ENHANCING STABILITY AND ACTIVITY OF SHORT COMPUTATIONALLY-DESIGNED ANTIMICROBIAL PEPTIDES THROUGH CHEMICAL MODIFICATION

Bruno Mendes[1], Glyn Barrett[1]

[1]*School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AH, U.K.*

Introduction

Short cationic peptides are valuable chemical templates for the design of clinically available antibiotics. The discovery and development of these peptides face numerous challenges, including time-consuming screening processes, toxicity, and stability issues. Computational methods have been integrated into the pipeline with successful outcomes.

Objectives

To identify novel candidates and expand treatment options in clinical settings, we explored the *in vitro* antibacterial potential and mode of action of computationally designed antimicrobial peptides. We also investigated the impact of chemical modifications on the activity and stability of potential candidates.

Methods

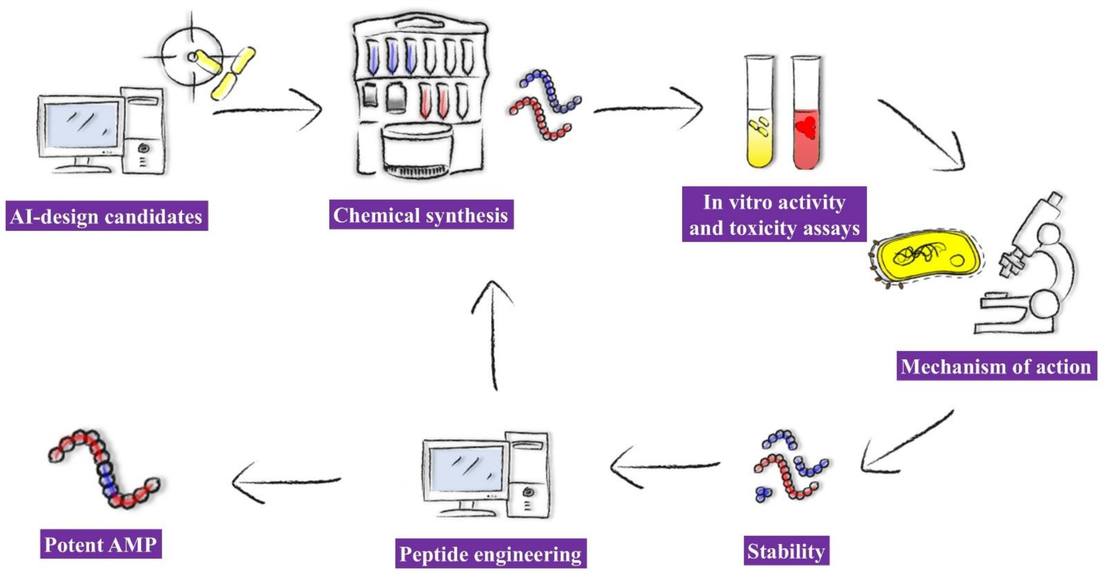

Figure 1. Schematic representation of the experimental procedure used to investigate the antibacterial, haemolytic and antibiofilm actions of computationally designed antimicrobial peptides. We used a computational-driven approach to assess the potential of arginine-rich peptides as an antimicrobial template. Finally, we designed chemically modified peptides based on the backbone of promising antimicrobial sequences.

Results and Discussion

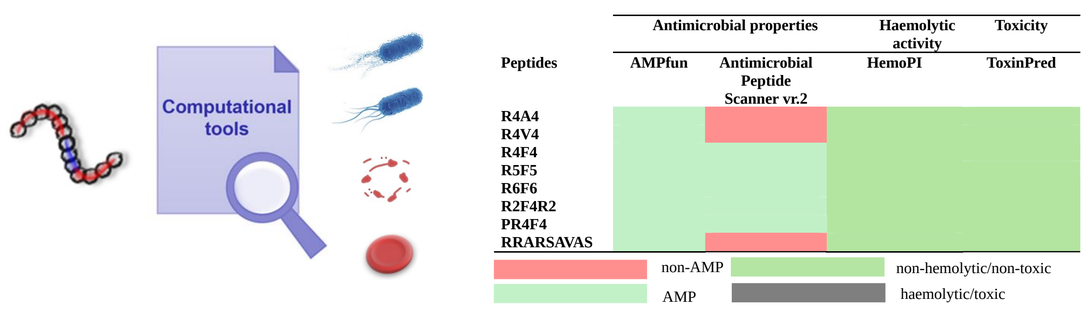

Figure 2. Computational approaches identify the antimicrobial potential and toxicity of peptide sequences. In our initial assessment, we combined computational tools for predicting toxicity (ToxinPred), antimicrobial (Antimicrobial Peptide Scanner vr.2 and AMPfun) and haemolytic (HemoPI, HemoPred) activities.

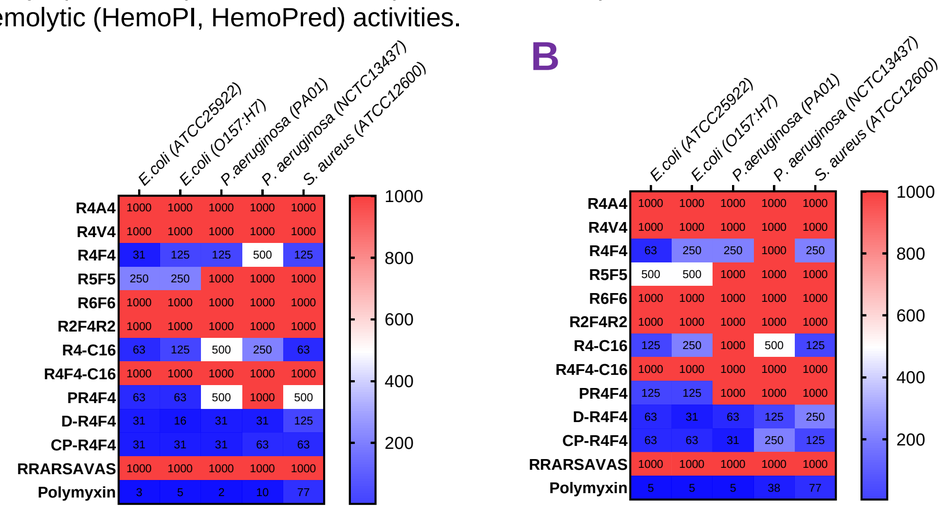

Figure 3. Arginine-based and engineered peptides showed broad-spectrum antibacterial effects. Heatmaps illustrate the **(A)** MICs and **(B)** MBCs of peptides and commercially available antibiotic (positive control) against Gram-positive and Gram-negative bacteria.

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Results and Discussion

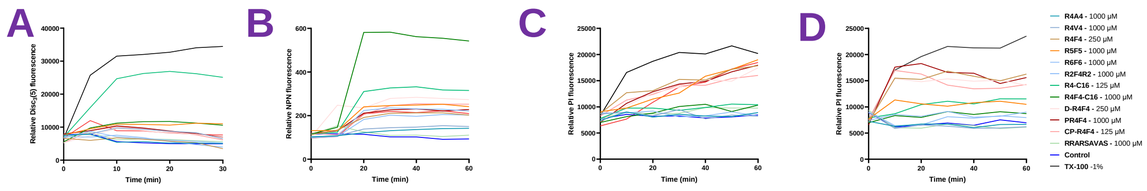

Figure 3. Membrane-disrupting activities of arginine-rich peptides. Representative curves of **(A)** cytoplasmatic membrane depolarisation, **(B)** outer membrane permeabilisation **(C,D)** inner membrane permeabilization of Gram-negative and Gram-positive bacteria, respectively.

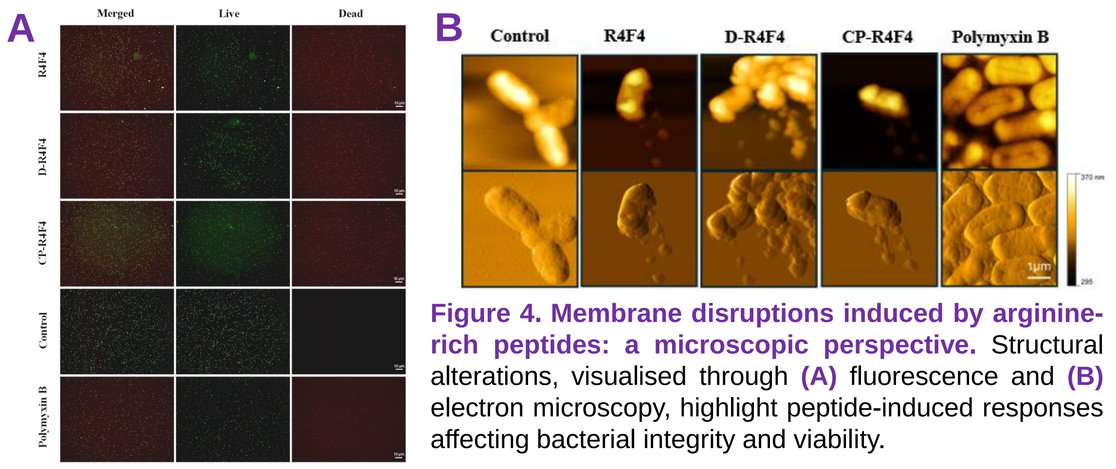

Figure 4. Membrane disruptions induced by arginine-rich peptides: a microscopic perspective. Structural alterations, visualised through **(A)** fluorescence and **(B)** electron microscopy, highlight peptide-induced responses affecting bacterial integrity and viability.

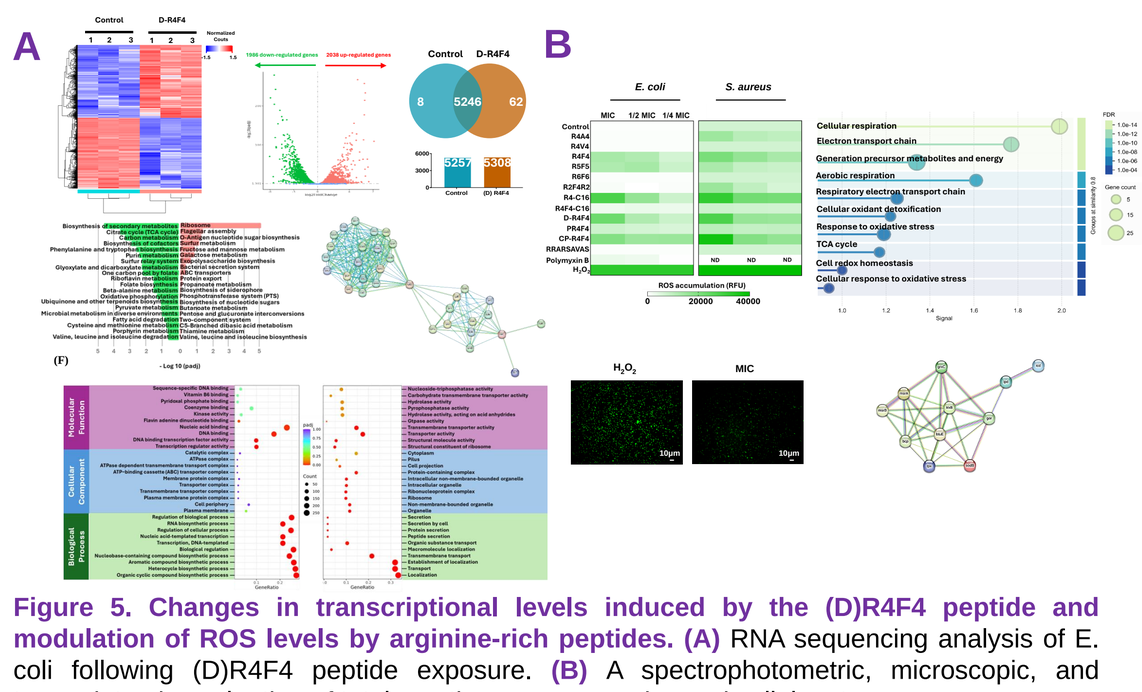

Figure 5. Changes in transcriptional levels induced by the (D)R4F4 peptide and modulation of ROS levels by arginine-rich peptides. (A) RNA sequencing analysis of E. coli following (D)R4F4 peptide exposure. **(B)** A spectrophotometric, microscopic, and transcriptomic evaluation of total reactive oxygen species and cellular stress response.

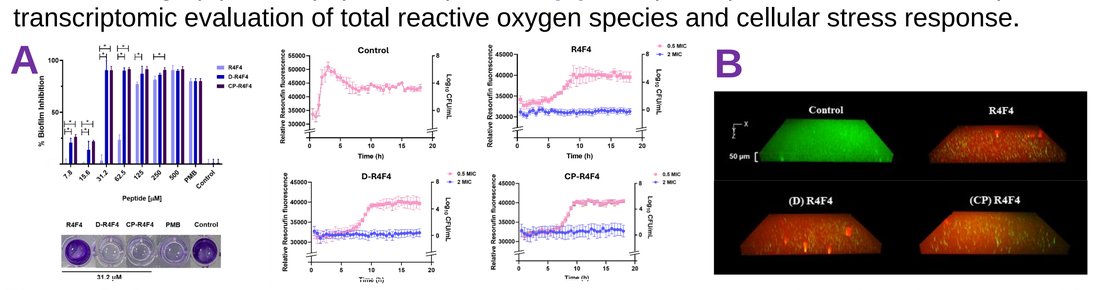

Figure 6. Impact of arginine-rich peptides on both stages of biofilm formation. (A) Quantification of crystal violet provides insights into antibiofilm properties of peptides. **(B)** Three-dimensional visualisation of pre-existing biofilm structural changes induced by peptide treatment.

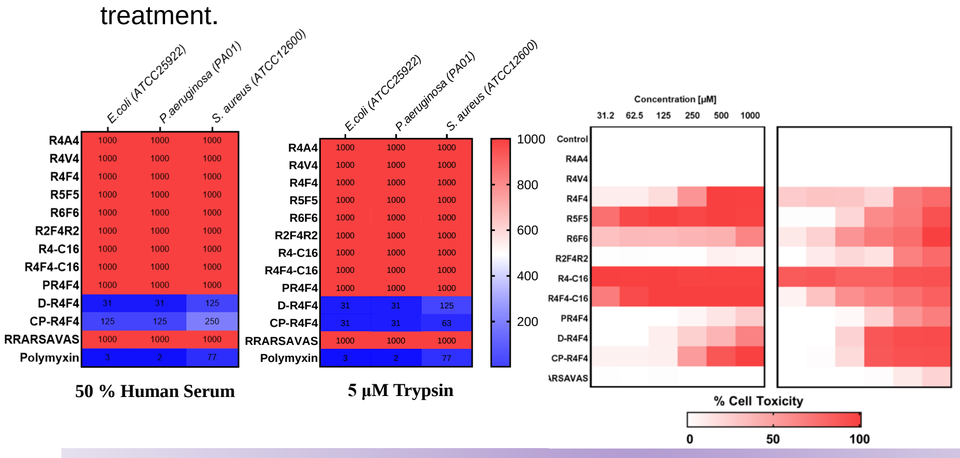

Figure 6. Monitoring the stability and toxicity of arginine-rich peptides. (A) Stability of peptides under protease-rich environments. **(B)** Toxicity of peptides in red blood cells and fibroblasts.

Conclusions

This study demonstrates the significant contributions of cyclisation and D-amino acid substitution approaches in enhancing the stability and activity of arginine-rich peptides. It represents an important step forward in developing peptide-based candidates, which could form the basis of future antibacterial interventions.

References

1 Larsson, D.G.J. et al. Antibiotic resistance in the environment. Nat Rev Microbiol 20, 257–269 (2022). 2 Miethke, M. et al. Towards the sustainable discovery and development of new antibiotics. Nat Rev Chem 5, 726–749 (2021). 3 Robles-Loaiza A.A. et al. Traditional and computational screening of non-toxic peptides and approaches to improving selectivity. Pharmaceuticals (2022).