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Supplemental Information

The Affinity of Cholesterol for Different Phospholipids Affects Lateral Segregation in Bilayers

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Supporting material

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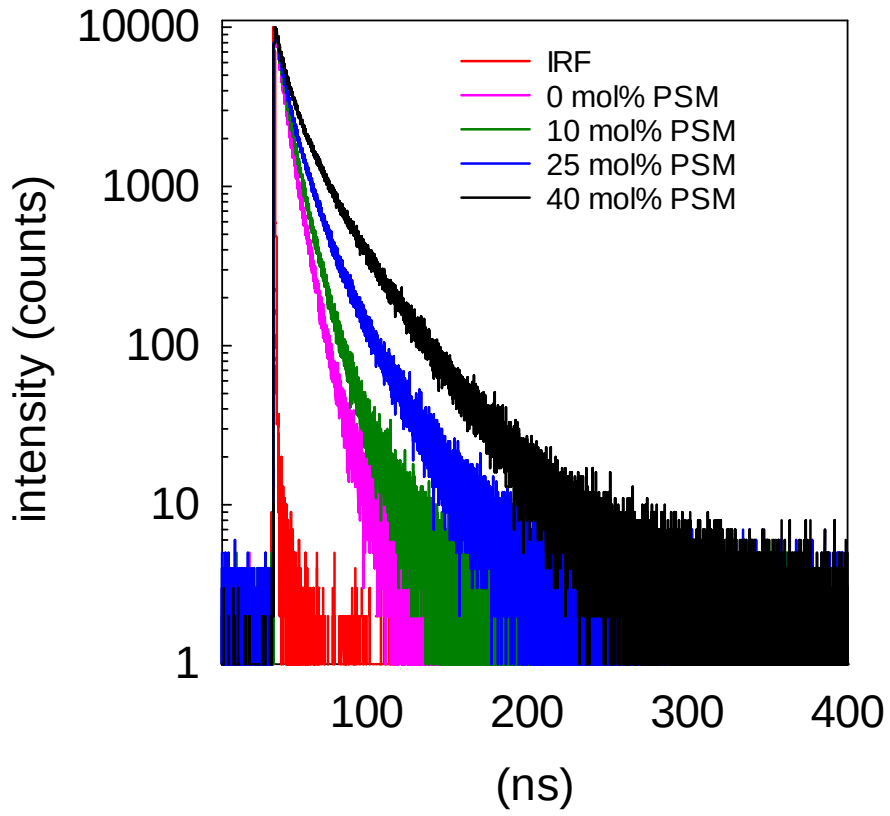

Figure S1. Representative fluorescence decays of tPA in POPC bilayers with 20 mol% cholesterol as a function of PSM concentration at 23 °C. IRF is the instrument response factor.

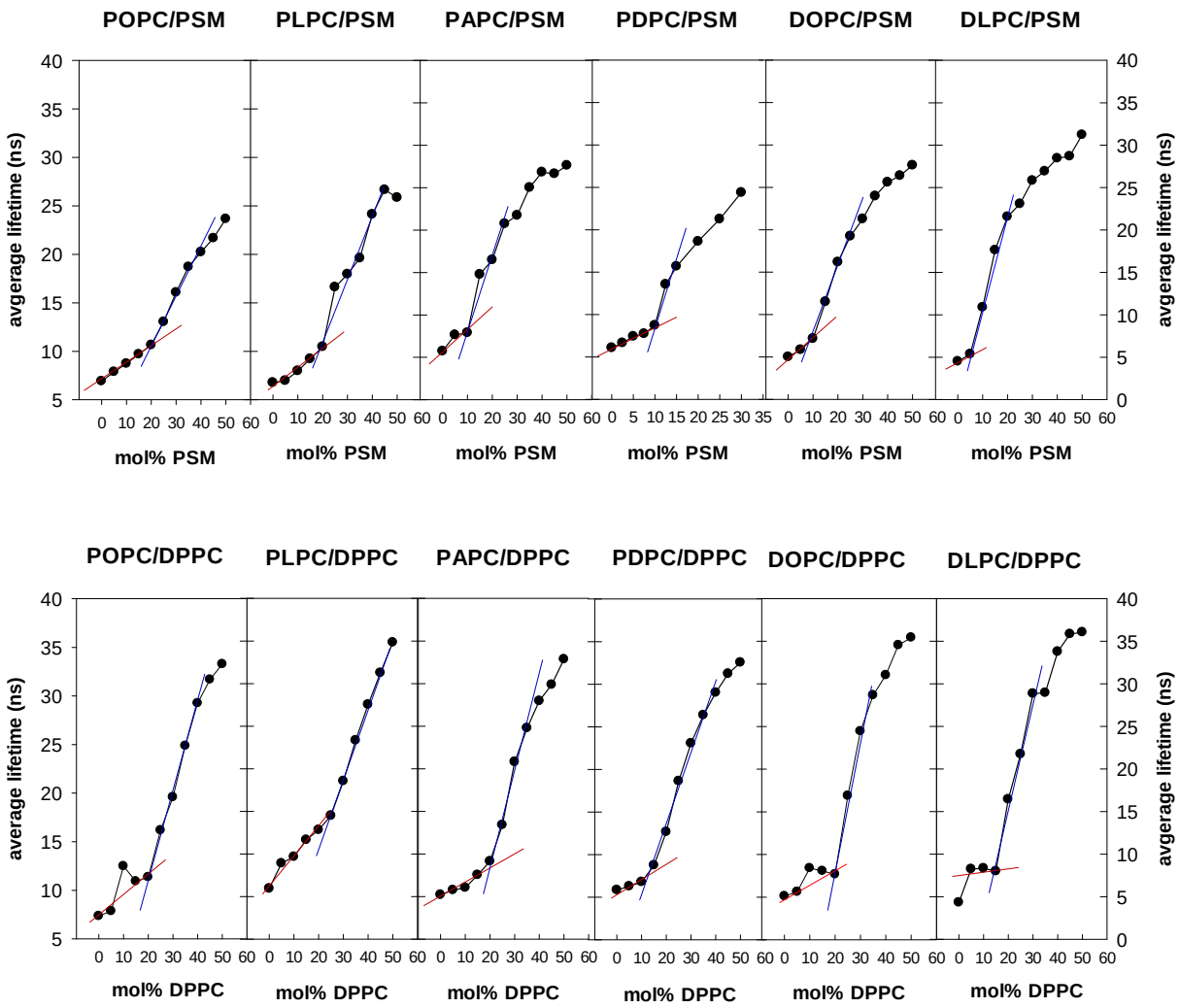

Fig S2. The average fluorescence lifetime of tPA in different phospholipid bilayers containg 20 mol% cholesterol. Representative data from experiments performed at 23°C are shown as a function of the mol fraction saturated lipid (PSM or DPPC). The determined l_o-domain onset in ternary bilayers are indicated as the points where the blue and red lines cross.

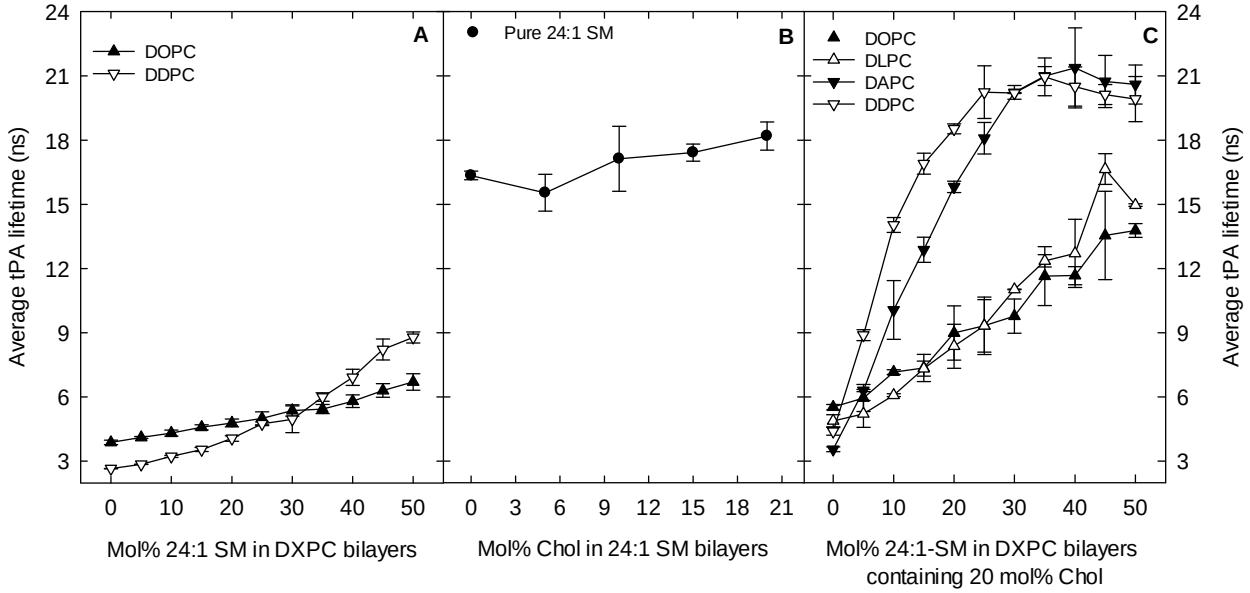

Figure S3. Lateral segregation with 24:1 SM by time-resolved fluorescence of tPA. Panel A) Addition of 24:1 SM to DOPC or DDPC bilayers. Panel B) Addition of cholesterol to pure 24:1 SM bilayers. Panel C) Addition of 24:1 SM to different polyunsaturated PL bilayers with 20 mol% cholesterol. All experiments were performed at 23 ^{o}C.

To gain further insight into how cholesterol can promote lateral segregation, experiments with an unsaturated SM, 24:1-SM was performed. This SM is in the fluid phase at 23 °C (1). The tPA lifetime measurements showed that neither addition of cholesterol nor unsaturated PLs to 24:1-SM lead to a discontinuity in the lifetime function, suggesting no formation of ordered domains (Fig. S2A and S2B). However, addition of 24:1-SM to bilayers with 20 mol% cholesterol and the polyunsaturated DAPC and DDPC lead to a marked increase in the average fluorescence lifetime of tPA, which reached a plateau around 30 mol% 24:1 SM(Fig. S2C). For comparison, no segregation was observed with DOPC or DLPC (Fig. S2C). The lifetimes at 30 mol% 24:1 SM were slightly higher than those observed in binary 24:1-SM/cholesterol bilayers (Fig. S2B), suggesting that the ordered domains were enriched in 24:1-SM and cholesterol. These results indicate that cholesterol can facilitate the segregation even of two unsaturated lipids if the degree of order in the acyl chains (and likely the relative cholesterol affinity for the two lipids) is large enough.

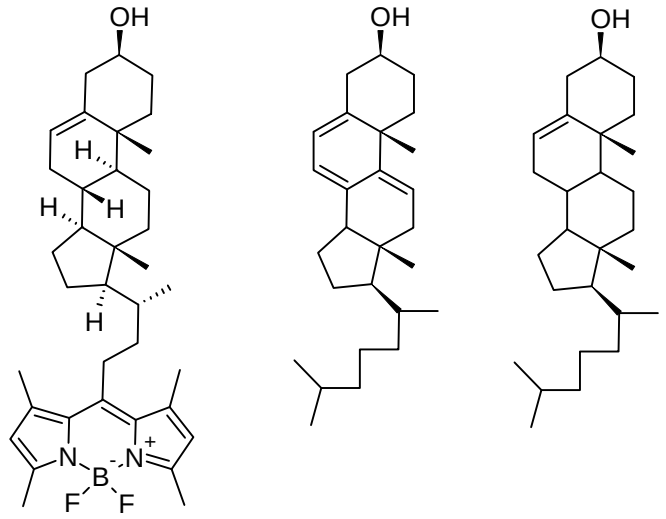

Figure S4. Structures of cholesterol and the cholesterol analogs used in the study.

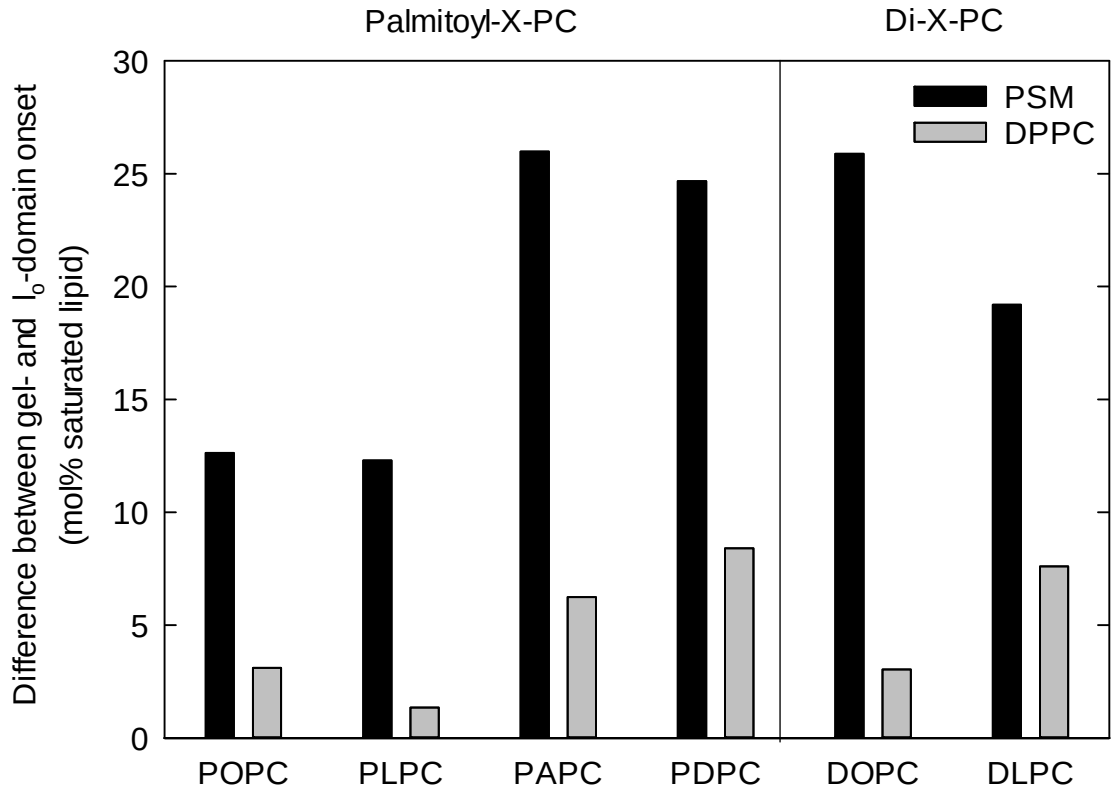

Figure S5. Determination of cholesterol promoted lateral segregation. Y-axis is gel-domain onset minus l_o-domain onset. Onsets were calculated from average fluorescence lifetimes of tPA as described in figure 1 and data taken from figure 2, 4 and (2). All experiments were performed at 23 ^{o}C.

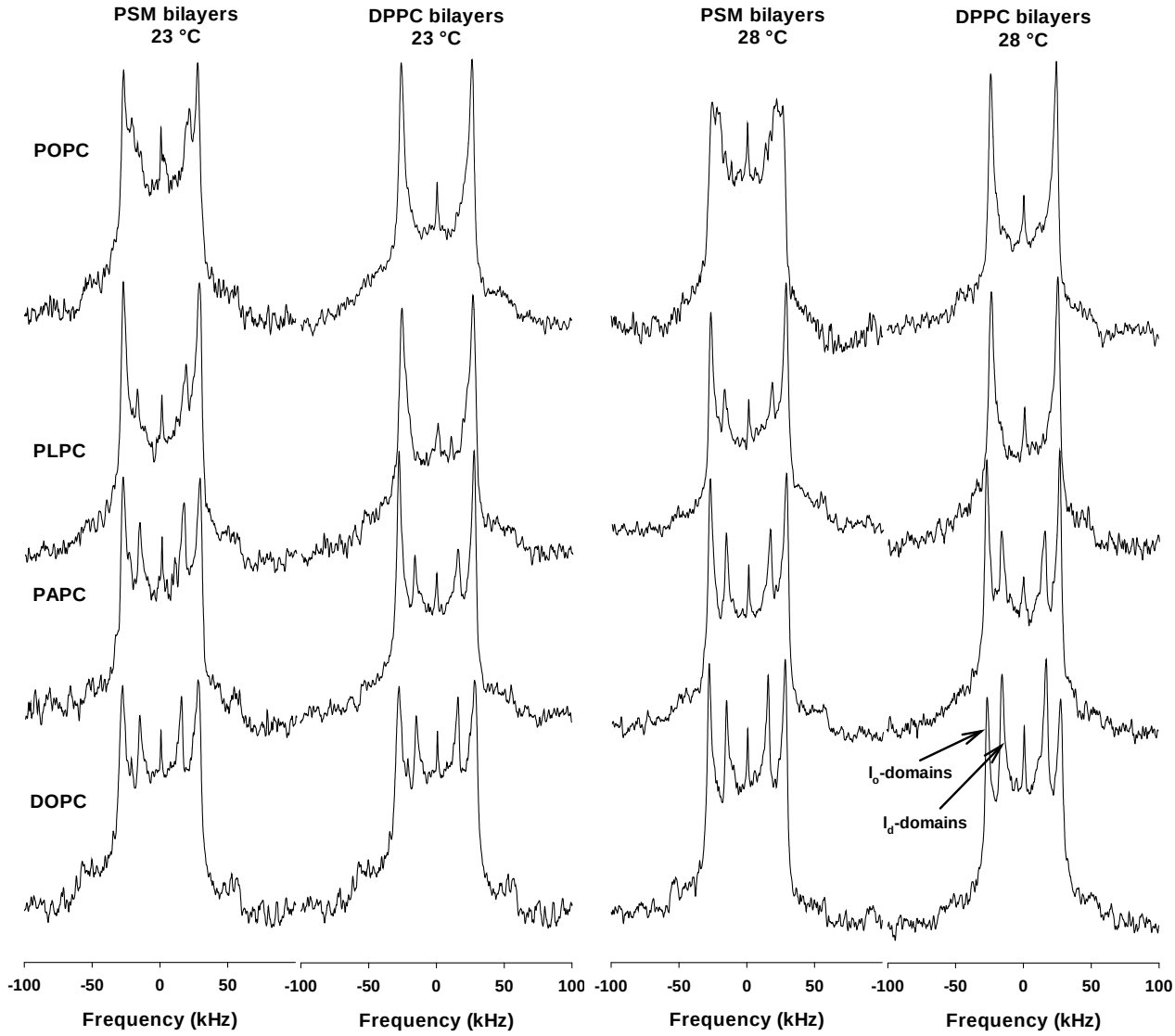

Figure S6. ^{2}H NMR spectra of ternary bilayers at 23 and 28 °C. Multilamellar vesicles were prepared to to 50 wt % H_2O containing one unsaturated lipid (POPC, PAPC or DOPC), a saturated lipid P(d_2)SM or DP(d_2)PC) and cholesterol mixed in the ratio 40/40/20.

Supporting references

1. Bjorkqvist, Y. J., J. Brewer, L. A. Bagatolli, J. P. Slotte, and B. Westerlund. 2009. Thermotropic behavior and lateral distribution of very long chain sphingolipids. Biochim. Biophys. Acta 1788: 1310-1320

2. Kullberg, A., O. O. Ekholm, and J. P. Slotte. 2015. Miscibility of Sphingomyelins and Phosphatidylcholines in Unsaturated Phosphatidylcholine Bilayers. Biophys. J. 109: 1907-1916