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Strongly Correlated Rafts in Both Leaves of an Asymmetric Bilayer

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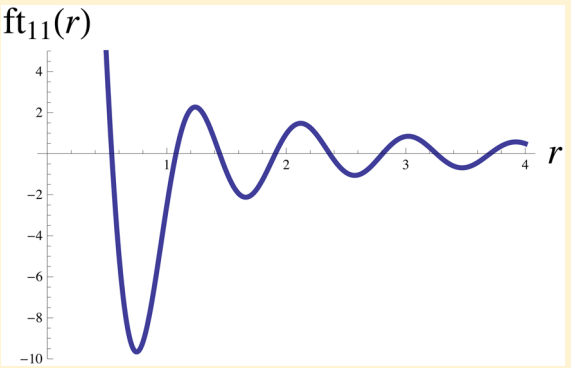
ABSTRACT: I consider a model of a bilayer characterized by two order parameters, one in each leaf. That of the inner leaf represents the difference in mole fractions of lipids with large spontaneous curvature, phosphatidylethanolamine (PE), and those with small spontaneous curvatures, phosphatidylcholine (PC) and phosphatidylserine (PS). Similarly, the order parameter in the outer leaf represents the difference in mole 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.



I'm afraid that the clarity of Professor Widom's lecture basically obscures the difficulty of the physics. -Leo Kadanoff

INTRODUCTION

According to the raft hypothesis,[1,2] the plasma membrane is heterogeneous, characterized by dynamic 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 mole 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] They are structured fluids characterized not only by a correlation length but also by an additional length set by the structure. A simple example is a

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system with water as a majority component, and oil as a minority component, to which a surfactant is added. Such a system can form a microemulsion consisting of droplets of oil in water. The size of the droplets is determined by the total volume of oil enclosed within them and the total area of the droplets which can be covered by the surfactant in the system. The distance between droplets is simply related to their size and the volume fraction of oil. The droplets are dynamic, responding easily to thermal fluctuations, because their surface free energy per unit area is reduced to a very small value by the presence of the surfactant. That the microemulsion is structured is detected in scattering experiments.

Because a microemulsion is characterized by dynamic domains of a well-determined size, it is natural to ask whether one could form in the plasma membrane and undergird the hypothesis of rafts. Presumably, a microemulsion 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 arise 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 those 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 brought about 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 2 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[21,22] 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 mole 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 mole 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 Figure 1, the raft

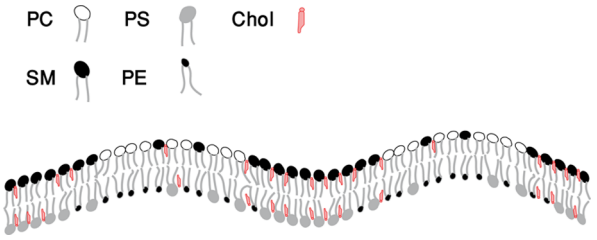

Figure 1. Regions rich in SM in the outer leaf and of PC and negatively charged PS in the inner leaf floating in a sea of PC in the outer leaflet and PE in the inner leaf. The PC in the inner leaf is not depicted.

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 rafts in the inner and outer leaves are strongly correlated, as can already be intuited from the figure, so that there is no need to posit an intrinsic coupling between them.

THEORY

I follow the earlier formulations of refs 21 and 23 with exceptions to be noted explicitly. I define an order parameter $\phi(\mathbf{r})$ in the inner leaf of the bilayer which represents the difference in local mole fractions in that leaf between PE on the one hand and PC and PS on the other. Similarly, define $\psi(\mathbf{r})$ as the difference in local mole fraction in the outer leaf between PC and SM. The free energy of a planar bilayer can then be written in the usual form[23]

$$F_{\text{plane}}[\phi, \psi] = \int \mathrm{d}^2 r \left[\frac{b_\phi}{2} (\nabla\phi)^2 + \frac{b_\psi}{2} (\nabla\psi)^2 + f_{\text{plane}} \right] \tag{1}$$

with the mean-field free energy density

$$\begin{aligned} f_{\text{plane}}[\phi, \psi] = {} & -J_\phi n\phi^2 + \frac{k_B T}{2} n[(1+\phi)\ln(1+\phi) \\ & + (1-\phi)\ln(1-\phi)] - J_\psi n\psi^2 \\ & + \frac{k_B T}{2} n[(1+\psi)\ln(1+\psi) \\ & + (1-\psi)\ln(1-\psi)] \end{aligned} \tag{2}$$

Here n is the areal density of lipids, k_B is Boltzmann's constant, and T is 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

$$F_{\text{curv}}[h] = \int \mathrm{d}^2 r \left[\frac{\kappa}{2} (\nabla^2 h)^2 + \frac{\gamma}{2} (\nabla h)^2 \right] \tag{3}$$

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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.

Finally, I assume that the difference in compositions in the inner leaf couples to the membrane curvature with a coupling strength Γ_ϕ. Similarly, the difference in compositions in the outer leaflet couples to the curvature with strength Γ_ψ. I assume implicitly that the membrane is of constant thickness so that the curvatures of the two leaves are the same locally.

$$F_{coupl}[\phi, \psi, h] = -\int d^2r(\Gamma_\phi\phi + \Gamma_\psi\psi)\nabla^2 h \tag{4}$$

Because the height variable $h(\mathbf{r})$ enters only quadratically into the total free energy $F_{tot}[\phi, \psi, h] = F_{planar}[\phi, \psi] + F_{curv}[h] + F_{coupl}[\phi, \psi, h]$, it can easily be eliminated. This is conveniently done in Fourier space in which

$$\phi(k) = \frac{1}{A}\int(\phi(r) - \bar{\phi})e^{-ikr}\, d^2r \tag{5}$$

where A is the area of the bilayer, and similarly for $\psi(k)$ and $h(k)$, with $\bar{\phi}$ and $\bar{\psi}$ being the average values of $\phi(\mathbf{r})$ and $\psi(\mathbf{r})$

$$\bar{\phi} \equiv \frac{1}{A}\int d^2r\phi(\mathbf{r}), \qquad \bar{\psi} \equiv \frac{1}{A}\int d^2r\psi(\mathbf{r})$$

Then, with

$$\int d^2r f_{plane}[\phi, \psi] = F_{tot}(\bar{\phi}, \bar{\psi}) + \frac{A^2}{(2\pi)^2}\int d^2k[a_\phi\phi(k)\phi(-k) + a_\psi\psi(k)\psi(-k)]$$

$$a_\phi = \frac{n}{2}\left[\frac{k_BT}{1-\bar{\phi}^2} - 2J_\phi\right] \tag{6}$$

$$a_\psi = \frac{n}{2}\left[\frac{k_BT}{1-\bar{\psi}^2} - 2J_\psi\right] \tag{7}$$

to second order in small quantities, one obtains

$$F_{tot} = F_{tot}(\bar{\phi}, \bar{\psi}) + \frac{A^2}{(2\pi)^2}\int d^2k\left\{\left[a_\psi + \frac{b_\psi}{2}k^2\right]\psi(k)\psi(-k) + \left[a_\phi + \frac{b_\phi}{2}k^2\right]\phi(k)\phi(-k) + \frac{1}{2}[\kappa k^4 + \gamma k^2]h(k)h(-k) + [k^2\Gamma_\phi\phi(-k) + k^2\Gamma_\psi\psi(-k)]h(k)\right\} \tag{8}$$

The value of $h(k)$ which minimizes F_{tot} is found to be

$$h_{min}(k) = -\frac{\Gamma_\phi\phi(k) + \Gamma_\psi\psi(k)}{\gamma + k^2\kappa} \tag{9}$$

Substituting this into eq 8, one obtains

$$F_{tot}[\phi, \psi] = F_{tot}(\bar{\phi}, \bar{\psi}) + \frac{A^2}{(2\pi)^2}\int d^2k(\phi(-k)\ \ \psi(-k))\begin{pmatrix} m_{11}(k) & m_{12}(k) \\ m_{21}(k) & m_{22}(k)\end{pmatrix}\begin{pmatrix}\phi(k)\\ \psi(k)\end{pmatrix}$$

$$m_{11}(k) = a_\phi - \frac{b_\phi k^2}{2(1+\kappa k^2/\gamma)}\left\{\left[\left(\frac{\Gamma_\phi^2}{b_\phi\gamma}\right) - 1\right] - \left(\frac{\kappa}{\gamma}\right)k^2\right\}$$

$$m_{12}(k) = m_{21}(k) = -\frac{(b_\phi b_\psi)^{1/2}k^2}{2(1+\kappa k^2/\gamma)}\frac{\Gamma_\phi}{(b_\phi\gamma)^{1/2}}\frac{\Gamma_\psi}{(b_\psi\gamma)^{1/2}}$$

$$m_{22}(k) = a_\psi - \frac{b_\psi k^2}{2(1+\kappa k^2/\gamma)}\left\{\left[\left(\frac{\Gamma_\psi^2}{b_\psi\gamma}\right) - 1\right] - \left(\frac{\kappa}{\gamma}\right)k^2\right\} \tag{10}$$

The structure factors of interest follow immediately

$$\langle\phi(k)\phi(-k)\rangle \equiv S_{11}(k) = nk_BT\frac{m_{22}(k)}{m_{11}(k)m_{22}(k) - m_{12}^2(k)}$$

$$\langle\psi(k)\psi(-k)\rangle \equiv S_{22}(k) = nk_BT\frac{m_{11}(k)}{m_{11}(k)m_{22}(k) - m_{12}^2(k)}$$

$$\langle\phi(k)\psi(-k)\rangle \equiv S_{12}(k) = \langle\phi(-k)\psi(k)\rangle \equiv S_{21}(k) = -nk_BT\frac{m_{12}(k)}{m_{11}(k)m_{22}(k) - m_{12}^2(k)} \tag{11}$$

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 Γ_ϕ, and the order parameter of the outer leaf is coupled to the same curvature with a strength proportional to Γ_ψ. Thus, the membrane couples the two order parameters with a wave-number-dependent coupling

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

Its strength will be obtained below.

RESULTS

I shall now estimate the parameters which enter the structure factors. First, I consider the strength of the coupling between composition and membrane to be[17,19]

$$\Gamma_\phi = \kappa_\phi\delta H_\phi \approx \frac{\kappa}{2}\delta H_\phi \tag{13}$$

$$\Gamma_\psi = \kappa_\psi\delta H_\psi \approx \frac{\kappa}{2}\delta H_\psi \tag{14}$$

where κ_ψ and κ_ϕ are the bending modulii of the monolayers and which, for the purpose of estimation, I approximate as $\kappa/2$, one-half that of the bilayer. As the order parameter ψ was defined as the difference of mole fractions in the outer leaflet of 1-palmitoyl, 2-oleoylphosphatidylcholine (POPC) and SM, it is reasonable to take δH_ψ as the difference of the spontaneous curvatures of the lipids weighted by their mole fractions. A similar statement applies to δH_ϕ

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$$\delta H_\psi = H_{0,\mathrm{PC,out}} y_{\mathrm{PC,out}} - H_{0,\mathrm{SM}} y_{\mathrm{SM}} \quad (15)$$

$$\delta H_\phi = H_{0,\mathrm{PE}} y_{\mathrm{PE}} - H_{0,\mathrm{PS}} y_{\mathrm{PS}} - H_{0,\mathrm{PC,in}} y_{\mathrm{PC,in}} \quad (16)$$

where $y_{\mathrm{PC,out}}$ is the POPC mole fraction of all phospholipids in the outer leaf and $H_{0,\mathrm{PC,out}}$ is its spontaneous curvature. Note that the spontaneous curvature of a lipid with a small headgroup 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:[24,25] $H_{0,\mathrm{PC,out}} = 0.022$, $H_{0,\mathrm{SM}} = -0.134$, $H_{0,\mathrm{PE}} = 0.316$, $H_{0,\mathrm{PS}} = 0.07$, $H_{0,\mathrm{PC,in}} = -0.022$. (The spontaneous curvature $H_{0,\mathrm{PS}}$ is that for DOPS.[25]) The mole fractions are[7] $y_{\mathrm{PC,out}} = 0.4$, $y_{\mathrm{SM}} = 0.4$, $y_{\mathrm{PS}} = 0.3$, $y_{\mathrm{PE}} = 0.5$, and $y_{\mathrm{PC,in}} = 0.15$. With these values, eqs 15 and 16 yield $\delta H_\psi = 0.062\ \mathrm{nm}^{-1}$ and $\delta H_\phi = 0.14\ \mathrm{nm}^{-1}$. For the bilayer bending modulus, I take[26] $\kappa = 44 k_B T = 181$ pN nm. Then, from eqs 13 and 14, I obtain $\Gamma_\phi = 12.7$ pN and $\Gamma_\psi = 5.6$ pN. For the energies b_ϕ and b_ψ, I take them to be equal to a value of $b = 5 k_B T = 20$ pN nm,[27] and for the surface tension, I use[28] $\gamma = 0.02$ pN/nm. It should be noted that this value includes the effect of the cytoskeleton on the tension. The dimensionless couplings are then

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

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

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

At the above temperature and with these parameters, I 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 Figures 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 characterized by structure, and is most susceptible to perturbations with a nonzero wave vector. Thus, it is a microemulsion.

The order-parameter order-parameter correlation functions $\langle \phi(r)\phi(0)\rangle - (\bar{\phi})^2$ and $\langle \psi(r)\psi(0)\rangle - (\bar{\psi})^2$ are simply the two-dimensional Fourier transforms of $S_{11}(k)$ and $S_{22}(k)$. In general, they behave at large distances like

$$\frac{\exp(-r/\xi)}{r^2}\cos(2\pi r/\lambda) \quad (19)$$

and are characterized by two lengths: the correlation length ξ and the wavelength λ. If ξ is considerably larger than λ, then one would see variations in the composition in both leaves of size on the order of $\lambda/2 \approx 75$ nm. If the correlation length is much smaller than λ, such oscillations would not be observed in a lipid-only system. (The correlation length is minimum at the disorder line.[29]) Even if $\xi < \lambda$, the peaks in the structure functions at nonzero wave vector would still reveal the microemulsion nature of the fluid.

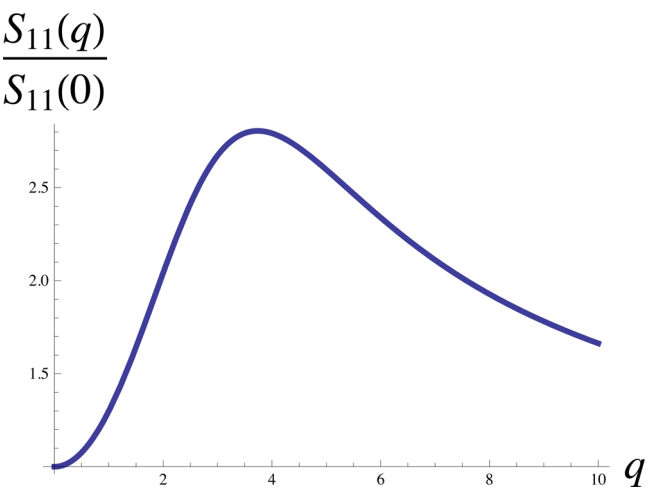

Figure 2. Structure function $S_{11}(q)/S_{11}(0)$ plotted as a function of the dimensionless wave vector $q = (\kappa/\gamma)^{1/2}k$.

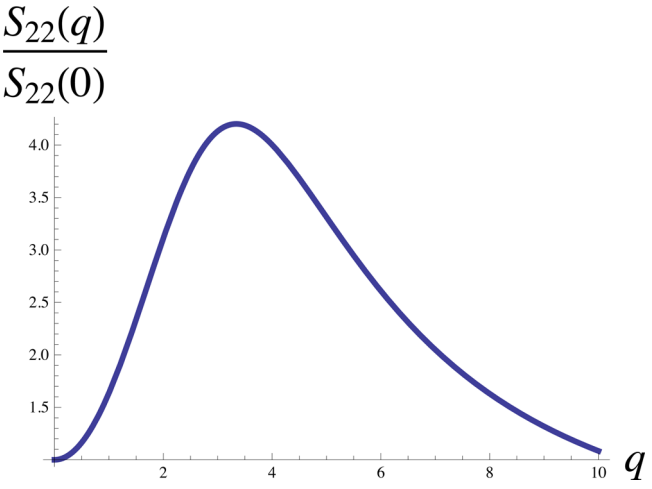

Figure 3. Structure function $S_{22}(q)/S_{22}(0)$ plotted as a function of the dimensionless wave vector $q = (\kappa/\gamma)^{1/2}k$.

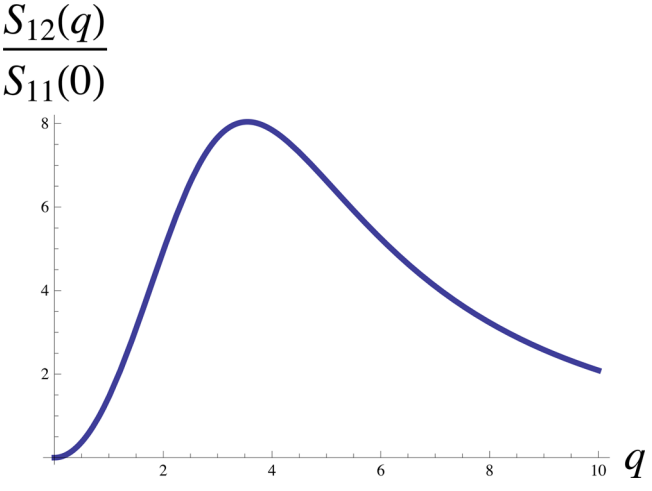

Figure 4. Structure function $S_{12}(q)/S_{11}(0)$ plotted as a function of the dimensionless wave vector $q = (\kappa/\gamma)^{1/2}k$.

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The pair 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, I obtain the transforms shown in Figures 5 and 6. One sees from these transforms that each leaf

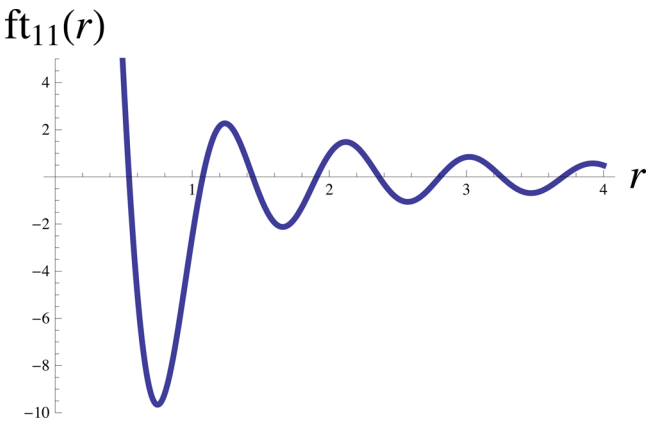

Figure 5. Two-dimensional Fourier transform, $\mathrm{ft}_{11}(r)$, of $S_{11}(k)/S_{11}(0)$ shown in Figure 2, plotted here as a function of r, the distance from the origin in units of $(\kappa/\gamma)^{1/2}$ = 95 nm. It is proportional to the pair correlation function $g_{11}(r)$.

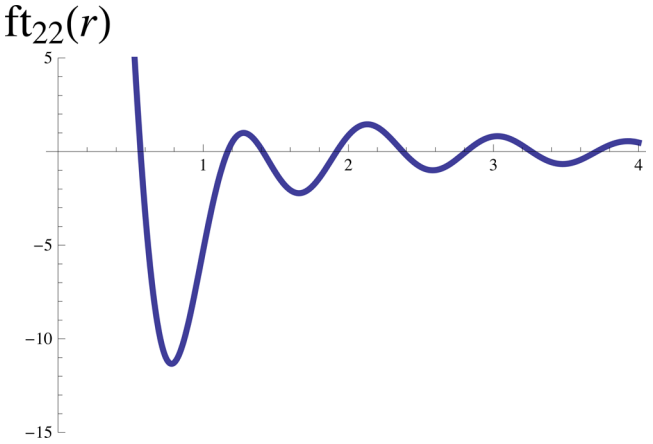

Figure 6. Two-dimensional Fourier transform, $\mathrm{ft}_{22}(r)$, of $S_{22}(k)/S_{22}(0)$ shown in Figure 3, plotted here as a function of r, the distance from the origin in units of $(\kappa/\gamma)^{1/2}$ = 95 nm. It is proportional to the pair correlation function $g_{22}(r)$.

is a microemulsion with a wavelength $\lambda \approx 100$ nm and a correlation length which is longer than that so that oscillations are clearly seen. I note that $\lambda/2 \approx 50$ nm obtained from the correlation function is smaller than that estimated solely from the position of the peak in the structure function, 75 nm. However, again, this size is certainly that of the putative size of rafts.

I now turn to the strength of the coupling between inhomogeneous regions in the two leaves which, from eq 12, can be written

$$\Lambda_c = \frac{\Gamma_\phi \Gamma_\psi}{\kappa} \frac{q^2}{1+q^2} \tag{20}$$

with $q = k(\kappa/\gamma)^{1/2}$. From Figures 2, 3, and 4, the value of the dimensionless wave vector q at the peak of the structure factors is about 4, so that the factor $q^2/(1+q^2)$ is almost unity. Using this and the forms for Γ_ϕ and Γ_ψ from eqs 13 and 14, I obtain the expression

$$\Lambda_c \approx \left[\left(\frac{\kappa_\phi \delta H_\phi^{\,2}}{2}\right)\left(\frac{\kappa_\psi \delta H_\psi^{\,2}}{2}\right)\right]^{1/2} \approx \frac{\kappa \delta H_\phi \delta H_\psi}{4} \tag{21}$$

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, I obtain $\Lambda_c \approx 0.39$ pN/nm = $0.09 k_B T$/nm^2. This is almost an order of magnitude larger than the coupling between domains calculated from models based on phase separation,[30,31] and measured in symmetric model membranes that do undergo phase separation.[32]

DISCUSSION

I 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 curvatures 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, I have shown that they are coupled more strongly than in phase-separated model membranes. Therefore, the model makes a testable prediction that regions rich in PE in the inner leaf of the plasma membrane should be anticorrelated with regions rich in SM in the outer leaf. Such correlations in the plasma membrane have not been probed as of yet. 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

$$q_\phi^* \approx \left(\frac{\Gamma_\phi^{\,2}}{\gamma b_\phi}\right)^{1/4}$$
$$q_\psi^* \approx \left(\frac{\Gamma_\psi^{\,2}}{\gamma b_\psi}\right)^{1/4} \tag{22}$$

From eqs 17 and 18, one obtains $q_\phi^* \approx 4.5$ and $q_\psi^* \approx 3.0$. Given that the coupling between leaves is nonzero, the maximum in the structure functions will be shifted, but as seen from Figures 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

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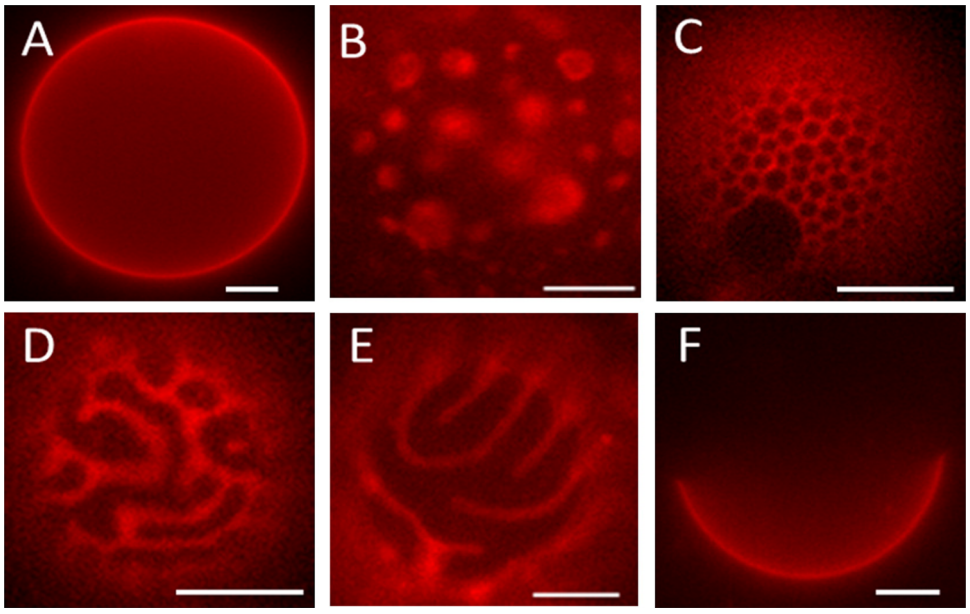

Figure 7. GUV patterns from four-component mixtures of DSPC, POPC, DOPC, and cholesterol as POPC is increasingly replaced by DOPC in going from A to F. I believe B, D, and E are microemulsions. Figure reproduced with permission from ref 36.

$$\frac{\lambda^*}{2} = \pi\left(\frac{\kappa}{\gamma}\right)^{1/2}\frac{1}{q^*} = 75\ \text{nm} \tag{23}$$

which is somewhat larger than that obtained from the Fourier transforms themselves, about 50 nm. I utilize eqs 13 and 14 and write $\kappa_\phi = (\kappa + \delta\kappa)/2$, $\kappa_\psi = (\kappa - \delta\kappa)/2$ to express the couplings $\Gamma_\phi = \delta H_\phi \kappa_\phi$, $\Gamma_\psi = \delta H_\psi \kappa_\psi$. I also take $b_\psi = b_\phi = b$. Then, the natural length scale can be written

$$\frac{\lambda^*}{2} = \sqrt{2}\,\pi\left(\frac{b}{\gamma \delta H_\psi \delta H_\phi}\right)^{1/4} \tag{24}$$

with corrections of order $(\delta\kappa/\kappa)^2$. It is interesting that the bending modulus has dropped out of this length scale even though it is clearly of importance both 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 wave vector $k^* = q^*(\sigma/\kappa)^{1/2}$ 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 $\delta H \approx 10^{-3}$/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 28 that includes the effect of the cytoskeleton, and takes the characteristic size to be π/k^*, then one obtains, even with so small a spontaneous curvature of $\delta H = 10^{-3}$/nm, a characteristic size of 500 nm. Given that using the position of the peak in the structure function overestimates the characteristic wavelength, and given uncertainties in the parameters and the calculation, such as the lack of the effect of fluctuations,[33] 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.[34,35]

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 a hexagonal array of one domain embedded in the other.[23,34,35] These phases are characterized by weak long-range order in which the correlation functions decay, not exponentially with distance as they do in a liquid but rather as power laws.[33] Microemulsions can be thought of as the phase which results when these modulated phases melt. A few examples of such melted modulated phases have appeared in the literature. Some of the clearest examples appear in symmetric membranes comprised of a quaternary mixture of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), POPC, and cholesterol.[36,37] In particular, Figure 2B, D, and E of ref 36 and Figures 2F, G, J, and K and 4 of ref 37 are clear examples of microemulsions. That from ref 36 is reproduced here as Figure 7. 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 symmetric GUVs of diphytanoylphosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), and cholesterol, shown here in Figure 8, and those of DOPC, DPPC, cholesterol, and fatty acids, Figure 2b of ref 38.

One observes in several of these micrographs that the morphologies indicate the energy per unit length between regions is very small. This is, again, characteristic of a microemulsion in two dimensions in which the energy per unit length between regions is of the order of a thermal energy per characteristic wavelength, λ^*. In contrast, the line tension between coexisting phases is of the order of a thermal energy per a few molecular lengths.

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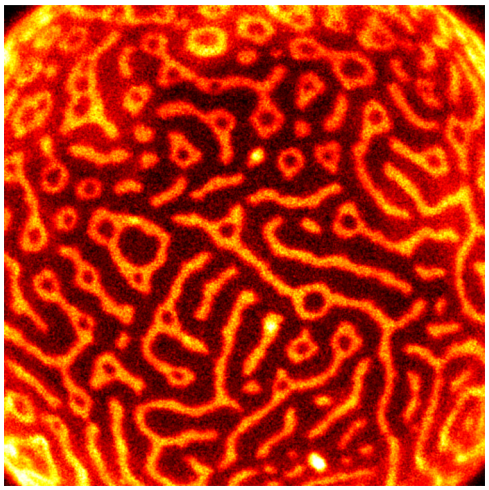

Figure 8. Image from a GUV of diameter about 100 μm which contains a ternary mixture of diphytanoylphosphatidylcholine, DPPC, and cholesterol. It appears on the cover of *Biophysical Journal*, July 2013, volume 105, and is used by permission. It was kindly provided by Aurelia Honerkamp-Smith and Sarah Keller.

CONCLUSIONS

I believe that microemulsions have been observed in symmetric model membranes. It would be of interest to determine whether the mechanism that brings them about is the coupling between membrane height and composition variations[17,18] discussed here. This could be done by varying the parameters that determine the natural length scale, eq 24. For example, the parameter $b = b_\phi = b_\psi$, the coefficient of the square gradient terms in the free energy, eq 1, is expected to decrease with increasing temperature. Hence, all other things being equal, the theory predicts that the characteristic size of the microemulsion regions should decrease with increasing temperature. That is, as the temperature increases, the energy cost of variations in the composition decreases; hence, one should make more such variations which implies that the characteristic length decreases. Similarly, if one increases the surface tension of the membrane, making it more taut, then the energy cost of a long-wavelength variation in membrane height increases, so that one should make fewer such variations and the characteristic length decreases. This is reflected in eq 24. One could also determine whether the domains are out of registry, as predicted by this theory for symmetric membranes.

Further, it should be observed that in all of the model systems noted above the characteristic wavelengths are larger than those expected in the plasma membrane. This may be due to a small surface tension of the model membranes that lack the cytoskeleton of the plasma membrane. An additional factor is that the differences in spontaneous curvatures of the lipids utilized, all PCs, are relatively small. It would be very interesting to study model membranes with ternary mixtures of cholesterol, PC, and PE for one would expect, in that system, the characteristic wavelengths to be appreciably smaller. If the characteristic lengths were too small to be observed optically, X-ray or neutron scattering experiments might well be able to observe the characteristic peak in the structure function at nonzero wave vector that is characteristic of microemulsions.

Such observations of microemulsions in symmetric model membranes make their occurrence in the asymmetric plasma membrane plausible. As I have argued, this occurrence would provide the natural length scale for domains in both leaves, and a strong coupling between them.

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Notes

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REFERENCES

(1) Simons, K.; Ikonen, E. Functional rafts in cell membranes. *Nature* **1997**, *387*, 569−572.

(2) Lingwood, D.; Simons, K. Lipid rafts as a membrane-organizing priciple. *Science* **2010**, *327*, 46−50.

(3) Brown, D.; London, E. Structure and origin of ordered lipid domains in biological membranes. *J. Membr. Biol.* **1998**, *164*, 103−114.

(4) Schroeder, R.; London, E.; Brown, D. Interactions between saturated acyl chains confer detergent resistance on lipids and glycophosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 12130−12134.

(5) Hjort Ipsen, J.; Karlstrom, G.; Mouritsen, O.; Wennerstrom, H.; Zuckermann, M. Phase equilibria in the phosphatidylcholine-cholesterol system. *Biochim. Biophys. Acta, Biomembr.* **1987**, *905*, 162−172.

(6) Veatch, S. L.; Keller, S. L. Seeing spots: Complex phase behavior in simple membranes. *Biochim. Biophys. Acta, Mol. Cell Res.* **2005**, *1746*, 172−185.

(7) Zachowski, A. Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. *Biochem. J.* **1993**, *294*, 1−14.

(8) Wang, T. Y.; Leventis, R.; Silvius, J. R. Fluorescence-based evaluation of the partitioning of lipids and lipidated peptides into liquid-ordered microdomains: A model for molecular partitioning into 'lipid rafts'. *Biophys. J.* **2000**, *79*, 919−933.

(9) Devaux, P.; Morris, R. Transmembrane asymmetry and lateral domains in biological membranes. *Traffic* **2004**, *5*, 241−246.

(10) Machta, B.; Papanikolaou, S.; Sethna, J. P.; Veatch, S. L. Minimal model of plasma membrane heterogeneity requires coupling cortical actin to criticality. *Biophys. J.* **2011**, *100*, 1668−1677.

(11) Veatch, S. L.; Sengupta, P.; Honerkamp-Smith, A.; Holowka, D.; Baird, B. Critical fluctuations in plasma membrane vesicles. *ACS Chem. Biol.* **2008**, *3*, 287−93.

(12) Widom, B. Lattice model of microemulsions. *J. Chem. Phys.* **1986**, *84*, 6943−6954.

(13) Gompper, G.; Schick, M. *Self-assembling amphiphilic systems*; Academic Press: San Diego, CA, 1994.

(14) Yamamoto, T.; Brewster, R.; Safran, S. Chain ordering of hybrid lipids can stabilize domains in saturated/hybrid/cholesterol lipid membranes. *Euro Physics Lett.* **2010**, *91*, 28002-1−28002-6.

(15) Hirose, Y.; Komura, S.; Andelman, D. Concentration fluctuations and phase transitions in coupled modulated bilayers. *Phys. Rev. E* **2012**, *86*, 021916-1−13.

(16) Palmieri, B.; Safran, S. Hybrid lipids increase the probability of fluctuating nanodomains in mixed membranes. *Langmuir* **2013**, *29*, 5246−5261.

(17) Leibler, S. Curvature instability in membranes. *J. Phys. (Paris)* **1986**, *47*, 507−516.

DOI: 10.1021/acs.jpcb.7b08890
J. Phys. Chem. B XXXX, XXX, XXX−XXX

(18) Leibler, S.; Andelman, D. Ordered and curved meso-structures in membranes and amphiphilic films. *J. Phys. (Paris)* **1987**, *48*, 2013−2018.
(19) Liu, J.; Qi, S.; Groves, J.; Chakraborty, A. Phase segregation on different length scales in a model cell membrane system. *J. Phys. Chem. B* **2005**, *109*, 19960−19969.
(20) Schick, M. Membrane heterogeneity: Manifestation of a curvature-induced microemulsion. *Phys. Rev. E* **2012**, *85*, 031902-1−031902-4.
(21) Shlomovitz, R.; Schick, M. Model of a raft in both leaves of an asymmetric lipid bilayer. *Biophys. J.* **2013**, *105*, 1406−1413.
(22) Shlomovitz, R.; Maibaum, L.; Schick, M. Macroscopic phase separation, modulated phases, and microemulsions: a unified picture of rafts. *Biophys. J.* **2014**, *106*, 1979−1985.
(23) Kumar, P. B. S.; Gompper, G.; Lipowsky, R. Modulated phases in multicomponent fluid membranes. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **1999**, *60*, 4610−4618.
(24) Kollmitzer, B.; Heftberger, P.; Rappolt, M.; Pabst, G. Monolayer spontaneous curvature of raft-forming membrane lipids. *Soft Matter* **2013**, *9*, 10877−10884.
(25) Fuller, N.; Benatti, C.; Rand, R. Curvature and bending constants for phosphatidylserine-containging membranes. *Biophys. J.* **2003**, *85*, 1667−1674.
(26) Evans, E. Bending elastic modulus of red blood cell membrane derived from buckling instability in micropipet aspiration tests. *Biophys. J.* **1983**, *43*, 27−30.
(27) Lipowsky, R. Budding of membranes induced by intramembrane domains. *J. Phys. II* **1992**, *2*, 1825−1840.
(28) Dai, J.; Sheetz, M. P. Membrane tether formation from blebbing cells. *Biophys. J.* **1999**, *77*, 3363−3370.
(29) Fisher, M.; Widom, B. Decay of correlations in linear systems. *J. Chem. Phys.* **1969**, *50*, 3756−3772.
(30) Putzel, G. G.; Uline, M. J.; Szleifer, I.; Schick, M. Interleaflet coupling and domain registry in phase-separated lipid bilayers. *Biophys. J.* **2011**, *100*, 996−1004.
(31) Haataja, M. P. Lipid-domain co-localization induced by membrane undultions. *Biophys. J.* **2017**, *112*, 655−662.
(32) Blosser, M. C.; Honerkamp-Smith, A. R.; Han, T.; Haataja, M. P.; Keller, S. L. Transbilayer colocalization of lipid domains explained via measurement of strong coupling parameters. *Biophys. J.* **2015**, *109*, 2317−2327.
(33) Toner, J.; Nelson, D. Smectic, cholesteric, and Rayleigh-Bernard order in two dimensions. *Phys. Rev. B: Condens. Matter Mater. Phys.* **1981**, *23*, 316−334.
(34) Kawakatsu, T.; Andelman, D.; Kawasaki, K.; Taniguchi, T. Phase transitions and shapes of two component membranes and vesicles I: strong segregation limit. *J. Phys. II* **1993**, *3*, 971−997.
(35) Taniguchi, T.; Kawasaki, K.; Andelman, D.; Kawakatsu, T. Phase transitions and shapes of two component membranes and vesicles II: weak segregation limit. *J. Phys. II* **1994**, *4*, 1333−1362.
(36) Konyakhina, T.; Goh, S.; Amazon, J.; Heberle, F.; Wu, J.; Feigenson, G. Control of a nanscopic-to-macroscopic transition:Modulated phases in four-component DSPC/DOPC/POPC.Chol giant unilamellar vesicles. *Biophys. J.* **2011**, *101*, L8−L10.
(37) Goh, S. L.; Amazon, J.; Feigenson, G. Toward a better raft model: Modulated phases in the four-component bilayer, DSPC/DOPC/POPC/CHOL. *Biophys. J.* **2013**, *104*, 853−862.
(38) Shimokawa, N.; Mukai, R.; Nagata, M.; Takagi, M. Formulation of modulated phases and domain rigification in fatty acid-containing lipid membranes. *Phys. Chem. Chem. Phys.* **2017**, *19*, 13252−13263.

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