Blocker escape kinetics from a membrane channel analyzed by mapping blocker diffusive dynamics onto a two-site model

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ABSTRACT

When a large solute molecule enters a membrane channel from the membrane-bathing electrolyte solution, it blocks the small-ion current flowing through the channel. If the molecule spends in the channel sufficiently long time, individual blockades can be resolved in single-channel experiments. In this paper, we develop an analytical theory of the blocker escape kinetics from the channel, assuming that a charged blocking molecule cannot pass through a constriction region (bottleneck). We focus on the effect of the external voltage bias on the blocker survival probability in the channel. The bias creates a potential well for the charged blocker in the channel with the minimum located near the bottleneck. When the bias is strong, the well is deep, and escape from the channel is a slow process that allows for time-resolved observation of individual blocking events. Our analysis is performed in the framework of a two-site model of the blocker dynamics in the channel. Importantly, the rate constants, fully determining this model, are derived from a more realistic continuum diffusion model. This is done by mapping the latter onto its two-site counterpart which, while being much simpler, captures the main features of the blocker escape kinetics at high biases.

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I. INTRODUCTION

Understanding blockage of ion channels of biological membranes by natural and synthetic compounds is important from both theoretical and practical points of view. On the one hand, the phenomenon of blockage gives a rich example of the behavior of a blocker molecule in confined systems involving multiple interactions with the protein residues lining the channel walls and, in the case of a charged blocker, with the transmembrane electric field. On the other hand, its practical value is clear from the fact that about 13% of all present drugs of the world pharmacopeia act as ion channel modifiers, with most of them working as channel blockers.¹

Site models of solute dynamics in the channel have been used to study channel-facilitated membrane transport by many authors.²

It has been shown that a reasonable quantitative description of the molecular translocation through channels can be obtained using this discrete chemical kinetic approach, in which the molecular transport is modeled by a sequence of transitions between neighboring sites. In particular, two-site models are widely used because of their simplicity. Here, for the first time, we use this approach to develop an analytical theory of blocker escape kinetics from the channel. In contrast to other authors who treat the rate constants characterizing these intersite transitions as free parameters, we derive them from a continuum diffusion model.³

The outline of this paper is as follows. Mapping of the diffusion model of the blocker dynamics in the channel onto its two-site counterpart is discussed in Sec. II. The two-site model is used in Sec. III to analyze the time dependence of the blocker survival probability in the channel. Some concluding remarks are made in Sec. IV.

II. THEORY

A. Continuum diffusion model

Consider a spherical blocker molecule entering a membrane channel at $t = 0$. It is assumed that blocker motion along the channel axis, directed along the x -coordinate, is diffusion in the potential of mean force, $U(x)$, with position-dependent diffusivity, $D_{ch}(x)$.⁴ This motion occurs between the channel entrance, located at $x = 0$, and the bottleneck, located at $x = L$, which is a reflecting wall for the blocker since it cannot pass through this point. The blocker dynamics in the interval $0 < x < L$ is described by the propagator (Green's function), $G(x, t)$, that satisfies the Smoluchowski equation

$$\frac{\partial G}{\partial t} = \frac{\partial}{\partial x} \left[D_{ch}(x) e^{-\beta U(x)} \frac{\partial}{\partial x} (e^{\beta U(x)} G) \right], \quad (2.1)$$

with $\beta = 1/(k_B T)$, where k_B and T are the Boltzmann constant and the absolute temperature, subject to the initial condition, $G(x, 0) = \delta(x)$, and radiation (partially absorbing) and reflecting boundary conditions at $x = 0$ and $x = L$, respectively,

$$\begin{aligned} D_{ch}(0) e^{-\beta U(0)} \frac{\partial}{\partial x} (e^{\beta U(x)} G) \Big|_{x=0} &= \kappa G(0, t), \\ \frac{\partial}{\partial x} (e^{\beta U(x)} G) \Big|_{x=L} &= 0. \end{aligned} \quad (2.2)$$

The trapping rate κ entering the boundary condition at $x = 0$ determines the probability that the blocker approaching the channel entrance from inside the channel leaves the channel and returns to the bulk solution. This trapping rate is given in Refs. 4(a) and 5

$$\kappa = \frac{4D_b}{\pi a}, \quad (2.3)$$

where D_b is the blocker molecule diffusivity in the bulk and a is the effective radius of the channel entrance, which is the difference between the radii of the channel opening and the blocker molecule.

The probability density, $\varphi(t)$, of the blocker lifetime in the channel is

$$\varphi(t) = \kappa G(0, t). \quad (2.4)$$

It can be shown⁶ that the mean blocker lifetime in the channel, τ , is given by

$$\tau = \int_0^\infty t \varphi(t) dt = \left(e^{\beta U(0)} / \kappa \right) \int_0^L e^{-\beta U(x)} dx. \quad (2.5)$$

To make this paper self-contained, we derive this expression for τ in Appendix A.

B. Two-site model

In this subsection, we discuss mapping of the blocker diffusive dynamics in the channel onto a two-site model described by the following kinetic scheme:

assuming that sites 1 and 2 are located at the channel entrance ($x = 0$) and at the bottleneck ($x = L$), respectively. The rate constants k_{out} and α_1 determine the fate of the blocker entering the channel, which either returns to the bulk solution or jumps to site 2. The rate constant α_2 is the inverse mean time it takes the blocker to pass from the reflecting wall to the channel entrance. According to the kinetic scheme in Eq. (2.6), the mean blocker lifetimes on sites 1 and 2, denoted by τ_1 and τ_2 , respectively, are

$$\tau_1 = \frac{1}{k_{out} + \alpha_1}, \quad \tau_2 = \frac{1}{\alpha_2}. \quad (2.7)$$

Being on site 1, the blocker escapes from the channel to the bulk solution with probability P_{out} and jumps to site 2 with probability $P_{in} = 1 - P_{out}$. As follows from the kinetic scheme, these probabilities are given by

$$P_{out} = \frac{k_{out}}{k_{out} + \alpha_1}, \quad P_{in} = \frac{\alpha_1}{k_{out} + \alpha_1}. \quad (2.8)$$

The rate constants k_{out} , α_1 , and α_2 fully determine the two-site model of the blocker dynamics in the channel. The expressions relating these rate constants with the continuum diffusion model of the blocker dynamics discussed above are (see the derivations in Appendixes B and C)

$$k_{out} = \frac{A}{(1 + A)\tau_1}, \quad \alpha_1 = \frac{1}{(1 + A)\tau_1}, \quad \alpha_2 = \frac{1}{\tau_2}, \quad (2.9)$$

where

$$A = k_{out} / \alpha_1 = P_{out} / P_{in} = \kappa e^{-\beta U(0)} \int_0^L (e^{\beta U(y)} / D(y)) dy, \quad (2.10)$$

$$\tau_1 = (1 + A)^{-1} \int_0^L (e^{\beta U(y)} / D(y)) dy \int_0^y e^{-\beta U(x)} dx, \quad (2.11)$$

$$\tau_2 = \int_0^L (e^{\beta U(y)} / D(y)) dy \int_y^L e^{-\beta U(x)} dx. \quad (2.12)$$

The two-site model [Eq. (2.6)] with the rate constants in Eq. (2.9) gives the same mean blocker lifetime in the channel, τ , as the continuum diffusion model. To show this, we note that according to the two-site model, the mean lifetime satisfies the following linear equation:

$$\tau = P_{out} \tau_1 + P_{in} (\tau_1 + \tau_2 + \tau). \quad (2.13)$$

Here, the first term describes those realizations of the blocker stochastic trajectory, which escape from the channel without touching the reflecting wall (i.e., without jumping to site 2) and the second term describes the rest of the trajectory realizations that touch the reflecting wall (visit site 2) at least once. Solving Eq. (2.13), we find

$$\tau = (1/P_{out}) (\tau_1 + P_{in} \tau_2) = (\alpha_1 + \alpha_2) / (k_{out} \alpha_2), \quad (2.14)$$

where the expressions for τ_1 , τ_2 , P_{out} , and P_{in} in terms of the rate constants [Eqs. (2.7) and (2.8)] have been used. Substituting into Eq. (2.14), the expressions for the mean lifetimes τ_1 and τ_2 and the probabilities P_{out} and P_{in} , obtained using Eqs. (2.8)–(2.12), one recovers the expression for τ in Eq. (2.5).

C. Biased cylindrical channel with position-independent diffusivity

In this subsection, we apply general relations obtained above to the special case of a cylindrical channel with position-independent diffusivity of the blocker, $D_{ch}(x) = \text{const} = D_{ch}$, assuming that the potential of mean force along the channel axis is $U(x) = -Fx$, where F is a constant force controlled by the external voltage bias applied to the channel. The focus is on the case where $F > 0$ so that the external bias creates a potential well for the blocker near the reflecting wall (see Fig. 1). The well depth, Δ , is related to the biasing force F and the length L by $\Delta = FL$. It is convenient to discuss the results presented in this section using the time scale t_L and two dimensionless parameters $\tilde{\Delta}$ and $\tilde{\kappa}$ defined as

$$t_L = L^2/D_{ch}, \quad \tilde{\Delta} = \beta FL, \quad \tilde{\kappa} = \kappa L/D_{ch} = 4LD_b/(\pi a D_{ch}). \quad (2.15)$$

Performing the integrations, we find that the rate constants k_{out} , α_1 , and α_2 given in Eq. (2.9) are

$$k_{out} = \frac{\tilde{\kappa} \tilde{\Delta} (1 - e^{-\tilde{\Delta}})}{t_L (\tilde{\Delta} - 1 + e^{-\tilde{\Delta}})}, \quad (2.16)$$

$$\alpha_1 = \frac{\tilde{\Delta}^2}{t_L (\tilde{\Delta} - 1 + e^{-\tilde{\Delta}})}, \quad (2.17)$$

$$\alpha_2 = \frac{\tilde{\Delta}^2}{t_L (e^{\tilde{\Delta}} - 1 - \tilde{\Delta})}. \quad (2.18)$$

The $\tilde{\Delta}$ -dependences of these rate constants are quite different. While the rate constant α_1 monotonically increases with $\tilde{\Delta}$, two other rate constants decrease. At strong bias (large $\tilde{\Delta}$), the rate constant α_2 becomes exponentially small, whereas the rate constant k_{out} decreases compared to its value in the absence of bias ($\tilde{\Delta} = 0$) by a factor of 2. As $\tilde{\Delta}$ vanishes or becomes much larger than unity, these rate constants reduce to

$$k_{out} = \frac{\tilde{\kappa}}{t_L} \begin{cases} 2, & \tilde{\Delta} = 0 \\ 1, & \tilde{\Delta} \gg 1, \end{cases} \quad (2.19)$$

$$\alpha_1 = \frac{1}{t_L} \begin{cases} 2, & \tilde{\Delta} = 0 \\ \tilde{\Delta}, & \tilde{\Delta} \gg 1, \end{cases} \quad (2.20)$$

$$\alpha_2 = \frac{1}{t_L} \begin{cases} 2, & \tilde{\Delta} = 0 \\ \tilde{\Delta}^2 e^{-\tilde{\Delta}}, & \tilde{\Delta} \gg 1. \end{cases} \quad (2.21)$$

The rate constant dependences on the dimensionless well depth $\tilde{\Delta}$ are illustrated in Fig. 2.

Substituting the obtained results for the rate constants k_{out} and α_1 into the general expressions for the probabilities P_{out} and P_{in} [Eq. (2.8)], we arrive at

$$P_{out} = \frac{\tilde{\kappa} (1 - e^{-\tilde{\Delta}})}{\tilde{\kappa} (1 - e^{-\tilde{\Delta}}) + \tilde{\Delta}}, \quad P_{in} = \frac{\tilde{\Delta}}{\tilde{\kappa} (1 - e^{-\tilde{\Delta}}) + \tilde{\Delta}}. \quad (2.22)$$

The limiting behavior of these probabilities, as $\tilde{\Delta}$ vanishes or becomes much larger than unity, is given by

$$P_{out} = \begin{cases} \tilde{\kappa}/(\tilde{\kappa} + 1), & \tilde{\Delta} = 0 \\ \tilde{\kappa}/(\tilde{\kappa} + \tilde{\Delta}), & \tilde{\Delta} \gg 1, \end{cases} \quad (2.23)$$

$$P_{in} = \begin{cases} 1/(\tilde{\kappa} + 1), & \tilde{\Delta} = 0 \\ \tilde{\Delta}/(\tilde{\kappa} + \tilde{\Delta}), & \tilde{\Delta} \gg 1. \end{cases} \quad (2.24)$$

As might be expected, the probability P_{in} increases with bias and approaches unity as $\tilde{\Delta} \rightarrow \infty$, meaning that practically every blocker molecule entering the channel gets trapped at the sufficiently strong bias. Correspondingly, the escape probability, $P_{out} = 1 - P_{in}$, decreases with bias and vanishes as $\tilde{\Delta} \rightarrow \infty$. At strong

FIG. 1. Cartoon of a blocker in a cylindrical channel and the potential well of depth $\Delta = FL$ created for the blocker by the biasing force $F > 0$ in the channel of length L available for the center of the blocking molecule.

FIG. 2. Rate constants k_{out} , α_1 , and α_2 , the upper, middle, and lower curves, correspondingly, multiplied by t_L defined in Eq. (2.15), as functions of the dimensionless well depth $\tilde{\Delta} = \beta FL$. The rate constants are calculated according to Eqs. (2.16)–(2.18). The parameter $\tilde{\kappa}$ entering Eq. (2.16) is taken as $\tilde{\kappa} = 200$. This is a reasonable estimate for this quantity, which can be obtained for realistic values of the physical parameters in Eq. (2.15).

bias, a blocker entering the channel mainly jumps from site 1 to site 2. This is quite opposite to the situation in the absence of bias. Here, at realistic channel parameters, a blocker entering the channel mainly escapes and returns to the bulk solution. This is a consequence of the fact that the dimensionless trapping rate, $\tilde{\kappa} = 4LD_b/(\pi aD_{ch})$, is noticeably larger than unity.

Finally, we find the mean blocker lifetime in the channel by performing the integration in Eq. (2.5). The result is

$$\tau = \frac{t_L}{\tilde{\kappa}\tilde{\Delta}}(e^{\tilde{\Delta}} - 1). \quad (2.25)$$

This mean lifetime monotonically increases with the well depth from $t_L/\tilde{\kappa}$ at $\tilde{\Delta} = 0$, approaching its asymptotic behavior $t_L e^{\tilde{\Delta}}/(\tilde{\kappa}\tilde{\Delta})$ at $\tilde{\Delta} \gg 1$.

III. BLOCKER SURVIVAL PROBABILITY IN THE CHANNEL

In this section, we use the two-site model to analyze how the blocker survival probability in the channel depends on bias. Consider a blocker molecule entering the channel at time $t = 0$. Let $p_i(t)$, $i = 1, 2$, be the probabilities of finding the blocker on site i at time t . According to the kinetic scheme in Eq. (2.6), these probabilities satisfy the rate equations,

$$\frac{dp_1(t)}{dt} = -(k_{out} + \alpha_1)p_1(t) + \alpha_2 p_2(t), \quad (3.1)$$

$$\frac{dp_2(t)}{dt} = \alpha_1 p_1(t) - \alpha_2 p_2(t), \quad (3.2)$$

subject to the initial conditions, $p_1(0) = 1$ and $p_2(0) = 0$. The blocker survival probability in the channel, $S(t)$, is the sum of the probabilities $p_i(t)$

$$S(t) = p_1(t) + p_2(t). \quad (3.3)$$

After the Laplace transformation, Eqs. (3.1) and (3.2) take the form

$$s\hat{p}_1(s) - 1 = -(k_{out} + \alpha_1)\hat{p}_1(s) + \alpha_2\hat{p}_2(s), \quad (3.4)$$

$$s\hat{p}_2(s) = \alpha_1\hat{p}_1(s) - \alpha_2\hat{p}_2(s), \quad (3.5)$$

where s is the Laplace parameter and $\hat{f}(s)$ denotes the Laplace transform of function $f(t)$, $\hat{f}(s) = \int_0^\infty e^{-st}f(t)dt$. By solving Eqs. (3.4) and (3.5), we find that the Laplace transforms of the probabilities $\hat{p}_1(s)$ and $\hat{p}_2(s)$ are

$$\hat{p}_1(s) = \frac{s + \alpha_2}{s^2 + s(k_{out} + \alpha_1 + \alpha_2) + k_{out}\alpha_2}, \quad (3.6)$$

$$\hat{p}_2(s) = \frac{\alpha_1}{s^2 + s(k_{out} + \alpha_1 + \alpha_2) + k_{out}\alpha_2}. \quad (3.7)$$

Then, the Laplace transform of the survival probability, $S(t)$, is

$$\hat{S}(s) = \hat{p}_1(s) + \hat{p}_2(s) = \frac{s + \alpha_1 + \alpha_2}{s^2 + s(k_{out} + \alpha_1 + \alpha_2) + k_{out}\alpha_2}. \quad (3.8)$$

This Laplace transform can be written as

$$\hat{S}(s) = \frac{s + \alpha_1 + \alpha_2}{(s + s_1)(s + s_2)} = \frac{1}{s_1 - s_2} \left(\frac{s_1 - \alpha_1 - \alpha_2}{s + s_1} + \frac{\alpha_1 + \alpha_2 - s_2}{s + s_2} \right), \quad (3.9)$$

where s_1 and s_2 are given by

$$s_{1,2} = \frac{1}{2} \left[k_{out} + \alpha_1 + \alpha_2 \pm \sqrt{(k_{out} + \alpha_1 + \alpha_2)^2 - 4k_{out}\alpha_2} \right]. \quad (3.10)$$

Inverting the Laplace transform in Eq. (3.9), we arrive at

$$S(t) = \nu e^{-s_1 t} + (1 - \nu) e^{-s_2 t}, \quad (3.11)$$

where the weight factors ν and $1 - \nu$ are

$$\nu = \frac{s_1 - \alpha_1 - \alpha_2}{s_1 - s_2}, \quad 1 - \nu = \frac{\alpha_1 + \alpha_2 - s_2}{s_1 - s_2}. \quad (3.12)$$

The dependences of the decay rates s_1 and s_2 on the bias strength, i.e., on $\tilde{\Delta}$, are shown in Fig. 3. One can see that while the low rate, s_2 , decreases with $\tilde{\Delta}$, the high decay rate, s_1 , practically does not change. The weight factor ν of the rapidly decaying exponential decreases with $\tilde{\Delta}$, approaching zero, as $\tilde{\Delta} \rightarrow \infty$. Correspondingly, the weight factor of the slowly decaying exponential $1 - \nu$ increases with $\tilde{\Delta}$, approaching unity, as $\tilde{\Delta} \rightarrow \infty$.

Thus, the external bias results in a slowdown of the blocker escape from the channel. This is illustrated in Fig. 4. At strong bias, $\tilde{\Delta} \gg 1$, the rate constant α_2 becomes much smaller than two other rate constants, $\alpha_2 \ll k_{out}, \alpha_1$ (see Fig. 2). As a consequence, the expressions for decay rates s_1 and s_2 [Eq. (3.10)] and the weight factors ν and $1 - \nu$ [Eq. (3.12)] simplify and take the form

FIG. 3. The decay rates s_1 and s_2 [Eq. (3.10)] multiplied by t_L , top and bottom curves, respectively, determining the time behavior of the blocker survival probability in the channel $S(t)$ [Eq. (3.11)] as functions of the dimensionless well depth $\tilde{\Delta} = \beta FL$. As in Fig. 2, we take $\tilde{\kappa} = 200$.

FIG. 4. Survival probability of the blocker molecule in the channel $S(t)$ [Eq. (3.11)] at different values of the biasing force and hence the dimensionless well depth $\hat{\Delta} = \beta FL$, increasing from 0 to 2.5 to 7 (lower, middle, and upper curves, respectively) as functions of the dimensionless time t/t_L . Increasing bias results in a slowdown of the decay kinetics of $S(t)$. As in Fig. 2, we take $\tilde{\kappa} = 200$. The inset shows the long-time “tails” of the curves in a finer scale for $S(t)$ and the logarithmic scale for the dimensionless time.

$$s_1 \approx k_{out} + \alpha_1, \quad s_2 = \alpha_2 \quad (3.13)$$

and

$$v \approx \frac{k_{out}}{k_{out} + \alpha_1} = P_{out}, \quad 1 - v \approx \frac{\alpha_1}{k_{out} + \alpha_1} = P_{in}. \quad (3.14)$$

As mentioned earlier, at sufficiently strong bias, a blocker entering the channel mainly jumps to site 2, corresponding to the long-living state. In this limiting case, the biexponential decay of the survival probability [Eq. (3.11)] becomes essentially single-exponential

$$S(t) \approx e^{-s_2 t} \approx e^{-\alpha_2 t}. \quad (3.15)$$

IV. CONCLUSIONS

This paper focuses on the reversible blockage of membrane channels by a large charged solute that enters the channel from the membrane-bathing solution and comes back because it cannot pass through a constriction region (bottleneck, Fig. 1). It is worth noting that this situation is very common and represented by many studied examples of ion channel blockage by natural and synthetic solutes.⁷ We show that diffusive blocker dynamics in the channel can be mapped onto a much simpler two-site model, which gives the same mean blocker lifetime in the channel as the diffusion model.

The two-site model predicts biexponential decay of the blocker survival probability in the channel. This is, undoubtedly, a simplification of the real blocker escape kinetics from the channel in the general case. It is important, however, to emphasize that this kinetics can be studied experimentally only at sufficiently high voltage biases when the blocker lifetime in the channel is long enough to be measured. The reason is that high bias creates a

deep potential well for the blocker in the channel with a minimum located near the bottleneck. As a consequence, the eigenvalue spectrum of the Smoluchowski equation [Eq. (2.1)] under such conditions has a gap, and the decay of the survival probability, $S(t)$, has two components, fast and slow. This feature of $S(t)$ is caught by the two-site model. In a sense, the model lumps together all eigenstates corresponding to high eigenvalues of the Smoluchowski equation into one, a quickly decaying effective state. Thus, the two-site model effectively separates these eigenstates from the first one that corresponds to the smallest in a magnitude eigenvalue, responsible for the slowly decaying component of the survival probability. The existence of the gap is a universal property of the eigenvalue spectrum of the Smoluchowski equation at sufficiently high voltage bias. Therefore, the above arguments can be applied to more realistic models with varying channel geometry, nonlinear potential of mean force, and position-dependent blocker diffusivity.

The two-site model is fully determined by the three rate constants [see the kinetic scheme in Eq. (2.6)]. We derive general expressions giving these rate constants in terms of quantities that describe blocker diffusive dynamics in the channel [Eqs. (2.9)–(2.12)]. These expressions significantly simplify for a cylindrical channel with position-independent intrachannel diffusivity [Eqs. (2.16)–(2.18)]. The latter expressions are used to analyze the blocker escape kinetics from the channel in Sec. III, with the focus on the kinetics dependence on the applied voltage bias. Our analysis demonstrates that the two-site model captures the main feature of the blockage process, namely, that the decay of the blocker survival probability in the channel slows down as the applied bias increases (Fig. 4). We found that the average time the blocker spends in the channel grows nearly exponentially with the bias [Eq. (2.25)]. We hope that both our general approach and obtained results will be of use in structure-guided search for the new drugs whose action is based on the phenomenon of ion channel blockage.^{7(c),7(d),8} It is worth noting that our analysis based on the diffusion equation is equally applicable not only to the blockage of membrane channels but also to the blockage of microfluidic devices.

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APPENDIX A: BLOCKER MEAN LIFETIME IN THE CHANNEL

In this appendix, we derive the expression for the mean blocker lifetime in the channel, τ , given in Eq. (2.5). The propagator $G(x, t)$ of the blocker entering the channel at $t = 0$ satisfies Eq. (2.1) with the initial condition $G(x, 0) = \delta(x)$ and the boundary conditions in Eq. (2.2). The blocker survival probability in the channel for time t , denoted by $S(t)$, is

$$S(t) = \int_0^L G(x, t) dx. \quad (A1)$$

The probability density of the blocker lifetime in the channel, $\varphi(t)$, given in Eq. (2.4) is related to the survival probability by the relation $\varphi(t) = -dS(t)/dt$. Using this, we can write the mean lifetime τ [Eq. (2.5)] in terms of the survival probability as

$$\tau = \int_0^\infty t\varphi(t)dt = \int_0^\infty S(t)dt. \quad (\text{A2})$$

Substituting $S(t)$ in Eq. (A1) into the above equation and introducing function $g(x)$ defined as

$$g(x) = \int_0^\infty G(x,t)dt, \quad (\text{A3})$$

we arrive at the expression for the mean lifetime τ in terms of function $g(x)$

$$\tau = \int_0^L g(x)dx. \quad (\text{A4})$$

To determine function $g(x)$, we integrate both sides of Eq. (2.1) as well as the boundary conditions in Eq. (2.2) over time from zero to infinity. As a result, we find that function $g(x)$ satisfies the equation

$$\frac{d}{dx} \left[D_{ch}(x) e^{-\beta U(x)} \frac{d}{dx} (e^{\beta U(x)} g) \right] = -\delta(x), \quad (\text{A5})$$

subject to the boundary conditions

$$D_{ch}(0) e^{-\beta U(0)} \frac{\partial}{\partial x} (e^{\beta U(x)} g) \Big|_{x=0} = \kappa g(0), \quad \frac{\partial}{\partial x} (e^{\beta U(x)} g) \Big|_{x=L} = 0. \quad (\text{A6})$$

Solving Eq. (A5) for $\varepsilon < x < L$, $\varepsilon \rightarrow 0$, and using the reflecting boundary condition at $x = L$, we obtain

$$g(x) = g(0) e^{-\beta(U(x)-U(0))}. \quad (\text{A7})$$

Finally, to find $g(0)$, we integrate both sides of Eq. (A5) from zero to ε and use the boundary condition at $x = 0$. This leads to the relation $\kappa g(0) = 1$, and hence, $g(0) = 1/\kappa$. Thus, we have

$$g(x) = \frac{1}{\kappa} e^{-\beta(U(x)-U(0))}. \quad (\text{A8})$$

Substituting this into Eq. (A4), we arrive at the expression for the mean lifetime τ given in Eq. (2.5) of the main text.

APPENDIX B: RATE CONSTANTS k_{out} AND α_1

In this appendix, we derive expressions for the rate constants k_{out} and α_1 given in Eq. (2.9) with A and τ_1 given in Eqs. (2.10) and (2.11). To do this, we take advantage of the fact that these rate constants can be expressed in terms of the mean lifetime on site 1, τ_1 [Eq. (2.7)], and the probabilities P_{out} and P_{in} [Eq. (2.8)] as

$$k_{out} = P_{out}/\tau_1, \quad \alpha_1 = P_{in}/\tau_1. \quad (\text{B1})$$

Thus, to find the rate constants, we need to know τ_1 , P_{out} , and P_{in} . To derive expressions for these quantities, consider a blocker entering the channel at $t = 0$. This blocker either escapes from the channel to the bulk solution without touching the reflecting wall at $x = L$ or touches this wall. The probabilities of these two outcomes, respectively, are P_{out} and $P_{in} = 1 - P_{out}$. We can find these

probabilities using the Smoluchowski equation [Eq. (2.1)] for the propagator $G(x,t)$ with the initial condition $G(x,0) = \delta(x)$, the radiation boundary condition at $x = 0$ in Eq. (2.2), and the absorbing boundary condition at $x = L$

$$G(L,t) = 0. \quad (\text{B2})$$

This replaces the reflecting boundary condition at $x = L$ in Eq. (2.2) and guarantees termination of the blocker trajectory as soon as it touches the reflecting wall for the first time.

The flux escaping from the channel to the bulk solution at time t , denoted by $f_{out}(t)$, is given by

$$f_{out}(t) = \kappa G(0,t). \quad (\text{B3})$$

The escape probability, P_{out} , is the integral of this flux over time from zero to infinity

$$P_{out} = \int_0^\infty f_{out}(t)dt = \kappa g(0), \quad (\text{B4})$$

where function $g(x)$ is defined by

$$g(x) = \int_0^\infty G(x,t)dt. \quad (\text{B5})$$

To find this function, we integrate Eq. (2.1), the radiation boundary condition at $x = 0$ in Eq. (2.2), and the absorbing boundary condition at $x = L$ in Eq. (B2) over time from zero to infinity. As a result, we find that function $g(x)$ satisfies the equation

$$\frac{d}{dx} \left[D_{ch}(x) e^{-\beta U(x)} \frac{d}{dx} (e^{\beta U(x)} g) \right] = -\delta(x), \quad (\text{B6})$$

subject to the boundary conditions

$$D_{ch}(0) e^{-\beta U(0)} \frac{\partial}{\partial x} (e^{\beta U(x)} g) \Big|_{x=0} = \kappa g(0), \quad g(L) = 0. \quad (\text{B7})$$

Solving Eq. (B6) for $\varepsilon < x < L$, $\varepsilon \rightarrow 0$, and using the absorbing boundary condition at $x = L$, we obtain

$$g(x) = C e^{-\beta U(x)} \int_x^L (e^{\beta U(y)} / D(y)) dy, \quad (\text{B8})$$

where C is a constant. We find this constant by integrating both sides of Eq. (B6) from zero to ε and using the radiation boundary condition at $x = 0$ in Eq. (B7). This leads to the equation

$$1 = C + \kappa g(0) = C(1 + A), \quad (\text{B9})$$

where constant A is defined in Eq. (2.10). Then, we have

$$C = 1/(1 + A), \quad (\text{B10})$$

and function $g(x)$ is given by

$$g(x) = (1 + A)^{-1} e^{-\beta U(x)} \int_x^L (e^{\beta U(y)} / D(y)) dy. \quad (\text{B11})$$

Eventually, we find the probabilities P_{out} and P_{in} by substituting $g(0)$ into Eq. (B4). The result is

$$P_{out} = \kappa g(0) = \frac{A}{1+A}, \quad P_{in} = 1 - P_{out} = \frac{1}{1+A}. \quad (\text{B12})$$

Following the same line of reasoning as in [Appendix A](#), we find that the mean blocker lifetime on site 1, τ_1 , is related to the function $g(x)$, defined in Eq. (B5), by the relationship

$$\tau_1 = \int_0^L g(x) dx. \quad (\text{B13})$$

Substituting here $g(x)$ in Eq. (B11) and changing the order of integration in the resulting formula, we arrive at the expression for the mean lifetime τ_1 given in Eq. (2.11) of the main text. Finally, substituting P_{out} and P_{in} in Eq. (B12) and τ_1 in Eq. (B13) into the formulas for the rate constants k_{out} and α_1 [Eq. (B1)], we recover the expressions for these rate constants in Eq. (2.9) of the main text with A and τ_1 given in Eqs. (2.10) and (2.11).

APPENDIX C: RATE CONSTANT α_2

According to Eq. (2.9), the rate constant α_2 is the inverse of the mean blocker lifetime on site 2, τ_2 , which is the mean first-passage time of the blocker from the reflecting wall to the channel entrance. To find this mean first-passage time, consider a blocker starting from the reflecting wall at time $t = 0$. The blocker propagator $G(x, t)$ satisfies the Smoluchowski equation [Eq. (2.1)] subject to the initial condition $G(x, 0) = \delta(x - L)$ and absorbing and reflecting boundary conditions at the channel entrance ($x = 0$) and reflecting wall ($x = L$), respectively,

$$G(0, t) = \frac{\partial}{\partial x} \left(e^{\beta U(x)} G(x, t) \right) \Big|_{x=0} = 0. \quad (\text{C1})$$

The absorbing boundary condition at $x = 0$ guarantees termination of the blocker trajectory as soon as it comes to the channel entrance for the first time.

Following the same line of reasoning as in [Appendix A](#), it can be shown that time mean first-passage time τ_2 can be written as an integral over x from zero to L of a function $g(x)$

$$\tau_2 = \int_0^L g(x) dx. \quad (\text{C2})$$

This function satisfies the equation

$$\frac{d}{dx} \left[D_{ch}(x) e^{-\beta U(x)} \frac{d}{dx} \left(e^{\beta U(x)} g \right) \right] = -\delta(x - L), \quad (\text{C3})$$

subject to the boundary conditions

$$g(0) = \frac{\partial}{\partial x} \left(e^{\beta U(x)} g \right) \Big|_{x=L} = 0. \quad (\text{C4})$$

We find function $g(x)$ by solving Eq. (C3). The result is

$$g(x) = e^{-\beta U(x)} \int_0^x \left(e^{\beta U(y)} / D(y) \right) dy. \quad (\text{C5})$$

Substituting this into Eq. (C2) and changing the order of integration in the resulting formula, we arrive at the expression for the mean lifetime τ_2 given in Eq. (2.12) of the main text.

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