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General Assembly of the SCS Division of Medicinal Chemistry & Chemical Biology 2022

J. L. Reymond[1,2]

[1]Universtiy of Bern, [2]SCS, President DMCCB

Report and information on DMCCB activities 2022 and 2023.

Discovery and characterization of ACT-284069, a double prodrug of the $P2Y_{12}$ receptor antagonist selatogrel

E. Caroff[1], F. Hubler[1], C. Gnerre[1], A. Treiber[1]

[1]Idorsia Pharmaceuticals Ltd

Atherothrombotic conditions are currently managed by prescribing aspirin in combination with P2Y12 inhibitors. Approved oral P2Y12 antagonists include clopidogrel, prasugrel, and ticagrelor. We have previously disclosed the discovery of selatogrel (ACT-246475A), a new, potent, selective and reversible P2Y12 antagonist [1]. Selatogrel is currently in clinical development for early treatment of suspected acute myocardial infarction and is formulated for subcutaneous administration [2].

Based on the superior therapeutic window of selatogrel as compared to clopidogrel and ticagrelor in a rat thrombosis model [1,3], our initial goal was to develop a new efficacious oral treatment with less bleeding complications than standard of care. To address the low bioavailability of selatogrel observed after oral administration during preclinical animal studies, we embarked in an optimization program to design selatogrel prodrugs. Herein we report the synthetic efforts, the characterization cascade, and the data leading to the identification of the prodrug ACT-284069.

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Exploring Bicyclic Diamines as Drug Scaffolds

A. L. Carrel[1], J. L. Reymond[1]*

[1]University of Bern, Freiestrasse 3, 3012 Bern

Drug discovery is in constant need of new molecules to develop drugs addressing unmet medical needs. To assess the chemical space available for drug design our group developed tools to enumerate, visualize and search chemical space. The Generated DataBases (GDBs) list billions of possible organic small molecules following simple rules of chemical stability and synthetic accessibility. [1a-1d]

Following the synthesis of triquinazine as a novel piperazine analog for drug design from our GDBs, we asked the question whether even simpler diamine scaffolds might still be unexplored. [2] Therefore, we enumerated all 1323 bicyclic ring systems containing only 5, 6 or 7-membered rings and decorated them with two nitrogen atoms. Such diamines are interesting as drug scaffolds due to their favorable solubility and pharmacokinetic profile compared to aromatic scaffolds, as recently demonstrated for the g-secretase modulator RO7185876. [3] To our surprise, more than half of our GDB diamine cores are not found as molecular cores when considering any possible substituents on the nitrogen atoms in public databases.

Here we present the synthesis of fused bicyclic diamines, all of which represent yet unknown scaffolds. Furthermore, we have used our polypharmacology browser PPB2 to predict possible targets for the GDB-inspired bicyclic diamines and their *N*-monobenzylated analogs. [4] Activity screening showed that one of the *N*-benzylated diamines is a micromolar inhibitor of neurotransmitter transporters. To progress in the synthesis, we have implemented AIZynthfinder and have found that about half of the diamines can be solved by the tool. [5] In particular, AIZynthfinder proposed a retrosynthesis for the bicyclic diamine discussed above, from the same precursor but giving access to the previously unexplored *syn*-diastereoisomer due to inverted reaction sequence and milder conditions.

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PET Imaging of Cannabinoid Type 2 Receptors – Discovery of [^{18}F]RoSMA-18-d$_6$

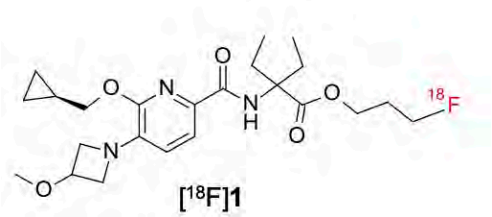
L. Gobbi[1]

[1]F. Hoffmann-La Roche - luca.gobbi@roche.com

Luca Gobbi[1], Ahmed Haider[2], Julian Kretz[1], Christoph Ullmer[1], Andreas Brink[1], Michael Honer[1], Dieter Muri[1], Adrienne Müller Herde[2], Francesco Spinelli[2], Hazem Ahmed[2], Kenneth Atz[1], Claudia Keller[2], Roger Schibli[2,3], Linjing Mu[2,3], Uwe Grether[1], Simon M. Ametamey[2]

[1]Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche, 4070 Basel, Switzerland; [2]Institute of Pharmaceutical Sciences, ETH Zurich, Vladimir-Prelog-Weg 4, 8093 Zurich, Switzerland; [3]Department of Nuclear Medicine, University Hospital Zurich, 8091 Zurich, Switzerland

The cannabinoid type 2 receptor (CB2) is a member of the GPCR cell surface receptors. CB2 has been recognized to be broadly involved in neuroinflammatory processes, but no suitable CB2-targeted probe is currently available for clinical use. In a collaborative medicinal chemistry program based on a partnership between academia and pharma industry, we have identified the pyridine-based CB2 PET radioligand [^{18}F]**1** with a binding affinity (K_i) of 6 nM for CB2 and a selectivity factor of 696 over the cannabinoid receptor type 1 (CB1) [1]. Despite the promising *in vitro* properties, [^{18}F]**1** exhibited only moderate *in vivo* specificity and a fast washout from rat spleen, an organ rich in CB2. In an effort to improve CB2 specificity and selectivity we synthesized 15 derivatives of [^{18}F]**1** and tested them for their binding affinities towards CB2 and CB1. As a result from this work, RoSMA-18 was identified as a novel tracer candidate displaying an affinity of 0.7 nM (K_i for CB2) and an exquisite selectivity factor of > 12'000 over CB1 [2]. [^{18}F]RoSMA-18 was synthesized with an average radiochemical yield of 10.6 ± 3.8% (n = 16) and excellent radiochemical purity (> 99%). Molar activities ranged from 52 - 65 GBq/µmol. Exceptional CB2 specificities were achieved in CB2-positive rat spleen by *in vitro* autoradiography (71 ± 2%) and *ex vivo* biodistribution (86 ± 2%), which are superior to any previously reported CB2 PET radioligand. The very high specificity was further demonstrated in CB2 knockout mouse spleen. PET experiments revealed specific and reversible CB2 binding of [^{18}F]RoSMA-18 in the CB2-positive rat spleen. Metabolite studies detected only intact [^{18}F]RoSMA-18 in the rat brain, however, *in vivo* defluorination was observed as evidenced by skull uptake. The defluorination was effectively prevented by replacing the hydrogen atoms in the fluoropropyl side chain with deuterium atoms to afford [^{18}F]RoSMA-18-d$_6$. RoSMA-18-d$_6$ showed a K_i value of 0.8 nM for CB2 and > 10 µM for CB1. Overall, these results suggest that [^{18}F]RoSMA-18-d$_6$ is a promising CB2 PET radioligand for clinical translation.



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YAP-TEAD protein protein interaction inhibitors. Discovery of the first class of small molecules efficiently disrupting the interaction by virtual screening and structure-based design

P. Furet[1]

[1]Novartis Institutes for BioMedical Research

The YAP-TEAD protein protein interaction is a therapeutic target of current interest in oncology to treat cancers associated with a dysregulation of the Hippo pathway. The very extended surface of interaction of the two proteins presents a formidable challenge for a small molecule inhibitor approach. Virtual screening allowed us to identify a weakly active hit binding to one of the two main sites of interaction of YAP at the surface of TEAD. By structure-based design it was possible to improve the potency of this hit by several orders of magnitude. The main features of this work that has led to the identification of the first representatives of a new class of cellularly active YAP-TEAD interaction inhibitors will be presented.

Bioactive Polymersomes Targeting Cancer-Associated Fibroblasts for Anticancer Therapy

D. Hürlimann[1,3], M. Skowicki[1,3], M. Kyropoulou[1,3], A. S. Gheata[2], W. P. Meier[1,3], S. Gerber-Lemaire[2]*, C. G. Palivan[1,3]*

[1]Department of Chemistry, University of Basel, BPR 1096, Mattenstrasse 24a, 4058 Basel, Switzerland, [2] Institute of Chemical Sciences and Engineering, EPFL, Batochime, 1015 Lausanne, Switzerland, [3]NCCR-Molecular Systems Engineering, BPR 1095, Mattenstrasse 24a, 4058 Basel, Switzerland

Despite constant development of anticancer therapies cancer remains a leading cause of mortality. Modern chemotherapies, as a main tool in anticancer treatment, have numerous side effects due to their lack of specificity or systemic toxicity. Innovative solutions in the field of nanomedicine can contribute to the development of more precise and effective therapeutics. Recently, the fibroblastic element in tumor microenvironments emerged as a valuable target for treatment and diagnosis of different types of cancer. Herein, we decorated polymersomes with a bioactive ligand to target fibroblast activation protein a (FAP). By shielding their content from their surrounding, polymersomes favor lower immunogenicity and prolonged activity. Polymersomes were prepared by the self-assembly of the amphiphilic diblock copolymer poly(dimethylsiloxane)-*block*-poly(2-methyl-2- oxazoline)(PDMS-*b*-PMOXA) followed by decoration with a bioactive ligand. The cellular uptake of decorated polymersomes in contrast to unmodified polymersomes significantly increased in a FAP positive human adenocarcinoma cell line (A549). These polymersomes display the ability to escape endosomes proving their potential as an efficient delivery carrier (Figure 1). Additionally, the insertion of the honeybee venom peptide melittin into the membrane offers stable pores to allow diffusion for a functional nanoreactor. Such decorated nanoreactors might prove their potential as an efficient delivery carrier.

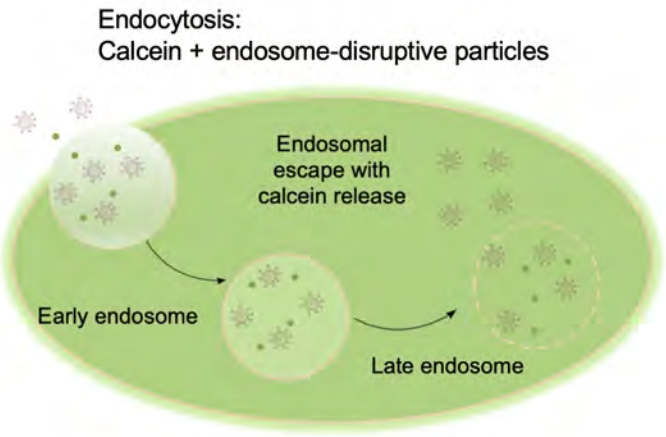

Figure 1: Endosomal escape of polymersomes decorated with a bioactive ligand.

Comparative metabolomics reveals the gene function of *kat-1* in *C. elegans*

M. Scheidt[1], S. Bandi[1], S. von Reuss[1]*

[1]University of Neuchâtel, Institute of Chemistry, Laboratory of Bioanalytical Chemistry, Avenue des Bellevaux 51, 2000 Neuchâtel

In the model organism *Caenorhabditis elegans* mutation of the mitochondrial 3-ketoacyl-CoA thiolase (*kat-1*) has been reported to affect lifespan [1]. Although *kat-1* has been assumed to be implicated in mitochondrial β-oxidation of fatty acids, its precise function has remained enigmatic. Comparative analysis of the *C. elegans* wildtype (N2) and *kat-1* mutant exometabolomes revealed some yet unidentified compounds (see Figure 1).

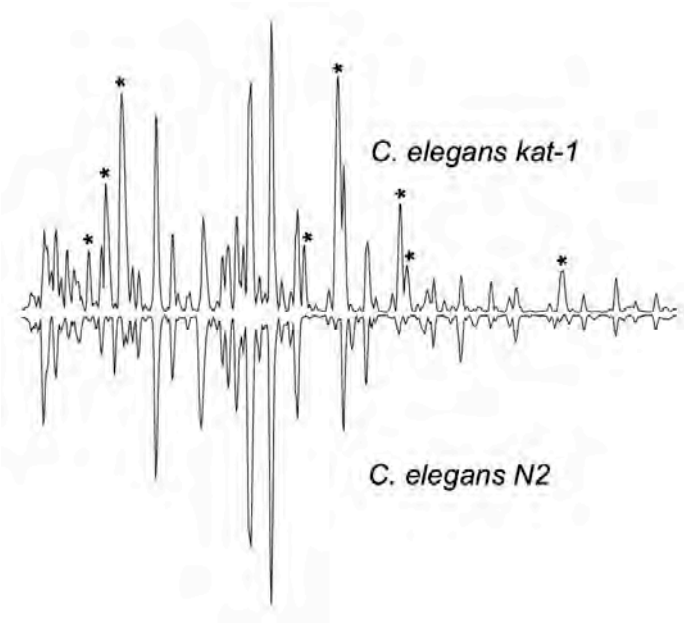

Figure 1: Comparative metabolomics of exometabolomes of a *kat-1* mutant (above) and a *N2* control (below). The markers indicate some of the metabolic changes found in HPLC-MS traces of the exometabolome.

Large scale cultivation, followed by fractionation, and NMR spectroscopy enabled the identification of several modular tiglyl-glucosides. Their upregulation in *kat-1* suggested a potential function in branched chain amino acid metabolism. Feeding experiments with the *C. elegans kat-1* mutant using L-[U-$^{13}C_5$]-valine, L- [U-$^{13}C_6$,^{14}N]-leucine, and L-[U-$^{13}C_6$,^{14}N]-isoleucine enriched *E. coli Δile Δleu Δval* highlighted diverse metabolites derived from the catabolism of L-isoleucine that are strongly upregulated in the *kat-1* mutant. Taken together, these results indicate that *kat-1* functions as a mitochondrial 2-methylacetoacetyl-CoA thiolase. Consequently, *C. elegans kat-1* might represent a suitable model system to study mitochondrial acetoacetyl-CoA thiolase (T2) deficiency, a rare disease in humans [2].

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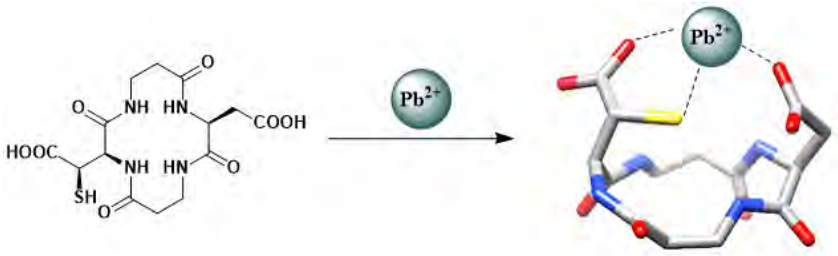
Cyclic Tetrapeptides: Novel Remedy for Lead Poisoning

T. A. Mohammed[1], C. M. Meier[1], L. Rulíšek[2], M. S. Shoshan[1]*

[1]University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zurich, [2]Czech Academy of Sciences, Institute of Organic Chemistry and Biochemistry, Flemingovo nµmeˇstí 2, 16610 Praha 6

Lead (Pb) is the most abundant toxic metal that causes significant ecological and medicinal issues. The primary approach to address Pb poisoning is by chelation therapy, in which a chelating agent that can coordinate and remove the poisonous metal is administered. To date, there is no ideal chelating agent for treating Pb toxicity, and the available chelating agents lack metal selectivity. Hence, they are highly toxic themselves and subsequently prohibited from treating the most affected population segments, including children and pregnant women.[1]

Nature harnessed peptides and proteins for handling metal poisoning.[2] Inspired by natural systems, we designed and synthesized a family of cyclic tetrapeptides.[3] We examined their ability to recover Pb-poisoned bacteria and human cells, where two candidates showed a prominent potency, outcompeting the current clinical chelating agents. Investigating the Pb-peptide complex of the lead ligand experimentally and computationally disclosed its surpassing metal affinity and selectivity. In addition, we carried out an *in vivo* mice study, which revealed its outstanding chelating ability and promising potential to be a novel antidote for Pb poisoning.



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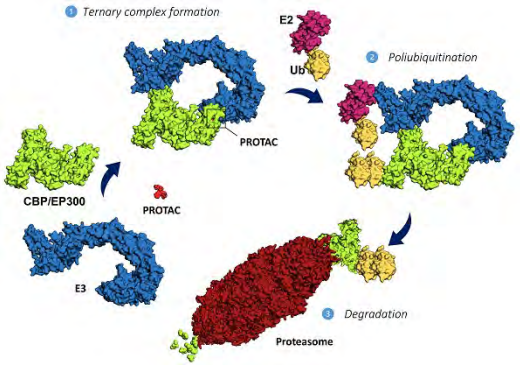
A rational and systematic approach to PROTACs development: identification of CBP/EP300 degraders

L. Palaferri[1], I. Cheng-Sánchez[1], K. Gosselé[1], C. Nevado[1]*

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

PROteolysis TArgeting Chimeras (PROTACs) are bifunctional molecules that simultaneously bind an E3 ligase and a protein of interest, inducing degradation of the latter via the ubiquitin-proteasome system. Despite their outstanding potential, the rational design of PROTACs remains challenging as several variables need to be tuned in parallel to obtain active degraders, and their large size and novel structure activity relationship (SAR) means that much of the knowledge surrounding the design of small molecule drugs cannot be applied. To tackle this problem, we systematically explored PROTAC SAR, leading to the rational design of degraders targeting CREB-binding protein (CBP) and E1A-associated protein (EP300) - two homologuous regulatory proteins crucial for enhancer mediated transcription and implicated in a wide range of human diseases. We established a novel synthetic route to improve the chemical accesibility of the CBP/EP300 ligand, and several cell-based assays including the measurment of cellular ternary complex formation for the first time for Cereblon (CRBN) and CBP. Through this we demonstrate that engagement of CRBN, rather than VHL or IAP, and a specific linker between the E3 and CBP binders are essential for PROTAC-mediated CBP degradation, and by doing so, we broaden the tools available to modulate CBP and EP300. Lessons learnt from this campaign, particularly the importance of cell-based assays beyond degradation to understand reasons for PROTAC inactivity, are widely applicable to other challenging targets and can assist in guiding the development of degraders whilst minimizing compound libraries.



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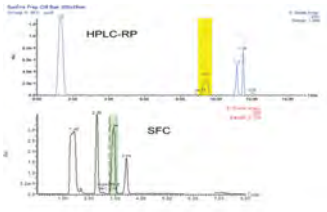
Achiral Supercritical fluid Chromatography (SFC) for the Purification of Pharmaceuticals

J. Reilly[1]

[1]Novartis Institute of Biomedical Research

With large quantities of small scale (<1000mg) compounds typically being needed to be purified and evaluated for discovery in-vitro and in-vivo testing regimes, a chromatographic purification technique needs to be easily integrated into discovery chemistry laboratories and highly reproducible. Within Separation Sciences departments in Pharmaceutical Industry there are a range of methodologies focused on the analytical separation and purification to isolate pure products. At Novartis, the integration of Supercritical fluid chromatography (SFC) for achiral separations has several advantages, such as faster run times, shorter equilibration times, reduced solvent consumption, and in preparative applications, fast solvent removal. SFC has rapidly become a very attractive alternative to Normal Phase and Reverse Phase purification for achiral samples and example separations are demonstrated



Single-molecule profiling of PRC1 ubiquitination dynamics in defined chromatin states

A. Teslenko[1], B. Fierz[1]*

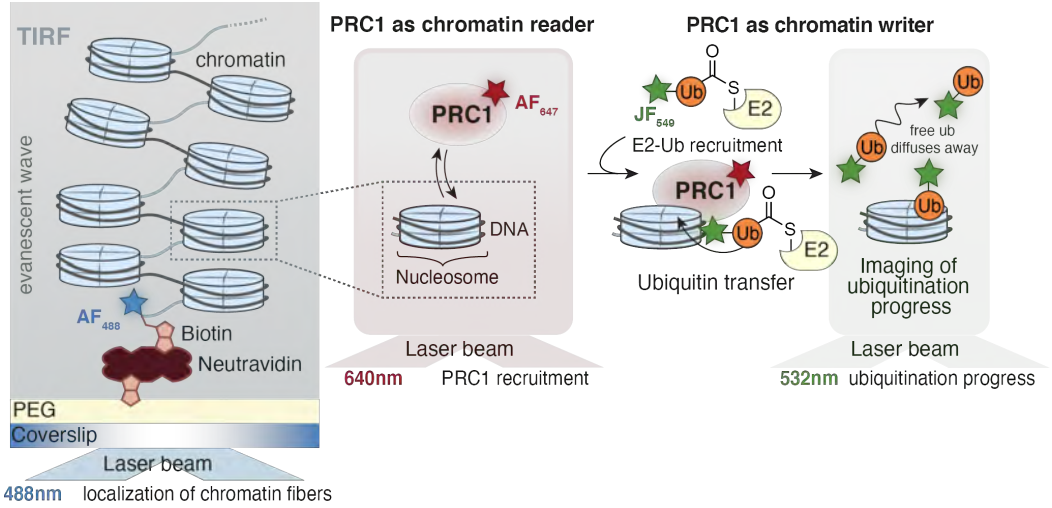
[1]Laboratory of Biophysical Chemistry of Macromolecules (LCBM), École Polytechnique Fédérale de Lausanne (EPFL)

Each cell of a eukaryotic organism carries universal genetic information despite its functional diversity. The DNA is organized as chromatin fiber, i.e. long arrays of nucleosomes. Cell-type-specific gene expression patterns have to be established to develop a diverse set of cell types in multicellular organisms.

Dynamic regulation of transcription is orchestrated by a large cohort of enzymes, among which chromatin modifiers or 'writers' install histone post-translational modifications (PTMs) controlling the recruitment of chromatin 'readers'. A specific subset of chromatin modifiers includes Polycomb group (PcG) proteins, which drive the inheritance of a repressed chromatin state during development and cell differentiation while preventing abnormal oncogenic transformations.

PcG members Polycomb Repressive Complex (PRC) 1 function as H2A-specific E3 ligases that establish gene repression. Variant PRC1 is involved in most of the ubiquitin deposition in the context of repressed chromatin, without the requirement of primary installation of additional PTMs. We hypothesize that chromatin modification, i.e. ubiquitination, by PRC1 is controlled by its recruitment dynamics on underlying chromatin and its subunit composition.

Here, we present a single-molecule method, which allows us to directly observe PRC1 binding dynamics on immobilized chromatin fibers in real-time. Consequently, we introduce ubiquitin-conjugating enzyme E2 which is loaded with fluorescently labeled ubiquitin. The preloaded E2-Ub is recognized by PRC1 driving H2AK119 ubiquitination. This allows us to gain a mechanistic view of 'reading' and 'writing' by PRC1 in real-time and elucidate its contribution to gene regulation.



Synthesis of short peptides in 384-well plates for generating large libraries of macrocyclic compounds

M. Schüttel[1], E. Will[1], C. Heinis[1]*

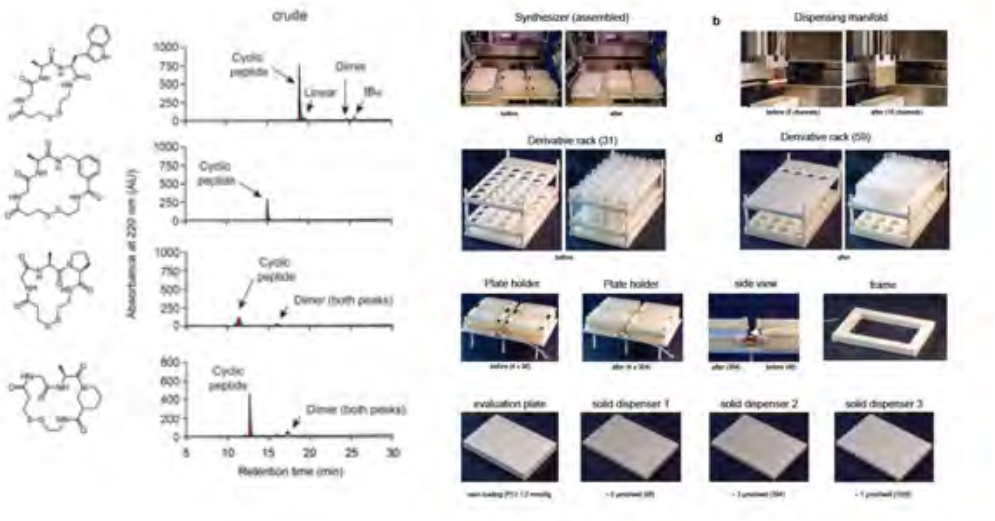
[1]École Polytechnique Fédérale de Lausanne (EPFL)

Macrocycles have raised much interest in the pharmaceutical industry due to their ability to bind challenging targets. However, the development of macrocyclic ligands to new disease targets is hindered by the limited availability of large, structurally diverse macrocyclic compound libraries for high-throughput screening.

In order to generate large libraries of macrocycles, our laboratory has recently introduced a new strategy in which "n" short linear peptides are combinatorially cyclized with "m" different chemical linkers to generate "m x n" different macrocyclic compounds.[1, 2] A bottleneck in applying the established strategy is the synthesis of large numbers of short peptides. The peptide synthesizer that we use – being the one having the highest throughput on the market - can produce 4 x 96 in four microtiter plates, which limits the synthesis to 384 peptides per run. The synthesizer holds reagent containers for a maximum of 31 different amino acids, which also limits the chemical and structural diversity of peptides that can be produced.

Herein, we have developed hard- and software to upgrade the microtiter plate based solid-phase peptide synthesizer. Now, the peptides can be synthesized in four 384-well plates, allowing the synthesis of 1,536 peptides in one run. The number of possible buildings blocks was increased from 31 to 395 derivatives. Moreover, we have developed practical tools for the rapid and reliable resin loading to 384-well plates. The short peptides synthesized in the 4 x 384-well plates were of high purity.

With the new peptide synthesis capacity, it has become possible to synthesize large combinatorial libraries comprising ten-thousands of macrocyclic compounds. The peptide synthesis in 384-well plates may be also attractive for other applications beyond macrocycle drug development, such as for antibody epitope scanning, epitope mimetic development, peptide ligand development, and peptide-substrate screening.



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Discovery of RG6006, a Tethered Macrocyclic Peptide Targeting Acinetobacter baumannii

P. Mattei[1]

[1]Small Molecule Research, pRED Therapeutic Modalities, Roche Innovation Center Basel, CH-4070 Basel - patrizio.mattei@roche.com

In the light of the rapidly rising number of people dying from bacterial infections and the lack of effective antibiotics, the Abx MCP project addresses the urgent need to fight multi-drug resistant bacteria.

The tethered macrocyclic peptides (MCPs) represent a structurally distinct compound class of antibiotics, which possesses a differentiated mode of action. Phenotypic high-throughput screening of a library of MCPs identified a hit, which was selectively targeting *Acinetobacter baumannii*. Medicinal chemistry efforts rapidly resulted in potent compounds, which were able to cure bacterial infections in mice, where established antibiotics failed.

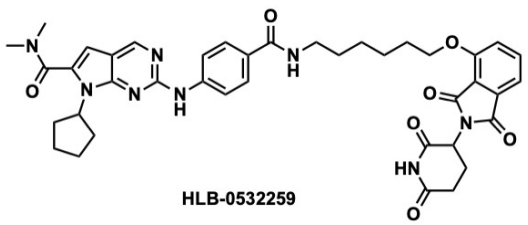
However, these compounds suffered from poor intravenous tolerability and multi-organ toxicity in rats. The lead optimisation was guided by consideration of the antibiotic drug-like space and supported by a customised plasma compatibility assay, producing highly efficacious compounds with improved intravenous tolerability and no organ toxicity. The development compound RG6006 is currently in phase 1 clinical trials, and if approved it would become the first antibiotic of a new class in more than 50 years to be used against infections caused by gram-negative bacteria.

Development of HLB-0532259: A First-in-Class Chemical Degrader of Aurora Kinase A and the N-Myc Transcription Factor

D. A. Harki[1]

[1]University of Minnesota, Department of Medicinal Chemistry, Minneapolis, MN 55455 United States

N-Myc is a master transcriptional regulator that has been called “undruggable” due to a lack of small molecule binding sites. However, unique molecular vulnerabilities have been identified that provide opportunities for chemical regulation. For example, in neuroblastoma, N-Myc is bound by Aurora kinase A (Aurora-A), which stabilizes N-Myc from proteolytic degradation. Genetic knockdown of Aurora-A in neuroblastoma confers the rapid proteolytic degradation of N-Myc by the ubiquitin proteosome system. Consequently, chemical degradation of Aurora-A provides an opportunity to affect N-Myc levels for oncology applications. Our laboratory has structurally modified the CDK4/6 inhibitor, ribociblib, to afford a high affinity Aurora-A ligand (K_d = 0.89 nM). Elaboration of our Aurora-A ligand into a Proteolytic Targeting Chimera (PROTAC) delivered the novel Aurora-A degrader, HLB-0532259.[1] Extensive biochemical, biophysical, cellular, and *in vivo* studies have resulted in the characterization of HLB-0532259 as a degrader of Aurora-A (DC_{50} = 14.4 nM in SK-N-BE(2) neuroblastoma cells) that concomitantly elicits the degradation of N-Myc ($DC_{50,\ app}$ = 179 nM in SK-N-BE(2) cells). Kinome profiling and tandem mass tag proteomics revealed that HLB-0532259 is highly specific and thereby confers a valuable chemical probe for mechanistic biochemical and cellular studies. In addition, HLB-0532259 possesses reasonable pharmacokinetic properties in mice ($t_{1/2}$ = 14.5 h, C_{max} = 169 ng/mL, AUC = 1993 ng·h/mL, and MRT = 16.1 h) and elicits tumor reduction *in vivo* in a murine xenograft model. Taken together, HLB-0532259 is a promising lead compound that is being further optimized in our laboratory for potency and physiochemical properties with an eye towards future clinical development. This presentation will highlight our development of HLB-0532259 and related analogues.



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Site-specific labeling of large RNAs for single-molecule FRET

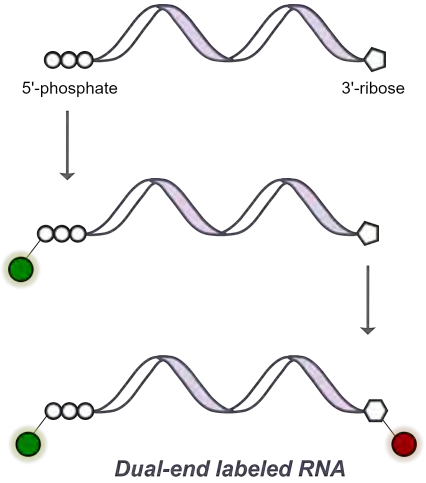
E. Ahunbay[1], S. Zelger-Paulus[1], R. K. Sigel[1]*

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

Catalysis in biological systems might be dominated today by protein enzymes, yet in the early world, RNA served as the principal biocatalyst.[1] Ribozymes, catalytic RNAs, undergo dramatic structural rearrangements to perfom their biological functions. Single-molecule Förster resonance energy transfer (smFRET) is an excellent method to study RNA conformational dynamics.[2] A prerequisite to any smFRET study is the strategic positioning of a fluorophore pair on the molecule of interest. Site-specific labeling of large RNAs is particularly challenging, especially when they are highly structured and catalytically active.[3]

We present a fast, efficient and covalent RNA labeling strategy that preserves the structural and functional integrity of the biomolecule. The RNA termini, the 5'-phosphate and the 3'-ribose, are chemically activated for bioconjugation with a FRET pair of fluorescent dyes. We replaced the proposed 5'-phosphoramidation[4] with an EDC-NHS activation step to achieve efficient labeling at shorter times and lower incubation temperatures, as well as pH control during subsequent dye coupling.[5]

We illustrate the strength of the chemical end-labeling on a highly dynamic and structured group II intron.[5] The previous state-of-the-art large ribozyme labeling method was hybridization-based and required modifications on the RNA structure.[3] Our covalent approach allows to tackle the structural complexity of the full-length intron and probe its folding and autocatalytic splicing dynamics at the single-molecule level via smFRET for the first time.



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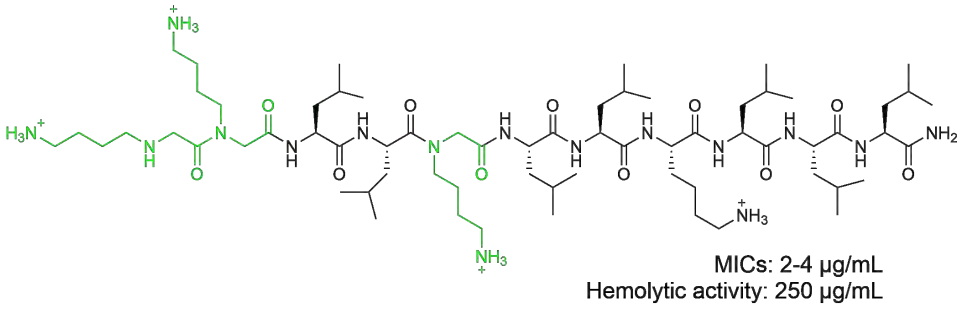
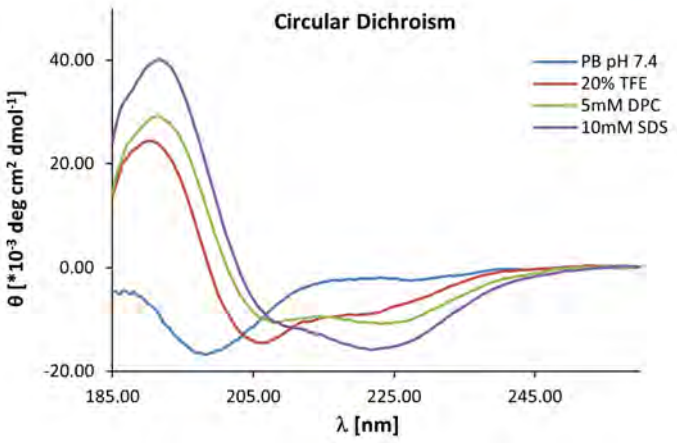
From mixed-chirality to mixed peptide-peptoids antimicrobial peptides to control multidrug resistant Gram-negative bacteria

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

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

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).





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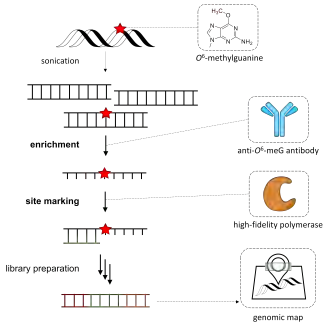
Genome-wide mapping of O^6-methylguanine

J. Büchel[1], C. Mingard[1], P. Reinert[1], S. Huber[1], S. J. Sturla[1]*

[1]ETH Zurich, Laboratory of Toxicology

The widely used chemotherapeutic drug temozolomide induces DNA alkylation such as O^6-methylguanine (O^6-MeG), a DNA adduct that causes mismatches upon replication leading to apoptosis by mismatch repair overload. However, O^6-MeG can be recognized and directly repaired by O^6-methylguanine-DNA methyltransferase (MGMT). Tumor cells with high MGMT expression tend to be resistant towards temozolomide. While identifying genomic patterns of temozolomide-induced DNA alkylation may lead to improved patient stratification for therapy, there are no methods established for specifically mapping the chemically modified nucleic acid O^6-MeG structure at single nucleotide resolution. To address this limitation, we developed a new method for precisely locating O^6-MeG in the whole genome, based on immunoaffinity enrichment with an anti-O^6-MeG antibody and subsequent stalling of a high-fidelity polymerase at the methylated site. Genomic DNA was exposed to temozolomide and O^6-MeG was mapped revealing favored formation of damage in certain trinucleotide sequence contexts. O^6-MeG was preferentially formed following purines while reduced formation was found after pyrimidines. Moreover, this DNA damage signature was highly similar to a mutational signatures extracted from tumor genomes from patients treated with temozolomide. These findings suggest that mapping O^6-MeG at single-nucleotide resolution provides a better insight to temozolomide-induced DNA damage in the context of drug resistance.



Overcoming aggregation in the chemical synthesis of c-Myc[86-143]

H. Bürgisser[1], R. Lescure[1], B. Tamás [1], E. T. Williams[1], N. Hartrampf[1]*

[1]Departement of Chemistry, University of Zürich

Chemical protein synthesis enabled by solid-phase peptide synthesis (SPPS) could provide peptide and protein samples with a virtually unlimited chemical space (including PTMs) by giving access to non-canonical amino acids and backbone modifications. Merrifield's SPPS method has remained the most popular synthesis method for chemical protein synthesis since its introduction in the 60s.[1] Since then, decades of improvement and optimization have increased the length of synthesized peptide chains of up to 50 amino acids.[2] Over this limit, Native Chemical Ligation (NCL) has been developed to join synthesized fragments, ultimately leading to the production of larger proteins.[3] Yet, generating soluble fragments in good yield and purity is challenging and requires extensive synthesis efforts. Several solutions have been developed to address the solubility problem of unprotected peptides in aqueous buffers, such as highly charged solubility tags. However, identifying and suppressing aggregation of the growing peptide chain during SPPS -one of the main contributors to decreased synthesis outcome- is still very challenging. H-bond formation between the amide backbone is thought to be a major contributor of aggregation, giving rise to β-sheet formation.[5] Backbone modifications can be introduced to reduce aggregation, but screening for suitable positions is time consuming. A deeper understanding of aggregation, as well as a general solution to improve aggregation are therefore urgently needed. We use flow-based peptide synthesis to investigate the sequence dependence of aggregation using in-line UV-Vis monitoring. Upon aggregation, the Fmoc groups are less accessible for deprotection resulting in a broadening of the deprotection peak. Analysis of numerous aggregating sequences has allowed for the development of a new synthetic strategy that was applied to multiple aggregating peptides such as GLP-1 and barstar[75-90] and was shown to increase not only the synthesis but also the solubility of the heavily aggregating peptide fragment c-Myc[86-143]. In the future, we aim to demonstrate the applicability of our strategy to additional peptides and will further investigate how it disrupts aggregation during synthesis, to ultimately increase our understanding on aggregation.

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Advanced NMR Methods Enable the Identification and Structure-Activity Relationship of K-Ras Ligands

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

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

Because of its high information content, Nuclear Magnetic Resonance (NMR) is a powerful technique for drug discovery. However, NMR is a very insensitive method, it needs expensive isotope labelling and it is complex to analyze. Therefore, high sample concentrations, long measurements, high magnetic fields and long data analysis are required.

The recent progress in the field made at the Riek group and Orts group, including photo-Chemical Induced Dynamic Nuclear Polarization (CIDNP)[1] and NMR Molecular Replacement (NMR^2)[2] address the limitations of NMR-based drug discovery. Using photo-CIDNP, the NMR signal is enhanced, which reduces the amount of sample and measurement time. Furthermore, NMR^2 provides the possibility to calculate protein-ligand complex structures without complicated assignment.

Here we show, how we used these advanced NMR techniques to discover molecules binding to the oncogenic protein K-Ras (G12V). We first performed a screening using the DSI-poised library (900 fragments). We found 130 hits, among which we have validated 60. Their affinity constants were determined, and binders in the low mM affinity range have been identified. Finally, we could obtain five structures of K-Ras-fragments complexes, which will allow medicinal chemists to rationally evolve these fragments into potent K-Ras inhibitors.

This work demonstrates as well, how the recent scientific advances empower NMR-based drug design to tackle new challenges. The introduction of photo-CIDNP NMR reduces the screening time by 100-fold, and the sample amount by 20-fold. We also showcase that photo-CIDNP can be used for affinity determination of the hits. On the other side, we present new NMR experiments that get rid of the expensive isotopic labeling (^{13}C, ^{15}N). These experiments provide the data to calculate complex structures with NMR^2, within a week, while state-of-the-art structure determination requires expensive labeled samples and takes months. Our goal is that NMR will be the gold standard for early drug discovery within the next year.

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

W. Cai[1], M. Mivelaz[1], B. Fierz[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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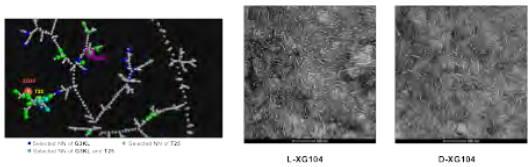
Stereorandomized Antimicrobial peptide dendrimers from Chemical Space against multidrug-resistant bacteria

X. Cai[1], M. Orsi[1], A. Capecchi[1], S. Javor[1], J. Reymond[1]*

[1]Departement für Chemie, Biochemie und Pharmazie, University of Bern

Nearest neighbours (NN) searching in virtual libraries has been a utility tool for optimizing antimicrobial peptide dendrimers (AMPDs).[1] AMPDs are promising candidates as antibacterial agents.[2] Our group previously discovered that stereorandomized AMPDs had reduced hemolysis while preserving antimicrobial activities.[3]Till now, NN searching has not been applied to search for new stereorandomized AMPDs in chemical space.

Herein, we selected 63 stereorandomized analogues of AMPD **T25** as nearest neighbour (NN) in chemical space by similarity using the MXFP fingerprint. Synthesis and testing of 63 stereorandomized analogues pointed to a new AMPD **XG45**. We then further modified this dendrimer by removing its N-termini to form **XG104** inspired by our previous pH dependent study.[4] Both **XG45** and **XG104** showed good potency against a panel of Gram-negative bacteria, they also showed good activity against **MRSA** at pH 8.0. Interestingly, the homochiral all L- and all D- versions of both **XG45** and **XG104** are not antibacterial but strongly hemolytic, this unusual finding indicates that stereorandomization not only can reduce hemolysis but also can confer antibacterial activity.



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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, Institute of Chemical Sciences and Engineering (ISIC), École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland, [2]Department of Biochemistry, University of Geneva, 1211 Geneva, Switzerland

Microtubules (MT) are dynamic polymers that are critical for cell regulation and organismal processes. MTs polymerize from a variety of tubulin isotypes and further carry post-translational modification (PTMs), in particular polyglycylation, poly-glutamylation (polyE) or detyrosination (ΔY) in the C-terminal tails. The combinations of isotypes and modifications generate a "tubulin code"[1], which acts as a fine regulator of MT function, whereas its dysregulation results in defects in development, fertility or disease. Due to a lack of methods to study tubulin PTMs, the mechanisms of action of many MT PTMs are still not well understood.

We recently developed a methodology to produce tubulins, which carry defined PTMs within their C-terminal tails via protein splicing[2], connecting synthetic peptides to recombinant tubulin proteins. This allowed us to explore the crosstalk between polyE and ΔY. However, in this study we installed the polyE modification via azide-alkyne Huisgen cycloaddition (click-chemistry[3]), resulting in a non-native analog. Here, I develop novel synthetic strategies to install native polyE branches on tubulin peptides. The installation strategy is done by fragment ligation of a carboxyl-protected polyE peptide and a fully-protected tubulin tail fragment. By using uncommon protection groups, such as cyanosulfurylide (CSY), we can selectively deprotect amino acids and also ensure the solubility of highly protected peptides, which are critical for further fragment ligation yield. This will allow us to produce fully native, modified tubulins for a detailed exploration of the tubulin code.

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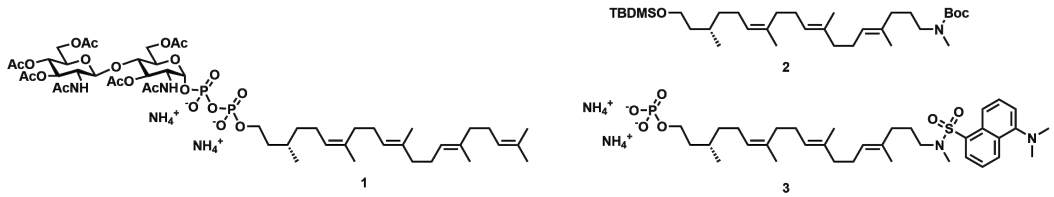
Fluorescent LLOs – Shedding light on OST and ALG enzymes

M. de Capitani[1], J. Reymond[1]*

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

Lipid Linked Oligosaccharides (LLOs) like **1** and their related compounds are important intermediates in the N-Glycosylation pathway in eukaryotes. This pathway is chiefly conducted by ALG and OST enzymes in the endoplasmatic reticulum. In recent years, our laboratory has provided numerous LLOs, inhibitors and related substrates for in-vitro investigations of both classes of enzymes. [1–4]

Extending on this work, we developed a synthetic approach to a fluorescent Dolichol-derived lipid meant to serve in synthetic LLOs. Given the intended role as substrate for in vitro studies, a reliable synthetic approach was required. In anticipation of the usually low yields in the pyrophosphate coupling just prior to final deprotection, the early stages of the synthesis also would need to be performed in multigram to decagram scale. Retrosynthetic analysis revealed lipid **2** as key intermediate, bearing two orthogonally protected end groups. From there, a variety of different N-linked fluorophore and O-linked phosphate-carbohydrate combinations are accessible.



Synthetic access to **2** was envisioned to be possible through either one of two major routes whose main difference lies in the order of lipid elongation and functionalisation. Opting for an early establishment of the N terminus followed by lipid elongation, a synthetic route was devised to give access to **2** in a 13 steps convergent synthesis starting from naturally occurring S-Citronellol and Farnesol. This sequence also featured a heavily modified Wittig-Schlosser reaction [5] and a regioselective oxidation of Farnesol [6] as key steps in establishing functionality and stereochemistry of the final product.

From this intermediate, N-functionalisation using Dansyl chloride and subsequent O-phosphorylation furnished the fluorescent lipid phosphate **3** from which the LLO analogous to **1** could be obtained in two steps to end a 22 step, 15 step longest linear sequence synthesis.

Building on this basis, a wide variety of compounds bearing modified sugars, phosphonate linkages or different fluorophores have been or are currently being prepared to serve as molecular probes.

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Frame Shifts Affect the Stability of Collagen Triple Helices

T. Fiala[1], E. P. Barros[2], M. Ebert[1], E. Ruijsenaars[2], S. Riniker[2], H. Wennemers[1]*

[1]Laboratory of Organic Chemistry, ETH Zürich, D-CHAB, Vladimir-Prelog-Weg 3, 8093 Zürich, Switzerland, [2]Laboratory of Physical Chemistry, ETH Zürich, D-CHAB, Vladimir-Prelog-Weg 2, 8093 Zürich, Switzerland

Collagen model peptides (CMPs), composed of proline–(4*R*)hydroxyproline–glycine (POG) repeat units, have been extensively used to study the structure and stability of triple-helical collagen – the dominant structural protein in mammals – at the molecular level. Despite the more than 50-year history of CMPs and numerous studies on the relationship between the composition of single-stranded CMPs and the thermal stability of the assembled triple helices, little attention has been paid to the effects arising from their terminal residues. Here, we show that frame-shifted CMPs, which share POG repeat units but terminate with P, O, or G, form triple helices with vastly different thermal stabilities. A melting temperature difference as high as 16 °C was found for triple helices from 20-mers Ac-$[OGP]_6OG$-NH_2 and Ac-$[POG]_6PO$-NH_2 and even triple helices of the constitutional isomers Ac-$[POG]_7$-NH_2 and Ac-$[GPO]_7$-NH_2 melt 10 °C apart. A combination of thermal denaturation, CD and NMR spectroscopic studies, and molecular dynamics simulations revealed that the stability differences originate from the propensities of the peptide termini to preorganize into a polyproline-II helical structure. Our results advise that care needs to be taken when designing peptide mimics of structural proteins as subtle changes in the terminal residues can have unexpected consequences on their properties. Our findings also provide a general and straightforward tool for tuning the stability of triple helices formed by CMPs for applications in synthetic materials and biological probes.

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

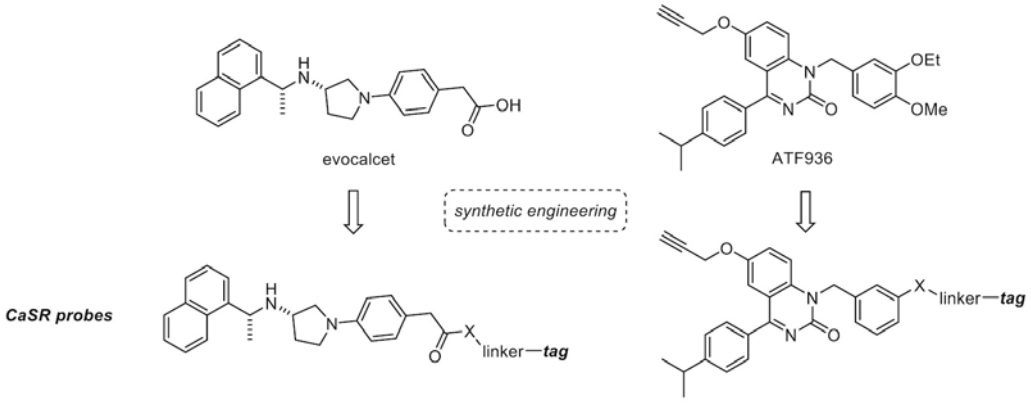
J. Fischer[1,3], D. Batora[1], L. Dick[1,3], R. Kaderli[2], J. Gertsch[1,3], M. Lochner[1,3]*

[1]Institute of Biochemistry and Molecular Medicine, University of Bern, Bühlstrasse 28, 3012, Bern, Switzerland, [2]Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, Freiburgstrasse, 3010 Bern, Switzerland, [3]Swiss National Centre of Competence in Research, NCCR TransCure, Bern, Switzerland

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 ATF936 and AXT914, and of evocalcet. In this context, we present our work on the synthesis of these probes and their preliminary biological assessment.



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Design and Biological evaluation of Drug-Like Inhibitors Targeting the Allosteric Site of the SARS-CoV-2 Main Protease Enzyme

M. A. Ghattas[1,2], L. Alzyoud[2], F. Mohamed[3], B. R. Ali[3], W. Rabeh[4], N. Atatreh[1,2]

[1]College of Pharmacy, Al Ain University, Abu Dhabi, UAE, [2]AAU Health and Biomedical Research Centre, Al Ain University, Abu Dhabi, UAE, [3]Department of Pathology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, UAE, [4]New York University Abu Dhabi, Abu Dhabi, UAE

With more than 240 million confirmed cases and near to 5 million deaths (as per WHO records), the COVID-19 pandemic is far from over and continues to impose challenges to almost every aspect of our daily lives. Vaccines seem to tackle some of these challenges, nonetheless, the continuously-evolving variants inform us about the necessity of developing new antiviral agents that can work in parallel with vaccines to put an end to this global crisis. We will be approaching this goal through targeting one of the most important enzymes in the SARS-COV-2 life cycle, the main protease enzyme (M^{pro}) [1]. There has been extensive research worldwide for the development of new M^{pro} inhibitors, most of which target the enzyme catalytic pocket. However, not much work has been conducted on the M^{pro} allosteric site, which we intend to target in this work, as it is believed to be less prone to mutations and more conserved among various coronaviruses strains. For the aforementioned reasons, we assessed all possible allosteric sites on M^{pro} dimer structure [2] and then we tackled the most promising sites via computer aided drug design by virtually screening >3.7 million ligands. Interestingly, several small inhibitors have been identified so far and experimental work is under development to determine their inhibition activity. Up to our knowledge, this is the first study for assessing allosteric sites and targeting them with a comprehensive computer-aided drug design approach. The findings from this work can help in the current battle against COVID-19 and, potentially against future corona viruses-related diseases.

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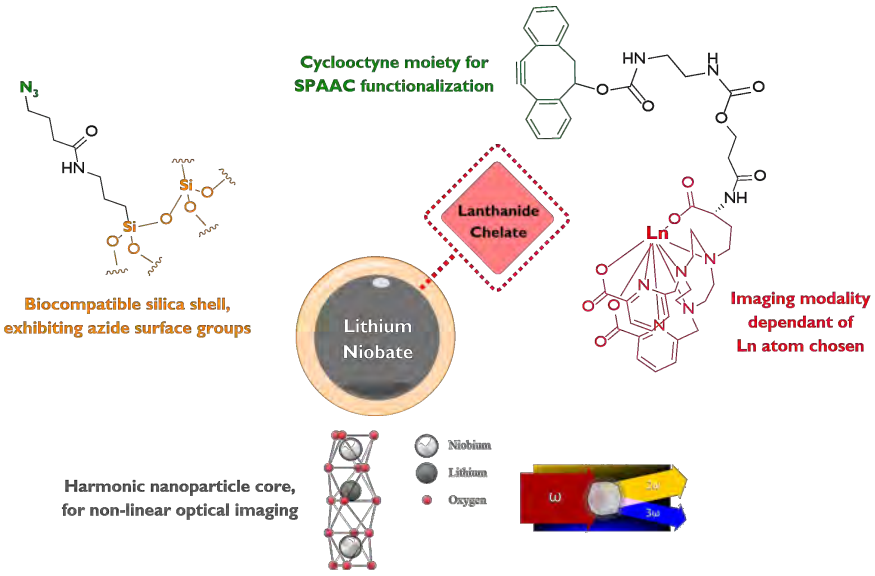
Lanthanide-Functionalized Lithium Niobate Nanoparticles for Multimodal Imaging

A. Gheata[1], A. Spada[1], S. Dumolard[1], M. Wittwer[1], P. Llussà[2], L. Bonacina[2], A. Chauvin[3], S. Gerber-Lemaire[1]*

[1]Institute of Chemical Sciences and Engineering, SCI-SB-SG, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland, [2]GAP-Biophotonics, University of Geneva, CH-1211 Geneva, Switzerland, [3]Institute of Chemical Sciences and Engineering, SCI-SB-MM, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Nanomaterials have attracted high interest in the biomedical field for their numerous favorable properties, including their large surface area, their potential for multi-functionalization, and their long blood circulation time compared to small molecules. Nanoscale systems integrating multiple imaging modalities allow for precise diagnosis, which could serve in the field of oncology to detect malignancies at an early stage of their development and thus increase the success rate of therapeutic interventions. The inherent shortcomings of individual imaging techniques can be overcome by their synergistic combination within a single nanoparticle platform, thereby providing simultaneously high spatial resolution, penetration depth and sensitivity. Harmonic nanoparticles (HNPs), are metal oxide nanomaterials characterized by a crystalline structure lacking inversion symmetry (e.g $LiNbO_3$, LNO). This unique property allows them to exhibit a non-linear optical response by generating second- and third- harmonic signals upon laser irradiation, with long-term photostability and spectral flexibility. These materials have thus emerged as attractive novel probes for optical bioimaging. Although advances in the field of optical imaging are now giving access to penetration depths in the order of the millimeter thanks to new laser sources, this modality is still not adapted for whole-body imaging. Magnetic resonance imaging (MRI) and X-ray computed tomography (CT) are more adapted for the acquisition of anatomical pictures due to their high tissue penetration capacity. We aim at functionalizing LNO HNPs with various lanthanide chelates to explore their multimodal capabilities. LNO HNPs are first coated in a silica shell using a water-in-oil microemulsion approach to improve their biocompatibility and display surface reactive azides for post-functionalization. The lanthanide chelates, designed with a cyclooctyne moiety, are then grafted on the coated LNO HNPs by strain-promoted azide-alkyne cycloaddition (SPAAC). Through the variation of the lanthanide atom, different imaging modalities can be achieved such as MRI with gadolinium, CT with ytterbium, and various luminescence wavelengths with europium and terbium. We thus aim at producing multimodal HNPs with complementary capabilities for cancer diagnosis.



Rapid Synthesis of Trifunctionalised Chemical Biology Probe from Unprotected L-Cysteine

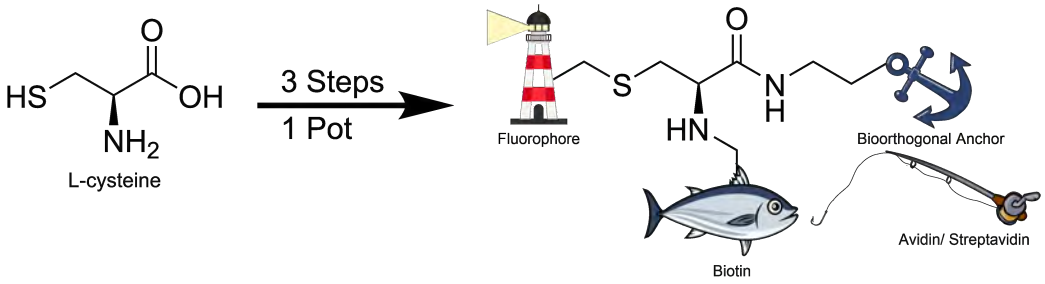
P. Grossenbacher[1], M. Lochner[1]*

[1]University of Bern, Institute of Biochemistry and Molecular Medicine, Bühlstrasse 28, 3012 Bern

Outline

Biotinylated or fluorescent probes are widely used in biochemistry labs. The use of biotinylated probes in combination with its binder avidin or streptavidin allows for selective pull-down of target proteins from lysates or other complex mixtures.[1] On the other hand, fluorescent probes are ubiquitously used to find and monitor targets in equally complex mixtures.[2] A polyfunctional chemical biology probe that combines several useful properties (e.g. biotin, fluorophore and bioorthogonal handle) can be a very powerful research tool in target identification and proteomic projects. Amino acids represent convenient scaffolds for multifunctional probe development but lengthy syntheses and protection/deprotection strategies can complicate access to such probes.

Here, we present a simple way to modify unprotected L-Cysteine with biotin, Si-Rhodamine, and a clickable azide linker in just three steps.



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Structure-based modelling of HIF2α warheads for PROTAC design

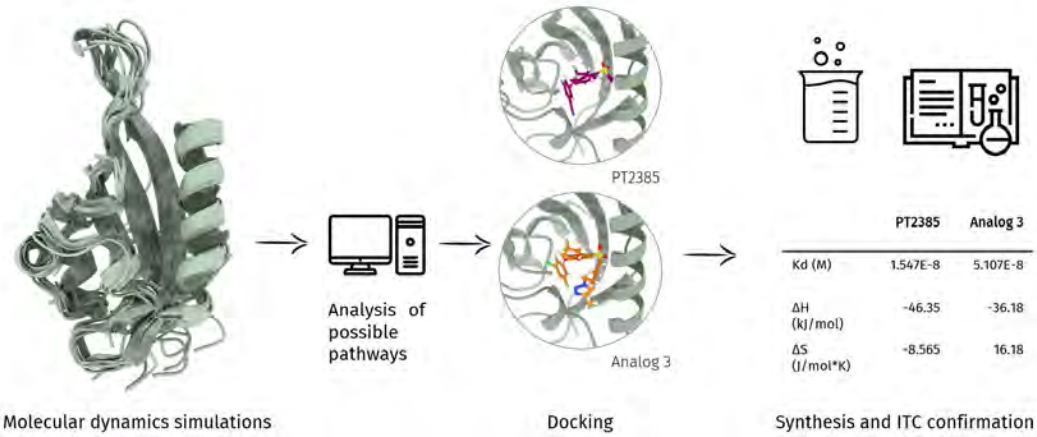
M. Héritier[1,4], C. Cecchini[1,4], V. Trush[2], F. L. Gervasio[3,4], F. Rastinejad[2], L. Scapozza[1,4]*

[1]Pharmaceutical Biochemistry Group, School of Pharmaceutical Sciences, University of Geneva, Rue Michel-Serve 1, 1206, Genève, [2]Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK, [3]Biomolecular and Pharmaceutical Modelling Group, School of Pharmaceutical Sciences, University of Geneva, Rue Michel-Servet 1, 1206, Genève, [4]Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva

We studied here the dynamics of HIF2α PAS-B domain, with the aim of designing and synthesizing a Proteolysis Targeting Chimera (PROTAC) to selectively degrade HIF2α, an upstream protein of the VEGF pathway involved in clear cell renal cell carcinoma[1, 2]. PROTACs consist of three parts: an E3 ligase recruiter, a small molecule that binds to the target and a linker.

In the holo form of HIF2α, the inhibitor PT2385 replaces water in the buried cavity with only minor residue rearrangement compared to the apo structure[3]. This suggests that HIF2α PAS-B domain can be found in a transient open conformation. The absence of open channels challenged the PROTAC design as we could not infer the direction of functionalization from the crystal structure.

We used molecular dynamics to understand how, where and with which amplitude HIF2α might open. The different simulations showed that the opening of the cavity seems to be more favorable at junction of AB loop, helix E and F. We then modelled the mutant HIF2α^{M252A} to create a channel at the said junction and tested different analogs of PT2385 by docking. We found that modified PT2385 with an azidobutane at the sulfonyl end (called analog 3) retrieves the original binding mode and reaches the solvent. After synthesis, we measure the binding affinity. While the K_d was similar, we noticed an increase of entropy in the process of binding compared to the original inhibitor. This effect might come from the partial closure of HIF2α and the loss of a crystal water molecule in the pocket. Future simulations will help us improve the PROTAC design in a rational manner and might provide a useful *in silico* protocol for similar cases.



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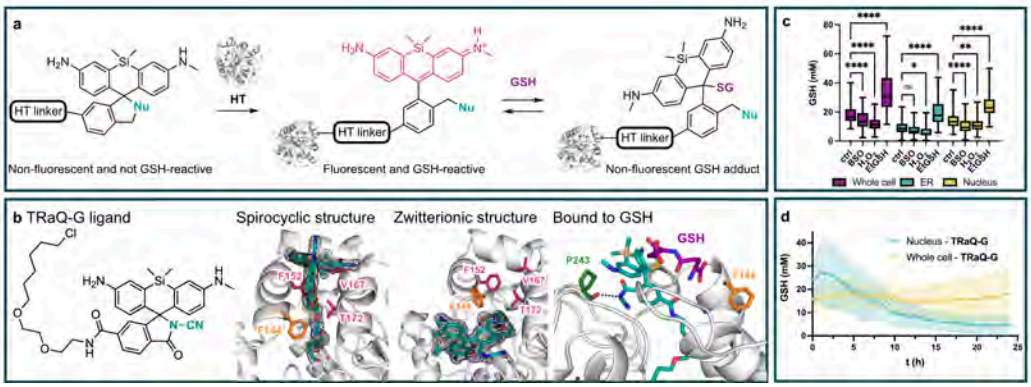
A Locally Activatable Sensor for Robust Quantification of Organellar GSH

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

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

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. Several significant advances have been made toward observing GSH dynamics using various sensors for live-cell imaging. Yet, a general tool to assess dynamic changes in GSH concentration featuring flexibility in terms of subcellular targeting or spectral range is still lacking. Here, we propose a **T**argetable **Ra**tiometric **Q**uantitative **G**SH sensor (**TRaQ-G**)[1] based on the previously reported reactivity of certain silicon rhodamines (SiRs) towards GSH.[2] We installed a nucleophilic group on our reporter fluorophore which leads in the unbound state to a spirocyclic, non-fluorescent conformation. Only upon binding to the HaloTag (HT) protein, the fluorescence, as well as the GSH-responsiveness, is switched on (panel a). The background is decreased, and the dye is prevented from dragging GSH while traveling to its target. The HT can be fused to a fluorescent protein which functions as a reference. This system allows for a robust ratiometric read-out, and the genetically encoded part of the sensor enables flexibility in terms of targeting and spectral range. To characterize our system, we performed *in vitro* experiments with purified protein, X-ray crystal structure analysis (panel b), molecular dynamics simulations (panel b), and live-cell imaging.
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 cells with our sensor (panel d). Our results indicate a separate nuclear GSH pool with an especially high concentration right before cell division which matches previous reports.[3]



a) Working principle of TRaQ-G. b) Spirocyclic structure of the TRaQ-G ligand, the corresponding crystal structures with HT in the spirocyclic form and the open form as well as a snapshot from a molecular dynamics simulation of the TRaQ-G GSH adduct. c) Response of TRaQ-G to modulations of the GSH concentrations in different organelles. d) Measured GSH concentration in synchronized HeLa cells in the course of the cell cycle.

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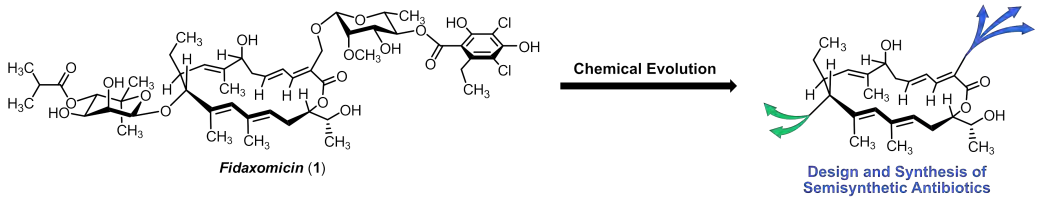
New Fidaxomicin Antibiotics: Combining Metabolic Engineering and Semisynthesis

E. Jung[1], M. Hunter[2], J. Costafrolaz[3], Y. Mattenberger[3], S. Dittmann[4], D. Schäfle[5], T. Griesser[5], P. Sander[5,6], S. Sievers[4], P. Viollier[3], R. Müller[2]*, K. Gademann[1]*

[1]Department of Chemistry, University of Zurich, Zurich, Switzerland, [2]HIPS, Department of Microbial Natural Products, Helmholtz Centre for Infection Research and Department of Pharmaceutical Biotechnology, Saarland University, Saarbrücken, Germany, [3]Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland, [4]Department of Microbial Physiology and Molecular Biology, Institute of Microbiology, Center for Functional Genomics of Microbes, University of Greifswald, Greifswald, Germany, [5] Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland, [6]National Center for Mycobacteria, University of Zurich, Zurich, Switzerland

Fidaxomicin (**1**, tiacumicin B, lipiarmycin A3)[1,2] constitutes a macrocyclic antibiotic which demonstrates potent activity against various Gram-positive bacteria through inhibition of RNA-polymerase (RNAP).[3,4] Fidaxomicin is the standard of care to treat *Clostridioides difficile* infections and it also features *in vitro* activity against resistant strains of *Mycobacterium tuberculosis* and *Staphylococcus aureus*, yet its unfavourable PK/PD profile prevents its application as a systemic antibiotic.

Structural evolution of a parent natural product is the primary source of new antibiotics. Even now, 50 years after the discovery of **1**, only a few derivates have been described to date. Our goal is the development of the next generation of fidaxomicin antibiotics unlocking new therapeutic applications.[5–7] We have developed a platform that allows for the selective replacement of each of the moieties decorating the macrocycle. In this presentation, we will present our synthetic strategy, the computationally-guided design of new derivatives, and explore their biological activities against various relevant pathogens.



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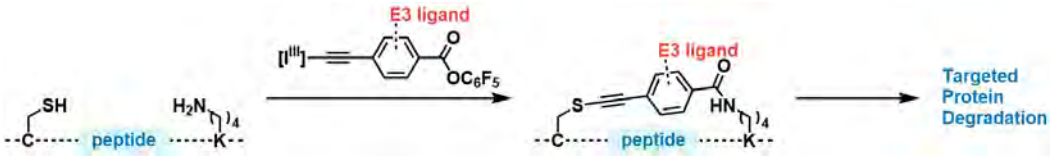
Stapled-Peptide PROTACs by Hypervalent Iodine Staples

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

[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. Indeed, 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 E3 ligand-loaded hypervalent iodine staples based on our previous report,[2] which readily transformed peptides into stapled-peptide PROTACs for degradation of undruggable proteins. Peptide stapling is known to stabilize α-helices and improve inappropriate properties for in vivo efficacy of peptides, namely low membrane permeability and low proteolytic stability. These staples modify peptides to have degradation activity as well as good physicochemical properties targeting intracellular environment at the same time. Thus, this tool would pave the way for rapid drugging of currently untouched proteins, even if small-molecule ligands are not available. We first chose to target the steroid receptor coactivator-1 (SRC-1) which interacts with transcription factors, with a reported SRC-1 degrader as a benchmark.[3]



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Aryltetralin lignans from *Hyptis brachiata* inhibiting human T-cell proliferation

M. Keller[1], M. Winker[2], N. Sperisen[1], M. Gupta[3], M. Hamburger[1], C. Gründemann[2]*, O. Potterat[1]*

[1]Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland, [2]Translational Complementary Medicine, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 80, 4056 Basel, Switzerland, [3]Centro de Investigaciones Farmacognosticas de la Flora Panamena (CIFLORPAN), Universidad de Panama, Apartado 0824-00172, Panama City, Panama, Facultad de Farmacia

Enhanced proliferation and activation of T lymphocytes is known to play a crucial role in the pathogenesis of autoimmune diseases and chronic inflammation. Current treatment options with immunosuppressant drugs often do not provide long-lasting relief of symptoms and show a gradual loss of efficacy over time, or they are accompanied by unwanted side effects [1]. Therefore, novel immunosuppressive lead compounds are needed.
For this purpose, an in-house library consisting of 600 extracts from Panamanian plants was screened for inhibition of human T-cell proliferation. As one of the hits, an ethyl acetate extract from the aerial parts of *Hyptis brachiata* (Lamiaceae) exhibited strong inhibitory effects. An HPLC-based activity profiling approach resulted in the targeted isolation of seven aryltetralin lignans, five arylnaphthalene lignans, two flavonoids, three triterpenes, and cinnamyl cinnamate. The aryltetralin lignans inhibited T-cell proliferation in a concentration-dependent manner (IC50s of 0.1 - 3 µM) without showing any signs of cytotoxicity at these concentrations. No relevant inhibition was observed for the arylnaphthalene lignans, flavonoids, and triterpenes. Further investigation using a cell cycle arrest assay revealed that the aryltetralin lignans potently inhibited mitosis similar to podophyllotoxin. This suggests an interaction with microtubule formation via an inhibition of tubulin polymerization

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A short motif goes a long way: How minimal ribozymes make large proteins redundant

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

The hepatitis delta virus (HDV) ribozyme catalyzes site-specific self-cleavage and was first discovered in the single-stranded circular RNA virus HDV [1]. HDV-like ribozymes (delta ribozyme; DRZ) share the conserved nested double-pseudoknot structure motif and have been found in all domains of life, including humans. Through a bioinformatic search using an adapted minimal active DRZ motif [2], we discovered hundreds of novel minimal DRZ sequences in bacteriophage genomes associated with the human microbiome. A subclass of these hits was identified to occur in direct conjunction with viral tRNA genes (Figure 1, bottom left). Correct tRNA processing is essential for their function and consequently protein biosynthesis. This includes the cleavage of 3'-trailers, which is known to be catalyzed by both endo- and exonuclease (ribo-)proteins (Figure 1, top left). With our discovery, we have found an additional, solely RNA-based factor that can site-specifically cleave tRNA 3'-trailers, thus making large protein enzymes redundant for this task. Studies of these novel tRNA-associated ribozymes will provide valuable insights into their function, thereby elucidating their biological and catalytic roles *in vivo*, which are still poorly understood.

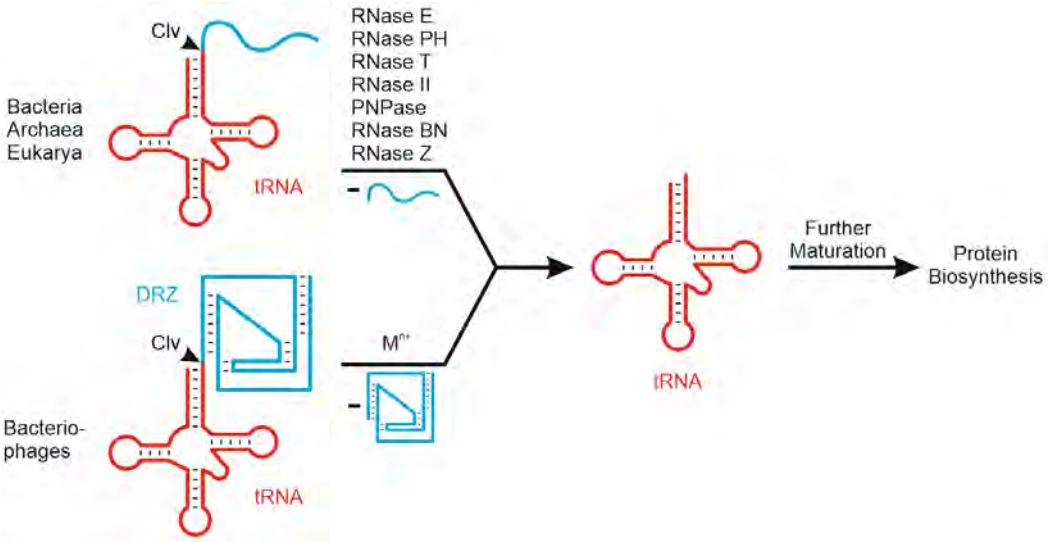

Figure 1: Processing of tRNA 3'-trailers in Bacteria, Archaea and Eukarya can be performed by several endo- and exonucleases (top). A novel mechanism was discovered in bacteriophages, where the 3'-trailer folds into a DRZ motif and site-specifically cleaves the trailer without additional protein factors (bottom).

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Engineering nonviral protein cages for delivery applications

M. D. Levasseur[1], D. Hilvert[1]*

[1]ETH Zürich

Throughout history, humankind has developed a broad collection of therapeutic agents to treat diseases. Their efficacy depends on (i) the ability to engage a relevant biological target, (ii) stability in patients, (iii) pharmacokinetic properties, and (iv) few off-target effects. Molecular systems that protect, transport, and deliver active drug molecules in targeted fashion can optimize these factors. For example, the recent clinical success of nucleic acid vaccines against SARS-CoV-2 was enabled by the development of viral vectors and lipid nanoparticles as specialized delivery platforms. However, given the large number of still untreated diseases and ongoing threats from countless pathogenic viruses and microorganisms, there is a great need for new flexible and robust delivery platforms.

Nonviral protein cages have many desirable characteristics for delivery applications. They come in a range of scaffold sizes and symmetries, are amenable to engineering of targeting and packaging properties, can be produced easily and cheaply, and often exhibit high physical stability, which can expedite global distribution. In this work, we chose structurally distinct protein cages as starting scaffolds and rationally engineered their properties to promote the delivery of therapeutically relevant molecules.

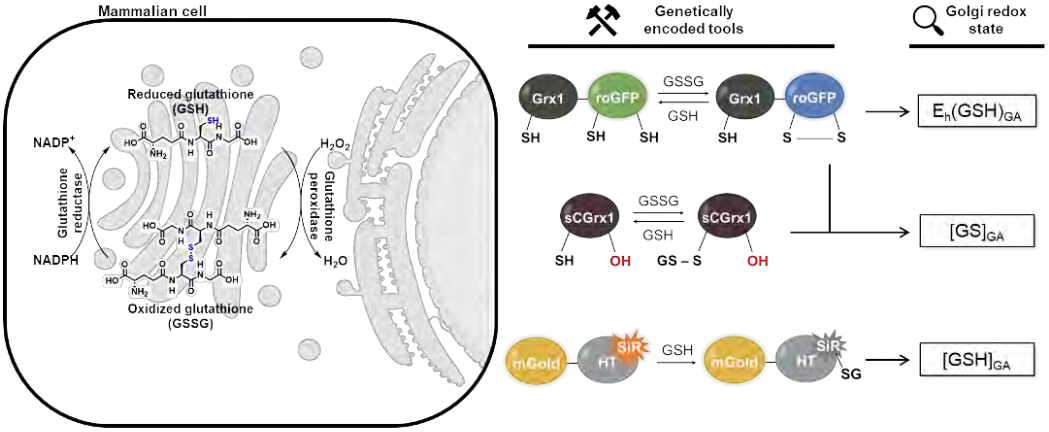
Determining the redox state of the Golgi apparatus by using genetically encoded tools

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

[1]Chemistry Department, University of Zürich, Winterthurerstrasse 190, Zürich, Switzerland, [2] Carla.mirovinals@uzh.ch

Redox homeostasis is essential for cell functioning and its study in a compartmentalized manner is key given that the redox pairs ratios differ 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) given their role in buffering the redox state of the cell.[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 recently published GSH ratiometric sensor called 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.



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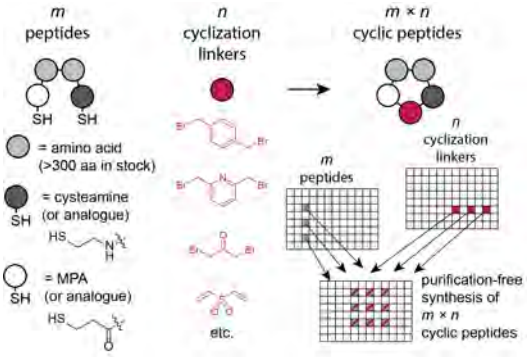
Purification-free synthesis and screening of thousands of cyclic peptides

A. L. Nielsen[1], A. S. Zarda[1], Z. Bognar[1], G. K. Mothukuri[1], C. Heinis[1]*

[1]Ecole Polytechnique Fédérale de Lausanne (EPFL)

Macrocyclic peptides provide an attractive modality for drug development due to their ability to bind feature-less protein targets, for which small-molecule binders are extremely difficult to obtain. However, synthesis of large macrocyclic libraries is currently limited by low-yielding cyclization reactions and the need for chromatographic purification of individual peptides. Therefore, there is an unmet need for methods that provide easier and faster access to macrocycle libraries applicable for high-throughput screening campaigns against challenging targets. Herein we report a method that allows for the synthesis of thousands of diverse macrocycles based on the cyclization of dithiol peptides with bis-electrophilic linkers. Synthesis is initiated by functionalizing cysteamine derivatives onto resin immobilized via a disulfide bridge. Following automated SPPS in 4×96-well format, side-chain protecting groups are removed with TFA mixture, which leaves only unprotected dithiol-peptide left on the resin. Subjection of a volatile reducing reagent leads to reductive release of crude dithiol peptides in excellent purity, which are cyclized with a plethora of linkers in a fully automated fashion applying automated liquid handling and acoustic droplet ejection in 384- or 1536-well plates to provide access to thousands of cyclic peptides. The strategy was utilized to create a focused library of 2,688 structurally diverse macrocycles for which one of the most potent thrombin inhibitors reported to date was identified. Conclusively, the method overcomes a major bottleneck in producing macrocyclic peptide libraries, as no purification steps are required for the synthesis of thousands of peptide macrocycles that can be directly used in screening campaigns.



Synthesis and biological evaluation of small-molecule inhibitors of Tumor Necrosis Factor Receptor 1 (TNFR1)

S. Pannilunghi[1,2], S. Tardy[1,2], A. Gouiller[1,2], M. Héritier[1,2], D. Pejoski[1,2], L. Scapozza[1,2]*

[1]School of Pharmaceutical Sciences, University of Geneva, Switzerland, [2]Institute of Pharmaceutical Sciences of Western Switzerland, Switzerland

Deregulation of the Tumor Necrosis Factor (TNF) pathway is responsible for the pathological onset of various autoimmune disorders and the perpetuation of chronic inflammation that adversely affects more than 50 million people worldwide.[1] TNFα interacts with two distinct trans-membrane receptors: TNFR1 and (TNFR2). Whereas TNFR1 signaling is principally pro-inflammatory and apoptotic, TNFR2 mediates the anti-inflammatory response and promotes cell survival and tissue regeneration. The current standard of care for a broad spectrum of chronic inflammatory conditions consists of using TNF inhibitors that directly prevent TNFα binding with both receptors. However, total TNF inhibition often leads to severe side effects, mainly caused by the impairment of the TNFR2-mediated response.[2] Indeed, achieving the selective inhibition of TNFR1 over TNFR2 would address a challenging, unmet medical need in the field of autoimmune and autoinflammatory diseases.

By exploiting a structural difference between TNFR1 and its related isoform, a library of small organic fragments was screened using the differential scanning fluorimetry (DSF) assay against the receptor. Further binding affinity tests, such as the nanoDSF assay and the microscale thermophoresis, were also conducted in parallel. The outcome of the screening process was the identification of one molecule presenting a dose-dependent destabilizing activity in the µM range of concentrations against the receptor. Based on this preliminary pharmacophore, we have synthesized several structural analogs that we tested on Hela cells, using the flow cytometry assay as a readout. Six of these molecules caused the dose-dependent disappearance of TNFR1 from the cell surface.

To develop analogs with higher affinity, we performed an accurate analysis of the chemical space around the region of interest (RoI), and we investigated the binding mode of the preliminary hit molecules. Following digestion of substance-bound TNFR1 with GLU-C endoproteinase, we determined by LC-MSMS analysis that our fragments are covalent binders of specific AAs in the RoI. To identify which residue is responsible for binding, we created several TNFR1 mutants in which different AAs were mutated separately. We demonstrated that while mutants retained their activity upon stimulation with the physiological ligand, cells expressing a particular TNFR1 mutation were less responsive to the selected molecules compared to cells expressing WT TNFR1 or other TNFR1 mutated forms.

Recently, we have assessed the inhibitory behavior of our hit molecules with the Lumit Immunoassay Cellular Systems. The results indicate that the disappearance of the receptor from the cell surface at increasing concentration of compounds is consistent with a decreased activation of the TNFR1-mediated inflammatory pathway.

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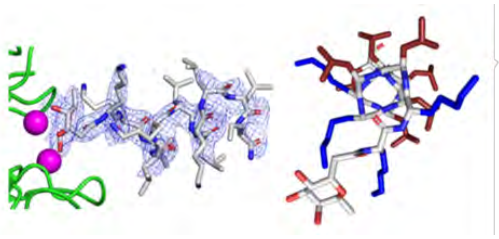
X-ray Structures of Mixed-chirality α-Helical Antimicrobial Peptides

H. Personne[1], A. Stocker[1], S. Javor[1], J. L. Reymond[1]*

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

Peptide α-helicity mostly depends on its amino acid sequence and is right or left-handed depending on amino acids chirality (respectively L- or D-). However, mixed-chirality sequences are usually unfolded. In case of antimicrobial peptides (AMPs), an amphiphilic α-helix is generally required to be active and research on mixed chirality AMPs is poorly documented. We recently reported the first X-ray crystal structures of mixed chirality short bicyclic and linear AMPs forming α-helices as complexes of fucosylated analogs with the bacterial lectin LecB (see figure).[1] Following up the study on our mixed-chirality peptide ln69, we discovered new chirality patterns also presenting α-helical conformation both in membrane-like environment and as helix bundle determined by X-ray crystallography in aqueous condition. Compared to their homochiral parent, these mixed chirality peptides display better stability in human serum, as well as, in selected cases, improved antimicrobial activity.



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Photopharmacology and smart biocompatible materials based on cyclic dipeptides.

Z. L. Pianowski[1,2], S. Kirchner[2], A. Leistner[2], P. Gödtel[2], A. Seliwjorstow[2], V. Schäfer[2]

[1]Institute of Biological and Chemical Systems-FMS KIT, [2]Institute of Organic Chemistry KIT

Cyclic dipeptides (CDP) are popular pharmacophores and interesting biocompatible structural motifs that share structural and recognition features with peptides and proteins. Gliotoxin or Spirotryptostatin are exemplary CDP-based bioactive compounds, while tadalafil, retosiban and plinabulin are CDP-based drugs. Many other CDPs serve e.g. as signaling molecules or in bacterial quorum sensing.

We have appended CDPs with molecular photoswitches, which resulted in photoswitchable hydrogelators. Here, we want to report on our biocompatible CDP-based supramolecular hydrogels that reversibly dissipate to fluids upon irradiation with green or red light. They can be used for light-triggered drug delivery systems, biocompatible 3D photoprinting, or as smart medium for tunable growth of organoids.

In the course of our research, we have also discovered novel biocompatible molecular photoswitches, i.a. switchable with the therapeutically relevant red light.

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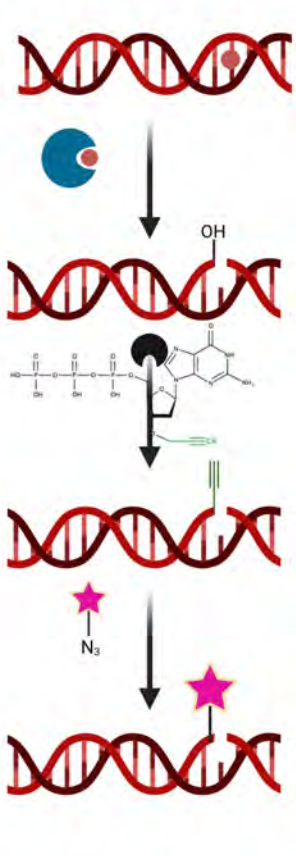
Fluorescence-guided Quantification and Mapping of DNA Damage in the Human Genome

N. J. Püllen[1], V. Takhaveev[1], N. K. Singh[1], A. Poetsch[2], H. Gahlon[1], S. J. Sturla[1]*

[1]ETH Zurich, Schmelzbergstrase 9, 8092 Zurich, Switzerland, [2]Technical Universty Dresden - Center of Biotechnology - Tatzberg 47-49, 01307 Dresden, Germany

Chemically induced DNA damage can accumulate in the genome or transform into mutations causing cancer or accelerated ageing. Major DNA damage types are single strand breaks, apurinic sites and oxidatively modified nucleobases. New DNA damage sequencing methods are rapidly emerging,[1] but accurate damage sequencing is impaired by naturally abundant generic damage sites and artifacts produced during sample processing and determining overall damage levels is technically difficult. Therefore, we developed a fluorescence-based method for rapid and cost-effective quantification of DNA lesions in the human genome. We accurately detected UV- and chemically induced oxidative lesions and apurinic sites in human cells and verified the results using mass spectrometry. We observed inherent differences in DNA damage levels between healthy and cancer cells. Furthermore, we applied our method to design a low-background sequencing protocol to map oxidative lesions and apurinic sites in the human genome. Single nucleotide resolution and the application of molecular barcodes allowed us to characterize the sequence context of damaged bases and damage distribution in genomic regions of functional importance. We found a depletion of damage in defined nucleobase sequences and transcriptionally active regions suggesting epigenetic functions and an active mechanism safeguarding these areas. The new fluorescence-based method was used to improve damage sequencing protocols and furthermore enables rapid quantification of a variety of common DNA damage types. Accurate damage sequencing and quantification paves the way for novel diagnostic tools and an in-depth understanding of the link between DNA damage and disease.



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The sulfonated Molybdenum Cofactor influences the Structure of the *moaA* riboswitch

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

[1]University of Zurich

The molybdenum cofactor (Moco) is a metal complex composed of a pterin moiety and a molybdenum coordinated via a dithiol bond, which is highly oxygen sensitive. The enzyme Xanthine Oxidase accommodates a sulfonated Moco molecule, which is less oxygen sensitive and only differs from Moco with one sulfur coordinated to the molybdenum instead of an oxygen.

We isolated sulfonated Moco from Xanthine Oxidase via heat treatment and showed with in-line probing that under anaerobic conditions sulfonated Moco induces a change on the *moaA* riboswitch structure. The *moaA* riboswitch is a highly conserved sequence of noncoding mRNA that upon binding of a specific cellular metabolite, which has been suggested to be Moco, changes its tertiary structure.[1] This change in structure controls the expression of downstream genes, displaying an exemplary representation of structure meets function.

Given that the isolated sulfonated Moco can only cause a structural change of the riboswitch under anaerobic conditions strongly suggests that it is indeed the oxygen sensitive molecule inducing the structural change. In earlier experiments, we showed that all stable biosynthetic precursors of Moco as well as synthetic structural analogs containing a pterin moiety showed no influence on the *moaA* riboswitch structure.[2] Our results strongly indicate that even though the substitution of a oxygen with a sulfur at the coordination sphere of the molybdenum does not seem to influence the recognition by the riboswitch at least one of the additional functional moieties of Moco besides the pterin, such as the complexed molydenum and/or the phosphate group, are necessary for the recognition by the riboswitch.

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Nanopore detection of carboxymethylated DNA bases

E. S. Sandell[1]

[1]Dept. of Health Science and Technology, ETH Zürich, Schmelzbergstrasse 9, 8092 Zürich, Switzerland

Carboxymethyl DNA adducts are chemical modifications associated with high red and processed meat consumption. [1] They can impede DNA replication machinery and induce mutations potentially leading to the development of colorectal cancer. Studies in polymerase knock-out cells indicate that carboxymethyl DNA adducts, such as O^6-CMG, can be bypassed by human DNA polymerases and induce mutations. [2, 3] However, the repair and distribution of DNA carboxymethylation and how it affects mutational distribution in the human genome is not well characterized, in part due to a lack of methods for single-base resolution detection of carboxymethyl adducts. In this work, we developed a nanopore-based approach for the detection and quantification of the carboxymethyl adduct O^6-CMG. With nanopore sequencing, nucleotides can be identified by measuring current changes as DNA passes through a nanopore. Thus, any structural modifications to DNA could be detected, making it an ideal but underutilized platform for detecting DNA lesions. We synthesized DNA oligonucleotides containing a methylated and carboxymethylated guanine and incorporated them into a plasmid. Characteristic signals for specific modified bases were observed with high coverage. Application of this approach to biological samples is expected to lead to better characterization of the chemical and molecular basis of carboxymethyl DNA adduct mutagenicity and elucidation of how the mechanisms of adduct formation and repair affect mutation distribution.

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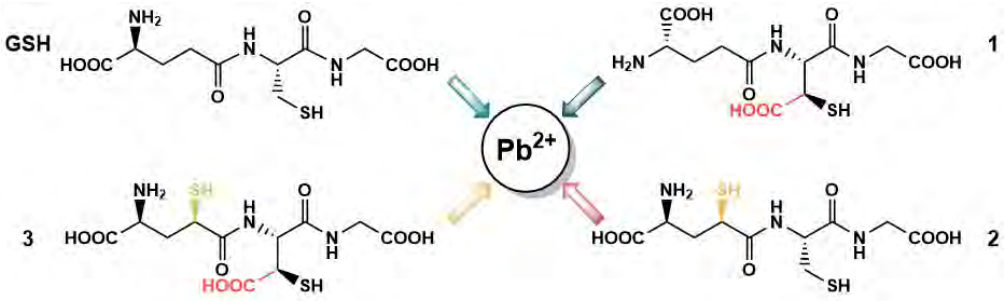
Thiolation and Carboxylation of Glutathione Synergistically Enhance Its Lead-Detoxification Capabilities

L. Sauser[1], T. A. Mohammed[1], L. Rulíšek[2], M. S. Shoshan[1]*

[1]University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zürich, [2]Czech Academy of Sciences, Institute of Organic Chemistry and Biochemistry, Flemingovo náměstí 2, 16610 Praha 6, Czech Republic

Lead (Pb) is a ubiquitous toxic metal capable of harming various organs.[1] The current treatment for Pb poisoning consists of chelation therapy that employs small molecules, such as ethylenediaminetetraacetic acid (EDTA) and dimercaptosuccinic acid (DMSA). Depletion of essential metal ions during treatment, inability to access intracellular Pb, and toxicity, represent the main constraints of Pb-specific chelation therapy.[2] Glutathione (GSH) is an abundant natural tripeptide harboring a diverse range of functions, among them interacting with essential and toxic metal ions. Although GSH weakly binds Pb, its capability to detoxify the metal ion is low. Here we present three analogues of GSH,[3] in which either the native cysteine (Cys; C), γ-glutamic acid (γGlu; γE), or both were replaced by two non-canonical amino acids (ncAAs) that are expected to induce Pb^{2+} capture. Two analogues showed an enhanced ability to detoxify Pb^{2+} ions in human cells, surpassing the current drugs. Complex formations between the analogues and Pb^{2+} ions were characterized using various experimental and computational tools. The study sheds light on their mode of action in vitro, revealing novel coordinations compared to GSH, metal selectivities, and enhanced binding affinities of the active anaolgues.[3]



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Homologous *N*-acyl ethanolamines from the *C. elegans* exometabolome

M. Scheidt[1], S. Bandi[1], S. von Reuss[1]*

[1]University of Neuchâtel, Institute of Chemistry, Laboratory of Bioanalytical Chemistry, Avenue des Bellevaux 51, 2000 Neuchâtel

Lipophilic *N*-acylethanolamines (NAEs) constitute conserved endogenous signaling molecules in plants, animals, and microorganisms [1]. The polyunsaturated anandamide (NAE 20:4) represents an endogenous ligand in cannabinoid signaling, whereas other NAEs activate a variety of alternative receptors [2,3]. In the model organism *Caenorhabditis elegans* some long chain NAEs have previously been identified, which are strongly enriched in the endometabolome [4].

Chemical analysis of the *C. elegans* exometabolome revealed a diversity of yet unidentified *N*-acyl ethanolamine derivatives. Large scale cultivation followed by fractionation and NMR spectroscopy facilitated the identification of 2-(β-glucosyl)-glyceryl *N*-acyl phosphoethanolamines carrying 20:5, 18:5, 16:2, *cyclo*-15:1, and 16:0_3-OH *N*-acyl moieties. Targeted DDA-MS/MS analysis revealed that these dominating components are accompanied by a diversity of homologous derivatives carrying *N*-acyl residues with 6 to 20 carbons. Comparative analysis of endo- and exometabolome extracts revealed that 2-*O*-glucosylation strongly facilitates the excretion of these metabolites, suggesting a potential function in interorganismal communication.

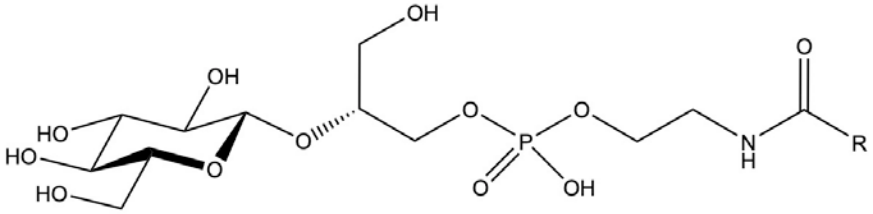

Figure 1 : Structure of the 2-(β-glycosyl)-glyceryl *N*-acyl phosphoethanolamines, R=C5-C19.

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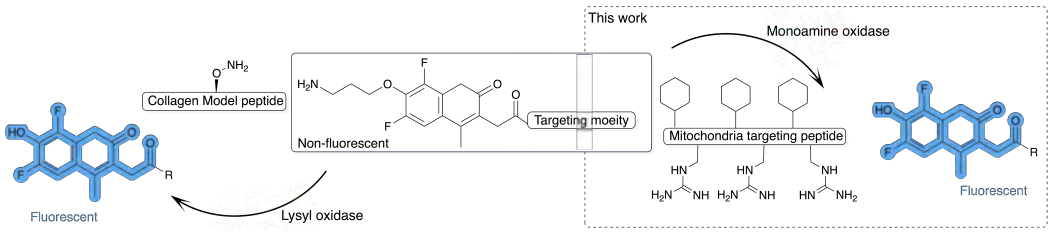
Turn-on Fluorescent Peptide Conjugates for the Detection of Human Monoamine Oxidase Enzymes

A. M. Schmitt[1], M. R. Aronoff[1], M. Li[1], H. Wennemers[1]*

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

Monoamine oxidase (MAO) enzymes catalyze the deamination of biogenic amines including neurotransmitters. MAOs exist as two isoforms (MAO-A and MAO-B) that are localized in the outer membrane of mitochondria. They differ in substrate and inhibitor specificity as well as in tissue distribution. MAO-A is closely linked to psychiatric disorders whereas MAO-B is involved in the development of neurodegenerative diseases.[1] Due to their crucial role in maintaining the balance of amines, tools to monitor the activity of these enzymes are important.[2]

Our group has recently developed an enzyme reactive fluorescent sensor that detects a related class of amino oxidases, lysyl oxidases (LOXs).[3] Here, we introduce the enzyme reactive fluorescent probe as a sensor for the detection of MAOs. Enzymatic assay revealed a preference of the sensor for MAO-B over MAO-A. Conjugation of the sensor with peptides that selectively localize in mitochondria[4], allowed for the delivery of the sensor to the location of MAO and activity studies in MCF-7 cells using confocal microscopy and fluorescence-activated cell sorting (FACS) analysis.



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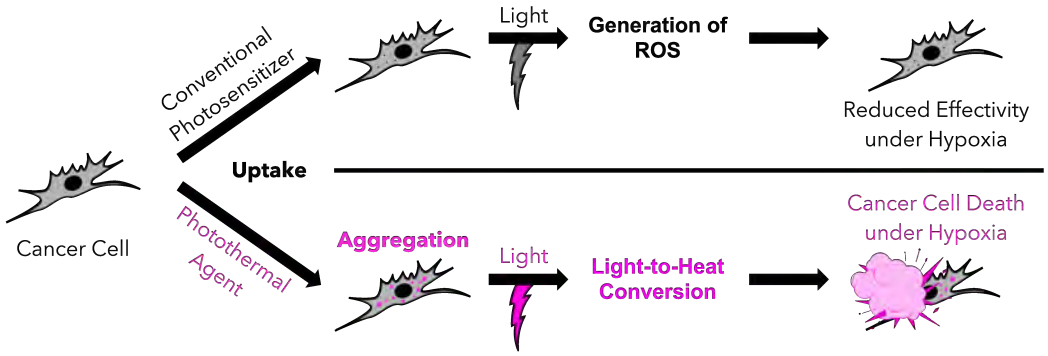
BODIPY-Based Photothermal Agents for Cancer Treatment

L. Schneider[1], M. Kalt[2], S. Koch[1], S. Sithamparanathan[1], V. Villiger[1], J. Mattiat[1], F. Kradolfer[1], S. Luber[1], M. Bonmarin[3], C. Maake[2,2], B. Spingler[1]*

[1]Department of Chemistry, University of Zurich, [2]Institute of Anatomy, University of Zurich, [3]ZHAW School of Engineering

Photodynamic therapy (PDT) has become a widely used therapeutic method for the treatment of a variety of premalignant and malignant diseases in the last couple of decades. Conventionally, PDT involves the application of a photosensitizer (PS) that is activated by light in the tissue to be treated. The mechanism of action (MOA) consists of the PS-mediated generation of reactive oxygen species (ROS) from its first excited triplet state.[1] This method has many potential applications due to the advantages it presents as a non-invasive therapy. However, a major drawback of this MOA is that solid tumours are invariably less well-oxygenated than non-malignant tissue. Alternative phototherapeutic treatment methods such as photothermal therapy (PTT) have emerged, applying functional biomedical and bioactive nanomaterials activated by light in the near-infrared (NIR) range to eliminate tumour cells via the generation of heat upon irradiation. Despite the advantages of PTT, drawbacks concerning biocompatibility, biodegradation, long-term toxicity, and threats of these nanomaterials to the environment remain unresolved.



Novel easily accessible BODIPY-based agents for cancer treatment are presented. In contrast to established PDT agents, the BODIPY-based compounds show photothermal activity and cytotoxicity is independent of ROS. The agents show high toxicity upon light irradiation and low dark toxicity in different cancer cell lines in 2D culture as well as in 3D multicellular tumour spheroids (MCTSs). The ratio of dark to light toxicity (phototoxic index, PI[2]) of these agents reaches values exceeding 830'000 after irradiation with energetically low doses of light at 630 nm. Under hypoxic conditions (0.2% O_2), which are known to limit the efficiency of conventional PSs in solid tumours, a striking phototoxic index of 360'000 was observed, indicating a photothermal MOA. Both phototoxic values are the highest reported to date. The oxygen-dependent MOA of established PSs hampers effective clinical deployment of these agents. We anticipate that small molecule agents with a photothermal MOA, such as BODIPY-based compounds, may overcome this barrier and provide a new avenue to cancer therapy.

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Impacts of DNA repair on anticancer drugs: synthesis and activity of novel acylfulvene analogs with aromatic groups

L. Slappendel[1], C. M. Aloisi[1], O. D. Schärer[2], S. J. Sturla[1]*

[1]ETH Zürich, [2]IBS Center for Genomic Integrity

Despite their wide use in cancer therapy, the efficacy and safety of DNA alkylating anticancer agents are hindered by cellular mechanisms of DNA damage repair. Acylfulvenes (AFs) are a class of experimental anticancer agents that alkylate DNA in the minor groove, giving rise to N3-adenine adducts as a basis of inducing cytotoxicity in cancer cells. However, by efficiently stalling RNA polymerase, AF-DNA adducts activate transcription-coupled nucleotide excision repair (TC-NER), and removal of the AF adduct by TC-NER causes drug resistance. We aim to exploit the favorable therapeutic properties of AFs but reduce repair-associated resistance by creating compounds with the potential to induce DNA-protein binding interactions that promote cell death also in repair-proficient cells. Therefore, we designed new AF analogs that link various apolar and aromatic groups with the core sesquiterpene structure of AFs comprised of a fused [6,5] ring system and reactive cyclopropyl group. These novel structures were synthesized in a one-step reaction from hydroxymethylacylfulvene by nucleophilic as well as nucleophilic aromatic substitution chemistries. The effects of the compounds were characterized in human cancer cell lines that were either proficient or deficient in NER factors. The new analogs were cytotoxic to human cancer cell lines ranging from 0.02 – 100 μM IC_{50} values, and repair-deficient cells were generally more sensitive. The results of this work indicate that chemical modification of AFs at the allylic alcohol position preserve the DNA-reactive nature of the compounds and suggests that stronger binding moieties towards the TC-NER repair proteins may promote cytotoxicity in repair-proficient cells.

Surface functionalization of harmonic nanoparticles with proteins for cancer active targeting

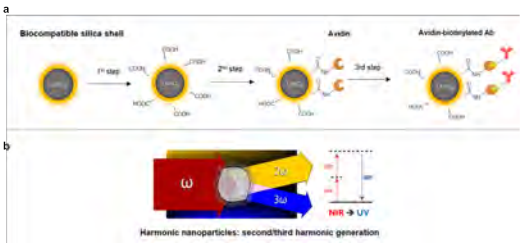
A. Spada[1], A. Gheata[1], E. Ansart[1], M. Wittwer[1], S. Gerber-Lemaire[1]*

[1]Institute of Chemical Sciences and Engineering, SCI-SB-SG, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Nowadays, cancer is a major health burden and has become the second leading cause of death in industrialized countries. Cancer mortality can be reduced through early detection and appropriate selective treatment. Respectively, traditional imaging techniques and cancer treatments lack sensitivity for early detection and show poor specificity, causing adverse side effects.[1] Nanomedicine has emerged as an optimal tool to support early diagnosis and targeted tumor therapy. Stimuli-responsive nanocarriers allow the simultaneous imaging, controlled release of payloads and active targeting, for a theranostic approach.[2]

We herein propose novel strategies for the surface functionalization of harmonic nanoparticles (HNPs, e.g. $LiNbO_3$, LNO), known for their efficient non-linear optical response by generation of second and third harmonic signals upon ultrafast laser irradiation.[3] These NPs can be functionalized with i) imaging probes for multimodal imaging; ii) photocaged cargos for controlled drug delivery; and iii) targeting ligands for active targeting of neoplastic cells and tumor sites. In this project, an inorganic silica-based coating was first achieved on LNO NPs to improve their biocompatibility and decorate their surface with reactive carboxyl groups. These were post-functionalized through amide bond covalent conjugation to avidin and neutravidin. Several quantification methods, such as fluorescence spectroscopy and BCA assays, were implemented to quantify the amount of grafted protein and assess the residual ability of the protein to subsequently bind biotinylated ligands. Finally, first attempts at immobilization of anti-EGFR biotinylated antibodies to the surface of avidin-functionalized NPs and characterization of the resulting conjugates are presented.

The long-term perspective of this project is to use protein-coated nanoparticles as theranostic nanoplatforms, focusing on the active targeting feature, through the use of monoclonal antibodies. Several grafting strategies, apart from avidin-biotin biotechnology, will be investigated. Overall, these nanocarriers, decorated with targeting ligands, anti-cancer drugs and imaging probes, will potentially combine early detection and *in vivo* cancer treatment.



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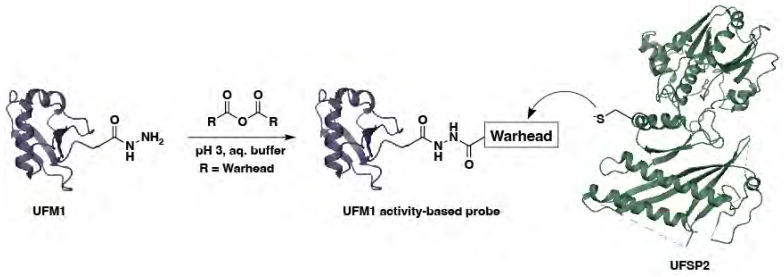
Facile Preparation of UFMylation Activity-Based Probes by Chemoselective Installation of Electrophiles at the C-terminus of Recombinant UFM1

K. A. Tolmachova[1], J. Farnung[1], J. Liang[2], J. E. Corn[2]*, J. W. Bode[1]*

[1]Laboratory for Organic Chemistry, Department of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zürich, Switzerland, [2]Institute of Molecular Health Sciences, Department of Biology, ETH Zürich, 8093 Zürich, Switzerland

Aberrations in protein modification with ubiquitin-fold modifier (UFM1) are associated with a range of diseases but the biological function and regulation of this post-translational modification, known as UFMylation, remains enigmatic. To provide activity-based probes for UFMylation, we have developed a new method for the installation of electrophilic warheads at the C-terminus of recombinant UFM1. UFM1 was expressed as an intein fusion in *E.coli* and a C-terminal UFM1 acyl hydrazide was readily produced by intein cleavage with hydrazine. Leveraging lower pH, at which nucleophilic side chains of the protein remain protonated, we have chemoselectively acylated the hydrazide C-terminus with a variety of carboxylic acid anhydrides. The protein remined folded, judged by the CD spectrophotometry, and only required gel filtration for purification. The resulting UFM1 activity-based probes show a range of tunable reactivity and high selectivity for proteins involved in UFMylation processes; structurally related E1s, E2s, and proteases associated with Ub or other Ubls were unreactive. The UFM1 probes were active in both cell lysates and in living cells. A previously inaccessible alpha-chloroacetyl probe was remarkably selective for covalent modification of the active site cysteine of de-UFMylase UFSP2 in cellulo.



Targeting RNA G-quadruplex to combat SARS-CoV-2

H. Wang[1]

[1]University of Zurich, Department of Chemistry

The ongoing pandemic of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poses a severe threat to human health globally with nearly half-billion laboratory-confirmed cases, including over 6 million global deaths by the end of March 2022[1]. Understanding the underlying mechanisms of infection and developing novel therapeutic strategies are urgently needed for the prevention and treatment of this disease. G-quadruplexes (G4s) are important noncanonical secondary structures formed within guanine-rich strands of regulatory genomic regions. Viral G4s are normally located in regulatory regions of the genome and implicated in the control of key viral processes[2]. Thus, targeting G-quadruplex sequences in the virus genome by G-quadruplex ligands could be a new approach to conquering virus infection[3].

It has been reported that there are conserved G-quadruplex sequences among SARS-CoV and SARS-CoV-2[4]. In our recent work, we evidence that these sequences could form stable RNA G4 structure *in vitro* and in live cells by various biochemical characterizations, including CD, ^{1}H NMR, and fluorescence imaging. We are solving the solution structure of RNA G4 from SARS-CoV-2 and screened for ligands that can stabilize G4. Further tests on the antiviral activities showed that these ligands are able to inhibit the growth of SARS-CoV-2 with IC_{50} values ranging from 2 to 7 μM in different cell infection models. This study provides a promising anti-SARS-CoV-2 strategy by targeting G-quadruplexes.

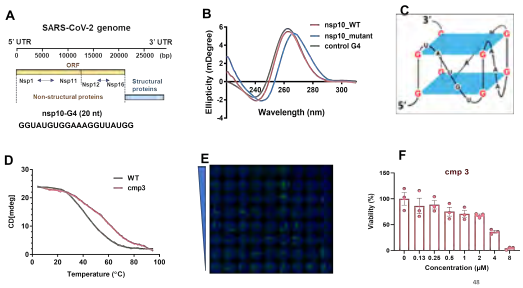


Figure 1. (A) SARS-CoV-2 genome and nsp10 G4 sequence. (B) CD spectra of nsp10 G4 and nsp10 G4 mutant 8A14A. (C) Nsp10 G4 model. (D) G4 ligands stabilize nsp10 G4. (E, F) G4 ligands exert anti-virus effect against SARS-CoV-2 with IC_{50} at μM level in different cell infection models.

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Flow chemical synthesis to study the regulatory role of post-translational modifications (PTMs) and PTM-crosstalk on c-Myc

E. Williams[1], M. Schuster[1], O. Zerbe[1], N. Hartrampf[1]

[1]Department of Chemistry, University of Zürich

The intrinsically disordered protein (IDP) c-Myc plays a crucial role in a broad range of cancers and is a highly promising target for the development of anticancer therapeutics. However, c-Myc has earned the reputation of being “undruggable”, due to its lack of defined binding pockets. A potential avenue to target c-Myc is through modulation of its protein-protein interactions (PPIs). These PPIs are often dependent on post-translational modifications (PTM); only occurring when specific PTMs are found on c-Myc. The tumour-suppressing protein Bin1 (Myc box-dependent-interacting protein 1) is known to interact with c-Myc in a PTM-dependent fashion. To date, this interaction has primarily been characterized using short fragments (less than 20 AA) of the MBI (Myc homology box I) of c-Myc, yet evidence suggests that Bin1 may bind to additional MBs and domains of c-Myc, which contain many other PTM hot-spots.

Current methods to obtain longer fragments of c-Myc, and PTM-variants thereof, are limited to recombinant expression, traditional solid-phase peptide synthesis (SPPS), or a combination of chemical and expressed protein ligation strategies. These methods are heavily limited by (a) difficulty in installing multiple, specific PTMs recombinantly, (b) the short half-life of c-Myc in biological systems due to rapid proteolysis, and (c) limitations of traditional SPPS to ~50 AA fragments. Recently, flow chemistry has been applied in automated SPPS for the rapid and linear synthesis of high-purity proteins up to 200 AAs in length. Therefore, to prepare a library of c-Myc PTM-variants comprising multiple MBs and PTM hot-spots, new technology is required. Such a library will enable the full characterization of the regulatory roles of PTMs on c-Myc, and the deconvolution of PTM-crosstalk on this IDP.

Herein, we describe the use of Automated Flow Protein Synthesis (AFPS) for the preparation of a library of PTM-variants of the c-Myc N-terminus (residues 1-84). Each c-Myc variant was isolated in high yield (up to 6% yield over ~200 steps) and purity (>95% by UHPLC). Protein NMR was then used to characterize the binding interactions of each synthetic c-Myc variant with expressed ^{15}N-labelled Bin1(SH3) protein. Chemical shift perturbations (CSPs) of ^{15}N-Bin1(SH3) were comparable between samples containing c-Myc$_{1\text{-}84}$ or the pT58 variant, indicating that phosphorylation at T58 is tolerated in the c-Myc:Bin1 complex. In contrast, CSPs of ^{15}N-Bin1(SH3) were minimal in the presence of c-Myc$_{1\text{-}84}$(pS62), indicating reduced binding.

In current and future work, we are studying additional modification sites and PTMs such as glycosylation and acetylation of c-Myc, including PTM-crosstalk, to continue building our understanding of c-Myc biology for anticancer therapies. Ultimately, we aim to develop this flow-based platform to study the role of PTMs in the regulation of other therapeutically relevant proteins.

Discovery and characterization of ACT-284069, a double prodrug of the $P2Y_{12}$ receptor antagonist selatogrel

E. Caroff[1], F. Hubler[1], C. Gnerre[1], A. Treiber[1]

[1]Idorsia Pharmaceuticals Ltd

Atherothrombotic conditions are currently managed by prescribing aspirin in combination with P2Y12 inhibitors. Approved oral P2Y12 antagonists include clopidogrel, prasugrel, and ticagrelor. We have previously disclosed the discovery of selatogrel (ACT-246475A), a new, potent, selective and reversible P2Y12 antagonist [1]. Selatogrel is currently in clinical development for early treatment of suspected acute myocardial infarction and is formulated for subcutaneous administration [2].

Based on the superior therapeutic window of selatogrel as compared to clopidogrel and ticagrelor in a rat thrombosis model [1,3], our initial goal was to develop a new efficacious oral treatment with less bleeding complications than standard of care. To address the low bioavailability of selatogrel observed after oral administration during preclinical animal studies, we embarked in an optimization program to design selatogrel prodrugs. Herein we report the synthetic efforts, the characterization cascade, and the data leading to the identification of the prodrug ACT-284069.

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