Strongly correlated rafts in both leaves of an asymmetric bilayer

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September 6, 2017

I’m afraid that the clarity
Of Professor Widom’s lecture
Basically obscures
The difficulty
Of the Physics

Leo Kadanoff
I consider a model of a bilayer characterized by two order parameters, one in each leaf. That of the inner leaf represents the difference in mol fractions of lipids with large spontaneous curvature, phosphatidylethanolamine, (PE), and those with small spontaneous curvatures, phosphatidylcholine, (PC), and phosphatidylserine, (PS). Similarly the order parameter in the outer leaf represents the difference in mol fractions of lipids with small spontaneous curvature, PC, and large curvature, sphingomyelin, (SM). Each order parameter is coupled to the variations in the height of the membrane that is assumed to be of constant thickness. The couplings are of different strength. I show that with reasonable parameters, a microemulsion is formed in each leaf, and that the two microemulsions are strongly coupled. Their characteristic size of domains is found to be on the order of 75 nm. In this picture, rafts consist of regions of SM in the outer leaf and PC and PS in the inner leaf, floating in a sea of PC in the outer leaf and PE in the inner leaf. I argue that microemulsions have been observed, but not identified as such, in model systems.
According to the raft hypothesis (1, 2), the plasma membrane is heterogeneous, characterized by domains of the order of 100 nm that serve as platforms for proteins, enabling them to aggregate and thereby function efficiently. These platforms are thought to be important to many cellular processes (3). The physical basis for the formation of these inhomogeneities is, however, unclear.

A common assumption is that such domains are the result of phase separation (4). One of the two coexisting phases is thought to be rich in saturated lipids and cholesterol. It is denoted “liquid ordered”, (lo). The other phase is assumed to be rich in unsaturated lipids, and is denoted “liquid disordered”, (ld) (5). This interpretation is supported by the fact that model membranes, consisting of cholesterol and relatively equal amounts of saturated and unsaturated lipids, do undergo phase separation (6). Applied to the plasma membrane, however, this assumption is fraught with difficulties. If there were phase separation, the domains would be expected to coarsen, as they do in model membranes, until only two macroscopic regions remained. Further, phase separation has never been observed in the mammalian plasma membrane. This absence is easily understood. The phase separation observed in model membranes is driven by the energetic cost of packing together the relatively straight saturated acyl chains with unsaturated chains whose cis double bond causes a kink in them. The outer leaflet of the plasma membrane is characterized by relatively equal amounts of saturated lipids, mostly sphingomyelin, and unsaturated ones. The inner leaflet, however, contains on the order of 0.05 mol fraction of sphingomyelin (7), the rest being unsaturated lipids. Hence phase separation will not occur in the inner leaf (8) and there will be no liquid-ordered phase in it (9). Consequently there can be no raft that spans the membrane in this picture.

A related proposal for the formation of rafts is that the inhomogeneities are critical fluctuations associated with a lo, ld miscibility transition that takes place at temperatures lower than biological ones (10). This argument is bolstered by the observation of phase separation in cell-derived giant plasma membrane vesicles (11). However the hypothesis shares the same difficulty with the one above; that there is so little saturated lipid in the inner leaflet of the plasma membrane that there can be no functional raft that spans both leaves.

A completely different idea is that the inhomogeneities are the manifestation of a microemulsion in the two-dimensional plasma membrane. Microemulsions in three-dimensions are, of course, well-known, and well-understood due to theoretical work by Widom (12) and others (13), but they are not well-known in membranes. I shall argue below that they have
been observed in several experiments, but have not been identified as such. Microemulsions could form in two dimensions if there were regions with two different properties, and if the free energy of the boundary between regions were small compared to thermal energies. The questions then arises as to what is the nature of the regions, and what is the mechanism driving down the boundary energy.

One suggestion was that the regions were, again, those rich in saturated lipids on the one hand, and rich in unsaturated lipids on the other. Further it was posited that the unsaturated lipids could reduce the energy per unit length between regions by orienting their tails at the boundary (14–16). Once again, the paucity of saturated lipids in the inner leaf of the plasma membrane vitiates the application of this idea to it.

An alternate hypothesis that can also lead to a microemulsion is that the two regions are characterized by lipids of rather different spontaneous curvature. The mechanism that drives down the energy between them is the coupling of lipid concentration and membrane shape; i.e the reduction of the bending energy of the membrane caused by a response of the local membrane curvature to the local spontaneous curvature of the lipids that comprise it (17, 18). This scenario was considered by Liu et al. (19) They estimated that to account for a raft of 100 nm, the difference in spontaneous curvature would have to be two orders of magnitude larger than the average spontaneous curvature estimated from experiment. Therefore, they concluded, this mechanism could not be responsible for the formation of rafts. I later argued (20), and will argue again below, that this conclusion is too pessimistic. It was then noted (21) that phophatidylethanolamine (PE), which has a rather large spontaneous curvature, is a major presence in the inner leaf of the plasma membrane, about 0.5 mol fraction of phospholipids. As the other major components, phosphatidylcholine, (PC), and phosphatidylserine, (PS), both have small spontaneous curvatures, one might expect a large coupling between these lipids and height variations of the membrane. If this were strong enough to bring about a microemulsion in the inner leaf of the plasma membrane, then a major problem in previous theories; i.e. the absence of a raft in the inner leaf, would have been overcome. How this raft in the inner leaflet would propagate to the outer leaf was addressed only by the incorporation of an intrinsic, unspecified, coupling between leaves.

In this paper, I note that the spontaneous curvature of sphingomyelin, SM, is also rather large compared to the other major component of the outer leaf, PC. Hence the outer leaf would also be expected to couple strongly to variations in the membrane height. Sphingomyelin makes up about 0.4 mol fraction of the phospholipids in the outer leaf (7). Thus an attractive
feature of a theory that posits that rafts are the result of a microemulsion, one brought about by the coupling of variations of lipid composition and membrane height, is that both leaves have significant differences in lipid spontaneous curvatures. As a consequence a raft is expected in both leaves. In this picture, illustrated schematically in Fig. 1 the raft consists of SM in the outer leaf and, opposite it in the inner leaf, phosphatidylcholine (PC) and phosphatidylserine (PS). The other region, the “sea” in which the raft floats, is comprised of PC in the outer leaf and, opposite it in the inner leaf, PE. I shall also show that the coupling between the inner and outer leaves of the raft are strongly correlated, as can already be intuited from the figure, so that there is no need to posit an intrinsic coupling between them.

1 Theoretical Model

I follow the earlier formulation of Ref. (21) with exceptions to be noted explicitly. I define an order parameter $\phi(r)$ in the inner leaf of the bilayer which represents the difference in local mol fractions in that leaf between PE on the one hand and PC and PS on the other. Similarly, define $\psi(r)$ as the difference in local mol fraction in the outer leaf between PC and SM. The free energy of a planar bilayer can then be written in the usual form

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

Figure 1: Regions rich in SM in the outer leaf and of PC and negatively-charged PS in the inner leaf floating in a sea of PC in the outer leaflet and PE in the inner leaf.
with
\[
  f_{\text{plane}}[\phi, \psi] = -J_\phi n \phi^2 + \frac{k_B T}{2} n [(1 + \phi) \ln(1 + \phi) + (1 - \phi) \ln(1 - \phi)],
\]
\[
  - J_\psi n \psi^2 + \frac{k_B T}{2} n [(1 + \psi) \ln(1 + \psi) + (1 - \psi) \ln(1 - \psi)].
\]

(2)

Here \( n \) is the areal density of lipids, \( k_B \) is Boltzmann’s constant and \( T \) the temperature. Also \( J_\phi < J_\psi \) are interaction energies, with their relative magnitudes reflecting the expectation that any miscibility transition temperature in the outer leaf would be greater than one in the inner leaf. No explicit coupling between order parameters is assumed.

The curvature free energy of the bilayer is taken to be
\[
  F_{\text{curv}}[h] = \int d^2r \left[ \frac{\kappa}{2} (\nabla^2 h)^2 + \frac{\gamma}{2} (\nabla h)^2 \right]
\]

(3)

where \( h(r) \) is the height of the bilayer from some reference plane and \( \kappa \) and \( \gamma \) are the bilayer bending modulus and surface tension respectively.

Finally we assume that the difference in compositions in the inner leaf couples to the membrane curvature with a coupling strength \( \Gamma_\phi \). Similarly the difference in compositions in the outer leaflet couples to the curvature with strength \( \Gamma_\psi \). I assume implicitly that the membrane is of constant thickness so that the curvatures of the two leaves are the same locally.

\[
  F_{\text{coupl}}[\phi, \psi, h] = -\int d^2r (\Gamma_\phi \phi + \Gamma_\psi \psi) \nabla^2 h
\]

(4)

Because the height variable \( h(r) \) enters only quadratically into the total free energy \( F_{\text{tot}}[\phi, \psi, h] = F_{\text{planar}}[\phi, \psi] + F_{\text{curv}}[h] + F_{\text{coupl}}[\phi, \psi, h] \), it can easily be eliminated. This is conveniently done in Fourier space in which
\[
  \phi(k) = \frac{1}{A} \int (\phi(r) - \bar{\phi}) e^{-ikr} d^2r,
\]

(5)

where \( A \) is the area of the bilayer, and similarly for \( \psi(k) \) and \( h(k) \), with \( \bar{\phi} \) and \( \bar{\psi} \) the average values of \( \phi(r) \) and \( \psi(r) \),
\[
  \bar{\phi} \equiv \frac{1}{A} \int d^2r \phi(r), \quad \bar{\psi} \equiv \frac{1}{A} \int d^2r \psi(r).
\]
Then
\[ F_{\text{tot}} = F_{\text{tot}}(\phi, \psi) + \frac{A^2}{(2\pi)^2} \int d^2k \left\{ \left[ a_\psi + \frac{b_\psi}{2} k^2 \right] \psi(k) \psi(-k) + \left[ a_\phi + \frac{b_\phi}{2} k^2 \right] \phi(k) \phi(-k) + \frac{1}{2} [\kappa k^4 + \gamma k^2] h(k) h(-k) + [k^2 \Gamma_\phi(-k) + k^2 \Gamma_\psi(-k)] h(k) \right\}, \tag{6} \]

where
\[ \int d^2 r \, f_{\text{plane}}[\phi, \psi] = F_{\text{tot}}(\phi, \psi) + \frac{A^2}{(2\pi)^2} \int d^2k [a_\phi \phi(k) \phi(-k) + a_\psi \psi(k) \psi(-k)], \tag{7} \]
\[ a_\phi = n \left[ \frac{k_B T}{1 - \phi^2} - 2 J_\phi \right] \tag{8} \]
\[ a_\psi = n \left[ \frac{k_B T}{1 - \psi^2} - 2 J_\psi \right]. \tag{9} \]

to second order in small quantities. The value of \( h(k) \) which minimizes \( F_{\text{tot}} \) is found to be
\[ h_{\text{min}}(k) = -\frac{\Gamma_\phi \phi(k) + \Gamma_\psi \psi(k)}{\gamma + k^2 \kappa}. \tag{10} \]

Substituting this into Eq. (6), we obtain
\[ F_{\text{tot}}[\phi, \psi] = F_{\text{tot}}(\tilde{\phi}, \tilde{\psi}) + \frac{A^2}{(2\pi)^2} \int d^2k (\phi(-k) \psi(-k) ) \begin{pmatrix} m_{11}(k) & m_{12}(k) \\ m_{21}(k) & m_{22}(k) \end{pmatrix} \begin{pmatrix} \phi(k) \\ \psi(k) \end{pmatrix} \]
\[ m_{11}(k) = a_\phi - \frac{b_\phi k^2}{2(1 + \kappa k^2/\gamma)} \left\{ \left[ \left( \frac{\Gamma_\phi^2}{b_\phi \gamma} \right) - 1 \right] - \left( \frac{\kappa}{\gamma} \right) k^2 \right\} \]
\[ m_{12}(k) = m_{21}(k) = -\frac{(b_\phi b_\psi)^{1/2} k^2}{2(1 + \kappa k^2/\gamma)} \left( \frac{\Gamma_\phi}{b_\phi \gamma} \right)^{1/2} \left( \frac{\Gamma_\psi}{b_\psi \gamma} \right)^{1/2} \]
\[ m_{22}(k) = a_\psi - \frac{b_\psi k^2}{2(1 + \kappa k^2/\gamma)} \left\{ \left[ \left( \frac{\Gamma_\psi^2}{b_\psi \gamma} \right) - 1 \right] - \left( \frac{\kappa}{\gamma} \right) k^2 \right\}. \tag{10} \]

The structure factors of interest follow immediately:
\[ < \phi(k) \phi(-k) > \equiv S_{11}(k) = nk_B T \frac{m_{22}(k)}{m_{11}(k)m_{22}(k) - m_{12}^2(k)} \]
\[ < \psi(k) \psi(-k) > \equiv S_{22}(k) = nk_B T \frac{m_{11}(k)}{m_{11}(k)m_{22}(k) - m_{12}^2(k)} \]
\[ < \phi(k) \psi(-k) > \equiv S_{12}(k) = < \phi(-k) \psi(k) > \equiv S_{21}(k) \]
\[ = -nk_B T \frac{m_{12}(k)}{m_{11}(k)m_{22}(k) - m_{12}^2(k)}, \tag{11} \]
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where the brackets denote an ensemble average.

As can be seen from $m_{12}(k)$, the coefficient in the free energy of $\phi(-k)\psi(k)$ and $\psi(-k)\phi(k)$, there is now a coupling between the order parameters of the inner and outer leaves. It arises because the order parameter of the inner leaf is coupled to the membrane curvature with a strength proportional to $\Gamma_\phi$, and the order parameter of the outer leaf is coupled to the same curvature with a strength proportional to $\Gamma_\psi$. Thus the membrane couples the two order parameters with a wave number-dependent coupling

$$\Lambda_c(k) = \frac{\Gamma_\phi\Gamma_\psi}{\gamma} \frac{k^2}{1 + \kappa k^2/\gamma}. \quad (12)$$

We shall estimate its strength below.

## 2 Results

We shall now estimate the parameters which enter the structure factors. First, we consider the strength of the coupling between composition and membrane to be (17, 19):

$$\Gamma_\phi = \kappa_{\text{mon}} \delta H_\phi = \frac{\kappa}{2} \delta H_\phi, \quad (13)$$

$$\Gamma_\psi = \frac{\kappa}{2} \delta H_\psi, \quad (14)$$

where $\kappa_{\text{mon}}$ is the bending modulus of a monolayer, which is one-half that of the bilayer. As the order parameter $\psi$ was defined as the difference of mol fractions in the outer leaflet of 1-palmitoyl, 2-oleoyl phosphatidylcholine (POPC) and SM, it is reasonable to take $\delta H_\psi$ as the difference of the spontaneous curvatures of the lipids weighted by their mol fractions. A similar statement applies to $\delta H_\phi$:

$$\delta H_\psi = H_{0,\text{PC},\text{out}} y_{\text{PC},\text{out}} - H_{\text{SM}} y_{\text{SM}},$$

$$\delta H_\phi = H_{\text{PE}} y_{\text{PE}} - H_{\text{PS}} y_{\text{PS}} - H_{\text{PC},\text{in}} y_{\text{PC},\text{in}}, \quad (15)$$

where $y_{\text{PC},\text{out}}$ is the POPC mol fraction of all phospholipids in the outer leaf, and $H_{0,\text{PC},\text{out}}$ is its spontaneous curvature. Note that the spontaneous curvature of a lipid with a small head group is negative if it is on the outer leaf, and is positive if it is on the inner leaf. Similarly, the spontaneous curvature of POPC in the outer leaf is of opposite sign from that in the inner leaf. The spontaneous curvatures, in nm$^{-1}$ are as follows (23, 24): $H_{0,\text{PC},\text{out}} = 0.022$, $H_{0,\text{SM}} = -0.134$, $H_{0,\text{PE}} = 0.316$, $H_{0,\text{PS}} = 0.07$. 


\( H_{0,\text{PC,\text{in}}} = -0.022 \). (The spontaneous curvature \( H_{0,\text{PS}} \) is that for DOPS (24)). The mol fractions are (7) \( y_{\text{PC,\text{out}}} = 0.4, y_{\text{SM}} = 0.4, y_{\text{PS}} = 0.3, y_{\text{PE}} = 0.5, y_{\text{PC,\text{in}}} = 0.15 \). With these values, Eqs. (15) and (16) yield \( \delta H_\psi = 0.062 \text{ nm}^{-1} \) and \( \delta H_\phi = 0.14 \text{ nm}^{-1} \). For the bilayer bending modulus, we take (25) \( \kappa = 44k_B T = 181 \text{ pN nm} \). Then from Eqs. (13) and (14) we obtain \( \Gamma_\phi = 12.7 \text{ pN} \) and \( \Gamma_\psi = 5.6 \text{ pN} \). For the surface tension, we use (26) \( \gamma = 0.02 \text{ pN nm} \) and for \( b_\phi = b_\psi = 5k_B T = 20 \text{ pN nm} \) (27). Therefore the dimensionless couplings are

\[
\frac{\Gamma_\phi}{(b_\phi \gamma)^{1/2}} = 19.9 \quad (17)
\]

\[
\frac{\Gamma_\psi}{(b_\psi \gamma)^{1/2}} = 8.9 \quad (18)
\]

Lastly we need \( a_\phi \) and \( a_\psi \) which are given in Eqs. (7) and (8). The quantity \( 2J_\psi \) is equal to the critical miscibility temperature of the outer leaf, which we take to be 300K. This is reasonable judging from critical temperatures of symmetric bilayers (6). For \( 2J_\phi \), the miscibility temperature of the inner leaf, we take 200 K (21). From the mol fractions given above, we obtain for the average order parameters \( \bar{\phi} = 0., \), \( \bar{\psi} = 0.05 \). With a density of lipids of \( n = 2 \text{ nm}^{-2} \) and a temperature \( T = 310 \text{ K} \), we obtain \( a_\psi = 0.13 \text{ pN nm} \) and \( a_\phi = 1.48 \text{ pN nm} \).

At the above temperature and with these parameters we find that the fluid phase is stable. The normalized structure function \( S_{11}(k)/S_{11}(0) \) of the inner-leaf order parameter, \( S_{22}(k)/S_{22}(0) \) of the outer-leaf order parameter, and the cross correlation \( S_{12}(k)/S_{11}(0) \) are shown in Figs. 2, 3 and 4 respectively. They are plotted as a function of the dimensionless wave vector \( q \equiv k(\kappa/\gamma)^{1/2} \). They all display a peak at \( k(\kappa/\gamma)^{1/2} \approx 4 \) which corresponds to a wavelength \( \lambda = 2\pi/k \) of 150 nm. These response functions show that this fluid system is most susceptible to perturbations with a non-zero wave vector. Thus it is a microemulsion.

The order-parameter order-parameter correlation functions are

\[
g_{11}(r) = \langle \phi(r)\phi(0) \rangle - (\bar{\phi})^2 \approx \langle \phi(r)\phi(0) \rangle, \quad (19)
\]

\[
g_{22}(r) = \langle \psi(r)\psi(0) \rangle - (\bar{\psi})^2 \approx \langle \psi(r)\psi(0) \rangle, \quad (20)
\]

where the approximation follows because \( \bar{\phi} \) and \( \bar{\psi} \) are so small. In general they behave at large distances like

\[
\frac{\exp(-r/\xi)}{r^2} \cos(2\pi r/\lambda), \quad (21)
\]
and are characterized by two lengths; the correlation length $\xi$ and the wavelength $\lambda$. If $\xi$ is considerably larger than $\lambda$, then one would see variations in the composition in both leaves of size on the order of $\lambda/2 \approx 75 \text{nm}$. If the correlation length is much smaller than $\lambda$, such oscillations would not be observed in a lipid-only system. (The correlation length is minimum at the disorder line (28)) But the peaks in the structure functions would still reveal the microemulsion nature of the fluid.

The correlation functions $g_{11}(r)$ and $g_{22}(r)$ are directly proportional to the two dimensional Fourier transforms of $S_{11}(k)$ and $S_{22}(k)$. From the structure factors given above for the system under discussion, we obtain the results shown in Figs. 5 and 6.

One sees from these correlation functions that each leaf is a microemulsion with a wavelength $\lambda \approx 150 \text{nm}$ and a correlation length which is longer than that so that the oscillations in the correlation function are clearly seen. In particular, Fig. 5 shows that if there is a PE-rich region at the origin of coordinates of the inner leaf, it extends for a distance of about 150 nm, beyond which it is more likely for PC and PS to be found, and beyond that once again it is more likely to find a PE-rich region. Note that the distance from the first minimum to the first maximum is on the order of 75 nm as
Figure 3: Structure function $S_{22}(q)/S_{22}(0)$ is plotted as a function of the dimensionless wavevector $q = (\kappa/\gamma)^{1/2}k$. 

\[
\frac{S_{22}(q)}{S_{22}(0)}
\]
Figure 4: Structure function $S_{12}(q)/S_{11}(0)$ is plotted as a function of the dimensionless wavevector $q = (\kappa/\gamma)^{1/2}k$. 
Figure 5: $g_{11}(r)$, the two-dimensional Fourier transform of $S_{11}(k)$ shown in Fig. 2, is plotted here as a function of $r$, the distance from the origin in units of $(\kappa/\gamma)^{1/2} = 95\text{nm}$. 
Figure 6: $g_{22}(r)$, the two-dimensional Fourier transform of $S_{22}(k)$ shown in Fig. 3, is plotted here as a function of $r$, the distance from the origin in units of $(\kappa/\gamma)^{1/2} = 95\text{nm}$. 
estimated above. Fig. 6 makes a similar statement about PC-rich regions in the outer leaf, with SM more likely to be found beyond it and another PC-rich region beyond that.

We now turn to the strength of the coupling between inhomogeneous regions in the two leaves which, from Eq. (12) can be written

$$\Lambda_c = \frac{\Gamma_\phi \Gamma_\psi}{\kappa} \frac{q^2}{1 + q^2},$$

(22)

with $q = k(\kappa/\gamma)^{1/2}$. From Figs 2, 3, and 4, the value of the dimensionless wavevector $q$ at the peak of the structure factors is about four so that the factor $q^2/(1 + q^2)$ is almost unity. Using this and the forms for $\Gamma_\phi$ and $\Gamma_\psi$ from Eqs. (13) and (14), we obtain the expression

$$\Lambda_c \approx \left[ \left( \frac{\kappa_{\text{mono}} \delta H_\phi}{2} \right) \left( \frac{\kappa_{\text{mono}} \delta H_\psi}{2} \right) \right]^{1/2} = \frac{\kappa \delta H_\phi \delta H_\psi}{4},$$

(23)

which shows the coupling between the order parameters of the two leaves as the geometric mean of the couplings of each order parameter to its respective leaf. Evaluating this coupling with the parameters above we obtain $\Lambda_c \approx 0.39$ pN/nm$= 0.09 k_B T$. This is almost an order of magnitude larger than the coupling between domains brought about by phase separation in model membranes (29–31).

3 Discussion

We have shown that by considering rafts in the plasma membrane to be the manifestation of a microemulsion brought about by the coupling of variations in composition and membrane height, one solves several problems that confront an explanation based on phase separation. First, and perhaps most important, is that rafts in both leaves occur quite naturally because both leaves contain lipids whose spontaneous curvature differ considerably. This is in contrast to the paucity of lipids in the inner leaf of the plasma membrane that are to bring about phase separation. Thus, rather than thinking of “rafts” and “sea” as regions of lo and ld, or saturated and unsaturated lipids, I suggest that one should rather think of regions of lipids with large spontaneous curvature, SM on the outer leaf, PE on the inner leaf, and other regions of lipids with small spontaneous curvature, PC on the outer leaf, PC and PS on the inner leaf. Second, in contrast to scenarios involving phase separation, it is obvious here how the rafts in the two leaves are
coupled. Further we have seen that they are coupled more strongly than in phase-separated model membranes. Third, and again in contrast to scenarios based on phase separation, the microemulsion picture provides a natural size for the inhomogeneities. It is not difficult to see from the form of the structure factors of Eq. (11) that if the two leaves were not coupled, then the structure factors $S_{11}$ and $S_{22}$ would have their maxima at dimensionless wave vectors

$$q^*_{\phi} \approx \left( \frac{\Gamma_{\phi}^2}{\gamma b_{\phi}} \right)^{1/4},$$

$$q^*_{\psi} \approx \left( \frac{\Gamma_{\psi}^2}{\gamma b_{\psi}} \right)^{1/4}. \quad (24)$$

From Eqs. (17) and (18), one obtains $q^*_{\psi} \approx 4.5$ and $q^*_{\phi} \approx 3.0$. Given that the coupling between leaves is non-zero, the maximum in the structure functions will be shifted, but as seen from Figs. 2, 3, and 4, the value at which the maxima in the structure functions occur is $q^* \approx 4$, comparable to the geometric mean $(q^*_{\phi} q^*_{\psi})^{1/2}$. The value $q^* = 4$ implies a natural size of the inhomogeneities

$$\lambda^* = \frac{\pi}{2} \left( \frac{\kappa}{\gamma} \right)^{1/2} \frac{1}{q^*} = 75 \text{ nm}, \quad (25)$$

which is certainly of the putative size of rafts. If one utilizes Eqs. (13) and (14) to express the couplings $\Gamma_{\phi} = \delta H_{\phi} \kappa / 2$, $\Gamma_{\psi} = \delta H_{\psi} \kappa / 2$, and takes $b_{\psi} = b_{\phi} = b$, then the natural length scale can be written

$$\frac{\lambda^*}{2} = \sqrt{2\pi} \left( \frac{b}{\gamma \delta H_{\psi} \delta H_{\phi}} \right)^{1/4}. \quad (26)$$

It is interesting that the bending modulus has dropped out of this length scale even though it is clearly of importance for the length scale of the membrane and for the strength of the coupling of the compositions to the membrane. Liu et al. (19) arrived at essentially the above result for the characteristic wavevector $k^* = q^* (\sigma / \kappa)^{1/2}$ and estimated a raft size as $2\pi / k^*$, rather than half this, and took the spontaneous curvature to be that of the average spontaneous curvature of a vesicle. They estimated this from experiment to be of order $\delta H \approx 10^{-3} / \text{nm}$. A value of the surface tension an order of magnitude smaller than that given above was utilized. They found, therefore, that the typical size, $2\pi / k^*$ was orders of magnitude larger.
than 100\,nm and therefore concluded that this mechanism was not at work in the plasma membrane. If one uses instead the larger tension of Ref. (26) and takes the characteristic size to be $\pi/k^*$, then one obtains, even with so small a spontaneous curvature of $\delta H = 10^{-3}/\text{nm}$, a characteristic size of 500 nm. Given the uncertainties in the parameters and the calculation, such as the lack of the effect of fluctuations (32), this value does not seem to provide a definite negative result for the applicability of the mechanism. More importantly, although the average spontaneous curvature of the vesicle might be on the order of inverse microns, the local spontaneous curvature can certainly be larger, as used above, and produce undulations in the membrane about its average shape (33, 34).

Lastly I turn to the question of whether microemulsions have ever been observed in experiment on membranes. I will now argue that they have been observed, but never identified as such. It is useful to recall that if the coupling between composition and curvature were sufficiently strong, the system would exhibit modulated phases, such as stripes of the two different regions, or an hexagonal array of one domain embedded in the other(22, 33, 34). These phases are characterized by weak long-range order in which the correlation functions decay, but not exponentially with distance as they do in a liquid, but rather as power laws (32). Microemulsions can be thought of as the phase which results when these modulated phases melt. A few examples of such modulated phases have appeared in the literature. Some of the clearest examples appear in the quaternary mixture of dioleoylphosphatidylcholine, DOPC, distearoylphosphatidylcholine, DSPC, POPC, and cholesterol (35, 36). In particular Fig 2, (B, D and E) of Ref. (35) reproduced here as Fig. 7, and Fig. 2 (F,G,J,K) and Fig. 4 of Ref (36), are clear examples of microemulsions. They are identified in the references, however, as modulated phases and to be within a two-phase region. I believe that both statements are incorrect, and that they are examples of microemulsions, disordered structured fluids that are single phases. Other examples appear in GUVs of diphytanoylphosphatidylcholine, DPPC, and cholesterol, Fig. 8 and those of DOPC, DPPC, cholesterol and fatty acids, Fig. 2(b) of Ref (37).

That the characteristic wavelengths in all of these model systems are larger than those expected in the plasma membrane may well be due to the fact that the differences in spontaneous curvatures of the lipids utilized, all PC’s, are relatively small. It would be very interesting to look at model membranes with ternary mixtures of cholesterol, PC, and PE for one would expect, in that system, the characteristic wavelengths to be appreciably smaller.
Figure 7: GUV patterns from four-component mixtures of DSPC, POPC, DOPC, and cholesterol as POPC is increasingly replaced by DOPC in going from A to F. I believe B, D, and E are microemulsions. Figure reproduced from Ref. (35).
Figure 8: Image from a GUV of diameter about 100 microns which contains a ternary mixture of diphytanoylphosphatidylcholine, DPPC, and cholesterol. It appears on the cover of the Biophysical Journal, volume 105, July 2013, and is used by permission. It was kindly provided by Aurelia Honerkamp-Smith and Sarah Keller.
4 Acknowledgments

I have benefited greatly from conversing and working with Ha Giang, David Allender, and Marcus Mueller. I am very pleased to offer this work in honor of one whom I admire greatly, Benjamin Widom.

References