Guiding Peptide Nanonet Formation and Antibacterial Activity via Hydrophobic Aromatic Ring Engineering for Sepsis Control

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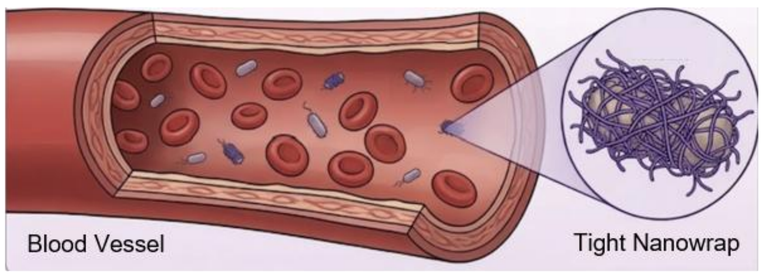
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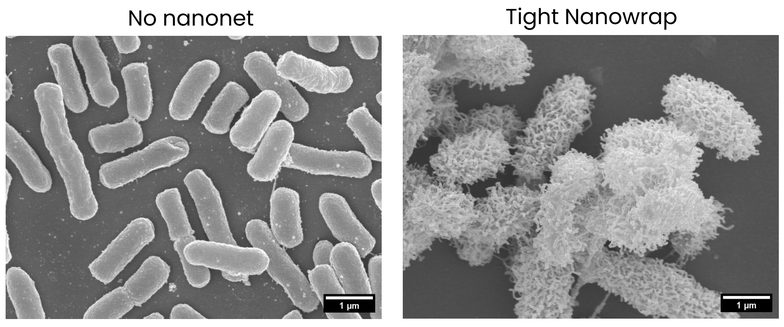
https://doi.org/10.17952/38EPS.2026.P217

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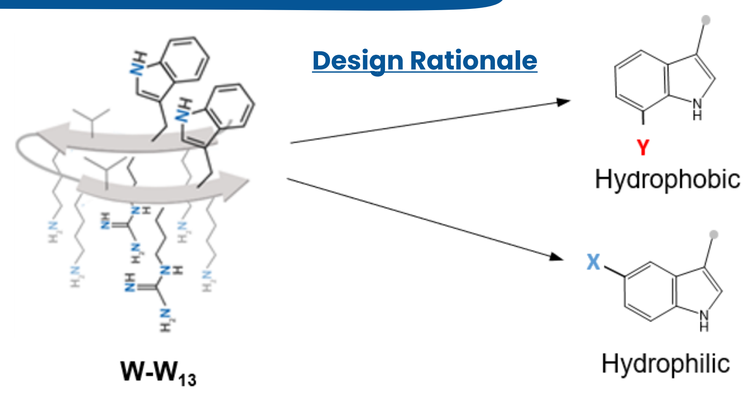
Introduction



Precise bacterial capture by Tight Nanowraps:
A potential new strategy against bacteremia and fatal sepsis.



P. aeruginosa PAO1 — **W-W$_{13}$**



- **Bacteria-responsive self-assembly:** β-hairpin peptides can self-assemble upon binding to bacterial surface components to form trap-and-kill nanonets [1].
- Tight Nanowrap: **Aromatic ring**-containing β-hairpin peptide **W-W$_{13}$** forms dense nanofibers that tightly encapsulate individual bacteria [2].

Research Questions:

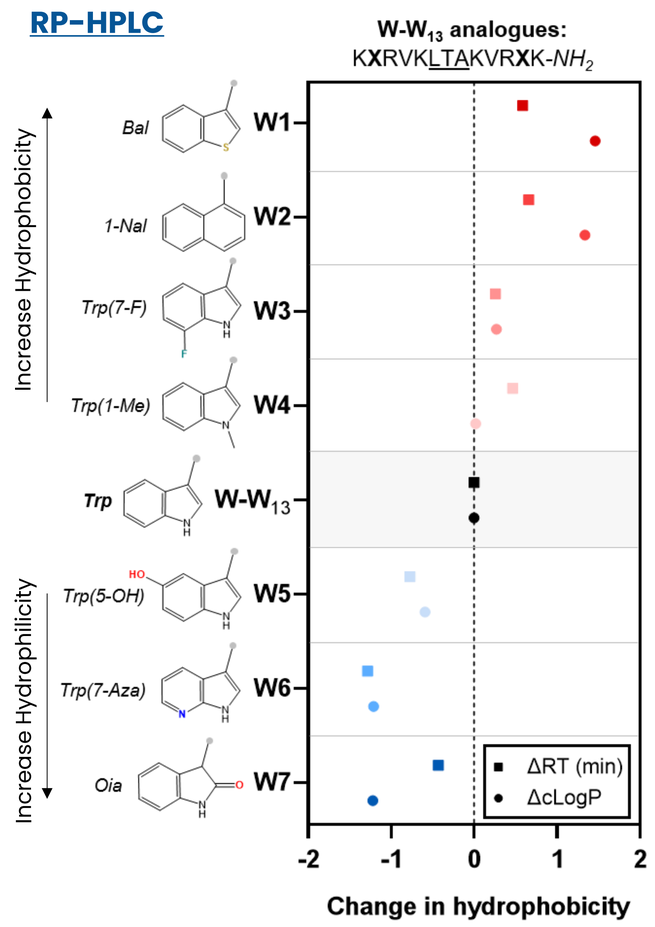
Aromatic ring as a scaffold: How chemical modification of the aromatic ring tunes nanonet morphology and activity remains unknown.

Increase hydrophilicity — Hydrogen bonding opportunity to stabilize nanofiber?

W-W$_{13}$

Increase hydrophobicity — Enhanced self-assembly and denser nanofibers?

Peptide Design

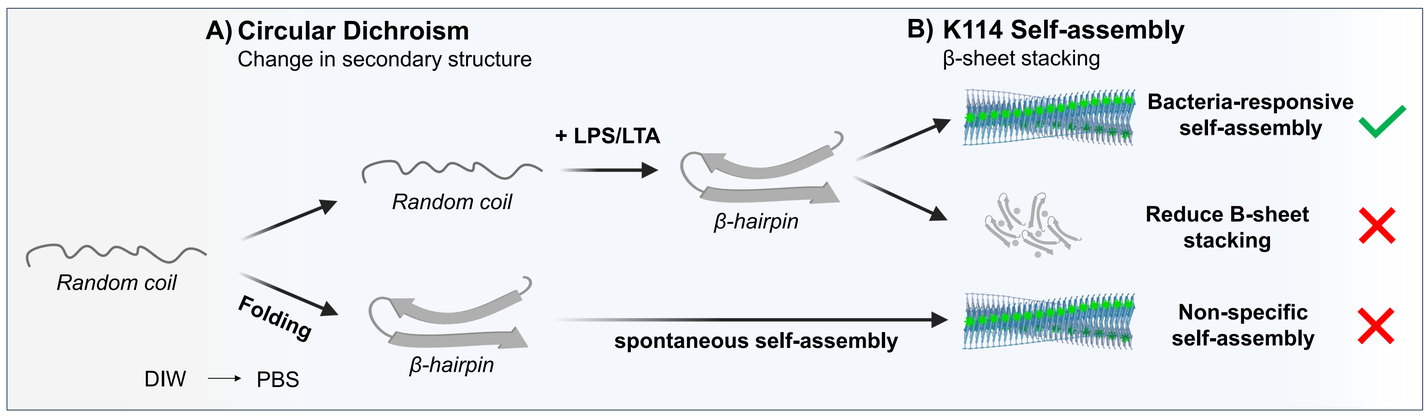


Small changes to the aromatic ring **alter peptide lipophilicity**, as measured by RP-HPLC and correlated with cLogP.

Aromatic Lipophilicity Impacts Self-Assembly and Activity

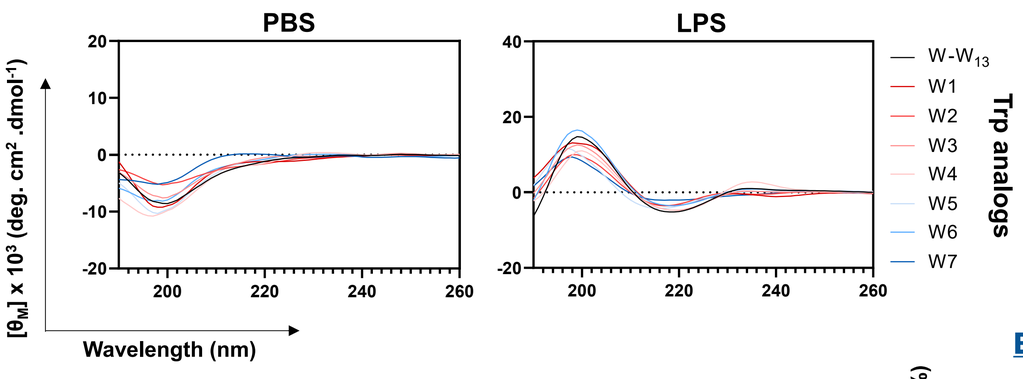
Cell-free Peptide Self-assembly Screening Workflow

*Only peptide candidates that **specifically** form nanofibers upon binding to bacterial LPS/LTA were selected for evaluation.



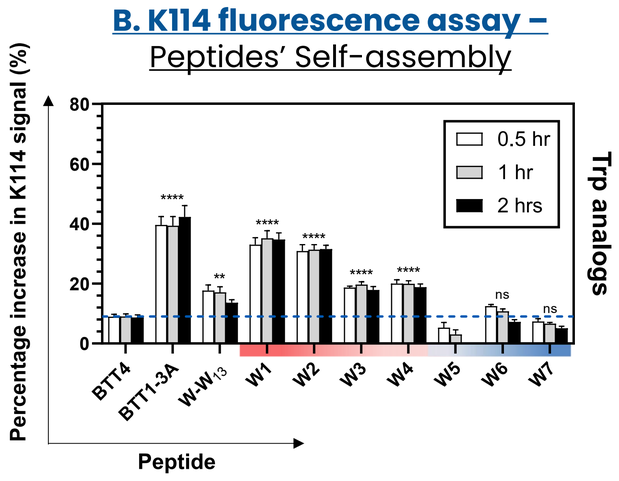
Legend: Fluorescent K114 / Quenched K114
LPS=Lipopolysaccharide
LTA=Lipoteichoic acid

A. Circular Dichroism - Peptides Secondary Structure



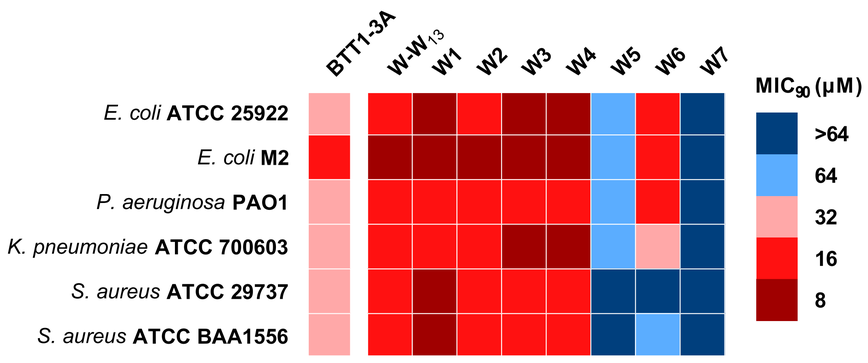
- Lipophilicity does not affect peptide secondary structure formation.
- Peptides remain as soluble random coils and fold into **β-hairpins** only upon binding to LPS specifically.

B. K114 fluorescence assay – Peptides' Self-assembly



- **Only hydrophobic aromatic Trp analogues** self-assemble in response to bacterial LPS.
- W3 and W4 show **similar self-assembly to W-W$_{13}$** (similar cLogP), while W1 and W2 with **higher hydrophobicity enhance nanofiber formation.**
- In contrast, **hydrophilic aromatic analogues significantly impair nanofiber formation.**

C. MIC Screening Assay– Antibacterial Activity



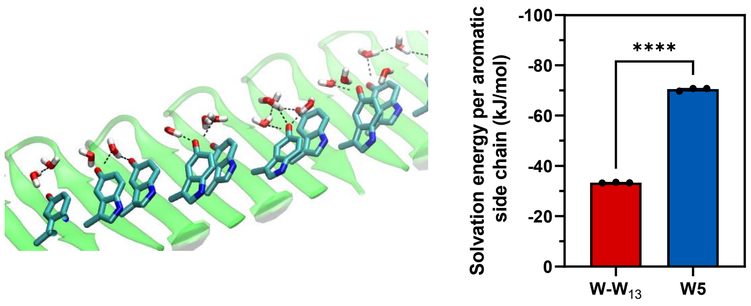
- Hydrophobic aromatic Trp analogues exhibit **broad-spectrum antibacterial activity.**
- W1, W3, and W4 showed a **2-fold increase** in bacterial killing.
- Hydrophilic aromatic Trp analogues **impaired antibacterial activity.**

Molecular Basis of Nanonet Formation

Scanning Electron Microscopy – Nanonet's Morphology

- Hydrophilic aromatic analogues (W5) **abolish nanonet formation.**
- W3 and W4 **maintain tight nanowrap** formation, similar to W-W$_{13}$.
- W1 and W2 nanofibers tend to self-assemble away from the bacterial surface.

Molecular Simulation – Mechanistic Studies



Weak π–π interactions and **strong water solvation** disfavor the intermolecular assembly of hydrophilic aromatic analogues (W5) into nanofibers.

Key Takeaways

- Hydrophobic aromatic analogues are critical for maintaining nanofiber assembly and antibacterial activity.
- Fine-tuning aromatic hydrophobicity modulates nanonet morphology and activity, with an optimal range for tight nanowrap formation.
- Hydrophilic aromatic modifications disfavor self-assembly due to weaker π–π stacking and strong water solvation.

References:
1. Tram, N. D. T., et. al., Bacteria-Responsive Self-Assembly of Antimicrobial Peptide Nanonets for Trap-and-Kill of Antibiotic-Resistant Strains. *Adv. Funct. Mater.* 2023, 33, 2210858.
2. Chen. W. M., et, al., Controlling Nanonet Morphology via Residue-Specific Modulation of β-Hairpin Peptide for Enhanced Bacterial Trapping. *Small* 2025, 2505823.

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