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Synthesis and biological investigation of new (–)-zampanolide analogues

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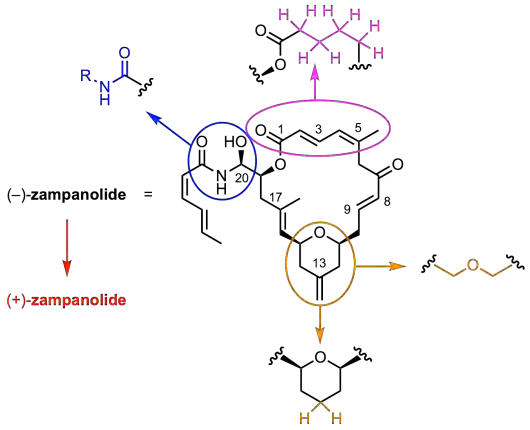
The continuous evaluation of new cytotoxic natural products offers significant potential for the discovery of new antitumor agents, which may overcome the multi-drug-resistance (MDR) problem. (–)-Zampanolide[1,2] is a marine macrolide, which exhibits low nM cytotoxicity against both drug-sensitive and MDR cancer cell lines.[3]

In 2013, a crystal structure of the complex between αβ-tubulin and (–)-zampanolide has been characterized.[4] Based on the crystal structure and existing SAR data, we have prepared new (–)-zampanolide analogues with reduced structural complexity and we have investigated the effects of these structural changes on microtubule-binding affinity and cellular potency.



For one analogue, we were also able to obtain an X-ray crystal structure in complex with β-tubulin. While this analogue had retained the potency of (–)-zampanolide almost completely, significant differences were found between the binding mode of the natural product and its modified congener. Finally, we have evaluated basic pharmacokinetic properties of selected compounds *in vitro*, as a basis for potential future *in vivo* applications.

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„Mini-Monoplant Technology for Pharmaceutical Manufacturing”

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Continuous processes are often related to the concept of process intensification as the unit operations are optimized and intensified by using the best in class technology. In other words, the equipment is selected to fit the optimal process rather than the process is fitted into the available equipment. However, this manner of operation is by far more complex and prone to extended R&D development time. Thus, for drug substances in clinical development phase, flow process steps or even full flow processes have not been considered until recently on a broad basis. A later or in-parallel developed continuous process or process step may encounter additional hurdles as it has to outperform the current process or process step significantly in order to justify a change in regulatory documents. Another important aspect that must be considered is the capital investment into new equipment upon scale up.

In this talk the concept of mini-monoplant technology will bediscussed and how the concept can provide a more attractive approach towards the development of continuous processes or process steps for API manufacturing. Some recent and published examples will be given to demonstrate the versatility of continuous processing in the field of API manufacturing.

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Synthesis and characterization of an adenosine A_1 receptor agonist with non-opioid analgesic properties based on Gα signaling bias

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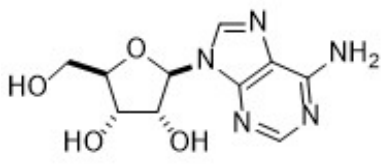
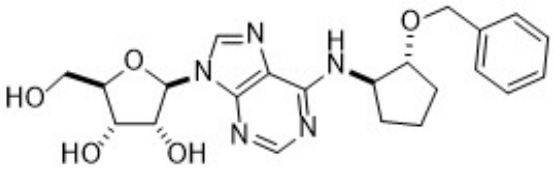
The four adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3) belong to the of G protein-coupled receptors (GPCRs). The purinergic signaling system plays an important role both in the central (CNS) and peripheral nervous system (PNS), but it is widely operational in other human tissues. For this reason, adenosine receptors (ARs) are linked to oncological, cardiovascular, neurological, respiratory and inflammatory disorders. Significant efforts were made by companies to develop agonist or antagonists against specific AR subtypes in order to treat diseases in the aforementioned areas. So far, no such drugs have been approved. GPCRs are one of the prominent target classes in medicinal chemistry and cover one third of all medicines, however, adenosine receptors (ARs) have particularly suffered as drug targets due to their propensity to couple to different Gα subunits, activating several downstream signaling pathway and therefore causing unwanted side effects. In the case of A_1R, its activation can cause cardiorespiratory side effects. The therapeutic limitations of A_1R due to its unselective coupling could be overcome by developing biased agonists: compounds that selectively activate one intracellular signaling pathway over others.



Adenosine



BnOCPA

Among the compounds synthesized in our laboratory, several showed A_1R subtype-selective agonist activity. Interestingly, compound BnOCPA stood out in that it is able to activate one particular G protein-mediated signaling pathway in a highly potent and selective manner. This exclusive Gα signaling bias was validated for the first time *in vivo*. BnOCPA properties were studied in a rat model of chronic neuropathic pain and showed that it is a potent and powerful analgesic without causing any side effects such as bradycardia, hypotension, respiratory depression, tolerance, dependence and abuse potential, as most opioid drugs. Therefore, BnOCPA represents a powerful to investigate biased GPCR signaling and it is also a lead structure of a new first-in-class analgesic drug.

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[2] J. Med. Chem. 2016, 59, 3, 947–964

Discovery and optimization of novel LpxC inhibitors for the treatment of serious Gram-negative infections

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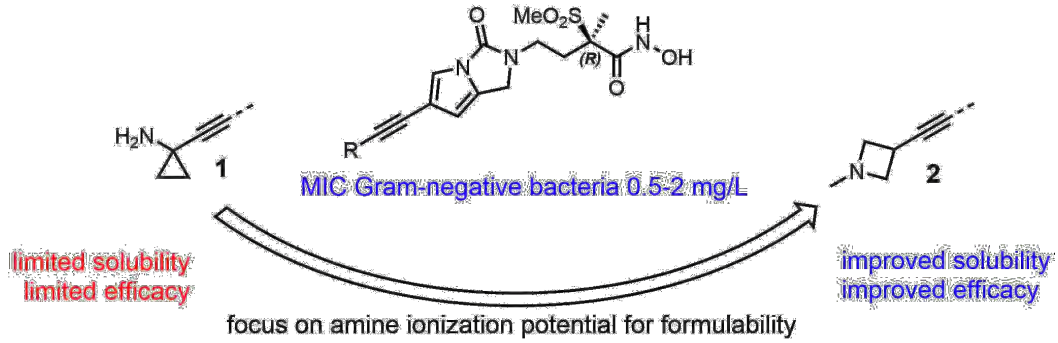
UDP-3-*O*-((*R*)-3-hydroxymyristoyl)-*N*-glucosamine deacetylase (LpxC) is as an attractive target for the discovery and development of novel antibacterial drugs addressing multi-drug resistant Gram-negative bacteria. After the discovery of novel inhibitors of LpxC, featuring a methylsulfone hydroxamic acid warhead harnessed on a 1,2-dihydro-3*H*-pyrrolo[1,2-c]imidazole-3-one scaffold, a structure-based lead generation afforded bis-alkyne compounds such as **1** with decent in vitro potency. However, their solubility and efficacy in vivo remained limited. The lead optimization program resulted eventually in the identification of a series of azetidines with high solubility and potent efficacy against Gram-negative pathogens in animal infection models. The presentation will describe the discovery of compound **1** and the dedicated optimization process that led to compound **2**.[1,2]



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Human and Chimp CPEB3 ribozymes: unexpected fold of the HDV-like ribozymes

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Cytoplasmic polyadenylation element binding (CPEB) proteins are involved in many vital processes including cell division, synaptic plasticity, learning and memory. A highly conserved, short mammalian ribozyme was found within an intron of the CPEB3 protein. This CPEB3 ribozyme is the third ribozyme confirmed in humans, besides the ribosome and the spliceosome, and belongs to the broad family of the hepatitis delta virus (HDV)-like ribozyme. All members of the HDV-like family have several features in common: (i) a nested double-pseudoknot structure, (ii) 5'-end self-cleavage activity, and (iii) a conserved cytosine in the catalytic core. However, their self-cleavage rates are highly divergent and it is assumed that the rates directly depend on the overall stabilization of the catalytic core. Since the first crystal structure of the cleaved HDV ribozyme in 1998 followed by structures of uncleaved, mutant-inhibited and ion-complexed forms, no three-dimensional structure of any other ribozyme of this family was published.

Here we present the first crystal structures of the cleaved human and chimp CPEB3 ribozyme in complex with the U1A spliceosomal protein. Theses sequences differ by only a single nucleotide but show a difference in cleavage rate by around ~1 order of magnitude. Our crystallographic data disclosed two highly similar structures with the proposed sophisticated double-pseudoknot fold. However, only the four helical regions P1, P2, P3 and P4 are present, whereas P1.1 consisting of only one Watson-Crick base pair and a U-U wobble is not formed. Instead, we observed an alternative interaction in which two copies of RNA base pair within the L3 region with each other. The dimer formation was also consistent with SEC-SAXS experiments and suggesting the highly dynamic behavior of the catalytic core. This is well in accordance with our NMR data of the cleaved wild-type human and chimp ribozyme in which P1.1 formation occurs only in the presence of at least 5 mM Mg^{2+} ions.

Discovery of Potent Selective $GABA_A$ Alpha5 Positive Allosteric Modulators (PAMs) for the treatment of neurological disorders

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$GABA_A$ receptors are ligand-gated chloride channels and the main mediators of inhibitory synaptic transmission in the human brain. There are 19 genes encoding for $GABA_A$ receptor subunits that assemble as pentamers, with the most common stoichiometry of two α, two β, and one γ subunit. The α5 subunit-containing $GABA_A$ receptors are of particular interest given their specific expression pattern[1] and physiological properties[2]. Multiple lines of evidence suggest that excessive neural activity in selected brain regions with consequent imbalance between excitatory/inhibitory neurotransmission underlie a variety of neurological disorders such as epilepsy, Autism Spectrum Disorder (ASD), Schizophrenia and Alzheimer's disease. The presentation will highlight our effort to identify highly potent, selective $GABA_A$ α5 PAMs from a program that already led to a clinical NAM asset (Basmisanil). Key medicinal chemistry concepts involved in the optimization of the ligands and structural determinants underlining the NAM-to-PAM switch will be disclosed. Finally, proof of concept studies with selected PAMs in disease-relevant animal models will be presented.

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The effects of chemical reactions on codon drift in DNA encoded libraries

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DNA encoded libraries connect small molecules with genetic information and became an important tool for drug discovery in both academia and industry [1]. They allow the construction of libraries with millions of different members that can be tested at the same time using, for example, affinity enrichments. A major disadvantage is the limited number of chemical transformations available that reduce the available chemical space [2], although there has been some significant progress on that in the recent years [3].

A key problem of reactions in DNA encoded reactions is always the side reactions they might undergo with the DNA tag, impairing the genetic information. A popular approach is the measurement of DNA amplifiability by utilizing qPCR [4]. More recently, characterization of reaction conditions according to their encoding fidelity has been shown [5]. Herein, we give insight into a third parameter that can be used to characterize reactions. Modifications of nucleobases can cause mutations and, as a result, the DNA used for encoding can change.

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Microcycle: An Integrated Design-Make-Test-Analyse Platform to Accelerate Drug Discovery

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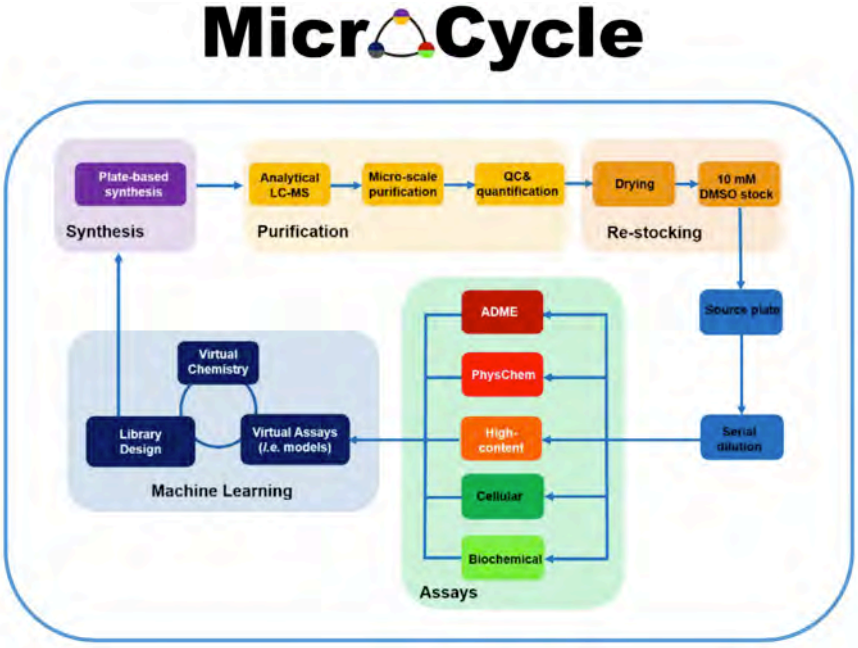
Microcycle is an integrated drug discovery platform which utilizes micro-scale chemistry, automated purification, real-time biological and physicochemical profiling in combination with machine learning driven compound design. This modular technology platform enables rapid access to high quality chemical matter, by integrating existing assay technology we deliver an array of profiling data to drive multi-parameter optimization in every learning cycle. This enhanced compound optimization process is generating knowledge for drug discovery projects in a timeframe never before possible. Our inspiration originates from a desire to enable fast and integrated translation of screening hits, into best in class chemical leads founded on deep collaborations with drug discovery teams.



Synthetic Collagen Heterotrimers by Conformational Design

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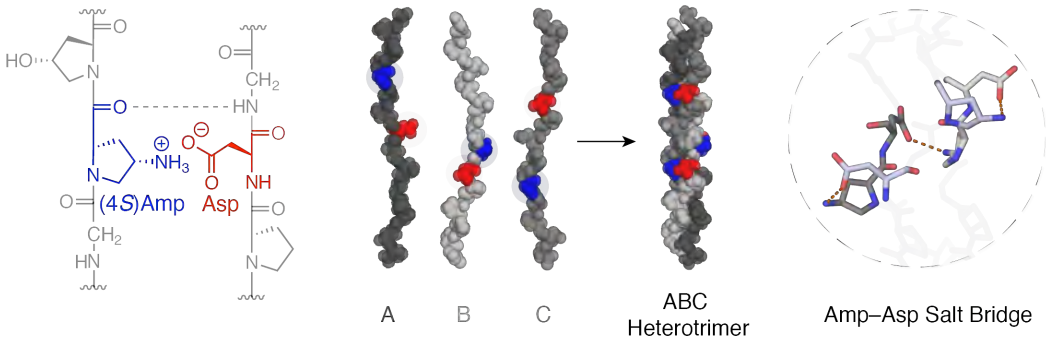
[1]Laboratory of Organic Chemistry, D-CHAB, ETH Zurich

Collagen is a key structural protein with a unique triple helical fold that comprises three protein chains. Collagen heterotrimers are composed of two or three different strands and assume essential biological functions by binding to transmembrane receptors *via* recognition motifs that are defined by the spatial arrangement of the strands.[1] Synthetic access to heterotrimers allows for determining unknown chain arrangements of natural collagen and for developing collagen-like materials for biomedical applications.[1,2] The synthesis of heterotrimers is, however, challenging since three different strands (A, B, C) can self-assemble into 3^3 = 27 triple helices of different composition and chain arrangement (ACB, AAB, CBC, *etc.*).[2] To achieve assembly of distinct peptides into only one triple helix, amino acids that selectively interact with each other need to be incorporated into neighboring strands. So far, Lys and Asp residues have been utilized to direct heterotrimer formation by salt bridges between the ammonium and carboxylate groups, but due to the conformational flexibility of the Lys side-chain these interactions are rather weak.[2]

Here, we present a salt bridge between (4*S*)-aminoproline (Amp) and Asp that guides heterotrimer formation.[3] As a result of the rigid conformation of Amp, the ammonium group forms a geometrically defined salt bridge with the carboxylate group of Asp in an adjacent strand of the triple helix. Three Amp–Asp pairs proved sufficient to assemble a unique 24-mer ABC triple helix with complete selectivity over the 26 competing helices. The position of the Amp and Asp residues can readily be adjusted to access any other type of heterotrimer (AAB, ABA, *etc*.). Our design was corroborated by an X-ray crystallographic structure of an ABB heterotrimer that provided detailed structural insights into heterotrimeric triple helices and, importantly, contributed to the understanding of the optimal conformation of a salt bridge between ammonium and carboxylate groups.

In summary, our study enables the rational design of heterotrimeric collagen with full structural control. These results open intriguing prospects for the development of synthetic collagen for biological applications.



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Stepwise Design of γ-Secretase Modulators

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[1]F. Hoffmann-La Roche Ltd, Roche Innovation Center Basel

We will present the design and synthesis of small and potent γ-Secretase Modulators (GSMs) starting from our most profiled lead compound RO6800020 using sequential structural replacements to improve desired properties, safety and potency.

Importantly, we also describe a novel phenyl ring bioisostere that affects dramatically the compound properties.

Our in-depth lead optimization strategy culminated in series of potent and in vivo active γ-secretase modulators devoid of safety flags with one compound being selected as an advanced candidate

High-affinity glycomimetic ligands for human Siglec-8

G. Conti[1,2], B. Kroezen[1], J. Cramer[1], B. Girardi[1], S. Rabbani[1], O. Schwardt[1], D. Ricklin[1], R. Pieters[2], B. Ernst[1]*

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We present here the identification of glycomimetics with low µM affinity towards the human Siglec-8. Siglec-8 is an immunoglobulin-type lectin solely expressed on eosinophils and mast cells, and weakly on basophils. Many pathological conditions are associated with altered functions and/or numbers of these cells, among which allergic inflammation and asthma[1]. Despite the only partially known biological mechanism of action, the pharmacological importance of Siglec-8 has been demonstrated as eosinophil apoptosis and inhibition of mast cell degranulation could be achieved by means of anti-Siglec-8 monoclonal antibodies or synthetic glycopolymers decorated with Siglec-8 ligands[2]. However, no small molecules targeting Siglec-8 have been described so far. Such molecules could be useful to better elucidate the apoptotic cellular pathway and potentially provide a new pharmacological approach for eosinophil and mast cell associated diseases.

The glycan epitope recognized by Siglec-8 is the tetrasaccharide 6'-sulfo sialyl Lewisx (6'S-sLe^x)[3]. While the sialic acid carboxylate and the sulfate group on the galactose are involved in two crucial salt bridges, fucose and glucosamine show minor contributions to binding. Therefore, not surprisingly, we discovered that the related disaccharide Neu5Ac-Gal6S represents the minimal binding epitope (Fig. 1). This disaccharide served as lead compound for our search of new ligands with improved affinity and drug-like properties. In addition, it has been recently reported that sulfonamide modifications at the 9-position of the sialic acid moiety lead to compounds with increased activity[4].

Applying different strategies, such as Gal6S replacement with non-carbohydrate moieties, bioisostere modifications, and extension of the glycerol side chain (Fig. 1), we synthesized a new series of glycomimetic structures. The best representative exhibits a low µM affinity, *i.e.* an almost 20-fold improved affinity compared to tetrasaccharide 6'S-sLe^x. ITC measurements revealed that binding of 6'S-sLe^x is punished with a substantial entropic penalty, whereas the disaccharide mimetics exhibit beneficial entropic and enthalpic contributions.

Our study made available potent small-molecule Siglec-8 antagonists, which can be used to further explored the biological role of Siglec-8.

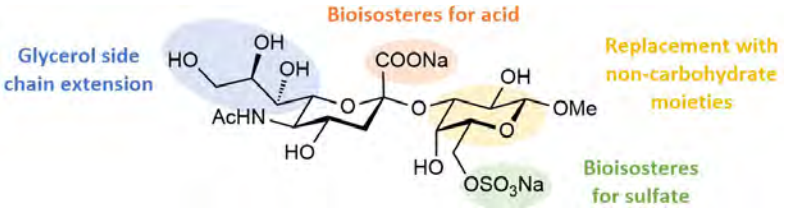

Figure 1. Chemical structure of Neu5Ac-Gal6S and the various modifications exploited for the discovery of high-affinity ligands.

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Target-based Identification and Optimization of Toll-like Receptor 7 and 8 Antagonists

T. Knoepfel[1], C. Betschart[1], R. Glatthar[1], E. Vangrevelinghe[1], T. Junt[1], S. Hawtin[1], J. Blank[1]

[1]Novartis Pharma AG, Basel

Toll-like receptors (TLRs) are an integral part of the innate immune system. They serve as both extracellular and endosomal sensors for a variety of pathogen-associated molecular patterns. Inappropriate activation of the endosomal TLRs 7 and 8 has been implicated in the pathogenesis of a series of autoimmune diseases, including lupus.

Previous hit generation strategies for identification of TLR modulators relied on cellular pathway assays, in which TLR expressing cells are stimulated with TLR specific agonists to elicit cytokines. While this approach has been successfully applied for decades, validation and optimization of hits from cellular screening campaigns can be time and resource intensive. In order to demonstrate target specificity a battery of counter-assays is required and rational optimization is complicated by the complexity of the assay system.

In this presentation we describe our efforts to identify and optimize TLR 7/8 antagonists with novel assays using the recombinant endosomal TLR 8 domain. A TLR 8 binding assay was set up using a labeled antagonist as probe and applied for high-throughput screening. The validated hits could be efficiently optimized in a structure-guided manner resulting in highly potent and selective TLR 7/8 antagonists with demonstrated in vivo efficacy after oral dosing.

Immunological Epitope Mapping of the SARS-CoV-2 Spike Protein

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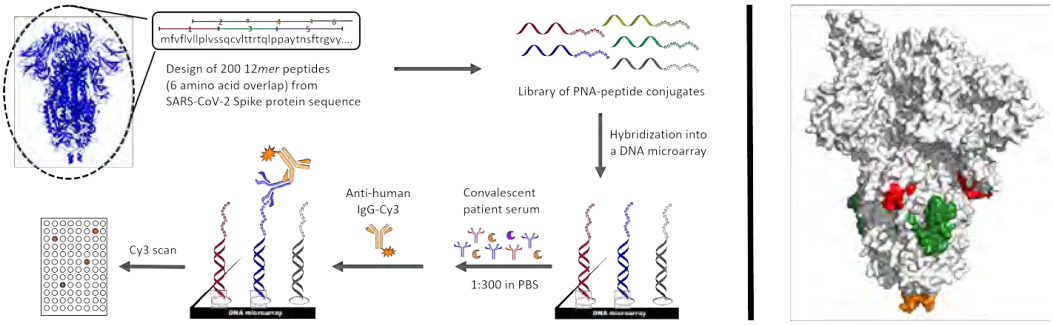
On December 2019 a novel infectious disease causing pneumonia was identified in the city of Wuhan (China). This new infectious disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has significantly impacted the economy, education and life of many countries in the world. The World Health Organization declared it a pandemic on the 11th March 2020. Mapping the epitope response of the immune system against the virus is of vital importance since it can lead to potential vaccines, more specific serological tests and to neutralizing antibodies which could be finally used as a treatment. In this work, using a peptide microarray, we have identified 3 immunodominant regions on the spike protein which are only present in COVID-19 convalescent patients' sera which could lead to potential neutralizing antibodies.



Secondary structure transitions in dephosphorylated phosvitin studied by circular dichroism spectroscopy

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Post translational modification of proteins such as phosphorylation are often associated with a protein's function in kinase-dependent signaling cascades.[1] However, the role of phosphorylation in highly-phosphorylated proteins such as phosvitin from chicken egg yolk,[2] where phosphorous accounts for 10% of its molecular weight, is still unclear. Li et al.[3] postulated that phosvitin is a phosphate storage in egg yolk. M. Sarem et al. have shown that phosvitin is capable of supporting hydroxyapatite growth suggesting a role in the skeletal development of chick embryos.[4] This capability depends on protein secondary structure, which itself is strongly pH-dependent.[5] As at least one of the secondary structure transition p*K*-values of phosvitin coincides with a p*K*-value of phosphate,[4] it is highly probable that the massive phosphorylation of phosvitin is a means to control or stabilize conformational status.

In this study, we manipulated the degree of phosvitin phosphorylation by enzymatic hydrolysis of phosphorylated amino acid side chains such as phosphoserine using potato phosphatase (acid optimum). To control the degree of dephosphorylation the phosphatase was immobilized on polymer beads and removed after varying reaction times. We employed circular dichroism (CD) spectroscopy to analyze the secondary structure of phosvitin and its dephosphorylated variants.

In comparison to native phosvitin, in a dephosphorylated variant, the transition p*K* between $P_{II}-$ disordered structure and β-sheet was increased from 1.6[4] to 3.1. Furthermore, different from native phosvitin where the pH-dependent equilibrium between the two conformations is established instantaneously, the transition in dephosphorylated phosvitin goes through a metastable intermediate indicated by transient band shifts in the CD-spectrum that can neither be attributed to $P_{II}-$ nor to β-sheet structure.

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Targeted Therapy for Neurological Disorders: A Novel, Orally Available, and Brain-Penetrant mTOR Inhibitor (PQR626)

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Mechanistic target of rapamycin (mTOR) is a key regulator of cell growth and survival. The mTOR pathway is dysregulated in many diseases including cancer and neurological disorders. Among Central Nervous Systems disorders, mTOR is implicated in Parkinson's, Alzheimer's and Huntington's disease and epilepsy. Rapamycin derivatives (rapalogs) and mTOR kinase inhibitors (TORKi) have recently been applied to alleviate epileptic seizures in Tuberous Sclerosis Complex (TSC).

Herein, we describe a pharmacophore exploration to identify a highly potent, selective, brain penetrant TORKi with optimized metabolic stability. An extensive investigation of the morpholine ring engaging the mTOR solvent exposed region led to the discovery of PQR626. PQR626 displayed excellent brain penetration as compared to everolimus and AZD2014 in rats and mice (brain:plasma ratio of ~ 1.8:1 for PQR626; ~ 1:61 for everolimus; ~ 1:25 for AZD2014).

PQR626 was well tolerated in mice (MTD: 100-150 mg/kg). A dose-range finding efficacy study in mice with a conditionally inactivated *Tsc1* gene in glia (*Tsc1*^{GFAP}CKO mice) showed a significant reduction of loss of *Tsc1*-induced mortality at 50 mg/kg PQR626 (p.o. BID, twice a day).

PQR626 overcomes the metabolic liabilities of PQR620[1], the a first-in-class brain penetrant TORKi showing efficacy in a TSC mouse model. The improved stability in human hepatocytes, together with the excellent brain penetration, safety profile and efficacy in *Tsc1*^{GFAP}CKO mice qualify PQR626 as a potential therapeutic candidate for the treatment of epilepsy and neurological disorders.

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NMR Studies of Hierarchical Protein Dynamics

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A fundamental challenge in biology is to understand the complex interaction between protein motion and protein function. In 2015 Lewandowski and coworkers have shown that temperature dependent magic angle spinning multinuclear solid state NMR relaxation measurements at temperatures ranging from 105 to 280K can provide a window into the hierarchy of dynamic processes in proteins.[1]

In order to better map the relaxations processes, the same measurements were performed at 9.4, 11.7, 14.1 and 18.8T (400, 500, 600 and 800MHz, same observables and temperature) with the same experimental protocol. At all field we are able to identify the same dynamic. Each motion can be characterized by three parameters including the energy of activation. Those information show transitions happening, where different motional process arises together, because their energy of activation is very close. And finally a comparison between GB1, SH3, Sendai virus Large protein Ntail (an Intrinsically Disordered Protein) and OmpG have been performed, and the differences can be highlighted specially on the side chain and solvent dynamic.

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One Fingerprint to Rule them All: Drugs, the Metabolome, and Biomolecules

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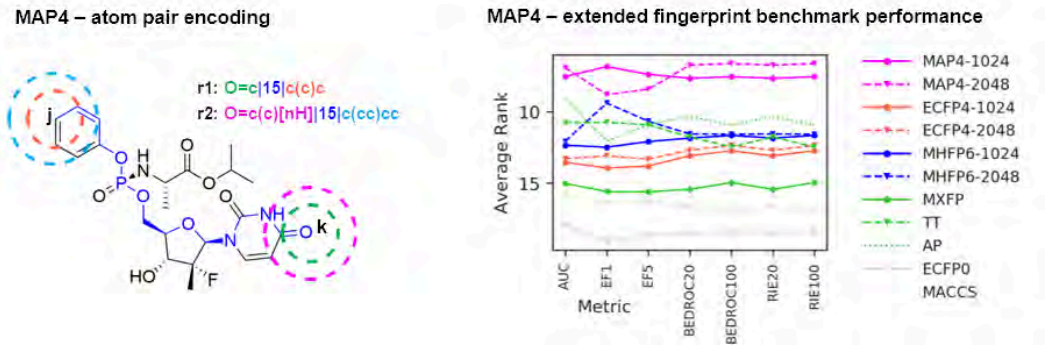
The description of molecules through molecular fingerprints is crucial in various cheminformatic approaches such as virtual screening and visualization. While substructure fingerprints[1,2] are known to perform best for small molecules and atom-pair fingerprints are preferable for large molecules,[3–6] no available fingerprint achieves good performance on both classes of molecules. Here we report a new fingerprint that combines substructure and atom-pair concepts: the MinHashed atom-pair fingerprint up to a diameter of four bonds (MAP4). MAP4 (https://github.com/reymond-group/map4) is suitable for drugs, the metabolome, and biomolecules, and it can be used to search (http://map-search.gdb.tools/) and visualize (TMAPs:[7] http://tm.gdb.tools/map4/) various databases.



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Targeting HIF-2α in Clear Cell Renal Cell Carcinoma with PROTACs

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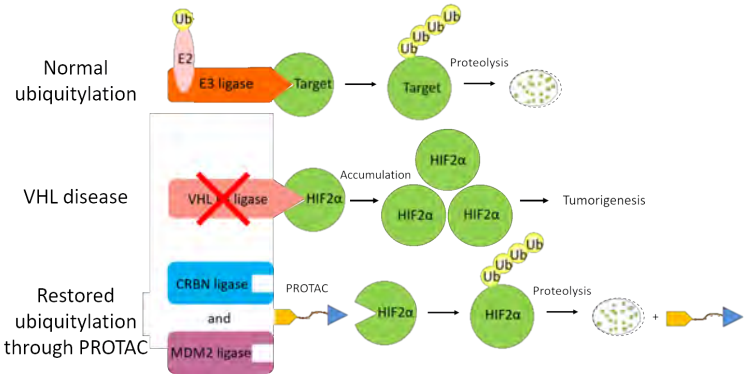
It has been shown that dysregulation of the Ubiquitin-Proteasome System (UPS) may result in the overexpression of oncogenic substrates.[1] In particular, mutations in Von Hippel-Lindau (VHL) tumor suppressor gene, which encodes for VHL E3 ubiquitin ligase, have been found in the vast majority of patients with clear cell renal cell carcinoma (ccRCC).[2] The loss of VHL ligase impairs the physiological proteolytic degradation of hypoxia-inducible factors (HIF-1α and HIF-2α), thus activating pathways that promote survival.[3] The growing evidence that HIF-2α plays a central role in ccRCC over HIF-1α fostered the development of HIF-2α antagonists.[4] Since the traditional use of small molecule inhibitors is related to resistance mechanisms, we want to take advantage of the promising targeted protein degradation approach to generate new HIF-2α degraders.[5] Essentially, Proteolysis Targeting Chimeras (PROTACs) are heterobifunctional molecules that bind specifically a target protein inducing its degradation through a given E3 ubiquitin ligase.[6] They showed to be a valid alternative in pharmacology to drive the knockdown of a large panel of proteins that are dysregulated in cancer and other diseases.[7] More specifically, our working hypothesis is to recruit other E3 ubiquitin ligases to restore the normal ubiquitylation and degradation of HIF-2α. Our previous work showed that, in the absence of VHL, cereblon ubiquitin ligase (CRBN) might induce selective degradation of HIF-1α and -2α. To pursue this path, we aim at generating new PROTACs recruiting another E3 ligase (MDM2), and having a potent HIF-2α inhibitor (PT2385) as a new warhead. Driven by molecular modelling, we have been able identify accessible points to functionalize the inhibitor with a linker. Because of its wide applicability and high compatibility, we chose the click chemistry as a linking strategy to connect the E3 recruiting moieties to the target of interest (TOI) ligands.[8] The data of the *in vitro* biological evaluation of the new degraders to prove their efficacy and toxicity will be presented. The potential of this approach relies on the possibility to combine different E3 recruiting moieties to the TOI ligands, thus generating a platform of new degraders with different degradation profiles towards HIF-2α.



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Preclinical Development of PQR514, a Highly Potent PI3K Inhibitor Bearing a Difluoromethyl-Pyrimidine Moiety

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The phosphoinositide 3-kinase (PI3K) – mechanistic target of rapamycin (mTOR) signaling pathway plays a key role in many cellular processes, including cell growth, proliferation and survival. Hyperactivation of the PI3K/mTOR pathway can occur at multiple levels of this signaling cascade, finally promoting cancer growth and progression. Therefore, PI3K inhibitors represent a valuable asset in cancer therapy. Recently, we have reported on PQR309 (bimiralisib), a brain-penetrant pan-PI3K inhibitor, which also moderately targets mTOR kinase. PQR309 is currently in phase II clinical trials for the treatment of lymphoma and solid tumors and first phase I clinical results have been disclosed.[1-2]

Herein, we have developed a novel anti-cancer agent, the potent pan-PI3K inhibitor PQR514, which is a follow-up compound for the phase II clinical compound PQR309. PQR514 has an improved potency both *in vitro* and in cellular assays with respect to its predecessor compounds. Pharmacokinetic studies of PQR514 showed good oral bioavailability and a minimal brain permeability, which suggests the possible application in the treatment of systemic tumors, with the advantage of avoiding putative neurological side effects. PQR514 showed superiority in the suppression of cancer cell proliferation, and demonstrated significant anti-tumor activity in an OVCAR-3 xenograft model, at concentrations ~8-fold lower than the parental Phase II inhibitor PQR309. On the basis of its remarkable PI3K affinity, favorable pharmacological parameters, safety profile, and *in vivo* antitumor efficacy, PQR514 qualifies as an optimized candidate for the treatment of systemic tumors.[3]

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High content screening on patient-derived cells to break drug resistance in melanoma targeted therapy

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Melanoma is the most fatal dermatologic cancer. Studies have shown that more than 50% of malignant melanomas exhibit the BRAF V600E mutation, leading to constitutive activation of the ERK pathway, and driving aberrant proliferation. Vemurafenib, a specific BRAF V600E inhibitor, has been approved in 2011 for the treatment of metastatic melanomas [1]. Despite high response rates, drug resistance appears within months, a classic problem in cancer targeted therapy. Studies on the mechanism of drug resistance suggest that the development of combination therapies is needed to address this problem. Novel inhibitors targeting aberrant proliferative signaling in melanoma are therefore urgently needed.

We established an innovative high content screening (HCS) assay based on multiplexed genetically-encoded fluorescent biosensors in patient-derived cells harboring the BRAF V600E mutation. These biosensors report on ERK and AKT activity at the single cell level. When coupled to high content microscopy and automated image analysis, our pipeline allows us to screen 25’000 purified compounds of synthetic and natural origin. We use this workflow to screen two kinds of libraries: 1. Compounds with known activity (FDA approved, known kinase and receptor inhibitors) and 2. Compounds with high structure diversity including a library of isolated natural products. This will allow us to 1. Explore the biology of the aberrant signaling networks implicated in melanoma, and 2. To identify novel compounds that directly targets oncogenic ERK/AKT signaling. This effort is expected to provide new leads to break drug resistance in targeted therapy in melanoma.

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Lipoprotein-mimickry through supramolecular protein engineering

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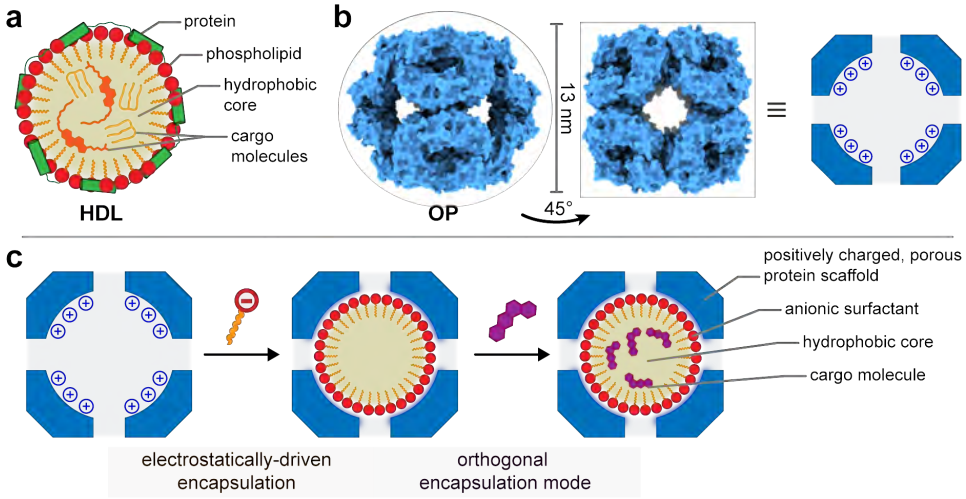
Expanding protein design to include other molecular building blocks has the potential to increase structural complexity and practical utility. Nature often employs hybrid systems, such as clathrin-coated vesicles, lipid droplets, and lipoproteins, which combine biopolymers and lipids to transport a broader range of cargo molecules. To recapitulate the structure and function of such composite compartments, we devised a supramolecular strategy that enables porous protein cages to encapsulate poorly water-soluble small molecule cargo through templated formation of a hydrophobic surfactant-based core. These lipoprotein-like complexes protect their cargo from sequestration by serum proteins and enhance the cellular uptake of fluorescent probes and cytotoxic drugs. This design concept could be applied to other protein cages, surfactant mixtures, and cargo molecules to generate unique hybrid architectures and functional capabilities.



Self-assembly of lipoprotein-mimetic capsids. (a) Cartoon depiction of a high density lipoprotein (HDL) particle, showing the charged phospholipids, proteins and hydrophobic cargo molecules. (b) Surface representations of the artificial protein cage **OP** viewed along the 2-fold (left) and 4-fold (right) symmetry axes and a cartoon cut away depicting the positively charged interior. (c) Two-tier encapsulation concept: electrostatic attraction drives the encapsulation of anionic surfactants, which phase separate due to their high effective concentration, forming micellar aggregates within OP cages. The hydrocarbon core of these stable protein-surfactant complexes then sequesters nonpolar small molecules by means of the hydrophobic effect.

Peptide Dendrimers Mimicking Glatiramer Acetate

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Multiple sclerosis (MS) is a chronic autoimmune neurodegenerative disease characterized by demyelinating lesions in the central nervous system (CNS). GLATiramer acetate (GA) is a first-line treatment drug which consist of large polypeptides with size distribution 4-11 kDa based on four amino acids coming from the name: Glutamic acid, Lysine, Alanine, Tyrosine. GA exhibit immunomodulatory properties and especially affects adaptive response on antigen presenting cells (APC). [1-2]

Here we used a chemical space guided approach [3-4] to design peptide dendrimer analogs of GA according to size and amino acid ratio. For instance, some of the dendrimers were chosen and synthetized by solid phase peptide synthesis (SPPS). Immunomodulatory properties of dendrimers were investigated on primary mononuclear cells in comparison with GA. Herein we discuss dependence of structural features of synthetised dendrimers on their ability to promote anti-inflammatory response on primary human monocytes.

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Immunological Epitope Mapping of the SARS-CoV-2 Spike Protein

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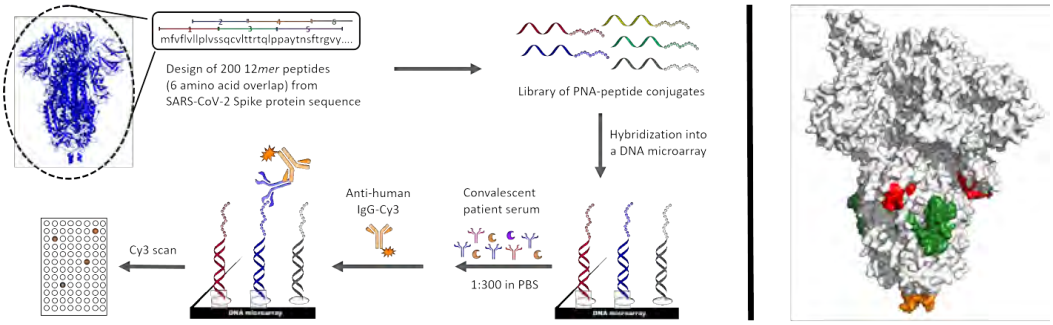
On December 2019 a novel infectious disease causing pneumonia was identified in the city of Wuhan (China). This new infectious disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has significantly impacted the economy, education and life of many countries in the world. The World Health Organization declared it a pandemic on the 11th March 2020. Mapping the epitope response of the immune system against the virus is of vital importance since it can lead to potential vaccines, more specific serological tests and to neutralizing antibodies which could be finally used as a treatment. In this work, using a peptide microarray, we have identified 3 immunodominant regions on the spike protein which are only present in COVID-19 convalescent patients' sera which could lead to potential neutralizing antibodies.



Increasing fluorophore brightness through self-labeling protein tags

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Self-labeling protein tags have become indispensable tools in fluorescence microscopy and are commonly used to label protein targets with small-molecule fluorophores in living cells. They allow the performance of advanced live-cell fluorescence microscopy including super-resolution experiments. Their use in combination with fluorogenic fluorophores, which only become fluorescent when bound to their protein target, reduces background fluorescence to a minimum, making them highly suitable for live-cell applications. A lot of effort has been invested in the development of fluorogenic fluorophores but so far, little attention has been paid to the self-labeling protein tag. Indeed, the protein surface is known to influence the fluorescence properties of fluorogenic fluorophores.

Here, we present the optimization of the self-labeling protein tag HaloTag with regard to the brightness of covalently bound rhodamine fluorophores. A screening based approach of HaloTag variants harbouring mutations at the protein-fluorophore interface allowed for the identification of both brighter and dimmer variants compared to parental HaloTag (Figure). Multiple rounds of directed evolution then led to the isolation of a variant that showed fluorescence intensity increases as high as 300% in combination with a variety of fluorogenic rhodamines *in vitro*. Mechanistic analysis via spectroscopic methods and X-ray crystal structure determination revealed that the brighter variants increased fluorescence intensities through changes in the local electrostatic potential. The measured trends were further confirmed in live-cell microscopy demonstrating the transferability to mammalian cells. The obtained results are crucial for live-cell microscopy and allow performing longer time-lapse experiments with lower laser intensities reducing photobleaching. In addition, it is anticipated that the enhanced HaloTag variant will be especially beneficial to super-resolution microscopy and live-cell tracking experiments.

New anti-inflammatory and pro-apoptotic photosensitizers against arthritis

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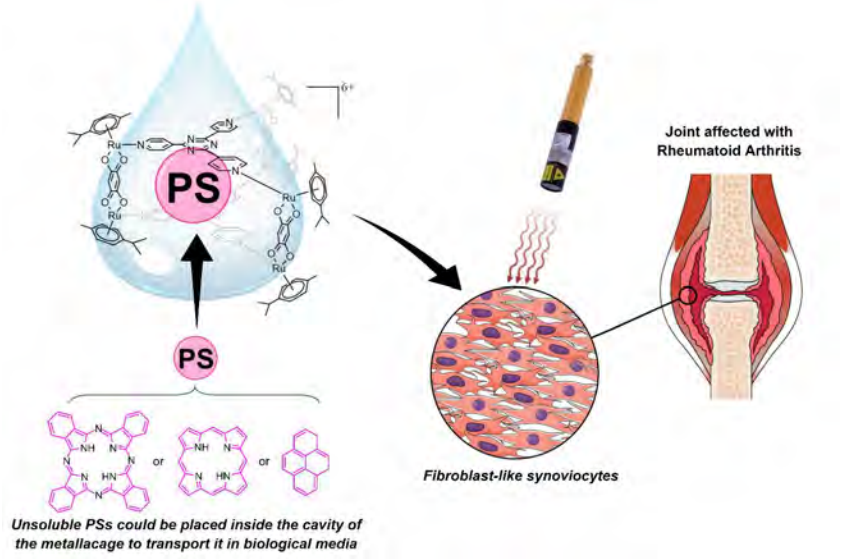
Contrary to popular belief, rheumatoid arthritis (RA) is not a disease only associated with aging, since it can also affect young people.[1] It is an autoimmune pathology that, although mainly affecting joints, can also attack other organs such as kidneys, lungs or heart. If left untreated, it can lead to a serious prognosis.[2] The most common treatment remains synovectomy, which is an invasive treatment and involves long periods of postoperative rehabilitation. In recent years, promising results have been achieved using non-invasive treatments such as anti-tumor necrosis factor drugs, Janus kinase inhibitors, and especially photodynamic therapy (PDT).[3] The latter involves a photoactive compound, a photosensitizer (PS), and light activation. The simplicity and non-invasiveness make PDT an ideal treatment to alleviate the pain or disability caused by RA. Unfortunately, conventional PSs often have some drawbacks related mainly to their low solubility in biological media and undesirable side effects such as light hypersensitivity.[4] We believe that it may be possible to solve the poor water solubility of PSs using ruthenium metallacages. These metallacages are soluble in biological media and have an inner cavity in which the PS can lodge. Such ruthenium metallacages have already been tested in vitro on cancer cells, demonstrating their potential.[5]



We have now designed new ruthenium metallacages and tested them as PDT agents against RA. The in vitro activity of these PS carriers in human fibroblast-like synoviocytes cells is promising. The anti-proliferative assays are excellent, and now the anti-inflammatory and pro-apoptotic activity of our new ruthenium metallacages-PSs are under investigation. Our most recent results will be presented.

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Fluorescent Probes for Measuring Membrane Tension, towards Super-Resolution Microscopy

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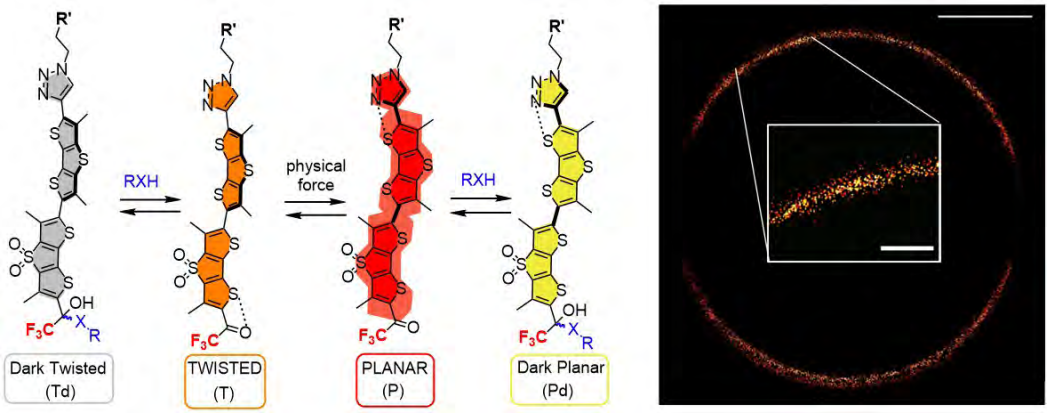
Planarizable push-pull probes ("Flippers", DTT-DTTO$_2$ derivatives) are highly mechanosensitive probes, which represent nowadays a hot topic for fluorescence bioimaging. The fluorescent properties of Flippers have allowed us to detect different distributions and/or small changes in the organization of the cellular membrane.[1] Currently, we have developed new Flipper derivatives with improved properties. These derivatives possess red shifted fluorescence, increased photostability and the possibility of "blinking", which is a dynamic off-on-off fluorescent mechanism that allows the probe to be applied in super-resolution microscopy experiments (SRM). Additionally, these Flippers are potentially useful for the staining different organelles, reaching higher resolutions and being more stable against time and irradiation.[2]



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Functionalization of harmonic nanoparticles for ultra-sensitive imaging and theranostic applications

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Cancer is among the leading causes of death worldwide, and as knowledge of the disease continues to grow there is an increasing interest towards precision medicine: more specifically towards the theranostics field, i.e. the development of targeted molecular probes combining specific diagnosis and treatment modalities. The theranostic paradigm involves merging, in a single agent, specific tumor biomarker targeting, multimodal imaging techniques which allow to overcome the inherent limitations of classical methods, and the controlled delivery of anticancer compounds. This strategy thus aims at high diagnosis sensitivity for earlier tumor detection and the reduction of off-target effects, which are critical factors in patient survival rates. In this context, inorganic nanoparticles emerge as promising tools owing to their surface properties, which are amenable to post-functionalization, and their imaging properties. We herein present the development of such theranostic tools based on harmonic nanoparticle (HNP) materials. These metal oxide nanoparticles, characterized by a crystalline structure lacking inversion symmetry (e.g. LiNbO3), exhibit a non-linear optical response by generating second- and third- harmonic upon laser excitation. A silica coating layer allows for improved biocompatibility of the inorganic HNPs and for the introduction of surface azide moieties, which were exploited for subsequent functionalization through bioorthogonal copper-free click reaction with a variety of cyclooctyne modified ligands. Erlotinib analogues and an inhibitor of fibroblast activation protein α were chosen for selective targeting of neoplastic cells and tumor microenvironment, respectively. In addition, the HNPs can be covalently conjugated to a gadolinium (III) chelate acting as a T1 MRI contrast agent, permitting multimodal imaging. Light-sensitive drug carriers were produced by the grafting of chemotherapeutics to the surface of HNPs through photosensitive tethers based on coumarinyl moieties. Irradiation of the HNPs with near-infrared (NIR) light allowed switching between imaging and treatment modalities by tuning of the excitation energy. Excitation at high wavelengths (> 1000 nm) was used for multi-harmonic detection, while lower wavelengths (~800 nm) resulted in the harmonic emission of ultraviolet light, inducing cleavage of the phototrigger and release of the therapeutic compound.

Figure: Schematic representation of the multifunctional theranostic nanoprobe

We are grateful to the French-Swiss Interreg Program for financial support (OncoNanoScreen project).

Novel Monovalent Ligands for Siglec-8, a Promising Target for Eosinophil and Mast Cell Related Disorders

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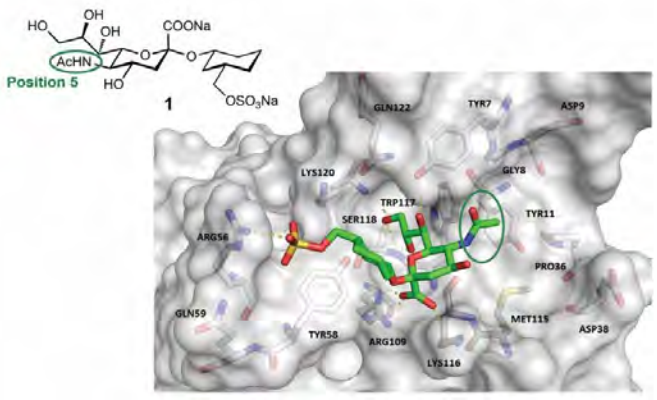
In this work, we present the design and synthesis of glycomimetic ligands for Siglec-8. Siglec-8 is a sialic acid binding lectin expressed exclusively on mast cells and eosinophils.[1,2] Binding of Siglec-8 with antibodies or glycan ligands results in apoptosis of eosinophils and inhibition of mast cells degranulation.[3,4] For this reason, Siglec-8 is gaining attention as a new interesting pharmacological target for the treatment of eosinophil or mast cell related diseases, such as asthma, chronic rhinosinusitis, hypereosinophilic syndromes, etc.[5] Recently, the NMR solution structure of the Siglec-8 lectin domain together with its preferred ligand, the tetrasaccharide 6′-sulfo sialyl Lewis^x, has been solved.[6] By investigating the main interactions between this ligand and the protein, we first identified the disaccharide 6-sulfo Sia-Gal as the minimal binding epitope for Siglec-8. Then, the replacement of the galactose by a hydroxymethyl-cyclohexane, while keeping the sulfate in the same position, led to the new lead compound **1**, which showed a 6-fold higher affinity to Siglec-8 compared to the parent disaccharide. Inspection of the binding mode of 1 by docking studies revealed an empty pocket close to position 5 of the sialic acid. We therefore performed a fragment-based virtual screening to explore this pocket in order to gain additional interactions. Furthermore, our docking studies indicated beneficial π-stacking interactions with aromatic residues in the binding pocket by replacing the cyclohexane with an aromatic ring.
Therefore, we synthesized a library of novel glycomimetics, introducing modifications both on the sialic acid and on the cyclohexane moiety. These novel compounds are expected to show better affinity towards Siglec-8 compared to our lead compound, paving the way to new prospectives for the treatment of eosinophil and mast cell associated disorders.



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AI-based design and synthesis of putative HtrA inhibitors

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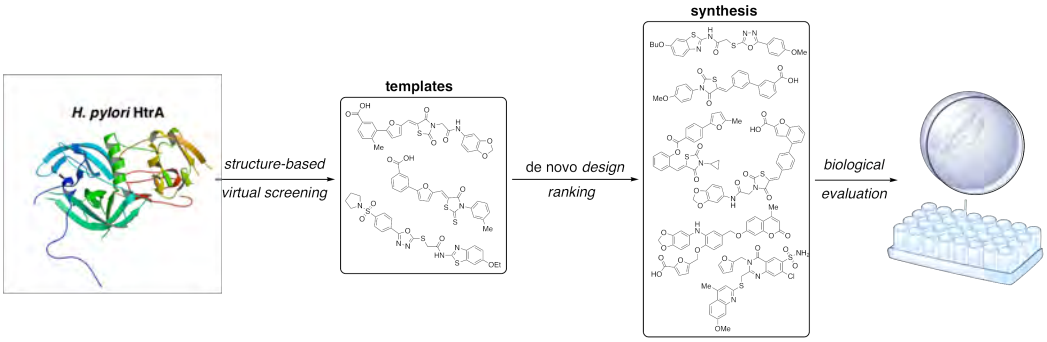
The development of antibiotics is one of the most pressing needs in current medicinal chemistry. Recently, the bacterial serine protease HtrA has been proposed as a potential target, in particular for the selective treatment of *H. pylori* infections, a main cause of gastritis and stomach cancer.[1] The first known inhibitors of HtrA were recently identified.[2]

We decided to expand upon those results by using Artificial-Intelligence-based approaches. AI in medicinal chemistry has experienced a renaissance in recent years. *De novo* design in particular enables us to rapidly explore chemical space and to generate novel scaffolds from known pharmaceutically active compounds.[3]

We chose three known HtrA inhibitors as templates for different algorithms. As a first step, we employed DINGOS, a rule-based method that generates synthetically accessible molecules from commercially available building blocks.[4] We generated and ranked a total of 30.000 putative active compounds. Seven compounds from the top 30 were chosen for synthesis and future biological evaluation. In most cases, we successfully followed the one- or two step routes proposed by the algorithm, with manual adjustments made when necessary. In many cases, the proposed starting materials were also synthesised, as they were commercially available only in very small quantities.



We are currently employing other rule-based approaches as well as deep learning methods in a similar fashion. We hope to contribute to the investigation of antibiotics targeting *H. pylori* HtrA by significantly increasing the number of known active inhibitors.

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Peptide Stapling via Selective Cysteine Alkynylation Using Hypervalent Iodine Reagents

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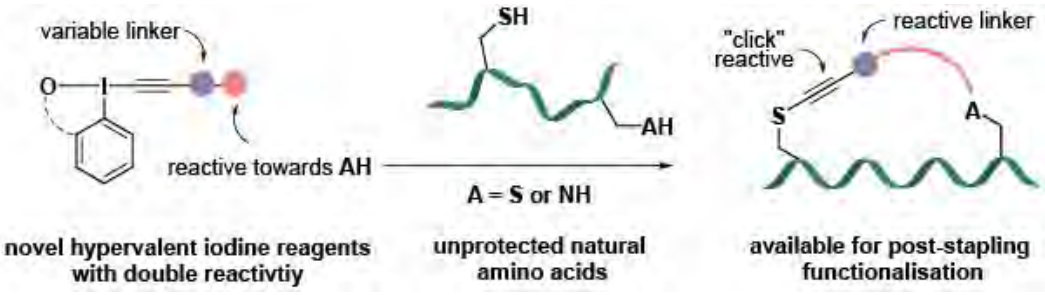
A stapled peptide is formed via a covalent linkage of two amino acid side chains. Such cross-linking can be used to stabilise short α-helices. This secondary structure is commonly mediating protein-protein interactions (PPIs). Thus, stapled peptides bearing helicity have a potential to inhibit PPIs, making them a desirable synthetic target.[1]

Selective cysteine alkynylation using hypervalent iodine reagents has been previously developed in our group.[2,3,4,5] This method has now been further extended to cysteine-cysteine and cysteine-lysine stapling by introduction of novel bi-functional reagents. Herein, we will present the recent progress towards our metal and protecting group free peptide stapling that allows for and easy access to diverse and functionalisable products. Discussion about this research will be possible during a zoom meeting on Tuesday 25.08.2020, 12h15-13h15.[6]



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Optimization of Antimicrobial Peptide Dendrimers Using a Genetic Algorithm

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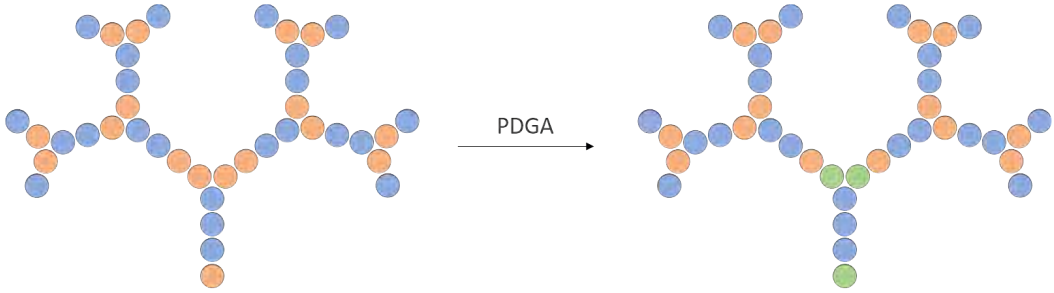
There is an urgent need for new strategies to fight multidrug resistant bacteria. Recently we reported antimicrobial peptide dendrimer (AMPD) G3KL with potent activities against *P. aeruginosa* and *A. baumannii*, two of the most problematic antibiotic-resistant nosocomial pathogens, and showed that this AMPD acts by a membrane disruptive mechanism.[1,2] Further optimization by a chemical space guided approach later uncovered analog T7 with a broader activity spectrum.[3] Here we computationally generated new analogs of AMPD T7 using the peptide design genetic algorithm (PDGA), a computational tool to generate topologically diverse peptides with high similarity to any molecule of interest.[4] Synthesis and testing of 60 PDGA analogs allowed us to identify a new AMPD with an even broader activity spectrum than T7 or G3KL against multidrug resistant Gram-negative strains while retaining good serum stability and negligible hemolysis.



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Synthesis and Reactivity of Colibactin Inspired 1,6-Michael Acceptor

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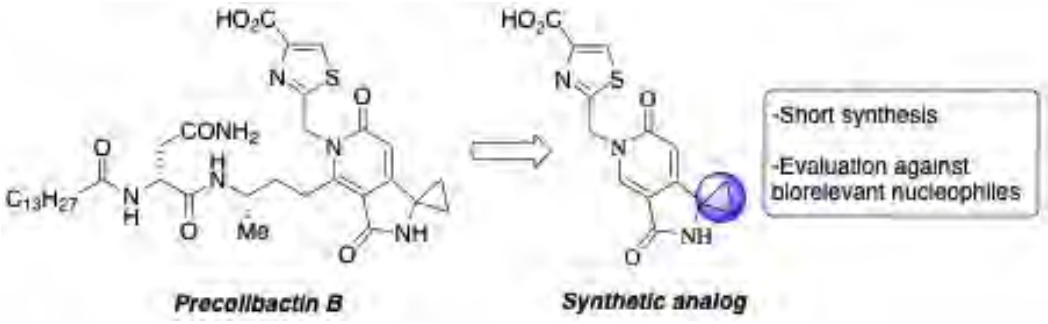
Double conjugated 1,6 Michael acceptor was synthesized based on metabolites isolated from human gut inhabitant *E. Coli*. Unexpectedly, the compound showed high stability towards nucleophiles and acidic conditions.



^{1}H HR-MAS NMR Based Metabolic Profiling of Cells in Response to Treatment with the Photosensitizer Chlorin e4 with and without Polymeric Carrier Systems

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Porphyrinic photosensitizers (PSs) are used in photodynamic therapy (PDT) of cancer and non-cancerous diseases due to their cytotoxic properties that are only unfolded in combination with light and oxygen. Polymer-based nanoparticles (NPs) are mostly used as delivery systems for PSs since they enhance porphyrin solubility and stability under physiologic conditions and can increase tumor uptake [1]. Both, the PS and the NP are implicitly required to exhibit low dark toxicity to avoid side effects and ensure a light directed localized therapy. Therefore, it is important to assess the in vivo tolerability of both, the PS in the dark and its polymeric delivery system.

Recently, we have shown that chlorin e6 type PSs including chlorin e4 (Ce4) are well encapsulated into polyvinylpyrrolidone (PVP) and into biodegradable block copolymer micelles formed by Kolliphor P188 (KP) [2-4]. While the cellular uptake of the PSs could be readily proved by fluorescence, little is known about the metabolic response of the cells towards the PSs and NPs in the dark. In this study we aim to address the question how PVP and KP-micelles affect the metabolic response of cancer cells after incubation with Ce4. High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy combined with multivariate statistical analyses was used to study the metabolic profile of cultured HeLa cells treated with Ce4 alone or encapsulated into PVP or KP at three different Ce4 concentrations.

47 metabolites could be assigned in the ^{1}H HR-MAS NMR spectra of lysed HeLa cells. A total of 39 ^{1}H spectra (corresponding to 6 controls, and 3 of each group of 10, 25 and 62.5 µM Ce4 alone or combined with PVP or KP, and pure PVP and KP) each subdivided into 259 bucket regions were analyzed. In principal component analysis (PCA), the cell samples that were incubated with carrier-free Ce4 were clearly separated from all other samples. The metabolic response was also correlated to the Ce4 concentration with the highest concentrations of Ce4 showing the strongest deviation from the control samples. Furthermore, both carriers, PVP and KP, attenuated the effect of Ce4 on the HeLa metabolites. Moreover, cell samples incubated with the pure carriers overlapped with the control samples indicating that they seem to be largely non-toxic. Contributions of single metabolites to the separation of Ce4 containing samples will be discussed in detail based on partial least squares (PLS) and PLS-discriminant analysis (PLS-DA) models and the corresponding loading plots.

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Model Peptide Studies of Ag^+ Binding Sites Inspired by the Silver Resistance Protein SilE

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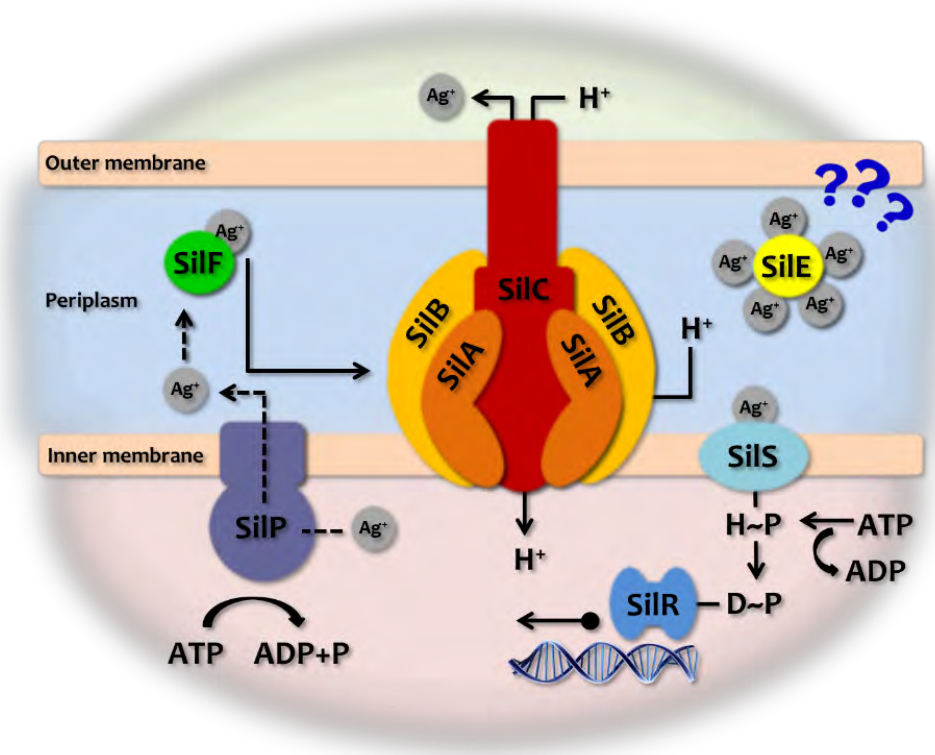
The silver cation Ag^+ and its compounds have been known for their antibacterial properties. [1] However, an increasing number of reports have highlighted the emergence of silver resistant bacterial strains isolated from burn care centers or silver contaminated media. The resistance is provided by the silver efflux pump, which contains eight proteins that act together to export silver ions. Among them, the SilE protein is the only one of which the role is still unknown, acting as a sponge for silver.

Model peptides were studied to identify Ag^+-binding sites of the bacterial silver resistance protein SilE. [2] The silver ions are binding in a linear coordination mode on histidine (His, H) and methionine (Met, M) residues. Following this study, the amino acids (AA) present between these two residues could influence the coordination of the His and Met residues with Ag^+. Thus replacing one AA by an other one could increase or decrease the stability of the complex Ag^+-HXXM (with X one AA). The goal of this study is to understand the contributions of different AA on the linear coordination of the His and Met residues with Ag^+.



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Synthesis and biological evaluation of PROTACs and destabilizers with putative anti-inflammatory activity

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In multicellular organisms, apoptosis is highly integrated with inflammation and it is crucial for physiological homeostasis and host defense against pathogens at the molecular level, Death Domain (DD) containing receptors, which are membrane proteins belonging to the Tumor Necrosis Factor receptors (TNFR) superfamily, are involved in both apoptotic and inflammatory signaling.(1) The tumor necrosis factor (TNF) is a cytokine playing a crucial role in the regulation of both innate and adaptive immune response and its deregulation is responsible for the pathological occurrence of various autoimmune disorders and perpetuation of chronic inflammation.(2) TNF exerts its functions thanks to the interaction with two distinct receptors: TNF receptor 1 (TNFR-1) and TNF receptor 2 (TNFR-2). While TNFR-1 is mainly in charge of the NF-κB mediated pro-inflammatory response and apoptosis induction, TNFR-2 promotes cell survival and tissue regeneration. From a structural point of view, their intracellular domains present distinct features that define them as representatives of the two main classes of the TNFR superfamily: the DD-containing receptors for TNFR-1 and the TRAF-interacting receptors for TNFR-2.(3) Ultimately, it is clear that targeting TNFR-1 DD could become a promising and selective therapeutic approach to neutralize the pro-inflammatory activity of TNF while maintaining the advantageous effects promoted by TNFR-2. In this perspective, we started addressing two main scientific questions: i) Is it possible to use the **“small-molecule” approach** to induce selective TNFR-1 inhibition in autoimmune and autoinflammatory diseases? ii) Can we combine the “small-molecule” approach with **PROTAC technology** for selective TNFR-1 degradation by the UPS machinery?

Based on a screening of 600 fragments against TNFR-1 DD, thirty-four new hits have been discovered. These thirty-four fragments have either stabilizing or destabilizing effects towards the target. This represents a very good starting point for developing small molecules specifically targeting TNFR-1, by combining molecular modeling, medicinal chemistry and *in vitro*-assays. In this poster, I will be presenting the first results towards reaching the aim of synthetizing and biological evaluating PROTACs and destabilizers with putative anti-inflammatory activity.

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Bio-inspired divergent synthesis of various sesquiterpene including Goyazensolide, its new cellular target and its therapeutic potential in cancer and virology.

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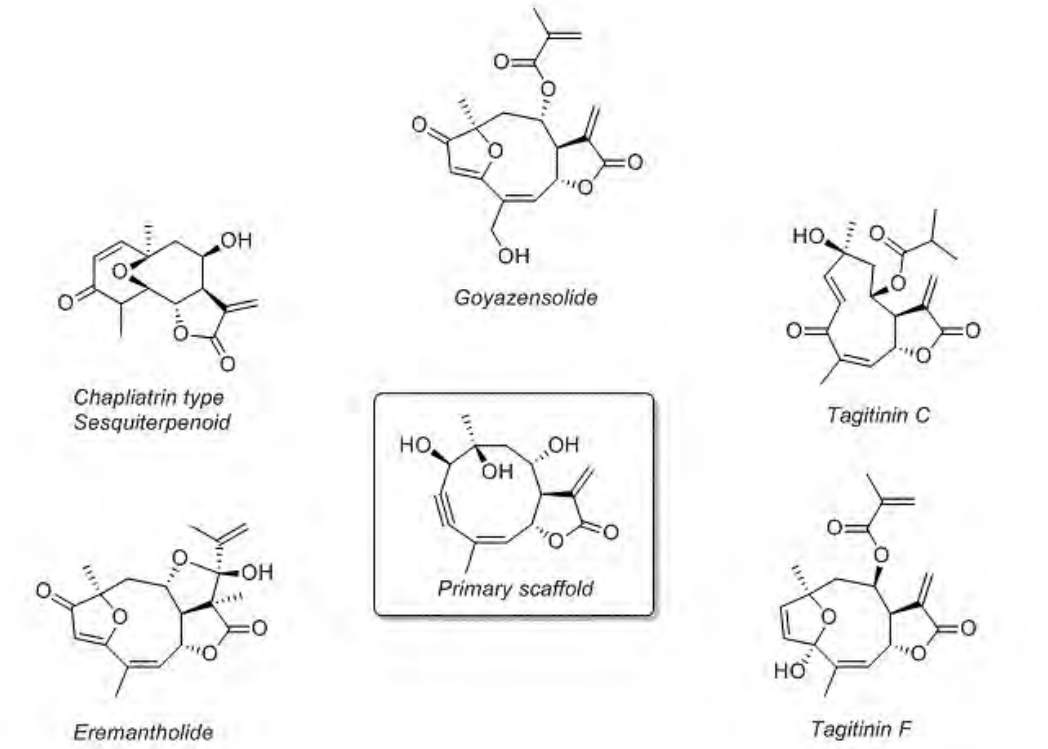
We have developed a divergent strategy, based in the merged of α-methylene-γ-butyrolactone formation and 10-membered ring construction in one step for the synthesis of dozens of natural products and synthetic analogs including furanoheliangolides, germacrane lactones and chapliatrin-type sesquiterpenoids. One of these natural products is Goyazensolide; which was initially isolated in *Eremanthus Goyazensis* during a research program to find schistosomicidal agents. We have also discovered a new target of Goyazensolide, to which it binds covalently and that perturbates the translocation of RASAL2, and perturbates the binding with influenza A NLS, what might lead to a therapeutic for viral treatment.



X-Ray Crystallographic Studies of Short Antimicrobial Helical Peptides against Multidrug Resistant Gram-Negative Bacteria

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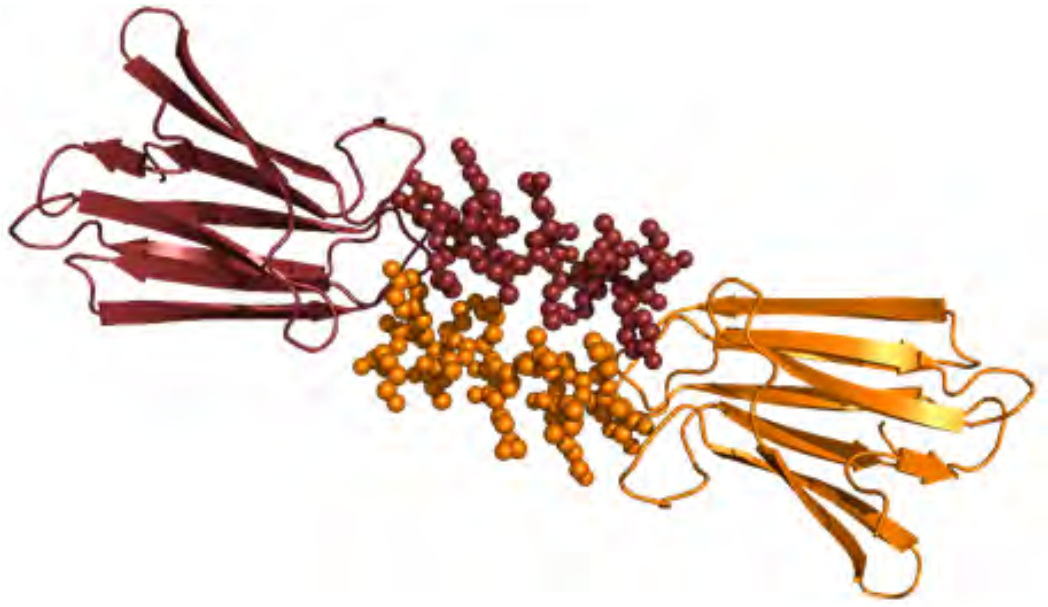
Multidrug resistant bacteria represent a global public health threat, calling for the development of new antibiotics. In our investigations of antimicrobial peptides (AMPs) to treat multidrug resistant Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* our group recently reported the first X-Ray crystal structures of short AMPs in form of N-terminally fucosylated analogs in complex with the microbial Lectin LecB [1]. These short peptides are disordered in aqueous solution but form helix bundles in the crystal, which probably represents the bioactive conformation at the bacterial membrane. Following the same approach, we later obtained the structure of short bicyclic antimicrobial peptides and discovered that they also contain an α-helical conformation aggregating to form helix bundles. To test whether the α-helix observed in our bicyclic AMP was enabled by the bicyclization, we investigated the parent linear 11-residue AMP sequence, and discovered that this sequence is also α-helical. Further optimization led to new very short linear AMPs with potent activities against a broad range of multidrug resistance Gram-negative bacteria, including *Klebsiella pneumoniae*.



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Solving the X-ray structure of a bio-artificial neurotransmitter receptor designed for the naked-eye recognition of dopamine

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The literature is constalleted with a wide variety of chemosensors against a plethora of analytes [1,2,3,4]. This seminal library is used to inspire chemists to improve them using chemical synthesis. However, their optimization via chemical synthesis is a difficult task which takes time without the guarantee of final success [2,3,4]. We show here that combinatorial chemistry, the use of first and second coordination spheres interactions and the displacement of indicators united within a protein cavity offers an easy-to-assemble colorimetric bio-chemical sensor [1]. It consists only of commercial chemicals. This colorimetric sensor is highly modular, cheap and evolvable. Its X-ray structure reveals the composition of its active site. This allows to design it rationally for the recognition of dopamine with the naked-eye. Our bio-sensor therefore resembles a biological receptor for the recognition of neurotransmitters. Its immediate high adaptability and ability to be evolved can be useful for the selective detection of a wide variety of analytes going from small molecules to microorganisms. This discovery therefore makes it possible to dream of new biotechnological or new immunotherapeutic applications.

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The effects of chemical reactions on codon drift in DNA encoded libraries

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DNA encoded libraries connect small molecules with genetic information and became an important tool for drug discovery in both academia and industry [1]. They allow the construction of libraries with millions of different members that can be tested at the same time using, for example, affinity enrichments. A major disadvantage is the limited number of chemical transformations available that reduce the available chemical space [2], although there has been some significant progress on that in the recent years [3].

A key problem of reactions in DNA encoded reactions is always the side reactions they might undergo with the DNA tag, impairing the genetic information. A popular approach is the measurement of DNA amplifiability by utilizing qPCR [4]. More recently, characterization of reaction conditions according to their encoding fidelity has been shown [5]. Herein, we give insight into a third parameter that can be used to characterize reactions. Modifications of nucleobases can cause mutations and, as a result, the DNA used for encoding can change.

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Antibiotic Delivery via Disulfide Exchange

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After the discovery of penicillin and hereby opening the “golden age” of antibiotic research, for the first time, many common bacterial diseases could be clinically addressed.[1] The bacterial diseases seemed to be permanently defeated by using a wide range of different classes of antibiotics. However, with the increase of antibiotic utilization, more and more pathogenic bacteria started to develop resistance to the inhibitory effect of antimicrobials. Nowadays, rapidly escalating bacterial resistance development causes serious concern for public health.[2] Therefore, there remains an urgent need for the development of new antibiotics. However, the number of approved antibiotics in the market has been decreasing over the decades. One of the reasons includes the failure of potential antibiotics to reach the target,[3] in particular for Gram-negative bacteria, or in biofilms. Therefore, several strategies have been developed for antibiotic delivery based on nanomaterials (self-therapeutic agents and carriers for antimicrobial cargo)[4] and modified living organisms.[5] However, there remains great interest in finding new strategies for drug delivery based on novel molecular mechanisms.

A number of dynamic covalent chemistry strategies have already been exploited, which can self-assemble, respond, and degrade in a controlled manner in mammalian cells.[6] One of them includes the utilization of cell surface thiols to increase cellular uptake.[7,8,9] Gram-positive and Gram-negative cells also contain thiol groups in their cell envelopes, which emerges as a potential target for dithiol-mediated uptake strategy. Moreover, there are several examples in the improvement of biofilm inhibition for the compounds contained dithiol moiety or after the introduction of those into known antimicrobial.[10]

In the pursuit of these goals, our group selected two antibiotics to be derivatized. Vancomycin and cephalosporin are two well-known and broadly used inhibitors of bacterial cell wall biosynthesis.[11] The aforementioned antibiotics were modified with dithiol-containing aliphatic chains, lipoic acid, and/or asparagusic acid.[8] All the obtained compounds were tested against several Gram-negative and Gram-positive bacteria, including MRSA and vancomycin-resistant Staphylococcus aureus (VRSA). Additionally, we studied the mechanism of action by microscopy visualization of Gram-negative bacteria mixed with fluorophore-labeled dithiol derivatives. Moreover, the synthesized compounds have the potential in the inhibition of biofilm formation.

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Exploring the role of CEMIP in Alport syndrome

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Alport syndrome (AS), a pediatric rare disease caused by mutations affecting type IV collagen, a major network-forming structural component of basement membranes, is one of the best characterized Mendelian human diseases affecting 1 in 5,000-10,000 individuals. AS leads almost inevitably to loss of renal function or end-stage renal disease (ESRD). Previous work has highlighted a major role for a collagen receptor tyrosine kinase called Discoidin Domain Receptor 1 (DDR1) in the onset and progression of AS. Because of the magnitude of renal damage reduction observed in DDR1 genetic deletion murine models, a series of DDR1 inhibitors have been developed by several research groups. Those DDR1 inhibitors, though highly selective, have however a limited clinical value. We therefore set to explore the protective mechanism downstream of DDR1 and, in a series of experiments, we identified an enzyme, CEMIP, selectively induced by DDR1 in presence and potentially mediating DDR1 protective role in AS. The overall aim of this project is to investigate the biology of DDR1/CEMIP/HA axis in Alport syndrome both in vitro and in vivo. Relevance of CEMIP will be tested in vitro with a series of mechanistic experiments exploring the role of CEMIP and HA fragments in inducing cell several autonomous behaviours. Molecular tools (e.g., a selective CEMIP inhibitor) will be developed to further investigate and characterize the DDR1/CEMIP/HA axis both in vitro and in vivo. Potentially these molecules will then be tested in Alport mice and hopefully exploitable to protect AS patients.

Structural investigation of the first unimolecular RNA G-quadruplex via NMR spectroscopy

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G-quadruplexes (G4s) are non-canonical nucleic acid structures, which are usually formed by guanine-rich DNA and RNA sequences.[1] The basic scaffold of a G4 are stacked G-quartets, which consist of four Hoogsteen base paired guanines and are stabilized by monovalent cations such as Na^+ and K^+.[1] G4s are supposed to play crucial regulatory roles in gene replication, translation and expression and are therefore handled as a promising target for chemotherapy. As G4s can adopt a large variety of different topologies, knowing their three-dimensional structures is an important step towards an efficient drug design. To date, most structural studies concentrate on DNA G4s, while the understanding of RNA G4s is quite poor.

NMR spectroscopy is a powerful tool to elucidate three-dimensional structures of biomolecules at atomic level including G4s.[2] However, mainly limited by the commercial availability of the isotope labelled RNA phosphoramidites, very few solution structures of RNA G4 have been solved so far (only 5 cases from the PDB databank) by NMR.[3] Particularly, solution structures of unimolecular RNA G4s has not been reported yet. As RNA is normally single-stranded *in vivo*, unimolecular RNA G4 structures would give an real insight into RNA G4 structures that occur *in vivo.*

In this work, we investigated a triple mutant of a 22mer RNA sequence at 5’ UTR of the BCL-2 proto-oncogene that forms a unimolecular G4 structure in the presence of K^+ ions. [4] Different from the site-specific labelling approach, which is commonly used to solve the structure of DNA G4s by NMR, we prepared various uniformly labeled RNA samples (e.g. ^{13}C,^{15}N(G)-RNA, ^{15}N(U)-RNA,…) by *in vitro* transcription. With the help of an adapted HNN-COSY experiment of the ^{15}N labelled (G)-RNA sample the hydrogen pattern of all G-quartets was determined and based on this scaffold a full assignment of this RNA quadruplex was achieved. This allowed for the elucidation of the first unimolecular RNA structure.

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Peptide dendrimers for cisplatin delivery to cancer cells

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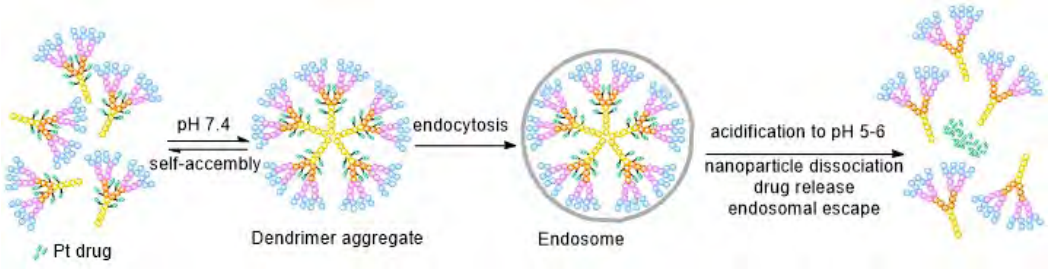
Cis-diammine-Platinum(II) (*cis*Pt) and 1,2-Diaminocyclohexane-Platinum(II) (DACHPt) are used in almost every cancer chemotherapy treatment, however these metal complexes cause lasting side-effects, which might be reduced by targeted delivery to cancer tissue in form of nanoparticles exploiting the enhanced permeation and retention effect.[1,2] Targeting of Pt has also been investigated using Pt(IV) complexes bound to cell penetrating peptides.[3] Here we investigate peptide dendrimers as possible delivery vectors for cisPt and DACHPt. Our group has developed a reliable solid-phase synthesis access to peptide dendrimers consisting of short dipeptide or tripeptide branches linked by lysine branching points.[4] We recently reported such peptide dendrimers with sequences tailored for siRNA delivery. These dendrimers consist of polycationic branches connected to a hydrophobic core and form nanoparticles at neutral pH which undergo endocytosis and disassembly upon acidification of the endosome, resulting in intracellular delivery of their siRNA cargo.[5] Here we adapted these peptide dendrimers by introducing multiple metal coordinating side chains enabling binding to cisPt and DACPt. Optimization of drug loading as well as hydrophobicity and charge distribution in the dendrimer structure allowed us to obtain macromolecular systems with improved in vitro cytotoxicity against HeLa cells compared to free drugs.



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Peptide-based vectors for delivery of pDNA encoding CRISPR/Cas9 in 3D tumor spheroids

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Gene therapy is a powerful technique that allows the treatment of many diseases at a molecular level through the introduction of genetic materials into cells. However, the transfection procedure is often very challenging, due to the lack of efficient delivery systems. [1]

Peptide dendrimers/lipid hybrid systems were showed to be efficient transfection reagents for DNA [2] and siRNA into HeLa cells [3]. Recently, these compounds have also been successfully applied in oligonucleotides delivery [4].

We are now exploring a new library of third generation peptide dendrimers in order to perform transfection of plasmid DNA coding for CRISPR-Cas9. The optimization of hydrophilicity, hydrophobicity and the introduction of non-natural amino acids in the structure allowed us to obtain systems displaying high pDNA transfection efficiency in absence of helper lipid. In particular, biological experiments showed high transfection efficiency -measured as GFP expression by FACS analysis- low cytotoxicity and low immunogenicity.

Furthermore, pDNA transfection experiments performed on 3D cellular spheroids showed promising transfection efficiency and low cytotoxicity, properties necessary for potential in vivo applications.

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