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The Ordered-Fluid Transition in Lipid Bilayers†

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The correlation between the basic functions of biological membranes including stability and flexibility, and the molecular properties of lipids forming the bilayer as the basic matrix of membranes is investigated. Stability is guaranteed by the two hydrophobic hydrocarbon chains and the hydrophilic polar head of lipids. The flexibility requirements, e.g. high transport rates, are related to the internal structure of the lipid bilayer and are guaranteed to some extent by demanding a fluid lipid phase at physiological temperatures. From a molecular theory of the ordered-fluid transition it is found that a double bond in one of the lipid chains is sufficient for this purpose. Transport rates may be increased further near the ordered-fluid transition, which is of first order, due to pretransitional effects arising from the vicinity to a hidden second order phase transition. These phenomena are studied within a Landau theory for the ordered-fluid transition. Optimal use of pretransitional increase of transport rates may be achieved by shifting the ordered-fluid transition towards a critical point. Proteins or cholesterol incorporated into a lipid bilayer are shown, again within the Landau theory, to act in such a way.

I INTRODUCTION

Lipids naturally occur in biological membranes, and artificial lipid bilayers are often studied as model systems for biological membranes. The question behind these studies is: In which way do lipids contribute to the function of biological membranes? To answer this question, let us start with a brief enumeration of the functions of biological membranes and then work out in which way lipids serve to fulfil these functions.

The functions of biological membranes are the following:

i) They enclose a space within which molecules are held together to react with each other, e.g. to produce proteins.

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ii) They transport substances through the membrane; food must enter and waste must be excreted.

iii) Enclosing a space, membranes represent an area, i.e. a place where molecules can stay and be met by others; the process of meeting between two reaction partners is easier if one of the two partners is bound to a surface.

iv) A two-particle reaction is even more likely to occur when both particles are confined onto the surface, the reaction then being controlled by two-dimensional diffusion; lateral transport along the membrane is a necessary condition for this mechanism.

v) Finally, the membrane has to have a variable form in order to fit into tissue.

These functions fall into two categories, one of stability and one of flexibility, the latter including transport and form variability. This combination of diametrically opposed properties stresses the uniqueness of the system, and immediately raises the question: How is a membrane to be constructed to achieve these functions?

The first possible way to build a membrane would be by an attractive interaction between the building elements. Ordinary walls are constructed this way, but membranes built this way would clearly lack the needed flexibility. The second possibility is that the building elements associate due to a repulsive interaction with the particles of the surrounding medium. Membranes constructed according to this mechanism can be flexible and, indeed, biological membranes are built this way. Because the surrounding medium is water, the membrane components must be hydrophobic. Purely hydrophobic particles in water, however, would form a separate phase and, to prevent this, the particles must also contain a hydrophilic part, which will maintain contact with water. All naturally occurring membrane components in fact have such hydrophobic and hydrophilic parts. For membrane proteins these regions are represented by certain amino acids. Lipids (more exactly phospholipids) have their hydrophobic part made of two hydrocarbon chains, which are between 14 and 18 carbon atoms long and one of which contains a double bond. The hydrophilic part of lipids resides in a polar head which contains an electric dipole or in some cases even a monopole, i.e., an electric charge. Cholesterol has a hydrophobic cycloaliphatic core and a polar OH group.

In water, these molecules associate with their hydrophobic parts side by side to avoid contact with water, the hydrophilic parts forming the interface. For lipids, the shielding of the chains from water is only possible on one side; therefore two monolayers associate to form a bilayer. The lipid bilayer may be regarded as the basic matrix of a membrane, in which the other components swim. Therefore, for our discussion of the origin of membrane

stability and flexibility, we can neglect the other components and restrict ourselves to a pure lipid bilayer.

II STABILITY OF LIPID BILAYERS

Let us first discuss the stability of a lipid bilayer. If lipid molecules are thrown into water, an equilibrium will be attained between monomers and different types of aggregates (Figure 1). The aggregates may be spherical, called micelles, or cylindrical, or planar, namely bilayers. The latter may be slightly curved to form closed shells called vesicles, which fulfil our requirement of enclosing a space. When are such bilayers stable? The underlying equilibrium may be divided into two equilibria, one between monomers and any type of aggregate, and one between the different types of aggregate.

The equilibrium between monomers and aggregates is governed by the hydrophobic interaction between lipid and water molecules, favouring aggregation, and by entropy which acts against aggregation. For sufficiently long hydrocarbon chains, the hydrophobic interaction dominates, and the equilibrium lies on the side of the aggregates. Theoretical and experimental evidence indicate that chains longer than 10 carbon atoms form stable aggregates.[1] Lipids in biological membranes have chains between 14 and 18 carbon atoms long, and thus guarantee stability against dissociation into monomers.

The equilibrium between the different forms of aggregation is governed by the residual hydrophobic interaction, which remains effective at the surface of the aggregates, tending to minimize the interface, and the hydrophilic interaction between lipid and water molecules, which tends to maximize the

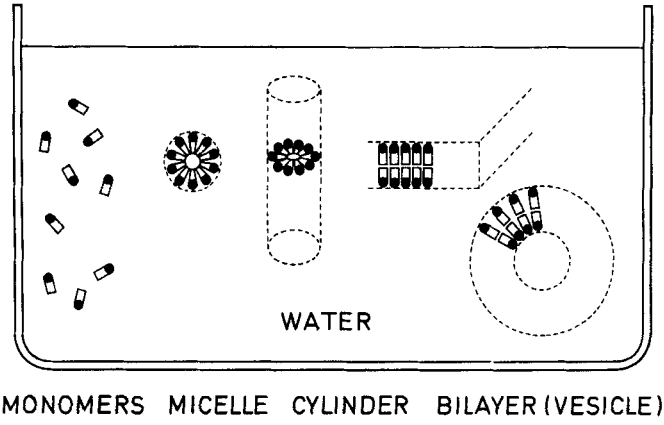

FIGURE 1 The possible forms of aggregation of lipid molecules in water.

interface. Obviously, an optimal value for the interfacial area per lipid molecule exists. This value is rather constant for different lipid molecules, approximately 60 $Å^2$, because the residual hydrophobic interaction is independent of chain length and the hydrophilic interaction varies only slightly for different polar heads.[2] The lipid molecules aggregate in a form to reach this optimal value. For the different forms of aggregation the actual interfacial area per molecule can easily be calculated, if the molecular volume and chain length are known. Evidently, the interfacial area is largest for micelles and smallest for bilayers. Considering molecules with a single chain, 14 carbon atoms long, one finds for micelles an interfacial area of 60 $Å^2$, equal to the optimal value. Single-chain molecules thus aggregate in the form of micelles. To obtain bilayers one needs a larger molecular volume, for which the actual interfacial area would be too large in the case of micelles or cylinders. For lipid molecules with two hydrocarbon chains the interfacial area is equal to the optimal value, if they aggregate in the form of bilayers.

The molecular properties of lipids needed to guarantee stability of pure lipid bilayers or biological membranes can be summarized as follows: The chains must be 14 to 18 carbon atoms long to prevent dissociation of the bilayer into monomers, and two chains per molecule are needed to ensure that the bilayer is the most stable form of aggregation.

Let us now turn to the molecular properties that guarantee flexibility of the membranes. This flexibility is a consequence of the internal structure of the membranes which will be discussed in the following.

III INTERNAL STRUCTURE OF LIPID BILAYERS

A Molecular Theory of the Ordered-Fluid Transition

Lipid bilayers exist in essentially two different states, a disordered or fluid phase at high temperatures and an ordered phase at low temperatures, with a sharp phase transition between them. The ordered state has

i) positional order—the chains form a two-dimensional crystalline lattice and are densely packed,

ii) orientational order—the chains are all oriented parallel, and

iii) conformational order—the chains are stretched or all-trans.

The fluid state has no positional order, the chains are no longer parallel, and the conformational order is distorted, the chains are bent by gauche states. The two different structures clearly lead to different degrees of membrane flexibility, e.g., the transport rates are low in the ordered phase and high in

the fluid phase. So flexibility demands membranes in the fluid phase. This requirement prompts the question: What determines the phase transition and how can it be fixed at a temperature below physiological temperatures? To answer this question, we need a molecular theory of the ordered-fluid transition.[3]

If different types of order change at a phase transition as in our case, that order which is the most cooperative is responsible for the phase transition. For long chains this is the orientational order, because if the orientation of one long chain is changed, many others have to follow, whereas if the position of a chain is changed, only a few neighbouring chains have to move slightly. The conformational order is not at all cooperative. So at the phase transition, the orientational order changes spontaneously, and the other types of order just follow. The orientational order will be described by the orientational order parameter

$$\langle S_n \rangle = \left\langle \frac{3\cos^2\theta_n - 1}{2} \right\rangle, \tag{1}$$

where θ_n is the angle between the preferred axis, usually the membrane normal, and the orientation of a chain at the nth carbon atom as illustrated in Figure 2. The angular brackets denote the thermodynamic average. To

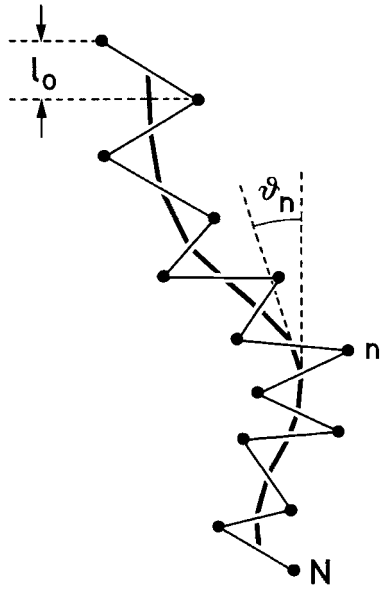

FIGURE 2 Geometry of a hydrocarbon chain to define the orientation of the nth segment.

study the phase transition it is sufficient to treat the order parameter averaged along a chain

$$\langle S \rangle = \frac{1}{N} \sum_{n=1}^{N} \langle S_n \rangle. \tag{2}$$

This order parameter corresponds to the order parameter of nematic liquid crystals.

The next step is to consider the molecular interactions relevant to orientational order. These are:

i) The van der Waals interaction, which tries to orient the chain segments parallel. It will be treated within a self-consistent field approximation analogous to the Maier-Saupe theory for nematics,[4]

$$V_{\text{vdWaals}} = -\Lambda \langle S \rangle \frac{3x_n^2 - 1}{2}, \tag{3}$$

Λ being the van der Waals interaction constant, $\langle S \rangle$ the self-consistent field, and $x_n = \cos \theta_n$.

ii) The steric interaction, tending to orient the segments into the membrane. It is related to the packing density in the following way: The volume v_{ch} of a chain is given by the area f per chain times the length l_0 of a segment projected into the membrane normal by the factor x_n and summed up along the chain. The lateral packing density $1/f$ then is given by

$$f^{-1} = \frac{l_0 N}{v_{\text{ch}}} \langle x \rangle,$$

where $\langle x \rangle$ is defined in analogy to Eq. (2). Assuming that v_{ch} is constant and that the packing density $1/f$ is an external parameter with a given value, $\langle x \rangle$ is also fixed at an externally given value. This condition we account for by the potential

$$V_{\text{st}} = -\Gamma x_n, \tag{4}$$

where Γ has to be chosen in such a way that the calculated $\langle x \rangle$ equals the externally given value.

iii) Finally, the intramolecular interaction between the segments, favouring stretched chains. It is treated within a continuum approximation describing the chains as continuous lines with bend elasticity,

$$V_{\text{el}} = \frac{M}{2} \left(\frac{\partial x_n}{\partial n} \right)^2, \tag{5}$$

M being the bend elastic constant.

Applying the techniques of statistical mechanics, the thermodynamic averages $\langle S_n \rangle$ and $\langle S \rangle$ can be calculated as functions of the external variables, temperature T and packing density $\langle x \rangle$, and the internal variables Λ, M, and chain length N.[3] The result for $\langle S \rangle$ is shown in Figure 3. For vanishing steric interaction, $\langle x \rangle = 0$, a first order phase transition is obtained due to the van der Waals interaction. This finding is analogous to the nematic-isotropic transition, but holds here for flexible molecules. The steric interaction increases the orientational order. Because this increase is stronger in the disordered phase than in the ordered phase, the discontinuity at the phase

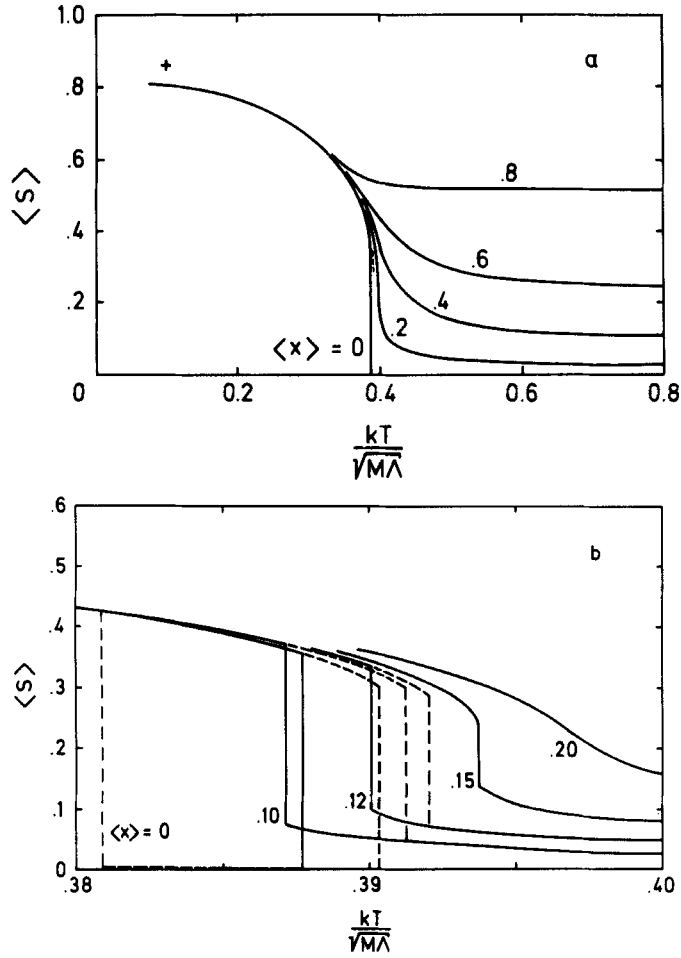

FIGURE 3 Temperature dependence of the orientational order parameter $\langle S \rangle$ for different values of $\langle x \rangle$ or the lateral packing density, for infinitely long chains; Figure 3b is an expansion of Figure 3a around the phase transition; dashed lines represent metastable states.

transition decreases, as seen in Figure 3b, until a second order phase transition or critical point is reached. Experimentally, such a behaviour has been observed in monolayer measurements, where the packing density can be fixed externally. For aqueous dispersions of lipids, the surface pressure is the external variable, which can be varied, e.g., by altering the net charge on the polar heads of ionizable lipids. This regulatory mechanism, however, is not very effective, since the hydrocarbon chains do not change their distance much with increasing electrostatic repulsion between the polar heads, but rather maintain their distance by tilting away from the membrane normal.[5] So in dispersions the chain lateral pressure and packing density are actually relatively constant parameters. The intramolecular interaction being non-cooperative has no influence on the qualitative behaviour of the system, but only on a quantitative aspect, as seen in the temperature scale $kT/\sqrt{\Lambda M}$. For $\langle x \rangle = 0$ and infinitely long chains, the phase transition occurs at $T_t = 0.39\sqrt{\Lambda M}/k$. Inserting values for Λ and M which are obtained from paraffins and polymethylenes, one gets $T_t = 254°\text{K}$. Marcelja,[6] solving the same problem by a computer simulation of the chain conformations, obtained $T_t = 262°\text{K}$, so our continuum approximation works very well. Concerning the variation of T_t with chain length, T_t is constant for long chains but decreases linearly for short chains. The experimental value for C16-ethanolamine is $T_t = 59°\text{C}$. To improve the agreement between theory and experiment, the value of Λ, which is not known very accurately, may be varied.

Let us now come back to the requirement for a flexible membrane, i.e., a fluid phase at physiological temperatures of 30–40°C. For chain lengths between 14 and 18 carbon atoms, which are necessary for the stability of biological membranes, and saturated chains the ordered-fluid transition temperature lies above physiological temperatures, and the membranes are in the ordered phase at 30–40°C. How then can T_t be decreased? Electrostatic regulation cannot be applied in biological membranes, because a high charge density would perturb other membrane processes. Λ being a constant, only the chain elasticity M can be varied. It is known that introduction of a double bond into a hydrocarbon chain makes the neighbouring single bonds more flexible and thus effectively decreases the elastic constant M. This leads to a lowering of T_t as required. Experimentally, one double bond decreases T_t by about 40°C. This solution is actually the way nature has chosen to ensure that membranes exist in the fluid phase: half of the chains in a biological membrane contain a double bond. A further problem then arises, because saturated and unsaturated chains tend to undergo phase separation. To avoid this, nature has inserted the double bond in one of the two chains of a lipid molecule.

So our conclusion on the molecular properties needed to guarantee the flexibility of biological membranes is: a lipid molecule must have one

saturated hydrocarbon chain and one with a double bond in order to maintain a fluid phase at physiological temperatures.

This conclusion is based on considering equilibrium order. Flexibility, e.g., transport, however, implies deviations from equilibrium. It is generally true that deviations from equilibrium are easier in a disordered phase than in an ordered phase as assumed above, but particular behaviour may arise in the vicinity of a phase transition. There the system becomes extremely soft and transport properties may show a pretransitional increase. These phenomena are discussed most conveniently within the framework of the Landau theory for phase transitions.

B Landau Theory of the Ordered-Fluid Transition

The Landau theory starts with an expansion of the free energy F of the system in powers of the order parameter,

$$F = -a_1\langle S\rangle + \tfrac{1}{2}a_2\langle S\rangle^2 - \tfrac{1}{3}a_3\langle S\rangle^3 + \tfrac{1}{4}a_4\langle S\rangle^4, \tag{6}$$

the coefficients a_i being unknown. The second and higher order terms are well-known from the Landau–deGennes theory of the nematic-isotropic phase transition.[4] Introducing the usual assumption that the coefficient a_2 vanishes at some temperature T^* corresponding to a second order transition, $a_2 = a_2'(T - T^*)$, the third order term actually leads to a first order transition at $T_t > T^*$. The first order term in the free energy arises due to the order imposed by the packing density. This order is characterized by the quantity $\langle x\rangle$, which may be regarded as a uni-directional order parameter in addition to the uni-axial order parameter $\langle S\rangle$, allowing for a term $\langle x\rangle^2\langle S\rangle$ in the free energy, so that $a_1 \sim \langle x\rangle^2$. The free energy Eq. (6), can also be derived from the molecular theory discussed above, simply by an expansion in powers of $\langle S\rangle$. From this approach, expressions for the coefficients a_i in terms of the external and internal parameters are obtained,

$$a_1 = \frac{\Lambda}{2}\langle x\rangle^2, \tag{7a}$$

$$a_2 = \Lambda\left(1 - \frac{2}{15}\frac{\Lambda M}{(kT)^2}\right), \text{ etc.} \tag{7b}$$

The expression for a_2 can be transformed to yield $T^* = 0.37\sqrt{\Lambda M}/k$, so that using the earlier values for Λ and M, the hypothetical second order transition temperature T^* is found about 10°C below the actual first order transition temperature T_t.

The equilibrium solution for $\langle S\rangle$ follows from the minimization of the free energy, $\partial F/\partial\langle S\rangle = 0$, and results as discussed within the molecular

theory. Now we continue by generalizing the expression for the free energy to take into account thermal fluctuations δS. We substitute $\langle S\rangle$ in Eq. (6) by $\langle S\rangle + \delta S$. The expansion around the equilibrium state leads to the energy expression

$$F = F(\langle S\rangle) + (\tfrac{1}{2}a_2 - a_3\langle S\rangle + \tfrac{3}{2}a_4\langle S\rangle^2)\delta S^2. \tag{8}$$

The zeroth order term represents the equilibrium free energy, the first order term has vanished due to the definition of equilibrium, and the second order term represents the fluctuation contribution. Applying the equi-partition theorem, we obtain for the strength of the fluctuations, if we furthermore restrict ourselves for simplicity to the case $\langle x\rangle = 0$ so that $\langle S\rangle = 0$ in the fluid phase,

$$\langle \delta S^2\rangle = \frac{kT}{a_2}. \tag{9a}$$

Actually the finite size of fluctuations must also be considered. Spatial inhomogeneities cost energy and usually a term $\Lambda\xi_0^2(\nabla S)^2$ is added to the free energy, ξ_0 representing a molecular distance. Then the fluctuations decay exponentially with a coherence length

$$\xi = \xi_0\sqrt{\frac{2}{a_2}}, \tag{9b}$$

under the same restriction $\langle x\rangle = 0$. Furthermore, fluctuations decay in time. Assuming as usual $\partial S/\partial t = v\,\partial F/\partial S$, where v is a transport coefficient, fluctuations decay exponentially with a relaxation time

$$\phi = \frac{1}{va_2}. \tag{9c}$$

So if we approach the ordered-fluid transition from above T_t, the system behaves as if it were approaching a second order phase transition at T^*, i.e. $\langle \delta S^2\rangle$, ξ, and ϕ increase according to Eqs. (9a)–(9c) and would diverge at T^*. But before reaching T^*, the system undergoes a first order transition and the divergence is not reached. Because the same behaviour occurs upon approaching the phase transition from below T_t, $\langle \delta S^2\rangle$, ξ, and ϕ show a cusp-like behaviour around the phase transition. Inserting the result from the molecular theory, Eq. (7b), into Eq. (9b), the coherence length ξ at the first order transition is estimated to be $5\xi_0$. The pretransitional increase is moderate, but may be detectable experimentally.[7]

Fluctuations of the orientational order in lipid bilayers have not yet been observed experimentally. Due to the close correlation between fluctuations and response functions, however, we also expect response functions to show

a pretransitional increase at the ordered-fluid transition. The most convincing experimental evidence for such an occurrence was provided by sound velocity measurements of Mitaku *et al.*[8] The sound velocity c is related to the compressibility κ *via* $c \sim \kappa^{-1/2}$. The density is coupled, although weakly, to the orientational order, so the compressibility as a response function is expected to show the cusp-like behaviour around the ordered-fluid transition, and the sound velocity therefore a symmetric decrease around T_t. Such a pretransitional behaviour was indeed found experimentally by Mitaku *et al.*, extending over 5°C on both sides of T_t. In addition, an abrupt change at T_t was observed due to the change of the equilibrium order at the first order transition. As expected, the fluid phase with its lower equilibrium order has a higher compressibility.

In a simple model for permeation of small particles through a membrane, one may visualize permeation to proceed *via* the creation of a hole by compression elsewhere in the surrounding lipid phase. Then permeation is governed by compressibility and should also show a maximum at the phase transition.[9] Experimentally, a maximum of the permeation of Na^+ ions at the ordered-fluid transition has been observed by Papahadjopoulos *et al.*[10]

This example of a pretransitional enhancement of permeation of particles through a lipid membrane indicates that the requirement of a flexible membrane may be optimized if the membrane is kept near the ordered-fluid transition due to the vicinity of a hypothetical second order transition. The pretransitional enhancement of transport properties may be utilized more extensively, if the ordered-fluid transition is shifted nearer to a second order transition or even becomes a second order transition itself. Such a mechanism will be discussed in the following section.

C Influence of Proteins and Cholesterol on the Lipid Phase

Particles such as proteins or cholesterol, when incorporated into a lipid bilayer, perturb the order of the lipid phase. In a simple approximation, we describe these particles as cylinders of radius R_0, the orientational order parameter at the surface of a cylinder being S_0 (Figure 4). From this boundary value the order parameter then falls off exponentially with the coherence length ξ to reach the unperturbed order parameter asymptotically. The lipid-protein or lipid-cholesterol interaction is contained solely in S_0. In the fluid lipid phase we expect an incorporated particle to act like a rod in a sea of disordered lipid molecules, and therefore the lipid molecules around a particle are more ordered, $S_0 > \langle S \rangle_{\text{fluid}}$. In the ordered phase, on the other hand, such a particle, with its uneven surface, decreases the high order of the surrounding lipid molecules, $S_0 < \langle S \rangle_{\text{ord}}$.[11] How does such a perturbation influence the lipid phase transition? To calculate this effect, we assume that

the particles are distributed homogeneously in the membrane, and the influence of the neighbouring particles on one particle is cylindrically symmetric. Then the perturbations from neighbouring particles superimpose to yield an order parameter profile as shown schematically in Figure 4. If, finally, we restrict ourselves to low particle concentrations, so that the half distance R between particles is much larger than the coherence length ξ, the overlapping can be neglected and we need consider merely one particle with its perturbation in the range R_0 to R.

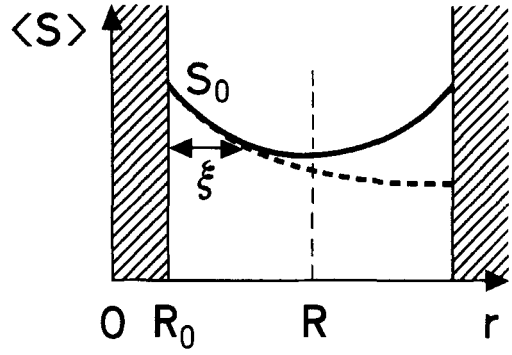

FIGURE 4 The perturbing effect of protein or cholesterol on the lipid orientational order parameter, for an isolated particle (– – –) and for the case of overlapping effects from neighbouring particles (——).

Applying again the Landau theory we have to minimize the free energy, Eq. (6), including the inhomogeneous term $\Lambda\xi_0^2(\nabla S)^2$. The resulting Euler–Lagrange variational problem can be solved analytically. The solution for the spatially averaged order parameter $\langle\bar{S}\rangle$ is illustrated in Figure 5, where for simplicity we confined ourselves to the case $\langle x\rangle = 0$. The order parameter

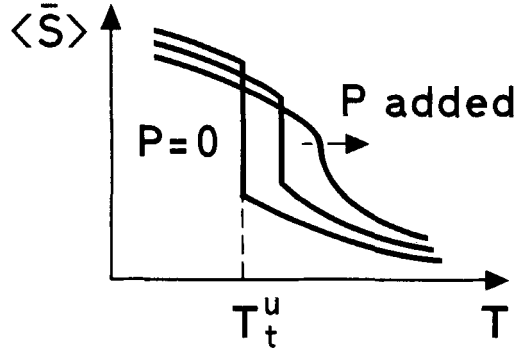

FIGURE 5 The temperature dependence of the spatially averaged lipid order parameter, for increasing concentration of protein or cholesterol.

upon addition of protein or cholesterol decreases in the ordered phase and increases in the fluid phase according to the above assumption on S_0. Consequently, the discontinuity at the phase transition becomes smaller, until it vanishes at a second order transition, a critical point. The shift δT_t in the phase transition temperature results as

$$\frac{\delta T_t}{T_t^u - T^*} = \left(\frac{\xi_t^u}{R}\right)^2 \left(2\frac{S_o}{S_t^u} - 1\right), \tag{10a}$$

for $R_0 = 0$. T_t^u, ξ_t^u, and S_t^u are the values of the temperature, the coherence length, and the order parameter of the ordered phase, respectively, at the unperturbed first order phase transition. The analogous result for the latent heat is

$$\frac{\delta Q}{Q^u} = -\left(\frac{\xi_t^u}{R}\right)^2 2\frac{S_o}{S_t^u}. \tag{10b}$$

The latent heat always decreases upon addition of protein or cholesterol, and the ordered-fluid transition becomes of weaker first order. The critical point is reached if $\delta Q = -Q^u$, thus fixing the critical distance R_c by $(\xi_t^u/R_c)^2 = S_t^u/(2S_0)$ or, using a finite particle radius $R_0 \approx \xi_t^u$, by

$$\left(\frac{R_0}{R_c}\right)^2 = \frac{2}{9(S_0)/(S_t^u) + 3/2}. \tag{11}$$

Numerical results from this expression agree with the results of a computer solution of the same problem by Owicki and McConnell.[12] The l.h.s. of Eq. (11) is the ratio of surface areas covered by incorporated particles and by lipids, equal to the ratio of molecular areas f times the molar particle/lipid ratio, $(R_0/R_c)^2 = (f_P/f_L)(P/L)_c$, thus fixing the critical particle concentration.

From the discussion of the pretransitional increase of fluctuations and response functions at the ordered-fluid transition in pure lipid bilayers, we know that this increase becomes more pronounced the closer the transition is to a second order transition. Consequently, upon incorporation of proteins or cholesterol into a lipid bilayer, the pretransitional increase of the lipid response functions at the ordered-fluid transition should become more and more pronounced, until the critical point is reached.

To compare this theoretical prediction with experimental observations we choose cholesterol as the incorporated particles, for which case the most complete set of data is available. For cholesterol we may assume $f_{Ch} = f_L$, and deduce S_0 from the observed constancy of T_t, i.e. $S_0 = S_t^u/2$ from Eq. (10a). Then Eq. (11) yields for the critical cholesterol–lipid mole ratio $(Ch/L)_c = 1/3$, or for the corresponding cholesterol mole fraction $X_c = 1/4$. So at 25% cholesterol we expect a critical point. Calorimetric data indeed

show the latent heat to vanish at about this cholesterol mole fraction, accompanied by a pretransitional increase of the specific heat, as expected for a response function.[13] Furthermore, a slowing down of the relaxation time on approaching the critical cholesterol concentration has been observed in dielectric relaxation measurements.[14] Finally, the lateral diffusion of lipid molecules was found to increase upon addition of cholesterol to lipid bilayers, although in these experiments the maximal diffusion constant was observed at a somewhat lower cholesterol content.[15]

This last example of lateral diffusion may show that membranes could optimize their transport properties, in general their flexibility functions, by incorporating particles in order to approach a critical point. It is known that many biological membranes contain about 30% cholesterol.

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