# Supplementary Information

# A combined pulse EPR and Monte Carlo simulation study provides molecular insight on peptide-membrane interactions

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## Experimental methods

*Materials for peptide synthesis*

The Rink amide MBHA resin and 9-fluorenylmethoxycarbonyl (Fmoc) amino acids for peptide synthesis were purchased from Calibochem-Novabiochem AG. Other reagents used for the synthesis included trifluoroacetic acid (TFA, Sigma), N,N-diisopropylethylamine (DIEA, Aldrich), methylene chloride (peptide synthesis grade, Biolab), dimethylformamide (peptide synthesis grade, Biolab), and benzotriazolyl-noxytris(dimethylamino) phosphonium hexafluorophosphate (BOP, Sigma).

*Peptide synthesis and purification*

Peptides were synthesized by a solid phase method on rink amide MBHA resin (0.68 mequiv) by using an ABI 433A automatic peptide synthesizer. The resin bound peptides were cleaved from the resins by a mixture composed of 95% trifluoroacetic acid (TFA, Biolab),1.25% triethylsilane (Tis, Fluka), 1.25% thioanisole (TA, Aldrich) and 2.5% ethandithiol (EDT, Aldrich), washed with dry ether (Biolab), and extracted with a mixture of 30% acetonitrile (Biolab) and 0.1% TFA, both in water. Peptide disulfide bonds were reduced with 5mM tris (2-carboxyethyl) phosphine Hydrochloride (TCEP·HCl tris, calbiochem) before purification. The peptides were purified by reversed phase (RP)-HPLC on a C18 reverse phase Vydac

analytical column (250 x 4.6mm, 300 Å pore size, 5 μm particle size). The column was eluted in 50 min, using a linear gradient of 20-70% acetonitrile in 0.1% TFA (v/v), at a flow rate of 0.6 ml/min. The purified peptides were shown to be homogeneous (>97%, by weight), by the analytical HPLC. The peptides were analyzed by electrospray mass spectroscopy to confirm their composition and molecular weight. The crude peptides were lyophilized and were stored in a freezer at -20ºC.

*Peptide spin labeling and purification*

The peptides were labeled with (MTSL) through attachment to the cystein SH group as reported earlier.[1] A tenfold molar excess of spin probe was added to the reaction buffer (0.1M phosphate buffer (PH 7.2) and 0.1M NaCl). The suspension was shaken for 12 hr, at room temperature. Excess spin label and unlabeled peptides were separated from the labeled peptide by RF-HPLC with the same analytical column that was used for the unlabeled peptide. Labeled peptides were analyzed using electrospray mass spectroscopy. The peptide solution was subdivided into aliquots that were lyophilized and were stored at -20ºC. The purified labeled peptides were shown to be homogeneous by analytical HPLC (>97%, by weight). Further purification was performed in case of unlabeled spin probes were detected by CW-EPR.

*DEER measurements*

The constant time four-pulse DEER experiment, $\pi/2\ (\nu_{obs})-\tau_1-\pi(\nu_{obs})-t-\pi(\nu_{pump})-(\tau_1+\tau_2-t)-\pi(\nu_{obs})-\tau_2-$echo was employed with a +x/-x phase cycle on the first pulse and averaging over 25 increments of $\tau_1$ ($\tau_1$ = 400 ns, $\Delta\tau_1$ = 8 ns) to suppress nuclear modulations. The echo was measured as a function of t, while $\tau_2$ was kept constant. The pump frequency, $\nu_{pump}$, was set to the center of the resonator bandwidth and the static magnetic field was set to the maximum of the nitroxide spectrum at the pump frequency. The observer frequency, $\nu_{obs}$, was set at 60 MHz higher than $\nu_{pump}$. The length of all mw pulses was 40 ns, and the dwell time was 20 ns. Typical numbers of shots per point and scan number were 30 and 50-600 respectively. Accumulation times for the data sets varied from 12 to 48 hr.

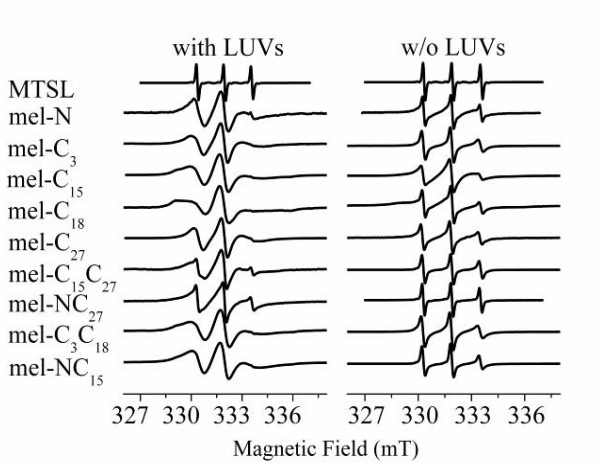



Fig. S1. X-band EPR spectra of melittin that is labeled at different locations along the peptide and of MTSL, in the presence *(left)* and absence *(right)* of vesicles.

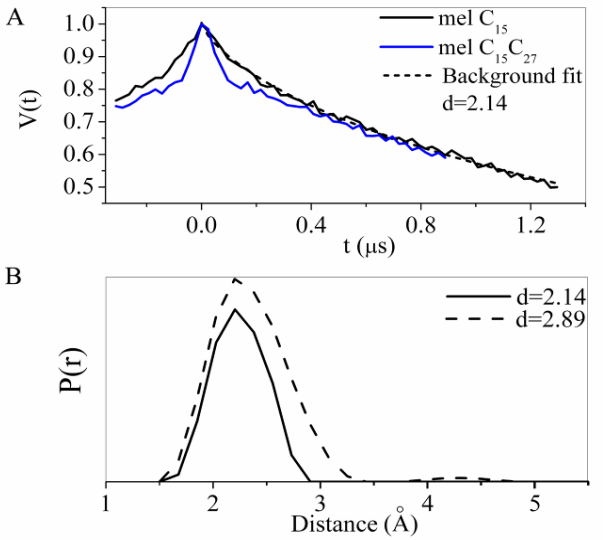



Fig. S2.: *(A)* DEER traces obtained from the singly labeled melittin, mel-$C_{15}$ *(black),* and the doubly labeled melittin, mel-$C_{15}C_{27}$ *(blue)*, both within DPPC/PG/$D_2O$ LUVs. The trace of mel-$C_{15}$ is fitted with an exponential decay with a dimensionality of 2.14. *(B)* Distance distributions P(r) obtained for mel-$C_{15}C_{27}$ after subtraction of background exponential decay with a dimensionality of d=2.14 *(solid curve)* and d=2.89 *(dashed curve).*

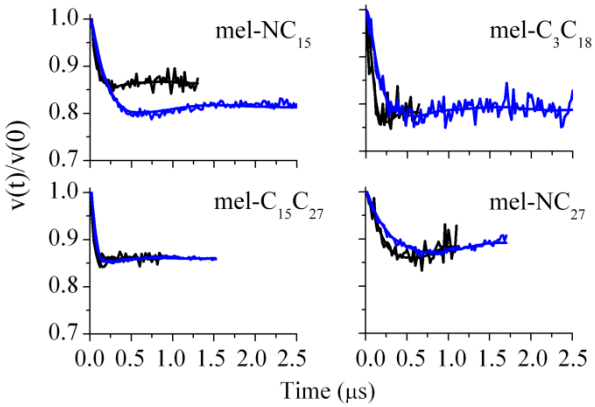



Fig. S3. Normalized DEER decays for the doubly labeled melittin peptides within DPPC/PG/$D_2O$ LUVs *(black)* and in $D_2O$/30% glycerol solution *(blue)* after background correction.

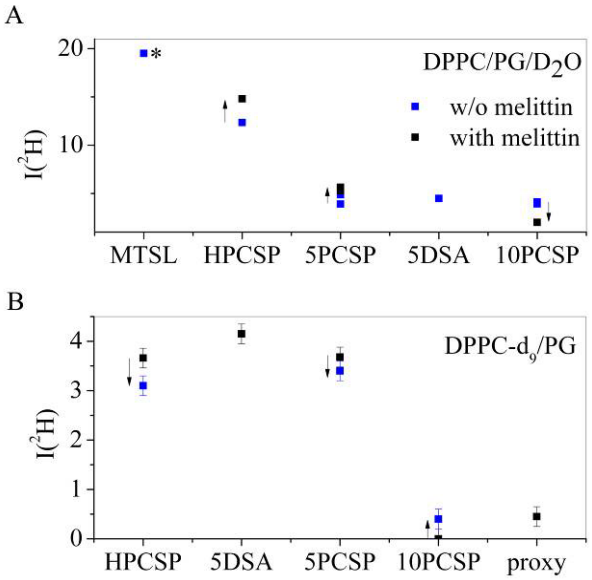



Fig. S4. **:** The I($^2$H) values of the spin probes examined in this work in *(A)* DPPC/PG/$D_2O$ and *(B)* DPPC-$d_9$/PG LUVs, with *(black squares)* and without *(blue squares)* melittin. MTSL in 70% $D_2O$ /30% glycerol (The value for is marked with *). The arrows designate the direction of the change in I($^2$H) value upon melittin addition. The standard errors are marked as bars in "B". The standard errors in "A" were smaller than the symbols.

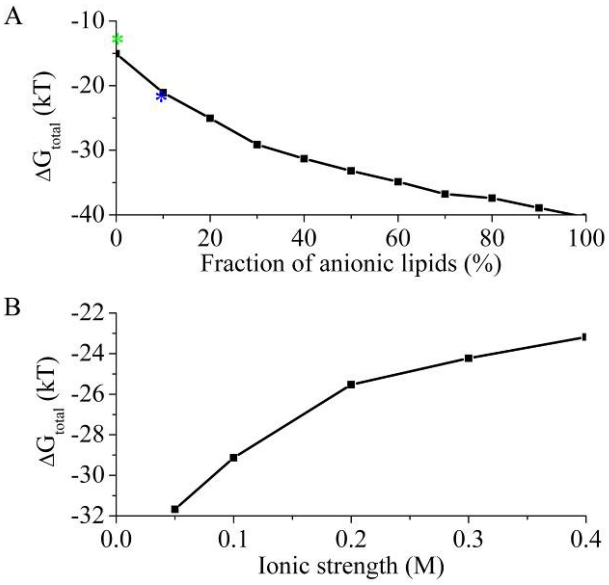



Fig. S5. The calculated free energy of melittin-membrane association as a function of: *(A)* the fraction of anionic lipids (at constant ionic strength of 0.1 M) and *(B)* the ionic strength (at constant acidic lipid fraction of 30%). The green asterisk marks the experimental value in zwitterionic membrane, and the blue asterisk the value calculated based on MD simulation in 10% acidic lipid.[2]

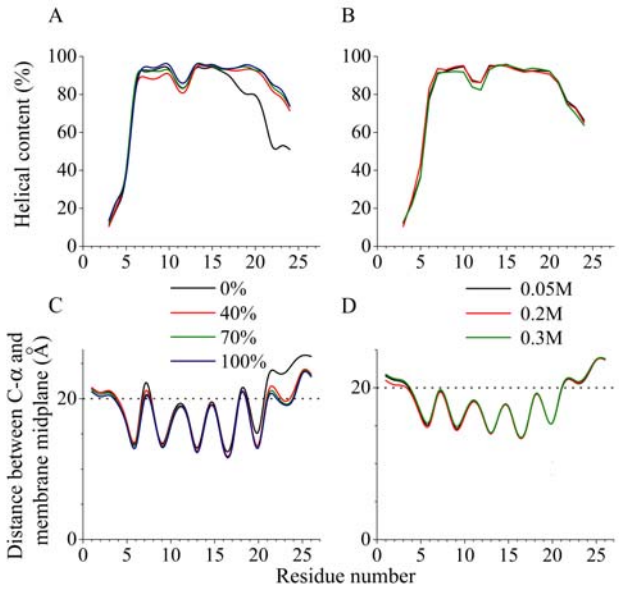



Figure S6. Melittin's helicity and location near the membrane is independent of the ionic strength and acidic lipid fraction. (A) The calculated (average) helicity along melittin's amino acid sequence at constant ionic strength of 0.1 M. Each curve marks the results obtained using different acidic lipid fraction according to the color legend. (C) The average location of the amino acids along the membrane normal at ionic strength of 0.1 M. The fraction of acidic lipids varied between 0-100% using the same color legend. (B) The calculated helicity along

melittin's amino acid sequence for acidic lipid fraction of 30%. The different curves mark the results at different ionic strengths according to the color legend. (D) The average location of the amino acids along the membrane normal at acidic lipid fraction of 30%. The curves mark the results at different ionic strengths according to the color legend.

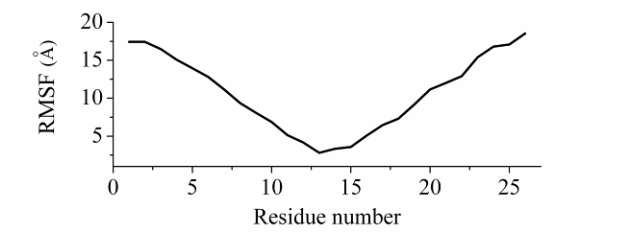
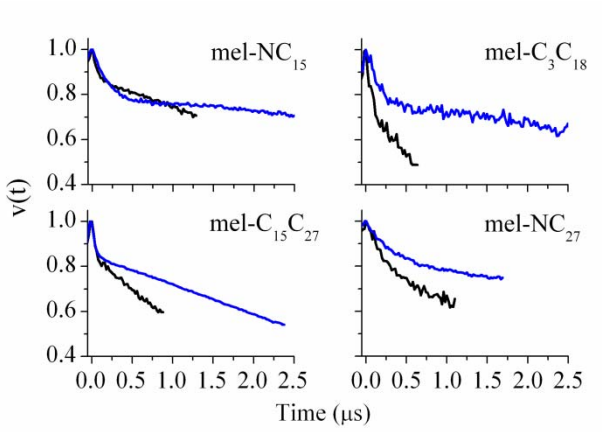



Fig. S7. Calculated RMS fluctuations of the α-carbon atoms of native melittin in association with a membrane composed of 30% anionic lipids at ionic strength of 0.1 M. The termini are significantly more mobile than the center.

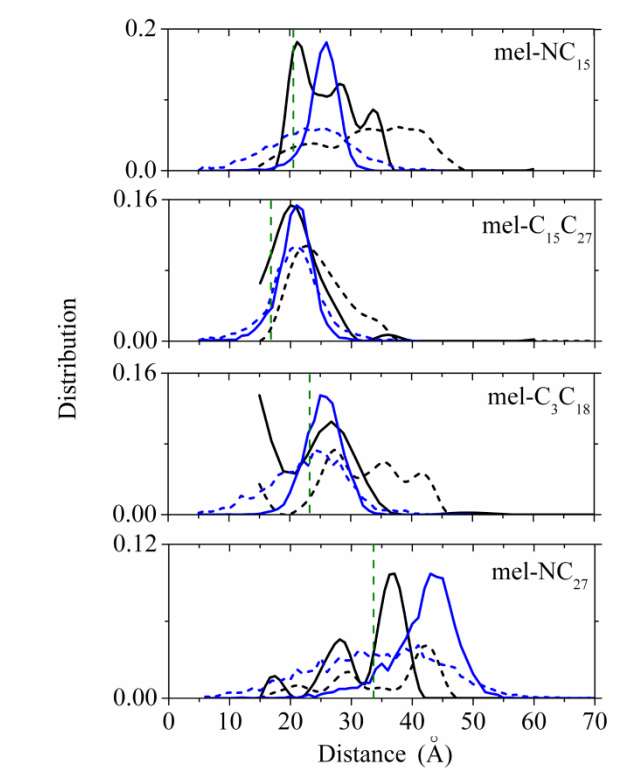
**References**

(1) Hubbell, W. L.; Gross, A.; Langen, R.; Lietzow, M. A. *Curr Opin Struct Biol* **1998**, *8*, 649.

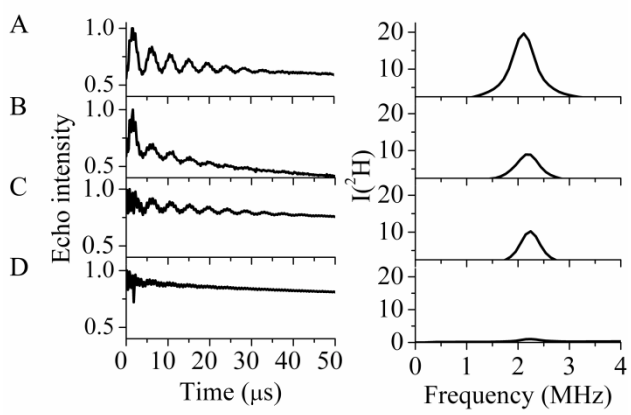
(2) Lazaridis, T. *Proteins* **2005**, *58*, 518.


mel-NC15
mel-C3C18
mel-C15C27
mel-NC27
v(t)
1.0
0.8
0.6
0.4
0.0 0.5 1.0 1.5 2.0 2.5
Time (μs)

F2

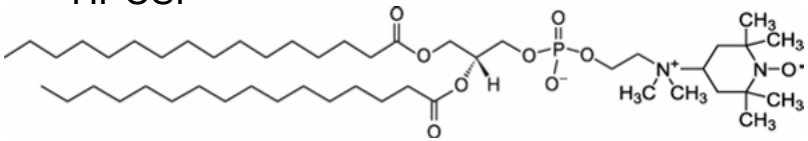

0.2
0.0
mel-NC15
0.16
0.00
mel-C15C27
0.16
0.00
mel-C3C18
0.12
0.00
mel-NC27
Distribution
0
10
20
30
40
50
60
70
Distance (Å)

F3

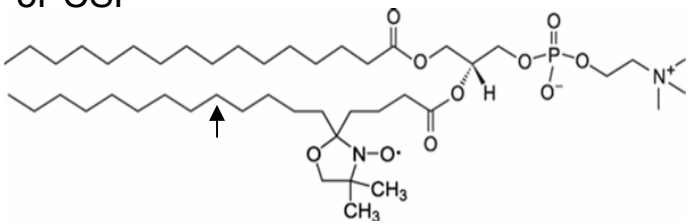

A
B
C
D
Echo intensity
1.0
0.5
0 10 20 30 40 50
Time (μs)
I(²H)
20
10
0
0 1 2 3 4
Frequency (MHz)

F4

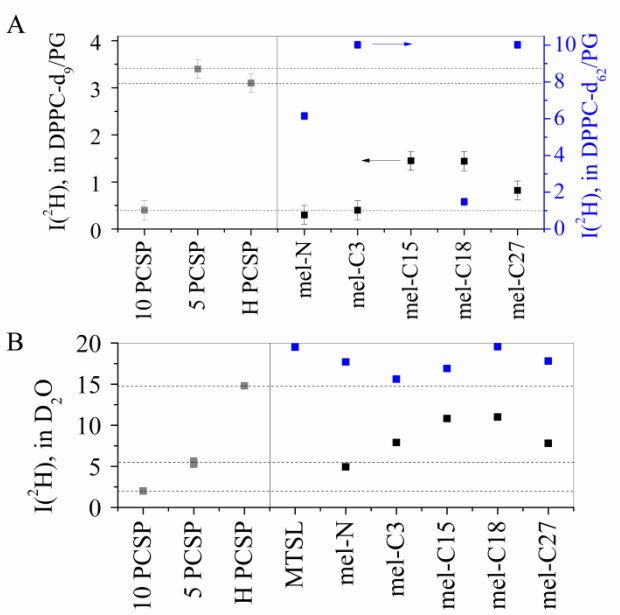
HPCSP



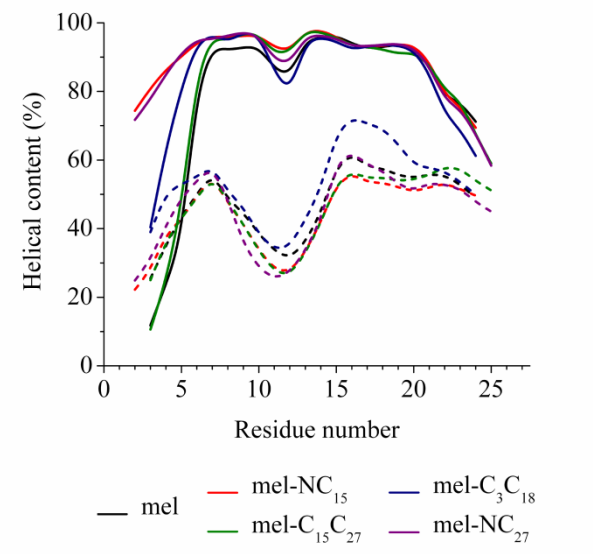
5PCSP



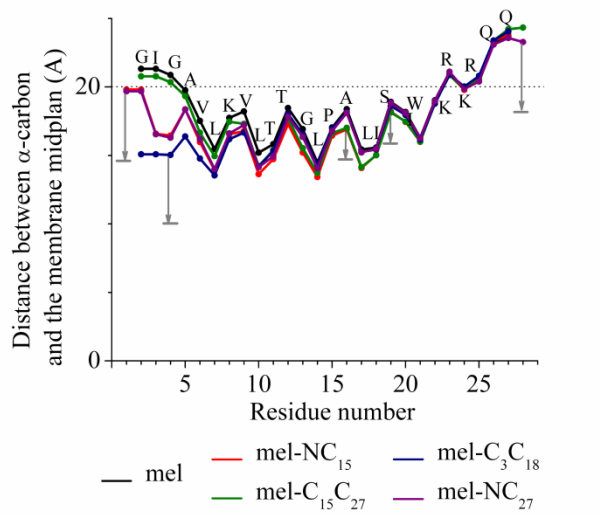
F5


A
I(²H), in DPPC-d9/PG
I(²H), in DPPC-d62/PG
10 PCSP
5 PCSP
H PCSP
mel-N
mel-C3
mel-C15
mel-C18
mel-C27
B
I(²H), in D2O
10 PCSP
5 PCSP
H PCSP
MTSL
mel-N
mel-C3
mel-C15
mel-C18
mel-C27

F6


Helical content (%)
100
80
60
40
20
0
0
5
10
15
20
25
Residue number
mel
mel-NC15
mel-C15C27
mel-C3C18
mel-NC27

F7


Distance between α-carbon and the membrane midplan (A)
20
0
5
10
15
20
25
Residue number
G I G A V K V L T T G L P A L L S W K K R R Q Q
mel
mel-NC15
mel-C15C27
mel-C3C18
mel-NC27

F8