Spontaneous Translocation of a Polymer across a Curved Membrane

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The translocation of a hydrophobic polymer across a *curved* bilayer membrane has been studied using Monte Carlo methods. It is found that for *curved* membranes the polymer crosses spontaneously and almost irreversibly from the side of lower curvature to the side of higher curvature. This phenomenon can be understood based upon the curvature-induced difference of lipid fluctuations between the two halves of the bilayer. The difference of fluctuations drives the polymer across the bilayer in order to maximize the conformational entropy of the polymer.

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Translocation of proteins across biological membranes is one of the mechanisms for protein import and export [1–4] into and from various cellular organelles and bacteria. Although much progress has been made in elucidating the biochemical basis of translocation of amphiphatic proteins into and across membranes, the physical scenario of this complex process is still not understood. In many cases the transport of proteins is a "spontaneous" process [4–7]. However, so far the spontaneous translocation process has been interpreted without taking into account the role of the dynamics of the lipids. It will be shown that the fluctuations of the lipids are of particular importance with respect to the question of the source for the necessary driving force completing the translocation from one to the other side of the membrane.

In the present paper we propose a simple physical mechanism which can promote spontaneous translocation. Based on results from Monte Carlo simulations we suggest that sites of membranes with high curvature are selective places where proteins may undergo spontaneous translocations across the membrane due to the difference of lipid-protein interaction in the two leaflets of the bilayer.

The translocation of proteins across a bilayer lipid membrane requires an input of energy. In some cases [4–7] this is provided essentially by the hydrophobicity of the protein and the concomitant processes of secondary and tertiary structure formation during or after its insertion into the membrane. If the polypeptide is a single-membrane-spanning molecule, then the spontaneous translocation process into a membrane consists of two coupled mechanisms [7], namely, the helix formation and the translocation from an aqueous phase into the hydrophobic bulk of a membrane. However, from a general point of view, the actual translocation event, which can be considered as an interdiffusion process, should take place independently of the conformational transition of the polymer. Hence it is of interest to consider the translocation separately from the helix formation. Investigations on the bare translocation process of a flexible polymer are reported in the present paper.

The efficiency of the translocation process may depend on various conditions and the properties of the two participants of this process, as the length, flexibility, composition, and hydrophobicity of the polymer, but also on the thickness of the bilayer, composition, and curvature of the membrane. In the present work we have focused our attention on the effects from the fluctuations of the lipids. One particularly fascinating aspect is related to the difference in fluctuations of the lipids in the two leaflets of a curved bilayer membrane [8]. Membranes with high stable curvatures are encountered at various intracellular membrane-bounded compartments [1,2,9]. There they may exhibit high curvatures at long cylindrical extrusions of the membrane or at the edges of folded membranes. There the typical ratio between the radius R of curvature and the thickness h of the membrane (≈ 40 Å) is in the order of $R/h \geq 3$. In the present study, we consider the influence of a membrane with fixed curvature on the transport of a polymer across a model lipid bilayer. Using simple models for polymer and membrane we apply Monte Carlo methods to monitor the translocation process.

The Polymer model.—As the polymer model we used a bead-spring model where $N = 20$ hard spheres of diameter $\sigma_P = 0.69$ are connected by harmonic springs of extension ℓ with spring constant $K = 5$ and potential $U(\ell) = K(\ell - \ell_0)^2$, where $\ell_{\min} < \ell < \ell_{\max}$, $U(\ell > \ell_{\max}) = U(\ell < \ell_{\min}) = \infty$, and $\ell_{\min} = 0.4, \ell_0 = 0.7, \ell_{\max} = 1.0$. Displacements of the hard spheres and extensions of the harmonic springs are accepted according to the Metropolis scheme. The average radius of gyration of the polymer is comparable to the width of one of the two leaflets of the bilayer membrane which is described below.

The Membrane model.—The bilayer lipid membrane is simulated using a simple coarse-grained model [8,10,11], which consists of N_L dumbbells tethered by one end to a penetrable interface, but are otherwise free to move from one to the other side of the interface and are allowed to diffuse in close proximity to the interface. Thus, each side of the lipid bilayer is comprised of dumbbells. A sketch of the model membrane is given in the inset of Fig. 1.

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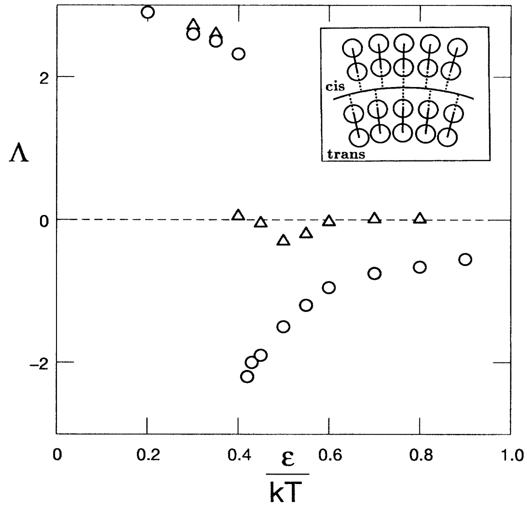

FIG. 1. Average penetration depth $\langle \Lambda \rangle$ versus adhesion parameter ε/kT. The open circles correspond to the curved membrane; the triangles correspond to the flat membrane. Inset: sketch of the model membrane.

There the dotted lines represent the tethers. The length of each tether may vary between the maximum extension $\lambda = 0.7$ and zero. Hence the maximal vertical movement of a lipid is limited by λ. The dumbbell is a coarse-grained representation of a lipid molecule, where the size fluctuation of the lipid tail is deliberately neglected and the mean extension of the tail is represented by a hard sphere of diameter σ_L. For simplicity we assume all lipids of the same type and of cylindrical shape and hence design the head group of the lipid by a hard sphere of the same diameter σ_L as the tail. The head and the tail of the dumbbell are connected at a fixed distance b (compare inset of Fig. 1). Hence the maximum width of the bilayer is $2\lambda + 2b + \sigma$. In the present model we use $N_L = 1000$, $\sigma_L = 1.1$, and $b = 1.32$. Since the length of the lipid is $b + \sigma_L$, the length to width ratio is 2.2, which is comparable to real lipids [12,13].

In the present work we used a rigid interface. Therefore, effects coming from large out-of-plane fluctuations of the membrane [13,14] have not been taken into account in the present model membrane. One possible way to include such undulations of the order of the width of the membrane itself would be to use a flexible interface [15] rather than a rigid one. However, since preliminary studies using a flexible interface have yielded similar results as for a rigid one, we have focused our study on using a rigid interface.

The geometry of the interface is a sphere of radius $R = 9.373$. Because of the curvature of the bilayer the numbers of lipids in each leaflet fluctuate and are self-consistently adjusted by flip-flop processes. The number densities of lipids, i.e., the number of lipids per interfacial area, are different in both layers, $\rho_{cis} = 0.55$ and $\rho_{trans} = 0.35$, for the outer half and the inner half, respectively. The average widths of the two halves are $h_{cis} = 1.47$ and $h_{trans} = 1.21$. The average orientations of the lipids with respect to the surface normal are $\langle \cos\theta \rangle_{cis} = 0.78$ and $\langle \cos\theta \rangle_{trans} = 0.75$, which are comparable to experimental values ≈ 0.7 [12]. It should be noted that the differences in the various quantities with respect to the inner and outer leaflets are caused by the curvature of the membrane, and they increase systematically with increasing curvature [8]. This is an important point and will be discussed again below with regard to the results of polymer translocation. For comparison, we have also simulated a flat bilayer of area $A \approx 1100$ with an average number density of lipids $\rho = 0.45$. The average width of each of the two halves is $h = 1.45$, and the average orientation is $\langle \cos\theta \rangle \approx 0.76$.

The dynamics of the membrane is achieved by randomly displacing the lipids. Each move is rejected if it leads to a violation of excluded volume conditions and/or the tethering conditions. The displacements of polymer and lipids are performed at equal rate. One Monte Carlo step is $2N_L + N$ attempted moves and is defined as one time unit.

The *hydrophobic effect* upon the polymer, which should drive the chain into the membrane, is described by an attractive potential $V(r)$ which is attributed between heads and tails of the lipid molecules and the monomers of the polymer. In reality, lipids are amphiphilic, and a hydrophobic residue would experience a free energy barrier on crossing the lipid head region. To speed up the translocation process, we have ignored this barrier. Presumably this will have no effect on a structureless polymer such as we consider here, but it may be important when polymers undergoing conformational transitions are included. We choose the Morse potential $V(r) = \varepsilon[\exp(-2\alpha(r - r_m)) - 2\exp(-\alpha(r - r_m))]$, where $\alpha = 4.0$. The potential is attractive for $r > r_0 = (\sigma_L + \sigma_P)/2$ and has a minimum at $r_m = r_0 + \ln 2/\alpha$ with $V(r_m) = -1.0$. Positional changes of polymers and lipids are accepted according to the Metropolis scheme.

The translocation of a polymer across a membrane as a function of adhesion strength ε/kT can be characterized by its mean penetration depth $\langle \Lambda \rangle$, which is the mean position of the center of mass of the polymer with respect to the interface of the bilayer. If $\Lambda > 0$ the polymer is located in the *cis* side (side of lower curvature), whereas for $\Lambda < 0$ the polymer is located in the *trans* side (side of higher curvature). Estimates of this quantity from Monte Carlo simulations are depicted in Fig. 1 for flat and curved membranes. At high temperatures ($\varepsilon/kT < 0.45$) the repulsion between polymer and lipids prevails and therefore $\Lambda > 0$. At very low temperatures ($\varepsilon/kT > 0.8$) the attraction between polymer and lipids dominates, and hence $\Lambda \rightarrow 0$ with decreasing temperatures. This behavior of the polymer is basically the same for both flat and curved membranes. However, at intermediate temperatures, approximately $0.45 < \varepsilon/kT < 0.8$, the average positions of the polymer differ significantly between the cases of flat and curved membranes.

In the case of the *curved* membrane a discontinuous transition of $\langle \Lambda \rangle$ is observed at $\varepsilon/kT \approx 0.45$ which indicates a spontaneous transport of the polymer from the *cis* to the *trans* side of the membrane. This phenomenon is absent in the case of the *flat* membrane, rather $\langle \Lambda \rangle \approx 0$, which reflects the fact that the polymer fluctuates symmetrically around the interface at $\Lambda = 0$. This interpretation is supported by the probability distribution $P(\Lambda)$, as depicted in Fig. 2, which shows that in the case of the *flat* membrane the polymer moves uniformly between the *cis* and the *trans* sides of the membrane with energetically and entropically preferred locations corresponding to the maxima in $P(\Lambda)$ in both halves of the membrane. In contrast, in the case of the *curved* membrane the polymer preferentially stays at the *trans* side. Therefore the transport of the polymer across the curved membrane corresponds to a "spontaneous translocation" process, whereas the event at the flat membrane corresponds to an "insertion" process.

Since the difference in the two mechanisms is related to the different curvatures of the membranes, it is conceivable that the structural asymmetry of the curved bilayer is responsible for the extraordinary transport property. One possible explanation for this effect could be related to the splay-induced larger available motional space of lipid heads at the *trans* side as compared to the *cis* side (compare sketch of the model membrane in Fig. 1), which would imply that the *trans* side is an energetically more favorable place than the *cis* side.

This would imply that translocation events should *not* happen if the effective hydrophobicity-induced attractive potential $V(r)$ between polymer and lipids is replaced by a *uniform* potential $V(z) = \varepsilon$ for $z < |h|$ and $V(z) = 0$ for $z > |h|$, where z is the distance of a monomer of the chain with respect to the interface between the two halves of the bilayer. The precise value of the width h, where $2h$ is the hydrophobic region of the membrane, is not very important and has been assumed to be $h = 1.5$, which is the average width of one-half of the bilayer. The result for the time evolution of the penetration depth $\Lambda(t)$ for this particular case at $\varepsilon/kT = 1.2$ is shown in Fig. 3. One observes a spontaneous translocation from the *cis* side ($\Lambda > 0$) to the *trans* side ($\Lambda < 0$), and a subsequent localization of the polymer at $\Lambda < 0$ for almost the whole time up to $t = 2 \times 10^6$ Monte Carlo time steps. This result demonstrates that in the present model membrane the translocation of a polymer is *not* energetically dominated and implies that translocation may take place for any type of potential which confines the polymer to the membrane with sufficient strength ε/kT.

Therefore, in addition, it is not very surprising that the total average energy $\langle U/N \rangle$ between polymer and lipids does not differ significantly in both cases, the curved and the flat bilayer, for all ε/kT (Fig. 4). This is also reflected by the average energy $U(z)$ of a monomer at position z, perpendicular to the membrane, which is almost the same in both cases, except that $U(z)$ for the curved membrane is symmetric around $z \approx -0.3$, instead of $z = 0$ as in the case of the flat membrane (inset of Fig. 4). Since the spontaneous translocation and the resulting asymmetry in $P(\Lambda)$ cannot be explained basically upon energetic considerations, it is conceivable that effects induced by entropy and fluctuations should hold the key for an explanation.

The main contribution to the entropy of the membrane is related to the orientational fluctuation of the lipids. Since the bilayer is curved, the fluctuation must be different in the two leaflets. The difference of fluctuations in the two leaflets of curved membranes is significant and increases systematically with increasing curvature

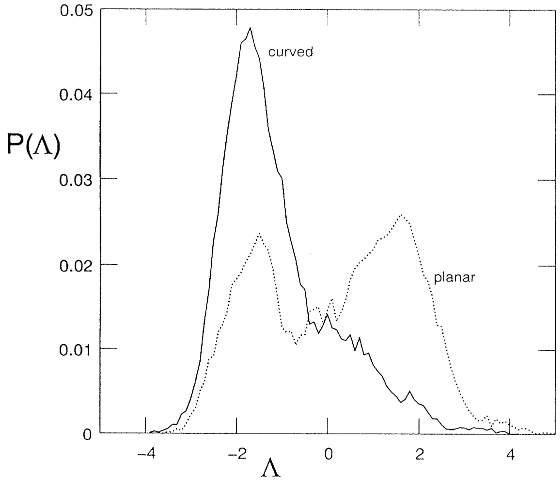

FIG. 2. Probability distribution $P(\Lambda)$ for adhesion parameter $\varepsilon/kT = 0.45$. The full and the dotted curves correspond to the curved and the flat membranes, respectively.

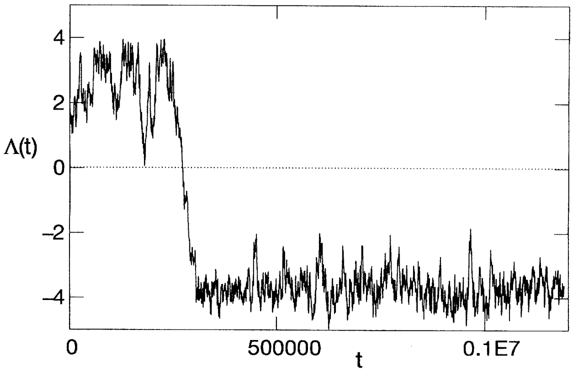

FIG. 3. Penetration depth $\Lambda(t)$ for the polymer versus time t in the case of a *curved* membrane at $\varepsilon/kT = 1.2$ with *uniform* hydrophobic potential $V(z) = \varepsilon$ at $z < |1.5|$.

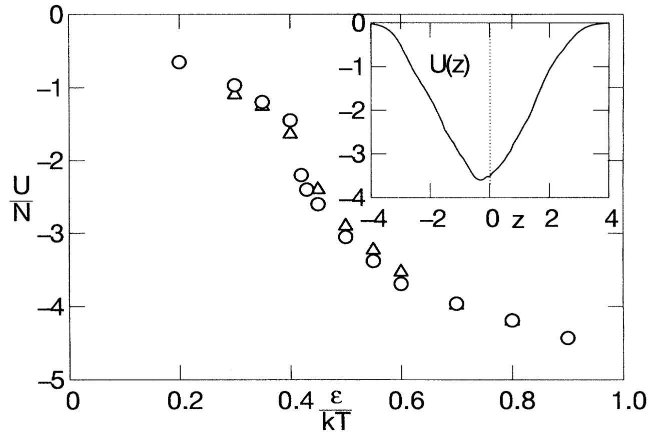

FIG. 4. Average adhesion energy $\langle U/N \rangle$ of the polymer versus adhesion parameter ε/kT. The circles correspond to the curved membrane; the triangles correspond to the flat membrane. Inset: $U(z)$, the average energy of a monomer of the chain at position z.

[8]. In the present lipid system, the fluctuations $\delta S = \sqrt{\langle S^2 \rangle - \langle S \rangle^2}$ of the orientational order parameter $S = \langle \cos\theta \rangle$ are approximately 2 times larger in the *trans* side than in the *cis* side; $\delta S/S = 0.018 \pm 0.003$ and 0.009 ± 0.002 for the *trans* and the *cis* side, respectively. Similar behavior is observed for the widths of the two halves with $\delta h/h = 0.025 \pm 0.003$ and 0.013 ± 0.002 for the *trans* and the *cis* side, respectively. The interpretation of the difference can be given based essentially on the asymmetry of available motional space of the lipids in the two layers. The splay of the lipids favors a stretching and hence a larger orientation of the outer lipids, whereas the inner lipids are in a region of lower density in the interior of the membrane with concomitantly greater orientational freedom. Therefore on average the heads are squashed toward the interface which leads to a smaller width of the inner leaflet and smaller orientational order and larger fluctuations [8].

The asymmetric entropical behavior of the two leaflets has some consequences for the translocation of the polymer. For low adhesion strength, say $\varepsilon/kT \ll 0.45$, the repulsion between polymer and lipids prevails and hence translocation is prohibited. With increasing adhesion strength the lipid-polymer interactions become more energetically dominated, and the polymer starts to penetrate into the membrane. Once experiencing the difference of fluctuations of the lipids in the two leaflets, the polymer prefers to move into an *entropically more favorable region* in order to maximize its conformational entropy. Consequently, the polymer moves into the region of higher entropy of the lipids and hence is driven toward the *trans* side which causes a spontaneous translocation of the polymer across the membrane and subsequent localization there.

The present work emphasizes the need to understand macromolecules interacting not only with perfectly flat membranes, but also with membranes of high curvature, and the need to understand the consequences of a gradient of lipid fluctuations across the membrane.

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[1] W. T. Wickner and H. F. Lodish, Science **230**, 400 (1985).
[2] S. J. Singer, Annu. Rev. Cell Biol. **6**, 247 (1990).
[3] S. M. Simon, C. S. Peskin, and G. F. Oster, Proc. Natl. Acad. Sci. U.S.A. **89**, 3770 (1992).
[4] F. Jähnig, Proc. Natl. Acad. Sci. U.S.A. **80**, 3691 (1983).
[5] D. M. Engelman and T. A. Steitz, Cell **23**, 411 (1981).
[6] R. E. Jacobs and S. H. White, Biochemistry **28**, 3421 (1989).
[7] M. Milik and J. Skolnick, Proc. Natl. Acad. Sci. U.S.A. **89**, 9391 (1992).
[8] A. Baumgärtner, J. Chem. Phys. **101**, 9060 (1994).
[9] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, *Molecular Biology of the Cell* (Garland Publishing, New York, 1994).
[10] E. A. Evans, Biophys. J. **14**, 923 (1974).
[11] A. G. Petrov and I. Bivas, Prog. Surf. Sci. **16**, 389 (1984).
[12] J. N. Israelachvili, S. Marcelja, and R. G. Horn, Q. Rev. Biophys. **13**, 121 (1980).
[13] R. B. Gennis, *Biomembranes: Molecular Structure and Function* (Springer, New York, 1989).
[14] W. Helfrich, J. Phys. (Paris) **46**, 1263 (1985).
[15] A. Baumgärtner and J.-S. Ho, Phys. Rev. A **41**, 5747 (1990); A. Baumgärtner, J. Chem. Phys. **98**, 7496 (1993).