

2017 - Posters



Synthesis of two-photon sensitive pro-fluorescent photo-removable groups: Application to the in vivo light induced gene expression using photoactivatable gene inducers.

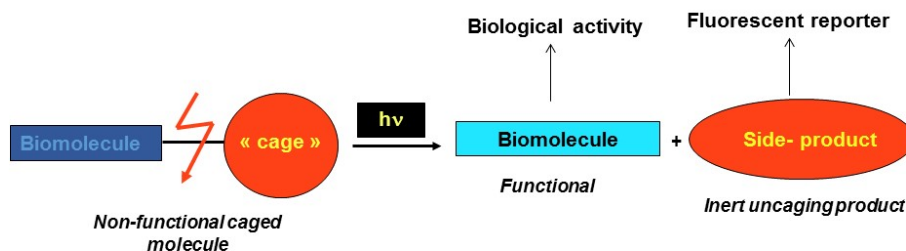
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Photoremovable protecting groups (PPGs) have been able to provide spatial and temporal control over the release of various biological effector (neurotransmitters and cell signaling molecules), this process is called uncaging¹ (Scheme 1).

The development of optical reporters of uncaging has only attracted little attention² as caged compounds and the uncaging secondary product exhibited similar low fluorescence in most cases.

Only one example has been developed by using caging groups that were designed to release a fluorophore as a side product. This was achieved with the o-hydroxycinnamate photoremovable group introduced by Porter's group³. In order to acutely monitor uncaging event in cells, we wish to develop new caging groups with fluorescence uncaging reporting properties. Based on the photolytical release mechanism of our two-photon sensitive photoremovable groups, we will design new non-fluorescent photoremovable groups based on ortho-nitrophenethyl derivatives which release a fluorophore as a side product.⁴ (Scheme 1)



Scheme 1: Mechanism of photo-irradiation (uncaging) of a PPG containing molecule

We aim to synthesize 2-photon sensitive pro-fluorescent photo-removable groups based on the *ortho*-nitrophenethyl moiety and study their biological application in gene expression by the help of photoactivatable gene inducer (biological effector).

The aim is to optimize a synthetic route for the synthesis of these photoremovable groups with high efficiency in 2-photon excitation, as soon as the synthesis is optimized we aim to develop new caging groups (different groups attached by extending the conjugation) with fluorescent uncaging reporting properties.

¹ Blanc, A.; Wirz, J.; Kostikov, A.; Popik, V.; Rubina, M.; *Chem. Rev.* **2013**, 113,119-191

² Labruere, R.; Alouane, A.; Le Saux, T.; Aujard, I.; Pelupessy, P.; Gautier, A.; Dubruille, S.; Schmidt, F., and Jullien, L.; *Angew. Chem. Int. Ed.* **51**, **2012**, 9344-9347.

³ Turner, A.D., Pizzo, S.V., Rozakis, G., and Porter, N.A.; *J. Am. Chem. Soc.*, **1988**, 110, 244-250.

⁴ Donato, L.; Mourot, A.; Bolze, F.; Goeldner, M.; Specht, A.; *Angew. Chem. Int. Ed.*; **2012**, 51(8), 1840-1843

Modulation of the desensitization process of P2X receptors by photoisomerizable molecules.

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P2X receptors are trimeric, non-selective cation channels activated by extracellular ATP. They are involved in important physiopathological roles, such as neuropathic pain or inflammatory pain. P2X receptors can exist in three different states: closed, open and desensitized. Despite their central function in human physiology, the molecular mechanism underlying the activation of P2X receptors remains unclear, especially the molecular motions that desensitize the channel. Here we used photo-switchable molecules to highlight an important region that can modulate desensitization. These molecules are able to open or close the channel by light, in the absence of ATP. They comprise a maleimide moiety for cysteine attachment at one extremity, a photoswitchable azobenzene in the middle, and a second maleimide (MAM) or a positive charge at the other extremity (MEA-TMA). The azobenzene allows cis/trans isomerization upon light irradiation at specific wavelengths, resulting in a dynamic change in the structure of the molecule. We show that when covalently attached at engineered cysteine mutants, these photoswitches dynamically occupy transmembrane cavities, resulting in the alteration of the desensitization process. These results reveal a new modulation site for desensitization.

Role of the transcription factor E2F6 in the recruitment of DNA methylation during mouse development

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The methylation on cytosines is an epigenetic modification that represses gene transcription when located on promoter-proximal CpG islands. During mouse embryonic development, a global methylome reprogramming event occurs. The genome is first demethylated after fertilization and is then *de novo* methylated after the embryo implantation. During this step, only 1% of promoter CpG islands gain methylation leading to the repression of the corresponding genes. The mechanism by which DNA methylation is specifically addressed to these CpG islands during development is unknown.

My thesis project is to investigate the role of the candidate transcription factor E2F6 in the targeting of DNA methylation to specific gene promoter during mouse development. To address this question, an E2f6 knock out mouse model is available in the laboratory and comparative methylome (using RRBS for Reduced Representation Bisulfite Sequencing) and transcriptome (using RNA-seq) between WT and E2f6 *-/-* samples were achieved to characterize the role of E2F6.

Preliminary data revealed that E2F6 is involved in the recruitment of DNA methylation at a small number of CpG-rich promoters of germline genes, a class of genes regulating gametic functions that need to be repressed in somatic cells. My data provide evidence that a sequence specific DNA binding transcription factor recruits DNA methylation during early embryogenesis. Further studies are needed to shed light on the mechanisms used by E2F6 to recruit DNA methylation and repress germline genes during mouse development.

Bioremediation of asbestos wastes

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The use of asbestos has been banned in France since 1997 due to its toxic effect on health. Currently, asbestos removal is a priority and we have to deal with huge amount of wastes. Only two treatments are in use, storage or transformation in glass by plasma technology. However, these methods do not eliminate the waste.

Asbestos fibers are mainly composed of silica and some metals (Fe, Mg, Al, Ni), which vary according to different species. Asbestos species possess different structures and properties. The difficulty to find a remediation process is associated with various asbestos structure and composition that react differently. The aim of my project is to develop a biotechnical process able to eliminate the toxicity of asbestos wastes by fibers dissolution. This process is based on magnesium and/or iron (essential growth elements for most bacteria) extraction from asbestos wastes. Two strategies are being considered. The first will involve bacterial siderophores, and more precisely the pyoverdine produced by *Pseudomonas* species. These molecules are microbial metabolites secreted in the environment during iron deficiency which possess a high affinity for ferric iron. The second way will involve direct contact of bacteria in the presence of asbestos wastes. The first way will allow iron extraction of asbestos whereas the second way will allow simultaneous extraction of iron and magnesium. Depending on results, a combination of these two processes could be combined for a better extraction.

Chronic oral intake of EPA:DHA 6:1 improves both the NO and EDH components of ageing-related endothelial dysfunction and vascular oxidative stress 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 EPA:DHA 6:1 being a superior formulation. The aim of the present study was to determine whether chronic intake of EPA:DHA 6:1 improves ageing-associated endothelial dysfunction, and, if so, to determine the underlying mechanisms.

Male Wistar rats (20 months-old) received daily 500 mg/kg of either EPA:DHA 6:1 (omega 3), corn oil (control), or water for 2 weeks. Young male Wistar rats (12 weeks-old) were used as control. Vascular reactivity was assessed using organ chambers.

In the main mesenteric artery, ageing was associated with an endothelial dysfunction characterized by a reduced relaxation to acetylcholine (ACh) involving a blunted NO-mediated component (in presence of indomethacin, UCL-1684 and TRAM-34) whereas the endothelium-dependent hyperpolarization (EDH, in presence of indomethacin and N^ω-nitro-L-arginine)-mediated component was abolished, and also by the induction of endothelium-dependent contractile responses (EDCF) to ACh (in presence of N^ω-nitro-L-arginine, UCL-1684 and TRAM-34) sensitive to indomethacin (a cyclooxygenase inhibitor). Endothelial dysfunction was associated with an increased level of vascular oxidative stress and expression of cyclooxygenase (COX-2) in the mesenteric artery. Chronic intake of EPA:DHA 6:1 improved both the NO- and EDH-mediated relaxations, and reduced EDCF, vascular oxidative stress and expression of COX-2 in aged mesenteric artery rings.

The present findings indicate that chronic intake of EPA:DHA 6:1 prevented the development of the ageing-related endothelial dysfunction in 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 most likely by preventing vascular oxidative stress.

Compact Polyelectrolyte Complexes as New Bioactive Biomaterials for Regenerative Medicine

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Nowadays, the development of new highly efficient biomaterials for regenerative medicine becomes of increasing importance. After the different lines of research on the bio inertness and biodegradability of these materials, the focus is now on their bioactivity. Indeed, besides the mechanical activity, the objective is now to create biomaterials which will be able to initiate a therapeutic treatment when placed in the biological environment.

A new technique of preparation of biomaterials by ultracentrifugation of synthetic polyelectrolytes has been described by J. B. Schlenoff (Florida State University) in 2009 (a). This method is based on the complexation of synthetic polyanions and polycations in the presence of salt, which acts as a plasticizing agent, forming after ultracentrifugation (Fig.1) a Compact Polyelectrolyte Complex (CoPEC) with very interesting mechanical and structural properties (b, c).

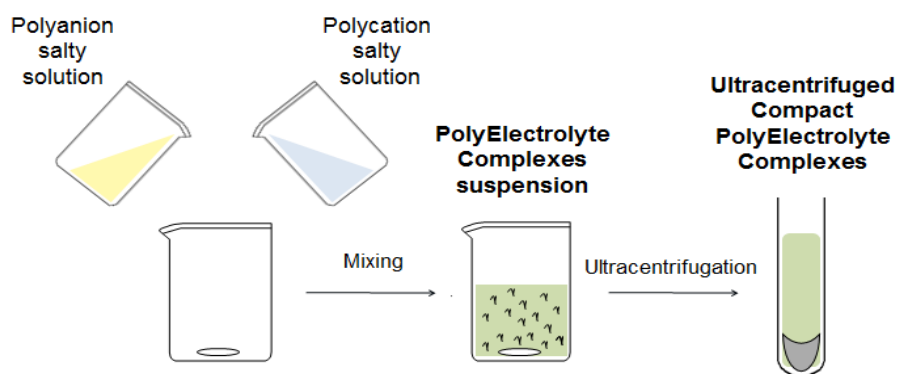


Figure 1: Representation of the preparation of a CoPEC by ultracentrifugation

Here, this technique was adapted to obtain CoPECs of natural polyelectrolytes: chitosan and alginate. Furthermore, these polyelectrolytes have been covalently modified using cyclodextrins. In this way, active principles can now be charged into the cyclodextrins and the final CoPECs prepared with these polyelectrolytes become bioactive.

First bioactive systems have been prepared using Piroxicam, an anti-inflammatory drug, as model API. *In vitro* biological studies conducted on macrophages showed that this kind of system is therapeutically efficient. Indeed, the amount of pro-inflammatory cytokines produced by activated macrophages can be reduced by using our systems while the cell viability is preserved.

Over time, this kind of bioactive biomaterials could constitute a new family of systems that would expand the list of therapeutic treatments available to fight a multitude of chronic diseases such as cancer or arthritis.

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Surface modification of fluorescent polymer nanoparticles by pluronic surfactant: tuning specific interactions with cells

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Fluorescent polymer nanoparticles (NPs) emerged recently as promising candidates for bio-imaging and theranostics due to their extreme brightness, small size, low toxicity and potential biodegradability.^[1] Ideally, NPs should feature controlled interactions and high specificity toward their target. To this end, we studied the use of pluronic F-127, an amphiphilic polymer containing polyethylene glycol, for reducing non-specific interactions and introducing specific interactions of 20 nm ultrabright polymer NPs.^[2,3] Fluorescence correlation spectroscopy and Förster resonance energy transfer allowed determining the number of pluronic molecules adsorbed per NP as well as the stability of the pluronic shell in biological media.

These NPs were then used to specifically label membrane proteins on living cells using the SNAP-tag technology.^[3] Adsorption of pluronic modified with a benzylguanine (BG) moiety gave NPs with a precisely controlled number of BGs. These NPs were used to label HeLa cells expressing adrenergic receptor fused to SNAP-tag, an engineered variant of the 20-kDa DNA repair protein alkylguanine-DNA-alkyltransferase (AGT) that covalently reacts with BG. The reaction leads to covalent labeling of the SNAP with our fluorescent NPs.

In this way simple adsorption of amphiphiles could be used to tune specific vs non-specific interactions of small polymer NPs with living cells.

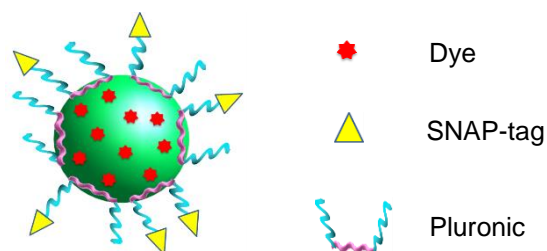


Fig. 1: Scheme of NPs with pluronic modified by SNAP-tag.

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An unexpected Role of the Basal Transcription Machinery in Limb Morphogenesis

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During my internship, I work on the wide project based on an understanding of the basal transcription machinery in embryogenesis, and more specifically on its transcription regulation role. Interestingly, the pre-initiation complex is not only required to initiate the transcription but also plays an important role in the transcription regulation. In the last study, my tutor, Stéphane Vincent, and its Ph.D. student, Paul Bardot, figured out that in absence of TAF10, a subunit of TFIID which is a general transcription factor, from E7 in mesoderm, the somitogenesis is not impaired but limb morphogenesis induction fails. More generally, lateral plate mesoderm (LPM) is more sensitive in absence of TAF10 than paraxial mesoderm (PM). Then Taf10-containing PIC in embryonic mesoderm plays some specific roles in lateral plate mesoderm integrity and commitment.

So, I interest in the specific role of the TAF10-containing pre-initiation complex (PIC) in limb morphogenesis in the mouse embryo. I use two models: T-Cre/+; Taf10^{f/f} embryos in which *Taf10* is deleted at E7 specifically in mesoderm and Rosa26CreERT2/R; Taf10^{f/f} mESCs in which *Taf10* can be deleted by the use of tamoxifen. I have two hypothesis: the first one is TAF-10 containing PIC is required for cell physiological behavior: proliferation, migration or differentiation. My second hypothesis is Taf10-less cells can't express certain limb morphogenesis factor and so the limb induction pathway is stopped.

Finally, I would like to know the TFIID composition in absence of TAF10 in LPM-like cells and PM-like cells and the TAF10-less PIC effect on transcription on these two different cell type to understand why TAF10-containing PIC is more important for LPM than PM and why it is required for limb morphogenesis induction at the molecular level.

Evaluation of the activity of CXCL12 neutraligands in different models of pain

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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 the CXCR4 antagonist, AMD3100 efficiently blocks this effect. However, AMD3100 displays several adverse side effects. An alternative strategy to block CXCL12 action is 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*. Here we used chalcone 4 to study the role of CXCL12 in different models of hyperalgesia such as acute or chronic administration of opiates (fentanyl or morphine), inflammatory pain (induced by carrageenan or CFA) and neuropathic pain (CCI, chronic constriction injury, model). We observed that in mice chalcone 4 prevents acute hyperalgesia induced by fentanyl or carrageenan. More importantly, this neutraligand is able not only to block long lasting hyperalgesia induced by chronic morphine or CFA injection but also to reverse it after establishment.

Furthermore, in a model of neuropathic pain, chalcone 4 was able to reverse the cold allodynia induced by the CCI surgery. 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, our results point out CXCL12 as a critical player in the development of hyperalgesia induced by opiates, inflammation or nerve injury and suggest that CXCL12 neutralizing compounds could represent interesting therapeutic tools for pain treatment.

Interactomic analysis of the GPCR-associated Sorting Protein GASP1 with the Ubiquitin Proteasome system

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Protein ubiquitination and its reverse reaction, deubiquitination, both play a major role at the level of the sorting endosomes in addressing G Protein-coupled Receptors (GPCRs) towards recycling or degradation pathways. GPCR-Associated Sorting Protein 1 (GASP1) interacts with the carboxy-terminal tail of numerous GPCRs and has been shown to mediate their post-endocytic sorting. But the molecular machinery involved in GASP1 function remains poorly characterized. GASP1, a large protein of 1395 residues, belongs to the GASP family : 10 proteins that share a conserved C-terminal domain of 250 amino acids (ref 1). In our previous study, we have identified a small repeated motif of 15 amino acids present in the central domain of several GASPs that is critically involved in the interaction with GPCRs (ref 2).

In the current study, we have tested the hypothesis that GASP1 could serve as a scaffold for proteins belonging to the Ubiquitin Proteasome System (UPS). We have used a library of vectors coding for half of the human UPS proteins and built to detect partner proteins using a high-throughput Gaussia luciferase Protein Complementation Assay (GPCA, ref 3,4). A reproducible luminescent signal of complementation was obtained in cell extracts co-transfected with GASP1 and 48 hits out of 589 screened proteins. Among the hits, there are fifteen E3 ubiquitin-ligases with RING domains, twenty-two proteins with BTB-POZ domains belonging to cullin complexes and five de-ubiquitinases. Several of the hit proteins are already suggested partners of GASPs and/or implicated in targeting GPCRs towards lysosomes. Using the GPCA complementation assay, we have further tested the specificity of these potential GASP1 partners against GASP2, GASP3 and GASP5 then investigated which domains of GASP1 are critical for each interaction. Taken together, our results indeed suggest a role of GASP1 as a scaffolding protein for the Ubiquitin Proteasome System.

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An anthocyanin-rich black currant extract prevents high glucose-induced senescence and dysfunction in cultured coronary artery endothelial cells

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The high glucose (HG)-induced premature endothelial senescence, in particular at atheroprone sites (i.e., bifurcations), has been suggested to contribute to endothelial dysfunction in diabetes. Indeed, high glucose promotes endothelial senescence characterized by oxidative stress and the down-regulation of the endothelial NO synthase (eNOS)-derived NO formation. The aim of the present study is to determine the ability of a black currant extract (BCE), a dietary source rich of anthocyanins, to prevent HG-induced endothelial senescence, and if so, to determine the underlying mechanisms.

Porcine coronary artery endothelial cells were cultured either under normal glucose (5 mM) or HG (25 mM) for 4 days in the absence or presence of BCE (1, 3 or 10 g/ml). BCE extract (310.0 ± 0.1 mg gallic acid equivalent/g) was prepared from a black currant juice containing 2.7 g gallic acid equivalent /L of polyphenols using a Sephadex LH-20 column. Senescence was evaluated using the senescence-associated β galactosidase (SA-gal) activity by flow cytometry and confocal microscopy. The expression of senescence-associated proteins and eNOS were analyzed by Western blot.

Exposure of coronary artery endothelial cells to HG increased SA-gal activity and the expression level of eNOS and the senescence marker p16, a cell cycle regulator. BCE prevented the HG-induced increased SA-gal activity and the overexpression of eNOS and p16.

These data indicate that BCE is able to prevent the induction of endothelial senescence in response to HG. These observations suggest that black currant anthocyanins in particular conjugated delphinidin and cyanidin, may help to prevent the HG-induced endothelial senescence and dysfunction.

Functional and structural studies of Human type II DNA Topoisomerases in Cancer treatment

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Topoisomerases are enzymes conserved from bacteria to higher eukaryotic organisms. Their main objective is to relax DNA topology and prevent topological stress as supercoils or precatenanes, during processes such as transcription or cell division. To relax DNA, topoisomerases catalyze a transient and reversible single (Type I) or double strand (Type II) break into DNA molecules [1]. Therapeutic molecules targeting this mechanism have been developed for cancer treatment [2]. Type II topoisomerases are present as 2 α and β isoforms in human. They share 70% sequence homology outside of their C-terminal domain. They both relax DNA but were identified in different cellular complexes. In the context of our project we try to understand the differences between the 2 isoforms using biochemistry and biophysics approaches. We are investigating the structure-function relationship of the C-terminal domain of both isoforms for which no structure is known.

We designed expression constructs and purified the two isoforms, from yeast and mammalian cells. We identified the phosphorylation and acetylation sites using mass spectrometry and characterized a specific position in the ATP binding pocket modified by an acetylation.

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Structure-Activity Relationship for the Discovery of a Novel, Selective and Stable CXCL12 Neutraligand with Anti-inflammatory Activity in a Murine Model of Allergic Airway Hypereosinophilia

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Chemokines are small proteins which play critical roles in the development and function of various tissues in vertebrates. They are associated with an extraordinary high number of diseases, including chronic inflammation (1), autoimmune diseases (lupus erythematosus), cancer, atherosclerosis or AIDS. We have recently opened a novel avenue for drug development in reporting a small molecule, “chalcone-4” that displays an original mechanism of action as it binds to the chemokine CXCL12, not to its two cognate receptors CXCR4 and CXCR7, and thereby neutralizes its biological activity (2). These molecules have been termed “neutraligands” by analogy with neutralizing antibodies, and proved to have therapeutic potential (3).

Herein, a systematic structure-activity relationship study around the first neutraligand of the CXCL12 chemokine, chalcone-4 (**1**), is reported. Modifications of the two aromatic rings and the central core of the hit chalcone show that pharmacomodulation of the chemotype is limited but possible. Several conformationally-restricted analogues retain high binding capacity towards CXCL12. Among them, pyrimidinone **57** (SRN-927), a newly patented neutraligand of CXCL12, displays a higher solubility than **1**. In addition, **57** is no more Michael acceptor and is much more stable in the presence of human liver microsomes than **1**. Combining improved chemical and physicochemical properties, **57** is more active than **1** in reducing eosinophil recruitment in a murine model of allergic airway hypereosinophilia, thus showing therapeutic potential for asthma.

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PARP3 in muscle stem cell differentiation

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Poly(ADP-ribosyl)ation is a post-translational modification of proteins mediated by Poly(ADP-ribose) polymerases (PARP, 17 members). Among them, PARP3 is well characterized for its specific role in double-strand break repair and mitosis. PARP3 was also shown to modulate transcription in the early stages of zebrafish development, and to participate in epithelial to mesenchymal transition and stemness in breast cancer cells.

Recent discoveries in the lab reveal an unexpected key role of PARP3 in muscle stem cell differentiation and provide important insights into the potential role of PARP3 in the repair and/or formation of new myofibers upon muscle injury or for the treatment of muscle dystrophies.

Retrieving the parameters of cryo electron microscopy dataset in the heterogeneous ab-initio case

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The Single-Particle Electron Microscopy (SPEM) is one of the main imaging modalities to study macromolecules. It enables, after reconstruction, to visualize the 3D volume of large macromolecules (1 000-10 000 atoms) with a resolution around the nanometer which is an important biological challenge. The SPEM dataset consists in a large number (10 000-1 000 000) of images, each including a tomographic projection of a single specimen of the same macromolecule. For each image, the parameters are unknown. Therefore, the reconstruction of a volume from the images obtained by SPEM is a difficult problem. If the case of a single object is well studied, the so-called heterogeneous case where the object has several states or a continuous set of states, is an active research field. In SPEM. We focus our work on the parameter estimation of continuous deformable objects without *a-priori* knowledge for *ab-initio* reconstruction methods.

We propose to estimate the parameters in a low dimensional space where the dataset is embedded by graph based non linear dimension reduction. The neighborhood graph embeds local representation of the dataset. When the dataset is too sparse or too noisy (As in SPEM) the graph contained outliers edges, called shortcuts, that compromise the low dimensional embedding. We developed a method that aims to detect and remove shortcuts in the neighborhood graph. Our method is based on the construction of a sparse graph that reveals the underlying structure of the dataset.

Evaluations on synthetic dataset shown that our shortcut detection outperform state of the art in both detection accuracy and time consumption.

Separation of delayed and parameterized sources

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This work is inspired by the study of galaxy kinematics where data are multi-spectral images. The aim is to detect the peaks present in spectrum and to estimate their characteristics (amplitude, spectral delay and width). Due to the Universe expansion and the internal gas motion, the peaks undergo different delays from one spectrum to its neighboring one (Doppler effect), therefore the peak tracking is mandatory. This inverse problem can be seen as a delayed source separation problem, where sources and mixtures are respectively associated to peaks and spectra. The challenge resides in the fact that the sources are highly correlated here. Thus, the state of the art methods are inadequate, since they suppose sources uncorrelation and independence.

In this work, we propose an original method that takes advantage of prior knowledge on the shape of the source signals. We assume that the sources can be modeled by parameterized functions, whose parameters correspond to shape information such as the width of an emission line. The separation of parameterized sources might appear to be simpler than blind source separation since the shape of the sources is known beforehand up to a few parameters. However, parameterized sources are often correlated especially if the sources are modeled with the same function. For instance, emission lines in the galaxy kinematic problem are modeled by Gaussian functions, yielding highly correlated sources.

An alternative least square scheme is proposed to estimate the source parameters: widths are estimated with the Levenberg-Marquardt algorithm. Whilst, delays and amplitudes are estimated with a sparse approximation algorithm designed for the delayed source separation model, where a source can appear at most one each mixture (an algorithm inspired from the Orthogonal Matching Pursuit algorithm). Beside, an additional interpolation step allow for continuous delay estimation. Numerical simulations demonstrate the effectiveness of the proposed algorithms compared to state-of-the-art methods for highly correlated Gaussian sources.

Polydiacetylenic Nanofibers (PDA-NFs) as new siRNA delivery vehicles for *in vitro* and *in vivo* transfections

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Diacetylenic surfactants based on a long C25 hydrocarbon chain are able to self-assemble into a variety of supramolecular forms. The diacetylenic system can be photo-polymerized upon UV irradiation forming so-called PDA (PolyDiAcetylenic) systems. Our group already described successful use of the micellar form of some of these surfactants for gene transfection (Morin et al. 2011), hydrophobic drug formulation (Neuberg et al. 2015) as well as for siRNA delivery (Ripoll et al. 2016).

Here we studied a specific supramolecular organization of achiral nanofibers (PDA-Nfs). Thanks to large scale *in vitro* screening on a model cell line (A549-luc which stably expresses a luciferase reporter gene) we could identify a positive hit molecule for siRNA delivery. We further optimized the formulation, obtaining controlled sized nanofibers. Notably, these highly stable polymerized fibers also exhibit unprecedented fluorescent properties, allowing for microscopic cellular uptake studies.

The best nanofiber batches were evaluated by Dr. Massfelder's group. They used the PDA-NFs to silence the developmental transcription factor Lim1, a new oncogene that they have recently identified in human kidney cancer, *in vitro* and *in vivo*.

The PDA nanofibers were evaluated as new delivery vehicles for anti-Lim1 siRNA, for the design of innovative therapeutic tools.

In vitro, the PDA-NF-siLim1 efficiently silenced by more than 90% the expression of the Lim1 oncogene in the 786-O human kidney cancer cell line. *In vivo*, in nude mice bearing subcutaneous 786-0 tumors, the i.p. administration of the nanofibers also efficiently inhibited by 75% Lim1 expression in tumors.

Taken together, we found that PDA-NFs are promising siRNA vectors. Further evaluation and optimization studies for *in vivo* RNA interference are underway.

Note : This PDA-Nanofiber system is patented in 2016 under : EP16305367

Efficient access to novel 3D-shaped scaffolds derived from aza-diketopiperazines

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To date, high throughput screening represents one of the most efficient methods to find new leads in medicinal chemistry. However, chemical libraries are mainly made up of unsaturated, aromatic and planar compounds which are less likely to succeed in clinical trials than saturated ones.[1] Indeed, increasing saturation in molecules results in more complex 3D-shaped structures with greater potential to better complement the spatial subtleties of target proteins and also to improve pharmacokinetic properties. In addition, it has been recently reported that the number of stereogenic centers increases from early hit discovery to late drug candidate stages, highlighting the importance of molecular complexity in drug development.[2]

In this context, for many years our group has been involved in the design of efficient synthesis of original and complex 3D-shaped molecules containing sp³ hybridized carbons and stereocenters with potential application in medicinal chemistry.[3] Herein, we will present a rapid and atom economical multicomponent synthesis of complex 3D-shaped aza-diketopiperazines (aza-DKPs). This one-pot process enabled the formation of six bonds and a controlled stereocenter from simple starting materials leading to the conception of a small chemical library of diversely substituted bicyclic azaDKPs.

In addition, we will discuss about the 3D molecular descriptors and “drug-likeness” properties of these novel scaffolds in order to highlight their originality in the chemical space of existing scaffolds and also their great potential in medicinal chemistry.[4]

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

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RF-amide neuropeptides act through 5 different GPCRs and are characterized by their conserved Arg-Phe-NH₂ C-terminus. They have been shown 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 hand, RF-amide related peptides (RFRPs)/NPFF1R system recently emerged as an inhibitor of reproduction in different species including mammals. A misregulation of this system may lead to a default of puberty and a disruption of the switch to different reproductive states of seasonal animals. Moreover, this receptor has also been implicated in the modulation of nociception and opiates analgesia. However, the study of the involvement of RFRPs/NPFF1R system in reproduction and other functions is severely limited by the absence of pharmacological tools, particularly selective antagonists.

In this study, we screened a large focused chemical library of compounds (> 2000 molecules) that mimic the RF-amide sequence and identified several hit molecules that bind NPFF receptors. Further optimization allowed us to identify several derivatives that display nanomolar affinity on NPFF1R and very weak binding potency on the other RF-amides receptors. From this study, we selected the best compound (cpd 8b) and showed that it efficiently blocks, in a dose-dependent manner, the inhibition of cAMP accumulation induced by activation of NPFF1R but not NPFF2R, with a pA₂=18nM. *In vivo*, this compound fully blocked the RFRP-3-induced hyperalgesia when injected centrally as well as fentanyl-induced hyperalgesia when injected subcutaneously while it had no effect on the reversion of morphine-induced analgesia by NPFF. In the hamster, cp 8b prevented the secretion of the luteinizing hormone induced by RFRP-3.

Altogether, our results indicate that we have identified a potent and selective antagonist of NPFF1R that will be very helpful to elucidate the role of RFRPs/NPFF1R system *in vivo*. Moreover, our data point out NPFF1R as a critical player in the modulation the adaptation associated with opiate administration.

pH responsive polydiacetylenic micelles allow for synergistic co-delivery of intracellular siRNA and anticancer drug

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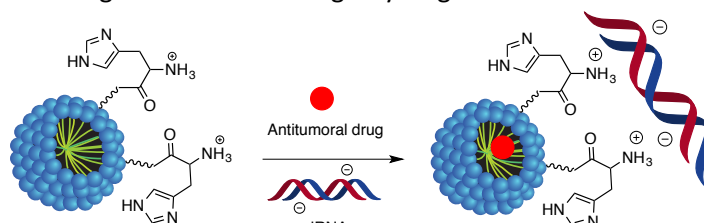
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The delivery of siRNA has revealed particularly interesting because of their great therapeutic potential in pathologies in which down-regulation of a specific mRNA lead to a beneficial effect. However as usual drugs, nucleic acid therapy also faces drug resistance issues, which could be overcome by adopting a drug-nucleic acid combination approaches.

To this end, the development of modern nanocarriers able to deliver simultaneous drugs has known 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 drugs¹.

To overcome this problem, our group developed a new generation of photopolymerized diacetylenic amphiphile micelles (PDA) able to efficiently deliver the siRNA into the cytoplasm by induce the so-called "proton sponge effect"². Our system reveals itself as a promising nanovector with up to 80 % of specific inhibition of gene in a reporter system but also a great carrier for hydrophobic and water insoluble drugs such as camptothecin (CPT)³. Then, we have extended this system to the co-delivery of these two therapeutic drugs to succeed having a synergistic effect in *in vitro* experiments.



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 tissues⁴, which is an attractive feature for *in vivo* studies.

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Charge-controlled nanoprecipitation for the preparation of fluorescent dye-loaded polymer nanoparticles

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Due to their flexibility, biocompatibility and ease of use, dye-loaded fluorescent polymer nanoparticles (NPs) have attracted growing interest in bioimaging over the past years [1]. The size control of these NPs is essential in order to obtain particles small enough to visualize and track small structures or molecules inside living cells.

In this study, we show that the presence of charged groups on polymers can be used to control the particle size. For this purpose, poly(lactide-co-glycolide) (PLGA), polycaprolactone (PCL) and poly(methyl methacrylate) (PMMA) were functionalized with charged groups such as carboxylate, sulfonate and ammonium, before being used for the preparation of NPs by nanoprecipitation. Using this approach the particle size could be decreased to less than 15 nm [2]. Moreover, these small NPs enabled encapsulation of high amounts of different chromophores, such as salts of a cationic dye with bulky hydrophobic counterions [3] or lanthanide complexes, leading to ultrabright NPs. Lastly, the adsorption of PEGylated Tween 80 and pluronic F-127 led to the formation of a thin shell on the particle surface ensuring the stabilization of our NPs in biological media.

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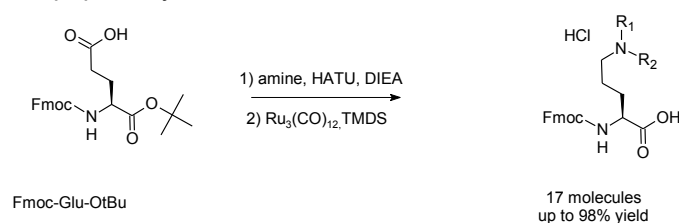
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Coupling reaction of non-natural basic amino acids

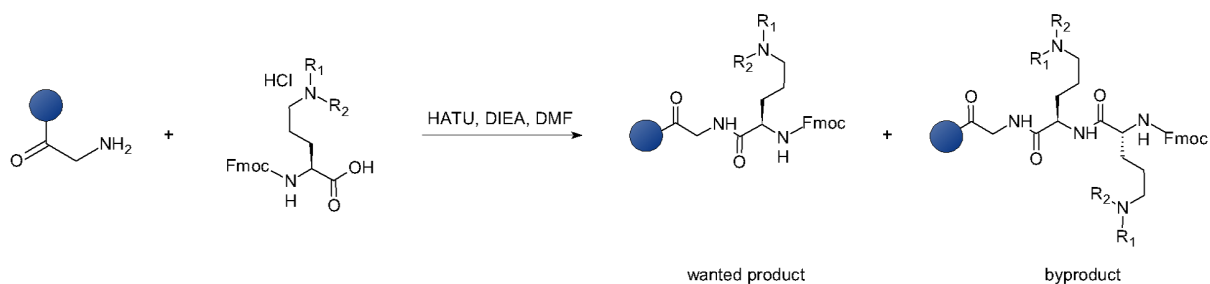
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We previously reported an efficient and selective Ruthenium-catalyzed amide reduction allowing the rapid production at the gram-scale, of original non-natural basic amino acids, readily available for liquid- or solid-phase peptide synthesis.^{1, 2}



These new amino acids show a particular reactivity in classical coupling reaction conditions, the basicity of the side chain is sufficient to deprotect the Fmoc group, leading to a second coupling reaction.



We performed an optimization process leading to conditions affording the desired peptide in high selectivity.

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Photophysics of Thienoguanosine Tautomers: Application to the labeling of HIV-1 (-) Primer Binding Site sequence

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The design and successful implementation of fluorescent nucleobase analogues is a precarious art because it requires minimal structural and functional perturbation upon substitution in oligonucleotides. Recently a fluorescent Guanosine analog, Thienoguanosine (thG) (1), was developed which showed respectable quantum yield as free probe and incorporated in single and duplex structures, and faithfully mimicked the W-C base pairing (2).

Dwelling into photophysics of free thG nucleoside revealed the existence of two ground state tautomers with shifted absorption and emission spectra (3). They were identified as thG-H1 and thG-H3 keto-amino tautomers by DFT calculation. Microenvironment sensitivity of both the tautomers revealed that the equilibrium between the two tautomers are dependent on the hydrogen bond ability of the solvent. When incorporated in (-)PBS, the (-)DNA copy of HIV-1 primer binding site, both tautomers are observed. But in duplex (-)/(+)PBS, dthG-H1 tautomer is favored forming a stable W-C base pairing which was also supported by MD calculations showing that dthG-H1 forms three canonical hydrogen bonds.

Meanwhile, comparison of matched with mismatched (-)/(+)PBS duplexes further revealed that the relative emission of thG-H3 can be used to detect single molecule polymorphism.

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Metal-Free, Dioxygenation of Styrenes with Molecular Oxygen in Water

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The environmental impact of chemical industry is a major issue for our society. One of the main research field is the development of alternatives to organic solvents, with expected outputs in terms of health, safety and environmental impacts. The development of organic synthesis in aqueous medium using surfactants appears as a promising ecofriendly strategy. Indeed, thanks to their amphiphilic nature, surfactants in water undergo spontaneous self-assembly into micelles, which act then as nanoreactors, as their hydrophobic cores play the role of reaction vessels in which the organic transformation involving water-insoluble reagents can occur.[1]

By employing Triton X-100 as a surfactant, tert-butyl hydroperoxide-mediated dioxygenation of styrene with molecular oxygen and N-hydroxyphthalimide was developed in water. The new method ran under mild (25 °C) and eco-friendly (O₂, water) conditions, a good yield (~80%) was obtained. It was also applicable for wide range of styrene derivatives with a variety of functional groups. Some of these synthesized compounds can be further deprotected, leading to interesting diols or alkoxyamines.

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Induction of immunogenic cell death by innovative antitumoral platinum(II) compounds

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Some cancer treatments like chemotherapeutic agents (anthracyclines, platinum derivatives,...) or different forms of radiotherapy cause a particular type of cell death able to activate the immune system: the immunogenic cell death (ICD). During this process, the exposition of the endoplasmic reticulum chaperone calreticulin, as well as the release of ATP and non-histone chromatin-binding protein high mobility group box 1 (HMGB1) serve as immunostimulatory damage-associated molecular patterns (DAMPs). These molecules are able to increase the immune response, as they are not exposed or released under physiological conditions. Recently, we focused on *N*-heterocyclic carbenes (NHC), a family of ligands able to stabilize transition metals, like platinum, to develop innovative metal-based antitumoral drugs.(1) Even if several research teams, including ours, had observed *in vitro* a cytotoxic activity of these platinum complexes (NHC-Pt) on cancer cells, they appeared to be inappropriate for *in vivo* experiments because of their weak solubility under biological conditions.(2-3) Hence, to overcome this limitation, we created multivalent cationic platinum compounds starting from linear polyethylenimine (PEI), a polymer used as transfection agent. First studies show that these NHC-Pt(II)-PEI particles induce apoptosis of tumor cells *in vitro* and *in vivo* in xenograft immunodeficient mouse model with no observable side effects.(1)

In order to evaluate the potential implication of the immune response on the NHC-Pt(II)-PEI *in vivo* effect, we treated immunocompetent mice bearing tumors with NHC-Pt(II)-PEI particles and we observed a significant antitumoral effect of the conjugates, in the same range than the clinical used platinum drug oxaliplatin, but with less side effects. We, then, evaluated if NHC-Pt(II)-PEI conjugates could induce ICD. First results indicate that the NHC-Pt(II)-PEI lead *in vitro* to the exposition of calreticulin at the surface of dying tumor cells. Moreover, it seems that our Pt(II) complexes are also able to generate the release of ATP by the cancer cells. These considerations suggest that our compounds induce ICD and activate the immune system.

Altogether, our results reveal the possibility of creating Pt(II) derivatives that can be used as chemotherapeutic agent by killing the tumor cells and as immunotherapeutic agent by triggering the antitumor immune response.

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UHRF1 inhibitors targeting the epigenetic pattern in cancer cells.

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UHRF1, a multi-domain protein was shown to serve as an essential epigenetic regulator for the faithful maintenance of DNA methylation pattern. UHRF1 recognizes specifically hemi-methylated DNA via its SET and RING-associated domain (SRA) that flips out the methylated cytosine (5-mC), and then recruits DNMT1 for the maintenance of methylation on the daughter strand.[1] UHRF1 also plays an important role in promoting proliferation, whereas its knockdown controls cell cycle transition, apoptosis. Many studies revealed that targeting UHRF1 by si-RNA, small molecules, and natural compounds in human cancer cell lines shows antitumor activities.[2]

In this work, we sought to develop and screen for small molecules targeting SRA domain, the 5-mC binding site of UHRF1, due to its critical role in recognizing hemi methylated DNA and recruiting DNMT1. The study has revealed one active compound with IC50 in the micromolar range that affected UHRF1 interaction with DNMT1 and their expression levels. This compound has also altered DNA methylation status by exhibiting anti-cancer activity with subsequent cell cycle arrest via up regulation of p53. Thus, our approach using chemical inhibitors of the UHRF1 might help us not only to disclose new clues for anticancer therapy, but also to better understand the role of UHRF1 in the replication of epigenetic codes.

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Novel Approach to Enhance The Metabolic Stability of GPCR Peptide Ligands by Means of Fluorocarbon Conjugates: Application to Apelin

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Over the past decade, peptides have shown an increasing interest for therapeutic applications. To date, 60 therapeutic peptides have been already approved by the FDA, 140 are currently under evaluation in clinical trials and 500 are in preclinical development [1]. In general, peptides are selective and efficacious signaling molecules that bind to specific cell surface receptors, such as G protein-coupled receptors (GPCRs) which represent a family of choice for the development of new therapeutics with more than 30% of all drugs presently on the market targeting these receptors [2]. However, peptides are often not directly suitable for use as convenient therapeutics because they have intrinsic weaknesses, including poor chemical and physical stability, and a short *in vivo* half-life due to rapid enzymatic degradation [3,4].

To enhance the metabolic stability of GPCR peptide ligands, we propose an unprecedented strategy based on the grafting of fluorocarbon chain (F-chain) onto the peptide. The idea was to induce a self-organization of the fluoro-peptide in aqueous solution resulting in the subsequent protection of the native peptide from enzymatic degradation. To demonstrate the efficacy of our approach the apelin 17 peptide, a neuro-vasoactive peptide which presents a short plasma half-life, was selected as model [5,6]. Different F-chains were then grafted onto apelin 17 following a solid-phase approach. The human plasma stability of the resulting fluoro-peptides was carefully investigated. To gain insight into the mechanism leading to the increase of human plasma stability, the physicochemical and plasma binding properties of the optimized fluoro-peptide LIT01-196 were then investigated. Finally, the *in vivo* evaluation of LIT01-196 in rats demonstrates the positive impact of F-chains to greatly improve the *in vivo* efficacy of the native peptide (see poster presented by Flahault *et al.*)

Alltogether, these promising results should open the route to a convenient, safe and general approach to greatly increase the metabolic stability of numerous native peptides for their *in vivo* use as pharmacological tools and/or therapeutic agents.

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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 16 years ago, the "Plate-forme de Chimie Biologique Intégrative 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.