Membrane interactions and pore formation by the antimicrobial peptide protegrin

Themis Lazaridis*, Yi He, and Lidia Prieto

Supplementary Material

All-atom explicit bilayer simulations of protegrin β-barrels

Using NAMD and the CHARMM36 force field we ran explicit simulations of octameric protegrin beta barrels in pores in the NCCN parallel and the NCNC parallel arrangements. Each beta barrel was simulated in two membranes: a 70% POPE and 30% POPG membrane and a 100% POPC membrane. Each membrane was first created and equilibrated for 0.9 ns using CHARMM-GUI, with dimensions 100 Å x 100 Å x 70 Å. In this membrane, we created a 13 Å radius pore eliminating the lipids in this region and adding water molecules to fill the space. This cylindrical pore was equilibrated for 1.5 ns constraining the positions of the lipid head-groups to prevent the pore from closing. The beta barrel structures were then added so that they lined the pore. These protein-pore systems were simulated for 100 ns, the first 2 ns with constraints on the backbone of the protein to let the lipids adapt to the protein complex. The final POPE/POPG system had 246 lipids (164 POPE and 82 POPG molecules), 10641 water molecules, 107 K^+ ions, and 73 Cl^- ions. The POPC system had 240 lipid molecules, 9990 water molecules, 27 K^+ ions, and 75 Cl^- ions. Unless otherwise indicated, the results shown are averages over the last 50 ns.

The initial structure for the NCNC)par beta barrel was the final structure from the implicit simulations (shown in Figure 6c). Since the NCCN)par beta barrel is not stable in our implicit simulations, for the explicit simulations we used the structure constructed by the constrained simulations, the same initial structure as the implicit membrane simulations.

Figure S1 shows top views of the final structures obtained in the four simulations. One can see that in both membranes the NCNC)par barrel is substantially more stable than the NCCN)par barrel. In the former, hydrogen bonding between the monomers is maintained throughout the barrel, but in the latter pairs of beta hairpins separate from the barrel and interactions with neighbor hairpins are broken, especially in the mixed membrane. The average *rmsd* with respect to the initial structure in both membranes is 4.5 ± 0.3 Å for the NCCN)par and 2.6 ± 0.1 Å for the NCNC)par barrel. It is worth noting that in a previous model of the NCCN)par barrel (1) hydrogen bonding seems to exist only within pairs, not between pairs of protegrin molecules (see Fig. 1 of ref. 2). A similar separation into

monomeric or dimeric subunits was reported in another simulation study of parallel and antiparallel NCCN barrels (2). Thus, the present results seem consistent with previous explicit simulations. The instability of the NCCN)par barrel is not as dramatic as in implicit simulations, but this may be due to the limited duration of the explicit simulations.

Figure S2 shows side views of the final structures in the mixed membrane. We do not observe the formation of a toroidal pore, only a bending of the membrane surface to adapt to the beta barrel structure. This effect is more pronounced in the NCCN)par barrel simulations and was also observed in previous simulations that started from a cylindrical pore (2).

Tang et al. (3) measured distances between protegrin C atoms and lipid P atoms by REDOR for four residues (see Table S1). For all of them they obtained 4.0-6.5 Å in anionic POPE/POPG membranes and 6.5-8.0 Å in zwitterionic POPC membranes. To compare with this experiment, we calculated the average minimum distance along the last 50 ns of the simulations between the P atom of the lipid head groups and C atoms of the beta barrel (Table S1). With the exception of ARG11, the P-C distances are longer than the experimental values for the NCNC)par arrangement of the barrel. In this configuration all Arg 4 residues line the pore lumen, therefore they are far from lipid. All Leu 5 and Val 16 are facing the membrane, but they are far from lipid head groups. The distances obtained with the NCCN)par barrel are more comparable to experiment, partly because in this arrangement half of all residues face the lipid. However, we still see some discrepancies with the NMR results: with an NCCN)par arrangement, we do not observe a systematic increase in the P-C distances when the membrane is zwitterionic with respect to the anionic membrane. If anything, the distances are shorter in the latter.

Table S1. Average of the minimum P-C distance along the last 50 ns of the simulation trajectory for the NCCN)par and NCNC)par beta barrels.

		NCCN)par		**NCNC)par**	
		POPE/POPG	POPC	POPE/POPG	POPC
ARG4	Cζ	4.4 ± 0.3	4.7 ± 0.5	10.1 ± 0.8	9.2 ± 0.6
	CA	6.5 ± 0.5	5.7 ± 0.6	7.7 ± 0.4	8.4 ± 0.3
	CO	6.2 ± 0.6	5.8 ± 0.7	8.2 ± 0.6	9.3 ± 0.4
ARG11	Cζ	4.2 ± 0.2	4.2 ± 0.1	4.2 ± 0.2	4.2 ± 0.1
	CA	5.9 ± 0.6	5.6 ± 0.6	5.7 ± 0.5	5.1 ± 0.2
	CO	7.0 ± 0.6	6.4 ± 0.6	5.6 ± 0.5	5.4 ± 0.3
LEU5	CA	6.8 ± 0.9	7.0 ± 0.8	9.3 ± 0.7	11.2 ± 0.5
VAL16	CO	8.5 ± 0.7	6.9 ± 0.5	8.6 ± 0.7	10 ± 1

FIGURE CAPTIONS

Figure S1. Final structures, after 100 ns explicit simulations, of the NCCN)par (a,b) and the NCNC)par (c,d) beta barrels in the POPE/POPG (a,c) and the POPC membrane (b,d). (This figure was made using VMD).

Figure S2. Final structures, after 100 ns explicit simulations in POPE/POPG membranes, of a) the NCCN)par beta barrel and b) the NCNC)par beta barrel. Lipid head groups are represented by spheres (P, tan and N, blue), lipid side-chains by silver lines, water molecules by black points, and the peptide barrel by VMD cartoon representation, each monomer colored in a different color.

REFERENCES

1. Langham, A. A., A. S. Ahmad, and Y. N. Kaznessis. 2008. On the nature of antimicrobial activity: A model for protegrin-1 pores. J Am Chem Soc 130:4338-4346.
2. Jang, H., B. Ma, R. Lal, and R. Nussinov. 2008. Models of Toxic beta-Sheet Channels of Protegrin-1 Suggest a Common Subunit Organization Motif Shared with Toxic Alzheimer beta-Amyloid Ion Channels. Biophysical Journal 95:4631-4642.
3. Tang, M., A. J. Waring, and M. Hong. 2007. Phosphate-mediated arginine insertion into lipid membranes and pore formation by a cationic membrane peptide from solid-state NMR. J Am Chem Soc 129:11438-11446.

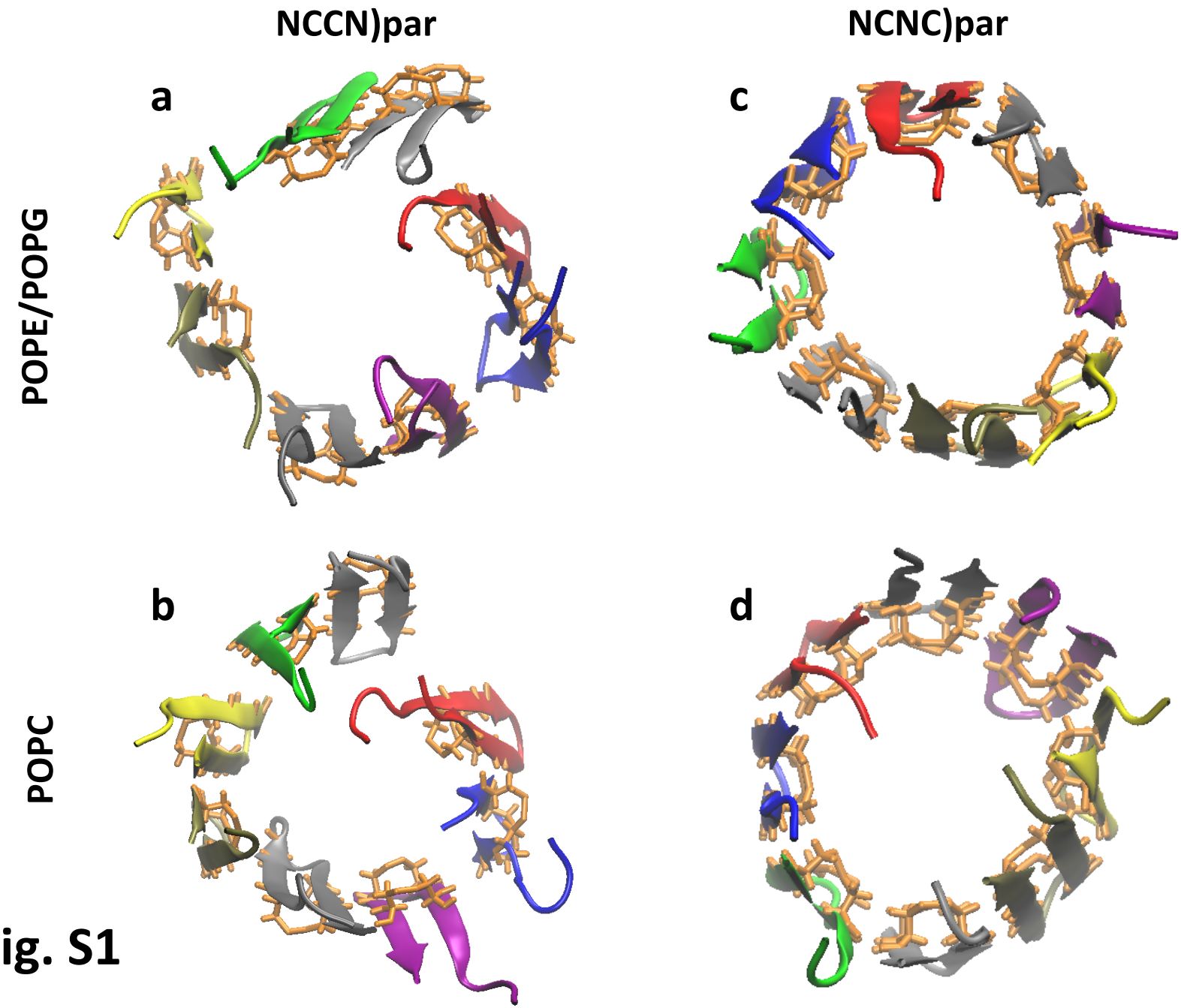
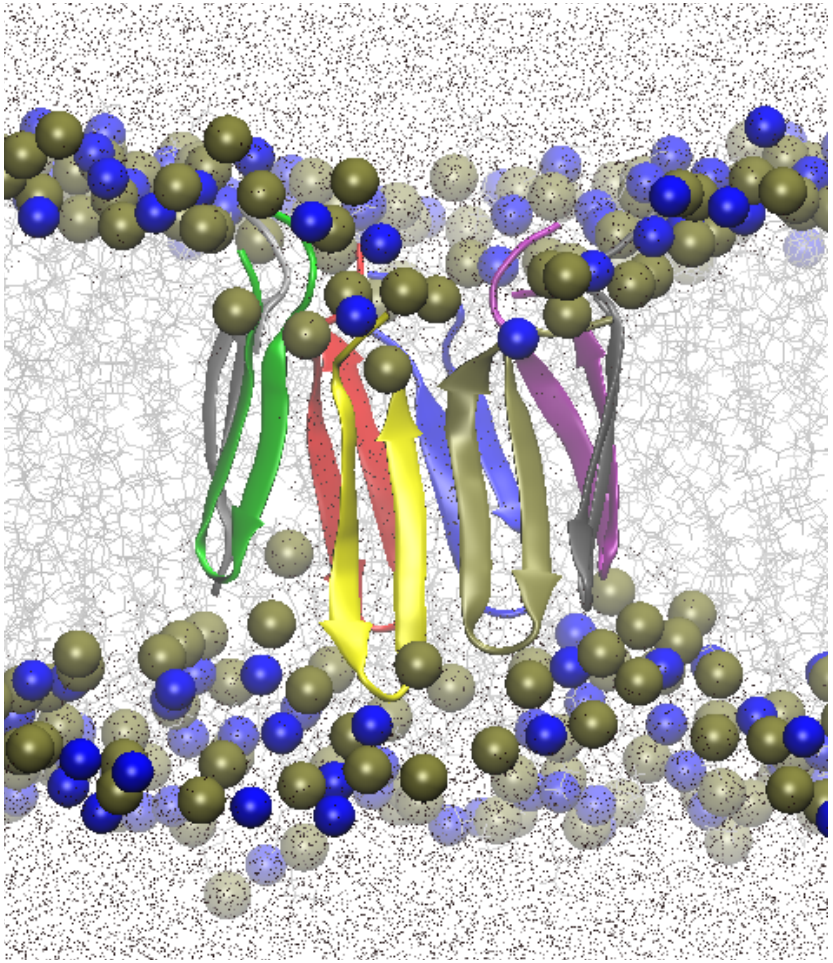


Fig. S1

a



b

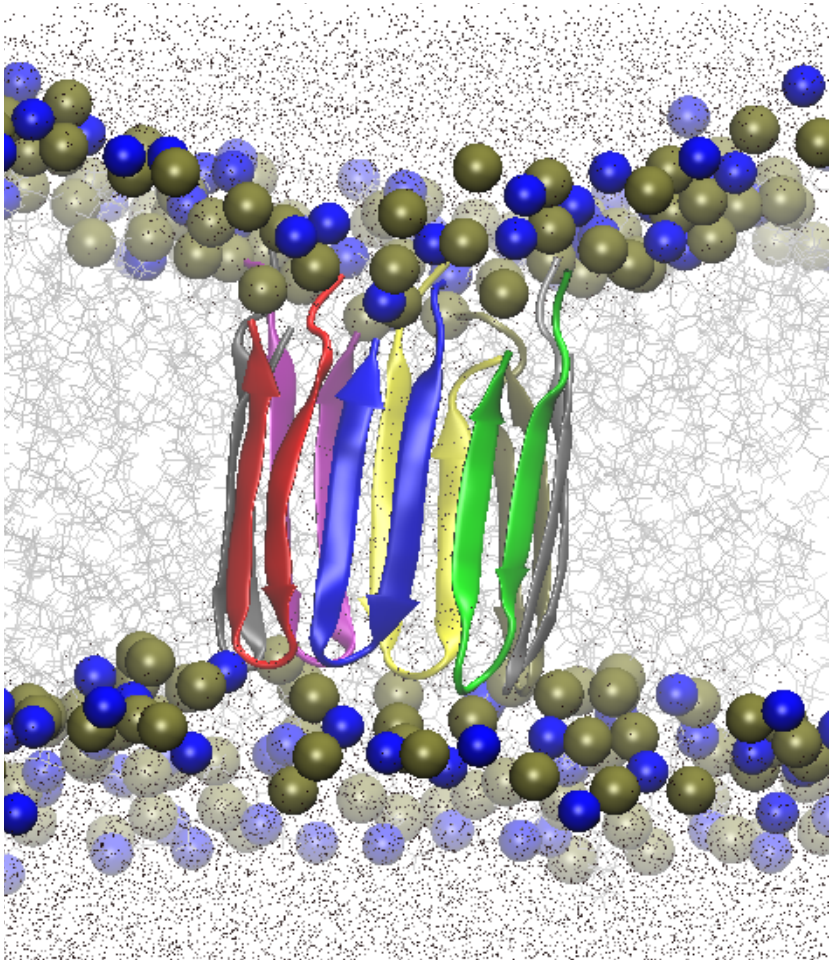


Fig. S2