System size effects on the free energy landscapes from molecular dynamics of phase-separating bilayers

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1 Simulation Details

System size	324			648			972		
Box dimension	x	y	z	x	y	z	x	y	z
Replica 1	116.323	116.024	66.34	158.584	164.268	66.112	198.21	190.454	65.9
Replica 2	118.467	119.067	65.493	159.915	154.015	68.398	196.82	189.72	66.631
Replica 3	119.016	114.615	67.081	156.536	159.436	66.549	192.698	197.197	65.509
Replica 4	117.241	113.441	68.094	161.742	158.942	66.4	191.791	192.192	66.596
Mean	117.762	115.787	66.752	159.194	159.165	66.865	194.88	192.391	66.159

System size	1944			3888			7776		
Box dimension	x	y	z	x	y	z	x	y	z
Replica 1	264.978	266.779	69.529	367.68	371.779	68.215	513.788	511.888	71.54
Replica 2	263.191	262.19	69.072	373.556	369.657	66.607	510.024	511.212	71.57
Replica 3	266.761	266.161	69.871	370.878	368.978	70.558	516.373	513.173	71.335
Replica 4	265.563	266.862	68.011	371.423	371.223	69.752	515.511	517.511	70.89
Mean	265.123	265.498	69.121	370.884	370.409	68.783	513.924	513.446	71.334

System size	10110		
Box dimension	x	y	z
Replica 1	585.942	583.184	70.644
Replica 2	585.119	590.418	70.869
Replica 3	579.708	579.307	72.342
Replica 4	580.175	580.475	75.137
Mean	582.736	583.346	72.248

Table S1: x, y, z dimensions of simulation boxes of different replicates of varying system sizes. All units are in Angstroms.

System Size	Replica	323 K		423 K	
		Mean	Std. Dev	Mean	Std. Dev
324	1	0.63	0.11	0.09	0.07
	2	0.59	0.17	0.07	0.07
	3	0.57	0.16	0.07	0.06
	4	0.56	0.18	0.04	0.05
	Summary	0.59	0.16	0.07	0.06
648	1	0.65	0.10	0.11	0.04
	2	0.6	0.07	0.09	0.04
	3	0.6	0.11	0.09	0.04
	4	0.63	0.11	0.08	0.05
	Summary	0.62	0.10	0.09	0.05
972	1	0.67	0.07	0.09	0.04
	2	0.68	0.09	0.09	0.05
	3	0.69	0.07	0.09	0.04
	4	0.64	0.07	0.08	0.03
	Summary	0.66	0.08	0.09	0.04
1944	1	0.7	0.04	0.09	0.02
	2	0.67	0.03	0.07	0.02
	3	0.646	0.05	0.08	0.03
	4	0.71	0.04	0.07	0.03
	Summary	0.68	0.04	0.08	0.03
3888	1	0.69	0.03	0.1	0.02
	2	0.67	0.03	0.1	0.02
	3	0.66	0.04	0.1	0.02
	4	0.63	0.03	0.09	0.02
	Summary	0.66	0.03	0.09	0.02
7776	1	0.65	0.02	0.09	0.01
	2	0.65	0.02	0.09	0.01
	3	0.64	0.03	0.09	0.01
	4	0.66	0.02	0.1	0.01
	Summary	0.65	0.02	0.09	0.01
10110	1	0.65	0.02	0.1	0.01
	2	0.67	0.03	0.1	0.01
	3	0.66	0.01	0.1	0.01
	4	0.64	0.02	0.10	0.01
	Summary	0.66	0.02	0.1	0.01

Table S2: Mean and variance in FLC estimated between 2 μs and 4μs of standard MD simulation. Summary for each system is computed using the "root-sum-squared" method to combine uncertainties from replicates

2 Standard Molecular Dynamics Analysis

2.1 Evolution of Individual Species Contribution to FLC

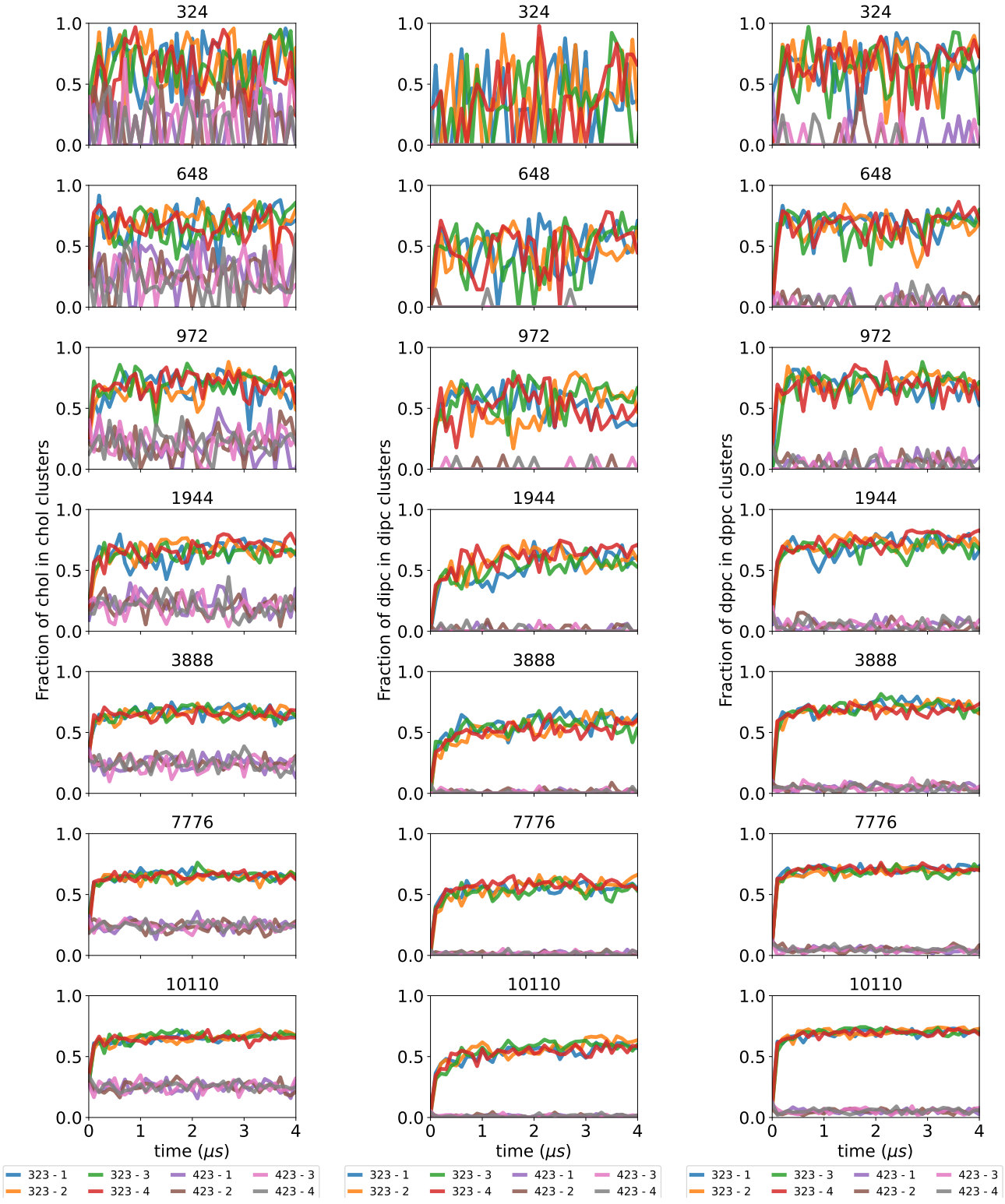

Figure S1: Evolution of Fraction of Lipid X in Lipid X cluster, where X corresponds to Cholesterol (Panel A), DIPC (Panel B), and DPPC (Panel C).

2.2 Cluster Count Analysis

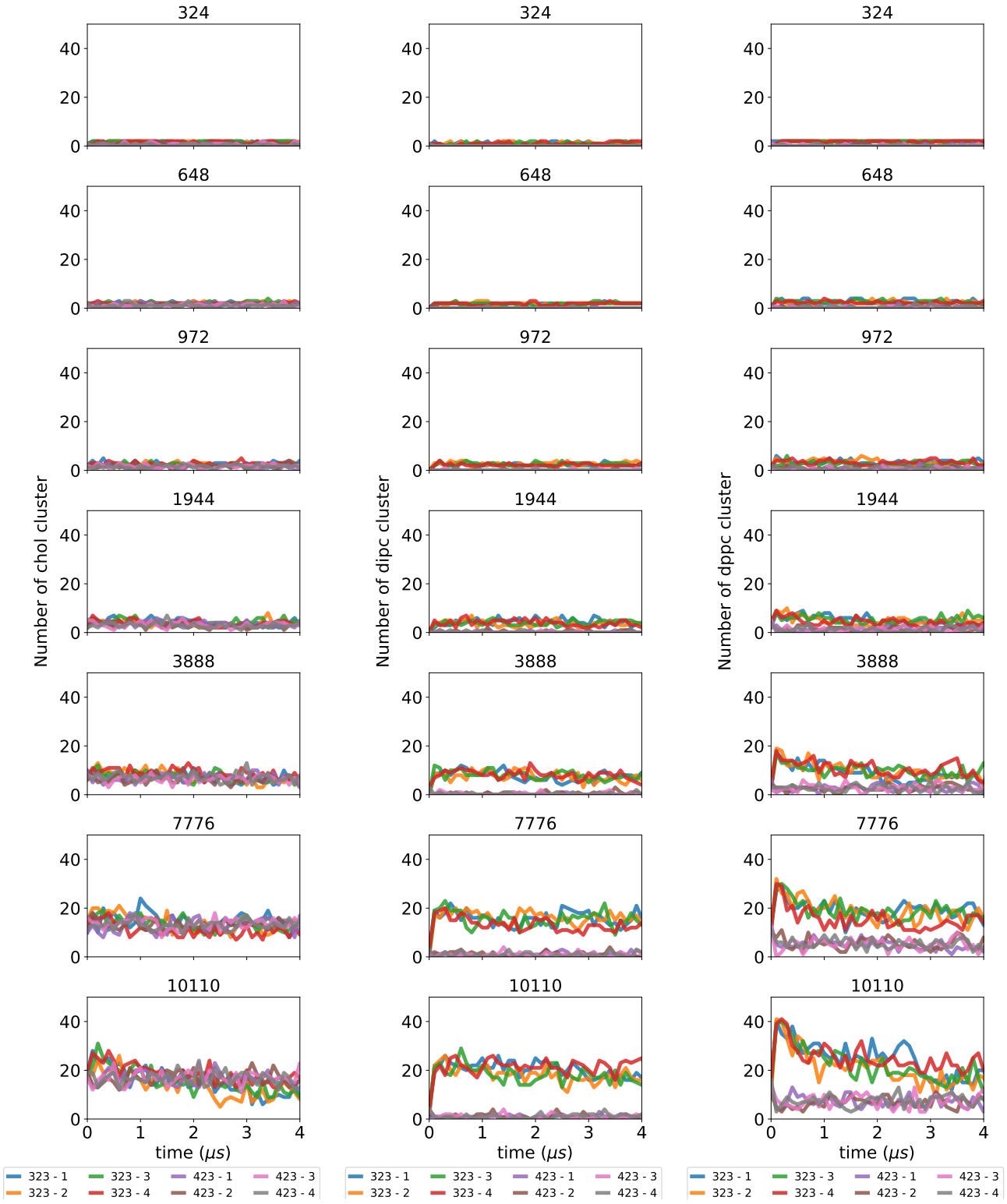

Figure S2: Tracking number of lipid X clusters, where X corresponds to Cholesterol (Panel A), DIPC (Panel B), and DPPC (Panel C).

2.3 Mean Number of Lipids in Clusters

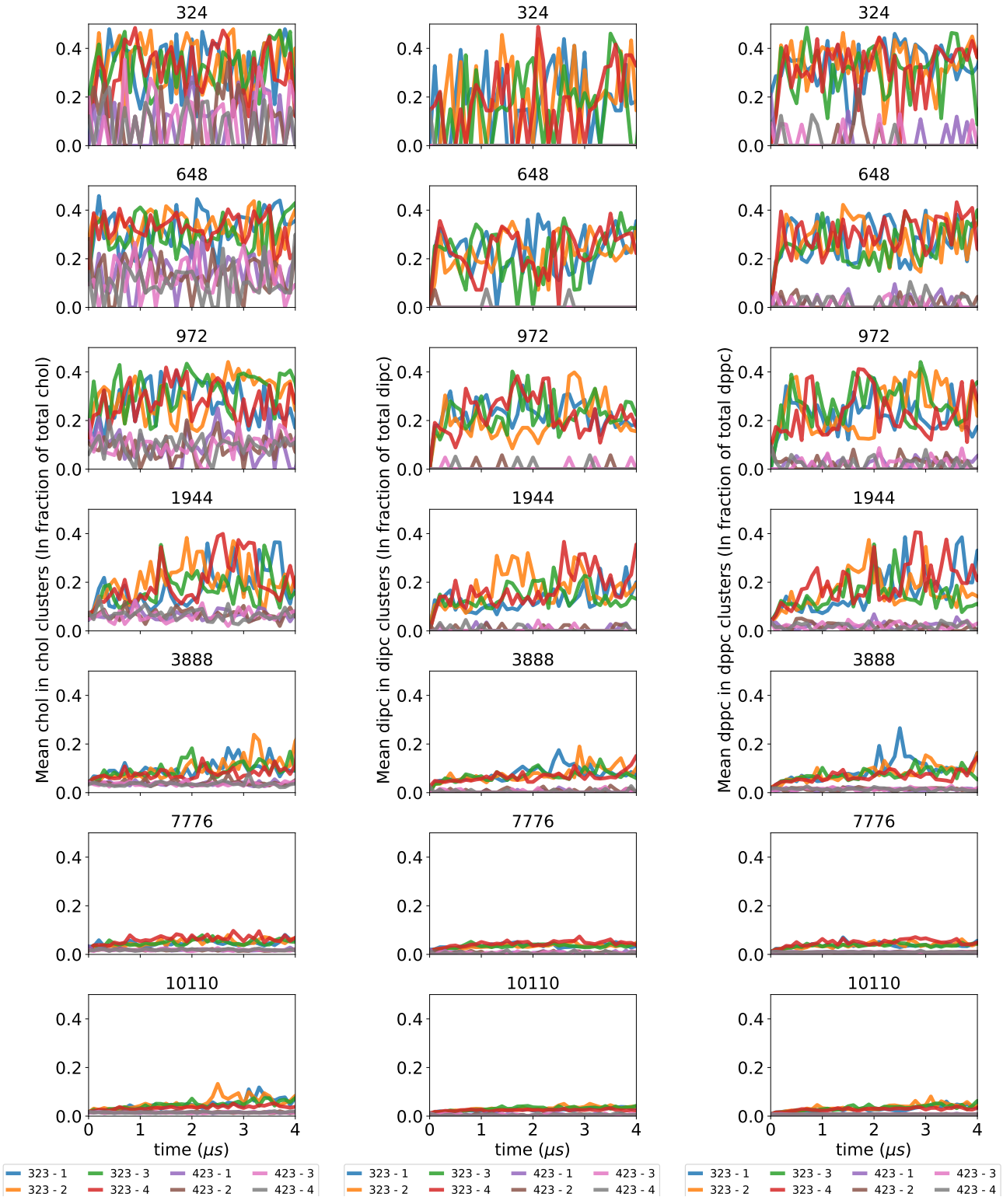

Figure S3: Tracking the mean number of lipid X in lipid X clusters and normalized to the total number of lipid X in the given system, where X corresponds to Cholesterol (Panel A), DIPC (Panel B), and DPPC (Panel C).

3 Weighted Ensemble Analysis

3.1 Contributions of Lipid Species Towards FLC

Figure S5 shows that the contributions of individual lipid species to FLC behave similarly to the overall FLC. However, this is not true for the smaller systems (see S4). This effect is more evident in the free energy landscape of FLC(DIPC), where the smaller systems not only fail to capture the two sub-basins in the mixed state but suggest that the most preferred state for DIPC in the system is not to cluster at all. The standard MD simulation only reports the possibility of one of three FLC(DIPC) basins (Supplementary Figure S1B).

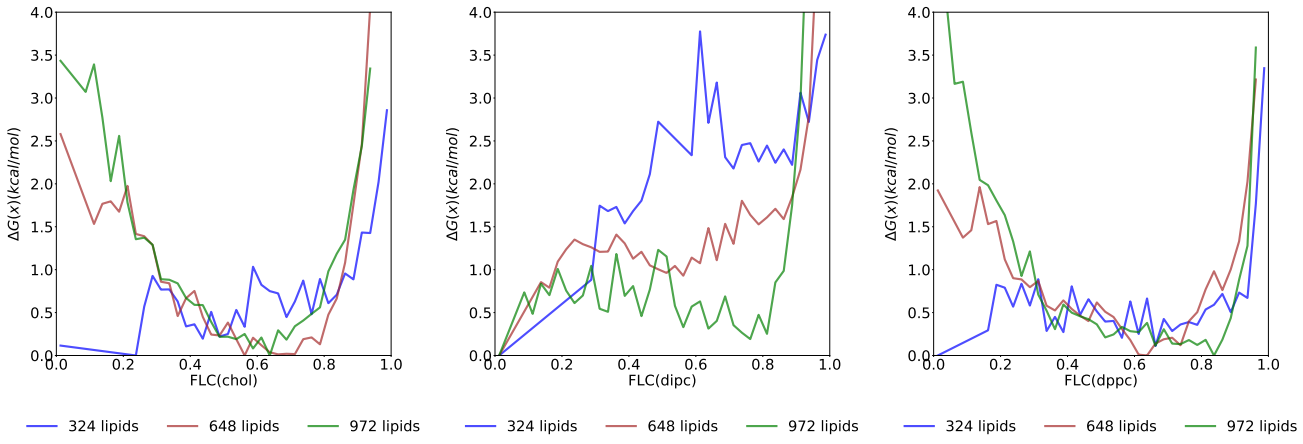

Figure S4: Free energy landscape as a function of Fraction of Lipid X in Lipid X cluster, where X corresponds to Cholesterol (Panel A), DIPC (Panel B), and DPPC (Panel C). For smaller system sizes

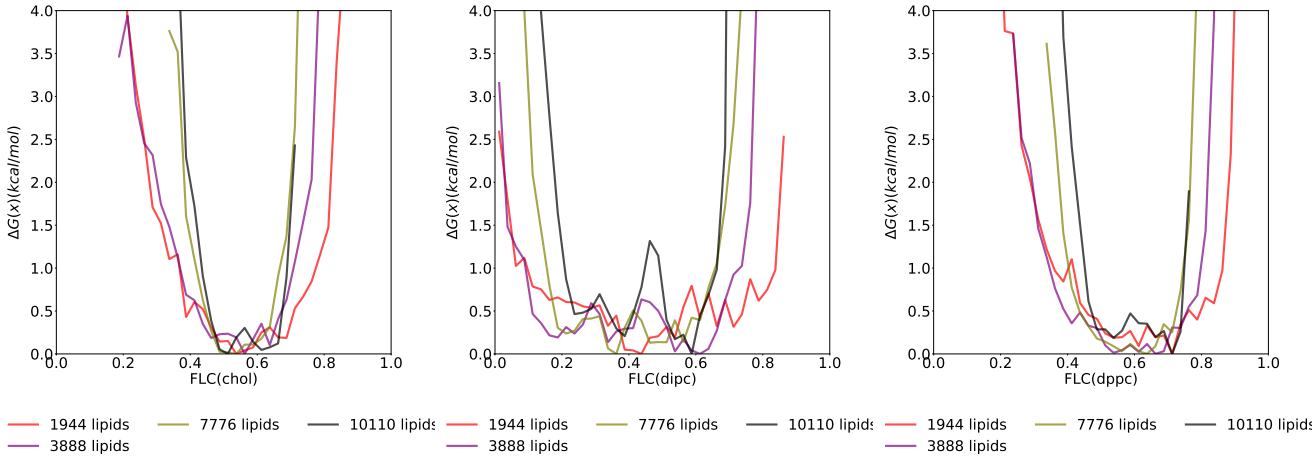

Figure S5: Free energy landscape as a function of Fraction of Lipid X in Lipid X cluster, where X corresponds to Cholesterol (Panel A), DIPC (Panel B), and DPPC (Panel C). For larger system sizes

3.2 Mean Number of Lipids in Clusters

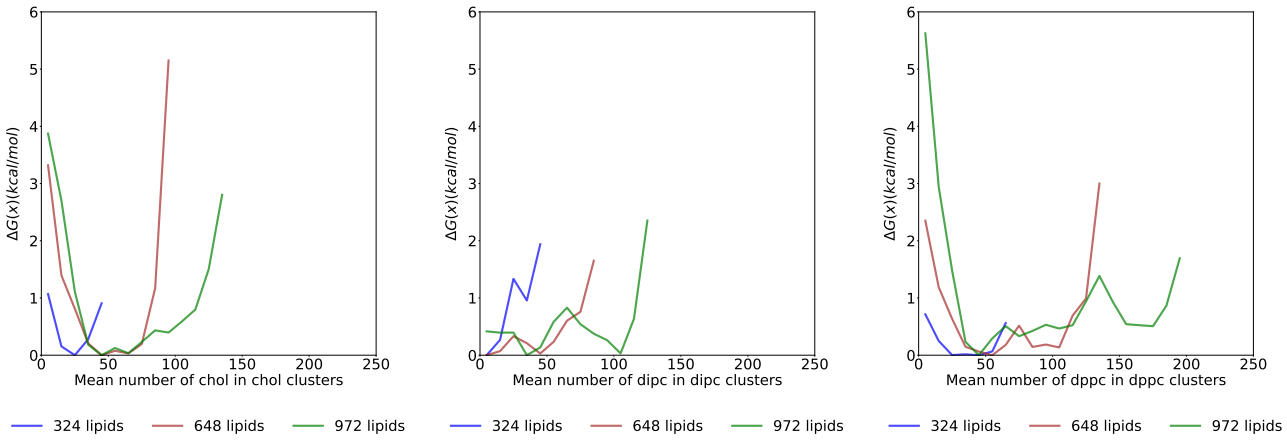

Figure S6: Free energy landscape as a function of the mean number of lipid X in a lipid X cluster, where X corresponds to Cholesterol (Panel A), DIPC (Panel B), and DPPC (Panel C).