Statistical Mechanics of Membrane Protein Conformation: A Homopolymer Model

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The conformation and the phase diagram of a membrane protein are investigated via grand canonical ensemble approach using a homopolymer model. We discuss the nature and pathways of α-helix integration into the membrane that result depending upon membrane permeability and polymer adsorptivity. For a membrane with the permeability larger than a critical value, the integration becomes the second order transition that occurs at the same temperature as that of the adsorption transition. For a nonadsorbing membrane, the integration is of the first order due to the aggregation of α helices. [S0031-9007(98)06428-X]

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Membrane proteins regulate signal transduction and ionic or macromolecular transport across biomembranes. Because of their unique roles in biological functions, their conformations and the folding pathways are important issues in biological physics no less than the corresponding aspects of globular protein folding. Folding of an integral membrane protein carries different characteristics compared to globular protein folding due to the hydrophobic environment of phospholipid membrane [1]. In a watery solvent, the outer surface of a globular protein is usually covered with hydrophilic segments, while the inner space is filled with hydrophobic segments to minimize the protein-solvent interaction energy. In contrast, a membrane protein has hydrophobic outer regions inside the membrane to minimize the protein-lipid interaction energy [2].

The three dimensional structures of a great variety of globular proteins are experimentally known. Yet the structures of only a few membrane proteins are resolved, because the proteins embedded in hydrophobic membranes are difficult to handle experimentally [2]. Since the structural determination of Bacteriorhodopsin [3], the idea has been widely accepted that the membrane proteins are predominantly made up of α helices induced by hydrogen bonding [4]. Unlike the globular proteins, the membrane proteins can adopt only a few basic structures such as α helix, allowing more tractable theoretical approaches for membrane proteins.

While a number of theoretical studies have been done separately on globular protein folding [5] and polymer adsorption on membranes [6], there are few efforts devoted to the folding of membrane proteins involving the surface adsorption [7]. In this Letter, we address this problem using the statistical mechanics via grand canonical ensemble approach. To extract the salient features of the conformations and their transitions from the intractable complexity characteristic of the real proteins, we introduce a simple but tenable model: a long homopolymer which undertakes a random walk outside the membrane regarded as planar and can interact with it via contact binding on its surface and penetration into its interior (Fig. 1), as will be detailed. Motivated by the fact that hydrogen bonding is very stable in the hydrophobic environments [8], we assume that α-helix structure is formed if and only if the segments are placed within the membrane. Here we neglect other secondary structures such as β sheets for simplicity. Another important observation to incorporate is that the α helices preferentially aggregate to form a thermodynamically stable structure, called α-helix oligomer, which is dominant over the dispersed α helices [9].

In our model, an α-helix column has a fixed number of hydrogen bonds n (implicitly representative of membrane thickness), with the statistical weight $W_H \sim \sigma_h^n \exp[-\beta(n\epsilon_h + \epsilon_a)]$, where $\beta = 1/k_B T$, $\sigma_h \equiv \exp(\Delta s_M/k_B) < 1$. The $\epsilon_h < 0$ and $\Delta s_M < 0$ are the energy and the entropy change associated with hydrogen bonding, and $\epsilon_a < 0$ is the aggregation energy per helix column. On the membrane surface, polymer segments are allowed to be adsorbed with the statistical weight for k segments given by $W_S \sim \sigma^k \exp(-\beta k \epsilon_s)$, where $\epsilon_s < 0$ is the segmental attraction energy, and

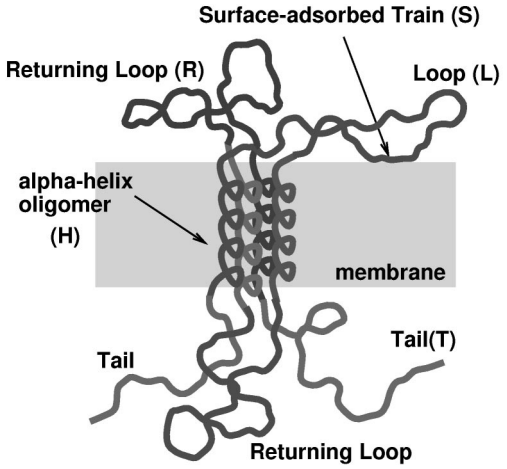

FIG. 1. Schematic figure of a membrane protein. Five different domains are indicated.

$\sigma \equiv \exp(\Delta s/k_B) < 1$ with Δs the segmental entropy change by adsorption. Because of chain connectivity, the domains other than the membrane-bound oligomer and surface-adsorbed trains consist of two end tails, loops starting or ending at surface trains, and loops that connect two helix columns (return to the starting point as the radius of an α helix will be neglected in this work) (Fig. 1). A tail of k segments has the statistical weight of random walk in half-space which departs from the membrane surface and ends up anywhere in the half space [10], $W_T \sim q^k/k^{1/2}$, where q is the segmental partition function. Loops have the statistical weights of random walks ending up anywhere on the surface, $W_L \sim q^k/k^{3/2}$, and of those returning to the starting point, $W_R \sim q^k/k^{5/2}$ [11].

We construct grand partition functions of five different kinds of domains as follows. The bound chains as a helix column and a surface-adsorbed train have, respectively, the partition functions

$$Q_H(z_M) = I_h z_M^n \sigma_h^n \exp[-\beta(n\epsilon_h + \epsilon_a)], \qquad (1)$$

$$\begin{aligned} Q_S(z) &= I_s \sum_{k=1}^{\infty} z^k \sigma^k \exp(-\beta k \epsilon_s) \\ &= I_s z\sigma \exp(-\beta\epsilon_s)/[1 - z\sigma \exp(-\beta\epsilon_s)], \end{aligned} \qquad (2)$$

where I_h, I_s are the nucleation or initiation parameters for an α helix and a surface-adsorbed train, respectively [12]. The z and z_M are the segmental fugacities outside and inside the membrane, respectively, which defines the chemical potential difference $\Delta\mu \equiv \mu_M - \mu = \beta^{-1}\log(z_M/z)$, a measure of membrane permeability determined by environmental effects such as membrane hydrophobicity. With $q = 1$, neglecting the irrelevant bulk contributions, the grand partition functions of a tail, a loop, and a returning loop are given by

$$Q_T(z) = A_T \sum_{k=1}^{\infty} z^k/k^{1/2} \equiv A_T g_{1/2}(z), \qquad (3)$$

$$Q_L(z) = A_L \sum_{k=1}^{\infty} z^k/k^{3/2} \equiv A_L g_{3/2}(z), \qquad (4)$$

$$Q_R(z) = A_R \sum_{k=1}^{\infty} z^k/k^{5/2} \equiv A_R g_{5/2}(z), \qquad (5)$$

where A_T, A_L, A_R are constants of the order of unity, and $g_m(z)$ is the polylogarithmic function of order m.

The total grand partition function of the membrane-bound polymer can now be calculated considering every possible conformation made of all the domains. To this end, consider the transfer matrices defined as

$$\mathbf{X} = \begin{bmatrix} Q_S & 0 \\ 0 & Q_H \end{bmatrix}, \qquad \mathbf{Y} = \begin{bmatrix} Q_L & Q_L \\ Q_L & Q_R \end{bmatrix},$$

which represent, respectively, the two membrane-bound domains (adsorbed-train and α-helix) and two types of loops (L and R) joining them. Introducing the matrix $\mathbf{B} = \mathbf{X}\sum_{p=0}^{\infty}[\mathbf{YX}]^p$, whose elements properly incorporate the interconnected arrays of all the domains between two tails (for example, the element B_{12} represents all the connected arrays starting from train and ending with helix), we finally get the total partition function $\mathcal{Q}$ and its diagrammatic representation (Fig. 2),

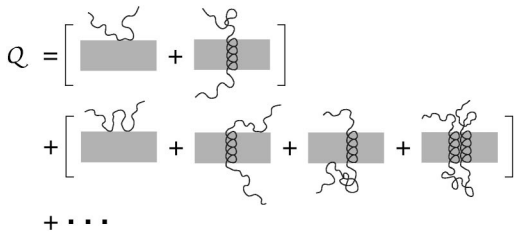

FIG. 2. Diagrammatic representation of the partition function.

$$\begin{aligned} \mathcal{Q} &= Q_T^2(B_{11} + B_{12} + B_{21} + B_{22}) \\ &= [Q_T^2(Q_S + Q_H)] \\ &\quad + [Q_T^2(Q_S^2 Q_L + Q_S Q_L Q_H + Q_H Q_L Q_S + Q_H^2 Q_R)] \\ &\quad + \text{terms with } p \geq 2. \end{aligned} \qquad (6)$$

Here we regarded the two ends of the polymer to be distinguishable. We will consider the thermodynamic limit, written as $\langle N\rangle \to \infty$, in order to define phase transitions that result. Then the partition function is reduced to

$$\mathcal{Q} \sim \begin{cases} Q_T^2, & T > T_c, \\ (1 - \lambda)^{-1}, & T < T_c, \end{cases} \qquad (7)$$

where λ is the largest eigenvalue of $\mathbf{YX}$, given by

$$\begin{aligned} \lambda &= \frac{1}{2}(Q_S Q_L + Q_H Q_R) \\ &\quad + \frac{1}{2}[(Q_S Q_L - Q_H Q_R)^2 - 4Q_S Q_H Q_L^2]^{1/2}, \end{aligned} \qquad (8)$$

and T_c is a critical temperature determined from $\lambda(z = 1, T = T_c) = 1$.

For $T > T_c$, the tail is the only allowed conformation, indicating that the polymer tends to be desorbed. For $T < T_c$, the surface trains and/or helices with loops and returning loops in between become dominant, which indicates the stability of the membrane-bound phase. The segmental fraction of each domain can be defined as $f_i \equiv \langle N_i\rangle/\langle N\rangle = \langle N\rangle^{-1}(\partial \log \mathcal{Q}/\partial \log Q_i)(\partial \log Q_i/\partial \log z)$, where $i = S, R, L$ represent three different types of domains considered (Fig. 1), and $f_H \equiv \langle N_H\rangle/\langle N\rangle = \langle N\rangle^{-1}(\partial \log \mathcal{Q}/\partial \log Q_H)(\partial \log Q_H/\partial \log z_M)$, for the helix domain. Depicted in Fig. 3 are the segmental fractions vs temperature, where all the energy parameters, scaled in units of $|\epsilon_s|$, are taken to be the same order of magnitude, and the entropies Δs and Δs_M are taken to be the order of unity, with $k_B = 1$ [13]. As shown in Fig. 3(a), the desorption-adsorption transition, which is of the second order as is known, takes place at $T = T_c$, where the order parameter, the surface-adsorbed segmental fraction (f_S),

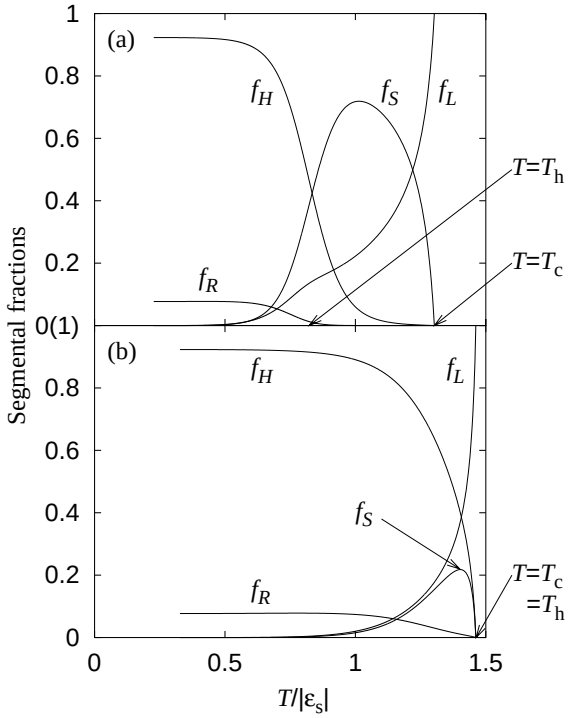

FIG. 3. Segmental fractions of a membrane-bound polymer vs temperature. The parameter values throughout this paper are selected as [13] $\sigma = \exp(-1)$, $\sigma_h = \exp(-2)$, $A_L = 1$, $A_R = 0.5$, $I_h = 0.01$, $I_s = 0.1$, $n = 12$, $\epsilon_a = -3$, where energies and temperature are in units of $|\epsilon_s|$. (a) $\epsilon_h^* \equiv \epsilon_h - \Delta\mu = -2$. Desorption-adsorption transition takes place at $T = T_c \simeq 1.30$ continuously, while the helix inclusion occurs at $T < T_h \simeq 0.82$. (b) $\epsilon_h^* = -3$ (more permeable membrane). Adsorption and helix inclusion occur simultaneously at $T = T_c = T_h \simeq 1.46$, but the helix structure becomes dominant over the adsorbed state at lower temperatures.

increases from zero continuously as T is lowered from T_c. Further lowering of the temperature drives the polymer integration in a form of α-helix aggregate at $T = T_h$, the helix inclusion temperature, which is defined by the local maximum of specific heat. The specific heat curve, shown in Fig. 4, clearly indicates that structural changes occur at both T_c and T_h. The specific heat diverges at $T = T_c$, and has a local maximum at $T = T_h$ indicating the helix formation that is a crossover [14].

As the value of $\epsilon_h^* \equiv \epsilon_h - \Delta\mu$, the energy of α-helix inclusion per segment, is lowered, or membrane permeability is increased, T_h approaches T_c, so that for the values of ϵ_h^* smaller than a critical value (about -2.6 using the parameters employed in Fig. 3), the helix inclusion is promoted to the second order transition with $T_h = T_c$. Figure 3(b) depicts the segmental fractions for this case ($\epsilon_h^* = -3$). It is shown that, in contrast to Fig. 3(a), helix formation dominates over adsorption below the common transition temperature. The phase diagram in Fig. 5 summarizes the foregoing discussions concerning the conformational phases and their transitions for wide range of ϵ_h^* and temperature.

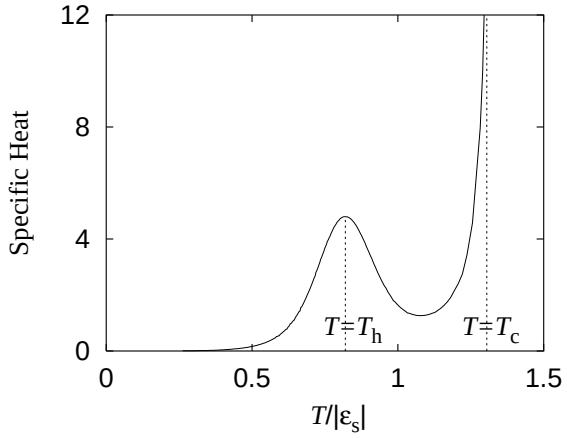

FIG. 4. Specific heat (in units of k_B) versus temperature. Parameters are the same as in Fig. 3(a).

The two temperatures appearing in our model, the desorption-adsorption temperature T_c and the helix inclusion temperature T_h, govern the pathways of our model-homopolymer integration into membranes. They are, respectively, similar to coil-to-globule transition temperature T_θ and folding transition temperature T_f in globular protein folding; like the globular phase, the adsorbed phase is indeed an intermediate state approaching the native folded structure [15]. Recently, Klimov and Thirumalai showed an evidence that globular protein folding time is scaled as $\tau \sim \exp[J|T_\theta - T_f|/T_\theta]$, where J is a model-dependent constant [16]. Even without considering the analogy, Fig. 5 suggests that a rapid α-helix integration can be attained for *permeable and adsorbing* membranes, with ϵ_h^* lower than a critical value where $T_c = T_h$. A detailed analysis of the free energy landscape and barrier crossing dynamics should confirm this highly plausible suggestion.

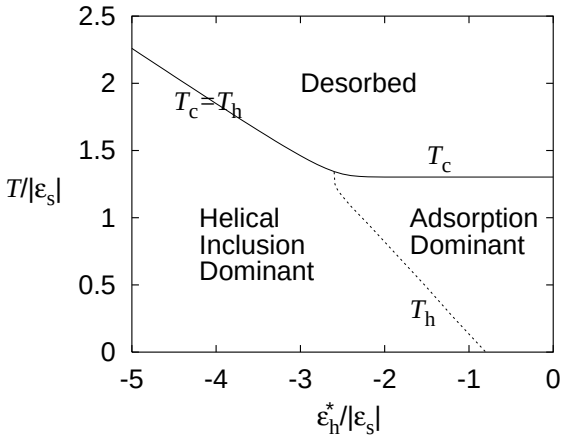

FIG. 5. Phase diagram of a membrane-bound polymer. The solid line indicates the second-order transition, while the dotted line indicates the crossover between adsorption and α-helix inclusion. Parameters are the same as in Fig. 3.

We now consider the situation of *nonadsorbing membrane* where Q_S, the partition function of surface-adsorbed domain vanishes (for example, I_s, the adsorption initiation parameter is zero). We then find the eigenvalue $\lambda = Q_H Q_R$, signifying that the partition function incorporates the conformations generated from recurrence of a helix and a returning loop in series. In this case, the polymer inclusion forming the α-helix oligomer is found to be the first order transition. At the transition temperature T_h, which is again determined by $\lambda(z = 1, T_h) = 1$, the order parameter, the fraction of segments in the α-helix oligomer (f_H), changes discontinuously from zero to

$$\Delta f_H = n/[n + g_{3/2}(1)/g_{5/2}(1)], \tag{9}$$

which incurs the segmental latent heat of dissociating the membrane-bound oligomer, $l_H = |\epsilon_h^* + \epsilon_a/n|\Delta f_H$. The reason why the transition should be discontinuous in the absence of adsorption is argued as follows. The inclusion accompanies the aggregation of helices, which restricts the loops to be closed between helix columns. Presence of long returning loops is suppressed entropically, and, also energetically in favor of the aggregate, which tends to be of significant fraction at the transition from the desorbed phase. This is in sharp contrast to the two second order transitions that can be obtained from our theory, the desorption-adsorption transition without helix inclusion ($\lambda = Q_S Q_L$) and the inclusion transition in forms of dispersed helices without aggregation and adsorption ($\lambda = Q_H Q_L$ with $\epsilon_a = 0$), where the loops can be transformed continuously into the adsorbed segments and helix columns, respectively. Similar discontinuous transition was reported in the helix-coil transition of double strand DNA [11,17]. For the biological processes without appreciable changes in temperature and volume, certain mechanisms of latent heat involving enzymatic activity could be essential to facilitate the first order transition [11]. Furthermore, the conclusions of this and foregoing paragraphs, if properly extended to an asymmetric membrane where one side is adsorbing and the other is not [18], imply that the rapid protein integration can be promoted only through the adsorbing side. It would be important to confirm this possibility as well as our results for symmetric membranes by experiments and/or simulations.

In summary, we studied various membrane-protein conformations and different pathways to the native structure of an α-helix aggregate as a function of temperature and membrane permeability. Two significant conclusions obtained are the following: (1) The polymer inclusion pathway is determined by membrane permeability above a critical value of which the adsorption and the helix inclusion converge, and (2) the nature of inclusion transition is determined by the availability of the polymer adsorption on membrane surface, due to the chain connectivity constraint. Although the important sequential heterogeneity and finite length effect involving chain stiffness of real proteins are neglected, our model gives some nonspecific features of membrane protein conformation, in particular, the roles of membrane hydrophobicity and segmental interaction with the membrane surface.

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