Polymer Translocation in Crowded Environments

Ajay Gopinathan\textsuperscript{1} and Yong Woon Kim\textsuperscript{2}
\textsuperscript{1}School of Natural Sciences, University of California, Merced, CA 95344, U.S.A.
\textsuperscript{2}Department of Physics and Materials Research Laboratory, University of California, Santa Barbara, CA 93106, U.S.A.

We study the effect of the crowded nature of the cellular cytoplasm on the translocation of a polymer through a pore in a membrane. By systematically treating the entropic penalty due to crowding, we show that the translocation dynamics are significantly altered, leading to novel scaling behaviors of the translocation time in terms of chain length. We also observe new and qualitatively different translocation regimes depending upon the extent of crowding, transmembrane chemical potential asymmetry, and polymer length.

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Transport of a variety of biopolymers across a dividing membrane is a fundamentally important process in biological systems \cite{1}. Examples include transfer of proteins across cellular membranes or endoplasmic reticulum \cite{2}, gene swapping through bacterial pili \cite{3} and DNA transport through nuclear pore complexes \cite{4}. Technological applications include gene delivery \cite{5} and DNA sequencing \cite{6}. A considerable amount of theoretical work has focused on both the basic physics \cite{7, 8, 9, 10, 11} underlying the translocation process and on how details such as polymer-pore interactions \cite{12}, intrinsic polymer structure \cite{13} and confinement \cite{14, 15} affect the dynamics of the process. One important aspect that has received very little attention is the effect of crowding on the translocation dynamics. Crowding due to macromolecular aggregates and other inclusions in the cellular cytoplasm can be as high as 50\% by volume \cite{16} and is known to have considerable influence on reaction rates, protein folding rates and equilibria in vivo \cite{17, 18}. A polymer threading its way through such a crowded environment is subject to a large entropic penalty which should dramatically affect the translocation dynamics. In this Letter we present the first systematic study of polymer translocation in terms of crowding. We show that the free energy penalty due to crowding has terms that scale sublinearly with the polymer length. The presence of non-linear terms in the free energy leads to qualitatively different translocation dynamics including novel power law scalings of the translocation time with polymer length as well as situations where the translocation time is nearly independent of the polymer length over several orders of magnitude.

We consider a Gaussian polymer of length $N$ (in units of the Kuhn length $b$) threading itself through a pore in a dividing membrane from cis (left) side to trans (right) side, as illustrated in Fig. 1. The pore is assumed to be small enough that it allows only one monomer to pass through at a time, with an effective diffusion constant $D_p$. Crowding is modeled by randomly distributed spherical obstacles, sterically interacting with the polymer, of radius $a$ and diffusion constant $D_o$ at a volume fraction $\phi_c(\phi_t)$ on the cis (trans) side. There could also be an excess chemical potential difference for monomers between cis and trans side, $\Delta \mu$. We now assume that we can treat the process quasi-statically with the polymer segments on both sides of the membrane being in equilibrium at all times. The validity of the assumption depends on the relative magnitudes of the three time scales in the problem: the total translocation time $\tau \sim (b^2/D_p)\tilde{\tau}$, the polymer relaxation time $\tau_R \sim (\Phi b^3/k_B T)N^2$ \cite{19}, and the timescale set by obstacle motion $\tau_o \sim \phi^{-2/3}/D_o$. Here $\tilde{\tau}$ is a dimensionless function that characterizes the translocation process and $\eta$ is the medium’s viscosity. Assuming equilibrium statistics for the polymer segments necessarily requires that $\tau_R \ll \tau \ll \tau_o$. In the presence of obstacles, this assumption remains valid in two different regimes, $\tau_o \ll \tau_R \ll \tau$ and $\tau_R \ll \tau \ll \tau_o$. The first regime corresponds to the situation where the obstacles diffuse fast enough that the “polymer segment + obstacles” system can be assumed to be at equilibrium (dynamic obstacles). In the second regime, the obstacles are essentially immobile on the translocation timescale and the polymer segment achieves equilibrium statistics in this static obstacle environment. It should be noted that if the pore friction is not high enough (i.e. $\tau_R \simeq \tau$) \cite{20} or if $\tau_R \leq \tau_o \leq \tau$ the quasi-static assumption breaks down leading to anomalous dynamics. In the regimes where the assumption re-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Schematic illustration of the translocation process of a polymer in the presence of crowding}
\end{figure}
mains valid we have a well defined free energy barrier whose form is governed by the polymer statistics, presence of the membrane, chemical potential gradient and the presence of crowding. Since the contributions to the free energy from factors other than crowding have been worked out before \[1,8\], we focus on the entropic penalty that arises from crowding.

Polymer configurations in the presence of static obstacles correspond to Brownian walk trajectories with a diffusion constant \(b^2/6\) that have survived to a time \(t = n\) with the obstacles playing the role of traps. The fraction of allowed polymer configurations is therefore identical to the presence of crowding. Since the contributions to the free energy barrier, \(F_{cr}^s\), due to static obstacles at volume fraction \(\phi\) are given by the Smoluchowski solution

\[
F_{cr}^s(n) = -\log S(n),
\]

where \(S(t)\) is the survival probability of an appropriate Brownian walker at time \(t\) \[18,20\]. For short times, \(S(t)\) is given by the Smoluchowski solution \(S(t) \sim \exp(-\kappa t)\) \[21,22\], while for long times it is dominated by walkers trapped in large void regions giving rise to the stretched exponential Donsker-Varadhan (DV) solution \(S(t) \sim \exp(-\lambda t^{5/6})\) \[23,24\], where \(\kappa\) and \(\lambda\) are constants that depend on trap radius, trap density and geometry. Using Eq. (1) and the exact solutions for \(S(t)\) \[21,23\], we can explicitly compute the free energy penalty for a chain of length \(n\) due to static obstacles at volume fraction \(\phi\), yielding

\[
F_{cr}^s(\phi,n) = \frac{1}{2} \left( \frac{b}{a} \right)^2 \phi \left( n + \sqrt{\frac{24a^2n}{\pi b^2}} \right) \quad \text{for} \quad n \ll N_x \]

\[
F_{cr}^s(\phi,n) = 2.6 \left( \frac{b}{a} \right)^{6/5} \phi^{2/5}n^{3/5} \quad \text{for} \quad n \gg N_x,
\]

where \(N_x \sim (a/b)^2\phi^{-2/5}\) represents the crossover polymer length from the Smoluchowski regime to the DV regime \[24\]. For the dynamic obstacle case, the “polymer + obstacles” system comes to equilibrium. The presence of the obstacles gives rise to a depletion induced attractive interaction between chain monomers that can lead to a collapsed polymer phase similar to that induced by poor solvent conditions \[20\]. Simulations \[27\] and analytical work \[28,29\] have shown that hard spheres can cause polymers to collapse if the sphere density is high enough. For the range of \(\phi\) (0.1 < \(\phi\) < 0.5) that we are interested in, the collapse occurs whenever \(a \geq 2b\). For our purposes it is then safe to assume that our Gaussian polymer is in a collapsed “dense globule” state with a volume \(Nb^3\). The free energy penalty is then given by the sum of the confinement entropic penalty (\(= \pi^2 R_c^2/6R^2\) where \(R \sim N^{1/3}\) is the confining radius) and the work done to create a cavity of volume \(Nb^3\) that is devoid of obstacles, which is known exactly from scaled particle theory \[30\]. The resulting free energy penalty for the chain due to dynamic obstacles reads as

\[
\begin{align*}
F_{cr}^d(\phi,\phi_c, N - n) + F_{cr}^s(\phi_n, n) + \frac{1}{2} \ln[n(N - n)] + n\Delta \mu
\end{align*}
\]

The general expression for total free energy, taking into account the presence of the dividing wall and chemical potential difference, of a chain with \(n\) monomers on the trans side and \(N - n\) on the cis side is then given by

\[
F_{tot}^s(n) = F_{cr}^s(\phi_c, N - n) + F_{cr}^s(\phi_n, n) + \frac{1}{2} \ln[n(N - n)] + n\Delta \mu
\]

for the static obstacle case and

\[
F_{tot}^d(n) = F_{cr}^d(\phi_c, N - n) + F_{cr}^d(\phi_n, n) + n\Delta \mu
\]

for the dynamic obstacle case. It is to be noted that the logarithmic term in Eq. (4), resulting from reduced chain configurations confined in a half-space, does not appear in Eq. (5) because \(F_{cr}^d\) already takes into account the entropic penalty associated with confining the chain to a dense globule. The translocation process can now be described by diffusion along the translocation coordinate \(n\) in the presence of a well-defined free energy barrier, \(F_{tot}^s(n)\). The dynamics of this process is governed by a Fokker-Planck equation which then allows one to compute the translocation time (mean first passage time for
the chain to diffuse across the pore \[7\]) as
\[
\tau_d(n) = \frac{b^2}{DP} \int_1^{N-1} dn \ e^{F_{tot}(n)} \int_1^n dn' \ e^{-F_{tot}(n')}.
\]
(6)
Since we have nonlinear terms in the free energy we first consider the behavior of \(\tau\) for a general free energy functional with a power law scaling, e.g., \(F(n) \sim n^\alpha\). Eq. \[9\] and saddle point approximations (in the large \(N\) limit) to do the integrals yield
\[
\tau \sim N^{2-\alpha} \quad \text{for} \quad F(n) \sim (N-n)^\alpha
\]
\[
\tau \sim \exp(N^\alpha) \quad \text{for} \quad F(n) \sim n^\alpha
\]
for translocation out of and into a crowded half-space, respectively. Thus we anticipate new exponents characterizing the scaling properties of the mean passage time with the number of monomers in the crowded medium.

We now consider, in detail, the impact of crowding on translocation dynamics in some physically interesting situations. The first example is that of a polymer escaping from a crowded environment, i.e., where the cis side is crowded and the trans side is not (\(\phi_c = 0\)). In the static case, the dominant contribution to the free energy has the form \(\phi_c^{2/5}(N-n)^3/5\) for a long chain and \(\phi_c(N-n)\) for a short chain (see Eq. \[2\]). The translocation times obtained by saddle point approximation scale as \(\phi_c^{-2/5}N^{7/5}\) and \(\phi_c^{-1}N\), respectively. Exact numerical evaluations of Eq. \[5\] confirm the predicted power law scalings with both \(N\) (Fig. \[2\]) and \(\phi_c\) (inset in Fig. \[2\]). In contrast to the dynamic case where the leading order behavior simply corresponds to having an effective “osmotic pressure” from obstacles, the driving force for translocation in randomly distributed immobile obstacles is weakened by the existence of rare, large voids on the cis side that sufficiently long polymers can explore. This is the physical origin for the novel exponents describing the scaling of \(\tau\) with respect to both \(N\) and \(\phi_c\) for a long enough chain in the static case.

Another situation of significant practical interest is translocation into a crowded environment driven by a chemical potential gradient. Here we take \(\phi_c = 0\) and \(\Delta\mu = -\mu\) that favors translocation into the crowded trans side. For the static obstacle case in the large \(N\) limit, the linear chemical potential term always dominates for any non-zero value of \(\mu\). Thus \(\tau \sim N\) in this limit, while, for \(\mu = 0\), \(\tau \sim \exp(N^{3/5})\) (from Eq. \[7\]). The situation is similar for the dynamic case but only if \(\mu\) exceeds a critical threshold \(\mu^*\) that is sufficient to overcome the osmotic pressure term (\(\mu^*\) is here defined by the value of the prefactor of the \(n\)-linear osmotic pressure term in \(F_{os}\)). For \(\mu < \mu^*\), \(\tau \sim \exp(N)\) for a long chain when obstacles are mobile. Fig. \[3\]a) and (c) clearly show these distinct limiting behaviors. The plots also reveal a striking phenomenon that seems to occur at intermediate length scales. Depending on the parameter values, there appear to be regimes spanning several orders of magnitude in polymer length, where the translocation time is nearly independent of \(N\). The reason for this can be understood by considering the form of the relevant free energy profile as a function of the translocation coordinate \(n\). The situation is particularly simple for the static obstacle case where there is a competition between a linear term (\(\sim n\)) due to the chemical potential and a sublinear term (\(\sim n^{3/5}\)) that comes from the crowding for a long chain. This gives rise to a free energy barrier whose height and position are nearly independent of the total length \(N\) followed by a “downward slope” all the way to \(n = N\). The time taken to surmount this barrier, which is independent of \(N\), is rate-limiting and hence effectively the total translocation time for polymer lengths shorter than the value of \(N\) at which the time taken to traverse the downward slope becomes comparable. At this point the scaling crosses over to being linear in \(N\). The situation for the dynamic obstacle case is similar except that the presence of two sublinear terms “softens” the plateau because barrier height and position are no longer independent of \(N\). Figs. \[3\]b) and (d) show the different translocation time scaling regimes in the \((\phi_c, \mu)\) phase space at a fixed \(N\). As one might expect, large values of \(\mu\) imply the dominance of the chemical potential, leading to \(\tau \sim N\), while low values give exponential behavior. The disappearance of the plateau regime for the dynamic obstacles at high concentrations is because the

FIG. 3: Translocation time \(\tau\) (in units of \(b^2/DP\)) vs. \(N\) for (a) static and (c) dynamic obstacles on the trans side (\(\phi_c = 0.3\), \(\phi_c = 0\)) for different values of chemical potential gradient, showing the asymptotic power law scalings as well as the plateau regime at intermediate length scales. Note \(\mu^*\) denotes the value of the prefactor of the \(n\)-linear term of \(F_{os}\) in Eq. \[2\] and \[4\], respectively. Different scaling behaviors (exponential, plateau, power law) of \(\tau\) in the \((\phi_c, \mu)\) phase space for (b) static and (d) dynamic obstacles at a fixed \(N = 10^7\).
in Fig. 4(a), even a minute amount of \( \Delta \mu \) gives rise to qualitatively different results. As seen however, the absence of a linear term in the long polymer smears out the plateau even more. In the static case we note however that here the presence of sublinear free gradient (compare Fig. 3(c,d) with Fig. 4(c,d)). It is to be noted however that here the presence of sublinear free energy contributions from both sides of the membrane smears out the plateau even more. In the static case however, the absence of a linear term in the long polymer limit gives rise to qualitatively different results. As seen in Fig. 4(a), even a minute amount of \( \phi_c \) leads to exponential translocation times at large polymer lengths despite substantial crowding on the cis side. This counterintuitive behavior arises because the free energy profile in this situation always has a barrier whose height scales to leading order as \( N^{3/5} \), which in turn implies exponential barrier crossing times.

In conclusion we have presented a systematic study of the impact of crowding on translocation dynamics. We have shown how crowding can lead to the emergence of novel exponents characterizing the scaling of translocation time with polymer length and crowding volume concentration. The existence of regimes where the translocation time depends very weakly on polymer length, apart from being of theoretical significance, suggests the possibility of designing filters for a tunable range of polymer lengths and also has implications for “synchronized” transport of proteins or nucleic acids from a wide range of sizes in the cellular context.

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![FIG. 4: Translocation time \( \tau \) (in units of \( b^2/D_P \)) as a function of chain length \( N \) for (a) static(\( \phi_c = 0.05 \)) and (c) dynamic obstacles(\( \phi_c = 0.2 \)) for different values of \( \phi_t \). Different scaling behaviors (exponential, plateau, power law) of \( \tau \) in the \((\phi_t, \phi_c) \) phase space for (b) static and (d) dynamic obstacles at a fixed \( N = 3 \times 10^4 \). No chemical potential gradient is applied (\( \Delta \mu = 0 \)).](image-url)