

Exploring interactions of Plantaricin NC8 α and β with lipid bilayer models using MD simulations

Sanjiv Kumar*, Emanuel Wiman, Abubakr Omer, Hazem Khalaf,
Torbjörn Bengtsson†

School of Medical Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden;
*sanjiv.kumar@oru.se; †torbjorn.bengtsson@oru.se

Introduction

The antimicrobial peptide (AMP) Plantaricin NC8 (PLNC8) $\alpha\beta$ produced by *Lactobacillus plantarum* is a two-peptide cationic bacteriocin belongs to the Class IIb subfamily. Previously, we have shown that PLNC8 $\alpha\beta$ inhibit Gram-negative pathogen (*P. gingivalis*) and are highly effective in killing Gram-positive pathogens (*S. aureus* and *S. epidermidis*) [1]. Present study is aimed at finding mechanism of action of these peptides using Molecular Dynamics (MD) simulations.

Table 1: Amino acid sequences of the peptides Plantaricin NC8 α and β

Peptides	Length	Sequence
$\text{PLNC8 } \alpha$ apo	47	MDKFEKISTSNLKEISGDLITTLKLWSSWGYLGKKARWNLKHPVQF
$\text{PLNC8 } \beta$ apo	55	MNNLKFSTLGKSSLSQEGGSVPTVYTLGKILWSAYKHKRTIEKSFNKGFYH
$\text{PLNC8 } \alpha$ active	29	DLTTKLWSSWGYLGKKARWNLKHPVQF
$\text{PLNC8 } \beta$ active	34	SVPTSVYTLGKILWSAYKHKRTIEKSFNKGFYH

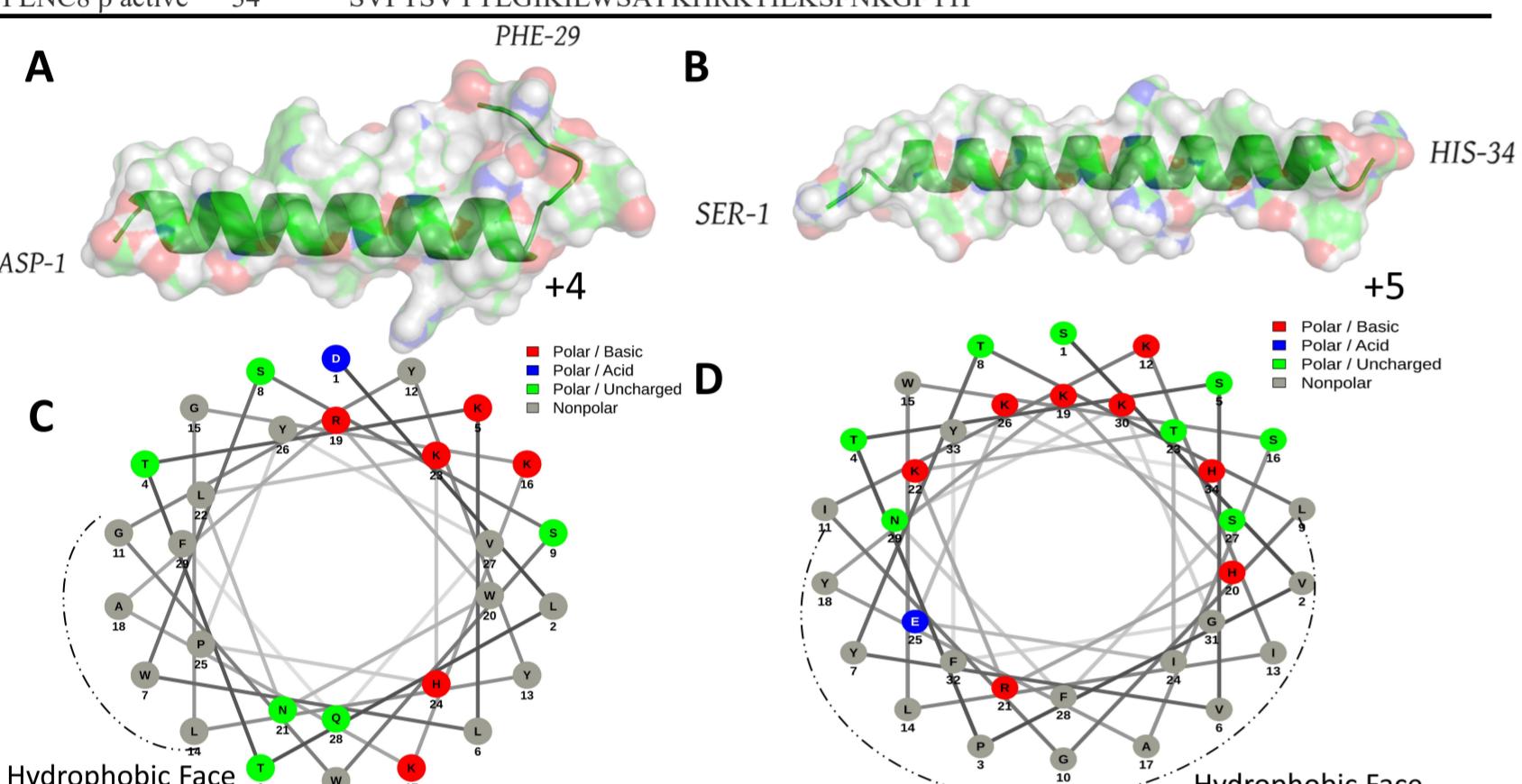


Figure 1: Models of (A) $\text{PLNC8 } \alpha$ & (B) $\text{PLNC8 } \beta$ generated by AlphaFold [4]. Helical wheel of (C) $\text{PLNC8 } \alpha$ & (D) $\text{PLNC8 } \beta$, generated by NetWheels [5]. Hydrophobic face is marked (dashed lines). Both peptides have net positive charge. N-terminus of peptides is hydrophobic and has transmembrane tendency.

Methods

No structural data is available for the peptides (Table 1), modelling of the peptides ($\text{PLNC8 } \alpha$ & $\text{PLNC8 } \beta$) were performed using AlphaFold 2 [4]. The lipid bilayer models mimicking Gram-positive and Gram-negative membranes were constructed using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) in the ratio of 3:1 and 1:3, respectively, using CHARMM-GUI server [2]. All-atom MD simulations of peptides with membrane models were performed using GROMACS (v 2022.2) [3] @ 310.15 K.

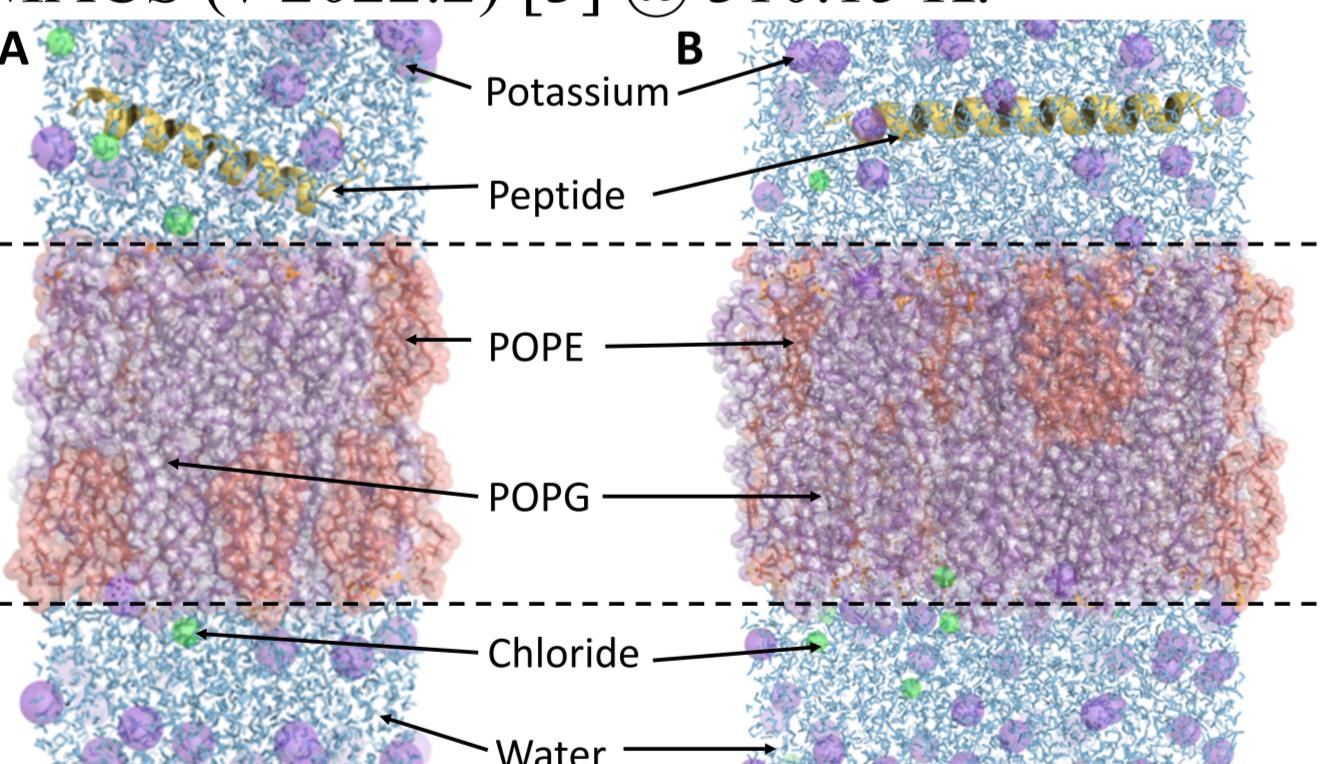


Figure 2: Example membrane models of (A) $\text{PLNC8 } \alpha$ & (B) $\text{PLNC8 } \beta$.

Results

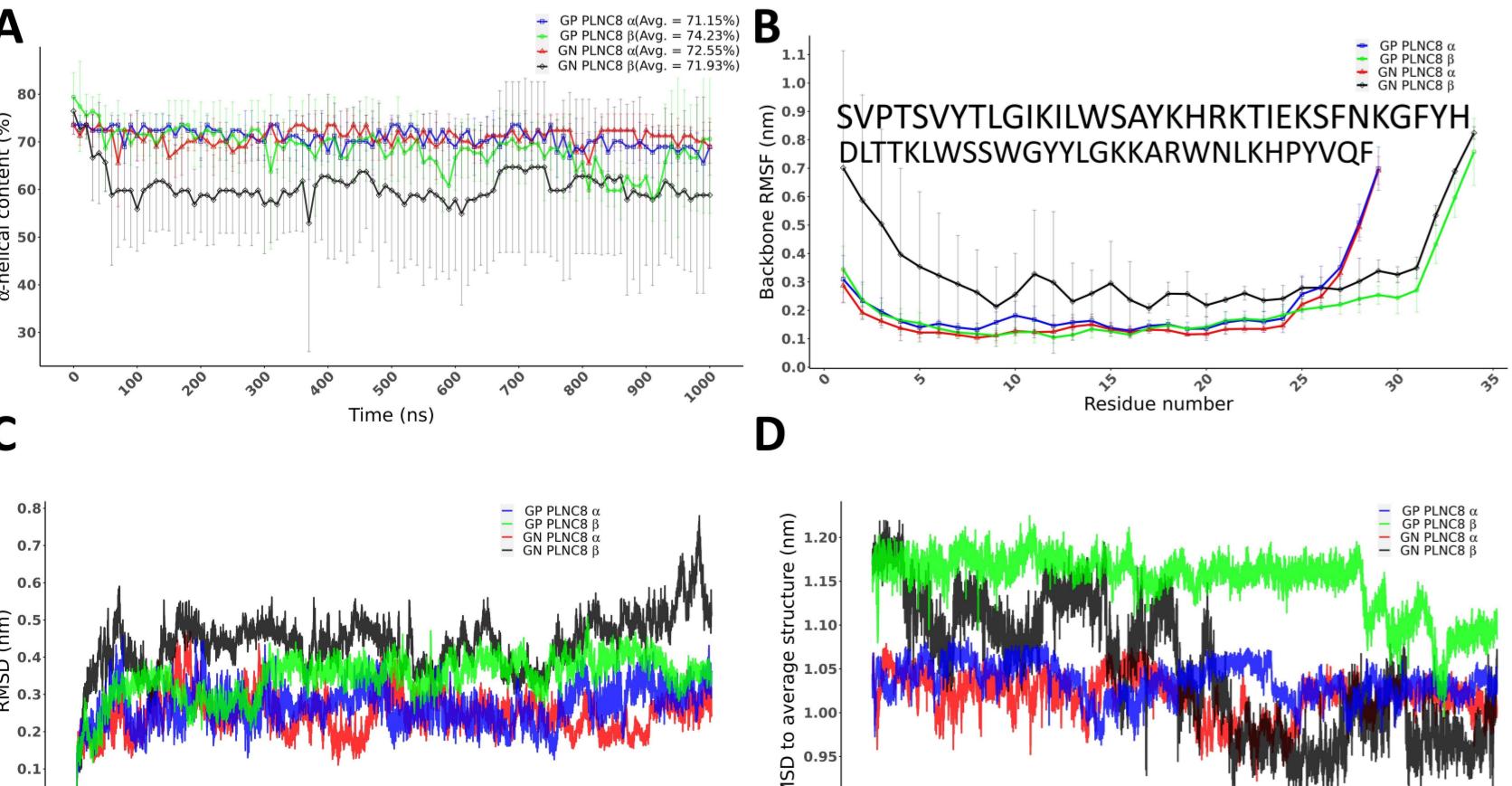


Figure 3: (A) α -helical content of peptides in different membrane model system during the 1000 ns of simulation. (B) RMSF, (C) RMSD and (D) RMSD to average structure of the peptides in different model systems. Error bars not shown for clarity.

- Both peptides retains α -helical conformation.
- RMSF shown fluctuating residues at both ends.
- RMSD increases but quickly stabilizes, fluctuates after 500 ns.
- RMSD to average structure remains stable changes after 500 ns.

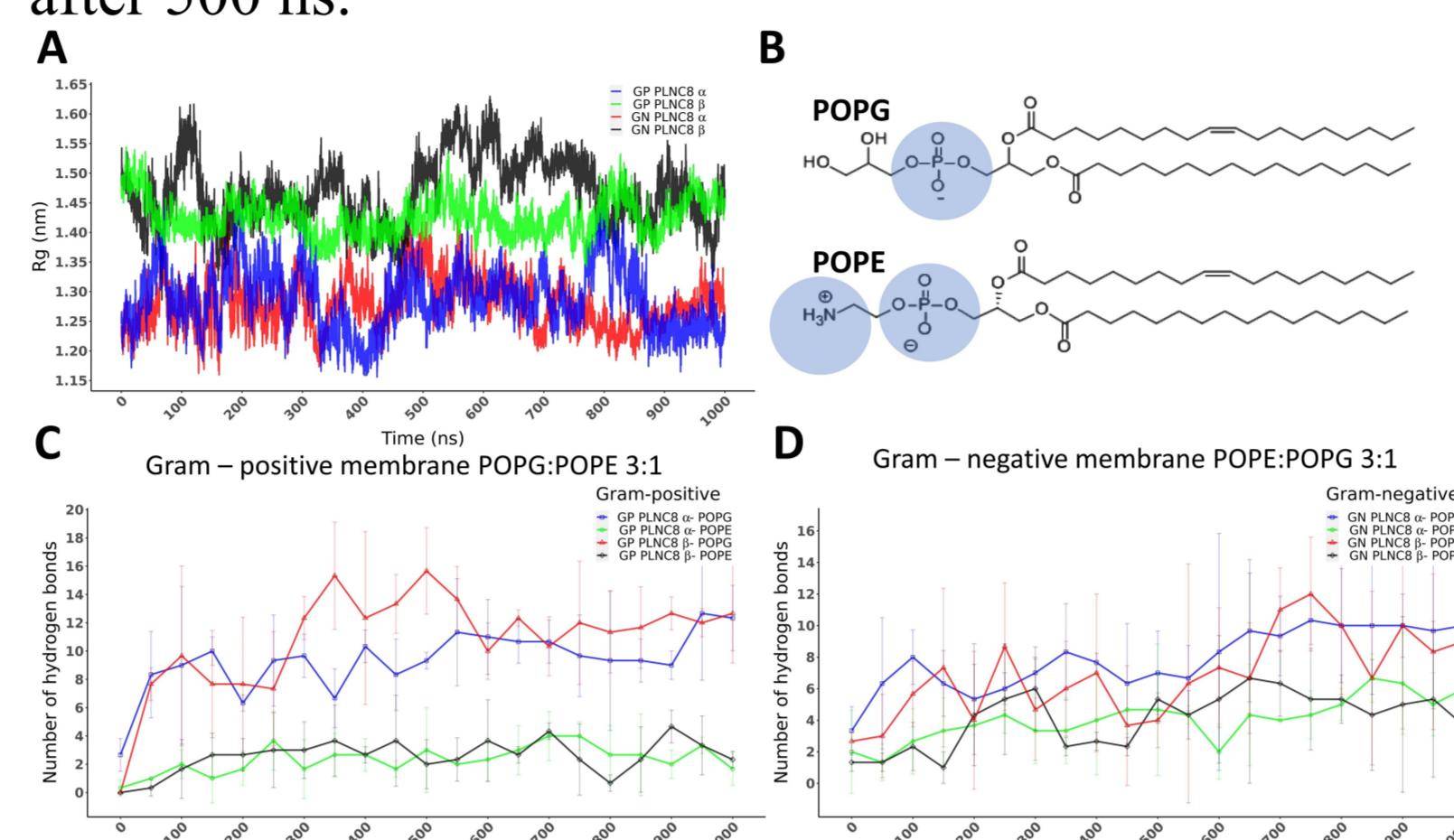


Figure 4: (A) The radius of gyration (R_g) of the peptides in different model systems. Error bars not shown for clarity. (B) Molecular structures of POPG and POPE. (C) Number of hydrogen bonds between the peptide and POPG, peptide and POPE in Gram-positive and (D) Gram-negative membrane system.

- Radius of gyration (R_g) (i.e., compactness) remains stable, fluctuates after 500 ns.
- Higher number of hydrogen bonds between peptides and Gram-positive membrane.
- Both peptides (positively charged) form higher number of hydrogen bonds with POPG (negatively charged) as compared to POPE (zwitterionic).

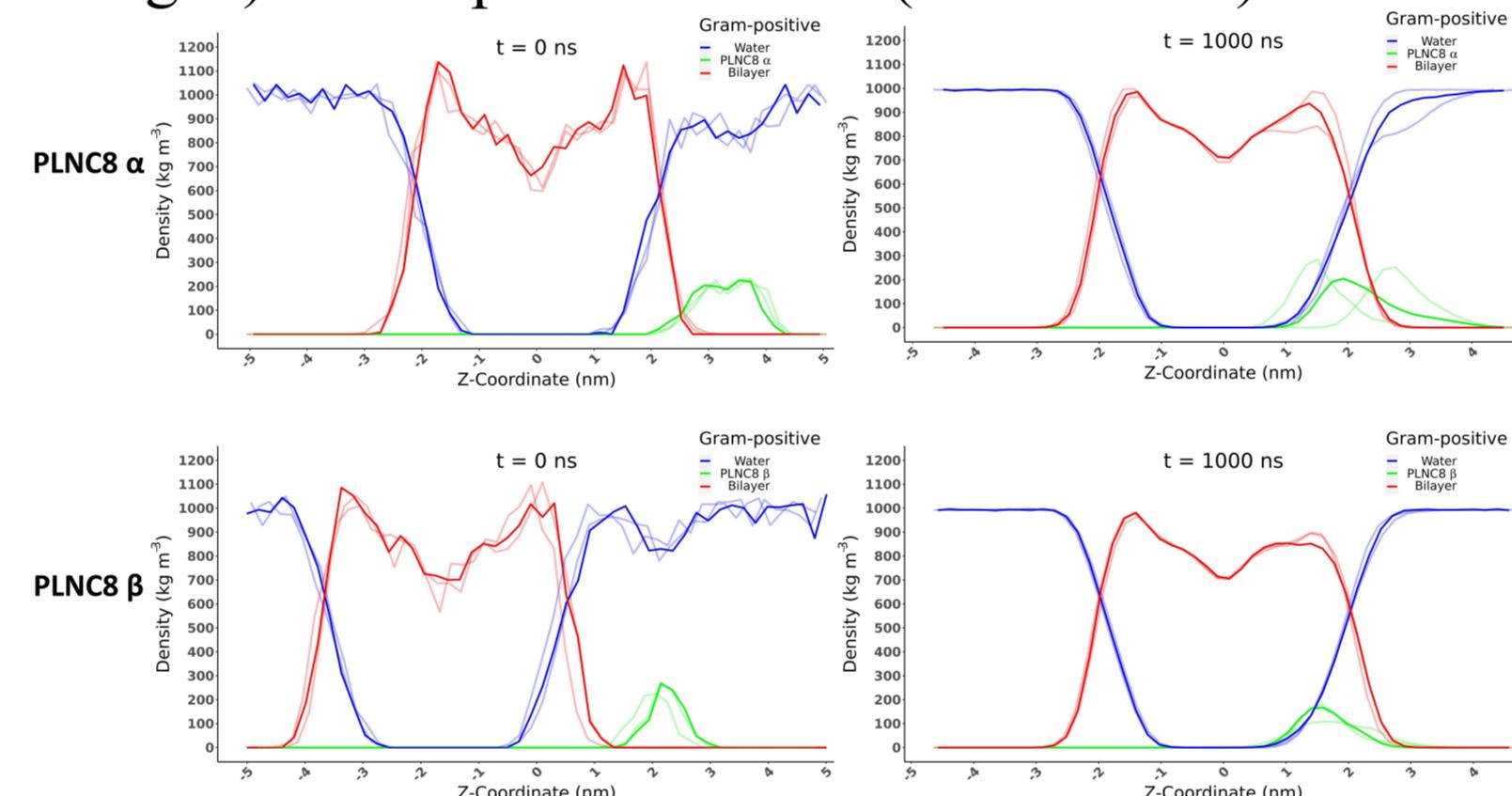


Figure 5: Density distribution of membrane components, water and peptide in Gram-positive membrane. Transparent lines are replicates.

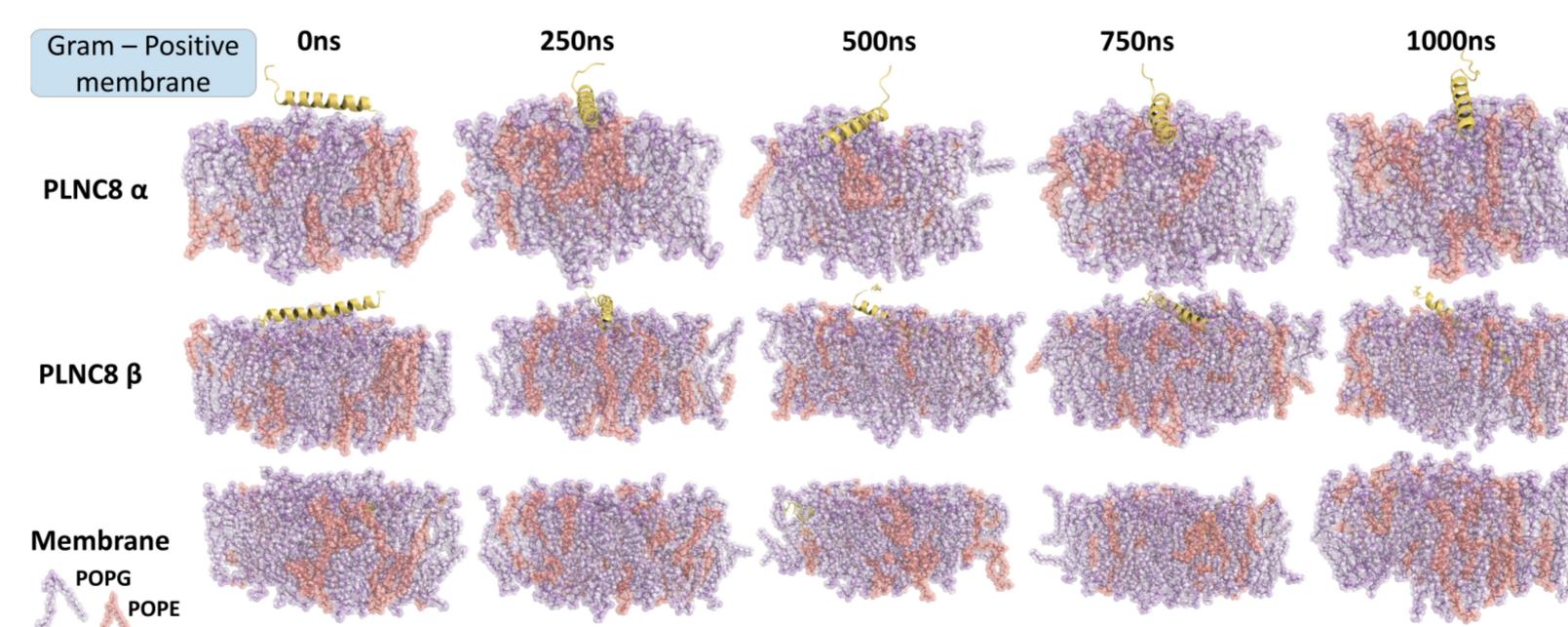


Figure 6: Representative snapshots of Gram-positive bilayer-peptide system at different time-points. Ions and water molecules are not shown for clarity.

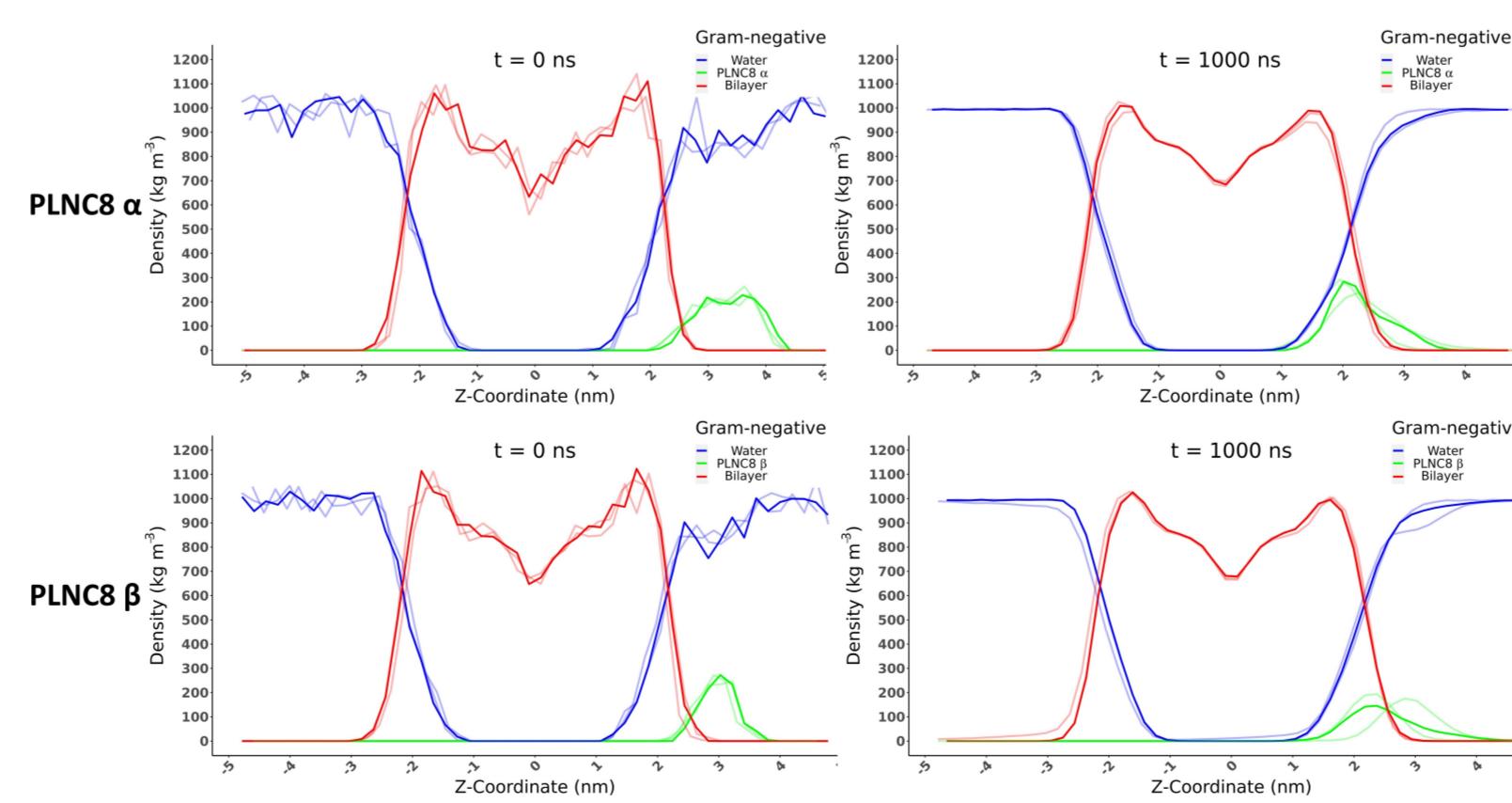


Figure 7: Density distribution of membrane components, water and peptide in Gram-negative membrane. Transparent lines are replicates.

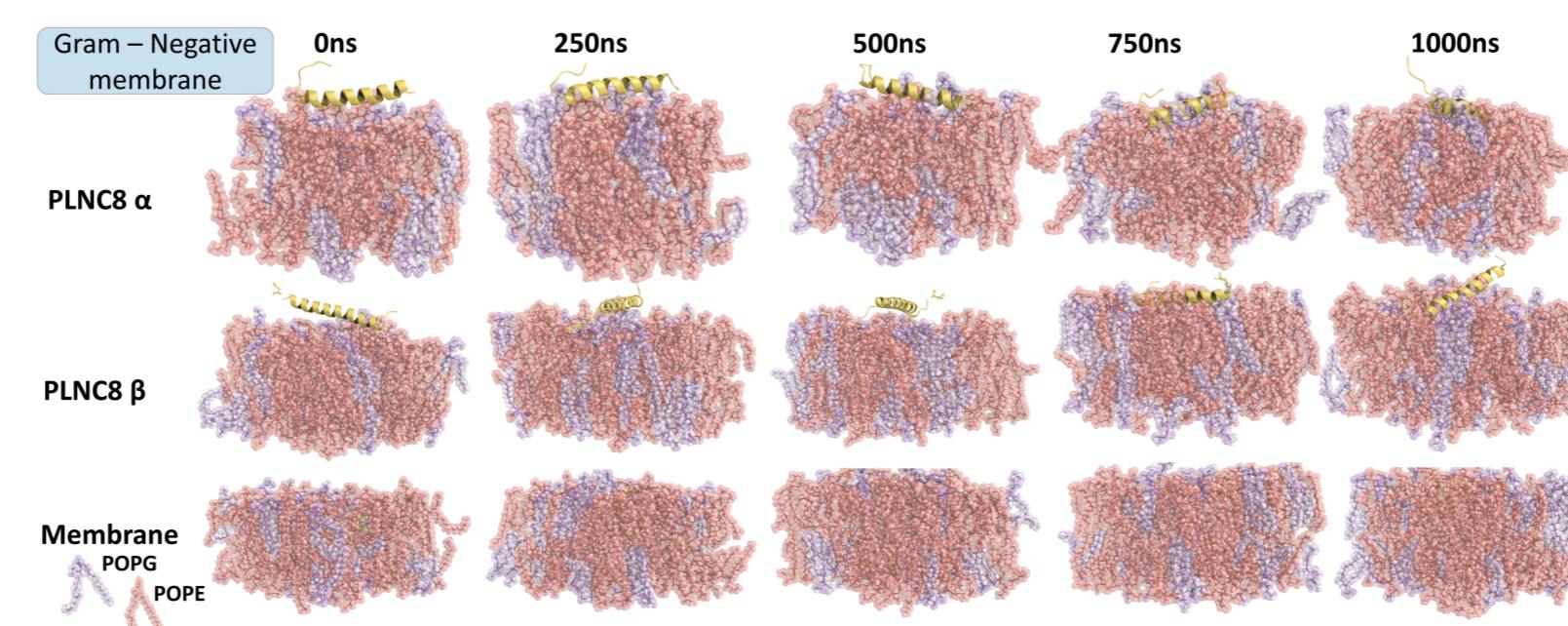


Figure 8: Representative snapshots of Gram-negative bilayer-peptide system at different time-points. Ions and water molecules are not shown for clarity.

- $\text{PLNC8 } \beta$ clearly enters into the membrane after 1000 ns in all three replicates.
- Both $\text{PLNC8 } \alpha$ and $\text{PLNC8 } \beta$ interact with the membrane at N-terminal.
- $\text{PLNC8 } \beta$ interact better with Gram-positive membrane as compared to Gram-negative membrane.

Conclusion

- N-terminus of both peptides is relatively hydrophobic with higher propensity towards the membrane
- Initial interaction with the membrane at the N-terminus.
- Net positive charge of +4 ($\text{PLNC8 } \alpha$) and +5 ($\text{PLNC8 } \beta$) interact with negatively-charged POPG as compared to zwitterionic POPE (electrostatic interactions).
- Both peptides form higher number of hydrogen bonds with POPG than POPE, more so in Gram-positive membrane as compared to the Gram-negative membrane (polar interactions).
- Density distribution shows higher penetration of $\text{PLNC8 } \alpha$ and β in Gram-positive membrane.

Acknowledgments

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