Polymer Translocation through a Pore in Membrane

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We construct a new statistical physical model of polymer translocation through pore in membrane treated as the diffusion process across a free energy barrier. We determine the translocation time in terms of chain flexibility yielding an entropic barrier, as well as in terms of the driving mechanisms such as transmembrane chemical potential difference and Brownian ratchets. It turns out that, while the chemical potential differences induce pronounced effects on translocation due to the long-chain nature of the polymer, the ratchets suppress this effect and chain flexibility.

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The process of polymer translocation into or across biomembranes is a problem of considerable importance to a multitude of biological functions. Proteins are transported across a cellular membrane and endoplasmic reticulum, while RNAs across a nuclear membrane after their synthesis \[1,2\]. There are similar macromolecular transport mechanisms in drug delivery, as well as in biotechnology of gene transfer \[3\] where it is fundamental to understand how DNAs can be incorporated into cells. It is a highly complex process with specificity involving conformational changes of the translocating polymers that can occur in both cis and trans sides as well as inside of the membrane.

Although the translocation apparatus have been suggested and examined empirically in a great variety \[4\], only recently there have been a few efforts to investigate quantitatively the driving force of translocation on physical grounds \[5,6\]. Baumgärtner and Skolnick \[5\] studied via simulation the translocation of polymer directly through lipid bilayer, driven by the concentration imbalance of lipids that exists at high-curvature regions in membrane. On the other hand, Simon, Peskin and Oster(SPO) \[6\] considered protein translocation through a translocation channel or pore, and postulated that its driving force is random thermal motion rectified by ‘ratchets’ which give rise to directional diffusion. The origin of this so called Brownian ratchets(BRs) is a chemical asymmetry, i.e., if specific predetermined segments of the protein cross the membrane, chemicals such as chaperones bind on the segments to prevent their backward diffusion to the cis side of the membrane.

SPO considered rigid proteins, leaving out the effects of three dimensional chain conformations and the associated flexibility and entropy. In this Letter we incorporate these important effects by considering the flexible-polymer model. Along with the BR mechanism, we also incorporate the more ubiquitous kind of asymmetry due to transmembrane chemical potential difference, which naturally exists in biomembranes, due to e.g., electrochemical gradients, membrane potentials and protein conformational changes. We aim at an analytic, quantitative theory on the basis of statistical physics of polymer and stochastic processes. To highlight these flexibility and asymmetry effects on translocation, we consider a simple, but tenable model for the membrane: a rigid wall of negligible thickness with a pore, which is assumed to be small enough to allow only a single segment passage. The interaction between membrane and polymer segments is considered to be only of steric origin, i.e., the segments cannot cross membrane except through the pore. We describe the translocation dynamics as a stochastic process crossing the free energy barrier calculated from chain configuration partition function. The translocation time, given as the mean first passage time for this barrier crossing, is obtained from the Fokker-Planck equation that we formulate below. The initial targeting of nascent chain to the pore is regarded as a separate process and is excluded in this study. The controversies over chain conformations and chaperone functions go beyond this investigation, which is mainly concerned with finding some nonspecific physical principles behind translocation.

**Free Energy Barrier of Polymer Translocation** — The conformation of a flexible polymer during its translocation is significantly affected by steric interaction with the membrane, leading to a reduction of the polymer entropy and increase of its free energy. We adopt, as our model, an ideal chain with \(N(\gg 1)\) Kuhn segments each with length \(b\). First consider a chain with \(n\) Kuhn segments with the initial segment anchored on a rigid wall introduced in \(yz\) plane. With the boundary condition(BC) that the other segments do not cross the surface, the \(G(r, r_0; n)\), the probability of finding the end segment at \(r\), given initial one at \(r_0\) on surface, is obtained using the image method \[8\]: it is given as the probability for all configurations in free space, the Gaussian distribution

\[
G_0(r, r_0; n) = \left(\frac{2\pi nb^3}{3}\right)^{-3/2} \exp\left[-\frac{3}{2}b^2 (r - r_0)^2 \right],
\]

minus the probability for the chain crossing the surface \(G_0(r, -r_0; n)\),

\[
G(r, r_0; n) = G_0(r, r_0; n) - G_0(r, -r_0; n)
= \left[\frac{2\pi nb^2}{3}\right]^{-3/2} \frac{6\epsilon\pi}{nb^2} \exp\left(-\frac{3r^2}{2nb^2}\right),
\]

where \(r_0 = (\epsilon, 0, 0)\), \(\epsilon\) is an arbitrarily small distance of the anchored segment from the surface. The steric constraint factor of chain, given as \(Z_S(n) = \int_{x>0} G(r, r_0; n) dr < 1\), scales as \(n^{-1/2}\). In the ab-
sence of the constraint the partition function is given by \( Z_B(n) \sim \exp(-\beta n\mu) \), where \( \beta = 1/k_BT \) and \( \mu \) is the chemical potential per segment defined by \( \mu = (\partial F(n)/\partial n) T \) in the limit \( n \to \infty \). The \( F(n) \) is the free energy given from the full partition function, \( F(n) = -k_BT \log[Z_S(n)Z_B(n)] = \frac{1}{2}k_BT \log n + \mu n + \text{const} \), where the constant term is independent of \( n \).

FIG. 1. Schematic figure of the configuration of a translating polymer.

The whole chain during translocation can be decomposed into two independent end-anchored chains each in the opposite half spaces. For the decomposition into an \( n \) and \( N-n \) segments as shown in Fig. 1, the total free energy is

\[
F(n) = F(n) + F(N-n) = \frac{1}{2}k_BT \log [n(N-n)] + n\Delta\mu + \text{const},
\]

where \( \Delta\mu \) is the excess chemical potential per segment of trans side relative to that of cis side. The free energy with \( \Delta\mu = 0 \) has a symmetric barrier of entropic origin which, for a long chain, is nearly flat except near \( n = 1 \) or \( n = N-1 \) (B of Fig. 2). As also shown in Fig. 2, for a very long chain, a minute chemical potential difference (e.g., \( \Delta\mu = 10^{-2}k_BT \)) can break the barrier shape symmetry and its contribution can dominate the free energy. This contribution, which does not appear for a polymer in homogeneous media, can yield pronounced effects on a translocating polymer we shall see below.

Stochastic Model for Translocation Dynamics — For the long-time scale behavior of translocation, we construct a coarse-grained description in terms of the translocated segment number (translocation coordinate) \( n \) adopted as a relevant stochastic variable and in terms of the associated free energy barrier. It can be treated as a diffusive random process, which is described by a Fokker-Planck equation for \( P(n,t) \), the probability distribution of \( n \),

\[
\frac{\partial}{\partial t} P(n,t) = \mathcal{L}_{FP}(n)P(n,t),
\]

where \( \mathcal{L}_{FP}(n) \) is the operator, \( \mathcal{L}_{FP}(n) = 1/b^2\partial/\partial n)D(n)\exp(-\beta F(n))\partial(\partial n)\exp(\beta F(n)) \). Here, \( D(n) \) is the chain diffusivity during translocation. In the case that the \( D \) remains constant, it is given by \( D = k_BT/\Gamma \sim N^{-\nu} \), where \( \Gamma \) is the chain friction proportional to \( N^\nu \). The exponent \( \nu \) is 1 if the hydrodynamic interaction between the segments is neglected (as in the Rouse model), and is 1/2 if it is included (as in the Zimm model) 3.

The mean first passage time \( \tau(n,n_0) \), which is defined as the time for diffusion from the coordinate \( n_0 \) to \( n \), is obtained by solving the equation \( \mathcal{L}_{FP}(n_0)\tau(n,n_0) = -1 \), where \( \mathcal{L}_{FP}(n_0) = 1/b^2\exp(\beta F(n_0))\partial(\partial n_0)D(n)\exp(-\beta F(n_0))\partial(\partial n_0) \). To obtain the translocation time for the case that only the front segment in trans side is ratcheted, we assign the reflecting and absorbing BCs respectively at \( n = 1 \) and \( n = N-1 \): \( J(n = 1,t) = -[(D(n)/b)(\partial/\partial n + \beta\partial F/\partial n)P(n,t)]_{n=1} = 0 \), and \( P(n = N-1,t) = 0 \). Under these BCs, the translocation time, defined by \( \tau \equiv \tau(N-1,1) \) is integrated to be

\[
\tau = b^2 \int_1^{N-1} dn \frac{1}{D(n)} e^{\beta F(n)} \int_1^n dn' e^{-\beta F(n')}.
\]

Let us first assume, for simplicity, that \( D \) does not change in the course of translocation. In case of rigid chain without chemical potential difference \( \Delta\mu = 0 \), the translocation time is simply reduced to \( \tau = L^2/2D \sim L^{2+\nu} \), the result for the one dimensional diffusion of a single Brownian particle. Here \( L = N\beta \) is length of the whole chain. To incorporate the chain flexibility effect, the free energy function in Eq. 3 should be included in Eq. 4, resulting in, for \( \Delta\mu = 0 \),

\[
\tau(\Delta\mu = 0) = \frac{\pi^2 L^2}{8} \frac{1}{2D} \sim L^{2+\nu}.
\]
While the length scaling behavior of the translocation time of flexible chain is same as that of rigid chain, the prefactor of $\pi^2/8$ indicates that the chain flexibility retards translocation by 23%. This trend is opposite to what SPO obtained, due to the entropy effect associated with the three dimensional chain conformation which they did not include [6]. The translocation time is proportional to $N^{3+\nu}$ and, remarkably, with $\nu = 1$ this scaling behavior is identical to that of chain reptation time in entangled polymer systems [11].

If there is a nonvanishing chemical potential difference, the translocation time can be calculated, having the analytical expressions for limiting cases,

$$
\tau(\mu^*) = \begin{cases} 
\frac{\pi^2}{8} \frac{L^2}{D} \frac{1}{2\nu} \frac{\Delta \mu^*}{\nu} \frac{1}{2\nu} \exp(\mu^*) \quad , |\mu^*| < 1 \quad (7-a) \\
\frac{\pi^2}{8} \frac{L^2}{D} \frac{1}{2\nu} \frac{\Delta \mu^*}{\nu} \frac{1}{2\nu} \exp(\mu^*) \quad , \mu^* \ll 1 \quad (7-b) \\
\frac{\pi^2}{8} \frac{L^2}{D} \frac{1}{2\nu} \frac{\Delta \mu^*}{\nu} \frac{1}{2\nu} \exp(\mu^*) \quad , \mu^* \gg 1 \quad (7-c)
\end{cases}
$$

where $\mu^* \equiv N\beta \Delta \mu$. When the chemical potential per segment is reduced on trans side, the translocation time as given by Eq. 7-a and Eq. 7-b encounters a crossover in the scaling behavior from $\tau \sim N^{3+\nu}$ to $\tau \sim N^{1+\nu}$ to $\tau \sim N^{1+\nu} \sim L^{2+\nu}$. As shown in Fig. 3, this crossover occurs around $\mu^* = 1$ corresponding to $\Delta \mu = k_B T/N$, a very minute chemical potential difference for a long chain. This remarkable sensitivity of translocation to chemical potential asymmetry is even enhanced for the opposite case of higher chemical potential on trans side. Consider, for example, a chain with $N = 10^3$, and $|\Delta \mu| = 10^{-2} k_B T$, then $|\mu^*| = 10$. While, this small chemical potential difference with negative sign, speeds up the polymer translocation by the factor $\tau(\mu^* = -10)/\tau(\mu^* = 0) = 0.18$, the one with positive sign slows it down by the factor of 1191. Regardless of chain flexibility, this extreme sensitivity, already implied in Fig. 2, is a cooperative phenomenon arising from chain connectivity; the segments respond all hand in hand (involving the scaling variable $\mu^* = N\beta \Delta \mu$ in Eq. 7) rather than as individuals (involving $\beta \Delta \mu$) to a driving asymmetry. This is reminiscent of the cooperative effect of a slight segmental bias that gives rise to fast protein folding as proposed by Zwanzig, Szabo and Bagchi [12].

The chain diffusivity can also change during translocation. Adopting the Rouse model, $D(n) = N^{-1}(nD_1^{-1} + (N - n)D_2^{-1})$ where $D_1$ and $D_2$ are the diffusivities of the whole chain in cis and trans sides respectively. The effect of the change, $\Delta D = D_1 - D_2$, on $\tau$ can be incorporated analytically, but the result does not affect the dramatic effect of $\Delta \mu$ discussed above. The relative insensitivity of $\tau$ to $\Delta D$ is obvious since, while $\Delta \mu$ appears exponentially, $D$ is involved inversely in Eq. 8. 

**Many-Ratchet Effect** — The BR mechanism, which was originally suggested by SPO as a non-specific driving mechanism for biased diffusion, assumes fast chemical binding of chaperones on chain entering the trans side of membrane [12]. The binding sites are assumed to be uniformly distributed with an interval of $\delta$ along the chain. To incorporate this ratchet mechanism within our model for the case of instantaneous action of ratchets without dissociation, the whole space of translocation coordinate is divided into intervals of length $\delta = L/M$, where $M$ is the number of binding sites. Then the range of $i$-th interval is $(i - 1)\alpha + 1 < n < i\alpha + 1$, where $i = 1, 2, 3, \ldots, M$, and $\alpha = N/M$ is the number of polymer segments in each interval. The dynamics is now consecutive translocation (uni-directional diffusion) of each interval subject to the free energy therein, as well as to the BCs at both borders of the interval, reflecting BC at the left border and the absorbing BC at the right. These BCs are written as, $J(n = (i - 1)\alpha + 1, t) = 0$ and $P(n = i\alpha + 1, t) = 0$, for all intervals.

Assuming $D$ is constant, the translocation time of the whole polymer is then $\tau = \sum_{i=1}^{M} \tau_i$, where

$$
\tau_i = \frac{b^2}{D} \int_{(i-1)\alpha + 1}^{i\alpha + 1} \text{d}n \text{e}^{-\beta F(n)} \int_{(i-1)\alpha + 1}^{n} \text{d}n' \text{e}^{-\beta F(n')}. \quad (8)
$$

FIG. 3. Translocation time (in units of $b^2/2D_0$, $D_0 = ND$) versus chain length $N$ for $\nu = 1$. (A: $\beta \Delta \mu = 0$, B: $\beta \Delta \mu = -10^{-4}$, C: $\beta \Delta \mu = -10^{-3}$, D: $\beta \Delta \mu = -10^{-2}$, E: $\beta \Delta \mu = -10^{-1}$, F: $\beta \Delta \mu = -1.0$, G: $\beta \Delta \mu = -10$. The crossover behavior from $\tau \sim N^3$ to $\tau \sim N^2$ occurs when $N$ is around $k_B T/|\Delta \mu|$.

FIG. 4. $\Omega(\mu^*, M)$ as a function of $M$ for different values of $\mu^* \equiv N\beta \Delta \mu$. ( $N = 1026$, and A: $\mu^* = 100$, B: $\mu^* = 10.74$, C: $\mu^* = 0$, D: $\mu^* = -10.74$, E: $\mu^* = -100$.) When $M$ is sufficiently large, $\Omega(\mu^*, M)$ goes to 1, the ratchet limit.
If the chain flexibility and $\Delta \mu$ are neglected, i.e. $F(n) = \text{const}$, it is reduced to $\tau = L\delta/2D = L^2/2DM$ naturally, the reduction by the factor of $1/M$ compared with $\tau(M = 1)$, as given by SPO. In general the translocation time can be written as

$$\tau = \frac{L^2}{2DM} \Omega(\mu^*, M).$$

(9)

Numerical integration for translocation time gives $\Omega(\mu^*, M)$ as depicted in Fig. 4, which clearly indicates that the ratchets suppress the chain flexibility, as well as the chemical potential difference regardless of its sign. Most striking is the approach of $\tau$ to that of rigid chain ($\Omega(\mu^*, M) = 1$), i.e., solely the ratchet result, when $M$ is very large, even with large negative value of $\mu^*$; it runs counter to the intuition, according to which, the negative chemical potential difference and the ratchet mechanism add up in series in speeding translocation.

This overriding effect of many ratchets can be better understood by considering the Langevin equation equivalent to the Fokker-Planck equation description,

$$b\Gamma \dot{n} = -\frac{1}{b} \frac{\partial F_R(n)}{\partial n} + \xi(t)$$

(10)

where $\xi(t)$ is the Gaussian, white noise connected to $\Gamma$ via fluctuation-dissipation theorem (FDT): $\langle \xi(t)\xi(0) \rangle = 2\Gamma k_B T \delta(t)$. Confining ourselves to the case in which the chain is rigid, the $F_R(n)$ shown in Fig. 5 is the free energy (ratchet potential) which effectively includes the BCs due to ratchets. For the reflecting and absorbing BCs we considered, the step height $h$ is infinity. (But for general consideration and more realistic ratchet activities, it can be put to be finite. The similarity of this ratchet potential to those employed for ratchet-driven motor proteins [11] is remarkable.) The FDT assures the approach to equilibrium, that is, under the potential $F_R(n)$ of Fig. 5, $n$ undergoes the rectified diffusion to the right, regardless of $\Delta \mu$. When $M$ is very large, so that $\Delta \mu = k_B T \mu^*/M$, the barrier height due to $\Delta \mu$ is very small, the global translocation dynamics become independent of the details of the local potential barriers, yielding the $\tau$ of many ratchets, $\tau = L^2/2DM$, or $\Omega(\mu^*, M) = 1$. A calculation shows that this result tends to be valid for finite values of $h$ larger than $k_B T$.

To summarize, we have investigated mechanisms affecting polymer translocation through a pore in membrane. It is found that, while chain flexibility, due to an entropic barrier it gives, does retard translocation, the ratchets speed it up, tending to reduce the flexibility and chemical potential effects to rigid chain behavior. The transmembrane chemical potential asymmetry, only with minute magnitude, is found to modulate dramatic changes in translocation behaviors of long polymers, which is a cooperative behavior arising from their chain connectivity.

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