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Microemulsions, modulated phases and macroscopic phase separation: a unified picture of rafts

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Abstract

We consider two mechanisms that can lead to an inhomogeneous distribution of components in a multicomponent lipid bilayer: macroscopic phase separation and the formation of modulated phases. A simple model that encompasses both mechanisms displays a phase diagram that also includes a structured fluid, a microemulsion. Identifying rafts with the inhomogeneities of this structured fluid, we see how rafts are related to the occurrence of macroscopic phase separation or the formation of modulated phases in other systems, and focus our attention on specific differences between them.

Keywords:

lipid bilayer, macroscopic phase separation, membrane, microemulsion, modulated phase, plasma membrane, raft, yeast vacuole.

Introduction

It is an interesting time in the study of rafts [1,2]. Domains of lipids and/or proteins have now been seen in a few systems [3,4]. Modulated phases have also been seen both *in vitro* [5,6] and *in vivo* [4]. These observations are almost certainly related to 'rafts', which are presumably

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domains, but ones of a small size, say 100 nm. The discussion of just how these various phenomena are related is unclear and often contradictory. We believe that there is, in fact, an underlying unity to all of these phenomena, and will explicate this view here.

Let us begin with a membrane with several components. Why should these components be non-uniformly distributed on the membrane? There are a couple of reasons.

Phase separation

The simplest explanation is that it is energetically more favorable for the components to interact with other molecules of the same species, A with A, B with B, etc., than to interact with those of different species. If energy were the only criteria, the components would indeed separate, but because the system is in contact with a thermal bath, entropy must also be considered, and it prefers that the components be mixed. As the temperature is reduced, however, the entropic contribution to the free energy is reduced accordingly so that eventually the system will undergo phase separation. A simple system that illustrates this is a ternary mixture of a saturated lipid, such as sphingomyelin, an unsaturated lipid, such as POPC (1-palmitoyl-2-oleoylphosphatidylcholine), and cholesterol [7,8]. Its phase diagram at 23°C is shown in Figure 1.

This system can undergo separation into two liquid phases. One of them is rich in the saturated lipid, the other in the unsaturated lipid. Cholesterol partitions between the two and is more prevalent in the saturated lipid-rich phase. The driving force of this phase separation is that the more ordered saturated chains do not pack well with the unsaturated ones whose *cis* double bond results in a kink in that chain. Because of this kink, chains will tend to overlap, which results in a large energy penalty. The number of configurations in which these expensive overlaps occur can be decreased, and those without such overlaps increased, if the saturated lipids form their own more dense, more ordered phase, and the unsaturated lipids form their own less dense, less ordered one. The attractive van der Waals force, which is long-ranged and is responsible for the condensation of the lipids into dense liquids, probably has little to do

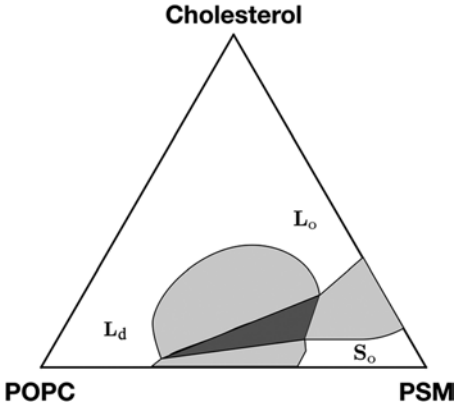

Figure 1. Phase diagram of a ternary mixture of POPC, sphingomyelin (PSM) and cholesterol at 23°C

There are two liquid phases, labelled L_o and L_d, and a gel phase, S_o. The three regions of coexistence between two phases are shown in light grey, and the triangular region of coexistence of all three phases is shown in dark grey. Reproduced with permission from [9].

with this transition into two lipid phases simply because it *is* long-ranged so that the relatively small density difference between the two phases results in a relatively small difference in the van der Waals energy. This phase separation, driven by packing considerations, also occurs in the simple binary system of saturated and unsaturated lipid. The phase rich in unsaturated lipids is a liquid, as in the ternary system, but in a binary system, the phase enriched in the saturated lipid is usually a well-ordered gel. It is seen in the lower right-hand portion of Figure 1, that is, for systems that consist primarily of sphingomyelin. If a relatively small amount of cholesterol is added to the binary system, it breaks up the order in the gel phase and brings about the coexistence of two liquid phases. There is a region in which all three phases, the two liquids and the gel, can coexist.

How many phases can coexist in such a system? We can derive the Gibbs phase rule as follows. The derivation is particularly simple because the system is a symmetric one, that is, the two leaflets are identical. The lipid bilayer can be thought of as consisting of two identical leaflets, of areas A that are separated by a semi-permeable barrier through which only energy and some components, such as cholesterol, can pass. Let the number of chemical species that are confined to the outer leaflet be denoted c_o. Again because of the symmetry, this is also the number of chemical species that are confined to the inner leaflet c_i. Finally, let the number of species that can exchange freely between the leaflets be c_x. A phase of the system is specified by the $c = c_o + c_x$ areal densities. Suppose that p phases coexist with one another. The system is specified by $p(c_o + c_x)$ areal densities and all phases are characterized by the same temperature. Thus the phase is specified by $p(c_o + c_x) + 1$ quantities. They are determined by the conditions of coexistence. These conditions consist of the requirement that each component has equal chemical potentials in all phases, a requirement that provides $(p - 1)(c_i + c_x)$ equations. Lastly, the surface tensions in the inner and outer leaflets must be the same in all coexisting phases. This provides another $(p - 1)$ constraints. The condition that the number of constraints be less than or equal to the number of unknown quantites yields the Gibbs phase rule that the number of coexisting phases must be less than, or equal to, $p \leq c_o + c_x + 2$. In the system of sphingomyelin, POPC and cholesterol, the former two components are found in the outer leaflet where they are confined, so $c_o = 2$. They are also found in the inner leaflet, where they are confined. Only cholesterol can pass from one leaflet to the other: $c_x = 1$. Hence the maximum number of phases that can coexist is five and they can do so only at one temperature. At an arbitrary temperature, the number of phases that can coexist must be less than or equal to four. That is the case in the phase diagram of Figure 1.

One can derive the Gibbs phase rule for the more general case of the asymmetric membrane in which the two leaflets are not identical. Then one finds that $p \leq c_o + c_i + c_x + 3$.

Modulated phases

There is another way in which different components can be made to segregate from one another. In this scenario, the system reduces its energy, not by having the components selectively interact with one another, but rather by having them each interact with something else, another component perhaps, or something less microscopic, such as the membrane curvature. For example, if one lipid component has a small head group compared with its tails, as is the case with DOPE (dioleoylphosphtidylethanolamine), and the other has a large head group comparable to its tails, as with DPPC (dipalmitoylphosphatidylcholine), then the former will

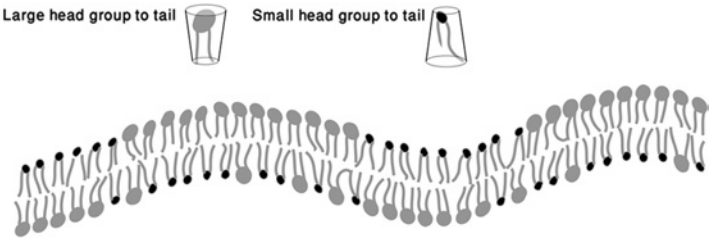

Figure 2. Coupling of curvature and composition
A lipid with a small head group, such as DOPE, goes preferentially to regions where the leaflet in which it is embedded are concave, causing the other lipid, such as DPPC, to go to regions in the same leaflet that are convex. Because concave regions in one leaflet are directly opposite convex regions in the other leaflet, anticorrelation of concentration fluctuations is expected in this bilayer.

prefer to occupy regions where the membrane bends inwards and cause the latter to occupy regions where the membrane bends outwards, as illustrated schematically in Figure 2. This tendency is expressed as a coupling between curvature and composition [10,11]. It is sufficiently important that its origin demands an explication.

For simplicity, let us consider a bilayer of which each leaflet consists of two components, A and B. Consider a point $\mathbf{r}$ on the bilayer and denote the concentration of A in the outer leaflet as $\phi_{\mathrm{A}}^{(o)}(\mathbf{r})$ and of B in the outer leaflet as $\phi_{\mathrm{B}}^{(o)}(\mathbf{r}) = 1 - \phi_{\mathrm{A}}^{(o)}(\mathbf{r})$. Similarly denote the concentrations in the inner leaflet as $\phi_{\mathrm{A}}^{(i)}(\mathbf{r})$ and $\phi_{\mathrm{B}}^{(i)}(\mathbf{r}) = 1 - \phi_{\mathrm{A}}^{(i)}(\mathbf{r})$. The bending energy of the bilayer can be written:

$$\int d^2r\, \kappa\big(H(\mathbf{r}) - c(\mathbf{r})\big)^2$$

where the bilayer bending modulus is κ, the local mean curvature of the bilayer is $H(\mathbf{r})$ and the local spontaneous curvature is $c(\mathbf{r})$. We assume that component A has a spontaneous curvature, c_A, which is different from that of component B, c_B. Taking into account the fact that when a bilayer bends in a certain direction the curvature of the inner and outer leaflets are opposite in sign, we can write the local spontaneous curvature as:

$$\begin{aligned} c(\mathbf{r}) &= c_{\mathrm{A}}\Big[\phi_{\mathrm{A}}^{(o)}(\mathbf{r}) - \phi_{\mathrm{A}}^{(i)}(\mathbf{r})\Big] + c_{\mathrm{B}}\Big[\phi_{\mathrm{B}}^{(o)}(\mathbf{r}) - \phi_{\mathrm{B}}^{(i)}(\mathbf{r})\Big] \\ &= (c_{\mathrm{A}} - c_{\mathrm{B}})\Big[\phi_{\mathrm{A}}^{(o)}(\mathbf{r}) - \phi_{\mathrm{A}}^{(i)}(\mathbf{r})\Big] \\ &\equiv (c_{\mathrm{A}} - c_{\mathrm{B}})\phi(\mathbf{r}) \end{aligned}$$

where the last line simply defines the order parameter $\phi(\mathbf{r})$ as the difference in the local concentrations of A in the outer and inner leaflets.

With this form for the local spontaneous curvature of the bilayer, its bending energy can now be written

$$\kappa\int d^2r\,\big(H(\mathbf{r}) - c(\mathbf{r})\big)^2 = \kappa\int d^2r\Big[H^2(\mathbf{r}) + (c_{\mathrm{A}} - c_{\mathrm{B}})^2\phi^2(\mathbf{r})\Big] - \Gamma\int d^2r\, H(\mathbf{r})\phi(\mathbf{r}) \qquad (1)$$

where $\Gamma \equiv 2\kappa(c_{\mathrm{A}} - c_{\mathrm{B}})$. The interesting term in this expression is the last one. It states that the system can gain energy if the local curvature, $H(\mathbf{r})$, and the local difference in compositions

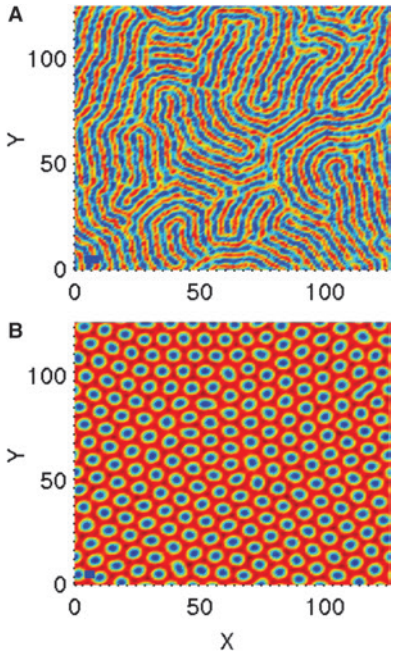

Figure 3. Representative configurations of two modulated phases
(**A**) A stripe phase and (**B**) a hexagonal phase obtained in simulations of a two–component bilayer [12]. Red indicates regions of large concentration of one component, blue indicates large concentrations of the other. Reproduced with permission from [12].

between outer and inner leaflets, $\phi(\mathbf{r})$, are correlated. In particular, the system gains energy if the lipids with the small head group go where a leaflet bends inwards and those with large head groups go where one bends outwards, as depicted in Figure 2. As a result of this coupling, the composition varies with the curvature of the membrane. It can become favorable for a flat membrane to spontaneously adopt a periodic, spatially varying curvature, which is accompanied by a periodic, spatially varying composition; that is, a modulated phase. The pattern of modulations depends on the average values of the A and B compositions. If the amounts of A and B are comparable, then a phase of stripes is produced, whereas if the amount of one component is much larger than the other, a phase consisting of small regions of the minority component hexagonally arranged in a sea of the majority component is brought about. Representations of these phases from simulations [12] are shown in Figure 3. As noted earlier, modulated phases have been observed both *in vitro* [5,6] and *in vivo* [4].

Length scales

Thus there are at least two mechanisms by which regions of one lipid component can be separated from regions of another. In general, both mechanisms would be expected to operate simultaneously. What distinguishes them? Can we say that any separation is due to one and not the other? In the case of a membrane that is flat on average, the answer is definitely yes. The two mechanisms bring about separation on very different length scales. In the case of phase separation, the two uniform phases separate completely and are, in principal, infinite in

extent. There is one interface between them. In contrast, in a modulated phase, there are many regions in which one component or the other dominates. These would be manifest in a scattering experiment that would display a series of peaks at integer multiples of a wave vector of magnitude $k^* = 2\pi/\lambda$, with λ the characteristic wavelength of the modulations. Because the composition is coupled to the membrane, it is not a surprise that the characteristic wavelength is determined by membrane properties: its bending modulus κ and its surface tension σ. In particular, the wavelength is on the order of $(\kappa/\sigma)^{1/2}$, a distance we shall simply refer to as D.

A schematic phase diagram for a system for which the total amounts of components A and B are comparable, is shown in Figure 4 in the plane of temperature, T, and strength, Γ, of the coupling between membrane height and composition fluctuations. There are four phases. At high temperatures, there is a fluid phase in which the ensemble average value of the order parameter, $<\phi(\mathbf{r})>$, vanishes. As the temperature is lowered in a system in which the coupling is not too large, $\Gamma < \Gamma_L$, the disordered phase undergoes a transition into two uniform phases, one enriched in one component, the other phase enriched in the other component. However if the coupling is large, $\Gamma > \Gamma_L$, the disordered system undergoes a transition to a modulated phase. At low temperatures the two uniform phases can coexist with the modulated phase along a triple line.

The disordered fluid phase is not without interest. Although the ensemble average value of the order parameter vanishes everywhere, $<\phi(\mathbf{r})> = 0$, its fluctuations, as measured by the structure function, are non-zero. At a temperature near the transition to two-phase coexistence, the fluctuations will be quite large, particularly those at very small wave vectors. The disordered phase exhibits fluctuating droplets whose composition resembles that of one or the other of the two uniform phases that are about to become stable. Their size is given by the correlation length, ξ. If the transition is a continuous one, the fluctuations at zero wave number diverge as the transition is approached, and the size of the droplets also diverges.

Similarly at temperatures near the transition to a modulated phase, the fluctuations at wave number k^* become large and, if the transition were continuous, would diverge as the transition was approached. The fact that fluctuations are large at wavelengths of non-zero

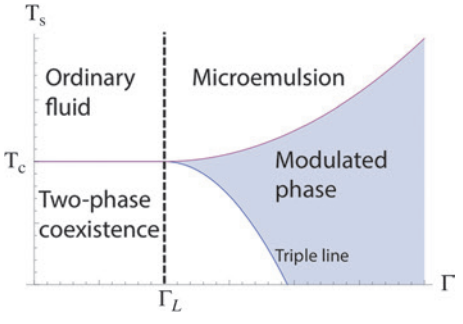

Figure 4. Schematic phase diagram of the model as a function of temperature T and coupling Γ

There are four phases. For any coupling there is at sufficiently high temperatures a fluid phase. At lower temperatures and small Γ the system separates into two fluid phases, liquid-ordered and liquid-disordered. This region is denoted 'two-phase coexistence' in the diagram. At sufficiently large **Γ**, a modulated phase occurs. The dashed line in the fluid phase is the Lifshitz line. To the left of it, the fluid is an ordinary one, whereas to the right of it, the fluid is a microemulsion.

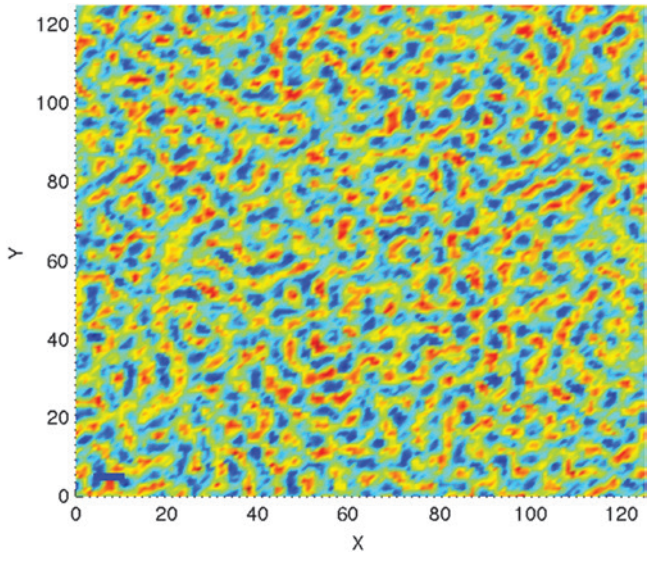

Figure 5. Representative configuration of a microemulsion that results from the melting of the stripe phase (shown in Figure 3a)
The results are from a simulation; reproduced with permission from [12].

magnitude has interesting consequences for the disordered phase. It means that although the disordered phase is characterized by fluctuating droplets whose size is of the order of the correlation length, ξ, within these droplets the composition is modulated with a wave number k^*. If the transition to the modulated phase were a continuous one, the size of the droplets would diverge as the transition was approached whereas the wave number k^* would remain finite. This means that the disordered phase in the vicinity of the modulated phase is a fluid that must be characterized by *two* lengths, ξ *and* k^*. Such a fluid is denoted a microemulsion [13]. A representation of this phase from simulation is shown in Figure 5.

The boundary in the phase diagram between a microemulsion and an ordinary fluid is arbitrary because there is no singularity in the free energy as one passes from one to the other, but a useful experimental marker is the line at which the peak in the structure factor begins to move off zero wave vector. It is denoted the Lifshitz line, and is shown as a dashed line in Figure 4.

The system on a closed vesicle

Thus far, we have considered the system on a membrane that fluctuates about a configuration that is flat. What changes when the system is put on a closed vesicle, like a cell, or a vacuole, one with a characteristic size R? Clearly if the characteristic size of the modulated phase, $D \equiv (\kappa/\sigma)^{1/2}$ is much smaller than R, there is little change in the modulated phases. But the uniform phases are changed a great deal because they can no longer be infinite in extent, and they sense the constraint of the finite size [14–18]. Instead of a separation into two domains, one A-rich, the other B-rich, the vesicle might separate into three, with A-rich domains at the two poles and a B-rich domain at the equator. There can be four domains, or some other number. The actual value is prescribed by a balance between the cost of the domain boundaries, which

favors few domains, and the energy gained from the coupling between concentration and curvature, which favors many domains. If the number of domains is not too large, their characteristic size will be a fraction of R, whereas if the number of domains is very large, the characteristic size will be much smaller than R. If this characteristic size between domains is comparable to the characteristic size of modulated phases, D, then there is no meaningful distinction between a modulated phase and a phase-separated one.

For completeness, we note the following: consider a single-component vesicle that has a shape on which the local mean curvature, $H(\mathbf{r})$, varies over the vesicle, a shape like that of a red blood cell, or a multi-lobed yeast vacuole, for example. Now add to the vesicle a second component, one with a different spontaneous curvature from that of the first. One would find that one of the two components would go preferentially to the regions of higher curvature. This can be seen from the coupling of curvature and composition (eqn 1). It was thought, therefore, that shape changes of vesicles could be a biological means of separating components [19]. Although the effect must certainly be present, its magnitude in the case in which the components are mixed in the absence of the varying curvature has not been measured. If the components are already phase separated, however, then one can demonstrate that regions of different curvature are indeed preferentially enriched in one component or the other [20,21].

Conclusions: putting it all together – vacuoles, rafts, the lot

We believe the above picture can encompass all of the experimental observations. Let us begin with yeast vacuoles in growing cells. These vacuoles show no domains and are multilobed in shape. We expect the concentration difference between components, $\phi(\mathbf{r})$, to smoothly follow the curvature, with the tips of lobes enriched in one component and the invaginations between lobes enriched in the other. That composition differences like this are not observed again indicates that this separation mechanism is weak when the components are not already phase separated. In the static phase of yeast, one vacuole usually grows to dominate the interior of the cell and becomes convex everywhere. Now the observation of Toulmay and Prinz [4] of domains on the vacuole, domains whose size D is comparable with R itself, indicates that the components do tend to phase separate, and the size of domains is of the order of the vacuole size itself. What of the plasma membrane of the yeast cell that shows numerous domains whose size is certainly smaller than R [3]? Presumably because of the presence of a cytoskeleton, the surface tension of the plasma membrane is larger than that of vacuoles, hence domains in the former are smaller than in the latter. The cytoskeleton is also observed to enhance domain formation [22], a phenomenon that might be due to its interaction with motor proteins [23] or to its effect in decreasing the entropy of mixing of the lipids, an entropy that opposes domain formation [24].

We turn now to the observations of Konyakhina et al. [5] and Goh et al. [6]. These experiments are reviewed in the present volume in Chapter 3 by Ackerman and Feigenson [24a]. The ternary mixture of DSPC (distearoylphosphatidylcholine), DOPC (dioleoylphosphatidylcholine) and cholesterol exhibits macroscopic liquid/liquid phase separation at a temperature of 23°C, whereas the ternary mixture of DSPC, POPC and cholesterol does not. Presumably this is because the two unsaturated chains of DOPC pack less well with the saturated chains of

DSPC than does the single unsaturated chain of POPC [25]. Konyakhina et al. [5] and Goh et al. [6] studied a system of the four components DSPC, DOPC, POPC and cholesterol and varied the amounts of DOPC and POPC. As noted, when the fraction of DOPC was large, liquid/liquid phase separation was observed, and when it was small and that of POPC was large, the system appeared to be uniform. Interestingly, in a range of relative DOPC concentrations, the system exhibited modulated phases. In terms of our unified picture, as embodied in Figure 4, this behaviour can be understood as follows. As the amount of DOPC is increased, the chain repulsion increases as does the tendency of the system to phase separate. Thus one is effectively lowering the temperature. A glance at Figure 4 shows that, for sufficiently large coupling Γ, if the system starts in a disordered phase and the temperature is lowered, it will pass through a modulated phase before exhibiting phase separation. This is just the behaviour observed in experiment [5,6]. Our scenario indicates that the initial uniform fluid phase is not a simple one, but rather is a microemulsion.

And now to 'rafts'. It has been emphasized by Devaux and Morris [26] that the great difference in compositions of the plasma membrane of mammalian cells must be part and parcel of any description of rafts. Whereas the outer leaflet has a large fraction of sphingomyelin in addition to unsaturated lipids, the inner leaflet is composed almost entirely of unsaturated lipids. Consequently, whereas symmetric bilayers with compositions mimicking that of the outer leaflet readily phase separate [7,8], those mimicking the composition of the inner leaflet do not [27]. It is expected, therefore, that if the plasma membrane, with its coupled inner and outer leaflets, undergoes any phase separation at all, the transition would occur at a much lower temperature than that of the former symmetric bilayer. In our schematic phase diagram, this means that the biological temperature T is above the phase separation temperature. What about the coupling of curvature to concentration? Two of us have suggested that it is the inner leaflet that is important [28]. One of the major classes of lipid components of the inner leaflet are the PEs (phosphatidylethanolamines), which have a small head group. This is reflected in the large negative spontaneous curvature of POPE (1-stearoyl-2-oleoyl-phosphoethanolamine), which is $-0.283\ \mathrm{nm}^{-1}$ [29]. It is also evidenced in the tendency of PEs to form inverted hexagonal phases [30].

The other major class, the phosphatidylserines, have a much smaller spontaneous curvature. They do not form inverted hexagonal phases at physiological pH [31]. Because of this large difference in spontaneous curvatures, the coupling between curvature and composition of the inner leaflet, Γ, is expected to be large. With a large Γ and a temperature, T, above that of phase separation, one sees from the phase diagram of Figure 4 that the system is expected to be either in a modulated phase or a fluid microemulsion. The dynamic nature of the inhomogeneities in a microemulsion would, in comparison with experimental observations, favor the latter. Thus we identify rafts with the inhomogeneities of a microemulsion. Because the inner and outer leaflets are coupled, these inhomogeneities are also exhibited in the outer leaflet [28].

In conclusion, one relatively simple picture can encompass the various scenarios that have been observed and can place them in context. To understand why one behaviour, separation into coexisting phases, formation of a modulated phase or formation of a microemulsion, is observed rather than another, the model causes one to focus on the mechanism that drives the formation of modulated phases. In particular, the suggestion that the mechanism is a coupling of membrane height and concentration fluctuations forces one to examine the relative compositions of the different systems, particularly how their compositions differ of those lipids with

quite distinct spontaneous curvatures. It also forces one to look at those circumstances that would change the length scale of modulations, such as whether a membrane has a cytoskeleton, and re-enforces the importance of determining whether the two leaflets of the particular membrane are similar in composition or not. Like any useful model, it indicates what questions we should be asking of the systems we study.

Summary

- There are several mechanisms that could explain a non-uniform distribution of components in a lipid bilayer.
- Simple repulsions between unlike molecules can bring about phase separation into coexisting, uniform, liquid phases.
- Modulated phases, characterized by alternating regions of a definite wavelength enriched in one component or another, can be formed by several mechanisms. One of these is the coupling of fluctuations in membrane height to fluctuations in membrane composition. The coupling is stronger if the components have very different spontaneous curvatures, as is the case with the components on the inner leaflet of the mammalian plasma membrane.
- A simple model shows that uniform liquid phases and modulated phases can occur near one another in the sense that small changes in interactions can take the system from one to the other.
- Both phase separation and modulated phases have been observed *in vitro* and in yeast, but not in the mammalian plasma membrane.
- A modulated phase 'melts' into a microemulsion, a disordered phase characterized by two lengths: the usual correlation length and a wavelength over which the composition varies. A microemulsion is a fluid with structure. Its fluctuating regions of a characteristic size are a good candidate for 'rafts'.
- Similar phenomena occur on closed vesicles or cells. If the rafts are much smaller than the cell size, the distinction between phase separation, modulated phases and microemulsions is clear.
- The model can explain a sequence of phases observed *in vitro*: from phase separation to modulated phase to a disordered phase. The model predicts that the disordered phase is a microemulsion.

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