

Supplement 3 - Explanation of dip in $q_0(\phi)$

Supplementary information for:

Liquid-liquid domains in bilayers detected by wide angle x-ray scattering

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In Fig. 3 in the main paper, for 1:1 DOPC/DPPC + 15% cholesterol, at temperatures of 30°C and below, q_0 decreases as a function of ϕ up to $\phi \sim 20^\circ$, and then begins to increase. There we noted that the minimum in the $q_0(\phi)$ plot can be accounted for if q_0 is smaller for the Ld phase than for the Lo phase. We can evaluate if this assumption is reasonable based on our q_0 values for DOPC/cholesterol and DPPC/cholesterol mixtures. Table S3.1 shows the q_0 and $d (=2\pi/q_0)$ values for DOPC/cholesterol and DPPC/cholesterol mixtures at 25°C. For 1:1 DOPC/DPPC + 15% cholesterol at 25°C, the compositions of the coexisting phases (the tie line endpoints) have been reported (1) to be: Ld = 57% DOPC / 34% DPPC / 9% cholesterol and Lo = 16% DOPC / 58% DPPC / 26% cholesterol. If we assume the q_0 value for the Ld composition is close to that of DOPC + 10% cholesterol ($q_0 = 1.36 \text{ \AA}^{-1}$) and the q_0 value for the Lo composition is close to that of DPPC + 25% cholesterol ($q_0 = 1.45 \text{ \AA}^{-1}$), the above explanation is consistent.

A larger q_0 value for the Lo phase (more cholesterol) in comparison with the Ld phase (less cholesterol) may seem counterintuitive based on our knowledge of binary mixtures of phospholipid and cholesterol. For binary mixtures of phospholipid and cholesterol, we have observed that q_0 decreases as a function of cholesterol concentration (see Table S3.1). Thus, we might expect the Lo phase (higher cholesterol content), not the Ld phase, to have a smaller q_0 . However, the Ld and Lo phases have different amounts of DOPC and DPPC, which appear to dominate the relative q_0 values.

Table S3.1. Values of q_0 and d for DPPC/cholesterol and DOPC/cholesterol mixtures at 25°C (for $I(q)$ plot with $\phi = 5-10^\circ$).

	mol% cholesterol	q_0^* (\AA^{-1})	$d = 2\pi / q_0$ (\AA)
DPPC	10	1.48	4.25
	15	1.46	4.30
	25	1.45	4.33
	40	1.38	4.55
DOPC	0	1.39	4.52
	10	1.36	4.62
	40	1.28	4.91

*Uncertainty on q_0 values is ± 0.01 .

REFERENCES

1. Veatch, S. L., O. Soubias, S. L. Keller, and K. Gawrisch. 2007. Critical fluctuations in domain-forming lipid mixtures. Proc. Natl. Acad. Sci. U. S. A. 104:17650-17655.