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

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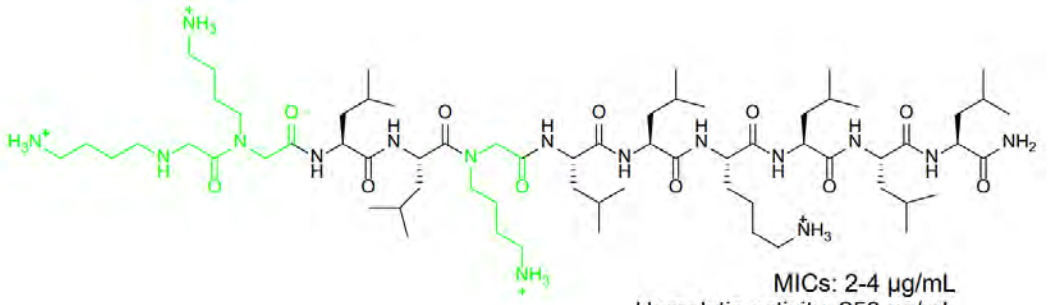
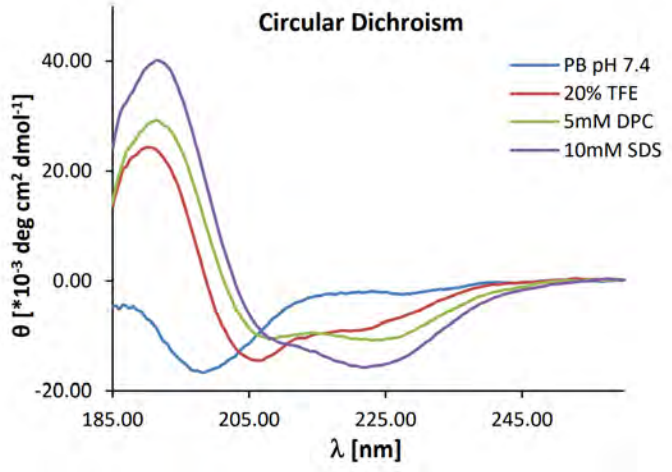
Membrane disruptive antimicrobial peptides (AMPs) such as polymyxin B offer an opportunity to control multidrug resistant (MDR) Gram-negative bacteria,[1] which are a leading cause of death in hospitals.[2] Recently we discovered that inverting the chirality of lysine amino acids in an 11-residues α-helical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





[1] N. Mookherjee, M. A. Anderson, H. P. Haagsman and D. J. Davidson, *Nat Rev Drug Discov*, **2020**, *19*, 311–332.
[2] C. J. Murray *et al.*, *The Lancet*, **2022**, *399*, 629–655.
[3] S. Baeriswyl, H. Personne, I. D. Bonaventura, T. Köhler, C. van Delden, A. Stocker, S. Javor and J.-L. Reymond, *RSC Chemical Biology*, **2021**, *2*, 1608–1617.
[4] T. Godballe, L. L. Nilsson, P. D. Petersen and H. Jenssen, *Chemical Biology & Drug Design*, **2011**, *77*, 107–116.

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

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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. Merrifield's SPPS method has remained the most popular synthesis method for chemical protein synthesis since its introduction in the 60s.[1] Since then, decades of improvement and optimization have increased the length of synthesized peptide chains of up to 50 amino acids.[2] Over this limit, Native Chemical Ligation (NCL) has been developed to join synthesized fragments, ultimately leading to the production of larger proteins.[3] Yet, generating fragments by SPPS in good yield and purity requires extensive synthesis efforts. A particular problem during the synthesis itself is aggregation, 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. H-bond formation between the amide backbone is thought to be a major contributor of aggregation, giving rise to β-sheet formation.[4] Backbone modifications can be introduced to reduce aggregation, but screening for suitable positions is time- and resource-intensive. A deeper understanding of aggregation, as well as a general solution to improve aggregation are therefore urgently needed. We use flow-based peptide synthesis to investigate the sequence dependence of aggregation using in line UV monitoring. Upon aggregation, the Fmoc groups are less accessible for deprotection resulting in a peak broadening effect as the deprotection efficiency decreases. Analysis of numerous aggregating sequences has allowed for the development of a "synthesis tag" (analogous to often used "solubility tags"). The tag was applied to multiple aggregation peptides, and was shown to increase not only the synthesis but also the solubility of the heavily aggregating peptide fragment c-Myc[86-143] belonging to the transactivation domain (TAD) of the intrinsically disordered transcription regulator c-Myc.[5] In contrast with the DNA binding domain, which has been the subject of extensive research and chemical synthesis efforts, the TAD and its regulation via PTMs is much less understood.[6] In the future, we aim to demonstrate the applicability of the tag to more peptides and will further investigate how it disrupts aggregation during synthesis, to ultimately increase our understanding on aggregation.

[1] Merrifield, R. B., *J. Am. Chem. Soc.* **1963**, 85 (14), 2149–2154.
[2] Hartrampf, N.; Saebi, A.; Poskus, M.; Gates, Z. P.; Callahan, A. J.; Cowfer, A. E.; Hanna, S.; Antilla, S.; Schissel, C. K.; Quartararo, A. J.; Ye, X.; Mijalis, A. J.; Simon, M. D.; Loas, A.; Liu, S.; Jessen, C.; Nielsen, T. E.; Pentelute, B. L. *Science* **2020**, 368 (6494), 980–987.
[3] Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. Science 1994, 266 (5186), 776–779.
[4] Huang, Y.-C.; Guan, C.-J.; Tan, X.-L.; Chen, C.-C.; Guo, Q.-X.; Li, Y.-M., *Org. Biomol. Chem.* **2015**, 13 (5), 1500–1506.
[5] Dang, C. V., *Cell* **2012**, 149 (1), 22–35.
[6] Calo-Lapido, R.; Penas, C.; Jiménez-Balsa, A.; Vázquez, M. E.; Mascareñas, J. L., *Org. Biomol. Chem.* **2019**, 17 (28), 6748–6752.

Chemical space guided design of antimicrobial peptide dendrimers

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Space Peptides is an AI drug discovery company based on 20 years of computational chemistry research from the group of Prof. Jean-Louis Reymond (University of Bern), which reported the AI-generated largest peptide database till date, mapped with molecular fingerprints to decode the chemical space.[1,2] Furthermore, a portfolio of AI-augmented technologies and state-of-the-art algorithms, empower the evaluation of billions of molecules to design molecules with desired target profiles faster and cheaper. Its workflow consists in a generative model supplemented with both machine learning and structure-based classifiers to predict a compound activity, selectivity and other properties relying on a modular approach with each module being optimized for its specific task and easily repurposable.[3] After several iterative rounds, the selected molecules are then synthesized on-demand at Space Peptides' in-house facilities and experimentally validated.

In particular, this workflow was applied to the discovery of novel antimicrobial resistance peptide dendrimers (AMPD) that showed a broad-spectrum activity against both Gram-negative and Gram-positive bacteria, while showing no sign of hemolysis against red blood cells. Interestingly, the AMPD developed showed high activity against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* including MDR clinical isolates, two critically relevant bacterial strains according to WHO list of target pathogens, and was further validated in vivo in two different infection murine model.[4,5]

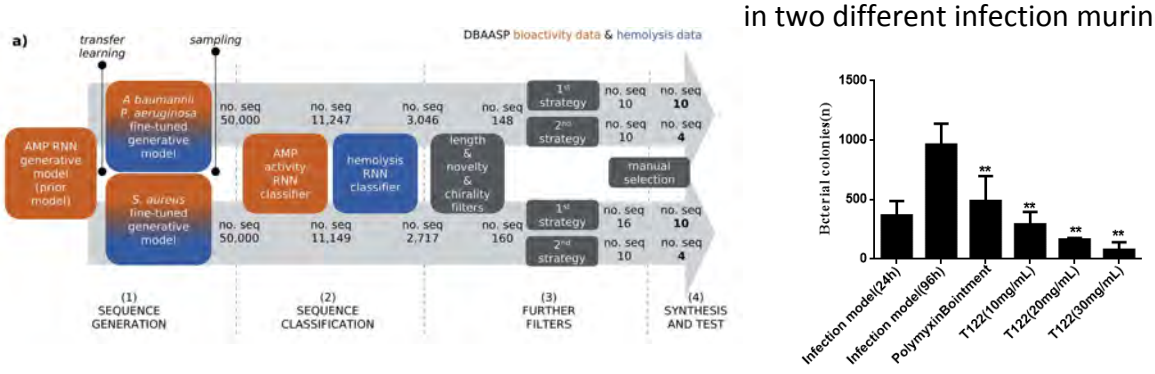

Fig. 1. a. Example of workflow used to design the AMPD. **b.** Selectivity and effectiveness of our AMPD in a murine model.

(1) Capecchi, A.; Probst, D.; Reymond, J. L. One Molecular Fingerprint to Rule Them All: Drugs, Biomolecules, and the Metabolome. *J. Cheminform.* **2020**, *12* (1), 1–15.
(2) Capecchi, A.; Reymond, J. L. Peptides in Chemical Space. *Med. Drug Discov.* **2021**, *9*, 100081.
(3) Capecchi, A.; Cai, X.; Personne, H.; Köhler, T.; van Delden, C.; Reymond, J. L. Machine Learning Designs Non-Hemolytic Antimicrobial Peptides. *Chem. Sci.* **2021**, *12* (26), 9221–9232.
(4) Siriwardena, T. N.; Stach, M.; He, R.; Gan, B. H.; Javor, S.; Heitz, M.; Ma, L.; Cai, X.; Chen, P.; Wei, D.; et al. Lipidated Peptide Dendrimers Killing Multidrug-Resistant Bacteria. *J. Am. Chem. Soc.* **2018**, *140* (1), 423–432.
(5) Siriwardena, T. N.; Capecchi, A.; Gan, B. H.; Jin, X.; He, R.; Wei, D.; Ma, L.; Köhler, T.; van Delden, C.; Javor, S.; et al. Optimizing Antimicrobial Peptide Dendrimers in Chemical Space. *Angew. Chemie - Int. Ed.* **2018**, *57* (28), 8483–8487.

Closing the loop, toward a fully automated pipeline for designing, making and testing bioactive molecules

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The ability to probe and alter interactions between macromolecules and small molecule entities with high specificity forms one of the foundations of modern life science. The design of small molecules binding to specific targets proceeds through iterative cycles of hypothesis formulation, molecule generation, biological testing, data analysis, leading to an updated structure-activity relationship hypothesis to initiate a new cycle. Traditionally, this process involves scientists and facilities from several disciplines with manual “hand-over” of the results from each step.

Recent developments in chemistry, biology, AI and robotics allow for the first time to build such an automated closed-loop design-make-test (DMT) environment to design bioactive molecules for life-science and clinical applications. Guided by modern AI algorithms, the platform will combine fully automated modules for molecular synthesis, purification, and activity testing, to enable the iterative variation and optimization of a bioactive molecule.

As a first step toward fully automated small molecules design, we will start with established automated solid phase chemistry for peptidic compounds, which provides a molecule class of broad biomedical relevance and structural variability. This involves, at the beginning, benchmarking existing methods and checking their applicability, in terms of computational time and prediction accuracy, in the context of a closed-loop environment with no human intervention. For the design and optimisation of peptidic sequences a combination of an active learning method, Bayesian optimisation, with structured-based methods implemented in the Rosetta software was explored. Preliminary results of the Major HistoCompatibility (MHC) complex class I show encouraging results. Further investigations will be pushed in that direction, including benchmarks and development for de novo peptide prediction, cyclisation and integration of nonstandard amino acid residues.

Identification of an autophagy modulating HBA1-derived peptide from human peptide libraries

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Background & Objective: The innate immune system is a powerful barrier against invading pathogens like viruses. Autophagy is an evolutionary ancient catabolic pathway and an integral part of innate immunity. It can directly target and eliminate intracellular viruses or viral components. However, successful pathogens, such as SARS-CoV-2 or Influenza viruses, have evolved strategies to not only circumvent but exploit the autophagic machinery to promote their replication. Thus, repressing autophagy is a potential anti-viral approach. However, currently available agents lack specificity and are accompanied by severe side effects in patients. Therefore, there is an urgent need for specific autophagy modulating compounds. Here, we screened human-derived peptide libraries to identify novel autophagy modulators.

Methods: Using a high-throughput FACS based approach, peptide libraries derived from human bone marrow and platelet lysates were analysed for their impact on the autophagic flux. After initial identification of bioactive fractions, these were iteratively sub-purified to eventually identify the containing bioactive peptide(s) by mass spectrometry. Identified peptides were then chemically synthesized and tested for their autophagy modulating activity.

Results: In two independent human peptide libraries derived from bone marrow and platelet lysate, a 22-amino acid long fragment of human haemoglobin A (aa 111-132) was identified that down-regulates autophagy in a dose-dependent manner with an EC50 in the mid micromolar range. The peptide could be generated by acidic peptidases from full length human haemoglobin. Analyzing various truncations revealed the C-terminus to be important for the autophagy reducing ability, whereas N-terminal modifications were well tolerated. Thus, a 12-mer was identified as the smallest active peptide.

Conclusion /Future Outlook: Our results identified a 12-mer as a promising autophagy inhibiting peptide. Current experiments will reveal its anti-viral potential and the physiological mechanism. In addition, SAR studies will be conducted to optimize the candidate peptide for future therapeutic approaches.

Investigating the feasibility of EPI-X4 analogs for targeting CXCR4-expressing tumors *in vivo*

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Aim/Introduction:

The overexpression of the C-X-C motif chemokine receptor (CXCR4) in more than 23 types of human cancer, followed by its contribution in tumor growth, angiogenesis, and metastasis designates it as an interesting target for theranostic interventions in oncology. The identification of an endogenous peptide antagonist of CXCR4, termed EPI-X4 [1], opens the space for the development of radiotracers for non-invasive molecular imaging and treatment of CXCR4 expressing cancers using the EPI-X4 as platform. We developed DOTA-conjugated EPI-X4 analogs (coded JMF-01 to JMF-07) and studied their CXCR4 targeting ability *in vitro* and *in vivo* labeled with ^{177}Lu. The ^{177}Lu-labeled conjugates were evaluated for their cellular uptake in GHOST-CXCR4+ cells, followed by SPECT/CT imaging in Jurkat xenografts at 1h post injection (p.i.). Among all conjugates, the ^{177}Lu-JMF-02 and -04 were identified as the most promising, based on their significantly higher tumor uptake, in comparison to others. SPECT/CT imaging showed rapid wash out from the body (only 5% of the injected activity remaining after 1h) for most of the analogs, with the exception of [^{177}Lu]Lu-JMF-04 and [^{177}Lu]Lu-JMF-07 (approx. 40-60% remaining in the body after 1h). [^{177}Lu]Lu-JMF-04 displayed high accumulation in the kidneys and distinguished uptake in the CXCR4-expressing tumors, while [^{177}Lu]Lu-JMF-07 was primarily concentrated in the liver. Our data validate the feasibility of developing EPI-X4 analog as radiotracers for CXCR4 *in vivo* targeting. SPECT/CT imaging revealed the strengths and limitations of EPI-X4-based platform and designated the lead analog, [^{177}Lu]Lu-JMF-04, for further optimization.

References:

1. Zirafi O et al., Cell Reports *11*, 737–747, 2015.

Elucidation of Binding Interactions and Mechanism of Rivastigmine Tartrate with dsDNA Via Multi-Spectroscopic, Electrochemical, and Molecular Docking Studies

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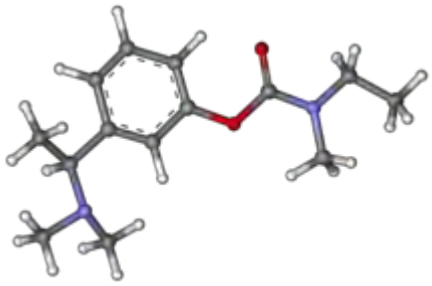
ABSTRACT

Rivastigmine tartrate (Scheme) is a noncompetitive carbamate acetylcholinesterase inhibitor that is carbamylated, long-acting, and reversible[1].



Scheme. The chemical structure of rivastigmine tartrate

It's used to alleviate Alzheimer's disease symptoms that range from mild to moderate[2]. Although various cholinesterase inhibitors have been researched for their DNA binding mode, the rivastigmine tartrate -dsDNA binding mechanism has not been investigated yet. As a result, both experimentally and theoretically, the binding mechanism and reversible interactions of rivastigmine tartrate with double-stranded fish sperm deoxyribonucleic acid (dsDNA) have been thoroughly investigated. Our overall purpose is to have a better understanding of the potential of cholinesterase inhibitor medications in order to find novel medications that target dsDNA and develop drugs with fewer adverse effects. We applied a variety of spectroscopic methods under physiological situations, including UV-Vis and fluorescence, spectroscopy, thermal denaturation, electrochemical and viscosity measurements, as well as molecular docking investigations. In addition, the interactions between rivastigmine tartrate and dsDNA were investigated computationally with the assistance of molecular docking and molecular dynamics simulations. Data from both experimental and theoretical results showed that Rivastigmine tartrate interacts with dsDNA through the groove binding mode[3].

References

1- P.N. Tariot. Alzheimer-Disease - An Overview, Alzheimer Disease & Associated Disorders, 8 (1994) S4-S11.
2- Alzheimer A. Über eine eigenartige Erkrankung der Hirnrinde. Allgemeine Zeitschrift fur Psychiatrie und Psychisch-gerichtliche, Medizin, 64 (1907) 146-148.
3- P. Şenel, S. Agar, Y.S. İş, F. Altay, A. Gölcü, M. Yurtsever, Deciphering the mechanism and binding interactions of Pemetrexed with dsDNA with DNA-targeted chemotherapeutics via spectroscopic, analytical, and simulation studies, Journal of Pharmaceutical and Biomedical Analysis, 209 (2022)114490.

Bifunctional Hypervalent Iodine Reagents for Cys-Cys and Cys-Lys Peptide Stapling

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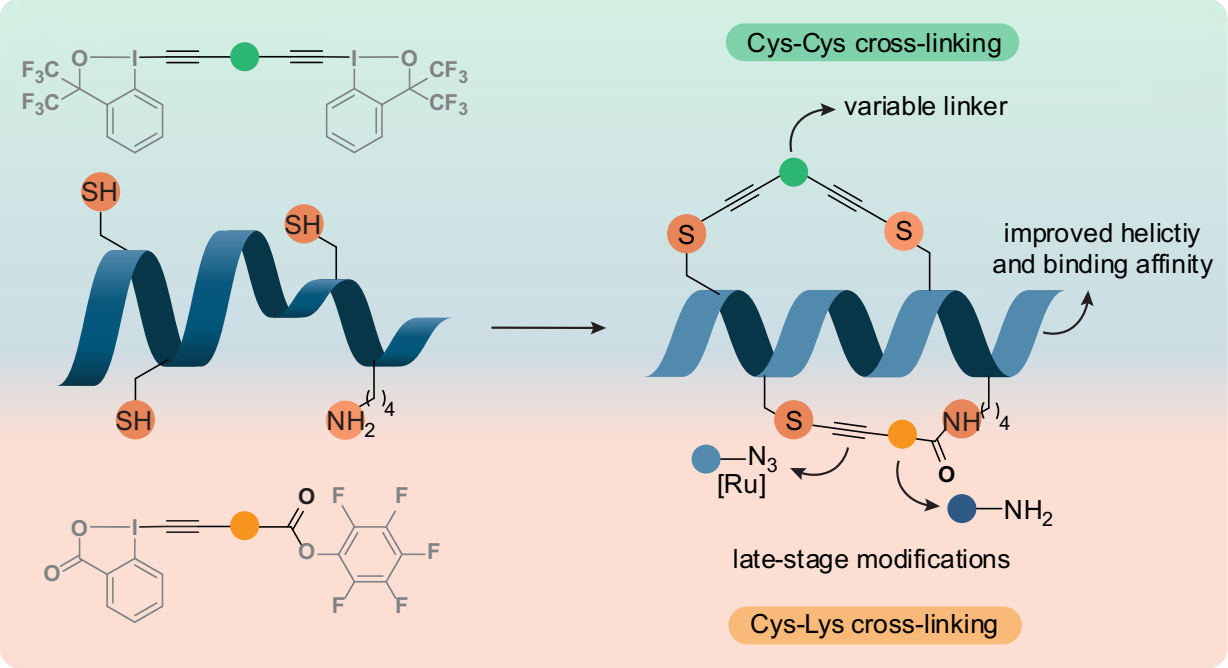
Protein-protein interactions (PPIs) are responsible for regulating many biological processes in our bodies. Due to the large binding site, often involving an α-helix, inhibition of PPIs can be difficult using small molecules.[1] In contrast, short helical peptides have a potential to bind to the protein targets. Nevertheless, isolated helical sequences lack the necessary structural rigidity, binding affinity and cell-permeability. Peptide stapling - covalent cross-linking of two amino acid side chains, can be used to improve these properties of helices. Therefore, an easy access to a wide range of structurally varied stapled peptides is crucial for the development of efficient inhibitors of PPIs.



Alkynylation of cysteines has been previously developed in our group using various hypervalent iodine reagents.[2] Their high reactivity and selectivity towards thiols was now used to development novel bifunctional tools for two-component cysteine-cysteine and cysteine-lysine peptide stapling.[3] This metal free method utilises unprotected peptides, providing excellent functional group tolerance, as well as, proximity driven selectivity. The obtained structurally diverse products can undergo post-stapling modifications via amidation of an activated ester, or via Ru "click" cycloaddition with the unique thioalkyne group present on the linker. Stapling of a peptide, derived from p53 protein, showed significant increase in helicity and binding affinity to MDM2 protein, a known cancer target.

[1] Y. H. Lau, P. de Andrade, Y. Wu, D. R. Spring, *Chem. Soc. Rev.*, **2015**, *44*, 91-102.

[2] E. M. D. Allouche, E. Grinhagena, J. Waser, *Angew. Chem. Int. Ed.*, **2022**, *61*, e202112287.

[3] J. Ceballos, E. Grinhagena, G. Sangouard, C. Heinis, J. Waser, *Angew. Chem. Int. Ed.*, **2021**, *60*, 9022-9031.

Preclinical development of the endogenous CXCR4 antagonist EPI-X4 for therapy of cancer and inflammatory diseases

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The CXCR4/CXCL12 axis plays an important role for several processes of the human body, including development, stem cell homeostasis and immune cell function. Aberrant CXCR4/CXCL12 signaling is involved in diverse pathologies such as cancer and inflammatory diseases. The **E**ndogenous **P**eptide **I**nhibitor of C**X**CR**4** (**EPI-X4**) is a 16 amino acid long fragment of human serum albumin, which has previously been identified in our lab. The peptide specifically binds to CXCR4, blocks CXCL12-induced signaling and migration and acts as an inverse receptor agonist. EPI-X4 is a promising candidate for the development of improved analogues for the therapy of CXCR4-associated diseases. We optimized the antagonistic activity of EPI-X4 by combining computational approaches and rational drug design. In addition, we applied different methods to prevent enzymatic degradation and to prolong systemic circulation time *in vivo*. Compared to the wild type peptide these newly developed EPI-X4 derivatives have a more than 1000-fold increased anti-CXCR4 activity, are stable for several hours in blood plasma and circulation *in vivo*, and are therapeutically active in different mouse models of inflammatory diseases, e.g. topical dermatitis and eosinophilic asthma, and cancer, e.g. Waldenström's macroglobulinemia and acute myeloid leukemia. In addition, therapeutic efficacies of lead derivatives are currently being evaluated in mouse models of other CXCR4-dependent disease like chronic lymphocytic leukemia, sepsis and rheumatoid arthritis.

A macrocyclic peptide that inhibits SARS-CoV-2 infection across variants by binding to a buried ternary site in the spike protein

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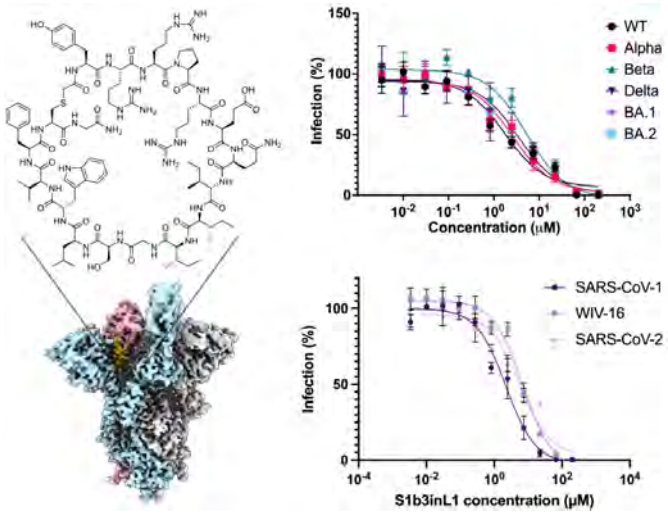
Using mRNA display under a reprogrammed genetic code we have found a macrocyclic peptide that is able to inhibit infection by SARS2-S pseudotyped VSV in cellular models of infection. This peptide retains activity across all variants of concern tested, as well as with spike protein from several different sarbecoviruses and against whole virus. A cryo-EM structure, with refinement guided by hydrogen-deuterium exchange footprinting, revealed this peptide to be binding in both the 'up' and 'down' configurations to a site in the S1b domain distal to ACE2. Molecular dynamics simulation further revealed binding in the 'down' state to be most stable, exploiting further interactions with the NTD and S2 domains in adjacent protomers. Alanine scanning shows that some of the residues involved in the quaternary interaction are crucial for inhibition, and so we hypothesize that this ternary binding site is responsible for activity. Phylogenetic analysis of coronavirus spike proteins and sequence entropy analysis shows this site to be highly conserved. Our work has thus revealed a potentially important new druggable site in the coronavirus spike protein, as well as a molecule that can exploit that site for all known SARS-CoV-2 variants of concern and several other sarbecoviruses, which may prove valuable for future pandemic preparedness.



Pyridyl-Ala in the third position of somatostatin receptor antagonists: regioisomeric substitution modulates their biodistribution

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The radiolabeled somatostatin receptor subtype 2 (SST2) antagonists [^{177}Lu]Lu-DOTA-JR11 (JR11 = pCl-Phe^1-cyclo(DCys^2-Aph(Hor)^3-DAph(Cbm)^4-Lys^5-Thr^6-Cys^7)-DTyr^8-NH_2) and [^{177}Lu]Lu-DOTA-LM3 (LM3 = pCl-Phe^1-cyclo(DCys^2-Tyr^3-DAPH(Cbm)^4-Lys^5-Thr^6-Cys^7)-DTyr^8-NH_2) are under clinical evaluation for the treatment of neuroendocrine tumor patients with remarkable success. These peptides differ in the amino acid in position 3, which is involved in a β-turn and reported to play a crucial role in modulating the receptor subtype selectivity and affinity of octreotide-based somatostatin agonists[1,2]. Therefore, the choice of the amino acid in this position is of particular interest. We investigated, among other non-natural amino acids, the role of the pyridylalanine, and more specifically of the three regioisomers, 2-pyridyl-alanine (2-Pal), 3-pyridyl-alanine (3-Pal) and 4-pyridyl-alanine (4-Pal) on SST2 targeting *in vitro* and *in vivo*. The three DOTA-conjugates DOTA-[2Pal^3]-LM3, DOTA-[3Pal^3]-LM3 and DOTA-[4Pal^3]-LM3 were labeled with Lu-177 and studied, comparatively to the reference radiotracer [^{177}Lu]Lu-DOTA-LM3, *in vitro* in HEK-SST2 expressing cells and *in vivo* in HEK-SST2 xenografts. All Pal-radiotracers compared well with [^{177}Lu]Lu-DOTA-LM3 in terms of affinity and SST2-mediated cellular uptake *in vitro*. Interestingly, differences were observed in SPECT/CT images acquired at 1h p.i.. [^{177}Lu]Lu-DOTA-[2Pal^3]-LM3 accumulates mainly in the tumor and displayed the best tumor-to-kidney ratios. [^{177}Lu]Lu-DOTA-[3Pal^3]-LM3 accumulated mainly in the kidney, while [^{177}Lu]Lu-DOTA-[4Pal^3]-LM3 accumulated in the kidneys and additionally in the abdomen.. Further *in vivo* investigations are in progress to assess the impact of the Pal regioisomers on the pharmacokinetics and SST2-tumor targeting *in vivo*.

References

1. Ginj M et al. Chemistry & Biology. 2006;13:1081
2. Hocart SJ et al. J Med Chem. 1998;41:1146

Array-Based Development of Ultra-Specific Peptidic Binders

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Protein–protein interactions (PPIs) mediated by intrinsically disordered protein regions play a fundamental role in cellular function and disease. We use synthetic chemistry to first identify peptidic protein binders that display outstanding specificity on proteomic level and then further exploit the same sites for the development of ultra-specific interaction modulators and antibody-superior fluorescent probes.

Promising sequences are first identified and mapped by determining and filtering whole-lysate interactomes of synthesised peptides [unpublished]. Stepwise *in vitro* evolution of excellent endogenous linear peptide binders is achieved by economic high-throughput synthesis [1] combined with the determination of thermodynamic [2] and kinetic [3] binding parameters. Applied to gephyrin the master regulator of fast synaptic inhibition [4], this workflow yielded *Sylites* – As fluorescent probes Sylites reveal inhibitory synapse ultrastructure in neurons and connectivity in brain slices [5]. When applied to hepatitis B virus capsid binders [6] our workflow yields compounds that interfere with Virus formation in live cells [unpublished]. Applied to Histone-tail sequences a variant of this workflow identifies isozyme-specific HDAC warheads [7].

Towards the goal to develop highly specific peptidomimetic binders with therapeutic potential we currently explore the combination of computer-based mimetic design, combinatorial synthesis and high-throughput evaluation in array format.

[1] C Schulte, V Khayenko, AJ Gupta, **HM Maric**. Low-cost synthesis of peptide libraries and their use for binding studies via temperature-related intensity change. *STAR Protocols*, **2021**, 2
[2] C Schulte, V Khayenko, NF Nordblom, F Tippel, V Peck, AJ Gupta **HM Maric**, *iScience*, **2021**, *101898*.
[3] C Schulte, A Solda, S Spänig, N Adams, I Bekic, W Streicher, D Heider, R Strasser, **HM Maric**. Multivalent Binding Kinetics Resolved by Fluorescence Proximity Sensing. *ResearchSquare,* **2022**.
[4] R Dos Reis, E Kornobis, A Pereira, F Tores, J Carrasco , C Gautier, C Jahannault-Talignani, P Nitschké, C Muchardt, A Schlosser, **HM Maric**, F Ango, E Allemand. Complex regulation of Gephyrin splicing is a determinant of inhibitory postsynaptic diversity. *Nature Communications,* **2022**, 13.
[5] V Khayenko, C Schulte, SL Reis, OAvraham, C Schietroma, R Worschech, NF Nordblom, S Kachler, C Villmann, KG Heinze, A Schlosser, O Schueler-Furman, P Tovote, CG Specht, **HM Maric**. *Angewandte Chemie*, **2022**, 61,30.
[6] Makbul C, Khayenko V, **Maric HM**, Böttcher B. Conformational Plasticity of Hepatitis B Core Protein Spikes Promotes Peptide Binding Independent of the Secretion Phenotype. *Microorganisms*. **2021**, 9(5).
[7] C Moreno-Yruela, M Bæk, AE Vrsanova, C Schulte, **HM Maric**, CA Olsen. Hydroxamic acid-modified peptide microarrays for profiling isozyme-selective interactions and inhibition of histone deacetylases. *Nature Communications*, **2021**, 62.

FAP-2286: head-to-head comparison of tumor radiation dose with small molecule-based FAP-targeting radioligands

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Fibroblast Activation Protein α (FAP)-targeting radioligands have recently demonstrated high diagnostic potential for a wide variety of tumors. Nevertheless, the therapeutic potential of this class of radio-therapeutics is impaired by its short tumor residence time, thus suboptimal tumor radiation dose. Several different strategies have been tested, such as dimerization, the use of albumin binders or the peptidic binder FAP-2286, which seemed to overcome this limitation in a preliminary *first-in-human* study. Nevertheless, a head-to-head comparison of these approaches has never been done. In this work, we aim to clarify the strengths and the limitations of the proposed FAP-binding radioligands, in comparison with the well-characterized molecules FAPI-46 and FAP-2286. *In vitro* inhibition properties of all the compounds assessed on isolated hFAP indicated sub-nanomolar inhibition for all the tested compounds. Cellular distribution, which was assessed in FAP-expressing cell lines (HT-1080.hFAP and HEK-293.hFAP) against wild-type cells, indicated that most of the compounds internalized inside the cells 4h after incubation, with the exception of the peptide ^{177}Lu-FAP-2286 which was mainly bound to the cell surface. *In vivo* biodistribution and SPECT/CT, assessed at different time points after injection of ^{177}Lu-labelled compounds, showed uptake for all the compounds, with a high and sustained uptake for the dimer up to 72h, while ^{177}Lu-FAP-2286 presented the lowest tumor uptake, but the best tumor-to-critical-organs ratios. In conclusion, while the dimeric FAPI-46 delivered the highest radiation dose at the tumor over time, the peptide ^{177}Lu-FAP-2286 was found to be the most tumor-selective ligand, spearing healthy tissues.

[1] Kratochwil C, Flechsig P, Lindner T, et al., J Nucl Med. 2019, 60, 801-805.
[2] Kaghazchi F, Aghdam RA, Haghighi S, et al., Clin Nucl Med. 2022, 47, E243-E245.
[3] Zboralski D, Osterkamp F, Simmons AD, et al., Ann Oncol. 2020, 31, S488.
[4] Li H, Ye S, Li Li, et al., Eur J Nucl Med Mol Imaging. 2022, 2705-2715.
[5] Xu M, Zhang P, Ding J, et al., J Nucl Med. 2022, 63, 952-958.

Cyclic Tetrapeptides: Novel Remedy for Lead Poisoning

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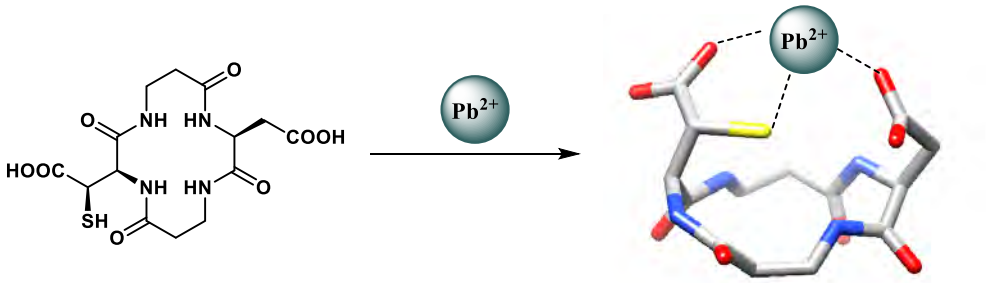
Lead (Pb) is the most abundant toxic metal that causes significant ecological and medicinal issues. The primary approach to address Pb poisoning is by chelation therapy, in which a chelating agent that can coordinate and remove the poisonous metal is administered. To date, there is no ideal chelating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

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



The lead peptide and its computed structure upon binding Pb^{2+} ion

[1] N. Rees, R. Fuller, *UNICEF and Pure Earth*, **2020**.
[2] C. Cobbett, P. Goldsbrough, *Annu. Rev. Plant Biol.*, **2002**, *53*, 159-182.
[3] T. A. Mohammed, C. M. Meier, T. Kalvoda, M. Kalt, L. Rulíšek, M. S. Shoshan, *Angew. Chem. Int. Ed.*, **2021**, *60*, 12381-12385.

Precision Engineered GPCR-Targeted Therapeutics: CCR2 Case Study

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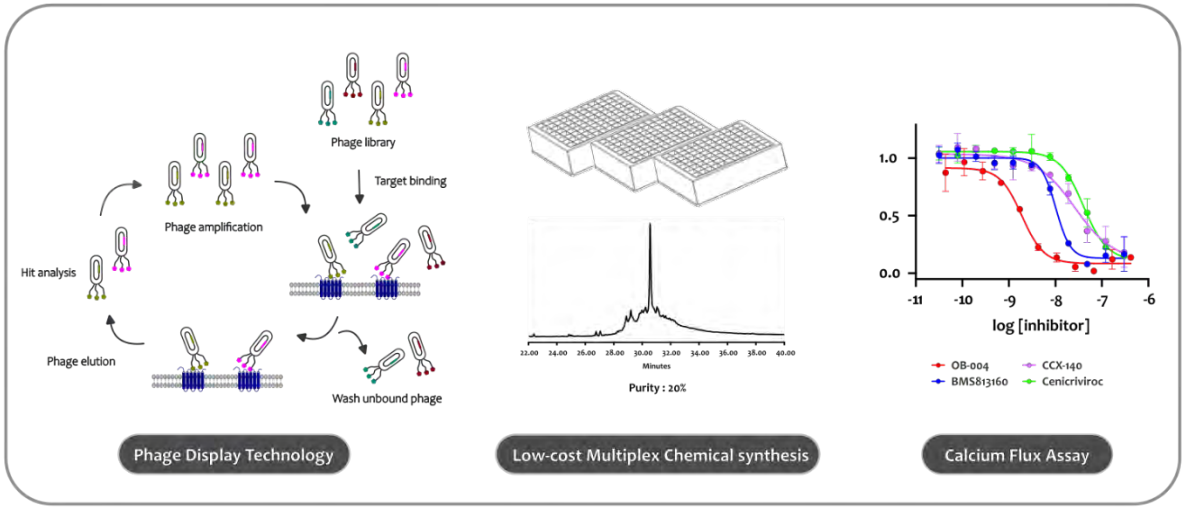
CCR2 is an undrugged GPCR linked to a broad range of serious diseases including cancer and atherosclerosis.[1, 2] Although many pharmaceutical companies have investigated the CCL2/CCR2 axis, there are only a few programs that have advanced into the clinic.[3] Here, we applied Orion Biotechnology's proprietary platform,[4] screening billions of engineered ligand analogs using phage display technology on CHO cells overexpressing CCR2, producing hits using low-cost multiplex chemical synthesis, and testing to identify high affinity and selective antagonist OB-004. Remarkably, OB-004 exhibited superior antagonist potency when compared to the leading small molecule competitors (BMS-813160, CCX-140 and Cenicriviroc) in both calcium mobilization assay (THP-1 cells; EC50 = 3.6 ± 0.8 nM) as well as in chemotaxis assay (human ex-vivo model). Currently, OB-004 is positioned for *in vivo* efficacy assessment in inflammation disease models.



[1] C. Shi *et al.*, *Nature Reviews Immunology* **2011**, *11*, 762-774.

[2] Q. Hao, J. V. Vadgama, P. Wang, *Cell Commun Signal* **2020**, *18*, 82.

[3] https://go.drugbank.com/bio_entities/BE0003387

[4] https://orionbiotechnology.com/science/

Selection of chemically-upgraded macrocyclic peptides by phage display

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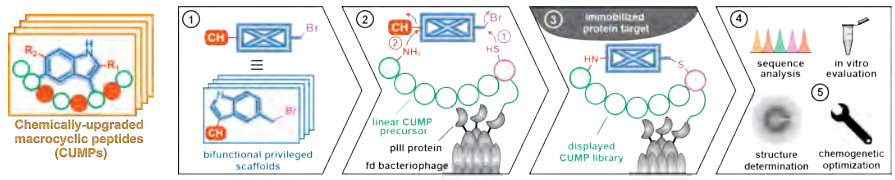
Macrocyclic peptides (MPs) show great potential for the development of pharmaceuticals and chemical probes. Aided by *in vitro* selection strategies that enable the efficient culling of vast libraries, the number of approved MP drugs has steadily increased over the past decade.[1] However, man-made MPs are typically ill-suited for therapeutic intervention, as they cannot undergo the same *chemogenetic* optimization mechanisms that are key for ameliorating the properties of naturally-occurring MPs.[2] In nature, organisms take advantage of the evolutionary algorithm to fine-tune not only the amino acid sequence, but also posttranslational processes such as the introduction of non-peptidic moieties for peptide macrocyclization. As a result, mimicking such a chemogenetic optimization in the laboratory is desirable in order to improve the effectiveness (and pharmacological properties) of man-made MPs.[3]



OUR WORKFLOW: (**1**) synthesis of privileged scaffolds featuring distinct cyclization handles; (**2**) evaluate CUMP formation with synthetic peptides and linear precursors displayed on the phage coat; (**3-4**) select CUMP binders against drug targets; (**5**) characterization and chemogenetic optimization of CUMPs.

To this end, we present an efficient two-step cyclization strategy to access chemically-upgraded macrocyclic peptides (CUMPs) via the programmed modification of a unique cysteine residue and an N-terminal amine. We demonstrated that this approach yields MPs featuring asymmetric cyclization units from both synthetic peptides and when linear precursors were appended onto a phage-coat protein. Finally, we showcased that our cyclization strategy is compatible with phage-display protocols and enables the selection of CUMP-binders against a model target protein from a naïve library.[4] We anticipate that the future selection of CUMPs by phage display for clinically relevant targets will enable a comprehensive exploration of a previously-unexplored chemical space and provide unique opportunities for drug discovery.

[1] a) E. M. Driggers, S. P. Hale, J. Lee, N. K. Terrett, *Nat. Rev. Drug. Discov.* **2008**, *7*, 608-624; b) E. Valeur, S. M. Gueret, H. Adihou, R. Gopalakrishnan, M. Lemurell, et al., *Angew. Chem. Int. Ed.* **2017**, *56*, 10294-10323; c) A. Zorzi, K. Deyle, C. Heinis, *Curr. Opin. Chem. Biol.* **2017**, *38*, 24-29; d) C. Morrison, *Nat. Rev. Drug. Discov.* **2018**, *17*, 531- 533; e) A. A. Vinogradov, Y. Yin, H. Suga, *J. Am. Chem. Soc.* **2019**, *141*, 4167-4181.

[2] a) D. P. Fairlie, J. D. Tyndall, R. C. Reid, A. K. Wong, G. Abbenante, et al., *J. Med. Chem.* **2000**, *43*, 1271-1281; b) D. Wang, W. Liao, P. S. Arora, *Angew. Chem. Int. Ed.* **2005**, *44*, 6525-6529; c) E. A. Villar, D. Beglov, S. Chennamadhavuni, J. A. Porco, Jr., D. Kozakov, et al., *Nat. Chem. Biol.* **2014**, *10*, 723-731; d) A. K. Malde, T. A. Hill, A. Iyer, D. P. Fairlie, *Chem. Rev.* **2019**, *119*, 9861-9914.

[3] a) C. J. White, A. K. Yudin, *Nat. Chem.* **2011**, *3*, 509-524; b) A. K. Yudin, *Chem. Sci.* **2015**, *6*, 30-49; c) L. R. Malins, J. N. deGruyter, K. J. Robbins, P. M. Scola, M. D. Eastgate, et al., *J. Am. Chem. Soc.* **2017**, *139*, 5233- 5241; d) R. Derda, M. R. Jafari, *Protein Pept. Lett.* **2018**, *25*, 1051-1075.

[4] T. R. Oppewal, J. Hekelaar, C. Mayer, *ChemRxiv*. **2021**, A Phage-Compatible Strategy to Access Macrocyclic Peptides Featuring Asymmetric Molecular Scaffolds as Cyclization Units. doi:10.26434/chemrxiv.13705618.v1 This content is a preprint and has not been peer-reviewed.

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

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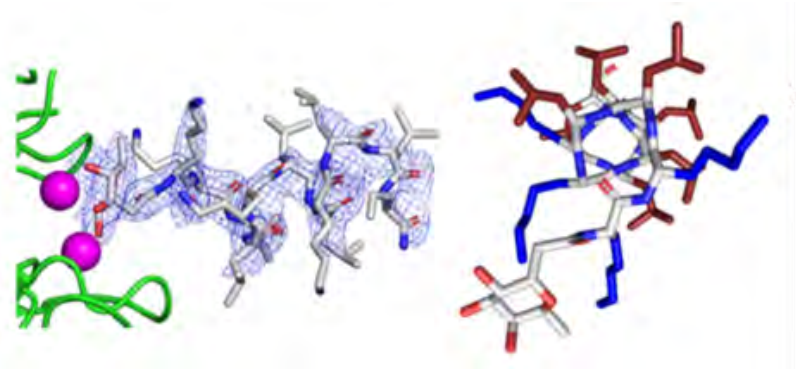
Peptide α-helicity mostly depends on its amino acid sequence and is right or left-handed depending on amino acids chirality (respectively L- or D-). However, mixed-chirality sequences are usually unfolded. In case of antimicrobial peptides (AMPs), an amphiphilic α-helix is generally required to be active and research on mixed chirality AMPs is poorly documented. We recently reported the first X-ray crystal structures of mixed chirality short bicyclic and linear AMPs forming α-helices as complexes of fucosylated analogs with the bacterial lectin LecB (see figure: X-ray structure of lectin-bound fucosylated AMP dln69, KKllKllKlll).[1] Following up the study on our mixed-chirality peptide ln69, we discovered new chirality patterns also presenting α-helical conformation both in membrane-like environment and in aqueous condition determined by X-ray crystallography. Compared to their homochiral parent, these mixed chirality peptides display better stability in human serum, as well as, in selected cases, improved antimicrobial activity.



[1] Stéphane Baeriswyl, Hippolyte Personne, Ivan Di Bonaventura, Thilo Köhler, Christian van Delden, Achim Stocker, Sacha Javor and Jean-Louis Reymond, *RSC Chem. Biol.*, **2021**, 2, 1608-1617

Stereoselective Peptide Catalysis in Complex Environments — From River Water to Cell Lysates[1]

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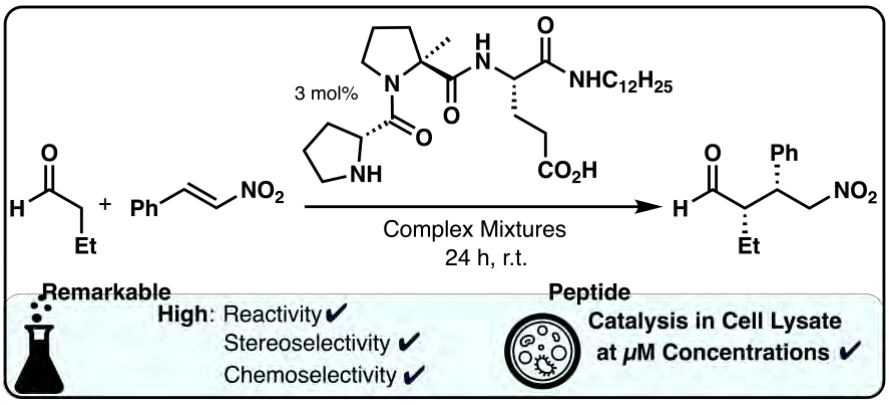
Peptides have been recognized as powerful catalysts for various reactions throughout the last two decades.[2],[3] Of these peptide catalysts, several are characterized by a high degree of stereoselectivity and reactivity. Similar to nature's catalysts, enzymes, they are also composed of amino acids but have a much lower molecular weight and could hence be considered 'minienzymes'. Whilst enzymes function splendidly at low concentrations in complex aqueous biological environments, peptide catalysts normally require pure organic solvents and high concentrations.[4a-h]

We were therefore intrigued by the question of whether a peptide catalyst could exhibit chemoselectivity in similar environments reminiscent of enzymes. Consequently, we probed the behavior of tripeptide catalysts in both hydrophobic and aqueous reaction media and further challenged the catalysts with complex reaction media, consisting of aqueous solutions, containing biomolecules, bearing functional groups that can coordinate or react with the catalyst, substrate, or intermediates. Finally, we subjected the peptide catalysts to the ultimate test by investigating their reactivity, chemoselectivity and stereoselectivity in cell lysate in micromolar concentrations, entering a range also typical for enzymes. Despite its relatively short length and small size, H-DPro-αMePro-Glu-$NHC_{12}H_{25}$ proved to be a conformationally well-defined tripeptide, able to catalyze C-C bond formations with high reactivity and stereoselectivity, independent of the solvent and its compound composition. In fact, this peptide yielded our desired product with excellent stereoselectivity (≥ 93% ee, d.r. 85:15 - 94:6) and yield (80 - 97%), even in cell lysate, a highly complex mixture with numerous compounds that could either react or coordinate to the catalyst, the substrates, or the reaction intermediates. These findings provoke the question of the potential role of peptide catalysis in nature and during the evolution of enzymes.



[1] T. Schnitzer, J.W. Rackl, H. Wennemers, *Chem.Sci.*, **2022**, DOI: 10.1039/d2sc02044k.

[2] A.J. Metrano, A.J. Chinn, C.R. Shugrue, E.A. Stone, B. Kim and S.J. Miller, *Chem.Rev.*, **2020**, 120, 20, 11479.

[3] H. Wennemers, *Chem.Commun.*, **2011**, 47, 12036.

[4] a) B. Lewandowski and H. Wennemers, *Curr.Opin.Chem.Biol.*, **2014**, 22:40–46. b) S. Juliá, J. Masana and J.C. Vega, *Angew.Chem.Int.Ed.*, **1980**, 19, 929. c) A. Weyer, D. Díaz, A. Nierth, N.E. Schlörer and A. Berkessel, *ChemCatChem*, **2012**, 4, 337. d) M. L. Lanxiang Shi, G. Li, S. Chen, W. Fang, Z. Ge, T. Cheng and R. Li, *Tetrahedron*, **2007**, 63, 7892. e) G. Maayan, M.D. Ward, K. Kirshenbaum, *Proc.Natl.Acad.Sci.* U.S.A., **2009**, 106, 13679. f) K. Akagawa, H. Akabane, Seiji Sakamoto and Kazuaki Kudo, Org.Lett., **2008**, 10, 2035. g) Baishakhi Sarkhel, Ayan Chatterjee, Dibyendu Das, J.Am.Soc., **2020**, 142, 4098. h) C.S. Foden, S. Islam, C. Fernández-García, L. Maugeri, T.D. Sheppard, M.W. Powner, Science, **2020**, 370, 865.

Turn-on Fluorescent Peptide Conjugate for the Detection of Human Monoamine Oxidases Enzymes

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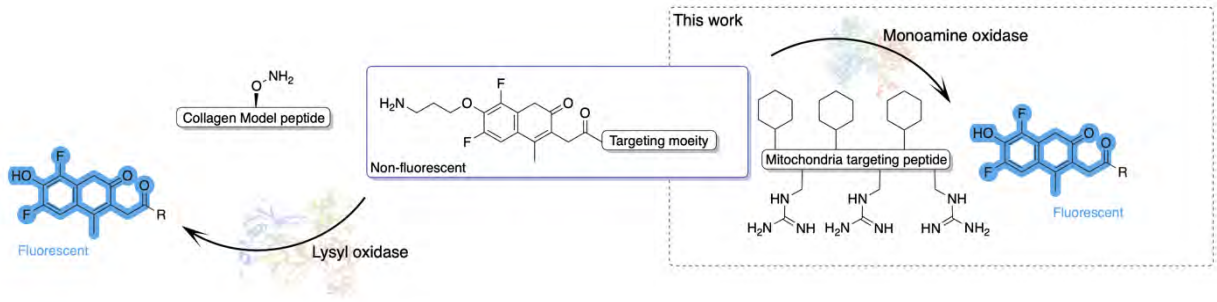
Monoamine oxidase (MAO) enzymes catalyze the deamination of biogenic amines including neurotransmitters. MAOs exist as two isoforms (MAO-A and MAO-B) that are localized in the outer membrane of mitochondria. They differ in substrate and inhibitor specificity as well as in tissue distribution. MAO-A is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

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



[1] a) A. W. K. Yeung, M. G. Georgieva, A. G. Atanasov, N. T. Tzvetkov, *Front. Mol. Neurosci.*, **2019**, *12*, 1-12. b) M. B. H. Youdim, D. Edmondson, K. F. Tipton, *Nat. Rev. Neurosci.*, **2006**, *7*, 295–309.
[2] X. Li, H. Zhang, Y. Xie, Y. Hu, H. Sun, Q. Zhu, *Org. Biomol. Chem.*, **2014**, *12*, 2033–2036.
[3] M. R. Aronoff, P. Hiebert, N. B. Hentzen, S. Werner, H. Wennemers, *Nat. Chem. Biol.*, **2021**, *17*, 865–871.
[4] K. L. Horton, K. M. Stewart, S. B. Fonseca, Q. Guo, S.O Kelley, *Chem Biol*. **2008**, *15*, 375-82.

Mono- and Multivalent Peptide Binding Resolved by Fluorescence Proximity Sensing and Temperature Related Intensity Change

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Protein-protein interactions (PPIs) are critical for virtually all physiological processes. PPIs involving short, linear motifs (SLiMs) play a major role in immunological recognition, signalling and regulation and provide attractive starting points for probing biological function and pharmacological intervention. Yet, state-of-the-art protein-peptide interaction characterization approaches exhibit limited throughput and sensitivity often due to surface immobilization, fluorescent labelling, and sample consumption.

Using the gephyrin protein, the master regulator of the inhibitory synapse, as benchmark, we exemplify the application two complementary biophysical phenomena for the kinetic and thermodynamic optimization of mono- and multivalent peptide architectures derived from SLiMs. First, a phenomenon called temperature related intensity change (TRIC) is applied for the identification and fine-mapping of low- and high-affinity protein interaction sites and the definition of sequence binding requirements. Validation by microarray-based studies and strong correlation with structural data establish TRIC as a quasi-label-free method to determine binding affinities of unmodified peptide libraries in high-throughput. [1], [2]

Secondly, we exemplify the use of Fluorescence Proximity Sensing (FPS) for the systematic kinetic and thermodynamic analysis of +100 peptides with varying combinatorial dimeric, tetrameric, and octameric architectures. Direct coupling of automated peptide synthesis to FPS measurements resolved on-rates, off-rates, and dissociation constants with high accuracy and low sample consumption compared to other complementary technologies. The dataset and its machine learning-based analysis deciphered the relationship of specific architectural features and binding kinetics and thereby identified binders with unprecedented protein inhibition capacity, thus, highlighting the value of FPS for the rational engineering of multivalent inhibitors. [3]

[1] C. Schulte et al., *iScience*, **2021**, 24, 101898
[2] C. Schulte et al, *STAR Protocols*, **2021**, 2, 100605
[3] C. Schulte et al., *Nat. Commun. Biol.*, **2022**, in revision

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

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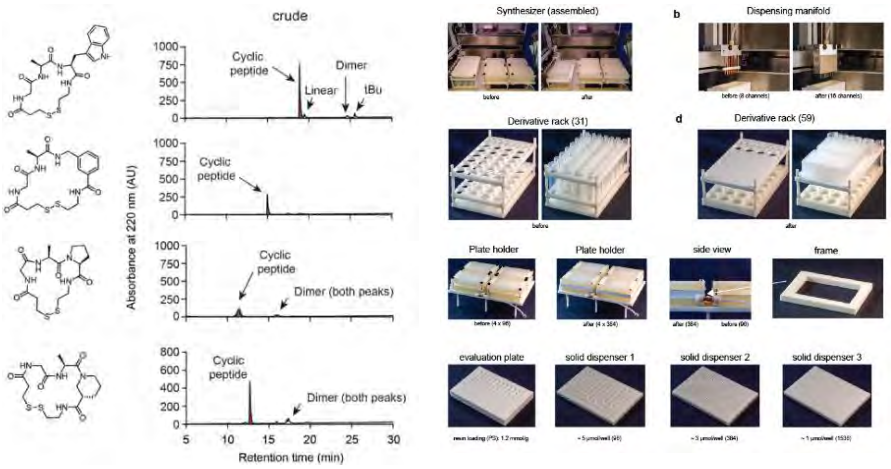
Macrocycles have raised much interest in the pharmaceutical industry due to their ability to bind challenging targets and the still small size that often allows crossing membranes to reach intracellular proteins. However, the development of macrocyclic ligands to new disease targets is hindered by the limited availability of large, structurally diverse macrocyclic compound libraries for high-throughput screening.

In order to generate large libraries of macrocycles, our laboratory has recently introduced a new strategy in which "n" short linear peptides are combinatorially cyclized or acylated with "m" different chemical reagents to generate "m x n" different macrocyclic compounds.[1,2,3] A bottleneck in applying the established strategy is the synthesis of large numbers of short peptides. The peptide synthesizer that we use – being the one having the highest throughput on the market - can produce 4 x 96 peptides in four microtiter plates, which limits the synthesis to 384 peptides per run. The synthesizer holds reagent containers for a maximum of 31 different amino acids, which also limits the chemical and structural diversity of peptides that can be produced.

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

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

Figure 1:



References:

[1] Sangram S. Kale, M. Bergeron-Blerk, Yuteng Wu, Mothukuri G. Kumar, Mahn V. Pham, Julien Bortoli, Jonathan Vesin, Xudong Kong, Joao Franco Machado, Kaycie Deyle, Patrick Gonschorek, Gerardo Turcatti, Laura Cendron, Alessandro Angelini and Christian Heinis, *Sci. Adv.* **2019**; 5: eaaw2851.

[2] Sevan Habeshian, Ganesh A. Sable, Mischa Schüttel, Manuel L. Merz, and Christian Heinis, *ACS Chem. Biol*. **2022**, 17, 181−186.

[3] Sevan Habeshian, Manuel Leonardo Merz, Gontran Sangouard, Ganesh Kumar Mothukuri, Mischa Schüttel, Zsolt Bognár, Cristina Díaz-Perlas, Jonathan Vesin, Julien Bortoli Chapalay, Gerardo Turcatti, Laura Cendron, Alessandro Angelini & Christian Heinis, *Nature Comm.* **2022**, 13, 3823.

A new mode of action of Teixobactin

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Teixobactin, produced by unculturable bacteria and considered the first novel antibiotic in 30 years, has aroused tremendous interest worldwide (L.Ling *et al*, *Nature*, 2015). While it was known that teixobactin targets an essential lipid (called Lipid II) in the bacterial plasma membrane, the mechanism of teixobactin remained obscure because of technical problems to study drugs in biological membranes.

Here, by combining solid-state NMR (ssNMR) with modern microscopy methods, we resolve the mechanism of teixobactin from the micrometer to the atomistic scale (Figure 1). Thereby, we show that teixobactin self-assembling into fibrils that destroy the bacterial membrane, Is a novel mechanism and a paradigm shift in understanding how antibiotics kill bacteria[1,2]. Advanced microscopy techniques (super-resolution microscopy and high-speed atomic force microscopy) disclosed that teixobactin forms large fibrillar structures on the bacterial surface. Strikingly, these fibrillar assemblies severely damage the membrane, killing the bacteria. Using comprehensive ssNMR studies, we solved the high-resolution structure of teixobactin-Lipid II complex in membranes. This structure shows that teixobactin specifically targets an immutable part of Lipid II, explaining why bacteria has major difficulty to develop resistance against the drug.

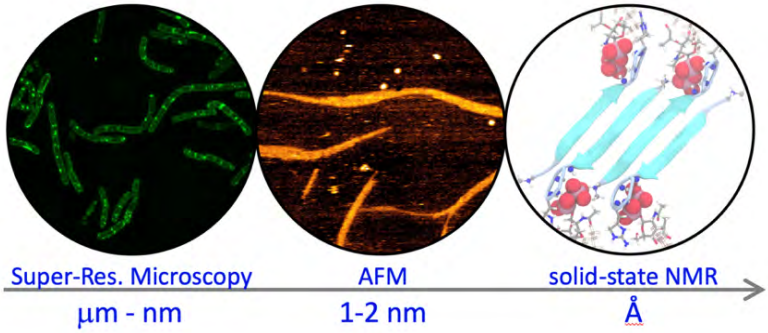

Fig 1. The killing mechanism from the micrometre to the atomistic scale.

Altogether, we have shown that teixobactin uses a novel dual killing mechanism by trapping Lipid II in an irreversible mesh of lethal fibrils that damage the membranes. This explains the excellent bactericidal activity of teixobactin and is a paradigm shift how antibiotics kill bacteria. This knowledge serves as a steppingstone towards the development of better antibiotics to tackle the urgent problem of antimicrobial resistance.

1) Shukla, R., Medeiros-Silva, J., Parmar, A., Vermeulen, B.J.A., Das, S., Paioni, L.A., Jekhmane, S., Lorent, J., Bonvin, A.M.J.J., Baldus, M., Lelli, M., Veldhuizen, E., Breukink, E., Singh, I., Weingarth, M. (2020) Nature Communications, 11, 2848, *Mode of action of teixobactins in cellular membranes*

2) Shukla, R., Lavore, F., Derks, M., Becker, L.M., Vermeulen, B., Wang, X., Kumar, R., Medeiros-Silva, J., Bonvin, AMJJ, Lorent, J., Lelli, M., Peoples, AJ., Spoering, AL., Ling, LL., Hughes, DE., Breukink, E., Lewis, K., Weingarth, M., *Teixobactin kills bacteria by a two-pronged attack on the cell envelope*, Accepted

Norleucine and its Occurence in Recent Drug Development Projects

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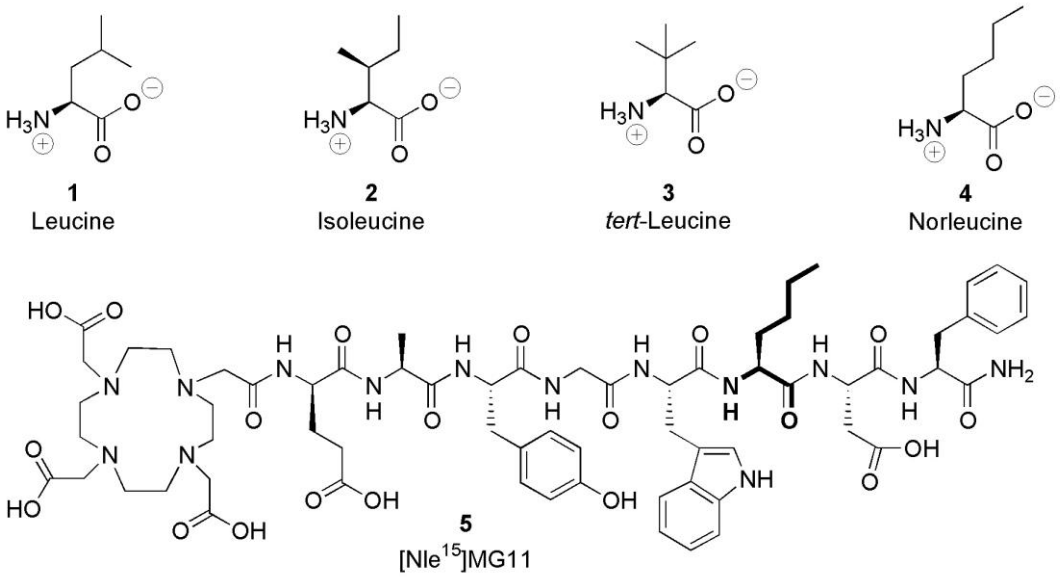
The well-known proteinogenic amino acids Leucine **1** and Isoleucine **2** are highly interesting tools for Chemical Education purposes and can be used *e.g.* in the discussion of different forms of isomerism, absolute stereochemistry and *Fischer*-type projections. The set of constitutional isomers, however, can be extended by the less known *tert*-Leucin **3** and Norleucine **4**, which are underrepresented in Chemical Education, but play a very important role particularly in the area of Drug Development. Following our general strategy to establish less familiar natural and unnatural amino acids as additional tools in basic Organic Chemistry teaching on universitary level, we have recently assembled useful informations about **3**[1] and **4**[2] and specified didactic topics and problems that could be addressed by using those compounds.



Within the presentation, concepts for the didactic use of **4** and its occurrence in recent Drug Development projects will be shown. One – but not the only – example will be [Nle^{15}]MG11 **5** (a chelator/peptide conjugate from radiotherapy),[3,4] for which the chemical structure is shown above.

[1] C. A. Vock, *CHIMIA*, **2020**, *74*, 825-827.
[2] C. A. Vock, *CHIMIA*, **2021**, *75*, 443-445; C. A. Vock, *CHIMIA*, **2021**, *75*, 554.
[3] N. M. Grob, S. Schmid, R. Schibli, M. Behe, T. L. Mindt, *J. Med. Chem.*, **2020**, *63*, 4496-4505.
[4] C. Müller, M. Behe, S. Geistlich, N. P. van der Meulen, R. Schibli, *CHIMIA* **2020**, *74*, 939-945.

Novel HTS-Compatible Peptide Discovery Platform for the Screening of Functionally Active Peptides

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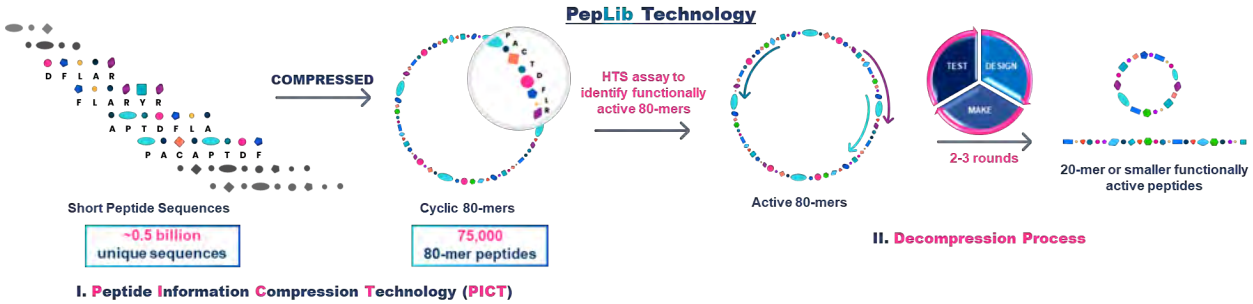
Affinity-based technologies like phage display and mRNA display platforms generate a non-quantifiable mixed library format, where each member is genetically tagged limiting their use to binding assays. Based on PepLib's proprietary Peptide Information Compression Technology (PICT), we have designed and synthesized a library of cyclic peptides that contain half a billion unique sequences. Each member is individually synthesized, head-to-tail cyclized, and purified in a tag-less manner to build a one well one compound library format, which breaks through the traditional limitations of display-based peptide screening technologies.



Moreover, the platform is capable of biochemical and cell-based functional screens making it suitable for difficult-to-drug membrane proteins. For example, a primary cell-based or biochemical screen is carried out to identify functionally active 80-mer peptide hits. Most active hit(s) are then taken through a 'decompression process' to convert 80-mer hit(s) into 20-mer or smaller (cyclic and/or linear) peptide leads.

* Lead and corresponding author

Highly diverse libraries of peptidic macrocycles enable the discovery of low-molecular weight PPI inhibitors

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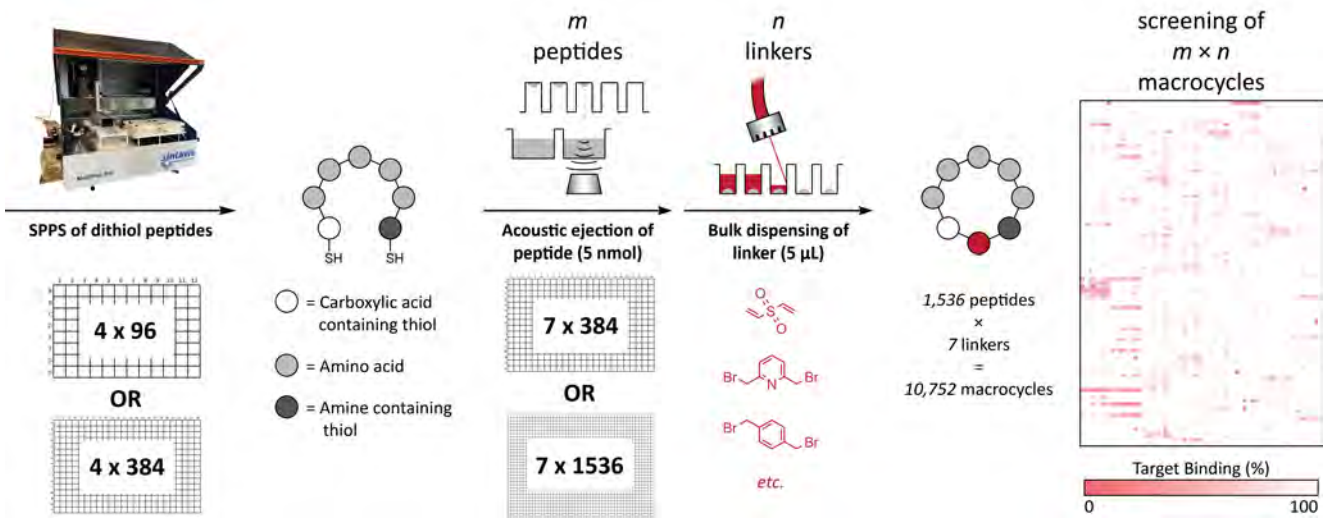
Macrocyclic compounds have received much attention from the pharmaceutical industry due to their ability to bind challenging disease targets, such as proteins without binding pockets or protein-protein interactions (PPIs).[1] Depending on their size and polarity, many macrocycles are also membrane permeable which allows for the targeting of intracellular proteins and can enable oral bioavailability. However, the development of macrocycle-based ligands to new targets is currently limited by a lack of sufficiently large macrocycle libraries and methods to generate them.

Herein, we present a method for the rapid synthesis and screening of 10,000+ macrocyclic peptides.[2,3,4] Thousands of short dithiol peptides comprising 3 to 5 amino acids are synthesized in 384-well plates on disulfide-anchored resin, then side chains deprotected while the peptides remain on solid-phase. After reduction of the disulfide bond, essentially pure peptides are obtained. Acoustic droplet ejection technology is subsequently used to distribute peptides for nanomole-scale, combinatorial cyclization with bis-electrophilic linkers. A library of 10,752 such macrocycles was screened against a protein-protein interaction to yield low-µM inhibitors.



[1] E. M. Driggers, S. P. Hale, J. Lee, N. K. Terrett, *Nat Rev Drug Discov*. **7**, 608–624 (2008).
[2] S. Habeshian, G. A. Sable, M. Schüttel, M. L. Merz, C. Heinis, *ACS Chem. Biol.* **17**, 181–186 (2022).
[3] S. S. Kale *et al.*, *Science Advances*. **5**, eaaw2851 (2019).
[4] S. Habeshian *et al.*, *Nat Commun*. **13**, 3823 (2022).

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

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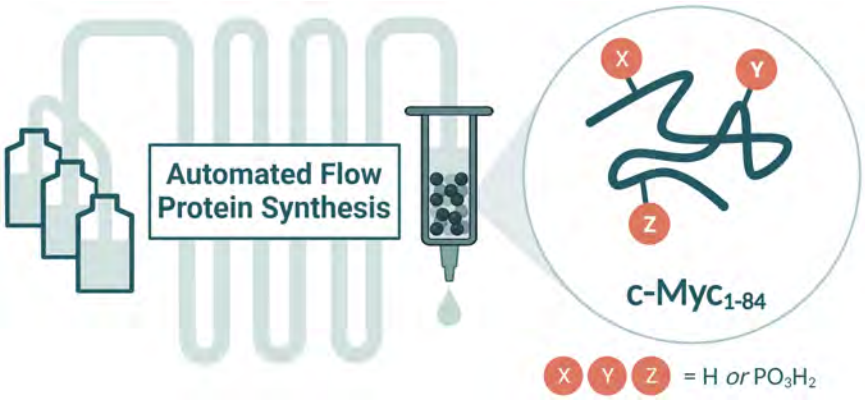
The intrinsically disordered protein (IDP) c-Myc is an oncogenic transcription factor with thousands of known and predicted binding proteins. Post-translational modifications (PTMs) on c-Myc, particularly in the N-terminal transactivation domain (TAD) play important roles in the regulation of c-Myc binding interactions and stability. However, details of the specific effects of each PTM and PTM-crosstalk on the interactions, functions, and stability of c-Myc have remained elusive due to the difficulty in obtaining PTM-variants of c-Myc for biochemical and biophysical assays.

Recently, automated fast-flow protein synthesis (AFPS) has been applied in the linear synthesis of proteins up to 200 AAs in length with high yield and purity.[1] Herein, we report the use of AFPS for the synthesis of a library of PTM-variants of the c-Myc N-terminus (residues 1–84), including phosphorylation at T58 or S62, glycosylation at T58, and combinations thereof. Each c-Myc variant was isolated in high yield (up to 6% yield over ~170 steps) and purity (>95% by UHPLC) in milligram quantities. These Myc_{1-84} PTM-variants were then screened against the known c-Myc interactor protein, Bin1, using NMR spectroscopy to validate our approach compared to the literature.[2] Myc_{1-84} induced significant CSPs in Bin1, and phosphorylation at T58 or S62, slightly reduced these CSPs but did not prevent Bin1 binding. Interestingly, short c-Myc fragments (residues 55–68), gave different results. It was found that phosphorylation at T58 of Myc_{55-68} barely affected binding to Bin1, whereas phosphorylation at S62 prevented binding altogether. These results support the hypothesis[2] of a secondary Bin1 binding site on c-Myc outside of residues 55–68, validating the continued study of c-Myc binding interactions with longer fragments (e.g. Myc_{1-84}) of c-Myc.

In current and future work, we are screening the Myc_{1-84} PTM-variants against a range of binding proteins and aim to elucidate the regulatory roles of PTMs and PTM-crosstalk on c-Myc. Ultimately, we seek to develop this flow chemistry-based platform to study the role of PTMs in the regulation of c-Myc and other biologically relevant proteins.



[1] N. Hartrampf, A. Saebi, M. Poskus, Z. P. Gates, A. J. Callahan, A. E. Cowfer, et al., *Science* **2020**, *368*, 980.
[2] C. Andresen, S. Helander, A. Lemak, C. Farès, V. Csizmok, J. Carlsson, et al., *Nucleic Acids Res.* **2012**, *40*, 6353.

Generating improved VIRIP-based HIV-1 gp41 FP inhibitors

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VIRus Inhibitory Peptide (VIRIP) was first identified by screening of a hemofiltrate-derived peptide library (Münch et al., 2007). It represents a naturally-occurring 20 amino acid fragment of α1-antitrypsin that block HIV-1 entry by preventing insertion of the conserved Fusion Peptide (FP) into the host cell membrane. An optimized analog (VIR-576) was safe and effective in a phase I/II clinical trial (Forssmann et al., 2010) and HIV-1 resistance to VIRIP-based inhibitors has a high genetic barrier (Müller et al., 2018). However, the necessity of i.v. injection of high doses of VIR-576 *in vivo*, likely due to degradation, tissue distribution and/or absorption, makes VIRIP-576 impracticable for use as an anti-HIV-1 agent in humans.

Based on the NMR structure of the complex between an optimized VIRIP derivative and the HIV-1 gp41 FP, we utilized ReaxFF molecular dynamics simulations for further optimization, ultimately obtaining active VIRIP derivatives consisting of 9 aa and a mass < 1 kDa. To facilitate functionalization, we synthesized and characterized VIRIP derivatives harboring cysteine residues at positions 7 to 10. Based on its high anti-HIV activity, we selected VIRIP(C9) as lead compound for further development. One way to increase serum stability and bioavailability of therapeutic peptides is to couple them with fatty acids. Conjugation of Palmitic acid to Lysine or the introduced cysteine residues did not compromise the antiretroviral activity of VIRIP. Further, coupling of VIRIP to Biotin(PEG), carrying a maleimide residue, allowed us to decorate tetrameric Neutravidin nanocarriers with VIRIP(C9) without impairing its antiviral activity.

VIRIP is an HIV-1 fusion inhibitor with the potential for clinical application. We further optimized VIRIP regarding size and the possibility for functionalization. VIRIP(9C)-Biotin coupled with Neutravidin keeps its ani-viral activity and provides the base for the further generation of multivalent FP inhibitors.