Membrane heterogeneity: Manifestation of a curvature-induced microemulsion

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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 in one leaf and the other. Because of this coupling, the most easily excited fluctuations can occur at non-zero wavenumbers. 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 towards modulation remains manifest in a liquid phase that exhibits 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.

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 or 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 a 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, the other rich in the third[7]. Hence rafts might occur in a two-phase region and simply be a 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 composition which 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 size of these fluctuations are proposed to be limited by the cytoskelton[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, which is 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 teranary systems that contain no lipids with one saturated and one unsaturated tail, as in the system dipalmitoylphosphatidylcholine (DPPC), dilauroylphosphatidylcholine (DLPC), and cholesterol[19].

Although it appears that one can rule out the presence in a biological membrane of a microemulsion which is induced by a line-active agent, this does not necessarily rule out the presence of a microemulsion formed by other means. 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 modulated phases 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 which are responsible for the modulated phases, and second, the characteristic size of the droplets in the two-dimensional microemulsion to which the 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 in one leaf and the other couple to fluctuations in the curvature of the membrane. That is, lipids with larger headgroups and smaller tails will be attracted preferentially to the outer leaf in regions where the membrane bulges outward, while lipids with smaller heads and larger tails will be 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 with the unsaturated lipid the other component. There are two order parameters representing the differences in mol fractions of these two components in the inner leaf, $\Phi_i(\mathbf{r})$, and in the outer leaf, $\Phi_o(\mathbf{r})$. It is convenient to consider the two linear combinations $\phi(\mathbf{r}) \equiv (\Phi_i(\mathbf{r}) - \Phi_o(\mathbf{r}))/2$, and $\psi(\mathbf{r}) \equiv (\Phi_i(\mathbf{r}) + \Phi_o(\mathbf{r}))/2$. 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

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

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

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

with κ the bending modulus and γ the surface tension. Lastly there is the coupling between the curvature, $\nabla^2 h$, and the difference in compositions between the two leaves, ϕ;

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

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

$$\begin{aligned} F_{tot} &= \int d^2k[(a + \frac{b}{2}k^2)\phi(k)\phi(-k) \\ &\quad +(a_\psi + \frac{b_\psi}{2}k^2)\psi(k)\psi(-k) \\ &\quad +\frac{1}{2}(\kappa k^4 + \gamma k^2)h(k)h(-k) \\ &\quad -\lambda(b\gamma)^{1/2}k^2 h(-k)\phi(k)]. \end{aligned} \tag{4}$$

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

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

This form of the free energy displays everything that is needed. First, the wavevector, 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

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

Thus the system is softest at a non-zero wavevector when $\lambda > 1$. This requirement is understood as follows. The coupling between the curvature and the difference in compositions favors a soft wavevector k^* that is non-zero. As a structure with such a wavenumber is curved, and thus of larger area than when flat, this bending is opposed by the surface tension γ. Further as regions of different compositions alternate, their occurrence is opposed by a line tension between such regions, a line tension 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 non-zero wavenumber, $k^* \neq 0$, 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 λ 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 non-zero 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 the earlier works[22,25], and their possible manifestations in coupled bilayers has 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 wavevectors 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 a fluid that still reflects a tendency towards order. That tendency is manifest in its composition fluctuations, which are strongest at a non-zero wavevector. 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 non-zero when $\lambda - 1 > 0$. The structure is also reflected in the correlation function, $g(\mathbf{r})$, which is the inverse Fourier transform of $S(\mathbf{k})$. In the interesting regime in which $(2a\kappa/b\gamma)^{1/2} > \lambda - 1 > 0$, and for small wavenumbers $\kappa k^2/\gamma < 1$ it is straightforward to show that $g(r)$ behaves, for large r like $g(\mathbf{r}) \approx r^{-1/2}\exp(-r/\xi)\sin(k_c r+\delta)$, with k_c and ξ given explicitly below and δ a phase of no interest. The exponential damping, with a characteristic correlation length ξ, implies that the system is disordered, 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, ξ, and characteristic wavenumber, k_c, are given by

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

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

The correlation length ξ 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 $\lambda > 1$ which indicates 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, the characteristic size of the fluctuating regions is on the order, 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 could display a microemulsion is strongly indicated by the recent observation in giant unilamellar vesicles of modulated phases 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 modulated phases is also evidenced by other results of this four-component system. With fixed mole fractions of DSPC and cholesterol, the relative mole fraction $\rho \equiv$ [DOPC]/([DOPC]+[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 ρ from small values should drive the system towards a modulated phase, and this is indeed what is observed. Similarly decreasing the value of ρ 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. Within the hypothesis I have proposed, this fluid, labeled "nanoscopic" by Konyakhina et al.[20] would be a microemulsion.

Additional support for the mechanism proposed here would be provided by an estimate of the value of λ which characterizes the strength of the coupling $\lambda(b\gamma)^{1/2}$ between the curvature and the difference of lipid mol 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 δH is the difference in spontaneous curvatures of cholesterol-rich raft domains and the phospholipid background. With the coupling b of Eq. (1) of order k_BT, this yields a simple expression for the dimensionless coupling $\lambda = [\kappa/k_BT][k_BT(\delta H)^2/\gamma]^{1/2}$. Liu et al. estimated δH to be on the order of 10^6m^{-1} and took $\gamma = 3.1 \times 10^{-6}$N/m and $\kappa = 400k_BT$. These values yield an estimate for λ of about 14 which would imply 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 $\kappa = 2.7 \times 10^{-19}Nm= 66k_BT$, 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 -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 would 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 fracture, as in ordinary microemulsions[33].

The hypothesis makes additional predictions. For example the ternary system POPC, DSPC, and cholesterol does not undergo macroscopic phase separation but does show nanodomains[34]. One also knows that the ternary system 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 would also increase the curvature difference. The dependence of the characteristic wavenumber of the domains, k_c Eq. (9), on surface tension suggests that the domain size, obtained experimentally by FRET, could be varied by controlling the tension in experiment[36].

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