Rational structure-based design of potent antimicrobial peptides using artificial intelligence

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Abstract

Antimicrobial peptides (AMPs), key components of the innate immune system, represent promising alternatives to conventional antibiotics. However, their therapeutic application against multidrug-resistant pathogens remains limited due to cytotoxicity, poor stability, and insufficient selectivity. Here, we present an integrated artificial intelligence (AI)-guided strategy combining computational peptide design with experimental validation to generate highly active AMPs with reduced toxicity. We developed a computational pipeline based on machine learning models capable of generatively designing short peptide sequences and identifying prototype–analog pairs with optimized antimicrobial activity, selectivity, and predicted stability. To validate the pipeline, selected peptides were synthesized and evaluated in vitro against clinically relevant multidrug-resistant pathogens, including *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecium*, and *Listeria monocytogenes*, using minimum inhibitory concentration assays. Nearly 90% of the tested peptides displayed broad-spectrum antimicrobial activity at low micromolar concentrations. Safety profiles were assessed through hemolysis assays and cytotoxicity studies in human HepG2 and MRC-5 cell lines, revealing several peptides with minimal cytotoxicity at therapeutically relevant concentrations. Mechanistic analyses suggested a predominantly membrane-disruptive mode of action, particularly against Gram-negative bacteria. In addition, serum stability assays demonstrated favorable stability under physiological conditions. Finally, selected peptide candidates were evaluated in vivo in a murine model of *A. baumannii* infection, where treatment resulted in reduced bacterial burden in infected organs, supporting their anti-infective potential and further development as therapeutic candidates.

Pipeline

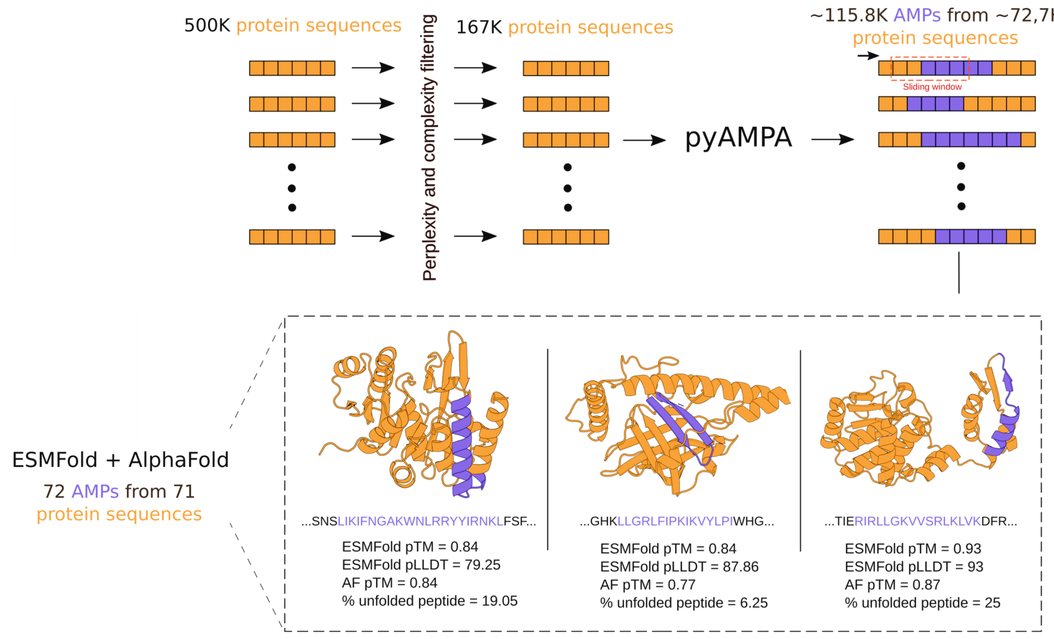


Figure 1. Proposed AI-guided workflow for the discovery of antimicrobial peptide candidates. Protein sequences are computationally processed to identify potential antimicrobial regions, which are subsequently evaluated based on their predicted properties and structural characteristics. The resulting candidates are prioritized for experimental characterization and validation

Structural Characterization

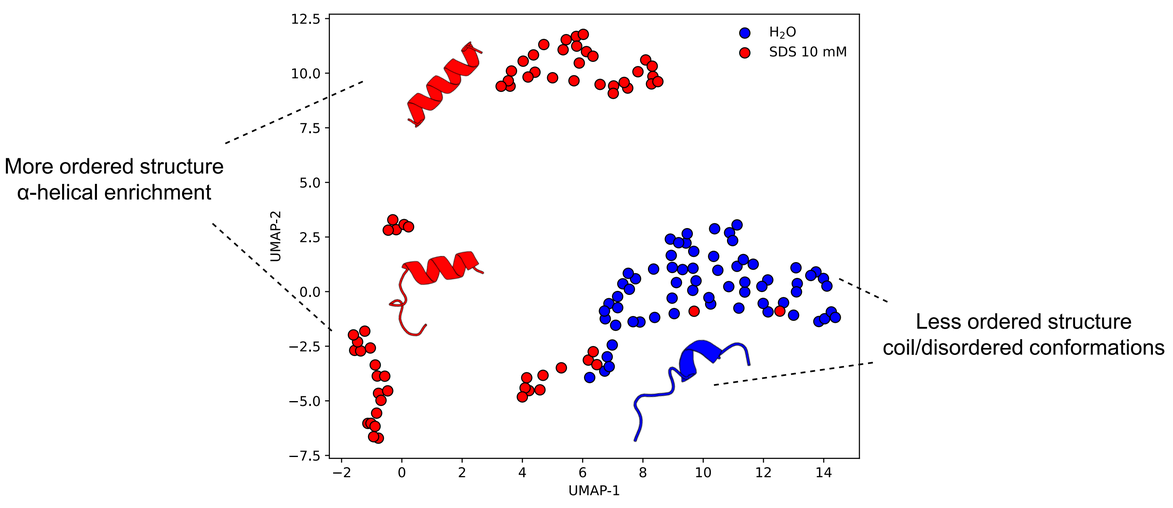


Figure 2. UMAP projection of circular dichroism spectra obtained for 66 experimentally validated AMPs in H_2O and SDS 10 mM. Peptides in aqueous solution clustered separately from those in SDS, indicating a pronounced environment-dependent conformational transition. The spectral shift observed in SDS suggests the acquisition of more ordered secondary structures, consistent with membrane-induced folding, likely toward α-helical conformations.

Antimicrobial activity

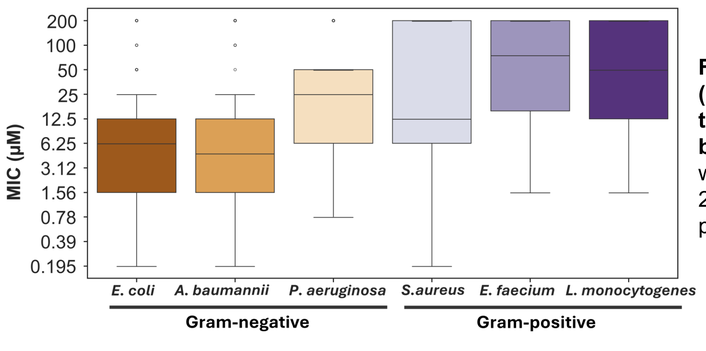


Figure 3. Minimum inhibitory concentrations (MICs) of 66 experimentally validated AMPs tested against six clinically relevant bacterial strains. 88% percent of peptides were active against at least one strain (MIC <= 25 μM). Overall, peptides displayed greater potency against Gram-negative species.

Biocompatibility and Cytotoxicity

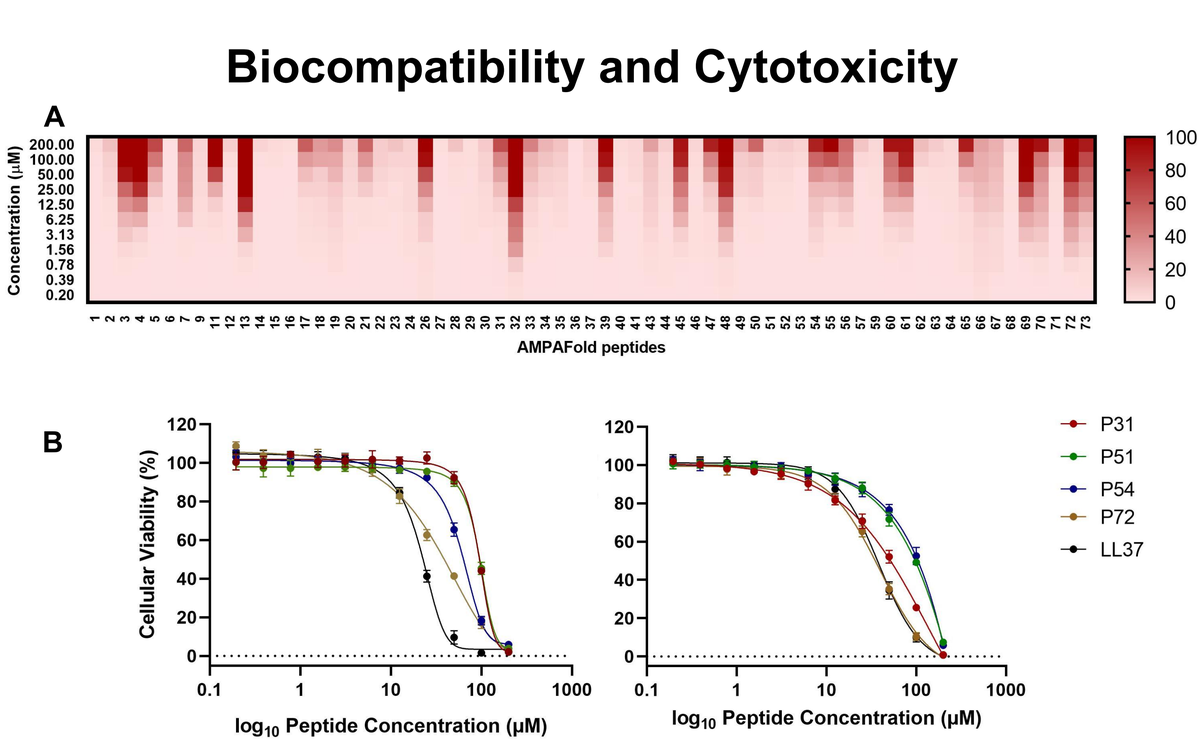


Figure 4. Biocompatibility and cytotoxicity evaluation of AI-designed antimicrobial peptides. (A) Heatmap showing the hemolytic activity of designed peptides in horse blood across increasing peptide concentrations. Color intensity represents the percentage of hemolysis, with darker red indicating higher hemolytic activity. (B) Dose–response cytotoxicity assays performed in MRC-5 human lung fibroblasts (left) and HepG2 human hepatocellular carcinoma cells (right), showing cellular viability as a function of peptide concentration. Several peptide candidates displayed low cytotoxicity and favorable biocompatibility profiles at therapeutically relevant concentrations. Data are presented as mean ± standard deviation.

Membrane Interaction and Stability Characterization

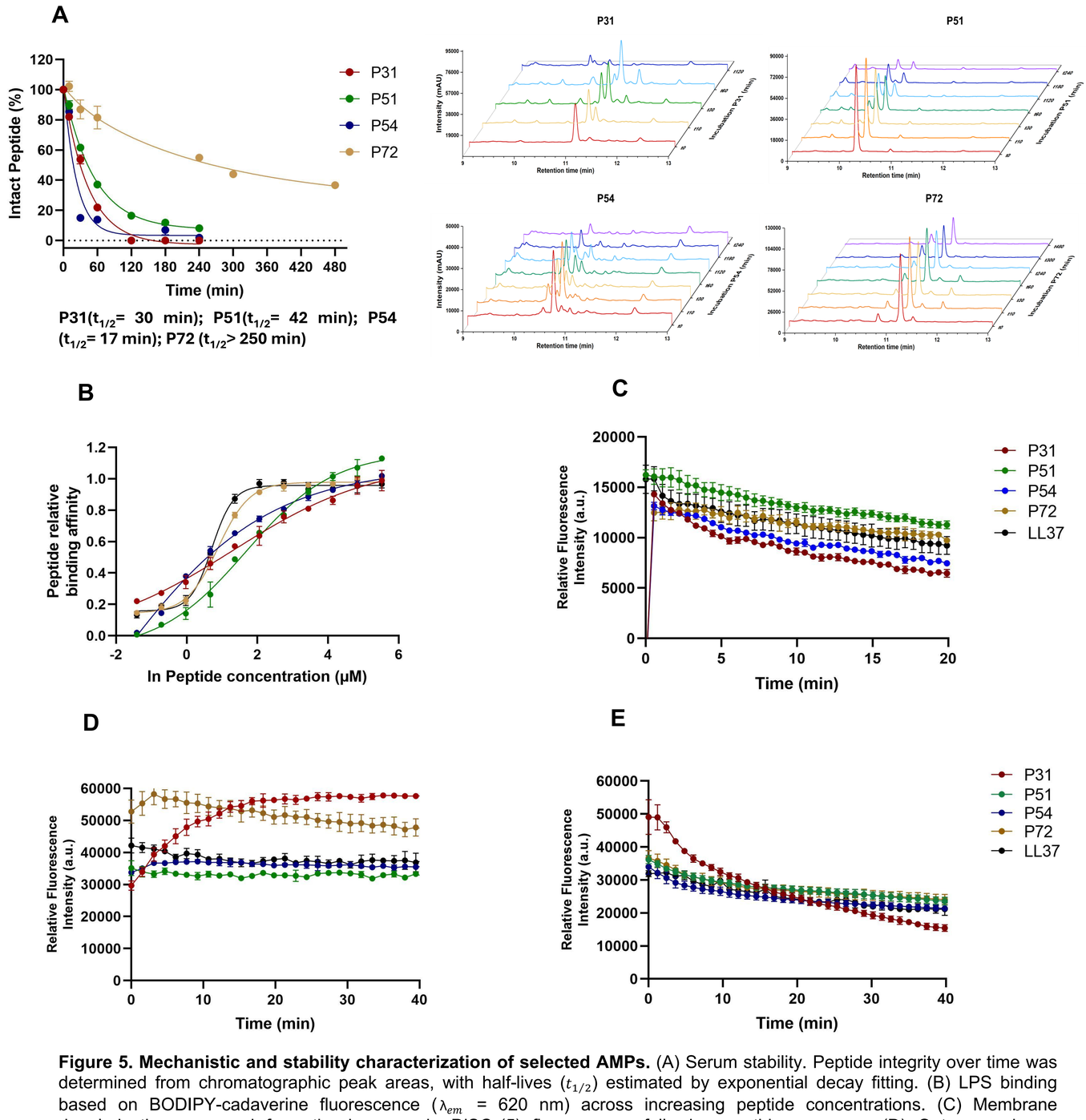


Figure 5. Mechanistic and stability characterization of selected AMPs. (A) Serum stability. Peptide integrity over time was determined from chromatographic peak areas, with half-lives ($t_{1/2}$) estimated by exponential decay fitting. (B) LPS binding based on BODIPY-cadaverine fluorescence (λ_{em} = 620 nm) across increasing peptide concentrations. (C) Membrane depolarization assessed from the increase in $DiSC_3(5)$ fluorescence following peptide exposure. (D) Outer membrane permeabilization and (E) Inner membrane permeabilization, determined by 1-N-phenylnaphthylamine (NPN) and propidium iodide (PI) fluorescence, respectively. Assays (B–E) were performed against *E. coli* ATCC 11775. LL-37 served as a positive control for all assays (B–E). Data are presented as mean ± standard deviation.

In vivo Validation

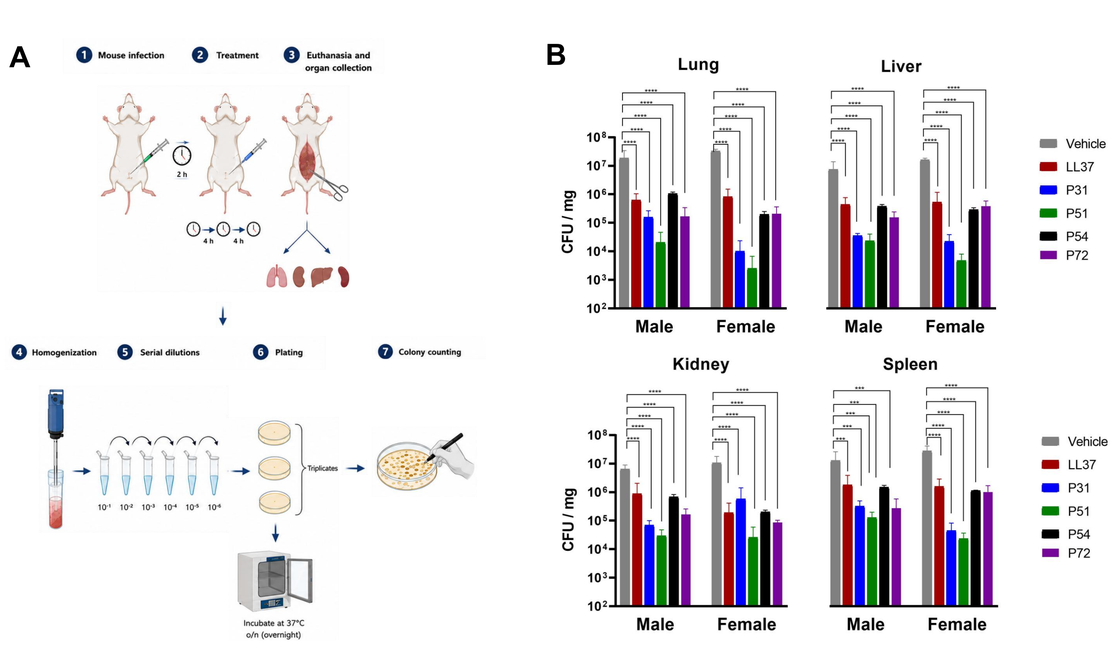


Figure 6. Anti-infective activity of peptides in an animal model. (A) Schematic of the sepsis mouse model used to assess AMPs (n = 6) against *A. baumannii* ATCC 15308. (B) Average CFU/mg in organs after 12 h treatment. Statistical significance compared to vehicle was determined using two-way ANOVA followed by Dunnett's test (denoted as ****P < 0.0001).

Conclusions

- An integrated AI-guided pipeline for the rational design of antimicrobial peptides was successfully developed and experimentally validated.
- The designed peptide candidates exhibited broad-spectrum antimicrobial activity against clinically relevant multidrug-resistant pathogens at low micromolar concentrations.
- Several peptides demonstrated low hemolytic activity, limited cytotoxicity in human cell lines, and favorable serum stability, supporting their potential therapeutic applicability.
- Mechanistic studies indicated membrane disruption as the predominant mode of antimicrobial action, particularly against Gram-negative bacteria.
- *In vivo* validation demonstrated significant reductions in bacterial burden for selected AMPs in infected organs, providing preclinical evidence of their anti-infective activity against *A. baumannii*.

References

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