Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Material

Simulations reveal that antimicrobial BP100 induces local membrane thinning, slows lipid dynamics and favors water penetration

Leandro R. Franco[1,†*], Peter Park[2,†], Hernan Chaimovich[2], Kaline Coutinho[1,#], Iolanda M. Cuccovia[2,#], Filipe S. Lima[3,#]

[†] L.R. Franco and P. Park contributed equally to this manuscript

[#] Correspondence to kaline@if.usp.br, filipe.slima2@ufpe.br and imcuccov@iq.usp.br

[1] Instituto de Física, Universidade de São Paulo, São Paulo, Brazil;

[2] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil;

[3] Departamento de Química Fundamental, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco, Recife, Brazil.

Contents:

[*] Present address: Department of Engineering and Physics, Karlstad University, Karlstad 65188, Sweden

Simulation set-up

	Simulated Systems	Peptide/Lipid	Water	Time (ns)
Bilayers	DPPC	0/128	54/Lipid	860
	DPPG		53/Lipid	1100
	PCPG	0/[64/64]	54/Lipid	1200
	PCPG*		52/Lipid	1200
Peptide with Bilayers	BP100 in DPPC	1/128	54/Lipid	2000
	BP100 in DPPG		53/Lipid	2000
	BP100 in PCPG		54/Lipid	2000
	BP100 in PCPG*		52/Lipid	2000

TABLE S1: All MD simulated systems carried out in this work at 1 bar and 323 K (above DPPC and DPPG transition temperatures). We used Slipids forcefield[1] for the lipids, ff99sb-ildn-NMR[2] for BP100 and TIP3P for the water. L-BP100 and α-BP100 indicate constraint-free simulations using linear and alpha helix initial conformations respectively.

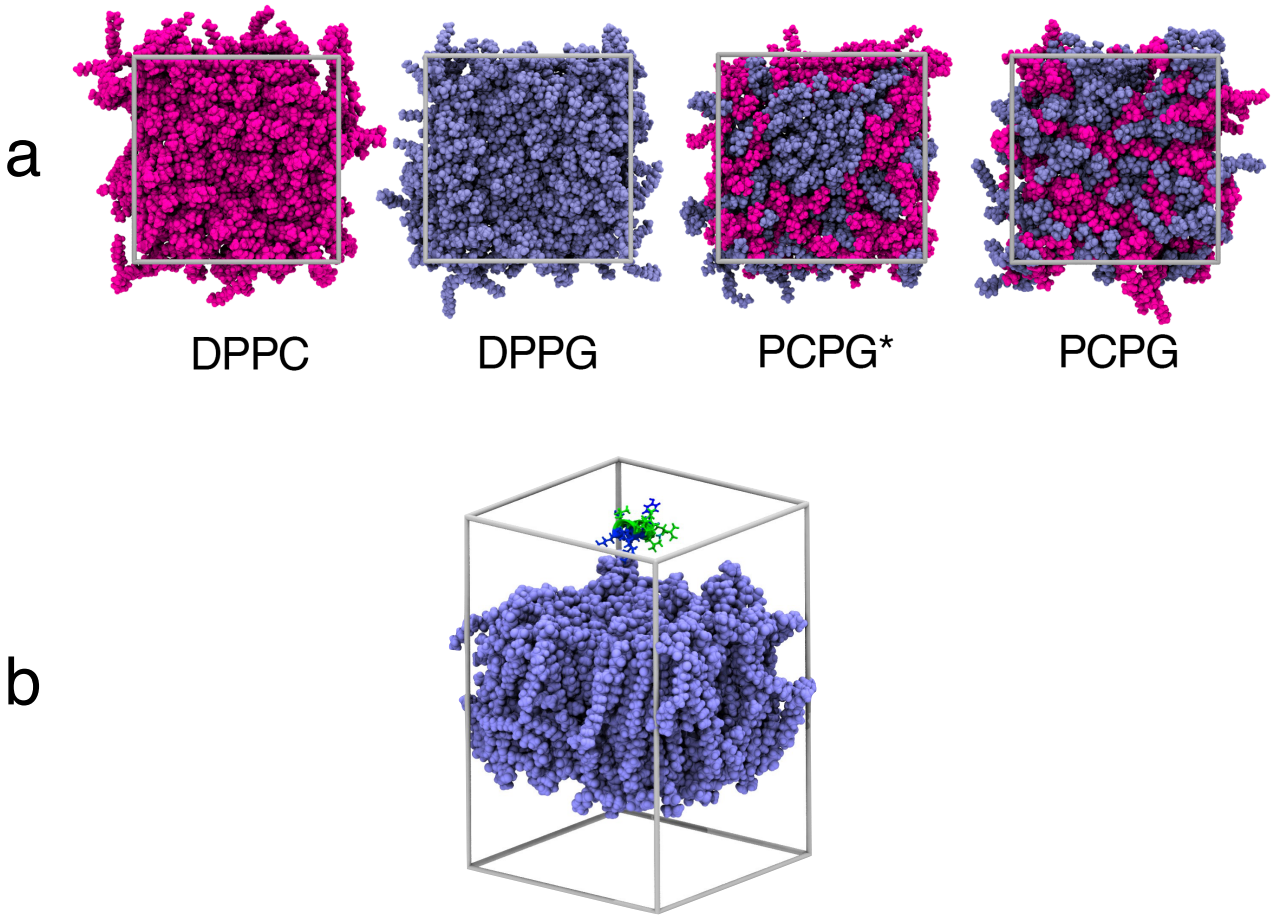


FIGURE S2: **(a)** Image of the simulated membranes seen from above with average dimensions of 6.2x6.2x9.7 nm for DPPC, 6.3x6.3x9.0 nm for DPPG, 6.2x6.2x9.5 nm for PCPG* and 6.2x6.2x9.4 nm for PCPG. **(b)** Image with example of a peptide-membrane initial configuration set-up. The water molecules and ions are not shown for clarity.

Peptide Flip

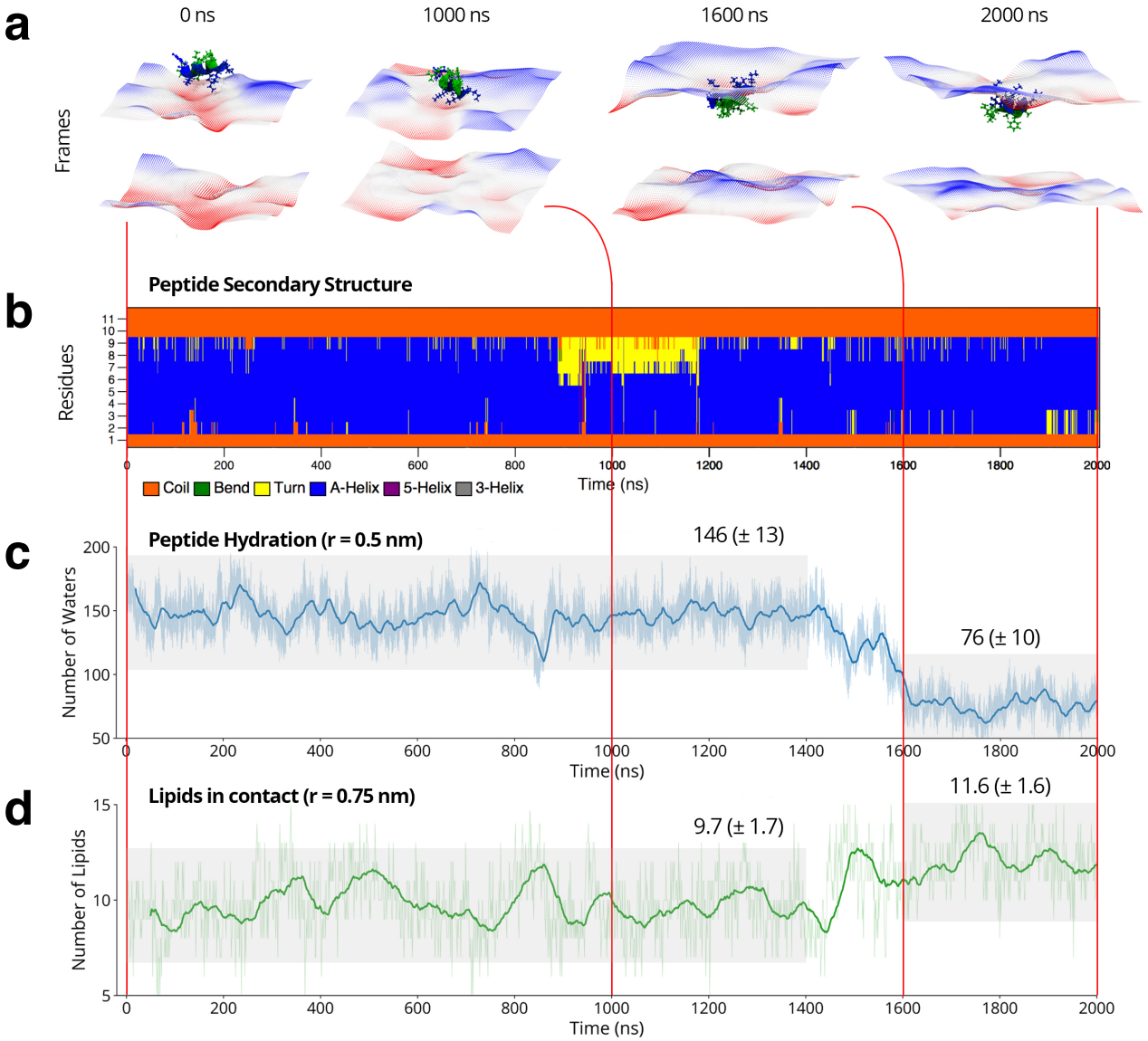


FIGURE S3: **Peptide flip. (a)** Snapshots from BP100 in DPPG simulation; positively charged residues are colored in blue and non-polar residues in green. Upper and lower grids were generated by averaging the positions of DPPG phosphorus atoms. **(b)** BP100 secondary structure along the simulation. **(c)** Peptide hydration analysis, calculating the number of waters around the peptide using a cut-off = 0.5 nm. **(d)** Number of DPPG lipids in close contact with BP100 using a cut-off = 0.75 nm. Initially, the peptide binds to the membrane with its positively charged facet facing the membrane, driven by electrostatic interactions. At approximately 1400 ns, BP100 flips turning its non-polar facet to the membrane hydrophobic core, burying the peptide inside the membrane, below the bilayer phosphates (in (a), see frames 1600 ns and 2000 ns). To this dynamic phenomenon, we designated as **peptide flip**. Alpha-helical structure preservation seems to be crucial for peptide flip (b), and the flip is accompanied by drastic peptide dehydration (c). We calculated 146 ± 13 (0-1400 ns) waters before peptide flip and 76 ±10 (1600-2000 ns) waters after the flip. A slight increase in the average number of lipids in close contact with BP100 was detected. We obtained 9.7 ± 1.7 (0-1400 ns) lipids before the flip and 11.6 ±1.6 (1600-2000 ns) lipids after the flip. Peptide flip occurred also in DPPC and PCPG* simulations. For more information, see ref 6.

Helicity

Helicity (%) - Last 100 ns	
BP100 in DPPC	45
BP100 in DPPG	72
BP100 in PCPG*	72
BP100 in PCPG	54

TABLE S4: **Helicity percentage averaged over the last 100 ns of each simulation.**

Membrane Thickness

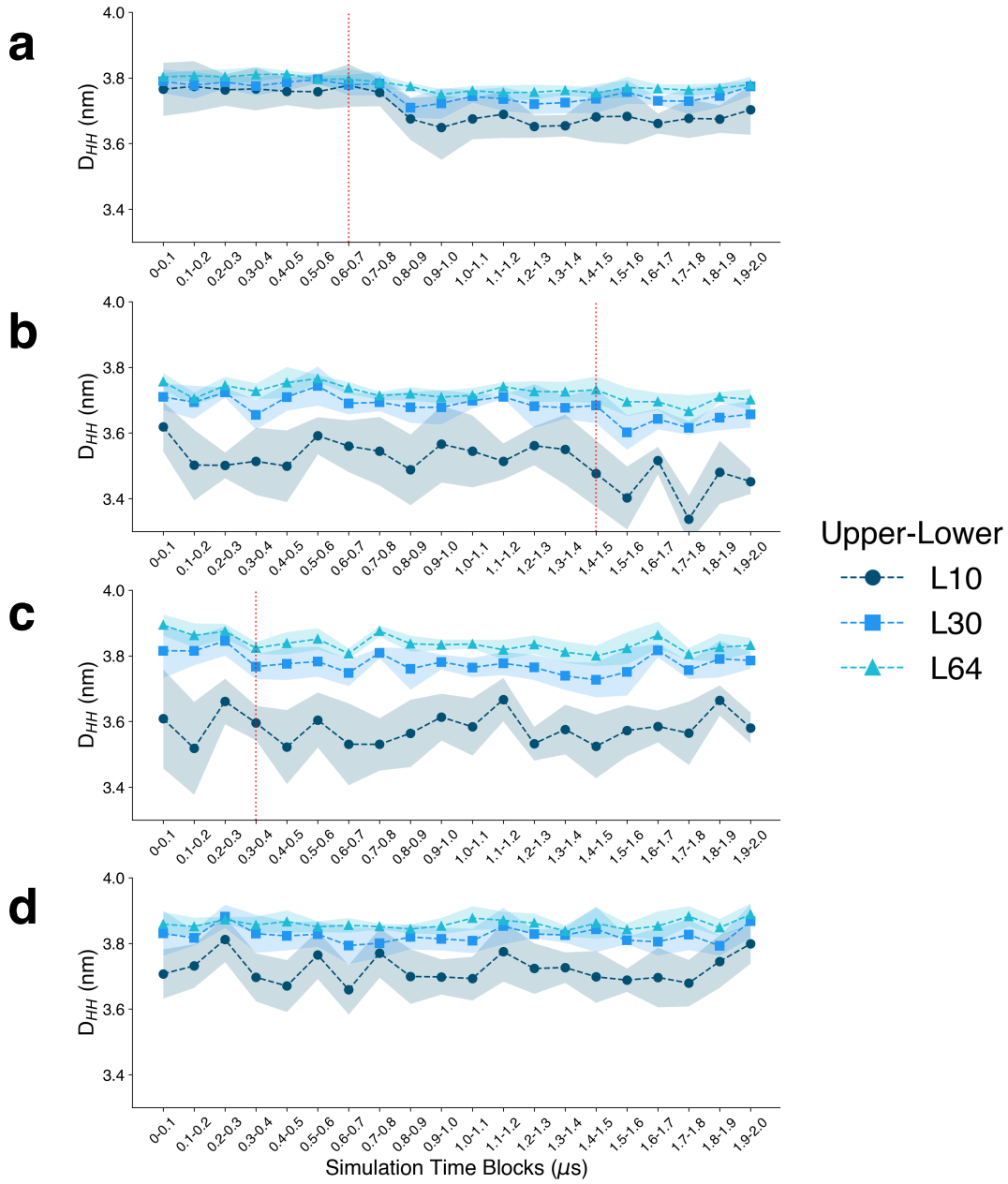
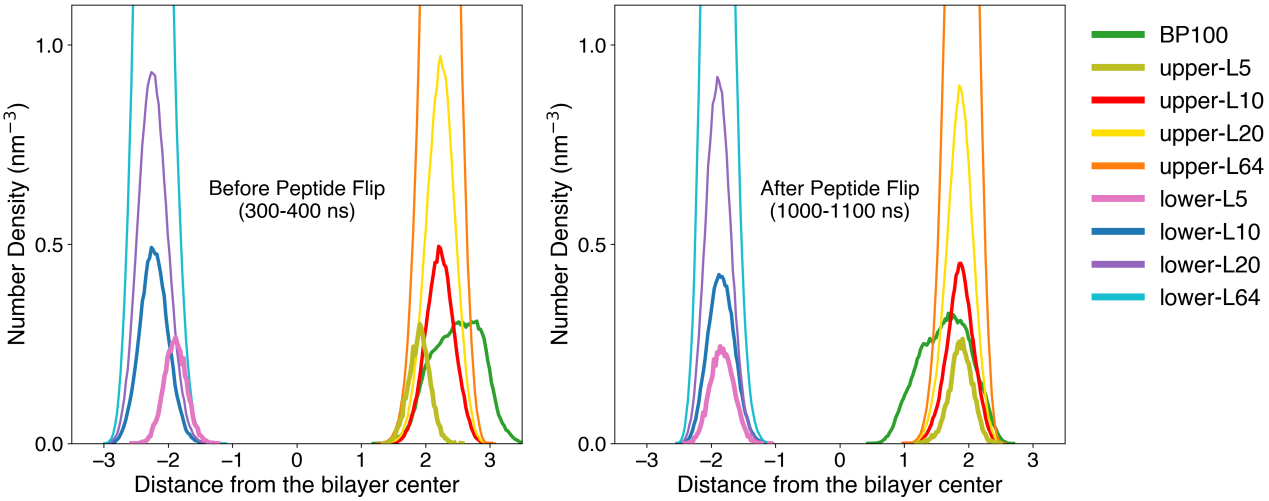


FIGURE S5: **BP100 causes local membrane thinning.** Graphs show membrane thickness throughout simulation time for simulations of α-BP100 in DPPC **(a)**, DPPG **(b)**, PCPG* **(c)**, and PCPG **(d)**. Bilayer thickness was obtained using SuAVE software, measuring the average distance between the upper and lower leaflets surface grids, generated by SuAVE. Each point represent data averaged over 100 ns of simulation. Standard deviations are represent as fillings and red dotted vertical lines indicate the peptide flip.

Number Density

(a) BP100 in DPPC - Before and after flip



(b) BP100 in DPPG - Before and after flip

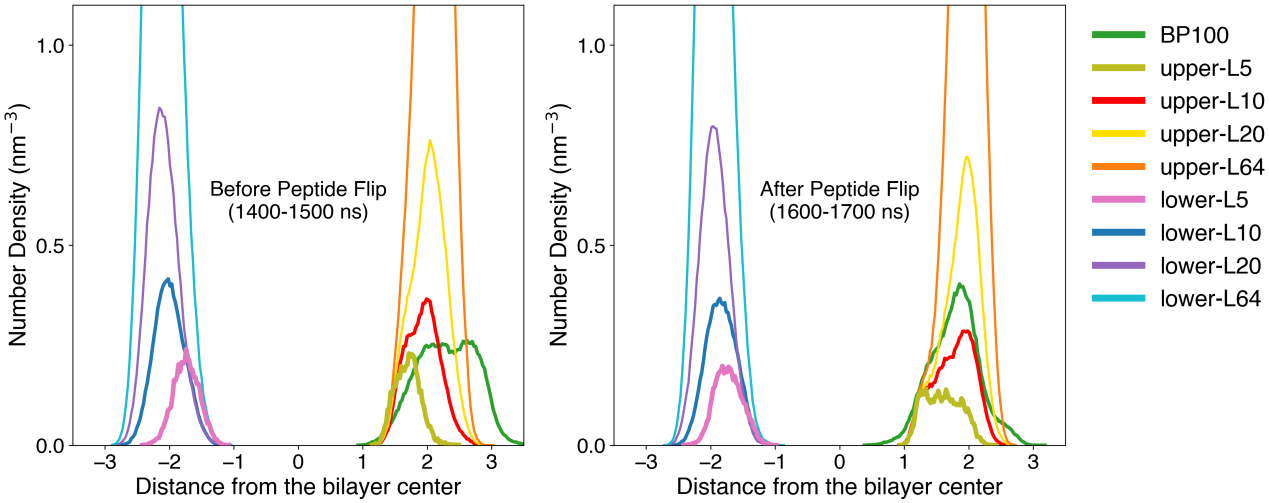
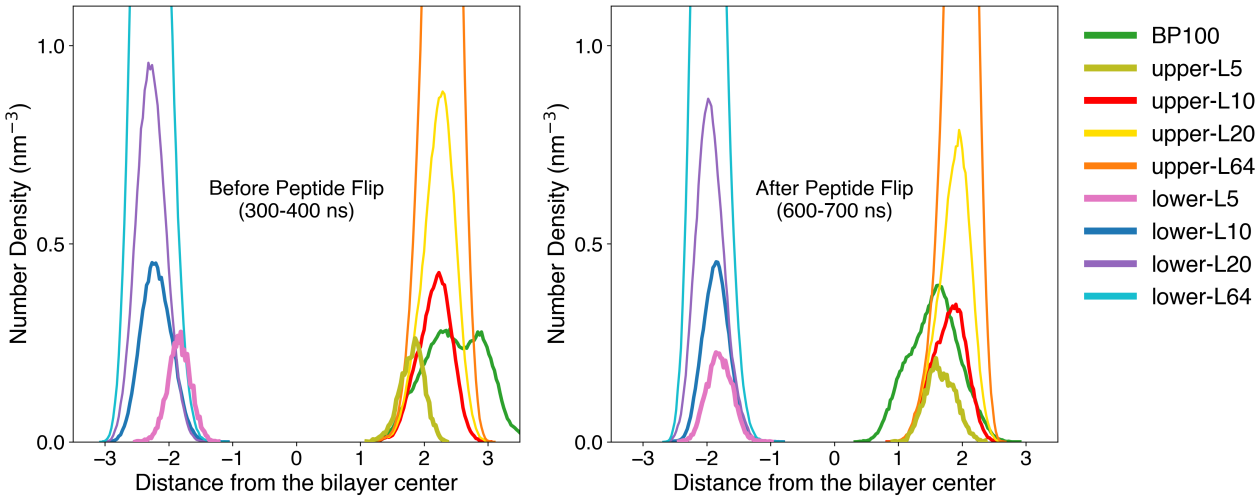


FIGURE S6: **Membrane curvature induced by peptide flip observed through number density analysis: (a) DPPC and (b) DPPG.** Number density graphs showing 100-ns-averaged positions of the peptide and lipids before and after peptide flip. In simulations with anionic lipids, membrane thinning is observed by the distance shortening between upper-L10 and lower L10.

(a) BP100 in PCPG* - Before and after flip



(b) BP100 in PCPG - No flip

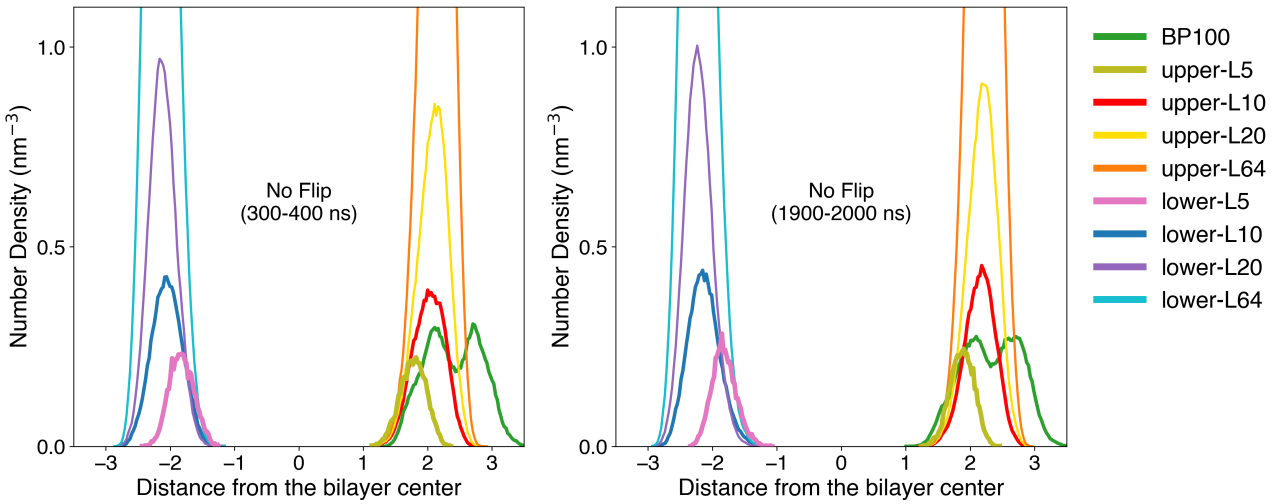


FIGURE S7: **Membrane curvature induced by peptide flip observed through number density analysis: (a) PCPG* and (b) PCPG.** Number density graphs showing 100-ns-averaged positions of the peptide and lipids before and after peptide flip. In simulations with anionic lipids, membrane thinning is observed by the distance shortening between upper-L10 and lower L10.

2D density maps

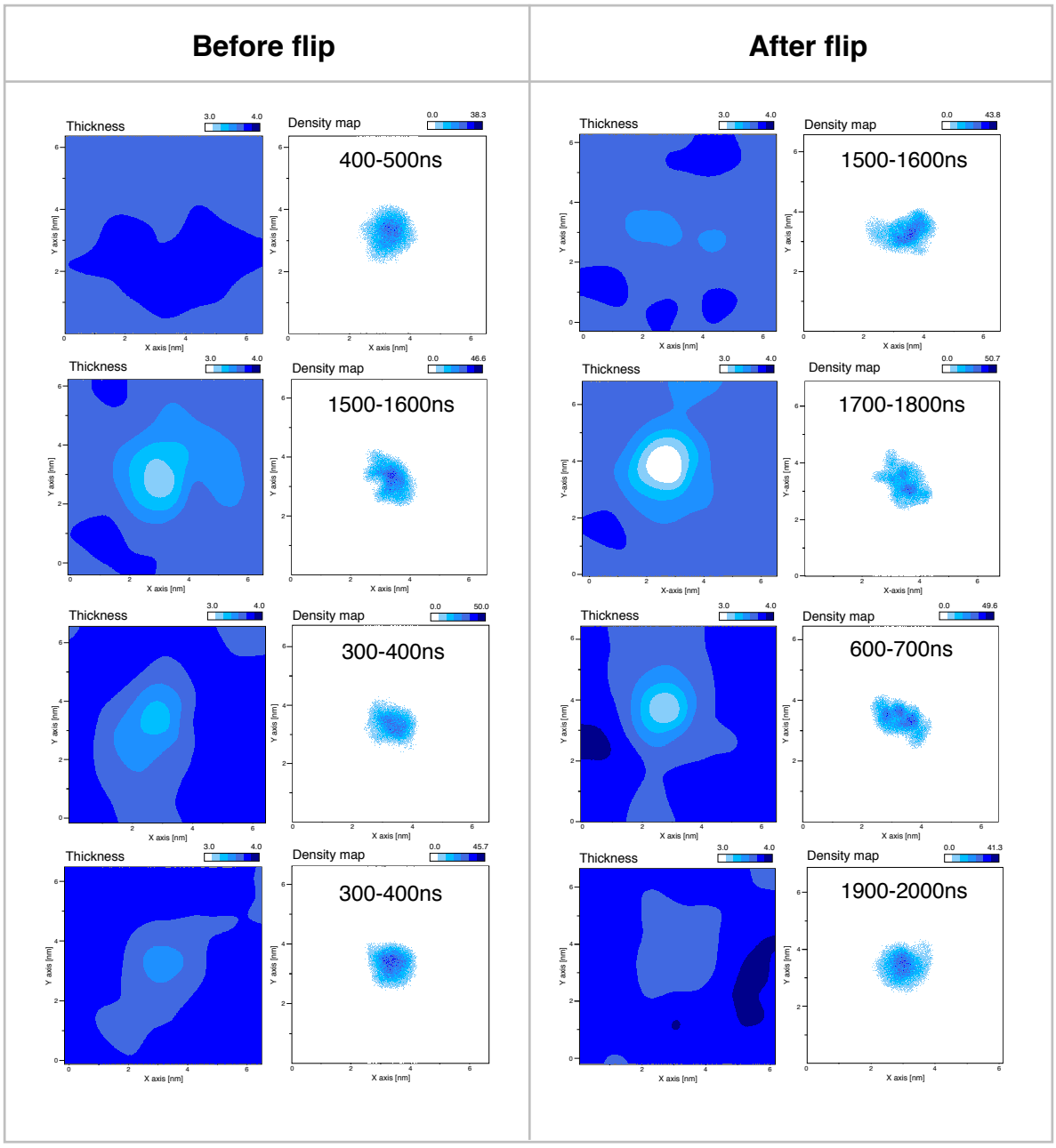
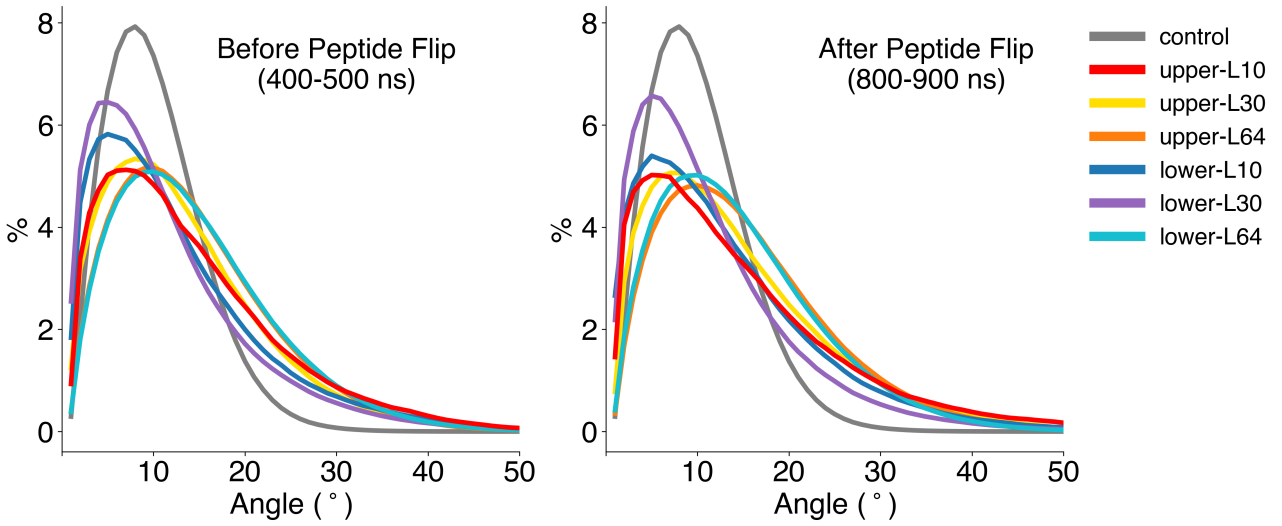


FIGURE S8: **Membrane thickness and peptide density in 2D mappings** are shown in pairs (before and after) for each simulation set: BP100 in DPPC **(a)**, in DPPG **(b)**, in PCPG* **(c)**, and in PCPG **(d)**. Note that peptide flip was found in all simulations except BP100 in PCPG (d). Thicknesses are scaled in nm and density maps in nm^3.

Angle Distribution

(a) BP100 in DPPC



(b) BP100 in DPPG

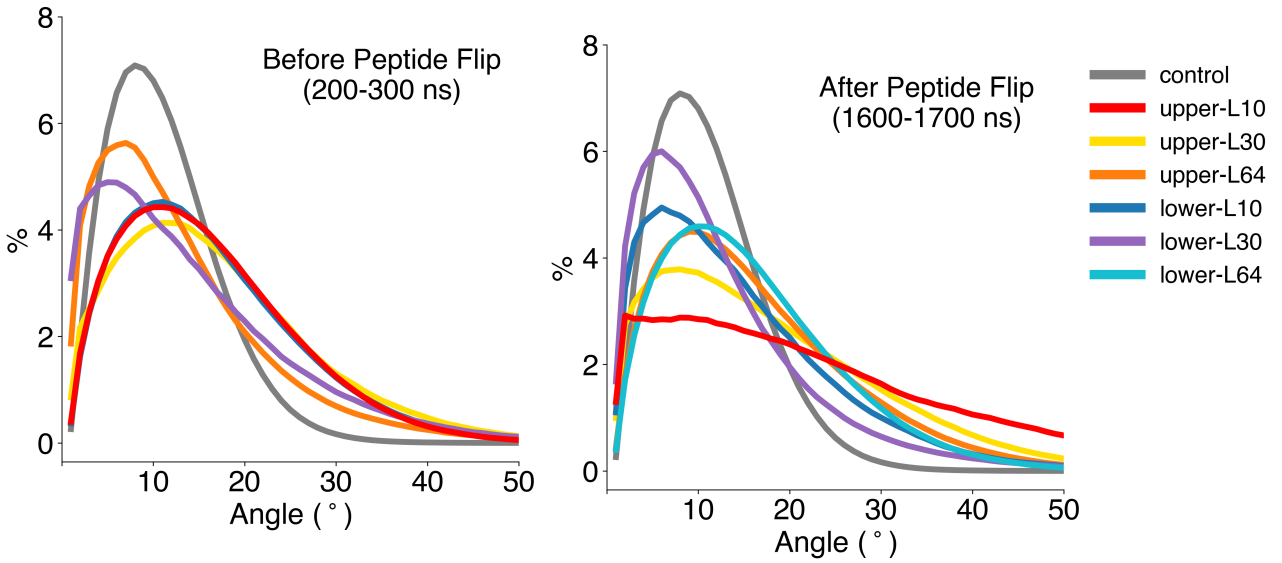
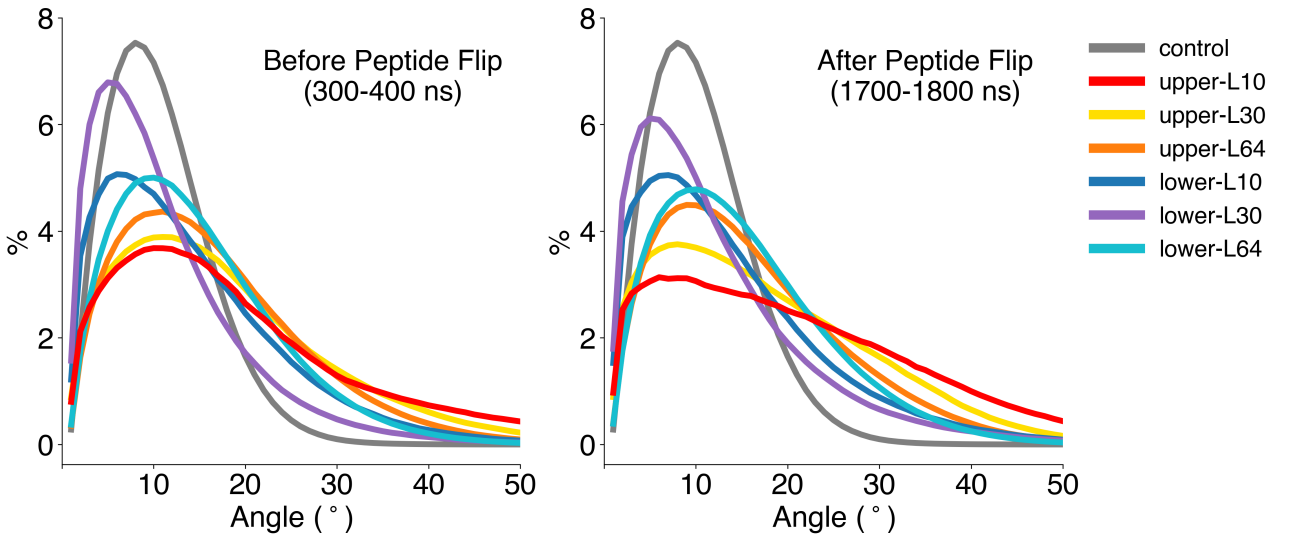


FIGURE S9: **Peptide flip induces negative membrane curvature.** Membrane surface curvature angle distribution for lipid groups in BP100 in DPPC **(a)** and in PCPG **(b)** simulations. The angle distribution was calculated using the SuAVE analysis software[3], measuring the angle between the normal vector of the surface rectangular grid partition and the z-axis. Higher angles indicate membrane curvature. To assess peptide flip influence on membrane curvature, membrane surface curvature angle distribution was calculated before and after peptide flip. It should be noted that in the BP100 in DPPC, a semi-flip was observed, due to the partial unfolding of BP100 (see reference 4 for details).

(a) BP100 in PCPG*



(b) BP100 in PCPG

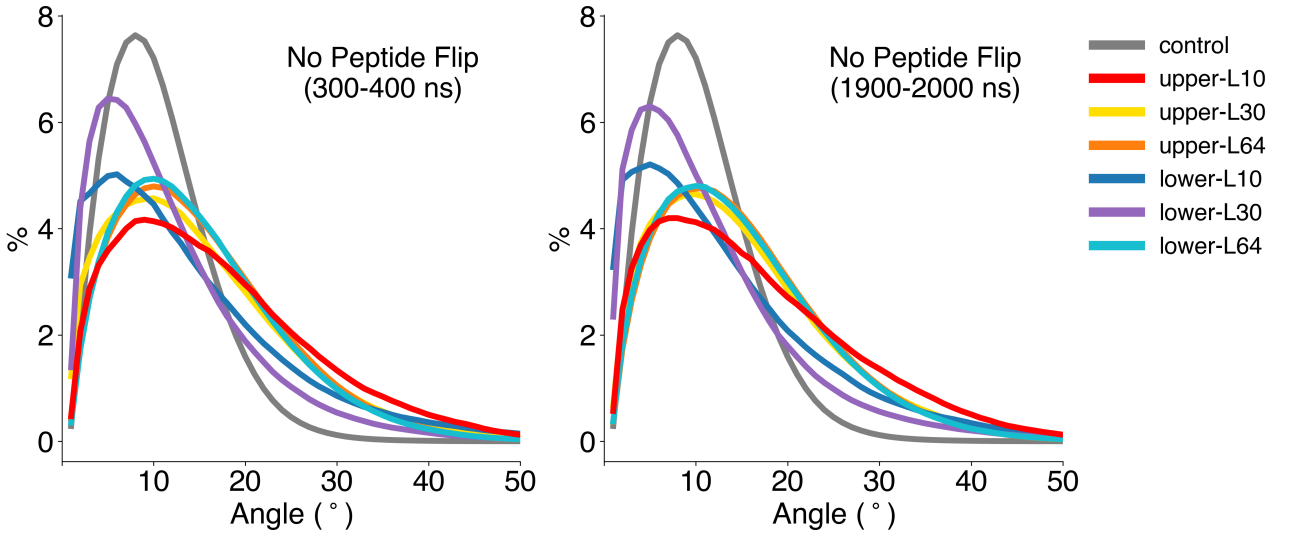


FIGURE S10: **Peptide flip induces negative membrane curvature.** Membrane surface curvature angle distribution for BP100 in PCPG* **(a)** and in PCPG **(b)**. In BP100 in PCPG*, flip occurred at ~1500 ns and no flip was observed in the PCPG simulation.

Order parameters (S_{CD})

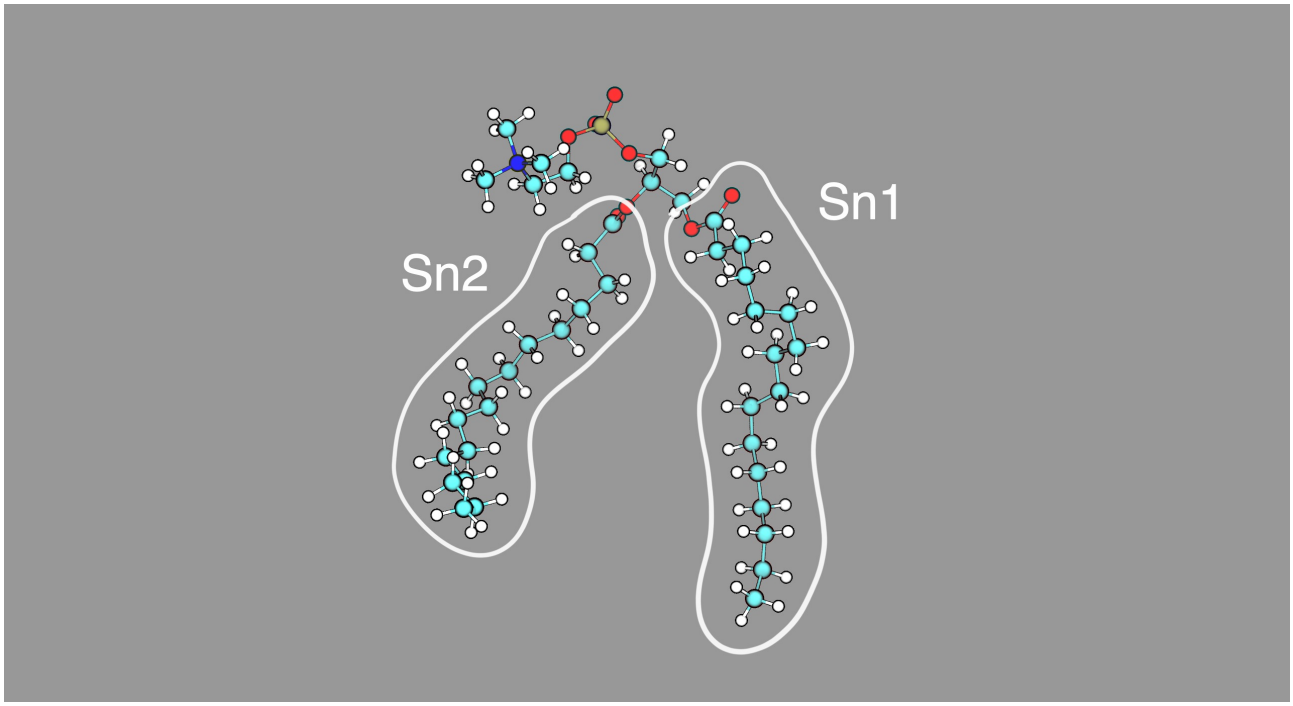


FIGURE S11: **Stereospecific numbering of phospholipids (Sn1 and Sn2) in DPPC.** For deuterium order parameter analysis, only sn1 acyl chain S_{CD} was computed. Carbon atoms are colored in cyan, Hydrogens in white, Oxygens in red, Nitrogen in blue, and Phosphorus in beige.

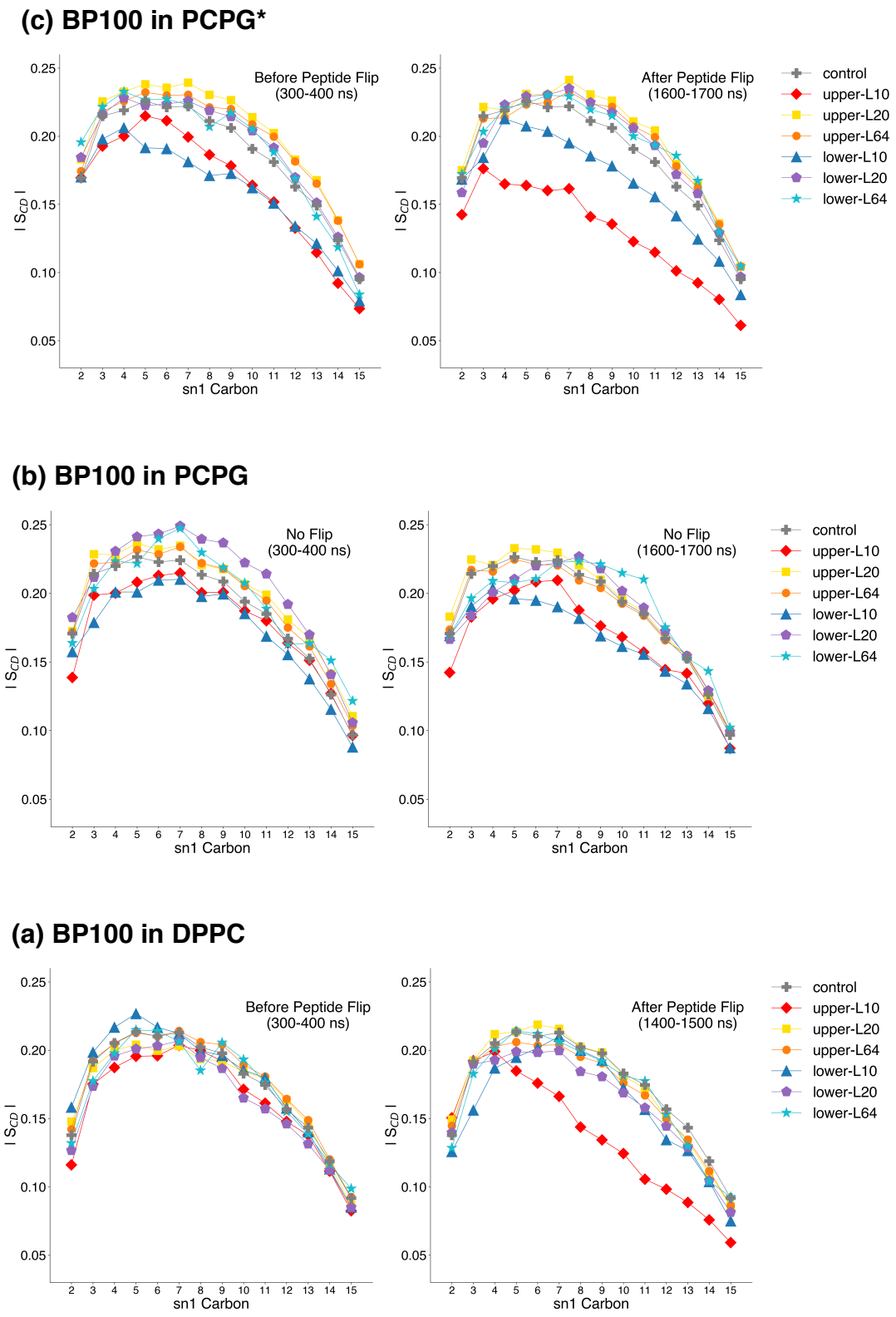


FIGURE S12: **Peptide flip decreases lipid order parameter.** Order parameters before and after flip for BP100 in DPPC **(a)** and PCPG* **(c)**, and beginning and end of the simulation for BP100 in PCPG **(b)**. Sn1 chain of lipids was used as representative for calculating lipid order parameters for L10, L20, and L64 for both monolayers. Order parameters were calculated in 100 ns time windows. Control data were obtained from pure membrane simulations, averaging order parameters from the last 800 ns of simulation. Semi-flip occurred at ~700 ns for BP100 in DPPC and full peptide flip at ~400 ns for PCPG*.

Lipid lateral diffusion coefficient (D_L)

Lipid Lateral Diffusion (10^{-7} cm^2s^{-1})						
	upper-L5	upper-L10	upper-L20	lower-L64	Control	Literature
DPPC	0.8 ± 0.3	0.9 ± 0.2	1.3 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.8[5],1.78[6], ~1.5[7], ~1.5[8]
DPPG	0.5 ± 0.2	0.6 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	0.9[9]
PCPG*	0.5 ± 0.2	0.6 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	
PCPG	0.6 ± 0.2	0.7 ± 0.3	1.1 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	

TABLE S13: **BP100 binding leads to local lipid slow down.** Lipid lateral diffusion coefficients were calculated from the slopes of the mean-square displacements (MSD) in the xy-plane of selected lipids. Lateral diffusion coefficients for pure membranes were used as control and values found in the literature are shown for comparison.

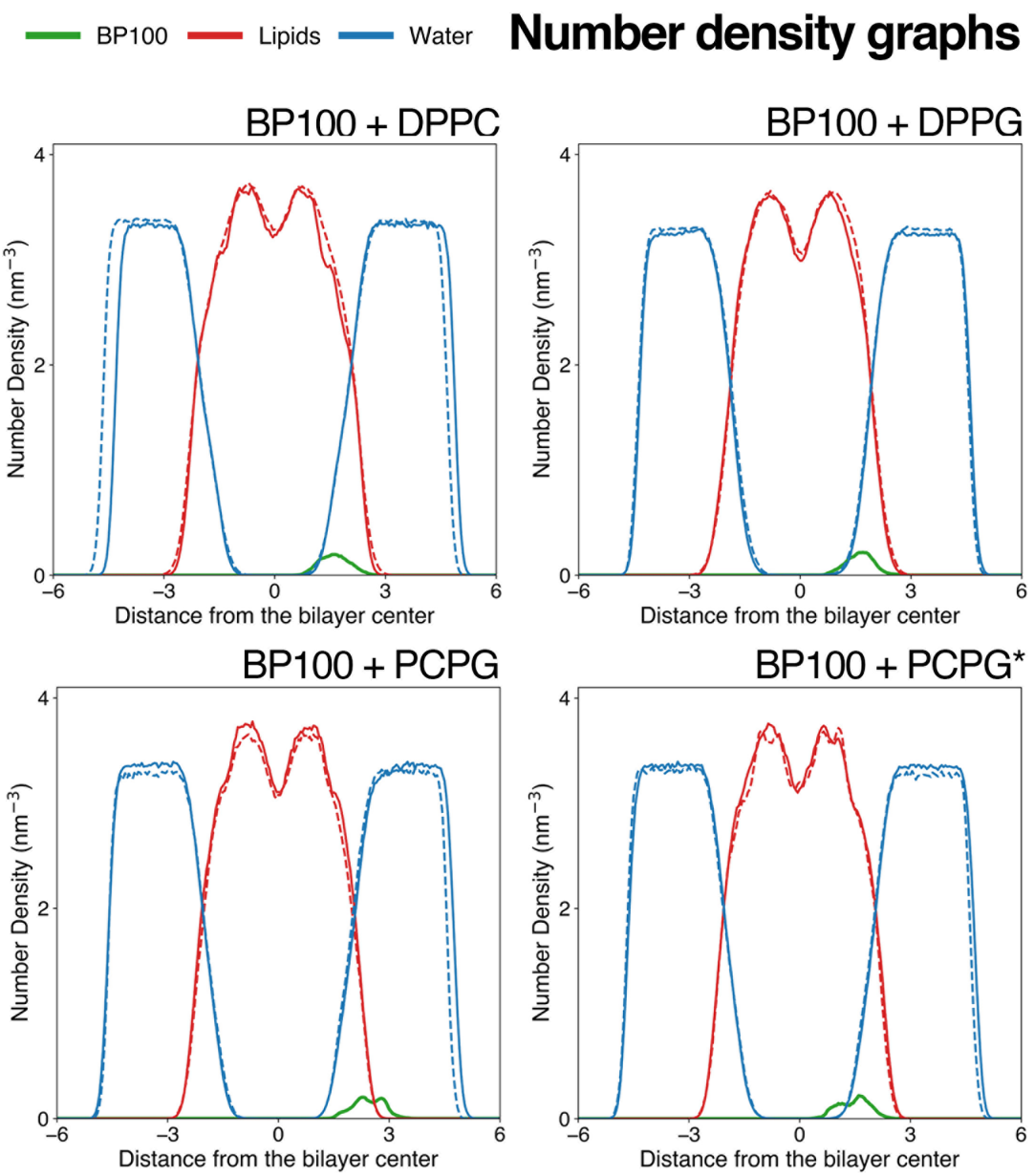


FIGURE S14: Number density profile of water and lipids for peptide/membranes systems (solid lines) and pure membrane systems (dashed lines) over the last 100 ns of simulations. Peptide flip was observed in DPPC, DPPG, and PCPG* simulations.

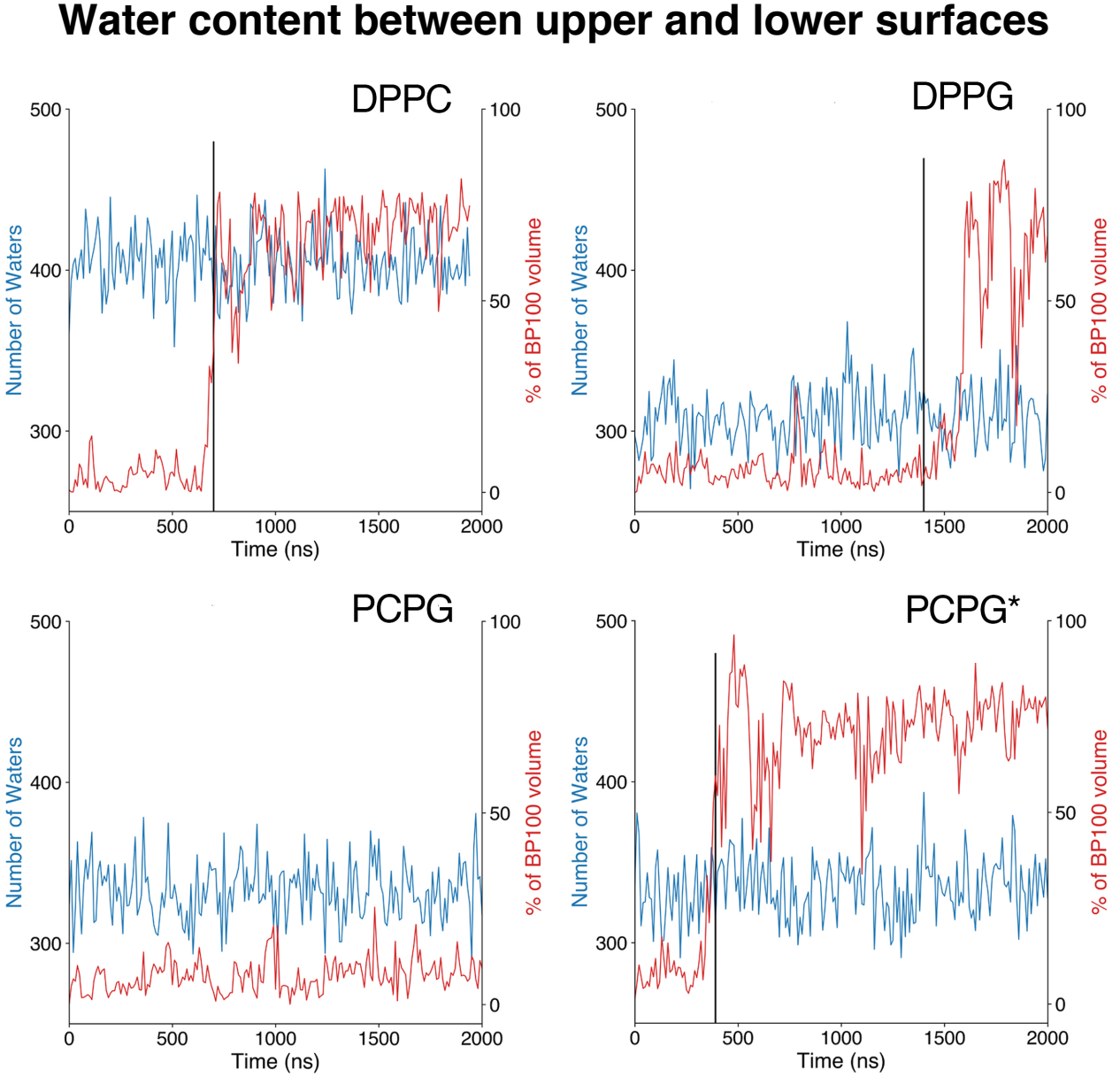


FIGURE S15: **Membrane hydration in peptide/membrane simulations.** The number of water molecules accounted between the upper and lower surface grids (R3 region) is shown for all peptide/membrane simulations, as well as the percentage of peptide volume insertion into the R3 region. Vertical lines indicate when peptide flip was observed and the average number of waters are shown on the top right corner in blue. For control systems, we computed an average of 390 ± 28, 307 ± 18, 341 ± 20, and 336 ± 17 water molecules for DPPC, DPPG, PCPG and PCPG* simulations, respectively, and 393 ± 31, 309 ± 18, 332 ± 18 and 334 ± 19 water molecules for the same membranes with the peptide binding.

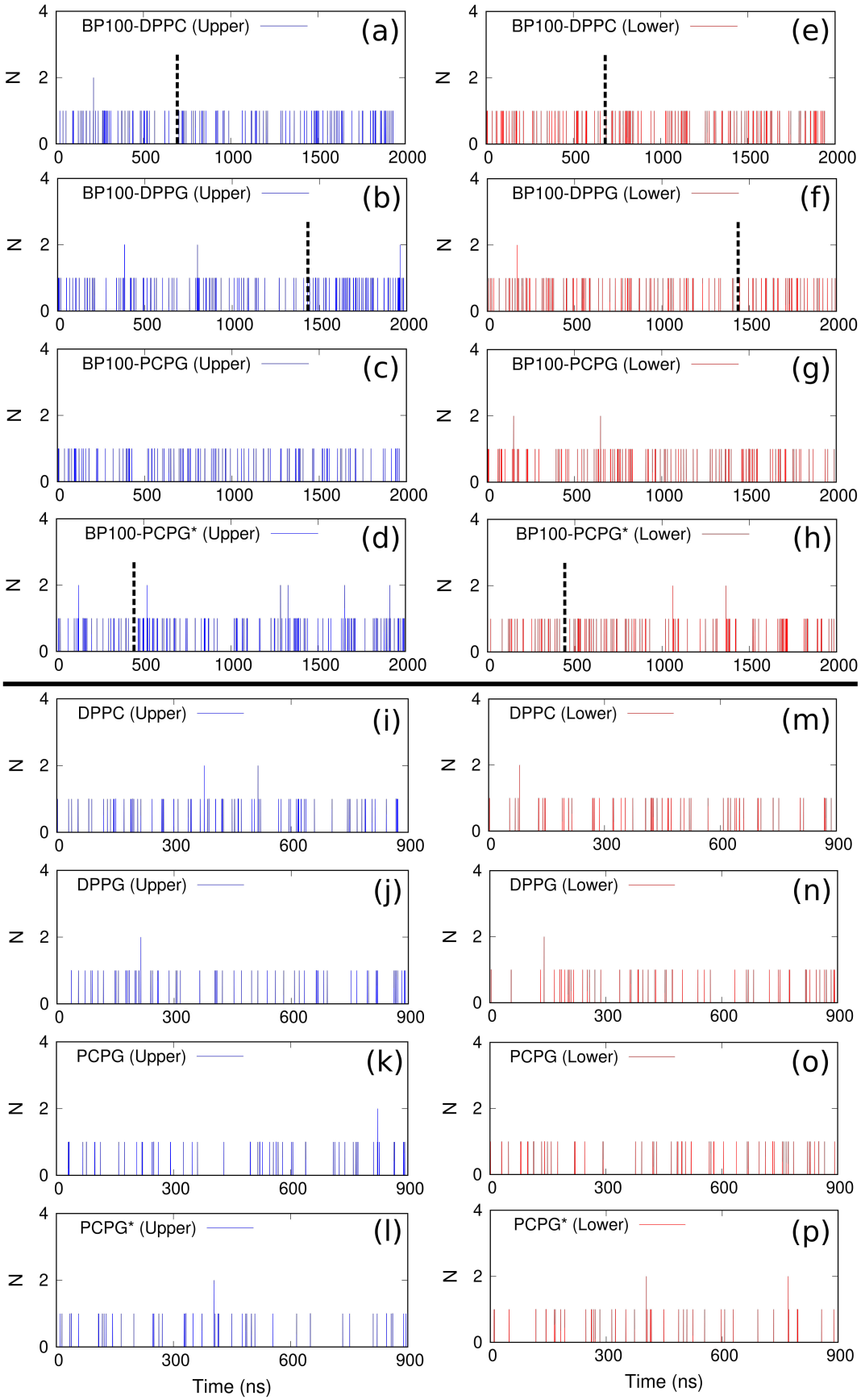


FIGURE S16: **Membrane core hydration in the R1 region.** Number of waters (N) detected between the upper (blue) and lower (red) R1 regions in simulations with **(a-h)** and without **(i-p)** BP100. BP100 was bound on the upper monolayer, above upper R2 (z > 0.5 nm). Black dashed vertical lines indicate when peptide flip took place. Analyses performed using a 10 ps resolution.

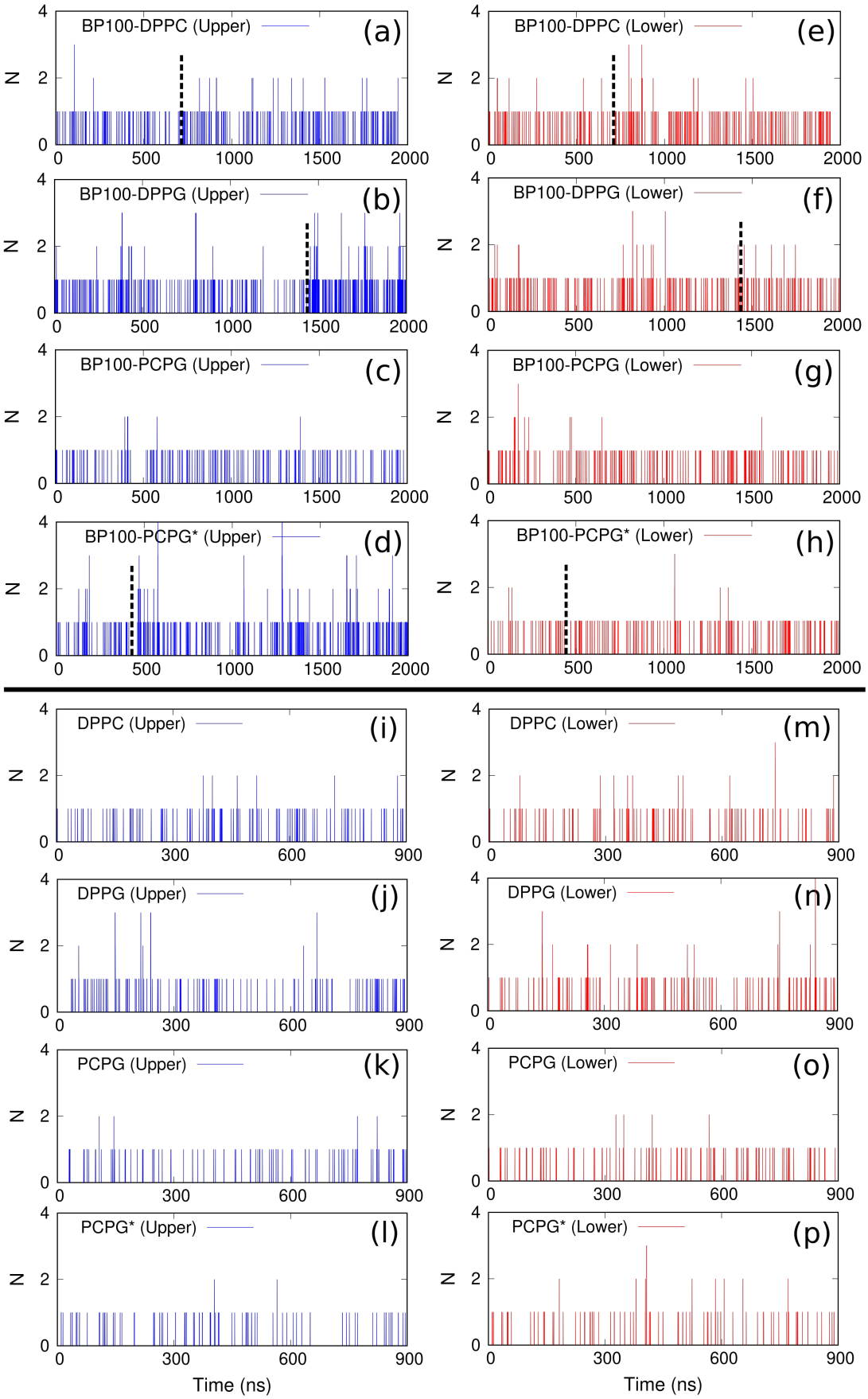


FIGURE S17: **Membrane core hydration in the R2 region.** Number of waters (N) detected between the upper (blue) and lower (red) R2 regions in simulations with **(a-h)** and without **(i-p)** BP100. BP100 was bound on the upper monolayer, above upper R2 (z > 0.5 nm). Black dashed vertical lines indicate when peptide flip took place. Analyses performed using a 10 ps resolution.

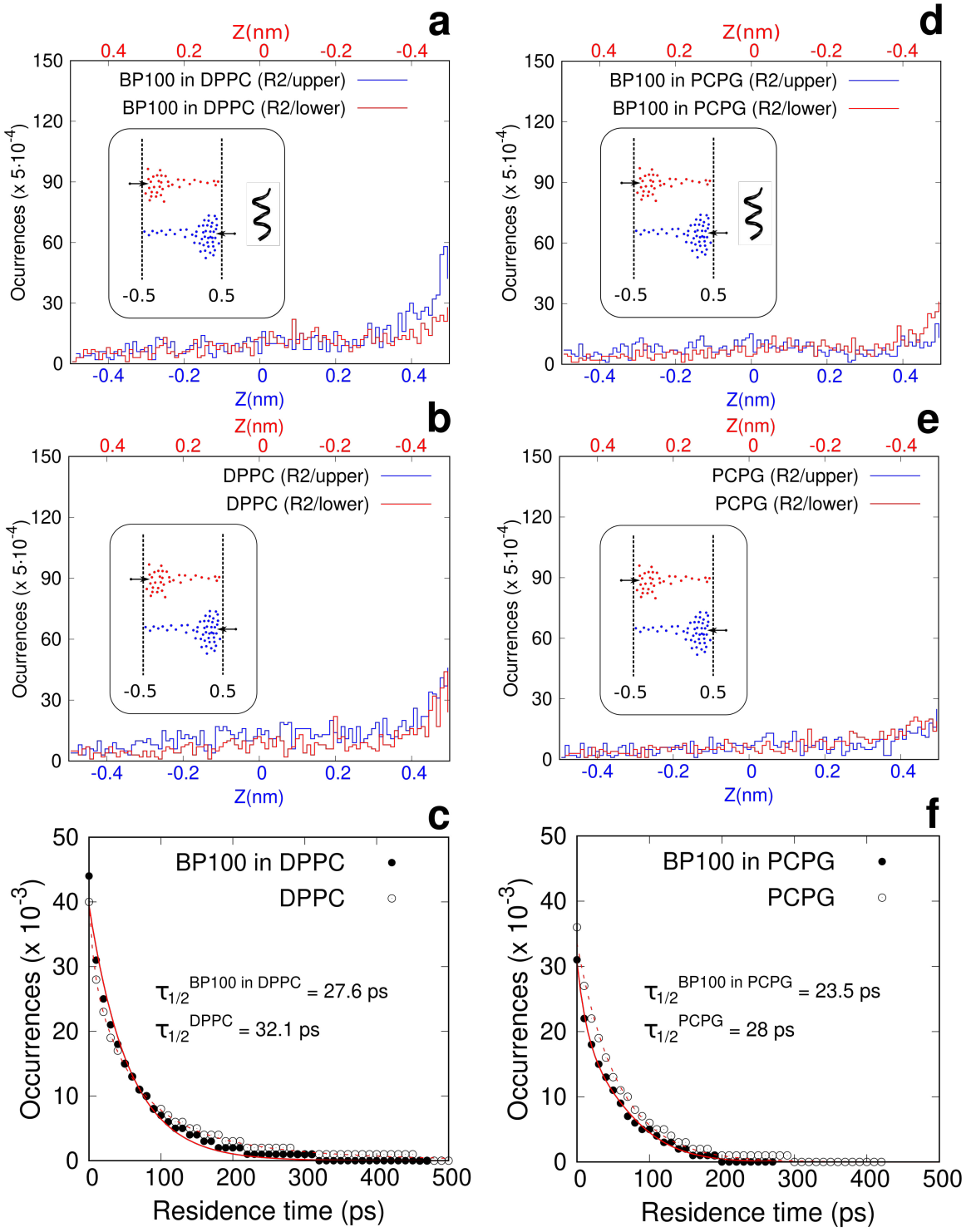


FIGURE S18: **Membrane core hydration (for BP100/DPPC, BP100/PCPG and controls) and average residence time of waters within R2 region. (a, b, d, e)** Histograms of the Z-axis of the center of mass of waters detected within the R2 region in simulations with (a and d) and without (b and e) peptide. Waters that access the R2 region coming from the upper monolayer (in blue) or from the lower monolayer (in red) are accounted separately. BP100 is bound on the monolayer above the upper R2 region ($z > 0.5$ nm). **(c, f)** Residence time of waters within R2 region are shown for the same systems, with the half-life ($\tau_{1/2}$) obtained from exponential fits. Analyzes performed over the last 500 ns of simulation using a time resolution of 10 ps.

BP100 IN MEMBRANES			N_{WF} and $P_W(N)$ in R1												
			$N_{WF}(N)$ with N waters				⟨N⟩				$P_W(N)$ ($x10^{-4}$)				
			N = 1	2	3	4					N = all		1	2	3
0 to 300 ns (First 300ns) $N_{TF} = 3x10^4$	DPPC	R1/Upper	40	1	0	0	1.02	± 0.02	DPPC	R1/Upper	13.7	± 0.7	13.3	0.3	0.0
		R1/Lower	38	0	0	0	1.00	± 0.02		R1/Lower	12.7	± 0.6	12.7	0.0	0.0
	DPPG	R1/Upper	27	0	0	0	1.00	± 0.03	DPPG	R1/Upper	9.0	± 0.7	9.0	0.0	0.0
		R1/Lower	29	1	0	0	1.03	± 0.02		R1/Lower	10.0	± 0.5	9.7	0.3	0.0
	PCPG*	R1/Upper	34	1	0	0	1.03	± 0.03	PCPG*	R1/Upper	11.7	± 1.0	11.3	0.3	0.0
		R1/Lower	24	0	0	0	1.00	± 0.02		R1/Lower	8.0	± 0.4	8.0	0.0	0.0
	PCPG (No Flip)	R1/Upper	37	0	0	0	1.00	± 0.01	PCPG	R1/Upper	12.3	± 0.6	12.3	0.0	0.0
		R1/Lower	43	1	0	0	1.02	± 0.02		R1/Lower	14.7	± 0.5	14.3	0.3	0.0
1700 to 2000 ns (Last 300ns) $N_{TF} = 3x10^4$	DPPC	R1/Upper	33	0	0	0	1.00	± 0.01	DPPC	R1/Upper	11.0	± 0.7	11.0	0.0	0.0
		R1/Lower	35	0	0	0	1.00	± 0.02		R1/Lower	11.7	± 0.5	11.7	0.0	0.0
	DPPG	R1/Upper	72	1	0	0	1.01	± 0.03	DPPG	R1/Upper	24.3	± 0.7	24.0	0.3	0.0
		R1/Lower	22	0	0	0	1.00	± 0.02		R1/Lower	7.3	± 1.0	7.3	0.0	0.0
	PCPG*	R1/Upper	65	8	1	0	1.14	± 0.02	PCPG*	R1/Upper	24.7	± 0.9	21.7	2.7	0.3
		R1/Lower	35	2	0	0	1.05	± 0.02		R1/Lower	12.3	± 1.1	11.7	0.7	0.0
	PCPG (No Flip)	R1/Upper	16	0	0	0	1.00	± 0.02	PCPG	R1/Upper	5.3	± 0.8	5.3	0.0	0.0
		R1/Lower	14	0	0	0	1.00	± 0.02		R1/Lower	4.7	± 1.0	4.7	0.0	0.0
0 to 2000 ns (Entire simulation) $N_{TF} = 2x10^5$	DPPC	R1/Upper	188	1	0	0	1.01	± 0.02	DPPC	R1/Upper	9.5	± 0.8	9.4	0.1	0.0
		R1/Lower	207	0	0	0	1.00	± 0.01		R1/Lower	10.4	± 0.7	10.4	0.0	0.0
		R1	395	1	0	0	1.00	± 0.02		R1	19.8	± 0.4	19.8	0.1	0.0
	DPPG	R1/Upper	236	5	0	0	1.02	± 0.02	DPPG	R1/Upper	12.1	± 0.8	11.8	0.3	0.0
		R1/Lower	157	1	0	0	1.01	± 0.01		R1/Lower	7.9	± 0.8	7.9	0.1	0.0
		R1	393	6	0	0	1.02	± 0.02		R1	20.0	± 0.8	19.7	0.3	0.0
	PCPG*	R1/Upper	272	8	0	0	1.03	± 0.02	PCPG*	R1/Upper	14.0	± 0.7	13.6	0.4	0.0
		R1/Lower	187	3	1	0	1.03	± 0.01		R1/Lower	9.6	± 0.8	9.4	0.2	0.1
		R1	459	11	1	0	1.03	± 0.02		R1	23.6	± 0.7	23.0	0.6	0.1
	PCPG	R1/Upper	176	0	0	0	1.00	± 0.02	PCPG	R1/Upper	8.8	± 0.6	8.8	0.0	0.0
		R1/Lower	189	2	0	0	1.01	± 0.01		R1/Lower	9.6	± 0.5	9.5	0.1	0.0
		R1	365	2	0	0	1.01	± 0.02		R1	18.4	± 0.5	18.3	0.1	0.0
PURE MEMBRANES															
0 to 900 ns (Entire simulation) $N_{TF} = 9x10^4$	DPPC	R1/Upper	94	2	0	0	1.02	± 0.02	DPPC	R1/Upper	10.7	± 0.3	10.4	0.2	0.0
		R1/Lower	70	1	0	0	1.01	± 0.01		R1/Lower	7.9	± 0.5	7.8	0.1	0.0
		R1	164	3	0	0	1.02	± 0.01		R1	18.6	± 0.5	18.2	0.3	0.0
	DPPG	R1/Upper	84	1	0	0	1.01	± 0.02	DPPG	R1/Upper	9.4	± 0.7	9.3	0.1	0.0
		R1/Lower	70	1	0	0	1.01	± 0.02		R1/Lower	7.9	± 0.8	7.8	0.1	0.0
		R1	154	2	0	0	1.01	± 0.03		R1	17.3	± 0.7	17.1	0.2	0.0
	PCPG*	R1/Upper	63	1	0	0	1.02	± 0.02	PCPG*	R1/Upper	7.1	± 0.6	7.0	0.1	0.0
		R1/Lower	56	2	0	0	1.03	± 0.01		R1/Lower	6.4	± 0.8	6.2	0.2	0.0
		R1	119	3	0	0	1.02	± 0.03		R1	13.6	± 0.7	13.2	0.3	0.0
	PCPG	R1/Upper	83	1	0	0	1.01	± 0.02	PCPG	R1/Upper	9.3	± 0.5	9.2	0.1	0.0
		R1/Lower	76	0	0	0	1.00	± 0.01		R1/Lower	8.4	± 0.4	8.4	0.0	0.0
		R1	159	1	0	0	1.01	± 0.02		R1	17.8	± 0.5	17.7	0.1	0.0

TABLE S19: **Number of frames with N water molecules, $N_{WF}(N)$, and probability of finding water molecules inside the hydrophobic core of membranes, $P_W(N)$ with N = 1 to 4, in region R1.** $N_{WF} = \Sigma\ N_{WF}(N)$; $\langle N \rangle = \Sigma\ N \times N_{WF}(N)/N_{WF}$; $P_W(N) = N_{WF}(N)/N_{TF}$, where $N_{TF} = \tau_{tot}/\Delta t_r$ is the total number of trajectory frames analyzed and $N_{WF}(N)$ is the number of frames with N water molecules within the hydrophobic core membrane R1 region. Using a time resolution of Δt_r = 10 ps, the analyzes were performed: (i) over the first and last τ_{tot} = 300 ns of the peptide/membrane simulations, which the peptide-flip effect in the upper and lower sub-regions were highlighted in green for DPPG and in yellow for PCPG*, and (ii) over the whole peptide/membrane (τ_{tot} = 2000 ns) and pure membrane simulations (τ_{tot} = 900 ns), which the values in the whole R1 region were highlighted in gray.

BP100 IN MEMBRANES			N_{WF} and $P_W(N)$ in R2												
			$N_{WF}(N)$ with N waters				⟨N⟩				$P_w(N)$ ($x10^{-4}$)				
			N = 1	2	3	4					N = all		1	2	3
0 to 300 ns (First 300ns) N_{TF} = $3x10^4$	DPPC	R2/Upper	64	2	1	0	1.06	± 0.02	DPPC	R2/Upper	22.3	± 1.1	21.3	0.7	0.3
		R2/Lower	62	6	0	0	1.09	± 0.03		R2/Lower	22.7	± 1.0	20.7	2.0	0.0
	DPPG	R2/Upper	63	2	0	0	1.03	± 0.04	DPPG	R2/Upper	21.7	± 1.3	21.0	0.7	0.0
		R2/Lower	66	5	0	0	1.07	± 0.03		R2/Lower	23.7	± 0.9	22.0	1.7	0.0
	PCPG*	R2/Upper	43	1	0	0	1.02	± 0.03	PCPG*	R2/Upper	14.7	± 1.8	14.3	0.3	0.0
		R2/Lower	29	0	0	0	1.00	± 0.02		R2/Lower	9.7	± 0.7	9.7	0.0	0.0
	PCPG (No Flip)	R2/Upper	58	0	0	0	1.00	± 0.02	PCPG	R2/Upper	19.3	± 1.1	19.3	0.0	0.0
		R2/Lower	64	9	1	0	1.13	± 0.03		R2/Lower	24.7	± 0.9	24.7	3.0	0.3
1700 to 2000 ns (Last 300ns) N_{TF} = $3x10^4$	DPPC	R2/Upper	69	4	0	0	1.05	± 0.03	DPPC	R2/Upper	24.3	± 1.2	23.0	1.3	0.0
		R2/Lower	62	0	0	0	1.00	± 0.03		R2/Lower	20.7	± 0.8	20.7	0.0	0.0
	DPPG	R2/Upper	222	38	2	0	1.16	± 0.04	DPPG	R2/Upper	87.3	± 2.2	74.0	12.7	0.7
		R2/Lower	36	1	1	0	1.08	± 0.03		R2/Lower	12.7	± 0.8	12.0	0.3	0.3
	PCPG*	R2/Upper	142	7	3	0	1.09	± 0.03	PCPG*	R2/Upper	50.7	± 1.9	47.3	2.3	1.0
		R2/Lower	54	0	0	0	1.00	± 0.02		R2/Lower	18.0	± 1.3	18.0	0.0	0.0
	PCPG (No Flip)	R2/Upper	33	0	0	0	1.00	± 0.03	PCPG	R2/Upper	11.0	± 0.7	11.0	0.0	0.0
		R2/Lower	36	0	0	0	1.00	± 0.03		R2/Lower	12.0	± 1.1	12.0	0.0	0.0
0 to 2000 ns (Entire simulation) N_{TF} = $2x10^5$	DPPC	R2/Upper	381	20	1	0	1.05	± 0.03	DPPC	R2/Upper	20.1	± 1.0	19.1	1.0	0.1
		R2/Lower	344	17	2	0	1.06	± 0.03		R2/Lower	18.2	± 0.9	17.2	0.9	0.1
		R2	725	37	3	0	1.06	± 0.03		R2	38.3	± 1.4	36.3	1.9	0.2
	DPPG	R2/Upper	761	78	14	0	1.12	± 0.04	DPPG	R2/Upper	42.7	± 1.3	38.1	3.9	0.7
		R2/Lower	341	18	3	0	1.07	± 0.03		R2/Lower	18.1	± 0.8	17.1	0.9	0.2
		R2	1102	96	17	0	1.11	± 0.04		R2	60.8	± 1.8	55.1	4.8	0.9
	PCPG*	R2/Upper	636	66	17	4	1.15	± 0.05	PCPG*	R2/Upper	36.2	± 2.0	31.8	3.3	0.9
		R2/Lower	297	7	2	0	1.04	± 0.02		R2/Lower	15.3	± 0.9	14.9	0.4	0.1
		R2	933	73	19	4	1.12	± 0.05		R2	51.5	± 2.3	46.7	3.7	1.0
	PCPG	R2/Upper	301	8	0	0	1.03	± 0.04	PCPG	R2/Upper	15.5	± 1.0	15.1	0.4	0.0
		R2/Lower	330	13	1	0	1.04	± 0.03		R2/Lower	17.2	± 1.0	16.5	0.7	0.1
		R2	631	21	1	0	1.04	± 0.04		R2	32.7	± 1.7	31.6	1.1	0.1
PURE MEMBRANES															
0 to 900 ns (Entire simulation) N_{TF} = $9x10^4$	DPPC	R2/Upper	159	5	0	0	1.03	± 0.03	DPPC	R2/Upper	18.2	± 1.8	17.7	0.6	0.0
		R2/Lower	129	9	1	0	1.08	± 0.03		R2/Lower	15.4	± 1.6	14.3	1.0	0.1
		R2	288	14	1	0	1.05	± 0.03		R2	33.7	± 1.8	32.0	1.6	0.1
	DPPG	R2/Upper	177	6	7	0	1.11	± 0.04	DPPG	R2/Upper	21.1	± 1.3	19.7	0.7	0.8
		R2/Lower	183	15	3	0	1.10	± 0.03		R2/Lower	22.3	± 1.0	20.3	1.7	0.3
		R2	360	21	10	0	1.10	± 0.04		R2	43.4	± 1.7	40.0	2.3	1.1
	PCPG*	R2/Upper	113	2	0	0	1.02	± 0.03	PCPG*	R2/Upper	12.8	± 1.1	12.6	0.2	0.0
		R2/Lower	106	11	1	0	1.11	± 0.03		R2/Lower	13.1	± 1.0	11.8	1.2	0.1
		R2	219	13	1	0	1.06	± 0.03		R2	25.9	± 1.6	24.3	1.4	0.1
	PCPG	R2/Upper	139	4	0	0	1.03	± 0.04	PCPG	R2/Upper	15.9	± 1.6	15.4	0.4	0.0
		R2/Lower	136	4	0	0	1.03	± 0.03		R2/Lower	15.6	± 1.1	15.1	0.4	0.0
		R2	275	8	0	0	1.03	± 0.04		R2	31.4	± 1.6	30.6	0.9	0.0

TABLE S20: **Number of frames with N water molecules, $N_{WF}(N)$, and probability of finding water molecules inside the hydrophobic core of membranes, $P_W(N)$ with N = 1 to 4, in region R2.** $N_{WF} = \Sigma\ N_{WF}(N)$; $\langle N \rangle = \Sigma\ N \times N_{WF}(N)/N_{WF}$; $P_W(N) = N_{WF}(N)/N_{TF}$, where $N_{TF} = \tau_{tot}/\Delta t_r$ is the total number of trajectory frames analyzed and $N_{WF}(N)$ is the number of frames with N water molecules within the hydrophobic core membrane R2 region. Using a time resolution of Δt_r = 10 ps, the analyzes were performed: (i) over the first and last τ_{tot} = 300 ns of the peptide/membrane simulations, which the peptide-flip effect in the upper and lower sub-regions were highlighted in green for DPPG and in yellow for PCPG*, and (ii) over the whole peptide/membrane (τ_{tot} = 2000 ns) and pure membrane simulations (τ_{tot} = 900 ns), which the values in the whole R2 region were highlighted in gray.

System	R1			R2		
Pure membrane	**Upper**	**Lower**	**Total**	**Upper**	**Lower**	**Total**
DPPC	60	58	118	180	175	355
DPPG	59	56	115	195	197	392
PCPG	39	40	79	98	102	200
PCPG*	44	46	90	108	110	218
Peptide/membrane						
BP100 in DPPC	69	64	133	198	176	374
BP100 in DPPG	79	56	135	215	194	409
BP100 in PCPG	41	43	84	99	105	204
BP100 in PCPG*	67	48	115	203	111	314
Difference (peptide/membrane – pure membrane)						
DPPC	9	6	15	18	1	19
DPPG	20	0	20	20	-3	17
PCPG	2	3	5	1	3	4
PCPG*	23	2	25	95	1	96

TABLE S21: Total amount of water molecules (with different simulation indexes) that have entered into the R1 and R2 regions in the last 500 ns of pure and peptide/membrane simulations using a time resolution of 10 ps, i.e. using $N_{TF} = 5x10^4$ frames. The differences between pure and peptide containing simulations are also shown for comparison.

References

1. Pfeiffer, W.; Henkel, T. H.; Sackmann, E.; Knoll, W.; Knoll, W. Local Dynamics of Lipid Bilayers Studied by Incoherent Quasi-Elastic Neutron Scattering. *Epl* **1989**, *8* (2), 201–206. https://doi.org/10.1209/0295-5075/8/2/016.

2. Lindblom, G.; Orädd, G. Lipid Lateral Diffusion and Membrane Heterogeneity. *Biochim. Biophys. Acta - Biomembr.* **2009**, *1788* (1), 234–244. https://doi.org/10.1016/j.bbamem.2008.08.016.

3. Filippov, A. V.; Rudakova, M. A.; Oradd, G.; Lindblom, G. Lateral Diffusion of Saturated Phosphatidylcholines in Cholesterol- Containing Bilayers. *Biophysics (Oxf).* **2007**, *52* (3), 307–314. https://doi.org/10.1134/S0006350907030098.

4. Lindblom, G.; Orädd, G.; Filippov, A. Lipid Lateral Diffusion in Bilayers with Phosphatidylcholine, Sphingomyelin and Cholesterol. An NMR Study of Dynamics and Lateral Phase Separation. *Chem. Phys. Lipids* **2006**, *141* (1–2), 179–184. https://doi.org/10.1016/j.chemphyslip.2006.02.011.

5. Korchowiec, B.; Stachowicz-Kuśnierz, A.; Korchowiec, J. The Role of DPPG in Lung Surfactant Exposed to Benzo[a] Pyrene. *Environ. Sci. Process. Impacts* **2019**, *21* (3), 438–445. https://doi.org/10.1039/c8em00497h.

6. Park, P. *et al.* Binding and Flip as Initial Steps for BP-100 Antimicrobial Actions. *Sci. Rep.* **9**, 8622 (2019) doi: 10.1038/s41598-019-45075-5.