Inclusions induced phase separation in mixed lipid film

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Abstract

The effect of rigid inclusions on the phase behavior of a film containing a mixture of lipid molecules is investigated. In the proposed model, the inclusion-induced deformation of the film, and the resulting energy cost are strongly dependent upon the spontaneous curvature of the mixed film. The spontaneous curvature is in turn strongly influenced by the composition of film. This coupling between the film composition and the energy per inclusion leads to a lateral modulation of the composition, which follows the local curvature of the membrane. In particular, it is shown that the inclusion may induce a global phase separation in a film which would otherwise be homogeneously mixed. The mixed film is then composed of patches of different average composition, separated by the inclusions. This process may be of relevance to explain some aspects of lipid-protein association in biological membranes.

Keywords Theoretical Biophysics, Statistical Mechanics, Transmembrane Proteins, Phase Separation.

I. INTRODUCTION

Our understanding of lamellar systems for which the basic element is a lipid bilayer has greatly progressed in recent years (Nelson et al., 1989; Safran, 1994; Sackmann and Lipowsky, 1995). Attempts to apply this knowledge to biological systems have lead to many exciting and fundamental questions, such as the morphology of vesicles, diffusion and transfer across membranes, or fusion and adhesion of membranes (Sackmann and Lipowsky, 1995). Among the systems of interest in biology are membranes containing proteins or other embedded molecules; the presence of such inclusions within the assembly leads to interesting new phenomena. Such complexes are of great importance in many areas of cell biology, since membrane proteins perform such fundamental functions as the formation of pores in the membrane, the transfer of signals through the membrane, or the binding of

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other proteins to the membrane (Alberts et al., 1994). These proteins can be polymer-like coils tethered to the cell membrane (peripheral proteins), or more rigid molecules embedded in the membrane (integral proteins). In the latter case, they are often mostly hydrophobic, and shield themselves from water contact when buried within the hydrophobic part of the lipid bilayer (see Fig. 1). In both cases, membrane proteins are known to locally disrupt the order in the fluid membrane. Following the pioneering work of Huang (Huang, 1986), a large body of work has been done on the physics of lipid film with inclusions (for a recent review, see Goulian, 1996). Several possible effects have been considered theoretically in order to describe the behavior of the membrane protein complexes, such as a change of the membrane rigidity and a tilt of the membrane at the location of the inclusion (Goulian, 1993), or a local expansion of the bilayer to accommodate the inclusion size (Mouritsen and Bloom, 1984-1993; Dan, 1993-1994). The latter seems of particular relevance for hydrophobic integral inclusions, and predicts that the deformation of the film around an inclusion extends over a length related to the molecular details of the membrane, and leads to non-monotonic interactions between neighboring inclusions. In addition, several works have highlighted the influence of inclusions (e.g. peptides) on the phase behaviour of lipid solutions (Morein et al., 1997, May and Ben-Shaul, 1998).

Biological membranes are often formed by a wide variety of lipid and surfactant molecules (Alberts et al., 1994). These mixed membranes have excited the imagination of many theorists over the past years, because a coupling between the composition and the shape of self assembled membranes promises many interesting phase transition phenomena (Leibler, 1986; Leibler and Andelman, 1987; Seul and Andelman, 1995; Safran et al., 1991, MacKintosh and Safran, 1993; Harden and MacKintosh, 1994; Tanigushi, 1996; Gelbart and Bruinsma, 1997). Most of those works exploit the coupling between film composition and curvature related characteristics of membranes to connect a lateral phase separation and a shape deformation of the membrane.

Despite extensive research, the role played by these different amphiphile species in real (biological) systems is yet unclear. Some evidence show that the composition of amphiphile of a given portion of a cell membrane is related to the membrane proteins present in the area (Alberts et al., 1994), a particular lipid environment being needed in order for certain proteins to function properly. Explanations invoking the lateral pressure exerted on a membrane protein by the lipid bilayer, as a regulatory phenomenon for its morphology has been proposed to explain this specificity (de Kruijff, 1997; Cantor, 1997a; Cantor, 1997b; Dan, 1998). On the other hand, the existence of lipid domains in biomembranes is of fundamental importance to understand specific membrane structures and functions. Domains of different compositions, which are in the fluid state under physiological condition, are expected to be formed by the interplay of interactions between lipids and membrane proteins, and the mixing properties of the lipids themselves (Melchior, 1986). These considerations have led to extensive experimental and theoretical research on the behavior of mixed film with proteins. Some work follow the concept of “hydrophobic matching”, and bear some similarities with the present work (Mouritsen and Bloom, 1993; Dumas et al., 1997 and reference therein). Several experimental studies have been performed to determined the influence of given proteins on the stability of mixed membranes (Mittler-Neher and Knoll, 1989), but they tend to focus mostly on electrostatic effects. Artificial mixed membrane are also useful for the study of specific peripheral and integral membrane proteins close to physiological conditions.
In this work, we investigate theoretically the consequences of the presence of inclusions on the mixing properties of a mixed lipid film, assuming that an inclusion imposes a given deformation to the membrane. The aim of this paper is to extend previous results for the deformation and interaction of fluid membranes with inclusions to bilayers containing two different species of lipid. Special emphasis is given to the occurrence of phase separation in a mixed membrane which would be homogeneously mixed in the absence of inclusion. The main results include the prediction that the local composition of the film around an inclusion is controlled by the deformation imposed on the film by the inclusion, and can vary widely. In our model, the control parameter is the difference between the thickness of the film and the height of the inclusion, but more complex couplings (slope, asymmetry..) could also be relevant, and could be treated following the same pathway.

As in the often used “Mattress model” (Mouritsen and Bloom, 1984-1993), the lipid molecules are modeled as spring with a given elasticity. In addition, an key ingredient of the present model is the concept of curvature energy developed by Helfrich (Helfrich, 1973), and of particular importance is the idea of spontaneous curvature of a lipid monolayer (Safran, 1994). It has been clearly demonstrated that the composition of a mixed monolayer of amphiphilic molecules influences strongly its mean spontaneous curvature (Milner et al., 1989). We use this coupling between composition and deformation of the membrane to derive the effect of a protein, not only on the local composition near the inclusion, but also on the average composition of a given patch of membrane. The two competing characteristics of the film, namely the stretching and curvature of the lipid molecules, define a characteristic length over which a membrane deformation vanishes. It defines also the range of the lipid mediated interactions between inclusions, which is of order 100Å in typical biological membranes. It should be noted that in our model, the membrane is always supposed to be in the fluid state. For this reason, this characteristic length is a constant and one does not observe the temperature dependence which has been predicted in relation to the gel-to-fluid transition in the membrane (Sperotto and Mouritsen, 1991). One advantage of our model over the mattress model is that our findings are expressed with physical quantities (bending rigidity, spontaneous curvature) that could be measure via independent experiments. Moreover, any more “exotic” deformations imposed by the inclusion (such as a tilt of the bilayer interface), or direct, specific interaction between the inclusion and one or the two types of lipid, can be introduced by a straightforward change of the boundary conditions. Finally, this model predicts a deformation of the membrane even if the inclusion hydrophobic core matches exactly the thickness of the film, providing that the lipid monolayer has a non zero spontaneous curvature.

The energy per inclusion being strongly influenced by the value of the average spontaneous curvature of the film (it even change sign upon changing the value of this parameter), it is argued that a “global” phase separation in the film may be induced by the presence of inclusions for a certain range of parameters. Our results show that some incompatibility between the two types of lipid is still needed to observe a phase separation in the membrane, but that the presence of inclusions may increase the critical temperature below which the membrane ceases to be an homogeneous 2-D fluid. The film is then divided into patches of very different average compositions, hence different mean spontaneous curvature, which create a nonrandom lipid distribution in the bilayer.
Most molecular models describe the equilibrium conformation and energy of a lipid bilayer as the interplay of three terms. Two surfaces terms, account for the interactions between the hydrophilic heads of the molecules at the water interface, and the surface tension of the film; the third, is a spring-like term for the stretching of the hydrophobic lipid chains due to packing constraints (Ben-Shaul and Gelbart, 1995). The minimization of this energy defines an optimum area per lipid head in the film, from which the equilibrium bending and stretching characteristics of the film can be derived. In the following, we will consider a two-dimensional symmetrical bilayer, and we focus only on the deformation of one monolayer, assuming that the inclusion induce deformations that are symmetric in each monolayer. The deformation energy per unit length can be written in the general form (Helfrich, 1973) (see Fig. 1 for notations):

\[
f = f_0 + \frac{1}{2}k(u - u_\infty)^2 + \frac{1}{2}\kappa u''^2 - \kappa c_0 u''
\]

where \( u \) is the deviation of the membrane surface from the flat state, \( k \) and \( \kappa \) are the stretching and bending modulii of one monolayer per unit length, and \( c_0 \) is the spontaneous curvature of the monolayer. Note that according to this formula, a positive spontaneous curvature tends to give a concave bend towards the outside of the monolayer. The reference energy, \( f_0 \), is the energy of a flat film. To within a constant, this energy can be written (Safran, 1994) \( f_0 = (1/2)\kappa c_0^2 \). It reflects the fact that the monolayer is forced to be flat for symmetry reasons, while it would rather adopt the curvature \( c_0 \). For a mixed lipid film, the three parameters \( k, \kappa \) and \( c_0 \) are functions of the local composition of the film. Rigorously speaking, these parameters also depend on the local area per lipid head, which is, in turn, coupled to the deformation. However, it will be shown in Section II that one need not consider such a coupling to reach a correct physical picture of the effect of the membrane inclusion.

In the next section, we will give a simplified presentation of previous results for a one-component lipid film. We then study mixed lipid films containing by two types of molecules of different spontaneous curvature. In section III, we derive the expression for the deformation of the film interfaces, the local composition of the film, and the interaction energy between two fixed inclusions. We study both the case of random mixing and the case where some specific interactions exist between the two types of lipid. In section IV, we discuss the possibility of a phase separation in mixed film. We show that the presence of inclusion always favors the demixing of the two components. Nevertheless, this effect alone is never strong enough to overcome the strong tendency for mixing which is a property of the reference energy of a flat film without inclusions. However, if there already exists a weak repulsive interaction between the two lipid species, the inclusion induced tendency to phase separation may result in such a separation in a film which would be homogeneous in the absence of inclusions.

## II. INCLUSION IN A PURE LIPID BILAYER

In this section, we review in a simplified way the results in Ref. (Dan, 1993) for a one component fluid bilayer with inclusions. The deformation of one monolayer interface, and
the resulting interaction energy between two immobile inclusions is calculated. For our purposes, we neglect the coupling between the deformation and the area per lipid molecule (Dan, 1993).

We assume that the only physical effect of the inclusion is to impose a given height of the interface at contact, reflecting the fact that the inclusion is hydrophobic (see Fig. 1). The energy per unit length $E_{\text{eq}}(1)$ is normalized using the displacement $\Delta = (u - u_\infty)/(u_0 - u_\infty)$, where $u_0$ is the height of the film at the inclusion and $u_\infty$ is the equilibrium thickness of the bilayer. The total energy of a film between two inclusions separated by $2L$ is:

$$\Delta F \equiv F - F_0 = \frac{k_B T L}{a} \Gamma \int_0^1 dx \left( \frac{\Delta'^2}{\tilde{\omega}^4} + \Delta^2 - 2C_0 \frac{\Delta''}{\tilde{\omega}^2} \right)$$

(2)

where $a$ is a molecular size, and $\Gamma = \frac{1}{2} k(u_0 - u_\infty)^2 a/(k_B T)$ measures the ratio between the typical stretching energy of the lipid tails and the thermal energy ($k_B T$). The normalized coordinate is $x = z/L$, and $\tilde{\omega} = \omega L$ with $\omega = (k/\kappa)^{1/4}$ being the inverse of the characteristic length of the film, $C_0 = c_0/((u_0 - u_\infty)\omega^2)$ is the normalized spontaneous curvature. The energy of the flat film becomes in these units: $F_0 = TL/a \Gamma C_0^2$ (note that in these units, $\tilde{\omega}$ varies linearly with the separation between the two inclusions). The functional minimization of this energy gives a differential equation for the equilibrium profile of the film interface:

$$\Delta^{(iv)} + \tilde{\omega}^4 \Delta = 0 \quad (3)$$

Two of the boundary conditions are physically fixed by the inclusions: $\Delta|_{x=0} = 1$ (to match the height of the inclusion) and $\Delta'|_{x=1} = 0$ (by symmetry; $x = 1$ is at the midpoint between the of two inclusions). Two other boundary conditions are set by minimization requirements, and read (Fox, 1950): $(\partial f/\partial \Delta')|_{x=0} = 0$ and $\partial_x(\partial f/\partial \Delta'')|_{x=1} = 0$, where $f(\Delta, \Delta'')$ is the energy density. Finally, the four boundary conditions are:

for $x = 0$ \begin{align*}
\Delta &= 1 \\
\Delta'' &= \tilde{\omega}^2 C_0
\end{align*}

for $x = 1$ \begin{align*}
\Delta' &= 0 \\
\Delta''' &= 0
\end{align*}

(4)

After proper integration by part and the use of the equations (4), the energy per inclusion Eq.(2) can be written as a function of derivatives of $\Delta$ at $x = 0$ only:

$$\Delta F = \frac{k_B T L}{a} \Gamma \left( \frac{C_0}{\tilde{\omega}^2} \Delta'(0) + \frac{1}{\tilde{\omega}^4} \Delta''(0) \right)$$

(5)

The deformation profile is easily derived:

$$\Delta(x) = \frac{(1 - iC_0)}{2} \frac{\cosh \left( \sqrt{i\tilde{\omega}} (1 - x) \right)}{\cosh \left( \sqrt{i\tilde{\omega}} \right)} + \frac{(1 + iC_0)}{2} \frac{\cosh \left( i\sqrt{i\tilde{\omega}} (1 - x) \right)}{\cosh \left( i\sqrt{i\tilde{\omega}} \right)}$$

(6)

and the interaction energy between two inclusions separated by a distance $2L$ is:

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1The case $u_0 = u_\infty$ is also of interest, since the inclusion allows for a discontinuity of the curvature of the membrane interfaces. This case can be studied straightforwardly from our results
\[ \Delta F = -\frac{k_B T L}{a} \Gamma (C_0^2 - 2C_0 - 1) \sin \sqrt{2\omega} + (C_0^2 + 2C_0 - 1) \sinh \sqrt{2\omega} \]

(7)

An estimate of the energy of interaction gives the value \( \Delta F \sim 5k_B T(\Delta u/h)^2 \) in the case of an hydrophobic mismatch \( \Delta u \) between the inclusion and a bilayer of thickness \( 2h \) (no spontaneous curvature), and the value \( \Delta F \sim 5k_B T(c_0 h)^2 \) for monolayers of spontaneous curvature \( c_0 \) and no mismatch. Both energies should be higher than \( k_B T \) and hence, represent relevant contributions to aggregation/dispersion of inclusion in membrane, in addition to any direct physical forces\(^2\).

The profile of the film interface and this energy are shown in Figs. 2&3 for typical values of the parameters. The perturbation decreases over a length \( \tilde{\omega}^{-1} \), which is also the range of the interaction between inclusions. It is a measure of the relative strength of the stretching and the bending modulii. If the stretching dominates, \( \tilde{\omega} \) is large and the profile decays rapidly to its asymptotic values, and conversely. The spontaneous curvature fixes the curvature at \( x = 0 \) (the inclusion is assumed to constraint only the height of the film at that point), and has a strong influence on the sign of the interaction energy, which can change upon changing \( C_0 \) (see Fig. 3). These results are very similar to the conclusions of Dan \textit{et al.} (Dan, 1993), which tends to suggest that a complete physical picture of the system can be obtained without considering the coupling between the film profile and the area per lipid molecule.

As will be shown in the next section, the phase behavior of a mixed lipid film is closely related to the variation of the energy of an homogeneous film with the spontaneous curvature \( C_0 \). From the expression of the energy Eq.(7), one can easily show that the energy of the film is non-monotonous, and reaches a maximum value at a given value of \( \tilde{\omega} \) for a spontaneous curvature:

\[ \bar{C}_0 = \frac{\bar{c}_0}{(\bar{u}_0 - \bar{u}_\infty)\omega^2} = -\frac{\sinh \sqrt{2\omega} \sin \sqrt{2\tilde{\omega}}}{\sinh \sqrt{2\omega} + \sin \sqrt{2\tilde{\omega}}} \]

(8)

### III. INCLUSION IN A MIXED LIPID BILAYER

We consider a mixed film of average composition \( \bar{\psi} \) of molecules \( A \) and \( 1-\bar{\psi} \) of molecules \( B \). For simplicity, we consider the case where these two types of molecule differ only in their spontaneous curvature \( c_A \) and \( c_B \) (their stretching and bending modulii being identical) and, as earlier, we need not consider any change in the area per lipid head\(^3\). We consider

\(^2\)For comparison, the magnitude of the Van der Waals attraction between two cylinders of radius \( R \) and separation \( L \) in a medium of Hamaker constant \( A \sim 20k_B T \) is \( F \sim A/6hR^{1/2}/L^{3/2} \sim k_B T \) (Israelachvili, 1992)

\(^3\)Practically, parameters such as the stretching and bending modulii are mostly influences by the hydrophobic tails of the lipids, while the spontaneous curvature results of the asymmetry between heads and tails (Israelachvili, 1992)
the free energy of the lipid film as a function of two order parameters: the displacement of the film interface $\Delta(x)$, and the variation of the film composition $\phi \equiv \psi(x) - \bar{\psi}$. The free energy can be written as a general Landau-Ginzburg expansion up to second order in these order parameters\(^4\), but all the terms can be motivated from microscopic considerations as described in the Appendix:

$$\Delta F = \frac{k_B T L}{a} \Gamma \int_0^1 dx \left\{ \frac{\Delta''^2}{\tilde{\omega}^4} + \Delta^2 - 2C_0 \frac{\Delta''}{\tilde{\omega}^2} - 2 \frac{C_1}{\tilde{\omega}^2} (\Delta'' + \mu) \phi + D\phi^2 + E\phi'^2 \right\}$$  \hspace{1cm} (9)

where the constants $D$ and $E$ are positive if there is no spontaneous phase separation in the film in the absence of foreign inclusions.

Such a coupling between the membrane deformation and local parameters of the film has already been formulated in previous works, in particular to describe curvature instability related to the presence of inhomogeneities in the membrane (Leibler, 1986). The physical significance of the two first parameters $\tilde{\omega}$ and $C_0$ has been given in the previous section. The three new parameters $C_1$, $D$, and $E$ can be explained physically with the aid of a molecular model for the bilayer (see Appendix). The parameter $C_1$ gives the coupling between the spontaneous curvature of the film and the local composition, and corresponds roughly to the difference of spontaneous curvature between the two kinds of molecule. The parameter $D$ includes the mixing entropy of the film, specific interactions between lipids, and spontaneous curvature terms, and the gradient term $E$ arises from interactions between the different lipids; if the free energy of mixing includes only entropic contributions, $E = 0$. The chemical potential $\mu$ is determined by the conservation relation: $\int_0^1 \phi dx = 0$.

In what follows, we first study the behavior of the film if the term in gradient of the composition vanishes in Eq.(9). Although not realistic, this treatment gives us some insight into the more complete case ($E \neq 0$), treated in the next subsection.

**A. Mixed bilayer without interaction between different species**

For simplicity, we first consider the case where there are no specific interactions between the two species and the gradient term in Eq.(9) vanishes ($E = 0$). The problem is then greatly simplified since the composition of the film can be readily expressed as a function of the deformation $\Delta(x)$ by minimizing Eq.(9). Invoking the conservation relation to determine the chemical potential, one obtains:

$$\mu[\Delta] = \Delta'(0) \quad \phi[\Delta] = \frac{C_1}{\tilde{\omega}^2 D} (\Delta''(x) + \Delta'(0))$$ \hspace{1cm} (10)

where the symmetry requirement $\Delta'(1) = 0$ has been used. The equilibrium free energy can then be expressed as a function of the interface profile only:

$$\Delta F = \frac{T L}{a} \Gamma \int_0^1 dx \left\{ \frac{\Delta''^2}{\tilde{\omega}^4} + \Delta^2 - 2C_0 \frac{\Delta''}{\tilde{\omega}^2} - \frac{\epsilon}{\tilde{\omega}^4} (\Delta'' + \Delta'(0))^2 \right\}$$ \hspace{1cm} (11)

\(^4\)We have omitted quadratic terms without clear physical meanings such as $\Delta''\phi'$.
where the difference between the two lipid species appears only in the perturbation parameter

\[ \epsilon = \frac{C_1^2}{D} \]  

(12)

The Euler-Lagrange equation and the boundary conditions for the profile are obtained by a functional minimization of (11):

\[ \Delta^{(iv)} + \frac{\tilde{\omega}^4}{(1 - \epsilon)} \Delta = 0 \]  

(13)

with the four boundary conditions obtained similarly to Eq. (4):

for \( x = 0 \) \[
\begin{cases}
\Delta = 1 \\
(1 - \epsilon) \Delta'' = \tilde{\omega}^2 C_0 + \epsilon \Delta'
\end{cases}
\]

for \( x = 1 \) \[
\begin{cases}
\Delta' = 0 \\
\Delta''' = 0
\end{cases}
\]

(14)

One can see from this set of equation that one of the main effects of the modulation of composition is to reduce the bending modulus of a monolayer, as was already observed in (Leibler, 1986). Hence, the modulation leads to a change of the characteristic length scale: \( \tilde{\omega}^* = \tilde{\omega}/(1 - \epsilon)^{1/4} \) and of the normalized spontaneous curvature: \( C_0^* = C_0/\sqrt{1 - \epsilon} \). One also notices a term involving \( \Delta'(0) \) in the boundary conditions. As the perturbation parameter \( \epsilon \) approaches 1, the effective bending rigidity of the film becomes small and the quadratic form of the energy in \( \Delta'' \) (which assumes a small curvature of the membrane everywhere) ceases to be valid. This phenomenon has been referred to as “Curvature Instability” in asymmetric lipid bilayers (Leibler, 1986). It has also been identified as the onset of the spontaneous formation of vesicles in mixed lipid bilayers (Safran et al., 1991; MacKintosh and Safran, 1993).

Eq. (13) coupled with the boundary conditions Eq. (14) at \( x = 1 \) imposes solutions of the form

\[ \Delta(x) = \sum_{i=1}^{2} A_i \cosh q_i (1 - x) \]

with \( q_1 = \sqrt{i} \frac{\tilde{\omega}}{(1 - \epsilon)^{1/4}} \)  \( q_2 = i \sqrt{i} \frac{\tilde{\omega}}{(1 - \epsilon)^{1/4}} \)

(15)

and corresponds to an interaction energy:

\[ \Delta F = \frac{k_B T L}{a} \Gamma \left( \frac{C_0}{\tilde{\omega}^2} \Delta'(0) + \frac{(1 - \epsilon)}{\tilde{\omega}^4} \Delta'''(0) \right) \]

(16)

quite close to the one derived for a pure lipid film (Eq. (5)).

The full expression of the energy with the proper value of \( \Delta \) and its derivatives is not given here. As expected, the energy per inclusions is always lower when modulations of the composition of the film are allowed.

B. Including interaction between different species

If the interaction between the different types of lipids are taken into account, the problem is complicated by the fact that the concentration \( \phi \) and the deformation \( \Delta \) are determined
by two coupled differential equations. The functional minimization of the energy (9) with respect to the two order parameters gives

\[ \Delta^{(iv)} + \tilde{\omega}^4 \Delta - \tilde{\omega}^2 C_1 \phi'' = 0 \quad E \phi'' - D \phi + \frac{C_1}{\tilde{\omega}^2} (\Delta'' + \mu) = 0 \]  \hspace{1cm} (17)

The boundary conditions are obtained similarly to Eq.(4), augmented by the conditions:

\( \left. \frac{\partial f}{\partial \phi'} \right|_{x=0,1} = 0 \) (Fox, 1950):

\[ \begin{array}{ll}
\Delta = 1 & \Delta'' = \tilde{\omega}^2 C_0 + \tilde{\omega}^2 C_1 \phi \\
\phi' = 0 & \end{array} \quad \text{for } x = 0 \]

\[ \begin{array}{ll}
\Delta' = 0 & \Delta'' = 0 \\
\phi' = 0 & \end{array} \quad \text{for } x = 1 \]  \hspace{1cm} (18)

The value of the chemical potential can be obtained by the integration of the second equation of (17), between \( x = 0 \) and \( x = 1 \):

\[ \mu = \Delta'(0). \]

With the help of the Euler-Lagrange equations and the boundary conditions, we can express the energy in a more compact form depending only on the profile at the inclusion. Remarkably, it has the same expression as for a one component film (Eq.(5)):

\[ \Delta F = \frac{k_B T L}{a} \Gamma \left( \frac{C_0}{\tilde{\omega}^2} \Delta'(0) + \frac{1}{\tilde{\omega}^4} \Delta''(0) \right) \]  \hspace{1cm} (19)

After the change of variable

\[ \phi(x) \to \Phi(x) \equiv \tilde{\omega}^2 C_1 \phi(x) - \epsilon \Delta'(0) \]  \hspace{1cm} (20)

Eq.(17) become

\[ \Delta^{(iv)} + \tilde{\omega}^4 \Delta - \Phi'' = 0 \quad \lambda^2 \Phi'' - \Phi + \epsilon \Delta'' = 0 \]  \hspace{1cm} (21)

where \( \lambda \equiv \sqrt{E/D} \) is a lengthscale related to the variation of the composition \( \phi \) (and \( \epsilon = \frac{C_1^2}{D} \)). The boundary conditions become

\[ \begin{array}{ll}
\Delta = 1 & \Delta'' = \tilde{\omega}^2 C_0 + \Phi + \epsilon \Delta' \\
\phi' = 0 & \end{array} \quad \text{for } x = 0 \]

\[ \begin{array}{ll}
\Delta' = 0 & \Delta'' = 0 \\
\phi' = 0 & \end{array} \quad \text{for } x = 1 \]  \hspace{1cm} (22)

The Eq.(21), coupled with the three boundary conditions, Eq.(22), for \( x = 1 \), imposes solutions of the form:

\[ \Delta(x) = \sum_{i=1}^{3} A_i \cosh q_i (1 - x) \quad \Phi(x) = \sum_{i=1}^{3} B_i \cosh q_i (1 - x) \]

\[ B_i = \frac{A_i q_i^2}{\omega^2 C_1 (1 - \lambda^2 q_i^2)} \]  \hspace{1cm} (23)

where the wave vectors \( q_i \) are defined by the equations:

\[ q_i = \sqrt{Q_i} \quad \text{with} \quad -\lambda^2 Q_i^3 + (1 - \epsilon) Q_i^3 - \lambda^2 \omega^4 Q_i + \omega^4 = 0 \]  \hspace{1cm} (24)

The three values of \( A_i \) are determined by the boundary conditions, Eq.(22), at \( x = 0 \). Fig. 2 shows typical deformation and lipid concentration, where it is easily seen that one of
the two species is “attracted” toward the inclusion. Such an example of surface enrichment has already been discussed in the context of the hydrophobic matching model (Mouritsen and Bloom, 1993), and is clearly expected in our situation as well. We would like however to reiterate here that all the results are determined by energy minimization and that there is no assumption of any particular composition near the inclusions, nor any length scale at the which the composition changes. From Eq. (19), the energy per inclusion takes the form:

$$\Delta F = -k_B T L \Gamma \sum_i \left( \frac{\tilde{\omega}^2 C_0 + q_i^2}{\tilde{\omega}^4} \right) q_i A_i \sinh q_i$$

(25)

The energy is now a function of four variables, namely the two length scale: $\tilde{\omega}^{-1}$ and $\lambda$, related to the variation of the displacement and the composition, the spontaneous curvature $C_0$, and the coupling between the spontaneous curvature and the composition of the film $\epsilon$. The explicit expression of the energy of the film is not given here, but it is clear from Eq.(9) that this energy always lies between the energy of an homogeneous film ($E \rightarrow \infty$ or $\phi = 0$), and the energy of a film without interaction ($E = 0$). It should be noted at this point that according to the molecular model given in the appendix, the two parameters $\epsilon$ and $\lambda$ are not independent. Fig. 3 shows the plot of the energy per inclusion, taking this coupling into account.

The full expression for the energy will be used in the next section to described the phase separation process.

IV. INCLUSION-INDUCED PHASE SEPARATION IN A MIXED LIPID FILM

In this section, we describe the demixing of the film into patches of different average composition, as driven by the presence of inclusions. We still restrict ourselves to two dimensional films, although the existence of a line tension between different phases in real (three dimensional) films, which can be avoided by the presence of an inclusion between two phases in a 2-D film, is usually an important factor for the size and shape of the different domains (see Fig. 1b). Since we are only interested in the influence of the inclusions as initiators of the transition, we do not consider any line tension term here. Such a term would also be present in the description of the phase separation in a mixed film without inclusions (Seul and Andelman, 1995).

In the previous sections, three different energies per inclusion have been calculated, with increasing accuracy and increasing complexity. The first energy, Eq.(7), is the energy of a simple, one component, lipid film. An approximation for the energy of a mixed film can be obtained from this equation by expressing the spontaneous curvature $C_0$ as a function of the average composition $\bar{\psi}$ of the film. This will be done in the next section. Secondly, the energy of a mixed film has been derived, Eq.(15,16), allowing for local variation of the composition, $\phi = \psi(x) - \bar{\psi}$, but neglecting the interactions between different species. From this calculation, a perturbation parameter $\epsilon$ has been defined (Eq.(12)). Finally, the interactions between different components have been taken into account to obtain the third energy, Eq.(23-25), which can be studied only via numerical analysis.

It is shown with a simple argument in the first paragraph below that although the inclusions always favor the demixing of the film, the strong tendency for mixing due to the
spontaneous curvature for the flat film always dominates the curvature effects. Hence, no phase separation is possible without repulsive interaction between the two different species. A full study of the phase separation when these interactions are taken into account is given in the next paragraph.

A. Phase separation in a mixed film - simplified view

We first study for simplicity the case where neither modulation of composition, nor interactions between different species, are taken into account. This means that the composition of the film is assumed to be everywhere equal to its average value $\bar{\psi}$. The total free energy of the film is the sum of two contributions: the reference energy of a flat film, including mixing entropy and mean field interaction (Appendix, Eq.(A6)), and the deformation energy due to the inclusion Eq.(7). Since we do not consider any specific interaction between lipid, the parameters $\Delta c$ and $J$ are set to zero in the reference energy, which becomes:

$$F_0 = \frac{k_BTL}{a} \left( \bar{\psi} \log \bar{\psi} + (1 - \bar{\psi}) \log (1 - \bar{\psi}) + \Gamma C_0(\psi)^2 \right)$$

with $C_0(\psi) = C_A\psi + C_B(1 - \psi)$, and where $\Gamma$ is the ratio of the stretching energy at the inclusion to the thermal energy (see Appendix).

One can see that this reference energy strongly favors mixing, since both the entropy term and the spontaneous curvature term are convex functions of the film composition.

The total energy is expressed as a function of $\bar{\psi}$ from Eq.(7):

$$F = \frac{k_BTL}{a} \left( \bar{\psi} \log \bar{\psi} + (1 - \bar{\psi}) \log (1 - \bar{\psi}) + \Gamma C_0^2 \left(1 - f(\bar{\omega})\right) \bar{\psi}^2 \right) + \alpha \bar{\psi} + \beta$$

where $0 < f(\bar{\omega}) \equiv \frac{\sin \sqrt{2\bar{\omega}} + \sinh \sqrt{2\bar{\omega}}}{\sqrt{2\bar{\omega}} \left(\cos \sqrt{2\bar{\omega}} + \cosh \sqrt{2\bar{\omega}}\right)} < 1$.

In this simple case, the non-entropic contribution of the energy is quadratic in the film composition $\bar{\psi}$. The quadratic term is composed of a positive part, which favor mixing and comes from the reference energy: $1/2\kappa c_0^2$, and the negative part (which does favor phase separation), due to the presence of inclusions. One can see that this term is always positive. This means that although the presence of the inclusion favor a phase separation, the reference energy of the flat film prevents the demixing.

According to Fig. 3, the energy is only slightly modified by a modulation of the local composition of the film. This is especially true for small separation between the inclusions (small $\bar{\omega}$), which is the only situation where the effect of the inclusion could compete with the reference energy. We thus expect our simplified view to still hold, at least qualitatively, when we consider the modulation of the film composition and the interactions between different species.
B. Phase separation in a mixed film - full treatment

In this section, we consider the full energy of a mixed film, including variations of the local composition of the film, and interactions between the two lipid species. For simplicity, we assume in what follows that the interactions between the \( A \) and \( B \) lipids do not affect the spontaneous curvature of a mixed film of a given composition. This means that \( \Delta c = 0 \) in Eq. (A.1), and the spontaneous curvature is then composed of a constant term and a term linear in \( \phi \):

\[
c_0(\psi) = c_0(\bar{\psi}) + c_1 \phi \quad \text{with} \quad c_1 = c_A - c_B.
\]

We have seen in Eq. (11) that the modulation of the film composition introduce the perturbation parameter:

\[
\epsilon = 1 + \frac{1}{2\Gamma C_1} \left( \frac{1}{\psi(1-\psi)} - J \right) \quad (28)
\]

where, as already stated, \( J \) is the interaction parameter between the two different lipids, \( C_1 = c_1/\omega^2(u_0 - u_\infty) \), and \( \Gamma = \frac{1}{2}k(u_0 - u_\infty)^2a/k_BT \) is the ratio between typical stretching and thermal energies. Although this parameter stems from a calculation where the interaction between lipids are not taken into account, it is reasonable to assume that this perturbation parameter is still pertinent to the more complex case where the full energy is considered (Eqs. (23-25)).

When trying to calculate the influence of the inclusions on the value of the critical demixing point, we run into the following problem. When modulations of the film composition are allowed, the so-called curvature instability develops as \( \epsilon \to 1 \) (see discussion following Eq. (14)). This occurs for the value \( J_{cr} = 1/(\psi(1-\psi)) \), which corresponds exactly to the position of the spinodal curve for a model two fluid mixture, and is probably close to the spinodal curve in our system as well. To properly describe the inclusion-induced demixing with the quadratic expansion Eq. (11), we need to be far enough from the onset of this instability. For that reason, we choose to show the evolution of the critical interaction energy \( J \) (the binodal curve) for a given average composition \( \bar{\psi} \) sufficiently far from \( \bar{\psi} = 1/2 \), in which case binodal and spinodal curves are clearly distinct, and the critical interaction parameter correspond to a value of \( \epsilon < 1 \).

As already mentioned, the problem of the failure of the quadratic theory for \( \epsilon \approx 1 \) is reminiscent of the curvature instability (Leibler, 1986). However, while those authors considered bilayers of fixed thickness, but allowed for an asymmetry between the two sides of the bilayer, we consider here a symmetrical bilayer allowed to change its thickness near a inclusions. The inclusion not only fixes locally the thickness of the bilayer, but also allow for a discontinuity of the curvature, which is impossible in membranes with no inclusions. If the bilayer is asymmetrical, an overall spontaneous curvature can exist, giving rise of rippled phase (Leibler, 1986) or spontaneous vesicles formation (Safran et al., 1991).

The binodal curve for a composition dependent energy \( F[\psi] \) is obtained from the so-called common tangent construction, leading to the two combined equations (Safran, 1994):

\[
\frac{F[\psi_1] - F[\psi_2]}{\psi_1 - \psi_2} = \left. \frac{\partial F}{\partial \psi} \right|_{\psi_1} = \left. \frac{\partial F}{\partial \psi} \right|_{\psi_2}
\]

which give the critical value of \( J = J_b \) for which regions of given composition \( \psi_1 \) are in chemical and mechanical equilibrium with regions of composition \( \psi_2 \).
If we assume an energy of the form: $F = \psi \log \psi + (1 - \psi) \log (1 - \psi) + (\alpha/2)\psi^2 + \beta \psi + \gamma$, the binodal curve is given by the equations:

\[
\alpha = -2 \log \frac{1 - \psi_1}{1 - 2\psi_1} \quad \text{and} \quad \psi_2 = 1 - \psi_1 \tag{30}
\]

A simplified expression of the binodal curve of a mixed film with inclusions can be obtained using the simple treatment of the previous section (Eq.(27)), and adding to the reference energy Eq.(26), a mean-field interaction term $\frac{1}{2}J\bar{\psi}(1 - \bar{\psi})$. Although somewhat artificial, since Eq.(27) results from a no-interaction calculation, this treatment gives a good approximation of the critical demixing interaction, as is shown in Fig. 4. The resulting expression for $J_b$ is:

\[
J_b = 2 \log \frac{1 - \psi_1}{1 - 2\psi_1} + 2\Gamma C_1^2 \left(1 - f(\bar{\omega})\right) \tag{31}
\]

where $f(\bar{\omega})$ is defined in Eq.(27). The condition $J > J_b$ (corresponding to phase separation) is shown on Fig. 4. It is satisfied only if the repulsion between the two components is strong enough to overcome \(i\) the entropy of mixing, and \(ii\) the increase of the spontaneous curvature energy if the flat membrane phase separates. The effect of the inclusions is simply to reduce the second requirement by a factor of $(1 - f(\bar{\omega}))$ (Eq.27). One clearly sees that the inclusions reduce the slope of the boundary line, and that the effect becomes stronger as the inclusions get close to each other.

The next step is to identify the effect of the inclusions on the variation of the full energy $F[\psi]$ (Eq.(25)). This expression is far too complex to be manipulated analytically. It is however possible to construct the binodal curve numerically using a procedure equivalent to solving the Eqs.(29): We use the Gibbs potential $G[\psi] = F[\psi] - \mu\psi$ where $\mu$ is the chemical potential of the solution. For a given composition $\psi_1 \ll 1/2$ (we choose $\psi_1 = 0.2$) we find the interaction $J$ and the potential $\mu$ for which \(i\) $G[\psi_1]$ is a minimum, and \(ii\) there exists a composition $\psi_2 \gg 1/2$ (practically $\psi_2 \simeq 1 - \psi_1$) for which $G[\psi_2]$ is a minimum. The numerical results are shown in Fig. 4 for a given value of the separation between inclusions, and agree well with the approximation given by Eq.(31). One notable difference is that the binodal curve now depends weakly on the absolute value of the spontaneous curvature (see the dependence on $C_B$ of Fig. 4), and not only on the difference $C_1 = C_A - C_B$. This is of course due to the fact that the energy Eq.(25) is not quadratic in the composition of the film, but involves more complex couplings between curvature energy, mixing entropy, and specific interactions.

V. SUMMARY

The deformation of a mixed lipid film caused by the presence of embedded inclusions has been predicted in term of the difference of spontaneous curvature between the lipid molecules present in the film. The local spontaneous curvature is related to the local composition of the film, and varies in order to match the film deformation. This additional degree of freedom reduces the energy per inclusion with respect to the energy of an homogeneous film, but does not modify it qualitatively. It is shown that the composition of the membrane around a given
inclusion is controlled by the deformation of the film. For instance, inclusions larger than the film thickness would tend to be surrounded by lipids of positive spontaneous curvature (see Fig. 2) while smaller inclusions, which would pinch the film would be surrounded by lipids of negative spontaneous curvature. This phenomenon could be partly responsible for the lateral segregation in biological membranes, where nonrandom lipid distributions around given membrane proteins have been experimentally demonstrated.

A particularly interesting phenomenon is the inclusion-induced phase separation in the mixed lipid film. Phase separation in a mixed fluid usually takes place when the repulsive interaction between the two components is strong enough to overcome the entropic mixing. In a mixed liquid film, there exists an additional effect due to the coupling between the spontaneous curvature and the composition of the film. At the simplest level, this coupling always favors mixing. We have shown in this paper that the presence of inclusions can alter significantly this phase behavior.

Consider an homogeneously mixed film composed of two different lipid species. If the (repulsive) molecular interactions between different constituents are increased, or the temperature of the sample lowered, a phase separation can occur where regions of different lipid composition are observed. We have shown that this phase separation can also be triggered by the deformation and composition modulation induced by intermembrane inclusions. As the density of inclusions in the film is increased, the distance between inclusions decreases up to the point where it reaches a critical value where the phase separation occurs. In previous works (Dumas et al. 1997), computer simulations have led to the conclusion that inclusions will preferentially be located at the interface between two coexisting phases. We claim here that the inclusions can actually be responsible for the very existence of the two phases.

The influence of the inclusions on the phase separation is shown in Fig. 4. They always favor the phase separation, but their effect is never strong enough to compete with the tendency of mixing of a flat film in the absence of other repulsive interactions, of strength $J$. Nevertheless, the presence of inclusions can lower significantly the critical interaction energy $J$ for which the phase separation occurs if the inclusions are close enough to each other (typically $\omega L < 5$ where $\omega$ is the inverse decay length of the deformation).

Our model is based on a unidimensional lipid membrane, which, as was already mentioned, is formally equivalent to considering infinitely long inclusions. This may seem a very restrictive assumption, however, an analogy to the (more realistic) situation where inclusions are closer to small cylinders (with a circular basis) in a 2D membrane is quite straightforward. As can be seen when one compares our results for a single component film (section II) with those obtained by the resolution of the full 2D problem (Dan 1994), a good picture of the 2D situation can be obtained by dividing the bending and stretching coefficient by a molecular size equivalent to the square root of the area per lipid head, and by multiplying the energies per inclusion by $2\pi$. This should be the case in our mixed film as well.

To conclude this work, we estimate the effect of the inclusions on the critical interaction parameter for typical lipids. According to Eq. (31), the inclusions reduce the interaction energy by the amount $2ΓC_1^2f(Ω)$, which, in natural units, becomes $κac_1^2/(k_BT)f(\omega L)$. One first remarks that the effect does not depend on the mismatch between the height of the inclusion and the thickness of the lipid bilayer. Even inclusions matching the thickness of the bilayer have an effect, since they allow for a discontinuity of the curvature of the layer (Dan, 1993). The first important parameter is the “healing length” for the deformation of
the bilayer $\omega^{-1}$. Because of the quarter power of the dependence of $\omega$ on the stretching and bending modulii of the lipid layers (see Eq.(2)), this length is only weakly dependent of the characteristics of the membrane: $\omega^{-1} = 0.5 - 2.5 \ h$ where $h$ is the thickness of one lipid monolayer $\sim 25$Å. The composition of lipid and protein molecules varies widely in biological membranes. Typical protein over lipid ratios are of the order of $1/50$ (Alberts et al., 1994), in which case the mean separation between proteins can be of order $50-100$˚A (the area per lipid head is typically $a^2 = 40$˚A$^2$). Hence, in these membranes with high protein concentration, we can reach values of $f(\omega L) \sim 1/2$. The spontaneous curvature of a monolayer is typically the inverse of its thickness: $c_1 \sim h^{-1}$, and its bending rigidity of order $\kappa \sim 25h \ k_B T$ (in our 2-D model). Hence, the presence of proteins in a lipid bilayer under biological conditions can reduce the critical interaction parameter $J_b$ by an amount $1.5 \ k_B T$, which corresponds to 30% of its value without inclusions. We can thus expect many situations where the proteins not only control the local composition of the membrane, but are also able to induce a major demixing of the lipid membrane.

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**APPENDIX A: ABOUT THE COEFFICIENTS IN THE L-G EXPANSION**

The coefficients in the Landau-Ginzburg expression, Eq. [2], can be related to molecular parameters of the film. If one considers that the spontaneous curvature of a monolayer arises principally from interactions between neighboring molecules, it can be expressed as (Safran, 1994):

$$c_0(\psi) = c_A \psi^2 + c_B (1 - \psi)^2 + (c_A + c_B + \Delta c) \psi (1 - \psi) \quad (A1)$$

where $\Delta c$ represents the contribution to the spontaneous curvature of neighboring $A$ and $B$ molecules. For a weak modulation of the composition $\phi \ll \bar{\psi}$, the expression becomes:

$$c_0(\bar{\psi} + \phi) = c_0(\bar{\psi}) + c_1 \phi - \Delta c \phi^2$$

with $c_1 = c_A - c_B + \Delta c (1 - 2 \bar{\psi}) \quad (A2)$

The expansion of the energy per unit length of a flat film gives

$$\frac{1}{2} \kappa c_0(\bar{\psi} + \phi)^2 = \frac{1}{2} \kappa \left( c_0(\bar{\psi})^2 + 2c_0 c_1 \phi + c_2 \phi^2 \right)$$

$$c_2^2 = c_1^2 - 2c_0 \Delta c \quad (A3)$$

The mixing energy per unit length, $f_m$, is written in the general form (Safran, 1994):

$$f_m = \frac{k_B T}{a} \left( \psi \log \psi + (1 - \psi) \log (1 - \psi) + \frac{1}{2} J \psi (1 - \psi) + \frac{1}{2} B |\nabla \psi|^2 \right)$$

$$J = \frac{J_b}{2} \left( \frac{a}{L} \right)^2 \quad (A4)$$

where $J$ is the interaction parameter between the two species, and the gradient is a derivative over the dimensionless variable $x = z/L$. 

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After expansion of all the terms up to the quadratic order in the deformation and the composition difference, the energy takes the form of Eq. (9), with

\[
C_\alpha = \frac{c_\alpha}{(u_0 - u_\infty)\omega^2} \quad D = C_2^2 + \Gamma S_2 \quad E = \Gamma \left( \frac{a}{L} \right)^2 \frac{J}{4} \quad \Gamma \equiv \frac{1}{2} \frac{k(u_0 - u_\infty)^2 a}{k_B T} \quad S_2 \equiv \frac{1}{2} \frac{1}{\psi(1 - \psi)} - \frac{J}{2}
\]  \quad (A5)

The reference energy is:

\[
F_0 = \frac{k_B T L}{a} \left( \psi \log \psi + (1 - \psi) \log (1 - \psi) + \frac{1}{2} J \psi(1 - \psi) + \frac{C_0(\psi)^2}{\Gamma} \right)
\]  \quad (A6)
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FIG. 1. (a) Schematic representation of a mixed lipid film with inclusions. The notations used in the text are described on the figure. (b) Difference between the two dimensional and three dimensional films for the phase separation process. In the 2D film, phase separation can occur without the existence of a line tension, a situation that is impossible in the 3D film. As noted in the next, the problem does not concern us as long as we are only interested in the effect of the inclusion on the phase separation.
FIG. 2. Effect of the inclusion on the deformation profile of a monolayer $\Delta(x)$ (thin curve, left axis) and on the composition of the film $\phi(x)$ (thick curve, right axis), with the distance to the inclusion $x$, for three different values of the spontaneous curvature $C_0 = -0.8$ (upper left), $C_0 = 0$ (upper right) and $C_0 = 0.8$ (lower). In this example, $C_1 > 0$, and the $A$ molecules move toward the regions of positive curvature.
FIG. 3. Variation of the interaction energy between two inclusions with the separation between inclusions $\tilde{\omega} = \omega L$ for the same three values of the spontaneous curvature. The thin curves are plotted using the expression of the energy for a pure lipid film (Eq.7), and the fat curve is using the energy of a mixed film (Eq.26), with $\Gamma = 1$ and $a/L = 1/20$. Note that the energy is attractive if $C_0 > 0$. 
FIG. 4. Phase separation diagram. (a) Using the simplified expression Eq.(32). The x-axis represents the spontaneous curvature difference between the two molecules: $C_1$ and the y-axis the repulsive interaction energy: $J$. The thick line represents the boundary for a film without inclusion and the other lines correspond to different separations between inclusions: $\tilde{\omega} = 1$, $\tilde{\omega} = 2$ and $\tilde{\omega} = 4$. (b) Comparison between the previous approximation (full line) and the result obtained numerically from Eq.(26) (dashed curves), for three values of the spontaneous curvature of the $b$-species $C_B$. The quality of the fit depends of $C_B$. 