Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

The effect of hydrostatic pressure on model membrane domain composition and lateral compressibility

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Supplementary Information

The peak areas from samples lying along the same tie line were used to determine the 'zero diffraction intensity' composition for each phase (which corresponds to the tie lie end points) by linear extrapolation of the normalised composition – peak area data. A custom built automated LabVIEW based programme was used to fit the L_o and L_d diffraction peaks simultaneously using double pseudo-Voigt functions. The de-convoluted peaks from each sample were assigned as either L_o or L_d based on the samples position along the tie line and the relative peak intensities. The DOPC, DPPC and cholesterol composition was then plotted as a function of the normalised area of each peak. Normalisation of the sum of the L_o and L_d peak areas to 1 was performed post-fitting and has been described previously.[1] We have demonstrated this fitting method for samples 2, 3 and 4 at atmospheric pressure and 100 MPa as shown in Figure S1 to calculate the composition of the L_o phase. The normalised area of the L_d diffraction peak is plotted as a function of (a) DOPC (b) DPPC (c) cholesterol composition, with a linear fit to this data where:

mol% lipid = (gradient x Peak Area) + y intercept

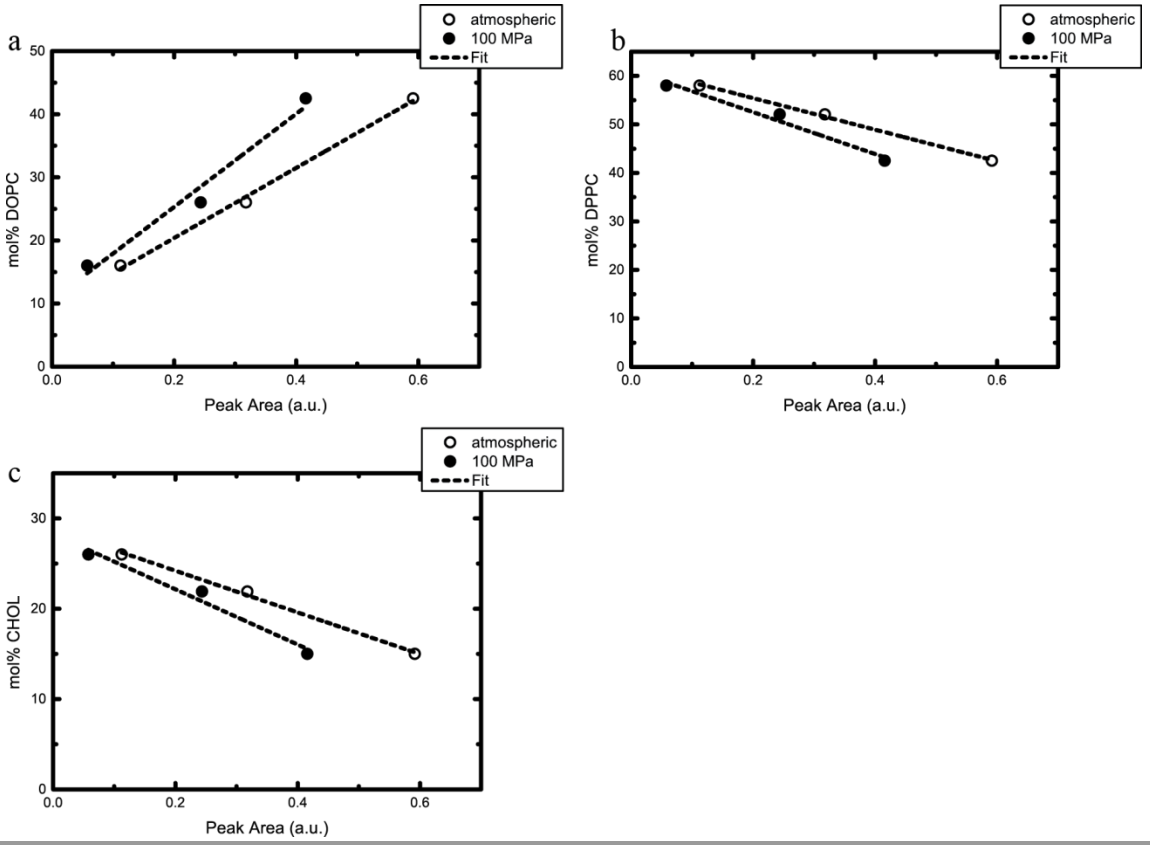

Figure S1. Plots of the L_d peak area used to find the compositions of the L_o phase (based on the tieline endpoint) for samples 2, 3 and 4.

We note that any changes in layer-to-layer registry due to pressure would lead to an equal reduction in the L_o and L_d registration within any given bilayer and is therefore accounted for through the normalisation post-fitting. Therefore, the level of registration or anti-registration would not influence the determination of the tieline endpoints or the assignment of the L_o and L_d phases. We also assume that the direction of the tie line does not change significantly with pressure, as discussed previously and supported by the linear relationship of peak area with lipid composition at all pressures.

When the L_d diffraction peak area is zero, the sample is solely composed of the L_o phase and hence the mol% of DOPC, DPPC and cholesterol in the L_o phase can be calculated using the y intercept from the linear fit.

The zero diffraction compositions for L_o and L_d were calculated separately to independently determine the two endpoints. For the tie line endpoint calculations only the first order diffraction peaks were used, given their significantly higher intensity than the second order peaks. Results for the tie line endpoint calculations, including the errors in the fits have been shown in Figure S2.

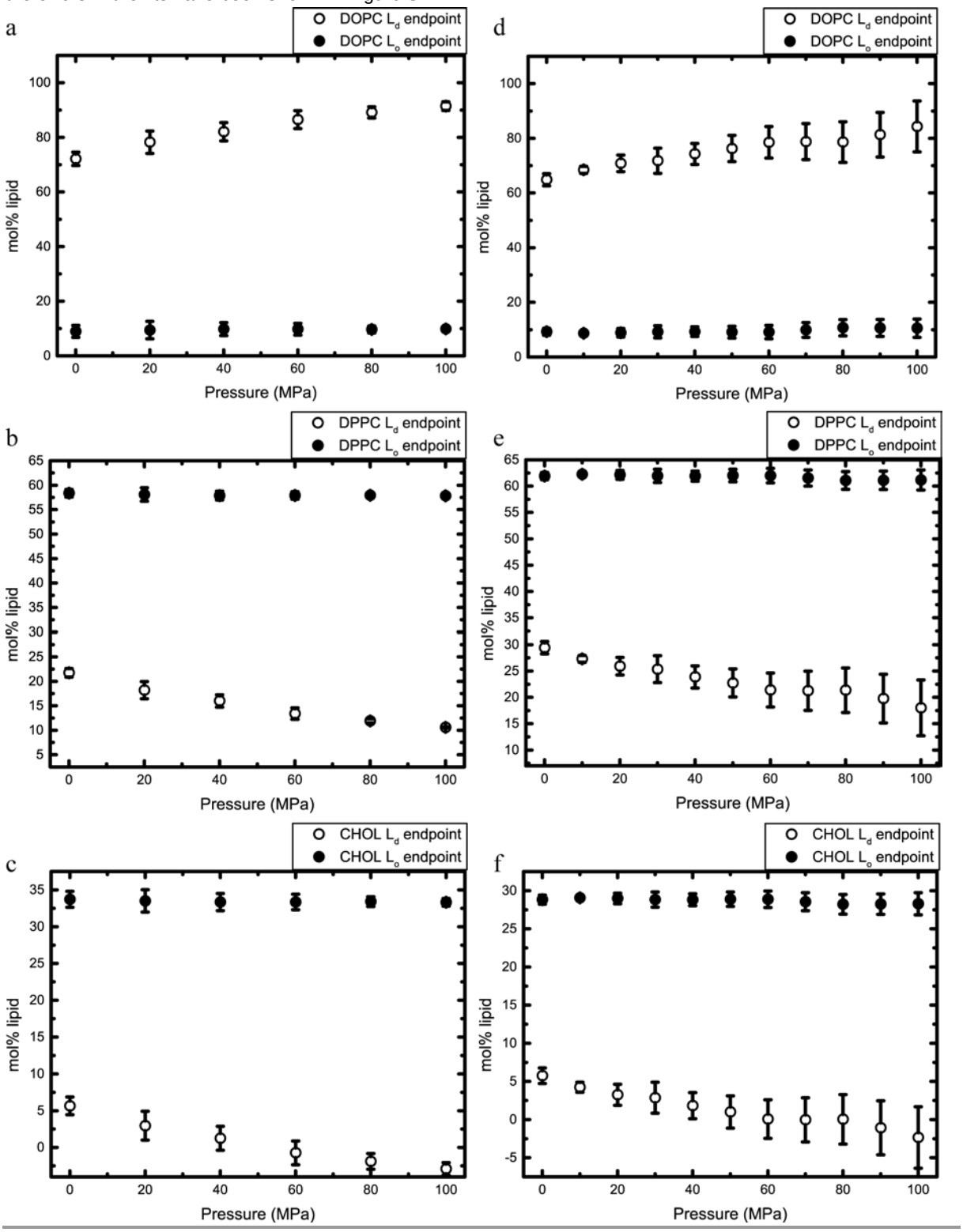

Figure S2. Calculated tie line endpoint compositions for samples 5 to 7 showing (a) DOPC (b) DPPC (c) CHOL and samples 1 to 4 showing (d) DOPC (e) DPPC (f) CHOL

1. P. Heftberger, B. Kollmitzer, A. A. Rieder, H. Amenitsch and G. Pabst, *Biophysical Journal*, 2015, **108**, 854-862.