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Affiches / Posters

Lipid emulsions used in parenteral nutrition-induced endothelial dysfunction in porcine coronary artery rings involves oxidative stress and cyclooxygenase-derived vasoconstrictors

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Lipid emulsions are used to provide a source of calories and essential fatty acids for patients requiring parenteral nutrition. They have been associated with hypertriglyceridemia, hypercholesterolemia and metabolic stress, which may promote the development of endothelial dysfunction. The aim of the present study was to evaluate the potential five different commercial lipid emulsions that affect the endothelial function of coronary arteries.

Porcine coronary artery rings were incubated with a lipid emulsion at 1% (v/v) for 30 min before the determination of vascular reactivity in organ chambers and measurement of the level of oxidative stress using the redox-sensitive fluorescent dye dihydroethidium (DHE). Incubation of coronary artery rings with either Lipidem®, Medialipid® or SMOFlipid® significantly reduced the bradykinin-induced endothelium-dependent relaxations affecting both the nitric oxide (NO) and endothelium-dependent hyperpolarization (EDH) components, whereas Intralipid® or ClinOleic® had no effect. In contrast, Lipidem® did not affect endothelium-independent relaxations to sodium nitroprusside. The endothelial dysfunction induced by Lipidem® was significantly improved by indomethacin, a cyclooxygenase (COX) inhibitor, and by inhibitors of oxidative stress (N-acetylcysteine, superoxide dismutase, catalase) and transition metal chelating agents (neocuproine, bathocuproine) . Lipidem® significantly increased the level of vascular oxidative stress as indicated by increased DHE signal throughout the arterial wall.

The present findings indicate that several, but not all lipid emulsions induce an endothelial dysfunction in coronary artery rings, involving both blunted NO- and EDH-mediated relaxations. The Lipidem®-induced endothelial dysfunction is associated with increased vascular oxidative stress and the formation of COX-derived vasoconstrictor prostanoids.

Novel ultra-bright fluorescent nanoparticles based on cationic BODIPY molecules

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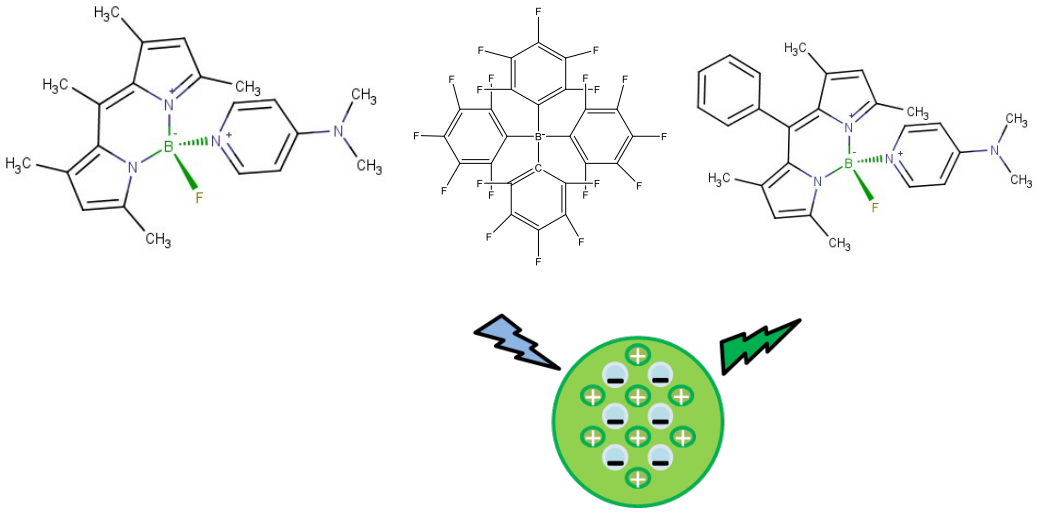
Fluorescent polymer nanoparticles are highly promising for bioimaging application, as they can be biodegradable and very bright. To achieve the high brightness one should load the polymer particles with highest possible concentration of dyes. However, one of the major problems of such systems is self-quenching of dyes at high loading. Another issue is encapsulation efficiency, which usually becomes lower with increasing the dye loading. Recently we showed that cationic rhodamine dye can be encapsulated with high efficacy and minimal self-quenching by using hydrophobic counterion[1]. The latter serves as insulator that separates the fluorophores in the polymer matrix, thus preventing their aggregation. Moreover, it also improves dye encapsulation.

In this work we present ultra-bright nanoparticles, which are made of biodegradable polymer PLGA (polylactide-co-glycolide) and doped with new BODIPY dyes. These dyes are attractive for encapsulation, because they feature high brightness and photostability. To improve encapsulation efficiency and prevent self-quenching of these dyes, we synthesized cationic analogues of BODIPY and further assembled them with hydrophobic counterions.



Using the counterion-assembled dyes concept we succeeded to create highly-loaded fluorescent nanoparticles about 50 nm in size. They present high quantum yields and photostability, they are brighter than commercial polymer nanoparticles and thus can become promising tools for bioimaging.

[1] Andreas Reisch, Pascal Didier, Ludovic Richert, Sule Oncul, Youri Arntz, Yves Mély, Andrey S. Klymchenko, *Nature Communications, 2014, 5, 4089.*

Analysis of the transcription machinery in the mouse embryo.

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Laszlo Tora lab : Chromatin modifications and transcription regulation during cellular differentiation.

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Formation of the preinitiation complex at the promoter consists in a multistep assembly of transcription factors and coactivators. The general transcription factor TFIID is essential for promoter sequence recognition and mediates RNA PolII binding. TFIID is a conserved macromolecular complex, composed of TBP and 13-14 TAFs. However, several evidences suggest the existence of different TFIID subpopulations in Hela cells. In addition, some TAFs are expressed in some restricted cell types and are associated to expression of a subset of genes. Thus, the transcription machinery represents an additional level of gene expression regulation. Interestingly, Taf10, which is also part of the coactivator SAGA, is known to have a differential requirement for transcription as Taf10 is essential in the embryo but not in the adult.

To study the transcriptional machinery in the mouse embryo, we use the presomitic mesoderm as a new paradigm. The presomitic mesoderm is a particular embryonic tissue where takes place the sequential segmentation of the anterio-posterior axis. This event is driven by signaling gradients coupled with oscillation of gene expression controlled by a molecular clock. Spatial and temporal conditional deletion of *Taf10* in the embryo does not impair immediately global transcription but blocks embryonic growth at E9.5 suggesting that transcription still occurs without Taf10. In order to characterize the complex that allows transcription in the absence of Taf10 we have developed both *in vivo* and *in vitro* approaches. In this project, we aim i) to characterize by mass spectrometry TFIID and SAGA complexes composition in the mouse embryo; ii) to assess how transcription is achieved in the absence of Taf10.

One-pot access to cyclooctatetraenes derivatives via palladium-catalyzed cascade reactions

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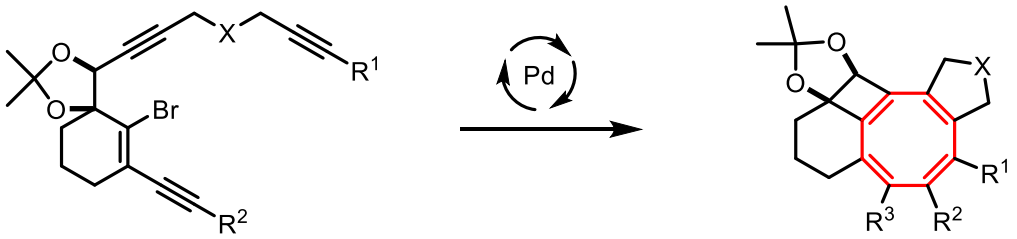
Cyclooctatetraenes are a fascinating class of molecules present in Nature[1] and which represent a great potential utility as building blocks for organic synthesis,[2] scaffolds for drug discovery, designed carbohydrate mimics,[3] ligands for metals catalysis (including asymmetric catalysts)[4] and electron transporters in Organic Light Emitting Diodes (OLED).[5]

Based on the expertise of our group in the field of palladium-catalyzed cascade reactions,[6] we have developed a versatile methodology leading to octasubstituted cyclooctatetraenes. This step-economical synthesis is a straightforward way to access cyclooctatetraene frameworks starting from original scaffold and creating four new C-C bonds in a one-pot operation.



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(3) Mehta, G.; Pallavo, K. *Chem. Commun.* **2002**, 2828-2829.
(4) For a review on substituted COTs being used as ligands see: a) Roesky, P.W. *Eur. J. Inorg. Chem.* **2001**, 1653-1660.
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Mild and efficient Ullmann-type arylation of primary amines in presence of D-Glucose under micellar conditions

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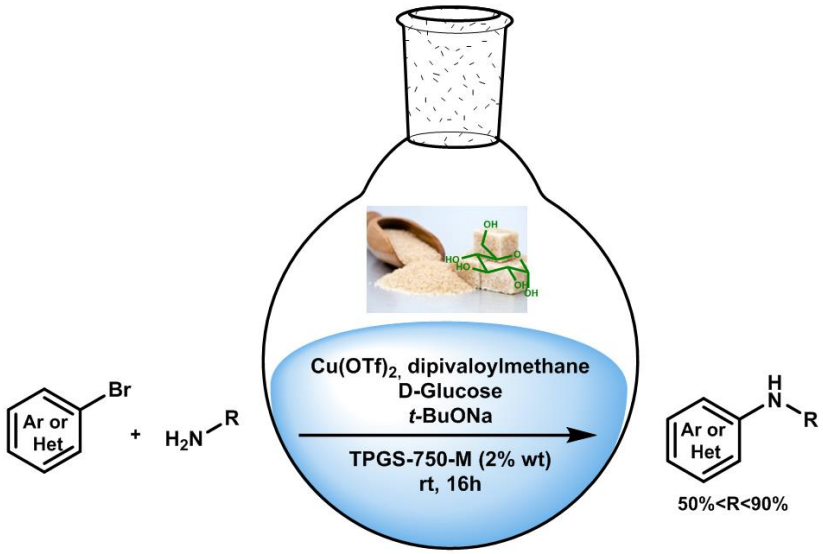
The transition-metal catalyzed formation of C-N bonds via cross-coupling reactions play an important role in the preparation of numerous products in biological, pharmaceutical and material sciences. Efficient palladium catalyzed amination (Buchwald-Hartwig reaction) has been developed in recent years and has been proved useful in both academic and industrial laboratories. For instance, with the right choice of the phosphine/palladium system, we described recently a mild *N*-arylation reaction under micellar conditions (TPGS-750-M as surfactant).[1]

Copper-catalyzed reactions are very attractive and more convenient than palladium from a synthetic point of view due to the low cost and low toxicity of this metal. In the last few years, remarkable progress has been made in the development of cross-coupling reactions promoted by copper. However, the copper catalyzed coupling in water has been less considered. That is why we decided to continue our effort on the Ullmann reaction. We described herein a mild copper mediated amination under micellar conditions. In particular, the commercially available dipivaloylmethane in combination with $Cu(OTf)_2$ and D-Glucose shows excellent performance for coupling aryl/heteroaryl bromide with primary amines.



[1] P. Wagner, M. Bollenbach, C. Doebelin, F. Bihel, J.-J. Bourguignon, C. Salomé, M. Schmitt *Green Chemistry* **2014**, *16*, 4170-4178.

Josiphos CyPF-^{t}Bu a highly efficient ligand for the Palladium-catalyzed amination of 3-chloropyridazines.

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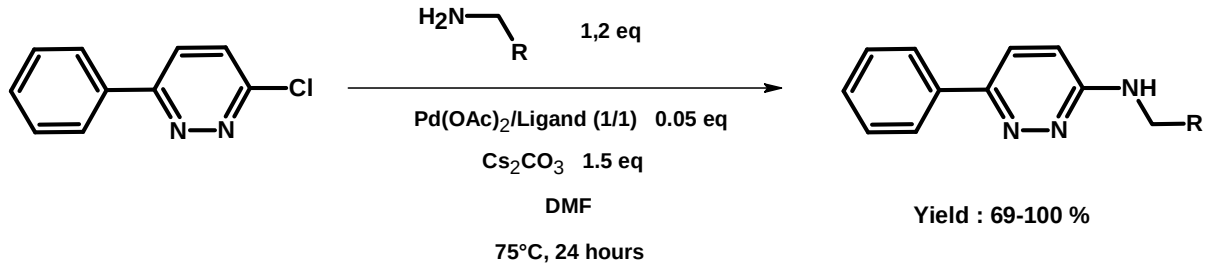
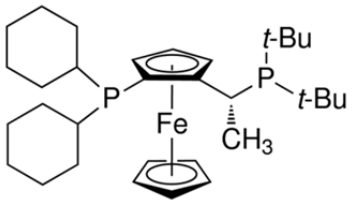
The 3-aminopyridazine derivatives have provided numerous pharmacologically active compounds acting as receptor ligands or enzyme inhibitors. Classical aminations of 3-chloropyridazines generally needs the use of a large excess of amine reagents, drastic conditions and give fair to moderate yields.

More recently, the palladium-catalyzed cross-coupling reactions of hetero(aryl) halides and NH substrates (i.e Buchwald-Hartwig amination) has emerged as an effective methodology for the construction of heteroarylamines. However, attempts to extend this reaction to the 3–chloropyridazines were unsuccessful with primary amine.[1]

We report here an efficient palladium catalyzed amination reaction of 3-chloropyridazine with primary amine. A variety of ligands, including monodendate and bidendate phosphines were investigated. A multivariate screening analysis of the variables (Pd catalysis, bases, solvents, reaction conditions, etc.) was performed on the 6-Ph-3-chloropyridazine and highlighted the efficacy of the Josiphos CyPF-^{t}Bu in combination with $Pd(OAc)_2$ for the formation of the C-N bond.



Ligand Josiphos CyPF-^{t}Bu



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1 Parrot I.; Ritter G.; Wermuth, C. G.; Hibert, M.; *Synlett*, 2002, 7, 1123-1127.

Functional and structural studies of two periplasmic binding proteins involved the iron uptake in *Pseudomonas aeruginosa*.

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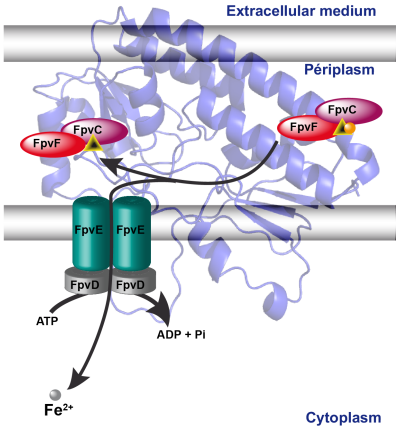
Pyoverdine (Pvd) is the main siderophore secreted by *Pseudomonas aeruginosa* PAO1 to obtain access to iron (Demange *et al.*, 1990). After extracellular iron chelation, Pvd-Fe uptake into the bacteria involves a specific outer-membrane transporter, FpvA (Poole *et al.*, 1993). Iron is then released in the periplasm by a mechanism involving no siderophore modification but probably iron reduction (Schalk *et al.*, 2002; Hannauer *et al.*, 2012a). The proteins involved in this dissociation step are currently unknown. The pyoverdine locus contains an operon of four genes, *fpvCDEF*. These genes encode an ABC transporter with the distinguishing characteristic of encompassing two periplasmic binding proteins, FpvC and FpvF, associated with the ATPase, FpvE, and the permease, FpvD (Brillet *et al.*, 2012). Deletion of these four genes partially inhibited cytoplasmic uptake of ^{55}Fe in the presence of pyoverdine and markedly slowed down the *in vivo* kinetics of iron release from the siderophore. This transporter is therefore involved in iron acquisition by pyoverdine in *P. aeruginosa*. Sequence alignments clearly showed that FpvC and FpvF belong to two different subgroups of periplasmic binding proteins. FpvC appears to be a metal-binding protein, whereas FpvF has homology with ferrisiderophore binding proteins. *In vivo* cross-linking assays and mass spectrometry experiments showed formation of complexes between FpvC and FpvF. In this project, our aims are to solve the 3D structure of these proteins, in the apo- and holo-forms, and to characterize the interaction between these partners. Thereafter, we will try to solve the 3D structure of the ABC transporter, FpvDE.



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Direct Nitrogen and Sulfur Cyanation by CuCN: A Practical Access to Cyanamides and Thiocyanates

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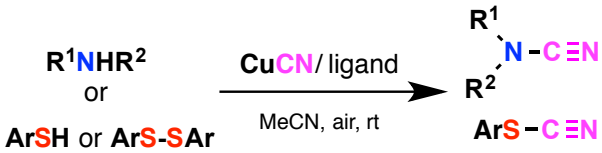
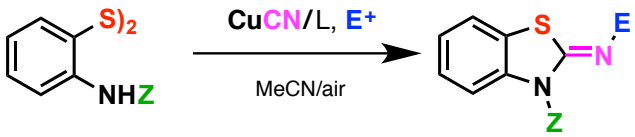
Cyanamides and thiocyanates are characterised by a specific X-C≡N function (X = N, S) that confers them a unique and valuable reactivity. They are found in various synthetic biomolecules or natural products, and they represent versatile intermediates or reagents in the synthesis of a large number of relevant compounds.

The main strategy used for the preparation of these compounds consists in the reaction of a nitrogen or sulfur nucleophile with an electrophilic cyanide. However, the high toxicity and moisture-sensitivity of this type of CN sources represents a drawback and decrease the practicability of this method.

In order to propose an efficient alternative approach, we have developed a mild and practical synthesis of cyanamides and thiocyanates based on an aerobic copper-mediated coupling between copper cyanide and diversely functionalized secondary amines, or aromatic thiols and disulfides.[1]



At the present, further studies are in course to develop domino transformations involving these substrates. Preliminary results were obtained in the synthesis of imino benzothiazoles *via* a domino *S*-cyanation/cyclization/*N*-functionnalization reaction.



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Endothelium-independent vasorelaxant effect of a *Berberis orthobotrys* root extract via inhibition of phosphodiesterases in the porcine coronary artery

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Berberis orthobotrys Bien ex Aitch. (Berberidaceae) is a plant indigenous of Pakistan that is locally used for the treatment of hypertension. The aim of the present study is to evaluate the vasoactive properties of a *Berberis orthobotrys* root extract and its fractions, and to determine the underlying mechanism [1].

The aqueous methanol extract induced similar relaxations in porcine coronary artery rings with and without endothelium, and the most potent fraction was the butanol fraction (BFBO). Analysis of BFBO by LC-MS indicated the presence of four major isoquinoline alkaloids including berbamine and berberine. In addition, a low concentration of BFBO (10 µg/ml) significantly potentiated the relaxations induced by cyclic GMP- and cyclic AMP-dependent relaxing agonists. In contrast, BFBO did not affect relaxations to endothelium-dependent vasodilators. BFBO at the concentration of 100 µg/ml significantly inhibited concentration-dependent contractions to KCl, $CaCl_2$, and U46619 in endothelium denuded rings.

Since BFBO potentiated cyclic GMP- and AMP-dependent relaxing agonists, we have assessed the ability of BFBO to inhibit the activity of different cyclic nucleotide phosphodiesterase (PDEs) isoforms. BFBO induced a concentration-dependent inhibition of cyclic GMP-hydrolyzing activity of basal PDE1, CaM-activated PDE1 and PDE5, and of cyclic AMP-hydrolyzing activity of PDE3 and PDE4 with IC_{50} values ranging from 40 to 130 µg/ml.

In conclusion, BFBO induced pronounced endothelium-independent relaxations and inhibited contractile responses by acting directly at the vascular smooth muscle in the coronary artery. Moreover, BFBO potentiated relaxations induced by both cyclic GMP- and cyclic AMP-dependent vasodilators, at least in part, by inhibiting several vascular PDEs, and in particular PDE4 and PDE5.

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Protective effect of a Protein Epitope Mimetic (PEM) CCR10 antagonist, POL7085, in an allergic model of asthma

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Potential involvement of the CCR10/CCL28 axis was recently reported in a murine model of allergic asthma[1]. If confirmed, blockade of the CCR10 receptor would represent an alternative to current asthma therapies. We evaluated the effect of a novel Protein Epitope Mimetic CCR10 antagonist, POL7085, in a murine model of allergic eosinophilic airway inflammation.

Nine week-old male Balb/c mice were sensitized and challenged to ovalbumin. POL7085, a CCR10 antagonist, was administered once daily 1 hour before each OVA challenge at 9 and 18µmol/kg intra-nasally (i.n.) *vs* dexamethasone i.n. (DEX; 2.3µmol/kg) *vs* vehicle.

In vehicle treated animals, allergen challenge induced airway hyperresponsiveness (AHR) in vehicle-treated animals as measured by whole body barometric plethysmography, and eosinophilia in bronchoalveolar lavage (BAL) fluid. POL7085 dose-dependently and significantly decreased AHR by 34±16%, and eosinophil numbers in BAL by 66±6%. In addition, the highest dose of POL7085 used significantly inhibited lung IL-5 expression (42±13 %), and lung collagen formation (43±11 %).

The Protein Epitope Mimetic CCR10 antagonist, POL7085, significantly and dose-dependently decreased allergen-induced airway hyperresponsiveness and inflammation after once daily local treatment. Blocking the CCR10 chemokine receptor therefore might become a potential novel therapeutic approach for treating asthma.

1- English et al, Immunol Lett; 2006;103: 92-100

Inhibiting proliferating cell nuclear antigen with antibodies delivered to the cytoplasm generates irreversible genome instability and cell death

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Antibodies are valuable tools for functional studies *in vitro*, but their use in living cancer cells remains almost unexplored because they do not naturally cross the cell membrane. We have generated a set of new monoclonal antibodies against human proliferating cell nuclear antigen (PCNA) and tested whether those that block DNA replication *in vitro* could also interfere with the cellular replication machinery after electrotransfer in living cells. This method of delivery allows all cells to be transduced without any loss of viability (1). Amongst the 8 monoclonal antibodies able to specifically interact with PCNA, as probed by immunofluorescence microscopy, two (2H3 and 4D6) were found to be present in the cell nuclei following electroporation. Although these two antibodies inhibited the incorporation of 5-ethynyl-2'-deoxyuridine into newly-synthesized DNA in cells, no drastic effect on cell growth was observed. However, when either 2H3 or 4D6 were co-delivered to the cells with moderate amounts of anti-PCNA siRNA that had no effect when used alone, both antibodies were able to induce cell growth suppression and subsequently cell death. Strikingly, under these conditions, the size of the nuclei increased up to 10 fold before the cells became detached from the plastic, a phenomenon analogous to the effect of hydroxyurea, which is known to promote replication stress. In addition, we found that the g-H2AX levels in the treated cells were considerably raised, demonstrating that the inhibitory anti-PCNA antibodies, whilst blocking replication fork progression, affect genome integrity by triggering numerous DNA double-strand breaks that cannot be cured by the DNA repair systems. This remarkable size increase of the nucleus before cell death mediated by the delivered anti-PCNA antibodies was also observed when the Fab molecules of either 2H3 or 4D6 were used to transduce chemoresistant cells. Thus, it is possible to induce DNA damage and genome instability in a variety of cancer cells by knocking-down the biological activity of PCNA with antibodies. This study may form the basis of a simple approach for identifying and validating the functional accessible sites of intracellular targets that are essential for cancer cell survival.

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Cytotoxic activity of bisacodyl on a 3D tumor-like model of human glioblastoma stem-like cells

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Glioblastomas (GBM) are the most malignant primary brain tumors. Cancer stem-like cells (CSCs), which are responsible for tumor maintenance and recurrence after treatment, have been identified in human GBM. They reside in specific niches, characterized by hypoxia and extracellular acidity, both favoring glioblastoma CSCs quiescence and drug resistance. Thus, to increase the efficacy of treatments against GBM, therapies should include molecules targeting those stem-like cells in the particular conditions prevailing in the tumor microenvironment.

We have established an *in vitro* model of GBM consisting in large size clonal GBM CSCs spheres (diameter ~ 1 mm). Those spheres exhibit several characteristics of tumors, namely cell heterogeneity with the presence of both proliferating and quiescent cells and existence of both a hypoxic zone and a necrotic center. Here we show that bisacodyl, a marketed laxative molecule previously identified in our laboratory by HTS and selected for its specific activity on GBM CSCs, is highly effective in the *in vitro* 3D GBM model. The molecule induces sphere disruption and death of the constituting cells. The presentation will highlight our recent findings on the mode of action of bisacodyl, a molecule with a high potential in combination cancer chemotherapy.

Microparticles from rat splenocyte induce endothelial senescence : Impact for islets transplantation.

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Abstract

Background: Microparticles (MPs) have emerged as a surrogate marker of endothelial injury and act as pro-inflammatory mediators in immune disorders and in islets graft rejection. When Tissue Factor (TF) is expressed at the vicinity of transplanted islets it favors a noxious inflammatory reaction leading to instant blood mediated inflammatory reaction (IBMIR) and graft loss. MPs bearing TF are released by inflamed endothelium and monocytes. MPs are also inducers of endothelial senescence. This study determined the possibility that rat splenocyte-derived MPs promote premature endothelial cells ageing and expression of TF.

Methods and results: MPs were produced from rat splenocyte (SR) stimulated, either by [5 μg/ml] LPS or [25 ng/ml; 1μM] PMA-ionophore A23187. Senescence was assessed by flow cytometry using C12FDG probe for the measurement of Senescence-Associated β-galactosidase activity. Exposure of young P1 primary endothelial cells to different concentrations of MPs-derived splenocyte (MP-SR) (30 nM) induced premature senescence, oxidative stress, and the up-regulation of p53, p21 and p16. The MP-SR induced oxidative stress involved NADPH oxidase subunit gp91 as detected by western blot. MP-SR promoted endothelial cell thrombogenicity through tissue factor up-regulation, shedding of pro-coagulant MPs, increased the SA-beta-gal activity and favored eNOS down-regulation. Treatment with SR-MPs (1-10-30 nM) for 24 h had no effect on apoptosis as detected by flow cytometry using PI-AV double labeling but increased the number of senescent cells detected by X-gal blue staining using microscopy.

Conclusions: MPs-derived SR induces premature endothelial senescence and thrombogenicity in young P1 ECs and promote up-regulation of TF. We previously showed that TF+-MPs prompt inflammatory reaction in insulin secreting cells. These new data could bring new hints on the role of pro-inflammatory, pro-oxidative and pro-thrombogenicity MPs in islets allograft transplanted.

Keywords: aging, thrombosis, microparticle, pancreatic islets, IBMIR

Development of imidazo[1,2-c]quinazolin-5-ones as 18kDa translocator protein (TSPO) ligand

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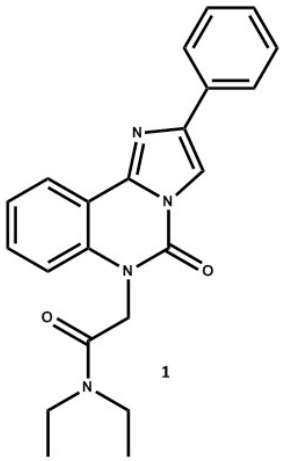
The 18kDa translocator protein (TSPO), which was initially named peripheral benzodiazepine receptor (PBR), is known to play a key role in the synthesis of steroids and neurosteroids, through the translocation of cholesterol from the outer to the inner mitochondrial membrane. Many studies recently highlighted the potential use of TSPO ligands for neuroprotection, neurogenesis and for treating dysfunctions of the nervous system. Moreover, radioligands of TSPO are already currently used as sensitive biomarkers of brain damage. We recently identified a new chemical family based on an imidazo[1,2-c]quinazolin-5-one scaffold, and acting as a TSPO ligand. Hit optimization led to compound 1 which exhibits a high affinity towards TSPO, and a good selectivity towards central benzodiazepine receptor. Moreover, compound 1 showed interesting functional effects, significantly increasing the production of pregnenolone by glioma cells.



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Study of the molecular mechanism of P2X pore dilation

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Abstract

P2X receptors are ligand-gated ion channels (LGICs) selectively permeable to cations. They are activated by extracellular ATP and are involved in many physiological processes such as neuromodulation, neuropathic pain or even platelet aggregation (1). P2X receptors are characterized by a trimeric structure. Following ATP binding, a rapid conformational change converts the ion-conducting pore from a closed to an open state, allowing sodium, potassium and calcium ions to flow through the open pore. Electrophysiological and fluorescence data have indicated that for some P2X receptors there is a second open state, which is permeable to larger cations, like *N*-methylglucamine (NMDG) (2). This mechanism, referred to as pore dilation, is thought to have a link with neuropathic pain (3). However, the molecular mechanism underlying this unique pore property remains ill-defined. We have used photoswitchable azobenzene-containing derivatives to investigate the dilation mechanism of P2X receptors with several engineered cysteine mutations. These molecules attached at specific sites within the pore domain can be switched between cis and trans configurations that subsequently induce helices displacement relevant to gating. Our data indicate that photoswitchable molecules are able to modulate the open state of the P2X2 receptor and possibly induce a dilated state.

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Regulation of poly(ADP-ribose) glycohydrolase function by phosphorylation

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Among post-translational modifications, poly(ADP-ribosyl)ation has emerged to be a crucial event in a wide range of processes from DNA damage signalling to regulation of chromatin structure and gene expression. While poly(ADP-ribosyl)ation of proteins occurs through the activity of a family of 17 proteins called the poly(ADP-ribose) polymerases (PARPs), the reversion of this modification is essentially driven by the degrading activity of poly(ADP-ribose) glycohydrolase (PARG). Although PARG is encoded by a single gene in the human genome, its regulation is finely tuned by several processes such as alternative splicing and translational re-initiation, generating at least five isoforms displaying various subcellular localisations and functions. Regulation of PARG function or activity by post-translational modifications has not been addressed so far.

The present work aims at deciphering the regulation of PARG function by phosphorylation. We have observed that a kinase activity co-purifies with PARG from cell extracts, PARG being itself a substrate for this phosphorylating activity. Proteomic studies identified several protein kinases involved in DNA damage response as PARG partners. For one candidate, *in vitro* phosphorylation assays using recombinant proteins validated that PARG is a specific target for phosphorylation. Our current work is focused on the identification by mass spectrometry of the phosphorylation site(s) within PARG, to subsequently generate unphosphorylable or phosphomimetic PARG mutants. Next, we will evaluate the influence of PARG phosphorylation status on its *in vitro* enzymatic activity. Through the generation of stable cell lines expressing these PARG mutants, we will evaluate the involvement of PARG phosphorylation on its function in DNA repair. We will also examine which PARG isoform is regulated by this phosphorylation. We expect that our results will shed light on the regulation of PARG that is needed to tightly control the level of PAR produced in response to DNA damage to avoid its detrimental accumulation.

Project funded by CNRS, Université de Strasbourg, LabEx Medalis, Ligue contre le Cancer (Equipe Labellisée), ANR.

MORPHOLOGICAL AND FUNCTIONAL ABNORMALITIES OF THE ENDOCRINE PANCREAS IN ELDERLY RAT: IMPACT FOR TRANSPLANTATION

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Introduction: In transplantation, donor age could contribute to chronic allograft failure. We investigated whether the morphological changes of islets and the proportion of insulin and glucagon secreted cells may reflect pancreas dysfunction during aging.

Materials and Methods: Pancreas were harvested from 12 or 52 weeks wistar rats (n=8 each). The morphology of islets was examined on 4 µm paraffin sections stained by Haematoxylin & Eosin. The surface of cells secreting insulin or glucagon was measured by immunofluorescence. Protein extracts from frozen pancreatic tissues were analyzed by western blots to study senescence and inflammatory markers. Blood samples withdrawn at moment of sacrifice in aged and young rats were analyzed for lipid profiles, glycemia and insulinemia.

Results: A higher proportion of small diameter islets (20-154 µm) was measured in aged vs. young rats (91 % vs. 87 %) whereas larger islets were doubled in young rats. In aged rats, the glucagon /islets surface ratio increased by 40 % while the insulin/islet surface ratio decreased by 10 %. In addition, Western blots indicated a trend to the up-regulation of P53, P21 and tissue factor. Plasma insulin was significantly elevated in aged rats (7.01±0.35 vs. 5.69±0.47 ng/ml $p < 0.05$). Cholesterol and LDL-cholesterol were also elevated.

Conclusion: Aging is associated with an altered islets size distribution range, and abnormal proportion of endocrine secreting cells. Senescence and inflammatory markers are majored with aging suggesting the determinant role of donor age in transplantation.

Novel Conjugation and Cleavage Techniques for Preparation of Bioconjugates

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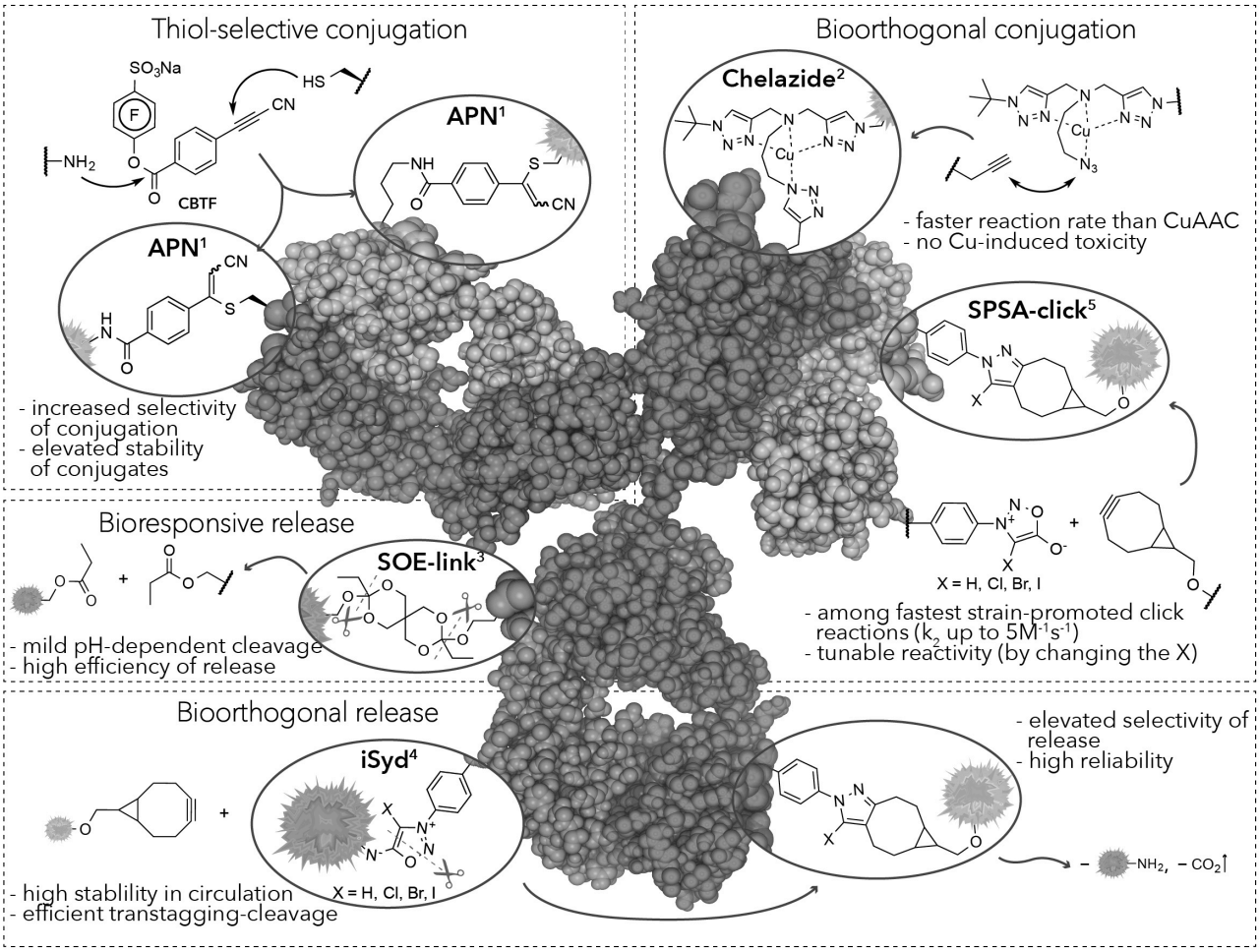
Bioconjugation involves linking of a biolmolecule with a molecule of interest in a covalent manner to form a novel complex having the combined properties of its individual components. Thus, natural or synthetic compounds with their individual activities can be chemically combined to create unique substances possessing carefully engineered characteristics.



With continuously increasing requirements of modern applications in terms of selectivity, stability, mildness, and preserving of biomolecule integrity, reliable chemoselective reactions on biomolecules are undoubtedly among the most demanded transformations.

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High throughput screening for selection of anti-HIV drugs

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The efficacy of the highly active anti-retroviral therapy (HAART) that is currently used for HIV-1 treatment is limited by HIV-1 mutations occurring in response to pharmacological pressure. Due to its key role in the viral life cycle and its highly conserved zinc fingers, the nucleocapsid protein (NC) is a profitable target for overcoming drug resistance. Currently developed NC inhibitors suffer from toxicity and limited specificity. Since the specific nucleic acid recognition and chaperone properties of NC are mediated through its hydrophobic platform at the top of the folded zinc fingers, this platform appears as an ideal target for new antiviral strategies with greater specificity.

Here we combined molecular modeling, organic synthesis and biophysical studies to find an efficient NC inhibitor. We examined 400 compounds that were previously selected by virtual screening for their ability to inhibit NC chaperone properties based on a NC-promoted cTAR DNA destabilization test. From the screening, 32 compounds were selected for IC50 titrations, based on their ability to induce more than 25% of inhibition at 100 μM concentration in the screening test. The IC50 values of these compounds were found to be in the range 8 – 900 μM. For the most active compounds, we performed additional mechanism-of-action studies. Taken together our results represent a good starting point for further development of anti-NC HIV drugs.

A fluorescent live imaging screening assay based on translocation criteria identifies novel cytoplasmic proteins implicated in GPCR signalling pathways

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Several cytoplasmic proteins that are involved in G protein-coupled receptor signalling cascades are known to translocate to the plasma membrane upon receptor activation, such as beta-arrestin2. Based on this example and in order to identify new cytoplasmic proteins implicated in the ON-and-OFF cycle of G protein-coupled receptor, a live-imaging screen of fluorescently-labeled cytoplasmic proteins was performed using translocation criteria.

The screening of 193 fluorescently-tagged human proteins identified 8 proteins that responded to activation of the tachykinin NK2 receptor by a change in their intracellular localization. Previously we have presented the functional characterization of one of these proteins, REDD1, that translocates to the plasma membrane.

Here we report the results of the entire screening. The process of cell activation was recorded on videos at different time points and all the videos can be visualized on a dedicated website. The proteins BAIAP3 and BIN1, partially translocated to the plasma membrane upon activation of NK2 receptors. Proteins ARHGAP12 and PKM2 translocated towards membrane blebs. Three proteins that associate with the cytoskeleton were of particular interest : PLEKHH2 rearranged from individual dots located near the cell-substrate adhesion surface into lines of dots. The speriolin-like protein, SPATC1L, redistributed to cell-cell junctions. The Chloride intracellular Channel protein, CLIC2, translocated from actin-enriched plasma membrane bundles to cell-cell junctions upon activation of NK2 receptors. CLIC2, and one of its close paralogs, CLIC4, were further shown to respond with the same translocation pattern to muscarinic M3 and lysophosphatidic LPA receptors.

This screen allowed us to identify potential actors in signalling pathways downstream of G protein-coupled receptors and could be scaled-up for high-content screening.

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Web page : http://www.gfpcdnalive-gpcr.cnrs.fr/

Black currant juice induces a redox-sensitive pro-apoptotic effect on leukemia Jurkat cells

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Abstract

Black currant, a rich source of anthocyanins, has been reported to exhibit an antiproliferative effect on several types of solid tumor cancer cells, including mammary, colon and hepatocellular carcinoma, but the active molecules and the precise mechanism of action remain unclear. The aim of the present study was to investigate the anticancer effect of black currant-derived products (juice (BCJ), extract (BCE) and anthocyanins) in Jurkat cells, and, if so, to characterize the underlying mechanism. Cell cycle and apoptosis were assessed by flow cytometry, the formation of reactive oxygen species by dihydroethidine, and protein expression by Western blotting. BCJ and BCE inhibited the proliferation and induced G_2/M phase cell cycle arrest and apoptosis in Jurkat cells associated with an increased expression of p73 and caspase 3, dephosphorylation of Akt and Bad, and down-regulation of UHRF1 and Bcl-2. A pro-apoptotic response was also observed in response to two major black currant anthocyanins, delphinidin-3-*O*-rutinoside and delphinidin-3-*O*-glucoside. The BCJ- and BCE-induced activation of caspase 3 was markedly inhibited by pretreatment with the antioxidant N-acetyl cysteine. BCE and the two active anthocyanins induced the formation of reactive oxygen species.
BCJ and its' major anthocyanins induced a redox-sensitive caspase 3-dependent apoptosis in Jurkat cells, involving a dysregulation of the Akt/Bad/Bcl-2 pathway.

Super resolution imaging of DNA using PAINT (Point Accumulation for Imaging in Nanoscale Topography)

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Imaging biological samples beyond the diffraction-limited resolution of conventional fluorescence microscopy has gained strong interest in recent years [1]. Optical imaging of single double-stranded DNA (dsDNA) molecule have been recently performed [2, 3].

Herein, we used Point Accumulation for Imaging in Nanoscale Topography (PAINT) technique to image Lambda phage DNA. PAINT is based on pinpointing the positions of single emitting fluorogenic probes, which are poorly emitting when freely diffusing in the sample but strongly enhancing their fluorescence emission upon binding to the target object [4]. We *PAINTed* DNA with different dyes to investigate their ability to generate super-resolution images and we developed analytical tools to provide a quantitative comparison of the photophysical properties of a large set of commercially available intercalating dyes. We also tested the effect of different imaging buffers on the imaging of DNA using YOYO-3 dye. We were able to achieve the pointing precision of 15-20 nm leading to a cross sectional FWHM (Full width at half maxima of Gaussian peak) of the dsDNA down to 50 nm, showing achievement of super resolution images of DNA.

The methodological approach we developed for obtaining super resolution images of DNA could be used in various biological application including DNA reparation, changes in DNA topology during interaction with proteins, characterization of artificial DNA nanostructures and nucleic acid packaging.

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Direct *in vivo* cell reprograming: when the food intake influences a natural cell reprogramming event in *C. elegans*

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While most cells acquire and maintain a differentiated identity and function during their lifespan, it has become clear in recent years that some cells can change their identity. This can occur not only after an experimental trigger, but also naturally. Indeed, we have described such a natural occurance of natural cell-type conversion, where rectal cell becomes a functional moto-neuron during the larval development of the nematode *C. elegans*. For this specific cell, transdifferentiation always occurs in 100% of WT worms at a precise time point. This process is a powerful model to describe in detail mechanisms responsible of direct cell reprogramming in a physiological context, at a single cell level.

Numerous genes involved in this direct reprogramming were discovered through genetic screens in the lab. During my PhD I cloned *lin-15A,* which encodes a zinc finger protein that binds nucleic acids, as an important gene to allow this rectal cell conversion. In *lin-15A* mutant, half of the worms present a transdifferentiation defect where the rectal cell does not convert and remains rectal during all the worm life. Surprisingly, food deprivation during larval development restores the transdifferentiation in *lin-15A* mutant. We have further identified genes which loss-of-function mutations can also lead to suppression of lin-15A transdifferentiation defects. Our hypothesis is that these genes represent a genetic pathway that counteracts cellular plasticity. The fact that both food deprivation and loss of these genes activity both lead to suppression of *lin-15A* defects suggests that they could either impact the same targets, or that caloric restriction activates a dedicated pathway that results in inactivation of these genes.

Our work aims at understanding how food deprivation impacts transdifferentiation, and its possible link with the genetic pathway that counteracts transdifferentiation.

Kinetic profiling of chemokine and chemokine receptor mRNA in a rat model of left ventricular hypertrophy

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Introduction. Left ventricular hypertrophy (LVH) is a risk factor for heart failure or death, involving cardiomyocyte growth, interstitial cell proliferation, inflammation and collagen deposition. Chemokines, interacting with GPCR chemokine receptors, attract and activate leukocytes to inflamed tissues. We hypothesised that chemokines could play a role in the development of LVH by promoting the recruitment of activated leukocytes leading to inflammation and by modulating the left ventricular remodelling during LVH.

Method. Male Lewis rats (100±10g) were randomly assigned to undergo banding of the suprarenal abdominal aorta (aortic banding, AB) or sham operation for 0, 1, 3, 5, 7 and 14 days (n=6). LVH development was assessed by the heart weight/body weight ratio (HW/BW), and by the surface and the cross-sectional thickness of left ventricles (LV) on paraffin-embedded sections stained with hematoxylin-eosin. Collagen was quantified by densitometry after Sirius Red staining. Integrity of RNA extracted from LV was assessed using the Bioanalyzer 6000 Nano (Agilent). Expression of 96 chemokines, chemokine receptors, cytokines and growth factors was quantified by qPCR using the Taqman Low Density Array (Applied Biosystems). Fold change was shown as $2^{-\Delta\Delta Ct}$ for AB *vs* sham.

Results. HW/BW progressively increased over time in AB *vs* sham (by 20.4±2.6% at D14, $p<0.05$). The LV surface, cross-sectional LV thickness and collagen deposition were increased in AB at D14 by 18.2±4.6%, 14.9±4.3% and 76.7±30.1% ($p<0.05$ for each), respectively. The positive control ANP was overexpressed in AB (26.1±12.6-fold at D14, $p<0.05$). As examples of the expression profile of all analysed 96 genes, chemokines CCL2, CCL3, CCL4 and chemokine receptors CCR1, CCR2, CCR5 were overexpressed in AB *vs* sham during the inflammatory phase of LVH development (D3-D5). CCL2 showed 13.3±8.9-fold overexpression at D3 and 2.5±0.5-fold at D5 ($p<0.05$ for both). IL-1β and NLRP3 expression was increased in this phase in AB rats, confirming an inflammatory phase. Later, during the hypertrophic phase (D7-D14), CCL19 and CCL21 showed overexpression (1.7±0.1-fold overexpression at D7 for CCL21, $p<0.05$). This phase is marked by increased TGFβ and collagen expression. Interestingly, CXCL16 and CX3CR1 expression was increased throughout the inflammatory and hypertrophic phases.

Conclusion. We describe the chemokine and chemokine receptors expression profile in rat LVH development over time. This kinetic study highlights the chemokines and receptors involved in the early inflammatory (D3-D5) and the later hypertrophic (D7-D14) phases of LVH development. These results can help understanding some of the molecular and cellular mechanisms underlying the development of LVH.

Characterization of pharmacological Dyrk1A kinase inhibitors for therapeutic use in Down Syndrome models

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The Down Syndrome or Trisomy 21 (DS), is due to an extra copy of the chromosome 21, and is the most frequent mental retardation which affects about 1 new born per 700 births.

Among the candidates implicated in DS intellectual disabilities, the Dual Specificity Tyrosine Phosphorylation regulated Kinase, Dyrk1a, found in the DS critical region of chromosome 21, is one of the most relevant. Indeed, several studies have shown a correlation between an increase of its kinase activity and the intellectual defects observed in DS models.

In order to go further understand the mechanisms underlying the impact of Dyrk1a dosage on the cognitive alterations, we used different trisomic mice models expressed Dyrk1a alone or with additional Hsa21 homologous genes and specific Dyrk1A inhibitors from ManRos Therapeutics.

We will present here the consequence of the treatment using Leucettine 41, a synthetic Dyrk1A inhibitor, when administered to several DS mouse models on the behaviour and cognition and on several activities of Dyrk1a. Further analysis of the phosphoproteome of DS mouse models treated or not with L41 unravels a few targets and pathways which are involved in the restoration of cognitive capacities of DS models. These results supported the potential of Dyrk1A inhibitor in therapeutic approach to ameliorate cognitive function in DS patients.

Synthesis of natural alkaloid cernumidine from L-arginine

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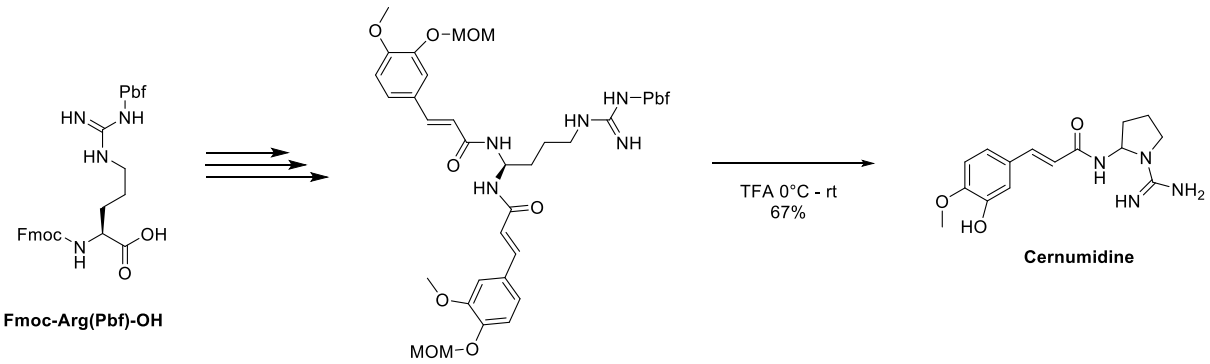
A cyclic guanidine alkaloid named cernumidine has recently been identified from extract of dried leaves of solanum cernuum vell., a endemic Brazilian medicinal plant. In vitro it was demonstrated that cernumidine decreases the generation of interleukin-8 with up to 50% reduction at a 50 mM concentration on colorectal carcinoma cells (HT-29).

Herein, we present an original synthetic route to prepare cernumidine from commercially available Fmoc-Arg(Pbf)-OH, the key reaction was consisted of deprotection-cyclisation of N,N-alkylidene-bis-amides in TFA to provide the cernumidine in good yield.



Cellular and Molecular Characterization of New anti-HIV-1 Molecules Targeting The NucleoCapsid Protein

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The Human Immunodeficiency Virus (HIV) is responsible of the Acquired Immunodeficiency Syndrome (AIDS), one of the most serious pandemic diseases of the modern era. Although current therapies targeting key processes of the HIV replication cycle are potent and selective, several clinical failures appeared due to the emergence of drug resistance. Hence, there is an urgent need for novel drugs and alternative therapeutic strategies. The main objective of the THINPAD project is to develop novel anti-HIV agents targeting the HIV nucleocapsid protein (NC), which plays key roles in HIV replication and is one of the most conserved within HIV strains. THINPAD aim is to screen by a virtual screening a chemical library for their potential interaction with NC and then combine *in-vitro* and *in-vivo* assays to test the antiviral activities of the selected hits. Our *in-vivo* infectivity-assay is based on the ability of the selected hits to inhibit the infection of HeLa cells by HIV-1 pseudoparticles mimicking the early phase of the virus infection cycle. In parallel, the cytotoxicity of the tested compounds is quantified using an MTT assay. This allows determining both the IC_{50} (Inhibitory Concentration at 50% of effect) and the CC_{50} (Cytotoxic concentration at 50%). So far, we tested around sixty molecules and the best IC_{50} determined is 0.3µM. In order to decipher the mechanism of action of the best hits, two assays were set up to identify which step of the viral infection cycle was targeted. First, the time of addition assay (TOAA) is a time-based approach which determines how long the addition of a compound can be postponed before losing its antiviral activity [1]. The step targeted by the tested molecule can be identified by comparing its relative position in the time scale to that of reference drugs. The second assay is based on the quantification of late Reverse Transcriptase products and DNA integration by quantitative PCR (qPCR) [2]. The relative proportions of these products can identify which step of the reverse transcription or integration process is inhibited. Finally, a third assay is under development to monitor the effects of the selected hits on Gag polyprotein assembly. To do so, we developed a FRET-FLIM based assembly assay which monitors Gag multimerization in transiently transfected cells [3]. This assay should inform us whether the selected hits can alter the assembly of Gag oligomers, their compaction, as well as their trafficking toward the plasma membrane.

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Pharmacological characterization of NPFFR1/GPR147 antagonists for the study of its role in the central control of reproduction

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Control of reproduction is driven by GnRH (Gonadotropin Releasing Hormone), which represents the main positive regulator of the reproductive system. Downstream, GnRH is able to stimulate synthesis and secretion of gonadotropins LH (Luteinizing Hormone) and FSH (Follicle-Stimulating Hormone), which will act on peripheral reproductive organs as ovaries. RF-amide peptides, characterized by their Arg-Phe-NH_2 C-terminal, and their G-protein coupled receptors have been demonstrated to be involved in the central control of reproduction. Notably, Kisspeptin/GPR54 system represents a potent activator of reproductive axis, stimulating GnRH neurons. On the other side, RF-amide related peptides (RFRPs)/NPFFR1 system seems to emerge as an inhibitor of reproduction in different species, including mammals. Especially, an implication of this system has been described in seasonal animal models as hamsters.

However, the study of the involvement of RFRPs/NPFFR1 system in reproduction is severely limited by the absence of pharmacological tools, particularly antagonists that selectively block NPFFR1 and not the other RF-amide receptors including NPFFR2 subtype and GPR54.

In collaboration with a team of medicinal chemists from Faculty of Pharmacy (Illkirch), we screened in binding assay with NPFFR1 and NPFFR2 receptor subtypes a large focused chemical library of compounds (> 2000 molecules) that mimic the RF-amide sequence, which is critically involved in the binding of RF-amide peptides to their receptors. From this screening we identified several derivatives that display nanomolar affinity on NPFFR1 and very weak binding potency on the other RF-amides. NPFFR1 and NPFFR2 are G_i coupled receptors, which prevents the accumulation of cAMP induced by G_s protein activation. We thus look at the effect of the identified compounds on inhibition of the cAMP accumulation in HEK293 cells stably expressing both receptors and GloSensor in order to evaluate their antagonist or agonist nature. From this study we have identified several compounds that efficiently block, in a dose-dependent manner, the inhibition of cAMP accumulation induced by activation of NPFFR1 but not NPFFR2.

Altogether, our results indicate that we have identified potent and selective antagonists of NPFFR1 that will be very helpful to elucidate the role of RFRPs/NPFFR1 system in the central control of reproduction in different models as mice or seasonal animals like hamsters and ewes.

Deciphering the functional and molecular differences between MTM1 and MTMR2 to understand two neuromuscular diseases

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My laboratory is working on the **MTM1** and **MTMR2** proteins that are involved in two distinct human pathologies, a **myopathy** and a **neuropathy**. MTM1 and MTMR2 belong to the myotubularin family and are **very similar**, but we still do not know why they lead to two different diseases.

Our recent results show that in yeast cells, **MTM1 and MTMR2 lead to different vacuolar morphologies** linked to **different subcellular localizations**: MTM1 is associated with membranes and its in vivo phosphatase activity induces a vacuolar enlargement, whereas MTMR2 is not associated with membranes and thus cannot dephosphorylate its lipid substrates and the yeast vacuoles remain fragmented. Interestingly, the **deletion of an N-terminal motif of MTMR2 restores the MTM1-like phenotype in yeast cells**. Thus, this specific motif seems to be **involved in functional differences between MTM1 and MTMR2**, opening a complete new field of investigation.

Based on this, the aim of my PhD project is to :

1) determine if the expression of the truncated-MTMR2 **restores the XLCNM-like phenotypes of the Mtm1 KO mice**. In parallel, I will study the **phosphorylation of MTMR2** on a serine residue close to the N-terminal motif of MTMR2. This phosphorylation has been shown to have an **impact on the localisation** of MTMR2 in HeLa cells, and our preliminary results in yeast cells are very promising. The next step is to study all variants of MTMR2 in **C2C12 cells** to see their effect on myoblasts and on the differenciation into myotubes.

2) identify a **potential therapeutic drug** able to render MTMR2 more similar to MTM1 by using a yeast-based screening of chemical libraries. Candidate drugs could then be used on XLCNM patient's muscles, where modified MTMR2 would compensate for non-functional MTM1 and thus **restore a normal muscle organization and contractility**. This achievement would constitute a feasible low-cost therapeutic strategy for this severe neuromuscular disease.

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Intake of an optimized omega 3 formulation EPA:DHA 6:1 prevents the angiotensin II-induced hypertension and endothelial dysfunction in rats

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Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to cause endothelium-dependent nitric oxide (NO)-mediated relaxations of isolated blood vessels, with an optimized ratio of EPA:DHA 6:1. The aim of the present study was to determine whether chronic intake of EPA:DHA 6:1 affects hypertension and endothelial dysfunction induced by angiotensin II (Ang II).

Male Wistar rats daily received 500 mg/kg of either EPA:DHA 6:1 (omega 3) or corn oil (control) for 5 weeks. After 1 week, rats underwent sham surgery (sham rats) or surgery with implantation of an osmotic mini-pump infusing Ang II (0.4 mg/kg/d) for 4 weeks.

Infusion of Ang II to rats induced a pronounced increase of systolic blood pressure, which reached 215.6 ± 8.5 mmHg compared to 136.8 ± 6.2 mmHg (n = 8) in the control group after 21 days. The hypertensive response to Ang II was markedly reduced in the omega 3 group (169.0 ± 7.8 mmHg) whereas the omega 3 treatment alone had no effect (136.0 ± 4.2 mmHg). In second branch mesenteric artery rings, relaxations to acetylcholine (Ach) were markedly reduced in the Ang II group affecting the endothelium-dependent hyperpolarization (EDH)-mediated component more than the NO-mediated component. Pronounced endothelium-dependent contractile responses to Ach were observed in the Ang II group compared to Control, which were abolished by a cyclooxygenase inhibitor. Chronic intake of EPA:DHA 6:1 prevented the Ang II-induced endothelial dysfunction both by improving the NO- and EDH-mediated relaxations and by reducing endothelium-dependent contractile responses to Ach.

The present findings indicate that chronic intake of EPA:DHA 6:1 prevented the development of hypertension and endothelial dysfunction induced by the infusion of Ang II to rats. The beneficial effect of EPA:DHA 6:1 is mediated by an improvement of both the NO- and the EDH-mediated relaxations as well as a reduction of endothelium-dependent contractile response.

In vivo study of Bin1, a novel and major gene predisposing to late-onset Alzheimer.

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Alzheimer's disease, characterized by dementia and memory loss, is the most common age-related neurodegenerative disorder. Indeed we can estimate more than 1,2 billion people will be affected in France in 2020. Although it has been already demonstrated that some gene mutation as in APP, PS1 and PS2 are responsible for the establishment of the Earl Onset Alzheimer's Disease and represent less of 10% of the cases, the causes for the Late Onset Alzheimer's Disease remain unclear.

We know that some risks factors such as the age, education, socio-professional activities can be determinant. Since several years genetics risks have been introduced like the ApoE4 loci. Recently genome-wide association studies linked the gene *BIN1* (Brinding Integrator 1) with this pathology. The BIN1 gene codes for the Amphiphysin 2 and is involved in endocytosis, trafficking and membrane remodelling. This gene, known to be involved in centronuclear myopathy, was also found by Chapuis *et al*, overexpressed in Alzheimer's patients brain and linked with the modulation of Tau aggregation in *drosophila* in 2013.

Our hypothesis is that BIN1, by its interaction with Tau, could enhance the propagation of the disease and constitutes an important therapeutic target. Based on these observations we have generated a murine model which overexpressed the human BIN1 gene and the human MapTau gene. These mice, and the double crossed line, will be characterized by behavioural tests to determine anxiety, spatial and conditioned learning at 3, 6, 9 and 12 months. Histological and molecular experiments will be done, including biological markers of Alzheimer such as neurofibrillary tangles and amyloid deposits.

Identification of minor components in plant extracts: the HPLC-MS-SPE-NMR system.

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This study reports the identification of two compounds in an algal extract using recently acquired equipment: a HPLC system coupled with a Mass Spectrometer, a SPE system and a NMR spectrometer. The LC-MS-SPE-NMR hyphenation technique is a powerful analytical tool used to identify metabolites or impurities at low concentration in various matrices. The aim of this system is to separate molecules with the HPLC and, using one or more UV or MS signals, to concentrate these molecules on SPE cartridges and analyse them by NMR.

Plant extracts are complex mixtures of molecules. Consequently, working with crude extracts is tricky, small amounts are available, and conventional isolation methods are very difficult to carry out. Structural elucidation of pure compounds is often difficult. The traditional method for identifying molecules is time and sample consuming. The coupling method showed is very helpful for plant extracts.

A crude extract is directly injected in the HPLC system. After chromatographic separations, a flow splitter is used to create split ratio from 95/5 (DAD detector/MS detector). Selected chromatographic peaks are trapped. In order to concentrate samples, several injections are performed and trapped on the same cartridge. Trapped peaks are dried with nitrogen gas and eluted with deuterated acetonitrile into a NMR spectrometer equipped with a capillary probe.

All standards NMR experiments are available, limitations lies in the Signal-to-Noise ratio. Only few 2D experiments may be recorded on very low-concentrated analytes.

This coupling system allows separation, concentration and characterization of minor compounds in crude extracts. With this technique, purification and separation times are significantly reduced. Using the coupling system is faster than traditional method, brings more structural information, and enables characterization of minor analytes.

Identification of novel myopathy genes and functional validation

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Congenital myopathies are severe inherited neuromuscular diseases. There are several forms of congenital myopathies that can be distinguished by particular histological abnormalities in muscle biopsies. The diagnosis is made from the results of the histopathological features and at the molecular level. Knowing the mutated gene is important for a better caring of the patient, genetic counseling and new therapeutic perspectives but also for studying pathological mechanisms. Unfortunately, about 40 % of the patients do not have a genetic diagnostic. The goal of my project is to identify new genes implicated in congenital myopathies and to validate the impact of the mutations on protein function. The strategy that will be used is first, to analyze data from whole-exome sequencing of patients excluded for mutations in known genes and family members by using a bioinformatic pipeline that has been validated before. Then, the impact of the mutations will be assessed in patient's cells or in muscle cells transfected with a cDNA carrying the identified mutation or by knock-down. The functional studies will be diversified, including localization and expression studies but also interaction with other proteins and other experiments according to the function of the studied protein. The importance of the new genes identified for the muscle function will also be assessed in animal models (zebrafish and/or mouse) with *in vivo* experiments like force measurement and molecular experiments to understand the pathological mechanisms of the mutated genes.

Metal-catalyzed amide reduction for the synthesis of non-natural basic amino acids

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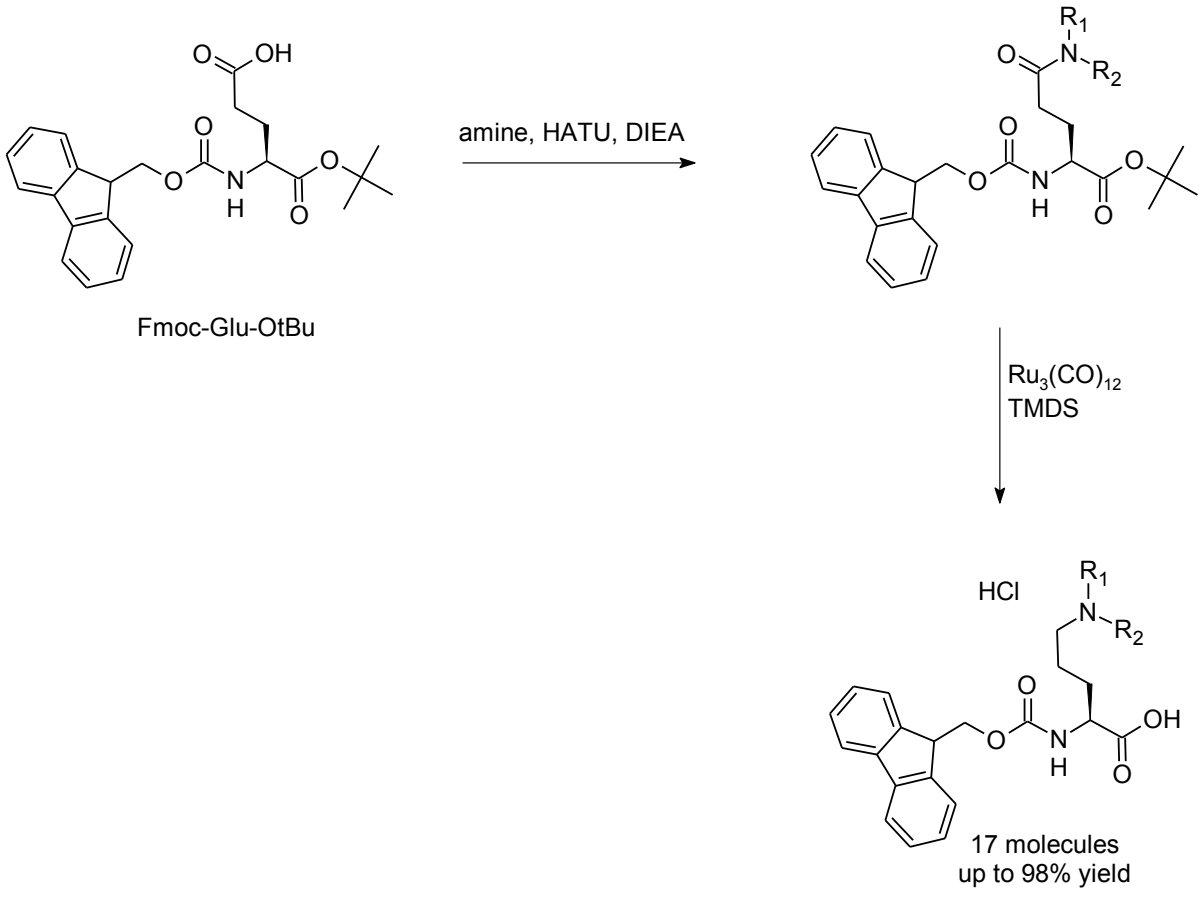
In the peptide there are only three natural basic amino acids (Arginin, Lysine, Ornithine), thus spacial exploration of the basic side chain is limited. That is why synthesis of non-natural basic amino acids would provide diversity, readily available to prepare new peptidomimetics which may improve interaction or selectivity with targeted protein.

Our work reports an efficient and selective Ruthenium-catalyzed amide reduction allowing the rapid production at the gram-scale, of original non-natural basic amino acids, readily available for liquid- or solid-phase peptide synthesis.



Physiopathology and preclinical approaches in centronuclear myopathies

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Keywords: centronuclear myopathies, Dynamin2, Myotubularin1, BIN1, gene therapy

Centronuclear myopathies (CNM) are a group of rare severe muscle diseases characterized by muscle weakness. Muscle biopsies from CNM patients show hypotrophic fibers with centralized nuclei. Three forms have been described: X-linked, Autosomal-recessive (AR) and Autosomal-dominant forms caused by mutations in Myotubularin (MTM1), Amphiphysin 2 (BIN1) and Dynamin 2 (DNM2) respectively. However, the physiopathological mechanisms are barely understood and no specific therapy is available.

Using a novel “cross therapy” approach, our group has shown that genetic reduction of Dnm2 in the *Mtm1* Knock-out (KO) mice restores a normal lifespan (from 1-3 months to 2 years) with improved muscle structure and function. Therefore, we aim to translate this proof-of-principal experiment by reducing Dnm2 expression using deliverable agents. Several shRNA sequences that target specifically mRNA were selected and screened on human embryonic Kidney (HEK) and/or mouse C2C12 myoblasts. The best candidates were then injected using AAVs into wild-type and *Mtm1* KO mice to confirm the reduction of Dnm2 detected in cells.

The overall goal is to understand the physiopathology of CNM, and to test different novel therapeutic approaches in preclinical trials.

Controlled switching of fluorescent organic nanoparticles

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Fluorescence Resonance Energy Transfer (FRET) based assays are widely used in biological research and their sensitivity can be drastically improved using ultrabright nanoparticles (NPs). Recently developed highly fluorescent and biodegradable polymer 40 nm NPs encapsulated with specially designed rhodamine B derivative dyes were ~6 times brighter than quantum dots[1]. Moreover, it was shown that the encapsulated dyes underwent cooperative blinking under the microscope due to fast dye-dye communication, which suggested that we could use these particles as efficient FRET donors. For this propose we co-encapsulated single acceptor molecules (Cy5 derivative) together with hundreds of donor dyes. And we found that only one acceptor gives FRET efficiency > 60% for NPs containing more than 700 donor dyes, which was accompanied with > 200-fold amplification of acceptor intensity. Single particle microscopy (TIRF) and fluorescence correlation spectroscopy (FCS) revealed a complete ON/OFF switching of the NPs emission by a single acceptor. This efficient FRET was used to create photoswitching NPs, where photochromic molecule served as an energy acceptor. That allows obtaining switching of NPs up to 19-fold, compared with 2.5-fold photoswitching for NPs without cooperative dye behavior. The high efficiency of NPs as an energy donor with consequent amplification of acceptor signal allowed us to detect binding of single acceptor molecule (Cy5 conjugated with biotin) to NPs surface, as well as its unbinding due to the desorption by streptavidin. The obtained results suggest these NPs as very efficient FRET donors in the construction of FRET-based nanoprobes for detection of biomolecules with single molecule sensitivity.

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PCBIS : Chemical libraries, biological models, technological tools and early ADMETox for laboratories

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High Throughput Screening (HTS) is the technology which best facilitates the search of new molecules with the potential of becoming the drugs of tomorrow. Until recently this expensive technology was only available in pharmaceutical companies.

Starting 15 years ago, the "Plate-forme de Chimie Biologique Integrative de Strasbourg" (PCBIS) developed the expertise in this field in order to be able to offer this technology in an academic context. One of our main goals is to offer our expertise to laboratories aiming to find new drugs to cure rare and/or neglected diseases. Our commitment to quality drove us to set up a quality management system granted by ISO 9001 and NFX 50-900 certifications.

The PCBIS's expertise and equipment necessary for new drug discovery is now proposed to academic laboratories, start-ups and industries interested in a fast paced approach to screening.

We also propose to train people and give an access to our technologies to interested laboratories.

We will show some of the tools that PCBIS can propose to the scientific community.

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t-BuXPhos: a highly efficient ligand for Buchwald-Hartwig coupling in water

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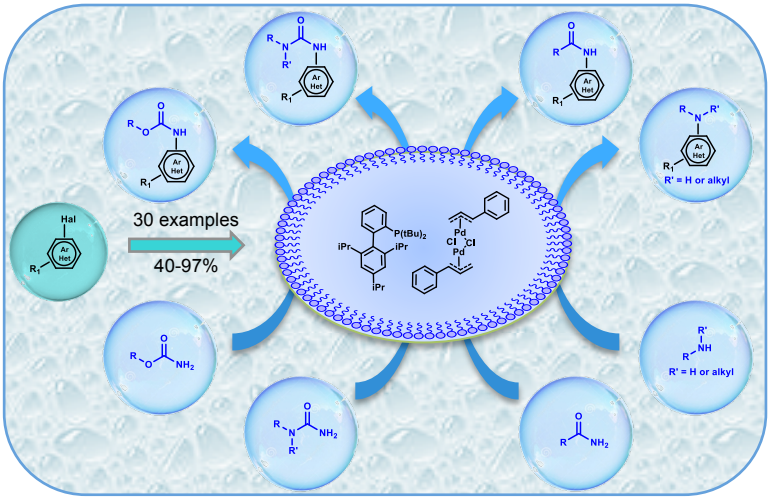
Actually, one of the most important challenges of the chemical industry is to produce compounds by using more environmentally friendly process. Although some chemicals reactions are already successfully developed in water, in general manner, the poor solubility of organic reagents and catalysts is as a strong limitation of this approach. To overcome this, it's possible to add into the media a small amount of surfactant, because thanks to their amphiphilic properties, they spontaneously gathered in a micellar form, acting like a nanoreactor. Since 2008, Lipshutz *et al.* have published a series of papers proving this concept for many transition-metal-catalyzed cross-coupling reactions, such as metathesis, Suzuki-Miyaura, Heck and Sonogashira reactions, using TPGS-750-M, a vitamin E derivative, as surfactant.

More recently, Lipshutz *et al.* have been rapidly expanding the range of application to the *N*-arylation Buchwald-Hartwig reaction. This reaction is important, because nitrogen-containing heterocyclic compounds are of considerable biological and chemical significance. In particular, Lipshutz and co-workers demonstrated that Takasago's cBRIDP ligand in combination with $[(allyl)PdCl]_2$ generates a highly catalytic system for aniline derivates and protected NH groups (carbamates, sulphonamides or ureas). However, this ligand failed when alternative amine classes are employed (benzamides, primary amines). This led us to explore other reaction conditions in order to broaden this scope of Buchwald-Hartwig reaction under micellar conditions. In particular, after an extensive ligand – palladium-catalyses – base screening, the combination *t*-BuXPhos – $[(cinnamyl)PdCl]_2$ – *t*-BuONa was identified as an universal palladium-based catalytic system for the *N*-arylation reaction. When applied to the synthesis of a known $NaV_{1.8}$ modulator, this method led to a significant improvement of the E-factor in comparison with classical organic synthesis.



The EPA:DHA 6:1-evoked endothelium-dependent NO-mediated relaxation in the coronary artery involves a copper-dependent pro-oxidant response triggering the PI3-kinase/Akt-mediated activation of eNOS

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Omega-3 fatty acid products containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to reduce the risk of cardiovascular disease, in part, by stimulating the endothelial formation of nitric oxide (NO), a potent vasoprotective factor. This study determined the mechanism leading to endothelial NO synthase (eNOS) activation in response to the highly active EPA:DHA 6:1 product.

Vascular reactivity was assessed using porcine coronary artery rings suspended in organ chambers, the level of oxidative stress in coronary artery sections using the redox-sensitive probe, dihydroethidine, and the phosphorylation level of target proteins in cultured coronary artery endothelial cells by Western blot analysis.

EPA:DHA 6:1 caused pronounced endothelium-dependent relaxations in porcine coronary artery rings. Relaxations to EPA:DHA 6:1 were slightly but significantly reduced by an eNOS inhibitor, not affected by inhibition of endothelium-dependent hyperpolarization and abolished by both treatments. Relaxations to EPA:DHA 6:1 were reduced by inhibitors of oxidative stress (MnTMPyP, PEG-catalase), an inhibitor of either Src kinase (PP2) or PI3-kinase (wortmannin), and intracellular copper chelating agents (neocuproine, tetrathiomolybdate) and were insensitive to cyclooxygenase inhibition (indomethacin), chelating agents for iron (desferroxamine), zinc (histidine), extracellular copper (bathocuproine). EPA:DHA 6:1 induced phosphorylation of Src, Akt and eNOS at Ser 1177; these effects were inhibited by MnTMPyP and PEG-catalase. EPA:DHA 6:1 induced the endothelial formation of ROS in coronary artery sections, this effect was inhibited by MnTMPyP, PEG-catalase, and intracellular copper chelating agents.

EPA:DHA 6:1 causes endothelium-dependent NO-mediated relaxations in coronary artery rings, and this effect involves an intracellular copper-dependent event triggering the redox-sensitive PI3-kinase/Akt pathway to activate eNOS by phosphorylation at Ser 1177.

Interaction of the HIV-1 nucleocapsid protein (NCp7) with the cellular protein NoL12: molecular and functional aspects

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The Human Immunodeficiency Virus-1 (HIV-1) nucleocapsid protein as a mature protein (NCp7) or as a domain of the polyprotein Gag plays several important roles in the viral life cycle, including virus assembly, viral genomic RNA encapsidation, primer tRNA placement, and enhancement of viral reverse transcription. NCp7 is a nucleic acid (NA) chaperone protein with two highly conserved zinc fingers. Due to its high conservation and its key roles in the viral cycle, NCp7 stands out as a promising target for anti-viral drugs. In order to find new clues to develop anti-NCp7 drugs, it is crucial to identify cellular proteins interacting with NCp7 and to understand the role of their interaction during the HIV-1 replication cycle.

Using co-immunoprecipitation, we have identified the protein NoL12 as an NCp7 protein partner. Human NoL12 (hNol12 called also Nop25 in the rat, Rrp17p in the yeast, viriato in *Drosophila*) is a 25kDa nucleolar RNA-binding protein highly conserved in mammals, yeast, and drosophila for which only a few data are available. It was described, in human and yeast, to be implicated in the processing of ribosomal RNA (rRNA) in the nucleolus and thus in the assembly and maturation of ribosomes.

Rrp17p, is an eukaryotic exonuclease involved in 5´-end maturation of the 60S subunit rRNAs, it accompanies the 60S subunit from the nucleolus to the nuclear periphery and even to the nuclear pore. The knockdown of Rrp17p is lethal for the yeast and can be rescued by hNoL12 expression. Hence, it is highly possible that hNoL12 shares some or all its cellular functions with Rrp17p.

Using confocal microscopy, hNoL12 and NCp7 were found to colocalize in the nucleolus. The hNoL12/NCp7 interaction was confirmed by FRET-FLIM, co-immunoprecipitation and in a yeast two-hybrid assay. The interaction domain was mapped, using deletion mutants of hNol12 tested for their co-immunoprecipitation with NCp7, to be localized on the exonuclease domain of hNoL12. The functional involvement of this interaction was underlined by the fact that a knockdown of hNoL12 in HeLa cells leads to a decreased infection by a non-replicative HIV-1 lentivirus.