

# Supplementary Materials

## Interleaflet Coupling and Domain Registry in Phase-Separated Lipid Bilayers

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### 1 Details of molecular mean field model

We consider a planar mixed lipid bilayer of hydrophobic thickness  $L$  composed of cholesterol, a lipid with two saturated hydrocarbon chains of 16 carbons, C16:0, such as dipalmitoylphosphatidylcholine (DPPC), and a lipid with two mono-unsaturated hydrocarbon chains of 18 carbons, C18:1, such as dioleoylphosphatidylcholine (DOPC). The chains are described by Flory's Rotational Isomeric States Model (1, 2) in which each dihedral angle is in one of three configurations: the lowest energy *trans*, or *gauche-plus* or *gauche-minus*, the latter two having an energy 500 cal/mol greater than that of the *trans* configuration. For saturated carbons these states are thermally populated. The double bond of the unsaturated chains is treated as a permanent *gauche* bond. We denote the total number of molecules  $N$ . There are  $N_i$  molecules in the inner leaflet, of which a fraction,  $x_{c,i}$ , are cholesterol,  $x_{s,i}$  saturated lipid, and  $x_{u,i}$  unsaturated lipid. Mole fractions  $x_{c,o}$ ,  $x_{s,o}$ , and  $x_{u,o}$  for the  $N_o$  molecules of the outer leaflet are similarly defined. We denote the fraction of lipids in the inner leaflet as  $\chi$ :

$$\chi \equiv \frac{N_i}{N}, \quad 1 - \chi = \frac{N_o}{N} \quad (1)$$

We now describe how the free energy of the bilayer is calculated within the model. We denote the free energy per molecule, in units of  $k_B T$ , by  $\tilde{f}$  to distinguish it from the areal free energy density  $f$  used in the text. They are of course related by  $f = a^{-1} \tilde{f}$  where  $a \equiv A/N$  and  $A$  is the area of the bilayer midplane. The free energy used in our model has the following components:

$$\tilde{f} = \tilde{f}_{\text{ideal}} + \tilde{f}_{\text{config}} + \tilde{f}_{\text{bond}} + \tilde{f}_{\text{Lagrange}} + \tilde{f}_{\text{surface}} \quad (2)$$

The first of these terms is the free energy contribution of the entropy of mixing, namely

$$\begin{aligned}\tilde{f}_{\text{ideal}} &= \sum_{\sigma=c,s,u} [\chi x_{\sigma,i} \ln(\chi x_{\sigma,i}) + (1-\chi)x_{\sigma,o} \ln((1-\chi)x_{\sigma,o})] \\ &- \ln a\end{aligned}\quad (3)$$

The second term in Eqn. (2) is due to the entropies and *gauche* bond energies of the configurations taken on by the molecules:

$$\begin{aligned}\tilde{f}_{\text{config}} &= 2\chi x_{s,i} \sum_{\alpha \in \Omega_{s,i}} P_{s,i}(\alpha) [\ln P_{s,i}(\alpha) + \beta \epsilon_{s,i}(\alpha)] \\ &+ 2\chi x_{u,i} \sum_{\alpha \in \Omega_{u,i}} P_{u,i}(\alpha) [\ln P_{u,i}(\alpha) + \beta \epsilon_{u,i}(\alpha)] \\ &+ \chi x_{c,i} \sum_{\alpha \in \Omega_{c,i}} P_{c,i}(\alpha) [\ln P_{c,i}(\alpha) + \beta \epsilon_{c,i}(\alpha)] \\ &+ 2(1-\chi)x_{s,o} \sum_{\alpha \in \Omega_{s,o}} P_{s,o}(\alpha) [\ln P_{s,o}(\alpha) + \beta \epsilon_{s,o}(\alpha)] \\ &+ 2(1-\chi)x_{u,o} \sum_{\alpha \in \Omega_{u,o}} P_{u,o}(\alpha) [\ln P_{u,o}(\alpha) + \beta \epsilon_{u,o}(\alpha)] \\ &+ (1-\chi)x_{c,o} \sum_{\alpha \in \Omega_{c,o}} P_{c,o}(\alpha) [\ln P_{c,o}(\alpha) + \beta \epsilon_{c,o}(\alpha)]\end{aligned}\quad (4)$$

Here  $\Omega_{s,i}$  is the set of molecular configurations of single saturated lipid chains in the inner leaflet, with analogous definitions for other components. A large number (of order hundreds of millions) of these configurations is generated on the basis of the Rotational Isomeric States Model, including *gauche/trans* isomerization, overall rotations of the molecules, and steric hindrances within a given chain configuration. The factors of 2 multiplying the terms corresponding to saturated and unsaturated lipids are due to the two chains present in each of these molecules.

The third term  $\tilde{f}_{\text{bond}}$  of Eqn. (2) contains attractive interactions between molecules which depend on the average local orientation of carbon-carbon bonds. These interactions are responsible for the liquid-gel and liquid-liquid phase transitions occurring in the model.

$$\tilde{f}_{\text{bond}} = - \sum_j \sum_k \beta J_{j,k} \frac{N_{j,\text{tails}} N_{k,\text{tails}}}{2aN^2 v_s} \int_0^L \langle \xi_j(z) \rangle \langle \xi_k(z) \rangle dz, \quad (5)$$

where the indices  $j$  and  $k$  specify both the type of molecule and the leaflet. The numbers of tails are defined in the obvious way; for example,  $N_{s,i,\text{tails}} = 2\chi x_{s,i}N$  whereas  $N_{c,o,\text{tails}} = (1 - \chi)x_{c,o}N$ . The quantity  $v_s$  is the volume assigned to a  $\text{CH}_2$  group, equal to  $27 \text{ \AA}^3$ . The interaction strengths  $J_{j,k}$  take on different values depending on whether the molecules indexed by  $j$  and  $k$  are lipids or cholesterol. We have used values determined in previous work (3) by qualitatively matching experimental phase diagrams. These values are  $J_{ll} = 1.9 \text{ cal/mol}$ ,  $J_{lc} = 0.85 J_{ll}$ , and  $J_{cc} = 0.80 J_{ll}$ . The integral in Eqn. (5) runs across the hydrophobic thickness of the bilayer. The quantities  $\langle \xi_j(z) \rangle$  are local order parameters which measure the extent to which carbon-carbon bonds located at a depth  $z$  in the bilayer are aligned with the bilayer normal. These order parameters are ensemble averages of microscopic quantities defined for each molecular configuration  $\alpha$ ; for example, for saturated lipids in the inner leaflet the microscopically defined quantity is

$$\xi_{s,i}(\alpha; z) \equiv v_s \sum_{k=2}^{n-1} \delta(z - z_{k,\alpha}) g(\hat{\mathbf{u}}_{k,\alpha} \cdot \hat{\mathbf{z}}), \quad \text{where} \quad (6)$$

$$g(x) \equiv \frac{2m+1}{2} (\cos^2 x)^m \quad (7)$$

The number of carbon atoms in the saturated lipid chain is given by  $n = 16$ . For a configuration  $\alpha$  of a saturated lipid chain,  $z_{k,\alpha}$  is the coordinate of the  $k$ th carbon atom in the direction normal to the bilayer, while  $\hat{\mathbf{u}}_{k,\alpha}$  is the unit vector pointing from carbon  $k - 1$  to carbon  $k + 1$ . The function  $g$  determines the average shape of lipid molecules; as in previous work (4) we use  $m = 18$  in order to match the correct area per molecule in a pure saturated lipid bilayer. Similar definitions as Eqn. (6) apply for unsaturated lipid chains, and for the short flexible hydrocarbon portion of the cholesterol molecule. Cholesterol's rigid ring structure is assigned a single directional vector, pointing along its length and contributing to the directional order parameter.

We arrive now at the term  $\tilde{f}_{\text{Lagrange}}$  in Eqn. (2), which accounts for the dense, liquid-like molecular packing in the hydrophobic interior of the bilayer. This constraint is enforced using a local Lagrange multiplier  $\beta\pi(z)$ :

$$\begin{aligned} \tilde{f}_{\text{Lagrange}} \equiv \int \beta\pi(z) \{ & 2\chi x_{s,i} \langle v_{s,i}(z) \rangle + 2(1 - \chi)x_{s,o} \langle v_{s,o}(z) \rangle \\ & + 2\chi x_{u,i} \langle v_{u,i}(z) \rangle + 2(1 - \chi)x_{u,o} \langle v_{s,o}(z) \rangle \\ & + \chi(1 - x_{s,i}) \langle v_{c,i}(z) \rangle + (1 - \chi)x_{c,o} \langle v_{c,o}(z) \rangle \\ & - a \} dz \quad (8) \end{aligned}$$

The volume  $\langle v_{s,i}(z) \rangle$  is the average volume contributed by saturated lipid chain configurations at a depth  $z$  within the bilayer. It is the ensemble average of the following microscopic quantity:

$$v_{s,i}(\alpha; z) \equiv v_s \sum_{k=1}^{n-1} \delta(z - z_{k,\alpha}) + v_{\text{CH}_3} \cdot \delta(z - z_{n,\alpha}), \quad (9)$$

where the volume  $v_{\text{CH}_3} = 54 \text{ \AA}^3$  of the terminal  $\text{CH}_3$  group has been taken into account. Similar definitions apply for unsaturated lipids and cholesterol, taking into account their structure; each half of the  $-\text{CH}=\text{CH}-$  segment of an unsaturated bond is given a volume  $v_{\text{CH}} = 0.8v_s$  while each of the 27 carbon atoms of cholesterol is assigned a volume  $v_c = 21 \text{ \AA}^3$ .

The final term,  $\tilde{f}_{\text{surface}}$ , of the free energy of Eqn. (2) takes into account in an implicit fashion the interactions between lipid head groups as well as the interactions between lipid molecules and the solvent. Intuitively, if the area per molecule becomes too large, this must be penalized due to contact between the solvent and the hydrophobic interior of the membrane. This penalty is incorporated into the free energy via the effective surface tension  $\Gamma$ :

$$\tilde{f}_{\text{surface}} = 2\beta\Gamma a \quad (10)$$

We use  $\Gamma = 0.12 k_B T / \text{\AA}^2$ , roughly the surface tension of an oil-water interface.

We have now defined each of the terms of Eqn. (2) in such a way that the free energy  $\tilde{f}$  is specified as a function of the composition of the bilayer (the inner leaflet fraction  $\chi$  and the mole fractions  $x_{\sigma,j}$ ), the area per molecule  $a$ , and the probabilities  $P_{\sigma,j}(\alpha)$  of the chain configurations. The latter are chosen so as to minimize the free energy; the resulting Boltzmann distribution may be easily derived by taking derivatives of Eqn. (2) with respect to  $P_{\sigma,j}(\alpha)$  for a given configuration  $\alpha$ . Thus the free energy  $\tilde{f}$  is defined in terms of the bilayer composition and area per molecule, except for the problem of choosing the Lagrange multiplier function  $\beta\pi(z)$  so as to make the contribution  $f_{\text{Lagrange}}$  vanish. This is in fact the main difficulty in calculating the free energy. It is solved by discretizing the thickness of the bilayer into a finite number of slabs of thickness  $\Delta z$  (we have used  $\Delta z = 1 \text{ \AA}$ ) and defining discretized versions of the average volumes such as  $\langle v_{s,i}(z) \rangle$  so that

the incompressibility condition can be written in a discrete form:

$$\begin{aligned}
& 2\chi x_{s,i}\langle v_{s,i}(k)\rangle + 2(1-\chi)x_{s,o}\langle v_{s,o}(k)\rangle \\
& + 2\chi x_{u,i}\langle v_{u,i}(k)\rangle + 2(1-\chi)x_{u,o}\langle v_{s,o}(k)\rangle \\
& + \chi x_{c,i}\langle v_{c,i}(k)\rangle + (1-\chi)x_{c,o}\langle v_{c,o}(k)\rangle \\
& = a\Delta z,
\end{aligned} \tag{11}$$

where  $j \in 1, 2 \dots, lz \equiv L/\Delta z$

The quantity  $\langle v_{s,i}(k)\rangle$ , for example, is the average volume contributed by saturated lipids in the inner leaflet to the slab with index  $k$ . We note that the area per molecule  $a$  and the hydrophobic thickness  $L$  of the bilayer are not independent, but are related by the assumption of incompressibility:

$$\begin{aligned}
La &= [\chi x_{s,i} + (1-\chi)x_{s,o}] v_{\text{sat}} \\
&+ [\chi x_{u,i} + (1-\chi)x_{u,o}] v_{\text{unsat}} \\
&+ [\chi x_{c,i} + (1-\chi)x_{c,o}] v_{\text{chol}},
\end{aligned} \tag{12}$$

where  $v_{\text{sat}}, v_{\text{unsat}},$  and  $v_{\text{chol}}$  are the total volumes of the hydrophobic portions of saturated lipids, unsaturated lipids, and cholesterol.

To summarize, the free energy is calculated as follows. Eqn. (11) gives a number  $lz$  of coupled nonlinear equations which are solved by standard methods. These equations involve quantities such as  $\langle v_{s,i}(k)\rangle$  which are calculated from ensemble averages over a large set of chain configurations, as described above. The discretized Lagrange multipliers  $\beta\pi(k)$  appear as the unknowns via the Boltzmann probability distribution. Once these multipliers are known, the free energy may be calculated from Eqn. (2).

We note that the spatial discretization of the  $z$  direction normal to the bilayer means that we only calculate free energies for bilayer thicknesses which are multiples of  $\Delta z$ . Since  $a$  and  $L$  are related by Eqn. (12), this means that we only calculate free energies explicitly for a discrete set of areal densities. Free energies at intermediate values are calculated by means of third-order spline interpolation. In particular, the area per molecule  $a$  at which the membrane is tensionless is found in this way. We have verified that different means of interpolation (for example, global polynomial interpolation) do not affect our results.

## 2 Probability distribution of mismatch area

Equation (19) of the paper gives the average mismatch area per unit projected interface length. We may further ask for the complete probability

distribution  $P(a)$  of mismatch areas, that is, the probability that the mismatch area  $A[y_1, y_2]$  of a configuration of the interfaces takes on the given value  $a$ . This can be done by writing  $P(a)$  in terms of path integrals over all trajectories of  $y_1(x)$  and  $y_2(x)$ , the curves delineating the phase boundaries in the two leaflets:

$$P(a) = \frac{\int \mathcal{D}y_1 \mathcal{D}y_2 \delta(A[y_1, y_2] - a) \exp(-\beta H_{\text{fluct}}[y_1, y_2])}{\int \mathcal{D}y_1 \mathcal{D}y_2 \exp(-\beta H_{\text{fluct}}[y_1, y_2])}, \quad (13)$$

where  $H_{\text{fluct}}$  is given in Eq. (8) of the paper. The delta function can be written

$$\delta(A - a) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \exp[-i\beta\tilde{\lambda}(A - a)] d\beta\tilde{\lambda}, \quad (14)$$

so that

$$\begin{aligned} P(a) &= \frac{1}{2\pi} \frac{\int_{-\infty}^{\infty} d\beta\tilde{\lambda} \exp(i\beta\tilde{\lambda}a) \int \mathcal{D}y_1 \mathcal{D}y_2 \exp(-i\beta\tilde{\lambda}A[y_1, y_2]) \exp(-\beta H_{\text{fluct}}[y_1, y_2])}{\int \mathcal{D}y_1 \mathcal{D}y_2 \exp(-\beta H_{\text{fluct}}[y_1, y_2])} \\ &= \frac{1}{2\pi} \int_{-\infty}^{\infty} d\beta\tilde{\lambda} \exp(i\beta\tilde{\lambda}a) \frac{Z(\tau_0, \lambda + i\tilde{\lambda}, T, L)}{Z(\tau_0, \lambda, T, L)} \\ &= \frac{1}{2\pi Z(\tau_0, \lambda, T, L)} \int_{-\infty}^{\infty} d\beta\tilde{\lambda} \exp(i\beta\tilde{\lambda}a) \exp[-\beta F(\tau_0, \lambda + i\tilde{\lambda}, T, L)] \\ &= \frac{1}{2\pi Z(\tau_0, \lambda, T, L)} \int_{-\infty}^{\infty} d\beta\tilde{\lambda} \exp(i\beta\tilde{\lambda}a) \cdot \\ &\quad \exp \left[ -\beta L \left[ \frac{3\pi}{8} \right]^{2/3} \left[ \frac{(k_B T)^2 (\lambda + i\tilde{\lambda})^2}{\tau_0} \right]^{1/3} \right], \end{aligned} \quad (15)$$

where the partition function,  $Z$ , and free energy  $F$ , are those encountered earlier in Eq. (9) of the paper. Because the exponent is dimensionless, one sees that there is a characteristic value,  $\lambda_c$ , of  $\lambda$ :

$$\lambda_c = \frac{8}{3\pi} \left( \frac{k_B T \tau_0}{L^3} \right)^{1/2}. \quad (16)$$

It is also convenient to define an area  $a_c \equiv k_B T / \lambda_c$ . The integral can be made dimensionless by defining the dimensionless interleaflet coupling parameters  $g \equiv \lambda / \lambda_c$  and  $\tilde{g} \equiv \tilde{\lambda} / \lambda_c$ .

$$P(a) = \frac{\beta \lambda_c}{2\pi} \exp(g^{2/3}) \int_{-\infty}^{\infty} d\tilde{g} \exp \left[ i\tilde{g}(a/a_c) - (g + i\tilde{g})^{2/3} \right] \quad (17)$$

In the limit of large  $g$ , the distribution is the Gaussian

$$P(a) \approx \frac{3\beta\lambda_c g^{2/3}}{2\pi^{1/2}} \exp \left[ -\frac{9}{4} g^{4/3} \left( \frac{a}{a_c} - \frac{2}{3g^{1/3}} \right)^2 \right], \quad (18)$$

from which we may again recover the average mismatch area of Eqn. (20) of the paper. We can likewise determine the tail of the probability distribution of mismatch areas in the limit where  $a$  is large using a saddle point approximation, with the result that

$$P(a) \propto \exp \left[ -\frac{a}{a_c} \left( \lambda_c + \frac{4}{27} \left( \frac{a_c}{a} \right)^3 \right) \right]. \quad (19)$$

Thus when the mismatch area  $a$  is sufficiently large, the tail of the probability distribution takes the form  $P(a) \propto \exp(-\beta\lambda a)$  assumed by Risselada and Marrink (5) in their estimate of the magnitude of the interleaflet coupling.

In general the integral in Eqn. (17) must be calculated numerically. In Fig. 1 we show a plot of the probability distribution of the scaled area,  $a/a_c$ , given by

$$\tilde{P}(a/a_c) \equiv a_c P(a) \quad (20)$$

for various values of the scaled interleaflet coupling parameter  $g$ . We note that there is a small interval of areas near the origin in which the distribution is extremely small. This is undoubtedly an entropic effect as there are few configurations with very small mismatch areas.

### 3 Details of lattice Monte Carlo simulation

The lattice model we have considered is simply two coupled square-lattice Ising models, also known as an Ising bilayer (6). The values of the spins in each square lattice are  $\sigma_i$  and  $\sigma'_i$ , where  $i = 1 \dots N^2$ . The spins take the values 1 (“ordered”) and  $-1$  (“disordered”) representing two components in a phase-separating binary mixture. The Hamiltonian is

$$\beta H [\{\sigma_i\}, \{\sigma'_i\}] = -J \sum_{\langle i,j \rangle} [\sigma_i \sigma_j + \sigma'_i \sigma'_j] - K \sum_i \sigma_i \sigma'_i, \quad (21)$$

where the first sum is taken over pairs of neighboring spins and corresponds to interactions within each leaflet of a bilayer, taking into account periodic boundary conditions. The second term is a coupling between the two square

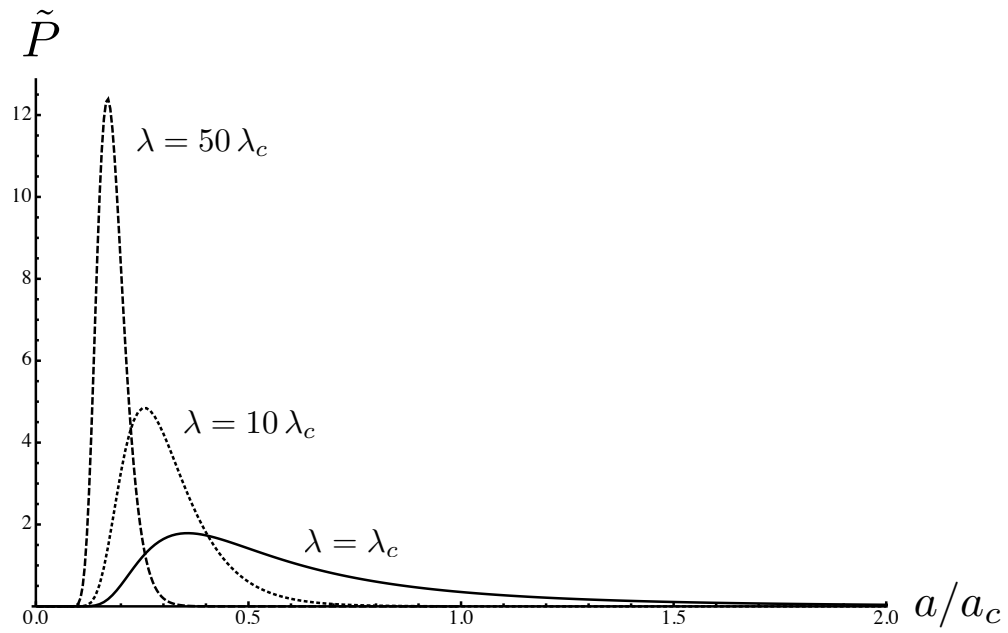


Figure 1: Probability distribution of scaled mismatch area,  $a/a_c$ , calculated numerically with the following values of the scaled interleaflet coupling parameter  $g \equiv \lambda/\lambda_c$ : 1 (solid), 10 (dotted), and 50 (dashed).



lattices and corresponds to the interleaflet coupling in a phase-separating bilayer. In our simulations we used  $N = 50$ ,  $J = 0.75$ , and  $K = 0.25$ .

We performed Monte Carlo simulations of the model described above. Attempted Monte Carlo moves consisted of switching the values of two neighboring spins within the same leaflet. The location and leaflet of the spins were chosen at random. Because the Monte Carlo moves consist of switching the values of spins, the total “magnetization” within each leaflet is conserved. This corresponds to the conserved average composition of a lipid bilayer. We always used an initial condition where half of the lattice (in both leaflets) was filled with “up” spins and the other half with “down” spins. A single Monte Carlo time step was defined to be  $2N^2$  attempted moves.

The mismatch area of a configuration is defined as the total number of spins in all the mismatch regions. A mismatch region is defined to be a connected region containing three or more spins in which the spins in the two leaflets have different values. Two spins are considered connected if they neighbor each other vertically or horizontally, but not diagonally. There are two types of mismatch regions, shown as two shades of gray in Fig. 6 of the paper; either the inner leaflet is ordered and the outer disordered, or vice versa. Neighboring mismatch regions of these two types are considered distinct. Our definition of a mismatch region requires that the region include at least three spins. The purpose of this requirement is to exclude small clusters of “ordered” spins in the bulk of the “disordered” phase, or vice versa, since these clusters are not mismatch regions at the interface between the phases. Changing or removing this criterion should not influence the statistics of large mismatch areas. The perimeter of a mismatch region is defined to be the number of spins in that region which border on spins outside it. The total perimeter of the mismatch area is the sum of the perimeters of the mismatch regions.

Figure 7 of the paper was generated as follows. Series of configurations were taken from eight simulations of the model, each consisting of one million MC steps. The first hundred thousand steps of each simulation were ignored to allow equilibration, as measured by the average mismatch area. Among the remaining configurations, every hundredth configuration was selected in order to obtain configurations which were nearly statistically independent. This produced  $8 \times 9000$  configurations. These configurations were binned according to their mismatch areas with bin widths of 10 (areas are dimensionless since they are numbers of spins). This produces the histogram shown as a solid line in Fig. 7 of the paper. Among configurations belonging to a given bin, the average perimeter was calculated and divided

by the average mismatch area to obtain the quantity plotted as a dashed line in the figure.

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