Mining bacteriophage endolysin structures for novel membrane-active antibacterial peptides

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CENTRAL IDEA

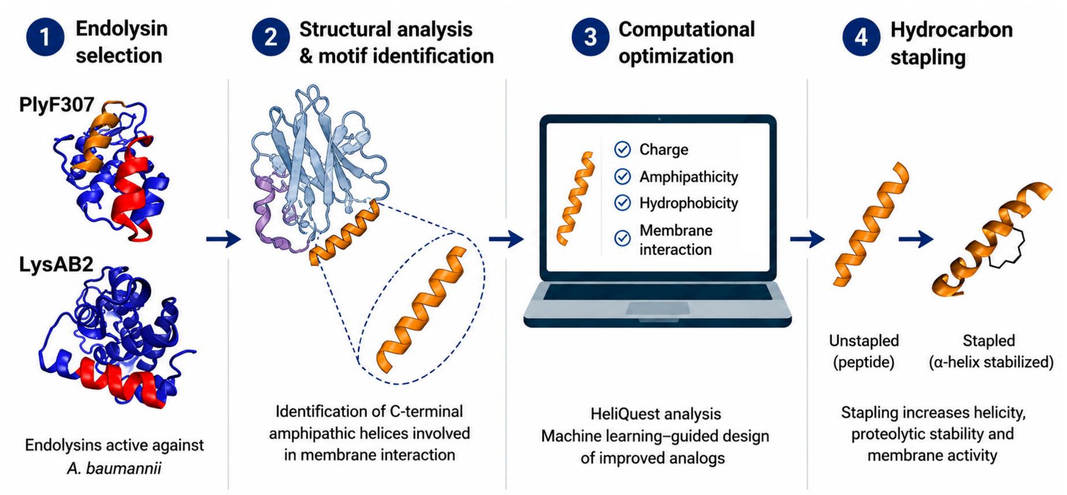
Can bacteriophage endolysins serve as blueprints for novel antimicrobial peptides?

We hypothesized that hidden amphipathic motifs within endolysin structures can be identified, rationally optimized and structurally stabilized to generate potent membrane-active antibacterial peptides.

RESULTS

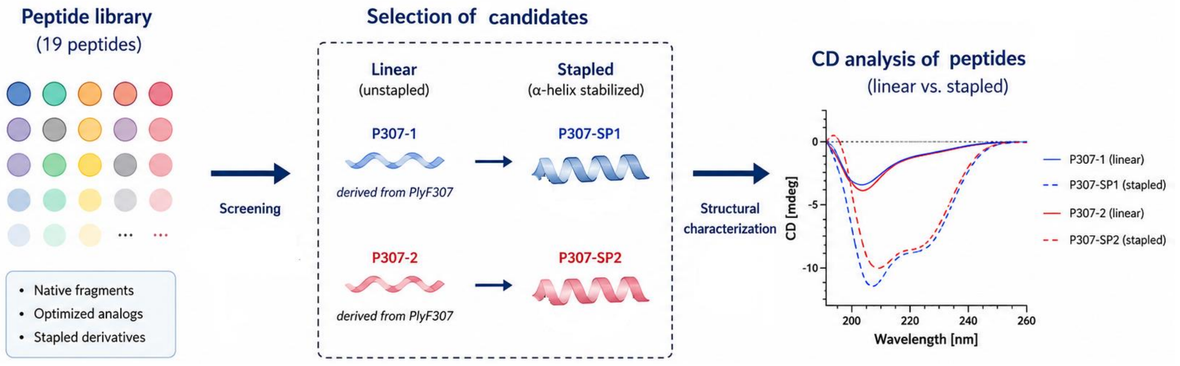
1. RATIONAL AND ML-GUIDED DESIGN

Structural analysis of PlyF307 and LysAB2 revealed amphipathic, positively charged motifs as potential templates for membrane-active peptides. ML-assisted design was then used to optimize their physicochemical properties.



2. FROM PEPTIDE LIBRARY TO LEAD CANDIDATES

Selected sequences were further optimized by hydrocarbon stapling to stabilize their α-helical conformation. This strategy yielded the most promising antibacterial candidates.



3. POTENT ANTIBACTERIAL ACTIVITY

The lead stapled peptides P307-SP1 and P307-SP2 displayed broad antibacterial activity (**MIC = 1–4 µM**), including potent activity against resistant *A. baumannii* and clinically relevant UPEC strains.

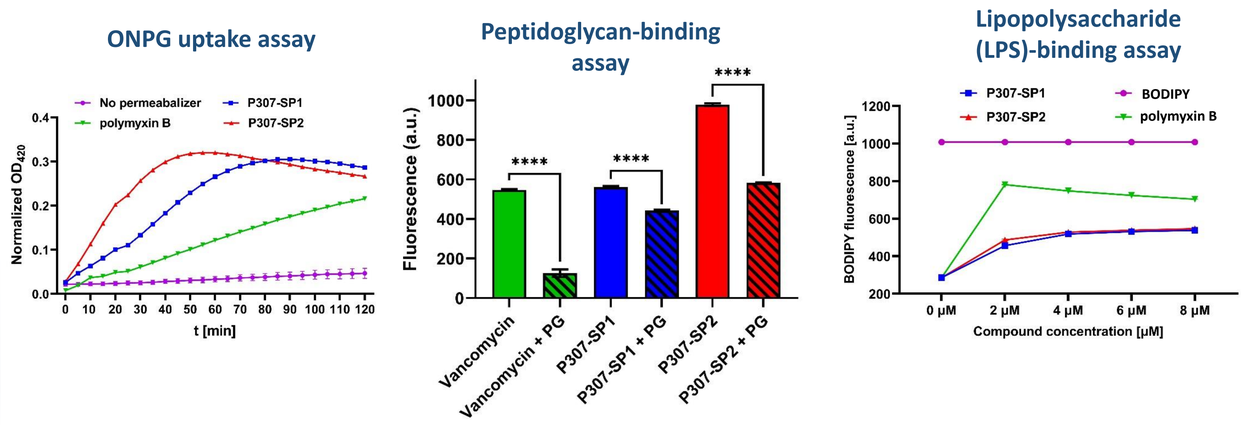
	Minimum inhibitory concentration (MIC) [µM]		
Bacterial strain	**P307-SP1**	**P307-SP2**	**Polymyxin B**
E. coli K-12 MG1655	2	4	0.25
E. coli AS19	2	2	1
E. coli O6:K15:H31 536 (UPEC)	2	4	-
E. coli ATCC 700928 (UPEC)	2	4	-
A. baumannii ATCC 19606	1	2	0.5
A. baumannii ATCC BAA-1605	2	2	0.5
A. baumannii ATCC 19606R	1	1	32
S. aureus ATCC 29213	2	2	>32
M. catarrhalis PCM 1902	2	2	0.5

1 µM (very potent)

2 µM (potent)

4 µM (moderate)

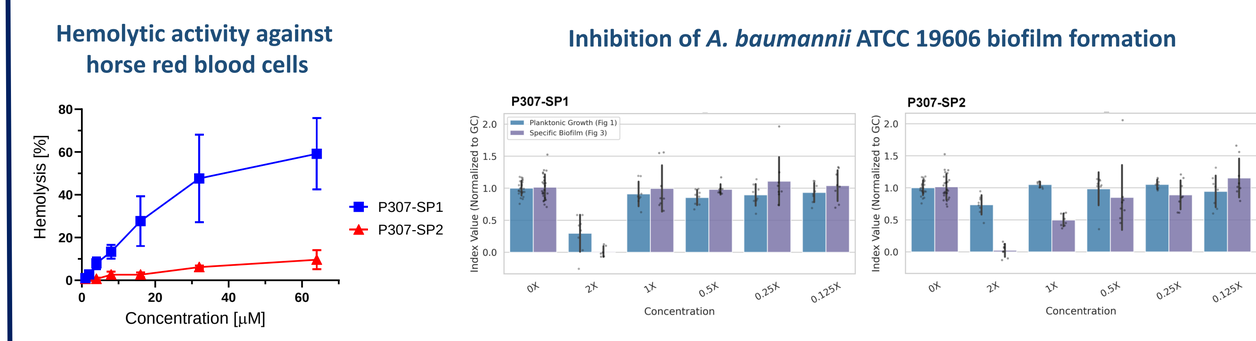
4. MEMBRANE-TARGETING MECHANISM



The lead peptides interact with bacterial envelope components and permeabilize the bacterial membrane. These results support a membrane-targeting mechanism underlying their antibacterial activity.

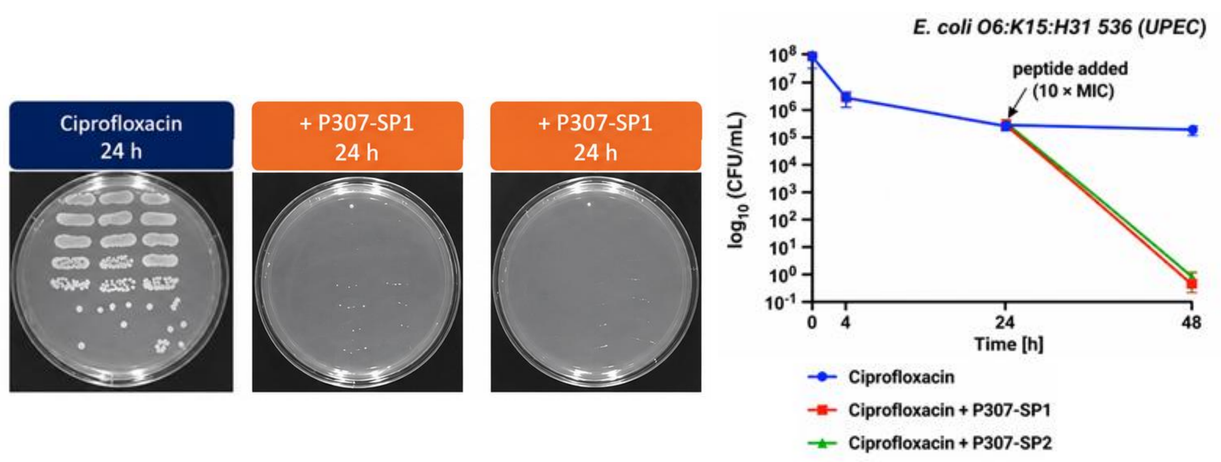
5. SELECTIVITY AND ANTIBIOFILM EFFECT

Despite their potent antibacterial activity, the lead peptides showed low hemolytic activity. Both peptides also reduced *A. baumannii* biofilm formation.



6. TOWARD DIFFICULT-TO-ELIMINATE POPULATIONS

The lead peptides retained antibacterial activity against persister cells, demonstrating their potential against difficult-to-eradicate bacterial populations.



TAKE-HOME MESSAGE

- Bacteriophage endolysins are a promising and underexplored source of membrane-active peptide sequences.
- Structure-guided and ML-assisted design enabled the identification and optimization of antibacterial peptides derived from endolysin motifs.
- Hydrocarbon stapling promoted α-helical structure and generated highly active peptide variants.
- The lead stapled peptides showed broad antibacterial activity, **with MIC values of 1–4 µM**, including activity against resistant *A. baumannii*.
- Their antibacterial activity is associated with membrane disruption, while maintaining low hemolytic activity.
- Beyond planktonic bacteria, the lead peptides retained activity against clinically relevant UPEC strains and difficult-to-eradicate bacterial populations, including persisters, and reduced *A. baumannii* biofilm formation.

From hidden endolysin motifs to potent antibacterial peptides active against planktonic cells, biofilms and persisters.

REFERENCES

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