Monte Carlo simulation studies of lipid order parameter profiles near integral membrane proteins

Maria M. Sperotto and Ole G. Mouritsen
Department of Structural Properties of Materials, The Technical University of Denmark, DK-2800 Lyngby, Denmark

ABSTRACT Monte Carlo simulation techniques have been applied to a statistical mechanical lattice model in order to study the coherence length for the spatial fluctuations of the lipid order parameter profiles around integral membrane proteins in dipalmitoyl phosphatidylcholine bilayers. The model, which provides a detailed description of the pure lipid bilayer main transition, incorporates hydrophobic matching between the lipid and protein hydrophobic thicknesses as a major contribution to the lipid-protein interactions in lipid membranes. The model is studied at low protein-to-lipid ratios. The temperature dependence of the coherence length is found to have a dramatic peak at the phase transition temperature. The dependence on protein circumference as well as hydrophobic length is determined and it is concluded that in some cases the coherence length is much longer than previously anticipated. The long coherence length provides a mechanism for indirect lipid-mediated protein–protein long-range attraction and hence plays an important role in regulating protein segregation.

INTRODUCTION

The modern view of a biological membrane is that of a cooperative system consisting of a lipid matrix, the lipid bilayer, where molecules such as proteins and cholesterol are embedded. When a protein is incorporated in the lipid bilayer it may perturb the physical properties of the surrounding lipids, such as their hydrocarbon chain conformational order. One of the main questions to answer is whether this perturbation is restricted to a small neighborhood around the protein (Hesketh et al., 1976; Jost and Hayes Griffith, 1980), as in the case of cytochrome oxidase (Jost et al., 1973), or if it has a long-range character as has been experimentally detected for bacteriorhodopsin (Rehorek et al., 1985). Because the order of the lipid chain is related to its hydrophobic thickness (Seelig and Seelig, 1974, 1980; Schindler and Seelig, 1975; Ipsen et al., 1990*a*), a protein in the membrane may induce a local lipid hydrophobic thickness variation which can be described by a characteristic decay length, ξ_P. Theoretical attempts to calculate ξ_P include phenomenological Landau theories (Schröder, 1977; Owicki et al., 1978; Owicki and McConnell, 1979; Jähnig, 1981*a* and *b;* Abney and Owicki, 1985) and mean-field modeling (Marčelja, 1976) which both consider the decay of the lipid order away from the protein by treating the protein as a rigid geometrical constraint on the nearest lipids. The rigid boundary condition for the lipid order parameter reflects the necessity of matching between the thickness (length) of the hydrocarbon chains and the thickness of the hydrophobic part of the protein in order to avoid the nonpolar moieties being exposed to the aqueous environment. Owicki et al. (1978) assumed that the decay is exponential and that it may be characterized by the decay length, ξ_P. It was shown (Owicki et al., 1978; Jähnig, 1981*a* and *b*) that ξ_P has a pronounced temperature dependence with a maximum at the gel-to-fluid main phase transition temperature of the lipid bilayer. Jähnig (1981*a*) argued that ξ_P is closely related to the correlation length ξ_L characteristic of the correlation of the lateral fluctuations in the pure lipid bilayer. In fact it was asserted (Jähnig 1981*a*) that $\xi_P \simeq \xi_L \simeq 15$ Å at the transition, i.e., the protein perturbation is felt only a few lipid molecular diameters away from the protein surface. Similar estimates were provided by other theories (Marčelja, 1976; Owicki et al., 1978). The determination of the decay length of the perturbations caused by the proteins, and its dependence on the temperature and the form and size of the protein, is the aim of the theoretical work presented in this article.

Here we are going to present detailed direct calculations of both correlation lengths, ξ_L and ξ_P, and show within a simple method how ξ_P depends on temperature and on form and size of the integral protein embedded in the lipid bilayer. In contrast to earlier work, the present work is based on a microscopic model which treats the membrane as a discrete molecular system by accounting in some detail for the conformational states of the lipid acyl chains, their statistics, and mutual specific interactions. Furthermore, we calculate the properties of this model by computer-simulation techniques which provide an accurate estimate of the thermal fluctuations. This is very important when the proper-

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ties in the phase transition region are considered. In this region Landau and mean-field theories (Marčelja, 1976; Jähnig, 1981*a* and *b*) are unreliable due to the suppression of the correlations in the lateral density fluctuations. The proteins are treated within our model as rigid objects presenting an excluded volume to the lipids. The protein is assumed to have a cylindrical shape characterized by a hydrophobic length and a circumference. The direct interaction between the lipids and the protein molecule is assumed to be short ranged including interface-contact hydrophobic interactions as well as an interaction term which accounts for a possible mismatch between the lipid bilayer and the protein hydrophobic thicknesses. Due to the detailed description of the lipid chains, a mismatch condition will force the lipids to wet the hydrophobic surface of the protein. This mechanism is the main determinant of the protein-induced perturbation of the lipid membrane investigated in the present work. Our main result is that ξ_P as well as ξ_L are much larger than previously anticipated.

MICROSCOPIC MODEL

The microscopic interaction model used in the present paper to describe lipid-protein interactions in membranes is related to the statistical mechanical models used by Pink and collaborators (Pink and Chapman, 1979; Lookman et al., 1982; Tessier-Lavigne et al., 1982; Caillé et al., 1980; Pink, 1984; MacDonald and Pink, 1987). The model is built on the Pink model (Pink et al., 1980) of the properties and the phase transition in pure lipid bilayers. However, rather than introducing purely microscopic phenomenological lipid-protein interaction constants, an attempt is made, in the spirit of the mattress model (Mouritsen and Bloom, 1984), to identify part of the lipid-protein interaction constants in terms of molecular properties, specifically hydrophobic thickness and hydrophobic matching between the lipid and protein hydrophobic thicknesses. Here we choose Pink's 10-state model (Pink et al., 1980) to describe the pure lipid bilayer phase behavior. This model accounts accurately for the most important conformational states of the lipids and their mutual interactions and statistics. Within the scope of this model the bilayer is considered as two monolayer sheets which are independent from each other, and each monolayer is represented by a triangular lattice on which sites the lipid acyl chains are arrayed. The model is hence a pseudo-two-dimensional lattice model which neglects the translational modes of the lipid molecules by focusing on the conformational degrees of freedom of the acyl chains. The neglect of the translational degrees of freedom of the acyl chains is justified by Doniach's estimate (Doniach, 1978) that their contribution to the enthalpy of the fluid-to-gel transition is negligible in equilibrium. Each acyl chain can take on one of 10 conformational states m, each of which is characterized by an internal energy E_m, a hydrocarbon chain length d_m, and a degeneracy D_m, which accounts for the number of conformations that have the same chain length d_m and energy E_m, where $m = 1, 2, \ldots 10$. The 10 states are derivable from the all-*trans* state in terms of *trans*-gauche isomerism. The state $m = 1$ is the nondegenerate gellike ground state, representing the all-*trans* conformation, whereas the state $m = 10$ is a highly degenerate excited state characteristic of the melted or fluid chain. The eight intermediate states are gellike states containing kink and jog excitations and they satisfy the requirement of low conformational energy and optimal packing. The conformational energies E_m are obtained from the energy needed for a gauche rotation (0.45×10^{-13} erg) relative to the all-*trans* conformation. The values of D_m are obtained from combinatorial considerations (Caillé et al., 1980) and those of the chain lengths are trivially related to the values of the cross-sectional areas A_m because the volume of an acyl chain is assumed to be invariant under temperature changes (Marčelja, 1974; Träuble and Haynes, 1971). The saturated hydrocarbon chains are coupled by nearest-neighbor anisotropic forces which represent both van der Waals and steric interactions. The interaction is formulated in terms of products of shape-dependent nematic factors. The lattice approximation automatically accounts for the excluded volume effects and, to a rough approximation, for the part of the interaction with water which brings the bilayer into existence. An effective lateral pressure, Π, of 30 dyn cm^{-1}, acting on the hydrophobic region of the bilayer due to the interaction among the polar moieties, is added to the model to assure bilayer stability (Marčelja, 1974). The Hamiltonian energy of the pure lipid bilayer model may then be written

$$\mathscr{H}_{\text{pure}} = \sum_i \sum_m (E_m + \Pi A_m)\mathscr{L}_{m,i} - \frac{J_0}{2} \sum_{\langle i,j \rangle} \sum_{m,n} J(d_m, d_n)\mathscr{L}_{m,i}\mathscr{L}_{n,j}, \quad (1)$$

where J_0 is the strength of the van der Waals interaction between neighboring chains, and $J(d_m, d_n)$ is an interaction matrix which involves both distance and shape dependence. The Hamiltonian is expressed in terms of site occupation variables $\mathscr{L}_{m,i}$: $\mathscr{L}_{m,i} = 1$ if the chain on site i is in state m, otherwise $\mathscr{L}_{m,i} = 0$. The model parameters are chosen to reproduce the transition temperature, T_m, for the pure DPPC (dipalmitoyl phosphatidylcholine) bilayer (Mouritsen et al., 1983).

The proteins are considered as regular objects which

occupy a number, n_P, of sites of the underlying triangular lattice. The lipid-protein interactions are included in the model Eq. 1 along the same lines as in the phenomenological mattress model of lipid-protein interactions (Mouritsen and Bloom, 1984; Sperotto and Mouritsen, 1988; Sperotto et al., 1989). In the mattress model the phase behavior of the pure lipid membrane is fed in from experimental thermodynamic data via a regular solution theory and the lipid-protein interactions are modeled phenomenologically by expanding around the infinite dilution limit (Mouritsen and Bloom, 1984). In the present microscopic model the cooperative behavior of the pure lipid membrane is modeled from first principles by the lattice model in Eq. 1. Within this lattice formulation, a protein molecule may occupy one or more lattice sites and its hydrophobic part is assumed to be hydrophobically smooth, rodlike, and with no appreciable internal flexibility. These assumptions hold in those cases where the protein structure is known and is normally used in protein modeling (Jähnig, 1981*a*; Sadler and Worcester, 1982; Sadler, et al., 1984). In this way the protein is characterized only by a cross-sectional area A_P, a circumference ρ_P and the half-length of the hydrophobic core, d_P. In the following, the term 'protein length' will refer to d_P.

The Hamiltonian of the model is then

$$\mathcal{H} = \mathcal{H}_{pure} + \mathcal{H}_{L\text{-}P}$$

$$\mathcal{H}_{L\text{-}P} = \Pi A_P \sum_i L_{P,i} + \gamma_{mis}\left(\frac{\rho_P}{Z}\right) \cdot \sum_{\langle i,j\rangle}\sum_m |d_{m,i} - d_P| \mathcal{L}_{m,i} L_{P,j} - \nu_{vdW}\left(\frac{\rho_P}{Z}\right)\sum_{\langle i,j\rangle}\sum_m \min(d_{m,i}, d_P)\mathcal{L}_{m,i}, L_{P,j}, \quad (2)$$

where Z is the coordination number of the lattice (=6) and $L_{P,i}$ is the protein occupation variable. $\mathcal{L}_{m,i}$, and $L_{P,i}$ must satisfy a completeness relation which holds for each lattice site. For example for a single-site protein the following is valid: $\Sigma_m \mathcal{L}_{m,i} + L_{P,i} = 1$. The parameter ν_{vdW} is related to the direct lipid-protein hydrophobic van der Waalslike interaction which is associated with the interfacial contact of the two species. The interaction parameter γ_{mis} is related to the hydrophobic effect. This interaction accounts for a possible existence of a "mismatch" between the protein and the lipid hydrophobic lengths. The values of the lipid-protein interaction parameters γ_{mis}, and ν_{vdW} have been chosen in accordance with those of the phenomenological mattress model (Sperotto and Mouritsen, 1988), but it is not expected that the parameter values in the microscopic and phenomenological models should necessarily be the same. In contrast to the phenomenological model, the microscopic model described in this article automatically includes the elastic contribution to the free energy of the bilayer, whereas it is explicitly included in the phenomenological model. The hydrophobicity of typical protein side chains is about half of that of the hydrocarbon chains (Tanford, 1973) and the parameter γ_{mis} in Eq. 2 will therefore depend on the phase of the system: if $\gamma_{mis} = \gamma^{\alpha}_{mis}$ [where α is a label referring to the lipid phase: α = g (gel) or α = f (fluid)] in the cases when $d_{m,i} < d_P$, then $\gamma_{mis} \simeq 2\gamma^{\alpha}_{mis}$ for $d_{m,i} > d_P$. In contrast, the parameter ν_{vdW} is considered phase independent because the lipid hydrophobic length is now consistently determined by the cooperativity of the microscopic model. In this respect, the phenomenological model approach to lipid-protein interactions and the microscopic approach are conceptually different. Hence, the microscopic model contains only two model parameters which in principle are unknown and in practice determined empirically by drawing upon the phenomenological mattress model (Sperotto and Mouritsen, 1988).

METHOD OF CALCULATION OF THE DECAY LENGTH

We have used standard Metropolis Monte Carlo simulation techniques (Mouritsen, 1990) to calculate the thermodynamic properties of the model both in the absence and in the presence of proteins. For the pure lipid bilayer we have determined the correlation length ξ_L by use of the cluster-size distribution function (Cruzeiro-Hansson and Mouritsen, 1988). The average linear size of the clusters of correlated lipid chains is a direct measure of ξ_L. For the lipid-protein system we have tested the exponential character of the decay of the lipid order near the proteins in order to directly estimate the decay length ξ_P and its temperature and protein-size dependence.

The cases of three different protein sizes have been considered: a protein occupying one lattice site (n_P = 1), a protein occupying seven sites (n_P = 7) in a regular hexagon, and finally a "barrier" of proteinlike sites ($n_P = \infty$) which would model a very big protein compared to the lipids. Integral proteins like gramicidin A (Chapman et al., 1977) or polypeptides (Davis et al., 1983) may be modeled by a seven-site protein, while in the case of phospholipids coming into contact with big proteins like the photosynthetic reaction center of *Rhodopseudomonas viridis* (Deisenhofer et al., 1985) or bacteriorhodopsin from *halobacterium halobium* (Henderson and Unwin, 1975), the barrier may be a reasonable model for such proteins. Because we are only interested here in the low-concentration regime, where the correlation between different proteins can be neglected, we can

treat the protein perturbations on the lipid bilayer as a single-particle effect and hence consider an ensemble of isolated stationary proteins embedded in the lipid matrix at mutual separations which are larger than the actual coherence length. For lipid lattices of 60 × 60 chains, we have placed four proteins in a regular fashion in the lattice in the case of $n_P = 1$ and 7. For $n_P = \infty$, the protein barrier is simply a slap of a single line of lattice sites along one of the canonical axes of the triangular lattice. The restriction to the low-concentration regime together with the assumption of immobile proteins also implies that there is no phase separation and only a negligible depression of the transition temperature.

It has *a priori* been assumed, and then verified later, that for each temperature, T, the mean lipid hydrophobic length, $\langle d_L(I)\rangle_T$, of the nearest layers (indexed by I) around one of the isolated proteins (see Fig. 1) are related to the decay length $\xi_P(T)$ as

$$\langle d_L(I)\rangle_T = \langle d_L^o\rangle + (d_P - \langle d_L^o\rangle)e^{-D(I)/\xi_P(T)}, \qquad (3)$$

where $\langle d_L^o\rangle$ is the temperature-dependent mean lipid chain length of the unperturbed pure lipid bilayer and $D(I)$ is the distance of the lipid from the protein. $D(I)$ is a multiple of D_o, the lipid chain diameter, which for convenience has been fixed to a mean value of 6.5 Å (Lewis and Engelman, 1983*a*), neglecting its temperature dependence. The estimation of $\langle d_L(I)\rangle_T$ proceeds as follows: after having allowed the system to equilibrate, the circularly averaged values of the lengths of the lipids in the first, second, third, . . . , twelfth layer around each of the immobilized proteins are calculated in an initial configuration, Ω_1. It was found sufficient to consider "distances" from each protein up to the 12th layer in order to reach the mean value of the lipid order parameter (i.e., lipid chain length) for the pure bilayer. These circularly averaged lipid lengths are then further averaged over 200 equilibrium configurations that are created from Ω_1 and "separated" by 100 Monte Carlo steps per site. This procedure yields then the equilibrium data $\langle d_L(I)\rangle_T$.

Assuming the validity of Eq. 3, the Monte Carlo data for $\langle d_L(I)\rangle_T$ and those for $\langle d_L^o\rangle$ at different temperatures allow an estimate of ξ_P by use of the following relation:

$$F(I) = \frac{D_o I}{\xi_P}, \qquad (4)$$

where

$$F(I) \equiv \ln\left(\frac{d_P - \langle d_L^o\rangle}{\langle d_L(I)\rangle_T - \langle d_L^o\rangle}\right). \qquad (5)$$

For each considered temperature, the values of the function at the left-hand side of Eq. 5 have been plotted versus I and their asymptotic behavior at large I and the corresponding decay length are determined by linear regression.

All the calculations refer to proteins with a hydrophobic length in the bracket $d_L^{o,f} < d_P < d_L^{o,g}$ where $d_L^{o,g}$ is the lipid length of the all-*trans*-chain conformation $d_L^{o,f}$ is that of the fully melted chain conformation. Because the 10-state model due to its discreteness allows for only a few different lipid chain hydrophobic lengths, the mismatch interaction term in the Hamiltonian may in some cases force the first layer around the protein to have, in the case of $d_P > \langle d_L^o\rangle$ ($d_P < \langle d_L^o\rangle$), a bigger (smaller) length than the one of the protein and the lipids in the second layer. This is obviously unphysical and an artifact of the model. In such cases the decay length has been calculated neglecting the first layer. Because the decay length is calculated over many layers this shortcoming of the discrete 10-state model has only negligible influence on the results.

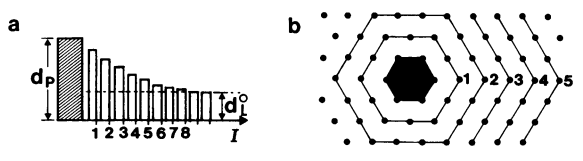

FIGURE 1 Lipid chain length profile (*a*) and lipid layers on a lattice (*b*) around an isolated protein. d_P is the protein hydrophobic length and d_L^o is the unperturbed lipid bilayer hydrophobic thickness.

RESULTS AND DISCUSSION

The equilibrium data, $\langle d_L^o\rangle$, for the unperturbed lipid bilayer are shown in Fig. 2. This figure clearly shows the dramatic change in hydrophobic bilayer thickness at the main transition. We have verified numerically, by a study of the specific heat function, that by the introduction of the proteins there is no discernable shift in the transition temperature. The variation of the membrane thickness at T_m is dramatic although effectively continuous. It is associated with strong lateral density fluctuations which manifest themselves in peaks in the response functions, e.g., the specific heat and the later compressibility, and a large correlation length of the fluctuations (Cruzeiro-Hansson and Mouritsen, 1988; Ipsen et al., 1990*b*). The transition is pseudocritical (Mitaku et al., 1983; Ruggiero and Hudson, 1989). The fluctuations show up microscopically by formation of clusters at each side of the transition. These clusters are domains of lipid chains which are predominantly in states characteristic of the opposite phase. This gives rise to a picture of dynamic membrane heterogeneity in which the average linear

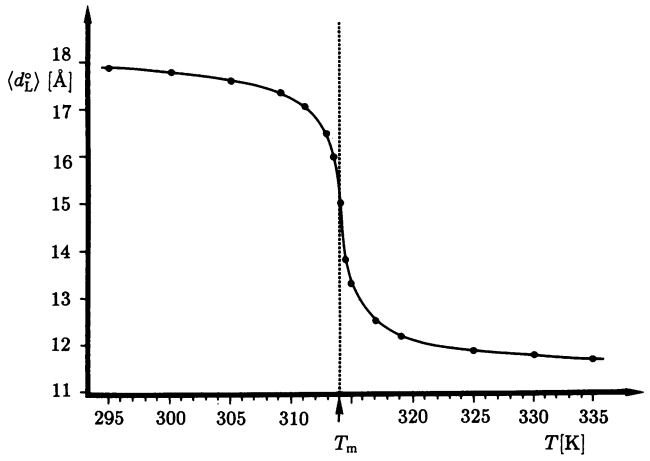

FIGURE 2 Temperature dependence of the equilibrium lipid chain length, $\langle d_L^o \rangle$, of the unperturbed DPPC bilayer. The transition temperature is $T_m = 314$ K.

size of the clusters is a measure of the coherence length, $\xi_L(T)$, of the fluctuations. The average linear cluster size which is determined from the complete cluster-size distribution function is shown in Fig. 3. It has a dramatic peak at the transition and it is seen that the correlation length gets very large as the transition is approached from either size. The value of $\xi_L(T)$ at the transition is much larger than anticipated by earlier theoretical estimates (Jähnig, 1981*a* and *b*; Owicki et al., 1978). $\xi_L(T)$ depends on the lipid species in question. For saturated phosphatidylcholines $\xi_L(T)$ increases as the acyl chain length is decreased (Ipsen et al., 1990*b*).

In most of the calculations performed on the lipid-protein system the values of the interaction parameters have been fixed to $\gamma_{mis} = 0.01 \times 10^{-13}$ erg Å^{-2} and $\nu_{vdW} = 0.03 \times 10^{-13}$ erg Å^{-2}. The projected area of the protein on the membrane plane is chosen to be n_P times the area of a single-site protein which has been fixed to 20.4 Å^2, i.e., the circumference is $\rho_P = 16$ Å.

To verify that $\langle d_L(I) \rangle_T$ satisfies the relation Eq. 3, the calculated values of $F(I)$, Eq. 5, have been plotted versus I for the case of the protein with $d_P = 18$ Å. The values of $\langle d_L^o \rangle$ needed for this calculation are taken from Fig. 2. The results are shown in Fig. 4 which contains two data sets corresponding to temperatures above and below the pure lipid bilayer transition temperature. Fig. 4 shows that, to a good approximation, $F(I)$ is linear in I. Therefore, the inverse of the slope of the solid straight line, which best interpolates the calculated values of $F(I)$ at each temperature, determines the values of $\xi_P(T)$. Obviously, the determination of ξ_P becomes more accurate as the temperature approaches the transition point and the decay length increases. For temperatures far away from T_m the decay is too fast to allow a determination of ξ_P in the discrete lattice model. Moreover, the discreteness in the selection of chain states puts a restriction on the accuracy of ξ_P. As the temperature gets very close to T_m, there is a distinct deviation from an exponential decay. This is likely to be a real effect caused by the fact that the bilayer transition is pseudocritical and dominated by large fluctuations (Ip-

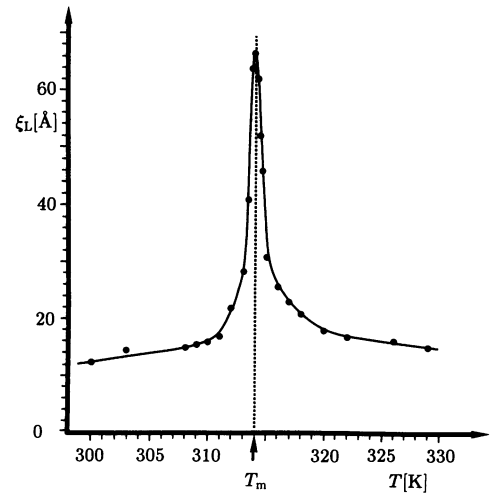

FIGURE 3 Temperature dependence of the coherence length, $\xi_L(T)$, of the lateral fluctuations in a pure DPPC bilayer.

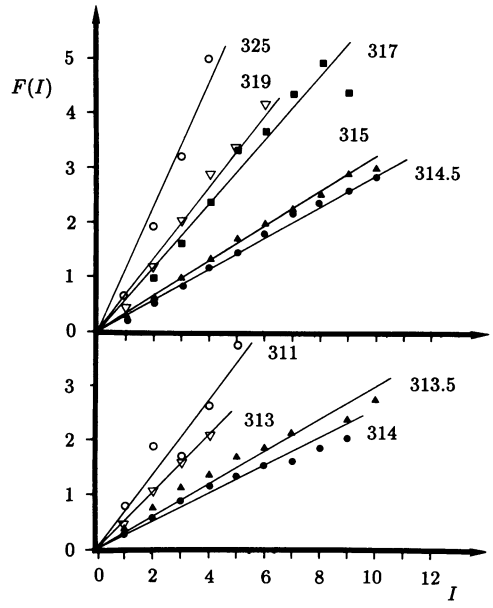

FIGURE 4 The function $F(I)$ in Eq. 5 shown at different temperatures above and below the transition temperature for the case of a seven-site protein with a hydrophobic length of $d_P = 18$ Å (cf. Fig. 5). The different data sets are labeled by the value of the temperature (in K).

sen et al., 1990*b*). This implies an effective algebraic decay at the transition point which will show up as an Ornstein-Zernike crossover behavior of the type $e^{-D(I)/\xi_P(T)}/D(I)^{1+\eta}$, where η is a critical-point exponent.

The temperature dependence of $\xi_P(T)$ in the case of seven-site proteins ($n_P = 7$) with different hydrophobic lengths, $d_P = 12$, 15, and 18 Å, is shown in Fig. 5. For all three proteins the decay length is found to have a dramatic temperature dependence with a sharp peak at the transition temperature. The shaded areas on Fig. 5 (as well as on Fig. 6) indicate a temperature region where $\langle d_L^o \rangle \simeq d_P$ very close to the protein and the decay cannot be resolved within the numerical accuracy to yield a reliable value of ξ_P. In a wide range of temperatures around T_m the protein-induced perturbance of the lipid bilayer is seen to extend beyond the first few molecular layers. The overall shape of $\xi_P(T)$ is very similar to that of the correlation length $\xi_L(T)$ of the pure lipid bilayer, cf. Fig. 3. For the protein parameters corresponding to those underlying Fig. 5, $\xi_P(T)$ is suppressed relative to $\xi_L(T)$. However, this is not generally true and the precise relation between $\xi_P(T)$ and $\xi_L(T)$ is not a simple one (Jähnig, 1981*a*). Whereas the presence of inert immobile impurities in a system with a first-order phase transition tends to increase the overall fluctuations in the system (e.g., as manifested in the specific heat and the lateral compressibility) and drive the system towards criticality (Jähnig, 1981*a*), the impurities (proteins) of the present system are not inert but couple directly to the order parameter of the system (the bilayer thickness). Because this coupling depends upon the value of the order parameter which is temperature dependent, cf. Eq. 2, the determining factor becomes a relation between d_P and the average lipid length at the transition, cf. Fig. 2. For the present system a protein length of $d_P = 15$ Å is close to the average lipid length at the transition. Comparison of the different cases in Fig. 5 shows that $\xi_P(T)$ is strongly suppressed at the transition for $d_P = 15$ Å relative to $\xi_P(T)$ for $d_P = 12$ and 18 Å. For the short protein the decay length is very long in the transition region, up to about seven molecular layers at T_m. Far away from T_m the decay length is in all cases very short, less than one molecular distance, in accordance with the vanishing lipid–lipid fluctuations.

In addition to the temperature dependence and protein-length dependence of $\xi_P(T)$ there is a strong dependence on the protein size (circumference ρ_P, i.e., n_P within the present model). This is demonstrated in Fig. 6 by data which refer to a single-site protein ($n_P = 1$), a seven-site protein ($n_P = 7$), and a protein barrier ($n_P = \infty$) for $d_P = 16$ Å. It is seen that the correlation length increases as the protein size is increased. The reason for this is that as n_P increases, the curvature of the protein decreases and the influence of a given part of the protein extends to more lipid sites. In this way a given lipid chain interacts with a greater portion of the protein hydropho-

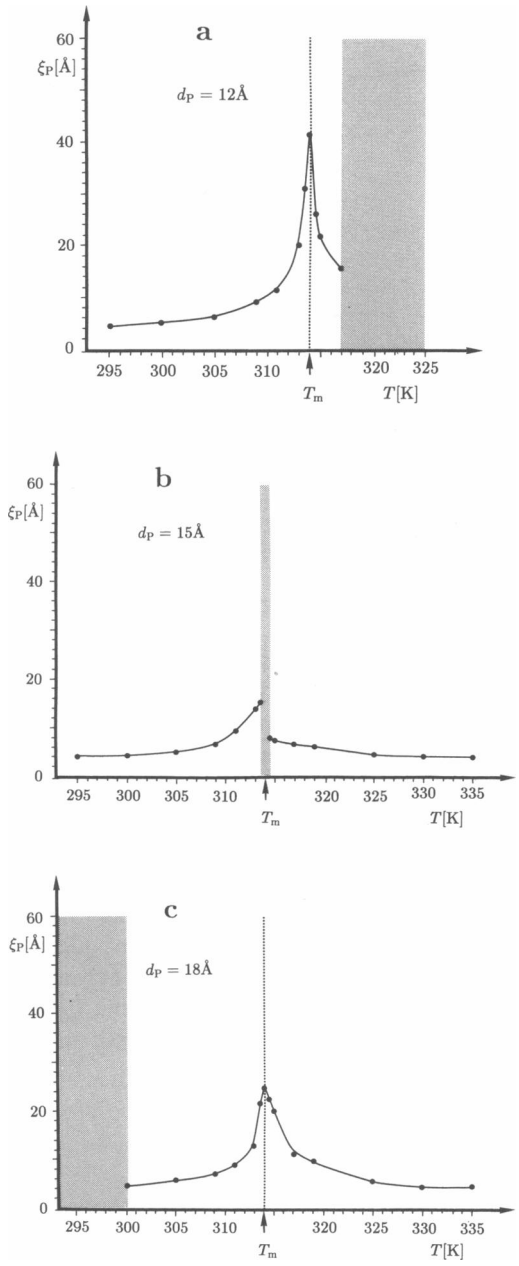

FIGURE 5 Temperature dependence of decay length, ξ_P, for a seven-site ($n_P = 7$) protein with a hydrophobic length of $d_P = 12$ Å (*a*), 15 Å (*b*), and 18 Å (*c*). The shaded areas indicate a temperature region where $\langle d_L^o \rangle \simeq d_P$ very close to the protein and the decay cannot be resolved within the numerical accuracy to yield a reliable value of ξ_P. The lipid-protein interaction parameters are chosen to be $\gamma_{mis} = 0.01 \times 10^{-13}$ erg Å^{-2} and $\nu_{vdW} = 0.03 \times 10^{-13}$ erg Å^{-2}.

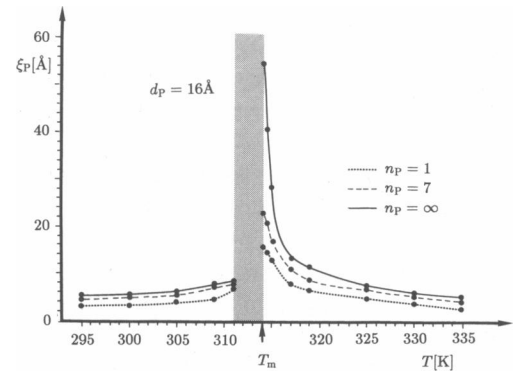

FIGURE 6 Temperature dependence of ξ_P for proteins with $n_P = 1$ (·····), $n_P = 7$ (– – – –), and $n_P = \infty$ (——). All three cases refer to protein lengths of $d_P = 16$ Å and the values of the interaction parameters are $\gamma_{mis} = 0.01 \times 10^{-13}$ erg Å^{-2} and $\nu_{vdW} = 0.03 \times 10^{-13}$ erg Å^{-2}.

bic surface the larger the protein is. Obviously this implies that nonsmooth variations on the surface of the protein will have a larger effect on the lipid environment the more comparable these variations are to the lipid length scale. In the case of a very large protein (the barrier, $n_P = \infty$), Fig. 6 demonstrates that the influence of the protein in this case extends to eight lipid layers.

Finally, some simulations were performed to investigate the influence of the van der Waalslike interaction parameter, ν_{vdW}, of Eq. 2 in the case of a protein with $d_P = 12$ Å. It found that $\xi_P(T)$ in this case is only slightly sensitive to the value of ν_{vdW} in the investigated range from 0 to 0.03×10^{-13} erg/Å^2. This is a consequence of the fact that the very short protein length chosen is shorter than $\langle d_L^o \rangle$ for most temperatures and the van der Waalslike interaction term in Eq. 2 is therefore approximately temperature independent. A different picture emerges for larger values of d_P. In that case the van der Waalslike attractive interaction will be effectively temperature dependent via $\langle d_L^o \rangle$ and $\xi_P(T)$ will then be dependent on the choice of ν_{vdW}.

CONCLUSION AND DISCUSSION

We have in this paper studied a model of lipid-protein interactions in membranes in the low-concentration regime and explored the effects due to a mismatch between the protein and lipid hydrophobic thicknesses. Within this model the integral protein is considered a hydrophobically smooth object of cylindrical shape presenting itself to the lipids as a rigid boundary condition, cf. Fig. 1. The properties of the model, which is a microscopic statistical mechanical model which accounts for the lipid bilayer main transition, have been calculated accurately using Monte Carlo computer-simulation techniques. In particular we have focused on the spatial density fluctuations in the membrane and the decay of the lipid order parameter profiles around the integral proteins. We have shown that this decay, which is induced by the mismatch, is of an approximate exponential form, cf. Fig. 4, and hence described by a coherence length, $\xi_P(T)$. We have found that $\xi_P(T)$ has a dramatic peak at the transition temperature, cf. Figs. 5 and 6, and related this peak to the corresponding behavior of the coherence length, $\xi_L(T)$ cf. Fig. 3, of the correlation of the fluctuations in the pure lipid bilayer. We have shown that the intensity of $\xi_P(T)$ is mainly determined by the difference between the hydrophobic protein length, d_P, and the mean lipid membrane hydrophobic thickness at the transition point. Moreover, we have found that the larger the protein circumference is, the larger is $\xi_P(T)$, cf. Fig. 6, i.e., the greater is the influence of the protein on its lipid environment.

In many cases the influence of the protein is felt by the lipids far beyond the first one or two lipid layers. In this respect our results for $\xi_P(T)$ near the transition are quantitatively different from the previous theoretical estimate, $\xi_P \sim 15$ Å (Jähnig, 1981*a* and *b*). This estimate was based on a mean-field calculation which is known to strongly suppress the fluctuations close to phase transitions. Furthermore, Jähnig's estimate assumes that $\xi_P(T) \simeq \xi_L(T)$ which we know from the present work may not be fulfilled. Two questions now arise: (*a*) what are the effects of the long coherence length on the overall state of the membrane, and (*b*) how can $\xi_P(T)$ be determined experimentally? Firstly, the long coherence length implies an enhancement of the lipid-mediated protein–protein interactions. When there is an overlap of the decay from neighboring proteins, it will induce an indirect attraction between the proteins (Marčelja, 1976; Mouritsen and Bloom, 1984). This may eventually lead to protein aggregation and lateral phase separation which, however, we do not consider here where the proteins are immobile and only present in a very low concentration. We are going to study the phase separation problem in a separate publication (Sperotto, M. M., and O. G. Mouritsen, 1991). Secondly, we address the question of an experimental measurement of $\xi_P(T)$. So far no technique has been devised which allows $\xi_P(T)$ to be determined directly. However, a number of spectroscopic techniques, including e.g., electron and nuclear magnetic resonance (Bloom and Smith, 1985; Devaux, 1983), fluorescence depolarization (Rehorek et al., 1985), and Raman scattering (Mendelsohn et al., 1981), have been used to give an indirect assessment of $\xi_P(T)$. The

interpretation of the spectroscopic measures are not without problems, however, because they each refer to their own time scale and they basically measure the properties of individual lipids or probes. They therefore often give conflicting results which have led to the long-standing argument about the possible presence of a so-called lipid annulus around the protein. Our calculation of an order parameter profile refers to a static thermodynamically averaged property with no information about the lipid diffusion characteristics. Moreover, the calculation leads to a decay (that is a gradual variation of the perturbation) whereas most interpretations are made on the basis of an annulus, i.e., a fairly sharply bounded region over which the influence of the protein can be felt by the lipid molecules within the time scale of the experiment. Most experiments have been performed on membrane preparations in the fluid phase for proteins which are close to being hydrophobically matched to the membrane (Bloom and Smith, 1985). Within our model this should imply that $\xi_P(T)$ is very small, in agreement with other recent model calculations on more refined models (Scott, 1986; Edholm and Johansson, 1987). To consider a specific example involving hydrophobic mismatch, we refer to the fluorescence anisotropy work by Rehorek et al. (1985) who studied bacteriorhodopsin in dimyristoyl phosphatidylcholine bilayers. Above the transitions these authors found that the perturbation (which is on the time scale of about 10^{-12} s) extends to at least 45 Å which they found disqualifies the earlier theoretical maximum of 15 Å (Jähnig, 1981*a* and *b*). However, the present model calculations have shown that $\xi_P(T)$ may in fact be much larger and not inconsistent with the reported large experimental value.

Our finding of an enhanced range of influence on the lipid environment of a membrane protein may have some bearing of the theoretical interpretation of selfdiffusion of lipids and proteins in membranes. It has been found in a hard-core model calculation (Saxton, 1989) and by an integral-equation theory with a soft potential (Abney et al., 1989) that the theoretical estimates of the diffusion constant is higher than that experimentally observed. Our finding of an unanticipated long decay length from a detailed and numerically accurate model calculation with a realistic potential implies that the influence of the proteins are felt longer out in the membrane which in turn would lead to a lower diffusion constant, in closer agreement with experiments.

In the present work we focused on a model for DPPC bilayers. Obviously our results are qualitatively applicable to other lipid bilayers as well. In fact a possible and interesting way of varying the mismatch is to study the same protein in bilayers of lipids with different acyl-chain lengths as was done for bacteriorhodopsin (Lewis and Engelman, 1983*b*) and photosynthetic reaction center proteins (Riegler and Möhwald, 1986; Peschke et al., 1987). The possibility also exists of directly manipulating $\xi_L(T)$ and hence indirectly $\xi_P(T)$ because it is known that for e.g., saturated phosphatidylcholines $\xi_L(T)$ increases as the acyl-chain length is decreased (Ipsen et al., 1990*b*). Finally, the mismatch may be varied by varying the protein length directly in model membranes incorporated with synthetic amphiphilic polypeptides (Huschilt et al., 1985).

The final conclusion of the results presented in this work is that, due to the strongly fluctuating nature of the main transition in lipid membranes, the perturbation of embedded integral proteins may be of a significant range and extend up to a large number of lipid layers, depending on the temperature and the degree of mismatch between the lipid and protein hydrophobic thicknesses.

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