Supplement 1 - Data fitting and errors

Supplementary information for:

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

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Table S1.1 summarizes the fitting data for 1:1 DOPC/DPPC + 15% cholesterol. The data and fits for this sample were shown in Fig. 2 in the paper. S_1 corresponds to $S_{x\text{-}ray}$ for the less ordered fraction and S_2 corresponds to $S_{x\text{-}ray}$ for the more ordered fraction. Uncertainties reflect 95% confidence intervals (see Materials and Methods of the accompanying paper (1)). For all the data, both the single and double order parameter fits were performed, and the fit was either accepted or rejected on the basis of plausibility and uncertainty in the parameters (discussed later in this supplement). For the double order parameter fits, P_1 and P_2 are shown, but as discussed in the Materials and Methods, we are cautious about interpreting these values as true phase fractions. The table also lists the reduced chi-square, defined as (2; p.188):

$$\chi^2_{\text{red}} = \frac{1}{\nu}\sum_i \left(\frac{y_i - f_i}{\sigma_i} \right)^2 . \qquad \text{(S1.1)}$$

where y_i is the observed value, f_i is the fitted value, ν is the degrees of freedom (# data points- # fitting parameters -1), and the uncertainty , σ_i, had essentially the same value, σ, for all the i data points. If the fit is good, χ^2_{red} should be approximately equal to 1.0. For convenient comparison, the σ's were chosen such that the reduced chisquare for the double order parameter fit was exactly 1; these values of σ agree with the uncertainties estimated from the noise in the data, ~ 0.5% of the maximum intensity in the $I(\phi)$ plot.

Table S1.1. Results of fits to $I(\phi)$ data for 1:1 DOPC/DPPC + 15% cholesterol.

T (˚C)	*m**	$S_{x\text{-}ray}$*	Phase Fractions	χ^2_{red}	Accept fit?
15	m= 11.5 ± 1.1	S= 0.86 ± 0.01		64.9	NO
	m_1= 3.69 ± 0.23 m_2= 30.6 ± 1.3	S_1= 0.52 ± 0.03 S_2= 0.95 ± 0.01	P_1= 0.67 ± 0.01 P_2= 0.33 ± 0.01	1.0	YES
20	m= 8.96 ± 0.86	S= 0.82 ± 0.02		168.4	NO
	m_1= 3.48 ± 0.12 m_2= 29.8 ± 0.9	S_1=0.50 ± 0.01 S_2= 0.95 ± 0.01	P_1= 0.72 ± 0.01 P_2= 0.28 ± 0.01	1.0	YES
25	m= 7.08 ± 0.58	S= 0.76 ± 0.02		88.4	NO
	m_1= 3.34 ± 0.14 m_2= 25.7 ± 1.1	S_1= 0.48 ± 0.02 S_2= 0.94 ± 0.01	P_1= 0.77 ± 0.01 P_2= 0.23 ± 0.01	1.0	YES
30	m= 5.15 ± 0.29	S= 0.66 ± 0.02		36.1	NO
	m_1= 3.16 ± 0.14 m_2= 20.1 ± 1.5	S_1= 0.46 ± 0.02 S_2= 0.92 ± 0.01	P_1= 0.85 ± 0.01 P_2= 0.15 ± 0.01	1.0	YES
35	m= 3.47 ± 0.07	S= 0.50 ± 0.01		2.3	YES
	m_1= 1.43 ± 3.52 m_2= 4.61 ± 2.01	S_1= 0.21 ± 0.56 S_2= 0.61 ± 0.18	P_1= 0.58 ± 0.36 P_2= 0.42 ± 0.36	1.0	NO
40	m= 3.25 ± 0.04	S= 0.47 ± 0.01		1.0	YES
	m_1=m_2	S_1=S_2=0.47	NA	NA	NA
45	m= 2.96 ± 0.05	S= 0.43 ± 0.01		1.0	YES
	m_1=m_2	S_1=S_2=0.43	NA	NA	NA

* If only one order parameter is given, the fit is a single parameter fit. If 2 order parameters are listed, the fit is the double order parameter fit. If "m_1=m_2" and "S_1=S_2" are listed, these parameters were the same to within ±0.01.

Fig. S1.1 illustrates the reasoning (outlined in the Materials and Methods) behind accepting the double order parameter fit for $T \leq 30$˚C and rejecting it for $T \geq 35$˚C for the $I(\phi)$ data for 1:1 DOPC/DPPC + 15% cholesterol. Fig. S1.1*A* shows that between 30˚C and 35˚C there is a large jump in the fractional uncertainties in $S_{x\text{-}ray}$ ($\Delta S/S$) for the double order parameter fit, particularly for the lower order parameter. This jump correlates with $I(\phi=80^\circ)$-I_{back} (where I_{back} is for the single order parameter fit) changing from negative (unphysical) to positive (see Fig. S1.1*B*). Also, there are very large values of χ^2_{red} for the single order parameter fit for $T<35$˚C and much smaller values for $T>30$˚C (see Fig. S1.1*C*). The same trends were observed for all the ternary mixtures studied.

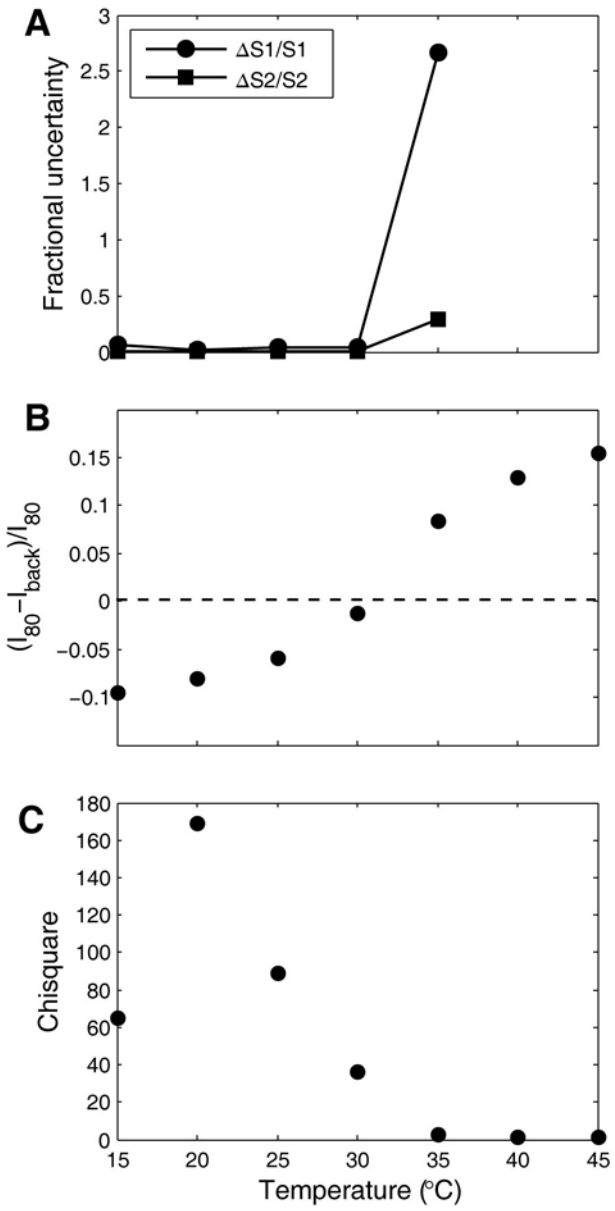

Figure S1.1. (*A*) Fractional uncertainty in $S_{x\text{-ray}}$ ($\Delta S/S$) as a function of temperature for double order parameter fits to the 1:1 DOPC/DPPC + 15% cholesterol $I(\phi)$ data. S_1 corresponds to $S_{x\text{-ray}}$ for the less ordered phase and S_2 corresponds to $S_{x\text{-ray}}$ for the more ordered phase. ΔS is half the total size of the 95% confidence interval. Points are not shown for 40˚C and 45˚C, as the fit gave $S_1=S_2$ at these temperatures. (*B*) shows $[I(80)- I_{back}]/ I(80)$ as a function of temperature for the same sample, where I_{back} is the background parameter for the single order parameter fit and $I(80)$ is the scattering intensity at ϕ=80˚. (*C*) shows χ^2_{red} as a function of temperature for the single order parameter fit.

For our data, we were able to reject one of the models (double or single order parameter fit) based on consideration of 1) plausibility of the best-fit parameters (e.g., is the background-subtracted data positive?) and 2) preciseness of parameters (e.g., are the confidence intervals reasonably narrow?) [3; see pp. 29-31 and p. 34]. In cases where two models fit the data reasonably with plausible best-fit parameters and narrow confidence intervals, statistical tests such as the "F test" (3; pp. 152-153) are often applied. Such tests depend on the degrees of freedom being a well-defined quantity to account for differences in the number of fitting parameters between the two models. In our case, the degrees of freedom is difficult to define because the number of data points can be made arbitrarily large or small simply by changing the ϕ bin width over which the data are integrated. In other words, our $I(\phi)$ data points are correlated.

REFERENCES

1. Mills, T. T., G. E. S. Toombes, S. Tristram-Nagle, D.-M. Smilgies, G. W. Feigenson, and J. F. Nagle. 2008. Order parameters and areas in fluid-phase oriented lipid membranes using wide angle x-ray scattering. Biophys. J. Submitted.
2. Bevington, P. R. 1969. Data Reduction and Error Analysis for the Physical Sciences. McGraw-Hill, New York.
3. Motulsky, H., and A. Christopoulos. 2004. Fitting Models to Biological Data Using Linear and Nonlinear Regression. Oxford University Press, Oxford.