Molecular Dynamics Simulations Reveal Leaflet Coupling in Compositionally Asymmetric Phase-Separated Lipid Membranes

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Supporting Information

I. Uncertainty and Autocorrelation

We performed autocorrelation measurements for each property reported, in order to select independent frames for analysis and determine statistically accurate uncertainty. We fit autocorrelation data to find the decay time τ, since independent frames are separated by at least 2τ.[1] Since different properties decorrelate at different rates, a separate measurement was made for each observable. In particular, bulk properties of a phase or bilayer change much more slowly that configurations of individual lipids.

For partition coefficient (Kp) measurements, τ reaches 72 ns, so frames each 144 ns were used. More details on Kp statistics are discussed below.

For leaflet and bilayer thickness measurements, τ is less than 4.8 ns, so frames each 9.6 ns were included. In the case of carbon density measurements, τ is approximately 9.6 ns, so frames each 19.2 ns were used. Order parameter, which adapts most quickly to changing conditions, was observed to always have $\tau < 0.72$ ns, so measured frames were found each 1.44 ns.

[1] Morales, J. J.; Nuevo, M. J.; Rull, L. F. Statistical error methods in computer simulations. Journal of Computational Physics 1990, 89, 432–438.

II. Transbilayer Partition Coefficient

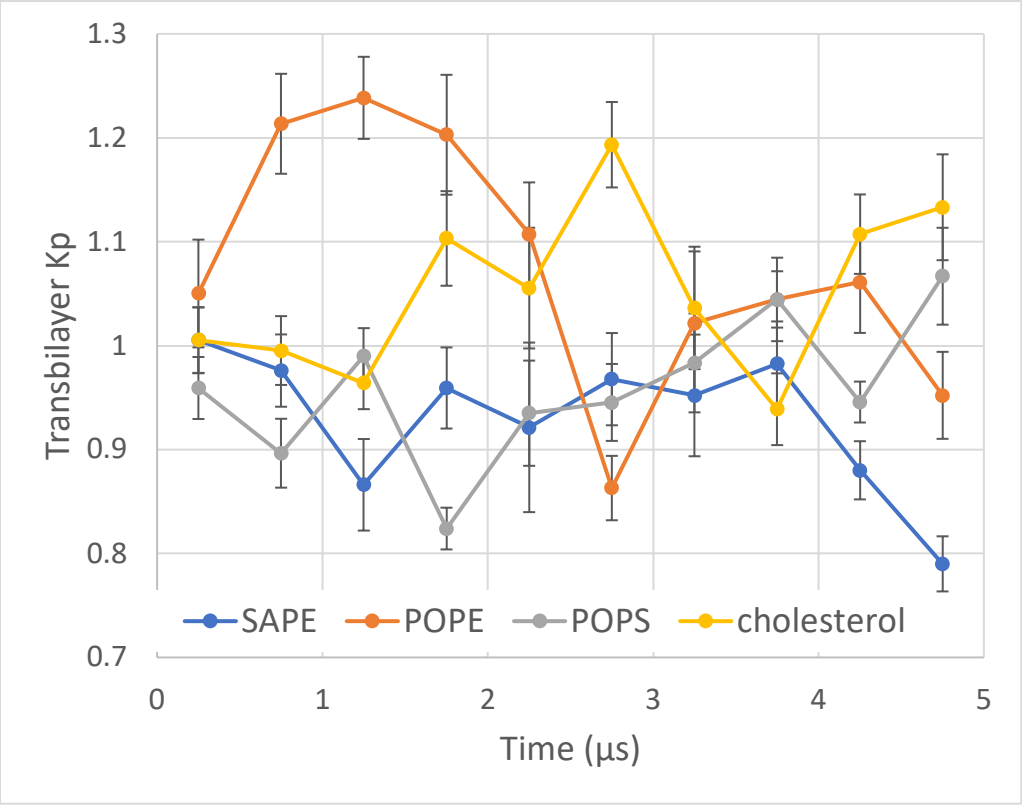

Figure S1. The transbilayer partition coefficient (Kp) over the full 5-µs simulation is shown for each of the cytoplasmic leaflet lipids in the complex asymmetric bilayer. Each point represents the Kp for a 500-ns window. Error bars represent standard error from five independent frames in each window. As described in the main article, the transbilayer Kp values do not converge within the simulation time and do not appear likely to converge in any amount of time accessible to simulation.

III. Leaflet Thickness

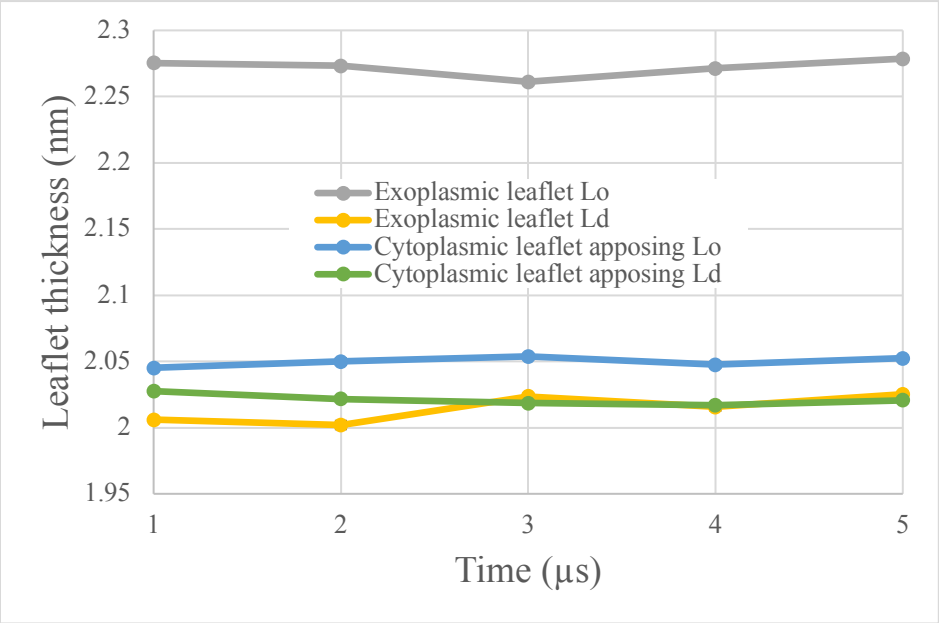

Figure S2. The thickness of each leaflet over the full 5-μs simulation is shown for the complex asymmetric bilayer. Unlike partition coefficient, thickness appears to reach equilibrium within 3-4 μs, as each point plots the average over the preceding μs. Due to the lack of variability, error bars, while shown, may be obscured by the line. The finding of a significant thickness difference between the cytoplasmic leaflet region apposing Lo (blue line) and the region apposing Ld (green line) is therefore robust when taken from the final microsecond, after convergence.

IV. Cholesterol Distribution

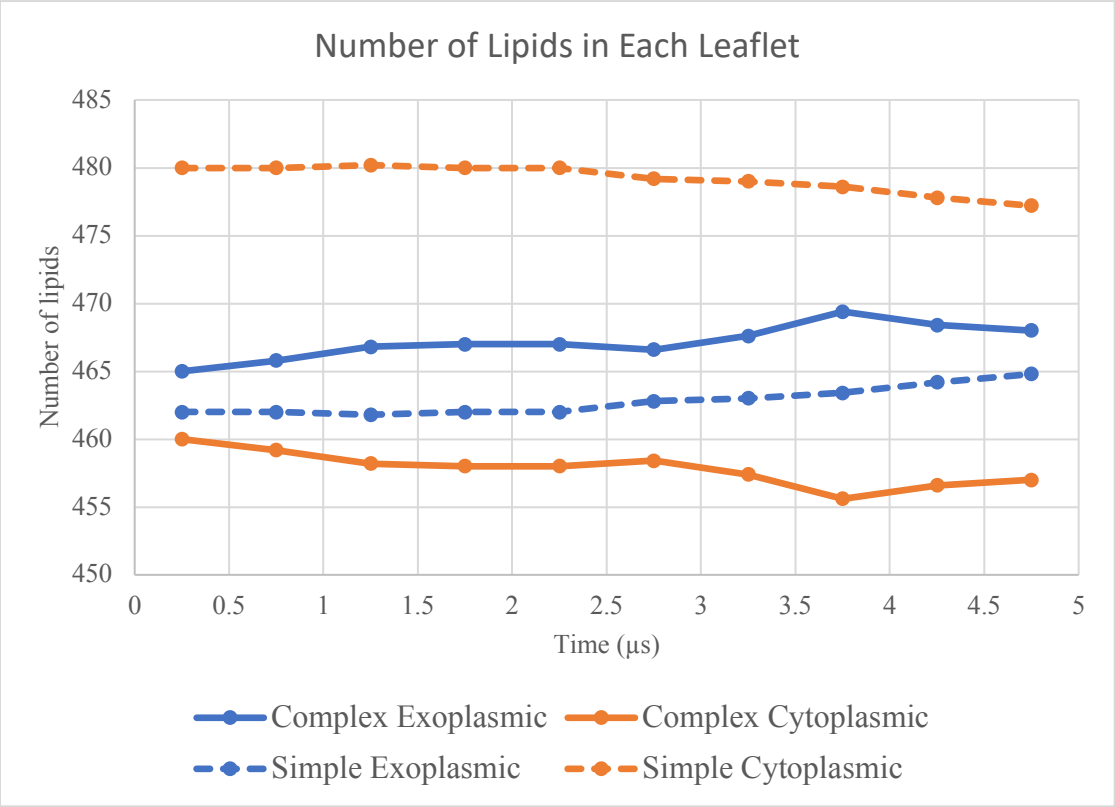

Figure S3. Cholesterol, due to its hydrophobicity and small size, can move between leaflets far more easily than phospholipids can. While most cholesterol flip-flops cause no net change in cholesterol concentration, as molecules are moving in both directions, there can be a net movement from one leaflet to the other. We find a small net movement of cholesterol in the asymmetric systems, suggesting they are not at a full equilibrium of composition. This drift may be explained in part due to cholesterol responding to compositional changes in the regions of the cytoplasmic leaflet, which are still ongoing (see above). In the asymmetric bilayers, there is a slight movement towards the exoplasmic leaflet over the 5-μs simulation, but this amounts to only about 1% of cholesterols in the cytoplasmic leaflet moving out. The graph above shows the number of lipids in each leaflet, averaged over each 500-ns window. Only cholesterols are observed to flip, so all changes represent a gain or loss of cholesterol.