

A permeation theory for single-file ion channels: One- and two-step models

Peter Hugo Nelson^{a)}*Department of Physics, Benedictine University, Lisle, Illinois 60532, USA*

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How many steps are required to model permeation through ion channels? This question is investigated by comparing one- and two-step models of permeation with experiment and MD simulation for the first time. In recent MD simulations, the observed permeation mechanism was identified as resembling a Hodgkin and Keynes knock-on mechanism with one voltage-dependent rate-determining step [Jensen *et al.*, PNAS **107**, 5833 (2010)]. These previously published simulation data are fitted to a one-step knock-on model that successfully explains the highly non-Ohmic current–voltage curve observed in the simulation. However, these predictions (and the simulations upon which they are based) are not representative of real channel behavior, which is typically Ohmic at low voltages. A two-step association/dissociation (A/D) model is then compared with experiment for the first time. This two-parameter model is shown to be remarkably consistent with previously published permeation experiments through the MaxiK potassium channel over a wide range of concentrations and positive voltages. The A/D model also provides a first-order explanation of permeation through the *Shaker* potassium channel, but it does not explain the asymmetry observed experimentally. To address this, a new asymmetric variant of the A/D model is developed using the present theoretical framework. It includes a third parameter that represents the value of the “permeation coordinate” (fractional electric potential energy) corresponding to the triply occupied state n of the channel. This asymmetric A/D model is fitted to published permeation data through the *Shaker* potassium channel at physiological concentrations, and it successfully predicts qualitative changes in the negative current–voltage data (including a transition to super-Ohmic behavior) based solely on a fit to positive-voltage data (that appear linear). The A/D model appears to be qualitatively consistent with a large group of published MD simulations, but no quantitative comparison has yet been made. The A/D model makes a network of predictions for how the elementary steps and the channel occupancy vary with both concentration and voltage. In addition, the proposed theoretical framework suggests a new way of plotting the energetics of the simulated system using a one-dimensional permeation coordinate that uses electric potential energy as a metric for the net fractional progress through the permeation mechanism. This approach has the potential to provide a quantitative connection between atomistic simulations and permeation experiments for the first time. © 2011 American Institute of Physics. [doi:10.1063/1.3580562]

I. INTRODUCTION

Potassium ion channels (K^+ channels) are a common component in the membranes of living organisms and are even encoded by some viruses. Located in the lipid bilayers of bacterial, archeal, plant, and animal cells, the purpose of a K^+ channel is the selective conduction of K^+ ions across the membrane. While the channel is open, this net charge transfer continues until the membrane capacitor charges to the Nernst potential or the channel closes. The transmembrane voltage generated by this charge transfer is involved in many biological processes ranging from cell volume regulation and homeostasis of bacteria to hormone secretion and the action potential of the human nervous system.^{1–3} Permeation of ions through K^+ channels is vital to biological organisms and all known K^+ channels are members of a single protein family. They have a highly conserved signature sequence that codes for the selectivity filter of the channel. The

selectivity filter is a short ~ 1.2 nm long ion-attracting tube that is highly selective for K^+ over Na^+ .² In this paper, we will focus on the movement of K^+ ions through this narrow structure.

In the first paper of this series, a simple theoretical framework was presented for ion channels that allowed for concerted translocation of all the ions contained within the selectivity filter. Within this framework a three-step permeation model was proposed based on the common structural features of the KcsA and gramicidin A channels. It was discovered that the Michaelis–Menten saturating behavior observed experimentally for many ion channels can be explained if the translocation step is assumed to be rapid (not rate limiting). In this rapid translocation (RT) model, permeation is sorption limited with either association and/or dissociation being the rate determining steps. A significant new feature of this RT model was that association is assumed to be voltage independent. This feature correctly predicted permeation behavior at high voltages (for the first time) while reducing the minimum number of adjustable parameters from eight to just three.⁴

^{a)} Author to whom correspondence should be addressed. Electronic mail: pete@circle4.com.

Motivated by the results of molecular simulations, this theoretical framework was extended in the second paper of this series to include concerted-association and concerted-dissociation transitions. This concerted-association/dissociation hypothesis allowed for the possibility that permeation might proceed according to just two steps.⁵ This two-step hypothesis is now supported by a large group of simulations,^{6–15} but no comparison of this two-step model with experiment has yet been conducted.

Subsequently a four-step model was proposed¹⁶ based on a free-energy landscape for KcsA permeation obtained from MD simulations.^{12,17} This four-step model was used to analyze permeation through both wild-type and mutant BK_{Ca} potassium channels. However, while this model (with 10 adjustable parameters) was able to successfully model permeation, the resulting free-energy diagram was qualitatively different from the free-energy landscape used to develop the model.

Recently, Jensen *et al.* performed extensive MD simulations of the Kv1.2 potassium channel. Analysis of hundreds of permeation events led them to the conclusion that permeation proceeded via a (one-step) Hodgkin and Keynes knock-on mechanism, in which two selectivity-filter bound ions are “knocked-on” by a third entering ion. They found that the formation of the three-ion “knock-on intermediate” was the rate-determining and voltage-dependent step. However, the current–voltage curves they obtained were qualitatively different from experiment exhibiting superlinear behavior, whereas sublinear behavior is observed experimentally.¹⁴

The central question to be addressed in this paper is this—how many steps are required to account for the qualitative features observed in single ion channel permeation experiments? To answer this question, we will investigate the simplest one- and two-step models of single-file permeation that can be developed within the present theoretical framework and how they can be used to relate molecular simulations to experimental measurements of permeation through real channels.

II. SINGLE-FILE PERMEATION MODELS

Models of multi-ion single-file permeation cannot be based on traditional Fickian diffusion theory. In single-file systems, the movements of all molecules in the file are highly correlated by the single-file constraint.¹⁸ Motion within the file can occur either via a vacancy mechanism (density fluctuations) or through concerted motion of the whole file. Permeation through K⁺ channels is currently thought to occur predominantly through concerted motion, although vacancy formation within the selectivity filter has been observed in some simulations.^{14,15,19,20} In what follows, we will focus on mechanisms that rely primarily on concerted motion of the entire file of ions and water molecules within the selectivity filter (although vacancy mechanisms can also be incorporated within the theoretical framework).

In this approach, the system is conceptually divided into three regions—the two bulk solutions on either side of the membrane and the selectivity filter region that separates them. Transport to the selectivity filter is characterized by a first-

order association rate constant k_a that includes diffusional and other resistances between the bulk solution and the selectivity filter (and thus should be considered as a mass transfer coefficient).^{21,22} The selectivity filter is modeled by a small number of discrete states. Transitions between these states are characterized by a multi-ion “permeation coordinate” that uses electric potential energy as a metric for the fractional progress through the net outward permeation of one ion.

A major simplifying assumption in all the permeation models considered in this paper is that the voltage drop is concentrated across the selectivity filter. This is equivalent to assuming that any transport processes outside the selectivity filter are not dependent on voltage and can be included in voltage-independent mass transfer coefficients (e.g., k_a and k_d). This assumption is supported by a simplified calculation of the voltage profile through the open K⁺ channel pore (calculated using a modified Poisson–Boltzmann theory) which predicts that the entire voltage drop will be concentrated across the selectivity filter (selectivity filter sites S₄ through S₀).¹² This same simplifying assumption was made in the first paper of this series,⁴ but strictly speaking, the requirement is simply that transport to the selectivity filter can be characterized by a voltage-independent rate constant.

A. Knock-on model

The knock-on mechanism was originally proposed by Hodgkin and Keynes long before the structure of the potassium channel was discovered. It provides a simple explanation for the experimentally observed flux ratio of radioactive tracer-labeled K⁺ permeation²³ (that occurs within the counter-permeation eigenmode of tracer-labeled binary permeation^{24,25}). It was based on the hypothesis that ions move through ion channels in a single file via a one-step mechanism. To test their idea, Hodgkin and Keynes built a mechanical model that had steel balls in two flat compartments separated by a narrow channel capable of containing a single file of balls.²³ Thermal motion of the balls was simulated by shaking the system using a motor. A ball colliding with one end of the file resulted in the immediate “knock on” of the ball at the other end of the file (into the opposite compartment), in a manner similar to the “Newton’s Cradle” executive toy.^{26,27} While the knock-on model successfully explains the very large experimental departures from the independence relation, Hodgkin and Keynes did not present any expression [such as Eq. (2) below] for the net ion flux (this expression was not required for their analysis).²³

For K⁺ channels, the knock-on mechanism can be summarized as shown in Fig. 1. The approach of an ion toward one side of the selectivity filter is coupled with the simultaneous exit of another ion from the other side of the filter.^{17,28} Within the present theoretical framework, this mechanism can be schematically represented as shown in Fig. 2. In this energy



FIG. 1. Hodgkin and Keynes knock-on mechanism. Permeation is a one-step process—after Bernèche and Roux. (See Ref. 17.)

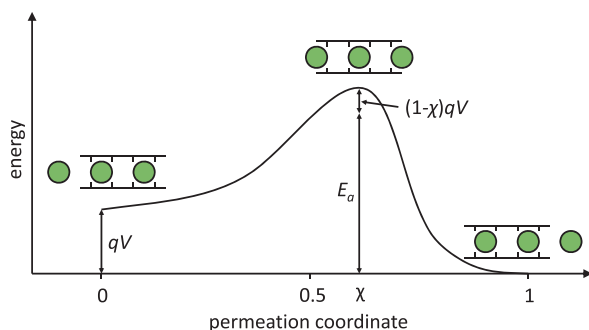


FIG. 2. Energetics of the knock-on model for a positive transmembrane voltage V . The permeation coordinate represents the fractional progress through outward permeation. qV is the electrical work done on the ions during a complete outward permeation transition. $(1 - \chi)qV$ is the increase in the transition state energy as a result of the transmembrane voltage V . E_a is the activation energy for $V = 0$.

diagram the permeation coordinate represents the fractional distance through the permeation process using electrical potential energy as a metric. A positive transmembrane potential V raises the electrical potential energy of a single positive ion on the inside (left-hand side of the figures) of the cell by an amount qV , where q is the electrical charge of the ion. As can be seen in Fig. 2, the effect of the potential V is to lower the activation energy for outward (left to right) permeation from E_a to $E_a - \chi qV$, where χ is the value of the permeation coordinate corresponding to the knock-on transition state. In what follows, it is convenient to define the dimensionless voltages, μ and λ , which are given by

$$\mu = \frac{\chi qV}{k_B T} \quad \text{and} \quad \lambda = \frac{(1 - \chi)qV}{k_B T}, \quad (1)$$

where χqV is the electrical work done by the transmembrane voltage V (as the system moves from the left-hand side of the diagram to the transition state) and $(1 - \chi)qV$ is the electrical work done by the transmembrane voltage (as the system moves from the transition state to the right-hand side of the diagram). The denominator is the thermal energy (Boltzmann constant times absolute temperature). e^μ is thus an electrical Boltzmann factor for the transition state that reflects the increase in the association rate due to the lowering of the activation energy (for outward permeation) by an applied positive voltage. That is, if the rate of outward permeation events is $k_a c_i$ in the absence of a transmembrane voltage ($V = 0$), then the outward rate is given by $k_a e^\mu c_i$ in the presence of a transmembrane voltage. k_a is the zero-volt association rate constant (mass transfer coefficient) and c_i is the K^+ ion concentration (activity) in the bulk inner solution. Similarly, the association rate in the inward direction is given by $k_a e^{-\lambda} c_o$, where c_o is the K^+ ion concentration in the bulk outer solution and $e^{-\lambda}$ is the electrical Boltzmann factor for an inward knock-on transition. Combining these forward and reverse rates, the net flux in the outward direction is

$$J = k_a (e^\mu c_i - e^{-\lambda} c_o). \quad (2)$$

For symmetric solutions, $c_i = c_o = c$, this expression reduces to

$$J = k_a c (e^\mu - e^{-\lambda}), \quad (3)$$

for which a symmetrical barrier ($\chi = 1/2$) further reduces to

$$J = 2k_a c \sinh \phi, \quad (4)$$

where

$$\phi = \frac{qV/2}{k_B T}. \quad (5)$$

Equation (4) has the same mathematical form as a single ion crossing a (symmetric) potential barrier in an electric field.²⁹ Thus, while the knock-on model accounts for single-file permeation, it produces a net flux (in the copermutation eigenmode) that is unaffected by the single-file nature of its permeation, analogously with other simple single-file permeation models.^{24,25} The knock-on model is thus the simplest single-file permeation model. The symmetric knock-on model has just one adjustable parameter k_a , which can be directly related to the “permeability” of the channel.²⁹

B. Connecting the knock-on model with simulation

In recent MD simulations, Jensen *et al.* found that conduction through the Kv1.2 potassium channel could be summarized by a Hodgkin and Keynes knock-on mechanism. For a channel in state $[S_4, S_2]$ (which denotes a selectivity filter occupied by two K^+ ions, one at crystallographic site S_4 and one at site S_2 , with water molecules usually occupying the remaining crystallographic sites) formation of a knock-on intermediate $S_5 [S_4, S_2]$ was found to be the principal rate-determining (and voltage-dependent) step (where S_5 denotes an ion located at crystallographic site S_5 outside the selectivity filter). Upon binding to site S_5 , the incoming ion causes a rapid concerted transition to state $S_5 [S_3, S_1]$ and then onto state $[S_4, S_2, S_0]$, with the permeation process being completed by the exit of the ion from site S_0 to return the selectivity filter to state $[S_4, S_2]$.¹⁴ Thus, in contrast to other simulations of K^+ channels,^{6–15} the predominant double-occupancy state of the selectivity filter is $[S_4, S_2]$ rather than $[S_3, S_1]$.

In order to test Jensen *et al.*'s hypothesis that these simulations can be explained by a knock-on model, Fig. 3 shows a weighted least-squares fit of Eq. (3) to the simulated potassium current–voltage data reported by Jensen *et al.* [their Fig. 1C]. The solid line was fitted to all the nonzero current data and resulted in $\chi = 0.95$ and $k_a = 4.5 \times 10^6 \text{ s}^{-1} \text{ M}^{-1}$.^{30,31}

The knock-on mechanism is a one-step process. Selectivity filter microstates that contain three ions are assumed to be short-lived transition states so that the selectivity filter has only one stable (stationary) state containing two ions—see Figs. 1 and 2. Most previous molecular simulations of K^+ channels appear to be inconsistent with this feature of the knock-on model as they show stable states with both double and triple occupancy of the selectivity filter.⁶ In addition, the knock-on model predicts [Eqs. (3) and (4)] that permeation through an ion channel between symmetric solutions will be directly proportional to the permeant ion concentration and that the current–voltage curves will generally be superlinear (i.e., conduction will be super-Ohmic as shown in Fig. 3).

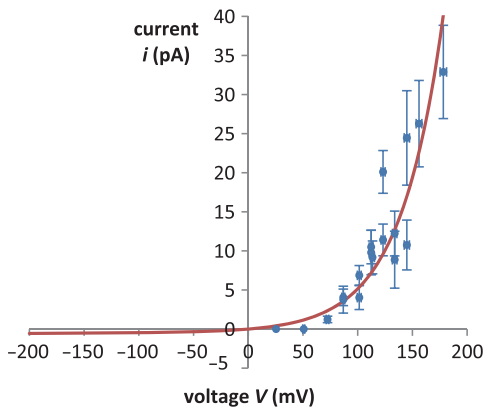


FIG. 3. Comparing the asymmetric knock-on model Eq. (3) with simulated single-channel Kv1.2 permeation data. The theoretical curve was fitted to all the nonzero current data resulting in $\chi = 0.95$ and $k_a = 4.5 \times 10^6 \text{ s}^{-1} \text{ M}^{-1}$. Simulation data (symbols) reproduced from Jensen *et al.* (See Ref. 14.)

Neither of these qualitative predictions are consistent with permeation experiments through K^+ channels.³

C. Association/dissociation (A/D) model

The problems with the knock-on model can be corrected by simply inverting the energy diagram in Fig. 2 to give Fig. 4. In this A/D model (originally proposed in the second paper of this series),⁵ the selectivity filter now has two stable (stationary) states as shown in Fig. 5. In state m the selectivity filter contains two K^+ ions and in state n it contains three K^+ ions. The net result is that permeation is now a two-step process, in which association needs not be followed immediately by dissociation. Association proceeds via a concerted “shunt-on” transition, in which the three ions (and any water molecules trapped between them) move together like the shunted carriages of a train. Similarly, dissociation occurs via a concerted mechanism where the helper ions “shunt off” the permeant ion.

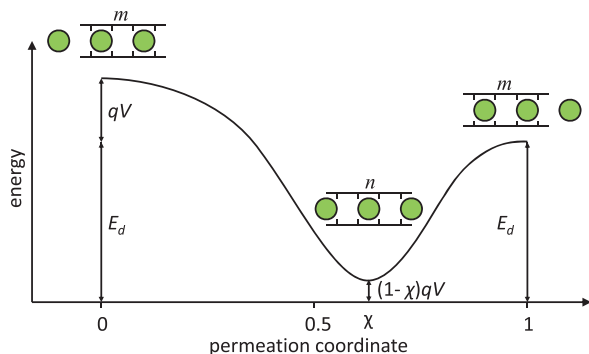


FIG. 4. Energetics of the A/D model for positive membrane voltage V . The two states of the selectivity filter are labeled n (triple occupancy) and m (double occupancy). The permeation coordinate represents the fractional progress through outward permeation. qV is the electrical work done on all of the ions during a complete outward permeation event. $(1 - \chi)qV$ is the increase in state n 's energy as a result of the transmembrane voltage V . E_d is the dissociation energy for $V = 0$.



FIG. 5. A/D mechanism. Permeation is a two-step process. During the association step all three ions move together in a concerted manner as the entering ion shunts-on the two helper ions to form the triply occupied state n . During the dissociation step all three ions move together in a concerted manner as the two helper ions shunt-off the permeant ion to return the channel to state m .

In a recent review,⁶ the results of a variety of molecular simulation techniques are reported as providing a coherent picture of permeation through K^+ channels that can be summarized by the following two states:⁶⁻¹³

$$[S_3, S_1] \leftrightarrow [S_4, S_2, S_0]. \quad (6)$$

According to this permeation scheme, outward permeation is a two-step process $[S_3, S_1] \rightarrow [S_4, S_2, S_0] \rightarrow [S_3, S_1]$ which matches the $m \rightarrow n \rightarrow m$ outward permeation process in Fig. 5.

de Haan *et al.* reported the first MD movie of permeation through K^+ channels.¹⁵ Their MD movie shows inward conduction through the KcsA channel at a negative voltage via transitions between three distinct states of the selectivity filter that can be identified as $m = [S_3, S_1]$, $n = [S_4, S_2, S_0]$, and $n' = [S_3, S_2, S_0]$. The selectivity filter transitions observed in the movie can be summarized by $n' \rightarrow m \rightarrow n' \rightarrow m \rightarrow n' \rightarrow n \rightarrow m \rightarrow n \rightarrow m \rightarrow n' \rightarrow n \rightarrow m \rightarrow n'$. The transitions between triply occupied states $n' \rightarrow n$ occur via a single-ion hop forming a vacancy at site S_3 . While these transitions can be easily incorporated within the present theoretical framework, they introduce additional parameters that are difficult to determine from permeation experiments (see discussion below). If the primed state n' is considered as a fluctuation within a lumped (unprimed) state n , then the de Haan *et al.* MD movie can be summarized by the sequence $m \rightarrow n \rightarrow m$ consistent with the A/D mechanism.³²

Within this model, the occupancy of the channel is represented by θ_n , which is the probability of finding the channel in state n . As there are only two possible occupancy states, the probability of finding the channel in state m is given by

$$\theta_m = 1 - \theta_n. \quad (7)$$

In this model it is assumed that dissociation is an activated process, whereas association is barrierless. Hence, for nonzero voltages, dissociation has an electrical Boltzmann factor but association does not.⁴ At the inner end of the selectivity filter, the outward flux is given by

$$J = k_a c_i \theta_m - k_d e^{-\lambda} \theta_n. \quad (8)$$

Similarly, at the outer end, the flux is

$$J = k_d e^{\lambda} \theta_n - k_a c_o \theta_m, \quad (9)$$

where k_a is the association rate constant (assumed to be the same at both ends of the filter for simplicity), c_i and c_o are the K^+ ion concentrations (activities) in the bulk inner and outer solutions, respectively, k_d is the zero-volt dissociation rate constant, and λ and μ are the dimensionless voltages defined in Eq. (1).

Association with the inner end of the selectivity filter is independent of voltage and occurs with a rate of $k_a c_i \theta_m$. The factor θ_m accounts for the assumption that the channel can only accept an ion while it is in state m . The second term in Eq. (8) is the rate at which ions dissociate from the selectivity filter into the inner solution. This is an activated process that must overcome a barrier of $E_d + \chi qV$. As a result, the dissociation rate is reduced by an electrical Boltzmann factor of $e^{-\mu}$ compared with the zero-volt dissociation rate of $k_d \theta_n$. The factor θ_n represents the probability that the channel is in state n —the state required for dissociation to occur.

Similarly, the first term in Eq. (9) represents activated dissociation over a barrier of magnitude $E_d - (1 - \chi)qV$ at a rate that is increased by an electrical Boltzmann factor of e^λ compared with 0 V. The second term represents the voltage-independent association from the bulk outer solution (with the selectivity filter in state m). For asymmetric solutions, Eqs. (7)–(9) can be solved for the net flux resulting in

$$J = \frac{k_d(c_i e^\lambda - c_o e^{-\mu})}{K_d(e^\lambda + e^{-\mu}) + (c_i + c_o)}, \quad (10)$$

where we have defined the parameter

$$K_d = \frac{k_d}{k_a}, \quad (11)$$

which is the (zero-volt) equilibrium dissociation constant. For symmetric solutions, $c_i = c_o = c$, Eq. (10) simplifies to

$$J = \frac{k_d c (e^\lambda - e^{-\mu})}{K_d (e^\lambda + e^{-\mu}) + 2c}, \quad (12)$$

which can be rewritten in the form of a Michaelis–Menten equation,

$$J = \frac{k_{\max} c}{K_M + c}, \quad (13)$$

where the voltage-dependent maximum permeation rate k_{\max} (cf. the velocity of an enzyme) is given by

$$k_{\max} = k_d \frac{e^\lambda - e^{-\mu}}{2} \quad (14)$$

and the voltage-dependent Michaelis concentration is given by

$$K_M = K_d \frac{e^\lambda + e^{-\mu}}{2}. \quad (15)$$

Equation (12) exhibits universal saturating behavior of the form⁴

$$j^* = \frac{c^*}{1 + c^*} = \theta_n, \quad (16)$$

where

$$j^* = \frac{J}{k_{\max}} \quad \text{and} \quad c^* = \frac{c}{K_M}. \quad (17)$$

According to Eq. (16) the reduced experimental flux j^* is a universal function of the reduced experimental concentration c^* , and j^* provides an experimental measure of the

channel saturation (occupancy θ_n), which is predicted by a voltage-dependent Langmuir isotherm of the form

$$\theta_n = \frac{c}{K_M + c}. \quad (18)$$

This asymmetric model reduces to the symmetric A/D model when $\chi = 1/2$ and Eq. (12) becomes

$$J = \frac{k_d c \sinh \phi}{K_d \cosh \phi + c}, \quad (19)$$

which can be written in the same Michaelis–Menten form as Eq. (13) with

$$k_{\max} = k_d \sinh \phi \quad \text{and} \quad K_M = K_d \cosh \phi, \quad (20)$$

where ϕ is given by Eq. (5). The symmetric A/D model has only two adjustable parameters.

Like the knock-on model, the A/D model is an intrinsically single-file permeation model that produces a net flux (in the copermeation eigenmode)^{24,25} that is unaffected by the single-file nature of permeation. In other words, the same Eqs. (10), (12) and (19) apply for any number of ions in state n (including $n = 1$). Hence, this same model can also be applied to one-ion channels. An interesting feature of this model is that it is actually simpler than traditional one-ion channel models (which assume that association is an activated process).^{3,29,33} The existence of this model also corrects the misconception that if a channel exhibits Michaelis–Menten kinetics it must be a one-ion channel—or conversely—if it is a multi-ion channel, then it should not exhibit Michaelis–Menten kinetics.^{3,34–41} Because of its simplicity, the A/D model is also particularly useful from a pedagogical perspective.⁴²

Equations (2) and (10) represent the predictions of the asymmetric knock-on and A/D models for asymmetric solutions. Setting the flux to zero in Eqs. (2) and (10) and solving for the resulting equilibrium voltage V_N , we obtain

$$V_N = \frac{k_B T}{q} \ln \frac{c_o}{c_i}, \quad (21)$$

which is the Nernst equation. Hence, both the knock-on and A/D models are consistent with equilibrium thermodynamics. This compliance is guaranteed because the permeation coordinate represents the electric potential energy of the system.

D. Connecting the A/D model with experiment

The predictions of the A/D model can be tested by comparing the predictions of Eq. (10) with a series of current–voltage data at a series of different asymmetric concentrations. As collecting this type of data presents experimental challenges,³⁶ it has been more common for permeation experiments to be conducted between symmetric solutions. These data can be compared with the predictions of Eq. (12) or (19) (see below). If these data are not available, then an even more limited test can be made by comparing the predictions of Eq. (13) with a series of current measurements at different (symmetric) concentrations at the same voltage. As k_{\max} and K_M only depend on voltage (and not concentration), Eq. (13) predicts that a plot of current (or conductance) versus concentration for *any* fixed voltage V will exhibit Michaelis–Menten

saturation behavior. This saturating behavior has previously been confirmed by a comparison of experimental currents with an empirical equation of the same form as Eq. (13) (at fixed voltage) for a wide variety of channels,³ including GlyR Cl⁻ channels⁴³ and sarcoplasmic reticulum K⁺ channels.³⁸ The A/D model is the simplest model that explains this experimental behavior.

For low voltages, the current–voltage curves predicted by the A/D model are approximately linear and Eq. (19) simplifies to an Ohmic approximation for the conductance

$$g \approx \frac{g_{\max} c}{K_d + c}, \quad (22)$$

where g_{\max} is the maximum (low-volt) conductance. Recently, the molecular properties of Kcv, a virus-encoded K⁺ channel, were investigated by Pagliuca *et al.* via single-channel recordings techniques.⁴⁴ The authors found that their results (for activities ranging from about 80 to about 1200 mM) could be summarized by an empirical equation of the same mathematical form as Eq. (22), with a maximum conductance of $g_{\max} = 362$ pS and a half-maximal conductance at a concentration of $K_d = 125$ mM. Hence, permeation through the Kcv K⁺ channel is also consistent with the predictions of the A/D model.

Schroeder and Hansen recently reported a series of single-channel current–voltage data for permeation through the MaxiK potassium channel between symmetric solutions, wherein the raw experimental data were corrected to account for unresolved rapid gating of the channel.⁴⁵ Figure 6 shows a least-squares fit⁴² of the symmetric A/D model to the positive-voltage “true” current reported by Schroeder and Hansen.⁴⁵ Figure 6(a) shows the experimental data in the current–voltage form and Fig. 6(b) shows the same data in the form of a corresponding occupancy states plot.⁴ The fit at positive voltages is remarkably good for such a simple model, indicating that the model provides a good explanation of the qualitative features of outward permeation through the MaxiK channel. The fitted model is symmetric and predicts that negative currents will mirror those at positive voltages. As shown in Fig. 6(a) this prediction is only approximately followed.

According to the model, the experimentally determined j^* shown in Fig. 6(b) represents the occupancy, θ_n , of the channel. Thus, j^* is an experimental measure of the channel saturation (probability of finding the selectivity filter in state n). As a result of the scaling (and the voltage dependence of the Michaelis concentration), the highest values of c^* for each experimental concentration correspond to the lowest voltages. As shown in Fig. 6(b) the MaxiK channel exhibits the universal saturating behavior predicted by the model with the channel generally having low occupancy under most of the conditions investigated experimentally.⁴⁶

Jensen *et al.* compared their MD simulations (of the Kv1.2 K⁺ channel)¹⁴ with the positive-voltage data previously published by Heginbotham and MacKinnon for the Shaker K⁺ channel.³⁷ Figure 7 shows a least-squares fit of the symmetric A/D model to this same positive-voltage data. The fit at positive voltages in Fig. 7(a) shows that the symmetric A/D model provides a reasonable first-order explanation of permeation through this channel. However, there is a

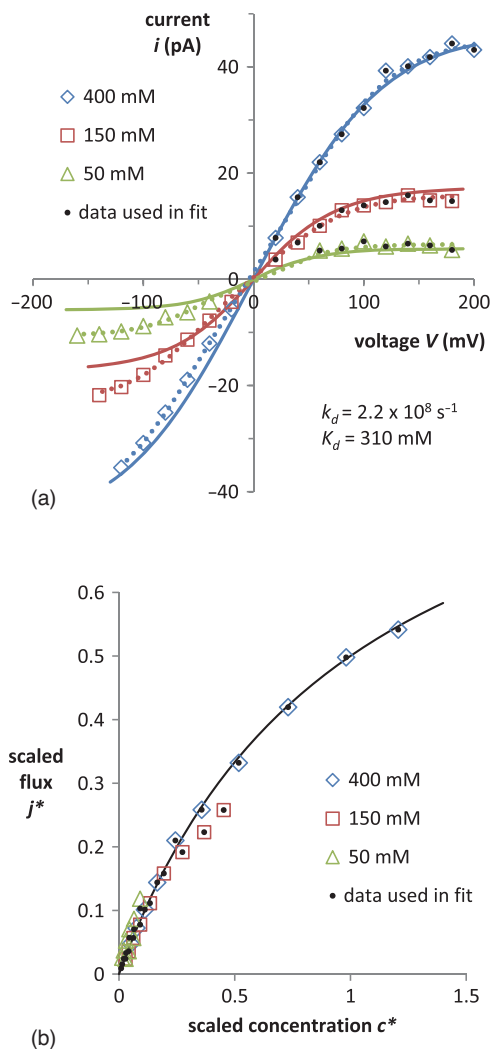


FIG. 6. (a) Comparing the two-parameter symmetric A/D model Eq. (19) with single-channel MaxiK permeation data. Theoretical curves (solid lines) were fitted to the $V > 0$ experimental data using Eq. (19), resulting in $k_d = 2.2 \times 10^8 \text{ s}^{-1}$ and $K_d = 310$ mM. Experimental data (symbols) and previous 15-parameter fit (dotted lines) reproduced (with permission) from Schroeder and Hansen. (See Ref. 45.) (b) Corresponding occupancy states plot of the positive-voltage data in Fig. 6(a). Within the A/D model, the experimental values of the scaled flux j^* represent the occupancy θ_n (degree of saturation) of the channel—see Eq. (16).

significant qualitative difference between the model and the experimental current–voltage curves. The positive-voltage experimental data are all approximately linear whereas the fitted model exhibits sublinear behavior at low concentrations and superlinear behavior at high concentrations.⁴⁷ The fitted model does successfully predict the approximately symmetric current–voltage behavior observed experimentally at the two highest concentrations (activities of $c = 1150$ mM and $c = 605$ mM), but the model significantly underestimates the negative current at lower concentrations (activities of $c = 325$, 206, 73, and 43 mM) suggesting that the Shaker K⁺ channel may exhibit more than one permeation mode (similar to the KcsA K⁺ channel).⁴⁷

As shown in Fig. 7(b), the A/D model can explain the basic saturating behavior of the scaled current as a function of scaled concentration. The experimentally determined

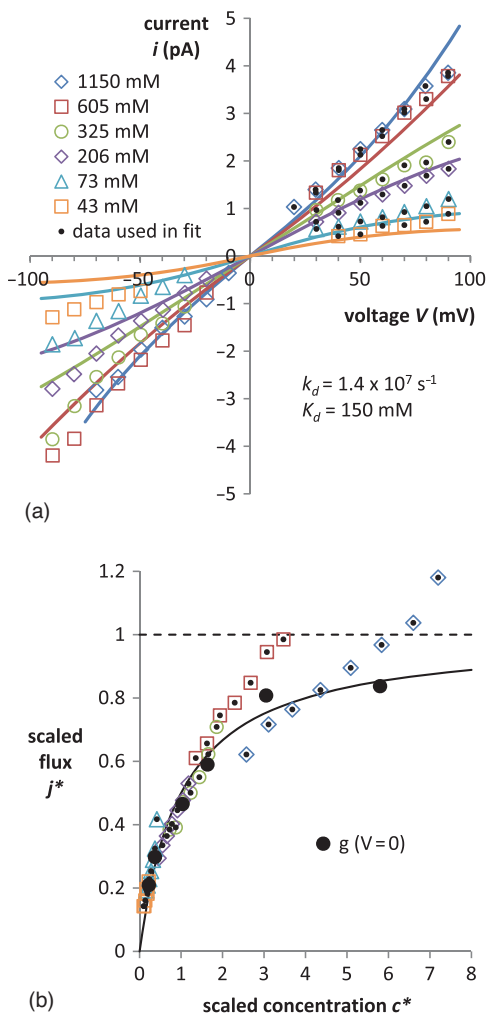


FIG. 7. Comparing the symmetric A/D model Eq. (19) with single-channel *Shaker* K^+ channel permeation data. (a) Theoretical curves (solid lines) were fitted to the $V > 0$ experimental data resulting in $K_d = 150$ mM, $k_d = 1.4 \times 10^7$ s $^{-1}$. Experimental data (symbols) reproduced (with permission) from Heginbotham and MacKinnon. (See Ref. 37.) (b) Corresponding occupancy states plot of the positive-voltage data in Fig. 6(a). Solid circles represent the zero-volt conductances reported by Heginbotham and MacKinnon that have been fitted to Eq. (22).

values of the zero-volt conductance $g(V = 0)$ (Fig. 1C of Heginbotham and MacKinnon³⁷) have been fitted to Eq. (22) and scaled by g_{\max} and K_d for comparison with the raw current–voltage data. As can be seen, the zero-volt conductance values generally correspond to the low-voltage data, consistent with the prediction of Eq. (20) that the voltage-dependent Michaelis concentration K_M has its minimum value at 0 V. The model does a good job of explaining the saturating behavior of the positive current at the lower concentrations (activities of $c = 325$, 206, 73, and 43 mM). However, there are systematic deviations in the slopes of the experimental data for the two highest concentrations in the corresponding occupancy states plot of Fig. 7(b), indicating that the occupancy of the channel (as measured by j^*) is overestimated at high concentrations. This also suggests that permeation at high concentrations may occur via a different permeation mechanism than at low concentrations.

E. Association barrier model

Traditional site-based models of ion channels have always included a substantial energy barrier for ion entry into the channel. They utilized Eyring rate theory and assumed that the transfer of an ion from an aqueous environment to the dehydrated environment of the selectivity filter would be an activated process in which the barriers on the outside of the pore had to be high (e.g., $8k_B T$) to ensure that ions do not enter faster than the maximum diffusion rate.^{3,48} While investigating a theoretical approach for predicting rate constants from more detailed structural information, Abad *et al.*⁴¹ used the energy profile from a traditional one site model as a test case. This energy profile is symmetric and has an energy barrier for association at each end. Abad *et al.* characterized this model using a parameter σ that represents the fractional width of the central binding site. The A/D model can be modified to include a similar association barrier. In this new “association barrier” model the association rate at the inner end of the channel is given by $k_a e^{\rho} c \theta_m$ and the dissociation rate at the outer end of the channel is given by $k_d e^{\eta} \theta_n$ etc., with $\rho = (1 - \sigma)qV/2k_B T$ and $\eta = \sigma qV/2k_B T$. For symmetric solutions, this model gives saturating behavior of the form of Eq. (13) with $K_M = K_d \cosh \eta / \cosh \rho$ and $k_{\max} = k_d (e^{\rho} e^{\eta} - e^{-\rho} e^{-\eta}) / (e^{\rho} + e^{-\rho})$, and it successfully predicts the double crossover effect observed by Abad *et al.*⁴¹ This new multi-ion model is thus formally equivalent to a traditional one-ion one site model within the copermeation eigenmode. However, when this association barrier model is fitted to the MaxiK channel data⁴⁵ (not shown), the best-fit value for the fractional width of the binding site is greater than $\sigma = 0.995$, indicating that the association barriers account for a negligible fraction of the electrical distance through the channel. This model results in an almost identical fit to the experimental data (not shown) with a less than 0.1% decrease in the sum of the squares of the differences, indicating that the inclusion of an association barrier is not supported by experiment for this K^+ channel.

F. Connecting the asymmetric A/D model with simulation and experiment

Recent MD simulations of permeation through the Kv1.2 potassium channel were described by Jensen *et al.* as resembling the Hodgkin and Keynes knock-on mechanism. As we have seen (in Fig. 3) their simulated conduction can, in fact, be successfully summarized quantitatively by the asymmetric knock-on model of Eq. (3). However the current–voltage curve predicted by the simulation (and the fitted knock-on model) is inconsistent with permeation experiments through real potassium channels suggesting that the energy landscape observed in the simulations is qualitatively incorrect. Notwithstanding this obvious discrepancy, the predominant states observed in the simulation might correspond to a permeation mechanism in real potassium channels.

In contrast to the simulations of Khalili-Araghi *et al.*,²⁰ Jensen *et al.*'s MD Movie S2¹⁴ shows just two dominant stationary states $m \equiv [S_4, S_2]$ and $n \equiv [S_4, S_2, S_0]$, and the whole of Movie S2 can be summarized by transitions between

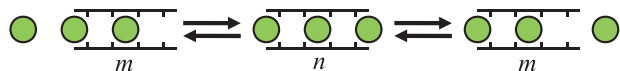
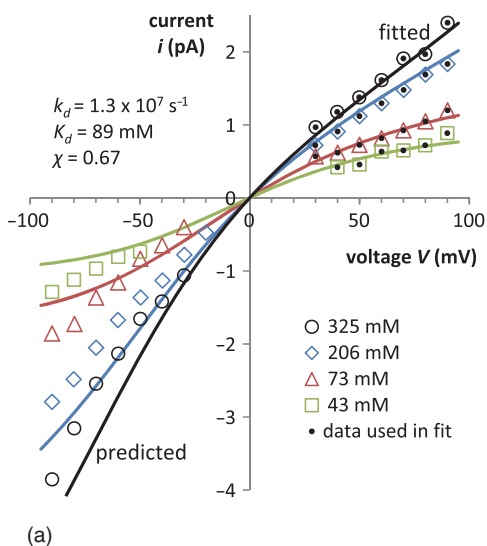
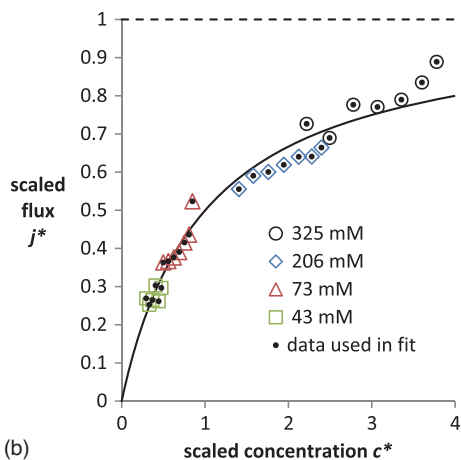


FIG. 8. Two-step asymmetric “shunt-on pop-off” variant of the A/D permeation model proposed here based on the MD simulations of Jensen *et al.* (See Ref. 14.)

these two states as shown in Fig. 8. The primary difference between this putative permeation mechanism and the original (symmetric) A/D model is that the double occupancy state m is clearly no longer in the middle of the selectivity filter suggesting that the fractional electrical distance will no longer be close to $\chi = 1/2$. Outward transitions from $m \rightarrow n$ occur at time indexes 0:01, 0:09, 0:22, 0:58, and 1:08 via an extended shunt-on transition. Outward transitions from $n \rightarrow m$ occur at time indexes 0:05, 0:21, 0:57, 1:01, and 1:07 via a “pop-off” transition wherein a single ion dissociates into the outer solution with no net movement of the remaining helper ions. At time index 1:04, a K^+ ion in the outer solution “pops-on” to

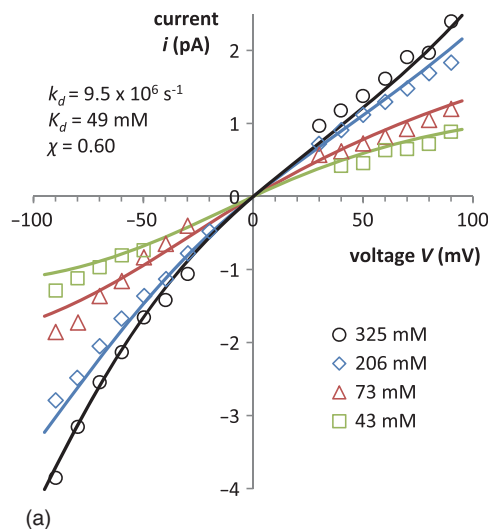


(a)

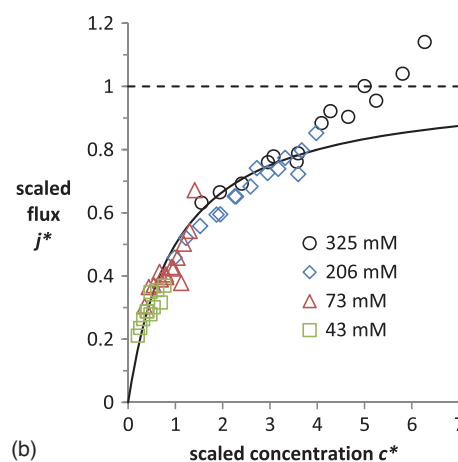


(b)

FIG. 9. Comparing the asymmetric A/D model Eq. (12) with single-channel *Shaker* K^+ channel permeation data. (a) Theoretical curves (solid lines) were fitted to the experimental data resulting in $K_d = 89$ mM, $k_d = 1.3 \times 10^7$ s $^{-1}$, and $\chi = 0.67$. Experimental data (symbols) reproduced (with permission) from Heginbotham and MacKinnon. (See Ref. 37.) (b) Corresponding occupancy states plot of the positive-voltage data in Fig. 9(a).



(a)



(b)

FIG. 10. Comparing the asymmetric A/D model Eq. (12) with single-channel *Shaker* K^+ channel permeation data. (a) Theoretical curves (solid lines) were fitted to both positive and negative experimental data resulting in $K_d = 49$ mM, $k_d = 9.5 \times 10^6$ s $^{-1}$, and $\chi = 0.60$. Experimental data (symbols) reproduced (with permission) from Heginbotham and MacKinnon. (See Ref. 37.) (b) Corresponding occupancy states plot of all the data in Fig. 10(a).

the outer end (site S_0) of the selectivity filter forming state n , in a transition that is the reverse of a pop-off transition. Thus, the whole of Movie S2 can be summarized by the asymmetric A/D model shown in Fig. 8.

As discussed above, it appears that the *Shaker* K^+ channel exhibits different permeation modes at high and low concentrations. To further investigate this hypothesis, Fig. 9 shows a least-squares fit of the asymmetric A/D model [Eq. (12)] to the positive-voltage single-channel data reported by Heginbotham and MacKinnon for the *Shaker* K^+ channel for the four lowest concentrations.³⁷ At positive voltages, the model successfully reproduces the approximately linear current–voltage behavior (with positive current intercepts). As shown in Fig. 9(b), the model also successfully explains the saturating behavior observed at positive voltages.

In addition to explaining the positive-voltage behavior, the model (fitted only to the positive data) successfully *predicts* that all of the negative currents have greater magnitude than the positive voltages and that the negative currents become super-Ohmic at high concentrations (see $c = 206$ mM

and $c = 325$ mM). This suggests that the model is capturing an essential asymmetry of permeation through the *Shaker* K^+ channel that is caused by the electrical dissociation distance being greater at the inner end of the selectivity filter.

Figure 10 shows a least-squares fit of the asymmetric A/D model [Eq. (12)] to all the data shown in Fig. 9. The correspondence between the model and experiment in Fig. 10(a) is improved at negative voltages, but the fit at positive voltages is noticeably worse for the 325 mM data at low voltages. As shown in Fig. 10(b) the fitted model also explains the saturating behavior of the channel over the whole voltage range investigated experimentally (except for scaled concentrations of $c^* > 4$, perhaps indicating the presence of another permeation mode that may begin to operate after the channel occupancy reaches $\theta_n = 0.8$).

III. DISCUSSION

The primary goal of MD simulations of K^+ channels is to gain insight into permeation. To this end, MD studies have provided verbal descriptions of permeation, schematic diagrams showing state transitions, multi-ion energy diagrams, and recently—movies of K^+ ions actually passing through the selectivity filter.^{2,6,14,15,20,28,49–51} The primary goal of permeation modeling is also to gain insight into K^+ channel permeation. However, in addition to providing verbal descriptions of permeation, schematic diagrams showing state transitions and multi-ion energy diagrams, the aim is to develop a simple mathematical model of permeation. If the model includes the essential features of the permeation process, then the conceptual framework it provides should be able to provide a simple explanation of the observed experimental behavior. In addition, the model will provide a self-consistent network of testable predictions for how the real system will behave under conditions different from those used to fit the model parameters, leading to the design of new experiments that can further test the model and our understanding of the permeation process.

MD simulations provide a fascinating opportunity for making connections between mathematical models and the underlying dynamics at atomic resolution, but significant challenges still remain. The most prominent of these is that apparently minor changes in the force field parameters (or other simulation conditions) can significantly affect the free energies of the ions—to the point where the permeation mechanism is changed. For example, Khalili-Araghi *et al.* and Jensen *et al.*, both performed simulations of permeation through the same K^+ channel (Kv1.2), but their simulations exhibited strikingly different permeation mechanisms even though they were both performed using variants of the same CHARMM force field.^{14,20} In addition, the ionic motion in the Khalili-Araghi *et al.* MD movies appears to be qualitatively different from that seen in the Jensen *et al.* MD movies. The state transitions in the Khalili-Araghi *et al.* MD movies are rather jerky (resembling a directed random walk), whereas the ionic motions during state transitions in the Jensen *et al.* movies are considerably smoother (being more like a single unidirectional transition). In addition, the current–voltage data provided by Jensen *et al.* are clearly non-Ohmic with

minimal conduction below 50 mV and super-Ohmic conduction above 50 mV. While these permeation data are consistent with a one-step permeation model, the simulation and the model based upon it are in qualitative disagreement with the experimental observation of approximately Ohmic behavior at both positive and negative voltages (see Fig. 7) indicating that the permeation mechanism in the simulation is not representative of the real channel behavior—even at a qualitative level.

In discussing their simulated current (Fig. 3), Jensen *et al.* suggested that the unexpected absence of current below 50 mV might be due to an unidentified energetic barrier to conduction that is present at low voltages and they fitted their permeation data to an empirical gating model.^{14,52,53} In contrast, the fitted knock-on model shown in Fig. 3 suggests that an association barrier (in the permeation pathway that extends over the majority of the permeation coordinate) may be responsible both for the absence of current in the simulated channel below 50 mV and the rapid voltage-dependent increase in current above 50 mV. The fitted knock-on model (with $\chi = 0.95$) also predicts minimal negative current (from a fit to $V > 50$ mV data) without requiring that the channel undergo any conformational change.

A. Symmetric A/D model

The symmetric A/D model in Eq. (19) is the simplest model that can simultaneously explain Ohmic behavior at low voltage and the universal Michaelis–Menten saturating behavior observed experimentally for many ion channels, including the MaxiK, *Shaker*, sarcoplasmic reticulum, and Kcv K^+ channels. The two-step A/D permeation mechanism (shown in Fig. 5) is also supported by a large group of MD simulations.^{6–15} Despite this extensive prior support, Fig. 6 shows the first published fit of this minimal saturating model to any experimental data.

The success of this A/D model, Eq. (19) in fitting the MaxiK channel data in Fig. 6 is quite remarkable for such a simple model. In addition to successfully explaining the Ohmic conductance below about 50 mV and the basic saturating behavior of the channel, the A/D model also provides a very simple physical explanation of the sub-Ohmic behavior observed experimentally at higher voltages in Fig. 6(a). According to the model, the scaled flux j^* shown in Fig. 6(b) is an experimental measure of the channel occupancy θ_n . As predicted by Eq. (20), the Michaelis concentration K_M has its lowest value at 0 V so that c^* has its highest value. As the voltage is increased, K_M increases and c^* decreases (at a fixed concentration). This effect is illustrated by the $c = 400$ mM data shown in Fig. 6(b), where K_M increases from $K_M = 310$ mM at 0 mV to $K_M = 7900$ mM at 200 mV. As a result, the channel occupancy decreases dramatically. Thus, the model predicts that the occupancy of state n will tend to zero at high voltages, and the association rate (at the inner end of the channel) will become the rate-limiting step (approaching $k_a c$), producing permeation that is independent of voltage and proportional to concentration. If this explanation is correct, then the approach to voltage-independent

permeation at high voltages is not a “saturation” phenomenon as was previously proposed,⁴⁵ instead it is caused by an “emptying out” of the selectivity filter (to state m).

B. Asymmetric A/D model

The asymmetric A/D model has an additional adjustable parameter χ for the value of the permeation coordinate corresponding to state n . As shown schematically in Fig. 4, state n need not correspond to a value of $\chi = 1/2$ even for a mechanism that appears to be spatially symmetric. The electrical distances for the two steps need not be the same due to structural differences outside of the selectivity filter or asymmetries within the selectivity filter. The shunt-on pop-off mechanism shown in Fig. 8 is clearly asymmetric from a mechanistic perspective. Association of an ion with the inner end of the selectivity filter shunts on each helper ion by two crystallographic sites. The inner helper ion moves from site S_4 to S_2 and the outer helper ion moves from site S_2 to S_0 . The net effect of this shunt-on transition is to move an ion from the inner bulk solution to site S_0 . If we assume that the five crystallographic sites are located at the same electrical distance from each other (and from the ends of the selectivity filter), then site S_0 is located 5/6th of the electrical distance through the channel and a value of $\chi = 5/6 = 0.83$ is expected from this mechanism. If we assume that sites S_4 and S_0 are located at half that distance from the bulk solutions then a value of $\chi = 9/10 = 0.90$ is expected. However, both of these values are significantly larger than the fitted values of $\chi = 0.67$ (Fig. 9) or $\chi = 0.60$ (Fig. 10) suggesting that the real *Shaker* permeation mechanism may be more like the shunt-on shunt-off mechanism in Fig. 5.

As shown in Fig. 9(b), the success of the asymmetric A/D model in predicting the saturating behavior of the *Shaker* K^+ channel seems fairly impressive at first glance, but care should be taken when interpreting fits to current–voltage data that appear linear. Any model that predicts the Michaelis–Menten saturating behavior of the form of Eq. (13) (with K_M and k_{\max} functions of voltage only), will predict the Ohmic behavior at low enough voltages.^{3,54–56} Since saturating and Ohmic behavior at low voltages can be accounted for by just two adjustable parameters in the A/D model, this is not a particularly stringent test for models that have three or more adjustable parameters. With more parameters it is possible to simultaneously adjust some of the parameters without significantly affecting the fit to the Ohmic region of the current–voltage curves, and the improvement in the fit provided by the extra parameters will be of questionable physical relevance. As an example, the experimental data for the viral-encoded Kcv channel are consistent with the A/D model, but the support provided is not conclusive.⁴⁴

However, while the positive-voltage experimental data in Fig. 9(a) are approximately linear, the current axis intercepts of linear fits to this data (not shown) are about 0.2 pA. When the asymmetric A/D model is fitted to the positive data it successfully *predicts* distinct qualitative changes in the negative voltage behavior (increased negative current and super-Ohmic behavior). This provides strong evidence that this model is

capturing a fundamental asymmetry in the permeation mechanism of the *Shaker* K^+ channel. This can be contrasted with the asymmetric RT model where a variety of negative voltage behaviors have been shown to be consistent with the same experimental positive-voltage data.⁴⁷

Earlier analysis of permeation through the KcsA channel using the three-parameter RT model is (of course) susceptible to the same criticism as any other saturating permeation model with more than a minimum of two parameters. The value of the third parameter is largely determined by the non-Ohmic portion of the current–voltage curves. Hence, any proposals made based on a fit of this model to experimental permeation data should be viewed with skepticism until they are confirmed by an additional independent evidence. For example, the proposal that K^+ permeation through KcsA can be separated into two permeation modes⁴⁷ is somewhat speculative. However, this hypothesis is now supported by subsequent independent experimental evidence. Thompson and Begenisich came to a similar conclusion based solely on experiments of external blockage of *Shaker* K^+ channels by tetraethylammonium.⁵⁷ They concluded that at high concentrations the K^+ channel exhibits an $n = 3$ permeation mechanism (with $n - 1 = 2$ ions in the blocked state). Whereas at low concentrations, the channel exhibited an $n = 2$ mechanism (with $n - 1 = 1$ ion in the blocked state). Additional support for this permeation mode switching is also provided by binding experiments⁵⁸ and the observation that water permeation through KcsA also undergoes a transition at $[K^+] \cong 200$ mM (that might be correlated to the number ions in the $n - 1$ states of the channel).⁵⁹ These experimental results also provide support for the separate analysis of the low concentration permeation mode of the *Shaker* K^+ channel data in Figs. 9 and 10.

C. Connections with simulation and experiment

A key concept in the present theoretical framework is the notion of a one-dimensional permeation coordinate, which is used in Figs. 2 and 4 to represent the fractional permeation progress using electric potential energy as a metric. This representation is an essential feature of the present theoretical framework that suggests a way to analyze the permeation mechanism(s) observed in MD simulations by calculating the electric potential energy of the microstates observed during permeation. This representation should facilitate identification of the key states and the steps between them and should also facilitate identification and physical interpretation of the parameter χ directly from the MD simulation.

The A/D model also makes a network of kinetic and thermodynamic predictions that can be tested by MD simulations (assuming that they are truly representative of the real system), thereby providing the desired connection between the nanoscopic details of molecular simulations and experimental results from real ion channels.^{60,61} The most basic prediction of the A/D model is that the permeation process can be broken down into two steps, such as those shown in Fig. 5. A large group of simulations appear to be consistent with this prediction.^{6–15} Equation (7) through Eq. (22) makes

quantitative predictions that have not (as yet) been tested via simulation. Equation (7) encapsulates the model assumption that only two states of the selectivity filter need to be considered in the permeation process. Each of the four terms of the right-hand sides of Eqs. (8) and (9) represent the four possible association/dissociation events predicted by the model and how they depend on concentration, voltage, and the occupancy of the channel. Each of these four terms makes detailed nanoscopic predictions that can be tested via simulation. According to the model, the two parameters k_d and K_d (or alternatively k_d and k_a) can (in principle) be determined from equilibrium (zero-volt symmetric solution) simulations. Equation (18) predicts how the channel occupancy varies with both concentration and voltage. This is a key nanoscopic prediction of the model that can be directly tested via simulation (and used to estimate K_d).

Finally, in addition to predicting selectivity filter transitions that *do* occur during permeation (and how their relative rates depend upon experimental conditions), the model also predicts which potential transitions *do not* occur. For example, the factor θ_m in Eqs. (8) and (9) reflects the assumption that association *cannot* occur while the selectivity filter is in state n . This assumption is responsible for the saturating behavior observed experimentally and can also be tested by MD simulation.

D. Modeling outlook

The modeling of multi-ion single-file ion channels has a long history and many approaches have been investigated.³ The present approach provides models with as few as one or two adjustable parameters that are much simpler than traditional models that typically have many more adjustable parameters.^{3,4} The simplifying assumptions employed are all supported by experiment or recent molecular simulations and the resulting theoretical framework does not suffer from the problems identified by Syganow and von Kitzing.⁶² In particular: a constant electric field is not assumed; concerted motions of ions inside the channel are explicitly included; large barriers are not required to hold the ions in place; and the energy profile along the permeation coordinate depends explicitly on channel occupancy.

As a result of fitting the association barrier model to the MaxiK data of Schroeder and Hansen⁴⁵ we discovered that no association barrier is necessary to explain the experimental data. In addition to adding an unnecessary parameter, the association barrier also introduces an exponential dependence of the association rate on voltage that is not supported by permeation experiments at very high voltages.^{63–65} In the association-barrier model, there is nothing to mitigate this error and the association barrier is eliminated in the fit. However, for more complex site-based models, such as those that have been traditionally applied to K^+ channels,³ there are other processes (e.g., translocation and dissociation from single- and double-occupancy states) that also depend exponentially on voltage. The presence of multiple parameters that affect the voltage dependence exponentially can provide a mitigating effect, resulting in an apparently satisfactory fit.

These canceling errors may explain many of the well-known problems with traditional site-based models of ion channels including a lack of clear physical meaning for the fitted parameters.^{4,16,34,35}

In order to avoid new misconceptions, it is important that any new phenomenological models be made as simple as possible by applying the principle of Occam's Razor to shave off any unnecessary parameters, so that attention can be focused on the essential model assumptions and their qualitative consequences. Given the success of models with just two or three parameters, the value of models with 10–15 parameters is unclear. As the ultimate purpose of phenomenological modeling is to gain insight into how the experimental system works, fitted parameters should not be added just to improve graphical summarization of the experimental data.⁶⁶ Experimental deviations from the predicted behavior often serve as an essential guide to further insights.

IV. SUMMARY AND CONCLUSION

This paper addresses the question of how many steps are required to explain experimental permeation through ion channels. A simple theoretical framework has been used to develop one- and two-step models of permeation that are supported by MD simulations. These models were then compared with the published current–voltage data from both simulation and experiment.

The simplest single-file model is the one-step knock-on model originally proposed by Hodgkin and Keynes.²³ In recent MD simulations, the observed permeation mechanism was identified as resembling the Hodgkin and Keynes knock-on mechanism.¹⁴ In the present paper, these simulation results have been found to be consistent with an asymmetric version of the knock-on model that was developed using the present theoretical framework. The success of this asymmetric knock-on model in explaining the simulation data suggests that the super-Ohmic behavior observed in the simulations is the result of a highly voltage-dependent association step. However, this conclusion and the simulation data upon which it is based are not representative of permeation experiments through real channels, which typically exhibit Ohmic behavior at low voltages.

Recently, experimental data from the MaxiK K^+ channel were analyzed using a 15-parameter permeation model and the sub-Ohmic behavior observed above 50 mV was described as a saturation phenomenon.⁴⁵ In the present paper, these previously published experimental results have been compared with the predictions of the simplest (two-parameter symmetric) version of the A/D model that was developed using the present theoretical framework. This is the first-ever successful comparison of a two-step single-file permeation model with experiment and the fit to the positive-voltage data is quite remarkable for such a simple model. The model provides a very simple explanation for the observed experimental behavior including the transition to sub-Ohmic behavior above 50 mV. In contrast to the previous explanation of these phenomena,⁴⁵ the A/D model suggests that they are caused by an emptying out of the selectivity filter to state m .

A key simplifying assumption in the A/D model is that the association step does not depend on voltage. This assumption was tested here by developing an “association-barrier” variant of the A/D model and then fitting it to the same MaxiK permeation data. In the fitting process, the voltage-dependence was essentially removed from the association step, providing further support for the hypothesis that association is independent of voltage in the MaxiK channel.

The symmetric A/D model was also fitted to the positive-voltage *Shaker* permeation data³⁷ and the model successfully explained the Ohmic behavior at low voltages and the Michaelis–Menten saturating nature of the zero-volt conductance observed experimentally, indicating that the symmetric A/D model provides a reasonable first-order explanation of permeation through the *Shaker* channel at physiological concentrations. To investigate the asymmetry apparent in the experimental data, a (three-parameter asymmetric) version of the A/D model was developed using the present theoretical framework. This model includes a single parameter χ representing the asymmetry of the channel. From a fit to the positive-voltage data (that appear linear), it was discovered that the model could successfully *predict* a change to super-Ohmic behavior at negative voltages (and high concentrations). This successful prediction of a qualitative change in the behavior suggests that the model is capturing an essential asymmetry in the channel using just one parameter χ . As a final test of the asymmetric A/D model, it was fit to both the positive and negative current–voltage data and (as expected) the resulting fit at negative voltages was improved.

Despite the recent focus on atomistic simulations, traditional mathematical models are still an important tool for investigating permeation through ion channels. They provide much more than just a verbal description of permeation, a schematic diagram showing state transitions or even a movie showing K^+ ions actually passing through the selectivity filter. A mathematical model provides a valuable abstraction of the real system that postulates the key features required to explain the experimental behavior. In addition to providing a conceptual framework for understanding the existing experimental data, a good mathematical model also provides a network of testable hypotheses that can motivate future research. Inevitably, simple mathematical models will fail to explain experimental details, but even these differences will usually provide valuable insights into how the real system behaves.

While the A/D model investigated here is supported by a large group of MD simulations, there has been no systematic attempt to connect simulation with the model (and hence with experiment). At its most basic level, the model predicts the key permeation steps that can be observed in atomistic simulations including potential events that should not occur (such as association with a channel already in state n). Key simplifying assumptions of the model are: that association is independent of voltage and can be characterized by a first-order rate constant; that dissociation is an activated process that can be characterized by a voltage-independent rate constant and an electrical dissociation distance; that the permeation coordinate can be used to summarize the energetics of the permeation process; and that the electrical dissociation distance is determined by the fractional electric potential energy of state

n . In principle, all of these assumptions can be confirmed by MD simulations.

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