Translocation of a Single Stranded DNA Through a Conformationally Changing Nanopore

O. Flomenbom and J. Klafter

School of Chemistry, Raymond & Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel

(Dated: 2nd February 2008)

We investigate the translocation of a single stranded DNA through a pore which fluctuates between two conformations, using coupled master equations. The probability density function of the first passage times (FPT) of the translocation process is calculated, displaying a triple, double or mono peaked behavior, depending on the interconversion rates between the conformations, the applied electric field, and the initial conditions. The cumulative probability function of the FPT, in a field-free environment, is shown to have two regimes, characterized by fast and slow timescales. An analytical expression for the mean first passage time of the translocation process is derived, and provides, in addition to the interconversion rates, an extensive characterization of the translocation process. Relationships to experimental observations are discussed.

PACS numbers: 87.14.Gg, 87.15.Aa, 87.15.He

INTRODUCTION

Translocation of biopolymers through a membrane pore occurs in a variety of biological processes, such as gene expression in eucaryotic cells, conjugation between procaryotic cells, and virus infection. The importance of translocation in biological systems and its applications, have been the motivation for recent theoretical and experimental work on this topic. In experiments one usually measures the time it takes a single voltage-driven single stranded DNA (ssDNA) to translocate through a hemolysin channel of a known structure. Since ssDNA is negatively charged (each monomer has an effective charge of $q$, where $q$ is the electron charge, and $z$ is controlled by the solution pH and strength), when applying a voltage the polymer is subject to a driving force while passing through the transmembrane pore part (TPP) from the negative (cis) side to the positive (trans) side. The presence of the ssDNA in the TPP blocks the cross-TPP current, one can deduce the FPT probability density function (pdf), $F(t)$, from the current blockade duration times.

Experiments by Kasianowicz et al. (1996), show $F(t)$ with three peaks. It was suggested that the short-time peak represents the non-translocated events, while the other two, longer-time peaks, represent translocation events of different ssDNA orientations. In addition, the times that maximize the translocation peaks were shown to be proportional to the polymer length and inversely proportional to the applied field. In experiments by Meller et al. (2001), $F(t)$ was shown to be mono-peaked, with a corresponding maximizing time that has an inverse quadratic field dependence. More recently, Bates et al. (2003) measured the FPT cumulative probability function (cpf), which is the probability to exit the channel until time $t$, $G(t) = \int_0^t F(s)ds$, in a field-free environment. $G(t)$ was approximated by two well separated timescales with the ratio of 1/20.

In previous theoretical works, the translocation of a ssDNA through a nanopore was described by statistical models that focused on calculating the free energy of the process as a function of the translocation state. The free energy contained terms that represent the entropy and the chemical potential of the polymer parts on both sides of a zero thickness membrane. The role of the membrane thickness was studied by Ambjörnsson et al. (2002), Solonika and Kolomeisky (2003), and Flomenbom and Klafter (2003a). The obtained free energy was used to calculate the mean first passage time (MFPT), which asymptotically was found to scale linearly with the polymer length for a field-biased process. This is the expected MFPT dependence of a Markovian biased random walk in a finite interval.

A different approach was suggested by Lubensky and Nelson (1999) and was further developed by Brezhkovskii and Gopich (2003), where a diffusion-convection equation was used to describe the translocation process, under the assumption that the polymer parts outside the membrane hardly affect the translocation. Brezhkovskii and Gopich (2003) showed that by changing the cis absorbing end to be partially absorbing, the mono-peaked $F(t)$ obtained by Lubensky and Nelson (1999) changes to a superposition of a decaying non-translocation pdf and a peaked translocation pdf. Using the fractional Fokker-Planck equation, Metzler and Klafter (2003) suggested an explanation for the slow relaxation time of the experimentally obtained $G(t)$. We have shown by using the master equation (ME) that $F(t)$ can be double or mono-
peaked, depending on the applied field and on the initial condition (Flomenbom and Klafter, 2003a).

In the approaches summarized above the structure of the pore is taken to be rigid, namely, governed by a single conformation. Although it is known that the α-hemolysin channel has a rigid structure that allows its crystallization (Song et al., 1996), during the translocation of a long polymer (larger than the pore length) with the same width that of the channel at some cross sections along the channel, small fluctuation in the channel structure may occur, which give rise to a more complex process than what was assumed so far. In this work we relax the assumption of a single pore conformation and introduce a second conformation coupled to the first one. In a continuum formalism, the process takes place in an effective two-dimensional system, where one dimension represents the translocation itself, and the second dimension represents the structural fluctuations. This picture is richer and is more realistic, since small structural changes in physiological conditions are known to occur in large biomolecules, certainly during interaction with other large biomolecules.

The function that represents best the translocation process is $F(t)$ (or its integral $G(t)$). The dependence of $F(t)$ on the system parameters, we learn about the important degrees of freedom which participates in the translocation process. The characteristics of $F(t)$ are the dependence of its shape, moments, and times that maximize its peaks on the system parameters. Using the generalized model that takes into account fluctuations in the pore structure, we calculate $F(t)$ and show that it can display one two or three peaks, depending on the applied voltage, the temperature and the interconversion rates between the two conformations. Analytical expressions for the MFPT are derived and related to the experimental findings. In addition, we calculate the cumulative probability $G(t)$ in the field-free limit, and show that it also provides valuable information about the system parameters. Thus, these tools help in gaining insight into the translocation of a polymer through a narrow pore, and in explaining the diversity of the experimental observations (Kasianowicz et al., 1996; Meller et al., 2001).

**THEORETICAL MODELLING**

**Basic Model**

The basic model we use to describe the translocation relies on a one-dimensional process. To use this simplification, we map the three-dimensional translocation process onto a discrete one-dimensional space containing $n (= N + d - 1)$ states separated from each other by a unit length $b$. The translocation takes place within a TPP of a length that corresponds to $d$ monomers. An $n$-state ME is introduced to describe the translocation of an $N$-monomer long ssDNA subject to an external voltage $V$ and temperature $T$. The occupation pdf of the $j$ state is $[P(t)]_j = P_j(t)$, where the state index $j$ determines the number of monomers on each side of the membrane and within the TPP ($m_j$). $P_j(t)$ satisfies the equation of motion:

$$\frac{\partial P_j(t)}{\partial t} = a_{j+1,j} P_{j+1}(t) + a_{j-1,j} P_{j-1}(t) - a_{j,j+1} P_{j+1}(t) - a_{j,j-1} P_{j-1}(t),$$

under absorbing boundary conditions on both sides of the membrane (the polymer can exit the TPP on both sides). Eq. 1 can be written in a matrix representation:

$$\frac{\partial \mathbf{P}(t)}{\partial t} = \mathbf{A} \mathbf{P}(t),$$

where the propagation matrix $\mathbf{A}$ is a tridiagonal matrix that contains information about the transitions between states in terms of rate constants, $a_{j,j\pm1}$, which are given by:

$$a_{j,j\pm1} = k_j p_{j\pm1}.$$  

Here $k_j$ is the rate to perform a step, $p_{j,j-1}$ ($p_{j,j+1}$) is the probability to move one state from state $j$ to the trans (cis) side, and $p_{j,j+1} + p_{j,j-1} = 1$.

$k_j$ is taken to be similar to the longest bulk relaxation time of a polymer (Doi and Edwards, 1986):

$$k_j = 1/(\beta \xi_p b^2 m_j^2) \equiv R/m_j^2; \quad \beta^{-1} = k_B T,$$

with two exceptions: the parameter $\xi_p$ represents the ssDNA-TPP interaction and cannot be calculated from the Stokes relation, and $\mu$ serves as a measure of the polymer stiffness inside the confined volume of the TPP, and is bounded by the conventional values (Doi and Edwards, 1986): $0 \leq \mu \leq 1.5$.

Assuming a quasi-equilibrium process, which enables using the detailed balance condition, and using the approximation $a_{j,j-1}/a_{j,j-1} \approx p_{j,j-1}/(1-p_{j,j-1})$, the probability $p_{j,j-1}$ is found to be:

$$p_{j,j-1} = (1 + e^{\beta \Delta E_j})^{-1}.$$  

The free energy difference between states, $\Delta E_j = E_{j-1} - E_j$, is computed considering three contributions: electrostatic, entropic, and an average attractive interaction energy between the ssDNA and the pore. More explicitly, $\beta \Delta E_j$ is given by $\beta \Delta E_j = \beta \Delta E_j^p + \delta_j$, where $\beta \Delta E_j^p \leq 0$ represents the effect of the field which directs towards the trans-side and $\delta_j > 0$ (for $j > d$) represents an effective directionality to the cis-side, which originates from the entropic factors and the average attractive interaction energy between the ssDNA and the pore. For a more detailed discussion see Flomenbom and Klafter (2003a).

Several features emerge from the simple one-dimensional model. For homopolymers, poly-$dn$u, where $nu$ stands for the nucleotide type, we estimate $\xi_p(A_{nu}) \approx 10^{-4}meV/\text{nm}^2$, $\xi_p(C_{nu}) = \xi_p(T_{nu}) = \xi_p(A_{nu})/3$ and $\mu(C_{nu})=1$, $\mu(A_{nu})=1.14$, $\mu(T_{nu})=1.28$. Here $A_{nu}$, $C_{nu}$ and $T_{nu}$ stand for adenine, cytosine and thymine nucleotides, respectively. Interestingly, $\xi_p$ is three order of magnitude larger than the bulk friction constant,
which is consistent with the role assign to \( \xi_p \) to represent the interaction between the polymer and the channel.

In addition, from the expressions for \( \beta \Delta E_j \) and \( p_{j-1,j} \), the important parameter \( V/V_C \equiv \beta \zeta_q |V(1+1/d) | \) comes out naturally. This ratio determines the directionality of the translocation, and, in particular, for \( V/V_C > 1 \) there is a bias towards the trans-side of the membrane.

**Translocation Through a Conformationally Changing Pore**

A more realistic description of the translocation can be obtained by taking into consideration fluctuations in the TPP, either spontaneous or interaction induced. Accordingly, we introduce an additional pore conformation which is represented by the propagation matrix \( B \). The changes in the pore conformation between \( A \) and \( B \) are controlled by the interconversion rates, \( \omega_A \) and \( \omega_B \). \( \omega_A (\omega_B) \) is the rate of the change from the \( A (B) \) to the \( B (A) \) pore conformation.

The physical picture of the process is that when the pore conformation changes, a different environment is created for the ssDNA occupying the TPP. This implies a change in \( \xi_p \) and \( \mu \). For a large polymer, \( N > d \), we take \( B \cong \lambda A \), where \( \lambda \) is a (dimensionless) parameter that represents the effect of the conformational change on \( \xi_p \) and \( \mu \). The parameter \( \lambda \) may be interpreted as a measure of an effective available volume in the TPP, when the amino acids residues protruding the TPP change their positions.

The equations of motion of the ssDNA translocation through the fluctuating pore, written in matrix representation, are:

\[
\frac{\partial}{\partial t}\begin{pmatrix}
\bar{P}(t;A) \\
\bar{P}(t;B)
\end{pmatrix} = \begin{pmatrix}
A - \omega_A & \omega_B \\
\omega_A & B - \omega_B
\end{pmatrix}\begin{pmatrix}
\bar{P}(t;A) \\
\bar{P}(t;B)
\end{pmatrix},
\]

where \( \bar{P}(t; i) \), \( i = A, B \) is the occupation pdf vector of configuration \( i \), \( \omega_i = \omega_I \), and \( I \) is the unit matrix of \( n \) dimensions.

As a general note we refer to the form of Eq. 6, which was used to study the resonant activation phenomenon [Bar-Haim and Klafter, 1999]. This phenomenon, which was first reported by Doering and Gadoua (1992), is the occurrence of a global minimum in the MFPT as a function of the interconversion rate for a system in which \( \omega_A = \omega_B \). Because of the assumption \( B = \lambda A \), the system investigated here cannot exhibit this phenomenon [Flomenbom and Klafter, 2004].

**DENSITY OF TRANSLOCATION TIMES**

**Parameters Tuning**

To study the translocation of ssDNA through a fluctuating pore, we start by computing \( F(t) \). Formally, \( F(t) \) is defined by

\[
F(t) = \frac{\partial (1 - S(t))}{\partial t}.
\]

Here, \( S(t) \) is the survival probability, namely, the probability to still have at least one monomer in the pore, and is given by summing the elements of the vector that solves Eq. 6; see Appendix A for details. Using the known values of \( \xi_p \) and \( \mu \) from the single conformation model, we examine in this subsection the effect of the parameters \( \lambda, \omega_A \) and \( \omega_B \) on \( F(t) \).

First, we check the effect of \( \lambda \) on \( F(t) \) for several limiting cases. For \( \lambda = 0 \), movement in any direction occurs only under the A conformation environment. The B conformation traps the polymer for a period of time governed by the interconversion rates. For \( \lambda = 1 \), namely, \( B = A \), the environmental changes do not affect the translocation, and the process reduces to a translocation through a single conformation. For \( \lambda > 1 \) the environmental changes enhance the process. In this paper we restrict ourselves to the range \( 0 \leq \lambda \leq 1 \).

The picture is less intuitive for intermediate values of \( \lambda \). Fig. 1 shows that by choosing \( \lambda \) properly, three peaks in \( F(t) \) can be obtained. In particular, as shown in the inset of Fig. 1, the range of \( \lambda \) values for which \( F(t) \) exhibits three distinct peaks is \( 0.1 \leq \lambda \leq 0.30 \). For the single conformation case we found that \( F(t) \) can be either mono or double peaked depending on \( V/V_C \), and on the initial state of the translocation \( x \). The short time peak represents the non-translocated events, while the long time peak represents the translocation events. The generalization for two pore conformations may yield two translocation peaks in addition to a short time non-translocation peak. Indeed, Fig. 1 supports the expected behavior for the limiting \( \lambda \) values, and shows that as \( \lambda \to 1 \), \( F(t) \) possess only one translocation peak, as well as for \( \lambda \to 0 \), where the B conformation peak spreads out towards larger times, which results in its disappearance.

Although Fig. 1 is obtained for a given value of the interconversion rates, our explanations regarding the \( F(t) \) transection process are

![Figure 1 Poly-dTtrc, F(t), for several values of λ, with: N=30, d=12, z=N + d/2, T=2°C, V/V_C=2, \( \omega_B=10^5 \) Hz, \( \omega=1 \), and \( \xi \approx 1/2 \). The left peak represents the non-translocated events, whereas the other two peaks represent translocation. Inset: The range for which \( \lambda \) yields three peaked \( F(t) \) is shown to be \( 0.1 \leq \lambda \leq 0.3 \), when given the above parameters.](image-url)
behavior for the limiting cases $\lambda=1,0$, are valid for any system conditions. This is demonstrated by calculating the MFPT (Appendices B and D). In Appendix B we show that when $\lambda=1$, the MFPT of the two configurations model reduces to that of the single conformation model. In Appendix D we show that for $\lambda=0$, the B conformation contribution for the MFPT is a term which is inversely proportional to the interconversion rate, $\omega_B^{-1}$.

Therefore, $\lambda$ serves as a tuning parameter that leads to either one or two actual translocation peaks in $F(t)$. The question of interest is how $\lambda$ depends on the system parameters. We assume a small field perturbation in the regime of biological interest $|0 < V / V_C | \leq 3$, using $V_C \approx 50mV$ [Flomenbom and Klafter (2003)], so that $\lambda(V)$ follows $\lambda \approx \lambda_0 + V / V_\lambda$, and keeping $\lambda(V) \leq 1$. Here $\lambda_0$ and $V_\lambda$ might be expansion coefficients, where $\lambda_0 \ll 1$ is implied from recent experiments [Bates et al. 2003], as we discuss later. The process can be viewed such that as the voltage increases, amino acids residues protruding the TPP that constitute obstacles for the translocating ssDNA clear the way. While the $\lambda$ dependence on the voltage is assumed here, its dependence on other system parameters (e.g., temperature and pH) is unknown and is folded into $V_\lambda$.

To check how interconversion rates affect $F(t)$, it is convenient to define two dimensionless parameters, $\omega \equiv \omega_A / \omega_B$ and $\omega_B / k$ (or $\omega_A / k$), where $k$ is the dominate rate of the A conformation for a sufficiently large $N$, $k = R / \rho \nu$. The first ratio set the dominance of a given conformation over its counterpart; namely, for $\omega \ll 1$ most of the translocation events take place in the A conformation. The second ratio gives an estimate of the number of moves done in a given conformation before a change in the pore structure occurs, and thus relates the ssDNA dynamics to the structural changes dynamics.

As shown in Fig. 2 and in the inset of Fig. 2, $F(t)$ exhibits two peaks corresponding to actual translocation only when $\omega \approx 1$. For $\omega \ll 1$ and $\omega \gg 1$ only one peak corresponding to an actual translocation survives. For all cases there is a peak representing non-translocation events. In addition, we find that for two translocation peaks to be obtained, the ratio $\omega_B / k$ (or $\omega_A / k$ due to $\omega \approx 1$) must fulfill $\omega_B / k \leq 10^{-3}$ (data not shown). The lower limit of the interconversion rates is inversely proportional to the order of the measurement time otherwise only one conformation will be detected.

Finally, we assume that the rate of the conformational changes is controlled mainly by temperature; namely, we take $\omega_A$ and $\omega_B$ as voltage independent in the regime of biological interest: $0 \leq V / V_C \leq 3$.

**Translocation Velocity**

To study further the translocation process, we check the voltage dependence of the times that maximize the peaks of $F(t)$, denoted as $t_{m,i}$ where $i=1,2,3$ (e.g. $t_{m,1}$ characterized the short time peak). In previous works [Flomenbom and Klafter (2003)], $t_{m,1}$ for one translocation peak was regarded as the most probable average velocity of the translocation (up to a multiplicative constant). We show below that our assumptions regarding the voltage dependence of the system parameters, yield either linear or quadratic dependence of the translocation velocity on the voltage, and can be used to explain the different experimental observations. For the single conformation case, we showed that $t_{m,2}^{-1}$ scales linearly with $V / V_C$, when the initial state of the translocation is near the cis-side of the membrane [Flomenbom and Klafter (2003)]. Fig. 3b shows that the linear scaling of $t_{m,2}^{-1}(V / V_C)$ persists. However, $t_{m,3}^{-1}(V / V_C)$ (Fig. 3c) displays a quadratic behavior, which is a consequence of the form of $\lambda(V)$, as discuss in the next section when computing the MFPT. On the other hand, setting $V_T = 120mV$ leads to one translocation peak, and to small deviations from linearity towards a weak quadratic behavior of $t_{m,2}^{-1}(V / V_C)$; see Fig. 4.
dependence of \( t \) early and quadratically on \( V \). The solid line is a polynomial fit. Inset: behave qualitatively the same as for the case \( V \).

Figure 3 a: \( t_{m,i} \) for poly-\( dT_{nu} \), as a function of \( V/V_C \) for the same parameters as in Fig. 1 and \( V_\lambda = 350mV \). \( t_{m,1} \) is almost independent of \( V_C/V \) in contrast to the pronounced dependence of \( t_{m,2} \) and \( t_{m,3} \). b-c: \( t_{m,2}^{-1} \) and \( t_{m,3}^{-1} \) depend linearly and quadratically on \( V/V_C \), respectively. The solid lines through the circles are polynomial fits.

Figure 4 \( t_{m,2}^{-1} \) for poly-\( dT_{nu} \) for the same parameters as in Fig. 3 except for \( V_\lambda = 120mV \). This value for \( V_\lambda \) leads to 0.625 \( \leq \lambda(V) \leq 0.875 \) and accordingly for one translocation peak. The solid line is a polynomial fit. Inset: \( t_{m,1} \) and \( t_{m,2} \) behave qualitatively the same as for the case \( V_\lambda = 350mV \).

a function of \( V_\lambda \). Thus, varying \( V_\lambda \) we obtain different behaviors of the translocation, which can be related to the different experimental observations.

**THE MFPT**

**Small Field Biased Translocation**

We now turn to calculate the MFPT, which allows for an analytical estimation of the characteristic times of the FPT pdf and cdf. In general, the \( m \) moment of \( F(t) \) is calculated by raising to the \( m \) power the inverse of the propagation matrix. For the two conformations translocation this matrix is given on the right hand side of Eq. 6.

After somewhat lengthly calculations, which are given Appendices B and C, the expression for the MFPT, \( < \tau > \), reads:

\[
< \tau > \approx \frac{1}{\lambda} \left[ (\lambda P_{A,0} + P_{B,0}) + \tau(\omega_A + \omega_B)/2 \right],
\]

where \( \tau \) is the MFPT for the single configuration model, and is given by Eq. C5, and \( P_{A,0}(P_{B,0}) \) is the probability that the process starts in conformation \( A \) (\( B \)). For \( P_{A,0} \) and \( P_{B,0} \) the equilibrium condition is assumed, \( P_{A,0} = \omega_A/(\omega_A + \omega_B) \) and \( P_{B,0} = 1 - P_{A,0} \).

Eq. 8 is valid for not too high fields, \( V/V_C \gtrsim 1 \), and the relations between the interconversion with \( k \) found in the previous section, \( \omega_A/k, \omega_B/k \ll 1 \). The first term in the brackets of Eq. 8, \( \lambda P_{A,0} + P_{B,0} \), represents the translocation peaks and can be compared with \( t_{m,2} \) and \( t_{m,3} \). The second term in the brackets, \( \tau(\omega_A + \omega_B)/2 \), represents the coupling time cost, and is of the order of \( o(10^{-2}) \) for voltages that obey \( V/V_C \gtrsim 1.5 \). Keeping the first term in Eq. 8, we have

\[
< \tau > \approx \frac{2\pi \xi \omega^2 \epsilon^2}{eV(1 + 1/d)} \frac{1}{V-V_C} \left( P_{A,0} + P_{B,0} \frac{V_\lambda}{V} \right) \tag{9}
\]

where \( x \approx N \) for a translocation process that starts near the cis-side of the membrane.

Eq. 9 provides a solid basis for the numerically obtained dependence of the translocation velocity on the voltage. \( < \tau > \) consists of two terms that can be attributed to the \( A \) (first term in the brackets) and \( B \) (second term in the brackets) pore conformations. For \( V_\lambda \approx 120mV \) we can replace the expression in brackets by one in the relevant voltages window. Thus, we find that \( < \tau > \approx \infty \left( V-V_C \right)^{-1} \), which implies that \( F(t) \) has one translocation peak for this choice of \( V_\lambda \). For higher values of \( V_\lambda \) and voltages of biological interest, the two terms in the brackets are separated. This leads to a term that represent the \( A \) conformation and scales as \( (V-V_C)^{-1} \), and a term that represents the \( B \) conformation that scales as \( \left[ V(V-V_C) \right]^{-1} \).

Accordingly, Eq. 9 captures the physical essence of the translocation of the ssDNA through the conformationally changing pore, under a relatively small field.

**Field-Free Translocation**

In recent field-free experiments by Bates et al. (2003), the cdf \( G(t) = \int_0^t F(s)ds \) was shown to have two regimes that were approximated by a fast and a slow timescales, \( \tau_1 \) and \( \tau_2 \), with the ratio \( \tau_1/\tau_2 \approx 1/20 \). Motivated by these experimental result, which implies ,within our approach, that \( \lambda_0 \) fulfills \( \lambda_0 \ll 1 \), we study in this subsection
the zero field translocation, \( V \to 0 \). We start by computing \( G(t) \) for a translocation process that starts at the middle state, \( x = n/2 \). This is the the same initial condition that was imposed in the experiments \cite{Bates2003}. As shown in Fig. 5 (full curve), \( G(t) \) displays two regimes, a fast increase at short times and a slow increase from intermediate to large times. Accordingly, we try the approximation

\[
G_{ap}(t) \approx 1 - (P_{A,0} e^{-t/\tau_1} + P_{B,0} e^{-t/\tau_2}).
\]  

(10)

Matching the first and the second moments obtained from \( F(t) \) and from the approximated \( F(t) \) we find that the characteristics timescales of \( G_{ap}(t) \) are (see Appendix D):

\[
\tau_1 = \tau(1 + 3\omega/2); \quad \tau_2 = \tau(1/2 + \omega) + 1/\omega_B, \quad \tau \leq 1/\omega_B.
\]  

(11)

which, when used in \( G_{ap}(t) \), lead to the dashed curve plotted in Fig. 5. Also shown, dotted curve, is a modified version of \( G_{ap}(t) \), where \( \tau_1 \to t_m \) is used in Eq. 10. Note that for the short times, \( t < \tau_1 \), the later approximation fits better \( G(t) \), but from intermediate times, \( t > 3\tau_1 \), \( G_{ap}(t) \) and \( G(t) \) coincide.

The two conformations model produces a temporal behavior that agrees with experimental observation, and provides a good explanation for it. In the limit, \( V \to 0 \), the \( B \) conformation acts as a trapping conformation; namely, the polymer is stuck in its position when subject to the environment due to the \( B \) conformation. Movement occurs only through the \( A \) conformation. As a result two regimes are obtained for \( G(t) \). The fast increase in \( G(t) \) at short times, is a consequence of exiting due to the \( A \) conformation (at either side of the membrane), while the slow saturation at longer times is a result of the release from the trapping in the \( B \) conformation.

In the inset of Fig. 5 we show both \( F(t) \), the approximate \( F(t) \), and the modified version of the approximation, which is obtained when using \( \tau_1 \to t_m \). Because the process starts in the middle state, \( x=n/2 \), \( F(t) \) has only one peak, which coincides with previous results \cite{Flomenbom2003}. Although the approximate \( F(t) \), or any other approximation of two exponentials with positive coefficients, does not exhibit a peaked shape, information about the maximal peak value of \( F(t) \) and the interconversion rates can still be extracted from \( G_{ap}(t) \) timescales by using Eq. 11. For example, the timescales suggested by Bates et al. (2003), imply that \( t_m \approx 165 \mu s \), and \( \omega_B \approx 300 Hz \).

**CONCLUSIONS**

The model introduced here describes the translocation of ssDNA through a fluctuating pore structure. As a consequence the ssDNA within the transmembrane pore is exposed to a changing environment, which can be reflected in the first passage times pdf, \( F(t) \). By computing \( F(t) \), comparing our results to experimental results, and using physical arguments, we obtained theoretically a behavior which was previously observed experimentally - \( F(t) \) having three peaks. This behavior is obtained by tuning the dimensionless parameter \( \lambda \), which controls the effect of the change in the pore structure on the translocating ssDNA, and the interconversion rates between the pore conformations, \( \omega_A \) and \( \omega_B \). In particular, \( \lambda \) has to fulfill \( 0.1 \leq \lambda \leq 0.30 \), and the interconversions rates have to be of the same order of magnitude, and much smaller than the typical rate of the \( A \) pore conformation, \( k, \omega_B/k \leq 10^{-3} \). This implies that the relaxation timescale of the ssDNA in the pore is much shorter than of the pore conformational changes timescale. From these relations the maximal values of the interconversion rates can be deduced from the value of \( k \), given by Eq. 3, to be: \( \omega_A \approx \omega_B = 10^2 Hz \).

We have been able to show, both numerically and analytically, that the times that maximize the \( F(t) \) actual translocation peaks, \( t_{m,i}, i = 2,3 \), and the MFPT, are inversely proportional to the first or the second power of the field, depending on \( V_A \). This emphasizes the crucial role of \( V_A \) on the translocation extracted functions, and may explain the different experimental results for \( F(t) \) discussed in the introduction, meaning that \( V_A \) is sensitive for the specific experimental set up, and biological conditions.

The probability to exit the channel until time \( t \), \( G(t) \), in a field-free environment, has been shown to have two regimes that can be approximated by two timescales, \( \tau_1 \) and \( \tau_2 \), which are about one order of magnitude apart, and are closely related to the, \( \tau \), \( t_m \), and the interconversion rates: \( \tau_1 = \tau(1 + 3/2\omega) \), or \( \tau_1 \to t_m \), and \( \tau_2 = \tau(1/2 + \omega) + 1/\omega_B \). From these relations the interconversion rates can be deduced when analyzing experimental data.
we acknowledge fruitful discussions with Ralf Metzler and with Amit Meller, and the support of the US-Israel Binational Science Foundation and the Tel Aviv University Nanotechnology Center.

APPENDIX A

In this appendix we introduce the formal solution of Eq. 6 and define the symbols used in next derivations. In general, $S(t)$ for a discrete system is given by summing the elements of the vector that solves the ME,

$$S(t) = \mathbf{U}_n e^{t \mathbf{D}} \mathbf{E}^{-1} \mathbf{P}(0|2n).$$

(A1)

Here $\mathbf{U}_n$ is the summation row vector of $2n$ dimensions, $\mathbf{P}(0|2n)$ is the initial condition column vector,

$$[\mathbf{P}(0|2n)]_j = (P_{A,0}\delta_{x,j} + P_{B,0}\delta_{x,n+j}),$$

(A2)

where $x$ is the initial state of the translocation process. The definite negative real part eigenvalues matrix, $\mathbf{D}$, is obtained through the similarity transformation: $\mathbf{D} = \mathbf{E}^{-1} \mathbf{H} \mathbf{E}$, which is given on the right hand side of Eq. 6, and $\mathbf{E}$ and $\mathbf{E}^{-1}$ are the eigenvectors matrix, and its inverse, of $\mathbf{H}$.

APPENDIX B

Here we calculate formally the MFPT $< \tau >$. The $m$ moment of $F(t)$ is given by: $< \tau^m > = \int_0^\infty t^m F(t) dt = m! \mathbf{U}_n (-\mathbf{H})^{-m} \mathbf{P}(0|2n)$. To calculate the inverse of the propagation matrix $\mathbf{H}$, which is given on the right hand side of Eq. 6, we use the projection operator [KlafTer and Silber, 1981; Zwanzig, 2001]: $\mathbf{Q_H} \equiv \mathbf{H}_{QQ} = \mathbf{A} - \omega_A$, $\mathbf{H}_{QZ} = \omega_B$, $\mathbf{H}_{ZQ} = \omega_A$, $\mathbf{H}_{ZZ} = \mathbf{B} - \omega_B$, and the identity, $\mathbf{I} = \mathbf{H_M}$, and obtain M blocks:

$$\mathbf{M}_{QQ} = [\mathbf{A}_{QQ} - \mathbf{A}_{QZ} (\mathbf{A}_{ZZ})^{-1} \mathbf{A}_{QZ}]^{-1} = \mathbf{A}^{-1} (\mathbf{B} - \omega_B)$$

$$\mathbf{M}_{QZ} = [\mathbf{A}_{QZ} - \mathbf{A}_{ZZ} (\mathbf{A}_{QQ})^{-1} \mathbf{A}_{QZ}]^{-1} = -\mathbf{A}^{-1} \omega_B,$$

where $\mathbf{M}_{QQ}$ and $\mathbf{M}_{QZ}$ are obtained in a similar way. Now, we can write the $m$ moment vector of $F(t)$ as

$$< \tau^m > = m! (-M)^m \mathbf{P}(0|2n),$$

(B2)

where $\mathbf{M}$ is given by

$$\mathbf{M} = \begin{pmatrix} \mathbf{A}^{-1} (\mathbf{B} - \omega_B) & -\mathbf{A}^{-1} \omega_B \\ -\mathbf{A}^{-1} \omega_A & \mathbf{A}^{-1} (\mathbf{A} - \omega_A) \end{pmatrix},$$

(B3)

and $\mathbf{C} = (\mathbf{B} - \omega_B - \omega_A)^{-1}$. For $m = 1$ in Eq. B2 we obtain the MFPT vector:

$$< \tau > = \begin{pmatrix} -\mathbf{A}^{-1} \omega_B & \mathbf{P}(0|n) \\ -\mathbf{A}^{-1} \omega_A & \mathbf{P}(0|n) \end{pmatrix},$$

(B4)

where $[\mathbf{P}(0|n)]_{j} = \delta_{x,j}$. Summing $< \tau >$ elements by using the summation row vector of $n$ dimensions $\mathbf{U}_n$, results in:

$$< \tau > = -\mathbf{U}_n \mathbf{C} \mathbf{P}(0|n)(\lambda P_{A,0} + P_{B,0}) +$$

$$+ \mathbf{U}_n \mathbf{A}^{-1} \mathbf{C} \mathbf{P}(0|n)(\omega_A + \omega_B).$$

(B5)

Note that the MFPT of the single $A$ conformation, $\tau$, is:

$$\mathbf{P} = -\mathbf{U}_n \mathbf{A}^{-1} \mathbf{P}(0|n),$$

which has a similar form as of the first term in Eq. B5 when choosing $\mathbf{C}^{-1}$ as the propagation matrix.

It is easy to verify that for $\lambda = 1$, $< \tau >$ reduces to the MFPT of the single conformation case, $\tau$. Rewriting Eq. B5 as

$$< \tau > = -\mathbf{U}_n \mathbf{A}^{-1} \mathbf{P}(0|n),$$

and substituting $\lambda = 1$ we find that

$$< \tau > = -\mathbf{U}_n \mathbf{A}^{-1} \mathbf{P}(0|n) = \tau.$$ (B7)

APPENDIX C

In order to obtain an explicit expression for the MFPT of the translocation in a weak field limit, we first rewrite Eq. B5 as

$$< \tau > = \hat{\tau}(\lambda P_{A,0} + P_{B,0}) + \hat{\sigma}^2 (\omega_A + \omega_B),$$

(C1)

where \( \hat{\tau} = -\mathbf{U}_n \mathbf{C} \mathbf{P}(0|n), \) and \( \hat{\sigma}^2 = \mathbf{U}_n \mathbf{A}^{-1} \mathbf{C} \mathbf{P}(0|n). \) We can further rewrite \( \hat{\tau} \) as \( \hat{\tau} = \sum_{s=1}^{\infty} \hat{\tau}_{s,x}. \) \( \hat{\tau}_{s,x} \equiv -\mathbf{C}_{s,x} \mathbf{P}(0|n). \) defines the mean residence time (MRT) spent in state $s$ before exiting the channel, given that the process started at state $x$ (Bar-Haim and KlafTer, 1998), and has the form [Huang and McColl., 1995]:

$$-(\mathbf{C})_{s,x} = \frac{\Delta(h^x) \Delta(h^{s+1-x}) \tau^{s-s} \hat{\tau}_{s,x}}{\Delta(h) \Delta(h^{s+1}) \hat{\tau}_{s,x}^2}; \quad s < x,$$

(C2)

where $\mathbf{C}_{s,x}$ for $s \geq x$, is obtained when exchanging $x$ for $s$ and $r$ for $l$ in Eq. C2. Here $h = [1 \pm (4\lambda r)^{1/2}] / 2$, $r = a p_1$, $l = a p_2$, $a = \lambda k / \hat{k}$, and $\hat{k} = \lambda k + \omega_B + \lambda \omega_A$. Thus, we find that $\hat{\tau}_{s,x}$ is a function of the parameter $a = [1 + (\omega_A + \omega_B) / \lambda] / k$, which obeys $0 \leq a \leq 1$, and is a measure of the difference between $\tau$ and $\hat{\tau}$. Using $\omega \approx 1$ and $\omega_A / k \approx 10^{-3}$ leads to $\omega \approx 1$ given $V / V_C \geq 1$, and accordingly to

$$\mathbf{C}_{s,x} = \mathbf{A}^{-1} \mathbf{P}(0|n) / \hat{k} = (\mathbf{A}^{-1})_{s,x} / \lambda.$$

(C3)

Eq. C3 implies

$$\hat{\tau} = \tau / \lambda.$$ (C4)

To obtain an explicit expression for $\tau$, it is convenient to use the independence approximation and replace $p_{j,j-1}$ and $k_j$ by state independent terms: $p_{j} = \frac{p_{j,j-1}}{p_{j+1,j}}$ and $k = \frac{k_j}{k_{j+1}}$. Then, we can write

$$\hat{\tau} = \tau / \lambda.$$ (C4)

To obtain an explicit expression for $\tau$, it is convenient to use the independence approximation and replace $p_{j,j-1}$ and $k_j$ by state independent terms: $p_{j} = \frac{p_{j,j-1}}{p_{j+1,j}}$ and $k = \frac{k_j}{k_{j+1}}$. Then, we can write

$$\hat{\tau} = \tau / \lambda.$$ (C4)
\[ 1 + e^{-(V/V_C+1)^{-1}} \] and \( k \). This approximation, which is valid for large polymers, \( N > d \), and becomes more accurate as \( N \) increases, leads to \( a_+ = p_+ k, \ a_- = (1 - p_+) k \), so that [Flomenbom and Klafter, 2003a]:

\[
\tau = \frac{\Delta(p^{n+1-x})p_+^x - \Delta(p^x) p_+^{n+1-x}(n + 1 - x)}{k\Delta p \Delta(p^{n+1})}, \quad (C5)
\]

where \( \Delta(p^x) = p_+^x - p_-^x \). In the limit of a not too large field, \( V/V_C \geq 1 \), Eq. C5 reduces to

\[
\tau \approx \frac{2x\xi_b^2d''}{z[q(1+1/d)]} \frac{1}{V - V_C}. \quad (C6)
\]

To compute \( \tilde{\sigma}^2 \) we rewrite \( \tilde{\sigma}^2 = \sum_{n=1}^\infty \tau_{s,s,x}^2 \), where \( \tau_s \) is given by Eq. C5 for \( x = s \), and \( \tau_{s,s,x} \) is given by Eq. C2. For \( a \approx 1 \) we have \( \tilde{\sigma}^2 = \tau^2/2\lambda \), where \( \tau^2 \) is the second moment of \( F(t) \) for the single A conformation case. The calculation of \( \tau^2/2 = \sum_{s=1}^\infty \tau_{s,s,x}^2 \) yields in the weak field limit \( V/V_C \geq 1 \):

\[
\frac{\tau^2}{2} \approx \frac{x(x-1)}{2(k\Delta p)^2} + \frac{xy^2(1-y) - y(1-y^2)}{(1-y)^2} + II,
\]

where \( y = p_-/p_+ \) and

\[
II = \frac{y^{n-1}}{y^n - 1} \left[ \frac{p_+^{x+1-n} - 1}{p_- - 1} - \frac{p_+^{x+1-n} - 1}{p_+ - 1} + n + 1 - \frac{x p_+^{n+1-x}}{(n + 1 - x) \frac{n + x}{2}} \right]. \quad (C7)
\]

Noticing that \( II \) represents the non-translocation events and vanishes for \( V/V_C \geq 1 \) as \( y^{n-x} \), we rewrite Eq. C7 up to a leading term in \( x \) as:

\[
\frac{\tau^2}{2} \approx \frac{x(x-1)}{2(k\Delta p)^2}. \quad (C9)
\]

Using \( \tau \approx (x/k\Delta p) \) valid for \( V/V_C \geq 1 \) [Flomenbom and Klafter, 2003a], Eq. C9 yields for a leading order in \( x \)

\[
\tilde{\sigma}^2 \approx \tau^2 / 2\lambda. \quad (C10)
\]

Substituting Eq. C4 and Eq. C10 into Eq. C1, Eq. 8 is obtained.

**APPENDIX D**

For the analysis of the field-free translocation we start by computing \( < \tau > \) and \( < \tau^2 > \). Substitute \( \lambda = 0 \) in Eq. B5, we obtain

\[
< \tau > = -\tilde{U} A^{-1} P_n(0) \left( \frac{\omega_A + \omega_B}{\omega_B} \right) + \frac{P_{B,0}}{\omega_B}. \quad (D1)
\]

which can be written as

\[
< \tau > = \frac{\tau(1 + \omega) + P_{B,0}/\omega_B}{\tau(1 + \omega) + P_{B,0}/\omega_B}. \quad (D2)
\]

Note that experiments suggest that \( \lambda_0 \ll 1 \), which leads to \( \lambda_0 k < \omega_B \), whereas \( \lambda_0 k \ll \omega_B \) is used for simplification, and enables the substitution of \( \lambda = 0 \) in Eq. B5.

To compute \( < \tau^2 > \), we have to calculate the blocks of \( M^2 \):

\[
M_{QQ}^2 = A^{-2}(1 + \omega); \quad M_{QZ}^2 = A^{-2}(1 + \omega) - A^{-1}/\omega_B; \quad M_{ZQ}^2 = A^{-2}(1 + \omega); \quad M_{ZZ}^2 = A^{-2}(1 + \omega) - A^{-1}2\omega/\omega_B + 1/\omega_B^2. \quad (D3)
\]

Substituting Eq. D3 into Eq. B2 and summing the vector elements, we obtain

\[
< \tau^2 > = \frac{\tau^2}{2} (1 + \omega)^2 + \frac{\tau^2}{2} (1 + \omega) \frac{P_{B,0}}{\omega_B} + \frac{P_{B,0}}{\omega_B}, \quad (D4)
\]

To get the relaxation timescales of \( G_{ap}(t), \tau_1 \) and \( \tau_2 \), we match \( < \tau > \) and \( < \tau^2 > \) obtained from

\[
F_{ap}(t) = \frac{P_{A,0}}{\tau_1} e^{-t/\tau_1} + \frac{P_{B,0}}{\tau_2} e^{-t/\tau_2}, \quad (D5)
\]

to the corresponding moments obtained from Eq. D2 and Eq. D4. This procedure yields:

\[
\tau_1 = < \tau > - \frac{P_{B,0} \tau_2}{P_{A,0}} \quad (D6)
\]

and

\[
\tau_2 = < \tau > + \left[ \frac{P_{A,0}}{P_{B,0}} \left( \frac{< \tau^2 >}{2} - < \tau > \right) \right]^{1/2}. \quad (D7)
\]

Substituting Eq. D2 and Eq. D4 into Eq. D7 results in

\[
\tau_2 = \tau(1 + \omega) + \frac{P_{B,0}}{\omega_B} + \frac{P_{A,0}}{\omega_B} \left[ 1 - \frac{\omega_B}{P_{A,0} P_{B,0}} \left( \frac{\tau^2}{2} - \tau^2 \right) \right]^{1/2}. \quad (D8)
\]

Expanding the square root in Eq. D8 to leading order and using Eq. D6, Eq. 11 is obtained.

**References**

Alberts B., K. Roberts, D. Bray, J. Lewis, M. Raff, and J. D. Watson. 1994. *Molecular Biology of The Cell*. Garland Publishing, Inc, NY & London.

Ambjornsson, T., S. P. Apell, Z. Konkoli, E. A. Di Marzio and J. J. Kasianowicz. 2002. Charged polymer membrane translocation. *J. Chem. Phys.*: 117, 4063-4073.

Bar-Haim, A., and J. Klafter. 1998. On mean residence and first passage times in finite one-dimensional systems. *J. Chem. Phys.*: 109, 5187-5193.
Bar-Haim, A., and J. Klafter. 1999. Escape from a fluctuating system: A master equation and trapping approach. Phys. Rev. E: 60, 2554-2558.
Bates, M., M. Burns and A. Meller. 2003. Dynamics of DNA Molecules in a Membrane Channel Probed by Active Control Techniques. Biophys. J.: 84, 2366-2372.
Berezkhkovskii, A. M., and I. V. Gopich. 2003. Translocation of Rodlike Polymers Through Membrane Channels. Biophys. J.: 84, 787-793.
Doering, C. R., and J. C. Gadoua. 1992. Reasonant Activation Over a Fluctuating barrier. Phys. Rev. Lett.: 69, 2318-2321.
Doi, M., and S. F. Edwards. 1986. The Theory of Polymer Dynamics. Clarendon press, Oxford.
Huang, Y., and W. F. McColl. 1997. Analytical inversion of general tridiagonal matrices. J. Phys. A: 30, 7919-7933.
Flomenbom, O., and J. Klafter. 2003a. Single Stranded DNA Translocation Through A Nanopore: A Master Equation Approach. Phys. Rev. E: 68, 041910-7.
Flomenbom, O., and J.Klafter. 2004. Insight into Resonant Activation in Discrete Systems. Phys. Rev. E: in press.
Kasianowicz, J. J., E. Brandin, D. Branton and D. W. Deamer. 1996. Characterization of individual polynucleotide molecules using a membrane channel. Proc. Natl. Acad. Sci.: 93, 13770-13773.
Klafter, J., and R. Silbey. 1980. Derivation of the Continuous-Time Random-Walk Equation. Phys. Rev. Lett.: 44, 55-58.
Lubensky, D. K., and D. R. Nelson. 2001. Driven Polymer Translocation Through a Narrow Pore. Biophys. J.: 77, 1824-1838.
Madigan, M. T., J. M. Matinko and J. Parker. 1997. Biology of Microorganisms. Prentice-Hall, International,Inc.
Meller, A. 2003. Dynamics of polynucleotide transport through nanometer-scale pores. J. Phys: Cond. Matt.: 15, R581-R607.
Meller, A., L. Nivon and D. Branton. 2001. Voltage-Driven DNA Translocations through a Nanopore. Phys. Rev. Lett.: 86, 3435-3438.
Metzler, R., and J. Klafter. When translocation dynamics becomes anomalous. Biophys. J.: 85, 2776-2779.
Muthukumar, M. 1999. Polymer translocation through a hole. J. Chem. Phys.: 111, 10371-10374.
Redner, S. 2001. A Guide to First-Passage Process. Cambridge University Press, Cambridge, UK.
Slonika, E., and A. B. Kolomeisky. 2003. Charged polymer membrane translocation. J. Chem. Phys.: 118, 7112-7118.
Song, L., M. R. Hobaugh, C. Shustak, S. Cheley, H. Balyley and J. E. Gouaux. 1996. Structure of Staphylococcal α-Hemolysin, a Hepatrameric Transmembrane Pore. Science: 274, 1859-1866.
Sung, W., and P. J. Park. 1996. Polymer Translocation through a Pore in a membrane. Phys. Rev. Lett.: 77, 783-786.
Zwanzig, R. 2001. Nonequilibrium Statistical Mechanics. Oxford University Press, NY, NY.