Supporting Information for

Interaction of an Antimicrobial Peptide with Membranes: Experiments and Simulations with NKCS

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TITLE RUNNING HEAD: Peptide-membrane interactions.

Theoretical calculations

The total free energy difference between a peptide in the aqueous phase and in the membrane (ΔG_{tot}) can be divided into several terms according to Eq. 1:[1]

$$\Delta G_{tot} = \Delta G_{con} + \Delta G_{def} + \Delta G_{Coul} + \Delta G_{sol} + \Delta G_{imm} + \Delta G_{lip} \quad (1)$$

The free energy terms and the approach taken to calculate them were previously described in details.[1,2] Generally speaking, ΔG_{con} is the free energy change due to membrane-induced conformational changes in the peptide. It can be calculated as:

$$\Delta G_{con} = \Delta E - T\Delta S \quad (2)$$

Where, ΔE is calculated as a sum of the internal energy changes between the water- and membrane-bound states of the peptides. The internal energy is derived from a statistical potential based on available 3D structures.[3,4] The energy function assigns a score (energy) to each conformation of the peptide according to its abundance in the PDB. Common conformations receive high scores (low energy) while rare conformations receive lower scores (higher energy). ΔS refers to the entropy changes between the states, while S in each state is determined by the distribution of the virtual bonds in the reduced peptide representation. As the virtual bonds for the first and last two amino acids are not defined, those are omitted from the calculation. ΔG_{def} is the free energy penalty associated with fluctuations of the membrane width around its resting (average) value of 30 Å.

ΔG_{Coul} stands for the electrostatic interactions between titratable residues of the peptide and the (negative) surface charge of the membrane. We calculate this energetic term using the Gouy-

Chapman theory, that describes how the electrostatic potential depends on the distance from the membrane surface in an electrolyte solution.[1] To this end, we considered the solution neutral, containing monovalent salt at a concentration of 0.1M. POPE surface potential was calculated as -2.1kT/e based on previous Z-potential measurements.[5] The protonation state of the side chains of the titratable residues in the solution was set according to pH=7.

ΔG_{sol} is the free energy of transfer of the peptide from water to the membrane. It accounts for electrostatic contributions resulting from changes in the polarity of the solvent, as well as for nonpolar (hydrophobic) effects, which result from both differences in the van der Waals interactions of the peptide with the membrane and aqueous phases, and from solvent structure effects. ΔG_{imm} is the free energy penalty resulting from the confinement of the external translational and rotational motion of the peptide inside the membrane. ΔG_{lip} is the free energy penalty resulting from the interference of the peptide with the conformational freedom of the aliphatic chains of the lipids in the bilayer.

The latter three terms are included in ΔG_{SIL} and are calculated based on a previously developed hydrophobicity scale.[1] The scale accounts for the free energy of transfer of the amino acids, located in the center of a polyalanine α-helix, from the aqueous phase into the membrane midplane. In order to avoid excessive penalty associated with charge transfer into the bilayer, the titratable residues were neutralized gradually when located closer to the membrane, so that a nearly neutral form was desolvated into the hydrophobic core.[1] Exceptionally, the charge was retained when the model was applied to calculate the interaction between a charged peptide and an anionic membrane, as described above.

Sampling protocol

To calculate the membrane interaction energy of each peptide, we simulated the peptides both in water and in membrane environments. The values were averaged over four different simulations of 900,000 Monte Carlo (MC) cycles each. In water simulations, the peptide was subjected solely to internal conformational modifications. In membrane simulations, additional external rigid body rotational and translational motions were also generated to allow the peptide to change its location in- and orientation with respect to the membrane. New peptide structures were generated by simultaneous perturbing the generalized coordinates.

The maximal step of the virtual backbone torsion angle was 3° and 0.5° for both the side-chain torsion angle and its angle with respect to the backbone. New configurations were generated by perturbing both the Euler angles that describe the peptide orientation by a maximal step of 5°, and the Cartesian coordinates of its geometric center by a maximal step of 0.5 Å. A detailed description of the sampling protocol is available in references.[1,2,6] Clustering of conformations and the calculation of the average helicity were carried out following the methodology described in reference. [6]

Table S1. Energy decomposition. Energy values are shown in kT as average ± standard deviation.

peptide	conformations	ΔG_{con}	ΔG_{SIL}	ΔG_{def}	ΔG_{Coul}
NKCS	inner	-2.3±1.0	-5.6±0.7	0.5±0.01	-14.2±0.1
	outer	4.2±0.9	0.0±0.1	0.7±0.1	-11.4±0.3
	all	-2.5±1.0	-4.7±0.8	0.5±0.01	-13.8±0.2
NKCS-[LP]	inner	-2.6±1.4	-1.9±0.2	0.5±0.01	-13.3±0.1
	outer	-0.3±1.0	0.1±0.1	0.6±0.01	-11.3±0.1
	all	-2.8±1.3	-1.2±0.2	0.5±0.02	-12.6±0.1
NKCS-[AA]	inner	-5.7±0.6	-14.4±0.3	0.5±0.01	-14.8±0.04
	outer	48.7±19.1	0.3±0.1	0.1±0.02	-10.2±0.1
	all	-5.7±0.6	-14.4±0.3	0.5±0.02	-14.8±0.1

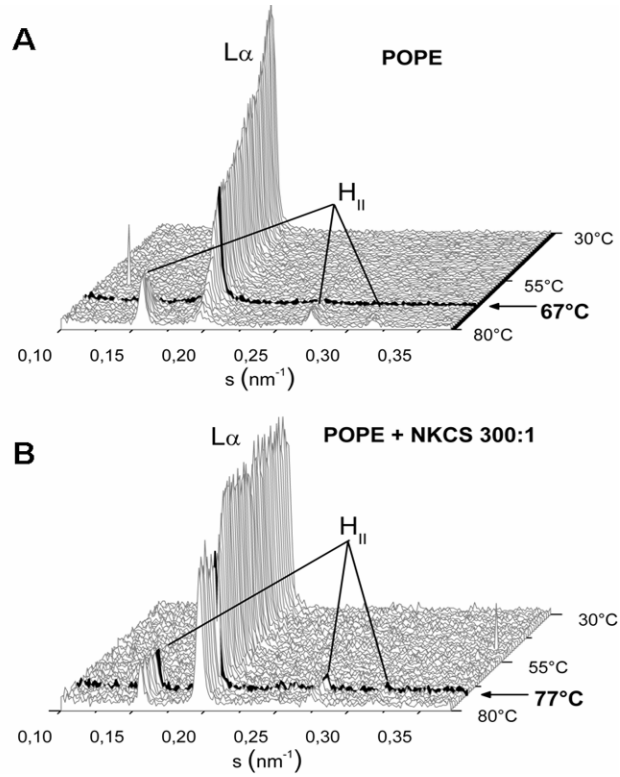

Figure S1. The influence of the peptide NKCS on the POPE phase transition temperature (10 mM sodium phosphate buffer pH 7.4). SAXS pattern for POPE (A) and POPE + NKCS (300:1) (B). The phases are indicated and the onset of the HII phase is highlighted.

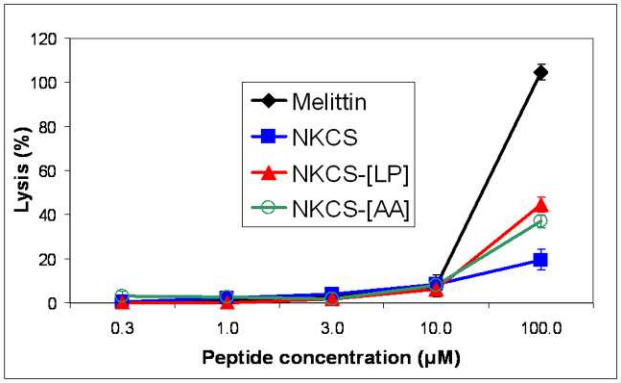

Figure S2. Hemolytic activity of the peptides in comparison to melittin.

References

(1) Shental-Bechor, D.; Haliloglu, T.; Ben-Tal, N. *Biophys J* **2007**, *93*, 1858-71.
(2) Kessel, A.; Shental-Bechor, D.; Haliloglu, T.; Ben-Tal, N. *Biophys J* **2003**, *85*, 3431-44.
(3) Bahar, I.; Jernigan, R. L. *Fold Des* **1996**, *1*, 357-70.
(4) Bahar, I.; Kaplan, M.; Jernigan, R. L. *Proteins* **1997**, *29*, 292-308.
(5) Willumeit, R.; Kumpugdee, M.; Funari, S. S.; Lohner, K.; Navas, B. P.; Brandenburg, K.; Linser, S.; Andra, J. *Biochim Biophys Acta* **2005**, *1669*, 125-34.
(6) Shental-Bechor, D.; Kirca, S.; Ben-Tal, N.; Haliloglu, T. *Biophys J* **2005**, *88*, 2391-402.