

Supplementary Information

Melittin can permeabilize membranes via large transient pores

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<i>Lipid</i>	<i>N_{peptide}</i>	<i>N_{lipids}</i>	<i>P/L</i>	# peptides in initial position				<i>T</i>	<i>t</i>
				↓ TM1	↑ TM2	S1	S2	[K]	[μs]
DOPC	16	192	1/12	16	-	-	-	333	3.7
	16	192	1/12	16	-	-	-	363	21.8
	16	192	1/12	16	-	-	-	393	27.0
POPC	16	192	1/12	16				393	8.2
DMPC:DMPG 3:1	18	360 (270:90)	1/20	18	-	-	-	363	5.7
DPPC:DPPG 3:1	18	216 (162:54)	1/12	18	-	-	-	393	10.8

Table S1. Summary of the all-atom MD simulation of Alamethicin in symmetric bilayers. *N_{peptide}* = number of peptides; *N_{lipids}* = number of lipids; *P/L* = peptide to lipid ratio; *T* = temperature; *t* = simulation length; TM1 = transmembrane alignment down; TM2 = transmembrane alignment up; S1 = upper leaflet surface aligned; S2 = lower leaflet surface aligned.

<i>Lipid</i>	<i>N_{peptide}</i>	<i>N_{lipids}</i>	<i>P/L</i>	# peptides in initial position				<i>T</i>	<i>t</i>
				↓ TM1	↑ TM2	S1	S2	[K]	[μs]
POPC	16	192	1/12	16	-	-	-	333	2.3
	16	192	1/12	16	-	-	-	363	1.5
	16	192	1/12	16	-	-	-	393	8.3
	18	216	1/12	9	9	-	-	363	1.8
	18	216	1/12	9	9	-	-	393	1.7
DMPC	16	192	1/12	4	4	4	4	343	11.3
	12	288	1/24	-	-	6	6	353	4.0
	14	288	1/21	4	-	10	-	363	4.0
	9	261	1/29	-	-	9	-	333	7.0

Table S2. Summary of the all-atom MD simulations of Melittin in symmetric bilayers. *N_{peptide}* = number of peptides; *N_{lipids}* = number of lipids; *P/L* = peptide to lipid ratio; *T* = temperature; *t* = simulation length; TM1 = transmembrane alignment down; TM2 = transmembrane alignment up; S1 = upper leaflet surface aligned; S2 = lower leaflet surface aligned.

<i>Lipid</i>	<i>T</i>	<i>Sim #</i>	<i>t</i>	<i>t_{open}</i>	<i>t_{close}</i>	<i>dt</i>	<i>ln k</i>	<i>Final peptide position #</i>			
	[K]		[ns]	[ns]	[ns]	[ns]	[ln (1/ns)]	S1	↓ TM1	↑ TM2	S2
288 DMPC P/L = 1/16	313	1	10248	200							
		1	41680	453	41425	40972	-10.6	0	6	12	0
	333	2	25167	15	24666	24651	-10.1	3	3	9	3
		3	12975	54	12925	12871	-9.5	3	7	5	3
		1	9800	63	7100	7037	-8.9	2	6	8	2
	343	2	10163	60	8812	8752	-9.1	3	5	6	4
		3	3751	53	1950	1897	-7.5	9	3	3	3
		1	964	58	600	542	-6.3	10	3	4	1
	353	2	1613	40	1300	1260	-7.1	10	3	3	2
		3	1861	70	1700	1630	-7.4	9	2	2	5
		1	1396	120	685	565	-6.3	10	3	3	2
		2	1238	53	400	347	-5.8	10	4	3	1
	363	3	2438	76	1166	1090	-7.0	5	6	5	2
		4	3455	68	1976	1908	-7.6	8	1	4	5
		1	1292	80	290	210	-5.3	8	3	2	5
	393	2	1279	71	103	32	-3.5	14	0	1	3

Table S3. Summary of the all-atom MD simulations of transient leakage of Melittin in asymmetrically loaded membranes of DMPC. *T* = temperature; *Sim #* = number of simulation; *t* = simulation length; *t_{open}* = time of pore opening; *t_{close}* = time of pore closure; *dt* = lifetime of pore; *ln k* = pore closure rate; S1 = upper leaflet surface aligned; S2 = lower leaflet surface aligned; TM1 = transmembrane alignment (up); TM2 = transmembrane alignment (down).

<i>Lipid</i>	<i>N_{peptide}</i>	<i>N_{lipids}</i>	P/L	<i>T</i>	<i>t</i>	<i>t_{open}</i>	<i>t_{close}</i>	<i>dt</i>	<i>ln k</i>	<i>Final peptide position #</i>			
				[K]	[ns]	[ns]	[ns]	[ns]	[ln (1/ns)]	S1	↓ TM1	↑ TM2	S2
DOPG	16	256	1/16	333	3772	802	2284	1482	-7.3	12	2	2	0
	16	224	1/14	353	5462					16	0	0	0

Table S4. Summary of the all-atom MD simulations of transient leakage of melittin in an anionic membrane (DOPG). *T* = temperature; *t* = simulation length; *t_{open}* = time of pore opening; *t_{close}* = time of pore closure; *dt* = lifetime of pore; *ln k* = pore closure rate; S1 = upper leaflet surface aligned; S2 = lower leaflet surface aligned; TM1 = transmembrane alignment (up); TM2 = transmembrane alignment (down).

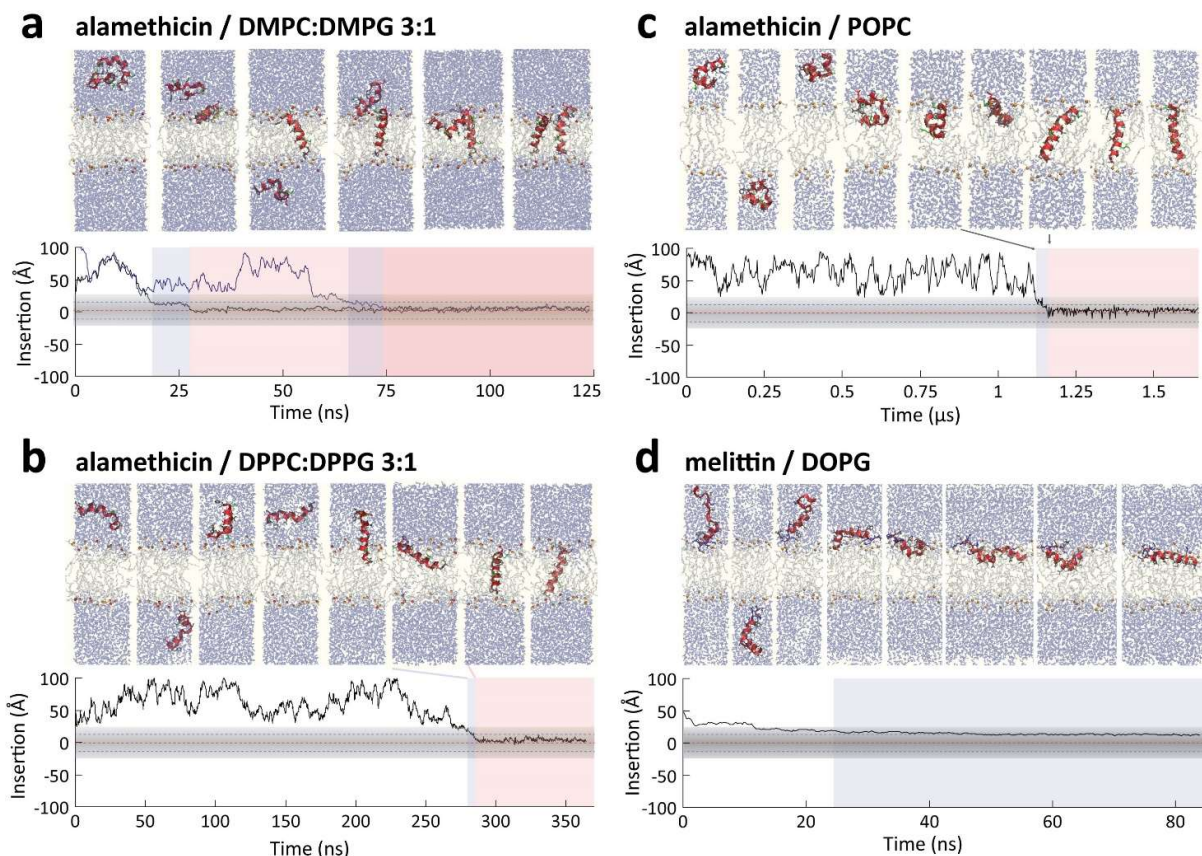


Fig. S1 | Generation of the initial conformation of alamethicin and melittin in various other lipid bilayers. **a-c** A single peptide (in the case of alamethicin in DMPC:DMPG it also works with two peptides) is placed into the water phase. It is then allowed to adsorb to the membrane, first to a surface aligned state (light blue shading). **d** Melittin remains in this interfacial state, which is aligned parallel to the membrane plane, in all simulations. In contrast, all alamethicin peptides subsequently insert transmembrane (light red shading). The membrane is indicated as a grey bar, with blue dotted lines the interfacial center-of-mass location of the peptides, and the red dotted line the membrane center. The final state of these simulations is assembled in a grid to generate the initial conformation for the multi-peptide simulations.

alamethicin / DPPC:DPPG 3:1

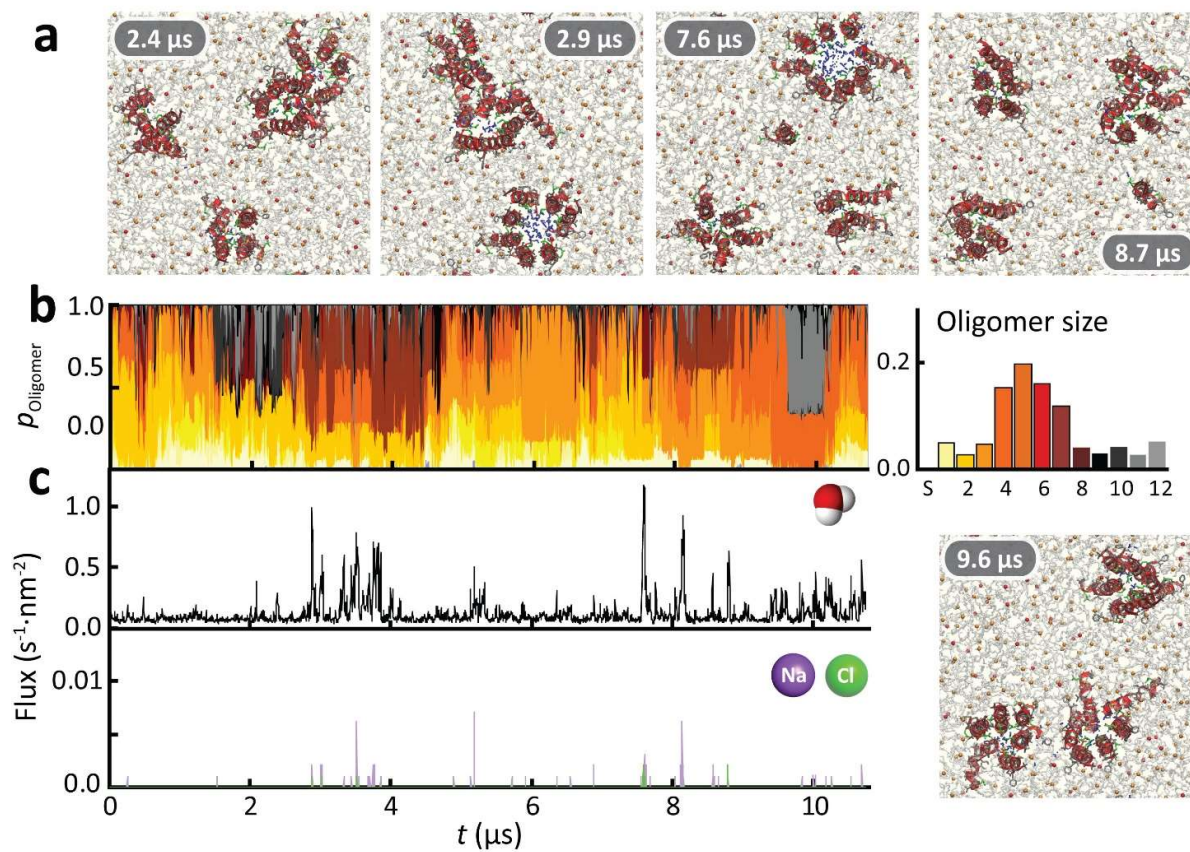


Fig. S2 | Simulation of alamethicin in a DPPC:DPPG 3:1 anionic bilayer. **a** Topview snapshots of the simulation box at various times. **b** Population of oligomers formed throughout the trajectory and overall distribution (S = surface (blue), yellow-black: increasing transmembrane oligomer size). **c** Transmembrane flux of water (top) and ions (bottom) throughout the simulation. Very large oligomers ($N > 8$) are usually smaller channels that have temporarily collided, rather than a large pore.

alamethicin / DMPC:DMPG 3:1

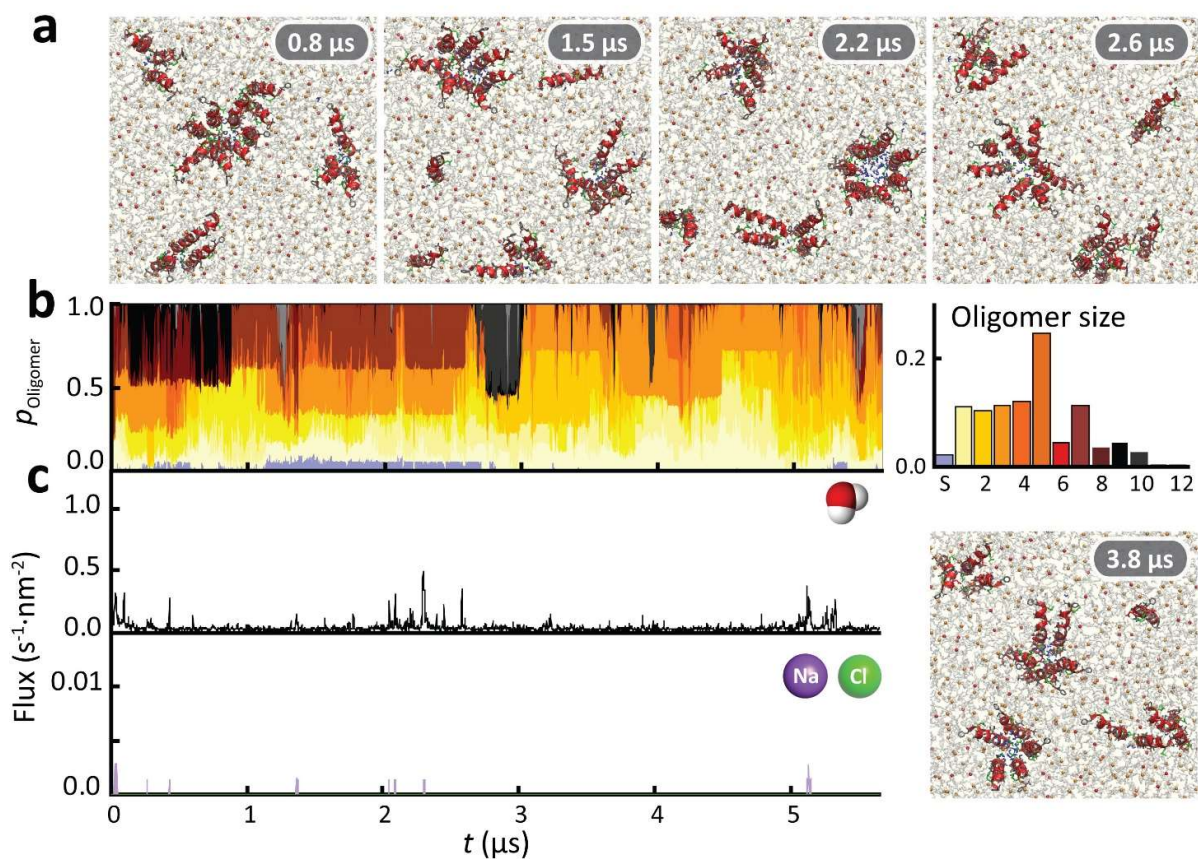


Fig. S3 | Simulation of alamethicin in a DMPC:DMPG 3:1 anionic bilayer. **a** Topview snapshots of the simulation box at various times. **b** Population of oligomers formed throughout the trajectory and overall distribution (S = surface (blue), yellow-black: increasing transmembrane oligomer size). **c** Transmembrane flux of water (top) and ions (bottom) throughout the simulation. Very large oligomers ($N > 8$) are usually smaller channels that have temporarily collided, rather than a large pore.

alamethicin / POPC

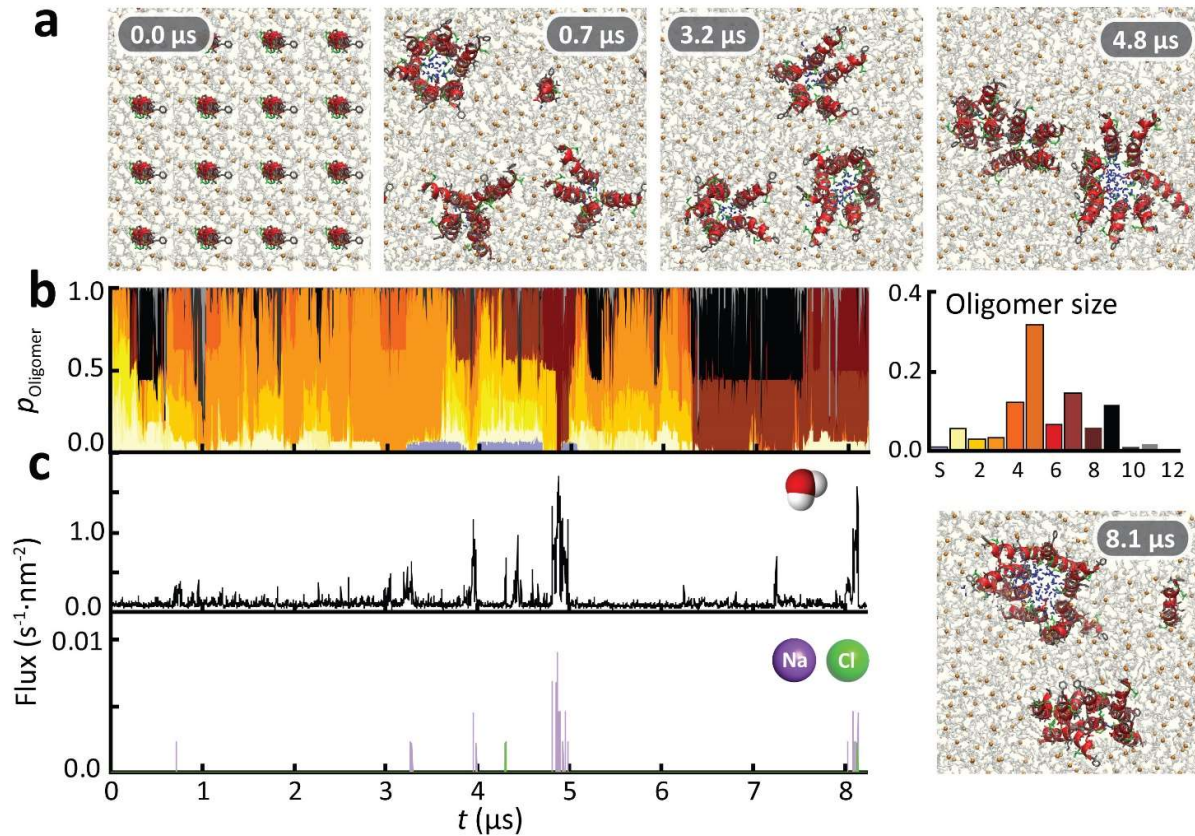


Fig. S4 | Simulation of alamethicin in a POPC bilayer. **a** Topview snapshots of the simulation box at various times. **b** Population of oligomers formed throughout the trajectory and overall distribution (S = surface (blue), yellow-black: increasing transmembrane oligomer size). **c** Transmembrane flux of water (top) and ions (bottom) throughout the simulation. Very large oligomers ($N > 8$) are usually smaller channels that have temporarily collided, rather than a large pore.

alamethicin / DOPC

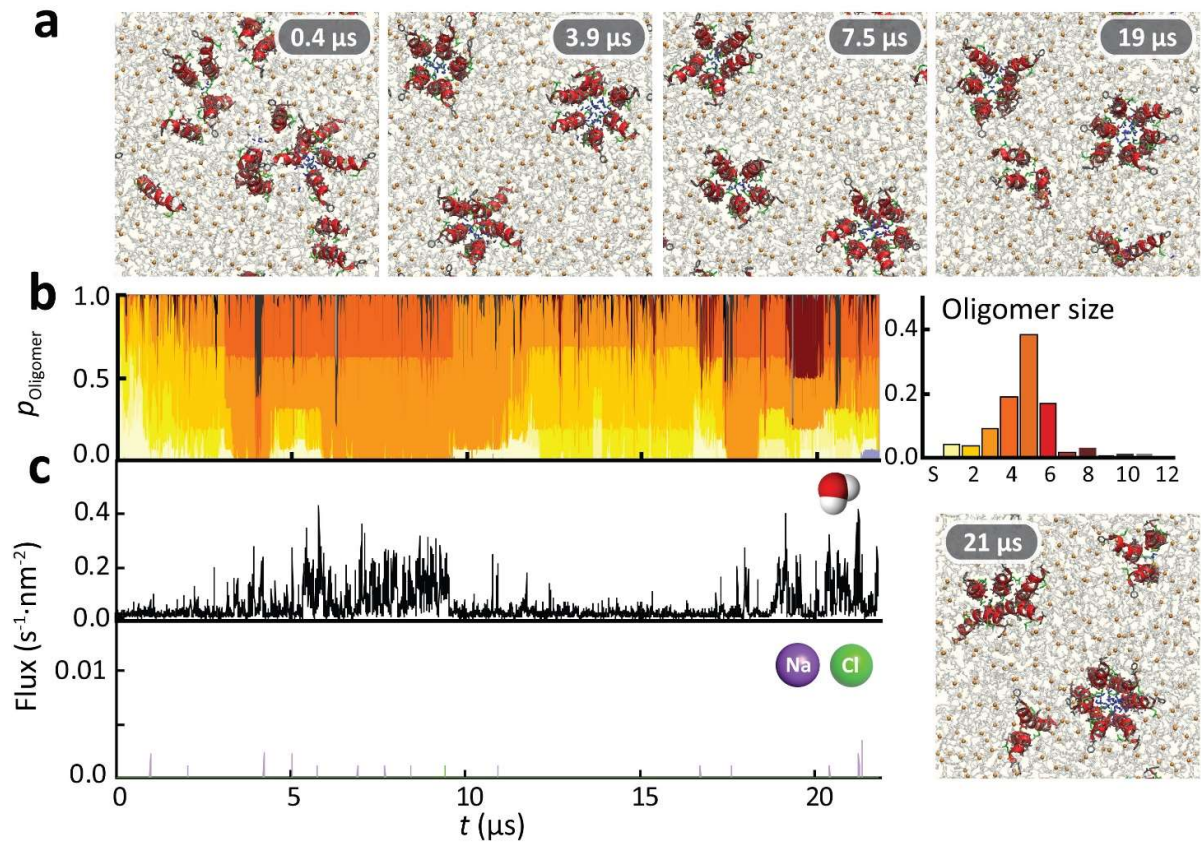


Fig. S5 | Simulation of alamethicin in a DOPC bilayer. **a** Topview snapshots of the simulation box at various times. **b** Population of oligomers formed throughout the trajectory and overall distribution (S = surface (blue), yellow-black: increasing transmembrane oligomer size). **c** Transmembrane flux of water (top) and ions (bottom) throughout the simulation. Very large oligomers ($N > 8$) are usually smaller channels that have temporarily collided, rather than a large pore.

melittin / POPC

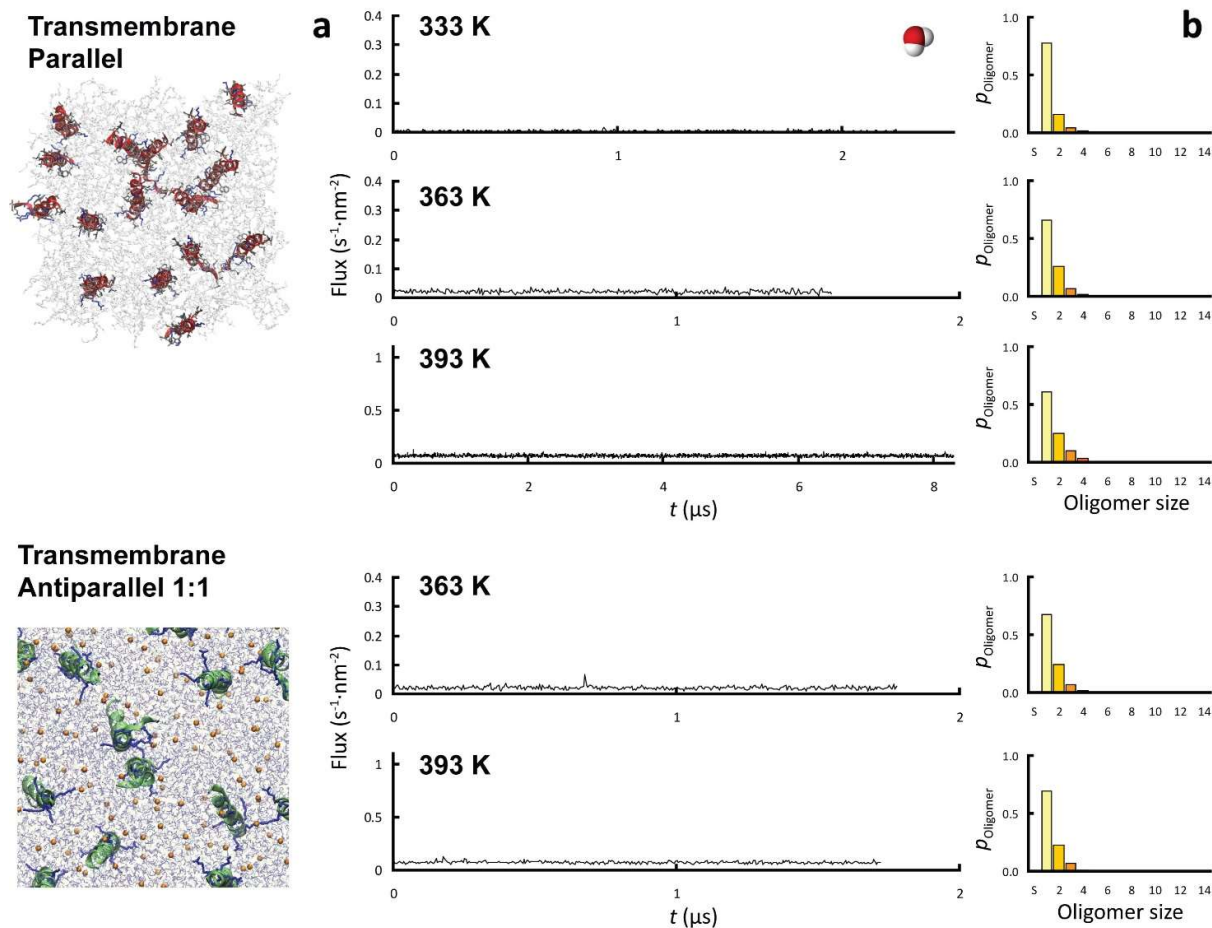


Fig. S6 | Simulation of transmembrane inserted melittin in POPC bilayers. All peptides were initially transmembrane inserted, either all parallel (top) or 1:1 antiparallel (bottom). The peptides do not aggregate in both cases. **a** Transmembrane water flux. No water pores form during the simulations and there is only tiny background leakage due to residual water in the membrane. **b** Distribution of oligomers. Peptides prefer single or occasionally dimeric and trimeric aggregates with no specific interactions and loosely packed contact interface. No higher order pores or oligomers form.

melittin / DOPG

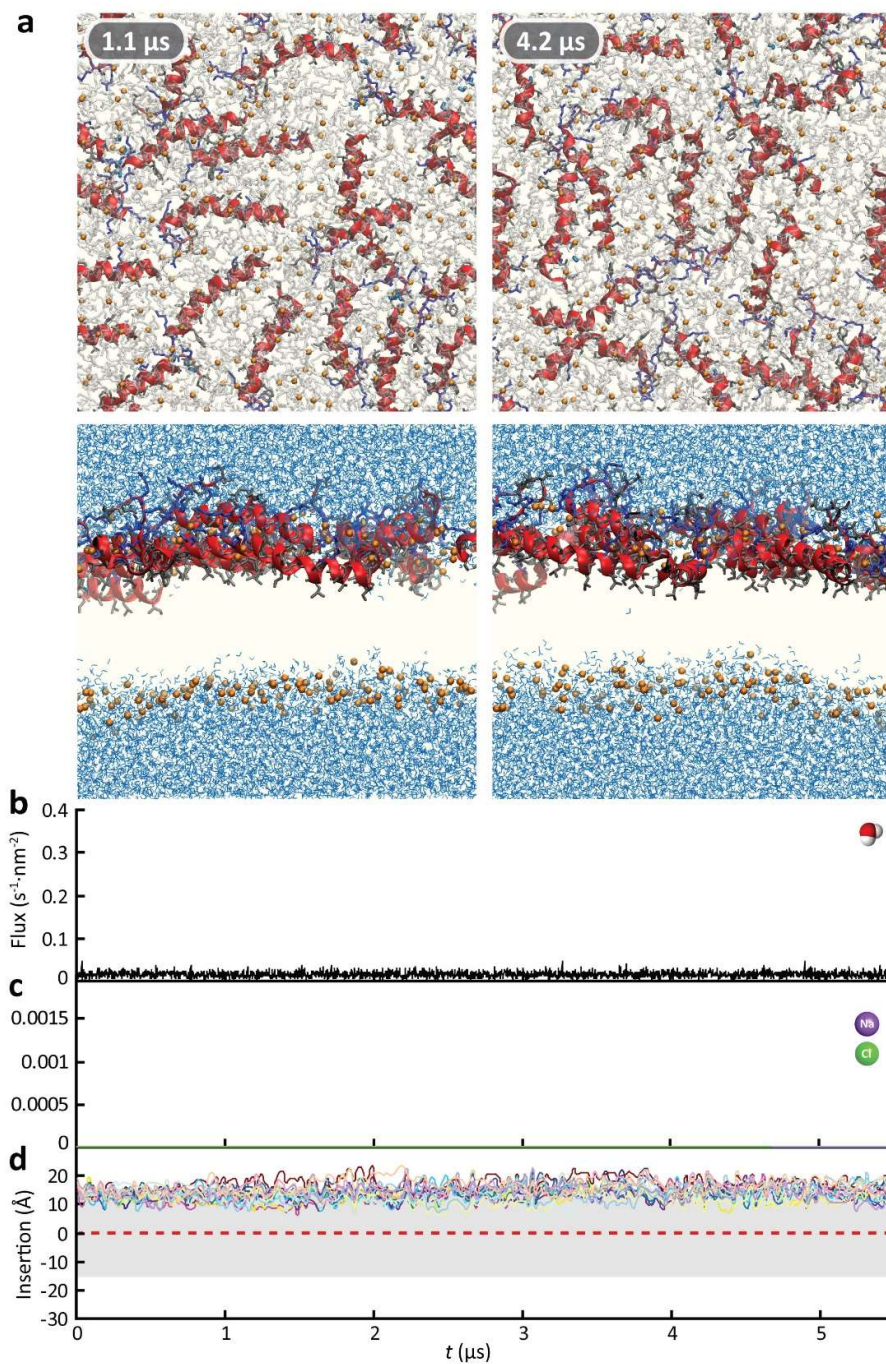


Fig. S7 | Simulation of melittin in a DOPG anionic bilayer. All peptides are initially on the surface in one leaflet, and 2 lipids have been removed per peptide. **a** Snapshots of the simulation. **b** Transmembrane water flux. No pores form, only residual water is observed in the membrane. **c** Transmembrane ion flux. **d** Insertion depth (center of mass) of all peptides (red dotted line = membrane center, grey bar = membrane).

melittin / DOPG

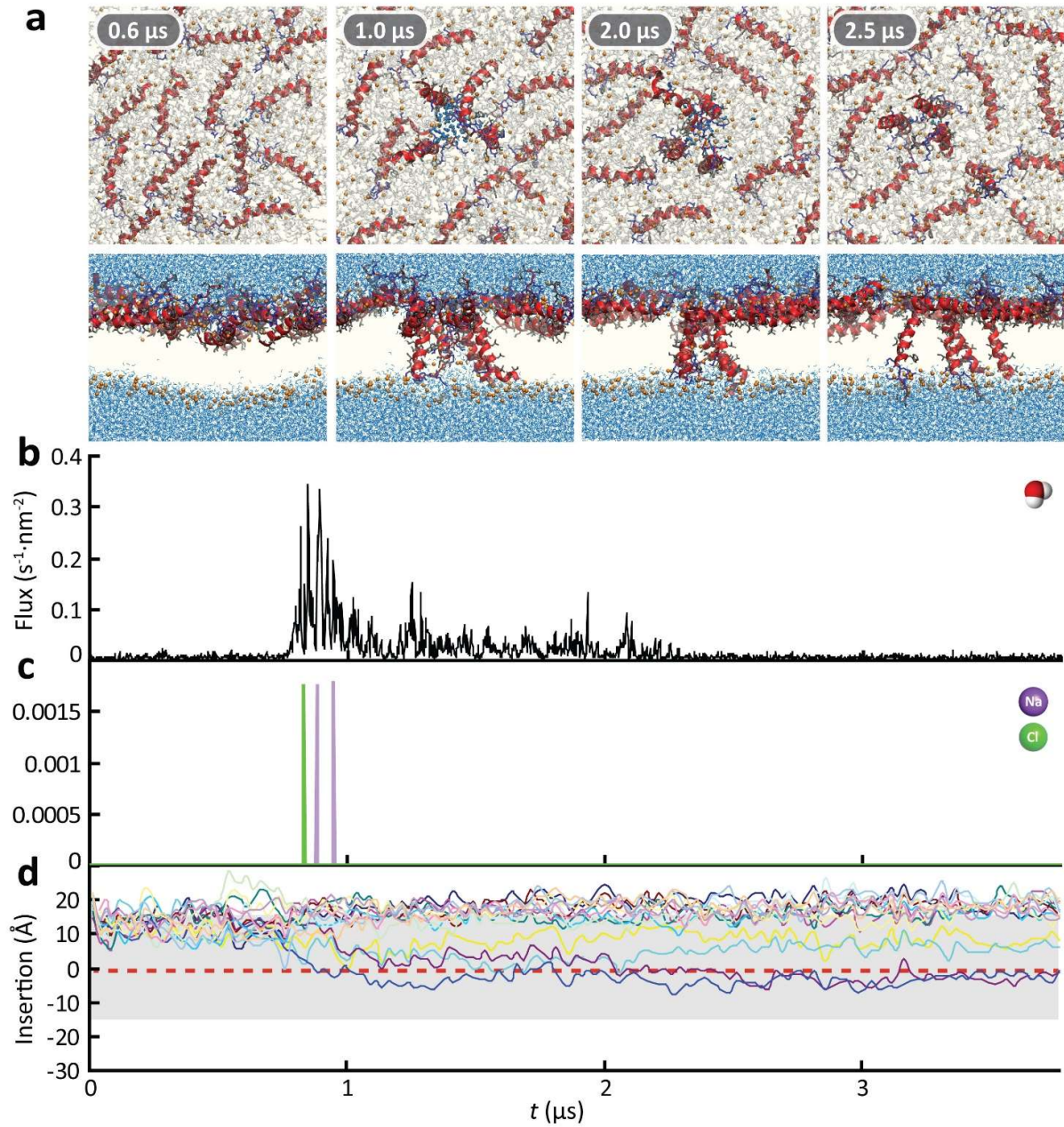


Fig. S8 | Simulation of melittin in a DOPG anionic bilayer. All peptides are initially on the surface in one leaflet, and no lipids have been removed. **a** A tiny toroidal pore consisting of 4 peptides opens after 0.8 μs , and closes at 2.2 μs . **b** Transmembrane water flux. **c** Transmembrane ion flux. **d** Insertion depth (center of mass) of all peptides (red dotted line = membrane center, grey bar = membrane).