Rencontre des Facultés de Biologie et de Chimie

7 nov. 2016

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Programme et Résumé des posters

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Journée des Facultés de Biologie et de Chimie

7 Novembre 2016

UPMC Amphi Charpak (Patio 22-33 SB02 - Campus de Jussieu)

(Déjeuner et posters caves Esclangon)

PROGRAMME	
9h00	**Accueil**
9h15	**Introduction par les directeurs des deux Facultés**
9h30	**Biomatériaux & Biopolymères** (Onnik Agbulut et Thibaud Coradin)
10h00	**Nanoparticules** (Christine Ménager et Anne-Marie Genevière)
10h30	***Pause café***
11h00	**Imagerie par spectrométrie de masse & Métabolome** (Gérard Bolbach et Richard Cole)
11h30	**"Mon poster en 3' "**
12h30	***Déjeuner & Posters***
14h30	**L'enseignement à l'interface biologie-chimie** (Sophie Louvet, Berni Hasenknopf, Caroline Dubacq, Giovanni Poli)
15h00	**Biologie synthétique** (Stéphane Lemaire et Ludovic Julien)
15h30	**L'équipe iGEM-UPMC**
15h45	**La chemobiologie** (Dominique Guianvarc'h et Philippe Grellier)
16h15	***Pause café***
16h45	**Chimie thérapeutique** (Anne Vessières-Jaouen et Alexandre Escargueil)
17h15	**Guidage axonal** (Alexandra Rebsam et Ahmed Hamraoui)

Comité d'organisation

V. Béréziat, **T. Coradin, C. Dubacq,**

L. Fensterbank, F. Francis, J.F. Lambert,

S. Lemaire, V. Maréchal, C. Ménager,

F. Peronnet, **S. Sagan**

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Sommaire

HSP90 regulation by RPAP3-PIH1D1 cochaperones during macromolecular complex assembly.

Julien Henri, Rénette Saint-Fort, Philippe Meyer
Sorbonne Universités, UPMC University Paris 6, CNRS UMR 8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie 75005 Paris, France.

Box C/D small nucleolar ribonucleoparticles, phosphatidyl-inositol 3-kinase-related kinases, telomerase and RNA polymerases are essential eukaryotic molecular machines. Biogenesis of these macromolecular complexes requires the intervention of the R2TP-HSP90 chaperone machinery. The Hsp90 molecular chaperone is responsible for the conformational activation and assembly of proteins that are essential for cell signaling and regulation. Many of these client proteins control functions involved in malignant transformation and the chaperone has attracted considerable interest as anticancer target. Maturation of client proteins is an ATP-dependent phenomenon where the chaperone conformational changes are coupled to the binding and hydrolysis of the nucleotide. This Hsp90 ATPase/chaperone cycle is regulated by a set of cochaperones proteins. The human R2TP cochaperone complex is composed of RUVBL1 and RUVBL2 AAA+ ATPases, PIH1D1 interacting platform and RPAP3 adaptator. RPAP3 cochaperone contains tetratricopeptide repeats (TPR) domains anchoring HSP70 and HSP90 to R2TP.
We focused our attention on the mechanisms of chaperones regulation by the RPAP3-PIH1D1 cochaperones and observed a modulation of Hsp90 activity by RPAP3. This modulation is correlated with the stabilizing interactions between the HSP90 dimer and RPAP3 as evaluated by FRET experiments. PIH1D1 associates with RPAP3, and both cochaperones closely interact with the HSP90 dimer. Finally, we integrated 3D structures, SAXS measurements and biochemical assays into a global model that supports an iterative mechanism of client complex assembly by the RPAP3-coordinated action of Hsp90.

1: von Morgen et al. (2015) Front Genet. 6:69.
2: Benbahouche et al. (2014) J Biol Chem 289:6236–6247.

FUNCTIONAL STUDIES OF BACTERIAL TSPO

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The 18 kDa translocator protein TSPO is a transmembrane protein that has been conserved through evolution from bacteria to mammals. It remains unknown if a unique function has also been conserved. Bacterial TSPO could regulate porphyrin content while mammalian TSPO could be involved in cholesterol transport, a key step in steroidogenesis. The latter has been recently questioned and the role of mammalian TSPO in porphyrin regulation has been suggested but remains poorly studied.

In this present study, we produced recombinant *Bacillus cereus* TSPO (BcTSPO) and performed its biochemical and biophysical characterization. Overexpression of BcTSPO in *E.coli* leads to the accumulation of tetrapyrrole containing proteins in inclusion bodies. The SDS purified BcTSPO still contains a small amount of tetrapyrrole revealed by an absorbance peak at 400 nm. The bound compound is not yet characterized. Is it a ligand or/and a breakdown product? In order to characterize the function of BcTSPO, we first show that this protein binds protoporphyrin IX (PPIX) using visible spectroscopy, NMR and fluorescence. Secondly, we noticed that BcTSPO decreases almost completely the fluorescence of bound PPIX upon UV irradiation. This suggests an enzymatic activity of BcTSPO that we are challenging to characterize.

Adaptation contributes to replicative senescence dynamics and genome instability

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Abstract

Failure to maintain telomeres leads to their progressive erosion at each cell division. This heterogeneous process eventually triggers replicative senescence, a pathway shown to protect from unlimited cell proliferation but also to increase genome instability. However, the mechanisms underlying its variability and its dynamics are not characterized. To investigate this issue, we used a microfluidics-based live-cell imaging assay to investigate replicative senescence in individual *Saccharomyces cerevisiae* cell lineages following telomerase inactivation. We show that most lineages experience an abrupt and irreversible transition from a replicative to an arrested state (type A lineages), contrasting with the idea of a progressive transition. Such a sharp switch is fully consistent with a mathematical model where the first telomere reaching a critical short length triggers senescence onset. However, many lineages also undergo frequent reversible Mec1-dependent cell-cycle arrests (type B lineages), some of them lasting more than 6 hours. Cells with this phenotype persist only at low frequency in bulk cultures, making them undetectable in conventional population-averaged assays. While we show that Rad51 and Pol32-dependent repair pathways participate in these reversible arrests, we also hypothesized that adaptation may contribute to this senescence dynamics. Here, we describe that the long reversible arrests in type B lineages are suppressed in a *cdc5-ad* mutant, which is an adaptation defective mutant of the polo-like kinase Cdc5. Furthermore, the *cdc5-ad* mutant strongly reduces the senescence-specific genome instability and alters the post-senescence survival patterns. Based on these results, we propose a model where telomere replication fragility, enhanced by telomerase inactivation, initiates repair and adaptation pathways, leading to genomic instability and post-senescence survival.

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- Bourgeron, T., Xu, Z., Doumic, M., and Teixeira, M.T. (2015). The asymmetry of telomere replication contributes to replicative senescence heterogeneity. Sci Rep 5, 15326.

Biocompatible magneto-thermoresponsive nanogels for controlled anticancer drug release by magnetic hyperthermia

E. Cázares Cortés[1], A. Espinosa[2], N. Griffete[1], C. Wilhelm[2], C. Ménager[1]

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Hybrid nanogels, composed of thermoresponsive polymers and superparamagnetic nanoparticles (MNPs) are highly interesting for biomedical applications, being able – as polymeric matrix – to uptake and release high quantities of drugs and – as MNPs – to heat when exposed to an alternative magnetic field (AMF) [1], [2].

This presentation focuses on the preparation and characterization of biocompatible, magneto-thermoresponsive nanogels based on oligo(ethylene glycol) (macro)-monomers (OEGMAs), methacrylic acid (MAA) and iron oxide nanoparticles (ɣ-Fe_2O_3) for magnetically triggered release of doxorubicin (DOX, anticancer drug). The spherical nanogels (320 nm at pH 5,5; 25°C), have a swelling/deswelling behavior at their volume phase temperature transition (50°C) in a physiological medium (pH 7,5), enhancing drug release above human body temperature (37°C). An *in vitro* study (physiological medium, temperatures from 4°C to 70°C) of nanogels (containing 72 µmol.L^{-1}, loading efficiency of 62%) shows that DOX release increases with global temperature (after 2h, 15% at 37°C vs. 24% at 50°C). DOX-loaded nanogels can be well stored at low temperature (after 1 month at 4°C, less than 20% of DOX release). When applying AMF (332 kHz; 17 mT), at 8 mmol.L^{-1} iron concentration, DOX release significantly increases without detecting a macroscopic heating (after 2h at 37°C, 31% vs. 15% without AMF). DLS and cryo-TEM reveal that nanogel size decreases under AMF, suggesting that enhancement drug release is due to a shrinking of polymer network by local heating. An *in vivo* study in PC-3 cancer cells shows that DOX-loaded magnetic nanogels have a stronger cellular internalization than free DOX. Moreover, cancer cell viability decreases from 74% vs. 56% when applying AMF (470 kHz, 18 mT, 2h) after nanogels incubation, thus enhancing DOX cytotoxicity.

These results demonstrate that P(OEGMAs-co-MAA) nanogels are excellent carriers for enhancing cellular internalization and drug release under AMF. Furthermore, this strategy may be combined with imaging (MRI) and magnetic targeting for a theranostic approach in cancer therapy.

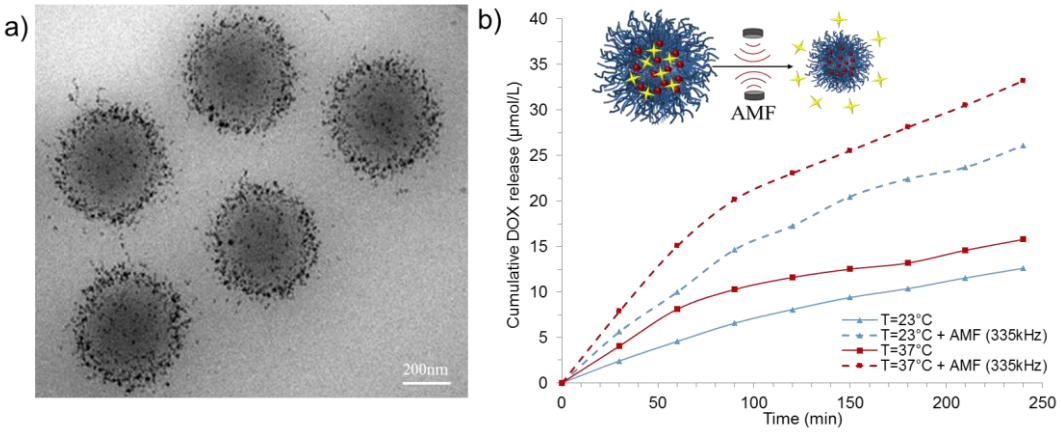

Figure a : Cryo-TEM image of P(OEGMAs-co-MAA) nanogels loaded with 60wt% of MNPs. Figure b : Cumulative DOX release profile (µmol/L) versus time (min) of DOX loaded P(OEGMAs-co-MAA)_MNPs60wt% nanogels ([Fe]=8.35mmol.L^{-1}, pH7.5, 0.1M hepes sodium buffer medium) at 23°C and at 37°C (human body temperature) without and with AMF (pulse: 332kHz; 7,6A; 17mT; 30min)

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A new N-acyl-homoserine lactone identified in the intestinal ecosystem is associated to normobiosis and has an anti inflammatory effect

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Inflammatory bowel diseases (IBD) are characterized by a chronical inflammation of the gut mucosa. IBD patients exhibit imbalance in gut microbiome called dysbiosis. Bacteria can use quorum sensing small signal molecules for cell-cell communication including inter-kingdom (prokaryote-eukaryote) communication. In various ecosystems, one of the most studied quorum sensing system relies on amphiphilic molecules derived from fatty acids called N-acyl-homoserine lactones (AHLs). We aimed to: i) detect presence of AHL in human gut microbiota, ii) investigate their features in IBD associated dysbiosis, iii) look for their impacton inflammation pathways on host cells.

Methods: Fecal samples from 49 IBD patients in remission (n= 24) and during flare (n= 25) and from 26 healthy subjects were analyzed. AHLs profile was determined for each sample by using HPLC coupled with tandem mass spectrometry. In parallel, fecal microbiota composition was assessed by quantitative PCR and Miseq 16S (V3-V5) sequencing. To assess the impact of the AHLs of interest on the intestinal inflammatory pathways, Caco2TC7 cells were cultured until confluence and then stimulated by IL1B at 25 ng/mL during 18h in a middle with increasing concentration of AHLs of interest (0, 1, 5, 10, 25, 50, 100 and 200 µM). The inflammatory response was measured by the level of IL8 in the supernatant using ELISA.

Results: We detected 10 different AHLs in human gut microbiota. We identified a prominent and never described AHL at m/z 294.17. High resolution mass spectrometry allow to determine its composition as 3oxoC12:2 AHL. Its presence was significantly associated with normobiosis. We observed lower levels of IL8 with 10 to 50 µM of 3oxoC12:2 AH after IL1B stimulation.

Conclusion: We were able to detect for the first time AHLs in gut ecosystem. 3oxoC12:2 AHL was prominent in normbobiosis. 3oxoC12:2 AHL exerts an anti-inflammatory effect of on enterocyte like caco2TC7 cells.

Title:

Plexin-A1 and Semaphorin-6D are involved in retinal axon fasciculation and targeting

Authors& affiliations:

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Abstract:

Most axons are fasciculated into nerves as they are guided towards their target and must defasciculate and exit this nerve in order to innervate their specific targets. However, how axon fasciculation and target innervation are regulated is still unclear. To better understand these mechanisms, we used retinal axons of the visual system because of their clear fasciculation in the optic tract and precise organization in their target. Retinal axons from the same (ipsilateral) and the opposite (contralateral) eyes gather in the optic tract and then innervate separate territories in the dorso-lateral geniculate nucleus (dLGN), forming an eye-specific map. We investigated the role of the guidance receptor Plexin-A1 and its ligand Semaphorin-6D (Sema6D) in these processes.

After anterograde tracing, Plexin-A1 -/- mice present fasciculation defect in the optic tract and ectopic retinal projections in the dLGN, without alterations in topographic mapping. A similar phenotype is observed in Sema6D -/- mice, suggesting that Sema6D is the ligand of Plexin-A1 for the appropriate target innervation by retinal axons. Both Plexin-A1 and Sema6D are expressed in retinal ganglion cells and dLGN during development. To determine where the expression of Plexin-A1 and Sema6D is required, we used *in utero* electroporation of Sema6D-shRNA to knock-down retinal expression of Sema6D before target innervation. Mice electroporated with Sema6D-shRNA present the same targeting defect in dLGN as Sema6D -/- mice. Thus, we showed that Sema6D expression in retina is essential for retinal axonal targeting. All these results suggest that Plexin-A1 and retinal expression of Sema6D are necessary for fasciculation and positioning of retinal axons as well as for their target innervation.

Design of Protein Mimetics by Dynamic Combinatorial Chemistry on Folded Peptidic Scaffolds

*Benjamin Zagiel,[1,2] Nathalie Eilstein,[3] Hélène Berthoumieux,[4] EmericMiclet, [1,2] Emmanuelle Sachon [1,2] and Roba Moumné[1,2]**

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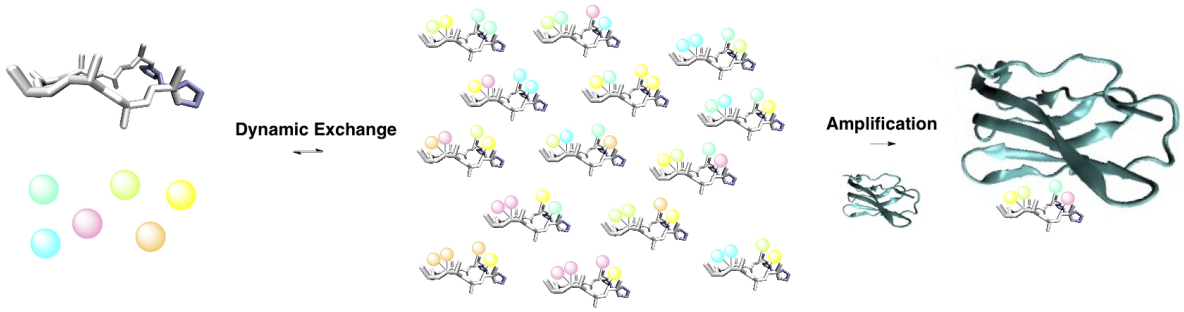
Protein-Protein Interactions (PPI) are central to all biological processes.[i] The design of compounds that specifically mimic proteins and inhibit their interactions is a foremost goal in chemical biology. Except the particular case of enzymes where the substrate binds a specific complementarily shaped cavity on the surface of the molecule, the recognition interfaces usually involve extensive, shallow and hydrophobic complementary surfaces. The interaction domains of proteins have often no particular folding and it is only in the presence of their partner that their bioactive conformation is reached. For all these reasons mimicking proteins with small "drug-like" compounds is rather tricky and many interactions involving proteins are described as "un-druggable".[ii] Historically, the most popular way to interfere with PPIs has been through the high-throughput screening of libraries of compoundsbut identification of hits is much rarer than with traditional target. Among small molecules, short peptides seem very attractive and underexploited mimetic. They occupy an intermediate molecular space (1-2 kDa) between that of traditional drug like compounds and the much larger "biologic" therapeutic antibodies. However, when removed from their native context, peptide segments usually fail to adopt the bioactive conformation and this conformational freedom upon binding to the target leads to entropic penalty that affect their affinity. In order to bring an alternative, we propose here to combine a small folded peptide scaffold and the use of dynamic combinatorial chemistry (DCC)[iii] to decorate this scaffold with functional groups involved in the recognition of a relevant target. The dynamic functionalization of a peptide scaffold would allow the rapid access to a large and complex library of well-ordered protein mimetic that are in equilibrium with each other and can be screened in a single step toward a relevant protein. Such a library of structurally defined peptides would be highly difficult to access by conventional method. Thus, this new strategy should allow to greatly accelerate the discovery of new bioactive peptides and drive the development of peptide drugs.



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Réactivation de l'AChE et états de protonation: Une approche QM/MM

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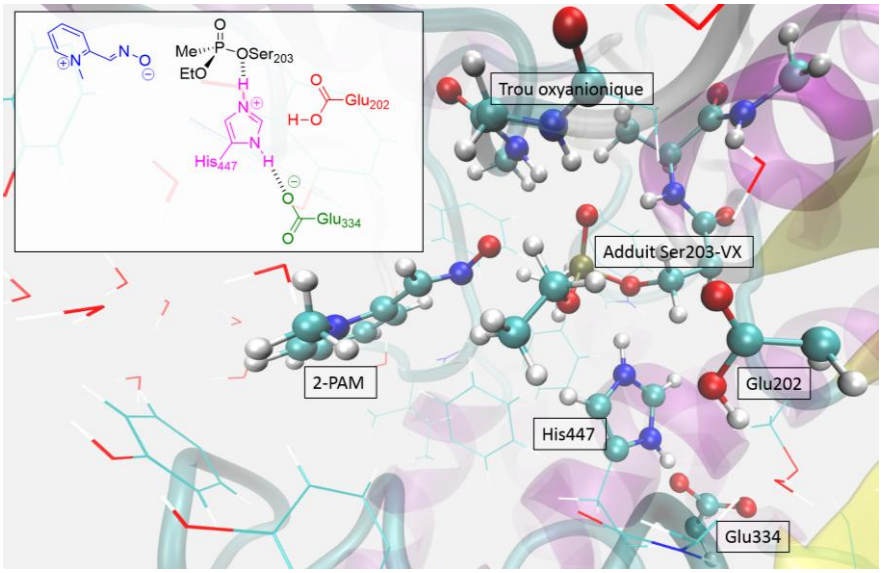
QM/MM, Chemshell, Acétylcholinestérase, réactivation, Transfer de proton, oxime



Structure optimisée du réactif de la réactivation par la 2-PAM de l'AChE inhibée par le VX.

L'acétylcholinestérase (AChE) est une sérine hydrolase qui a pour rôle la médiation du signal aux synapses cholinergiques.[1] Cette enzyme est sujette à l'inhibition par les composés organophosphorés (OP), notamment, mais pas seulement, les gaz de combat comme le Sarin, le VX, le Tabun et le Soman. L'inhibition de l'AChE induit une suractivation des synapses cholinergiques qui conduit à la mort par arrêt des fonctions respiratoires.[2] Cette inhibition covalente n'est pas spontanément réversible et les réactivateurs connus ont une activité limitée.[3] Les outils de la chimie théorique permettent de simuler les processus chimiques qui sous-tendent le retour de l'activité enzymatique. Nous avons utilisé une méthode additive hybridant la mécanique quantique à une description en mécanique classique (B3LYP-D3:CHARMM) pour modéliser la réactivation par la 2-PAM de l'AChE inhibée par le VX. Ces simulations ont notamment porté sur l'état de protonation d'un glutamate à proximité du site actif, le Glu202. Il sera montré que ce résidu à un rôle clé, inconnu jusqu'à maintenant, sur la réactivation de l'AChE. La déprotonation de la 2-PAM, un élément important pour la faisabilité de la réactivation, a aussi été explorée dans les deux états de protonation du Glu202. Ces simulations permettent d'apporter une vision plus large des effets des états de protonation des résidus du site actif sur la restauration de l'activité enzymatique.

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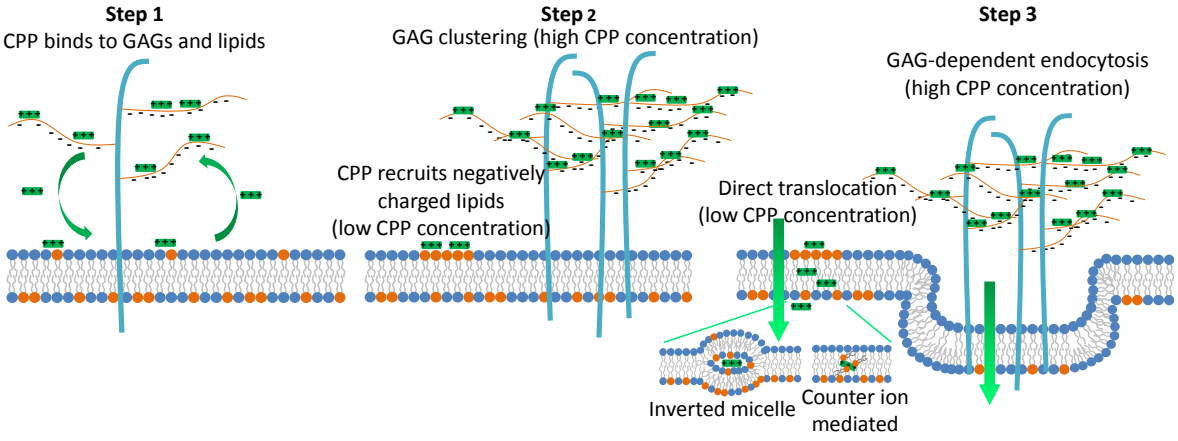
Contributions des résidus tryprophanes pour l'internalisation des « cell-penetrating peptides » riches en arginine.

Astrid Walrant, Laboratoire des Biomolécules, UMR 7203 UPMC CNRS ENS

Les peptides pénétrants ou cell-penetratingpetides (CPPs) ont la capacité de pénétrer dans les cellules selon un mécanisme ne dépendant pas d'un récepteur. Ce sont des séquences peptidiques courtes, riches en résidus basiques, en particulier Arg. Leur mécanisme d'internalisation est toujours sujet à débat mais il maintenant admis qu'ils peuvent entrer dans les cellules par endocytose ou translocation directe à travers la membrane plasmique. Ces différentes voies d'entrées suggèrentque ces peptides peuvent interagir avec différents types de partenaires membranaires à la surface de la cellule, comme les phospholipides membranaires et les glycosaminoglycanes.
Le peptide R6W3-1 (RRWWRRWRR) est un CPP très efficace, issu d'une étude structure/activité sur un des premiers CPP identifiés, la Pénétratine. Il a été montré que les résidus Trp de la pénétratine sont indispensables pour son internalisation. De même, la mutation des Trp en Leu ou Phe dans R6W3-1 compromet les propriétés d'internalisation de ce peptide. Afin d'étudier plus finement le rôle des Trp, nous avons synthétisé une série de peptides de neuf résidus contenant des Arg et des Trp à diverses positions. Nous avons évalué l'internalisation de ces peptides dans des cellules CHO et étudié leurs interactions avec différents types de partenaires membranaires afin de mieux comprendre les mécanismes moléculaires gouvernant l'internalisation de ces peptides.



Bio-functionalized magnetic nanoparticles for remote control of differentiation and oriented growth of neuronal cells

Emilie Secret[1], Elie Balloul[2],Aude Michel[1], Cornelia Monzel[2], Maxime Dahan[2], Jérôme Fresnais[1], Christine Ménager[1],Jean-Michel Siaugue[1]

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Neurodegenerative disorders, such as Parkinson's, Alzheimer's or Huntington's diseases are among the most common group of medical conditions in the world, and are expected to surpass cancer by 2040.[1] However no cure exists for such diseases at that time. Cell replacement therapy is among the most promising approach to treat neurodegenerative disorder. In the work presented here, part of the MAGNEURONEuropean project,[2]we used magnetic nanoparticles that are bio-functionalized to trigger neurons' differentiation and growth along the directionof use of the external magnet gradient. Mature neurons would in term be re-implanted in the patient brain to replace degenerated neurons.

To this goal, maghemite (γ-Fe_2O_3) nanoparticles were synthesized by an inverse co-precipitation process.[3] These nanoparticles were then used to synthesize γFe_2O_3@SiO2 core-shell nanoparticles with size, charge and magnetization adjustedto obtain a good colloidal stability, render them injectable in cells, and facilitate intracellular motion.These magnetic nanoparticles (MNPs) were then functionalized with a HaloTag ligand in order to interact specifically with proteins able to trigger different pathways in the cell. MNPs were then microinjected in the cell andshowed intra-cellular biased diffusion toward the micro-magnet. The magnet can then be used to displace target proteins attached to the MNPs inside the cell, and trigger signaling events such as actin polymerizationatparticular subcellular localizations[4,5]

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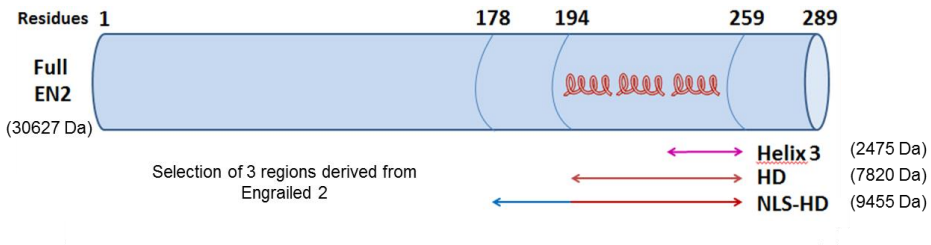
QUANTIFICATION OF THE INTERNALIZATION EFFICACY OF HOMEOPROTEINS AND DERIVED-CELL PENETRATING DOMAINS

Sébastien Cardon, Laura Molina, Alain Joliot, Fabienne Burlina, Gérard Bolbach, Olivier Lequin, Ludovic Carlier, Sandrine Sagan

Homeoprotein (HP) transcription factors and HP-derived homeodomains (HD) have the characteristic of being secreted and internalized by eukaryotic cells. The internalization mechanism of these proteins and peptides is not yet fully understood.

At the molecular level, it was found that the 16 amino acid long third helix of HD provides the driving force for internalization. Interaction with cell-surface, which is the first step in the internalization process, predominantly relies on carbohydrates and was reported to be predominant for HD, Penetratin, and HPs binding to distinct cell types.



The aim of the work was to compare the entry of HPs, HDs and derived-cell-penetrating peptides by absolute quantification of the internalized species. We chose Engrailed 2 as a representative HP. The first step consisted in the production and purification of the Full HP Engrailed 2, and two fragments of this protein, HD and an extended HD sequence incorporating a nuclear localization sequence (NLS-HD). Thereafter the secondary structure of these 3 sequences, free in solution, or in interaction with model membranes or carbohydrates has been studied by circular dichroism. The interaction strength between proteins and membrane phospholipids or GAG is a point which can enter in the internalization mechanism. We have made isothermal titration calorimetry (ITC) to determine all thermodynamic parameters between our different proteins and lipids (POPG LUV) or Heparin like GAG mimic.

Inorganic Biological and Cellular Chemistry

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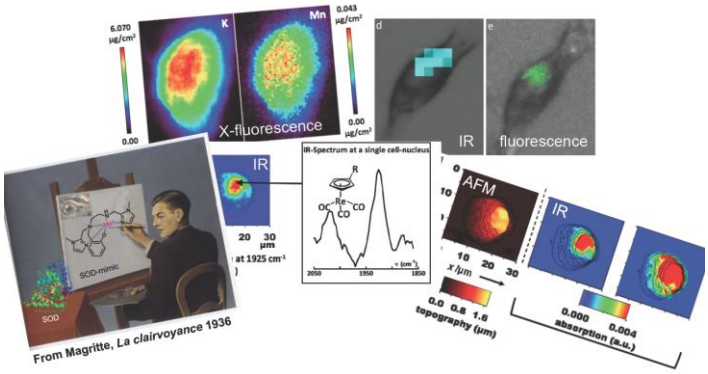
Inorganic biological and cellular chemistry deals with the study of metal complexes in a biological context. At LBM, we gather complementary expertise for these studies: organic synthesis from peptide chemistry to organometallic chemistry, inorganic synthesis, bio-inorganic chemistry, physical-chemistry, spectroscopies, imaging and cell-biology.
Two main projects are currently being developed at LBM in inorganic biological and cellular chemistry, **metal-based antioxidant** and **metal-based probes**, for which the group is involved from the design to the applications in biology. Innovative techniques to investigate their speciation, quantify them and determine their cellular-location are used, leading to key information about their behavior inside cells. Controlling inorganic complexes inside cells is an emerging field that we call "inorganic cellular chemistry" as it involves translation of knowledge acquired in round-bottom flask to cells.

(1) Superoxide dismutases are proteins involved in cell protection against oxidative stress. We design efficient antisuperoxide complexes called SOD mimics in a bio-inspired approach.[1] We develop studies aiming at the characterization of their bio-activity in cells in combination with their speciation and imaging in cells using micro-X-fluorescence of the metal ion.[2]

(2) Metal-CO derivatives can be designed as multimodal probes, with fluorescence, IR-modality,[4-7] but also, as we have shown very recently, X-fluorescence modality.[9] These probes are based on a single molecular core enabling multimodal imaging, both at the sub-cellular level and tissue level, and we have coined the term SCoMPI for these *Single Core Multimodal Probe for* Imaging.[5-9] One of the interests of IR is its easy implementation for direct quantification.[8] These SCoMPIs are thus very promising to tag small molecules for imaging and quantification in biological media.



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Casting Light on Intrinsically Disordered Proteins by Exchange with Hyperpolarized Water

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Abstract: Hyperpolarized water can selectively enhance rapidly exchanging protons in Osteopontin (OPN), a metastasis-associated intrinsically disordered protein (IDP), at near-physiological pH and temperature. The transfer of magnetization from hyperpolarized water is limited to solvent-exposed residues and therefore selectively enhances signals in (^{1}H, ^{15}H) correlation spectra. Binding to the polysaccharide Heparin induces unfolding of preformed structural elements in OPN.

Approach to Ferrocenyl-Podophyllotoxin Analogs and their Evaluation as Anti-Tumor Agents

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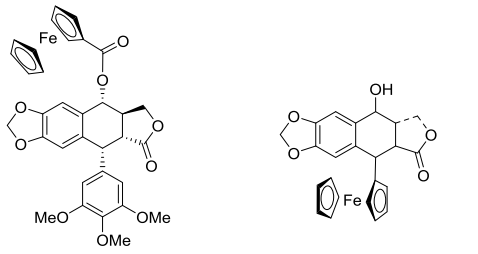
Podophyllotoxin is a natural product endowed of a high antimitotic activity and a high affinity for tubulin.[1] Its action results in the cessation of cell division, inducing cell death. However, its high toxicity restrains its use as drug. To overcome this drawback, several chemical modifications of the native podophyllotoxin have been made. However, to date, no reports have so far been directed toward incorporation of a metallocene moiety.

The search for new organometallic drugs is a central field in drug discovery, including the domain of cancer therapy. In particular, metallocenyl moieties are known to increase the selectivity of drugs toward cancer cells, the conjugate organometallic compound reducing the damage of healthy tissues, yet permitting the desired antimitotic and cytotoxic effects of the active principle. [2]

We report here the synthesis of ferrocene-containing podophyllotoxin analogs and preliminary antiproliferative tests.



ferropodophyllotoxin **1** ferropodophyllotoxin analogs **2**

Targeted ferrocenyl analogs of podophyllotoxin

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Synthesis of ganglioside GM3 analogues with biological activities

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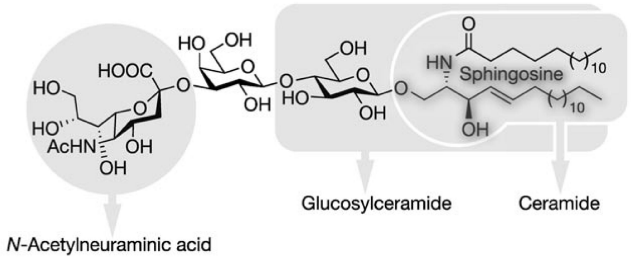
Ganglioside GM3 belongs to glycosphingolipid family, containing sialic acids. It's one of the essential components of plasma membrane rafts. Studies also indicated that ganglioside GM3 involves in many cellular functions, such as proliferation, adhesion, motility and differentiation. So the research for new GM3 analogues appears to be a logical matter of research recently.



Structure of ganglioside GM3

In this study, the sialic acid was activated by sialyl xantes form, and we adopted a short and practical route to 3-*O*-benzoyl azidosphingosine from the commercial D-(+)-galactose. Especially, the different monosaccharides with free 6-OH were obtained by enzymatic hydrolysis. The key step was α-sialylation between glycoside acceptor and a sialyl xanthate by phenylsulfenyl triflate as a promoter to provide the α-glycoside in a high stereoselectivity with good yield.

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Amino-methyl coumarin as a potential SERS@Ag probe for the evaluation of protease activity and inhibition

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Abstract
Proteases are found deregulated in many diseases including cancer and neurodegenerative diseases. They thus represent good therapeutical targets for the development of inhibitors mainly small organic molecules. Peptide substrates containing fluorogenic groups constitute central tools for the monitoring of protease activities and inhibitor screening platforms. Amino-methyl coumarin (AMC) is a well-known fluorogenic group that functionalized a huge number of peptide substrates used for kinetics *in vitro* but also *in vivo*. However, either autofluorescence or quenching of the AMC fluorescence could compromise selection and accurate evaluation of these inhibitors. It is thus needed to explore alternative spectroscopic tools to unravel these limitations. Here, we investigate for the first time whether AMC could constitute a valuable SERS probe in the presence of Creighton' silver colloids under 532nm excitation to monitor protease activity and to evaluate inhibitors. The kallikrein-related peptidase 8 (KLK8) was used as model of proteolytic enzyme. BTEM analysis was successfully used to validate the present SERS approach.

Keywords: silver colloids, coumarin, concentration, BTEM, protease

Analysis of a DNA-peptide complex using photocross-linking coupled to mass spectrometry (MS)

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Photoaffinity labeling is a powerful tool enabling the crosslink of a protein to its ligand through the formation of a covalent bond. The recent developments in MS have gained renewed interest to this method especially for the study of protein-nucleic acid interactions. The setup of such a strategy relies on the tedious choice of the most suitable photoreactive group to crosslink efficiently the target protein. In this work different photoreactive probes have been synthesized and incorporated into a 30-mer oligonucleotide (ON) sequence containing a biotin tag at the 3' extremity allowing purification of the covalent complexes once formed under UV irradiation by activation of the photoreactive probe.

The different photoreactive probes were compared for conditions and efficiency of the photoactivation reaction with a purified protein as a model (human protein histone H3), using electrophoretic mobility shift assays coupled to a ^{32}P detection and quantification. The photoprobes benzophenone and 4-thiothymine (4-ST) displayed the best properties in terms of efficiency and specificity providing the most appropriate photoactive probes for our future study aiming at identifying protein-ON partners.

We first worked on the development of a methodology to analyze the synthesized 30-mer ON as well as covalent peptide-ON heteroconjugates which are difficult to observe by MS due to the different physico-chemistry properties of the two entities. For this methodology study, we are working with a model basic peptide issued from the N-terminus of the histone protein H3 (namely ARTK peptide : ARTKQTARKSTGGKAPRK(Biotin)Q-$CONH_2$) and the 30-mer ON (5' TTGCACTCTCTTGACGXACTGTCCAGCTTC Biotine 3') containing the 4-ST photoprobe in position X. Covalent peptide-ON complexes have been obtained by UV irradiation at 365 nm and analyzed using MALDI-TOF linear negative ions mode. Crystallization and incorporation of the heteroconjugates within the MALDI matrix have been optimized, resulting in highly reproducible crystals leading to much resolved and intense signals in the relatively high mass range 9500-12000 m/z.

Another point is that the photochemistry of the 4-ST photoreactive probe is not well-described for the DNA-protein interactions. It is thus necessary to better characterize adducts in order to be able to search for such modification in future interactomic studies. We are therefore investigating by MS the specificity of the 4-ST photoprobe towards the different aminoacid residues. First results are encouraging, showing selectivity of the 4-ST photoprobe for positively charged residues and most probably Lysine residues.

Design of polymer-based theranostic agents for imaging and targeted delivery of antitumor metallodrugs

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Abstract

The aim of the project is to design polymer-metallodrug conjugates with unprecedented theranostic properties.(1,2) The nanocarrier strategy is meant to improve the metallodrug therapeutic efficacy, reduce side effects by targeted drug delivery and allow optical monitoring of the metallodrug both *in vitro* and *in vivo*. Indeed, although organometallic half-sandwich complexes based on ruthenium(II) (3) iridium(III) (4,5) and rhodium(III) (5) have proved to be highly effective cytotoxic/antitumor agents,(6,7) they often suffer from toxicity, poor water solubility, poor bioavailability, drug resistance, unspecific drug delivery and equivocal mechanism of action. The project intends a) to design, synthesize and accurately characterize polymer conjugates having a well-defined nanostructure, b) to control the number of conjugated theranostic agents, c) to demonstrate their preferential accumulation at target sites and to prove their antitumor activity. Two strategies are envisaged to synthesize the theranostic metallodrug nanocarriers: the multicomponent polymer drug conjugate and drug-initiated polymerization and formulation of nanoconjugates. In the course of our nanocarrier synthesis, we will also study the antitumor capacities of our organometallic half-sandwich metallodrugs tethered, through the arene, to the imaging reporter. This will be the first example of metallodrug-fluorophore conjugates using such tethering strategy. The ease of access to tethered metallodrugs will be useful in the screening of anticancer metallodrugs. This strategy is also anticipated to provide important information on the mechanism of action of these antitumor agents

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DESIGN AND SYNTHESIS OF CYCLODEXTRIN-BASED SUPRAMOLECULAR POLYMERS FOR NEW siRNA TRANSFECTION SYSTEMS

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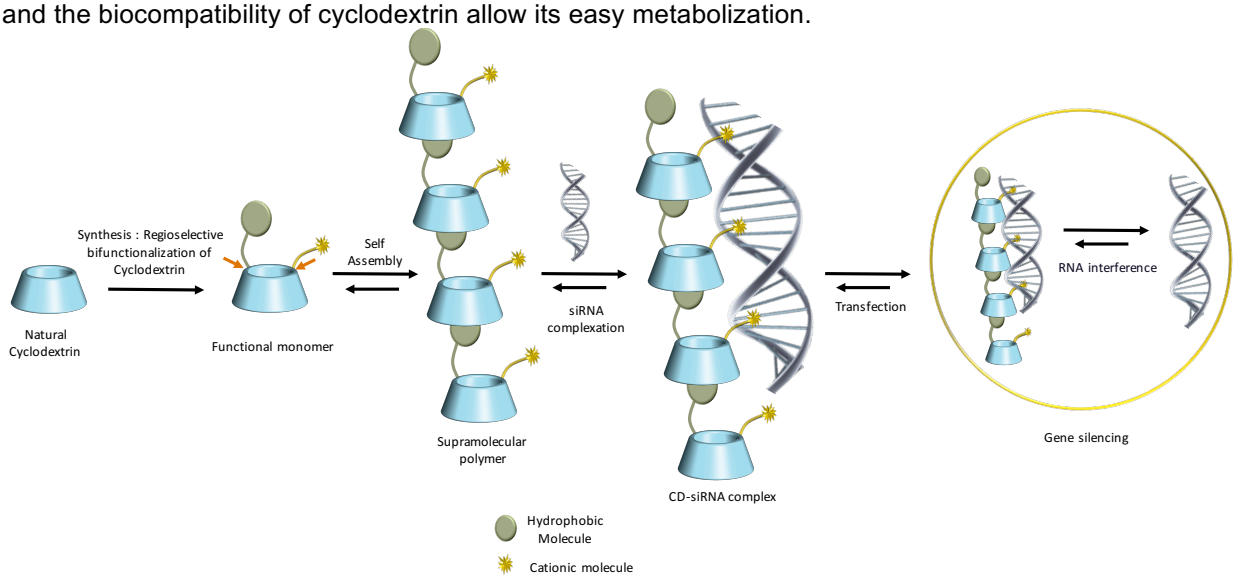
The polyfunctionnalysation of cyclodextrin has always been a real challenge in organic synthesis.[1] Advances have been made in developing methods to address it, [2], [3], [4] and to open new opportunities such as the organization in three dimensions of groups conjugated to cyclodextrin. Here we report the synthesis of a family of cationic cyclodextrin derivatives. This first structure will serve as a platform to form supramolecular polymers able to transfect siRNAs (small interfering RNA).
Since the discovery of siRNAs[5] and their ability to trigger gene silencing in mammalian cells,[6] development of RNA interference for the treatment of diseases, have given rise to great interest.[7] Because of their anionic and hydrophilic nature as well as their size and their instability in the bloodstream, their intracellular delivery still remains an obstacle for their application as drug.[8] Therefore, a sophisticated delivery system becomes an indispensable component to realize siRNA-based therapeutics. Such vectors can be cationic polymers able to neutralize the negative charges of siRNAs and allow the penetration of cells. But their use *in vivo* is often accompanied by a real toxicity due to the polymeric character of cations.
To avoid such toxicity, we disclose here a new delivery system which consists in a supramolecular assembly of a cationic-cyclodextrin-adamantane conjugates. The non-covalent behavior of our system, and the biocompatibility of cyclodextrin allow its easy metabolization.



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SYNTHESIS OF GLYCOSYL-CARBASUGARS FOR N-GLYCAN PROCESSING MECHANISTIC STUDY AND POTENTIAL INHIBITION OF ENDO-ALPHA-MANNOSIDASE

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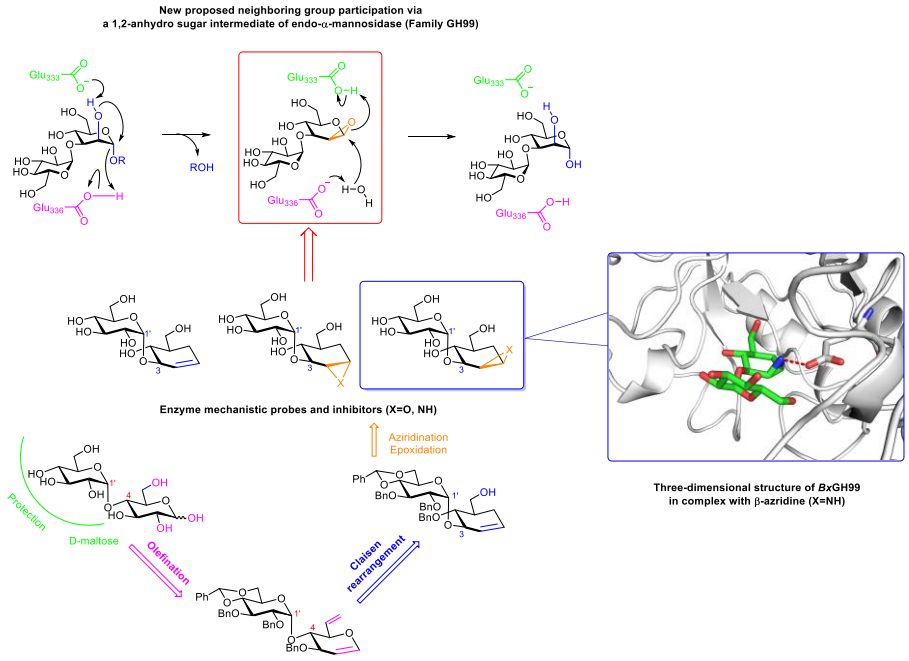
N-linked glycans play key roles in protein folding, stability and function.[1] Endo-α-mannosidase of family GH99 is a unique glycosidase which is involved in N-linked glycans processing pathway. Beside the classical N-glycans biosynthetic pathway, Endo-α-mannosidase can let the glycanproteins enter the Golgi apparatus without folding control.[2] Endo-α-mannosidase as a retaining glycosyl hydrolase requests an enzymatic nucleophile close to the reactive anomeric center to be functional, but the resolution of the X-ray structure by G. J. Davies shows the absence of such a nucleophile! Instead, a carboxylate is placed next to the 2-OH of the mannose, consequently, the authors proposed a totally new mechanism for a glycohydrolase involving the participating of the 2-OH and the formation of a unique transient epoxide.[3] Therefore, we proposed and synthesized five glycosyl-carbasugars from natural sugar D-maltose, four of which comprise an aziridine or an epoxide cycle, as mechanistic probes to structurally characterize the enzyme-ligand complex and investigate its catalytic mechanism.[3] Since our glycosyl-carbasugars are designed to mimic the transition-state structure, they might also be powerful inhibitors.



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Characterization of the Calcium Oxalate Polyhydrates Using IR, NMR and DFT

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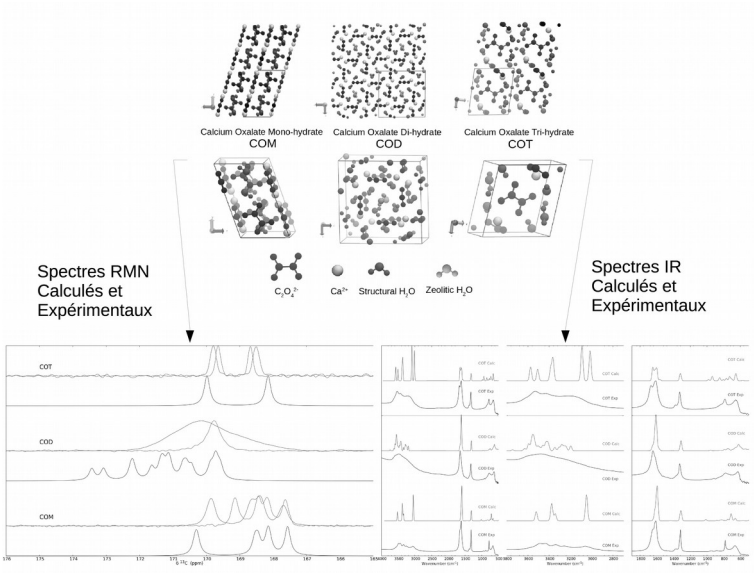
The calcium oxalates polyhydrates are the main constituent of kidney stones[1,2]. They are classified according to their hydration state: monohydrate (CaC2O4.H2O)[3], dihydrate (CaC2O4.2H2O) and trihydrate[4] (CaC2O4.3H2O).These three polyhydrates were studied theoretically and experimentally by IR and NMR. The morphologies of the respective polyhydrates were also studied by electronic spectroscopy and compared with the theoretical predictions based on the Gibbs-Wulff relation.



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Dissolution Dynamic Nuclear Polarization of Deuterated Molecules

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We present novel means to hyperpolarize deuterium nuclei in $^{13}CD_2$ and $^{13}CD_3$ groups at cryogenic temperatures. The method is based on cross-polarization from ^{1}H to ^{13}C, and does not require any radio-frequency fields applied to the deuterium nuclei. After rapid dissolution, a new class of long lived spin states can be detected indirectly by ^{13}C NMR in solution. These long lived states results from non-equilibrium states that involves two or three equivalent deuterons with spin $I = 1$ in case of $^{13}CD_2$ and $^{13}CD_3$ respectively. Although the lifetimes T_{STI} are shorter than $T_1(C_z)$, they can exceed the life-time $T_1(D_z)$ of deuterium Zeeman magnetization by a factor of more than 20.

New design for artificial metalloenzymes based on hybrid β-lactoglobulin/prochiral complexes systems

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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 complexesof prochiral hemi-labile ligands since, once embedded within the protein host, they could be forced to adopt a specific stereoconfiguration. This would in turn make possible to bring the chirality centers closer to the catalytic metal center and, therefore, to increase the enantioselectivity of catalyzed reactions.

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

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Nouveaux hétérocycles fluorescents pour la détection des protéines carbonylées associées au vieillissement et à l'inflammation

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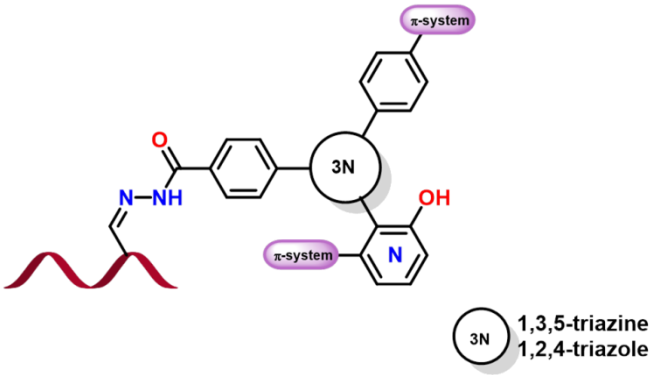
La découverte de nouvelles sondes fluorescentes pour l'analyse de mécanismes biologiques impliquant le stress oxydant reste un enjeu important pour l'étude de différentes pathologies associées au vieillissement. L'objectif est de développer de nouveaux fluorophores pour l'analyse des protéines carbonylées via la technologie 2D Oxy-DIGE[1]. A partir d'une famille de molécules originales 4H-pyrido[e][1,3]oxazin-4-ones[2], nous avons entrepris de synthétiser de nouvelles sondes fluorescentes.



Nouvelles sondes fluorescentes modulables pour la détection des protéines carbonylées

La technique 2D-Oxy DIGE, développée par l'équipe du Pr. Friguet, est une technologie qui permet de détecter et de quantifier les protéines carbonylées présentes dans les cellules à travers l'étude des protéomes. Il est possible d'étudier plusieurs lots de cellules et ainsi de pouvoir les comparer au moyen d'une analyse bidimensionnelle, selon le pH et le poids moléculaire. Pour réaliser ces analyses, il est nécessaire de marquer les protéines avec des fluorophores commerciaux. Ces derniers sont stables à différents pH et possèdent une bonne sensibilité optique mais possèdent certains défauts. En effet, leurs mauvaises solubilités en milieu biologique et leurs faible déplacement de Stokes représentent un frein à la 2D-Oxy DIGE. Les phénomènes de superposition résultant obligent l'utilisation de filtres spéciaux et ne permettent pas d'éliminer l'autofluorescence. Dans le but de répondre à ces problèmes, de nouveaux hétérocycles fluorescents modulables ont été synthétisés.

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