A new insight into diffusional escape from a biased cylindrical trap

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Recent experiments with single biological nanopores, as well as single-molecule fluorescence spectroscopy and pulling studies of protein and nucleic acid folding raised a number of questions that stimulated theoretical and computational investigations of barrier crossing dynamics. The present paper addresses a closely related problem focusing on trajectories of Brownian particles that escape from a cylindrical trap in the presence of a force F parallel to the cylinder axis. To gain new insights into the escape dynamics, we analyze the “fine structure” of these trajectories. Specifically, we divide trajectories into two segments: a looping segment, when a particle unsuccessfully tries to escape returning to the trap bottom again and again, and a direct-transit segment, when it finally escapes moving without touching the bottom. Analytical expressions are derived for the Laplace transforms of the probability densities of the durations of the two segments. These expressions are used to find the mean looping and direct-transit times as functions of the biasing force F . It turns out that the force-dependences of the two mean times are qualitatively different. The mean looping time monotonically increases as F decreases, approaching exponential F -dependence at large negative forces pushing the particle towards the trap bottom. In contrast to this intuitively appealing behavior, the mean direct-transit time shows rather counterintuitive behavior: it decreases as the force magnitude, $|F|$, increases independently of whether the force pushes the particles to the trap bottom or to the exit from the trap, having a maximum at $F = 0$. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.5002127>]

I. INTRODUCTION

Our interest in the problem analyzed in this paper is motivated by our recent single-molecule experiments with protein chain dynamics in nanopores.^{1–8} Specifically, we studied the capture and escape of the densely negatively charged C-terminal tails of cytosolic proteins by nanopores of β -barrel channels. Among studied objects were tubulin dimers,^{1–4} α -synucleins,^{5,6,8} and even artificial constructs⁷ assembled from albumin molecules and covalently attached negatively charged peptides mimicking C-termini of cytosolic proteins. Naturally occurring nanopores of α -Hemolysin⁹ and the Voltage-Dependent Anion Channel¹⁰ (VDAC) from the outer mitochondrial membrane (MOM) were purified and then reconstituted into planar lipid membranes. A cartoon of such an experiment with α -synuclein and VDAC is shown in Fig. 1.

α -Synuclein is a small 14-kDa intrinsically disordered cytosolic protein.¹¹ It is the major component of the Lewy bodies characteristically observed in the brains of Parkinson's disease patients, but it is also abundant in normal cells of the central nervous system, where it constitutes up to 1% of the total cytosolic protein.¹² α -Synuclein's functional role in both healthy and diseased cells remains unknown, thus explaining why its newly described interaction with the mitochondrial

channel⁶ could be of importance. In solution, it exists in the disordered form, but can adopt an α -helical structure in its N-terminal domain upon binding to lipid membranes.¹¹ The densely negatively charged C-terminal tail of the bound α -synuclein remains unstructured and is available for interactions with nanopores.

The capture and escape of the C-terminal tails of cytosolic proteins by the channel nanopores was monitored by measuring dynamic obstruction of the ionic current through these channels. It was demonstrated that the escape of the tail from the nanopore is strongly voltage dependent. In particular, in the presence of a biasing electric field of about 50 mV, the escape takes many orders of magnitude more time than that extrapolated to zero field. Interestingly, at transmembrane potential differences of several millivolts, which, due to the membrane small thickness, recalculate into the electric fields of about 10^6 V/m, the tail capture is barely observable. This and higher fields are necessary for the tail to overcome significant entropy barrier since the number of conformations available for the tail inside the nanopore is well below the number available in free solution outside the pore. The tail lifetime in the nanopore is also a function of the tail length, charge, and chemical composition.

The simplest model describing the escape from a cylindrical trap in the presence of a force F parallel to the cylinder axis is one-dimensional biased diffusion of a point particle on an interval terminated by reflecting and absorbing endpoints. The particle starts from the reflecting end of the interval

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FIG. 1. Schematic representation of reversible capture of the α -synuclein unstructured C-terminus (shown in red) by the VDAC nanopore. The rest of the alpha-synuclein molecule, shown as a green helix, is bound to the membrane surface preventing C-terminus translocation. The characteristic time of the terminus escape from the nanopore is very sensitive to the transmembrane electric field because of its significant negative charge (15 elementary charges).

and disappears as soon as it touches the opposite absorbing endpoint for the first time.

Here, to gain both qualitative and quantitative understanding of the escape dynamics, we study trajectories of Brownian particles in a cylindrical trap. Our analysis is based on the observation that any escape trajectory can be divided into two segments: a looping segment, when the particle unsuccessfully tries to escape returning, after each attempt, to the trap bottom, and a direct-transit segment, when it finally escapes moving to the exit without returning to the bottom of the trap. This simple observation makes it possible to look at the escape process from a new perspective and to gain deeper insight into the process dynamics. The focus is on the durations of these two fragments of the escape trajectory, their probability densities and mean values as functions of the biasing force F . We derive the Laplace transforms of the probability densities of the direct-transit and looping times, and use them to find the mean values of these times. It turns out that, counterintuitively, the mean direct-transit time has a maximum at $F = 0$ and decreases with $|F|$, independent of the force direction, that is, whether the force is pushing the particle to the trap bottom or to the exit from the trap, whereas the mean looping time is a monotonic function of F . These analytical results are in perfect agreement with the Brownian dynamics simulations of the escape process.

To illustrate the above-mentioned force dependences of the direct-transit and looping times, in Fig. 2 we show three examples of simulated escape trajectories that are divided into two segments: looping parts, when the particle returns

to the reflecting bottom (shown in red), and direct-transit parts, when the particle escapes from the trap without returning to the bottom (shown in green). These examples illustrate the decrease in the duration of the looping segments of the trajectories, as the applied force increases, and insensitivity of the duration of the direct-transit segments to the force polarity.

We expect that the results of the present study will have broad applications in other areas of biophysics, where single-molecule studies are becoming a routine used for precise quantification of interactions between and within different nanoscale objects of biological origin. Specific examples include investigations^{13–51} of folding of proteins and nucleic acids by single-molecule fluorescence spectroscopy and pulling experiments, focused on rare and very fast barrier-crossing events. These studies have brought interest in the physics of such processes to its new heights. Interestingly, the latest computational studies of the barrier crossing dynamics have demonstrated that the increase in the barrier height accelerates direct transitions.^{24,40}

II. THEORY

The problem of escape from a cylindrical trap is essentially one-dimensional. Therefore, we model the escape process as one-dimensional diffusion on an interval terminated by reflecting and absorbing end points, located at $x = 0$ (the trap bottom) and $x = L$ (the trap exit), respectively, in the presence of a uniform biasing force F . The particle starts from the

FIG. 2. Escape trajectories of single particles obtained in Brownian dynamics simulations at different values of the dimensionless applied force $\tilde{F}$ [see the definition below Eq. (3)] split into the looping (red) and direct-transit (green) parts. Panel (a) shows an escape trajectory for the force $\tilde{F} = -4$ that pushes the particle towards the reflecting bottom of the trap. Trajectories for the no bias case, $\tilde{F} = 0$, and for $\tilde{F} = 4$, where the force drags the particle out of the trap, are shown in panels (b) and (c). Time scales are the same for all three panels. These trajectories are chosen to highlight the main messages of the article: the mean direct-transit time monotonically decreases with $|\tilde{F}|$, independent of the force direction, exhibiting maximum at $\tilde{F} = 0$, whereas the mean looping time monotonically decreases with the force, as might be expected.

reflecting boundary at $x = 0$ and is terminated at the first touch of the absorbing boundary at $x = L$. To find the probability densities of the direct-transit and looping times, it is convenient to consider an auxiliary problem in which the boundary at $x = 0$ is partially absorbing. Let $G_\kappa(x, t)$ be the propagator of a particle starting from this boundary at time $t = 0$. This propagator satisfies

$$\frac{\partial G_\kappa}{\partial t} = D \frac{\partial}{\partial x} \left[e^{\beta F x} \frac{\partial}{\partial x} (e^{-\beta F x} G_\kappa) \right], \quad (1)$$

subject to the initial condition $G_\kappa(x, 0) = \delta(x)$ and boundary conditions $G_\kappa(L, t) = 0$ and $D \partial G_\kappa(x, t) / \partial x|_{x=0} = \kappa G_\kappa(0, t)$. Here D is the particle diffusivity, $\beta = 1/(k_B T)$, with k_B and T denoting the Boltzmann constant and absolute temperature, and the subscript κ at the propagator indicates the trapping rate entering the partially absorbing boundary condition at $x = 0$.

The probability flux escaping from the interval at time t through the perfectly absorbing boundary at $x = L$, denoted by $f_L(t|\kappa)$, is $f_L(t|\kappa) = -D \partial G_\kappa(x, t) / \partial x|_{x=L}$. Using this flux, we can find the probability, $P_L(\kappa)$, that the particle escapes from the interval through this boundary, $P_L(\kappa) = \int_0^\infty f_L(t|\kappa) dt$. The conditional probability density, $\varphi_L(t|\kappa)$, of the particle lifetime in the interval, conditional on that it escapes through the perfectly absorbing boundary, is defined as the ratio of the escaping flux to the escape probability, $\varphi_L(t|\kappa) = f_L(t|\kappa) / P_L(\kappa)$. As $\kappa \rightarrow \infty$, this probability density reduces to the probability density $\varphi_{dtr}(t)$ of the direct-transit (dtr) time,

$$\varphi_{dtr}(t) = \lim_{\kappa \rightarrow \infty} \varphi_L(t|\kappa), \quad (2)$$

which is the quantity of interest.

Solving Eq. (1), one can find that the Laplace transform of this probability density is given by

$$\hat{\varphi}_{dtr}(s) = \int_0^\infty e^{-st} \varphi_{dtr}(t) dt = \frac{z \sinh(\tilde{F}/2)}{(\tilde{F}/2) \sinh(z)}, \quad (3)$$

where s is the Laplace parameter, $\tilde{F} = \beta FL$ is the dimensionless biasing force, and $z = \sqrt{sL^2/D + (\tilde{F}/2)^2}$. One can see that $\hat{\varphi}_{dtr}(s)$ is a symmetric function of F . As a consequence, $\varphi_{dtr}(t)$ is also symmetric, $\varphi_{dtr}(t)|_{-F} = \varphi_{dtr}(t)|_F$. The time dependence of this conditional probability density can be found by numerically inverting its Laplace transform $\hat{\varphi}_{dtr}(s)$. Alternatively, one can obtain $\varphi_{dtr}(t)$ as an infinite series using the eigenfunction expansion.¹⁹ Although the independence of the direct-transit time probability density of the force direction at first sight seems surprising, it can be rationalized as a consequence of the time reversibility of diffusion trajectories.⁵² Indeed, if we take the set of direct-transit segments of escape trajectories of duration t , which contribute to $\varphi_{dtr}(t)|_F$, and invert the course of time, we obtain the set of direct-transit segments contributing to $\varphi_{dtr}(t)|_{-F}$.

The mean direct-transit time, $\langle t_{dtr} \rangle$, defined as $\langle t_{dtr} \rangle = \int_0^\infty t \varphi_{dtr}(t) dt$, can be found from the small- s expansion of the Laplace transform in Eq. (3), using the relation $\langle t_{dtr} \rangle = -d\hat{\varphi}_{dtr}(s)/ds|_{s=0}$. The result is

$$\langle t_{dtr} \rangle = \langle t_{FP} \rangle|_{F=0} \left[(\tilde{F}/2) \coth(\tilde{F}/2) - 1 \right] / (\tilde{F}/2)^2, \quad (4)$$

where $\langle t_{FP} \rangle|_{F=0} = L^2/(2D)$ is the mean first-passage (FP) time from the reflecting boundary of the interval of length L to its absorbing boundary in the absence of bias.⁵³ The F -dependence of $\langle t_{dtr} \rangle$ is illustrated in Fig. 3, which shows that the mean direct-transit time is a symmetric function of F that monotonically decreases as the absolute value of the biasing force increases. Its maximum value at $F = 0$ is $\langle t_{dtr} \rangle|_{F=0} = (1/3) \langle t_{FP} \rangle|_{F=0} = L^2/(6D)$. The asymptotic behavior of $\langle t_{dtr} \rangle$, as $|F| \rightarrow \infty$, is given by $\langle t_{dtr} \rangle|_{|F| \rightarrow \infty} = 2 \langle t_{FP} \rangle|_{F=0} / |\tilde{F}| = L/(D\beta|F|)$. This asymptotic behavior has a transparent physical interpretation. As might be expected, in this limiting case $\langle t_{dtr} \rangle$ is the ratio of length L and particle drift velocity $D\beta|F|$.

We would like to note here that though the problem is essentially one-dimensional, we found that it is informative to illustrate our analysis by the trajectories obtained in two-dimensional simulations, as we hope is clear from the insets in Fig. 3. These insets show the looping and direct-transit segments of the trajectories at applied forces of $\tilde{F} = -12$ (left panel) and $\tilde{F} = +12$ (right panel). It is seen that while the looping segments (red) are drastically different for the opposite force directions, the direct transit segments (green) seem to be similar. This similarity is a consequence of the identity of the distributions of the direct-transit times at opposite polarities of the force of the same magnitude [see the discussion below Eq. (3)].

To find the distribution of the looping time we use the fact that the total duration of the escape trajectory, which is the first-passage time from the reflecting to the absorbing boundary, t_{FP} , is the sum of the durations of its looping (l) and direct-transit segments, t_l and t_{dtr} , $t_{FP} = t_l + t_{dtr}$ (see Fig. 2). Then the

FIG. 3. The dimensionless mean direct-transit, $\langle \tilde{t}_{dtr} \rangle$, looping, $\langle \tilde{t}_l \rangle$, and first passage, $\langle \tilde{t}_{FP} \rangle$, times given by Eqs. (4), (9), and (10) and shown in green, red, and blue, respectively, as functions of the dimensionless biasing force. The tilde above t indicates that the time is measured in units of $\langle t_{FP} \rangle|_{F=0} = L^2/(2D)$. Triangles and circles are the simulation results. The insets show typical escape trajectories at the biases of opposite polarities, $\tilde{F} = \pm 12$. As for the symbols and curves, red and green traces correspond to the looping and direct-transit segments of the full trajectories, respectively.

probability density of the first-passage time, t_{FP} , $\varphi_{FP}(t)$, is the convolution of the probability densities and of the direct-transit [$\varphi_{dtr}(t)$] and looping [$\varphi_l(t)$] times,

$$\varphi_{FP}(t) = \int_0^t \varphi_{dtr}(t-t')\varphi_l(t')dt'. \quad (5)$$

The Laplace transform of this relation is $\hat{\varphi}_{FP}(s) = \hat{\varphi}_{dtr}(s)\hat{\varphi}_l(s)$, and hence we have

$$\hat{\varphi}_l(s) = \hat{\varphi}_{FP}(s)/\hat{\varphi}_{dtr}(s). \quad (6)$$

The probability density $\varphi_{FP}(t)$ is nothing else than the probability density $\varphi_L(t|\kappa)$ at $\kappa=0$. Indeed, when $\kappa=0$, the boundary at $x=0$ is reflecting, and, therefore, the escape probability through the absorbing boundary is unity, $P_L(\kappa=0)=1$. As a consequence, the probability density of the first-passage time is simply the flux through the absorbing boundary at time t , $\varphi_{FP}(t) = f_L(t|\kappa=0)$. One can find this flux and hence the probability density of the first-passage time by solving Eq. (1) with the reflecting boundary condition at $x=0$. The result is

$$\hat{\varphi}_{FP}(s) = \frac{ze^{\tilde{F}/2}}{z \cosh(z) + (\tilde{F}/2) \sinh(z)}. \quad (7)$$

Substituting this and $\hat{\varphi}_{dtr}(s)$ in Eq. (3) into Eq. (6), we arrive at

$$\hat{\varphi}_l(s) = \frac{\tilde{F} \sinh(z)}{(1 - e^{-\tilde{F}}) [z \cosh(z) + (\tilde{F}/2) \sinh(z)]}. \quad (8)$$

The mean looping time, $\langle t_l \rangle = \int_0^\infty t\varphi_l(t)dt$, can be obtained from the small- s expansion of $\hat{\varphi}_l(s)$, $\langle t_l \rangle = -d\hat{\varphi}_l(s)/ds|_{s=0}$. This leads to

$$\langle t_l \rangle = 2 \langle t_{FP} \rangle|_{F=0} [1 + e^{-\tilde{F}} + \tilde{F} (1 - \coth(\tilde{F}/2))] / \tilde{F}^2. \quad (9)$$

The force dependence of $\langle t_l \rangle$, presented in Fig. 3, shows that the mean looping time monotonically decreases with F . In the absence of bias, this mean time is $\langle t_l \rangle|_{F=0} = (2/3) \langle t_{FP} \rangle|_{F=0} = L^2/(3D)$. Thus, at $F=0$, the mean looping time is twice longer than the mean direct-transit time, $\langle t_l \rangle|_{F=0} = 2 \langle t_{dtr} \rangle|_{F=0}$. The asymptotic behavior of the mean looping time, as $F \rightarrow -\infty$, is given by $\langle t_l \rangle|_{F \rightarrow -\infty} = 2 \langle t_{FP} \rangle|_{F=0} / \tilde{F}^2 = 2/[D(\beta F)^2]$ and $\langle t_l \rangle|_{F \rightarrow -\infty} = \langle t_l \rangle|_{F \rightarrow \infty} e^{|\tilde{F}|}$. The exponential increase of $\langle t_l \rangle$ as $F \rightarrow -\infty$ is an expected consequence of the fact that in this limiting case the particle moves in a deep potential well, and to escape, it has to overcome a high energy barrier. The ratio of the two mean times, $\langle t_l \rangle / \langle t_{dtr} \rangle$, decreases from infinity to zero as F increases from $-\infty$ to ∞ .

Figure 3 also presents the force dependence of the mean first-passage time, $\langle t_{FP} \rangle$, which is the sum of the mean looping and direct-transit times,

$$\langle t_{FP} \rangle = \langle t_l \rangle + \langle t_{dtr} \rangle = 2 \langle t_{FP} \rangle|_{F=0} (\tilde{F} - 1 + e^{-\tilde{F}}) / \tilde{F}^2. \quad (10)$$

Thus, the mean first-passage time is determined by the mean looping time when $F \rightarrow -\infty$, whereas, when $F \rightarrow \infty$, it is determined by the mean direct-transit time. The relation in Eq. (10) is a special case of the general relation between the moments of the first-passage, looping, and direct-transit times,

$$\langle t_{FP}^n \rangle = \sum_{k=0}^n \frac{n!}{k! (n-k)!} \langle t_l^k \rangle \langle t_{dtr}^{n-k} \rangle, \quad (11)$$

which follows from Eq. (5) and the fact that $t_{FP} = t_l + t_{dtr}$.

In Fig. 4 we show the probability densities $\varphi_{FP}(t)$, $\varphi_{dtr}(t)$, and $\varphi_l(t)$. The solid curves are drawn by numerically inverting the Laplace transforms in Eqs. (3), (7), and (8) at different values of the biasing force, $\tilde{F} = \pm 12$ and $\tilde{F} = 0$. The probability densities obtained from Brownian dynamics simulations are

FIG. 4. Panels in (a)–(c) rows show the probability densities $\varphi_{FP}(t)$, $\varphi_{dtr}(t)$, and $\varphi_l(t)$, respectively, as functions of the dimensionless time, $2Dt/L^2$, at $\tilde{F} = -12$ (left), $\tilde{F} = 0$ (middle), and $\tilde{F} = 12$ (right). Solid curves are the theoretically predicted dependences; bars are the simulation results. The theoretical curves were obtained by numerically inverting the Laplace transforms in Eqs. (3), (7), and (8).

shown by bars. One can see excellent agreement between our analytical and simulation results. One can also see that $\varphi_{FP}(t)$ is close to $\varphi_l(t)$ at $\tilde{F} = -12$, whereas at $\tilde{F} = 12$ $\varphi_{FP}(t)$, is close to $\varphi_{dir}(t)$.

III. CONCLUDING REMARKS

To summarize, the escape of Brownian particles from a cylindrical trap in the presence of a biasing force is analyzed by splitting the escape trajectory into the looping and direct-transit segments. One of the goals of this study is to reveal the “fine structure” of the escape dynamics and to develop intuition on the conditional quantities, namely, mean looping and direct-transit times, arising in our analysis. We show that, as might be expected, the mean looping time grows exponentially with the force when the force is large and negative, that is, directed towards the trap bottom (reflecting boundary); when a sufficiently large force is applied in the escape direction, the mean looping time decreases quadratically with the force. However, the effect of the force on the direct-transit time is independent of whether the force is pushing the particle back into the cylindrical trap or dragging it out. The mean direct-transit time and its distribution are symmetric about the biasing force direction. This symmetry is closely related to the identity of distributions of the direct uphill and downhill translocation times for particles traversing membrane channels.⁵²

Our approach to the problem provides new insights into the physics of stochastic escape from traps by allowing one to look at the escape processes from a novel perspective. This approach is proposed in our recent paper,⁵⁴ which considers one-dimensional escape trajectories in the presence of a potential with the focus on the dependences of the mean direct-transit and looping times on the shape of the potential. Here, this approach is used to study the dependence of these mean times on the biasing force. In addition, for the first time, analytical expressions for the Laplace transforms of the conditional probability densities of the direct-transit and looping times are derived. Though performed for a simple model system, the proposed methodology can be used for better understanding of the particle dynamics at the nanoscale in general, including more complex systems and confined geometries. It may also be helpful in the examination of barrier-crossing processes in protein and nucleic acid folding^{13–51} and nanopore-based analysis of proteins and peptides.⁸

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