Interdisciplinary approach towards rational design of resistance breaking albicidin derivatives

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Albicidin (**Figure 1**) is a natural occurring antibiotic produced by the phytopathogen *Xanthomonas albilineans* for defending itself against other bacteria. It shows antibacterial activity at nanomolar concentrations against several ESKAPEE organisms as well as a broad range of Gram-positive and -negative strains by inhibiting DNA gyrase activity. In 2015, the structure of albicidin was elucidated revealing a modular structure of six building blocks connected by peptide bonds.[1] With these characteristics, albicidin is a promising lead structure for rational drug design.
In 2023 we elucidated the binding mechanism of albicidin to the DNA gyrase. In contrast to many other known gyrase inhibitors, albicidin binds in an asymmetrical manner to the gyrase tetramer, intercalating from one side into the DNA strand break with its A-building block. By wedging the GyrA/GyrA' interface with its C-terminus, albicidin traps the enzyme in a transitional state and prevents religation of the DNA.[2] These structural insights into the binding mode paved the way for rational design of more active derivatives. Variations in the A-building block (methyl coumaric acid, MCA) showed to increase the inhibitory effect of albicidin and its hydrophilicity. Substitution of MCA by methylquinoline or benzothiazole showed to best combination of strong gyrase binding, hydrophilicity and antibacterial activity.[3]

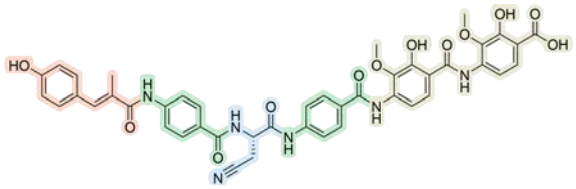

Figure 1 Structure of albicidin wild type

Several resistances against albicidin have been described in various strains. These are, amongst others different albicidin sequestering transcription factors (i.e. AlbA[4], AlbB[5] and STM3175[6]), endoproteases (e.g. AlbD[7]) and mutations in the nucleoside Tsx channel[8], entry point of albicidin. By generating several mutations in AlbA, we recently showed that high affinity albicidin binding and high transcription activation do not ensure protection of the cell suggesting a more complex mechanism. Additionally, a tool to assess the ability of albicidin derivatives to evade the resistance factor AlbA was established.[9] Interestingly, some of the recently published A variations, besides enhanced gyrase activity, showed antibacterial activity in the presence of AlbA. In accordance with the findings from Kosol *et al.*, evasion of AlbA by albicidin and activation of the transcription by AlbA showed no clear correlation.[3]

[1] S. Cociancich, A. Pesic, D. Petras, S. Uhlmann, J. Kretz, V. Schubert, L. Vieweg, S. Duplan, M. Marguerettaz, J. Noëll, I. Pieretti, M. Hügelland, S. Kemper, A. Mainz, P. Rott, M. Royer, R. D. Süssmuth, *Nat Chem Biol,* **2015**, *11*, 195–197.
[2] E. Michalczyk, K. Hommernick, I. Behroz, M. Kulike, Z. Pakosz-Stępień, L. Mazurek, M. Seidel, M. Kunert, K. Santos, H. Von Moeller, B. Loll, J. B. Weston, A. Mainz, J. G. Heddle, R. D. Süssmuth, D. Ghilarov, *Nat Catal,* **2023**, *6*, 52–67.
[3] M. Kulike-Koczula, K. Hommernick, L. B. Ghimire, S. Kosol, L. Zborovsky, M. Seidel, N. Sattler, A. Mainz, J. B. Weston, D. Ghilarov, R. D. Süssmuth, *Chemistry A European J,* **2025**, *31*, e202500162.
[4] M. J. Walker, R. G. Birch, J. M. Pemberton, *Mol Microbiol,* **1988**, *2*, 443–454.
[5] W. V. Basnayake, R. G. Birch, *Microbiology (Reading),* **1995**, *141 (Pt 3)*, 551–560.
[6] M. Saathoff, S. Kosol, T. Semmler, K. Tedin, N. Dimos, J. Kupke, M. Seidel, F. Ghazisaeedi, M. C. Jonske, S. A. Wolf, B. Kuropka, W. Czyszczoń, D. Ghilarov, S. Grätz, J. G. Heddle, B. Loll, R. D. Süssmuth, M. Fulde, *PLoS Biol,* **2023**, *21*, e3002186.
[7] L. Zhang, R. G. Birch, *Plant Biology,* **1997**.
[8] R. G. Birch, J. M. Pemberton, W. V. S. Basnayake, *Journal of General Microbiology,* **1990**, *136*, 51–58.
[9] S. Kosol, L. Rostock, J. Barsig, T. Tabarelli, K. Hommernick, M. Kulike, T. Eulberg, M. Seidel, I. Behroz, L. Kleebauer, S. Grätz, A. Mainz, R. D. Süssmuth, *Chem. Sci.* **2023**, *14*, 5069–5078.

Stereorandomized Oncocins with Preserved Ribosome Binding and Antibacterial Activity

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We recently demonstrated that solid-phase peptide synthesis using racemic amino acids yields stereorandomized peptides comprising mixtures of all possible diastereomers as homogeneous, single-mass products that can be purified by HPLC. We also showed that stereorandomization modulates the activity, toxicity, and stability of membrane-disruptive cyclic and linear antimicrobial peptides (AMPs) as well as dendrimers.[1]

In this study, we investigated whether stereorandomization is compatible with target-binding peptides, using the proline-rich AMP Oncocin which targets the bacterial ribosome. We found that stereorandomization of up to nine C-terminal residues preserved ribosome binding and antibacterial activity, including efficacy against drug-resistant strains, and resistant to serum degradation. Remarkably, fully stereorandomized Oncocin retained antibacterial activity in dilute growth media that promotes peptide uptake, despite lacking ribosome binding, suggesting an alternative mechanism of action. These findings demonstrate that stereorandomization can be compatible with target-binding peptides and may offer insights into their mechanisms of action.[2]

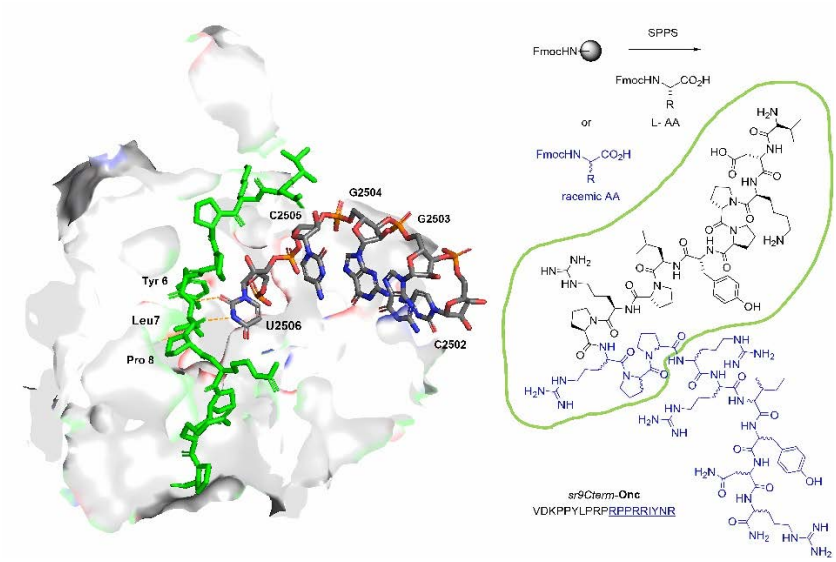

Figure 1. Partial stereorandomization of oncocin preserves ribosome binding.

[1] Siriwardena, T. N., *et al.* Stereorandomization as a Method to Probe Peptide Bioactivity. *ACS Cent. Sci.* **2021**, *7*, 126–134.
[2] Gan, B. H., *et al.* Stereorandomized Oncocins with Preserved Ribosome Binding and Antibacterial Activity. *J. Med. Chem.* **2024**, *67*, 19448–19459.

Unravel the biosynthesis of an antifungal compound produced in *Burkholderia cepacia* complexes

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An antifungal compound AFC-BC11 is a natural secondary metabolite produced in the strain *Burkholderia pyrrocinia* BC11 (formerly *B. cepacia* BC11). In general, these strains belong to the *Burkholderia cepacia* complex (BCC) and are known to be human pathogens that cause respiratory problems in patients with cystic fibrosis (CF).[1] However, it is also well known that *Burkholderia* produces various secondary metabolites with antibacterial, antifungal, insecticidal and herbicidal effects.[2] For example, *B. pyrrocinia* is an effective biological control agent against *Rhizoctonia solani*-induced root rot in poinsettias.[3,4]

Recently, the complex structure of the light-sensitive antifungal lipopeptide AFC-BC11 was elucidated. In a series of different *in vitro* reconstitution experiments the assembly of the backbone of AFC-BC11 was elucidated and the role of various enzymes associated with the biosynthetic gene cluster were characterized. The BGC of AFC-BC11 contains over 25 different genes involved in the biosynthesis and transport of the compound. Despite the peptide-like structure, features of a classic modular non-ribosomal peptide synthetase (NRPS), including a condensation domain (C) that catalyzes peptide bonds, are not present. In one of the various *in vitro* reconstitutions, a ketoacyl synthase (KAS) III-like protein was shown to induce peptide formation and release of the peptide chain from the protein itself.

[1] D. Speert, D. Henry, P. Vandamme, M. Corey, E. Mahenthiralingam, *Emerg. Infect. Dis.*, **2002**, 8, 181–187.
[2] L. Chiarini, A. Bevivino, C. Dalmastri, S. Tabacchioni, P. Visca, *Trends Microbiol.*, **2006**, *14* (6), 277–286.
[3] J. Parke, D. Gurian-Sherman, *Ann. Rev. Phytopathol.*, **2001**, *39*, 225–258.
[4] J. Jia, S. Lu, *Microorganisms*, **2024**, *12* (1), 100.

Nanobody-Derived Peptidomimetics as Selective μ-Opioid Receptor Ligands

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The μ-opioid receptor (μOR), a prototypical member of the G protein-coupled receptor (GPCR) family, is the molecular target of opioid analgesics such as morphine and fentanyl. Due to the limitations and severe side effects of currently available opioid drugs, there is considerable interest in developing novel modulators of μOR function. In a recent study, we reported NbE to selectively binds to and antagonizes the μOR [1]. However, the macromolecular size of NbE may limit its tissue distribution, and consequently, the therapeutic potential of NbE. To address this, we focused on the design and characterization of peptide analogues (peptidomimetics) derived from the core interaction region of NbE. The shortest peptide was composed of the four residues that form the β-turn segment at the tip of CDR3, and the subsequent peptides were of increasing length. Here we report that head-to-tail cyclic peptides demonstrate improved affinity for μOR, inhibit μOR-mediated signaling and retain μOR selectivity. This project shows the structural optimizations that lead to peptides with antagonistic activity comparable to the parental nanobody NbE.

[1] J. Yu *et al.*, "Structural basis of μ-opioid receptor targeting by a nanobody antagonist," *Nat. Commun.*, vol. 15, no. 1, p. 8687, Oct. 2024, doi: 10.1038/s41467-024-52947-6.

Leech-Inspired Bivalent Peptides for Multi-Target Modulation of Host-Defense Responses

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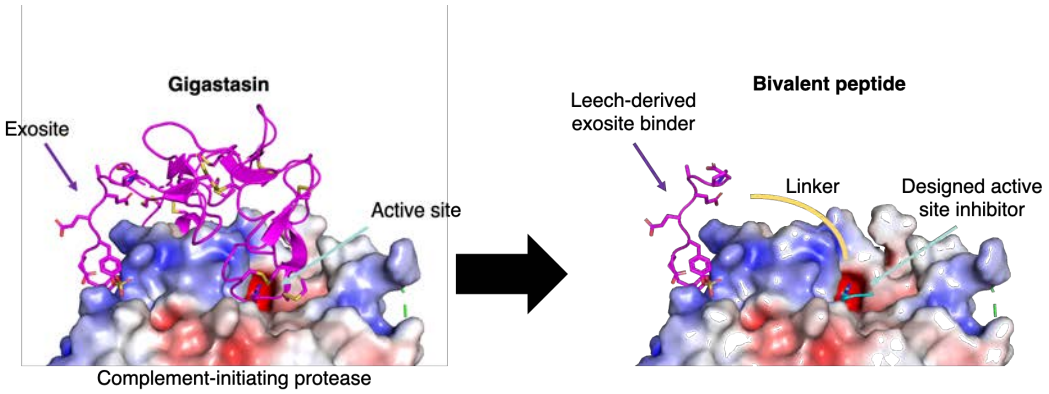
The complement and coagulation systems are blood-based proteolytic cascades that are essential for innate immunity and maintaining hemostasis. Though tightly controlled by the action of various serine proteases, dysregulation of both systems can occur simultaneously and lead to thromboinflammation - creating a need for new therapeutic strategies.[1] Leeches have evolved to secrete proteins that inhibit complement and/or coagulation through simultaneous blockade of multiple proteases, but issues regarding production, PK, and immunogenicity may limit their direct application as therapeutics. Inspired by nature, we have utilized structural insights of gigastasin, a protein from the giant Amazon leech, in complex with a protease target as a template to design bivalent peptides capable of mimicking its mechanism of action.[2]



We first designed macrocyclic peptides capable of blocking the protease active site in a substrate-like binding mode. These peptides were then conjugated to a sulfated peptide derived from gigastasin that blocks a functional exosite on the proteases. The resulting bivalent peptides, termed gigalirudins, synergistically inhibit complement activation at low micromolar concentrations in human serum via both the classical (antibody-mediated) and lectin (glycan-induced) pathways, by blocking homologous serine proteases. The atomistic control of peptides can enable precision tuning of target selectivity profiles beyond the constraints of the natural protein. Therefore, we employed structure-guided tuning of the active-site inhibitor to develop the SCIP-34 macrocyclic peptide. With nanomolar C1s inhibition and exquisite selectivity amongst the off-target proteases, SCIP-34 was demonstrated to be a specific inhibitor of the classical complement pathway in human serum. Macrocyclic peptides with various target selectivity profiles will be grafted into the bivalent gigalirudin scaffold, with the aim to create a family of peptides with precisely defined target selectivity profiles against blood proteases. Collectively, this work illustrates the potential of designing peptides inspired by naturally-evolved proteins – yielding functionality difficult to achieve using de-novo discovery efforts.

[1] E. Rawish, et. al., Br. J. Pharmacol., **2021**, 178(**14**), 2892-2904.
[2] S. Pang, et. al., J Immunol, **2017**, 199(**11**), 3883-3891.

Investigating Ligand Binding and Activation of the NPFFR1

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The neuropeptide FF receptor 1 (NPFFR1) belongs to the family of G protein-coupled receptors (GPCRs) and is involved in the perception of pain and the regulation of the hypothalamic-pituitary gonadal axis.[1] The receptor can be activated by several neuropeptides carrying a C-terminal RF-amide motif, including neuropeptide FF (NPFF) and neuropeptide VF (NPVF). NPFFR1 is discussed as a potential target for the treatment of pain and cardiovascular diseases. However, selective targeting is challenging due to the highly conserved binding pocket in related GPCRs, including the NPFFR2. Recently we identified the small molecule hederagenin as selective NPFFR1 antagonist, highlighting mechanisms for subtype-selectivity.[2] Investigating receptor activation and ligand binding in more detail is crucial for the development of selective therapeutics.

We present a bioluminescence resonance energy transfer (BRET)-based ligand binding assay for the NPFFR1, using a nanoluciferase (Nluc)-tag at the N-terminus of the receptor as energy donor and fluorescently-labeled peptides as acceptor. This cell based assay does not only allow quantification of ligand binding, but also gives information on the orientation of the bound peptide, as an appropriate proximity of donor to acceptor is required for efficient energy transfer. Our results indicate distinct binding modes of NPFF and NPVF at the NPFFR1, despite their high sequence identity. Investigating the binding modes of different ligands at the NPFFR1 does not only contribute to the understanding of ligand-receptor interactions and subtype selectivity but also facilitates rational drug design targeting nociceptive signaling pathways and cardiovascular diseases.

[1] S. Ayachi, F. Simonin, *Front. Endocrinol.*, **2014**, *5*, 158.
[2] H. Lentschat, F. Liessmann, C. Tydings, T. Schermeng, J. Stichel, N. Urban, M. Schaefer, J. Meiler, A. G. Beck-Sickinger, *Angew. Chem. Int. Ed.*, **2024**, *64*, e202417786.

Assembly and Oligomerization Dynamics of Short Peptides: Insights from LL-37 Fragments and Diphenylalanine Derivatives

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The self-assembly behavior of short peptides is fundamental to their biological functions and potential therapeutic applications. This talk integrates insights from two investigations: one examining the oligomerization dynamics of LL-37 truncated fragments using α-hemolysin nanopores, mass spectrometry, and molecular dynamics simulations, and the other exploring the assembly transitions of diphenylalanine (FF) and its analogs. The former reveals that LL-37 fragments form transient oligomeric states, with their stability and dynamics elucidated at the single-molecule level. The latter demonstrates that FF and its derivatives undergo a transition from monomeric forms to oligomer equilibrium and nanocluster assemblies, influenced by subtle modifications in peptide structure. Collectively, these findings underscore the critical role of peptide sequence and environmental conditions in dictating assembly pathways, offering valuable perspectives for the design of peptide-based nanomaterials and therapeutics.

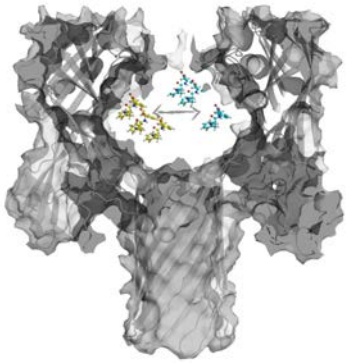

Figure 1. The structural model of α-hemolysin nanopore and oligomers formed by short peptides

References:

[1] Liu, C., Dan, Y., Yun, J., Adler-Abramovich, L., & Luo, J, ACS nano, **2025**, *19*, 13250–13263.
[2] Liu, C., Henning-Knechtel, A., Österlund, N., Wu, J., Wang, G., Gräslund, R. A. O., Kirmizialtin, S., & Luo, J, Small (Weinheim an der Bergstrasse, Germany), **2023**, *19*, e2206232.

Characterization and structure elucidation of class III and IV synthetases, proteases, and precursor peptides in lanthipeptides

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Lanthipeptides are an important group of ribosomally synthesized and post-translationally modified peptides produced by bacteria that exhibit potent antimicrobial, antiviral, morphogenic and antinociceptive properties. These properties make them potential therapeutic candidates.

The genes for their biosynthesis are organized in clusters and code for precursor peptides, modifying enzymes and transport proteins. Lantibiotic synthetases are specialized enzymes involved in the biosynthesis of lanthipeptides, a class of peptide antibiotics characterized by the presence of unusual amino acids such as labionin, lanthionine and methyllanthionine (Figure 1).

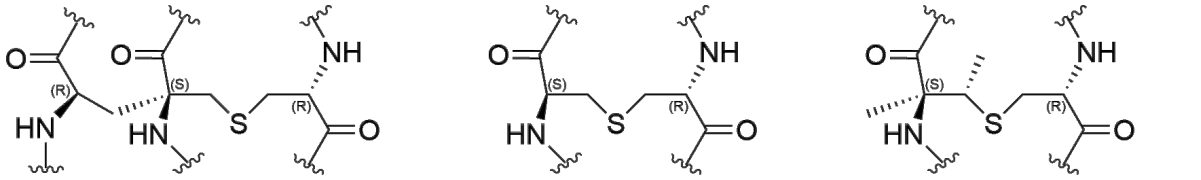

Figure 1: Chemical structure of unusual amino acids labionin, lanthionine and methyllanthionine.

Lantibiotics are categorized into five major classes based on their structure and the type of modification they undergo during biosynthesis. Our work focuses on classes III and IV. These two classes assign each catalytic step to a specialized domain and thus include a kinase (phosphorylation), a lyase (elimination) and a cyclase domain (thioether formation). In addition, class IV enzymes require Zn^{II} as a cofactor.

The aim of the project is to investigate homologous lanthipeptide gene clusters in different strains with regard to the biosynthetic pathway and the structure of the precursor peptide, the protease and the modification enzyme.

[1] J. D. Hegemann and R. D. Süssmuth, *RSC Chem. Biol.* **2020**, *1*, 110–127.
[2] Y. Goto, A. Ökesli, and W. A. van der Donk, *Biochemistry* **2011**, *50*, 891–898.
[3] Arnar Sigurdsson et al. , *Angew. Chem. Int. Ed.* **2023**, *62*, e202302490.
[4] Sara M. Eslami and Wilfred A. van der Donk, *ACS Bio Med Chem* **2024**, *4*, 20–36

Nature-Inspired Protection: Engineering Complement-Resistance via Surface-Tethered M22 Peptidomimetics

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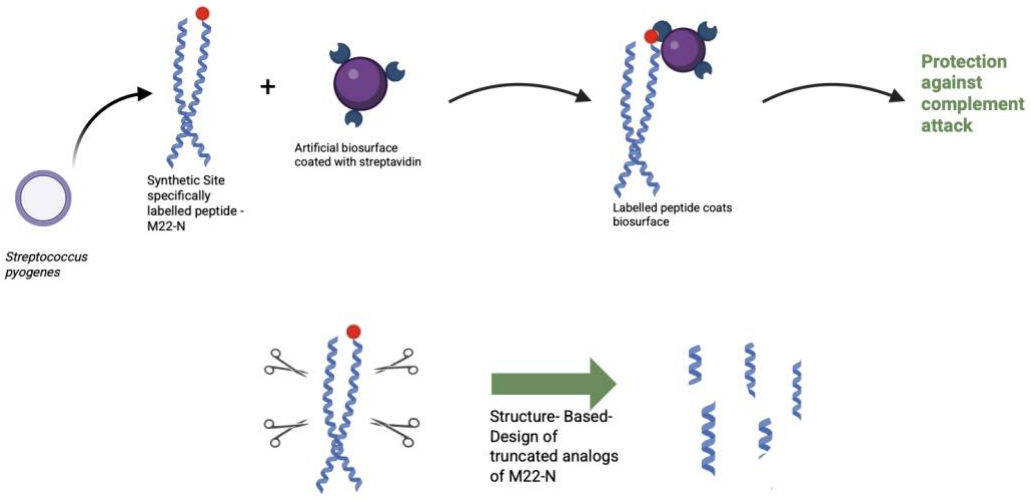
The complement system plays a crucial role in immune defense, contributing to both innate and adaptive immunity. However, because complement can also harm healthy human cells, its activity is tightly regulated by various membrane-bound and soluble factors. Complement activation can also trigger unintended attacks on non-self surfaces, such as transplanted organs. Preventing complement-mediated injury to cells—for instance, during transplant rejection—is a pressing clinical challenge, yet current therapeutic options have shown limited success. This work aims to take inspiration from nature to develop new strategies to protect biosurfaces from complement-mediated attack.

Several pathogens have been identified to recruit a potent soluble **inhibitor** of the Classical and Lectin pathway of the Complement System called C4b Binding Protein (C4BP), bypassing thus the complement system attack when entering the human body. [1] *Streptoccocus pyogenes* binds to C4BP via a hyper-variable domain in its M protein (M22), escaping complement-mediated opsonization and phagocytosis. Previously, a synthetic 52 aminoacids homodimeric peptide derived from M22 (M22-N), has been shown to bind to C4BP. [2] Therefore, we synthesized site specifically labelled M22-N to enable capture onto artifical biosurfaces, mimicicking the natural presentation on bacterial cells. Flow cytometry analysis demonstrated that the M22-N-decorated surface can efficiently recruit C4BP from normal human serum, and prevents complement mediated attack of the model biosurface. Furthermore, we also characterized target interaction kinetics between immobilized M22-N and soluble C4BP. Based on these insights, our goal is to utilize structure guided design to develop shorter, more stable M22 peptidomimetics, with improved physicochemical properties suitable for surface coatings. Therefore, we aim to develop a new artificial coating strategy for mitigating complement damage of biosurfaces, by mimicking mechanisms employed in nature.



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[1] Ermert, D., & Blom, A. M. (2015). C4b-binding protein: The good, the bad and the deadly. Novel functions of an old friend. *Immunology Letters*, *169*, 82–92. https://doi.org/10.1016/j.imlet.2015.11.014

[2] Engberg, A. E., Sandholm, K., Bexborn, F., Persson, J., Nilsson, B., Lindahl, G., & Ekdahl, K. N. (2009). Inhibition of complement activation on a model biomaterial surface by streptococcal M protein-derived peptides. *Biomaterials*, *30*(13), 2653–2659. https://doi.org/10.1016/j.biomaterials.2009.01.001

The natural diversity of acyltransferases reveals versatility and specificity in the synthesis of gene-encoded lipopeptides

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Lipopeptides are lipid–peptide conjugates that can be naturally produced or chemically synthesized. They exhibit diverse bioactive functions, which are strongly dependent on the chain length and identity of the lipid appendage and are widely used in health care and industrial applications [1]. Despite their versatility, the production and diversification of lipopeptide compounds continue to be a challenge for state-of-the-art biological and chemical synthesis. To offer a streamlined and adaptable route to lipopeptides, we exploit biocatalysts from ribosomally synthesized and post-translationally modified peptide (RiPPs) natural product pathways. The primary structure of RiPPs is determined by a genetically encoded precursor protein, which is modified by post-translational enzymes co-encoded together in biosynthetic gene clusters [2]. The crucial lipidation reaction is catalyzed by a novel class of maturases of the GCN5-related N-acetyltransferase (GNAT) superfamily, which introduce medium-to-long chain-length fatty acids onto the side chain amino group of lysines or arginine-derived ornithines (Fig. 1) [3].

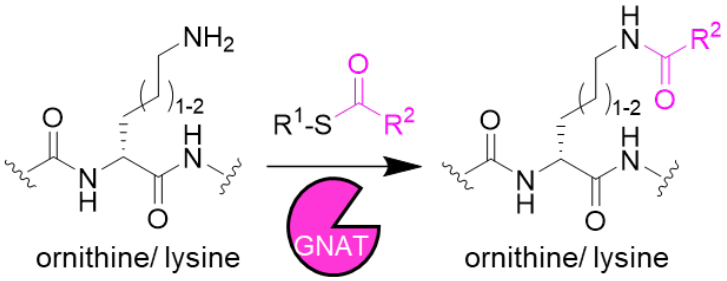

Fig. 1. Transfer of fatty acids to the sidechain of ornithines and lysines by RiPP GNATs.

Here we present a survey of the natural diversity of this GNAT family and their corresponding gene-encoded lipopeptides. By characterizing the substrate scope of selected GNATs with their precursor peptides, we could show that various fatty acids of C10 to C18 chain lengths can be specifically introduced into diverse peptide sequence contexts. Ongoing research efforts focus on developing this enzyme class as biocatalysts for custom peptide engineering of bioactive lipopeptides. We envision that this synthetic biology platform will facilitate the targeted and library-based production of lipopeptides for human health, including antimicrobial lipopeptides and non-ribosomal lipopeptide mimics.

[1] Hamley, I. W., *Chem. Comm.*, **2015**, *51* (41), 8574-8583.
[2] Montalbán-López, M., et al., *Nat. Prod. Rep.*, **2021**, *38*, 130-239.
[3] Hubrich, F. & Bösch, N. M., et al., *PNAS*, **2022**, *119* (3), e2113120119.

Peptide Coatings on Living Cell Surfaces – Combining Metabolic Glycoengineering with Click Chemistry

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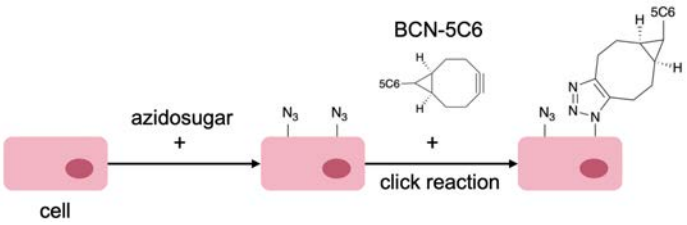
Contact of biomedical surfaces with the human complement system can cause severe inflammation and loss of function, resulting in severe risks for the health of patients. To mitigate the danger of complement activation, soluble complement regulators can be recruited to such vulnerable surfaces. Factor H (FH) is a natural regulator of complement amplification that natively recognizes host cells. The cyclic peptide 5C6 binds to FH and, when coated on surfaces, has been shown to impair complement activation.[1] However, a lack of biocompatible peptide coating strategies has restricted the evaluation of 5C6-coatings on living surfaces such as allo- and xenotransplant models. Herein, we demonstrated the modification of human endothelial cells (HMEC-1) and porcine endothelial cells (PIEC) with 5C6 using metabolic glycoengineering and click-chemistry. Additionally, we demonstrate that the protection of complement-sensitive bacteria with peptide coatings can serve as a complement-activating surface model for therapeutics screening.



Decoration of living cell surfaces with 5C6 could be accomplished using metabolic glycoengineering in combination with clickable peptides without impairing cell viability. Upon 5C6 coating, FH was recruited onto endothelial cells, as evaluated with flow cytometry and verified by fluorescence microscopy. Coating of complement-sensitive surfaces led to reduced complement biomarkers in the case of PIEC and increased survival of coated bacteria when compared to control cells.
Our modification strategy validates the application of peptide coatings on living surfaces to recruit complement regulators and impair immune-mediated complications. Due to the facile modification with strained alkynes, different peptides and tethering strategies can be assessed in the established test systems. The expansion of the screening platform to living cell surfaces can help to evaluate protective peptide coatings and improve the therapeutic outcome of allo- and xenotransplantations.

[1] C. Bechtler et al., *Acta Biomater.*, **2023**, *155*, 123-138.

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

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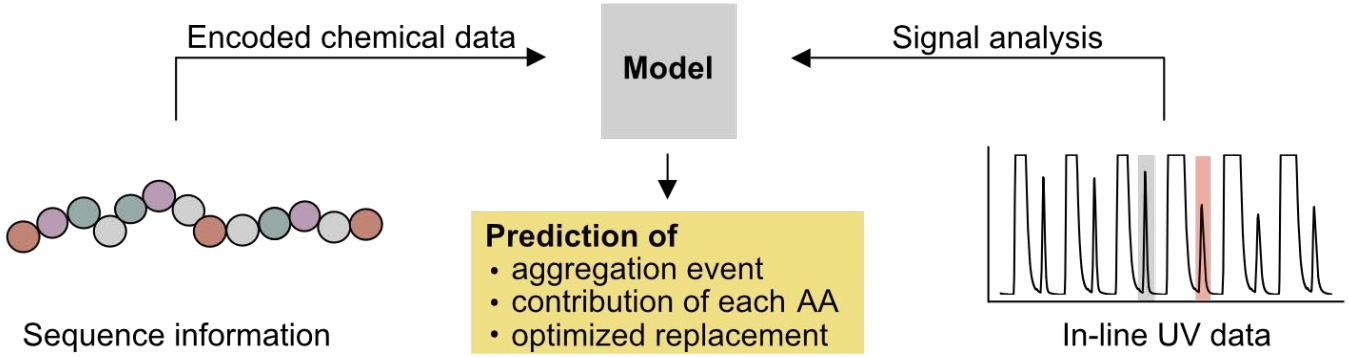
Peptide aggregation is a long-standing challenge in chemical peptide synthesis, limiting its efficiency and reliability. Although data-driven methods have enhanced our understanding of many sequence-based phenomena, no comprehensive approach addresses so-called “non-random difficult couplings” (generally linked to aggregation) during solid-phase peptide synthesis. Here, we leverage existing peptide synthesis datasets, supplemented with newly acquired experimental data, to build a predictive model that deciphers the role of individual amino acids in triggering aggregation. First, we identified and experimentally validated composition-dependent aggregation as a stronger predictor than sequence-based patterns. This insight enabled the development of a composition vector representation, allowing insights into the aggregation propensities of individual amino acids. Applying an ensemble of trained models, we predict the aggregation properties of peptides and recommend optimized synthesis conditions. By elucidating each individual amino acid’s influence, this method holds the potential to accelerate synthesis optimization through existing data, offering a robust framework for understanding and controlling peptide aggregation.



[1] Tamás B, Alberts M, Laino T, Hartrampf N. Amino Acid Composition drives Peptide Aggregation: Predicting Aggregation for Improved Synthesis. ChemRxiv. **2025**; doi:10.26434/chemrxiv-2025-wjbmv

Overcoming the Species Specificity of Cyclic Peptide Compstatin and Analogs: mRNA Display Screening for the Identification of Translationally-Active Analogs

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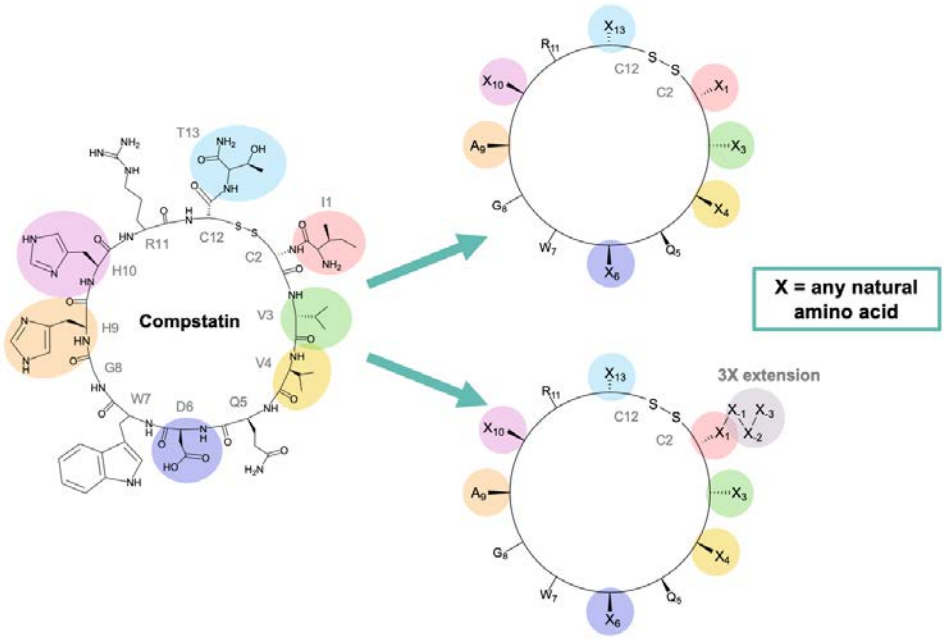
Compstatin is a cyclic tridecapeptide acting as a protein-protein interactor inhibitor for complement component C3. Studies have been performed to optimize its activity and specificity, leading to the approval of its analog pegcetacoplan (Empaveli/Syfovre, Apellis) and to the clinical testing of Cp40/AMY-101 (Amyndas) for complement-mediated diseases. The main limitation of these peptides is represented by their species specificity, reduced to humans and non-human primates, and significantly affecting the possibility of performing preclinical studies in murine and rodent animal models. Therefore, development of new compstatin analogs with wider species tolerance is crucial for more thorough translational research.



Consequently, a mRNA display library encoding a compstatin analog with randomized amino acids at fixed positions has been screened in alternating rounds of selection against rat and human C3. The presence of a N-terminal extension of three random amino acids has additionally been tested to estimate the potential improvement in affinity. Sequences retrieved from next-generation sequencing will be tested in SPR and ELISA to assess the affinity and inhibition efficacy of the selected peptides.

[1] Sahu, A., B.K. Kay, and J.D. Lambris, *Inhibition of human complement by a C3-binding peptide isolated from a phage-displayed random peptide library.* J Immunol, 1996. **157**(2): p. 884-91.
[2] Lamers, C., et al., *Insight into mode-of-action and structural determinants of the compstatin family of clinical complement inhibitors.* Nat Commun, 2022. **13**(1): p. 5519.
[3] Qu, H., et al., *Novel analogues of the therapeutic complement inhibitor compstatin with significantly improved affinity and potency.* Mol Immunol, 2011. **48**(4): p. 481-9

[4] Sahu, A., D. Morikis, and J.D. Lambris, *Compstatin, a peptide inhibitor of complement, exhibits species-specific binding to complement component C3.* Mol Immunol, 2003. **39**(10): p. 557-66.

Proteomics Analysis of the Effects of a Therapeutic Peptide, G3, on Glioblastoma

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Glioblastoma is a lethal type of brain tumour killing most patients within 17 months and it accounts for ~50% of all brain tumours. There are few effective options for patients and in the US alone there are ~15,000 new cases per year. Peptide-based therapeutics are gaining momentum in oncology due to their precision and reduced off-target toxicity. The G3 peptide has demonstrated anticancer potential in several tumour types, yet its applicability in glioblastoma (GBM), a highly heterogeneous and aggressive brain tumour remains under-explored. GBM consists of glioblastoma stem-like cells (GSCs) and differentiated glioblastoma cells (DGCs), and the GSC are usually the reason for death after chemotherapy. We explored the potential of G3 targeting these subpopulations and determined its therapeutic potential.

In this study, label-free quantitative proteomics was employed to characterize the protein-level changes induced by G3 treatment in GSCs and DGCs. STRING and Gene Ontology analyses were used to explore the subcellular localization and cellular compartment enrichment of significantly regulated proteins. G3-treated GSCs showed upregulation of proteins associated with extracellular compartments and organization, suggesting enhanced interactions with the tumour microenvironment. In contrast, upregulated proteins in DGCs were enriched in intracellular compartments such as lysosomes, endoplasmic reticulum, vacuoles and vesicles, indicating shifts in vesicle trafficking and membrane-associated processes.

These results show that G3 induces distinct proteomic responses in different GBM cell populations, pointing to its ability to influence both the cellular microenvironment and intracellular signalling pathways. This dual action highlights the therapeutic promise of peptides like G3—not only in broadly suppressing tumour activity but also in targeting specific subpopulations within the tumour. Together, these insights support the further development of G3 and similar peptides as flexible tools in GBM treatment, including potential use in combination therapies.

[1] M. Price, C. Ballard, J. Benedetti, C. Neff, G. Cioffi, K. A. Waite, C. Kruchko, J. S. Barnholtz-Sloan, Q. T. Ostrom, *Neuro Oncol* **2024**, *26*, vi1, DOI: 10.1093/NEUONC/NOAE145.
[2] H. Acar, S. Srivastava, E. J. Chung, M. R. Schnorenberg, J. C. Barrett, J. L. LaBelle, M. Tirrell, *Adv Drug Deliv Rev* **2017**, *110–111*, 65, DOI: 10.1016/J.ADDR.2016.08.006.
[3] R. Hadianamrei, M. A. Tomeh, S. Brown, J. Wang, X. Zhao, *J Colloid Interface Sci* **2022**, *607*, 488, DOI: 10.1016/j.jcis.2021.08.200.
[4] S. Cirillo, M. A. Tomeh, R. N. Wilkinson, C. Hill, S. Brown, X. Zhao, *ACS Appl Mater Interfaces* **2021**, *13*, 49713, DOI: 10.1021/acsami.1c14761.

Origamins — Hypermodified peptides in human symbionts and pathogens

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Origamins are a new family of ribosomally synthesized and post-translationally modified peptides (RiPPs). The core genes of an origamin cluster encode a precursor peptide with an interlocked NX_5N motif, a radical SAM epimerase, and an *N*-methyltransferase. The encoded enzymes produce hypermodified peptides containing an alternating LD-amino acid pattern, with all asparagine side chains being *N*-methylated. For a *Polymorphum gilvum* cluster, only three encoded maturases introduce up to 43 posttranslational modifications. NMR studies have shown that the final hypermodified peptide adopts a *β*-helical secondary structure, and the first bioactivity studies suggest cytotoxic bioactivity of this compound.

Genome mining based on epimerases and the interlocked NX_5N motif revealed that origamin clusters are enriched in human-associated bacteria, predominantly within the gut microbiome. Interestingly, origamin clusters were identified in both important commensal microorganisms (e.g., *Bifidobacterium longum*, *Actinomyces oris*) and known human pathogens (e.g., *Clostridium* sp., *Escherichia coli*) with clinical relevance. Little is understood about the ecological function of these hypermodified peptides and their role in the human microbiome. Most origamin clusters include an additional immunity protein, which has been shown to be crucial for avoiding the autotoxicity of the final product for its producing organism. This provides initial indications that some of the products might serve as lead compounds for new narrow-spectrum antibiotics. Additionally, the production of potential antimicrobial peptides by symbionts is particularly interesting, as it will enhance our understanding of the complex network involved in defending against pathogens.

The goal of the project is to investigate the ecological role of the origamin natural products in the human body. Therefore, we aim to heterologously express the clusters to study the dependencies of the different maturation steps *in vivo* and obtain the modified peptides for bioactivity testing and structural analysis.

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

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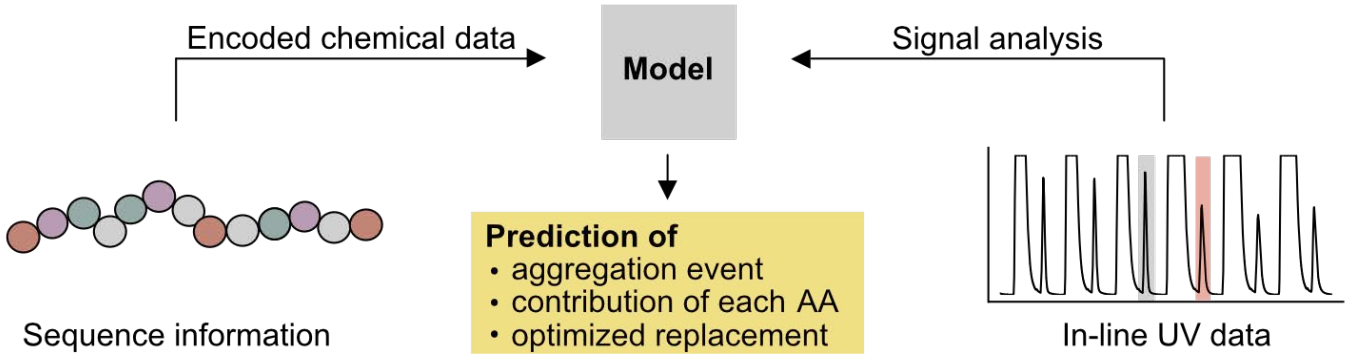
Solid-phase peptide synthesis (SPPS) is a standard technique for peptide production, however, so called "non-random difficult couplings" often limit efficient amino acid coupling. While data-driven approaches have improved our understanding of sequence-dependent behaviors, there is still no comprehensive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



[1] Tamás, B.; Alberts, M.; Laino, T.; Hartrampf, N.; Amino Acid Composition drives Peptide Aggregation: Predicting Aggregation for Improved Synthesis; *ChemRxiv* **2025**. *doi:10.26434/chemrxiv-2025-wjbmv.*

Peptide Therapeutics Forum 2025

Discovery of an endogenous type-I Interferon inhibiting peptide

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The interferon (IFN) system is an integral part of innate immunity and a powerful barrier against invading pathogens such as viruses. However, aberrant or chronic activation of the IFN system causes detrimental inflammation, driving pathology of virus-induced cytokine storms, chronic infections or auto-inflammatory diseases like type I interferonopathies. Currently, there are only nonspecific immunosuppressive compounds in the clinics, such as corticosteroids or JAK/STAT inhibitors, which cause severe side effects, or costly but specific neutralizing antibody treatments. Thus, there is a dire need for novel specific innate immune downmodulating compounds.

Here, we discover a 10 amino acid peptide from a human hemofiltrate peptide library called DF01. DF01 is derived from the mucosa-specific BPI fold-containing family B member 1 (BPIFB1). Transcriptomics analyses show a remarkable specificity of the peptide against type I interferon signaling, with the closely related type II IFN and NfκB being unaffected. DF01 inhibits IFN-β induced signaling with an IC_{50} in the low micromolar range (IC_{50} = 74μM). Localization analysis revealed that the peptide is taken up by the cell. Interestingly, the peptide forms amyloid-like structures *in vitro,* as shown in electron microscopy and confirmed via amytracker staining. Chemical denaturation of the secondary structure using hexafluoro-2-propanol results in a loss of function.

In summary, our data identify DF01 as an endogenous, highly specific anti-type I IFN peptide, which forms amyloid-like structures. Due to its specificity, it may be developed into a future therapeutic approach against type I IFN-dependent auto-inflammatory diseases such as type I interferonopathies.

Novel Peptide Targeting CXCR4: Rational Design and characterization through Computational Modeling

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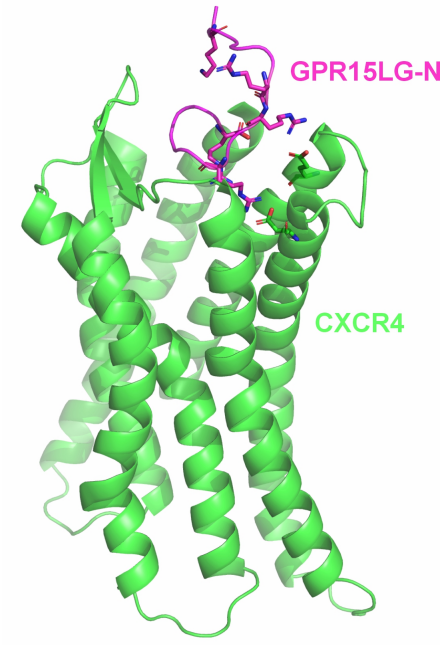
CXCR4 regulates immune cell trafficking and cancer metastasis. Recent evidence suggests a connection between the G Protein-Coupled Receptor 15 Ligand (GPR15LG) and CXCR4 signalling, prompting us to design GPR15LG-derived peptides to target CXCR4. Starting from the full-length GPR15LG, we performed Gaussian accelerated molecular dynamics (GaMD) simulations to explore its binding to CXCR4. These results guided the design of an N-terminal peptide derivative, GPR15LG-N, which exhibited conserved binding to CXCR4. This binding was characterized by the interactions of $R12_{GPR15LG-N}$ and $R14_{GPR15LG-N}$ with $D262_{CXCR4}$ and $E277_{CXCR4}$, respectively. By combining potential of mean force (PMF) maps, obtained through energy reweighting, with predictions from PPI-Affinity, our machine learning-tool, we ranked the peptide variants and identified key residues at the interaction interface. Our integrated *in silico* approach, combining GaMD simulations with machine learning-based binding affinity predictions, allows identifying and engineering peptide derivatives towards therapeutic applications.



Trypstatin as a Novel TMPRSS2 Inhibitor with Broad-Spectrum Efficacy Against Corona and Influenza Viruses

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Respiratory viruses such as SARS-CoV-2 and influenza virus rely on host proteases, notably TMPRSS2, for cell entry, positioning TMPRSS2 as an attractive antiviral target. Here, we describe the identification and characterization of Trypstatin, a 61-amino acid Kunitz-type protease inhibitor identified from a screening of a human hemofiltrate-derived peptide library. Trypstatin potently inhibits TMPRSS2 and related proteases, with half-maximal inhibitory concentrations in the nanomolar range. Notably, Trypstatin demonstrates superior potency compared to aprotinin, a protease inhibitor currently administered by inhalation for the treatment of influenza virus infections. *In vitro* assays demonstrated that Trypstatin

Figure 1: Structure of Trypstatin (PDB 4u30)

effectively blocks spike-mediated entry of SARS-CoV-2, SARS-CoV-1, MERS-CoV, and hCoV-NL63, as well as hemagglutinin-driven entry of influenza A and B viruses. In primary human airway epithelial cultures, Trypstatin markedly reduced SARS-CoV-2 replication and maintained activity in the presence of airway mucus. *In vivo*, intranasal administration of Trypstatin to SARS-CoV-2-infected Syrian hamsters significantly lowered viral titers and mitigated clinical symptoms. These results underscore Trypstatin's promise as a broad-spectrum antiviral agent targeting TMPRSS2-dependent respiratory viruses.

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

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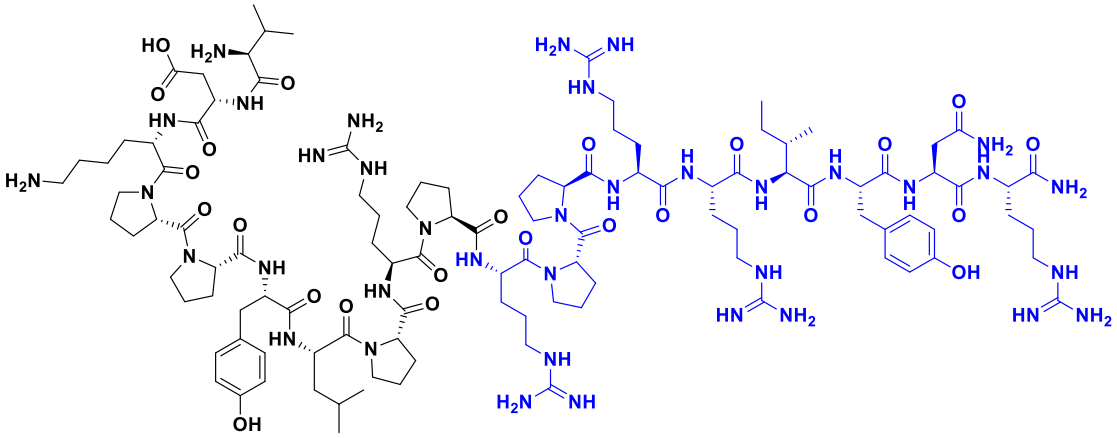
The escalating prevalence of multidrug-resistant Gram-negative bacterial infections necessitates the exploration of novel antimicrobial agents. Proline-rich antimicrobial peptides (PrAMPs) have emerged as promising candidates due to their unique mechanisms of action. Among them, Oncocin (VDKPPYLPRPRPPRRIYNR-NH_2), a synthetic derivative inspired by peptides from the milkweed bug Oncopeltus *fasciatus*, has demonstrated potent antibacterial activity against *K. pneumoniae* and *E. coli* by inhibiting bacterial ribosome (Figure 1).[1,2]

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



L-Oncocin: MIC *(A.baumannii): 64 µg/mL*
L-Oncocin-peptoid hybrid: MIC *(A.baumannii)*: *4 µg/mL*

Figure 1. Chemical structure of L-Oncocin.

[1] Seefeldt, A. C., *et al*. The proline-rich antimicrobial peptide Onc112 inhibits translation by blocking and destabilizing the initiation complex. *Nat. Struct. Mol. Biol.*, **2015**, *22*, 470–475.
[2] Roy, R. N., *et al*. The mechanism of inhibition of protein synthesis by the proline-rich peptide oncocin. *Nat. Struct. Mol. Biol.*, **2015**, *22*, 466–469.
[3] Gan, B. H., *et al*. Stereorandomized Oncocins with Preserved Ribosome Binding and Antibacterial Activity. *J. Med. Chem.* **2024**, *67*, 19448-19459.

N-Ethyl Cysteine linkerfor improved Solid-Phase Peptide Synthesis and Native Chemical Ligation

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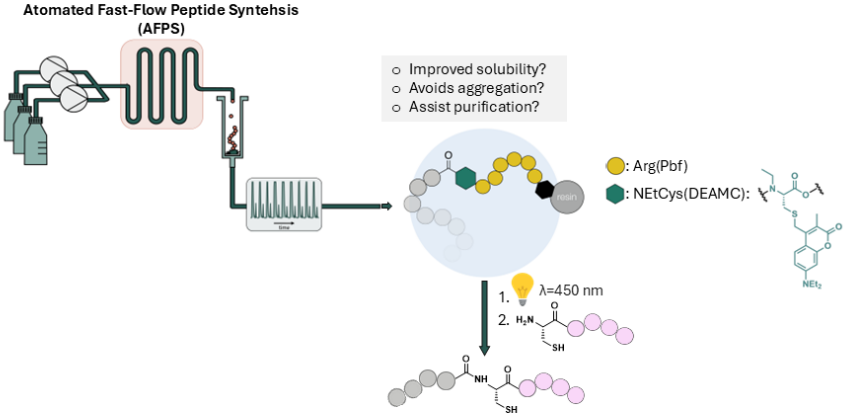
Solid-phase peptide synthesis (SPPS) and native chemical ligation (NCL) are key tools for peptide and protein synthesis. Recent advances such as Automated Fast-Flow Peptide Synthesis (AFPS)[1] have significantly reduced synthesis time and expanded the accessible length of synthetic peptides. However, poor solubility and sequence-dependent aggregation during synthesis often complicate purification and reduce coupling efficiency, limiting access to certain peptides. To address this, the SynTag strategy was developed, combining six arginines [(Fmoc-Arg(Pbf)-OH)$_6$ or R$_6$] with the Dawson linker (Fmoc-MeDbz-OH) to improve solubility, purification, and enable downstream NCL.[2] Still, MeDbz requires activation with *p*-nitrophenyl chloroformate, a step prone to side reactions and impurities.

We have developed a simple, additive-free on-resin cyclization method based on N⟶S acyl migration and intramolecular NCL using N-Ethyl-Cysteine (NEtCys), enabling efficient synthesis of cyclic peptides in good yield and purity without thiol additives or activating reagents.[3] Given that *N*-alkylated amino acids like NEtCys can reduce aggregation by influencing peptide conformation,[4] we propose an NEtCys-R$_6$ linker as an alternative to SynTag. To ensure orthogonality, NEtCys is protected with the photolabile DEAMC group,[5] which also enhances UV detection for LC purification. After purification, DEAMC is removed by 450 nm light, triggering the N⟶S acyl shift under mildy acidic conditions to form a thioester for further ligation or cyclization. This method is fully orthogonal to SynTag and broadens the chemical synthesis toolbox for accessing aggregation-prone peptides.



(1) 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.; *Science* **2020**, *368* (6494), 980–987.
(2) Bürgisser, H.; Williams, E. T.; Lescure, R.; Premanand, A.; Jeandin, A.; Hartrampf, N. *J. Am. Chem. Soc.* **2024**, 146, 50, 34887–34899
(3) Serra, G.; Posada, L.; Hojo, H. *Chem. Commun.* **2020**, *56* (6), 956–959.
(4) Jwad, R.; Weissberger, D.; Hunter, L. *Chem. Rev.* **2020**, *120* (17), 9743–9789.
(5) Kambanis, L.; Chisholm, T. S.; Kulkarni, S. S.; Payne, R. J. *Chem. Sci.* **2021**, *12* (29), 10014–10021.

Exploring microproteins through chemical protein synthesis: KRASIM as KRAS interaction partner

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Microproteins are single-domain proteins typically less than 100 amino acids long, encoded by small Open Reading Frames (smORFs) of long, supposedly non-coding RNAs (lncRNA). Largely overlooked for many years, microproteins are now garnering increasing attention due to their involvement in a wide range of biological functions in both humans and plants. Consequently, they are being recognized for their potential as therapeutic targets.[1] Flow-based protein synthesis enables the synthesis of microproteins and uniquely modified derivatives thereof, with the potential to include non-canonical amido acids, post-translational modifications (PTMs) and bio-orthogonal handles.[2] In 2022, the microprotein KRAS Interacting Microprotein (KRASIM) was identified by Ribosome Profiling as a 99 amino acids microprotein likely containing disordered and α-helical structural elements. Notably, it was reported as a KRAS interaction partner in hepatocellular carcinoma (HCC) cells, preventing the further down-stream signaling of the ERK pathway, and affecting RAS levels as well as cell growth upon overexpression.[3] However, the specific protein-protein interactions of KRASIM remain unvalidated by biophysical studies to date. Herein, we set out to synthesize full-length KRASIM as well as truncated versions thereof by flow-based protein synthesis, and evaluate the postulated interaction with KRAS by various biophysical assays. Our current data points toward an interaction between KRAS and the full-length microprotein KRASIM[1–99], but not truncated versions thereof. Further characterization of the interaction with RAS and potential additional interaction partners is ongoing with the ultimate goal of elucidating the biological role of KRASIM in KRAS regulation and cancer progression.

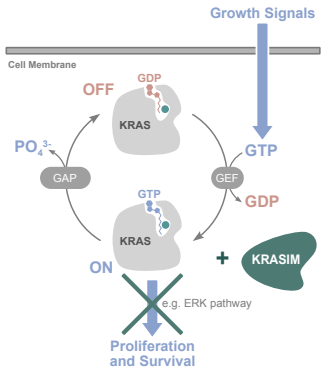

Figure 1 The reported KRASIM:KRAS interaction in HCC cells supposedly interferes with the down-stream signaling of the ERK pathway, preventing cell proliferation and survival. GEF= guanine nucleotide exchange factor, GAP = GTPase activating protein, GDP = guanosine diphosphate, GTP=guanosine triphosphate

[1] a) Bhati, K. K.; Blaakmeer, A.; Paredes, E. B.; Dolde, U.; Eguen, T.; Hong, S. Y.; Rodrigues, V.; Straub, D.; Sun, B.; Wenkel, S. *Cell. Mol. Life Sci.* **2018**, *75*, 2529; b) Hassel, K. R.; Brito-Estrada, O.; Makarewich, C. A. *IScience* **2023**, *26*, 106781.
[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**, *6494*, 980.
[3] Xu, W.; Deng, B.; Lin, P.; Liu, C.; Li, B.; Huang, Q.; Zhou, H.; Yang, J.; Qu, L. *Sci. China Life Sci*. **2020**, *63*, 529.

Computational engineering of peptides against prion induced toxicity

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Prion diseases are neurodegenerative disorders associated with the conversion of the cellular prion protein (PrP^{C}) into a pathologic conformer (PrP^{Sc}).

A proposed therapeutic approach to prevent the toxic transformation is to develop monoclonal antibodies that bind to PrPC and stabilize its structure[1]. Over the past years, peptide-based therapeutics are taking over the global market due to their high bioavailability, good efficiency, and specificity[2]. In particular, cyclic peptides have a long in vivo stability, while maintaining a robust antibody-like binding affinity, reduced accumulation propensities, cross the brain-blood-barrier and work on their targets very selectively[2].

Here, we introduce and computationally validate a novel approach toward the de novo design of cyclic peptides against prion-induced toxicity by combining rational design with molecular dynamics simulations[2]. First, we rationally design cyclic peptides starting from the crystal structures of PrP^{C} in complex with monoclonal antibodies. Next, we employ molecular dynamics simulations to probe the structural stabilities of the individual peptides and to determine their binding affinities to the cellular prion protein.

This work sheds light on the physical and structural mechanisms of PrPC-peptide interactions and offers the molecular basis for the development of new therapeutics against neurodegenerative diseases.

REFERENCES:

1. Polymenidou, M. et al. The POM Monoclonals: A comprehensive set of antibodies to non-overlapping prion protein epitopes. PLoS One 3, (2008).

2. *manuscript in preparation*