Electronic Supplementary Material (ESI) for Soft Matter.

Lipid Bilayer Thickness Determines Cholesterol's Location in Model Membranes

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Supplementary Information

^{2}H NMR

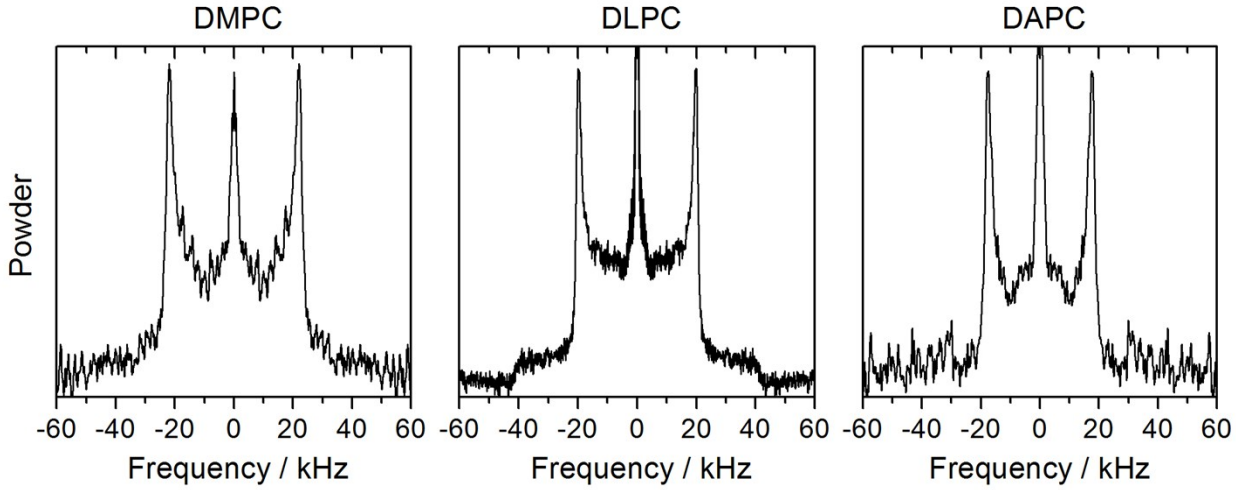

Figure S1 ^{2}H NMR spectra for 10 mol% [3α-$^2H_1$]cholesterol in aqueous multilamellar dispersions of DLPC, DMPC and DAPC (50 wt% in 50 mM Tris, pH 7.5) at 30°C. The spectra are plotted in powder pattern format that were obtained by conventional FFT. The experiments were conducted on resonance, and the out-of-phase channel was zeroed, improving the signal-to-noise by a factor of $\sqrt{2}$ for the spectra that were reflected about their central (resonant) frequency. Residual ^{2}HHO together with [3α-$^2H_1$]cholesterol in isotropic phospholipid phase produced during sample mixing are responsible for the central spike, which was ignored for data analysis.

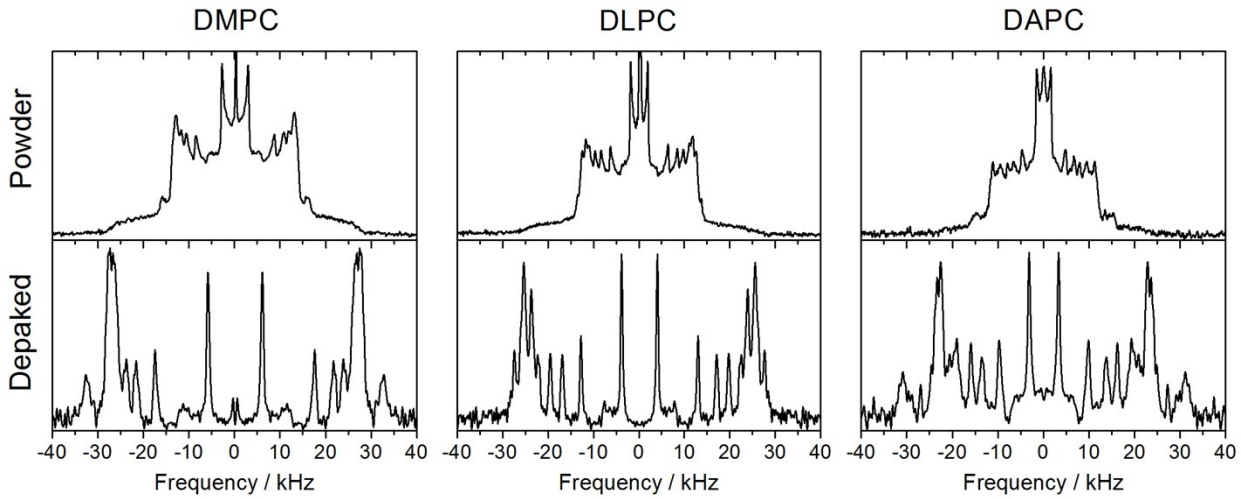

Figure S2 ^{2}H NMR spectra recorded at 30 °C with LA-d_{23} (5 mol%) in 50 wt% aqueous multilamellar dispersions of DMPC, DLPC and DAPC in 50 mM Tris (pH 7.5). The upper panels are powder patterns and the lower panels are FFT depaked. In processing the intrinsically noisy depaked data, the out-of-phase channel was zeroed before Fourier transformation. The resultant spectra were reflected about the central frequency and possess an improvement in signal/noise of a factor of $\sqrt{2}$.

MD simulations

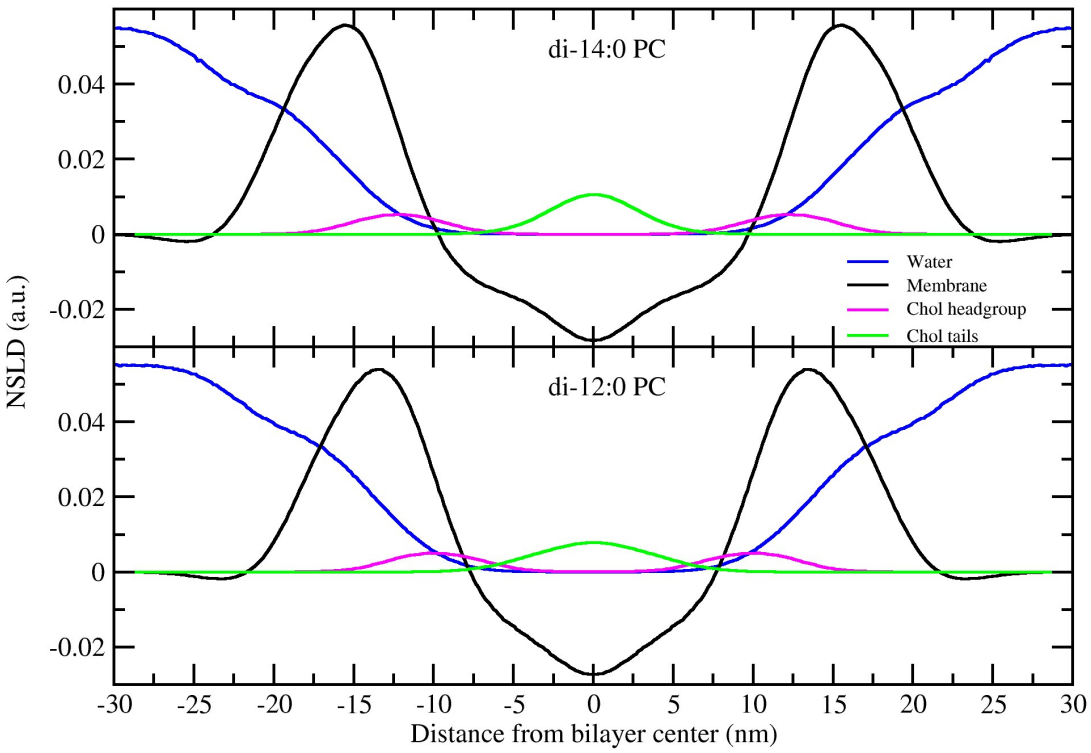

Figure S3 NSLD profiles of bilayers containing cholesterol calculated from MD simulations for DMPC + 10 mol % cholesterol (upper panel), and DLPC + 10 mol % cholesterol (lower panel). Shown are profiles of the lipid (black), water (blue), [2,3,4]-carbons of the cholesterol headgroup (purple), and [25,26,27]-carbons of the cholesterol tail (green).

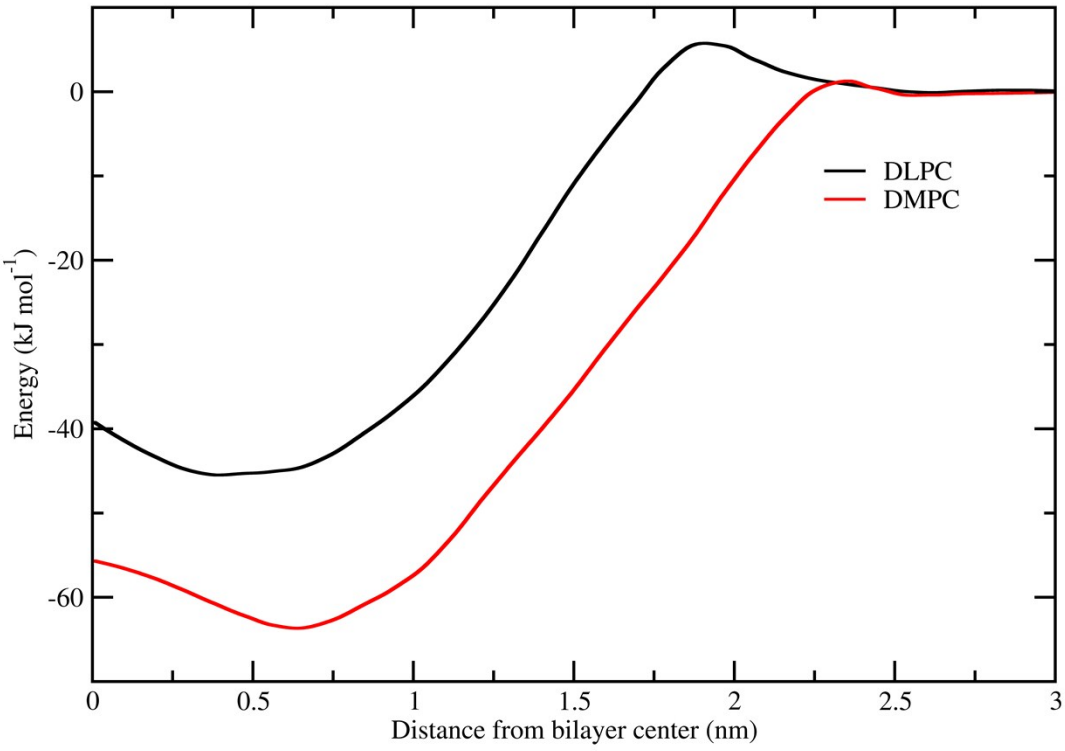

Figure S5 Potentials of mean force calculations reveal a larger energy barrier at the center of the membrane for cholesterol in DMPC (red) compared to DLPC (black) bilayers. The energy barrier for cholesterol flip-flop is 8 kJ/mol in DMPC bilayers, compared to 5.4 kJ/mol in DLPC bilayers.

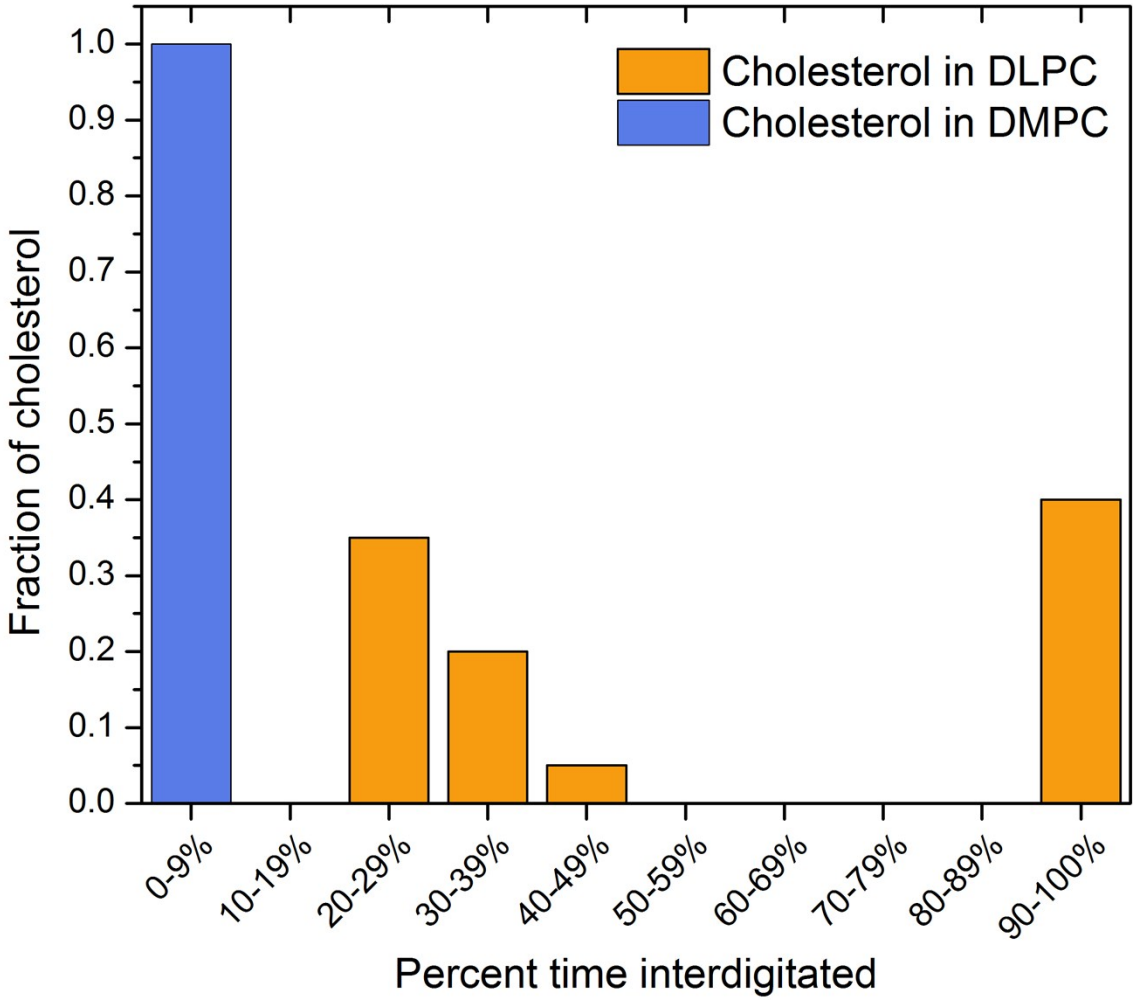

Figure S5 MD results illustrating cholesterol's propensity for interdigitation in DLPC (orange bars) and DMPC (blue bars) bilayers. 20 cholesterol molecules were incorporated into the simulated bilayer. A cholesterol molecule is defined as being interdigitated when at least 25% of cholesterol atoms are present in each bilayer leaflet, simultaneously. The percentages indicated by the plot represent the amount of time cholesterol spends interdigitated, with the size of each bar corresponding to the number of cholesterol molecules.

References

1) S. Jo, H. Rui, J.B. Lim, J.B. Klauda and W. Im, Cholesterol Flip-Flop: Insights from Free Energy Simulation Studies. *J. Phys. Chem. B,* 2010**, 114**, 13342-13348.

2) W.F.D. Bennett, J.L. MacCallum, M.J. Hinner, S.J. Marrink and D.P. Tieleman, Molecular View of Cholesterol Flip-Flop and Chemical Potential in Different Membrane Environments. *J. Am. Chem. Soc.,* 2009**, 131**, 12714-12720.