Supporting Information

Clustering of Stearic Acids in Model Phospholipid Membranes Revealed by Double Electron-Electron Resonance

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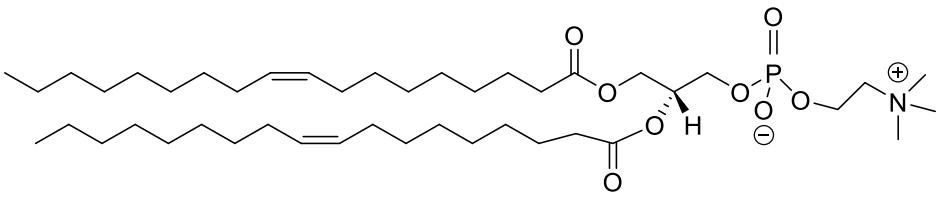
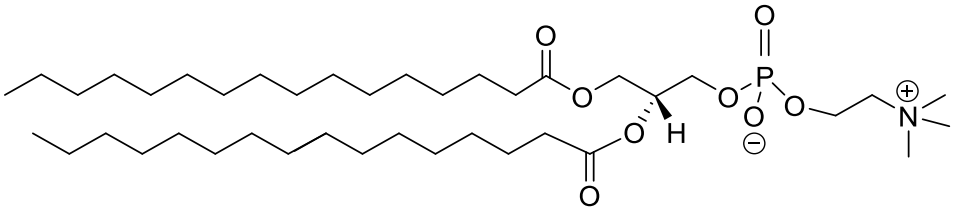
Content:

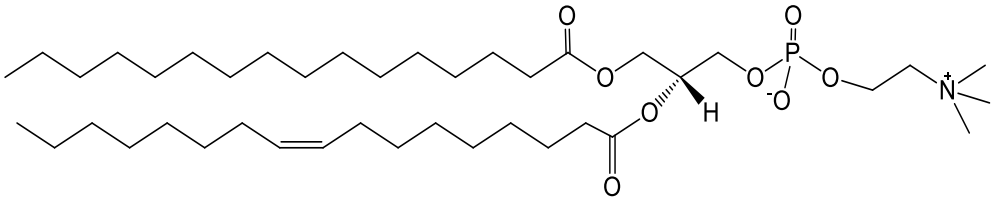
Chemical structures



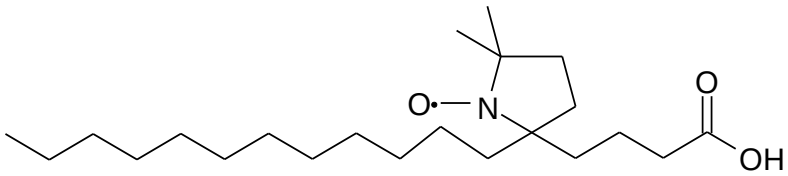
DOPC



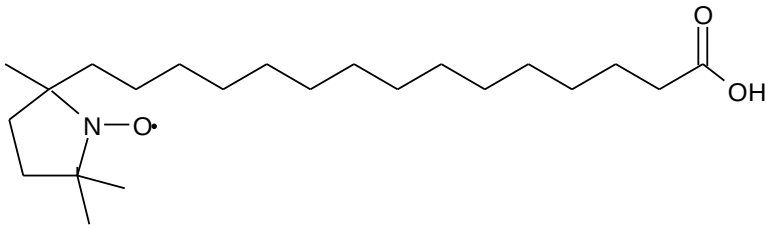
DPPC



POPC



5-DSA



16-DSA

Scheme S1.

Echo-detected EPR spectrum and excitation frequencies

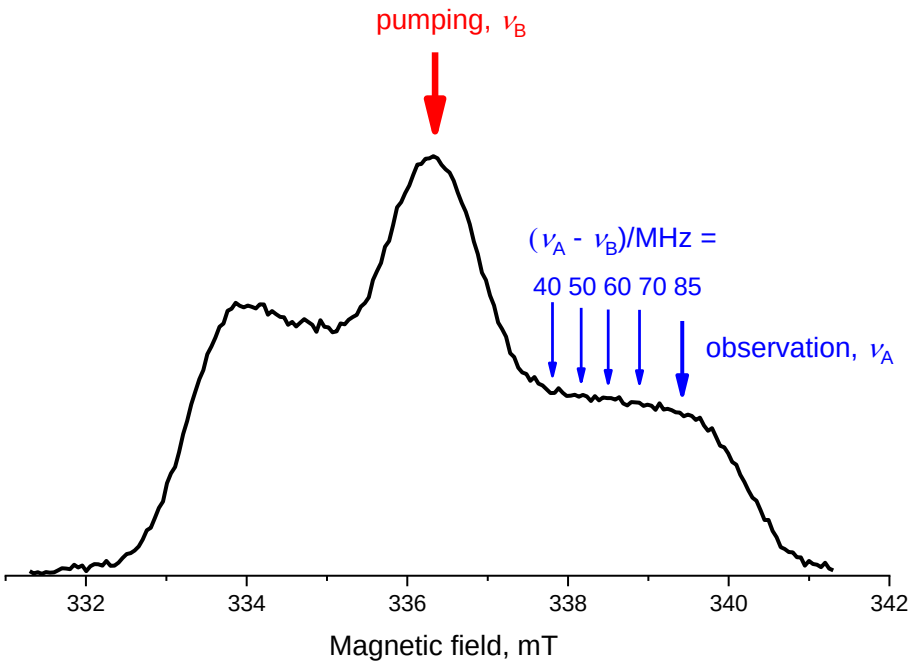


Figure S1. Echo-detected EPR spectrum obtained with scanning magnetic field and fixed time delay τ (120 ns) between two microwave pulses in the pulse sequence $(\pi/2)_{\nu A}$ - τ – $\pi_{\nu A}$ – *echo*. The arrows show field positions for observation at microwave frequency ν_A and excitation at microwave frequency ν_B. The sample is 0.5 mol % 5-DSA in the POPC bilayer, temperature 80 K.

DEER signal correction upon the pumping pulse passage through the detecting pulses

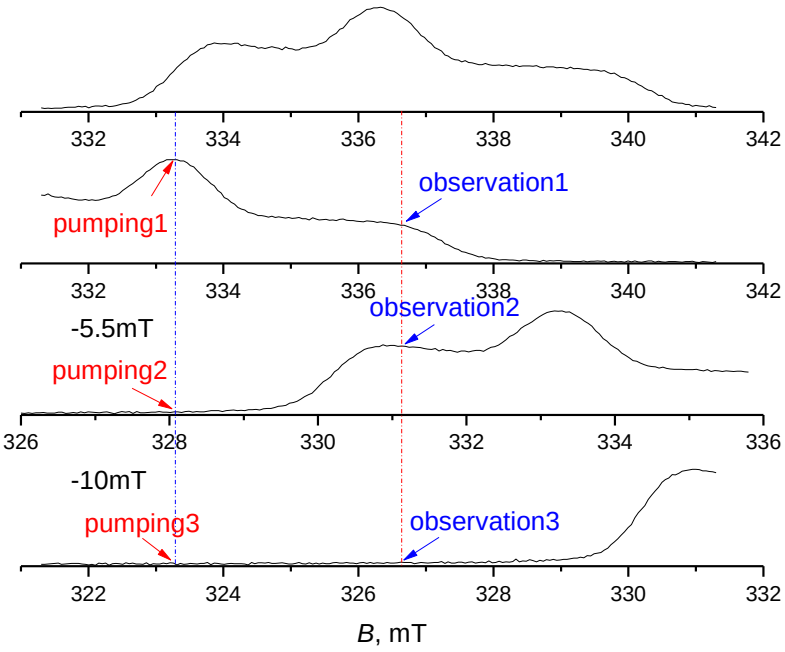


Figure S2. Calibration measurements at three different magnetic field positions.

The corrected *V*(t) time dependence is obtained by relation:[35]

$$V(t) = \frac{V_1(t) - V_3(t)}{V_2(t) - V_3(t)}$$

(35) Milov, A. D.; Grishin, Y. A.; Dzuba, S. A.; Tsvetkov, Y. D. Effect of Pumping Pulse Duration on Echo Signal Amplitude in Four-Pulse PELDOR. *Appl. Magn. Reson*. **2011**, *41*, 59-67.

Examples of the original DEER time traces

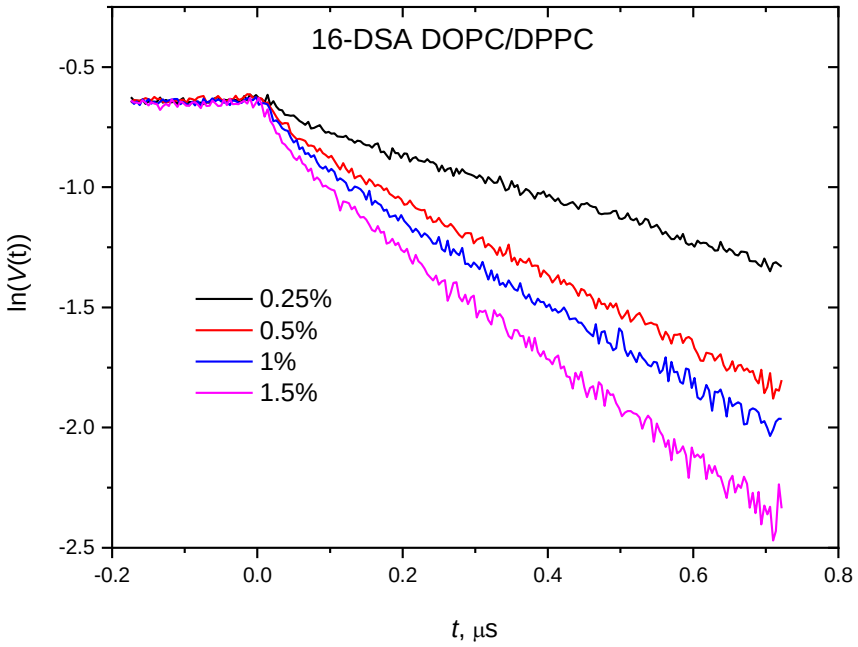


Figure S3. Original three-pulse DEER time traces for different 16-DSA content in DOPC/DPPC (1:1) bilayer. Temperature is 80 K.

CW EPR spectra in POPC bilayer

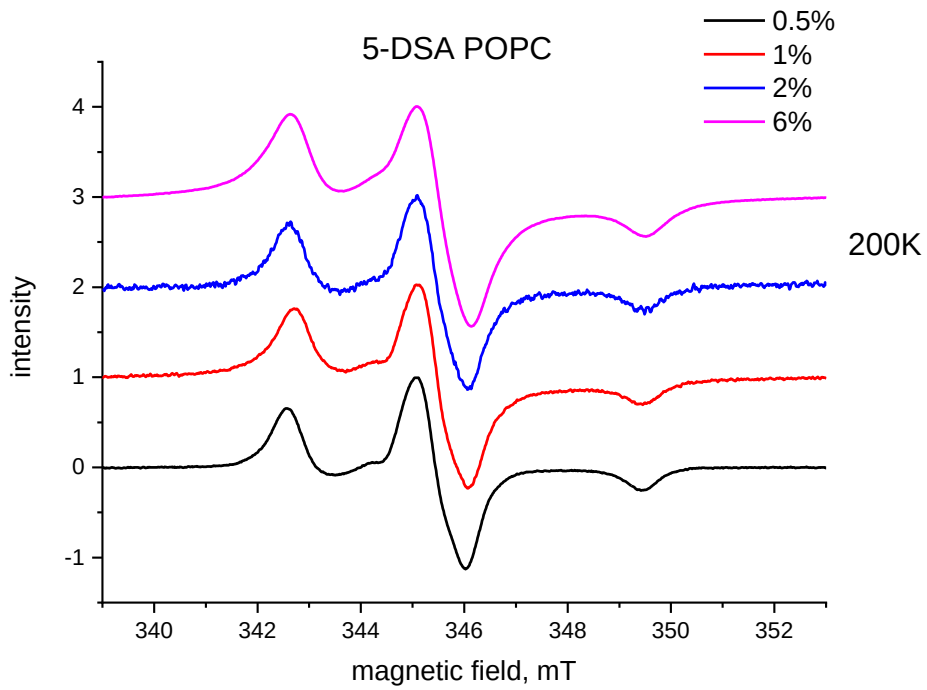


Figure S4. CW EPR spectra taken at 200 K for 5-DSA at different concentrations in the POPC bilayers. Spectra are normalized to the same intensity of the central component and vertically shifted.

Checking orientational selectivity

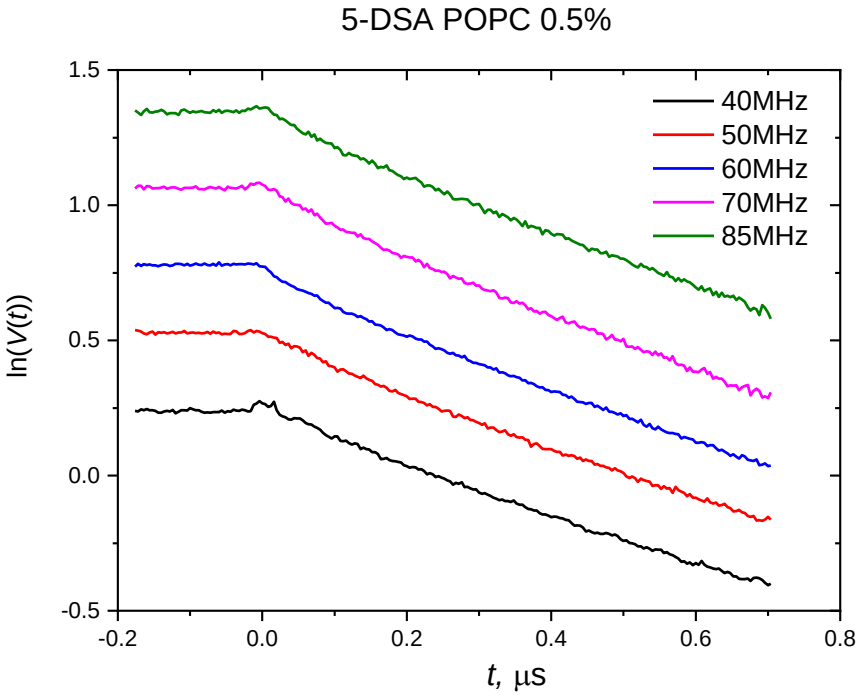


Figure S5. DEER time traces for different $\nu_A - \nu_B$ frequency offsets (see Figure S1). The sample is 0.5 mol % 5-DSA in POPC bilayer.

Calibration experiment for p_B determination

The samples are 5 mM 16-DSA and 5 mM 5-DSA in ethanol/methanol (95:5) glass.

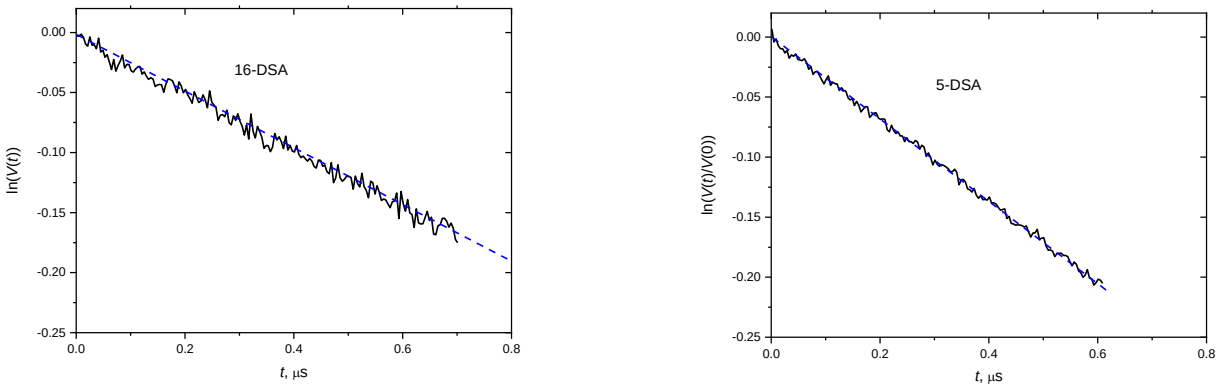


Figure S6. DEER time traces for 5mM 16-DSA (left) and 5-DSA (right) in ethanol/methanol (95:5) glass at 80 K. The dashed straight lines present linear approximations

Using formula for uniform three-dimensional distribution

$$V_{3D}(t) = V(0)\exp(-\frac{8\pi^2}{9\sqrt{3}}\frac{g^2\mu_B^2}{\hbar}Cp_Bt),$$

from the slope tangents we obtain p_B = 0.049 for 16-DSA and p_B = 0.068 for 5-DSA.

Simulations of DEER signal decay for spin labels in two planes

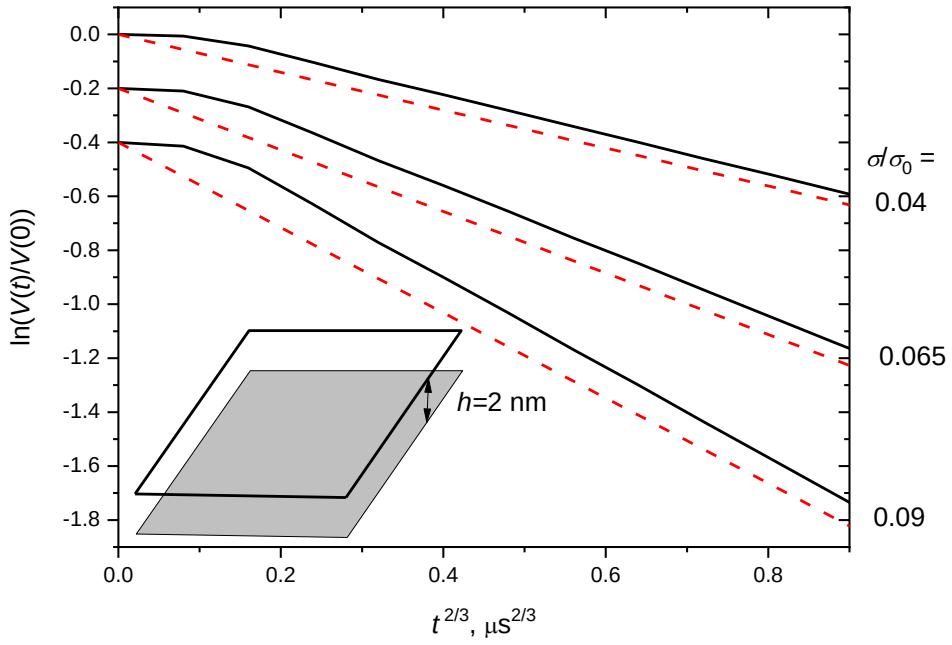


Figure S7. DEER time trace calculated[23] for spins randomly distributed in two infinite parallel planes separated by the distance h = 2 nm, for three different local concentrations. Dashed lines show the cases when h = 0 (the two planes coincide). Curves are vertically shifted for better visualization.

(23)Kardash, M.E.; Dzuba, S.A. Lipid-mediated Clusters of Guest Molecules in Model Membranes and their Dissolving in Presence of Lipid Rafts. *J. Phys. Chem. B* **2017**, *121*, 5209-5217.