CRITICAL EFFECTS FROM LIPID-PROTEIN INTERACTION IN MEMBRANES

I. THEORETICAL DESCRIPTION

FRITZ JÄHNIG, *Max-Planck-Institut für Biologie, 74 Tübingen, Federal Republic of Germany*

ABSTRACT The ordered-fluid phase transition in lipid membranes is described within the framework of the Landau theory, a general theory for phase transitions. The long-range order of lipids is characterized by the orientational order of the hydrocarbon chains implying the ordered-fluid transition to be of first order. Approaching the transition from either side, thermodynamic fluctuations and response functions are shown to increase, e.g. the specific heat, the lateral compressibility, the permeability, and the lateral diffusion coefficient. A protein molecule incorporated in the membrane is represented as a boundary condition on the lipid order at the protein surface. In the surrounding lipid the perturbation falls off exponentially with the coherence length. Assuming the protein molecules to be distributed homogeneously in the membrane plane and the boundary condition to be temperature independent, the protein-induced shift of the transition temperature and the latent heat is calculated. The latent heat decreases linearly with the protein concentration until it vanishes at a critical point. The critical protein concentration is determined by the ratios of the coherence length to lipid and protein radii in the membrane plane. At the critical point the lipid specific heat, lateral compressibility, permeability, and lateral diffusion coefficient become maximal.

INTRODUCTION

One of the most exciting discoveries in the physics of many-particle systems during the last decades was the universality of their behavior in the vicinity of phase transitions. Qualitatively, universality refers to the onset of cooperative or long-range order at a phase transition that is preceded by the pretransitional increase of fluctuations of this long-range order. This universality finds its expression in a universal description of phase transitions known as the Landau theory. Here the different systems are only distinguished by different order parameters, which characterize their long-range order (1). Quantitatively, universality refers to distinct variations of order and order fluctuations with temperature. The order parameter decreases as $(T^* - T)^x$, where T^* is the critical temperature; the strength of the fluctuations, their spatial extension described by the coherence length and their relaxation time, as well as the response functions increase as $|T^* - T|^{-x}$, with different critical exponents x. First of all, for any given system these exponents are not independent of each other, but are correlated by universal scaling laws. The simplest set of exponents that fulfill the scaling laws are the classical ones of the original Landau theory for superconductors. In general, the exponents for any system depend only on the dimensionality of the system and on the number of independent components of the order parameter; they do not depend on the microscopic details (2).

In artificial lipid membranes, as well as in biological membranes, a phase transition also occurs. It is the ordered-fluid transition, and one is tempted to ask whether or not this phase transition fits into the universal scheme. What is the long-range order; are there pretransitional fluctuations and diverging response functions; do these show a power-law behavior? An additional problem arises, namely, that the ordered fluid transition is of first order, whereas universality is typical for phase transitions of second order. There are, however, cases of first-order transitions such as the liquid-gas transition under noncritical conditions or the nematic-isotropic transition, which are also subject to this universality, although in a slightly modified way. For example, they can be described by the Landau theory, and the classical critical exponents apply. Our aim will therefore be to derive a Landau description of the ordered-fluid transition.

In a first step, we treat the one-component system, pure lipid membranes, and derive the static and dynamic effects at the genuine ordered-fluid transition. In the next step, the alteration of the lipid phase transition by proteins or cholesterol is studied, both of which are present in biological membranes. This can still be achieved within the framework of the Landau theory, if proteins and cholesterol are represented as boundary conditions for the lipid long-range order. The most interesting result is that the ordered fluid transition can become a critical point at a certain protein or cholesterol concentration. The dynamics of the membrane are then drastically enhanced, and such important membrane functions like permeability and lateral diffusion are increased.

The ordered-fluid transition under the influence of proteins or cholesterol has already been studied theoretically. Closely related to the present work is the study of Owicki et al. (3, 4). These authors, however, did not dwell upon the dynamic pretransitional behavior, thermodynamic fluctuations, and response functions. Moreover, all the results were obtained by computer analysis, which tends to obscure their physical meaning. More sophisticated theories starting from molecular interactions have been worked out by Marčelja (5) and by Pink and Chapman (6). Even more computer processing is involved in these theories.

Detailed experimental results on the lipid phase transition and its alteration by proteins or cholesterol exist. Upon comparison with the results of the Landau theory they can be given a unified interpretation. Because everyone in this field is not willing to go through the theory but might be interested in a qualitative interpretation of the experimental results, this is presented in a separate subsequent paper.

THE ORDERED-FLUID TRANSITION IN PURE LIPID MEMBRANES

Static Lipid Order

The Landau theory for phase transitions starts with the definition of the order parameter characterizing the macroscopic or long-range order that arises spontaneously at the phase transition. At the ordered-fluid transition two types of long-range order arise: the lateral positional order and the orientational order of the lipid-hydrocarbon chains. The longer ranged order is responsible for the phase transition. In the case of long chains, this is the orientational order—if one long chain changes its orientation, many others have to follow suit, whereas if the position is changed, only a few chains have to be displaced. Thus the

orientational order changes spontaneously at the ordered-fluid transition, and the positional order changes as a consequence of that.

The orientational order of flexible chains such as the lipid-hydrocarbon chains is a superposition of internal or conformational order and rigid body order, and since the internal order varies along the chains, the orientational order must be specified for each CH_2 segment. The instantaneous orientation of a segment n is described by the angle θ_n between its direction and the preferred direction, the inward membrane normal. The segment orientation undergoes statistical fluctuations because of thermal motion. The degree of its average orientation can be obtained from temporal averages which, for a system of many identical particles, are equal to averages over all particles. The average of $\cos\theta_n$, $\overline{\cos\theta_n} = x_n$, is a measure of the orientation of the segment into the direction of the inward membrane normal, specifying the unidirectional order of the segment. To characterize the orientational order, unidirectional order alone is not sufficient. If we imagine a segment n, which is oriented along the inner membrane normal as frequently as opposite to it (evidently $x_n = 0$) some order is nonetheless still present. The segment still has a finite uniaxial order, which is described by an average of $\cos^2\theta_n$, namely $S_n = (3\,\overline{\cos^2\theta_n} - 1)/2$. By definition, x_n and S_n are 1 for complete order and 0 for complete disorder.

For the purposes of constructing a theory for the phase transition we can restrict ourselves to averages over all segments along the chains, $x = N^{-1}\Sigma_1^N x_n$ and $S = N^{-1}\Sigma_1^N S_n$ with N being the total number of segments. The average unidirectional order x is coupled to the positional order represented by the lateral packing density $\nu = 1/A$, A denoting the area per chain. To derive this coupling relationship we note that the volume of a chain is $V = ANlx$, l is the length of a segment. Introducing the segment density $\rho = N/V$, we obtain

$$\nu = \frac{1}{A} = \rho l x. \tag{1}$$

The segment density ρ is approximately constant, so that the packing density varies linearly with the strength of the unidirectional order. This implies that a change in the orientational order, e.g. at the phase transition, is accompanied by a change in the positional order as stated above.

The quantities x and S describe the strength of the corresponding orders, the complete order parameters furthermore specify their spatial symmetry. The order parameter for the unidirectional order is a vector $x_i = xn_i$, where n_i is a unit vector pointing in the preferred direction. The order parameter for the uniaxial order is a symmetric traceless tensor $S_{ij} = S(3n_in_j - \delta_{ij})/2$, known from liquid crystals (7).

Having defined the relevant order parameters, the Landau theory proceeds by expanding the free energy density f of the system in powers of the order parameters. Thus we have to construct scalar terms from x_i and S_{ij}. A linear term in S_{ij} is possible only in combination with x_ix_j (since $S_{ii} = 0$, summation over repeated indices implied), whereas higher order terms in S_{ij} including a cubic one are possible without x_i. Inserting the above expressions for x_i and S_{ij} the scalar terms can be evaluated (e.g. $x_ix_jS_{ij} = xS$) and one obtains

$$\tilde{f} = -a_1\tilde{S} + \frac{1}{2}a_2\tilde{S}^2 - \frac{1}{3}a_3\tilde{S}^3 + \frac{1}{4}a_4\tilde{S}^4, \tag{2}$$

where the coefficients a_α are functions of $\tilde{x}$, e.g. $a_1 \sim \tilde{x}^2$, and the tilde distinguishes arbitrary values from equilibrium values. The equilibrium values of the order parameters follow from the conditions that the free energy is minimal and, if external forces are acting, that these are balanced by internal forces. In our case these conditions take the form $\partial\tilde{f}/\partial\tilde{S} = 0$ and $\partial\tilde{f}/\partial\tilde{A} = -\pi$, π representing the external surface pressure, which holds the membrane together and originates in the hydrophobic interaction between the hydrocarbon chains and the surrounding water. Application of Eq. 2 to the first condition leads to

$$-a_1 + a_2 S - a_3 S^2 + a_4 S^3 = 0, \tag{3}$$

yielding a solution $S(x)$ which minimizes the free energy. To evaluate the second condition we replace A by x using Eq. 1 and obtain a solution $x(S,\pi)$. Taken together, we formally get the equilibrium order parameters as functions of the external surface pressure, $S(\pi)$ and $x(\pi)$, or $A(\pi)$. The latter functional dependence is accessible in monolayer experiments, where π can be varied externally; in aqueous dispersions, however, π is fixed. In the further analysis, for simplicity we only treat the uniaxial order $S(\pi)$, so we are only concerned with the equilibrium condition Eq. 3, in which the coefficients a_α are now functions of π.[1]

Additionally, the coefficients depend upon the other external variable, the temperature. This dependence is introduced in the usual way by assuming $a_2 = a_2'(T - T^*)$ with $a_2' > 0$, whereas the other coefficients are temperature independent and $a_{1,3,4} > 0$. Then the free energy behaves qualitatively as shown in Fig. 1, and the order parameter as shown in Fig. 2. For small a_1 corresponding to small π the phase transition is of first order, as a consequence of the cubic term that arose from the symmetry of the uniaxial order. This is best seen in the

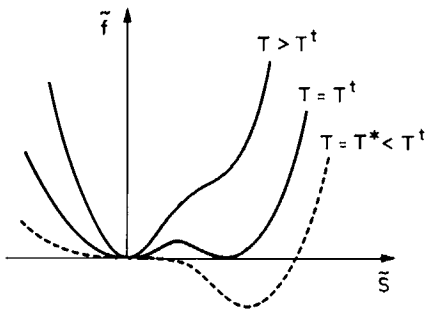

FIGURE 1

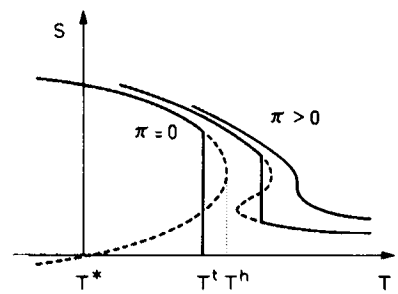

FIGURE 2

FIGURE 1 The free energy as a function of the order parameter above, at, and below the phase transition temperature T^t.

FIGURE 2 Temperature dependence of the order parameter for different values of the surface pressure π. Dashed lines indicate nonequilibrium solutions.

[1]Owicki et al. (3, 4) used the area A per lipid molecule (more exactly the difference $A^t - A$) as the single order parameter and the surface pressure as the external variable, whereas the orientational order was not considered. The positional order changed spontaneously at the phase transition, in contrast to the arguments presented above. Since their order parameter is a scalar, the phase transition similarly resulted as a first-order transition, in qualitative agreement with our description.

limiting case $a_1 = 0$. There the order parameter follows from Eq. 3 as $S^f = 0$ in the fluid phase and

$$S^o = \frac{a_3}{2a_4}(1 \pm \sqrt{1 - 4a_2a_4/a_3^2}) \tag{4}$$

in the ordered phase. The transition temperature is derived from the condition that the free energies of the fluid and ordered phase are equal, $f^o = f^f$, leading to

$$a_2^t = \frac{2a_3}{9a_4} \quad \text{or} \quad T^t = T^* + \frac{2a_3^2}{9a_2'a_4} \tag{5a}$$

and

$$S^t = \frac{2a_3}{3a_4}. \tag{5b}$$

The cubic term shifts the phase transition from a second-order transition at T^* to a first-order transition at T^t with a discontinuity S^t in the order parameter. The latent heat follows from the entropy $\sigma = -\partial f/\partial T$ as $q = T^t(\sigma^f - \sigma^o)$ and is equal to

$$q = \frac{1}{2}a_2'T^tS^{t2}. \tag{5c}$$

With increasing a_1, the surface pressure acts to increase the orientational order in the fluid phase more effectively than in the ordered phase (Fig. 2). Thus the discontinuity of the order parameter at the phase transition is decreased. At a critical value of the surface pressure the discontinuity vanishes, and the transition becomes of second order or a critical point. In aqueous dispersions, the actual value of the surface pressure lies below the critical value and the ordered-fluid transition is "weakly first order."

The coefficients a_α can be determined from experiment using relations such as Eqs. 5a-c. They may also be deduced theoretically from the molecular interactions, within the framework of a microscopic theory of lipid-chain order. Such a theory has been articulated by the present author, treating the chains as continuous lines with bend elasticity which interact via van der Waals forces and steric hindrance (8). For long chains ($N \to \infty$) and in lowest order of x, the result for the a_α (normalized to yield the free energy per segment) is

$$a_1 = \frac{1}{2}\Lambda x^2 \tag{6a}$$

$$a_2 = \Lambda\left[1 - \frac{2}{3 \cdot 5}\frac{M\Lambda}{(kT)^2}\right] \tag{6b}$$

$$a_3 = \frac{2}{3 \cdot 5 \cdot 7}\Lambda\left(\frac{M\Lambda}{(kT)^2}\right)^2 \tag{6c}$$

$$a_4 = \frac{4 \cdot 13}{27 \cdot 125 \cdot 7}\Lambda\left(\frac{M\Lambda}{(kT)^2}\right)^3, \tag{6d}$$

where M is the bend elasticity constant, Λ the van der Waals interaction constant, x expresses the effect of steric hindrance[2], and k is the Boltzmann constant. As required in the Landau theory, the coefficients $a_{1,3,4}$ depend weakly on temperature, whereas a_2 varies approximately as $T - T^*$ with $kT^* = (2/15\ M\Lambda)^{1/2}$. The phase transition temperature T_t, for $a_1 = 0$ according to Eq. 5a, is given by $kT^t = 0.4\ (M\Lambda)^{1/2}$. For numerical estimates, we insert M = 2.5 kcal/mol and Λ = 0.7 kcal/mol and obtain T^t = 262°K and $T^t - T^*$ = 20°C.

Fluctuations and Response

Order parameter fluctuations and response represent deviations of the order parameter from its equilibrium value. In a strict sense, this implies that the order parameter is no longer defined as an average over infinitely long times or over all chains covering all fluctuations, but only over the fast local fluctuations. Then the order parameter undergoes slow, long-range fluctuations that are highly cooperative and thus are very sensitive to the phase transition.

Order parameter fluctuations and response can be studied within the framework of the Landau theory. The free energy density, Eq. 2, has to be generalized in two ways. First, since deviations from equilibrium in general occur as spatial inhomogeneities and since inhomogeneities require energy, a gradient term $1/2\ b[\nabla S(\mathbf{r})]^2$ is added in Eq. 2, $\mathbf{r}$ being a vector in the membrane plane and b another phenomenological coefficient. Second, one has to account for the external perturbation by adding a term $-S\delta\phi$, $\delta\phi$ representing the external force. The free energy density then is expressed as

$$\tilde{f}(\mathbf{r}) = -a_1\tilde{S}(\mathbf{r}) + \frac{1}{2}a_2\tilde{S}(\mathbf{r})^2 - \frac{1}{3}a_3\tilde{S}(\mathbf{r})^3 + \frac{1}{4}a_4\tilde{S}(\mathbf{r})^4 + \frac{1}{2}b[\nabla\tilde{S}(\mathbf{r})]^2 - \tilde{S}(\mathbf{r})\delta\phi(\mathbf{r}), \quad (7)$$

and the free energy is given by $\tilde{F} = \int \tilde{f}(\mathbf{r})d\mathbf{r}$.

To study thermal fluctuations ($\delta\phi = 0$), the variation of the free energy in the neighborhood of equilibrium is calculated by substituting $\tilde{S}(\mathbf{r}) = S + \delta S(\mathbf{r})$, δS representing a small deviation from equilibrium, and expanding the free energy in powers of δS. The linear term vanishes due to the definition of equilibrium, so that in lowest order the free energy density δf of the fluctuations is quadratic in δS. We consider an infinitely extended membrane; this eliminates boundary conditions, and the uncoupled variables are the Fourier components $\delta S(\mathbf{q}) = \int \delta S(\mathbf{r}) \exp(\mathbf{iqr})d\mathbf{r}$. In terms of these the free energy density is obtained as

$$\delta f(\mathbf{q}) = \frac{1}{2}(a_2 - 2a_3S + 3a_4S^2 + Lq^2)|\delta S(\mathbf{q})|^2 \equiv \frac{1}{2}(K + Lq^2)|\delta S(\mathbf{q})|^2. \quad (8)$$

K denotes the curvature, for $q = 0$, of the free energy parabola around the equilibrium state (Fig. 1). The temperature variation of K in the fluid phase: for $a_1 = 0$, is given by $K = a_2 \sim T - T^*$, i.e., with decreasing temperature K decreases as if approaching a hypothetical

[2]In the fluid phase far from the phase transition, higher order terms of S can be neglected and the Landau theory, Eq. 3, like the molecular theory lead to $S \sim x^2$. Within the framework of the molecular theory this implies that the chain order in the fluid phase is determined by steric hindrance, whereas van der Waals forces are unimportant. Therefore, the criticism expressed recently by Dill and Flory (9) about the absence of steric hindrance and the overemphasis of van der Waals forces in the molecular theory of the present author (8) does not seem to be justified. The van der Waals forces in this theory are responsible for the phase transition, Eq. 6b, which is not considered in the work of Dill and Fory. The relation $S \sim x^2$ has already been derived by de Gennes (10) from symmetry arguments.

second-order transition at T^*. Before reaching this second-order transition, however, the actual first-order transition intervenes and K remains nonzero. Analoguously, in the ordered phase K decreases with increasing temperature as if approaching another hypothetical second order transition, the high-temperature inflection point T^h of $S(T)$ in Fig. 2. In the realistic case $a_1 > 0$, K behaves similarly. The decrease on both sides of the phase transition becomes more pronounced, since the lower and upper inflection points of S lie closer to the phase transition. Thus on approaching the phase transition from either side the free energy parobola becomes flatter and deviations from equilibrium are facilitated.

The strength of the thermal fluctuations follows from the equipartition theorem $\langle \delta f(\mathbf{q}) \rangle = 1/2\, kT$ as

$$\langle |\delta S(\mathbf{q})|^2 \rangle = \frac{kT}{K(1 + \xi^2 q^2)} \tag{9a}$$

with

$$\xi^2 = \frac{b}{K}. \tag{9b}$$

The magnitude of the fluctuations increases if the phase transition is approached, as shown qualitatively in Fig. 3. The fluctuations in real space are obtained by a two-dimensional integration of Eq. 9a yielding

$$\langle \delta S(\mathbf{r}) \delta S(\mathrm{o}) \rangle \sim \left(\frac{\xi}{r}\right)^{1/2} e^{-r/\xi} \qquad \text{for } r \gg \xi. \tag{10}$$

The fluctuations fall off exponentially in the membrane plane with the coherence length ξ (apart from the slowly varying factor $r^{-1/2}$). According to Eq. 9b the coherence length also increases on approaching the phase transition.

A remark on the effective dimensionality of a lipid membrane is appropriate here. As mentioned previously, the chain order is described comprehensively by the segmental order parameter $S_n(\mathbf{r})$. An additional gradient term acting along the membrane normal should then be included in the free energy, Eq. 7. This leads to a further coherence length $\xi_\parallel$ for the decay of fluctuations along the membrane normal. This coherence length also describes the decrease

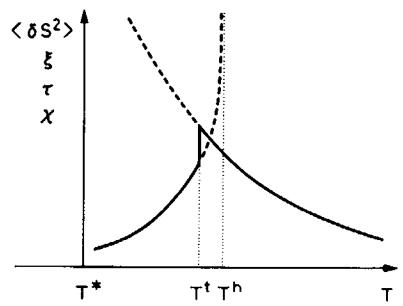

FIGURE 3 The pretransitional increase of the strength of the order parameter fluctuations $\langle \delta S^2 \rangle$, their coherence length ξ, and relaxation time τ, and the response function χ, in the case $\pi = 0$ for comparison with the temperature dependence of the order parameter given in Fig. 2.

of the segmental order parameter along the chains, if S_n is fixed at a given value at the glycerol backbone. $\xi_\parallel$ is known as the persistence length, and in the molecular theory referred to earlier (8) is given by the bend elastic constant M, $\xi_\parallel = lM/(3kT) \approx 1.4l$. Thus $\xi_\parallel$ is much smaller than the thickness $Nlx \approx 0.7Nl$ of a chain layer, and therefore the chains behave as a three-dimensional system and not as a two-dimensional one (11). This obviates problems of two-dimensional melting (12), and allows us to apply the Landau theory in the usual form for three-dimensional systems.

The kinetics of fluctuations is described in the simplest approximation by a linear relation between the flux and the restoring force, namely $\partial\tilde{S}/\partial t = -\eta\partial\tilde{f}/\partial\tilde{S}$ with η representing a transport coefficient. In lowest order of δS, this leads to an exponential decay $\delta S(t) \sim \exp(-t/\tau)$ with the relaxation time

$$\tau = \frac{1}{\eta K(1 + \xi^2 q^2)}. \tag{11}$$

The transport coefficient η may be assumed essentially invariant at the phase transition, so that the temperature dependence of the relaxation time is again governed by the energy curvature K and hence exhibits a slowing down of fluctuations at the phase transition.

Turning to response functions the simplest of them is the specific heat c. With $c = T\partial\sigma/\partial T$ and the former expression for the entropy, $\sigma = -1/2\, a_2' S^2$ in the case $a_1 = 0$, one obtains

$$c = \begin{cases} \dfrac{a_2'^2 T}{2a_4}\left[1 + \left(\dfrac{4S}{3S^t} - 1\right)^{-1}\right] & \text{for } T < T^t \\ 0 & \text{for } T > T^t. \end{cases} \tag{12}$$

The Landau theory is known to underestimate the specific heat. The actual pretransitional increase in the specific heat is larger than predicted by Eq. 12 for the ordered phase, and exists also in the fluid phase.

Next we study the response in the uniaxial order, when a small external force $\delta\phi$ is applied. The response δS is linearly related to the force, $\delta S = \chi\delta\phi$ thereby defining the response function χ. An example for an external force acting on the uniaxial order is a magnetic field $H_i = Hn_i$ along the membrane normal. In analogy to the coupling between the uniaxial (S_{ij}) and unidirectional (x_i) order parameter the corresponding coupling term in the free energy density, Eq. 7, is $S_{ij}H_iH_j$ so that $\delta\phi = H^2$. Hence the response function χ is observable as the magnetic birefringence.

To calculate χ, the equilibrium condition $d\tilde{F}/d\tilde{S} = 0$ has to be evaluated, where $d\tilde{F}/d\tilde{S}$ is a functional derivative with respect to the function $\tilde{S}(\mathbf{r})$. This leads to the Euler-Lagrange equation $\partial\tilde{f}/\partial\tilde{S} - \nabla \cdot \partial\tilde{f}/\partial\nabla\tilde{S} = 0$, which after insertion of Eq. 7 becomes

$$-a_1 + a_2\tilde{S}(\mathbf{r}) - a_3\tilde{S}(\mathbf{r})^2 + a_4\tilde{S}(\mathbf{r})^3 - b\nabla^2\tilde{S}(\mathbf{r}) = \delta\phi(\mathbf{r}). \tag{13}$$

Using again $\tilde{S}(\mathbf{r}) = S + \delta S(\mathbf{r})$ and restricting ourselves to the lowest order in δS we obtain for the Fourier transform of χ

$$\chi(\mathbf{q}) = \frac{1}{K(1 + \xi^2 q^2)}. \tag{14}$$

The response function is simply related to the fluctuations, Eq. 9a. $\langle |\delta S(\mathbf{q})|^2 \rangle = kT\chi(\mathbf{q})$, and again behaves as shown in Fig. 3.

Another example for a response function deals with the unidirectional order: x is proportional to the lateral packing density ν, upon which the surface pressure π acts as the external force. The response in ν to a small variation in π is similarly governed by a linear relation, $\delta\nu/\nu = \kappa\delta\pi$, the response function κ representing the lateral compressibility. If the same treatment as above is applied to the unidirectional order, $\kappa(\mathbf{q})$ is found to behave qualitatively the same as $\chi(\mathbf{q})$, Eq. 14.

The power-law behavior of fluctuations and response functions is easily derived in the fluid phase for $a_1 = 0$, when $K = a_2 \sim (T - T^*)$. Then one obtains, for $\xi q \ll 1$,

$$\langle |\delta S(\mathbf{q})|^2 \rangle \sim (T - T^*)^{-1} \tag{15a}$$

$$\xi \sim (T - T^*)^{-1/2} \tag{15b}$$

$$\tau \sim (T - T^*)^{-1} \tag{15c}$$

$$\chi \sim (T - T^*)^{-1}. \tag{15d}$$

The critical exponents are the classical ones. They apply in our case, because for a first-order transition the pretransitional effects occur relatively far from the hypothetical second-order transition at T^*, and far from T^* the so-called classical regime is reached. There the fluctuations are still relatively weak and interactions between them can be neglected (as done above), leading to the classical exponents.

The coefficient b can be determined, for example, from measurements of the coherence length ξ, whose temperature dependence would furthermore permit the determination of T^* (or its analogue for $a_1 > 0$). To estimate ξ, we introduce a length $\xi' = b/(a_2' T^*)$ in Eq. 9b to obtain, in the fluid phase for $a_1 = 0$, $\xi(T) = \xi' [T^*/(T - T^*)]^{1/2}$. The length ξ' is a typical microscopic length of the system, e.g. the chain diameter of about 4 Å. Then at the phase transition, with $T^* \approx 300°\text{K}$ and $T^t - T^* \approx 20°\text{K}$, $\xi^t \approx 15$ Å.

Dissipative response functions or transport coefficients such as the permeability and the lateral diffusion coefficient are more complicated quantities, and we restrict ourselves to a semiquantitative treatment. It is intuitively clear that diffusion of particles in a membrane (including permeation) is easier in the fluid than in the ordered phase. One may assume that for diffusion to occur in the ordered phase small regions of lipid must become fluid. This requires an activation energy proportional to the difference between the free energies per lipid molecule in the fluid and ordered state, i.e. for the diffusion coefficient $d \sim \exp [-\gamma(F^f - F^o)/kT]$. This activation energy is high far below the phase transition, but vanishes at T^t (and remains zero above T^t), thus d increases strongly with temperature in the ordered phase. In the vicinity of the phase transition, however, a further mechanism may be visualized, especially for the diffusion of small particles. Here large deviations of the lipid order are possible without an activation barrier, because the free energy minimum is flatter (Fig. 1). The lateral compressibility is strongly increased, and diffusion may proceed simply via compression of the surrounding lipids. The temperature dependence of d is then determined by the temperature dependence of the lateral compressibility or the response function $\chi(\mathbf{q})$, Eq. 14. A local compression implies relatively large q values, but since the

coherence length does not diverge at T^t, we may assume $\xi q < 1$, so that

$$d \sim \frac{1}{K}. \tag{16a}$$

Thus lateral diffusion and permeation do exhibit a pretransitional increase. Because in the ordered phase d increases already due to the activated process, the pretransitional effect is visible more clearly in the fluid phase, where in analogy to Eqs. 15

$$d \sim (T - T^*)^{-1}. \tag{16b}$$

Permeation has already been treated along similar lines by Nagle and Scott (13) and by Doniach (14).

THE INFLUENCE OF PROTEINS ON THE ORDERED-FLUID TRANSITION

Static Lipid Order

A protein molecule or another particle such as cholesterol incorporated into a lipid bilayer perturbs the lipid order. The protein will act predominantly on the orientational order of the surrounding lipid chains and influences their positional order only indirectly via the coupling between orientational order and packing density. As in the case of pure lipid membranes, we describe the orientational order of the chains by the uniaxial order for a fixed surface pressure and do not consider the unidirectional order and the packing density. Furthermore, we allow the protein to alter locally the degree of the uniaxial order, the order parameter S, but not the preferred axis of orientation (which would lead to similar consequences). Thus the protein imposes a certain order parameter S_0 on the lipid order at the protein-lipid interface, which in general is different from the unperturbed order parameter S_u (S_u is identical with the former S). The perturbation of the lipid order at the protein surface gradually disappears in the surrounding lipid phase, with a characteristic length of the order of the coherence length for fluctuations. This behavior is shown in Fig. 4, where for simplicity the protein is represented as a cylinder of radius R_0. Similar models have been applied to superconductors sandwiched between normal conductors and to nematic films on a solid surface (15).

The boundary value S_0 reflects the lipid-protein interaction proper, whereas the spatial decay of the perturbation reflects lipid-lipid interaction. S_0 will be treated as a phenomenological parameter, but some qualitative arguments about its magnitude can be given. In the fluid lipid phase, a protein irrespective of its surface acts as an obstacle for the orientational fluctuations of the disordered lipid chains. Therefore the chains at the protein surface undergo weaker fluctuations; they are more ordered. The preferred axis of their orientation may also differ from the unperturbed one, but this possibility is neglected in our model. Thus we expect $S_0 > S_u^f$. In the ordered phase, a protein due to its uneven surface perturbs the parallel order of the chains thereby creating more space for orientational fluctuations, thus $S_0 < S_u^o$. Note that the latter conclusion could not be drawn, if the protein were regarded as an exact cylinder; the representation as a cylinder is used only to specify the cross-sectional area of the protein in the membrane plane.

The spatial decay of the perturbation of lipid order around a protein can be calculated within our description of lipid long-range order. The total free energy $\tilde{F}$ including the

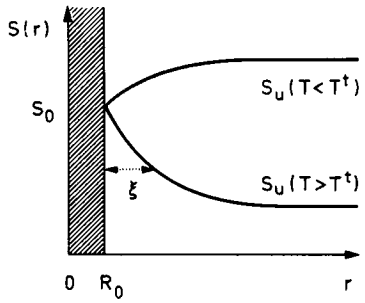

FIGURE 4

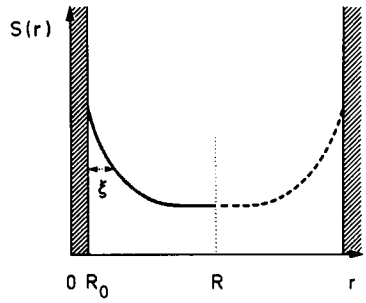

FIGURE 5

FIGURE 4 Radial variation of the order parameter around a protein molecule of radius R_0, at temperatures below and above the phase transition temperature T^t.

FIGURE 5 Geometry in the membrane plane to account for neighboring protein molecules, R denoting the half-distance radius, in the case $\xi \ll R$.

inhomogeneous term, Eq. 7, is minimized. This leads to Eq. 13 for $S(\mathbf{r})$ ($\delta\phi = 0$), which has to be solved under the cylindrical boundary condition $S(R_0) = S_0$. Upon linearization in the perturbation $S_0 - S_u$ a decay in the form of Eq. 10 is obtained (16). If the condition $S_0 - S_u \ll S_u$ does not hold, linearization is not allowed, and an exact analytic solution is not possible. Owicki et al. (3), however, showed by means of a computer solution, that the exponential decay function

$$S(r) = S_u + (S_0 - S_u)e^{-(r-R_0)/\xi_p} \tag{17}$$

is a good approximation. The decay parameter ξ_p is determined by minimization of the total free energy $\tilde{F}$, Eq. 7, with respect to ξ_p. Restricting ourselves to the fluid phase and $a_1 = 0$, we obtain the condition

$$\left(a_2 - \frac{8}{27}a_3S_0 + \frac{1}{8}a_4S_0^2\right)\frac{1}{R_0}\xi_p^3 + \left(a_2 - \frac{4}{9}a_3S_0 + \frac{1}{4}a_4S_0^2\right)\xi_p^2 - b = 0. \tag{18}$$

If the whole first term and S_0 in the second term are omitted, ξ_p equals the coherence length $\xi = (b/a_2)^{1/2}$ for fluctuations, Eq. 9b. The first term arises from the cylindrical symmetry and lowers ξ_p relative to ξ (this more pronounced decrease is expressed by the factor $r^{-1/2}$ in Eq. 10). The terms in S_0, on the other hand, raise ξ_p. Both shifts are small and furthermore cancel to a large extent, so that within an error of 10% one can set $\xi_p = \xi$. This is also true for the ordered phase. Thus the perturbation of lipid order due to a protein decays exponentially with the coherence length.

Knowing the perturbation exerted by one protein molecule on the lipid order, we can proceed to calculate the alteration in the lipid phase transitional behavior due to protein molecules. For this purpose further simplifying assumptions have to be introduced. (*a*) We assume that the protein molecules are distributed homogeneously in the membrane plane. Thus phase separation between lipid and protein is excluded, although this is known to occur frequently below the phase transition. (*b*) We extend the cylindrical geometry applied to one protein to describe the neighboring protein molecules around one protein as another cylinder with the boundary order parameter S_0, illustrated in Fig. 5. The half-distance R between the

two cylinders is determined by the protein concentration. At high protein concentration, the perturbations of the lipid order by neighboring boundaries overlap, which may lead to an alteration of the boundary order parameter. This would represent a lipid-mediated protein-protein interaction as studied by Marčelja (5) and Schröder (16). (*c*) We restrict ourselves, however, to low protein concentrations, where the overlap is negligible, $R \gg \xi$. Then we have to consider only one protein molecule and the surrounding lipid region between R_0 and R.[3] (*d*) Finally, we assume the boundary order parameter S_0 to be temperature independent.

The total free energy F of lipid per protein in the equilibrium state is derived by integration of Eq. 7 in cylindrical coordinates within the boundaries R_0 and R, after insertion of $S(r)$ from Eq. 17 with $\xi_p = \xi$. The result is

$$F = \left(-a_1 S_u + \frac{1}{2} a_2 S_u^2 - \frac{1}{3} a_3 S_u^3 + \frac{1}{4} a_4 S_u^4\right)\pi(R^2 - R_0^2) + 2\pi b(S_0 - S_u)^2\left(2\frac{R_0}{\xi} + 1\right), \quad (19)$$

where higher order terms in $S_0 - S_u$ have been neglected. The first term represents the unperturbed energy F_u, the second the energy due to the perturbation by the protein. The transition temperature again follows from the condition that the free energies of the ordered and fluid phase are equal. In the ensuing analysis we restrict ourselves to the case $a_1 = 0$ with $S_u^f = 0$. The above condition leads to the equation

$$\left(\frac{1}{2} a_2^t - \frac{1}{3} a_3 S_u^t + \frac{1}{4} a_4 S_u^{t2}\right)(R^2 - R_0^2) = b\left(2\frac{R_0}{\xi^t} + 1\right)\left(2\frac{S_0}{S_u^t} - 1\right). \quad (20)$$

For $R \to \infty$, the treatment reduces to that of a pure lipid membrane with the solutions Eqs. 5a and b for a_2^t and S_u^t. If the transition temperature T^t is altered by protein, the quantities a_2^t, S_u^t, and ξ^t in Eq. 20 are altered. The shift $\Delta T^t = T^t - T_u^t$ will be calculated in lowest order of these variations, so that variations on the right-hand side of Eq. 20 can be neglected. Note that a variation of the coherence length is a higher order effect and does not enter. After substitution of $b = \xi^{t2} a_2'(T_u^t - T^*)$ according to Eq. 9b one obtains

$$\frac{\Delta T^t}{T_u^t - T^*} = \frac{\xi^{t2}}{R^2 - R_0^2}\left(2\frac{R_0}{\xi^t} + 1\right)\left(2\frac{S_0}{S_u^t} - 1\right). \quad (21a)$$

This result finds an especially simple interpretation in the case $R_0 = 0$, where

$$\frac{\Delta T^t}{T_u^t - T^*} = \left(\frac{\xi^t}{R}\right)^2\left(2\frac{S_0}{S_u^t} - 1\right). \quad (21b)$$

The shift ΔT^t is normalized to $T_u^t - T^*$, the only inherent temperature difference of the system, and is determined by two factors. The first one, $(\xi^t/R)^2$, is a geometric factor equal to the ratio of perturbed to unperturbed area in the membrane plane. In the case of a planar boundary condition, to which Eq. 21a reduces in the limit $R_0 \to \infty$, this factor would result as ξ^t/R; the area ratio would be replaced by the length ratio. This case is realized in nematic thin films (15). The second factor $(2S_0/S_u^t - 1)$ accounts for the strength of the boundary

[3] In the limit of no overlap, translational diffusion of the protein molecules is irrelevant. The opposite case of large overlap and diffusing impurities has been studied by Imry and Wortis (17) in the context of rounding of a first-order transition by impurities.

condition in an obvious way: if S_0 lies exactly between the order parameters of the ordered and fluid phase at the phase transition, i.e., $S_0 = S_u^t/2$, the shift vanishes. For higher values of S_0 the transition temperature is increased, for lower values it is decreased.

The geometric factor in Eq. 21a can be expressed in terms of the protein/lipid molar ratio P/L, since $(R_0/R)^2 = f_P P/(f_P P + f_L L)$ with f_P, f_L, P, and L denoting the molecular areas and particle numbers of protein and lipid, respectively. Using furthermore $f_P/f_L = (R_0/R_L)^2$, R_L the lipid radius, Eq. 21a is transformed into

$$\frac{\Delta T^t}{T_u^t - T^*} = \frac{P}{L}\left(\frac{\xi^t}{R_L}\right)^2\left(2\frac{R_0}{\xi^t} + 1\right)\left(2\frac{S_0}{S_u^t} - 1\right). \tag{21c}$$

The factor $(\xi^t/R_L)^2(2R_0/\xi^t + 1)$ simply represents the number of boundary lipids per protein of radius R_0.

Under the same assumptions, the total latent heat can be derived from the total entropy $\Sigma = -\partial F/\partial T$. Using Eq. 19 and replacing the temperature derivative by partial derivatives with respect to a_2 and S_u (the further temperature-dependent variable ξ obeys $\partial F/\partial \xi = 0$ at equilibrium) one obtains for the ordered phase with S_u from Eq. 4

$$\Sigma^o = -\left[(\partial F_u/\partial a_2)\, a_2' + (\partial \Delta F/\partial S_u)\, a_2'(a_3^2 - 4a_2a_4)^{-1/2}\right]. \tag{22}$$

In the fluid phase, the entropy would equal zero. To be more realistic, we assume $\partial S_u/\partial T$ in the fluid phase at T_t to be the same as in the ordered phase at T_t, thus $\sum^f$ to be given by the second term in Eq. 22. The first term yields the unperturbed latent heat Q_u, Eq. 5c with a prefactor $(R^2 - R_0^2)/\pi$, while the second terms yield the shift ΔQ as

$$\frac{\Delta Q}{Q_u} = -2\frac{\xi^{t2}}{R^2 - R_0^2}\left(2\frac{R_0}{\xi^t} + 1\right) \tag{23a}$$

or in terms of the protein/lipid molar ratio

$$\frac{\Delta Q}{Q_u} = -2\frac{P}{L}\left(\frac{\xi^t}{R_L}\right)^2\left(2\frac{R_0}{\xi^t} + 1\right). \tag{23b}$$

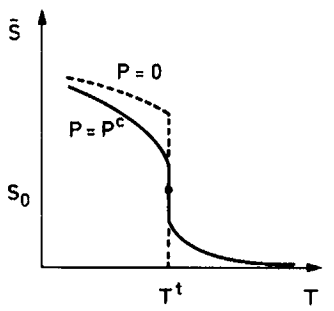

FIGURE 6

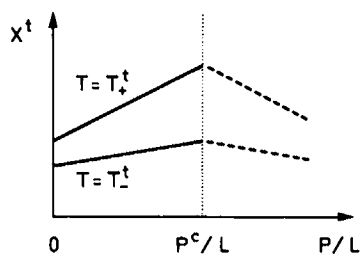

FIGURE 7

FIGURE 6 Temperature dependence of the spatially averaged order parameter without protein and at the critical protein concentration, for $\pi = 0$ and $S_0 = S_u^t/2$ implying constant T^t.

FIGURE 7 Critical increase of the response function with the protein/lipid ratio at T^t, if T^t is approached from the ordered ($T = T_-^t$) or the fluid ($T = T_+^t$) phase.

The latent heat always decreases linearly with the number of protein molecules, irrespective of the boundary condition S_0. This decrease is a consequence of the intermediacy of S_0 to the order parameters of the unperturbed ordered and fluid phase. The average orders of the two phases then approach each other (Fig. 6). Hence, the change of the order at the phase transition becomes smaller with increasing protein concentration, and the latent heat decreases. Quantitatively, the spatially averaged order parameter follows from Eq. 17 as

$$\bar{S} = S_u + 2\frac{P}{L}\left(\frac{\xi}{R_L}\right)^2\left(\frac{R_0}{\xi} + 1\right)(S_0 - S_u). \tag{24}$$

In the ordered phase, $\bar{S}$ decreases with the protein/lipid ratio, in the fluid phase it increases, as shown in Fig. 6. The temperature dependence of the shift is determined by $\xi(T)$, hence the shift extends over the temperature region of the pretransitional effects in pure lipid membranes.

If the latent heat vanishes, a critical point is reached. The corresponding condition $\Delta Q = -Q_u$, inserted in Eq. 23b, yields the critical lipid/protein ratio as

$$\frac{L}{P^c} = 2\left(\frac{\xi^t}{R_L}\right)^2\left(2\frac{R_0}{\xi^t} + 1\right). \tag{25a}$$

The lipid quantities ξ^t and R_L entering this expression do not vary much for different lipids, thus the critical point for any lipid-protein system is mainly determined by the protein radius R_0. Using as typical values $\xi^t \approx 15$ Å and $f_L \approx 60$ Å^2 corresponding to $R_L \approx 5$ Å, one obtains

$$\frac{L}{P^c} = 18\left(1 + \frac{2}{15}R_0[\text{Å}]\right). \tag{25b}$$

In the limit $R_0 = 0$, this expression yields $L/P^c = 18$, whereas for $R_0 \approx \xi^t \approx 15$ Å one gets $L/P^c = 54$. For the critical temperature shift Eq. 21c yields

$$\frac{\Delta T^c}{T_u^t - T^*} = \frac{1}{2}\left(2\frac{S_0}{S_u^t} - 1\right). \tag{26}$$

An upper limit for ΔT^c is obtained from the condition $S_0 \leq S_u^t$, leading with the earlier value $T_u^t - T^* = 20$°C to $\Delta T^c \leq 10$°C.

The result for the critical point can be compared with the numerical result of Owicki and McConnell (4). In the case $R_0 = \xi^t$, they obtained for the fractional area covered by protein at the critical point $(R_0/R^c)^2 = 0.16$. For the same case, Eq. 25a yields $L/P^c = 6f_p/f_L$ and thus $(R_0/R^c)^2 = 1/7 = 0.14$, which agrees well with the result of Owicki and McConnell (4). In their calculation, the overlap between the perturbations of neighboring proteins was formally taken into account, in contrast to our treatment. The agreement between the results confirms that the overlap can, in fact, be neglected, and the protein-induced alteration of the lipid-phase transition arises from the single-particle effect of protein on lipid order. The corresponding assumption was $\xi^t/R \ll 1$, which according to the above result $\xi^t/R^c = 1/\sqrt{7}$ is valid up to the critical point.

Fluctuations and Response

If a phase transition approaches a critical point, this is expressed most drastically as an increase of thermal fluctuations and response functions. The pretransitional effects are the more pronounced, the closer the phase transition is to the critical point, as discussed for pure lipid membranes. As an example, we first consider the specific heat $C = T\partial\Sigma/\partial T$. The total entropy Σ in the ordered phase is given by Eq. 22, and the derivative with respect to temperature can be taken as indicated. The result for the protein-induced shift ΔC in the ordered phase at T^t is, under the same assumptions as for the shift of the transition temperature,

$$\frac{\Delta C^t}{C_u^t} = \frac{P}{L}\left(\frac{\xi^t}{R_L}\right)^2\left(2\frac{R_0}{\xi^t}+1\right)3\left(1-\frac{4}{3}\frac{S_0}{S_u^t}\right). \tag{27}$$

As expected, the specific heat increases with the protein concentration. The geometric factor is the same as for ΔT^t, Eq. 21c. The S_0 term, however, is different and approximately proportional to $1 - S_0/S_u^t$, so that it vanishes for $S_0 \approx S_u^t$. For $S_0 = S_u^t/2$, or $\Delta T^t = 0$, the increase at the critical point is $\Delta C^c/C_u^t = \frac{1}{2}$, the specific heat increases maximally by 50% because of protein. The Landau theory underestimates the specific heat, however.

The calculation of the response function χ is not as straight-forward as for the specific heat. The uncoupled variables are no longer given by the Fourier components, because of the boundary condition. We restrict ourselves to a homogeneous external force $\delta\phi$ and assume a response of the form $\delta S(r) = \delta S_u(1 - e^{-(r-R_0)/\xi})$ implying $\delta S(R_0) = 0$ and $\delta S(r \gg \xi) = \delta S_u$. Thus we consider a deviation of the order parameter from equilibrium, which is spatially constant far away from the protein but leaves the boundary condition fixed. Actually, the coherence length may be increased but for the calculation of the response function this is a higher order effect. To calculate δS_u we insert $\delta S(r)$ in Eq. 13 and integrate from R_0 to R in order to get the spatially averaged response function X. The right-hand side of Eq. 13 yields $\delta\phi$ $(R^2 - R_0^2)/2$, on the left-hand side δS_u can be taken out of the integral, so that as before we arrive at a linear relation $X^{-1}\,\delta S_u = \delta\phi$, with

$$X^{-1} = \frac{2}{R^2 - R_0^2}\int_{R_0}^{R}(a_2 - 2a_3S_u + 3a_4S_u^2 - b\nabla^2)(1 - e^{-(r-R_0)/\xi})\,r dr. \tag{28}$$

The resulting protein-induced shift ΔX, at the phase transition, and again assuming $a_1 = 0$, is

$$\frac{\Delta X^t}{X_u^t} = \frac{5}{2}\frac{P}{L}\left(\frac{\xi^t}{R_L}\right)^2\left(2\frac{R_0}{\xi^t}+1\right)\begin{cases}1-\dfrac{S_0}{S_u^t} & \text{for } T \longrightarrow T^t \text{ from below}\\[2ex] 2\dfrac{S_0}{S_u^t} & \text{for } T \longrightarrow T^t \text{ from above.}\end{cases} \tag{29}$$

The response function increases with the protein concentration on both sides of the phase transition; the earlier pretransitional increase is enhanced. The geometric factor is the same as before, whereas the S_0 term implies that the shift ΔX^t vanishes in the ordered phase if $S_0 = S_u^t$ and in the fluid phase if $S_0 = 0 = S_u^f$, as for the specific heat. This implies that if the boundary

order parameter is equal to the unperturbed order parameter, the protein does not function as a perturbation upon the lipid phase. The variation of X at higher protein concentrations, especially the expected decrease of X above the critical protein concentration, is not described by this lowest order approximation. At the critical point, Eq. 29 leads to

$$\frac{\Delta X^c}{X_u^t} = \frac{5}{4}\begin{cases} 1 - \dfrac{S_0}{S_u^t} & \text{for } T \to T^c \text{ from below.} \\ 2\dfrac{S_0}{S_u^t} & \text{for } T \to T^c \text{ from above.} \end{cases} \tag{30}$$

For $S_0 = S_u^t/2$, when the transition temperature remains constant, the increase of X^t with protein concentration at constant temperature directly reflects the critical effect. This is shown in Fig. 7. Typically X^t increases by a factor of two. As discussed for pure lipid membranes, the lateral compressibility behaves like X, and is thus expected to vary with protein concentration as in Fig. 7. The critical increase of the latter has already been studied by Bates et al.[4] when they extended the model of Owicki et al. (3, 4).

Formally, the increase of the response function X corresponds to a decrease of the free energy curvature K, Eq. 14, or a shift of the second-order transition temperature T^* towards the actual transition temperature T^t, Eq. 15d. Owing to the close correlation between response functions and thermal fluctuations, formulated in Eqs. 15, fluctuations are also enhanced when protein is added. Their strength, coherence length, and relaxation time increase like the response function in Fig. 7.

Finally, the dissipative response function permeability and lateral diffusion, whose pretransitional increase is expressed by Eq. 16b, are also increased by proteins. These processes involve more localized perturbations of the lipid order than the fluctuations treated above, but qualitatively their variation with protein concentration is again as in Fig. 7. Thus one obtains the result that proteins, on the one hand, increase the lipid order in the fluid phase and, on the other hand, facilitate membrane transport.

CONCLUSION

We have shown that the ordered-fluid transition in lipid membranes fits into the universal scheme for phase transitions, statically as well as dynamically. The long-range orientational order of the lipid chains changes spontaneously at the phase transition, which because of the symmetry of the uniaxial orientational order is of first order. Above the phase transition the order remains nonzero due to the surface pressure acting as an external ordering field. Thermodynamic long-range fluctuations, their strength, coherence length and relaxation time, and response functions such as the lateral compressibility exhibit a pretransitional increase with classical critical exponents. Their behavior mimics the approach to a second-order transition, which is interrupted by the intervention of the first-order transition thus avoiding a divergence of the pretransitional effects. Transport coefficients such as the permeation and lateral diffusion of small particles also become maximal at the phase transition.

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[4]Bates, E.H., S. Marčelja, and J. Wolfe. Elastic response of bilayers with intrinsic proteins. Preprint.

Proteins or cholesterol are represented as boundary conditions on the lipid orientational order, distributed homogeneously in the membrane plane. The perturbation of the lipid order at the protein surface falls off exponentially with the lipid coherence length. The magnitude of the boundary condition is assumed to be temperature independent, increasing the lipid order in the fluid phase and decreasing it in the ordered phase. With increasing protein concentration, therefore, a critical point is reached, defined by the vanishing of the latent heat. The approach to the critical point is accompanied by an increase of thermal fluctuations and response functions including permeability and lateral diffusion. Where the boundary condition is equal to the unperturbed value on one side of the phase transition, fluctuations and response are unaltered in this phase, whereas in the other phase they are increased all the more. In any case, fluctuations and response do not become divergent, which points out the special role of a critical point evoked by boundary conditions.

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