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Siglec-8 – A Novel Target For Asthma

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Sialic acid-binding immunoglobulin-like lectins (siglecs) are members of the immunoglobulin family. They are predominantly found on cells of the immune system. Up to now, 15 siglecs have been identified.[1] Among them is Siglec-8, which is selectively expressed on human eosinophils and regulates their survival.[2-4] Crosslinking Siglec-8 with specific glycan ligands or antibodies induces apoptosis.[5] In some diseases, such as asthma or chronic rhinosinusitis, an excessive amount of eosinophils is produced, causing an inflammatory reaction. Thus, targeting Siglec-8 provides a unique opportunity to control such allergic reactions.

Using a glycan array, the tetrasaccharide 6'-sulfo-sLe^x was identified as a ligand of Siglec-8.[6] However, it exhibits poor drug-like properties and its synthesis is laborious and of high complexity. Therefore, we synthesized a number of mono- and oligosaccharides derived from the scaffold of 6'-sulfo-sLe^x and determined their affinities for Siglec-8.

Herewith, the potent disaccharide Neu5Acα2-3(6-*O*-sulfo)Gal was identified, displaying a relative IC_{50} of 2.2 with respect to the natural 6'-sulfo-sLe^x. Moreover, the affinity of the disaccharide ligand was significantly reduced when small modifications to this structure were made. For example, when the *O*-glycosidic bond was changed from α2-3 to a α2-6 linkage, the sulfate-group was removed or only the corresponding monosaccharide fragments were evaluated, almost no binding to Siglec-8 was observed.

These results suggest that the scaffold of Neu5Acα2-3(6-*O*-sulfo)Gal contains the essential epitope of 6'-sulfo-sLe^x required for Siglec-8 binding. Based on this information, a new family of Siglec-8 ligands is currently synthesized.

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Chemically defined chromatin and protein engineering via EPL to study histone ubiquitination on the single molecule level

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The epigenetic landscape, essentially defined by histone post-translational modification marks (PTMs) and different DNA methylation states, is crucial for proper packaging of DNA into eukaryotic cells and dynamic regulation of DNA during transcription, DNA damage repair (DDR) and DNA replication. Many histone PTMs have been associated with an accessible, decondensed chromatin state (acetylation, H2B ubiquitination)[1] or a rather dense state of chromatin and chromatin silencing (H3K9/27 methylation). In the past, proteins that interact with PTMs have been studied with biochemical methods providing low dynamic resolution and suffering from ensemble averaging.

Here, we aim to reveal the dynamic behavior of effector protein interactions with chromatin fibers of defined modification states on the single-molecule level, using total internal fluorescence microscopy (TIRFM). Our laboratory has extensive expertise in semi-synthesis of chromatin arrays containing a defined set of PTMs. Via expressed protein ligation (EPL)[2] of recombinantly expressed histones and PTMs on a synthetic histone tail we achieve traceless modification of histones, as H3K9me3 or H2BK120ub[1]. We combine this approach with an EPL based labelling strategy to attach ultra-bright fluorophores to expressed proteins. Thus, we are able to perform single-molecule measurements (*in vitro*) using a precisely defined system of labelled proteins and chromatin arrays. In particular, we used this system to examine different binding models of HP1α protein to chromatin.

We aim to extend our method to study enzymatic protein-chromatin interactions by focusing on histone ubiquitination. We fluorescently label a key E3 ubiquitin ligase in the the DDR, RNF168, to directly measure its dynamic association with the repressed or active chromatin state with different PTM signatures, on the single-molecule level. In parallel we will determine ubiquitination kinetics in a separated emission channel using photoactivatable fluorophores. This will put us in the position to measure chromatin state dependent ubiquitination events and determine the contribution of other PTMs/ubiquitin marks towards ubiquitin installation on chromatin.

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Engineering of a specific probe for the visualization and analysis of bivalent epigenetic marks in living cells

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In embryonic stem (ES) cells, promoters of developmentally important genes are marked with the combination of repressive (H3 lysine 27 methylation, H3K27me3) and activating (H3 lysine 4 methylation, H3K4me3) post-translational modifications (PTMs) of histones, called bivalent marks. Bivalent chromatin domains are believed to play an important role in maintaining the pluripotency of ES cells but have been observed in cancer cells as well. Bivalent domains have thus far mostly been studied by methods involving fixed, non-living cells or requiring lysis of large number of cells and by employing specific antibodies.

Here, we combine chemical chromatin synthesis (using expressed protein ligation) and protein design to develop a genetically encoded probe that binds specifically to bivalent nucleosomes. This probe is designed to weakly interact with singly modified nucleosomes but it strongly binds to nucleosomes having both H3K4me3 and H3K27me3 marks. This sensor platform allows us to image co-existing H3K4me3 and H3K27me3 at the level of single live cells, to follow bivalent chromatin through the cell-cycle and differentiation, and to re-write the histone code.

We thus designed, expressed and optimized a series of genetically encoded multivalent protein probes consisting of a sensor (fluorescent protein), inserted between two histone PTM binding domains which cooperatively detect the bivalent marks. The binding properties of these constructs were assessed *in vitro* for different trimethyl-marks, using a library of chemically synthesized nucleosomes carrying defined combinations of H3K4me3 and H3K27me3.

Expression and subsequent observation by fluorescence confocal microscopy of functional constructs allowed the localization of bivalent chromatin in stem cells and cancer cells. These tools now allow us to directly monitor the dynamics of bivalent PTMs in living cells.

The Power of the 'SCS': Improving the Pharmacological Properties of Peptide Therapeutics

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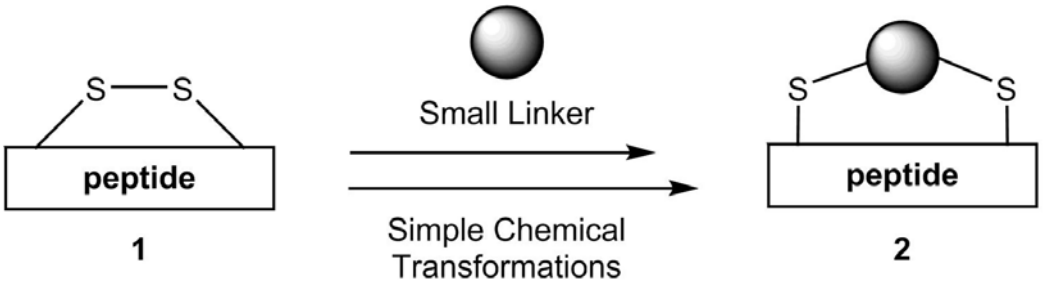
Cyclic peptide therapeutics (1) fill the gap between small molecules (5000 Da) as a novel class of drugs, combining the advantages of both in terms of high selectivity, bioavailability, synthetic accessibility and low toxicity.[1] However, their fast clearance times pose a serious limitation to their utility.[2] Several strategies exist to stabilize these peptides through local or global constraints, side chain functionalisation or amide bond surrogates.[3],[4]



The replacement of easily reducible disulfide bonds, which are commonly found in several peptide structures, by adding a linker moiety,[5] would allow us to obtain non-reducible and supposedly more stable analogues (2). We aim to maintain or even improve the pharmacological properties of known peptide drugs without negatively affecting their receptor selectivity and activity.

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An “in vivo” temperature dependence study of the protoporphyrine IX delayed fluorescence lifetime while measuring the oxygen partial pressure.

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Oxygen partial pressure (pO2) in biological tissues is central in the destruction mechanisms at play during radiotherapy and photodynamic therapy (PDT). Thus, monitoring this parameter in real time can be useful to predict the outcome of these therapeutic approaches.

The triplet state of most porphyrin -photosensitizers (PSs) used in PDT is quenched by molecular oxygen. Consequently, measurement of this state’s lifetime yields information on the surrounding pO2 via the Stern-–Volmer relationship [1]. Unfortunately, the very low brightness of these PSs’ phosphorescence lifetime makes it difficult to use it in clinical settings. Our original approach is to measure the lifetime of the delayed fluorescence (DF) resulting from inverse intersystem crossing (iISC) transition taking place between the triplet and singlet states of the PS. By discriminating the prompt fluorescence (PF) and the –DF of an endogenous PS with an optical fiber-based time-gated system, Piffaretti and *al.*[1] reported the first real-time *in vivo* monitoring of the radiative dose in PDT. This study was based on the measurement of the protoporphyrin IX (PPIX) DF lifetime, a PS in extensive clinical use. Our team also reported a correlation between pO2 depletion and vascular damages induced by PDT[3].However, temperature is known to have a significant effect on the Stern-Volmer constants in most situations. This is likely to be important in the case of PPIX’s DF since the iISC is a thermal process[4]. Consequently, the temperature dependence of DF lifetime may contribute to the fluctuations reported by Piffaretti and *al.*[3] and must be known to clearly define the parameters linking PpIX’s DF lifetime to vascular damages. We investigated the temperature influence on PPIX’s DF lifetime *in vivo* and *in vitro*.

The *in vivo* study was carried out by measuring PPIX’s DF lifetime after topical application of amino-levulinic acid, a PPIX precursor, on the chorio-allantoic membrane of fertilized chick’s eggs at various physiological temperatures. Our results give insights on improvements of PDT dosimetry, in particular when the treated organ is subject to relatively important temperature fluctuations, such as cutaneous tissue.

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HP1α dynamic binding to different compaction states of chromatin

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The genetic material of eukaryotic cells is tightly packed into the nucleus in the form of chromatin. The building blocks of chromatin are nucleosomes, composed of 145-147 basepairs of DNA wrapped around eight histone proteins, which display post-translational modifications (PTMs) on their tails, such as methylation, acetylaion, phosphorylation or ubiquitination. Effectors or reader proteins are known to dynamically interact with these histone modifications. One example is the heterochromatin protein 1 (HP1) family, which binds to a specific PTM, histone H3 trimethylated at lysine 9 (H3K9me3), in a multivalent fashion and is involved in chromatin compaction, gene repression, telomere maintenance and DNA repair.

A single-molecule method using total internal reflection microscopy was recently developed in our lab to measure HP1α dynamics with chemically modified chromatin arrays, carrying defined levels of H3K9me3[1]. From these measurements, binding to individual chromatin arrays was observed to be very variable, possibly reflecting the different compaction states of the chromatin fibers.

In order to reveal the effect of fiber conformation on multivalent chromatin binding, we probe the binding dynamics of HP1α to chromatin in various compaction states. We thus engineered H3K9me3 containing chromatin in a condensed state, through the addition of histone H1, and in a decondensed state, by inserting histone H4 containing an acetylated lysine 16.

HP1α interaction studies on the single-molecule scale currently allow us to directly probe the effects of chromatin fiber compaction on effector binding dynamics. Taken together, these results will lead to a greater understanding of molecular mechanisms involved in epigenetic gene repression.

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Tackling Malaria by Inhibiting the SHMT Enzyme

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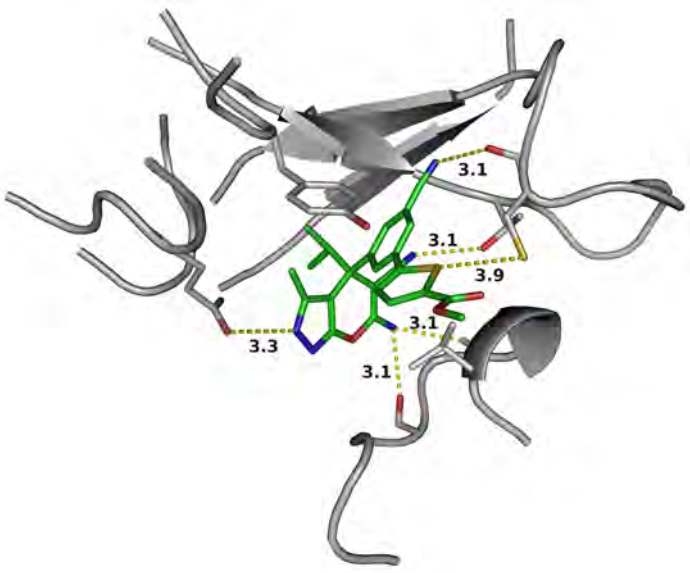
Malaria is mainly caused by the parasite *Plasmodium falciparum*. Due to the emergence of drug-resistant strains, there is an urgent need of novel treatment. The folate cycle containing several enzymes was identified as promising target; some of them are already addressed by antimalarials.[1] However, inhibition of serine hydroxymethyl transferase (SHMT), a key enzyme of the folate cycle, has not been investigated so far. *A. Thaliana* SHMT inhibitors, based on a pyrazolopyran scaffold, from an herbicide optimization program at BASF-SE demonstrated promising antimalarial activity on *P. falciparum* and *P. vivax*.[2] Because of pharmacokinetic limitations no significant activity in the *P. berghei* mouse model could be achieved.[3] The binding mode was resolved by several X-ray crystal structures of *Pv*SHMT-ligand complexes.[3] Based on the high similarity of *P. vivax* and *P. falciparum* SHMT, the X-ray co-crystal structures can be utilized for 3D modeling to design small drug-like molecules against *Pf*SHMT. In this work the development of novel inhibitors is focused on improving selectivity and liver microsomal stability while keeping high potency. In that perspective, variation of the moieties on the pyrazolopyran core and derivatization of the exit-vector are investigated.



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Investigation of an engineered AaLS-13 capsid and Identification of the encapsulation pathway for GFP (+36) by high mass MALDI MS analysis

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Proteins that self-assemble into nanocontainers are of emerging scientific interest for drug development.
Here, an engineered variant of Lumazine synthase from *Aquifex aeolicus* (AaLS-13) that self-assembles into a capsid was studied. Coulomb attraction between supercharged GFP (+36) and the capsid modified with additional negative charges on the lumen favors cargo encapsulation. We report on the successful implementation of native ESI-MS and MALDI-MS, to map the subunit composition at high molecular weight. The subunit stoichiometry of the AaLS-13 capsid was determined to be composed of only pentamer subunits. The capsid structure of AaLS-13 proposed earlier by Wörsdörfer *et al.* organizing into a regular icosaheder in the T=3 state, could not be confirmed, due to additionally required hexamer subunits [1]. Our results suggest the protein to build up a regular T=7d icosaheder capsid. High-mass MALDI-MS combined with crosslinking with glutaraldehyde (GA) is suggested to monitor the encapsulation pathway of supercharged GFP(+36) into the capsid. The results obtained showed capsid stabilization upon addition of supercharged GFP. Further, encapsulation of GFP (+36) strongly shifts the equilibrium towards capsid formation. The time scale of this reaction was determined to be out of range for crosslinking followed by high-mass MALDI analysis.

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Fluorine Scan at the Active Sites of Rhodesain and Human Cathepsin L: Enhanced Binding Affinity by Stacking of Fluorinated Phenyl Rings on Flat Dipeptide Fragments

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Amide bonds are ubiquitously present in proteins. However, exploitation of their planar π-surface for ligand binding remains to be further developed.[1] Harder *et. al* showed that for efficient ligand–amide bond interactions (1) favorable dipole moment orientation between various heterocycles and *N*-methylacetamide (antiparallel alignment) and (2) reduced electron density of the heterocycle were required.[2] Because of its high electronegativity and low polarizability, fluorine represents an ideal tool to (1) tune the electron density of benzene derivatives without introducing steric hindrance upon H/F replacement, and (2) introduce large dipole moment variations. Following these guidelines, we performed a fluorine scan of 20 inhibitors for two cysteine proteases, rhodesain and human cathepsin L, which both feature a flat dipeptide moiety in their respective pockets. We identified that multiple fluorination of the inhibitors led to an increased binding affinity for both rhodesain and human cathepsin L, resulting in low nanomolar inhibitors. Binding affinities were determined by fluorescence-based assays. We believe that these findings will not only be helpful to guide future development of inhibitors of rhodesain, a target against human African trypanosomiasis, but also to any other medicinal chemistry program whose target possesses exposed amide bonds.

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O^6-Alkylguanine Post-lesion DNA synthesis by Y-family DNA polymerase ζ characterized with synthetic nucleosides

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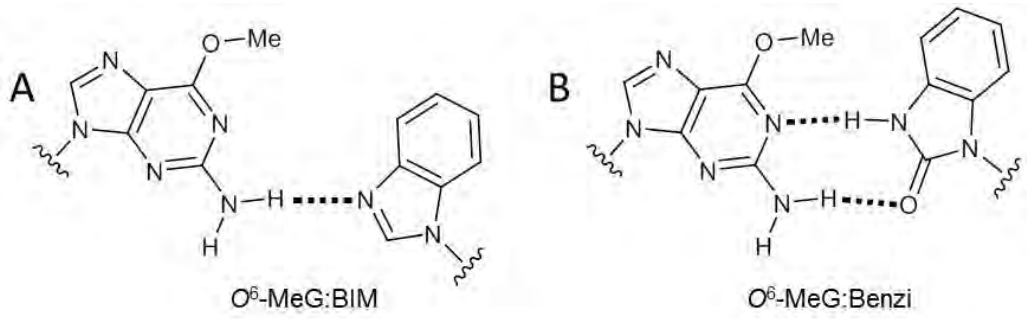
DNA polymerase ζ (Pol ζ) is a low-fidelity B-family polymerase involved in DNA damage tolerance and genomic stability by catalyzing the synthesis of DNA in the presence of DNA adducts.Pol ζ can bypass such chemical damage to the structure of DNA bases, but is in most cases inefficient at inserting a base opposite the adduct. In contrast, it seems to be very effective in performing post-lesion DNA synthesis (PLS), which is the extension step immediately following lesion bypass. Thus, Pol ζ may contribute to mutagenesis by fixing mismatches created by error-prone insertion.

In an effort to understand the mechanism of PLS for alkylated DNA of relevance to carcinogens that induce O^6-alkylguanine adducts, which are known to induce mutations, we used adduct-directed synthetic nucleoside probes Benzi and BIM (Figure 1) with altered size and hydrogen bonding possibilities to test the structural requirements for Pol ζ PLS. Rates where characterized for DNA synthesis of terminal primer template mismatches comprised of G, O^6-methylguanine (O^6-MeG) and O^6-(carboxymethyl)guanine (O^6-CMG) paired opposite either canonical nucleotides or synthetic bases. When the adduct was paired at the template:primer terminus with a synthetic base with correct H-bond donor alignment with the adduct, extension was similar to canonical mismatch variations, whereas when the paired base had no minor-groove H-bond donor, extension was hindered. The rates of PLS were related with biophysical properties of the duplexes, and lesion/mismatch extension was found to be slowed in the presence of modification relative to an undamaged construct. These data provide new insight regarding contributions of hydrogen bonding interactions to promoting extension past O^6-MeG and O^6-CMG catalyzed by Pol ζ.



O^6-MeG:BIM O^6-MeG:Benzi

Figure 1. Hydrogen bonding capacity for nucleoside probes paired opposite O^6-MeG (A) BIM opposite O^6-MeG and (B) Benzi opposite O^6-MeG.

Structural Characterization of Oligoproline

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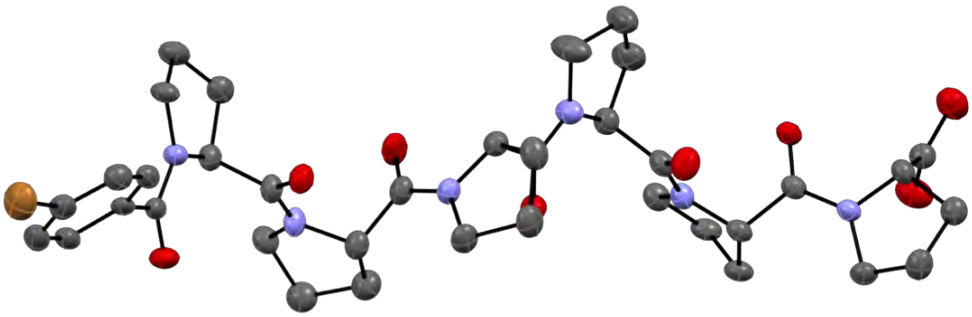
Oligoprolines have found wide applications as molecular scaffolds in various fields of chemistry and biology due to their well-defined and rigid conformation. In aqueous environments oligoprolines adopt a left-handed Polyproline II (PPII)-helix with every third residue stacked on top of each other. This secondary structure is the third most common in proteins and is widely adopted by collagen, the most abundant protein in mammals.
Herein, we present a detailed analysis of the first high-resolution crystal structure of an oligoproline adopting a PPII-helix.



The crystal structure provided deep insights into the structural parameters of the PPII-helix. It revealed that neighboring amide bonds interact with each other and that water is not a prerequisite to form a PPII-helix.
Furthermore we determined the persistence length of oligoproline using EPR-spectroscopy. The obtained data are crucial for the use of oligoprolines as molecular scaffolds.

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Cell Penetrating Peptides Based on an Oligoproline Scaffold

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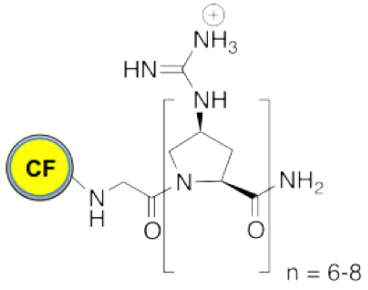
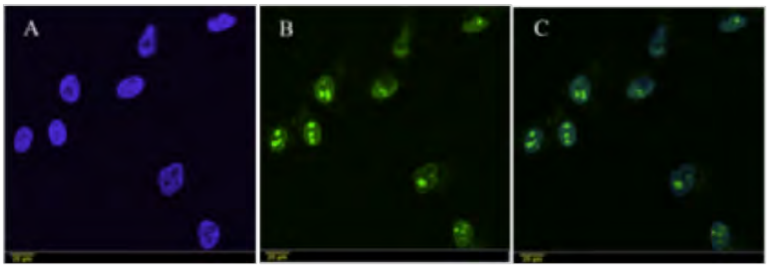
The efficient uptake of drugs, biomolecules and imaging agents into cells is still limited by the poor translocation through the plasma membrane of eukaryotic cells. Cell Penetrating Peptides (CPPs) based on cationic and amphipilic oligoproline structures are promising tools to address this issue.[1,2] To exploit the unique properties of oligoprolines as rigid and functionalizable scaffolds (Polyproline II helix)[3] we designed well-defined guanidine decorated oligoprolines and explored their cell permeability properties. Oligoprolines with varying chain lengths, charge densities, as well as helicities were prepared. Their uptake into human cervical cancer (HeLa) cells was evaluated by flow cytometry. The quantitative uptakes of the peptides were compared to established CPPs like the Tat-peptide and oligoarginines. The intracellular localization of the peptides was analyzed by confocal microscopy. Additionally the stability of the oligoprolines was tested in human blood serum and trypsin.





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Oligoprolines as Scaffolds for Tumor Targeting withHybrid Bombesin Analogues

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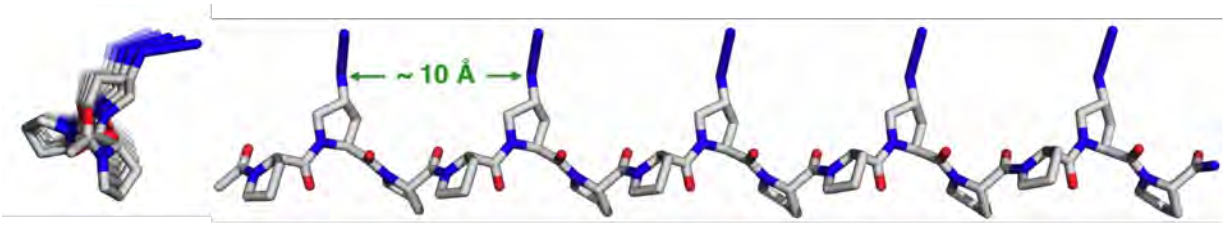
In aqueous environments oligoprolines adopt the well-defined polyproline II helix already at short chain lengths.[1] Incorporation of 4-azidoproline residues into the sequence provides reactive sites in defined distances that can easily be functionalized through, *e.g.*, click chemistry.[2]



Hybrid ligands consisting of an oligoproline scaffold equipped with a bombesin-based agonist and antagonist exhibit extraordinary tumor uptake properties in prostate carcinoma.[3] The hybrid ligands showed significantly higher uptakes *in vitro* and *in vivo* compared to monovalent and divalent controls. The defined distance between the recognition motives proved to be important for high, specific, and long lasting uptakes. Based on these initial findings we are now designing modified ligands to achieve higher uptakes and a deeper understanding of how the uptake is accomplished on the molecular and cellular level.

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Impact of minor groove alkylation on transcription by RNA polymerase II

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Minor-groove binding chemicals can alkylate the 3-position of Adenosine, giving rise to adducts such as 3-methyl-adenine (3MeA), which is the major DNA adduct from methyl lexitropsin, an alkylating agent with potential utility in the therapy of gliomas. The effective cytotoxicity of minor groove alkylation may be reduced, however, by repair mechanisms like transcription-coupled nucleotide excision repair (TC-NER), which is activated when RNA polymerase II (RNAPII) stalls at DNA damage sites. In this study we describe the behavior of RNAPII in the presence of modified DNA including a depurination-resistant analog of 3MeA, 3-deaza-3-methyl-adenine (3dMeA). We performed transcription assays with human RNAPII derived from HeLa nuclear extract and primer extension of ternary elongation complexes with purified yeast RNAPII. Our results show that although RNAPII efficiently synthesizes past 3dMeA, a significant reduction in the formation of full-length product was observed in comparison to transcription of an unmodified DNA template. Moreover, RNAPII promoted the mutagenic incorporation of cytosine opposite 3dMeA, a phenomenon that may lead to transcriptional mutagenesis in cells. Additional data from studies concerning how steric and H-bonding properties of minor groove modifications modulate these processes will be presented. Taken together, these results provide new chemical insight concerning how RNAPII copes with DNA alkylation, and this information is relevant for how tumor cells may resist chemotherapy.

Novel azobenzene-derived visible light photoswitches for biological applications

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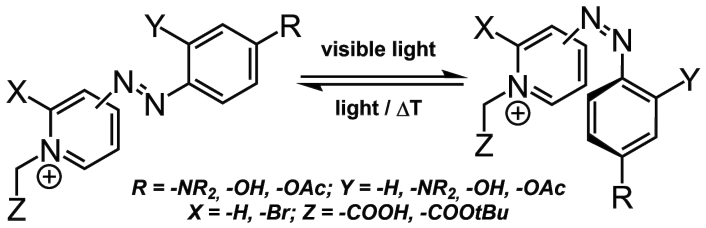
Molecular photoswitches are compounds that undergo reversible structural changes upon irradiation with light. After covalent or non-covalent attachment to biopolymers they enable photomodulation of their structure or activity. Azobenzene derivatives are most common molecular photoswitches applied in biological systems. However, unmodified azobenzene is switched with UV light, insoluble in water and moderately resistant on intracellular reducing environment. Therefore structural modifications are developed to increase the scope of applications, especially inside living cells and organisms.



We have synthesized several arylazopyridinium salts which absorb in the visible wavelength range, are well-soluble in aqueous buffers and tolerate glutathione at physiological concentrations. We will present our most recent results and discuss the scope of applications of our compounds for biological systems.

Bacterial Resistance to Silver: The Role of SilE Protein

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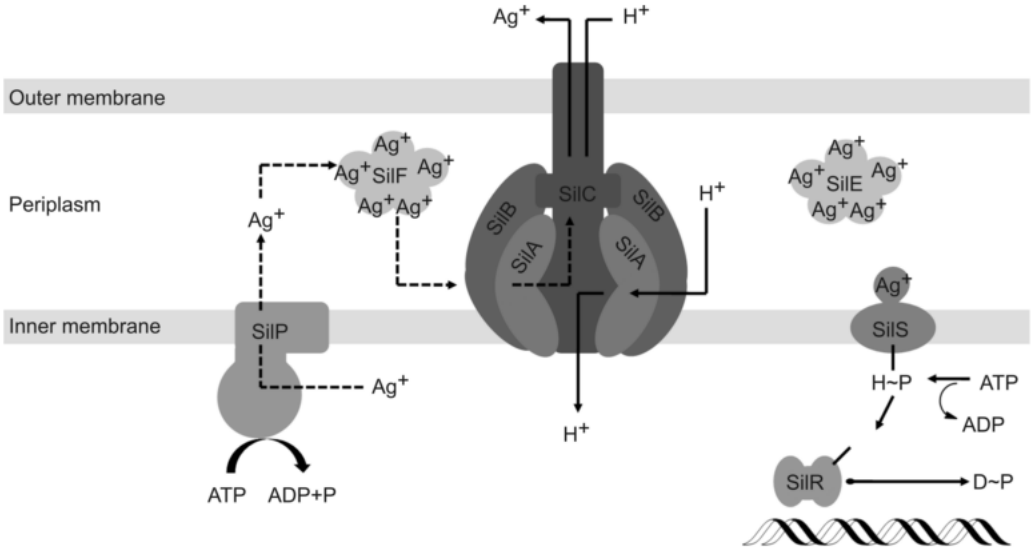
Silver has been used for hundreds of years for its antimicrobial properties. Since the emergence of many multi-resistant bacterial strains against classical antibiotics, the research of new silver compounds is now at its apogee. Nowadays, a lot of researches are focused on compounds with slow- and stimuli-responsive- release of Ag^+. While these drugs have been shown to be highly able to kill bacteria, some of these pathogens have developed a resistance to high concentrations of Ag^+.

This resistance is provided by the plasmid pMG101, which encodes for eight proteins that act together in an efflux pump system to deal with silver ions. Among these, the SilE protein is the only one of which its mode of action is actually unknown.



To identify the role of SilE in this bacterial machinery, two approaches have been intended in our group. While one way is to study the interaction of the whole protein with silver ions, the other is based on a bottom-up approach, investigating the interaction of silver ions with short peptide sequences of this protein.

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The use of phosphorylated peptides to explore the folding properties of the protein tau required for AT8 antibody recognition

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Paired helical filaments (PHFs) resulting from the hyperphosphorylation of the protein tau are major components of the Alzheimer disease. They contribute to the formation of intraneuronal neurofibrillary tangles. Accordingly, the phosphorylation of the serine 202 (Ser-202) and the threonine 208 (Thr-208) seem relatively specific. These two residues are, indeed, key for the recognition of the AT8 antibody [1], which is widely used for post mortem diagnosis of Alzheimer's disease. Although it is well established that the epitope of AT8 is located around the Ser-202 and the Thr-205, the folding characteristics associated with the recognition of this region with the AT8 are not known.

By using NMR spectroscopy in combination with scaled molecular dynamics (SMD) on different phosphorylated peptides corresponding to the 192-212 fragment of tau, we studied the secondary structure of the AT8 epitope of tau under its hyperphosphorylated form [2]. We observed that the AT8 epitope forms a turn that is induced by the phosphorylated threonine 205 (pThr-205) and the glycine 207 (Gly-207). More precisely, we detected hydrogen interactions between the phosphate group of the pThr-205 and the amide proton of the Gly-207. This interaction was stabilized by electrostatic contacts between the pSer-202/pThr-205 side chains and the Arg-209/Arg211. These results were compared with those obtained from the hyperphosphorylated full-length version of tau obtained by using the recombinant CDK2/CycA3 kinase [2].

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Pollen induced asthma - could small molecules in pollen exacerbate the protein-mediated allergic response?

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Plant pollen are known to be strong airborne elicitors of asthma in humans. *In vitro* data and clinical studies corroborate the involvement of small surface proteins present on the pollen grain. They modulate the immune system through IgE cross-linkage causing airway inflammation, and obstruction due to constriction of airways. At the physiological level, relaxation and constriction of airways is regulated by mechanisms involving proteins such as the lipid kinase PIP5Kγ and the cation channel TRPA1. While the role of proteins is well established, a possible contribution of small molecules present in pollen to the clinical outcome of asthma has not been explored up to now. Therefore, we analyzed and compared the phytochemical profiles of pollen originating from thirty plant species causing varying degrees of pollen allergenicity. Profiling was performed with high performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry (ESIMS), photodiode array (PDA) and evaporative light scattering (ELSD) detectors, and supported by microprobe nuclear magnetic resonance (NMR) spectroscopy and spectrophotometric analysis. The presence of conjugated polyamines, such as N^1,N^5,N^{10}- tricoumaroylspermidine, N^1-caffeoyl-N^5,N^{10}-dicoumaroylspermidine and N^1,N^5,N^{10},N^{15}-tetracoumaroylspermine was a characteristic feature of pollen from Asteraceae (*Ambrosia* and *Artemisia* ssp.), and compounds with Michael acceptor properties were also mainly present in pollen of these species. Polyamines such as spermine and spermidine are modulators of the lipid kinase PIP5Kγ[1], and sesquiterpene lactones activate cation channel TRPA1[2]. Thus, the possible contribution of these small molecules in the exacerbation of airway constriction should be explored in more detail.

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Search for alternatives to copper in organic farming: Fungicidal activity of a Juncus effusus medulla extract and its active constituent, dehydroeffusol, against downy mildew and apple scab

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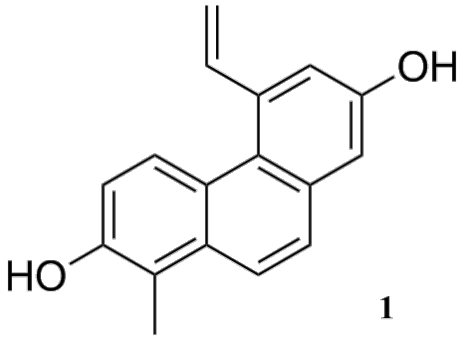
Copper has been used since the 19th century for the control of plant diseases, and is still permitted in organic agriculture out of this tradition. In recent years, the utilization of copper has been criticized due to an unfavourable ecotoxicological profile [1]. Even though the amounts of copper have been significantly reduced, copper input is still above uptake by plants, and results in accumulation in the soil. Therefore, considerable efforts have been made in organic agriculture to identify ecologically safer substitutes.

In this context, an in-house library of plant and fungal extracts was screened *in vitro* for an inhibitory effect against several plant pathogens (fungi, oomycetes, bacteria) [2]. Hits were further assessed on grapevine and apple seedlings. Active constituent(s) were identified by a procedure referred to as HPLC-based activity profiling which combines biological activity data with chemoanalytical information. Structure elucidation was performed by a combination of ESI-MS and NMR spectroscopy.

As one of the hits, the ethyl acetate extract of *Juncus effusus* L. (Juncaceae) medulla showed strong inhibitory activity against *Venturia inaequalis* (apple scab) and *Plasmopara viticola* (grapevine downy mildew), with mean minimal inhibitory concentrations (MIC) (100%) of 35 µg/mL and 25 µg/mL, respectively. In a secondary assay on grapevine leaf discs inoculated with *P. viticola*, 94% inhibition was observed at a concentration of 0.5 mg/mL. When tested on grapevine and apple seedlings at a concentration of 0.5 mg/mL, the growth of these fungi was, on average, inhibited with 98% and 84% efficacy, respectively. The active constituent was identified as dehydroeffusol (**1**) [3], and showed mean MICs of 12 µg/mL against *V. inaequalis*, and 4.1 µg/mL against *P. viticola*, *in vitro*. Subsequent *in vivo* assessment of the pure compound revealed inhibition rates of 82% on grapevine seedlings, and 86% on apple seedlings at a concentration of 32 µg/mL. Our results demonstrate that plants can provide promising opportunities for the replacement of copper in organic farming.



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Natural and semisynthetic antitrypanosomal sesquiterpene lactones from Anthemis nobilis

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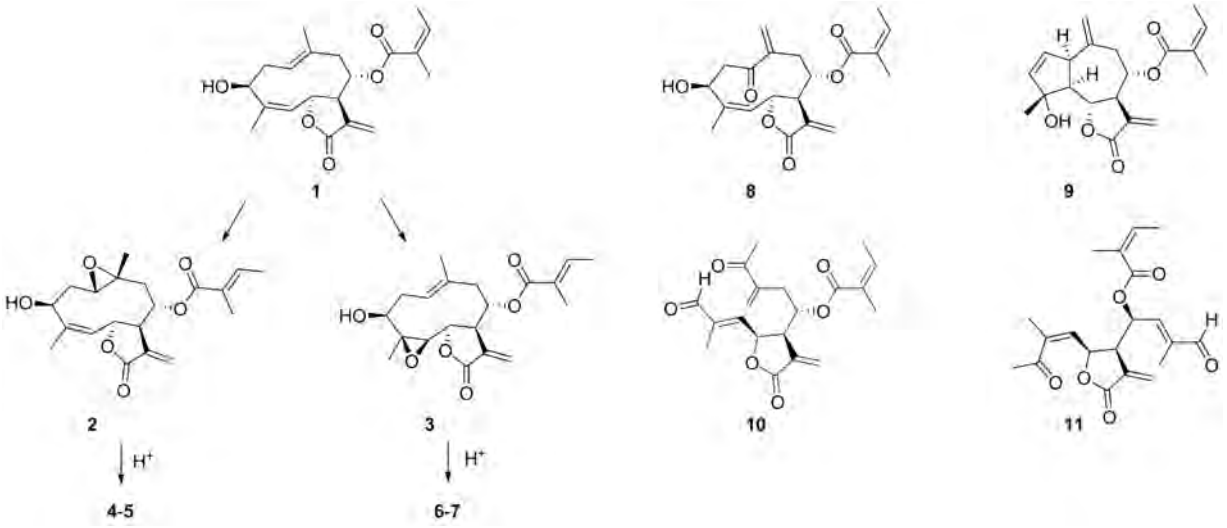
Trypanosomatid parasites of the genera *Trypanosoma* and *Leishmania* are responsible for neglected tropical diseases for which new, safer, and affordable drugs are urgently needed. [1] Natural products from plants have been instrumental in developing drugs against protozoal diseases, e.g. artemisinin and quinine, and still represent an important source for leads with trypanocidal activity. [2] In previous studies sesquiterpene lactones (STLs) were found to possess promising antitrypanosomal activity. [3] We recently reported on the synthesis, by biomimetic acid catalysis, of a focused library of antitrypanosomal compounds generated from nobilin (**1**)**,** the major STL in Roman chamomile (*Anthemis nobilis*) flower cones. [4] We here applied the same biomimetic approach to the 1(10) and 4(5) epoxides of **1** (**2** and **3**) and obtained a series of furanoheliangolide STL derivatives (**4**-**7**). Moreover, further phytochemical investigation of *A. nobilis* afforded a series of minor STLs, four of which (**8**-**11**) were new compounds. All the above mentioned STLs showed high antitrypanosomal activity (IC_{50s} 0.13-0.82±0.01 µM and 0.14-10.85±0.01 µM against *T. b. rhodesiense* and *L. donovani*, respectively), although with low selectivity. SAR studies are currently ongoing to identify structural and physico-chemical features responsible for activity.



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Antagonizing Bacterial Adhesion – Hit Identification by a Dynamic Combinatorial Chemistry Approach

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In Dynamic Combinatorial Chemistry (DCC) substance libraries are formed from reversibly reacting building blocks. These libraries are under thermodynamic control, and their composition can be influenced by the applied conditions. For instance, in the presence of a receptor protein, those members of the library binding to the target are up-regulated, leading to a shift of the composition of the library, i.e. an amplification of high-affinity ligands at the expense of weak binders.[1,2]

Urinary tract infections belong to the most common bacterial infections and are mainly caused by uropathogenic *Escherichia coli* (UPEC). The infection cycle is initiated by the binding of UPEC via the lectin FimH to uroplakin 1a, a mannosylated glycoprotein on bladder epithelial cell.[3,4] This interaction can be prevented with aryl mannosides leading to elimination of bacteria with the bulk flow of urine and thus presenting an alternative treatment strategy to classical antibiotics.[5-8]

We here present a DCC approach for the bacterial lectin FimH. Aryl glycosides with an aldehyde moiety in the aglycone are reacted reversibly with hydrazides to generate a library of acylhydrazones. The synthesis is carried out in the absence and presence of the FimH. The library is then rendered pseudostatic by increasing the pH, making it suitable for HPLC analysis. Comparing the library compositions obtained in the presence and in absence of the lectin allows for quantitative affinity ranking of the corresponding acylhydrazones.

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It’s better to bend than to break

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The attachment of uropathogenic *E. col*i to host cells is mediated by the adhesin FimH which is located at the tip of bacterial type 1 pili. The carbohydrate recognition domain of FimH forms an extended hydrogen bond network with the hydroxyl groups of aryl α-D-mannosides. In addition, the so-called tyrosine-gate hosts the aryl aglycone.

Herein we describe the molecular interaction between flexible seven membered ring (septanose) monosaccharides that deform upon binding to the rigid FimH to accommodate to the shape of the binding pocket. The interaction was firstly evaluated in a competitive binding assay and molecular docking experiments. Subsequent isothermal titration calorimetry (ITC) measurements, molecular dynamics (MD) simulations and NMR chemical shift perturbation (CSP) experiments revealed that the difference in affinity between *n*-heptyl α-D-mannoside and the corresponding septanose derivative was solely due to an induced conformational change (entropy cost) of the septanose ligand. Finally, the cocrystal structure confirmed the binding mode of the septanose to FimH.

Combining different analytical methods considerably improved our understanding of FimH-ligand interaction and will guide the design of therapeutically relevant FimH antagonists.

Keywords: FimH, mannosides, NMR, molecular dynamics, thermodynamics, X-Ray.

Development of small molecular tools for the cellular study of adenosine A_1 receptors

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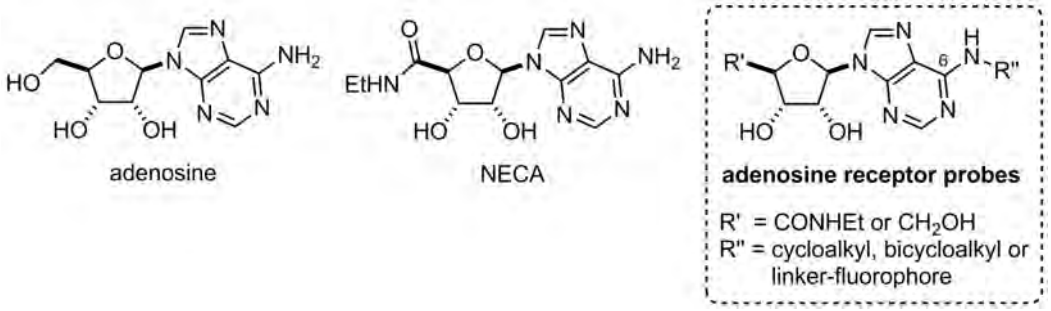
The adenosine receptors are members of the G-protein coupled receptor (GPCR) family which represents the largest class of cell-surface proteins mediating cellular communication. As a result, GPCRs are formidable drug targets and it is estimated that approximately 30% of the marketed drugs act through members of this receptor class. There are four known subtypes of adenosine receptors: A_1, A_{2A}, A_{2B} and A_3. The adenosine A_1 receptor, which is the subject of this presentation, mediates the physiological effects of adenosine in various tissues including the brain, heart, kidney and adipocytes. In the brain for instance, its role in epilepsy and ischemia has been the focus of many studies.[1]

Previous attempts to study the biosynthesis, trafficking and agonist-induced internalisation of the adenosine A_1 receptor in neurons using fluorescent protein-receptor fusion constructs[2] have been hampered by the sheer size of the fluorescent protein (GFP) that ultimately affected the function of the receptor. We have therefore initiated a research programme to develop small molecule fluorescent agonists that selectively activate the adenosine A_1 receptor.

Our probe design is based on the endogenous ligand adenosine and the known unselective adenosine receptor agonist NECA (Figure). We have synthesised a small library of non-fluorescent adenosine derivatives that have different cyclic and bicyclic moieties at the N^6 position of the purine ring and have evaluated the pharmacology of these compounds using a yeast-based assay.[3] This analysis revealed compounds with interesting behaviour, i.e. exhibiting adenosine A_1 subtype-selectivity or G-protein bias. Thus, these compounds can be potentially used as tool compounds in their own right for cellular studies of the adenosine A_1 receptor. Furthermore, we have also linked fluorescent dyes to the purine ring and discovered fluorescent compounds that can activate the adenosine A_1 receptor.



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Fluorescent probes for the cellular study of the 5-HT$_3$A receptor – synthesis and evaluation of near-infrared probes

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The 5-HT$_3$ receptor is a ligand-gated ion channel (LGIC) and a member of the Cys-loop family of receptors. Fluorescent probes are powerful tools to study transmembrane proteins such as the 5-HT$_3$. We previously reported[1],[2] a fluorescein derivative that showed high affinity (1.9 nM) and that was used as a tracer in fluorescence polarization and flow cytometry binding assays.

A near-infrared fluorescent probe was used for in-vivo imaging of the 5-HT$_3$R in a live mouse[2] and stained receptors in the abdominal cavity, in intestines, but also in salivary glands. The unexpected presence of 5-HT$_3$ receptors in mouse salivary glands was confirmed by Western blots. This probe was also used in single molecule spectroscopy studies of the receptor[3]. Herein we report a straightforward synthesis of the SiRhodamine methyl and lactone fluorophores their live cells evaluation and the evaluation of their granisetron conjugates.

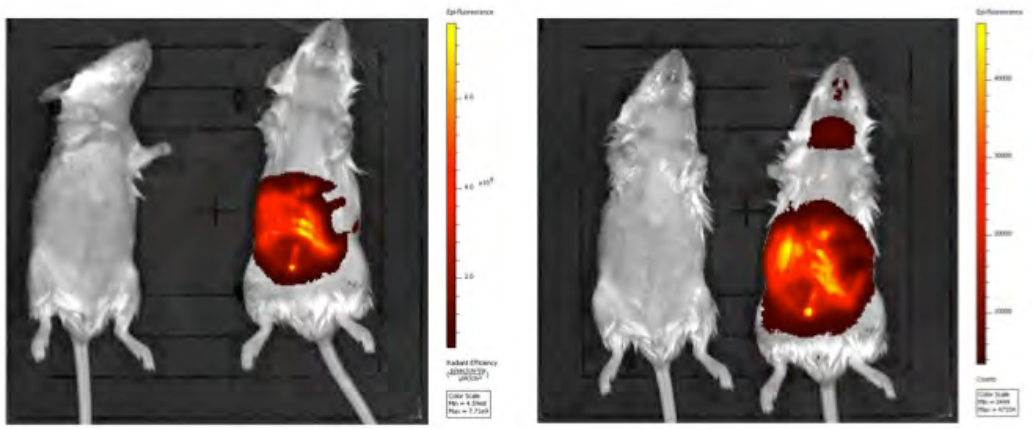

Fig. 1. 10 mg/kg i.p. of G-SiR (640/680 nm) life imaging with anesthetized mice, 5 minutes post injection and 30 minutes post injection.

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Peptide dendrimer as SiRNA transfection reagent

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RNA interference (RNAi) allows effective and specific silencing as described by Tuschl and co-workers in their proof-of-principle experiment demonstrating that synthetic double stranded small interfering RNA (SiRNA) could achieve sequence-specific gene knockdown in a mammalian cell line by promoting the degradation of complementary mRNA via RISC complex[1]. Potential therapeutic applications of RNAi are crucially dependent on the delivery of SiRNA into the cytosol to avoid that this step becomes a bottleneck.Naked or chemically modified SiRNA delivery is of limited application and therefore nanoparticles encapsulating SiRNA molecules have been investigated as a more general method to bring SiRNA into cells.
We have previously explored a collection of peptide dendrimers for the transfection of plasmid DNA and found efficient reagents that obeyed structure-activity relationships.Of crucial importance was the distribution of cationic charges across the three dendrimer generations and the use of DOTMA/DOPE as lipids (Figure 1, A)[2].

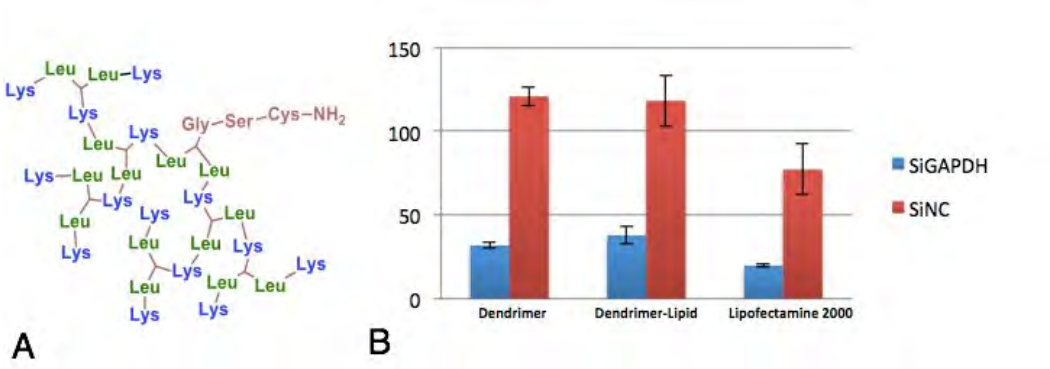

Figure 1. The chemical structure of the peptide dendrimer G3-KL1,2,3 used in DNA transfection (A). SiGAPDH expression following a transfection in presence of 10 % serum of the lead compounds and lipofectamine 2000 in HeLa cells compared to untreated cells (B).

We are now exploring peptide dendrimers as delivery agents for SiRNA. In this project a library of 100 peptide dendrimers were prepared by SPPS and their gene silencing ability investigated. The parameters necessary for efficient gene silencing have been discovered and optimized to lead to an only amino acid and a lipid-conjugated dendrimer (Figure 1, B). The biological experiments included treatment of HeLa cells by the new transfection agents and SiRNA targeting GAPDH (SiGAPDH) or scrambled (SiNC) in the absence and presence of serum. The knockdown efficiency was measured by monitoring enzyme activity of GAPDH.

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Excess Electron Transfer in DNA Containing a Pyrenyl Donor and Multiple Stable Phenanthrenyl Base-Surrogates

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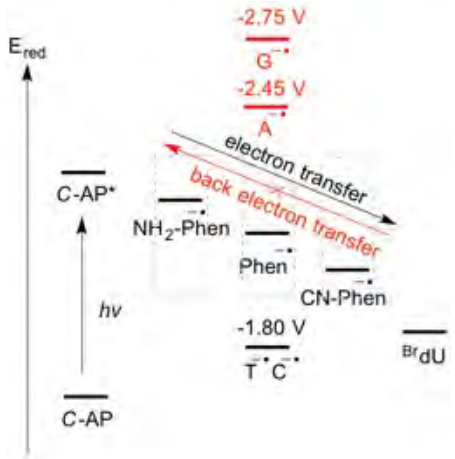
The reductive electron transfer along DNA attracted considerable interest in the last two decades and is a promising feature for molecular electronics. A system based of a p-stacked *C*-nucleoside pyrenyl donor (*C*-AP), multiple phenanthrenyl based base surrogates as electron carriers and 5-bromodeoxyuracil (^{Br}dU) as and kinetic electron trap was synthesized in order to study the excess electron transfer (EET). Gradually lowering the lowest unoccupied molecular orbital (LUMO) of the sequentially installed electron carriers is envisioned to enhance the electron transfer efficiency by disfavoring the back electron transfer.



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Antimicrobial Cyclic Peptides with L,D- architecture Targeting Pseudomonas aeruginosa

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Membrane active antimicrobial peptides (AMP) with unusual topologies offer an attractive opportunity to address multi-antibiotic resistant bacteria, in particular Gram negative Pseudomonas aeruginosa (PA), which is one of the most problematic pathogens in the clinic.[1] Six and eight residue cyclic D,L- α-peptides have been reported to show antimicrobial activities against Gram positive strains such as S. aureus [2]. We asked the question whether this architecture could also be used to obtain AMPs targeting PA. A modified synthesis using a simple thioether ligation for cyclization was employed to prepare cyclic 6- to 12-residues peptides with alternating D- and L-residues, leading to the identification of two potent tryptophan containing cyclic peptides with strong activity against PA. CD spectroscopy shows that these active cyclic peptides undergo specific conformational changes in membrane environment compared to inactive analogs. Moreover, a red shift in the UV-VIS spectrum in SDS solution indicates that tryptophan residues form J-aggregates, suggesting aggregation in the membrane of bacteria as the mechanism of antibacterial action.

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Synthesis of a Geminal Difluorinated Tricyclic Nucleoside Analog

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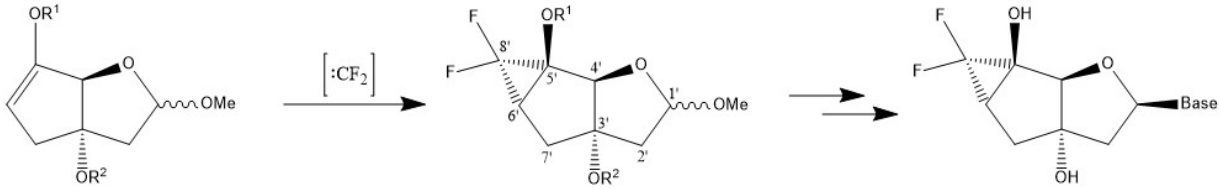
In recent years fluorine substituted compounds have become particularly attractive in medicinal chemistry due to the bio-stability enhancing properties of the fluorine atom. [1] This properties do not only pertain to small molecules but also to oligonucleotides. This led us to synthesize and investigate therapeutic antisense oligonucleotides bearing a fluorine substituent at the C6' position of a bicyclic [3] or tricyclic [4] as well as at the C2' position [5] of a tricyclic nucleotide analog. In all cases these oligonucleotides were found to be good RNA mimics. Here we will show and discuss the synthesis of a C8' gem-difluoro modified tricyclic nucleoside analog. The key step in the synthetic pathway was the treatment of a bicyclic silyl enol ether with a difluoro carbene precursor reagent to form the gem-difluoro cyclopropane ring of the tricyclc sugar backbone with the desired exo-configuration.



R^1 = TES or TBDMS
R^2 = TES, TMS or Ac

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Synthesis of photo-crosslinking probes and their application for the site-selective chemical modification of the 5-HT_3 receptor

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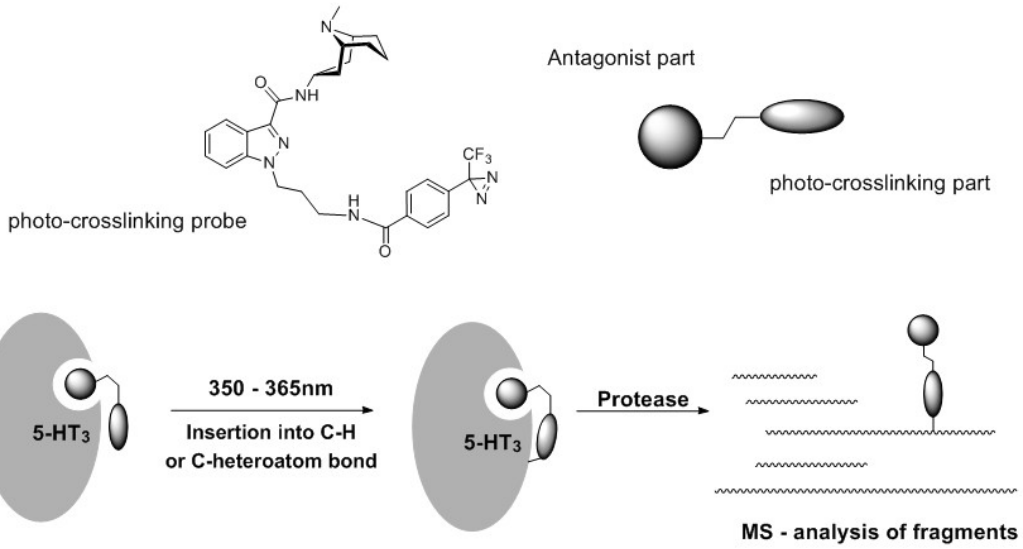
The 5-HT_3 receptor (5-HT_3R) is an important ion channel responsible for the transmission of nerve impulses in the CNS and PNS and is activated by the endogenous agonist serotonin (5-hydroxytryptamine, 5-HT). The 5-HT_3R is the only serotonin receptor belonging to the Cys-loop superfamily of neurotransmitter receptors. Different structural biology approaches can be applied, such as crystallization and x-ray analysis.[1] However, characterizing the exact ligand binding site(s) of these dynamic receptors remains challenging. The use of photo-crosslinking probes is an alternative validated approach and allows identification of regions in the protein that are important for the binding of small molecules.

We designed our probes based on the core structure of the 5-HT_3R antagonist granisetron, an FDA approved drug used for the treatment of chemotherapy-induced nausea and vomiting.

We synthesized a small library of photo-crosslinking probes by conjugating diazirines and benzophenones via various linkers to granisetron. We were able to obtain several compounds with diverse linker lengths and different photo-crosslinking moieties that show nanomolar binding affinity for the orthosteric binding site. Furthermore we established a stable h5-HT_3R expressing cell line and a purification protocol to yield the receptor in high purity. Several experiments showed unambiguously that we are able to photo-crosslink our probes with the receptor site-specifically. The functionalised protein was analysed by Western blot and MS-analysis.[2] This yielded the exact covalent modification site, corroborating current ligand binding models derived from mutagenesis and docking studies.



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Design of 3D Protein Fingerprint and its Application to Map the Protein Data Bank

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The RCSB Protein Data Bank (PDB) provides public access to experimentally determined 3D-structures of biological macromolecules (proteins, peptides and nucleic acids).[1] Although various tools are available to explore the PDB for analogs of specific proteins, the overall structural diversity available in PDB is difficult to perceive.[2-6] Inspired by fingerprint based similarity searches which can be performed within seconds and greatly facilitate the exploration of small molecule databases,[7] we have adapted our recently reported fingerprint-browser[7] and fingerprint-mapplet[8] concepts to enable the visualization and similarity search of PDB in form of a “PDB-Mapplet” application. PDB structures are encoded in a 136-bit atom pair 3D protein fingerprint (3DP) counting categorized atom pairs at increasing through-space distances.[9] This fingerprint perceives molecular shape and groups PDB structures according to 3D-features such as folds rather than by primary sequence. The related 136-dimensional molecular shape space is visualized by principal component analysis of a secondary 200-dimensional satellite similarity space[10] and representation of the (PC1,PC2)-plane. This plane covers 98 % of data variability and distributes PDB structures according to size and shape.

The PDB-Mapplet consists of a main window displaying interactive “google-maps-like” color-coded PC-maps, and locate or search for nearest neighbours is available for a given pdb entry. Each PDB structure is displayed as a cartoon image and can be inspected closer as 3D-model by opening a secondary Jmol visualisation window. The PDB-Mapplet offers an unprecedented insight into the diversity of PDB-structures and allows perceive relationships between proteins otherwise difficult to identify.

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QPD-BA, A Precipating Dye For Monitoring Hydrogen Peroxide In Living Cells

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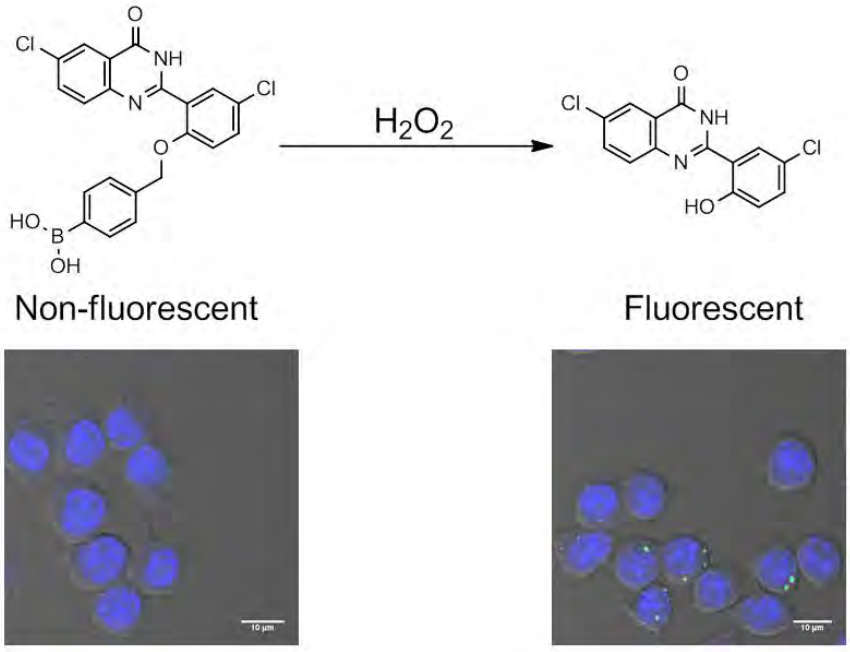
Reactive oxygen species such as hydrogen peroxide serve a variety of functions in living systems, including protein modification, signaling, immune and growth responses[1][2]. Several fluorescent probes selectively detecting hydrogen peroxide based on boronate oxidation have been reported [3].



Thus a quinazolinone precipitating dye (QPD) was modified with an immolative boronic acid trigger. Cleavage of the boronic acid moiety in the presence of hydrogen peroxide induced the precipitation of QPD which could be visualized in living cells.

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Protein Glycoconjugation by bioorthogonal click chemistry

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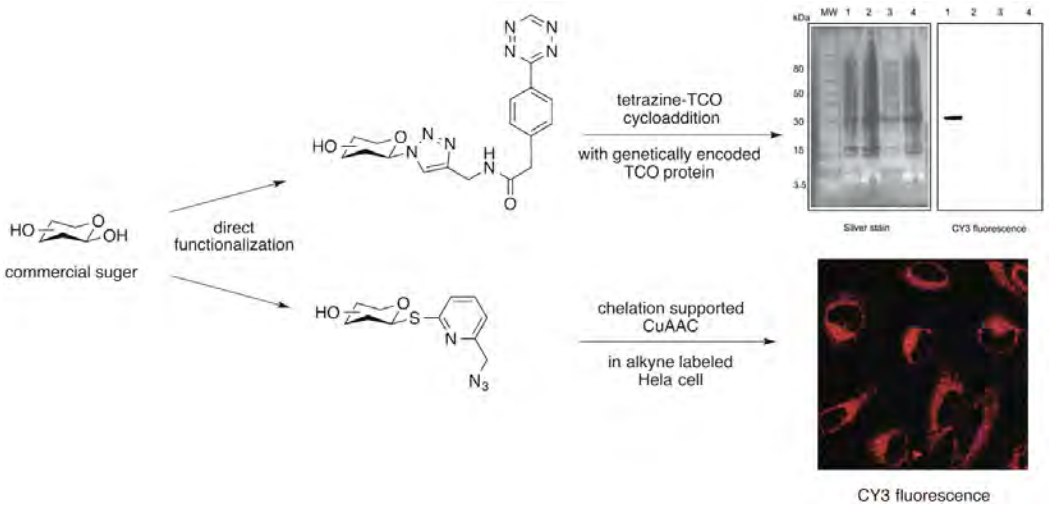
[1]University of Geneva, [2]Technical University Munich, [3]Ecole Polytechnique Fédérale de Lausanne (EPFL), [4]Medical Research Council

Protein glycosylation constitute an important post-translational modification and the glycans of glycoprotein have been specifically associated with numerous biological processes. We developed biocompatible methods to chemoselectively glycosylate protein in the lysate or live cell situation. Tetrazine-glycan conjugate allowed site-selectively glycosylation with genetically encoded*trans*-cyclooctene (TCO) protein in E coli pellet[1)]. Similarly, CuAAC based glycosylation proceeded in live cell under low copper concentration (50mM,5 min reaction) in alkyne labeled Hela cell (metabolic labeling of homopropargylglycine)[2)].



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Platinum-Porphyrin Conjugates as Highly Phototoxic Agents against Human Cancer Cells

B. Spingler[1], A. Naik[1], R. Rubbiani[1], G. Gasser[1]

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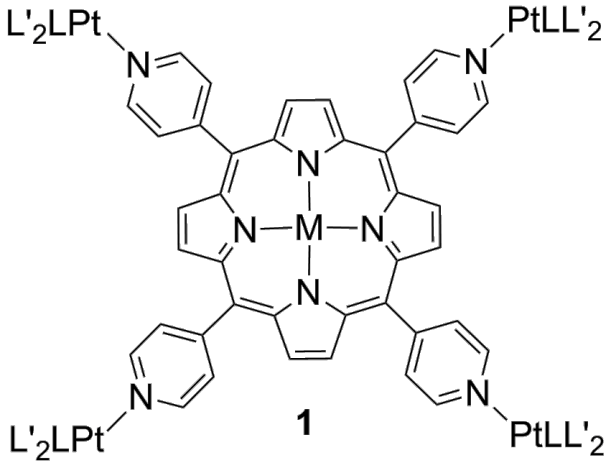
Despite the extended use of porphyrin complexes in PDT [1], tetraplatinated porphyrins have so far not been studied for their anticancer properties in the presence of light [2]. This is in contrast to ruthenated tetrapyridyl porphyrin complexes which have been synthesized [3] and tested for their PDT ability in animal models [4].
We would like to report about the synthesis of novel tetraplatinated porphyrins **1** as well as their photophysical characterization and *in vitro* light-induced anticancer properties [5]. The quantum yield of $^1O_2$ (Φ) production upon light irradiation was found to be between 0.42 and 0.54. The dark and light toxicity against human cancerous and non-cancerous cell lines (MRC-5, HeLa, A2780 and CP70) was determined by the resazurin assay. IC_{50} values were obtained after 4 h incubation, followed by 15 min irradiation at either 420 nm or 575 nm respectively. These platinum-porphyrin conjugates **1** had only minor dark toxicity, however upon visible light irradiation, IC_{50} values down to 19 ± 4 nM could be observed. These values correspond to an excellent phototoxic index (PI = IC_{50} dark / IC_{50} light) of greater than 5000. The influence of the absence / presence of different metals inside the porphyrin upon photophysical and pharmacological properties will be discussed as well.



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RNA internal loop: suitable binding site for metallo-intercalators?

E. Alberti[1], M. Coogan[2], D. Donghi[1]*

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Metal complexes are widely used for therapeutic and diagnostic applications, and their interaction with DNA has been extensively studied [1,2]. However, besides DNA, there are several other possible biological targets, including RNA, which is particularly attractive owing to its involvement in several biological processes and its complex structural diversity [3]. Our project deals with the study of the RNA interaction of a family of Re(I)dppz complexes with potential as bio-imaging agents, whose DNA binding properties were already studied [4]. It was also shown that their cellular uptake varies upon changing their axial ligand and that they can accumulate in DNA and RNA rich regions [4]. However, there are no information on their RNA interaction. Therefore we are investigating the behaviour of one of these complexes, namely $[Re(CO)_3(dppz)(3\text{-}CH_2OH\text{-}Py)]^+$, in the presence of two different RNA constructs containing common RNA secondary structural features. The first construct is a 27 nucleotide long RNA, derived from the mitochondrial group II intron ribozyme *Sc*.ai5γ [5], which contains a GU wobble, an internal and a terminal loop. The second one is an analogous short RNA that does not contain the internal loop and is used to probe a preferential interaction for this feature. A similar DNA sequence has also been used in the binding assays. Moreover, the behaviour of a classical ruthenium complex, known to act as DNA molecular "light switch" [6], was also studied for the sake of comparison. The RNA/DNA binding was studied by UV/Vis and fluorescence titrations, as well as ethidium bromide fluorescence displacement assays.Also, NMR experiments were performed to localize the interaction site. Interestingly, our NMR data suggest, in addition to an overall generalized binding, a localized interaction at the RNA internal loop. Further experiments are currently being performed to better understand the nature of this interaction.

Financial support by the Swiss National Science Foundation (Ambizione fellowship PZ00P2_136726 to DD), by the University of Zurich (including the Forschungskredit grant FK-13-107 to DD) and within the COST Action CM1105 is gratefully acknowledged.

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Secrets of in vitro RNA folding and splicing revealed byfluorescent PNA labels

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RNA is a versatile and functionally highly diverse molecule involved in numerous crucial processes within biological systems. It's mostly known for its catalytic activity and role as carrier of genetic information. The latter comprises the maturation of RNA, of which RNA splicing is an essential process not yet completely understood.

A model system of high interest is the wild type group II intron ai5 from *Saccharomyces cerevisiae*. The self-catalytic property of this ribozyme can *in vitro* be induced by a high non-physiological concentration of Mg2+ ions, whereas *in vivo* it requires the help of chaperone proteins [1]. Visualization of the *in vitro* splicing event under physiological conditions was enabled by native Polyacrylamide Gel Electrophoresis (PAGE) and Förster Resonance Energy Transfer (FRET) experiments using fluorescent Peptide Nucleic Acid (PNA) labels [2]. PNAs are synthetic molecule with high affinity to nucleic acids and a low mismatch tolerance in complementary PNA/nucleic acid duplexes [3]. Based on these *in vitro* experiments, we want to go further towards *in vivo* studies of the splicing process and improve the comparability of *in vitro* and *in vivo* experiments on RNA macromolecules.

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RNA and Oxaliplatin: Investigation of Possible Platinum Binding Sites

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Nucleic acids are important intracellular targets for many drugs. For example, platinum(II) anticancer drugs are known to primarily exert their activity upon covalent binding to DNA purine nitrogens [1]. In this context, to better understand their mechanism of action, several studies have been dedicated to the characterization of platinum-DNA adducts [2]. However, also RNA is an important biomolecule, with many important functions *in vivo*. The disruption of these processes can have serious consequences, and it has been already reported that some RNA related activities can be inhibited upon platinum drug administration. Still, only little is known on the effect of platinum drugs on RNA structure and biology [3, 4].

In order to understand how platinum drugs affect RNA structure, we are studying the interaction of a 27 nucleotide long RNA construct, derived from the mitochondrial group II intron ribozyme *Sc.*ai5*γ* [5] and used as model system, with oxaliplatin. The latter is an FDA approved anticancer drug used in clinics worldwide [6]. After having found the optimal conditions to obtain and isolate monoplatinated RNA samples, we are now investigating which are the possible platinum binding sites, using a combination of NMR studies as well as hydrolytic and enzymatic digestions of ^{32}P 5`- and 3`-end labelled samples.

Financial support by the Swiss National Science Foundation (Ambizione fellowship PZ00P2_136726 to DD), by the University of Zurich and within the COST Action CM1105 is gratefully acknowledged

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Studies on the mode of action of cationic β-hairpin antibiotics

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With increasing bacterial resistance to existing antimicrobial drugs, the need for new antibiotics active against drug-resistant microorganisms is becoming a pressing issue. Conformationally restrained peptidomimetics based on naturally occurring antimicrobial peptides of the innate immune system are gaining in importance, and are interesting starting points in the discovery of new biologically active molecules. Our research focuses on β-hairpins, a recurrent motive that participates in many important biological processes. Several antimicrobial peptides from the innate immune system have been identified having this structural motif, making the synthesis and use of stable structurally constrained peptides that mimic these of great interest. The peptidomimetics contain 12 residues linked to a hairpin-stabilizing D-Pro-L-Pro template, which can be synthesized by solid phase peptide synthesis. Earlier efforts to discover new cyclic cationic antimicrobial peptides that adopt stable β-hairpin structures led to the discovery of L27-11, which has a potent and selective antimicrobial activity against *Pseudomonas spp.* Following the synthesis and screening of libraries of β-hairpin peptidomimetics, several other candidates with good antimicrobial properties and low toxicity were also identified.

As in the case of L27-11, which was shown to target the outer membrane protein LptD and to inhibit its key role in outer membrane biogenesis, understanding the mechanisms of action of these new antimicrobial peptidomimetics is a priority, to identify new targets that could be used to fight against bacterial resistance. In this work, we describe one novel peptidomimetic antibiotic, showing potent activity against *E. coli*. Combining several known techniques to methodically analyse the potential targets in this bacterium, we were able to obtain evidence for a likely mechanism of action. The influence on membrane integrity was examined by following the uptake of Sytox and release from cells of β-lactamase and β-galactosidase. Radiolabelled precursors were used to assess effects on the biosynthesis of macromolecular components of the cell (proteins, RNA, DNA and cell envelope). Various microscopy techniques (transmission and scanning EM and optical STED), photoaffinity labelling and proteomic studies were used to highlight possible targets within the cell. Although his general approach was applied to one antimicrobial peptidomimetic, because of its potent antimicrobial action against Gram-negative bacteria, it could also be employed to systematically analyse targets of other new antimicrobial peptidomimetic compounds.

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Towards in vivo splicing of group II intron ai5γ

M. Gulotti-Georgieva[1], S. Zelger-Paulus[1], R. K. O. Sigel[1]*

[1]University of Zurich

The group II intron family represents a large class of non-coding RNA elements found in bacteria and organellar genomes of fungi, plants, and lower eukaryotes. Group II introns are known to undergo self-splicing during mRNA maturation. This occurs in a two-step mechanism, where catalysis of successive trans-esterification reaction leads to autocatalytic excision of the intron and concomitantly to exon ligation. Both steps are reversible rendering group II introns mobile genetic elements [1]. *In vivo* the splicing process is supported by cofactors. Our research focuses on the autocatalytic group II intron ai5γ from *Saccharomyces cerevisiae.* For its folding and splicing *in vivo* it requires the assistance of the DEAD-box protein Mss116, an ATP dependent helicase [2]. Intensive studies over the past decades have concentrated on a truncated (modified) intron model, whereas we are interested in the wild type molecule. Both *in vitro* and *in vivo* splicing process of the full length construct will be investigated in the presence and absence of Mss116. Two well-known methods will be applied: native Polyacrylamide Gel Electrophoresis (PAGE) and single-molecule Förster Resonance Energy Transfer (smFRET). The fluorescence labeling of the target molecule on specific positions will be performed either using DNA oligogonucleotides or alternatively, Peptide Nucleic Acids (PNAs), both carrying fluorescence dyes. Our results will lead to a detailed understanding of the wild type group II intron ai5γ folding and splicing pathway.

Financial support by the ERC and the University of Zurich is gratefully acknowledged.

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Characterization of group II intronsretrohoming site at the single molecule level.

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Group II introns are catalytically active RNA molecules: "*Ribozymes*". They cleave single RNA or DNA strand via two transesterification reactions. Group II introns are strictly dependent on Mg^{2+} for proper folding and catalysis [1]. In this study, we used smFRET to characterize the interaction between d3'EBS1* (Exon Binding Site 1) hairpin and its cognate IBS1* (Intron Binding Site 1). The cognate was either an RNA (IBS1*) or DNA (dIBS1*) fragment [2]. These two interactions, i.e. d3'EBS1*-IBS1* and d3'EBS1*-dIBS1*, mimic the recognition interaction required for splicing and for *retrohoming* of group II introns, respectively. NMR studies of these two constructs revealed three binding pockets for M^{2+} [3-4]. We combine structural information from NMR with kinetic and structural data from smFRET to compare our findings of the d3'EBS1*-dIBS1* construct with those of the d3'EBS1*-IBS1* [5].

Our results indicate that the interaction of the d3'EBS1* hairpin with both cognates displays a specific requirement for Mg^{2+} ions at physiological concentration. However, unlike the d3'EBS1*-IBS1* interaction, d3'EBS1*-dIBS1* requires Mg^{2+} ions to promote docking of dIBS1* to d3'EBS1*. In addition, our results show that the two interactions differ slightly in their conformation of the bound state, in a qualitative agreement with the NMR results performed on the same structures [3-4]. The kinetic analysis revealed that the presence of Mg^{2+} leads to a pronounced heterogeneity in the kinetic of both interactions. We corroborate the finding that heterogeneity in the case of by d3'EBS1*-IBS1* results from an incomplete occupancy of M^{2+} binding pockets by Mg^{2+} and Ca^{2+} ions [5] and we propose that kinetic heterogeneity of d3'EBS1*-dIBS1* interaction results as well from an incomplete occupancy of M^{2+} binding pockets by Mg^{2+} ions.

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Correlation between structure and antimicrobial activity of chitosan-alkyl thiomers – a biological study

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Chitosan is a natural polymer consisting of N-acetyl-D-glucosamine and β-1,4-linked-D-glucosamine units. Its intrinsic antimicrobial properties lead this compound to be a promising material for many clinical applications.[1] In our previous work we discovered that functionalization of chitosan with thioglycolic acid produces an excellent antimicrobial agent.[2] In this study, the relation between structure and antimicrobial activity of different chitosan-thiomers has been investigated, particularly we explored the role of the alkyl-chain length of the thio-substituent.

Chitosans with different degrees of deacetylation (87.5-94.2 %) and molecular weights (25-250 KDa) have been used as starting material for the synthesis of chitosan-thioglycolic acid, -thiolactic acid, -mercaptopropionic acid, -2-methyl-3-sulfanylpropanoic acid, -mercaptobutyric acid, -mercaptohexanoic acid and -8-mercaptooctanoic acid. After the chemical characterization of all the chitosan-thiomers their antimicrobial activity was screened against four different bacterial strains, i.e. two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram positive (*Streptococcus sobrinus* and *Streptococcus mutans*) with a reliable and optimized MTT viability assay. Further microbiological tests, including minimal inhibitory and bactericidal concentration determination, disk diffusion test and plate counting were performed.

Our study showed that Gram positive strains were more affected by the treatment with the chitosan-thiomers compared to the Gram negative ones. On the other hand, Gram negative bacteria were more sensitive to chitosan-thioglycolic acid and chitosan-mercaptopropionic acid compared to the other synthesized compounds.

This study emphasizes that the functionalization of chitosan, together with the molecular weight and the degree of the deacetylation, may affect the antimicrobial activity. It is clear that a meticulous characterization is a required step for correlating the structure and the antimicrobial activity, especially for polymeric compounds.

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Peptide Shuttle System to Deliver PNAs to their Place of Action

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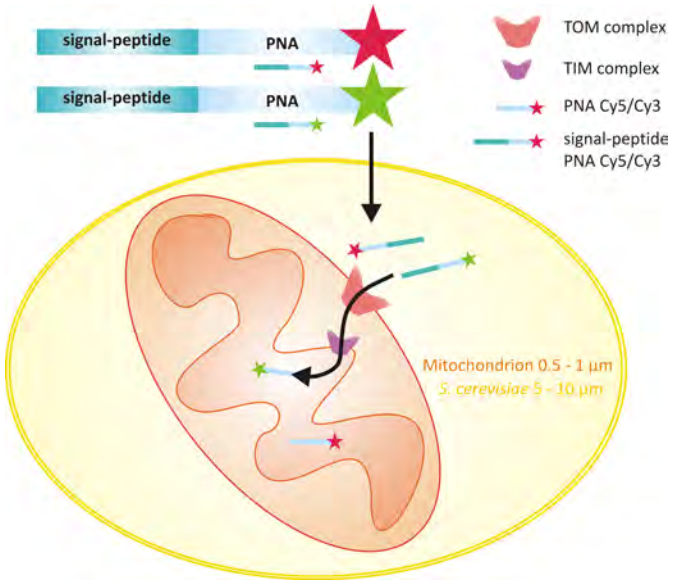
[1]University of Zurich

We are exploring a specific process during RNA maturation called splicing and trying to understand the folding mechanism of the involved RNA under native conditions. Thereby, we are applying a new labelling strategy for RNA by using hybridization probes with an unnatural backbone [1]. Peptide nucleic acids (PNAs) are an excellent alternative to DNA oligonucleotides, since they are highly specific and affine to RNA and less prone to enzymatic degradation. Towards *in vivo* splicing studies, it is essential to find an ideal shuttle system to deviler the fluorescent PNA probes to their final intracellular destination, namely to the mitochondrial matrix of *S. cerevisiae*. Hence two lipid bilayers have to be overcome, the cell membrane and the mitochondrial membrane (Figure). RNA complementary PNAs can be designed as follows: i) an N-terminal signal peptide to facilitate a directed transport into the mitochondria [2] and ii) a C-terminal fluorophore to visualize the RNA. These labels have to be actively imported through the outer (TOM) and the inner membrane channels (TIM23) of the mitochondria. After successful entry, the signal-peptide is proteolytically cleaved. We aim to use this strategy to introduce our fluorescently labelled PNA into mitochondria, to specifically target the groupIIintron and to investigate the splicing mechanism by FRET. Here we are presenting the first step towards a deeper comprehension of RNA-driven processes occurring in living cells.



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Roles of the continuous internal water pathway in G-protein-coupled receptors activations

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More than 800 human GPCRs allow the selective detection of extracellular signals as diverse as photons, odorants, flavors, nucleotides, hormones, neurotransmitters – revealing GPCRs fundamental role in signal transduction. As they regulate many central physiological processes and are thus implicated in many diseases, GPCRs are among the most important targets for modern medicines. In spite of this medical importance and the recent progress in elucidating the 3D structures of various GPCRs, central questions how these receptors recognize extracellular chemical signals and transfer them across the cellular membrane to finally evoke an intracellular response are largely unresolved at a molecular level, mainly because the different steps during signal transmission are not directly accessible by experiments. In this context we are concentrating on central questions of GPCR mediated cellular signalling using molecular dynamics simulations and structural modelling. Our work revealed for the first time, in atomic detail, the entire process of transmembrane signalling of various GPCRs: we found that ligand binding induces a series of conformational changes within a GPCR which opens a gate inside the receptor for water molecules entering the internal region of the receptor and subsequently driving conformational switches within the receptor which finally led to the activation of a G protein on the intracellular side of the receptor. The principle of uncovered internal water channels has successfully applied to the design sub-nM active compounds for two GPCRs targets.

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Molecular Mechanism of Ruthenium and Gold Anticancer Agents in the Allosteric Regulation of the Nucleosome Core Particle (NCP)

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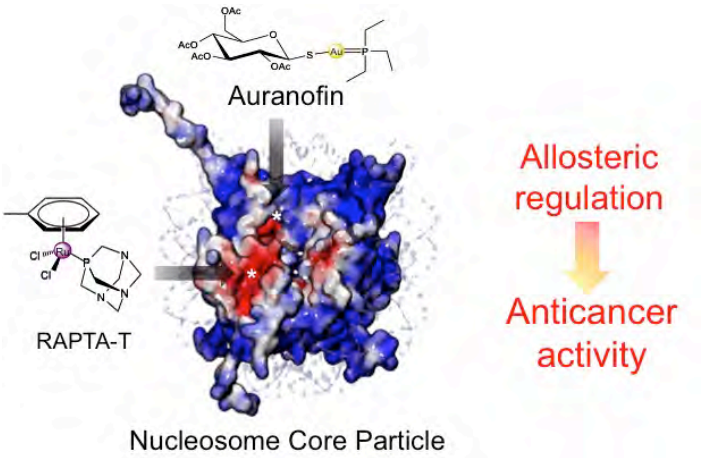
Metal-based antitumor drugs have emerged as effective chemotherapeutic agents.[1,2]In particular, Ru-based RAPTA (Ru(II), Arene, PTA = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane) compounds appear to interfere with transcription, resulting in apoptosis.[3] Besides, the gold-based compound Auranofin – a clinically approved antiarthritic drug – has shown significant antiproliferative properties in in vivo and in vitro models.[4] Here, we report the molecular mechanism of Auranofin and Rapta-T (T = Toluene) in the allosteric regulation of histone proteins of nucleosome core particles (NCP), the basic repeating unit of chromatin. High-resolution crystal structures indicate that Auranofin and Rapta-T bind at two sites of the NCP histone core that are ~35 Å apart. In spite of this, biochemical and quantitative ICP/MS experiments show a ~3 fold increased binding of Auranofin when in combination with RAPTA-T, therefore suggesting a synergistic effect of the two compounds. Integrating these experimental results with microsecond molecular dynamics (MD) simulations, we are able to propose at the atomistic level the cooperative binding mechanism of Auranofin and Rapta-T. Our results show that the allosteric communication between the ruthenium and gold sites occurs through a series of subtle conformational changes within the protein framework, which are transmitted via coupled motions of the histone components. These findings depict a novel mechanism for the allosteric regulation of the NCP. Overall, our study provides novel insights on the mechanism of action of Ru(II) and Au(I) anticancer agents, potentially leading to the discovery of new therapeutic strategies against cancer.



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Long-Lived States of Pairs of Fluorine-19 Nuclei: a new Tool for Ligand-Protein Screening

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NMR is routinely used in pharmaceutical industry for screening drug candidates by ranking their affinities for protein targets. We have exploited the enhanced contrast afforded by Long-Lived States (LLS) for drug screening [1]. Because T_{LLS} is greater than T_1, the contrast between the signal intensities of free and bound ligands is dramatically improved, which allows one to reduce the concentrations of both ligands and protein targets. LLS can be used to screen and determine the dissociation constants K_D of molecular fragments that bind weakly to protein targets. We used our LLS method for screening weakly binding fragments against Heat Shock Protein Hsp90, a target for cancer treatment. By exploiting the LLS behavior of a spy molecule, we demonstrated that it is possible to measure dissociation constants K_D as large as 12 mM [2]. This corresponds to a very weak binding regime, where most other biophysical techniques fail, including other NMR methods based on the observation of ligands. This weak binding regime is crucial for fragment-based binding studies (FBB).

More recently, we have combined LLS for drug screening with ^{1}H dissolution-DNP to enhance the sensitivity. This allows one to reduce the concentrations of ligands and the protein trypsin to 120 µM and 1.4 µM respectively [3]. We observed dramatic differences between the spectra of ligands in the presence or absence of trypsin. The presence of a stronger ligand displaces a weaker “spy” ligand, which allows one to determine the dissociation constants K_D of the strong ligand by monitoring the signals of the spy ligand. We demonstrated that it is possible to perform experiments with a very dilute 12 µM solutions of ligands, obtaining similar signal-to-noise ratios in one-shot DNP experiments as after several hours of acquisition using conventional experiments without DNP.

We are now exploring LLS involving pairs of ^{19}F nuclei to study binding phenomena. The ^{19}F spectra do not suffer from any overlapping signals. In a custom-designed fluorinated ligand that binds trypsin, we have observed T_{LLS}= 2.6 s and T_1= 0.6 s, hence a promising ratio T_{LLS}/T_1 is greater than 4. We found a dramatic effect on the LLS lifetime T_{LLS} of 800 µM of the fluorinated ligand: an 85% contrast has been observed between signals derived from T_{LLS} with/without 2µM trypsin. The titration of the fluorinated ligand against trypsin gave a dissociation constant K_D = 106 µM. This fluorinated ligand can be used as spy molecule in competition experiments, which allows one to rank the affinities of arbitrary ligands that do not contain any fluorine.

Comparison of ^{18}F-labeled alpha and gamma-conjugated folate derivatives for tumor imaging using positron emission tomography (PET)

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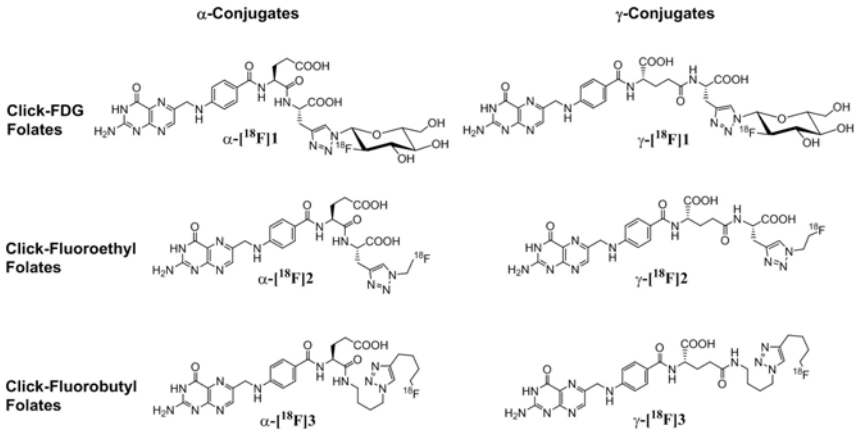
The folate receptor (FR) is upregulated in various epithelial cancer types (FRα-isoform), whereas healthy tissues show only restricted expression of FR-α. FR-targeted imaging is therefore a promising approach for the detection of FR-positive cancer tissue. Folic acid has two carboxylic functionalities at the alpha (α) and gamma (γ) positions which are accessible for chemical modification. We present the (radio)syntheses, biological evaluation and comparison of three pairs of fluorine-18 labeled α- and γ-conjugated folate derivatives.



Click chemistry was used for the syntheses of the three different pairs of folate regioisomers (α/γ-FDG-, α/γ-fluoroethyl- and α/γ-fluorobutyl-folates). All synthesized folate regioisomers exhibited nanomolar binding affinities to the FR (IC_{50} values in the range of 1 to 3nM) which were determined using FR-expressing KB tumor cells. PET and biodistribution studies revealed similar KB-tumor uptake for both the α-regioisomers and their corresponding γ-isomers of all three pairs of folate-conjugates (α-[^{18}F]**1**: 10.9 ± 0.52% ID/g, γ-[^{18}F]**1**: 9.05 ± 2.12% ID/g; α-[^{18}F]**2**: 12.5 ± 1.04% ID/g, γ-[^{18}F]**2**: 7.24 ± 0.99% ID/g; α-[^{18}F]**3**: 3.73 ± 0.62% ID/g, γ-[^{18}F]**3**: 3.22 ± 1.05% ID/g). In contrast, liver uptake was more than two times higher for the γ-conjugated derivatives compared to their corresponding α-derivatives (α-[^{18}F]**1**: 3.01 ± 0.48% ID/g, γ-[^{18}F]**1**: 8.37 ± 1.19% ID/g; α-[^{18}F]**2**: 2.77 ± 0.57% ID/g, γ-[^{18}F]**2**: 8.66 ± 1.19% ID/g; α-[^{18}F]**3**: 0.90 ± 0.22% ID/g, γ-[^{18}F]**3**: 2.25 ± 0.15% ID/g). A significant difference between the α- and γ-regioisomers was also observed for the kidney uptake values, whereby the γ-regioisomers showed a 50% lower uptake compared to their α-analogues. These results clearly demonstrated that the site of conjugation on the glutamyl moiety of folic acid has a significant impact on the *in vivo* behavior of radiofolates but not on their *in vitro* FR-binding affinity.

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Human Biomonitoring Bridging Gaps between Medicine and Environment

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Keywords: environmental quality, human health, human biomonitoring.

Bringing the two schools of clinical medicine and environmental sciences is always challenging. Elevated and escalated diseases related to the deterioration of environmental quality call for some research areas in between. These areas can address the roots of the environmental problems (identification of contaminants, natural and anthropogenic contribution, new sensitive and selective analytical tools, contaminant Chemodynamics, transfer to the food chain, etc.) and occurrence in the human body showing short or long term symptoms up to the gene or molecular levels. Human biomonitoring (HBM) is a very powerful tool on exposure sciences. Briefly, HBM is defined as the measurement of concentrations of chemicals or their metabolites in human biological samples such as blood, serum, urine, saliva, breast milk, nails, hair or body parts and tissues. It includes Environmental Health Surveillance and Environmental Health Tracking.

The complexity of environment and the emerging contaminants should be associated with dynamic tools for monitoring. Theory (modeling, computation and simulation) is not driven by or linked to the experimental work and coordination is absent or minimum. A very strong roadmap should be known to bridge the area of environmental deterioration and human health. Qualified and certified national laboratories and competent staff are needed to target ultra-trace inorganic and organic contaminants from sources to fate. Contaminant speciation, metabolites, isotopes and derivatives are examples. The presentation covers new meanings of environmental monitoring and highlits risks asscoaited with contaminants.

Palladium-mediated Suzuki-Miyaura coupling: an efficient method for the formation of therapeutically relevant protein conjugates

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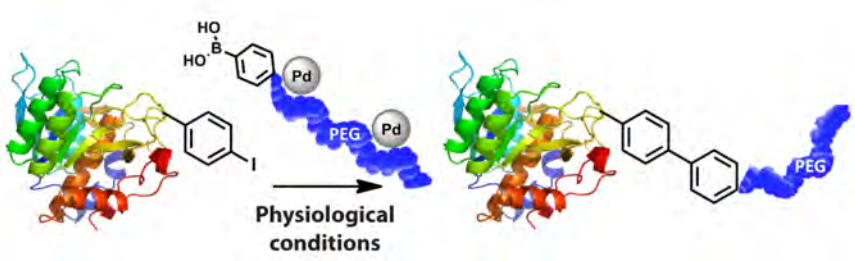
[1]Université Paris-Sud, [2]University of Oxford

Chemical protein modification is a powerful tool for probing natural systems structure and function and generating protein conjugates with new or improved properties.[1] Owing to their broad functional group tolerance and biological compatibility, palladium-catalyzed reactions targeting unnatural amino acids emerged as attractive strategies for site-specific protein modification.[2]

The efficiency and robustness of these reactions was recently demonstrated for the PEGylation of proteins, a widespread approach to improve the stability and pharmacokinetic properties of protein drugs. The development of palladium-mediated *Suzuki-Miyaura* coupling at genetically encoded or chemically installed halogenated amino acids enabled the efficient synthesis of structurally defined protein-PEG conjugates under physiological conditions.[3] Thanks to the liganding properties of the PEGylated boronic acids involved, this biotechnologically relevant reaction proceeds in a “self-liganded” manner, using the simplest palladium salts as catalyst, without the need for exogenous ligands.[3]

Given the biorthogonality and the high specificity of the *Suzuki-Miyaura* reaction as well as the remarkable properties of palladium in biological settings, and its low associated toxicity, exciting possibilities emerge for the application of this reaction to more complex biological systems.[4]



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Enzyme catalyzed sulfur-carbon bond formation byergothioneine biosynthetic sulfoxide synthase

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[1]University of Basel

EgtBs form a class of non-heme iron enzymes, which catalyze oxygen-dependent sulfur-carbon bond formation between low molecular weight thiols and *N*-α-trimethyl histidine as the central step in ergothioneine biosynthesis (Fig. 1). The crystal structure of EgtB from *Mycobacterium thermoresistibile* in complex with gamma-glutamylcysteine and *N*-α-trimethyl histidine implicate both substrates and three histidine residues as ligands in an octahedral iron binding site. In the secondary coordination sphere we identified a tyrosine residue which may serve as a hydrogen-bond or proton donor to an iron (III)-superoxo or -peroxo species. Mutation of this residue to phenylalanine produced a variant with 300-fold reduced sulfoxide synthase activity. Instead, this protein catalyzes thiol dioxygenation with an efficacy that rivals naturally evolved cysteine dioxygenases (CDO). Comparison of the EgtB and the CDO structures reveals two very similar active sites within two entirely unrelated protein folds. EgtB likely forms the same iron (III)-superoxo intermediate as described for CDO, but strategic placement of a tyrosine residue as catalytic acid promotes sulfur-carbon bond formation and prohibits thiol dioxygenation.

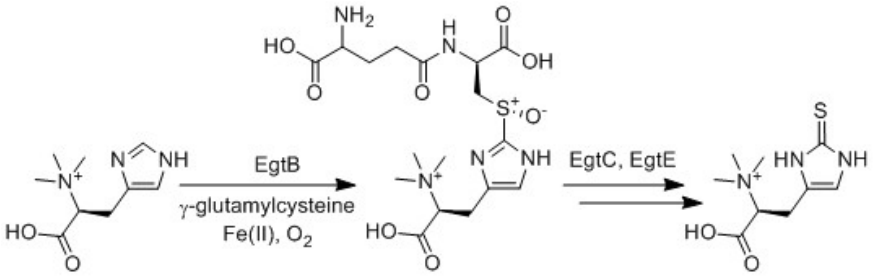

Figure 1. Biosynthesis of ergothioneine in *M. thermoresistible* via sulfoxide intermediate.

Electrostatic Effect Of Halogenation On The Thermodynamic Stability Of Rapid Insulin Analogs

K. El Hage[1], M. A. Weiss[2], M. Meuwly[1]*

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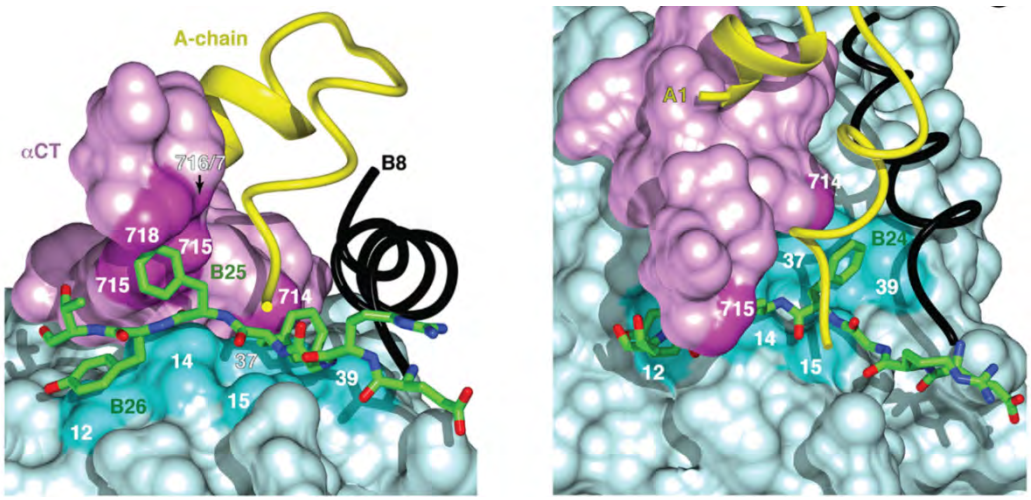
Insulin analogs are designed containing amino acid substitutions at or near the classical dimerization surfaces of the zinc insulin hexamer [1,2]. One of these amino acids is Phe^{B24}, an invariant aromatic anchor at this interface and site of a human mutation causing diabetes mellitus *(See figure below [3])*. A recent study showed that a nonstandard aliphatic mutagenesis at this position can optimize the molecular properties of therapeutic proteins and lead to accelerated hexamer disassembly while preserving native receptor binding affinity and biological activity [3].These findings motivated us to investigate further substitutions by halogenated aromatic rings. The effect of these mutations on the conformational ensemble and on the differential solvation free energy is evaluated using thermodynamic integration with the CHARMM force field and results are compared to experimental data. In addition, we used the multipolar module [4] that allows us to study the critical dependence of the halogen-induced electrostatic dipole on the position of the halogenation (ortho, meta or para) and its role in adding solvation free energy changes. Preliminary results show, that in addition to local chain stabilization, a global thermodynamic stabilization of 1 kcal/mole takes place reflecting enhanced enthalpic interactions via weakly polar interactions by the modified aromatic ring and a more favorable solvation free energy.



Since insulin provides a model for the therapeutic application of protein engineering, the resulting differential effects of these modifications can help provide a molecular strategy to augment protein stability while preserving rapid action.

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Preventing aggregation of porphyrinic photosensitizers using a biodegradable triblock copolymer

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Photodynamic therapy (**PDT**) is a form of phototherapy using nontoxic light-sensitive compounds that are exposed selectively to light, upon which they become toxic to targeted malignant cells (phototoxicity). Over the last few decades porphyrins have gained much interest as promising photosensitizers but their intrinsic hydrophobicity and tendency to aggregate in aqueous solution represent limiting factors which decrease their PDT efficacy. One approach to overcome these problems is the use of drug delivery vehicles [1]. Among those vehicles, some polymers like polyvinylpyrrolidone (PVP) have received much attention, notably because they are nontoxic as well as soluble in water. It was also shown that the biological activity and phototoxicity of porphyrins can be enhanced by attaching an amino acid to form porphyrin amino acid conjugates [2].
The aim of this study was to probe the propensity of the biodegradable triblock copolymer Kolliphor P188 to disaggregate various chlorin e6 amino acid conjugates (xCE) [3].
The interaction and loading between the copolymer Kolliphor P188 and the xCE conjugates was probed using NMR spectroscopy as a main tool. The results indicate that Kolliphor P188 is able to monomerize all xCE conjugates in aqueous buffer at room temperature. NMR diffusion data suggest that the loading depends on the structure of the amino acid residue.

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Why are Vesicles of the Artificial 1,3-Diamidophosholipid Pad-PC-Pad Mechanosensitive?

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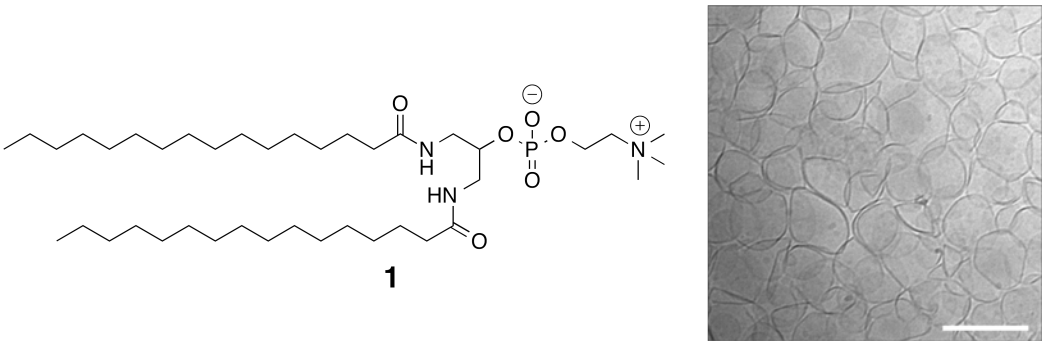
We have shown that vesicles of the artificial phospholipid Pad-PC-Pad (**1**) are lenticularly shaped and mechanosensitive.[1]Temperature, ultrasound, enzymes, pH-changes, redox-reactions or photo-reactions are all well known as trigger for cargo release from phospholipid liposomes.[2]On the other hand mechanosensitvity is a fresh concept in targeted drug delivery. If vesicles of **1** are exposed to increased shear-stress they release their content. The change in shear stress in blocked arteries could be used as a trigger for targeted drug delivery for the acute treatment of a myocardial infarction patient.



1

Figure 1: left: chemical structure of the 1,3-diamidophospholipid Pad-PC-Pad (**1**), right: cryo transmission electron micrograph of extruded unilamellar Pad-PC-Pad vesicles, the scale bar is 200 nm.

Mechanosensitiviy is based on the unnatural 1,3-fatty acyl chain substitution pattern found in **1**which allows a tail interdigitation of the opposing membrane leaflets. The interdigitation increases the lateral stiffness of the bilayer in a vesicle and rapid cooling from temperatures above T_m leads to the formation of lenticularly shaped vesicles. To proof this hypothesis and to gain a deeper understanding of the mechanosensitivity of phospholipid vesicles we synthesized different artificial lipids showing the natural 1,2- or the unnatural 1,3-fatty acyl chain substitution pattern each with and without the ability to form hydrogen bonds. Preliminary results indicate that hydrogen bonding is less important for mechanosensitivity than the 1,3-substitution pattern in Pad-PC-Pad.

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Addressing the tempormandibular joint disorder

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The temporomandibular joints (TMJ) are the joints connecting the lower jaw (mandible) to the skull and regulating the action of the mandible. Temporomandibular joint disorder (TMD) is a generic term designating the dysfunction of the TMJ. TMD is caused by a broad range of causes such as injuries of the jaw or the TMJ, disc displacement, osteoarthritis, reumathoid arthritis, teeth grinding. The current treatment ranges from physical therapy to surgical replacement of the joint, with the typical treatment being the administration of a painkiller with all the known side effects.[1]

The work presented here aims at fulfilling the need for a mid- to long-term alternative to the parental or oral administration of a painkiller. The idea is to design and study an *in situ* crosslinking hydrogel containing drug loaded liposomes for a long-term and on demand drug delivery system. Periodate oxidized dextran is reacted with amine bearing liposomes and polyethylenimine in order to form a hydrogel. The chemistry and biophysical properties of this gel will be presented.

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NMR investigation of the human RNA BCL2 G-quadruplex: restricting folding dynamics

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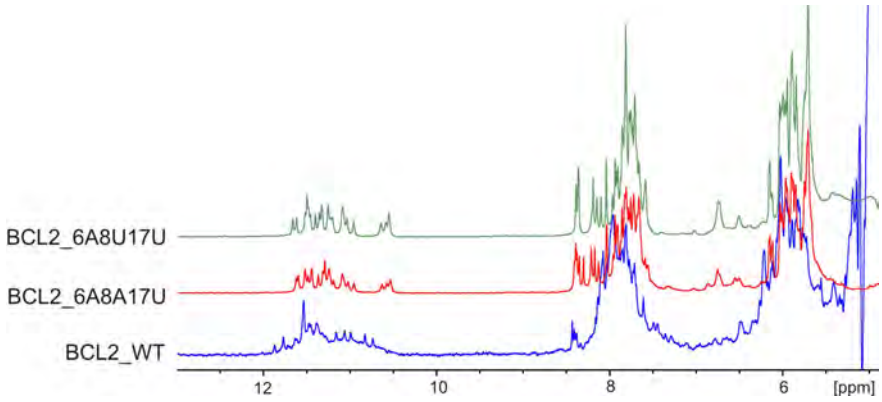
A 25 nucleotide guanine rich sequence (5'-$G_5CCGUG_4UG_3AGCUG_4$-3') was found in the 5'-unstranslated region of the mRNA of the human BCL2 proto-oncogene (B-Cell Lymphoma/Leukemia-2). This sequence is prone to fold into a specific non-canonical secondary structure, a so-called G-quadruplex (G4). Interestingly, the presence of this G4 structure has shown to down-regulate the expression of the bcl2 protein *in cellulo* [1]. The BCL2 family of proteins play a crucial role in the regulation of apoptosis [2]. For instance an aberrant expression of the bcl2 protein has been isolated in several human cancers. Therefore, the understanding of this biologically relevant RNA G4 sequence is of high interest for anticancer therapy.

1D ^{1}H-NMR spectra of the 25-nt BCL2 G4-forming sequence in the presence of K(I) ions revealed the formation of multiple G4 structures. To reduce the dynamics of the system, the sequence was shortened to yield a more restricted 22-nt sequence (5'-$G_3CCGUG_4UG_3AGCUG_3$-3'). Our results showed the stabilization of mainly one G4 structure upon the addition of K(I), defined as unimolecular, intramolecular and parallel-stranded. However, additional NMR data evidenced the presence of two different but non-equally populated species in solution. This fact hampers NMR investigations. Thus, further stabilization of the 22-nt BCL2 construct was performed by mutational studies. The aim is to isolate one single G4 conformation, which allows us to determine the solution structure of this G-quadruplex by NMR. The triple mutated sequences BCL2_6A8U17U and BCL2_6A8A17U successfully stabilize one single G-quadruplex structure with different loop isomers. Preliminary NMR data shows promising results regarding the structural elucidation of the BCL2 G-quadruplex.



Financial support by the Fundacion Ramon Areces and Forschungskredit (A.D.M.), the Swiss National Science Foundation (R.K.O.S.), the COST Action CM1105 (Swiss State Secr. Edu. Res. Innov. to RKOS) and by the University of Zurich is gratefully acknowledged.

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Metal ion dependency and multimerization behavior of biologically relevant human RNA G-quadruplexes

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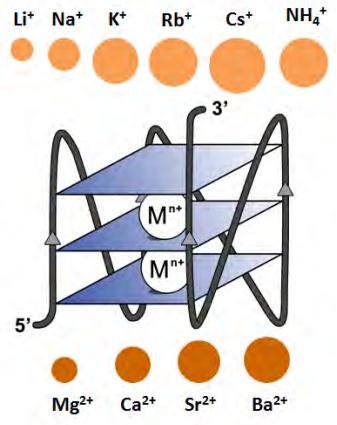
Guanine-rich nucleic acids fold into non-canonical helical structures, called G-quadruplexes (G4), and formed by stacking of guanine planes. RNA G4s appear in regulatory regions of the transcriptome, suggesting they play a role *in vivo*, and indeed their presence in human cells was proven [1]. We study a sequence from the 5' UTR of the NRAS oncogene mRNA (5'-$G_3AG_4CG_3UCUG_3$-3'), shown to inhibit translation *in vitro* and to be accessible for antitumor targeting [2, 3].

G4 motifs need charge-balancing metal ions to fold and remain stable.We investigated the influence of the metal ion type and concentration on G4 folding by following two G4 sequences with several mono- and divalent metal ions (see figure): the NRAS and the telomeric-repeat containing RNA (TERRA, 5'-$(UUAG_3)_4$), the most studied G4 RNA so far [4].

Circular dichroism showed that Na^+, K^+, Sr^{2+} and Ba^{2+} promote G4 folding, while only K^+ and Sr^{2+} yield further stabilized G4s, as seen by a significant increase in melting temperatures. Further addition of K^+ and Sr^{2+} leads to unspecific interactions and cation-mediated multimers, evidenced by polyacrylamide gel electrophoresis, dynamic light scattering and mass spectrometry. Comparison with published DNA G4s shows G4 RNAs to be more stable, more prone to multimerize and less Na^+-sensitive. These studies delve into the under-studied RNA G4 motifs and their folding behavior, relevant towards possible functions *in vivo*.



Financial support by the COST Action CM1105, an ERC Starting Grant 2010 (R.K.O.S.), the SNSF (R.K.O.S.), a UZH Forschungskredit (FK-13-090 to H.G.M.) and by the UZH is gratefully acknowledged. We are grateful to Mr. A. Marchand, Dr. F. Rosu and Dr. V. Gabelica (Univ. of Bordeaux) for MS measurements, and Dr. B. Klejevskaja and Prof. R. Vilar (Imperial College London) for collaboration regarding PAGE gels.

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Structure - antimicrobial activity relationships of chitosan-alkyl thiomers

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[1]University of Zurich

Chitosan is a biopolymer consisting of N-acetyl-D-glucosamine and β-1,4-linked-D-glucosamine units with wide potential for clinical applications[1] mainly based on its antimicrobial properties. Recently, a new versatile antimicrobial agent has been synthesized by Geisberger at al. [2], functionalizing chitosan with thioglycolic acid through amide bond formation. In this study, the relationship between the structure and the antimicrobial activity has been explored synthetizing chitosan-alkyl thiomers with different alkyl-chain length.

Chitosans with different degrees of deacetylation (87.5-94.2 %) and molecular weights (25-250 KDa) have been functionalized with thiol-bearing compounds adapting the literature protocols[3] to achieve different degrees of substitution. The series encompasses chitosan-thioglycolic acid, -thiolactic acid, -mercaptopropionic acid, -2-methyl-3-sulfanylpropanoic acid, -mercaptobutyric acid, -mercaptohexanoic acid and -8-mercaptooctanoic acid. All the thiomers were characterized by the following techniques: ^{1}H-NMR, FT-IR, Ellman test, GPC, elemental analysis and ζ-potential measurements. The antimicrobial activity of the title compounds was screened against four different bacterial strains, namely two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram positive (*Streptococcus sobrinus* and *Streptococcus mutans*) with a reliable and optimized MTT assay. For the most promising compounds further microbiological studies (minimal inhibitory and bactericidal concentration determination, disk diffusion test and plate counting) were performed.

Chitosan-thiomers showed higher antimicrobial activity against Gram positive strains compared to the Gram negative ones. Besides, Gram negative strains were more affected by chitosan-thioglycolic acid and chitosan-mercaptopropionic acid compared to the other thiomers.

This study gives good evidences that antimicrobial activity may be influenced by the functionalization, together with the molecular weight and the degree of the deacetylation. Moreover, an accurate characterization of the new polymers is required to establish systematic structure-antimicrobial activity relationship.

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Real-time Characterisation of a Large, Catalytic and Dynamic RNA by Single Molecule Microscopy.

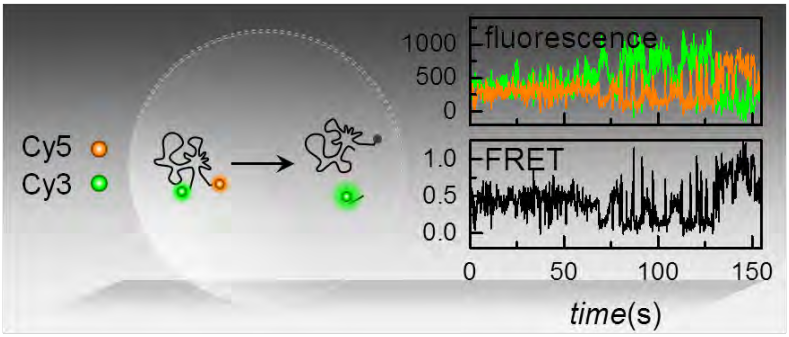
M. C. Hadzic[1], S. Zelger-Paulus[1], R. K. O. Sigel[1]*

[1]University of Zurich

Group II introns are large natural RNA sequences able to self-excise from the nucleic acid strand via a process called *splicing*, allowing the molecule to fold into a functional conformation. Although tertiary structures and autocatalytic properties of engineered constructs have been unveiled along with bulk and single molecule techniques [1,2], there is little knowledge available about the correlation of splicing events with conformational dynamics, which renders this mechanistic picture incomplete. The present study intends to lift the veil on these folding choreographies and on their role in the reaction efficiency.

The ai5γ group IIB intron from the yeast *Saccharomyces cerevisiae* flanked by its original 45 nt 5'-exon was fluorophore-tagged on two sites according to different labelling schemes [3]. Single molecules were trapped into small and surface-immobilized phospholipid vesicles, affording to track the 5' cleavage reaction and its products as well as conformational changes by following the distance-dependant energy transfer (smFRET) from the donor (Cy3) to the acceptor (Cy5) fluorophore.

By means of electrophoresis, the splicing reaction was proven to be inhibited at room temperature under ideal salt concentrations but spontaneously triggered by temperature increase to 40 °C, later extended to sm-FRET investigations. Monoencapsulation and subsequent imagining of large fluorophore-bilabelled systems was successful. Data were evaluated with our recently developed and freely available graphical user interface, allowing complete data evaluation [4]. Our sm-FRET results reveal detailed insights in streaming a biochemical process at the single molecule level, by correlating conformational motion with biological activity.



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Pore size matters - A crowding study of ribozyme folding and activity

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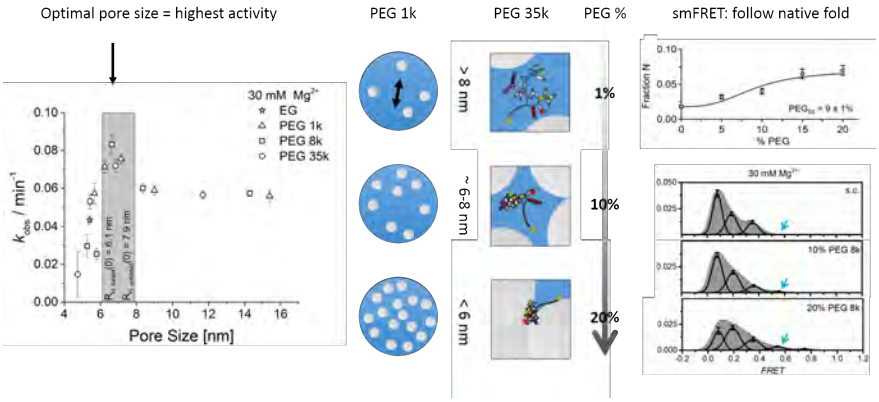
Catalytic RNAs, like the group IIB intron ribozyme of *S. cerevesiae*, are known to require a high magnesium(II) concentration to show folding and function *in vitro* [1]. In contrast, *in vivo* conditions are characterized by a highly crowded cellular environment and much lower ion concentration. Nowadays, molecular crowding agents like poly(ethylene)glycol (PEG), are a widespread tool to mimic cellular crowding and thus, to reach near physiological conditions [2]. However, particular physical and/or chemical properties explaining the crowders influence are mostly not understood. In this study, we gain new insights on how polymer properties like viscosity, pore size etc. influence the activity and folding of a large RNA.
We combined bulk activity assays and single-molecule Förster Resonance Energy Transfer (smFRET) experiments to investigate the influence of a crowding agent on group II intron folding. A screening of PEG volume fraction (%) and molecular weight (MW) was further used to elucidate the impact of different polymer properties on the ribozyme activity.
Our results revealed that upon the influence of a crowding agent, a compaction of the underlying RNA structure is dependent on the volume fraction of the crowding co-solute. Activity assays in the presence of different PEG MW and % unveiled an optimal pore size in terms of catalytic activity, which coincides with the dimension of the folded ribozyme. In summary, an increasing density of the crowding environment shifts the RNA conformations towards the most compact state, but the ribozyme is only active if the crowders network matches its size [4]. We therefore interpret the most compact state as necessary, but not sufficient, to keep the ribozyme active.



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The binding mechanism between a B_{12}-specific RNA and its ligand coenzyme B_{12}

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A remarkable characteristic of riboswitches, short RNA elements involved in gene regulation, is their ability to interact directly and specifically with their ligand, mostly being a cellular metabolite. In this context, the interaction between the 202 nt long *btuB* riboswitch with its ligand coenzyme B_{12} (AdoCbl) is highly interesting due to the complexity of both binding partners.[1, 2]

We present here a comprehensive thermodynamic and kinetic study of the *btuB*-AdoCbl binding mechanism combining extensive isothermal titration calorimetry (ITC) experiments with surface plasmon resonance (SPR) and dynamic light scattering (DLS). The ITC data retrieved from measurements at different conditions were assembled to a global thermodynamic treatment to generate accurate K_D and ΔH-values. Our data propose the existence of two distinct *btuB* species, both binding AdoCbl with high affinity, and their ratio being temperature dependent. This finding was confirmed by SPR. DLS measurements and gel-shift assays conducted at various temperatures complete this study as complementary methods. Based on our data we propose a complex binding mechanism involving a preorganization of the *btuB* riboswitch into two distinct binding-competent forms [3], however similar in their overall, global structure.

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Hydrogels with short peptides and their composites with metal nanoparticles

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For centuries it is known that silver possesses antimicrobial properties and it was regularly used for the treatment of burns, wounds and several bacterial infections. But with the emergence of antibiotics it was nearly forgotten for almost 50 years[1,2].
Nowadays, due to the unique physical and chemical properties of silver nanoparticles and the rising concern regarding infectious diseases induced by multidrug-resistant bacteria, metallic silver has made a remarkable comeback as a potential antimicrobial agent[2].
Hereby, we propose a silver nanoparticle hydrogel system which can be used for medical purposes against multidrug-resistant bacteria.
Peptide self-assembled hydrogels are a promising class of soft biomaterials for cell culture, regenerative medicine, or drug delivery applications[3].
These low molecular weight compounds assemble by weak non-covalent interactions and are responsive to different physicochemical stimuli[4].
In our case the hydrogel system (see Figure 1) itself can synthesize silver nanoparticles due to its hydrazide end group.
The aim is therefore to synthesize the dipeptide hydrogel Boc(βAla)$_2$NHNH$_2$ and to analyze its chemical, physical and mechanical characteristics with or without the incorporation of silver and to test its suitability for biological applications.

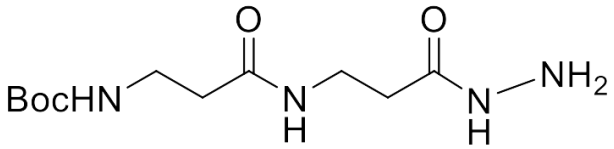

Figure 1: The dipeptide Boc(βAla)$_2$NHNH$_2$

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SCS Division of Medicinal Chemistry: What does the DMCCB do?

Y. Auberson[1,2]

[1]President of the Division of Medicinal Chemistry and Chemical Biology, [2]Novartis Pharma AG

The DMCCB comprises about 750 scientists interested in medicinal chemistry, chemical biology and related fields of research. It organizes scientific events such as the Basel mini-symposiums, the Medicinal Chemistry and Chemical Biology session of the SCS annual meeting, and every second year co-organizes the Frontiers in Medicinal Chemistry congress together with the German GDCh. It also offers a high-quality, bi-annual Medicinal Chemistry Course which takes place in Leysin. Beyond Switzerland, the DMCCB interacts with other country organizations to represent the swiss medicinal chemistry and chemical biology community.

Objectives
The aims of the DMCCB are

- to foster a worldwide network of medicinal chemists, chemical biologists and scientists working in related fields
- to facilitate contacts with leading experts in our field
- to organize symposia, seminars and advanced training courses
- to network and encourage the exchange of ideas

DMCCB is a member of the European Federation of Medicinal Chemistry (EFMC)

Scope

- Dedicated scientific sessions at the SCS Fall Meetings
- A bi-annual minisymposium on a cutting edge topic
- Joint conferences with the medicinal chemistry and chemical biology divisions of chemical societies in neighboring countries
- The bi-annual Swiss Course on Medicinal Chemistry, held in a superb Alpine setting. SCS members benefit from reduced fees.

Upcoming Events

- FMC 2015 - Frontiers in Medicinal Chemistry
  Sep 14-16, 2015,Antwerp, Belgium
- The expanding Toolbox of Medicinal Chemistry;From Chemical Biology to Clinical Applications
  October 16, 2015,Parc des expositions et congres, Dijon, France
- Lift Basel Conference 2015
  October 29-30, 2015,MarkthalleBasel
- Changing Paradigms in Drug Development
  February 3, March 24, May 27, September 15 and November 17, 2015, Bern

Website: http://scg.ch/dmccb

Discovery and optimisation of a CREBBP Bromodomain ligand.

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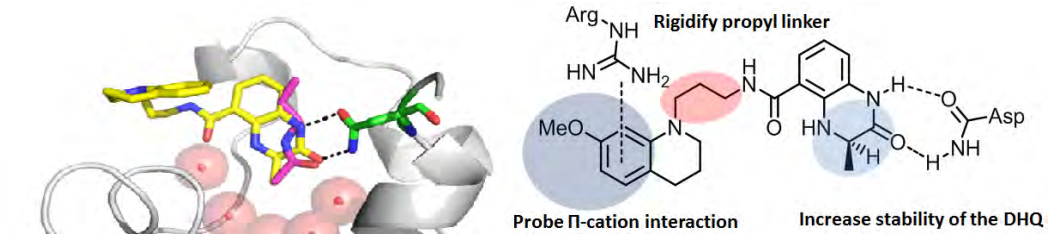
Epigenetic transfer of information is linked to dynamic DNA methylation and post-translational modifications on histones, the small, positively-charged proteins, around which DNA is stored. Specific combinations of these marks, and their crosstalk, can mediate context-dependent effects on transcription, a concept known as the “histone code”. Bromodomains are acetyl-lysine “readers” of the histone code, which interact with acetylated lysine residues. This protein-protein interaction results in changes in gene expression levels and leads to specific downstream effects. [1-3]

The human bromodomain family consists of 61unique proteins, which are divided into eight different subfamilies, according to sequence similarity. Most research has been focused on the bromodomain and extra C- terminal (BET) family, which has yielded probe compounds such as I-BET762, currently in clinical trials. Only a few ligands have been reported for other bromodomain families than BET. Herein we report the discovery [4] and structure-guided development of potent, CREBBP inhibitors. We have focused on the rigidification of the linker between an acetyl lysine mimic and its tail, which fits in an induced cation-π pocket. This cation-π interaction was probed with different electronics of the π-system and showed correlation between the electronic properties and the measured potency. The stability of the acetyl-lysine mimic moiety was also considered in this most recent work by modification of the dihydroquinoline system.



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LC-MS/MS for determination of brain uptake and target mediated differential PK of PDE10A PET tracer candidates

G. Luca[1], M. Honer[1], R. Alvarez Sanchez[1], C. Flament[1], A. Flohr[1], H. Schaffhauser[1], D. Muri[1], T. Hartung[1], E. Borroni[1]

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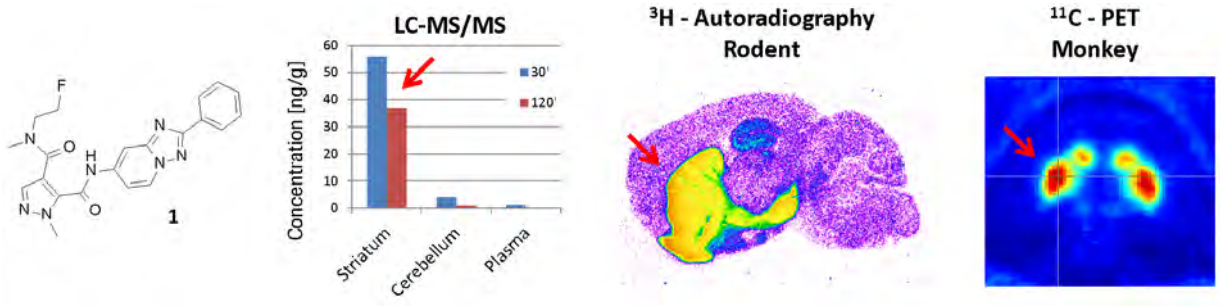
Positron emission tomography (PET) is a non-invasive imaging technique routinely used to support the development of novel drugs to treat neurological and psychiatric disorders. A prerequisite for any PET study is the availability of a radiotracer for the biological target of interest.

At the onset of an internal program to identify inhibitors for the phosphodiesterase PDE10A, no such PET tracer was accessible. We thus started a discovery program, to ensure a validated PET tracer would be available in time for the early development phases of the drug molecule. A LC-MS/MS bio-analytical method [1] was used to rapidly profile 38 unlabeled high affinity PDE10A inhibitors. Rats were intravenously injected with a 'tracer dose' of 10µg/kg of unlabeled compound and samples of plasma, striatum (high PDE10A expression) and cerebellum (low PDE10A expression) were collected after 30 and 120 min. This method allowed identification of tracer candidates with i) high brain uptake, ii) favorable target mediated differential PK (striatum *vs.* cerebellum concentration ratio) and iii) appropriate washout kinetics within the typical time-window of a PET scan (*i.e.* concentration ratio at 120 and 30 min below 1).

Promising candidate structures were radiolabeled with tritium and carbon-11 or fluorine-18 and profiled in autoradiography experiments in rodents as well as PET studies in the non-human primate. A retrospective analysis of the results confirmed good agreement between the LC-MS/MS results and the outcome of the autoradiography and PET experiments.

The LC-MS/MS method was further used to assess and exclude the presence of brain penetrant radiometabolites for the advanced tracer candidate [^{11}C]RO5548119 (**1**).

This work finally lead to the discovery of [^{11}C]RO5548119 as a PET tracer for the visualization and quantification of PDE10A in rodents and non-human primates.



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Synthetic Nucleotides Reduce Human DNA Polymerase η-mediated Synthesis Over a Cisplatin DNA Cross-link Adduct

A. Nilforoushan[1], A. Furrer[2], L. Wyss[1], B. Van Loon[2], S. Sturla[1]*

[1]ETH Zurich, [2]University of Zurich

DNA cross-link adducts from the chemotherapeutic drug cisplatin block replicative DNA polymerases and induce apoptosis. However, these adducts are bypassed by human DNA polymerase η (hPol η) in the process of translesion DNA synthesis. Therefore, inhibiting hPol η has been suggested as a strategy for improving the efficacy of platinum drugs. We hypothesized that synthetic nucleotides with a propensity to be inserted opposite a DNA adduct but block extension could impede DNA synthesis past the platinum cross-link adduct. In this study, we characterized the influence of synthetic nucleoside triphosphates on hPol η-mediated DNA synthesis. An over ten-fold variation in the incorporation efficiency was observed when varying the hydrogen bonding potential of the synthetic base. Comparing reaction rates with molecular models suggested that incorporation appeared to be promoted by the potential to form an additional hydrogen bond between templating base and incoming dNTP. Additionally, increasing concentrations of synthetic nucleotides reduced the amount of DNA product independent of the template under full-length DNA synthesis conditions. This process is a unique example of inhibiting the progress of hPol η on a platinated DNA template. These data indicate how chemical changes to nucleotide triphosphates impact platinum-DNA adduct bypass by hPol η[1].

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Discovery of G Protein-Coupled Bile Acid Receptor 1 (GPBAR1, TGR5) Agonists as Antiinflammatory Agents

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[1]Novartis Pharma AG

Many inflammatory mediated diseases can be treated today, yet for many of them there is still an unmet need for alternative therapies that offer a benefit in terms of overall efficacy, ease of administration and an improved side-effect profile. To this end, significant research is being conducted towards identifying and dissecting selective biological pathways that are key drivers for human disease.

GPBAR1 (also known as TGR5) is a G protein-coupled receptor (GPCR), which triggers intracellular signals upon ligation by various bile acids. The receptor has been studied mainly for its function in energy expenditure and glucose homeostasis, and there is little information on the role of GPBAR1 in the context of inflammation. After a high-throughput screening campaign, we identified a series of isonicotinamides as non-steroidal GPBAR1 agonists. We optimized this series to potent derivatives that are active on both human and murine GPBAR1. These agonists inhibited the secretion of the pro-inflammatory cytokines TNF-alpha and IL-12, but not the anti-inflammatory IL-10 in primary human monocytes. These effects translate in vivo, and we show with one compound that LPS induced TNF-alpha and IL-12 release in mice is inhibited. The response was GPBAR1 dependent, as demonstrated using knockout mice. Furthermore, agonism of GPBAR1 stabilized the phenotype of the alternative, non-inflammatory, M2-like type cells during differentiation of monocytes into macrophages.

Overall, our results illustrate an important regulatory role for GPBAR1 agonists as controllers of inflammation and highlight the potential benefit of GPBAR1 agonists for the treatment of Th1 driven autoimmune diseases.

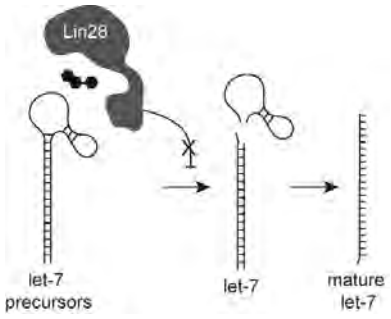
From pre-miRNA labeling to the identification of small molecule modulators of microRNA biogenesis: application to the cancer associated pre-let-7/lin28 interaction.

U. Pradere[1], M. Roos[1], H. Towbin[1], J. Scheuermann[1], D. Neri[1], C. Ciaudo[1], J. Hall[1]*

[1]ETH Zurich

MiRNA are a large class of non-coding regulatory RNAs repressing the expression of target messenger RNAs.[1] Post-transcriptional regulation of miRNA biogenesis by RNA Binding Proteins (RBPs) such as Lin28, hnRNP A1, Smad, KHSRP and p53 binding miRNA precursors (pri- and pre-miRNA) is increasingly recognized as an important element controlling miRNA maturation.[2] Malfunctioning of this mechanism causes miRNA misexpression which is significant in some human cancers, and therefore represents a promising novel target for drug development.

We present as part of an on-going program addressing RNA drugability, the development of a novel, carefully optimized Fluorescence Resonance Energy Transfer (FRET) based method which allowed the identification of small molecule inhibitors of the let-7 – Lin28 interaction[3] involved in cancer. A flexible labeling strategy of pre-miRNAs[4] allowed the preparation of multiple RNA FRET acceptors. By varying the position on the pre-miRNA, the number (mono-, bis-) and the nature (Cy3, BHQ-1) of the chromophore acceptor, significant improvement of the energy transfer led to a prominent FRET window. Assay miniaturization to 384 wells format allowed the screen of a library of 16000 small molecule compounds. From the 14 best hits, follow-up cellular assays including RT-qPCR and luciferase reporter assay identified one compound as a potent inhibitor of the pre-miRNA – RBP interaction able to restore let-7 levels in cancer cells. Mechanistic investigation using a biotinylated derivative revealed it as protein-targeting antagonist. Treatment of stem cells with the compound led to a change in morphology of murine embryonic stem cells consistent with its proposed mechanism of action.



We believe this approach will be broadly applicable to other RNA – RBP partners involved in (patho)physiological mechanisms offering the opportunity to identify inhibitors of such interactions. Moreover, compounds emerging from such screens represent valuable tools for further biological investigation of the target and possible lead structures for future drug development.

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Fluoroquinolone containing inhibitors of bacterial topoisomerases with a novel mode of action

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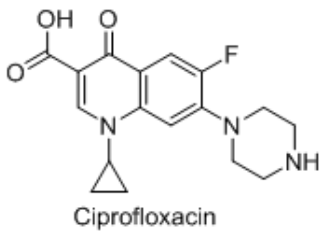
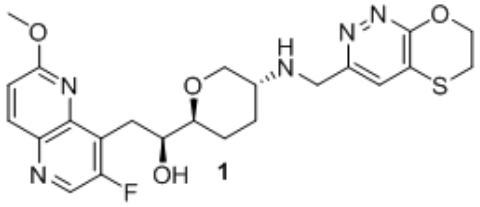
The bacterial topoisomerases Gyrase and TopoisomeraseIV are well validated targets in antibiotic research and discovery. The Fluoroquinolones (FQ, eg. Ciprofloxacin) are potent inhibitors of these targets and are an important weapon in the battle against infections. Unfortunately their utility is lately being limited due to emerging resistance. Over the last years several companies have discovered molecules that inhibit topoisomerases by a novel mode of action and are therefore devoid of cross-resistance with clinically used antibiotics. Especially the *novel bacterial topoisomerase inhibitors* (NBTI) such as **1** have shown promising properties.[1]

We report here the synthesis of compounds (**A**) containing a quinolone moiety as well as a pharmacophore present in NBTI's. Depending on the substitution, these FQ-NBTI hybrids have different modes of action. Very interesting is the improved activity on Gram negative bacteria. The presentation will focus on the characterization of the compounds in terms of enzymatic and cellularactivities.



Ciprofloxacin



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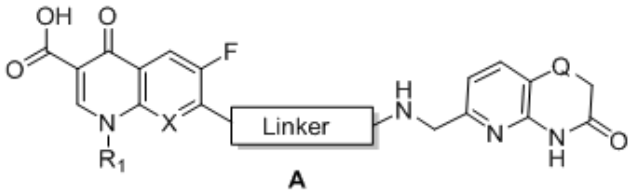

A

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Siglec-8 – A Novel target For Asthma.

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Sialic acid-binding immunoglobulin-like lectins (siglecs) are members of the immunoglobulin family. They are predominantly found on cells of the immune system. Up to now, 15 siglecs have been identified.[1] Among them is Siglec-8, which is selectively expressed on human eosinophils and regulates their survival.[2-4] Crosslinking Siglec-8 with specific glycan ligands or antibodies induces apoptosis.[5] In some diseases, such as asthma or chronic rhinosinusitis, an excessive amount of eosinophils is produced, causing an inflammatory reaction. Thus, targeting Siglec-8 provides a unique opportunity to control such allergic reactions.

Using a glycan array, the tetrasaccharide 6'-sulfo-sLe^x was identified as a ligand of Siglec-8.[6] However, it exhibits poor drug-like properties and its synthesis is laborious and of high complexity. Therefore, we synthesized a number of mono- and oligosaccharides derived from the scaffold of 6'-sulfo-sLe^x and determined their affinities for Siglec-8.

Herewith, the potent disaccharide Neu5Acα2-3(6-*O*-sulfo)Gal was identified, displaying a relative IC_{50} of 2.2 with respect to the natural 6'-sulfo-sLe^x. Moreover, the affinity of the disaccharide ligand was significantly reduced when small modifications to this structure were made. For example, when the *O*-glycosidic bond was changed from α2-3 to a α2-6 linkage, the sulfate-group was removed or only the corresponding monosaccharide fragments were evaluated, almost no binding to Siglec-8 was observed.

These results suggest that the scaffold of Neu5Acα2-3(6-*O*-sulfo)Gal contains the essential epitope of 6'-sulfo-sLe^x required for Siglec-8 binding. Based on this information, a new family of Siglec-8 ligands is currently synthesized.

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SMN2 splicing modifier for the treatment of Spinal Muscular Atrophy (SMA)

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[1]F. Hoffmann-La Roche AG

Spinal muscular atrophy (SMA) is the leading genetic cause of mortality in infants and toddler and currently only palliative treatments are available. It is caused by the reduced expression of the survival of motor neuron (SMN) protein due to loss of functional SMN1 gene and alternative splicing of exon 7 in the SMN2 gene.

At the end of 2011, PTC Therapeutics, the SMA Foundation and F. Hoffmann-La Roche, Ltd entered into a unique three party collaboration to develop urgently a life changing treatment for children with SMA.

The strategy was to identify orally available novel small molecules that specifically modify SMN2 splicing in SMA patient-derived cells, increasing the production of full length SMN2 mRNA and consequently functional SMN protein production. Upon oral administration, the SMN protein level was restored in two mouse models of SMA, and subsequently, a dramatic increase in the life-span of delta 7 mice, a model of severe SMA, was achieved. This program is currently in clinical trials.

From bulk to single molecule - Point mutations reveal specific intra domain interactions essential for group II intron ribozyme folding

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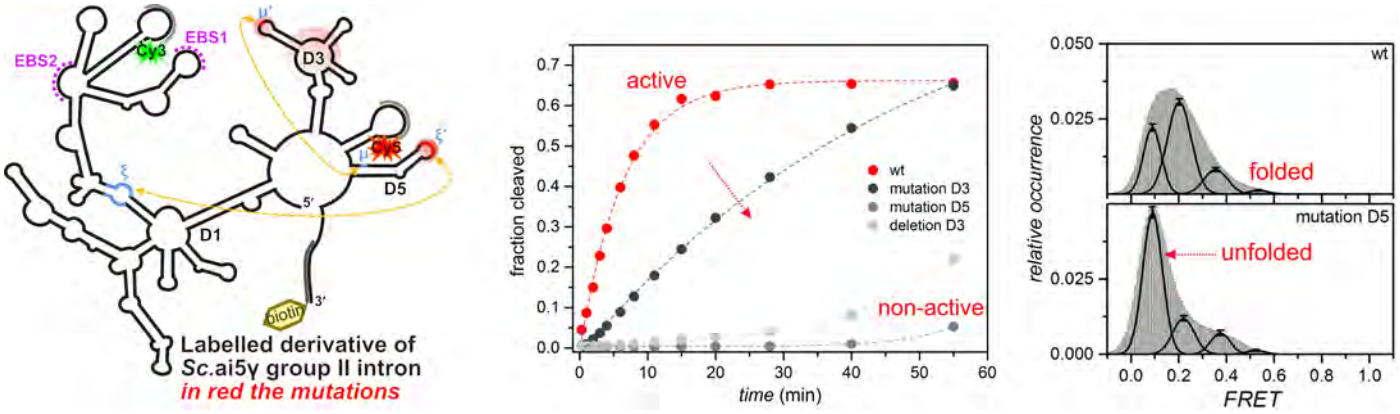
[1]University of Zurich, [2]University of Birmingham

Group II introns are among the largest ribozymes known. Their structural analysis suggests that they have evolved into ribonucleoproteins generating the eukaryotic nuclear spliceosome. They are found in the genome of bacteria, plants and lower eukaryotes [1]. These self-splicing ribozymes are active upon formation of specific long-range tertiary interactions that define a precise conformation influenced by co-factors such as Mg^{2+} [2,3]. We study the folding pathway of a truncated but active Sc.ai5γ group II intron through point mutations in the RNA sequence in positions essential for inter-domain docking. Combining bulk activity assays and single molecule Fluorescent Resonance Energy Transfer (smFRET) experiments we test the effect of these mutations on the catalytic activity and the folding pathway of this ribozyme. In both, bulk and single molecule experiments, different mutations have distinct effects on both activity and folding. In particular smFRET allowed us to quantify the differences in the relative population of a certain conformation attributed for splicing activity, especially a clear shift towards the unfolded conformations when the catalytic domain (D5) is mutated (Figure, right) [4]. Although even a drastic mutation still allows for the folding into the most compact state, addressed to be the active one, activity assays show that no activity is present if the interaction D5-D1 is disturbed. We introduced high Mg^{2+} concentration and crowding environment, however, the mutation cannot be compensated and the catalytic activity restored [5]. From the obtained results, we can assign a change in conformation/FRET state to a particular event in the folding, providing us with a deeper understanding of the actual active state. Targeting specific domains, in particular single motifs, drastically decreases the activity making group II introns a specific target for drugs. Furthermore, their capability to reinsert into the genome may ultimately be applicable for gene therapy.



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Photoaffinity labeling in Chemoproteomic: Cyanopindolol and a small molecule mediated reprogramming of mESCs as successful case study.

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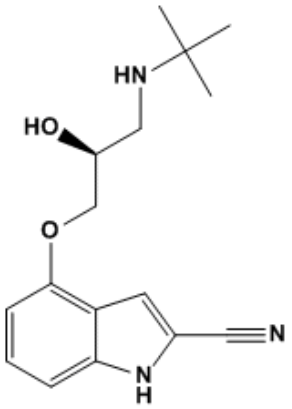
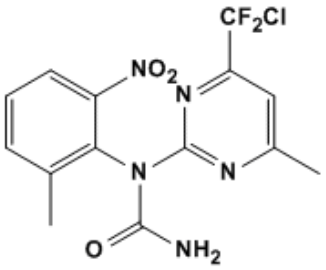
Knowing the cellular targets of drugs is crucial if the process of drug discovery is to be made more efficient. Identifying the full spectrum of targets associated with a bioactive small molecule can lead to faster optimization, understanding of off-target side effects and the ability to minimize possible toxicities early on in the process. We have developed a robust and unbiased method of probing the proteins that bind to the small molecule of interest in a biologically relevant setting.

We propose to present two show cases for which we are able to successfully unravel the target and/or the MoA of the molecule of interest in two separated context: one being the well-known Cyanopindolol and the other being a small molecule modulator of mESC reprogramming.



Cyanopindolol



SM1

[1] Laure Bouchez*et al.Article in preparation.*

Refining the understanding of the catalytic mechanism of DNAzymes

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DNAzymes (or DNA enzymes) are single-stranded DNA molecules that are capable of catalyzing chemical reactions.[1] Unlike their natural counterparts, the ribozymes and proteinaceous enzymes, DNAzymes have no precedent in nature and are all identified by SELEX and related combinatorial methods of *in vitro* selection.[2] In this context, DNAzymes 8-17 and 10-23 are two well-known and widely used ribonucleases that are capable of cleaving a broad variety of mRNA substrates with high catalytic efficiencies (k_{cat}/K_M › 10^8 $min^{-1}M^{-1}$).[3] In the absence of any X-ray structures, numerous studies have been dedicated to investigate on the folding and catalytic mechanism of these DNAzymes, underscoring the importance of conserved residues and the M^{2+} cofactors.[4, 5] Here, we show that minor grove interactions might play a significant role in the catalytic mechanisms of DNAzymes.[6] Indeed, substitution of dA units of the catalytic core of these DNAzymes by an analog were the capacity at forming interactions in the minor groove was suppressed, revealed to be an adequate tool for investigating these fragile interactions.

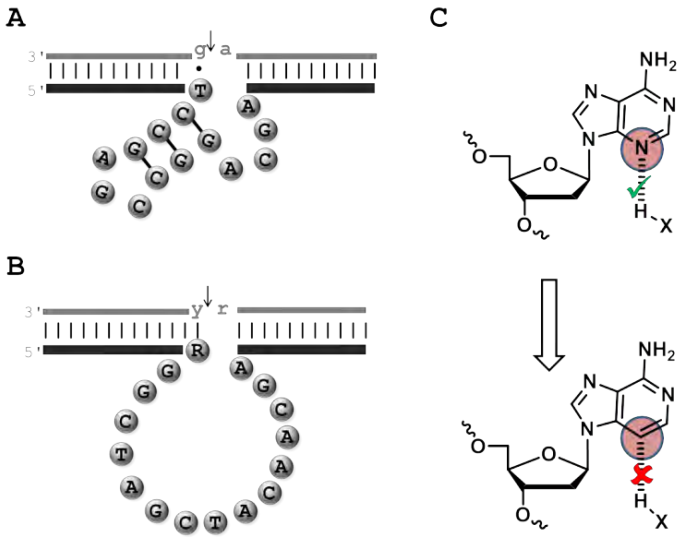

Figure 1. Hypothetical secondary structures of DNAzymes 8-17 (A) and 10-23 (B); substitution of the *N*3-atoms in the catalytic cores (C).

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Strigolactones and their potential role in modern agriculture

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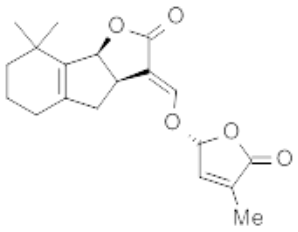
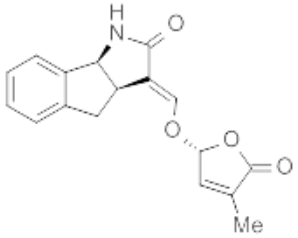
[1]Syngenta Crop Protection AG

Nowadays, the importance of abiotic stresses has been strongly recognized as threat for crop production both in public and industrial research organizations. New technologies able to mitigate the stress caused by the main abiotic stresses (i.e. drought, salinity, cold and heat) represent a substantial opportunity to contribute to a sustainable increase of agricultural production. Very recently, strigolactones have been conclusively identified as phytohormones and their role in controlling plant architecture and germination has been unveiled. The progresses achieved in this field are culminating in the identification of the molecular receptors involved in the signal transduction mechanism. The exact mechanism of the mode of action of strigolactones still remains to be fully elucidated and we were interested to gain some insight into the mechanism of action of strigolactones by selectively modifying the reactivity of the lactone C-ring. Therefore, we will present the synthesis of 5-deoxystrigol and synthetic analogue strigolactam and their surprisingly good activity on the germination of *Orobanche cumana* parasitic weed seeds.



5-deoxystrigol



strigolactam

M.Lachia, P.-Y. Dakas, A. De Mesmaeker, *Tetrahedron Lett.* **2014**, *55*, 6577-6581
M.Lachia, Hanno C. Wolf, Pierre M. J. Jung, Claudio Screpanti, A. De Mesmaeker,*Bioorg. Med. Chem. Lett.* **2015**, *25,*2184–2188

Guineensine as a Novel Inhibitor of Endocannabinoid Reuptake

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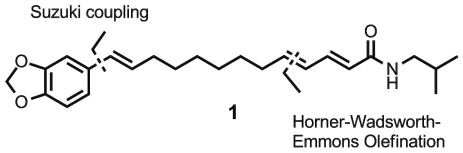
[1]ETH Zurich, [2]University of Bern

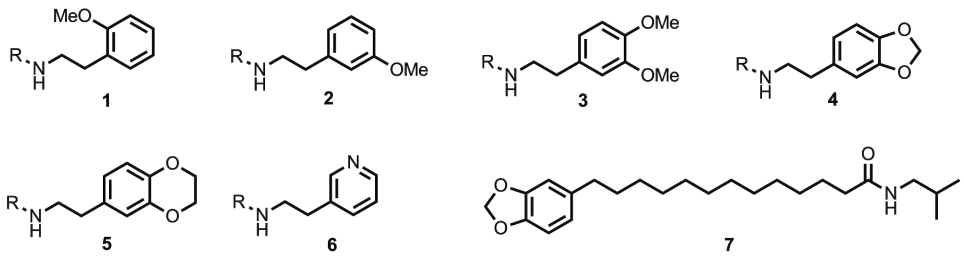
Guineensine (**1**) is a plant-derived natural product that can be isolated from *Piper nigrum* and was recently shown to be a novel nanomolar inhibitor (EC_{50} = 290 nM) of the cellular reuptake of the endocannabinoid anandamide.[1]



Scheme 1: Structure of guineensine (**1**) and key retrosynthetic disconnections.

In order to gain insight into the structure-activity-relationships for guineensine(**1**) we first developed an efficient total synthesis of the natural product itself. Key steps were a Suzuki coupling and a Horner-Wadsworth-Emmons Olefination (Scheme 1). Based on this chemistry, we then prepared a number of analogs of **1** (such as compounds **2**-**7**) and we assessed their capacity to inhibit anandamide upuptake *in vitro*.

In this contribution we will discuss the details of the synthesis of **1** and guineensine analogs and we will disclose the first SAR data for this new type of endocannabinoid uptake inhibitors.



Scheme 2: Amide part analogs of guineensine (**1** - **6**) and saturated guineensine (**7**).

[1] Simon Nicolussi et al., *Pharmacological Research***2014**, *80*,52-65.