Theoretical study of protein–lipid interactions in bilayer membranes
(boundary lipid/liquid crystals/phase transitions)

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ABSTRACT An analysis is given for the perturbation of the order and composition of lipid bilayers near an intrinsic membrane protein. Two cases are examined: the protein influences the lipid order (i.e., “fluidity”), and the protein associates with one component of a lipid mixture preferentially. The order perturbation is studied as a function of temperature and lateral pressure by using Landau–de Gennes theory and a variational procedure. It is concluded that, for a given lateral pressure, the greatest amount of boundary lipid is present at the lipid phase-transition temperature. A critical point for the phase transition occurs, near which the amount of boundary lipid increases dramatically. The composition perturbation is modeled in a binary lipid mixture by using a simple regular solution theory. The perturbation is found not to extend much beyond the directly bound layer of lipids unless the solution is near a critical mixing point.

An intrinsic membrane protein must alter the properties of nearby lipids in the bilayer. This effect has been reported in several magnetic resonance studies of reconstituted protein–membrane systems (1–6), and the perturbed lipids have been called boundary lipids. Protein–lipid interactions are obviously essential for membrane function. It is of interest to know how far from the protein the boundary lipid extends and what factors control this extent. This paper gives a simple theoretical analysis that seeks semiquantitative answers to these questions.

THEORY FOR BOUNDARY LIPIDS

Order Parameter and Lateral Pressure. The theoretical treatment below deals largely with the main phase transition in phospholipid bilayers. As the temperature is raised through the transition temperature, Tc, lateral molecular motions and the disorder of the acyl chains increase abruptly. For a more detailed discussion of this solid → fluid phase transition, and references to earlier work, see Jacobs et al. (7).

We focus on the temperature dependence of the area per molecule in the bilayer. For a representative phospholipid, dipalmitoyl phosphatidylcholine, near Tc0 the thickness of the fluid bilayer is 30% less than that of the solid (8), whereas the volume is 4% greater (9). Using these data, we estimate that the surface area is A1 ≈ 67 Å² per molecule in the fluid phase and A3 ≈ 48 Å² per molecule in the solid phase near Tc. For dipalmitoyl phosphatidylcholine, the solid is the intermediate or P′ phase, and the thickness and area are referred to the global plane of the bilayer.

A macroscopic order parameter, u, is defined in terms of the area:

\[ u = \frac{(A_3 - A_1)}{(A_3 - A_0)} \]

In the phase transition, u changes from 1 to 0. Farther from Tc0, du/dT < 0, but the effect is comparatively small.

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In other systems the order parameter is usually defined so as to equal zero in the limit of high temperatures. An example is the spectroscopic order parameter, S, for rod-like molecules in liquid crystals (10), which is, in addition, restricted to values between \(-\frac{1}{2}\) and \(+\frac{1}{2}\). In contrast, \(u = 0\) refers to a more arbitrary state, and \(|u| > 1\) is allowed. Despite these differences, u and S should be strongly correlated in bilayers.

We choose this definition of u for two reasons. First, it simplifies the adaptation of Landau–de Gennes theory (10) to lipid bilayers. Second, linking u to A facilitates treating the effects of the (external) lateral pressure \(\pi\). If the bilayer is treated as a two-dimensional system, \(-\pi\) is the area derivative of the Helmholtz free energy.

Several studies of lateral pressure in lipid monolayers have been made (11–13). It was suggested (13) that, for many physical properties, monolayers with \(\pi \sim 47\) dyne/cm (for dipalmitoyl phosphatidylcholine) approximate the corresponding bilayers. In typical bilayer preparations, \(\pi \sim 0\); presumably, the area dependence of the energy of the van der Waals interaction between monolayers in a bilayer is roughly equivalent to an internal lateral pressure of \(\pi \sim 47\) dyne/cm.

It should be possible to vary \(\pi\) experimentally in bilayers. For example, \(\pi < 0\) could be produced by osmotic imbalance in vesicles. Also, surface charge in phospholipid bilayers leads to an electrostatic analog of \(\pi\).

Landau–de Gennes Theory. Following the liquid crystal studies of de Gennes (10), we represent that part of the Gibbs free energy density that depends on the order parameter as a truncated Taylor expansion in u about \(u = 0\):

\[ G(u) = -\pi(A_3 - A_1)u + \frac{1}{2}\alpha(T - T^*)u^2 - \frac{1}{2}\beta_3u^3 + \frac{1}{2}\gamma_4u^4. \]

[2]

The term \(-\pi(A_3 - A_1)u\) is the u-dependent part of \(\pi A_3\); \(A_3\) and \(A_1\) refer to \(T = T_c^0\) and \(\pi = 0\). For these values of \(T\) and \(\pi\), the following relationships hold:

\[ \Delta u^0 = \frac{2B}{(3C)} \]

\[ \Delta H^0 = aT_c^0(\Delta u^0)^2/2 \]

\[ T_c^0 - T^* = 2B^2/(9aC). \]

\[ \Delta u^0 \] is the experimental transition discontinuity in u (= 1 here), and \(\Delta H^0\) is the experimental transition enthalpy (zero superscripts signify \(\pi = 0\)). Additional information to help fix the parameters \(a, T^*, B, \) and C could be obtained from the experimental dependence of \(T_c\) and \(u\) on \(\pi\) if these data were known.

\[ G(u) \] is a function of \(T\) and \(\pi\); the variation of the local and global minima of \(G(u)\) with \(T\) and \(\pi\) describes a first-order

Abbreviation: \(T_c^0\), transition temperature.

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phase transition between states of low and high \( u \). For the special case \( B = 0 \), the transition is second-order (14).

At \( \pi = 0 \) the temperature limits for the metastability of the fluid and solid phases are \( T^* \) and \( T^{**} = T^* + (B^2/4A) \), respectively.

It is convenient to divide Eq. 2 through by \( a(T_c^0 - T^*) \), to simplify by using Eq. 3, and to express the results in reduced units:

\[
\tilde{C}(u) = \frac{C(u)}{a(T_c^0 - T^*)} = \tilde{\pi} u + \frac{1}{2} \tilde{T} u^2 - u^3 + \frac{1}{2} u^4 \quad [4]
\]

Fig. 1 shows the \( \pi \)-dependence of \( u \) at \( T_c \), of \( T^* \) and of the metastability limits. A critical point is given by this mean field model, with \( u_{\text{crit}} = 0.5 \), \( \tilde{T}_{\text{crit}} = 1.5 \), and \( \tilde{\pi}_{\text{crit}} = 0.25 \).

*Elastomorphic deformations.* The effect of spatial variations in \( u \) on the free energy is approximated by an elastic energy term (15) which is quadratic in \( u \): \( D|\nabla u|^2/2 \). If the gradient is taken in reduced distance units,

\[
\tilde{r} = r[a(T_c^0 - T^*)/D]^{1/2},
\]

then \( D \) is no longer explicitly present, so that the total (reduced) free energy density becomes

\[
\tilde{C}_{\text{tot}}(u, \nabla u) = -\tilde{\pi} u + \frac{1}{2} \tilde{T} u^2 - u^3 + \frac{1}{2} u^4 + \frac{1}{2} |\nabla u|^2. \quad [6]
\]

**Application to Boundary Lipid.** The protein–lipid interface is represented as a circle of radius \( r_0 \) imposing circular symmetry on the system. Next, \( u(r_0) \) is fixed at some value \( u_{\text{eq}} \) which, for example, may be chosen to match the hydrophobic surface of the protein to the thickness of the membrane at the interface. For large \( r \), \( u \sim u_B \), in which \( u_B \) is the bulk equilibrium value in the absence of the protein. The problem then is to determine the function \( u(\tilde{r}) \) that minimizes the free energy:

\[
\int_0^{\tilde{r}_{\text{max}}} C_{\text{tot}}[u(\tilde{r}), u'(\tilde{r})]2\pi d\tilde{r} = \text{minimum}. \quad [7]
\]

Here, \( u' = du/d\tilde{r} \). Through the Euler–Lagrange equation (16), this reduces to solving the following differential equation:

\[
u'' + \frac{1}{\tilde{r}} u' + \tilde{\pi} - \tilde{T} u + 3u^2 - 2u^3 = 0 \quad [8]
\]

\[
u(\tilde{r}_0) = u_0 \quad u(\infty) = u_B.
\]

This boundary value problem can be solved numerically. An alternative to solving it exactly is to insert a simple variational trial function for \( u(\tilde{r}) \) and adjust its parameters to get the lowest free energy consistent with that functional form. The simple decaying exponential function

\[
u(\tilde{r}) = u_B + (u_0 - u_B) \exp[-(\tilde{r} - \tilde{r}_0)/\tilde{\lambda}] \quad [9]
\]

is generally an excellent approximation. The radial correlation length \( \tilde{\lambda} \) is the root of a cubic equation.

**Results.** Fig. 2 depicts the radial order profiles about a protein for several temperatures, with \( \tilde{\pi} = 0 \). Representative points from the approximate variational solution are seen to lie close to the exact solutions.

Fig. 3 represents \( \tilde{\lambda} \) for a wider range of \( \tilde{T} \) and \( \tilde{\pi} \). At constant \( \tilde{\pi} \), \( \tilde{\lambda} \) is largest near the transition temperature; the largest of all values of \( \tilde{\lambda} \) are in the critical region. At constant \( \tilde{T} \), increasing \( \tilde{\lambda} \) lowers \( \tilde{\lambda} \) in the solid and raises it in the fluid phase.

In other calculations, not discussed here in detail, the relationship between protein radius \( r_0 \) and \( \tilde{\lambda} \) was studied. \( d\tilde{\lambda}/dr_0 > 0 \), but the dependence is fairly weak; doubling \( r_0 \) typically increases \( \tilde{\lambda} \) by 10–20%. Using \( r_0 = 0.1 \)–10.

We can make order-of-magnitude estimates of reduced quantities for various physical conditions. By comparison to earlier applications of Landau–de Gennes theory, \( r/\tilde{r} \) is likely to be of molecular dimensions, say 10 \( \tilde{\lambda} \). If \( T_c^0 - T^* \ll 20 \), one expects a measurable temperature dependence of the amount of boundary lipid, as was suggested by Dahlquist et al. (3). Taking \( \Delta H^0 = 10 \text{ kcal} (41.9 \times 10^3 \text{J})/\text{mol} \), \( A_T - A_s \sim 20 \text{ nm}^3 \), and
THEORY FOR LIPID MIXTURES

Biological membranes typically contain a mixture of lipids, and some membrane proteins bind one lipid species preferentially (17–19). We have made the following calculations to study how far the alteration of the local lipid composition can extend from the protein.

Following the approach used by Cahn and Hilliard (20) for alloys, we treat the membrane as a fluid binary mixture of lipids. The dependence of the free energy density on the mole fraction, \( x \), of one of the components is approximated by a simple regular solution theory (21):

\[
\Gamma(x) = Wx(1-x) + kT[x\ln x + (1-x)\ln(1-x)].
\]

The parameter \( W \) is a measure of the nonideality of the solution; usually \( W > 0 \). This is a mean field theory with a critical mixing point; \( x_{\text{crit}} = 0.5, T_{\text{crit}} = W/(2k) \).

The effect on \( \Gamma \) of spatial variation in \( x \) is taken to be a quadratic term, \( E[\nabla x]^2 \). The integral representing the specific interfacial free energy (20) for the same geometry as before is minimized by (numerically) solving the Euler-Lagrange equation:

\[
x'' + \frac{1}{r}x' + 2(x - x_B) + \frac{\hat{T}}{x_B(1-x)} \ln \frac{x(1-x_B)}{x_B(1-x)} = 0.
\]

Here \( x_B \) is \( x \) in the bulk mixture, \( \hat{T} = kT/W \), and \( \hat{r} = r/(E/W)^{1/2} \).

With a lattice representation of the mixture (21) and a constant concentration gradient, we estimate \( (E/W)^{1/2} \approx 3 \, \text{Å} \) for lipids such as phosphatidylcholines. This is independent of the value of \( W \). We make no estimates of \( W \) except to note that complete miscibility of the components requires \( W < 2kT \approx 1.2 \, \text{kcal/mol at room temperature} \).

The main result of the calculations is that the perturbation of composition does not extend much beyond the first layer of bound lipid unless the system is close to critical. This can be seen in the sample composition profiles presented in Fig. 4.

For \( x_0/x_B \approx (1-x_0)/(1-x_B) \approx 1 \), Eq. 11 is well approximated by a Bessel equation. The solution then is

\[
x(t) = (x_0 - x_B) \cdot K_0(\hat{r}/\hat{\xi})/K_0(\hat{r}_0/\hat{\xi}) + x_B \quad \text{[12]}
\]

where \( K_0 \) is the modified Bessel function of the second kind and the radial correlation length \( \hat{\xi} \) is given by

\[
\hat{\xi}^{-2} = \frac{\hat{T}}{[x_B(1-x_B)]} - 2.
\]

Near the critical point, \( \hat{\xi} \) diverges as \( (\hat{T} - \hat{T}_{\text{crit}})^{-1/2} \) and \( x_B - x_{\text{crit}}^{-1} \). For comparisons, see Fisk and Widom (22).

DISCUSSION

We have described simple methods of approximating the order and composition of membrane lipids near intrinsic membrane proteins. Use of the known phase behavior of bulk lipid bilayers embeds the protein–lipid problem in a context that is better understood. The Landau–de Gennes treatment can reproduce, at least qualitatively, most of the existing experimental data on boundary lipids. We cite examples below.

The boundary lipid is observed to be either less or more ordered (or “fluid”) than the bulk lipid, depending on the composition and phase of the system (1–6). Assuming that such order is monotonically related to \( u \), this corresponds to the choice of \( u_0 \) relative to \( u_B \) in the theory. A temperature dependence of the amount of boundary lipids, as suggested by Dahlquist et al. (3), arises naturally from the theory.

The calculations likewise are consistent with the calorimetric results of Curatolo et al. (23) that membrane proteins can broaden the lipid phase transition and lower its \( \Delta H \). The protein-induced increase in \( T_\text{c}^0 \) observed by these workers does not follow from the theory, except insofar as the temperature dependence of the amount of boundary lipid is not symmetric about \( T_\text{c}^0 \).

The interpretation of the experimental data is not trivial. For example, when the content of membrane protein is high, the (unknown) lateral distribution of the proteins may significantly influence the amount and properties of the boundary lipid. Also, the precise population of molecules observed as boundary lipid depends somewhat on the experimental method used.

Theoretically, lipid–protein interactions cause lipid-mediated
protein–protein interactions when two proteins are close enough for their boundary lipids to overlap (24–26). In the present model, proteins with similar values of \( u_0 \) (or \( x_0 \)) attract each other because of the resultant reduction of the total boundary lipid. These interactions are selective. For example, two proteins with \( u_0 - u_0' \) (or \( x_0 - x_0' \)) of opposite sign repel at all separations. Thus, the state of the bulk lipids modulates the protein–protein interaction through \( u_0 \) (or \( x_0 \)).

**Weaknesses of Theories.** The theories in this paper are mean field analyses, and they are heir to all the approximations inherent in such treatments of phase transitions and critical phenomena (10). Also, treating the membrane as a continuum is a poor approximation when correlation lengths are smaller than molecular diameters.

Using a single-order parameter in any bilayer membrane theory gives only a very simple account of a complex system. For example, we do not distinguish among contributions to the area from chain conformation, chain tilt, and membrane undulations (i.e., the \( F'' \) phase).

Our choice of the zero of \( u \) leads to some incorrect predictions, principally that \( du/dT > 0 \) when \( u < 0 \). Because we are more concerned with a qualitative discussion of boundary lipid than a comprehensive treatment of the state of the bulk bilayer, this is not a major problem here. It can, however, be ameliorated at the cost of complicating the free energy equations.

One way is to define \( u \propto \) bilayer thickness. Neglecting volume changes, then \( A \propto 1/u \); this is less convenient than the direct relationship we have used. Alternatively, one could add a term to \( \pi \), a constant positive quasi-external lateral pressure from the van der Waals interactions between monolayers. The reduced equation for \( G \) is unchanged, but the relationships of the variables to experiment are more complicated. The physically relevant states always would have \( \pi > 0 \) and, therefore, \( u > 0 \), (see Fig. 1A).

**Other Theories.** Marčelja (27) has published a microscopic mean field model of order in lipid bilayers based on chain conformation. He has applied this to the boundary lipid problem, treating the protein–lipid interaction as a constant contribution to the molecular field of the lipids touching the protein (24). Sample calculations showed the boundary lipid extending several molecular layers out from the protein, the system being greater for \( T \sim T^* \) than for \( T \sim T^* + 10 \). Our results generally agree with this. The area for each lipid molecule was fixed at the experimental bulk value, so \( \pi - A \) effects were not investigated.

Schröder (25) described a method of incorporating lipid–protein interactions into a preexisting mean field treatment of lipid bilayers. The protein acted formally as an external field on the lipids. A linear approximation to the resulting differential equation describing the perturbation of lipid order was solved by using linear response theory. The spatial order profile about a circular protein was found in terms of modified Bessel functions with arguments scaled by the correlation length for order fluctuations in the lipids.

In both the above studies (24, 25), the attractive lipid-mediated interaction between two identical proteins was demonstrated.

Jähnig (26) also has developed a microscopic mean field bilayer model based on chain conformation. The area dependence of the free energy entered not directly as \( \pi A \) but as a steric potential depending on the bilayer thickness. In the limit of low thickness, the model reduced (28) to a Landau–de Gennes theory, with results for the bulk bilayer similar to those obtained independently here.

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