Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information for Rapid Diffusion of Cholesterol Along Polyunsaturated Membranes via Deep Dives

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Simulated Systems & General Results

Table S1: List of all simulated CG systems and the calculated diffusion coefficients. Composition shows the total numbers of lipid components in the two leaflets ("CHOL" refers to cholesterol). Temp. shows simulation temperatures, and Time the unscaled simulation times. The numbers in names refer to the width of the flat-bottom potential which restraints CHOL flip–flops (see Methods). In the asymmetric membranes, the number of the non-DOPC lipid was adjusted when necessary to prevent the buckling of the membrane. For these asymmetric membranes, only the diffusion coefficient of the non-DOPC lipid is provided, yet the DOPC values are plotted in Fig. 7 in the main text. Also note that the "CG-DOPC-DOPC system" is symmetric, yet has the same size as the asymmetric membranes and therefore serves as a control for finite-size effects. The total simulation time of the CG systems was 600 µs.

Name	Composition	Temp. (K)	Time (µs)	$D_{\text{Phospholipid}}$ (10^{-7} cm^2/s)	D_{CHOL} (10^{-7} cm^2/s)
(A) Free Cholesterol with Varying Level of Chain Unsaturation					
CG-DOPC	520 DOPC + 56 CHOL	298	75	4.28 ± 0.14	5.35 ± 0.12
CG-DLiPC	520 DLiPC + 56 CHOL	298	75	7.03 ± 0.02	11.86 ± 0.52
CG-DAPC	520 DAPC + 56 CHOL	298	75	8.24 ± 0.00	17.91 ± 0.46
CG-DDPC	520 DDPC + 56 CHOL	298	75	8.27 ± 0.05	18.50 ± 0.68
(B) Cholesterol Restrained by a Flat-Bottom Potential					
CG-DLiPC-FB-08	520 DOPC + 56 CHOL	298	10	7.29 ± 0.15	9.06 ± 0.51
CG-DLiPC-FB-10	520 DOPC + 56 CHOL	298	10	7.30 ± 0.02	10.95 ± 0.90
CG-DLiPC-FB-12	520 DOPC + 56 CHOL	298	10	6.82 ± 0.08	11.10 ± 0.26
CG-DLiPC-FB-14	520 DOPC + 56 CHOL	298	10	6.98 ± 0.11	11.11 ± 0.15
CG-DLiPC-FB-16	520 DOPC + 56 CHOL	298	10	7.33 ± 0.10	11.64 ± 0.24
CG-DAPC-FB-08	520 DOPC + 56 CHOL	298	10	8.57 ± 0.12	12.81 ± 0.69
CG-DAPC-FB-10	520 DOPC + 56 CHOL	298	10	8.31 ± 0.00	15.51 ± 0.82
CG-DAPC-FB-12	520 DOPC + 56 CHOL	298	10	8.15 ± 0.09	16.43 ± 0.48
CG-DAPC-FB-14	520 DOPC + 56 CHOL	298	10	8.23 ± 0.05	19.64 ± 0.97
CG-DAPC-FB-16	520 DOPC + 56 CHOL	298	10	8.22 ± 0.06	18.29 ± 0.97
(C) Asymmetric Membranes with Varying Level of Chain Unsaturation					
CG-DOPC-DOPC	1040 DOPC + 112 CHOL	298	50	4.31 ± 0.09	5.02 ± 0.06
CG-DOPC-DLiPC	520 DOPC + 520 DLiPC + 112 CHOL	298	50	7.44 ± 0.12	6.74 ± 0.59
CG-DOPC-DAPC	520 DOPC + 520 DAPC + 112 CHOL	298	50	7.67 ± 0.07	6.64 ± 0.04
CG-DOPC-DDPC	520 DOPC + 468 DDPC + 112 CHOL	298	50	6.74 ± 0.14	7.50 ± 0.28

Table S2: List of all simulated AA systems and the calculated diffusion coefficients. Composition shows the total numbers of lipid components in the two leaflets ("CHOL" refers to cholesterol). Temp. shows simulation temperatures, and Time the simulation times. In the last group, "C" refers to one of the cholesterols being maintained in the membrane core in a flat-lying conformation. For these systems, D_{CHOL} is the diffusion coefficient of the restrained cholesterol. In the asymmetric membranes, the number of the non-DOPC lipid was adjusted to compensate for the differing areas per lipid. For these asymmetric membranes, only the diffusion coefficient of the non-DOPC lipid is provided, yet the DOPC values are plotted in Fig. 7 in the main text. The total simulation time of the AA systems was 26 µs.

Name	Composition	Temp. (K)	Time (µs)	$D_{\mathrm{Phospholipid}}$ (10^{-7} cm^2/s)	D_{CHOL} (10^{-7} cm^2/s)
(A) Free Cholesterol with Varying Level of Chain Unsaturation					
AA-DOPC-310	260 DOPC + 28 CHOL	310	1	0.88 ± 0.05	1.04 ± 0.01
AA-DLiPC-310	260 DLiPC + 28 CHOL	310	1	1.26 ± 0.14	1.56 ± 0.40
AA-DAPC-310*	260 DAPC + 28 CHOL	310	1	1.62 ± 0.07	2.14 ± 0.40
AA-DDPC-310	260 DDPC + 28 CHOL	310	1	1.80 ± 0.01	2.29 ± 0.05
AA-DDPC-310-Large	520 DDPC + 56 CHOL	310	1	1.81 ± 0.01	2.44 ± 0.38
(B) DAPC with Free Cholesterol at Varying Temperatures					
AA-DAPC-285	260 DAPC+ 28 CHOL	285	1	0.58 ± 0.03	0.67 ± 0.06
AA-DAPC-298	260 DAPC + 28 CHOL	298	1	1.01 ± 0.08	1.36 ± 0.10
AA-DAPC-310*	260 DAPC + 28 CHOL	310	1	1.62 ± 0.07	2.14 ± 0.40
AA-DAPC-320	260 DAPC + 28 CHOL	320	1	2.28 ± 0.01	3.09 ± 0.74
AA-DAPC-333	260 DAPC + 28 CHOL	333	1	3.24 ± 0.15	4.60 ± 0.52
(C) One Cholesterol Restrained into the Interleaflet Region at Varying Temperatures					
AA-DAPC-C-285	260 DAPC + 28 CHOL	285	2	0.60 ± 0.02	4.27 ± 0.34
AA-DAPC-C-298	260 DAPC + 28 CHOL	298	2	1.03 ± 0.08	3.80 ± 0.53
AA-DAPC-C-310	260 DAPC + 28 CHOL	310	2	1.63 ± 0.03	11.41 ± 2.37
AA-DAPC-C-320	260 DAPC + 28 CHOL	320	2	2.20 ± 0.18	12.01 ± 1.74
AA-DAPC-C-333	260 DAPC + 28 CHOL	333	2	3.22 ± 0.14	17.59 ± 4.27
(D) DAPC with Free Cholesterol with Varying Cholesterol Concentrations					
AA-DAPC-310*	260 DAPC + 28 CHOL	310	1	1.62 ± 0.07	2.14 ± 0.40
AA-DAPC-20	232 DAPC + 56 CHOL	310	1	1.43 ± 0.05	1.67 ± 0.08
AA-DAPC-30	204 DAPC + 84 CHOL	310	1	1.16 ± 0.05	1.30 ± 0.18
AA-DAPC-40	176 DAPC + 112 CHOL	310	1	0.97 ± 0.02	1.05 ± 0.05
AA-DAPC-50	144 DAPC + 144 CHOL	310	1	0.63 ± 0.01	0.65 ± 0.01
(E) Asymmetric Membranes with Varying Level of Chain Unsaturation					
AA-DOPC-DLiPC	130 DOPC + 125 DLiPC + 28 CHOL	298	1	1.16 ± 0.03	1.25 ± 0.41
AA-DOPC-DAPC	130 DOPC + 120 DAPC + 28 CHOL	298	1	1.55 ± 0.01	1.59 ± 0.55
AA-DOPC-DDPC	130 DOPC + 115 DDPC + 28 CHOL	298	1	1.66 ± 0.01	1.61 ± 0.74

*The same simulation

Table S3: Numbers of flip–flops observed in different systems and the corresponding flip–flop rates. The error estimate shows standard error.

System	Flip–Flops	Rate (μs^{-1})
AA Simulations		
AA-DAPC-285	3	0.12 ± 0.11
AA-DAPC-298	10	0.35 ± 0.13
AA-DAPC-310*	29	1.04 ± 0.23
AA-DAPC-320	38	1.36 ± 0.34
AA-DAPC-333	80	2.86 ± 0.54
AA-DOPC-310	0	0
AA-DLiPC-310	3	0.11 ± 0.06
AA-DAPC-310*	29	1.04 ± 0.23
AA-DDPC-310	51	1.82 ± 0.54
AA-DDPC-310-L	96	1.71 ± 0.40
AA-DAPC-310*	29	1.04 ± 0.23
AA-DAPC-20	34	0.61 ± 0.13
AA-DAPC-30	17	0.20 ± 0.06
AA-DAPC-40	11	0.10 ± 0.04
AA-DAPC-50	2	0.01 ± 0.01
AA-DOPC-DLiPC	1	0.04 ± 0.19
AA-DOPC-DAPC	8	0.29 ± 0.71
AA-DOPC-DDPC	8	0.29 ± 0.71
CG Simulations		
CG-DOPC	29158	7.0 ± 0.1
CG-DLiPC	228585	55.2 ± 0.1
CG-DAPC	592099	142.9 ± 0.2
CG-DDPC	458686	110.7 ± 0.1
CG-DOPC-DOPC	37709	6.9 ± 0.42
CG-DOPC-DLiPC	78599	14.32 ± 0.74
CG-DOPC-DAPC	73660	13.42 ± 0.81
CG-DOPC-DDPC	101735	18.54 ± 0.13

*The same simulation

Results from Symmetric Coarse-Grained Simulations

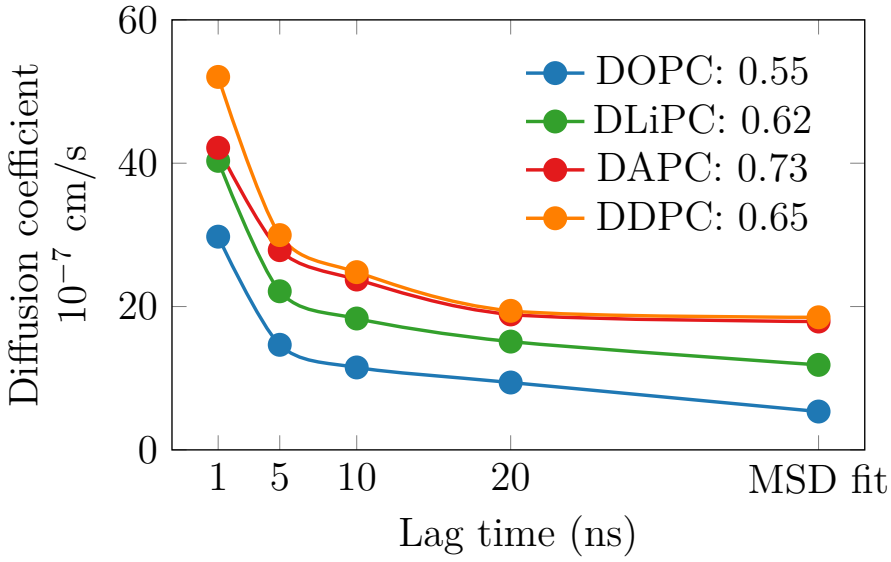

Figure S1: Diffusion coefficients extracted from displacement distributions over different lag times, as well as from long-time MSD fits assuming normal diffusion. This highlights the seemingly faster kinetics at short time scales. The inset shows values of the subdiffusion exponent α extracted from the displacement distribution fits at different lag times (see Methods).

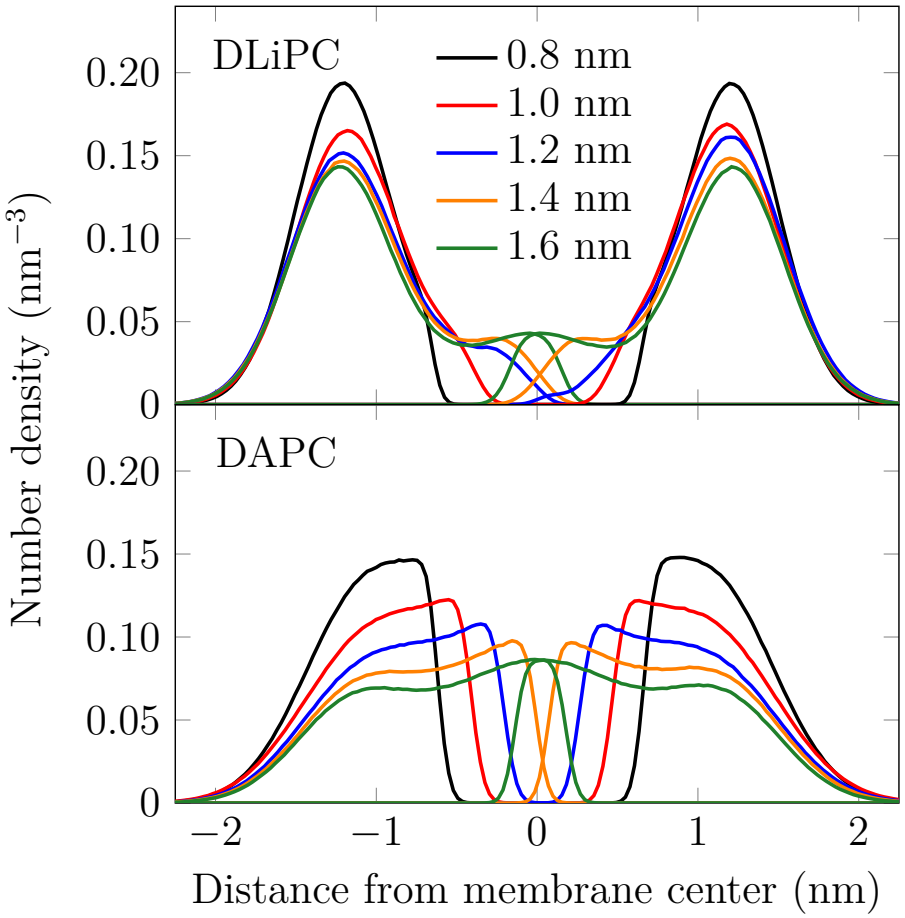

Figure S2: Number density of CHOL hydroxyl bead in CG simulations with flat-bottom potential applied to it (see Methods). Different colors correspond to different widths of the flat part of the potential. Profiles for both leaflets are drawn separately using the same color. It is evident that for values between 0.8 nm and 1.2 nm result in essentially no hydroxyl groups residing in the membrane core. For values of 1.4 nm and 1.6 nm, the core is populated but complete CHOL flip–flops cannot take place.

Results from Symmetric Atomistic Simulations

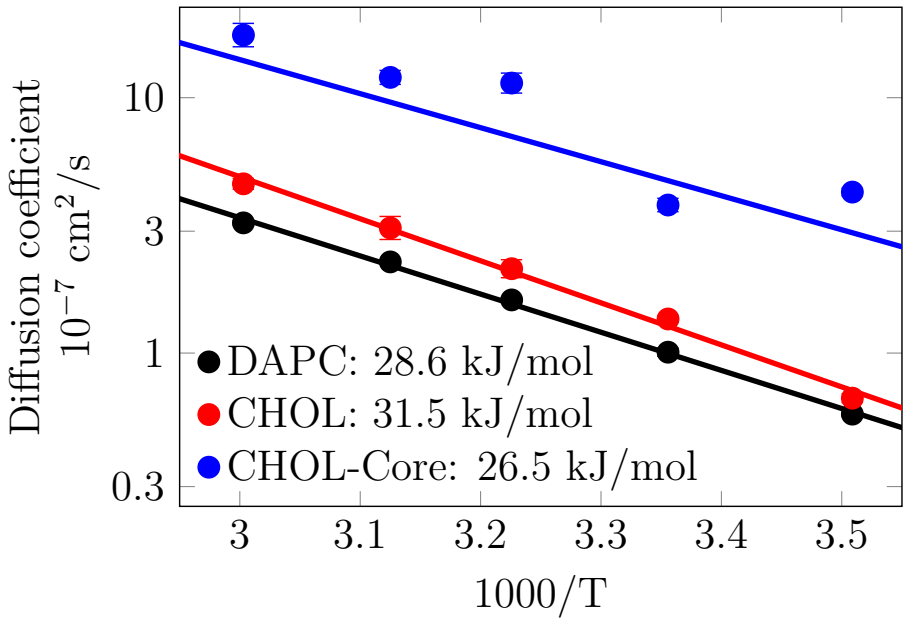

Figure S3: Diffusion coefficients of free DAPC, free CHOL, and CHOL restricted to the membrane core (CHOL-Core) as a function of inverse temperature. The data are shown in the Arrhenius manner (see Methods), and the activation energies are given in the legend.

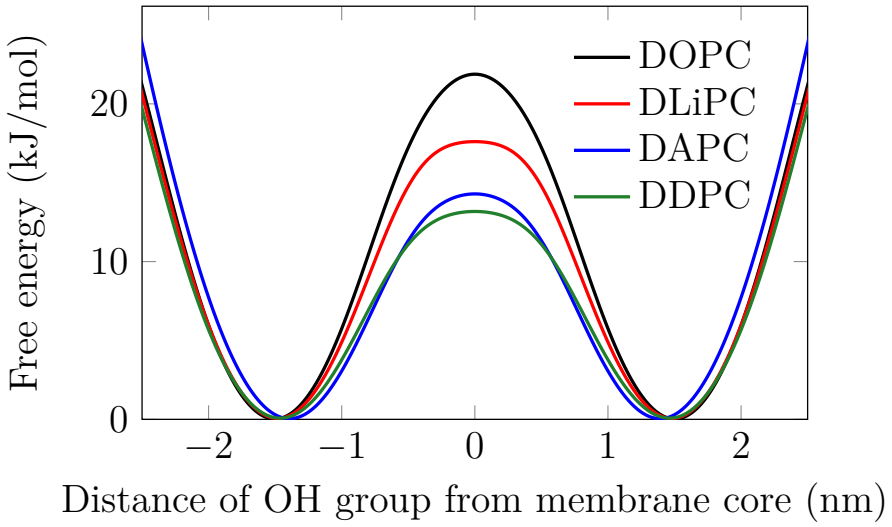

Figure S4: Free energy profiles of CHOL flip–flop extracted from the density profiles of its hydroxyl oxygen as a function of lipid chain unsaturation from the AA simulations (see Methods). The region of highest density is assigned a value of zero.

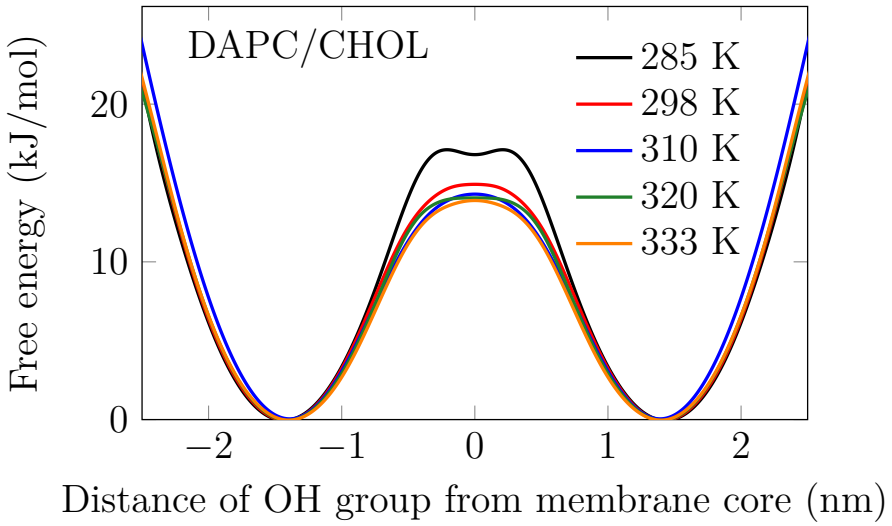

Figure S5: Free energy profiles of CHOL flip–flop extracted from the density profiles of its hydroxyl oxygen as a function of temperature from the AA DAPC/CHOL simulations (see Methods). The region of highest density is assigned a value of zero.

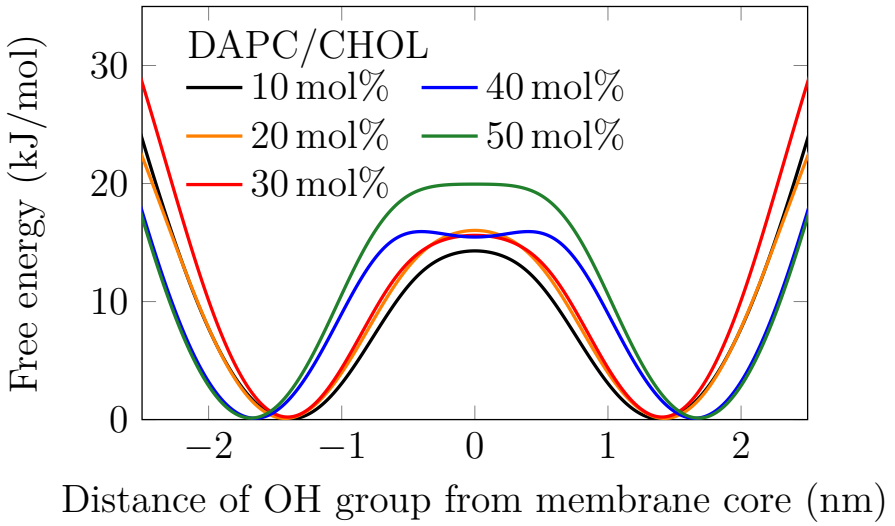

Figure S6: Free energy profiles of CHOL flip–flop extracted from the density profiles of its hydroxyl oxygen as a function of cholesterol concentration from the AA DAPC/CHOL simulations (see Methods). The region of highest density is assigned a value of zero.

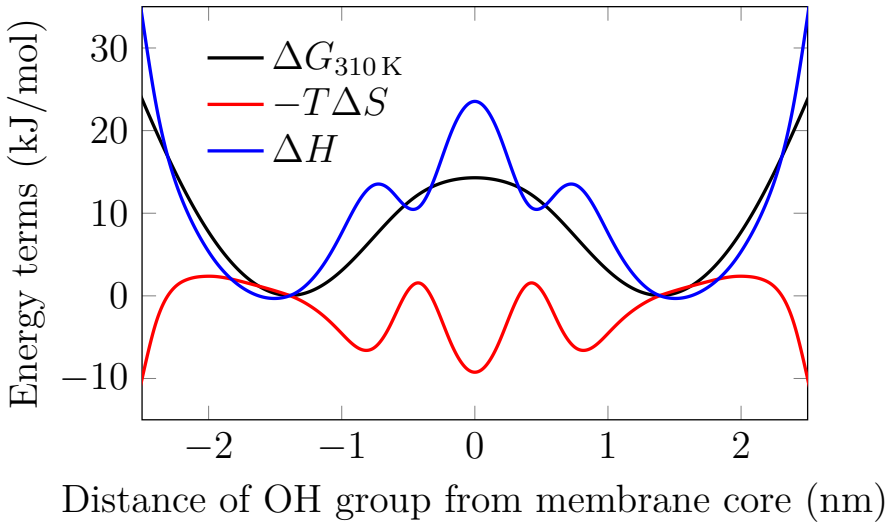

Figure S7: The decomposition of CHOL flip–flop free energy profiles into entropic and enthalpic components (see Methods).

Results from Asymmetric Membranes

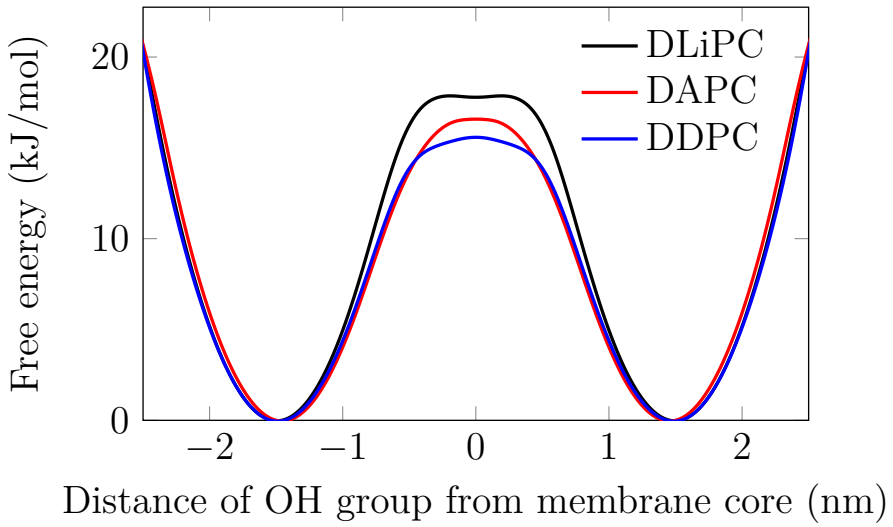

Figure S8: Free energy profiles of CHOL flip–flop extracted from the density profiles of its hydroxyl oxygen from the asymmetric atomistic membranes. The legend refers to the non-DOPC phospholipid in the membrane. The region of highest density is assigned a value of zero. As discussed in the text, the CHOL concentrations in the leaflets have likely not equilibrated during the 1 µs simulations.