Supporting Information

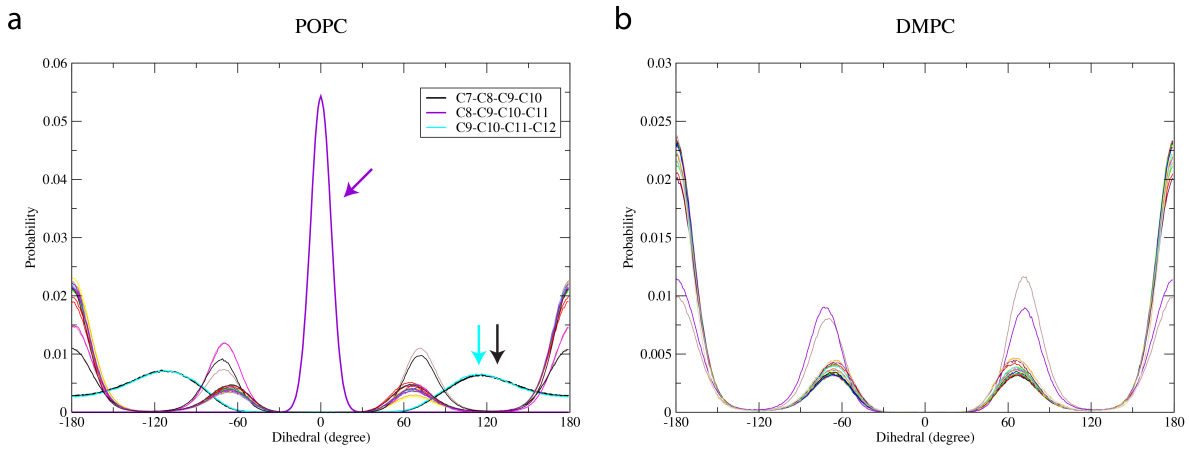


Figure S1: Probability distribution of lipid tail dihedrals of POPC (a) and DMPC (b). The calculation was performed using the last 20 ns of the 70-ns cMD simulations of the two bilayers. The arrows highlight three dihedrals involving the double bond in the sn-2 oleoyl chain of POPC: C7-C8-C9-C10, C8-C9-C10-C11, and C9-C10-C11-C12.

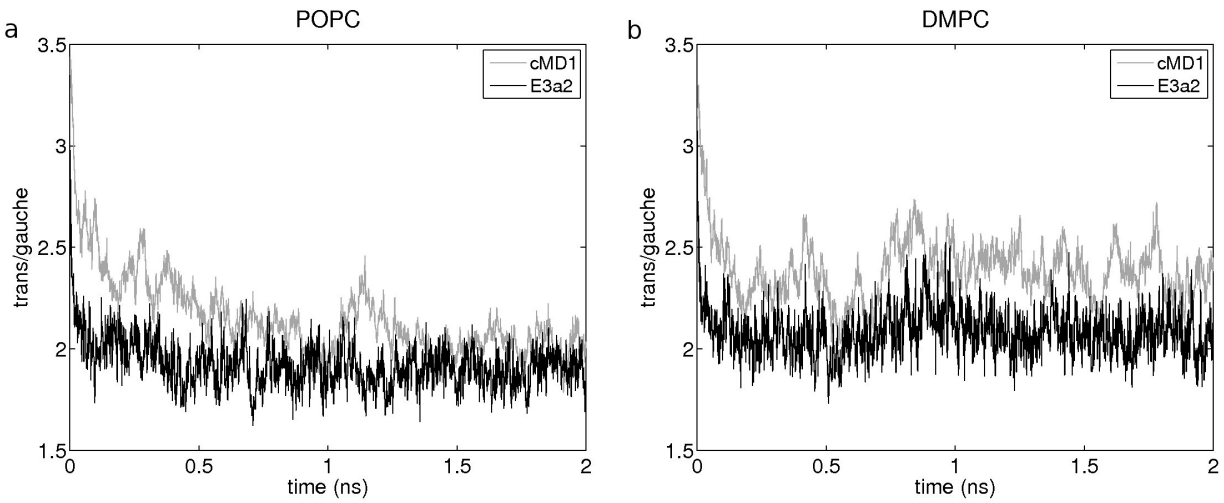


Figure S2: The *trans/gauche* ratio of POPC (a) and DMPC (b) in selected cMD (grey) and aMD (black) simulations.

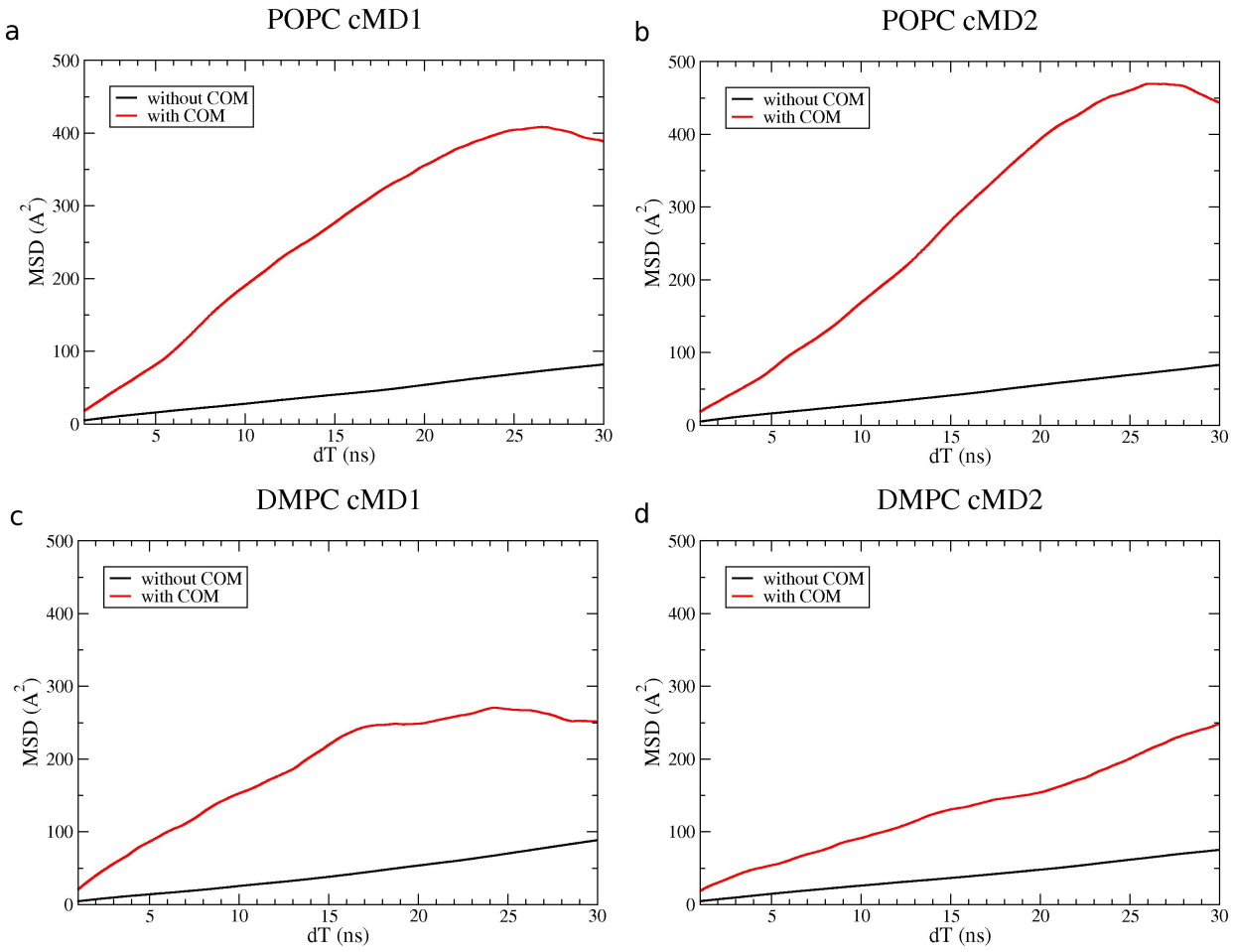


Figure S3: The mean square displacement (MSD) of POPC and DMPC calculated with the center-of-mass (COM) of each monolayer removed (black) or kept (red). The linear relationship between MSD and the time interval t clearly "breaks down" around $t = 25$ ns when monolayer COMs are kept, while such a relationship is maintained till $t \approx 55$ ns when monolayer COMs are removed (not shown). Using MSD data from t=1 ns to t=20 ns, we calculated the lateral diffusion coefficients, D1 and D2, of the cMD1 and cMD2 simulations, respectively. Their difference ($|D1 - D2|/(D1 + D2)$) is 1.6% (POPC) and 7.4% (DMPC) when monolayer COMs are removed, and 6.2% and 26.4% when monolayer COMs are kept. The latter set of numbers reflects the large fluctuations in the monolayer COMs. As small systems tend to have artificially enhanced COM motions, large fluctuations between different simulations are expected. For this reason, we chose to remove monolayer COMs from the calculation of D to ensure better statistical precision when comparing different simulation results.

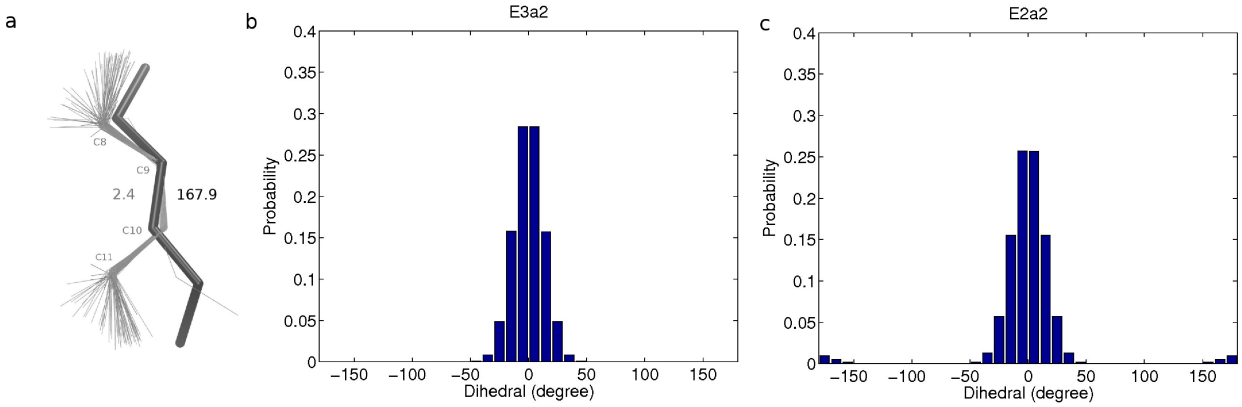
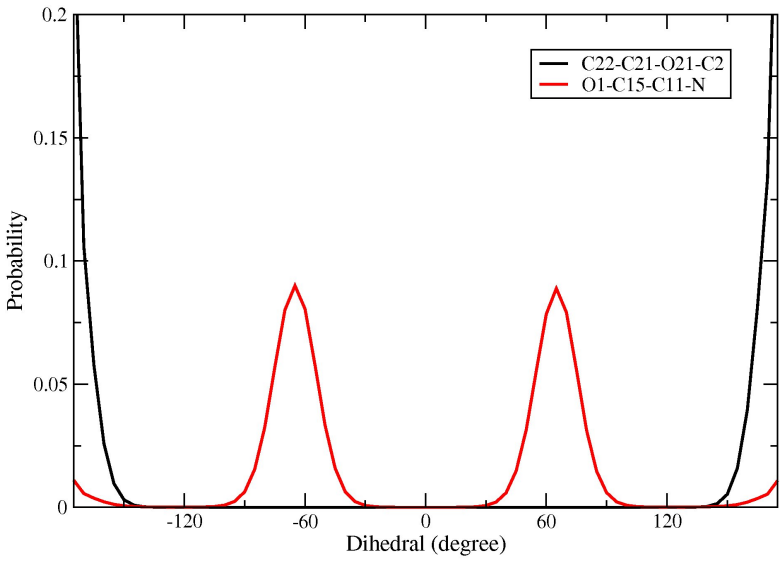


Figure S4: Conformations of the double bond C9-C10 in the sn-2 oleoyl chain of POPC. (a) Snapshots of the C8-C9-C10-C11 dihedral during the E2a2 simulation. While most lipid molecules maintain the *cis-* conformation (grey thin lines), the *trans-* conformation (black thick line) is observed occasionally. Typical values of the dihedral C8-C9-C10-C11 in the *cis-* and *trans-* conformations are shown. (b, c) Probability distribution histograms of the dihedral C8-C9-C10-C11 in the simulations E3a2 (b) and E2a2 (c). The *trans-* conformation ($\chi < -150^\circ$ or $\chi > 150^\circ$) can be identified in the E2a2 histogram.



Dihedral	cMD1	cMD2	E1	E2a3	E3a2	E3a3
C21-O21-C2-C1	8329	8469	10073	10965	11855	11069
O21-C2-C1-O2	205	196	693	1293	2141	1493
C1-O2-P1-O1	1278	1292	1913	2388	2908	2532
O2-P1-O1-C15	1406	1432	2092	2607	2975	2719
P1-O1-C15-C11	2301	2305	3679	5063	6016	5427
O1-C15-C11-N	96	94	1095	2162	2757	2392
C31-O31-C3-C2	4691	4745	5533	6074	6926	6337
O21-C2-C3-O31	70	69	243	474	879	566
C3-C2-O21-C21	8259	8407	10022	10904	11797	11009

Figure S5: Barrier-crossing events of the POPC headgroups in cMD and aMD simulations. Top: Probability distributions of selected headgroup dihedrals in the POPC cMD2 simulation. The distribution profiles are used to define barrier-crossing events for each dihedral: C22-C21-O21-C2 has only one state, and no barrier-crossing is defined; O1-C15-C11-N has three states (1: $\chi < -150^\circ$ or $\chi > 150^\circ$, 2: $30^\circ < \chi < 100^\circ$, 3: $-100^\circ < \chi < -30^\circ$), and a barrier-crossing event is defined as the transition from any one state to a different state. Bottom: The number of barrier-crossing events for 12 POPC headgroup dihedrals. The calculation was performed using the 70-ns cMD and aMD trajectories (saved every 1 ps). Three dihedrals have only one state, and are not included in the table. The analysis was also performed for DMPC, which produced similar results (data not shown). All the atoms are named according to the CHARMM27r force field (1, 2).

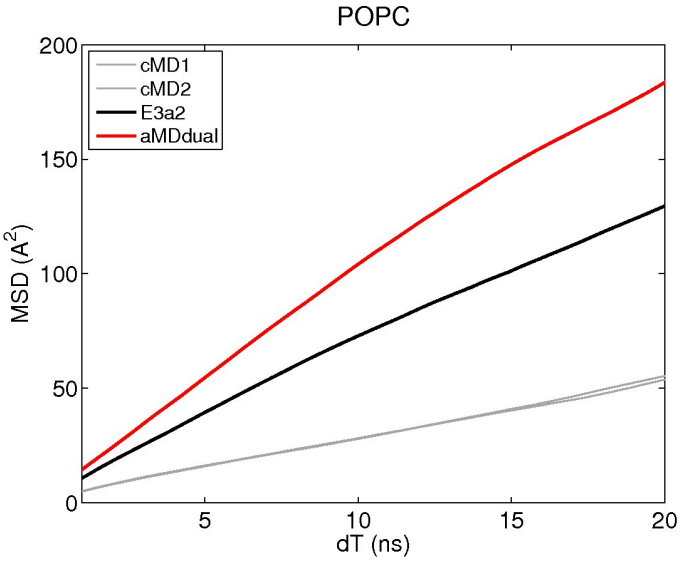


Figure S6: The mean square displacement (MSD) of POPC calculated from the E3a2 and aMDdual simulations. The cMD results are shown in thin grey lines for reference. The lateral diffusion coefficient of POPC in the aMDdual simulation is 22.0×10^{-8} cm^2/s, approximately 45% larger than the result of the E3a2 simulation. The parameters used in the aMDdual simulation are adopted from a previous aMD study (3).

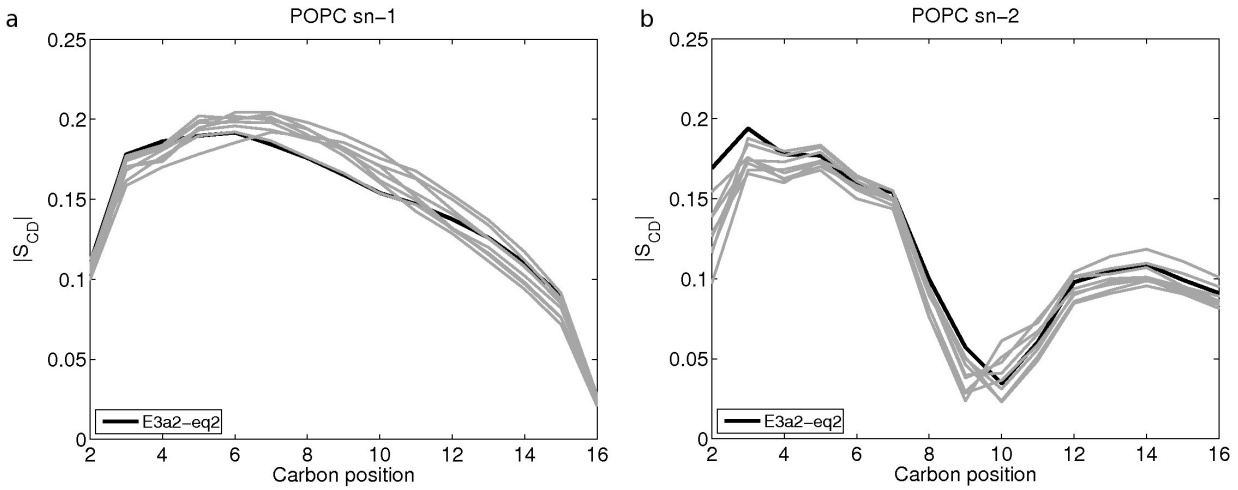


Figure S7: Order parameter S_{CD} of POPC calculated from the last 5 ns of the 20-ns E3a2-eq simulation (black). For comparison, the results obtained using a 5-ns block for the last 20 ns of the cMD1 and cMD2 simulations are shown in eight grey lines.

References

1. Klauda, J.; Brooks, B.; MacKerell, A. D.; Venable, R. and Pastor, R., *J. Phys. Chem. B*, 2005, **109**, 5300–5311.

2. Klauda, J.; Pastor, R. and Brooks, B., *J. Phys. Chem. B*, 2005, **109**, 15684–15686.

3. de Oliveira, C.; Hamelberg, D. and McCammon, J., *J. Phys. Chem. B*, 2006, **110**, 22695–22701.