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Efficient encapsulation of cyanine dyes with minimized self-quenching: bright fluorescent polymer nanoparticles

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Among the various fluorescent tools for bioimaging, fluorescent nanoparticles are particularly attractive due to their high brightness and capacity to bear multiple functional units. However, their main problem is aggregation caused quenching (ACQ) of fluorophores encapsulated at high concentrations, which limits their brightness.

As it was recently reported by our group, bulky hydrophobic counterions can strongly decrease ACQ of cationic alkyl rhodamine B in polymer nanoparticles and improve the dye loading.[1, 2] Such counterions not only separate dyes from each other, decreasing quenching, but also assemble them into supramolecular structures exhibiting cooperative behavior due to exciton diffusion.

In the current work we checked whether the counterion concept could be extended to other cationic dyes. We have chosen cyanine dyes, which represent the dye family that covers probably the largest spectral range from green to NIR while exhibiting very large absorption coefficients. Dioctadecyl-substituted cyanines were used, where an inorganic counterion was replaced with different bulky counterions of the tetraphenylborate family with different levels of fluorination. The obtained salts were encapsulated at concentrations up to 50nmol/mg (dye/polymer) into 40-nm nanoparticles of the biodegradable polymer poly(lactide-*co*-glycolide) (PLGA). It was found that counterions can improve the fluorescence quantum yield of cyanines loaded at >100 molecules per particle. Importantly, the most promising counterions were those with the largest size and highest fluorination level, whereas highest quantum yield was observed for cyanines with shorter electronic conjugation chain. The obtained results suggest that the counterion approach is universal for the preparation of ultrabright dye-loaded NPs.

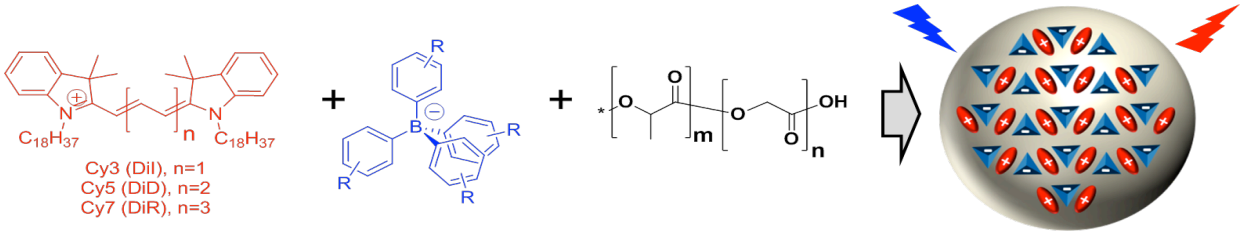

Figure. Scheme of preparation of ultrabright polymer nanoparticles loaded with cyanine dyes with the help of bulky counterion.

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Glucosyloxybenzyl derivatives of (*R*)-2-Benzylmalic acid from the aerial parts of *Arundina graminifolia* (D.Don) Hochr (Orchidaceae)

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Benzyl ester glycosides of natural organic acids such as citric [1], malic [2] or tartaric acid [3]are known to be specific secondary metabolites occurring in the orchid family. A rapid RP-HPLC-DAD-HRMS screening of the ethyl acetate extract from *Arundina graminifolia* aerial parts, suggested the presence of these compounds due to their high molecular weight as well as their MS fragmentation patterns [4].Thus, a multiple step fractionation from the ethyl acetate extract led to the isolation of seven new glucopyranosyloxybenzyl derivatives of the (*R*)-2-benzylmalic acid, named Arundinosides A-G. Their structures were elucidated by means of extensive spectroscopic experiments. The structure novelty has the acetic and cinnamic esters distributed over two sugars in Arundinoside A and B, whereas those functionalities were previously reported on the C-2 glucose only [5]. On the other hand that the (*R*)-2-benzylmalic acid, here regarded as the centrepiece of the backbone structure, could also be found on the C-2 glucose in Arundinoside C. This particular class of glycosides was detected in this well-studied Asian orchid for the first time.

Numerous studies have reported malic acid derivatives as promising drug agents for neurodegenerative diseases [6,7]. We therefore investigated the neuroprotective potential of these 2-benzylmalic acid glycosides on beta-amyloid-induced toxicity, using a PC12 cell model [8]. Only Arundinoside A showed an interesting neuroprotective activity. A comparison of structural similarities of the tested compounds suggests that cinnamic(s) ester(s) could play a major role for the activity of Arundinoside A.

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Characterization and functional insights into the basal transcription machinery during development

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The pre-initation complex formation requires RNA Pol II recruitment at the promoter by general transcription factors. However, different sets of core promoter complexes have been described and are associated to distinct subsets of genes (Muller et al, 2010). In particular, the multi subunit transcription factor IID (TFIID) which recognizes the core promoter sequences, including the TATA box by the TATA-binding protein (TBP), is not conserved in all cells (Maston et al, 2012; Deato et al, 2008; Zhou et al, 2013; Muller et al, 2013). Canonic TFIID complex is composed of TBP and 13 TBP associated factors (TAFs). Indeed, some cells present different sets of TAFs and can also lack the TAF10 subunit. TAF10 is a scaffold and ubiquitous protein, shared between TFIID and the coactivator SAGA. But, TAF10 has a differential requirement for the core promoter apparatus depending on the cellular context and the development stage. TAF10 is necessary for transcription during embryonic development but dispensable in the adult (Mohan et al, 2003; Taratakis et al, 2005; Metzger et al, 1999; Indra et, 2005). In order to characterize the features of the basal transcription machinery during development and differentiation, we developed *in vivo* and *in vitro* approaches based on *Taf10* conditional deletion. Both conditional, in the presomitic mesoderm, and ubiquitous deletion of *Taf10* in the embryo at E9.5, induce a growth arrest. *Taf10* deletion in mouse embryonic stem cells (mESCs) recapitulate the phenotype observed *in vivo* with a proliferation impairment, thus offering an interesting alternative model for further biochemical analyses. Moreover, *Taf10* seems to be required for TFIID and SAGA complete assembly in mESCS and its absence leads to a dramatic decrease of transcription initiation in mESCs. Current investigations aim to determine TFIID and SAGA composition during development and differentiation as well as the influence of *Taf10* deletion on developmental gene expression.

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Ultra small gold nanoparticles-antibody conjugates for imaging applications by high-resolution electron microscopy

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Electron microscopy (EM) and the high selectivity of binding of antibodies allow to map with high resolution target proteins inside cells. This approach requires to prepare opaque antibodies to electrons. These probes are generally obtained by conjugating antibodies with 6-10 nm gold particles, making them easily detectable by EM after cell fixation. However, such probes are not suitable for immunolabelling intracellular epitopes in the dynamic cell context. Our project is to develop an immunolabelling method for tracking antigens in living cells. More precisely, we are developing nanoparticle-antibody probes circumventing specific problems of this approach.

To allow diffusion within the cytosol, we have synthesized innovative 2 nm gold nanoparticles and studied their conjugation to an antibody directed against the nuclear protein RNA polymerase II.

The implementation of this project has been carried out in the following manner : $(Fab')_2$ fragments have been prepared from IgG by papain digestion. For the linkage of the antibody moiety to the 2nm gold particles, the disulfide bonds of the $(Fab')_2$ were reduced with TCEP and the Fab' fragments with reduced thiol groups were incubated with activated gold particles Our first results show that this new method of Fab'-gold particle conjugate preparation is simple to implement in the laboratory and does not affect the binding activity of the Fab'.

Development of inhibitor of phosphodiesterase 5 (PDE5) for the treatment of neuropathic pain

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Neuropathic pain is a chronic pain caused by damage or disease affecting the somatosensory nervous system. The major diseases concerned by those pains are diabetes, cancers and infectious diseases such as HIV and shingles. A survey estimated the prevalence of chronic pain with neuropathic characteristics to be around 6% of general population.[1] Nowadays, no specific treatment has been developed to cure this pain. Indeed, antidepressants and anticonvulsant drug are used, but they aren't effective for everyone, and they often cause important side effects.

Recent works in the literature highlighted the efficacy of sildenafil, a well-known inhibitor of phosphodiesterase 5 (PDE5, (CI_{50}=3,3nM)), in chronic constriction injury (CCI) model.of neuropathic pain.[2] Sciatic nerve injury is associated with the development of hyperalgesia 14-16 days after the nerve ligation. Sildenafil produced a significative decrease in pain threshold after 12 days of treatment. Based on this observation, another inhibitor of PDE5, the MY5445 (CI_{50}=0,6µM), has been tested by Dr. Barrot and showed the same effect. Encouraged by those results, a structure-activity relationship (SAR) study has been realized on the MY5445 as depicted in Figure 1. In particular we determine the importance of 1) the benzo group, 2) the possible substitution on both phenyl groups, 3) the homologation, and 4) ring opening leading to benzamide derivatives. Finally, starting from the benzamide **3** different semi rigid derivatives were prepared (Figure 2). The chemical access to the different compounds and the structure activity relationship of this study will be presented.

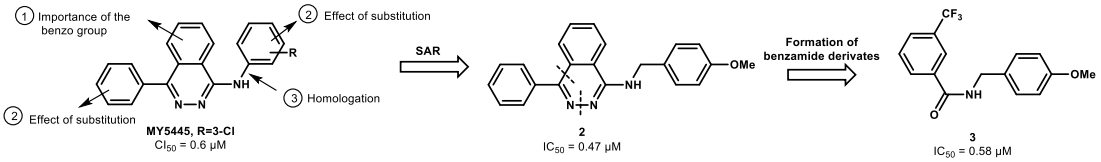

Figure 1 : SAR study

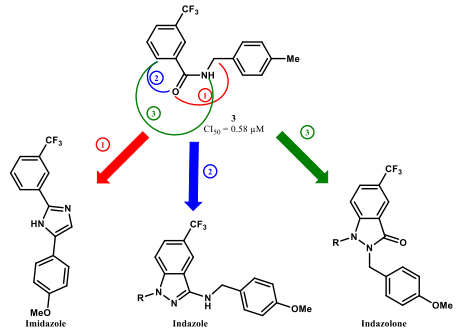

Figure 2 : Conception and preparation of semi rigid analogues of **3**

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Comparative Study of the Structural and Physicochemical Properties of Aza-DKP

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One of the main challenges in drug discovery is the identification of novel scaffolds for therapeutic applications, easy to modulate to optimize their biological and physicochemical properties. High throughput screening is nowadays one of the most efficient methods to find new leads in medicinal chemistry. However, chemical libraries are more often made up with aromatic compounds which are planar and with low stereochemical complexity.[1] These kinds of scaffold with high content of sp2 and sp hybridized carbons do not fit with the spatial configuration required for interaction with most of the biological receptors.[2] Therefore, there is a crucial need to enrich the chemical diversity of screening libraries to facilitate the discovery of new original drugs.

In this context, we herein describe a promising scaffold hopping of 2,5-diketopiperazines (DKP), a well-known and privileged scaffold in medicinal chemistry, affording underprivileged aza-diketopiperazines (aza-DKP).[3–5] As this original scaffold could offer new opportunities for chemical biology and drug discovery by exploring a new chemical space, we evaluated the potential of aza-DKP as a replacement of DKP for a drug discovery purpose in order to develop innovative molecules and improve their physicochemical parameters.

X-ray study and ^{1}H NMR analyses have shown that the structure of aza-DKP is rigid like its 2,5-DKP analog, which is an asset for drug discovery as it can substantially improve the binding affinity. Moreover, the 3-dimensional structure of aza-diketopiperazine is suitable for the introduction of several functional groups at four different positions. Noteworthy, the replacement of one C_{α}-stereogenic center by a nitrogen atom led to a scaffold with improved properties such as a significantly increased water solubility and microsomal stability drawing new promising avenues for the exploitation of aza-diketopiperazines in medicinal chemistry.

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Surface functionalization of 2nm gold nanoclusters for biomedical applications

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Gold nanoparticles may have biological applications.[1] Herein we are interested in the synthesis and evaluation of 2nm diameter gold clusters surrounded by a protected but still reactive layer of organic molecules. By using a novel thiolated molecule, we were able to prepare an aqueous-soluble and highly homogeneous nanocluster, which migrate in polyacrylamide gel as a single band. Besides its unique monodisperse property, this nanoclusters can be functionalized by reaction with thiolated molecules. Our objective is to evaluate the reactivity of the gold nanocluster toward thiolated compounds and to exploit its multi-anchorage potential.

At first, we evaluate, the kinetic of functionalization in aqueous conditions using glutathione as the thiolated molecules. Analysis of the experiments shows the reaction to be fast and 18 molecules can be attached per gold particles within 200 minutes. We also show that other peptides can be linked onto the surface and keep their functionalities. Altogether, these data demonstrated that this novel gold nanoparticle may be a scaffold for tethering multiple molecules and may lead to novel therapeutic or biomedical applications. We wish in particular to explore the use of this gold particle to prepare antibody-drug conjugates.

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

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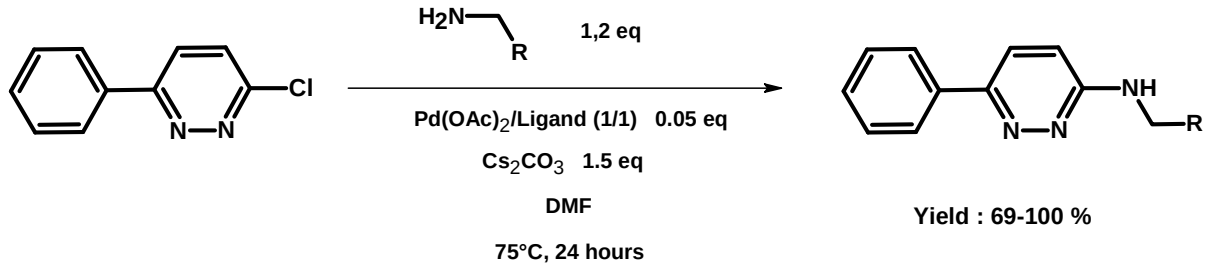
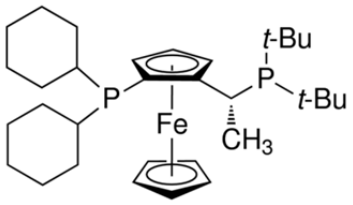
The 3-aminopyridazine derivatives have provided numerous pharmacologically active compounds acting as receptor ligands or enzyme inhibitors. Classical aminations of 3-chloropyridazines generally needs the use of a large excess of amine reagents, drastic conditions and give fair to moderate yields.

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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Detection of Faint Extended Sources in Hyperspectral Data and Application to HDF-S MUSE Observations

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Circum-Galactic Medium surrounding galaxies has been punctually detected, but its morphology remains largely unknown. The Multi-Unit Spectroscopic Explorer (MUSE) spectro-imager provides for the first time both spectral and spatial resolution to spatially map such features.

The problem lies in the statistical detection of faint spatially-extended sources in massive hyperspectral images such as provided by MUSE, and has not been previously handled.

We present a statistical detection method based on hypothesis testing tackling this problem. The proposed strategy is step-by-step validated over alternative ways with simulations. Then, results on MUSE observations are presented.

Optimizing upconverting nanoparticles for FRET-based assays

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The growing interest for luminescent nano-particles (NPs) is explained by their unique ability to act as nanoscale reporters, which find a tremendous number of applications in biological imaging and sensing. Even though these objects show brighter fluorescence and higher photostability compared to classical fluorescent organic dyes, their use in biological applications is still challenged by the background noise generated by the autofluorescence of surrounding media. To avoid this problem, the ideal strategy is to use lanthanide upconverting nanoparticles (UCNPs) that display anti-Stokes luminescence, where the emitted photons are of higher energy than the excitation photons [1]. Additional features that make them ideal nanoscale reporters include high photostability and non-blinking emission.

Among the applications found by luminescence in the life science, the process of Förster Resonance Energy Transfer (FRET) is a unique research tool [2], as it allows resolving distances in the 2-10 nm range. As a result, FRET can be used to quantify biomolecular interactions and conformational changes in biomolecules. The remarkable photophysical properties of UCNPs have been already exploited in FRET assays using various types of acceptors ranging from small organic dyes to semiconductor quantum dots [3]. In order to optimize UCNPs for FRET-based assays, methods to quantify their behavior on the level of individual particles must be developed.

In this work, we compare several methods to investigate the behavior of systems based on FRET between biocompatible water-solubilized UCNPs and organic dyes coupled to their surface. The results show that the behavior of these systems deviates from classical FRET model. We anticipate that this work will pave the way for usage of UCNPs in quantitative biological sensing, notably single-molecule assays.

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A Turn-On Dual Emissive Nucleobase Analogue Sensitive to Mismatches and Conformations of DNA

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Nucleic acids are the genomic templates, crucial for encoding proteins that are essential for all the cellular functions. Mutations or variations in the genome sequence produce malfunctioned proteins and cause diseases including cancer.[1-2] This kind of variation in the genome sequence is known as single nucleotide polymorphism (SNP), which occurs at every few hundred to thousand base pairs in genomic DNA. Besides, SNPs serve as markers for identifying disease causing genes for early diagnosis and treatment. Hence, the development of a simple and accurate detection of oligonucleotide mutations with high specificity and sensitivity is a burgeoning research area. Very recently we have reported a dual emissive hydroxychromone based nucleoside analogue (TCeU) sensitive to hydration and mismatches in DNA.[3]

Herein, we have come up with a new furyl based hydroxychromone derivative, FCeU, sensitive to SNPs and conformations of DNA. Solvent dependent emission study confirms that FCeU shows single emission band in water and dual bands in other solvents due to modulation of its excited state proton transfer (ESIPT) process. However, when it is incorporated in DNA, the probe exhibits different photophysical properties in single and double stranded DNA. The T* band which is not at all observed in water appears in ssDNA due to switching on of the ESIPT process. When the probe sequence is annealed with its complementary target sequence with varying opposite nucleobase, its T* band intensity is found to be sensitive to the opposite nucleobase. On the other hand this dual emission behavior is not seen in fully matched B form duplexes. Noticeably, this important T* band again reappears in the A form of DNA/RNA hybrid duplexes. This on-off-on ESIPT mechanism which is sensitive to hybridization, conformations and mismatches of DNA is very interesting and can be employed as a probe for in-depth understanding of enzyme induced structural and conformation changes in nucleic acids.

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Localization of HIV-1 protein NCp7 in the nucleolus investigated by Super-Resolution microscopy

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Nucleocapsid (NCp7) is a key protein of type 1 Human immunodeficiency virus (HIV-1), which is involved in both early and late stages of the virus life cycle. One of its most attractive features is the highly conserved structure among different HIV subtypes and viral strains that makes NCp7 a promising target for anti-viral drug development and therefore a subject of intense investigations [1]. Recently, confocal studies [2][3] revealed a strong accumulation of NCp7 in the nucleoli – site of ribosome synthesis, known to be targeted by many other viruses in order to promote their transcription/translation and even regulate the cell cycle [4]. The nucleolus is subdivided in three domains corresponding to different stages of ribosome biogenesis.

Hence, the aim of this work is to determine the precise localization of NCp7 in the nucleolus, using super-resolution microscopy technique PALM [5], in order to understand its roles and functions in this subnuclear compartment. To this end, HeLa cells were transiently transfected with constructs codding NCp7 tagged with the photoactivable fluorescent protein mEos2 and then the localization of NCp7-mEos2 was compared to the one of three markers of nucleolar domains.

The results suggest that nucleocapsid protein localizes mainly in the granular component of the nucleolus – place of the last stages of ribosome assembly and where numerous nucleolar and ribosomal proteins concentrate. Further colocalization and single particle tracking experiments have to be done to study the interaction of NCp7 with its possible nucleolar partners.

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CCR5 DIMER: CHARACTERISATION OF INTERFACE

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The type 5 C-C chemokine receptor (CCR5) is the major co-receptor of HIV-1. Although experimental data in the literature reveals evidences for CCR5 dimerization, the dimerization mode is not known. [1] In order to determine which residues are involved in contact between protomers, we combined biochemical crosslink and molecular modeling. [2]

Introduction of cystein and subsequent disulfure bridge formation between protomers suggest the involvement of several transmenbrane helix (TM) residues in a symmetrical organization of the dimer. The modeling of CCR5 dimer based on crystallographic structure of CXCR4 and mu opioid receptors is in agreement with two dimerization modes involving mostly TM4/TM5 and TM5/TM6, respectively. [3-4]

Dimer stability was assessed by molecular dynamics (MD) simulations of these two dimers embedded in a hydrated lipid bilayer, supporting the existence of two interfaces of dimerization. CCR5 dimerization is inhibited if a lysine residue is introduced at specific position in TM5 (as demonstrated by FRET experiments and RUSH assays). Surprisingly, the antiviral drug maraviroc restores partly the dimerization of mutants. MD simulations of monomeric CCR5, free or bound to maraviroc, indicate that the binding of maraviroc modifies the receptor dynamics and induces changes at the protein surface. Thus conformational changes may favor the formation of a new dimer interface. This dimeric organization was experimentally trapped using DSP covalent bridging of protomers.

Altogether, our data support at least three dimeric forms of CCR5: two interfaces involve TM5 and a third one not yet modeled.

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Pharmacological blockade of CXCL12/CXCR4 signaling prevents the development of hyperalgesia in mice.

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CXCL12 is a chemokine involved in pain and inflammation mechanisms through its receptor CXCR4. Intracerebroventricular or intraplantar injections of this chemokine induces hyperalgesia and CXCR4 antagonist AMD3100 efficiently blocks this effect. However, this compound also displays several adverse side effects. An alternative strategy to block CXCL12 action is to use neutralizing compounds that interact directly with the chemokine to prevent its interaction with CXCR4. Chalcone 4 has been shown to display CXCL12 neutralizing activity both in vitro and in vivo. Particularly, this compound was used to demonstrate the involvement of CXCL12 in different diseases including asthma and Whim syndrome.

Here we used chalcone 4 to study the role of CXCL12 chemokine in different models of hyperalgesia such as acute or chonic administration of opiates (fentanyl or morphine) and inflammatory pain (induced by carrageenan or CFA). We observed that in mice chalcone 4 prevents the fentanyl-induced hyperalgesia at doses ranging from 30 to 100 mg/kg. This effect was similar to that of AMD3100 and was not observed with chalcone 1, a compound that is closely related to chalcone 4 but does not display CXCL12 neutralizing activity. In a model of inflammatory pain, chalcone 4, at 100mg/kg, was also able to block the development of hyperalgesia. In addition, in a model of long lasting inflammation induced by CFA and in a model of opioid-induced hyperalgesia with chronic morphine administration, chalcone 4 fully reversed this installed hyperalgesia. Finally, we found that mRNA expression levels of several inflammatory genes were modified in spinal cord and/or DRG upon fentanyl administration and that these modifications are blocked by co-administration of chalcone 4.

Altogether, these results point to CXCL12 as a critical player in the adaptations associated with opiate administration.

Five BIN1s are better than nothing

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In skeletal muscle Amphiphysin 2 / BIN1 is an N–BAR protein which plays a key role by regulating membrane dynamics and remodelling. BIN1 is involved in three different myopathies: myotonic dystrophy (#160900) with *BIN1* altered splicing and autosomal recessive and dominant centronuclear myopathies (CNM)(#255200 and 160150 respectively) with *BIN1* mutations. Thus, BIN1 functions are of main importance for skeletal muscle. Total and muscle-specific loss of BIN1 is neonatal lethal (*Bin1*-/- mice), due to late muscle development defects and an inability to feed. However, the impact of BIN1 overexpression on skeletal muscle organisation and ultrastructure is not known.

The goal of this project is to characterize the role of BIN1 in skeletal muscle. To complement our understanding of BIN1 role we are using transgenic human BIN1 mice (*BIN1*Tg) where *BIN1* is 5-fold over-expressed in skeletal muscle compared to the endogenous murine *Bin1*. The overexpression of BIN1 doesn't impact in mice lifespan, whole body strength and muscle strength. Ultrastructure analysis reveals normal sarcomere organization, although sporadic T tubules have abnormal localization, in line with BIN1 known function on membrane curvature. Additionally, the level of proteins involved in other CNM forms, as the phosphoinositides phosphatase myotubularin (MTM1) and the large GTPase dynamin 2 (DNM2), is not modified by the overexpression of BIN1 in mice. Moreover, the overexpression of human *BIN1* in the *Bin1*-/- mice rescues the neonatal lethality, arguing that human BIN1 has similar roles and interactors than mouse BIN1 in a murine context.

In the future we will analyse in more details the localization and interactors of BIN1 in muscle and a possible genetic/functional link between BIN1, DNM2, and MTM1 in order to assess if they are part of the same pathway regulating membrane dynamic.

Identification of genes implicated in congenital myopathies

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Congenital myopathies are genetic disorders characterized by distinctive morphological abnormalities in skeletal muscle fibers. They define a class of severe muscle diseases with a strong impact on patient survival and quality of life. The main subclasses include nemaline myopathy (with protein aggregates or rod), cores myopathy (central core or multi-minicore, well demarcated areas devoid of mitochondria) and centronuclear myopathy (centralization of nuclei). A large number of genes has already been described for causing congenital myopathies. However, about half of the patients do not have a genetic diagnosis supporting the implication of a large number of yet unidentified genes.

Massively parallel sequencing offers an unbiased and integrated approach to accelerate the identification and characterization of the genetic basis of congenital myopathies. Myocapture is a consortium of research teams, clinicians and sequencing platform working together to characterize the clinical, histological and genetic data of patients. The strategy was to sequence 1000 exomes of patients and their family, previously excluded for known myopathy-causing gene. For exome data analysis, efficient bio-informatic pipelines have been developed in house and shown to be very powerful to identify the mutations responsible for the disease.

Within this project, we studied a non-consanguineous Franco-Lebanese family with three affected children suffering from severe neonatal hypotonia, swallowing troubles and weak limb reflexes. Structural abnormalities on biopsy were not specific of any classical congenital myopathy. We sequenced the exome of the six family members, the two parents and their four children and filtered the variants according to a recessive mode of inheritance. All affected members carried two variants in SCN4A, a sodium channel highly expressed in muscle, compatible with a compound heterozygous segregation. A missense mutation in a well conserved amino-acid was transmitted by the father and a mutation affecting an essential splice donor site was transmitted by the mother. The mutations were well covered and confirmed by Sanger sequencing.

This example of integrated approach helped to expand the phenotype of diseases associated with mutations in SCN4A, previously described in other diseases such as congenital myotonia and potassium-related periodic paralysis. We identified SCN4A as a gene causing a new type of myopathy characterized by a clinical improvement over time and an overlap between classical congenital myopathy and dystrophy on muscle biopsy. Thus, this integrated clinic-molecular approach refines the classification of myopathies.

The identification, validation and characterization of novel implicated genes in congenital myopathies such as SCN4A will allow the development of novel diagnosis protocols to improve genetic counseling, including eventual prenatal or pre-implantation diagnosis. Moreover, the identification of novel genes is an important step for the discovery of new therapeutic targets.

FlAsH-PALM Imaging of cellular distribution of HIV-1 Nucleocapsid Protein

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FlAsH-PALM is a pointillist superresolution fluorescent microscopy technique based on the localization of individual fluorophores that allows imaging the structures smaller than the diffraction limit [1,2]. The labeling of the proteins by genetically encoded tetracystein tag combined with a permeant FlAsH or ReAsH fluorophore provides a specific labeling with minimal perturbation of the biological system [3]. We applied the FlAsH-PALM technique to visualize the Nucleocapsid protein 7 of Human immunodeficiency virus 1 (HIV-1) during the early steps of the viral cycle. For this purpose a HeLa cells were infected with a virus like particles containing TC-tagged NCp7 molecules. The size of a HIV-1 is ~100nm the superresolution microscopy is thus perfectly adapted to follow the virions in the infected cells.

NCp7 is a viral chaperone of nucleic acids. In each virion the RNA dimer is covered by ~2500 molecules of NCp7. It has been proposed that during the reverse transcription and the synthesis of the genomic DNA, the majority of NCp7 molecules are released into the cytoplasm and only a small part enters into the nucleus with the virus [4]. This hypothesis is plausible; however this redistribution of NCp7 during the infection has never been directly evidenced.

Our results show that 8 hours post infection the NCp7-TC shows a punctuate localization in the infected cells. These puncta represent individual virions. In the cytoplasm the size of these particles is comprised between 110 and 120 nm corresponding to the size measured for the viral particles deposited on the coverslid. In the nucleus the NCp7 clusters are significantly smaller (~50-60 nm). This decrease of the size can be explained by the partial release of NCp7 molecules from the virion during the global remodeling of the viral particle prior to the entry to the nucleus.

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Development of a pharmacophoric deconvolution method to accelerate the discovery of antiplasmodial molecules from Rhodophyta

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In natural-product research, the bioassay-guided isolation is usually used to find new bioactive compounds. However, this strategy is time-consuming, onerous and sometimes leads to well-known molecules[1]. A new method combining 2D-NMR techniques and bioactivity assay is developed with the aim of accelerating the discovery process of new bioactive natural products. Based on differential analysis of 2D-NMR spectra (DANS), the method allows identifying molecular fingerprints related to biological activity (pharmacophoric fingerprint) within a complex natural mixture. This method combined with the HPLC-DAD-MS-SPE-NMR hyphenated technique leads to the reliable structural identification of the activity-bearing compounds[2]. This analytical strategy will be used to identify new antimalarial molecules from active red algae extracts with original mechanism of action. These molecules could be used in combination with current antimalarials to avoid or to delay resistance occurrence. Previously, it was shown that red algae are a source of antiplasmodial products[3]. The parasite *Plasmodium* possesses a relict organelle, the apicoplast, which is a plastid from a secondary endosymbiosis of a red alga[4]. Because of this particular evolutionary past, we hypothesized that red algae molecules could interfere with apicoplastic biosynthesis pathways in *Plasmodium* and inhibit its development.

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Programmation of the α5β1 integrin expression in glioma stem cells confers aggressiveness to glioblastoma

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Background: Glioblastomas (GBM) represent the most aggressive brain tumor with 15 months median survival after diagnosis because of a recurrence of the disease. Indeed, some cells are able to spread out of tumor beyond the limits of the resection. Understanding the mechanisms causing this process is a new challenge to prevent GBM cells from invading the host tissue and increasing life expectancy of patients. We previously discovered a correlation between integrin α5β1 expression and glioblastoma grade and patient survival. Recent studies showed that GBM contains self-renewing, tumorigenic cancer stem cells (GSCs) implicated in tumor initiation, recurrence and therapeutic resistance. Our goal is to determine if integrin α5β1 plays a role in GSCs behavior and agressiveness.

Methods: Two GSCs were used in this study, NCH644 et NCH421k. GSCs are cultured either as neurospheres in DMEN medium supplemented with growth factors (EGF and bFGF) or as adherent differentiated tumoral cells after addition of serum in the medium. Expression of proteins was analyzed by Western Blots with specific antibodies and mRNAs by RT-qPCR with specific primers. Several markers either associated with stemness (CD133, CD44, nestin, sox2...) or with differentiation in glial, neuronal or oligodendroglial lineages (GFAP, Tuj, BMP...) were investigated as well as the expression of integrins. Phenotypical characteristics are evaluated by in vitro proliferation and migration assays and by the capacity of GSC to initiate tumors in vivo when xenografted in nude mice. NCH421k cell line was also engineered to express α5 integrin by transduction of a lentiviral vector containing the human integrin gene.

Results: The two GSC lines did not express α5 integrin when cultured in neurosphere medium (undifferentiated form). However, when forced to differentiate, only NCH644 cells express the integrin and this expression confered proliferative and migratory advantages in vitro and an increase in tumor aggressiveness in vivo. Similar results (enhanced proliferation/migration and tumorigenesis) were obtained when α5 expression was forced in NCH421k cells suggesting that the integrin was indeed involved. Interestingly, expression of α5 integrin in NCH421k spheroids induced the loss of proneural stemness markers (nestin, sox2, oct4 and CD133) without increasing the differentiation markers (GFAP, tuj). However the mesenchymal stem cell markers CD44, serpine1, AXL, cMet were dramatically increased.

Conclusions: Although α5β1 integrin does not appear as a glioma stem cell marker, its expression is differentially observed when stem cells differentiate to form the bulk tumor in vitro and in vivo. Forced expression of α5 integrin in stem cells seemed to induce a switch from proneural to mesenchymal stem cells, these latter already proposed to be more aggressive in patients. Our results confirm that α5β1 integrin is an important player in glioblastoma aggressiveness and that it may represent a pertinent therapeutic target.

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

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Left ventricular hypertrophy (LVH) is a risk factor for heart failure and death, characterized by cardiomyocyte growth, interstitial cell proliferation and leukocyte infiltration. We hypothesized that chemokines, interacting with GPCR chemokine receptors, might play a role in LVH development through promoting recruitment of activated leukocytes and/or modulating left ventricular remodeling. In a kinetic study (0, 1, 3, 5, 7 and 14 days) using the left ventricular hypertrophy model induced by banding of the suprarenal abdominal aorta in Lewis rat, we highlight the expression of chemokines and chemokine receptors mRNA in left ventricles from aorta-banded (AB) *vs* sham-operated animals.

Two phases were clearly distinguished in our model. An inflammatory phase occurred at D3-D5 characterized by significant overexpression of inflammatory genes such as IL-1ß (3.4±1.5-fold at D3), TNFa (1.9±0.6-fold at D3), NLRP3 (1.9±0.5-fold at D3) and the RelA subunit of NF-kB (1.7±0.4-fold at D5). This inflammatory phase was followed by hypertrophic phase at D7-D14, where the endogenous hypertrophic marker ANP was significantly overexpressed (34±20-fold at D7 and 18±9-fold at D14) and the heart weight/body weight ratio was significantly increased (15.9% at D7 and 20.4% at D14). At this time, no cardiac dysfunction was detectable by echocardiography.

Global analysis of the mRNA expression of 36 chemokines and 20 chemokine receptors analyzed by Taqman Low Density Array (Applied Biosystems) showed a peak mean-fold change difference between AB and sham-operation in the inflammatory phase (D3-D5), while remaining constant at D0, D1, D7 and D14. Among these genes, we identified a significant mRNA overexpression at D3 of monocyte chemotactic proteins CCL2 (12.2±7.2-fold), CCL7 (7.1±3.8-fold) and CCL12 (2.6±1.2-fold), of macrophage inflammatory proteins CCL3 (3.8±1.9-fold), CCL4 (1.8±0.6-fold) and CCL9 (2.0±1.0-fold) as well as of their receptors CCR2 (4.4±2.3-fold), CCR1 (3.3±1.1-fold), CCR5 (3.0±1.3-fold). During the hypertrophic phase mRNA expression returned to baseline levels.

Hence, this study of the first exhaustive mRNA expression kinetics of chemokines and chemokine receptors in LVH reports early expression of monocyte/macrophage-related chemokines and receptors during development of left ventricular hypertrophy in the rat, followed by regulation of inflammation when hypertrophy takes place.

MSK1 kinase as potential therapeutic target to combat obliterative bronchiolitis

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Obliterative bronchiolitis (OB) is the manifestation of chronic allograft rejection, and the first cause of death in lung-transplant patients, with no effective treatment available. OB is characterized by airway epithelium degradation and obstruction of small airways with inflammatory infiltrate and fibrosis. MSK1 is a nuclear kinase, which phosphorylates Ser276 of the p65-subunit of NFkB in inflammation. MSK1 might play a role in OB *via* NFkB activation.

Heterotopic tracheal allotransplantation in the mouse induced an increased expression of MSK1 mRNA (6±2-fold at D21) and its activity (39±19-fold increase of IL-6 production at D21), which were unmodified in isografts. MSK1 inhibitors, H89 (10mg/kg/day), fasudil (30mg/kg/day) *vs* solvent (DMSO 5%) (I.P.) reduced the tracheal fibroproliferative occlusion by 43±7 and 31±13%, respectively, on D21. Tracheal sections revealed inhibition of the epithelial degradation in allografts by 45±10 and 28±9% on D7, and reduction of infiltration of CD3+ cells (92±4 and 91±5%) and DCs (64±7% by H89), suggesting that MSK1 inhibition might be a potential strategy to combat OB.

Since these MSK1 inhibitors could also act through inhibition of other kinases, we screened for new inhibitors of MSK1 activity in a panel of newly synthesized compounds, existing drugs or virtually selected molecules by HTRF and luminescence on a NFkB peptide mimetic. We detected one molecule, compound S, with IC50=0.6±0.1µM, compared to H89 (IC50=0.3±0.04µM) and fasudil (IC50=1.5±0.2µM), to be used as a pharmacological tool to validate MSK1 as a good target. In human primary lung fibroblasts stimulated with IL-1b, compound S inhibited IL-6 production with an IC50=1.04±0.03µM, compared to H89 with IC50>10µM. In our OB model of heterotopic tracheal transplantation in vivo, compound S inhibited the epithelial degradation at D7 by 35±11%. We have thus obtained compound S as a new MSK1 inhibitor to be evaluated also at D21 on the fibroproliferative occlusion during OB.

Photopolymerized micelles of diacetylene amphiphile: physical characterization and cell delivery properties

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A series of polydiacetylene (PDA) - based micelles were prepared from diacetylenic surfactant bearing polyethylene glycol, by increasing UV-irradiation times. These polymeric lipid micelles were analyzed by physicochemical methods, electron microscopy and NMR analysis. Photopolymerization leads to more stable structures without structural or morphological changes. Dynamic surface tension measurements at various concentrations of non-polymerized and polymerized micelles allowed us to deduce the quantity of polymerized vs. unpolymerized molecules inside the photopolymerized micelles (PM). This observation also correlates with DOSY NMR analysis of the PMs showing discrete populations for polymerized and unpolymerized surfactant. Even high polymerization times lead to incomplete polymerization of lipids, with remaining unreacted monomer surfactant.

The PDA micelles were further evaluated in biological assays for the intracellular delivery of hydrophobic compounds, with the aim of delivering poorly soluble anti-cancer drugs. Cellular delivery of fluorescent dye (NR12S, as a hydrophobic fluorescent molecule model) suggests that adjusting the polymerization state is crucial to reach the full in vitro potential of PDA-based delivery systems.

This work highlights the delivery potential of PEGylated polydiacetylene micelles, and their potential use as tools for drug delivery. It thus appears that adjusting polymerization time of micelles enables fine-tuning of the intracellular delivery/cytotoxicity ratio. The micelles obtained after short irradiation times seem to be an excellent compromise between efficiency and toxicity for intracellular delivery applications.

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Chronic treatment with an aqueous extract of *Phyllanthus amarus* prevents hypertension and endothelial dysfunction in Doca-salt rats

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Phyllanthus amarus (Euphorbiaceae family) has been reported in traditional medicine to possess beneficial effects in the management of hypertension. In different animal models of hypertension, an impairment of the vascular function has been linked to an endothelial dysfunction. Therefore, the aim of the present study was to determine if an aqueous extract of *Phyllanthus amarus* (AEPA) obtained by decoction was able to prevent hypertension and endothelial dysfunction in Doca-salt rats, and, if so, to clarify the underlying mechanism.

Male Wistar rats were randomly assigned into the control group, the AEPA group (100 mg/kg/day, by gavage), the Doca-salt group (50 mg/kg, s.c, per week), and the Doca-salt + AEPA group (100 or 300 mg/kg/day, by gavage). Doca-salt-treated rats were allowed free access to water containing 1% NaCl. Systolic blood pressure (SBP) was determined by tail-cuff plethysmography twice a week, in the morning during 5 weeks. Vascular reactivity using main mesenteric artery rings was assessed in organ chambers. Dihydroethidine (DHE) and immunofluorescence methods were used for the determination of the vascular formation of reactive oxygen species (ROS) and the expression of levels of proteins, respectively.

After 5 weeks, SBP increased significantly in Doca-salt hypertensive rats. It was significantly lowered by the treatment with AEPA (100 or 300 mg/kg/day) by 24 and 21 mmHg respectively. In mesenteric artery rings, the phenylephrine induced contractile response was increased significantly in comparison to the control group. After treatment by AEPA, the contractile effect was shift to the right. Both the NO-mediated (assessed in the presence of Indomethacin and Tram plus Apamin) and the EDH-mediated (assessed in the presence of Indomethacin and N^G-nitro-L-arginine) respectively relaxation to acetylcholine, were significantly reduced in the Doca-salt group, compared to the control group. Fluorescence study showed that the endothelial dysfunction was associated with reduced expression levels of Cx37, an increased expression of eNOS and the formation of ROS in main mesenteric arteries. The antihypertensive effect of AEPA was related to an improvement of the blunted of the NO- and EDH- mediated relaxation, and an increase of vascular oxidative stress and the modulation expression of levels of target proteins in Doca- salt rats.

Altogether, our study shows that AEPA is able to act as antihypertensive agent, and to prevent endothelial dysfunction in Doca-salt hypertensive rats in part by preventing vascular oxidative stress.

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 16 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 NF X50-900 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.

www.pcbis.fr

Pathophysiology of tubular aggregate myopathies

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Tubular aggregate myopathies (TAM) are progressive muscle diseases characterized by abnormal accumulations of membrane tubules in muscle fibers. Our team identified dominant mutations in *STIM1* in TAM. STIM1 is the main calcium sensor of the endo/sarcoplasmic reticulum (ER/SR). Following a stimulus, calcium (Ca2+) is released from the ER/SR into the cytoplasm where it triggers muscle contraction. Upon store depletion, STIM1 unfolds, oligomerizes and activates the ORAI1 channel to allow Ca2+ entry. This mechanism is known as store-operated calcium entry (SOCE). We demonstrated that STIM1 mutations lead to an impairment of Ca2+ homeostasis in TAM myoblasts. However, the physiopathology of tubular aggregates and the link between STIM1 mutations and muscle dysfunction need to be unraveled.

We aim to decipher the sequence of physiological events that leads from *STIM1* mutations to the formation of tubular aggregates and to muscle dysfunction in cellular and animal models.

In order to study the impact of STIM1 mutations on clustering and ORAI1 recruitment and opening, C2C12 murine myoblasts or Hela cells were transfected with mutant or wild-type (WT) STIM1 constructs. Unlike WT STIM1, mutant STIM1 constitutively clusters and recruits ORAI1. Calcium level measurements showed a higher basal Ca2+ level and a higher cytoplasmic Ca2+ increase after addition of exogenous Ca2+ in patient's myoblasts and in cells transfected with mutant STIM1. Together it strongly suggests STIM1 mutations lead to a constitutive activation of SOCE.

There is currently no mammalian model for TAM. We therefore generated AAVs harboring WT or mutant STIM1, and injected them into the tibialis anterior of WT mice. We are currently assessing the impact of STIM1 mutations on muscle structure and function by histological and ultra-structural analyses at different time-points post transduction. To establish a correlation between the molecular and cellular alterations, the muscle function and the development of the disease, a second cohort of transduced mice will undergo specific muscle force measurements.

In conclusion, we aim to shed light on the physiological mechanisms leading to TAM and identify actionable cellular defects that could be rescued at the cellular level and in the animal model.

Dynamic, structural and functionnal studies of P2X receptors through unnatural amino acid incorporation.

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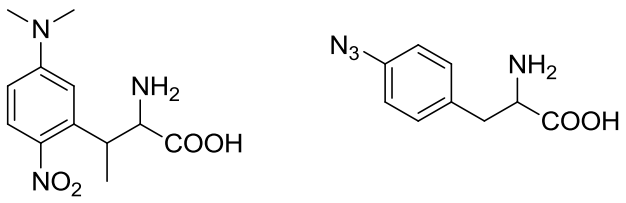
The opening of ligand gated ion channels, in response to agonist binding, is an important process in biology, because it allows the regulation of ion flows through the plasmic membrane of cells. Understanding this extremely fast mechanism at the atomic scale is a major challenge and new tools are needed to be designed and developed for investigation. Publications of atomic structures of P2X receptor in the closed state and the activated state, bound by ATP (adenosine-5'-triphosphate), represent for this purpose a big step forward. These structures allow to partially understand the molecular mechanisms responsible for the opening of the channel. However, these structures are motionless and don't give access to information about the dynamics of conformational changes in the protein.

The emergence of methods that allows the specific incorporation of non natural amino acids in the protein sequence through the cellular components, leads to new approaches to study this type of protein. Indeed, new functionalities, or site specific probes can be added by the incorporation of unnatural amino acids. Two strategies are currently being developed in our laboratory, with the aim of obtaining new information about P2X receptors, through two different unnatural amino acids.

First, a photocleavable amino acid is developed, to lead to controlled photo proteolysis in the sequence of P2X receptors, after incorporation using a tRNA suppressor of STOP codon chemically amino-acylated. This will then allow for structure-function studies.

Secondly, a photoreactive amino-acid, capable of undergoing bio-orthogonal reactions (p-azido-L-phenylalanine), incorporated using an orthogonal tRNA suppressor/aminoacyl tRNA synthetase pair, will be used as an anchor for fluorescent probes. Following this, FRET (fluorescence resonance energy transfer) analysis should allow us to have access to dynamic and structural information.



Structure of the photocleavable amino acid developed in this study and structure of the p-azido-L-phenylalanine.

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Pharmacological blockade of CXCL12/CXCR4 signaling prevents the development of hyperalgesia in mice.

GAO Lijia, DOIGNON†Christophe, HABED Adlane, VOROS Sandrine

ICube, Illkirch

The goal of our research is to devise a flexible, yet accurate, method for surface reconstruction using multiple endoscopic cameras/viewpoints in the abdominal cavity. Such method would assist the practitioners in minimally invasive surgery. To achieve this goal, multiple steps need to be investigated: multi-view feature extraction and correspondences, camera self-calibration and surface reconstruction. In this poster presentation, we will focus on the problem of camer self calibration ; i.e. recovering the internal geometry of all cameras involved.

The outline of a novel method we are developing will be presented.

Mote clés : endoscopy, self-calibration, DAQ,ALQ, LMIs, Branch-and-Prune, Global optimization

In vivo genome editing as a therapeutic strategy for myopathies

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Centronuclear myopathies (CNM) are rare muscle disorders belonging to the large group of congenital myopathies. Heterozygous mutations in the *DNM2* gene cause the autosomal form of CNM and unfortunately, to date, there is no specific treatment for CNM. Theoretically, the most upstream therapeutic approach is to correct the disease-causing mutation on the DNA. This approach would particularly be indicated for dominant mutations that are not leading to a loss-of-function, as in the case of gain-of-function *DNM2* mutations. I am using CRISPR-associated RNA guided endonuclease Cas9 technology. My project aim is to establish a proof of concept for allele-specific genome editing *in vitro* and *in vivo* as a therapeutic approach for CNM. We propose 2 strategies based on allele-specific correction: The first consists in correcting the mutation and restoring the WT allele. The second strategy consists in inactivating the mutated allele via the non-homologous end-joining (NHEJ).

For the *in vitro* step, patients' immortalized fibroblasts (and later primary cells or iPS) are transfected with Cas9-EGFP and sgRNA constructs and the repair template, and clones are selected and assessed for correction efficiency by Sanger sequencing. With this approach, *DNM2* sequence was successfully targeted in HeLa cells and clones were obtained with both precise genome modification (HDR) and KO (NHEJ).

The final aim of this project is to correct the disease-causing mutation in vivo using AAV-Cas9 injection in postnatal KI *Dnm2* R465W mice and assessing muscular phenotyping and fibers ultrastructure analysis.

The specificity of CRISPR/Cas9 tool remains one of the main safety concern because genetic modification is permanent and "off-target" mutations may have devastating consequences. To assess "off-target" toxicity, I will sequence top-10 bio-informatically predicted sites, and ultimately perform genome sequencing.

This project should provide a proof-of-concept for the use of genome editing to correct mutations leading to myopathy *in cellulo* and *in vivo.* Corrected cells may be further used for cell therapy, while the efficiency of *in vivo* correction will be fine-tuned to reach maximum rescue impact.

Using yeast Saccharomyces cerevisiae to better understand two neuromuscular diseases

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The **X-linked centronuclear myopathy** (XLCNM) and the **Charcot-Marie-Tooth neuropathy type 4B** (CMT4B) are neuromuscular diseases caused by mutations in lipid phosphatases: the myotubularin **MTM1** for the XLCNM and the myotubularin-related protein **MTMR2** for CMT4B. Although they are involved in two distinct pathologies, MTM1 and MTMR2 are ubiquitously expressed in human tissues and very similar in sequence, catalytic function and domain organization.

The aim of this project is to decipher the molecular specificities of the myotubularin MTM1 and the myotubularin-related protein MTMR2, to better understand why they are involved in two very different diseases.

One molecular specificity of MTMR2 could be the **S58 phosphorylation site**, on the N-terminal part of the protein. The site is absent in MTM1. The phosphorylation has an impact on the localization of MTMR2 in HeLa cells. The localization of MTM1 and MTMR2 could be crucial for their specific functions. Thus, we decided to study this phosphorylation site in our model: yeast ***Saccharomyces cerevisiae***.

pH responsive polydiacetylenic micelles allow for synergic co-delivery of intracellular siRNA and anti-cancer drug

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In recent years, nucleic acid therapeutics such as small interfering RNA (siRNA) have become particularly interesting because of their great therapeutic potential in pathologies in which down-regulation of a specific mRNA leads to a beneficial effect.[1] They are already involved in various clinical trials for the treatment of several diseases such as cancers, viral infections as well as genetic disorders. [2-4] However as with usual drugs, siRNA treatment is also facing resistance issues, which could require drug-nucleic acid combination approaches.

To this end, the development of modern nanocarriers such as micelles, liposomes or even nanoparticles able to deliver simultaneously drugs and nucleic acids has shown an increasing interest. Nevertheless, this remains a tough challenge because of the significant differences that exist in the physicochemical properties of the two types of drugs. [5]

To overcome this problem, our group developed a new generation of photopolymerized diacetylenic amphiphile micelles (PDA), which contain a hydrophilic head able to efficiently deliver a siRNA into the cytoplasm and a hydrophobic core, which can accept small drugs.

Our system reveals itself as a promising nanovector for siRNA delivery with up to 80 % of specific inhibition of gene expression in a reporter system, but also a great carrier for hydrophobic and water-insoluble anti-cancer drugs, such as paclitaxel (PXL) or camptothecin (CPT).

Once the nanocarrier optimized, we have extended this system to the co-delivery of a siRNA targeting a endogenous gene (PLK1) and the small hydrophobic therapeutic drug CPT. Our results show that we succeeded in having a synergic effect on the HeLa tumor cell line.

Moreover, due to the nanometric size range of these objects (<100 nm), the micelles should better diffuse through blood vessels and reach deeper into the tumor tissues, [6] which are attractive features for *in vivo* studies that will be performed in the near future.

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Real-Time Marker Tracking For MRI-guided Percutaneous Procedures In Radiology

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This thesis aims to develop an automatic uninterrupted 3D view of the percutaneous path of the instrument during the interventional surgery. The proposed system detects and tracks the interventional instrument to automatically guide the MRI scanner to a desired location, showing a 3D view aligned with the percutaneous path. To achieve the 3D view, the interventional path of the instrument is detected and tracked in consecutive MR image frames. This is carried out by using the state of art object classification and visual tracking methods to control the MR image acquisition in real-time. We provide the most suitable methods for visual tracking and object classification under medical imaging setups, based on their performance obtained from our extensive survey on numerous state of art methods. Furthermore, we also propose and demonstrate the complete pipeline of MR image alignment, starting from image acquisition to fully automatic working model in real-time. The proposed working model of the system is duly tested with 1.5 Tesla Siemens MRI machine at Strasbourg's Civil Hospital.

In addition, a system quality assessment method is proposed which not only evaluates the visual tracking but also the overall quality of the MR image alignment system. The automatically adjusted 3D view of the interventional path produced by this system provides the radiologist a greater insight of the anatomy around the percutaneous path, saving time and increasing accuracy of the surgical operation. Hence the research carried out during the thesis has an academic as well as a social impact.

Study of Myotubularin, a phosphatase responsible for centronuclear myopathy by using as a model the yeast *Saccharomyces cerevisiae*.

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Keywords: Myopathy, myotubularins, MTM1, phosphoinositides, PH-GRAM domain, yeast

Centronuclear myopathy (CNM) is a genetic muscular disease characterized at the histological level by nuclei at the center of the myofibers instead of the periphery. Mutations in three genes (MTM1, DNM2 and BIN1) are associated with this pathology. MTM1 on the X chromosome affects males and codes for an enzyme called myotubularin. Myotubularin is a phosphoinositide lipid phosphatase. The cellular processes controlled by MTM1 at the muscular level are still unknown. MTM1 study in human cells is complicated due to the presence of 14 homologues. Thus, we used the unicellular eukaryotic yeast model *Saccharomyces cerevisiae* to study MTM1. Yeast has a similar organellar organization as human cells and encodes only 1 myotubularin, termed Ymr1 (yeast myotubularin related 1).

To better understand the cellular function of MTM1, we are studying the two main domains of this protein: the PH-GRAM N-terminal lipid binding domain and the C-terminal catalytic phosphatase domain. The two domains were tagged with different fluorescent reporters (GFP and mCherry) and either expressed or co-expressed into yeast cells. The yeast cells were observed by fluorescence microscopy to analyze the cellular localization and function of these MTM1 domains. We also tested two CNM patient mutants affected into the PH-GRAM domain.

The results show that intermolecular interactions between the N-terminal PH-GRAM and the C-terminal catalytic domain are required to restore a catalytically active MTM1 phosphatase.

L. Amoasii, D. Bertazzi, K. Hnia, H. Tronchere, B. Rinaldi, G. Chicanne, B.S. Cowling, A. Ferry, B. Payrastre, J. Laporte, S. Friant (2012) Phosphatase-dead myotubularin ameliorates x-linked centronuclear myopathy phenotypes in mice. *PLoS Genetics* 8(10):e1002965

Thienoguanosine efficacy surmounting long standing 2-Aminopurine's deficiencies

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The design and successful implementation of fluorescent nucleobase analogues is a precarious art because it requisites minimal structural and functional perturbation upon substitution in oligonucleotides. The long pursued fluorescent isomorphic nucleotide, 2-Aminopurine (2Ap), has been used to countless assays, though it suffers from significant loss of quantum yield and a rather complex fluorescent decay along with large amounts of dark species upon incorporation in oligonucleotides. Recently, thienoguanosine (thG) [1] was designed as an analog of Guanosine (G) to overcome the barriers of 2Ap. Primarily, we focused on identifying and characterizing the tautomers of free thG nucleoside and their microenvironment dependency. Later, we characterize their potency to adapt the conformations and dynamics of the G7 residue when substituted in minus strand primer binding site (-)PBS DNA or double stranded (-)/(+)PBS. Steady-state analysis of thG in different solvents suggested the presence of two ground-state tautomers having distinct absorption and emission maxima. Through quantum chemical calculations, the existence of two energetically stable keto-amino tautomers, thG-H1 and thG-H3, differing by the position of H-atom was confirmed. In addition, MD calculations were performed to observe the conformational and thermodynamic effects of deoxyguanosine (dG) replacement by dthG-H1 and dthG-H3 in the 3D structure of ΔP(-)PBS DNA. Moreover, it was observed that the emission of dthG-H3 is dependent on the nature of the mismatched opposite base and could be used to predict single molecule polymorphism, which is near to impossible if performed with 2Ap. In further contrast to 2Ap as substituted at G7 position in (-)PBS within (-)/(+)PBS, dthG also illustrated better structural isomorphicity by forming thermodynamically stable Watson-Crick base pairing. Moreover, a twice higher quantum yield in duplex form and a rather simple fluorescent lifetime decay [2] with marginal amount of dark species allow it to faithfully monitor the conformations and dynamics of substituted G7 residue, notably better than 2Ap.

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Reorganizations of model lipid membranes induced by Shiga toxin

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The glycosphingolipid globotriaosylceramide (also called Gb3 – fig. 1) is a plasma membrane receptor and plays a crucial role in many diseases, e.g. Fabry's disease, colorectal cancer and bacterial invasion. Shiga toxin from *Shigella dysenteriae* requires this receptor for binding and internalization in target cell[1]. The pentameric B-subunit of Shiga toxin (StxB - fig. 2) can be uptaken into living cells as well as into artificial model membranes (e.g. Giant unilamellar vesicles - GUV). Before the uptake, StxB forms clusters on the outer leaflet of such membranes and then induces tubular endocytic structures, even in the absence of cellular proteins [2] (fig. 3). During the first steps of this formation process, reorganization of the bilayer membrane occurs and the rigidity and lipid consistence of the membrane domains involved in the uptake process changes.

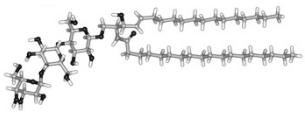

Figure 1. – Gb3

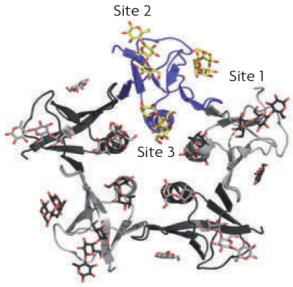

Figure 2. – Shiga toxin (B subunit)

To study such reorganizations, different Gb3 receptor lipid species are used with StxB. Membrane reorganizations induced by StxB are followed on artificially prepared liposomes (giant and large unilamellar vesicles), containing different compositions of Gb3 receptors by using environmental sensitive membrane probes, based on 3-hydroxoflavone derivatives [3] associated to fluorescence techniques (steady state, time-resolved, confocal microscopy, fluorescence lifetime imaging microscopy).

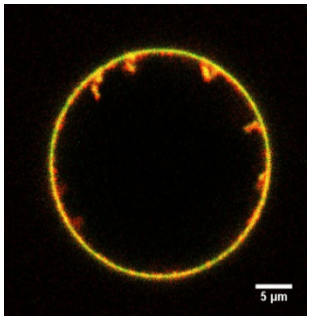

Figure 3. – StxB induces tubular invaginations on GUV (Confocal image)

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A new mechanism for DNA Gyrase inhibition by an aminocoumarin

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Type 2 DNA topoisomerases, such as DNA Gyrase and human topoisomerases Top2a/b, are essential proteins regulating DNA topology during cell division, chromosome replication, and transcription. They are part of large macromolecular complexes of DNA interacting machineries, targets for widely used antibiotics and chemotherapeutic agents which induce the formation of ternary complexes between enzymes and drugs. Being irreversible, these complexes are converted into DNA breaks, leading to cell death [1].

We investigate the structure/function relationships of the eukaryotic and prokaryotic type 2 DNA topoisomerases through a combination of biochemistry and structural biology. More specifically, efforts are being made to characterize the bacterial DNA Gyrase from *T. thermophilus and E. coli* by X-ray crystallography and Single Particle CryoEM reconstructions to try deciphering the protein architecture in complex with DNA and drugs [2]. In this context, I will present results obtained by X-ray crystallography regarding a new mechanism for DNA Gyrase inhibition by coumermycin, an aminocoumarin drug.

The structures of the Gyrase ATPase domains of *T. thermophilus* and *E. coli* in complex with coumermycin were solved at 1.95 Å and 2.35 Å, respectively. They present a different inhibition pattern than other Gyrase ATPase domains targeted by aminocoumarins, addressing new possibilities for drug design [3] and biotechnological tools improvement [4].

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Conception, synthesis and bioactivity evaluation of structural analogues of anti-*Leishmania* anthranoïds from *Psorospermum* genus (Hypericaceae) used in traditional medicine

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Leishmaniasis is a vector-borne disease caused by the protozoa Leishmania, which are spread by a variety of sand fly species[1]. It is mostly occurring in East-African and Asian countries and it is directly linked to poverty. With 1.3 million new cases and 20 000 to 30 000 deaths by year, leishmaniasis is a major cause of morbidity and mortality. As available drugs show important adverse effects and become less and less efficient due to drug-resistant appearance, it is urgent to find new active compounds with original mechanism of action. The huge chemical diversity of natural compounds makes them a target of choice for the discovery of new treatments.

A previous ethnopharmacological study allowed selecting three *Psorospermum* species for the strong antileishmanial activity of their bark extracts[2]. Then phytochemical studies led to the isolation and characterization of several anthranoid compounds with an interesting in vitro activity against *Leishmania* amastigotes. The selectivity of the activity seemed to be directed by the nature of the anthranoid moiety and the position and the nature of the substitution groups. However, when purified these compounds are easily oxidized and the activity of both main and degradated compounds has to be further investigated. To evaluate the structure-activity relationship, structural analogues of the active natural compounds are currently synthetized. Natural anthranoids analogues will also be isolated and characterized by using original separation methods as Centrifugal Partition Chromatography. Controlled degradation assays are in progress to evaluate the stability of these compounds and the activity of the degradation products. Finally, to find out the mechanism of action implicated *in silico* approaches and proteomic studies will be performed, by using fluorescent probes and affinity chromatography.

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Study of Elk3 regulation of glycolytic genes in hypoxia

Naimah Zein, Bohdan Wasylyk

IGBMC - France

Hypoxia is a condition in which cells are deprived of an adequate oxygen supply, and is involved in various pathological and physiological processes.Factors involved in the hypoxia responsecan be expected to be therapeutic targets. These factors include the transcription regulators HIF1α and Elk3. HIF1α is degraded in normoxia, but is stabilised in hypoxia where it regulates angiogenesis and apoptosis. Elk3regulates HIF1αby various mechanisms [1-3]. Elk3 ChIP-seq experiments in our laboratory [4] identifiedElk3 peakslinked to genesinvolved in various processes, including EGR1 (cell differentiation), FIH, VhL and PHDs(hypoxia), PAI-1 (cell migration) [1] and ENO1(glycolysis) [5]. I am focussing on the potential role of Elk3 in the regulation of genes involved in glycolysis, using PC-3M-pro4-luc2 cells as a model system. PC-3M-pro4-luc2 are prostate cancer cells that efficiently form metastases in mice and express luciferase to facilitate in vivo imaging. Furthermore, the laboratory has stable clones expressing shRNAs directed against Elk3 that will be useful for my experiments.

Using ChIPexperiments, I have shown that there are high levels of Elk3 on the Egr1 promoter, compared to several other prostate cell lines that express much higher levels of Elk3 at the RNA level. I have confirmed that Elk3 is efficiently and stably downregulated at the RNA level in a number of clones that express different Elk3 shRNAs. I am now testing whether the decrease in Elk3 expression affects expression of genes involved in glycolysis, focussing on the genes that have significant proximal Elk3 peaks in the ChIP-seq experiment [5].

These studies will lead tothe characterization of the functional interactions between Elk3 and HIF1α on the promoters of glycolytic genes, and in the longer term to the understandingof the pathophysiological role of Net in hypoxia, glycolysis and metastasis.

Targeted demethylation of a tumor suppressor gene in breast cancer cells

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DNA cytosine methylation (5-methylcytosine, 5mC) is a dynamic and reversible epigenetic mark. It takes place on CpG dinucleotides in mammals and can inhibit gene promoter activity. This process is catalysed by DNA methyltransferases (DNMT). Inversely, TET (Ten eleven translocation) family proteins play a role in DNA demethylation. TET proteins (TET1, TET2, TET3) catalyse the hydroxylation of 5mC in 5-hydoxymethylcytosine (5hmC), an intermediary demethylation molecule. Cancer cells are characterised by frequent promoter hypermethylation leading to transcriptional repression of tumour suppressor genes. Because of its reversible nature, DNA methylation is a target of choice in epigenetic therapies of cancer. However, current DNMT inhibitors act in a global and non-specific manner, leading to side effects and toxicity in normal cells. Here we present the development of a strategy to reprogram the epigenetic state of a single target gene in a breast cancer cell line. We have engineered chimeric proteins containing a DNA specific targeting module (TALE domain) targeting the tumor suppressor gene *SERPINB5* and a TET catalytic domain allowing the hydroxylation and demethylation of methylated cytosines. The results showed here are the first step on the optimization of a targeted gene reprogramming strategy.

We have validated a TALE-TET construction capable of demethylating and inducing the re-expression of the gene *SERPINB5* in MDA-MB-231 cells. We are currently working on the creation of a stable MDA-MB-231 line coding for an inducible TALE-TET construction targeting *SERPINB5* promoter. These cells will allow us to study the kinetics of the hydroxylation and demethylation processes, the specificity of the demethylation and the persistence of the demethylation. Moreover, we want to decipher the link between hydroxymethylation and gene expression and the consequences on the viability of cancer cells.