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Supporting Material

**Two Conserved Residues Are Important for Inducing Highly
Ordered Membrane Domains by the Transmembrane Domain of
Influenza Hemagglutinin**

Mingtao Ge and Jack H. Freed

Supplementary Materials

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Figure SF1

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						
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
DPPTC	2.0084	2.0064	2.0020	6.00	6.00	36.45
DMPC/DMPG/Chol=60/10/30						
5PC	2.0090	2.0060	2.0024	5.40	6.20	33.30
14PC	2.0088	2.0064	2.0024	4.80	5.20	33.30
DMPC/Chol=70/30						
14PC	2.0088	2.0064	2.0024	4.80	5.10	33.40
Liver lipid total extrat (LLE)						
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
DPPTC	2.0086	2.0064	2.0020	5.40	5.40	36.60

S1. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 5PC in DMPC/DMPG/Chol = 40/30/30 MLVs vs. the concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^7 s^{-1})$	S_0	relative population
0	3.80	4.47	0.52	
1/400	3.89	4.07	0.52	
1/200	3.55	3.63	0.53	
1/150	3.80	3.98	0.53	
1/100				
comp.1	4.07	4.17	0.55	0.79
comp.2	6.46	64.6	0.61	0.21
			avg. 0.56	
1/88				
comp.1	4.07	4.17	0.55	0.74
comp.2	6.46	64.6	0.64	0.26
			avg. 0.58	
1/75				
comp.1	4.17	4.27	0.55	0.68
comp.2	10.5	105	0.69	0.32
			avg. 0.60	

S2. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of DPPTC in DMPC/DMPG/Chol = 40/30/30 MLVs vs. concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^8 s^{-1})$	S_0
0	6.43	5.11	0.412
1/400	6.61	5.26	0.415
1/200	6.17	4.94	0.427
1/100	5.75	4.60	0.437
1/75	5.89	4.65	0.476

S3. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 14PC in DMPC/DMPG/Chol = 40/30/30 MLVs vs. concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^8 s^{-1})$	$R_{\parallel}(10^9 s^{-1})$	S_0
0	1.23	1.95	0.24
1/400	1.02	1.62	0.24
1/200	1.07	1.70	0.25
1/100	1.26	2.00	0.27
1/75	1.48	2.34	0.27

S4. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 5PC in DMPC/DMPG/Chol = 60/10/30 MLVs vs. the concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^7 s^{-1})$	S_0	relative population
0	3.72	3.80	0.51	
1/400	3.98	4.08	0.52	
1/200	3.55	3.63	0.53	
1/150	3.63	3.72	0.53	
1/100				
comp.1	3.24	3.31	0.55	0.81
comp.2	10.0	100.0	0.64	0.19
			avg. 0.57	
1/88				
comp.1	3.55	3.63	0.53	0.89
comp.2	25.1	251.0	0.60	0.11
			avg. 0.53	
1/75	2.95	3.02	0.52	

S5. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 14PC in DMPC/DMPG/Chol = 60/10/30 MLVs vs. concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^8 s^{-1})$	$R_{\parallel}(10^9 s^{-1})$	S_0
0	3.39	3.39	0.25
1/400	2.29	2.29	0.25
1/200	2.34	2.34	0.25
1/100	2.45	2.45	0.26
1/75	2.29	2.29	0.25

S6. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 14PC in DMPC/Chol = 70/30 MLVs vs. concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^8 s^{-1})$	$R_{\parallel}(10^9 s^{-1})$	S_0
0	3.31	5.25	0.36
1/400	2.88	4.57	0.35
1/200	2.40	3.80	0.34
1/100	2.88	4.57	0.29
1/75	2.75	4.37	0.29

S7. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 5PC in LLE MLVs vs. the concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^7 s^{-1})$	S_0	relative population
0	6.76	6.92	0.43	
1/400	6.46	6.60	0.43	
1/200	6.46	6.60	0.43	
1/150	6.61	6.76	0.44	
1/100				
comp.1	6.61	6.76	0.43	0.79
comp.2	19.1	191.0	0.57	0.21
			avg. 0.45	
1/88				
comp.1	6.46	6.60	0.42	0.79
comp.2	13.2	132.0	0.57	0.21
			avg. 0.45	
1/75				
comp.1	6.76	6.92	0.43	0.74
comp.2	14.1	141.0	0.60	0.26
			avg. 0.48	

S8. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of DPPTC in LLE MLVs vs. concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^8 s^{-1})$	S_0
0	5.62	4.47	0.181
1/400	4.79	3.80	0.182
1/200	5.25	4.17	0.185
1/150	4.37	3.47	0.193
1/100	5.50	4.37	0.205
1/88	4.90	3.89	0.214
1/75	4.57	3.63	0.217

S9. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 14PC in LLE MLVs vs. concentration of TM-wt incorporated at pH 7 and 37° C

TM-wt/lipids (M/M)	$R_{\perp}(10^8 s^{-1})$	$R_{\parallel}(10^8 s^{-1})$	S_0
0	1.38	2.75	0.13
1/400	1.45	2.88	0.13
1/200	1.51	3.02	0.13
1/150	1.55	3.09	0.14
1/100	1.66	3.31	0.14
1/88	1.48	2.95	0.14
1/75	1.78	3.55	0.14

S10. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of DPPTC in DMPC/DMPG/Chol = 40/30/30 vs. concentration of mut1 at pH 7 and 37° C

mut1/lipids (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^8 s^{-1})$	S_0
0	6.91	5.50	0.412
1/400	6.76	5.37	0.419
1/200	6.91	5.50	0.420
1/150	6.91	5.50	0.416
1/100	6.91	5.50	0.419
1/88	6.91	5.50	0.419
1/75	6.76	5.37	0.416

S11. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 5PC in DMPC/DMPG/Chol=40/30/30 vs. concentration of mut1 at pH 7 and 37° C

mut1/lipids (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^7 s^{-1})$	S_0
0	3.98	4.07	0.52
1/400	4.27	4.37	0.52
1/200	3.63	3.71	0.53
1/150	3.80	3.89	0.53
1/100	3.63	3.71	0.53
1/88	3.72	3.80	0.53
1/75	3.80	3.89	0.53

S12. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 14PC in DMPC/DMPG/Chol=40/30/30 vs. concentration of mut1 at pH 7 and 37° C

mut1/lipids (M/M)	$R_{\perp}(10^8 s^{-1})$	$R_{\parallel}(10^9 s^{-1})$	S_0
0	1.20	1.90	0.24
1/400	1.17	1.86	0.24
1/200	1.02	1.62	0.25
1/150	0.98	1.55	0.25
1/100	0.96	1.52	0.25
1/88	1.10	1.74	0.25
1/75	1.17	1.86	0.24

S13. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of DPPTC in LLE vs. concentration of mut2 at pH 7 and 37° C

mut2/LLE (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^8 s^{-1})$	S_0
0	5.62	4.47	0.184
1/400	5.37	4.27	0.178
1/200	5.50	4.37	0.185
1/150	6.31	5.01	0.179
1/100	6.03	4.79	0.179
1/88	5.37	4.27	0.176
1/75	4.68	3.72	0.179

S14. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 5PC in LLE vs. concentration of mut2 at pH 7 and 37° C

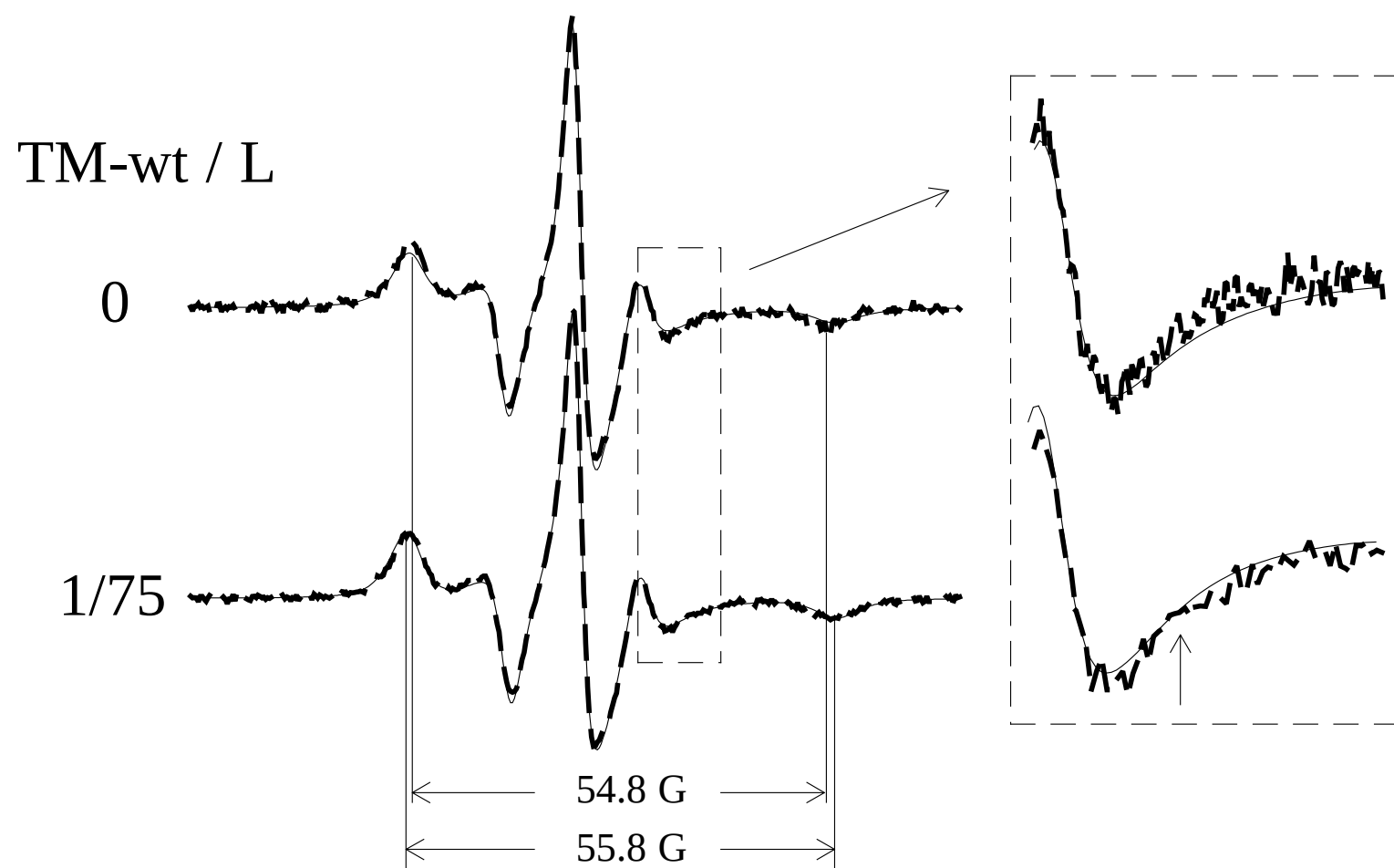
mut2/LLE (M/M)	$R_{\perp}(10^7 s^{-1})$	$R_{\parallel}(10^7 s^{-1})$	S_0
0	6.76	6.92	0.43
1/400	6.92	7.08	0.43
1/200	6.92	7.08	0.44
1/150	6.76	6.92	0.44
1/100	6.76	6.92	0.43
1/88	6.76	6.92	0.43
1/75	6.76	6.92	0.43

S15. Rotational diffusion rates $R_{\perp}, R_{\parallel}$ and order parameter S_0 of 14PC in LLE vs. concentration of mut2 at pH 7 and 37° C

mut2/LLE (M/M)	$R_{\perp}(10^8 s^{-1})$	$R_{\parallel}(10^8 s^{-1})$	S_0
0	1.58	3.16	0.13
1/400	1.58	3.16	0.14
1/200	1.55	3.09	0.13
1/150	1.48	2.95	0.13
1/100	1.55	3.09	0.13
1/88	1.58	3.16	0.13
1/75	1.58	3.16	0.13

Caption for figure SF1

Experimental (thick dashed line) and calculated (thin continuous line) ESR spectra of 5PC in MLVs of DMPC/DMPG/Chol=40/30/30 at pH 7 and 37°C are superimposed. The upper one is the spectrum from the vesicles without TM-wt, while the lower one from the vesicles containing TM-wt with molar ratio TM-wt/lipids = 1/75. The outer peak separation of the two spectra is 54.8 G and 55.8 G, respectively. A part of the high field peak of the spectra is magnified in the inserted box. An inflection in the lower spectrum is marked by an arrow, which indicates a feature of two-component spectrum.



Supplementary Figure 1