

On a novel rate theory for transport in narrow ion channels and its application to the study of flux optimization via geometric effects

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We present a novel rate theory based on the notions of splitting probability and mean first passage time to describe single-ion conduction in narrow, effectively one-dimensional membrane channels. In contrast to traditional approaches such as transition state theory or Kramers theory, transitions between different conduction states in our model are governed by rates which depend on the full geometry of the potential of mean force (PMF) resulting from the superposition of an equilibrium free energy profile and a transmembrane potential induced by a nonequilibrium constraint. If a detailed theoretical PMF is available (e.g., from atomistic molecular dynamics simulations), it can be used to compute characteristic conductance curves in the framework of our model, thereby bridging the gap between the atomistic and the mesoscopic level of description. Explicit analytic solutions for the rates, the ion flux, and the associated electric current can be obtained by approximating the actual PMF by a piecewise linear potential. As illustrative examples, we consider both a theoretical and an experimental application of the model. The theoretical example is based on a hypothetical channel with a fully symmetric sawtooth equilibrium PMF. For this system, we explore how changes in the spatial extent of the binding sites affect the rate of transport when a linear voltage ramp is applied. Already for the case of a single binding site, we find that there is an optimum size of the site which maximizes the current through the channel provided that the applied voltage exceeds a threshold value given by the binding energy of the site. The above optimization effect is shown to arise from the complex interplay between the channel structure and the applied electric field, expressed by a nonlinear dependence of the rates with respect to the linear size of the binding site. In studying the properties of current-voltage curves, we find a double crossover between sublinear and superlinear behaviors as the size of the binding site is varied. The ratio of unidirectional fluxes clearly deviates from the Ussing limit and can be characterized by a flux ratio exponent which decreases below unity as the binding site becomes wider. We also explore effects arising from changes in the ion bulk concentration under symmetric ionic conditions and the presence of additional binding sites in the hypothetical channel. As for the experimental application, we show that our rate theory is able to provide good fits to conductance data for sodium permeation through the gramicidin A channel. Possible extensions of the theory to treat the case of an asymmetric equilibrium PMF, fluctuations in the mean number of translocating ions, the case of fluctuating energy barriers, and multi-ion conductance are briefly discussed. © 2009 American Institute of Physics. [DOI: [10.1063/1.3077205](https://doi.org/10.1063/1.3077205)]

I. INTRODUCTION

Ion channels are membrane proteins which enable selected ions to permeate membranes at near diffusion-limited rates. They play a key role in the membranes of excitable cells¹ and their malfunction is associated with a number of diseases.² From a biophysical perspective they are of interest as they offer the possibility of developing a rigorous theoretical approach to the relationship between structure and physiological transport function for a key class of membrane protein. In particular, the question of how the structure of ion channels is related to the large transport rates observed (despite very high ion selectivity) is of significant interest. The

reverse question, namely, how the morphologies of characteristic conductance curves for channels with different structures differ from each other, is also of physiological importance.

A number of theoretical and computational approaches have been used to explore selectivity and permeation of ions through channels,^{3–5} including equilibrium Monte Carlo,⁶ molecular dynamics (MD),⁷ and Brownian dynamics⁸ simulations. These approaches have been very productive, but calculations for ion transport properties (i.e., permeation and selectivity) from channel structures remain a challenging task due to the complexity of the channel proteins and the computational limitations for the implementation of the associated interactions, preventing one from realistically modeling ion transport processes beyond the nanosecond time

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scale. Hybrid approaches combining both computer simulations and the theory of stochastic processes may prove useful to access larger time scales (involving a repeated series of ion translocation events) and thus improve our current understanding of structure-function relationship in ion channels. In this context, one of the possible research strategies is to use a potential of mean force (PMF) obtained via detailed atomistic simulations of protein structure as an input to mesoscopic transport models, i.e., continuum electrodiffusion or hopping models.

Traditional one-dimensional (1D) hopping models for single-ion transport in narrow channels are based on Arrhenius-like rates given by the product of exponentials of the corresponding energy barriers (in $k_B T$ units) times frequency factors.^{1,9,10} In the framework of Arrhenius theory for first-order kinetics, the frequency factor is the number of attempts per unit time made by the reactant to reach the activated state, while the exponential factor gives the fraction of successful attempts. For ion transport, the nature of the frequency factors and its possible dependence on the geometry of the PMF and the diffusion coefficient profile governing the ion's motion are subtle questions, to the extent that in the absence of a detailed, first-principles theoretical framework the frequency factors are often regarded as free parameters to fit experimental data.

Following Eyring's work on the theory of dilute gas phases,^{11,12} frequency factors were often set equal to a universal factor $k_B T/h$ (where $h=6.626 \times 10^{-34}$ J s is the Planck constant) to deal with a number of problems in chemical kinetics. However, in the case of ion channels this simple ansatz turns out to greatly overestimate the transport rate, as it fails to account for the strong friction experienced by ions in the solvent within the restrained channel geometry. A more refined approach valid in the limit of moderate to strong friction was provided by Kramers' pioneering work,¹³ who obtained frequency factors given by the curvature of the potential at both its minimum (metastable state) and its maximum (top of the energy barrier). Kramers' result was originally obtained from the solution of a differential equation, but it can also be recovered from the notion of mean first passage time (MFPT), i.e., the time needed to reach a certain point in space for the first time via diffusive motion. Indeed, there is an intimate relationship between the rate of escape of a particle over a barrier top and the inverse of the MFPT.¹⁴⁻¹⁹ The latter can be expressed in terms of a double integral over exponential functions of the PMF extending over an interval containing both the minimum and the maximum. A steepest descent approximation of this integral involving a second-order (harmonic) expansion around these two points eventually yields Kramers' expressions. The approximation can be further improved by considering higher order terms in the spatial derivatives of the PMF.^{17,20}

However, this kind of expansion is not suitable to describe situations in which the potential displays sharp ledges between the minimum and the maximum²¹ or where knowledge of the potential is limited to a small set of fairly distant points and one has no reasonable basis to perform smooth, higher order interpolation. In such cases, linear interpolation may be a helpful tool to test the role of the potential geom-

etry in terms of a few length and energy scales. For a piecewise linear PMF and a piecewise constant diffusion coefficient the integral for the MFPT (and thus the escape rate over the barrier) can be computed exactly; the resulting explicit analytic expressions allow one to study the effect of the individual potential slopes on the escape rate over the full barrier.

Kramers' original calculation concerned a situation in which the particle is allowed to escape on one side of the potential well only, since a confining, infinite potential wall is found on the other side of the asymmetric well. The situation in narrow channels is different, as ions can usually escape over the left and the right barriers characterizing a given binding site. Kramers' theory can be easily extended to account for escape from such a metastable state in a double-humped potential and, as one would anticipate, the result is that the total escape rate is equal to the sum of the partial escape rates over each of the potential barriers.

In the present work, we show that each of these partial rates are limiting expressions of more general transition rates which can be written as products of the total escape rate (associated with the inverse MFPT to reach either of the two maxima) and the so-called splitting probability, i.e., the probability to attain the maximum on one side of the well before reaching the one on the other side. It is well known that the splitting probability obeys a differential equation governed by the backward Fokker-Planck operator for the underlying diffusion process whose solution can be expressed as a ratio of two integrals involving exponential functions of the potential.¹⁴

In an interesting work, Cooper *et al.* used the MFPT to construct rates for transitions over a single energy barrier.²¹ In contrast, the rates we shall present here are valid for the case where a competition between transitions over two barriers takes place and, as already mentioned above, they make additional use of the splitting probability. Such rates are defined even in the case of a nondifferentiable potential profile (such as a piecewise potential) and can be used for numerical or analytical computations of the ionic current. As it turns out, one can find exact analytic solutions for the rates when the PMF is described by a set of linear polynomials. We shall use these results as well as the constraints imposed by the principle of microscopic reversibility at equilibrium (detailed balance) to explore the role of geometry in the framework of a simple stochastic conduction model displaying surprisingly rich behavior. In particular, we shall see that there is an optimum size of the binding site domain which maximizes the ionic current provided that the applied voltage exceeds a certain threshold value. This optimization effect is fundamentally different from the one reported recently by Kolomeisky,²² where flux optimization with respect to the binding energy of the site was observed under asymmetric ionic conditions. At the level of the current-voltage curves, we also find an interesting double crossover between sublinear and superlinear behaviors when σ (the fraction of the channel length occupied by a binding site) is varied. Beyond these two striking results, our approach may prove to be a useful tool to bridge the gap between atomistic channel structure and macroscopic ion flux measurements. In order to

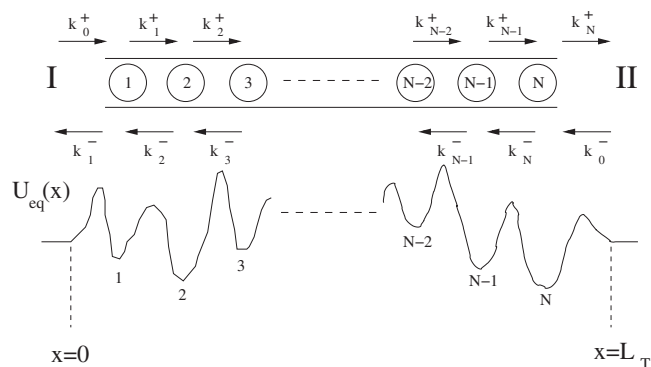


FIG. 1. Schematic view of the model for single-ion conduction and correspondence between the states and the equilibrium PMF $U_{eq}(x)$: The channel ends (at equal equilibrium potential) are located at $x=0$ and $x=L_T$, where L_T is the effective channel length.

illustrate this, we show how our rate theory can be used to fit conductance data for the gramicidin A (gA) channel.

II. MASTER EQUATION FORMULATION

The theoretical approach in this section draws considerably on Refs. 22 and 23. Following Ref. 22, we consider ion transport between two reservoirs I and II with respective ion concentrations C^I and C^{II} . Both reservoirs are connected through a narrow (≈ 1 D) membrane channel of total effective length L_T as depicted in Fig. 1. The channel is assumed to hold at most one ion at all times. Single ions enter the channel from the left (right) reservoir I (II) and can be found in relatively stable positions at N not necessarily identical binding sites. Thus, during ion transport, the channel switches between an empty state and states where a single ion is at a given site. Let us denote by P_0 the probability of the empty state and by P_n the probability that the channel holds an ion at the n th site. The above definition of the conduction states implicitly assumes that there are significant energy barriers separating each of the N states so that a description in terms of a hopping model pertains. This requires separation of time scales, implying that the mean relaxation time of the degrees of freedom associated with the electrostatic and the entropic contributions to the PMF is very different from the dwelling time of an ion in any of the metastable states in the reservoirs and the binding sites. Under these conditions one can define a static PMF $U(x)$ (for simplicity expressed in $k_B T$ units in what follows) describing the interactions arising from the channel structure, the solvent, and possibly other ions in solution moving on a different time scale and use a hopping model to describe ion translocation through jumps between metastable states corresponding to local minima of $U(x)$. The determination of this function lies beyond the scope of this paper and can be done by simultaneously solving the equations for the electrostatic potential and the transport equations via self-consistent methods (e.g., Poisson-Nernst-Planck-like theories²⁴); an alternative approach we shall follow here is to use a given $U(x)$, obtained, e.g., from the outcome of MD simulations of possible ion trajectories) as input to an adequate transport model. If the computed $U(x)$ turns out to display well iden-

tified binding sites, then a hopping model can indeed be regarded as a suitable approach.

The total PMF $U(x)$ “seen” by an ion is the superposition of two contributions, i.e., $U(x) = U_{eq}(x) + U_{noneq}(x)$, where $U_{eq}(x)$ is the equilibrium PMF due to the intrinsic channel structure and the ion equilibrium distribution and $U_{noneq}(x)$ is a transmembrane potential arising from a non-equilibrium constraint such as a difference in bulk concentration $\Delta C = C^I - C^{II}$ (the possibility considered in Ref. 22) or an applied voltage V .

A change in V or ΔC will modify $U(x)$, and the positions of the minima and maxima will be shifted with respect to their equilibrium locations [=extrema of $U_{eq}(x)$]. Besides, for a sufficiently complex geometry of $U_{eq}(x)$ or $U_{noneq}(x)$, some shallow wells corresponding to intermediate substates between the binding sites could appear as a result of applying the perturbation. However, we shall assume that the latter is weak compared to the typical binding energy of the sites and that its spatial variation is sufficiently smooth. Under these conditions, the shift of the pre-existing potential wells can be neglected in good approximation and no additional shallow states that would complicate the description and question the validity of the hopping model will arise.²⁵

Let us further assume that the hopping dynamics is Markovian, restricted to nearest-neighbor jumps and governed by the master equation

$$\frac{dP_n}{dt} = k_{n-1}^+ P_{n-1} + k_{n+1}^- P_{n+1} - (k_n^- + k_n^+) P_n, \quad (1)$$

where $n = 1, \dots, N$ and $k_n^\pm$ is the (time-independent) Markov rate (measured in s^{-1}) for jumps between state n and state $n \pm 1$. Equation (1) is subject to periodic boundary conditions, i.e., one sets $P_{N+1} = P_0$ and $k_{N+1}^- = k_0^-$ in the N th equation.

The above Markovian approximation is justified, as the dwelling time of the ions in each metastable state is assumed to be long enough to thermally wash out the memory of their previous trajectory. The explicit form of the rates and its dependence on $U(x)$ is crucial to determine the conduction properties of the model and will be discussed in detail in the next section. Besides, one has the (obvious) normalization condition

$$\sum_{n=0}^N P_n = 1. \quad (2)$$

The quantities $k_n^+ P_n \equiv j_n^+$ and $k_{n+1}^- P_{n+1} \equiv j_{n+1}^-$ appearing in Eq. (1) can respectively be interpreted as unidirectional probability fluxes over the energy barrier separating states n and $n+1$. In the stationary regime, i.e., when all the time derivatives are zero, one sees from the above set of equations that the net flux $j_n^+ - j_{n+1}^-$ over any barrier is the same everywhere and equal to a constant, say, j .

Using Eq. (1) and the normalization condition (2), one can solve for the steady state probabilities by means of diagrammatic cycle flux methods^{29,30} and subsequently obtain an expression for j in terms of the rates. Alternatively, one can take advantage of a mapping to a single particle hopping model on a 1D periodic lattice with $N+1$ sites²² whose dy-

namic properties are exactly known.²³ The solution for the stationary flux in terms of the rates can be written as the difference between the flux in positive direction j^+ and the flux in negative direction j^- . It reads as

$$j = j^+ - j^- = \frac{1}{\left[\sum_{n=1}^{N+1} r_n\right] \prod_{n=1}^{N+1} k_n^+} \left[\prod_{n=1}^{N+1} k_n^+ - \prod_{n=1}^{N+1} k_n^- \right], \quad (3)$$

where

$$r_n = \frac{1}{k_n^+} \left[1 + \sum_{k=1}^N \prod_{j=1}^k \left(\frac{k_{n+j}^-}{k_{n+j}^+} \right) \right] \quad (4)$$

(again, the subindices in the products and the sums are evaluated modulo $N+1$; in particular, one takes $k_{N+1}^\pm = k_0^\pm$).

III. TRANSITION RATES

To start with, let us set $k_0^+ = k_{\text{on}}^{\text{I}}$ and $k_0^- = k_{\text{on}}^{\text{II}}$ for clarity. A traditional distinction between $k_{\text{on}}^{\text{I,II}}$ and the other rates (describing transitions whose outgoing states are binding sites) is that the former depend both on bulk concentrations and $U(x)$ [see Eqs. (13) and (14) in Sec. III B], whereas the latter depend only on $U(x)$. This simplified picture, which we shall also retain here, assumes that $U(x)$ does not depend on the absolute bulk concentrations $C^{\text{I,II}}$ but only on the concentration difference ΔC .³¹

A. Explicit form of the rates for jumps from binding sites

As already anticipated in Sec. I, we intend to go beyond Eyring's classical transition state theory [which overestimates these rate constants by writing them as exponentials of energy barriers times a universal factor $(k_B T)/h$] as well as Kramers' theory (which only incorporates the PMF geometry via the curvature at adjacent extrema). To this end, let us make the heuristic assumption that the overdamped motion of an ion about the n th well on time scales short compared with the mean dwelling time in the well is properly described by a Smoluchowski equation of the form

$$\frac{\partial P(x,t)}{\partial t} = D_n \frac{\partial}{\partial x} \left[\frac{\partial U(x)}{\partial x} P(x,t) + \frac{\partial}{\partial x} P(x,t) \right], \quad x_n^- \leq x \leq x_n^+, \quad (5)$$

where $P(x,t)$ is the probability density to find the ion between x and $x+dx$ inside the n th well at time t and $x_n^{-(+)}$ denotes the coordinate of the maximum located to the left (right) of the energy minimum at $x=x_n$.³³ We shall characterize diffusional transport by a constant effective value of the diffusion coefficient D_n .

The splitting probability $R(x_n^-|x)$ associated with Eq. (5), i.e., the probability to reach the maximum at x_n^- before the maximum at x_n^+ starting from a given position x inside the well, obeys the differential equation¹⁴

$$D_n \left[\frac{\partial^2}{\partial x^2} - \frac{\partial U(x)}{\partial x} \frac{\partial}{\partial x} \right] R(x_n^-|x) = 0, \quad x_n^- \leq x \leq x_n^+, \quad (6)$$

subject to the boundary conditions

$$R(x_n^-|x_n^-) = 1, \quad R(x_n^-|x_n^+) = 0. \quad (7)$$

The solution of Eq. (6) with Eq. (7) for the special case $x = x_n$ reads

$$R_n \equiv R(x_n^-|x_n) = \frac{\int_{x_n^-}^{x_n^+} dx \exp(U(x))}{\int_{x_n^-}^{x_n^+} dx \exp(U(x))}. \quad (8)$$

On the other hand, the escape rate from the well is $\tilde{k}_n \equiv (2\tau_n)^{-1}$ [this is just the inverse of the lowest eigenvalue of the Fokker–Planck operator associated with Eq. (5) (Ref. 17)], where τ_n is the average time to reach either maxima starting from x_n . This characteristic time is given by the following expression:^{14,16}

$$\tau_n = D_n^{-1} \left\{ \int_{x_n^-}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y)) - R_n \int_{x_n^-}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y)) \right\}. \quad (9)$$

Thus, the partial escape rates associated with jumps to the minima at x_{n-1} and x_{n+1} will, respectively, be given by the total jump rate $\tilde{k}_n$ times the corresponding splitting probability:

$$\tilde{k}_n^- = R_n \tilde{k}_n, \quad (10a)$$

$$\tilde{k}_n^+ = (1 - R_n) \tilde{k}_n. \quad (10b)$$

As it should be, if $U(x)$ is assumed to be sufficiently smooth, standard expansions of the above integrals show that in the limit of large friction [small D_n or large value of the energy barriers $\Delta U_n^\pm \equiv U(x_n^\pm) - U(x_n)$] the rates (10) reduce to the usual Kramers' result featuring the corresponding energy barrier height and the curvature of the total potential at both x_n and the corresponding maximum $x_n^\pm$ (see the proof in the Appendix):

$$k_n^{\text{Kr},-} = \frac{D_n}{2\pi} \sqrt{|U''(x)|_{x=x_n^-} U'''(x)|_{x=x_n^-}} \exp(-\Delta U_n^-), \quad (11a)$$

$$k_n^{\text{Kr},+} = \frac{D_n}{2\pi} \sqrt{|U''(x)|_{x=x_n^+} U'''(x)|_{x=x_n^+}} \exp(-\Delta U_n^+). \quad (11b)$$

In the general case, though, the rates will depend on all details of the potential geometry via the above integrals, which are well defined even in the case of a PMF with discontinuous derivatives (see Sec. IV).

The above rates hold for the case in which one has a sharp initial condition, i.e., the ion starts exactly at $x=x_n$. However, thermal fluctuations will in general make the ion jiggle about the minimum. Therefore, an ion will be at any position $x_n^- < x < x_n^+$ with a probability given by the Boltzmann distribution. Thus, a more correct approach consists in taking as the rates to be used the thermal averages of $\tilde{k}_n^\pm$, i.e.,

$$\bar{k}_n^- = Z_n^{-1} \int_{x_n^-}^{x_n^+} \tilde{k}_n^-|_{x_n=x} \exp(-U(x)) dx, \quad (12a)$$

$$k_n^+ = Z_n^{-1} \int_{x_n^-}^{x_n^+} \tilde{k}_n^+|_{x_n=x} \exp(-U(x)) dx, \quad (12b)$$

where $Z_n = \int_{x_n^-}^{x_n^+} \exp(-U(x)) dx$. One can easily show that the total escape rate $k_n \equiv k_n^+ + k_n^-$ obeys the inequality $k_n \geq \tilde{k}_n$, i.e., escape is facilitated by thermal fluctuations about the minimum. However, in the limit of large barriers (or the biologically less relevant one of low temperatures) the ion sojourn probability will display a sharp peak at $x=x_n$ and the partial rates $\tilde{k}_n^\pm$ will rapidly become indistinguishable from their thermal averages $k_n^\pm$ and converge to the well-known Kramers' expressions (11).

B. Entry rates

The process of ion entry into the channel is rather complex and not understood in detail. We shall therefore adopt a less detailed approach to model the entry rates $k_{\text{on}}^{\text{I,II}}$. In what follows, we shall hypothesize that the process of entering the channel from the bulk can be decomposed into a diffusive motion from the bulk solution to the vicinity of the barriers at each channel end and a subsequent activated jump over the latter.^{34–37} This approach is pertinent in cases where the potential drop inside the bath is negligible with respect to its change in the channel (see the example of the gA channel in Sec. V). The channel end can be viewed as a partially absorbing target of complex geometry which is reached by the ions through diffusion. Capture by the target requires surmounting the entry barrier. Smoluchowski's theory for diffusion to a partially absorbing target shows that the entry rates are of the form³⁵

$$k_{\text{on}}^{\text{I}} = \kappa^{\text{I}} \exp(-(U(x_1^-) - U(0))), \quad (13a)$$

$$k_{\text{on}}^{\text{II}} = \kappa^{\text{II}} \exp(-(U(x_N^+) - U(L_T))), \quad (13b)$$

where $x=0$ and $x=L_T$ are the coordinates of both channel ends (see Fig. 1) and the kinetic parameters

$$\kappa^{\text{I,II}} = \lambda^{\text{I,II}} D_{\text{bulk}} C^{\text{I,II}} \quad (14)$$

model the diffusive approach to the channel ends. In Eq. (14), D_{bulk} is the diffusion coefficient of ions in solution and $\lambda^{\text{I,II}}$ is a prefactor which depends on the geometry of the reservoir and the target (=channel end). This implies that the rate constants can be written in terms of their equilibrium counterparts as follows:

$$k_{\text{on}}^{\text{I}} = k_{\text{on}}^{\text{I,eq}} \exp(-(U_{\text{noneq}}(x_1^-) - U_{\text{noneq}}(0))), \quad (15a)$$

$$k_{\text{on}}^{\text{II}} = k_{\text{on}}^{\text{II,eq}} \exp(-(U_{\text{noneq}}(x_N^+) - U_{\text{noneq}}(L_T))), \quad (15b)$$

where the equilibrium rates take the form

$$k_{\text{on}}^{\text{I,eq}} = \kappa^{\text{I}} \exp(-(U_{\text{eq}}(x_1^-) - U_{\text{eq}}(0))), \quad (16a)$$

$$\begin{aligned} k_{\text{on}}^{\text{II,eq}} &= \kappa^{\text{II}} \exp(-(U_{\text{eq}}(x_N^+) - U_{\text{eq}}(L_T))) \\ &= \kappa^{\text{II}} \exp(-(U_{\text{eq}}(x_N^+) - U_{\text{eq}}(0))). \end{aligned} \quad (16b)$$

C. Role of detailed balance and connection of the model with simulations and ion flux measurements

The equilibrium rates for transitions between different states are not independent, but rather related to each other by the condition of microscopic reversibility (detailed balance). This condition requires that in the absence of applied voltage, $j=0$ everywhere, implying $j_n^{\text{I,eq}} = j_{n+1}^{\text{I,eq}}$. Thus, one has the $N+1$ equations

$$k_n^{\text{I,eq}} P_n^{\text{eq}} = k_{n+1}^{\text{I,eq}} P_{n+1}^{\text{eq}}. \quad (17)$$

Following Ref. 22, we now use $P_{n+1}^{\text{eq}}/P_n^{\text{eq}} = \exp(-U_{\text{eq}}(x_{n+1}) + U_{\text{eq}}(x_n))$ to express the set of N diffusion coefficients $\{D_n\}$ and one of the equilibrium entry rates (say, $k_{\text{on}}^{\text{II,eq}}$) in terms of the other entry rate (say, $k_{\text{on}}^{\text{I,eq}}$).³⁸ If an analytic expression for $U_{\text{eq}}(x)$ is available (e.g., from a spline fit to the output of a MD simulation^{4,5}), the integrals in the rates can be computed numerically. Alternatively, one can also find analytical expressions for the integrals by using piecewise linear fits to approximate the equilibrium PMF. As will be seen in the next section, this procedure allows one to compute explicit expressions for the equilibrium rates for hopping from binding sites. One is thus left with a single free parameter $k_{\text{on}}^{\text{I,eq}}$, which, however, must take values compatible with the experimental range of the diffusion coefficients. Typically, the values for the latter are significantly smaller than D_{bulk} , dropping down to $0.1D_{\text{bulk}}$ or even less (guideline values of D_{bulk} for different ions can be found in, e.g., Ref. 39).

Once the diffusion coefficients and $k_{\text{on}}^{\text{II,eq}}$ have been determined in terms of $k_{\text{on}}^{\text{I,eq}}$, the resulting expressions can subsequently be used in Eqs. (9) and (10) together with the full PMF $U(x)$ obtained from simulations for particular values of V and/or ΔC to compute the associated nonequilibrium rates. Again, explicit analytic solutions are only possible if $U_{\text{noneq}}(x)$ is approximated by piecewise linear functions, as previously done for $U_{\text{eq}}(x)$ (see also next section). We emphasize that, for realistic applications to ion channels, this task requires as a necessary input the outcome of a previous numerical computation of the transmembrane potential $U_{\text{noneq}}(x)$, which is in general a nontrivial function of V or ΔC .⁴⁰ However, the highly idealized case of the constant field approximation for the potential drop along the restricted channel geometry (leading to the Goldman-Hodgkin-Katz equation in the framework of electrodiffusion models) works surprisingly well in a number of cases, as seen from transmembrane potential calculations for the gA channel and the open-state KcsA channel (see Refs. 41 and 42, respectively).

Once the two contributions to the full PMF $U(x)$ are known, one is in the position to calculate the ion flux j in terms of $k_{\text{on}}^{\text{I,eq}}$ for a given value of V or ΔC . The associated ionic current I and the conductance $\mathcal{G} = I/V$ follow straightforwardly from the relation $I = Zej$, where Z is the ion valency. The entry rate $k_{\text{on}}^{\text{I,eq}}$ can then be used to obtain least-squares fits matching a series of experimental characteristic curves for I and $\mathcal{G}$. One can, for instance, consider the case of symmetric ionic conditions and study how I or $\mathcal{G}$ change with the applied voltage V or with bulk ion concentration C when V is held constant (this latter case is explored in Sec.

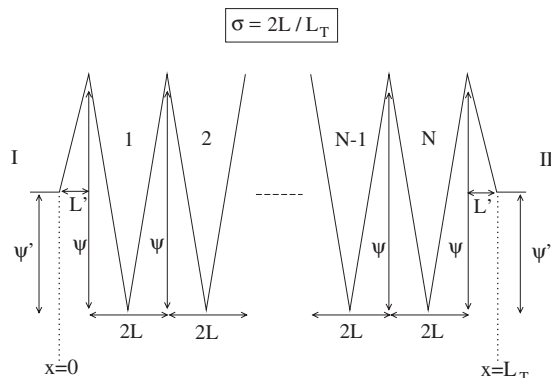


FIG. 2. Symmetric sawtooth equilibrium PMF with definitions of the length parameters and the (dimensionless) barrier heights.

V). Alternatively, one can change the ion concentration asymmetrically and study $I(\Delta C)$ or $\mathcal{G}(\Delta C)$, and so on. As a model test, the diffusion coefficients obtained from the best fit value of $k_{\text{on}}^{\text{I,eq}}$ can be compared with realistic values for diffusion coefficients in the interior of the channel.

Other quantities of interest are also expressible in terms of the rates, e.g., the ratio of unidirectional fluxes γ , defined as

$$\gamma = \frac{j^+}{j^-} = \frac{\prod_{n=0}^N k_n^+}{\prod_{n=1}^{N+1} k_n^-} = \frac{k_{\text{on}}^{\text{I}} \prod_{n=1}^N k_n^+}{k_{\text{on}}^{\text{II}} \prod_{n=1}^N k_n^-}. \quad (18)$$

In 1949 Ussing proposed the flux ratio criterion, an important experimental test to assess flux coupling. This test consists of measuring γ via tracer ion experiments⁴³ and reveals possible coupling between j^+ and j^- , typically associated with ion-ion interactions (deviations from the independence principle⁴⁴) or active transport mechanisms. In the absence of these, γ would be expected to take the value

$$\gamma_{\text{Ussing}} = \left(\frac{C^{\text{I}}}{C^{\text{II}}} \right) \exp(ZeV/k_B T). \quad (19)$$

Departures from this ratio are usually characterized by a law of the form $\gamma = \gamma_{\text{Ussing}}^\delta$ where δ (the so-called flux ratio exponent) is mostly found to be larger than unity.⁴⁵ However, we shall shortly see a theoretical example where flux ratio exponents lower than unity can be obtained by a proper modulation of the geometry of the binding sites.

IV. A SIMPLE SYSTEM LEADING TO COMPLEX BEHAVIOR: THE SYMMETRIC SAWTOOTH EQUILIBRIUM PMF

A. General framework

Our theory may find applicability for a wide range of narrow channels. One example is the gA channel,⁴⁶ which will be dealt with in the next section. However, complex behavior may already arise in much simpler systems. Let us, e.g., consider the special case of a hypothetical channel with the symmetric sawtooth potential illustrated in Fig. 2. The potential profile consists of a succession of N symmetric binding sites separated by $N-1$ energy barriers formed by two opposite linear slopes of height ψ and horizontal length L . Entry of the ions from the bulk reservoirs into the channel

is regulated by two additional barriers of horizontal length L' and height $\psi - \psi'$ (which is given by the energy difference in $k_B T$ units between the hydrated state of the ion in the bulk solution and the solvated bound state in the channel). The total length of the channel is thus $L_T = 2NL + 2L'$. Let us also introduce $\sigma \equiv 2L/L_T$, which is the fraction of the channel occupied by each binding site. This quantity will play a key role to assess geometric effects later on.

The analytic form of the equilibrium PMF around, say, the n th binding site, is given by the piecewise linear function

$$U_{\text{eq}}(x) = \frac{\psi}{L}(x_n - x), \quad x_n^- \leq x < x_n, \quad (20a)$$

$$U_{\text{eq}}(x) = \frac{\psi}{L}(x - x_n), \quad x_n \leq x < x_n^+, \quad (20b)$$

where $x_n^- = x_n - L$ and $x_n^+ = x_n + L$. Following our previous convention, one has $x_1^- = L'$ and $x_N^+ = L_T - L'$ (see Fig. 2). In order to further simplify the discussion, we shall assume that the binding energies ψ are sufficiently large so that the approximation $k_n^\pm \approx \tilde{k}_n^\pm$ holds. Computation of the equilibrium rates in terms of the diffusion coefficients and the potential slope parameters via Eqs. (9) and (10) then becomes a simple matter. A straightforward calculation yields

$$k_n^{+, \text{eq}} = k_n^{-, \text{eq}} = \frac{D_n}{G(\psi)L^2}, \quad (21)$$

where $G(\psi) = 4[(\exp(\psi) - \psi - 1)/\psi^2]$. The conditions of detailed balance (17) immediately show that due to the symmetry of the potential all diffusion coefficients must be equal, i.e., $D_n \equiv D$, also implying equality of all equilibrium rates $k_n^{\pm, \text{eq}} \equiv k^{\text{eq}}$.

Let us now introduce a nonequilibrium constraint in the system by applying an electric field $\mathcal{E}$ (say, by placing electrodes in the bulk solutions). One then has $U_{\text{noneq}}(x) = (Ze/k_B T)\chi(x)$, where $\chi(x)$ is an effective voltage profile. In certain cases (see Sec. V), it is a reasonable approximation to neglect the spatial variation of the transmembrane potential inside the bulk solutions with respect to the voltage drop across the channel itself. Voltage effects are then surprisingly well described by a profile of the form

$$\chi(x) = 0, \quad x < 0, \quad (22a)$$

$$\chi(x) = -\mathcal{E}x, \quad 0 \leq x < L_T, \quad (22b)$$

$$\chi(x) = -V, \quad L_T \leq x, \quad (22c)$$

where $\mathcal{E}$ is a constant effective electric field and $V \equiv \mathcal{E}L_T$ is the applied voltage (equal to minus the voltage drop across the channel). For simplicity, the reference value of the voltage inside reservoir I has been taken to be zero. With the above convention, if $V > 0$, $\chi(x)$ will decrease when ions move from the left to the right, resulting in a flux in the positive direction of the x axis.

Applying a not too large piecewise linear voltage ramp of the form (22) to the channel will tilt the PMF, but it will not alter the locations of the minima and the maxima (the

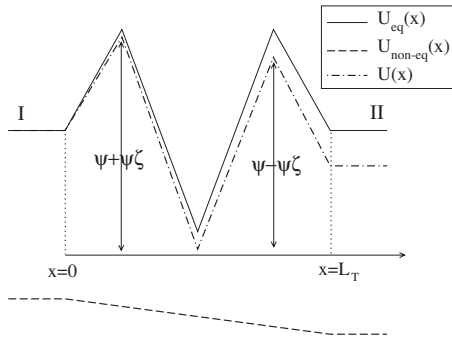


FIG. 3. Effect of a tilting voltage on the symmetric sawtooth PMF for the case $N=1$.

effect of the tilting voltage is shown in Fig. 3 for the case $N=1$. From Eqs. (9) and (10), one then gets the following expressions for the nonequilibrium rates:

$$k_n^\pm \equiv k^\pm = \frac{D\psi}{2L^2} \frac{(1 \pm \zeta)(1 \mp \zeta)^2(1 - e^{\psi(1 \pm \zeta)})}{(e^{\psi(1+\zeta)} + e^{\psi(1-\zeta)}) \left(1 + \frac{2}{\psi} - \zeta^2\right) - 2\frac{e^{2\psi}}{\psi} - 2 - \frac{2}{\psi} + 2\zeta^2}, \quad (23)$$

where we have introduced the dimensionless parameter

$$\zeta \equiv \frac{Ze\mathcal{E}L}{k_B T \psi} = \left(\frac{ZeV}{k_B T \psi}\right) \frac{L}{L_T} = \left(\frac{Ze}{2k_B T}\right) \left(\frac{V}{\psi}\right) \sigma. \quad (24)$$

This quantity is the relative change in the barrier heights when a tilting field is applied (see Fig. 3).

B. Single site case

In the following subsections we shall focus on a comprehensive analysis of the above model for the case $N=1$, i.e., a channel with a single binding site. From Eq. (17) and the equality of the equilibrium rates we have

$$k_{\text{on}}^{\text{I,eq}} P_0^{\text{eq}} = k_{\text{on}}^{\text{eq}} P_1^{\text{eq}}, \quad (25a)$$

$$k_{\text{on}}^{\text{II,eq}} P_0^{\text{eq}} = k_{\text{on}}^{\text{eq}} P_1^{\text{eq}}. \quad (25b)$$

Dividing Eq. (25b) by Eq. (25a) we find equality of the equilibrium entry rates

$$k_{\text{on}}^{\text{II,eq}} = k_{\text{on}}^{\text{I,eq}} \equiv k_{\text{on}}^{\text{eq}}. \quad (26)$$

On the other hand, the diffusion coefficient $D_1 \equiv D$ can be easily obtained from Eq. (26), the equilibrium condition $P_1^{\text{eq}}/P_0^{\text{eq}} = \exp(-\psi')$, the explicit form of the equilibrium rates (21) and, say, Eq. (25a). One gets

$$D = k_{\text{on}}^{\text{eq}} \exp(-\psi') G(\psi) L^2. \quad (27)$$

In order to make the analytical study more transparent, we now seek to obtain simplified expressions for the nonequilibrium rates (23) valid for large ψ , i.e., the limit in which a description in terms of the hopping model is best justified. The first step is to set $1 - \exp(\psi(1 \pm \zeta)) \approx -\exp(\psi(1 \pm \zeta))$ in the numerator of the right-hand side and then multiply both numerator and denominator by $\exp(-\psi)$. In the limit $\psi\zeta \ll 1$ the denominator function is then clearly dominated by the

term $2(\exp(\psi)/\psi)$ and the other terms can all be neglected. One then gets the approximation

$$k^\pm \approx \left[\frac{D\psi^2}{4L^2} \exp(-\psi) \right] (1 \pm \zeta)(1 \mp \zeta)^2 \exp(\pm \psi\zeta). \quad (28)$$

On the other hand, in the large ψ limit Eq. (27) gives

$$D \approx 4k_{\text{on}}^{\text{eq}} \exp(\psi - \psi') \frac{L^2}{\psi^2}. \quad (29)$$

Inserting this into Eq. (28) we obtain the approximated rates

$$\hat{k}^\pm \equiv k_{\text{on}}^{\text{eq}} \exp(-\psi') (1 \pm \zeta)(1 \mp \zeta)^2 \exp(\pm \psi\zeta). \quad (30)$$

Next, we focus on the nonequilibrium entry rates. In the present symmetric case Eq. (15) becomes

$$k_{\text{on}}^{\text{I}} = k_{\text{on}}^{\text{eq}} \exp(ZeVL'/k_B TL_T) = k_{\text{on}}^{\text{eq}} \exp(\psi\zeta(1 - \sigma)/\sigma), \quad (31a)$$

$$k_{\text{on}}^{\text{II}} = k_{\text{on}}^{\text{eq}} \exp(-(ZeVL'/k_B TL_T)) = k_{\text{on}}^{\text{eq}} \exp(-\psi\zeta(1 - \sigma)/\sigma), \quad (31b)$$

with

$$k_{\text{on}}^{\text{eq}} = \kappa \exp(-(\psi - \psi')) \quad (32)$$

[see Eqs. (14) and (16)]. This kind of dependence ensures a well behaved flux which vanishes as the binding energy ψ becomes very large. As discussed in the previous section, the parameter $\kappa \equiv \kappa^{\text{I}} = \kappa^{\text{II}}$ will in general depend on L' (and thus on L for a fixed value of L_T) via the parameter $\lambda \equiv \lambda^{\text{I}} = \lambda^{\text{II}}$ in Eq. (14). However, in what follows we shall make the simplifying assumption that this dependence is weak ($\lambda \approx \text{const}$) and can therefore be ignored for any practical purpose. Besides, one does not *a priori* expect significant qualitative changes in the model when λ displays a polynomial dependence on L , since the behavior of the entry rates is dominated by the L dependence of the exponential factors.

C. Qualitative behavior of the flux: Onset of the optimization effect

The computation of the ion flux in terms of the rates is now carried out by setting $N=1$ in Eq. (3). This substitution yields

$$j = \frac{k_{\text{on}}^{\text{I}} k^+ - k_{\text{on}}^{\text{II}} k^-}{k_{\text{on}}^{\text{I}} + k^+ + k_{\text{on}}^{\text{II}} + k^-}. \quad (33)$$

In order to investigate the role of the binding site geometry we hold L_T fixed and study the behavior of I as a function of σ . Figure 4 illustrates the characteristic behavior of the ion current I as a function of σ for different values of V and typical values of the relevant length and energy scales (see figure caption for the detailed numerical values). For voltages below a certain critical value V_{crit} ($=27$ mV for the parameter values corresponding to Fig. 4), the current decreases monotonically with increasing σ (see curve in the inset of Fig. 4). In contrast, for $V > V_{\text{crit}}$ one can clearly see that I displays a maximum for an optimum size σ_{opt} of the binding site.

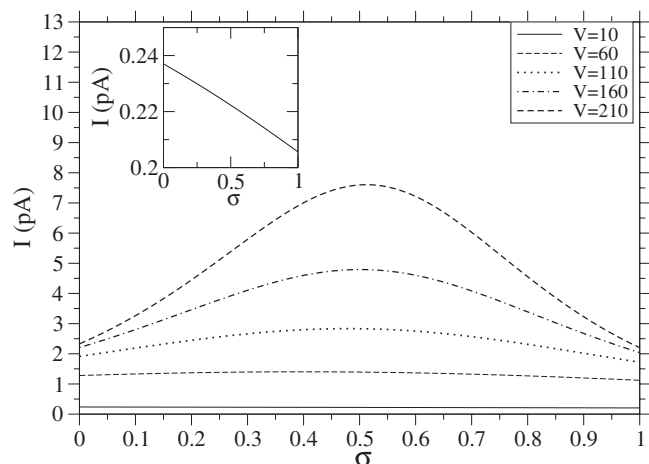


FIG. 4. Ionic current I vs σ for different values of the applied voltage V . The corresponding values of V are given in mV in the legend. The other parameter values are $\psi=7.5$, $\psi'=0$, $k_{\text{on}}^{\text{eq}}=1.5 \times 10^7$, $L_T=20$ Å, $T=293.15$ K, and $Z=1$ (monovalent ions). The inset shows a close-up view of the curve for $V=10$ mV.

For voltages well above V_{crit} , one can neglect the ion flux in the negative direction and approximate the total flux j by the equation

$$j \approx j^+ = \frac{k_{\text{on}}^I k^+}{k_{\text{on}}^I + k^+ + k_{\text{on}}^{\text{II}} + k^-}. \quad (34)$$

We hereby assume that $\psi\zeta \ll \psi$ still holds (so that the hopping model retains its validity), but the value of ζ is nevertheless large enough to make the unidirectional flux in the negative direction j^- negligible. In this limit, the backward rates $k_{\text{on}}^{\text{II}}$ and k^- become negligibly small, and we can further approximate the flux as follows:

$$j \approx \frac{k_{\text{on}}^I k^+}{k_{\text{on}}^I + k^+} = \left(\frac{1}{k_{\text{on}}^I} + \frac{1}{k^+} \right)^{-1}. \quad (35)$$

In the domain of validity of this approximation, the inverse rates $(k_{\text{on}}^I)^{-1}$ and $(k^+)^{-1}$ display a nonlinear dependence on σ , as seen from the expressions (23) and illustrated in Fig. 5. The figure also shows that in the high voltage regime the sum of the inverse forward rates displays a clear minimum as a function of σ . In contrast, for weak voltages k_{on}^I and k^+ vary almost linearly with σ , whereas the backward rates $k_{\text{on}}^{\text{II}}$ and k^- (which are no longer negligible in this limit) change

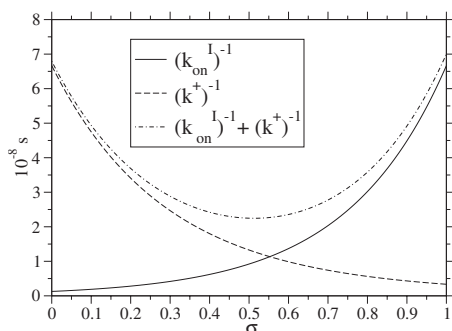


FIG. 5. σ dependence of the inverse forward rates and its sum in the strong voltage limit ($V=200$ mV): The values for ψ , ψ' , $k_{\text{on}}^{\text{eq}}$, L_T , T , and Z are the same as in Fig. 4.

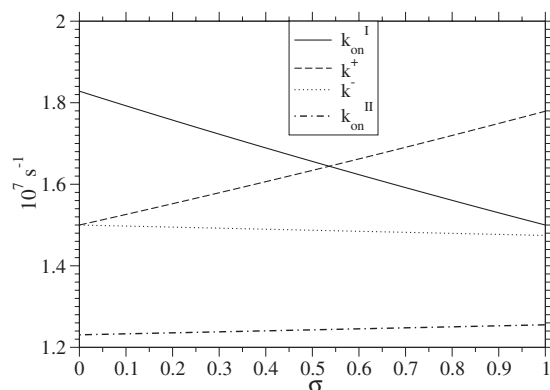


FIG. 6. σ dependence of the forward and backward rates in the weak voltage limit ($V=10$ mV): The quasilinear behavior of the rates leads to a similar behavior of the sum and to a monotonic behavior of the current with respect to σ . The values for ψ , ψ' , $k_{\text{on}}^{\text{eq}}$, L_T , T , and Z are the same as in Fig. 4.

very slowly and can essentially be regarded as constant (see Fig. 6). This implies that in this regime the denominator of Eq. (33) behaves linearly and the numerator quadratically, the net result being quasilinear σ dependence of the rates and absence of the nonmonotonic behavior brought out by the quantitative analysis in the next subsection. We thus see that the onset of the optimization effect is a direct consequence of the nonlinear coupling to both the applied field and the parameters describing the geometry of the binding site.

At this stage, a comment on the analogy of Eq. (35) and Ohm's law is in order. It is well known that traditional rate models obey Ohm's law for weak voltages. A straightforward analysis shows that our model also displays this behavior (see next subsection). However, the form of Eq. (35) suggests that some kind of analogy with Ohm's law may also apply for large voltages. Indeed, from Eq. (35) one has

$$I = Zej = \frac{V}{\mathcal{R}^I + \mathcal{R}^+} \quad (36)$$

with

$$\mathcal{R}^I \equiv \frac{V}{Ze} \frac{1}{k_{\text{on}}^I} \quad \text{and} \quad \mathcal{R}^+ \equiv \frac{V}{Ze} \frac{1}{k^+}. \quad (37)$$

These quantities (proportional to the inverse jump rates) can be regarded as a couple of circuit resistors placed in series and characterizing ion passage over each of the two energy barriers. Note, however, that $\mathcal{R}^I$ and $\mathcal{R}^+$ are voltage dependent. Besides, the resistance of a usual Ohmic resistor is proportional to its length, whereas $\mathcal{R}^I$ and $\mathcal{R}^+$ display a complicated dependence on the geometry of the barriers, i.e., they change via the parameters L and L' even if the total length $L_T/2 = L + L'$ of each equivalent "resistor" is held constant.

In passing, let us also note that there is an interesting structural analogy between Eq. (35) and the formula obtained in Ref. 22 for the flux induced by a bulk concentration difference ΔC . In both cases, the inverse rates $(k_{\text{on}}^I)^{-1}$ and $(k^+)^{-1}$ are associated with the ion translocation times for each of the two barriers.

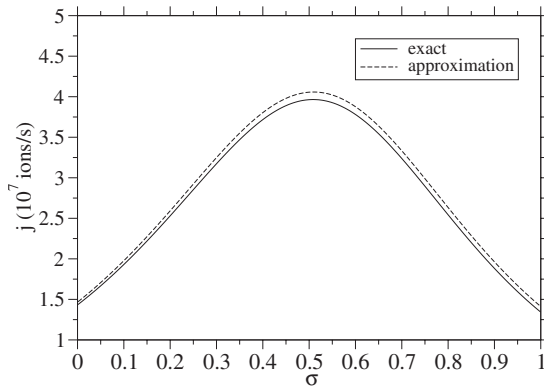


FIG. 7. Comparison of the exact flux (33) with the analytical approximation (38): We have chosen $V=190$ mV and the same values for ψ , ψ' , $k_{\text{on}}^{\text{eq}}$, L_T , T , and Z as in Fig. 4.

To conclude this subsection, let us derive an approximate formula for the flux in the large ψ limit. To this end we make use of the approximation (30) for the rates. One then gets the limiting expression

$$j \approx \frac{k_{\text{on}}^I \hat{k}^+}{k_{\text{on}}^I + \hat{k}^+} = \frac{k_{\text{on}}^{\text{eq}} e^{-\psi\zeta} (1+\zeta)(1-\zeta)^2}{e^{\psi'-2\psi\zeta} + (1+\zeta)(1-\zeta)^2 e^{-\psi\zeta/\sigma}} = \frac{\kappa e^{-\psi(1+\zeta)} (1+\zeta)(1-\zeta)^2}{e^{-2\psi\zeta} + (1+\zeta)(1-\zeta)^2 e^{-\psi'-(\psi\zeta/\sigma)}}, \quad (38)$$

which indeed displays a maximum as a function of σ . Figure 7 shows a comparison between the exact expression for the flux given by Eq. (33) and the approximation (38) for $V=190$ mV. The approximation overestimates slightly the flux, but the overall behavior is very well reproduced. For larger voltage values the agreement becomes even better, but the use of a hopping model starts to become questionable.

D. Calculation of σ_{opt} and the critical voltage

We now turn to the calculation of the optimum length σ_{opt} corresponding to maximum flux and the critical voltage V_{crit} above which flux optimization takes place. To begin with, let us replace the exact rates $k^{\pm}$ in Eq. (33) with the large ψ approximation (28). Taylor expanding the resulting expression about $V=0$ one gets

$$j = \frac{Dk_{\text{on}}^{\text{eq}} e^{-\psi(\psi L_T/2 - L)\psi}}{4k_{\text{on}}^{\text{eq}} L^2 + D e^{-\psi}\psi^2} \frac{ZeV}{k_B T L_T} + \mathcal{O}(V^3) \approx k_{\text{on}}^{\text{eq}} \frac{e^{-\psi'}}{1 + e^{-\psi'}} (\psi - \sigma) \frac{\zeta}{\sigma} + \mathcal{O}\left(\left(\frac{\zeta}{\sigma}\right)^3\right), \quad (39)$$

where we have retained the (Ohmic) linear term and the first nonlinear correction term only and we have invoked Eq. (29) in the last step. The above expression for the flux leads to the zero-voltage differential conductance

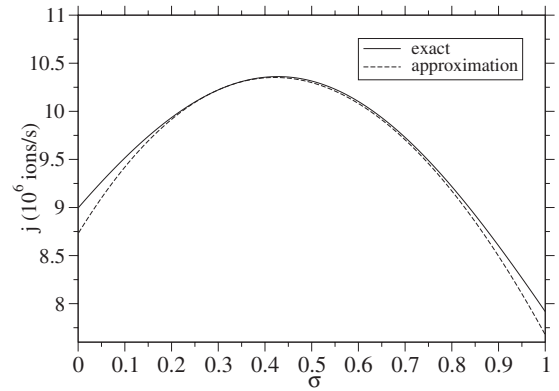


FIG. 8. Comparison between the exact result (33) for the flux and the approximate formula (41) valid in the limit of moderate voltages ($V=70$ mV). Same values of ψ , ψ' , $k_{\text{on}}^{\text{eq}}$, L_T , T , and Z as in Fig. 4.

$$\mathcal{G}_0^{\text{diff}} \equiv \left. \frac{\partial I}{\partial V} \right|_{V=0} = Z e \left. \frac{\partial j}{\partial V} \right|_{V=0} = \frac{e^{-\psi'}}{1 + e^{-\psi'}} \left(\frac{1}{2} - \frac{\sigma}{2\psi} \right) \frac{Z^2 e^2 k_{\text{on}}^{\text{eq}}}{k_B T}, \quad (40)$$

which decreases linearly with σ .

As expected from the discussion in the previous subsection, the first-order approximation for the flux in the applied voltage given by Eq. (39) decreases monotonically with increasing σ , and no optimum size for the binding site is found (see curve in the inset of Fig. 4). In order to find such an optimization effect, it is necessary to increase the voltage so that higher order corrections come into play.

As it turns out, the form of the third-order term in the expansion (39) is greatly simplified if one assumes $\psi'=0$. This assumption, which we shall use henceforth, does not really imply a loss of generality, as a shift in ψ' has the sole effect of renormalizing $k_{\text{on}}^{\text{eq}}$ [see Eq. (32)]. With $\psi'=0$ Eq. (39) becomes

$$j \approx \frac{k_{\text{on}}^{\text{eq}}}{2} (\psi - \sigma) \frac{\zeta}{\sigma} + \frac{k_{\text{on}}^{\text{eq}}}{4} \left[(\psi^2 - \psi + 1)\sigma^3 - \psi^2(\psi + 1)\sigma^2 - \psi \left(\frac{\psi}{2} - 1 \right) \sigma - \frac{\psi^3}{6} \right] \left(\frac{\zeta}{\sigma} \right)^3. \quad (41)$$

The right-hand side of this equation can be shown to display a maximum as a function of σ . Figure 8 shows the behavior of the flux as a function of σ computed both from the exact formula (33) and from Eq. (41) for $V=70$ mV. The agreement is excellent for intermediate values of σ and somewhat worse for values in the vicinity of zero and 1, but the qualitative behavior is well reproduced.

In order to compute the value σ_{opt} yielding maximum flux, we require

$$\left. \frac{\partial j}{\partial \sigma} \right|_{\sigma=\sigma_{\text{opt}}} = 0. \quad (42)$$

If one uses the third-order approximation (41) for the flux, one gets a second-order equation for σ_{opt} . The physical solution of the latter is

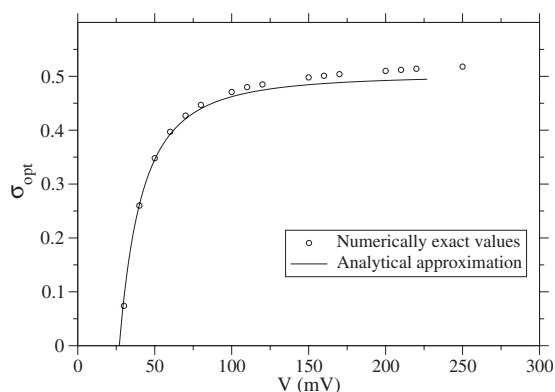


FIG. 9. Behavior of σ_{opt} as a function of the applied voltage: The dots correspond to numerically exact values of σ_{opt} . The solid line corresponds to the analytic formula (43) valid for voltage values slightly above V_{crit} . Same values of ψ , ψ' , $k_{\text{on}}^{\text{eq}}$, L_T , T , and Z as in Fig. 4.

$$\sigma_{\text{opt}} = \frac{\psi}{6(1 - \psi + \psi^2)} \left[2\psi^2 + 2 - \left[4\psi^4 - 12\psi^3 + 26\psi^2 - 18\psi + 10 + \frac{96k_B^2 T^2}{Z^2 e^2 V^2} (1 - \psi + \psi^2) \right]^{1/2} \right]. \quad (43)$$

Figure 9 shows the behavior of σ_{opt} as a function of the applied voltage V computed both from Eq. (43) and from the appropriate numerical solution of Eq. (42). The numerically exact solution for the latter equation was found by substituting the expressions (23) and (27) for the rates and the diffusion coefficient in the analytic expression (33) for the flux, deriving with respect to σ and finding the real root in the interval $[0, 1]$ of the resulting equation for different values of the voltage. As it turns out, the third-order approximation given by Eq. (43) yields very good results and even captures qualitatively the saturation effect displayed by the exact solution for larger values of the voltage.

Equation (42) can also be solved with respect to the applied voltage, allowing one to express it in terms of σ_{opt} :

$$V = \frac{4k_B T}{Ze \sqrt{2\psi - 1 - 4\left(\psi + \frac{1}{\psi}\right)\sigma_{\text{opt}} + 6\left(1 - \frac{1}{\psi} + \frac{1}{\psi^2}\right)\sigma_{\text{opt}}^2}}. \quad (44)$$

Taking the limit $\sigma_{\text{opt}} \rightarrow 0$ in this expression gives an analytic expression for the critical voltage:

$$V_{\text{crit}} = \frac{4k_B T}{Ze} \frac{1}{\sqrt{2\psi - 1}} \approx \frac{4k_B T}{Ze} \frac{1}{\sqrt{2\psi}}. \quad (45)$$

Clearly, as the binding energy ψ decreases, V_{crit} increases, and the voltage window where optimization with respect to σ is observed becomes larger.

E. Current-voltage curves: Double transition between sublinear and superlinear behaviors

In the previous subsections we have studied the dependence of the ionic current on the binding site geometry for a given applied voltage. This can be justified *a posteriori* in view of the obtained results, which involve an interesting maximization phenomenon of the current. It is also of interest for the question on how the structure of ion channels may

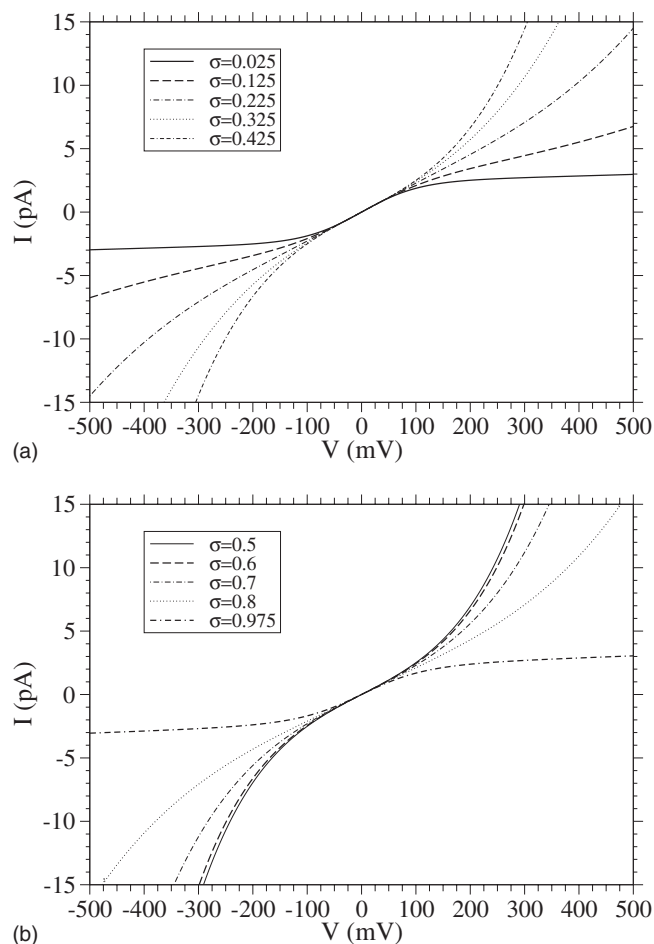


FIG. 10. Ionic current vs applied voltage V for different values of σ . Same values of ψ , ψ' , $k_{\text{on}}^{\text{eq}}$, L_T , T , and Z as in Fig. 4. As σ is increased, the curves undergo a first transition from sublinear to superlinear behavior (a) and then a second transition from superlinear back to sublinear (b).

have evolved to optimize transport. However, the reverse question, i.e., the shape of current-voltage curves for channels with different structures (and hence PMF geometries; in our case σ) is undeniably closer to the physiological experiments.

In the framework of our model we observe an interesting double crossover of the current-voltage curves from sublinear behavior (characterized by voltage saturation) to superlinear behavior and back. This double transition is illustrated in Figs. 10(a) and 10(b). For very small values of σ (≈ 0.025) one can see that the resulting current-voltage curves are sublinear and display saturation at sufficiently large values of the voltage (but still compatible with the condition $\zeta \ll 1$). For example, saturation becomes perceptible from 100 to 150 mV for the $\sigma = 0.025$ curve in Fig. 10(a). When σ is increased slightly to values $\sigma \approx 0.1$ the characteristic curves become rapidly superlinear [see Fig. 10(a)] and they remain as such up to very large values of σ . However, if σ is increased beyond 0.95 and up to values very close to 1 (i.e., if the binding site becomes very large and the entry barriers very thin and steep), the characteristic curves suddenly become sublinear again [see Fig. 10(b)]. Note that complex current-voltage curve behavior (both superlinear and sublinear behaviors) has been observed experimentally

for, e.g., gA channels⁴⁷ for which channel modification (by fluorination) and changes in lipid bilayer environment may both alter current-voltage curves. While our hypothetical sawtooth model is by no means a realistic approach to the gA channel (especially so, since we consider here the case of a single binding site rather than the complex double-humped equilibrium PMF of the gA channel), it emphasizes the drastic effect that small geometry differences may have on channel transport properties.

For reasons of space, we shall refrain from a detailed theoretical explanation for this case and limit ourselves to mention that the behavior for large voltages is again given by the approximate expression for the forward flux (35). Taking Eqs. (23) and (31) as a starting point one can show that for σ in the vicinity of 0 or 1, one of the inverse forward rates decreases almost linearly and the other one decreases exponentially with increasing voltage, whereas for intermediate σ the decrease in both quantities is exponential. This behavior can be shown to lead to saturation of j^+ and I in the former case and to superlinear growth in the latter.

F. Behavior of the flux ratio exponent

Next, let us investigate how the ratio of the unidirectional fluxes γ is affected by changes in the binding site geometry. To begin with, from Eq. (23) we note that the exact ratio of the forward and backward escape rates from the site is

$$\frac{k^+}{k^-} = \frac{(1 - \zeta)(1 - e^{\psi(1+\zeta)})}{(1 + \zeta)(1 - e^{\psi(1-\zeta)})}. \quad (46)$$

On the other hand, the ratio of unidirectional fluxes can be obtained from Eq. (18) by setting $N=1$. Combining the resulting equation with Eqs. (46) and (30) we get the exact result

$$\gamma = \frac{k_{\text{on}}^I k^+}{k_{\text{on}}^{II} k^-} = \frac{e^{2\psi\zeta/\sigma - 2\psi\zeta}(1 - \zeta)(1 - e^{\psi(1+\zeta)})}{(1 + \zeta)(1 - e^{\psi(1-\zeta)})}. \quad (47)$$

Applying the definition

$$\delta \equiv \frac{\ln(\gamma)}{\ln(\gamma_{\text{Ussing}})}, \quad (48)$$

we find

$$\begin{aligned} \delta &= \frac{1}{\psi\zeta L_T} \ln(\gamma) \\ &= 1 + \frac{1}{\psi\zeta L_T} \ln\left(\frac{1 - \zeta}{1 + \zeta}\right) + \frac{1}{\psi\zeta L_T} \ln\left(\frac{e^{-2\psi\zeta/\sigma} (1 - e^{\psi(1+\zeta)})}{1 - e^{\psi(1-\zeta)}}\right). \end{aligned} \quad (49)$$

This result is in excellent agreement with the δ values corresponding to the slopes of the curves in Fig. 11. It is seen that the flux ratio exponent decreases with increasing value of δ . Due to the prefactor $(1 - \zeta)/(1 + \zeta)$ in the right-hand side of Eq. (46) the ratio k^+/k^- does not increase as fast as $k_{\text{on}}^I/k_{\text{on}}^{II}$ decreases when σ increases. Thus, the difference between forward and backward fluxes becomes smaller as the binding site becomes larger and flatter.

Let us once again focus on the relevant large ψ behavior of Eq. (49). As ψ becomes large the argument in the loga-

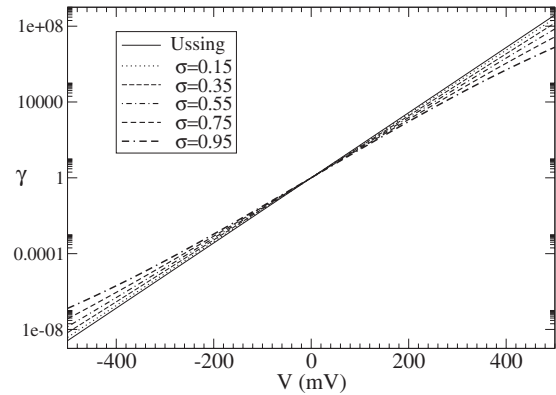


FIG. 11. Semilogarithmic plot of the flux ratio as a function of the applied voltage for different values of σ ; the Ussing limit is obtained in the limit $\sigma \rightarrow 0$: Same values of ψ , ψ' , $k_{\text{on}}^{\text{eq}}$, L_T , T , and Z as in Fig. 4.

rithm of the last term of the right-hand side approaches 1, i.e., this contribution becomes negligible. On the other hand, we can use the expansion

$$\ln\left(\frac{1 - \zeta}{1 + \zeta}\right) = -2\zeta - \frac{2}{3}\zeta^3 + \mathcal{O}(\zeta^5) \quad (50)$$

in the second term. Retaining only up to the third-order term in ζ , we get the first two correction terms about the Ussing limit $\delta_{\text{Ussing}} = 1$:

$$\delta = 1 - \frac{\sigma}{\psi} - \frac{\sigma\zeta^2}{3\psi}, \quad (51)$$

i.e., the flux ratio exponent decreases linearly for small values of σ with a voltage-dependent cubic correction in σ which becomes significant for larger values of σ . Figure 12 shows a comparison between numerically exact values of δ obtained from the exact result (47) fits obtained by retaining up to the linear term and the third-order term in Eq. (51). Excellent agreement of the third-order expansion with the exact result is found even for values of σ close to 1.

G. Concentration effects

In this subsection, we shall explore the effect of changing the bulk concentration. We shall restrain ourselves to the case of symmetric ionic conditions $C^I = C^{II} \equiv C$, where cur-

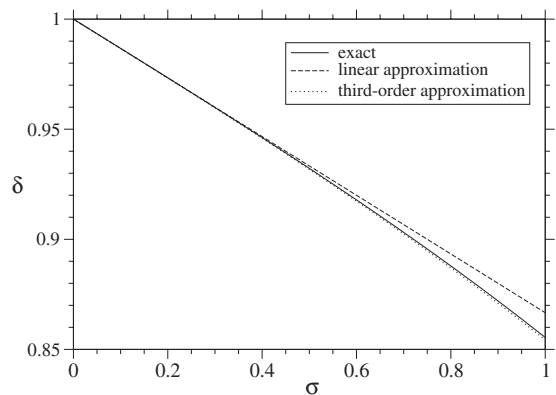


FIG. 12. Flux ratio exponent δ as a function of σ : Same values of ψ , ψ' , $k_{\text{on}}^{\text{eq}}$, L_T , T , and Z as in Fig. 4.

rent flows only if an external voltage is applied. However, there are no obstacles of principle that would prevent one from dealing with the case of asymmetric ionic conditions, such as is seen physiologically, arising from a concentration difference ΔC (see Ref. 22).

As already anticipated at the beginning of Sec. III, we shall assume that $U(x)$ does not depend on C . Hence, the only effect of a change in concentration is to modify the entry rates $k_{\text{on}}^{\text{I,II}}$ via Eq. (14).⁴⁸ As discussed previously, for sufficiently large V the current will display a maximum as a function of σ . In this regime, an increase in the bulk concentration at a fixed voltage turns out to have a similar effect as an increase in voltage at fixed concentration, i.e., it shifts σ_{opt} toward larger values (see Fig. 13). As expected, for a fixed value of σ the flux increases with increasing concentration.

Regarding the behavior of the current as a function of concentration, one can see from the proportionality of the entry rates to concentration and the structure of Eq. (33) that the corresponding I - C curves display Michaelis–Menten behavior and eventually saturate to a value I_s which is very sensitive to changes in the binding site length (Fig. 14). For a fixed value of C , the current does not necessarily increase with increasing σ , but the saturation current I_s does. How-

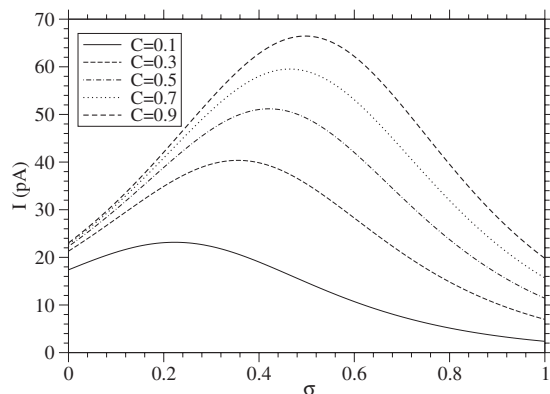


FIG. 13. I vs σ for different values of the concentration C . The concentration values are given in the legend in M . The maximum value of the current is shifted to larger values of σ as C increases. Here we have set $k_{\text{on}}^{\text{eq}} = 1.5 \times 10^6 C$. The value of the applied voltage is $V = 200$ mV. Same values of ψ , ψ' , L_T , T , and Z as in Fig. 4.

ever, the concentration values at which I_s is attained are in some cases well above the physiologically realistic range.

H. Two-site system

Finally, we explore the effect of increasing the number of binding sites of the system on the ionic current. For $N=2$ the analytic expression for the flux becomes

$$j = \frac{k_0^+ k_1^+ k_2^+ - k_2^- k_1^- k_0^-}{k_0^+ k_2^+ + k_0^+ k_2^- + k_0^- k_2^- + k_0^+ k_1^+ + k_0^- k_1^- + k_0^- k_1^+ + k_1^+ k_2^+ + k_1^- k_2^- + k_1^- k_2^+}. \quad (52)$$

In the particular case of the symmetric sawtooth PMF subject to a linear voltage drop, we get

$$j = \frac{k_{\text{on}}^{\text{I}}(k^+)^2 - k_{\text{on}}^{\text{II}}(k^-)^2}{2k^+ k_{\text{on}}^{\text{I}} + k^- k_{\text{on}}^{\text{I}} + 2k^- k_{\text{on}}^{\text{II}} + k^+ k_{\text{on}}^{\text{II}} + (k^+)^2 + k^- k^+ + (k^-)^2}. \quad (53)$$

For given values of $k_{\text{on}}^{\text{eq}}$, ψ , and σ (which only ranges from zero to 1/2 in this case), introducing an extra binding site has only the quantitative effect of decreasing the flux (see Fig. 15). However, both the optimization effect with respect to σ and the sublinear-superlinear-sublinear transition of the current-voltage curves (data not shown) are robust with respect to the introduction of the extra binding site. Preliminary results suggest that if N is further increased, the analytic expressions for the flux become increasingly cumbersome, but no qualitatively new effects arise (data not shown).

V. APPLICATION TO SODIUM PERMEATION IN THE GRAMICIDIN A CHANNEL

Once the properties of the above hypothetical channel have been discussed, let us now turn to our next goal, namely, the reproduction of conductance data for a real system with the help of our hopping model. The system we have chosen is the gA channel, for which experimental tracer stud-

ies of sodium permeation⁵⁰ and the observed Michaelis–Menten behavior of the conductance versus concentration curves suggest that single-ion occupancy is the predominant state.

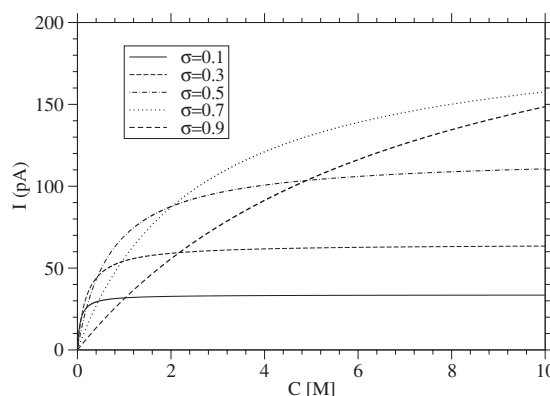


FIG. 14. Saturation behavior of the I - C curves: Here we have set $k_{\text{on}}^{\text{eq}} = 1.5 \times 10^6 C$. The value of the applied voltage is $V = 200$ mV. Same values of ψ , ψ' , L_T , T , and Z as in Fig. 4.

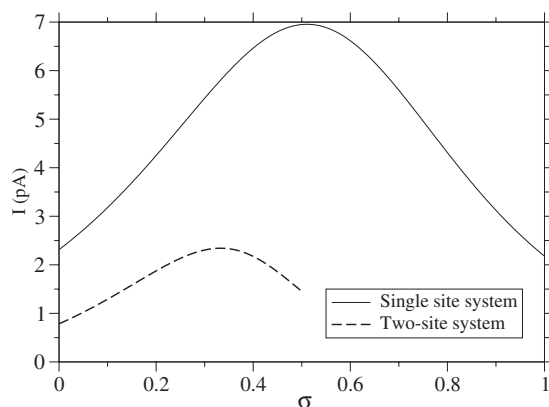


FIG. 15. Comparison of the current vs σ curves for the single site and the two-site system. The corresponding value of V is 200 mV. Same values of ψ , ψ' , L_T , T , and Z as in Fig. 4.

Several studies suggest that under symmetric ionic conditions $C_{\text{Na}^+}^{\text{I}} = C_{\text{Na}^+}^{\text{II}} = C_{\text{Na}^+}$ the one-ion equilibrium profile $U_{\text{eq}}(x)$ is symmetric and consists of two entrance barriers at each channel mouth and two relatively deep binding sites separated by a central energy barrier. To the best of our knowledge, free energy profiles obtained from MD simulations for Na^+ remain incomplete,⁵¹ although alchemical transformation free energy calculations have been used to explore Na^+ versus K^+ selectivity at selected points along the K^+ pathway.⁵² Because of these shortcomings we shall use for illustrative purposes an equilibrium profile $U_{\text{eq}}(x)$ obtained in Ref. 41 from a fit of the electrodiffusion equation to the experimental conductance data of Russell *et al.*⁵³ rather than from a rigorously computed PMF. The dashed line displayed in Fig. 16 corresponds to $U_{\text{eq}}(x)$ in $k_B T$ units; it is seen that the height of the exit barriers between the binding sites and the reservoirs is $\sim 6.1 k_B T$, while the height of the central barrier is $\sim 4.2 k_B T$. These values are fairly large with respect to $k_B T$ and therefore ensure that for not too strong applied voltages and typical values of the (effective) diffusion coefficient $D \equiv D_1 = D_2$ in the binding sites (for which the bulk value $D_{\text{bulk}} = 1.33 \times 10^{-9} \text{ m}^2/\text{s}$ can be expected to be an upper bound) the dwelling time of ions in each well is long compared to their characteristic reciprocal jump frequency [given by $(\nu^{kr})^{-1} = (2\pi/D) \times (|U''(x)|_{x=x_{\min}} U''(x)|_{x=x_{\max}})^{-1/2}$ in Kramers' approximation].⁵⁴ Thus, a description in terms of a hopping model

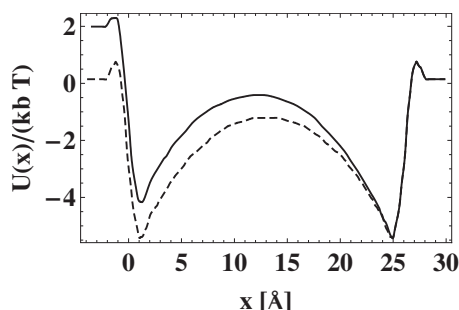


FIG. 16. Equilibrium profile $U_{\text{eq}}(x)$ (dashed line) vs full profile $U(x)$ including the nonequilibrium contribution due to the transmembrane potential (solid line). $U(x)$ corresponds to the value of 37.5 mV for the applied voltage.

seems *a priori* a reasonable alternative to a continuum electrodiffusion approach on a sufficiently long time scale.

We now proceed to discuss the validity of the continuum Smoluchowski equation at time scales long with respect to the jump frequency but short with respect to the mean exit time. We expect this approach to provide a suitable description if the condition $\lambda_{\text{thermal}} \nabla U \ll U_B$ holds, where $\lambda_{\text{thermal}} \sim D \sqrt{3m_{\text{Na}^+}/k_B T}$ is the ionic thermal mean free path, U_B is the typical height of the energy barrier, and $\nabla U \equiv L_B^{-1} \int_{x_{\min}}^{x_{\max}} U'(x) dx$ is the mean free energy gradient (average over the spatial extent L_B of the barrier). As can be seen from Fig. 16, in our case the typical value of $U_B/\nabla U$ describing the spatial extent of the barriers for the channel exit is $\sim 2.2 \text{ Å}$ while the length of the central barrier is $\sim 12 \text{ Å}$. In Ref. 41, Chiu and Jakobsson determined D directly from a fitting procedure by a steepest descent method rather than from a detailed balance condition. They found $D = 9.4 \times 10^{-11} \text{ m}^2/\text{s}$, roughly one order of magnitude below D_{bulk} . Using this value along with $m_{\text{Na}^+} = 22.99 \text{ amu}$ and $T = 293.15 \text{ K}$, one finds $\lambda_{\text{thermal}} \sim 0.158 \text{ Å}$. This value is clearly below the characteristic length of the barriers, implying that the condition of validity of the diffusional approach is fulfilled.

Next, let us examine what happens when a weak applied voltage is applied. In such a case, the energy profile displays an asymmetry as a result of the superposition of $U_{\text{eq}}(x)$ with a nonsymmetric contribution $U_{\text{noneq}}(x)$. Calculations by Jordan *et al.*⁴⁰ suggest that, in general, a significant transmembrane potential drop takes place inside the bath. To a good approximation, the relative potential drop is independent of the applied voltage. According to Ref. 41, for the particular case of the gA channel geometry ($L_T \sim 30$ and 4 Å diameter) the potential drop on each side of the bulk solution outside the channel is 5% in the range of physiological Na^+ concentrations. For a voltage value of, say, 40 mV this results in a transmembrane potential of the order of 2 mV on each side, which can be neglected in good approximation. This justifies the Smoluchowski-like description based on an essentially diffusion-limited approach of ions to each of the entry barriers [see Eq. (13)]. With regard to the potential drop across the channel itself, Chiu and Jakobsson assumed a linear dependence on position⁴¹ based on the calculations in Ref. 40 [reminiscent of the constant field approximation expressed by Eq. (22)].

The solid line in Fig. 16 corresponds to the potential profile $U(x)$ computed in Ref. 41 for an applied voltage $V = 37.5 \text{ mV}$ and symmetric ionic conditions (strictly speaking, this curve corresponds to the fixed value $C_{\text{Na}^+}^{\text{I,II}} = 1M$, but in Ref. 41 it is shown to vary very little for symmetric concentration changes in the experimental range). The above profile was obtained by fitting a cubic spline to the corresponding curve in Fig. 2(b) of Ref. 41. Using the piecewise analytic expressions provided by the spline fits, we were able to compute numerical values for the nonequilibrium rates $k_{1,2}^{\pm}$ via evaluation of Eq. (12) and a standard routine for multiple integration, as well as to obtain k_{on}^{I} and $k_{\text{on}}^{\text{II}}$ in terms of $k_{\text{on}}^{\text{I,eq}}$ by means of Eq. (15) (note that in the present case $k_{\text{on}}^{\text{I,eq}} = k_{\text{on}}^{\text{I}} \equiv k_{\text{on}}^{\text{eq}}$ and thus $\lambda^{\text{I}} = \lambda^{\text{II}} \equiv \lambda$, a direct consequence of the symmetric equilibrium PMF). The resulting expressions for

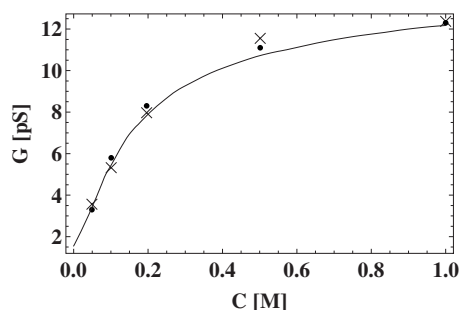


FIG. 17. Conductance vs concentration curve for sodium in the gA channel. The crosses correspond to the experimental data; the solid line represents a least-squares fit to these data based on the continuum model. Finally, the dots represent the theoretical points which get closest to the experimental ones after determining the optimum value $\lambda_{\min}$.

the rates were subsequently plugged into Eq. (52) to evaluate the flux. Calculating the channel conductance $\mathcal{G} = Zej/V$ in terms of $k_{\text{on}}^{\text{eq}}$ is then a straightforward task.

We are now in the position to attempt a fit to experimental data. In principle, one could use a series of nonequilibrium profiles corresponding to different voltage values to fit $\mathcal{G}$ - V curves, but this is not very interesting for the conventional gA channel, since the conductance varies very little with voltage. Instead, we shall therefore turn to the experimentally available $\mathcal{G}$ - C curves. As already mentioned, the latter display saturation with increasing concentration, a feature also displayed by our model.

Figure 17 displays a set of experimental concentration-conductance points (C_n, G_n) from Ref. 53 (represented by crosses) as well as a least-squares fit $\mathcal{G}^{\text{cont}}(C)$ to these experimental points obtained from the continuum electrodiffusion approach (solid line). The experimental conductance values were taken by an equal number of measurements at both $V_0 = 25$ mV and $V_2 = 50$ mV. Unfortunately, no theoretical energy profiles for each of these two voltage values are available to us; however, the solid curve displayed in Fig. 16 corresponds to $V_1 = 37.5$ mV, i.e., the mean value of V_0 and V_2 . Since changes in $U(x)$ are not drastic in this weak voltage range, we expect that this profile can be used to obtain a reasonable approximation to the actual conductance values.

In order to obtain a set of theoretical $(C, \mathcal{G})$ points, let us first make the replacement $k_{\text{on}}^{\text{eq}} = \lambda D_{\text{bulk}} C \exp(-(U_{\text{eq}}(x_1^-) - U_{\text{eq}}(0)))$ in the expression for $\mathcal{G}$ provided by the hopping model. Since $\exp(-(U_{\text{eq}}(x_1^-) - U_{\text{eq}}(0)))$ and D_{bulk} are known, one gets from here a theoretical expression $\mathcal{G}^{\text{hopp}}$ in terms of λ and C only. One can then compute the conductance value $\mathcal{G}_n^{\text{hopp}} \equiv \mathcal{G}^{\text{hopp}}(C_n)$ corresponding to each concentration value C_n in terms of λ and subsequently compute the value $\lambda_{\min}$ which minimizes the sum of squared distances $(1/N_p) \sum_{n=1}^{N_p} (\mathcal{G}_n^{\text{hopp}} - \mathcal{G}_n)^2$ by finding the appropriate solution of the equation

$$\frac{\partial}{\partial \lambda} \sum_{n=1}^{N_p} (\mathcal{G}_n^{\text{hopp}} - \mathcal{G}_n)^2 = 0, \quad (54)$$

where the number of points N_p is equal to 5 in the present case. Finally, one can substitute the optimum value of $\lambda_{\min}$ back into $\mathcal{G}_n^{\text{hopp}}$, numerically evaluate the set of points

$(C_n, \mathcal{G}_n^{\text{hopp}})$, and compare both with the experimental points and with the fit provided by the continuum model.

The dots plotted in Fig. 17 represent the points $(C_n, \mathcal{G}_n^{\text{hopp}})$. It is seen that the agreement with the experimental data is good. The overall deviations are comparable to the ones displayed by the continuum fit. While the latter seems to work better in the low concentration regime, points at higher concentrations are better described by the hopping model.

VI. SUMMARY OF RESULTS AND DISCUSSION

We have presented a novel rate theory for ion transport in narrow channels which allows one to establish a relationship between the channel structure (as represented by its equilibrium PMF) and conductance measurements from single channel recordings. In contrast to traditional hopping models, the analytic form of the jump rates we propose accounts for the full geometry of the energy barriers. The integrals appearing in the expressions for the rates can be computed numerically if an analytic expression for a PMF is available, as done for the cubic spline fits obtained from the experimental conductance data of the gA channel. On the other hand, any numerical PMF can be approximated arbitrarily well by a set of linear polynomials from which one can subsequently derive exact analytic expressions for the rates and the ion flux. Our approach then becomes a powerful tool to study the relationship between geometry and conductance in narrow channels. Admittedly, the piecewise approximation becomes cumbersome with increasing complexity of the PMF. However, even for such a case the computational cost is small compared to the cost of estimating the PMF itself, a necessary input for the model. Furthermore, adaptive methods may be tailored to the problem at hand to obtain more transparent analytic results; one could, e.g., use a larger number of interpolation polynomials in the regions where ion sojourn probabilities are highest and less accurate fits far away from the local minima.

The above rate theory has been applied to a simple model for ion channel transport where the equilibrium PMF is given by the simplest piecewise linear potential displaying minima, i.e., a symmetric sawtooth potential. Despite its simplicity, the model displays several interesting features, such as the onset of flux optimization and transitions between sub-linear and superlinear current-voltage curves when the parameter σ describing the binding site geometry is varied. Besides, our model gives rise to flux ratio exponents which decrease monotonically below the Ussing limit $\delta = 1$ as the binding site becomes wider. Finally, we have seen that saturation effects due to changes in bulk concentration are strongly influenced by the geometry of the binding site and that the qualitative behavior of the conductance curves is robust with respect to the introduction of new binding sites.

The results obtained in this work can be generalized in many ways. Possibly, the most straightforward extension is considering the case of an equilibrium PMF given by an asymmetric sawtooth potential. Research in this direction is underway and will be the object of a forthcoming publication; preliminary results indicate that in the appropriate volt-

age regime the ionic current displays complex nonmonotonic behavior, including the onset of both maxima and minima of the current as a function of σ . Coexistence of binding sites with different geometries will also lead to a complicated behavior of the current.

A further interesting generalization concerns the study of fluctuations about the mean number of translocating ions $M(t)$. The relationship between the latter and the ion flux is given by the equation $j = \lim_{t \rightarrow \infty} (d\langle M(t) \rangle / dt)$, where the angular brackets denote average over all possible translocating events. Likewise, one can take as a measure for fluctuations in ion transport the dispersion⁵⁵ $\mathcal{D} = (1/2) \lim_{t \rightarrow \infty} (d(\langle M(t)^2 \rangle - \langle M(t) \rangle^2) / dt)$, which is also expressible in terms of the jump rates.²³

Another situation worthy of study is the case of binding sites located at fixed positions but separated by fluctuating barriers arising from degrees of freedom commensurate with the time scale of ion transport;⁵⁶ an example for such a system is e.g., the CIC family of chloride channel and transporter proteins, for which the distinction between a channel and a transporter is somewhat blurred, in that gating-like transitions seem to occur on the same time scale as ion transport.^{57,58} Admittedly, the curvilinear pores and the asymmetric location of the side chains in these systems suggests that, unless one is able to find an appropriate effective coordinate, a 1D theory will not be suitable in this case. However, assessing the properties of the case with a fluctuating 1D potential profile may pave the way for the understanding of more realistic, higher dimensional systems.

For very fast potential fluctuations between, say, two different PMF states 1 and 2, it is possible to define an average PMF seen by the ion and to use it to compute the average rates $k_n^\pm$. In case of fast fluctuations (but whose characteristic switching time is still long compared to the time needed by an ion to relax inside a binding site), transport can no longer be described by an average potential; in this case, the theory of “flashing” ratchets (i.e., PMFs subject to multiplicative fluctuations) shows that the mean rates are $k_n^\pm \approx p_1 k_{n,1}^\pm + p_2 k_{n,2}^\pm$ where p_1 and p_2 are the (in general concentration- and voltage-dependent) stationary probabilities to find the PMF in either of the states characterizing the dichotomous process at hand.^{59,60} In contrast, for slower fluctuations, rather than the rate it is the MFPT and the splitting probability which are obtained by linear superposition, i.e., $\tau_n \approx p_1 \tau_n^1 + p_2 \tau_n^2$ and $R_n \approx p_1 R_n^1 + p_2 R_n^2$. The resulting expressions can be injected into Eqs. (9) and (10) to compute the rates $k_n^\pm$ and subsequently the mean ion flux.

Finally, a far more difficult extension of the theory concerns the description of multi-ion conduction, i.e., concerted motion of several ions through the channel on commensurate time scales. This task is relevant to the case of, e.g., potassium channels, for which structural data provide direct evidence of a multi-ion pore.⁶¹ In such systems, the effect of ion-ion interactions cannot in general be described by a one-variable PMF resulting from a mean-field-like approximation based on the separation of time scales. Instead, multi-ion states as well as a multivariate (=higher dimensional) PMF describing the free energy of multi-ion states characterized by a set of 1D coordinates $\vec{x} \equiv (x_1, x_2, \dots)$ for the positions of

the different ions 1, 2, ... inside the narrow channel need to be used. However, even in the case of strong ion-ion correlations, a Markovian description for transitions between states can be retained if the ion waiting times in the different binding sites are sufficiently long.

In the higher dimensional case, transitions may involve simultaneous motion of ions which take short-lived intermediate positional configurations with higher energy than the outgoing and the incoming state. Such configurations correspond to barriers associated with saddle points rather than to maxima as in the 1D case, and the system can in general escape a given state over more than two barriers. This complicates the analytic description in the framework of our model, since it is no longer possible to find an exact solution of the differential equations fulfilled by the MFPT to a saddle point and the conditional probabilities to overcome a given energy barrier among all the ones surrounding a given multi-ion state. One possibility to overcome this difficulty is the use of lattice-based formulations of the Fokker–Planck equation^{62,63} which allow one to compute ion fluxes numerically. If the PMF is sufficiently smooth, another possibility is to use higher dimensional Kramers’ theory,^{18,64} implying that the transition rate from a multi-ion state n to another one m would read as

$$k_{n \rightarrow m} = w_{n \rightarrow m} \exp(-\Delta U_B^{n \rightarrow m}). \quad (55)$$

Here, $\Delta U_B^{n \rightarrow m}$ is the free energy difference between the outgoing metastable state n [corresponding to a minimum of $U(\vec{x})$ whose location is given by the spatial coordinates $\vec{x}_n$] and the top of the lowest free energy barrier separating the states n and m (corresponding to a saddle point $\vec{x}_{n \rightarrow m}^S$).⁶⁵ The frequency factor $w_{n \rightarrow m}$ can be expressed in terms of the determinant $\mathcal{H}$ of the Hessian matrix of the free energy $U(\vec{x})$ (evaluated both at $\vec{x}_n$ and $\vec{x}_{n \rightarrow m}^S$) and the growth rate λ_+ of a small deviation from the saddle point along the unstable manifold.^{18,64} The rate λ_+ can be computed from the transposed of the dynamical matrix $\mathbf{M} = \mathbf{D} + \mathbf{A}$ (where $\mathbf{D}$ is the diffusion matrix and $\mathbf{A}$ is the inertial part of the transport matrix due to the applied nonequilibrium perturbation) and $\mathcal{H}$ evaluated at $\vec{x}_{n \rightarrow m}^S$. Following the procedure illustrated above for the 1D case, the detailed balance condition could be used to express a certain number of matrix entries in $\mathbf{D}$ in terms of the various equilibrium entry rates, which would subsequently allow one to compute nonequilibrium quantities as a function of the remaining free parameters.

ACKNOWLEDGMENTS

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APPENDIX: KRAMERS' LIMIT OF TRANSITION RATES

To begin the proof, let us explicitly write down the transition rate $\tilde{k}_n^-$ by making use of Eqs. (9) and (10):

$$\tilde{k}_n^- = \frac{D_n}{2} \left(R_n^{-1} \int_{x_n^-}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y)) - \int_{x_n^-}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y))^{-1} \right). \quad (\text{A1})$$

Let us first examine the behavior of the splitting probability (8) for large barriers $\Delta U_n^\pm \gg 1$. In this case, the major

contribution to the integral $\int_{x_n^-}^{x_n^+} dx \exp(U(x))$ in the numerator of Eq. (8) stems from the vicinity of the potential maximum at $x = x_n^+$, whereas the major contributions to the integral in the denominator arise from the vicinity of the maxima at $x = x_n^-$ and $x = x_n^+$. If $U(x)$ is sufficiently smooth, one can use the harmonic approximation to expand the integrand about the maxima as follows:

$$\exp(U(x)) \approx \exp(U(x_n^\pm)) \exp\left(\frac{1}{2} U''(x)|_{x=x_n^\pm} (x - x_n^\pm)^2\right). \quad (\text{A2})$$

One thus has

$$\begin{aligned} R_n^{-1} &= 1 + \frac{\int_{x_n^-}^{x_n^+} dx \exp(U(x))}{\int_{x_n^-}^{x_n^+} dx \exp(U(x))} \approx 1 + \frac{\exp(U(x_n^-) - U(x_n^+)) \int_{x_n^-}^{x_n^+} dx \exp\left(\frac{1}{2} U''(x)|_{x=x_n^-} (x - x_n^-)^2\right)}{\int_{x_n^-}^{x_n^+} dx \exp\left(\frac{1}{2} U''(x)|_{x=x_n^+} (x - x_n^+)^2\right)} \\ &\approx 1 + \frac{\exp(U(x_n^-) - U(x_n^+)) \int_{x_n^-}^{\infty} dx \exp\left(\frac{1}{2} U''(x)|_{x=x_n^-} (x - x_n^-)^2\right)}{\int_{-\infty}^{x_n^+} dx \exp\left(\frac{1}{2} U''(x)|_{x=x_n^+} (x - x_n^+)^2\right)}. \end{aligned} \quad (\text{A3})$$

In the last step, we have respectively extended the integration boundaries to $+\infty$ and $-\infty$. This is a good approximation because the contribution of the integrands in the intervals $[x_n, \infty)$ and $(-\infty, x_n]$ is comparatively very small. The underlying idea is of course to make use of the well-known analytic result:

$$\begin{aligned} \int_{z_0}^{\infty} dz \exp(-\alpha(z - z_0)^2) &= \int_{-\infty}^{z_0} dz \exp(-\alpha(z - z_0)^2) \\ &= \frac{1}{2} \int_{-\infty}^{\infty} dz \exp(-\alpha(z - z_0)^2) \\ &= \frac{1}{2} \sqrt{\frac{\pi}{\alpha}}, \quad \alpha > 0. \end{aligned} \quad (\text{A4})$$

Thus, we get the auxiliary result

$$R_n^{-1} \approx 1 + \exp(U(x_n^-) - U(x_n^+)) \sqrt{\left| \frac{U''(x)|_{x=x_n^+}}{U''(x)|_{x=x_n^-}} \right|}. \quad (\text{A5})$$

In order to further simplify the expression for $\tilde{k}_n^-$ we now make the approximation

$$\begin{aligned} \int_{x_n^-}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y)) &\approx \int_{x_n^-}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y)) \\ &\approx \int_{x_n^C}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y)), \end{aligned} \quad (\text{A6})$$

where x_n^C is an appropriately chosen cut-off value slightly

below x_n^+ (see Fig. 18). In the limit of large barriers, this is a good approximation, since the functions $\int_{x_n^-}^x dy \exp(-U(y))$ and $\exp(U(x))$ become simultaneously large only in the vicinity of x_n^+ (see Fig. 18). Using Eqs. (A6) and (A5) in Eq. (A1), we then get

$$\tilde{k}_n^- \approx \sqrt{\left| \frac{U''(x)|_{x=x_n^-}}{U''(x)|_{x=x_n^+}} \right|} \frac{D_n \exp(-(U(x_n^-) - U(x_n^+)))}{2 \int_{x_n^C}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y))}. \quad (\text{A7})$$

The x -function $\int_{x_n^-}^x dy \exp(-U(y))$ varies very slowly in the short interval $[x_n^C, x_n^+]$; we may therefore replace it with its

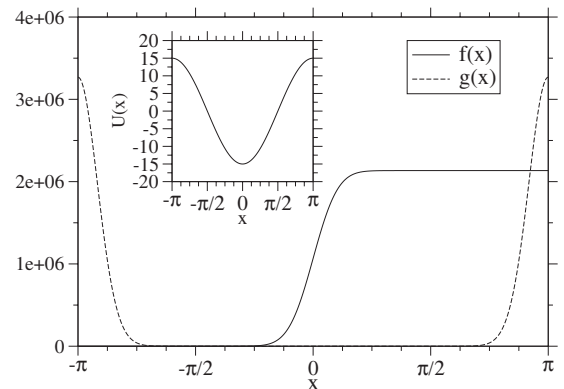


FIG. 18. Comparison between $f(x) \equiv \int_{x_n^-}^x dy \exp(-U(y))$ and $g(x) \equiv \exp(U(x))$ in the interval $[x_n^-, x_n^+]$. We have taken the values $x_n^- = -\pi$, $x_n = 0$, $x_n^+ = \pi$, and the symmetric function $U(x) = U_0 \cos(x + \pi)$ with $U_0 = 15$ (note, however, that the arguments in the Appendix hold also for slightly asymmetric functions). $U(x)$ is depicted in the inset. For the chosen value of U_0 one can see from the figure that $x_n^C \approx 11\pi/16$. An increase in U_0 shifts x_n^C toward x_n^+ .

value at x_n^+ . Hence, in this limit the integral in the denominator of Eq. (A7) factorizes and it can be approximated as follows:

$$\begin{aligned} & \int_{x_n^C}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y)) \\ & \approx \int_{x_n^C}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^{x_n^C} dy \exp(-U(y)) \\ & \approx \exp(\Delta U_n^+) \int_{x_n^C}^{x_n^+} dx \exp\left(\frac{1}{2} U''(x) |_{x=x_n^+} (x - x_n^+)^2\right) \\ & \quad \times \int_{x_n^-}^{x_n^C} dx \exp\left(-\frac{1}{2} U''(x) |_{x=x_n^-} (x - x_n^-)^2\right). \end{aligned} \quad (\text{A8})$$

In the last step we have used Eq. (A2) in the first integral after the exponential and a similar expansion about the minimum x_n in the second integral. One can now replace the lower bounds in the integrals with $-\infty$ and the upper bound of the second integral with $+\infty$. Subsequent use of Eq. (A4) yields

$$\begin{aligned} & \int_{x_n^C}^{x_n^+} dx \exp(U(x)) \int_{x_n^-}^x dy \exp(-U(y)) \\ & \approx \frac{\pi \exp(\Delta U_n^+)}{\sqrt{|U''(x)|_{x=x_n^+} U''(x)|_{x=x_n^-}}}. \end{aligned} \quad (\text{A9})$$

Inserting this expression into Eq. (A7) then immediately yields Kramers' expression (11a). A similar derivation leads to Eq. (11b) for the rate of hopping into the well to the right.

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