RNAPII non paused genes are preferentially downregulated in the striatum of Huntington's disease mice

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Huntington's disease (HD) is a progressive neurodegenerative disease, affecting preferentially the striatum. Transcriptional dysregulation is an early and central mechanism of HD pathogenesis. We hypothesized that the mechanism underlying transcriptional dysregulation in HD might include alterations of chromatin structure at regulatory regions. To evaluate this hypothesis, we examined the level at chromatin of RNAPII and H3K27 acetylation (H3K27ac), a histone mark associated with active enhancers and promoters. We used chromatin immunoprecipitation followed by massive parallel sequencing (ChIP-seq) on the striatum of HD R6/1 transgenic and control mice. Differential analysis of the RNAPII and H3K27ac ChIP-seq data between R6/1 and control striatum revealed many significant changes. Importantly, the enriched biological processes, cellular components and molecular functions were predominantly involved in neuronal functions. By using the SeqMINER programme, we found that the profiles of RNAPII and H3K27ac at genes were highly correlated. The data revealed that a high RNAPII pausing index (i.e. a high level of RNAPII at TSS relative to gene body) correlated with a localization of H3K27ac restricted to the promoter region. In contrast, a low RNAPII pausing index correlated with a distribution of H3K27ac covering the gene body, suggesting that RNAPII non-paused genes are regulated by large enhancers. Gene ontology analysis indicated that RNAPII non-paused genes were predominantly involved in neuronal processes. To further analyze our ChIP-seq data, we performed RNA-seq experiments using the striatum of R6/1 and control mice. The results were consistent with previous transcriptomic data using the striatum of HD mice and patients. Changes were extensive and deregulated genes were predominantly down-regulated and highly enriched in genes involved in neuronal functions. Interestingly, combined analysis of ChIP-seq and RNA-seq data showed that RNAPII non-paused genes were preferentially down-regulated, indicating that the probability of a gene to be down-regulated is determined by its transcription dynamics. In addition, H3K27ac and RNAPII at down-regulated genes were significantly decreased in R6/1 striatum compared to controls. Our results suggest that preferential down-regulation of non-paused genes in HD striatum leads to alteration of neuronal functions, due to decreased H3K27ac at enhancer regions. Therefore, targeting H3K27 might be an effective therapeutic strategy.

Lipid emulsions used in parenteral nutrition induce endothelial dysfunction in porcine coronary artery rings: Role of oxidative stress and cyclooxygenase-derived vasoconstrictors

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

Porcine coronary arteries were incubated with lipid emulsions 1% v/v for 30 minutes before vascular reactivity determination in organ chambers and oxidative stress level using the redox-sensitive fluorescent dye dihydroethidium (DHE). Coronary artery rings incubation with Lipidem®, Medialipid® or Smoflipid® (mixture of medium- and long-chain triglyceride), but not with Intralipid® or Clinoleic® (long-chain triglycerides), significantly reduced the bradykinin-induced endothelium-dependent relaxations mediated by both nitric oxide (NO) and endothelium-dependent hyperpolarization (EDH). In contrast, Lipidem® did not affect endothelium-independent relaxations to sodium nitroprusside. The endothelial dysfunction induced by Lipidem® was significantly improved by indomethacin, a cyclooxygenase (COX) inhibitor, and by inhibitors of oxidative stress (n-acetylcysteine, superoxide dismutase, catalase) and transition metal chelating agents (bathocuproine, tetrathiomolybdate, desferrioxamine and L-histidine,). Lipidem® significatly increased the vascular oxidative stress as indicated by increased DHE signal throughout the arterial wall. The present findings indicate that several but not all lipid emulsions induce an endothelial dysfunction in coronary artery rings, involving both blunted NO- and EDH-mediated relaxations. The Lipidem®-induced endothelial dysfunction is associated with increased vascular oxidative stress and the formation of COX-derived vasoconstrictor prostanoids.

Rational design of ligands targeting GPCR heterodimers – application to V1B CRHR1 dimer in the treatment of stress, anxiety and depression

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G protein-coupled receptors (GPCRs) are a family of membrane proteins whose function is to activate specific cellular signaling pathways in response to extracellular stimuli. They are involved in many physiological and pathological processes and are the target of 40% of drugs currently on the market [1]. In the past decades, many studies have demonstrated that some GPCRs are able to cross-react, forming homo-, hetero-dimers and even higher ordered oligomers [2].

Arginine vasopressin receptor 1B (V1BR) and Corticotropin-releasing factor receptor 1 (CRFR1) are involved in the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Both receptors are activated in the hippocampus by two peptide hormones: arginine-vasopressin (AVP) for V1BR and corticotropin-releasing factor (CRF) for CRFR1. Once activated by their respective hormones, these two receptors synergistically regulate adrenocorticotropic hormone (ACTH) release from the pituitary gland, as a functional adaptation to stress. Experimental data suggests that V1BR and CRFR1 receptors can form heterodimers with a pharmacology differing from that of isolated receptors [3]. One can assume that there is a physiopathological relevance for their physical association.

In order to demonstrate this association, heterobivalent ligands targeting both receptors in their heterodimeric forms are currently being designed in our laboratory (D. Bonnet). Finally, *in vitro* and *in vivo* studies will be conducted by our biological partners (G. Guillon, IGF Montpellier).

This poster will summarize all the steps performed to design heterobivalent ligands:

- Homology modeling of the V1B receptor
- Molecular docking of several known V1B receptor antagonists
- Consistency of docking results with SAR (Structure Activity Relationship) data
- Studying of the available CRFR1 X-ray structure [4] by molecular dynamics to confirm that a bivalent ligand will effectively be able to access the binding pocket.

In a next step, we will model the V1BR-CRFR1 dimer, on the basis of available site directed mutagenesis data, to depict the dimer interface and propose chemical linkers needed to join previously docked antagonists in their respective pocket and therefore design the final heterobivalent ligands.

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[1] Overington JP, Al-Lazikani B, Hopkins AL (2006). How many drug targets are there? *Nat Rev Drug Discov,* 5, 993-996

[2] Hiller C, Kühhorm J, Gmeiner P (2013). Class A G-Protein-Coupled Receptor (GPCR) Dimers and Bivalent Ligands. *J Med Chem,* 56, 6542-6559

[3] Murat B, Devost D, Andrés M, Mion J, Boulay V, Cobani M, Zingg HH, Guillon G. (2012) V&b and CRHR1 receptor heterodimerization mediates synergistic biological actions of vasopressin and CRH. *Mol Endocrinol*, 26, 502-250

[4] Hollenstein K, Kean J, Bortolato A, Cheng RK, Doré AS, Jazayeri A, Cooke RM, Weir M, Marshall FH. (2013) Structure of class B GPCR corticotropin-releasing factor receptor 1., Nature, 499, 438-443

Role of the TFIID subunit TAF10 in transcription during mouse development

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Somite formation, called somitogenesis, during vertebrate segmentation is controlled at the spatial and temporal levels. Segments are progressively added from the anterior tip of the presomitic mesoderm (PSM): in mouse, a pair of somite is added every two hours. Such process relies on molecular oscillations of genes related to the Notch, Wnt and Fgf pathways. This is thought to occur by the cyclic transcription of repressors, leading to the periodic activation of these pathways. As somitogenesis requires a precise regulation of gene expression, it represents an interesting experimental model to study transcription. Here we focus on the core machinery of transcription during this process. The pre-initiation complex formation requires RNA Pol II recruitment at the promoter by general transcription factors (GTFs), including the TFIID complex. Canonical TFIID complex is composed of TBP and 13 TBP associated factors (TAFs), but there are some indications that there is some flexibility in the composition of this complex. In particular, TAF10 is necessary for transcription during embryonic development and dispensable in the adult. To understand its role during somitogenesis, we combine embryo studies using *Taf10* mouse mutant lines and mouse embryonic stem cells (mESC) differentiated in a PSM-like state. *In vivo*, induction of *Taf10* deletion by tamoxifene injection at E7.5 impairs embryonic growth at E9.5. Unexpectedly, whereas *Taf10*-null murine F9 embryonal carcinoma cells or *Taf10* depleted inner cell mass undergo apoptosis, the absence of *Taf10* in mESC is viable but impairs proliferation. Moreover, differentiation into PSM-like cells is not altered by the loss of *Taf10*. Future investigations aim to further determine what the role of TAF10 is, and more generally, how TFIID composition varies during development.

PARP3 affects the relative contribution of homologous recombination and nonhomologous end-joining pathways

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Poly(ADP-ribose) polymérase 3 (PARP3, also known as ARTD3) is a newly characterized member of the PARP family that catalyzes the reaction of ADP-ribosylation, a key post-translational modification of proteins involved in different signaling pathways such a DNA damage, cell cycle progression, transcription or energy metabolism. Recent studies identified PARP3 as a novel actor in cell response to DNA damage (Boehler et al. PNAS 2011). Here we define PARP3 as a specific player in cellular response to DSBs (double strand breaks). The toxic DSB lesions are repaired by either homologous recombination (HR) and classical or alternative nonhomologous end-joining (C-NHEJ versus A-EJ). Because these pathways compete for the repair of DSB, the choice of the appropriate repair pathway is pivotal. Among the mechanisms that influence this choice, DNA end-resection plays a critical role by driving cells to HR while accurate C-NHEJ is suppressed. We first confirm that PARP3 is involved in DSBs repair and promotes C-NHEJ. We next demonstrate that PARP3 together with Ku80 limits DNA end-resection and consequently helps to drive the choice between HR and NHEJ pathways. The depletion of PARP3 impairs the recruitement of Ku80 to laser-induced DNA damage sites and controls the balance between 53BP1 and BRCA1 foci formation in response to DNA damage. Together, these events induce an increase of DNA end-resection and a defect in C-NHEJ. Upon PARP3 knockdown, HR is reduced and there are mutagenic resection-mediated deletions during A-EJ. Together these results identify PARP3 is a key actor in the balance between HR and NHEJ pathways.

Functional and Structural studies of the cellular complexes associated to human DNA topoisomerases, targets of chemotherapeutics agent.

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Type 2 DNA topoisomerases (Top2) are essential proteins that regulate DNA topology during cell division, replication, transcription and chromosome replication. The human Top2 is targeted by anti-cancer agents used in chemotherapy. Most of them induce the formation of ternary complexes between Top2-DNA and the drug. These complexes are then converted into DNA breaks leading to cell death. In human cells, two isoforms are encoded by different genes. The α isoform is overexpressed during cell proliferation and is a hallmark in certain types of cancer. The β isoform was discovered later and several studies suggest that this isoform could be involved in the development of secondary malignancy after treatment by anti-topo compounds such as etoposide. The two isoforms share high sequence similarity and a similar structural organization but mostly diverge on their C terminal domain. The structure and the functional role of this domain is not well understood since no structural information on this domain alone or in the context of the full length enzyme is available yet.

Furthermore, the Top2 enzymes are part of large cellular complexes whose role in disease processes are still to be elucidated. The identification of potential drug targets among proteins associated with the Top2 complexes could help to elaborate efficient multi-therapy and decrease the secondary effect and malignancy of drugs. In this context, we combine the proteomic analysis of the cellular partner of Top2 enzyme and the structural analysis of these complexes. We are using cryo electron microscopy to analyse the full length Top2α enzyme in complex with DNA and drugs, and a chemoproteomic approach to identify the Top2 cellular complexes targeted by drugs.

We will present here the production in yeast and the purification of the two isoforms of this large enzyme (340 KDa) that are prepared for functional and structural studies, and the analysis of their post-translational modifications. Our recombinant enzyme is used to validate the chemical probes derived and synthetized by our collaborators in organic chemistry prior to pulldown experiments on cancer cell lines. This project is the first step to characterize large complexes centered around Top2 and targeted by drugs. We will also present our preliminary study on the three dimensional reconstruction of the full length enzyme complex with DNA and etoposide using single molecule reconstruction by cryo electron microscopy.

Role of alpha5 beta1 integrin in EGFR oncogenic activity in glioblastoma

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Introduction:

Glioblastoma multiforme (GBM) is the most common primary brain tumor. Amplification and mutation of the epidermal growth factor receptor (EGFR) is detected in about 50% of patients with GBM. Despite the well-recognized oncogenic activity of EGFR, clinical trials using anti-EGFR therapies for the treatment of GBM reveal limited efficacy. Our team previously showed that overexpression of the fibronectin receptor, alpha5 beta1 integrin, is associated with a poor prognosis for patients and that the integrin triggers resistance to chemotherapy. It is well established that integrins can cross-talk with tyrosine kinase growth factor receptors to promote cell growth survival and migration. The aim of my thesis is to characterize the functional interaction between EGFR and alpha5 beta1 integrin in GBM cells and to determine its potential involvement in resistance to anti-EGFR targeted therapy.

Results:

Using three different glioblastoma cell lines, we first showed by western blotting that cell attachment to fibronectin potentiates EGF signaling (Erk/MAPK and PI3K/Akt pathways). To assess whether EGFR activity might be modulated by alpha5 beta1 integrin, we genetically modified U87-MG cell line to modulate alpha5 integrin expression. Our data indicate that alpha5 beta1 integrin overexpression potentiates EGFR signaling cascade. Importantly, we showed that integrin/EGFR signaling crosstalk prevent Erk inactivation by a clinically approved anti-EGFR drug (mAb CetuximabTM/ErbituxTM).

We next examined the impact EGFR/alpha5 signalling on crosstalk using 2D cell proliferation assays. The expression level of alpha5 had no impact on cell proliferation or cell sensitivity towards Cetuximab of GefitinibTM (an anti-EGFR TKI). By contrast with experiment on culture plates, overexpression of alpha5 increased cell growth in 3D soft agar anchorage-independent colony formation assay. While growth of alpha5 integrin low expressing cells were inhibited by CetuximabTM , we surprisingly observed that low dose of CetuximabTM increased colony size of U87 overexpressing the integrin. Finally, using Boyden chamber assay (chemotaxis induced by serum), we showed that combination of CetuximabTM and an alpha5 selective antagonist (an RGD derived chemical compound K34c) was required to efficiently block U87 MG cell migration.

Conclusion:

Our results revealed that alpha5 beta1 integrin potentiate EGFR signaling and may trigger resistance to anti-EGF therapy in GBM cells. Future experiments will show if antagonists targeting alpha5 beta1 can sensitize GBM to CTX therapy to impair glioma cell invasion, growth and survival. Ongoing studies based on immunofluorescence assay and confocal microscopy, have focused on EGFR and alpha5beta1 localisation in U87 MG cells. We will address the putative formation of a physical complex between EGFR/alpha5 beta1 integrin by FRET-FLIM microscopy on cell expressing genetically encoded fluorescent proteins. We will thus test the impact of EGFR and integrin antagonists on EGFR/alpha5 beta1 complex formation in GBM and determined in the future if this molecular complex is expressed in tumor samples.

Buchwald–Hartwig reactions in water using surfactants

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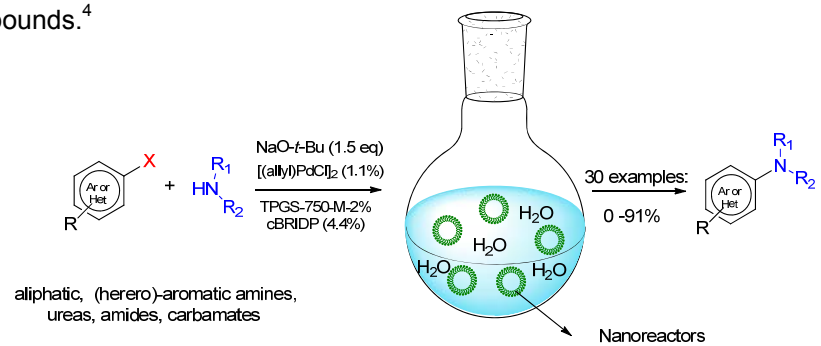
Nitrogen-containing heterocyclic compounds are key structural cores of various bioactive natural products, medicinally important compounds, and organic materials and thus, of great biological and chemical significance. Transition-metal assisted amination of aryl (heteroaryl) halides has been developed as the most viable and direct method leading to the syntheses of a large variety of substituted amines in recent years. With an ever-increasing deployment of these reactions in synthesis, the development of cross-coupling catalysts should be coordinated with the principles of green chemistry.

Conducting transition metal-catalyzed cross-coupling chemistry in water instead of organic solvent has a number of potential benefits in terms of cost, environmental impact, safety, and impurity profiles. Increasing focus on the "green-ness" of chemical processes has further promoted recent development in this field. One solution that has been applied to a broad range of transition-metal-catalyzed processes is the use of small amounts of a nanomicelle-forming amphiphile in water which provides a lipophilic medium where cross-coupling reactions can take place. Recently, Lipshutz[2] developed safer surfactants, polyoxyethanyl-α-tocopheryl succinate (TPGS-750-M), to serve as nanoreactors. In particular, the second-generation surfactant, TPGS-750-M, forms nanomicelles in water, that are lipophilic on the inside and hydrophilic on the outside, and allowed efficient Pd-catalyzed cross-coupling reactions. Unfortunately, for the interesting Buchwald–Hartwig cross-coupling reactions, only *N*-arylation of aniline derivatives has been reported.[3] The scope and limitations of the Buchwald–Hartwig reaction in a micellar medium, as described earlier by Lipshutz, by reacting various (hetero)aryl halides with diverse nitrogen coupling partners will be presented on a wide variety of *N*-containing compounds.[4]



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[2] (a) Lipshutz, B. H.; Ghoraib, S. *Aldrichimica Acta.* **2008**, *41*, 59-72; (b) Lipshutz, B. H.; Ghoraib, S. *Aldrichimica Acta* **2012**, *45*, 3-18

[3] (a) Lipshutz, B. H. ; Chung, D. W.; Rich, B. *Org. Lett.*, **2008**, *10*, 3793. (b) Lipshutz, B. H.; Ghorai, S.; Abela, A. R.; Moser, R.; Nishikata, T.; Duplais, C.; Krasovskiy, A.; Gaston, R. D.; Gadwood, *J. Org. Chem.* **2011**, *76*, 4379.

[4] Salomé, C.; Wagner, P.; Bollenbach, M.; Bihel, F.; Bourguignon, J.-J.; Schmitt, M. *Tetrahedron* **2014** (accepted)

General linear models for group studies in diffusion tensor imaging

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The degenerative diseases of the human central nervous system are an important clinical issue. Neurodegenerative diseases affect the cerebral white matter integrity, which may now be probed *in vivo* using diffusion tensor imaging (DT-MRI) [1]. Characterizing those pathologies and its evolution provides new information for the diagnosis and the prognosis of doctors. It can be done by comparing a group of healthy subjects *vs* cohorts of patients, according to neuroimaging data, clinical data and cognitive scores.

It usually performed on scalar images derived from DT-MRI such as Fractional Anisotropy (FA) or Mean Diffusivity (MD) using either region of interest-based analysis, the voxel-based analysis framework provided in SPM [3] or the tract-based spatial statistics (TBSS) method [4] provided in the FSL library. However, none of these methods can handle all the information contained in the tensor images and thus cannot detect all kind of changes, such as modification of the diffusion orientation. Some methods proposed to perform the comparison using multivariate statistical tests on several scalar indices simultaneously or to compare eigenvalues or eigenvectors of diffusion tensors [5]. However, these approaches cannot include clinical data in the statistical analysis. To the best of the author's knowledge, only the work of Zhu *et al.* [6] has carried out multivariate regression analysis and statistical testing on diffusion tensors. The framework proposed in [6] is versatile and accounts for the positive definiteness of diffusion tensor by estimating a generalized linear model on a Riemannian manifold. Unfortunately, the regression step suffers from being computationally intensive.

In this context, we extended the general linear model, as implemented in SPM [3], to tensor images. This model has the advantage of being both versatile and computationally effective. We investigated the relevance of the whole tensor information compared to scalar indices only by a simulation framework based on DT-MRI acquisitions of healthy subjects in which different kinds of lesions have been introduced. Results on a cohort of patients suffering from neuromyelitis optica (NMO) are also analysed [7].

1. C. Yu, F.Lin, K. Li, T. Jiang, W. Qin, H. Sun and P. Chan, « Pathologenesis of normal-appearing white matter damage in neuromyelitis optica : Diffusion-Tensor MR Imaging », Radiology, vol. 246, no. 1, pp. 222-228, 2008.
2. M. Cercignani, *Strategies for Patient-Control Comparison of Diffusion MR Data*, Oxford University Press, 2010.
3. W.D Penny, K.J Friston, J.T Ashburner, S.J Kirbel and T.E Nichols, *Statistical Parametric Mapping : The Analysis of Functional Brain Images*, Elsevier LTD, Oxford, 2006.
4. S.M Smith, M. Jenkinson, H. Johansen-Berg, D. Rueckert, T.E Nichols, C.E Mackay, K.E Watkins, O. Ciccarelli, M.Z Cader, P.M Matthews, T.E.J Behrens, « Tract based spatial satistics : voxelwise analysis of multi-subjects diffusion data », NeuroImage, vol. 31, no. 4, pp. 1487-1505, 2006.
5. A. Schwartzman, R.F Dougherty, J.E Taylor, « Group comparison of eigenvalues and eigenvectors of diffusion tensors », *Journal of the American Statistical Association*, vol. 105, no. 490, pp. 588-599, 2010.
6. H. Zhu, Y. Chen, J.G Ibrahim, Y. Li, C. Hall, W. Lin, « Intrinsic regression models for positive-definite matrices with applications to Diffusion Tensor Imaging », *Journal of the American Statistical Association,* vol. 104, no. 487, pp. 1203-1212, 2009.
7. A. Bouchon, V. Noblet, F. Heitz, J. Lamy, F. Blanc, J.P Armspach, « General linear models for group studies in diffusion tensor imaging », ISBI 2013 in press.

Assistance to High Intensity Focused Ultrasound (HIFU) therapy: Real-time motion compensation using ultrasound imaging

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HIFU therapy is a very promising non-invasive and non-ionizing method for ablation of tumors[1]. HIFU therapy relies on the absorption of the acoustic energy at the focal region, leading to localized tissue heating and necrosis subsequently. Some applications of this therapy such as ablation of prostate tumors and of uterine fibroids are already used in clinical routine. Other applications such as tumor ablation in intra-abdominal organs are still under research. The major problem of intra-abdominal HIFU therapy is organ motion, which is principally due to breathing, thus preventing the focused ultrasound beam from targeting correctly the tumor. The main solution to overcome this limitation is to perform HIFU sonication only during the patient apnea periods. However, this solution is associated with substantially longer treatment times and decreased efficiency. In this context, we are developing an all-in one robotized HIFU solution with the active compensation in real-time using ultrafast ultrasound imaging. A fast speckle cross correlation method[3] is used to estimate motion in real-time. Ultrasound signals were acquired using a research ultrasound imaging system (Verasonics, WA, USA). This method was tested with different imposed sinusoidal motions. The satisfying results allowed us to validate the 1D motion estimation. The compatibility between the HIFU device and the ultrasound imaging scanner was addressed by using an alternating imaging and sonication sequence. HIFU sonication was performed on a piece of chicken breast in order to verify the efficiency of this HIFU/imaging sequence. The 1D motion estimation combined with the HIFU sonication was validated as well. The ongoing development is the conception of the visual servoing in order to keep the distance between the HIFU transducer and the tumor constant during the whole treatment.

1 O. Al-Bataineh, J. Jenne, and P. Huber, “Clinical and future applications of high intensity focused ultrasound in cancer,” Cancer Treatment Reviews, vol. 38, no. 5, pp. 346–353, 2012.

2 A. Muller, L. Petrusca, V. Auboiroux, P. J. Valette, R. Salomir, and F. Cotton, “Management of Respiratory Motion in Extracorporeal High-Intensity Focused Ultrasound Treatment in Upper Abdominal Organs: Current Status and Perspectives,” Cardiovasc Intervent Radiol, vol. 36, no. 6, pp. 1464–1476, Dec. 2013.

3 J. Luo and E. Konofagou, “A fast normalized cross-correlation calculation method for motion estimation” Ultrasonics, Ferroelectrics and Frequency Control, IEEE Transactions on, vol. 57, no. 6, pp. 1347–1357, 2010.

A cell biology view of the siderophore pyochelin iron uptake pathway in *Pseudomonas aeruginosa*

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ABSTRACT

In order to get access to iron, Pseudomonas aeruginosa produces two major siderophores, pyoverdine (PVD) and pyochelin (PCH). PVD is a chromopeptide and its biosynthesis was shown to be highly organized in the bacteria: in the cytoplasm, PVD appears to be assembled at the inner membrane and particularly at the pole of the bacterium. (Guillon et al. 2012)
Pyochelin (PCH) is the condensation product of salicylate and two molecules of cysteine, which are cyclized and undergo several modifications during the assembly process. To investigate the cellular organization of PCH pathway (siderophore biosynthesis and iron uptake), we generated *P. aeruginosa* mutant strains producing native levels of the proteins involved in this process, fused to either eYFP or mCherry. We focused on PchE, an NRPS involved in PCH biosynthesis, the inner membrane Pch-Fe importer FptX and the AraC regulator PchR. We first confirmed that FptX is involved in PCH-Fe uptake across the inner membrane and determined its affinity for this ferri-siderophore complex. In a second step, we used the strains expressing the fluorescent proteins to study the expression of these proteins in the absence and presence of iron (switch off of the PCH pathway). At last, epifluorescence microscopy investigation showed a uniform distribution of FptX all over the inner membrane and for PchE, a membrane-bound distribution, which is apparently concentrated at the bacterial cell poles. All theses new data may help a better understanding of the PCH pathway and contribute to the understanding of the iron uptake mechanism in *Pseudomonas aeruginosa*.

1. Guillon, L.; El Mecherki, M.; Altenburger, S.; Graumann, P.; Schalk, I. J. *Environmental Microbiology,* **2012.**

PPIome – 3D mapping of protein-protein interfaces. Druggable cavity detection and ligand design

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Modulating protein-protein interactions by low molecular-weight ligands is a novel and promising approach in drug discovery, opening novel therapeutic avenues and extending the scope of applicability of currently known macromolecular targets. Detection and characterization of protein-protein interfaces (PPi) is a key but at the moment there are few tools to study protein-protein interfaces. Nowadays we can only detect a PPi but not characterize their properties for drug design.

Thus our work is to develop a software which can analyze and characterize PPis. The development of this software contains three main parts. We have to detect first the biological interface. In protein structures we can observe two types of interface, biological and crystallographic. It is essential to separate them to work only with the biologically relevant ones. This separation is determined by a machine-learning model generate with known data.
Next we characterize interactions between proteins with an in-house tool [1]. It describes all non-covalent interactions between two chains of a protein by type (hydrogen bond, aromatic interaction, etc.).
The final part is the detection of allosteric cavities. We developed a method to detect cavities up to 8Å around PPis and to calculate a druggability index using Volsite [2]. Detecting a cavity means to determine all possible interactions between a protein and a ligand (not determined). We use a regularly spaced grid in a buried part of the surface of the protein, each cell of it having a specific property, that we call ligand site. The druggability value is calculated by a Support Vector Machine model. It shows if a cavity can host a drug or not. All these information characterize a protein-protein interaction.

Our project aims at charting, for the first time, the ensemble of all druggable protein-protein interfaces of known 3D structures as well as their allosteric binding sites. With all these data we wish to screen commercial compound libraries to find novel compounds that can interact, stabilize or inhibit protein-protein interactions.

1. DESAPHY J., RAIMBAUD E., DUCROT T., ROGNAN D. J.Chem. Inf. Model. 2013, 53, 623-637.
2. DESAPHY J., ASDIMOUSA K.; KELLENBERGER E., ROGNAN D. j.Chem. Inf Model. 2012, 52, 2287-2299.

Unravelling the mechanism of action of chalcone 4, a CXCL12 neutraligand, in asthma

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The chemokine receptor CXCR4 and its ligand CXCL12 are involved in the recruitment of immune and inflammatory cells in the airways, as demonstrated by use of agents blocking either CXCL12 or CXCR4. We recently discovered a new class of agents with neutralizing properties of the chemokine CXCL12, preventing its interaction with its CXCR4 receptor [1-3]. We here highlight the unique relationship between pharmacokinetics and anti-asthma activity of the CXCL12 neutraligand, chalcone 4, in a murine model of asthma. Chalcone 4 significantly reduced the levels of CXCL12 as measured by ELISA in lung homogenates (-20%). 123I-chalcone 4, displaying the same anti-asthma activity as chalcone 4, used for SPECT imaging confirms that chalcone 4 rapidly leaves the lung (<30 min) to be rapidly excreted in the bile and urine. Pharmacokinetics studied by UHPLC-MS/MS shows half-life of chalcone 4 in the lung (<5 min) and in blood stream (<10min). We show that this unusual pharmacokinetics is related to the activity of chalcone 4: the neutraligand binds the chemokine CXCL12 in the lung to transfer it to the blood (1000-fold increase in plasma 5 min after i.n. administration) where from it is gradually removed (<10min). Chalcone 4 administered i.n. before each allergen challenge reduces asthma features in the asthma model: decreased airway hyperresponsiveness as measured either by whole body plethysmography (-45±7%) or Flexivent® (-37±5%), decreased eosinophilia (-54±2%), IL-5 (-75±6%) and mucus (-84±2%) in BALF and decreased lung collagen (-78±8%). IgE in plasma and IL-4 in BALF were not changed, indicating a downstream effect on sensitization. In addition, chalcone 4 reduced recruitment of pro-inflammatory M1 macrophages (-62±4%), and their production of TNFα in response to CXCL12 (-98±4%). In conclusion, we have clearly demonstrated the original and innovative mode of action of the CXCL12 neutraligand, chalcone 4, as reducing the local concentration of CXCL12 to abolish airway hyperresponsiveness, inflammation and remodeling in asthma.

1- Hachet-Haas M, et al., Small neutralizing molecules to inhibit actions of the chemokine CXCL12. J Biol Chem. 2008; 283(34):23189-99.
2- V. Gasparik, et al., Prodrugs of a CXC Chemokine-12 (CXCL12) Neutraligand Prevent Inflammatory Reactions in an Asthma Model in Vivo. ACS Med. Chem. Lett. 2012; 3 (1), pp 10–14.
3- F. Daubeuf, et al., An antedrug of the CXCL12 neutraligand blocks experimental allergic asthma without systemic effect in mice. J Biol Chem. 2013; 288(17):11865-76.

IChem : a novel toolkit to detect, describe and compare protein/ligand complexes in the Protein Data Bank

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Three-dimensional (3D) structures of protein-ligand complexes provide crucial information to better understand molecular rules governing living cells and assist rational drug design. The exponential growth of crystallographic structures within the Protein Data Bank lead to the need for new ways to simplify protein-ligand information. Among the applications that will benefit from these representations is a more precise description of protein binding sites, the selection of potential hits for a target, and an improved optimization of lead compounds. We herewith present a new set to tools, IChem, that aim to generate numerous descriptions of protein-ligand complexes, depending on the goal.

Without information about the protein function or potential ligands, it is necessary to characterize as much as possible it structure. IChem is able to detect all cavities generated by protein atoms by using a regularly spaced pharmacophoric grid points that describes both shape and expected non-bonded interactions of potential protein/ligand binding sites. This first tool called Shaper[1] helps to infer the protein function by measuring the similarity of its known or potential ligand-binding pockets to a collection of functionally annotated binding sites. Moreover, the description tool (VolSite) predicts the druggability of a binding site, i.e. the ability of a binding site to bind with high affinity an orally bioavailable druglike compound.

When ligands are known for a particular protein, one can describe their non-bonded interactions in order to infer a potential binding mode. IChem represent each interaction (hydrophobic, aromatic, hydrogen bond, ionic bond, metal complexation) with points on the interacting ligand atom, the interacting protein atom, and the geometric center of both interacting atoms. This new description, called InterGen[2], is used to infer the protein function but also help in selecting new compounds that shares the same binding mode. Applied to docking tools, one can test *in-silico* the ability of compounds to fit a protein-binding site and filter them according to their ability to reproduce the same binding mode than one or multiple ligands of that a target.

Optimizing lead molecules is a difficult task since equilibrium must be kept between potency and physiochemical/metabolic properties. One way to reduce toxicity is to exchange one part of the lead compound with a bioisosteric group. By using a subset of the Protein Data Bank (called sc-PDB [3]), we splitted all ligands into fragments and assigned them their local interactions with the protein binding site. All fragments are compared against all others in order to retrieve fragments pairs sharing the same binding mode but being structurally dissimilar. We retrieve around 10000 bioisosteric pairs that will help medicinal chemist in their optimization process.

[1] DESAPHY J., AZDIMOUSA K. KELLENBERGER E., ROGNAN D., *J. Chem. Inf. Model.* 2012, 52, 2287–2299

[2] DESAPHY J., RAIMBAUD E., DUCROT T, ROGNAN D., *J. Chem. Inf. Model.* **2013**, 53, 623–637

[3] MESLAMANI J. ROGNAN D., KELLENBERGER E, *Bioinformatics*. **2011,** 27, 1324-6

Nucleophilic Substitution of Azide Acting as a Pseudo Leaving Group: One-Step Synthesis of Various Aza Heterocycles

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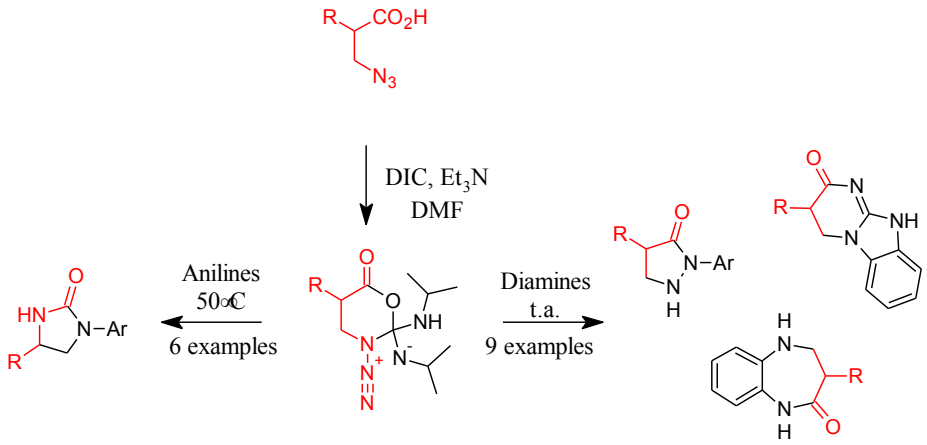
Since the first description of phenyl azides, organic chemistry involving organic azides has been extensively developed, leading to the synthesis of amino acids as well as a diverse range of aza heterocycles. Over the last decades, organic azides have been reported to exhibit a wide range of chemical reactivities. Because of their 1,3-dipolar character, azides react with electron-deficient compounds at N1 and with electron-rich compounds at N3, providing access to [3 + 2] cycloaddition reactions with unsaturated substrates. Organic azides can also behave as pseudonitrenes and allow for the formation of a new bond to nitrogen N1, with loss of molecular nitrogen. In contrast, the pseudohalogenic character of aliphatic azides is less well-known. Although azides have been reported to undergo elimination to form alkenes, to our knowledge no nucleophilic substitution has ever been reported of an azido group in an aliphatic chain. Herein, we describe the unexpected reactivity of 3-azidopropionic acid with standard coupling reagents such as carbodiimides, opening access to a variety of aza heterocyclic systems under mild conditions.



We have shown for the first time that an aliphatic azido group can act formally as a pseudo leaving group in a nucleophilic substitution under mild conditions. This reaction proceeds through an original mechanism, opening a highly efficient access to a variety of aza heterocycles, including mono-, di- and tricycles.

Intracellular localization of the HIV-1 Gag (NC) during the assembly of the viral particle: a quantitative fluorescence microscopy approach

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KEY WORDS: Gag, NCp7, TSG101

The main structural protein of the HIV-1 particle corresponds to a polyprotein referred to as Pr55Gag or Gag. This protein is composed of various domains such as matrice (MA), capsid (CA), nucleocapsid (NCp7) and p6 and self polymerised to form the viral particle1. The main domains of Gag involved in this oligomerisation are the CA and the nucleocapsid. Moreover, this polymerization requires two platforms in the infected cell: the genomic RNA and a lipid bilayer membrane, such as the plasma membrane. To complete virion assembly, trafficking and ultimately budding, Gag hijacks cellular proteins of the cytoskeleton and the ESCRT (Endosomal Sorting Complex Required for Transport) like TSG1013. Till now, a panel of biochemical, fluorescence microscopy and in vitro models, showed that the assembly process is initiated in the cytoplasm by the formation of low order Gag oligomers. Then, during their translocation to the PM, membrane interaction was shown to promote higher order Gag multimerization and subsequent particle assembly in cells.

The aim of this work was to use quantitative cell imaging microcopy to describe the role of NCp7 in Gag-Gag oligomerization and during the interaction between Gag and the cellular partners, TSG101. By FRET, we were able to follow these complexes at the level of the plasma membrane and in the cytoplasm, in agreement with the role of these proteins during the formation of the viral particle. Additionally, this interaction was found to be dependent on the NCp7 region of HIV-1 Gag.

1- Christopher P. Jones, Siddhartha A. K. Datta, Alan Rein,2Ioulia Rouzina, and Karin Musier-Forsyth, JOURNAL OF VIROLOGY, Feb. 2011, p. 1594–1603.

2- Wesley. Sundquist and Hans-Georg Kra¨ usslich, Cold Spring Harb Perspect Med. 2012 July.

3- Yoshiko Usami, Sergei Popov, and Heinrich G. Göttlinger, J Virol. 2007 June; 81(12): 6614–6622.

4- De Rocquigny, H, Hocine Gacem, Pascal Didier, Jean Luc Darlix and Yves Mely. Springer Ser Fluorescence (2012).

Development of a monoclonal neutralizing antibody targeting S100b to reduce airway responses

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Respiratory diseases (RD) like asthma and chronic bronchitis are extremely complex and invalidating diseases common in the general population. RD represents an important economic burden. The main features of RD are exaggerated inflammatory response and airflow obstruction. Many genetic factors and pathways have been implicated leading to therapeutic treatment. Unfortunately, severe forms of respiratory diseases are refractory to current treatments.

Our group has previously shown that the S100 calcium binding protein B (S100b) is expressed in the lungs following airway stimulation with a very dynamic profile similar to pro-inflammatory cytokines. After challenge, expressing cells accumulate near the upper airway epithelial and muscular cells suggesting a potential role of S100B in controlling the muscular contraction observed during the airway hyper-responsiveness. Using either knock-out approach or therapeutic antibodies, interfering with the S100b function strongly modulates the lung responses. More interestingly, challenging the airways using acute lung injury or allergic asthma model is partially prevented by therapeutic polyclonal neutralizing antibody against S100b.

We propose here to go further by producing a monoclonal antibody directed specifically against S100b and to evaluate its activity. We used AbDesigner software to select 2 immunogenic peptides (Ck20 and LC22) specific to S100B. The peptides were coupled to ovalbumin and injected into BALB/c mice. After 4 immunizations, mice were sacrificed and B lymphocytes were isolated from spleen and fused with an immortal cell line to generate Hybridoma. 5 clones Ck20 and 10 LC22 were selected. The monoclonal antibodies were purified and validated for the detection of recombinant human S100b (by ELISA and western Blotting) and endogenous S100b (Immunofluorescence and flow cytometry on Hela cells). We are currently assessing the potential neutralizing effects of the antibodies on the activation of NF-κB signaling which is the major cellular pathway activated by S100B. We also aim to determine the effect of antibody on the interaction of S100b with its receptor RAGE. These tests will allow us to select the clones to be tested in mice.

Convergent approaches to find out new leishmanicidal lead molecules

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Chemotherapy is the main tool to control leishmaniases, a family of parasitic diseases with various clinical manifestations[1]. Of the two main forms, visceral and cutaneous, the visceral form is fatal in 85 - 90% of untreated cases[2]. The treatment of leishmaniases requires expensive medicines with several side effects. In addition some chemoresistances start appearing[3].

Psorospermum genus is known to be traditionally used for the treatment of parasitic diseases in Cameroon[4]. Extracts from these species have been screened and fractionated, leading to the isolation of several prenylated anthranoids with strong *in vitro* antileishmanial properties. In an effort to improve the activity and the selectivity of these molecules, total synthesis protocol has been developed to obtain different analogs. Compounds with the same core have also been purified from plant material and will be further used for hemisynthesis.

Anthranoids diversely substituted from the French Chemical Library are currently screened to highlight Structure-Activity Relationships. These informations will guide the synthesis of new bioactive derivatives.

These convergent approaches are expected to increase *in vitro* activities of the lead compounds that could be further tested in animal models and used as pharmacological tools to investigate new therapeutic targets.

Notes :

[1] Sundar S, Chakravarty J - Leishmaniasis: an update of current pharmacotherapy - *Expert Opin Pharmacother* - 2013 - **14**(1) : 53-63.

[2]Stockdale L, Newton R - A review of preventative methods against humanleishmaniasis infection. - PLoS Negl Trop Dis. 2013 - **7**(6)

[3] Vanaerschot M, Huijben S, Van den Broeck F, Dujardin JC. - Drug resistance in vector-borne parasites : multiple actors and scenarios for an evolutionary arms race - *FEMS Microbiol Rev* - 2013.

[4] Adjanohoun JE & *al*.- Contribution to Ethnobotanical and Floristic Studies in Cameroon - 1996 - CSTR/OUA.

pH dependent photophysical profile of oxyluciferin for biomedical applications

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Understanding the emission mechanism behind firefly bioluminescence is significant for numerous applications as in biochemistry, bio-imaging, analytical chemistry and many more. The mysterious flash of light produced by the firefly results from chemical excitation and excited state relaxation of the optically active part, *oxyluciferin*. In particular, the chemistry and photophysics behind the color tuning mechanism of the firefly bioluminescence is still a strongly debated question. A possible explanation relies in the chemical equilibrium achieved by double deprotonation and keto-enol tautomerisation.

To unravel the emission mechanism and to get information about the spectral contribution from individual components of oxyluciferin, we investigated the photophysical properties of structurally modified synthetic derivatives of firefly oxyluciferin over a range of physiologically relevant pH. To this aim, we used a variety of techniques including steady state and time resolved spectroscopy with keto, enol and neutral derivatives of oxyluciferin in different chemical environments. From the spectral deconvolution of these three derivatives, it was possible to understand the emission mechanism of the natural firefly oxyluciferin.

Optical control of P2X receptor gating

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Ligand-gated ion channels (LGICs) are transmembrane proteins, which ensure the selective permeation of ions through the plasma membrane and thus are essential to control the excitability of cells. LGICs comprise three different superfamilies of receptors differing in their oligomeric assemblies. Among these, the trimeric P2X receptors, which are activated by ATP, are involved in various physiological or pathological processes such as neuromodulation, neuropathic pain or vascular remodeling (1). Crystal structures were solved recently (2) but they give little insight into molecular mechanisms involved in channel gating and thus complementary approaches have to be developed to adress this issue. Here we present a new method, which uses azobenzene-containing derivatives as photoisomerizable crosslinkers to control channel gating. We have engineered a P2X receptor to obtain a photocontrol of the receptor : it can be opened by irradiation at 580nm and closed at 365nm in the absence of its native ligand. The cis-trans isomerization of the covalently tethered bis-maleimide azobenzene-containing derivatives to engineered cysteine mutants induces movements of the transmembrane helices similar to that induced by the endogenous ligand. With these results we confirm expansion of the extracellular part of the channel during opening. Thus this approach could be used to explore molecular motions of distant regions relevant to activation, and can be extended to any membrane-embedded proteins.

1. Lemoine, D., et al., Ligand-gated ion channels: new insights into neurological disorders and ligand recognition. Chem Rev., 2012. 112 (12) : p. 6285-318.

2. Hattori, M., and Gouaux, E., Molecular mechanism of ATP binding and ion channel activation in P2X receptors. Nature, 2012. 485 : p. 207-12.

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

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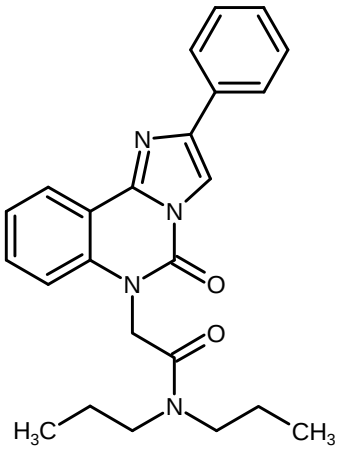
The 18kDa translocator protein (TSPO), which was initially named peripheral benzodiazepine receptor (PBR), is known to play a key role in the synthesis of steroids and neurosteroids, through the translocation of cholesterol from the outer to the inner mitochondrial membrane. Many studies recently highlighted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



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DNA epigenetic modification monitored by new fluorescence based tools

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DNA methylation is a key epigenetic mechanism, involved in numerous biological functions. As a result, alteration of the DNA methylation pattern is involved in a variety of diseases, including cancer, neuro-degenerative disorders and metabolic diseases [1-2]. DNA methylation is a multicomponent reaction, in which UHRF1 protein plays an important role by recognizing hemimethylated CpG sites through its SET and RING Associated (SRA) domain. To further understand the role of UHRF1 in methylated DNA recognition, we used a recently developed fluorescent nucleoside analogue, based on 2-thienyl 3-hydroxyhcromone (3HCn) nucleobase, a multi-parametric and ratiometric fluorescent probe [3]. Due to an excited state intramolecular proton transfer (ESIPT), this fluorescent nucleobase exhibits two excited state forms, namely the initially excited normal (N*) and the tautomeric (T*) forms which are highly sensitive to the environment changes occurring on interaction of the SRA domain with the labeled DNA. By labeling methylated and non methylated DNA sequences at various positions by this fluorescent nucleoside, we evidenced position dependent changes of fluorescence as well as changes in the ratio of their two bands on SRA binding. Most interestingly, comparison of corresponding methylated and non methylated DNA sequences revealed that the fluorescent nucleobase was sensitive to the methylation state of cytosines and could be used to monitor the SRA-induced flip of the methylated cytosine.

References:

1. *Burdge, G.C. and K.A. Lillycrop, Annu Rev Nutr, 2010.* ***30****: p. 315-39.*
2. *Jones, P.A. and S.B. Baylin, Cell, 2007.* ***128****(4): p. 683-92.*
3. *Dziuba, D., et al., A Journal of the American Chemical Society, 2012. 134(24): p. 10209-10213.*

Keywords: Epigenetics, DNA methylation, methylcytosine, 3-hydroxychromone, ESIPT.

A biomarker for asthma and Chronic Obstructive Pulmonary Disease (COPD)

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The G protein-coupled receptors (GPCRs) constitute a large family of 380 proteins with seven transmembrane domains involved in many functions in the organism. Our study proposes to determine whether the modulation of expression of GPCRs could be used as a biomarker for diagnosis and/or prognosis of inflammatory diseases and/or of effectiveness of treatments in two chronic pulmonary inflammatory diseases: asthma and chronic obstructive pulmonary disease (COPD). In these chronic diseases, the inflammatory profile is totally opposed: mast cells, eosinophils and Th2 lymphocytes are recruited in asthma, whereas macrophages, neutrophils and Th1 and CD8 cells infiltrate the airways in COPD.

We assessed the full expression profile of the 380 GPCRs transcriptome in leukocytes of patients with well characterized asthma or COPD compared to controls selected among relatives (brother, sister, parents, children) for patients with asthma and in the immediate environment (neighbor, spouse/husband) for COPD patients. For each patient and control, a blood sampling on EDTA was collected and leucocytes circulating are separated with the total filtration of red blood cells (LeukoLOCK^{TM}). For each cell sample, RNA was extracted, purified (RN*easy mini kit* – Qiagen), quantified (NanoDrop®, Qubit^{TM}) and its quality validated by measuring RNA integrity Number (RIN) (Bioanalyzer 2100) for good (included if RIN ≥ 8) or bad (rejected) quality. The expression of GPCRs was quantified by qPCR using microfluidic cards 384 wells (TaqMAn TLDA®).

We first show in 10 patients versus controls one GPCR gene and one only that was differentially expressed between asthma and COPD as compared with their respective controls. GPRAB mRNA expression was increased in COPD and decreased in asthma. Also, GPRAB expression was found to be age-dependent, with an expression decreasing with age in all study groups. By increasing the number of patients, we confirm that the expression of GPRAB is age-dependent, is over-expressed in COPD patients as compared to asthma patients and controls, and decreased in asthma compared to COPD patients and controls. Since cigarette smoke is the main cause of occurrence of COPD, we also analysed the effect of cigarette smoke on GPRAB expression. Results show that ex-smokers with COPD maintain their phenotype of an overexpression of GPRAB.

In conclusion of this ongoing study, we submit GPRAB as a biomarker to differentiate asthma and COPD, two inflammatory diseases of the airways, which are now difficult to differentially diagnose since only clinical parameters are taken into account.

Intracellular distribution of HIV-1 nucleocapsid protein

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The nucleocapsid protein (NCp7) of HIV-1 is a small basic protein of 55 amino acid residues. NCp7 is characterized by two zinc fingers that bind zinc with high affinity [1]. NCp7 as a mature protein or as a domain of the Gag polyprotein plays a key role in reverse transcription of the viral RNA, integration of the proviral DNA into the host genome, selective packaging of the viral genome RNA, and processing and maturation of the new viral particle [2-3]. Though it is well demonstrated that numerous NCp7 functions rely on its interaction with nucleic acids, the interaction of NCp7 with host cell components remains largely unexplored. For identifying the potential ligands of NCp7 in the cell, we electroporated inside the cells, quantum dots (semiconductor CdSe nanocrystal) conjugated to NCp7 molecules. Using single particle tracking (SPT) technique, we monitored the movements of QD-NCp7 inside the cell. SPT indicated that QD-NCp7 particles exhibited active directed movement. Furthermore, using high resolution STORM and TEM microscopy, we further evidenced that NCp7 likely binds to actin filaments and cellular RNAs.

1. Mely Y et al., Zinc binding to the HIV-1 nucleocapsid protein: a thermodynamic investigation by fluorescence spectroscopy Biochemestry. 1996. 35(16).p5175-5182

2. Thomas JA and Gorelick RJ Nucleocapsid protein function in early infection processes Virus Res. 2008.134(1-2). P39-63

3. Darlix et al., Flexible nature and specific functions of the HIV-1 nucleocapsid protein J. Mol Biol. 2011. 410(4). p65-581

Substituted flavones: a promising scaffold in the fight against malaria

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Malaria is a tropical disease due to apicomplexan parasites of the genus *Plasmodium*. These parasites are transmitted through the bite of a female *Anopheles* mosquito and infect human erythrocytes. Five species of *Plasmodium* can infect human, *P. falciparum* being the most deadly. Malaria was characterized in 2004 by Doctors Without Borders as a "*weapon of mass destruction that nobody cares*". Despite progress realized since in the management of the disease, especially through the WHO Global Malaria Action Plan, *Plasmodium* is still responsible of approximately 650.000 deaths per year, the majority of which affects the African children under 5 years[1]. In addition, resistant strains of *Plasmodium* exist for most of the treatments available, and recently for the most active drug, artemisinin[2]. Thus, there is an urgent need for new treatments with original scaffold, to counter those resistances. For this purpose, we are developing new synthetic antimalarial agents with an original structure inspired by nature. The isolation of an active biflavonoid from *Campnosperma panamense* (Anacardiaceae, IC_{50} = 450 nM, *Plasmodium falciparum* K1 resistant strain), which present an interesting selectivity index (SI = 130, L6 cells)[3] led us to the development of simplified synthetic analogs. Structure Activity Relationship (SAR) study is still in progress, but several active compounds have already been synthesized. Among these, MR70 is partially active *in vivo* at a dosing regimen of 100 mg/kg on a murine model of *Plasmodium* (*P. berghei* ANKA – unpublished data). The aim is to pursue the SAR study and to understand how these compounds are acting on the parasite. Differential metabolomics analysis of the parasite versus the treated parasite is in progress by solid NMR in order to identify the metabolic pathways affected by our compound (*coll. Prof. J. Izzie Namer,* Service de Biophysique et Médicine Nucléaire, Hôpitaux Universitaires de Strasbourg). In order to highlight some potential apicomplexan-specific mechanism of the drug, this analysis will be transposed to another apicomplexan parasite, *Toxoplasma gondii*, responsible of toxoplasmosis. This will help us to characterize the target(s) involved in MR70 antiplasmodial activity and thus to optimize its activity more efficiently.

1. World Malaria Report, World Health Organization, 2013
2. Aung Pyae Phyo et al., 2012. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. The Lancet, 379, 9830, 1960 – 1966.
3. Weniger et al., 2004. A bioactive biflavonoid from Campnosperma panamense. Fitoterapia, 75, 764 – 767

Genetics and pathophysiologic mechanisms of tubular aggregates myopathies

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Congenital myopathies have an estimated prevalence of 1:25 000, affect children as well as adults in all populations. They are potentially lethal and encompass more than 10 etiologies varying in symptoms, complications and treatment options. They can be distinguished by the predominance of particular histological signs on muscle biopsies.

Tubular aggregate myopathies (TAM) are progressive muscle disorders with an autosomal recessive or dominant inheritance. They are associated with muscle pain and weakness and typically display abnormal accumulations of membrane tubules in muscle fibers. Our team recently identified *STIM1* as the first gene implicated in TAM. STIM1 is a major regulator of calcium homeostasis and it was demonstrated that the identified mutations strongly impact on the calcium level in TAM myoblasts. The nature, formation and pathogenicity of the tubular aggregates are not defined and the link between STIM1 mutations and muscle dysfunction is not understood. Moreover, the genetic basis for a majority of TAM families is not known, precluding genetic counseling and improved disease management. This project aims to identify novel implicated genes, to characterize the pathological mechanism underlying the muscle disorder, and to assess rescue strategies in models.

Nanostructuration of two-photons phototrigger

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Photolabile protecting groups are efficient tools for revealing intimate dynamic processes in cells[1]. Thus, new advances have been made in neurosciences by controlled photoliberation of neurotransmitters, yielding to a better understanding of the neuronal circuitry. A fine spatio-temporal control of the release of a biologically active substance can be afforded by a two-photon (TP) induced photocleavage. In that case the excited state is reached by TP absorption, which is related to the third order nonlinearity of the chromophore. Due to the quadratic dependence of the TP absorption versus light intensity, the effect is confined at the focus of the laser beam (with an excitation volume in the order of 1 mm^3). In addition to this high spatial control, the use of a pulsed IR laser for the TP excitation is less photodamageable to cells and tissues, and allows a deeper penetration in living tissues than the UV light classically used in one-photon uncaging.

Many efficient two-photons sensitive cages has been synthetized in our laboratory, based on an electron donor-acceptor biphenyl system used as key component (EANBP-GABA[2,3], PENB-Glu[3]). We propose here to overcome a step with nano-structuration of such systems. By joining several caging systems on a central core, in a non-conjugated way, we should obtained new caging platforms with new pharmacodynamics, photophysical and photochemical properties and allowing a higher concentration gap for the caged compound after irradiation.

We present the synthesis and photochemical characterization of PENB non-conjugated dimer and tetramer. Unfortunately the photochemical properties of such systems are not more efficient than the ones of the monomer due to cage-cage interactions. Size and rigidity of the central pattern seems to be the solution to keep individual photochemical efficiency of each cage. Calculations showed that the use of PAMAM as core for such systems would minimize cage–cage interactions.

1. Kramer, R. H., Mourot, A. & Adesnik, H. Optogenetic pharmacology for control of native neuronal signaling proteins. *Nat. Neurosci.* **16,** 816–823 (2013).
2. Specht, A. *et al.* The donor–acceptor biphenyl platform: A versatile chromophore for the engineering of highly efficient two-photon sensitive photoremovable protecting groups. *Photochem. Photobiol. Sci.* **11,** 578 (2012).
3. Donato, L. *et al.* Water-Soluble, Donor-Acceptor Biphenyl Derivatives in the 2-(o-Nitrophenyl)propyl Series: Highly Efficient Two-Photon Uncaging of the Neurotransmitter γ-Aminobutyric Acid at λ=800 nm. *Angew. Chem. Int. Ed.* **51,** 1840–1843 (2012).

Efficient DNA and siRNA Transfection Mediated by a proline mutant of the C-Terminal Domain of HIV Viral Protein R

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The accessory genes of lentiviruses are not essential for viral replication in tissue culture but can be critical for the establishment of a productive infection in their natural hosts. The *vpr* gene is found in human immunodeficiency virus type 1 (HIV-1) and HIV-2, and encodes the 15-kDa viral protein R (Vpr), which is produced late in the virus life cycle and is assembled into the virion through binding to Gag. A variety of activities have been associated with Vpr. Among these latter, Vpr has been implicated in transport of the viral pre-integration complex into the nucleus, a property that may help HIV-1 infect non-dividing cells. In the absence of other HIV-1 proteins, Vpr is localized predominantly in the nucleus, although it does not contain a typical nuclear localization signal. Genetic and structural studies have assigned the various functions of Vpr to overlapping domains within the molecule. Nuclear accumulation of Vpr was reported to depend on the presence of α-helices in both the N- and C-terminal halves of the protein. The N-terminal helices are believed to be involved in incorporation of Vpr into virions. The C-terminal domain, which is rich in basic amino acids, contains elements essential for G_2 arrest and nucleic acid binding activities. Considering the karyophilic properties of Vpr as well as its ability to bind nucleic acids, we explored in the last years the possibility of using Vpr as a DNA transfection agent. We found that C-terminal fragments of Vpr (Vpr52-96 ; Vpr55-82) but not the whole protein, are able to deliver DNA efficiently into different cell lines. It was the first example of a peptide derived from a natural protein displaying such a high transfection activity in the absence of auxiliary agents such as chloroquine. In the present work, we have tested the capacity of different Vpr derived peptides to deliver plasmid DNA and siRNAs into human cell lines. Our results indicate that a proline mutant of Vpr55-82 possesses very good siRNA and DNA transfection properties.

Physiopathology and preclinical approaches in centronuclear myopathies

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

Our group has shown that reduction of *Dnm2* in the *Mtm1* Knock-out (KO) that dies between 1-3 months restores a normal lifespan (2 years) with improved muscle structure and function. Therefore, I aim to translate this proof-of-principal experiment by reducing Dnm2 expression using RNA interference. I selected 14 shRNA sequences that target Dnm2 mRNA and I am currently screening Dnm2 reduction using mouse C2C12 myoblasts. The best candidates will be integrated in Adeno-Associated virus (AAVs) and will be injected to *Mtm1* KO mice to confirm the observations found by genetic crossing.

The second part of my project involves developing a mouse model for the AR form of CNM. *Bin1* KO mice are lethal at birth. Therefore, I aim to develop a conditional *Bin1* KO model that will recapitulate the AR form of CNM, and in which novel therapies can be tested.

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

Ultrabright switchable organic nanoparticles

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The development of bright fluorescent organic nanoparticles (NPs) is of particular interest for bio-imaging and medical diagnostics. Highly fluorescent NPs developed so far, like quantum dots and silica nanoparticles are not biodegradable and relatively toxic. The additional challenge in the field is to switch ON/OFF fluorescence of NPs through a robust mechanism of Fluorescence Resonance Energy Transfer (FRET), which would open possibilities to numerous applications in biological sensing. Dye-doped fluorescent organic nanoparticles (NPs), due to their potential biodegradability and low toxicity, are very attractive tools for using as fluorescent labels and as building blocks for high sensitive sensors. The group of the Laboratory of Biophotonic and Pharmacology of the Strasbourg University recently developed highly fluorescent and biodegradable polymer (PLGA, poly (lactic-co-glycolic acid) nanoparticles featuring high cooperativeness of the encapsulated dyes. The specially designed rhodamine B derivative dye was encapsulated into 35-nm particles of PLGA at high concentration with minimal self-quenching. FRET phenomena were studied in NPs containing large amount of donor molecules and few acceptors (Cy5 derivative dyes) located either inside NPs or at its surfaces. These studies leaded to development of new NPs featuring ON/OFF switching, which so far was not reported for organic nanoparticles. We found that FRET efficiency was >50% when only one acceptor was located inside NP bearing >100 donor molecules, which allowed >100-fold amplification of the acceptor emission comparing with the direct excitation. Single particle microscopy (total internal reflection fluorescence microscopy (TIRF) and fluorescence correlation spectroscopy (FCS)) revealed a complete ON/OFF switching of the NPs emission by a single acceptor. In the case, when the relatively polar Cy5-based acceptor is adsorbed on the surface of NPs, to achieve >50%of FRET efficiency, up to 10 acceptor molecule were needed. The high amplification of the acceptor fluorescence through FRET up to 200-fold was achieved for NPs with 5wt% of the donor. The obtained results suggest these NPs as very efficient FRET donors in the construction of FRET-based nano-probes for detection of biomolecules with single molecule sensitivity.

Chloroform extract of *Phyllanthus amarus* potentiates relaxation induced by isoproterenol on porcine coronary artery rings

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Phyllanthus amarus (PA) (Family: Euphorbiaceae) is currently used in African traditional medicine to treat cardiovascular disorders such as hypertension [1]. Several studies have reported that aqueous extracts of *Phyllanthus amarus* induce hypotensive effects [2]. We have previously prepared a chloroform extract (CF) after liquid-liquid fractionation of an aqueous extract of *Phyllanthus amarus* (AEPA) using successively cyclohexane (CHF) and, then, chloroform (CF). Experiments evaluating the vasoreactivity of the different extracts in isolated porcine coronary artery rings has indicated that PA extract and its fractions induced relaxation with the following order of potency CF > CHF > AEPA. In this study, we have investigated the mechanism underlying the vasorelaxant activity of the CF.

Coronary artery rings precontracted by U46619 were exposed to cumulative concentrations of the CF. In some experiments, rings were incubated for 30 min with a pharmacological inhibitor before being contracted with U46619.

CF induced similar relaxations in coronary artery rings with endothelium and in those without endothelium starting at concentrations of or greater than 0.01 mg/ml and reaching maximal relaxation at 0.3 mg/ml. In addition, CF (0.03 mg/ml; inducing about 20% relaxation) significantly shifted to the left the concentration relaxation curve to isoproterenol (a beta-adrenergic agonist receptor). In contrast, CF affected neither relaxations to forskolin (an activator of adenylyl cyclase) nor those to sodium nitroprusside (a nitric oxide donor). Propranolol (a non-selective antagonist of beta-adrenergic receptors) and SQ22536 (an adenylyl cyclase inhibitor) did not inhibit CF-induced endothelium-independent relaxations.

Altogether, these findings indicate that the chloroformic fraction of *Phyllanthus amarus* contains the most active compounds for inducing vasorelaxation by acting directly at the vascular smooth muscle. CF is able to enhance isoproterenol-induced vasorelaxation, this effect involves neither interaction with beta-adrenergic receptors nor with the adenylyl cyclase pathway.

References:

[1] Patel et al., 2011, *J. Ethnopharmacol.* 138, 286-313.

[2] Amaechina et al., 2007, *Acta Pol. Pharm.* 64, 547-552.

Dynamic imaging (tracking) of cytoplasmic and nuclear HIV-1 complexes

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The HIV-1 nucleocapsid protein Ncp7 is a small basic protein containing two zinc fingers. Most of NCp7 functions are linked to its nucleic acids chaperone activity. NCp7 is implicated in several steps of the virus cycle. It was described as an actor of the viral reverse transcription transforming the RNA containing reverse transcription complex (RTC) into a DNA containing preintegration complex (PIC). NCp7 is also implicated in HIV-1 genome integration into the host cell genome as underlined by the defects of integration observed with viruses containing NCp7 point mutants. Due to its high degree of conservation NCp7 stands out as a potential target for anti-viral drug development. However the NCp7 association with the HIV-1 genomic RNA and DNA along its journey to the nucleus and the mechanism of entry of NCp7into the nucleus are still subjects to debate. Therefore studying NCp7 behaviors in infected cells and its interaction with cellular proteins is crucial for the understanding of the roles of NCp7 during the HIV replication cycle. The aim of this study is to track NCp7 in infected cells using high resolution microscopy (PALM, STED). In order to do so, we prepared lentiviral pseudoparticles labeled with the bis-arsenical fluorescein derivative FlAsH attached to the NCp7 protein tagged with a tetracystein (TC) motif. The tetracystein tag (CCRECC) coding sequence was fused at the C-terminus of the NCp7 in the plasmid encoding Gag-Pol used to produce replicative defective lentiviral vectors (NCp7-TC). Those NCp7-TC viruses retained a level of infectivity comparable to the wild type (WT) ones. After labeling with FlAsH the purified particles were observed by Atomic force microscopy (AFM), the particles size distribution was comprised between 80 and 100 nm in diameter which is comparable to the WT particles. The colocalization of Flash signal and P24 capsid was confirmed using immunofluorescence confirming the formation of viral particles. Those labeled particles were used to infect HeLa cells and were observed inside the cells by confocal microscopy. Wild type particles labeled with FlAsh were used as a control for non specific labeling. The FlAsH signal, in the case of NCp7-TC particles, was much higher than the one of the WT labeled particles indicating that the Flash NCp7-TC labeling is specific.

HeLa cells will be observed at several times after infection with NCp7-TC viruses. To do so, the positions of all the intracellular particles will be determined according to the nucleus and the cell plasma membrane using confocal and PALM STED microscopes. The localizations will be compared to the one obtained with viruses labeled on the integrase protein known to be associate with the viral genome till the integration step.

Molecular Insights into the formation of the nucleotide excision repair complex revealed on undamaged chromatin

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Nucleotide excision repair (NER) is one of the several processes that a cell uses to preserve genome integrity. It's aimed to remove helix-distorting lesions, such as cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6- 4PP) generated by UV light. It acts through two related sub-pathways, the global genome repair (GGR) that removes lesions from the entire genome, and transcription coupled repair (TCR) that acts on actively transcribed genes. The normal process begins by the recognition of the lesion by XPC/hHR23B in GGR, or by stalled RNA pol II in TCR, followed by the recruitment of TFIIH that opens the DNA around the lesion through its helicase activities contained in XPB and XPD, two of its ten subunits. Next, XPA, RPA and the two endonucleases XPF/ERCC1 and XPG are recruited to generate cuts at the 3' and 5' sides of the lesion, respectively. Finally, the resynthesis machinery fills the resulting DNA gap[1]. Here we studied the recruitment of what we called the pre-incision complex (PInC) composed of XPC/hHR23B, TFIIH, XPA, RPA, XPF/ERCC1 and XPG, in the absence of DNA lesions. Indeed, recent studies showed that these NER factors assemble on chromatin during transcription process and in absence of any genotoxic stress[2], raising questions about the mechanism of PInC formation. Using a LacO/LacR system[3], we observe a sequential and ordered self-assembly of the PInC operating upon immobilization of individual NER factors on undamaged chromatin and mimicking that functioning on a DNA damage. Using this system, we reveal molecular insights into NER including that the recruitment of the TFIIH subunit TTDA, involved in Trichothiodystrophy group A disorder (TTD-A), is key in the PInC completion as it recruits XPA through a protein domain depleted in TTD-A patients. These results suggest that immobilization of NER factors on chromatin is sufficient to trigger PInC formation through protein-protein interactions, independently of DNA damage.

1. Kamileri, I., Karakasilioti, I. & Garinis, G. A. Nucleotide excision repair: new tricks with old bricks. *Trends Genet.* **28,** 566–573 (2012).
2. May, N. L. *et al.* NER Factors Are Recruited to Active Promoters and Facilitate Chromatin Modification for Transcription in the Absence of Exogenous Genotoxic Attack. *Mol. Cell* **38,** 54–66 (2010).
3. Soutoglou, E. & Misteli, T. Activation of the cellular DNA damage response in the absence of DNA lesions. *Science* **320,** 1507–1510 (2008).

High-resolution dose-response screening using droplet-based microfluidic

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Droplet-based microfluidic allows us to obtain dose-response curves at high resolution (over 10,000 points). Based on the Taylor-Aris dispersion law, a wide range of concentrations of the test compounds is encapsulated in just a few picoliter droplets and activity within each droplet is analysed at a rate of several hundred droplets per second. This technology can be applied to the search fo new inhibitors of enzymes involved in many diseases as for example, kinases.

An ABC transporter with two periplasmic binding proteins involved in iron acquisition in *Pseudomonas aeruginosa*.

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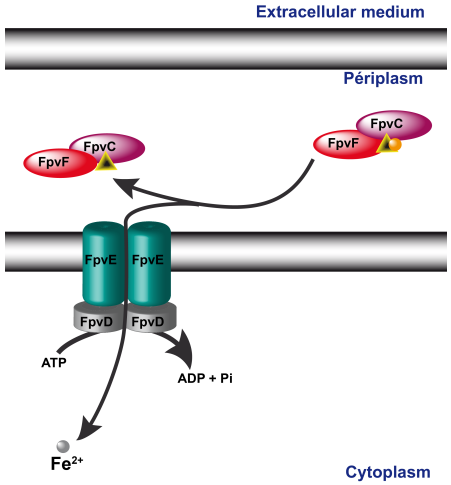
Pyoverdine (Pvd) is the main siderophore secreted by *Pseudomonas aeruginosa* PAO1 to obtain access to iron (Demange *et al.*, 1990). After extracellular iron chelation, Pvd-Fe uptake into the bacteria involves a specific outer-membrane transporter, FpvA (Poole *et al.*, 1993). Iron is then released in the periplasm by a mechanism involving no siderophore modification but probably iron reduction (Schalk *et al.*, 2002; Hannauer *et al.*, 2012a). The proteins involved in this dissociation step are currently unknown. The pyoverdine locus contains the *fpvCDEF* operon, which contains four genes. These genes encode an ABC transporter of unknown function with the distinguishing characteristic of encompassing two periplasmic binding proteins, FpvC and FpvF, associated with the ATPase, FpvE, and the permease, FpvD. Deletion of these four genes partially inhibited cytoplasmic uptake of ^{55}Fe in the presence of pyoverdine and markedly slowed down the *in vivo* kinetics of iron release from the siderophore. This transporter is therefore involved in iron acquisition by pyoverdine in *P. aeruginosa*. Sequence alignments clearly showed that FpvC and FpvF belong to two different subgroups of periplasmic binding proteins. FpvC appears to be a metal-binding protein, whereas FpvF has homology with ferrisiderophore binding proteins. *In vivo* cross-linking assays and incubation of purified FpvC and FpvF proteins showed formation of complexes between both proteins. These complexes were able to bind in vitro PVDI-Fe or apo PVDI. This is the first example of an ABC transporter involved in iron acquisition via siderophores, with two periplasmic binding proteins interacting with the ferrisiderophore.



Demange, P., A. Bateman, C., Mertz, A., Piemont, Y., Abdallah, M. A. (1990). Biochemistry **29**(50): 11041-11051.
Hannauer, M., Braud, A., Hoegy, F., Ronot, P., Boos, A., Schalk, I. J. (2012). Environ Microbiol **14**(7): 1696-1708.
Poole, K., Neshat, S., Krebes, K., Heinrichs, D. E. (1993). J Bacteriol **175**(15): 4597-4604.
Schalk, I. J., Abdallah, M. A., Pattus, F. (2002). Biochemistry **41**(5): 1663-1671.

IMPReSs: Protein production platform of the School of Biotechnology of Strasbourg.

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Capitalizing on the solid expertise developed in several groups of the institute, the IMPReSs (Integral Membrane Protein Research and Services) platform has been launched in 2012. It proposes a panel of optimized methods and tools for the production, the purification and the analysis of difficult-to-handle proteins, including membrane proteins such as G protein-coupled receptors (GPCRs), bacterial transporters, viral glycoproteins, but also antibody fragments and other cytosoluble proteins.

The platform is endorsed by the CNRS french governmental research organization and operates either via manufacturing (CMO) or research contarcts (CRO). The platform provides a consistent set of services spread over 6 representative milestones ranging from gene to purified protein thematic modules.

Bornert, O. et al., Curr. Protoc. Protein. Sci. 2012. 67, 29-32.
Brillet, K. et al., Acta Crystallogr. D Biol. Crystallogr. 2009. 65, 326-331.
Desplancq, D. et al., Biotechnol. Appl. Biochem. 2008. 51, 53-61.

Cytochrome P450 induction assay with cryopreserved hepatocytes

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Cytochrome P450 induction is a new *in vitro* assay available at PCBIS – TechMed[ILL] platforms. Cytochromes P450 (CYP) are the major enzymes involved in drug metabolism. These enzymes are subject to induction by chemicals, occasioning drug-drug interactions: if a cytochrome P450 is induced by a compound, it may increase the metabolism of a co-admistered drug that is substrate of the induced enzyme, resulting in a decrease in efficacy. The assay identifies the potential of test compounds to induce CYP1A2, CYP2B6 or CYP3A4, using plateable cryopreserved human hepatocytes.

After two days of incubation with cell culture medium in a 24-well plate, hepatocytes are exposed to test compounds at one ore more concentrations, based ideally on the expected therapeutic concentrations, alternatively on solubility. Following two days of exposure, CYP enzymatic activities are determined using appropriate substrates and quantified by determining the quantity of metabolites by LC-MS/MS. Negative control (vehicle) and positive controls (known inducers) are included in the assay.

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

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

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

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

New Cationic Phospholipid-Detergent Conjugates for Nucleic Acid Delivery to the Lung

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Our ongoing research objectives deal with therapeutic gene delivery to pulmonary tissues. Indeed, lung disorders such as cystic fibrosis, chronic obstructive pulmonary disease (COPD) and asthma are among the more representative causes of mortality and morbidity worldwide, and identifying powerful and cost-effective treatments is a matter of high priority. In this case, local pulmonary gene delivery may be especially advantageous as it reduces systemic side effects and do not lead to en-route interactions with serum proteins.

Within the last few years, we designed, synthesized and evaluated the transfection potential of a new family of synthetic cationic lipids that are built on the chemical basis of endogenous diacylglycerophosphocholines (PCs), with the view to limit *in fine* the toxicity of the nucleic acid carrier. These cationic DOPC conjugates were also designed so they loose their cationic charge upon hydrolysis either under a chemical or enzymatic stimulus, in a time-controlled manner. Depending on the structure of the cationic lipids, we identified a window of stability, in which the cationic lipids display high transfection activity in various cell lines with low toxicity.

As an extension of this concept, we were then interested in covalently conjugating DOPC and a membrane active species (*i.e.* a detergent) through a pH-labile linker so that the detergent cannot be depleted from the transfection particle at physiological pH. We thus investigated the ability of such cationic vectors to mediate transfection in cultured cells. Interesting results obtained with Triton-X100 as the detergent encouraged us to further explore this direction, by varying the nature of the detergent. This finally led us to identify a versatile and innocuous vector for DNA and siRNA delivery, resulting from the conjugation of DOPC with $C_{12}E_4$ as the detergent.

We recently assessed the ability of this conjugate to efficiently deliver pDNA to pulmonary tissues in mice. We were pleased to observe that optimized lipoplex formulations led to higher transfection level than GL67, the cationic lipid developed by Genzyme and which is the current “gold-standard” for *in vivo* gene delivery to the lung.

Fully Regiocontrolled Polyarylation of Pyridine

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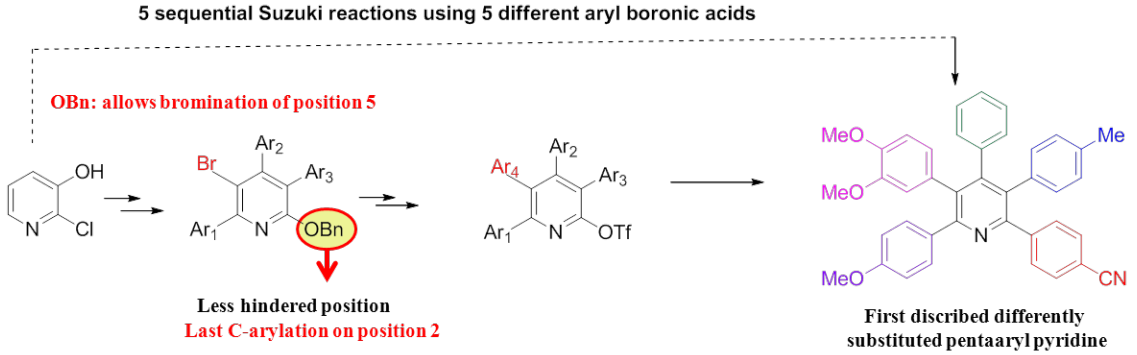
Pyridine derivatives represent an important class of six membered heterocycles prevalent in a number of biologically active natural products, as well as in various pharmaceuticals. They also present strong interest in many areas of chemistry. In particular they are useful building blocks for preparation of chiral ligands and also new materials with important photo or electrochemical properties. Several strategies have been developed to control polysubstitution on pyridine. However these methods offered limited chemical diversity and sometimes failed due to chemical hindrance, in particular for tetra or penta substituted pyridines or gave limited yields. We describe in this work a versatile strategy allowing regiocontrolled aryl substitutions at the five positions of the pyridine ring

Starting from-2-chloro-3-hydroxypyridine, this strategy involves a set of 5 sequential but fully regiocontrolled Suzuki-Miyaura reactions, and highlighted the 2-OBn pyridine protecting group as a key intermediate. The 2-OBn pyridine plays a double role i) it still allowed additional bromination at position 5 and ii) it could yield a reactive OTf specie for a last C-arylation step on the less hindered 2-position of tetraarylpyridine. Photophysical properties of the novel compounds are described



References: Fully Regiocontrolled Polyarylation of Pyridine, *J. Org. Chem.*, **2014**, 79, 908-918.