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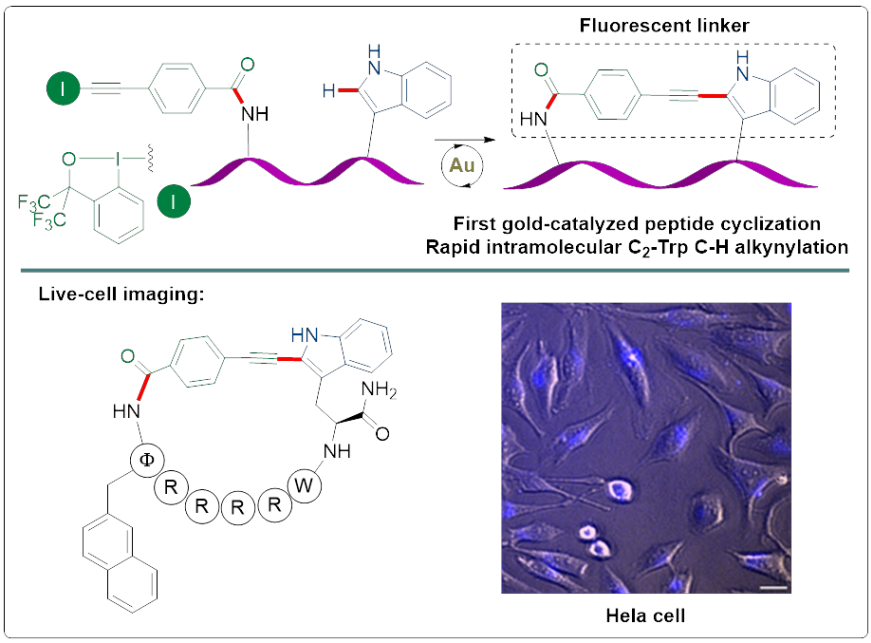
The synthesis of fluorescent cyclic peptides via gold(I)-catalyzed macrocyclization

X. Liu[1], W. Cai[2], B. Fierz[2], J. Waser[1]*

[1]Laboratory of Catalysis and Organic Synthesis (LCSO), Institute of Chemical Sciences and Engineering (ISIC), EPFL, [2]Laboratory of Biophysical Chemistry of Macromolecules (LCBM), Institute of Chemical Sciences and Engineering (ISIC), EPFL

Rapid and efficient cyclization methods, forming novel 3D macrocycles, are still urgently needed. We presented here the first gold(I)-catalyzed peptide macrocyclization of peptide-EBXs (ethynylbenziodoxolones). This reaction was carried out in the presence of protecting group free peptide sequences enabled by simple and commercial gold catalyst ($AuCl{\cdot}Me_2S$). The method displayed rapid reaction rate (within 10 min), wide functionality tolerance (26 unprotected peptides were tested) and good yield. This unique highly conjugated cyclic peptide linker, formed through alkynylation, can be directly applied to cell imaging without further attachment of fluorophores.



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LipMetE (Lipophilic Metabolism Efficiency) as a Simple Guide for Half-Life and Dosing Regimen Prediction of Oral Drugs

G. Cecere[1,2]

[1]Roche Pharmaceutical Research and Early Development, [2]Roche Innovation Center Basel

The in vivo half-life is a key property of every drug molecule, as it determines dosing regimens, peak-to-trough ratios and often dose. However, half-life optimization can be challenging due to its multifactorial nature, with in vitro metabolic turnover, plasma protein binding and volume of distribution all impacting half-life. We here propose that the medicinal chemistry design parameter Lipophilic Metabolism Efficiency (LipMetE) can greatly simplify half-life optimization of neutral and basic compounds. Using mathematical transformations, examples from preclinical GABAA projects and clinical compounds with human pharmacokinetic data, we show that LipMetE is directly proportional to the logarithm of half-life. As the design parameter LipMetE can be swiftly calculated using the readily available parameters LogD, intrinsic clearance and fraction unbound in human liver microsomes or hepatocytes, this approach enables rational half-life optimization from the early stages of drug discovery projects.

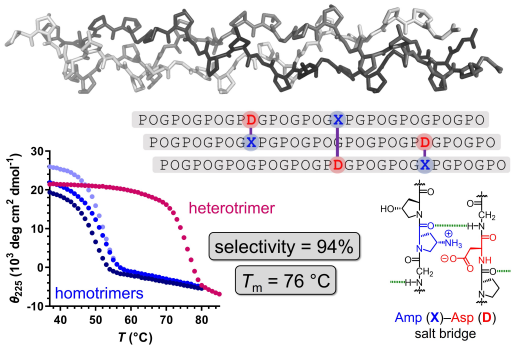
Cecere, G. et al., *ACS Med. Chem. Lett.*, **2022**, *13*, 1444−1451
DOI: https://doi.org/10.1021/acsmedchemlett.2c00183

A General Strategy to Access Hyperstable and Ultrashort Collagen Heterotrimers

T. Fiala[1], V. Islami[1], P. Bittner[1], R. Heeb[1], C. Söll[1], R. Zenobi[1], H. Wennemers[1]*

[1]Laboratory of Organic Chemistry, ETH Zurich, D-CHAB, Vladimir-Prelog-Weg 3, 8093, Zurich, Switzerland.

Most types of natural collagen are heterotrimers comprised of three strands that assemble into a triple helix. Despite the significance of collagen as a biomaterial – one third of all proteins in mammals is collagen – the isolation of pure collagen is a challenge due to the large size, crosslinking and numerous heterogenous post-translational modifications. Collagen model peptides (CMPs) have emerged as synthetic surrogates for investigating collagen structure and developing biological materials and probes. However, the selective assembly of CMPs into heterotrimers is challenging since a combination of three different CMPs can form up to 27 distinct triple helices. In this study, we demonstrate that salt bridges between (4*S*)-aminoproline (Amp) and aspartate (Asp) can be employed as a robust and universal driving force for the selective assembly of collagen heterotrimers. By introducing a single Amp-Asp salt bridge between each pair of strands, we assembled the shortest stable supramolecular collagen heterotrimer (with 17-mer strands) as well as the most stable selectively assembled collagen heterotrimer (32-mer strands, melting temperature 76 °C) reported to date. Our work provides a modular toolbox for the development of heterotrimeric collagen-based materials and probes.



***De novo* design of *talo*-configured lactones as a new class of highly potent, selective, and orally bioavailable galectin-3 inhibitors**

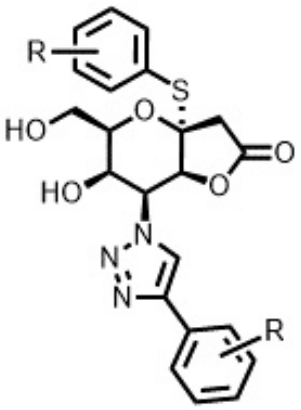
L. Remeň[1], C. Zumbrunn[1], C. P. Sager[1], A. Mac Sweeney[1], E. Hühn[1], J. Gatfield[1], M. H. Bolli[1]

[1]Idorsia Pharmaceuticals Ltd, Hegenheimermattweg 91, 4123 Allschwil, Switzerland

Galectins - a family of glycan-binding proteins - are involved in many different biological processes, such as inflammation, apoptosis, angiogenesis, fibrosis, immunomodulation, or tumor proliferation. Their carbohydrate-recognition domain (CRD) binds to a b-galactopyranose unit present in many glycoproteins on the cell surface. Crystal structures of galectin-3 with various ligands have revealed that its CRD is characterized by a relatively large but shallow, polar, crater-shaped, and solvent accessible binding site that accommodates many water molecules. Upon binding of an oligosaccharide containing a galactose moiety, some of these water molecules are replaced by the galactose OH-groups in a highly directional fashion, some of them playing an integrative role in the stabilization of the ligand-protein complex. The galectin-3 binders known today all represent galactopyranose derivatives. While they incorporate modifications at the 1, 2, and 3-position of the galactopyranose moiety, they strictly conserve the highly directional interactions of the galactose 4-OH and 6-OH groups with the protein. Because of that, all potent galectin-3 binders have at least two OH-groups conserved, a feature that can limit their permeability across biological membranes.

For the above-mentioned reasons, the design of small, potent and orally bioavailable galectin-3 inhibitors remains a great challenge in medicinal chemistry.

Based on these observations, we concluded that a detailed understanding of the binding mode at an atomic level and precise information about the hydrogen-bond network of the ligand-protein complex are essential for a successful design of potent and selective galectin-3 inhibitors. We therefore based our de novo design of galectin-3 inhibitors on X-ray crystallography, isothermal titration calorimetry and molecular dynamics simulations, and combined these methods with physico-chemical and DMPK characterization of the prepared molecules. In our talk, we will illustrate the path that led to the discovery of talo-configured lactones as a new series of highly potent, selective, and orally bioavailable galectin-3 inhibitors.

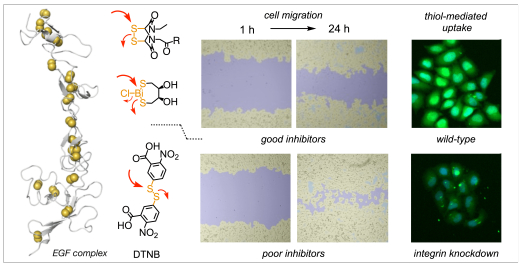


Inhibition of Cell Motility by Cell-Penetrating Dynamic Covalent Cascade Exchangers - Integrins Participate in Thiol-Mediated Uptake

F. Coelho[1], S. Saidjalolov[1], D. Moreau[2], O. Thorn-Seshold[3], S. Matile[1]

[1]Department of Organic Chemistry, University of Geneva, 1211 Geneva, Switzerland, [2]Department of Biochemistry, University of Geneva, 1211 Geneva, Switzerland, [3]Department of Pharmacy, Ludwig-Maximilians University of Munich, 81377 Munich, Germany

Thiol-mediated uptake (TMU) is the process of internalization facilitated by the continuous exchange (CAX) between activated disulfides and exofacial thiols on transmembrane proteins. This process can be inhibited by using hydrophilic surface-thiol-reactive agents preventing any other molecule to participate in any CAX. TMU cascades are a complex mechanism which is not yet well understood due to its dynamic nature and fleeting intermediates. Recently, we demonstrated that TMU is not a single target process but rather involves a network of transmembrane proteins meshed in a dynamic fashion.[1] The most relevant example is the direct dependence of asparagusic acid to transferrin receptor for TMU.[2] These results drove our attention to thiol/disulfide rich transmembrane proteins that might be potentially involved in TMU. Integrins, a family of glycoprotein receptors responsible for mediating the process of cell migration and evasion, viral uptake, and wound healing, were speculated to be part of TMU. The activation of the integrin is caused by the change of conformation after redox exchange between Protein Disulfide Isomerase (PDI) and the thiol/disulfide-rich leg of the b-subunit of integrin. Inspired by this, we tested our TMU inhibitor library in a cell migration assay. Results show that migration was indeed inhibited in three different cell lines and across three different coatings. The antimigratory activities exceeds that of Ellman's reagents and correlates globally with their abilities to penetrate cells.[3] Most importantly, knockdown experiments support the conclusion that the integrins are involved in TMU as in the antimigratory properties. Now, we found that the integrin superfamily is an exchange partner to participate in TMU - biggest finding since the transferrin receptor (the only known until now). These results thus (a) introduce dynamic covalent cascade exchange chemistry to the control of cell motility, (b) expand the CAX drug discovery space from anti-viral toward anti-thrombotic and anti-tumor potential, and (c) they confirm integrins as exchange partners in the dynamic TMU networks that deliver matter into cells. Opens doors to further advances in the field of drug delivery.



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Discovery of ligands for TRIM58, a novel tissue selective E3 ligase

K. Hoegenauer[1]

[1]Novartis Institutes for Biomedical Research

Redirecting E3 ligases to neo-substrates leading to their proteasomal disassembly, known as targeted protein degradation (TPD), has emerged as a promising alternative to traditional, occupancy driven pharmacology. Although the field has expanded tremendously over the last years, the choice of E3 ligases remains limited, with an almost exclusive focus on CRBN and VHL. Here, we report the discovery of novel ligands to the PRY-SPRY domain of TRIM58, a RING ligase that is specifically expressed in erythroid precursor cells. A DSF screen, followed by validation using additional biophysical methods, led to the identification of the TRIM58 ligand **TRIM-473**. A basic SAR around the chemotype was established by utilizing a competitive binding assay employing a short FP peptide probe derived from an endogenous TRIM58 substrate. The X-ray co-crystal structure of TRIM58 in complex with **TRIM-473** gave insights to the binding mode and potential exit vectors for bifunctional degrader design.

Rapid photoradiosynthesis and preclinical evaluation of theranostic radioimmunoconjugates using Lutetium-177

S. Klingler[1], J. P. Holland[1]*

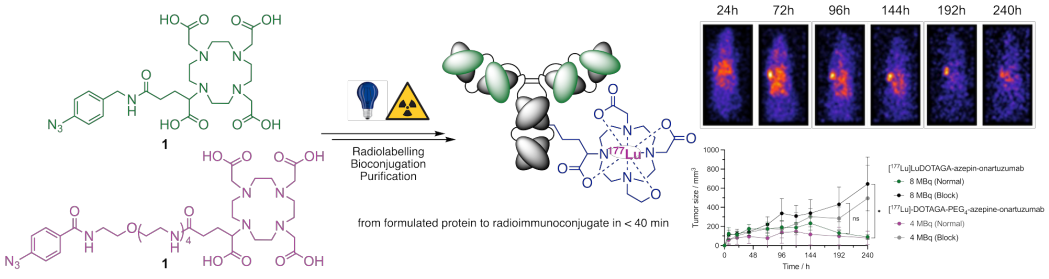
[1]Universtiy of Zurich, Winterthurerstrasse 190, 8057 Zurich

The conventional radiosynthesis of ^{177}Lu-radiopharmaceuticals makes use of analogues of the *aza*-macrocyclic chelate DOTA. The radiolabelling of such compounds typically requires heating to achieve efficient radiolabelling in short reaction times, good radiochemical yield (RCY), radiochemical conversion (RCC) and molar activity.[1] However, high temperatures are incompatible with proteins such as monoclonal antibodies (mAbs). We recently developed a fast photoradiochemical process which can produce ^{89}Zr-radiolabelled mAbs under mild conditions in less than 15 minutes, starting from clinical-grade protein samples with full formulation.[2,3] Here, we adapt the photoradiochemical process for the work with ^{177}Lu to access potential theranostic radiotracers.

The radiolabelling of the photoactivatable chelates DOTAGA-ArN_3 (**1**) and DOTAGA-PEG_4-ArN_3 (**2**) were achieved with RCYs >99% at 70 °C for 15 min before photochemical bioconjugation to proteins was performed at ambient temperature. Photochemical conjugation reactions on protein samples including human serum albumin (HSA), and the monoclonal antibodies trastuzumab (formulated as Herceptin) and onartuzumab (formulated as Met-mAb) and gave RCYs between 47-54% for HSA and 20-32% for the preparation of ^{177}Lu-labelled mAbs in formulation. The entire two-step process, including radiosynthesis, purification and formulation, can be completed in

[^{177}Lu]LuDOTAGA-azepin-onartuzumab and [^{177}Lu]LuDOTAGA-PEG_4-azepin-onartuzumab were administered to mice bearing subcutaneous MKN-45 tumours and the pharmacokinetic profile of the tracers were studied by temporal *in vivo* g-ray imaging up to 10 days and by *ex vivo* biodistribution studies at 3 and 10 day after administration and showed high uptake of 25.3 ± 3.9 and 21.66 ± 2.0 %ID/g respectively. The tumours showed a significant decrease in size at 10 days after treatment versus the blocking group.

DOTAGA aryl azides **1** and **2** grant access to a fast and reliable radiosynthetic route for ^{177}Lu-radioimmunoconjugates without exposing the protein to heat or other extreme conditions. The tracers produced following this procedure have been tested *in vivo* and showed selective accumulation in target tissue and indicated their efficacy against this model of gastric adenocarcinoma.



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TYMIRIUM® Technology: The Discovery of Cyclobutrifluram

T. Luksch[1], O. Loiseleur[1]

[1]Syngenta Crop Protection AG, Research Chemistry, Schaffhauserstrasse, 4332 Stein, Switzerland

The damage caused by plant-parasitic nematodes leads to severe agricultural yield losses worldwide. To tackle the threat of nematodes, a discovery program was started at Syngenta which led to the development of TYMIRIUM® technology, a new nematicide and fungicide that obtained first registrations in 2022.

The active ingredient is Cyclobutrifluram, a chiral phenyl-cyclobutyl-pyridineamide, which displays broad spectrum efficacy against the plant parasitic nematodes and soil-borne diseases – particularly the *Fusarium* species.

We describe the discovery of this molecule from hit identification through lead exploration and lead optimization phases.

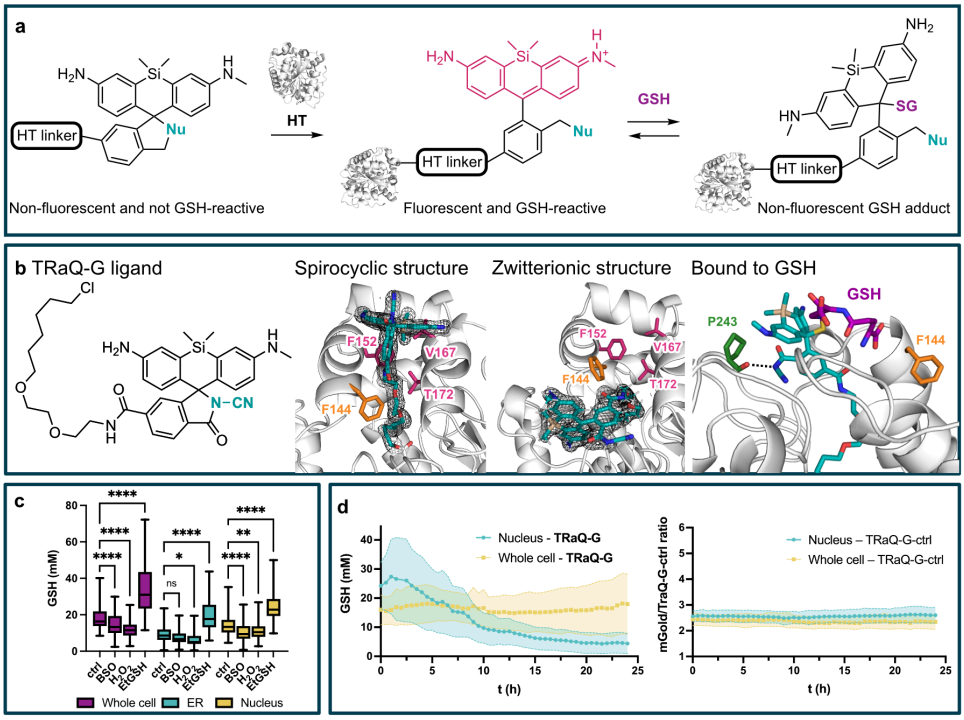
This includes the establishment of the nematicide screening platform together with the generation of a focused nematicide screening library, and the rapid identification of the mode of action for the lead series. Target identification using biochemical and genetic methods, allowed the development of an *in vitro* assay for SAR analysis and the development of homology models for structure-based design. This established a design and analysis toolbox for accurate molecular design coupled to synthesis. Our collaborative, multi-disciplinary approach led to the discovery of TYMIRIUM® technology.

A Locally Activatable Sensor for Robust Quantification of Organellar GSH

S. Emmert[1,2], G. Quargnali[1,2], S. Thallmair[3]*, P. Rivera-Fuentes[1,2]*

[1]Department of Chemistry, University of Zurich, 8057 Zurich, Switzerland, [2]Institute of Chemical Sciences and Engineering, EPF Lausanne, 1015 Lausanne, Switzerland, [3]Frankfurt Institute for Advanced Studies, 60438 Frankfurt am Main, Germany

Glutathione (GSH) is the main determinant of intracellular redox potential and participates in multiple cellular signaling pathways. Achieving a detailed understanding of intracellular GSH trafficking and regulation depends on the development of tools to map GSH compartmentalization and intra-organelle fluctuations. Here, we propose a **T**argetable **Ra**tiometric **Q**uantitative **G**SH sensor (**TRaQ-G**)[1] for live-cell imaging based on the previously reported reactivity of certain silicon rhodamines towards GSH.[2] A nucleophilic group on our reporter fluorophore leads in the unbound state to a spirocyclic, non-fluorescent conformation. Only upon binding to the HaloTag (HT) protein, the fluorescence and the GSH-responsiveness is switched on (panel a). Fusing HT to a fluorescent protein and a targeting peptide, allows for a robust ratiometric read-out and flexible targeting. Our structural analysis including X-ray crystal structures and molecular dynamics simulations (panel b) allowed us to reason the sensitivity of our sensor molecule towards GSH. We confirmed that our sensor reliably responds to artificially induced changes in GSH concentration in several subcellular compartments (panel c). Furthermore, we studied GSH regulation during the cell cycle by imaging synchronized HeLa cells with our sensor (panel d). Our results indicate a separately regulated nuclear GSH pool with an especially high concentration right before cell division which matches previous reports.[3]



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Discovery of Novel Allosteric EGFR L858R Inhibitors for the Treatment of Non-Small-Cell Lung Cancer

A. Ricci[1], U. Obst-Sander[1], G. Jaeschke[1]*

[1]F. Hoffmann-La Roche Ltd, Roche Pharmaceutical Research and Early Development, Therapeutic Modalities, Roche Innovation Center Basel, Grenzacherstrasse 124, Basel 4070, Switzerland

Addressing resistance to third-generation EGFR TKIs such as osimertinib via the EGFR C797S mutation remains a highly unmet need in EGFR-driven non-small-cell lung cancer (NSCLC). The discovery of novel allosteric inhibitors such as EGFRai-51 and EGFRai-57 to overcome EGFR C797S-mediated resistance in patients harboring the activating EGFR L858R mutation are presented. These allosteric EGFR inhibitors demonstrate robust tumor regression in a mutant EGFR L858R/C797S tumor model. Additionally, EGFRai-57 demonstrates superior efficacy in combination with osimertinib compared to the single agents in an H1975 EGFR L858R/T790M NSCLC xenograft model. These data highlight the potential of using EGFRai-57 as a single agent against EGFR L858R/C797S and EGFR L858R/T790M/C797S and as combination therapy for EGFR L858R- and EGFR L858R/T790M-driven NSCLC.

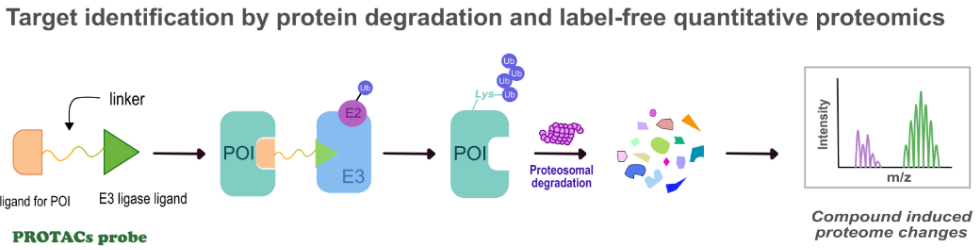
Ulrike Obst-Sander, Antonio Ricci, Georg Jaeschke et al., *J. Med. Chem.,* **2022**, 65, 19, 13052–13073.

PROTACs as a target deconvolution tool of Hedgehog Pathway Inhibitor 1

M. Bagka[1], S. Hoogendoorn[1]*

[1]University of Geneva, Department of Organic Chemistry

The Hedgehog (Hh) signaling pathway is pivotal for embryonic development of vertebrates, however abnormal activation can lead to tumorigenesis. The current FDA approved drugs targeting the Hh pathway act on the upstream protein Smoothened and suffer from acquired resistance, rendering essential to find new inhibitors with a different target[1] . Hedgehog Pathway Inhibitor 1 (HPI-1) was reported as a downstream inhibitor by Hyman et al and has subsequently been shown to have anti-cancer activity, but its cellular target has remained elusive for many years[2].



To reveal the molecular targets of HPI-1 we designed a proteolysis-targeting chimeras (PROTACs) approach, that involves heterobifunctional molecules consisting of two-headed small ligands which bind to two different proteins; an E3 ligase and a target protein (POI) that is to be degraded through the ubiquitin-proteasome system[3]. The POI can eventually be identified by mass spectrometry-based proteomics. Using this strategy, we discovered the BET bromodomains as the targets of HPI-1, extending the scope of PROTACs as a novel target deconvolution technique.

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Leveraging historical small molecule data from the Novartis portfolio: From chemical series evolution to predictive modelling

M. Beckers[1], N. Sutrm[1], N. Fechner[1], N. Stiefl[1]

[1]Novartis Pharma AG

In this contribution, we plan to give an overview of our recent activities towards reconstructing the historical landscape of chemical series used in past projects in the Novartis compound archives and how we leveraged this data to build predictive models. In the first part, we will present how we reconstructed the chemical series using clustering methods and show the subsequent statistical analyses of the dataset. These analyses cover the time evolution of structural properties, ADMET and target activities during optimization of the chemical series [1]. Next, we will show how we used this data to build machine learning models for the prediction of small molecule developability. To this end, we further extended the dataset and annotated the terminal development stages the compounds reached. Using these historical milestones together with the recently developed MELLODDY model [2], we built a deep neural network that takes the predicted ADMET profile of a compound as input and returns a number which describes the likelihood of transitioning beyond in vivo PK studies [3]. The resulting score, which we termed bPK score, showed strong performance in discrimination of development candidates from non-development candidates (AUC>0.8). Interestingly, on these internal and public datasets other compound scoring techniques did not show any discriminative performance anymore. We will discuss the retrospective application to MedChem projects and show recent examples of prospective usage.

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Small Ribozymes in Gut Bacteriophages and Their Potential Role in Microbiome-Virome Interactions

K. Kienbeck[1], L. Malfertheiner[2], S. Zelger-Paulus[1], S. Johannsen[1], C. von Mering[2]*, R. K. Sigel[1]*

[1]Department of Chemistry, University of Zurich, Zurich, Switzerland, [2]Department of Molecular Life Sciences, University of Zurich, Zurich, Switzerland

Hepatitis delta virus (HDV)-like ribozymes belong to the class of small self-cleaving RNA enzymes, which catalyze a site-specific internal transesterification while exhibiting diverse sequences and structures [1]. However, the biological functions of these ribozymes, particularly minimal examples, remain largely unknown [2].

We computationally discovered thousands of minimal HDV-like ribozymes in bacteriophage genomes associated with the human gut. *In vitro* validation was performed on selected examples associated with viral tRNAs, coined theta ribozymes. We propose a novel biological function of these ribozymes, suggesting their involvement in tRNA maturation and the lytic-lysogenic switch of specific phages (Figure 1). Investigating the catalysis and regulation of theta ribozymes sheds light on the poorly understood implications of the microbiome-virome interactions of the human gut and their impact on health and disease.

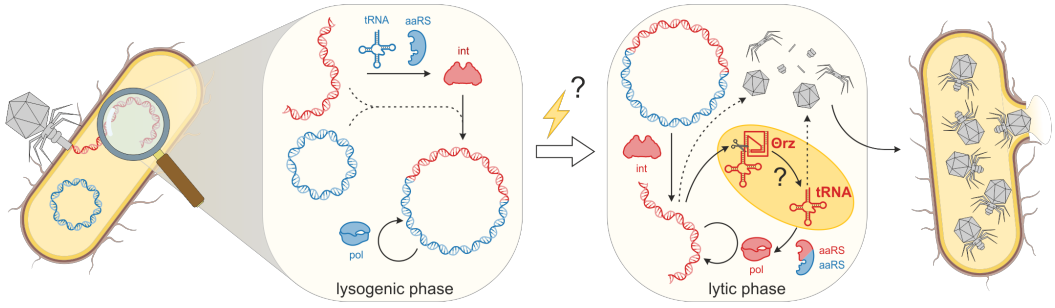

Figure 1: Proposed phage infection cycle involving theta ribozymes. aaRS: aminoacyl-synthetase; int: integrase; Θrz: theta ribozyme; pol: polymerase.

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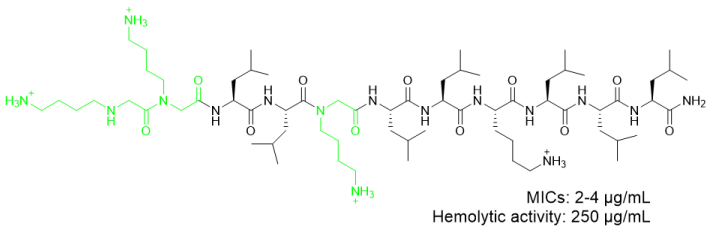
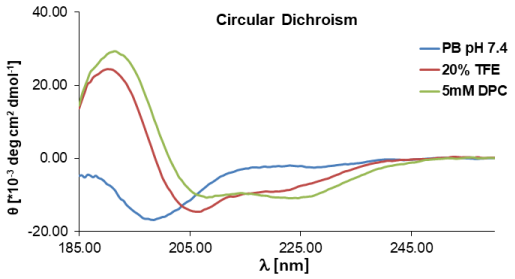
Antimicrobial peptide-peptoid hybrids to control multidrug resistant Gram-negative bacteria

E. Bonvin[1], J. L. Reymond[1]*

[1]University of Bern, Department of Chemistry, Biochemistry and Pharmaceutical Sciences

Membrane disruptive antimicrobial peptides (AMPs) such as polymyxin B offer an opportunity to control multidrug resistant (MDR) Gram-negative bacteria,[1] which are a leading cause of death in hospitals.[2] Recently we discovered that inverting the chirality of lysine amino acids in an 11-residues α-helical AMP with strong activity against these bacteria preserved its α-helical folding and activity while abolishing its hemolytic properties and serum instability.[3] Inspired by several reports of using peptoid building blocks to tune AMP activity,[4] we investigated if our AMP activity might also be tolerant to peptoid substitutions. Our investigations revealed several peptide-peptoid hybrids with preserved α-helical folding and antibacterial activity, but increased serum stability and reduced hemolysis compared to the parent all-L AMP sequence (Figure). Additionally, even if helicity was lacking, several hybrids including the full peptoid displayed strong antibacterial effect under dilute medium conditions, typically used for proline-rich antimicrobial peptides,[5] suggesting a transition from membrane disruption to intracellular targets.





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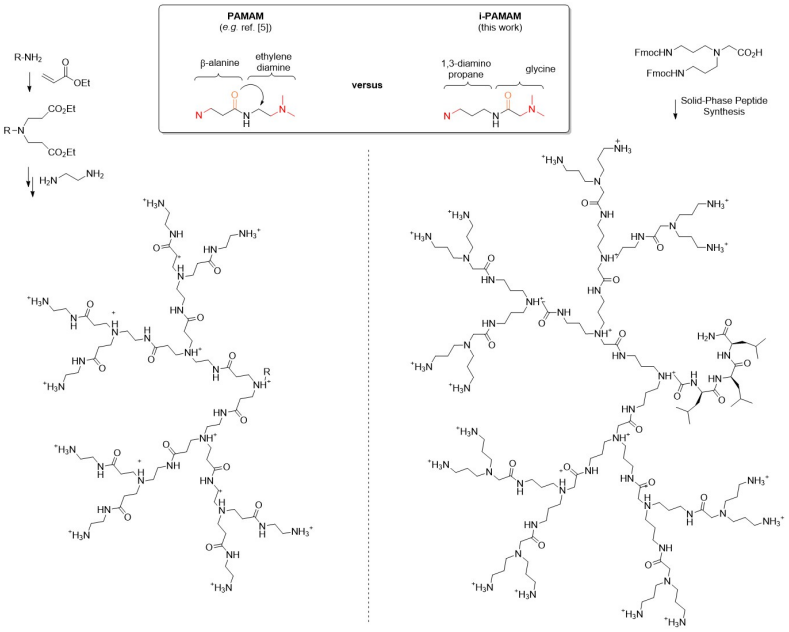
Redesigning PAMAMs: Antimicrobial Inverse-Polyamidoamine (i-PAMAM) Dendrimers

E. Bonvin[1], J. L. Reymond[1]*

[1]University of Bern, Department of Chemistry, Biochemistry and Pharmaceutical Sciences

Polyamidoamine (PAMAM) dendrimers have shown various interesting properties ranging from technology to medicine.[1-5] However, PAMAM dendrimers suffer from an intrinsic instability due to the presence of the β-alaninyl-amidoethylamine branch in their structure, which easily undergoes retro-*Michael* reaction.[6] For this reason, we redesigned the branches of PAMAM dendrimers by moving the carbonyl group of β-alanine across the amide bond. This modification transforms the ethylene diamine unit into glycine and the β-alanine into 1,3-diaminopropane, removing the possibility of a retro-*Michael* reaction, and resulting in inverse PAMAM (i-PAMAM) dendrimers. Contrary to the preparation of PAMAMs in solution and the difficulties encountered during their purification, our strategy gave us access to solid-phase peptide synthesis at high temperature by iterative coupling and deprotection of the commercially available *N,N*-bis(N′-Fmoc-3-aminopropyl)glycine. Good purity was reached after preparative reverse phase HPLC and no degradation of our i-PAMAMs could be detected over time. To demonstrate this new class of dendrimers, we synthesised potent so far non-membrane disruptive antimicrobial dendrimers with activities against both *Gram*-negative and *Gram*-positive bacteria.[7]



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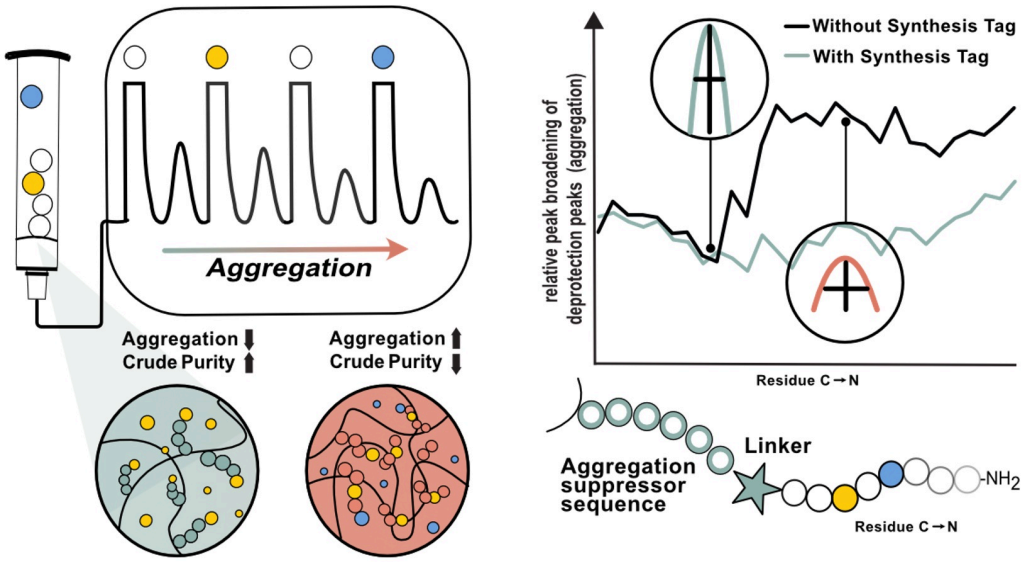
Chemical synthesis of c-Myc transactivation domain using a synthesis/solubility tag

H. M. Bürgisser[1], R. Lescure[1], A. Jeandin[1], A. Premanand[1], E. T. Williams[1], N. Hartrampf[1]*

[1]University of Zurich, Departement of Chemistry

Chemical protein synthesis enabled by solid-phase peptide synthesis (SPPS) provides peptide and protein samples with a virtually unlimited chemical space (including PTMs) through incorporation of non-canonical amino acids and backbone modifications. Decades of improvement and optimization have increased the length of synthesized peptide chains of up to 50 amino acids.[1] Over this limit, Native Chemical Ligation (NCL) has been developed to join synthesized fragments, ultimately leading to the production of larger proteins.[2] Yet, generating fragments by SPPS in good yield and purity requires extensive synthesis efforts. A particular problem during the synthesis itself is the aggregation of the resin-bound peptides, which is highly sequence dependent. While several solutions have been developed to address the aggregation problem, identifying and suppressing its cause is still very challenging. A deeper understanding of aggregation, as well as a more general solution to this problem, are therefore urgently needed. Our flow-based fast peptide synthesizer (AFPS) with in-line UV analysis has the capacity to monitor aggregation during synthesis. Combining our results from screening various linkers and amino acid sequences resulted in the development of a versatile “synthesis tag”. The tag reduced aggregation for several “difficult peptides”, yielding significantly improved crude purities as well as enhanced peptide solubilities. As an application of the method, we now use our “synthesis tag” in the synthesis of the heavily aggregating transactivation domain (TAD) of the intrinsically disordered transcription factor MYC. [3]



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From screening to structure - Boosting NMR-based drug discovery with hyperpolarization and label-free structure determination

M. Bütikofer[1,2], F. Torres[2], G. Stadler[1], T. Segawa[1], H. Kadavath[1], R. Riek[1]*, J. Orts[3]*

[1]Laboratory of Physical Chemistry, ETH Zürich, [2]NexMR GmbH, [3]University of Vienna, Department of Pharmaceutical Sciences

Nuclear Magnetic Resonance (NMR) is the gold standard for fragment-based drug discovery (FBDD). However, due to its low sensitivity and tedious analysis, it needs high sample concentrations and has a low throughput. We present the use of photo-Chemically Induced Nuclear Polarization (photo-CIDNP) [1] and NMR Molecular Replacement (*N*MR²) [2] to establish a workflow from screening to structure-activity relationship for FBDD, overcoming the above-mentioned limitations. Photo-CIDNP drastically reduces the time and amount of material needed and *N*MR² makes protein-fragment structures accessible within a few days without the need for isotope labelling.

A photo-CIDNP fragment library is screened against the cancer target PIN1. Photo-CIDNP enables accelerated measurements of a few seconds. Furthermore, we demonstrate the possibility to screen at nanomolar concentrations and cryogen-free benchtop NMR spectrometers within a few minutes. Hyperpolarization also enables to determine the affinity of the hits within minutes. We present a new method analogous to STD-NMR to measure the affinity of protein-ligand interactions with photo-CIDNP. Several examples are presented. Finally, we show the implementation of a T_1, T_2-filtered 2D-NOESY pulse sequence to measure fragment-protein distance restraints. *N*MR² does not need a protein assignment and the distance restraints are directly converted to a protein-fragment complex structure. More than 10 complex structures of the oncogenic protein K-Ras have been elucidated this way.

This study shows how new NMR methods are implemented into a FBDD workflow. Lowering the target concentration for screening and K_D measurements into the nanomolar regime, moving away from cryogenic magnets, and elucidating structures without isotope labelling could help to tackle new targets that were up to now out of reach for NMR and medicinal chemists.

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Fluorescent labeling of cellular DNA for an exploration of in-situ chromatin structure

W. Cai[1]

[1]École Polytechnique Fédérale de Lausanne (EPFL), SB ISIC LCBM, Station 6, CH-1015 Lausanne, Switzerland

The regulation of gene expression controls diverse biological processes. Specific gene regulation depends on the three-dimensional (3D) folding of the genome, the establishment of close enhancer contacts, and a specific spatial organization of the chromatin fiber. How these contacts between different gene loci are mediated is still poorly understood on a molecular level. Although current methods based on fluorescence in situ hybridization (FISH) can resolve chromatin structure at the ~ 10 kilobase (kb) scale, it requires DNA denaturation for probe hybridization which will lead to the disruption of nucleosome-level fine structure. Another choice is the staining of chromatin via antibodies or dyes, which is milder but lacks locus-specific. Here we propose a new approach to resolve these issues. In this approach, chemical biology and single-molecule imaging are combined to achieve the observation of locus-specific 3D folding of the genome in a living cell.

The aim of our investigation is to implement a novel imaging approach to reveal chromatin structure at defined genomic loci by a combination of chemical biology and super-resolution imaging. Here, we are incorporating modified nucleosides, including ethynyl-deoxyuridine (EdU) and azidomethyl-deoxyuridine (AmdU) into a DNA sequence at high density[1]. We then fluorescently label this DNA using copper-catalyzed or strain-promoted click chemistry. This enabled super-resolution imaging with 3D stochastic optical reconstruction microscopy (STORM) of DNA strands in vitro. In a second step, we then transfect EdU or AmdU-tagged into cells and integrate the labeld DNA strands into the native chromatin using the piggyBac transposon system[2]. There, the labeled DNA will be chromatinized, followed by labeling and 3D super-resolution imaging. Together, this system will enable important insights into the conformational ensemble of a defined chromatin locus in cells, and provides a deeper understanding of the relationship between gene architecture and expression regulation.

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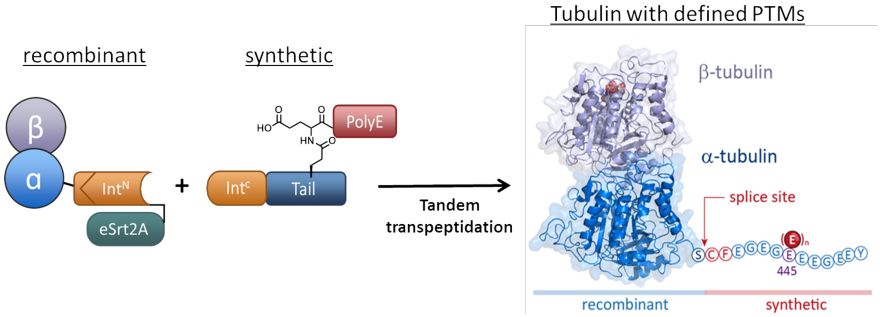
Production of Semisynthetic Tubulin with Definable Post-Translational Modification

P. Chang[1], E. Ebberink[1], S. Fernandes[2], C. Aumeier[2], B. Fierz[1]*

[1]Laboratory of Biophysical Chemistry of Macromolecules, Ecole Polytechnique Fédérale de Lausanne (EPFL), [2]Department of Biochemistry, University of Geneva

Microtubules, a vital constituent of the cytoskeleton, carry various post-translational modifications (PTMs) that are essential for regulating essential cellular mechanisms. However, the precise mechanisms underlying how PTM patterns govern the functions of microtubules are not well understood, mostly due to a lack of methods to generate tubulin with well-defined PTMs. Our previous work showed a promising semi-synthetic approach to preparing α-tubulin with polyglutamylation (polyE) on their C-terminals. Building upon this, this work aims to improve the method for producing semi-synthetic α- and β-tubulin by incorporating two key advancements. The first improvement involves the use of a building block approach to conjugate the branched polyglutamic acid and the α-tubulin tail via a native amide bond. The second improvement involves the development of a method to modify the β-tubulin tail, offering a potential solution to orthogonally tailor both α- and β-tubulin tails. In future work, we plan to investigate the mechanism behind the activation of the vasohibin complex through polyglutamylation and determine whether polyglutamylation also activates MATCAP. Additionally, we plan to use the semi-synthetic tubulin to identify microtubule-associated proteins (MAPs) that are sensitive to polyglutamylation in cell lysates, by using photoaffinity proteomics.



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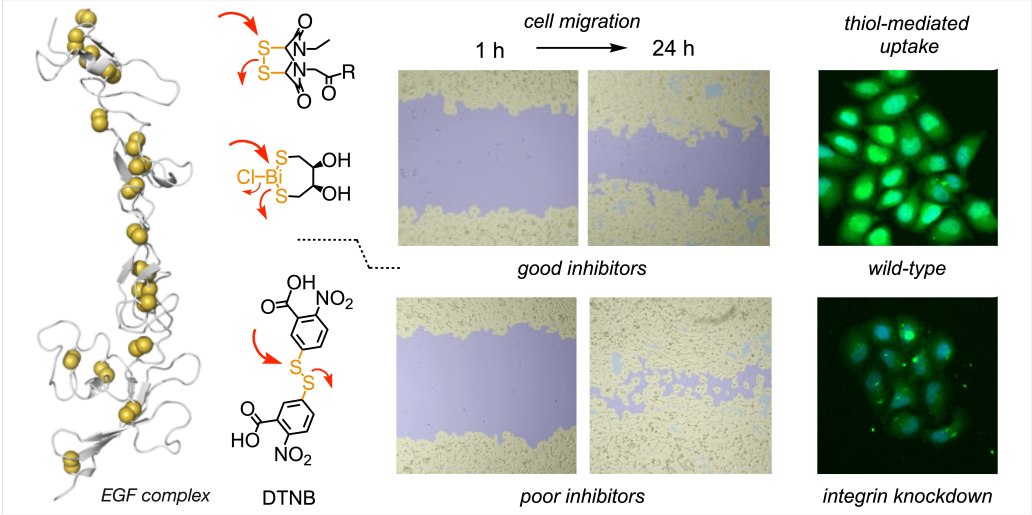
Inhibition of Cell Motility by Cell-Penetrating Dynamic Covalent Cascade Exchangers - Integrins Participate in Thiol-Mediated Uptake

F. Coelho[1], S. Saidjalolov[1], D. Moreau[2], O. Thorn-Seshold[3], S. Matile[1]

[1]Department of Organic Chemistry, University of Geneva, 1211 Geneva, Switzerland, [2]Department of Biochemistry, University of Geneva, 1211 Geneva, Switzerland, [3]Department of Pharmacy, Ludwig-Maximilians University of Munich, 81377 Munich, Germany

Thiol-mediated uptake (TMU) is the process of internalization facilitated by the continuous exchange (CAX) between activated disulfides and exofacial thiols on transmembrane proteins. This process can be inhibited by using hydrophilic surface-thiol-reactive agents preventing any other molecule to participate in any CAX. TMU cascades are a complex mechanism which is not yet well understood due to its dynamic nature and fleeting intermediates. Recently, we demonstrated that TMU is not a single target process but rather involves a network of transmembrane proteins meshed in a dynamic fashion.[1] The most relevant example is the direct dependence of asparagusic acid to transferrin receptor for TMU.[2] These results drove our attention to thiol/disulfide rich transmembrane proteins that might be potentially involved in TMU. Integrins, a family of glycoprotein receptors responsible for mediating the process of cell migration and evasion, viral uptake, and wound healing, were speculated to be part of TMU. The activation of the integrin is caused by the change of conformation after redox exchange between Protein Disulfide Isomerase (PDI) and the thiol/disulfide-rich leg of the b-subunit of integrin. Inspired by this, we tested our TMU inhibitor library in a cell migration assay. Results show that migration was indeed inhibited in three different cell lines and across three different coatings. The antimigratory activities exceeds that of Ellman's reagents and correlates globally with their abilities to penetrate cells. Most importantly, knockdown experiments support the conclusion that the integrins are involved in TMU as in the antimigratory properties.[3] Now, we found that the integrin superfamily is an exchange partner to participate in TMU - biggest finding since the transferrin receptor (the only known until now). These results thus (a) introduce dynamic covalent cascade exchange chemistry to the control of cell motility, (b) expand the CAX drug discovery space from anti-viral toward anti-thrombotic and anti-tumor potential, and (c) they confirm integrins as exchange partners in the dynamic TMU networks that deliver matter into cells. Opens doors to further advances in the field of drug delivery.



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Unlocking the high-throughput potential of peptidomimetic diversification

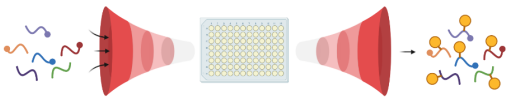
N. De Sadeleer[1], A. Nielsen[1], S. Ballet[2]*, C. Heinis[1]*

[1]Institute of Chemical Sciences and Engineering, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland., [2]Departments of Chemistry and Bioengineering Sciences, Vrije Universiteit Brussel, 1050 Brussels, Belgium.

The pharmaceutical industry currently faces a productivity crisis, prompting a renewed interest in peptide therapeutics a highly promising drug modality for challenging targets.[1] Recent advancements in the field have emphasized the remarkable potential of peptidomimetics, surpassing the limitations of their native counterparts by expanding the chemical space beyond the confined twenty canonical amino acids.

A particularly effective strategy employed to enrich chemical and structural diversity involves the lateral diversification of individual residues or peptides.[2] This approach can potentially be strengthened by the power of high-throughput experimentation (HTE), which exponentially enhances the output of a single step when applied across an entire library. Unfortunately, only a handful of studies have thus far reported successful high-throughput lateral diversification of amino acids and peptides.[3-5] Hence, the primary objective of this research endeavor is two-fold: to address the discrepancy in available methods and to acquire insights into the inherent limitations entailed by HTE.

To this goal, two well-established techniques for diversification were selected: the renowned Suzuki-Miyaura cross-coupling and the highly-efficient copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). This work embraces the optimization of both transformations on individual amino acids at low nanomole scale within the ambit of a high-throughput environment. Subsequently, the feasibility of our methodology has been validated by demonstrating proof-of-concept through the lateral diversification of cyclic in-house peptide mimetics in a 384-well format.



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Single-molecule studies elucidate the 5'exon binding mechanism for group II intron splicing

B. Fazliji[1], K. M. Egger[1], S. Zelger-Paulus[1], R. K. Sigel[1]*

[1]University of Zurich

Group II introns are large ribozymes found in bacteria and eukaryotic organelles, capable of self-splicing. However, the precise intron-exon binding mechanism to detect the cleavage site remains unclear.[1-3] To elucidate the binding process, we employed single-molecule Förster Resonance Energy Transfer (smFRET).

By gradually increasing Mg^{2+} concentrations, we investigated the role of metal ions in intron folding. Our smFRET analysis revealed two folding states: a high FRET state consistent with the predicted conformation, indicating a tight intron-exon binding, and a dominant low FRET state. Mg^{2+} facilitates tertiary interactions and 5'exon binding.[4,5] However, even at high Mg^{2+} concentrations, the low FRET prevailed. These findings validate the existing 3D model and unveil a prevalent low FRET state. We conclude that the group II intron primarily adopts this low FRET state, temporarily binding the 5'exon to achieve the necessary folding state for subsequent cleavage. To elucidate the binding mode, we perturbed specific contacts, demonstrating the connection between structural changes, reduced cleavage activity, and the function of group II introns.

Our study unveils novel insights into the conformational dynamics of group II introns, deepening our understanding of the binding and functional mechanisms in self-splicing ribozymes.

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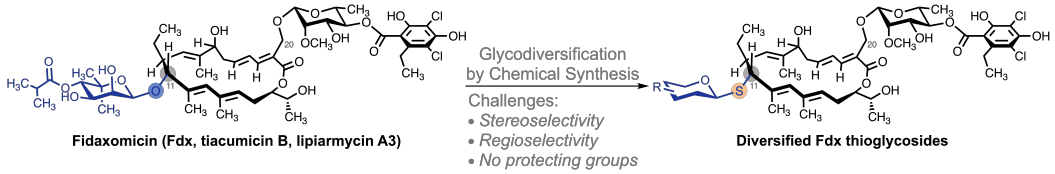
Semi-Synthesis of Thioglycoside Derivatives of the Natural Product Antibiotic Fidaxomicin

I. Ferrara[1], A. Altorfer[1], M. D. Salim[1], G. Chesnokov[1], S. Dittmann[2], S. Sievers[2], K. Gademann[1]*

[1]Department of Chemistry, University of Zurich, Zurich, Switzerland, [2]Department for Microbial Physiology and Molecular Biology, Institute of Microbiology, Center for Functional Genomics of Microbes, University of Greifswald, Greifswald, Germany

Antibiotic treatments can disturb the gut flora and can give rise to the proliferation of pathogens. The pathogen *Clostridioides difficile* (*C. diff.*) constitutes the most frequent cause of nosocomial infections, and can evoke diarrhea, but in severe cases also lead to sepsis or death.[1,2] The repertoire of treatment options is limited to mainly the antibiotics fidaxomicin, vancomycin, and metronidazole. Out of these, the natural product macrolactone fidaxomicin (Fdx, tiacumicin B, lipiarmycin A3) is the gold-standard treatment due to low recurrence-rates and high selectivity for *C. diff*.[1–4] The limited treatment options along with the potential for resistance development emphasize the need for research on antibiotics targeting *C. diff*. Our aim is to access structural diversification of the Fdx parent compound to slow-down resistance development of *C. diff.* and to optimize the PK/PD profile.

Fdx targets the bacterial RNA polymerase inhibiting the transcription initiation process, culminating in bactericidal activity.[5,6] Structurally, Fdx comprises a core aglycon linked to carbohydrate moieties at the C11- and C20-position. The D-noviose-derived sugar at C11 binds deep within the relatively narrow binding site for Fdx. In this work, we will present our strategy to access glycodiversification at C11 under mild conditions leaving the rest of the molecule intact. In addition, we will discuss how we avoid the use of protecting groups on Fdx, the regio- and stereochemical outcome of these reactions and present the synthetic strategy to access non-classical 1-thio-noviose derivatives. Further, the biochemical characterization of the new Fdx derivatives will be presented.



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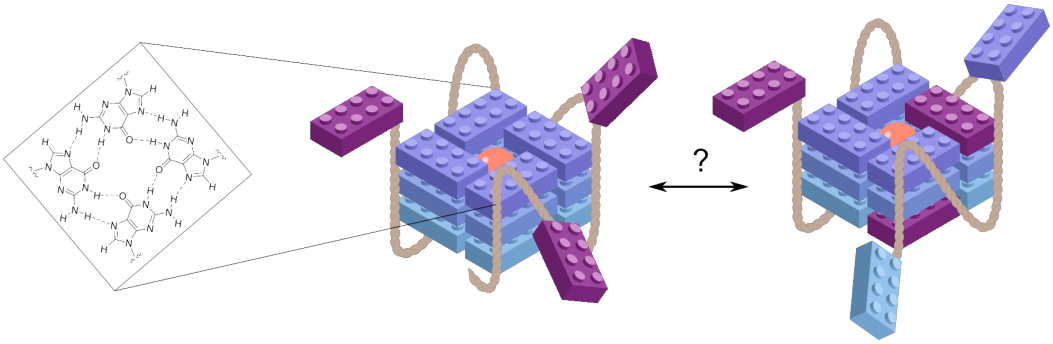
Characterising RNA G-quadruplex structure

C. Ferreira Rodrigues[1], Z. Wang[1], S. Johannsen[1], R. K. Sigel[1]*

[1]University of Zürich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zürich

In guanine-rich DNA and RNA sequences, non-canonical nucleic acid structures called G-quadruplexes can form. While DNA G-quadruplexes have been extensively studied since their discovery, RNA G-quadruplexes have gained attention due to their involvement in gene regulation, such as the G-quadruplex in the 5'-untranslated region of the B-cell lymphoma gene 2 (*BCL2*). Abnormal expression of Bcl-2 has been associated with various diseases [1]. However, the mechanism of regulation remains unknown.

G-quadruplexes are composed of a cyclic arrangement of four guanines through Hoogsteen hydrogen bonds, resulting in a single plane [2]. Typically, a G-quadruplex consists of three stacking planes connected through the backbone by flexible loops.



RNA G-quadruplexes adopt a parallel orientation of the backbone strands due to the ribose sugar's C3'-endo preference [3]. The exchange of guanines between the planes and loops contributes to the high structural dynamics. By strategically mutating the G-quadruplex sequence, the dynamics can be minimized, enabling the elucidation of individual conformers. Using NMR is an ideal method for analysing their structure, characterising their dynamics, and providing insights into their mechanism of regulation.

Through detailed analysis of the various mutant structures by our newly developed NMR-based approach, valuable insights into essential structural features were obtained. Understanding these characteristic elements is crucial for elucidating the regulatory mechanisms and designing future drug treatments.

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Developing Molecular Tools for the Study and Detection of Calcium-Sensing Receptor

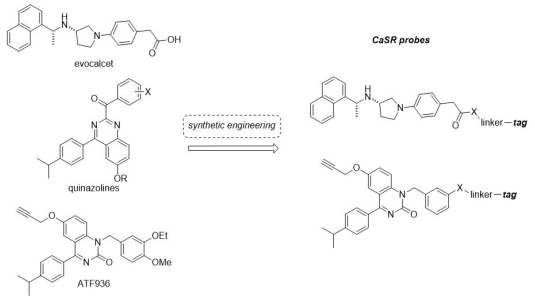
J. Fischer[1], D. Batora[1], L. Dick[1], R. Kaderli[2], J. Gertsch[1]*, M. Lochner[1]*

[1]Institute of Biochemistry and Molecular Medicine, University of Bern, [2]Department of Visceral Surgery and Medicine, Inselspital Bern

Background: The calcium-sensing receptor (CaSR) is a G protein-coupled receptor that plays a central role in the regulation of calcium homeostasis in humans.[1] It is highly expressed in parathyroid glands, pancreatic endocrine cells and kidneys. Impaired expression or function of CaSR causes several diseases and enlarged parathyroid glands, in particular, can lead to a pathological shift of calcium homeostasis and necessitate surgical removal in some cases.[2] Thus, the accurate pre- and intraoperative localisation of parathyroid glands is essential to avoid persistent complications that can significantly impair the patient's quality of life.[3] Molecular tools currently used in the clinic are not specific to the parathyroid glands and false-positive and false-negative readouts are common. Several small compounds and peptides have been developed to target and modulate CaSR as allosteric ligands, some of which are used in the clinic as so-called calcimimetic drugs to increase CaSR activity (e.g. cinacalcet, evocalcet and etelcalcetide).

Aim: To develop synthetic molecular probes for the study, modulation and localisation of the CaSR in cells and tissue.

Methods and Results: To this end, we have synthesised derivatives and conjugates of calcilytics (i.e. negative allosteric CaSR modulators), such as quinazolines and quinazolinones, and derivatives of evocalcet (positive allosteric modulator). In this context, we present our work on the synthesis of these probes and their preliminary biological assessment.



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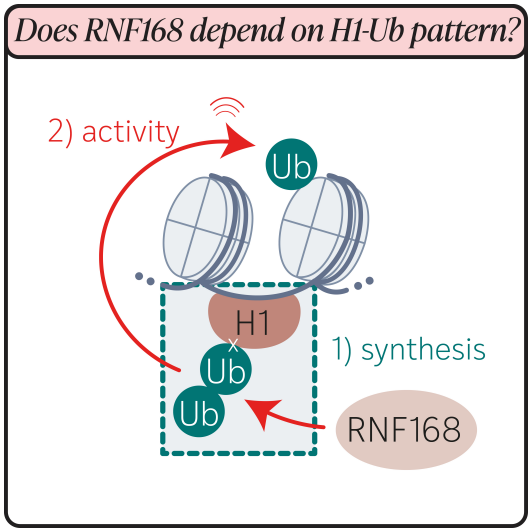
A chemical biology approach to decipher chromatin ubiquitylation by RNF168

P. Franz[1], C. Delvaux de Fenffe[2], B. Fierz[1]*

[1]EPFL, [2]Utrecht University

Recognition, integration and propagation of post-translational modifications (PTMs) on histone proteins play a crucial role in the DNA damage response. In particular, the ubiquitylation cascade mediated by the E3 ubiquitin ligase RNF168 is central in promoting homologous recombination (HR) and nonhomologous end-joining (NHEJ) following DNA double-strand breaks. RNF168 is recruited to DNA damage sites by binding to ubiquitylated linker histone H1 [1], where it further ubiquitylates H2AK13-15 [2], thereby acting as reader and writer of chromatin PTMs. However, the exact mechanisms underlying RNF168 regulation remain elusive, as the limited availability of specifically poly-ubiquitylated H1 restricts mechanistic research on a molecular level.

Therefore, we develop an approach employing expressed proteins, chemical derivatization, and in vitro reconstitution strategies to poly-ubiquitylate H1, with site- and chain-length specificity. Thus, we tightly control and systematically vary the chromatin fiber modification state. Using such ubiquitylated 'designer chromatin', we found that the ubiquitylation activity of RNF168 correlates with the chromatin ubiquitylation state. Currently, we are working on incorporating synthetic ubiquitylated H1 in cells via bead loading [3] to investigate the dependence of RNF168 activity in a physiological environment. This will reveal the chromatin-state-dependent activity of RNF168 in DNA damage repair pathways on a molecular level.



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Matched Pair Theranostics with ^{99m}Tc and ^{188}Re-DETA-N-Onartuzumabfor the c-Met Receptor.

J. Genz[1], S. Klingler[1], J. P. Holland[1]*

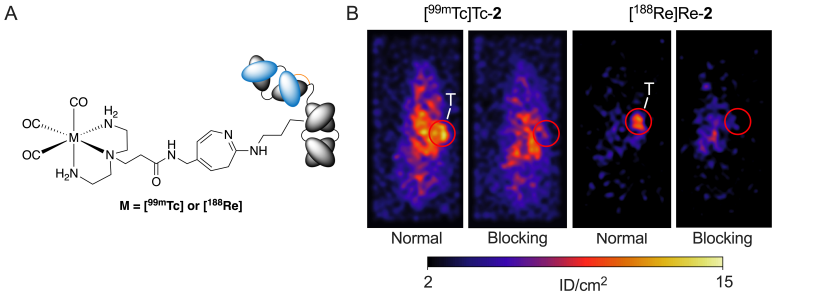
[1]University of Zurich, Wintherthurerstrasse 190, 8057 Zurich

Highly specific targeting vectors such as monoclonal antibodies (mAbs), enable a personalized radiotherapy that limits damage to the surrounding tissues.[1] The linkage between a biomolecule and the radionuclide can be made by employing rapid photoreactions.[2] The use of the matched pair ^{99m}Technetium ($t_{1/2}$ = 6 h, γ-ray = 141 keV [89%]) and ^{188}Rhenium ($t_{1/2}$ = 17 h, β_{max} = 2.12 MeV and γ-ray = 155 keV [15%]) have high potential for cancer therapy.

Herein, we report the synthesis, characterization, protein conjugation, and ^{99m}Tc/^{188}Re-radiolabeling studies on the cancer-specific mAb onartuzumab (MetMAb), with the novel photoactivatable DETA-N ligand (Figure A). Photochemical protein ligation reactions with onartuzumab were performed in water under ambient conditions in 15 min. Planar γ-ray scintigraph imaging (γ-eye, Bioemtech, Greece) was performed on female athymic nude mice bearing subcutaneous MKN-45 xenograft between 0 h and 24 h post-radiotracer injection (Figure B). Biodistribution experiments were performed after 24 h and 72 h. The tumor specificity of ^{99m}Tc/^{188}Re-onartuzumab was assessed *in vivo* by competitive inhibition (blocking) studies.

The photoradiosynthesis of [^{99m}Tc/^{188}Re][Tc/Re(CO)$_3$(DETA-N)]-onartuzumab was accomplished by irradiating the reaction mixture with 395 nm light for 15 min. Purification by size-exclusion methods yielded the radiolabeled antibody in a RCP over 99% (^{99m}Tc and ^{188}Re) and an overall RCY of 17% (^{99m}Tc) and 18% (^{188}Re). Tumor uptake reached 20.20±4.05%ID g^{-1} for ^{99m}Tc-onartuzumab and 22.13±3.11%ID g^{-1} for ^{188}Re-onartuzumab after 24 h and 20.21±1.47 %ID g^{-1} for ^{188}Re-onartuzumab after 72 h in the normal groups. Blocking experiments confirmed tumor specificity with a reduction in tumor uptake of ~70% at the time points for ^{99m}Tc and ^{188}Re.

[^{99m}Tc/^{188}Re][Tc/Re(CO)$_3$(DETA-N)]-onartuzumab is a promising candidate for further use in theranostic studies of tumors presenting high expression of the c-MET receptor.



A: Structure of [$^{99m/188}$Re][Tc/Re(CO)$_3$(DETA-N)]-onartuzumab; B: Szintigraphs of the mice (left: ^{99m}Tc, right:^{188}Re).

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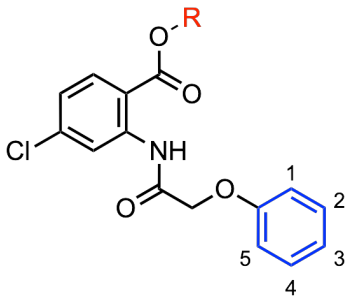
Development of novel 4-chloro-2-(2-phenoxyacetamido)benzoic acid based TRPM4 inhibitors

C. E. Gerber[1], P. Grossenbacher[1], S. A. Singer[1], M. Lochner[1]*

[1]Institute of Biochemistry and Molecular Medicine, Faculty of Medicine, University of Bern, Switzerland

TRPM4 is a non-selective monovalent cation channel, activated by an intracellular increase in Ca^{2+} concentration. The channel depolarizes cells by conducting $Na^+ > K^+ >> Cs^+ > Li^+$ from the extracellular space into the cytosol. [1] Mutations of TRPM4 have been associated with cardiovascular and neuronal diseases [1] and only a few small molecules are known for their ability to inhibit TRPM4 [2, 3]. The goal of this project is to conduct an SAR-study on the core scaffold structure (Figure) of the three most potent inhibitors reported in literature (CBA, NBA and LBA) [2] with the intention to further improve the inhibitory potency and physicochemical properties for its use as a chemical probe. Such compounds would be valuable tools in biomedical research, e.g. as blockers in electrophysiology and *in vivo* animal model studies, or as ligands in cryo-EM studies with TRPM4.



Useful SAR-trends were gained by the synthesis of a compound library and subsequent evaluation of their TRPM4 inhibitory activity. A HEK293 cell-based *in vitro* Na^+-influx assay developed in-house was adapted for this purpose [2]. Several new analogues with sub-micromolar potencies have been discovered, with the best compound showing a 3-fold increase of inhibitory potency compared to NBA and a 7.5-fold increase of inhibitory potency compared to CBA.

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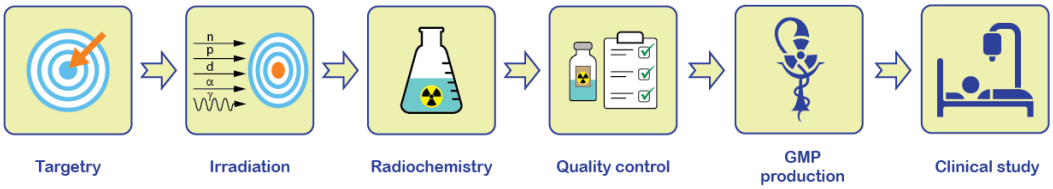
Paving the way to provide the β⁻-emitting ^{161}Tb radionuclide for clinical studies: challenges and lessons learned

P. V. Grundler[1], C. Favaretto[1,4], Z. Talip[1], U. Köster[2], J. Zeevaart[3], S. Geistlich[1,5], R. Schibli[1,5]*, N. P. van der Meulen[1]*

[1]Paul Scherrer Institute, Villigen-PSI, Switzerland, [2]Institut Laue-Langevin, Grenoble, France, [3]South African Nuclear Energy Corporation (Necsa), Brits, South Africa, [4]Nuclear Medicine Department, University Hospital Basel, Switzerland, [5]Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland

Targeted radionuclide therapy can be a potent means to treat cancer, as exemplified, among others, by therapeutic agents based on the β⁻-emitting radionuclide ^{177}Lu. It has been determined that ^{161}Tb has a decisive advantage over ^{177}Lu because it co-emits significant amounts of conversion and Auger electrons. The least energetic of these electrons, characterized by their short penetration path (≤ 1 µm) but high linear energy transfer, are expected to be selectively lethal for small tumors and even single cells as encountered in the metastatic progression of the disease.

To conduct clinical studies with ^{161}Tb, it needs to be reliably available on a regular basis and in a quality that meets the stringent requirements of the pharmacopeia and the medicinal control authorities. To reach this goal several steps, as shown in the following scheme, need to be taken.



It begins with sourcing the target material with the adequate chemical and isotopic composition for the irradiation, followed by partnering with irradiation facilities with high neutron fluxes (up to ≈$1\cdot10^{15}$ *n* $cm^{-2}s^{-1}$) to generate the radionuclide via the desired ^{160}Gd(n,γ)^{161}Gd→^{161}Tb nuclear reaction. A robust separation procedure, adapted to the remote handling of highly radioactive material, needed to be devised. In this case, this encompasses not only engineering challenges but also the notoriously difficult task of quantitatively separating two neighboring lanthanides (Gd and Tb), knowing that the desired ^{161}Tb is diluted in a ≈10'000-fold excess of the starting material [1]. Successful chromatographic separation is critical to achieving high radiochemical purity (≥ 99.0%, separation yield ≈80%) of the final product considering that the vector molecule to be radiolabeled is present in the micromolar range with only a minimal excess over the purified radionuclide. In addition, high radionuclidic purity (^{161}Tb ≥ 99.9%) is essential to minimize unnecessary radiation dose to the patient by other Tb radioisotopes or impurities [2]. Finally, the formulation of the final product must take place under Good Manufacturing Practice (GMP) conditions and pass the prescribed tests before release for use in humans.

With concerted efforts of a multidisciplinary team, the challenges have been mastered and all requirements met **for ^{161}Tb to become the first radionuclide fully developed in Switzerland to enter the clinical trial stage** as the SSTR antagonist ^{161}Tb-DOTA-LM3.

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Discovery & synthesis of mucosal glycans for attenuating virulence in pathogens

R. Hevey[1]

[1]Dept. Pharmaceutical Sciences, University of Basel

Glycans (i.e., carbohydrates) are an important family of natural products which coat all cell surfaces and play essential roles in cell signalling and function. Many diseases are characterized by changes in glycan structures, suggesting their potential utility as a therapeutic target.

The mucosal barrier is well-established to play an important role in microbiota development and as a first line of host defence. Although this has traditionally been attributed to the physicochemical properties of mucus, recent reports indicate that mucin glycoproteins (the main protein component of mucus) and their associated glycans can regulate gene expression and are capable of attenuating virulence in diverse, cross-kingdom pathogens, including Gram-positive bacteria, Gram-negative bacteria, and fungi.

With mucins displaying several hundred distinct glycans, we sought to identify the discrete glycan structures responsible for this novel gene regulation. Individual mucin O-glycans are not commercially available, are not amenable to automated synthesis, and given their overlapping physical and chemical properties cannot be isolated as pure compounds from natural sources using current technologies.

Therefore, through a multi-centre collaborative effort (full list of contributors in [1-3]) we have been actively: (i) characterizing complex mucin O-glycan pools to identify structures most likely to display biological activity; (ii) developing a synthetic approach to obtain individual mucin O-glycans in sufficient quantity for functional analysis [2]; and (iii) assessing the virulence attenuating capabilities of individual glycans in diverse pathogens [1,3]. Within this framework, we have successfully identified specific structures that suppress virulence phenotypes in the fungal pathogen *Candida albicans* (e.g., filamentation, biofilm formation), and regulate pathogenicity in *Vibrio cholerae* (e.g., reduced cholera toxin production), with potency comparable to native mucin glycan pools.

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Deciphering the intricate structure and dynamic behavior of the minimal HDV-like ribozyme Drz-Mtgn-1

S. Jana[1], S. Johannsen[1], R. Sigel[1]*

[1]University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zurich

Hepatitis delta virus (HDV)-like ribozymes are a class of small self-cleaving RNAs spread across different life forms and play crucial roles in various biological processes [1]. The self-cleavage reaction relies on an acid-base mechanism in which a conserved cytosine in the catalytic core activates the 2'-hydroxyl group adjacent to the phosphodiester bond for nucleophilic attack. Although ribozymes in this family vary widely in length, nucleotide composition, and cleavage rates, they all share the same nested double pseudoknot structure that resembles the prototypical HDV ribozyme. While the HDV ribozyme has been extensively studied regarding its structure, catalytic mechanisms, and biological significance, the same level of understanding is still lacking for most group members. However, this knowledge is essential to identify the structural elements and specific nucleotides that tune the dynamics and, thus, the catalytic reaction of this ribozyme family.

Here, we study the ribozyme Drz-Mtgn-1, which is derived from the human gut microbiome and exhibits a unique bell-shaped self-cleavage activity in the presence of divalent metal ions. This ribozyme belongs to the minimal HDV-like ribozymes lacking a non-essential catalytic activity domain [3]. Our goal is to determine the three-dimensional structure of Drz-Mtgn-1 and decipher its complex interplay with metal ions using NMR spectroscopy. Initial NMR studies reveal that Drz-Mtgn-1 ribozyme folds into the nested double pseudoknot structure, even without divalent metal ions. In the next step, we aim to localize specific metal ion binding sites using chemical shift change experiments, paramagnetic line broadening studies, and direct detection of NOE contacts between RNA and metal probes.

This study will shed light on the structural basis, and the role of metal ions in the dynamics of the self-cleavage reaction and help connect the gap regarding ribozyme's structure and function.

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Antibiotics conjugated small tag for bacterial labeling and monitoring of drug uptake/efflux

K. Jantarug[1], V. Tripathi[2], B. Morin[3], A. Iizuka[3], D. Bumann[2], N. Khanna[3], P. R. Fuentes[1]*

[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland, [2]Biozentrum, University of Basel, Spitalstrasse 41, 4056 Basel, Switzerland, [3] Department of Biomedicine, University of Basel, Hebelstrasse 20, 4031 Basel, Switzerland

The NCCR AntiResist is developing novel *in vitro* conditions mimicking patient samples for drug screening and development. However, to create efficient patient-like conditions, the physiological properties of single-cell bacteria need to be studied. To detect bacteria in patient samples and study the drug uptake and efflux in bacteria, we functionalized antibiotics of interest to have a small tag, for example, a relatively small fluorophore or a Raman tag. These small tags would allow us to visualize bacterial infections in patient samples and monitor the uptake and efflux of antibiotics of interest with decreased interference in cell permeability, chemical and biological properties. As a first example, we modified the antibiotic vancomycin with a silicon-rhodamine-based fluorophore, Janelia Fluor 669 (JF669), to label *Staphylococcus aureus (S. aureus)* in patient samples. Using this probe (VanJF699), we could detect *S. aureus* in human cells with low background, minimal non-specific binding, and using a suitable wavelength for human tissue imaging. Additionally, VanJF669 could be used for super-resolution bacterial imaging. In a second example, we explored even smaller tags to minimize the interference of the tag with antibiotic trafficking. Raman tags were utilized instead of small fluorophores. Alkyne and azide moieties could exhibit Raman signals around 2100 cm^{-1}, which are in a silent region of human cells. We modified trimethoprim to have an alkyne or azide that would allow us to monitor the efflux and uptake by confocal Raman microscopy. Regarding these small tag-conjugating antibiotics, *E. coli* treated with trimethoprim-alkyne can be imaged by Stimulated Raman scattering (SRS) microscopy showing Raman signal around 2143 cm^{-1}. In the future, we will use our probes to understand the physiological properties of bacteria at the single-cell level and accelerate the development of patient-like conditions.

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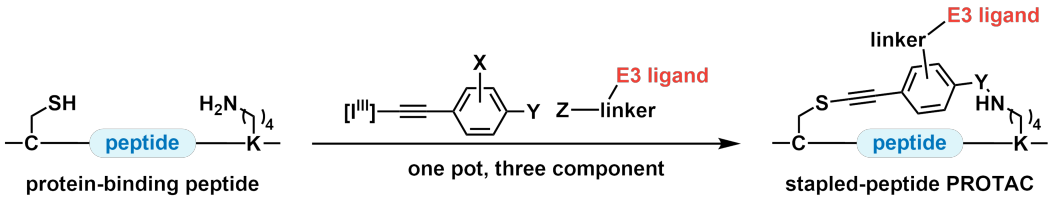
Stapled-Peptide PROTACs by Hypervalent Iodine Staples

Y. Kamei[1,2], E. Delavictoire[1], B. Fierz[2]*, J. Waser[1]*

[1]Laboratory of Catalysis and Organic Synthesis, Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, [2]Laboratory of Biophysical Chemistry of Macromolecules, Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne

Protein–protein interactions (PPIs) are deemed undruggable due to the lack of well-defined binding pockets on corresponding proteins. Peptides can target undruggable proteins by mimicking PPIs. The development of peptide-based inhibitors of PPIs is one of the major topics in current medicinal chemistry. PROTAC (proteolysis targeting chimera) technology is an emerging modality to degrade pathological proteins.[1] Most of the PROTACs are synthesized by linking two small-molecule ligands: a POI (protein of interest) ligand and an E3 ligand. However, the development of small-molecule ligands for undruggable proteins is in itself challenging. Thus, the availability of POI ligands is hampering the development of PROTACs for undruggable proteins.

We developed trifunctional hypervalent iodine staples based on our previous report.[2] This reagent is first assembled with E3 ligand-linker conjugate and then readily transforms protein-binding peptides into stapled-peptide PROTACs in a one-pot three-component manner. Our platform would rapidly provide diverse analogues and pave the way for drugging currently untouched proteins, even if small-molecule ligands are unavailable. We are working on the degradation of transcriptional coactivators, applying this methodology.



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Synthetic Molecular Motor Activates Drug Delivery from Polymersomes

M. Korpidou[1], A. Guinart[2], D. Doellerer[2], G. Pacella[3], M. C. Stuart[2], I. A. Dinu[1,4], G. Portale[3], C. Palivan[1,4]*, B. L. Feringa[2,3]*

[1]Department of Chemistry, University of Basel, Mattenstrasse 24A, BPR 1096, 4058, Basel, Switzerland., [2]Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands., [3]Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands., [4]NCCR-Molecular Systems Engineering, Mattenstrasse 24a, BPR 1095, Basel, Switzerland

The development of stimuli-responsive, smart delivery systems in nanomedicine arises from the limitations, side effects and unsolved needs of current drug delivery. [1] In our study, we developed a highly spatiotemporal controlled delivery system with tunable release profile. [2] A hydrophobic synthetic molecular rotary motor is embedded in the hydrophobic domain of a PDMS-*b*-PMOXA diblock copolymer, resulting in a responsive self-assembled system. The release of a fluorescent dye with high efficiencies is triggered by the successful incorporation of the motor and its selective activation by low energy visible light (λ = 430 nm, 6.9 mW). Moreover, the system presents a responsive behavior due to its ability to turn on and off on demand over sequential cycles and even low concentrations of the photo-responsive unit are shown to effectively promote release. Our system was further investigated under relevant physiological conditions, encapsulating the FDA approved drug, Pemetrexed and tested on a lung cancer cell line. When compared to free given drug, similar levels of cell viability are observed, highlighting the potential of our platform to deliver functional drugs on request with high efficiency. This work is an important step in the field of next generation smart delivery systems exploiting the application of synthetic molecular machines.

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Phospholipid Bicelles as Topical Delivery Systems for Porphyrinic Photosensitizers

D. C. Krummenacher[1], I. Gjuroski[1], S. Kässmeyer[2], P. Vermathen[3], J. Furrer[1], M. Vermathen[1]*

[1]University of Bern, Department of Chemistry, Biochemistry and Pharmaceutical Sciences, Bern, Switzerland, [2]University of Bern, Department of Clinical Research and Veterinary Public Health, Bern, Switzerland, [3]University of Bern, MRM, Department of BioMedical Research and Neuroradiology, Bern , Switzerland

Topical photodynamic therapy (PDT) serves as a minimally invasive and safe treatment approach for various superficial skin diseases such as actinic keratosis [1]. PDT treatment of skin employs the delivery of a precursor (5-ALA) that is converted into the active form, the photosensitizer (PS) protoporphyrin IX, followed by targeted irradiation. This causes the PS to undergo a photochemical reaction, producing reactive oxygen species, which induce oxidative damage to surrounding cells [2]. Directly applied porphyrinic PSs are interesting candidates for topical PDT due to their absorbance range in the therapeutic window [3]. However, these lipophilic compounds require solubilization to remain active for PDT, thus a carrier is necessary [4]. Bicelles are biocompatible disc-shaped phospholipid nanocarriers. Their lipid bilayer offers an environment for lipophilic drug molecules [5]. Smaller than liposomes, bicelles might facilitate penetration into the skin surface. The aim of this current project is to investigate bicelles as a topical drug delivery system for porphyrinic PSs.

Bicelles with differently combined phospholipids are loaded with PSs (Chlorin e4, m-THPP, or m-THPC). During the bicelle production the PSs are encapsulated. The resulting system is characterized using NMR spectroscopy and dynamic light scattering (DLS). With NMR spectroscopy the bicelle formation, drug encapsulation efficiency and the size of the system can be determined. DLS is used to measure the size and stability of the empty bicelles. With the successful formulations in vitro skin penetration tests are performed to investigate the penetration of PS into the skin. Furthermore, cell uptake studies are conducted to quantify PS uptake in keratinocytes.

Results so far show that with the right phospholipid combination PS can efficiently be encapsulated into small bicelle systems and the PS remains solubilized in the formulation. Depending on the delivery system and the PS an average skin penetration ranged from 100 ng/cm^2 – 860 ng/cm^2 has been achieved employing porcine ear skin as model, comparable to other vesicular delivery systems [6].

To increase the penetration efficiency the addition of various chemical penetration enhancers to the bicelle system is planned. Due to the biological variations of the skin samples, the results of the penetration experiments show a high variability. In coming efforts, we aim to address this concern with experiments using an artificial human skin model. Furthermore, microscopic localization of the PS in the skin or skin model are planned

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A HaloTag-based Gene Reporter System for Live-Cell Imaging and High-Throughput Screening

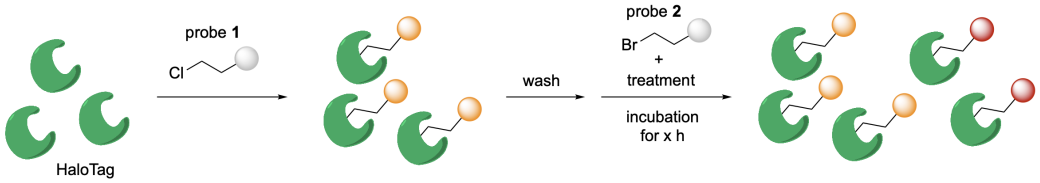
H. Lämmermann[1], J. Nguyen[2], G. Turcatti[2], M. Chambon[2], F. Kuttler[2], J. Bortoli Chapalay[2], P. Rivera-Fuentes[1,2]*

[1]University of Zurich, [2]EPFL

Gene expression monitoring is a powerful tool to elucidate stress response mechanisms and dynamics. We developed a gene reporter system for BiP (binding immunoglobulin protein), a key mediator of the endoplasmic reticulum (ER) stress response.[1]

Upon accumulation of unfolded proteins in the ER, the unfolded protein response (UPR) is activated, which consists of three pathways that are initiated by IRE1, PERK, and ATF6.[2] The target BiP is a chaperone that assists protein folding. It is involved in the activation of the UPR as well as induced via the ATF6 pathway.[3]

Our system is based on the co-expression of the target BiP and the self-labeling HaloTag protein[4] in a stable cell line. The amount of HaloTag that is produced thus corresponds to the amount of BiP. By labeling HaloTag with two fluorogenic compounds with different spectral properties—a rhodamine and a silicon rhodamine—two gene expression levels can be time-stamped and visualized in a single pulse-chase experiment, providing additional information and increasing the robustness of the system. The method is suitable for live-cell fluorescence imaging as well as flow cytometry. Moreover, we are able to employ the system in high throughput screening experiments, searching for novel inducers of BiP expression.



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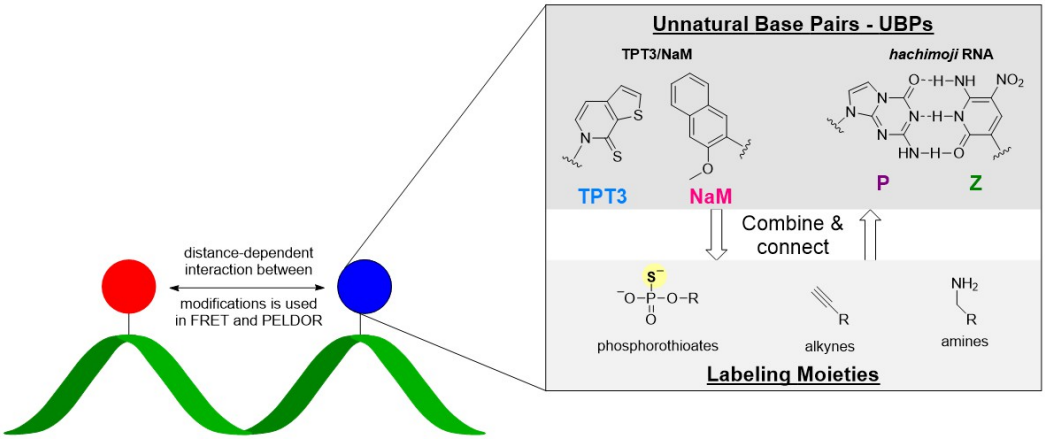
Advancing RNA Research: A Novel Approach for High-Yield Synthesis and Labeling of Long RNA Strands

N. S. Lindlar[1], S. Zelger-Paulus[1], R. K. Sigel[1]*

[1]University of Zurich, Department of Chemistry

The increasing recognition of RNA in scientific research has prompted the need for advanced methodologies.[1] However, studying RNA proves challenging, as traditional protein research techniques offer limited insights.[2] To understand the functional dynamics of RNA, molecular rulers such as PELDOR (Pulsed Electron Electron Double Resonance) or FRET (Förster Resonance Energy Transfer) are employed on µs-s time scales.[3] Yet, these methods require the presence of multiple modifications at specific sites on the RNA molecule, which is a significant hurdle.



In this work, we propose a solution by combining two classes of unnatural base pairs with modifiable moieties. Leveraging enzymatic synthesis for RNA production and well-established chemical reactions for labeling, we anticipate achieving high yields of long RNAs (>100 nt). The synthetic bases NaM/TPT3[3] and *hachimoji* RNA[4], previously employed in RNA synthesis, serve as our unnatural base pair candidates. In addition, we incorporate alkynes[3] and phosphorothioates[5] as modifiable moieties, well-known in bio-orthogonal chemistry. Our proposed method represents a promising approach to overcome the limitations of current techniques and enable efficient synthesis and labeling of long RNA strands.

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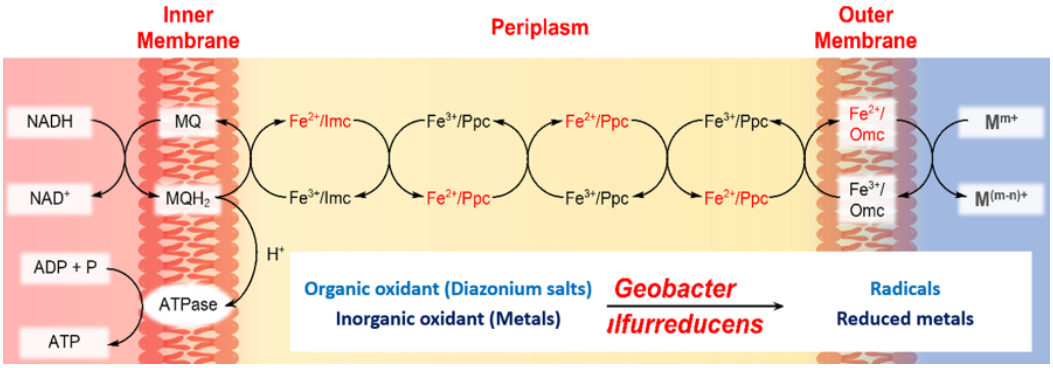
Electron transfer processes in *Geobacter sulfurreducens*

S. Majouri[1], M. Karamash[1], K. M. Fromm[1]*, B. Giese[1]*

[1]University of Fribourg, Department of Chemistry, Chemin du musée 9, 1700 Fribourg

Geobacter sulfurreducens reduces many metal ions by transporting electrons from the inside of the bacterial cell to the outside using different type of cytochromes that act as electron shuttles [1]. The kinetic study of the reduction of silver and other metal ions has revealed some first insights into the chemistry of bacterial homeostasis and extracellular electron transfer (EET) processes, however the mechanism of transfer of electrons from one cytochrome to another remains to be investigated [2].

More investigations are needed to understand the EET mechanism. In our study, the reduction of some inorganic and organic oxidants such as aryl diazonium salts are investigated. We are particularly interested in the interactions between the generated radicals from the reduction of the diazonium salts and the different cytochromes. Results obtained from protein labelling and modification using generated radicals from redox reaction between the cytochromes of *Geobacter sulfurreducens* and diazonium salts will be discussed.



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Scaling-up enzyme immobilization: efficiency and productivity of two model systems

V. Marchini[1], D. Roura Padrosa[1]*

[1]InSEIT AG, Gesellschaftsstrasse 42, 3012-CH, Bern

Enzymatic reactions have received increased attention over the years, owing to the incontrovertible necessity for sustainable processes [1]. Indeed, biocatalysts fulfil the demand for greener reactions thanks to their valuable properties, while providing simpler synthetic routes with higher selectivity than the traditional hazardous methods. In addition, enzyme immobilization onto solid supports is an essential tool to allow their reusability and to preserve stability over several operational cycles [2]. Nevertheless, immobilization of biocatalysts is usually considered a small-scale process at the research level, while larger scale is required in order to achieve considerable amounts of the desired product. Moreover, this technique is still very unpredictable, meaning that significant experimental effort is involved during the screening process, since it relies on a trial-and-error approach. To overcome this issue, bioinformatic tools, like the package CapiPy, allows scientists to rationalize the experimental design for more time and costs effective studies [3,4]. In this work, it has been demonstrated that scaling up is possible and efficient, without any major loss in terms of immobilization and productivity of the enzymatic reactions performed in batch and in continuous flow systems. For this proof of concept, two different enzymes were chosen together with two well-established reactions, previously reported in scientific publications. Consequently, two high-value compounds were obtained, starting from relatively cheap molecules. As further outcome, costs and profits were calculated to prove the efficiency of the biocatalytic scalability not only from the productivity perspective but also from a financial one.

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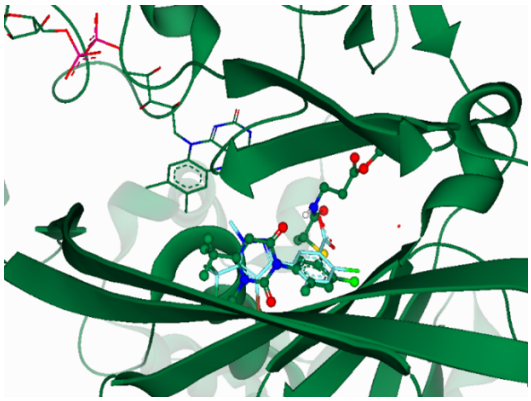
Design, synthesis and screening of herbicidal activity of new protoporphyrinogen oxidase-inhibitors (PPO) overcoming resistance issues.

R. L. Mattison[1], J. Frackenpohl[1], B. Bollenbach-Wahl[1], J. Freigang[1], I. Heinemann[1], H. Helmke[1], E. Gatzweiler[1], G. Lange[1], H. Jakobi[1]

[1]CropScience Division, Weed Control, Bayer AG, Industriepark Höchst, D-65926 Frankfurt am Main

Whilst there are several methods to control weeds, which continuously plague farmers around the globe, theapplication of small molecular compounds is still the most effective technology to date. Plants can evolve to become resistantto PPO-inhibitors, a class of herbicides in commercial use since the 1960s. It is therefore essential to continuously develop newherbicides based on this mode-of-action with enhanced intrinsic activity, an improved resistance profile and favourable phys-icochemical properties. Based on an Amaranthus PPO crystal structure and subsequent modelling studies, halogen-substitutedpyrazoles have been investigated as isosteres of uracil-based PPO-inhibitors. By combining structural features from the commercial PPO-inhibitors tiafenacil and pyraflufen-ethyl and by investigat-ing receptor-binding properties, we identified new promising pyrazole-based lead structures showing strong activityin vitroandin vivoagainst economically important weeds of the *Amaranthus* genus: *A. retroflexus*, and resistant *A. palmeri* and *A. tuberculatus.* The present work covers a series of novel PPO-inhibiting compounds that contain a pyrazole ring and asubstituted thioacetic acid sidechain attached to the core phenyl group. These compounds show good receptorfit in line withexcellent herbicidal activity against weeds that plague corn and rice crops with low application rates. This, in combination withpromising selectivity in corn, have the potential to mitigate and affect weeds that have become resistant to some of the currentmarket standards. Remarkably, some of the novel PPO-inhibitors outlined herein show efficacies against economically impor-tant weeds that were superior to recently commercialized and structurally related tiafenacil.



Superposition of the wild-type *Amaranthus* structure with tiafenacil (dark green) and the targeted molecule **15a.**

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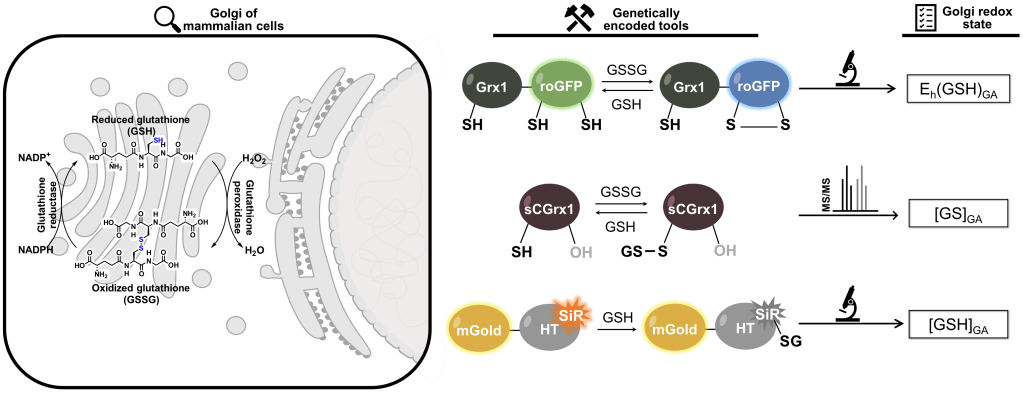
Characterization of the Glutathione Redox State of the Golgi Apparatus

C. Miró-Vinyals[1], S. Emmert[1,2], G. Grammbitter[2], P. Rivera-Fuentes[1]*

[1]Chemistry Department, University of Zürich, CH-8057, Zürich, Switzerland, [2]Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédéral de Lausanne, CH-1015, Lausanne, Switzerland.

Redox homeostasis is essential for cell functioning and its study in a compartmentalized manner is key due to the differentiated redox pairs ratios in each organelle.[1] The redox state of an organelle can be determined by quantifying the redox potential (E_h) and the concentration of a given redox pair. In our case, we chose to study glutathione (GSH) and its oxidized counterpart (GSSG) because of their high cellular concentration (mM).[1] The Golgi apparatus (GA) is a central organelle responsible for protein glycosylation and protein sorting and its dysfunction is linked to cancer and neurodegenerative diseases. It is considered one of the most oxidizing organelles in the cell, yet there are no reports of redox potential nor values for absolute GSH+GSSH concentrations in the Golgi.[2]

In this work, we determined the redox state in the Golgi by using different and independent genetically encoded tools. A redox-sensitive green-fluorescent protein (roGFP1-iE)[3] allowed us to calculate the $E_h(GSH)_{GA}$. Together with a single-cysteine glutaredoxin (sCGrx1p)[4] we calculated the total GSH+GSSG concentration in the organelle ($[GS]_{GA}$). Finally, using a GSH sensor (TRaQ-G),[5] consisting of a fusion protein of mGold and HaloTag (HT) conjugated to a GSH-reactive silicon rhodamine (SiR), we were able to calculate the absolute GSH concentration ($[GSH]_{GA}$). These results allowed us to present for the first time a quantitative redox profile in the Golgi apparatus and allow for further enlightenment on how the redox state is maintained in the specified organelle.



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How Ring-Size, Stereochemistry and Substituents Modulate the Activity of a Nanomolar JAK1 Inhibitor

K. Mulliri[1], K. Meier[1], J. Reymond[1]*

[1]University of Bern

We investigate the generated database (GDBs)[1], to access novel, challenging and unknown molecules with high potential in medicinal chemistry. These in sillico libraries contain billions of molecules created following several designs rules producing a huge chemical space[2]. We have selected and synthesized a tricyclic diamine dubbed triquinazine, which is used as the core of one of the most potent and selective Janus Kinase Inhibitors to date (IC_{50} = 1.0 nM for JAK1) **KMC 420**[3] (Fig 1). Inspired by these results we are synthesizing a series of compounds by diversifying the diamine core of our lead compound **KMC 420**. Our synthetic approach involves a seven-step linear sequence, that allows for the control of the ring size and diastereochemistry. Through the deconstruction of triquinazine, we obtained a diverse set of analogues, focusing on those closely resembling the core structure. Stereochemistry was crucial for bioactivity where a one pot two step Mitsunobu, Staudinger protocol was implemented to obtain the compounds with the desired stereochemistry. Further functionalization of the amines with the deazapurine and four other selected functionalities, furnished more then 20 final compound which were tested for biological activities. **KM-174** showed to be selective on JAK1 with IC_{50} = 25 nM, improving the understanding JAK1 pharmacology.

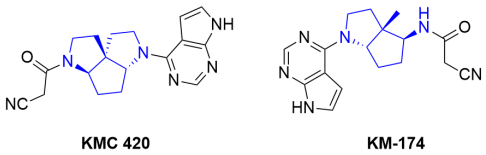

Fig 1. **KMC 420**, triquinazine inhibitor, IC_{50} = 1 nM and **KM-174**, IC_{50} = 25 nM on JAK1**.**

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Characterization of Amyloid β aggregation via Supercritical Angle Fluorescence and Raman microscopy and spectroscopy

N. S. Münch[1], S. Seeger[1]*

[1]University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zürich

The two main forms of the Amyloid β (Aβ) peptide, Aβ1-40 and Aβ1-42 play an important role in Alzheimer's disease (AD), one of the main neurodegenerative diseases related to aging. The most important consequence of AD is the degradation of neural cells due to the aggregation of Aβ into oligomers and fibrils with a subsequent accumulation outside the neurons [1]. Both Aβ peptides play a different role in the AD process and in neurotoxicity. Therefore, a comparison of the aggregation of both peptides is crucial.

In this work, we studied the aggregation of Aβ *in vitro*, directly on the lipid bilayer and compare the surface with the bulk solution, using Supercritical Angle Fluorescence (SAF) [2] and Raman (SAR) [3] microscopy and spectroscopy. The advantage of Raman over fluorescence is the label-free method with the resolution of the peptide's secondary structure. The experiments clearly show Aβ1-42 aggregates stronger at the surface where more α-helices are present, whereas Aβ1-40 forms mostly β-sheets. In addition, we studied the effect of calcium ions and confirmed a structural change of the peptides.

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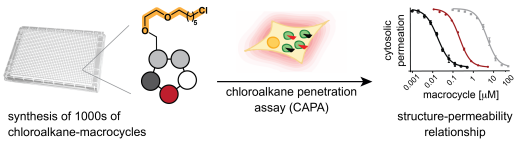
Assessing the cellular permeability of peptidic macrocycles in high-throughput

A. Nielsen[1], C. Bartling[2], K. Strømgaard[2], C. Heinis[1]*

[1]Institute of Chemical Sciences and Engineering, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland., [2]Department of Drug Design and Pharmacology, University of Copenhagen, DK-2100 Copenhagen, Denmark.

Small cyclic peptides provide an attractive modality for drug development due to their ability to bind challenging targets and their potential to cross membranes for reaching intracellular proteins. In our laboratory, we have recently developed methods to synthesize and screen large combinatorial libraries of small cyclic peptides.[1-3] For example, "*m*" short linear peptides containing thiol groups at both ends were combinatorially cyclized with "*n*" bis-electrophilic linker reagents to obtain *m* × *n* cyclic peptides that were screened in microwell plates as crude products. While the approaches yielded ligands to several disease targets, not all of them were membrane permeable. A full picture of the membrane permeability of the newly developed format of peptidic macrocycles, and factors that determine their permeability, was lacking.

In this work in progress, we have taken advantage of the chloroalkane penetration assay (CAPA), that has recently emerged as a robust method to determine cytosolic permeability of chloroalkane-tagged biomolecules.[4] We have established a method to synthesize thousands of diverse chloroalkane-tagged peptidic macrocycles to determine their cytosolic permeability using CAPA. This has given us a new insight into the structure-permeability relationships of an unprecedented number of macrocycles and provides a clearer picture of what features govern permeability of macrocyclic compounds in cellular systems.



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Machine Learning Guided Exploration of Antimicrobial Peptide Chemical Space

B. Olcay[1], M. Orsi[1], J. L. Reymond[1]*

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

Antimicrobial peptides play a crucial role in combating antimicrobial resistance. The sequence of KKLLKLLKLLL (ln65), a short membrane disrupting but hemolytic linear undecapeptide discovered, and its over 30 diastereomers have been investigated by our group to date and many promising sequences in terms of antibacterial activity have been discovered. However, there are 2048 possible diastereomers that could be investigated. Based on this, our goal is to discover the chemical space of possible 2048 diastereomers using machine learning and our high-capacity parallel synthesizer.

Our proposed method integrates computational design with a semi-automated synthesizer. We compare three design approaches: random sequence selection, genetic evolution through single point mutations of active sequences, and machine learning-guided design using GPT-3 fine-tuned on experimental data. The iterative process involves synthesizing novel peptide sequences by our parallel synthesizer, which operates at a high temperature, resulting in significantly shorter reaction times and is capable of synthesizing 48 sequences simultaneously. Our testing protocol consists of testing the antibacterial activity on 5 bacterial strains and testing the hemolytic activity on human red blood cells (hRBCs). And finally, the activity and hemolysis data obtained are used to refine computational designs for subsequent iterations.

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Determination of Biomarkers in Liver Disease by In vivo and Ex vivo NMR

C. onyia[1], P. Vermathen[1], M. Vermathen[2], P. Vermathen[1]*

[1]University of Bern, MRM, Department of Biomedical Research and Neuroradiology, Bern, Switzerland, [2]University of Bern, Department of Chemistry, Biochemistry and Pharmaceutical Sciences

INTRODUCTION Chronic liver diseases, in particular non alcoholic fatty liver disease and steatohepatitis(NAFLD/NASH), is becoming prevalent in association to obesity. NAFLD is characterized by excessive fat accumulation in the liver, while NASH refers to a hepatic disease where fat accumulation is complicated by hepatic inflammation, liver cell injury and liver fibrosis. The pathogenic mechanism and early diagnoses of NAFLD is poorly established in humans and this currently pose a major public health concern. The gold standard to reach the diagnosis of chronic liver disease relies on liver biopsy. The overall aim of this project is to identify biomarkers by in vivo and ex vivo NMR that can accurately determine patients at risk of severe liver disease and to assess the potential impact of treatment strategies. Metabolomics is established as an investigational tool that provides rich information on metabolic disturbances in human disease and in this sub-study, we make use of High-Resolution Magic Angle Spinning (HR-MAS) NMR[1] spectroscopy which is a powerful analytical tool for investigating the metabolic state of an intact tissue.

AIM We aim at determination of metabolic and lipid biomarkers from liver biopsies of NAFLD and NASH patients.

METHOD As an initial step we are currently utilizing control liver tissues obtained from pig. This was prepared for HR-MAS spectroscopy to generate a measurement protocol aimed at facilitating a comprehensive determination of lipid and metabolic profiles and physical properties, and in the future will be used to study liver biopsies from patients with liver diseases. Different NMR methods include 1D NMR with T2 filter for small molecules, diffusion weighted spectra for lipids, and more advanced methods for determination of physical properties including T1- and T2- determination, diffusion-constant determination (with different diffusion times Δ), and spinning speed variation. The metabolomical analyses, spectral analyses and metabolite identification of the acquired NMR data, will be correlated to biochemical and physiological results and will allow a comparison of metabolite and lipid compositions between ex vivo and in vivo methods.

RESULTS The measurement protocol was established and we will use it for the samples from patient biopsies. 1D and 2D HR-MAS NMR spectra were acquired from pig liver, indicating numerous signals corresponding to lipids and metabolites. With the help of databases and own reference spectra, numerous resonances could be assigned to specific metabolites. By applying different spectral filters, we successfully separated lipids from small molecules metabolites. Additionally, we determined the diffusion coefficients of lipids, providing insight on lipids mobility in the tissue microenvironment. To understand the organization and structural properties of the lipid deposits, we aim at calculation of lipid droplet sizes by using diffusion NMR applying different diffusion times.

DISCUSSIONThe separation of the lipid signals from the metabolite signals allows a detailed analysis of their individual contributions in liver metabolism. The result from the ex vivo and in vivo data will be correlated with findings from liver pathology results to accurately identify a non-invasive biomarker which can be used as drug target in chronic liver diseases.

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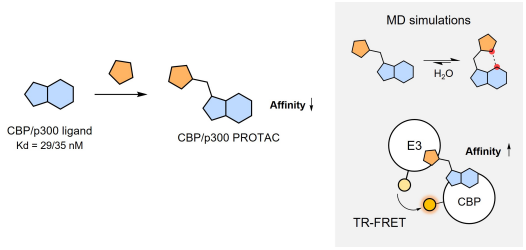
CBP/p300 Degraders: When Cooperativity Overcomes Affinity

L. Palaferri[1], K. Gosselé[1,2], I. Cheng-Sánchez [1], E. Laul[1], A. Müller[2], Y. Li[2], A. Caflisch[2]*, C. Nevado[1]*

[1]University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zurich, Switzerland, [2]University of Zurich, Department of Biochemistry, Winterthurerstrasse 190, 8057 Zurich, Switzerland

PROteolysis TArgeting Chimeras (PROTACs) are bifunctional molecules that simultaneously bind an E3 ligase and a protein of interest, forming a ternary complex that enable degradation of the target protein via the ubiquitin-proteasome system.[1] Compared to small molecules - which typically inhibit a single protein site - PROTAC-mediated degradation enables loss of function of the whole target protein. This effect is particularly useful for multidomain proteins, like the homologous coactivators CBP and p300, crucial for enhancer mediated transcription and implicated in a wide range of human diseases.[2]

Starting from binders of the CBP/p300 bromodomain identified by our group during a previous screening,[3] we developed a series of PROTACs, which despite being efficient degraders show decreased affinity in biochemical assays. To further investigate this unexpected behaviour, we assessed the ability of our compounds to form a ternary complex by TR-FRET and performed molecular dynamics simulations to understand whether their intramolecular folding could affect degradation. Our results show that the unique conformation assumed by our best CBP/p300 degraders is key to ternary complex formation, underling how a cooperative PROTAC can be an extremely efficient degrader despite having poor biochemical affinity.



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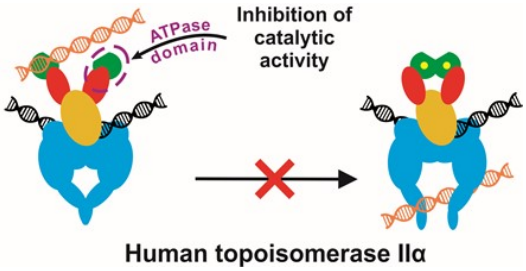
Advancing Type II DNA Topoisomerase Research through QM/MM Simulations and Development of Catalytic Inhibitors

A. Perdih[1,3], M. Janežič[1], M. Ogrizek[1], K. Bergant Loboda[1,3], B. Herlah[1,3], K. Valjavec[1], G. Wolber[2]

[1]National Institute of Chemisty, Hajdrihova 19, 1001 Ljubljana, Slovenia, [2]Freie Universität Berlin, Institute of Pharmacy, Königin-Luise-Straße 2-4, 14195 Berlin, Germany, [3]Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

Human DNA topoisomerase IIα (htIIα) is one of the established anticancer targets due to its role in cell proliferation process. It catalyzes topological changes of the DNA molecule, essential for progression of transcription and translation, and its level is higher in rapidly dividing cells. There are several ways to tackle htIIα, and agents targeting it are divided into two groups. Among the established group of topo II poisons, some molecules are used in clinical practice, such as doxorubicin and etoposide. Due to the frequent occurrence of serious side effects, especially cardiotoxicity and induction of secondary tumors, as well as the observed resistance to topo II poisons, further drug development efforts were initiated, trying to take advantage of other inhibition paradigms available in the topo IIα catalytic cycle. This has led to the development of catalytic inhibitors of htIIα that offer new opportunities to revisit this established target and inhibit it via alternative inhibition mechanisms. Such molecules could, in principle, have an improved safety profile with comparable anticancer efficacy [1].

In our research, we are using available structural information about the htIIα ATPase domain to gain insight into the enzymatic mechanism of ATP hydrolysis and to design novel catalytic htIIα inhibitors that would target the ATP binding site [2]. With multiscale QM/MM calculations and a point mutation study, we investigated the catalytic mechanism of ATP hydrolysis and showed it favors the dissociative substrate-assisted mechanism, with Lys376 acting as a stabilizing residue [3]. Next, we used htIIα as a model target to validate a new virtual screening strategy that incorporates dynamic components of molecular recognition and expanded the known chemical space of flavonoid-based htIIα catalytic inhibitors [4]. Finally, we also discovered a class of substituted 4,5'-bithiazoles acting as ATP competitive htIIα catalytic inhibitors. These molecules arrested the cell in G1 phase, affected cell proliferation, and did not cause DNA double-strand breaks, thus displaying potential for further development to efficient and potentially safer cancer therapies that exploit an alternative topo II inhibition paradigm [5].



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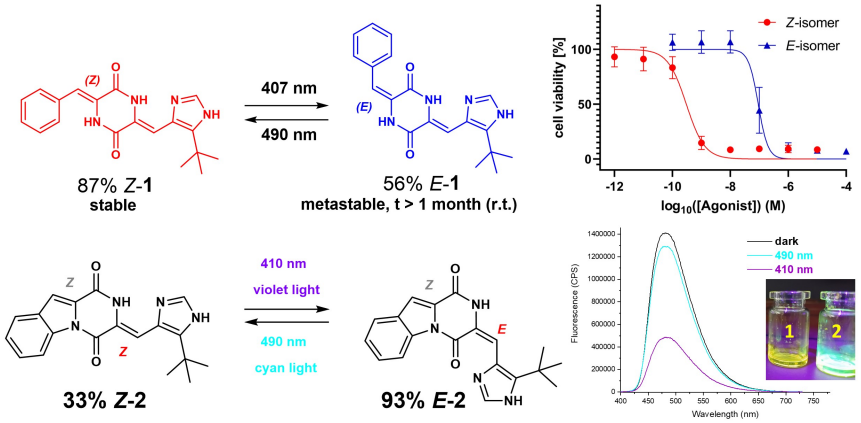
Hemipiperazines: peptide-derived photoswitches with low-nanomolar toxicity

Z. Pianowski[1], S. Kirchner[1], A. Leistner[1], P. Gödtel[1], A. Seliwjorstow[1]

[1]Institute of Biological and Chemical Systems – FMS, Karlsruhe Institute of Technology, Hermann-von-Helmholtz Platz 1, 76344 Eggenstein-Leopoldshafen b. Karlsruhe, Germany

Cyclic dipeptides (CDP) are common structural motifs in biology, potent pharmacophores, and important constituents of numerous supramolecular systems based on hydrogen bonding.[1] Light-triggered photomodulation of their properties, e.g. upon merger with molecular photoswitches,[2,3] opens the way for photopharmacology applications, or producing smart materials.

Upon investigation of light-triggered release of CDP drugs from photochromic supramolecular hydrogels [4], we have discovered that plinabulin (*Z*-**1**) – a low-nM antimitotic agent - reversibly photoisomerizes to its thermally stable photoisomer (*E*-**1**) with significantly lower activity, which can be isolated and used as a photoactivated pro-drug.[5] Moreover, its previously unreported photochromic system – hemipiperazine – constitutes a new class of molecular photoswitches [2,3] with broad application potential, ranging from smart materials to photopharmacology. We have examined basic photophysical properties of the isolated hemipiperazine photochrome.[5,6] Finally, the "locked" plinabulin **2** exhibits enhanced fluorescence, and reversible photomodulation of the fluorescence level upon photoisomerization - which may in turn find applications e.g. in super-resolution microscopy.[5]



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Sequence and Structure Selectivity of Human Lysyl Oxidase-Like 2 (LOXL2)

L. M. Poller[1], M. C. Deen[1], T. Fiala[1], E. Schönbächler[1], R. Heeb[1], H. Wennemers[1]*

[1]Laboratory of Organic Chemistry, ETH Zurich, D-CHAB, Vladimir-Prelog-Weg 3, 8093, Zurich, Switzerland

Collagen is the dominant structural protein in mammals and its maturation is vital for the integrity of organs and wound healing.[1,2] A key intermediate of collagen biosynthesis and maturation is the so-called tropocollagen.[1] The collagen assembly comprises a proline-rich triple-helical domain and two terminal telopeptide domains which are not assembled into higher-order structures.[1] Tropocollagen undergoes cross-linking into fibrils and fibers. The cross-linking process is induced by the lysyl oxidase enzyme family (LOX and four LOX-like enzymes).[1,2] These copper amine oxidases catalyze the conversion of lysine residues (Lys) to aldehyde-containing allysines that spontaneously undergo aldol and related reactions to form cross-links.[2,3] While the LOX-mediated cross-linking is crucial for the mechanical properties of the extracellular matrix, excessive LOX activity is associated with fibrotic and malignant diseases.[2,3] The isoform LOXL2 is of particular interest as a therapeutic target as it is over-expressed in many types of cancers.[3] In this work, we decipher the sequence and structure selectivity of LOXL2. We will present the selectivity of LOXL2 for Lys derivatives, including Lys-containing single-stranded and triple-helical collagen model peptides.

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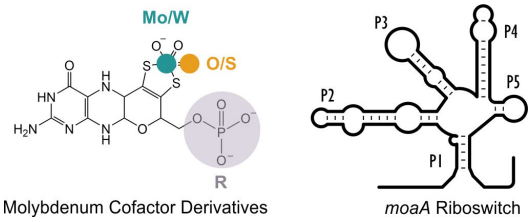
Exploring the Binding of natural Molybdenum Cofactor Derivatives to the *moaA* Riboswitch

M. Reichenbach[1], S. Gallo[1], R. K. Sigel[1]*

[1]Department of Chemistry, University of Zurich

Riboswitches are mRNA sequences in the 5'-untranslated region that change their structure upon binding of a specific metabolite, thereby regulating the expression of downstream genes. The *moaA* riboswitch has been predicted to respond to the molybdenum cofactor (Moco)[1]. The instability and high oxygen sensitivity of Moco prevented to unambiguously demonstrate this interaction to this date. By footprinting experiments under anaerobic conditions, we however showed that the Moco-derivative released from Xanthine Oxidase (S-Moco) induces a structural change of the *moaA* riboswitch.

Riboswitch classes recognize small modifications of the metabolite triggering them, using different recognition mechanisms involving aromatic moieties or charged functional groups. We know that the pterin moiety alone is not enough to be recognized by the *moaA* riboswitch [2]. We therefore want to test, which naturally ocuring Moco derivatives are recognized by this hardly investigated riboswitch. We are especially interested in the specificity with respect to the phosphate group as well as the identity of the metal centre.
While in-line probing is the state of the art to investigate a structural change of RNA in an isolated system, its long incubation time is hindersome when working with oxygen sensitive and unstable Moco derivatives. We are therefore working on establishing alternative methods to test for binding and structural change.



Molybdenum Cofactor Derivatives *moaA* Riboswitch

Acknowledgements

Financial support from the Swiss National Science Foundation (RKOS), the UZH Forschungskredit (MR) and the University of Zurich is gratefully acknowledged.

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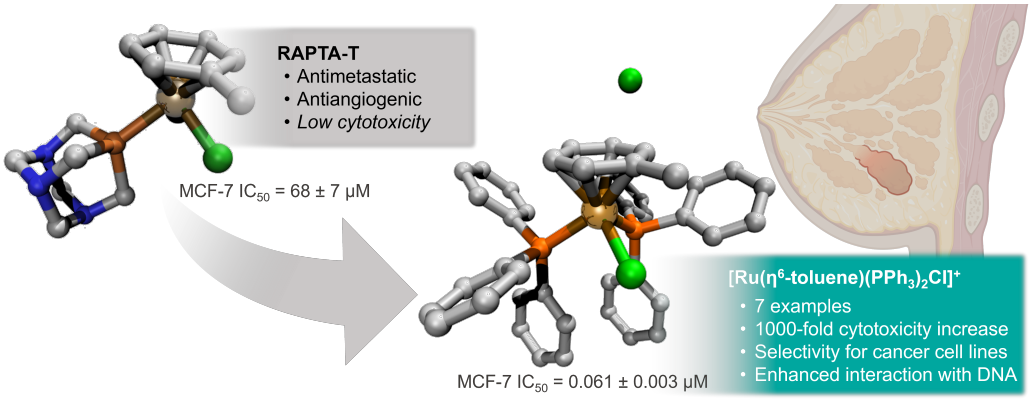
Fine-tuning the cytotoxicity of ruthenium(II) arene compounds to enhance selectivity against breast cancers

J. Romano-deGea[1], S. A. Pereira[1,2], M. M. Saraiva[2]*, P. J. Dyson[1]*

[1]Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne (CH), [2]LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto (PT)

Ru(η^6-arene)(PTA)Cl_2 (PTA = 1,3,5-triaza-7-phosphaadamantane), or RAPTA, complexes have arisen (together with other ruthenium compounds) as alternatives to platinum-based drugs in cancer therapy [1]. RAPTA compounds exhibit reduced general toxicity [2] and have demonstrated efficacy against breast cancer by suppressing metastasis [3], tumorigenicity [4], and inhibiting the replication of the human tumour suppressor gene BRCA1 [5]. However, their limited cytotoxicity, and therefore comparatively high dosing, has hindered their translation to the clinic.

We synthesised and explored the activity of a series of RAPTA-like ruthenium(II) arene compounds against two breast cancer cell lines and identified [Ru(η^6-toluene)(PPh_3)$_2$Cl]Cl as a potential therapeutical candidate with nearly a 1000-fold increase in cytotoxicity compared to RAPTA-T. The compound was further studied, revealing its remarkable stability and enhanced interaction with the minor and major grooves of DNA.



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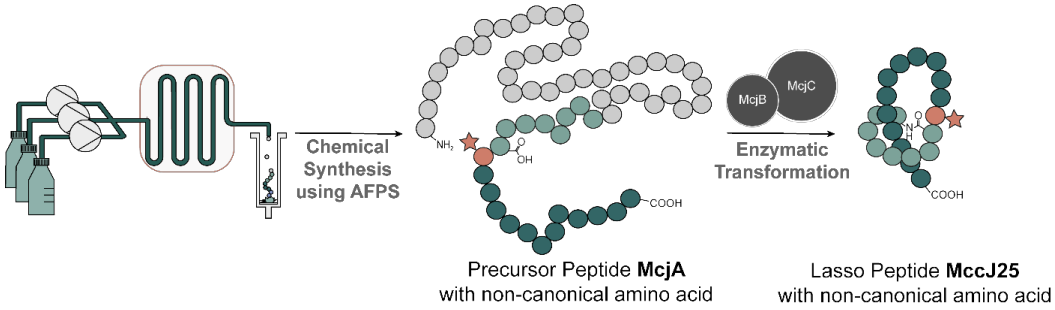
Expanding the Chemical Space of Lasso Peptides: Enzymatic Maturation of Synthetic Peptide Precursors

K. Schiefelbein[1], R. Striga[2], J. Lang[3], M. Schuster[1], O. Zerbe[1], Y. Li[2], N. Hartrampf[1]*

[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich (Switzerland), [2]Molécules de Communication et Adaptation des Microorganismes, UMR 7245 CNRS, Muséum National d'Histoire Naturelle, CP 54, 57 rue Cuvier, 75005 Paris (France), [3]Department of Geography, University of Zurich, Winterthurerstrasse 190, 8057 Zurich (Switzerland)

Lasso peptides are a class of ribosomally synthesized and post-translationally modified peptides (RiPPs), and many display antimicrobial, antiviral, and antitumor activity.[1] Their biological activities and their excellent stability against heat treatment and enzymatic digestion make them potential therapeutic agents, and chemical modifications would be desirable to explore this potential.[2] A chemical synthesis, however, is challenging because of their unique knot-like structure, and therefore, the most prominent member of their class – Microcin J25 (MccJ25), which shows activity against Gram-negative bacteria[3] – has not been chemically synthesized to date.

Here, **we use flow-based peptide synthesis in combination with *in vitro* enzymatic maturation to investigate the promiscuity of the processing enzymes and give access to several chemically modified MccJ25 derivatives including non-canonical amino acids.** We confirm lasso-formation by ion-mobility mass spectrometry, and perform antimicrobial assays to obtain additional information about the influence of these chemical modifications. Incorporating non-canonical amino acids will expand the chemical space; this allows for rational drug design and enables grafting onto this scaffold to synthesize lasso peptide libraries.



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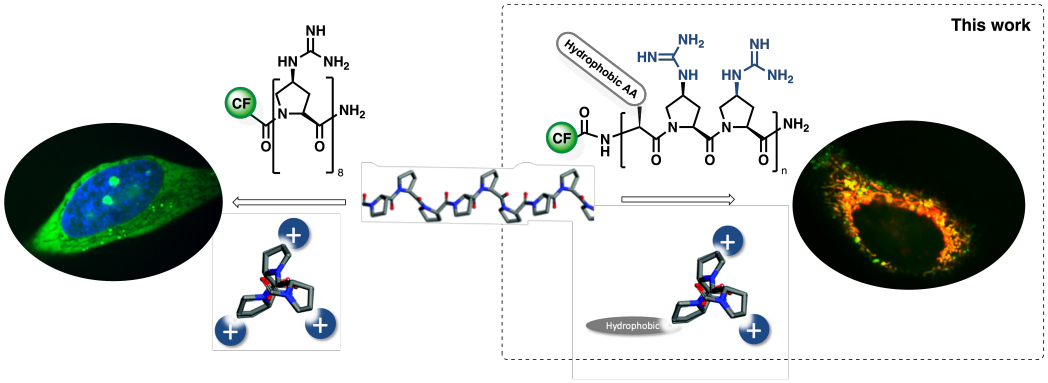
Amphipathic proline-rich cell penetrating peptides for mitochondria targeting

A. M. Schmitt[1], H. Wennemers[1]*

[1]Laboratory of Organic Chemistry, ETH Zürich, Vladimir-Prelog-Weg 1-5/10, Zürich, 8093, Switzerland

Cell penetrating peptides (CPPs) cross the lipophilic barrier of the cellular membrane and serve as delivery vectors to translocate cargo into cells.[1] They can also be useful for target-specific delivery, for example, of bioactive molecules to a specific cellular organelle. Here, targeting mitochondria constitutes an important goal since mitochondria dysfunction is associated with many diseases, including neurodegenerative and auto-immune diseases, diabetes, and cancer.[2] Selective delivery of bioactive compounds to mitochondria is challenging due to the dense and hydrophobic double membrane.[3]

Our group developed a cytoplasm and nucleus penetrating oligoproline (Z_8) CPP, which exhibits higher cellular uptake in comparison to more flexible peptides (e.g. octaarginine).[4] Here, we showcase that oligoproline peptides with hydrophobic amino acids installed at every third position allow mitochondria targeting. Selectivity is achieved by the PPII helix conformation with two cationic faces and one hydrophobic face enabling the crossing of the mitochondria membranes. The localization of the amphipathic peptides inside cells was evaluated by confocal microscopy, and the cellular uptake efficiency by fluorescence-activated cell sorting (FACS).



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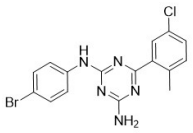
Triggered Release of a potent LPAAT-β inhibitor from inactive prodrugs to kill cancer cells

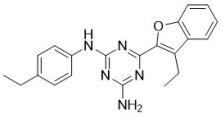
C. Schuppisser[1], S. Yasmin[1], M. Poirier[1], G. T. Giuffredi[1], H. Personne[1], J. L. Reymond[1]*

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

Lysophosphatidic acid acyltransferase β (LPAAT-β) is an enzyme controlling signaling pathway in mammalian cells and a potential cancer target.[1] In our hands however, the enzyme appeared to be similarly expressed in several cancer and non-cancer cells, and the nanomolar LPAAT-β inhibitors CT32228 and aminotriazine (**1**) appeared to be equally cytotoxic to both cell types.[2] Herein we report our investigation of a prodrug strategy to target **1** to cancer cells. By a structure-activity relationship study, we identified two potential vectors for connecting **1** to a cleavable linker. We then elaborated substituent and linker chemistry and obtained a redox-triggered release of active analogs of **1** from peptide-conjugated prodrugs.



CT32228



LPAAT-β inhibitor **(1)**
IC_{50} = 51 nM

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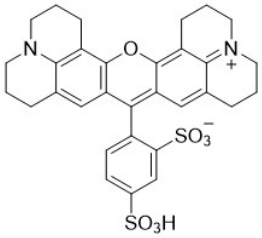
Astrocyte–specific targeting and kinase inhibition of the TNFR1 pathway

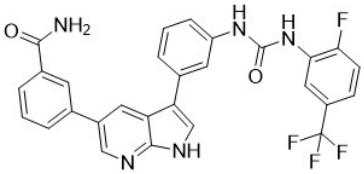
C. Schuppisser[1], R. De Ceglia[2], I. Zalachoras[2], A. Volterra[2]*, J. L. Reymond[1]*

[1]Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland, [2]Department of Fundamental Neurosciences, University of Lausanne, Rue du Bugnon 9, 1005 Lausanne, Switzerland

Astrocytes, the most abundant subtype of glial cells, have important roles in metabolic support of the neurons i.e. regulation of blood flow, detoxification and clearance of synapses.[1] Additionally, astrocytes are active players in synaptic functions by releasing gliotransmitters as the cytokine TNFα. Therefore, the consequences of disruption of astrocytic supportive functions or gliotransmission could play a significant role in human neuronal diseases. TNFα transforms astrocytes into a neurotoxic phenotype and elevated levels of TNF are found in several human brain diseases including Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis, trauma and stroke.[2]



(1)



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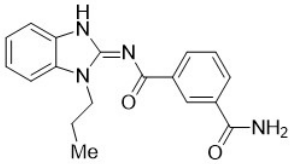

(3)

In this context, we aim to specifically inhibit upstream and downstream kinases of the TNFR1 pathway in astrocytes to gain insight in their function and for further physiological studies. Astrocytes can be specifically labelled by sulforhodamine 101 **(1)** via the thyroid hormone transporter OATP1C1.[3] Here we report the design, synthesis and cellular activity of linked sulforhodamines to RIPK1 **(2)** and TAK1 **(3)** inhibitors via cleavable and non-cleavable linkers.

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Fragment Screening and Fast Nanomolar Detection on a Benchtop NMR Spectrometer Boosted by Photoinduced Hyperpolarization

G. R. Stadler[1], T. F. Segawa[2], M. Bütikofer[1], V. Decker[3], S. Loss[4], B. Czarniecki[4], F. Torres[1,5]*, R. P. Riek[1]*

[1]Institute for Molecular Physical Science, ETH Zürich, [2]Laboratory of Physical Chemistry, ETH Zürich, [3]Bruker BioSpin GmbH, Ettlingen, Deutschland, [4]Bruker Switzerland AG, Fällanden, [5]NexMR GmbH, Schlieren

Nuclear magnetic resonance (NMR) spectroscopy has a variety of applications in drug discovery such as screening, determination of binding affinity, epitope mapping, and complex structure determination. The low sensitivity of NMR can be overcome by using photo-chemically induced dynamic nuclear polarization (photo-CIDNP), thereby reducing measurement time and consumption of protein and small molecules for screening.

We designed a dedicated photo-CIDNP fragment library of 212 compounds for screening on a high-field (600 MHz) NMR spectrometer, using low micromolar concentrations and single-scan experiments of a few seconds. [1]

The polarization yield obtained by photo-CIDNP increases inversely proportional to the magnetic field, facilitating the use of low-field benchtop magnets. Benchtop NMR spectrometers are about 20-fold cheaper than high-field spectrometers, require little maintenance, and their permanent magnets do not require cryogenic or helium cooling.

We show that photo-CIDNP-based fragment screening is possible on a cryogen-free low-field benchtop NMR spectrometer. We present a photo-CIDNP miniscreen with 30 compounds against the cancer target PIN1 measured on an 80 MHz NMR spectrometer. [2] The experiments were measured in only 3 minutes per sample using 500 µM compound and 10 µM protein concentrations and verified the fast detection of low-millimolar binders. While the concentrations used are comparable to a state-of-the-art NMR screening on high-field, the measurement time could be reduced by 5 to 10-fold. Binding could also be observed at lower concentrations down to 50 µM ligand and 1 µM protein. The detection limit for one compound was 100 nM after 6 minutes. The estimated measurement time at this concentration and field without hyperpolarization would be 450’000 hours.

The performance of screening in comparison to state-of-the-art high-field NMR reveals the advantages of our approach regarding costs and simplicity of execution. These results demonstrate the potential of photoinduced hyperpolarization to enable life science applications on cheap low-field permanent magnets and open the door to broader use of NMR in the drug discovery community.

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Development of a self-optimizing platform for flow-based peptide synthesis

B. Tamás[1], P. L. Willi[1], N. Hartrampf[1]

[1]Department of Chemistry, University of Zurich

Despite improvements in data analysis techniques, data- and ML-based optimization is not yet widespread in chemistry due to the need to collect data of adequate quality and quantity. Automated fast-flow peptide synthesis (AFPS), with its rapid reaction rates and already integrated analytical tools (in line UV-Vis), would be well suited to explore this toolbox. Although reaction conditions for solid-phase peptide synthesis (SPPS) were optimized over decades, SPPS is sequence-dependent, and events such as often-observed aggregation can lead to a decreased synthesis outcome. The occurrence of aggregation is thus a major remaining challenge in batch- and flow-SPPS, and the impact of various parameters, such as protecting groups, the sequence itself, and linkers, needs to be better understood. We therefore developed improved data-based methods to exploit the generated in-line UV data in multiple ways to reduce aggregation: We developed a new approach to identify aggregation from UV data, which enables the recovery of lost or low-quality data while simplifying the prediction for future machine learning applications. Furthermore, we developed a real-time self-optimizing algorithm allowing quick intervention upon aggregation detection. These two methods serve as the initial steps in creating a more complex machine learning-based system that aims to eliminate sequence dependence in SPPS.

Combinatorial design of nanoparticles for efficient delivery of therapeutic biomacromolecules through the blood brain barrier

C. Wu[1], N. Santacroce[1], E. Laprévotte [2], Y. Dudal [2], L. Suter-Dick [1,3], P. Shahgaldian[1]*

[1]University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Muttenz, Switzerland, [2]Perseo-Pharma AG, Muttenz, Switzerland, [3]Swiss Center for Applied Human Toxicology (SCAHT), Basel, Switzerland

The blood-brain barrier (BBB) is a crucial biological filter made of endothelial cells mediating the communication between the blood and the central nervous system. This unique barrier effectively blocks the entry of harmful substances from the bloodstream. However, it also imposes strict limitations on the passage of therapeutic proteins, *e.g.,* enzymes and monoclonal antibodies, thereby impacting their therapeutic benefits when targeting the brain. To address this challenge, a variety of nanoparticulate drug transporters have been designed and several targeting moieties have been shown to favor their transport through the BBB. However, there is a lack of a systematic study that enables the screening of optimized parameters for nanoparticles (NPs) in designing such carriers. Herein, we developed a novel method of NP combinatorial surface modification by utilizing copper(I)-catalyzed azide alkyne cycloaddition reaction. This method enables an efficient chemical modification of the surface of NPs with a variety of ligands for facilitating screening experiments. In specific, these NPs consist of a silica core with an immobilized model protein, protected in an organosilica shield. For investigating the capability of the combinatorially modified NPs to cross the BBB, we utilized an *in vitro* BBB model system. The results showed that the NPs exhibited good biocompatibility when incubated with human brain endothelial cells (hCMEC/D3) for 24 hours. In addition, screening experiments based on this novel method identified lead formulations with combinatorial modification of glucose, transferrin and carboxy-, amino-functions, which significantly increased delivery efficiency without compromising BBB integrity. Taken together, we have established a platform that enables to investigate the impact of NPs' surface modification on their interactions with cells. This approach provides a valuable avenue for screening and selecting formulations with improved delivery efficiency.

Chemically Enhanced Antisense Oligonucleotides: A Molecular Approach for Treating Autosomal Dominant Tubulointerstitial Kidney

S. Sjöström[1], J. Lake[2], M. Mariniello[2], G. Schiano[2], A. Kokanovic[2], A. Hill[1], O. Devuyst[2], J. Hall[1]*

[1]Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zürich, Switzerland, [2]Institute of Physiology, University of Zurich, Switzerland

Uromodulin, a predominant urinary protein, is produced by epithelial cells of the thick ascending limb (TAL) in the renal nephrons[1,2]. Mutations in the *Umod* gene, responsible for uromodulin production, can lead to autosomal dominant tubulointerstitial kidney disease (ADTKD) – a prevalent hereditary cause of progressive chronic kidney disease with limited treatment options[3–5]. To tackle this problem, we constructed a chemically enhanced antisense oligonucleotide (ASO) library to inhibit the translation of *Umod* mRNA and prevent its toxic accumulation. The design of our gapmer ASOs focused on two targets: the wild type (wt) sequence and a single nucleotide polymorphism (SNP) associated with severe ADTKD. The wt-targeting library is formulated to decrease overall uromodulin levels, providing a broad-spectrum approach to treat known ADTKD-UMOD cases. On the other hand, the SNP-targeting library aims to serve as a precision molecular tool to study SNP-discrimination and allele-selective oligonucleotide therapies, although with a narrower therapeutic scope. We identified a lead ASO that selectively silences an adverse disease-causing SNP (R185S) in vitro. Additionally, in vivo investigations into the ASO's silencing potency and bio-distribution were conducted post-subcutaneous administration in mice. Results show a promising uptake into the TAL cells and moderate silencing of *Umod* mRNA. This study demonstrates the potential for Umod as a molecular target of therapy and the TAL cells as a site of accumulation for this class of drugs.

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RNA-PROTACs targeting aggregate prone RNA-Binding Proteins

C. Weller[1], J. P. Becker[1], J. Hall[1]*

[1]Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zürich, Switzerland; 2Institute of Physiology, University of Zurich, Switzerland

Proteolysis Targeting Chimeras (PROTACs) are bifunctional molecules that exploit the Ubiquitin-Proteasome system to degrade proteins. These chimeric compounds consist of a ligand for the target protein and a ligand for a Ubiquitin E3 ligase [1]. By employing PROTACs, proteins previously considered "undruggable," such as non-enzymatic or structural proteins, can now be effectively targeted for degradation [2]. The versatility of PROTACs extends beyond cellular mechanism studies, as several PROTACs have progressed into clinical trials, showcasing their therapeutic potential [3].

Despite the significant role played by RNA-binding proteins in the onset of numerous diseases, developing conventional drugs to effectively target them has proven challenging [4]. One such protein of interest is Tar DNA-binding protein 43 (TDP-43), an RNA-binding protein involved in splicing regulation, primarily located in the nucleus. In conditions like amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTLD), TDP-43 undergoes abnormal post-translational modifications, leading to cytosolic aggregation and eventually death of motoneurons [5]. Similarly, mutations in the C-terminal region of the Fused in Sarcoma (FUS) protein are associated with ALS and can result in cytoplasmic aggregation of FUS [6]. Hence, it is crucial to explore methods for selectively degrading aberrant cytosolic forms of TDP-43 and FUS.

Previously, our laboratory introduced the concept of RNA-PROTACs, demonstrating their efficacy in inducing degradation of the RNA-binding protein Lin28 [7]. The initial RNA-PROTAC utilized a chemically modified 7-mer oligonucleotide to target Lin28. In this study, we present an enhanced version of the RNA-PROTAC employing a chemically modified RNA structure to target a regulatory protein. Furthermore, we report our initial progress in developing an RNA-PROTAC specifically designed to target cytosolic aggregated RNA-binding proteins.

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Installation of Electrophiles onto the C-terminus of recombinant ubiquitin and ubiquitin-like proteins

K. A. Tolmachova[1], J. Farnung[1], J. W. Bode[1]*

[1]ETH Zurich, Vladimir-Prelog-Weg 3, 8093 Zurich

Ubiquitin and related ubiquitin-like proteins (Ubls) influence a variety of cellular pathways including protein degradation and response to viral infections. The chemical interrogation of these complex enzymatic cascades relies on the use of tailored activity-based probes (ABPs). We report the preparation of ABPs for ubiquitin and a range of Ubls, including NEDD8, SUMO2, ISG15 and UFM1 by selective acyl hydrazide modification. Acyl hydrazides of Ubls are readily accessible by direct hydrazinolysis of Ubl-intein fusions. The suppressed pKa and superior nucleophilicity of the acyl hydrazides enables their selective modification at acidic pH with carboxylic acid anhydrides. The modification proceeds rapidly and efficiently, and does not require chromatographic purification or refolding of the probes. We modified Ubl–NHNH2 with various thiol-reactive electrophiles that couple selectively with E2s and DUBs. The ease of modification enables the rapid generation and screening of ubiquitin probes with various C-terminal truncations and warheads for the selection of the most suitable combination for a given E2 or DUB.