Atomistic Simulations Reveal Differential Insertion of a Novel Human Thrombin-Derived Peptide (FRL16) in Gram-Negative Bacterial Membranes

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1. Introduction

AMPs offer resistance-limiting antibiotic alternatives[1], with human defensins charting a new path. Defensins generally encode their antimicrobial activity in special sequences that translate to secondary structural features, important for membrane activity[2]. A novel thrombin-derived peptide **FRL16** disrupts bacterial membranes, and an atomistic MD simulations reveal its structural dynamics and insertion mechanism.

2. Protein-membrane

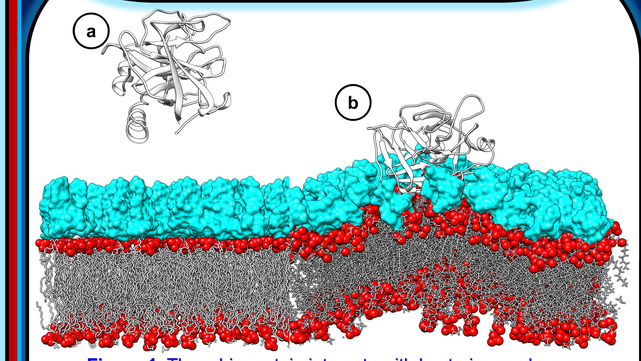


Figure 1: Thrombin protein interacts with bacteria membrane

The human thrombin protein associates with the membrane surface, where its insertion perturbs lipid organization and alters membrane structure.

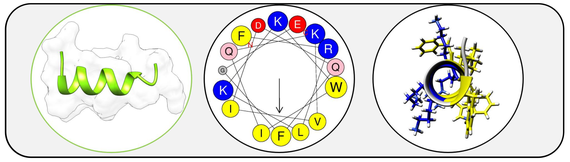
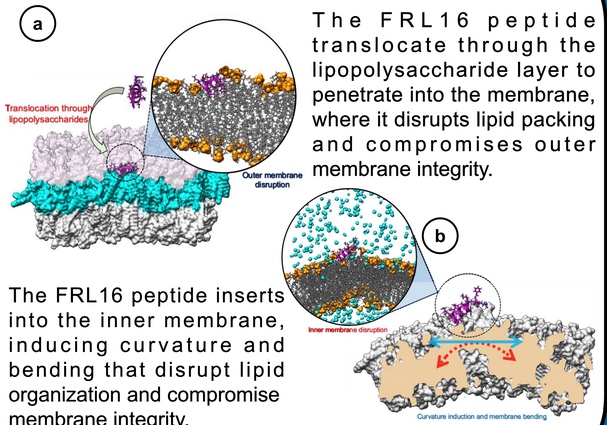


Figure 2: FRL16 crystal structure and amino acid distribution

α-Helical antimicrobial peptide FRL16, showing its amphipathic helix and residue distribution

3. FRL16-membrane



The FRL16 peptide translocate through the lipopolysaccharide layer to penetrate into the membrane, where it disrupts lipid packing and compromises outer membrane integrity.

The FRL16 peptide inserts into the inner membrane, inducing curvature and bending that disrupt lipid organization and compromise membrane integrity.

Figure 3: FRL16 bypass dense LPS layer (a); interaction with outer membrane (b).

4. Membrane disruption

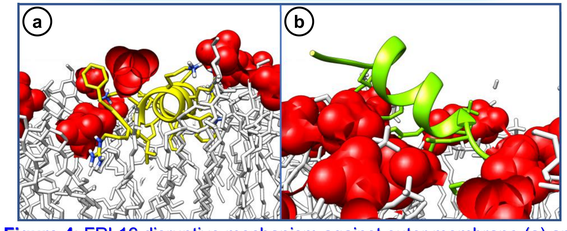


Figure 4: FRL16 disruptive mechanism against outer membrane (a) and inner membrane (b).

(a) Intersection of FRL16 within the outer membrane, showing distortion and displacement of surrounding phospholipid headgroups. **(b)** Interaction of FRL16 with the inner membrane, illustrating peptide insertion and perturbation of lipid packing.

5. Tilt and rotation

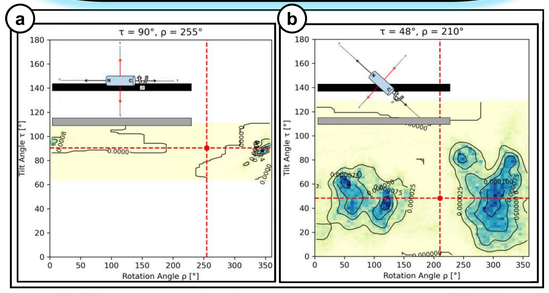


Figure 5: Tilting and rotational orientations assumed by FRL16 against Gram-negative outer (a); inner membranes (b).

(a) Tilting and rotational orientations assumed by FRL16 against G(-)-OM, **(b)** Tilting and rotational orientations assumed by FRL16 against G(-)-IM.

6. Phospholipid diflection

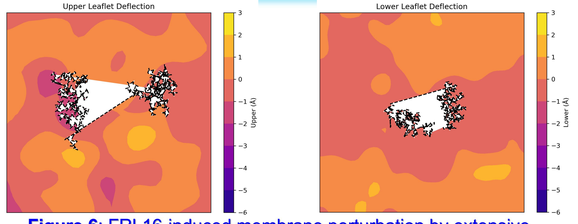


Figure 6: FRL16-induced membrane perturbation by extensive phospholipid deflection leading to pore formation in both leaflets.

FRL16 induces phospholipid deflections confirming bilayer disruption and consistent with its carpet-to-toroidal mechanism.

7. Pore size and water influx

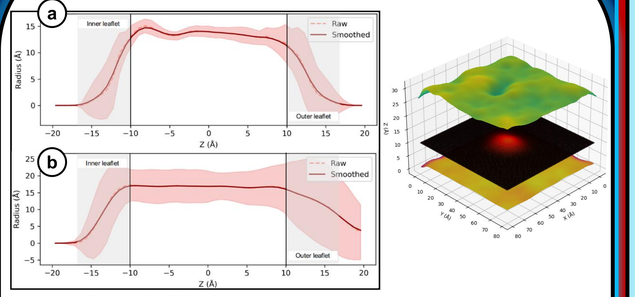


Figure 7: FRL16 creates a stable transmembrane pore

(a) Pore radius in the Gram-negative outer membrane (OM) after FRL16 insertion. **(b)** Pore radius in the Gram-negative inner membrane (IM) after FRL16 insertion.

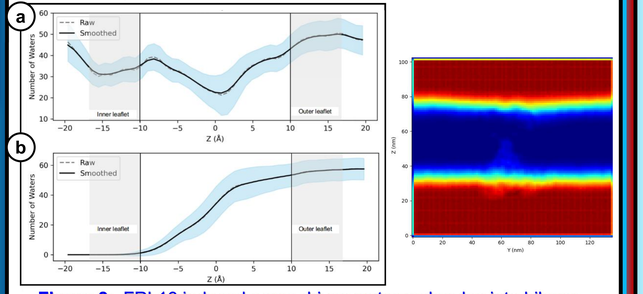


Figure 8: FRL16 induced pores drives water molecules into bilayer.

(a) Hydration of G(-)-OM due to FRL16 disruptive mechanism and **(b)** Hydration of G(-)-IM due to FRL16 disruptive mechanism. Hollow cleft created and water found in the hydrophobic core of the meembrane

8. Conclusion

FRL16 penetrates Gram-negative membranes, inducing lipid disorder, pore formation, and water influx through distinct tilt and residue-specific interactions. These mechanisms highlight FRL16 as a promising scaffold for peptide-based antimicrobials.

9. References

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[2] Jarczak J, Kościuczuk EM, Lisowski P, Strzałkowska N, Jóźwik A, Horbańczuk J, Krzyzewski J, Zwierzchowski L, Bagnicka E. Defensins: Natural component of human innate immunity. Hum. Immunol. 74, 1069–1079 (2013). doi.org/10.1016/j.humimm.2013.05.008