Tension-Induced Morphological Transition in Mixed Lipid Bilayers

S. Komura and N. Shimokawa
Department of Chemistry, Faculty of Science,
Tokyo Metropolitan University, Tokyo 192-0397, Japan

D. Andelman
School of Physics and Astronomy, Raymond and Beverly Sackler Faculty of Exact Sciences,
Tel Aviv University, Ramat Aviv 69978, Tel Aviv, Israel
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Raft domains in biological cell membranes are associated with membrane signaling pathways and have attracted great deal of interest in recent years [1, 2]. Due to the complexity of biological membranes, a minimal model to investigate rafts (or more precisely, domain formation) consists of three-component lipid bilayers containing saturated and unsaturated lipids (e.g., sphingomyelin and DOPC) as well as cholesterol. These three-component systems have been investigated both at the air-water interface and in artificially vesicles [3, 4]. It was observed that these multi-component systems exhibit complex phase separation and appearance of domains.

Recently, Rozovsky et al. reported on the morphology and dynamics of superstructures in such a ternary mixture [5]. A giant unilamellar vesicle is placed in contact with a lipid bilayer that is supported on top of a silica surface. Fluorescence microscopy is used to monitor the creation and temporal evolution of domains on the adhering vesicle. In a number of cases, the upper part of the vesicles exhibit a stripe pattern of quite well-characterized stripe thickness but without any long-range stripe orientation. It is important to note that due to the large size of the vesicle, the vesicle upper part is far and in no contact with the adhering surface. As the adhesion process evolves in time, the stripe pattern transforms quite abruptly into another type consisting of an ordered hexagonal lattice of circular domains. The transformation of the lamellae into circular domains is seen first as an instability of the stripe tip. Eventually the entire stripes are replaced by circular domains having roughly the same diameter as the original stripe width. As time progresses even further, the vesicle looks more tense, and the hexagonal pattern of circular domains becomes more disordered.

The aim of our Comment is to point out that the observed sequence of the stripe to hexagonal morphological transition in mixed bilayers can be attributed to an enhanced membrane surface tension that is induced by the vesicle adhesion on the solid surface.

It is known that surface tension can induce adhesion of a vesicle onto a substrate or to another vesicle [6]. The reason being that surface tension of the membrane suppresses thermal fluctuations and promotes its adhesive properties. A self-consistent treatment of such a problem predicted the conditions for tension-induced adhesion [6]. On the other hand, it was reported that the spreading of red blood cells on a substrate produces finite surface tension which can even cause them to rupture [7]. More recent experiments on the pulling of nanotubes from adhered vesicles revealed that the membrane tension strongly increases during elongation [8]. In view of these works, we explore the relation between an increase
in surface tension and lateral morphological transitions that can occur on the membrane plane.

The main ingredient of our model is to introduce a coupling between local composition on the membrane plane and the local curvature \[9\]. We do not consider a closed form vesicle of a finite area and volume. We rather model a planar and extended membrane. Its shape is described by a displacement variable \(\ell(x, y)\), relative to the reference \(x-y\) plane. The local composition (assuming a simple binary mixture of two components) is similarly denoted by \(\phi(x, y)\). The total free energy is written in terms of these two local fields. For simplicity, we consider only small undulations above the \(x-y\) plane in the form of the following expansion \[9\]:

\[
H\{\ell, \phi\} = \int d^2 x \left[ \frac{1}{2} \sigma (\nabla \ell)^2 + \frac{1}{2} \kappa (\nabla^2 \ell)^2 + \frac{1}{2} b (\nabla \phi)^2 + \frac{1}{2} a_2 \phi^2 + \frac{1}{4} a_4 \phi^4 + \Lambda (\nabla^2 \ell) \phi \right],
\]

where \(\sigma > 0\) and \(\kappa > 0\) are the surface tension and bending rigidity of the membrane, respectively. The other coefficients, \(b, a_2, a_4\) and \(\Lambda\) are phenomenological ones. The parameter \(b > 0\) is related to the line tension between different domains, \(a_2 \sim T - T_c\) is the reduced temperature (\(T_c\) is the critical temperature). Below the phase transition it is negative and we need to include in the expansion a positive 4\(^{th}\) order term, \(a_4 > 0\), for stability purposes. Finally, \(\Lambda\) is the coupling constant. This coupling term represents the situation where the spontaneous curvature of the membrane depends on the local composition. For simplicity, the other coupling term \((\nabla^4 \ell) \phi\) (higher order in Fourier space) as well as higher-order coupling terms are omitted here.

Starting from the above free energy, we first integrate out the \(\ell\)-field in Fourier space, and the effective free energy is approximated as the Brazovskii free energy \[9\]:

\[
H_{\text{eff}}\{\phi\} \approx \int d^2 x \left[ \frac{1}{2} B (\nabla \phi)^2 + \frac{1}{2} C (\nabla^2 \phi)^2 + \frac{1}{2} a_2 \phi^2 + \frac{1}{4} a_4 \phi^4 \right],
\]

where

\[
B \equiv b - \frac{\Lambda^2}{\sigma}, \quad C \equiv \frac{\Lambda^2 \kappa}{\sigma^2}.
\]

In deriving the above expression, we performed an expansion up to 4\(^{th}\) order in the wavevector \(q\) (or equivalently up to 4\(^{th}\) order in differential operator in real space). The homogeneous disordered phase becomes unstable with respect to the modulated phase when the coupling constant is large enough so that \(\Lambda^2 > b\sigma\). Note that the sign of \(\Lambda\) can also be negative as
the results depend only on $\Lambda^2$. The condition to have finite unstable $q$ vectors is simply that $B < 0$. The most unstable wavevector is then given by $q^* = (-B/2C)^{1/2}$.

We recall that the mean-field free energies of the stripe and the hexagonal phases have been used in Ref. [9] in order to construct the membrane phase diagram (see Fig. 1 of Ref. [9]). Only the most unstable wavevector $q^*$ (single-mode approximation) is used in the calculation, which is justified for the region close to the homogeneous critical point. There are four distinct phases: disordered (D), stripe (S), hexagonal (H), and inverted hexagonal phases. These phases are separated by two-phase coexistence regions (first-order phase transitions). The calculated phase diagram is in agreement with experiments performed on mixtures of lipids in Langmuir monolayers at the air-water interface [11].

In order to see clearly the role of surface tension $\sigma$, we cannot use directly the phase diagram of Ref. [9]. Instead, we construct a new phase diagram in terms of two different reduced variables defined by [10]

$$\Omega \equiv \frac{a_2}{a_4 \phi_0^2}, \quad \Sigma \equiv \frac{b\sigma}{\Lambda^2}. \tag{4}$$

In the above, $\phi_0 = \langle \phi \rangle$ is the average composition that is fixed by the chemical potential. We note that $\Omega$ is independent of the surface tension parameter $\sigma$, and that the only variation of $\sigma$ enters in the second variable, $\Sigma \sim \sigma$.

Figure 1 is the corresponding phase diagram in the $(\Omega, \Sigma)$ plane when $M \equiv (4\kappa a_4)^{1/2} \phi_0/\Lambda$ is kept constant. In the figure, we chose $M = 0.1$. Regions of coexistence are omitted in this phase diagram for clarity purposes. Thus, the transition lines indicate the locus of points at which the free energies of different phases cross each other. The stripe phase (S) appears when $\Sigma \sim \sigma$ is small. The dashed arrow in Fig. 1 indicates a horizontal path along which $\Sigma$ (or equivalently the surface tension $\sigma$) increases for a fixed value of $\Omega$. Clearly, along this path the stripe phase (S) transforms into the hexagonal phase (H). As $\Sigma$ increases even further, there will be another phase transition into the homogeneous disordered phase (D). Such a sequence of the pattern transformation, S → H, upon increasing the surface tension is consistent with that observed in the mixed lipid bilayers (see Fig. 1 in Ref. [5]). This is our main result. We consider that the enhanced surface tension during the adhesion process is responsible for the morphological evolution of the domain shapes in the adhering vesicle. It should be noted that although the calculated phase diagram depends on the value of $M$, the sequence of the modulated structures remains unchanged.
We discuss now our results and their relationship with experiment. In Ref. [5], the vesicles are shown to become more tensed as the adhesion process evolves. This results in a reduced area of the vesicle (see Fig. 1 of Ref. [5]). In the case of mixed vesicles, the spontaneous curvature arises from the asymmetry in the composition between the inner and outer leaflets comprising the bilayer membrane. Such an effect is represented in the last coupling term of Eq. (1). Although the evidence of the asymmetric composition between the inner and outer leaflets has not been reported in Ref. [5], it certainly exists in biological cells due to an active mechanism. Asymmetry in the composition might also occur in situations where the two leaflets experience a different environment. In an adhering vesicle, for example, only the outer leaflet is in contact with the surface. More detailed theories dealing with the bilayer nature of the membranes are given in Refs. [12, 13]. It should be noted, however, that these works as well as the present one consider only an averagely flat and extensive membrane, rather than closed vesicles of finite area and volume. In the previous experiments of Ref. [4], similar stripe and hexagonal patterns have been shown but no similar temporal evolution was reported. This may be because the vesicles in Ref. [4] were suspended in an aqueous solution (rather than adhering on a surface), and their surface tension is not increasing as function of time. It is also entirely not clear how much the patterns and domains seen on the vesicular surface are truly equilibrium ones or just long-lived metastable ones.

Finally, we comment on the dynamical aspect of the observed morphological transition. In the last decade, kinetics of phase transitions between different ordered mesophases have been studied intensively, especially, for block copolymer systems [14]. Among various works, Nonomura and Ohta investigated the morphological transition between the lamellar and the hexagonal phases in two dimensions [15]. Their dynamical equation is based on the time-dependent Ginzburg-Landau equation using a free energy which includes a long-range repulsive interaction term. This free energy is known to be similar to the one used in the present work.

In the simulation, the transition is induced by changing the temperature. Note that in our Fig. 1 a change in temperature is simply a vertical scan where \( \Omega \sim a_2 \sim T - T_c \) changes, while \( \Sigma \sim \sigma \) (as well as \( M \) or \( \phi_0 \)) are kept fixed. The vertical scan can also lead to a crossover between the stripe and hexagonal phases. In the experiments [4], on the other hand, the temperature is kept fixed and we believe that the origin of the transition relies on the temporal increase in the surface tension as detailed above. Despite the difference
in the control parameters (either temperature or surface tension), the time evolution of
the structure in the simulation is very similar to that observed for the lipid mixtures. For
example, the stripes undergo a pearling phenomenon and the hexagonal domains appear
at the grain boundaries of the lamellar structure [15]. We hope that more quantitative
measurements of the surface tension during the adhesion process will shed light on this
intriguing phenomena and will lead to further theoretical investigations.

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    and the reduced average concentration \( m \equiv (4Ca_4/B^2)^{1/2} \phi_0 \), where the numerical prefactors
    of “2” in Eq. (10) of Ref. [9] are corrected. In terms of the above parameters, we have \( \Omega = \varepsilon/m^2 \)
    and \( \Sigma = 1 - (M/m) \), where \( M \) is defined in the text.
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FIG. 1: Mean-field phase diagram in the $(\Sigma, \Omega)$ plane for $M = 0.1$. $\Sigma$ represents the reduced surface tension and $\Omega$ the curvature-composition coupling parameter, as defined in Eq. (4). There are three different phases: the stripe phase (S), the hexagonal phase (H), and the disordered (homogeneous) phase (D). These phases are separated by the first-order transition lines. For simplicity we show here by solid lines only the crossover in the free energies while avoiding plotting two-phase regions. The effect of increasing the surface tension is represented by the dashed arrow.