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Theory for the bending rigidity of protein-coated lipid membranes

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Abstract

We study the bending rigidity of protein-coated lipid bilayer membranes by a continuum Landau theory. For up–down symmetric membrane proteins, the bending rigidity (κ) of the membrane is found to increase linearly with protein fraction (ϕ) and coupling strength (λ) due to the coupling between membrane curvature and local protein fraction. We estimate the coupling strength as $\lambda \propto \kappa a_p^2/a_l^2$, where a_p and a_l are the lateral sizes of proteins and lipids, respectively. Estimated values of λ/κ are given for various combinations of membrane proteins and lipids. We show that the presence of membrane proteins leads to thinning of the effective membrane thickness and reduces the mean layer spacing of a stack of bilayers.

Keywords: Bending rigidity; Lipid membranes; Landau theory; Thermal fluctuation; Interlamellar spacing

1. Introduction

Living cells are enclosed by a plasma membrane which distinguishes the cells' contents from the environment. The plasma membrane consists of various lipids and proteins – the relative amount of proteins to lipids is usually about 1 : 50. Lipid molecules in water can self-assemble into a bilayer membrane about 40 Å thick, while protein molecules are usually embedded in the lipid bilayer to shield nonpolar side groups of their amino acid residues from contact with the surrounding water. The shape of the plasma membrane is subject to a local deformation in exo- or endocytosis or a global deformation during mytosis. These structural deformations of membranes are determined by mechanical properties of the membrane, such as bending rigidity.

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Therefore, the study of physical properties of both protein-coated and protein-absent membranes is of physical and biological importance.

Recently, lamellar structures have received considerable experimental attention. Many model systems, such as pure or mixed lipid bilayers, have been prepared and well studied by various experimental methods [1–6]. On the other hand, theories based on curvature elasticity and statistical mechanics have satisfactorily explained many physical properties of these model systems. Early theory [7] predicts a very small bending rigidity of lipid bilayers, $\kappa \approx 10^{-19}$ J (which is consistent with experimental values of egg lecithin and other biological model membranes [8]), so that thermal fluctuation of the membrane is important at the lengthscale of microns. The amplitude of thermal fluctuation is mostly controlled by bending rigidity and lateral tension. Theoretical work by Helfrich [9] as well as by Peliti and Leibler [10] has shown that thermal fluctuation is not only controlled by bending rigidity, but also reduces the effective bending rigidity of a macroscopic membrane with respect to its bare value at the microscopic scale.

Many of the previous theoretical studies focus on the physical properties of pure lipid membranes. Recently, there are increasing interests in studying physical properties of mixed systems [11–19]. The additional degrees of freedom due to the complexity of mixed systems lead to many interesting behaviors. For example, the coupling between local lipid composition and Gaussian curvature of the membrane can enhance topological changes of two-component lipid vesicles [15]. Research results in this area have provided a better understanding of many important biological processes such as exo- and endocytosis. Another interesting mixed system is protein-coated lipid membranes which also attract considerable theoretical attention [13,14,16–21]. Those membrane proteins not only provide extra biological functions for the membrane, but also change the physical properties of the membrane. Nevertheless, much of such systems is still unclear. For example, recent experiments show that membrane proteins tend to reduce both the effective membrane thickness [1,2] and the mean layer spacing [3] of a stack of bilayers. These effects might be relevant for the myelin sheaths to maintain their compact architecture [22]. Further study in this area not only can improve our understanding on biological membranes, but also have potential pharmaceutical applications.

In this paper, we study the bending rigidity of protein-coated lipid bilayer membranes by a phenomenological Landau model that includes a coupling between local protein fraction and membrane curvature square. This coupling can be understood in a simple way resulting from the molecular geometry of proteins to the curve surface where they stay, as shown in Fig. 1. The geometry of protein molecules in Fig. 1(b) is simplified as a cylinder embedded in the lipid matrix since the polypeptide chain usually forms an α helix to maximize the hydrogen bonding between peptides as it crosses the bilayer. In general, transmembrane proteins could have more than one α helix embedded in the bilayer. Since the surface area of proteins is much larger than that of lipids, it is more difficult to bend a protein-coated lipid membrane [Fig. 1(b)] than to bend a pure lipid membrane [Fig. 1(a)]. The coupling strength is roughly proportional to the ratio of proteins' area to lipids' area. For small values of protein fraction, we predict that the bending rigidity of the membrane increases linearly with protein

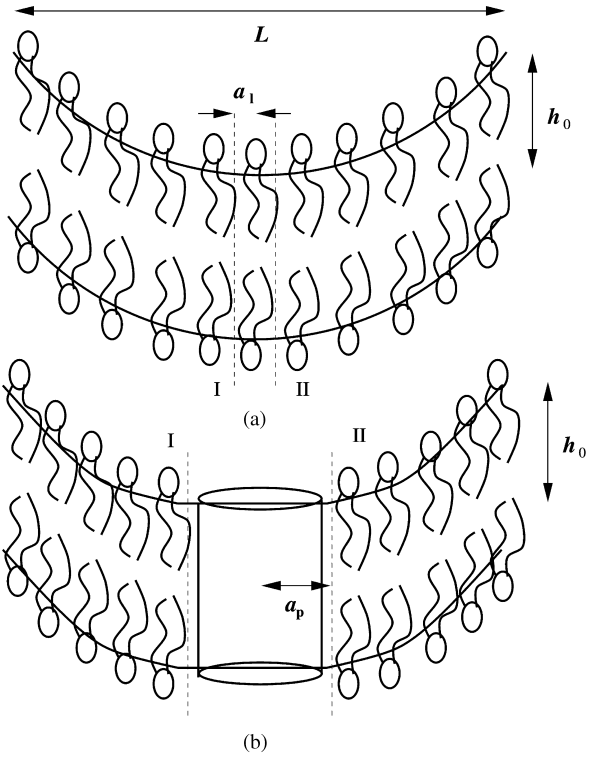

Fig. 1. Geometry of a protein-absent lipid bilayer membrane (a) and a protein-coated lipid bilayer membrane (b) under a bending moment (upward). Since the surface area of proteins is much larger than that of lipids, it is easier to bend (a) than to bend (b).

fraction ϕ and coupling strength λ due to this coupling. Thus protein-coated membranes provide a good model system to study the transition of flexible membranes to semi-flexible membranes by slowly increasing protein fraction. We show that both the effective membrane thickness and the mean layer spacing of a stack of lamellae decrease with the protein fraction. Such a squeezing effect might be important in maintaining the compact architecture of the native myelin sheaths [22].

2. The model

For a protein-coated lipid membrane with vanishing surface tension, the Gibbs free energy in our model can be expressed as

$$G[\Omega] = F[\Omega] - \mu \int_{\Omega} \mathrm{d}A\phi(\mathbf{r}) \,, \tag{1}$$

where Ω represents the whole surface of the membrane, $F[\Omega]$ is the free energy of the membrane, and $\phi(\mathbf{r})$ is the local protein fraction. Here μ is a Lagrange multiplier which can be adjusted to achieve the desired fraction of proteins. For a nearly flat

membrane, the curvature energy of the system can be expanded in terms of mean curvature and Gaussian curvature [7]. For small values of local protein fraction ϕ, the free energy of the membrane including mixing entropy and interactions among lipids and proteins can be expanded in a power series of ϕ. Therefore, to the lowest orders, we can express $F[\Omega]$ as

$$F[\Omega] = \frac{\kappa}{2}\int_{\Omega} \mathrm{d}A[2H(\mathbf{r})]^2 + \lambda\int_{\Omega} \mathrm{d}A\, H^2(\mathbf{r})\phi(\mathbf{r}) + \frac{t}{2}\int_{\Omega} \mathrm{d}A\, \phi^2(\mathbf{r})\,, \tag{2}$$

where κ is the bending rigidity, $H(\mathbf{r})$ is the mean curvature of the membrane, λ is the coupling constant between mean curvature square and local protein fraction, and t is the reduced temperature. In this paper, we limit our discussion to nearly uniform membranes, and an integrated Gaussian curvature energy has been ignored in Eq. (2) since it is a constant for a fixed topology. The last two terms come from the expansion of the ϕ-dependent part of the free energy. Here, we have assumed no phase separation of lipids and proteins (i.e., $t > 0$). Since, the shape of many transmembrane proteins which undergo thermal rotation is roughly cylindrical, by symmetry the coupling between ϕ and H should be small enough to be ignored. In general, if the proteins are not up–down symmetric, there is a characteristic softening of the membrane due to the coupling between protein fraction and mean curvature of the membrane [11,4]. A more general form of the model free energy of membranes with internal variables can be found in the reference of Leibler and Andelman [11]. Since, the effect of ϕH coupling has been thoroughly discussed in previous papers and since the effect of ϕH^2 coupling is always ignored, we will focus on the stiffening effect of the latter term for simplicity.

Minimizing the Gibbs free energy in Eq. (1) with respect to ϕ and keeping only the lowest order terms in curvature, we have

$$G[\Omega] = \frac{\kappa_p}{2}\int_{\Omega} \mathrm{d}A[2H(\mathbf{r})]^2 - \frac{\mu^2}{2t}A_{\mathrm{all}} \tag{3}$$

and the optimized value of ϕ is given by

$$\phi(\mathbf{r}) = \frac{1}{t}[\mu - \lambda H^2(\mathbf{r})]\,. \tag{4}$$

Here $\kappa_p = \kappa + 2\lambda\mu/t$ is the effective bending rigidity of protein-coated membranes and A_{all} is the total surface area of the membrane. The second term in Eq. (3) is a constant which is independent of membrane curvature. Eq. (4) shows that, for $\lambda > 0$, the protein fraction decreases with $H^2(\mathbf{r})$ due to the shape of proteins which disfavors the regions with large curvature. Since the membrane is nearly flat, the average protein fraction (ϕ) is roughly μ/t. The effective bending rigidity of protein-coated lipid membranes increases linearly with protein fraction and the coupling strength λ.

To estimate the value of the coupling constant λ, we calculate the extra bending energy associated with a rigid flat disk that is embedded in a 1-D sinusoidally bent membrane, as shown in Fig. 2 (half-wavelength). The membrane in Fig. 2 is divided into three regions. For regions I and III, the membrane shape is described by

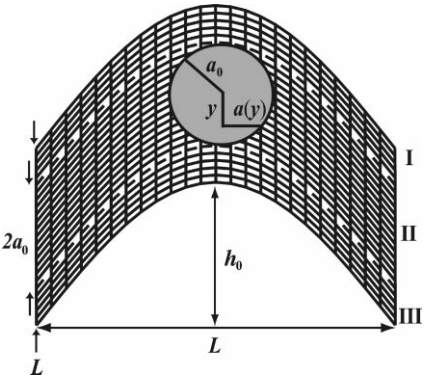

Fig. 2. A schematic representation of a protein-coated lipid membrane which is bent into a sinusoidal shape.

$h_1(x, y) = h_0 \sin(qx)$, where $q = \pi/L$. For region II, the membrane shape is described by

$$h_2(x, y) = \begin{cases} h_0 \sin[q(y)x] & 0 \leqslant x \leqslant L/2 - a(y)\,, \\ h_0 & L/2 - a(y) \leqslant x \leqslant L/2 + a(y)\,, \\ h_0 \sin[q(y)(L - x)] & L/2 + a(y) \leqslant x \leqslant L \end{cases} \tag{5}$$

and

$$q(y) = \frac{\pi}{L - 2a(y)}\,, \tag{6}$$

where $a(y) = \sqrt{a^2 - y^2}$. The curvature energy of such a membrane can be expressed as

$$\begin{aligned} E_b &= \frac{\kappa}{2} \int_{-L/2}^{L/2} \int_0^L [\nabla^2 h(x, y)]^2 \, \mathrm{d}x \, \mathrm{d}y \\ &= \kappa \int_a^{L/2} \int_0^L [\nabla^2 h_1(x, y)]^2 \, \mathrm{d}x \, \mathrm{d}y + 2\kappa \int_0^a \int_0^{L/2 - a(y)} [\nabla^2 h_2(x, y)]^2 \, \mathrm{d}x \, \mathrm{d}y \\ &= \frac{\kappa \pi^4 h_0^2}{4L^2} \left(1 + \frac{3\phi a_p^2}{a_l^2}\right)\,, \end{aligned} \tag{7}$$

where L is the projected length of the membrane and h_0 is the bending amplitude. Compared to a pure lipid membrane with the same projected length and amplitude [Fig. 1(a)], the extra bending energy is $(3\pi^4 h_0^2 \kappa\phi/4L^2)[(a_p^2/a_l^2) - 1]$. Equivalently, we have

$$\lambda \simeq \frac{3}{2}\kappa \left(\frac{a_p^2}{a_l^2} - 1\right) \propto \kappa \frac{a_p^2}{a_l^2}\,, \tag{8}$$

for $a_p \gg a_l$. A list of estimated values of λ for various protein-coated membranes is given in Table 1 [23–27]. Among those proteins, gramicidin A is a β helix dimer [24], the subunit of a porin trimer is a β sheet [25], acetylcholine receptor is an α helix pentamer [26] , and connexon is an α helix hexamer [27]. Here we assume that thermal

Table 1
The ratio of coupling strength to bending rigidity for various combinations of membrane proteins and lipids

Proteins λ/κ Lipids [23]	Gramicidin A [24] (3 nm^2)	Porin trimer [25] (43 nm^2)	Acetylcholine receptor [26] (56 nm^2)	Connexon [27] (98 nm^2)
DPPC (0.45 nm^2)	8.5	141.8	185.2	325.2
DOPC (0.72 nm^2)	4.8	88.1	115.2	202.7
Egg PC (0.62 nm^2)	5.8	102.5	134.0	235.6
Sphingomyelin (0.42 nm^2)	9.2	152.1	198.5	348.5

energy is too small to distort the proteins' structure. For a connexon-coated lipid membrane with a fraction $\phi = 0.2\%$, the bending rigidity of the membrane is doubled. We note that our results are consistent with those of a two-component theory which gives $\lambda = (\kappa' - \kappa)/2$, where κ' is the bending rigidity of a pure protein membrane. However, κ' is ill defined and cannot be measured experimentally nor calculated theoretically. Thus our model provides a much better description of protein-coated membranes.

3. Results and discussions

In the Monge representation, the mean curvature H can be approximated by $\frac{1}{2}\nabla^2 h(x, y)$, where $h(x, y)$ is the height fluctuation of the membrane relative to its average position, as shown in Fig. 3. By the equipartition theorem, the ensemble average of square Fourier component of height fluctuation is given by $\langle |h_{\mathbf{q}}|^2 \rangle_{\text{en}} = k_B T/(\kappa_p q^4)$, where k_B is the Boltzmann's constant and T is the temperature. The mean-square height fluctuation is thus inversely proportional to protein fraction

$$\langle h^2(\mathbf{r}) \rangle_{\text{en}} = \frac{1}{A_{\text{proj}}} \sum_{\mathbf{q}} \langle |h_{\mathbf{q}}|^2 \rangle \sim \frac{k_B T A_{\text{proj}}}{\kappa + 2\lambda\mu/t} , \tag{9}$$

where A_{proj} is the projected surface area of the membrane on the x–y plane. For small values of protein fraction, the height fluctuation of the membrane decreases linearly with protein fraction.

For the membrane, the normal–normal correlation function ($g_n(\mathbf{r}) \equiv \langle [\mathbf{n}(\mathbf{r}) - \mathbf{n}(0)]^2 \rangle_{\text{en}}$) can be expressed as $k_B T \log(r/a_l)/[4\pi(\kappa + 2\lambda\mu/t)]$, where $\mathbf{n}$ is the normal unit vector. Thus g_n is inversely proportional to protein fraction, which indicates that the correlation between orientation vectors of proteins (or α helices) increases with protein fraction. The normal vectors decorrelate at a distance r larger than the persistence length where $g_n(r) \sim 1$. The persistence length ξ of the protein-coated membrane can then be defined by

$$\xi = a_l \exp\left[\frac{4\pi(\kappa + 2\lambda\mu/t)}{k_B T}\right] . \tag{10}$$

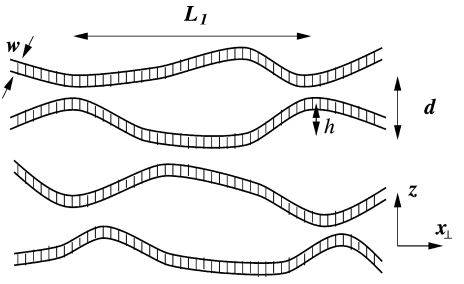

Fig. 3. Fluctuating membranes in a stack of lipid bilayers of bilayer thickness w and mean separation distance between neighboring membranes d. L_1 is mean free length between successive collisions of neighboring membranes in the $x_\perp$ direction, $h(x, y)$ is the height fluctuation of each membrane at position (x, y).

Eq. (10) shows that the persistence length ξ increases exponentially with protein fraction. Thus protein-coated lipid membranes also provide a two-dimensional analogue of a polymer with continuously variable stiffness. By slowly increasing protein fraction, one can study the transition of flexible membranes to semi-flexible membranes.

Moreover, the total surface area $A_{\rm all}$ is related to its projection $A_{\rm proj}$ by $A_{\rm all} = \langle 1/n_z\rangle_{\rm en} A_{\rm proj}$, where n_z is the z-component of the normal vector $\mathbf{n}(\mathbf{r})$. For a weakly rippled membrane, the crumpling ratio is roughly $\langle 1/n_z\rangle_{\rm en} = 1 - k_B T \ln(a_l q_{\rm min})/4\pi\kappa_p$, where $q_{\rm min}$ is the lower cutoff wave number. For a single bilayer, $q_{\rm min}^{-1}$ is about the size of the system, $\sqrt{A_{\rm proj}}$. For a stack of bilayers with layer spacing d as shown in Fig. 3, $q_{\rm min}^{-1}$ is the mean free length L_1 between successive collisions of neighboring membranes. From Eq. (9), the mean free length is approximately given by $L_1 \sim d\sqrt{\kappa_p/k_B T}$. Since the membrane is crumpled due to thermal fluctuation, the effective membrane thickness $w_{\rm eff}$ is greater than the real bilayer thickness w by a factor of $\langle 1/n_z\rangle_{\rm en}$. Therefore, in a stack of bilayers, we have

$$w_{\rm eff} = w\left[1 - \frac{k_B T}{4\pi\kappa_p}\ln\left(\frac{a_l}{d}\sqrt{\frac{k_B T}{\kappa_p}}\right)\right]$$
$$\simeq w\left[1 + \frac{k_B T}{4\pi\kappa}\ln\left(\frac{d}{a_l}\sqrt{\frac{\kappa}{k_B T}}\right)\right] - \frac{w\lambda k_B T}{4\pi\kappa^2}\left[2\ln\left(\frac{d}{a_l}\sqrt{\frac{\kappa}{k_B T}}\right) - 1\right]\phi. \quad (11)$$

Eq. (11) shows that the effective membrane thickness decreases linearly with protein fraction. For a stack of lipid membranes at room temperature, the slope is of the order 10 Å. The correction to the slope due to the change of interlamellar space d in the presence of proteins is negligible (of the order 0.1 Å). Recent experiments on peptide/lipid systems also show similar behavior in membrane thickness as peptide fraction increases. This effect has been observed in both alamethicin/DPPC (diphytanoyl phosphatidylcholine) and Magainin-2/[POPC (palmitoyloleoyl phosphatidylcholine) + POPS (palmitoyl oleoyl phosphatidylserine)] mixture systems [1,2]. The experimental observations of membrane thinning occur only at wet regions where thermal fluctuation is important, but not at dry regions where thermal fluctuation can be ignored [2].

Clearly, suppression of thermal fluctuation due to embedded proteins as discussed in this paper plays an important role in membrane thinning. This is not addressed in the original model in the reference of Ludtke et al. [1]. At higher values of peptide fraction, the experimental data show a slight increase in membrane thickness with peptide fraction after reaching a minimum. This might indicate that the effect of thermal fluctuation is highly suppressed and the local stretching of bilayer thickness due to embedded peptides, as suggested in the reference of Dan et al. [11], becomes important at high peptide fractions.

We further discuss the effect of inserted membrane proteins on the mean layer spacing of a stack of bilayers. For a stack of bilayers, the total interaction energy per unit area of two parallel layers can be written as [28,29]

$$V(d) = V_W(d) + V_s(d) + V_H(d) + V_E(d)\,, \tag{12}$$

where d is the mean layer spacing. The first term in Eq. (12), $V_W(d)$, is the van der Waals interaction energy resulting from different polarizabilities of the lipid and the water molecules and can be written as [30]

$$V_W(d) \approx -\frac{W}{12\pi}\left[\frac{1}{d^2} - \frac{2}{(d+w)^2} + \frac{1}{(d+2w)^2}\right], \tag{13}$$

where the Hamaker constant W is of the order 10^{-21} J. The second term, $V_s(d)$, is an effective steric interaction resulting from thermal undulations of the membranes as proposed by Helfrich and can be expressed as [31]

$$V_s(d) \approx \frac{3(k_BT)^2}{\pi^2(\kappa + 2\lambda\phi)(d-w)^2}\,. \tag{14}$$

The hydration energy, $V_H(d)$, has the empirical form [30]

$$V_H(d) \approx A_H \exp(-d/\lambda_H)\,, \tag{15}$$

with typical values $A_H \simeq 0.2$ J/m^2 and $\lambda_H \simeq 0.3$ nm. The unscreened long-range electrostatic energy, $V_E(d)$, for charged membranes is given by [32]

$$V_E(d) \approx E/d \tag{16}$$

for sufficiently large d and the coefficient E increases with the surface charge density. The mean layer spacing can be obtained by minimizing $V(d)$ in Eq. (12). Fig. 4 shows the mean layer spacing as a function protein fraction for $E = 0$, 10^{-24}, and 2×10^{-24} J/nm. Here we have used $W = 5\times10^{-21}$ J, $w = 4$ nm, $k_BT = 5\times10^{-21}$ J, $A_H = 2\times10^{-19}$ J/nm^2, $\lambda_H = 0.3$ nm, $\kappa = 10^{-18}$ J, and $\lambda/\kappa = 102.5$. As shown in Fig. 4, the mean layer spacing decreases exponentially with protein fraction for both charged and uncharged membranes. Those three sets of data can be well fitted by the formula

$$d = d_0(E) + b(E)\exp[-c(E)\phi]\,, \tag{17}$$

with $d_0 \simeq 5.88, 5.94, 6$ nm, $b \simeq 1.64, 1.74, 1.87$ nm, and $c \simeq 316, 334, 351$ for $E = 0$, 10^{-24}, and 2×10^{-24} J/nm, respectively. For uncharged membranes ($E = 0$), d_0 is the layer spacing set by balancing the van der Waals interaction and the hydration

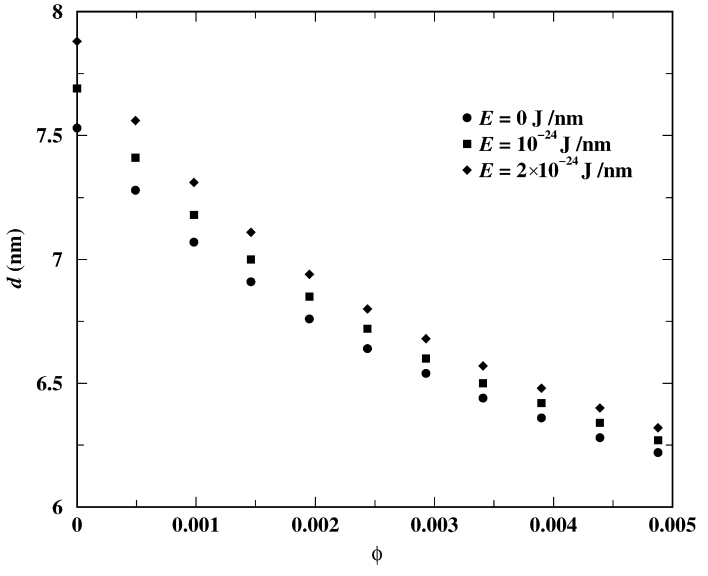

Fig. 4. The mean layer spacing of a stack of lamellae as a function of protein fraction at $E = 0, 10^{-24}$, and 2×10^{-24} J/nm.

energy, while b is the extra layer spacing due to Helfrich repulsion and c is proportional to the coupling constant λ. For charged membranes, all three coefficients (d_0, b, and c) increase with E. We conclude that adding membrane proteins on a stack of membranes will lead to the squeezing of the interlamellar space and the squeezing effect is more significant for charged membranes than for uncharged membranes. Recent experiments on a ternary system made of a nonionic surfactant, dodecane, and water, in the absence and upon insertion of a transmembrane protein also show similar squeezing behavior [3].[1] Such a squeezing effect might be important in maintaining the compact architecture of the nerve myelin sheaths [22].

4. Conclusions

To conclude, we have analyzed a continuum Landau mean-field theory for the effect of the coupling between local protein fraction and membrane curvature. The coupling strength λ is roughly proportional to a_p^2/a_l^2, where a_p and a_l are the lateral sizes of transmembrane proteins and lipids, respectively. As a result of this coupling, the bending rigidity of the membrane increases linearly with protein fraction and thermal fluctuations of such systems are greatly suppressed. The persistence length of the membrane increases exponentially with the protein fraction, and height fluctuation is inversely proportional to protein fraction. These effects can be observed by optical

[1] We note that the Bragg spacing in Fig. 3 of Ref. [3] can also be well fitted by Eq. (17).

microscopy in unilamellar giant vesicles. The orientation of α helices is predicted to be more correlated, or more oriented in the z-direction, at higher values of protein fraction. This effect can be tested by circular dichroism spectrum. For a stack of lamellae, both the effective membrane thickness and the interlamellar space decrease with protein fraction. The effective thickness of the membrane is shown to decrease linearly with protein fraction with a slope of the order of 10 Å. Also, the squeezing effect of interlamellar space is enhanced for charged membranes. These effects can be measured by neutron or X-ray scattering in a stack of bilayers.

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