Supporting Materials

The interaction between influenza HA fusion peptide and transmembrane domain affects membrane structure

Alex L. Lai† and Jack H. Freed†*

†Department of Chemistry and Chemical Biology, Cornell University, Ithaca, N.Y. 14853

Supporting Materials

Figure S5 Plot of $\Delta\Delta S_0$ of DPPTC, 5PC and 14PC versus 23-mer FP concentration.

Methods Two-Component Fitting Strategy.

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)
DMPC/DMPG/Chol=40:30:30						
DPPTC	2.0084	2.0064	2.0020	6.00	6.00	36.45
5PC	2.0090	2.0060	2.0024	5.40	6.20	33.20
14PC	2.0088	2.0064	2.0020	4.80	5.20	33.20

S1. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in pure lipid vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0*	δS_0** (uncertainty from fitting)	δS_0*** (ave over experiments)
0	6.14	5.12	0.412	0.0021	0.008
0.125	6.21	5.54	0.413	0.0034	0.007
0.25	6.32	5.95	0.450	0.0014	0.005
0.50	6.17	5.01	0.451	0.0009	0.006
1.0	6.85	5.40	0.451	0.0012	0.007
2.0	6.42	5.81	0.451	0.0014	0.006

*The $R_{\perp}$, $R_{||}$ and S_0 are the average of three experiments on WT.

** The uncertainty of from the fitting, δS_0, is obtained from those of C_{20} and C_{22} and their uncertainties, and represents the maximum uncertainty obtained in the repeated experiments.

** *The δS_0 from the average over three experiments is the standard deviation from the repeated experiments.

S2. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in pure lipid vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	3.80	4.47	0.512	0.0017	0.007
0.125	3.41	4.28	0.512	0.0016	0.006
0.25	3.79	4.60	0.546	0.0023	0.006
0.50	2.98	4.52	0.556	0.0017	0.005
1.0	3.17	4.54	0.557	0.0016	0.007
2.0	3.16	4.73	0.557	0.0018	0.007

S3. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in pure lipid vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	1.28	1.94	0.247	0.0009	0.005
0.125	1.15	1.85	0.247	0.0023	0.004
0.25	1.04	1.57	0.246	0.0012	0.006
0.50	1.05	1.56	0.247	0.0031	0.006
1.0	1.10	1.62	0.248	0.0029	0.005
2.0	1.09	1.77	0.247	0.0015	0.004

S4. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in pure lipid vesicles vs. P/L ratio of WT FP at 37°C, pH7

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	6.24	5.25	0.412	0.0027	0.006
0.125	6.25	5.43	0.413	0.0042	0.005
0.25	6.70	5.59	0.438	0.0031	0.006
0.50	6.67	5.60	0.439	0.0051	0.005
1.0	6.75	5.94	0.44	0.0022	0.006
2.0	6.75	5.89	0.44	0.0035	0.004

S5. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in pure lipid vesicles vs. P/L ratio of WT FP at 37°C, pH7

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	3.55	3.94	0.511	0.0017	0.007
0.125	3.56	3.89	0.512	0.0032	0.006
0.25	4.04	4.17	0.531	0.0025	0.005
0.50	3.92	3.94	0.531	0.0008	0.008
1.0	3.75	4.01	0.533	0.0012	0.005
2.0	3.74	4.01	0.532	0.0019	0.004

S6. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in pure lipid vesicles vs. P/L ratio of WT FP at 37°C, pH7

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	1.35	1.84	0.242	0.0009	0.006
0.125	1.25	1.91	0.242	0.0031	0.004
0.25	1.35	1.94	0.243	0.0012	0.004
0.50	1.35	1.94	0.243	0.0034	0.006
1.0	1.34	1.85	0.243	0.0026	0.008
2.0	1.35	1.84	0.243	0.0021	0.006

S7. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in 0.5% TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	6.17	4.74	0.431	0.0038	0.006
0.125	6.24	4.23	0.452	0.0025	0.008
0.25	6.71	4.59	0.501	0.0051	0.007
0.50	6.58	4.21	0.514	0.0035	0.006
1.0	6.98	4.12	0.531	0.0019	0.007
2.0	6.95	4.18	0.531	0.0038	0.006

S8. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	3.45	3.58	0.532	0.0032	0.005
0.125	3.86	3.37	0.544	0.0009	0.006
0.25	3.94	3.26	0.575	0.0012	0.007
0.50	4.01	3.25	0.589	0.0023	0.005
1.0	4.12	3.26	0.603	0.0032	0.008
2.0	4.12	3.27	0.605	0.0016	0.007

S9. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	1.10	1.75	0.251	0.0025	0.005
0.125	1.20	1.79	0.257	0.0008	0.005
0.25	1.38	1.92	0.272	0.0036	0.006
0.50	1.42	2.03	0.284	0.0027	0.007
1.0	1.43	2.03	0.285	0.0050	0.008
2.0	1.42	2.03	0.286	0.0050	0.007

S10. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in 0.5% TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH7

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	6.16	5.24	0.428	0.0015	0.004
0.125	6.38	5.01	0.448	0.0049	0.003
0.25	6.49	4.88	0.496	0.0032	0.005
0.50	6.50	4.76	0.502	0.0009	0.006
1.0	6.55	4.65	0.511	0.0017	0.004
2.0	6.52	4.58	0.513	0.0021	0.003

S11. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH7

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	3.57	3.68	0.531	0.0035	0.005
0.125	3.57	3.86	0.545	0.0020	0.005
0.25	3.79	3.49	0.576	0.0020	0.004
0.50	3.68	3.85	0.585	0.0017	0.003
1.0	3.68	3.91	0.591	0.0016	0.006
2.0	3.89	3.78	0.592	0.0012	0.007

S12. Rotational diffusion rates $R_\perp$ and $R_{||}$, and order parameter S_0 of 14PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH7

peptide/lipid (%)	$R_\perp$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0	δS_0 (uncertainty from fitting)	δS_0 (ave over experiments)
0	1.06	1.75	0.251	0.0021	0.007
0.125	1.08	1.73	0.256	0.0015	0.006
0.25	1.89	1.70	0.276	0.0023	0.007
0.50	1.74	1.59	0.287	0.0019	0.006
1.0	1.89	1.49	0.288	0.0009	0.005
2.0	1.85	1.44	0.288	0.0011	0.004

S13. Rotational diffusion rates $R_\perp$ and $R_{||}$, and order parameter S_0 of DPPTC in pure lipid vesicles vs. P/L ratio of G1S FP at 37°C, pH5

peptide/lipid (%)	$R_\perp$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0*
0	6.19	4.99	0.412
0.125	6.23	4.79	0.412
0.25	6.51	5.07	0.445
0.50	6.42	4.86	0.443
1.0	6.80	4.95	0.444
2.0	6.58	4.96	0.446

* The $R_\perp$ and $R_{||}$ and S_0 are the average of two experiments on the mutants.

S14. Rotational diffusion rates $R_\perp$ and $R_{||}$, and order parameter S_0 of 5PC in pure lipid vesicles vs. P/L ratio of G1S FP at 37°C, pH5

peptide/lipid (%)	$R_\perp$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
0	3.50	4.20	0.512
0.125	3.71	4.08	0.512
0.25	3.99	4.38	0.535
0.50	3.96	4.22	0.536
1.0	3.93	4.27	0.536
2.0	3.93	4.36	0.536

S15. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in pure lipid vesicles vs. P/L ratio of G1S FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0
0	1.31	1.89	0.247
0.125	1.20	1.88	0.245
0.25	1.18	1.75	0.246
0.50	1.19	1.74	0.245
1.0	1.21	1.73	0.247
2.0	1.21	1.80	0.247

S16. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in 0.5% TMD reconstituted vesicles vs. P/L ratio of G1S FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
0	6.16	5.18	0.431
0.125	6.31	5.27	0.431
0.25	6.60	5.39	0.457
0.50	6.54	4.88	0.459
1.0	6.76	5.01	0.461
2.0	6.73	5.16	0.461

S17. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of G1S FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
0	3.68	3.63	0.532
0.125	3.49	3.61	0.533
0.25	3.79	3.37	0.567
0.50	3.31	3.54	0.569
1.0	3.42	3.57	0.571
2.0	3.51	3.52	0.571

S18. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of G1S FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0
0	1.08	1.75	0.251
0.125	1.14	1.76	0.254
0.25	1.61	1.81	0.253
0.50	1.57	1.80	0.253
1.0	1.64	1.74	0.254
2.0	1.62	1.71	0.254

S19. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in pure lipid vesicles vs. P/L ratio of G1V FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
0	6.19	5.05	0.412
0.125	6.23	4.83	0.412
0.25	6.57	4.95	0.419
0.50	6.49	4.61	0.421
1.0	6.82	4.59	0.423
2.0	6.67	4.64	0.423

S20. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in pure lipid vesicles vs. P/L ratio of G1V FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
0	3.57	4.05	0.512
0.125	3.64	3.93	0.512
0.25	3.84	4.15	0.513
0.50	3.66	4.01	0.513
1.0	3.73	4.05	0.515
2.0	3.84	4.11	0.515

S21. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in pure lipid vesicles vs. P/L ratio of G1V FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0
0	1.17	1.86	0.247
0.125	1.20	1.87	0.246
0.25	1.45	1.81	0.247
0.50	1.45	1.82	0.247
1.0	1.47	1.80	0.246
2.0	1.46	1.85	0.247

S22. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in 0.5% TMD reconstituted vesicles vs. P/L ratio of G1V FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
0	6.16	5.12	0.431
0.125	6.30	5.25	0.431
0.25	6.53	5.53	0.436
0.50	6.47	5.15	0.437
1.0	6.74	5.42	0.436
2.0	6.64	5.55	0.437

S23. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of G1V FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
0	3.62	3.77	0.532
0.125	3.56	3.76	0.533
0.25	3.94	3.58	0.534
0.50	3.60	3.75	0.534
1.0	3.61	3.78	0.531
2.0	3.60	3.75	0.534

S24. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of G1V FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0
0	1.21	1.78	0.251
0.125	1.14	1.77	0.251
0.25	1.33	1.75	0.253
0.50	1.30	1.72	0.252
1.0	1.37	1.68	0.251
2.0	1.35	1.67	0.253

S25. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in pure lipid vesicles vs. P/L ratio of W14A FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
0	6.19	5.00	0.412
0.125	6.23	4.83	0.412
0.25	6.51	5.09	0.423
0.50	6.42	4.91	0.425
1.0	6.80	5.03	0.425
2.0	6.59	5.04	0.425

S26. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in pure lipid vesicles vs. P/L ratio of W14A FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
0	3.50	4.21	0.512
0.125	3.71	4.09	0.512
0.25	3.99	4.39	0.514
0.50	3.97	4.23	0.513
1.0	3.94	4.28	0.514
2.0	3.93	4.37	0.513

S27. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in pure lipid vesicles vs. P/L ratio of W14A FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0
0	1.23	1.89	0.247
0.125	1.23	1.88	0.247
0.25	1.37	1.76	0.248
0.50	1.39	1.75	0.244
1.0	1.39	1.74	0.245
2.0	1.39	1.81	0.246

S28. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in 0.5% TMD reconstituted vesicles vs. P/L ratio of W14A FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
0	6.17	5.18	0.431
0.125	6.31	5.28	0.433
0.25	6.60	5.42	0.439
0.50	6.54	4.89	0.444
1.0	6.77	5.03	0.442
2.0	6.74	5.20	0.443

S29. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of W14A FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
0	3.69	3.63	0.532
0.125	3.49	3.62	0.538
0.25	3.79	3.38	0.549
0.50	3.33	3.55	0.557
1.0	3.43	3.59	0.558
2.0	3.53	3.53	0.557

S30. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in 0.5% TMD reconstituted vesicles vs. P/L ratio of W14A FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0
0	1.17	1.75	0.251
0.125	1.12	1.76	0.251
0.25	1.47	1.81	0.259
0.50	1.40	1.81	0.264
1.0	1.50	1.76	0.264
2.0	1.47	1.74	0.264

S31. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in 0.5% K183E TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
0	6.19	5.15	0.412
0.125	6.25	5.30	0.413
0.25	6.62	5.52	0.450
0.50	6.54	5.09	0.451
1.0	6.83	5.33	0.451
2.0	6.74	5.47	0.451

S32. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in 0.5% K183E TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
0	3.62	3.74	0.512
0.125	3.56	3.69	0.512
0.25	3.84	3.58	0.546
0.50	3.50	3.66	0.556
1.0	3.58	3.69	0.557
2.0	3.66	3.67	0.557

S33. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in 0.5% K183E TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0
0	1.16	1.82	0.247
0.125	1.14	1.84	0.247
0.25	1.43	1.85	0.246
0.50	1.39	1.87	0.247
1.0	1.47	1.84	0.248
2.0	1.45	1.85	0.247

S34. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of DPPTC in 0.5% L187A TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^8 s^{-1}$)	S_0
0	6.16	5.02	0.412
0.125	6.29	4.78	0.413
0.25	6.49	4.95	0.450
0.50	6.42	4.67	0.451
1.0	6.74	4.67	0.451
2.0	6.58	4.70	0.451

S35. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 5PC in 0.5% L187A TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^7 s^{-1}$)	$R_{||}$ ($10^7 s^{-1}$)	S_0
0	3.56	4.08	0.512
0.125	3.63	4.00	0.512
0.25	3.94	4.14	0.546
0.50	3.76	4.10	0.556
1.0	3.76	4.14	0.557
2.0	3.77	4.19	0.557

S36. Rotational diffusion rates $R_{\perp}$ and $R_{||}$, and order parameter S_0 of 14PC in 0.5% L187A TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_{\perp}$ ($10^8 s^{-1}$)	$R_{||}$ ($10^9 s^{-1}$)	S_0
0	1.23	1.82	0.247
0.125	1.20	1.79	0.247
0.25	1.34	1.71	0.246
0.50	1.35	1.68	0.247
1.0	1.37	1.64	0.248
2.0	1.36	1.67	0.247

S37. Populations, rotational diffusion rates $R_\perp$, and order parameter S_0 of 5PC in 1% TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_\perp$ ($10^7 s^{-1}$)	S_0	Population (%)
0			
Comp.1	4.10	0.55	75
Comp.2	6.46	0.59	25
0.5			
Comp.1	4.23	0.52	63
Comp.2	6.95	0.69	37
1.0			
Comp.1	4.44	0.52	48
Comp.2	6.85	0.71	52

S38. Populations, rotational diffusion rates $R_\perp$, and order parameter S_0 of 5PC in 2% TMD reconstituted vesicles vs. P/L ratio of WT FP at 37°C, pH5

peptide/lipid (%)	$R_\perp$ ($10^7 s^{-1}$)	S_0	Relative population
0			
Comp.1	4.10	0.54	70
Comp.2	9.14	0.68	30
0.5			
Comp.1	3.94	0.47	59
Comp.2	10.2	0.72	41
1.0			
Comp.1	3.54	0.42	50
Comp.2	10.5	0.73	50

S39. Populations, rotational diffusion rates $R_\perp$, and order parameter S_0 of 5PC in DMPC/DMPG/Chol vesicles with 1% TMD + 1% FP at 25°C, 30°C, and 37°C, pH5

Temperature	$R_\perp$ ($10^7 s^{-1}$)	S_0	Population (%)
25°C			
Comp.1	3.29	0.56	45
Comp.2	5.34	0.75	55
30°C			
Comp.1	4.12	0.55	48
Comp.2	6.19	0.72	52
37°C			
Comp.1	4.44	0.52	48
Comp.2	6.85	0.71	52

S40. Typical Correlation Matrixes of the fittings (A) 5PC in 1% FP in DMPC/DMPG/Chol MLV, pH5 and (B) 1% FP in 1% TMD reconstituted membranes, pH5.

A)

	RBAR	C20	C22
	1.0000	0.0947	-0.1602
		1.0000	0.3433
			1.0000

B)

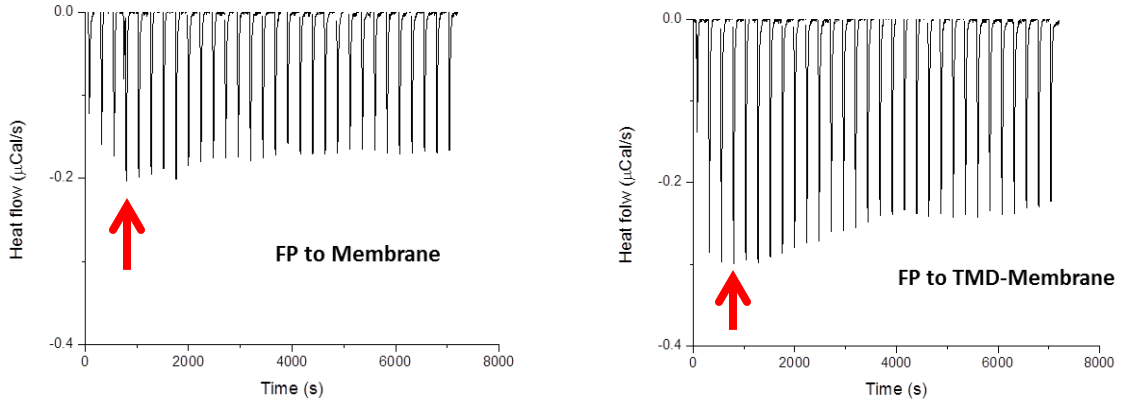
	RBAR(1)	RBAR(2)	C20(1)	C20(2)	SITE1	SITE2
	1.0000	-0.2719	0.3495	0.2801	0.2490	-0.3088
		1.0000	-0.3500	-0.1323	-0.3668	0.2719
			1.0000	-0.2403	0.4784	-0.1855
				1.0000	-0.0520	-0.1969
					1.0000	-0.6079
						1.0000

S41. Thermodynamic parameters of fusion peptide binding to lipid bilayers composed of POPC/POPG (4:1) at pH 5.

Titration	**ΔH (lipid)** kCal/mol	**ΔH (lipid +TMD)** kCal/mol	**ΔH (FP-TMD)** kCal/mol
WT FP → WT-TMD	–16.08 ± 0.38	–21.75 ± 0.54	-5.67
G1S FP → WT-TMD	–15.93 ± 0.57	–17.38 ± 0.33	-1.45
G1V FP → WT-TMD	–12.65 ± 0.40	–11.99 ± 0.27	0.66
W14A FP → WT-TMD	–13.89 ± 0.61	–17.08 ± 0.34	-3.19
ΔG1 FP → WT-TMD	–9.57 ± 0.32	–9.72 ± 0.11	-0.15
WT-FP → K183E TMD	-16.08± 0.38	-20.71 ± 0.14	-4.63
WT-FP → L187A TMD	-16.08± 0.38	-18.01 ± 0.43	-1.93

Figure S1 Binding of FPs to lipid only or TMD-reconstituted POPC:POPG=4:1 SUVs at 37°C by isothermal titration calorimetry.

A) Measurement of enthalpy change by titrating WT FP to a large excess of lipid and TMD. Left, WT-FP to lipid only SUVs; right, WT FP to TMD-reconstituted SUVs. We used the data starting from the 4th injection (arrow) to get rid of the unstable initial injections.



B) Reaction enthalpy of each injection during the titration, blue WT FP to WT-TMD reconstituted membrane; brown, WT FP to K183E TMD reconstituted membrane; green, WT FP to L187A TMD reconstituted membrane; purple, WT FP to lipid only membranes.

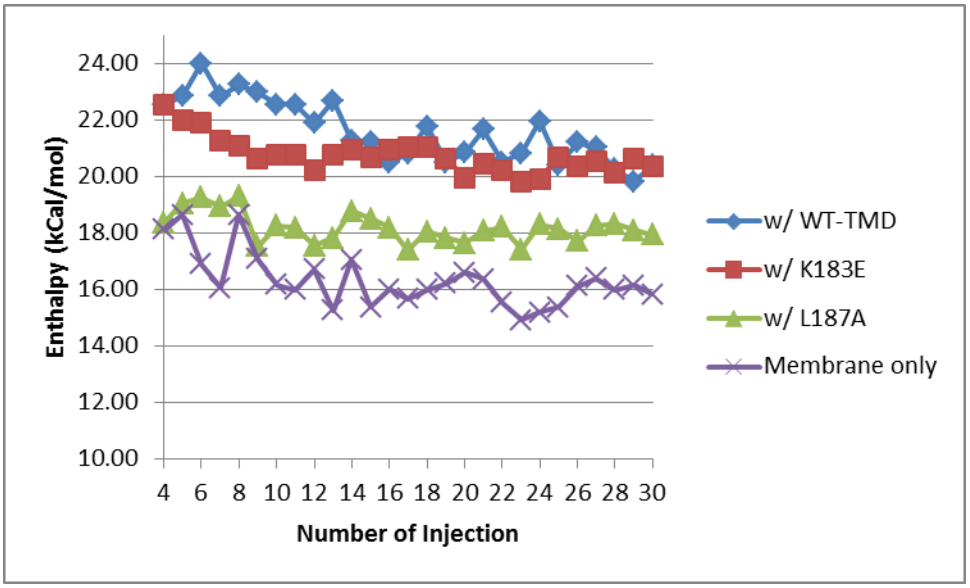


Figure S2 ESR Spectra of 5PC in DMPC:DMPG:Chol=40:30:30 MLVs with 1% TMD, 1% TMD + 0.5% FP and 1% TMD+1% FP, and 2% TMD recorded at 37°C. The outer peak separations of the spectra are 53.86 G, 54.45 G, 55.33 G, and 54.94 G, respectively.

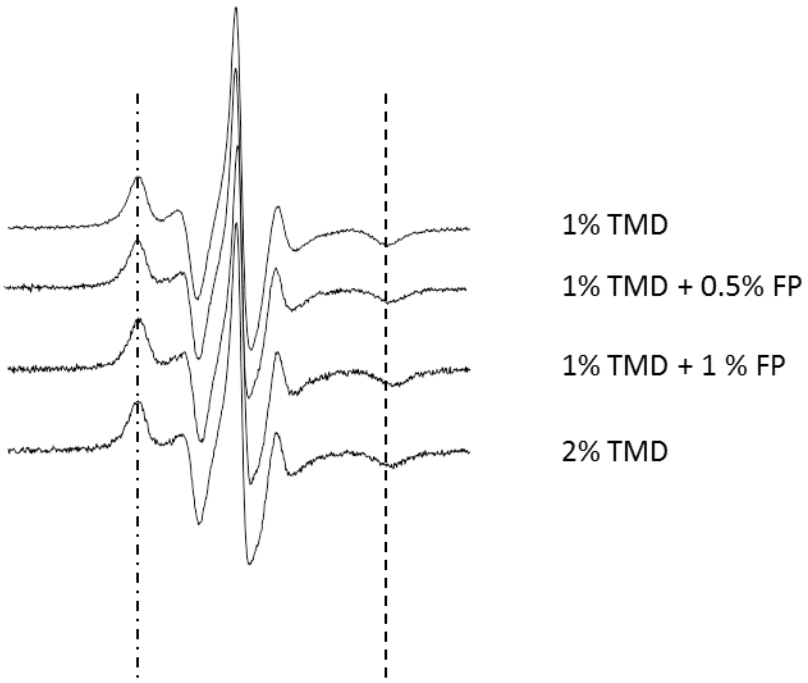
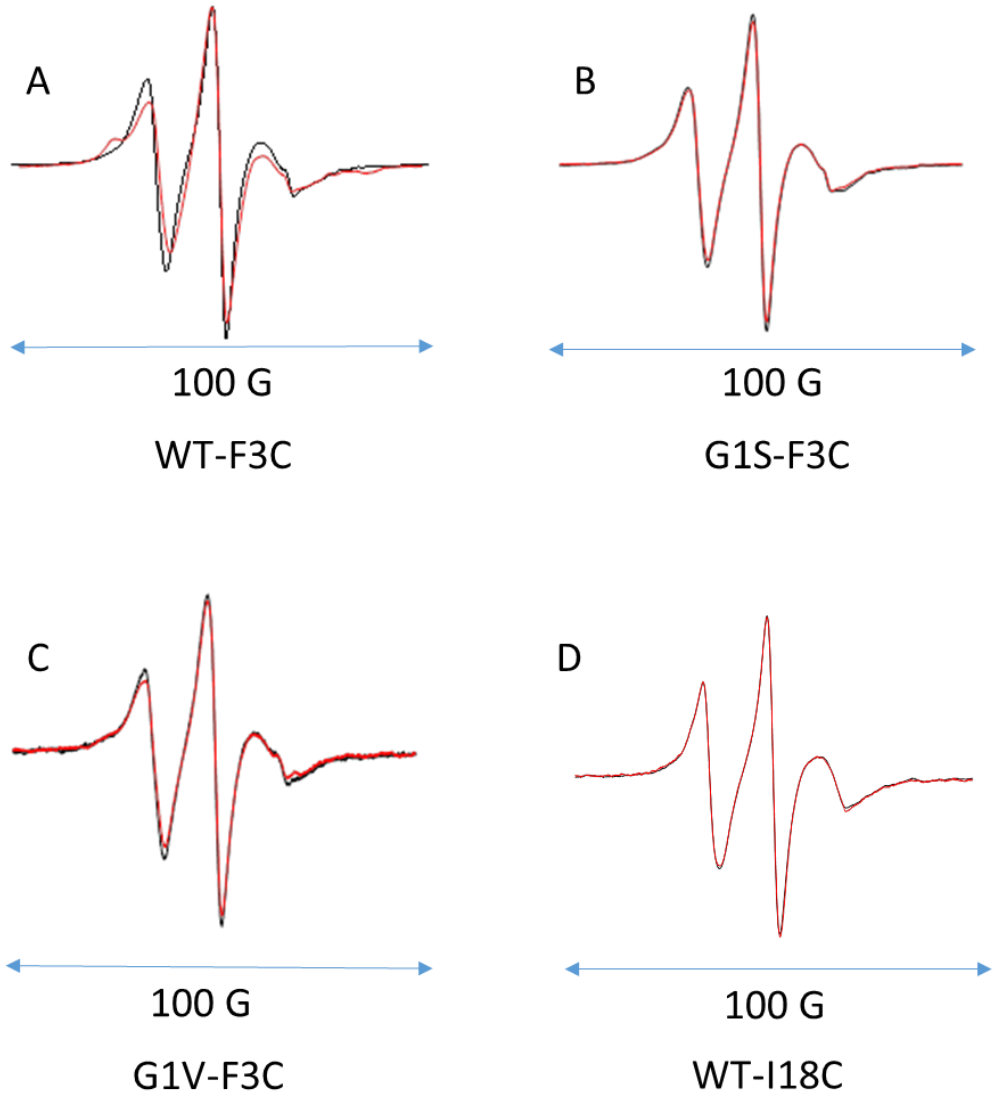


Figure S3 **ESR Spectra of WT-F3R1 (A), G1S-FP-F3R1 (B), G1V-F3R1 (C) and WT-I18R1 (D) in DMPC:DMPG:Chol=40:30:30 LUV's without (black) and with (red) 0.5% TMD reconstituted at RT.**

Figure SF3



-------- pure lipid membrane
-------- TMD-reconstituted membrane

Figure S4. Representative ESR spectra of spin-labeled lipids in DMPC:DMPG:Chol=40:30:30 MLVs, showing the changes upon FP binding. A-C), the spectra of DPPTC (A), 5PC (B) and 14PC (C) in 0.5% TMD-reconstituted membranes with 0.125% WT FP (black) and 0.5% WT FP (red). D-F), the spectra of DPPTC (D), 5PC (E) and 14PC (F) in 0.5% TMD-reconstituted membranes with 0.125% G1V FP (black) and 0.5% G1V-FP (red). G-I), comparing the spectra of DPPTC (G), 5PC (H) and 14PC (I) in TMD-reconstituted membranes (black) and pure lipid membranes (red) with 0.5% WT FP. The differences are magnified by 2x in the insets.

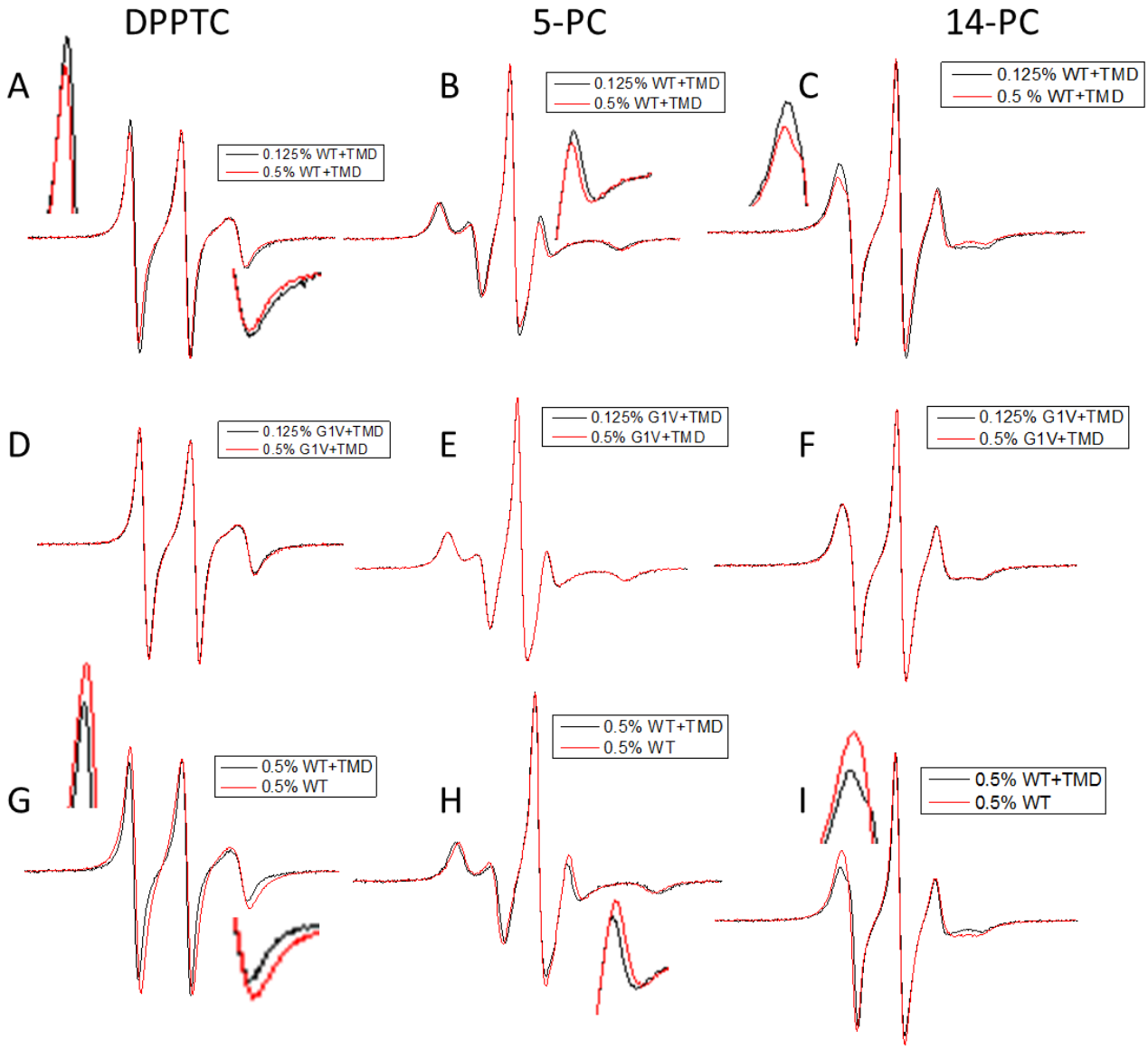
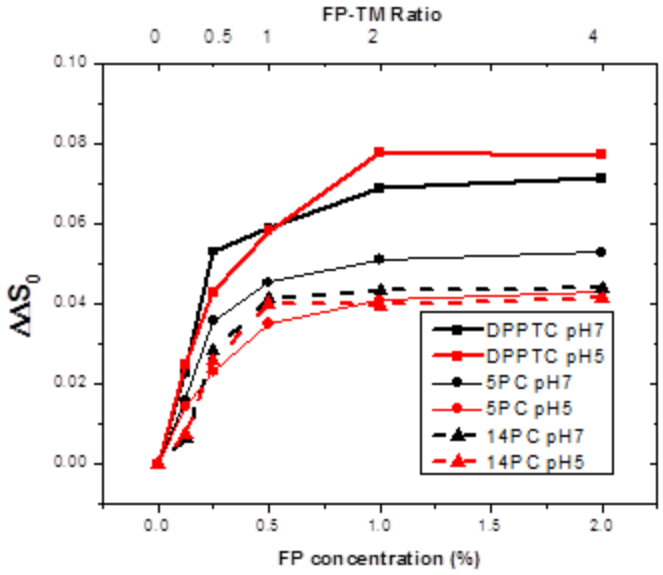


Figure S5. Plot of $\Delta\Delta S_0$ of DPPTC (solid thick line), 5PC (solid thin line) and 14PC (dashed thick line) versus 23-mer FP concentration in DMPC:DMPG:Chol=40:30:30 MLV with 0.5% TMD. Black, pH7, red, pH5.



Supporting Methods

Two Component Fitting Strategy

The fitting strategy for two components described below was previously described in Ref 17. Initially the membranes were assumed to consist of a single phase, and the ESR spectra were analyzed as having only one spectral component as described in the main text. Then the parameters that would converge to the best fit were used as a "seed" or initial parameter set for the major component, and the second "seed" parameters were from the previous experiments of similar systems showing two components (Ref 3). The two sets of seed parameters were used to test the possibility of the existence of a second component. We compared the best fits of the one and two components by their respective χ^2, and their correlation matrix and we examined the detailed features of the final simulation compared to the experimental spectrum (Ref 17). We found that the C_{22}'s were always small (-0.1 to 0.1) and only had a modest effect on the predicted spectrum, so we repeated the fitting using the C_{22}'s obtained in the fitting of both components. In the repeated fitting the values of S_0 changed by no more than ±0.01 from the original fitting. We report in Table S40 a typical correlation matrix for fitting to the reduced number of parameters. This fitting procedure provided a consistent set of results, yielding reproducible S_0's and fractions of the two components both in fitting each experiment using several sets of seed values to initiate the fitting as well as over the fitting of three independent experiments. The correlation coefficients are less than 0.35 (Table S40) and the observed effect of C_{22} variation of the final value of S_0 was less than 0.01. This fitting procedure provided a consistent set of results in terms of reproducible S_0's and fractions of two components obtained in the fitting. We repeated the experiments on these two-component samples at two additional temperatures (25 and 30 °C) in addition to the original temperature (37 °C). Fitting these spectra yields the trend in R_{bar} and S_0 (Table S39) which we expected based on our previous work (Ref 19) and little change in fractions of the components (also expected) . The uncertainty in the S_0 for both components is smaller than 0.03 and the uncertainty in the percentages of the components is smaller than ±1.5%.