Theories of Equilibrium Inhomogeneous Fluids

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Abstract

I review two theoretical explanations for the existence of inhomogeneities in a fluid bilayer, such as the mammalian plasma membrane, which one might well expect to be homogeneous. The first is the existence of a phase separation. If biologically relevant temperatures are below the critical temperature of the separation, then these inhomogenieties are simply inclusions of one phase within the other. One has to understand, however, why macroscopic separation is not seen in the plasma membrane. If biologically relevant temperatures are above the critical temperature, then the inhomogenieties could be ascribed to critical fluctuations. There are difficulties with this interpretation which I note. The second possible interpretation is that the dynamic heterogeneities are evidence of a two-dimensionl microemulsion. Several mechanisms which could give rise to it are discussed. Particular attention is paid to the coupling of membrane height fluctuations to composition differences. Such a mechanism naturally gives rise to a length scale which is of the correct order of magnitude for the domains postulated to exist in the plasma membrane.

1 Introduction

With such great interest in the hypothesis that the mammalian plasma membrane is characterized by inhomogenieities, or "rafts", of a characteristic size on the order of 100 nm, (1, 2), it is incumbent upon us to understand how such distinct regions could come about. Why should a fluid be heterogeneous; more specifically, why should a biological membrane be heterogeneous? What mechanism overcomes the entropic tendency for all components to mix uniformly? There are not many candidates for such a mechanism that one can invoke, and even fewer if one assumes the membrane to be in thermal equilibrium, as I shall. Non-equlibrium processes are discussed elsewhere in this volume. Furthermore I will concentrate on a pure lipid bilayer, and ignore the possible effects that proteins could have on bringing about non-uniformity in a lipid system. I focus on the two mechanisms most often discussed. The first is simple phase separation, which has been observed recently in different membranes, those of yeast vacuoles (3).

2 Phase Separation and Associated Critical Fluctuations

Consider first a system containing only one species of lipid, say the saturated dipalmitoylphosphatidylcholine, (DPPC). At high temperatures, its acyl chains explore many configurations in which the chains are not at all straight but are rather disordered, a disorder characterized by the appearance of thermally-excited kinks, i.e. gauche bonds. As a consequence, the chains do not pack well together. The system is in a liquid phase. As the temperature is lowered, the number of these thermally excited gauche bonds decreases. At the main-chain transition temperature, the number of these bonds decreases discontinuously; the chains become more ordered and pack together better. The system is in the gel phase.

The chains of a mono-unsaturated lipid, such as dioleoylphosphatidylcholine, (DOPC), are characterized by a permanent kink at the site of the *cis* double bond. As a consequence, it is more difficult for these chains to pack together and they are always more disordered than saturated chains of the same length at the same temperature. Hence the temperature of their main-chain transition is lower than that of the system of saturated chains.

Now consider a two-component mixture of DPPC and DOPC. Due to the presence of DOPC with its disordered chains, it is more difficult for the DPPC to order. When the temperature is lowered sufficiently for ordering

to occur, the saturated lipids expel many of the unsaturated ones resulting in the coexistence of two phases: a DPPC-rich gel phase and a DOPC-rich liquid phase. The transition is a first-order one; that is, there is a difference in the densities of DPPC and DOPC in the two phases. That chain-packing is indeed the mechanism which drives the transition is borne out by calculations on microscopic models of the lipids that describe very well the configurations of the lipid chains (4). The results are in good agreement with experiments which observe the phase transition in such systems (5).

The addition of cholesterol to this mix changes things in an interesting way. The rather rigid cholesterol molecule does not insert itself well in between the tightly packed tails of the gel phase. Hence its presence tends to disorder it. With the addition of enough cholesterol, the DPPC-rich gel phase melts to a DPPC-rich liquid, one quite distinct from the DOPC-rich liquid which coexisted with the gel phase. So now the ternary system can exhibit two different liquid phases. Not only do they differ in composition, but they also differ in the degree of order of the acyl chains. As noted above, the chains of the DOPC-rich liquid are rather disordered. Those of the saturated DPPC-rich liquid are more ordered. Furthermore now that the cholesterol can insert itself between the less-tightly packed chains of the DPPC-rich fluid, its rigidity tends to further order those chains. Because of the difference in the average configuration of chains in the two fluids, they are denoted liquid-ordered, and liquid disordered, respectively (6). A typical ternary phase diagram, this one for the system of the saturated lipid, palmitoylsphingomyelin (PSM), the unsaturated lipid, palmitoyloleloylphosphatidylcholine (POPC), and cholesterol (7) is shown in Fig. 1. It exhibits all three phases; gel, denoted S_o in the figure, liquid-ordered, (L_o), and liquid-disordered, (L_d). There is a region in which all three phases coexist. The two liquid phases become one at a critical point. Again, the above explanation for the phase behavior is supported by a theoretical calculation (8) which embodies these ideas, treats the chains accurately, and produces a ternary phase diagram with the same general features as that in Fig.1. Once the origin of the phase behavior is understood, it can be reproduced by simpler models which replace the many coordinates needed to specify a chain configuration by a single order parameter (9). Even more simply, one can restrict that order parameter to only two values thereby dividing the saturated chains into just two classes; ordered, representing chains with few gauche bonds, and disordered, representing chains with more. Because one does not control the number in each class, the configurations freely interchange with one another (10). Molecular Dynamics simulations of coarse-grained models of ternary mixtures of cholesterol, a saturated, and an unsaturated lipid,

while not attempting to obtain the whole phase diagram, do find the new and interesting feature of these ternary systems, namely, the coexistence of two liquid phases (11, 12).

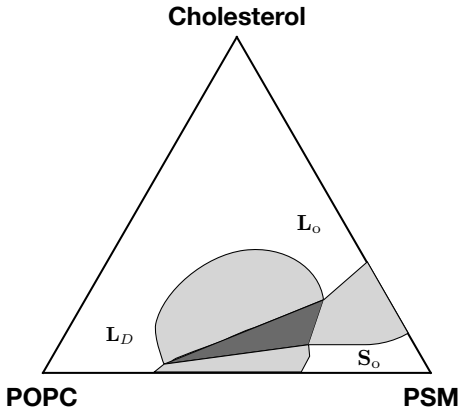

Figure 1: Phase diagram of a ternary mixture of POPC, sphingomyelin, here labeled PSM, and cholesterol. There are two liquid phases, labeled L_o and L_d, and a gel phase, S_o. The three regions of coexistence between two phases are shown in light gray, and the triangular region of coexistence of all three phases is shown in dark gray. After reference (7)

As noted, for the particular temperature, 23°C, for which the phase diagram in Fig. 1 was determined, there is a critical point at a particular concentration of the components. As such a point exists at nearby temperatures as well, there is a critical line in the phase diagram when temperature is included as a variable. The behavior of a system near a critical point has been studied intensely and is well understood. In particular, the one-phase fluid near the critical point is characterized by droplets with compositions corresponding to either the L_o or L_d phase and of a size characterized by a correlation length ξ which diverges as the critical point is approached. In particular, if the composition of the system corresponded to one on the critical line at a temperature T_c (in degrees Kelvin) then, as the temperature approached T_c, the correlation length would diverge as

$$\xi(T) = \xi_0 \left(\frac{T - T_c}{T_c} \right)^{-\nu}, \tag{1}$$

where for a lipid bilayer ξ_0 is of the order of a few nm and the critical exponent $\nu = 1$. That this should be the case for the critical point of a

miscibility transition of a lipid bilayer follows from the modern theory of critical phenomena; in particular that the miscibility transition in the two-dimensional bilayer is characterized by a one-component order parameter and is therefore in the universality class of the two-dimensional Ising model of which the exact critical behavior was famously solved by Lars Onsager (13). Nevertheless it was nice to have this strong expectation confirmed by experiment (14).

The phenomenon of macroscopic liquid-liquid phase separation provides a plausible explanation for the origin of inhomogeneities, or "rafts," in the plasma membrane: they are droplets of one phase immersed in a background of the other. Presumably if one waited long enough, these droplets would coalesce into a macroscopic phase so that one would observe the coexistence of two phases as one does in the case of yeast vacuoles (3).

But nothing like liquid-liquid phase separation is seen in the plasma membrane. Why is this? Several answers have been proposed. One is that while the transition does occur, the macroscopic separation of phases which should accompany it is prevented by the underlying cytoskeleton which forms a network of corrals (15). The size of these corrals was found to be of the order of 300 nm, large enough to hold a raft, so this idea explains the absence of macroscopic phase separation, but preserves the idea of a phase transition being the cause of the inhomogeneities.

A second line of argument (16) is that integral membrane proteins or attachments of the membrane to the cytoskeleton favor one of the phases over the other and, occurring randomly across the membrane, destroy the transition just as a random field is known to do in the two-dimensional Ising model (17). This argument would seem to doom an explanation of rafts in terms of phase separation.

A third possible reason for the lack of macroscopic phase separation is that, while the biological system can undergo a miscibility phase transition, the critical temperature is below that of relevant body temperature. However if the latter is sufficiently close to a critical transition, there are large fluctuations as noted above, and these could be identified as rafts (18, 19). The effect of a cytoskeleton-like network is to cut off fluctuations larger than the mesh size of the network, but smaller fluctuations remain and, again, could be identified as the much-sought after inhomogeneities (20). This explanation does require that the system, at a biological temperature T, be close to the line of critical transitions. If the transition were at a temperature $T_c = T - \Delta T$, then the correlation length $\xi(T)$ at T would be larger

than the typical size of a lipid, ξ_0, by a factor

$$\frac{\xi(T)}{\xi_0} = \frac{T - \Delta T}{\Delta T}. \tag{2}$$

Note that there is no "characteristic size" of the fluctuations. Rather their size depends upon how close the system is to its critical point. If $\xi(T)$ were to be 30 nm, an order of magnitude larger than $\xi_0 \approx 3$nm, then the system would need be about 30 °C above a critical transition. But it is more than that; the system at biological temperature would have to have *almost the same composition* as the system which is critical at the lower temperature. This is *a priori* unlikely and one must argue that the cell regulates its composition in order to be near the critical transition. There is no evidence that this is, or is not, the case for the plasma membrane. The evidence from giant plasma membrane vesicles (18). isolated from living cells and carrying no cytoskeleton, is reviewed elsewhere in this volume by Cicutta and Veatch. Another problem which must be addressed if one favors criticality as the origin of rafts is that a miscibility transition seems to be characteristic only of the exoplasmic leaf of the plasma membrane. Lipid bilayers in which both leaves have a composition characteristic of the cytoplasmic leaf of the plasma membrane do not exhibit phase separation (21). That is because the lipids in the cytoplasmic leaf are almost all unsaturated. There are too few saturated lipids to bring about a phase of their own. Therefore if phase separation were to occur in the plasma membrane, composition differences in the cytoplasmic leaf would be small. As a consequence of that, there would be little distinction between "raft" and "sea", hence no useful mechanism for conveying information from one leaf to the other. The same argument would apply to fluctuations near a critical point.

In addition to the three possible reasons given above for the lack of a macroscopic phase separation in the plasma membrane, there is also a fourth: macroscopic phase separation is not seen because the plasma membrane is not near a miscibility phase transition. As noted above, whereas the exoplasmic leaf is expected to display a tendency to undergo phase separation, the cytoplasmic leaf is not. Any coupling between the exoplasmic and cytoplasmic leaves will tend to drive the system away from phase separation temperatures characteristic of the exoplasmic leaf, an effect seen experimentally (22, 23) and understood theoretically (24, 25).

In closing this section, I can summarize by saying that nothing like the phase separation observed in yeast vacuoles is seen in the plasma membrane of mammalian cells. Large Molecular Dynamics simulations of the plasma membrane do not see such separation (26). Whether associated critical

fluctuations will prove to be the origins of rafts remains to be seen, and the case is open. Personally I remain a skeptic on this, both because the cell would have to regulate its composition to bring it near a critical transition, and because it is not clear that there would be much of an effect in the cytoplasmic leaf. That lack would defeat the purpose for which rafts were proposed.

3 Modulated Phases and Associated Microemulsions

For those of us who had worked on the theory of inhomogeneous fluids in three-dimensional systems (27), the idea that rafts could be associated with two-dimensional microemulsions was an appealing one. After all, microemulsions are characterized by regions, or droplets, which have a characteristic size and which are dynamic, fluctuating objects. In the best-known bulk system of oil, water, and amphiphile, the latter, as its name implies, loves both of the former, gaining energy by sitting between them. Because it likes the oil and water to mix, it increases the region of phase space in which they do so, driving down the miscibility transition temperature (28). Further it reduces the surface tension between coexisting phases. If enough amphiphile is added, the energy of such interfaces is driven to zero and a modulated phase, one of lamellae or cylinders or droplets, appears in which there is an extensive amount of oil-water interface. The observation of modulated phases, or perhaps melted versions of them in lipid bilayers (29–31), and in giant plasma membrane vesicles strengthens the idea that rafts could be identified with a microemulsion.

The problem with identifying rafts with the droplets of a microemulsion is that there is no obvious amphiphile that loves both saturated and unsaturated lipids. In particular, cholesterol is certainly not. One knows this because the addition of cholesterol to a one-phase region of a mixture of saturated and unsaturated lipids brings about phase separation, i.e. raises the miscibility transition (32), a clear indicator that the cholesterol prefers one component to the other, and hence benefits if the two components separate. Safran and co-workers (33, 34) proposed that the common unsaturated lipids, those with one saturated tail and one unsaturated tail, which they called "hybrid" lipids, could be *both* a major component of the liquid-disordered phase as well as an amphiphile which would like to sit between that phase and the saturated-rich liquid-ordered phase. The idea is that at an $L_o - L_d$ interface, the hybrid lipid will reduce its energy if its sat-

urated leg is oriented toward the L_o phase and its unsaturated leg toward the L_d phase. This leads naturally to a model in which the hybrid lipids are modeled by two-dimensional vectors (35). Such models have been explored extensively (33–37) and have been reviewed recently (38, 39).

There is no doubt in my mind that the mechanism works in principle, but one must believe that the energy gain in *orienting* a hybrid lipid at a $L_o - L_d$ interface is substantial, comparable to the repulsive interaction between saturated and unsaturated lipids itself. Further, recent experimental evidence appeared that indicates that hybrid lipids do not play a unique role as an amphiphile in lipid bilayers (40).

But if there is no amphiphile in the lipid bilayer, is it possible to bring about modulated phases and microemulsions in them by some other means? The answer to this question is, yes, there is. It is well known that there are many mechanisms, several not employing an amphiphile, that can bring about modulated phases in many different kinds of systems (41). One that is of particular interest for lipid bilayers is the coupling of lipid curvature to height fluctuations of the membrane (42–44). The basic idea is that in a height fluctuation, the membrane will bend outward in some places, and bend inward in others. Lipids with a large head group and small tail will move toward the former regions whereas lipids with a small head group and large tails will move to the latter. If the coupling between the fluctuations and composition is sufficiently strong, the system will form modulated phases (45). It follows that the system can also support a microemulsion because a microemulsion can be viewed simply as a melted modulated phase (46).

In order for the coupling to be strong, it is clear that there must be a significant difference between the spontaneous curvatures of the lipids. Unfortunately this is not the case in the exoplasmic leaf. The major lipid components of this leaf are phosphatidylcholine, (PC), and sphingomyelin (SM), both of which have similar, small spontaneous curvatures (47). But in this regard the cytoplasmic leaf is quite another story. Its major components are phosphatidylserine, (PS), and phosphatidylethanolamine, (PE). The first, again, has a small spontaneous curvature, but that of PE is large in magnitude because of the small PE head group. Thus the difference in spontaneous curvatures of the two components is large. If there is any hope that this mechanism brings about a microemulsion in the plasma membrane, it seems that it will be due to a coupling of height fluctuations to composition differences in the cytoplasmic leaf. That there are composition differences in this leaf will be conveyed to the exoplasmic leaf by coupling between the leaves. The exoplasmic leaf will respond presumably because, as we have seen, its composition is such that it is near a phase separation which implies

that the response of its lipids to perturbations in composition is large. In this way the system brings about a raft in *both* of its leaves (48). This is in contrast to the problem I noted above, that a raft initiated by phase separation in the outer leaf is not expected to have much effect on the composition of the inner leaf.

A theoretical description of the system is readily formulated. We denote by $\phi(\mathbf{r})$ the local difference in mol fraction of PS and PE in the inner leaf, and by $\psi(\mathbf{r})$ that of SM and PC in the outer leaf. We assume equal numbers, N, of lipids in the two leaves which have equal areas A. The local, planar, free energy functional per unit area of the bilayer can be written in the usual form (45)

$$\begin{aligned} f_{plane}[\phi(\mathbf{r}),\psi(\mathbf{r})] &= -J_\phi n\phi^2 + \frac{k_BT}{2}n[(1+\phi)\ln(1+\phi)+(1-\phi)\ln(1-\phi)], \\ &- J_\psi n\psi^2 + \frac{k_BT}{2}n[(1+\psi)\ln(1+\psi)+(1-\psi)\ln(1-\psi)] \\ &- \Lambda\phi\psi, \end{aligned} \tag{3}$$

where k_B is Boltzmann's constant and T the temperature, $n \equiv N/A$ is the areal density of lipids, and Λ is a energy of coupling between the leaves, The quantities $J_\psi > J_\phi > 0$ are interaction energies, and the inequality ensures that the outer leaf is closer, in temperature, to a phase separation than is the inner. The total free energy of the planar bilayer is then

$$F_{plane}[\phi,\psi] = \int d^2r \left[\frac{b_\phi}{2}(\nabla\phi)^2 + \frac{b_\psi}{2}(\nabla\psi)^2 + f_{plane}\right], \tag{4}$$

where b_ϕ is related to the energy per unit length between regions rich in PS and those rich in PE, and b_ψ is similarly related to the energy per unit length between regions rich in SM and those rich in PC.

The elastic free energy of the bilayer is taken to be (49)

$$F_{curv}[h] = \int d^2r \left[\frac{\kappa}{2}(\nabla^2 h)^2 + \frac{\gamma}{2}(\nabla h)^2\right] \tag{5}$$

where $h(r)$ is the height of the bilayer from some reference plane and κ and γ are the bilayer bending modulus and surface tension respectively. The latter is the tension related to the membrane's response to normal, i.e. perpendicular to the membrane, strain (50, 51). It is often referred to as the "frame tension". It is the quantity that can be obtained from tether-pulling experiments.

Now one couples the curvature of the bilayer to the difference in mol fractions of PS and PE in the inner leaflet:

$$F_{coupl}[\phi, h] = -\Gamma \int d^2r \ \phi(\mathbf{r})\nabla^2 h(\mathbf{r}). \tag{6}$$

The total free energy, $\tilde{F}_{tot}[\phi, \psi, h]$ is then $\tilde{F}_{tot} = F_{plane} + F_{curv} + F_{coupl}$.

It is appropriate at this point to mention related work of Friederike Schmid and collaborators (52, 53). Suppose that one wanted to study bilayers containing PE in the laboratory. Most likely they would be symmetric bilayers. In such a case, the curvature of the bilayer would couple to composition differences in *both* leaves. One can picture PE-rich regions opposite one another bending inward on both leaves. It is essentially this situation described by Schmid. But again, this differs from the asymmetric plasma membrane in which the PE is almost completely in the inner leaf.

Returning to the total free energy above, we minimize it with respect to the height variable $h(\mathbf{r})$ for a given distribution of the membrane components, one specified by $\phi(\mathbf{r})$ and $\psi(\mathbf{r})$. After doing so, we express the resulting free energy in terms of the Fourier transforms of $\phi(\mathbf{r})$ and $\psi(\mathbf{r})$, and obtain

$$\begin{aligned} F_{tot}[\phi, \psi] &= \int d^2r \ f_{plane} + \frac{A^2}{(2\pi)^2} \int d^2k \left[\frac{b_\psi}{2} k^2 \psi(k)\psi(-k) + \right. \\ &\left. \frac{b_\phi}{2} \left\{ 1 - \frac{(\Gamma^2/b_\phi\gamma)}{1 + \kappa k^2/\gamma)} \right\} k^2 \phi(k)\phi(-k) \right]. \end{aligned} \tag{7}$$

Note that the free energy to bring about spatial variations in the order parameter $\phi(\mathbf{r})$, which had been $[b_\phi/2][\nabla\phi]^2 \propto [b_\phi/2]k^2\phi(k)\phi(-k)$, is reduced by its coupling to the height fluctuations. Of most interest to us is a disordered, fluid phase, for which the ensemble average values of all quantities are constant, independent of position. To examine the fluctuations in that phase, we expand $\phi(r)$ and $\psi(r)$ about their average values $\bar{\phi}$ and $\bar{\psi}$, and then expand the free energy, $F_{tot}[\phi, \psi]$, about that of the uniform fluid phase to second order in these deviations. The result is (54)

$$\begin{aligned} F_{tot}[\phi, \psi] &= F_{tot}(\bar{\phi}, \bar{\psi}) \\ &+ \frac{A^2}{(2\pi)^2} \int d^2k \left[\left\{ a_\phi + \frac{b_\phi}{2} \left[1 - \frac{(\Gamma^2/b_\phi\gamma)}{(1 + \kappa k^2/\gamma)} \right] k^2 \right\} \phi(k)\phi(-k) \right. \\ &+ \left. \left(a_\psi + \frac{b_\psi}{2} k^2 \right) \psi(k)\psi(-k) - \Lambda\phi(k)\psi(-k) \right], \end{aligned} \tag{8}$$

where

$$\begin{aligned} a_\phi &= \frac{n}{2}\left[\frac{k_BT}{1-\bar{\phi}^2} - 2J_\phi\right] \\ a_\psi &= \frac{n}{2}\left[\frac{k_BT}{1-\bar{\psi}^2} - 2J_\psi\right]. \end{aligned}$$

The quantity a_ψ, with the dimension of energy per unit area, measures how far the temperature T is from the critical temperature, $2J_\psi/k_B$, of a symmetric, uncoupled (i.e. $\Lambda = 0$), bilayer with equal average compositions of SM and PC ($\bar{\psi} = 0$). A similar statement applies to a_ϕ.

One can see what the fluid phase is like by examining the structure functions

$$\begin{aligned} S_{\phi\phi} &\equiv \langle\phi(k)\phi(-k)\rangle, \\ S_{\psi\psi} &\equiv \langle\psi(k)\psi(-k)\rangle, \\ S_{\phi\psi} &\equiv \frac{\langle\phi(k)\psi(-k) \ + \ \psi(k)\phi(-k)\rangle}{2}, \end{aligned}$$

which are all measurable, in principle, by means of scattering. The brackets denote an ensemble average. The results are (37, 55)

$$S_{\phi\phi} = \frac{2g_\psi}{4g_\phi g_\psi - \Lambda^2} \tag{9}$$

$$S_{\psi\psi} = \frac{2g_\phi}{4g_\phi g_\psi - \Lambda^2} \tag{10}$$

$$S_{\phi\psi} = \frac{\Lambda}{4g_\phi g_\psi - \Lambda^2} \tag{11}$$

where

$$g_\phi(k) = \Lambda\left\{\frac{(b_\phi/2\Lambda)(\kappa/\gamma)k^4 - (b_\phi/2\Lambda)[(\Gamma^2/b_\phi\gamma) - 1]k^2}{1 + \kappa k^2/\gamma} + \frac{a_\phi}{\Lambda}\right\} \tag{12}$$

$$g_\psi(k) = \Lambda\left\{\frac{b_\psi}{2\Lambda}k^2 + \frac{a_\psi}{\Lambda}\right\}. \tag{13}$$

From this, one sees that there is a characteristic length in the system, $(\kappa/\gamma)^{1/2}$ which originates from the properties of the membrane. Let us pause and evaluate this length for the plasma membrane. Both the bending modulus, κ, and the surface tension, γ, of the plasma membrane have been measured several times. The values obtained vary by an order of magnitude due to the use of different cell lines and methods of measurement. Results for

the bending modulus range from 1.8×10^{-19}Nm to 1.6×10^{-18}Nm, while those for the surface tension fall between 10^{-6}N/m and 10^{-5}N/m. I choose values from a recent measurement (56): $\kappa = 4.1\times10^{-19}$ Nm and $\gamma = 0.8\times10^{-5}$N/m. This yields a characteristic length of 226 nm which is certainly of the correct order of magnitude of the phenomena one is trying to explain. This does not mean that it is the correct explanation, but at least it indicates that it is not obviously wrong.

The structure functions tell us the response of the system to fluctuations in the order parameters; i.e. they are essentially susceptibilities to perturbations in the order parameter at a certain wavelength. The essence of the phase diagram can be obtained from them. There are four phases. At high temperatures and for concentration-curvature couplings Γ which are not too large, the system is in a disordered fluid phase. As the temperatures is lowered, the system undergoes a transition to two coexisting fluid phases. In one, assuming that the inter leaf coupling $\Lambda > 0$, the inner leaf is rich in PS, and the outer in SM, while in the other phase, the inner leaf is rich in PE, and the outer in PC. In the disordered phase in the vicinity of the transition, all structure functions are peaked at $k = 0$, and as the transition is approached, all structure functions diverge. If the temperature is lowered for large couplings Γ, then the system makes a transition to a modulated, striped, phase. Just above the transition, the structure functions take their maximum values at some $k^* > 0$ and diverge as the transition is approached. These two lines of continuous transitions meet at a Lifshitz point which occurs at some coupling Γ_{Lif}. At lower temperatures, the modulated phase coexists with the two fluid phases along a triple line. A phase diagram is shown in Fig. 2 as a function of the temperature-like variable $a \equiv a_\phi$ and $\tau \equiv [\Gamma_{Lif} - \Gamma]/(b_\phi\gamma)^{1/2}$.

The disordered fluid is of particular interest, and its nature can again be determined by examining the structure functions, in particular, $S_{\phi\phi}(k)$. When the coupling, Γ, between curvature and composition is small, the peak in $S_{\phi\phi}(k)$ occurs at $k = 0$, and the fluid is an ordinary one. The density-density correlation function, which is the Fourier transform of the structure function, is characterized by a single length, the correlation length, over which correlations decay. But for larger values of Γ, the peak in $S_{\phi\phi}(k)$ occurs at some non-zero value of k indicating that the fluid is most susceptible to fluctuations which vary in space. The density-density correlation function is characterized by *two* lengths, and behaves like an exponentially-damped oscillatory function. The scale of the damping is the correlation length and the additional length is the wavelength of the oscillation in space of the fluctuations. It is the same length which characterizes the nearby

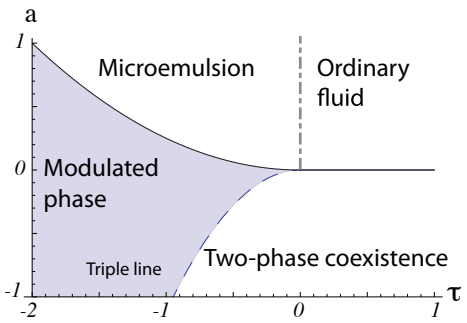

Figure 2: Phase diagram of the model as obtained from mean-field theory which does not include effects of fluctuations. The phases are shown as a function of the temperature-like parameter, a, and the coupling, τ. Solid lines denote continuous transitions, while the dashed line denotes a triple line, i.e. three-phase coexistence. The dashed-dotted line is the Lifshitz line separating the ordinary disordered fluid from the microemulsion.

modulated phase. When the modulated phase melts, the fluid to which it melts "remembers" the characteristic length scale. This is analogous to the melting of a solid to a liquid; the liquid's density-density correlation function clearly shows that the first few neighbors are at about the same distance that they were in the solid. It is this disordered fluid which clearly has structure which is denoted a *microemulsion*. There is no phase transition between the ordinary disordered fluid and the microemulsion, no singularity in the free energy. Thus the boundary in the phase diagram between these two fluids is an arbitrary one. A common, and experimentally accessible, definition is the locus of points at which the peak in a structure factor moves off of zero wave vector. That locus is called the Lifshitz line. It is denoted in Fig. 2 by the dashed-dotted line. Note that within this phase diagram, obtained via a mean-field theory, the microemulsion and the regions of two-phase coexistence are not contiguous; that is, there is no phase transition from the one to the other. It is always the ordinary fluid that undergoes phase separation.

Some of the effects of thermal fluctuations on this phase diagram have been investigated by simulations (57, 58). The effects are seen in Fig. 3. Of interest is that the microemulsion and two-phase coexistence are now close to one another in the sense that one can go from one to the other via a first-order transition (59). This phase diagram presents a simple explanation for the observation of the sequence of phase separation, followed by a modulated phase, followed by a disordered fluid in a four-component lipid mixture (29).

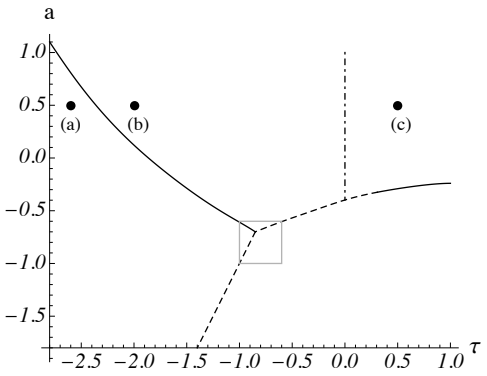

Figure 3: Phase diagram of the model including fluctuations as a function of the two parameters a and τ. Dashed lines denote first-order transitions, solid lines continuous ones. Phase boundaries within the boxed region are extrapolations from the regions outside. The parameter b_ϕ has been set to 4.0. The dashed-dotted line is the Lifshitz line. The dots a, b, and c indicate the systems whose representative configurations are shown in Fig. 4

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Representative configurations within the modulated phase, the microemulsion, and the ordinary fluid are shown in Fig. 4. That the microemulsion (b) is a melted version of the modulated phase (a) is clear. Similarly one see that as the parameters change to bring the system from (b) to the ordinary fluid (c), the amount of contrast, that is the difference in the order parameter, between neighboring droplets decreases.

4 Conclusion

Because the proposition (1, 2) that the plasma membrane is inhomogeneous, characterized by "rafts" rich in sphingomyelin and cholesterol, has attracted so much attention, one would certainly like to understand the physical basis for them. Assuming that the phenomenon is an equilibrium one, I have reviewed the two most likely explanations; 1) that they arise from a phase transition, or at least the proximity to one, or 2), that they are the manifestation of a microemulsion brought about by one of several possible mechanisms, also reviewed. So does either apply to the mammalian plasma membrane? Many questions must be resolved and these pose experimental challenges. Are there critical fluctuations in the plasma membrane itself as opposed to those fluctuations observed in giant plasma membrane vesicles? If the

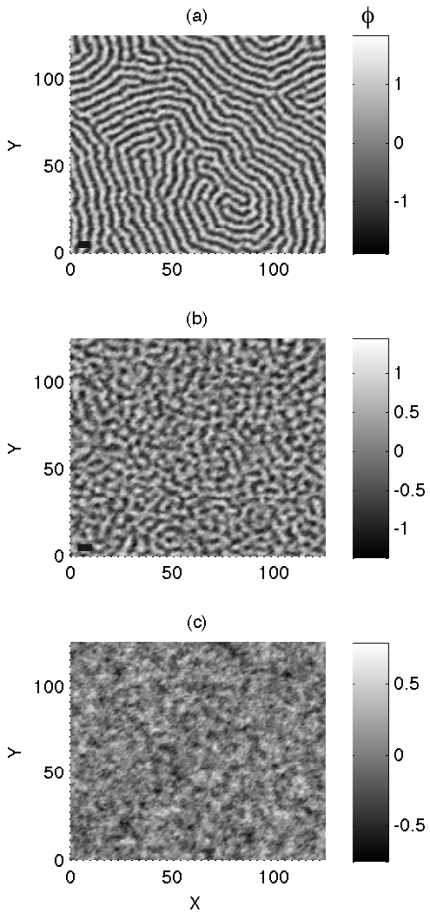

Figure 4: Representative configurations from different phases of the system. The parameter b_ϕ is set to 4. a) The location of the system is $a = 0.5$ and $\tau = -2.6$. and the system is in the stripe phase. b) The location of this system is $a = 0.5, \tau = -2.0$. The system is a microemulsion. c) At $a = 0.5$ and $\tau = 0.5$. The system is an ordinary fluid. These three systems are indicated in the phase diagram of Fig. 3.

plasma membrane were characterized by a microemulsion, how would one know it? One might think that a microemulsion, which has been predicted (57) to be one of the phases which has been observed *in vitro* (29) in micron-sized GUVs could be detected by neutron scattering experiments which are capable of determing the structure function. That of a microemulsion is characterized by a peak at a non-zero wave vector. But thus far, scattering experiments have only been carried out on small vesicles of a radius of tens of nanometers. A peak at non-zero wavevector was indeed observed in that system, but was interpreted by the authors as arising from the presence of circular domains of a system at coexistence (60). The interpretation is not unique however.

As I have noted, a system which exhibits phase separation could be very close, in its parameters, to another which exhibits a microemulsion. This is interesting as it prods one to compare the lipid composition of a system which clearly shows phase separation, like a yeast vacuole (3), to one in which the mechanism causing inhomogeneities is not clear, like the plasma membrane. One might well hope that comparative studies of the lipid compositionof these systems will resolve the very basic issue underlying the concept of rafts.

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