Point of VIEW

Understanding Membranes

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ABSTRACT The fluorescence intensity fluctuations of fluorescent lipid probes seen in giant plasma membrane blebs tell an important story about the physical state of the lipids from animal cell membranes. The fluctuations are associated with lateral liquid–liquid phase separation observed in these same membrane blebs at lower temperatures. Remarkably, the intensity fluctuations in the membrane blebs are found to be the same as those expected for the theoretical 2D Ising ferromagnet!

The accompanying Letter by Veatch *et al*. on page 287 describes a benchmark study of the lipids in bilayer membranes composed of animal cell lipids (*1*). The bilayers are formed as giant plasma membrane vesicles (GPMVs) that bleb from rat basophile leukemia cells. The investigators use a low concentration of fluorescent lipid probe to infer composition and density changes. A time- and spatial-image analysis is made of these fluctuations. The membranes show a phase transition between 15 and 25 °C, below which there is a liquid–liquid phase separation. The image analysis of the membrane fluorescence both above and below the miscibility phase transition enables the measurement of critical exponents. The Veatch Letter has a companion paper by Honnerkamp-Smith *et al*. (*2*), a study of a defined model bilayer system. The reader is referred to this companion paper for additional discussion of the physical chemistry of bilayers near miscibility critical points. Both papers report critical exponents that belong to the universality class of the 2D Ising ferromagnet. But what are critical exponents, what is the Ising model, and how could these be related to animal cell membranes lipids?

In 1944, Lars Onsager solved the theoretical 2D Ising problem, one of the great challenges of statistical physics (*3*). We can describe the problem by means of Figure 1. Consider a 2D infinite square lattice, in each cell of which an A or a B molecule is placed, and where the total number of A molecules is equal to the total number of B molecules. Assume that neighboring A and B molecules repel each other with energy $\varepsilon > 0$. At high temperatures, $kT \gg \varepsilon$, entropy dominates the free energy, so the arrangement is essentially random, as in Figure 1, panel a. At low temperatures, $kT \ll \varepsilon$, the repulsions of A and B dominate, and there is a phase separation as in Figure 1, panel b. The transition between the high-temperature disordered state and the low-temperature phase separated state takes place at the critical temperature T_c, for which a distribution of molecules is sketched schematically in Figure 1, panel c. At the critical temperature, a strong but not perfect correlation exists among the molecular positions. That is, even for distances that are large compared with the lattice spacing, an A molecule is most likely to be surrounded by A molecules. These correlations rapidly become weaker as temperature is increased above the critical temperature; this dependence has been the subject of extensive theoretical study (*4*). A distance ξ describes this correlation. In the Ising model, ξ is proportional to $(T - T_c)^{-1}$ where $T - T_c$ is the temperature above the critical temperature. In this case, the critical exponent is -1. This same temperature dependence of the correlation length is found in the experiment of Veatch *et al*.

The authors find another Ising critical exponent (7/4) that describes the temperature dependence of the zero frequency component of the structure factor. In the schematic phase separation shown in Figure 1, panel b, a rather well-defined boundary exists between the A- and B-rich regions, even on the molecular distance scale. An energy is associated with this boundary, and the energy per unit length

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10.1021/cb8000974 CCC: $40.75

Figure 1. Mixtures of equal numbers of A and B molecules for which the free energy involves only the entropy of mixing and a nearest neighbor repulsion between dissimilar molecules. This picture is equivalent to the ferromagnetic Ising model. Temperatures shown are above (panel a), below (panel b), and at (panel c) the critical temperature T_c. At the critical temperature, the correlation length is infinite and the composition correlation function is much longer range than the lattice spacing, features that are not evident in this schematic drawing.

of boundary is the line tension. As the critical temperature is approached from below, this tension approaches zero. Again, the observed temperature dependence is characterized by an Ising critical exponent.

A reader of this work may or may not be amazed at these results. A theoretical physicist might not be amazed on the grounds that a 2D system with only short-range interactions would be expected to belong to the Ising universality class. A physical chemist would certainly be somewhat amazed in that the bilayer membrane is not strictly 2D, but involves two coupled monolayers, with the possibility of bending fluctuations that could be particularly significant in the critical region. And the biochemist would surely be amazed to learn that a bilayer membrane composed of dozens or even hundreds of different lipid components might show properties in common with those calculated for a seemingly primitive mathematical model with two chemical components.

Unfortunately, the universality of critical behavior disguises that in which the chemist and biochemist are most interested: the nature and specificity of the intermolecular interactions. This point is illustrated schematically in Figure 2, which is to be compared with Figure 1, panel b. Figure 2 again imagines a model that starts off with two molecular components, A and B, but here, A and B undergo a reversible reaction to produce a third component, C. When component C is immiscible with components A and B, there is a separation into two phases, one rich in C and the other rich in A and B. It has been shown that at least for some lattice models, such complex formation can be mapped on the Ising model (*5*). Thus, critical exponents do not distinguish the repulsive interaction between A and B in the first case and the attractive interaction between A and B in the second case. Conformity to the model does require that the important intermolecular interactions be short range.

Veatch *et al.* have also analyzed the time dependence of the critical fluctuations in the GPMVs, and using these results they have determined the gradient diffusion coefficient of the fluorescent lipid probe. As expected, this gradient diffusion coefficient goes to zero as the critical temperature is approached. Again, the quantitative results fit with predictions of the 2D Ising model. The absence of structural molecular information in these equilibrium and dynamic

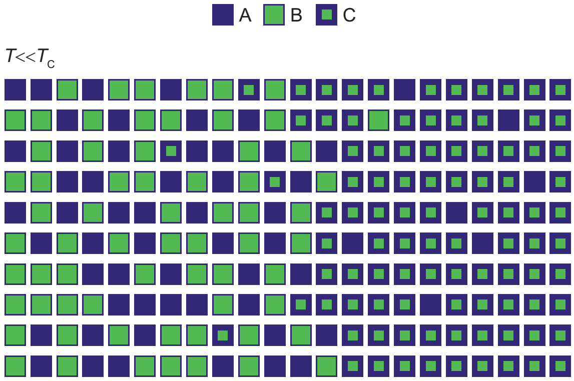

Figure 2. A phase separation can also arise when two components, A and B, undergo a reversible chemical reaction to produce a product, C, that is immiscible with A and B. Complex formation of this type also involves only short-range interactions and is expected to conform to the Ising model, with the same critical properties as the example in Figure 1.

measurements is a result of the fact they involve distances that are large compared to the lattice spacing.

As remarked above, the work by Veatch *et al.* discussed here provides a benchmark in the field of membrane physical chemistry and biophysics. It raises the bar in the sense that proposed theoretical models for the physical chemical properties of bilayers should be consistent with this Ising model. I suggest that this is particularly true for theoretical analyses of the lateral diffusion of lipids, where there is likely to be a crossover in diffusive behavior when $\xi/\lambda \sim 1$, where λ is the fluctuation wavelength (*6*).

Our view is that this outstanding paper, together with its companion paper, shows productive directions for future research in the physical chemistry and cell biology of membranes. In their discussion Veatch *et al.* have emphasized the proximity of the critical temperatures to cell growth temperature and the fluctuations in composition. In addition, I would emphasize that understanding these critical properties is one step along the way to understanding weak but nonetheless specific lipid−lipid and lipid−protein interactions.

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