

# Domain formation in cholesterol/phospholipid membranes exposed to adhesive surfaces or environments - Electronic Supplementary Information (ESI)

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## 1 Compositions of spectator phases

### 1.1 Equations for compositions of spectator phases

When the unbound membrane segment undergoes phase separation, its mole fraction  $X^{[1]}$  can vary within the interval  $X_\beta \leq X^{[1]} \leq X_\alpha$  whereas the corresponding chemical potential difference  $\Delta\mu^{[1]}$  stays at the constant value  $\mu_{\alpha\beta} + \Delta U^{[1]}$ , see Fig. 4. It now follows from the chemical equilibrium condition  $\Delta\mu^{[2]} = \Delta\mu^{[1]}$  that the mole fraction  $X^{[2]} = X_*^{[2]}$  of the spectator phase within the bound membrane segment satisfies the equation

$$\mu_{\alpha\beta} + \Delta U^{[1]} = \Delta\mu^{[2]}(X_*^{[2]}) = G(X_*^{[2]}) + \Delta U^{[2]} \quad (1.1)$$

or

$$\mu_{\alpha\beta} = G(X_*^{[2]}) + \Delta U^{[2]} - \Delta U^{[1]} = G(X_*^{[2]}) + \Delta U \quad (1.2)$$

with the affinity contrast  $\Delta U$ .

Likewise, when the bound membrane segment undergoes phase separation, its mole fraction  $X^{[2]}$  can vary within the interval  $X_\beta \leq X^{[2]} \leq X_\alpha$  whereas the corresponding chemical potential difference  $\Delta\mu^{[2]}$  stays at the constant value  $\mu_{\alpha\beta} + \Delta U^{[2]}$ , see Fig. 4. It then follows from the chemical equilibrium condition  $\Delta\mu^{[1]} = \Delta\mu^{[2]}$  that the mole fraction  $X^{[1]} = X_*^{[1]}$  of the spectator phase within the unbound membrane segment satisfies the equation

$$\mu_{\alpha\beta} + \Delta U^{[2]} = \Delta\mu^{[1]}(X_*^{[1]}) = G(X_*^{[1]}) + \Delta U^{[1]} \quad (1.3)$$

or

$$\mu_{\alpha\beta} = G(X_*^{[1]}) + \Delta U^{[1]} - \Delta U^{[2]} = G(X_*^{[1]}) - \Delta U. \quad (1.4)$$

### 1.2 Compositions of spectator phases for negative $\Delta U$

The two equations (1.2) and (1.4) imply that the mole fractions  $X_*^{[2]}$  and  $X_*^{[1]}$  satisfy the inequalities

$$0 < X_*^{[2]} \leq X_\beta(T) \quad \text{and} \quad X_\alpha(T) \leq X_*^{[1]} < 1 \quad \text{for} \quad \Delta U > 0 \quad (1.5)$$

as one can also conclude from the graphical construction in Fig. 4. Furthermore, inspection of this figure shows that these mole fractions approach the values

$$X_*^{[2]} \approx X_\beta(T) \quad \text{and} \quad X_*^{[1]} \approx X_\alpha(T) \quad \text{for small} \quad \Delta U > 0 \quad (1.6)$$

as well as

$$X_*^{[2]} \approx 0 \quad \text{and} \quad X_*^{[1]} \approx 1 \quad \text{for large} \quad \Delta U > 0. \quad (1.7)$$

### 1.3 Small positive values of $\Delta U$

For small positive values of the affinity contrast  $\Delta U$ , the mole fraction  $X_*^{[2]}$  approaches the limiting value  $X_*^{[2]} \approx X_\beta(T)$ . In order to determine the leading correction term, we expand  $\Delta\mu^{[2]}(X_*^{[2]})$  in eqn (1.1) around  $X_*^{[2]} = X_\beta$ , which leads to

$$\Delta\mu^{[2]} \approx \Delta\mu^{[2]}(X_\beta) + (X_*^{[2]} - X_\beta) \left( \frac{\partial \Delta\mu^{[2]}}{\partial X_*^{[2]}} \right)_{X_\beta}. \quad (1.8)$$

Inserting the identity  $\Delta\mu^{[2]}(X_\beta) = \mu_{\alpha\beta} + \Delta U^{[2]}$ , we obtain the asymptotic equality

$$\Delta\mu^{[2]} \approx \mu_{\alpha\beta} + \Delta U^{[2]} + (X_*^{[2]} - X_\beta) \left( \frac{\partial \Delta\mu^{[2]}}{\partial X_*^{[2]}} \right)_{X_\beta} \quad (1.9)$$

for small deviations  $X_*^{[2]} - X_\beta$ . Finally, replacing  $\Delta\mu^{[2]}$  in eqn (1.1) by the expansion in eqn (1.9), the mole fraction  $X_*^{[2]}$  of the bound spectator phase is found to behave as

$$X_*^{[2]} \approx X_\beta - \frac{\Delta U}{(\partial \Delta\mu^{\text{bo}} / \partial X^{\text{bo}})_{X_\beta}} \quad \text{for small} \quad \Delta U > 0. \quad (1.10)$$

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Likewise, an expansion of  $\Delta\mu^{[1]}(X_*^{[1]})$  in eqn (1.3) around  $X_*^{[1]} = X_\alpha$  leads to

$$X_*^{[1]} \approx X_\alpha + \frac{\Delta U}{(\partial\Delta\mu^{\text{un}}/\partial X^{\text{un}})_{X_\alpha}} \quad \text{for small } \Delta U > 0. \quad (1.11)$$

Inspection of the expressions (1.10) and (1.11) shows that the temperature-dependence of the mole fractions  $X_*^{[2]}$  and  $X_*^{[1]}$  is now contained in the binodal lines  $X = X_\beta(T)$  and  $X_\alpha = X_\alpha(T)$  as well as in the partial derivatives  $\partial\Delta\mu/\partial X$  taken at these binodal lines. The latter derivatives can be brought into a more explicit form as follows.

Using the Gibbs-Duhem relation

$$X_a \frac{\partial\mu_a}{\partial X_a} + X_b \frac{\partial\mu_b}{\partial X_a} = 0 \quad (1.12)$$

for a binary mixture with  $X_a = X$  and  $X_b = 1 - X$ , the partial derivatives  $\partial\Delta\mu/\partial X$  of the relative chemical potential as defined by eqn (14) in the main text attain the form

$$\frac{\partial\Delta\mu}{\partial X} = \frac{\partial\mu_a}{\partial X} \left( 1 + \frac{A_a}{A_b} \frac{X}{1-X} \right) \quad (1.13)$$

which reduces to the simpler expression

$$\frac{\partial\Delta\mu}{\partial X} = \frac{1}{1-X} \frac{\partial\mu_a}{\partial X} \quad \text{for } A_a = A_b. \quad (1.14)$$

For the binary phospholipid/cholesterol mixtures considered here, the liquid-liquid coexistence regions are located at mole fractions  $X \lesssim 0.25$ , see Fig. 2. Therefore, a simple estimate for the quantity  $\partial\mu_a/\partial X$  at  $X = X_\beta$  and  $X = X_\alpha$  can be obtained from ideal solution theory with

$$\mu_a \approx \mu_a^o + k_B T \ln(X), \quad (1.15)$$

which leads to

$$\left( \frac{\partial\mu_a}{\partial X} \right)_{X_\beta} \approx \frac{k_B T}{X_\beta} \quad \text{and} \quad \left( \frac{\partial\mu_a}{\partial X} \right)_{X_\alpha} \approx \frac{k_B T}{X_\alpha}. \quad (1.16)$$

Furthermore, using dimensional analysis, we can always write

$$\left( \frac{\partial\mu_a}{\partial X} \right)_{X_\beta} = c_\beta \frac{k_B T}{X_\beta} \quad \text{and} \quad \left( \frac{\partial\mu_a}{\partial X} \right)_{X_\alpha} = c_\alpha \frac{k_B T}{X_\alpha}. \quad (1.17)$$

with dimensionless coefficients  $c_\beta$  and  $c_\alpha$  that should be of order one because of the relations in eqn (1.16).

Inserting the expressions in (1.17) into (1.10) and (1.11), the mole fractions  $X_*^{[2]}$  and  $X_*^{[1]}$  are found to behave as

$$X_*^{[2]} \approx X_\beta - c_\beta X_\beta (1 - X_\beta) \Delta U / (k_B T) \quad \text{for small } \Delta U > 0 \quad (1.18)$$

and

$$X_*^{[1]} \approx X_\alpha + c_\alpha X_\alpha (1 - X_\alpha) \Delta U / (k_B T) \quad \text{for small } \Delta U > 0. \quad (1.19)$$

The asymptotic equalities in (1.18) and (1.19) are identical to those in eqn (49) and eqn (55) of the main text.

#### 1.4 Compositions of spectator phases for positive $\Delta U$

As explained in Section 5.3 of the main text, the mole fractions  $X_*^{[1]}$  and  $X_*^{[2]}$  satisfy the symmetry relation

$$X_*^{[2]}(T, -\Delta U) = X_*^{[1]}(T, +\Delta U), \quad (1.20)$$

which is equivalent to

$$X_*^{[1]}(T, -\Delta U) = X_*^{[2]}(T, +\Delta U), \quad (1.21)$$

for all values of  $T$  and  $\Delta U$ .

A combination of eqn (1.21) with eqn (1.18) then leads to the asymptotic behavior

$$X_*^{[1]} \approx X_\beta - c_\beta X_\beta (1 - X_\beta) |\Delta U| / (k_B T) \quad \text{for small } \Delta U < 0. \quad (1.22)$$

Likewise, a combination of eqn (1.20) with eqn (1.19) implies

$$X_*^{[2]} \approx X_\alpha + c_\alpha X_\alpha (1 - X_\alpha) |\Delta U| / (k_B T) \quad \text{for small } \Delta U < 0. \quad (1.23)$$

The asymptotic equalities in eqn (1.22) and eqn (1.23) are identical with those in eqn (56) and eqn (50) of the main text.

## 2 Membranes exposed to many environments

We now consider membranes exposed to  $M$  different environments and, thus, partitioned into  $M$  different segments  $\mathcal{S}^{[m]}$  with  $m = 1, 2, \dots, M$ . As explained in Sect. 7 of the main text, we can choose the index  $m$  in such a way that the relative affinities  $\Delta U^{[m]}$  are ordered according to

$$\Delta U^{[1]} < \Delta U^{[2]} < \dots < \Delta U^{[M]}, \quad (2.1)$$

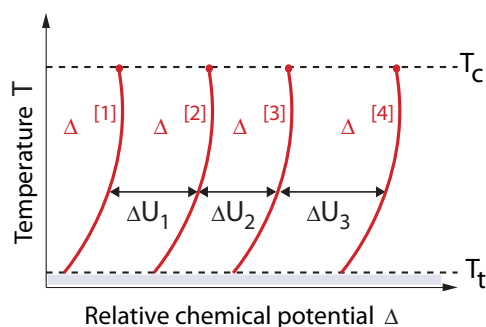
which leads to  $M - 1$  affinity contrasts

$$\Delta U_m \equiv \Delta U^{[m+1]} - \Delta U^{[m]} \quad \text{for } m = 1, 2, \dots, M - 1. \quad (2.2)$$

In the semigrand canonical ensemble, we have  $M$  phase transitions along the demixing curves  $\Delta\mu^{[m]} = \mu_{\alpha\beta}^{[m]}(T)$  with

$$\mu_{\alpha\beta}^{[m]}(T) = \mu_{\alpha\beta}(T) + \Delta U^{[m]} \quad \text{for } m = 1, 2, \dots, M \quad (2.3)$$

and  $T_t < T \leq T_c$ . Thus, the phase diagram in the  $(\Delta\mu, T)$ -plane now consists of  $M$  curves that are shifted against each other by the affinity contrasts  $\Delta U_m$  as shown in Fig. S1.



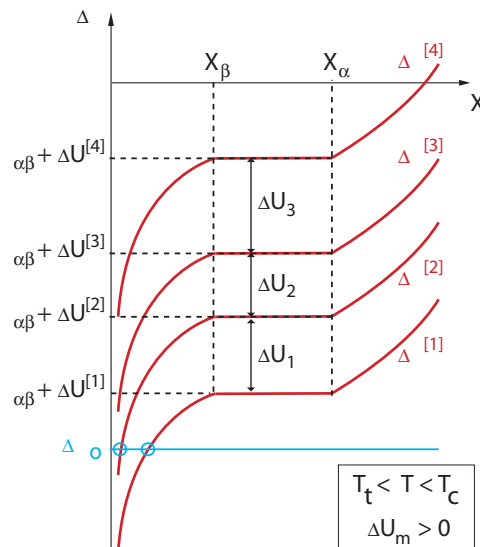
**Fig. S1** Phase diagram for two-component membrane exposed to  $M = 4$  different environments as a function of relative chemical potential  $\Delta\mu$  and temperature  $T$  with  $T_t < T \leq T_c$ . Segment  $\mathcal{S}^{[m]}$  phase separates along the demixing line  $\Delta\mu = \Delta\mu^{[m]}(T)$ . The demixing lines  $\Delta\mu^{[m+1]}(T)$  and  $\Delta\mu^{[m]}(T)$  are separated by the affinity contrast  $\Delta U_m$ . Each demixing line ends in a critical point.

In order to determine the compositions of the  $M$  segments, we now start from the chemical equilibrium between the  $M$  segments, which is described by the  $M - 1$  equations

$$G(X^{[1]}) + \Delta U^{[1]} = G(X^{[2]}) + \Delta U^{[2]} = \dots = G(X^{[M]}) + \Delta U^{[M]}. \quad (2.4)$$

The solution of these equations can again be obtained graphically as shown in Fig. S2, which generalized the graphical construction for  $M = 2$  in Fig. 4 to an arbitrary value of  $M$ . This graphical solution directly shows that the compositions of all segments as described by the mole fractions  $X^{[m]}$  are all different for  $\Delta U_m > 0$  and ordered according to

$$X^{[M]} < X^{[M-1]} < \dots < X^{[2]} < X^{[1]}. \quad (2.5)$$



**Fig. S2** Graphical solution of eqn (2.4) with  $M = 4$  for  $T_t < T < T_c$  and positive affinity contrasts  $\Delta U_m$ . The possible solutions can now be obtained from the intersections of the  $M$  relative chemical potentials  $\Delta\mu^{[m]}$  (red curves) with a horizontal line (light blue) corresponding to constant  $\Delta\mu = \Delta\mu_o$ . For  $\Delta\mu_o = \mu_{\alpha\beta}(T) + \Delta U^{[m]}$ , segment  $\mathcal{S}^{[m]}$  undergoes phase separation whereas the other  $M - 1$  segments attain different spectator phases.

As a consequence, when segment  $\mathcal{S}^{[m]}$  undergoes phase separation, the other  $M - 1$  segments attain uniform spectator phases that differ in their compositions.