Controlling Nanonet Morphology via Residue-Specific Modulation of β-hairpin Peptide for Enhanced Bacterial Trapping

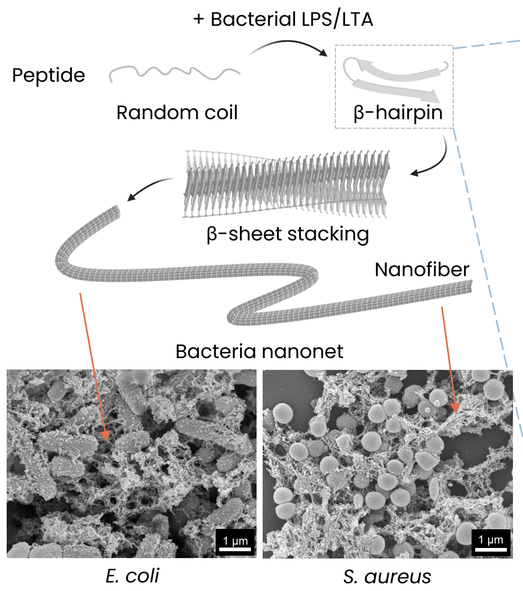
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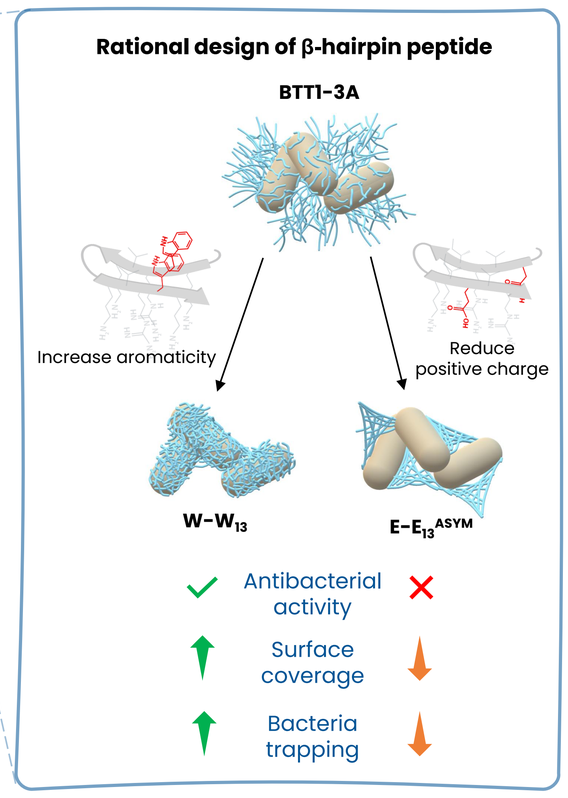
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Introduction



- **Self-assembling peptides** can form nanonets to control bacterial infections and combat antibiotic resistance.
- Our previously designed synthetic peptide, **BTT1-3A**, forms nanonets that trap-and-kill bacteria [1], but its **loose** architecture may limit trapping efficiency.
- In this work, we demonstrate that rational peptide design can **modulate nanonet morphology** and enhance antibacterial and trapping activity [2].



Design Rationale

Aromatic residues may promote membrane interaction to form **tight** trap-and-kill nanonets.

Reduced net positive charge may result in **loosely** trapping nanonets without effective killing.

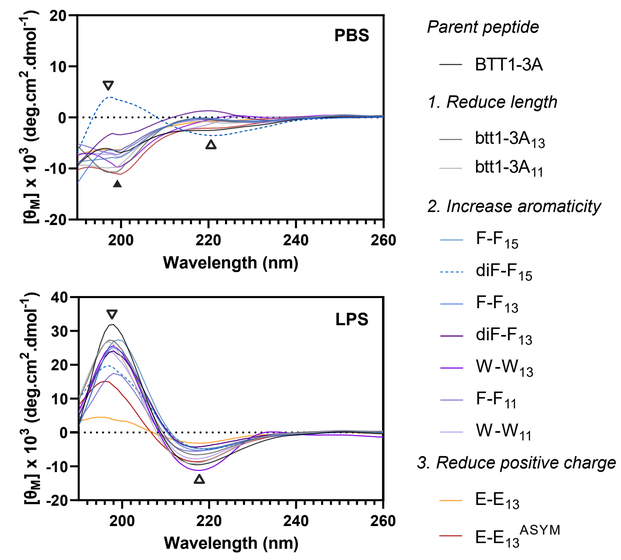
Peptide Designs

Peptide	Sequence	Length	Charge
BTT1-3A	LKLKLKLTAKLKLKL	15	7
1. Reduce length			
btt1-3A$_{13}$	KLKLKLTAKLKLK	13	7
btt1-3A$_{11}$	LKLKLTAKLKL	11	5
2. Increase aromaticity			
F-F$_{15}$	LK**F**RVKLTAKVR**F**KL	15	7
diF-F$_{15}$	LK**F**R**F**KLTAK**F**R**F**KL	15	7
F-F$_{13}$	K**F**RVKLTAKVR**F**K	13	7
diF-F$_{13}$	K**F**R**F**KLTAK**F**R**F**K	13	7
W-W$_{13}$	K**W**RVKLTAKVR**W**K	13	7
F-F$_{11}$	**F**RVKLTAKVR**F**	11	5
W-W$_{11}$	**W**RVKLTAKVR**W**	11	5
3. Reduce positive charge			
E-E$_{13}$	**E**VRIKLTAKIRV**E**	13	3
E-E$_{13}^{ASYM}$	EVRIKLTAKI**E**VR	13	3

Acronym: lipopolysaccharide (LPS), lipoteichoic acid (LTA), phosphate buffer saline (PBS)

1 Bacteria-induced peptide folding

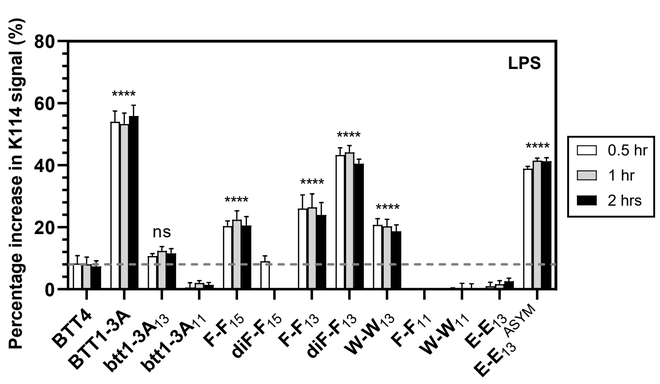
Circular dichroism spectroscopy



- All peptides remained as random coils (▲) in PBS, except diF-F$_{15}$, which adopted a β-sheet structure (△).
- All peptides folded into **β-sheets** upon binding to bacterial LPS, indicating their **responsiveness to bacterial presence.**
- This bacteria-triggered folding enables **targeted self-assembly** only at infection sites.

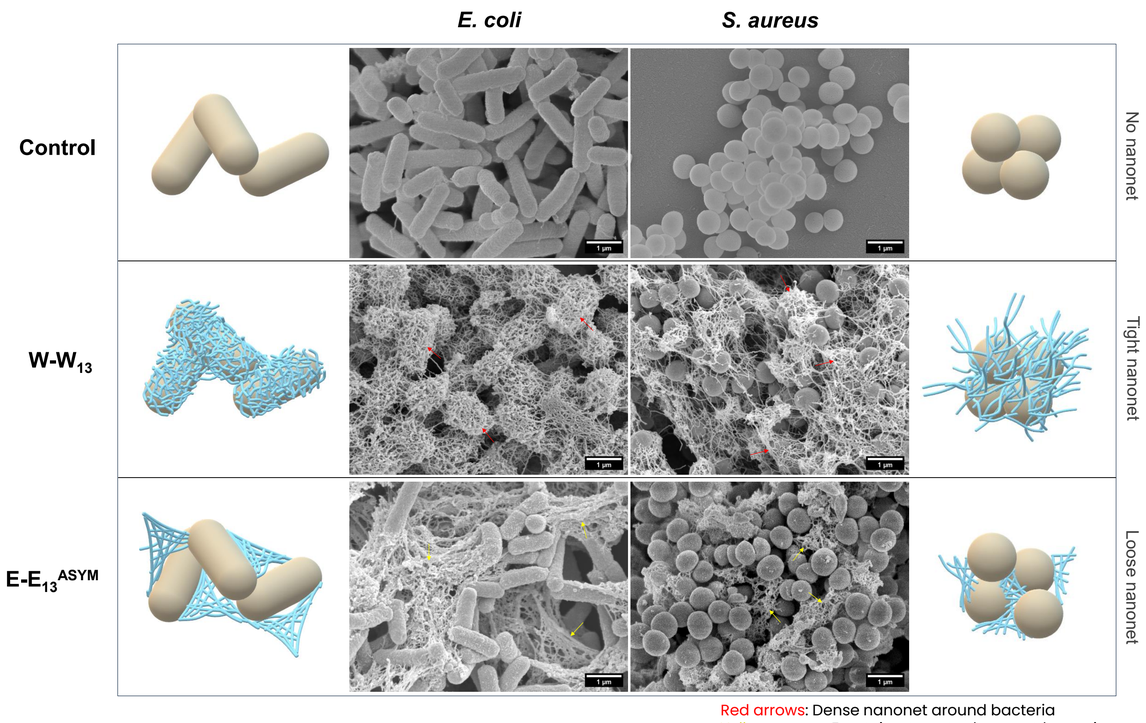
2 Bacteria-responsive nanonet formation

K114 fluorescence assay



- K114 dye bind specifically to **cross β-sheet** structure and fluoresces, confirming self-assembly upon binding to bacterial LPS.
- BTT1-3A, F-F$_{15}$, F-F$_{13}$, diF-F$_{13}$, W-W$_{13}$ and E-E$_{13}^{ASYM}$ **rapidly self-assemble** after binding to LPS within 30min.
- **π–π stacking** and **electrostatic interactions** can stabilize the β-hairpin and promote self-assembly in the shorter 13-mer peptide.

Scanning electron microscopy

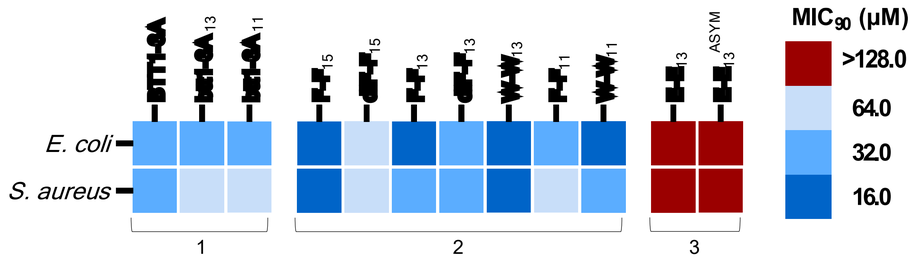


Red arrows: Dense nanonet around bacteria
Yellow arrows: Extensive nanonet between bacteria

- W-W$_{13}$ forms **dense, interwoven nanofibers** on individual *E. coli* surface, forming **tight nanonets** that fully encage the bacteria, but shows lower surface coverage on *S. aureus*.
- E-E$_{13}^{ASYM}$'s nanofibers tend to **self-interweave** into extensive nanonets, with **minimal surface coverage** on *E. coli* and *S. aureus*, resulting in **loose** bacterial trapping.

3 Bacterial trapping efficacy

Minimum inhibitory concentration (MIC) assay

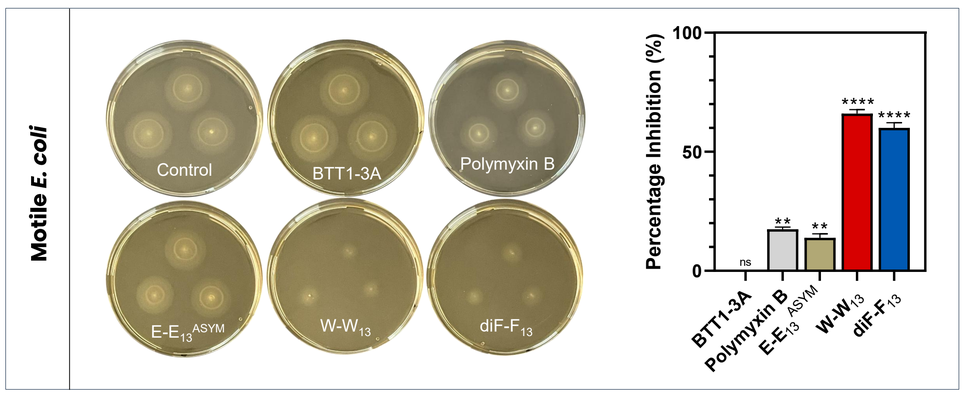


MIC$_{90}$: Lowest concentration of peptide that kill 90% of the bacteria

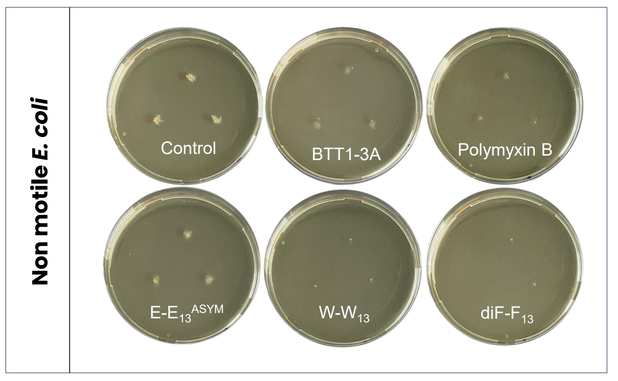
- W-W$_{13}$ and F-F$_{15}$ showed 2-fold **improved killing** of *E. coli* and *S. aureus* while retaining nanonet formation.
- Reducing net positive charge in E-E$_{13}^{ASYM}$ **abolished antibacterial activity** despite preserved nanonet formation.
- High positive charge and aromaticity are critical for balancing nanonet formation and antibacterial function of β-hairpin peptides.

Soft agar motility assay

Nanonet-trapped bacteria were seeded in soft agar to assess post-entrapment escape



- Motile *E. coli* move freely and proliferate in soft agar, forming a clear motility halo.
- Kill-only antibiotic (Polymyxin B): *E. coli* survive by **escaping** from high-concentration zones.
- Loose trap-and-kill nanonet (BTT1-3A): Kills bacteria but **ineffective trapping** allows escape.
- Tight trap-and-kill nanonet (W-W$_{13}$ & diF-F$_{13}$): Effectively trap and **inhibit bacteria spread**.
- Loose trap-only nanonet (E-E$_{13}^{ASYM}$): Slows movement only but fails to kill bacteria.



- Non-motile *E. coli* (ΔflhD mutant) were **more susceptible** to polymyxin B and loose BTT1-3A nanonets due to impaired escape.
- W-W$_{13}$ and diF-F$_{13}$ effectively blocked the growth of non-motile bacteria.
- Without antibacterial activity, E-E$_{13}^{ASYM}$ nanonets failed to prevent bacterial outgrowth and potential spread.

Key takeaways

- **Design-Driven Assembly:** Rational modification of β-hairpin side strands controls nanonet morphology through amino acid side chain chemistry.
- **Tight vs. Loose Nanonets:** W-W$_{13}$ forms dense, surface-bound nanonets that effectively limit bacterial spread, while E-E$_{13}^{ASYM}$ forms loose trap-only nanonet.
- **Motility Matters:** Bacterial motility reduces antibiotic effectiveness; tight nanonets that encage and restrict motile bacteria offer a promising alternative strategy.

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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