On the non-canonical structure of keratin-derived antimicrobial peptides (KAMPs) from molecular dynamics simulations

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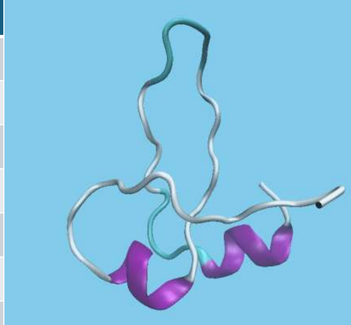
1. Introduction

- The rise of antimicrobial resistance (AMR), due to the misuse of currently available antimicrobials and lack of novel antibiotics discovery,[2] has challenged the capabilities of modern healthcare infrastructure.[1]
- Antimicrobial peptides (AMPs) have gained significant traction as an alternative class of therapeutics due to their selective cytotoxicity against microorganisms and slower development of AMR.[3]
- The structure, size, and charge[4] of AMPs directly influence their mechanism of action against cell membranes. However, little is known about their structure, conformation, aggregation, and especially antimicrobial activity mechanism.

2. AMPs of interest

- KAMPs: Derived from human cytokeratin 6A of corneal epithelial cells. Active against both gram-negative (*Pseudomonas aeruginosa*) and gram-positive bacteria (*Streptococcus pyogenes*).[5-7]
- Pw-Antibac12$_3$: Derived from chicken feather lysates. Active against gram-positive, multi-drug resistant *Staphylococcus aureus*.[8]

Peptide	Sequence	# of residues	Net charge
KAMP-10	GGLSSVGGGS	10	0
KAMP-10G2A	GALSSVGAGS	10	0
KAMP-18C	R^+AIGGGLSSVGGGSSTIK^+	18	+2
KAMP-18N	AIGGGLSSVGGGSSTIK^+Y	18	+1
KAMP-19	R^+AIGGGLSSVGGGSSTIK^+Y	19	+2
KAMP-19scr	IR^+GSVTISGYSGGLK^+GSAG	19	+2
KAMP-36	YGSGLGVGGGFSSSSGR^+AIGGGLSSVGGGSSTIK^+YT	36	+2
Pw-Antibac12$_3$	CNE^-PCVR^+QCQD^-SR^+VVIQPSPVVVTLPGPILSSFPQNTAVGSSTSA	45	0



Pw-Antibac12$_3$

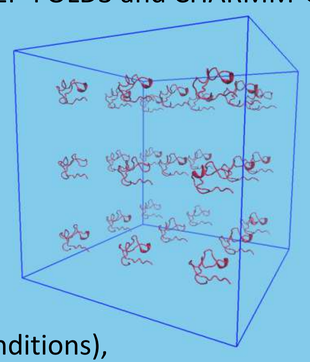
3. Scope

- Determine the secondary structure of KAMPs and Pw-Antibac12$_3$ in dilute aqueous solutions using detailed molecular simulations (MD).
- Compare predicted results with experimental data and models available in the literature.
- Examine the effect of peptide concentration on their conformation.
- Elucidate the dynamics and mechanism of their self-aggregation.
- Elucidate the role of amino acid side chain composition in the aggregation mechanism.

4. Simulation protocol

- ❖ Generation of initial configurations using PEP-FOLD3 and CHARMM-GUI
- ❖ Energy minimization
- ❖ Thermal equilibration (NVT Ensemble for solutions, NVT + NPT for membranes)
- ❖ Production MD runs (NPT Ensemble)

- GROMACS
- CHARMM36 FF
- T = 310.15 K
- P = 1 atm
- Peptide mass fractions: 0.2% w/w (dilute conditions), 5% w/w (semi-dilute conditions)



Initial configuration of KAMP-36 under semi-dilute conditions. Water and ions have been omitted.

5. Secondary structure distributions

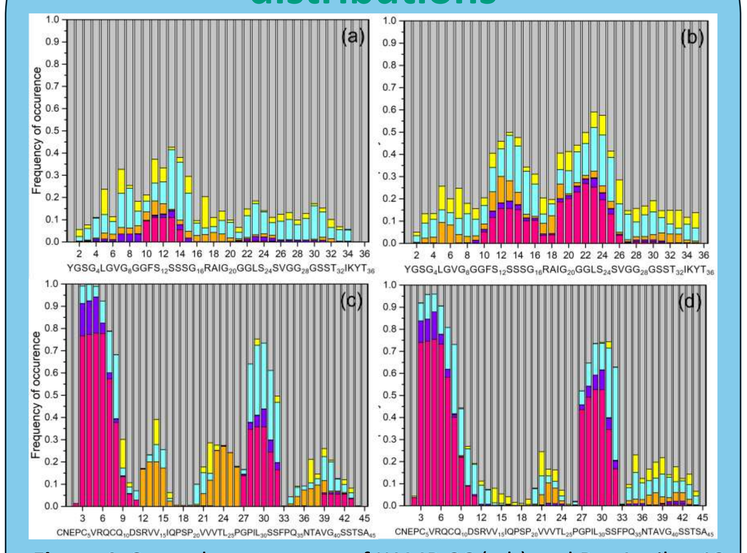


Figure 1. Secondary structure of KAMP-36 (a,b) and Pw-Antibac12$_3$ (c,d), in dilute (a,c) and semi-dilute (b,d) conditions.

- The dominant secondary structure is non-canonical (> 85%).
- Low helical content for KAMPs in dilute conditions (< 3%).[5]
- Aggregation promotes α-helical content.
- Formation of β-bridges which stabilize the clusters.

6. Aggregation dynamics

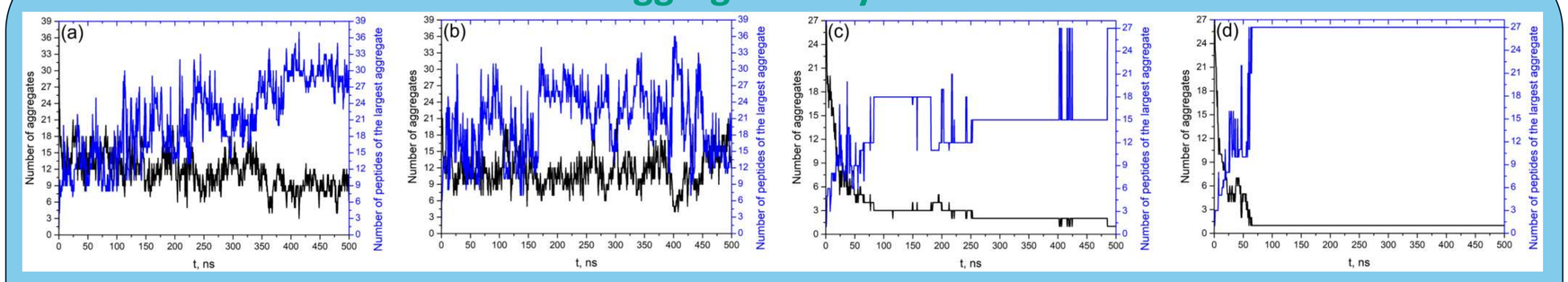


Figure 2. Self-aggregation of (a) KAMP-10, (b) KAMP-10G2A, (c) KAMP-36, and (d) Pw-Antibac12$_3$.

- Steady increase of aggregation number.
- KAMP-10G2A: Glycine to alanine substitution in KAMP-10 does not favor aggregation.
- KAMP-36 and Pw-Antibac12$_3$ self-associate into a single cluster.
- Process is the fastest in Pw-Antibac12$_3$.

7. Contact maps

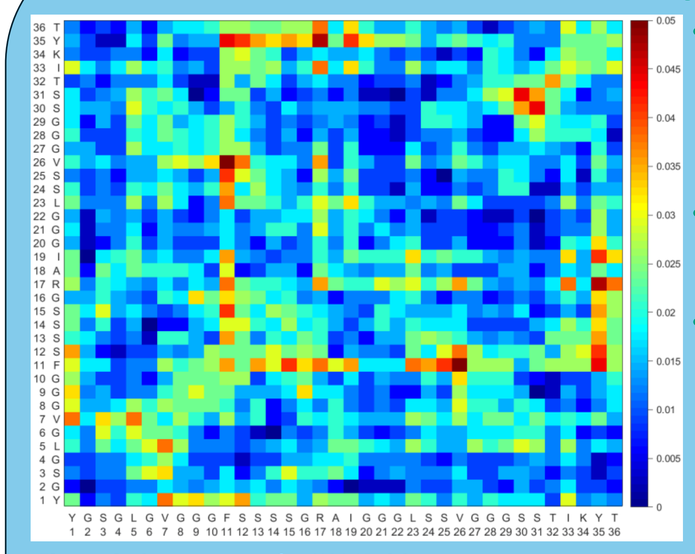


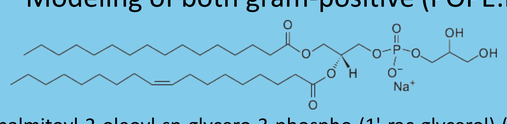
Figure 3. Contact map for KAMP-36.

- To gain insights into the interactions within the biggest cluster, we have calculated the number of inter-peptide contacts per residue.
- At each time frame, the biggest cluster and the peptides that constitute it are identified.
- Using a geometric criterion (cutoff of 0.35 nm) for the centers of mass, we calculate the inter-residue distance between all possible residue pairs in the biggest cluster.

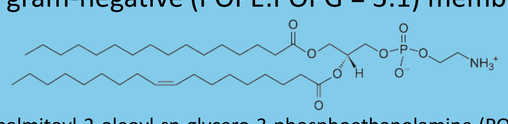
- ✓ High density of phenylalanine-valine and arginine-tyrosine contacts.
- ✓ Chemistry plays an essential role in aggregation propensity.

8. Interactions of KAMPs with model bacterial membranes

- Modeling of both gram-positive (POPE:POPG = 1:3) and gram-negative (POPE:POPG = 3:1) membranes.

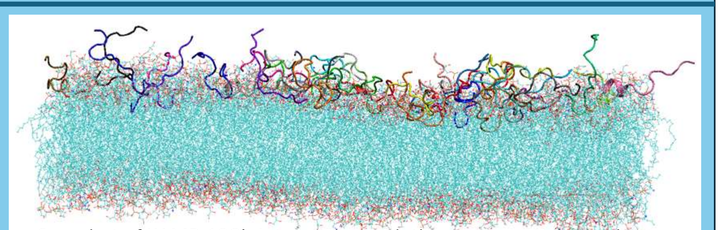


1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG)



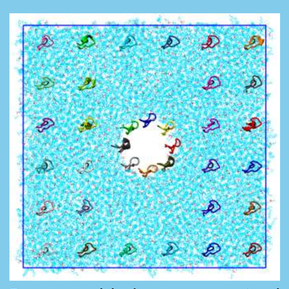
1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE)

- Use of most promising KAMPs (KAMP-10 and KAMP-18C).[5-7]
- Large scale simulations (2 μs for each system).
- Peptide-to-lipid ratio (P/L) = 1:30.
- No spontaneous pore formation observed yet.

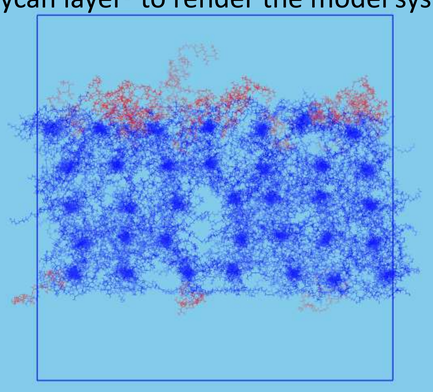


Snapshot of KAMP-18C's interactions with the gram-positive membrane.

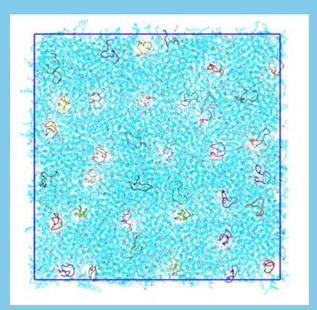
- Since the spontaneous pore formation by KAMPs needs long times to be observed, we have constructed systems with pre-assembled pores to assess their stability in time.
- Systems with KAMPs embedded into the membrane have also been constructed to observe their diffusion and aggregation in the membrane's hydrophobic environment.
- Also, incorporation of a peptidoglycan layer[9] to render the model system more realistic.



Pre-assembled pore comprised of 8 KAMP-18C molecules. 28 more peptides have been placed above the membrane.



Diffusion of KAMPs (red) through the peptidoglycan mesh (blue).



Grid of 36 KAMP-18C molecules initially embedded into the membrane.

9. Conclusions

- Confirmation of non-canonical structure and low helical content of KAMPs that was first documented by Tam et al.[5]
- MD-predicted structure of Pw-Antibac12$_3$ agrees with the molecular model of Paul et al.[8]
- Aggregation depends heavily on peptide amino-acid sequence and chemistry.
- Hydrogen bonding patterns determine the stability of the cluster (evident by the formation of β-structures during aggregation).
- Aromatic residues (phenylalanine & tyrosine) form more intermolecular contacts than smaller ones (glycine, serine, and valine).

10. Future work

- Study of other KAMPs with respect to their aggregation dynamics and membrane interactions.
- Incorporate the peptidoglycan layer model provided by Pokhrel et al.[9] above the gram-positive membrane.
- Elucidate the effect of temperature on pore stability.

11. Acknowledgements

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12. References

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