# Membrane heterogeneity: Manifestation of a curvature-induced microemulsion

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To explain the appearance of heterogeneities in the plasma membrane, I propose a hypothesis which begins with the observation that fluctuations in the membrane curvature are coupled to the difference between compositions of one leaf and the other. Because of this coupling, the most easily excited fluctuations can occur at nonzero wave numbers. When the coupling is sufficiently strong, it is well-known that it leads to microphase separation and modulated phases. I note that when the coupling is less strong, the tendency toward modulation remains manifest in a liquid phase that exhibits a transient structure of a characteristic size, that is, it is a microemulsion. The characteristic size of the fluctuating domains is estimated to be on the order of 100 nm, and experiments to verify this hypothesis are proposed.

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## I. INTRODUCTION

Certainly, one of the most interesting models of the plasma membrane is that rather than being homogeneous, it is characterized by aggregates of saturated lipids and cholesterol which float, like rafts, in a sea of unsaturated lipids [1]. An impressive array of experiments support this hypothesis [2], and limit the size of such aggregates in mammalian cells to the order of tens to hundreds of nanometers [3–5]. Experiments also limit the lifetime of the aggregates so that they are more readily described as dynamic domains [6]. The hypothesis remains controversial, however, due in part to the lack of a firm physical basis for the appearance of such domains.

A few explanations have been put forth. One arises from the fact that model membrane mixtures of cholesterol and saturated and unsaturated lipids readily undergo separation into two liquid phases, one rich in the first two components and the other rich in the third [7]. Hence, rafts might occur in a two-phase region and simply be the domain of one phase surrounded by the other. The small size of the domains could then be attributed to the effects of the cytoskeleton [8]. One difficulty with this hypothesis is that it is known that a bilayer of a composition that mimics the inner leaf of the plasma membrane does not undergo phase separation [9] and that the coupling of such a leaf to another which does tend to phase separate produces a bilayer in which the miscibility transition either occurs at a greatly reduced temperature or is eliminated entirely [10,11].

A second, related hypothesis is that the inhomogeneities occur in a one-phase region and are simply those fluctuations associated with a nearby critical point of two-phase coexistence [12]. Again, the sizes of these fluctuations are proposed to be limited by the cytoskeleton [13]. This hypothesis is not only subject to the criticism that there may be no miscibility phase transition nearby but also to the observation that fluctuations near a critical point exhibit little difference between their composition and that of the background from which they arise. Consequently, they would not easily discriminate between different proteins, the raison d'être for the raft hypothesis itself.

A third hypothesis is that the fluctuating domains are simply the signature of a microemulsion brought about by the presence of a line-active agent [14]. The difficulty with this proposal is that there is no obvious component to act as such an agent, one which would be attracted to the interface between the two phases. In particular it is clear that cholesterol is not line active as it prefers the phase rich in saturated lipids. Its initial addition to a single liquid phase brings about a phase separation [15], that is, it *raises* the miscibility transition temperature rather than lowers it as a line-active agent would. As cholesterol is not line active, it is posited that the unsaturated lipids, which in biological membranes usually have one saturated as well as one unsaturated tail [16], can in fact play a dual role: as a component of one of the two phases and as a line-active agent between the phases [17]. A detailed model which encapsulates this idea and explores the effects on this microemulsion of the coupling between the two leaves has been explored by Hirose *et al.* [18]. However, such a model cannot explain the observation of nanoscopic domains in ternary systems that contain no lipids with one saturated and one unsaturated tail as in the system containing dipalmitoylphosphatidylcholine (DPPC), dilauroylphosphatidylcholine (DLPC), and cholesterol [19].

As this last example illustrates, nanoscopic domains can be brought about by means other than the action of a line-active agent. Indeed, microemulsions almost invariably appear in any system which manifests modulated phases; they are, in general, the liquid phase to which modulated phases melt. It is for this reason that the recent observation of modulations of composition in giant unilamellar vesicles mimicking biological membranes [20] is so interesting; it implies that such membranes could well display a microemulsion. The questions that arise then, concern first, the nature of the interactions within the membrane that are responsible for the modulations and, second, the characteristic size of the droplets in the two-dimensional microemulsion to which modulated phases melt. A plausible scenario for the interactions which give rise to the modulated phases was proposed in the seminal work of Leibler [21] and of Leibler and Andelman [22], and I remind the reader in the next section of this mechanism which couples the local difference in mole fractions of different lipids to the local curvature. There I also estimate the characteristic size of the droplets in the microemulsion expected in a bilayer with a cytoskeleton and find it to be on the order of

 

100 nm. Therefore, I propose that rafts can be interpreted as the characteristic droplets of a curvature-induced microemulsion. Possible experiments to verify this hypothesis are proposed.

## II. THE BILAYER WITH COUPLED CURVATURE-COMPOSITION FLUCTUATIONS

The proposal that fluctuations in curvature and composition are coupled goes back to Leibler [21] and Leibler and Andelman [22]. Their work has been extended explicitly to bilayers [23–25], and I follow the last of these here. The basic physical idea is simple. The biological membrane consists of a plethora of distinct lipids with different spontaneous curvatures. Because of this variation in lipid architecture, fluctuations in the difference between local compositions of one leaf and the other couple to fluctuations in the curvature of the membrane, that is, lipids with larger headgroups and smaller tails are attracted preferentially to the outer leaf in regions where the membrane bulges outward while lipids with smaller heads and larger tails are attracted to the inner leaf in the same regions. This affinity is directly observed in experiment [26].

For simplicity I consider the cholesterol and saturated lipid as one component in a binary system and the unsaturated lipid as the other component. There are two order parameters representing the differences in mole fractions of these two components in the inner leaf $\Phi_i(\boldsymbol{r})$ and in the outer leaf $\Phi_o(\boldsymbol{r})$. It is convenient to consider the two linear combinations $\phi(\boldsymbol{r}) \equiv [\Phi_i(\boldsymbol{r}) - \Phi_o(\boldsymbol{r})]/2$ and $\psi(\boldsymbol{r}) \equiv [\Phi_i(\boldsymbol{r}) + \Phi_o(\boldsymbol{r})]/2$. The phenomenological free energy consists of three pieces. The first is the free-energy functional of the planar, coupled bilayer which to second order in the order parameters can be written

$$F_{\text{plane}} = \int d^2r \left[\frac{b}{2}(\nabla\phi)^2 + a\phi^2 + \frac{b_\psi}{2}(\nabla\psi)^2 + a_\psi\psi^2\right]. \tag{1}$$

The second piece is the curvature free energy, written here in the Monge representation in terms of $h(\boldsymbol{r})$, the height deviation from the planar configuration

$$F_{\text{curv}} = \int d^2r \frac{1}{2}[\kappa(\nabla^2 h)^2 + \gamma(\nabla h)^2], \tag{2}$$

where $\kappa$ is the bending modulus and $\gamma$ is the surface tension. Lastly, there is the coupling between the curvature $\nabla^2 h$ and the difference in compositions between the two leaves $\phi$

$$F_{\text{coupl}} = \lambda(b\gamma)^{1/2} \int d^2r(\nabla^2 h)\phi, \tag{3}$$

where $\lambda$ is a dimensionless coupling constant. In terms of the Fourier transform functions, the total free energy, up to second order, is

$$F_{\text{tot}} = \int d^2k \Big[\left(a + \frac{b}{2}k^2\right)\phi(k)\phi(-k) + \left(a_\psi + \frac{b_\psi}{2}k^2\right) \times \psi(k)\psi(-k) + \frac{1}{2}(\kappa k^4 + \gamma k^2)h(k)h(-k) - \lambda(b\gamma)^{1/2}k^2 h(-k)\phi(k)\Big]. \tag{4}$$

Within mean-field theory, one minimizes the free energy with respect to the membrane shape $\delta F_{\text{tot}}/\delta h(k) = 0$ and substitutes the resulting height $h[\phi(k)]$ into the free energy [Eq. (4)] to obtain

$$F_{\text{tot}} = \int d^2k \left\{a + \frac{b}{2}\left[1 - \frac{\lambda^2}{(1 + \kappa k^2/\gamma)}\right]k^2\right\}\phi(k)\phi(-k) + \left(a_\psi + \frac{b_\psi}{2}k^2\right)\psi(k)\psi(-k). \tag{5}$$

This form of the free energy displays everything that is needed. First, the wave vector $k^*$ at which the composition difference between the two leaves is softest, i.e., at which it shows the largest response, is the value of $k$ that minimizes the coefficient of $\phi(k)\phi(-k)$. It is

$$k^* = \begin{cases} 0, & \text{for} \quad \lambda < 1, \\ \left(\frac{\gamma}{\kappa}\right)^{1/2}(\lambda - 1)^{1/2}, & \text{for} \quad \lambda > 1. \end{cases} \tag{6}$$

Thus, the system is softest at a nonzero wave vector when $\lambda > 1$. This requirement is understood as follows. The coupling between the curvature and the difference in compositions favors a soft wave vector $k^*$ that is nonzero. As a structure with such a wave number is curved and thus of larger area than when flat, this bending is opposed by the surface tension $\gamma$. Furthermore, as regions of different compositions alternate, their occurrence is opposed by a free energy per unit length between such regions, an energy which is proportional to the coefficient $b$. Thus, the curvature coupling to the composition, measured in terms of the competing tensions, must be large, i.e., $\lambda > 1$. The particular consequence of this tendency to display a structure characterized by a nonzero wave number, $k^* \neq 0$, is determined by the coefficient of $\phi(k^*)\phi(-k^*)$ itself, which is equal to

$$a\left[1 - \frac{b\gamma}{2\kappa a}(\lambda - 1)^2\right]. \tag{7}$$

When $\lambda$ is not only greater than unity but is also greater than $1 + (2a\kappa/b\gamma)^{1/2}$, the coefficient of $\phi(k^*)\phi(-k^*)$ is negative so that the ensemble-average value of $\phi(k^*)$ is nonzero in equilibrium, that is, the system undergoes microphase separation. The resulting phase exhibits either stripes or a triangular array of domains. The possible occurrence of these phases was emphasized by earlier works [22,25], and their possible manifestations in coupled bilayers have recently been explored [27]. Indeed, these structures have been observed in some simulations of bilayers as predicted [28,29]. Within mean-field theory, transitions between all phases are of first order except at a critical point which can occur when the average compositions of the two leaves are identical. However, even this transition is driven first-order by the large fluctuations in the directions of the wave vectors characterizing the ordered phases [30] so that all transitions are of first order.

The observation that I emphasize here is that if the system tends toward order but not so strongly as to manifest that order in microphase separation, that is, if $1 + (2a\kappa/b\gamma)^{1/2} > \lambda > 1$, then the system will exist in a fluid phase, but this fluid will still reflect a tendency toward order. That tendency is manifest in its composition fluctuations which are strongest at a nonzero wave vector. Its structure is reflected in the structure factor $S(k)$ where $S(k)^{-1}$ is the coefficient of $\phi(k)\phi(-k)$. The structure factor has a peak at $k^*$ which is nonzero when $\lambda - 1 > 0$. The structure is

also reflected in the correlation function $g(\boldsymbol{r})$, which is the inverse Fourier transform of $S(\boldsymbol{k})$. In the interesting regime in which $(2a\kappa/b\gamma)^{1/2} > \lambda - 1 > 0$ and for small wave numbers $\kappa k^2/\gamma < 1$, it is straightforward to show that $g(r)$ behaves for large $r$ like $g(\boldsymbol{r}) \approx r^{-1/2}\exp(-r/\xi)\sin(k_c r + \delta)$ with $k_c$ and $\xi$ given explicitly below and $\delta$ a phase of no interest. The exponential damping, with a characteristic correlation length $\xi$, implies that the system is disordered, i.e., a liquid. The oscillatory function introduces an *additional* length $k_c^{-1}$, and this shows that the liquid is structured. It is this property of a liquid, to display structure at a length scale in addition to that of the correlation length, that is characteristic of a microemulsion. The correlation length $\xi$ and characteristic wave number $k_c$ are given by

$$2\xi^{-2} = \left[\left(\frac{2a\kappa}{\lambda^2 b\gamma}\right)^{1/2} - \frac{1}{2}\left(1 - \frac{1}{\lambda^2}\right)\right]\left(\frac{\gamma}{\kappa}\right), \tag{8}$$

$$2k_c^2 = \left[\left(\frac{2a\kappa}{\lambda^2 b\gamma}\right)^{1/2} + \frac{1}{2}\left(1 - \frac{1}{\lambda^2}\right)\right]\left(\frac{\gamma}{\kappa}\right). \tag{9}$$

The correlation length $\xi$ is equal to the characteristic distance $k_c^{-1}$ at the Lifshitz line at which $\lambda = 1$. Consequently, the microemulsion structure is strongly damped. However, the correlation length is larger than the characteristic distance for $\lambda > 1$, indicating that characteristic oscillations in the fluid are manifest before being damped out. From Eq. (9) we see that the characteristic distance $k_c^{-1}$ is on the order of or larger than $(2\kappa/\gamma)^{1/2}$. As typical values of the bending modulus and the tension of a membrane in the presence of a cytoskeleton are [31] $\kappa \approx 2.7 \times 10^{-19}$ Nm and $\gamma \approx 2 \times 10^{-5}$ N/m, respectively, the characteristic size of the fluctuating regions is on the order of or greater than $10^{-7}$ m, or 100 nm. This mean-field estimate indicates that the proposed mechanism could account for regions of the observed size.

## III. DISCUSSION

I have proposed that inhomogeneities in the plasma membrane and those observed in model membranes are microemulsions brought about by the coupling of curvature to the difference in composition of the two leaves. This hypothesis of a microemulsion avoids the difficulties associated with ascribing such inhomogeneities either to phase separation or to the fluctuations associated with a critical point. As noted earlier, that a biological membrane can display a microemulsion is strongly indicated by the recent observation in giant unilamellar vesicles of modulations of composition in a four-component system consisting of distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), 1-palmitoyl 2-oleoylphosphatidylcholine (POPC), and cholesterol [20]. That curvature is strongly indicated as the mechanism responsible for bringing about the modulations is also evidenced by other results of this four-component system. With fixed mole fractions of DSPC and cholesterol, the relative mole fraction $\rho \equiv [\mathrm{DOPC}]/([\mathrm{DOPC}] + [\mathrm{POPC}])$ was varied. One expects that the difference in spontaneous curvature between DSPC and DOPC is greater than that between DSPC and POPC. Therefore, increasing the fraction $\rho$ from small values should drive the system towards a modulated phase, and this is indeed what is observed. Similarly, decreasing the value of $\rho$ from the modulated phase is expected to cause it to become unstable to a fluid phase, one which would appear uniform to fluorescence microscopy. Again, this is what is observed.

Additional support for the mechanism proposed here is provided by an estimate of the value of $\lambda$, which characterizes the strength of the coupling $\lambda(b\gamma)^{1/2}$ between the curvature and the difference of lipid mole fractions. It must be on the order of unity for a microemulsion to occur. Leibler and Andelman [22] in their original paper, and later Liu *et al*. [32], reasonably assume that the energy per unit length $\lambda(b\gamma)^{1/2}$ should be set equal to $\kappa\delta H$ where $\delta H$ is the difference in spontaneous curvatures of cholesterol-rich raft domains and the phospholipid background. With the coupling $b$ of Eq. (1) on the order of $k_B T$, this yields a simple expression for the dimensionless coupling $\lambda = [\kappa/k_B T][k_B T(\delta H)^2/\gamma]^{1/2}$. Liu *et al*. estimated $\delta H$ to be on the order of $10^6$ m$^{-1}$ and took $\gamma = 3.1 \times 10^{-6}$ N/m and $\kappa = 400\, k_B T$. These values yield an estimate for $\lambda$ of about 14, implying that such membranes should always display a modulated phase, contrary to experiment. This negative result would support their conclusion, which they reached by a slightly different argument, that the coupling between curvature and concentration fluctuations could not explain raft phenomena. However, if one utilizes the same difference in spontaneous curvature, the larger surface tension $\gamma = 2 \times 10^{-5}$ N/m of Dai and Sheetz [31], and the same reference's smaller bending modulus $\kappa = 2.7 \times 10^{-19}$ Nm $= 66 k_B T$, then one obtains the estimate $\lambda = 0.94$. This shows that it is certainly plausible that the coupling has the correct order of magnitude to bring about the existence of a microemulsion.

The coupling that is assumed in this paper to produce the microemulsion, one between curvature and the *difference* in the compositions between the two leaves, predicts that saturated lipid-rich and lipid-poor regions in the two leaves are *anticorrelated*. This is in line with the results of coarse-grained simulations of the ternary mixture of diarachidoylphosphatidylcholine, dilinoleoylphosphatidylcholine, and cholesterol [29] of which the first two components differ markedly in curvature. The simulations show a modulated stripe phase in which the stripes in the two leaves are indeed anticorrelated. This anticorrelation has interesting consequences for the microemulsion. For example, an area in the outer leaf rich in saturated lipids and cholesterol would, due to the damped oscillations in composition, be bordered by a region rich in unsaturated lipids. These areas are anticorrelated with regions of the inner leaf, i.e., the above areas in the outer leaf face in the inner leaf a region rich in unsaturated lipids bordered by one rich in saturated lipids and cholesterol. It would be most interesting, of course, to determine whether domains either in the microemulsion or modulated phases are indeed anticorrelated. Perhaps this could be accomplished in the modulated phases by tagging the lipids on the inner and outer leaves of the vesicles with different dyes. Another possibility would be to observe images obtained from the system after being subjected to freeze fracturing as in ordinary microemulsions [33].

The hypothesis leads to additional predictions. For example, the ternary system of POPC, DSPC, and cholesterol does not undergo macroscopic phase separation but does show nanodomains [34]. One also knows that the ternary system of

POPC, DPPC, and cholesterol does not undergo macroscopic phase separation [35]. As the difference in spontaneous curvature of the two cholines in the latter is certainly larger than in the former, one would expect nanodomains to be present, and this can be ascertained by Förster resonance energy transfer (FRET). It would also be of interest to consider the ternary systems in which DPPC is replaced by dimyristoylphosphatidylcholine or dilauroylphosphatidylcholine as these compounds would also increase the curvature difference. The dependence of the characteristic wave number of the domains $k_c$ [see Eq. (9)] on the surface tension suggests that the domain size, obtained experimentally by FRET, could be varied by controlling the tension in experiments [36].

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