HIV gp41 Fusion Peptide Increases Membrane Ordering in a Cholesterol-Dependent Fashion

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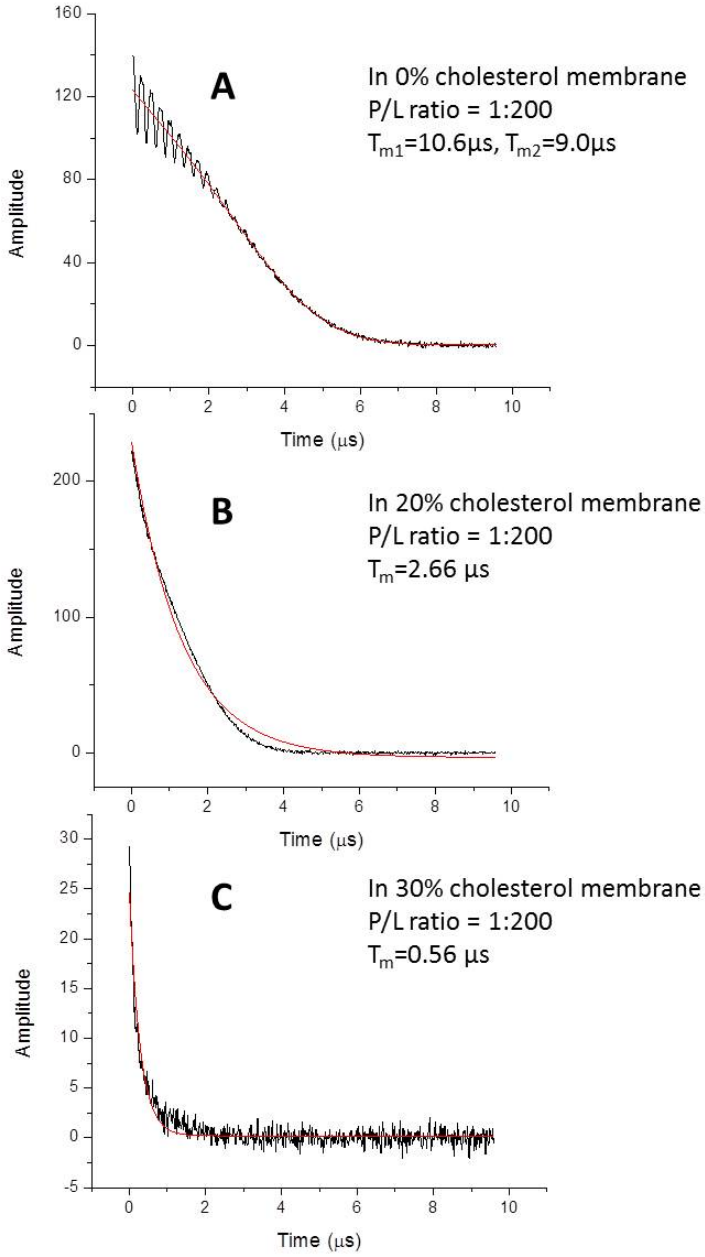
Supporting Materials

Supporting Figure SF 1

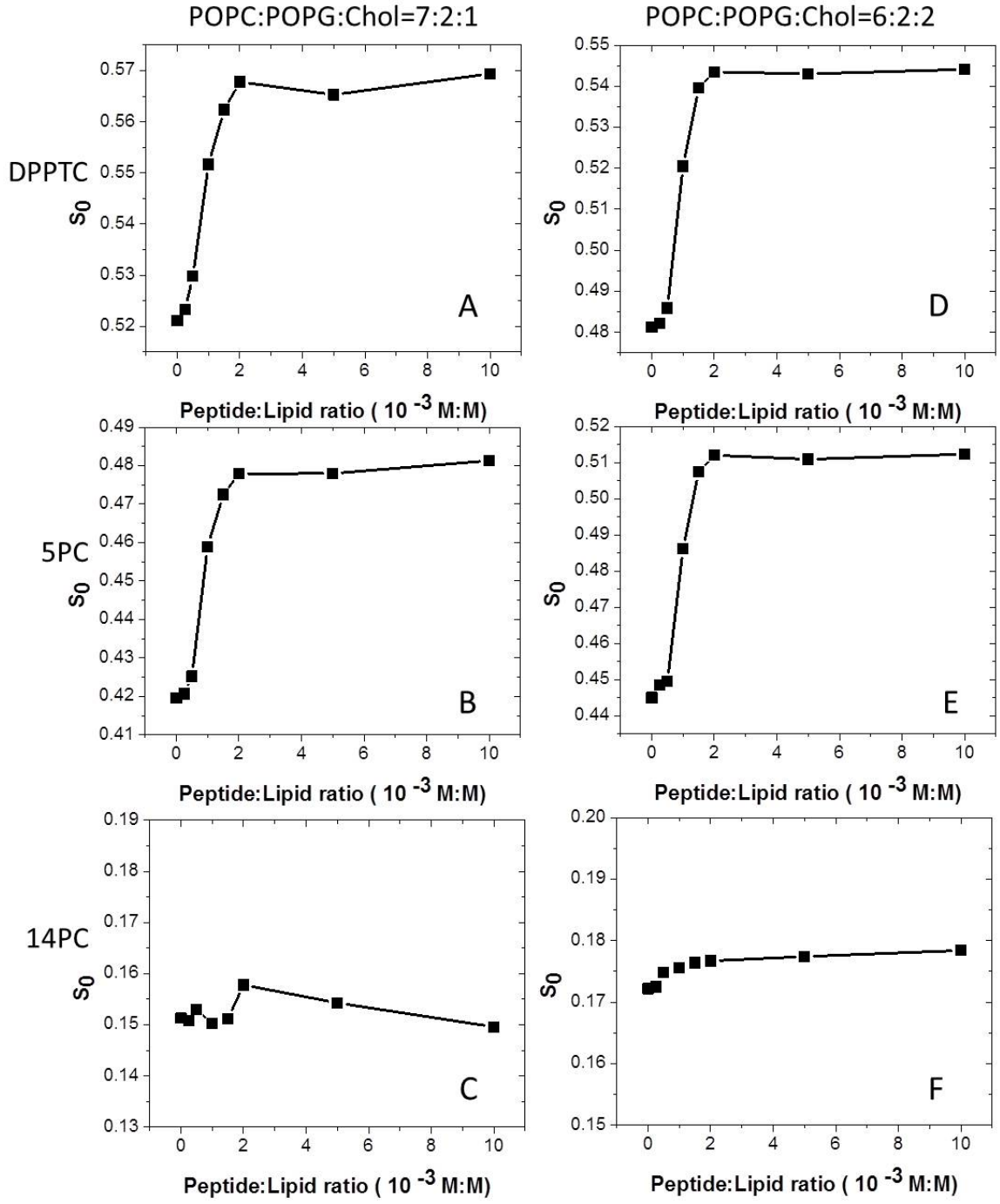


Plot of ESR spin-echo experiments on spin-labeled HIV FP A15R at 1:200 P/L ratio in POPC/POPG membranes containing 0% (A), 20% (B) and 30% (C) cholesterol performed at 17.3 GHz and 60K, showing amplitude of echo vs. t, the time between 90° and 180° pulses. The black curves are experimental and the red curves are fits to yield values of T_m, phase memory times.

Pulsed ESR Measurements

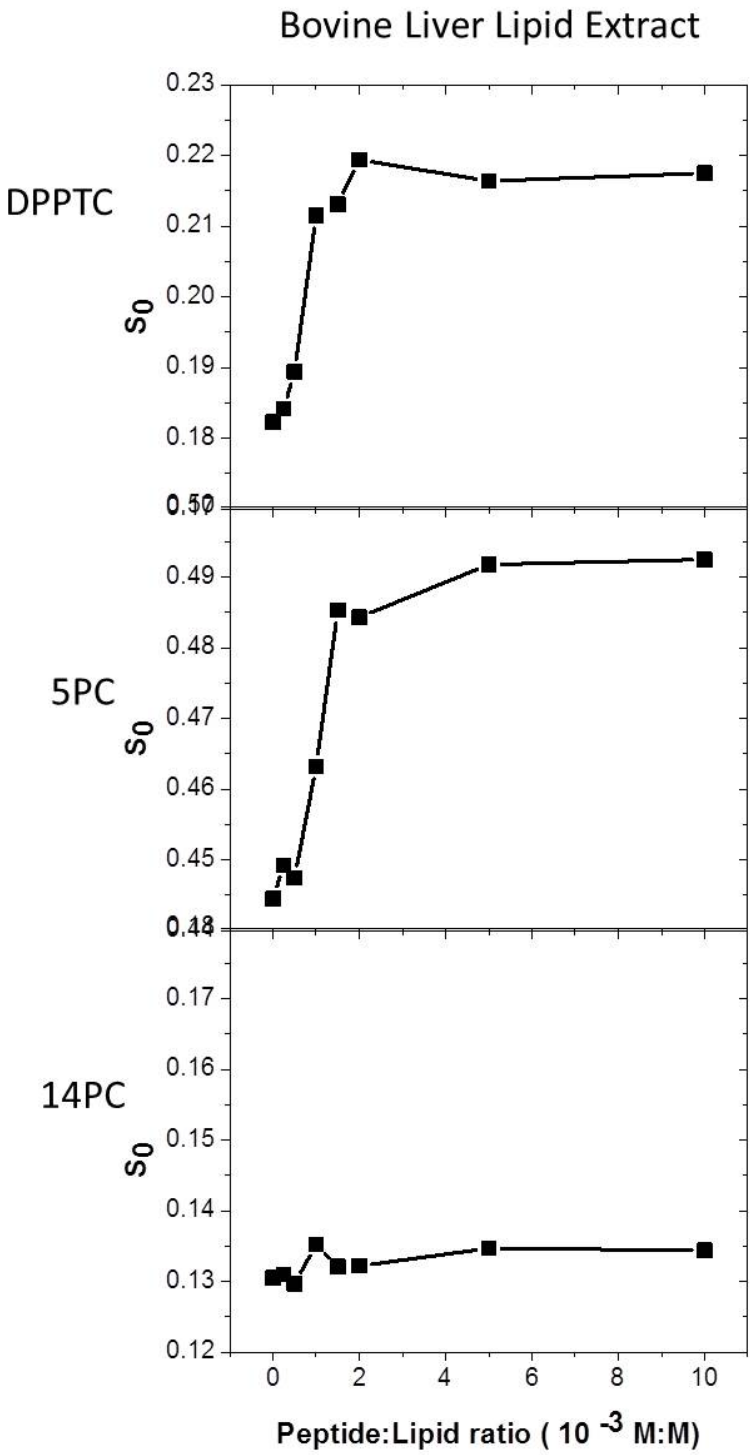
The spin echo experiments shown in Figure SF1 to measure the relaxation of spin-labeled HIV FP A15R in POPC/POPG membranes containing 0% (A), 20% (B) and 30% (C) cholesterol in 1:200 peptide:lipid ratio were performed at 17.3 GHz and 60K using a home-built Ku-band pulsed ESR spectrometer(1). The phase memory time, T_m was calculated by fitting the amplitude of the primary echo vs. time curve using Origin software (OriginLab Corp., Northampton, MA). While the relaxation curves in 30% and 20% cholesterol were well fit using a single exponential decay $V(t)=V_0\exp(-2t/Tm)$, the relaxation curve in 0% cholesterol was best fit by multiplying by another exponential with a cubic in time to yield $V(t)=V_0\exp(-2t/Tm_1)\cdot\exp[(-2t/Tm_2)^3]$, where the latter exponent is due to nuclear spin diffusion and related effects. The T_m for 30% cholesterol ($T_m\approx0.56\mu s$) is over an order of magnitude faster than those for 0% cholesterol ($T_{m1}\approx10.6\mu s$), and five times faster than that for 20% cholesterol $T_m\approx2.7\mu s$). The shorten T_m of the FP in 30% cholesterol membranes is largely due to the modulation of dipolar interactions between spin labels on aggregated peptides. Such a short T_m precluded us from observing any dipolar oscillations in the DEER experiments we performed. DEER experiments on 0% and 20% membranes did not show any evidence for dipolar oscillations from static dipolar interactions, as expected when the singly labeled peptides are well separated and in random arrays. Therefore, we do not show any of these uninformative DEER results.

Supporting Figure SF 2



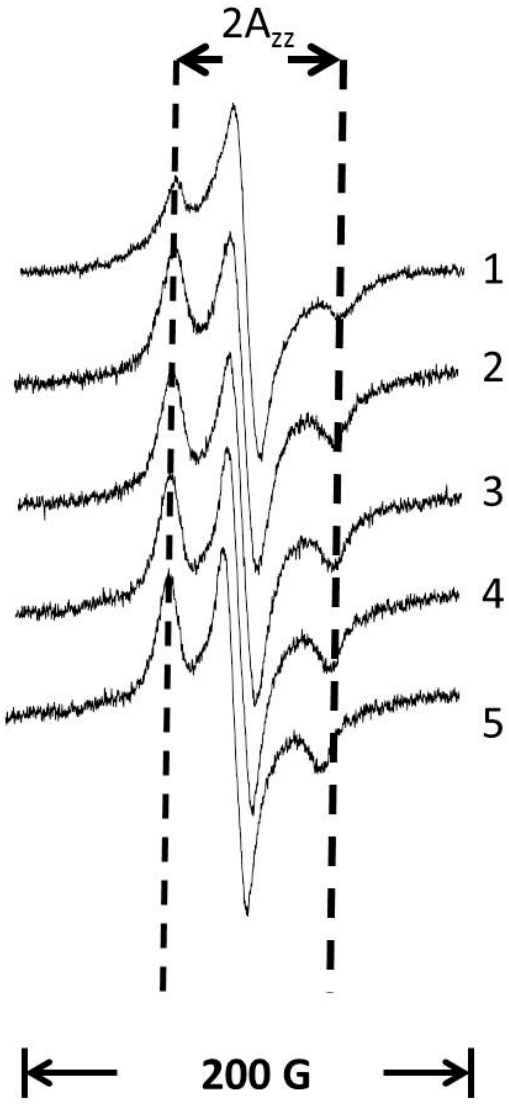
Plot of order parameters of DPPTC (A,D), 5PC (B,E), and 14PC (C,F) in POPC:POPG:Chol =7:2:1 (A-C) and POPC:POPG:Chol=6:2:2 (D-E) mixture versus P/L ratio of HIV FP at 25°C.

Supporting Figure SF3



Plot of order parameters of DPPTC, 5PC, and 14PC in LLE lipid mixture versus P/L ratio of HIV FP at 25°C.

Supporting Figure SF4



ESR spectra of A21R mutant in pH7 buffer (1), POPC:POPC=4:1 (2), POPC:POPG:Chol=7:2:1 (3), POPC:POPG:Chol=6:2:2 (4) and POPC:POPG:Chol=5:2:3 (5) MLVs recorded at 90K. The dashed lines indicate the alignment of $2A_{zz}$ splitting.

S0. G- and A- tensor components used for the simulations

System	g_{xx}	g_{yy}	g_{zz}	A_{xx}(G)	A_{yy}(G)	A_{zz}(G)
POPC:POPG=4:1						
DPPTC	2.0084	2.0062	2.0020	6.00	5.90	35.90
5PC	2.0089	2.0059	2.0025	5.50	5.50	33.20
14PC	2.0086	2.0059	2.0024	5.50	5.45	33.00
POPC:POPG=7:2:1						
DPPTC	2.0083	2.0060	2.0021	5.80	6.00	36.50
5PC	2.0088	2.0051	2.0024	5.40	5.80	33.20
14PC	2.0087	2.0059	2.0024	5.40	5.40	33.00
POPC:POPG=6:2:2						
DPPTC	2.0085	2.0063	2.0020	5.70	6.20	36.40
5PC	2.0088	2.0057	2.0025	5.40	6.10	33.30
14PC	2.0090	2.0060	2.0026	5.40	6.20	33.30
POPC:POPG=5:2:3						
DPPTC	2.0085	2.0064	2.0020	5.60	6.20	36.30
5PC	2.0087	2.0059	2.0022	5.40	5.90	33.50
14PC	2.0090	2.0063	2.0024	5.20	6.00	33.50
Liver lipid extract (LLE)						
DPPTC	2.0086	2.0064	2.0020	5.40	5.40	36.60
5PC	2.0086	2.0064	2.0024	5.40	5.40	34.70
14PC	2.0088	2.0064	2.0020	5.00	5.00	32.80

S1. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
Pure lipid	8.25	3.69	0.552
0.25	7.93	4.27	0.553
0.50	8.34	4.49	0.559
1.00	7.25	3.56	0.588
1.50	7.98	4.12	0.603
2.00	7.33	4.59	0.605
5.00	7.21	3.85	0.603
10.00	7.34	4.71	0.605

S2. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	3.07	4.52	0.359
0.25	3.07	4.59	0.358
0.50	3.05	4.44	0.363
1.00	3.13	4.12	0.397
1.50	3.32	4.03	0.411
2.00	3.35	4.10	0.412
5.00	3.29	4.13	0.413
10.00	3.21	4.25	0.412

S3. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	5.54	1.54	0.146
0.25	5.54	1.53	0.145
0.50	5.55	1.49	0.143
1.00	5.62	1.52	0.151
1.50	5.71	1.42	0.151
2.00	5.75	1.43	0.151
5.00	5.72	1.39	0.151
10.00	5.72	1.43	0.151

S4. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	8.54	1.27	0.602
0.25	8.57	1.28	0.602
0.50	8.44	1.31	0.607
1.00	8.51	1.45	0.629
1.50	8.32	1.39	0.648
2.00	8.43	1.71	0.651
5.00	8.39	1.63	0.651
10.00	8.36	1.59	0.651

S5. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	4.92	1.90	0.292
0.25	5.23	1.78	0.298
0.50	5.35	1.72	0.302
1.00	5.22	1.68	0.311
1.50	5.43	1.82	0.358
2.00	5.79	1.86	0.361
5.00	5.83	1.73	0.363
10.00	5.94	2.01	0.361

S6. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	5.73	1.56	0.070
0.25	5.60	1.64	0.070
0.50	5.96	1.45	0.070
1.00	6.11	1.38	0.071
1.50	6.04	1.47	0.071
2.00	6.61	1.27	0.070
5.00	6.51	1.28	0.072
10.00	6.58	1.29	0.071

S7. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	6.06	1.29	0.427
0.25	6.08	1.32	0.434
0.50	6.14	1.32	0.448
1.00	6.21	1.35	0.476
1.50	6.22	1.38	0.508
2.00	6.29	1.33	0.514
5.00	6.31	1.40	0.524
10.00	6.32	1.45	0.523

S8. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
Pure lipid	8.52	5.57	0.493
0.25	8.54	5.53	0.501
0.50	8.61	5.55	0.536
1.00	8.73	5.97	0.573
1.50	8.86	5.32	0.591
2.00	9.02	5.21	0.588
5.00	9.32	5.19	0.612
10.00	9.28	5.22	0.613

S9. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	4.65	2.24	0.264
0.25	4.64	2.16	0.271
0.50	4.83	1.93	0.272
1.00	4.85	1.86	0.291
1.50	5.11	1.78	0.296
2.00	5.32	1.66	0.303
5.00	5.39	1.65	0.345
10.00	5.54	1.54	0.347

S10. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
Pure lipid	0.292	1.39	0.558
0.25	0.298	1.39	0.559
0.50	0.302	1.43	0.561
1.00	0.311	1.44	0.583
1.50	0.358	1.52	0.594
2.00	0.361	1.54	0.601
5.00	0.363	1.54	0.625
10.00	0.361	1.57	0.628

S11. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
Pure lipid	6.71	9.81	0.411
0.25	6.99	9.03	0.413
0.50	6.87	9.53	0.415
1.00	6.24	9.32	0.446
1.50	6.35	9.47	0.451
2.00	6.55	9.78	0.461
5.00	6.41	9.42	0.481
10.00	6.80	9.56	0.482

S12. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	4.48	2.49	0.152
0.25	4.56	2.41	0.151
0.50	4.52	2.38	0.162
1.00	4.44	2.52	0.201
1.50	4.82	2.09	0.216
2.00	4.91	2.13	0.219
5.00	4.34	2.71	0.275
10.00	4.47	2.79	0.284

S13. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in LLE dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	5.59	4.83	0.182
0.25	4.98	3.72	0.184
0.50	5.23	4.12	0.189
1.00	5.33	4.34	0.212
1.50	5.20	4.01	0.213
2.00	4.88	4.32	0.219
5.00	4.87	4.45	0.216
10.00	4.62	4.21	0.218

S14. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in LLE dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
Pure lipid	6.72	7.03	0.445
0.25	6.88	7.23	0.449
0.50	6.82	7.89	0.447
1.00	6.45	7.33	0.463
1.50	6.50	7.52	0.485
2.00	6.67	7.35	0.484
5.00	6.30	7.75	0.492
10.00	6.34	7.68	0.493

S15. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in LLE dispersions vs. P/L ratio of gp41 FP at 37°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	1.22	2.37	0.131
0.25	1.10	2.32	0.131
0.50	1.32	2.59	0.130
1.00	1.54	2.75	0.135
1.50	1.32	2.88	0.132
2.00	1.56	2.72	0.132
5.00	1.62	2.69	0.135
10.00	1.65	2.54	0.134

S16. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP V2E mutant at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
Pure lipid	8.25	3.69	0.552
0.25	8.27	3.34	0.553
0.50	8.54	3.45	0.560
1.00	8.95	3.82	0.561
1.50	8.31	3.61	0.572
2.00	8.21	3.74	0.574

S17. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP V2E mutant at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	3.07	4.52	0.359
0.25	3.58	4.76	0.357
0.50	3.24	4.87	0.358
1.00	3.35	4.63	0.358
1.50	3.71	4.49	0.359
2.00	3.21	4.56	0.360

S18. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in POPC:POPG=4:1 dispersions vs. P/L ratio of gp41 FP V2E mutant at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	5.54	1.54	0.146
0.25	5.52	1.63	0.143
0.50	5.48	1.75	0.145
1.00	5.30	1.82	0.146
1.50	5.37	1.77	0.147
2.00	5.63	1.69	0.148

S19. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP V2E mutant at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	6.06	1.29	0.427
0.25	6.07	1.32	0.429
0.50	6.21	1.46	0.431
1.00	6.33	1.34	0.440
1.50	6.23	1.35	0.460
2.00	6.25	1.35	0.460

S20. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP V2E mutant at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
Pure lipid	8.52	5.57	0.493
0.25	8.37	5.99	0.500
0.50	8.36	5.83	0.501
1.00	8.72	5.71	0.507
1.50	8.85	5.35	0.507
2.00	8.60	5.75	0.508

S21. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in POPC:POPG:Chol=5:2:3 dispersions vs. P/L ratio of gp41 FP V2E mutant at 25°C

peptide/lipid (10^{-3})	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
Pure lipid	4.65	2.24	0.264
0.25	4.62	2.21	0.263
0.50	4.73	2.33	0.262
1.00	4.25	2.41	0.264
1.50	4.30	2.59	0.266
2.00	4.27	2.60	0.264

Supporting References

1. Borbat, P. P., E. R. Georgieva, and J. H. Freed. 2013. Improved Sensitivity for Long-Distance Measurements in Biomolecules: Five-Pulse Double Electron-Electron Resonance. The journal of physical chemistry letters 4:170-175.