



13th International Meeting on Antimicrobial Peptides
Faculty of Science, University of Split, Croatia
September 2 -4, 2026

Conference Co-Chairs:

Prof. Larisa Zoranić

Prof. Tomislav Rončević

University of Split



**University
of Split**

Conference Co-Organiser:

Prof. Alex Tossi, University of Trieste



#IMAP2026



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An international conference hosted by the Faculty of Science, University of Split and held under the auspices of The Protein & Peptide Science Group of the Royal Society of Chemistry and supported by the European Peptide Society

Wednesday September 2

08:00 Registration

09:15 **Welcome & Opening of Conference: Larisa Zoranic and Tomislav Roncevic**

AI / non-AI Supported Database Mining

Chair: Edwin Veldhuizen

09:30 L1: Antimicrobial Peptide *In Silico* Optimization Enables the Generation of Stabilized Bioinspired Products

Octavio Franco, Universidade Católica de Brasília

09:50 L2: AI-guided discovery of serum-synergistic antimicrobial peptides (SeSy-AMPs) active against multidrug-resistant *Escherichia coli*
Kai Hilpert, City St. George's University of London

10:10 L3: *In silico* design and experimental validation of novel peptides in the leaderless bacteriocin class; building a significant pipeline of novel antibiotic candidates
Vicky Bennett, Amprologix Ltd.

10:30 R1: Repurposing Anti-*Candida* Peptides Against WHO Priority Yeast Pathogens *Nakaseomyces glabrata* and *Candidozyma auris*
Ahmad Alhanich, Queen's University of Belfast

10:40 **Coffee**

AMP Biomaterials and on Surfaces

Chair: Carles Mas-Moruno

11:10 L4: Surface Immobilized Antimicrobial Peptides to Prevent Medical Device Infections
Jayachandran Kizhakkedathu, University of British Columbia

11:45 L5: Host Defence Peptide derived Self-Assembling Hydrogels to Combat Ocular Infections
Sanhita Roy, L.V. Prasad Eye Institute

12:05 L6: ESKAPEEs versus Cyclic AMPs: How to win the battle on surfaces
Marina Rautenbach, Stellenbosch University

12:40 **Lunch & Poster Session 1** (odd number posters)

Chair: Jayachandran Kizhakkedathu

14:40 L7: Multifunctional Biomaterials: Integrating Cell-Instructive and Antimicrobial Peptides for Tissue Regeneration
Carles Mas-Moruno, Universitat Politècnica de Catalunya

15:15 L8: Poloxamer P407 as a Multifunctional Adjuvant Enhancing Antimicrobial, Antibiofilm, and Anti-Inflammatory Activity of Colistin and P13#1
Ilaria Di Nardo, University of Naples "Federico II"

15:35 L9: Polymer-Based Nanoassemblies as Robust Delivery Platforms for Antimicrobial Peptides: Structural and Microbiological Characterization
Thomas Vogelaar, Roskilde University

15:55 **Coffee**

Non-ribosomal AMPs

Chair: Alethea Tabor

16:25 L10: Stereochemistry vs linker: exploration of ring C analogues of antimicrobial peptide nisin
Fiona Kinis, UCL

16:45 L11: Optimisation of Linear Non-Ribosomal Peptides that Target Gram-Negative Bacteria
Mary Lagan, Queen's University of Belfast

17:05 **Close of session**

17:30 **Group Photograph**

18:00 **Welcome Reception - Skybar**

Thursday September 3

AI / non-AI Supported Database Mining

Chair: Kai Hilpert

09:00 L12: From Sequence to Target Hypothesis: Systematic Docking of AMPs Against Essential Bacterial Cell Surface Proteins
Inanc Birol, University of British Columbia

Prediction, Design and Synthesis of AMPs

Chair: Lidia Feliu

09:35 L13: Structure-Guided Peptide Design with AMPAFold: Enabling Reliable Antimicrobial Peptide Discovery Beyond Sequence Space
Marc Torrent Burgas, Universitat Autònoma de Barcelona

09:55 L14: Random Peptide Mixtures as Antimicrobials: Bridging Synthetic Design and Natural Diversity
Zvi Hayouka, The Hebrew University of Jerusalem

10:15 L15: Peptide Synthesis: Spoiler Alert – It's Complicated
Samantha Bann, Queen's University of Belfast

10:35 R2: Synthesis and Antimicrobial Activity of Cyclic Temporin L Analogues via Non-Conventional Guanidinium-Based Functionalization
Ida Boccino, University of Napoli "Federico II"

10:45 **Coffee**

Chair: Kai Hilpert

11:15 L16: Early maturation of host antimicrobial peptide defences is associated with host-pathogen coexistence
Phil Jervis, Queen Mary University of London

11:35 R3: From Mitochondrial Targeting to Microbial Killing: Twinkle-Derived Peptides as a New Class of AMPs
Emanuela Galatola, University of Napoli "Federico II"

11:45 L17: From Food for Food: Prediction and Identification of Novel Food-Derived Antimicrobial Peptides for Food Preservation
Monika Pieschetsrieder, Universität Erlangen-Nürnberg

12:05 R4: Strategic Residue Substitution in Tolaasin for the Synthesis of New Bioactive Derivatives
Matthias Vanheede, Ghent University

12:15 R5: Black Vulture Gut-Derived LAB as a Source of Natural Antimicrobials Against Foodborne

Pathogens

Ana Clara Candelaria Cucick, University of Sao Paulo

12:25 **Lunch & Poster Session 2** (even number posters)

Mode of action

Chair: Marina Rautenbach

14:25 L18: Antimicrobial Peptides in Action: A Kinetic Perspective

Federico Carneri, Sapienza University of Rome

14:45 R6: A cetacean PrAMP bridging distinct ruminant peptide features to target ESKAPE pathogens.

Mario Mardirossian, University of Trieste

14:55 L19: Esculentin-Derived AMPs as Adjuvants to Overcome Multidrug Resistance in KPC-Klebsiella pneumoniae

Carlo Vetrano, Sapienza University of Rome

15:15 R7: Hybrid proline-rich antimicrobial peptides derived from Api137 and Chex1-Arg20 with dual modes of action

Paula Schliebe, Leipzig University

15:25 R8: Mechanistic Insights into Hemozoin Formation Inhibition by the Tyrocidines

Wian Vermeulen, Stellenbosch University

15:35 L20: Exploring amphiphilicity and hydrophilicity in antimicrobial peptide star polymers and their impact on biological activity

Thomas Roads, University of Melbourne

15:55 **Coffee**

Chair: Alex Tossi

16:25 R9: Functional Proteomics Reveals PilR As A Novel Molecular Target Of Temporin A In *Pseudomonas Aeruginosa*

Angela Di Somma, University of Napoli 'Federico II'

16:35 R10: Molecular Dynamics Simulations of Proline-rich Antimicrobial Peptides in their Ribosomal Binding Sites

Renke Itzenga, Leipzig University

16:45 R11: Evaluating the Mechanism of Uptake and Antimicrobial Efficacy of the Proline-Rich Antimicrobial Peptide PR-39

Hitha Muthirakkal, MS Ramaiah University of Applied Sciences

Applications of AMPs in Therapeutics

Chair: Lisa Ryan

16:55 L21: Novel Therapeutic Strategies Using AMPs to Treat and Prevent Infectious Diseases

Gill Diamond, University of Louisville School of Dentistry

17:30 L22: Weaponizing Bacterial Aminopeptidases for species-selective antibiofilm peptides

Clarissa Melo Czekster, University of St. Andrews

17:50 **Close of Session**

19:15 **Conference Dinner (pre-reserved only)**

Friday September 4

Applications of AMPs in Therapeutics, cont.

Chair: Gill Diamond

09:30 L23a / L23b: Cecacins from the chicken caecal microbiome: translational applications in bovine mastitis and tuberculosis

Linda Oyama, Queen's University of Belfast

10:00 R12: Therapeutic potential and safety challenges of antimicrobial peptides and peptidomimetics against ESKAPEE Pathogens: a systematic review with quantitative analysis

Elias Shiferaw Mekonen, University of New South Wales

AMP Structure and Membrane Interactions

Chair: Larisa Zoranic

10:10 L24: Expanding Microscale Thermophoresis to Study Peptide Interactions with Bacterial Membranes

Tomislav Rončević, University of Split

10:30 R13: Biophysical Insights into the Selective Membrane Interaction and Permeabilization Induced by the Antimicrobial Peptide RKW

Alessandra Porritiello, University of Naples "Federico II"

10:40 **Coffee**

AMP Structure and Membrane Interactions, cont.

Chair: Tomislav Rončević

11:10 L25: Translocation of Amphiphilic Peptides Across Cell Membranes

Robert Vácha, Masaryk University

11:30 L26: Structural engineering of stapled membrane-targeting antibacterial peptides

Joanna Trylska, University of Warsaw

11:50 R14: Design of membrane-selective peptides by evolutionary molecular dynamics

Alejandro Hernandez Tanguma, Masaryk University

12:00 **Lunch**

13:00 L27: Membranolytic versus non-membranolytic mode of action of two antimicrobial latarcin peptides from spider venom

Marnie Baumeister, Karlsruhe Institute of Technology (KIT)

13:30 L28: The hydrophobic profile along the helix axis influences the pore-forming activity of cationic amphipathic helices

Erik Strandberg, Karlsruhe Institute of Technology (KIT)

13:50 **End of session**

14:00 **Panel Discussion 'The Future of AMPs and AMP research'**

- Edwin Veldhuizen (chair / facilitator)
- Inanc Birol
- Jayachandran Kizhakkedathu
- Marina Rautenbach
- Alex Tossi

14:45 **Presentation of Prizes & Announcement of IMAP 2027**

15:00 **Close of Meeting**

Rapid Oral Communications

These are indicated by R numbers in the programme. The authors will also present their work as a poster, with the same R number. Please visit these posters during the poster sessions.

AI / non-AI Supported Database Mining

L1: Antimicrobial Peptide *In Silico* Optimization Enables the Generation of Stabilized Bioinspired Products

Octavio L. Franco^{1,2}

¹ S-Inova Biotech, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, Mato Grosso do Sul, Brazil; ² Centro de Análises Proteômicas e Bioquímicas, Programa de Pósgraduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília, Distrito Federal, Brazil; ocf Franco@gmail.com

Antimicrobial peptides (AMPs) are compelling candidates to address antimicrobial resistance due to their multifaceted mechanisms of action. Yet, mounting evidence shows that bacteria can adapt to AMP pressure, underscoring the need for next-generation designs that combine potency with enhanced stability. Here, we describe a digital optimization pipeline that integrates genetic algorithms with artificial intelligence to generate bioinspired AMPs from genome-derived sequence spaces. This strategy yielded distinct peptide classes, including cationic-residue-enriched prototypes and hydrophobic counterparts, and enabled rapid prioritization via an engineered luminescent *Pseudomonas* screening platform. Lead peptides exhibited low-micromolar bactericidal activity against multiple resistant bacteria and were further characterized with respect to structure, mechanism, and biotechnological potential. Most candidates were unstructured in aqueous solution but underwent a coil-to-helix transition in hydrophobic environments, as corroborated by NMR in dodecylphosphocholine micelles, which revealed α -helical conformations. Notably, the most potent peptides were rationally cyclized through disulfide bond formation, establishing a conformational scaffold that preserves the effective helical length while enhancing structural constraint. Mechanistic studies indicated membrane disruption associated with hyperpolarisation rather than depolarisation. To advance translational performance, we are also implementing peptide lipidation to increase plasma stability and exposure, while incorporating aminobutyrate residues to reduce proteolytic susceptibility without compromising antimicrobial function, maintaining activity for more than 24 h. Finally, scalable production strategies compatible with market translation are discussed. Overall, this work demonstrates how computational exploration of natural product-inspired sequence space, coupled to stabilisation chemistries, can deliver short, potent, and durable peptide antibiotics relevant to human and animal health.

L2: AI-guided discovery of serum-synergistic antimicrobial peptides (SeSy-AMPs) active against multidrug-resistant *Escherichia coli*

Isabelle Zawadzki¹, Anat Yanai^{2,3}, Alex Vasquez¹, Nuria Valles Serret¹, Emre Demirsoy², Berke Ucar², Teagan Parkin⁴, Aaron Fernandes¹, Mark Gurin¹, Amerachi Okorie¹, Daniel Osborn⁵, Axel Nohturfft⁶, Jayachandran Kizhakkedathu⁷, Ralf Mikut⁸, Caren Helbing⁴, Inanc Birol^{2,9} and Kai Hilpert¹

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Photography

As well as the group photograph, photographs will be taken throughout the meeting and may be passed to the RSC and/or EPS and used to publicise this and future meetings. Please inform the registration desk if you do not wish to appear in any photographs.

Blood Research and Life Sciences Institute, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; ⁸ Institute for Automation and Applied Informatics, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany; ⁹ Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

Multidrug-resistant *Escherichia coli* is a major clinical challenge, particularly in bloodstream infections where rapid bacterial clearance is essential and treatment options are increasingly restricted. Antimicrobial peptides (AMPs) offer a promising alternative modality because they can act rapidly and may target bacterial membranes or other essential cellular structures. However, many AMP candidates perform well in standard broth assays but lose activity under physiologically relevant conditions, especially in the presence of serum. This serum penalty can arise from proteolysis, protein binding, altered ionic conditions, aggregation, or other host-environment interactions.

To address this limitation, we screened artificial intelligence (AI)-prioritised peptide libraries directly under serum-containing conditions rather than relying only on conventional broth-based minimum inhibitory concentration assays. This approach enabled the identification of serum-synergistic antimicrobial peptides (SeSy-AMPs), a distinct class of peptides whose antibacterial activity is retained or enhanced in the presence of human serum. Several SeSy-AMP candidates showed potent activity against multidrug-resistant *E. coli*, including activities substantially stronger in serum than in standard medium.

These findings suggest that screening peptides in physiologically relevant environments can reveal activity profiles that are missed by conventional antimicrobial discovery workflows. The discovery of SeSy-AMPs therefore provides a new route for identifying peptide candidates with potential relevance for severe Gram-negative infections, including bacteraemia. More broadly, this work highlights the importance of integrating AI-guided design with biologically relevant screening conditions to improve the translational value of antimicrobial peptide discovery.

L3: *In silico* design and experimental validation of novel peptides in the leaderless bacteriocin class; building a significant pipeline of novel antibiotic candidates

Jack Kay¹, Vicky Bennett², Rutendo Mupfumi³, Scott Baxter¹, Ian Fotheringham¹, Gordon Barker², Mathew Upton^{2,3}

¹ Ingenza Ltd, Edinburgh, UK; ² Amprologix Ltd, Plymouth, UK; ³ University of Plymouth, Plymouth, UK; vicky.bennett@amprologix.com

The 4-Helix Bundle (4-HB) bacteriocin class share common topology and physicochemical properties. Of the several members of this class reported as showing antimicrobial activity, epidermicin NI01 has progressed to late-stage preclinical evaluation for treatment of methicillin-resistant *Staphylococcus aureus* (MRSA)1,2.

To identify novel therapeutic candidates within this class, a combination of sequence and structure-based homology search strategies were used to mine public databases for putative 4HBs. A Variational Autoencoder (VAE) model was also developed to generate de novo, non-naturally occurring 4-HB sequences.

Candidates were produced using solid phase synthesis and were screened for antimicrobial activity against a panel of clinically relevant pathogens including *Acinetobacter baumannii*, MRSA, and vancomycin resistant *Enterococcus faecalis* (VRE). Subsequently, the VisABLE® recombinant expression platform was used to isolate 22 of the top targets from *Pichia pastoris*.

In total, 234 naturally occurring 4-HB candidates were identified through database mining, while the VAE model generated >90

synthetic sequences with predicted physicochemical properties and structural folds consistent with the 4-HB class. Antimicrobial activity screening validated both methods as efficient tools for the discovery of 4-HB bacteriocins.

Peptides identified through both database mining and the VAE model displayed potent activity against Gram positive and Gram negative pathogens, with minimum inhibitory concentrations as low as 1-2 µg/ml for MRSA, 2 µg/ml for VRE, and 4 µg/ml for *A. baumannii*.

These encouraging findings demonstrate the utility of both approaches for antimicrobial discovery, and support further investigation of the promising 4-HB bacteriocins identified here as therapeutic candidates to address the urgent need for new antimicrobials.

[1] Bennett, V., Barker, G., Sharp, A., Birkett, V., Johnson, J., Tourlomousis, P., and Upton, M. JAC – AMR, 2026 [in review]; [2] Upton, M., Barker, G., Bennett, V., Fotheringham, I., and Currie, H. EMJ Microbiol Infect Dis, 2025;6[1]:36-38

R1: Repurposing Anti-Candida Peptides Against WHO Priority Yeast Pathogens *Nakaseomyces glabrata* and *Candidozyma auris*

Ahmad Alhanich¹; Edel Hyland¹; Stephen Cochrane²; Linda Oyama¹

¹ School of Biological Sciences, Queen's University Belfast, UK; ² School of Chemistry, Queen's University Belfast, UK

Invasive *Candida* infections remain a major clinical challenge due to rising antifungal resistance and few drug classes. WHO-priority *Nakaseomyces glabrata* and *Candidozyma auris* are of concern due to increasing incidence and reduced susceptibility to current therapies.

Here, we aimed to identify, repurpose, and design AMPs active against *C. albicans* as templates against *N. glabrata* and *C. auris*. From the APD3 Database ('Anti-Candida'/'Anti-Fungal'), we removed duplicates and manually curated for efficacy vs. *C. albicans* (technical control), length, toxicity, cysteine content, and modification requirements. After criteria application, 77 of 964 initial peptides remained. Sixty-seven were synthesized (>97% purity) and screened.

Using the broth microdilution method, we tested activity against *C. auris* (DSM 105986, 21092), *N. glabrata* (DSM 24506, 70614), and *C. albicans* (DSM 11946). Haemolysis was assessed against human red blood cells. Peptides were more active against *N. glabrata* than *C. auris*. Eight peptides had activity ≤64 µg/mL against ≥1 strain (excluding those active only against *C. albicans*); 9 were active across all five strains. AP03957 and AP04223 were the most activity with MICs of 64 µg/mL against *N. glabrata* strains and 128 µg/mL against *C. auris* strains. Nine peptides were non-haemolytic within our tested range (HC50 >1024 µg/mL) and constituted the top performing peptides tested, yielding therapeutic indices of 16 (*N. glabrata*) and 8 (*C. auris*).

These results identify repurposable AMP scaffolds and a rapid discovery framework for next-generation antifungals against WHO priority yeast pathogens.

[1] World Health Organization (2022). WHO fungal priority pathogens list to guide research, development and public health action. World Health Organization; ISBN 9789240060241; [2] Oliveira, N.G., Souza, C.M., Buccini, D.F., Cardoso, M.H. and Franco, O.L. (2025). Antimicrobial peptides: structure, functions and translational applications. Nature Reviews Microbiology, [online] 23(687-700). doi:https://doi.org/10.1038/s41579-025-01200-y; [3] Wang, G., Li, X. and Wang, Z. (2015). APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Research, 44(D1), pp.D1087–D1093. doi:https://doi.org/10.1093/nar/gkv1278.

AMP Biomaterials and on Surfaces

L4: Surface Immobilized Antimicrobial Peptides to Prevent Medical Device Infections

Jayachandran N. Kizhakkedathu

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The long-term prevention of biofilm formation on the surface of indwelling medical devices and implants remains a challenge. In this

presentation, I describe novel substrate independent coating approaches to prevent bacterial colonization and biofilm prevention with long-term activity. Building on our research on substrate independent hydrophilic polymer coatings, we enhanced their antibiofilm and antibacterial activities using novel antimicrobial peptides (AMP). Optimal antimicrobial coatings are arrived through a screening of diverse polymer and antimicrobial peptide compositions which resist bacterial colonization, and are able to kill both surface adhered as well as planktonic bacteria. The surface coatings showed high biocompatibility in vitro in number of models including mammalian cultured cells and blood. I also describe some example of biomedical applications of AMP coatings including the development of self-sterilizing blood bags and infection resistant urinary catheters.

L5: Host Defence Peptide derived Self-Assembling Hydrogels to Combat Ocular Infections

Sanhita Roy^{1,2}, Bibrita Bhar^{1,2}, Saumya Mishra^{1,2}

¹ Prof. Brien Holden Eye Research Centre, LV Prasad Eye Institute, Hyderabad, India; ² Dr. Chigurupati Nageswara Rao Ocular Pharmacology Research Centre, LV Prasad Eye Institute, Hyderabad, India; sanhita@lvpei.org

Fungal keratitis, often termed as “silent pandemic” is a leading cause of vision loss in India and globally. The major etiological agents are *Fusarium* or *Candida*, often showing limited response to current antifungal therapies. The aim of this study is characterization of self-assembling hydrogels derived from antimicrobial peptide S100A12, that exhibit antifungal property. The short peptide sequences were designed, and subjected to gelation by solvent-switch method, and was further characterized by biophysical methods like FTIR spectroscopy, SEM, rheometry, or cryo-EM. The antifungal efficacy against ocular isolates of *Fusarium solani* was evaluated both in vitro and in vivo using murine model. Cytotoxicity of hydrogels was assessed using corneal epithelial cells. The most potent peptide was chosen based on in situ gelation in phosphate buffer. The β-sheet formation of peptide hydrogel was indicated by shift in the amide I band by FTIR. The porous, fibrillar hydrogel network was confirmed by SEM imaging, while cryo-EM revealed self-assembling of peptide into filamentous nanostructures. The peptide-hydrogel also exhibited anti-fungal property and inhibited fungal growth comparable to amphotericin B, the commonly used antifungal drug. The peptide-hydrogel remained stable in simulated tear fluid over two weeks and demonstrated minimal cytotoxicity. In murine model of *Fusarium* keratitis, topical application of the peptide-hydrogel caused a significant reduction in corneal opacity and fungal burden. This study confirms that peptide hydrogels derived from host defence peptides can be effectively engineered to undergo self-assembly into structurally stable hydrogels with potent antifungal activity with scope to develop biomaterial-based interventions for fungal keratitis.

L6: ESKAPEEs versus Cyclic AMPs: How to win the battle on surfaces

Marina Rautenbach

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The global life-threatening rise of multi-antibiotic resistance affects the medical, industrial and agricultural sectors. Curbing the spread of resistant pathogens from the environment to hosts would have a major impact in reversing antimicrobial resistance (AMR). Antimicrobial peptides (AMPs) have accumulated very little resistance over millennia and are natural alternatives to antibiotics, biocides and bio-surfactants. A group of antimicrobial cyclodecapeptides (CDPs), the tyrocidines and analogues, was shown to have potential in the creation of robust self-sterilising materials and surfaces. However, they have a weak activity against Gram-negative bacteria and tend to form inactive oligomers in certain depositing solutions. To win the battle against surface- and material-associated microorganisms, we optimised the depositing/absorbing solution and utilised synergistic mixtures with an analogous CDP, gramicidin S. The CDP-treated textiles and surfaces showed variable to excellent broad-spectrum sterilising activity, killing 103-107 cells per cm². In an application trial of one month, bus seats in a public bus were sprayed with the synergistic CDP mixtures in a nanocellulose formulation to form a layered film on the textile

surface. The CDP-nanocellulose coating remained active with >99% reduction in the microbial load after each of the five challenges. Our small victory in the surface battle showed the potential of antimicrobial CDPs to protect soft textiles from recurrent microbial fouling for at least a month.

L7: Multifunctional Biomaterials: Integrating Cell-Instructive and Antimicrobial Peptides for Tissue Regeneration

Carles Mas-Moruno

Universitat Politècnica de Catalunya

Improving the biointegration of biomedical implants with surrounding tissues represents a major goal in biomaterials science. However, implant surfaces that support tissue growth may also favor bacterial colonization. Biomaterial-associated infections and biofilm formation can have devastating consequences for patients, representing a growing and unmet healthcare challenge. Conversely, strategies to inhibit bacterial colonization may exert cytotoxic effects or interfere with host-cell functions, compromising biomaterial-tissue integration. Moreover, conventional antibiotics can impose strong selective pressure on bacterial populations, contributing to the emergence and spread of antimicrobial resistance.

These challenges highlight the need for multifunctional biomaterials capable of simultaneously supporting tissue regeneration and controlling infection. Combining cell-instructive and antimicrobial peptides (AMPs) within implant surfaces offers a promising strategy. Our research focuses on combining the RGD sequence, which promotes key eukaryotic cell responses, with the AMP LF1-11, derived from lactoferrin and exhibiting broad-spectrum antibacterial activity.

In this talk, I will discuss the development of multifunctional peptides integrating these complementary activities. First, their application to metallic implants, particularly titanium, will be presented. Their translation to three-dimensional systems, such as hydrogels, will then be explored. Finally, recent studies investigating the synergistic combination of AMPs with antibacterial nanotopographies will be discussed.

L8: Poloxamer P407 as a Multifunctional Adjuvant Enhancing Antimicrobial, Antibiofilm, and Anti-Inflammatory Activity of Colistin and P13#1

Ilaria Di Nardo¹, Andrea Bosso¹, Ida Palumbo¹, Rosanna Culurciello¹, Maria Cristiano¹, Rosaria Schettini², Irene Izzo², Valeria Cafaro¹, Elio Pizzo¹, Eugenio Notomista¹

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Poloxamer P407 is a non-ionic surfactant extensively used as a pharmaceutical excipient and drug delivery agent [1]; however, its potential as an adjuvant to enhance antimicrobial efficacy remains poorly characterized. In this study, we systematically investigated the impact of P407 on the antimicrobial, antibiofilm, and immunomodulatory activity of colistin and the antimicrobial peptoid P13#1 [2] against *Pseudomonas aeruginosa* (PAO1 and the cystic fibrosis clinical strain RP73) and a representative panel of ESKAPE pathogens.

P407 markedly enhanced the antimicrobial activity of colistin in a concentration-dependent manner, causing up to 64-fold reductions in minimum inhibitory concentration (MIC) values. Instead, P407 exerted a slightly weaker and strain-dependent effect on the antimicrobial activity of P13#1, with modest reductions in the MIC values against Gram-negative bacteria and a more pronounced effect against some Gram-positive strains resulting particularly effective against *Staphylococcus aureus* 6538P. Intriguingly, no enhancement was observed for tobramycin or vancomycin, suggesting a selective mechanism of action. Bactericidal assays confirmed that P407/colistin and P407/P13#1 combinations retained bactericidal activity (MBC = MIC). A three-component mixture, colistin/P13#1/P407, showed high antimicrobial activity with colistin apparent MIC values in the low ng/mL range.

Notably, P407 enhanced the antibiofilm activity of colistin and P13#1 measured by the XTT assay, significantly decreasing the antibiotic

concentration needed to completely eradicate the biofilm. Also in this case, the three-component mixture demonstrated superior efficacy, achieving complete biofilm eradication at further reduced doses.

Very intriguingly, P407 exhibited low intrinsic anti-inflammatory and lipopolysaccharide (LPS) neutralization activity which proved to be additive to those of colistin and P13#1.

Collectively, these findings identify P407 as a promising multifunctional adjuvant capable of potentiating antimicrobial, antibiofilm, and anti-inflammatory activity, reducing therapeutic doses, thereby supporting its potential application in advanced antimicrobial therapies.

[1] Giuliano et al. ACS Applied Bio Materials. (2025). Doi: 10.1021/acsabm.4c01452; [2] Cafaro et al. Pharmaceuticals. (2023). Doi: 10.3390/ph16101386.

L9: Polymer-Based Nanoassemblies as Robust Delivery Platforms for Antimicrobial Peptides: Structural and Microbiological Characterization

Thomas D. Vogelaar^{1,2}, Paraschiva-Catalina Irazi¹, Håvard Jensen^{1,2}

¹ Department of Science and Environment, Roskilde University, Roskilde, Denmark; ² Department of Chemistry, University of Oslo, Oslo, Norway

To tackle the escalating global threat of antibiotic resistance, it is essential to improve the pharmacological properties of antimicrobial peptides (AMPs) as promising alternatives to conventional antibiotics [1]. In addition to developing new peptide libraries [2] and focusing on optimization to improve AMP performance, we also work on developing effective delivery strategies to improve targeting and reduce side effects, such as cytotoxicity and enzymatic breakdown [3].

In this work, we investigate a range of AMP-loaded nanoparticle platforms, including self-assembled poly(ethylene oxide)-*b*-poly(ϵ -caprolactone) (PEO-*b*-PCL) and poly(ethylene oxide)-*b*-poly(lactic-co-glycolic acid) (PEO-*b*-PLGA) systems, as well as lipid-polymer hybrids combining DPPC lipids with these block copolymers. A diverse set of AMPs, such as polymyxins, LL-37, Temporin A, and newly designed short lysine-rich lipopeptides and TM peptoids, is efficiently encapsulated into stable nanoparticle formulations.

Using small-angle X-ray scattering combined with analytical modeling, we resolve distinct morphologies and internal structures across the delivery systems, highlighting clear differences between polymer-only and polymer-lipid hybrids. Notably, polymer-lipid assemblies form elongated, anisotropic vesicular structures, indicating strong coupling between lipid curvature and polymer organization, while with only polymer, rod-like micelles are formed. Encapsulation therefore, depends strongly on peptide chemistry: polymer-only systems favor hydrophobic AMPs such as Temporin A, whereas polymer-lipid hybrids, due to their hydrophilic interior, accommodate a much broader range of AMPs.

Functional antimicrobial studies, including killing kinetics, MIC determinations, and disk diffusion assays, demonstrate that encapsulated AMPs retain activity and show slower release kinetics. Overall, this study highlights polymer-based nanoassemblies as tunable carriers where nanoscale morphology governs peptide encapsulation, localization, and antimicrobial efficacy.

[1] Okeke, I. N., et al. (2024). The Lancet, 403(10442), 2426-2438; [2] Irazi, P. et al. (2026). Manuscript in preparation; [3] Vogelaar, T. D. et al. (2024). Biomacromolecules, 25(7), 4267-4280.

Non-ribosomal AMPs

L10: Stereochemistry vs linker: exploration of ring C analogues of antimicrobial peptide nisin

F. L. Kinnis¹, W.T. P. Darling², L. H. E. Wiese³, M. Erdélyi² & A. B. Tabor¹

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Nisin is a promising antibacterial macrocyclic peptide with broad range activity against Gram-positive bacteria. It targets lipid II, an

essential component of bacterial membranes, and forms a stable pore composed of eight nisin and four lipid II molecules [1]. The central ring C of nisin is of particular interest as it is believed to be crucial for both pore assembly and membrane penetration, and may undergo a conformational change when entering the nonpolar membrane environment. Antimicrobial activity was abolished in a nisin mutant in which a disulfide bond ((L)Cys13-(L)Cys19) replaced the thioether bridge in ring C; however, in this mutant, the stereochemistry at residue 13 was also inverted [2]. In order to determine the importance of the stereochemistry and ring size, the solution conformation ensembles of two ring C analogues (Figure 1 b, c, d) in aqueous and non-polar solvents, mimicking the membrane environment, has been determined. This was done through deconvolution of the population-averaged NMR data using the NMR Analysis of Molecular Flexibility In Solution (NAMFIS) algorithm [3]. These solution conformation studies will provide a unique insight into whether synthetic nisin analogues can adopt suitable conformations for optimum membrane permeability, providing the necessary driving force for the formation of the membrane-spanning nisin:lipid II pores.

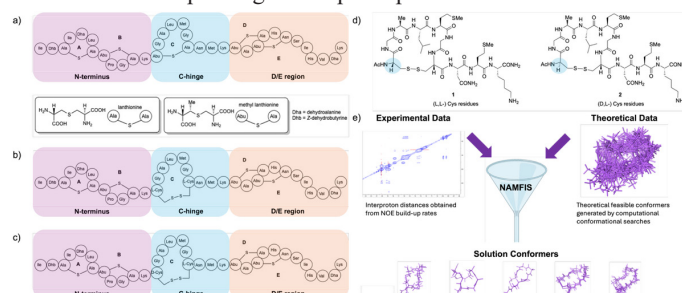


Figure 1. (a) Structure of full-length nisin. (b) Structure of full-length analogue 1. (c) Structure of full-length analogue 2. (d) Chemical structure of analogues 1 and 2 of the nisin C-hinge region. (e) Workflow of NMR data deconvolution using NMR Analysis of Molecular Flexibility in Solution (NAMFIS) algorithm.

This work was supported by the BBSRC and RSC under grant C25-3623282013.

[1] E. Breukink and B. de Kruijff, *Nat. Rev. Drug. Discov.*, 2006, 5, 321-323; [2] C. van Kraaij, E. Breukink, H.S. Rollema, R.S. Bongers, H.A. Kesters, B. de Kruijff, and O.P. Kuipers, *Eur. J. Biochem.*, 2000, 267, 901-909; [3] L.H.E. Wieske, S. Peintner, and M. Erdélyi, *Nat. Rev. Chem.*, 2023, 7, 511-524.

L11: Optimisation of Linear Non-Ribosomal Peptides that Target Gram-Negative Bacteria

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Antimicrobial-resistance (AMR) is an increasing risk to human health. New antimicrobial agents are urgently needed to help combat the problem, otherwise our current quality of life will dramatically decrease. A potential avenue for development is the modification of known antimicrobial agents. This is appealing as it is less risky and time-consuming than the development of entirely novel antibiotics. The modification of existing antimicrobial agents, such as the tridecaptins, polymyxins and ornicidines, forms the core of this project. Ion chelators have long been used in medicine with a wide range of applications.¹ More recently, the attachment of ion chelators to existing antimicrobial peptides (AMPs) has been reported, with promising results. Chelators can act as antimicrobials because bacteria require metal ions such as iron, potassium and sodium for survival.² Bacteria import iron using siderophores, which tightly bind Fe^{3+} and transport it into the cell, where the iron is released under reducing conditions.³ This natural uptake pathway can be exploited: attaching chelating groups to AMPs allows the peptide to enter bacterial cells via active transport rather than mere passive diffusion. This strategy can enhance antimicrobial activity, broaden the spectrum, and reduce toxicity,⁴ giving rise to sideromycin-type antibiotics.

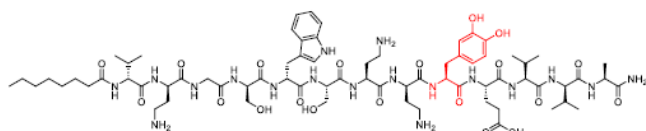


Figure 1. TridecaptinA2 with ion chelator incorporated in position 9.

(1) P. A. Wahidiat, M. Yosia, T. T. Sari and P. Amalia Wahidiat, 2018, vol. 50; (2) S. L. Begg, Portland Press Ltd, 2019, preprint, DOI: 10.1042/BST20180275; (3) D. Repac Antić, M. Parčina, I. Gobin and M. Petković Didović, MDPI, 2022, preprint, DOI: 10.3390/antibiotics11081105; (4) S. Renard, S. Versluys, T. Taillier, N. Dubarry, C. Leroi-Geissler, A. Rey, E. Cornaire, S. Sordello, J.C. B. Carry, O. Angouillan-Boniface et al., *J Med Chem*, 2023, 66, 16869-16887.

AI / non-AI Supported Database Mining

L12: From Sequence to Target Hypothesis: Systematic Docking of AMPs Against Essential Bacterial Cell Surface Proteins

Inanc Birol

University of British Columbia

Prediction, Design and Synthesis of AMPs

L13: Structure-Guided Peptide Design with AMPAFold: Enabling Reliable Antimicrobial Peptide Discovery Beyond Sequence Space

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Artificial intelligence has transformed macromolecular design, from small molecules to entire genomes. Despite major progress in protein engineering, the role of structure in peptide design remains underappreciated, even though peptides often need to adopt defined conformations to engage receptors or membranes. Yet most antimicrobial peptide (AMP) prediction methods still operate primarily in sequence space and do not explicitly include three-dimensional information. Recently, we showed that structure is essential for capturing the conformational features required for AMPs to recognize bacterial molecular targets [1] and is a major determinant of AMP prediction accuracy. Hence, neglecting structural information substantially increases false-positive rates, particularly for AMPs embedded within intrinsically disordered proteins. [2]

To address this limitation, we developed AMPAFold, a framework for AMP design that natively integrates structure prediction into activity evaluation. AMPAFold prioritizes peptides according to their ability to fold under structuring conditions relevant to antimicrobial function. Peptides designed with AMPAFold displayed broad antimicrobial activity, with 92% of synthesized candidates proving active. Consistently, active peptides folded in membrane-mimetic environments such as lipid micelles, and foldability correlated with antimicrobial activity. These peptides disrupted bacterial membranes, interacted strongly with lipopolysaccharide in Gram-negative bacteria, and several candidates also showed extended serum half-life together with low-to-moderate cytotoxicity.

Most importantly, in a mouse model of sepsis, selected peptides reduced bacterial burden by more than 3 log CFU across multiple tissues without detectable toxicity. Together, these results identify structure-aware design as a critical principle for AMP discovery, with clear potential to expand the antimicrobial pipeline against resistant bacterial infections.

This work was supported by the Spanish Ministry of Economy and Innovation under grant PID2023-152706GB-I00 funded by MICIU/AEI/10.13039/501100011033 and by FEDER, UE and by the Agencia de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) under grant 2025 LLAV 00017

[1] R. Bello-Madruga, J. Valle, J. Gómez, L. Comas, M.N. Larrosa, J.J. González, M.A. Jiménez, D. Andreu and M. Torrent, *Molecular Systems Biology* 2025, 23, 889-910. [2] R. Bello-Madruga and M. Torrent, *Comput Struct Biotechnol J* 2024, 23, 972-981.

L14: Random Peptide Mixtures as Antimicrobials: Bridging Synthetic Design and Natural Diversity

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The rapid rise of antimicrobial resistance demands innovative strategies that overcome the limitations of conventional antibiotics. Here, we present two complementary peptide-based approaches: synthetic random peptide mixtures (RPMs) and naturally occurring antimicrobial peptides derived from proteasomal degradation. Our synthetic RPM platform generates highly diverse peptide ensembles with randomized sequences all contain hydrophobic and cationic amino acids. This design mimics the structural and functional diversity of natural host-defense peptides without relying on a single sequence. RPMs exhibit potent, broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, including multidrug-resistant strains, and demonstrate great efficacy and safety profiles in mouse model [1]. Their primary mechanism involves rapid disruption of bacterial membranes, and their intrinsic heterogeneity reduces the likelihood of resistance development. In parallel, we have uncovered a previously unrecognized role of the proteasome as a source of antimicrobial peptides. Peptides generated during intracellular protein degradation display intrinsic antimicrobial activity, revealing a novel layer of innate immune defense. These proteasome-derived peptides contribute to host protection by directly inhibiting microbial growth and influencing host–pathogen interactions [2]. Together, our findings highlight peptide diversity as a unifying principle for antimicrobial function. By integrating synthetic combinatorial design with naturally generated peptide repertoires, our work provides new mechanistic insights and establishes a foundation for the development of next-generation, resistance-resilient antimicrobial therapeutics.

1. Jonathan Z Lau, Shanny Hsuan Kuo, Yael Belo, Einav Malach, Bar Maron, Hannah E Caraway, Myung Whan Oh, Yi Zhang, Nahed Ismail, Gee W Lau, Zvi Hayouka. Antibacterial efficacy of an ultra-short palmitoylated random peptide mixture in mouse models of infection by carbapenem-resistant *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy* 67 (11), e00574-23, 2023. 2. Goldberg K., Lobov A., Shmueli M.D, Yakir I, Weizman T, Antonello P., Ulman A., Sheban D., Laser E., Kramer M, Shteinvil R, Chen G., Ibraheem A., Sysoeva V, Fishbain-Yoskovitz V., Abramov A., Ogneva K., Amidor S., Bootz-Maoz H., Kuo S., DeZorella N, Kacen A., W. Lau G., Yissachar N., Hayouka Z. and Merbl Y. Cell-autonomous innate immunity by proteasome-derived defence peptides. *Nature*. 2025;639(8056):1032-1041.

L15: Peptide Synthesis: Spoiler Alert – It's Complicated

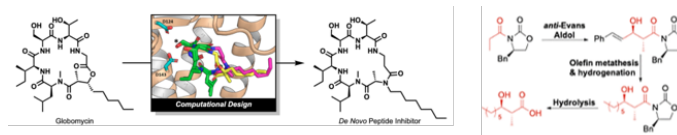
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Antimicrobial resistance (AMR) is a growing threat to human health and global economies, resulting in approximately 6 million deaths per annum at a cost of €1.5 billion in healthcare expenses and productivity losses[1,2]. A key contributing factor to AMR is the decades long void in novel antibiotic discovery, alongside the reluctance for industrial investment into Research and Development (R&D). Fortunately, research into antimicrobial peptides (AMPs) as potential therapeutics has received growing attention in the past few decades and there are now approximately 100 clinically approved therapeutic peptides on the market[3].

Luckily, there is an abundant pool of naturally occurring antimicrobial peptides that can be leveraged for antibiotic R&D. These parent compounds can be chemically to improve their antimicrobial and pharmacokinetic properties. In this work, rational chemical intervention strategies were investigated for the AMPs tridecaptin[4], globomycin[5,6], and the ornicidine family[7-9], with the aim to improve activity and address performance shortcomings.

While the blueprints appeared straightforward, practical implementation proved significantly more complex. Some approaches worked and others failed for no obvious reason, confirming that rational design doesn't always translate to experimental feasibility.



- [1] Davido B., Ny S., van Lingen C. et al. *The Lancet Regional Health – Europe*, 2025; 61. [2] Naghavi M., Vollset S., Ikuta K. et al. *The Lancet*, 2024; 404, 1199–1226. [3] Rosson E., Lux F., David L. et al. *Int. J. Pharm.*, 2025: 675,125555. [4] Bann S. J., Ballantine R. D. et al. *J. Med. Chem.* 2019; 62, 22, 10466–10472. [5] Craven, T. W., Nolan, M. D. et al. *ACS Chem Biol* 2024, 19 (5), 1125–1130. [6] Bann, S. J. & Cochrane, S. A. *RSC Med Chem* 2025, 16 (1), 373–378. [7] Al Ayed, K., Ballantine, R. D. et al. *Chem Sci* 2022, 13 (12), 3563–3570. [8] Ballantine, R. D., Al Ayed, K. et al. *RSC Med Chem* 2022, 13 (12), 1640–1643. [9] Ballantine, R. D., Al Ayed, K. et al. *J Med Chem* 2023, 66 (8), 6002–6009.

R2: Synthesis and Antimicrobial Activity of Cyclic Temporin L Analogues via Non-Conventional Guanidinium-Based Functionalization

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Temporin L (TL) is a potent 13-mer peptide. Its broad antimicrobial activity is associated with significant cytotoxicity, which has motivated extensive efforts to mitigate this limitation.[1] We have recently reported the rational design and synthesis of a new library of compounds starting from a lactam-cyclized analogue of TL (cyc-TL).[2] The new library was conceived to improve activity against Gram-negative bacteria and incorporates non-canonical amino acids bearing guanidino groups either as pendant substituents of side chains, in combination with lactam cyclization, or as part of an unconventional guanidino-based cyclic bridge (Figure 1).[3] These strategies highlight how conformation and distribution of positive charges modulate peptide properties, leading to enhanced antimicrobial efficacy against Gram-negative bacteria through a membrane-lytic mechanism of action. Selected analogues displayed multi-fold improved MIC values against representative Gram-negative strains, together with reduced cytotoxicity toward HaCaT cells and erythrocytes. Notably, cyclic peptides featuring the guanidino-based bridge showed a marked enhancement in proteolytic stability, identifying this motif as a particularly effective design element. Overall, this presentation will define a structure-property framework for the development of next-generation cyclic TL analogues.

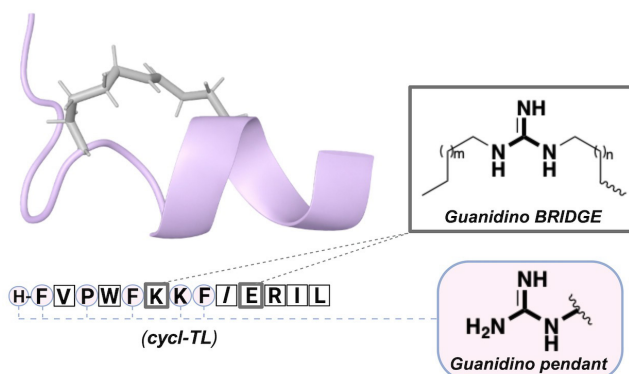


Figure 1. Structural modifications herein presented and applied to cycl-TL peptide sequence to improve antimicrobial properties.

- [1] L.D. D'Andrea et al. *Int. J. Mol.* 2023, 24, 5426; [2] R. Bellavita, et al. *J. Med.Chem.* 2021, 64, 11675–11694; [3] R. Bellavita et al. *J. Med. Chem.* 2025, 68, 25038–25060.

L16: Early maturation of host antimicrobial peptide defences is associated with host-pathogen coexistence

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Epizootic outbreaks of invasive pathogens, such as the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), are increasingly recognised as a global threat to biodiversity. However, not all susceptible host populations are equally impacted by these disease outbreaks. While some populations experience extirpation or declines driven by intense infections and high mortality rates, others can transition to enzootic coexistence with the pathogen¹.

The role of antimicrobial peptide (AMP) defences in effectively resisting novel pathogens in wildlife has received limited attention. We used mass spectrometry-based peptidomics (with a transcriptomic reference) to characterise the complex peptidomic mixtures secreted by the skin of wild amphibians. This high throughput method allowed us to investigate whether skin antimicrobial peptides predict the disease dynamics in four wild populations of Common midwife toad (*Alytes obstetricans*) in the Pyrenees mountains¹. We find that:

- Alyteserin AMPs show a variable ability to inhibit Bd in-vitro
- Contrary to expectations, Alyteserin AMPs appear to inhibit Bd through an intracellular mechanism
- Populations of *A. obstetricans* which are successfully coexisting with Bd develop their AMP defences earlier in life.

Our results show that even intrinsically susceptible amphibian species can possess AMPs which allow populations to recover following a severe Bd epizootic.

[1] Jervis, P.A., Rosa, G.M., Bates, K.A., Karu, K., Murtza, A., Edwards, S., Hopkins, K., Michaels, C., Jordine-Morgan, T., Nylander, C., Sergeant, C., Skelly, E.J., Harrison, X.A., Rhodes, J., Tapley, B., Bosch, J., Garner, T.W.J., Fisher, M.C., Tabor, A.B. Early maturation of host antimicrobial peptide defenses is associated with host-pathogen coexistence. (In-press- Nature Chemical Biology).

R3: From Mitochondrial Targeting to Microbial Killing: Twinkle-Derived Peptides as a New Class of AMPs

Emanuela Galatola¹, Alessandra Porritiello², Monica Ambrosio², Bruna Agrillo², Marta Gogliettino², Marco Balestrieri², Ennio Cocca² and Gianna Palmieri²

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In recent years, antimicrobial peptides (AMPs) have attracted considerable interest as a promising alternative to conventional drugs for fighting multidrug-resistant pathogens, due to their broad-spectrum activity and diverse mechanisms of action [1]. Nevertheless, the de novo design of AMPs that combine a high therapeutic index with strong stability, resistance to degradation, and optimal bioavailability still represents a significant and complex challenge.

In this context, two novel peptides (TAMP1 and TAMP2) were rationally designed starting from the mitochondrial signal peptide sequence (MTS) of the Twinkle helicase derived from the Antarctic marine ray-finned fish *Nothothenia coriiceps* [2], and deeply characterised.

Results demonstrated a robust antimicrobial efficacy against a large panel of both Gram-negative and Gram-positive bacteria, among spoilage and pathogenic microorganisms, with MBC values in the low micromolar range. Molecular modelling revealed a canonical amphipathic α -helix structure for both peptides, with iso-oriented side chains for the three Trp in TAMP2.

Their conformational and structural features were investigated by using circular dichroism (CD) and fluorescence spectroscopy across a wide range of pH and temperatures, providing insight into their stability under diverse environmental conditions. Moreover, peptide stability under physiological salt conditions and proteolytic resistance were assessed using RP-HPLC.

Therefore, TAMP1 and TAMP2 represent the first AMPs derived from Twinkle helicase MTS and a new promising antimicrobial peptide class to be used in food preservation and human health.

[1] Jiaqi X, Weiguo F, Jiaye W, Ruichen W, Bowen Z, Letao B, Zhe-Sheng C, Hui Y, Leming S, Drug Resistance Updates, 2023, 68- 100954; [2] Tyynismaa H, Sembongi H, Bokori-Brown M, Granycome C, Ashley N, Poulton J, Jalanko A, Spelbrink JN, Holt JJ, Suomalainen A. Hum Mol Genet. 2004 Dec 15;13(24):3219-27. Epub 2004 Oct.

L17: From Food for Food: Prediction and Identification of Novel Food-Derived Antimicrobial Peptides for Food Preservation

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¹ Department of Chemistry and Pharmacy, Food Chemistry, Friedrich-Alexander Universität Erlangen-Nürnberg, Germany, monika.pischetsrieder@fau.de; (MLH: affiliation during the study) ² Food Hygiene and Technology, Centre for Food Science, University of Veterinary Medicine Vienna, Austria

Antimicrobial peptides can be a key in food production to avoid bacterial spoilage and pathogens. Food derived bioactives offer the advantage that they are classified as "food-grade" not requiring safety evaluation. The study aimed to identify therefore novel antimicrobial peptides from food sources for the potential use as preservatives [1]. Thus, chick pea protein was hydrolyzed by the digestive protease chymotrypsin. Untargeted protein profiling of the hydrolysates by LCMSMS led to the identification of 1,279 peptides from 103 precursor proteins. Antimicrobial activity was predicted for 21 peptides by APD, PepCal, I-Tasser and NetWheels considering mainly net charge, hydrophobicity, helical structure and water solubility. The predicted antimicrobial peptides were synthesized and 18/17 of them were bacteriostatic against *Escherichia coli*/*Bacillus subtilis*. Legumin-like protein₃₄₄₋₃₆₁ (Leg 1) and Legumin A-like protein₂₆₃₋₂₈₀ (Leg2) were most potent with MIC of 62.5 μ M (*E. coli*) and 15.6 μ M (*B. subtilis*). Leg1 and Leg2 inhibited the growth of 14 strains of food spoilage bacteria and food pathogens with $\leq$ 82,000-fold higher activity compared to approved small molecule preservatives and a similar activity as nisin an approved bacterial peptide preservative. Leg1 was stable during meat storage with MBC values of 125 and 15.6 μ M against *E. coli* and *B. subtilis*. [2]

Conclusion: Peptide profiling of food sources, virtual screening and experimental testing of predicted antimicrobial peptides led to the identification of Leg1 and Leg2 with promising beneficial properties for food preservation. Both peptides can be released in the human digestive system after the consumption of chick pea. Consequently, a shortened risk assessment may apply for approval.

[1] Heymich M-L, Friedlein U, Trollmann M, Schwaiger K, Böckmann RA, Pischetsrieder M (2021) Food Chem. 347, 128917; [2] Heymich M-L, Srirangan S, Pischetsrieder M (2021) Foods 10(6), 1192

R4: Strategic Residue Substitution in Tolaasin for the Synthesis of New Bioactive Derivatives

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Tolaasin, an 18 residue cyclic lipodepsipeptide, is the main virulence factor of *Pseudomonas tolaasii*, and causes brown blotch disease in edible mushrooms, indicating antifungal activity [1]. More recently, cytotoxicity against cancer cell lines was also demonstrated. While experimental data supports a mode of action involving direct interaction of tolaasin with cellular membranes, molecular level understanding of the features of tolaasin involved and how these ultimately lead to cell lysis are not well understood [2,3].

Given these intriguing anticancer (and antifungal) properties, a total synthesis route towards tolaasin was successfully developed. After achieving this, we became interested in generating a library of synthetic analogues to further investigate structure-bioactivity relationships

However, the solid-phase peptide synthesis (SPPS) of tolaasin is hindered by several challenging coupling steps, primarily due to the presence of the non-proteinogenic amino acid Z-dehydrobutyrine (Z-Dhb) in its sequence. These difficulties significantly reduce the overall yield, resulting in limited amounts of the final peptide.

To overcome this, we introduced a strategic yet straightforward amino acid substitution, replacing these Z-Dhb residues. This modification led to the development of a synthetic tolaasin analogue that retains bioactivity comparable to the native compound. Importantly, this substitution substantially reduces synthetic complexity while markedly improving yields, thereby enabling the efficient generation of a library of analogues for comprehensive bioactivity and biophysical studies. Bioactivity screening of a library of derivatives provides a first insight in the molecular determinants essential for bioactivity.

[1] Nutkins, J.C., et al. *J. Am. Chem. Soc.* 1991, 113, 2621-2627; [2] Brodey, C.L., et al. *Physiological Mol. Plant Pathology* 1991, 39, 57-70; [3] Steigenberger, J., et al. *Front. Mol. Biosci.* 2022, 9:1064742.

R5: Black Vulture Gut-Derived LAB as a Source of Natural Antimicrobials Against Foodborne Pathogens

Ana Clara Candelaria Cucick^{*1}, Tais Mayumi Kuniyoshi¹, João Victor dos Anjos Almeida², Fernando Moises Mamani Sanca¹, Carlos Emilio Cabrera Matajira¹, Elionio Galvão Frota¹, Iago Blanco¹, Luis Fabio Silveira³ and Ricardo Pinheiro de Souza Oliveira¹

¹Faculty of Pharmaceutical Sciences, University of São Paulo (USP), São Paulo, Brazil. ²Department of Agricultural and Environmental Biotechnology, São Paulo State University (UNESP), College of Agricultural and Veterinary Sciences, Jaboticabal, SP, Brazil. ³Institute of Museology, University of São Paulo (USP), São Paulo, Brazil; anaclara.candelaria@usp.br

Underexplored microbiotas represent promising reservoirs of antimicrobial-producing microorganisms. Black vultures (*Coragyps atratus*), scavenging birds exposed to high microbial loads, may harbor competitive gut microbiota with biotechnological potential. This study aimed to bioprospect bacteriocin-producing lactic acid bacteria (LAB) from the gut of *C. atratus* and evaluate their activity against foodborne pathogens. Oxford Nanopore 16S rRNA sequencing of intestinal samples from two animals revealed *Campylobacter* spp. and *Lactobacillus* spp. as the most abundant genera. Guided by this profile, LAB were isolated from proximal, medial, and distal intestinal segments using selective MRS medium under microaerophilic conditions. Neutralized and heat-treated cell-free supernatants were screened for bacteriocin-like inhibitory substances (BLIS). Antimicrobial activity against *Listeria monocytogenes* ATCC 15313 and *Staphylococcus aureus* ATCC 29213 was assessed by agar diffusion and growth inhibition kinetics, and hemolytic activity was evaluated on sheep blood agar. Among 46 LAB isolates, 16 inhibited *L. monocytogenes*, and six exhibited a non-hemolytic (γ) profile. Growth kinetics showed that LBM-M4 displayed strong inhibition for up to 20 h, suggesting a bactericide effect. LBM-D30 and LBM-P39 also demonstrated marked inhibitory activity, whereas LBM-P31, LBM-P38, and LBM-P42 showed no effect against *L. monocytogenes*. Against *S. aureus*, LBM-M4, LBM-P39, and LBM-P42 exhibited significant inhibition over 22 h. MALDI-TOF MS identified the most active isolates as *Enterococcus faecium*, *Enterococcus hirae*, *Limosilactobacillus reuteri*, and *Lactiplantibacillus plantarum*. These findings highlight the black vulture gut microbiota as a valuable source of bacteriocin-producing LAB with potential applications in food preservation and alternative antimicrobial strategies.

Acknowledgements: This work was financially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP #2024/17310-7; #2020/15599-9 #2024/23506-1), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq # 304635/2025-1).

Mode of action

L18: Antimicrobial Peptides in Action: A Kinetic Perspective

Federico Carneri,¹ Emiliano Franci,¹ Angelo Toto,² Cassandra Troiano,¹ Maria Rosa Loffredo,² Bruno Casciaro,² Maria Luisa Mangoni,² Michelle Digman,³ Stefano Gianni,² Gianfranco Bocchinfuso,¹ Daniela Roversi^{1*} and Lorenzo Stella¹

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Biophysical studies on the interaction of antimicrobial peptides (AMPs) with artificial membranes are pivotal in developing models of the mechanisms of pore formation. However, quantitative data on the mechanism of AMP killing are scant. Previously, we quantitatively investigated the AMP interaction with both real cells and model systems, shedding further light in their mechanism of action.^{1,2} We applied the same approach to kinetic investigation, showing that AMP-induced *E. coli* killing occurs within minutes. To gain deeper insights into the sequence of events leading to Gram-negative bacterial death, we characterized AMP-*E. coli* interactions across different timescales, ranging from milliseconds to minutes. Therefore, we exploited stopped-flow experiments at sub-bactericidal peptide concentrations using a dansyl-labeled analogue of the PMAP-23 AMP, showing that the first step involved in the interaction is the binding to the outer membrane, which occurs in less than one second. Subsequently, steady-state fluorescence kinetic experiments showed that the second step is the outer-membrane permeabilization. Perturbation of the *E. coli* inner membrane, followed by propidium iodide kinetics, occurs after the onset of bacterial killing. Therefore, we have for the first time clarified the following sequence of events involved in AMP-*E. coli* interaction: AMPs bind to the outer bacterial membrane in less than one second and start killing bacteria before perturbing the inner membrane. Ultimately, AMPs localize in both the cytosol and the cell membrane, as demonstrated by confocal laser scanning microscopy experiments. Overall, these results highlight how kinetic studies can disclose the detailed sequence of events in bacterial killing by AMPs.

[1] Roversi, D. et al. *ACS Chem. Biol.*, 2014, 9, 2003–2007; [2] Carneri, F. et al. *J. Colloid Int. Sci.*, 2025, 83, 1078–1086.

R6: A cetacean PrAMP bridging distinct ruminant peptide features to target ESKAPE pathogens

Di Stasi A¹, Janani S², Lamarca F¹, Morici M³, Berger M³, Theodorou M³, D'Amore A¹, Peggion C², Biondi B⁴, Scocchi M¹, Wilson DN³, Mardirossian M¹

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The rise of antimicrobial resistance has driven interest in proline-rich antimicrobial peptides (PrAMPs), which act through intracellular mechanisms with limited membrane disruption. Genomic analyses have identified novel cetacean-derived PrAMPs (cePrAMPs), thereby expanding the scope of this peptide family to include not only terrestrial mammals and arthropods, but also cetaceans [1,2]. One of these, Lip1, shows similarity to the bovine Bac7(1–35) peptide, particularly in the N-terminal portion. Interestingly, it also shows similarity in the C-terminus to the recently discovered ruminant PrAMP, Rumacidin1. Like other class 1 PrAMPs, this latter peptide relies on blocking protein synthesis; however, it displays a different pattern of interaction with the ribosomal exit tunnel compared to other mammalian PrAMPs [3]. In this study, we investigated the structure-activity relationships of Lip1 in order to understand how its combination of Bac7(1–35) and Rumacidin1 sequences outperforms both peptides, providing a broader antimicrobial spectrum that encompasses all ESKAPE pathogens. We also investigated whether improvements in antimicrobial activity are due to its effect on ribosomes or the bacterial envelope.

[1] Mardirossian et al. (2018) *Cell chemical biology*; [2] Sola et al. (2020)

L19: Esculentin-Derived AMPs as Adjuvants to Overcome Multidrug Resistance in KPC-Klebsiella pneumoniae

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Antimicrobial resistance (AMR) is a major concern for public health worldwide. In this context, carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) represents one of the most critical ESKAPEE pathogens due to its extensive multidrug-resistant (MDR) profile and the scarcity of effective therapeutic options [1]. This scenario has driven interest in antimicrobial peptides (AMPs), which combine membrane-targeting activity with a lower propensity for resistance development.

Here, we investigated the antibacterial efficacy of a panel of esculentin-1a-derived AMPs—Esc(1–21), its diastereomer Esc(1–21)-1c, and their α -aminoisobutyric acid (Aib)-containing analogs—against clinical isolates of KPC-Kp [2]. Minimum inhibitory concentrations (MICs) were determined to assess intrinsic activity. In parallel, we evaluated the combinatorial effects of these peptides with ceftazidime (CAZ), a third-generation cephalosporin whose clinical effectiveness has been severely compromised by the spread of KPC-producing strains. Mechanistic insights into peptide/CAZ synergy were obtained by evaluating outer and inner membrane permeabilization via quantification of β -lactamase activity, released into the extracellular medium, and the increase in fluorescence of Sytox Green probe, respectively.

Our results show that Esc peptides retain significant activity against KPC-Kp clinical isolates, with MIC values in the low micromolar range. Importantly, peptide–antibiotic combinations displayed synergistic effects, notably reducing the effective concentration of CAZ and restoring bacterial susceptibility to the antibiotic. Mechanistic studies indicate that AMP-induced membrane perturbation enhances antibiotic uptake, suggesting a self-reinforcing mechanism in which CAZ-mediated cell wall damage further destabilizes the bacterial envelope, promoting additional peptide and antibiotic entry and amplifying membrane disruption and antibacterial activity.

Overall, these findings highlight the potential of esculentin-derived AMPs as adjuvants to restore antibiotic efficacy, offering a promising strategy to counteract AMR in high-risk Gram-negative pathogens [3].

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[1] Giacobbe DR et Al. (2023). Ann Med 55: 101-113.

10.1080/07853890.2022.2152484; [2] Loffredo MR et Al. (2024). ACS Infect Dis 10: 2403-2418. 10.1021/acsinfecdis.4c00130; [3] Taheri-Araghi S (2024) Front Microbiol 15: 1390765. 10.3389/fmicb.2024.1390765

R7: Hybrid proline-rich antimicrobial peptides derived from Api137 and Chex1-Arg20 with dual modes of action

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The spread of multi-resistant bacteria represents a major clinical challenge, requiring novel antimicrobial agents with new mechanisms of action. The proline-rich antimicrobial designer peptides

Onc112, Api137 and Chex1-Arg20 exhibit strong activity against various bacterial strains and primarily target the 70S ribosome. While their binding sites at the 70S ribosome partially overlap, they differ in binding orientation and mechanism. Api137, whose C-terminus is crucial for its mode of action, inhibits translation termination by trapping release factors and preventing their recycling [1]. In contrast, Onc112 prevents accommodation of A-site tRNA thereby inhibiting the onset of translation elongation. The activity of Onc112 is mostly driven by its N-terminal residues. [2] Despite sequence similarities to Onc112, Chex1-Arg20 likely follows a third unknown mechanism [3]. To combine these distinct mechanisms, hybrid peptides were designed by linking the N-terminal sequence of Chex1-Arg20 (Chex-RPD-KRPYL motif) with C-terminal sequence of Api137 (-HPRL motif), aiming to enable both modes of action and reduce the likelihood of resistance development. Our newly designed hybrid peptides show potent antibacterial activity, with MIC values up to 4 μ g/mL against *E.coli* as well as the ability to inhibit in vitro translation, in contrast to Chex1-Arg20. Competitive binding assays using cf-Api137 and cf-Chex1-Arg20 indicate that the hybrid peptides do not fully occupy the Api137 binding site but partially overlap. Substitution of a histidine residue critical for Api137 binding [4] with arginine further supports binding at the Chex1-Arg20 site. Cryo-EM structures of peptide–ribosome complexes provide further insights into their mechanisms of action.

[1] Florin, T.; Maracci, C.; Graf, M.; Karki, P.; Klepacki, D.; Berninghausen, O.; Beckmann, R.; Vázquez-Laslop, N.; Wilson, D.N.; Rodnina, M.V.; Mankin, A.S. Nat Struct Mol Biol., 2017, 24; [2] Roy, R.N.; Lomakin, I.B.; Gagnon, M.G.; Steitz, T.A. Nat Struct Mol Biol., 2015, 22; [3] Brakel, A.; Krizsan, A.; Itzenga, R.; Kraus, C.N.; Otvos, L.; Hoffmann, R. Int. J. Mol. Sci. 2022, 23; [4] Lauer, S.M.; Reepmeyer, M.; Berendes, O.; Klepacki, D.; Gasse, J.; Gabrielli, S.; Grubmüller, H.; Bock, L.V.; Krizsan, A.; Nikolay, R.; Spahn, C.M.T.; Hoffmann, R. Nat Commun., 2024, 15.

R8: Mechanistic Insights into Hemozoin Formation Inhibition by the Tyrocidines

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Plasmodium falciparum (Pf) remains the most lethal malaria species and dominates in Africa – a region burdening 95% of global deaths [1]. The parasite's persistence is driven by its complex life cycle, multidrug resistance to all commercial antimalarials, and several critical virulence factors, including hemozoin formation [2]. As byproduct of haemoglobin catabolism, toxic free haem is sequestered into inert hemozoin biocrystals [3,4]. Consequently, disruption of this essential process remains an attractive antimalarial strategy.

Given current drug resistance, antimicrobial peptides (AMPs) offer promising alternatives due to their fast modes of action (MoA) which may circumvent resistance development [5]. The tyrocidine (Trc) AMP family has potent antimalarial activity and shares a target with chloroquine (CQ) [5,6], a quinolone inhibiting hemozoin formation. Microscopically, amorphous and fragmented hemozoin crystals were observed in Trc-treated Pf [5], leading us to investigate hemozoin inhibition as a potential MoA.

In chemico, Trcs exhibited equimolar or superior potency compared to CQ. Competition assays suggest a dual inhibitory mechanism: the Trcs appear to inhibit haem dimerization by coordinating to the labile axial site of the central Fe²⁺ and by perturbing lipid-mediated nucleation. Mass spectrometry and UV-Vis spectroscopy confirmed these Fe²⁺-binding interactions and established affinities. Integrating this with biological data (IC₅₀) and toxicity (HC₅₀), a structure-activity-relationship analysis revealed that reduced steric bulk, coupled with increased hydrophobicity and hydrogen-bonding potential are key features for hemozoin inhibition and Pf selectivity. Together these findings provide a robust framework for pharmacophore mining and the development of peptidomimetic antimalarials.

1. World Health Organization (2025). Available at: <https://www.who.int/teams/>

global-malaria-programme/reports/world-malaria-report-2025; 2. Thu, A. M., Phyto, A. P., Landier, J., Parker, D. M. & Nosten, F. H. FEBS J 284, 2569–2578 (2017). DOI: 10.1111/febs.14127; 3. Ncokazi, K. K. & Egan, T. J. Anal. Biochem. 338, 306–319 (2005). DOI: 10.1016/j.ab.2004.11.022; 4. Egan, T. J. Mol. Biochem. Parasitol. 157, 127–136 (2008). DOI: 10.1016/j.molbiopara.2007.11.005; 5. Leussa, A. N. N. & Rautenbach, M. Antibiotics 11, (2022). DOI: 10.3390/antibiotics11060801; 6. Rautenbach, M., Vlok, N. M., Stander, M. & Hoppe, H. C. Biochim. Biophys. Acta - Biomembranes 1768, 1488–1497 (2007). DOI: 10.1016/j.bbmem.2007.01.015.

L20: Exploring amphiphilicity and hydrophilicity in antimicrobial peptide star polymers and their impact on biological activity

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As even last-line-of-defence antibacterials begin to lose effectiveness, antimicrobial resistance is rapidly advancing as one of the most significant threats to global health. Structurally Nanoengineered Antimicrobial Peptide Polymers (SNAPPs) have emerged as a promising new class of antibacterial agent that can kill without developing resistance. In this work the role of amphiphilicity in these polymers is explored, expanding on the range of amino acids used to form the peptide arms in the star structure (Fig. 1). Hydrophilic SNAPPs are investigated for the first time, removing the amphiphilic character entirely. A library of SNAPPs with varying hydrophobic and hydrophilic amino acids, as well as arm numbers and arm lengths, is prepared. The antibacterial activity of these SNAPPs is assessed against Gram-negative and Gram-positive bacteria to compare how amphiphilicity and hydrophilicity affects effectiveness and mechanism of action. This work finds for the first time that hydrophilic SNAPPs are equally effective as amphiphilic SNAPPs and show a different mechanism of action against Gram-negative *Escherichia coli*. Biocompatibility studies show how these characteristics effect SNAPP toxicity, with hydrophilic SNAPPs generally showing improved biocompatibility haemocompatibility. Hydrophilic SNAPPs are a promising new direction for antimicrobial star polymers, with comparable performance and lower toxicity compared to traditional SNAPPs.

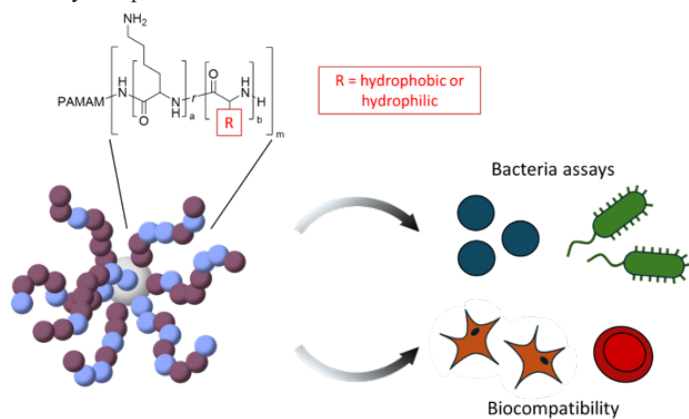


Figure 1: Investigating the bacteria activity and biocompatibility of hydrophilic and amphiphilic SNAPPs

R9: Functional Proteomics Reveals PilR As A Novel Molecular Target Of Temporin A In *Pseudomonas Aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen whose ability to form biofilms plays a key role in chronic infections and antibiotic resistance [1]. Antimicrobial peptides (AMPs) represent promising

anti-biofilm agents, although their molecular targets are often poorly characterized. Here, we investigated the mechanism of action of Temporin A (Temp-A), a frog-derived AMP with negligible antibacterial activity against *P. aeruginosa*, focusing on its anti-virulence properties [2].

Temp-A significantly inhibited biofilm formation and reduced pyoverdine production without affecting bacterial growth. In addition, the peptide markedly impaired swimming and swarming motility, suggesting interference with early stages of biofilm development.

To understand the molecular basis of this activity, a functional proteomics approach combining pull-down assays was applied, leading to the identification the putative protein interactors. Bioinformatic analysis revealed a strong enrichment of proteins involved in flagellar assembly, type IV pilus biogenesis, and type VI secretion system, indicating a multi-target effect on virulence-related pathways. Among these, PilR, a key transcriptional regulator of pili and flagella, was identified as a potential target. For the first time, recombinant PilR was successfully produced and purified. Molecular docking predicted a stable Temp-A/PilR complex, which was experimentally confirmed by fluorescence spectroscopy, yielding a dissociation constant (K_d) of ~24 μM. These findings demonstrate that Temp-A exerts anti-biofilm activity by targeting regulatory networks controlling motility and adhesion, providing new insights into AMP mechanisms and identifying PilR as a novel anti-virulence target.

[1] Pachori, P., Gothalwal, R., & Gandhi, P. (2019). Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. *Genes & diseases*, 6(2), 109-119. [2] Canè, C., Tammara, L., Duilio, A., & Di Somma, A. (2024). Investigation of the mechanism of action of AMPs from amphibians to identify bacterial protein targets for therapeutic applications. *Antibiotics*, 13(11), 1076.

R10: Molecular Dynamics Simulations of Proline-rich Antimicrobial Peptides in their Ribosomal Binding Sites

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Proline-rich antimicrobial peptides (PrAMPs), like the insect-derived apidaecins and oncocins, are a promising class of antimicrobial agents against multidrug resistant bacteria. PrAMPs are actively transported into the cell, where they target protein synthesis at the ribosome. While oncocin analogs bind inside the peptide exit tunnel and peptidyl transferase center blocking elongation,[1] apidaecin-derived peptides bind to at least two sites in the peptide exit tunnel, inhibiting translation termination and ribosome assembly.[2-4]

In this work, models of different PrAMPs in the different ribosomal binding sites were built using the latest amber ff19SB and OL3 protein and RNA forcefields. Additional parameterization of non-canonical residues was performed to accurately represent the binding of the tested PrAMPs. Fully atomistic Molecular Dynamics (MD) simulations were performed to further understand their behavior in these binding sites, and to identify residues important for binding in each site. These results will enable synthesis and testing of derived PrAMPs, targeting either specific modes of action, or broader activity, balancing different modes of action.

[1] Seefeldt, A. C. et al. *Nat Struct Mol Biol* 22, 470–475; 10.1038/nsmb.3034 (2015); [2] Florin, T. et al. *Nat Struct Mol Biol* 24, 752–757; 10.1038/nsmb.3439 (2017); [3] Lauer, S. M. et al. *Nat Commun* 16, 567; 10.1038/s41467-025-55836-8 (2025); [4] Lauer, S. M. et al. *Nat Commun* 15, 3945; 10.1038/s41467-024-48027-4 (2024).

R11: Evaluating the Mechanism of Uptake and Antimicrobial Efficacy of the Proline-Rich Antimicrobial Peptide PR-39

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Antimicrobial resistance (AMR) is a silent pandemic, characterised by pathogens showing an increased ability to thrive even in the presence of commonly used antibiotics. Antimicrobial peptides (AMPs) offer great potential for development as novel antibiotics. Unlike conventional antibiotics, AMPs target conserved bacterial structures and essential pathways while demonstrating low toxicity to mammalian cells.[1]

Proline-rich AMPs (PrAMPs) like Apidacein, are intracellular acting. They typically rely on inner membrane transporters, such as SbmA, to gain entry into Gram-negative bacteria, where they exert their activity by targeting the ribosome.[2] However, PR-39, a 39-residue porcine PrAMP is possess a high number of arginine residues, and thereby possesses a very high net charge which may enable it to function as a cell-penetrating peptide, entering bacteria independently of specific inner membrane transporters. Critically, while the efficacy of most PrAMP and cathelicidins is highly salt-dependent [3], PR-39 retains consistent efficacy regardless of salt concentration, demonstrating resilience under physiologically relevant conditions. Most strikingly, PR-39 maintains potent efficacy against laboratory strains of *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, as well as MDR clinical isolates harbouring multiple drug resistances for which treatment options are critically limited.

In this study, we investigate the mechanism of cellular uptake of PR-39, its independence from inner membrane transporters, and whether multi-drug efflux pumps can neutralise its activity. We further examine its serum stability, synergistic potential with existing antibiotics, and cytotoxic profile collectively positioning PR-39 as a compelling novel therapeutic candidate against drug-resistant infections.

1. Cezara Bucataru, Corina Ciobanasu, Antimicrobial peptides: Opportunities and challenges in overcoming resistance, Microbiological Research, Volume 286, 2024, 127822, ISSN 0944-5013; 2. Baliga, C.; Brown, T.J.; Florin, T et al; Proc. Natl Acad. Sci. USA 118, e2026465118 (2021); 3. Bluhm MEC, Schneider VAF, Schäfer I, Piantavigna S, Goldbach T, Knappe D, Seibel P, Martin LL, Veldhuizen EJA and Hoffmann R (2016) N-Terminal Ile-Orn- and Trp-Orn-Motif Repeats Enhance Membrane Interaction and Increase the Antimicrobial Activity of Apidaceins against *Pseudomonas aeruginosa*. Front. Cell Dev. Biol. 4:39.

Applications of AMPs in Therapeutics

L21: Novel Therapeutic Strategies Using AMPs to Treat and Prevent Infectious Diseases

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While antimicrobial peptides show promise in initial studies, it has been difficult to develop them into actual direct therapeutic agents. We are examining two different strategies to overcome the hurdles of using AMPs as antimicrobial agents. The first is to enhance antimicrobial activity by inducing expression of LL-37 with vitamin D. *In vitro* studies show that the active form of vitamin D can strongly induce mRNA levels of the CAMP gene in multiple cell types. Pre-treatment of cells with active vitamin D increases antibacterial and antiviral activity in epithelial cells. Furthermore, injection of vitamin D into mice reduces the levels of bacteria in the airway, and delivery of vitamin D on the gums of mice prevents the development of periodontal disease. The second approach is to develop small molecule mimetics of AMPs as antimicrobials. *In vitro* studies show that N-substituted glycine oligomers ("peptoids") based on the structure of LL-37 exhibit potent activity against bacteria, fungi and enveloped viruses. *In vivo*, topical delivery prevents herpes simplex virus-1 lesions on the lips of infected mice, and subcutaneous injection clears systemic infection of the fungus *Candida auris*. These two approaches can lead to the development of AMP-based therapies for microbial infection.

L22: Weaponizing Bacterial Aminopeptidases for species-selective antibiofilm peptides

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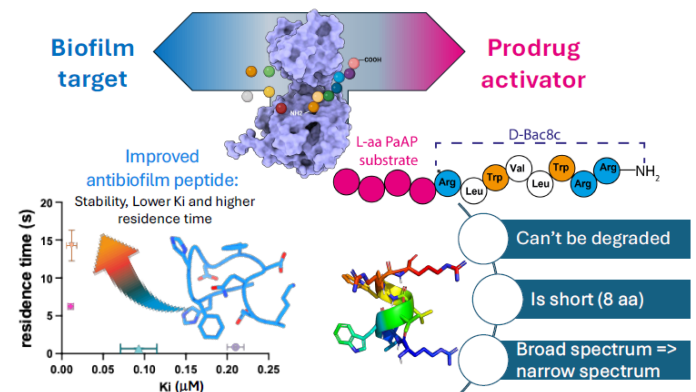
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Bacteria invest heavily in extracellular proteases and aminopeptidases to survive in biofilms.[1] However, these enzymes remain largely underexploited in antimicrobial peptide design.[2, 3]

Focusing on the secreted aminopeptidase PaAP from *Pseudomonas aeruginosa*, one of the most abundant proteins in the biofilm extracellular matrix[4], we show how detailed mechanistic understanding enables enzyme inhibition or its deployment against the bacterium. Structural and kinetic dissection of PaAP revealed an autoinhibitory C-terminal peptide and a significant promiscuity towards unstructured peptide substrates[5], guiding the rational and AI-enabled design of nM potency cyclic peptides that trap it in an inactive state and disrupt late-stage biofilms.

We then exploited PaAP's abundance and efficiency to selectively activate a prodrug AMP.[6] By grafting preferred aminopeptidase substrates onto antimicrobial peptides, we created inactive precursors that are cleaved by PaAP within *P. aeruginosa* biofilms. Effective activation required more than charge masking: prodrugs had to be explicitly matched to aminopeptidase substrate preferences to achieve rapid, species-selective killing. The result is a peptide prodrug that exploits bacterial metabolism itself as a trigger, reshaping resistance trajectories relative to the parent antimicrobial peptide. We successfully converted a broad spectrum antimicrobial peptide into a narrow spectrum compound, deployed for activation only by *P. aeruginosa*.

Together, these findings position extracellular aminopeptidases as exploitable control points for peptide therapeutics. Acting as targets or activators, we propose a general strategy for designing peptides that respond to the enzymatic logic of infection rather than fighting against it.



PaAP can be exploited (left) as a target in biofilms; we developed enhanced inhibitors based on target residence time or (right) as a species-specific activator of prodrugantimicrobial peptides acting only on *P. aeruginosa*.

1. Wauters, M. et al. Virulence 16, 2581889 (2025). 2. Galdino, A.C.M. et al., in Pathophysiological Aspects of Proteases, S. Chakraborti, N. S. Dhalla, Eds., pp. 381-97. 3. Galdino, A.C.M. et al. Curr Protein Pept Sci 20, 471-87 (2019). 4. Esoda, C.N. et al. mBio 10, (2019). 5. Harding, C.J. et al. Nat Chem Biol 19, 1158-66 (2023); 6. Gong, Q. et al. bioRxiv, 2026.03.29.715093 (2026).

L23a / L23b: Cecacins from the chicken caecal microbiome: translational applications in bovine mastitis and tuberculosis

Gama Dominic was allocated an oral presentation for her abstract 'Novel Cecacin peptides from the chicken ceca-microbiome show anti-tuberculosis and combinatorial activity'. Unfortunately, she was not able to obtain a visa to attend the conference. Consequently, some of her work will be included in Linda Oyama's talk which has been specially extended. You can see all of Gama's work in her poster (P34) that is being presented by Linda Oyama in today's poster session.

Cecacin-9: A chicken microbiome-derived antimicrobial peptide with potent *in vivo* efficacy and a favourable safety profile as a residue-free alternative to antibiotics for bovine mastitis

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Bovine mastitis, predominantly caused by *Staphylococcus aureus* and *Escherichia coli*, is the most economically significant infectious disease of dairy cattle [1,2], costing the global dairy industry an estimated USD 35 billion annually [3]. Increasingly difficult to treat due to antimicrobial resistance and biofilm formation, it presents both a veterinary and One Health challenge through the potential for food chain transmission of resistant bacteria.

To address this unmet need, we identified Cecacins, a novel class of antimicrobial peptides from the chicken caecal microbiome. The lead candidate, Cecacin-9 (CHK-9), is an 11 amino acid cationic peptide, net charge +4 and 58% hydrophobicity, with activity against key mastitis pathogens, including multidrug resistant *S. aureus* and *E. coli*, at MICs of 4 to 32 µg/mL. CHK-9 rapidly disrupts membranes, synergises with existing antimicrobials, and shows potent antibiofilm activity. Cytotoxicity testing showed no toxicity against five human cell lines, A498, BEAS 2B, Caco 2, HepG2, and HaCaT, with CC50 values exceeding 1,000 µg/mL. Haemolysis was only 26.4% at 1,000 µg/mL, giving a therapeutic index of 31.25 to greater than 250.

Circular dichroism showed that CHK-9 is intrinsically disordered in aqueous solution but adopts a partial alpha helical conformation in dodecylmaltoside, with a CD₂₂₂/CD₂₀₈ ratio of 0.69, minima at 207.6 and 222 nm, and a positive peak at approximately 195 nm. In contrast, CHK-9 remained largely unstructured in dodecylphosphocholine, a eukaryotic membrane mimetic, supporting its selective antimicrobial activity and low mammalian cytotoxicity.

In a murine mastitis model, a single intramammary dose of CHK-9 at 500 µg/gland significantly reduced the bacterial load of *S. aureus* ATCC 29737 (Newbould) compared to the untreated control. CHK-9 was also effective against *E. coli* ATCC 25922 at 2,000 µg/gland, demonstrating broad spectrum activity against key mastitis pathogens in its natural, unmodified form.

A safety trial in dairy cows where 500 mg of CHK-9 were infused confirmed no adverse effects on body temperature, feed intake, milk production, or inflammatory markers (including haptoglobin and somatic cell counts). CHK-9 remained detectable in milk only up to 10 hours post-infusion, suggesting potential for shorter withdrawal periods than conventional antibiotics and positioning CHK-9 as a compelling, residue-free alternative therapeutic for bovine mastitis.

1. Tomanić D, Samardžija M, Kovačević Z. Alternatives to Antimicrobial Treatment in Bovine Mastitis Therapy: A Review. *Antibiotics* (Basel). 2023 Mar 30;12(4):683. doi: 10.3390/antibiotics12040683. PMID: 37107045; PMCID: PMC10135164; 2. Wang K, Cha J, Liu K, Deng J, Yang B, Xu H, Wang J, Zhang L, Gu X, Huang C, Qu W. The prevalence of bovine mastitis-associated *Staphylococcus aureus* in China and its antimicrobial resistance rate: A meta-analysis. *Front Vet Sci*. 2022 Nov 10;9:1006676. doi: 10.3389/

fvets.2022.1006676. PMID: 36439336; PMCID: PMC9687384; 3. Kappes A, Tozooni T, Shakil G, Railey AF, McIntyre KM, Mayberry DE, Rushton J, Pendell DL, Marsh TL. Livestock health and disease economics: a scoping review of selected literature. *Front Vet Sci*. 2023 Sep 19;10:1168649. doi: 10.3389/fvets.2023.1168649. PMID: 37795016; PMCID: PMC10546065.

R12: Therapeutic potential and safety challenges of antimicrobial peptides and peptidomimetics against ESKAPEE Pathogens: a systematic review with quantitative analysis

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Background: Antimicrobial peptides (AMPs) investigated as alternatives to conventional antibiotics against multidrug-resistant pathogens. However, clinical translation is constrained by safety concerns, that compromise therapeutic selectivity. This systematically evaluates the potency, safety, stability, and *in vivo* efficacy of AMPs and peptidomimetics against ESKAPEE pathogens.

Methods: A literature search was performed across PubMed, Embase and Scopus (January 2000 and May 2025), yielding 47 studies. Data on minimum inhibitory concentrations (MIC), toxicity, selectivity indices (SI), stability and *in vivo* efficacy were extracted and synthesised using pooled geometric means (GM) and categorical summaries.

Results: Analysis of 136 peptide entries (14.7% natural AMPs, 51.5% synthetic AMPs, and 33.8% peptidomimetics) established potency dominance by natural AMPs, particularly against Gram-negative pathogens (*Escherichia coli*: GM MIC 1.16 µM for natural AMPs, 6.58 µM for synthetic AMPs and 27.45 µM for peptidomimetics). Against Gram-positive pathogens, peptidomimetics showed superior potency compared to synthetic AMPs. Safety profiles consistently favoured peptidomimetics, demonstrating significant higher SI value (e.g., GM SI 28.03 vs 10.82 for synthetic AMPs against *Enterococcus faecium*). Natural AMPs carried the highest risk of toxicity. High serum protein binding was observed for several natural AMPs (e.g., Polymyxin B: 99%), and divalent cations reduced activity. *In vivo* studies demonstrated promising therapeutic efficacy with favourable safety, though Polymyxin B consistently produced nephrotoxicity.

Conclusions: Clinical viability of AMPs involves a potency-safety trade-off. Natural AMPs are the most potent whilst peptidomimetics provide optimal balance with acceptable efficacy, low toxicity and enhanced stability. Structural engineering, improved formulation and targeted delivery are for clinical translation.

AMP Structure and Membrane Interactions

L24: Expanding Microscale Thermophoresis to Study Peptide Interactions with Bacterial Membranes

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Antimicrobial peptides (AMPs) active against bacteria are broadly classified into membrane-permeabilizing and non-membrane-permeabilizing peptides.[1] However, this distinction is often not absolute, as many AMPs exhibit hybrid mechanisms of action or transition between modes depending on the mechanistic landscape, including peptide concentration, lipid composition of the bilayer, peptide-to-lipid ratio, temperature, ionic strength, and pH.[2] In most cases, the initial step of AMP activity involves interaction with the bacterial membrane, even for peptides that do not primarily disrupt membrane integrity. These interactions are traditionally investigated using biophysical techniques such as surface plasmon resonance, isothermal titration calorimetry, and nuclear magnetic resonance, typically employing simplified model systems based on synthetic lipid bilayers.[2,3]

Here, we report the development of a novel approach that enables direct investigation of peptide-membrane interactions in live Gram-positive and Gram-negative bacteria. To this end, we implemented microscale thermophoresis (MST), an immobilization-free technique that quantifies molecular interactions by measuring the movement of molecules in microscopic temperature gradients.[4] The method relies on fluorescence signal modulation arising from microenvironmental changes that occur upon interaction between a ligand (unlabelled molecule) and a fluorescently labelled target.[5] While MST has recently been applied to study AMP interactions with model membranes[6], we demonstrate its applicability to intact bacterial cells. Using fluorescently labelled bacteria as targets, we successfully assessed the interaction of helminth-derived AMPs with live bacterial membranes. More broadly, this protocol provides a versatile platform for investigating interactions between bacteria and AMPs or other antimicrobial agents, with potential applications in both fundamental research and clinical contexts.

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[1] T. Rončević, J. Puizina, A. Tossi, "Antimicrobial Peptides as Anti-Infective Agents in Pre-Post-Antibiotic Era?" *International Journal of Molecular Sciences* 2019, 20, 5713; [2] S. Guha, J. Ghimire, E. Wu, W. C. Wimley, "Mechanistic Landscape of Membrane-Permeabilizing Peptides" *Chemical Reviews* 2019, DOI 10.1021/acs.chemrev.8b00520; [3] M.-T. Lee, "Biophysical characterization of peptide-membrane interactions" *Advances in Physics: X* 2018, 3, 1408428; [4] M. Asmari, R. Rati, H. A. Alhazmi, S. El Deeb, "Thermophoresis for characterizing biomolecular interaction" *Methods* 2018, 146, 107–119; [5] M. Jerabek-Willemsen, T. André, R. Wanner, H. M. Roth, S. Duhr, P. Baaske, D. Breitsprecher, "MicroScale Thermophoresis: Interaction analysis and beyond" *Journal of Molecular Structure* 2014, 1077, 101–113; [6] P. Rainsford, F. G. Rylandsholm, M. Jakubec, M. Silk, E. Juskewitz, J. U. Ericson, J.-S. Svendsen, R. A. Engh, J. Isaksson, "Label-free measurement of antimicrobial peptide interactions with lipid vesicles and nanodiscs using microscale thermophoresis" *Sci Rep* 2023, 13, 12619.

R13: Biophysical Insights into the Selective Membrane Interaction and Permeabilization Induced by the Antimicrobial Peptide RKW

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Antimicrobial peptides (AMPs) are key components of the innate immune system and have emerged as promising candidates for next-generation antibiotics due to their broad-spectrum activity, including efficacy against multidrug-resistant bacteria. These peptides are typically small, cationic molecules that interact with negatively charged bacterial membranes, a fundamental step of their mechanism [1]. However, their clinical application remains limited, primarily due to potential cytotoxicity toward host cells. Therefore, detailed biophysical studies on model membranes are critical for clarifying AMP-membrane interactions, representing a fundamental prerequisite for designing safe and potent antimicrobial treatments. This study investigates the interaction of antimicrobial peptide RKW with model lipid vesicles mimicking prokaryotic and eukaryotic membrane compositions [1]. RKW exhibited a strong preference for negatively charged bacterial membrane models, especially those representing Gram-negative bacteria, while showing minimal affinity for zwitterionic eukaryotic-like membranes. These findings suggest that electrostatic interaction is the primary driving force behind membrane selectivity. Fluorescence spectroscopy and quenching experiments revealed that RKW localises mainly at the membrane interface, likely adopting a parallel orientation relative to the bilayer surface. Furthermore, RKW induced substantial leakage of carboxyfluorescein from bacterial model vesicles, indicating a potent membrane permeabilization, as also evidenced by dynamic light scattering analyses. To evaluate the peptide's toxicity, *in vivo* experiments were conducted using the *Caenorhabditis elegans* as a model, which

revealed no negative impact on survival, reproduction, motility, or development of this organism. Further studies are currently underway, including microscale thermophoresis experiments, to validate the model membrane findings in the presence of Gram-negative and Gram-positive bacterial cells.

[1] Agrillo, B.; Ambrosio, M.; Ambrosio, R.L.; Gogliettino, M.; Balestrieri, M.; Porritiello, A.; Peruzzi, M.F.; Mancusi, A.; Nicolais, L.; Palmieri, G. <https://doi.org/10.3390/biom15070989>

L25: Translocation of Amphiphilic Peptides Across Cell Membranes

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Cell membranes act as semi-permeable barriers that restrict the entry of large or hydrophilic molecules into cells, limiting the development of new drugs. Peptides with the right properties can bypass the cellular machinery and spontaneously translocate across membranes. However, it remains elusive what these "right" peptide properties are. We developed a new collective variable describing the translocation process, which allowed us to calculate the translocation free energy profiles of various peptides and demonstrate that amphiphilic peptides can spontaneously translocate across the membrane. We have shown that membrane asymmetry could reduce the translocation barrier. However, even in these cases, the translocation free energy barrier remained significant. We therefore investigated whether the barrier could be reduced by transmembrane proteins. Using two very different models, we consistently show that transmembrane proteins with hydrophilic residues in the hydrophobic core of the membrane can enhance peptide translocation. We have shown that there are natural membrane proteins with such properties, scramblase or insertase. These proteins facilitate the translocation of lipids or the insertion of proteins into the membrane, and we have shown that they share the common mechanism of local membrane deformation, which also reduces the translocation barrier of amphiphilic peptides. Therefore, scramblases and insertases could be exploited for more efficient drug delivery systems, especially those involving the translocation of amphiphilic peptides into the cell.

L26: Structural engineering of stapled membrane-targeting antibacterial peptides

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Short cationic antimicrobial peptides (AMPs) are attractive scaffolds for the development of novel antimicrobials, yet their activity is limited by conformational instability and susceptibility to proteolysis. We present a combined experimental and computational approach for the rational design of biostable, membrane targeting AMPs using hydrocarbon stapling [1] to constrain peptide secondary structure in a membrane-active conformation. We focused on lysine- and leucine-rich peptides and derivatives of known antimicrobial and cellpenetrating sequences, including (KFF)₃K and anoplin.

Stapling of anoplin, a natural 10-residue peptide from wasp venom, stabilized its helical conformation, enhanced protease resistance, increased membrane disruption, and induced antibacterial activity with minimal inhibitory concentrations (MICs) of 4 μM [2]. Staple position governed selectivity: anoplin stapled at positions 2–6 was potent against Gram-negative bacteria, including drug-resistant strains, whereas anoplin stapled at positions 5–9 preferentially targeted Gram-positive organisms.

We next converted the cell-penetrating peptide (KFF)₃K – inactive against bacteria – into an AMP via stapling. Stapling stabilized its membrane-active conformation and promoted membrane insertion [3]. Stapled (KFF)₃K analogues inhibited methicillin-resistant *Staphylococcus aureus* and β-lactam-resistant *Escherichia coli* at MICs of 4–8 μM while remaining non-hemolytic.

Finally, we designed de novo lysine- and leucine-rich peptides, whose stapling yielded MICs of 2–4 μM against antibiotic-resistant clinical isolates, without hemolytic or cytotoxic effects [4]. Molecular dynamics simulations showed how stapling stabilizes the amphipathic helix and accelerates membrane insertion.

We further conjugated stapled peptides with aminoglycosides (neomycin and amikacin) using either noncleavable linkers via click chemistry or disulfide linkers cleavable in the bacterial cytoplasm [5]. The resulting conjugates were active against strains resistant to the parent antibiotics.

Collectively, these results establish hydrocarbon stapling as a versatile strategy for engineering biostable, selective AMPs and antibiotic conjugates with activity against drug-resistant pathogens.

[1] L. D. Walensky, G. H. Bird, *J. Med. Chem.*, 57:6275, 2014; [2] M. Wojciechowska, J. Macyszyn, J. Miszkiewicz, R. Grzela, J. Trylska, *Front. Microbiol.*, 12:772038, 2021; [3] J. Macyszyn, P. Chyży, M. Burmistrz, M. Lobka, J. Miszkiewicz, M. Wojciechowska, J. Trylska, *Sci. Rep.*, 13:14826, 2023; [4] M. Lobka, I. Siekierska, P. Chyży, M. Burmistrz, J. Macyszyn, R. Grzela, M. Wojciechowska, J. Trylska, *Eur. J. Med. Chem.*, 290:117445, 2025; [5] J. Macyszyn, M. Burmistrz, A. Mieczkowski, M. Wojciechowska, J. Trylska, *ACS Omega*, 8:19047, 2023

R14: Design of membrane-selective peptides by evolutionary molecular dynamics

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Antimicrobial peptides (AMPs) are a viable alternative to conventional antibiotics due to their broad-spectrum activity and reduced resistance propensity [1]. One of the primary targets of AMPs is the microbial cell membrane, which they usually disrupt by forming pores on it [2]. However, the clinical application of membrane-binding AMPs is often limited by poor selectivity, resulting in cytotoxicity toward human cells. Consequently, achieving selective membrane targeting remains a key challenge in AMP design, and in general membrane-binding peptides.

Here, we present a computer-aided design strategy for selective membrane-binding peptides. Peptide sequences were designed through evolutionary optimization driven by differences in membrane-binding free energy between two membrane models, computed using coarse-grained molecular dynamics simulations. The optimization process was initialized from random sequences constrained solely by their hydrophobic moment. The resulting peptides exhibited enhanced selectivity in coarse-grained computational membrane models.

[1] Cabral et al. *Antibiotics*. 2025, 14(11), 1115. [2] Pál, et al. *Nature Communications*. 2019, 10, 4538.

L27: Membranolytic versus non-membranolytic mode of action of two antimicrobial laticin peptides from spider venom

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Laticins (Ltc) are a group of 7 linear antimicrobial peptides isolated from the venom of the spider *Lachesana tarabaei* [1]. Ltc1 and Ltc2a show structural and functional similarities such as high charge, folding into an α -helix, and high antimicrobial activity but display pronounced differences in their mode of action and membranolytic side effects. Oriented circular dichroism and solid-state 15N-NMR show that these differences are correlated to the preferred alignment of the peptide in the membrane [2]. Ltc1 is found to be oriented parallel to the membrane surface, and it is non-membranolytic. On the other hand, Ltc2a has a pronounced tendency to tilt into the membrane, and it shows considerable membranolytic side effects. Thus, we hypothesized that a tilted amphipathic helix (Ltc2a) operates via a membranolytic mode, whereas an amphipathic helix on the bilayer surface (Ltc1) uses a non-membranolytic route for antimicrobial action. To test this hypothesis, polar residues in the hydrophobic sector

of Ltc1 (K10, R15, K19), which are absent in Ltc2a, were individually or in combination replaced with hydrophobic residues. Our results demonstrate that a contiguous hydrophobic sector devoid of polar residues, and with a certain twist around the helix axis, is indeed able to induce tilt and membranolytic side effects. On the other hand, the presence of polar residues in the hydrophobic sector promotes a largely non-membranolytic mode of antimicrobial action. These results will be discussed to highlight the different modes of action for these two closely related antimicrobial peptides present in the same spider venom.

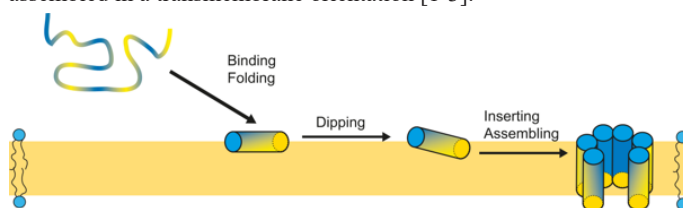
[1] Dubovskii PV, Vassilevski AA, Kozlov SA, Feofanov AV, Grishin EV, Efremov RG. *Cell Mol Life Sci*. 2015;72:4501-22; [2] Wadhvani P, Sekaran S, Strandberg E, Bürck J, Chugh A, Ulrich AS. *Int J Mol Sci*. 2021;22,10156.

L28: The hydrophobic profile along the helix axis influences the pore-forming activity of cationic amphipathic helices

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KIA21 (also known as MSI-103) is a cationic amphipathic helical antimicrobial peptide with the repetitive sequence [KIGAKIA]₃-NH₂. It is able to permeabilize membranes, as demonstrated by antimicrobial activity, hemolysis, and vesicle leakage experiments [1-3]. KIA21 has been proposed to form toroidal pores in lipid bilayers, with the helices assembled in a transmembrane orientation [1-3].



It can be assumed that the peptide must dip one terminus into the membrane as a first step to insert and form pores. Given its charged N-terminus and hydrophobic C-terminus, KIA21 presumably tilts into the membrane with its C-terminus first. To test this hypothesis, we made either the N- or the C-terminal part of the helix more hydrophobic by changing the order of the amino acids, but without changing the overall amino acid composition of the peptide. A more hydrophobic C-terminus was indeed found to give a more membrane-active peptide, with higher hemolytic activity and leakage. On the other hand, a more hydrophobic N-terminus (and thus less hydrophobic C-terminus) has the opposite effect, fully supporting our hypothesis. Another KIA-like peptide with the sequence [IAGKIAK]₃-NH₂, modified to carry a Lys at the C-terminus, was found to be less active [4], which also supports the hypothesis, since the charge should make it energetically less favorable to dip the C-terminus into the membrane. However, when the hydrophobicity of its C-terminus was increased, the peptide got more active again, showing that local hydrophobicity can compensate for the charge effect.

[1] A Grau-Campistany, E Strandberg, P Wadhvani, J Reichert, J Bürck, F Rabanal, AS Ulrich (2015). *Sci Rep* 5, 9388; [2] A Grau-Campistany, E Strandberg, P Wadhvani, F Rabanal, AS Ulrich (2016). *J Phys Chem Lett* 7, 1116-1120; [3] E Strandberg, D Bentz, P Wadhvani, AS Ulrich (2020) *Sci Rep*, 10, 4710; [4] E Strandberg, D Bentz, P Wadhvani, J Bürck, AS Ulrich (2020) *Biochim Biophys Acta*, 1862, 183243.

Friday at 14:00

Panel Discussion 'The Future of AMPs and AMP research'

- Edwin Veldhuizen (chair / facilitator)
- Inanc Birol
- Jayachandran Kizhakkedathu
- Marina Rautenbach
- Alex Tossi

Poster Abstracts

Poster and Rapid Oral prizes sponsored by



Posters will be displayed throughout the meeting. Poster authors should attend their posters during the poster session on Wednesday (odd numbers) or Thursday (even numbers).

Final poster judging will take place during the lunch break on Friday, so posters should not be removed before the end of the lunch break on Wednesday.

The poster competition is open to all PhD student and postdoc poster authors.

The Rapid Oral prize judging will be based on the quality of the oral presentation and the associated poster.

Posters associated with rapid oral communications should also not be removed until the end of the lunch break on Friday. Authors of Rapid Oral communications should attend their poster during the poster session of the day of their Rapid Oral presentation, unless their presentation is scheduled for Friday, in which case they should be beside their poster during the Thursday poster session.

P1: AI-driven discovery and optimization of outer membrane-permeabilizing peptides

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The rise of multidrug-resistant Gram-negative pathogens is partially driven by their highly impermeable outer membrane (OM), which blocks antibiotic uptake. We have identified a class of innate, genome-encoded outer membrane-permeabilizing peptides (OMPPs) that selectively disrupt the OM, synergizing with otherwise ineffective antibiotics such as mupirocin and vancomycin in *Escherichia coli*. Experimental characterization has resulted in OMPPs with up to a 2000-fold potentiation of antibiotics, largely driven by the work outlined in my colleague Robin Rydbergh's abstract submission (P16). We are now developing an AI-driven framework to accelerate the discovery and optimization of new candidates.

Our computational approach addresses the challenge of data scarcity in peptide engineering. We developed SCARSE, a versatile AI framework that learns meaningful patterns from a small number of characterized peptides and leverages this knowledge to improve experimental outcomes [1]. We found that as few as 50 characterized peptides were sufficient to accurately predict the performance of future iterative peptide optimization efforts. This provides researchers with a practical tool for assessing whether AI-driven peptide engineering is

likely to be effective for a given dataset of peptides. As experimental data from our expanding peptide panel accumulates, our AI models will be iteratively retrained, refining the ranking of potential OMPP candidates. This computational pipeline will eventually be adapted to support the discovery of mycolic acid layer-permeabilizing peptides (MAPPs) in mycobacterial systems. By integrating experimental validation with machine learning, we aim to establish a scalable workflow for the rational design of antibiotic-potentiating peptides.

[1] Andrekson, L., Rydbergh, R., Mercado, R., & Wenzel, M. (2026). AI-guided discovery for low-resource peptide engineering using evolutionary scale modeling. *bioRxiv*, 2026-06. <https://doi.org/10.64898/2026.06.25.734678>.

P2: Study of the interactions of a biomimetic, elastin-inspired recombinant polypeptide with the bacterial proteins

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Recombinant fusion biotechnology is a powerful approach for producing antimicrobial peptides (AMPs), which can contribute to fight potentially pathogenic microorganisms. In our lab, fusion carriers inspired to elastin were employed to produce model antimicrobial fusion constructs exploiting their unique thermo-responsive behavior. [1] A new biomimetic elastin-based carrier variant named UELP4 was recently developed. Unexpectedly, the carrier itself showed significant antibacterial activity, especially towards the Gram-negative strains tested. [2] Notably, it has been described that elastin hydrolysates possess strong capacity to inhibit bacterial growth, pointing to the presence of hidden antimicrobial sequences within elastin. [3]

Outer membrane permeability in the Gram-negative *Escherichia coli* ATCC 8739 in the presence of UELP4 was assessed by employing the fluorescent probe N-Phenyl-1-Naphthylamine (NPN). To dive deeper into this context, we applied an optimized methodology that integrates limited proteolysis with mass spectrometry [4], providing a comprehensive approach to exploring UELP4-target protein interactions involved in its antimicrobial activity. Among the few identified candidates, the PqiC protein (Intermembrane Transport Lipoprotein) emerged as an interesting target. Indeed, this outer membrane-associated lipoprotein, a component of the paraquat operon, contributes to membrane integrity, suggesting a possible involvement in the mechanism of action of UELP4 [5].

Overall, our approach represents a valuable strategy to undertake the first steps toward unveiling the mechanisms of the antibacterial activity of cryptic sequences nested in structural proteins like elastin.

[1] Bandiera, A. et al., *Biomacromolecules*, 2023, 24(11): 5277-5289. doi: 10.1021/acs.biomac.3c00782; [2] Colomina-Alfaro, L., et al., *Int. J. Biol. Macromol.*, 2024, 283(Pt 1): 137682. doi: 10.1016/j.ijbiomac.2024.137682; [3] Al-Ghanimi, G. M. M., and Alrubei, A. M. S., *Iraqi Journal of Agricultural Sciences*, 2024, 55, 422-431, DOI: <https://doi.org/10.36103/8w3ft36>; [4] Capuano, A., et al., *Food Chem.*, 2025, 1: 479:143838. doi: 10.1016/j.foodchem.2025.143838; [5] Cooper, B. F. et al., *EMBO Rep.*, 2024, 25 :82-101. doi: 10.1038/s44319-023-00014-4.

P3: Immobilization of short antifungal peptides on contact lens

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Purpose: Fungal keratitis is a leading cause of blindness worldwide. *Candida albicans* being one of the major causative organisms and is included in the high priority pathogen list recently published by WHO. Immediate intervention is needed considering the currently available therapies are either limited or ineffective. Host Defense Peptides (HDP) are envisaged as potential antimicrobial candidates that can help us to combat drug resistant pathogen.

Methods: SA-XV a 15 mer fragment was designed from S100A12 and its antifungal property and mode of action against *C. albicans* was determined by colony forming units, surface plasmon resonance and scanning electron microscope. SA-XV was also immobilized onto soft silicone hydrogel contact lenses using EDC chemistry and checked for its antifungal property. Various physical parameters like optical transmittance, transparency, thickness and contact angle were also checked.

Results: We found that SA-XV inhibited more than 99% of *C. albicans* at a concentration of 60 μ M. SA-XV was found to bind to membrane, translocating into the cytoplasm, binding to DNA, and arresting cell cycle, finally causing death by apoptosis. The immobilized SA-XV onto contact lenses inhibited 90% of *C. albicans* with complete membrane rupture. Further, the immobilization did not alter physical parameters like transparency, optical transmittance, thickness or contact angle. The immobilized lenses were found to be biocompatible and non-cytotoxic upon incubation with human corneal epithelial cells.

Conclusions: SA-XV retains its antifungal property like the parent peptide with multifaceted mode of action both in soluble form and when immobilized into contact lenses. Therefore SA-XV can be considered as a potential candidate to treat fungal keratitis with the increasing antifungal resistance.

[1] Agarwal R, Biswas K, Agrawal A, Shankar NN, Kundu S, Roy D, Son D, Harikishore A, Yennamalli RM, Lee D, Bhunia A. SA-XV, a 15-amino acid fragment of host defense peptide S100A12, targets mitochondria and is protective against fungal infections. *Journal of Biological Chemistry*. 2025 Oct 1;301(10).

P4: Structural and functional contributions of an unstructured, disulfide-bridged C-terminus to helminth antimicrobial peptides

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Most helminth-derived antimicrobial peptides (AMPs) share distinctive sequence and structural characteristics, providing an opportunity to identify evolutionarily conserved determinants of antimicrobial function [1,2]. Structural predictions revealed that flatworm peptides with a C-terminal CXXXC disulfide-forming motif consistently adopt a common architecture consisting of an N-terminal α -helix followed by a predominantly disordered C-terminal tail, reminiscent of the human AMP LL-37. To investigate the functional significance of this architecture and the cysteine motif, we combined all-atom molecular dynamics simulations with complementary experimental approaches, using the mesco-2 peptide from *Mesocestoides corti* and the taenimycin peptide family from *Taenia solium* as representative examples. Our results demonstrate that the disulfide bridge in mesco-2 modulates peptide conformational dynamics and the kinetics of bacterial killing, highlighting its role in regulating peptide function. The flexible C-terminal tail also appears to contribute to peptide association, with this behavior being strongly influenced by the presence of the disulfide bridge. In contrast, analyses of the taenimycin family suggest that the same motif may serve a distinct structural or functional role that remains to be elucidated. Together, these findings identify the conserved helix–tail architecture and the CXXXC motif as characteristic features of flatworm AMPs and provide new insights into how these structural elements shape peptide dynamics, intermolecular interactions, and antimicrobial activity.

[1] Rončević T, Gerdol M, Pacor S, Cvitanović A, Begić A, Weber I, et al. *Int J Mol Sci*. 2024;25:11690. doi:10.3390/ijms252111690; [2] Budimir A, Stojan I, Primorac K, et al. *Sci Rep*. 2026;16:14472. doi:10.1038/s41598-026-44795-9.

P5: The creation of sustainable self-sterilising nanocelluloses functionalised with natural antimicrobial cyclic decapeptides and a 2-aminoimidazole derivative

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Targeting the indirect contact-spread of pathogens via surfaces in public spaces is a crucial tactic in preventing the spread of microbial pathogens. In this study crystalline nanocellulose (CNC) and cellulose nanofibers (CNF) were utilised as substrates for developing antimicrobial surface treatments. To ensure these nanoformulations have durable, broad-spectrum activity against the relevant pathogens, we focussed on three antimicrobial preparations, namely gramicidin-S (GS), tyrocidines (Trcs), and a small-molecule derivative of 2-aminoimidazole (2-AI), termed BT-1. Both GS and the Trcs are basic, cyclic decapeptides (CDPs) with potent antimicrobial activity, produced naturally by soil bacteria. The Trcs have potent activity against a wide range of Gram-positive bacteria [1,2], fungi [3,4] and against selected Gram-negative bacteria [4–7]. They also show robust activity on treated surfaces, including cellulose-type materials [8]. GS show potent activity against both Gram-positive and -negative bacteria [9]. 2-AI derivatives have shown the ability to inhibit biofilm formation [10,11]. Low MICs were observed for NCs with Trcs against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli* and *Acinetobacter baumannii* ($\pm$ 15–25 μ g/mL). GS shows comparable activity against Gram-negative pathogens in both gel and film NCs. Formulations combining Trcs with BT-1 and/or GS led generally to synergism (FIC index < 0.8) against *E. coli* and *A. baumannii* in the gel and film-type NCs. We also found that all three compounds bind with high affinity to CNF and the Trcs to CNC. This evidence points to the reliable inhibition of key pathogens using NCs as a substrate for antimicrobial peptides and small molecules.

[1] Dubos RJ, Hotchkiss RD & Coburn AF (1942). *J Biol Chem* 146, 421–426; [2] Spathelf BM & Rautenbach M (2009). *Bioorg Med Chem* 17, 5541–554; [3] Mach B & Slayman CW (1966). *Biochimica et Biophysica Acta (BBA) - General Subjects* 124, 351–361; [4] Arnold Vosloo J, Beims H, Allsopp MH, van Rensburg W, von der Ohe W, Steinert M & Rautenbach M (2017). *Apidologie* 48, 833–844; [5] Chopra S, Torres-Ortiz M, Hokama L, Madrid P, Tanga M, Mortelmans K, Kodukula K & Galande AK (2010). *Journal of Antimicrobial Chemotherapy* 65, 2598–2601; [6] Troskie AM, de Beer A, Vosloo JA, Jacobs K & Rautenbach M (2014). *Microbiology (United Kingdom)* 160, 2089–2101; [7] Troskie AM, Rautenbach M, Delattin N, Vosloo JA, Dathe M, Cammue BPA & Thevissen K (2014). *Antimicrob Agents Chemother* 58, 3697–3707; [8] Van Rensburg W & Rautenbach M (2022). *Antibiotics* 11, 174; [9] Kondejewski LH, Farmer SW, Wishart DS, Hancock REW & Hodges RS (1996). *Int J Pept Protein Res* 47, 460–466; [10] Belardinelli JM, Li W, Martin KH, Zeiler MJ, Lian E, Avanzi C, Wiersma CJ, Nguyen TV, Angala B, de Moura VCN, Jones V, Borlee BR, Melander C & Jackson M (2022). *Int J Mol Sci* 23; [11] Rogers SA & Melander C (2008). *Angew Chem Int Ed* 47, 5229–5231.

P6: SAR Study Of The Human Host-Defence Peptide LL-37

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Host defence peptides are an important component of innate immunity, acting as a first line of defence against infections. Among these natural antibiotics, the cathelicidin family [1] of antimicrobial peptides (AMPs) is widely distributed throughout vertebrates. Primates have a single cathelicidin, LL-37 [2] in humans and RL-37 [3] in *Macaca mulatta*, both of which have been extensively studied because of their diverse biological activities.

LL-37 adopts an amphipathic α -helical structure under physiological conditions and acts as a pore-forming oligomer on bacterial membranes. In contrast, RL-37 remains largely disordered until membrane contact, where it acts as a helical monomer through a carpet-like mechanism. The ability to form intramolecular salt bridges in the helical conformation appears to be a major determinant of these distinct structural and mechanistic behaviours [3].

To investigate the sequence features underlying these differences, we used predictive approaches to design minimally modified RL-37 analogues with enhanced structural stability, as well as destabilized LL-37 variants. A library of minimal variants was generated and their conformational stability assessed.

Structural properties were then correlated with antibacterial activity and cytotoxicity towards host immune cells. Selected analogues were further characterized to define their antimicrobial mechanism through bacterial membrane permeabilization[3] assays, and to evaluate immunomodulatory functions, including cytotoxicity and induction of pro-inflammatory responses.

Our results reveal a direct relationship between peptide structural stability and biological function, demonstrating how conformational properties influence both antimicrobial activity and host immune responses. These findings provide a framework for interpreting the sequences of over 200 primate sequences collected by genome mining approaches.

1. Tossi et al. Front. Drug Discov. (2024), Sec. Anti-Infective Agents doi: 10.3389/fddsv.2024.1458057; 2. Xhindoli et al. BBA: Biomembranes (2016) doi: 10.1016/j.bbmem.2015.11.003; 3. Pacor et al. Peptide Science (2018) doi: 10.1002/pep2.24087.

P7: Making materials matter: How metals and matrix properties influence peptide surface activity

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The rapid rise in antimicrobial resistance, along with the inability of conventional antibiotics to treat bacterial infections, has resulted in a shift toward 'green' biocides to combat these infections. Antimicrobial peptides (AMPs) are one such 'green' biocide, and the frontrunner in this regard. While many AMPs show promise against a range of bacterial and fungal pathogens, their real-world performance often drops when applied to different surfaces and materials. The loloatins (Lols) are a family of antimicrobial cyclic decapeptides produced by *Brevibacillus laterosporus* [1], and our studies have shown that the Lols have strong surface activity on polystyrene against the Gram-positive *Staphylococcus aureus*, the Gram-negative *Acinetobacter baumannii*, as well as anti-fungal activity against *Candida albicans*. However, we found that changes in the peptide-seeding solution and depositing matrix resulted in activity changes and even a complete loss of activity. In the analogous tyrocidine family, adding metal ions in formulation, such as zinc, calcium or silver, typically enhanced antibacterial activity against several bacterial pathogens [2–5]. However, our study showed that Fe^{2+} salts reduce the surface activity of these families of cyclodecapeptides. Ion-mobility mass spectrometry revealed that Fe(II) causes the peptides to form peptide- Fe(II) complexes instead of pairing into amphipathic dimers which is considered the membrane active moieties. This supported that dimers and possibly higher-order oligomers, rather than the more cationic monomeric peptide- Fe(II) complexes, are the key drivers of antimicrobial surface activity by these peptides. While activity is lost on materials other than polystyrene, incorporating peptide-silver nanoparticles or zinc oxide may offer a future strategy for recovery of activity on surfaces.

1. Gerard JM, Haden P, Kelly MT, Andersen RJ (1999) Loloatins A-D, cyclic decapeptide antibiotics produced in culture by a tropical marine bacterium. J Nat Prod 62:80–85; . Leussa Nyango-Nkeh Adrienne by, Rautenbach M (2014) Characterisation of small cyclic peptides with antilisterial and antimalarial activity, <http://hdl.handle.net/10019.1/86161>; 3. Van Rensburg W, Rautenbach M (2015) Characterization of natural antimicrobial peptides adsorbed to different matrices, <http://hdl.handle.net/10019.1/97929>; 4. Borrageiro C (2023) Formulation of natural cyclodecapeptides for surface sterilisation, <http://hdl.handle.net/10019.1/127096>; 5. De Villiers C, Rautenbach M (2023) Metal and tyrocidine nano-assemblies to create broad-spectrum metal-peptide formulations, <http://hdl.handle.net/10019.1/127255>.

P8: Dissecting antimicrobial peptide mechanisms through LPS structural variability in *Escherichia coli*

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The increasing prevalence of multidrug-resistant Gram-negative bacteria has intensified the search for alternative antimicrobial strategies, positioning antimicrobial peptides (AMPs) as promising candidates. However, the determinants governing their activity remain incompletely defined, particularly the role of lipopolysaccharide (LPS), the main interaction interface between AMPs and the bacterial outer membrane. How structural variations in LPS influence peptide activity and contribute to differential susceptibility across bacterial species is still largely unknown.

In this study, we perform a systematic and comparative analysis of AMP activity across *Escherichia coli* strains displaying defined variations in LPS composition (D21, D21e7, D21e19, D21f1 and D21f2), which represent a gradient of LPS truncation from a complete structure to progressively reduced core oligosaccharide regions. These modifications alter key physicochemical properties of the bacterial surface, including charge distribution, membrane organization, and permeability, providing a controlled framework to assess peptide–membrane interactions. A diverse panel of antimicrobial peptides with distinct physicochemical properties and proposed mechanisms of action is evaluated, including representative peptides such as magainin, indolicidin, cecropin, melittin and Bac8c, among others.

By combining complementary approaches to assess antimicrobial activity, membrane perturbation, and structural alterations at the cellular level, we aim to establish direct relationships between LPS architecture and peptide mechanism of action. Preliminary observations indicate that LPS truncation modulates bacterial susceptibility in a peptide-dependent manner, supporting the existence of both LPS-dependent and LPS-independent modes of action. This integrative framework provides mechanistic insight into how outer membrane composition governs peptide performance.

Taken together, this work contributes to explaining variability in AMP effectiveness across Gram-negative bacteria and supports the rational design of next-generation peptides with improved and predictable activity against diverse bacterial targets.

P9: Developing Antimicrobial Peptide Drug Delivery Based on Protein Nanoparticle Display

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Antimicrobial peptides (AMPs) are promising alternatives to antibiotics for combating antimicrobial resistance [1]. However, their clinical application is limited by poor stability and cytotoxicity [1]. While nanocarrier-based delivery systems have been explored, protein-based assembly platforms remain relatively underdeveloped [2].

Here, we present a protein-based delivery system using a self-assembling encapsulin nanocompartment as a scaffold to display the model AMP LL37. A fusion protein was engineered by linking LL-37 to the encapsulin shell protein via a linker and a protease cleavage site, enabling enzyme-responsive activation of the peptide.

Fusion constructs with different linker designs have been successfully expressed in both *Escherichia coli* cell-free protein synthesis and in vivo systems. The cell-free platform was used for rapid screening of antimicrobial activity across different variants, and selected candidates are being further evaluated for protease-triggered activation. In parallel, recombinant expression in *E. coli* has enabled production and purification of promising candidates, supported by a stable purification workflow.

Ongoing studies include structural characterization, antimicrobial activity assays, enzyme responsiveness, and safety evaluation. This work presents proof of concept towards establishing a platform for controlled presentation and activation of AMPs using protein nanocompartments.

[1] Asif, Fahim, Sabbir Uz Zaman, Md. Kamrul Hasan Arnab, Moynul Hasan, and Md. Monirul Islam. 2024. <https://doi.org/10.1016/j.microb.2024.100051>; [2] Zheng, Sainan, Yuhuan Tu, Bin Li, et al. 2025. <https://doi.org/10.1186/s12967-025-06321-9>.

P10: Time-resolved single-cell imaging of antimicrobial peptide-induced membrane permeabilization in gram-negative bacteria

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The increasing prevalence of multidrug-resistant bacteria highlights the need for new antimicrobial treatments and rapid methods to evaluate their activity and mechanism of action [1]. Antimicrobial peptides (AMPs) are promising alternatives to conventional antibiotics, with many AMPs acting through disruption of bacterial membranes. Since the membrane of Gram-negative bacteria is a major barrier to drug uptake [2], rapid methods for assessing membrane-targeting activity can facilitate the development and characterization of new antimicrobial candidates.

Here, we present a microfluidic method for studying the effect of AMPs on the membranes of live *Escherichia coli*. Microfluidic chips allow manipulation of small liquid volumes and, when combined with fluorescence microscopy, enable real-time observation of individual bacterial cells [3]. Membrane permeabilization is monitored using Sytox Orange (Invitrogen™), a cell-impermeant nucleic acid dye that becomes fluorescent after entering cells with compromised membranes and binding to DNA.

Based on the previous studies [4, 5], we investigated seven AMPs by monitoring immobilized live *E. coli* cells exposed to a medium containing both the AMP and Sytox Orange. The Sytox Orange fluorescence dynamics revealed differences between predominantly lytic AMPs and the remaining peptides. Time-lapse fluorescence imaging allowed comparison of the onset time and intensity of the Sytox Orange signal for each peptide at the single-cell level.

This method provides a simple and efficient approach for assessing the membrane-disrupting activity of antimicrobial peptides and evaluating new antimicrobial candidates.

[1] Theuretzbacher U. *Nature Reviews Microbiology* 23, 8: 491-508 (2025). doi: 10.1038/s41579-025-01169-8 [2] C. Maher, K. A. Hassan. *mBio* 14: e01205-23 (2023). doi: 10.1128/mbio.01205-23 [3] J. K. Nunes, H. A. Stone. *Chemical Reviews* 122, 7: 6919-6920 (2022). doi: 10.1021/acs.chemrev.2c00052 [4] H. Choi, N. Rangarajan, J. C. Weisshaar, *Trends in microbiology* 24, 111-122 (2016), doi: 10.1016/j.tim.2015.11.004 [5] C. Landon, Y. Zhu, M. Mustafi, J. B. Madinier, D. Lelievre, V. Aucagne, A. F. Delmas, J. C. Weisshaar, *International Journal of Molecular Sciences* 23, 2057 (2022), doi: 10.3390/ijms23042057

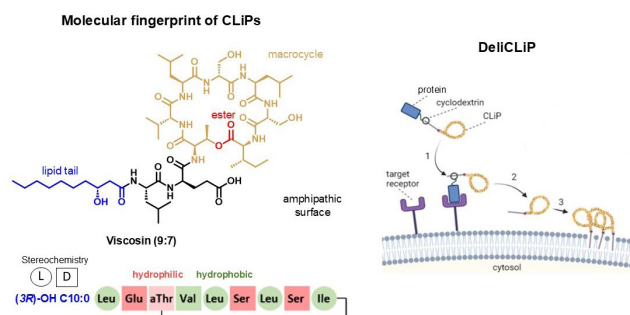
P11: A Delivery System for Bioactive Cyclic Lipopeptides: Toward Improved Solubility and Selectivity

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Cyclic lipopeptides (CLiPs) are amphipathic compounds with diverse biological activities against microbial pathogens and cancer cells [1]. Their membrane-targeting mechanism of action makes them an attractive class of bioactive molecules with potential to overcome resistance to current therapeutic agents. However, their clinical translation is often hampered by poor aqueous solubility and limited selectivity. To address these challenges, we are focused on developing a delivery system, named DeliCLiP, designed to mitigate formulation limitations

of CLiPs while enhancing their biological performance. The platform comprises cyclodextrins (CDs) as carrier molecules, capable of forming inclusion complexes with poorly water-soluble CLiPs, and targeting moieties for selective delivery. This poster focuses on synthesizing the key building blocks of the DeliCLiP platform.



As a model poorly soluble CLiP, we are investigating viscosin, a member of the Viscosin (9:7) family with activity against Gram positive bacteria. Viscosin consists of a nonapeptide containing a seven-residue macrocycle. In addition to native viscosin, seven analogues with different lipid modifications, including single and double lipid tails and adamantane moieties, were designed and synthesized to investigate their impact on CD complexation and membrane interaction. To further enhance selectivity, CD-protein conjugates were developed through first the synthesis of CD-linker building blocks. The linker design is based on the in-house developed 5HP2O technology (OBCR) [2], which provides enhanced stability to both the building blocks and the resulting conjugates.

Overall, this strategy aims to improve CLiP solubility and enable controlled delivery of bioactive lipopeptides at biologically relevant concentrations in the vicinity of the target membrane.

[1] Geudens, N., J. C. Martins. *Frontiers in Microbiology*, 9 (2018); [2] De Geyter, E. et al. *Chemical Science* 12, 5246-5252 (2021).

P12: Structural characterization and mode of action of larvicidal peptides from *Xenorhabdus indica* 157-C against *Anopheles arabiensis*

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The emergence and spread of insecticide resistance among malaria vectors threaten the effectiveness of current vector control strategies, highlighting the need for novel larvicidal compounds [1,2]. *Xenorhabdus* spp., the bacterial symbionts of entomopathogenic nematodes, produce a diverse range of secondary metabolites with potent insecticidal activity [3, 4]. This study aimed to isolate, characterize and investigate the mode of action of larvicidal metabolites produced by *Xenorhabdus indica* 157-C against *Anopheles arabiensis* larvae. Whole-genome sequencing and biosynthetic gene cluster analysis identified a fabclavine biosynthetic gene cluster, while liquid chromatography tandem mass spectrometry (LC-MS/MS) and nuclear magnetic resonance (NMR) enabled the structural characterization and putative identification of the active compounds as fabclavine derivatives. Bioassays demonstrated potent larvicidal activity against *An. arabiensis* larvae, with the most active peptide exhibiting a 48 h LC₅₀ of 3.12 µg/mL. The mode of action was investigated using detoxification enzyme assays and confocal laser scanning microscopy. Collectively, these findings identify fabclavine-derived peptides from *X. indica* 157-C as promising mosquito larvicides and provide a foundation for their development as environmentally sustainable tools for malaria vector control.

1. World Health Organization. 2025. World malaria report 2025: addressing the threat of antimalarial drug resistance. Geneva: World Health Organization;

2. World Health Organization. 2018. Global report on insecticide resistance in malaria vectors: 2010–2016. Geneva: World Health Organization; 3. Goodrich-Blair, H. and Clarke, D.J., 2007. Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabdus*: two roads to the same destination. *Molecular microbiology*, 64(2), pp.260–268; 4. Dreyer, J., Malan, A.P. and Dicks, L.M., 2018. Bacteria of the genus *Xenorhabdus*, a novel source of bioactive compounds. *Frontiers in Microbiology*, 9, p.3177.

P13: Effect of antimicrobial peptide position on the mechanical and antibacterial properties of silk-elastin-like protein (SELP)-based biomaterials

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The global antimicrobial resistance (AMR) crisis presents major implications for the performance of medical devices and implants, where bacterial colonisation and biofilm formation can lead to persistent infections. Antimicrobial peptides (AMPs) are promising alternatives to conventional antibiotics due to their broad-spectrum activity and reduced propensity to develop resistance. However, their direct application is limited by toxicity, instability and rapid degradation. To tackle some of these issues, recombinant silk-elastin-like proteins (SELPs) provide a versatile biomaterial platform for the manufacture of antimicrobial materials with tunable mechanical properties, temperature-responsive self-assembly, and biocompatibility. [1] [2] [3]

In this project, we fuse SELP with the antimicrobial peptide CM4, a cecropin family AMP derived from *Bombyx mori*. [4] Specifically, we investigate how sequence design, protein structure, and material processing conditions influence the antibacterial activity of the resulting biomaterials. This modular approach enables precise control over the AMP position within the protein sequence, which, in turn, influences material properties. The SELP-CM4 recombinant proteins were expressed in *Escherichia coli* and purified using inverse transition cycling (ITC), a technique leveraging the lower critical solution temperature (LCST) behaviour of elastin-like repeats in SELPs. The product successfully formed stable protein films under aqueous casting conditions with ethanol annealing. Antibacterial activity was evaluated using both solution-based minimum inhibitory concentration (MIC) testing and solid surface-based bacterial viability assays. Our results provide guidelines for the design and manufacture of protein-based materials fused to AMPs with an enhanced combination of mechanical and antibacterial properties.

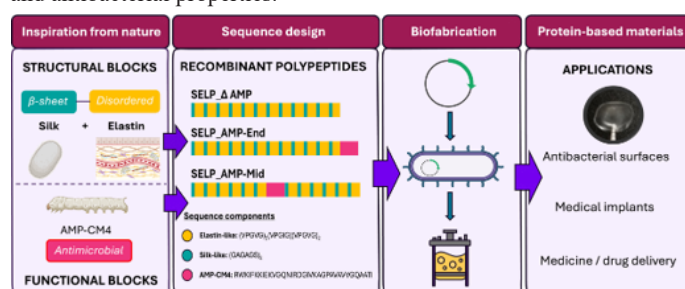


Figure 1. Workflow for developing SELP-AMP hybrid biomaterials.

[1] Costa, A. da; Machado, R.; Ribeiro, A.; Collins, T.; Thiagarajan, V.; Neves-Petersen, M. T.; Rodríguez-Cabello, J. C.; Gomes, A. C.; Casal, M. ACS Publications. <https://doi.org/10.1021/bm5016706>; [2] Xia, X.-X.; Xu, Q.; Hu, X.; Qin, G.; Kaplan, D. L. *Biomacromolecules* 2011, 12 (11), 3844–3850. <https://doi.org/10.1021/bm201165h>; [3] López Barreiro, D.; Houben, K.; Schouten, O.; Koenderink, G. H.; Thies, J. C.; Sagt, C. M. J. *ACS Applied Materials & Interfaces* 2024. <https://doi.org/10.1021/acsami.4c17903>; [4] Li, J.-F.; Zhang, J.-X.; Li, G.; Xu, Y.-Y.; Lu, K.; Wang, Z.-G.; Liu, J.-P. *Food Funct.* 2020, 11 (8), 7245–7254. <https://doi.org/10.1039/D0FO01031F>.

P14: Impact of an N-Terminal D-Amino Acid Substitution on the Membrane Interaction and Antibacterial Efficacy of Piscidin-2 Peptide

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Antimicrobial peptides (AMPs) hold particular interest in the field of antimicrobial research [1–3] due to their potential as a novel class of antimicrobial agents and as an alternative to conventional antibiotics. In addition, stereochemical modification represents a powerful strategy for optimizing the therapeutic potential of AMPs [4]. Here, we present a comprehensive structural and biophysical characterization of ecPis-2s peptide and its epimerized analog, D-ecPis-2s, featuring a single D-F substitution. Although both peptides possess identical net charges and global hydrophobicity, our results reveal that the D-substitution acts as a conformational switch that fundamentally alters the membrane interaction dynamics and antimicrobial efficacy. Circular dichroism and solution nuclear magnetic resonance (NMR) spectroscopy demonstrate that D-ecPis-2s maintains similar overall helical character, but it adopts a distinctive N-terminal structural bend compared to the wild-type. Structural refinements and topology studies based on solid-state NMR data elucidated a peptide topology in which the D-configuration promotes a deeper sequestration of the N-terminal aromatic triad (F1–F3) into the hydrophobic membrane core. This aromatic anchor effectively drives the peptide backbone and consecutively the aromatic side chains into the bilayer, resulting in enhanced membrane tension, faster pore-forming kinetics, and a more robust disruption of lipid packing. These structural features correlate directly with the increased antimicrobial potency across a wide range of bacterial targets, including multidrug-resistant strain. Therefore, our findings provide a molecular rationale for the superior performance of D-epimer and establish a framework for the rational design of membrane-active therapeutics through targeted stereochemical modifications.

1. Li, X., Zuo, S., Wang, B., Zhang, K. & Wang, Y. Antimicrobial Mechanisms and Clinical Application Prospects of Antimicrobial Peptides. *Molecules* 27, 2675 (2022); 2. Kapil, S. & Sharma, V. D-Amino acids in antimicrobial peptides: a potential approach to treat and combat antimicrobial resistance. *Can. J. Microbiol.* 67, 119–137 (2021); 3. Bechinger, B. & Gorr, S.-U. Antimicrobial Peptides: Mechanisms of Action and Resistance. *J. Dent. Res.* 96, 254–260 (2017); 4. Torres, M. D. T., Sothiselvam, S., Lu, T. K. & de la Fuente-Nunez, C. Peptide Design Principles for Antimicrobial Applications. *J. Mol. Biol.* 431, 3547–3567 (2019).

P15: Combatting the silent pandemic: Synthesis of potent antimicrobial nanosilver formulations from cyclodecapeptides

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The global rise in antimicrobial resistance (AMR), driven by antibiotic misuse and the persistence of drug tolerant biofilms, presents a major challenge across medical, agricultural and industrial sectors [1,2]. Stagnation in novel antibiotic development has prompted renewed interest in repositioning and reformulating established antimicrobial agents [3]. Tyrocidines, including tryptocidines, are cyclic decapeptides with potent antimicrobial and biofilm disrupting properties [4,5]. However, their broader application is limited by haemolytic activity and reduced efficacy against Gram-negative bacteria. Combining tyrocidines with silver nanoparticles (AgNPs) may enhance their antimicrobial potency [4,6], although the effects of silver interactions and nanoparticle formation on their biophysical properties remain poorly understood.

This study investigated the irradiation dependent synthesis, biophysical properties and antibacterial activity of tyrocidine-silver nanoparticle (AgNP)/Ag⁺ formulations. Variables considered during synthesis optimisation included peptide identity and aromatic composition, peptide to silver ratio, irradiation wavelength, intensity and exposure time. Mass spectrometric analysis confirmed peptide composition, with some changes in structure in the presence of Ag⁺ and upon irradiation. Spectrophotometric and fluorescence analyses indicated the laser-induced changes according to peptide identity and irradiation

conditions. Selected formulations produced spectral changes, such as surface plasmon resonance consistent with AgNP formation, following irradiation.

Antibacterial activity against *Escherichia coli* and *Acinetobacter baumannii* was evaluated using a resazurin based solid surface assay. The formulations displayed a potent increase in activity but differences in their antimicrobial profiles according to peptide identity and irradiation conditions. However, antimicrobial enhancement did not always correspond with the strongest spectroscopic evidence of nanoparticle formation. These findings suggest that peptide structure and synthesis conditions influence silver interactions and biological activity. Enhanced activity may therefore reflect multiple peptide-silver interactions rather than AgNP formation alone. This work supports the continued development of tyrocidine-silver formulations for antimicrobial coatings and related surface applications.

1. Ahmed SK, Hussein S, Qurbani K, Ibrahim RH, Fareeq A, Mahmood KA, Mohamed MG. Antimicrobial resistance: impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health*. 2024;2:100081; 2. Patel R. Biofilms and antimicrobial resistance. *Clinical Orthopaedics and Related Research*. 2005;(437):41–47; 3. Aggarwal M, Patra A, Awasthi I, George A, Gagneja S, Gupta V, et al. Drug repurposing against antibiotic resistant bacterial pathogens. *European Journal of Medicinal Chemistry*. 2024;279:116833; 4. de Villiers C. Metal and tyrocidine nano-assemblies to create broad-spectrum metal-peptide formulations [thesis]. Stellenbosch: Stellenbosch University; 2023; 5. van Rensburg W, Rautenbach M. Creating robust antimicrobial materials with sticky tyrocidines. *Antibiotics*. 2022;11(2):174; 6. Kumar V, de Villiers C, Rautenbach M. Tryptocidine C encapsulated nano-silver assemblies with potent antibacterial activity. *Journal of Molecular Liquids*. 2025;433:127747.

P16: Development and evaluation of bacterial envelope-permeabilizing peptides to potentiate antibiotic activity.

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Bacterial envelope permeability remains a major obstacle to antibiotic efficacy. We aim to develop peptides that selectively permeabilize these barriers and thereby potentiate existing antibiotics without causing cytotoxicity or hemolysis. In *Escherichia coli*, 50 candidate outer membrane permeabilizing peptides (OMPPs) have been identified, of which 30 have been characterized. These peptides were tested for their ability to potentiate mupirocin, which otherwise poorly crosses the outer membrane, with one peptide facilitating a 1000-fold potentiation. Complementary dye-based assays characterized their outer- and inner-membrane activities. Most tested OMPPs also showed species selectivity and low cytotoxicity and hemolytic activity under the conditions examined. Initial studies of mycolic acid permeabilizing peptides (MAPPs) in *Mycobacterium tuberculosis*, *Mycobacterium marinum* and *Mycobacterium smegmatis* have likewise demonstrated antibiotic potentiation, supporting extension of this strategy to mycobacterial permeability barriers.

A major current focus is rapid expansion of the OMPP dataset to enable iterative peptide prediction and optimization. Assay workflows are being optimized for higher throughput and evaluated for reproducibility, while preparations are underway to adapt methods to robotic liquid-handling platforms.

In parallel, we investigate how peptide features relate to biological activity by mapping OMPPs within antimicrobial-peptide representation spaces using Uniform Manifold Approximation and Projection (UMAP) of protein language-model peptide embeddings. By integrating these representations with experimental outcomes, we aim to identify structure–activity patterns and generate mechanistic hypotheses for peptide permeabilization and selectivity.

For a more detailed overview of the project's low-data machine-learning strategy and *in silico* optimization framework, please see the poster by my colleague L. Andrekson (P1).

P17: A new generation of food preservatives: a broad-spectrum antimicrobial peptide active against foodborne viruses

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Foodborne viruses are a major cause of gastrointestinal, hepatic, and neurological diseases worldwide, representing a significant challenge for food safety. Consequently, there is growing interest in developing bioactive compounds able to prevent viral contamination of food products. Among these, antimicrobial peptides (AMPs) are promising candidates owing to their broad antimicrobial activity and potential antiviral properties, although their effectiveness against foodborne viruses remains largely unexplored. In this study, we investigated the antiviral activity of known and newly designed AMPs against two clinically relevant non-enveloped RNA viruses, hepatitis A virus (HAV) and murine norovirus (MNV-1), a surrogate for human norovirus. Several peptides displayed virucidal activity against one or both viruses. Notably, the decapeptide RiLK1 exhibited the greatest efficacy, reducing viral infectivity by up to 1.4 log for HAV and 1.2 log for MNV-1 in virus pre-treatment assays. Mechanistic studies demonstrated that RiLK1 directly interacts with viral particles, possibly impairing their attachment to host cell receptors and subsequent infection. This finding is particularly relevant because activity against non-enveloped viruses is uncommon among AMPs, whose mechanisms of action are typically associated with viral envelope disruption or inhibition of membrane fusion [1]. To further elucidate the structural determinants of antiviral activity, a RiLK1 analogue (RiLK3), carrying lysine-to-arginine substitutions, was rationally designed and evaluated [2]. Despite a comparable positive charge, RiLK3 showed a marked loss of antiviral activity and was ineffective against HAV. These results highlight the importance of amino acid composition and residue distribution in governing antiviral efficacy. Overall, our findings provide new insights into the structure–activity relationships of antiviral AMPs and support the development of peptide-based preservatives as innovative tools to control foodborne viral contamination.

1. Galatola E, Agrillo B, Gogliettino M, Palmieri G, Maccaroni S, Vicenza T, Proroga YTR, Mancusi A, Di Pasquale S, Suffredini E, et al. *Molecules*. 2024; 29(10):2305. doi.org/10.3390/molecules29102305; 2. Agrillo B, Porritiello A, Gratinio L, Balestrieri M, Proroga YT, Mancusi A, Cozzi L, Vicenza T, Dardano P, Miranda B, Escrivá PV, Gogliettino M and Palmieri G (2023). *Front. Microbiol.* 14:1244325. doi: 10.3389/fmicb.2023.1244325

P18: Evaluation of the global and intracellular effect of human histone H1.2-derived peptide in *Pseudomonas aeruginosa* via Differential and Functional Proteomic

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Antimicrobial resistance (AMR) represents an escalating global health crisis that urgently necessitates the development of innovative alternative therapeutics, including human host-defense peptides. Beyond their canonical structural role in chromatin organization, extranuclear histones are increasingly recognized as essential components of the innate immune system, exhibiting potent antimicrobial properties. Prior investigations established that a human Histone H1.2-derived fragment exerts selective, non-lytic antibacterial activity against the critical ESKAPE pathogen *Pseudomonas aeruginosa* (*P. aeruginosa*) [1]. This activity induces a post-transcriptional down-regulation of the Sec translocon and the β -barrel assembly machinery (BAM)

complex, both of which are critical for cell envelope homeostasis[2]. To elucidate the specific intracellular target driving this global effect, we employed a targeted functional proteomics approach utilizing biotinylated H1.2 as bait. This strategy successfully identified the RNA chaperone Hfq, a master regulator of bacterial post-transcriptional networks[3], as the principal interacting partner. Furthermore, in silico molecular docking simulations confirmed this physical interaction, demonstrating highly favorable binding affinities between the H1.2 peptide and Hfq ($\Delta G = -9.4$ kcal/mol and $K_d = 1.4 \cdot 10^{-7}$ M). By specifically targeting Hfq, H1.2 putatively disrupts essential regulatory sRNA-mRNA networks, causing a translational arrest[4]. This mechanism supports the downstream of the membrane biogenesis system observed in previous differential proteomics analysis. To definitively validate these findings, future objectives entail producing recombinant Hfq for *in vitro* binding assays, followed by *in vivo* studies evaluating targeted protein synthesis inhibition. Finally, this research underscores the power of functional proteomics in deciphering complex host-pathogen interactions, reinforcing H1.2 as a highly promising therapeutic candidate for combating multidrug-resistant infections.

[1] Jaber N., et al., Manuscript in preparation, 2026; [2] Alvira S., et al., eLife, 2020, 9, e60669; [3] Bandyra K.J., et al., Biochimie, 2012, 94(7), 1480-1489; [4] Carpousis A.J., Annual Review of Microbiology, 2007, 61, 71-87.

P19: Discovery, isolation, and bactericidal activity of Spat1 - a novel cyclotide from *Spigelia anthelmia* (Loganiaceae)

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Cyclotides are ultra-stable cyclic cystine-knot mini-proteins with promising antimicrobial properties. Here we report Spat1, a 30-residue bracelet cyclotide from *Spigelia anthelmia*, the first confirmed cyclotide in Loganiaceae and an extension of cyclotide phylogeny. Spat1 was enriched by optimized SPE and HPLC, identified by MALDI-TOF MS, and sequenced by combined proteolytic digests and amino acid analysis. In rapid bactericidal assays, Spat1 killed *Bacillus subtilis* with an LC99.9 of 20 μ M within 1 h and caused rapid membrane permeabilization, but showed no measurable activity against *Staphylococcus aureus* or *Escherichia coli* at concentrations up to >400 μ M. Mechanistic assays indicate that its activity is independent of lipoteichoic acid binding and is most consistent with direct interactions with cytoplasmic membrane phospholipids, likely phosphatidylethanolamine, supported by low LTA affinity and AlphaFold/AfCycDesign models predicting the canonical cyclotide fold and amphipathic surface topology. Spat1, therefore, provides a tractable scaffold for structure-activity studies aimed at broadening the antimicrobial spectrum and highlights the convergent emergence of cyclotides across plant lineages.

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P20: Human ezrin peptide 1: Structural characterisation and function implications

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Understanding the structure of a peptide provides essential insight into the understanding of its structure. To that end, this study aims focussed on the structural characterisation of human ezrin peptide 1 (HEP-1) from a loop-region in the human ezrin protein. HEP-1 has been approved for human use as a oral and topical treatment for viral infection

Russia since 2001 [1,2]. Recent anecdotal reports indicate its use is in treatment of viral diseases like hepatitis C and severe acute respiratory syndrome coronavirus 2. HEP-1 and the well-known antimicrobial peptide LL-37's share biological functions such as immunomodulation and inflammatory control [3]. Interestingly, HEP-1 and LL-37 have a high degree of identity in the amino acids responsible controlling the biological response as well as some similarity spanning other regions of the peptide. This suggests that HEP-1 may be able to exert its effect in a similar manner to LL-37 due to these structural similarities. To that end ion mobility mass spectrometry, circular dichroism and molecular modelling was performed to unravel the major aspects of HEP-1's *in-vacuo* and *in-solution* structure. From this we found that HEP-1 has a distinct conformational difference is between charge states, similar to that seen for LL-37. Both peptides also share α -helical sequences with regions having more flexibility. HEP-1's effect on IL-6 production compared to LL-37 in the A549 human lung cancer cell line no significant differences between the two peptides further strengthening their structure-function relationship.

[1] Ataullakhanov, R., Holms, R., Katlinsky, A., Deryabin, P., Narovlyansky, A., Mezentsseva, M. et al. (2002) Immunomodulator Gepon suppresses the replication of Hepatitis C Virus (HCV) in human cell culture in vitro Antibiotics and Chemotherapy 47, 9–11; [2] Holms, R. D., and Ataullakhanov, R. I. (2021–10–28) Ezrin Peptide Therapy from HIV to COVID: Inhibition of Inflammation and Amplification of Adaptive Anti-Viral Immunity International Journal of Molecular Sciences 2021, Vol. 22, Page 11688 22; [3] Leite, M. L., Duque, H. M., Rodrigues, G. R., Da Cunha, N. B., and Franco, O. L. (2023) The LL-37 domain: A clue to cathelicidin immunomodulatory response? Peptides 165, 171011

P21: A predictive approach to developing new antimicrobial peptoids

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We present the preliminary validation of the antimicrobial activity of a series of peptoid (oligo-N-substituted glycine) trimers that considered 35 different peptoid residues possible in each position. Antimicrobial sequences were improved over eight cycles of machine learning (ML) selection and coarse-grained molecular dynamics (MD) simulations of membrane interactions, enabled by a flexible approach for generating peptoid MD parameters we recently developed [1, 2]. A small subset of peptoids spanning a range of predicted activities were then synthesised using solid phase synthesis and tested in bacterial assays against *Escherichia coli*, methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Sequences with high predicted activity generally contain contrasting hydrophobic, hydrophilic and aromatic sidechains. The results of the experimental assays followed the predicted scores closely. While current MD simulations are limited to predicting trimer sequences due to computational constraints, we speculate that many peptoid sequences could behave in a manner similar to the repetitive amphiphilic “kff” trimer motif originally reported by Patch and Barron, where increasing the number of motif repeats from 1 to 4 increased the antimicrobial effect of the peptoid [3]. By combining ML and MD *in silico* methods in combination with traditional lab-based methods, the development time for peptoids even with a large basis set of residues can be greatly reduced. This will also reduce the materials required for synthesis and experiments, while also opening the door to a more directed and efficient approach for validating functional sequences.

[1] Swanson, H.W.A., A. Van Teijlingen, K.H.A. Lau, and T. Tuttle, Physical Chemistry Chemical Physics, 2024. 26(6): p. 4939-4953; [2] Swanson, H., K. Lau, and T. Tuttle, JOURNAL OF PHYSICAL CHEMISTRY B, 2023. 127(49): p. 10601-10614; [3] Patch, J. and A. Barron, JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, 2003. 125(40): p. 12092-12093.

P22: Unveiling *S. aureus* biofilm architecture through comparative mechanical and optical bioimaging: atomic force vs. confocal microscopy

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Biofilm maturation is an open matter of debate in biological and medical sciences. Understanding biofilm maturation requires imaging approaches capable of resolving structural organization across multiple spatial scales. In this work, we present a correlative imaging framework that integrates confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM) to characterize methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms during maturation. CLSM using SYTOTM9, TO-PROTM-3 and wheat germ agglutinin (WGA) staining enabled three-dimensional reconstruction of hydrated biofilms and quantitative assessment of biofilm thickness, biomass distribution, membrane integrity and PNAG exopolysaccharide localization.

Younger biofilms exhibited greater thickness and more compact cellular organization, whereas mature biofilms were thinner and more spatially heterogeneous, with more widely redistributed extracellular matrix components. Complementary AFM imaging after fixation provided nanoscale topographical characterization of partially dehydrated biofilm surfaces, revealing a transition from densely packed smooth bacterial assemblies to matrix-embedded cells with ageing. While volumetric CLSM analysis revealed substantial PNAG accumulation in early-stage biofilms, AFM did not reveal a continuous extracellular layer at the surface, highlighting the importance of hydration to the outer layer of the biofilm.

Quantitative comparisons of optical and mechanical scanning probe data unravels the structural diversity of biofilm microenvironment. This multi-imaging approach establishes a robust workflow for correlating three-dimensional biofilm architecture with nanoscale surface morphology and demonstrates the value of integrating complementary microscopy techniques for comprehensive structural analysis of microbial communities over biofilm maturation.

P23: Taenimycins reveal hidden multifunctionality in helminth-derived antimicrobial peptides

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Throughout their life cycles, parasitic worms occupy diverse, bacteria-rich niches where antimicrobial peptides (AMPs) serve as key components of defense against microbial pathogens [1, 2]. In this study, using genome and transcriptome mining, we identified taenimycins, a previously undescribed family of AMPs originating from the helminth *Taenia solium* (pork tapeworm). Based on expression profiles and the tripartite organization of the mature sequence, one parent peptide, taenimycin(1-39), was selected for further functional characterization. Its mature sequence comprises N-terminal helix, disulfide-bridged loop, and flexible C-terminus containing RGD integrin-binding motif. To define the structure-activity relationships of these domains, the parent peptide, its four fragments, and linearized analogue were synthesized and evaluated. Generally, taenimycins displayed broad and potent antibacterial activity against standard bacterial strains and clinical isolates at submicromolar to low micromolar concentrations, with corresponding differences in mechanisms of action revealed with atomistic-level molecular dynamics and flow cytometry, binding affinity and cytotoxicity against human cells. Results

indicate that, except for the inactive C-terminal fragment, all peptides interact with and disrupt bacterial membranes through distinct mechanisms, with only N-terminal fragment taenimycin(1-23) and linearized analogue forming larger pores. Interestingly, although lacking antimicrobial activity, the RGD-containing C-terminal fragment inhibited host cell adhesion via integrin engagement at concentrations below those associated with antimicrobial or cytotoxic effects. These results identify helminth-derived sequences as an underexplored source of modular bioactive molecules and expand the functional landscape of AMPs beyond direct antimicrobial activity.

[1] Mladineo I, Rončević T, Gerdol M, Tossi A. Helminthic host defense peptides: using the parasite to defend the host. Trends in Parasitology. Elsevier Ltd; 2023. p. 345–57. doi:10.1016/j.pt.2023.02.004 PubMed PMID: 36890022; [2] Rončević T, Gerdol M, Mardirossian M, Maleš M, Cvjetan S, Benincasa M, et al. Anisaxins, helical antimicrobial peptides from marine parasites, kill resistant bacteria by lipid extraction and membrane disruption. Acta Biomater. 2022 Jul 1;146:131–44. doi:10.1016/j.actbio.2022.04.025 PubMed PMID: 35470073.

P24: Biopolymer-coated liposomes for delivery of antimicrobial peptides to the oral cavity – a structural study using small angle x-ray scattering.

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Close to 3.7 billion people are estimated to be affected by oral diseases globally, many in which are associated with infections [1]. The simultaneously increasing global threat of antimicrobial resistance urges development of novel therapeutics [2]. One promising solution is the use of antimicrobial peptides due to their low resistance rates and multifaceted mechanisms of action [3]. However, drug delivery of antimicrobial peptides for local treatment in the oral cavity presents challenges related to low retention times and enzymatic degradation [4,5]. One approach to overcome these challenges is the use of liposomes, consisting of phospholipids that self-assemble into a bilayer forming a nanosized lipid vesicle which can further be modified with biopolymer coatings [4]. Biopolymers are attractive in such formulations due to their biocompatibility, biodegradability and potential contribution to mucoadhesion, stability and antibacterial activity [6,7].

The aim of this work is to characterize liposomes coated with various biopolymers suited for drug delivery to the oral cavity, as well as characterization of the kinetics of the coating process. Liposomes were prepared through thin-film hydration and extrusion before they were coated and biopolymers either through electrostatic deposition or mixing using a stopped flow set-up. Small angle X-ray scattering (SAXS) measurements were carried out at the European Synchrotron Radiation Facility in Grenoble, France. Static and time-resolved SAXS data for liposomes coated with chitosan, alginate and sodium hyaluronate will be presented. The next steps include further development and implementation of scattering models for characterization of biopolymer-coated liposomes, encapsulation of antimicrobial peptides and mucoadhesion studies.

[1] World Health Organization: WHO; 2025. [2] Ferraz MP. Societies. 2024;14(9):187. [3] Nielsen JE, Bjørnstad VA, Pipich V, Jenssen H, Lund R. J Colloid Interface Sci. 2021;582:793–802. [4] Hiorth M, Mihailovic L, Adamczak M, Goycoolea FM, Sarkar A. Biotribology. 2023;35–36:100239. [5] Hemmingsen LM, Giordani B, Paulsen MH, Vanić Ž, Flaten GE, Vitali B, et al. Biomater Adv. 2023;145:213238. [6] Opris O, Mormile C, Lung I, Stegarescu A, Soran M-L, Soran A. Appl. Sci. 2024;14(4):1383. [7] Pasarin D, Ghizdareanu A-I, Enascuta CE, Matei CB, Bilbie C, Paraschiv-Palada L, et al. Polymers. 2023;15(3):782.

P25: Discovery and functional validation of antimicrobial peptides from the ayurvedic medicinal plants *Aegle marmelos* and *Moringa oleifera*

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Antimicrobial resistance remains a significant global health challenge¹, driving the need for new antimicrobial agents with alternative mechanisms of action. In this context, plants are a rich yet underexplored source of antimicrobial compounds². Here, two understudied Ayurvedic plants, *Aegle marmelos* and *Moringa oleifera*, were investigated as potential reservoirs of novel antimicrobial peptides (AMPs).

Genomic data from *A. marmelos* (ASM3044987v1) and *M. oleifera* (ASM2139783v1) were retrieved from NCBI3. Open reading frames (ORFs) were predicted using Augustus⁴. For *A. marmelos*, AMP candidates were identified using Py.AMPA5, whereas for *M. oleifera*, MACREL6 was used for AMP prediction. Candidate peptides (≤ 22 amino acids) were further screened using AMPA and CAMP tools, and additional criteria, such as including AMP probability, blood toxicity and half-life criteria, were used to prioritise the selected AMPs. While BLAST was used to determine the novelty of the predicted peptides.

From >10,000 predicted candidates, stepwise prioritisation yielded 21 and 81 AMPs from *Aegle marmelos* and *Moringa oleifera*. These peptides averaged 15 amino acids in length. Thirteen *M. oleifera* AMPs (Moringanins) showed strong activity (MIC 2–32 $\mu\text{g/mL}$), with Moringanin-49 active against *Pseudomonas aeruginosa* (MIC 4 $\mu\text{g/mL}$), Moringanin-27 against *Acinetobacter baumannii* (MIC 2 $\mu\text{g/mL}$), and Moringanins 9–18 against EMRSA-15 (MIC 8 $\mu\text{g/mL}$). Two *A. marmelos* AMPs exhibited moderate activity (MICs of 128–256 $\mu\text{g/mL}$) against *Enterococcus faecalis*, Methicillin-sensitive *Staphylococcus aureus*, and *Escherichia coli*. Low sequence similarity to known AMPs suggests high novelty, highlighting their potential against drug-resistant pathogens.

[1] Antimicrobial Resistance Collaborators. The Lancet. 2022; 399(10325): 629–655. DOI: [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0); [2] Woo S, et al. Natural Product Reports. 2023; 40(7): 1271–1290; [3] Rangwala SH, Kuznetsov A, Ananiev V, Asztalos A, Borodin E, Evgeniev V, et al. Genome Research. 2021; 31(1): 159–169; [4] Stanke M, Tzvetkova A, Morgenstern B. Genome Biology. 2006; 7(Suppl 1): S11; [5] Ramos-Llorens M, et al. mSystems. 2024; 9(7): e01358-23; [6] Santos-Junior CD, et al. PeerJ. 2020; 8: e10555.

P26: Globular domain of linker histone H1 boosts chemokine induced signaling and immune cell chemotaxis

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Background: Histones are released by neutrophils at sites of inflammation where they help immobilize and kill invading pathogens. However, the broader role of histones in pathogen protection remains elusive.

Methods: In vitro cell signaling and 3D chemotaxis assays using human cell lines and primary human immune cells. Fluorescence polarization, NMR and chemical shift perturbation studies of protein-protein interactions.

Results: We show that beyond antimicrobial activity, the linker histone H1.0 boosts immune activation. Specifically, its globular domain potentiates the activity of the chemokines CCL19 and CCL21 through their receptor CCR7, leading to increased signaling and immune cell chemotaxis. The globular domain has a net charge of +9 and interacts with the acidic N-terminal region of CCR7, providing an electrostatic basis for chemokine boosting activity. Using different charge variants of the globular domain, we show that its boosting activity can be modulated by altering both net charge and the position of charge. Linear peptides with similar net charge derived from the C-terminal tail of H1 lacked chemokine boosting activity, indicating that beyond net charge, an optimal spatial arrangement are required.

Conclusions: Our findings suggest that histones may act as enhancers of chemokine signaling, leading to increased directed migration of immune cells towards sites where histone concentrations are elevated

P27: Pathogen-specific antimicrobial activity prediction with biological large language model-based methods

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Driven by the rise of antimicrobial resistance, antimicrobial peptides (AMPs) have emerged as promising therapeutics capable of targeting multidrug-resistant pathogens. Because identifying AMPs and their specific targets requires costly and labour-intensive wet-lab experiments, *in silico* methods to prioritize candidates are highly valuable. However, current computational methods often lack pathogen specificity or fail to incorporate crucial targeted proteomic and genomic contexts. To bridge this gap, we developed triAMPh, a robust, zero-shot framework for pathogen-specific peptide bioactivity prediction. triAMPh integrates a heterogeneous graph attention network-based link predictor (HLP), Extreme Gradient Boosting, and a multilayer perceptron trained on features from biological large language models (bLLMs). Our novel HLP constructs a knowledge graph that maps peptides and pathogens as distinct nodes, connected by similarity and bioactivity edges. The model extracts information through semantic traversals, prioritizing neighbouring nodes and their biological contexts. Benchmarking shows that triAMPh provides unbiased, peptide- and pathogen-centered zero-shot predictions, matching or outperforming state-of-the-art methods across all eight metrics except precision. Ultimately, triAMPh offers a powerful computational tool to accelerate wet-lab AMP discovery while demonstrating the capability of bLLMs to capture complex, pathogen-specific bioactivity patterns.

P28: Direct Detection of Cyclic Lipopeptides in Bacterial Culture via NMR

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Antibiotic resistance is a growing problem, and new types of antimicrobial compounds are needed. Cyclic lipodepsipeptides (CLiPs) are a promising class of natural products produced by bacteria from various genera, including *Pseudomonas*. Their initial discovery from screening bacterial cultures is typically relying on liquid chromatography–mass spectrometry (LC-MS), which requires sample extraction or even purification prior to analysis. Moreover, molecular mass alone is often insufficient to confirm that a candidate hit is in fact a peptide, since other natural product classes can have similar masses.

To address these limitations, we aimed to explore an NMR-based screening approach that detects CLiPs directly from bacterial culture. Cultures are grown with a ¹⁵N-labeled nitrogen source and the presence of peptides is monitored by scanning for the presence of N–H signals characteristic for backbone amides, using 2D ¹H–¹⁵N BEST-HSQC spectra acquired from samples taken directly from the culture. This method eliminates the need for extraction and purification, and the backbone N–H fingerprint provides direct structural evidence of peptide character, resolving ambiguities inherent to mass-based detection alone, provided sufficient quantities of peptide are produced to generate signals above the LOD (Limit of detection).

As a proof of concept, we applied this approach to two different *Pseudomonas* strains, producing the CLiP - Orfamide. Resolving interpretable amide N–H fingerprints required addition of ACN-d₃ to improve peptide solubilization within the culture matrix. Spectra from the two strains showed consistently aligned peaks, demonstrating reproducible detection of the shared Orfamide fingerprint across independent samples. The potential application and limitations of the approach will

be discussed.

[1] De Roo, V.; Verleysen, Y.; Kovács, B.; De Vleeschouwer, M.; Muangkaew, P.; Girard, L.; Höfte, M.; De Mot, R.; Madder, A.; Geudens, N.; Martins, J. C. *Microbiol. Spectr.* 2022, 10 (4), e01261-22. DOI: 10.1128/spectrum.01261-22.

P29: Investigation into the nephrotoxicity of the novel antimicrobial peptide Teixobactin

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The nephrotoxicity of cationic antibiotics is an important limitation to their therapeutic use, yet the mechanisms determining renal toxicity of these compounds remains elusive. This project aims to gain a deeper understanding of the rules and mechanisms underlying the nephrotoxicity of (poly)cationic drugs, using the novel antibiotic Teixobactin (Txb)[1] and last-resort antibiotic Polymyxin B (PmxB)[2] as model compounds. Nephrotoxicity was investigated in conditionally immortalized proximal tubule epithelial cells (ciPTECs)[3] using PrestoBlue and lactate dehydrogenase (LDH) assays, and in kidney organoids using immunostaining and LDH measurements.

After 72 h exposure, Txb produced IC₅₀ values of 220 µM, while PmxB showed an IC₅₀ of 139 µM after 24 h in ciPTECs. An increase in cell viability non-megalin-expressing ciPTECs, indicating that megalin is involved in the mechanism of toxicity, mainly the internalization of the drugs. LDH measurements for PmxB indicated higher apparent viability than observed by PrestoBlue, suggesting metabolic inactivation may occur prior to substantial membrane damage. In kidney organoid models, Txb exposure resulted in a decrease in the total number of LTL-positive proximal tubule cells, observed by immunostaining and confocal microscopy. Furthermore, Txb exposure caused a 2.5-fold increase in LDH release compared with untreated organoids.

Together, these findings demonstrate nephrotoxic effects of Txb and PmxB in both cellular and kidney organoid models. While binding of PmxB to megalin has previously been established[4], whether Txb interacts with megalin remains unknown. Further investigation of the interaction between Txb and megalin, using a library of Txb analogues, will help elucidate the molecular determinants underlying nephrotoxicity and identify structural features that may contribute to renal toxicity.

Literature references: [1] Ling, L. L., Schneider, T., Peoples, A. J., et al. (2015). *Nature*, 517, 455–459, [2] Poirel, L., Jayol, A., Nordmann, P. (2017). *Clinical Microbiology Reviews*, 30(2), 557–596, [3] Wilmer, M. J., Masereeuw, R. et al. (2010). *Cell and Tissue Research*, 339, 449–457, [4] Ali, Z., et al. (2017). *Antimicrobial Agents and Chemotherapy*, 61(3), e02391-16

P30: Twinkle-derived antimicrobial peptides from the Antarctic fish *Nothothenia coriiceps*: a new potent weapon against bacteria pathogens

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This abstract is closely related to that one presented by Emanuela Galatola (rapid oral communication R3). Both contributions originate from the same research topic, offering complementary analysis and perspectives on the mitochondrial-targeting sequence of Twinkle helicase from the Antarctic fish *Nothothenia coriiceps* use as scaffold for the rational design of a new class of antimicrobial peptides: TAMP-1 and TAMP-2.

Peptides conformational and structural properties were investigated by using spectroscopy techniques as circular dichroism and fluorescence spectroscopy, at different pH (2.0, 7.0, 11.0) and temperatures (4, 37 and 90 °C), providing insight into their stability under diverse environmental conditions.

Furthermore, molecular dynamic simulations were performed for an anionic lipid bilayer having the composition of the inner membrane of *Escherichia coli* and interacting with six copies of TAMP1 or TAMP2 peptides, to investigate their modes of interactions with bacterial membranes. This analysis showed the propensity of these peptides to be rapidly adsorbed onto the bacterial membrane, with TAMP2 able to penetrate more deeply into the leaflet.

These findings were further corroborated by bacterial growth kinetics analyses [1] which provided insight into the temporal dynamics of peptide activity. TAMP1 exhibited limited activity against *Staphylococcus aureus*, whereas TAMP2 showed broader potency, with the lowest micromolar MIC/MBC values against *Escherichia coli* and *S. aureus*. At sub-MIC concentrations, TAMP2 did not fully suppress proliferation of Gram-negative bacteria but significantly delayed growth. This behavior suggests that the peptide could exert sublethal stress on bacterial cells, representing a promising alternative to conventional antibiotics.

[1] Rončević, T.; Gerdol, M.; Pacor, S.; Cvitanović, A.; Begić, A.; Weber, I.; Krce, L.; Caporale, A.; Mardirossian, M.; Tossi, A.; Zoranić, L. *Int. J. Mol. Sci.* 2024, 25, 11690.

P31: Synergistic peptide-biocide formulations: combining cyclic decapeptides and DDAC to overcome antimicrobial resistance

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The global surge in antimicrobial resistance (AMR) demands innovative strategies capable of circumventing conventional tolerance mechanisms. While didecyltrimethylammonium chloride (DDAC) is a widely used biocide, its extensive application has increasingly selected for plasmid- and efflux-mediated resistance among nosocomial pathogens [1, 2]. To address this, we investigated the biochemical synergy between DDAC and the natural cyclic decapeptides (CDPs) gramicidin S (GS) and tyrocidines (Trc). These CDPs were selected due to their broad-spectrum, membrane-disruptive activity, and low propensity for inducing resistance. By combining reduced concentrations of DDAC with the CDPs in synergistic mixtures, we sought to optimise membrane-disruptive efficacy while minimising the selective pressure driving DDAC resistance.

Quantitative checkerboard assays were conducted to evaluate interaction profiles against key Gram-positive and Gram-negative organisms, including *Staphylococcus aureus*, *Escherichia coli*, *Serratia marcescens*, and *Acinetobacter baumannii*. The co-formulations exhibited potent synergistic antimicrobial activity, substantially lowering the minimum inhibitory concentrations required for pathogen eradication on hard surfaces. Furthermore, evaluating these optimised formulations within functionalised polyester wipes (soft material) confirmed high peptide stability, active retention, and sustained antimicrobial efficacy upon contact transfer. These results highlight engineered CDP-surfactant co-formulations as potent, resistance-resilient solutions for durable surface decontamination and environmental infection control in clinical settings.

[1] J.Y. Maillard, *Microbiol. Spectr.* 6 (2018); [2] E. Meade, M.A. Slattery, M. Garvey, *Pathogens* 10 (2021) 598.

P32: Novel Cecacin peptides from the chicken ceca-microbiome show anti-tuberculosis and combinatorial activity

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb) accounted for ~10.7 million new cases and 1.23 million deaths in

2024 [1]. Novel anti-TB regimens are of high priority to improve the efficiency of current treatments, reduce treatment duration, and combat antimicrobial resistance in Mtb strains. Here, we used a double auxotrophic Mtb strain, *Mycobacterium tuberculosis* H37Rv mc26206 [2] to investigate the anti-TB activity of novel chicken ceca microbiome-derived antimicrobial peptides (AMPs), Cecacins (9, 55, 56, 77, 94 and 103). We assessed the anti-TB activity of peptides alone and in combination with conventional antibiotics Bedaquiline (BDQ), Isoniazid (INZ) and Rifapentine (RFP). We recorded powerful anti-TB activity with minimum inhibitory concentrations (MICs) of 1 µg/mL for Cecacin-55, 2 µg/mL for Cecacin-77, 94, 103 and 4 µg/mL for Cecacin-9 and 56. The fractional inhibitory concentration index (FICI) of Cecacins with INZ were predominantly within the indifferent range ($0.5 < \text{FICI} < 4$). RFP exhibited indifferent interactions with Cecacin-9 and 56. However, most peptides, except Cecacin-77 and 94, demonstrated synergy ($\text{FICIs} \leq 0.5$) with BDQ. Cecacin-56 showed the strongest synergy ($\text{FICI} = 0.24$), with an 8-fold reduction in BDQ MIC and a 16-fold reduction in peptide MIC. Additionally, Cecacins exhibited low haemolytic activity against human erythrocytes and minimal cytotoxicity across mammalian cell lines in a resazurin-based assay conducted on human kidney (A498), lung (BEAS-2B), intestinal (Caco-2) and liver (HepG2) cells. Cecacins-9, 56 and 103 showed highest therapeutic index (>250) with $\text{CC}_{50} > 1000$. Ongoing work is evaluating the efficacy Cecacins and Cecacin-antibiotic combinations in macrophage models, focusing on intracellular clearance and host cell safety.

[1]. WHO, Global tuberculosis report 2025; [2]. Sambandamurthy, V.K., et al., Vaccine, 2006. 24(37-39): p. 6309-20.

P33: A Synergistic AMP-Based Strategy Against *Pseudomonas aeruginosa*: From Identification to Translational Application

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The increasing prevalence of antimicrobial resistance in respiratory pathogens such as *Pseudomonas aeruginosa* calls for novel therapeutic strategies. Antimicrobial peptides (AMPs) are promising candidates due to their diverse mechanisms of action and potential for synergistic interactions (1). Here, we investigated the activity of a peptide combination composed of the membrane-active peptide Temporin A (TA) and the proline-rich peptide B7-005 (2) in the context of pulmonary infections.

Checkerboard assays demonstrated synergistic interaction between TA and B7-005 against multiple *P. aeruginosa* strains ($\text{FICI} \leq 0.5$). This potentiation effect was dependent on the order of administration of the two compounds. In parallel, cytotoxicity assays on bronchial epithelial cells indicated a favorable selectivity profile for B7-005, while the combination induced moderate metabolic effects. Serial exposure experiments showed no increase in MIC values or evidence of cross-tolerance, indicating a low propensity for resistance development. Importantly, the peptide combination retained strong antibacterial activity in artificial sputum medium and significantly reduced the viability of preformed biofilms, supporting its effectiveness under physiologically relevant conditions. In addition to antimicrobial activity, TA promoted wound closure in bronchial epithelial cells, and this effect was maintained in combination with B7-005, suggesting added regenerative potential. Preliminary in vivo and advanced airway epithelial co-culture models further confirmed a reduction in bacterial burden with the combined treatment. Overall, these findings support the TA-B7-005 combination as a promising strategy for the treatment of difficult-to-treat *P. aeruginosa*-induced pulmonary infections.

Acknowledgments: This research was funded by the following grants: 2022XFFTH5 Project of the Italian Ministry of University "Progetti di Ricerca di Rilevante Interesse Nazionale—PRIN2022" and from Fondazione Italiana per la Ricerca sulla Fibrosi Cistica FFC6/2025. Delegazione FFC Ricerca di Dongo Como, Delegazione FFC Ricerca di Moncalvo- Asti and Gruppo di

sostegno FFC Ricerca di Scauri- Minturno. GC was supported by the PNRR PhD scholarship (ex M.D. 118/2023).

1) Cesar Augusto Roque-Borda et al. (2026) Pharmacological Reviews 78:100104; 2) Cappella Giacomo et al. (2026) Antibiotics 15, 412 <https://doi.org/10.3390/antibiotics15040412>

Friday at 14:00

Panel Discussion 'The Future of AMPs and AMP research'

- Edwin Veldhuizen (chair / facilitator)
- Inanc Birol
- Jayachandran Kizhakkedathu
- Marina Rautenbach
- Alex Tossi

Poster Session Instructions

Posters may be attached to the boards using pins (thumb tacks) or Velcro dots which will be supplied. Poster boards will be numbered; please see the index below for your poster number.

Posters may be put up from 08:30 on Wednesday September 2nd. Please try to ensure that your poster is in place by the first coffee break, scheduled for 10:40.

Posters will be available for viewing during the refreshment and lunch breaks throughout the meeting.

The main poster sessions are as follows

Poster Session 1 (odd number posters): Wednesday September 2nd, 12:40 – 14:40

Poster Session 2 (even number posters): Thursday September 3rd, 12:25 – 14:25

Poster authors are expected to be next to their posters during their allocated session. The poster sessions are combined with the lunch breaks, so please try to get your lunch quickly on the day of your session.

Posters must be left in position until the end of lunch on Day 3, Friday September 4th; all posters must be removed by 15:00 on Day 3.

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Bezuidenhout, Sherwin	Making materials matter: How metals and matrix properties influence peptide surface activity	7
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Early Stage Researcher Competition for Posters and Rapid Oral Presentations

There will be two Competitions for Early Stage Researchers (PhD students and Post-Docs) kindly sponsored by the Protein & Peptide Science Group of the Royal Society of Chemistry.

Poster Prizes

1st place: €150, 2nd place: €100

Rapid Oral Communication Prizes

1st place: €150, 2nd place: €100

Prizes will be awarded at the end of the meeting.



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The Protein & Peptide Science Group of the Royal Society of Chemistry aims to maintain the logical progression of molecular science through chemistry into biochemistry, biology, medicine, plant and food science. Thus, it has an interdisciplinary approach, representing the protein science interests of chemists, molecular biologists, structural biologists, peptide chemists and biologists, as well as medics and members of the biotechnology, food and pharmaceutical industries.

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- Edwin Veldhuizen (chair / facilitator)
- Inanc Birol
- Jayachandran Kizhakkedathu
- Marina Rautenbach
- Alex Tossi

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