CHIMIA

CHIMIA 2014, Volume 68
ISSN 0009-4293
www. chimia.ch

SCS
Swiss Chemical
Society

Supplementa to Issue 7-8/2014

SCS Fall Meeting 2014
Poster Abstracts
Session of Medicinal Chemistry & Chemical Biology

September 11, 2014
University of Zurich, Irchel Campus
http://scg.ch/fallmeeting2014

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Unravelling the RNA binding of Rhenium(I)-dppz complexes

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Luminescent metal complexes belonging to the d^6 family, like Ru(II), Ir(III) and Re(I), have been extensively studied as potential bio-imaging agents due to their suitable photophysical, spectroscopic and electrochemical properties [1,2]. Unexpectedly, beside a wide study of their DNA interaction, there is a lack of information on their RNA binding properties.
In particular the metallo-intercalators Rhenium(I)-dppz complexes have been already described as potential imaging agents, and their DNA binding properties have been reported [3]. It was shown that their accumulation within the cell varies upon changing their axial ligand, typically pyridine derivatives. The various axial ligands, without affecting the optical properties, drive the accumulation in different cell compartments, including RNA-rich regions [3].
For these reasons we decided to investigate the interaction of various mononuclear Rhenium(I)-dppz complexes with several RNA constructs, containing the most common secondary structural features. Both the effects of varying the RNA model and the axial ligand of the rhenium complexes are evaluated to rationalize the structural origin of interaction preferences.
We are at the moment investigating the changes of the optical properties of one of these complexes upon interaction with a 27 nuclelotide long RNA construct, derived from the mitochondrial group II intron ribozyme Sc.ai5γ [4]. The study is carried out by means of UV/Visible and fluorescence spectroscopy with the final aim of evaluating the effect of structural features contained in the construct on the binding properties. Furthermore, since the NMR structure in solution of this RNA construct is known [5], NMR studies are in progress to determine the site of interaction and to give further insights into RNA-Rhenium(I) complexes non-covalent binding.

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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Labeling the bacterial outer membrane transporter LptD using an antimicrobial peptide by chemical cross-linking.

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[1]University of Zurich

The increasing resistance of bacteria to conventional antibiotics has resulted in a considerable effort to develop new antimicrobial compounds with new mechanisms of action. A new family of antimicrobial ß-hairpin-shaped peptidomimetics show antimicrobial activity in the nanomolarrange specifically against Gram-negative *Pseudomonas* sp. Interestingly the outer membrane protein LptD, required for LPS transport to the cell surface, has been identified as the target by a forward genetic screen for resistance in *Pseudomonas aeruginosa* andby photo-affinity labelling experiments with a photo-active peptide analogue[1]. The aim of this research is the optimization of the labelling in order to investigate the interaction between LptD and the peptide antibiotic in more detail. Recently, peptides containing 3,4-dihydroxyphenylalanine (DOPA) have been shown after oxidation to covalently cross-link to target proteins. This method is based on the known oxidation chemistry of DOPA-containing proteins common in mollusks [2,3].

In this work, analogues of the antimicrobial ß-hairpin antibiotics were chemically synthesized, with DOPA substituting for a tryptophan residue, and with a biotin tag linked to a glutamic acid side chain for detection of cross-linking to the target LptD. The antimicrobial activity of the DOPA-derivatives were typically about one hundred fold lower than that of the parent antibiotic, and one-tenth of that of the previously used photo-probe as determined by minimum inhibitory concentration assays.

Despite the decreased activity compared to the parent compound, the DOPA-derivative could be successfully cross-linked to LptD. Cross-linking was inhibited by excess antimicrobial peptide, proving the specificity of the labelling. The labelling efficiency was estimated to be higher than cross-linking using the photoaffinity technique. The labelling with the DOPA-derivative was also carried out on *P. aeruginosa* PAO1 cells. The DOPA-peptide labels LptD *in vivo* with a reduced selectivity compared to the photo-active peptide. In conclusion, a new labeling probe was successfully developed, which may be a valuable tool for future investigations on the binding site of the antimicrobial peptide in the target protein LptD.

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Two-Photon Uncaging as a More Versatile Alternative to Photodynamic Therapy

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Photodynamic therapy uses photosensitizers to create reactive species in cancer cells, mostly singlet oxygen, and ultimately kills them.[1] This fantastic application of fundamental photochemistry is already in clinical use with porphyrin based agents such as Photofrin. Nevertheless, the concept has drawbacks: 1) It relies on the presence of oxygen in the tissue, while cancerous tissue often has reduced oxygen levels; 2) It cannot be used for localized diseases apart from cancer, since it can only kill cells, not influence cellular processes in a constructive way. Recently, our group reported on an alternative strategy to use light to influence biological pathways.[2] To this end, bioactive compounds were inactivated by attachment of a “cage”, which can then be removed by irradiation with light in the UV-A range. With this approach, all kinds of drug-like agents can be activated. However, light in the UV-range can damage biological structures and penetrates only very little into tissues, thereby limiting its applicability. More recently, two-photon uncaging, has gained significant interest.[3] The technique is based on the near-simultaneous absorption of two photons in the near-IR range, leading to deeper tissue penetration and better spatial localization. Herein, we present the development of a targetable two-photon caging platform which can be used to trigger biological functions with great spatial and temporal control. Furthermore, we demonstrate the concept in an *in vitro* assay.

This work was supported by the Swiss National Science Foundation (Professorship N° PP00P2_133568 and Research Grants N° 200021_129910 and N° 200020_146776 to G.G.), the University of Zurich (G.G.) and the Stiftung für Wissenschaftliche Forschung of the University of Zurich (G.G.). The authors thank Klemens Koziol and Peter Hamm for help with two-photon uncaging experiments.

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A Multi-fingerprint Polypharmacology browser for ChEMBL

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[1]University of Berne

Drug discovery is increasingly influenced by the availability of very large databases of drug-like molecules, comprising millions of compounds from commercial or from theoretical sources awaiting biological evaluation, as well as large collections of bioactive compounds with annotated activities. Working with such large databases requires efficient tools to browse through very large lists of molecules, in particular to rapidly identify structurally similar molecules. Recently we showed that databases up to billions of molecules can be classified using simple descriptor sets such that similarity searches are completed within seconds, with optional browsing via interactive color-coded principal component maps if a query molecule is not available. The search principle was recently extended to design a multi-fingerprint browser for the ZINC database allowing to rapidly identify analogs of any screening hit and perform clustering to compose focused sets for hit confirmation. Here we report the extension of this multi-fingerprint approach to the problem of polypharmacology searches, i.e. how to find out if a newly identified bioactive molecule is closely related to molecules with documented bioactivity and therefore likely to interact with the corresponding biological target. Our polypharmacology browser searches within seconds through 717 groups of at least 20 bioactive molecules with documented activity against a biological target, as listed in ChEMBL, to identify analogs of any query molecule using nine different fingerprints or fingerprint combination, and displays results groups by targets as lists of bioactive compounds, which allows one to directly estimate whether the identified similarity is meaningful in the examined context. Compared to previous reports of related poly-pharmacology predictors, our browser application is much more versatile and gives more direct access to the chemical structures underlying the polypharmacology prediction. An application of the browser to target deconvolution in the framework to a nano-molar cytotoxic compound identified in cell-based phenotypic screen will be discussed.

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Guineensine as a Novel Inhibitor of Endocannabinoid Reuptake

Ruben Bartholomäus[1], Simon Nicolussi[2], Ana Simao[1], Jürg Gertsch[2], Karl-Heinz Altmann[1] *

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

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

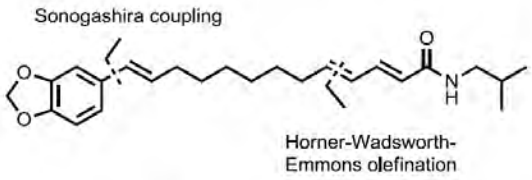
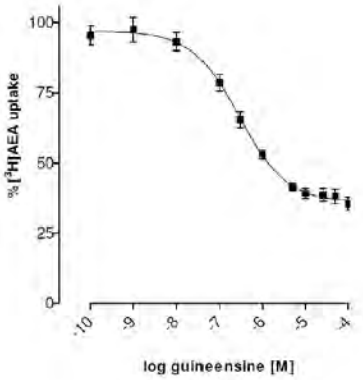


Figure 1: Structure of guineensine



A preliminary goal of the project is to gain knowledge about structure-activity relationships. Therefore we synthesized various head group analogs of guineensine (Figure 2, A). The most potent compound was found to be 3,4-methylene-dioxyphenyl derivative **4**.

The saturated compound **7** (Figure 2, B) reveals that the presence of the double bonds is crucial in terms of anandamide reuptake inhibition.

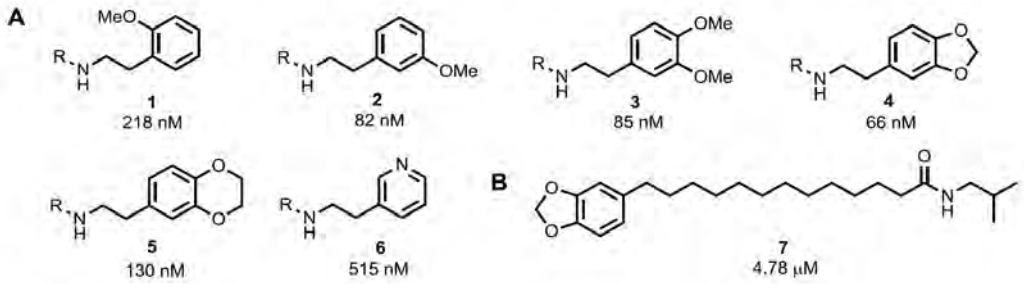


Figure 2: Synthesized headgroup derivatives (A) and saturated guineensine (B)

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Synthesis and SAR of New des-THP Analogs of (-)-Dactylolide and (-)-Zampanolide

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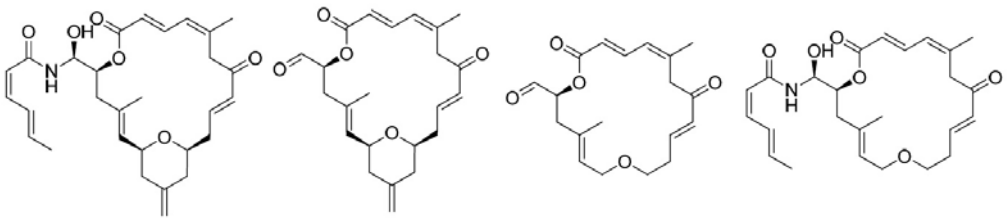
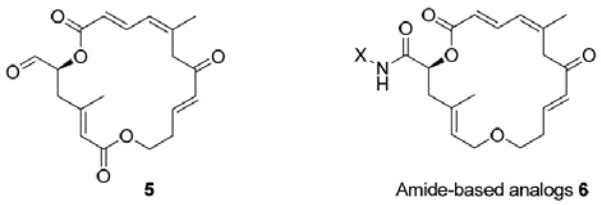
[1]ETH Zurich

(-)-Dactylolide is the non-natural enantiomer of the marine natural product (+)-dactylolide and it also constitutes the core structure of another secondary metabolite, (-)-zampanolide. The latter was first isolated from the marine sponge *Fasciospongia rimosa* in 1996[1] and it was found to exhibit significant antiproliferative activity. More recently, (-)-zampanolide was shown to be a potent microtubule-stabilizing agent that binds covalently to β-tubulin in the taxane site. [2]



We have previously shown, that derivatives of either (-)-dactylolide (**2**) or (-)-zampanolide (**1**) that lack the tetrahydropyran (THP) ring embedded in the macrolide core structure (i. e. **3** or **4**, respectively), are still active against various human cancer cell lines.[3] To further investigate the role of acyclic THP replacements, we are currently pursuing the synthesis of additional des-THP analogs of (-)-dactylolide and (-)-zampanolide. As a first example this presentation discloses the synthesis of ester-based analog **5**. In addition, we have prepared a series of side chain-modified analogs of **4**; in particular, we have investigated structures of type **6** where the hemi-aminal moiety is replaced with an amide functionality. This contribution will discuss the details of the synthesis of **5** and analogs of type **6** and provide first data on the biological activity of these compounds.



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Miniaturized Assays for Point-Of-Care Therapeutic Drug Monitoring

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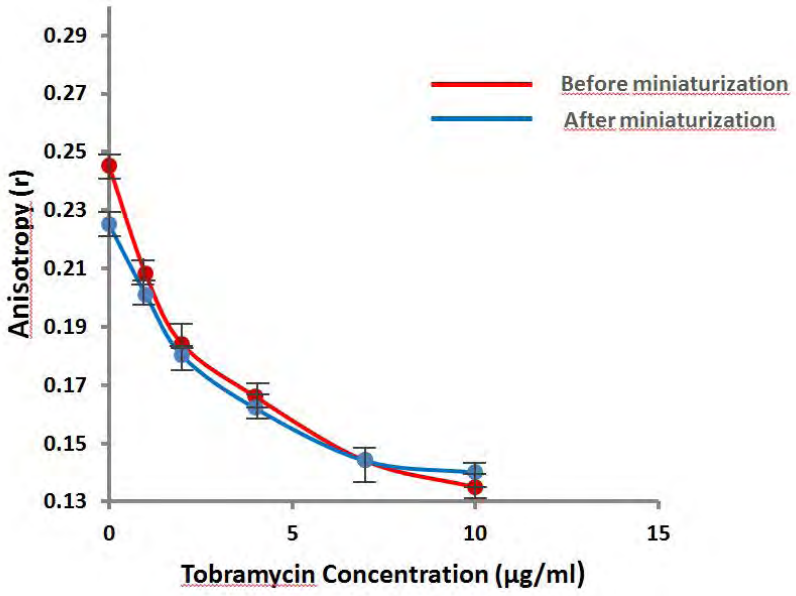
Many modern therapeutics involved for instance in the treatment of infections, cancer or in post-transplant therapies require Therapeutic Drug Monitoring (TDM) owing to their narrow therapeutic range. Currently this process is demanding for the patient, as several milliliters of blood are required, slow and costly, as the sample need to be transferred to a central laboratory, and suffer of limited efficacy, as the results are difficult to interpret for a non-specialist.To overcome these problems, we
aim at providing a simple, rapid and sensitive solution by developing a compact and cost-effective Point-Of-Care (POC) drug quantification device based on miniaturized competition immunoassays. First results have demonstrated the feasibility of downsizing Fluorescence Polarization immunoassays and shown that the two prototypical drugs tobramycin and tacrolimus, an antibiotic and an immunosuppressant, can be quantified using minute amounts (only 20 µl) of human blood. For tobramycin, the assay could be further miniaturized down to just one µl of human serum while preserving its performance.



Moreover, the assays could be transposed onto custom-made FP instruments as a first step towards a Point-Of-Care Therapeutic Drug Monitoring device

Cell membrane association of the 295-311 fragment of the estrogen receptor α

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[1]Université Pierre et Marie Curie, Paris, [2]Université de Crète

The estrogen receptor α (ERα) is a transcription factor that interferes with many physiopathological processes. It is activated in a ligand (estradiol)-dependent (or independent) manner through mechanisms occurring, at least partially, at the membrane. To date, four estrogen-sensitive membrane proteins have been identified: the full length ERα (ER66), the ER36 and ER46 truncated forms, and the protein GPR30. The ER36, ER46 and ER66 proteins are located at the cell membrane, and more precisely at its inner face after a palmitoylation step at the key cysteine 447. GPR30 is engulfed within the phospholipidic bilayer in a heptatransmembrane-related mechanism. We have recently identified a positively charged region at the surface of the ERα (K299RSKK303 motif, third NLS), which is located ~10Å from the Cys-447. This structural characteristic, which has also been reported for the Rho-GTPase family, could be involved in the stabilization of the protein at the membrane. To explore this possibility, we have synthesized an ERα derived peptide containing this positive motif (ERα17p, H-P295LMIKRSKKNSLALSLT311-OH) and we have studied its ability to interact with membrane models. This interaction was studied by using phospholipidic bilayers (vesicles composed of neutral or cationic phospholipids). We used differential scanning calorimetry (DSC), fluorescence techniques and circular dichroism . Our approach was extended to both ER+ and ER- breast cancer cells. By using a fluorescent version of our peptide, we showed that it also interacts with cell membranes. The use of estradiol-BSA-FITC, which is specific for membrane estrogen binding sites, reveals that this interaction is independent from the presence of ER. As membrane estrogen receptors are involved in the activation kinase pathways and the proliferation of cancer cells, the PLMIKRSKKNSLALSLT sequence may counteract the association of ER with membranes and may open new perspectives for the control of hormone-dependent breast cancer.

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Synthesis of Diphosphoinositol Polyphosphates

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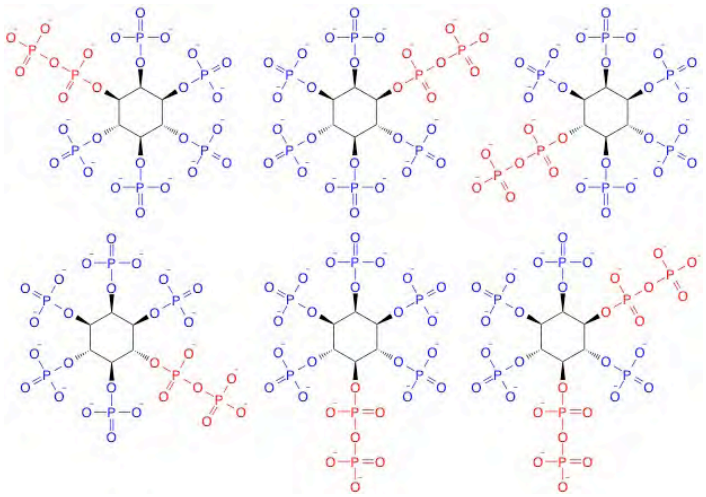
Diphosphoinositol polyphosphates (X-PP-InsP_5) represent a novel group of secondary messengers that regulate diverse important cellular processes.[1] The low abundance of X-PP-InsP_5 makes isolation procedures difficult, while enzymatic preparations failed to produce large quantities of material and are not suited for the synthesis of the non-natural enantiomers. The *myo*-inositol scaffold gives rise to symmetric and non symmetric phosphorylated patterns and an efficient total synthesis offers unique possibilities to study the structure and function of X-PP-InsP_5.



Different syntheses of enantiomerically pure inositol polyphosphates have been reported, but X-PP-InsP_5 are not commercially available and the quality of earlier preparations has been called into question.[2] Asymmetric phosphorylation is an outstanding way to introduce a phosphate group in a specific position in the structure of *myo*-inositol. We will report a novel total synthesis of unsymmetric diphosphoinositol polyphosphates using aC_2-symmetric phosphoramidite to desymmetrize different inositol derivatives. With the application of the new phosphorylating reagent we are able to target all four possible unsymmetric X-PP-InsP_5 and two possible X,Y-$(PP)_2$-InsP_4, allowing us to generate interesting derivatives as tools for in-depth biological studies.[3]

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Structural patterns associated with the recruitment of holocalmodulin by ERα

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[1]Université Pierre et Marie Curie, Paris, [2]Université Libre de Bruxelles

Classically, estrogen-dependent transcription requires the liganded estrogen receptor α (ERα) under its phosphorylated and dimerized form. Before acting as a transcription factor on estrogen-response elements (ERE), it recruits coactivatory proteins belonging typically to the p160 family. The p160 binding groove is located at the surface of the ERα, along its helix H3. Holocalmodulin (Ca2+-calmodulin, CaM) activates ERα-dependent transcription and behaves, therefore, as an ERα co-activator. By using specific ERα antibodies in CaM-Sepharose binding assays, we have shown that antibodies associating with the hinge region of the ERα inhibit its interaction with CaM. Referring to the primary sequence of typical CaM ligands, we have hypothesized that the 285-311 region of ERα, which encompasses key basic residues involved in CaM association, could be responsible for the formation of the complex. Accordingly, we have shown by using isothermal calorimetry and tryptophan-based fluorescence that the peptide corresponding to the 285-311 region of the ERα associates with holocalmodulin, only, with a 1:2 stoichiometry in the micromolar range, a feature that has also been observed by mass spectrometry. Likewise, we have shown by circular dichroism that this association was accompanied by a random-to-helix structuring of the peptide. Interestingly, our NMR study revealed that the interaction of two peptides corresponding to the 285-311 region of ERα with holocalmodulin was atypical and requires the two lobular domains (Fig. 1).

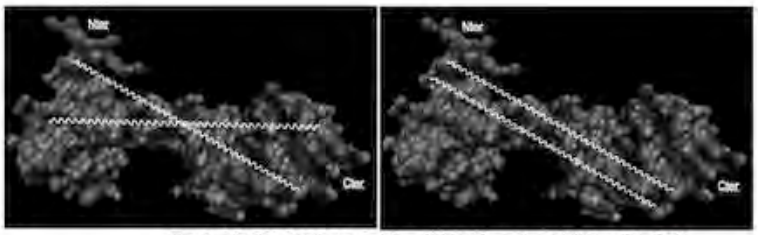

Fig.1. Modes of binding of the 285-311 region of ERα with CaM.

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Tackling Antibiotic Resistance by Transcription Repressor Inhibitory Compounds

Mathieu Chellat[1], Luka Raguz[2], Mark Sephton[1], Patrik Züger[2], Michael Brand[2], Rainer Riedl[1] *

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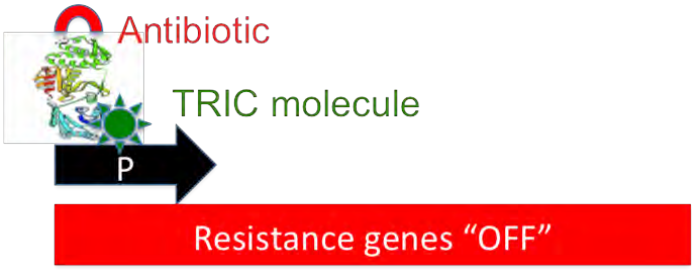
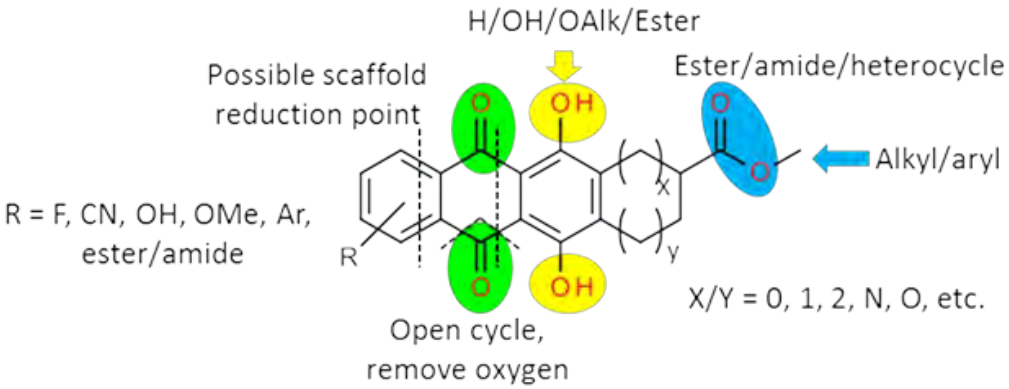
The emergence of bacterial pathogens that no longer respond to antibiotics is a global threat to public health according to the World Health Organization WHO.[1] New therapies to combat antibiotic resistances are therefore urgently needed.[2]
Researchers at the ZHAW and the FHNW are collaborating with the biopharmaceutical company BioVersys AG in order to tackle antibiotic resistance. They have identified the need for certain types of small organic molecules, called Transcription Repressor Inhibitory Compounds (TRICs).



In contrast to a wide range of traditional antibiotics for which bacteria have developed resistance, the TRICs do not interfere with the bacterial metabolism but work on the bacteria’s genetic level. These TRICs switch off the bacterial defense program and the original antibiotic can kill the bacteria again.



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Chitosan thiomers for antimicrobial applications

Matteo Croce[1], Simona Conti[1], Greta Ricarda Patzke[1] *

[1]University of Zurich

Chitosan is a natural linear polycationic biopolymer consisting of N-acetyl-D-glucosamine and b-1,4-linked-D-glucosamine and it is obtained by partial alkaline deacetylation of chitin as the second most abundant polysaccharide in nature. During the past decades, many applications were developed based on this biocompatible and biodegradable polymer such as for wound dressings, food coating, wastewater treatment and drug delivery.[1] Chitosan thiomers were also developed as efficient drug carrier due to their peculiar biological and chemical characteristics such as mucoadhesive properties.[2] Furthermore, they were also tested as antimicrobial compounds with promising results.[3]

In order to address the constant increasing threat of antibiotic resistant bacteria and to investigate their action mechanism, new efficient complexes are required. In this context, we have synthesized a series of different thiolated compounds through the formation of amino and amidine bonds with the free amino groups of the chitosan. The functionalized biopolymers were characterized with a wide variety of analytical techniques, and their biological properties were tested on different types of microorganisms and on cancer cells in order to evaluate the relationship between chemical structures and potential antibacterial and anticancer behavior.

Due to their chelating properties, the synthesized polymers were also used as a matrix to incorporate biologically active polyoxometalates[4] and/or transition metals, creating nanoparticles that would be tested as promising agents against bacteria, cancer cells and viruses.

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Antiprotozoal Compounds from Drypetes gerrardii

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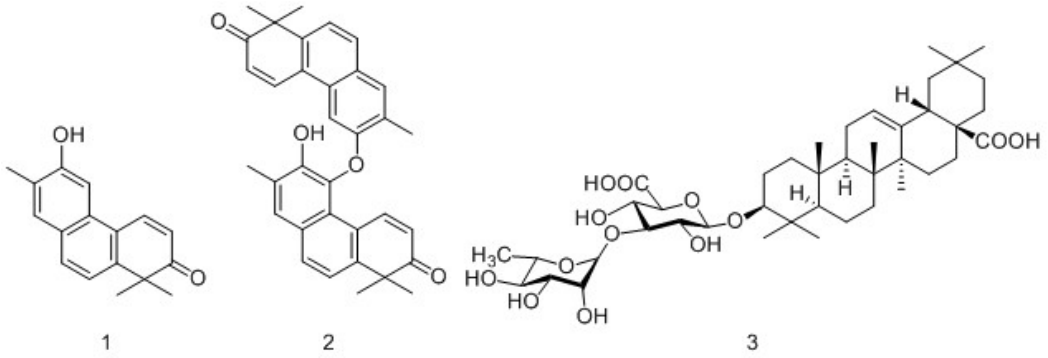
[1]University of Basel, [2]Swiss Tropical and Public Health Insitute, Basel

In an *in vitro* screen of 206 extracts from South African medicinal plants, the CH_2Cl_2/MeOH (1:1) stem extract of *Drypetes gerrardii* Hutch. var *gerrardii* (Putranjivaceae) inhibited *Plasmodium falciparum* and *Leishmania donovani* (IC_{50}s of 0.50 and 7.31 mg/mL, respectively). In addition, the CH_2Cl_2/MeOH (1:1) extract of the leaves showed activity against *Trypanosoma brucei rhodesiense* (IC_{50} of 12.1 mg/mL).

The active constituents were tracked by HPLC-based activity profiling, and isolated by preparative and semi-preparative RP-HPLC chromatography. Their structures were established by HR-ESIMS, and 1D and 2D NMR (^1H, ^{13}C, COSY, HMBC, HSQC, and NOESY).



From the stem extract, a new phenantrenone (**1**) and its dimer (**2**) were isolated. Compound **1** showed potent *in vitro* activity against *P. falciparum* (IC_{50} of 0.9 µM) with a selectivity index (SI) of 71, as calculated from cytotoxicity data in L-6 cells. These data qualified **1** for *in vivo* assessment in the *Plasmodium berghei* mouse model, but the compound turned out to be inactive. Compound **2** also exhibited good *in vitro* antiplasmodial activity (IC_{50} of 1.2 µM) and selectivity (SI of 53). From the leaf extract, the saponin putranoside A (**3**) was isolated and identified. Compound **3** showed weak *in vitro* trypanocidal activity, with an IC_{50} of 18.0 µM, and a SI of 4.[1]

Oligoprolines as Scaffolds for Tumor Targeting with Hybrid Bombesin Analogues

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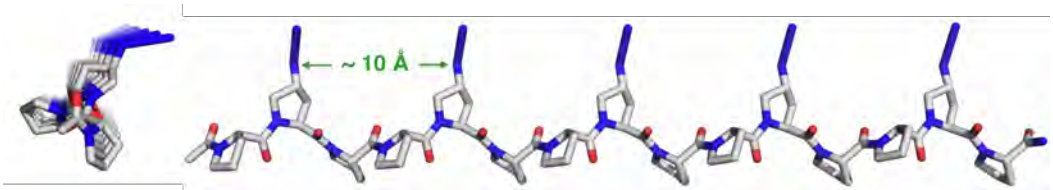
In recent years, oligoprolines have emerged as an attractive example of a molecular scaffold suitable for controlling the spatial arrangement of various compounds in medicine and material sciences.[1] In aqueous environments oligoprolines adopt the well-defined polyproline II (PPII) helix already at chain lengths as short as six residues.[2] Within this left-handed secondary structure every third proline residue is stacked on top of each other in a distance of approximately 1 nm.[2] Incorporation of 4-azidoproline (4-Azp) residues into this helix provides reactive sites located in desired distances from each other that can easily be functionalized by Cu(I)-catalyzed Huisgen's 1,3-dipolar cycloaddition (click reaction) with terminal alkynes or through Staudinger reduction followed by acylation.[3]



Previous studies within our group showed that hybrid ligands consisting of an oligoproline scaffold equipped with a bombesin-based agonist and antagonist as recognition motives exhibit extraordinary tumor uptake properties in prostate carcinoma.[4] The hybrid ligands showed significantly higher tumor uptake *in vitro* and *in vivo* compared to not only monovalent but also divalent controls. Notably, the defined distance between the recognition motives proved to be important for high, specific, and long lasting tumor uptakes. Based on these initial findings we are now designing modified oligoproline-based ligands to achieve yet higher tumor uptakes and a deeper understanding of how the uptake is accomplished on the molecular and cellular level.

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Repair of Protein Radicals by Antioxidants

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[1]ETH Zurich, [2]Macquarie University, Australia

Aerobic organisms are continuously subjected to partially reduced oxygen species (PROS). Under conditions of oxidative stress, this may lead to biological damage. Proteins are significant targets of PROS and increased levels of protein oxidation products are markers for biological damage. Protein radicals are precursors of these products. They are located on amino acid residues of the protein and, in the presence of oxygen, may yield protein peroxyl radicals ($PrOO^{\cdot}$) and hydroperoxides (PrOOH). All these species are considered to propagate damage. Endogenous antioxidants, such as ascorbate or urate, are part of the antioxidant defenses of cells and tissues and may repair proteins.

We studied the reactions of these antioxidants with amino acid and protein radicals.

Ascorbate and urate rapidly repair $Trp^{\cdot}$ and $Tyr^{\cdot}$ in proteins[1-4] and reduce the peroxyl radicals of Gly, Ala and Pro derivatives.[5] They also react, but considerably slower, with the C-centered radicals in Gly, Ala, Pro derivatives and α-methylalanine (preliminary results).

In vivo, ascorbate and urate may prevent biological damage because they: (a) repair $Trp^{\cdot}$ and $Tyr^{\cdot}$ in proteins, and (b) reduce $PrOO^{\cdot}$ to the corresponding PrOOH. Most likely, *in vivo*, ascorbate and urate do not inhibit the reaction of C-centered amino acid radicals with O_2. Ascorbate may be the main repair agent in cells and tissues that contain high ascorbate concentrations, such as in the lens and brain; urate may be important for repair in tissue compartments with higher urate concentration, such as in plasma and saliva.

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New insights into the folding and NMR structure of the human RNA BCL2 G-quadruplex

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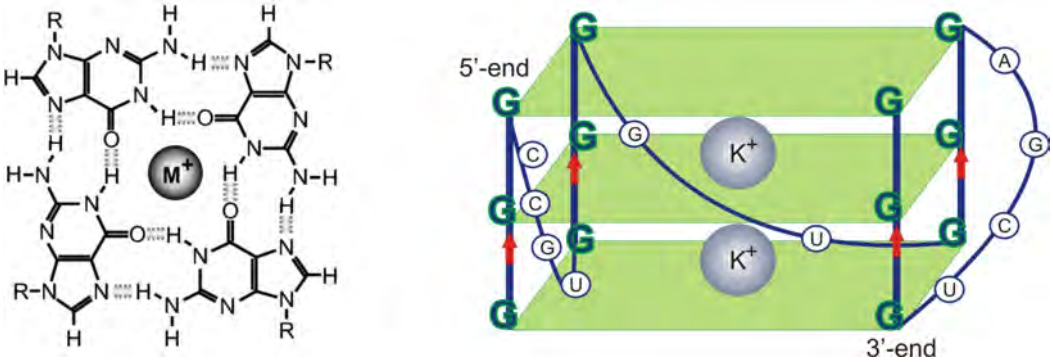
Guanine-quadruplexes (G4) are secondary non canonical structures of nucleic acids that result from the stacking of several guanine quartets, each quartet being a planar association of four guanines by cyclic Hoogsteen hydrogen bonds. A metal ion is located in the centre, between two adjacent quartets, stabilizing the structure [1] (see Figure). RNA G4 structures are believed to be actively involved in the translational regulation of several oncogenes [2]. This fact makes RNA G4 interesting targets for the development of novel antitumoral drugs [3]. Furthermore, the presence of RNA G4 structures in the cytoplasm of human cells has been recently evidenced [4].

The BCL2 (B-Cell Lymphoma/Leukemia-2 proto-ongene) RNA G4 structure has been shown to modulate the *in vitro* translation of certain proteins encoded by the BCL2 gene and over-expressed in several types of cancers [2,5]. In this work, the stability and topology of the BCL2 RNA G4 is evaluated in the absence and presence of potassium ions by Thermal Difference Spectra, UV melting, Circular Dichroism (CD) and CD melting experiments. The G-quadruplex formation is also observed by monitoring the imino proton region of ^{1}H NMR spectra. All the data support the stabilization and formation of a parallel G-quadruplex structure upon addition of KCl. However, time evolution experiments suggest the existence of more than one G-quadruplex stable conformation.



Financial support by the Fundacion Ramon Areces (A.D.M.), the Swiss National Science Foundation (R.K.O.S.), within 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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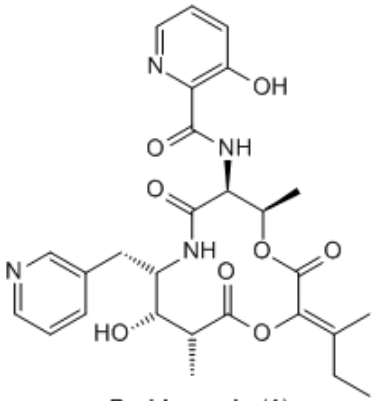
Dihydropyridomycins as New Antitubercular Agents: Synthesis and SAR Studies

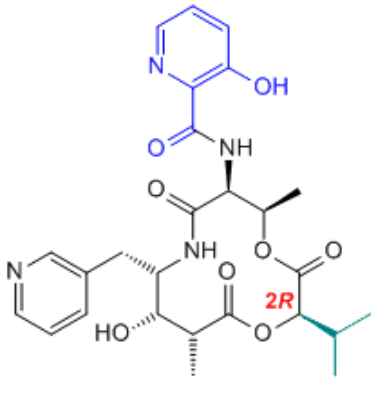
Maryline Dong[1], Oliver P. Horlacher[1], Rafael Schrof[1], Ruben Hartkoorn[2], Stewart T. Cole[2], Karl-Heinz Altmann[1] *

[1]ETH Zurich, [2]EPFL Lausanne

Pyridomycin (**1**) is a bacterial natural product with significant *in vitro* anti-tubercular activity.[1] The molecular target of **1** has recently been identified as the NADH-dependent enoyl-ACP reductase InhA, which is also the target of the clinically used anti-TB drug isoniazid.[2]



Pyridomycin (1)



2

We have previously shown that the dihydropyridomycin analog **2** retains most of the antibacterial activity of **1**, while the corresponding 2*S* isomer was substantially less potent.[3] Based on this initial finding, we have investigated variants of **2** with alternative alkyl substituents attached to C2 in an *R* configuration. Likewise, we have investigated dihydropyridomycin analogs where the hydroxypicolinic acid moiety was replaced by acyl residues incorporating aromatic, heteroaromatic and non-aromatic ring structures. This contribution will discuss the synthesis of these new dihydropyridomycin analogs and their antibacterial and InhA-inhibitory activity.

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Enzymatic C-H bond cleavage probed by deuterium kinetic isotope effects

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C-H bond activation is often a key step in the synthesis of complex molecules, be it in biosynthesis or in lab-based synthetic organic chemistry and accomplishing these important chemical transformations with small molecular entities remains a challenge. There is a great interest in new methodologies, since functionalization of complex hydrocarbon scaffolds is something that synthetic chemists do on a daily basis. The problem of selectivity and the unreactive nature of these bonds, due to the high bond strength, pose constant obstacles in the design of new reactions. Small molecule catalysts rely on metalloorganic complexes in order to achieve selective C-H bond cleavage. In the realm of nature, enzymes are very efficient at catalyzing such reactions with the help of suitable cofactors, which can either be metallic such as an iron-heme complex, or non-metallic such as an SAM radical. We study C-H bond activation by an enzyme which catalyzes such a reaction on a methylene group without the apparent need of any metallic or non-metallic cofactors. Kinetic isotope effects measured with enantioselectively deuterated peptide substrates show that C-H bond cleavage is the rate limiting step in this reaction, which is efficient as well as highly stereoselective.

FimH antagonists as novel approach for the prevention and treatment of Urinary Tract Infections

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[1]University of Basel

Urinary tract infections (UTIs), one of the most common infections worldwide with both a high rate of incidence and recurrence, affect millions of people each year and are associated with high medical costs. The primary causes of UTIs are uropathogenic *E. coli* (UPEC), which initiate the infection cycle by adhering to urothelial cells. The adhesion is mediated by the bacterial lectin FimH expressed on type 1 pili of UPEC and is based on the interaction between FimH and high mannose glycoproteins present on urothelial cells. This interaction is shear enhanced which means that FimH mediates weak binding at low shear stress and strong adhesion when shear stress is high [1]. This observation of different mannose binding phenotypes is due to FimH being present in two distinct affinity states, a high and a low affinity state. Various α-D-mannopyranoside derivatives have demonstrated FimH antagonism and offer a therapeutic approach to prevent and treat UTIs as an alternative to the conventional treatment with antibiotics [2], which has become progressively unavailing due to the increasing rates of antibiotic resistance. We present several promising FimH antagonists and relate their capability of blocking the initial adhesion step of UPEC to the conformational heterogeneity of FimH.

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Impact of Sulforaphane on Cytotoxicity of PR-104A in Human Colon Cells

Melanie Erzinger[1], Katrin Hecht[1], Simona Constantinescu[1], Nadine Sobotzki[1], Niko Beerenwinkel[1], Bernd Wollscheid[1], Shana Sturla[1] *

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PR-104A is an experimental anticancer drug and the first hypoxia-activated nitrogen mustard alkylating agent to enter clinical trials. It relies on reductive activation to be effective in the human body. Two activation mechanisms have been proposed: Besides being directly activated under hypoxic conditions, the aldo-keto reductase 1C3 (AKR1C3) was shown to be responsible for PR-104A activation under aerobic conditions. By increasing the activity and the amount of AKR1C3 in cancer cells, the function of PR-104A may be promoted, possibly leading to improved therapy outcome. Sulforaphane (SF) is a bioactive food component that is derived from cruciferous vegetables, for example from broccoli. It is known to modulate drug metabolizing enzymes like AKR1C3, mostly by the interaction with the Nrf2/Keap1-pathway. In this study, we investigated the impact of SF on AKR1C3 enzyme levels as well as activities and linked these to changes in PR-104A cytotoxicity. To compare the response in colon cancer cells and healthy colon epithelial cells we did the analysis in different cell models, namely HT29, HCT116 (cancer) and HCEC (healthy) cells. With a combination of molecular approaches such as quantitative proteomic techniques and activity probes we observed an up to 7-fold increase in AKR1C3 levels and 2.3-fold increase in activity in SF-treated colon cancer cells but not in HCEC cells. Furthermore, this increase appeared to contribute to increasing the cytotoxicity of PR-104A in cancer cells, whereas in the healthy colon cells SF might even have a protecting effect. Results that support a mechanism explaining how SF can modulate drug metabolizing enzymes and enhance the cytotoxicity of bioreductive anticancer drugs selectively in cancer cells over non-cancerous cells will be presented.

HPLC activity based profiling of Swartzia simplex and targeted MPLC isolation of its antifungal diterpenes

Quentin Favre-Godal[1], Emerson Ferreira Queiroz[1], Stéphane Dorsaz[2], Samad Nejad Ebrahimi[3], Laurence Marcourt[1], Matthias Hamburger[3], Jean-Luc Wolfender[1] *

[1]University of Geneva, [2]CHUV, University of Lausanne, [3]University of Basel

The dichloromethane extract of the root bark of *Swartzia simplex* var. grandiflora (Fabaceae) presented a noticeable antifungal activity in a bioautography assay against *Candida albicans* [1]. To localize the active compounds, biological profiling for antifungal activity was performed using at-line HPLC-microfractionation in 96-well plates and subsequent antifungal bioautography [2]. LC-HRMS was used for the dereplication in parallel to this procedure. The analytical HPLC-PDA conditions were transferred geometrically to a preparative medium-pressure liquid chromatography column (MPLC-UV) using chromatographic calculations. This gradient transfer ensure that the same selectivity and elution order was kept between the analytical and the preparative scale and provided an efficient isolation of the active compounds at the milligram scale. Using this approach 13 compounds were isolated, 6 of them were found to be new natural products. The structures of the isolated secondary metbolites were elucidated by classical spectroscopic methods including 2D NMR and HR-MS. The absolute configuration of some compounds was established by comparing experimental and calculated ECD spectra.

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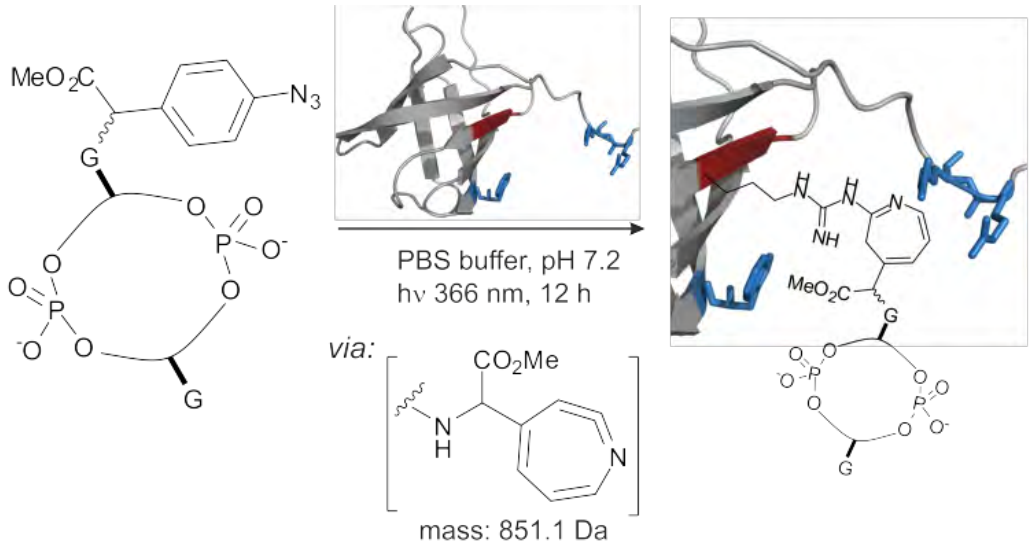
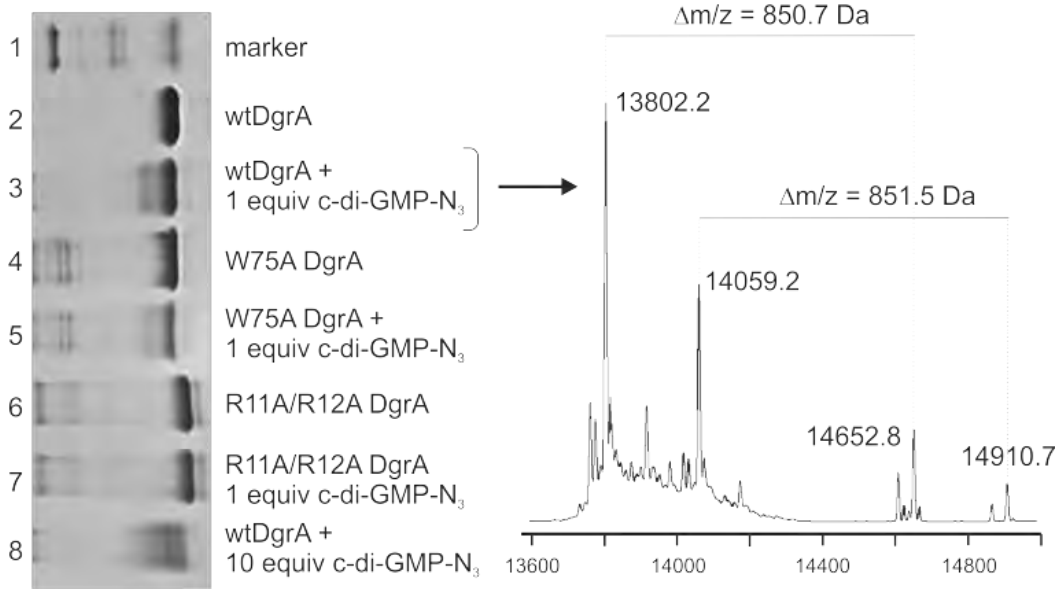
Catalytic carbene transfer allows the direct customization of cyclic dinucleotides

Na Fei[1], Dennis Gillingham[1] *

[1]University of Basel

We describe a simple method for the direct modification of nucleobases in cyclic dinucleotides (CDN), important signalling molecules in both prokaryotes and eukaryotes involving cell growth and immune response[1-2]. The method tolerates all members of the cyclic dinucleotide family and the modified c-di-GMP with ary azide tag was used to introduce useful side-chains such as fluorophores and proved to be a selective cross-linking probe that is efficient enough to determine binding sites in CDN receptors.





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Antifungal and acetylcholinesterase inhibitors from Croton heliotropiifolius

Marcos Ferreira Queiroz[1], Emerson Ferreira Queiroz[1], Claudia Avello Simoes Pires[1], Muriel Cuendet[1], Vanderlan Bolzani[2], Jean-Luc Wolfender[1] *

[1]University of Geneva, [2]São Paulo State University. brazil

The ethanolic extract of the stem bark of *Croton heliotropiifolius* Kunth (Euphorbiaceae) showed significant inhibition of acetylcholinesterase and antifungal activity against *Candida albicans* on thin layer chromatography bioautographic assays [1,2]. In order to target the isolation of the active compounds at a large scale, HPLC-activity-based-microfractionation in 96 well plates was used in a first step to localize the active compounds. Different regions of the HPLC-UV chromatogram were linked to the acetylcholinesterase inhibition and antifungal activities. Some active compounds were dereplicated by HPLC-PDA-ESI-MS and UHPLC-TOF-HRMS.The target isolation of the active compounds was performed by medium pressure liquid chromatography (MPLC-UV) and semi-preparative HPLC. Using this approach, nine compounds were isolated, one of them being a new indole alkaloid derivative.

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Investigating C(6')-butylamide tricyclo-DNA as a means for endosomal escape

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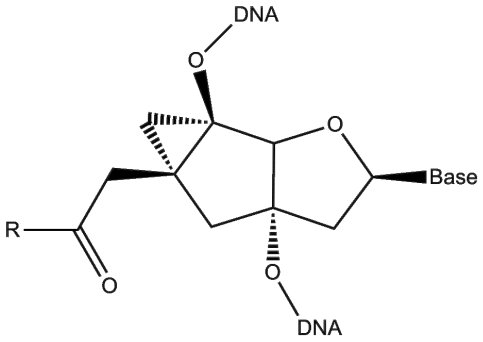
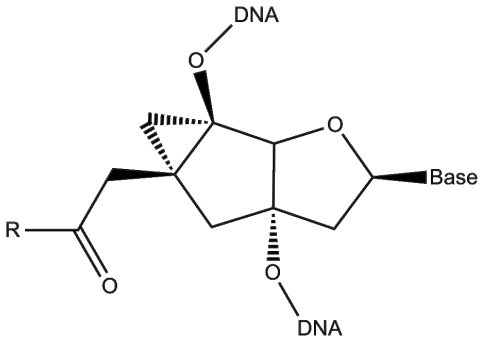
[1]University of Berne, Department of Chemistry and Biochemistry

Regulation of RNA expression through antisense therapy is a potential form of treatment for various genetic diseases that are currently not treatable with drugs (cancer, Duchenne Muscular Dystrophy…) [1]. However, major obstacles such as poor cellular uptake and slow release after endocytosis have arisen [2]. In this context, we previously demonstrated that our hydrophobic palmityl-functionalized tricyclo-DNA (tc-DNA **1**) was able to enter cells without the use of any transfection agent [3]. In addition, studies have shown that cell-pentrating peptides bearing blocks of hydrophobic residues and guanidinium functions can efficiently escape the endosome [4]. Consequently, combining both approaches, we modified the tricyclic sugar entity by introducing an alkylamide moiety at position C(6') (tc-DNA **2**). With this respect, we report the synthesis of a hydrophobic 6'-butylamide tricyclo phospharimidite, its incorporation into oligonucleotides and the cellular uptake properties of the afore-mentioned oligonucleotides.



$R=O(CH_2)_{15}CH_3$

1



$R=N(CH_2)_3CH_3$

2

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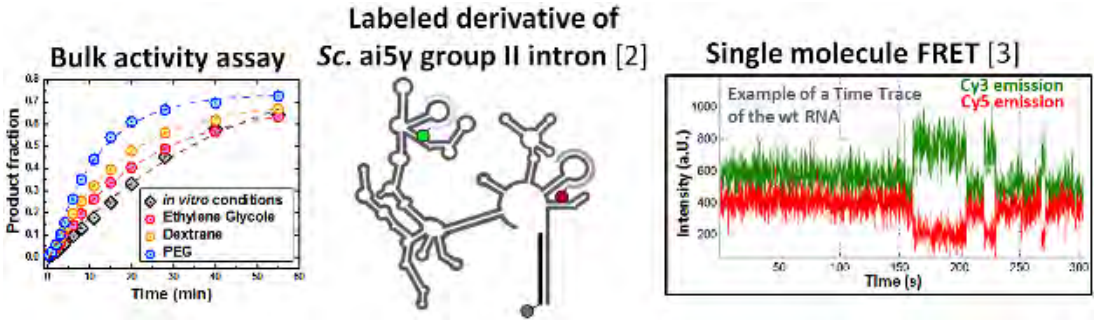
Close to near physiological conditions – A study under crowded conditions of group II intron ribozyme folding

Erica Fiorini[1], Richard Börner[1], Roland K.O. Sigel[1] *

[1]University of Zurich

Nowadays, the addition of molecular crowding agent to investigate their influence on the structure and function of macromolecules is a widespread tool to mimic the cellular environment [1]. Previously, folding studies have been carried out under conditions optimized for maximum catalytic activity *in vitro*: i.e. elevated temperature and high concentrations of cations, while all biological processes take place in the presence of lower ion concentrations and in a highly crowded cellular environment. We have combined bulk activity assays and single-molecule Förster Resonance Energy Transfer (smFRET) experiments to test if the presence of crowding agents (different molecules and concentrations) can stabilize the folding of a group II intron ribozyme at near physiological concentration of Mg^{2+}. Our studies reveal that upon the addition of crowding agents the ribozyme is able to reach more packed and quasi native folds at low Mg^{2+} concentrations. Moreover, the activity assays reveal that the concentration of Mg^{2+} required to obtain the fully active ribozyme is reduced to concentrations close to the physiological values in the presence of crowding agents.

Financial support by the ERC (to R.K.O.S.) and the University of Zurich is gratefully acknowledged.



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Strategic targeting of multiple water-mediated interactions in the design of potent and selective MMP-inhibitors

Thomas Fischer[1], Rainer Riedl[1] *

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Matrix metalloprotease-13 (MMP-13) is one of the enzymes belonging to the zinc-dependent endopeptidase family and is involved in angiogenesis as well as in tissue remodeling. An over activity of MMPs can lead to various pathological processes such as rheumatoid arthritis or tumor growth and metastasis.[1, 2]

We developed potent and selective inhibitors of MMP-13 by targeting multiple water-mediated interactions using structure based design and organic synthesis. After the generation of a novel active scaffold, we targeted conserved water molecules within the active site to form hydrogen bond networks to the target protein, aiming at increasing the inhibitor activity.[3] This resulted in a MMP-13 inhibitor with nanomolar potency (IC_{50}: 490 nM) and a very appealing selectivity profile against antitargets such as MMP-2 and MMP-14.

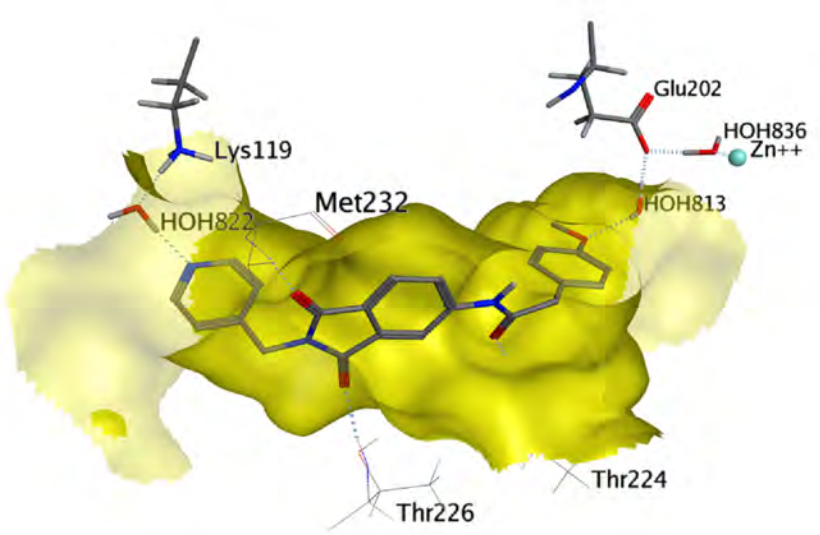

Fig.1: Interaction map: hydrogen-bonding network to structural water molecules in addition to direct hydrogen bonding to Thr 224, Thr 226 and Met 232.

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Functionalized Proline-Rich Peptides as Selective Binders of c-diGMP

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In recent years, the importance of bis-(3',5')-cyclic dimeric guanosine monophosphate (c-diGMP) as an ubiquitous secondary messenger in bacteria that plays crucial roles in the regulation of biofilm formation, cell cycle progression and virulence of several pathogens has been recognized.[1] Furthermore, its absence in higher order eukaryotes makes it an attractive target for the development of antibiotics.[1]

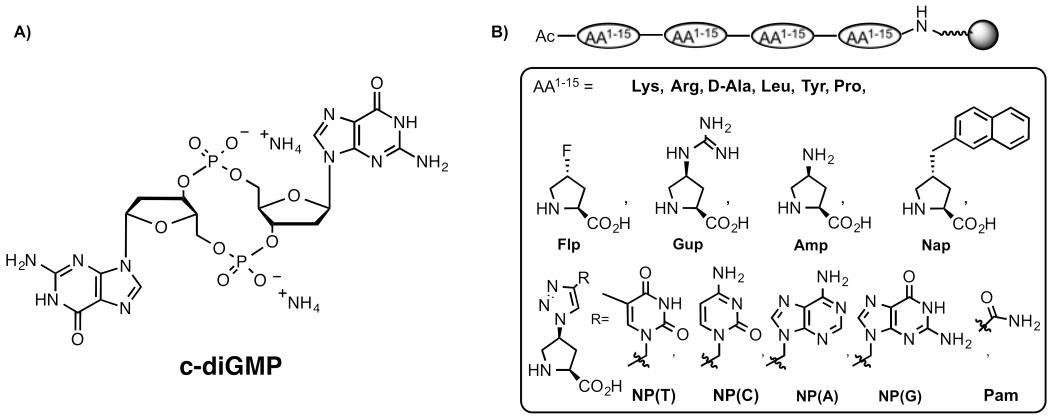

Figure 1: A) c-diGMP. B) Schematic representation of the split-and-mix peptide library.

A major challenge in the development of efficient binders for RNA is the limitation to electrostatic and aromatic stacking that typically are non-specific interactions. To address this issue we have prepared and screened a proline-rich split-and-mix[2] peptide library against c-diGMP. Using this technology, we have developed tetrapeptides that selectively bind to c-diGMP with affinities of $\Delta G \approx -5$ kcal/mol ($K_d \approx 180$ µM). We are currently using our lead sequence as a platform for the development of tighter binding peptides through the incorporation of new unnatural amino acids.

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Engineering an artificial carboxysome using capsid forming lumazine synthase

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Multiple bioreactions take place simultaneously within membrane bound organelles in eukaryotes and proteinaceous microcompartments in bacteria. These subcellular structures contain sets of enzymes co-involved in metabolic pathways [1]. Both natural and non-natural protein containers are extensively studied and engineered in the laboratory for applications in e.g. biocatalysis, bioimaging and drug delivery. Recently, our group developed an encapsulation system through design and evolution of the capsid forming protein lumazine synthase from *Aquifex aeolicus* (AaLS) [2, 3, 4]. Here, we seek to employ this encapsulation system to develop an artificial organelle that emulates the metabolic activity of the carbon fixating organelle of autotrophic bacteria, the carboxysome. Using an electrostatic tagging strategy, the two carboxysomal enzymes, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase (CA) were successfully targeted into AaLS capsid *in vitro*. Kinetic analysis of this simple carboxysome model suggests that RuBisCO benefits from a high local concentration of its substrate CO_2 which is provided by the co-encapsulated CA, leading to enhanced carbon fixation. In the next stage of the project, artificial carboxysomes will be assembled *in vivo* in *E. coli*, followed by optimization of carboxysome activity through directed evolution.

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Structural determination of the core region of the group II intron Sc.ai5γ and the role of the divalent metal ions in folding and structure

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Group II introns are among the largest natural ribozymes and are able to catalyze their own excision from RNA [1]. They have a common ancestry to the eukaryotic spliceosome which makes it interesting to study their structure and mechanism. Group II introns having a complex secondary structure consisting of six domains, depend on divalent metal ions for both folding and catalytic activity [2]. Situated at the core of the group II intron, a small region in domain 1 (D1), consisting of a three-way junction with the κζ motif folds first of the whole intron [3, 4]. This κζ region also acts as a scaffold for domain 5 (D5) to achieve the catalytically active structure. We have already studied this small part of D1 from the yeast mitochondrial group II intron *Sc*.ai5γ in the presence of Mg^{2+} ions [5]. However, our experimental results showed that D5 docking was not achieved probably due to non-native intradomain A-minor interactions [5]. We hypothesize that the extension of D1κζ (D1κζext) by adding the adjacent coordination loop will discrupt this A-minor motive. The coordination loop is expected to disrupt the intradomain interaction and thus allowing D1κζ/D5 docking. To reach our final goal we first focus on the structural characterization of the D1κζext region in solution by NMR spectroscopy. Our construct of interest is 57 nt long, which consists of the κ region and the coordination loop. Our first results showed that the three-way junction is still stabilized by Mg^{2+} ions, while interestingly the coordination loop adopts a defined structure in the absence of Mg^{2+} ions.

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Single-molecule studies on a biologically relevant RNA G-quadruplex

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Guanine-rich nucleic acid sequences aggregate into non-canonical, potasium-sensitive helical structures, called G-quadruplexes [1]. These sequences are thought to play a role *in vivo* [2, 3], being highly enriched in regulatory regions of DNA and RNA. RNA G-quadruplexes, often located in regulatory, non-coding regions of messenger RNAs (mRNAs) of oncogenes, offer new possibilities as novel antitumor targets. For our work, we chose NRAS (Neuroblastoma RAS viral oncogene homolog), an oncogene that is overexpressed in some types of leukemias and melanomas. Its mRNA contains a 18-nt G-quadruplex sequence in the 5' untranslated region, which has been shown to inhibit translation *in vitro* [4]. We are working in setting up a system to visualize the NRAS RNA via single-molecule Förster Resonance Energy Transfer (smFRET). We are interested in elucidating the dynamics and kinetics of the RNA G-quadruplex formation and dissociation, as well as observing possible folding or unfolding intermediates.

Finantial support within the COST Action CM1105, by an ERC Starting Grant 2010 (R.K.O.S.), a *Forschungskredit* of the University of Zurich (grant no FK-13-090, H.G.M.), by the Swiss National Science Foundation (R.K.O.S.) and by the University of Zurich is gratefully acknowledged.

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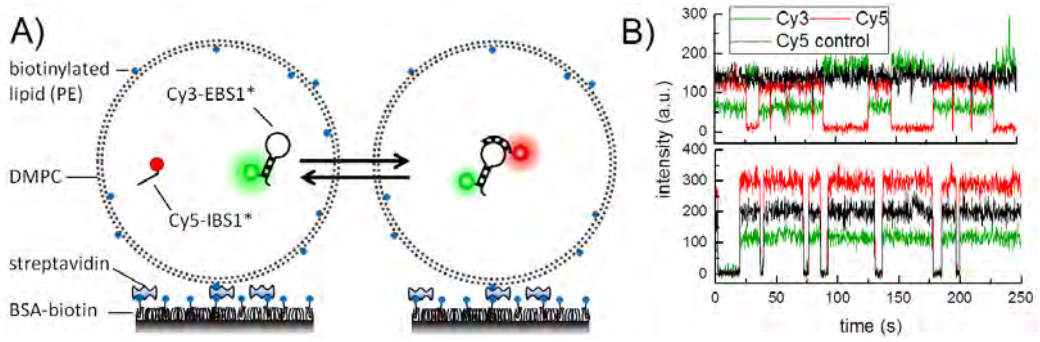
Following inter- and intramolecular dynamics of single encapsulated RNA molecules by FRET spectroscopy

Mélodie Hadzic[1], Sebastian König[1], Danny Kowerko[1], Roland K.O. Sigel[1] *

[1]University of Zurich

Single molecule FRET (Förster resonance energy transfer) is a state-of-the-art technique to investigate molecular dynamics, revealing sparse spatial configurations and kinetic heterogeneities hidden in bulk experiments. In bimolecular reactions, direct surface immobilisation of one reactive element allows long observation time but might bias the authenticity of the response. Co-encapsulation in surface-tethered vesicles, ensures both free diffusion and constant proximity of the reactants. After experimental procedure optimisation, we co-encapsulated - 1:1 ratio - a previously studied RNA duplex [1] (**Figure A**) and followed the successive associations/dissociations over time by 2-colour sm-FRET (top, **Figure B**). As encapsulation permitted to keep both RNAs in focal position - regardless if the duplex is formed or dissociated - we could alternatively collect the constant Cy5 emission from direct excitation by alternating laser pulses (black in **Figure B**). Hereby, we detected a new type of event unknown from the previous surface tethered studies, for which all fluorescence are quenched simultaneously (bottom, **Figure B**). Significance tests [2] based on the evaluation of the cross-sample variability, testify most probably of a new conformation displaying Cy3-Cy5 distance lower than 2 nm.

Single vesicle spectroscopy of small RNA systems - later extended to the large autocatalytic S. Cerevisiae ai5γ group II intron - as well as the development of Matlab-based analysis software [2,3], are both integral parts of my research.



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Synergistic folding and potency increase in an antimicrobial peptide pair against Pseudomonas aeruginosa

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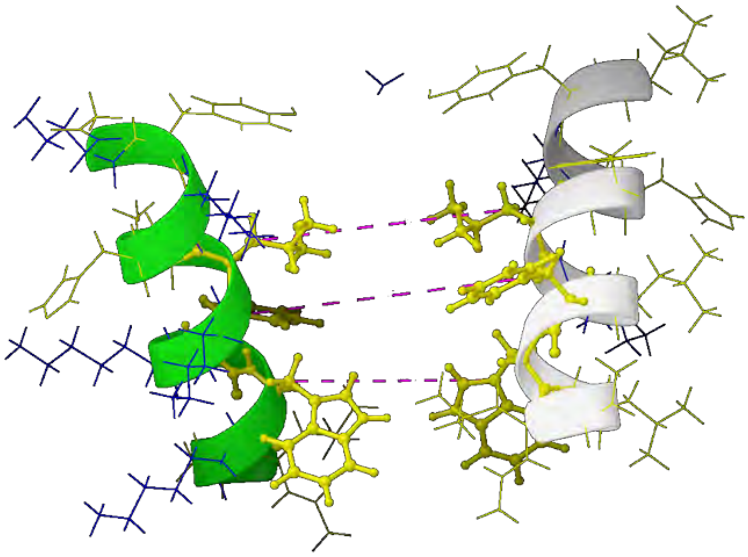
The Gram negative opportunistic pathogen *Pseudomonas aeruginosa* (PA) is one of the main causes of fatal hospital infections due to resistance to multiple antibiotics. Herein we report the discovery of potent antimicrobial peptides (AMPs) against PA. These AMPs were obtained by combinatorial diversification of a naturally occurring 13-mer AMP, which led to multiple analogs and a detailed structure-activity landscape showing that the new AMPs interact with the microbial membrane and fold to an a-helical conformation leading to membrane disruption. Several analogs with improved activity and peptide pairs acting synergistically were identified. The synergy is triggered by direct interaction between the two peptides with the more hydrophobic and structured component inducing folding of the less structured one to accelerate membrane interaction and increase activity against PA. Such synergistic folding and membrane disruption might represent a canonical mechanism in naturally occurring AMP mixtures.



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Novel fluorescent agonists for the A_1 adenosine receptor

Jennifer Hemmings[1], Graham Ladds[2], Anthony Knight[2], Bruno Frenguelli[3], Martin Lochner[1] *

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The adenosine receptors are members of the GPCR family and there are four sub-types: A_1, A_{2A}, A_{2B} and A_3. The A_1 adenosine receptor is involved in a range of processes in the CNS, including epilepsy and ischaemia. Despite its important role, relatively little is known about the trafficking of this receptor in neurons.[1] An agonist conjugated to a fluorophore would provide insight.

In collaboration with the University of Warwick, UK we have started to design and synthesise selective fluorescent compounds. In the first part of this project we have prepared a number of novel intermediates, with interesting activity at the A_1 adenosine receptor. The synthesis and initial biological results will be presented.

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Evaluation of Scoring Functions for QSAR within the S1´ Selectivity Loop of MMP-13

Stefan Höck[1], Rainer Riedl[1] *

[1]Zurich University of Applied Sciences, ZHAW

The discovery of potent and selective matrix metallo proteinase (MMP) inhibitors represents the key prerequisite for the modulation of this family of validated therapeutic targets in order to create treatment options for patients [1]. Modern medicinal chemistry projects require intense use of cheminformatics and computational chemistry for the design and discovery of novel drug molecules. We present a systematic evaluation of different scoring functions using OpenEye's Docking Toolkit [2] for the analysis of quantitative structure activity relationships (QSAR) of MMP-13 small molecule inhibitors binding into the selectivity loop of the target protein by a non-zinc binding motif. Our findings will allow for the targeted design of novel MMP inhibitors for the treatment of MMP-related disease pathologies such as cancer or rheumatoid arthritis.

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Towards Phosphoanhydrides via P^{III} chemistry

Alexandre Hofer[1], Gregor Cremosnik[1], Henning Jessen[1] *

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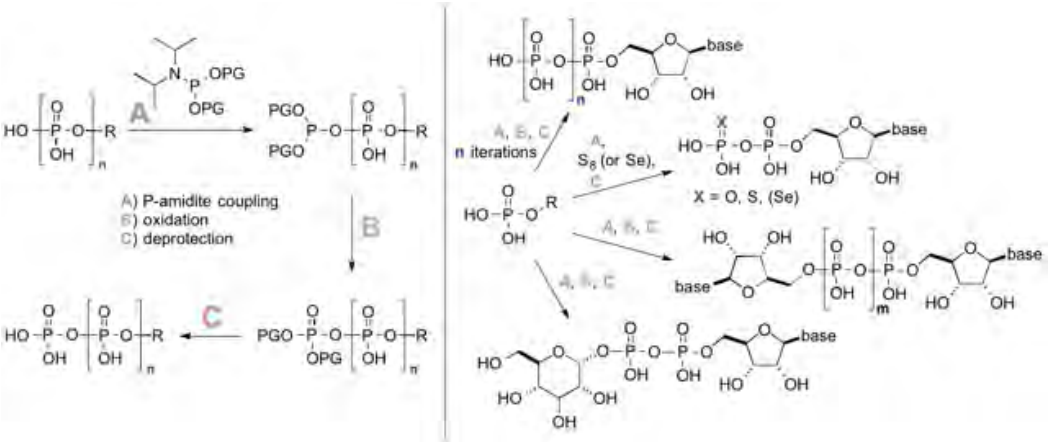
Phosphate esters and anhydrides are omnipresent in living systems, for instance as linking components in nucleic acids, as the energy carrying moieties of ATP, or as modifications for the regulation of protein activity[1]. The development of new synthetic routes to introduce and modify such functional groups is therefore likely to improve the accessibility to a wide range of natural products and molecules that can be used as tools for the investigation of various biological processes.[2]

We recently developed a straightforward method for the elongation of phosphate chains on nucleoside oligophosphates, which is based on couplings of P-amidites with nucleotides[3]. The three reaction steps (coupling, oxidation, deprotection) are fast, highly selective, and quantitative, what allows to precipitate the products in high purity and avoids the need of tedious purification steps[4].



This method could be modified in order to introduce thiophosphates at different positions in nucleotides. The use of amidites bearing only one protection group and another organic moiety furthermore allows the preparation of dinucleoside polyphosphates and nucleotide sugars in a similarly convenient method[5].

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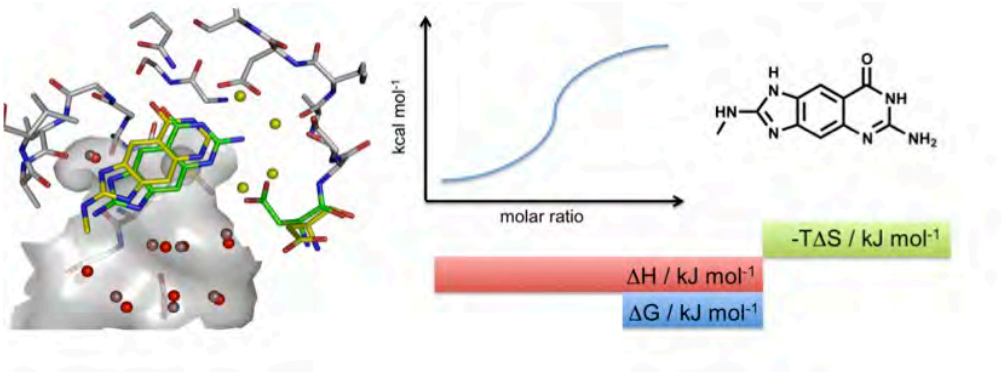
How ITC, Mutagenesis, and pKa Calculations Trace the Locus of Charge in Ligand Binding to a tRNA-Binding Enzyme

Christoph Hohn[1], Luzi Barandun[1], Manuel Neeb[2], Florian Immekus[2], Gerhard Klebe[2], François Diederich[1] *

[1]ETH Zurich, [2]Philipps-Universität Marburg

Shigellosis, caused by the *Shigella* bacterium, is an intestinal infection. The emergence of multidrug-resistant strains demands the development of novel drugs. *t*RNA−guanine transglycosylase (TGT), one enzyme involved in the virulence mechanism, has been identified as a drug target and inhibition was shown to reduce virulence dramatically. Due to the structural similarity of the active sites, the *Z. mobilis* TGT has been utilized as a model system.



A *lin*-benzoguanine scaffold was previously identified as the central core of a series of highly potent inhibitors.[1] The high affinity can be explained by a charge-assisted hydrogen bond, which could recently be investigated in more detail by isothermal titration calorimetry (ITC).[2] Comparison of a series of *lin*-benzoguanines and *lin*-benzohypoxanthines reveals significant changes in the binding mode, inhibition constant, and pK_a values. The binding modes were resolved by several X-ray crystal structures of TGT–ligand complexes.[3]

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Modified nucleoside triphosphates: synthetic tools for chemical biology

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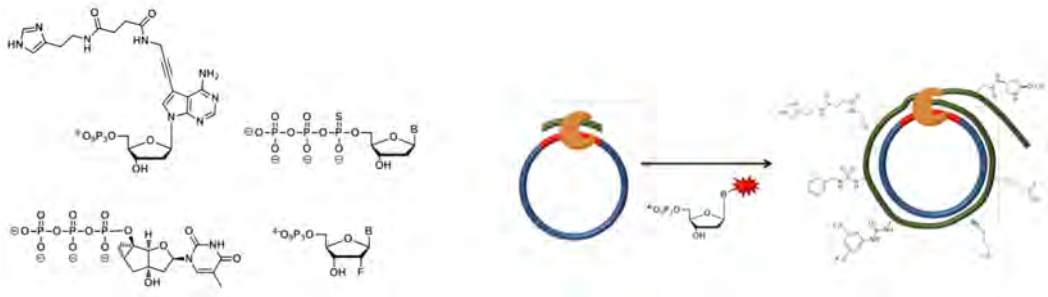
[1]University of Berne

The chemical alteration of nucleic acids is of interest for numerous potential practical applications including the development of antisense and antigene agents.[1-3]The traditional method, which uses suitably modified phosphoramidite building blocks as vectors, is restricted to certain functionalities due to the conditions imposed by solid-phase synthesis and as well to rather short sequences.[4,5] On the other hand, modified nucleoside triphosphates (dNTPs) have advanced as a mild and versatile method for the introduction of modifications into nucleic acids.[6-8] Indeed, provided that the dNTPs are substrates for DNA polymerases, virtually any functionality can be introduced. In addition, dNTPs are compatible with enzymatic amplification techniques and can be applied in SELEX for the generation of aptamers and catalytic nucleic acids.[9]Here, the synthesis of dNTPs equipped with side chains capable of organocatalysis[10] and amino acids that mimic the catalytic triad of proteases[11]is presented (see Figure).
Moreover, the compatibility of these modified dNTPs with DNA polymerases was established, further showing that their simultaneous polymerization will increase the chemical space that can be explored during in vitro selection of DNAzymes with hitherto unknown reactivities.[12] In addition, the use of Terminal deoxynucleotidyl Transferase (TdT) allows for the template independent polymerization of modified dNTPs, further underscoring the versatility of these nucleoside analogues. Finally, these analogues were used in rolling circle amplification (RCA)[13]



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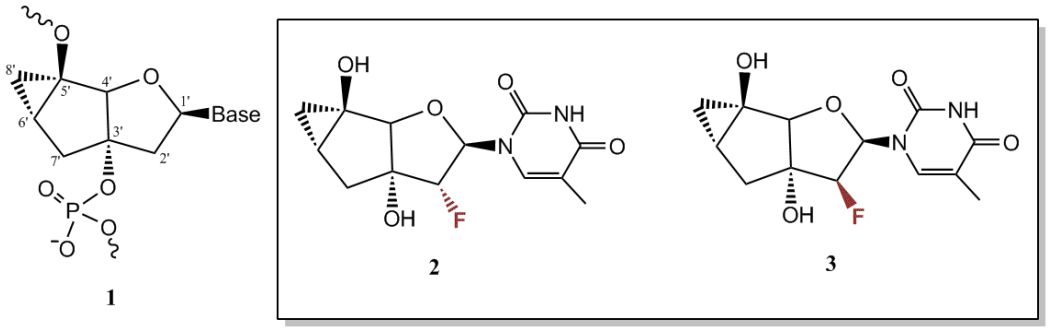
Synthesis and Pairing Properties of 2’-Fluoro-Tricyclo-DNA

Alena Istrate[1], Christian Leumann[1] *

[1]University of Berne

A wide range of modified nucleosides have been synthesized to improve target affinity, nuclease resistance, cellular uptake and distribution of biologically active oligonucleotides. Oligonucleotides containing 2’-deoxy-2’-fluoronucleotides were recently found to have high binding affinity to its complementary DNA and RNA and improved stability towards enzymatic digestion. [1, 2]
In earlier work we have developed the conformationally constrained oligonucleotide analog tricyclo-DNA (tc-DNA, **1**) which shows improved binding properties to complementary RNA and is stable in serum. [3] Based on these findings we recently became interested in the synthesis and characterization of 2’-fluorinated analogs of tc-DNA.



Here we report on the synthesis of two novel fluorinated tricyclo nucleosides, 2’-α-F-tc-T (**2**) and 2’-β-F-tc-T(**3**), and their incorporation into DNA oligonucleotides. The stability of 10-mer duplexes containing modified units was studied by thermal denaturation experiments. Compared to unmodified DNA, oligonucleotides with 2’-F-α-tc-T units bind with higher affinity to their DNA and RNA complements. Preparation of the modified oligonucleotides for the cellular uptake experiments is currently in progress.
br

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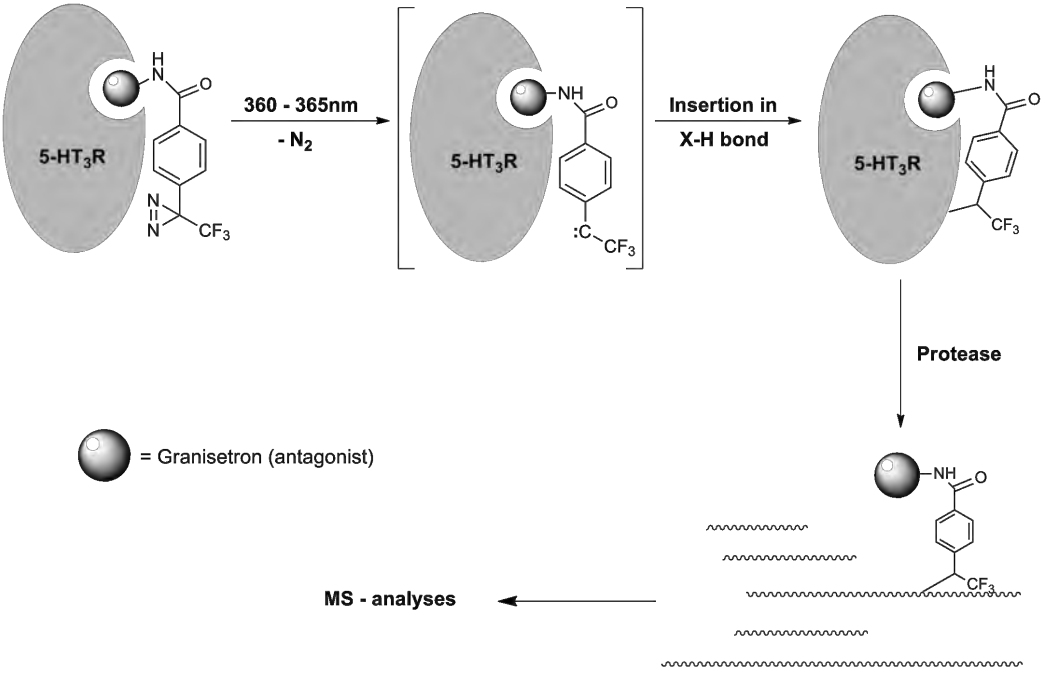
Site-selective Chemical Modification of the 5-HT3 Receptor with Newly Developed Photo-Crosslinking Probes

Thomas Jack[1], Marc-David Ruepp[1], Andrew J. Thompson[2], Oliver Mühlemann[1], Martin Lochner[1] *

[1]University of Berne, [2]University of Cambridge

The 5-HT_3 receptor (5-HT_3R) is an important ion channel responsible for the transmission of nerve impulses in the central nervous system.[1] It is difficult to characterize transmembrane dynamic receptors with classical structural biology approaches like crystallization and x-ray. The use of photoaffinity probes is an alternative approach to identify regions in the protein that are important for the binding of small molecules. Therefore we synthesized a small library of photoaffinity probes by conjugating photophores via various linkers to granisetron which is a known antagonist of the 5-HT_3R. We were able to obtain several compounds with diverse linker lengths and different photolabile moieties that show nanomolar binding affinities for the orthosteric binding site.[2] Furthermore we established a stable *h*5-HT_3R expressing cell line and a purification protocol to yield the receptor in a high purity. Currently we are investigating the photo crosslinking of these ligands with the 5-HT_3R.



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Stereochemical bias introduced during RNA synthesis modulates the pharmacological properties of phosphorothioate siRNAs

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An established means of improving the pharmacokinetics properties of oligoribonucleotides (ORNs) is to exchange their phosphodiester linkages for phosphorothioates (PS). However, this strategy has not been pursued for small interfering RNAs (siRNAs). The PS-group is chiral at phosphorous (*R*p/*S*p centers).We have performed a detailed study of the coupling mechanism and showed the influence of different activators on the ds-ratio.Specifically, we analyzed the ds-ratios of all possible 16 PS-dinucleotide motifs of RNA to give a picture of Rp- to Sp- distribution in siRNAs. Since Rp-/Sp-diastereoisomers have different chemical and biological properties e. g. affinity, activity and stability [1][2], we show uniform PS-modified siRNAs with different population of stereochemically-biased may provide a useful compromise of their pharmacokinetics and pharmacodynamics properties in RNAi therapeutics.

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The influence of Mg^{2+} ions on single RNA tertiary contact seen at single molecule level

Mokrane Khier[1], Sebastian König[1], Danny Kowerko[1], Roland K.O. Sigel[1] *

[1]University of Zurich

The enormous potential of RNA for biotechnological and medical applications requires more than ever a clear picture and fundamental understanding of how RNA folds and acts. As the RNA folding process is inescapably related to the surrounding metal ions, understanding metal ion-RNA interactions is a key step for understanding the RNA folding process [1].

We are applying single molecule Förster Resonance Energy Transfer (smFRET) to characterize the influence of metal ions on a single RNA tertiary contact, consisting of an RNA hairpin, known as EBS1* (Exon Binding Site 1), interacting with its cognate (Intron Binding Site 1). The cognate was either an RNA (IBS1*) or DNA (dIBS1*) fragment [2]. Our results show that the two interactions differ slightly in the conformation of their bound state, in a qualitative agreement with the NMR results performed on the same structures [3,4]. In parallel, the thermodynamic and kinetic analysis indicates that the affinity of EBS1* toward the RNA cognate is almost two orders of magnitude higher than its affinity toward the DNA one, independent from the nature of metal ions in the solution. However, in the case of the EBS1*-IBS1* interaction, the presence of Mg2+ leads to a pronounced heterogeneity in the kinetic of this interaction due to the presence of at least two kinetic rates describing the dissociation process, a finding that was not observed for the same interaction in the presence of high amount of K+ nor in the case of EBS1*-dIBS1* in the presence of both Mg2+ and high amount of K+.

Based on our results and previous work that had pointed out at least three potential binding sites for metal ions in EBS1*-IBS1* [4], we propose a model for the interaction where the kinetic heterogeneity seen in the presence of Mg2+ results from the different combination of occupying the three binding pockets within EBS1*-IBS1* structure.

Financial support from the ERC to R. K. O. S, the SNF to R. K. O. S and the University of Zurich is gratefully acknowledged.

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Betaines: the missing link in understanding fungal physiology and metabolism

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Betaines are characterized as neutral compounds which combine a non-protic cationic and an anionic function. The most studied natural example is glycine betaine which is a ubiquitous metabolite in microorganisms, plants and animals. Glycine betaine serves as cellular osmoprotectant, as methyl donor in one-carbon metabolism and possibly in signal transduction. Much less is known about betaines based on amino acids with functional side chains. For example, aromatic amino acid betaines such as ergothioneine or hypaphorine are also present in many life forms and, because of their redox active side chains, they may play additional roles in redox homeostasis. Our discovery of the biosynthetic origin of several such aromatic betaines in bacteria and fungi allows us to explore this little known but seemingly important class of secondary metabolites.

Novel Kinase Inhibitors for PKA and PKB targeting the phosphate-loop

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The wide occurrence of more than 500 kinases in the human genome and it's important roles in diseases such as cancer and diabetes make kinases to an attractive target in drug design.[1] Protein Kinase A (PKA) and B (PKB, also called akt) play important roles in the regulatory processes of glycogen, sugar, and lipid metabolism, and are furthermore involved in signal transduction processes. Selectivity for one specific kinase has been extensively discussed in antitumor strategies and is of high interest for the pharmaceutical use.[2,3] PKA is a widely used model for many kinases, as it is a well known enzyme with good availability and handling properties. To study PKB affinities a PKA triple mutant, a so called PKAB3[4] is often used as a model to mimic the active pocket.

In our research, we have used structure-based design and 3D modeling as tools for inhibitor design. The novel inhibitors show single-digit-nanomolar affinity against PKA and the recently resolved co-crystal structures enable us to obtain insights into the P-loop of PKA and PKB to investigate the interactions. All tested inhibitors (n = 32) were ATP-competitive inhibitors, interacting with the hinge-region of the active pocket, reaching the ribose pocket and targeting the phosphate-loop.

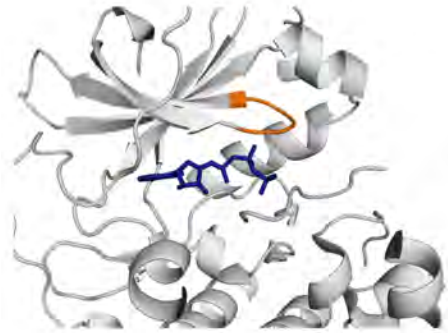

Fig. 1: co-cristal structure of PKB (grey) with AMP-PNP (blue). In orange the glycine-rich loop is indicated. PDB-code: 106K[5]

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Synthesis of Inhibitors and Probes for the Cellular Study of Glutamate Transporters

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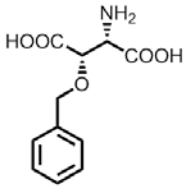
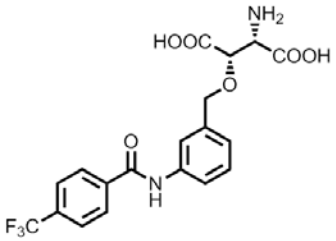
Glutamate transporters of the SLC1 family play a central role in removing released glutamate from the synaptic cleft, terminating excitatory neurotransmission.[1] Because a high extracellular glutamate concentration causes excitotoxicity, glutamate transporter dysfunction is associated with neurodegenerative disease (e.g. amyotrophic lateral sclerosis, Alzheimer's disease) and ischemic damage (e.g. after a stroke). Information about the substrate and small molecules binding sites of glutamate transporters is rather sketchy and would be highly beneficial to design new potential drugs**.**

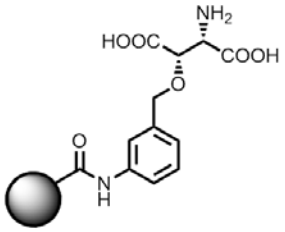
To expand our knowledge of glutamate transporter substrate and inhibitor binding sites, a rapid synthesis of analogues of L-*threo*-β-benzyloxyaspartate (**L-TBOA**) and (2*S*,3*S*)-3-[3-[4-(trifluoromethyl) benzoylamino] benzyloxyaspartate (**TFB-TBOA**), was developed and utilized for the preparation of photoaffinity probes (**PA-probes**).[2]



L-TBOA



TFB-TBOA



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Modulation of Y-family DNA polymerase-mediated translesion synthesis by nucleotide analogs detected by a fluorescence-based method

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[1]ETH Zurich, [2]Michigan Technological University

Translesion DNA synthesis (TLS) enables cells to avoid the cytotoxic stalling of replicative DNA polymerases at chemotherapy-induced DNA lesions like O6-methyl-guanosine from temozolomide, N7-guanine-crosslinks from cisplatin and N3-methyl-adenosine from methyl lexitropsin, thereby leading to drug resistance. Overcoming drug-resistance by inhibiting TLS is a therapeutic strategy that requires the development of suitable analytical methods for the identification of inhibitors. Existing strategies have proven successful in identifying new inhibitors from high-throughput screens, but involve the displacement of a fluorescent DNA strand upon DNA polymerase primer elongation therefore specific DNA sequences and several nucleotide incorporation events are required. Since polymerase-mediated DNA synthesis involves the release of inorganic pyrophosphate (PPi), we established a universal and fast method for monitoring the progress of DNA polymerases based on the quantification of PPi with a fluorescence-coupled assay that involves an enzyme-coupled conversion of the non-fluorescent substrate Amplex Red to the red-fluorescent resorufin (PiPer^{TM}), which we coupled to *in vitro* primer extension reactions (PE-PiPer). For PE-PiPer, the DNA polymerase primer extension was quenched prior the fluorescence readout, enabling the evaluation of DNA polymerase inhibition and kinetics. The approach was validated for primer extension reactions with DNA Polymerase IV (Dpo4) from *Sulfolobus Solfataricus* and natural DNA templates by comparison with conventional gel-based electrophoresis. Subsequently, we sought to evaluate nucleotide analogs-mediated Dpo4 and human Pol η replication inhibition over natural DNA as well as DNA carrying O6-methyl-guanine, intrastrand cisplatin crosslink or 3-deaza-3-methyl-adenine lesions. Our results show a lesion-specific modulation of DNA polymerase activity that was reduced up to about 25% in presence of nucleotide analogs. The PE-PiPer method enables the study of DNA polymerase activity compatible with high-throughput settings and allows the discrimination of small alterations in the activity of these enzymes over natural or modified templates, providing a tool to screen for DNA polymerase inhibitors.

Probing DNA Structures and Dynamics with Fluorescent Nucleoside Analogs

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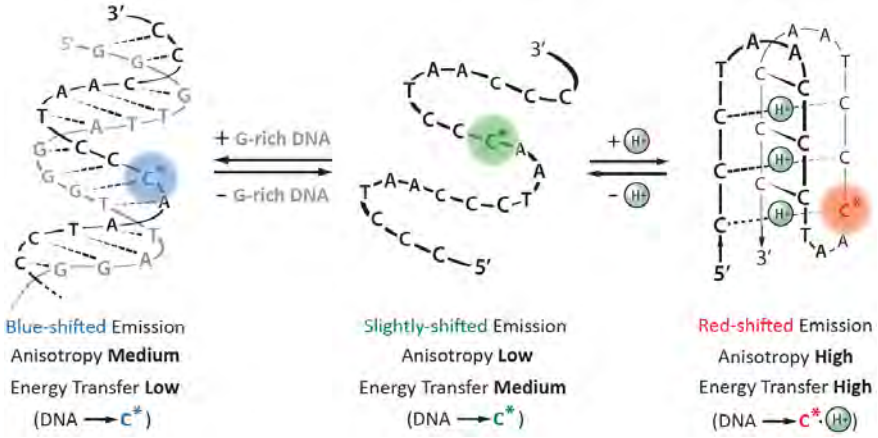
DNA is regarded by most as a uniform double helix and a passive library of genetic information. DNA structure is, however, highly dynamic and it can fold into a wide variety of secondary structures, such as G-quadruplex and *i*-motif DNA. However, very little is known about the potential biological relevance of these non-duplex DNA structures *in vivo*. Specific probes capable of reporting such structures will provide important new tools for expanding our current understanding of DNA biology, and may provide new leads for anti-cancer agents. Characterization of DNA folding and dynamics can be accomplished using spectroscopic analysis of a fluorescent probe that mimics a natural nucleobase. The heterocycles commonly found in nucleic acids are practically non-emissive, but they are amenable to diverse modifications that endow fluorescence properties. In this work, a stereoselective *N*-glycosylation was developed to give access to newly designed, fluorescent nucleoside analogs [1]. These molecules exhibit solvatochromic and push-pull fluorescence due to twisted intramolecular charge transfer states [2]. A fluorescent cytosine analog has been implemented in oligonucleotides (see Figure) and is capable of reporting DNA secondary structures as well as real-time strand displacement reactions.



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Sphingoid Base Analogs as Biochemical Tools

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[1]University of Fribourg

Sphingolipids are ubiquitous components of eukaryotic cell membranes, where they play important roles in intracellular signaling and in membrane structure. The sphingoid base shows species-specific structural differences: straight-chain C18 sphingoid base building blocks in mammals and budding yeast, shorter C14 bases in flies. We have synthesized the nematode-specific C17 iso-branched sphingoid base and its 1-deoxy (spisulosine) analog (Figure 1) to better understand sphingolipid physiology in the context of intestinal development and resistance to anoxia.

Figure 1: Targeted iso-branched sphingoid bases

The project impressively shows species-specificity for sphingolipids as straight-chain sphingoid bases are non-functional in worms and iso-branched sphinganine shows strong toxicity in budding yeast. The synthesis of this sphingoid base from nematodes and its 1-deoxy analogue will be crucial to study their function in development and anoxia resistance.

Discovery of Fucose/Galactose Heteroglycopeptide Dendrimers as Dual Biofilm Inhibitors Targeting Pseudomonas aeruginosa Lectins LecA and LecB

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[1]University of Berne

The gram-negative bacteria *Pseudomonas aeruginosa* is among the top five organisms causing lung, urinary tract and kidney infections. It is one of the main causes of antibiotic-resistant nosocomial infections today. Previous studies have shown that biofilm formation is partly mediated by the galactose-specific lectin LecA (PA-IL) and the fucose-specific lectin LecB (PA-IIL).[1] The possibility of inhibiting LecA and LecB has generated wide interest in the development of carbohydrate-based inhibitors of these lectins. The first multivalent *P. aeruginosa* biofilm inhibitors, **FD2** targeting LecB (Figure 1a) [2] and **GalAG2** targeting LecA [3][4]were reported in our group.

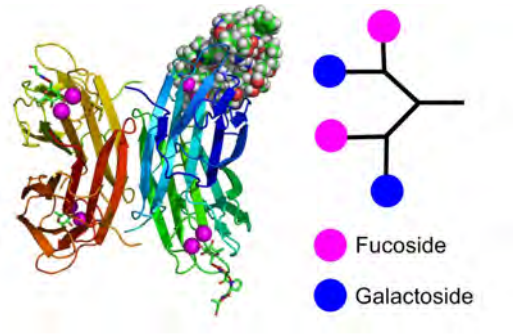

Figure 1: a) Model of the dendrimer FD2-LecB complex obtained by molecular dynamics and energy minimization. [Ca2+] ions are in magenta and indicate the location of the fucose binding pocket. Three binding sites are shown with the resolved fucosyl residues of c-Fucoside KPL peptide and the fourth site is modelled with dendrimer FD2. b) Schematic structure of heterogylcopeptide dendrimers.

Here, we hypothesized than heterogylcopeptide dendrimers combining two fucosides and two galactosides within two adjacent arms (Figure 1b) can achieve lectin binding. Binding to lectins LecA and LecB was measured by Isothermal Titration Calorimetry. Heterogylcoclusters binds both lectins with very high affinity; K_d values in the nanomolar range were measured. These data let suppose the occurrence of a cluster effect. Moreover these compounds show good activities in biofilm inhibition and biofilm dispersion assays.

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New highlights on the interaction mechanism between coenzyme B_{12} and the btuB riboswitch

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[1]University of Zurich

The *btuB* riboswitch is a bacterial RNA sequence able to control gene expression of a B_{12} carrier protein by specifically binding coenzyme B_{12}[1]. The B_{12} metabolite can interact with this large RNA through different moieties. Our research focuses on understanding this binding mechanism, investigating the influence of B_{12} differently modified derivatives. Previous work already demonstrated the importance of the adenosyl moiety for the affinity of the metabolite to the RNA, meanwhile the corrin ring is determinant for the correct structural rearrangement of the riboswitch[2,3]. Moreover, the presence of a specific binding pocket for the adenosyl moiety was identified in recent X-ray structures of B_{12}-riboswitches[4,5]. To further elucidate the role of a correct H-bonding and electrostatic network for the B_{12}-RNA interaction, new B_{12} derivatives modified on the corrin ring sidechains *b* and *e* have been synthesized. Many primary amide sidechains are protruding from the corrin ring of the B_{12} metabolite. We modified these sidechains to study how the presence of a carboxylic group or a secondary/tertiary amidic groups influences the structural rearrangement of the *btuB* riboswitch. To investigate the impact of these chemical modifications, we exploited in-line probing assays.

The experiments performed in this work confirmed the importance of the adenosyl moiety for a high binding affinity to the RNA. Chemical modifications on the sidechain *e* seem to affect the structural rearrangement of the RNA but not the affinity. Meanwhile, the presence of a secondary amide on the sidechain *b* increases the affinity to the riboswitch and leads to differences in the cleavage pattern of the RNA. The negative charge in the corrin ring sidechains usually decreases the binding affinity and leads to differences in the RNA structural rearrangement.

Financial support by the European Research Council (ERC starting grant 2010 259092-MIRNA to R.K.O.S.), the Swiss State Secretariat for Education, Research and Innovation (COST Action CM1105) and the University of Zurich is gratefully acknowledged.

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Chemical Basis for Modulating Human DNA Polymerase η-mediated Bypass and Extension Past the Major Cisplatin-DNA Adduct

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

Cisplatin is a widely used chemotherapeutic agent that binds to DNA, forming cross-link adducts that block DNA replication and induce apoptosis, but replicative bypass catalyzed by the human Y-family DNA polymerase η (hPolη) leads to platinum drug resistance. Therefore, hPolη has been suggested as a drug target for improving cisplatin efficacy and despite some inhibitors of hPolη already discovered, very little is known about how these compounds interact with the polymerase or by which mechanism they inhibit it. We propose that nucleoside analogs with a propensity to be inserted opposite DNA damage and block extension of DNA synthesis can be used as tools for elucidating the kinetic, structural, and functional basis of bypass of platinum-DNA cross-link adducts. In this study we tested two nucleoside triphosphates that vary in hydrogen-bonding potential as hPolη substrates and characterized their influence on hPolη-mediated DNA synthesis. The nucleotide analogs were found to be incorporated by hPolη opposite natural as well as platinum cross-linked DNA templates. Steady-state kinetics of incorporation was determined and showed that the analog bearing an additional carbonyl group is incorporated over 10-fold more efficiently on both DNA templates. Furthermore, full-length DNA synthesis experiments were carried out to assess the ability of the nucleotide analogs to inhibit product formation. Regardless of the template, increasing the concentration of synthetic nucleotides reduced the amount of product, suggesting that the nucleotide analogs influence the processivity of hPolη. These data provide new insight regarding how small chemical changes to nucleotide triphosphates have a significant influence on platinum adduct bypass by hPolη and can serve as a starting point for generating novel therapeutics that overcome platinum drug resistance in cancer therapy.

Thermosome – a cage protein for targeted delivery of macromolecules

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[1]University of Basel, [2]University of Fribourg

Protein cages such as viral capsids, heat shock proteins and chaperonins are a new nanotechnology-based delivery platform with a broad field of application. Several studies show the potential for the delivery of drugs and contrast agents by protein cages.[1,2] However, the capability of protein cages to encapsulate and deliver drugs is most often restricted to smaller molecules. In this study, we show *in vitro* targeted delivery of macromolecules by the protein cage thermosome (THS), a chaperonin from *T. acidophilum*.[3] The advantage of THS over other protein cages is that it features large pores to the cavities of THS, where macromolecules can be accommodated.

In a first example dendritic cationic poly(amidoamine) (PAMAM) were conjugated to cysteines which are only present in the interior of the cavity. This cationic polymer is able to bind and stabilize small interfering RNA (siRNA) by electrostatic interactions.[4] siRNA is degraded rapidly by RNase, e.g. in serum. Our experiments show binding and stabilization of siRNA by the THS-PAMAM complex. Additionally, we could show that THS-PAMAM was able to deliver the oligonucleotide into cells *in vitro* and that an siRNA silencing effect was achieved.

A second approach aims to use the THS as delivery vehicle of pharmaceutically active peptides (PAP), which can be conjugated into the cavities via a disulfide bond. This redox-sensitive linker can be reduced in cells, which would lead to the release of PAP would and actuate their pharmaceutical action. THS was modified on its outer surface with the targeting ligand vEGF to achieve cell-targeting or with the cell penetrating peptide TAT to enhance the cellular uptake. vEGF modified THS showed specific uptake by HUVEC cells and with TAT a 75-fold increase of cell uptake was observed. Studies are underway to determine the efficiency of PAP delivery with the surface-modified protein cage.

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Site-specific post-synthetic oligonucleotide labeling for single-molecule studies

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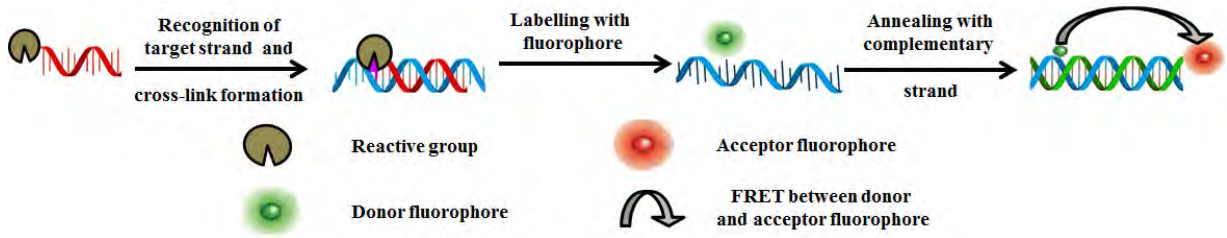
[1]University of Zurich

Single molecule spectroscopy has been used intensively to investigate mechanisms in living cells [1]. This technique requires the sample to be functionalized to achieve dye conjugation in a site-specific manner. Currently, our research focuses on a new labeling strategy for the site-specific functionalization of long oligonucleotides.

The newly developed strategy is based on a modular system consisting of three parts: (i) a short oligonucleotide recognition sequence required to sense the specific position in the target DNA template by annealing of the two strands, (ii) a reactive group that is specifically designed to generate the desired functionalization, as well as optionally (iii) a cleavable part inserted between the complementary sequence and the reactive group to cleave the intermediately formed covalent cross-link between the modified oligonucleotide and the target DNA. Among the new functional groups that we were able to introduce so far are alkyne, aldehyde, and thiol groups. After coupling of a fluorophore to the new functional group, such a modified oligonucleotide can be used, e.g., for FRET studies.



Financial support by the Swiss National Science Foundation (EF), the Forschungskredit of the University of Zurich (DE), as well as the COST Action CM1105 is gratefully acknowledged.

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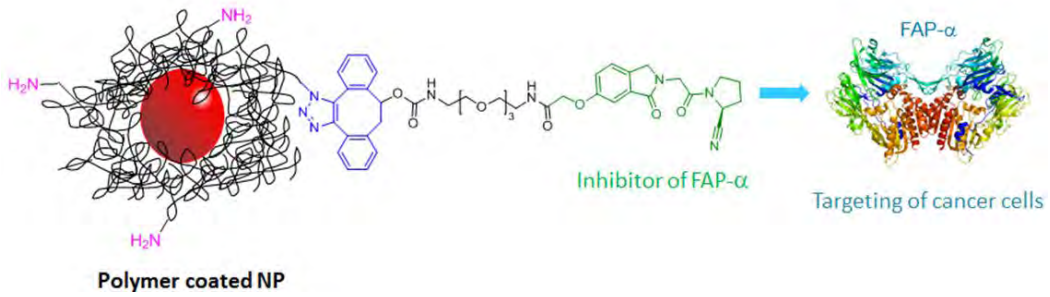
Functionalization of second harmonic nanoparticles with inhibitors of prolyl-endopeptidases for cancer cells labelling and imaging

Solène Passemard[1], Davide Staedler[1], Lucienne Juillerat-Jeanneret[2], Sandrine Gerber[1] *

[1]EPFL Lausanne, [2]CHUV, University of Lausanne

Medical imaging is a major tool for the prevention and detection of cancer. The development of functionalized inorganic nanoparticles (NPs) combining recognition of cancer specific molecular biomarkers with multimodal imaging capabilities is expected to provide a qualitatively new level of sensitivity and accuracy for the detection of malignant diseases. We report herein the preparation of polymer coated metal oxide NPs and their conjugation to inhibitors of prolyl endopeptidases for the detection and labelling of breast and lung cancer cells. In particular, the targeting of fibroblast activation protein-α (FAP-α), which is a cell surface antigen of reactive fibroblasts in cancer but is not found in healthy tissues,[1] was envisaged for the discrimination between tumor and healthy cells. Bismuth iron oxide (BFO) harmonic nanoparticles were conjugated to inhibitors of FAP-a based on a Gly-Pro sequence,[2] through bioorthogonal copper-free click reaction.



The ability of the resulting nanomaterials for the selective labelling of cancer cells and for their imaging by multiphoton microscopy was demonstrated *in vitro*. We also established that this methodology can involve other inorganic oxide nanoparticles (eg: Fe_3O_4 NPs) and can be universal to other types of targeting molecules (eg: c(RGDfK)).

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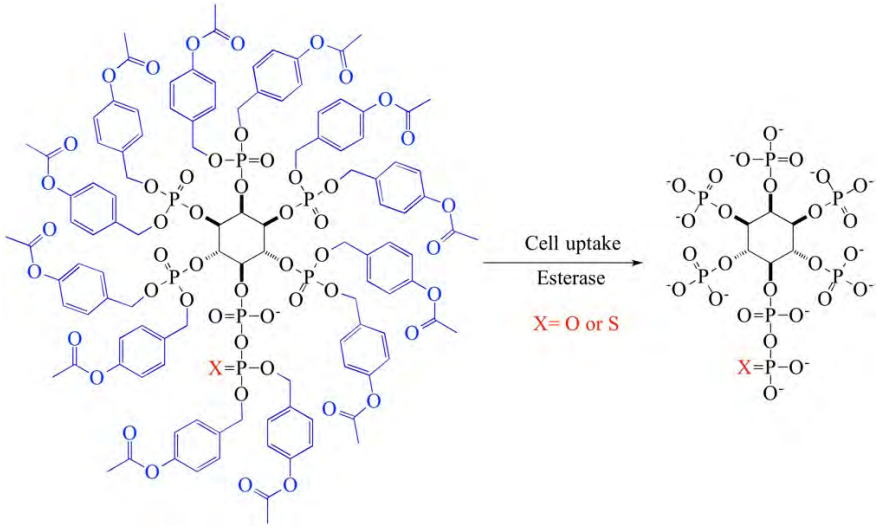
Development and Application of Diphosphoinositol Polyphosphate Analogs

Igor Pavlovic[1], Henning Jacob Jessen[1]

[1]University of Zurich

Recently, the second messengers diphosphoinositol polyphosphates moved into sharp focus of research as their importance in different cellular processes like apoptosis, cytoskeleton dynamics or telomere length regulation became obvious.[1]Phosphorylations of proteins, metabolites and cofactors are omnipresent in nature and consequently phosphates and phosphoanhydrides provide unique binding scaffolds for molecular recognition.5-PP-InsP_5 can act as phosphate donor by donating β-phosphate to different proteins *in vitro*.The phosphate transfer from 5-PP-InsP_5 generates a protein pyrophosphate. To discriminate it from regular phosphorylations, this process has been dubbed transphosphorylation.[2]This novel posttranslational modification is poorly understood *in vitro* and not yet confirmed *in vivo*. Therefore new chemical biology approaches are highly desirable. Studying 5-PP-InsP_5 and its processes in living cells using synthetic analogs is a significant challenge. The natural product contains up to 13 negative charges and is thus membrane impermeable. We are now ableto modify 5-PP-InsP_5 with different interesting tags and protecting groups.

Here, we present the synthesis and biological studies of 5-PP-InsP_5 analogs with biolabile protecting groups[3] that will facilitate *in vivo* studies as they enable cellular uptake of highly charged compounds.[4]



Cell-permeable prodrugs of 5-PP-InsP_5 can be efficiently released by different mamalian tissue extracts such as brain and liver (*In vitro*) as well as taken up by different mammalian cell lines (*In vivo*). This releases and uptake can be analyzed and quantified by PAGE and followed by staining with fluorescent dyes.[5]

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Identification of a peptide issued from the hinge region of the ERα and inducing apoptosis

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[1]Université Pierre et Marie Curie, Paris, [2]Université de Crète, [3]Université Libre de Bruxelles

The hinge region of the estrogen receptor α (ERα) is a key platform for the recruitment of co-activators and protein partners involved in the turnover of this receptor. The 295-311 region of ERα, a part of its autonomous activation function (AF2a), is subjected to post-translational modifications and is in charge of the recruitment of calmodulin, Hsp70 and ubiquitin ligase E6AP. Thus, this motif appears as an important regulatory platform for ERα function. We have tested the effect of ERα17p on the growth of ERα-negative (MDA-MB-231, SKBR-3) and -positive (MCF-7, T47D) breast cancer cells. Remarkably, ERα17p was pro-apoptotic at 10 µM after 6h incubation, independently from estrogen binding sites including both ERα and other membrane estrogen receptors. Likewise, it modifies the migration of breast cancer cells as well as actin distribution through mechanisms implying PI3K/Akt, Rho/ROCK and p38/MAPK signalling cascade. In a second part of our study, we tested the action of ERα17p in vivo. We used BalbC-/- mice xenografted with ERα-negative MDA-MB-231 breast cancer cells. Two weeks after the implantation of the tumor cells, animals were treated with ERα17p (intraperitoneal injection, 3 times a week, 0.5 mL of ERα17p 50 µM, i.e., 1.5 mg/kg body weight). Remarkably, tumor growth was lowered in treated mice (from 41.5 ± 3.61 mm3 to 139.4 ± 3.61 mm3) when compared to untreated mice (from 59.2 ± 5.24 mm3 to 323.9 ± 62.9 mm3), over a four week period. It should be stressed that ERα17p did not alter hepatic functions, as stressed by histological examination of liver biopsies and ASAT / ALAT activity measurements.2 In conclusion, ERα17p is a peptide that exerts significant inhibitory effects on the growth of estrogen-negative breast tumors through apoptotic / necrosis processes. Moreover, it interferes with cell migration and modifies the actin network. These in vivo effects are in accordance with computational studies and several in vitro effects. Hence and according to our preliminary pre-clinical investigations, ERα17p could be a promising tool for the understanding of novel actions of the estrogen receptor itself in ER-positive and -negative breast cancer, through direct or paracrine pathways.

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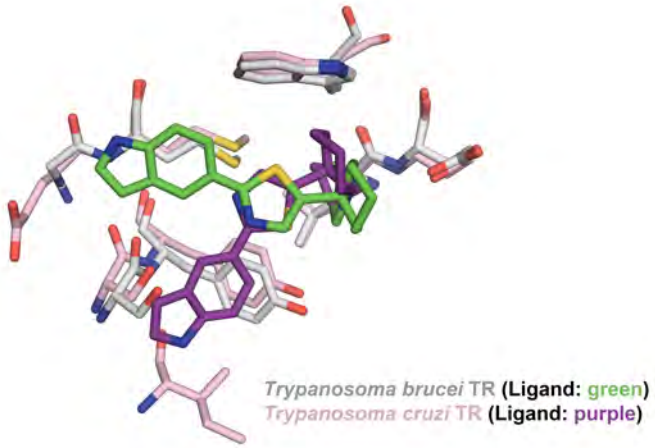
Marilena Kampa, Vassiliki Pelekanou et al. *Journal of Cellular Biochmistry*, **2011**, 112, 3786-3795

Structure-based Design of a New Lead Generation and Inhibition of the Anti-trypanosomal Target Trypanothione Reductase.

Elke Persch[1], Nina Hentzen[1], Steve Bryson[2], Emil. F. Pai[2], R. Luise Krauth-Siegel[3], François Diederich[1] *

[1]ETH Zurich, [2]University of Toronto, [3]Heidelberg University

The flavoenzyme trypanothione reductase (TR) is essential for the thiol redox metabolism of the protozoan parasites causing human African trypanosomiasis, Chagas' disease and Leishmaniasis and therefore has been validated as promising drug target.[1] In a multidimensional approach, our group recently revealed the binding mode of a series of small molecule TR inhibitors, which show slight differences in binding depending on the enzyme species.[2] Herein, we report the careful analysis of the co-crystal structures followed by the structure-based design and synthesis of a new rational lead generation. The biological evaluation of the ligands gives further insight into the molecular recognition of the inhibitors and the characteristics of each enzyme species.



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Efficient in vitro encapsulation of charged molecules by engineered AaLS protein containers

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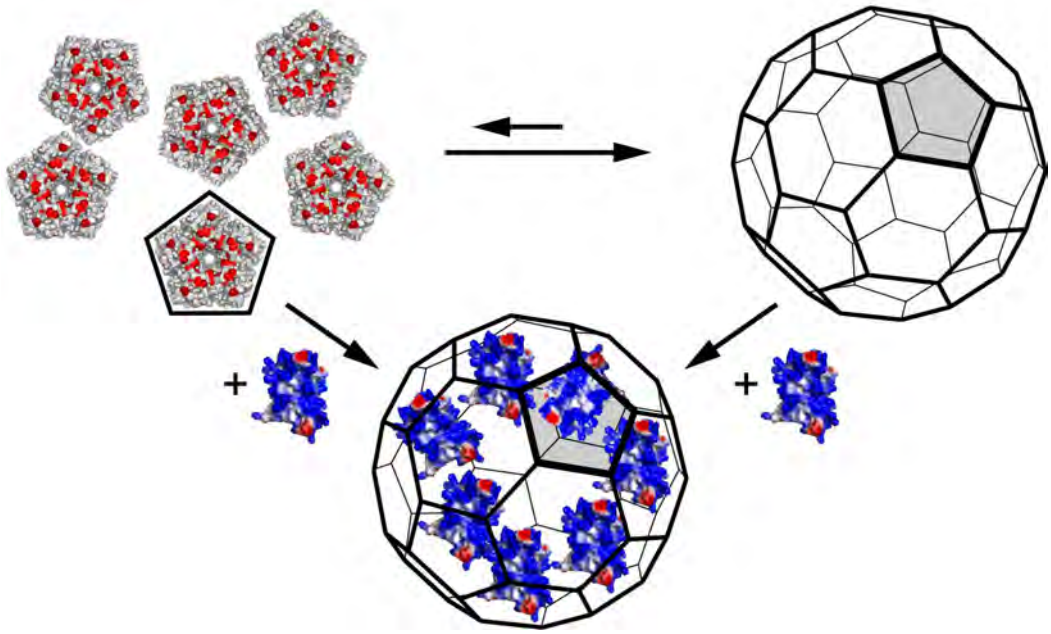
[1]ETH Zurich

Protein containers derived from viruses, mineral transporters, or enzymatic complexes find increasing number of applications in nanotechnology. They also serve for research on basic mechanisms of encapsulation processes, which is important to understand compartmentalization inside living organisms.
In our lab, we engineered a non-viral protein container (*Aquifex aeolicus* lumazine synthase - AaLS) to encapsulate charged molecules by introducing oppositely charged residues which drive selective electrostatic binding of the guests *in vivo*. Here we present our results on conditions and efficiency of *in vitro* encapsulation by engineered AaLS variants.



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Discovery of a new class of neuropeptitde S receptor antagonists

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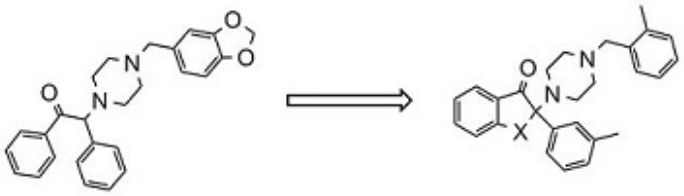
[1]Actelion Pharmaceuticals Ltd, Allschwil

Neuropeptide S (NPS) is a small peptide (20 amino acids) whose sequence and possible physiological function were first described in 2004 [1]. It binds to a G-protein-coupled receptor (NPSR) expressed especially in the brain: in the hypothalamus, thalamus, amygdala and certain cortical regions. NPSR -/- mice display reduced arousal in different paradigms, suggesting a physiological role for this receptor as a central regulator of arousal. Small molecule antagonists of NPSR could therefore offer novel therapeutic approaches to insomnia and possibly help to treat arousal induced stress-related disorders [2].
High-throughput screening of our in-house compound collection for human NPSR antagonists by means of a FLIPR assay revealed aminoketone **1** as a hit with an IC_{50} = 170 nM.



1

2a (X=CO)
2b(X=CH_2)

A medicinal chemistry effort to increase the chemical stability, to control the integrity of the chiral center and to obtain patentable analogues of **1** delivered indanones of type **2a** and indan-1,3-diones of type **2b.** Eventually, a detailed and consequent structure activity relationship (SAR) could be established and potential proof of concept compounds suitable for *in vivo* experiments were discovered.

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Development of a FRET-based high-throughput screen to identify antagonists of the Lin28/pre-let-7 interaction: a promising new target for cancer.

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[1]ETH Zurich

MiRNA are a large class of regulatory RNAs repressing the expression of target messenger RNAs. Post-transcriptional regulation of miRNA biogenesis by trans-acting factors binding miRNA precursors (pri- and pre-miRNA) is increasingly recognized as an important element controlling miRNA maturation. The dysregulation in expression of several miRNA is linked to various human cancers, and therefore represents a promising novel target for potential drugs.

In human cancer, the let-7 miRNA family exerts a tumor suppressor function. Lin28b is an RNA binding protein (RBP) which binds to pre-let-7 miRNAs through a GGAG motif in the terminal loop region and inhibits their processing depleting cells of mature-let-7. We hypothesized that the inhibition of such an interaction in cancer cells would restore let-7 levels and normal cell function.

Here, we present as part of our on-going program addressing RNA drugability, the development of a novel FRET based high-throughput screening assay for the identification of lin28/pre-let-7 small molecule antagonists. We believe this approach can be adapted and applied to a wide variety of RNA – RBP interactions for other miRNA-related diseases.

Parts of this work were funded by grants from the ETH Zürich (ETH-01 11-2) and the Krebsforschung Schweiz (KFS-02648-08-2010).

A modular LHC built on the DNA three-way junction

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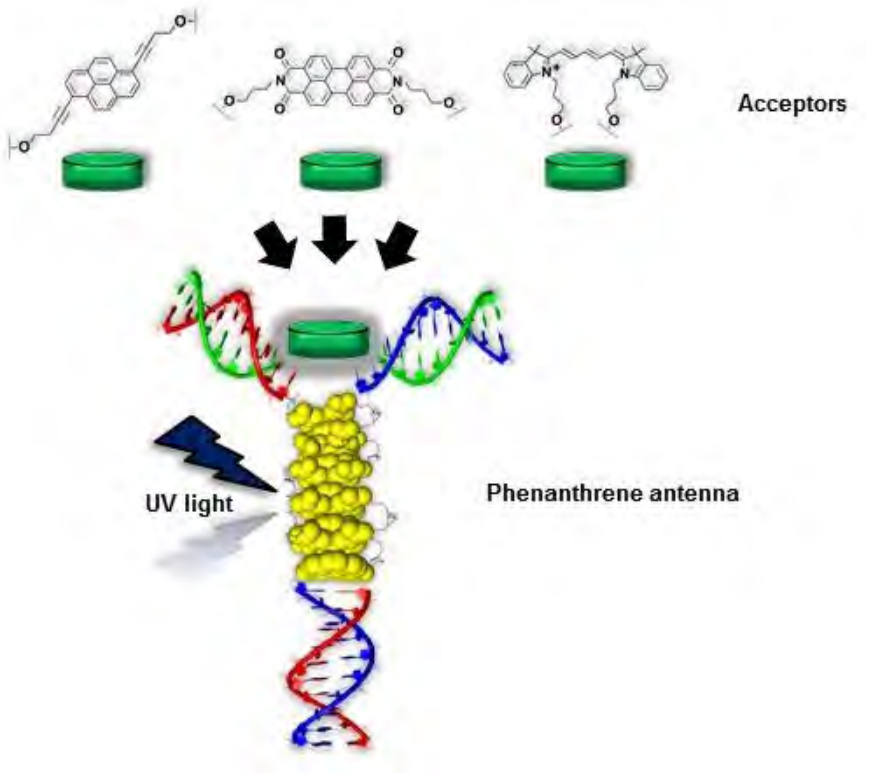
[1]University of Berne

Over the past years, in numerous studies the DNA double helix serves as a scaffold for the controlled arrangement of functional molecules, including a wide range of different chromophores [1]. Other nucleic acid structures like the DNA three-way junction have been exploited for this purpose as well. Recently, the successful development of DNA-based light-harvesting antenna systems have been reported [2]. Herein, we describe the use of the DNA three-way junction (3WJ) as a versatile scaffold for the modular construction of an artificial light harvesting complex (LHC). The LHC is based on a modular construction in which a phenanthrene antenna is located in one of the three stems and the acceptor is brought into proximity of the antenna through the annealing of the third strand. Phenanthrene excitation (320 nm) is followed by energy transfer to pyrene (resulting in exciplex emission), perylenediimide (quencher) or a cyanine dye (cyanine fluorescence) [3].



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Unusual Dimeric Antitrypanosomal Flavonoids from Arrabidaea brachypoda

Claudia Quitino da Rocha[1], Emerson Ferreira Queiroz[2], Cassio Santana Meira[3], Wagner Vilegas[1], Milena Botelho Pereira Soares[3], Laurence Marcourt[2], Jean-Luc Wolfender[2] *

[1]Universidade Estadual Paulista (UNESP), Brazil, [2]University of Geneva, [3]Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (Fiocruz), Brazil

Chagas disease is a parasitic disease caused by the flagellate protozoan *Trypanosoma cruzi*. This disease is transmitted to humans mainly in rural endemic areas through the infected feces of triatomine insects. However, when infected people from rural regions began to migrate to cities the disease was then spread to different non-endemic areas [1].The World Health Organization (WHO) reports that globally approximately 10 million people are infected by *T. cruzi*, and more than 25 million people are at risk of infection in endemic countries [2]. The aqueous ethanol extract of the roots of *Arrabidaea brachypoda*, a Brazilian medicinal plant, exhibited significant *in vitro* activity against *Trypanosoma cruzi*, the parasite responsible for Chagas disease. Targeted isolation of the active constituents led to the isolation of three unusual dimeric flavonoids. The structures were elucidated using UV, NMR and HRMS analysis, as well as by chemical derivatization. The anti-*T. cruzi* activity and cytotoxicity toward mammalian cells were determined for these substances. Two compounds exhibited selective activity against the trypomastigotes and also inhibited the parasite invasion process and its intracellular development in host cells with similar potencies to benznidazole. In addition one compound reduced the blood parasitemia of *T. cruzi*-infected mice. This study has revealed that these two dimeric flavonoids represent potential anti-*T. cruzi* lead compounds for further drug development.

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Synthesis and biological evaluation of methylated tetrabenazine derivatives

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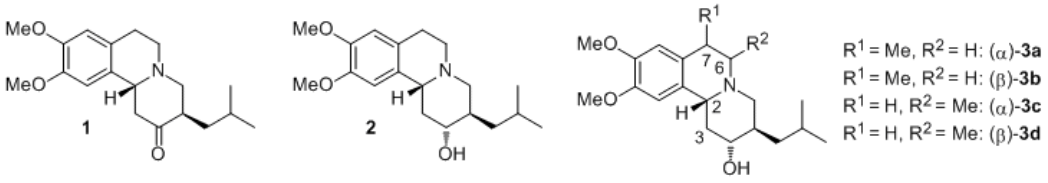
[1]ETH Zurich, [2]University of Berne

The benzoquinolizine tetrabenazine (TBZ (**1**)) is an inhibitor of the vesicular monoamine transporter type 2 (VMAT2), which is approved for the treatment of hyperkinetic movement disorders like Chorea Huntington.[1] *In vivo*, TBZ (**1**) is rapidly metabolized to α-dihydrotetrabenazine (**2**) as the pharmacologically active form.[2] The SAR for **1** has been investigated to some degree,[1] but the existing SAR information is rather limited and no modifications to the central tetrahydropyridine ring have been reported so far.



In order to address the effect of substituents at the C6/C7 positions on VMAT2-inhibitory activity, we have prepared the methylated a-dihydrotetrabenazine derivatives **3a-d** and we have assessed their VMAT2-inhibitory activity. The syntheses involved the formation of the C2-C3 bond by RCM-based ring-closure and subsequent acid-catalyzed addition of the N-atom to the ensuing double bond as the key steps.[4]

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Cell permeability of polycationic oligoprolines

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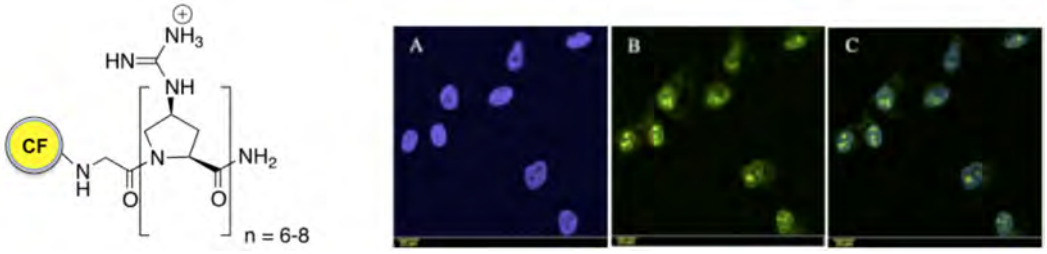
[1]ETH Zurich

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 molecular 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 fluorescence-activated cell sorting (FACS). The quantitative uptakes of our guanidylated oligoprolines were compared to established CPPs like the Tat-peptide and oligoarginines. The intracellular localization of the peptides was analyzed by confocal microscopy using the Hoechst 33342 marker as a nucleus stain. Additionally the stability of the oligoprolines in human blood serum and trypsin was evaluated.

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Target Identification and Optimization of the Novel Notch Inhibitor I3

Viktoria Reinmüller[1], Rajwinder Lehal[1], Sina Reckel[1], Oliver Hantschel[1], Romain Hamelin[1], Jieping Zhu[1], Freddy Radtke[1] *

[1]EPFL Lausanne

Notch signaling represents a type of cell-to-cell communication that plays a critical role in both embryonic development and adult tissue homeostasis.[1] Over-activation of the Notch cascade has been associated with cancer, including T-cell leukemia [2] and breast cancer [3]. Studies in mice and humans over the last two decades highlighted the critical need of targeting the Notch pathway in order to treat human malignancies. Current approaches to target Notch signaling are limited to small molecule γ-secretase inhibitors (GSIs) and functional inhibitory antibodies against Notch receptors and ligands.[4] Other therapeutic strategies including small inhibitory peptides are still in development. The lack of effective therapeutic possibilities to directly counteract deregulated Notch signaling justifies the development of novel inhibitors. We recently identified the novel small molecule Notch inhibitor I3 by screening chemical compound libraries in a luciferase reporter assay. *In vitro* and *in vivo*, I3 shows a significant Notch inhibitory effect. However, the mode of action is still unknown. For this purpose, we synthesized a comprehensive library of I3 derivatives in order to systematically investigate the structure-activity relationship. This enabled us not only to identify further Notch-inhibiting compounds, but also revealed at which position the molecule preferably tolerates an affinity tag, such as biotin. Currently we are using a biotinylated derivative of I3 to perform pull down experiments combined with mass spectrometry analysis to identify the target proteins of I3. Preliminary results support our hypothesis that I3 functions in the nucleus by interfering with proteins of the Notch transcription complex.

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MMP-inhibitor development with 3D in vitro cell-based assays

Rainer Riedl[1] *, Deborah Hohl[1], Jan Lanz[1], Thomas Fischer[1], Markus Rimann[1], Ursula Graf-Hausner [1]

[1]Zurich University of Applied Sciences, ZHAW

The family of matrix metalloproteinases (MMPs) belongs to the zinc-endopeptidases and plays a crucial role in reconstructing the extracellular matrix (ECM). In healthy individuals MMPs are highly regulated whereas in the pathological case MMPs are overexpressed which leads to several diseases (atherosclerosis, liver fibrosis, cancer, etc).[1]

At ZHAW a series of highly potent and selective MMP-13 inhibitors has been developed.[2] To evaluate the biological relevance of these inhibitors cell-based test systems are mandatory. In drug development standard monolayer 2D cell culture is used to screen for novel compounds. In order to monitor cell migration and/or cell invasion from a primary tumor into the surrounding tissue 3D cell culture systems are gaining momentum.[3] Therefore, we developed a novel assay based on 3D tumor micro tissues. Micro tissues with different cancer cell lines are produced in hanging drops and transferred into a hydrogel providing ECM molecules and mimicking the surrounding tissue. In this way the cell migration from the micro tissue into the ECM can be monitored and controlled by the addition of MMP-inhibitors. This test system will serve as a metastasis model since MMP-inhibitors are said to have a huge potential to inhibit metastasis.

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Identification of sirtuins modulators: new scaffolds and overall strategy

Lucie Ryckewaert[1], Claudia Avello Simoes Pires[1], Pierre-Alain Carrupt[1] *

[1]University of Geneva

Among the eighteen histone deacetylases isoforms (HDACs) found in human, class III HDACs, also known as sirtuins, are promising targets for treating neurodegenerative conditions and cancer [1,2]. Indeed, EX-527 (SEN0014196, Siena Biotech), the most potent and selective SIRT1 inhibitor discovered so far, is currently into phase II clinical development for the treatment of Huntington's disease [3,4].

As a starting point, a highly diverse chemical library generated *in silico* was harnessed. It was built with lipophilicity values (log P) and molecular weights coherent with 'drug-like' compounds [5]. This library was supplemented with natural compounds in order to maximize chemical space coverage. Overall, more than 600 compounds were tested *in vitro*, using human recombinant enzyme SIRT1. Then, hits generated were checked for their selectivity towards SIRT2, another SIRT isoform possessing the closest catalytic domain [6]. Cytotoxicity of selected hits was then assessed into both HEK293 and HeLa cells. Based on this combination of data, their cellular mechanism was explored through carefully selected targets implicated either in cancer or neurodegenerative pathologies.

Herein, we expose a decision tool based on unconventional early considerations such as physico- and pharmacochemical properties into the hit discovery process. Downstream combination of bench testing for sytematic profiling of hits are also described. This decision tool which is oriented towards therapeutic applications has already enabled to identify two new scaffolds that modulate SIRT1 with IC_{50} values in the low µM range.

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HPLC activity based profiling of Conchocarpus fontanesianusand targeted MPLC isolation of its antifungal compounds

Rodrigo Santana cabral[1], Emerson Ferreira Queiroz[2], Laurence Marcourt[2], Maria Cláudia Marx Young[1], Jean-Luc Wolfender[2] *

[1]Instituto de Botânica SMA/SP Núcleo de Pesquisa em Fisiologia e Bioquímica, Brazil, [2]University of Geneva

Conchocarpus fontanesianus (A. St.-Hil.) Kallunki Pirani (Rutaceae), popularly known as pitaguará, is a Brazilian native and endemic species distributed in the Atlantic Rain Forest. The dichloromethane extract of the stem bark of this plantshowed significant antifungal activity against *Candida albicans* on thin layer bioautographic assays [1]. In order to isolate the active compounds, bioassay-guided fractionation was undertaken using HPLC-microfractionation to localize the active compounds [2].This HPLC-based antifungal activity profiling enabled the efficient localisation of different active metabolites. Dereplication procedure based on LC-HRMS did not allow an unambiguous identification of these compounds that were putatively new. The targeted isolation of the active and the novel compounds was performed by medium pressure liquid chromatography (MPLC-UV) and semi-preparative HPLC. Using this approach, 15 compounds were isolated, among them two unusual alkaloids and two new limonoids. The structures of the isolated compounds were elucidated by classical spectroscopic methods including 2D NMR and HR-MS. The natural products obtained have also served as standard for a comprehensive metabolite profiling of various organs of this plant collected in different region for a complete survey of its phytochemical composition.

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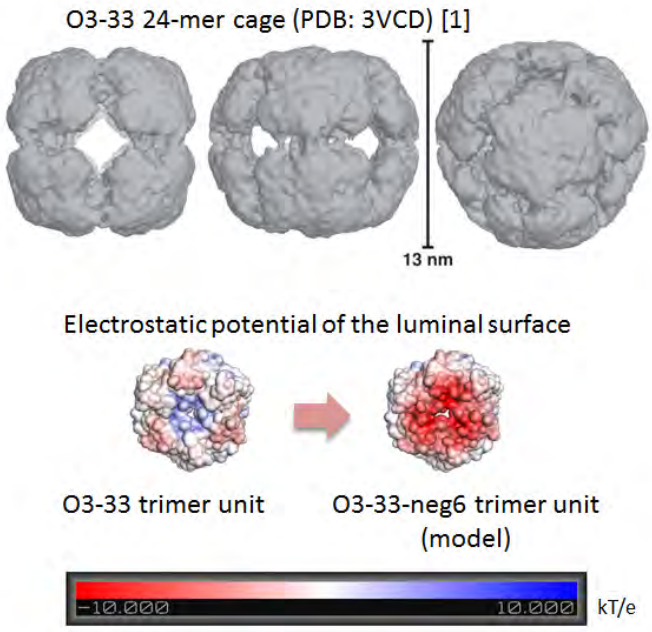
Exploration of Encapsulation Strategies for an Artificial Protein Cage O3-33

Eita Sasaki[1], Neil P. King[2], Tobias Beck[1], David Baker[2], Donald Hilvert[1] *

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

O3-33 is a computationally designed protein, which self-assembles into a 24-mer cage structure with octahedral symmetry.[1] To investigate its self-assembly and macromolecular encapsulation properties, we have made a series of mutants possessing different numbers of negatively charged amino acids on the interior surface. One of the variants, O3-33-neg6, was selected as the best variant to interact with guest proteins bearing positive-surface charge such as supercharged GFP(+36).[2] Encapsulation properties between O3-33-neg6 and GFP(+36) were further studied by size-exclusion chromatography followed by UV-vis spectroscopy, X-ray crystallography, and cryo-EM (electron microscopy) single particle reconstruction. The data suggest that GFP(+36) molecules can fit inside the large pores (ca. 3.4 nm) of the protein cage, and the interaction is very stable even in a buffer with 1M NaCl. In addition, directed evolution based on the polyarginine-tagged HIV-protease detoxification system [3] was applied to O3-33-neg6. Results of the evolution and self-assembly properties of the O3-33 variants in the presence and absence of positively charged guest molecules under different NaCl concentrations will be presented and discussed.



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Carbohydrate-based tRNA−Guanine Transglycosylase Inhibitors

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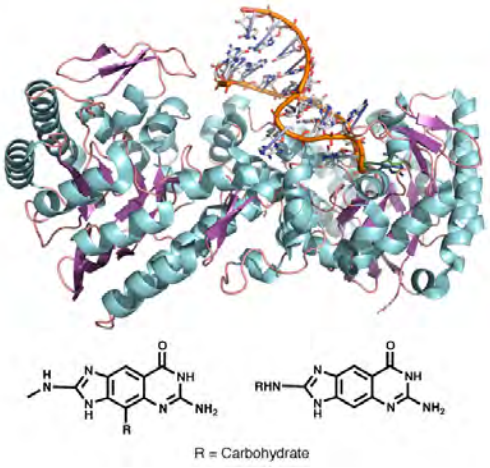
[1]ETH Zurich, [2]Philipps-Universität Marburg

Shigellosis is a severe inflammatory bowel disease caused by the pathogen *Shigella*.[1] Due to the high homology of its active site to *Zymomonas mobilis* *t*RNA–guanine transglycosylase (TGT), we consider the inhibition of TGT as a suitable target to prevent bacterial invasion.[2]Therefore, this research focuses on the structure-based design and synthesis of suitable inhibitors of TGT. Previous work employed a *lin*-benzoguanine scaffold (see figure) containing ribose derivatives targeting the ribose-34 binding site. The inhibitors showed high affinities and proved to replace water molecules of a conserved water cluster and furthermore, to uptake and mimic interactions in a phosphate binding site.[3]



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Synthesis of multifunctional ligands for bioceramic coating; towards functional cell-engineered bone implants.

Vladislav Semak[1], Françoise Borcard[1], Stéphanie Prior[1], Sandrine Gerber[1] *

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Tissue engineering of permanent implants is a promising approach for the treatment of large bone defects. While many efforts are currently devoted to the development of porous bioceramics mimicking both the structure and composition of bones, their integration into human tissue remains a challenging issue. In particular, failure to develop a functional vasculature within the material scaffold and fibrotic overgrowth at the implantation site often compromise long term functionality of bone substitute materials. To suppress the foreign body reaction and subsequent rejection of the implant, a narrow contact between bioceramic surface and surrounding tissue must be ensured.[1] Furthermore, trans-scaffold migration and in-scaffold survival of human bone-derived cells are mandatory for development of cell engineered permanent implants. Multifunctional ligands **A** and **B** (Figure 1) for bioceramic coating were designed: *i*) to initiate the adhesion of human endothelial cells and osteoblast progenitors within the material scaffold and *ii*) to reduce fibrosis by inhibition of fibroblast activation protease-α (FAP-α), which is cell surface antigen of reactive fibroblasts.

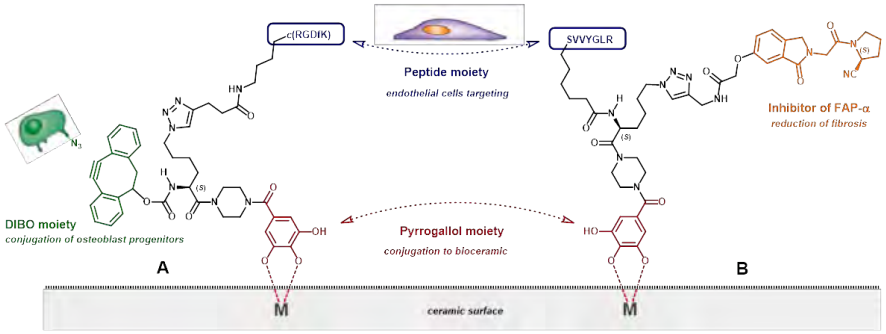

Figure 1 Structure of ligand **A** and **B**.

Chemical ligands **A** and **B** are based on three active subunits. The pyrrogallol moiety, which was established as a robust anchoring group to the inorganic matrix of bioceramics,[2] is a common pattern for both ligands. Ligand **A** is designed to allow a biocompatible copper-free [3+2] cycloaddition reaction between an activated cyclooctyne (DIBO) and azido-modified human fetal osteoblasts.[3] Adhesion of endothelial cells promoting further vascularization will be favoured by two oligopeptides. The cyclic peptide *c*(RGDfK) mimics cell adhesion proteins and binds to integrins;[4] and short SVVYGLR sequence promote migration and tube formation of entothelial cells.[5] In addition, ligand **B** displays a low molecular weight inhibitor of FAP-α.[6] In this contribution, chemical synthesis and preliminary biological evaluation of ligands **A** and **B** will be presented.

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Studies on the Chemistry and Biology of Fragin

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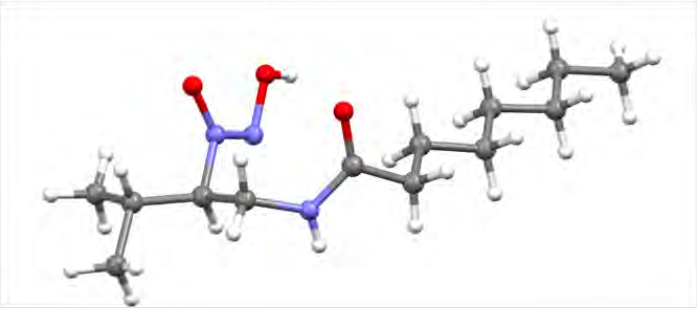
[1]University of Basel, [2]University of Zurich

Fragin was isolated from *Pseudomonas fragi* in 1967[1] and its constitution was established by total synthesis[2] and X-ray crystallography.[3] To date, its absolute configuration has not yet been established. The natural product possesses an unusual *N*-nitroso-*N*-hydroxylamine functional group, which is present only in few natural products, and exhibits a range of biological activities such as growth inhibitor of algae and lettuce seeds, antifungal, antimicrobial and anticancer properties.[4]



Fragin

In this communication, we report our studies on the chemical and biological properties of this natural product.

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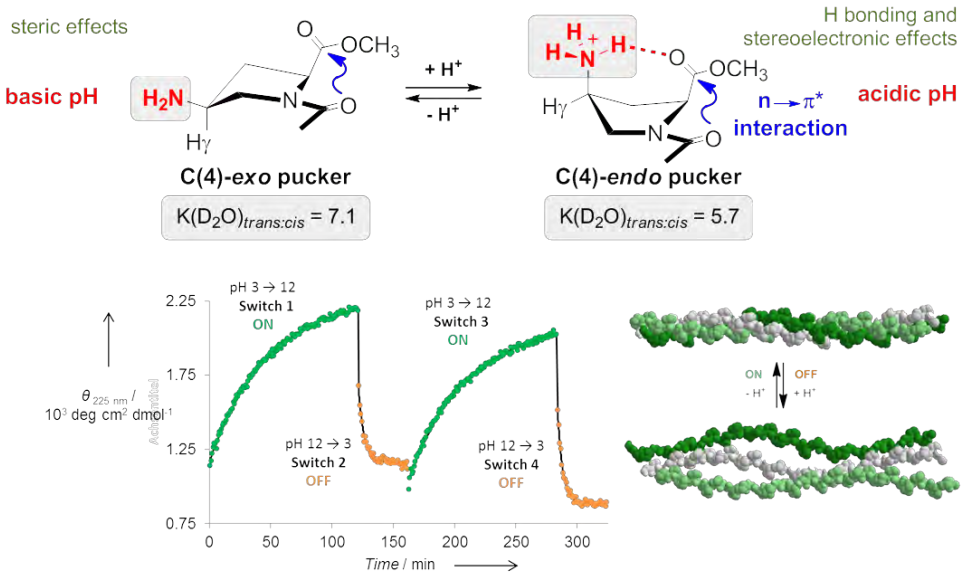
Switchable Proline Derivatives: Tuning the Conformational Stability of the Collagen Triple Helix by pH Changes

Christiane Siebler[1], Roman S. Erdmann[1], Helma Wennemers[1] *

[1]ETH Zurich

Proline residues are crucial for the conformational and functional properties of many natural peptides and proteins. They play important roles in, *e.g.*, protein folding and signal transduction.[1] Proline (Pro) and (4*R*)-hydroxyproline (Hyp) are also the predominant amino acids within the structural protein collagen which is responsible for the high stability of skin and bones.[2]Derivatives that influence the conformational properties of proline containing peptides and proteins are therefore important. Several C(4)-substituted derivatives with conformational properties that are insensitive to changes in the surrounding medium have been used to tune the functional and conformational properties of peptides and proteins.Our group contributed azidoproline derivatives that allow for functionalization via triazolyl or amide groups to this field.[3,4]
Within the presentation we show that (4*S*)-aminoproline is a pH-sensitive probe for tuning the conformational properties of peptides and proteins. Whereas the conformation is controlled at basic pH by steric effects,stereoelectronic gauche effect combined with transannular H bonding determine the properties at acid pH.[5]



The resulting pH-triggered flip of the ring pucker and the formation/release of a transannular H bond were used to switch the formation of collagen triple helices on and off reversibly.[5]

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Fluorescent probes for the cellular study of the 5-HT_3A receptor - development of binding assays and in-vivo imaging

Jonathan Simonin[1], Thomas Jack[1], Marc-David Ruepp[1], Jürg Gertsch[1], Andrew Thompson[2], Martin Lochner[1] *

[1]University of Berne, [2]Cambridge

The 5-HT_3 receptor is a ligand-gated ion channel (LGIC) and a member of the Cys-loop family of receptors. Transmembrane proteins, such as the 5-HT_3 receptor, are challenging to study by X-ray diffraction, fluorescent and photoaffinity probes can yield structural and functional information of native receptors. Herein we report eight fluorescent 5-HT_3 receptor ligands and describe their spectroscopic and binding properties. The ligands were tested on purified receptors or those expressed on live cells. A fluorescein derivative showed the highest affinity with radioligand binding (1.9 nM) and was used as a tracer in fluorescence polarization and flow cytometry binding assays. Cysteine substitutions of eight important amino acids in the 5-HT_3 receptor binding site were used to compare the orientation of granisetron and the fluorescein conjugate of granisetron (G-FL). A near-infrared fluorescent probe was used for in-vivo imaging of the receptor in a live mouse.

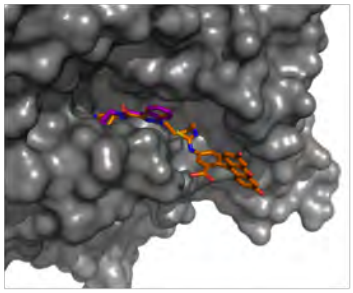

Fig.1 G-FL and granisetron docked into 5-HT binding protein (5-HTBP).

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Drug monitoring and obstructive sleep apnoea diagnosis by in vivo breath analysis

Pablo Martinez-Lozano Sinues[1], Lukas Bregy[1], Robert Dallmann[2], Esther Schwarz[3], Malcolm Kohler[3], Renato Zenobi[1] *

[1]ETH Zurich, [2]University of Zurich, [3]University Hospital Zurich

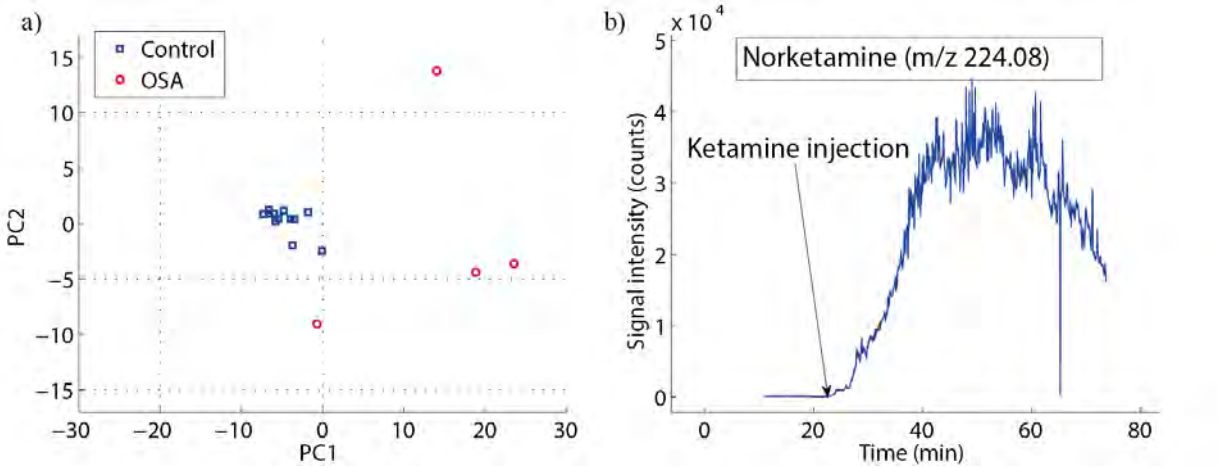
Introduction: Exhaled breath contains relevant metabolites that may reflect the biochemical activity within a subject. However, in contrast to other biofluids (e.g. plasma), the analysis of breath remains far less explored. Here we present some recent examples of how real-time breath analysis may contribute to the fields of disease diagnosis and drug monitoring.

Methods: We modified the entrance of a commercial quadrupole time-of-flight (Qtof) mass spectrometer to allow for the real-time analysis of breath via secondary electrospray ionization-mass spectrometry. We have studied i) differences between obstructive sleep apnoea (OSA) and case controls; ii) breath levels of ketamine and its main metabolites in mouse models.

Results: Diagnosis of OSA. We found a panel of discriminant features that allowed for the accurate prediction of disease/non-disease states. The figure (left, a) shows the score plot of principal component analysis of controls and OSA, suggesting a distinct breath signature for the latter.
Drug monitoring. The figure (right, b) displays the time trace of norketamine (the main ketamine metabolite) following injection of ketamine. Our method is in accordance with the literature on the mouse pharmacokinetics for ketamine, but provides much higher time resolution.

Conclusions: We conclude that the real-time mass spectrometric analysis of exhaled metabolites may contribute to address some of the most relevant clinical pharmacological problems, which are currently investigated through the analysis of body fluids other than breath.

Novel aspect: In vivo monitoring of exhaled compounds related to OSA and ketamine



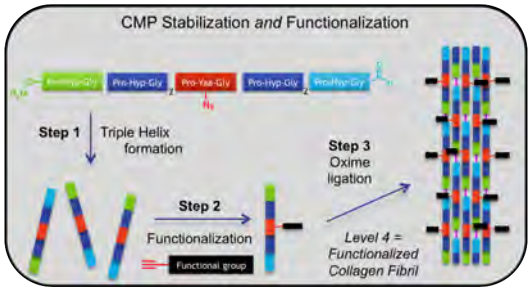
A New Route Towards Synthetic Collagen Based Materials: Oxime Criss-links To Stabilize Collagen Model Peptides

Linde Smeenk[1], Helma Wennemers[1] *

[1]ETH Zurich

Collagen is an abundant protein mostly found in human skin and bones. It provides stability in these tissues and also modulates cellular activities and regulation processes. The complex structure of collagen is responsible for its general stability and elastic properties. Mutations in the amino acid sequence of collagen often destabilize this structure, resulting in a number of diseases such as osteogenesis imperfectand Ehlers-Danlos syndrome [1]. Furthermore, the isolation process of natural collagen is still suffering from heterogeneity, potential immunogenicity, and loss of structural integrity [2]. Synthetic routes towards easily accessible collagen model peptides (CMPs) are therefore an interesting alternative, but remain challenging because the current routes cannot yet provide CMPs that show similar fibril length or mechanical properties as seen in natural collagen [3].

With this project, we aim to develop a new route towards stabilized CMPs using oxime ligation. This bio-orthogonal reaction is fast, does not require a catalyst and results in relatively small covalent linkages. Introduction of the required functional groups (e.g. the aldehyde and the alkoxyamine) in the side chain or at the N- and C-terminus of CMPs will be presented. Furthermore, secondary structures of the peptides, together with their ability to form higher ordered structures, are analyzed by UV and CD spectroscopy, as well as CD thermal denaturation studies. Ultimately, we hope to combine oxime stabilization with current optimized functionalization techniques in our group (e.g. click chemistry and amidation reactions [6]), to guide further development of synthetic functionalized collagenous materials for biomedicine and nanotechnology.



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Visible-Light-Induced Annihilation of Human Tumor Cells with Platinum-Porphyrin Conjugates

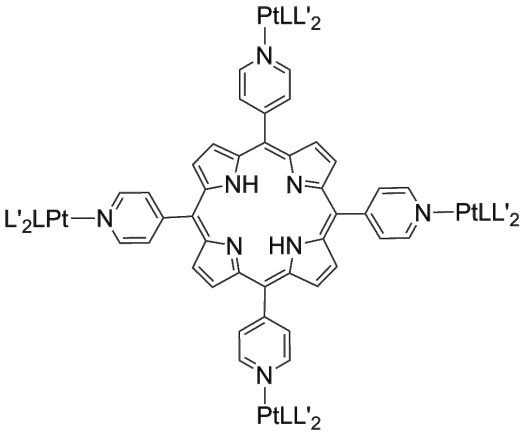
Bernhard Spingler[1], Anu Naik[1], Riccardo Rubbiani[1], Gilles Gasser[1] *

[1]University of Zurich

Despite the extended use of porphyrin complexes in PDT [1], tetraplatinated porphyrins have so far not been studied for their anticancer properties [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 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 MTT assay. IC_{50} values were obtained after 4 h incubation, a washing step, followed by 15 minutes irradiation at either 420 nm (6.95 Jcm^{-2}) or 575 nm, (6.23 Jcm^{-2}) respectively. These platinum-porphyrin conjugates had only minor dark toxicity, while 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. After 4 h incubation in HeLa cells, incubation of a tetraplatinum-porphyrin conjugate leads to a concentration of about 105 ng Pt in the nucleus per mg protein in the cell. Strikingly, the use of this conjugate increased the nuclear platinum content by more than 30-fold compared with cisplatin. This is obviously only partially a consequence of porphyrin conjugate having 4 platinum centers versus one in cisplatin; the main reason being that the conjugate is a more efficient platinum importer into the cell nucleus than cisplatin.

Taken together, all these favourable characteristics imply that tetraplatinated porphyrin complexes may be worth being explored as novel PDT anti-cancer agents *in vivo*.



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Influence of RNA structure on RNA-protein binding

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RNAs play key roles in many biological processes, including disease mechanisms [1]. A major challenge in RNA biology is understanding the interaction of RNA with RNA binding Proteins (RBPs) [2, 3].
The human RBFOX family plays an important role in alternative splicing. Its RNA recognition motif (RRM) is known to bind to single stranded RNA [4], however its consensus motif GCAUG is often present in highly structured forms containing single and double stranded regions.
We investigated whether RBFOX can bind to its consensus motif when embedded in a hairpin structure. We used a combination of melting experiments, commonly used algorithms and surface plasmon resonance spectroscopy to determine how the stability of RNA hairpins affects the affinity of the RBFOX RRM for its binding site.

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Lipid self-assembly and its applications

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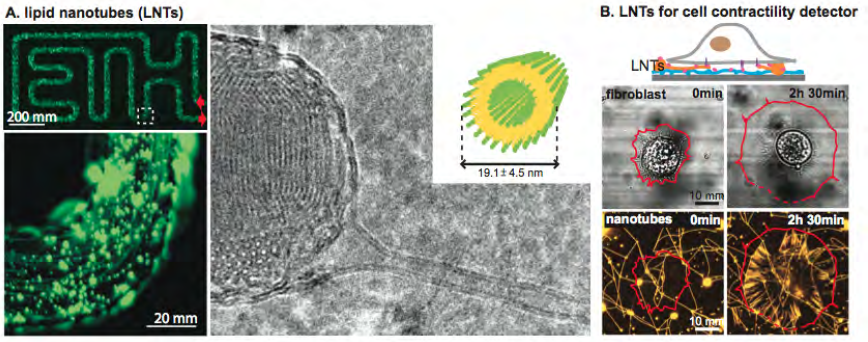
Biological nanofabrication by self-assembly is the sustainable technology that could potentially inspire and supplement the conventional clean-room-based lithography techniques. Our lab, which started in April 2014 at the University of Geneva, focuses on studying the mechanism of lipid self-assembly and develops applications in the field of biomedical engineering and electronics. Previously we have discovered that the main component of bacterial cell membranes, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), self-assembles into single-wall nanotubes (Figure A).[1] The lipid nanotubes (LNT) were visualized by fluorescence microscopy and transmission electron microscopy. The diameter of the tube is 20 nm and they can be patterned on surfaces either by microfluidic systems or by micromanipulators. We also study the transformation of vesicles into supported lipid bilayers (*i.e.* planar lipid bilayers on solid surfaces, SLB) because there is a demand to develop versatile SLB formation techniques for membrane protein studies. We have demonstrated several tricks to form SLBs with many types of lipids on different substrates. For example, the mechanical forces imposed by the ice formation can trigger the rupture of adsorbed vesicles into SLBs.
Such self-assembled lipid structures are tested for the use in different applications. We applied surface-bound self-assembled LNTs as force detectors to visualize the contractile activity of spreading cells (Figure B).[2] The newly generated LNTs upon cell spreading exhibited fingerprints characteristic for cell types and conditions. The system can be used as a label-free cell toxicity assay since the loss of contractility is an early sing for cell death. SLBs created with developed methods are used for electrophysiology. A SLB was formed over a single 800-nm pore in a Si/Si_3N_4 chip by pre-filling the pore with a polyelectrolyte multilayer (PEM) to enable the spontaneous fusion of 50-nm liposomes over the pore.[3] The single channel activities of the pore-forming peptide melittin were recorded over a period of two and a half weeks. The 200–times longer lifetime of the artificial cell membrane compared to the patch clamp method revealed a new feature of the peptide, the time-dependent stabilization effect upon bias application.



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Benzimidazole-derived nucleosides in DNA synthesis as probes for O^6-alkylguanine adducts

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[1]Department of Health Sciences and Technology, ETH Zurich

Exposure to alkylating agents such as dietary *N*-nitroso compounds can generate DNA lesions, i.e. O^6-alkylguanine adducts. Bulky DNA adducts can stall replicative DNA polymerases, however, specialized translesion DNA polymerases can bypass the lesion resulting in damage tolerance. Nucleotide analogs are known as potential substrates for natural and damaged templating DNA in replication, but less is known regarding how polymerases deal with synthetic nucleobases as templates in DNA synthesis. In this study, we explored the ability of a thermostable Taq DNA polymerase mutant (from *Thermus aquaticus*) to bypass O^6-methylguanine (O^6-MeG) adducts in single nucleotide incorporation experiments. Benzimidazole-derived synthetic nucleotides were tested as specific substrates for incorporation opposite O^6-MeG vs non-damaged G. Steady-state kinetic efficiencies of synthetic nucleotide incorporation were determined for replication past natural templating bases. Moreover, we also explored how Taq polymerase mutant can deal with non-natural nucleobases in a template for DNA replication in single-nucleotide incorporation and steady-state kinetic experiments. The new knowledge gained from these data regard what structural features of DNA base pairs are important for efficient enzyme catalysis whether particular chemical modifications reside in the templating or incoming base position.

Chemical biology of Inositol polyphospahtes

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Inositol polyphosphates are derived from the carbocyclic D-*myo*-inositol that is phosphorylated in distinct patterns resulting molecules are crucially important cell signaling molecules that are linked to series of signaling events such as ion-channel function,vesicle trafficking,apoptosis,transcriptional regulation,motility,cell proliferation and energy transformation (1,3,5).

The most abundant inositol *pyro*phosphate 5-PP-InsP_5 (IP_7) does not exceed sub-micro molar concentrations in most cells and very difficult to purify from biological resources. Our recently developed cell permeable IP_7 analog allows us to artificially increase IP_7 concentration in living cells. We found that cell permeable IP_7 analog has been efficiently released by different mamlian tissue extracts such as brain and liver (*Ex-vivo*) as well as taken up by different mammalian cells (*In-vivo*).These uptakes were analyzed and quantified by recently discovered polyacrylamide gel electrophoresis (PAGE) method (2,4,6). Resolve products were visualized by staining with different cationic metachromatic dyes such Toludine blue and DAPI which are very efficient to binds with phosphate groups (2,5,6). This techniques has enormously improved the *in vivo* study and allows to detect less than 100 pmoles of inositol phosphates (IPs) (6).

Application of new cell-permeable IP_7 analogs in living systems with spatiotemporal control over signal generation contributes for the development of a novel approach to study associated signaling coplexicityand protein localization in obesity research.

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Sulfonamide Inhibitors of 2-Methylerythritol 2,4-Cyclodiphosphate Synthase (IspF) from Arabidopsis thaliana and Plasmodium falciparum.

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Malaria, caused by parasites of the genus *Plasmodium*, is responsible for over 600’000 deaths per year.[1] The rise of multidrug-resistant strains demands for the development of new antimalarials with novel mode of action.

IspF is involved in the non-mevalonate biosynthetic pathway of IPP and DMAPP, which are isoprenoid precursors. This pathway presents a promising target for for the development of new drugs and has been validated as a target for the treatment of malaria.[2] Humans utilize the mevalonate pathway for the biosynthesis of isoprenoids, whereas many bacteria, plants and some protozoa, such as *P. falciparum* exclusively obtain isoprenoids via the non-mevalonate pathway.

In this pathway, the enzyme IspF catalyzes the cyclization of diphosphocytidyl-2-methylerythritol 2-phosphate into 2-methylerythritol 2,4-cyclodiphosphate.

The inhibition of plant IspF enzyme from *A. thaliana* was screened against 40’000 compounds. A disulfonamide was identified as possible inhibitor. Several derivatives were synthesized, the most active showing one-digit micromolar IC_{50} values against *P. falciparum* IspF.

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Factors influencing the uptake of biotinylated ruthenium complexes for in vivo catalysis in E.coli

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To be able to perform olefin metathesis reactions *in vivo*, ruthenium complexes must efficiently penetrate cells without being inhibited or adhering to cellular components. Cells expressing streptavidin were used to bind biotinylated ruthenium complexes. The amount of ruthenium taken up by the cells was determined by optical emission spectroscopy. Factors such as the concentration of ruthenium complexes, various biomolecules, ATP inhibitors, DMSO and cultivation conditions were investigated. Optimized cultivation-, incubation- and washing conditions will be presented, yielding high Ru-uptake levels in *E. coli*.

New structure-activity relationship studies on bombesin-based tracers for tumor targeting

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The bombesin receptor subtype 2 (BRS2) is a receptor overexpressed in a variety of clinically relevant tumors including prostate, and breast cancer. In this context, bombesin-based radiopharmaceuticals hold great promise for imaging and peptide receptor radionuclide therapy of BRS2-expressing tumors. We have shown that the use of 1,4-disubstituted 1,2,3-triazoles as amide bond surrogates leads to an enhanced stability and improved tumor uptake of a bombesin-based radiopeptide *in vivo*.[1] In order to improve the targeting properties of our tracer, we set out to conduct a structure activity relationship (SAR)-guided study on the bombesin binding domain BBN(6-14). We herein wish to report the synthesis, and *in vitro* and *in vivo* evaluation of novel radiolabeled bombesin analogs with promising biological characteristics for radiotracer development.

We have successfully synthesized a series of novel bombesin conjugates. Labelling with Lu-177 was achieved in excellent radiochemical yields and purity (95%). The influence of amino acid substitutions within the binding sequence of BBN(6-14) on critical parameters such as cell internalization kinetics, metabolic stability, receptor affinity and occupancy, as well as tumor uptake in xenografted nude mice has been studied.

The combination of a careful optimization of the amino acid sequence of BBN(6-14) with our novel stabilization strategy led to the identification of novel radiopeptides with promising characteristics for application in nuclear medicine for targeting BRS2-expressing tumors.

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Investigation of the structure of LecA and multivalent ligands with crystallography and MD simulation

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The increasing number of infections with antibiotic resistant bacteria in hospitals is a serious problem for current medicines. The ability to form biofilms is of the mechanisms bacteria use to acquire resistance against many treatments. Among the many different bacteria is also the pathogen *Pseudomonas aeruginosa(1),* this bacteria also form biofilms. It is an opportunistic pathogen which can be fatal for people with weak immune system. This problem leads to the initiative to discover new targets and new drugs against *Pseudomonas aeruginosa.*

LecA(2) is a homo-tetrameric protein which is known to be crucial for the formation of biofilm and to explain the pathogenicity of P*seudomonas aeruginosa*. There are some high affinity multivalent(3,4) ligands already published. These compounds are galactosides with different sorts of linkers to connect the galactosyl groups and to make additional contacts. Here we wanted to understand how LecA and the ligands interact and how this explains the different strength of bindings. Some of these multivalent ligands were investigatedby us with crystallography and later we studied with MD simulation the system to gain a deep insight in the binding mode and behavior of this ligands. This study helps to explain why this type of ligands shows such a strong binding to the LecA protein. Finally, this allows it to see how these drugs could be optimized in the sense of structure based drug design.

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Iron phosphate nanoparticles do not impair membrane integrity or metabolic activity in intestinal cell lines

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Fortifying food with highly bioavailable nanostructured iron compounds has the potential to better combat iron deficiency, a major global public health issue, however their absorptive pathway and safety remains unclear. In this study, we tested the hypothesis that the presence of mucus and the transformational status of cells influence the potential cytotoxicity of iron phosphate nanoparticles ($FePO_4$-NPs) to human intestinal epithelial cells in vitro. By flame spray pyrolysis (FSP), we produced amorphous $FePO_4$-NPs with a specific surface area (SSA) of 100 m^2/g and 190 m^2/g which corresponds to an average particle diameter of 20 nm and 10 nm, respectively. Food-grade silica nanoparticles (SiO_2-NP) were used as negative control for toxicity, soluble $FeSO_4$ as control for the biological effect of iron and fine powder $FePO_4$ (SSA 28 m^2/g) as control for the effect of larger $FePO_4$ particles. Human colon adenocarcinoma derived (HT29, HT29-MTX) and noncancerous immortalized human colon epithelial cells (HCEC) were exposed to 0.1-500 µg/mL $FePO_4$-NPs or control compounds for 24 or 48 h. Membrane integrity was assessed based on the release of LDH and metabolic activity was evaluated with the MTS assay. Membrane integrity was not impaired upon NP treatment, only exposure to high doses of commercial fine powder FePO4 showed minimally increased LDH release in HCEC cells. Regarding metabolic activity of HCEC cells, it was markedly reduced upon exposure to high doses of commercial fine powder $FePO_4$, whereas $FeSO_4$ and $FePO_4$-NPs (SSA 100 m^2/g) had a less profound effect. These results give a first indication that these $FePO_4$-NPs are not acutely toxic to intestinal epithelial cells.

Metabolomic profiling of bovine cumulus cells and oocytes during in-vitro maturation of cumulus-oocyte complexes

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Oocytes are unique cells carrying the potential to develop into a complex living being after fertilization. *In-vitro* fertilization of oocytes plays an important role in clinical settings as well as for research purposes. Despite their widespread use these assisted reproductive techniques are still limited in their success rates. One major bottleneck is the reduced developmental competence of oocytes matured under *in-vitro* conditions (IVM). Detailed unravelling of metabolism during maturation provides the unique opportunity for evidence-based adjustments of culture conditions during IVM. Cumulus cells accompany oocytes, forming the cumulus-oocyte-complex (COC), which hold a close bidirectional communication with each other by gap-junctions and paracrine signaling. Central glucose metabolism has shown to play a fundamental role for the oocyte to reach metaphase II and acquire developmental potential. Parallel metabolomic profiling of cumulus cells and oocytes collected before (oocytes in prophase I (PI), compact cumulus complexes (CP)) and after in-vitro maturation (oocytes in metaphase II (MII), expanded cumulus complexes) will be conducted using matrix-assisted laser desorption/ionization tandem mass spectrometry in a negative mode using 9-aminoacridine as matrix. This analytical system has shown to be capable to study central metabolism of model organisms even on single cell level [1,2]. In first preliminary experiments this technology was transferred to analyze methanol cell extracts of pooled cumulus cells and oocytes. These COCs were matured in groups and separated in pure cumulus and oocyte cell extracts. Cumulus and oocytes extracts of COCs before (PI, CP) and after maturation (PI, CP) were analyzed in first promising measurements that indicate that the chosen technology will be capable to provide novel insights in metabolism of bovine COCs during IVM.

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3-Alkoxy-pyrrolo[1,2-b]pyrazolines as novel selective androgen receptor modulators (SARMs) with unique physicochemical properties for transdermal administration

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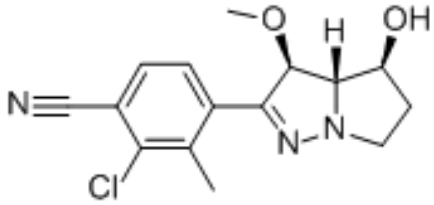
Drug discovery efforts have identified selective androgen receptor modulators (SARMs) that exert strong anabolic effects on skeletal muscle and bone, but minimal androgenic effects on reproductive tissues such as prostate.[1] To date, only oral SARMs have been developed, and their clinical efficacy is limited by adverse events such as induction of liver transaminases or interference with lipoprotein homeostasis.[2,3] Such effects may be avoided by parenteral routes that minimize Cmax-driven liver exposure, providing more constant drug levels to the body. The present work describes the synthesis and characterization of 3-alkoxy-pyrrolo[1,2-b]pyrazolines that possess adequate physicochemical properties for transdermal administration. Compound **1** binds to human androgen receptor (AR) with a Ki of 0.45 nM with great selectivity over other nuclear hormone receptors and potently activates AR in a C2C12 muscle cell reporter gene assay with an EC50 of 0.5 nM. It showed excellent aqueous solubility of 1.3 g/L at pH7.4 and an in silico model as well as a customized parallel artificial membrane permeability assay (PAMPA) indicated good skin permeation. Indeed, when measuring skin permeation through excised human skin an excellent flux of 2 µg/(cm2*h) was determined without any permeation enhancers. In a two-week Hershberger model using castrated rats, **1** showed dose-dependent effects fully restoring levator ani muscle weight at 0.3 mg/kg/day after subcutaneous administration with high selectivity over prostate stimulation. The structure-activity relationship of **1** and its binding mode in the AR ligand binding domain will be discussed.



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Crystal Structure of an Oligoproline PPII-Helix

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Polyproline II (PPII) helices are, together with *α*-helices and *β*-sheets, the most frequently occurring secondary structures in peptides and proteins.[1] It is for example adopted by collagen, the most abundant protein in mammals. Oligoprolines are the parent peptides and eponyms for this left-handed helix. Whereas, crystal structures of collagen and several proteins with PPII helical domains have been obtained, crystallisation of oligoprolines has so far been elusive. The only experimental structure information of oligoprolines with PPII helicity stems from X-ray powder diffraction,[2,3] FRET,[4,5] Raman spectroscopy,[6] and NMR studies.[5] The lack of a crystal structure of the parent peptide of the PPII helix has led to a lot of debate about the factors that are critical for stabilizing this secondary structure.
In this study we are presenting structural details of the single-crystal obtained of an oligoproline adopting a PPII-Helix.[7] Thorough analysis of the crystal structure revealed that non-covalent interactions between the carbonyl groups are a key feature for the stabilization of the PPII-helix. Furthermore, the structure showed that there is a clear correlation between this interaction and the exo/endo ring puckering of the pyrrolidine rings. Bridging water molecules at the peptides backbone have been discussed to play a major role of stabilization for the PPII-Helix since this conformation is predominant in aqueous environment. In the crystal structure no water molecules are incorporated, indicating that bridging water molecules do not play an important role for the stability of an oligoproline adopting a PPII-helix.

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A synthetic nucleotide analog enables polymerase-mediated amplification of DNA containing promutagenic O^6-alkylguanine adducts

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Exposure to dietary and environmental alkylating agents, i.e. nitrosamines can generate promutagenic O^6-alkylguanine adducts. Current methods for detection of these physiologically low occurring DNA adducts lack both adduct- and sequence specificity. In order to overcome these problems, developing new tools that enable the amplification of O^6-alkylguanine adducts would allow studies investigating DNA damage levels and cancer etiology.
In this work, we explored the polymerase-mediated lesion bypass of the model bulky O^6-benzylguanine (O^6-BnG) adduct with benzimidazole-derived non-canonical nucleotide analogs. Nucleotide analogs BIMTP and BenziTP were synthesized and tested as substrates for various thermostable polymerases in single nucleotide incorporation and full-length primer extension studies. We identified a *Taq* polymerase mutant that specifically incorporated BenziTP opposite O^6-BnG vs G. Furthermore, the synthetic nucleotide was required to promote full-length extension of alkylated DNA in the presence of natural dNTPs. Steady-state kinetic incorporation efficiencies were determined for DNA replication mediated by this *Taq* mutant. Finally, the combination of *Taq* polymerase mutant, synthetic nucleotide analog BenziTP, and natural dNTPs enabled the amplification of O^6-BnG containing DNA in multiple cycles (Figure).

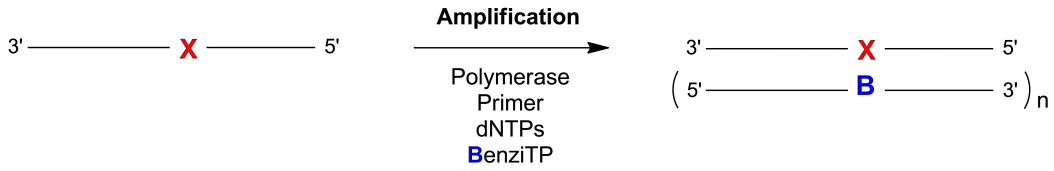

Figure: Polymerase-mediated amplification of templating damaged DNA (X = O^6-benzylguanine) with a synthetic nucleotide analog BenziTP (= B) resulting in amplified B-containing product.

A unifying framework for protein amyloid self-assembly: from protein-protein interactions to large-scale structures

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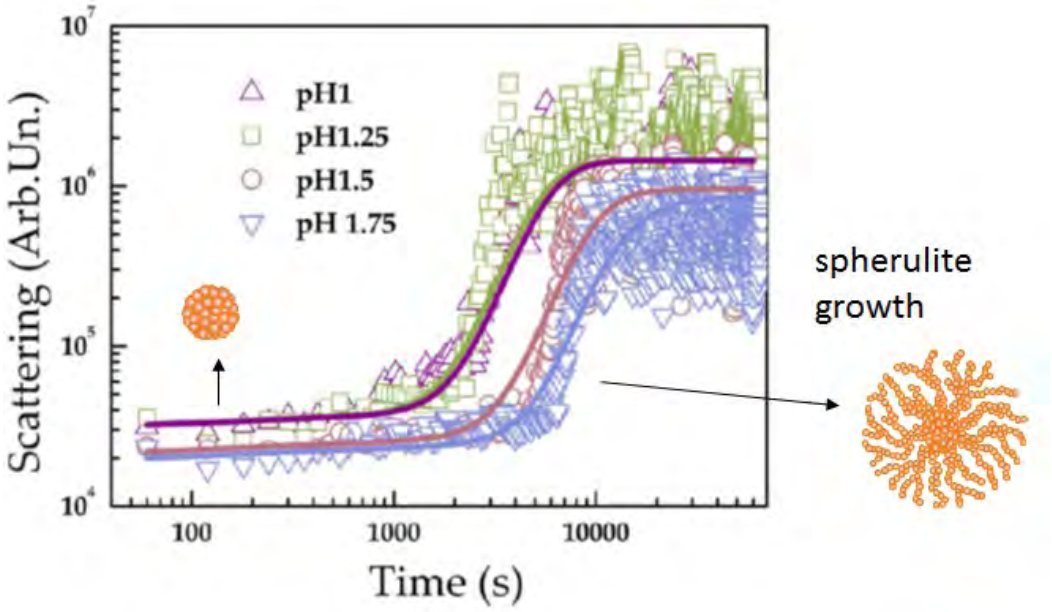
A unifying theoretical framework able to predict self-assembly kinetics, structural changes and self-assembled morphologies based on the underlying protein-protein interactions has not been lacking. Using a novel statistical mechanical approach (Foderà et al PRL 2013) as the starting point, we are able to quantitatively investigate the connection between the early-stage protein-protein interactions (electrostatic, hydrophobic, hydration and entropic contributions) and the development of large-scale structures both in terms of mechanisms (conformational changes, nucleation and templated aggregation) and in terms of 3D molecular-level arrangements in the large-scale aggregates.



V. Fodera', A. Zaccone, et al. Phys. Rev. Lett. 111, 108105 (2013).

A new labelling strategy to visualize an RNA splicing process

Susann Zelger-Paulus[1], Anita G. Schmitz[1], Gilles Gasser[1], Roland K.O. Sigel[1] *

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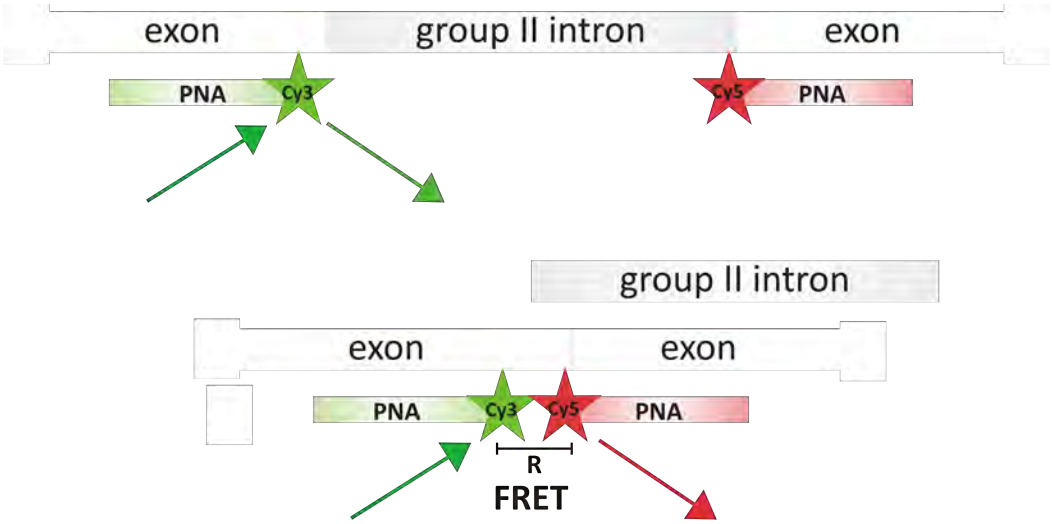
The labelling procedure of large nucleic acids (200 bp) at specific regions is quite challenging due to a lack of specific binding sites in the sequence. A common label strategy is the use of complementary DNA oligonucleotides bearing the required fluorophores. However, Peptide nucleic acid (PNA) hybridization probes with an unnatural backbone are an excellent alternative to DNA oligonucleotides since they have the advantage of being highly specific and affine to RNA and less prone to enzymatic degradation.[1] The latter is of high important with regards to further *in vivo* studies. We are focusing on the visualization of the splicing process of a catalytic active RNA which takes place during RNA maturation. The RNA called group II intron ai5γ originated from *Saccharomyces cerevisia* folds in a defined three dimensional structure while at the same time actively induces its self-cleavage. [2,3] The splicing process was investigated by native fluorescent gel studies and bulk FRET (Förster Resonance Energy Transfer) measurements using PNA labels. Thereby, two PNAs carrying the relevant FRET dyes, either Cy3 or Cy5, are hybridized to the intron flanking regions. FRET only occurs when the intron is self-spliced as the exons are ligated and the two fluorophrores attached to the PNA come nearby (Figure). With regard towards *in vivo* studies of the splicing mechanism, this approved labelling strategy is very promising.



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Probing Capsid Dynamics with Protein FRET

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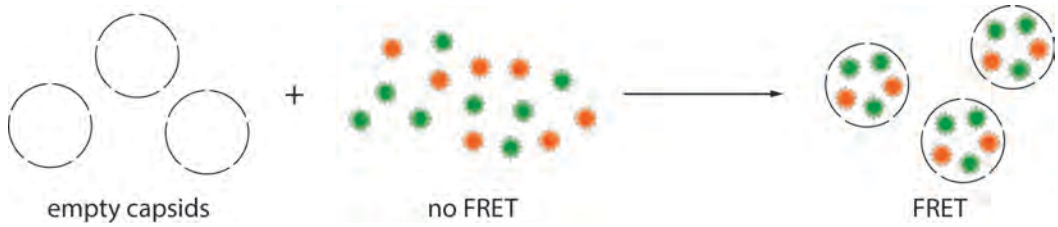
Lumazine synthase from *Aquifex aeolicus* (AaLS) is a capsid-forming protein that was recently engineered and subsequently evolved to accommodate positively charged cargo [1,2]. Ongoing work aims to employ the variant AaLS-13 as a reaction chamber or delivery vehicle. In order to investigate encapsulation behavior, capsid dynamics and capsid permeability to small molecules, we have developed a pair of positively charged fluorescent proteins that exhibit resonance transfer upon encapsulation.



Time-dependent FRET measurements allow us to follow the kinetics as well as the extent of cargo encapsulation. Using this technique in combination with gel filtration and electron microscopy, we are exploring new capsid variants with a range of dynamic properties tailored for various applications.

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