Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Micropatterned model membrane with quantitatively controlled separation of lipid phases

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1. Observation of the phase separation process:

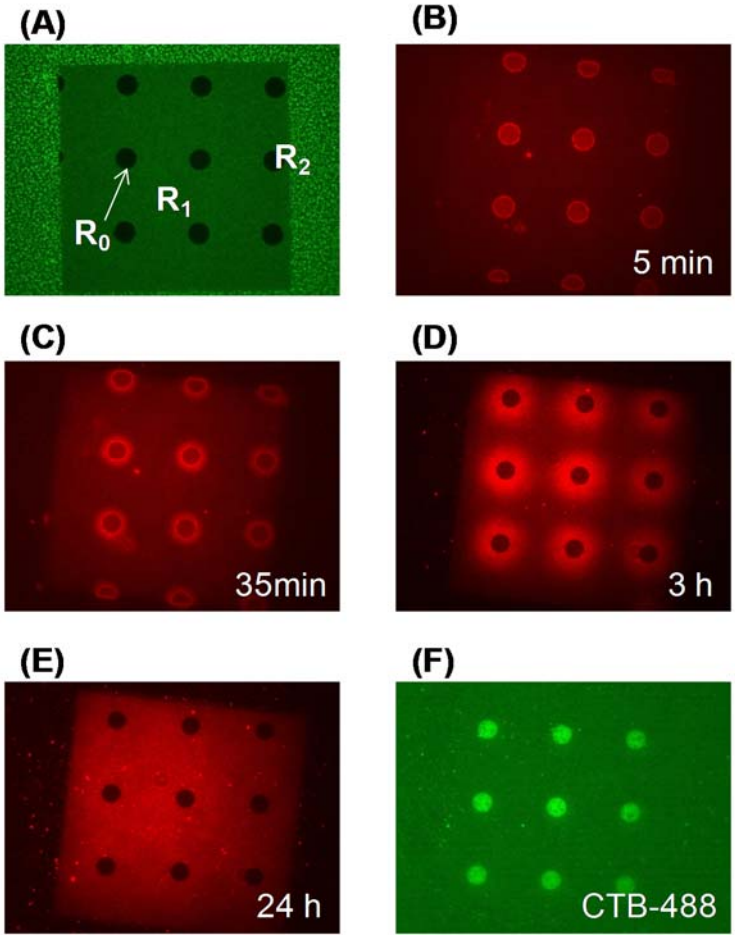

Figure S1: The phase separation process was observed by the total internal reflection fluorescence microscopy (TIR-FM). (A) Fluorescence of polymerized DiynePC (epi-fluorescence). R_0, R_1, and R_2 are polymer-free region, partially polymeric region, fully polymeric region, respectively. (B)-(E) TIR-FM observation of TR-PE after the introduction of DOPC/ SM/ Chol/ TR-PE/ G_{M1}. The elapsed time after the introduction of lipid membrane is shown. (F) The accumulation of L_o phase in R_0 was confirmed with CTB-488.

2. Parameters for controlling the phase separation:

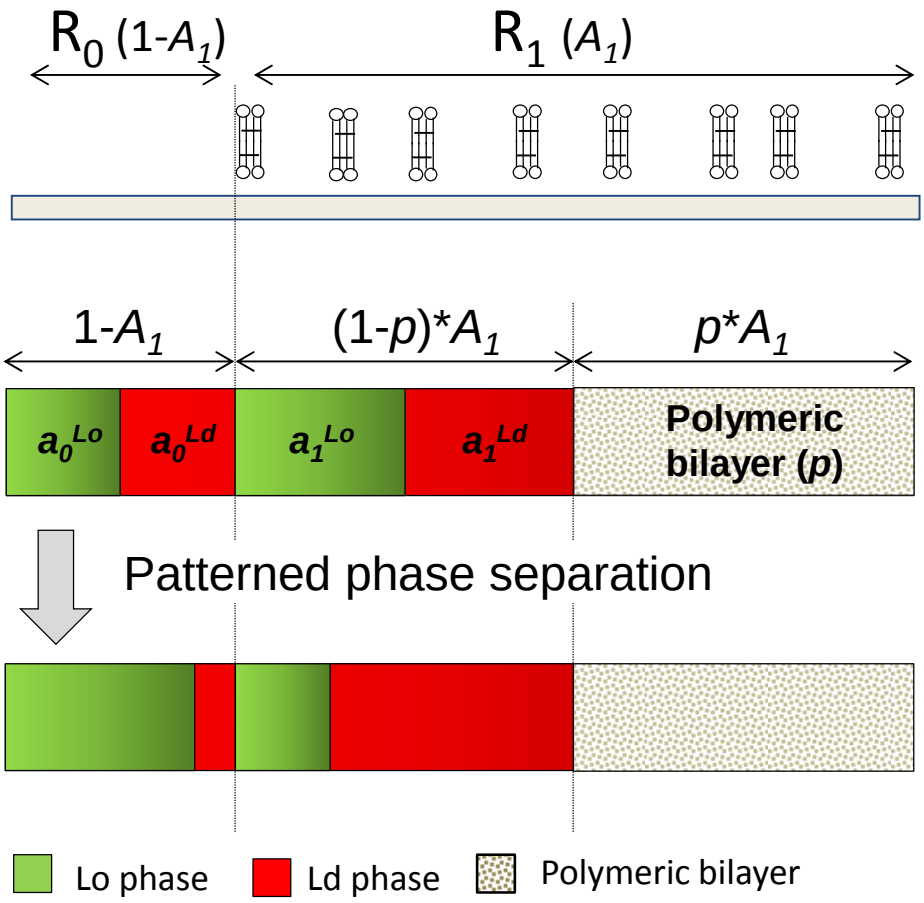

Figure S2: Schematic representation of L_o/ L_d distributions in the patterned membrane. Fluid bilayer is incorporated into the polymer-free region (R_0) and partially polymeric region (R_1). The area fraction of R_1 is designated as A_1. The coverage of polymeric bilayer in R_1 is designated as p. The area fractions of L_o and L_d phases in R_0 and R_1 are designated as a_0^{Lo}, a_0^{Ld}, a_1^{Lo}, and a_1^{Ld}, respectively ($a_0^{Lo} + a_0^{Ld} = a_1^{Lo} + a_1^{Ld} = 1$). At the beginning, L_o/ L_d compositions in R_0 and R_1 are the same ($a_0^{Lo} = a_1^{Lo}$, $a_0^{Ld} = a_1^{Ld}$). After incubation, L_d phase is enriched in R_1 and L_o phase is enriched in R_0 ($a_0^{Lo} > a_1^{Lo}$, $a_0^{Ld} < a_1^{Ld}$).

3. FRAP measurement of the membrane after phase separation:

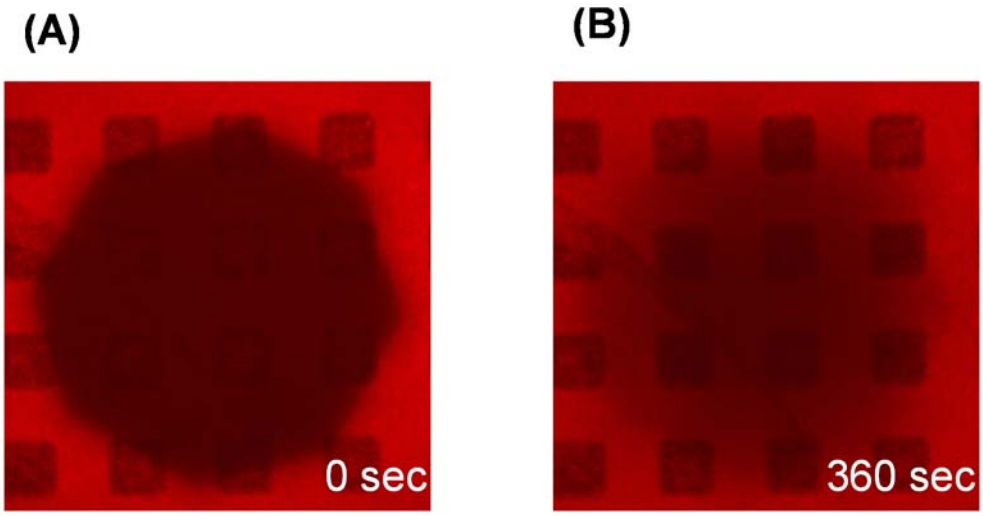

Figure S3: FRAP measurement of the bilayer after phase separation of DOPC/ SM/ Chol/ TR-PE/ G_{M1}: TR-PE was photobleached and the fluorescence recovery with time was observed. The boundary of photobleached area became unclear with time, suggesting the lateral diffusion of lipid molecules in R_1. The size of corrals was 10 μm.

4. Line profiles of TR-PE and CTB-488 in the membrane:

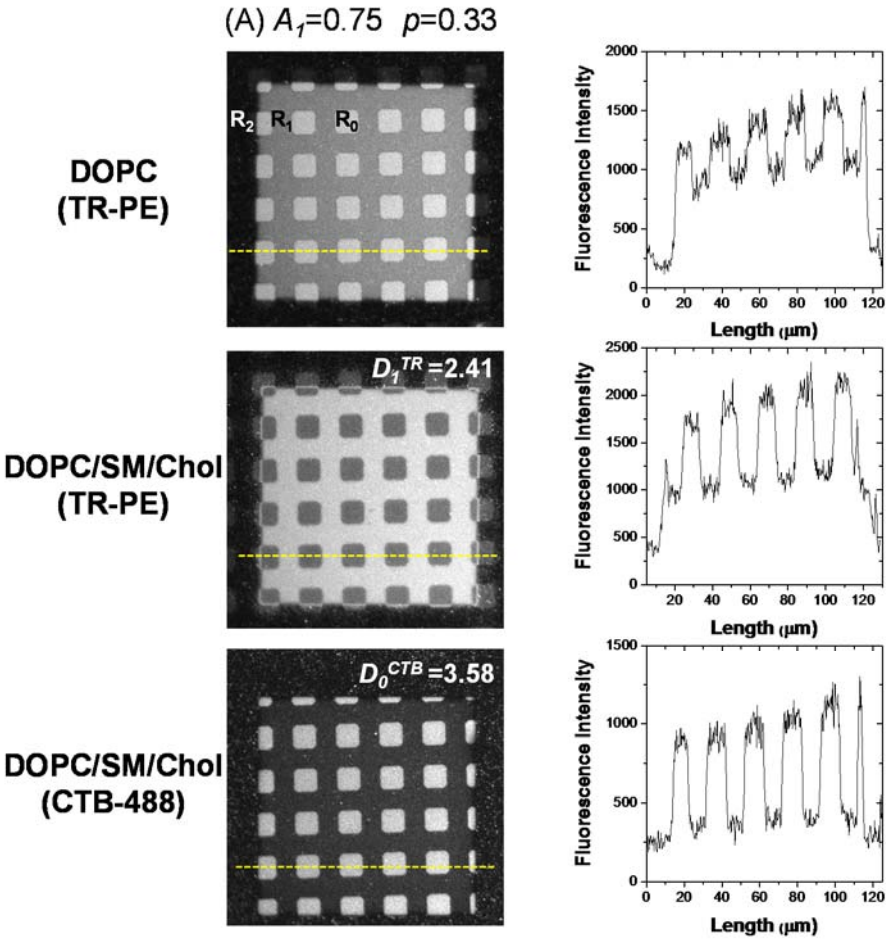

Figure S4: Fluorescence intensity profiles of TR-PE and CTB-488 after the phase separation of DOPC/ SM/ Chol/ TR-PE/ G_{M1}: Micrographs of the patterned bilayer (left) and the intensity profiles (right). The micrographs are the same as Figure 2(A). The size of corrals was 10 μm.

5. Model of the polymer-induced phase separation:

To account for the effect of polymeric bilayer on the phase separation, we formulated a model assuming random distribution of polymeric bilayer domains in R_1 and accumulation of L_d domains around the polymeric domains. We hypothesize that a part of L_d phase is accumulated around polymeric bilayers (we call it bound L_d domains) and the rest of L_d phase (free L_d domains) is randomly mixed with L_o domains in R_0 and R_1 (Figure S5). We assume that the amount of bound L_d domains is proportional to the area fraction of polymeric bilayers in R_1 (p). (This assumption is valid if polymeric bilayer domains are uniform in size and the domains are not connected with each other.) The area fractions of L_o and L_d phases in the patterned membrane can be described by Eqs. (1)-(3) (Figure S2). The total area of the patterned membrane (R_0 and R_1) corresponds to unity. The parameter q represents the gross area fraction of L_o phase, which is determined by the lipid composition.

Total area of fluid bilayer: $A^{Lo} + A^{Ld} = 1\text{-}A_1 p$ (1)

Total area of L_o phase: $A^{Lo} = (1\text{-}A_1 p)\, q$ (2)

Total area of L_d phase: $A^{Ld} = (1\text{-}A_1 p)\,(1\text{-}q)$ (3)

The amount of bound and free L_d domains in R_1 can be expressed as follows.

Bound L_d domain (in R_1): $A_b{}^{Ld} = r\, A_1 p$ (4)

Free L_d domain (in R_0 and R_1):

$A_f{}^{Ld} = 1 - A_1 p - A^{Lo} - r A_1 p = 1 - A_1 p(1+ r) - (1\text{-}A_1 p)\, q$ (5)

The character *r* represents the proportionality between the areas of bound L_d domains and the area of polymeric bilayer. The physical meaning of *r* is analogous to the coherence length around the lipid bilayer domains (i.e. the degree of ordering in mobile lipids at the boundaries), [1] although its length should be determined by the thickness mismatch at the boundary and bending elasticity of the fluid bilayer.

Since free L_d domains are randomly mixed with L_o domains, the area fraction of L_o and L_d phase in R_0 can be determined by Eqns (6) and (7). a_0^{Lo} and a_0^{Ld} are area fractions of L_o and L_d phases *within* R_0 ($a_0^{Lo} + a_0^{Ld} = 1$) (It should be noted that a_0^{Lo} and a_0^{Ld} have a different definition compared with A^{Lo} and A^{Ld}.).

Area fraction of L_o in R_0: $$a_0^{Lo} = \frac{A^{Lo}}{A^{Lo} + A_f^{Ld}} = \frac{(1-A_1 p)q}{1-A_1 p - r A_1 p} \quad (6)$$

Area fraction of L_d in R_0: $$a_0^{Ld} = 1 - a_0^{Lo} = \frac{1-A_1 p - r A_1 p - q + A_1 pq}{1-A_1 p - r A_1 p} \quad (7)$$

The area fractions of L_o phase in R_1 can be calculated by subtracting the area fraction of L_o domains in R_0 $(1 - A_1)a_0^{Lo}$ from the gross L_o area fraction, A^{Lo}.

Area fraction of L_o in R_1:

$$a_1^{Lo} = \frac{A^{Lo} - (1-A_1)a_0^{Lo}}{A_1(1-p)} = \frac{(1-A_1 p)q - (1-A_1)\frac{(1-A_1 p)q}{1-A_1 p - r A_1 p}}{A_1(1-p)} = \frac{(1-A_1 p)(1-p-rp)q}{(1-p)(1-A_1 p - r A_1 p)} \quad (8)$$

This leads to the area fraction of L_d in R_1:

$$a_1^{Ld} = 1 - a_1^{Lo} = \frac{(1-p)(1-A_1 p - r A_1 p - q + A_1 pq) + (1-A_1 p)rpq}{(1-p)(1-A_1 p - r A_1 p)} \quad (9)$$

Therefore, the enrichment of L_d phases in R_1 compared R_0 is expressed with the following equation:

$$\frac{a_1^{Ld}}{a_0^{Ld}} = \frac{(1-p)(1-A_1p-rA_1p-q+A_1pq)+(1-A_1p)rpq}{(1-p)(1-A_1p-rA_1p)} \times \frac{1-A_1p-rA_1p}{1-A_1p-rA_1p-q+A_1pq}$$

$$= 1+\frac{(1-A_1pq)rpq}{(1-p)(1-A_1p-rA_1p-q+A_1pq)} \quad (10)$$

We fitted Eq. 10 to the experimental results in Figure 3(A) by varying the *r* value. Figure S6 shows the fitting results. The fitted *r* values were 0.27 and 0.14 for A_1 = 0.75 and 0.95, respectively. In the case of A_1 = 0.95, we also show the simulation result with *r* = 0.27. Although the fitting result was rather poor due to the strong influence from the data points near the limit at infinity, the model qualitatively reproduces the experimentally observed dependency of D_1^{Ld} on *p*. The model underestimates D_1^{Ld} for small *p* values. The discrepancy between the model and experimental results could stem from several factors such as Ostwald ripening of domains in R_0. The model does not take into account the spontaneous growth of L_o domains. In the partially polymerized region (R_1) the growth of domain size is hindered by the polymeric bilayer domains (obstacles). As a result, L_o domains may preferentially expand in the polymer-free region (R_0). Another factor is the area fraction of bound L_d domains (A_b^{Ld}), which may not proportionally increase with the area fraction of the polymeric bilayer (*p*) for higher values of *p*. As the number of polymeric bilayer domains increases, the total length of polymeric bilayer boundary may become smaller with respect to the occupied area due to the overlap and connection of the domains. It should result in decreased accumulation of L_d domains in R_1. In spite of these limitations, the agreement of the model with the experimental results underlines the

important role played by the polymeric bilayer domains.

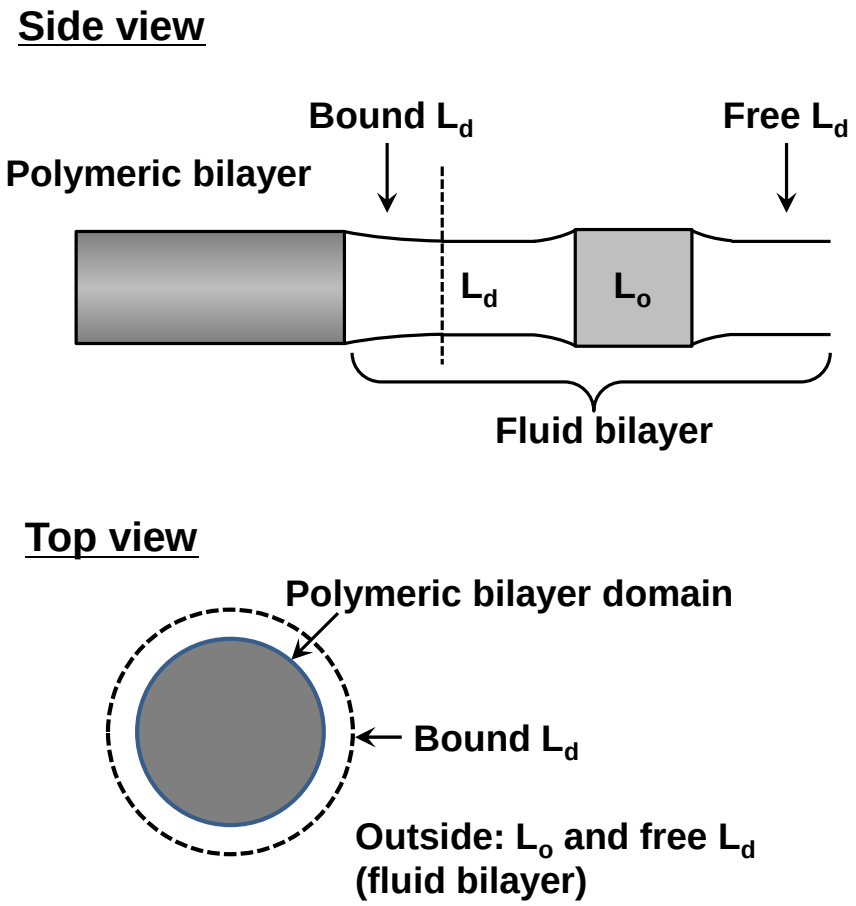

Figure S5: Schematic illustration of the polymer-induced phase separation. Due to the finite thickness mismatch at the polymer-fluid bilayer junction, the fluid bilayer is expected to be deformed. We hypothesized that L_d domains preferentially accumulated around a polymeric bilayer domain because of their lower bending moduli, and formed a circular region of bound L_d domains.

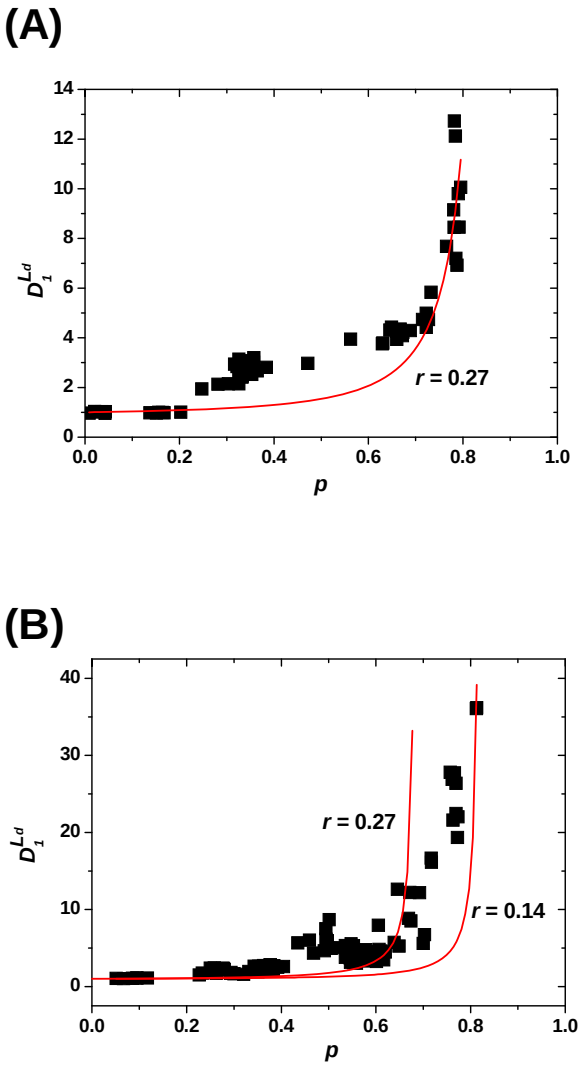

Figure S6: Enrichment of L_d phase in R_1 (D_1^{Ld}) in samples with varied polymeric bilayer fractions in R_1 (p). The experimental results (black dots) and the model (red lines) were compared. (A) $A_1 = 0.75$; (B) $A_1 = 0.95$. The r values (proportionality between the amount of bound L_d domains and polymer) were fitted and compared with the experimental results.

Reference

(1) Almeida, P. F. F.; Vaz, W. L. C.; Thompson, T. E. Lateral diffusion and percolation in 2-phase, 2-component lipid bilayers - topology of the solid-phase domains inplane and across the lipid bilayer. *Biochemistry* 1992, *31*, 7198-7210.