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News and Activities of the DMCCB

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class="sppb-addon-title">Introcuton of the SCS Division of Medicinal Chemistry & Chemical Biology
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The DMCCB comprises about 750 scientists interested in medicinal chemistry, chemical biology and related fields of research. Beyond Switzerland, the DMCCB interacts with other country organizations to represent the Swiss medicinal chemistry and chemical biology community. The Division is also a member of the European Federation of Medicinal Chemistry (EFMC).

Introduction and promotion of events in the field of MedChem and ChemBio

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Novel macrocyclic NLRP3 inhibitors

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Innate immunity is the first and immediate line of defense against invading endogenous or exogenous pathogens. Here, the NOD-like receptor family pyrin domain containing 3 (NLRP3) acts as a sensor that detects a broad range of danger signals [1]. Upon its activation, pro-caspase-1 is cleaved to form mature caspase-1, leading to subsequent release of IL-1 β and Gasdermin N-terminal fragments, and ultimately to inflammation and pyroptosis [2].

Aberrant activation of NLRP3 due to e.g. persistent tissue damage, misfolded proteins or crystal deposits has been linked to multiple chronic inflammatory disorders such as cryopyrin-associated period syndrome (CAPS), Parkinson's disease, gout and numerous others [3]. Hence, there has been an increasing interest in NLRP3 inhibitors as therapeutics. A first generation of NLRP3 inhibitors bearing a sulfonylurea core such as MCC950 (developed by Pfizer) were discovered by phenotypic screening, however its mode of action was only elucidated later [4]. Based on MCC950 second-generation inhibitors were developed, aiming to overcome some liabilities such as moderate potency and drug induced liver injury (DILI) [5]. During the optimization of these (second-generation) inhibitors, conformational studies lead to the identification of novel macrocycles [6]. Here we report the discovery and optimization of this class of NLRP3 inhibitors.

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Chemical Synthesis and Chaperone Peptide Mediated Folding of Human Nerve Growth Factor by Expressed KAHA Ligation

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Nerve growth factor is a powerful protein for treating central nervous system diseases, but its therapeutic potential is limited by a severe pain-inducing side effect. This adverse effect arises from insufficient receptor selectivity and can, in principle, be mitigated through side chain mutations or modifications, making it a suitable target for chemical protein synthesis. Despite its small size (13 kDa), the chemical synthesis of NGF has been challenging due to its exceptional hydrophobicity and the requirement for a 104-residue N-terminal chaperone peptide for folding. Here, we present the chemical synthesis of NGF using α -ketoacid-hydroxylamine (KAHA) ligations, featuring recombinant expression of the chaperone peptide and its chemoselective conversion to a C-terminal α -ketoacid.^[1] A novel solubility tag, SOLACE, and ester-forming KAHA ligations enabled the assembly of linear proNGF (25 kDa) from three synthetic and one recombinant segment. Controlled folding and disulfide bond formation mediated by the chaperone peptide followed by proteolytic cleavage yielded biologically active synthetic NGF. This work establishes a platform for engineering NGF variants with tailored receptor selectivity and offers a general strategy for the semisynthesis of neurotrophins and related proteins that also require long chaperone peptides for proper folding.

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MicroCycle: An Integrated and Automated Platform to Accelerate Drug DiscoveryS. Racine¹¹Novartis Pharma AG, Basel; sophie.racine@novartis.com

We herein describe the development and application of a modular technology platform which incorporates recent advances in plate-based microscale chemistry, automated purification, in situ quantification, and robotic liquid handling to enable rapid access to high-quality chemical matter already formatted for assays. In using microscale chemistry and thus consuming minimal chemical matter, the platform is not only efficient but also follows green chemistry principles. By reorienting existing high-throughput assay technology, the platform can generate a full package of relevant data on each set of compounds in every learning cycle. The multiparameter exploration of chemical and property space is hereby driven by active learning models. The enhanced compound optimization process is generating knowledge for drug discovery projects in a time frame never before possible.

Towards fully integrated ultrafast drug discovery by NMR

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Hyperpolarized nuclear magnetic resonance (NMR) holds immense promise for drug discovery. We are excited to present a fully automated, end-to-end, fragment-based lead discovery ultrafast NMR platform leveraging photoinduced hyperpolarization.

Utilizing our recently developed photoinduced fragment library and our fully automated light-coupled NMR platform (Cryolight), we can screen up to 700 samples daily.(1, 2) The Cryolight technology is plug-and-play, enabling the automation of hyperpolarized NMR fragment screening and affinity determination using a sample changer. Cryolight performs equally on low-field benchtop spectrometers (60-80 MHz), an economical alternative to high-field NMR that does not require liquid helium or dedicated facilities.(3, 4)

Hyperpolarized fragments can be screened within a few seconds at low micromolar concentrations, enabling a throughput of several hundred samples per day.(2) In conjunction with our automated data analysis software, results are available in real time. Furthermore, ligand-based binding epitopes can be inferred directly from screening data, revealing early structural information that can be used for docking experiments. In the next step, we show how the affinity of a protein-ligand interaction can be obtained within 15 minutes using the light-induced NMR method, which allows the characterization of up to 50 systems daily.(5) Due to the strong sensitivity enhancement of up to 1000-fold for the fragments, hyperpolarization can also reveal structural information of the bound ligand or the complex. We tested our platform on several targets, including PIN1, K-Ras, and PKG1, for which we present our findings.

In summary, we present the first fully operational hyperpolarized NMR drug discovery platform, comprising screening, affinity determination, and structure determination, compatible with commercially available automated high and low field spectrometers.

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Development of a Late-Stage Derivatization Platform

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Late-stage functionalization offers the discovery chemist the promise of preparing many relevant analogues of a lead compound with maximum synthetic efficiency. We aimed to realize this promise by setting up an automation-compatible platform for preparing multiple useful derivatives from a lead compound using a lynchpin functionalization strategy. First, we identified thianthrenation and borylation as attractive C-H functionalization methods to install a versatile reactive handle on a late-stage intermediate. We demonstrate the applicability of these reactions in the context of several representative drugs and agrochemicals [1] and, for borylation, developed a reaction screen to identify catalysts capable of regiodivergent functionalization [2]. We next identified suitable reaction conditions to convert the thianthrene and boronate intermediates into 6-12 diverse derivatives useful for structure-activity relationship studies and optimized the conditions such that the reactions could be run in parallel on an automated synthesis platform. We demonstrate that a systematic late-stage functionalization and derivatization approach can be a useful tool in optimization campaigns and share some of our experience in applying this challenging chemistry as a platform.

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Grafting Substrates-of-Interest to Cell-Penetrating Polydisulfides: a Covalent Bioconjugation for Traceless Intracellular Delivery

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Cell-penetrating polydisulfides (CPDs) emerged in 2014 as traceless alternatives to cell penetrating peptides (CPPs) as a tool for intracellular delivery.^[1] Replacement of the original peptidic scaffold with a disulfide chain allows CPDs to enter cell by thiol-mediated uptake (TMU) and be subsequently depolymerized by intracellular GSH. CPDs have been usually grown from thiolated cargoes successfully delivered in the intracellular environment.^[2] Despite many efforts, such grafting-from approach only works at high concentrations, above any biological utility. We have recently unlocked the possibility of grafting thiolated cargoes to the CPDs through a dynamic and covalent process operating at concentration < 1 μ M. Although a broad screening of cargoes is still currently ongoing, this new bioconjugation opens the possibility of making CPDs the final traceless TMU tag, which has been missing for decades.

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Turning Processes into Automation for High-Speed InnovationG. Lee¹

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The driver for innovation within chemical biology is the demand and growing need for novel active ingredients. This is accompanied by the search for targeted molecular diversity and novel experimental routes resulting in an increase of synthetic work with the same amount of human resources. The only way to overcome these challenges are flexible, modular, automated solutions for library synthesis to accelerate research and development.

With Chemspeed's extensive portfolio and over 25 years of experience, we provide innovative solutions to overcome challenges and bottlenecks within the lab. Such as the modular combination of tools like liquid handling, gravimetric dispensing, shaking and heating, the entire screening workflow can be automated. Additionally, we present case studies using e.g. parallel synthesis with online NMR analysis, and showcase the concept of connected laboratories, the Design-Make-Test-Learn (DMTL) cycle.

Evolving a human capsid protein for mRNA delivery

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PNMA2 is a human protein derived from the Ty3 retrotransposon that forms capsid-like nanocages and does not intrinsically package any nucleic acids, however it has previously been engineered to package RNA with low efficiency^[1]. In this project we apply protein engineering and directed evolution to repurpose PNMA2 for therapeutic mRNA packaging and delivery. We have engineered PNMA2 variants to package their own mRNA 'genome', constructed mutagenised plasmid libraries using error-prone PCR, expressed and purified capsid variants, and selected for RNase-resistant assemblies to enrich for RNA cargo protection and packaging efficiency, all according to a previously published method^{[2][3]}. Ongoing rounds of evolution will be guided by long-read sequencing and cryo-EM guided structural analysis, offering insight into both functional optimization and potential evolutionary trajectories of retroelement-derived proteins. Additionally, PNMA2 has an external 'spike' domain, which was shown to be immunogenic in mice^[4]. Through truncating the spike domain, we are evaluating its role in capsid assembly in order to engineer a less immunogenic capsid, also with the possibility to replace it and functionalise the surface of the capsid for enhanced delivery. In future work, we aim to evolve PNMA2 variants for improved cellular uptake, endosomal escape and cargo release. This approach not only expands the toolbox of RNA delivery technologies, but also provides a platform for exploring the evolution of virus-like capsids in a controlled, synthetic context.

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Discovery of novel IL-17 inhibitors by scaffold morphing strategy

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The proinflammatory cytokine interleukin IL-17 (IL-17) plays an important role in the immune response to fungal and bacterial infections.¹ An overexpression of IL-17 is associated with various autoimmune and inflammatory diseases including psoriasis, psoriatic arthritis and ankylosing spondylitis.² The role of IL-17 in these diseases was validated by the clinical use of IL-17 neutralizing monoclonal antibodies like secukinumab (Cosentyx).³ Recently, many organizations conducted research aimed at discovery of low molecular weight IL-17 inhibitors.⁴ In this talk, our work towards identification of structurally diverse small molecule IL-17 inhibitors will be presented. The focus will be on the scaffold morphing activities aiming to mask or replace the amides in the known IL-17 scaffold.

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Design and Evolution of L/D-Microproteins Against XIAP to Induce Apoptosis of Cancer Cells

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Recognition L/D-microproteins, reported in this study are small, compact, atactic, protein-like peptides that function as antibody mimics. These L/D-microproteins are designed to comprise all the key advantages of antibodies, such as high affinity and long serum half-life, while reducing size and immunogenicity. They are designed using mutational molecular dynamics (MMD) and synthesized using automated solid-phase peptide synthesis (SPPS) and by the CuAAC click reaction to enhance structural and metabolic stability. The recognition L/D-microproteins are designed to target the caspase 3/7 binding site (BIR2 domain) and the caspase 9 binding site (BIR3 domain) of the intracellular cancer protein X-linked inhibitor of apoptosis (XIAP). Overexpression of XIAP is observed in tumours leading to increased cell survival and resistance to chemotherapeutics.¹ Inhibition of XIAP's interaction with caspases induces apoptosis, making this approach beneficial for targeting tumour cells. Five XIAP domains have been structurally determined, but the structure of full-sized XIAP is not available. Hence, the spatial organization of these domains is not known. This study reports the expression of the full-size protein XIAP. The XIAP protein was fused with maltose-binding protein (MBP) to stabilise it for expression in *E. coli*, the full-length XIAP-MBP was successfully isolated. MBP was subsequently cleaved from XIAP using TEV protease.² The complete structure of XIAP was further investigated using photo-crosslinking, which may enable more precise designs of inhibitors against XIAP.³ To assess the designed and synthesized recognition L/D-microproteins, binding and activity assays were performed. Preliminary results show binding to the target protein XIAP, validating our structural model and suggesting the potential for developing more potent inhibitors targeting XIAP and other proteins of interest for the treatment of various diseases.

Fig. 1: An overview of the project, highlighting the computational design of microproteins, SPPS, protein expression of XIAP-MBP, and affinity assays, aimed at inducing apoptosis in cancerous tumour cells.

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Discovery and Optimization of Macrocyclic Allosteric Inhibitors of the MET KinaseA. Goergler¹¹F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124 4070 Basel, Switzerland

The MET kinase, along with its oncogenic alterations, is a clinically validated target for the development of anticancer medicines. The emergence of resistance to first-generation inhibitors necessitates the development of new compounds able to inhibit both wild-type enzyme and resistant mutants. In this presentation, we will describe our hit-finding efforts aimed at discovering allosteric MET inhibitors and the subsequent optimization of one class of allosteric type III sulfonamide inhibitors. Starting from a DNA-encoded library technology (DEL^T) hit, and guided by ligand-bound x-ray co-crystal structures, this process led to highly potent macrocyclic compounds with promising molecular properties. These compounds could serve as starting points for the design of next-generation MET inhibitors.

Thiol-Selective Radiolabelling of Antibodies Using Light-Activated Tetrazoles

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Introduction

In 1967, Huisgen reported the unique photochemical reactivity of tetrazoles, demonstrating their ability to undergo light-induced cycloaddition with alkenes.^[1] Subsequent investigations revealed their capacity to interact with biologically relevant molecules upon photoactivation, opening new avenues in chemical biology.^[2] Building on this foundation, we explored the application of photoactive tetrazoles for protein radiolabelling, leveraging their rapid reaction kinetics and high chemoselectivity to develop an efficient strategy for photoradiosynthesis and bioconjugation.

Methods

The study focused on the synthesis, radiolabelling, and bioconjugation of ¹⁷⁷Lu-DOTAGA to the monoclonal antibody onartuzumab using a PEG-functionalised photoactivatable tetrazole. Radiolabelling was performed at 80 °C for 15 minutes and monitored by radio-iTLC and radio-HPLC. Photoconjugation under mild aqueous conditions (pH 8, room temperature, 15 min) with 365 nm irradiation was first validated using human serum albumin (HSA), then applied to onartuzumab. Thiol-selective labelling was examined by comparing unmodified and Traut's modified onartuzumab versus a control study using maleimide-blocked HSA. Following purification, the radiolabelled antibodies were evaluated *in vivo* by γ -scintigraphy and biodistribution studies in mice bearing MKN-45 xenografts. In parallel, PEG-tetrazole was synthesised and characterised by NMR, HPLC, and HR-MS. Its photochemical behavior was studied under 365 nm irradiation in aqueous-organic buffer with isolation of several key products. Reactivity with model nucleophiles was monitored by UV-Vis, fluorescence, HPLC, and HR-MS, highlighting selectivity toward thiol-containing species at pH 10.5.

Results and Discussion

Radiochemical studies showed high conjugation yields (83–86%) of the ¹⁷⁷Lu-labelled tetrazole complex with HSA. In contrast, substantially lower yields (25–30%) were obtained with blocked HSA or with native MetMAb, attributed to the lack of available thiol groups on these proteins. Following thiol functionalisation with Traut's reagent, a marked increase in conjugation efficiency was observed, underscoring the critical role of sulfhydryl groups in enabling efficient and selective bioconjugation.

To investigate the underlying mechanism, photochemical studies were performed by using the isolated PEG-tetrazole. Upon irradiation at 365 nm, the compound underwent photolysis with nitrogen release, forming a highly nitrile imine intermediate, which, in photolysis experiments in buffered solution revealed a clear kinetic preference for thiol nucleophiles. Mechanistic insight was supported by UV/Vis, fluorescence, HPLC, and HRMS analyses, confirming the thiol-targeting nature of the reaction.

Conclusion

This work establishes the photoinduced reactivity of tetrazoles with free sulfhydryl groups as a robust and chemoselective platform for site-specific functionalisation and radiolabelling of proteins. This approach significantly enhances radiolabelling efficiency, particularly for monoclonal antibodies where thiol sites can be introduced in a controlled manner using established chemical or enzymatic processes. The methodology is

Synthesis of GDB Derived Bicyclic Diamines as Scaffolds for Medicinal ChemistryG. Baldoni¹, J. Reymond^{1*}¹Department of Chemistry, Biochemistry and Pharmaceutical Sciences DCBP, University of Bern

The Generated Data Base (GDB), curated by the Reymond group, offers stable, feasible, 3D-shaped chiral molecules, enabling exploration a vast chemical space, providing promising scaffolds for Medicinal Chemistry. (1) These molecules are innovative due to their polycyclic system, high sp³-hybridized carbon fraction and the presence of many quaternary carbons. (2)(3) This project exploits a subset of the GDB, listing 1139 mono- and bicyclic saturated diamine scaffolds containing 5, 6 or 7 membered rings, including 680 novel accessible scaffolds (Figure 1A). Herein I discuss initial synthetic steps for preparing a target subset of these diamines based on Diels-Alder and ring expansion reactions. As a proof-of-concept molecule 1 (Figure B) was recently synthesised in the group, showing potent inhibition of monoamine transporter, with selectivity to norepinephrine and dopamine transporter (IC₅₀<100 nM).(4) Moreover, the amine functional group within the cyclic scaffolds grants straightforward functionalisation with desired moieties.

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Expanding the RNA packaging capacity in evolved nucleocapsids

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RNA-based therapeutics have rapidly emerged as transformative tools in medicine, highlighted by the success of RNA vaccines. However, the efficient delivery of RNA molecules remains a significant challenge, necessitating the development of novel delivery vehicles. Recently, the Hilvert group engineered NC4, a protein capsid derived from lumazine synthase—a cage-forming bacterial enzyme that initially lacked RNA-binding affinity. NC4 was evolved to encapsulate 2 to 3 copies of its own encoding RNA, achieving a packaging capacity of approximately 2'500 nucleotides [1].

In single-stranded RNA (ssRNA) viruses, RNA binding and packaging are mediated by arginine-rich motifs that interact with specific nucleotide sequences [2]. Notably, the charge of the capsid's lumen has been shown to correlate with the amount of RNA encapsulated [2]. Drawing inspiration from this principle, we introduced targeted point mutations within NC4's arginine-rich motif to modulate its RNA packaging capacity. We show that we can expand NC4's packaging capability from 2'500 nt to 4'000 nt by this charge modulation.

To assess the versatility of the charge-engineered NC4 variants, we used a previously developed in vitro assembly protocol that enables capsid formation around synthetic RNA transcripts [3]. This platform provides a controlled system to systematically evaluate how capsid luminal charge influences RNA encapsulation and supports the design of RNA carriers capable of packaging transcripts of varying lengths.

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Accessing therapeutic natural products via a bio-based approach

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Botanical extracts have been used in traditional medicine for centuries, however many times the source plants are protected or difficult to access. We are discovering new, laboratory-cultivable sources for bioactive natural products typically used in medical supplements. In this work, we aim to provide an alternative, more sustainable production system for these therapeutic compounds while also revealing the molecular mechanisms behind their production.

Fast, efficient and chemoselective radio-ADC preparation through tetrazole photochemistry

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Harnessing the innate power of biomolecules is a challenging task that requires specialised chemical reactions that function under conditions favourable to the biomolecular targets. In the production of radioactive bioconjugates, this is conventionally accomplished through thermal reactions or by using reactive intermediates that differ in efficiency and shelf life, leading to unwanted variability. There is a continual need for reactions that demonstrate enhanced yields, reduced reaction times, better biocompatibility, and increased selectivity; photochemistry provides innovative and alternative approaches to address these issues (Figure 1). [1] Tetrazoles are compounds that have been studied since the 1960s, experiencing fluctuating levels of popularity over time. They undergo photolysis, forming nitrile imines, which are potent electrophiles, following N₂ extrusion (Figure 2). [2, 3]

This presentation specifically emphasises understanding the underlying chemistry of these compounds and their reactivity towards proteins. It was found that electrophilic addition to thiolates proceeds with remarkable efficiency, selectivity and high rates. Photo-reactivity and kinetics were carefully monitored using model compounds through optical and NMR spectroscopy, HPLC-MS, and product isolation. Subsequently, radiolabelled tetrazoles were employed to functionalise biomedically relevant proteins, such as monoclonal antibodies (mAbs). To this end, they were tested for the synthesis and biodistribution of ¹⁷⁷Lu-radiolabelled mAbs to be used in future cancer therapy endeavours.

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Volatile-mediated interaction between plant-associated beneficial microorganisms and phytopathogenic fungi

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Many beneficial microorganisms contribute to plant resistance towards biotic and abiotic stresses. Recently, there has been growing evidence that plants are protected from diseases by their microbiome and the volatile organic compounds (VOCs) they emit could have a major role in this process.

Emission of VOCs is an important means of communication among microorganisms. These volatiles have various effects, they contribute to the stabilization of microbial communities, they can attract or repel different species, promote growth or display antimicrobial properties. Recently, several pieces of evidence have shown that the volatilome emitted by a microorganism depends on the volatiles of its surrounding. Thus, the volatilome of two different microbes grown together is different from the sum of the two individuals grown separately. These differences may include inhibition and promotion of various compounds, and the production of new compounds. However, the set up used so far to highlight these kinds of interactions has several drawbacks, including artificial volatile overaccumulation, potential oxygen limitation and the impossibility to assign a producer to the compounds newly emitted during the interaction.

To solve this problem, we have developed a solution enabling us to trap the whole volatilome of an organism and to use it to expose another one unilaterally. By combining this method with comparative Gas Chromatographic–Mass Spectrometry (GC-MS), it is thus possible to study the volatile-mediated interactions more precisely by identifying more easily the compounds responsible for the changes in the volatilome and the emitter of any newly produced compound.

We have used this new procedure to study volatile-mediated interactions between the biocontrol fungus *Trichoderma simmonsii* and the two plant pathogens *Botrytis cinerea* and *Fusarium oxysporum*. Our results show that the perception of each pathogen's volatilome triggered a specific response, resulting in significant changes in the VOCs emitted by *Trichoderma*. *Trichoderma*'s volatilome modulation was overall stronger when exposed to the VOCs from *Fusarium* than to the VOCs from *Botrytis*, which correlated with increased siderophore production when co-incubated with this fungus. Our newly developed method will not only help to better understand volatile-mediated interactions in microbes but also to identify new molecules of interest that are induced by VOCs, as well as the putative-inducing signals themselves.

Novel Oncocin–Peptoid Hybrids Show Potent Antibacterial Activity Against Multidrug-Resistant *A. baumannii*

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The escalating prevalence of multidrug-resistant Gram-negative bacterial infections necessitates the exploration of novel antimicrobial agents. Proline-rich antimicrobial peptides (PrAMPs) have emerged as promising candidates due to their unique mechanisms of action. Among them, Oncocin (VDKPPYLPRPRPPRIYNR-NH₂), a synthetic derivative inspired by peptides from the milkweed bug *Oncopeltus fasciatus*, has demonstrated potent antibacterial activity against *K. pneumoniae* and *E. coli* by inhibiting bacterial ribosome (Figure 1).^[1,2]

We have recently demonstrated that stereorandomization is compatible with target binding peptides to the C-terminal region of Oncocin. It preserved ribosome binding and antibacterial effects including activities against drug-resistant bacteria and protected against serum degradation.^[3] Following up on this progress, we focus on the modification of the last 9 C-terminal residues by incorporating mixed peptide/peptoid chains, along with random chirality alterations. Preliminary biological assays reveal that some analogues exhibit broadened antimicrobial activity, including potent effect against *A. baumannii*, a strain against which Oncocin was inactive, while maintaining strong activity against *K. pneumoniae* and *E. coli*, and preserving the mechanism of action of the parent Oncocin peptide.

L-Oncocin: MIC (*A.baumannii*): 64 µg/mL

L-Oncocin-peptoid hybrid: MIC (*A.baumannii*): 4 µg/mL

Figure 1. Chemical structure of L-Oncocin

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Evolving a human capsid protein for mRNA delivery

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PNMA2 is a human protein derived from the Ty3 retrotransposon that forms capsid-like nanocages and does not intrinsically package any nucleic acids, however it has previously been engineered to package RNA with low efficiency^[1]. In this project we apply protein engineering and directed evolution to repurpose PNMA2 for therapeutic mRNA packaging and delivery. We have engineered PNMA2 variants to package their own mRNA 'genome', constructed mutagenised plasmid libraries using error-prone PCR, expressed and purified capsid variants, and selected for RNase-resistant assemblies to enrich for RNA cargo protection and packaging efficiency, all according to a previously published method^{[2][3]}. Ongoing rounds of evolution will be guided by long-read sequencing and cryo-EM guided structural analysis, offering insight into both functional optimization and potential evolutionary trajectories of retroelement-derived proteins. Additionally, PNMA2 has an external 'spike' domain, which was shown to be immunogenic in mice^[4]. Through truncating the spike domain, we are evaluating its role in capsid assembly in order to engineer a less immunogenic capsid, also with the possibility to replace it and functionalise the surface of the capsid for enhanced delivery. In future work, we aim to evolve PNMA2 variants for improved cellular uptake, endosomal escape and cargo release. This approach not only expands the toolbox of RNA delivery technologies, but also provides a platform for exploring the evolution of virus-like capsids in a controlled, synthetic context.

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Guanine by guanine: Decoding the BCL2 RNA G-quadruplex conformation landscape

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G-quadruplexes (G4s) are intricate secondary structures formed in guanine-rich regions of RNA and DNA. These motifs consist of the stacking of multiple cyclic arrangements of four guanines through Hoogsteen hydrogen bonding.^[1] Generally, G4-forming sequences contain more guanines than are required for G4 formation, allowing for guanine exchange between the core and the loop regions. In the 5' untranslated regions of mRNAs, G4s are implicated in translational regulation.^[1] One such example is the highly conserved sequence of a G4 in *BCL2* mRNA positioned 42 nucleotides upstream of the translation start site. This G4 has been linked to the repression of translation of the Bcl-2 protein. Bcl-2, an anti-apoptotic protein, is tightly regulated and has been associated with cancer, Alzheimer's, and Parkinson's diseases.^[2] Therefore, understanding the structure and dynamic behaviour of this G4 is an important step in uncovering how the G4 contributes to the regulation of Bcl-2 expression.

The wild-type *BCL2* G4 sequence, comprising 18 guanines, adopts multiple G4 conformations in dynamic equilibrium. This project aims to understand the conformational landscape by investigating the role and impact of each guanine on the overall structure of the G4. In addition, we explore the interplay between the conformers to understand how structural heterogeneity may influence function. A deeper understanding of both the structure and dynamics offers valuable insights into G4 RNA-based regulation and shape the development of novel therapeutic strategies

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Development of novel orthosteric-allosteric bitopic ligands to study adenosine A₁ receptor

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Compounds, that covalently link two pharmacophore units via spacers are called bitopic ligand and can be divided into homobivalent (same pharmacophore) and heterobivalent (different pharmacophores).^[1] Simultaneously targeting the orthosteric and allosteric binding sites of G protein-coupled receptors (GPCRs) has the potential to increase affinity, potency, subtype selectivity and/or G protein selectivity.^[2,3] Different examples of orthosteric-allosteric bitopic ligands have been described for the adenosine A₁ receptor (A₁R), where the endogenous agonist adenosine is attached to various thiophene-based positive allosteric modulators (PAMs), demonstrating biased A₁R signalling (subtype and G protein selectivity). However, these ligands are also active at the adenosine A_{2B} receptor subtype.^[3,4] Adenosine receptor subtypes (A₁R, A_{2A}R, A_{2B}R and A₃R) are involved in various pathologies, e.g. cancer and cardiovascular diseases, and therefore interesting targets for developing new treatments with different pharmacological mechanisms.^[5]

The aim of this project is to develop novel orthosteric-allosteric bitopic A₁R ligands. To potentially achieve biased signalling, phenyloxycyclopentyladenosine derivatives (PhOCPA) are used as orthosteric ligand. PhOCPAs were discovered as highly potent and selective A₁R agonists in our group.^[6] The -position of the phenoxy group tolerates various substituents and is therefore ideal for functionalisation with linker-PAM conjugates. Different combinations of linkers and PAMs will be explored to identify an optimal bitopic ligand. Here, we describe the multistep-synthesis of these heterobivalent bitopic ligands.

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Structural and Dynamic Insights into Minimal HDV-like Theta Ribozymes

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Ribozymes sharing both the nested double pseudoknot structure and catalytic mechanism of the hepatitis delta virus (HDV) ribozyme have been identified across diverse organisms. Yet, the biological functions of most HDV-like ribozymes remain unknown. Using a bioinformatic motif search, our group recently discovered a subclass – termed theta ribozymes (Θrz) – positioned adjacent to viral tRNA 3' ends, suggesting a role in tRNA maturation.^[1]

Characterized by extensive sequence diversity and minimal conservation, theta ribozymes exhibit variable self-cleavage rates with distinct metal-ion dependencies. To uncover the structural and dynamic origins of these differences, we employ NMR spectroscopy, focusing on the formation of the catalytic core in both pre- and post-cleaved states.

Our initial structural analysis centers on Θrz5, the fifth most abundant variant identified. Its activity in the self-scission reaction exhibits a characteristic bell-shaped Mg^{2+} dependence with a marked reduction of the cleavage rate in the presence of Ca^{2+} ions. Using [¹H,¹H]-NOESY and HNN-COSY experiments, we confirmed the predicted secondary structure of the cleaved Θrz5 ribozyme through imino proton and hydrogen-bond analysis. Slow exchange dynamics were observed near the catalytic center, indicating conformational changes that are significantly restricted upon the addition of divalent metal ions. Moreover, in the presence of Mg^{2+} and Ca^{2+} ions, several chemical shift changes involving key nucleotides around the active site were also noted.

Appearing predominantly in bacteriophages associated with the mammalian gut, theta ribozymes are deeply implicated in phage biology and microbiome dynamics, underscoring the significance of our novel structural insights into their metal-dependent behavior. Exploring the relationship between conformational dynamics and ribozyme activity promises to further advance our understanding of these intriguing molecular systems.

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Novel bio-based solvents for SPPS

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The growing demand for therapeutic peptides highlights the need for more sustainable synthetic methods.¹ While solid-phase peptide synthesis (SPPS) is the standard for peptide production, it suffers from high process mass intensity (PMI), largely due to excessive solvent use—particularly *N,N*-dimethylformamide (DMF), a carcinogenic and reprotoxic solvent now restricted in the EU.²⁻⁴ We present bio-based alternatives to DMF for SPPS, evaluating solubility of Fmoc-protected amino acids and activators, as well as resin swelling. Peptides, including difficult sequences, were successfully synthesized using these greener solvents for coupling, deprotection and intermediate washing steps. While overall solvent reduction remains a challenge, replacing DMF marks a significant step toward more sustainable SPPS.

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Steps towards radioligand therapy of prostate cancer in a mouse modelJ. Genz¹, S. Kichou^{1*}, S. Prytuliak^{1*}, J. P. Holland^{1*}¹University of Zurich

Peptides and peptide-like molecules serve as highly specific targeting vectors, allowing for personalized radiotherapy that minimizes harm to surrounding tissues.^[1,2] Building on the success of the recent block-bluster drug ¹⁷⁷Lu-PSMA-617 (Pluvicto®),^[3] we aimed to adapt the prostate specific membrane (PSMA) targeting vector with the generator produced therapeutic nuclide ¹⁸⁸Re and the imaging nuclide ^{99m}Tc. The radionuclide ¹⁸⁸Re ($t_{1/2}$ =17 h, $E_{\max}(\beta)$ =2.12 MeV, E_{γ} =155 keV (15%)) holds potential for effective cancer therapy while ^{99m}Tc ($t_{1/2}$ = 6 h, γ -ray = 141 keV [89%]) facilitates diagnostic imaging.

We present the synthesis, characterization and ¹⁸⁸Re radiolabeling of three novel radioligands consisting of mercapto acetyl diglycine (MAG₂) as the chelating unit, a non-PEG, a PEG₂ or a PEG₄ linker, and the binding motive of PSMA-617 (¹⁸⁸Re-MAG₂-PEG_n-PSMA; n = 0, 2, or 4) (Figure 1). Cellular binding and competitive binding experiments were performed on PSMA expressing PC-3 PIP and non-PSMA expressing PC-3 cells to investigate specificity and binding affinities. Planar γ -ray scintigraphy imaging was performed on female athymic nude mice with subcutaneous PC-3 PIP and PC-3 xenografts at intervals from 1 to 24 hours post-injection of the radiotracer, and biodistribution analysis was completed after 1, 4, and 24 h for ¹⁸⁸Re-MAG₂-PEG_{0,2}-PSMA (Figure 2). The tumor-specific binding of the radiotracer was verified *in vivo* through competitive inhibition (blocking) studies.

Radiosynthesis of ¹⁸⁸Re-MAG₂-PEG_n-PSMA (Figure 1) yielded the complexes in radiochemical purities (RCP) over 95% and a decay-corrected radiochemical yields (RCY) of over 50%. In mice with PC-3 PIP xenografts, tumor uptake of ¹⁸⁸Re-MAG₂-PEG_{0,4}-PSMA reached 7.58±1.54 %ID/g and 4.31±1.11 %ID/g respectively after 4 hours in the normal groups. Blocking studies showed tumor specificity, reducing tumour uptake by over 50%.

Three novel PSMA specific radiotracers were successfully synthesized. Cellular experiments showed high affinity and specificity of ¹⁸⁸Re-MAG₂-PSMA for target engagement. Animal experiments confirmed the favourable pharmacokinetic behaviour of ¹⁸⁸Re-MAG₂-PSMA which shows potential as a candidate for future radiotherapy studies targeting tumors with high expression of PSMA.

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Development and physicochemical characterization of novel anthranilic anilide-based TRPM4 channel inhibitors identified by SAR study

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TRPM4 is a non-selective, voltage-gated monovalent cation channel, activated by an increase in intracellular Ca^{2+} concentration. The channel depolarizes cells by conducting Na^+ , K^+ , Cs^+ , or Li^+ [1]. TRPM4 is widely expressed in various tissues and associated with cardiovascular or neuronal diseases [1]. In addition, the channel is highly expressed in various types of cancer and contributes to cancer hallmark functions [2]. This renders TRPM4 a promising target in future drug development avenues. Only a few small molecules are known to inhibit TRPM4, including 9-phenanthrol, anthranilic acid derivatives such as flufenamic acid or the previously discovered, more potent and selective, anthranilic anilide-based compounds CBA, LBA and NBA [3]. The goal of our project was to conduct a SAR-study on the anthranilic anilide-based core structure of the two inhibitors CBA and NBA, [3] with the intention to further improve the inhibitory potency and physicochemical properties of this compound class. Such novel TRPM4 inhibitors can serve as improved chemical probes in biomedical research, e.g. as blockers in electrophysiology and *in vivo* animal model studies, or as ligands in cryo-EM studies of TRPM4. We conducted a systematic SAR-study by chemical synthesis of a compound library and subsequent evaluation of TRPM4 inhibition of its members using a HEK293-hTRPM4 overexpressing cell-based fluorescence Na^+ -influx assay. Several derivatives with sub-micromolar potencies have been discovered in this fluorescence assay and our most potent compounds were validated in whole-cell patch-clamp electrophysiology measurements using human colon cancer cells (HCT116), which highly express TRPM4 endogenously. Our best compound exhibited an 18-fold increase in inhibitory potency (IC_{50}) at TRPM4 compared to NBA, and our study revealed valuable structure-activity-relationships for further optimizations. In addition, we measured the aqueous solubility in PBS (pH = 7.4) and lipophilicity ($\log(D)_{7.4}$), as well as determined solid and solutions structures of selected anthranilic anilides to further rationalize their mode of action.

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Exploring Actinomycetes as Biocontrol Agents for Late Blight Management in Potato and Tomato

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The widespread use of chemical pesticides for crop protection has raised significant environmental and health concerns, driving the urgent need for sustainable alternatives. These agrochemicals contribute to soil degradation, biodiversity loss, and the emergence of resistant pathogens, necessitating the exploration of eco-friendly solutions for plant disease management. Actinomycetes have demonstrated great potential as biocontrol agents due to their ability to produce specialized metabolites with antimicrobial properties and to enhance plant growth and resilience. Potato (*Solanum tuberosum*), one of the world's most important staple crops, is highly susceptible to various pathogens, leading to severe yield losses. Among them, *Phytophthora infestans*, the causative agent of late blight disease, remains one of the most devastating threats to global potato production. Traditional late blight management relies heavily on fungicides, which pose environmental risks and are increasingly ineffective due to the rise in pathogen resistance. This study aims to evaluate the biocontrol potential of 200 actinomycete strains isolated from the rhizosphere of potato plants in Switzerland. Their antagonistic activity will be assessed *in vitro* against a broad range of potato pathogens using a high throughput screening method. Promising antagonistic strains will be further tested *in planta* against late blight infection in potato and tomato plants to assess their protective effects under natural conditions, as well as their potential to enhance plant resistance. By identifying effective actinomycete strains with biocontrol properties, this research will contribute to the development of sustainable plant disease management strategies. Our findings will provide valuable insights into harnessing actinomycetes as biocontrol agents, offering a promising approach to reducing reliance on chemical pesticides in modern agriculture.

Phosphorylation of alcohol sidechains to investigate PTM crosstalk of oncogenic MYC

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MYC is an intrinsically disordered protein (IDP) that acts as a regulator of gene transcription and is deregulated in over 50% of human cancers.^{1,2} Its activity is largely modulated by phosphorylation and the protein-protein interactions (PPIs) they induce.³ Changes in phosphorylation could be responsible for switching from a controlled (normal) state to a deregulated (cancer) state.⁴ To date, mainly small fragments of MYC with minimal phosphorylations have been isolated and studied.⁵

It is challenging to obtain peptides with multiple selective phosphorylations using the typical methods of a phosphorylated building block or P(III) global phosphorylation. The SPPS building block approach suffers from incomplete couplings that lead to decreased yield, limiting the number of phosphorylated residues that can be incorporated, and high costs. Meanwhile, P(III) phosphoramidite methods require a harsh oxidation step that can be incompatible with oxidation prone residues and destructive to the peptide.⁶ To address this issue, a novel method for the on-resin (poly)phosphorylation of Ser, Thr & Tyr sidechains has been developed. This approach utilizes previously discovered, inexpensive and commercially available P(V) reagents, eliminating the need for oxidation and expensive SPPS building blocks.

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Producing the radionuclide ^{149}Tb for preclinical investigations

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Terbium radioisotopes have been termed the “Swiss Army knife” of nuclear medicine. There are four radioisotopes which can potentially be used for diagnosis or therapy [1]. Among them, ^{149}Tb is one which emits both α - and β^+ -particles ($T_{1/2}=4.1$ h, $E_{\alpha} = 3.98$ MeV (16.7%), 28 μm range in tissue), implying it can be imaged for positron emission tomography as well as therapy [2]. Targeted alpha therapy (TAT) leverages on the high linear energy transfer (LET) of α -particles on malignant tissues, delivering well-localized lethal doses to cancerous lesions.

Currently, the most efficient route to obtain ^{149}Tb is by spallation of a tantalum target induced by a high-energy proton beam and coupled to online mass separation, a process which can be performed at the ISOLDE facility at CERN [3]. To conduct preclinical *in vitro* and *in vivo* investigations high specific activity, radiochemical and radionuclidic purities are required.

The product collected from spallation and mass separation was a mixture of isobars and pseudo-isobars e.g. nuclides of the same mass as well as monoxides of nuclides of the mass 133 ($133+16 = 149$), respectively, embedded in a Zn film deposited on an inert metal (Au, Pt, Ta) foil. Therefore, a subsequent chemical separation is essential to meet the quality requirements for preclinical investigations. The samples with ^{149}Tb were sent from ISOLDE/CERN to the Paul Scherrer Institute's Center for Radiopharmaceutical Sciences (CRS) to perform radiochemical separations in a shielded cell. The zinc film was dissolved off the foil using 0.5 M HNO_3 . The solution pH was subsequently adjusted to ~ 1.5 with 1.0 M NH_3 . The solution was then passed through a chromatographic column filled with a macroporous cation exchange resin (Sykam GmbH, Germany). The separation on this column was performed with 0.07 M to 0.11 M α -hydroxyisobutyric acid (α -HIBA) in a gradient elution process. the eluted ^{149}Tb fraction was concentrated on an extraction resin (Triskem, France), where α -HIBA was removed, and the activity obtained in less than 1 mL 0.05 M HCl . An activity concentration of up to 300 MBq/mL was achieved in this way. Activities of up to 400 MBq were collected at end of separation. The radionuclidic purity was evaluated by γ -spectrometry with a high purity germanium detector. Other than the daughter nuclides of ^{149}Tb , no impurities were detected. The radiochemical purity was assessed by radiolabeling a ligand (DOTA-based) and determining the yield by HPLC, with RCP>95% at 20 MBq/nmol consistently achieved [3].

Another production campaign is planned for 2025 the last before CERN's long shutdown (LS3), where the ISOLDE facility will be under maintenance for approximately 2.5 years. Following the encouraging results obtained so far, the activities to be produced in 2025 will be used for further preclinical investigations at CRS.

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tRNA modifications and their importance in the proto-ribosomal translation system

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Ribonucleic acids (RNAs) play a key role in the theories about the origin of life. They function both as carriers of information and catalysts in reactions which puts them in the spotlight of these theories. Considering a complex mixture of the early prebiotic soup and the presence of amino acid-modified nucleotides found in contemporary transfer-RNAs (tRNAs), considered as molecular fossils, we investigate a putative RNA-peptide world as a complementing concept to the RNA-world¹. Recently RNA-templated amino acid transfer between two complementary strands containing the modified RNA nucleobases (m¹)nm¹U and (m¹)aa¹A and peptide chain elongation could be demonstrated in our group². The increased chemical space of RNA modified by amino acids could have been a driving force to evolution. Moreover, catalytically active RNA ribozymes raise special interest in the prebiotic context. It has been shown that there exist small aminoacylating ribozymes recognizing the tRNA 3'-terminal CCA motif and that this concept can be applied to aminoacylate shorter oligonucleotides³. To expand the way towards a more complex ribosomal translation machinery, the combination of the templated peptide synthesis with amino acid loading by ribozymes is under investigation. Moreover, we chemically try to attach the amino acid via an ester bond to the acceptor RNA. It is of interest what structural role ribozymes played in prebiotic catalysis during evolution in an RNA-peptide world. Therefore, the hammerhead ribozyme is used to screen the effect of non-canonical motifs on catalytic efficiency.

Fig.1 Flexizyme aminoacylation of an acceptor strand with the recognition motif CCA.

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pH-Dependent Cellular Uptake of CPPs – Guanidinium versus Oxyguanidinium ProlineR. Heeb¹, L. Massaad^{1*}, H. Wennemers^{1*}¹ETH Zurich, Laboratory of Organic Chemistry, Vladimir-Prelog Weg 3, 8093 Zürich, Switzerland
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Cancer is one of the main causes of death worldwide and, due to its inherent complexity and resistance to conventional therapies, often not treatable. Current therapeutics are often limited by poor cellular uptake or toxicity and reduced efficacy, thus, hindering clinical use. Cell-penetrating peptides (CPPs) have the potential for specific tumor targeting as well as efficient cargo delivery into the tumor cells.[1]

The acidic microenvironment typically found in pathological conditions is an opportunity for the selective and efficient uptake of tumor targeting CPPs. However, CPPs developed for this purpose so far suffer from limitations such as aggregation, endosomal entrapment and uptake efficiency.[2-4]

The Wennemers group has developed cationic oligo guanidinium proline (e.g., Gup₈) CPPs, which exhibit higher cellular uptake and low endosomal entrapment compared to previously established peptides (e.g., octa arginine).[5] The higher cell penetration is due to the rigidity of the PPII helical structure and the resulting defined spatial charge arrangement. Retaining the concept of the spatial charge arrangement, we developed a new tumor-selective CPP based on oxyguanidinium prolines. The advantage of this novel peptide is a lower pK_a of the oxyguanidinium functionality allowing for environment dependent protonation and cellular uptake. Our studies revealed a pK_a of ~7 for the Ac-(2*S*,4*S*)-Gop-OMe and a preference for the C4-*endo* pucker like the Ac-(2*S*,4*S*)-Gup-OMe, and different secondary structures dependent on temperature, solvent and pH. Further, this oxyguanidinium-based CPP has a higher cellular uptake at acidic than neutral pH. These results open exciting prospects for the development of novel tumor-selective delivery agents.

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An enzymatic approach to fluorinated pharmaceuticals

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Fluorine plays a crucial role in pharmaceutical molecules and is currently present in 25% of all approved drugs due to its effects on pharmacokinetics and pharmacodynamics^[1–3]. Furthermore, ¹⁸F isotopes are commonly used for radiolabeling in PET scans because of their favorable half-life of 110 min^[4]. Installation of fluorine via synthetic routes can be challenging because of its strong electronegative properties, lack of methods and catalysts, as well as slow reaction rates for radiolabeling^[5–7]. In nature only one known enzyme mechanism has been found to catalyze C-F bond formation. The fluorinase enzyme (FlA, EC 2.5.1.63), first identified in *Streptomyces cattleya* in 2002,^[8] facilitates the conversion of *S*-adenosyl-L-methionine (SAM) to 5'-fluoro-5'-deoxyadenosine (5'-FDA). Sixteen native FlA homologs have since been identified^[9], several of which accept a limited number of non-native substrates^[10–14]. The reaction proceeds via an S_N2-like mechanism under room temperature and aqueous conditions which contrasts the harsh conditions, specialized solvents and catalysts synthetic C-F bond formation often requires^[10,15]. This work applied an interdisciplinary approach combining computational modeling and enzyme engineering for the development of enzymatic fluorination strategies for newly synthesized substrates. The expected output is the expansion of chemical space around enzymatically accessible fluorinated therapeutics.

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Diastereomeric Optimization of Indolicidin for the Discovery of Novel Antimicrobial Peptides

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Antimicrobial peptides (AMPs) are considered promising candidates for developing antibacterial agents¹. Indolicidin (ILPWKWPWWPWR-NH₂) is a linear AMP known for its moderate, broad-spectrum activity against bacteria. However, its clinical application is hindered by significant hemolytic activity and limited antibacterial potency². Consequently, there is growing interest in developing indolicidin analogues with enhanced antibacterial efficacy and reduced toxicity.

Recent studies from our group have demonstrated that diastereomeric optimization is an effective strategy to improve the bioactivity of peptides, leading to the development of optimized AMPs. Siriwardena et al. highlighted the importance of stereochemical purity in determining AMP properties, showing that stereorandomization can maintain antibacterial activity while reducing hemolytic effects³. Similarly, Personne et al. incorporated D-amino acids into a linear α -helical undecapeptide, producing stereoisomeric analogues with improved antibacterial properties and lower cytotoxicity⁴.

Building on these findings, this project aims to develop diastereomeric analogues of indolicidin by substituting L-amino acids with their D-enantiomers. The resulting peptides will undergo further biological and pharmacological evaluation to explore their cellular mechanisms of action and drug-like properties.

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Synthesis and characterization of covalent inhibitor probes targeting transcription-coupled nucleotide excision repair

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Cellular DNA repair pathways, such as transcription-coupled nucleotide excision repair (TC-NER), can drive chemotherapeutic drug resistance through the removal of DNA adducts formed by DNA alkylating drugs. Thus, preventing repair, such as via TC-NER inactivation, may promote drug efficacy but strategies for TC-NER inhibition are limited. An approach to impede the excision of the damaged nucleotide would be to inhibit the activity of one of the TC-NER endonucleases ERCC1-XPF or XPG, via attachment of a covalent inactivator to known alkylating agents. To explore this strategy, in the present work we synthesized structures consisting of a covalent inhibitor attached to an oligonucleotide. In a first stage, a covalent inhibitor library was designed, combining three different protein reactive groups, namely vinyl sulfonamides, acrylamides and sulfonylfluorides with three linkers of distinct lengths. One of the distinct linkers was commercially available, whereas the other two linkers could be synthesized with good yields of up to 78%. These three linkers were subjected to a one-step reaction with the protein reactive groups. The reaction with the sulfonylfluorides yielded moderate results, with product yields ranging from 43% to 46%. In contrast, the formation of acrylamide inhibitors was effective for a single linker, yielding a yield of 57%. However, lower yields were observed for the two other linkers (27% and 39%). The sulfonamide inhibitor formation exhibited the lowest yield among all covalent inhibitor reactions, with yields ranging from 14% to 37%. This small covalent inhibitor library, consisting of nine covalent inhibitors, was then attached to a modified oligonucleotide bearing an alkyne group through copper catalyzed azide alkyne cycloaddition, generating nine different candidate covalent inhibitor probes. These candidate covalent inhibitor probes provide a novel instrument with which to examine their interactions with nucleophilic amino acids, such as cysteine and lysine. Furthermore, these candidate covalent inhibitor probes can be utilized to investigate the inhibition potential of the protein-reactive groups towards NER.

Fold to Cleavage: Key Structural Elements of the Minimal HDV-like Ribozyme drz-Mtgn-1

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HDV-like ribozymes are self-cleaving RNA molecules discovered across a wide range of organisms,^[1] suggesting important roles in diverse biological contexts. The prototype Hepatitis Delta Virus (HDV) ribozyme, which adopts a compact nested double pseudoknot fold, has been extensively studied and serves as a model for this class.^[2] Despite substantial variation in sequence and length, HDV-like ribozymes share a common overall architecture that enables them to catalyse the same self-cleavage reaction, relying on only a few conserved nucleotides and motifs located within or near the catalytic core.^[3]

Among this family, the minimal HDV-like ribozyme drz-Mtgn-1 is particularly noteworthy. It lacks the P4 stem loop and displays a distinct bell-shaped self-cleavage activity profile in the presence of divalent metal ions.^[4] Originally identified in a human intestinal metagenomic phage genome, drz-Mtgn-1 shares many structural features with over a thousand recently discovered theta ribozyme, which cleave at the 3'-end of bacteriophage tRNAs and are proposed to function in 3'-trailer tRNA processing.^[5] While structurally similar, the specific biological role of drz-Mtgn-1 remains elusive. To date, no structural data exists for any member of this minimal ribozyme class, leaving a significant gap in our understanding of how their compact architecture supports their activity.

To investigate the structural basis of catalysis in these minimal systems, we employed NMR spectroscopy with a sophisticated ²H/¹³C/¹⁵N isotope labelling strategy to study the structure of drz-Mtgn-1. We confirmed the predicted nested double pseudoknot and characterized several key structural motifs involved in forming the catalytic core. Additional titration experiments varying pH and metal ion concentrations indicate underlying conformational dynamics that may be important for catalytic activity.

This study provides the first structural insights into a minimal HDV-like ribozymes, aiming at illuminating the structural framework and dynamics of key nucleotides while also clarifying the role of metal ions in regulating the distinct self-cleavage activity. By tackling these aspects, we seek to close the gap between the ribozyme's structure and its functionality.

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Functionalization and Longevity of a Deuterated Silicon-Rhodamine Fluorophore for Application in Calcium Sensing Receptors and Adenosine Receptor Imaging

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Fluorophores are indispensable tools in modern bioimaging, as they enable the real-time visualization of dynamic cellular processes.^[1] However, conventional fluorophores are limited by issues such as photobleaching, spectral shifts, and short fluorescence lifetimes. These factors restrict their effectiveness in long-term live-cell imaging. Introducing deuterated amines to silicon rhodamines (SiR^[2]) significantly enhances the photostability and quantum yield of the fluorophore while reducing irreversible bleaching.^[3]

In this project, we address these issues by synthesizing a novel deuterated SiR-fluorophore variant for its conjugation with GPCR-active substances and evaluation of its stability. A fluorescent probe of Evocalcet (EvoSiR6-*d*₁₂), a clinically approved drug for the treatment of secondary hyperparathyroidism (SHPT), showed enhanced specificity and potency towards the calcium-sensing receptor (CaSR) during previous studies in our lab. Using our novel deuterated SiR fluorophore, we aim to develop an imaging probe with improved photo-physical properties.^[4]

Furthermore, we project connecting the novel fluorescent dye will to an adenosine receptor agonist that is selective for the A₁ subtype (A₁R). There is currently a lack of A₁R-selective, fluorescent agonist probes. Phenyloxycyclopentyladenosine (PhOCPA) derivatives exhibit high A₁R selectivity^[5], suggesting that this scaffold may be utilised as a ligand in the development of selective fluorescent adenosine receptor probes (e.g. PhOCPA-SiR-*d*₁₂). These efforts could possibly make it a promising candidate for advanced bioimaging tasks, including fluorescence lifetime imaging microscopy (FLIM) and long-term tracking of receptor dynamics in live cells and tissues.

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Design, Synthesis and Biological Evaluation of Chemo-radiotheranostic Chimera Molecules Fusing Prostate Targeting ^{99m}Tc Complexes with Potential Antimitotic Drugs

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Radiotheranostic compounds are at the forefront of new advances in nuclear medicine for both diagnosis and treatment of cancers.^[1] Whilst molecularly targeted radiotracer therapy shows great promise as an alternative treatment strategy, off-target dosimetry concerns mean that classic chemotherapy remains the primary standard of care.^[2] Here, we aimed to combine the high sensitivity of gamma ray imaging with the controlled delivery of potent cytotoxic drug molecules by combining ^{99m}Tc complexes with both prostate specific lysine-urea-glutamate ligands and the antimitotic agent (*R*)-ispinesib to create a first-in-class chemo-radiotheranostic chimera.

As a baseline radiotracer a prostate specific membrane antigen (PSMA) targeting group was functionalized with a norbornene unit as a 'click' handle to couple it to a $\text{TACN-}^{99m}\text{TcO}_3^+$ -core.^[3] And for chemo-radiotheranostic application a chimera analog was synthesized with the anti-Eg5 drug (*R*)-ispinesib, attached via a cathepsin B cleavable spacer. The radiolabeling via Tc-mediated 'click' reaction was conducted in water (80°C, pH 7-8) and allowed for easy labelling with an overall radiochemical yield of >53% (over two steps) and a radiochemical purity of >99% for both radiotracer and chimera. The cellular binding and IC₅₀ of the baseline radiotracer ($\text{TACN-}^{99m}\text{TcO-PSMA}$) were tested on PC3-PIP(+) cells, demonstrating specific binding. The pharmacokinetics and tumor targeting was evaluated in mice bearing subcutaneous PC3-PIP(+) xenograft using non-invasive planar gamma ray scintigraphy imaging, *ex vivo* biodistribution analysis and metabolite profiling with data compared against control compounds showing high tumor uptake (19.73±4.22 %ID/g after 4 hours) which was reduced by >80% in blocking control groups (3.16±0.62 %ID/g). The $\text{TACN-}^{99m}\text{TcO-PSMA-Ispinesib}$ chimera molecule was so far successfully synthesized, fully characterized and radiolabeled. Preliminary biological test shows that the free cargo (*R*)-ispinesib exhibits high potency towards the PC3-PIP(+) cells (GI₅₀ ≈ 0.13 nM). Additional biological tests are underway.

The TcO_3 -based labelling strategy is an ideal match labelling complex bioactive drug molecules and the resulting $\text{TACN-}^{99m}\text{TcO-PSMA}$ radiotracers were very stable and showed high tumour-specificity both *in vitro* and *in vivo*. Combining this with highly potent drugs such as (*R*)-ispinesib we hope to provide a new entry point toward chimeric chemo-radiotheranostic agents.

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Theta Ribozymes: Making the Cut in tRNA Maturation

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RNA is capable of storing genetic information while simultaneously catalyzing chemical reactions. Internal transesterification of the phosphate-ribose backbone is an abundant example carried out by the class of small self-cleaving ribozymes, such as the Hepatitis Delta Virus (HDV) ribozyme [1]. The site-specific catalysis is enabled by a double-nested pseudoknot. This motif is shared among the family of HDV-like ribozymes, which spans thousands of examples across the entire tree of life. Despite their abundance, biological functions clearly assigned to specific examples remain strikingly rare.

We tackled this question using an interdisciplinary approach combining bioinformatics and chemical biology, resulting in the discovery of *theta* ribozymes: minimal HDV-like ribozyme variants that cleave viral tRNA 3'-trailers *in vitro* [2]. Our analyses revealed that the predominant habitat of *theta* ribozymes are *Caudoviricetes* bacteriophages inhabiting mammalian gastrointestinal microbiomes. We propose that these ribozymes play a crucial role in the phage life cycle and consequently the delicate balance of the mammalian gut microbiome.

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Two-Color Timestamping of Gene Expression with a Chemigenetic Reporter System

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Stress response pathways rely on coordinated gene expression regulation. In the endoplasmic reticulum, the unfolded protein response plays an important role in maintaining the homeostasis of protein folding and processing. We developed a chemigenetic reporter assay that allows monitoring of BiP/GRP78 expression, a key regulator of the unfolded protein response. Our system is based on the co-expression of the self-labeling protein HaloTag via an internal ribosome entry site. HaloTag allows for flexibility in labeling color as well as labeling timepoint. We designed a two-color timestamping strategy that provides improved read-out sensitivity. The reporter system can be used in live-cell imaging as well as flow cytometry. We applied it in a high-content screening experiment in which we identified several new promising activators of BiP/GRP78 expression.

Figure 1. Schematic representation of chemigenetic reporter system. Insertion of the sequence IRES-HaloTag-T2A-PuroR into the genome downstream of BiP/GRP78 leads to the co-expression of the protein of interest BiP/GRP78, the reporter HaloTag, and the selection marker puromycin N-acetyltransferase (PuroR) as separate proteins. The expression level of HaloTag, corresponding to the level of BiP/GRP78, can be visualized by fluorescent labeling.

Click-chemistry-aided quantitation and sequencing of oxidized guanines and apurinic sites uncover their transcription-linked strand bias in human cells

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DNA modifications drive aging, neurodegeneration, carcinogenesis, and chemotherapy drug action. Accurate mapping of diverse DNA modifications with single-nucleotide precision in complex genomes remains challenging. We upgraded click-code-seq, a click-chemistry-aided DNA-modification mapping strategy previously used in yeast, for its first application to human genomes to sequence DNA oxidation and depurination [1]. We developed a companion fluorescence assay, click-fluoro-quant, to rapidly quantify common DNA modifications, and novel adapters to minimize false positives and assess modification frequency. To assess the accuracy and robustness of the method, we validated the upgraded click-code-seq using plasmids containing site-specific DNA modifications. We uncovered that endogenous DNA oxidation in a human cell line mirrors cancer mutational signatures linked to reactive oxygen species. The chemotherapy drug ifofulven preferentially induces depurination in ApA dimers and promoters. Notably, oxidized guanines and apurinic sites, both ifofulven-induced and endogenous, are depleted in gene transcribed strands, with the strand bias increasing with gene expression. This work substantially advances click-code-seq for deciphering the impacts of key modifications in human DNA on cellular physiology and toxicological responses.

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Applying New Methods for Multi-Color Labeling of Catalytically Active RNAs

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RNA is an elusive and complex molecule to study due to its dynamic nature and intricate structure. By labeling it with two or more fluorescent dyes, researchers can visualize its interactions and conformational changes using single-molecule Förster Resonance Energy Transfer (smFRET). This technique measures the distance-dependent energy transfer between the dyes, offering valuable insights into RNA's structural dynamics at the molecular scale. A class of very dynamic RNAs are ribozymes, i.e. RNA molecules performing a chemical reaction. Within this group, small variants (e.g., Twister or HDV ribozyme, ~30-70 nt) as well as very large representatives (group II intron, ~kb) are found. Our goal is to find and apply a labeling method suitable for the multi-color labeling of such large ribozymes. This would enable us and other researchers to study the dynamics of the folding and reaction steps of these molecules of interest, something that is difficult to achieve at the moment.

The first milestone, establishing a method for single-color labeling, has been reached in 2024 by Haslecker *et al.* by creating SegModTex (Segmental labeling and site-specific Modifications by Template-directed eXtension).[1] We present our work on reaching the second milestone: dual-color labeling of a small ribozyme ready to use for smFRET applications. Whereas multiple methods for RNA labeling exist in the literature, every one of these needs to be adjusted and perfected for the class of RNA molecules that will be targeted. We therefore present our work on how to mitigate common issues, such as secondary structure formation in the template, length of the target RNA, working with a chemically active class of RNA, and improving the yield in multi-step reactions.

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Binding or Unwinding? - The role of an RNA binding protein in ribozyme activity

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Ribozymes are non-coding RNAs folding into a specific, 3-dimensional structure to carry out chemical reactions. For an efficient reaction, some ribozymes rely on protein assistance. While the effect of proteins on ribozyme activity can easily be assessed by *in vitro* assays, understanding how the protein promotes activity is not. In this study the protein belongs to a helicase-family and can unwind double stranded RNA. We hypothesise that the increase in activity is the result of either protein binding and the stabilisation of the structure, or unwinding of misfolded regions. To identify the exact mechanism we combine photoisomerization-related fluorescence enhancement (PIFE) with Förster resonance energy transfer (FRET), two single-molecule techniques.

The initial goal is to establish and validate a combined PIFE-FRET methodology, employing the model system of a double-stranded RNA. The fluorescently labelled RNA is immobilized on a surface mounted in a TIRF microscope setup. The recorded intensity increase in the presence of the protein hints at a potential translocation along ssRNA. Subsequently, the interaction of the protein with the ribozyme will be studied using the established PIFE-FRET measurements.

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Total synthesis of a plant-derived glycoside that attenuates virulence in *Pseudomonas aeruginosa*

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P. aeruginosa is a detrimental lung pathogen, commonly associated with hospital-acquired infections. It is a major cause of mortality in patients with cystic fibrosis or a compromised immune system. It is associated with a high risk of developing drug resistance, and therefore, the World Health Organization has identified it as a pathogen for which novel therapeutic approaches are urgently needed.

Recently, the screening of a natural product library identified a novel glycoside structure (**1**) which was capable of attenuating virulence in *P. aeruginosa*. To further characterize this unique activity, we carried out the total synthesis of glycoside **1** and related derivatives to facilitate a structure-activity relationship study. This natural product, isolated from a plant extract, consists of a trisaccharide core structure and a geranylgeraniol diterpenoid aglycone. Although both the diterpenoid tail and the single acetylated rhamnose unit presented unique synthetic challenges, we successfully designed and carried out the total synthesis of this complex natural product and related analogues. An investigation of their biological activity is currently underway.

Key words: synthesis, glycoside, *P. aeruginosa*, virulence, infections

Ligand-directed bioconjugation of native protein with hypervalent iodine-based Ethynylbenziodoxolones (EBXs) Reagents.

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Site selective labeling of proteins is essential to improve diagnostics and therapeutics tools. While many site-selective labeling methods have been developed, they often require genetic modification of the protein of interest (POI). In contrast, affinity-driven chemistry enables the modification of native, non-tagged proteins with chemical probes in physiological contexts.¹

By combining the chemoselectivity of hypervalent iodine-based Ethynylbenziodoxolones (EBXs) and peptidomimetics to target protein-protein interactions (PPIs) we were able to label site selectively Cys 434 of the KELCH domain of KEAP.^{2,3} EBX could be used either as a traceless reagent to introduce reactive handles such as azides via ligand-directed chemistry or as a protecting group that could later be removed.⁴

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Synthetic Lipid-Linked Oligosaccharide for Structural Studies of Alg6

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N-linked glycosylation is a crucial post-translational modification that drives the formation of structurally diverse N-glycans.[1] Central to this pathway are lipid-linked oligosaccharides (LLOs), which serve as key substrates for glycosylation enzymes such as oligosaccharyltransferase (OST) and members of the ALG (asparagine-linked glycosylation) family.[2,3] To facilitate the study and application of this biosynthetic route, our laboratory has developed simplified LLO precursors. Among the substrates evaluated, GlcNAc₂-PP-Dol₂₅ (**1**) has proven to be the most efficient for enzymatic synthesis of LLO analogues.

The synthesis of compound **1** involves a lengthy sequence and challenging purification steps, due to its sensitivity to acidic and basic conditions. We have previously optimized this synthetic route, ensuring a reliable supply for biochemical investigations.[4] While highly effective for most enzymatic studies, **1** does not provide sufficient resolution in cryo-EM studies of ALG6 ternary complex. To overcome this limitation, we are currently developing a lipid analogue that more closely mimics the native dolichol backbone. GlcNAc₂-PP-Dol₃₀ (**2**) incorporates an additional isoprenyl unit and features predominantly *Z* double bonds, consistent with the structure of the native dolichol. For this, different strategies are being explored for the construction of this lipid chain. These structural refinements are expected to enhance the stability of the enzyme–substrate complex, thereby enabling higher-resolution cryo-EM studies of ALG6.

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Sustainable dipolar aprotic solvents

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Dipolar aprotic solvents are widely used in pharmaceutical and medicinal chemistry due to their versatile solvation properties and non-hydrogen-bond-donating nature. However, conventional solvents in this category, such as DMF, NMP and DMAc, pose significant environmental and health risks. Existing bio-based alternatives, including dihydrolevoglucosenone (Cyrene), gamma valero lactone (GVL), and propylene carbonate, suffer from limited versatility due to reactivity issues and / or high boiling points (> 205 °C), restricting their recyclability. Consequently, there is an urgent need for non-toxic, bio-based, stable, recyclable and cost-effective alternatives.

Here we introduce novel dipolar aprotic solvents derived from glycerol, a widely available and underutilized waste product from the biodiesel industry. These solvents green profile is demonstrated by a simple synthesis under mild conditions, employing heterogeneous catalysis and utilizing crude glycerol as a feedstock.

With a boiling point below 190 °C, one of these new solvent candidates is distillable and recyclable, while its high flash point ensures it does not require classification as a flammable substance. To assess its performance, we evaluated its efficacy in pharmaceutically relevant reactions (Menshutkin, amidation and Baylis-Hillman) by comparing reaction rate constants. This new solvent performed comparably or better than conventional dipolar aprotic solvents (Fig 1A), aligning with its charge density profile similarity to DMF (Fig 1B). Toxicological and biodegradability assessments are currently ongoing, while a preliminary techno-economic analysis suggests a minimum selling price of 2.72 USD/kg, which is comparable to existing dipolar aprotic solvents.

Figure 1: Performance of one new solvent candidate and selected other solvents in Menshutkin alkylation model reaction, against relevant Kamlet-Taft parameter (A) Sigma profiles of charge density of the new solvent compared to DMF (B) (AMS COSMO).

Collagen Cross-Linking: Structure and Sequence Selectivity of Lysyl Oxidase-Like 2

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The remodelling and maturation of collagen, the dominant structural protein in mammals, is crucial for the integrity of organs and wound healing.^{1,2} A key process is the cross-linking of collagen strands triggered by the post-translational oxidative deamination of lysine to allysine residues through lysyl oxidases (LOXs). Excessive LOX activity is, however, implicated in fibrotic and malignant diseases which are associated with more than one third of deaths worldwide.¹⁻³ Understanding the role of LOXs in specific tissues could facilitate addressing these diseases. Surprisingly little is, however, known about the selectivity of LOXs.

In this study, we deciphered structure and sequence effects on aldehyde formation by LOXL2. This LOX isoform is of particular interest as it is over-expressed in many cancers.⁴ Using fluorogenic enzyme activity assays, we investigated proline-rich peptides, comprising a lysine residue, that mimic either an intact or disrupted triple-helical structure, as found in connective tissue disorders.⁵ Our findings revealed the importance of the lysine-containing peptide sequence with a remarkable impact of charged residues on LOXL2 activity. We also show that LOXL2 is most active on single-stranded proline-rich peptides, as present in pathologically disturbed collagen triple helices.⁶

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Advancing DELSTAR: Sequencing PolyA Tails to Evaluate Binder Affinity of Enriched DNA-Encoded Library Members

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DELSTAR is a novel DNA-encoded library (DEL) selection strategy in which binding events are recorded directly into binder DNA via polyadenylation by Terminal deoxynucleotidyl transferase (TdT), fused to the target protein [1]. Upon ligand binding to the target protein, proximity between TdT and the DEL member leads to the addition of a polyA tail at the 3' end of the binder DNA, with tail length correlating with the ligand's binding affinity. These elongated, polyA-tailed members can be efficiently separated from non-binders using magnetic poly(dT)25 beads, enabling enrichment of ligands across a wide affinity range, including weak and moderate binders often missed by classical methods. However, the binding strength information stored in the polyA tail remains inaccessible until the tail lengths are precisely measured. In this work, I am developing a sequencing-compatible library preparation workflow specifically designed for DELSTAR-enriched members to enable reliable polyA tail length determination. Initial efforts focus on optimizing enzymatic processing and adapter ligation steps during library preparation to ensure accurate sequencing of polyA-tailed DNA, addressing challenges posed by long homopolymeric stretches. This ongoing work aims to expand the utility and precision of DELSTAR, ultimately facilitating its broader application in early-stage drug discovery.

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From stable to labile: Fine-tuning the off-target uptake of radiotracersS. Prytuliak¹, C. Berton^{1*}, J. Genz^{1*}, J. P. Holland^{1*}¹University of Zurich, Department of Chemistry, 8057 Zurich, Switzerland

One of the major challenges of molecularly targeted radiotherapy is to reduce off-target uptake of the radioconjugates in the healthy organs. We modified our radiotracer designs to incorporate enzymatically cleavable linkers that help eliminate the radioactivity from background tissues via controlled tissue-specific metabolism. Proof-of-concept models were tested with the monoclonal antibody onartuzumab and a lys-urea-glutamate radiotracer targeting the prostate specific membrane antigen (PSMA) to address dose limiting kidney uptake.^[1,2] ⁶⁸Ga and ¹⁷⁷Lu radiotracers bound to the PSMA-targeting unit or to onartuzumab were designed to incorporate the MWK tripeptide sequence, a known substrate for neprilysin expressed in the kidney. Stability studies were tested *in vitro* using homogenised porcine renal cortex. Radiotracer studies in mice bearing either PC-3-PIP (for PSMA studies) or MKN-45 xenografts (expressing c-MET for onartuzumab experiments) were performed using a combination of non-invasive positron emission tomography, gamma-ray scintigraphy, *ex vivo* biodistribution analysis and HPLC analysis of biological fluids. Both the ⁶⁸Ga-DOTA-MWK-PSMA and ¹⁷⁷Lu-DOTAGA-MWK-onartuzumab were prepared with high radiochemical yields, radiochemical purity, and molar activities. *In vitro* assays indicated high stability of the conjugates in the absence of enzymes or tissue lysates. Imaging and biodistribution experiments with ¹⁷⁷Lu-DOTAGA-MWK-onartuzumab showed high and specific tumour uptake (41.9±7.5%ID/g at 72h) but also showed high accumulation in the kidney. While the radioconjugate underwent rapid decomposition in tissue lysate *in vitro*, no renal excretion was observed *in vivo*. For ⁶⁸Ga-DOTA-MWK-PSMA high and specific tumour uptake was observed (12.1±3.5%ID/g at 4h) with PET image contrast improving out to 4 hours. HPLC analysis of urine samples revealed one new metabolite peak which was confirmed by independent synthesis to be the MWK cleavable byproduct. Whilst the MWK peptide sequence proved effective at modulating the pharmacokinetic profile of a ⁶⁸Ga-PSMA targeted small-molecule, it failed to enhance kidney clearance using the ¹⁷⁷Lu-antibody model. Future studies will explore alternative cleavable-linker/enzyme combinations.

Figure 1. A: Positron-emission tomography of a tumour-bearing mouse at 1 h and 2 h p.i. B: HPLC analysis of the intact tracer, urine metabolites, suspect metabolite, and free ⁶⁸Ga. C: Ex vivo biodistribution of ¹⁷⁷Lu-DOTAGA-MWK-onartuzumab (72 h p.i.) and ⁶⁸Ga-DOTA-MWK-PSMA (4 h p.i.). D: In vivo distribution of ⁶⁸Ga-DOTA-MWK-PSMA compound 1 h and 2 h p.i., from PET images.

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Synthesis of novel polycyclic diamine scaffolds derived from tropinoneA. Puckett¹, J. Reymond^{1*}¹Department of Chemistry, Biochemistry and Pharmacy University of Bern

Small molecule drugs often consist of rigid scaffolds equipped with reactive handles, typically amino groups functionalized with pharmacophores. To explore previously unknown chemical space, we can use generated databases (GDBs), which are a large collection of generated chemical scaffolds adhering to principles of synthetic feasibility and chemical stability. Comparing the generated databases (GDB) with biologically active small molecules in ChEMBL reveals that many scaffolds, even structurally simple ones, have never been synthesized (1)(2)(3).

From the GDB we have found interesting families of tricyclic and bicyclic chemical scaffolds which can be accessed synthetically from tropinone (Fig 1). In this poster we describe the simple synthesis to obtain these novel poly-cyclic diamine scaffolds from tropinone which can be easily functionalized to explore differentiated medicinal chemistry space.

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Medicinal Chemistry Functionalization of Brexazine, a Chiral Tricyclic Diamine Mimetic from the GDB Chemical Space

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The generated databases (GDBs) are a collection of possible molecules up to a certain size which are filtered by rules of synthetic feasibility and chemical stability. A large number of these molecules are novel, intrinsically chiral, 3D-shaped and have never been synthesized^[1]. As such, they are a great source for new building blocks for medicinal chemistry.^[2] A recent example for such a building block is brexazine (see Figure 1) which we synthesized from a commercially available norbornene. Brexazine is a tricyclic diamine that we envision to be used as a diamine mimetic with novel topology and exit vectors.^[3] We have worked on various applications for this building block which we will present here.

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RNA-Inhibitor Interactions using Multisite smFRET

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For around fifty years, structure determination methods have provided static snapshots of biomolecule–small molecule interactions with ever-increasing resolution. However, these techniques fall short in capturing how such interactions influence the dynamic structural rearrangements within biomolecules, such as domain-specific movements critical to their function^[1].

To address this limitation, we employ two-colour single-molecule Förster Resonance Energy Transfer (smFRET) with multiple labeling positions, enabling the measurement of multiple distances within a biomolecule, tackling a major limitation of 2 colour smFRET, which is single distance information^[2]. As a model system, we investigate group II introns—large self-cleaving RNA molecules that serve as paradigms to study mRNA splicing. These introns are absent in humans but are present in essential housekeeping genes of human-pathogenic fungi, making them promising antifungal targets with minimal side-effects^[3].

We focus on Intronistat B, a small-molecule inhibitor that binds to the evolutionarily conserved catalytic core of group II introns, disrupting their splicing activity^[4,5]. Using smFRET, we track RNA structural rearrangements in the presence of the inhibitor, revealing interesting results that shed light on the binding mode of the RNA-small molecule interaction. Furthermore, the study highlights smFRET techniques as powerful tools for exploring RNA-inhibitor interactions and provides valuable insights that can guide rational design of novel antifungals.

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Micromapping Thiol-Mediated UptakeS. Saidjalolov¹, F. Coelho^{1*}, N. Sakai^{1*}, S. Matile^{1*}¹Department of Organic Chemistry, University of Geneva, 1211 Geneva, Switzerland

The cell membrane is the natural biological barrier of the cell that maintains its integrity, i.e. shape and size, and assures an exclusive exchange with the outer matrix through several internalization processes. Thus deciphering its complete mechanism to exploit to its full potential remains a challenging task. Among the various studied internalization pathways, thiol-mediated uptake (TMU) has been recently given a lot attention due to its efficient transport of a wide range of substrates. This efficiency can be assigned to the dynamic covalent cascade exchange (CAX) between a disulfide rich molecule, and exofacial thiols of transmembrane proteins on the cell surface. The covalently bound substrate then crosses the membrane by either fusion, endocytosis, direct translocation or membrane deformation (toroidal elastic). Despite the recent advances over the last years, the mechanism of uptake is still poorly understood.^[1] This partially might be explained by the dynamic nature of the process that lacks of any steady state that might be exploited for better understanding. Furthermore, it needs to be taken into consideration that TMU is a multi-target process as concluded by previous studies,^[2] thus adding a layer of complexity to the uptake process. Here, combining two complementary methods of analysis, *i.e.* micromapping and HTS pattern generation, we discovered and confirmed 3 new quasi-orthogonal targets^[3] and many mores.^[4]

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**Optimization and Synthesis of Fully Stereopure PS-2'MOE ASOs via the Reiterative COW Protocol -
The Nusinersen Case Study -**

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Antisense oligonucleotides (ASOs) are a versatile class of therapeutics used to modulate gene expression, offering promise for conditions such as cancer, neurodegenerative diseases, among others. Chemical modifications, including phosphorothioate (PS) backbones and 2'-O-methoxyethyl (2'MOE) groups, enhance their stability, binding affinity, and therapeutic efficacy. Notably, PS bonds in ASOs are typically synthesized as uncontrolled, random stereomixes, which can impact their overall performance and consistency in therapeutic applications. Recent studies have shown that stereopure PS bonds can further improve ASO stability and biological activity, making them a focus for next-generation therapeutic development. Building on the patented iterative Coupling-Oxidation (Sulfurization)-Wash (COW) protocol developed by F. Hoffmann-La Roche, we demonstrate its application for the first time in the synthesis of fully stereopure PS-2'MOE ASOs.

Ascaroside production in the extremophile *Halicephalobus mephisto*

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The nematode *Halicephalobus mephisto* is the first multicellular organism found at deep subsurface levels [1]. It can withstand heat and high pressure and represents an extremophile nematode [1]. Ascarosides represent highly conserved signaling molecules in nematodes, which regulate a wide range of inter- and intraorganismal interactions [2, 3].

In order to characterize nematode chemical communication in extreme environments, we investigated ascaroside production in *H. mephisto*. Here, we report the identification of several species-specific asc-C5 derivatives (see Figure 1) and their biosynthetic origin. Using a combination of ESI-MS/MS data and stable-isotope labeling allowed for the characterization of new modular ascarosides in *H. mephisto*. Unambiguous structure assignment was achieved by total synthesis. The modular asc-C5 derivatives integrate different primary metabolic pathways, including C8 fatty acid metabolism, which has not been reported before.

Taken together, our results demonstrate the utility of ESI-MS/MS fragmentation data to propose putative ascaroside structures to be established by total synthesis. Our results confirm that ascaroside signaling in bacterivorous nematodes is highly species-specific and further demonstrates for the first time that it also includes extremophiles such as *H. mephisto*.

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Nuclear Delivery of Inositol Pyrophosphate Prometabolites Mediated by Short Cationic Peptides

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Inositol pyrophosphates (PP-InsPs) are crucial signaling molecules in eukaryotic cells. These highly phosphorylated inositol derivatives play key roles in regulating various cellular processes such as cell proliferation, apoptosis, vesicle trafficking, and ion channel activity to insulin secretion.^[1] While PP-InsPs localize predominantly in the cytoplasm, recent studies revealed their presence in the nucleus, suggesting additional nuclear functions for PP-InsPs.^[2]

The high turnover between different isomers and the low intracellular concentration of PP-InsPs complicate their study. Therefore, to understand the role of these highly phosphorylated molecules, tools are needed to deliver and manipulate the level of PP-InsPs inside cells. Existing methods are restricted to delivering PP-InsPs to the cytoplasm.^[3] Here, we present a novel prometalite strategy that uses a photocage-protected PP-InsP, allowing for visualization within cells and spatiotemporal control over phosphate turnover. Additionally, we conjugated the modified PP-InsP to a short cationic cell penetration peptide to promote nuclear localization^{[4],[5]} and subsequently released the native form of PP-InsP to investigate its effect in the nucleoli.

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Chemoselective Probes for Natural Products Discovery

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The discovery of novel bioactive natural products (NPs) has been crucial for developing the human health system. However, the field suffers from the high cost of the isolation and extraction process and the rediscovery of known compounds. To overcome those issues, several technologies have emerged, such as using well-designed derivatization agents to target a specific class of NPs.^[1,2] Those strategies can be combined with an enrichment system composed of a chemoselective probe to catch, enrich, and release a particular class of NPs. Herein, we present a novel strategy for the enrichment of amine-containing NPs^[3] and a methodology to profile isonitrile NPs in a complex mixture.^[4]

Catch, enrich, and release underivatized amine-containing natural products

A chemoselective probe for the catch, enrichment, and release of the amine-containing NPs has been designed and exploited the selectivity of the protease legumain to deliver the underivatized compound as a product. In brief, the amine-containing NPs react with the chemoselective probe using a standard amide coupling procedure (catch). Next, the matrix composed of unreacted compounds is removed by exploiting the high affinity of streptavidin for biotin (enrichment). In this case, a derivative of biotin, biotin sulfone, was used to ease the removal of the modified probe from the resin. Finally, the underivatized amine-containing NPs are obtained by selective cleavage of the amide bond by the protease legumain (release).

Profiling of isonitrile-containing natural products

Isonitrile-containing natural products (NPs) are a significant class produced by various organisms. However, identifying these compounds is challenging due to the difficulty of detecting isonitriles via NMR and MS, and the functional group's reactivity. We developed a selective detection approach for isonitrile NPs to address these challenges using chlorooxime ligation specificity.^[5] Our chemoselective probe features a chlorooxime warhead linked to a bromoaryl, serving as both MS and UV tags. This probe can react directly with crude extract mixtures, forming a hydroxyamino amide product exclusively with isonitrile-containing NPs.

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Light-activated ortho-nitrobenzyl alcohols for the conjugation of ^{89}Zr -DFO to onartuzumab for tumor imaging

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^{89}Zr -radiolabelled monoclonal antibodies (mAbs) play a central role in the diagnosis and monitoring of cancer treatment *via* non-invasive positron emission tomography (PET) imaging. Methods that provide access to ^{89}Zr -mAbs in a simple, fast, and efficient manner from the native mAb are highly desirable. To this end, we report the synthesis of a desferrioxamine (DFO) ligand decorated with an *ortho*-nitrobenzyl alcohol (NBA)¹ motif (DFO-NBA) for light-activated conjugation to the gastric cancer-targeting mAb onartuzumab² for *in vitro* and *in vivo* characterization, Figure 1.

DFO-NBA was synthesized in three steps. ^{89}Zr -radiolabeling was followed by TLC and HPLC. Photo-conjugation to the mAb was carried out using 365 nm light for 15 min under aqueous conditions (pH 8.1, 23 °C), and followed by TLC and size-exclusion chromatography (SEC). Tumor specificity was determined using cellular binding assays *in vitro*. The radiotracer pharmacokinetic profile and tumor targeting was then assessed by PET imaging in female athymic nude mice bearing subcutaneous MKN-45 xenografts. Biodistribution experiments were performed after 72 h. The tumor specificity of ^{89}Zr DFO-NBA-onartuzumab was assessed *in vivo* by competitive inhibition (blocking) studies.

^{89}Zr -DFO-NBA was synthesized with a radiochemical conversion (RCC) >99%. Photo-conjugation gave ^{89}Zr -DFO-NBA-onartuzumab in a crude RCC of 76% where purification by SEC gave a radiochemical purity of >99% and isolated radiochemical yield of 48%. Subsequent imaging and biodistribution studies showed high tumor uptake and specificity. After 72 h, a tumor uptake of $13.8 \pm 1.9\% \text{ID g}^{-1}$ was observed in the normal group and by comparison with blocking experiments tumor specificity was confirmed with ~44% tumor uptake reduction.

NBA provides a simple and fast method for the conjugation of ^{89}Zr -DFO to native onartuzumab with favorable radiochemical conversions and yields compared with other light-activated handles³. We continue to explore the photochemical reactivity of NBA, and other functional groups, to access new classes of bioconjugate bonds.

Figure 1 Cartoon depiction of the light-activated conjugation of ^{89}Zr -DFO-NBA to the targeting mAb onartuzumab.

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Synthesis of a mucosal glycan library for investigating pathogen virulence

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The alarming increase in antimicrobial resistance necessitates the development of new therapeutics with novel mechanisms of action. Recent studies have uncovered mucosal glycans as potent down-regulators of virulence in several pathogens, including the opportunistic *Pseudomonas aeruginosa*¹, but the discrete structures responsible for this activity and their mechanism(s) of action have yet to be elucidated. Obtaining individual glycans from mucus is hampered by physicochemical similarities among structures and mucosal glycans are not amenable to automated synthesis. Therefore, we have successfully developed a highly convergent and scalable synthetic approach to assemble target mucin glycans that were identified as top candidates in down-regulating virulence². The approach incorporates an orthogonal protecting group strategy in monosaccharide building blocks to obtain a library of complex glycans in multi-milligram scale for investigating pathogen virulence. It has been suggested that mucosal glycans attenuate *P. aeruginosa* virulence through the protein sensor RetS³. Therefore, recombinant RetSp was produced and evaluated as a target for mucosal glycan binding.

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Amino Acid Composition drives Peptide Aggregation: Predicting Aggregation for Improved Synthesis

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Solid-phase peptide synthesis (SPPS) is a standard technique for peptide production, however, so called “non-random difficult couplings” often limit efficient amino acid coupling. While data-driven approaches have improved our understanding of sequence-dependent behaviors, there is still no comprehensive solution for addressing non-random difficult couplings—events often associated with aggregation during SPPS. We therefore combined publicly available analytical datasets from flow-SPPS with new analytical data to develop a predictive model to investigate how individual amino acids contribute to aggregation. For short- to medium-sized peptides, we found that aggregation is more accurately predicted by amino acid composition than by their specific sequence, a result we confirmed through experimental validation. Next, we introduced a composition-based vector representation to assess the aggregation tendencies of individual residues. An ensemble of trained models was then used to predict peptide aggregation and to suggest optimized synthesis conditions. Our approach offers a data-driven framework to address aggregation challenges in SPPS and provides a powerful tool for synthesis planning, ultimately eliminating the need for trial-and-error synthesis optimization.^[1]

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Optimizing scandium-44 production and automated radiolabeling for potential clinical applications in nuclear medicine

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Scandium-44 is a radionuclide with a half-life of 4.04 hours [1] and high positron emission efficiency ($E_{\beta^+av} = 630.2$ keV, $E_{\gamma} = 1157$ keV, $I = 99.9\%$) [2]. These characteristics make it a promising candidate for use in nuclear medicine, particularly in positron emission tomography (PET) imaging. It can be produced via the $^{44}\text{Ca}(p,n)^{44}\text{Sc}$ nuclear reaction using enriched ^{44}Ca targets. However, challenges such as variability in pH, chemical purity, and the activity concentration of the final product across different production runs have been observed, limiting its reproducibility [4]. To enhance radiolabeling yields and overall reliability, additional optimizations were necessary. This research seeks to optimize the production, purification, and radiolabeling procedures for ^{44}Sc , with the objective of enhancing its use in preclinical and clinical applications.

Bench experiments were conducted to assess resin systems, such as DGA, DOODA, and Amberchrom resins, for the optimal separation of Sc from Ca in large excess, using hydrochloric acid for Sc elution. To concentrate Sc in a suitable solution for radiolabeling, AG MP-50 macroporous cation exchange resin was employed using buffered solutions. Parameters such as pH and column height were evaluated to optimize the Sc elution yield in minimal volume. The most effective radiochemical separation method was then applied to irradiated targets. The chemical and radionuclidic purity of the ^{44}Sc final product was evaluated using ICP-MS, HPLC, and γ -ray spectrometry. Radiolabeling efficiency was assessed with DOTATOC labeling, both manually and using an automated module system. Additionally, procedures for radiolabeling with other molecules are under development using the automated module system.

Significant advancements have been made in the production and separation of ^{44}Sc , resulting in a more reproducible process and higher purity. The integration of automated systems improves the potential of ^{44}Sc production at medical facilities with small cyclotrons. Future studies will focus on upscaling and further automating both the ^{44}Sc production and radiolabeling processes.

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Bioconjugated metal complexes for cancer therapy: from synthesis to anticancer evaluation

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Cancer is a leading cause of death worldwide, responsible for millions of deaths each year. The development of anticancer drugs that specifically target tumour sites is of utmost importance in cancer chemotherapy and is currently being investigated as a strategy to overcome one of the major drawbacks of traditional chemotherapeutic agents namely, systemic side effects that can damage organs and tissues not directly affected by cancer.[1]

Following the discovery of cisplatin and its antiproliferative properties, late transition metal complexes have been extensively studied as anticancer agents, demonstrating high cytotoxic potential in both *in vitro* and *in vivo* models.[2]

In this context, the conjugation of relevant biomolecules to drugs is a widely used strategy to improve drug selectivity toward tumour sites, thereby reducing side effects.[3,4] In this contribution, we present our current research project focused on the synthesis of bio-conjugated late transition metal complexes and their application as anticancer agents. In particular, the project explores the use of Fischer-type carbenes as robust linkers between metals and biomolecules, ranging from traditional imidazolylidenes to mesoionic carbenes.

The conjugation strategies rely on simple and efficient reactions, including Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC), which provides suitable mesoionic carbene (MIC) precursors, as well as photoreactions that exploit the specific reactivity of aryl azides toward nucleophilic attack by nitrogen-based nucleophiles in proteins and antibodies.

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Optimizing RNA-PROTAC Potency through Phosphorothioate Stereochemistry

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RNA-proteolysis-targeting chimeras (RNA-PROTACs) are a novel drug modality designed to degrade RNA-binding proteins (RBPs) [1]. Since RNA is naturally unstable, it requires chemical modifications for stability. One such modification is the phosphorothioate (PS) group, where a sulfur atom replaces an oxygen in the phosphate backbone [2]. PS groups enhance biodistribution by interacting with circulating proteins like serum albumin [3] [4]. PS modification creates a chiral center at the phosphorus atom, and several studies have demonstrated the impact of PS stereochemistry on biological activity [5].

We are developing a method to investigate how PS stereochemistry affects protein binding. RBFOX1, a well-characterized protein with a single RNA-binding domain, serves as a model system [6]. Surface plasmon resonance (SPR), a real-time kinetic technique, will provide insight into these interactions.

Our next step is to conjugate stereopure PS RNA-binding elements (RBEs) to VH-032, a well-established von Hippel–Lindau (VHL) ligase recruiter. The resulting stereopure PS RNA-PROTACs will then be evaluated in cellular assays. Through this work, we aim to demonstrate that controlling PS stereochemistry can enhance the stability, selectivity, and potency of RNA-PROTACs.

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RNA-PROTACs to induce degradation of TDP-43

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Proteolysis targeting chimeras - or PROTACs – are heterobifunctional molecules that can redirect the endogenous ubiquitin-proteasome system to induce degradation of protein of interests. The induced proximity between the target protein and the E3 ligase by the PROTAC allows transfer of ubiquitin from the E3 ligase onto a surface lysine of the target protein [1]. PROTACs are a new strategy to degrade and therefore inhibit proteins that are difficult to inhibit with conventional small molecules due to lack of small molecule binding pockets within these proteins [2]. RNA-binding proteins usually do not have such a small molecule binding site that could modulate their function, but RNA-binding proteins can bind RNA sequence specifically and with high affinity [3].

We have developed RNA-PROTACs by conjugating the binding sequences of RNA-binding proteins to small molecules or peptides that interact with E3 ligases. The first-generation RNA-PROTACs specifically targeted RNA-binding proteins with short, conserved RNA-binding motifs. These RNA-PROTACs, utilizing modified RNA conjugated to E3 ligase recruiters, successfully induced proteasome-dependent degradation of LIN28 and RBFOX1[4]. The second-generation RNA-PROTACs are designed to induce degradation of RNA-binding proteins associated with neurodegenerative diseases.

TDP-43 is a nuclear RNA-binding protein that is essential for the correct splicing of thousand of mRNAs [5]. In amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), TDP43 is mislocalized to the cytoplasm, where it accumulates and aggregates. These TDP-43 aggregates, along with a nuclear loss of function, lead to degeneration of motoneurons [6].

Here, we present rational design of a RNA-PROTAC against TDP-43. Based on the NMR structure of TDP-43 with RNA [7], a oligonucleotide-warhead was synthesized. A library of bioconjugates recruiting different E3 ligases was synthesized and tested in cell systems.

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Sustainable Bioproduction of Tetrahydroisoquinoline Alkaloids: Mining and Engineering Gene Clusters from Uncultured Microbial Communities

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Over the past 40 years, nearly half of all newly approved drugs have been derived from natural products [1], which often have complex structures that are challenging to synthesize via traditional synthetic methods [2]. Many of these natural products are produced by large megasynthase enzymes. For instance, non-ribosomal peptide synthetases (NRPS) build complex peptide scaffolds by incorporating and modifying amino acids through the coordination of various enzymatic domains [3]. One notable class of NRPS-derived natural products are the antitumor tetrahydroisoquinoline (THIQ) alkaloids, which exhibit anticancer activity via DNA alkylation and disruption of cell cycle [4]. These compounds share a common core structure that is decorated by a suit of tailoring enzymes that vary broadly across different microbial producers [5-7]. Via a metagenome mining approach, we have identified two novel biosynthesis pathways from uncultured bacteria, or microbial dark matter, which putatively produce new THIQ compounds with unique tailoring enzymes. We aim to validate the functionality of these newly identified pathways and thereby broaden the chemical space around these natural anticancer agents. Our biochemical knowledge will facilitate the expansion of this compound class via protein engineering and lead to the development of a sustainable biotechnology platform for producing THIQ analogs for anticancer therapeutic development.

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Antisense therapy targeting uromodulin aggregation in rare kidney disease

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Autosomal dominant tubulointerstitial kidney disease (ADTKD) caused by mutations in *UMOD*, the gene encoding uromodulin, is responsible for 2% of kidney failures worldwide. It is marked by toxic gain-of-function mutations that cause uromodulin to aggregate within cells, leading to cellular stress and progressive kidney dysfunction. We present a comprehensive proof-of-concept study demonstrating allele-selective antisense oligonucleotide (ASO) therapy against the pathological c.553C>A (p.R185S) *UMOD*-mutation. A library screen of mRNA-targeting ASOs in cell culture, identified the optimal sequence for selective silencing at both RNA- and protein levels. Knockdown of pathological uromodulin was confirmed in a trial intervention of a ADTKD-*UMOD* mouse model, but showed limited effect on toxic high-molecular-weight (HMW) aggregates. Longer dosing regimen however, achieve reduction of the toxic HMW-UMOD in mutant mice without impacting protein levels in WT counterparts. At the therapeutic endpoint ~320,000 ASO molecules per cell were detected, in the renal thick ascending limb (TAL). ASOs were taken up unidirectionally, at the epithelial TAL cells' basolateral membrane, and accumulated in the nucleus – the primary site of RNA target engagement – within 24 h. The kidneys of treated mice had less tubular uromodulin and reduced inflammation, while showing improved handling of uric acid, suggesting that reduction in aggregate uromodulin translates to clinically relevant changes. This work demonstrates the feasibility of RNA interference for therapeutic reduction of toxic aggregates, and offers a roadmap for drug development against ADTKD-*UMOD*, a devastating disease without current treatment options.

Dichalcogenide Fidaxomicin Derivatives to Probe Thiol-Mediated Uptake into Bacteria

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The natural product fidaxomicin (Fdx) is a narrow-spectrum antibiotic clinically prescribed for the treatment of *Clostridioides difficile* infections. However, limited cellular uptake reduces its therapeutic potential, particularly against Gram-negative bacteria and mycobacteria. In this study, we investigated Thiol-Mediated Uptake (TMU)^[2] to promote the delivery of Fdx into bacterial cells. We synthesized a library of Fdx derivatives bearing cyclic dichalcogenide moieties, and evaluated their antimicrobial properties against *C. difficile* and *Mycobacterium tuberculosis*. Remarkably, the synthetic Fdx derivatives retained strong levels of antibacterial activity, and the disulfide-containing analogs outperformed their all-carbon control counterparts in many instances. We then developed a systematic study to investigate the mechanistic impact of the introduced disulfide functionalities by conducting experiments with TMU inhibitors, and quantifying intracellular accumulation in *Mycobacterium bovis* BCG, a model organism for *M. tuberculosis*, via LC-MS/MS. While complete disentanglement of the factors influencing activity was not feasible, features such as compound stability and lipophilicity were identified as significant contributors. Overall, the superior performance of disulfide analogs suggests that differences in cellular entry or intracellular processing, potentially related to TMU, are involved. This work highlights that TMU remains a viable approach for modulating uptake of therapeutic agents into bacterial cells

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Towards a sustainable bio-based route for production of Trabectedin scaffoldsE. Moore¹, A. E. Fraley^{1*}¹Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, Eidgenössische Technische Hochschule (ETH) Zürich

Trabectedin (Ecteinascidin 743, ET-743) is a marketed drug, under the name Yondelis®, used against soft tissue sarcoma (1). It was first identified from the tunicate *Ecteinascidia turbinata* and, more precisely, made by a symbiotic bacterium ‘*Candidatus Endoecteinascidia frumentensis*’ (2). ET-743 is part of a diverse family of compounds characterized by tetrahydroisoquinoline (THIQ) rings (3,4). However, it is the only member to be marketed as an approved drug. Due to low yields from the current production methods (5), we aim to develop a biocatalytic production system for ET-743 to increase production efficiency and allow for facile access the therapeutically relevant analogs. In this regard, the biosynthetic development falls into three categories: i.) early-stage modification of precursors, ii.) THIQ core formation, and iii.) late-stage diversification. Across the THIQ family, the early-stage modification of the tyrosine precursor is consistent, and we have validated enzymatic reactions catalyzing both C- and O-methylation, as well as hydroxylation by a unique class of heme proteins (6). The formation of the THIQ core depends on a non-ribosomal peptide synthetase that catalyzes two iterative Pictet-Spengler reactions combining the tyrosine with an additional amino acid. In the case of *EtuA2* from ET-473 biosynthesis, cysteine incorporation is proposed to facilitate a late-stage cyclization to form a thioester bond in a mechanism under investigation in this work. This reconfigured THIQ then undergoes a final Pictet-Spengler reaction to incorporate an additional modified tyrosine and build the characteristic tri-THIQ scaffold of ET-743. We envision that these newly characterized enzymes will pave the way toward a biotechnology platform for the sustainable production of the anticancer therapeutic ET-743 and its previously inaccessible analogs.

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