Supporting Information for Publication
Cholesterol and POPC segmental order parameters in lipid membranes: Solid state ^{1}H$-^{13}$C NMR and MD simulation studies

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Gaussian distributions for the cholesterol tilt angle

We use the $|S_{\text{CH}}|$ value measured from the C_3 cholesterol segment for the present analysis. This segment has been used before for this type of analysis since it may be selectively deuterated. Any other carbon in the ring structure could have been chosen instead though. For C_3, the magnitude of the order parameter, $|S_{\text{CH}}|$, measured is 0.39 (see Figure 5 in the main article) and by assuming that S_{CH} is negative, i.e., that the tilt should be closer to an orientation perpendicular than parallel to the membrane plane, we get S_{CH} equal to -0.39. For defining the S_β value in C_3, $S_\beta = 1/2\langle 3\cos^2\beta - 1\rangle$ where β is the angle between the CH bond and the director axis (here the director axis was defined as the vector which connects C_{17} to C_3), we could simply assume that the axial bond is always perpendicular to the director axis - this would give S_β=-0.5 - however, molecular vibrations are present, thus we chose to use S_β=-0.46 as determined from the MD simulation. Using then S_{CH} and S_β in $S_\alpha = S_{\text{CH}}/S_\beta$ one obtains S_α equal to 0.85. Finally we use S_α in Equation 7 (main article) to find numerically the possible parameters α_0 and σ.

Calculation of maximum sizes for phase coexistence domains

Cross sections of 2D R-PDLF spectra for $C_{9'}$ at the *sn*-2 position, measured from POPC/cholesterol MLVs with 15 (A), 34 (B) and 50 mol % (C) cholesterol are shown in Figure 9. According to the phase diagram from fluorescence studies [1], samples A, B, and C should be close to pure L_d, L_d/L_o coexistence, and pure L_o phases, respectively. The line shape from the superposition of spectra from samples A and C (red line) is shown together with the measured line shape for sample B. Clearly, the experimental NMR result differs from the superposition and has a single-phase line shape. In order to get a single-phase NMR line shape from a two-phase system, the molecular exchange between the different domains needs to be much faster than the characteristic spectral time, τ, which is related to the NMR frequencies measured. For the dipolar field spectra as in Figure 1, this spectral time is equal to the inverse of the ^{1}H-^{13}C dipolar coupling difference between A and C, $\tau_{\text{CH}} = 1/\Delta d_{\text{CH}}$, which is around 2 ms (here we chose segment $C_{9'}$ since it has the maximum Δd_{CH} observed) . By using the measured diffusion coefficient of 22×10^{-12} m^2/s for POPC/cholesterol MLVs with 40 mol% cholesterol [2] and a diffusion time, t equal to τ_{CH}, together with the diffusion equation, we get $\langle r^2\rangle = 4tD \approx 2\times10^5$nm^2, i.e the domains must be much smaller than 500 nm. In addition, also a single phase line shape is obtained on the ^{2}H NMR spectra of these MLVs [3]. Since the spectral time is around one order of magnitude shorter in ^{2}H NMR experiments ($\Delta\nu_{\text{CD}} = \nu_{\text{CD}}^{\text{max}}/d_{\text{CH}}^{\text{max}} \times \Delta d_{\text{CH}}$, with $\nu_{\text{CD}}^{\text{max}} = (3/4)(e^2qQ/h) = (3/4)\times 170$ kHz and $d_{\text{CH}}^{\text{max}} = 21.5$ kHz), by using this shorter spectral time instead we find that if lateral domains in POPC/cholesterol bilayers exist they must be much smaller than 100 nm.
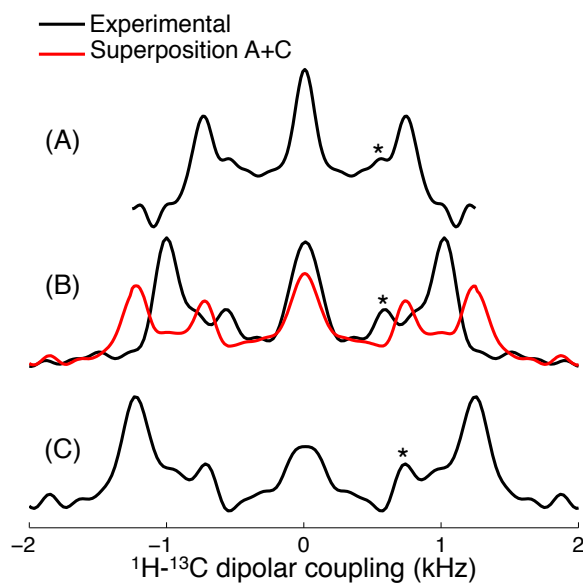
Figure 1: Cross sections (solid lines) from the 2D R-PDLF spectra of POPC/cholesterol multi-lamellar vesicles with 15 mol% (A), 34 mol% (B) and 50 mol% (C) cholesterol at the chemical shift of the $C_{9'}$ segment of the phospholipid sn-1 chain. The red line shows the hypothetical line shape for sample B in the case of L_d/L_o phase coexistence with large phase domains and slow exchange in comparison with the spectral time, $\tau \approx 1/\Delta\nu = 2$ ms. (*) Spurious peak from the R-PDLF experiment.

References

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