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

Supplementary Materials

Part 1. Optical microscopy, bright field microscopy and fluorescence microscopy pictures of phase separated ternary mixture lipid multilayer domains.

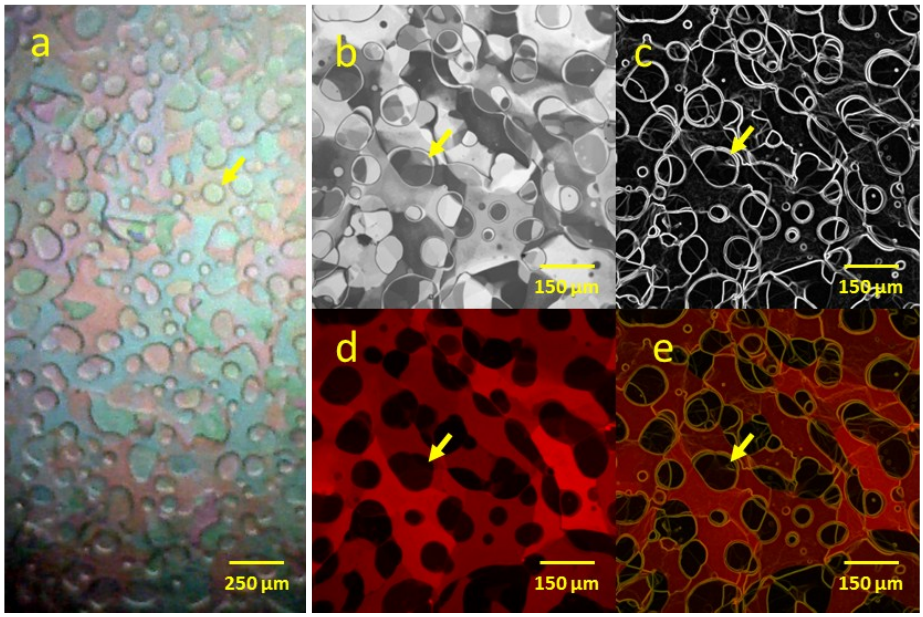

FIG. S1. Optical microscopy, bright field microscopy and fluorescence microscopy pictures of phase separated ternary mixture lipid multilayer domains, measured under 96% RH (saturated K2SO4 solution as reservoir in the sample chamber). After analyzing a large number of fluorescence microscopy images and bright field images, we found that the surface morphology, e.g., edges and heights, had a strong correlation with the domain distribution. DiI16 fluorescence dye was used in the fluorescence microscopy sample in the above image (d), which preferentially partitions into the L_d phase. DiIC18 and β-BODIPY fluorescence dyes were also used in our fluorescence microscopy studies to confirm the identification of phases. The edges in the bright field images are mainly correlated with the phase boundaries of the L_o and L_d domains; furthermore, the higher height regions of the sample surface are mostly correlated with the Lo rich regions and the lower height regions (concaves) are mostly correlated with the L_d rich regions. Figure shows optical microscope image (a), bright field image (b) and fluorescence microscope image (d) of the same sample with 16% cholesterol. (c) is the differential calculation of (b), which shows the edges of surface morphology. Overlapping (c) with the fluorescence image from (d), we obtain the image shown in (e). In this overlapped image, we can clearly see this strong correlation.

Part 2. Table for simulation parameters

Table S1. Simulation parameters of the non-anomalous swelling curve of L_d phase and L_o phase. The simulation uses Eq.(3) in the manuscript, and fits to the normal swelling part of the data.

	A_{fl} $(10^6 dyn/cm^2)$	λ_{fl} (Å)	A_h $(10^9 dyn/cm^2)$	λ_h (Å)	H $(10^{-14} erg)$	$D_B^{'}$ (Å)
L_d	**6.46**	**5.8**	**0.67**	**2.0**	**4.82**	**48.0**
L_o	**0.25**	**6.0**	**0.69**	**2.0**	**2.03**	**55.4**

Part 3. Calculation of the mismatch energy

The following amounts to a simplified model to account semi-quantitatively for the effects of domain wall mismatch.

1. Approximate the hydrophilic-hydrophobic periods as rectangular waves, as shown in Figure S2. Give hydrophilic part (head + water + head) the value one, and the hydrophobic part (tail) value zero. The blue rectangular wave f_d simulates the periods for L_d phase, while the red dotted rectangular wave f_o simulates the periods for the L_o phase, which has slightly longer chains. We assume that there is a constant mismatch energy of λ per unit length when the hydrophilic regions of one phase overlap with hydrophobic regions of the other phase, and zero otherwise.

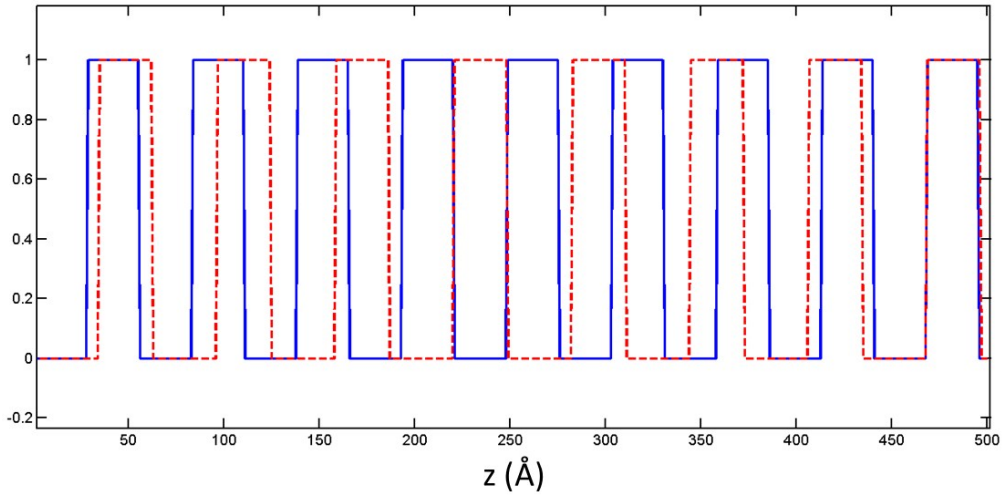

FIG. S2. Rectangular wave simulation of hydrophilic-hydrophobic periods of both the L_d phase (blue) and the L_o phase (red). Value one represents hydrophilic, while value zero represents hydrophobic.

2. Define d as the d-spacing, d_t as the tail region thickness, d_w as the hydrophilic region thickness (head + water + head), so that $d_w + d_t = d$. The numbers taken for Figure S2 are from 99% RH, where d = 55.5 Å is the d-spacing for the L_d phase, d′ = 63.6 Å is the d-spacing for the L_o phase. d_t = 28.5 Å is the tail region thickness for the L_d phase, d_t' = 34.7 Å is the tail region thickness of the L_o phase. These parameters remain constants with RH.

3. Calculation of mismatch energy:

$$\Delta E = \lambda \int_0^L dz\left[f_d(1-f_o) + f_o(1-f_d)\right] = \lambda \int_0^L dz\left[f_d + f_o - 2f_d f_o\right] \quad (S1)$$

Fourier expand f_d, f_o as

$$f = \frac{1}{2}a_o + \sum_{n=1}^{\infty} a_n \cos\left(\frac{2\pi}{d} nz\right) \quad (S2)$$

where

$$a_o = \frac{4d_w}{d}, \quad (S3)$$

$$a_n = \frac{2}{d}\int_{-\frac{d}{2}}^{\frac{d}{2}} f(z)\cos(\frac{2\pi}{d}nz)dz. \quad (S4)$$

So the mismatch free energy can be calculated as

$$\Delta E = \lambda\left\{L\left[\frac{2d_w}{d}+\frac{2d_w^{'}}{d^{'}}-\frac{8d_w d_w^{'}}{dd^{'}}\right]-\frac{2}{\pi^3}\sum_{n,n^{'}=1}^{\infty}\frac{(-1)^{n+n^{'}}}{nn^{'}}\sin\left(\frac{2\pi n}{d}d_w\right)\sin\left(\frac{2\pi n^{'}}{d^{'}}d_w^{'}\right)\right.$$
$$= \lambda\{f1+f2\} \quad (S5)$$

Where $$f1 = L\left[\frac{2d_w}{d}+\frac{2d_w^{'}}{d^{'}}-\frac{8d_w d_w^{'}}{dd^{'}}\right] \quad (S6)$$

$$f2 = -\frac{2}{\pi^3}\sum_{n,n^{'}=1}^{\infty}\frac{(-1)^{n+n^{'}}}{nn^{'}}\sin\left(\frac{2\pi n}{d}d_w\right)\sin\left(\frac{2\pi n^{'}}{d^{'}}d_w^{'}\right)\left[\frac{sin\left(2\pi\left(\frac{n}{d}+\frac{n^{'}}{d^{'}}\right)L\right)}{\left(\frac{n}{d}+\frac{n^{'}}{d^{'}}\right)}+\frac{sin\left(2\pi\left(\frac{n}{d}-\frac{n^{'}}{d^{'}}\right)L\right)}{\left(\frac{n}{d}-\frac{n^{'}}{d^{'}}\right)}\right] \quad (S7)$$

According to the above equation, we can simulate the mismatch free energy for $L = nd \approx n'd'$. See Figure S3 for n=10 and 7:

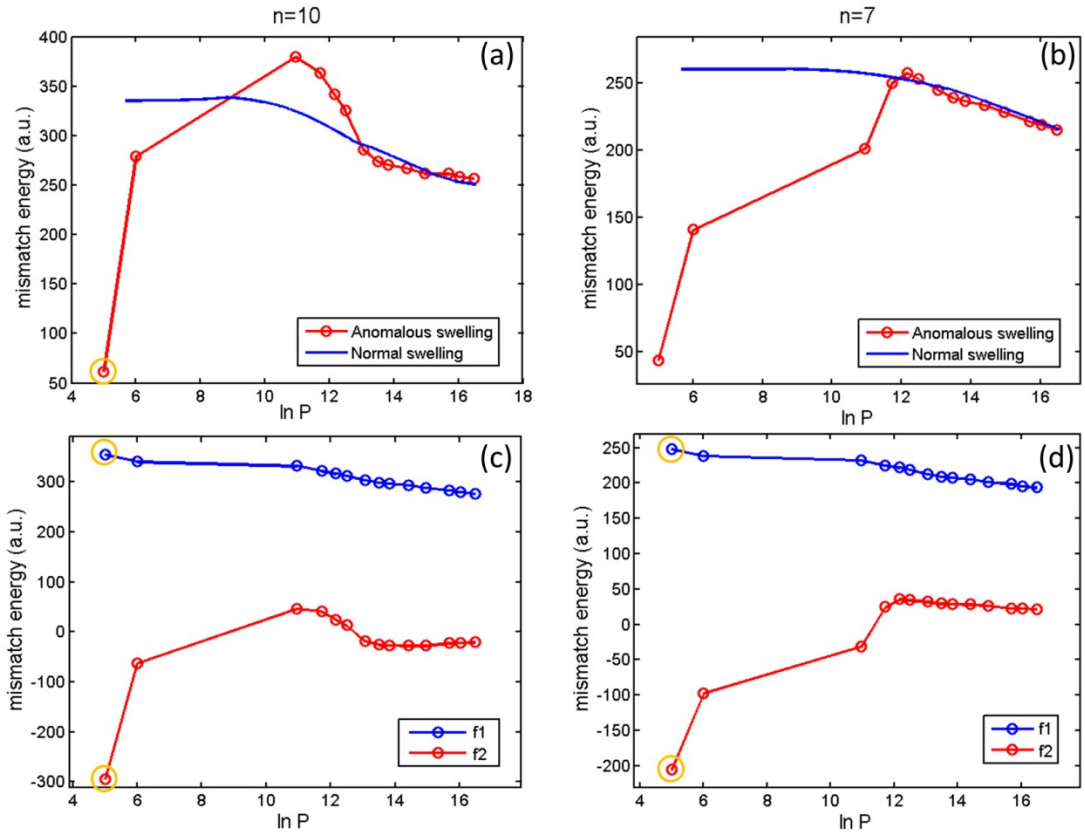

FIG. S3. Simulated mismatch energy with 10 bilayers (a, c) and 7 bilayers (b, d). (a, b) are plotting (f1+f2) vs. ln P for anomalous swelling (red) and normal swelling (blue). The normal swelling curve is simulated with the normal swelling d-spacings as shown solid curve in Figure 7(b). The anomalous swelling curve is simulated with the anomalous swelling experimental d-spacings shown as the data points in Figure 7(b). (c, d) are plotting the breakdown of f1 and f2 for the anomalous swelling in (a, b). The left most point circled in yellow is again the simulated data of $d = d' = 71.5$ Å, which is not an actual data point.

Note that the relative humidity increases from right to left of x-axis, as ln P decreases. The data for ln P <10 are really the data for 100% RH, where ln P goes to negative infinity, therefore these ln P are nominal labels just for plotting. In the simulation, we can see that the oscillation term f2 contributes in the overall mismatch energy, and lowers the anomalous swelling mismatch energy. In the 7 bilayer case (Figure S3 (b, d)), the energy of the anomalous swelling is mostly lower than the normal swelling. This because with low number of bilayers, the anomalous swelling caused the two periods to go out of phase slower (takes more bilayers) than the normal swelling case, therefore lowers the energy. The simulation suggests that system needs to re-adjust its periods every 7 layers or so to lower the energy.