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Engineered Protein Cages for RNA Delivery

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[1]EPFL-SB-ISIC-LIBN

Recent advances in RNA therapies, notably the mRNA vaccines for COVID-19, have emphasized the critical role of efficient gene delivery methods. While lipid nanoparticles and viral vectors have proven their efficacy, engineered virus-like protein cages stand out as a potential alternative. Engineered protein cages have distinct advantages: they are constructed from the ground up, allowing complete flexibility for functional engineering [1]. Given their protein-based nature, they can also undergo optimization through directed evolution techniques. In this presentation, we will share our latest results highlighting enhancements in the areas of assembly and RNA packaging for the evolved protein cage NC4 [2,3].

[1] *M. Hori, *A. Steinauer, S. Tetter, J. Hälg, E.-M. Manz, D. Hilvert, Nature Communications, 2024, accepted.

[2] *S. Tetter, *N. Terasaka, *A. Steinauer, R.J. Bingham, S. Clark, A.P. Scott, N. Patel, M. Leibundgut, E. Wroblewski, N. Ban, P.G. Stockley, R. Twarock, D. Hilvert, Science, 2021, 372, 1220-1224.

[3] *T.G.W. Edwardson, *M.D. Levasseur, S. Tetter, A. Steinauer, M. Hori, D. Hilvert, Chemical Reviews, 2022, 122, 9145-9197.

Identification of a peripherally restricted MAGL inhibitor which shows promising activity in disease models of visceral pain and IBD

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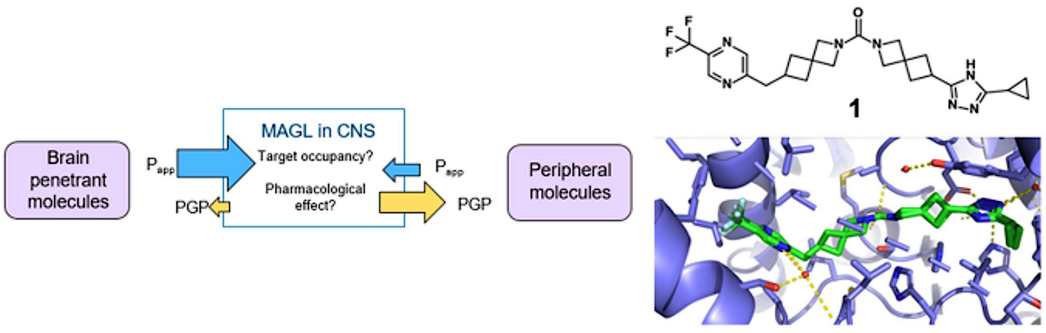
Monoacylglycerol lipase (MAGL), an intracellular serine hydrolase responsible for the hydrolysis of monoacylglycerols to free fatty acids and glycerol, is a key enzyme involved in endocannabinoid regulation. [1] Pharmacological inhibition of MAGL has been investigated as a therapeutic approach for indications including multiple sclerosis, Alzheimer's disease and Parkinson's disease. A diverse set of chemical matter is available, the majority of which has been optimized for CNS-indications, and thus exhibits brain penetrance. [2] MAGL inhibition also has potential therapeutic benefit in non-CNS indications such as inflammatory pain, IBD, fibromyalgia, osteoarthritis, and endometriosis, however development of MAGL inhibitors for these indications is complicated by the risk of CNS-mediated adverse events such as anxiety. The development of peripherally-restricted ligands would enable a high target occupancy to be obtained in a peripheral tissue of interest, without significant engagement of the target in the CNS.

We aimed to generate peripherally restricted MAGL inhibitors starting from existing high-quality brain penetrant ligands. The initial strategy to exploit compounds which had high P-gp efflux ratios was unsuccessful, as significant brain exposure still occurred. [3] Analysis of physicochemical data and PK studies allowed us to develop a strategy for the optimization towards peripheral restriction based on balancing P-gp efflux and passive permeability (P_{app}). Graph deep learning predictions of these key properties were used to guide medicinal chemistry design, resulting in the rapid identification of a structurally diverse set of potent and orally bioavailable tool molecules which demonstrated peripheral target engagement in rodents – an increase in 2-AG was seen in colon, but not in brain. Further optimization with focus on peripheral restriction in humans resulted in selection of a candidate, **1**, a highly-selective, orally administered reversible MAGL inhibitor which demonstrated a high degree of peripheral restriction, and efficacy in various models of visceral pain and IBD.



[1] A. Gil-Ordóñez *et al.*, *Biochem Pharmacol.*, **2018**, *157*, 18-32.
[2] G. Bononi *et al.*, *Expert Opin Ther Pat.* **2021**, 31, 153-168.
[3] S. E. Skerratt *et al.*, *J Med Chem.* **2016**, *59*, 10084-10099.

Expanding the Chemical Space of Lasso Peptides by Combining Flow Synthesis and Enzymatic Transformation

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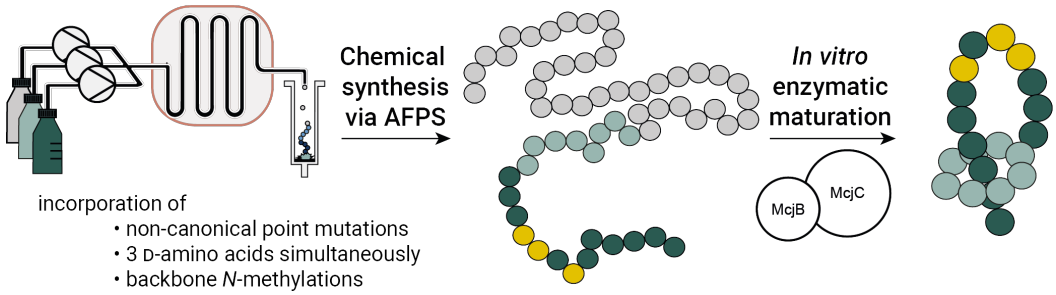
Lasso peptides are a subclass of ribosomally synthesized and post-translationally modified peptides (RiPPs) classified by their conformationally constrained lariat knot-like structure. Their remarkable enzymatic, thermal, and chemical stability combined with a variety of biological activities make them attractive scaffolds for drug development.[1] The total chemical synthesis of lasso peptides such as microcin J25 (MccJ25) remains elusive. Therefore, expanding the chemical space of MccJ25—an antagonist of Gram-negative bacteria—beyond the range of biological methods is challenging.[2] The in vitro transformation of non-natural precursor peptides could enable the production of MccJ25 derivatives.[3] **In this study, we employ flow-based peptide synthesis to obtain chemically modified precursor peptides and enzymatically transform them, *in vitro*, into correctly folded lasso peptides.**



The recombinant expression of the processing enzymes, McjB and McjC, was extensively optimized to facilitate the transformation of modified precursor peptides to multiple analogs of MccJ25, including the incorporation of non-canonical tyrosine and histidine residues. A biologically active analog with three D-amino acids and an MccJ25 derivative containing backbone *N*-methylations were produced, showcasing the versatility of our chemoenzymatic strategy. This method affords access to chemically modified lasso peptides previously inaccessible by strictly biological methods, thus increasing the scope of potential modifications to improve therapeutic properties.

[1] J. D. Hegemann, M. A. Marahiel, *Cyclic Peptides*, **2017**; 206–224

[2] F. J. Piscotta, J. M. Tharp, W. R. Liu, A. J. Link, *Chemical Communications*, **2015**, 51 (2), 409–412.

[3] K. P. Yan, Y. Li, S. Zirah, C. Goulard, T. A. Knappe, M. A. Marahiel, S. Rebuffat, *ChemBioChem*, **2012**, 13 (7), 1046–1052.

Antimicrobial peptide-peptoid hybrids

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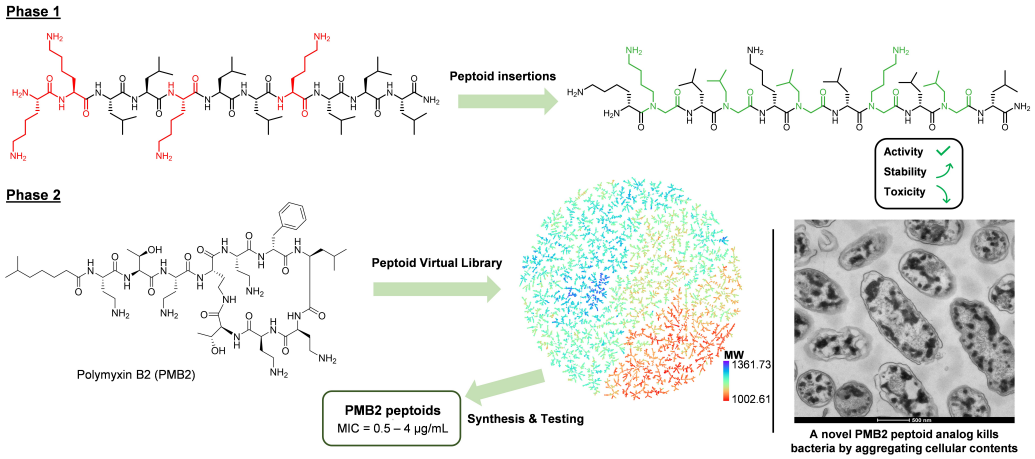
Many antimicrobial peptides (AMPs) and related natural products are promising starting points to develop new antibiotics to address the antimicrobial resistance crisis.[1,2] AMPs must be optimized for lower toxicity and increased resistance to proteases, which can be partly achieved by substituting peptide for peptoid (N-alkylated glycines) building blocks.[3,4] Introducing peptoid units into peptides furthermore often enhances their cell permeability, probably due to the elimination of the polar N-H bond from their scaffold.[5] Along these lines, we recently showed that gradual peptide to peptoid replacements in a linear AMP could initiate a transition in the mode of action from membrane-disruption toward intracellular targeting. This mechanistic shift could be observed with even less than 40 % of peptoids in the sequence.[4] In a second phase, we set out to test if redesigning macrocyclic antimicrobial peptides as peptoids might lead to new antibiotic compounds with improved properties at the example of polymyxin B2, a natural product used as last resort antibiotic against multidrug resistant bacteria but with problematic toxicity. For this exploration, we used a computational tool, previously reported as the peptide design genetic algorithm (PDGA), capable of generating peptide sequences in the proximity of any molecule in a defined chemical space.[6] Interestingly, some of the macrocyclic peptide-peptoid hybrids generated by the PDGA exhibited high potency against a panel of MDR bacteria, with high stability against proteolysis and low toxicity.



[1] C. J. Murray et al., The Lancet, 2022, 399, 629–655.

[2] Antimicrobial resistance, https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance, (accessed March 28, 2024).

[3] T. Godballe, L. L. Nilsson, P. D. Petersen and H. Jenssen, Chemical Biology & Drug Design, 2011, 77, 107–116.

[4] E. Bonvin, H. Personne, T. Paschoud, J. Reusser, B.-H. Gan, A. Luscher, T. Köhler, C. van Delden and J.-L. Reymond, ACS Infect. Dis., 2023, 9, 2593–2606.

[5] Y.-U. Kwon and T. Kodadek, J. Am. Chem. Soc., 2007, 129, 1508–1509.

[6] A. Capecchi, A. Zhang and J.-L. Reymond, J. Chem. Inf. Model., 2020, 60, 121–132.

Two-for-One Biosynthesis: Functional Biosynthetic Stereodivergence in a Gene Cluster via a Dihydrosydnone *N*-oxide

J. Ren[1], A. Mathew[2], M. Rodriguez Garcia[2], T. Kohler[1], O. Blacque[1], A. Linden[1], S. Sieber[1], L. Eberl[2]*, K. Gademann[1]*

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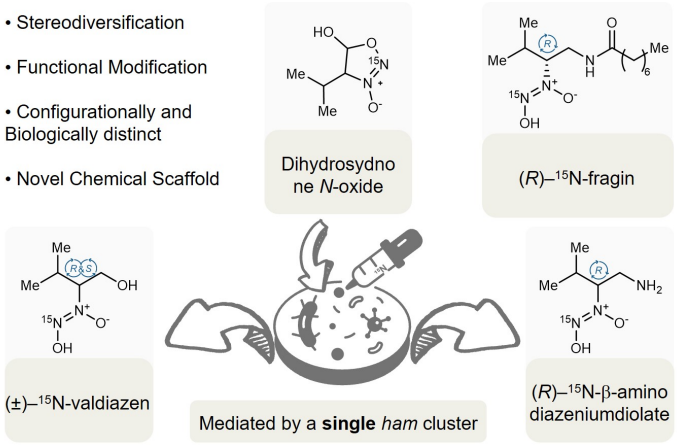
Chirality plays a critical role in the biochemistry of life and often only one enantiomeric series is observed.[1],[2] Only few natural products have been obtained as racemates, e.g. the quorum-sensing signal valdiazen produced by *Burkholderia cenocepacia* H111.[3] In this talk, I will showcase our investigation of a unique biosynthetic pathway governed by the *ham* cluster in H111. As evident from gene knockout, stable isotope feeding, and mass spectrometry experiments, we discovered that a dihydrosydnone *N*-oxide heterocycle is the pivotal intermediate leading to stereodivergence. This biosynthesis encapsulates non-enzymatic racemisation via keto-enol tautomerisation and stereoselective transformation via enzyme-mediated dynamic kinetic resolution. This novel mechanism underpins the production of configurationally and biologically distinct metabolites from a single gene cluster. Our findings highlight the intricate design of the intertwined biosynthetic pathway, providing a deeper understanding of microbial secondary metabolism and communication.[4]



[1] G. T. M. Bitchagno; V.-A. Nchiozem-Ngnitedem; D. Melchert; S. A. Fobofou, *Nat. Rev. Chem.* **2022**, 6, 806.
[2] D. G. Blackmond, *Cold Spring Harb. Perspect. Biol.* **2019**, 11, a032540.
[3] C. Jenul; S. Sieber; C. Daeppen; A. Mathew; M. Lardi; G. Pessi; D. Hoepfner; M. Neuburger; A. Linden; K. Gademann; L. Eberl, *Nat. Commun.* **2018**, 9, 1297
[4] **J. Ren**; A. Mathew; M. Rodriguez Garcia; T. Kohler; O. Blacque; A. Linden; L. Eberl; S. Sieber; K. Gademann, *BioRxiv*, **2024**, 04.29.591611.

Discovery of functional active low-molecular weight antagonists of IL-1ß

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[1]Global Discovery Chemistry

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that plays a critical role in the regulation of the immune response and the development of various inflammatory diseases. IL-1β directed antibodies have shown clinical benefit. In this presentation, we disclose our efforts towards the discovery of IL-1β low-molecular weight binders that interfere with IL-1β signaling. Several different approaches were applied in parallel, such as ^{19}F NMR fragment-based screening, DNA-encoded library technology, peptide discovery platform and virtual screening. These activities resulted in the identification of new chemical entities exploiting three different sites on IL-1β, all of them inhibiting the interaction with the IL-1R1 receptor.

Local environment in biomolecular condensates modulates enzymatic activity across length scales

M. Gil-Garcia[1], A. Benítez-Mateos[2], M. Papp[1], F. Stoffel[1], C. Morelli[1], K. Normak[1], K. Makasewicz[1], L. Faltova[1], F. Paradisi[2], P. Arosio[1]*

[1]Department of Chemistry and Applied Biosciences, Institute for Chemical and Bioengineering, ETH Zurich, Zurich, Switzerland, [2]Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Bern, Switzerland

The mechanisms that underlie the regulation of enzymatic reactions by biomolecular condensates and how they scale with compartment size remain poorly understood. Here we use intrinsically disordered domains as building blocks to generate programmable enzymatic condensates of NADH-oxidase (NOX) with different sizes spanning from nanometers to microns. These disordered domains, derived from three distinct RNA-binding proteins, each possessing different net charge, result in the formation of condensates characterized by a comparable high local concentration of the enzyme yet within distinct environments. We show that only condensates with the highest recruitment of substrate and cofactor exhibit an increase in enzymatic activity. Notably, we observe an enhancement in enzymatic rate across a wide range of condensate sizes, from nanometers to microns, indicating that emergent properties of condensates can arise within assemblies as small as nanometers. Furthermore, we show a larger rate enhancement in smaller condensates. Our findings demonstrate the ability of condensates to modulate enzymatic reactions by creating distinct effective solvent environments compared to the surrounding solution, with implications for the design of protein-based heterogeneous biocatalysts.

M. Gil-Garcia, AI. Benítez-Mateos, M. Papp, F. Stoffel, C. Morelli, K. Normak, K. Makasewicz, L. Faltova, F. Paradisi, P. Arosio, *Nature Communications*, 2024,15(1):3322

Development and Manufacture of highly potent products at Lonza Visp Development Services

E. González-Fernández[1]

[1]Lonza AG, API Development Services, Lonzastrasse, 3930 Visp, Switzerland

At Lonza, we enable a healthier world by supporting our healthcare customers on the path to commercialization of innovative medicines. The Lonza Visp site is the largest and oldest site in a global network of more than 30 sites globally, and includes a number of industry-leading facilities specializing in the development and manufacture of products for pharmaceutical applications. Among these are facilities able to produce highly potent substances that mostly find their application in cancer treatment, either as “classical” APIs or as payloads in anti-body drug conjugates. The Highly Potents Lab production unit at Development Services in Visp specializes in handling such highly potent substances, supporting drug substance development and supply from tox batch synthesis until commercial production. Activities and tasks are guided by phase appropriate procedures, based on a robust safety and quality strategy and technical expertise, thus enabling efficient and timely fulfilment of customers’ needs throughout the clinical life cycle of such compounds.

Semi-synthetic nucleosomes to probe sirtuin activity on chromatin

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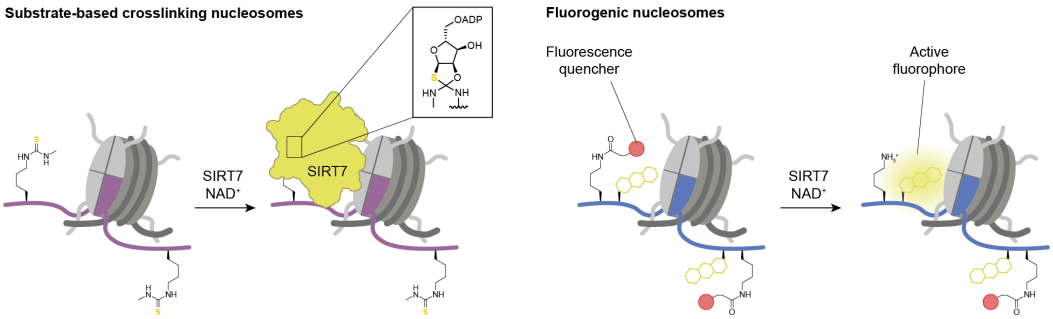
[1]Laboratory of Chemical Biology of Macromolecules (LCBM), ISIC, SB, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland, [2]Laboratory of Biological Electron Microscopy (LBEB), IPHYS, SB, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

SIRT7 regulates gene expression via deacetylation of the histone proteins that pack DNA into chromatin. In cancer, SIRT7 maintains genes involved in cancer progression and metastasis, and it is thus regarded as an anticancer target. The deacetylase activity of SIRT7 depends on the presence of nucleic acids, and it is especially activated by the structure of the nucleosome, *i.e.* a histone octamer core wrapped by 147 bp of DNA. This feature appears to be mediated by the N- and C-terminal domains of SIRT7 through mechanisms yet unknown. Here, we aim to elucidate the molecular mechanisms that drive SIRT7 activity in cancer and provide a platform for the development of inhibitors. First, we developed semi-synthetic nucleosomes with thiourea warheads at known SIRT7 substrate positions. These nucleosomes act as mechanism-based covalent inhibitors and trap the catalytic conformation of SIRT7 bound to the nucleosome. The resulting complexes were studied by cryoEM and revealed multivalent interactions of the catalytic and N-terminal domains of SIRT7 with the nucleosome core and both DNA gyres. Importantly, differences in binding to each substrate position allowed us to identify mutants with altered substrate selectivity. Second, we built nucleosomes with a fluorophore and a SIRT7 substrate that acts as FRET quencher. This system is therefore fluorogenic, and permits measuring SIRT7 activity in real time, which will be applied to high-throughput screening and mechanistic studies by single-molecule fluorescence microscopy. In summary, we have developed powerful nucleosome-based tools for the mechanistic study of SIRT7 and other deacetylases, and to help in the development of cancer therapeutics.



A Far-Red Fluorescent Probe to Visualize Gram-Positive Bacteria in Patient Samples

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

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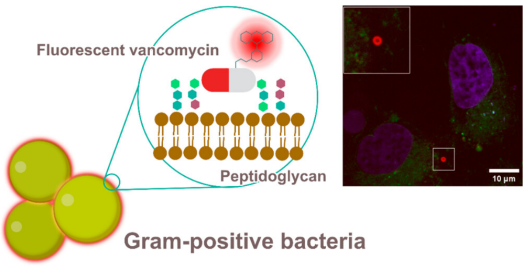
Staphylococcus aureus (S. aureus) is Gram-positive bacteria that can cause several serious infections. The treatment stills be challenged because of their multidrug-resistant species for example methicillin-resistant Staphylococcus aureus (MRSA).[1] The persistence of *S. aureus* in deep tissue leads to the difficulty of diagnosis and treatment.[2] Herein, we conjugate the red fluorophore, Janelia fluor 669 (JF_{669}), to vancomycin that can treat *S. aureus* including MRSA for the detection of *S. aureus* in patient samples. We utilize the specific binding of vancomycin with a terminal D-Ala-D-Ala peptide sequence on the bacterial peptidoglycan layer to label a red fluorophore to the cell wall of bacteria.[3] JF_{669} is suitable for human tissue because of its minimal background and red laser excitation. Vancomycin containing JF_{669} (Van-JF_{669}) can detect the *S. aureus* infected in a patient sample. Their fluorescence exhibits a higher signal-to-noise ratio compared to commercially available fluorescent vancomycin, vancomycin-BODIPY in the labeling of *S. aureus* in a patient sample. Furthermore, Van-JF_{669} shows the blinking activity. Therefore, we can use their blinking activity for super-resolution imaging of bacteria. The STORM imaging of bacterial cell wall provides a higher resolution than confocal images. Our Van-JF_{669} probe was also applied for several microbiological studies, for example, fixed- and live-cell imaging of *S. aureus*, labeling of *S. aureus* in patient samples preserved by FFPE, detection of *S. aureus* in macrophages-*S. aureus*- co-cultured and flow cytometry analysis of *S. aureus* infected cells. Regarding these applications, this probe is a powerful tool for the detection of *S. aureus* in patient samples and various microbiological studies.



[1] F. D. Lowy, N. Engl. J. Med. 1998, 339, 520–532.

[2] S.-M. Lim, S. A. R. Webb, Anaesthesia 2005, 60, 887–902.

[3] P. J. Loll, P. H. Axelsen, Annu. Rev. Biophys. Biomol. Struct. 2000, 29, 265–289.

AI-Enabled Synthesis Prediction in Modern Medicinal Chemistry

K. Atz[1], D. F. Nippa[1], A. T. Müller[1], U. Grether[1], R. E. Martin[1], G. Schneider[2]

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The synthesis of novel chemical matter remains a crucial challenge in small molecule drug discovery, often being the critical bottleneck impacting time and costs. Especially, C-H activation reactions on late-stage lead molecules usually require high throughput experimentation (HTE) screening to identify suitable conditions and substrates. [1] Machine learning methodologies, specifically methods that enable efficient learning on three-dimensional (3D) molecular structures, have proven instrumental in various domains of chemistry. [2] We demonstrate prospective applications of graph transformer neural networks (GTNN) to C-H alkylation and borylation reactions. We illustrate the importance of 3D information in predicting regioselectivity and quantify the impact of electronic information on predictive accuracy (Figure 1). [3,4] Moreover, we show how trained GTNNs enable efficient in silico library screening to identify suitable substrates. [5] Our approach is now routinely and successfully applied at Roche to assess binary reaction outcome, reaction yield, and regioselectivity for frequently applied reactions in drug discovery projects. The interdisciplinary approach highlights the potential of combining machine learning with high-throughput screening to streamline the compound synthesis process in drug discovery.

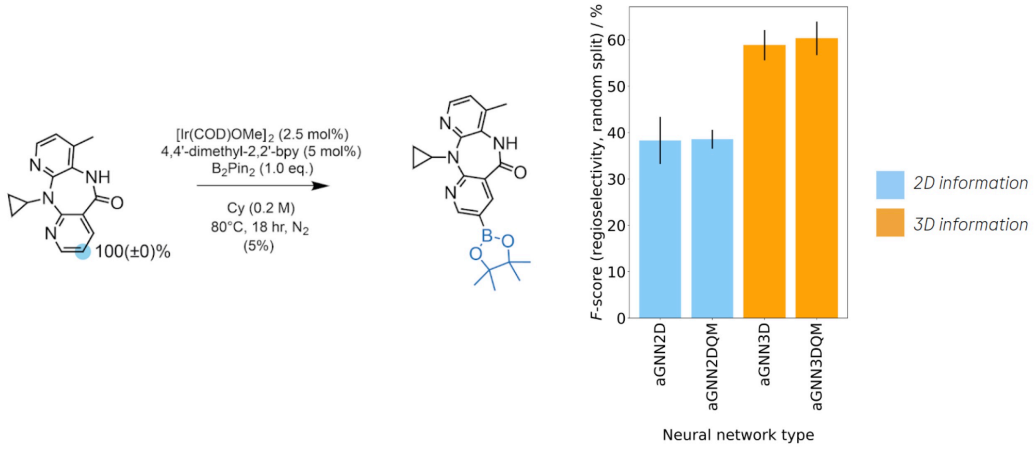

Fig. 1. Left: Prospective application of regioselectivity prediction models to the drug nevirapine. Right: Performance of the investigated atomistic GNNs including 2D (blue) and 3D (orange) information.

[1] D. F. Nippa et. al. *Chimia* 76 (3), 258-260 (**2022**).
[2] K. Atz, F. Grisoni and G. Schneider, *Nat. Mach. Intell.*, 3, 1023–1032 (**2021**).
[3] D. F. Nippa, K. Atz, et al., *Nat. Chem.*, 16, 239–248 (**2024**).
[4] D. F. Nippa, A. Müller, K. Atz et. al. *ChenRxiv*, **2024**
[5] D. F. Nippa, K. Atz, et al., *Communs. Chem.*, 6, 256 (**2023**).

Structural and Functional Analysis of the BCL2 RNA G-quadruplex

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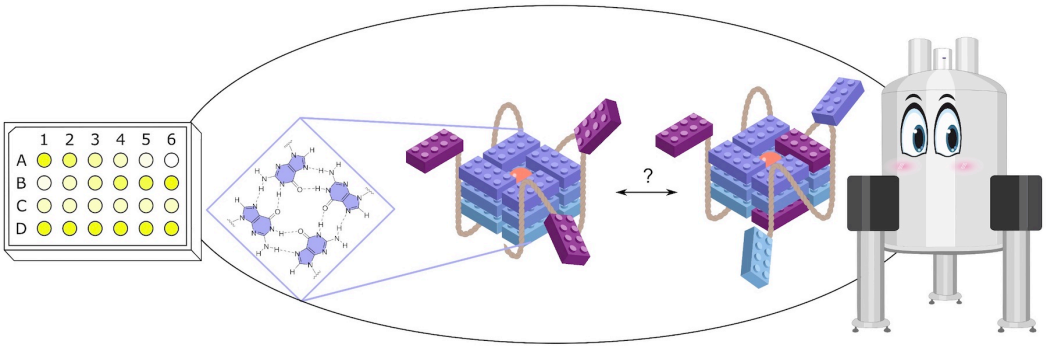
G-quadruplexes are intricate structures formed in guanine-rich sequences within RNA and DNA. These structures are characterisedby the arrangement of four guanines in a coplanar cycle through Hoogsteen hydrogen bonds, stacking on top of each other to form a G4 core.[1] Generally, these sequences contain surplus guanines than are required for G4 formation, frequently triggering dynamics between the core and the loops through guanine exchange processes, which might be connected to their biologicalfunction. The formation of RNA G-quadruplexes in the 5' untranslated region has emerged as an important regulatory factor in gene expression. We focus on the *BCL2* RNA G-quadruplex, a highly conserved sequence linked to the modulation of Bcl-2 protein expression.[2] The Bcl-2 protein, known for its anti-apoptotic function, is tightly regulated, and its dysregulation has beenimplicated in numerous diseases, including cancer.[3]



Our research aims to elucidate the structural details and dynamic behaviour of the *BCL2* RNA G-quadruplex using Nuclear Magnetic Resonance (NMR) spectroscopy. The wild-type *BCL2* G4 sequence, comprising a total of 15 guanines, adopts multiple G4 structures. Using NMR, we confirmed the presence of 2 conformations in the wild-type sequence. Strategic mutation of the guanines forced the G4 into a single conformation, facilitating initial structure determination.[4] By systematically reintroducing the removed guanines, we assessed the impact of each guanine on the structural and dynamic properties of the G-quadruplex sequence and parsed out the second conformation. Additionally, we want to explore the impact of these various mutants with luciferase assays to study the effect on the regulation of the Bcl-2 protein expression. Understanding how these mutations affect the structure and regulatory mechanisms of the *BCL2* RNA G-quadruplex is critical for exploring its therapeutic potential.

[1] Agarwala, S. Pandey, S. Maiti, *Org. Biomol. Chem.* **2015**, *13*, 5570.
[2] Shahid, A. Bugaut, S. Balasubramanian, *Biochem.* **2010**, *49*, 8300.
[3] J. McDonnell, P. Troncoso, S. M. Brisbay, C. Logothetis, L. W. Chung, J. T. Hsieh, S. M. Tu, M. L. Campbell, *Cancer Res.* **1992**, *52*, 6940.
[4] Wang, C. Ferreira Rodrigues, S. Jurt, A. Dominguez-Martin, S. Johannsen, R. K. O. Sigel, *in preparation* **2024**.

Discovery of NP3-562: a novel, orally bioavailable NLRP3-inhibitor

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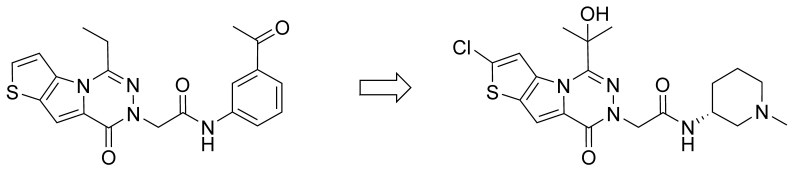
[1]Novartis Pharma AG, Basel, Switzerland

NLRP3, also known as cryopyrin, is an intracellular molecular sensor recognizing a broad range of danger signals.[1] Upon activation, NLRP3 forms a multimeric complex called inflammasome that leads to caspase-1 activation and subsequent release of pro-inflammatory cytokines IL-1ß and IL-18. NLRP3 plays an important role in inflammation and is implicated in various pathological conditions such as cryopyrin-associated autoinflammatory syndromes (CAPS), gout, atherosclerosis, type 2 diabetes, Alzheimer's disease and cancer.[2] To identify new chemical starting points that differ from the known sulfonylurea-based NLRP3-inhibitor MCC950, also known as CRID3 or CP-456,773, we performed a phenotypic high-throughput screening (HTS) that delivered a highly promising hit (**1**). Its optimization led to the discovery of **NP3-562**,[3] displaying a good overall profile including an improved potency in the whole blood. As revealed by the x-ray structure of this molecule bound to NLRP3-NACHT domain, the binding mode of this scaffold differs slightly from that of the sulfonylurea-type of compounds.[4] Good pharmacokinetic profile of **NP3-562** allowed for testing the compound *in vivo* and demonstrated a full inhibition of IL-1ß release at 30 mg/kg oral dose in acute mouse peritonitis model. Altogether, using HTS we have discovered a novel, tricyclic NLRP3-binding scaffold with the potential for developing new medicines against NLRP3-inflammasome driven diseases.



1 (hit)
IL-1β (THP-1): 0.63 μM
IL-1β (whole blood): >31 μM
HT-Sol. (pH 6.8): 4 μM
Clint HLM: 299 μL/min/mg

NP3-562
IL-1β (THP-1): 0.090 μM
IL-1β (whole blood): 0.21 μM
HT-Sol. (pH 6.8): >1000 μM
Clint HLM: 38 μL/min/mg

[1] Karen V. Swanson, Meng Deng, Jenny P.-Y. Ting, *Nat. Rev. Immunol.*, **2019**, 19, 477– 489.

[2] Hallie M. Blevins, Yiming Xu, Savannah Biby, Shijun Zhang, *Front. Aging Neurosci.*, **2022**, 14, 879021.

[3] Juraj Velcicky, Philipp Janser, Nina Gommermann, Silke Brenneisen, Slavica Ilic, Eric Vangrevelinghe, Nikolaus Stiefl, Andreas Boettcher, Christelle Arnold, Claire Malinverni, Janet Dawson, Renata Murgasova, Sandrine Desrayaud, Karen Beltz, Alexandra Hinniger, Carien Dekker, Christopher J. Farady, Angela Mackay, *J. Med. Chem.* **2024**, 67, 1544–1562.

[4] Carien Dekker, Henri Mattes, Michael Wright, Andreas Boettcher, Alexandra Hinniger, Nicola Hughes, Sandra Kapps-Fouthier, Jörg Eder, Paulus Erbel, Nikolaus Stiefl, Angela Mackay, Christopher J. Farady, *J. Mol. Biol.* **2021**, 433, 167309.

Trabectedin derails transcription-coupled nucleotide excision repair to induce DNA breaks in highly transcribed genes

V. Takhaveev[1], K. Son[2], V. Mor[2], H. Yu[2,3], E. Dillier[1], N. Zilio[4], N. J. Püllen[1], D. Ivanov[2], H. Ulrich[4], S. J. Sturla[1]*, O. D. Schärer[2,3]*

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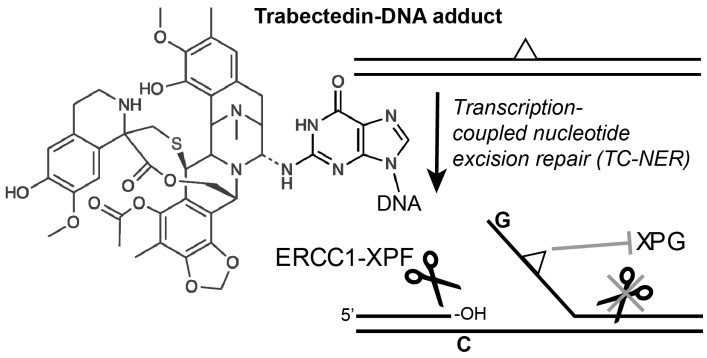
Drug resistance often results from DNA-alkylating anticancer agents failing to induce cell death in tumors due to DNA repair. Trabectedin is a tetrahydroisoquinoline alkaloid marine natural product used as an anticancer drug. Unusually, it is more toxic to cells that are active in the transcription-coupled nucleotide excision DNA repair pathway (TC-NER). To unlock the potential of trabectedin and inform its application in precision oncology, we need to understand how the drug's mechanism of toxicity relies on TC-NER activity, which is a strong contrast compared to other alkylating agents. Here, we determined that abortive TC-NER of trabectedin-DNA adducts forms persistent single-strand breaks (SSBs) as the adducts block the second of the two sequential NER hydrolytic incisions of DNA strands. We mapped the 3'-hydroxyl groups of SSBs originating from the first NER incision at trabectedin lesions, recording TC-NER on a genome-wide scale. Trabectedin-induced SSBs primarily occur in transcribed strands of active genes and peak near transcription start sites. Frequent SSBs are also found outside gene bodies, connecting TC-NER to divergent transcription from promoters. This work [1] advances the use of trabectedin for precision oncology and for studying TC-NER and transcription.



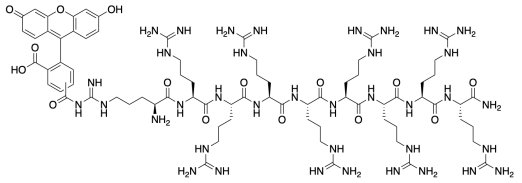
[1] Son, K., Takhaveev, V. et al. Nat Commun, 2024, 15, 1388.

Stereochemistry of Cell Penetrating Peptides

Y. Amer[1], J. Reymond[1]*

[1]Reymond Research Group

Peptides are linear oligomers of amino acids, almost exclusively of L-amino acids. Nevertheless, mixed-chirality sequences also occur, either in natural products mostly from microbial origin, such as the cyclic peptide Gramicidin S,[1] or in designed peptides such as our recently reported antimicrobial undecapeptide **ln69**.[2] Both of these peptides are membrane disruptors and the mixed chirality appears to play a key role in their activity/toxicity profile. Here we asked the question whether mixed chirality might also affect the properties of cell penetrating peptides (CPPs), focusing on the case of the arginine nonapeptide which is well-known as a drug delivery peptide.[3] We prepared a series of nona-arginine diastereomers by solid-phase peptide synthesis which we labeled at their N-terminus with 5(6)-carboxyfluorescein to trace cellular uptake.[4] The results of this study will be presented in the poster.



(1) Gause, G. F.; Brazhnikova, M. G. Gramicidin S and Its Use in the Treatment of Infected Wounds. *Nature* **1944**, *154* (3918), 703–703. https://doi.org/10.1038/154703a0.
(2) Personne, H.; Paschoud, T.; Fulgencio, S.; Baeriswyl, S.; Köhler, T.; van Delden, C.; Stocker, A.; Javor, S.; Reymond, J.-L. To Fold or Not to Fold: Diastereomeric Optimization of an α-Helical Antimicrobial Peptide. *J. Med. Chem.* **2023**, *66* (11), 7570–7583. https://doi.org/10.1021/acs.jmedchem.3c00460.
(3) Dubikovskaya, E. A.; Thorne, S. H.; Pillow, T. H.; Contag, C. H.; Wender, P. A. Overcoming Multidrug Resistance of Small-Molecule Therapeutics through Conjugation with Releasable Octaarginine Transporters. *Proceedings of the National Academy of Sciences of the United States of America* **2008**, *105* (34), 12128–12133. https://doi.org/10.1073/pnas.0805374105.
(4) Eggimann, G. A.; Buschor, S.; Darbre, T.; Reymond, J. L. Convergent Synthesis and Cellular Uptake of Multivalent Cell Penetrating Peptides Derived from Tat, Antp, pVEC, TP10 and SAP. *Org. Biomol. Chem.* **2013**, *11* (39), 6717–6733. https://doi.org/10.1039/c3ob41023d.

Synthesis of GBD Derived Bicyclic Diamine as Interesting Scaffold for Medicinal Chemistry

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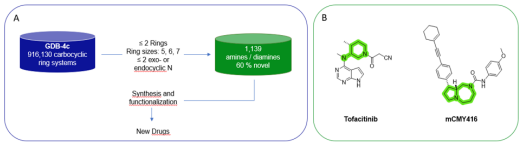
The Generated Data Base (GDB), curated by the Reymond group, offers stable, feasible, 3D-shaped chiral molecules, enabling exploration a vast chemical space, providing promising scaffolds for Medicinal Chemistry. These molecules are innovative due to their complex polycyclic system, high sp^3-hybridized carbon fraction and the presence of many quaternary carbons. 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 scaffolds. Herein I discuss initial synthetic steps for preparing a target subset of these diamines based on Diels-Alder and ring expansion reactions. (*Figure 1A)*. The amine functional group within the cyclic scaffolds grants straightforward functionalisation with desired moieties. Many small molecule cores in literature features this interesting structural motifs, namely, Tofacitinib (*Figure 1B*), an anti-inflammatory drug, bears one edo and one exo cyclic amine while mCMY416, an anti-infective lead, contains a bicyclic scaffold.



[1] Ruddigkeit, L.; Van Deursen, R.; Blum, L. C.; Reymond, *J. Chem. Inf. Model.* **2012**, *52* (11), 2864–2875.

[2] Lovering, F.; Bikker, J.; Humblet, C. *J. Med. Chem.* **2009**, *52* (21), 6752–6756.

[3] Ence, C. C.; Uddin, T.; Borrel, J.; Mittal, P.; Xie, H.; Zoller, J.; Sharma, A.; Comer, E.; Schreiber, S. L.; Melillo, B.; Sibley, L. D.; Chatterjee, *ACS Infect. Dis.* **2024**, acsinfecdis.4c00170.

A versatile “Synthesis Tag” (SynTag) for the chemical synthesis of aggregating peptides and proteins

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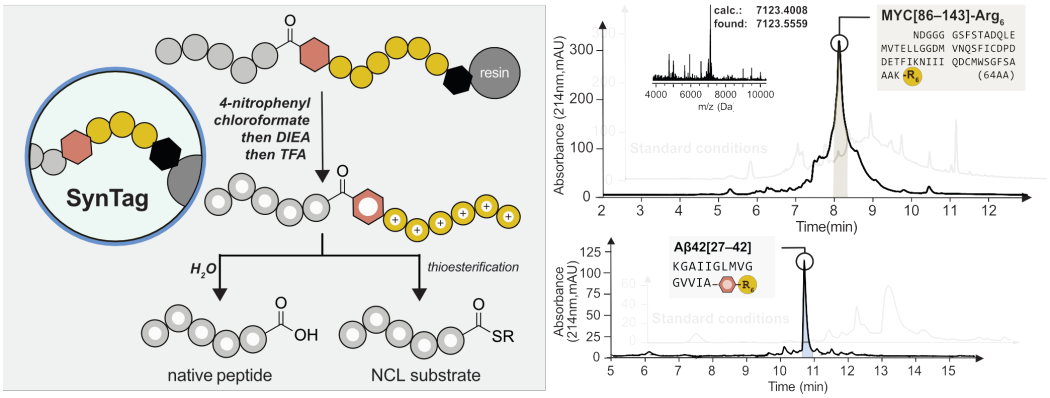
Chemical protein synthesis enabled by solid-phase peptide synthesis (SPPS) provides peptide and protein samples with a virtually unlimited chemical space (including PTMs) through incorporation of non-canonical amino acids and backbone modifications. Decades of improvement and optimization have increased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



[1] N. Hartrampf, A. Saebi, M. Poskus, Z.P, Gates, A.J. Callahan, A. E. Cowfer, S. Hanna, S. Antilla, C.K, Schissel, A.J. Quartararo, X. Ye, A. J. Mijalis, M.D. Simon,A. Loas, S. Liu, C. Jessen, T.E. Nielsen, B. L. Pentelute, Science 2020, 368 (6494).

[2] Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. Science 1994, 266 (5186), 776–779.

[3] Bürgisser, H.; Williams, E. T.; Lescure, R.; Premanand, A.; Jeandin, A.; Hartrampf, N. ChemRxiv September 5, 2023.

Synthesis and activity of novel acylfulvene analogs appended with covalent reactive groups

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Despite their widespread use in cancer therapy, the efficacy and safety of DNA alkylating anticancer agents are limited by cellular DNA damage repair mechanisms. Acylfulvenes (AFs), a class of experimental anticancer agents, alkylate DNA in the minor groove, creating N3-adenine adducts that induce cytotoxicity in cancer cells. However, AF-DNA adducts efficiently stall RNA polymerase, activating transcription-coupled nucleotide excision repair (TC-NER), which removes the AF adducts and leads to drug resistance. Our goal is to exploit the therapeutic properties of AFs while reducing repair-associated resistance by developing compounds that induce DNA-protein interactions, promoting cell death even in repair-proficient cells. In our lab we previously designed AF analogs by linking various apolar and aromatic groups to the core sesquiterpene structure of AFs, comprising a fused [6,5] ring system and a reactive cyclopropyl group. These compounds exhibited cytotoxicity to human cancer cell lines, with IC50 values ranging from 0.02 to 100 μM, and repair-deficient cells were generally more sensitive. As these results suggest that stronger binding moieties targeting TC-NER repair proteins may enhance cytotoxicity in repair-proficient cells, we aimed to generate new AF analogs capable of covalently engaging with nucleophilic lysine residues. We thus designed and synthesized a series of AF derivatives consisting of a spirocyclic cyclohexanone core appended with various covalent reactive groups. These derivatives will be profiled for cytotoxicity to human cell lines proficient and deficient in TC-NER. We anticipate that these data will inform the design further iterations of AF analogs and provide insight into the mechanism of TC-NER.

3D dynamic hydrogel matrix for cultivation of intestinal organoids

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Intestinal organoids (IOs) mimic the architecture and physiology of various tissues of the small and large intestines. They are used for disease modelling, tissue engineering and to study drug interactions. The current gold standard for the culture and propagation of IOs, is Matrigel, a 3D-extracellular matrix derived from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma. However, there are several disadvantages, primarily that they are derived from mouse tumors, which limit human clinical applications and they suffer from batch-to-batch variability. Moreover, intestinal cells are exposed to a dynamic environment in the human body, thanks to the peristaltic (wave-like) motion of the smooth muscle. However, the state-of-the-art organoid cultivation with Matrigel can not mimic these dynamic processes, limiting the morphology and function of cultured organoids.

We propose a new hydrogel matrix, which can mimic the dynamic behaviour of intestinal tissue by taking advantage of catch bond forming protein complexes, a so-called CatchGel. Catch bonds are mechanoresponsive protein complexes whose strength of interaction increases under applied shear stress, rather than breaking as in traditional 'slip' bonds. Furthermore, they are often responsive to the chemical environment allowing their mechanical behaviour to be more effectively tuned. One of the strongest known catch bonds is the complex between serine-aspartate repeat-containing protein G (sdrG) from S. epidermidis and the β-strand of the ECM protein fibrinogen (Fgβ). This complex can form interactions with rupture forces ranging from a few hundered pN (slip) to over 2 nN (catch) and is also highly dependent on the calcium concentration. By functionalising polysaccharide chains with either sdrG or Fgβ, we created physically cross-linked hydrogels whose properties were mediated by the mechanical and chemical sensitivities of the catch bond complex. The advantages of this hydrogel are, therefore: 1) physical stimulation through catch-bonds, 2) lower batch variability and defined composition, due to rational engineering of materials, and 3) biocompatibility, due to naturally derived source materials.

Our engineered hydrogel was compared with Matrigel to investigate its effects on intestinal organoid propagation and differentiation using immunofluorescent staining/imaging and flow cytometry. The physical and mechanical properties were also characterized at the microscale using atomic force microscopy and at the macroscale, using rheology and small-angle X-ray scattering, to understand the difference between local and bulk responsive behaviours and correlate these properties to the outcomes of organoid culture experiments.

[1] Milles, L. F., Unterauer, E. M., Nicolaus, T. & Gaub, H. E. Calcium stabilizes the strongest protein fold. Nat. Commun., 2018, 9, 1–10.
[2] Huang, W., Le, S., Sun, Y., Lin, D. J., Yao, M., Shi, Y. & Tan, J. Mechanical Stabilization of a Bacterial Adhesion Complex. J. Am. Chem. Soc., 2022, 144, 16808–16818.
[3] Campillo Poveda, M., Drurey, C., & Maizels, R. M. Generation, culture, and stimulation of small intestinal murine organoids in parasitology research. STAR Protocols, 2023, 4, 4.
[4] Chanet S, Martin AC. Mechanical force sensing in tissues. Prog Mol Biol Transl Sci., 2014, 126, 317-352.
[5] Caliari, S., Burdick, J. A practical guide to hydrogels for cell culture. Nat Methods, 2016, 13, 405-414.

Catalytic improvement of a metalloenzyme using hydrophobic tuning with non-canonical amino acids

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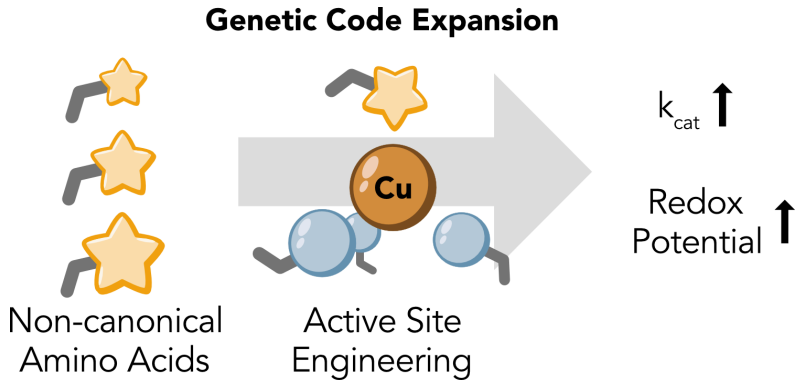
Enzymes evolved in nature through changes of their sequences using the 20 canonical amino acids (cAA) and posttranslational modifications. However, nature only samples a small fraction of the chemical space. By introducing non-canonical amino acids (ncAAs), the chemical space of enzymes can be expanded and new reactivities can be observed.[1,2] This approach is especially interesting for metalloenzyme engineering, because the AAs that are affiliated with metal binding are limited and the exchange of AAs in the first coordination spheres with other canonical AAs usually have detrimental effects.[3] However, with the use of ncAAs, the properties of metal binding AAs can be tuned more carefully to enhance the properties of the enzymes.

Our goal is to expand the available tools of enzyme engineering, particularly metalloenzymes, using ncAA-expanded directed evolution and to discover more about trends in (metallo)enzyme activity and engineering. As a proof-of-concept, we demonstrate engineering of a copper oxidase by ncAA-expanded directed evolution.

Therefore, we developed genetic code expansion tools for the incorporation of three ncAAs bearing highly hydrophobic side chains, and the incorporation efficiencies were quantified with the green fluorescent protein (GFP). These new tools were then harnessed to fine-tune the hydrophobic environment of a copper oxidase. We demonstrate that two of these ncAAs can be efficiently incorporated near the copper center, yielding variants with increased redox potentials and improved catalytic parameters. We characterized the impact of the mutations on the structure, reduction potentials, catalytic parameters, and substrate scope.



[1] Ivana Drienovská, Ana Rioz-Martínez, Apparao Draksharapua, Gerard Roefles, Chem. Sci., **2015**, 6, 770-776.

[2] Moritz Pott, Takahiro Hayashi, Peer R. E. Mittl, Anthony P. Green, Donald Hilvert, *J. Am. Chem. Soc.*, **2018**, 140, 1535-1543.

[3] Anna C. Olbrich, Jan N. Schild, Vlada B. Urlacher, *J. Inorg Biochem*, **2019**, 201, 110843.

Development of novel bitopic and bifunctional ligands to study adenosine A_1 recepotors

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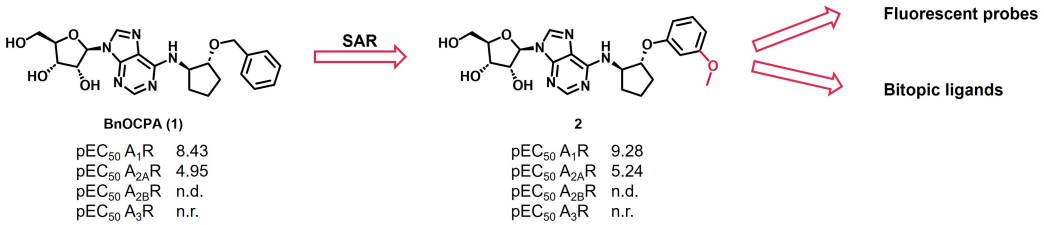
The adenosine receptor (AR) subtypes, named A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R, are involved in cancer, cardiovascular, respiratory, inflammatory, and neurological diseases and are therefore interesting targets for the development of new treatments with different pharmacological mechanisms.[1] Each AR subtype activates multiple signalling pathways depending on its interaction with different G proteins. A_1R and A_3R couple to $G_{i/o}$ proteins (G_{i1}, G_{i2}, G_{i3}, G_{oa}, G_{ob} and G_z), [2] so not only subtype selectivity but also G protein selectivity (biased signalling) is important. BnOCPA (**1**) turned out to be the first A_1R- and G protein-selective agonist (signalling only through G_{ob}), producing distinct physiological effects.[3]

One aim of this project is to develop A_1R-selective fluorescent probes with an agonist scaffold to monitor signalling processes in cells or to use as fluorescent tracers in displacement assays. On the other hand, by linking positive allosteric modulators (PAMs) to an agonist, we aim to generate bitopic A_1R ligands and potentially achieve biased A_1R signalling. Compound **2** was identified in previous structure-activity relationship (SAR) studies based on BnOCPA having higher potency, selectivity, and affinity for A_1R.[4] In *meta*-position, different substitutions were tolerated, so this position was chosen for the attachment of linkers to add a fluorescent dye or PAMs. Here we describe the multistep synthesis of these fluorescent probes and bitopic ligands.



[1] J.-F. Chen, H. K. Eltzschig, B. B. Fredholm, *Nature Reviews Drug Discovery,* **2013**, 12, 265–286.
[2] K. A. Jacobson, D. K. Tosh, S. Jain, Z.-G. Gao, *Frontiers in Cellular Neuroscience*, **2019**, 13, 124.
[3] M. J. Wall *et al.*, *Nature Communications*, **2022**, 13, 4150.
[4] B. Preti *et al.*, *Journal of Medicinal Chemistry*, **2022**, 65, 14864–14890.

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

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TRPM4 is a non-selective monovalent cation channel, activated by an intracellular increase in Ca^{2+} concentration. The channel depolarizes cells by conducting $Na^+ > K^+ >> Cs^+ > Li^+$ from the extracellular space into the cytosol. [1] Mutations of TRPM4 have been associated with cardiovascular and neuronal diseases [1] and only a few small molecules are known for their ability to inhibit TRPM4, including 9-phenanthrol, anthranilic acid derivatives such as flufenamic acid or the previously discovered anthranilic anilids CBA, NBA and LBA [2, 3]. The goal of our project is to conduct an SAR-study on the core scaffold structure (Figure 1) of the two inhibitors CBA and NBA [2] with the intention to further improve the inhibitory potency and physicochemical properties. 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.

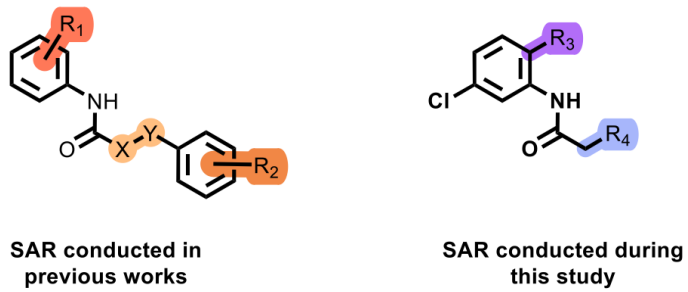

Figure 1: Anthranilic anilide scaffold and conducted SAR-studies.

We conducted a systematic SAR-study through the chemical synthesis of an extensive compound library and subsequent evaluation of their TRPM4 inhibitory activity using a HEK293-TRPM4 overexpressing cell-based fluorescent Na^+-influx assay. Several new analogues with sub-micromolar potencies have been discovered, with the most active compound showing a 3.3-fold increase of inhibitory potency compared to NBA and a 7.5-fold increase of inhibitory potency compared to CBA. We are currently complementing these findings with patch-clamp electrophysiology measurements using human colon cancer HCT-116 cells, which highly express TRPM4 endogenously. Our study revealed valuable structure-activity-relationships for further optimizations, and we are currently measuring physicochemical properties such as solubility, lipophilicity ($log(D)_{7.4}$) and determining solid and solutions structures of our hit compounds to further rationalize our findings.>

[1] I. Mathar, G. Jacobs, M. Kecskes, A. Menigoz, K. Philippaert, R. Vennekens. (2014). TRPM4. In: B. Nilius, V. Flockerzi (eds) Mammalian Transient Receptor Potential (TRP) Cation Channels. Handbook of Experimental Pharmacology, vol 222. Springer, Berlin, Heidelberg.

[2] L. C. Ozhathil, C. Delalande, B. Bianchi, G. Nemeth, S. Kappel, U. Thomet, D. Ross-Kaschitza, C. Simonin, M. Rubin, J. Gertsch, M. Lochner, C. Peinelt, J.-L. Reymond & H. Abriel, *British Journal of Pharmacology*, **2018**, *175(12)*, 2504-2519.

[3] Z. M. Kovács, C. Dienes, T. Hézső, J. Almássy, J. Magyar, T. Bányász & N. Szentandrássy, *Pharmaceuticals*, **2022**, *15(1)*, 81.

Discovery of indolicidin diastereomers as antimicrobial agents

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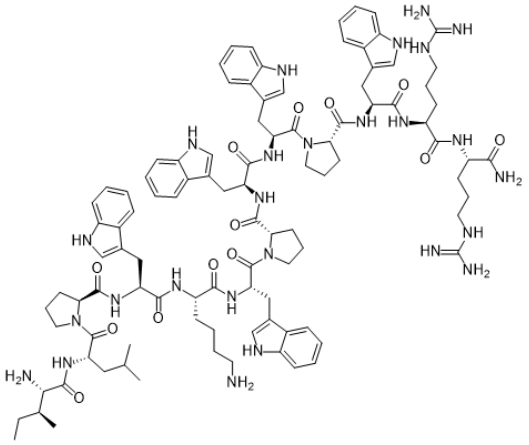
Due to the multiple mechanisms, antimicrobial peptides (AMPs) are regarded as reliable resources for antibiotic against multidrug resistance (MDR)[1]. Indolicicin (ILPWKWPWWPWRR-NH_2) is a linear AMP displaying extensive antibacterial activities. However, severe hemolysis and limited antibacterial ability are the obstacles in therapeutical application[2]. As a result, discovering indolicidin analogues with improved therapeutic index is a significant solution.



Recently, several interesting works in our group confirmed that diastereomeric optimization is an available strategy to modify peptides bioactivities and lead to optimized AMPs. Siriwardena *et al.* demonstrated stereochemical purity plays a critical role in the properties of AMPs[3]. Stereorandomization preserves antibacterial effect and decreases hemolysis. Personne *et al.* introduced D-residues in a α-helical linear undecapeptide to obtain diastereomers with improved antibacterial effect and reduced toxicity[4]. Based on these works, diastereomeric optimization might provide a promising method to obtain effective and safe indolicidin derivatives. This project focuses on replacing the L amino acids with single or multiple D amino acids to obtain diastereomeric indolicidin analogues. Preferred peptides will be tested at further biological and pharmaceutical experiments to investigate cellular mechanism and drug-like property.

[1] M. Magana, M. Pushpanathan, A. L. Santos, L. Leanse, M. Fernandez, A. Ioannidis, M. A. Giulianotti, Y. Apidianakis, S. Bradfute, A. L. Ferguson, A. Cherkasov, M. N. Seleem, C. Pinilla, C. de la Fuente-Nunez, T. Lazaridis, T. Dai, R. A. Houghten, R. E. W. Hancock, G. P. Tegos, The Lancet. Infectious Diseases, 2020, 20, 216−230.
[2] M.E. Selsted, M.J. Novotny, W.L. Morris, Y.Q. Tang, W. Smith, J.S. Cullor, Journal of biological chemistry, 1992, 267, 4292–4295.
[3] T. N. Siriwardena, B. H. Gan, T. Köhler, C. van Delden, S. Javor, J.-L. Reymond, ACS Central Science, 2021, 7, 126−134.
[4] H. Personne, T. Paschoud, S. Fulgencio, S. Baeriswyl, T. Köhler, C. van Delden, A. Stocker, S. Javor, J.-L. Reymond, Journal of Medicinal Chemistry, 2023, 66, 7570−7583.

A Deep Learning Model for Predicting the Thermal Stability of Collagen Triple Helices

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Collagen model peptides (CMPs) are key tools for investigating the physico-chemical properties of collagen, the most abundant mammalian protein, as well as designing synthetic collagen-based materials and probes. Like collagen, CMPs self-assemble into a triple-helical structure that is characterized by its melting temperature (T_m). The T_m is highly dependent on the CMP sequence. Because of the colossal number of possible sequence variations, computational methods are necessary for deciphering the trends in the thermal stability of collagen triple helices. Despite several attempts to predict T_m values from CMP sequences, no computational models integrate all key aspects that influence the T_m to achieve accurate predictions. Here, we design a deep-learning model that dissects the pairwise intra- and interstrand interactions in CMPs and uses them to accurately predict T_m values from CMP sequences. The model is trained with 733 published homo- and heterotrimeric CMP sequence–T_m pairs, augmented to 4290 input values through known effects of terminal functional groups. Our approach comprehensively deals with critical aspects affecting the experimental T_m such as frame shifts, capping groups, and heating rates. The best-fit model shows high accuracy both for the training ($R^2 > 0.98$) and validation ($R^2 > 0.98$) sets, indicating a high degree of generalization to a broad range of CMPs. Our model represents a general tool that will aid the design of collagen triple helices with desired stability.

Chemical Probe to Visualize Bacterial Physiology: A ratiometric pH Sensor for Gram-positive and Gram-negative Bacteria

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Fluctuating environments can lead to phenotypic heterogeneity within a clonal bacteria population. Especially in response to antibiotics, phenotypic heterogeneity plays a crucial role. Bacterial cells that may escape antibiotic exposure without undergoing genetic changes are known as persister cells, which have been linked to relapsing and chronic infections.[1] Persister formation can be induced by low pH exposure, for instance in acidic lysosomes of human immune cells.[2] Moreover, persisters have a more acidic intracellular pH than clonal cells, which will be susceptible to antibiotics.[3] To understand how individual cells interact with their environment and how they adapt to pH stress induced by the host, methods to analyze the physiology of single cells are required.

In this work, we report a ratiometric, fluorescent probe to sense cytoplasmatic pH in bacteria on a single-cell level. Our probes are based on hemi-cyanine dyes and show high uptake into Gram-positive and Gram-negative bacteria. The probes react selectively with OH^- over other nucleophiles present in biological systems. While under acidic conditions the probes emit in the red spectrum (ex/em 600/675 nm), upon reaction with OH^- the emission becomes more blue-shifted (ex/em 415/505 nm), allowing a ratiometric determination of the pH. The reaction to pH changes is both reversible and rapid, allowing for a real-time tracking of pH fluctuations. Importantly, the observed pH sensitivity range allows for monitoring acidifications and changes close to physiological pH in bacterial cells. Thus, our probes might be a promising tool for detecting phenotypic heterogeneity within bacterial populations.

[1] J. W. Bigger, *Lancet* **1944**, 244, 497–500.
[2] N. Leimer, C. Rachmühl, M. Palheiros Marques, A. S. Bahlmann, A. Furrer, F. Eichenseher, K. Seidl, U. Matt, M. J. Loessner, R. A. Schuepbach, A. S. Zinkernagel, *J. Infect. Dis*. **2016**, 213, 305–313.
[3] O. Goode, A. Smith, A. Zarkan, J. Cama, B. M. Invergo, D. Belgami, S. Caño-Muñiz, J. Metz, P. O'Neill, A. Jeffries, I. H. Norville, J. David, D. Summers, S. Pagliara, *mBio* **2021**, 12, e00909-21.

A HaloTag-based Gene Expression Reporter System for Live-Cell Imaging

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

Our system is based on the co-expression of the protein of interest BiP and the self-labeling protein HaloTag[2] in a stable cell line. By using two different fluorogenic HaloTag ligands, two timepoints of gene expression can be visualized simultaneously in a pulse-chase-like experiment, providing additional information and increasing the robustness of the system. The method is suitable for live-cell fluorescence imaging as well as flow cytometry. Moreover, we are able to employ the system in high-content screening experiments, searching for novel inducers of BiP overexpression.

[1] C. Hetz, K. Zhang, R. J. Kaufman, *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 421–438.

[2] G. V. Los, L. P. Encell, M. G. McDougall, D. D. Hartzell, N. Karassina, C. Zimprich, M. G. Wood, R. Learish, R. F. Ohana, M. Urh, D. Simpson, J. Mendez, K. Zimmerman, P. Otto, G. Vidugiris, J. Zhu, A. Darzins, D. H. Klaubert, R. F. Bulleit, K. V. Wood, *ACS Chem. Biol.* **2008**, *3*, 373–382.

Engineering a Zinc-Dependent Phosphotriesterase with a Non-Canonical Histidine Derivative

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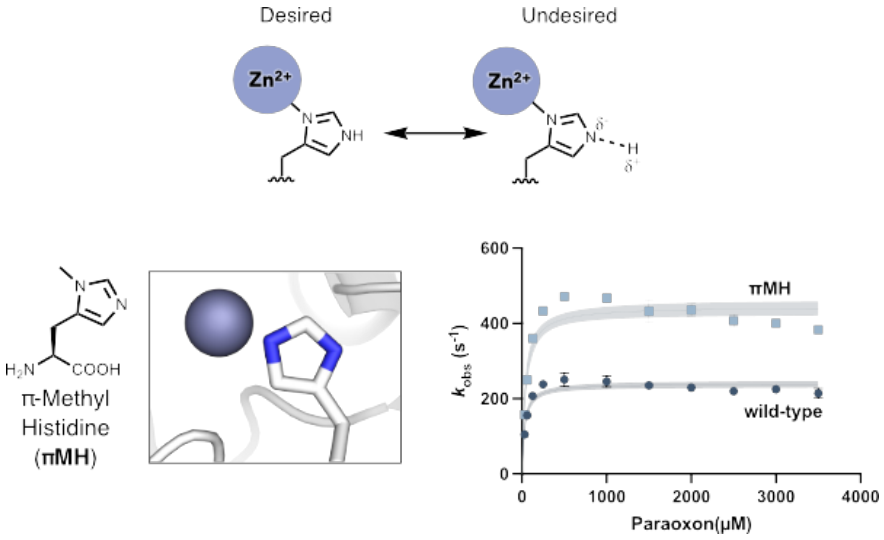
Many hydrolytic enzymes rely on the Lewis acidity of the Zn2+-ion to lower the pK_a of a zinc-bound water molecule for their catalytic activity.[1] In these zinc-dependent hydrolytic enzymes, the metal-coordinating histidine residues are crucial to stabilize the zinc-hydroxide species and charged transition states. Studies on carbonic anhydrase have highlighted that accumulation of a negative charge on such a histidine residue (eg. by deprotonation) decreases the activity of the enzyme drastically by raising the pK_a of the zinc-bound water.[2] Based on this observation, we formulated a hypothesis that the prevention of the negatively charged histidinate state could have a beneficial impact on the activity of zinc-dependent hydrolytic enzymes. To test this hypothesis, we aimed to incorporate a methylated histidine analog into a zinc-coordinating position of a hydrolytic enzyme using amber suppression—a common methodology for incorporation of non-canonical amino acids (ncAAs).[3]

When investigating a phosphotriesterase from *Pseudomonas diminuta*[4] as model enzyme, we found evidence that the replacement of histidine by π(pi)-methyl histidine within the catalytic zinc-binding site results in a 1.9 fold increase in the catalytic rate for the hydrolysis of paraoxon at pH 7. From preliminary results we propose the reason for this increased activity to be a decrease in pK_a of the zinc bound water, compared to the wild-type enzyme.



[1] M. L. Zastrow, V. L. Pecoraro, *Biochemistry* **2014**, 53 (6), 957–978.
[2] C. Huang, C. A. Lesburg, L. L. Kiefer, C. A. Fierke, D. W. Christianson, **Biochemistry** *1996*, 35 (11), 3439–3446.
[3] C. C. Liu, P. G. Schultz, *Annu. Rev. Biochem*. **2010**, 79 (1), 413–444. [4] O. Khersonsky, R. Lipsh, Z. Avizemer, Y. Ashani, M. Goldsmith, H. Leader, O. Dym, S. Rogotner, D. L. Trudeau, J. Prilusky, P. Amengual-Rigo, V. Guallar, D. S. Tawfik, S. J. A. Fleishman, Mol. Cell 2018, 72 (1), 178-186.e5.

Ligand-directed bioconjugation on native protein with hypervalent iodine-based Ethynylbenziodoxolones (EBXs) reagents

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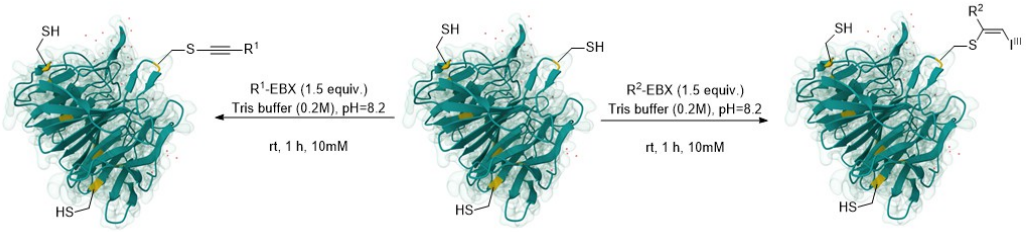
Site selective modifications of proteins is essential to improve diagnostics and therapeutics tools. If chemoselective transformations have been successful for nucleophilic amino acids, single site-selective modification still remains challenging on native protein. To overcome this problem, ligand-directed bioconjugation were developed and applied to form antibody drug conjugates (ACD). [1]

Our group reported the use of hypervalent iodine-based Ethynylbenziodoxolones (EBXs) reagents for bioconjugation on cysteine and tyrosine residues on proteins. [2] We present here single site-selective bioconjugation on native protein with ligand-directed EBXs reagents. These reagents offer the possibility to form S-alkynylated product or stable S-vinylbenziodoxolones (S-VBX) bioconjugates that can be further functionalized.



[1] Yue Zeng, Feng Tang, Wei Huang, *Angew. Chem. Int. Ed*. **2022**, 61, e202204132

[2] Romain Tessier, Javier Ceballos, Beat Fierz, Jerome Waser, *Chem*. **2019**, 5, 2243-2263

Synthesis of modified carbohydrates as glyco-donors aiming complex glycans synthesis and glycopeptides

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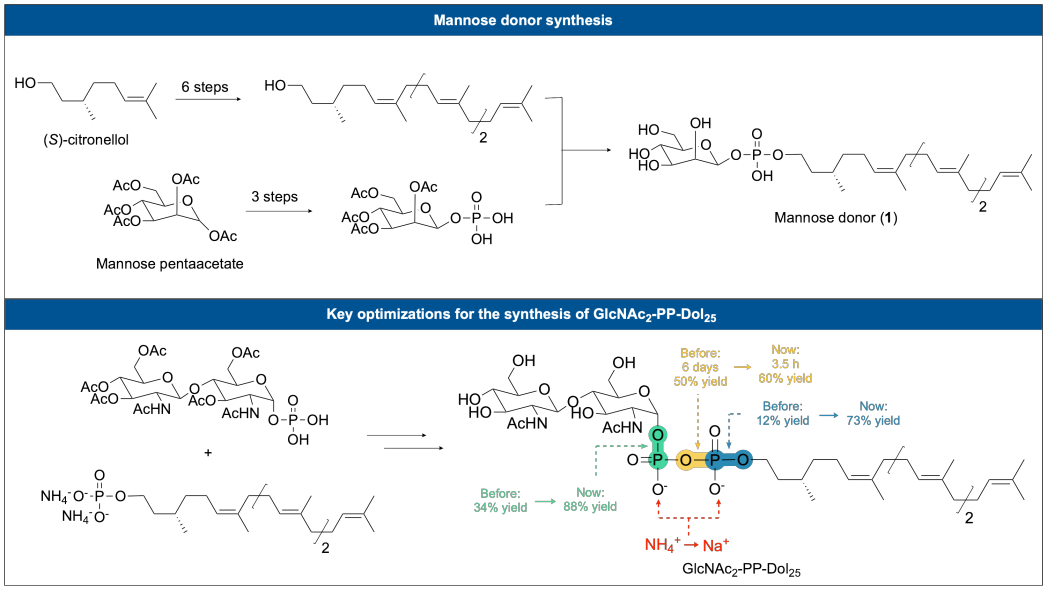
Lipid-linked oligosaccharides (LLOs) are pivotal in *N*-protein glycosylation, a crucial post-translational modification facilitating a broad spectrum of *N*-glycan structures.[1] In eukaryotes, this biological process is governed by various enzymes such as ALG (asparagine-linked glycosylation) and OST (oligosaccharyltransferase), which use LLO as substrates.[2] Our laboratory has developed simplified LLOs precursors, which are converted to synthetic LLOs through enzymatic synthesis employing glyco donors such as dolichyl phosphomannose (**1**).[3] Recently, we have also optimized the synthesis and purification of dolichyl diphosphochitobiose ($GlcNAc_2$-PP-Dol_{25}), providing a reliable method for preparing new LLO analogues.[4]

Extending on this work, we aim to synthesize modified glyco substrates capable of being recognized by ALG enzymes to produce complex oligosaccharides. Ultimately, these synthesized glycans hold the potential for producing glycopeptides, offering promising avenues for further exploration in glycoscience and biotechnology.



[1] A. S. Ramírez, K. P. Locher, Glycobiology 2023, 1–12.

[2] A. S. Ramírez, M. de Capitani, G. Pesciullesi, J. Kowal, J. S. Bloch, R. N. Irobalieva, J.-L. Reymond, M. Aebi, K. P. Locher, , Nat Commun 2022, 13, 7296.

[3] J. S. Bloch, G. Pesciullesi, J. Boilevin, K. Nosol, R. N. Irobalieva, T. Darbre, M. Aebi, A. A. Kossiakoff, J.-L. Reymond, K. P. Locher, Nature 2020, 579, 443–447.

[4] M. A. Meirelles, J.-L. Reymond, Helvetica Chimica Acta 2023, 106, e202300171.

Protein Engineering with Genetic Code Expansion

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Protein engineering is an invaluable tool that has revolutionized our understanding of fundamental biological processes, our approach to develop tools to study biology, and our ability to develop therapeutics and biocatalysts. Directed evolution is a highly effective tool to tailor the function of proteins that mimics Darwinian evolution of proteins and other biomolecules in a laboratory setting[1]. Although vast, the chemical space of proteins is restricted by the natural protein alphabet and the availability of co-factors. By exploiting orthogonal tRNA-synthetase/tRNA (aaRS/tRNA) pairs, such as the pyrrolysine system found in some bacteria and archaea[2], codon suppression techniques can be used to site-specifically incorporate non-canonical amino acids (ncAAs) into proteins. This technique has been used to encode a wide range of non-natural chemistries into proteins and expand their functionality beyond those found in nature[3]. We envision that genetic code expansion could be leveraged to streamline directed evolution workflows. We present progress towards a platform for low-cost, low-waste protein engineering leveraging genetic code expansion. Specifically, advances in the discovery and engineering of novel aaRS/tRNA pairs and their application in genetic code expansions are presented.

[1] Michael S Packer, David R Liu, D. R, *Nat Rev Genet*, **2015**, 16, 379–394.
[2] Alexandre Ambrogelly, Sotiria Palioura, Dieter Söll, *Nat Chem Biol*, **2007**, 3, 29–35.
[3] Wei Wan, Jeffrey M Tharp, Wenshe R Liu, *Biochim Biophys Acta*, **2014**, 1844, 1059–1070.

Examining the interaction between thiolate protected metal clusters and lipid bilayers

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The understanding of the interactions of monolayer protected metal clusters with biological membranes is of fundamental importance for applications of these systems in medicine and for biosensing. At the same time, using metal clusters imposes some restrictions such as toxicity[1] and undesirable immunological responses. In fact, the chemical properties and biological effects of monolayer protected metal clusters can critically depend on the size of the clusters, the protecting ligand layer and chirality. Therefore, different kinds of coatings are used to reduce the negative impacts of metal clusters and increase the beneficial interaction with biological systems. Although thiol groups play an important role for biological systems and have been used as a ligand for the preparation of metal clusters in many areas, the effect of thiolate protected metal clusters on the cell membrane is not well understood.

In our study, lipid bilayers are used as a model of the cell membrane. Different methods have been reported to prepare lipid bilayers[2]. We use several methods to prepare lipid bilayers on solid substrates including Langmuir Blodgett/Schaefer combination and vesicle fusion. We study interaction between different types of thiolates protected clusters and bilayer membranes, by atomic force microscopy (AFM), ellipsometry, quartz crystal microbalance (QCM) and attenuated total reflection infrared (ATR-IR) spectroscopy in a microfluidic and temperature-controlled flow-through cell. We furthermore make use of polarized light to study the orientation of the phospholipid bilayer and their order in presence and absence of metal clusters.

Our research findings show that the use of the Langmuir Blodgett method is beneficial for the preparation of at monolayer of phospholipids, but the use of combinations of methods for the preparation of the bilayer is not appropriate. On the other hand, the vesicle fusion method resulted in high quality phospholipid bilayers. ATR-IR spectroscopy proved to be a potential tool to study the interaction between the monolayer protected metal clusters and the phospholipid bilayers providing detailed molecular-level information.

[1] Bhattacharya, S.R., et al., ACS Applied Materials & Interfaces, 2022. **14**(26): p. 29521-29536.

[2] Paracini, N., et al., ACS Applied Materials & Interfaces, 2023. **15**(3): p. 3772-3780.

Similar Structure, Different Effects: Investigation of Pt(II) Complexes as DNA-Targeting Antibiotics against Gram-positive Bacteria

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Antimicrobial resistance (AMR) has emerged as one of the most significant health problems of the 21st century. In 2019, 4.95 million deaths were associated with bacterial AMR, and it was the direct cause of 1.27 million of those. It is forecasted to be the leading cause of mortality by 2050, with approximately 10 million annual deaths, more specifically its potential to increase up to 2.4 million deaths in high-income countries from 2015 to 2050, and up to 5.2 million deaths in Western Pacific region between 2023 and 2030[2]3Despite the pressing demand for the development of new antibiotics, the existing drug pipeline proves insufficient in addressing the urgent need. In 2021, only 27 new potential drugs, aimed at combatting bacterial infections were in clinical trials, which just 6 of them was described as innovative based on the criteria of the World Health Organization (WHO)[3]. These circumstances have created a need for the exploration and implementation of novel and inventive approaches in medicinal chemistry. Although metals have been used for medical purposes stretching back thousands of years, cisplatin was a paradigm shift in modern medicine as one of the first metal-containing anticancer drugs[4]. Three years before cisplatin was discovered, Rosenberg and his coworkers introduced the ionic species of Pt(IV) complexes inhibiting growth and cell division by stimulating filamentous formation in Escherichia coli[5]. In one of the largest systematic studies in the field conducted by the Community for Open Antimicrobial Drug Discovery (CO-ADD), around a thousand metal complexes have been screened and a promising number of active compounds contained platinum[6]. Recently, our group conducted a study on 1,5-cyclooctadiene (COD) complexes of Pt, which led to the discovery that $PtCODCl_2$ exhibits broad activity against Gram-positive bacteria and low in vivo toxicity[7]. To obtain further structure and activity insights on this compound class, the COD ring was modified, and the respective Pt(II) complexes were synthesized. Since the structure of compounds are similar to gold standard Pt-based chemotherapy drugs, the effects of cisplatin and lead compounds were compared in mode of action and cellular uptake studies. It was found out that the lead compounds have different biological activities than Pt drugs against Gram-positive bacteria.

[1] Tang, K. W. K.; Millar, B. C.; Moore, J. E. 2023. Antimicrobial Resistance (AMR). *Br J Biomed Sci*, *80*, 11387.
[2] Murray, C. J. et al. 2022.Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *The Lancet*, *399* (10325), 629–655
[3] World Health Organization (2023). https://www.who.int/westernpacific/news/item/13-06-2023-antimicrobial-resistance-expected-to-cause-5.2-million-deaths-in-the-western-pacific-by-2030.
[4] Franz, K.j., Metzler-Nolte, N.(2019. *Chem. Rev. 119, 727-729.*
[5] Rosenberg, B., Van Camp, L., Grimley, E.B., Thomson, A.J. 1967. *J. Biol. Chem*. *242*, 1347-1352.
[6] Frei, A. et al. 2020. *Chem. Sci., 11,* 2627–2639.
[7] Frei, A. et al. 2021. *ChemMedChem*, *16*, 3165–3171.

Evaluating Lysyl Oxidase Activity with Turn-On Fluorescent Probes

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Remodelling and maturation of collagen, the dominant structural protein in mammals, is crucial for the integrity of organs and wound healing.[1,2] These processes include post-translational cross-linking of collagen strands triggered by the oxidation of lysine residues through lysyl oxidases (LOXs). This enzyme family consists of five isoforms - lysyl oxidase and four lysyl oxidase-like enzymes. LOXs catalyze the oxidative deamination of lysine residues in the telopeptide domain of collagen and are important for the mechanical properties of the extracellular matrix (ECM).[1] Excessive LOX activity is, however, associated with fibrotic and malignant diseases which are estimated to account for around 45% of deaths in developed countries.[3] A comprehensive investigation of LOX activity is therefore important for a deeper understanding of normal physiological versus pathological processes. The current standard activity assay detects hydrogen peroxide, the by-product of the oxidative deamination reaction, and lacks specificity.[4] Our group has recently developed an enzyme-reactive sensor that detects LOX *in vitro*, *in vivo* and in tissue sections.[5] In this work, we developed a quick and straightforward assay for measuring LOX activity, based on the turn-on of a coumarin-based sensor. We have examined various analogs of the activity-based probe and evaluated their selectivity for LOX isoforms over related amine oxidases. We anticipate that our tools will be valuable for the screening of drug candidates targeting LOXs and deciphering the role of LOXs' in healthy and diseased states.

[1] Sylvain D. Vallet, Sylvie Ricard-Blum, *Essays in Biochemistry*, **2019**, *63*, 349-364.

[2] Matthew D. Shoulders, Ronald T. Raines, *Annual Review of Biochemistry*, **2009**, *78*, 929-958.

[3] Neil C. Henderson, Florian Rieder & Thomas A. Wynn, *Nature*, **2020**, *587*, 555-566.

[4] Fernando Rodriguez-Pascual, Tamara Rosell-Garcia, *Analytical Biochemistry*, **2022**, *639*, 114508.

[5] a) Matthew R. Aronoff, Paul Hiebert, Nina B. Hentzen, Sabine Werner, Helma Wennemers, *Nature Chemical Biology*, **2021**, *17*, 865-871. b) Paul Hiebert, Giuseppe Antoniazzi, Matthew Aronoff, Sabine Werner, Helma Wennemers, *Matrix Biology*, **2024**, *128*, 11-20.

Nano-carrier Encapsulated Chlorin e6 and its Derivatives as Photosensitizers for Dermal Application: Investigating their Incorporation into Lipid Carriers and Skin Penetration

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Photodynamic Therapy (PDT) employs porphyrin photosensitizers (PSs) to generate reactive oxygen species (ROS), effectively targeting diseased tissues [1,2]. However, the development of efficient PSs is challenged by their hydrophobic nature and tendency to aggregate [2]. These issues often necessitate the use of carrier systems to improve their solubility to targeted delivery in biological environments [2]. This current project aims to investigate the most suitable carriers for the topical application of PSs for superficial skin cancer treatment. This led to exploring phospholipid (PL) bicelles, i.e., small disk-shaped nanostructures that self-assemble from mixtures of long- and short-chain lipids components, serving as passive structural carriers to host hydrophobic drugs [3]. To probe the impact of PS polarity, three Chlorin Ce6 (Ce6) derivatives with increasing degrees of esterification were studied. The aim was to incorporate Ce6 and its derivatives into bicelles, to minimize aggregation, and enhance skin uptake. The incorporation of Ce6 into bicelles was monitored by NMR spectroscopy. NMR NOESY and diffusion-ordered spectroscopy (DOSY) were applied to prove successful PS encapsulation [4]. After encapsulating PSs in bicelles, the skin penetration was evaluated with a Franz diffusion experiment using porcine skin to assess the effectiveness of topical applications. Moreover, treated skin samples were embedded in paraffin, cut into slides, and imaged by widefield imaging using a customized filter cube (Cytation 10, Agilent BioTek, Basel, Switzerland) aiming to see the Ce6 fluorescence in the skin tissue at its emission peak at 680-685 nm wavelength.

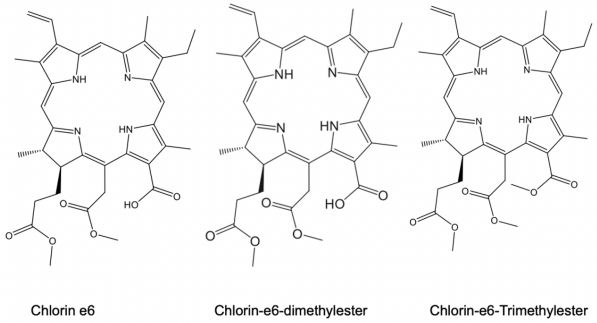

Figure1. Chlorin e6 and its derivatives, ranking from least to most hydrophobicity.

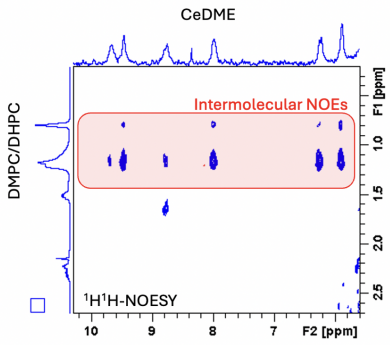

Figure2. H^1-H^1 NOESY spectrum of DMPC (long chain lipids) and DHPC (short chain lipids) and Chlorin-e6-dimethylester (CeDME).

[1] Udrea, A. M.; Smarandache, A.; Dinache, A.; Mares, C.; Nistorescu, S.; Avram, S.; Staicu, A. *Pharmaceutics*, **2023**, *15* (8), 2124.

[2] Kou, J.; Dou, D.; Yang, L. *Oncotarget*, **2017**, *8* (46), 81591–81603

[3] Sut, T. N.; Valle-González, E. R.; Yoon, B. K.; Park, S.; Jackman, J. A.; Cho, N.-J. *Applied Materials Today*, **2021**, *22*, 100947.

[4] Gjuroski, I.; Furrer, J.; Vermathen, M. *Molecules*, **2021**, *26* (7), 1942.

Synthesis of novel bicyclic diamine scaffolds derived from tropinone.

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Small molecule drugs often consist of rigid scaffolds equipped with reactive handles, typically amino groups which can be functionalized. 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 GDBs with biologically active small molecules in ChEMBL reveals that many scaffolds, even structurally simple ones, have never been synthesized (1). Here we discuss the synthesis of a novel family of tricyclic and bicyclic amino containing chemical scaffolds which can be accessed synthetically from tropinone and can be easily functionalized to explore differentiated medicinal chemistry space.

[1] Ye Buehler and Jean-Louis Reymond**,** *J. Chem. Inf. Model.* **2023**, 63, 20, 6239–6248.

Single-Molecule Peptide and Protein Identification using Fluorescence Blinking Fingerprints

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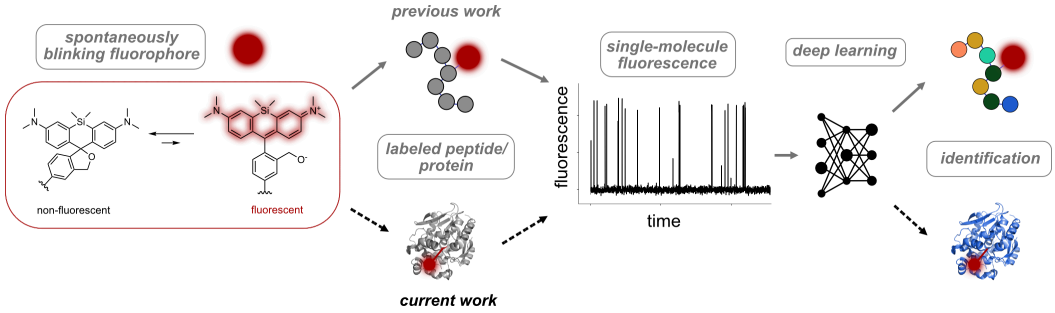
The analysis of the proteome is complicated by the presence of isoforms, post-translational modifications (PTMs), and the insufficient correlation between the abundance of proteins and the transcriptomic or genomic information. As the current state-of-the-art, mass spectrometry-based proteomics methods remain limited in their sensitivity and dynamic range compared to the established single-molecule approaches in genomics and transcriptomics.[1] In particular, single-molecule identification of peptides and proteins would enable the analysis of biomarkers that are present in very small quantities, for example in diluted clinical samples, single cells, or isolated organelles.[1] In our previous work, we provided the proof-of-concept of a fundamentally new approach to identify single peptide molecules.[2] The approach, termed blinkognition, exploits the emission of a spontaneously blinking fluorophore to capture information on the chemical environment of the probe.[3] We use single-molecule fluorescence measurements and a deep learning model based on convolutional, gated-recurrent unit layers to identify the peptide of interest in a targeted manner. By implementing Monte Carlo dropout, we obtain an uncertainty measure for classification which we use for filtering out low-quality traces. The method has been shown to differentiate between synthetic peptides of different sequences as well as variable numbers and positions of the PTMs: phosphorylation and epimerization. Blinkognition is presently constrained to targeted studies in well-defined settings.[2]



Our current work focuses on the applicability of blinkognition to full proteins. To test the method on pure protein samples different labeling and immobilization steps were required. For labeling, we synthesized a cysteine-reactive spontaneously blinking fluorophore. Immobilization was approached by encapsulating the proteins into liposomes. Preliminary results indicate that the method can distinguish unrelated proteins, proteins of similar function, and even different sites on the same protein. We envision that the technology is adaptable to molecules beyond peptides and proteins depending on available conjugation strategies. Moreover, the method has the potential for further refinement in experimental and analysis aspects to improve the accuracy and extend the applicability to more complex samples.

[1] Javier Antonio Alfaro et al., *Nat. Methods*, **2021**, 18, 604–617.
[2] Salome Püntener, Pablo Rivera-Fuentes, *J. Am. Chem. Soc*., **2023**, 145, 1441–1447.
[3] Shin-nosuke Uno et al., *Nat. Chem*., **2014**, 6, 681–689.

Synthesis of Chiral Tricyclic Piperazine Scaffolds from the GDB Database

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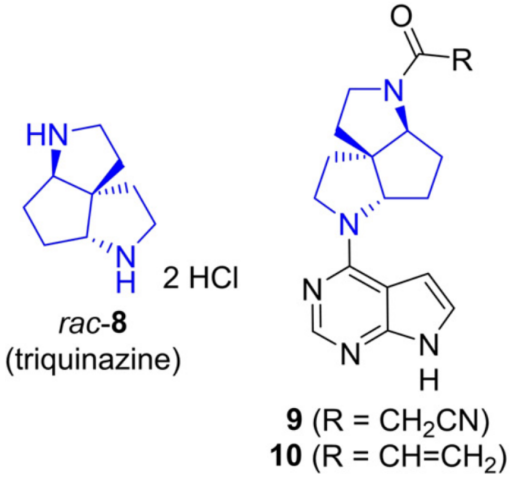
The generated databases (GDBs) are a collection of possible molecules up to a certain size which are filtered by rules of synthetic feasibility and chemical stability. Interestingly, a large number of these molecules are novel, intrinsically chiral, 3D-shaped and have never been synthesized. (1) As such the GDBs are a valuable source of new scaffolds for medicinal chemistry, which is why we are interested in exploring these databases synthetically. In the past this has already yielded interesting scaffolds such as triquinazine which has been used to discover a nanomolar and selective inhibitor of Janus Kinase 1 (see Figure 1). (2)



Building on this success, in our ongoing research we have further explored the GDBs to identify additional interesting cases of novel chiral tricyclic scaffolds.
These scaffolds are synthesized to be used in a medicinal chemistry context.

[1] K. Meier, S. B¨uhlmann, J. Ar´us-Pous, J.-L. Reymond, CHIMIA 74, 241–241, issn: 2673-2424 (Apr. 2020).
[2] K. Meier, J. Ar´us-Pous, J.-L. Reymond, Angewandte Chemie International Edition 60, 2074–2077, issn: 1521-3773 (2021).

Peptide-stabilized gold nanoparticles

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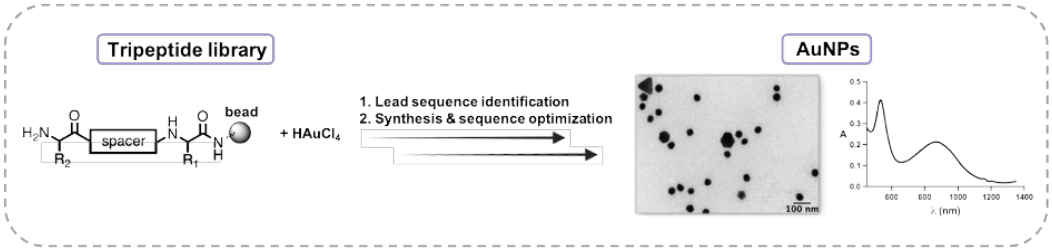
Gold nanoparticles (AuNPs) have gained significant attention for therapy, imaging, and biosensing due to their unique properties that can be tailored by varying their size, shape, and coating. In particular, the use of AuNPs in photothermal therapy has emerged as a promising tool for cancer treatment. However, the synthesis of monodisperse near-infrared light-absorbing anisotropic AuNPs with ligands that offer modularity and ease of modification is challenging. Commonly used methodologies rely on complex multi-step procedures involving numerous additives, such as reducing agents and surfactants.[1] Peptides offer multiple advantages for the formation and stabilization of AuNPs compared to traditional ligands. We envisioned that a peptide should be able to a) reduce gold precursors, b) facilitate the formation of anisotropic structures, and c) stabilize the emerging AuNPs. Furthermore, the modularity of peptides combined with their ease of modification should enable the development of functionalized nanoparticles tailored to specific applications. Building on previous work on peptide-stabilized AgNPs[2] and PdNPs,[3] we used a split-and-mix tripeptide library to identify peptides promoting gold nanoparticle formation without the need for external reducing agents. The screening identified lead peptide sequences and allowed, through the further optimization of the peptide structure and the nanoparticle synthesis conditions, the production of stable anisotropic AuNPs with absorption in the near-infrared region. We envision that these peptide-coated AuNPs will be valuable for use in photothermal therapy.



[1] Lu Wang, Morteza Hasanzadeh Kafshgari, Michel Meunier, *Adv. Funct. Mater*., **2020**, 30, 2005400.
[2] Kirsten Belser, Tünde Vig Slenters, Conelious Pfumbidzai, Grégory Upert, Laurent Mirolo, Katharina M. Fromm, Helma Wennemers, *Angew. Chem. Int. Ed.,* **2016**, 55, 8542-8545.
[3] Stefano Corra, Urszula Lewandowska, Edmondo M. Benetti, Helma Wennemers, *Angew. Chem. Int. Ed.*, **2017**, 40, 138-144.

Beyond static structures: The dynamic impact of Intronistat B on fungal ribozymes

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Human pathogenic fungi are a leading cause of mortality and current antifungals are limited by their toxicity to human cells[1]. The self-splicing activity of introns in housekeeping genes is essential for the survival of many pathogenic yeasts[2]. Intronistat B, a small molecule inhibitor, targets the conserved active site of group II introns, hindering their splicing in a sequence-unspecific manner[3]. Given that group II introns are absent in humans but crucial for pathogens, they represent a promising target for the antifungal development with reduced human cell toxicity. While crystal structures of ribozymes provide static snapshots of inhibition, they fall short on capturing dynamic processes such as structural rearrangements during catalysis[4]. To address this, we investigate the impact of Intronistat B on the folding and splicing of the group II intron *Sc*.ai5γ.

To track individual ribozymes in real time, we use single-molecule Förster resonance energy transfer (smFRET) microscopy. Interestingly, the results revealed conformational stabilizations that potentially halt the ribozyme folding process critical for splicing. These findings shed light on the dynamic aspects of the inhibition mechanisms of Intronistat B, aiding the rational design of novel antifungal agents. Additionally, the results validate smFRET as powerful tools for probing RNA-inhibitor interactions, advancing the field of RNA biophysics and drug development.

[1] J. Branco, I. M. Miranda, A. G. Rodrigues, Journal of Fungi 2023, 9, 80.

[2] J. Nosek, M. Novotna, Z. Hlavatovicova, D. W. Ussery, J. Fajkus, L. Tomaska, Molecular Genetics and Genomics 2004, 272, 173.

[3] O. Fedorova, G. E. Jagdmann, R. L. Adams, L. Yuan, M. C. Van Zandt, A. M. Pyle, Nat Chem Biol 2018, 14, 1073.

[4] I. Silvestri, J. Manigrasso, A. Andreani, N. Brindani, M. De Vivo, M. Marcia, bioRxiv 2023.

Amphipathic proline-rich cell penetrating peptides for mitochondria targeting

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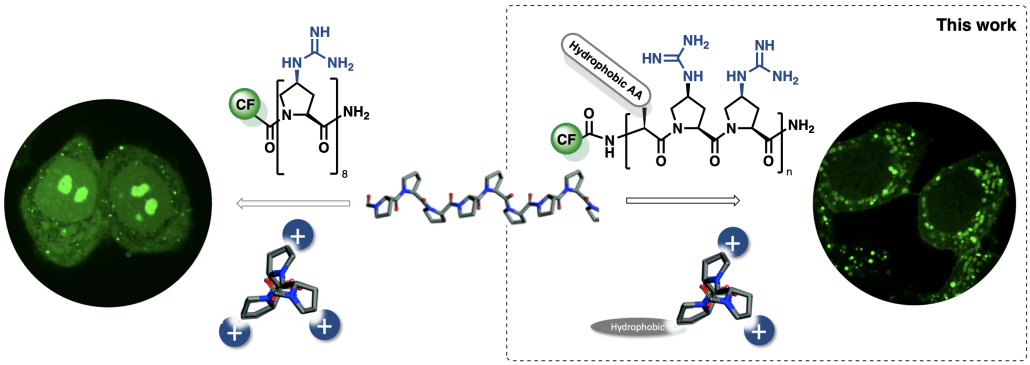
Cell penetrating peptides (CPPs) cross the lipophilic barrier of the cellular membrane and serve as delivery vectors to translocate cargo into cells.[1] They can also be useful for target-specific delivery, for example, of bioactive molecules to a specific cellular organelle. Here, targeting mitochondria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

Our group developed a cytoplasm and nucleus penetrating oligoproline (Z_8) CPP that exhibits higher cellular uptake than more flexible peptides (e.g., octaarginine).[3] Here, we showcase that oligoproline peptides with hydrophobic amino acids installed at every third position allow mitochondria targeting. Selectivity is achieved by the PPII helical conformation with two cationic faces and one hydrophobic face enabling the crossing of the mitochondria membranes. The localization of the amphipathic peptides inside cells was evaluated by confocal microscopy and the cellular uptake efficiency by fluorescence-activated cell sorting (FACS).



[1] U. Langel, Cell-Penetrating Peptides*, Methods and Protocols,* **2011,** Humana, Totowa, NJ

[2] Schenkel, L. C.; Bakovic, M.; *Int. J. Cell Biol.* **2014**, 1–13.

[3] Nagel, Y. A.; Raschle, P. S.; Wennemers, H.; *Angew. Chemie Int. Ed.* **2017**, *56*, 1, 122–126

Development of lysine-targeting DNA Probes to disrupt XPG-mediated Nucleotide Excision Repair for cancer therapy.

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Cellular repair mechanisms are crucial for cell maintenance and survival. Dysfunction in these pathways can lead to cell lethality, making repair inhibitors valuable in cancer treatment. [1, 2] For example, PARP inhibitors targeting homologous recombination repair are approved and used in clinic for cancer treatment. [2] We hypothesize that nucleotide excision repair (NER) inhibitors may serve as another valuable approach to target DNA repair for cancer therapy.[3] During NER, the TFIIH protein complex unwinds DNA to verify the lesion, followed by incisions made by endonucleases XPF-ERCC1 and XPG, facilitating lesion removal and gap filling by a DNA ligase. [4] Despite significant progress in understanding NER, gaps remain, particularly regarding the interaction between DNA damage and repair proteins, and strategies for repair inhibition. We aimed to design a DNA probe to elucidate the interaction mechanism between DNA damage and the XPG endonuclease, hypothesizing that inhibiting XPG could impair NER, offering a potential cancer therapy target. Our study focused on the XPG endonuclease, which is proximal to the damage site and contains a critical lysine residue in its active site. [5] As a proof of concept, we designed a library of DNA probes with covalent warheads targeting lysine residues. The chosen warheads—acrylamide, sulfonyl fluoride, and sulfonamides—were attached to the DNA probe via a CuAAC reaction, "clicking" the warheads onto the DNA sequence with a terminal alkyne. We evaluated the reactivity of these probes with isolated lysine and conducted initial in vitro NER assays to assess repair inhibition.

(1)Chatterjee, N.; Walker, G. C. Mechanisms of DNA damage, repair, and mutagenesis. *Environ Mol Mutagen* **2017**, *58* (5), 235-263. DOI: 10.1002/em.22087 From NLM.

(2)Lord, C. J.; Ashworth, A. PARP inhibitors: Synthetic lethality in the clinic. *Science* **2017**, *355* (6330), 1152-1158. DOI: 10.1126/science.aam7344 From NLM.

(3) D'Souza, A.; Blee, A. M.; Chazin, W. J. Mechanism of action of nucleotide excision repair machinery. *Biochemical Society Transactions* **2022**, *50* (1), 375-386. DOI: 10.1042/bst20210246 (acccessed 5/29/2024).

(4) Schärer, O. D. Nucleotide excision repair in eukaryotes. *Cold Spring Harb Perspect Biol* **2013**, *5* (10), a012609. DOI: 10.1101/cshperspect.a012609 From NLM.

(5) Tsutakawa, S. E.; Sarker, A. H.; Ng, C.; Arvai, A. S.; Shin, D. S.; Shih, B.; Jiang, S.; Thwin, A. C.; Tsai, M. S.; Willcox, A.; et al. Human XPG nuclease structure, assembly, and activities with insights for neurodegeneration and cancer from pathogenic mutations. *Proc Natl Acad Sci U S A* **2020**, *117* (25), 14127-14138. DOI: 10.1073/pnas.1921311117 From NLM.

Assessing gut microbiota's role in phytoestrogen bioactivation

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Isoxanthohumol (iXN), a prenylated polyphenol found naturally in hops and in dietary supplements for the relief of postmenopausal symptoms, is biotransformed via *O*-demethylation by the gut microbiome to 8-prenylnaringenin (8-PN), the most potent phytoestrogen known to strongly interact with estrogen receptors and potentially disrupt endocrine signaling. The potential adverse effects of 8-PN are poorly understood due to the difficulty in determining systemic concentrations of these gut microbial metabolites. To address this, we developed a human-specific physiologically based kinetic (PBK) model designed to predict the biologically available levels of hop-derived polyphenols and their microbial metabolites in human blood and target tissues based on realistic exposure scenarios. We integrated metabolic rates obtained from glucuronidation assays using hepatic and intestinal S9 fractions and the kinetic rate of microbial metabolism in anaerobic fecal fermentation. The conversion of iXN to 8-PN was quantified using liquid chromatography-mass spectrometry and these values were used to derive iXN metabolism. These data were used to establish a new kinetic model incorporating the gut microbiome as a distinct metabolic compartment, allowing the assessment of inter-individual variability in 8-PN formation depending on microbiome composition. We then performed in vitro cellular assays to evaluate estrogenic activity and further assess the safety of hop polyphenols. The outcomes advance our understanding of the toxicokinetics of phytoestrogens and provide a robust framework for the quantitative assessment of gut microbial metabolites in chemical safety assessment.

Potent Inducers of Paraptosis Through Electronic Tuning of Michael Acceptors

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Paraptosis is a non-apoptotic programmed cell death (PCD), that does not present the typical morphology observed in apoptosis. [1,2] This PCD is characterized by cytoplasmic vacuolation caused by endoplasmic reticulum (ER) and mitochondria swelling, it can be accompanied by protein and Ca^{2+} homeostasis disruption and activation of the unfolded protein response of the endoplasmic reticulum (UPR^{ER}). [1,2] Moreover, paraptotic cells do not exhibit DNA fragmentation or caspase activation. [1] The mechanisms underlying paraptosis are not well understood, but there are a few reports that showed some paraptosis-inducing targets like the insulin-like growth factor I receptor (IGFIR), [1,2] GDP-dissociation inhibitor beta (GDI2), [3] the USP10 (a member of the ubiquitin-specific protease family of cysteine proteases), [4] among others. Since some cancer cells have developed resistance to anti-cancer therapies due to defective apoptosis, non-apoptotic programmed cell death mechanisms, such as paraptosis, have gained importance in cancer therapy.

In this study, we developed a set of Michael acceptors that induced paraptosis in three types of mammalian cell lines (HEK293, HeLa, and MDA-MB-231). This process was indicated by cytoplasmic vacuolation resulting from ER dilation and did not involve caspase activation. We identified a highly potent compound with an IC_{50} of 1.3 ± 0.3 μM. This compound caused ER and mitochondria swelling, superoxide production, and high reactivity towards cysteines. Additionally, initial results from a proteomic analysis revealed six specific proteins distinct from the previously reported paraptosis-inducing targets. These proteins could potentially serve as new targets for paraptosis activation, but further validation is needed.

[1] S. Sperandio, I. de Belle, D.E. Bredesen, Proceedings of the National Academy of Sciences, **2000**, 97, 14376 –14381.
[2] S. Hanson, A. Dharan, J. P V, S. Pal, B. G. Nair, R. Kar, N. Mishra, Frontiers Pharmacology, -, 14, 1159409
[3] Y. Sun, H. Zheng, L. Qian, Y. Liu, D. Zhu, Z. Xu, W. Chang, J. Xu, L. Wang, B. Sun, L. Gu, H. Yuan, and H. Lou, JACS Au, -, 3, 2749-2762
[4] J. Y. Kim, D. M. Lee, H. G. Woo, K. D. Kim, H. J. Lee, Y.-J. Kwon, K. S. Choi, Scientific Reports, -, 9, 4909.

Non-invasive in vivo Determination of Potassium in Human Muscle by ^{39}K MR Spectroscopy on a 7T MR Scanner. A Feasibility Exercise Study.

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Introduction: Potassium is classified by the FDA as "a nutrient of public health concern" because of under consumption which can lead to hypertension, kidney diseases and neuromuscular dysfunction.[1,2] Existing methods to determine potassium levels are invasive since urine and serum level correlate poorly with tissue concentrations. Recently, high field 7T MRI and MR spectroscopic imaging (MRSI) has been reported to be feasible for quantification of muscle tissue ^{39}K levels.[3] This opens new possibilities for investigating quadrupolar nuclei like potassium with low sensitivities by MR methods, which was not possible before.

Aim: The goal of this project is to establish non-invasive and spatially resolved MR-methods for determination of tissue potassium concentration. In a first feasibility study the sensitivity to detect potassium changes is evaluated in human muscles during exercise in the MR Scanner in real-time.

Methods: A double tuned (^{1}H, ^{39}K) surface coil was used for all experiments. The coil was fixed to the thigh and the upper leg immobilized while the lower leg performed exercise against a stretch-band. Initial exercise measurements were performed using a simple non-localized FID sequence and only the sensitive volume of the coil as localization. Subsequently a real-time experiment during exercise was performed employing a spatially resolved MRSI sequence in addition to localization by the sensitive volume of the coil.

Results and Discussion: The initial experiments using a non-localized FID sequence demonstrated consistently a decrease in tissue potassium levels of about 5% during exercise which recovered quickly during rest. However, potential changes in fiber structure, muscle shape, and muscle movement during exercise may impact the tissue amount in the sensitive volume of the coil and thus the signal yield. Therefore, in order to exclude a potentially artificial finding, a localized sequence was employed in an additional measurement during exercise which allows ^{39}K determination of defined volumes located solely within muscle tissue, thus minimizing the effect of potential muscle shape and position changes.

Conclusion and Outlook: The feasibility study demonstrates that potassium changes can be determined non-invasively and spatially resolved in human muscles. Further experiments on human muscle are needed to validate previous findings by improving the experimental setup and improving spatially resolved measurements. Following the skeletal muscle experiments, potassium levels will be investigated for the first time in human liver which is clinically highly relevant.

[1] 2015-2020 Dietary Guidelines for Americans.

[2] Viera A. J., Wouk N., *Am. Fam. Physician* **2015**, *92* (6), 487–495.

[3] Gast L. V., Völker S., Utzschneider M., Linz, P., Wilferth T., Müller M., Kopp C., Hensel B., Uder M., Nagel A. M., *Magn. Reson. Med.* **2021**, *85* (1), 239–253.

Probing the Penetration of Encapsulated Photosensitizers into Skin Models

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Porphyrinic photosensitizers (PSs) are used in the selective treatment of oncologic and non-oncologic diseases including skin cancer. However, most porphyrinic PSs have hydrophobic properties and exhibit low dermal absorption [1]. Bicelles, made by combining long chain phospholipids (PLs) forming a bilayer and short chain PLs forming the rim, provide with their small size and hydrophobic interior a viable option for topical delivery of hydrophobic molecules [2]. The PSs 5,10,15,20-tetrakis(*m*-hydroxyphenyl)-porphyrin and -chlorin (mTHPP and mTHPC) are NMR-invisible in water due to aggregation (*Figure 1A*). NMR resonance appearance indicates their successful encapsulation into bicelles. Therefore, quantitative NMR allowed us to determine the encapsulation efficiency and loading capacity: 100 mM dihexanoyl- and dimyristoyl-*sn*-phosphatidylcholine (DHCP/DMPC) bicelles could successfully encapsulate concentrations up to 5 mM and 2.5 mM for mTHPP and mTHPC, respectively, with a stability over a period of at least 7 days. To test the penetration of PS into the skin, Franz diffusion experiments were performed using porcine skin, onto which the encapsulated mTHPP was applied. Subsequent extraction showed uptake of mTHPP into the epidermis of around 100 ng/cm2. Fluorescence microscopy of embedded skin showed PS penetration into the stratum corneum. To further increase the absorption quantity and depth of PSs into the skin, experiments with bicelles in combination with penetration enhancers are planned. As standardizable alternatives to porcine skin, two artificial human skin models are currently being developed. The collagen model uses a collagen scaffold containing fibroblasts (Fb), onto which keratinocytes (Kc) are seeded. The vascularized skin model is based on a fibroblast derived 3D extracellular matrix (ECM) scaffold, which is built up by first seeding Fb, then endothelial cells (Ec) for vascularization and finally seeding Kc on top (*Figure 1B*). In both models, the crosstalk between Fb and Kc is necessary for proper keratinocyte maturation [3].

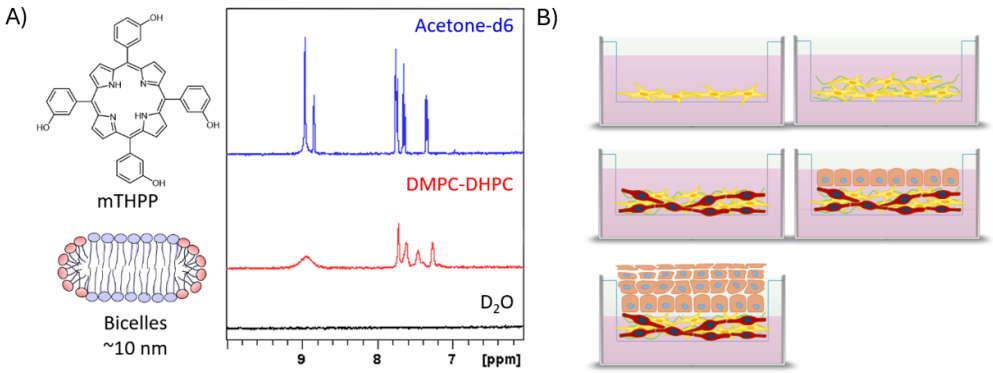

Figure 1. A: Left: mTHPP structure and scheme of a bicelle with the short PLs in red and the long PLs in blue. Right:¹HNMR spectra of mTHPP in acetone-d6 (blue), encapsulated in bicelles (red) and in D_2O (black). B: Step-by-step preparation of the vascularized artificial skin. The yellow cells represent the Fibroblasts (Fb), the red ones represent the endothelial cells (Ec) and the orange ones the Keratinocytes (Kc). As soon as the Kc are added, the medium (pink) is reduced as visible on the changed medium levels.

[1] N. Dragicevic-Curic, S. Gräfe, V. Albrecht, and A. Fahr *Journal of Photochemistry and Photobiology B: Biology*, 2008, 91, 41–50.
[2] E. J. Dufourc, *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 2021, 1863, 183478.
[3] M. Jevti? *et al.*, *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 2020, 1867, 118722.

Photosensitizers in Targeted Photodynamic Therapy of Colorectal Cancer

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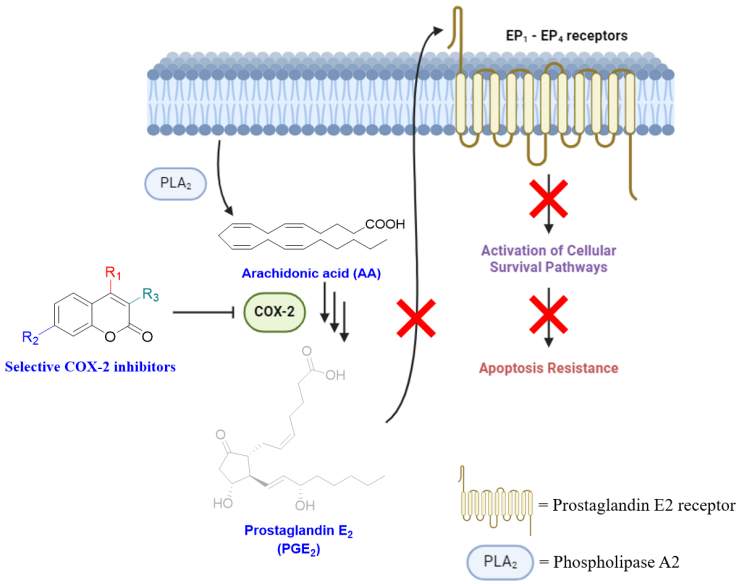
Colorectal cancer (CRC) ranks third in global cancer incidence and second in mortality rates. While conventional treatments like surgery, radiation and chemotherapy aim to eliminate tumors, they often come with side effects such as nausea, fatigue and organ damage. Photodynamic therapy emerges as a promising alternative, utilizing a photosensitizing agent activated by a specific wavelength to maximize its local efficacy. Its targeted and minimally invasive nature hold significant potential for CRC treatments.



Cyclooxygenase-2 (COX-2), an enzyme driving prostaglandin synthesis and inflammation, plays a pivotal role in CRC progression, making it an attractive therapeutic target.[1,2] Our research focuses on developing novel photosensitizers that absorb light in the red and selective to COX-2. Docking experiments have demonstrated the high affinity of coumarin derivatives for the COX-2 active site, and have identified three positions (3, 4 and 7) that can be exploited for optimization. Therefore, we have synthesized and characterized a series of functionalized coumarin-based inhibitors, and determined *in silico* their binding affinity to COX-2. In the future, *in vitro* assays will be conducted to evaluate their effectiveness on cancer cells, as well as isothermal titration calorimetry to confirm their binding affinity for COX-2.

[1] Lan W, Yuhuan Z, Ying H, Qi Z, Zhenfu W, Hongjuan L, Shiguo S, Xin C, Yongqian X, *J. Mater. Chem. B*, **2021**, *9*, 2001-2009.

[2] Negi RR, Rana SV, Gupta V, Gupta R, Chadha VD, Prasad KK, Dhawan DK, *Asian Pac. J. Cancer Prev.*, **2019**, *20*, 1675-1681.

A Drug-like Library of Structurally Diverse Spirocyclic Saturated N-Heterocycles

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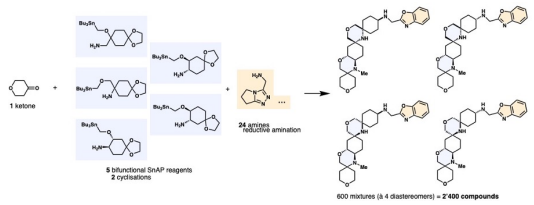
Saturated N-heterocycles are of great interest in drug discovery. Herein, we present the design, preparation, and ADMET property profiling of a shape-defined library of diverse, substituted dimorpholines. The distinct library components contain multiple stereocenters, spirocyclic and fused rings, resulting in rigid shapes with a generally sp3 rich character. We have previously demonstrated the construction of a limited number of trimorpholines though iterative synthesis where the choice of the bifunctional reagents allows access to distinct three-dimensional shapes. This work utilizes this methodology to synthesize a library of dimorpholines based on a cyclic ketone, 5 bifunctional SnAP reagents which are combined in two iterations to dimorpholines and further diversified in a reductive amination step with 24 amines. This combination of building blocks results in 600 potential mixtures comprising of 4 diastereomers, therefore, a total of 2'400 compounds.



Experimental ADMET property profiling of a selection of the compound library showed a high solubility, a distribution from low to high metabolic stability in microsomes, mostly low CYP inhibition, and low to high permeability. These findings confirm the potential of this compound class for biological applications.

F. Saito, N. Trapp and J. W. Bode, *J. Am. Chem. Soc*. **2019**, 141, 5544–5554