Supplementary information for "Cholesterol tilting drives phase separations in lipid bilayer membranes"

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1 Expressions for the coefficients in eq. (1)

The cholesterol molecules in a monolayer exhibit cohesive interactions with cholesterol molecules in the nearest neighbor. The energy arising from these interactions has the form

$$E = -\frac{U_0}{2}\sum_{i,j=i\pm1}\int dl_i \int dl_j \left[\frac{\sigma_{cc}^{12}}{r_{ij}^{12}} - 2\frac{\sigma_{cc}^{6}}{r_{ij}^{6}}\right], \tag{1}$$

where we assumed that the cohesive interactions are between the chain segments of two nearest neighbor chains and that these interactions are represented by Lennard-Jones potentials. The integral is along the chain length of cholesterol molecules. We first calculate the energy of the interactions between cholesterol molecules aligned in a 1d lattice with lattice spacing r_0, see Fig. 1 of the main text. Expanding eq. (1) with respect to $\sin\theta$ leads the form of eq. (1) in the main text. The expressions for the coefficients, α_0, α_2, and α_4, have the forms

$$\alpha_0 = \alpha_0^{\rm rep}\left(\frac{\sigma_{cc}}{r_0}\right)^{10} - 2\alpha_0^{\rm att}\left(\frac{\sigma_{cc}}{r_0}\right)^{4} \tag{2}$$

$$\alpha_2 = \alpha_2^{\rm rep}\left(\frac{\sigma_{cc}}{r_0}\right)^{10} - 2\alpha_2^{\rm att}\left(\frac{\sigma_{cc}}{r_0}\right)^{4} \tag{3}$$

$$\alpha_4 = \alpha_4^{\rm rep}\left(\frac{\sigma_{cc}}{r_0}\right)^{10} - 2\alpha_4^{\rm att}\left(\frac{\sigma_{cc}}{r_0}\right)^{4} \tag{4}$$

with

$$\alpha_0^{\rm rep} = \int_{-l/2r_0}^{l/2r_0} du \int_{-l/2r_0}^{l/2r_0} du' \frac{1}{(1+(u-u')^2)^6} \tag{5}$$

$$\alpha_2^{\rm rep} = 168\int_{-l/2r_0}^{l/2r_0} du \int_{-l/2r_0}^{l/2r_0} du' \frac{(u-u')^2}{(1+(u-u')^2)^8} \tag{6}$$

$$\alpha_4^{\rm rep} = 8064 \int_{-l/2r_0}^{l/2r_0} du \int_{-l/2r_0}^{l/2r_0} du' \frac{(u-u')^4}{(1+(u-u')^2)^{10}} \quad (7)$$

and

$$\alpha_0^{\rm att} = \int_{-l/2r_0}^{l/2r_0} du \int_{-l/2r_0}^{l/2r_0} du' \frac{1}{(1+(u-u')^2)^3} \quad (8)$$

$$\alpha_2^{\rm att} = 48 \int_{-l/2r_0}^{l/2r_0} du \int_{-l/2r_0}^{l/2r_0} du' \frac{(u-u')^2}{(1+(u-u')^2)^5} \quad (9)$$

$$\alpha_4^{\rm att} = 960 \int_{-l/2r_0}^{l/2r_0} du \int_{-l/2r_0}^{l/2r_0} du' \frac{(u-u')^4}{(1+(u-u')^2)^7}. \quad (10)$$

X-ray diffraction experiments suggest that the cholesterol molecules in a monolayer are aligned in a hexagonal lattice. We thus calculate the interaction energy when the cholesterol molecules are arranged in a hexagonal lattice. We treat the case that all the cholesterol molecules in this lattice tilt by the same tilt angle θ and azimuthal angle ϕ. In this case, the interaction energy due to the cohesive interactions has the form

$$U_{\rm 2d} = J_{\rm cc}\left[6\alpha_0 + \frac{3}{2}\alpha_2 \sin^2\theta + \frac{9}{16}\alpha_4 \sin^4\theta\right]. \quad (11)$$

Therefore, the energy of cholesterol molecules arranged in a 2d hexagonal lattice has a similar form as the energy of cholesterol molecules arranged in 1d lattice, except for the constants that are multiplied to the coefficients, α_2 and α_4.

For $l/r_0 \gg 1$, α_2 has an asymptotic form

$$\alpha_2 \simeq \left[\frac{693}{256}\pi\left(\frac{\sigma_{\rm cc}}{r_0}\right)^{11} - \frac{15}{4}\pi\left(\frac{\sigma_{\rm cc}}{r_0}\right)^{5}\right]\frac{l}{\sigma_{\rm cc}} - 4\left[\left(\frac{\sigma_{\rm cc}}{r_0}\right)^{10} - 2\left(\frac{\sigma_{\rm cc}}{r_0}\right)^{4}\right]. \quad (12)$$

The first term represents "bulk energy" that is proportional to the chain length l and the second term is "end energy" that results from end-effects: In the limit $r_0 \gg \sigma_{\rm cc}$ where we expect the chains to tilt due to the large head spacing, the bulk term gives a negative contribution to α_2 while the end effect gives a positive contribution. Thus, when $l/\sigma_{\rm cc}$ is large enough, the bulk term dominates and $\alpha_2 < 0$ which results in a tilted phase. The crossover between the small head spacing regime (the dotted curve in Fig. 1 of the main text) and the intermediate head spacing regime (the solid curve in Fig. 1 of the main text) occurs at $r_0/\sigma_{\rm cc} = (231/320)^{1/6} \simeq 0.947$, where α_2 approaches to a finite value (zero bulk energy) for $l/\sigma_{\rm cc} \to \infty$. Eq. (12) implies that, for the case of $r_0/\sigma_{\rm cc} = 1$ (like the solid line in Fig. 1 of the main text), α_2 is zero when the chain length is $l/r_0 = 1024/(267\pi) \simeq 1.22$; this is the chain length, at which α_2 changes sign (when one changes the chain length). The numerical calculations in Fig. 1 of the main text suggest that this chain length is slightly smaller, but this deviation results from the higher order terms than those shown in eq. (12). This asymptotic analysis shows that the competition between the bulk energy

and the end effect results in the non-monotonous behaviors of α_2 shown in Fig. 1 of the main text. For $l/r_0 \ll 1$, α_2 has an asymptotic form

$$\alpha_2 \simeq \left(\frac{l}{\sigma_{\rm cc}}\right)^4 \left[28\left(\frac{\sigma_{\rm cc}}{r_0}\right)^{14} - 16\left(\frac{\sigma_{\rm cc}}{r_0}\right)^{8}\right]. \tag{13}$$

This implies that the crossover between the intermediate head spacing regime (the solid curve in Fig. 1 of the main text) and the large head spacing regime (the broken curve in Fig. 1 of the main text) occurs at the head spacing $r_0/\sigma_{\rm cc} = (7/4)^{1/6} \simeq 1.10$.

2 Derivation of the mixing free energy (eq. (3))

We derive **the mixing free energy** of a membrane containing lipids, cholesterol monomers, and cholesterol dimers, eq. (3) in the manuscript. One approach is to follow the formalism described in ref. [3]. However, here we use a simpler argument using Boltzmann's expression for the entropy

$$F_{\rm mix} = -T \log W, \tag{14}$$

where T is the absolute temperature in the unit of Boltzmann constant, W is the total number of molecular configurations, and $F_{\rm mix}$ is mixing free energies.

A bilayer membrane is modeled as two lattices of M sites in each leaflet that is fully populated by lipids and cholesterol molecules. The number of cholesterol dimers in this membrane is N_d. The numbers of lipids and cholesterol monomers are N_l^+ and N_c^+ in the outer leaflet and are N_l^- and N_c^- in the inner leaflet, respectively. These imply two relationships $M = N_d + N_c^+ + N_l^+$ and $M = N_d + N_c^- + N_l^-$. The number of configurations obtained by arranging the cholesterol dimers has the form

$$\frac{M!}{N_d!(M-N_d)!}. \tag{15}$$

The numbers of the configurations obtained by arranging lipids and cholesterol monomers in the rest of the lattice sites have the forms

$$\frac{(M-N_d)!}{N_l^+!N_c^+!} \tag{16}$$

and

$$\frac{(M-N_d)!}{N_l^-!N_c^-!} \tag{17}$$

for the outer and inner leaflets, respectively. Finally, the total number of configurations W is derived in the form

$$W = \frac{M!}{N_d!(M-N_d)!}\frac{(M-N_d)!}{N_l^+!N_c^+!}\frac{(M-N_d)!}{N_l^-!N_c^-!}. \tag{18}$$

The mixing entropy is derived by substituting W into eq. (14) and by using Sterling's formula in eq. (3) in the main text **with** $\psi_d = N_d/M$, $\psi_c^+ = N_c^+/M$, $\psi_c^- = N_c^-/M$, $\psi_l^+ = N_l^+/M$, **and** $\psi_l^- = N_l^-/M$.

3 Free energy terms that are independent of the tilt angle

In eq. (2) of the main text, we neglected those interaction energies that are independent of the tilt angles of cholesterol dimers. These terms have the form

$$\begin{aligned} f_{\rm ind} &= -\frac{J^0_{\rm dd}}{2}\psi_d^2 - \frac{J^0_{\rm cc}}{2}\psi_c^{+2} - \frac{J^0_{\rm cc}}{2}\psi_c^{-2} - \frac{J^0_{\rm ll}}{2}\psi_l^{+2} - \frac{J^0_{\rm ll}}{2}\psi_l^{-2} \\ &\quad -J^0_{\rm cd}(\psi_c^+ + \psi_c^-)\psi_d - J^0_{\rm ld}\psi_d(\psi_l^+ + \psi_l^-) \\ &\quad -J^0_{\rm cl}\psi_c^+\psi_l^+ - J^0_{\rm cl}\psi_c^-\psi_l^-. \end{aligned} \tag{19}$$

$J^0_{\rm dd}$, $J^0_{\rm cc}$, and $J^0_{\rm ll}$ are the energies of dimer-dimer interactions, monomer-monomer interactions, and lipid-lipid interactions, respectively. $J^0_{\rm cd}$, $J^0_{\rm cl}$, and $J^0_{\rm ld}$ are the energies of monomer-dimer interactions, monomer-lipid interactions, and lipid-dimer interactions.

The two opposing leaflets of the bilayer membrane are symmetric; this implies that the cholesterol monomer concentration in one leaflet is equal to that of the other leaflet, $\psi_c^+ = \psi_c^-$. The lipid concentrations of these leaflets are also equal, $\psi_l^+ = \psi_l^-$. Therefore, the total concentration Φ_c ($\equiv \psi_c^+ + \psi_d = \psi_c^- + \psi_d$) of the cholesterol monomers and dimers in each leaflet is constant. With these relationships, eq.(19) is rewritten in the form

$$\begin{aligned} f_{\rm ind} &= \left(-\frac{J^0_{\rm dd}}{2} - J^0_{\rm cc} + 2J^0_{\rm cd}\right)\psi_d^2 - 2(J^0_{\rm ld} - J^0_{\rm cl})\psi_d \\ &\quad +2(J^0_{\rm cc} - J^0_{\rm cd} + J^0_{\rm ld} - J^0_{\rm cl})\psi_d\Phi_c \\ &\quad -2\Phi_c^2\left(\frac{J^0_{\rm cc} + J^0_{\rm ll}}{2} - J^0_{\rm cl}\right) \end{aligned} \tag{20}$$

The constant term and the linear terms of Φ_c were not written out in eq. (20) because these terms affect neither dimerization nor precipitations.

The free energies in eq. (20) arise from the interactions between constituent molecules without tilting (with respect to the membrane normal). In the absence of tilting, the interaction energies are insensitive to the states of cholesterol molecules (one cholesterol dimer or two cholesterol monomers in two opposing leaflets). Therefore, we approximate the energies of the dimer-dimer interactions as being twice the energies of the monomer-monomer interactions ($J^0_{\rm dd} = 2J^0_{\rm cc}$) and the energies of the interactions between one dimer and one monomer as being equal to the interactions between two monomers ($J^0_{\rm cd} = J^0_{\rm cc}$). Lipid-dimer interactions have the same energies as lipid-monomer interactions too ($J^0_{\rm ld} = J^0_{\rm cl}$). These approximations simplify eq. (20) in the form

$$f_{\rm ind} = -2\Delta J_{\rm ind}\Phi_c^2 \tag{21}$$

with

$$\Delta J_{\rm ind} = \frac{J^0_{\rm cc} + J^0_{\rm ll}}{2} - J^0_{\rm cl}. \tag{22}$$

This implies that, in the case of $\Delta J_{\rm ind} > 0$, cholesterol molecules may precipitate even in mixed monolayers at a temperature $\Delta J_{\rm ind}/2$. However, cholesterol molecules also have an affinity to associate with saturated lipids, e.g. sphingomyeline and dipalmitoyl phosphatidylcholine [1]; $\Delta J_{\rm ind}$ can be negative or positive, but we here treat the case that its value is very small because the X-ray diffraction experiments by Zibrat et al. [2] do not suggest the precipitation of cholesterol molecules in monolayers. Values of $\Delta J_{\rm ind}$ that are large and negative may suppress the precipitation of cholesterol crystallites even in bilayer membranes; thus this shifts the critical temperature. In the main text, we did not take into account this effect because this does not change the physics behind the precipitation of cholesterol crystals from bilayer membranes.

4 Derivation of the generalized law of mass action (eq. (5))

We derive the form of the (generalized) law of mass action from the free energy, eq. (2) in the main text. At first, we derive the tilt angle θ_d of cholesterol dimers by minimizing the free energy (eq. (2) in the main text) with respect to θ_d. Because of the symmetry of the bilayer membrane, its outer and inner leaflets have equal concentration of cholesterol monomers ($\psi_c^+ = \psi_c^-$). The same symmetry is true for the concentration of lipids in the outer and inner leaflets ($\psi_l^+ = \psi_l^-$). We denote the total cholesterol concentration as Φ_c ($= \psi_c^+ + \psi_d = \psi_c^- + \psi_d$). For $\psi_d < \psi_{\rm th}$, the tilt angle of cholesterol dimers is zero, whereas, for $\psi_d > \psi_{\rm th}$, this tilt angle θ_d has the form

$$\begin{aligned} \sin^2\theta_d &= -\frac{1}{\alpha_4\psi_d}\left[\left(\alpha_2 - \frac{2J_{\rm cd}}{J_{\rm dd}}\right)\psi_d + \frac{2J_{\rm cd}}{J_{\rm dd}}\Phi_c + \frac{2J_{\rm ld}}{J_{\rm dd}}(1-\Phi_c)\right] \\ &= -\frac{1}{\alpha_4}\left(\alpha_2 - \frac{2J_{\rm cd}}{J_{\rm dd}}\right)\frac{\psi_d - \psi_{\rm th}}{\psi_d} \end{aligned} \tag{23}$$

with

$$\psi_{\rm th} = -\frac{2J_{\rm cd}\Phi_c/J_{\rm dd} + 2J_{\rm ld}(1-\Phi_c)/J_{\rm dd}}{\alpha_2 - 2J_{\rm cd}/J_{\rm dd}}. \tag{24}$$

These equations imply that the cholesterol dimers exhibit cooperative dimerization when their concentration becomes larger than a threshold value $\psi_{\rm th}$.

Substituting eq. (23) into eq. (2) (in the main text) leads to an expression for the free energy as a function of only ψ_d and Φ_c. The concentration of the cholesterol dimers is derived by the minimization of the free energy with respect to ψ_d. Because of $\psi_c^+ = \psi_c^- = \Phi_c - \psi_d$, see also sec. 3, the condition of the

minimum of the free energy has the form

$$\begin{aligned} \frac{d}{d\psi_d} f &= \frac{\partial}{\partial \psi_d} f - \frac{\partial}{\partial \psi_c^+} f - \frac{\partial}{\partial \psi_c^-} f \\ &= 0, \end{aligned} \tag{25}$$

where the dependence of f on ψ_d , ψ_c^+, ψ_c^-, ψ_l^+, ψ_l^- was not written out explicitly. This represents the equality of chemical potentials between two cholesterol monomers (in each of the opposing leaflets) and a cholesterol dimer. Eq. (25) leads eq. (5) in the main text, where the equilibrium "constant" $K(\psi_d, \Phi_c)$ has the forms

$$K(\psi_d, \Phi_c) = \mathrm{e}^{-f_0/T} \tag{26}$$

for $\psi_d < \psi_{\mathrm{th}}$ and

$$K(\psi_d, \Phi_c) = \mathrm{e}^{-f_0/T + \frac{1}{2T} J_{\mathrm{dd}} \alpha_4^{-1} (\alpha_2 - 2J_{\mathrm{cd}}/J_{\mathrm{dd}})^2 (\psi_d - \psi_{\mathrm{th}})} \tag{27}$$

for $\psi_d > \psi_{\mathrm{th}}$.

A numerical calculation of the fraction of cholesterol dimers is shown in Fig. 3 of the main text. The fraction of cholesterol dimers is negligibly small for cholesterol concentrations that are smaller than a threshold value, and this jumps to approximately 1 for larger cholesterol concentrations. Corresponding to this threshold behavior for dimer formation, the tilt angle of cholesterol dimers is relatively small for cholesterol concentrations that are smaller than the threshold and jumps to a larger angle for larger concentrations. It is interesting to note that eq. (1) (of the main text) predicts that tilting transitions (by changing temperature) are second order phase transitions, with free energy arising from orientational entropy. However, the tilting transitions of cholesterol molecules in bilayer membranes (by changing either temperature or lipid compositions) are predicted to be first order phase transitions because cholesterol molecules tilt only when they form dimers.

References

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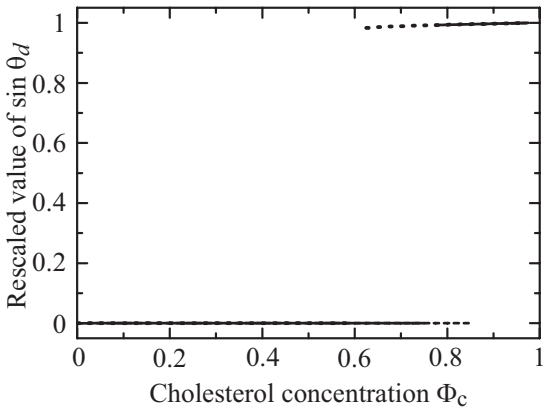

Figure 1: **The value of** $\sin\theta_d$ **(rescaled by** $\alpha_4/(\alpha_2 - 2J_{\rm cd}/J_{\rm dd})$**) of cholesterol dimers is shown as functions of the total cholesterol concentration,** Φ_c**, at the rescaled temperature** $\alpha_4 T/(J_{\rm dd}(\alpha_2 - 2J_{\rm cd}/J_{\rm dd})^2) = 0.03$ **(dotted),** 0.04 **(solid), and** 0.05 **(dashed). The values used are** $-2(J_{\rm ld} - J_{\rm cd})/(\alpha_2 J_{\rm dd} - J_{\rm cd}) = 0.05$**,** $-2J_{\rm cd}/(\alpha_2 J_{\rm dd} - J_{\rm cd}) = 0$**, and** $f_0/T = 5.0$**, where these parameters correspond to Fig. 3 of the main text.**