How capture affects polymer translocation in a solitary nanopore

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In silico studies identify and differentiate characteristic distributions of the mean first passage time due to single file translocation from those due to translocation of different types of folds and provide direct evidence of the interpretation of the experimentally observed folds [M. Gershow and J. A. Golovchenko, Nat. Nanotechnol. 2, 775 (2007) and Mihovilovic et al., Phys. Rev. Lett. 110, 028102 (2013)] in a solitary nanopore. Finally, we show a new finding—that a charged tag attached at the 5′ end of the DNA enhances both the multi-scan rate and the uni-directional translocation (5′ → 3′) probability that would benefit the genomic barcoding and sequencing experiments.

I. INTRODUCTION
Efficient capture of a dsDNA is the first step for its subsequent translocation through the nanopore that is central for sequencing, genome mapping, and other multiplexed nanopore sensing applications. A voltage is applied across the nanochannel to electrophoretically drive a dsDNA or other biopolymers through the nanopore or a nanochannel. The characteristic current blockade data are then deconvoluted to reconstruct the translocated species. The capture rate in a nanopore device is a function of several adjustable parameters, the pH of the electrolyte, the diameter and shape of the nanopore, the strength of the external electric field, and the length of the biomolecule and its effective charge. Evidently, a fundamental understanding of the dependencies of the capture rate on these factors is essential to improve the efficiency and quality of a nanopore device. A large majority of these items have been addressed experimentally. The theoretical studies have either concurred or validated some of these dependencies.

Correspondingly, simulation studies have revealed a more detailed picture of the capture process and helped toward the construction of a unifying theory of capture and translocation. Nanopore translocation consists of three distinct processes. Diffusion of a polymer toward the pore, which progressively acquires characteristics of drift as it approaches the pore; its eventual capture, often after multiple attempts; and finally, the successful threading through the nanopore, which we call translocation. A large fraction of the earlier simulation studies was done assuming the polymer is already captured at the pore entrance and that a localized field exists only inside the pore that drives the biopolymer from the cis to the trans side. One of the major focuses of these theoretical and computational studies was directed to find how the mean first passage time (MFPT) \( \langle \tau \rangle \) depends on the chain length assuming a power law dependence \( \langle \tau \rangle \sim N^\alpha \), where \( \alpha \) is the translocation exponent. However, the diffusion and the drift of the polymer, and its eventual capture in the pre-translocation phase getting ready to be translocated itself, are very rich in physics as has been demonstrated in...
FIG. 1. A series of simulation snapshots (a)–(f) in progressive time show the translocation process of the polymer in the presence of localized force bias applied in the nanopore thickness. τ is the total translocation time of the chain. (g)–(l) The same polymer configuration is released from the equipotentials surface d = 4.0 away from the pore in the presence of the electric field, which extends beyond the nanopore. During the capture process, the polymer makes a few unsuccessful attempts shown in (h) and (j) before one end threads into the nanopore. (k) and (l) show the translocation of the captured polymer through the nanopore under the electric field gradient.

recent experimental studies. Gershow and Golovchenko studied Kilo-base-pair (kbp) long dsDNA and studied capture-recapture probability as a function of the delay time (which approximately translates to different distances from the pore) of the voltage reversal. Mihovilovic et al. made a detailed study of how the capture of a folded configuration affects its translocation property. Wanunu et al. found that for a fixed concentration, the capture rate increases with the contour length up to a critical length beyond, which it saturates. They further observed that a salt gradient increases the capture probability. Jeon and Muthukumar conducted a similar study on the dependence of the salt-concentration gradient and pore-polymer interaction using an α-haemolysin protein pore. These experimental studies have motivated more simulation studies on the capture problem. Slater and co-workers used different approaches to define the capture radius and studied the effect of the time dependent field on the capture process. Vollmer and deHaan studied the change in the shape of the polymer as it approaches the pore by the ratio of gyration radii along the longitudinal and transverse direction. Moreover, with the advancement of computational methods, “all-atom-MD” simulations reveal intricate details such as the importance of dielectrophoresis in polymer capture and the non-trivial effect of nanopore temperature gradient on the translocation speed. In a recent paper, Choudhary et al. used the “Steric Exclusion Model” to compute the current traces showing the capture and transport mechanism in different nanopore geometries.

FIG. 2. Polymer radius of gyration $\sqrt{\langle R^2_g \rangle}$ as a function of radial distance of the first monomer $r(N_0)$ from the pore. The major/minor axes of the ellipse are the transverse/longitudinal ($R_{g\perp}$/$R_{g\parallel}$) components of the radius of gyration respectively. The average $R_g$ is denoted by the magenta dotted line. $R_g$ at different phases: (a) diffusion dominated, (b) drift dominated and capture, and (c) translocation and escape are presented in the inset of ellipses and compared against a unit circle in black. $e$ and $A$ denote the eccentricity and the area of the ellipses, respectively. The nanopore membrane location is denoted by the dotted line, and the drift region is identified from the diffusion dominated region by marking the inflection point of $\sqrt{\langle R^2_g \rangle}$.
In this article, we report a few new results on further details of the capture process and how the capture affects the translocation properties of a biopolymer. A few results have been influenced by and complement previous studies, and a few others which we believe add to our overall understanding of the capture and translocation characteristics. We primarily consider three factors; the extended electric field outside the pore, the initial release point of the polymer, and the DNA persistence length on the capture and translocation probability keeping the length and the field strength constant and present the following key results. (i) We revisit the effect of the electric field and demonstrate how an extended field influences the capture process as opposed to a field that strictly resides inside the nanopore. The polymer makes multiple attempts in the presence of an extended field (Fig. 1), similar to Kramer’s barrier crossing problem and increases the capture probability, as recently theoretically discussed by Grossberg and co-workers. (ii) Following Vollmer et al., we use the ratio of the gyration ellipses to monitor the shape of the polymer, which shows a signature as the diffusive motion acquires characteristics of drift as the polymer approaches the pore (Fig. 2). (iii) We further show that releasing the polymer close to the pore mouth from stronger equipotentials has a higher success rate of capture (Fig. 3) and shorter capture time (Figs. 4(a)–4(d)) and that (iii) the release distance affects the formation and translocation of hairpin structures (Fig. 4). (iv) We provide further details and validate different modes of folded and unfolded configurations from the reversal of the electric field (Fig. 5). (v) Finally, we show that an effective way to increase the cation probability is negligible after achieving a critical persistence length. (v) We then demonstrate that the single-file capture can be enhanced drastically by stiffening the polymer (Fig. 6) and the hairpin translocation probability is negligible after achieving a critical persistence length. (v) Finally, we show that an effective way to increase the unidirectional capture rate is to attach charged tags at one end of the DNA, which breaks the degeneracy promoting a higher rate of unidirectional capture with the increasing stiffness (Fig. 7).

FIG. 3. (a) Capture probability $P_{\text{cap}}(\Phi, t)$ as a function of time for the polymer released from different equipotentials $\Phi = 0.322$ (green empty circle), 0.157 (magenta empty triangle), 0.101 (orange empty diamond), and 0.071 (pink empty diamond). In each case, the solid colored line represents a fit to Eq. (9) with $A_{\text{cap}} = 1$ shown in Table I. (b) Dependence of the capture probability $P_{\text{cap}}(\Phi, t)$ as a function of the equipotential $\Phi$. The line through the points is described by a function $A \text{tanh}(B \Phi)$. The graphs (c)–(f) show the capture time distributions for different equipotentials. In each case, $\mu$ and $\sigma$ correspond to the distribution average and standard deviation, respectively. The black envelopes show the exponential fits of the distributions with the averages marked by the dashed lines.

FIG. 4. (a)–(d) show the rendition of different ways of polymer threads through the nanopore and translocates. The capture location $x$ denotes the normalized monomer index $m/N$, which first threads into the nanopore; (a) the single file capture events where either of the ends gets captured are denoted as type 1 translocation. (b) and (c) show the type 2-1 event in which polymer is captured at any random location except at the ends and in the symmetric location. (d) Type 2 events indicate the symmetrical capture cases. (e)–(h) denote the same using the actual coordinates from BD simulation for a fully flexible polymer. End-to-end distance $R_{\text{end}}$ of distributions of a polymer at the moment of capture at the nanopore orifice after being released from four different equipotential distances $d = 4.0, 8.0, 12.0, \text{and} 16.0$ are shown in (i)–(l) sub-plots. The black envelopes denote the exponentially modified Gaussian fits of the distributions. Average and standard deviation of the distributions are marked in $\mu$ and $\sigma$, respectively, and corresponding $(R_{\text{end}})$ are shown in the colored dashed lines, while the blue line represents the average end-to-end distances of the starting configurations. After capture, the mean first passage time histograms (m)–(p) follow Gaussian shape with average $\mu$ and standard deviation $\sigma$ are obtained from single file translocation (type 1) events for the same equipotential release distances. The type 2-1 translocations are faster than type 1 events and are shown in silver histograms, which are also predominant occurrences for higher $d$. Type 2 events are relatively rare, and the bar plots in the insets represent the occurrence of these three types of capture events on a percentage scale.

TABLE I. Type 1, Type 2-1, and Type 2 events are quantified in terms of the occurrence of the capture location $x$, the end-to-end distance $R_{\text{end}}$, and the type of events. Type 1 events are defined as both ends of the polymer thread through the nanopore and translocates. Type 2 events indicate the symmetrical capture cases. Type 2-1 events denote the case where either of the ends gets captured or the polymer is captured at any random location except at the ends and in the symmetric location. (a)–(d) show the rendition of different ways of polymer threads through the nanopore and translocates. The capture location $x$ denotes the normalized monomer index $m/N$, which first threads into the nanopore; (a) the single file capture events where either of the ends gets captured are denoted as type 1 translocation. (b) and (c) show the type 2-1 event in which polymer is captured at any random location except at the ends and in the symmetric location. (d) Type 2 events indicate the symmetrical capture cases. (e)–(h) denote the same using the actual coordinates from BD simulation for a fully flexible polymer. End-to-end distance $R_{\text{end}}$ of distributions of a polymer at the moment of capture at the nanopore orifice after being released from four different equipotential distances $d = 4.0, 8.0, 12.0, \text{and} 16.0$ are shown in (i)–(l) sub-plots. The black envelopes denote the exponentially modified Gaussian fits of the distributions. Average and standard deviation of the distributions are marked in $\mu$ and $\sigma$, respectively, and corresponding $(R_{\text{end}})$ are shown in the colored dashed lines, while the blue line represents the average end-to-end distances of the starting configurations. After capture, the mean first passage time histograms (m)–(p) follow Gaussian shape with average $\mu$ and standard deviation $\sigma$ are obtained from single file translocation (type 1) events for the same equipotential release distances. The type 2-1 translocations are faster than type 1 events and are shown in silver histograms, which are also predominant occurrences for higher $d$. Type 2 events are relatively rare, and the bar plots in the insets represent the occurrence of these three types of capture events on a percentage scale.

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different captured configurations eventually translocate through the nanopore.

II. COARSE-GRAINED MODEL AND LANGEVIN DYNAMICS SIMULATION

Our coarse-grained (CG) model of a dsDNA consists of 256 beads of diameter of \( \sigma \) that mimics a 4 μm long λ-phage DNA with 48 bp resolution associated with a single bead. We use a short range Lennard-Jones (LJ) potential, 

\[
U_{LJ}(r) = 4\epsilon \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^{6} + \epsilon \quad \text{for} \quad r \leq 2^{1/6}\sigma
\]

\[
= 0 \quad \text{for} \quad r > 2^{1/6}\sigma. \tag{1}
\]

to model the excluded volume interaction between two beads separated at a distance \( r \), where \( \epsilon \) is the strength of the LJ potential. The connectivity between two neighboring monomers is constructed using the finitely extensible nonlinear elastic (FENE) spring potential,

\[
U_{\text{FENE}}(r_{ij}) = -\frac{1}{2}k_{F}R_{0}^{2}\ln \left( 1 - \frac{r_{ij}^{2}}{R_{0}^{2}} \right). \tag{2}
\]

Here, \( r_{ij} = |r_i - r_j| \) is the distance between two consecutive monomer beads \( i \) and \( j \) at \( r_i \) and \( r_j \), \( k_{F} \) is the spring constant, and \( R_{0} \) is the maximum allowed separation between two connected monomers. An angle dependent three body interaction term is introduced between successive bonds, which accounts for the chain stiffness \( \kappa \),

\[
U_{\text{bend}}(\theta_i) = \kappa (1 - \cos \theta_i) \tag{3}
\]

and \( \theta_i \) is the angle between the bond vectors \( \vec{b}_{i-1} = \vec{r_i} - \vec{r}_{i-1} \) and \( \vec{b}_i = \vec{r}_{i+1} - \vec{r}_{i} \), respectively. For a homopolymer chain the persistence length \( \ell_p \) in three dimensions (3D) is given by

\[
\frac{\ell_p}{\sigma} = \kappa / k_{B} T. \tag{4}
\]

A cylindrical nanopore of diameter of 2\( \sigma \) is constructed by removing out particles from the center of a 2\( \sigma \) thick wall consisting of immobile LJ particles. We use the Langevin dynamics simulation following the equations of motion for the \( i \)-th monomer,

\[
m\ddot{r}_i = -\nabla \left( U_{LJ} + U_{\text{FENE}} + U_{\text{bend}} + U_{\text{wall}} \right) - \Gamma \dot{r}_i + \dot{\eta}_i. \tag{5}
\]

Here, \( \dot{\eta}_i(t) \) is the Gaussian white noise with zero mean at temperature \( T \) and satisfies the fluctuation–dissipation relation in \( d \) physical dimensions (here \( d = 3 \)),

\[
\langle \dot{\eta}_i(t) \cdot \dot{\eta}_j(t') \rangle = 2k_{B} T \delta(t - t'). \tag{6}
\]

We express length and energy in units of \( \sigma \) and \( \epsilon \), respectively. The parameters for the FENE potential in Eq. (2), \( k_{F} \) and \( R_{0} \), are set to \( k_{F} = 30\epsilon / \sigma \) and \( R_{0} = 1.5\sigma \), respectively. The friction coefficient and the temperature are set to \( \Gamma = 0.7\sqrt{\epsilon \sigma / m} \) and \( k_{B} T / \epsilon = 1.0 \). The numerical integration of Eq. (5) is implemented using the algorithm introduced by van Gunsteren and Berendsen.\(^{26}\)

The process of capture is defined as \textit{a posteriori} as follows: A monomer may arrive at the orifice of the nanopore but eventually drifts away without getting captured [see Fig. 1(b)]. Therefore, we only take into account those processes where a monomer arrives at the nanopore and eventually translocates [such as Fig. 1(i)] through the nanopore. Depending on the relative position of the monomer along the chain—it is possible that a folded conformation gets captured and translocated as discussed in Sec. III C [Figs. 4(a)–4(d)]. We keep track of separate statistics of these different capture events.

III. RESULTS

Unless otherwise specified, we choose a polymer of length \( N = 256 \) and each monomer carries a charge \( |q_i| = 1.0 \). We release the electrified polymers from different equipotentials \( \Phi \) referenced using the vertical distances \( d / \sigma = 4, 8, 12, \) and 16 from the pore (corresponding to the equipotential values \( \Phi = 0.322, 0.157, 0.101, \) and 0.071 in MD units respectively, see Table I). The release configuration of a polymer is such that the first monomer remains on the equipotential \( \Phi \), and the rest of the chain conformation remains in the lower equipotential levels away from the pore. The equipotentials are calculated numerically but exactly with appropriate boundary conditions and closely resemble those using the analytic expression by Farahpour \textit{et al.} \(^{37}\) Far away from the pore, the equipotentials are almost concentric circles but close to the pore become elliptical. We consider a fully flexible chain and chains of persistence length corresponding to \( \kappa = 3, 6, \) and 9 respectively. The initial locations of

\[\text{FIG. 5. The series of snapshots shows the multi-capture process of a single polymer by altering the voltage bias. (a) The first attempt of threading from the cis side (b) hairpin capture (type 2-1) and (c) translocation from the cis side to the trans side. (d) After the reversal of the E-field, (e) the same polymer gets captured from the bottom side of the pore. The hairpin loop structure is shown in the inset and at first both ends translocate at the same time. (f) Single end translocation begins after the unwinding of the loop.}\]
A. Translocation in a localized electric field

All of our results except Figs. 1(a)–1(f) are presented for an electric field that extends beyond the pore. To contrast those results, we show the results for an equilibrated chain for four Rouse relaxation times with an initial configuration placing a few beads inside the nanopore those experience the localized electric field strictly inside the pore. At the start of the translocation process, the beads inside the nanopore experience a downward pulling force while the tension the polymer are then chosen by uniform random sampling for a given equipotential. In addition, for certain cases, we simulated a chain length $N = 512$. For the first translocation event, we choose the negative $y$-axis to be the translocation axis (Fig. 1), the captures occur at $y = 0$, and the electric field deep inside the pore is directed along the $-y$ axis [Fig. 1(b)]. Both the diameter and width of the nanopore are chosen to be $2\sigma$ that also allows translocation of the folded configurations. For the multiple scans, the electric field is reversed accordingly across the pore.

FIG. 6. (a) Hairpin captures probability for different stiffnesses of the polymer. By increasing the chain stiffness, the single file capture (Type 1 events) represented by the blue circles (○) increases while reducing the type 2-1 event denoted by the magenta triangles (△). The symmetric capture rate (type 2 events) remains non-zero up to the chain persistence length $\ell_p = 6$ and goes to zero for stiffer chains. (b)–(e) figures show the distributions of capture locations (in reduced units) for different persistence lengths. (b) For a fully flexible polymer, capture distribution is broader, type 2-1 event occurrence has finite translocation probability along with the type 1 events. The capture location distributions for stiffer chains are shown in (c) $\ell_p = 3.0$, (d) $\ell_p = 6.0$, and (e) $\ell_p = 9.0$, respectively. With the stiffening of the chain hairpin capture, probability significantly reduces down improving the single file capture rate.

FIG. 7. (a) The scan time duration of a homopolymer in magenta circles (○) and polymer with end tags in blue diamonds (♦) as a function of the polymer persistence length. (b) Homopolymers of different stiffnesses are captured and scanned multiple times and the capture percentage of 3′ and 5′ end terminus are shown in yellow and pink bars. (c) The same bar diagrams are depicted for a polymer with charged tags located at the 5′ ends.

### TABLE I

| $d$ | $\Phi$ | $\mu$ | $\lambda_{\text{cap}} = \frac{1}{\mu}$ | $\tilde{\lambda}_{\text{cap}}$ |
|-----|--------|-------|--------------------------------------|----------------------------|
| 4   | 0.322  | 1361.76 | $7.343 \times 10^{-4}$ | $7.618 \times 10^{-4}$ |
| 8   | 0.157  | 2431.66 | $4.413 \times 10^{-4}$ | $4.112 \times 10^{-4}$ |
| 12  | 0.101  | 3636.57 | $2.749 \times 10^{-4}$ | $2.858 \times 10^{-4}$ |
| 16  | 0.071  | 5352.53 | $1.868 \times 10^{-4}$ | $1.897 \times 10^{-4}$ |
front propagates through the chain backbone in the opposite direction of translocation that first uncoils the chain. The chain is quickly sucked into the pore when the tension front hits the last bead. This entire process is demonstrated in Figs. 1(a)–1(f).

**B. Capture in an extended electric field**

However, the process in Figs. 1(a)–1(f) does not resemble an experimental situation. In an experimental situation, the polymer is first released into the solution executes a drift-diffusion motion and gets captured aided by the extended electric field beyond the pore shown in Figs. 1(g)–1(l). We use the Finite Element method to solve the Poisson equation to solve the electric field around the solitary nanopore. Unlike the case of a localized E-field, the extended E-field elongates the polymer along the field vector. The field gradient, which is the strongest at and near the pore also helps the capture process. Vollmer and de Haan have shown that the radius of gyration of the gyration ellipse deforms differently depending on the polymer size and the Péclet number. We use this idea but monitor the average radius of gyration \( \sqrt{R_g^2} \) for the entire processes from capture to translocation that shows the shape of the polymer under the influence of an extended electric field. We characterize this dynamic deformation process by comparing the transverse and longitudinal radius of gyration as the polymer drifts toward the pore. We define the longitudinal and the transverse gyration radii as

\[
\langle R_g^z \rangle = \sqrt{\langle R_g^2 \rangle}, \quad \text{(7a)}
\]

\[
\langle R_g^x \rangle = \sqrt{\langle R_g^2 \rangle + \langle R_g^2 \rangle}, \quad \text{(7b)}
\]

\[
\langle R_g^x \rangle / \langle R_g^x \rangle, \quad \text{(7c)}
\]

and construct a radius of gyration ellipse using Eq. (7c). The radius of gyration is monitored as a function of the radial distance of the first bead \( r(N_0) \) of the polymer from the pore orifice as shown in Fig. 2. We perform average over 1000 successful captured configurations (released from the equipotential \( \Phi = 0.071 \)) to get the average \( \sqrt{\langle R_g^2 \rangle} \) as a function of \( r(N_0) \). Far away from the pore, the polymer remains almost unaffected by the electric field, resembling the equilibrium configuration \( \langle R_g^z \rangle \approx \langle R_g^x \rangle \). Usually, the polymers wonder diffusively (see Fig. S1 in the supplementary material) before it drifts along the field lines and \( \langle R_g^z \rangle \) extends over \( \langle R_g^x \rangle \) enhancing the eccentricity of the gyration ellipse. The gyration ellipse eccentricity increases steadily until the polymer translocates through the nanopore. Another alternative way of characterizing the deformation of the gyration ellipse is to monitor the center of mass of the polymer, which we show in Fig. S2 in the supplementary material. Specifically, how the electric field affects the post-translocational conformations is further discussed in Sec. III E.

**C. Polymer drift and capture at the nanopore**

The process of capture requires the polymer to overcome the potential barrier by adjusting its conformational entropy resembling Kramer’s escape problem. The capture is a non-equilibrium process where field strength dominates over the diffusion near the pore. This leads to a directed motion of either end of the polymer until the polymer gets captured. During simulation, the equilibrated DNA polymers are released from different equipotentials. To implement that, we first determine the potential \( \Phi \) at a vertical distance \( d \) from the nanopore orifice and locate other points on the same equipotential surface. We use these equipotentials as the starting locations of the DNA polymer as this would be easier to compare and perform in the experiments. We define the capture probability \( P_{cap}(\Phi, t) \) of a polymer at the pore at time \( t \) released from an equipotential \( \Phi \) at time \( t = 0 \) as

\[
P_{cap}(\Phi, t) = \frac{N_p(\Phi, t)}{N_p(\Phi, 0)}
\]

where \( N_p(\Phi, t) \) is the number of polymers captured at time \( t \) at the pore and \( N_p(\Phi, 0) \) is the number released at the beginning at time \( t = 0 \) from an equipotential \( \Phi \). The capture of polymers at the pore from an equipotential are independent events (as they are released sequentially one after another), thus following a “memoryless” Poisson process and time between capture events resembles an exponential distribution. This has been measured experimentally also in a single nanopore context. Therefore, theoretically, the capture probability is the cumulative distribution function of the exponential distribution,

\[
P_{cap}(\Phi, t) = 1 - \exp(-\lambda_{cap} t).
\]

The cumulative capture probabilities for different equipotentials obtained from Brownian dynamics (BD) simulation are shown in Fig. 3(a). \( P_{cap}(\Phi, t) \) increases with time and follows Eq. (9). It is important to note that the values of the prefactor \( \lambda_{cap} \) to best fit Eq. (9) in Fig. 3(a) are almost the same as the values \( \lambda_{cap} = \frac{1}{\tau} \) obtained from the capture time distributions [Figs. 3(c)–3(f)] as shown in Table I.

Figure 3 (a) also shows the dependence of the capture time on different equipotentials. A larger value of the equipotential (closer to the pore) helps a faster capture. To get improved statistics, we monitor the journey of 1000 independent fully flexible polymers after the initial release from randomly chosen coordinates on an equipotential \( \Phi \) until they translocate. By increasing the release distance \( d \) (lowering the strength of the equipotential \( \Phi \)), our study shows that there is a finite probability [shown in Fig. 3(b)] that polymers drift away from the pore as the field strength becomes weaker at the distant equipotential surfaces. One can use this graph to define a capture radius. Though a polymer is captured, its motion barely follows the curvature of a single field line going straight through the nanopore. A successful capture often requires multiple failed attempts but with an increasing number of failed attempts, the probability of capture also gets enhanced as the polymer gyration radius gets compressed by the E-field and remains in the vicinity of the nanopore opening. Figures 3(c)–3(f) show the capture time distributions, which sum up the polymer journey from release to capture including the failed attempts. With increasing \( d \), the average capture time increases, which suggests a longer wonder time when released from a weaker potential. The process of capture is Poissonian in nature and the distribution follows the shape of an exponential distribution where the mean and standard deviation are almost identical. It is worth mentioning that the exponential distributions of Figs. 3(c)–3(f) produced
D. Modes of translocation

Storm et al. introduced the nomenclatures for polymers threading into the pore with three different conformations (type 1, type 2-1, type 2) events, depending on the relative location of the chain with respect to the pore. Mihovilovic et al. further studied and quantified translocation of these folded conformations. We observed these separate events in our simulation shown in Figs. 4(a)–4(h). In type 1 events, one end of the polymer threads [Figs. 4(a) and 4(e)], while in type 2-1, a random location capture occurs. The symmetric threading is a type 2 event [Figs. 4(b), 4(c), 4(f), and 4(g)]. Not only do we observe these threading conformations in our simulation studies but also our simulation provides the fraction of events belonging to these three categories shown Figs. 4(m)–4(p) insets. For a closer inspection, we observe these threading conformations in our simulation movies. In addition, from Fig. 6(e), it is evident that only beyond \( \ell_p = 6.0 \), type 1 capture probability is higher than hairpin capture (type 2-1 and type 2) probability, and \( \ell_p = 6.0 \) serves as a critical point between these two events. To understand how the chain stiffness affects the capture process in the presence of a field gradient, we have studied the capture location distributions shown in detail in Figs. 6(a)–6(d). For a fully flexible chain (\( \ell_p = 0 \)), all three types of capture occur but with the increased stiffness, as expected, the single file captures become predominant. It is important to note that for \( \ell_p = 3.0 \), Fig. 6(c)—that corresponds to the persistence length of a dsDNA under most experimental conditions, the distributions of the capture locations obtained from the BD simulation closely resemble those obtained experimentally by Mihovilovic et al. Moreover, our BD simulation indicates that the pronounced peak (at low values of x) corresponding to the type 1 capture might be a general feature of the polymer capture mechanism as it is observed in the experiment and the “all-atom-MD” simulation.

We further verify that for a longer chain with \( N = 512 \), the capture location distributions for different stiffness are qualitatively similar; however, the probability of the type 1 capture increases compared to the other two types of events. This enhancement of type 1 events with the increased chain length is more prominent for the fully flexible polymer and does not alter the distribution as such for the cases with higher persistence lengths, where type 1 is the only predominant event.

E. Post-translocation compression

Translocation being a faster process in the presence of an electric field gradient, the polymer configuration gets compressed in the post translocation phase. Figure 2 demonstrates the compression factor as the area of the gyration ellipse decreases by more than 20% compared to its pre-translocation stage. After translocation, both the eccentricity and area of \( (R_g) \) ellipse remain constant but the fluctuation in \( (R_g) \) increases (the pink cloud in Fig. 2) as it enters into the diffusive domain.

F. Multiple recaptures of a translocated polymer

It is the faster speed of the translocating DNA in a solitary nanopore that makes the current blockade measurements noisy for sequencing purposes. To overcome this issue, multiple recaptures of the same molecule can be a viable option that relies on increased statistics, hence, enhancing the accuracy of the measurement. In our simulation setup, we reverse the voltage bias after a successful translocation as the center of mass of the polymer moves 20\( \alpha \) away from the pore and study the polymer dynamics as a function of its persistence length. During the multi-capture events, statistics are collected for six independent runs, each containing 100 scans unless the polymer drifts away. Figure 5 demonstrates the recapture events for a polymer having the persistence length \( \ell_p = 3.0 \). Our study shows that even in the extremely narrow nanopore limit (pore diameter of 2\( \sigma \)), hairpin capture probability dominates over the single file translocation events for semi-flexible polymers (see the simulation movies). In addition, from Fig. 6(e), it is evident that only beyond \( \ell_p = 6.0 \), type 1 capture probability is higher than hairpin capture (type 2-1 and type 2) probability, and \( \ell_p = 6.0 \) serves as a critical point between these two events. To understand how the chain stiffness affects the capture process in the presence of a field gradient, we have studied the capture location distributions shown in detail in Figs. 6(a)–6(d). For a fully flexible chain (\( \ell_p = 0 \)), all three types of capture occur but with the increased stiffness, as expected, the single file captures become predominant. It is important to note that for \( \ell_p = 3.0 \), Fig. 6(c)—that corresponds to the persistence length of a dsDNA under most experimental conditions, the distributions of the capture locations obtained from the BD simulation closely resemble those obtained experimentally by Mihovilovic et al. Moreover, our BD simulation indicates that the pronounced peak (at low values of x) corresponding to the type 1 capture might be a general feature of the polymer capture mechanism as it is observed in the experiment and the “all-atom-MD” simulation.

We further verify that for a longer chain with \( N = 512 \), the capture location distributions for different stiffness are qualitatively similar; however, the probability of the type 1 capture increases compared to the other two types of events. This enhancement of type 1 events with the increased chain length is more prominent for the fully flexible polymer and does not alter the distribution as such for the cases with higher persistence lengths, where type 1 is the only predominant event.

G. Enhancement of capture rate by end tagging

Attaching a "charged tag" to a dsDNA provides additional information about its translocation dynamics through a nanopore. A possible experimental realization for tagging the dsDNA with a positive charge has been demonstrated. In our simulation, we enhance the charge content of the last six monomers of one end (let us call it 5’ end) by three times of a normal monomer (\( q_m = 3q \)) and apply the same recapture method to demonstrate the effect of end tagging on the capture and translocation process. We first monitor the scan time duration (capture time + translocation
time) of a homopolymer varying its persistence length. Figure 7(a) (magenta circles) shows that the scan time increases non-linearly with an increase in the chain stiffness and the error bar in scan time widens, which signifies a large variation in capture time for the stiffer chains. To reduce the uncertainties of capture, we use the end tagging method [see the insets of Fig. 7(a)] and blue diamonds confirm that end tags not only conclusively lower the scan time for the stiffer chains but the error range also gets reduced.

In reality, without the data post-processing, it is almost impossible to know which end of the polymer threads into the pore during each recapture scan. By charge tagging the 5’ end, we potentially break the degeneracy as the tags with high charge content are prone to enter into the nanopore first due to the stronger pull of the electric field. Figure 7(b) shows that the capture probability of the 3’ and 5’ ends is almost equal for a homopolymer even with different stiffnesses, hence degenerate. Our simulation confirms that these degeneracies are broken and the 5’ end has a significantly higher probability of entrance when tags are present at the 5’ end as shown in Fig. 7(c). This tagging prioritizes the 5’ → 3’ uni-directional translocations from either side of the pore during multi-recapture and scans, which can potentially benefit the single nanopore bar-coding and sequencing experiments where uni-directional reads would be preferred. Moreover, one can make the end terminus oppositely charged, which would, in principle, further promote the uni-directional translocations due to the favorable attractive interaction at one end and a repulsive force on the other end in the presence of an extended electric field.

IV. DISCUSSION AND CONCLUSION

In this article, we report the details of the capture and translocation of a DNA polymer as it meanders its way to the pore entrance from different equipotentials. Capture from different equipotentials affects the conformations at the pore entrance as well as the translocation speed. Our studies reveal the details of multiple failed attempts and eventual success in the presence of an extended E-field, quantify and differentiate accurately the characteristics of translocation of both straight and folded conformations. We analyze further details of the folded coordinates in reference to the entire chain. Based on these results, one can make the polymer stiffer to decrease the folded configuration and, hence, improve the accuracy of the experimental protocol using a solitary nanopore. We also demonstrate the model is capable of reproducing all the details of an actual experiment and Mimovich et al. We introduce a charge tag at one end of the dsDNA and demonstrate that the scan time and its variability are significantly reduced especially for the stiffer chains and uni-directional capture, probability increases by lifting the degeneracy of which end threads through the nanopore during the multi-capture process. We expect this work will promote future experimental studies on repeated scanning of a biopolymer through the nanopore and can be useful for a large community involved in various types of biopolymer translocations.

SUPPLEMENTARY MATERIAL

The details of the first bead r(N0) motion as a function of the BD-time and the change in the polymer’s radius of gyration as a function of the center of mass movement are included in the supplementary material.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Swarnadeep Seth: Data curation (equal); Formal analysis (equal); Visualization (equal); Writing – original draft (equal); Writing – review & editing (equal). Aniket Bhattacharya: Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal)

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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