Electronic Supplementary Information

Impact of Antimicrobial Peptides on *E. coli*-mimicking Lipid Model Membranes: correlating structural and dynamic effects using scattering methods

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Experimental section:

Differential Scanning Calorimetry (DSC)

Thermal analysis was performed using the TA Instruments "nano-DSC" instrument for solutions, which allows detection of heat flows on a µJ/s scale. The heating rate was 2°C/min and samples were scanned from 10 to 70°C. The Tris buffer was measured separately using the same settings and the buffer curve was subtracted from the thermograms using the NanoAnalyze software. The measured power was converted to specific heat capacity C_p in kJ/mol/K. The enthalpy values were obtained by integration of the area under the phase transition peak.

Scattering model used to analyse small angle X-ray scattering (SAXS) data:

From fit analysis of SAXS data we can extract detailed information on the structure of the membrane in large unilamellar vesicles (LUVs).[1-4] The significant difference in electron density (ED) between the head- and tail-groups of the lipid and water provides a significant sensitivity to changes in the contrast in X-ray scattering. It has previously been shown[5, 6] that the coherent scattering from LUVs, where the size of the vesicles and the thickness of the bilayer are well separated, can be described by the separated form factor (SFF) approximation:

$$I_{lip}(Q) = n \cdot S(Q)\left|F_{TS}(Q)\right|^2\left|F_{FB}(Q)\right|^2 \quad (1)$$

where n is the number of scatterers, defined as

$$n = \frac{\phi}{V_{lipid} \cdot P_{agg}} \quad (2)$$

with ϕ being the volume fraction and V_{lipid} the total volume of a phospholipid given by $V_{lipid} = M_{lipid}/(N_A \cdot d_{lipid})$. N_A is Avogadro's number, M_{lipid} is the molecular weight and d_{lipid} is the density. P_{agg} is the number of phospholipids in each lipid vesicle, i.e. the aggregation number of the vesicle given by

$$P_{agg} = \frac{4\pi\left(R_{shell}\right)^3 - 4\pi\left(R_{shell} - t_{shell}\right)^3}{3V_{tail}} \quad (3)$$

where R_{shell} is the outer radius of the vesicles, t_{shell} is the thickness of the bilayer and V_{tail} is the volume occupied by each double tail of the phospholipid.

S(Q) is the structure factor accounting for interaction between particles (in our case $S(Q) = 1$ because all liposome samples are sufficiently diluted), $F_{TS}(Q)$ is the form factor of an infinitely thin spherical shell (containing information on the radius of the lipid vesicles and the polydispersity), and $F_{FB}(Q)$ is the form factor of a flat bilayer sheet (containing information on the bilayer thickness and the distribution of the phospholipids segments across the bilayer).

The flat bilayer form factor can be expressed[7] as

$$|F_{FB}(Q)| = \int_{-D_i}^{D_0} \Delta\rho(z) e^{iQz} dz = \sqrt{(F_{cos}^{\ 2} + F_{sin}^{\ 2})} \quad (4)$$

where $\Delta\rho$ is the difference in the scattering length densities (SLDs) of the membrane and the solvent, and $F_{cos}^{\ 2}$ and $F_{sin}^{\ 2}$ are the real and the imaginary parts of $F_{FB}(Q)$.[4] The integral extends over the full bilayer thickness from the inner distance D_i to the outer distance D_o.

Following Kučerka and co-workers[4], we parse the phospholipids into the following segments: hydrocarbon tail group (HC), carbonyl+ glycerol (CG) (common for all three phospholipid) and outer part of head group (PC/G).

The volume probability distributions of the components are described by Gaussian functions[3]

$$P_n(z) = \frac{c_n}{\sqrt{2\pi}} \left(exp\left[-\frac{(z + z_n)^2}{2\sigma_n^2} \right] + exp\left[-\frac{(z - z_n)^2}{2\sigma_n^2} \right] \right) \quad (5)$$

where σ_n and z_n are the width and position of the distribution, respectively, and $c_n = V_n/(A_L\sigma_n)$. V_n is the volume of the group n and A_L is the area per lipid, which is equal to the integrated area under the curve.

The hydrocarbon groups (HC) are modelled using a half period squared sine/cosine function to account for the asymmetry in the bilayer, e.g. potential differences in the segmental distribution of the inner and the outer HC group [1]

$$P_{HC}(z) = \begin{cases} sin\left(\frac{z - z_{MN_i} + \sigma_{MN_i}\pi}{2\sigma_{MN_i}\ \ \ 2} \right)^2 \\ for\ z_{MN_i} - \sigma_{MN_i} \le z < z_{MN_i} + \sigma_{MN_i} \\ 1\ for\ z_{MN_i} + \sigma_{MN_i} \le z < z_{MN_o} - \sigma_{MN_o} \\ cos\left(\frac{z - z_{MN_o} + \sigma_{MN_o}\pi}{2\sigma_{MN_o}\ \ \ 2} \right)^2 \\ for\ z_{MN_o} - \sigma_{MN_o} \le z < z_{MN_o} + \sigma_{MN_o} \end{cases} \quad (6)$$

where $z_{MN_{i,o}}$ is the 0.5-probability value for the HC group and $2\sigma_{MN_{i,o}}$is the width of the squared sine/cosine functions. The volume probability distribution of the methylene groups (CH_2) can be expressed separately as

$$P_{CH_2}(z) = P_{HC}(z) - P_{CH_3}(z) \quad (7)$$

These expressions for the distributions of the lipid tails comply with spatial conservation consideration[1, 3] as the height of the expression for $P_{HC}(z)$ is equal to one in the central hydrocarbon region as there is no water present in this region of the membrane.

The volume probability distribution of the water is chosen to be the last group and the spatial conservation requirement is applied to give

$$P_w(z) = 1 - \sum_n P_n(z) \quad (8)$$

where $n = CH_3^{i,o}, CH_2^{i,o}, CG^{i,o}, HG^{i,o}$.

The total volumes of the head group and hydrocarbon chain, as well as the area per lipid, were constrained according to values from reported molecular dynamics simulation of DMPC[8], DMPG[9] and DMPE[10] phospholipids.

Because a small amount of PEGylated DMPE lipids was used to stabilize the lipid vesicles against aggregation, the scattering from the PEG chains was included in the fit model for SAXS/SANS data. The PEG chains on the inner and outer leaflet of the lipid bilayer have a Gaussian random coil confirmation and can therefore be described by the analytical model, previously described by Arleth et al.[11] See Nielsen et al. for details on how the PEG contribution, $I_{PEG}(Q)$ is included to the SDP model.[12]

To be able to use the analytical scattering models to quantitatively describe the interaction between antimicrobial peptides and lipid vesicles, the peptide was introduced as an additional pseudo-parsing group across the bilayer and modelled as an additional Gaussian function in the volume probability (Eq. 5) as formerly published by Nielsen et al.[12, 13] The integral under the curve was scaled by the total volume fraction of added peptides and the fraction of peptide bound to the liposomes, f_{bp}, in the following way

$$c_{pep} = \frac{V_p \cdot f_p \cdot f_{bp}}{A_L \cdot \sigma_{peptide}} \quad (9)$$

Further, to account for the changes in contrast as a result of the peptide potentially integrating into either the head-region, tail-region of the phospholipids or somewhere in the interface between the two areas of the bilayer, the difference in contrast is weighed by a fraction, f_{p_tail}, which gives the fraction of peptide in the tail region

$$\Delta\rho_p(z) = f_{p\_tail} \cdot \left(\rho_p - \rho_{CH_2}\right) + (1 - f_{p_{tail}}) \cdot (\rho_p - \rho_w) \quad (10)$$

where $\rho(p)$, $\rho(CH_2)$ and $\rho(w)$ are the SLDs of the peptide, methylene groups, and water, respectively.

The f_{p_tail} is expressed as the integral of the overlap of the peptide Gaussian function with the half period squared sine/cosine function expressing the volume probability of the HC groups in the following way

$$f_{p\_tail} = \frac{\int_{z_{inter}}^{z_{CH_2}+\sigma_{CH_2}} P_{HC}dz + \int_{Z_p-5\sigma_{pepetide}}^{z_{inter}} P_p dz}{\int P_p dz} \quad (11)$$

where z_{inter} is the intersect between the two overlapping curves found numerically by the Brent-Dekker method[14] and P_{HC} is the function described in Eq. 6. P_p is the Gaussian function expressing the volume distribution of the peptide (details in reference[12])

The form factor for the flat bilayer including the peptides is

$$\left|F_{FB_{pep}}(Q)\right| = \int_{-D_i}^{D_0} \Delta\rho(z)e^{iQz}dz = \sqrt{\left((F_{\cos,lipid} + F_{\cos,peptid})^2 + (F_{\sin,lipid} + F_{\sin,peptid})^2\right)} \quad (12)$$

where

$$F_{cos,peptide} = \left| c_{pep}\sigma_{peptide}\Delta\rho_p \cos\left(Qz_{peptide}\right) \cdot \exp\left[\frac{\left(Q\sigma_{ppeptide}\right)^2}{2}\right]\right| \quad (13)$$

and

$$F_{sin,peptide} = \left| c_{pep}\sigma_{peptide}\Delta\rho_p \sin\left(Qz_{ppeptide}\right) \cdot \exp\left[\frac{\left(Q\sigma_{peptide}\right)^2}{2}\right]\right| \quad (14)$$

To account for potential free peptide chains not bound to the lipid vesicles, an additional term was added to the model

$$I_{fp}(Q) = \varphi \cdot (1 - f_{bp}) \cdot \Delta\rho_p^2 \cdot V_p \cdot F_{chain}(Q) \quad (15)$$

Where φ is the total volume fraction and $F(q)_{chain}$ is the form factor of a Gaussian chain expressed by the Debye formula.[15]

To account for formation of mixed peptide-lipid micelles due to solubilisation of the vesicles upon peptide addition the model was modified to include a fraction of micelle scattering.[13]

$$I_{micelle}(Q) = \frac{n_{micelle}}{P_{agg\_micelle}} F_{micelle}(Q) \quad (16)$$

where $n_{micelle}$ is defined as

$$n_{micelle} = (Mconc_{lipid} \cdot f_{micelle} + Mconc_{peptide} \cdot f_{P_{micelle}})/V_{micelle} \quad (17)$$

Where $Mconc_{lipid}$ and $Mconc_{peptide}$ is the total molar concentration of lipids and peptides respectively, and $f_{micelle}$ and $f_{P_{micelle}}$ is the fraction of the lipids and peptides incorporated in the micelles respectively, and $V_{micelle} = \frac{4}{3}\pi(r_{micelle} + D)^3$ where $r_{micelle}$ is the core radius and D is the thickness of the shell..

$P_{agg_{micelle}} = V_{core}/\left(f_{PL} f_{core} V_p + V_{tail} \cdot (1 - f_{PL})\right)$ is the aggregation number per micelle scaled by f_{PL} which is the ratio of peptide to lipid in the micelles, and f_{core} is the fraction of peptide chain incorporated in the core, where $V_{core} = \frac{4}{3}\pi(r_{micelle})^3$ and $F_{micelle}(Q)$ is the form factor for spherical core-shell micelle with defined as:

$$P_{micelle}(Q) = \int_0^{\pi/2} \left[\Delta\rho_{shell} V_{micelle} A_{sphere}(Q r_{micelle}) + (\Delta\rho_{core} - \right. \quad (18)$$

where $\Delta\rho_{shell}$ is the difference in the SLDs of the shell and the solvent, and $\Delta\rho_{core}$ is the difference in the SLDs of the core and the solvent, $A_{sphere}(x) = 3[\sin(x) - x\cos(x)]/x^3$. $\Delta\rho_{shell}$ and $\Delta\rho_{core}$ were determined from a weighted average of the peptide and lipids using a fitting parameter describing the fraction of the peptide in the core, f_{core}, as such:

$$\Delta\rho_{shell} = \frac{Z_{lipidhead} + Z_{peptide} \cdot (1 - f_{core})}{f_{PL} \cdot (1 - f_{core}) \cdot V_p + V_{head} \cdot (1 - f_{PL})} \quad (19)$$

$$\Delta\rho_{core} = \frac{Z_{lipidtail} + Z_{peptide} \cdot f_{core}}{f_{PL} \cdot f_{core} \cdot V_p + V_{tail} \cdot (1 - f_{PL})} \quad (20)$$

where Z_i is the number of electrons in the group i.

The full expression for the intensity, including peptide in the bilayer, the PEGylation, the free peptide chains and mixed micelles is then

$$I = n\left(F_{TS}(Q)^2 F_{FB_{pep}}(Q)^2 + I_{PEG}(Q)\right) + I_{fp}(Q) + I_{micelle}(Q) \quad (21)$$

In the fit analysis, we allowed the concentration to vary slightly due to uncertainties in the determination of the exact value during the sample preparation.

Results:

Table S1. Fit results from SAXS data on DMPE (75%), DMPG (22.5%), DMPE-PEG (2.5%) liposomes with and without addition of indicated peptide at different ratios. All data measured at 37°C.

	Fraction of peptide	Bilayer thickness [Å]	Volume headgroup	Volume CH_2	$Z_{peptide}$ [Å]	$\sigma_{peptide}$ [Å]	$f_{micelles}$	Ratio P/L in micelles	f_{bp}	σ_{SD}
Liposomes	-	45	256	24.6	-	-	-	-	1	0.36
Aurein 1.2	1:20	44	255	25.0	0	10	0.48	0.05	1	0.38
	1:50	44	257	24.7	-1	10	0.05	0.01	1	0.36
	1:100	46	258	24.6	0	10	-	-	1	0.36
Indolicidin	1:20	45	281	24.5	14	5	0.04	0.05	1	0.36
	1:50	45	273	24.6	24	6	0.02	0.02	1	0.36
	1:100	45	266	24.6	23	6	-	-	1	0.36
LL-37	1:20	47	272	25.3	5	11	0.09	0.05	1	0.43
	1:50	46	262	25.0	18	13	0.04	0.02	1	0.38
	1:100	46	260	24.8	25	10	0.01	0.01	1	0.38
Lacticin Q	1:20	-	-	-	-	-	-	-	1	-
	1:50	45	280	25.2	9	7	-	-	1	0.36
	1:100	45	272	25.0	11	7	-	-	1	0.36
Colistin	1:10	45	256	24.6	-	-	-	-	0	0.36
	1:20	45	256	24.6	-	-	-	-	0	0.36
	1:50	45	256	24.6	-	-	-	-	0	0.36
	1:100	45	256	24.6	-	-	-	-	0	0.36

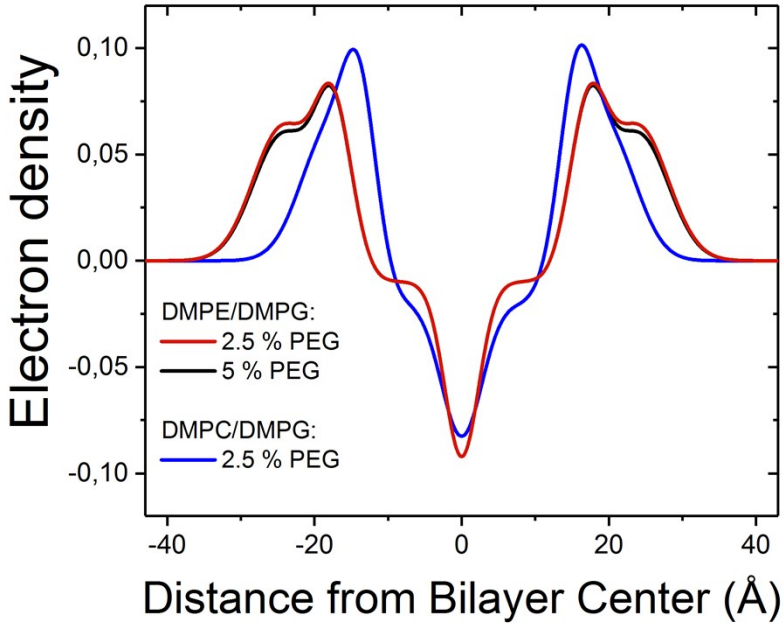

Figure S1. Electron density profiles calculated from the fit parameters of SAXS data on DMPE/DMPG lipid vesicles with 2.5 and 5% PEG and DMPC/DMPG vesicles with 2.5% PEG.

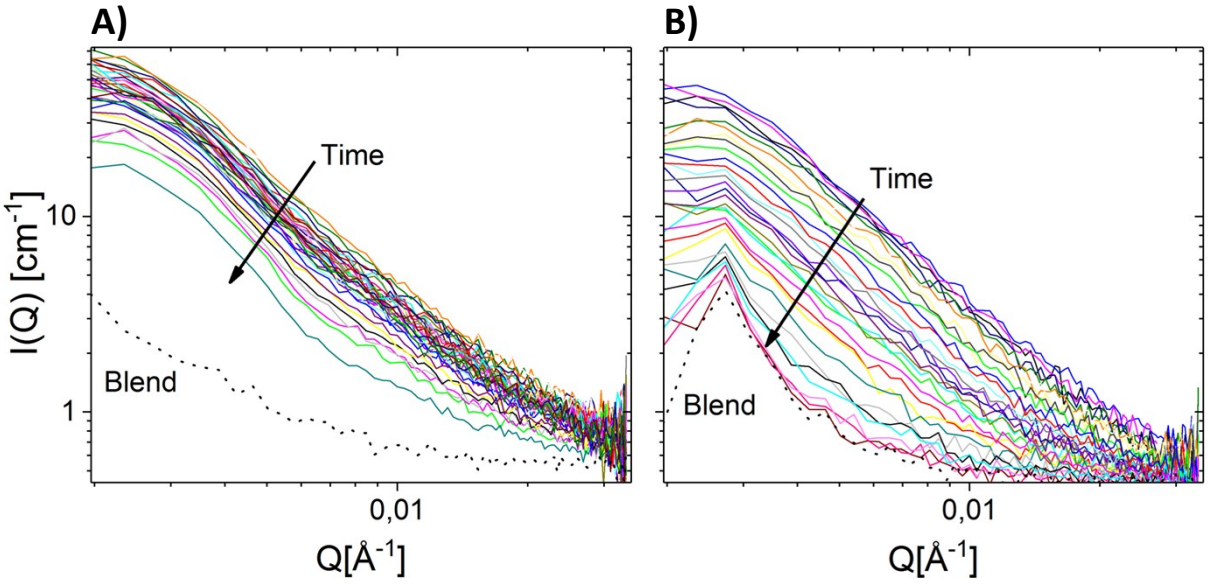

Figure S2. TR-SANS data on pure DMPE/DMPG liposomes (A) and DMPE/DMPG liposomes with Indolicidin (B) showing the decrease in scattering intensity over time due to lipid exchange and flip-flop.

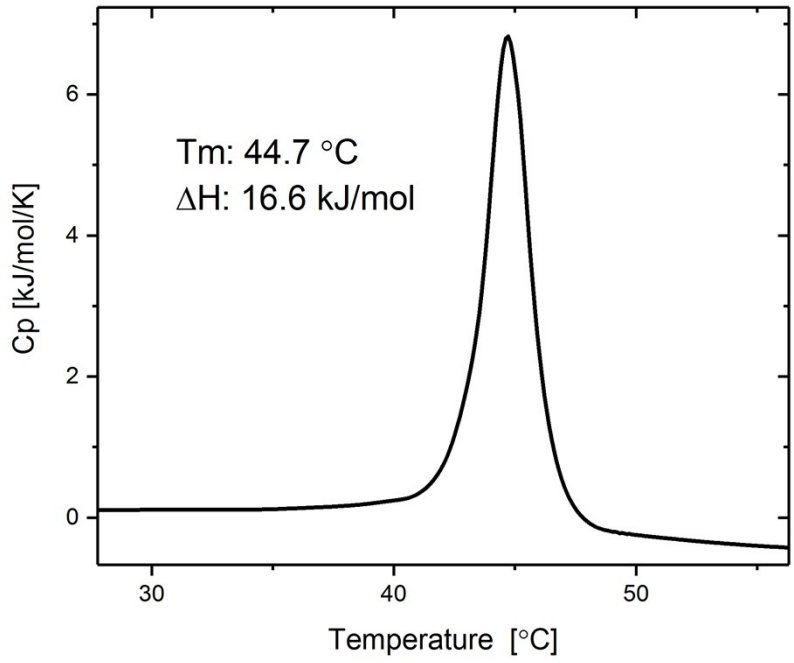

Figure S3. Nano-DSC data on DMPE (75%), DMPG (22.5%), DMPE-PEG (2.5%) liposomes showing the phase transition of the lipid bilayer.

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