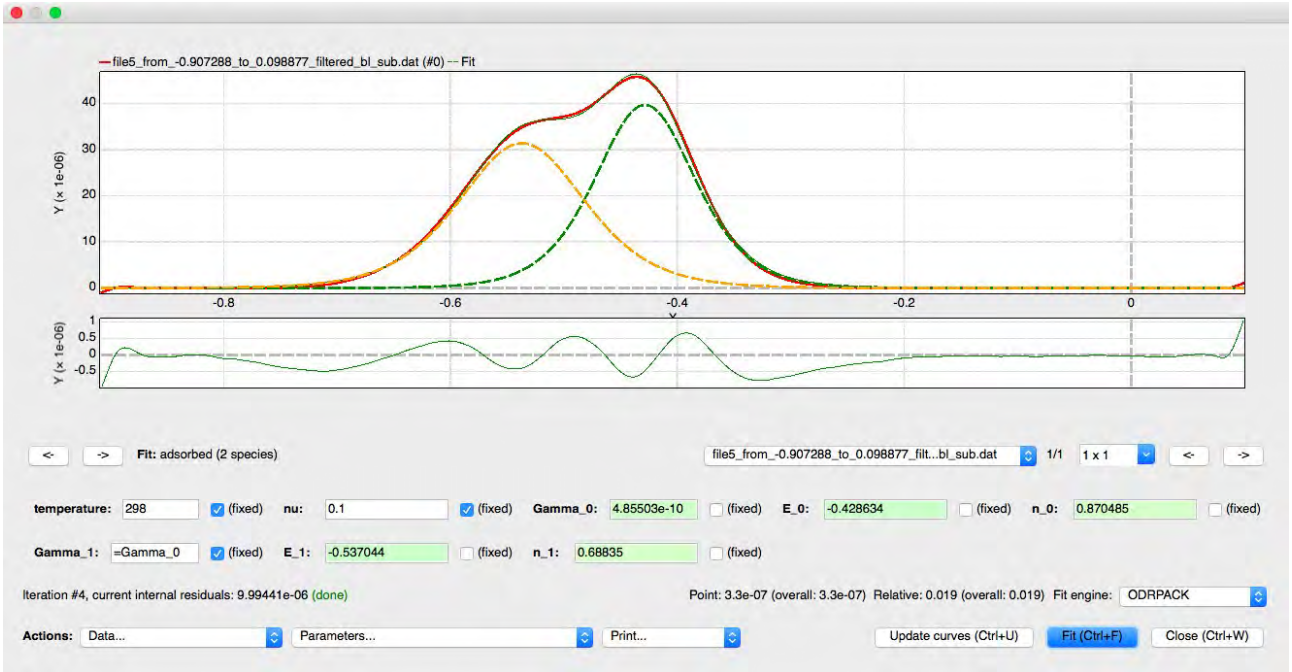

Figure: screenshot of the fit interface of QSoas showing a fit of a voltammogram of two distinct adsorbed non-catalytic species

We present QSoas, successor to the SOAS program[2]. QSoas is an open-source, cross-platform, general-purpose data analysis program written in C++ and Ruby. It features all the basic data analysis tools: filtering, baseline subtraction, arithmetic manipulations and so on. It is fully scriptable, which makes it easy to process hundreds of data files in seconds. Assuredly its strongest points are the facilities it provides for data fitting (figure), and in particular global fits with several data sets. Even if it was written initially to process electrochemical data, it is not specific to electrochemistry, but will be useful to anyone that needs fast data processing and flexible data fitting. More information available at http://www.qsoas.org

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Identification of nitrogen-containing residues in the vicinity of protein-bound semiquinones by selective ^{15}N labeling and high resolution EPR

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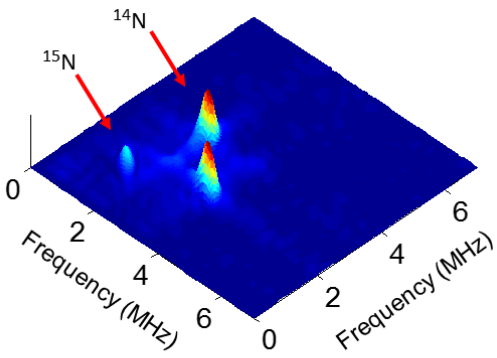
Isoprenoid quinones are liposoluble electron and proton carriers used in the vast majority of prokaryotic bioenergetic chains. Classified in either low or high redox potentials, quinones react with bioenergetic enzymes at specific quinone processing sites or Q sites. Deciphering the chemical processes catalyzed by Q-sites is challenging especially when these sites couple two one-electron transfer steps to the net release or uptake of two protons, as it occurs in dissociable Q sites. Indeed, this requires resolving the different binding modes of quinone, quinol and of the reactive semiquinone (SQ) intermediate.

We use *E. coli* nitrate reductase A (NarGHI) as a model enzyme to understand the role of the protein environment in tuning enzyme reactivity towards quinones of low (i.e. menaquinones, MK) and high (i.e. ubiquinones, UQ) redox potential. NarGHI is a membrane-bound heterotrimeric enzyme that couples the oxidation of quinols at a periplasmically-oriented Q site (named Q_D) to the cytoplasmic reduction of nitrate into nitrite [1].



HYSCORE spectrum of menasemiquinone in selectively ^{15}N-labeled NarGHI

Most interestingly, this complex was shown to stabilize the semiquinone catalytic intermediate of both MK and UQ [2]. High resolution pulsed EPR spectroscopy was used to explore the local environment of the protein-bound radicals with the use of NarGHI-enriched inner membrane vesicles containing endogenous quinones [2-4]. To directly assign the previously detected ^{14}N-SQ interactions to specific nitrogen nuclei in the Q_D site [2,3], we have developed a selective ^{15}N labeling strategy that relies on the construction and use of auxotrophic *E. coli* strains towards specific amino acids. This approach allowed us for the first time to specifically probe the previously proposed role of nitrogen-containing residues in SQ binding [2,4] and thus to refine our current model of SQ binding mode in NarGHI [4]. Overall, our experimental strategy combining genetics, biochemistry, site-directed mutagenesis, redox potentiometry and high resolution EPR methods allowed us to attain an unprecedented level of structural understanding of distinct semiquinone binding at a single Q site.

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Fungal Copper Monooxygenases for biomass conversion: structural and mechanistic investigation by EPR spectroscopy

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Lignocellulose is the main component of plant cell walls and represents a high potential renewable resource for the production of second generation biofuels. The cleavage of lignocellulosic biopolymers by cellulase hydrolysis is the first fundamental step in biofuel production process. However lignocellulose recalcitrance to this hydrolysis step makes difficult and expensive its use for biofuel production.
Lytic polysaccharide monooxygenases (LPMOs) are a novel class of copper enzymes that boost the efficiency of cellulases resulting in increased hydrolysis yields[1]. In LPMOs of AA9-type, copper ion shows a peculiar six-coordination involving two histidine side chains and the N-terminal amino-group of the N-ter histidine forming a T-shape His-brace (Fig1)[2]. In addition this His tend to be methylated (N$^{\tau}$Me) when AA9 are expressed in fungal hosts. Nevertheless, the mechanism of LPMOs activity on lignocellulose remains unclear[3].
In order to give novel insights into the mechanistic aspects of the LPMOs enzymatic activity, the AA9H LPMO from *Podospora anserina* was expressed[4] and we evaluated its activity on different substrates (carboxymethyl-cellulose and oligosaccharides). EPR analysis were also performed on AA9H to characterize the active site, which contains a type II Cu(II) ion. Moreover, the mechanistic aspects of the interaction between AA9H and its substrates were addressed by rapid freeze quench techniques coupled to EPR spectroscopy to detect radical intermediates expected to be generated in the catalytic cycle.
In parallel, biomimetic copper(II) complexes, which are used as model of LPMO active site, have been synthesized and characterized for a better comprehension of the enzyme reactivity.

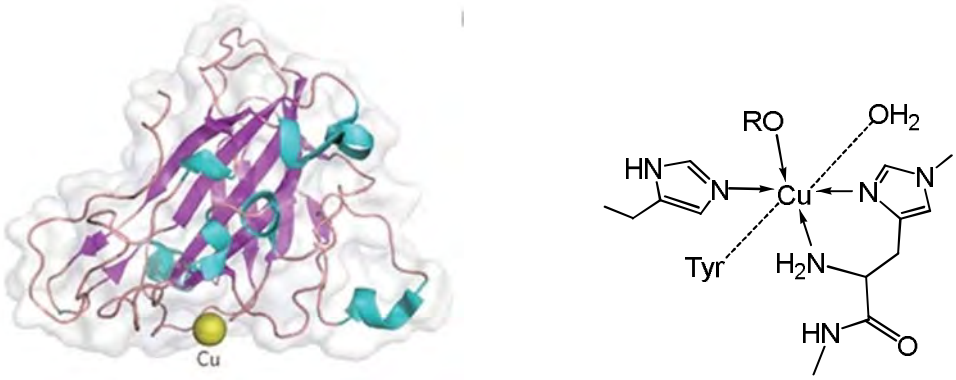

Figure 1 : Crystallographic structure of AA9-type LPMO and of the active site[2]

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Multifactors influenced nanostructures

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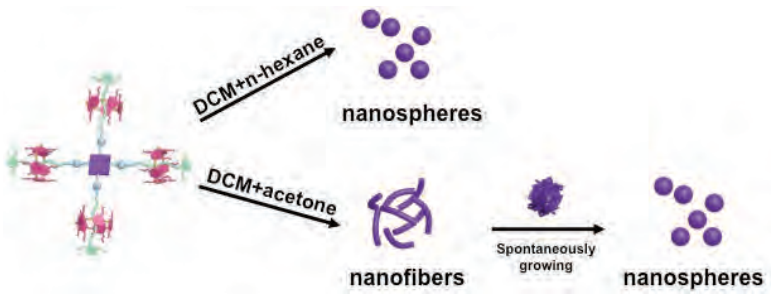
Nowadays, nanostructures are catching more and more attention because of their influence on the final performance in the aspect of chemistry, molecular science and nanodevices. Carefully designed molecules with the aid of weak interactions may aggregate in some specific way and thus generate nanostructures with desirable size and shape. In the past few years, chemists are been trying to get a better understanding on the formation and structural transition of the molecular nanostructures and many efforts have contributed to not only the obtention of different organic nanostructures but also the insight of the factors that may have influence on the nanostructures. Pillararene, first reported by Gribble, G. W.[1], is a promising molecule due to its high symmetry and rigid structures compared with ether crowns and some other classic macrocycles. Neutral rotaxanes based on pillararenes have been well developed[2] and it is appropriate to look into the formation of the molecular nanostructures through pillararene-based rotaxanes.

A novel star-shaped [5]rotaxane with zinc porphyrin as core was designed and synthesised. The introduction of zinc porphyrin helps the molecules to easily aggregate and form ordered nanostructures. Furthermore, the characterization of the porphyrin aggregation has been well investigated,[3] which may help to reveal the formation procedure of the nanostructures indirectly. It was noted that not only the polarity of the solvents but also pillararene have an influence in the formation of the nanostructures. In a mixture of dichloromethane and *n*-hexane, the morphology of the [5]rotaxane was spheres while in a mixture of dichloromethane and acetone, it formed nanofibers. More interestingly, the morphology in dichloromethane and acetone exhibited a spontaneous growing from nanofibers into nanospheres after fifty days. Meanwhile, the presence of the pillararene was crucial for the formation of the ordered nanostructures. Without pillararene, the compound formed unordered nanostructures. The presence of the pillararene decreased the π-π stacking interactions between two porphyrins and made the long soft alkane chains rigid in the meantime. Thus, we described a multifactors influenced nanostructures.



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Ciblage spécifique d'organites, détection et quantification intracellulaire par des sondes fonctionnalisables

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Les questions de la détection intracellulaire, de la localisation et de la quantification en milieu cellulaire de complexes de métaux de transition sont aujourd'hui assez peu explorées. Elles sont pourtant fondamentales car il est nécessaire de s'assurer que les composés biologiquement actifs pénétrant dans l'organisme atteignent leur cible. Pour ce faire, il est possible de greffer des sondes qui modifieraient peu les propriétés physico-chimiques de la molécule marquée. Dans ce but, des complexes de rhénium triscarbonyle ont été développés comme sondes multimodales.

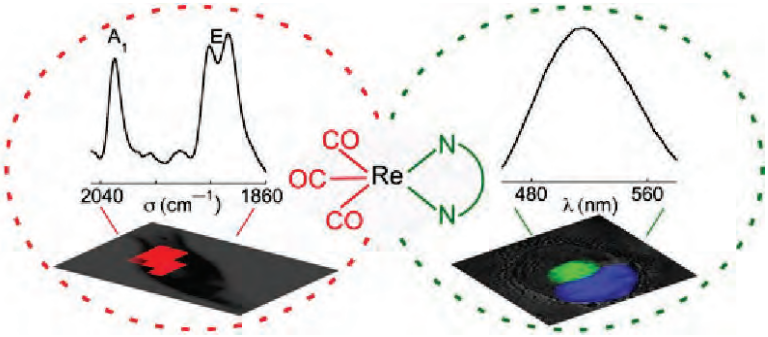

Figure 1 : SCoMPI et l'imagerie bimodale

Ces sondes, appelées SCoMPI pour « Single Core Molecular Probes for Imaging » sont fonctionnalisables, c'est-à-dire qu'elles peuvent être conjuguées à des molécules d'intérêt ou à des peptides ciblant des organites cellulaires. afin de les observer par microscpectroscopie IR et de fluorescence[1-3]. Il est possible de cibler et de marquer différents types d'organites intracellulaires tels que l'appareil de Golgi, les mitochondries[4] ou encore le noyau[5,6], et de les observer par différentes techniques d'imagerie (infrarouge, fluorescence, fluorescence X).

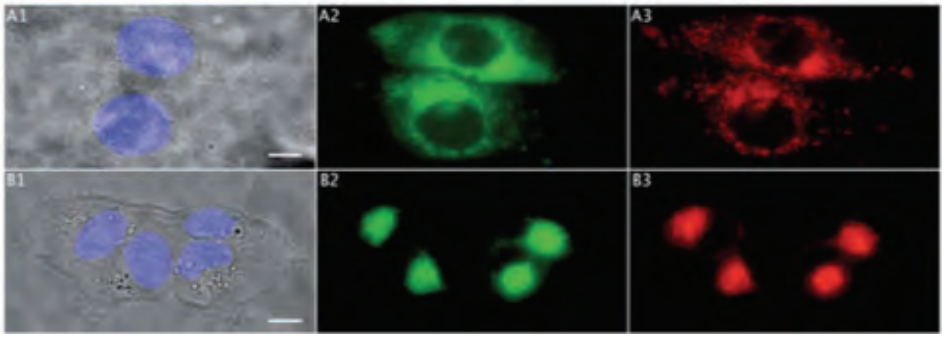

Figure 2 : Études de colocalisation (A) Deux cellules MCF-7 de cancer du sein incubées avec une sonde SCoMPI (25 mM, 1 h), (B) Quatre cellules MDA-MB-231 de cancer du sein, mêmes conditions d'incubation : (A1 et B1) Éclairage direct (barre : 10 mm) et le noyau marqué au DAPI (bleu), (A2 et B2) Sonde en vert, (A3 et B3) Marqueur de Golgi en rouge

1. Sylvain Clède, François Lambert, Christophe Sandt, Slavka Kascakova, Miriam Unger, Etienne Harté, Marie-Aude Plamont, Rénette Saint-Fort, Ariane Deniset-Besseau, Zoher Gueroui, Carol Hirschmugl, Sophie Lecomte, Alexandre Dazzi, Anne Vessières and Clotilde Policar, *Analyst.,* **2013**, *138*, *35*, 5627 – 5638.
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A new probe to determine the affinity constant of Cu(II) for the amyloid-β peptide

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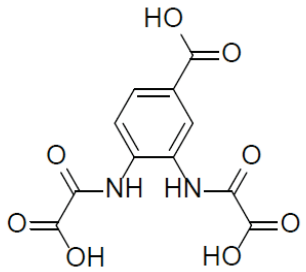
Alzheimer's disease is the most common neurodegenerative disease, with no known cure. Brain of Alzheimer's patients exhibits intracellular neurofibrillary tangles as well as extracellular senile plaques, consisting of insoluble fibrillar aggregates of the amyloid-β peptide (Aβ). Aβ peptide is able to bind Cu(II) and we want here to determine with which affinity. We have thus designed a new spectroscopic dye (see scheme below) to straightforwardly evaluate by UV-Vis the Cu(II) binding affinity towards Aβ and a wide panel of modified peptides.T his data has long been controversial in the literature.[1,2] In addition to the determination of Cu(II) affinity for Aβ, the studies on modified peptides allows us to confirm the coordination of Cu2+ to the Aβ peptide.[3]



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Financial support by ERC-2014-StG-638712 aLzINK grant is gratefully acknowledged.

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Des métalloenzymes artificielles pour une chimie tandem.

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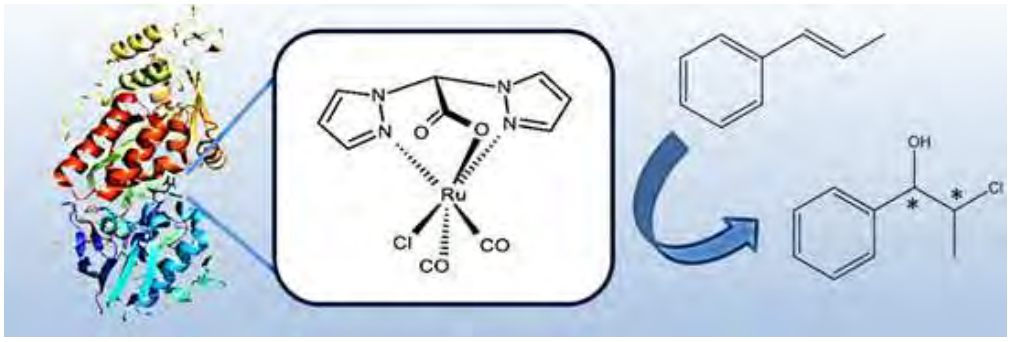
La prise de conscience de l'impact des activités anthropiques sur l'environnement et la santé humaine a conduit l'équipe BioCE, en collaboration avec l'équipe BioCAT, toutes deux du Laboratoire de Chimie et Biologie des Métaux de Grenoble, à mettre au point de nouveaux catalyseurs d'oxydations respectant les principes de chimie verte :[1] les métalloenzymes artificielles.[2] En combinant les avantages de la biocatalyse et de la catalyse homogène, de nouvelles alternatives aux solvants polluants et aux produits chimiques dangereux ont été trouvées.[3] Dans ce contexte, nous avons décidé d'incorporer un complexe de ruthénium (II), mimant le site actif des oxygénases et capable de réaliser des époxydations en milieu organique,[4] au sein de la protéine NikA. Ce nouvel hybride présente une réactivité différente du complexe parent, offrant des conditions d'oxydation douces pour la production de chlorhydrines, en milieu aqueux. L'utilisation de ce nouveau catalyseur permet d'obtenir ces synthons chiraux de façon chimio- et régio-sélective. Ces résultats illustrent l'avantage d'employer les métalloenzymes artificielles comme catalyseur : la cavité protéique active et stabilise le complexe inorganique tout en améliorant la sélectivité de la réaction. Afin de développer ces systèmes, une stratégie d'évolution dirigée du site actif de la protéine sera mise en place afin d'améliorer l'énantiosélectivité de la réaction.



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Biosynthesis of lignans, using an intriguing stereo-selective radical coupling control

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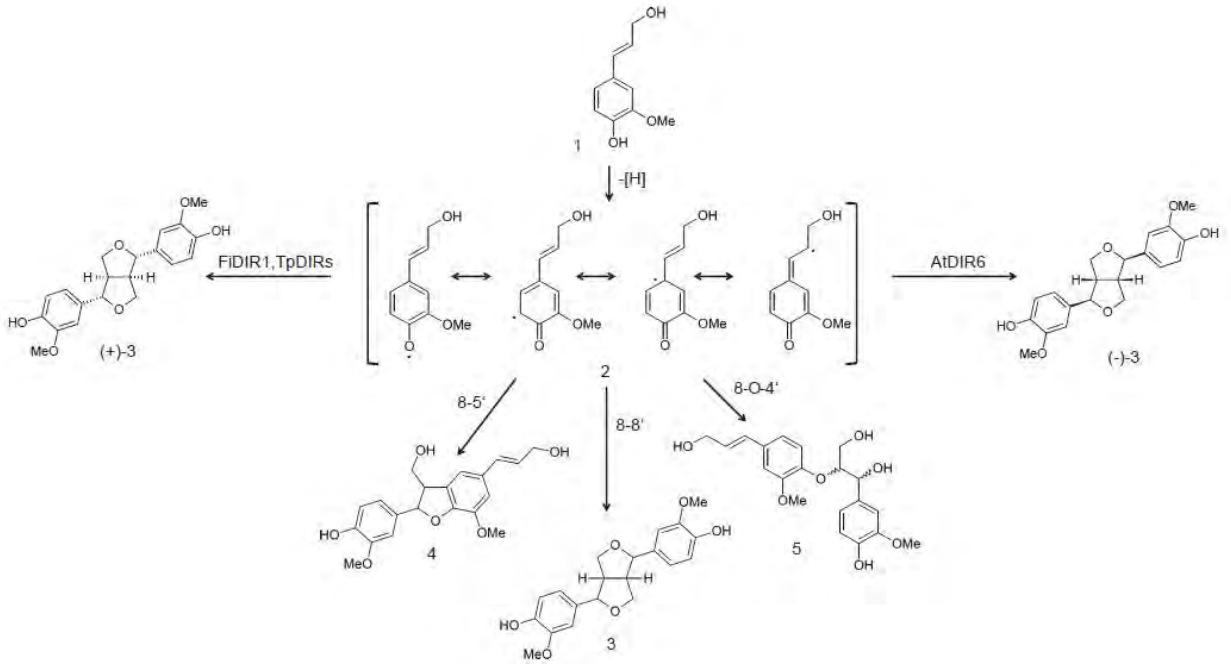
Natural products as lignans in plants, are usually product in optically pure form. However, how this process is realized is not well understood. In presence of one electron oxidant, the coupling of monolignols leads to non-specific radical-radical coupling to form many racemic lignans. However, the coupling of two oxydized form of coniferyl alcohool monolignols is directed in a stereo-selective manner in different plants (site une revue). A protein, called Dirigent Protein (DIR) is responsible of the formation of only the pure product, form of pinoresinol.[1] Nowadays some of DIRs are known and they can form (+) or (-)-pinoresinol.[2] Until now the exact mechanism on how substrate interact with DIRs and how DIRs orient them in a stereo-selective coupling is poorly understood. Investigation of the DIRs mechanism could allow us to develop new interesting biocatalysts.



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Formation of DIR mediated (stereoselective) versus non specific (racemic) coupling products of coniferyl alcohol radicals

Our goal is to study and understand the mechanism of DIRs, starting from their biochemistry characterization to their application in catalysis. The reaction mechanism is not known. How do they capture, bind and orient their phenoxyl substrate? At which step of the radical substrate formation DIR orient the reaction? What are the determinants of the specificity in the substrate-binding pocket of DIR? In this poster we will present our results about the production of the AtDIR6 from *Arabidospis thaliana* in *Pichia pastoris* as well as the results of oxidation coniferyl alcohol. Then, in the goal to decorticate the steps of the reaction, substrate analogues are required. These synthesis will also be presented.

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Synthesis, characterization, and catalytic activity of 2-hydroxo and 2-mercaptopyridine-N-oxide complexes of molybdenum [VI]

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Despite most of molybdenum-containing enzyme sharing similar metal centres (molybdopterin), they are able to catalyse a large variety of crucial biochemical processes. All the molybdoenzymes from the DMSO reductase family includes desoxo Mo^{IV}, $[Mo^{IV}(dt)_2(XR)]^-$ 1, and monooxo Mo^{VI} complexes, $[Mo^{VI}O(dt)_2(XR)]^-$. Considerable progress has been made in the preparation of desoxo Mo^{IV} synthetic mimics, but monooxo Mo^{VI} are challenging to isolate 23. In contrast, molybdenum complexes associated with monooxo Mo^{IV} and dioxo Mo^{VI} are more stable. Such complexes, including different types of N-, O-, S- donor ligands, have been shown to mimic some enzymatic functions of DMSO reductases, such as the reduction of N-oxide and sulfoxide 234. However, no nitrate or carbon dioxide reduction catalysis has been reported so far for this type of complexes.

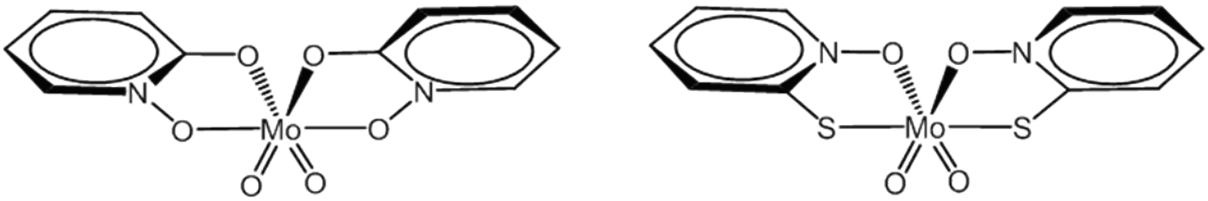

Figure 1. Chemical representation of the complexes $[MoO_2(OPNO)_2]$ and $[MoO_2(SPNO)_2]$

We report on the preparation of two new dioxo Mo^{VI} complexes, $[MoO_2(OPNO)_2]$ and $[MoO_2(SPNO)_2]$, which were characterized by X-Ray crystallography, IR, NMR, and UV-vis 5. The redox chemistry of the complexes was investigated using either electrochemistry or common reducing agents (monitoring the reaction by UV-vis and NMR). The ability of the complexes to catalyse various reduction processes relevant to the DMSO reductase family (sulfoxide, nitrate, carbon dioxide) is being investigated by the same means. These data will be presented and structure-activity relationships will be discussed.

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Intégration de complexes de manganèse antioxidants (modèle de la catalase) dans des nanoparticules mésoporeuses de SiO_2 et hybride silice/résol.

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Les complexes dinucléaires de manganèse(III) comportant le motif [Mn(μ-O)(μ-RCOO)2Mn] sont connus pour être des bons modèles de la Catalase de manganèse, un enzyme qui permet la décomposition d'espèces nocives pour les systèmes biologiques, tels que le peroxyde d'hydrogène (H_2O_2).[1,2] Le peroxyde d'hydrogène, tant que molécule neutre, peut rentrer dans la cellule avec une certaine facilité, ce qui d'une forme indirecte peut générer des radicaux hydroxyle (·OH), qui sont des espèces responsables de la dégradation de l'ADN. Pour cette raison, de tels systèmes du manganèse pourraient trouver des applications biomédicales comme antioxydants afin de diminuer le « stress oxydant » dans l'organisme.

Cependant, ils ne sont pas très solubles dans l'eau, ce qui limite leurs applications comme possibles antioxydants.[1] L'incorporation de ces complexes au sein de nanoparticules mésoporeuses permettrait de travailler dans l'eau et leur activité catalytique pourrait être améliorée. Des études d'incorporation sur silices mesoporeuses de complexes du type [{MnL(NN)}2(μ-O)(μ-RCOO)2]X2, où NN est une amine tel que la bipyridine (bipy) ou la phénantroline, phen, et X un contre ion (nitrate ou perchlorate), ont montré que l'activité antioxydant de ces systèmes est augmentée et qu'il est possible de travailler en solution aqueuse.[3] Ces systèmes hybrides Mn-silice se présentent comme des poudres, ce qui permet l'application des ces systèmes comme catalyseurs. Pour des applications en tant qu'antioxydants, des particules plus petites sont nécessaires afin de pouvoir injecter éventuellement ces systèmes dans le sang.

Nous travaillons actuellement sur la conception de nouveaux vecteurs à base de nanoparticules mésoporeuses de silice ou hybride resol/silice.[4,5] La synthèse de ces deux types de nanoparticules semble concluante avec des particules de diamètres adaptés à l'application (50 à 180 nm) et modulable, une bonne monodispersité en taille et stables en solution aqueuse avec l'aide de tensio-actif (CTAB). Les particules resol/silice sont en outre un sytème biphasique de réseaux polymère inorganiques et organiques intriqués singulier dont le mécanisme de formation et de croissance reste à étudier.

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The mechanism of protection of catalysts supported in redox hydrogel films

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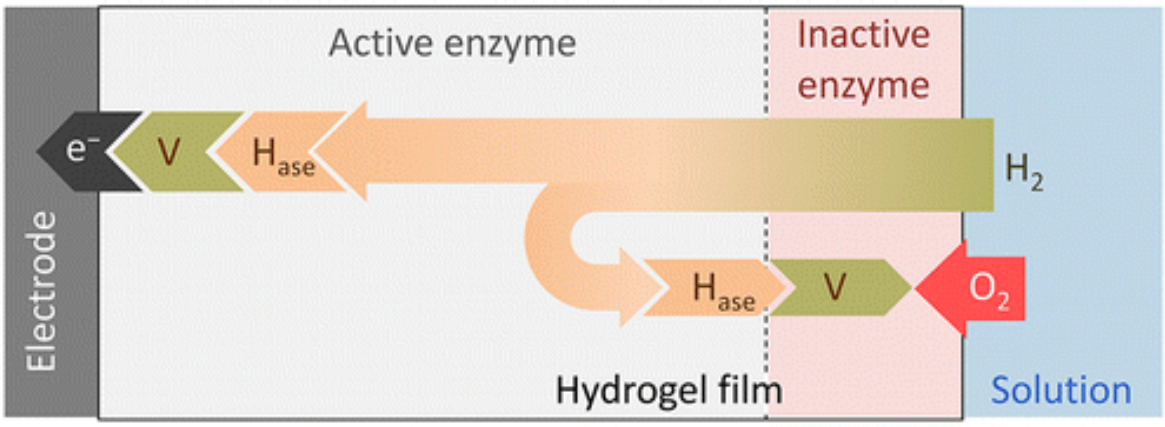
The use of synthetic inorganic complexes as supported catalysts is a key route in energy production and in industrial synthesis. However, their intrinsic oxygen sensitivity is sometimes an issue. Some of us have recently demonstrated that hydrogenases, the fragile but very efficient biological catalysts of H_2 oxidation, can be protected from O_2 damage upon integration into a film of a specifically designed redox polymer. Catalytic oxidation of H_2 produces electrons which reduce oxygen near the film/solution interface, thus providing a self-activated protection from oxygen.[1] Here, we rationalize this protection mechanism by examining the time-dependent distribution of species in the hydrogenase/polymer film, using measured or estimated values of all relevant parameters and the numerical and analytical solutions of a realistic reaction-diffusion scheme. Our investigation sets the stage for optimizing the design of hydrogenase-polymer films, and for expanding this strategy to other fragile catalysts.



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