Adhesion-induced phase separation of multiple species of membrane junctions

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A theory is presented for the intermembrane junction separation induced by the adhesion between two biomimetic membranes that contain two different types of anchored intermembrane junctions (receptor-ligand complexes). The analysis shows that several mechanisms contribute to the phase separation of the membrane junctions. These mechanisms include the following. (i) The elasticity of the membranes mediates a short-ranged nonlocal interaction between the junctions due to the height difference between type-1 and type-2 junctions. This is the main factor that drives the phase separation. (ii) When type-1 and type-2 junctions have different flexibilities against stretch and compression, the “softer” junctions are the “favored” species, and aggregation of the softer junctions can occur. (iii) The thermally activated shape fluctuations of the membranes also contribute to the phase separation by inducing another nonlocal interaction between the junctions and renormalizing the binding energy of the junctions. The combined effect of these mechanisms is that when phase separation occurs, the system separates into two domains with different relative and total junction densities.

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I. INTRODUCTION

Adhesion of membranes is responsible for cell adhesion, which plays an important role in embryological development, immune response, and the pathology of tumors [1]. In many cases, membrane adhesion in biological systems is mediated by the specific attractive interactions between complementary pairs of ligands and receptors which are anchored in the membranes [2]. At the same time, the adhesion between multicomponent biomembranes or biomimetic membranes is also intimately related to domain formation [3–11]. When the membrane adhesion is mediated by the specific lock-and-key type of bonds between the anchored ligands and receptors, i.e., intermembrane junctions (for simplicity, from now on I shall use the term junctions for these ligand-receptor complexes), adhesion-induced lateral phase separations have been observed in many experiments in biomimetic systems [3–5]. Theoretical models and Monte Carlo simulations [6–11] have also shown similar phase separation behavior in various systems.

So far, studies on adhesion-induced lateral phase separation have focused on the case when the system has a single type of junction. The presence of the glycol proteins anchored in the membranes (i.e., repellers), and the interplay between generic interactions (for example, van der Waals, or electrostatic interactions) and specific ligand-receptor interactions are believed to enhance this phase separation. However, in biological systems membrane adhesion is often mediated by more than one type of junction, and the adhesion-induced phase separation of the membrane junctions is believed to play an important role in some biological processes. For example, a key event governing a mature immune response when T lymphocytes interact with antigen-present cells is the formation of immunological synapses. An immunological synapse is a patch of membrane adhesion region between a T cell and an antigen-present cell, where the TCR–MHC-peptide complexes aggregate in the center with a LFA-1–ICAM-1 complex rich region surrounds it [12–14]. Since a complete understanding of the physical mechanism behind this type of adhesion-induced phase separation of multispecies membrane junctions is still unavailable, in the present work I develop a theoretical model to study the equilibrium properties of such systems.

This paper is organized as follows. In Sec. II, I discuss a coarse grained model for the adhesion of two membranes due to the formation of two types of junctions. To concentrate on the effect of the differences between type-1 and type-2 junctions, the glycolcalyx and the generic interactions between the membranes are not considered in this model. Furthermore, I assume that the membranes are bound to each other due to the formation of the membrane junctions. Hence I will not discuss another interesting problem of the unbinding transition. An approximate solution of this model which neglects the fluctuations of membrane-membrane distance (the “hard membrane” solution) is studied in Sec. III. This simplified solution already reveals several mechanisms that are important to the phase behavior of the system. For example, when type-1 and type-2 junctions have the same flexibility but with sufficiently large height difference, membrane adhesion induces a phase separation that is driven by the height difference of the junctions. In this situation the membranes separate into a type-1-junction-rich domain and a type-2-junction-rich domain. On the other hand, when the junctions have different flexibilities and the height difference is not very large, membrane adhesion can induce an aggregation of the “softer” junctions, i.e., the membranes separate into two domains, both of which are rich in softer junctions. The general situation is that both mechanisms contribute to the adhesion-induced phase separation. When phase separation occurs, the system separates into two domains with different membrane-membrane distances because of the height mismatch of the junctions, and the total number of softer junctions in the system is greater than the total number of stiffer junctions due to the effect of softer junction aggregation.

The fact that the hard membrane solution assumes that the
membrane-membrane distance is a constant that has neglected some interesting physics of the system. For example, an interesting feature of the hard membrane solution is that when phase separation occurs, the total junction density, i.e., $\phi_1 + \phi_2$ ($\phi_a$ is the density of type-$a$ junctions), is the same in domains that have different values of $(\phi_1 - \phi_2)/\phi_1 + \phi_2$ (the relative densities of the junctions). However, when the effects of nonconstant membrane-membrane distance, and the thermally activated fluctuations of the membranes are discussed in Sec. IV, this “interesting result” no longer holds. The fluctuation analysis in Sec. IV shows that, first, the thermally activated fluctuations of the membranes are effects of nonconstant membrane-membrane distance, and also shows that, when the hard membrane solution of the nonconstant membrane-membrane distance is a constant has neglected all the direct interactions between the junctions, which are mediated by the membrane elasticity and thermally activated fluctuations of the junction densities and membrane-membrane distance. These interactions are not included in the simple physical picture provided by the hard membrane solution. As a result of these effects, when phase separation occurs, domains with different values of $(\phi_1 - \phi_2)/\phi_1 + \phi_2$ also have different values of $\phi_1 + \phi_2$. The fluctuation analysis also shows that, when the hard membrane solution of the junction densities is small, or when the junctions are very short or very soft, the membrane fluctuations are sufficiently large such that the present analysis cannot provide the complete physical picture for the system. This criterion shows under what conditions one needs a numerical simulation of the model to provide a better picture of the physics in this system. Section V summarizes this work. The Appendix discusses the details of the fluctuation analysis around the hard membrane solution.

II. THE MODEL

To focus on the physics of adhesion-induced phase separation, I will not discuss the binding-unbinding transition but only consider the case when the membranes are bound to each other due to the presence of the junctions. The system is shown schematically in Fig. 1. The heights of the membranes measured from the reference plane (i.e., the $x$-$y$ plane) are denoted as $z_1(r)$ and $z_2(r)$, respectively, where $r=(x,y)$ is a two-dimensional planar vector. There are two types of anchored receptors in membrane 1, and two types of anchored ligands in membrane 2. Type-$a$ receptors ($a$ is 1 or 2) form specific lock-and-key complexes with type-$a$ ligands; these are the junctions that mediate the membrane adhesion. The density of type-$a$ junctions at $r$ is $\phi_a(r)$, and the densities of free type-$a$ receptors and ligands at $r$ are denoted by $\psi_{Ba}(r)$ and $\psi_{La}(r)$, respectively. The binding energy of a type-$a$ junction is denoted by $E_{Ba}$.

The effective Hamiltonian of the system can be written as

$$ H = \int d^2r \left\{ \frac{\kappa}{2} [\nabla^2 h(r)]^2 + \frac{\gamma}{2} [\nabla h(r)]^2 + \sum_{a=1}^{2} \frac{\Lambda_a}{2} \phi_a(r) \right\} 
\times [h(r) - h_a]^2 - \sum_{a=1}^{2} \phi_a E_{Ba} \right\}, $$

where $\gamma = \frac{\kappa l_0^2}{4\kappa}$ is the dimensionless surface tension, $\Lambda_a = \lambda_a l_0^2$ is the dimensionless junction flexibility, and all in-plane lengths and heights are scaled by $\sqrt{a}$ and $\sqrt{l_0^2\kappa} = l_0$, respectively.
The effective interaction free energy between the junctions due to the membrane-junction coupling is obtained by integrating over $h(\mathbf{r})$,

$$F_c[\phi_\alpha] = -\ln \left( \int D[h] e^{-\mathcal{H}\phi_\alpha} \right).$$

Thus, in the spirit of density functional theory, the total free energy of the system is provided by

$$F = F_c + F_s,$$

where

$$F_s = \sum_{\alpha=1}^2 \int d^2r [\phi_\alpha(\mathbf{r})(\ln \phi_\alpha - 1) + \psi_{\alpha L}(\mathbf{r})(\ln \psi_{\alpha L} - 1)]$$

is the contribution from the entropy of the junctions, receptors, and ligands. Here I have assumed that $\phi_\alpha \ll 1$, $\psi_{\alpha L} \ll 1$. In principle, once $F_c$ is calculated, the equilibrium distribution of the junction density is determined by minimizing the total free energy of the system under the constraint that the total numbers of the receptors and ligands in the system are fixed, i.e.,

$$\int d^2r [\phi_\alpha(\mathbf{r}) + \psi_{\alpha L}(\mathbf{r})] = N_{R\alpha},$$

$$\int d^2r [\phi_\alpha(\mathbf{r}) + \psi_{\alpha L}(\mathbf{r})] = N_{L\alpha},$$

here $N_{R\alpha}$ and $N_{L\alpha}$ are the total numbers of type-$\alpha$ receptors and ligands in each membrane when the membranes are completely detached.

### III. “HARD MEMBRANE” SOLUTION

Since the integral in Eq. (3) cannot be carried out exactly, in this section I discuss an approximate solution in which $\phi_{\alpha}$ and $h(\mathbf{r})$ are independent of $\mathbf{r}$. In this approximation, $F_c$ can be easily calculated by looking for the saddle point in the integrand. This is equivalent to neglecting the fluctuations of the membrane-membrane distance, therefore I call this mean-field approximate solution the “hard membrane” solution. To simplify the notation, I define $\Lambda_+ = \Lambda_1 / \Lambda_2$, $\phi_\pm = (\phi_1 \pm \phi_2)/2$, and let $h_1 = h_0 - \Delta h$, $h_2 = h_0 + \Delta h$. Thus the hard membrane solution of the membrane-membrane distance can be expressed by

$$h = h_0 - \frac{\Lambda_+ \phi_+ + \Lambda_- \phi_-}{\Lambda_+ \phi_+ + \Lambda_- \phi_-} \Delta h = h_0 - l_M.$$

Notice that $l_M$ depends on the junction densities. After substituting $h$ back to the Hamiltonian, the effective interaction free energy between the junctions, $F_c$, can be expressed by its saddle-point value

$$F_c = \int d^2r \left\{ \Lambda_+ \phi_+ + \Lambda_- \phi_- \frac{1}{2} \left[ (F^c_M + \triangle h^2) \right] 
+ (\Lambda_+ \phi_+ + \Lambda_- \phi_-) \mu_M \Delta h - (E_+ \phi_+ + E_- \phi_-) \right\},$$

where $E_+ = E_{B1} \pm E_{B2}$. It is clear that there is an interaction between the junctions due to the membrane adhesion. To minimize the total free energy under the constraints in Eq. (6), it is convenient to work in the grand canonical ensemble and define the free energy $G$ of the system under constant chemical potentials,

$$G = F_c + F_s - \sum_{\alpha} \mu_{R\alpha} \int d^2r (\phi_\alpha + \psi_{\alpha L}) - \sum_{\alpha} \mu_{L\alpha} \int d^2r (\phi_\alpha + \psi_{\alpha L}).$$

The chemical potentials, $\mu_{R\alpha}$, $\mu_{L\alpha}$ are determined by fixing the total number of receptors and ligands in the system. However, for convenience I will proceed the discussion in the grand canonical ensemble. After some straightforward algebra, $G$ is expressed as

$$G = \int d^2r \phi_+ \{ g(\phi) + 2(\ln \phi_+ - 1) - \mu_+ \} + G_\phi,$$

where

$$g(\phi) = -\frac{\Delta h^2}{2} \Lambda_+ (\lambda + \phi)^2 + (1 + \phi) \ln (1 + \phi)$$

$$+ (1 - \phi) \ln (1 - \phi) - \mu_+ \phi = f(\phi) - \mu_+ \phi,$$

$$\lambda = \Lambda_- \lambda_+ \phi_\pm = \lambda_+ \phi_\pm, \text{ and } \mu_\pm = (\mu_{R\alpha} + \mu_{L\alpha}) \pm (\mu_{R\alpha} + \mu_{L\alpha}) E_{B1} \pm E_{B2} - (\triangle h^2/2) \Lambda_+.$$ $G_\phi$ includes terms that only depend on $\psi_{R\alpha}$ and $\psi_{L\alpha}$, they are decoupled from the other terms, hence from now on I neglect $G_\phi$. From Eq. (10), it is clear that in the hard membrane solution the phase behavior of the junctions is governed by $\Delta h^2 \Lambda_+ \lambda$, and $\mu_\pm$. Minimizing $g(\phi)$ leads to the equilibrium value of $\phi$, and later I will show that there can be a phase separation in $\phi$. On the other hand, $\phi_\pm$ is determined by $\delta G / \delta \phi_\pm = 0$. From Eq. (10), $\phi_\pm$ can be expressed by

$$\phi_\pm = \exp \left[ \lambda_\pm \phi \frac{1}{2} g(\phi) \right].$$

Because in equilibrium $\phi$ is determined by minimizing $g(\phi)$, $g(\phi)$ takes a single value even when there is a phase separation in $\phi$. Therefore in the hard membrane solution $\phi$ is single valued even when the system separates into two domains with different values of $\phi$. This is a natural result of the approximation in the hard membrane solution in which all spatial correlations are negated. Since in this approximation the “spreading pressure” of the two-dimensional gas of junctions is the same as an ideal gas [16], the equilibrium condition requires that total junction density the same in both phases. In the following section I will show that, when the effects of fluctuations around the hard membrane solution are
taken into account, the analysis reveals a renormalization of the binding energy of the junctions and (nonlocal) interactions between the junctions which are mediated by the membrane elasticities and thermally activated membrane fluctuations. As a result, these spatial correlations modify the spreading pressure of the junctions such that it is not the same as the simple ideal-gas relation, and the true equilibrium solution of $\phi_+\equiv \phi$ is not single valued in the regime where phase separation happens. Thus, the fact that $\phi_+$ is single valued in the hard membrane solution is an artifact of the approximation that assumes constant junction densities and membrane-membrane distance.

Now I discuss the hard membrane solution of $\phi$. To emphasize different roles played by $\Delta_n^2 \Lambda_+$ and $\lambda$, I begin the discussion with the special case when $\lambda=0$, i.e., when both types of junctions have the same flexibility. In this case the important parameter of the theory is $\Delta_n^2 \Lambda_+$, and $g(\phi)$ has a very simple form

$$g(\phi) = -\frac{\Delta_n^2 \Lambda_+}{2} \phi^2 + (1+\phi) \ln(1+\phi) + (1-\phi) \ln(1-\phi) - \mu_- \phi.$$  

(13)

This form is exactly the same as the Flory-Huggins theory for binary mixtures [17], where phase separation occurs when $\Delta_n^2 \Lambda_+ > 2$ and the phase coexistence curve is a straight line at $\mu_- = 0$. This phase coexistence curve ends at a critical point $\mu_- = 0$, $\Delta_n^2 \Lambda_+ = 2$. The physics in this special case $\lambda=0$ is clear: the difference in junction height drives a phase separation, and this separation only occurs when the factor $\Delta_n^2 \Lambda_+$, a combination of junction height difference and junction flexibility, is sufficiently large. On the phase coexistence curve, the system separates into $\phi_1$-rich and $\phi_2$-rich domains, and the system is symmetric under $\phi \rightarrow -\phi$.

Next I discuss the more general case $\lambda \neq 0$, i.e., the junctions have different flexibilities. Figure 2 shows the shape of $g(\phi)$ with different values of $\mu_- \equiv \mu$ when $\lambda=0.2$ and $\Delta_n^2 \Lambda_+ = 1.998$. Notice that this is the case when $\Delta_n^2 \Lambda_+ < 2$, i.e., there is no phase separation if $\lambda=0$. Nevertheless, Fig. 2 clearly shows that $g(\phi)$ has two local minima, both at negative $\phi$, and phase coexistence occurs when $\mu_- = -0.4045$. Since this is the case when $\lambda > 0$, i.e., type-2 junctions are “softer” than type-1 junctions, double minimum at $\phi = \phi_1$ means that the softer junctions tend to aggregate, when phase coexistence occurs, a domain with high $\phi_2$ $- \phi_1$ coexists with a domain with small $\phi_2 - \phi_1$. For the choice of parameters in Fig. 2, the density of type-2 junctions is higher than the density of type-1 junctions in both domains.

Another effect of nonzero $\lambda$ can be seen in Fig. 3, where $g(\phi)$ for different values of $\lambda$ is shown at $\Delta_n^2 \Lambda_+ = 2.04 > 2$. It shows that $g(\phi)$ is symmetric in $\phi$ when $\lambda=0$ but asymmetric in $\phi$ for nonzero $\lambda$, i.e., the symmetry under $\phi \rightarrow -\phi$ no longer exists when the junctions have different flexibilities. Comparing to $\lambda=0$ case, in the case when $\lambda > 0$, the minima of $g(\phi)$ are shifted towards smaller $\phi$ values, i.e., the softer junctions are easier to be formed. Notice that different from the example in Fig. 2, in Fig. 3 when phase coexistence occurs the membranes separate into $\phi_1$-rich and $\phi_2$-rich domains, but the softer junctions (in this case type-2 junctions) are the “favored” species, i.e., the total number of the softer junctions in the system is greater than the total number of the stiffer junctions. From these two examples of nonzero $\lambda$, I conclude that in general the experimentally observed junction separation induced by membrane adhesion is actually a result of the combined effect of the aggregation of softer junctions and the separation of the junctions due to the mismatch of junction heights.

In the neighborhood of $\Delta_n^2 \Lambda_+ = 2$, $\lambda=0$, the equilibrium value of $\phi$ is small compared to unity, therefore the phase diagram of the system in this regime can be studied by expanding $g(\phi)$ around $\phi=0$,

$$g(\phi) = r_2 \phi^2 + r_3 \phi^3 + r_4 \phi^4 - \mu_- \phi + \text{const.} + O(\phi^5),$$

(14)
FIG. 4. Schematic representation of the phase coexistence curves near \( \Delta^2 h \Lambda_+ = 2 \) for \( n < 1 \) for \( n = 0 \) (thick solid line), \( n = \pm \lambda_1 \) (thick short-dashed lines), and \( n = \pm \lambda_2 \) (thick long-dashed lines). \( \lambda_1 > \lambda_2 > 0 \). The thin dashed curve is the position of the end points of the phase coexistence curves; this is given by \( \Delta^2 h \Lambda_+ = 2(1 - 9 \lambda^2/4) \), \( \mu_- = -2 \lambda \). The curves move towards the \( \lambda = 0 \) phase boundary as \( \Delta^2 h \Lambda_+ \) increases because the effect of junction height mismatch becomes more important.

\[
\frac{\Delta^2 h}{2} \Lambda_+ \lambda^2 (1 - \lambda^2)^2, \quad (15)
\]

\[
\tilde{\mu}_- = \mu_- + \frac{\Delta^2 h}{2} \Lambda_+ \lambda (2 - \lambda^2),
\]

\[
\text{const.} = \frac{\Delta^2 h}{2} \Lambda_+ \lambda^2, \quad (15)
\]

and \( O(\phi^5) \) is the contribution from terms of order \( \phi^5 \) and higher. The phase diagram in the neighborhood of \( \Delta^2 h \Lambda_+ = 2, \lambda = 0 \) is plotted schematically in Fig. 4, where the phase coexistence curve for \( \lambda = 0 \) ends at a critical point \( \Delta^2 h \Lambda_+ = 2, \mu_- = 0 \), and the end points of the phase coexistence curves for \( \lambda \neq 0 \) occur at the triple root of \( \partial g/\partial \phi = 0 \). Straightforward calculation leads to the position of the end points of the phase coexistence curves at

\[
\Delta^2 h \lambda^2 = 2(1 - 9 \lambda^2/4) + O(\lambda^4),
\]

\[
\mu_- = -2 \lambda + O(\lambda^3). \quad (16)
\]

This shows how the smallest value of \( \Delta^2 h \Lambda_+ \) above which phase separation can occur decreases as the difference of junction flexibilities increases. The phase coexistence curves move towards the \( \lambda = 0 \) phase boundary as the value of \( (\Delta^2 h/2) \Lambda_+ \) increases. This is because as \( (\Delta^2 h/2) \Lambda_+ \) increases, the effect of junction height mismatch becomes more important, and the difference in the junction flexibilities becomes less important.

Although the hard membrane solution is a very simplified analysis of the model, it nevertheless, reveals interesting physics of the phase separation due to membrane adhesion. First of all, the height difference between different types of junctions is not the only factor that is important for the junction distribution. It is only when the flexibilities of both types of junctions are the same that the difference in the junction height is the most important factor in the phase separation. If the system consists of junctions with different flexibilities, the softer junctions are more favored, and the phase separation is a result of the interplay between the aggregation of the softer junctions and the separation of the junctions due to the height difference. Therefore, the smallest value of \( \Delta^2 h \Lambda_+ \) above which phase separation can occur is smaller for systems with larger \( |\lambda| \). However, the approximations in the hard membrane solution neglect the spatial correlations in the system, this lead to the result that \( \phi_+ \) is the same in both domains when phase separation occurs. The analysis, which includes the elasticity of the membranes and the thermal fluctuations around the hard membrane solution, in the following section will show that the membrane elasticity and thermally activated fluctuations modify the hard membrane solution, and the true value of \( \phi_+ \) is not the same in both phases when phase separation occurs. Thus, thermal fluctuations have to be considered in order to gain the full understanding of the nature of the membrane-adhesion-induced interactions between the junctions.

IV. BEYOND “HARD MEMBRANE” SOLUTION

As mentioned in the preceding section, the hard membrane solution neglects the effects of nonuniform membrane membrane distance and junction densities, and therefore fails to take the effects of membrane-mediated nonlocal interactions between the junctions into account. This is reflected in the fact that the hard membrane solution predicts a single valued \( \phi_+ \) in both domains when phase coexistence occurs. To study these membrane-mediated effects, in this section I include the fluctuations of membrane-membrane distance and junction densities by expanding the free energy of the system around the hard membrane solution. In the following I denote the true membrane-membrane distance as

\[
h(r) = h_0 + l_M + \delta l(r) = h_M + \delta l(r), \quad (17)
\]

and the densities of the junctions are expressed by

\[
\phi_\alpha = \phi_M + \delta \phi(r). \quad (18)
\]

Here \( \delta l \), \( \delta \phi_\alpha \) are the deviations of the true values of \( h \) and \( \phi_\alpha \) from their hard membrane solutions, \( \phi_M \) and \( h_M \) are the hard membrane solution of \( \phi_\alpha (r) \) and \( h(r) \), respectively. In this expansion, the coarse-grained Hamiltonian can be expressed as

\[
H = H_M + H_0 + H_1 + H_\phi, \quad (19)
\]

where \( H_M \) is \( H_M, \phi M, \phi H, M \).

\[
H_0 = \int d^2 r [\frac{1}{2} (\nabla^2 \delta l)^2 + \frac{1}{4} \nabla (\nabla \delta l)^2 + h_M (\Lambda_1 \delta \phi_1 + \Lambda_2 \delta \phi_2) + \Delta h (\Lambda_1 \delta \phi_1 - \Lambda_2 \delta \phi_2)] |\delta l| \quad (20)
\]

includes terms that are bilinear in \( \delta l \) and \( \delta \phi_\alpha \).
FIG. 5. Membrane-mediated interaction revealed by the Gaussian approximation. This interaction comes from the bilinear coupling between $\delta l$ and $\delta \phi_h$. (a) A small region that has higher density in the junctions with greater natural height (or lower density in the junctions with smaller natural height) induces a positive $\delta l$. Two regions with positive $\delta l$ can reduce the bending elastic energy of the membranes by moving close to each other. Similarly, a region with negative $\delta l$ attracts another region with negative $\delta l$ due to the cost of membrane bending energy. (b) A small region with positive $\delta l$ repels a region with negative $\delta l$ because of the bending elastic energy cost of the high-curvature region between them.

\[ H_1 = \frac{1}{2} \int d^2r (\Lambda_1 \delta \phi_1 + \Lambda_2 \delta \phi_2)(\delta l)^2 \]  

is the nonlinear coupling between $\delta l$ and $\delta \phi_h$, and $H_\phi$ includes terms that are linear in $\delta \phi_h$.

First I discuss the contribution from $H_0$, i.e., the Gaussian fluctuations around the hard membrane solution. In this Gaussian approximation, $F_c$ has acquired two correction terms that can be expressed by

\[ -\frac{1}{2} \sum_m \ln q^4 + \Gamma q^2 + m_+ \]

\[ -\sum_q \frac{|\lambda M \delta m_+ + \Delta h \delta m_-|^2}{q^4 + \Gamma q^2 + m_+} , \]  

for convenience I have defined $m_+ = \Lambda_1 \phi_1 M + \Lambda_2 \phi_2 M$, and $\delta m_+ = \Lambda_1 \delta \phi_1 + \Lambda_2 \delta \phi_2$. The first term is independent of $\delta \phi_h$, therefore I neglect it in the rest of the discussion. The second term is a membrane-mediated nonlocal interaction between the junctions. This interaction has two characteristic lengths: $m_+^{-1/4}$ is the distance it takes for a perturbation in membrane-membrane distance to relax back to its hard membrane solution due to the membrane bending rigidity, another length is $\Gamma^{-1/2}$, for lengths greater than $\Gamma^{-1/2}$ the elasticity of the membrane is dominated by the surface tension of the membrane, and the contribution from the bending rigidity is negligible. In the rest of this paper, I focus on the case when $\Gamma < \sqrt{m_+}$, in which the membrane bending rigidity is the dominant effect that drives a perturbation in $h$ back to $h_M$, thus the contribution from surface tension of the membranes is negligible. To understand the nature of the nonlocal interaction, it is convenient to transform the second term to real space. Calculations in the Appendix show that, when $\Gamma < \sqrt{m_+}$, the second term in the real space has the form that is derived in Eq. (A3),

\[ \int d^2r \int d^2r' \frac{\Delta h^2}{8 \pi \sqrt{m_+}} G(|r - r'|m_+^{1/4}) \]

\[ \times \left[ \left( 1 - \frac{m_-}{m_+} \right) \Lambda_1 \delta \phi_1(r) - \left( 1 + \frac{m_-}{m_+} \right) \Lambda_2 \delta \phi_2(r) \right] \]

\[ \times \left[ \left( 1 - \frac{m_-}{m_+} \right) \Lambda_1 \delta \phi_1(r') - \left( 1 + \frac{m_-}{m_+} \right) \Lambda_2 \delta \phi_2(r') \right] \]  

where $G(x)$ is a Meijer G function [18], $G(x)$ is vanishingly small for $x > 5$. Equation (23) shows that this membrane-mediated interaction is attractive between junctions of the same type, and repulsive between junctions of different types. This interaction is short ranged with a characteristic length $m_+^{-1/4}$. Also notice that the contribution from $H_1$ is proportional to $\Delta h^2$, i.e., there is no membrane-mediated interactions in the level of Gaussian approximations when the junctions have the same height. The physical picture of this interaction can be seen from Fig. 5. A small perturbation of the junction density from the hard membrane solution induces a deviation of membrane-membrane distance from $h_M$, and there is a membrane bending energy associated with any given distribution of nonuniform membrane-membrane distance. To reduce the bending energy, a region with positive $\delta \phi_{1(2)}$ attracts a region with positive $\delta \phi_{1(2)}$ in order to reduce the elastic energy cost of a “pit” or a “bump” between these two regions due to the nonuniform $h(r)$. Similarly, a region with positive $\delta \phi_1$ repels a region with positive $\delta \phi_2$, in order to reduce the bending energy cost due to the high curvature configuration between these two regions. This also explains the fact that these interactions vanish when both types of junctions have the same height, i.e., $\Delta h = 0$. A similar kind of membrane-mediated nonlocal interaction between the junctions is discussed in the celebrated paper by Bruinsma, Goulian, and Pincus [19], where in their “van der Waals regime,” the competition between the potential minimum due to the van der Waals interaction between the membranes and another potential minimum due to the stiff membrane junctions results in a strong interaction between the junctions. Although there are only one type of junctions in the system discussed in Ref. [19], the interaction between the junctions in Ref. [19] and the present case share the same physical mechanism, i.e., the bending elasticity of the membranes mediates this interaction.

Another type of nonlocal interactions between the junctions can be studied by considering the effect of nonlinear couplings between $\delta l$ and $\delta \phi_h$. This is done by including the contributions from $H_1$ perturbatively to one-loop order. The resulting effective interaction free energy between the junctions, $F_c$, now has the form

\[ F_c = F_M + F_G + F_{\text{loop}} + H_\phi \]  

where $F_M$ is the hard membrane solution of $F_c$, $F_G$ is the contribution from terms that are bilinear in $\delta l$ and $\delta \phi_h$, and $F_{\text{loop}}$ is the contribution from the nonlinear couplings between $\delta l$ and $\delta \phi_h$ to one-loop order. The details of the calculations for $F_{\text{loop}}$ are discussed in the Appendix. When $\Gamma < \sqrt{m_+}$, the result (up to terms quadratic in $\delta \phi_h$) is provided by Eqs. (A5), (A7), and (A9),
Here the first term is a “renormalization” of the chemical potentials of the junctions due to membrane fluctuations. This term effectively reduces the binding energies of the junctions. The fact that the renormalization of the chemical potential for the softer junctions is less significant compared to that for the stiffer junctions is because the membrane fluctuations are energetically less costly for the softer junctions. The second term is a fluctuation-induced nonlocal interaction between the junctions, and higher-order terms are neglected. Notice that, as discussed in the Appendix, the second term in Eq. (25) is actually an approximate form of the much more complicated true result; it provides the correct asymptotic behavior of the true result at large and small \( q \) limits in the case when \( \Gamma < \sqrt{m_+} \). Similar to the case of Gaussian approximation, when the fluctuation-induced interaction between the junctions is expressed in real space, one finds that the interaction between the junctions is nonlocal, short ranged, and has a characteristic length on the order of \( m_+^{1/4} \). Since \( F_{\text{loop}} \) is nonvanishing even when \( \Delta_\theta = 0 \), it is clear that the thermal fluctuation of the membrane-membrane distance is the mechanism that induces the nonlocal interactions between the junctions in \( F_{\text{loop}} \). This is similar to, but not the same as, the interaction between the junctions in the “Helfrich regime” discussed in Ref. [19]. In Ref. [19], the interaction between the junctions in the Helfrich regime comes from the collisions between the membranes. Here in the one-loop calculation the interaction between the junctions comes from the fluctuations of the membrane-membrane distance around the hard membrane solution; the effect of membrane collisions is not included.

When the fluctuations around the hard membrane solution are taken into account to one-loop order, the total free energy of the system to second order in \( \delta \phi_a \) can be expressed by

\[
G = F_{\text{loop}} + \frac{1}{16 \sqrt{m_+}} \int d^2q \left[ \sum_{\alpha = \pm} \frac{1}{2 \phi_{\alpha M}} |\delta \phi_{\alpha}(q)|^2 \right] + \int d^2q \left( \frac{\Delta^2}{(2\pi)^2 q^2 + m_+} \right) \Lambda_1 \delta \phi_1(q) \]

\[
- \left( 1 + \frac{m_-}{m_+} \right) \Lambda_2 \delta \phi_2(q) \]

\[
- \frac{1}{16 \sqrt{m_+}} \int d^2q \frac{1}{(2\pi)^2 q^2 + 4m_+} |\Lambda_1 \delta \phi_1(q)| \]

\[
+ \Lambda_2 |\delta \phi_2(q)|^2 \]

\[
\times \left| \Lambda_1 \delta \phi_1(q) + \Lambda_2 \delta \phi_2(q) \right|^2 \quad (25)
\]

Here the first term on the right-hand side is the hard membrane solution, the second term comes from the entropy of the junctions, the third term is the nonlocal interaction between the junctions due to Gaussian fluctuations, the fourth and fifth terms come from the nonlinear couplings between \( \delta \ell \) and \( \delta \phi_a \). Notice that the contribution from \( H_{\phi} \) does not appear in the total free energy of the system, it cancels with the linear terms in the expansion of the entropy of the junctions. This is because \( \phi_{\alpha M} \) minimizes the hard membrane free energy, therefore in the expansion around the hard membrane solution, terms that are linear in \( \delta \phi \) cancel each other. The contribution from one-loop calculation, however, includes terms that are linear in \( \delta \phi_a \) because they come from the nonlinear couplings between \( \delta \phi_a \) and \( \delta \ell \). An important consequence of the presence of these terms is that, in general, the equilibrium values of \( \delta \phi_1 \) and \( \delta \phi_2 \) are non-zero due to the membrane fluctuations. Therefore when a phase separation occurs, the values of \( \phi_1 + \phi_2 \) are different in domains with different values of \( \phi \).

To discuss the correction of \( \phi_a \) and \( h(r) \) due to the thermally activated fluctuations, it is convenient to express Eq. (26) as

\[
G = F_{\text{loop}} + \sum_{\alpha = \pm} \int d^2q \frac{\Delta^2}{(2\pi)^2 q^2 + m_+} M_{\alpha M}(q) \delta \phi_a(q) \delta \phi_a(q), \quad (27)
\]

where

\[
\delta \mu_a = \frac{\Lambda_a}{16 \sqrt{m_+}},
\]

\[
M_{11}(q) = \frac{\Delta^2_h}{2 \phi_{1 M}} \left( 1 - \left( \frac{m_-}{m_+} \right)^2 \right) \Lambda_1^2
\]

\[
- \frac{1}{16 \sqrt{m_+}} q^2 + 4m_+.
\]

\[
M_{22}(q) = \frac{\Delta^2_h}{2 \phi_{2 M}} \left( 1 + \left( \frac{m_-}{m_+} \right)^2 \right) \Lambda_2^2
\]

\[
- \frac{1}{16 \sqrt{m_+}} q^2 + 4m_+.
\]

\[
M_{12}(q) = M_{21}(q) = \frac{\Delta^2_h}{q^2 + 4m_+} \left[ 1 - \left( \frac{m_-}{m_+} \right)^2 \right] \Lambda_1 \Lambda_2
\]

\[
- \frac{1}{16 \sqrt{m_+}} q^2 + 4m_+.
\]

(26) Now \( \delta \phi_a(q) \) can be expressed by \( \delta \mu_a \) and \( M_{\alpha \beta} \).
\[ \delta \phi_1(q) + \delta \phi_2(q) = \delta(q) \times \frac{-1}{2 \det \mathbf{M}} \left\{ (M_{22} - M_{21}) \delta \mu_1 ight. \\
+ (M_{11} - M_{12}) \delta \mu_2 \}, \]
\[ \delta \phi_1(q) - \delta \phi_2(q) = \delta(q) \times \frac{-1}{2 \det \mathbf{M}} \left\{ (M_{22} + M_{21}) \delta \mu_1 ight. \\
- (M_{11} + M_{12}) \delta \mu_2 \}, \]

where \( \det \mathbf{M} = M_{11}M_{22} - M_{12}M_{21} \). This rather complicated expression shows that, besides \( \mu, \lambda \), and \( \Delta^2 \Lambda \), the 
answer to the question of which domain has higher total junction density when the phase coexistence occurs also depends 
on the values of \( \Delta \Lambda \) and \( \phi_{aM} \) (to determine \( \phi_{aM} \), one needs to know the value of \( \mu \)) in this paper I shall not discuss the 
details of the values of \( \phi_1 + \phi_2 \) for different given parameters in the theory, but simply comment that when \( \det \mathbf{M} \) is 
positive, the phase diagram of the hard membrane solution is not modified by the thermal fluctuations. However, when 
\( \det \mathbf{M} < 0 \), the hard membrane solution is not stable at any finite temperature. The result in Eq. (29) also provides some 
criteria for the current analysis. For example, when the fluctuations are large, the deviation from hard membrane solution 
can no longer be treated by perturbation theory. This is true when \( \delta \phi_{aM} \sim O(1) \). Since \( \delta \phi_{aM} \) becomes large for 
small \( \det \mathbf{M} \), which occurs at small \( m_+ = \Lambda_1 \phi_{1M} + \Lambda_2 \phi_{2M} \), I conclude that the perturbation theory breaks down at small 
 junction densities. Finally, I point out that the collisions between the membranes are also neglected in the present 
analysis. This approximation is valid when the fluctuation of membrane-membrane distance is not large, i.e., when 
\[ \frac{\sqrt{\langle (\delta l)^2 \rangle \mu_0}}{h_M} = \left( \int \frac{d^2q}{(2\pi) \gamma^2} \frac{1}{q^4 + m_+^2} \right)^{1/2} \frac{1}{h_M} \]
\[ \approx \frac{1}{4m_+^{1/4}h_M} \equiv O(1). \]

In the regime where \( m_+^{1/4}h_M = (\Lambda_1 \phi_{1M} + \Lambda_2 \phi_{2M})^{1/4}h_M \) \( \approx O(1) \), i.e., when the junction densities are small, or the 
when junctions are very soft, or when the junctions are very “short,” the contributions from membrane collisions should 
be taken into account for a complete analysis of this system. Thus, when the membrane fluctuations or the membrane 
collisions become important, numerical simulations [20] or other methods that take the full membrane fluctuations into 
account should be applied to study the physics of this system.

V. SUMMARY

I have discussed the phase separation of multiple species membrane junctions induced by membrane-membrane adhesion 
with a continuum theory. In the hard membrane approximation, where the membrane-membrane distance and junction 
densities are assumed to be constants, I find that \( \Delta^2 \Lambda \) and \( \lambda \) are the important parameters that govern the phase 
separation. When \( \lambda = 0 \), both types of junctions have the same flexibility, and the phase separation is driven by the 
height difference of the junctions. Under this condition the phase separation is very similar to the Flory-Huggins theory 
for a binary mixture. Phase separation occurs when \( \Delta^2 \Lambda > 2 \) and \( \mu = 0 \), the phase coexistence curve ends at a critical 
point \( \mu = 0, \Delta^2 \Lambda = 2 \). When \( \lambda \neq 0 \), the junctions have different flexibilities, and the softer junctions are easier 
to form than the stiffer junctions. Therefore the softer junctions have a tendency to aggregate. In this more general case, 
the height difference and the junction flexibility difference both drive the phase separation, thus the phase separation 
can occur at \( \Delta^2 \Lambda < 2 \).

The Gaussian fluctuations around the hard membrane solution reveals a membrane-mediated nonlocal interaction 
between the junctions. This interaction is short ranged, which decays with a characteristic length \( (\Lambda_1 \phi_{1M} + \Lambda_2 \phi_{2M})^{-1/4} \). It is attractive between the same type of junctions, but repulsive between different types of junctions. 
The strength of this interaction is proportional to \( \Delta^2 \Lambda \), and it is due to the membrane bending energy cost between regions 
with different junction densities. Perturbation theory to one-loop order shows other effects of thermal fluctuations, this 
includes a renormalization of the chemical potential of the junctions, which effectively reduces the binding energies of 
the junctions, and a nonlocal interaction between the junctions which is independent of \( \Delta^2 \Lambda \). The fact that the contribution 
from one-loop calculation is nonvanishing even when junctions of type-1 and type-2 have the same height indicates that 
this contribution is a result of thermal fluctuations of the membranes. Hence it is nonvanishing at all finite temperatures. 
The current analysis also shows that when perturbation theory holds the thermal fluctuations do not modify the hard 
membrane phase diagram, they only modify the equilibrium junction densities in each phase. However, when the contribution 
from one-loop calculation becomes very large, the hard membrane solution is qualitatively incorrect, and the effect of thermal fluctuations is a dominant factor. This can occur at very low junction densities. The Gaussian fluctuations 
of the membrane-membrane distance also provide another limit of the present analysis: the mean squared fluctuations of the membrane-membrane distance should be small compared to \( h_M \). As a result, the analysis in this paper does not provide the complete physical picture of the system for very soft or very short junctions, either.

In summary, mean field and fluctuation analysis of a simple coarse grained model for adhesion-induced phase 
separation of multiple species of membrane junctions is studied in this paper. This model shows rich behaviors that 
capture much of the physics of multispecies membrane junction separation induced by adhesion. I show that not only the difference of junction height, but also the difference of junction flexibilities, and the membrane-mediated interactions between the junctions play important roles in the phase separation. The fluctuation analysis also shows that current analysis does not provide the complete physical picture for systems with very soft or very short junctions, or in the situation when the junction densities are extremely low, where the thermally activated membrane fluctuations or the Helfrich repulsion between the membranes become important interac-
tions in the system [19]. In this regime, numerical simulations [7,20] should provide valuable information on the distribution of the junctions, as well as a complete picture of the phase diagram, which includes the binding-unbinding transition between the membranes, and adhesion-induced phase separations.

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APPENDIX

In this appendix, I discuss the details of some calculations mentioned in the text. For simplicity I define

\[ \delta m_+(r) = \Lambda_1 \delta \phi_1(r) + \Lambda_2 \delta \phi_2(r), \]

\[ \delta m_-(r) = \Lambda_1 \delta \phi_1(r) - \Lambda_2 \delta \phi_2(r). \]

First, an integral that is very useful for the rest of this appendix is calculated:

\[
\int \frac{d^2q}{(2\pi)^2} \frac{A(q)B(-q)}{q^4 + m_+} \equiv \int d^2r \int d^2r' \int \frac{d^2q}{(2\pi)^2} \frac{A(r)B(r') e^{iq \cdot (r-r')}}{q^4 + m_+} \\
= \int d^2r \int d^2r' \left( \frac{1}{\sqrt{m_+}} \right) \\
\times \int_{0}^{\infty} dx \frac{x J_0(x |r-r'| m_+^{1/4})}{2\pi x^4 + 1} A(r)B(r') \\
= \frac{1}{8\pi \sqrt{m_+}} \int d^2r \int d^2r' G(|r-r'| m_+^{1/4}) A(r)B(r'),
\]

where \( G(x) = \text{Meijer } G \) function [18]. Also,

\[ G(x) \approx \begin{cases} \pi, & x \ll 1 \\ 0, & x \gg 5. \end{cases} \]

The shape of \( G(x) \) is plotted in Fig. 6.

Now I consider the nonlocal interaction between the junctions in the Gaussian approximation. Neglecting the first term of Eq. (22), the second term can be expressed by

\[
\begin{align*}
F_G &= -\sum_q \frac{|\lambda_q \delta m_+(q) + \Delta \delta m_-(q)|^2}{q^4 + \Gamma q^2 + m_+} \\
&= -\int \frac{d^2q}{(2\pi)^2} \frac{|\lambda_q \delta m_+(q) + \Delta \delta m_-(q)|^2}{q^4 + \Gamma q^2 + m_+} \\
&= -\int d^2r \int d^2r' \frac{\Delta^2}{8\pi \sqrt{m_+}} G(|r-r'| m_+^{1/4}) \\
&\times \left[ \left( 1 - \frac{m_-}{m_+} \right) \Lambda_1 \delta \phi_1(r) - \left( 1 + \frac{m_-}{m_+} \right) \Lambda_2 \delta \phi_2(r) \right] \\
&\times \left[ \left( 1 - \frac{m_-}{m_+} \right) \Lambda_1 \delta \phi_1(r') - \left( 1 + \frac{m_-}{m_+} \right) \Lambda_2 \delta \phi_2(r') \right],
\end{align*}
\]

where the last expression holds when \( \Gamma < \sqrt{m_+} \), and the integral in Eq. (A2) is used to calculate the Fourier transformation from the momentum space to the real space. The range of this membrane-mediated interaction between the junctions is determined by the shape of \( G(x) \), which sets the length scale of this interaction to \( m_+^{-1/4} \).

Next I show that the interaction between the junctions due to the contribution of \( H_1 \) is of the form in Eq. (25). The effective interaction free energy between the junctions is

\[
F_e = -\ln \left[ \int D[h] e^{-H_M - H_0 - H_1 - \phi} \right] \\
= H_M + H_\phi - \ln \left[ \int D[\delta l] e^{-H_0 - H_1} \right],
\]

where the contribution of \( H_1 \) is included by a one-loop calculation,

\[
F_e = H_M + H_\phi + (H_1)_0 - \frac{1}{2} \left( (H_1)_0^2 - (H_1)_0 \right) \\
= F_M + F_G + F_{loop} + H_\phi.
\]
\[ F_M = H_M, \quad F_G = -\ln\left[ \int D[\delta l]e^{-H_0}\right], \]

and

\[ \langle O \rangle_0 = \frac{\int D[\delta l]Oe^{-H_0}}{\int D[\delta l]e^{-H_0}} \tag{A6} \]

for any \( O \). The calculation of \( \langle H_1 \rangle_0 \) is straightforward, which in the case \( \Gamma < \sqrt{m_+} \) leads to

\[ \langle H_1^2 \rangle_0 - \langle H_1 \rangle^2_0 \approx \frac{1}{8\sqrt{m_+}} \int d^2q \frac{|\delta m_+(q)|^2}{q^4 + 4m_+} = \frac{1}{64\pi \sqrt{m_+}} \int d^2r \int d^2r' G(|r - r'|)(4m_+)^{1/4} \left[ \Lambda_1 \delta \phi_1(r) + \Lambda_2 \delta \phi_2(r) \right] \]

Putting \( \langle H_1 \rangle_0 \) and \( \langle H_1^2 \rangle_0 - \langle H_1 \rangle^2_0 \) together leads to the resulting expression of \( F_c \) in the one-loop order, which is given in Eq. (24) and Eq. (25). The real space form of \( \langle H_1^2 \rangle_0 - \langle H_1 \rangle^2_0 \) also shows that the membrane fluctuation induced interaction between the junctions is short ranged with a characteristic length on the order of \( m_+^{-1/4} \).