JCI 2015

Communications orales / Talks

Microparticles released by senescent endothelial cells promote premature endothelial senescence associated with an impaired NO formation and oxidative stress

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Circulating levels of endothelial microparticles (EMPs) are elevated in vascular diseases such as coronary artery disease, peripheral vascular disease or rejection after heart transplantation. EMPs, besides being relevant biomarkers, might also contribute to the development of endothelial dysfunction and vascular damage. This study examined whether the induction of endothelial senescence is associated with EMPs shedding, and if senescence-related EMPs promote endothelial senescence and prothrombotic changes of the endothelial cell. Replicative senescence was induced by serial passaging of primary cultures of porcine coronary artery endothelial cells (ECs) up to the third passage (P3). Cells retained normal phenotype of ECs at P1 and P2 whereas at P3 an increased proportion exhibited features of senescence with cell cycle arrest in the G0/G1 phase and senescence-associated β-galactosidase (SA-β-gal) activity. In addition, senescence was associated with increased EMP shedding and no detectable apoptosis. Exposure of ECs at P1 to EMPs collected from the conditioned medium of ECs at P3 induced cell cycle arrest and increased SA-β-Gal activity in target cells. Furthermore, EMPs isolated from ECs at P3 increased cellular oxidative stress and the expression of the senescence markers p53 and its downstream targets p21 and p16. Moreover, EMPs reduced the ability of ECs to inhibit platelet aggregation and up-regulated TF expression and activity at EC cell membrane. The present findings indicate that endothelial senescence favors the increased shedding of EMPs, which, in turn, promote premature senescence and an endothelial prothrombotic phenotype. The response to senescence-related EMPs involves oxidative stress, the up-regulation of p53 and p21, and a reduced formation of nitric oxide and up-regulation of TF. Our data further suggest that EMPs released by senescent endothelial cells may contribute to the development of an endothelial dysfunction and thrombogenicity.

New contrast agents for targeted Biomedical Imaging

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In spite of the progresses of the imagers' efficiency (which used for biomedical diagnosis in order to detect pathogens and specific cellular, tissular and molecular targets), notably X-ray and optical modality, their use and potentials are still dramatically limited by the low efficiency and toxicity of contrast agents.[1] This study presents the development of new contrast agents overcoming these limitations, based on non-toxic nano-emulsions highly loaded in contrasting materials, intended to fluorescence tomography and/or computed tomography (CT) preclinical imaging. The success of the formulation of such contrast agents relies on several interdependent challenges: *(i)* Designing efficient and cost-effective contrast that are easy to synthesize and that can be loaded at high concentrations in nanoparticles. *(ii)* Developing formulations of the contrast agents without organic solvents and specific mechanical device. *(iii)* Adjusting the nanoparticle surface to allow high stability of the nanoparticles (at least several months), good bioavailability and efficient targeting. *(iv)* Minimal toxicity of the contrast agent.[2]

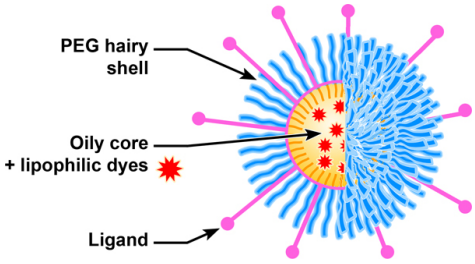

Figure 1 Schematic representation of nano-emulsion droplet covered by PEG layer (blue) and ligands (pink). The droplet core is generally oily and can be loaded with hydrophobic fluorescent dyes

This study deals with the development of new generations of contrast agent for biomedical imaging based on nano-emulsions templates. Compared to common and clinical contrast agents, nano-emulsions brings new real advantages like a long circulation in blood, the control of the biodistribution and pharmacokinetics, and the absence of toxicity. Contrast agents were formulated as lipid nano-emulsions that consisted in a lipid core, surrounded by a non-ionic surfactant layer (see Fig. 1). The lipid core comprised lipophilic molecules either grafted with iodine compounds for X-ray contrast, and/or solubilized fluorescent dyes with high loading ratio. The surface of the nano-droplets was fully covered by a hydrophilic polymer, like PEG, aiming at reducing the recognition by immune system, increasing the circulating time in the blood stream, and thus allowing a better control of the in vivo behavior. Moreover, we have developed several approaches to functionalize the droplet surface by grafting ligands

CONCLUSION

Nano-emulsions is a simple system that presents a great potential as contrast agent. In the present abstract, we showed that nano-emulsions are not only very suited for the formulation of CT contrast agents, but also that changing simple parameter like the nature of oil, we can target the imaging properties.

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Implication of alpha5 beta1 integrin in resistance to anti-EGFR therapies 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. Clinical trials using anti-EGFR therapies for the treatment of GBM reveal limited efficacy. Overexpression of the fibronectin receptor, α5β1 integrin, is associated with a poor prognosis for patients and the integrin triggers resistance to chemotherapy. 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 α5β1 integrin in GBM cells and determine its potential involvement in resistance to anti-EGFR targeted therapy.

Results:

We genetically modified a glioblastoma cell line, to overexpress (U87-a5+) or repress (U87-a5-) a5 integrin expression. We first examined the impact of EGFR/a5 crosstalk on cell migration with anti-EGFR drugs (Cetuximab® and Gefitinib®) used in clinical trials. Using Boyden chamber assay with a fibronectin coating, we showed that U87-a5+ cells are resistants to Cetuximab® and Gefitinib® activity. By contrast, the loss of α5 integrin sensitizes U87 MG cells towards anti-EGFR drugs. Inhibition of a5 integrin with specifics inhibitors (RGD mimetic antagonist and antibody) restores the sensitivity of U87-a5+ cells toward Cetuximab® demonstrating the importance of a5 integrin in resistance to anti-EGFR drugs in glioblastoma. Next, we examined the impact of a5 integrin expression on collective cell migration of spheroids. We examined the propensity of cells to migrate out of spheroids onto fibronectin coating. The addition of Gefitinib® during cell migration reduces by half the migration of a5 deficient cells compared to control cells. By contrast, U87-a5+ cells are totally resistant to anti-migratory activity of Gefitinib®. We then tested the implication of a5 integrin in EGFR trafficking in U87-MG cells resistant to Gefitinib® action during cell migration. Gefitinib® treatment induces a translocation of a5b1 integrin from focal adhesions to intracellular vesicles which are not identified for the moment. Moreover, the addition of Gefitinib® modifies the EGFR localization in U87-a5+ spheroids but not in U87-a5- spheroids.

Conclusion:

Using two models of migration, we showed that α5 integrin drives U87-MG cells resistance to anti-EGFR drugs. A drug association targeted EGFR and α5β1 might be a new therapeutic option to overcome resistance in brain tumors which overexpress a5b1 integrin. Moreover, Gefitinib® treatment increases the internalization of a5b1 integrin and the EGFR trafficking seems to be implicated in resistance to anti-EGFR drugs in glioblastoma cells.

Optical dissection of gating in P2X receptors

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P2X receptors are ligand-gated ion channels activated by extracellular ATP. They form trimeric pores selective to cations and are involved in different physiological and pathological functions, including neuromodulation, neuropathic pain and vascular remodeling (1). Crystal structures were solved recently (2) but they give little insight into the dynamic motions involved in channel gating. Complementary functional approaches have thus to be developed to address this issue. Here we present a new method, which uses photoisomerizable azobenzene-containing crosslinkers as "molecular rulers" to investigate channel gating. We have engineered P2X receptors to obtain optical control of the channel: they can be opened and closed by irradiation in the absence of their native ligand. The *cis-trans* isomerization of the covalently tethered bis-maleimide azobenzene-containing derivatives between substituted cysteine residues induces movements of the transmembrane helices that are similar to those induced by ATP. With these valuable experimentally derived interatomic distances, we confirm the X-ray predicted expansion of the extracellular part of the channel during opening and further identify a new mechanism of channel gating. Our novel approach can thus be extended to any membrane-embedded proteins for investigating physiological dynamic motions.

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Palladium-catalyzed synthesis of bridged Phe-Gly dipeptide to access novel ligands of Translocator Protein 18kDa (TSPO).

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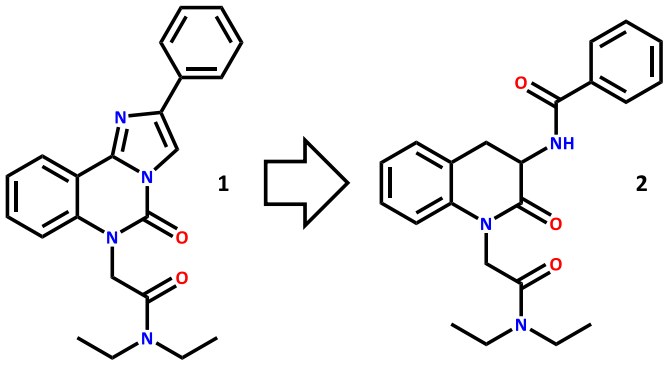
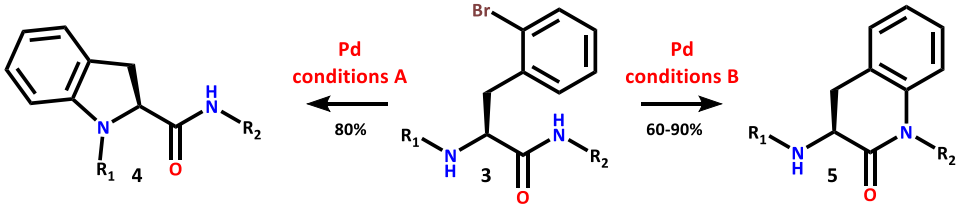
We identified compound NCS1008 (**1**) as a good ligand of the Translocator Protein 18kDa (TSPO). As many ligands of this protein, the druggability of NCS1008 is pretty poor as this compound is planar and contains a high number of aromatic rings leading to low water solubility.

With the goal to improve druggability, we designed a bridged Phe-Gly dipeptide (**2**) which presents the same pharmacophoric pattern as NCS 1008. This non-natural rigidified dipeptide constitutes a new unplanar scaffold for TSPO. The bridged dipeptide is obtained through an intramolecular Buchwald-Hartwig cross-coupling reaction starting from a 2-bromophenylalanine-glycine derivative (**3**), and we developed conditions that induce chemoselectivity leading to either indoline (**4**) or 3,4-dihydroquinolinone (**5**) derivatives. Performed under mild conditions, no racemization was observed during cyclization. A preliminary mechanistic study was performed to identify the parameters leading to the cyclization chemoselectivity.

Following an unconventional strategy, we started from a planar heterocyclic compound to design a novel unplanar bridged dipeptide, affording a new class of TSPO ligands.





Interaction of Gag (NCp7) in human immunodeficiency virus HIV-1 with ribosomic protein RpL7: complex characterization and role(s) in replicative cycle

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Gag is a polyprotein composed by various domains such as matrix (MA), capsid (CA), nucleocapsid (NCp7) and p6 as well as peptides SP1 and SP2 surrounding NC. Gag interact with gRNA *(1)* to initiate it dimerization, a mandatory step for the production of infectious particles and also interacts with large scale of cellular proteins (ALIX, Staufen…). Among them, our team was interested by the human ribosomal protein called RpL7, a multifunctional protein playing important roles in ribosome biogenesis and other extra-ribosomal activities like regulation of translation*(2)*. We demonstrated that Gag interacts with RpL7 by coIP and yeast 2-Hybrid throught the NCp7 domain of Gag with the N- and C- terminal domains of RpL7 in an RNA independent manner. We also confirmed the presence pf RpL7 in viral particles. To characterize the role of this complex an *in vitro* study was initiated, using a model measuring nucleic acid chaperone activity *(3)*; we demonstrate that RpL7 actively chaperones cTAR-dTAR duplex while Gag was less efficient in similar conditions. More importantly, we show that Gag-RpL7 accelerate this hybridization. In conclusion, we discovered and characterized a new cellular partner of Gag, the RpL7. We propose a model whereby Gag could recruit a cellular protein to offset the low level of Gag chaperone activity and help in gRNA dimerization, key step of encapsidation of viral genome.

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Identification and characterization of a novel GPR103 antagonist

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Chronic pain is a common health issue that remains difficult to treat. Opiates represent the standard treatment used in clinic but their chronic use leads to the development of several adverse side effects including hyperalgesia (enhancement of the pain perception) and tolerance (decrease of the analgesic effects over time). It has been proposed that stimulation of opioid receptors triggers activation of anti-opioid systems that in turns produce hyperalgesia thus diminishing the net analgesic effect of the opioid agonist (tolerance). This process has been evidenced *in vivo* both in rats and in man where acute and prolonged opioid treatments induce a long lasting hyperalgesia. Anti-opioid receptor antagonists could therefore represent a promising strategy for limiting the development of pain hypersensitivity and analgesic tolerance associated with chronic opiates treatments. Several systems have been shown to display anti-opioid properties including RF-amide neuropeptides family and their receptors. These peptides have an Arg-Phe-NH2 motif at their C-terminal and act through 5 specific GPCRs (G protein coupled receptors). However, pharmacological tools, particularly antagonists, are missing for several of these receptors including the receptor of 26RFa GPR103.

In collaboration with a team of chemists, we therefore decided to identify a GPR103 small ligand capable of antagonizing the effect of 26 RFa both *in vitro* and in *vivo*. In a first step, we screened a chemical library of RF-amide derivatives (± 2000 molecules) on recombinant human GPR103 receptor stably expressed in CHO cells. From this screening procedure we found several molecules that display significant affinity for GPR103. We further characterized the *in vitro* pharmacological profile of these hits and identify RF10 that display a good affinity (< 100 nM) for GPR103 as well as a high selectivity and antagonist activity at this receptor. In a second part, we tested the effect of RF10 *in vivo* in a mouse model. We measured the pain sensitivity to heat after the injection of either 26RFa (2.5 nmol, i.c.v.) alone or coadministered with RF10 (1 nmol, i.c.v.). As expected, we observed that 26RFa induced significant hyperalgesia while RF10 completely prevented hyperalgesia induced by this peptide. Altogether, our results indicate that RF10 represents a good pharmacological tool to study the involvement of GPR103 in the modulation of nociception and opiates analgesia.

New antimalarial flavones: from screening to *in vivo* proof of concept

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Malaria is the deadliest parasitic disease with almost 600.000 deaths every year[1]. The parasite (*Plasmodium sp.*) is transmitted by a female *Anopheles* mosquito during a blood meal. It undertakes then a complex life cycle in humans: first in the liver, then in the red blood cells. This erythrocytic cycle is responsible for the symptoms like fever, sweat and shivering. Death occurs in complicated cases due to severe anemia, kidney failure, or coma.

Only a few drugs cure malaria, and on top of that the parasite is resistant to most of them. The first line treatment—artemisinin—is not an exception: in 2008, Noedl and colleagues reported the emergence of partial resistance to artemisinin in South-East Asia[2]. By the past, progress in decreasing the mortality has already been reversed because of resistance spreading from Asia to Africa. Artemisinin resistance seems to follow the same march: resistance has already spread to Myanmar near the Indian frontier[3]. To conduct its malaria eradication plan by 2050, WHO needs new fast acting drugs with original mechanisms of action.

After the isolation of an active biflavonoid from *Campnosperma panamense* (Anacardiaceae, IC_{50} = 480 nM *in vitro* on *P. falciparum* K1 multi-resistant strain)[4], we developed novel simplified synthetic analogs (MR series) with improved pharmacological and pharmacokinetic profiles. Two of them (MR70 and MR87) exhibit a partial *in vivo* antimalarial activity. They reduce parasitaemia by 35% to 70% respectively on day 4 on a murine model (*P. berghei* ANKA, dosing regimen of 100 mg/kg for 4 days). But these compounds showed no significant improvement in terms of survival.

MR70 is parasiticidal on early blood stages of *P. falciparum* in less than 30 minutes. Interestingly, these stages are specifically the ones that are resistant to artemisinin[5]. Further investigation is needed to optimize *in vivo* activity and to understand the underlying mechanism(s) of action of these compounds.

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Role of Toll like receptor 7 and plasmacytoid dendritic cells in mouse models of experimental arthritis

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Introduction: Among immune cells, plasmacytoid dendritic cells (pDCs) are the main type I IFN producers following activation (i.e. upon viral challenge). These cells, which are also characterized by their expression of the endosomal TLRs (TLR3, 7/8 and 9) are central in the initiation of the inflammatory response and as such, are involved in the etiology of several chronic inflammatory diseases. Surprisingly however, anti-inflammatory effects of type I IFN were also considered [1], especially IFN β, which is known for its beneficial effect in murine models of rheumatoid arthritis (RA) [2]. In addition, circulating pDCs depletion in RA patients was observed [3], suggesting a protective role for these cells. In this work, we aimed at a better characterization of the role of these cells in murine models of RA to clarify these contradictory observations.

Methods: Induction of arthritis was made by arthritogenic (K/BxN) serum transfer [4] and upon repeated injection of heterologous collagen (CIA) [5]. Symptoms were evaluated by visual scoring and measurement of the joints with a caliper. IKAROS $^{L/L}$ mice are hypomorphic mutants in a C57Bl/6 background which exhibit reduced numbers in peripheral pDCs [6]. DBA/1 mice were used in the CIA model. Cellular infiltrates were analyzed by FACS and cytokines expression with ELISA and RTqPCR. Bone histological evaluation used TRAP staining.

Results: IKAROS $^{L/L}$ (pDC-deficient) mice showed exacerbation of inflammatory and arthritic symptoms after arthritogenic serum transfer (as seen upon measurements and scoring, quantification of pro-inflammatory cytokines, histological analysis of the erosion). In wild-type animals, topical application of a TLR7 agonist which induces Type I IFN production reduces articular inflammation in K/BxN and CIA arthritis models and concomitant pDCs recruitment at inflammatory sites. Our results suggest a beneficial role of pDCs in arthritis

Conclusion: Alternative pDC depletion models (genetic, Ab-mediated) as well as Tlr7$^{-/-}$ mice will be investigated to confirm these results. Our data, which suggest possible interactions between pDCs and other cells present in the inflamed, arthritic joint, could lead to new therapeutics options.

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Genetic analysis of epidermal cell mechanical properties during *C. elegans* embryonic elongation

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The contribution of mechanical forces to development is gaining wider acceptance. But the detailed molecular mechanism by which cells sense and respond to forces remains elusive. To investigate those issues, we focus on *C. elegans* embryonic elongation, a process controlled by cell shape changes transforming a ball of cells into a tube-shaped animal. *C. elegans* embryonic elongation consists of two phases. First, it is driven by the epidermal actomyosin. Second, muscles promote elongation through a recently reported mechanotransduction pathway involving CeHDs, which mechanically link muscles to the epidermis.

PAK-1 (p21-activated kinase) is a key regulator of both phases: it is a good starting point to further dissect the molecular landscape of elongation. To reach this aim, we have two approaches. First, we carried out a systematic search for genetic interactions by an RNAi screen, targeting 356 genes in a *pak-1(Ø)* mutant. Second, we looked for potential PAK-1 interactors by a yeast two-hybrid screen. We tested the *in vivo* relevance of the most interesting candidate, the α-spectrin SPC-1. We established a genetic interaction between the two genes, showing that double mutants display a novel elongation defect, whereby they retract to a lima bean-like shape after reaching the 1.5-fold stage. Retraction is not observed if muscles are defective, suggesting it is induced by muscle twitching. Spinning disk time-lapse analysis showed that the elastic properties normally displayed by epidermal cells in response to muscle twitching input are affected in double mutants. To further confirm these results, we are using laser ablations in the dorsoventral epidermis to measure epidermal viscoelastic properties.

Consistent with the genetic interaction, *in vivo* expression studies revealed a co-localization between the two proteins at the apical level of the epidermal cells. Furthermore, we found that the SH3 domain of SPC-1 interacting with PAK-1 *in vitro* is essential for its function *in vivo*, and that loss of SPC-1 disturbed PAK-1::GFP localization.

To define why embryos retract, we examined important elongation players and found that in the double deficient embryos the actin cytoskeleton and the CeHDs are getting gradually affected as the retraction appears and evolves.

Finally, we performed a supplementary genetic screen that allowed us to reproduce the retraction in different genetic contexts. Thereby we have identified novel molecular players acting together with *spc-1* and *pak-1*, triggering a retraction.

Altogether, we found that the SPC-1—PAK-1 interaction is important for *C. elegans* embryonic elongation. Moreover, we suggest it modulates the elastic properties of dorso-ventral epidermal cells submitted to external mechanical stress, helping to stabilize them between consecutive inputs.

Physiopathology of Tubular aggregate myopathy

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Tubular aggregate myopathies (TAM) are progressive muscle disorders with an autosomal dominant inheritance and characterized by abnormal accumulations of membrane tubules in muscle fibers. Our team identified STIM1 as the first gene implicated in TAM. STIM1 regulates calcium homeostasis through a mechanism known as store-operated calcium entry (SOCE). Upon stimulation, calcium is released from the sarcoplasmic reticulum to the cytoplasm, where it triggers muscle contraction and acts as a second messenger controlling growth and differentiation. In case of calcium store depletion, STIM1 unfolds, oligomerizes and thereby activates the calcium entry channel ORAI1 to trigger extracellular calcium entry. We demonstrated that the identified STIM1 mutations strongly impact on the calcium level in TAM myoblasts. However, the nature, formation and pathogenicity of the tubular aggregates are not yet defined and the link between STIM1 mutations and muscle dysfunction is not understood.

My PhD project aims to investigate the physiopathological mechanisms underlying TAM in both cellular and animal models. In order to analyze the formation and physiological impact of the tubular aggregates, I used correlative light-electron microscopy (CLEM) to monitor the aggregates in muscle cells overexpressing wild type or mutant STIM1 constructs. I observed that the STIM1 clusters correspond to stacks of membrane layers, and I will next compare and quantify the clusters size and membrane composition at different time points post transfection in order to assess the timeframe and phases of tubular aggregate formation.

There is currently no mammalian model for tubular aggregate myopathy. I therefore plan to use the AAV system to analyze the impact of different STIM1 mutations on muscle structure and function in $STIM1^{+/skm-}$ (= heterozygous knockout in skeletal muscle) mice. I generated STIM1 AAV constructs harboring different mutations that will be injected in the tibialis anterior and I will perform histological, ultrastructural and immunofluorescence analyses on the muscle at different time points post-transduction to decipher the sequence of events leading to TAM. In order to correlate molecular and cellular alterations with disease development and muscle function, the transduced mice will furthermore undergo general and specific force measurements in response to nerve and muscle stimulation at different time points post-transduction.

In conclusion, my project is expected to provide new insights in the physiological mechanisms leading to calcium-related muscle dysfunction and TAM, and might suggest targets for therapeutic approaches.

Cyclohydrocarbonylation-Based Strategy towards Novel *N*-Heterocyclic Scaffolds Derived from Aza-diketopiperazines

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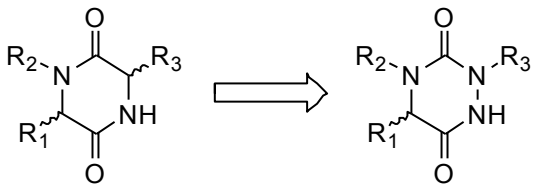
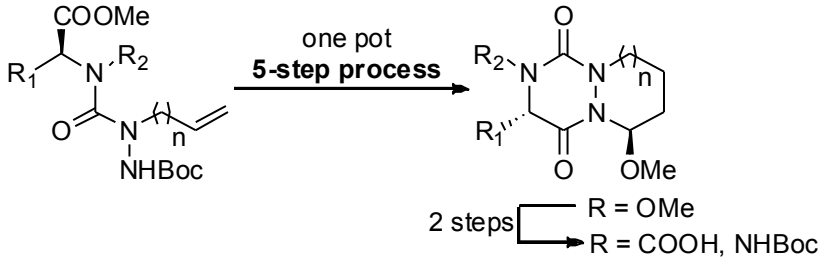
Privileged structures represent an ideal source of new biologically active molecules. Among these compounds, 2,5-diketopiperazines (DKP) are represented in a large array of structurally diverse natural products and display interesting therapeutic properties[1,2]. As reported for aza-peptides[3], the replacement of one Cα-stereogenic center by a planar nitrogen could have a profound impact in both the chemical and biological properties of DKP and could offer new potential opportunities for drug discovery and chemical biology.



Herein, we will present an efficient diversity-oriented and stereoselective synthesis of novel heterobicyclic and tricyclic compounds derived from this scaffold. To access such structures, we have explored a strategy based on Rh(I)-catalyzed hydroformylative cyclohydrocarbonylation (CHC)[4] of allyl-substituted aza-DKP. To facilitate the diastereoselective access to novel aza-DKP platforms, a one pot reaction combining 5-step process was also envisaged starting from allyl carbazate.



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Development of design tools for biosystem engineering

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Over the past fifteen years, synthetic biology, a new scientific field at the interface between biotechnologies and engineering sciences has developed rapidly. The goal of synthetic biology is to create new biological functions by a rational assembly of artificial or natural biological parts [1].

Along with the improvement of technological material and processes, focus should be put on the improvement of design tools (*in silico* simulations) and methodologies. As this rational approach used to design genetic networks is very similar to the one used in microelectronics, our team chose to take advantage of the valuable know-how of microelectronics scientists aquired with 40 years of experience in creating adapted design tools [2]. By using electronic formalism, we work on modeling biological systems and adapting the generic electronic workflow to synthetic biology by enriching existing tools to support multi-domain design with biological models or by developing new tools for synthetic biology.

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Isomorphic fluorescent nucleoside analogs: applications in protein/nucleic acid interactions

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Monitoring site-selective conformational changes in nucleic acids by fluorescence techniques is highly challenging due to the lack of appropriate fluorescent nucleic acid analogs. The most explored nucleoside analog, 2-aminopurine (2Ap),[1] has been used in countless assays though it suffers from very low quantum yield, especially when included in double strands. Moreover, its residual emission frequently does not represent biologically relevant information.

To circumvent these limitations, a nearly perfect fluorescent substituent of the guanine base thienodeoxyguanosine ($d^{th}G$) was designed.[2,3] Using steady-state and time-resolved fluorescence spectroscopy, we compared the ability of 2Ap and $d^{th}G$ to substitute and provide faithful structural and dynamical information on a key G residue in the (−) DNA copy of the HIV-1 primer binding site sequence. $d^{th}G$ fluorescent nucleoside, in contrast to 2Ap, was found to fully preserve the stability of the labeled hairpin and the duplex, and also showed advantageous photophysical properties. In further contrast to 2Ap, the fluorescently detected $d^{th}G$ species represent the predominantly populated G conformers that allow studying their relevant dynamics. Being able to perfectly substitute G residues, $d^{th}G$ opens a large area of possible applications and will be particularly used in studying the annealing mechanism of (−)/(+) PBS DNA, an HIV-1 reverse transcription key step.

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IChem-PIC – 3D mapping and classification of protein-protein interfaces.

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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 begins by the detection of biological interfaces. In protein structures we can observe two types of interface, biological and crystallographic. It is essential to separate them to retain the biologically relevant ones. This separation is determined by a machine-learning model generated from known x-ray structures.

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 (hydrophobic, hydrogen bond, ionic bond and aromatic interaction). We used a Random Forest algorithm to separate 300 known protein-protein Interfaces in two classes. We optimized the model parameters by cross-validation and test it on 4 external data sets. The first one is composed by 100 PPis manually curated in the lab. The 3 others are data sets from the literature currently used for the study of PPi. We remove all redundancy on the 4 sets.

Our Model shows that some descriptors (number of protein-protein interactions, proportion of fully buried hydrophobic contacts) are key to distinguish biological from crystallographic interfaces. The model is robust and presents an accuracy between 75 and 90% depending on the external data set used.

We herewith present a novel computational approach (available online) to distinguish between biologically-relevant and crystallographic protein-protein interfaces. IChem-PIC is the only approach able to predict with the same accuracy the two categories of PPi.

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.

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The effect of urapidil, an alpha-1 adrenoceptor antagonist and a 5-HT_{1A} agonist, on vascular tone of isolated porcine coronary and pulmonary arteries, rat aorta, and human pulmonary artery

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While being effective and well tolerated, urapidil (Eupressyl®, Takeda Pharmaceutical Company Limited, Osaka, Japan) an antihypertensive drug has restrained indications due to its incompletely known pharmacodynamics. Recent evidence suggests that urapidil may also be of interest in the treatment of hypertension associated with preeclamptic toxaemia and in hypertensive patients with respiratory disease. Urapidil acts as a selective α_1-adrenoreceptor antagonist and as an agonist of 5-HT_{1A} receptors. On the other hand, serotonin has been shown to induce endothelium-dependent relaxations of several types of blood vessels involving 5-hydroxytryptamine (5-HT) receptors. Therefore, the aim of the present study was to determine the ability of urapidil to cause relaxations of different types of blood vessels including the pig coronary and pulmonary artery, the rat aorta and the human pulmonary artery, using vascular reactivity studies.

36 pulmonary and coronary arteries from pigs, 22 aortae from rats and 9 human pulmonary arteries were cut into rings, which were suspended in organ chambers containing oxygenated Krebs bicarbonate solution. The endothelium was removed mechanically in some rings. The pig rings were contracted with U46619, a thromboxane mimetic, the aortic rings with endothelin-1, and the human pulmonary artery rings with U46619 before construction of a concentration-relaxation curve either to urapidil or serotonin.

Serotonin but not urapidil and the 5-HT_{1A} receptor agonist 8-OH-DPAT, induced a concentration-dependent relaxation in the porcine coronary and pulmonary artery rings with an intact endothelium (P <0.05). Urapidil (10^{-5} M) markedly inhibited phenylephrine-induced contraction in rat aortic rings with and without endothelium with a more pronounced effect in rings without endothelium. Both serotonin and 8-OH-DPAT did not induce relaxation of rat aortic rings with endothelium. Serotonin and phenylephrine but not urapidil caused concentration-dependent contractions in human pulmonary artery rings.

In conclusion, urapidil in contrast to serotonin failed to cause relaxation of porcine coronary and pulmonary artery rings. The present findings, while confirming that urapidil is a potent inhibitor of α_1-adrenoceptor-induced contraction, did not support a role of 5-HT_{1A} receptor activation in the control of the vascular tone in the three types of blood vessels studied, in response to urapidil. Moreover, the inhibitory effect of urapidil is more pronounced in rings without endothelium suggesting that urapidil may target preferentially arteries with an endothelial dysfunction. Such an effect might be of particular interest in managing hypertension associated with preeclamptic toxaemia and pulmonary disease.

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