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Supplemental Information

Sphingomyelin Acyl Chains Influence the Formation of Sphingomyelin- and Cholesterol-Enriched Domains

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Supporting material

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Methods

Equilibrium partitioning of CTL between mβCD and large unilamellar vesicles

The equilibrium partitioning of CTL between mβCD and large unilamellar vesicles (LUVs) was performed as described in a previously published protocol (1). In brief, LUVs were made by extruding multilamellar vesicles with defined lipid composition, through filters with 200 nm pores (Whatman International, Maidstone, UK). These LUVs (final concentration 50 μM) were mixed with different amounts of mβCD (from 0 to 1.0 mM)in a total volume of 2.5 mL. The resulting samples were incubated 2 h at 55 ºC after which the anisotropy of CTL was measured at the same temperature. The obtained anisotropy values were converted the molar concentration of CTL, C_{CTL}^{LUV}, according to

$$C_{CTL}^{LUV} = C_{CTL}\frac{(r_i - r_{CD})}{(r_{LUV} - r_{CD})} \quad [1]$$

where $C_{CTL}$is the total concentration of CTL in the samples, $r_{LUV}$is the anisotropy of CTL in the specific PL bilayer, $r_i$is the CTL anisotropy in the sample, and r_{CD} is the anisotropy of CTL in the CTL–mβCD complex. The molar fraction partition coefficients (K_X), describing the equilibrium partitioning of CTL between the different PL bilayers and mβCD, was calculated by plotting the calculated molar concentrations of CTL in the LUV bilayers against the mβCD concentration and fitting the obtained curves with the following equation:

$$C_{CTL}^{LUV} = \frac{C_L - C_{CTL} + (C_{CD})^n/K_X}{2} \times \left(\sqrt{1 + 4\frac{C_L C_{CTL}}{[C_L - C_{CTL} + (C_{CD})^n/K_X]^2}} - 1\right) \quad [2]$$

Here, C_L is the PL concentration, C_{CD} is the cyclodextrin concentration, and C_{CTL}^{LUV} is the cholesterol concentration in lipid bilayers. The relative partitioning coefficient K_R was calculated by dividing the K_X obtained with different PC samples with the K_X obtained from PSM samples.

Results

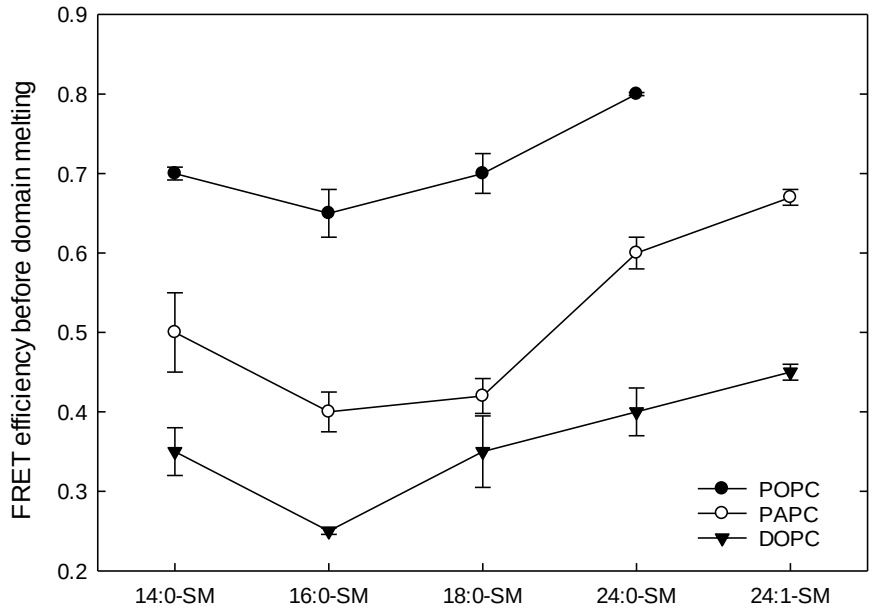

Figure S1. FRET efficiency before lo domain melting in different PC:SM:Cholesterol bilayers. Samples composed of unsaturated PC:SM:cholesterol (40:40:20) were prepared. The F0 samples contained 0.5 mol% DPH, and the F samples contained 0.5 mol% DPH and 2 mol% Rho-DOPE. The FRET efficiencies were measured at different temperatures with different lipid systems so that it was measured before the domain melting in all systems.

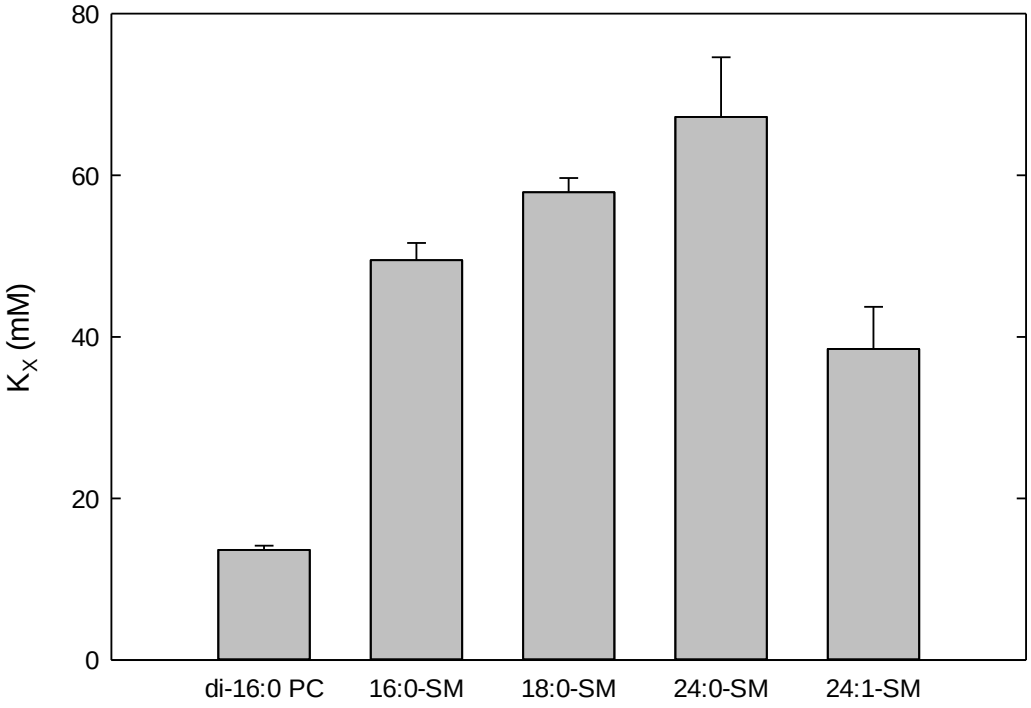

Figure S2. Equilibrium partitioning of CTL between mβCD and LUVs composed of different SMs. DPPC was included for comparison. All measurements were performed at 55 °C to ensure fluid membranes. n ≥3 + SD.

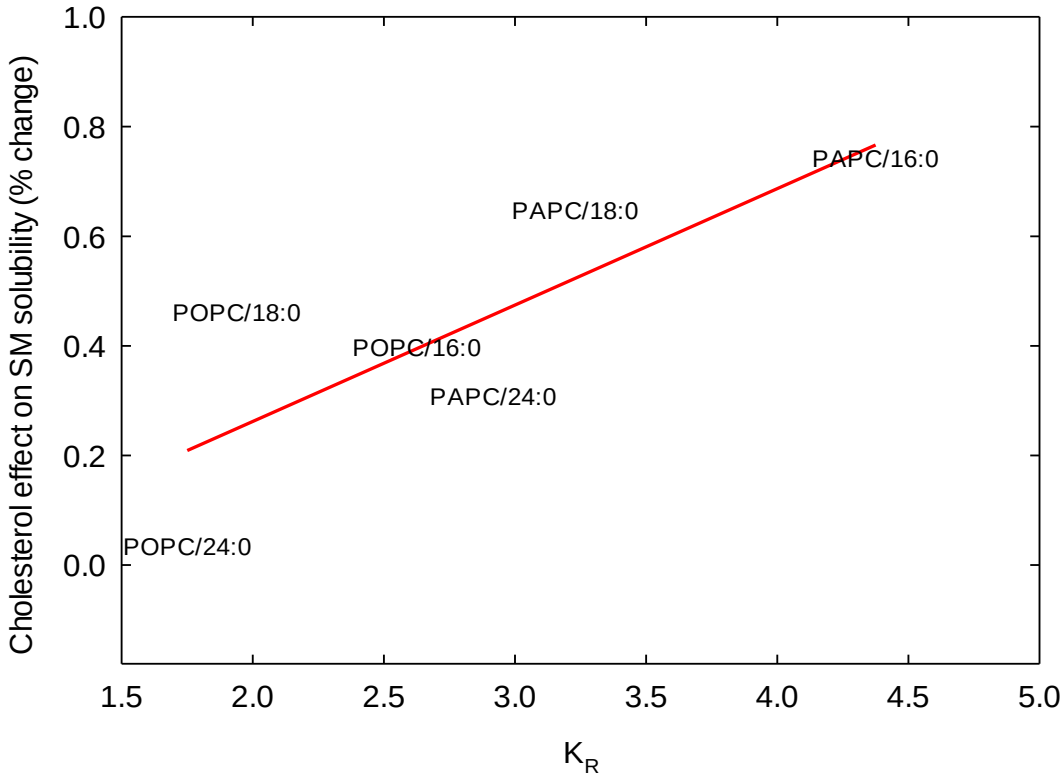

Figure S3. Correlation between relative cholesterol affinity (K_R)and the SM solubility in the fluid disordered phase. The change in SM solubility due to cholesterol addition was plotted against the measured relative partitioning coefficients of CTL for the different phospholipid in the different systems. The graph shows data from the present study, as well as from our previously published results (2, 3).

References

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2. Engberg, O., V. Hautala, T. Yasuda, H. Dehio, M. Murata, J. P. Slotte, and T. K. Nyholm. 2016. The Affinity of Cholesterol for Different Phospholipids Affects Lateral Segregation in Bilayers. Biophys J 111:546-556.
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