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# Poster Abstracts
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3001 Bern
Switzerland
info@scg.ch
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# Bacterial Resistance to Silver: The Role of SilE Protein

V. Chabert[1], K. M. Fromm[1]*

[1]University of Fribourg

Silver has been used for hundreds of years for its antimicrobial properties. Since the emergence of many multi-resistant bacterial strains against classical antibiotics, the research of new silver compounds is now at its apogee. While these drugs have been shown to be highly able to kill bacteria, some of these pathogens have developed a resistance to high concentrations of $Ag^+$. This resistance is provided by the plasmid pMG101, which encodes for eight proteins that act together in an efflux pump system to deal with silver ions. Among these, the SilE protein is the only one of which its mode of action is actually unknown.

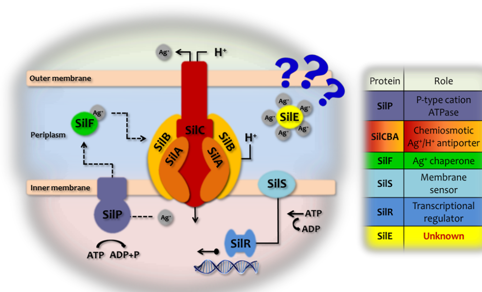


**Figure 1**: Proteins products of pMG101 silver resistance genes.

To identify the role of SilE in this bacterial machinery, two approaches have been intended in our group. While one way is to study the interaction of the whole protein with silver ions, the other is based on a bottom-up approach, investigating the interaction of silver ions with short peptide sequences of this protein. By NMR studies of these peptide models, we were able to highlight a potential methionine participation in the complexation of $Ag^+$ by SilE.

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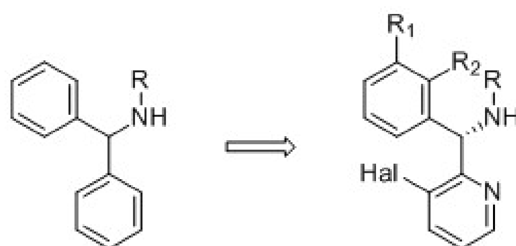
# The discovery of a potent and orally available Dot1L inhibitor

C. Ragot[1], C. Mura[1], F. Stauffer[1]

[1]Novartis Institutes for BioMedical Research, Basel

DOT1L is a histone methyltransferase that has emerged as an interesting new onconlogic target for mixed lineage leukemia[1]. A new series of DOT1L inhibitors has been identified by HTS and optimized by structure-guided design to lead to subnanomolar derivatives. Those novel DOT1L inhibitors, structurally un-related to cofactor SAM, have shown the potential to achieve good oral bioavailability. The benzhydrylamino fragment present in the original hit has evolved into a desymmetrized moiety with preorganization, optimal filling of the pharmacophore space induced by the ligand and reduced CYP450 metabolism at the benzylic position.




The synthetic access to a fully decorated potent DOT1L inhibitor will be presented and the role of its structural features will be discussed in the context of achieving suitable potency and *in vivo* exposure in rodent for preclinical investigation.

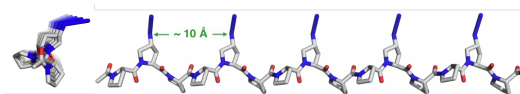
[1] C.-W. Chen, S.A. Amstrong. *Exp. Hematol.* **2015**, *43(8)*, 673-684.

## Oligoprolines as Scaffolds for Tumor Targeting with Hybrid Bombesin Analogues

S. Dobitz[1], C. Kroll[1], R. Mansi[2], F. Braun[2], H. Mäcke[2]*, H. Wennemers[1]*

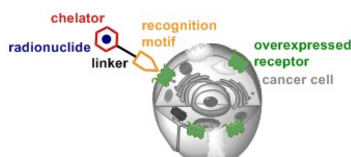
[1]ETH Zurich, [2]University Hospital Freiburg

In recent years, functionalizable oligoprolines have emerged as attractive molecular scaffolds for the introduction of moieties in defined spatial arrangements for applications in medicinal and material sciences.[1-3] In aqueous environments oligoprolines adopt the well-defined polyproline II (PPII) helix already at chain lengths as short as six residues.[4] Within this left-handed secondary structure every third proline residue is stacked on top of each other in a distance of ~ 0.9 nm (Scheme 1).[5] Incorporation of 4-azidoproline (4-Azp) residues into the sequence provides reactive sites in defined distances from one another that can easily be functionalized through click chemistry or Staudinger reduction followed by acylation.[6]




**Scheme 1** Oligoproline with 4-azidoproline (4-Azp) residues in every third position.[6]

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 (Scheme 2).[3] The hybrid ligands showed significantly higher tumor uptakes *in vitro* and *in vivo* compared to monovalent and divalent controls. Notably, the defined distance between the recognition motives proved to be important for high, specific, and long lasting tumor uptake. 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.




**Scheme 2** Recognition motives for receptors overexpressed on cancer cells labeled with radionuclides for diagnostic cancer imaging and targeted radionuclide therapy.[3]

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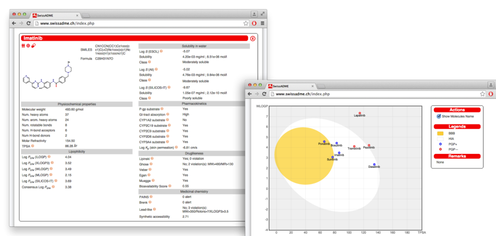
# Potency is not enough. SwissADME: a web tool to support medicinal chemists in the pharmacokinetic optimization of small molecules.

A. Daina[1], O. Michielin[1,2], V. Zoete[1]

[1]SIB Swiss Institute of Bioinformatics, Molecular Modeling Group, [2]UNIL-CHUV, Department of Oncology

Undeniably, a molecule must be intrinsically potent to be effective as a drug. However, it has also to reach its protein target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biological events to occur.
Thus, efficient drug discovery generally involve assessment of absorption, distribution, metabolism, and excretion (ADME). This is achieved earlier and earlier in the drug discovery process, when the number of chemical structures is huge but physical samples availability is limited. In that context, computer models are considered as a valid alternative to experimental procedures to estimate ADME of small molecules. We present here the SwissADME web tool (www.swissadme.ch) that gives medicinal chemists access to a collection of robust, fast and intuitive predictive models categorized as:

- *Physicochemical properties*, with an emphasis on lipophilicity computed by a variety of methodologies to enable a consensus approach, including the in-house physics-based iLOGP[1].
- *Druglikeness*, estimated by simple rules to evaluate oral bioavailability.
- *Pharmacokinetics*, which predicts several ADME behaviors (e.g. substrate of P-glycoprotein or cytochromes P450) by binary classification models relying on physicochemical descriptors.
- *Medicinal chemistry* that gives a score for synthetic accessibility and leadlikeness of molecules together with structural alerts for problematic fragments (e.g. metabolically unstable, chemically reactive or aggregators).



Recently, we added an intuitive 2D graphical model, called the *BOILED-Egg* able to predict two crucial ADME behaviors simultaneously: gastrointestinal absorption and brain access of molecules[2].
SwissADME is part of the SwissDrugDesign project of the Molecular Modeling Group at the SIB Swiss Institute of Bioinformatics (refer to the dedicated poster).

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# Microwave assisted synthesis of the antimicrobial peptide dendrimer G3KL

T. N. Siriwardena[1], M. Heitz[1], T. Darbre[1], J.-L. Reymond[1]*

[1]University of Bern

There is an urgent need to develop new antibiotics to control multidrug resistant (MDR) bacteria, in particular the Gram-negative opportunistic pathogen Pseudomonas aeruginosa, which represents a major cause of life threatening infections in hospitals today. Recently we reported peptide dendrimer G3KL as a novel antimicrobial peptide with activity against P. aeruginosa and other Gram-negative strains including MDR clinical isolates.[1-3] Here we report a preparative scale synthesis of G3KL on solid support by microwave assisted synthesis. The doubling of reagent amounts after each branching point required an optimized multiple coupling procedure. Compared to manual synthesis, the automated synthesis allows to double isolated yields while reducing synthesis time by 15-fold and waste production by 3-fold.

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3. Abdel-Sayed, P.; Kaeppeli, A.; Siriwardena, T.; Darbre, T.; Perron, K.; Jafari, P.; Reymond, J.-L.; Pioletti, D. P.; Applegate, L. A. Anti-Microbial Dendrimers against Multidrug-Resistant P. Aeruginosa Enhance the Angiogenic Effect of Biological Burn-Wound Bandages. *Sci. Rep.* **2016,** *6*, 22020.

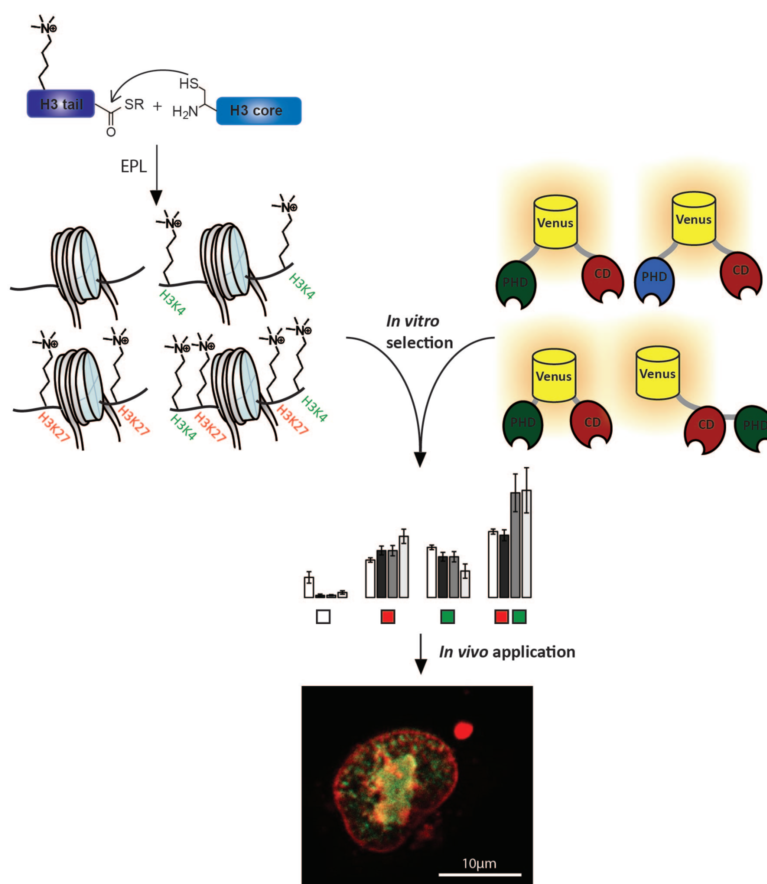
# Development of specific probes for the visualization of bivalent epigenetic marks in live cells

A. Delachat[1], O. Pundel[1], N. Guidotti[1], H. Pick[1], B. Fierz[1]*

[1]EPF Lausanne

Single-cell methods to visualize histone post-translational modifications (PTMs) patterns in living cells are currently missing. One of the most widely studied histone PTMs pattern consists of the gene transcription activating mark trimethyl Lysine 4 on histone H3 (H3K4me3) and the repressive mark H3K27me3. This PTMs combination decorates promoters of developmentally important genes in embryonic stem cells and are also found in some cancer cell lines. As these two marks have opposing effects, this type of chromatin was called bivalent chromatin. Bivalent domains were proposed to keep genes silenced but poised for activation. Currently, no single cell method exist to visualize bivalent marks in living cells.

We have developed genetically encoded probes which binds specifically to bivalent nucleosomes to reveal the co-existence of H3K4me3 and H3K27me3 at the level of single live cells. The probes consist of a fluorescent protein fused to two binding domains, a PHD zinc finger and a Chromodomain (CD). The probes specificity was determined using a library of differently modified semi-synthetic nucleosomes. For this purpose, methylated histone H3 was produced via Expressed Protein Ligation (EPL), which allows to ligate a synthetic peptide to a recombinant protein in a traceless manner. The most specific probe was selected and expressed in mouse embryonic stem cells. Subsequent observation by fluorescence confocal microscopy allowed the visualization of bivalent chromatin in live cells.



# Eudesmane sesquiterpenes from Verbesina lanata with inhibitory activity against major agricultural pathogens

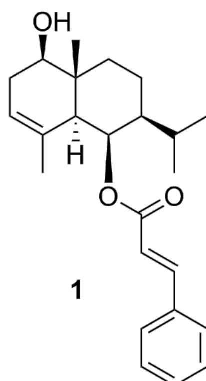
J. Ramseyer[1], B. Thuerig[2], M. De Mieri[1], H. Schärer[2], L. Tamm[2], O. Potterat[1], M. Hamburger[1]*

[1]University of Basel, [2]Research Institute of Organic Agriculture

In organic agriculture, there is a growing demand to replace copper by environmentally safer substitutes. In this context, an in-house library of more than 3000 extracts of plant and fungal origin was screened against the major plant pathogens *Venturia inaequalis*, *Phytophthora infestans*, and *Plasmopara viticola*.

As one of the hits, the ethyl acetate extract from inflorescences of *Verbesina lanata* Rob. Greenm. (Asteraceae) showed significant inhibitory activity *in vitro* against *V. inaequalis* and *P. viticola*, with $MIC_{100}$ values of 125 and 64 µg/mL, respectively.

Using an approach referred to as HPLC-based activity profiling which combines biological activity data with chemo-analytical information, the activity could be correlated with a series of lipophilic compounds in the HPLC chromatogram. Preparative isolation by a combination of chromatographic techniques, including silicagel column chromatography and preparative HPLC, afforded several compounds which were identified by ESI-MS and NMR analysis as eudesmane sesquiterpenes. The major compound, 6β-cinnamoyloxy-1β-hydroxy-eudesm-3-ene (**1**), showed strong antifungalactivity, with $MIC_{100}$ of 33 µg/mL against *V. inaequalis* and 4 µg/mL against *P. viticola*. Our results demonstrate that plant derived compounds could provide potential alternatives to copper in organic farming.



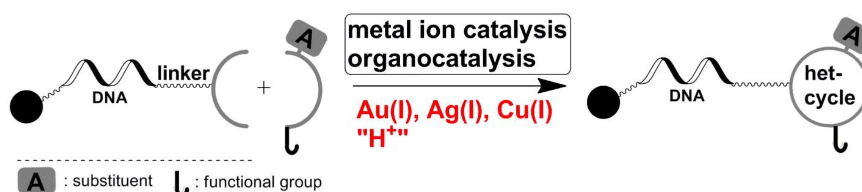
# Development of Synthesis Methodology for DNA-encoded Chemical Libraries

A. Brunschweiger[1]

[1]Technische Universität Dortmund

The selection of DNA-encoded small molecule libraries (DELs) is an attractive technology for target-based identification of bioactive compounds.[1] DELs are synthesized through iterative, combinatorial organic preparative chemistry and enzymatic encoding steps. Thus, chemical reactions applied to DEL synthesis strictly need to be DNA-compatible. Synthetic transformations are currently restricted to mostly carbonyl and Pd-catalyzed C-C cross-coupling reactions. This restriction defines a challenge for organic chemists: Development of synthesis methodology for DELs is desperately needed to expand chemical space covered by these libraries.

We developed a solid support-based synthesis strategy that broadens the range of applicable catalytic methods.[2] Among the catalytic systems that are now available for DEL synthesis are organocatalysts, and transition metal ions such as Au(I), Ag(I), and Cu(I). Our strategy opened access to substituted and functionalized heterocyclic scaffold structures as encodable DNA-conjugates from simple, readily available starting materials. For example, application of transition metal catalysts furnished DNA-heterocycle conjugates through [3+2] cycloaddition reactions. Some of the newly synthesized DNA-conjugated heterocycles display structural motifs from natural products, while others represent core structures of clinical candidates or approved drugs. All heterocyclic scaffolds enable subsequent DNA-encoded combinatorial library synthesis by well-described, robust reactions.




Solid phase-based transition metal ion- and organocatalyzed synthesis of DNA-heterocycle conjugates serving as starting points for encoded library synthesis.

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# Molecular interactions in crystal packing of dipeptide gels

A. Holzheu[1], A. Crochet[1], A. Apicella[2], K. Fromm[1]*

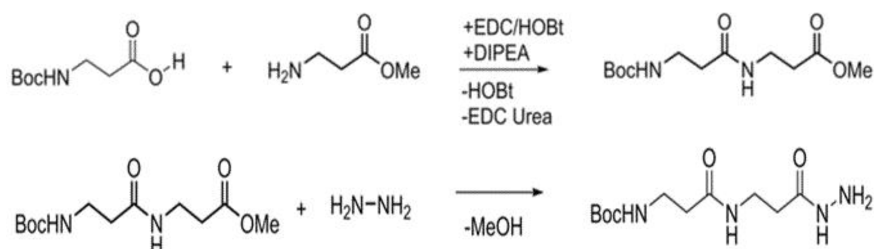
[1]University of Fribourg, [2]Fribourg

In general peptide hydrogels are a promising class of soft biomaterials for cell culture, regenerative medicine, or drug delivery applications having advantages in biocompatibility, biodegradability and injectability[1,2]. So far, many different longer peptide hydrogel systems like Max1 and $P_{11}$-2[2] are well studied but the publications about dipeptide hydrogels are limited. Dipeptide gels have certain advantages over longer peptide gels being less cost intensive, more versatile and easier to synthesize in high quantities. The most commonly studied one is the Fmoc-Phe-Phe dipeptide[3]. The major driving force of the self-assembly of such peptides is proposed to be π-π stacking. Other forces known to play a role are hydrophobic interactions, ionic interactions, hydrogen bonding and electrostatic interactions[4,5]. Nevertheless, a better understanding of the self-assembly process would allow a more rational design for specific applications.

In our work, we found that a dipeptide based on two β-alanine groups ($Boc(\beta ala)_2N_2H_3$), hence excluding π-π stacking, was able to form a gel-like state. In order to explore this phenomenon, we started to intensively characterize this system.

To obtain the dipeptide $Boc(\beta ala)_2N_2H_3$ a standard liquid phase synthesis was used (Fig.1). $^1$H-NMR, $^{13}$C-NMR, as well as a melting point analysis was performed to verify the purity of the product. Gel formation protocols were developed with the solvents ethyl acetate, acetonitrile and dioxane. The obtained gels were visually analyzed by SEM and AFM. Their structure was analyzed via XRD and SAXS. Furthermore, rheometric - as well as thermal analysis are ongoing.

Finally, we aim to solve the solid state structure of the dipeptide either by XRD or by x-ray single crystal diffraction. With that information at hand, we will understand the packing of the molecules and will be able to perform modelling regarding their self-assembly process. In addition, slight structural modifications in the dipeptide are planned to specifically target the different driving forces of assembly.

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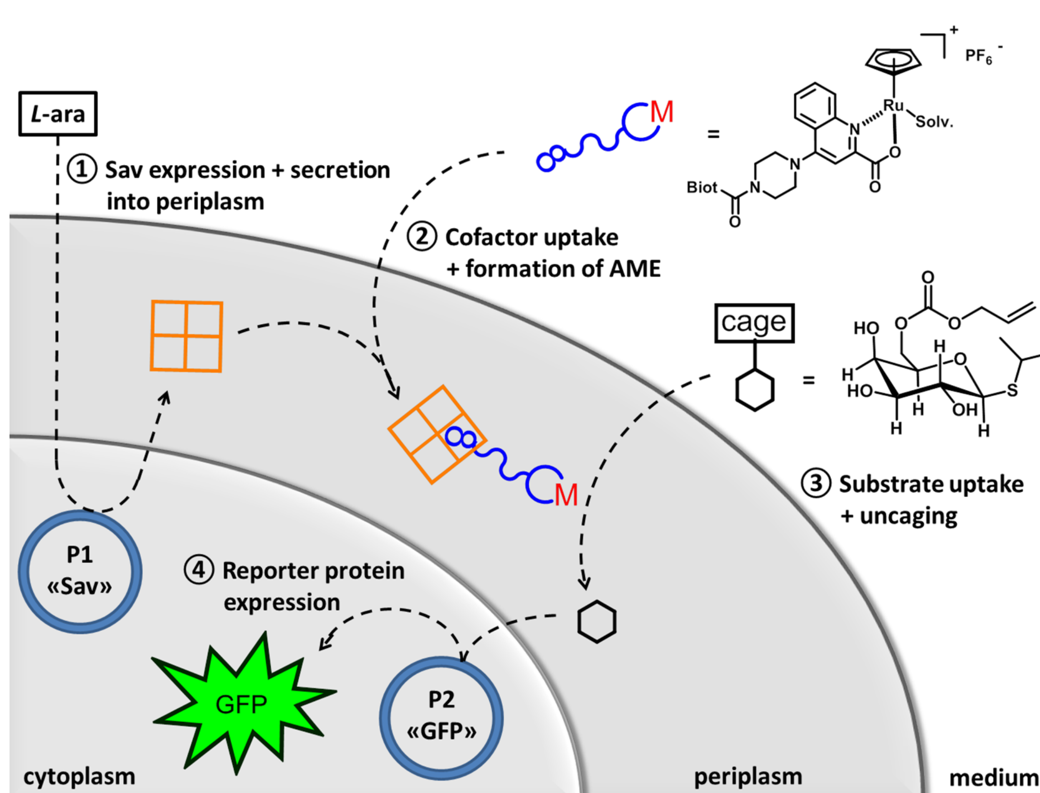
# In vivo Assay for Artificial Metalloenzyme Evolution

F. Schwizer[1], T. Heinisch[1], T. R. Ward[1]*

[1]University of Basel

Artificial metalloenzymes (AME)[1] allow to extend the reaction scope of natural enzymes, enabling unprecedented chemical transformations in biological systems. The performance and substrate scope of such AME can be tuned by chemical optimization of the metal cofactor or by genetic engineering of the host protein.[2] Incorporation of these AME in cellular environments bears the potential to construct powerful catalytic cascades and the development of high-throughput assays for AME evolution.

Herein, we present a two-protein *in vivo* assay consisting of an artificial allylic alkylase based on the biotin streptavidin technology and a natural reporter protein. Streptavidin (Sav) is expressed and secreted into the periplasm of *E.coli*. By incorporation of the biotinylated ruthenium cofactor [CpRu(Biot-Quinoline)][3] the AME is generated, which can uncage an allyl carbonate protected IPTG. The resulting uncaged IPTG inducer leads to the over-expression of a fluorescent readout (GFP), thus potentially allowing high-throughput screening of AME activity by fluorescence assisted cell sorting (FACS).

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# Orally Bioavailable Antimalarial 4(1H)-Quinolone Prodrugs with Single-Dose Cures

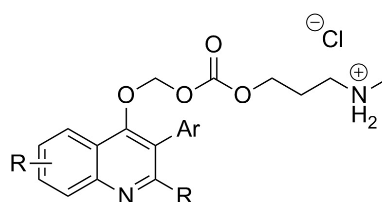
F. Brockmeyer[1], A. Monastyrskyi[2], A. LaCrue[2], T. Mutka[2], D. Kyle[2]*, R. Manetsch[1]*

[1]Northeastern University Boston, [2]University of South Florida

Malaria is estimated to have caused 438,000 deaths and 214 million cases of the disease globally in 2015. Four strains of *Plasmodium* parasite cause malaria in humans and the disease is transferred by *Anopheles* mosquitos. Though mortality rates are down 47% globally since 2000 and significant progress has been made in the quest for eradication, reported occurrences of resistance against current therapeutics threaten to reverse that progress. Longstanding treatment chloroquine has seen resistance since the 1950's, with resistance becoming widespread in the 70's and 80's. Artemisinin, the current main line of defense against malaria, is used in artemisinin combination therapies (ACTs) in order to curtail resistance, though at last count, artemisinin resistant parasites have been reported in 5 countries of the Greater Mekong sub region. In order to curb further resistance, it is essential that new antimalarial compounds be brought through the pipeline.

For approximately half a century, 4(1*H*)-quinolones such as endochin or ICI 56,780 were known to be causal prophylactic and potent erythrocytic stage agents in avian but not in mammalian malaria models.Hit-to-lead optimization of endochin lead to 4(1*H*)-quinolones ELQ-300 and P4Q-391, which target the liver, the blood as well as the transmitting stages of the parasite. Despite entering preclinical development, ELQ-300 did not enter phase I trials due to limited aqueous solubility and high crystallinity.[1]

To overcome these limitations, we designed and developed a prodrug approach containing an amino group linked to the parent 4(1*H*)-quinolone by an acetal carbonate group. Different reaction conditions were found to attach the prodrug moiety selectively onto the oxygen or the nitrogen of the 4(1*H*)-quinolone scaffold. The resulting O-alkylated prodrugs P4Q-1290 and P4Q-1291 were profiled for physicochemical properties such as chemical stability and aqueous solubility. The prodrugs are stable at low pHs and start releasing the parent drug independently of any enzyme activity at a pH level of about 7. Furthermore, prodrugs P4Q-1290 and P4Q-1291 were highly efficacious in *in vivo* efficacy assays displaying single-dose cures at low doses.




general structure of 4(1*H*)-quinolone prodrugs

The new discoveries are significant as mitochondrial inhibitors have the potential to advance the malaria elimination campaign by blocking parasite development in the blood and liver, as well as preventing transmission to mosquitoes.

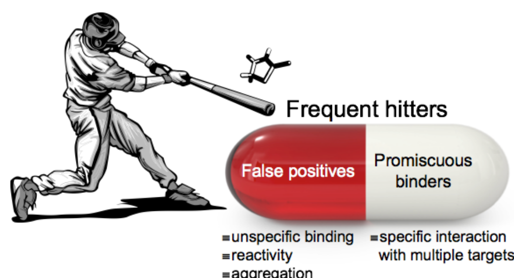
[1] Aaron Nilsen, Alexis LaCrue, Karen L. White, Isaac P. Forquer, R. Mattew Cross, Jutta Marfurt, Micahel W. Mather, Michael J. Delves, David M. Shackleford, Fabian E. Sáenz, Joanne M. Morrisey, Jessica Steuten, Tina Mutka, Yuexin Li, Grennady Wirjanata, Eileen Ryan, Sandra Duffy, Jane Xu Kelly, Boni F. Sebayang, Anne-Marie Zeeman, Rintis Noviyanti, Robert E. Sinden, Clemens H. M. Kocken, Ric N. Price, Vicky M. Avery, Inigo Angulo-Barturen, Maria B. JiménezDíaz, Santiago Ferrer, Esperanza Herreros, Laura M. Sanz, Francisco-Javier Gamo Benito, Ian Bathurst, Jeremy N. Burrows, Peter Siegl, R. Kiplin Guy, Rolf W. Winter, Akhil B. Vaidya, Susan A. Charman, Dennis E. Kyle, Roman Manetsch*, Michael K. Riscoe*,*Sci. Transl. Med.*, **2013**, 5, 177ra37.br

# De novo drug design revisited

G. Schneider[1]

[1]ETH Zürich

Good compounds are overlooked for various reasons. Computers can help by examining molecular features no chemist can see. Identifying promising candidates (positive design) is equally important as eliminating the bad apples to avoid undesired effects (negative design) as early as possible in the drug discovery process. While medicinal chemists excel in optimizing hits to eventually become lead structures and enter clinical trials, the computer's domain is to rapidly sift through many millions of molecules to discard the bulk before any screening assay is performed with the selected hits that remain after thorough *in silico* scrutiny. "Deep learning" methods may help in this endeavor by sifting through chemical space while considering available bioactivity data for navigation. In fact, recent technological advances in both computer hardware and software have enabled a renaissance of "de novo" design of molecules with desired pharmacological properties. We will present our current perspective on the concept of automated molecule generation by highlighting chemocentric methods that may capture druglike chemical space, consider ligand promiscuity for hit and lead finding, and provide fresh ideas for the rational design of customized screening compound libraries. Recent applications of automated *de novo* design methods will be presented that suggest innovative, synthetically accessible small compounds mimicking structurally more complex natural products.

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**Gut microbes and probiotics anaerobically transform carcinogenic dietary heterocyclic amines to metabolites with altered toxicity**

J. Zhang[1], C. Engels[1], M. Schneider[1], M. . Fekry[2], C. Lacroix[1], S. Sturla[1]

[1]Department of Health Sciences and Technology, ETH Zurich, Switzerland, [2]Cairo University

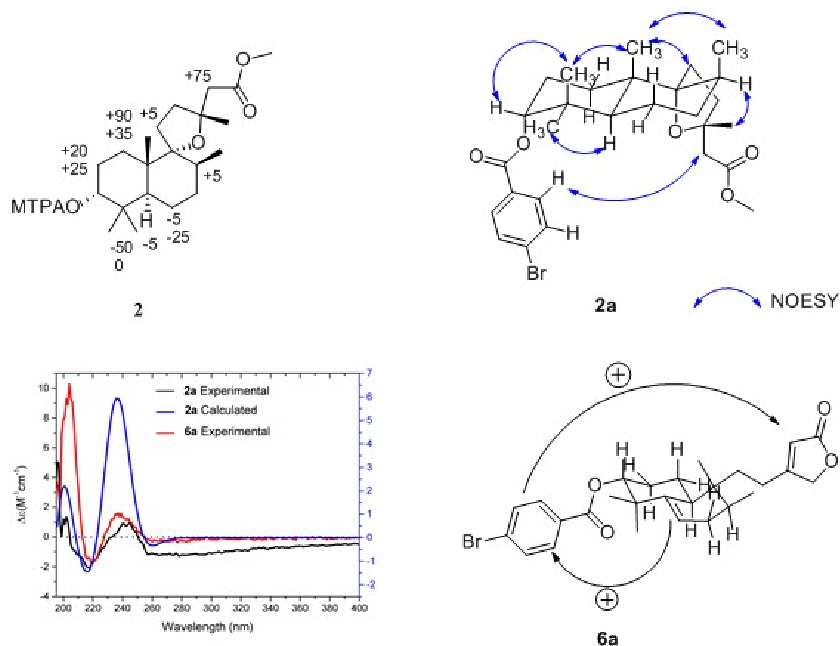
The human gut harbors a diverse community of microbes that can alter the potency of carcinogens in the colon, the chemical basis of bacterial mediated biotransformations is not well established. In this study, human gut microbes were tested for their ability to biotransform carcinogenic heterocyclic amines (HCAs). Three species belonging to *Eubacterium* and *Lactobacillus* species were observed to biotransform heterocyclic amines, and their proficiency in the presence of complex gut microbiota was confirmed using an intestinal fermentation model mimicking human proximal and distal colon microbiota. The chemical structures of the transformation products were characterized, and glycerol was found to be essential, providing a basis for insight regarding the mechanism of the transformation. The biological impact of the observed biotransformation was investigated by cell-based assays, supporting that biotransformed products have altered toxicological potencies relative to their precursors.These results suggest that dominant species of gut microbiota could biotransform dietary carcinogens and modulate their toxicity effects, a possible basis of individual susceptibility of individuals to toxicants and help in developing personalized nutrition-based disease prevention strategies.

**Assignment of absolute configuration in labdane and clerodane diterpenoids**

M. De Mieri[1], K. Du[2], M. Neuburger[1], D. Ferreira[3], M. Hamburger[1]*

[1]University of Basel, [2]University of the Free State, [3]University of Mississippi

Labdane and clerodane diterpenoids represent a large group of secondary metabolites that have shown interesting biological activities.[1] Structurally, they have four or five contiguous stereocenters on the decalin system. According to biosynthetic arguments,[2] natural labdane diterpenoids possess *trans*-fused decalin moieties, although both C-5/C-10 enantiomers (*syn*- and *ent*-forms) have been reported. On the other hand, clerodane-type diterpenoids can exhibit both *trans*- and *cis*-ring fusion.[3] For both compound classes, the stereochemistry at C-8 and C-9 can be either *cis* or *trans*, thereby allowing many diasteroisomeric combinations. Severely overlapping NMR signals, and the lack of strong chromophores render the stereochemical assignment of these compounds a challenging task, as documented by numerous discrepancies in the literature. We have employed different approaches (microscale derivatization, selective NMR experiments, ECD calculation, modified Mosher's method)[4] to assign the absolute configuration of a selected series of diterpenoids. The approach described here, and our data may be helpful for future unambiguous identification of structurally related compounds.

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## EgtB from Ergothioneine Biosynthesis - Mechanistic and Evolutionary Insights using Protein Crystallography

A. Stampfli[1,2], F. Seebeck[1]*

[1]Department of Chemistry, Universität Basel, [2]Biozentrum, Universität Basel

EgtB is an iron dependent mono-oxygenase that catalyses the central step in the biosynthesis of Ergothioneine, a thiourea derivative of histidine with antioxidant properties. While Ergothioneine is synthesized by relatively few organisms, it has been found in many species, including humans.We aim to understand the mechanistic details of dioxygen activation and control of reactivity at the iron centre of EgtB, a sulfoxide synthase. We believe that active site acidity is responsible for tuning the reactivity of a presumed ferric superoxo species, steering it towards sulfoxide synthase activity, thereby determining the reaction fate. Comparison of two homologues - their kinetics and active site architectures, has provided insight into the catalytic mechanism of these systems. Structural characterisation has also allowed us to probe their evolutionary relationships. Such an evolutionary perspective allows us to scope nature's diversification of this reaction chemistry and active site architecture. This in combination with a detailed mechanistic understanding of the features that control dioxygen activation and reactivity will provide an opportunity for engineering iron oxygenases with tailor made reactivities.

## Labelling strategies for studying the folding and splicing of the wild type group II intron ai5g

M. Gulotti-Georgieva[1], M. Zhao[1], F. Steffen[1], R. K. O. Sigel[1]*

[1]University of Zurich

The group II intron family represents a large class of non-coding RNA elements found in bacteria and lower eukaryotes. *In vitro,* group II introns are known to undergo $Mg^{2+}$ dependent self-splicing during mRNA maturation [1]. Under physiological conditions the intron folding and splicing is assisted by a cofactor, namely the DEAD-box ATP dependent helicase Mss116 [2]. The focus of our research is the study of these two mechanisms in the wild type group II intron ai5γ from *Saccharomyces cerevisiae.* We are investigating the intron dynamics during the mRNA maturation at physiological conditions and in presence of the coenzyme Mss116. Intensive studies over the past decades have concentrated on a truncated (modified) intron model, whereas we are interested in the wild type molecule. *In vitro* folding and splicing mechanism of the full length intron will be investigated in the presence and absence of Mss116 by two main methods: fluorescent native polyacrylamide gel electrophoresis (PAGE) and single-molecule Förster resonance energy transfer (smFRET). We are currently establishing the labeling strategy for visualizing both processes. On the one hand we are labelling the intron target molecule on specific positions for pursuing the folding, on the other hand the flanking exons for splicing studies. As hybridization probes DNA oligonucleotides or alternatively peptide nucleic acids (PNA) [3], both carrying fluorescence dyes, will be used. Another possibility is the direct labeling of ai5γ at specific single nucleotides [4]. Taken together, our labeling system will allow the investigation of both folding and splicing processes at the same time.

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

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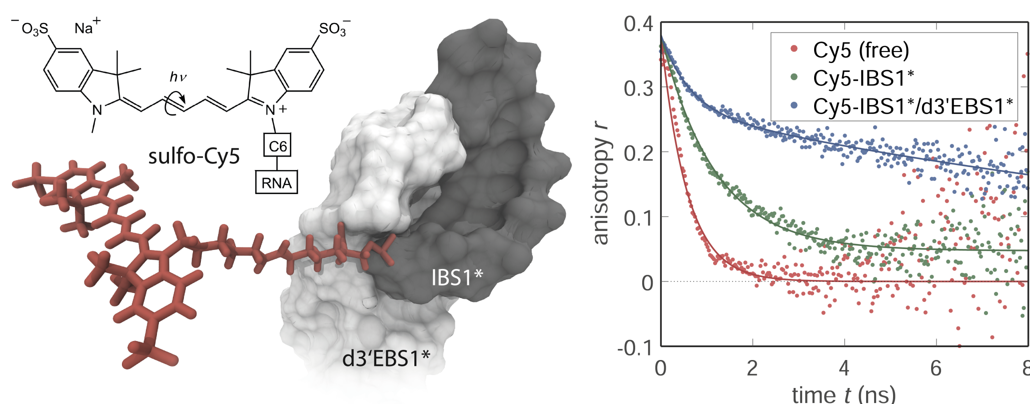
# Extending carbocyanine photophysics to the realm of RNA

F. Steffen[1], R. K. O. Sigel[1], R. Börner[1]

[1]University of Zurich

Labeling of nucleic acids for single-molecule fluorescence spectroscopy and imaging applications is usually achieved by introducing extrinsic, organic probes into the biomolecule. Among these, cyanines and derivatives are often the dyes of choice (Fig. left) [1,2]. Their photophysics has been intensively investigated in the context of DNA [3]. Little is known however about the interactions with secondary and tertiary structure motifs commonly found in RNA. To this end, we explored the influence of the nucleobase identity, RNA conformation and metal ion abundance on the fluorescence lifetime, quantum yield and dynamic anisotropy of the fluorophore [4].




The polymethine chain of carbocyanines makes the molecule act as a photoswitchable sensor of the local microenvironment that toggles between a fluorescent all-*trans* and a dark mono-*cis* isomer (Fig. left). A combination of molecular dynamics simulation and time-correlated single photon counting (Fig. right) allowed us to disentangle different tumbling modes and visualize them on an atomic level. We could demonstrate that the mobility of the fluorophore is governed not only by the sequence composition of the RNA, but to a large extent by structural features in vicinity of the dye. Awareness of such sterical constraints contributes to the interpretation of single-molecule Förster resonance energy transfer experiments.

Financial support from the European Research Council (MIRNA 259092, to R.K.O.S.), SystemsX.ch (to R.K.O.S), the Swiss National Science Foundation (to R.K.O.S) and the Forschungskredit of the University of Zurich (FK-14-096 to R.B.) are gratefully acknowledged.

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# RNA G-quadruplex formation within the BCL2 human oncogene: towards its structural determination.

A. Dominguez-Martin[1], R. K. O. Sigel[1]

[1]University of Zurich

RNA sequences can adopt different secondary structures. G-quadruplexes (G4) are non-canonical tetrahelical structures formed from planar arrangement of guanines in nucleic acids. These structures have recently raised a great interest since they are believed to contribute to genomic stability by serving as regulatory elements. The RNA BCL2 (B-Cell Lymphoma/Leukemia-2) quadruplex sequence is involved in the translational regulation of the anti-apoptotic protein BCL2, over-expressed in different types of cancer. Therefore, the study of this motif is of interest for new therapeutic approaches.

In the quadruplex sequence of the native 5'-UTR of the BCL2 RNA several structures co-exist in solution. The folding dynamics of this sequence was studied and progressively restricted yielding two specific mutants with single G-quadrudruplex conformation [1]. NMR structural studies reveal that defined dimeric structures or monomeric structures can be formed dependent on the RNA and metal ion concentration and the annealing conditions.

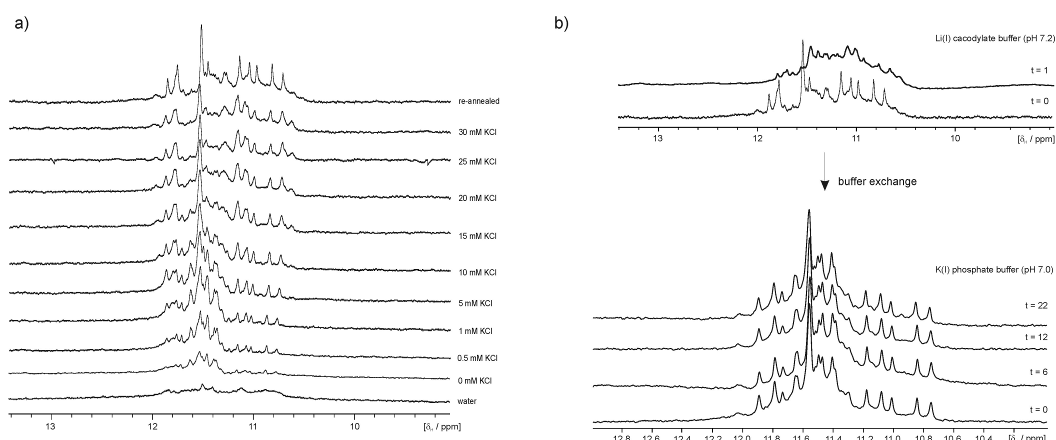


*Figure:* a) K(I) titration of the 22-mer BCL2 RNA sequence; b) Time dependece NMR spectra of the 22-mer BCL2 RNA G4 dependent on metal ions.

*Acknowledgements:* Financial support by the UZH Forschungskredit (A.D.M.), the Swiss National Science Foundation (R.K.O.S.), the European Research Council (R.K.O.S.), the SBFI (COST Action CM1105; R.K.O.S.) and the University of Zurich is gratefully acknowledged.

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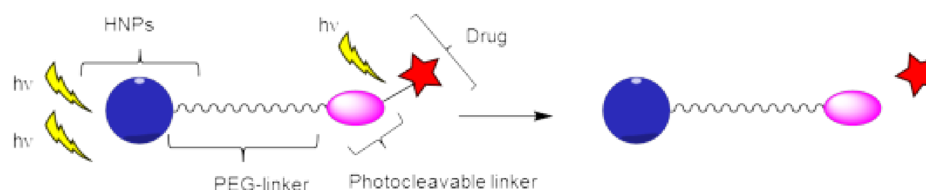
# Functionalization of second harmonic generation nanoparticles for theranostic applications

J. Vuilleumier[1], R. De Matos[1], S. Passemard[1], L. Bonacina[2], S. Gerber[1]*

[1]Ecole Polytechnique Fédérale de Lausanne, Institute of Chemical Sciences and Engineering, Batochime, CH-1015 Lausanne, Switzerland, [2]University of Geneva, GAP-Biophotonics, CH-1211 Geneva, Switzerland

Nowadays, cancer is the leading cause of death in developed countries. The emergence of new multimodal nanodevices for *in vivo* imaging offers the perspective of cancer detection at a very early stage.[1] The recent progress in nanotechnologies has generated high expectation that nanomaterials could provide unprecedented contrast agents in imaging set-ups and multifunctional platforms for drug delivery.[2] In this context, harmonic nanoparticles (HNPs), which are composed by non-centrosymmetric materials, can be easily imaged by their second harmonic generation signal in multiphoton imaging platforms.[3]

We recently disclosed efficient protocols for the biocompatible coating [4] and post-functionalization of bismuth ferrite ($BiFeO_3$, BFO) HNPs as well as their favorable properties for targeted imaging of human cancer cells and tissue.[5] We report therein the conjugation of BFO HNPs to caged molecular cargos through a photocleavable linker based on coumarinyl and *o*-nitrobenzyl derivatives. Excitation of these functionalized HNPs in the visible or near IR region generated second harmonic UV emission [6] and subsequent selective release of the conjugated drug models.

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# Investigation of the interaction between 5-HT$_3$R and its modulators: progress in understanding the agonist binding site

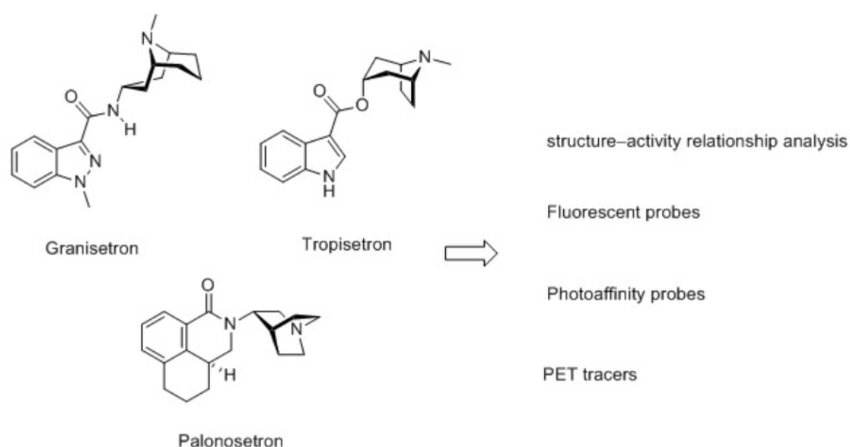
M. Lochner[1], T. Jack[1], J. Simonin[1], M. Leuenberger[1], P. Rüefli[1], Y. Bur - Cecilio Hechavarría[1], M.-D. Ruepp[1], A. J. Thompson[2]

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

The serotoninergic system plays an essential role in the brain. Nerve cells that use serotonin as neurotransmitter are among the most branched and cross-linked. They are responsible for the modulation of several processes of the CNS. Therefore, the serotoninergic system is the target for the therapy of different diseases like depression, pain, schizophrenia and chemotherapy induced nausea and vomiting. Additionally, several drugs of abuse, like LSD, DMT, Mescalin and Psilocybin are acting directly on this system.

After serotonin (5-hydroxytryptamine, 5-HT) is released into the synaptic cleft, the natural agonist binds to different receptors. Seven classes of receptors have been described too date, most of them are GPCRs (5-HT$_{1-2,4-7}$R), only the 5-HT$_3$R is an ion channel belonging to the Cys-loop superfamily of transmembrane receptors. The 5-HT$_3$R is assembled from 5 subunit positioned around the central pore. It is permeable to sodium, potassium and calcium. Recently, the crystal structure of the mouse 5-HT$_3$R was solved and reported using x-ray analysis.[1] This structure was stabilized by nanobodies, deeply binding into the orthosteric binding site, that leaves unanswered questions in the understanding of the pharmacology of the receptor.

Since few years our research focused on the study of the structure and pharmacology of 5-HT$_3$R. We report here our most recent results. Different approaches were applied. Starting from FDA approved antagonists we synthesised several probes (fluorescent,[2] photoaffinity[3] and PET[4]) and their behaviour was investigated. Most of them show very high binding affinity (low nM or even pM) and could be used as tools in microscopy, PET and proteomic analyses. Furthermore we established a stable *h*5-HT$_3$R expressing cell line and a purification protocol to yield the receptor in a high purity.

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# Peptide dendrimer as SiRNA transfection reagent

M. Heitz[1], T. Darbre[1], J.-L. Reymond[1]*

[1]University of Bern

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

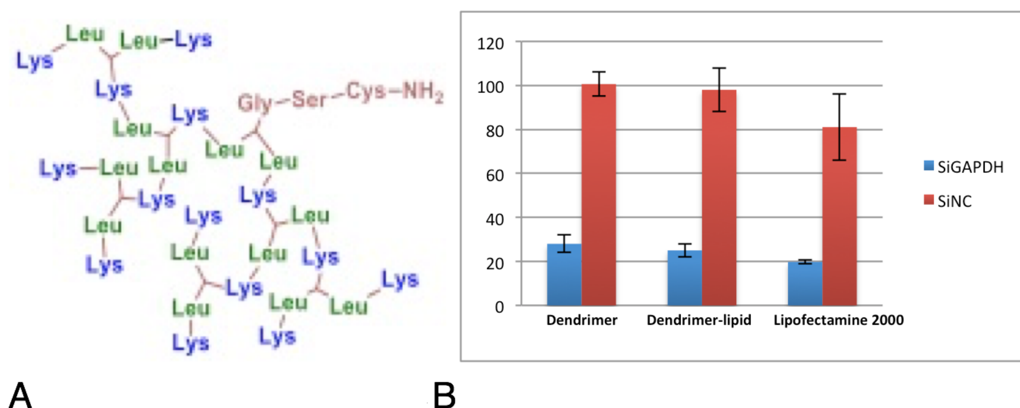


**Figure 1.** The chemical structure of the peptide dendrimer G3-KL1,2,3 used in DNA transfection (A). GAPDH expression following transfection of the lead compounds and lipofectamine 2000 in HeLa cells compared to untreated cells (B).

We are now exploring peptide dendrimers as delivery agents for SiRNA. In this project a library of 100 peptide dendrimers were prepared by SPPS and their gene silencing ability investigated. The parameters necessary for efficient gene silencing have been discovered and optimized to lead to an only amino acid and a lipid-conjugated dendrimer (Figure 1, B). The biological experiments included treatment of HeLa, CHO and HEK cells by the new transfection agents and SiRNA targeting GAPDH (SiGAPDH) or scrambled (SiNC) in the absence and presence of serum. The knockdown efficiency was measured by monitoring enzyme activity of GAPDH and quantification of GAPDH mRNA level. These potent compounds were then coupled to a fluorophore that maintain the overall knockdown efficiency and therefore allow intracellular colocalization between labeled SiRNA and dendrimer.

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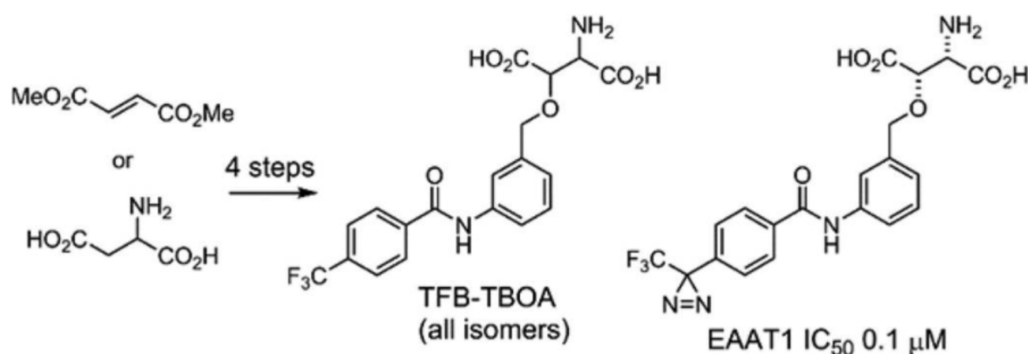
# New insights into glutamate transport in brain: Concise asymmetric synthesis of TFB-TBOA and photoaffinity probes

M. Leuenberger[1], A. Ritler[1], V. Aerni[1], S. G. Metzger[1], M.-D. Ruepp[1], M. Lochner[1]*

[1]University of Bern

In brain, glutamate is responsible for fast excitatory transmission of action potentials from one nerve cell to the next. To guarantee maximal efficiency the signal must be immediate and short. A long exposure of neurons to the effect of glutamate can damage the cells, because the neurotransmitter is toxic in the long term as it can start undesired cellular pathways ending in apoptosis. It is commonly accepted that several degenerative diseases, like Amyotrophic Lateral Sclerosis (ALS), Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD), are linked to this excitotoxicity. Therefore, one crucial point is to understand and investigate this process and the proteins that modulate this very narrow equilibrium between essential function and the aberrant secondary effect of glutamate. A family of transporters (the excitatory amino acid transporters, EAATs, belonging to the SLC1 superfamily) is responsible for the clearing of the neurotransmitter from the synapse. The investigation of the mechanism of action of these proteins could be beneficial for a better understanding of the death of neurons and could help to improve existing therapies.

In our research we focused on the synthesis of TFB-TBOA, a known high potent inhibitor of EAATs, and different photoaffinity probes derived thereof. We present here our significantly improved enantioselective synthesis and the inhibitory effect of our compounds on human EAAT1, EAAT2 and EAAT3.[1]

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# Polyoxometalate-chitosan nanocomposites for medical applications

M. Croce[1], S. Conti[1], C. Maake[1], G. R. Patzke[1]*

[1]University of Zurich

Polyoxometalates (POMs) are negatively charged metal oxide clusters whose biological activity against cancer cells, viruses and microorganisms was widely investigated with promising results.[1,2] Despite their efficacy *in vitro*, they are not yet suitable for clinical applications due to their cytotoxic side effects. However, POM encapsulation in a biocompatible matrix, e.g. carboxymethyl-chitosan, leads to a drastic reduction of the overall cytotoxicity.[3,4] In this work biologically relevant POMs, such as $K_6[P_2W_{18}O_{62}]$, $[Mo_8O_{26}]^{4-}$,and $(NH_4)_{17}[Na_2Sb_9W_{21}O_{86}]$, were synthesized and then encapsulated with chitosan through ionotropic gelation. Their characteristics have been evaluated by dynamic light scattering, scanning electron microscopy and FT-IR spectroscopy. The cytotoxicity and the antimicrobial activity of the polyoxometalates, pristine or encapsulated, was investigated on mammalian cells (HeLa cells) and on bacteria (*E. coli*, *S. sobrinus* and *S. mutans*), respectively. On HeLa cells some of the composites showed higher cytotoxicity compared to the pristine POM, as it was observed for N-trimethyl chitosan nanocomposites.[5] Generally, on bacteria the nanocomposites were always more effective than the pristine components, probably because of the combined antimicrobial effect of the chitosan matrix and the POM. A triggered release of polyoxometalates is currently under development.

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# Design, synthesis and pharmacological characterization of novel store-operated calcium channel (SOCE) modulators

A. Schild[1], D. Tscherrig[1], N. Wenger[1], B. Rajesh[1], B. Lüscher[1], M. A. Hediger[1], M. Lochner[1]*

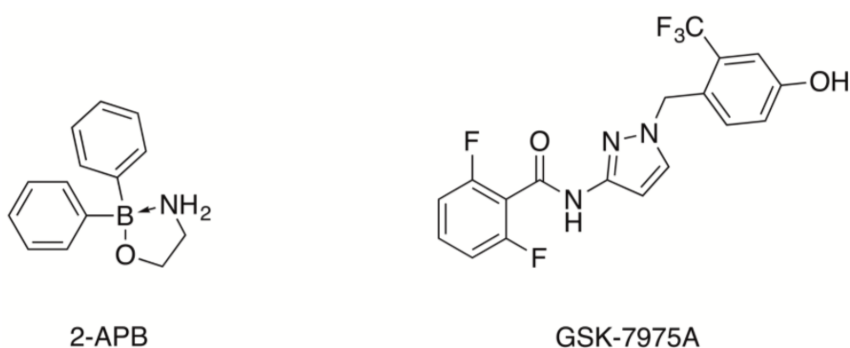
[1]University of Bern

Calcium is vital for the regulation of various physiological functions such as cell differentiation, proliferation, muscle contraction neurotransmission and fertilization. In order to accomplish calcium signaling, cells maintain a very low concentration of free calcium in the cytoplasm (~5x10-8 M). The endoplasmic reticulum (ER) is the main intracellular calcium store and its refilling process is known as store operated calcium channel entry (SOCE). [1]

The depletion of the calcium stored in the ER is detected by the calcium sensors located on the stromal interaction molecule (STIM). This induces an oligomerization of STIM which then interacts with the cytosolic part of Orai, a protein located in the plasma membrane. The STIM-Orai complex promotes calcium entry into the ER, thus causing a calcium-release activated calcium current (CRAC).

The upregulation of STIM and Orai enhances SOCE and has been associated with several cancers. In addition genetic studies revealed that patients deficient in STIM1 or Orai1 function suffer from severe immunodeficiency and autoimmune diseases, while gain-of-function mutations in STIM1 and Orai1 have been associated with bleeding disorders and muscular defects. Potent and selective SOCE modulators could represent a new avenue for therapies.

Previously studied SOCE modulators exhibit either low selectivity for SOCE or have low potency. In this work, analogues of the known SOCE modulators 2-APB, DPB162-AE, GSK-7975A and Synta 66 were synthesized. The aim of the introduced modifications was to enhance the performance of the modulators, as well as to provide modulators with the ability to covalently bind to the target for identification of their binding sites.

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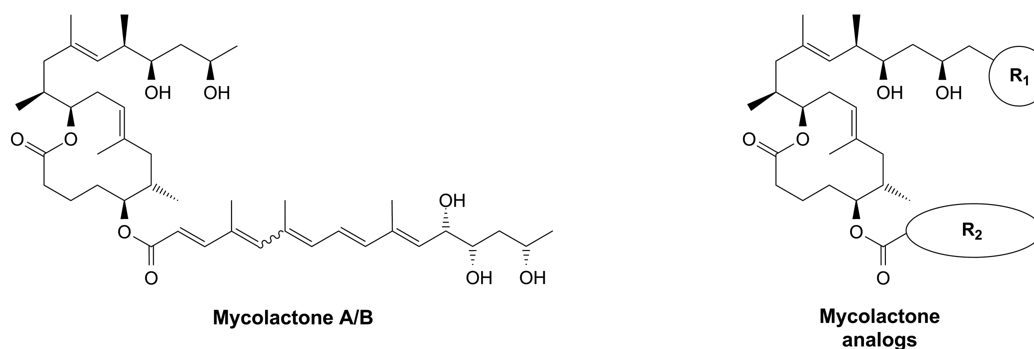
# Total Synthesis, Target Evaluation and Structure-Activity Studies of Mycolactone and its Analogs

M. Gehringer[1], P. Gersbach[1], R. Bieri[2], N. Scherr[2], G. Pluschke[2]*, K.-H. Altmann[1]*

[1]ETH Zurich, [2]Swiss TPH Basel

Mycolactones are a group of macrolides excreted by the human pathogen *Mycobacterium Ulcerans*,that exhibit cytotoxic, immunosuppressive and analgesic properties. The most prominent member of the mycolactone family is mycolactone A/B, the causative agent for the neglected skin disease Buruli ulcer.[1] Despite extensive research in several academic laboratories, the mechanisms of action of mycolactones are a matter of debate and it remains unclear whether the 4',5'-*cis*- or the respective *trans*-derivative is the major contributor to bioactivity.




Aiming to identify the targets of mycolactone A/B, several analogs with tags at the upper and the lower side chain (R1 or R2, respectively) were prepared. These conjugates were used in target fishing experiments, which have led to novel insights into the mechanism of action of mycolactone. Furthermore, several derivatives with structural modifications at both side chains have been prepared and structure-activity-relationships were studied.

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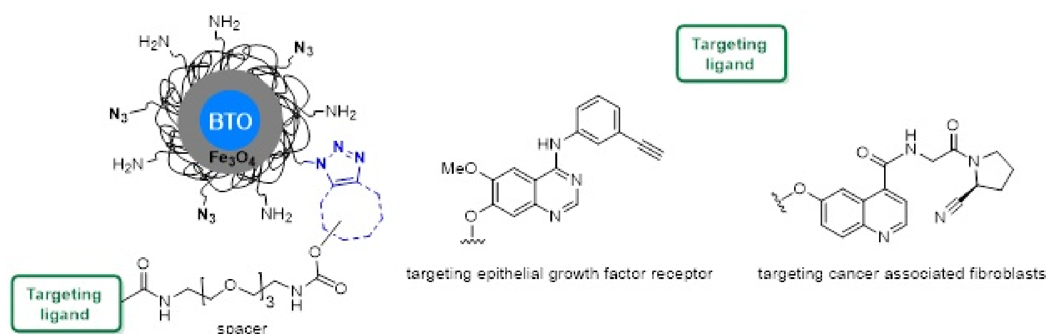
# Functionalization of second harmonic nanoparticles for targeted tumor imaging and multimodal cancer diagnosis

R. De Matos[1], S. Passemard[1], D. Staedler[2], S. Constant[3], L. Bonacina[4], S. Gerber[5]*

[1]Ecole Polytechnique Fédérale de Lausanne, Institute of Chemical Sciences and Engineering, Batochime, CH-1015 Lausanne, Switzerland, [2]TIBIO Sagl, CH-1131 Tolochenaz, Switzerland, [3] Epithelix, CH-1228 Plan les Ouates, Switzerland, [4]University of Geneva, GAP-Biophotonics, CH-1211 Geneva, Switzerland, [5]Ecole Polytechnique Fédérale de Lausanne, Institute of Chemical Sciences and Engineering, Batochime, CH-1015 Lausanne

The recent and rapid progress in nanotechnologies has paved the way for the investigation of nanomaterials in clinical settings for early detection, diagnosis and targeted treatment of cancer, which represents a major health burden in developed countries.[1] The ability to produce inorganic nanoparticles of tunable size and composition, combined with their surface properties suitable for chemical functionalization have generated intense efforts to develop novel theranostic tools based on multifunctional nanomaterials.[2]

In this work, we present the functionalization of core-shell BaTiO3@Fe3O4 (BTO-IO) nanoparticles (NPs) with molecular payloads targeting cancer cells and cancer associated cells biomarkers, for multimodal imaging applications. In particular, analogues of Erlotinib and inhibitors of fibroblast activation protein a were evaluated for their selective association to cancer cells and were further conjugated to poly(ethylene glycol) coated BTO-IO NPs through click reaction.[3]




Taking advantage of the second harmonic generation properties of the core and of the magnetic properties of the shell, the resulting nanomaterials were evaluated for their ability to be detected in multiphoton imaging platforms and to be used as contrast agent for magnetic resonance imaging.

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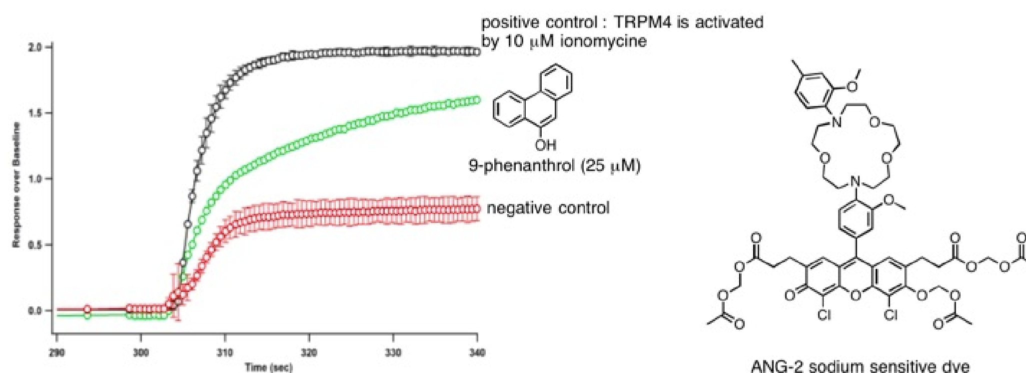
# Discovery of TRPM4 ion channel inhibitors

C. Delalande[1], L. Ozhatil[1], B. Bianchi[1], H. Abriel[1], J.-L. Reymond[1]*

[1]University of Berne

The expression of the cation channel TRPM4 has been reported in many different cell types but its physiological role and involvement in diseases are still elusive. Mutations in the gene coding for TRPM4 have been identified in patients with cardiac conduction disturbance[1]. In the last few years, 9-phenanthrol, the only selective TRPM4 inhibitor reported, has been of great help to characterise TRPM4 currents in biological tissues, and study the effect of inhibition of the channel[2]. However, this compound is not very potent (IC50 ~ 10 µM), has poor water solubility, and lacks selectivity against close analogs such as TRPM5. New TRPM4 inhibitors would therefore be useful to further study the involvement of TRPM4 in physiological processes. A small compound library selected through several rounds of ligand-based virtual screening (LBVS)[3], [4] was evaluated using a fluorescent-based sodium influx assay. Validation of the six potential TRPM4 inhibitors yielded 2 hits candidate, 162 and 487, sharing the same phenoxy acetamido-anthranilic acid core. Structure-activity relationship of these compounds is under investigation. Both anthranilic acid and phenoxy moiety were varied.

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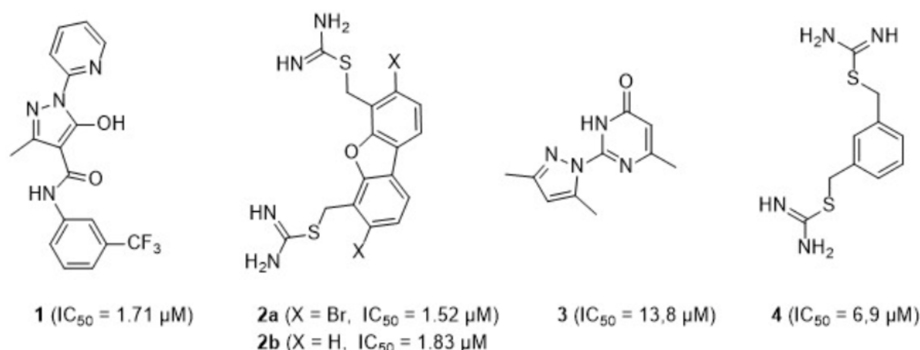
# Identification and improvement of modulators of Divalent Metal Ion Transporter 1 (DMT1)

M. Poirier[1], A. Embaby[1], J. Pujol-Giménez[1], M. A. Hediger[1], J.-L. Reymond[1]*

[1]University of Bern

DMT1 is a proton coupled iron transporter which plays an essential role in iron homeostasis, and whose deregulation is linked to hemochromatosis. Our aim is to develop small molecule inhibitor of DMT1 which can be used further for physiological studies or future developments in therapeutic applications. Starting with inhibitors **1** and **2a/b** previously reported as DMT1 inhibitors, we performed ligand-based virtual screening (LBVS) in the ZINC database using our recently reported shape and pharmacophore similarity algorithm xLOS,[1] and purchased over 200 pharmacophore analogs, which led to the identification of hit compounds **3** and **4**.[2] Compound **3** proved to act as a non-competitive inhibitor of DMT1 and was therefore investigated further. While analogs of **3** could not be identified from commercial catalogs, the compound was readily prepared by condensation of the parent hydrazine with acetylacetone, which enabled the synthesis of a series of analogs from which an optimized inhibitor reaching the activity of the parent literature compound **1** was identified. This new inhibitor has a significantly smaller size and therefore a better ligand efficiency compared to **1** or **2**. Additional approaches to the discovery of DMT1 inhibitors will also be presented including screening of a library of fragment-like 3D-shaped compounds derived from the chemical universe database GDB-17.[3]




**1** ($IC_{50}$ = 1.71 µM) **2a** (X = Br, $IC_{50}$ = 1.52 µM) **2b** (X = H, $IC_{50}$ = 1.83 µM **3** ($IC_{50}$ = 13,8 µM) **4** ($IC_{50}$ = 6,9 µM)

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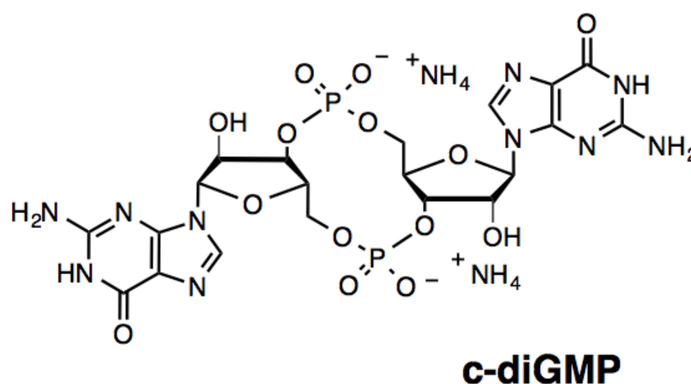
# Functionalized Proline-Rich Peptides as Selective Binders of c-diGMP

C. Foletti[1], R. A. Kramer[1], K. H. Bleicher[2], H. Wennemers[1]*

[1]ETH Zurich, [2]F. Hoffmann-La Roche AG

In recent years, the importance of bis-(3',5')-cyclic dimeric guanosine monophosphate (c-diGMP) as an ubiquitous secondary messenger in bacteria has been recognized. This messenger plays crucial roles in the regulation of biofilm formation, cell cycle progression and virulence of several pathogens.[1] Furthermore, its absence in higher order eukaryotes makes it an attractive therapeutic target. Development of c-diGMP inhibitors has focused on targeting either the proteins responsible for its synthesis or its receptors, both resulting in selectivity challenges due to protein redundancy.[2]




We have prepared and screened a proline-rich split-and-mix peptide library against c-diGMP. Using this technology, we have obtained tetrapeptides that bind c-diGMP and have further modified the library to improve binding. Currently, we are investigating the biofilm inhibition of our peptides on the clinical relevant bacteria, *P.aeruginosa,* and have obtained promising biological activity.[3]

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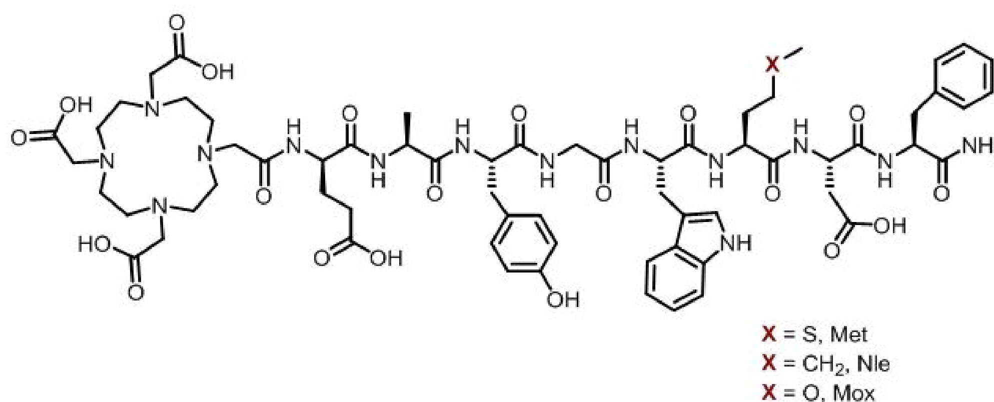
# Substitutions of Methionine on DOTA-Minigastrin 11 : Evaluation of two non-natural amino acids

N. Grob[1], M. Béhé[2], R. Schibli[2,1], T. L. Mindt[1,3]*

[1]ETH Zürich, [2]Paul Scherrer Institute, Villigen, [3]Ludwig Boltzmann Institute of Applied Diagnostics, Vienna

Minigastrin 11 (MG11) is a trunctuated analogue of minigastrin, a regulatory peptide with high affinity and specificity towards the cholecystokinin 2 receptor (CCK2R), which is overrexpressed in various forms of cancer[1]. By conjugation of the peptide to 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), it can be radiolabelled with radionuclides (e.g., $^{177}$Lu) and used for *in vivo* tumour imaging and peptide receptor therapy[2]. However, the high temperatures needed for radiometal complexation in combination with ionising radiation leads to oxidation of the thioether functionality of Methionine[3]. The formation of sulfoxides results in low radiochemical purities and loss of affinity of the peptide towards the CCK2R. Two analogues of MG11 with non-oxidizable amino acids as substitute for Methionine were investigated.




The poster will discuss the synthesis of the three MG11 analogues and the evaluation of their physiochemical properties. Receptor affinities ($IC_{50}$), cell internalisation rates, and hydrophilicities (logD) of the three peptide conjugates were investigated *in vitro*. The ultimate goal of this project is to identify an analogue of MG11 with similar biological behaviour but resistant to oxidation.

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# A close look to the interaction of a metallo-intercalator and an RNA internal loop via NMR

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

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

In the field of luminescent metal complexes with potential as bio imaging agents, the interaction with DNA was extensively studied. [1] On the contrary, RNA has received only little attentionto date, although it is a very challenging and interesting molecule. Indeed, it shows a wide structural variety and its importance in many biological processes is well acknowledged. [2] In this context, we aimed at understanding the interaction of $[Re(CO)_3(dppz)(3\text{-}CH_2OH\text{-}Py)]^+$ [3] with short RNA sequences, using as main technique NMR spectroscopy. A comparison to the behaviour of the well-known $[Ru(bpy)_2(dppz)]^{2+}$ "light switch" complex [4] was also performed. We used two different RNA constructs, a first one (RNA-l) that is 27-nucleotide long and contains a GU wobble, an internal and a terminal loop [5] and a second one (RNA-h) that is an analogous short RNA that does not contain the internal loop. Interaction assays were also performed on a DNA sequence similar to RNA-h. Mono and bi-dimensional NMR experiments, such as $^1H$, $[^1H,^1H]$-NOESY, $[^1H,^1H]$-TOCSY, $[^1H,^{15}N]$-HSQC and DOSY spectra were used to assess the binding and localise the interaction. All the data collected suggest that there is a preferential interaction localized at the RNA internal loop. Experiments at different temperature are currently being performed to further investigate the dynamics of the system. The interaction was confirmed by other techniques, such as fluorescence spectroscopy, including ethidium bromide displacement assays, and Job Plot analyses.

***Acknowledgments***

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 FK-15-080 to EA) and within the COST Action CM1105 is gratefully acknowledged.

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# Metal ion interactions in ncRNAs revealed by smFRET

R. Börner[1], R. K. O. Sigel[1]*

[1]University of Zurich

More than two decades investigating nucleic acids and ribonucleic acids (RNA) by single molecule Förster Resonance Energy Transfer (smFRET) have passed [1]. It was observed that sample heterogeneity in structure and function of RNA molecules including folding intermediates, kinetic subpopulations, and interconversion rates of conformational states is an often observed characteristic on the sm level. Further, metal ions play a crucial role in RNA folding and dynamics, as well as RNA/RNA or RNA/DNA interactions. We reviewed recent advances in the characterization of the role of metal ions in folding and function of nucleic acid structures by means of smFRET [1].

We further describe the entire workflow of smFRET data analysis: (i) Single molecule detection, (ii) fluorescence time trace generation, (iii) correction for background and spectral crosstalk, (iv) discretization of time-dependent FRET efficiencies, and (v) selection of thermodynamic and/or kinetic models. Accordingly, we provide a comprehensive account on the various today available data analysis strategies in camera-based smFRET experiments and seek to facilitate data analysis through a quantitative assessment of the robustness of the existing methods via MC simulations [2]. We apply our recently developed method to excess the sample heterogeneity via bootstrapping [3]. The workflow is exemplified by the metal ion-depending folding and dynamics of the group IIB intron from *S. cerevisiae* in a crowding environment [4] and RNA-RNA binding kinetics of this ribozyme's 5'-splice site formation on the sm level [5].

Financial support from the European Research Council (MIRNA N° 259092 to R.K.O.S.), the Swiss National Science Foundation (to R.K.O.S.), and the Forschungskredit Grant of the University of Zurich (FK-14-096 to R.B.) are gratefully acknowledged.

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# HPLC-based activity profiling for $GABA_A$ receptor modulators from Searsia pyroides leaves using a validated larval zebrafish locomotor assay

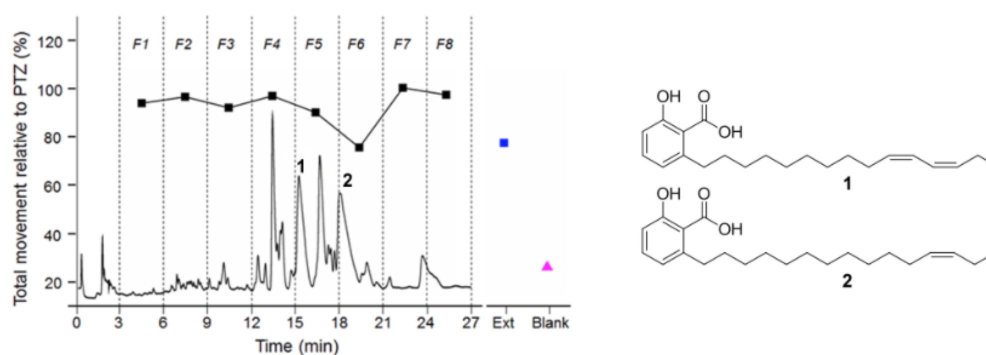
F. Moradi-Afrapoli[1], S. N. Ebrahimi[1], H. van der Merwe[2], M. Smiesko[3], M. Hamburger[1]*

[1]Division of Pharmaceutical Biology, University of Basel, Switzerland , [2]Department of Chemistry, University of the Free State, South Africa, [3]Division of Molecular Modeling, University of Basel, Switzerland

Gamma-aminobutyric acid type A ($GABA_A$) receptors are key inhibitory neurotransmitter receptors in the central nervous system (CNS). They are target for numerous clinically important drugs used to treat anxiety, insomnia and epilepsy. We previously identified a series of allosteric $GABA_A$ receptor agonists with the aid of HPLC-based activity profiling, whereby activity was tracked with an electrophysiological assay in *Xenopus* oocytes.[1] To accelerate the discovery process, we now established an approach for HPLC activity profiling using a behavioral model with zebrafish larvae.[2]

The assay uses 7-day post fertilization larvae in a 96-well format. Larval convulsions are provoked by the pro-convulsant $GABA_A$ receptor antagonist pentylenetetrazol (PTZ), and $GABA_A$ receptor agonistic extracts and compounds are identified through a decrease in larvae locomotor activity. We have validated the assay with the aid of representative GABAergic compounds and extracts.[3]

The validated assay was used for tracking GABAergic compounds in South African medicinal plants which were previously identified as active in the *Xenopus* oocyte assay. A dichlomethane extract from *Searsia pyroides*, traditionally used for treatment of epilepsy, significantly reduced PTZ-provoked activity in zebrafish larvae. The extract was separated by analytical HPLC, and 3-minute micro-fractions were collected. Lowering of locomotor activity was observed fractions 5 and 6. Anacardic acid derivatives **1** and **2** were identified in the active fractions of the extract. Structure elucidation was achieved by HRMS and microprobe NMR spectroscopy. Both compounds showed activity in the zebrafish larvae model. Anacardic acid derivatives are reported here for the first time as positive $GABA_A$ receptor modulators.

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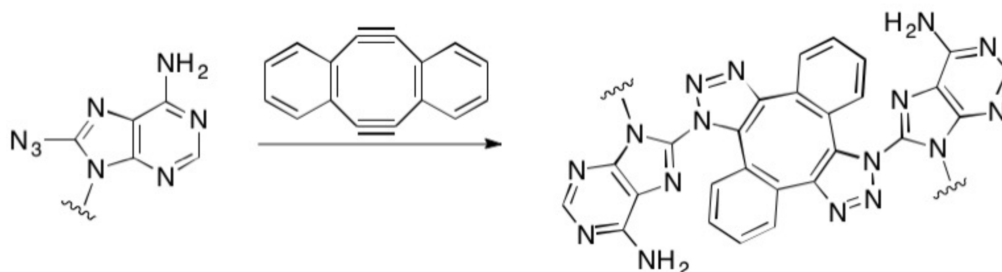
# Click Chemistry for DNA Interstrand Crosslinking

Z. Harati-Taji[1], N. W. Luedtke[1]*

[1]University of Zurich

DNA interstrand crosslinkers are highly toxic agents that trigger cell-death by covalently linking DNA strands and preventing transcription and replication, processes which require DNA strand separation. Such molecules, including *cis* platin and the nitrogen mustard cyclophosphamide, are widely used as chemotherapeutic drugs. [1][2]

Interstand crosslinks (ICLs) occur between native nucleobases in the DNA and, although highly lethal, form inefficiently. Introduction of a nucleoside analog with bioorthogonal functionality would allow the formation of specific ICLs and attenuation of the reactivity and efficiency of the cross-linking agent. Click chemistry provides a reliable and selective method for site-specific reactivity. In particular, strain-promoted click chemistry is highly efficient without the need for a copper catalyst. [3] The Sondheimer diyne, with two strained alkynes, provides a means of covalently linking two clickable molecules. [4] This diyne could act as a crosslinking agent in the presence of azide-modified nucleobases on two DNA strands. To this end, we have proposed the incorporation of the azide-modified deoxyadenosine below into an oligonucleotide sequence and subsequent introduction of the diyne to form an interstrand crosslink. *In vitro*, the azide-diyne double click reaction proceeds at the rate of 0.0490 ± 0.0046 $M^{-1}s^{-1}$ to give a fluorescent product.

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# Pre-organization of Selectin Antagonist: Novel Structural Modifications

N. Varga[1], P. Zihlmannn[1], T. Mühlethaler[1], M. Smiesko[1], B. Ernst[1]*

[1]University of Basel

Selectins form a family of three cell adhesion molecules (E-, P-, and L-selectin), which in case of an inflammatory stimulus, trigger the extravasation of leukocytes through the endothelium to reach the site of inflammation.[1] However, excessive infiltration of leukocytes into the adjacent tissue can lead to acute and chronic reactions, as observed in reperfusion injuries, stroke or rheumatoid arthritis.[2] Therefore, inhibition of selectins is regarded as a promising pharmaceutical option.

Sialyl Lewis$^x$ (sLe$^x$) is the minimal carbohydrate epitope of physiological selectin ligands. The pharmacophores of sLe$^x$ are well pre-organized[3], and to describe their spatial arrangement two dihedral angles have been utilized. The *core conformation* describes the arrangement of the fucose moiety and galactose moiety, while the *acid orientation* describes the orientation of the carboxylic acid contributed by N-acetyl neuraminic acid.[4]By replacing parts of the sLe$^x$ structure with less polar fragments, high-affinity sLe$^x$ mimetics were identified.[5] In the present study we show novel structural modifications to stabilize and pre-organize the pharmacophores of sLe$^x$ derived selectin antagonists.

Core conformation
Acid orientation

sLe$^x$

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## Target-Driven Dynamic Combinatorial Chemistry – Potentials and Pitfalls as Exemplified on a Bacterial Adhesin

P. Frei[1], L. Pang[1], D. Eris[1], M. Silbermann[1], T. Mühlethaler[1], O. Schwardt[1], B. Ernst[1]*

[1]University of Basel

Dynamic combinatorial chemistry (DCC) generates substance libraries starting from reversibly reacting building blocks. These libraries are under thermodynamic control and their composition in equilibrium state is affected by the conditions applied. For instance the presence of a target protein can stabilize ligands bound to the protein, leading to a shift in the composition of a library. This target-directed DCC has the potential to rank library members according to their affinities. (1, 2)

We studied acylhydrazone libraries formed from aldehydes reacting reversibly with hydrazides. The goal was to identify inhibitors of FimH, a bacterial adhesin crucial for the development urinary tract infections (UTI). (3, 4) FimH can be inhibited with biaryl mannosides (5, 6), which we used as lead structure in our library design.

The acylhydrazones can be monitored by UV-HPLC due to their strong absorption at 310 nm. Alterations in the composition of a library equilibrated in presence of FimH can be determined by comparison with the library equilibrated in absence of the target protein. However, sample preparation prior to HPLC analysis and ratio of building blocks can substantially influence the outcome and thereby the information content of the experiment.

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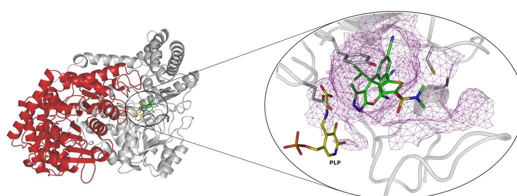
# Inhibition of SHMT: A Neglected Enzyme from the Folate Cycle

G. Schwertz[1], M. Witschel[2], M. Rottmann[3], R. Bonnert[4], P. Chaiyen[5], P. Chitnumsub[5], K. White[6], F. Diederich[1]*

[1]ETH Zurich, [2]BASF AG, [3]Swiss Tropical and Public Health Insitute, Basel, [4]Medicines for Malaria Venture, [5]National Center for Genetic Engineering and Biotechnology, Thailand, [6]Monash Insitute of Pharmaceutical Sciences

Malaria is mainly caused by the parasite *Plasmodium falciparum*. Due to the emergence of drug-resistant strains, there is an urgent need of novel treatment. The folate cycle contains several enzymes, and was identified as a promising target. Indeed few antimalarials already address this pathway.[1] However, inhibition of serine hydroxymethyl transferase (SHMT), a key enzyme of the folate cycle, has not been investigated so far. *A. Thaliana* SHMT inhibitors, based on a pyrazolopyran core, from an herbicide optimization program at BASF-SE demonstrated promising antimalarial activity on *P. falciparum* and *P. vivax*.[2] Our pioneering work on the inhibition of SHMT shed the light on this novel antimalarial target.[3] The binding mode was resolved by several X-ray crystal structures of *Pv*SHMT-ligand complexes.[3] Based on the high similarity of *P. vivax* and *P. falciparum* SHMT, the X-ray co-crystal structures can be utilized for 3D modeling to design small drug-like molecules against *Pf*SHMT. Nevertheless pharmacokinetic limitations of our lead compound prevented any *in vivo* activity in the *P. berghei* mouse model. In this work the development of novel inhibitors is focused on improving liver microsomal stability while keeping high *in vitro* potency. In that perspective, subtle modification of the scaffold and derivatization of the exit-vector led to promising candidates for further *in vivo* efficacy evaluation.

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# THE MOCO RIBOSWITCH FROM E.COLI

F. Amadei[1], S. Gallo[1], R. Börner[1], R. K. O. Sigel[1]*

[1]University of Zurich

Molybdenum is an essential metal for life and it has been found in several enzymes using the redox properties of molybdenum to catalyze fundamental reaction in the metabolic cycles of carbon, nitrogen and Sulphur [1]. To carry out its bioactivity molybdenum(VI) needs to be stabilized. In all molybdoenzymes, a part from nitrogenase, molybdenum is coordinated to a tricyclic pyranopterin forming the molybdenum cofactor (Moco) [2]. Moco by itself is highly dioxygen sensitive and can only be produced biosynthetically. In all taxonomic groups, Moco is biosynthesized by a well-known conservative four-step pathway involving different enzymes. The regulation of those is predicted to be controlled by a specific Moco-binding RNA, the so-called Moco riboswitch [3].

Riboswitches are non-coding RNA, which selectively bind cellular metabolites. Upon binding of the metabolite a conformational rearrangement of the RNA (switch) regulates the translation of the coding part of the mRNA. So far there is no study on the interaction of the Moco riboswitch and any kind of metabolites. We therefore investigate this system *in vitro* to reveal whether the Moco riboswitch is capable to interact specifically with Moco or one of its precursors and to undergo the expected structural change. We isolated the *moaA* gene from *E. coli* by cloning and produced the Moco riboswitch by *in vitro* transcription. Subsequently the Moco riboswitch was studied by UV/Vis, melting studies, native PAGE and circular dichroism giving a comprehensive view on the influence of metal ions such as potassium(I) and magnesium(II) on the RNA folding. The direct interaction between the Moco riboswitch and different metabolite candidates will be studied by in-line probing assays and other RNA footprinting assays [4] yielding a proof of the regulation capabilities of this promising riboswitch candidate.

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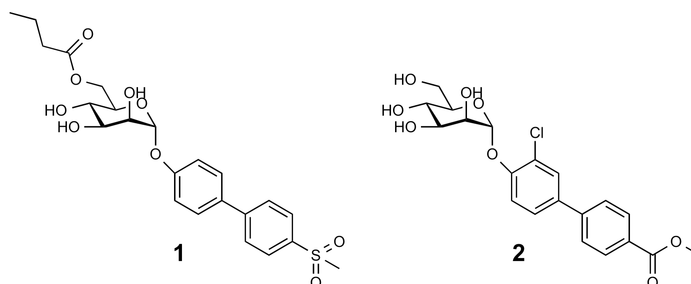
# The Influence of the Ester Prodrug Hydrolysis upon Bioavailability of FimH Antagonists

P. Dätwyler[1], J. Bezençon[1], A. Sigl[1], S. Kleeb[1], W. Schönemann[1], B. Ernst[1]*

[1]University of Basel

Urinary tract infections (UTIs) are among the most common infectious diseases and are mainly caused by uropathogenic *Escherichia coli* (UPEC). Increasing antibiotic resistance of UPEC requires new therapeutic approaches for oral treatment.[1] The first step of the pathogenesis, the adhesion of UPEC to urothelial cells, is mediated by FimH, a lectin located at the tip of bacterial type 1 pili. FimH recognizes mannosylated glycoproteins on urothelial cells and is therefore a potent target for the treatment and prevention of UTIs. Recent progress in the development of FimH antagonists resulted in highly affine, biphenyl α-D-mannopyranosides with nanomolar affinities.[2,3]

A major drawback of carbohydrate derivatives is their hydrophilicity, leading to insufficient oral bioavailability. Bioavailability can be improved by aliphatic ester prodrugs. After hydrolysis, the metabolically stable active principle is then renally excreted, reaching its target in the bladder. However, various esters are hydrolyzed by different carboxylesterases (CES). Prodrug **1** is highly permeable[4], but the small acyl moiety is preferentially hydrolyzed by carboxylesterase 2 (CES2) expressed in enterocytes, whereas prodrug **2** with an ester function in the aglycone is no longer hydrolyzed in enterocytes but in hepatocytes, which express carboxylesterase 1 (CES1).

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# Berberine dimer as a turn-on fluorescent G-quadruplex ligand by its conformational switching

M. Tera[1,3], T. Hirokawa[2], K. Sugahara[3]

[1]University of Zurich, [2]Molecular Profiling Research Center for Drug Discovery, [3]Suntory Foundation for Life Sciences

G-quadruplexes (G4) are thought to be important factors for telomerase inhibition and transcriptional/translational modulations.[1] Bioinformatic analyses imply the human genome and mRNA to contain a multitude of G4-forming sequences, and their analysis requires selective and detectable ligands. As two molecules of fluorescent berberine (BBR) are coordinated to telomeric G4 in their co-crystals,[2] we designed hydrocarbon-linked BBR-analog dimers, as we expected the alignment of two BBR chromophores to avoid Watson-Crick base pair intercalation, which should result in high selectivity towards G4 (Fig. 1). An alkene-cis C2 BBR dimer showed the highest affinity (Kd ≤ 2.6 nM) and selectivity (~ 900-fold vs. duplex) towards G4. The intrinsic "light-up" fluorescence properties of this BBR dimer allowed a selective visualization of various G4s in gel without using additional bulky fluorescence dyes, which suggested, combined with the observed lack of conformational change of the ligand, future applications in *in vitro* detection systems.[3]

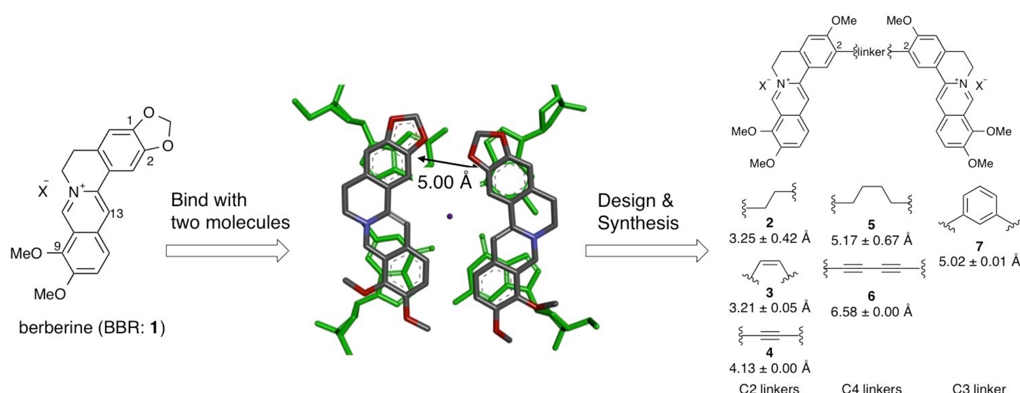


**Fig. 1** Chemical structures of BBR (**1**) and BBR dimers (**2-7**) bridged at the 2-C position with C2-C4 linkers. Two molecules of BBR bind to the G-quartet in a head to head manner (PDB ID: 3R6R).[2] The distances between the 2-C positions of the dimers are estimated on the basis of computational calculations.

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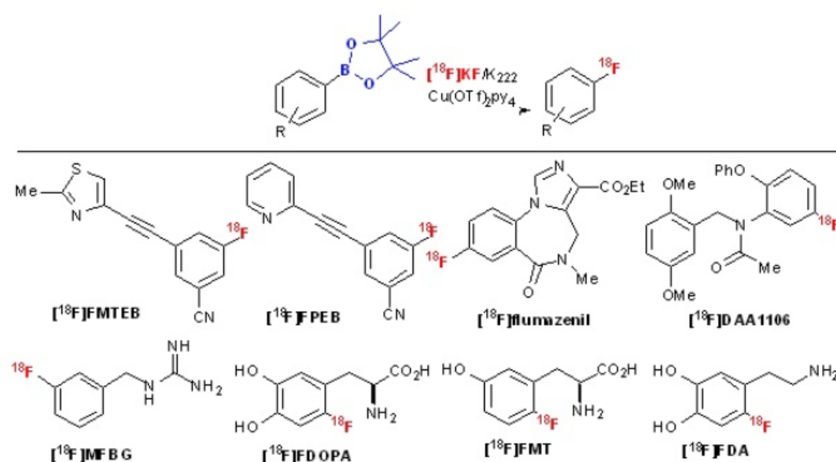
# Radiosynthesis of Eight Radiotracers via Cu-mediated $^{18}$F-Fluorination of Aryl Boronic Esters on a Clinically Relevant Scale

S. Preshlock[1], S. Calderwood[1], S. Verhoog[1], M. Tredwell[1], S. Gruber[1], T. C. Wilson[1], N. J. Taylor[1], M. Huiban[2], V. Gouverneur[1]*

[1]University of Oxford, Chemistry Research Laboratory, 12 Mansfield Rd, OX1 3TA, [2]Imanova, Burlington Danes building Imperial College, London Hammersmith Hospital, Du Cane Road, London W12 0NN

Positron emission tomography (PET) is a commonly used molecular imaging technique with applications in oncology, cardiology, neurology, as well as fundamental clinical research.[1] $^{18}$F is a widely used PET radioisotope due to its advantageous properties (half-life 109.8 min, 96.9% positron emission).[2] Our group has developed a novel strategy for the $^{18}$F-labeling of electron rich, neutral and deficient arylboronate pinacol esters which utilizes a commercially available Cu complex and [$^{18}$F]fluoride.[3] We have recently re-optimized this methodology for the preparation of eight clinically relevant radiotracers; [$^{18}$F]FMTEB, [$^{18}$F]FPEB, [$^{18}$F]flumazenil, [$^{18}$F]DAA1106, [$^{18}$F]MFBG, [$^{18}$F]FDOPA, [$^{18}$F]FMT, and [$^{18}$F]FDA. This study demonstrates that a range of radiotracers used in (pre)clinical studies is within reach applying a single reaction. These advances indicate that the process is robust and amenable to broad use in PET radiochemistry facilities and compatible for the preparation of a diverse array of highly functionalized molecules.

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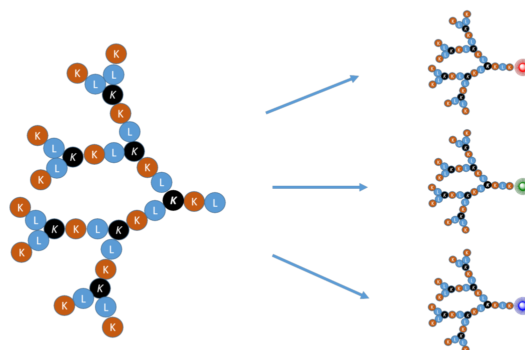
# Fluorescent Labeling of the Antimicrobial Peptide Dendrimer G3KL to Investigate its Mechanism of Action against Pseudomonas aeruginosa

B. H. Gan[1], T. Darbre[1], J.-L. Reymond[1]*

[1]University of Bern

We recently showed that peptide dendrimer G3KL (KL)8(KKL)4(KKL)2(KKL) exerts strong antimicrobial activity against multidrug resistant clinical isolates of the Gram negative bacteria A. Baumanii and P. aeruginosa[1],[2]. G3KL was further shown to have positive impact in burn wound-healing processes and pro-angiogenic effect[3]. G3KL has a strong membrane disrupting activity on fluorescein-loaded large unilamellar vesicle consisting of phosphatidyl glycerol that mimics bacterial membrane. Nevertheless its mechanism of action against *P. aeruginosa* remains to be elucidated. Molecular imaging probes that possess native antibacterial activity of Polymixin B has been shown to be a promising approach in understanding the mechanism of action of the antimicrobial[4]. Here, we show a systematic modification of G3KL at the C-terminus with different fluorophores to generate an active probe that mimics native G3KL activity. The reaction was carried out on solid phase peptide synthesis (SPPS) by introducing one extra lysine at the C-terminus of the peptide dendrimer G3KL leading to G3KLK (KL)8(KKL)4(KKL)2(KKLK), where the fluorophore was attached on the side chain of the additional lysine. Surprisingly, the coupling of 5(6)-carboxyfluorescein and dansyl moiety on the additional lysine lead to the formation of a stable amide bond and sulfonamide bond respectively. Most interestingly, the minimum inhibitory concentration (MIC) of both derivatives was retained at 4 µg/ml as compared to native G3KL. Inversely, coupling of rhodamine B gave poor yields and strongly decreased antimicrobial activity. Cytotoxicity of the native G3KL and its fluorescent derivatives have also been addressed. Interestingly, 5(6)-carboxyfluorescein labeled peptide dendrimer showed similar toxicity to native G3KL in eukaryotic cells, inversely, the dansylated analogue showed more toxicity. These labeled peptide dendrimers may enable a visualization of the membrane deformation of the bacteria by using a confocal microscope.



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# Impact of pathogen inactivation technologies on platelets: cell functionality and redox proteome.

G. Sonego[1,2], M. Prudent[1], M. Abonnenc[1], D. Crettaz[1], J. Tissot[1], N. Lion[1]*

[1]Transfusion InterRégionale, [2]University of Lausanne

**OBJECTIVES**: Pathogen inactivation technologies (PITs) are routinely used in blood banks to process platelet concentrates (PCs). PITs take advantage of the anucleate nature of platelet and combine UV light and photochemical molecules to block genetic material replication during room temperature storage (i.e. storage period of 7 days). Although they increase PCs safety against bacteria, viruses and parasites, PITs have been shown to accelerate the platelet metabolism and reduce their *in vitro* functionality. The molecular mechanisms at the origin of this behavior are still unclear. One of the suspected causes is the oxidative stress generated during pathogen inactivation. Indeed it is proven that PITs release reactive oxygen species (ROS) by sensitization of molecular oxygen. Uncontrolled ROS do not only target DNA or RNA (the scope here), but can also target lipids and proteins and may be involved in platelet reduced functionality. Considering that ROS are part of the activation/aggregation signaling pathway of platelets, a broad strategy was adopted to expand the knowledge in order to inform the transfusion medicine community.

**METHODS**: A two arms study where buffy coat-derived PCs were treated within amotosalen/UVA (n=3), riboflavin/UVB (n = 6) or UVB alone (n=3) and were compared to untreated (n = 6) PCs along storage. *In vitro* functionality (flow cytometry) and metabolism (*in vitro* assays) were measured at different time points, as well as the protein changes by 2D-PAGE (i.e. only untreated and riboflavin/UVB PCs enter 2D-PAGE study). Moreover, protein oxidation has been studied by mass spectrometry and proteomics. First, model peptides and recombinant proteins were used to classify oxidations under similar PITs conditions and build-up a database of oxidations (i.e. on W, Y, H, C amino acids). Second, shot gun proteomics for total soluble platelet protein extract was analyzed against the previous database of oxidation by label free technique (n=3). The last part of this comprehensive work is currently focusing on the ROS signaling role in redox cysteines regulated pathways. Indeed, with biotinylated probes, the fixation of sulfenic acid state, followed by enrichment, allowed to deeply understand ROS dependent platelet activation pathways.

**RESULTS**: A large palette of oxidations was found to be possible on model peptides containing W, Y, H, C residues. Quantifications showed an increased oxidative effect for riboflavin/UVB treatment compared to amotosalen/UVA. Simultaneously, in our experimental conditions, riboflavin/UVB-treated PCs showed the most pronounced functional and metabolic differences compared to untreated and amotosalen/UVA-treated PCs. Additionally, UVB alone showed a similar impact to riboflavin/UVB-treated PCs, indicating UVB as a fundamental factor to: accelerate glycolysis, decrease platelet deformability, increase pre-activation P-selectine exposure and mitochondrial membrane depolarization, increase GPαIIbβ3 activation and adhesion to fibrinogen coated wells. 2D-PAGE presented mild proteome mapping differences upon PCs treatment. Nevertheless, a few proteins showed product age independent p*I* shift or spot intensity changes. Particularly, glyoxalase containing domain 4 (GLOD4) was present in a new isoform only upon treatment. In addition, LC-MS/MS analysis of the total extract towards the database of oxidation showed poor differences between treated and untreated PCs. In view of these results we thought that the impact of the oxidation could be more subtle and reversible. By consequence we have focused on the signal translation by the sulfenylation of redox cysteines. Important proteins involved in platelet activation/aggregation, adhesion or cytoskeletal regulation presented the –SOH tag (PLEK, GPαIIbβ3, VASP, FERM3…)and could potentially be affected by oxidative stress.

**CONCLUSION**: This global non-targeted approach while aims for understanding PITs molecular impact on platelets behavior and resting state, tries to acquire further knowledge on platelet redox proteomic regulation, and might explain the clinical data. Finally this approach should deserve and participate to better transfusion medicine practices.

# Probing the cellular uptake and response of porphyrinic photosensitizers in polymeric nanoparticles by fluorescence measurements and $^{1}$H HR-MAS NMR based metabolic profiling of HeLa cells

S. Pfister[1], I. Gjuroski[1], D. Nydegger[1], M. Hädener[1], G. Diserens[1], P. Vermathen[1], J. Furrer[1]*, M. Vermathen[1]*

[1]University of Bern

Polymer-based nanoparticles are considered as suitable drug delivery vehicles for porphyrinic photosensitizers in photodynamic therapy (PDT) since they enhance porphyrin stability and prevent porphyrin self-association in aqueous solutions [1]. Previously, we have shown by $^{1}$H NMR spectroscopy that the photosensitizer serine-chlorin e6 (SerCE) is disaggregated upon insertion into either the polymer polyvinylpyrrolidone (PVP) or into polymer micelles consisting of Kolliphor P188 (KP188) [2, 3].

The aim of the current study was to probe and compare the impact of the carrier systems, i.e. PVP and KP188, on the cellular uptake of SerCE and on the cellular response towards SerCE treatment in the dark. For this, fluorescence detection using the ImageStream system and $^{1}$H High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy combined with multivariate statistical analysis were applied. The HR-MAS technique allows detection of small compounds in semi-solid material such as live cells. Monitoring of the small metabolites in HeLa cells after incubation with SerCE and PVP as transport vehicle showed alterations especially in metabolites derived from lipid components. Uptake of SerCE into HeLa cells was tested using the ImageStream system. The fluorescence data indicated that the uptake was decreased if Kolliphor P188 was present together with SerCE compared to SerCE alone. The results will be compared to the corresponding data obtained with KP188 (by HR-MAS NMR) and PVP (by ImageStream) in order to assess their properties and suitability as delivery vehicle for chlorin e6 based photosensitizers in PDT.

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# Polymer Delivery Systems of Porphyrin Photosensitizers Monitored by NMR Spectroscopy

I. Gjuroski[1], S. Pfister[1], J. Furrer[1]*, M. Vermathen[1]*

[1]University of Bern

Polymer nanoparticles are known delivery systems for porphyrin photosensitizers in photodynamic therapy (PDT). Photolon®, which is a polyvinylpyrrolidone (PVP) complex with chlorin e6 (Ce6), has already gained approval for medical application in PDT. Polymer carrier nanoparticles improve porphyrin monomerization, solubility and stability under physiological conditions, overcoming the consequences of their intrinsic tendency for aggregation thereby increasing the efficiency of PDT.

Previously we have shown that amino acid derivatives of chlorin e6 (xCE) are encapsulated into polyvinylpyrrolidone [1] and Kolliphor P188 (KP) [2] by using NMR spectroscopy techniques. We have also studied their aggregate structures, and monitored their interactions with membrane models [3].

In this study, various 1D and 2D NMR techniques were recorded to characterize the polymer-porphyrin ensemble, with a focus on the structural characterization, disaggregation capabilityand loading capacity of the polymers. The NMR data reveal that PVP binds stronger to amino acid derivatives of chlorin e6 compared to KP (stable complex vs equilibrium). In addition, the cellular uptake of serine-chlorin e6 has been followed by fluorescence detection after cell incubation with serine-chlorin e6 encapsulated either into PVP or into a KP polymer matrix.

Further studies are currently in progress to compare the effect of PVP and Kolliphor P188 as delivery system for SerCE on the metabolic response of HeLa cells.

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# Efficacy of Block Copolymers to Disaggregate and Encapsulate Porphyrins Monitored by NMR Spectroscopy

I. Gjuroski[1], S. Pfister[1], J. Furrer[1]*, M. Vermathen[1]*

[1]University of Bern

Since porphyrin compounds exhibit intrinsic phototoxicity, tumor accumulation and low dark toxicity, they play an important role as photosensitizers in photodynamic therapy (PDT). However, porphyrinic photosensitizers have the tendency to aggregate in aqueous solutions thereby potentially decreasing the PDT efficiency. Porphyrin-polymer systems represent an elegant way to cope with this problem. Polymer-carrier systems such as Photolon®, which is a polyvinylpyrrolidone (PVP) - chlorin e6 (Ce6) complex, have already gained approval for medical application in PDT. [1, 2]

Previously, we have reported and characterized by NMR spectroscopy a series of naturally derived porphyrin and chlorin e6 derivatives with a focus on their propensity to form aggregates as well as their interactions with membrane models. [3, 4]

The aim of the present study is to investigate the efficacy of several block copolymer systems to disaggregate the porphyrinic model compounds to determine their loading capacity in biological media using NMR spectroscopy as the main technique. The results point out that the selected block copolymers are capable to monomerize amino acid conjugates of chlorin e6. However, the results also suggest the existence of different binding motives and different equilibria between the porphyrinic photosensitizer and the block copolymer carrier molecules. These differences may have a considerable impact on the pharmacokinetic properties of the corresponding delivery systems.

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# Can Nanoparticle Carrier Systems decrease the Reactivity of Porphyrinic Photosensitizers towards Serum and Cytosolic Proteins?

M. Vermathen[1], L. Sauser[1], I. Gjuroski[1], J. Furrer[1]*

[1]University of Bern

Since porphyrinic photosensitizers (PSs) can accumulate in diseased tissue, become only toxic when irradiated by light, and can transfer energy to oxygen forming highly reactive singlet oxygen, they have been used in Photodynamic therapy (PDT) of cancer and other non-cancerous diseases for several years. [1] Porphyrinic PSs of interest are usually hydrophobic structures and their intrinsic capability of self-aggregation in aqueous solution represents limiting factors, which reduce their PDT efficiency. Porphyrin conjugates have therefore become the focus of our current research to overcome these drawbacks, to increase the cellular uptake and ultimately the selectivity towards cancer cells. [2-3]

Part of the reasons for low productivity of anticancer porphyrinic PSs development is a limited knowledge about the mode, in which metabolic state the molecule penetrates the tumor cell and how much is inactivated. Given that porphyrinic PSs are administered intravenously, special consideration should therefore be given to interactions with macromolecular blood components. In this context, binding to the serum proteins albumin or transferrin on one hand, and to cytosolic proteins on the other hand, appear to be the most important issues, because such interactions determine also the deactivation of the PS, the excretion and differences in efficacy, activity, and toxicity.

In this contribution, interactions of a model porphyrinic PS, serine-chlorin e6 (SerCE), and of SerCE-PVP (polyvinylpyrrolidone) and SerCE-Kolliphor® complexes with the serum proteins albumin and transferrin and the cytosolic proteins cytochrome C, myoglobin, and ubiquitin have been investigated using NMR spectroscopy. The results reveal that the porphyrinic PS SerCE reacts immediately with the five proteins investigated, while PVP is able to prevent SerCE to react with those proteins, especially with albumin. The results with Kolliphor appear contrasting: the reaction is slow with myoglobin and immediate with albumin while reaction with the other proteins is also prevented. As such, the formation of nanoparticle systems with PVP seems to protect porphyrinic photosensitizers more efficiently than Kolliphor from interacting with proteins and may therefore promote intact cell entering of the PS.

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**Rapid-Acting Insulin Analog Engineering using Multipolar Force Fields: Stabilization of a Protein Crevice by Halo-Aromatic Substitutions**

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

[1]University of Basel, [2]Case Western Reserve University, Cleveland, Ohio

Insulin analogs are designed containing amino acid substitutions at or near the classical dimerization surfaces of the zinc insulin hexamer [1]. One of these amino acids is $Phe^{B24}$, an invariant aromatic anchor at this interface that lies within a ß-strand at the edge of a protein crevice and site of a human mutation causing diabetes mellitus [2].The utility of halogenic substitutions in medicinal chemistry has stimulated their investigation in protein design [3]. The effect of such mutations on the conformational ensemble, the thermodynamic stability and on the activity is evaluated using free energy calculations and MD simulations with the CHARMM mulipolar force field [4]. Results suggest that in a singly halogenated aromatic ring regio-specific inductive effects may modulate multiple surrounding electrostatic (weakly polar) interactions, thereby amplifying potential changes in stabilityof 1 kcal/mole. Experimental evaluation of these analogs is in accordance with such calculations. Since, insulin provides a model for the therapeutic application of protein engineering, the resulting differential effects of these modifications can help provide a molecular strategy to augment protein stability while preserving rapid action.

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# Spectroscopic and structural studies of hisitidine-rich metallothioneins

J. Habjanic[1], O. Zerbe[1], E. Freisinger[1]*

[1]University of Zurich

Metallothioneins (MTs) are ubiquitous metalloproteins characterized by a high percentage of cysteine residues and a low molecular weight. As a consequence of their metal binding abilities they are important co-players in various physiological processes as metal ion homeostasis and detoxification. Although, all MTs are sharing these properties, MTs from different kingdoms of life show a high diversity in their amino acid sequences and 3D structures.

For bacterial MTs, structural investigations revealed several unique features when compared to the mammalian counterparts, i.e. the contribution of histidine residues to metal ion binding, a higher percentage of secondary structural elements, as well as the occurence of aromatic amino acids.[1]

The sequencing of numerous bacterial strains in the last decade let to the identification of several MT encoding genes in *Pseudomonas* species. The corresponding amino acid sequences revealed the presence of unusually high amounts of histidine residues and generally a high diversity in the primary structure. Accordingly, these MT sequences can be divided into three subgroups based on the total number of amino acids, the number of His residues, as well as the overall charge of the respective protein. At the same time they show a rather conserved Cys distribution patterns consisting of an N-terminal CxCxxCxC motif, a central YCC/SxxCAxxH stretch, as well as an C-terminal Cxxxx(x)CxC part.

We are investigating, how differences found in the primary structure of these novel bacterial MTs influence function and 3D structure, including protein fold and the metal clusters.

Financial support by the Forschungskredit of the University of Zurich (JH) is gratefully acknowledged.

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# Further insights into the cadmium specificity of Neclu_MT1

M. Perinelli[1]

[1]University of Zurich

Metallothioneins are a large super-family of metalloproteins ubiquitously presents in all living beings. They are characterized by a small size and a very high content of cysteine residues in their sequences. These residues confer a large coordination capacity towards most of the transition metal ions to the MTs. The characteristics of these proteins have been widely studied in the past years, with a particular interest in their detoxification and antioxidative properties. For example, the aquatic fungus *Heliscus lugdunensis* can survive in polluted spring water with heavy metal ion concentrations as high as 25 µM $Cd^{II}$ and 30 mM $Zn^{II}$). It was found that this fungus expresses the metalloprotein Neclu_MT1, which is with 25 amino acids one of the smallest MTs known.

Further studies have shown that this is the only MT for which induction of MT expression exclusively by $Cd^{II}$, but not by $Zn^{II}$, ions was observed so far.[1] This is despite the fact that the eight cysteine residues and one C-terminal histidine in principle allow the complexation of a wide range of metal ions. One interesting feature of the protein is its concentration-dependent metallation with $Zn^{II}$ ions: While a monomeric $Zn_2Cys_6His$ species is observed at a protein concentration of 5 µM, at 25µM or higher a monomeric $Zn_3Cys_8His$ species is formed. However, $Cd^{II}$ coordination is not affected by the protein concentration givin a metal load of three in each case, although conformational rearrangement of the ligands at high pH value can occur. [2]

Therefore the aim of our studies is to perform potentiometric investigations in order to better understand the ligand pattern of Neclu_MT1 and determine the individual protonation and complexation constants for each subsequent metal ion binding step.

*Project funding by Swiss National Science Foundation to EF is gratefully acknowledged.*

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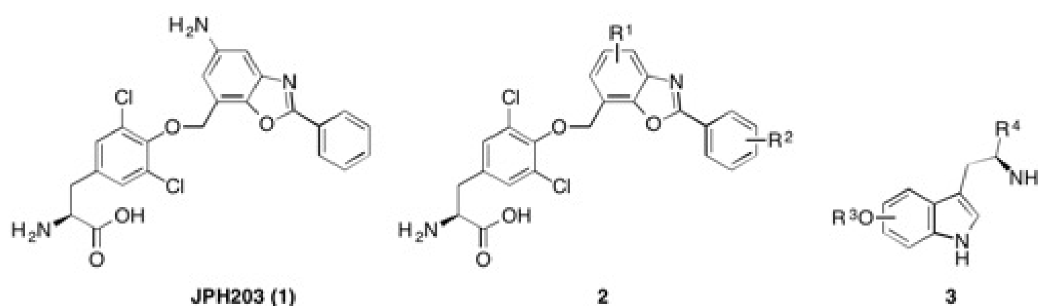
# Synthesis and Biological Acitivity of New SLC7A5/LAT1 Inhibitors

J. Graff[1], J. Gertsch[2], K.-H. Altmann[3]

[1]ETH Zürich, [2]University of Bern, [3]ETH Zurich

The L-type amino acid transporter SLC7A5/LAT1 is a member of the solute carrier protein family which is responsible for the transport of hydrophobic amino acids with bulky side chains. SCL7A5/LAT1 is overexpressed on a number of different tumor cells and, therefore, has emerged as a potential target for anticancer drug discovery.[1] So far, only one potent inhibitor of this transporter has been reported in the literature, namely JPH203 (KYT-0353) (**1**), which has been derived from the substrate amino acid L-Tyr through extensive modification.

We have now prepared a series of analogs of JPH203 with substituents at the 2-position of the benzoxazole ring (**2**), in order to probe the effects of changes in the steric and electronic properties of the remote part of the JPH203 side chain on transporter inhibition.[2] In addition, we have studied a series of analogs derived from the substrate amino acid L-Trp (**3**), which are characterized by extensions of the indole side chain at the 5- or 6-position and we have investigated the feasibility of replacing the carboxylate group by a bioisosteric tetrazole moiety. This contribution will discuss the synthesis of these modified amino acids and the atcivity of the compounds as LAT1 inhibitors.

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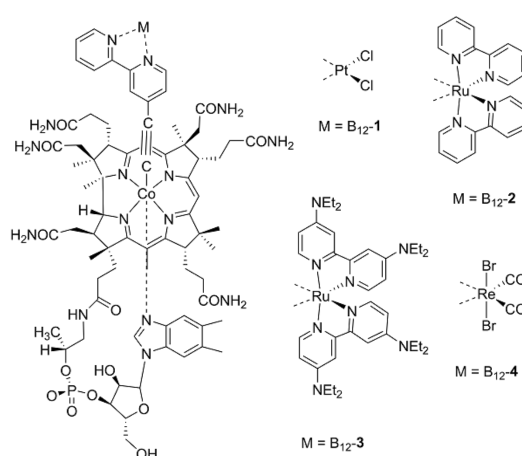
# Active metal-based drugs delivery using vitamin $B_{12}$ as a carrier

R. Jérémie[1], F. Zobi[1]*

[1]University of Fribourg

Cobalamin (Cbl, vitamin $B_{12}$) is a water-soluble vitamin of primary importance to the metabolism of every cell in the human body. Once ingested, it is actively carried by proteins and ultimately internalized into the cells by a receptor-mediated endocytosis. Confined inside the cell, the cobalt center of Cbl undergoes a series of enzymatic reductions that triggers the release of the β-axial ligand. Over the past few years, this feature has been explored in order to use vitamin $B_{12}$ against cancer cells in the manner of a Trojan horse. Indeed, the ability of the cyanide ligand in CNCbl to bridge metal centers has been successfully used to attach metal complexes[1]. Based on recent advances in organometallic chemistry applied to Cbl[2,3], a new structural design was developed and resumed herein. This approach offers the advantage of: 1. a better comparison between the cytotoxity of the complexes before their attachment to Cbl and after their release inside the cells; 2. a broader range of imaginable motifs; 3. an increased water solubility of the complexes.
Thus far, a series of fours vitamin derivatives of Pt ($B_{12}$-**1**), Ru ($B_{12}$-**2** and$B_{12}$-**3**) and Re ($B_{12}$-**4**) were prepared and characterized (see Image). As a common structure, the attached complexes exhibit a bipyridine modified at the para position with a simple alkyne. The latter serves as a point of attachment between the cobalt of Cbl and the anticancer metal complexes. Chemical reduction using either cobaltocene or Zinc showed that the four complexes are released entirely from Cbl.
In term of serum stability, both ruthenium derivatives showed no evidence of human serum albumin binding after 24h. On the other hand, the free fraction of the Pt derivative was measured at 61% and 40% for the Re in the same period. $B_{12}$-**4** and$B_{12}$-**3** showed comparable/lower cytotoxicity to that of cisplatin, while $B_{12}$-**1** was less effective and $B_{12}$-**2** essentially non-cytotoxic. To measure the affinity of the derivatives with Cbl carrier proteins (TCII and IF), a fluorescent $B_{12}$[4] was prepared in order to perform competitive displacement assays . Preliminary data indicate that our derivatives are recognized by these transport proteins.

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# Exploitation of the allosteric relationship between RAPTA-T and Auranofin on the Nucleosome Core Particle in the design of novel anti-cancer agents.

L. Batchelor[1], E. Paunescu[1], G. Palermo[1], U. Röthlisberger[1], C. A. Davey[2], P. Dyson[1]*

[1]EPF Lausanne, [2]Nanyang Technological University (Singapore)

Dinuclear metal complexes have emerged as a promising class of biologically active molecules that display interesting anti-cancer activity and properties. As a consequence, both homo- and hetero-bimetallic combinations are being explored. The allosteric relationship between RAPTA-T, a ruthenium(II) moiety, and Auranofin, a gold(I) drug, on the nucleosome core particle (NCP) has been exploited to design and synthesize a series of homo-bis-ruthenium (II) complexes and hetero-ruthenium(II)-gold(I) complexes. The design is based on crystallographic and computational data with the aim of simultaneously bind to the sites of the parent drugs, Auranofin and RAPTA-T, on the NCP.[1],[2]

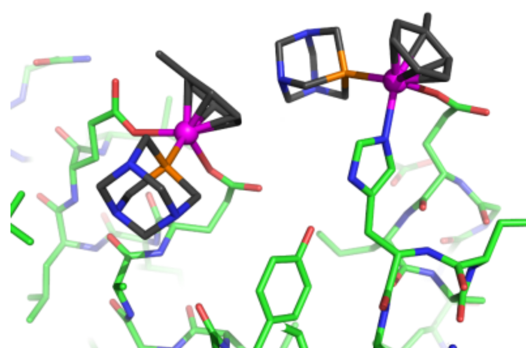

Figure 1 shows a crystal structure of the binding sites of the RAPTA-T moiety on the histone component of the NCP. A series of bis-ruthenium(II) complexes has been synthesized with varying lengths of polyethylene glycol linkers to provide an array of distances to span the 13Å between the metal centres. The complexes synthesized so far have exhibited cytotoxicity in the low to mid micromolar range against A2780, A270cisR and HEK298 cell lines with mild correlational observed between linker length and cytotoxicity. The focus of ongoing work is on synthesizing a series of hetero-ruthenium(II)-gold(I) complexes with high water solubility that may have greater potential as novel anti-cancer agents.

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# Endogenous Formation of N-Nitroso Compounds by Gut Microbiota

S. Sieber[1], C. Lacroix[2], S. Sturla[1]*

[1]Department of Health Sciences and Technology, Institute of Food, Nutrition and Health, Laboratory of Toxicology, 8092 Zürich, Switzerland, [2]Department of Health Sciences and Technology, Institute of Food, Nutrition and Health, Laboratory of Food Biotechnology, 8092 Zürich, Switzerland

A diet rich in red meat has been linked to a higher probability of developing colorectal cancer and *N*-nitroso compounds (NOCs), composed of *N*-nitrosamines and *N*-nitrosamides, are suspected to be a key factor.[1] NOCs are carcinogenic and require oxidation at the alpha position of the nitroso group to trigger spontaneous decomposition and formation of highly reactive diazonium ions that can alkylate DNA and induce mutations (figure 1).

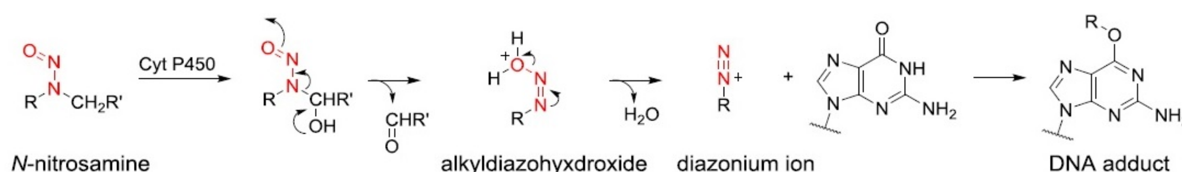


Figure 1: Mechanism of *N*-nitrosamines activation

Analysis of human feces suggest that most secreted NOCs are formed endogenously.[2] It is hypothesized that gut microbes promote endogenous nitrosation, but the structures of these compounds and their mechanism of formation are not known. We established a model system using a bacterium, known to promote the nitrosation reaction and to be present in the gut, to identify the putative NOCs formed endogenously. Additionally, we used a screening strategy based on the conversion of morpholine into *N*-nitrosomorpholine to identify gut microorganisms, which have the capacity to promote the nitrosation of food derived compounds.

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## A thioether ligated cyclic antimicrobial undecapeptide with D,L- architecture targeting multidrug resistant Pseudomonas aeruginosa

I. Di Bonaventura[1], R. He[1], J.-L. Reymond[1]*

[1]Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland

Cyclic antimicrobial peptides such as polymyxin represent a versatile and clinically useful class of antibiotics to treat infections by multidrug resistant bacteria, in particular *Pseudomonas aeruginosa* which represents a major cause of life threatening infections in hospitals today. By exploring amphiphilic cyclic peptides formed by double thioether ligation of a linear precursor peptide with alternating D and L residues, we identified the cyclic undecapeptide **RH14** with activity against *P. aeruginosa*, including multidrug resistant clinical isolates. **RH14** perturbs bacterial membranes and lipid vesicles and forms supramolecular peptide-lipid aggregates observable by atomic force microscopy (AFM). The unusual D,L-architecture and *p*-xylene double thioether bridge contribute to stability and activity and allow for a straightforward synthesis rendering **RH14** an attractive starting point for further optimization by combinatorial chemistry approaches.

# Studying histone ubiquitination on chemically defined chromatin

A.L. Bachmann[1], L. Bryan[1], B. Fierz[1]*

[1]EPF Lausanne

Chromatin, the entity of DNA and histones including post-translational modification marks (PTMs), is crucial for proper packaging of DNA into eukaryotic cells and dynamic regulation of DNA accessibility. During DNA damage response (DDR), histone ubiquitination plays a key role in mediating downstream signaling. Importantly, the E3 ubiquitin ligase RNF168 is reported to interact with the nucleosome acidic patch to ubiquitinate H2AK13/15 [1]. H2AK13ub/15ub is then recognized by DDR factors such as 53BP1 and BRCA, which govern downstream DNA damage repair.

Here, we aim to understand the detailed mechanism of ubiquitination in DDR as a function of the chromatin state. Assembly of chemically defined chromatin fibers containing the de-compacting mark H4K16ac or no PTMs, which results in a compact state, enabled us to investigate histone ubiquitination in dependence of the chromatin state. We observed enhanced ubiquitination activity in H4K16ac chromatin fibers, when compared to fibers without any modifications. An anticancer drug, RAPTA-C, was found to interact with the nucleosome acidic patch [2]. Due to the requirement of the acidic patch for RNF168 activity, we tested if RAPTA-C might mediate its anticancer characteristic via DDR-dependent ubiquitination. We indeed found reduced ubiquitination activity for RNF168 in the presence of RAPTA-C, whereas a control compound (RAED-C) did not interfere. In our laboratory, we established a method to investigate the dynamic behavior of effector protein interactions with chemically defined chromatin fibers using single-molecule total internal fluorescence microscopy (smTIRFM) [3]. This allows us to characterize RNF168 recruitment as a function of chromatin accessibility and the properties of the acidic patch. Together, these studies result in a mechanistic understanding of chromatin ubiquitination in a chromatin context.

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### The role of substrate hydrogen bonding in the non-heme iron enzyme EgtB

R. Burn[1], K. Goncharenko[1], F. Seebeck[1]*

[1]University of Basel

Ergothioneine is an important cellular antioxidant that occurs in many bacteria, most fungi and also in human tissue. The central step in ergothioneine biosynthesis is catalyzed by the non-heme iron enzyme EgtB. This enzyme mediates oxygen dependent sulfur – carbon bond formation between cysteine or γ-glutamyl cysteine and trimethyl histidine. In the active site of EgtB the two substrates are linked through a hydrogen bond between their amino acid moieties. Due to geometric constraints this interaction must break in the course of the reaction. To elucidate the precise sequence of elementary steps during EgtB catalysis we examined the contributions of this hydrogen bond in substrate binding and transition state stabilization using substrate analogs and kinetic analysis.

# Design and synthesis of highly potent and upmost selective acetylbenzenes as CREBBP ligands

A. Dolbois[1], A. Unzue[1], A. Caflisch[2]*, C. Nevado[1]*

[1]University of Zurich, Chemistry department, [2]University of Zurich, Biochemistry department

Disfunctional levels of acetylated lysine residues in DNA interacting proteins have beenrecently connected to numerous diseases like cancers, inflammation, viral infectionsand metabolic disorders.[1] Bromodomains are protein modules (c.a. 110 amino acids)that specifically recognise the acetylated lysine motif and have therefore become thefocus of potential therapeutic applications.[2] Out of 61 different bromodomains identifiedin humans, BRD4(1) and the BET family have been the most investigated so far,leading to inhibitors already in phase II of clinical trials.[3] In sharp contrast, the biologicalrelevance of other bromodomains, like the CREBBP/EP300, remains unclear.

Originating from an in-silico fragment based approach, our group has successfullydesigned, synthesized and biologically characterized a series of acetylbenzenederivatives as potent and selective CREBBP ligands.[4] Based on the informationobtained from the X-Ray structure of the parent compound in complex with CREBBP,compounds with improved potency and unprecedented selectivity against BRD4(1)have been identified.[5] These unique compounds are being further optimized (Figure 1)in terms of solubility, cell permeability and PK/PD properties to enable their use aschemical probes in biologically relevant in vitro and in vivo systems.

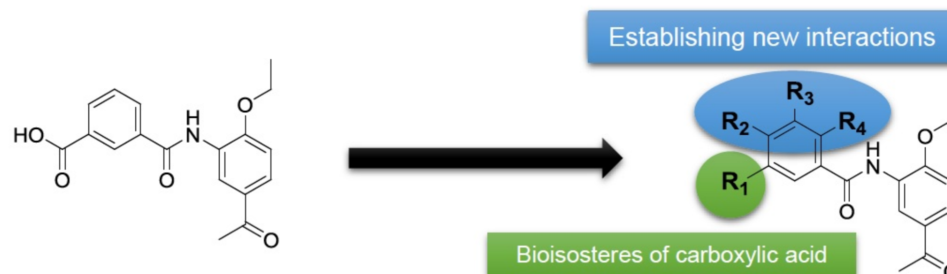


**Figure 1: Possible modifications to improve the potency and selectivity of acetylbenzene derivatives**

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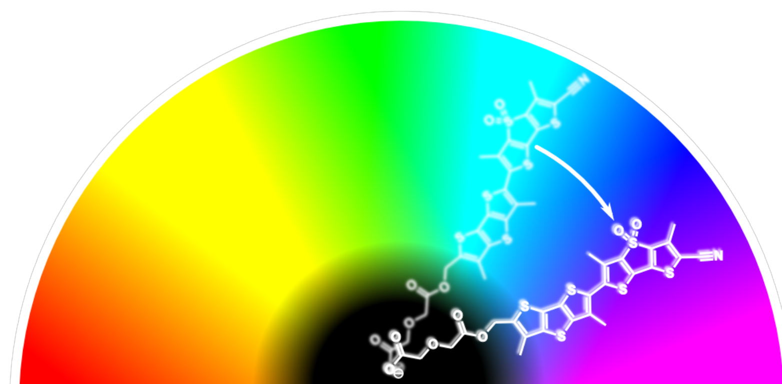
# Fluorescent Flippers on a Monolayer

F. Neuhaus[1], A. Zumbühl[1]*, S. Matile[2]*

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

Measuring the membrane tension via a mechanosensitive fluorescent probe requires a direct correlation between the surface pressure and the induced color change. Here we analyze the Flipper dithienothiophene planarizable push-pull probe in a monolayer at the air/water interface using fluorescence microscopy and grazing-incidence angle X-ray diffraction. An increase of the lateral membrane pressure leads to an organization of the probe and a clear change in color hue.



# Following the splicing process of an encapsulated group II intron by single-molecule FRET

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

[1]University of Zurich

The progress in fluorescence microscopy over the past decade allows the observation of inter- and intramolecular dynamics of single biomolecules. In particular, structural motion of a single biomolecule can be observed by following over time the Förster resonance energy transfer between two fluorophores attached to the molecule (smFRET). Our interest lies in characterizing the cleavage of an engineered construct of the group II intron *ai5γ* of S*accharomyces cerevisiae* (*Sc.ai5γ*, 932 nt), resulting in to the cleavage. This self-splicing RNA is located within a housekeeping gene, and is cleaved during RNA maturation. To visualize the conformational dynamics of the RNA by smFRET and during the process, two probes carrying each a fluorescent dye are hybridized to the two flanking regions, the 5'end being cleaved. PNAs are an excellent alternative to DNA hybridization probes due to their higher affinity and specificity to RNA and to their stability against enzymatic degradation [1]. Wide-field illumination smFRET experiments are usually performed on surface-immobilized molecules, preventing the free motion of the molecule [2]. To allow the RNA to freely diffuse and thus mimicking close to in vivo conditions, we encapsulate the large molecule into surface-immobilized vesicles. Furthermore, after cleavage, the products remain inside the vesicles and can be kept under observation. The encapsulation combined with PNA labelling, constitutes a new strategy to characterize structurally and dynamically large and catalytic RNAs.

Financial support by the ERC (R.K.O.S), the University of Zurich and the UZH Forschungskredit (S.Z.P, M.C.A.S.H. and R.B.) is gratefully acknowledged.

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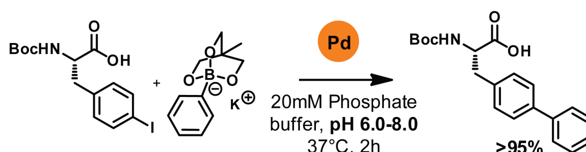
# Palladium nanoparticles for the specific modification of native proteins

A. Dumas[1], A. Peramo[1], D. Desmaële[1], P. Couvreur[1]

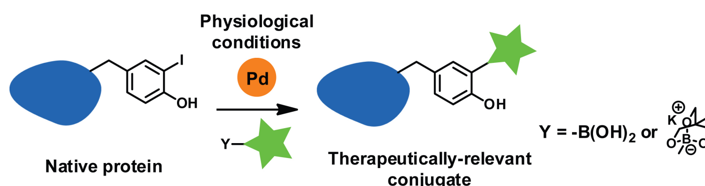
[1]Université Paris-Sud

Chemical transformations that can be performed selectively under physiological conditions are highly desirable tools to manipulate proteins and generate conjugates with new or improved properties.[1] Owing to their broad functional group tolerance and biological compatibility, palladium-catalyzed *Suzuki-Miyaura* reactions emerge as attractive strategies for the formation of functional protein conjugates.[2] In addition, the low toxicity associated with palladium opens perspectives for specific protein modifications of therapeutic interests.[3]

Palladium nanoparticles stabilized by polymers demonstrated excellent catalytic activity for the modification of halogenated amino acids through *Suzuki-Miyaura* cross-coupling reactions in water. Interestingly, up to 98% conversion into the coupled amino acid could be achieved in 2 h at 37°C using stable, water-soluble cyclic triolborates as organometallic partners in the presence of only 1 mol% of palladium.[4]




These nanocatalysts demonstrated the ability to modify thyroglobulin, a naturally iodinated protein involved in the production of thyroid hormones, presenting the first example of cross-coupling reaction on a native protein. Given the stability, selectivity and remarkable properties of palladium nanoparticles in biological settings, these systems open exciting therapeutic perspectives involving the manipulation of naturally-occurring proteins in living systems.[5]

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# Polarizing solids (HYPSO) for DNP applications

M. Cavaillès[1], D. Baudouin[1], A. Bornet[2], S. Jannin[2], A. Lesage[3], G. Bodenhausen[4], L. Emsley[3], C. Copéret[5], C. Thieuleux[1]*

[1]CPE Lyon, [2]EPF Lausanne, [3]Centre de RMN à très hauts champs, [4]ENS Paris, [5]ETH Zurich

Dynamic Nuclear Polarization is a method to increase the nuclear spin polarization of molecules by 30'000 or more.[1] The large increase in NMR MRI signal enhancement have enabled many new applications, in particular in vivo MR metabolic imaging where the metabolization of a tracer can be tracked.[2] The method relies on dynamic nuclear polarization at cryogenic temperature followed by a fast melting (dissolution) to produce the room temperature solution of highly polarized spins. Our group has recently developed HYperPolariszing SOlids (HYPSO) which consist in highly porous silica-based materials having persistent radicals, the polarizing agent, homogeneously attached at their surface. They can be used to hyperpolarize a large variety of molecules without using any undesirable glassing agents that are typically used for standard DNP sample preparation. Furthermore, the materials can be easily filtered off to produce pure hyperpolarized solutions. In addition, any radical can be incorporated into a HYPSO, allowing both direct 13C polarization (trityl radical), as well as 1H - › 13C Cross Polarization (CP)[3] using nitroxyl radical. Using CP, hyperpolarization of [1-13C]-pyruvate for instance can be performed in 10-30 minutes only leading to 13C polarization of ca. 25% after dissolution[PNAS].

Here we describe new generations of HYPSO and bring insights in the effect of material parameters on DNP performance. The effect of the link between the radicals and the solid, the presence of methyl groups, Cu ions and dioxygen on the DNP performance are explored as well as the overall porous architecture of the material. The latest generation of material, HYPSO-3, based on a cubic structuration (SBA-16) having 3D interconnected pore network give rise to 1H polarization as high as 65%, with a potential of 13C polarization of up to 55%.

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# Antimicrobial Activity and Stability of Diastereomeric Peptide Dendrimers

S. Baeriswyl[1], T. N. Siriwardena[1], T. Darbre[1], J.-L. Reymond[1]*

[1]University of Bern

The development of new antimicrobial agents has really slowed down over the past years. Therefore it is of the utmost importance to develop new antimicrobial agents effective against multidrug-resistant strains of bacteria, such as *P.aeruginosa*, which can cause life-threatening infections on immuno-depressed patients in hospitals. Recently, our group has designed and optimized stable and non-toxic antimicrobial peptide dendrimers that are highly active against *P.aeruginosa*, highly stable against proteases and non-hemolytic[1], [2], [3]. Here we report the synthesis and study of a library of diastereomeric analogs of G3KL displaying (D)-amino acid analogues at different positions in the dendrimer sequence. It appeared that changes in the chiral content can be used for the optimization of the properties of antimicrobial peptide dendrimers, including MIC and stability amongst others. This strategy can give a major boost in the discovery of new potent antimicrobial agents.

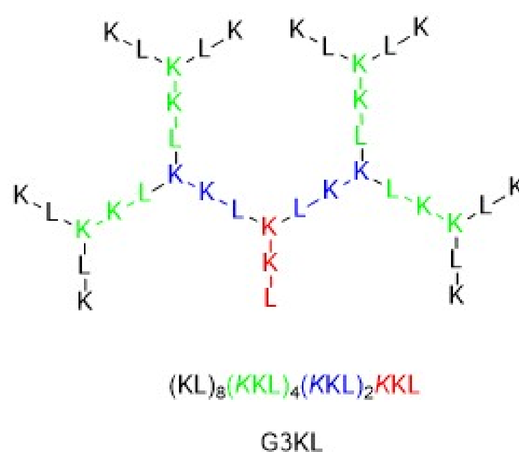


**Figure 1:** Structure of the antimicrobial peptide dendrimer G3KL.

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### Fluorescent modified DNA to detect frameshift mutation

F. D. Berger[1], S. Sturla[1], R. Manderville[2]

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

The NarI restriction site sequence, 5'-G1G2CG3CC-3', is a known mutational hotspot to induce -2 deletion. Upon modification at the G3 position, the -2 deletion is greater than at other guanine sites. Fluorescent modified nucleic acids can act as fluorescent molecules to monitor DNA mutagenicity or base conformation. F-biphenyl-dG is an environmentally sensitive fluorescent probe that has the ability to elucidate microenvironmental fluctuations such as polarity and viscosity variations. We synthesized a 22mer sequence containing the NarI sequence with the F-biphenyl-dG at the G3 position to probe the utility of fluorescence in order to detect a -2 base deletion. We annealed the modified sequence to a full length complementary strand and a truncated strand to mimic the slipped mutagenic intermediate (SMI duplex). We compared the thermal melting data and the fluorescence of the SMI duplex with the full length duplex. The stability of the SMI duplex is similar to the full length complement, however the fluorescence increases by almost 3.5-fold. When the -2 base deletion is mimicked, the adduct is flipped into the helix and due to stacking interactions of the adduct the fluorescence increases. Thus, oligonucleotides containing the F-biphenyl-dG modification are ideal to monitor -2 base deletions using fluorescence.

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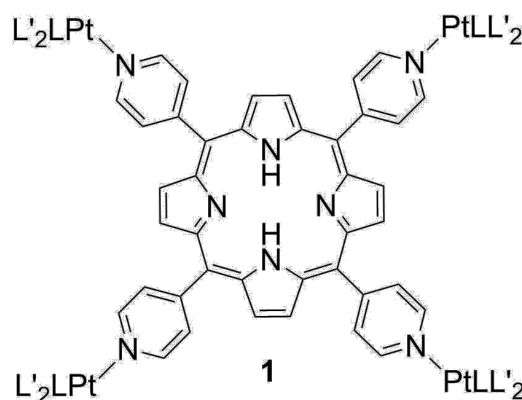
# Platinum-Porphyrin Conjugates in Photodynamic Therapy

M. Larocca[1], B. Spingler[1]*

[1]Department of Chemistry, University of Zurich

Our group has reported recently about the very promising *in vitro* light-induced anticancer properties of novel tetraplatinated porphyrins **1** [1]. The dark and light toxicity against human cancerous and non-cancerous cell lines (MRC-5, HeLa, A2780 and CP70) was determined by the resazurin assay. $IC_{50}$ values were obtained after 4 h incubation, followed by 15 min irradiation at either 420 nm or 575 nm respectively. These platinum-porphyrin conjugates **1** had only minor dark toxicity, however upon visible light irradiation, $IC_{50}$ values down to 19 ± 4 nM could be observed. These values correspond to an excellent phototoxic index (PI = $IC_{50}$ dark / $IC_{50}$ light) of greater than 5000. We have now started to study a similar system, inspired by the work of Therrien, who reported that ruthenium complexes of tetra(3-pyridyl)porphyrin needed less than a tenth of the light dose than the 4-pyridyl isomer to exert the same phototoxicity [2,3].

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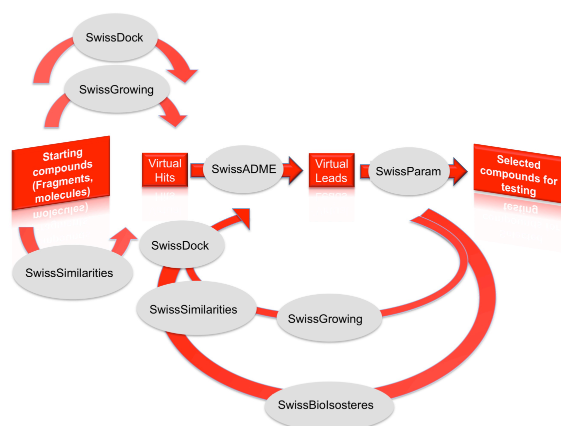
# SwissDrugDesign

V. Zoete[1], A. Daina[2], D. Haake[1], C. Bovigny[2], O. Michielin[3]*

[1]SIB Swiss Institute of Bioinformatics, [2]Swiss Institute of Bioinformatics, [3]Swiss Institute of Bioinformatics / Molecular Modeling Group

Drug discovery has been profoundly changed by the use of computational methods that help making rational decisions at the different steps of the process. A typical *in silico* drug design pipeline may be seen as the interplay between several main activities, e.g. hit finding, lead identification and optimization, ADME and toxicity estimation, and eventually selection of the molecules to be tested experimentally. The SwissDrugDesign project is an ambitious initiative that aims at providing a large collection of web-based tools covering all aspects of computer-aided drug design. Several components of SwissDrugDesign are already online. SwissDocks[1], is a web service dedicated to the docking of small molecules to protein active sites. SwissParam[2] provides topology and force field parameters for drug-like molecules for use with Molecular Mechanics packages. SwissSidechain[3,4] gathers information about hundreds of commercially available non-natural sidechains for peptide design. The SwissBioisostere[5] database collects more than 4.5 millions molecular replacements for lead optimization. SwissTargetPrediction[6-8] allows the prediction of possible targets of a query small molecule. SwissSimilarity allows the rapid ligand-based virtual screening of drugs, bioactive and commercial molecules, as well as of 205 million of virtual compounds readily synthesizable from commercially available synthetic reagents. SwissADME[9,10] calculates physicochemical parameters for small molecules in relation with pharmacokinetic, pharmacodynamic and druglikeness properties. Other tools are currently in development, including SwissGrowing, a program for automatic structure-based ligand design. The interoperability between these tools and the simplicity of use will create a comprehensive web-based environment able to assist the user through a complete computer-aided drug design pipeline.

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## A new Epitope Mimetic of the MPER in HIV-1 gp41

M. Morin[1], E. Stiegeler[2], N. Friedrich[2], T. Reinberg[3], K. Moehle[1], S. Hansen[3], A. Marrero Nodarse[1], A. Trkola[2], A. Plückthun[3], J. A. Robinson[1]*

[1]Department of Chemistry, University of Zurich, Zurich, Switzerland, [2]Institute of Medical Virology, University of Zurich, Zurich, Switzerland, [3]Institute of Biochemistry, University of Zurich, Zurich, Switzerland

Protein epitope mimetics (PEMs) are synthetic peptides designed to mimic the 3D structure of a protein or peptide, corresponding to the interaction surface with their biological target. Their design has been made possible by advances in synthetic chemistry as well as X-ray crystallography and NMR spectroscopy, which has led to an increase in available protein structures. Conformationally constrained synthetic epitope mimetics are of interest in vaccine design, where they can be used to focus immune responses on a specific region of interest of a protein.

Here new PEM molecules have been designed as potential HIV-1 vaccine candidates, focusing on the membrane proximal external region (MPER) of gp41. In order to design MPER mimetics, we have used crystal structures of anti-HIV-1 neutralizing antibodies (nAbs) bound to epitopes in the MPER.

The mimetics were designed by grafting the epitopes recognized by nAbs onto a helical template. The mimetics were synthesized and characterized by cross-linking and spectroscopic studies. As a first step to validate the epitope mimetics as vaccine candidates, we have used the Designed Ankyrin Repeat Protein (DARPin) technology to select DARPins able to bind specifically and with high affinity to the mimetics. Two MPER specific DARPins have been identified in this way, which also bind to gp41 on the virus and block HIV-1 infection. Interestingly, both DARPins show an extraordinary breadth of neutralization activity, which exceeds that currently seen with known broadly neutralizing monoclonal antibodies. Epitope mapping studies have identified residues in the mimetic that are important for binding to the DARPins and highlight a new site of vulnerability on the viral spike protein. These studies validate the PEM molecule as an accurate structural mimetic of a key epitope on the virus, and suggest that the mimetic may have exceptional properties as an HIV-1 vaccine candidate.

## Modifying Phenotypes by Chemical Cell Surface Engineering

I. P. Kerschgens[1], K. Gademann[1]*

[1]Universität Zürich, CH-8057 Zürich, Switzerland

Synthetic biology transformed science by its ability to engineer biosynthetic pathways and thereby modifying phenotypes of many organisms. However, this approach displays clear limitations in that expression is often difficult and potentially lethal phenotypes cannot be generated. We present in this communication a new approach based on chemical cell surface engineering, which in principle allows for (1) the use of non-genetically modified organisms, (2) full reversibility, (3) high spatial and temporal control of phenotype generation (4) fast trait introduction, (5) and finally, for the introduction of new-to-nature functionality in living systems.

The unicellular algae *Chlamydomonas reinhardtii* represents a very important model organism in biology ("green yeast").[1] Using two flagella, the phototropic algae are able to swim to a light source driven by their phototactic responses. Due to these properties the organism has been utilized earlier as a molecular motor[2] by immobilization of a polystyrene bead on the surface.[3]

Our goal consisted in the generation of an antibiotic phenotype of *C. reinhardtii*, therefore turning this phototroph into an antibacterial cell, which is able to kill Gram-positive bacteria. The attachment of the antibiotic to the surface was realized by a 4-hydroxy oligoproline anchor, which binds to the surface of the algae by non-covalent interactions developed earlier by Whitesides and co-workers.[3] Installation of a fluorophore allowed for visualization on the surface. Finally, the antimicrobial activity of *C. reinhardtii* after surface modification was investigated, fueling the concept of molecular prosthetics[4] and thereby creating an antimicrobial living material.[5]

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# Chemical Synthesis of Pollen Tube Attractant, Tf LURE1 protein

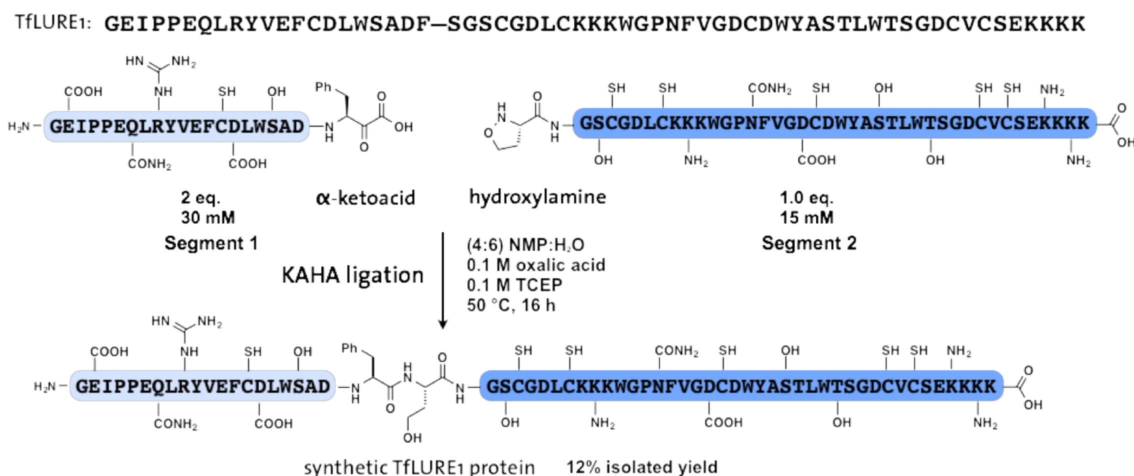
S. Oishi[1], N. Kamiya[2], M. Kanaoka[2], T. Higashiyama[1,2], J. W. Bode[1,3]*

[1]Institute of Transformative Bio-Molecules (ITbM), Nagoya University, Japan, [2]Graduate School of Science, Nagoya University, Japan, [3]Laboratorium für Organische Chemie ETH-Zürich, Switzerland

LURE proteins play important role in plant reproduction. Ovules express and secrete LURE proteins to attract male pollen tubes for fertilization.[1] Amino acid sequences of LURE proteins vary widely in plant species. Thus, LURE proteins would be one of the keys of species-specific reproduction in plant.

TfLURE1 protein found in *Torenia fournieri*, has 6 cysteine residues in 62 amino acids. These cysteine form disulfide bonds that is essential for the bioactivity. We report chemical synthesis of TfLURE1 using a-ketoacid-hydroxylamine (KAHA) ligation.[2]

Peptide segments were synthesized by Fmoc solid phase peptide synthesis. Segment 1, TfLURE1 (1-21), has a-ketoacid at the C-terminus. Segment 2, TfLURE1 (22-61), bears 2-oxaproline at its N-terminus (Scheme 1). KAHA ligation between these two peptide segments gave synthetic TfLURE1 protein (Ser22Hse). After the refolding process with redox buffer, the obtained synthetic protein exhibited comparable bioactivity to the recombinant proteins in bioassay. We will also report synthesis of an ortholog protein, TcLURE1 and their chimeric proteins.




Scheme 1. Synthesis of TfLURE1

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# Synthetic Study of SUMO E2 Enzyme (Ubc9)

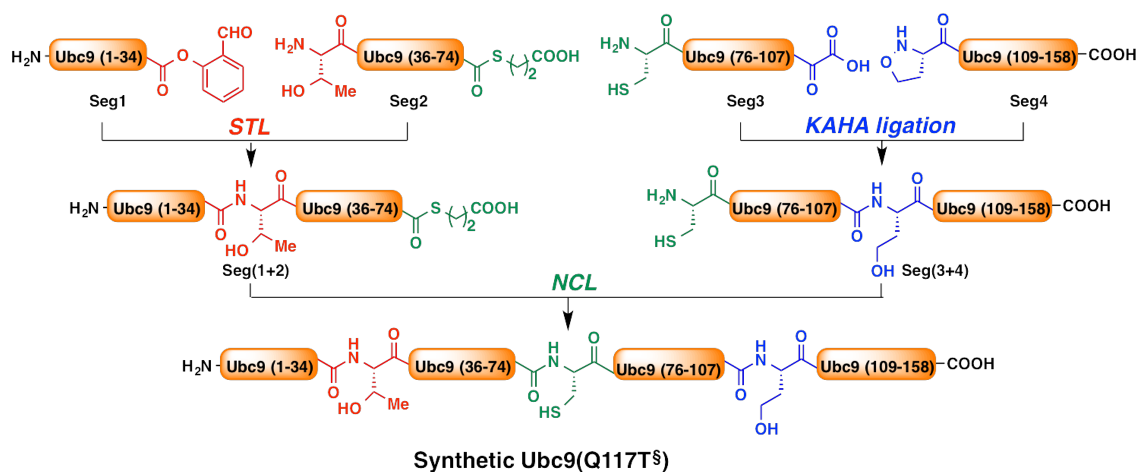
Y. Zhang[1], S. Oishi[1], J. W. Bode[1,2]*

[1]Institute of Transformative Bio-molecules (ITbM), Nagoya University, Japan, [2]Laboratorium für Organische Chemie, ETH-Zürich, Zürich, Switzerland

SUMO E2 (Ubc9) is a conjugating enzyme for sumoylation, which is an important post-translational modification involved in distinct cellular processes ranging from cell-cycle progression to gene expression.[1] To study its biological role, we aim to develop a rapid and efficient chemical synthetic strategy for Ubc9 and its derivatives with functional groups, such as photoaffinity probes.

Ubc9 is composed of 158 amino acids. Due to the size limitation of SPPS, we divided it into four segments (Scheme 1). These four segments will be assembled by the combination of the three current ligation method: serine and threonine ligation (STL),[2] the α-ketoacid-hydroxylamine (KAHA) ligation[3] and native chemical ligation (NCL).[4] It will be the first time to combine the three ligation methods to synthesize a protein. Since these three ligation methods utilize different functional group partners, we hypothesize that these four peptide segments will be assembled in a controlled manner without tedious protection-deprotection steps, and Ubc9 will be efficiently synthesized. This rapid synthetic strategy will also enable us to produce different functionalized protein derivatives only by replacing one peptide segment with another.

In this poster session, we will report the progress of our total chemical synthesis of Ubc9.




**Scheme 1. Synthetic route of Ubc9**

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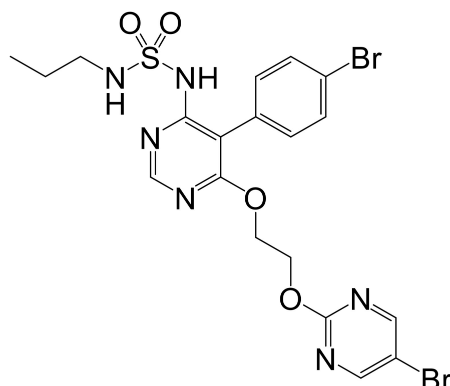
# The Discovery of Macitentan – A Standard Medicinal Chemistry Approach?

M. H. Bolli[1]

[1]Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, CH-4123 Allschwil, Switzerland




The dual endothelin receptor antagonist (ERA) macitentan (Opsumit®) is an approved drug for the treatment of pulmonary arterial hypertension.[1-4] The discovery efforts that led to its identification were motivated by the observation that bosentan, the first approved dual ERA, had several shortcomings. Based on this knowledge, our discovery efforts focused on three key compound properties: its in vitro potency on the endothelin receptors, its in vivo efficacy in reducing the mean arterial pressure (MAP) in hypertensive Dahl salt sensitive rats and its lack of interference with the hepatic bile salt transport in a rat model. Hence, in vitro assays for the two endothelin receptors served as a first filter to prioritize compounds for further profiling. More importantly, however, results obtained from in vivo experiments assessing compound efficacy and safety were clearly driving the discovery process. This 'in vivo'-driven approach was possible because both the pharmacological model as well as the liver safety assessment were easily accessable and allowed rapid and relevant testing of a relatively large number of compounds. Macitentan is about 100-fold more potent on $ET_A$ and approximately ten times more efficacious in reducing MAP in Dahl S rats than bosentan and shows no sign of interfering with the hepatic bile salt transport. It was selected for clinical development after testing about 2500 compounds in vitro and almost 400 compounds in vivo. This talk shall not only describe the process that led to the discovery of macitentan and highlight some of its key properties but also illustrate how this successful approach can be applied to other drug discovery programs.

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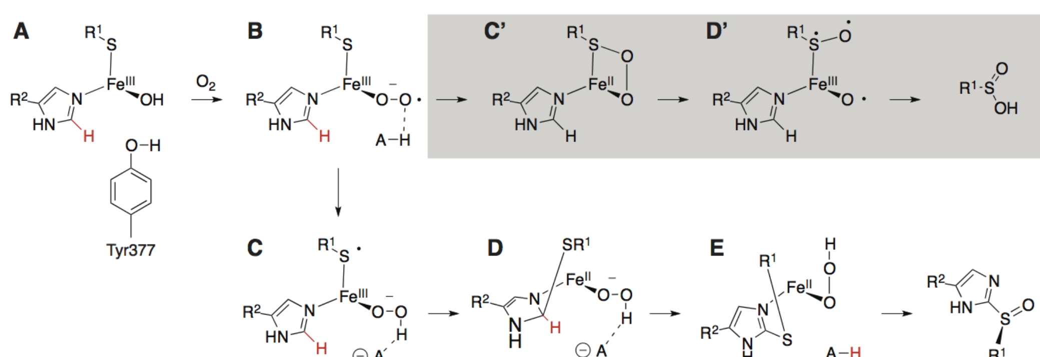
# Deciphering the catalytic mechanism of the sulfoxide synthase EgtB

K. Goncharenko[1], F. P. Seebeck[1]*

[1]University of Basel

EgtB from *Mycobacterium thermoresistibile* is an unusual non-heme iron enzyme that catalyzes the formation of a sulfur-carbon bond between cysteine and N-alpha-trimethylhistidine. Based on the crystal structure of this enzyme, compounded with kinetic characterization of the wild type enzyme and active site mutants we devised a model for the catalytic mechanism of this enzyme (Figure). This model predicts that the rate-limiting step includes oxidation of the substrate thiolate to a thiyl radical. To test this proposition we engineered a hydrogen bond interaction to this thiolate. This intervention does not change substrate binding but significantly reduces $k_{cat}$. In this presentation we discuss these observation in view of our general understanding of biocatalytic sulfur-carbon bond formation.

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# Discovery and Development of the Highly Potent, Highly Selective Cathepsin S Inhibitor RG7625 for the Treatment of Autoimmune Diseases

W. Haap[1]

[1]Roche Pharma Research & Early Development, Innovation Center Basel, F. Hoffmann–LaRoche Ltd., Grenzacherstr. 124, 4070 Basel, Switzerland, wolfgang.haap@roche.com

The lysosomal cysteine protease cathepsin S plays an important role in antigen presentation by degrading the invariant chain fragment p10 to CLIP. This CLIP fragment is associated to the major histocompatibility complex MHCII. After exchange of CLIP by antigens the MHCII/antigen complex is transported to the surface on antigen presenting cells such as microglia, dendritic and B-cells. This complex may be recognised by e.g. T-cells which subsequently become activated. If this process is disturbed, occasional loading of MHCII by self antigens may occur followed by an autoimmune response. Therefore, inhibition of cathepsin S may be an effective treatment of autoimmune diseases.

This presentation will cover the medicinal chemistry optimization of a series of cathepsin S inhibitors culminating in the identification of RG7625 as a highly potent and highly selective cathepsin S inhibitor. Aspects of structure based design, enzyme kinetics and multi dimensional optimisation will be highlighted. The preclinical profiling of RG7625 and clinical Phase I data will be outlined as well.

## A sticky interaction: Optimizing the hydrophobic stacking between the tyrosine gate of the bacterial lectin FimH with antagonists

B. Fiege[1], R. P. Jakob[1], S. Kleeb[1], R. C. Preston[1], P. Zihlmannn[1], X. Jiang[1], S. Rabbani[1], O. Schwardt[1], T. Maier[1]*, B. Ernst[1]*

[1]University of Basel

FimH is the main virulence factor of uropathogenic *E.coli* (UPEC) causing urinary tract infections (UTI) in humans. FimH attaches to mannosylated glycoproteins on urothelial cells preventing the clearance from the bladder and enabling infection of the host cells. Mannose-based antagonists have been developed to block this initial attachment step and thereby prevent UTI as a highly desired alternative to antibiotics treatment. Here we describe the rational development of FimH-antagonists with optimized interaction profiles for oral treatment of UTI.[1,2]
Special care was paid to the optimization of pharmacokinetic parameters and at the same time maintaining a high binding affinity.[2,3] The interaction between the tyrosine gate of FimH with hydrophobic aglycones of the antagonists was found to be crucial and could be analyzed by NMR spectroscopy and X-ray crystallography. NMR signals of key residues in the binding loop containing tyrosine 48 were identified as sensitive reporter for the conformation of the tyrosine gate. Finally, ITC profiles delivered full thermodynamic descriptions of the interactions guiding the further improvement of the antagonists.

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# The discovery of a potent and orally available Dot1L inhibitor

F. Stauffer[1], H. Möbitz[1], C. Scheufler[1], R. Tiedt[1], A. Weiss[1], K. S. Beyer[1], K. Calkins[1], M. Kiffe [1], C. Gaul[1]

[1]Novartis Institutes for BioMedical Research, Basel

Dot1L is responsible for the methylations of lysine 79 of histone 3 (H3K79), with the H3K79me2 mark being associated with active transcription. Under physiological conditions, Dot1L is critical for normal hematopoiesis, however, misdirected catalytic activity (methyltransferase) is believed to be causative for certain acute leukemias. Several oncogenic fusion proteins including MLL-ENL, MLL-AF4 and MLL-AF9 aberrantly recruit Dot1L to ectopic loci, leading to local hypermethylation of H3K79 and misexpression of genes (including HoxA9, Meis1) which drive the leukemic phenotype[1]. Inhibition of the methyltransferase activity of Dot1L in MLL-rearranged leukemias (mixed lineage leukemia, MLL) is predicted to reverse ectopic H3K79 methylation, leading to repression of leukemogenic genes and tumor growth inhibition. The recent quest for Dot1L inhibitors is spearheaded by Epizyme and culminated in the discovery of EPZ-5676, a SAM-competitive, nucleoside-containing Dot1L inhibitor, which is currently being evaluated in MLL patients in Phase 1b clinical trials. The agent is administered by continuous intravenous (i.v.) infusion due to its physicochemical properties largely inherited from SAM cofactor from which it is derived. Herein, we will describe a structurally completely novel (non-SAM like) series of Dot1L inhibitor that is not interacting in the SAM cofactor binding pocket. This series was optimized to match the exquisite potency of the current clinical candidate and to achieve oral bioavailability with a suitable exposure profile in mouse.

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## Optimization of 1,4-Disubstituted Benzodiazepines as Selective and Brain Penetrant Triple Calcium T-Channel Blockers

R. Siegrist[1], D. Pozzi[1], M. Kessler[1], C. Roch[1], R. Moon[1], O. Bezençon[1]

[1]Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, 4123 Allschwil, Switzerland

Epilepsy affects more than fifty million people worldwide. Despite the availability of around thirty antiepileptic drugs (AED), used alone or in combination, 30% of the epileptic patients are not seizure free. More importantly, the current treatments affect only the symptoms and no available AED can prevent epileptogenesis. In this context, inhibition of the calcium T-channels offers a promising new way to treat this disorder [1,2].

At Actelion, our search for potent and selective brain penetrant triple blockers started with an HTS campaign and 1,4-disubstituted benzodiazepine hits were identified. Despite showing low nanomolar activity, these hits suffered notably from poor physico-chemical properties and were metabolically unstable. Herein we report the SAR studies and optimization of this class of compounds, leading to the discovery of a potent lead derivative with improved solubility and DMPK properties. The optimized compound showed interesting in-vivo efficacy in a model of absence epilepsy [3].

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# A FUC/LecB system to crystallize versatile nucleic acid structures

P. Röthlisberger[1], C. Leumann[2], M. Hollenstein[1]*

[1]Institut Pasteur, [2]University of Bern

Over the last few decades, oligonucleotides consisting of base-modified nucleic acids found interest in applied material science.[1] In order to fully understand the properties of such oligonucleotides it is advantageous to know the exact three-dimensional structure. Functional nucleic acids, consisting of aptamers, DNAzymes and ribozymes have found applications in biosensing,[2] diganostics[3] and gene therapeutics.[4] A shortcoming of functional nucleic acids in *in vivo* applications is degradation by exonucleases, the requirement of high $M^{2+}$ concentration to attenuate catalytic activity and a challenging cellular delivery. The chemical alteration of the functional nucleic acids to circumvent such problems without impeding their activity is a commonly used method. The knowledge of the exact structural arrangement allows a more specific chemical intervention and could therefore lead to more potent therapeutics of diagnostics. Herein, we report on the X-ray structural analysis of modified DNA duplexes and functional nucleic acids. In this context, we have developed a method for the elucidation of the three-dimensional structures that involves the derivatization of the oligonucleotide with an L-fucose (FUC) residue to enable a non-covalent conjugation to the protein lectin B (LecB) that serves as robust co-crystallization agent.[5]

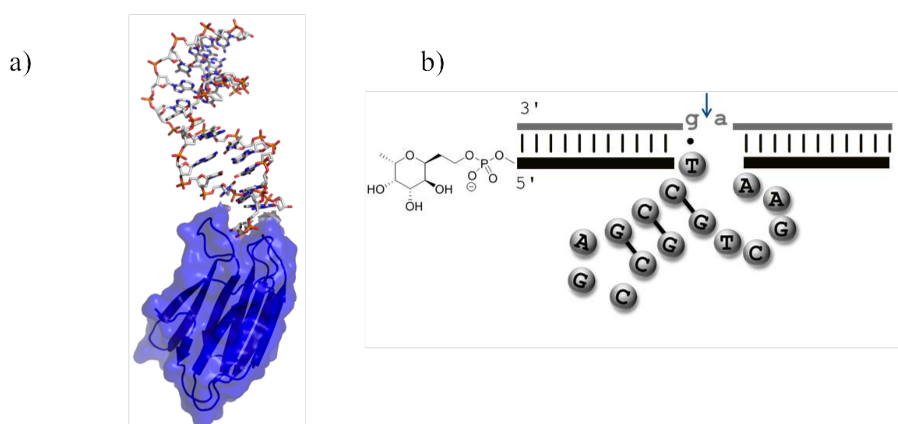


*Figure 1.* a) Representation of a DNA duplex crystallized with the FUC/LecB system; b) Schematic representation of a fucosylated DNAzyme binding to an RNA substrate.

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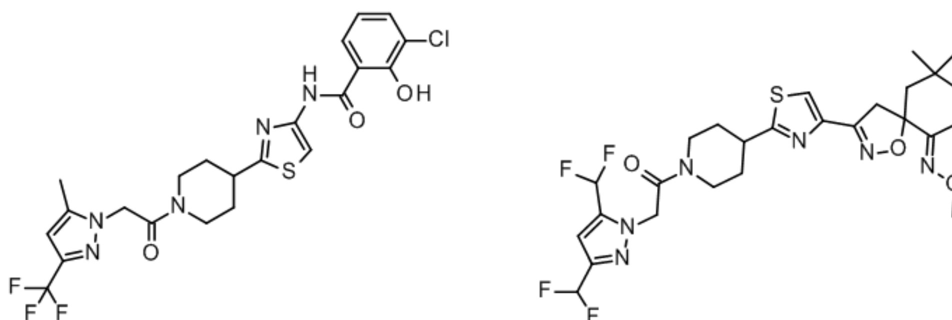
# Synthesis and oomycete fungicidal activity of a new family of inhibitors targeting an oxysterol binding protein

M. Pouliot[1]

[1]Syngenta Crop Protection

Inhibitors targeting oxysterol binding protein have shown excellent fungicidal activity against late blight and downy mildew, plant diseases caused by oomycete pathogens. Oxathiapiprolin, discovered by DuPont researchers, have been the first compound of this class to reach the market and is commercialized by both DuPont and Syngenta under the trade names Zorvec$^{TM}$ and Orondis$^{TM}$ respectively. In this talk, we would like to present research done in Syngenta on oomycete fungicide inhibiting oxysterol binding protein. The synthesis and antifungal activity of new classes of bicyclic and spirocyclic isoxazolines will be presented, along with that of the *N* -thiazol-4-yl-salicylamide class which distinguished itself by its unique capacity at controlling damping-off disease caused by *Pythium ultimum*.

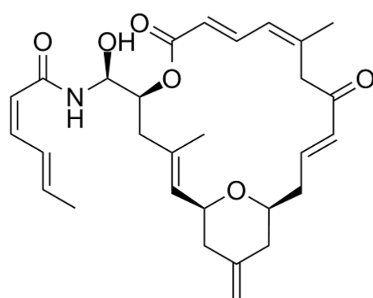


# Stereoselective Synthesis and Biological Evaluation of Highly Potent New (-)-Zampanolide Derivatives

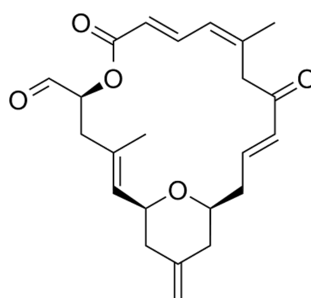
T. Brütsch[1], J. Miller[2], K.-H. Altmann[1]*

[1]ETH Zürich, [2]Victoria University of Wellington, School of Biological Sciences

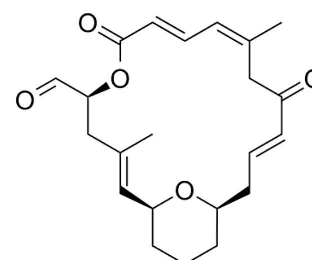
(-)-Zampanolide (**1**) is a polyketide natural product with a highly unsaturated macrolactone core structure and an uncommon N-acyl hemiaminal-linked side chain.[1] (-)-Zampanolide (**1**) is a microtubule-stabilizing agent that binds covalently to the luminal taxane site in b-tubulin through reaction of C9 with His$^{229}$.[2] We have previously shown that the removal of the the exo-methylene group at C13 in the related (-)-dactylolide **2** (leading to compound **3**) was well tolerated in terms of antiproliferative activity.[3]



**(-)-Zampanolide (1)**
$IC_{50}$ (A549) = 3.2 nM

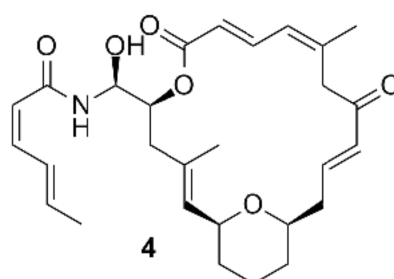


**(-)-Dactylolide (2)**
$IC_{50}$ (A549) = 301 nM



**3**
$IC_{50}$ (A549) = 149 nM

We have now also prepared 13-desmethylene-(-)-zampanolide (**4**) (from **3**) and we have found this analog to exhibit low nM antiproliferative activity. We have thus used **4** as the basis for extended SAR studies that have assessed the role of individual methyl groups and degree of unsaturation of the macrocyclic core structure. These studies were enabled by a newly developed stereoselective method for the installment of the N-acyl hemiaminal side chain.



**4**

This contribution will discuss the synthesis of a number of new (-)-zampanolide derivatives and will give new insights into the importance of specific structural features for antiproliferative and microtubules-stabilizing properties of **4**.

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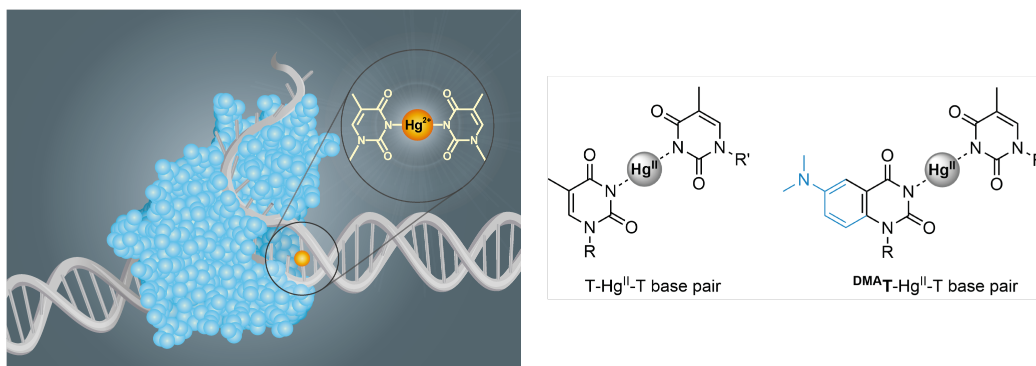
# High Kinetic Stability of T-Hg$^{II}$-T and DNA Polymerase Inhibition

O. P. Schmidt[1], G. Mata[1], N. W. Luedtke[1]*

[1]University of Zurich

The mechanisms responsible for the infamous cytotoxic and mutagenic activities of Hg$^{II}$ are only partly understood and are potentially the result of mercury-DNA interactions.[1] *In vitro*, T-T mismatches in duplex DNA stoichiometrically bind Hg$^{II}$ ions to give T-Hg$^{II}$-T base pairs, which exhibit similar thermal stabilities and structural dimensions as T-A base pairs in duplex DNA.[2]




We have utilized the fluorescent thymidine analog **$^{DMA}$T** to characterize the kinetic and thermodynamic parameters of Hg$^{II}$ binding to discrete T-T sites in duplex DNA.[3,4] **$^{DMA}$T** fluorescence quenching was used for the first reported kinetics study of T-Hg$^{II}$-T association and dissociation. The on- and off-rates of mercury were surprisingly slow, with association rate constants ($k_{on}$) = 0.8 – 9.0 x 10$^4$ M$^{-1}$s$^{-1}$, and dissociation rate constants ($k_{off}$) = 1.5 – 9.0 x 10$^{-4}$ s$^{-1}$; giving equilibrium dissociation constants ($K_d$) in the range of 8 – 50 nM. With half-lives ranging from 0.3 to 1.3 h in duplex DNA, T-Hg$^{II}$-T base pairs exhibit high kinetic stabilities that can inhibit enzymatic DNA synthesis and strand-displacement reactions at biologically relevant concentrations. Our results demonstrate that T-Hg$^{II}$-T base pairs are kinetically distinct from T-A base pairs and therefore have the potential to disrupt DNA metabolism *in vivo*.[4]

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# Discovery of a Potent and Selective Reversible BTK Inhibitor for the Treatment of Autoimmune Diseases

R. Pulz[1]

[1]Novartis Institutes for Biomedical Research

Bruton's Tyrosine Kinase (BTK) is a cytoplasmic tyrosine kinase and a member of the TEC kinase family. It is expressed selectively in a subset of immune cells, including macrophages, mast cells, platelets and B cells. BTK is a key regulator of B cell antigen receptor signalling in B cells and of Fc receptor signalling in mast cells and macrophages. Based on a strong genetic and pharmacological validation, it is likely that a BTK inhibitor will have a positive impact on autoimmune diseases which are driven by autoreactive B cells and immune-complex driven inflammation, like e.g. Rheumathoid Arthritis (RA). In this presentation, we describe the discovery, optimization and preclinical characterization of highly selective reversible BTK inhibitors. Combination of an internal HTS hit with a tail fragment led to a potent and selective lead compound. A co-crystal structure of the lead with BTK proved binding to a specific inactive conformation of BTK underlying the excellent kinase selectivity of the scaffold. Unfortunately, the lead exhibited poor physicochemical properties resulting in low oral bioavailability in rat. We will describe SAR studies focussing on improving the physicochemical properties of the lead by reducing molecular weight, aromaticity and lipophilicity. In addition, a key *t*Bu-group was replaced in order to increase metabolic stability. Finally, the optimization yielded a development candidate with a good balance of potency, physicochemical and PK properties, combined with an excellent preclinical safety profile. The still low solubility of the compound could be mitigated by a tosylate salt, which provided adequate exposure of the compound in relevant preclinical safety species. The compound showed efficacy in two animal models depending on the B cell receptor pathway (sheep red blood cell model) and the Fc receptor pathway (therapeutic collagen induced arthritis).

**Investigations for New Therapeutic Targets for Neurodegenerative Disease.**

E. Crane[1], K. Gademann[2,1]*

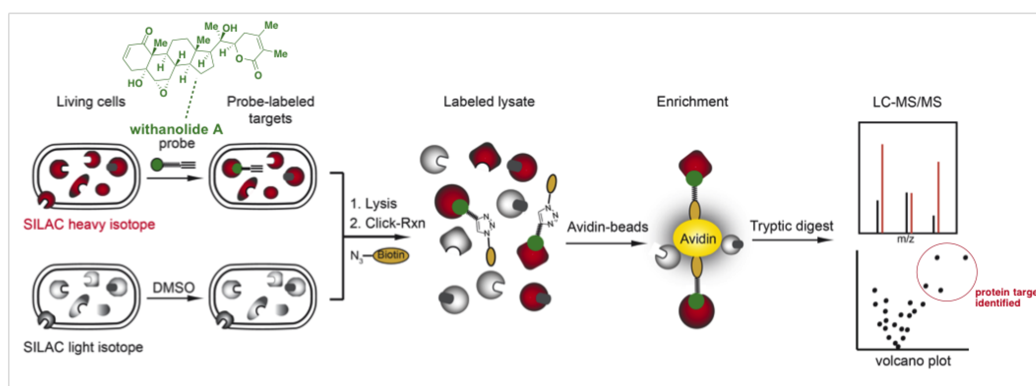
[1]University of Basel, [2]University of Zürich

Additional co-authors:

W. Heydenreuter[3], K. Beck[1], P. Strajhar[1], J. Vomacka[3], M. Smiesko[1], E. Mons[1], L. Barth[1], A. Vedani[1]*, A. Odermatt[1]*, S. Sieber[3]*

[1]University of Basel, [3]Technical University of Munich

In our quest to identify new potential therapeutic targets for neurodegenerative diseases, we initiated activity-based protein profiling (ABPP) studies with the neuritogenic natural product, withanolide A. Molecular probes were designed based on known reactivities of this scaffold from prior synthetic studies and six novel compounds were successfully prepared. Target profiling of these compounds revealed several relevant and novel hits in the context of neurodegeneration. Further investigations of these hits through molecular modeling with *VirtualToxLab* and several different types of *in vitro* assays were completed. As a result, new targets of relevance to the mechanism of action of withanolide A are suggested, providing more insight into these neuritogenic pathways.

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