

## A permeation theory for single-file ion channels: Concerted-association/dissociation

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In a recent paper,<sup>1</sup> a kinetic model was proposed for permeation in open single-file ion channels containing either  $n$  or  $(n-1)$  ions in their selectivity filters. The rate-limiting step was assumed to be association and/or dissociation of the  $n$ th ion via (single ion)–(water molecule) exchanges. In this letter, a generalization of the model is proposed, wherein association/dissociation occurs via concerted motion of all  $n$  ions (and any water molecules trapped between them).

Numerous molecular dynamics (MD) simulations have suggested that entry of a third  $K^+$  ion into the selectivity filter of the KcsA ion channel is accompanied by movement of the two  $K^+$  ions already in the selectivity filter.<sup>2,3</sup> Similar concerted-association/dissociation transitions have also been observed in Brownian dynamics simulations.<sup>2</sup> At first glance, this concerted motion appears to be quite different from the (single ion)–(water molecule) exchange mechanism of the recently proposed kinetic model,<sup>1</sup> because during (single ion)–(water molecule) exchange, the helper ions need not move. In this letter, the effects of incorporating concerted-association/dissociation into the kinetic model are investigated.

Figure 1(a) shows a simplified permeation mechanism motivated by the MD simulations of Bernèche and Roux.<sup>4</sup> This simplified permeation mechanism is comprised *entirely* of concerted motions during association/dissociation, and as such, it provides an extreme test case for analyzing the effect of concerted-association/dissociation within the kinetic approach. In this simplified model, the helper ions are assumed to be normally located in the middle of a doubly occupied selectivity filter. During association, the helper ions are displaced to the far end of the selectivity filter by the entering ion, and all  $n$  ions move in a concerted manner together with any water molecules trapped between them. The  $i$  and  $o$  states of the original kinetic model<sup>1</sup> are combined into a single lumped state  $m$  (where  $\theta_m = \theta_i + \theta_o$ ). If, as in the original model, it is assumed that the association step is accompanied by a decrease in the (electrochemical) potential of the system, then the association step occurs with a voltage-independent rate of  $\nu_a[S]\theta_m/2$ .<sup>5</sup>

During dissociation, all three ions move in a concerted manner together with any water molecules trapped between them. Hence, in the generalized kinetic model the electrical dissociation distance should be interpreted as the sum of the electrical distances moved by all ions while the (electrochemical) potential of system is increasing, i.e.,

$$\delta = \varepsilon_1 + \varepsilon_2 + \varepsilon_3, \quad (1)$$

where  $\varepsilon_i$  are the electrical distances traveled by each ion as the third ion dissociates into the bulk aqueous phase. Thus,  $ze_o\delta V$  once again represents the electrical work done on the system by the electric field during a dissociation event (where  $ze_o$  is the charge of a single ion).

In the simplified (symmetric selectivity filter<sup>6</sup>) model of Fig. 1(a), the electrical dissociation distance has a value of  $\delta = \frac{1}{2}$ , because the net effect of dissociation (and association) is to move a single ion halfway across the transmembrane potential.<sup>7</sup> The kinetic mechanism of Fig. 1(a) is thus formally identical to the original model,<sup>1</sup> but with just two adjustable parameters ( $\nu_a$  and  $k_d$ ). If the physical distances in Fig. 1(a) are proportional to the electrical distances,<sup>8</sup> then the helper ions move electrical distances of  $\varepsilon_1 = \varepsilon_2 = 1/6$ , and the substrate ion travels an electrical distance of  $\varepsilon_3 = 1/6$ .

Figure 1(b) shows a simplified representation of the concerted association/dissociation mechanism proposed by Morais-Cabral *et al.*<sup>9</sup> This mechanism represents a generalization of the mechanism in Fig. 1(a), because the helper ions must translocate to the far end of the selectivity filter before association of the  $n$ th ion can take place. During translocation, the two helper ions move electrical distances  $\zeta_1$  and  $\zeta_2$ , respectively, where

$$\ell = \zeta_1 + \zeta_2 \quad (2)$$

and all the electrical distances sum to unity, i.e.,

$$2\delta + \ell = 1. \quad (3)$$

The kinetic model of Fig. 1(b) is formally identical to the original model<sup>1</sup> having the same three adjustable parameters  $\nu_a$ ,  $k_d$ , and  $\delta$ .

If the physical distances in Fig. 1(b) are proportional to the corresponding electrical distances, then  $\varepsilon_1 = \varepsilon_2 = \varepsilon_3 = 1/10$  and  $\zeta_1 = \zeta_2 = 2/10$ . The experimental value of  $\delta = 0.18^{1,10,11}$  is significantly smaller than  $\delta = 3/10$ . However, smaller values of  $\delta$  can be inferred, if (on average) the ions travel shorter electrical distances while pushing the third ion out of the channel.

Figure 2 shows alternate permeation mechanisms suggested by the simulations of Bernèche and Roux.<sup>4</sup> In these mechanisms, association/dissociation of the  $n$ th ion occurs via (single ion)–(water molecule) exchanges at the outermost end(s) of the selectivity filter (consistent with the original permeation model<sup>1</sup>). While the kinetic model of Fig. 2(a) is identical to the original model,<sup>1</sup> the kinetic model of Fig. 2(b) includes an additional state  $m$ . It is straightforward to

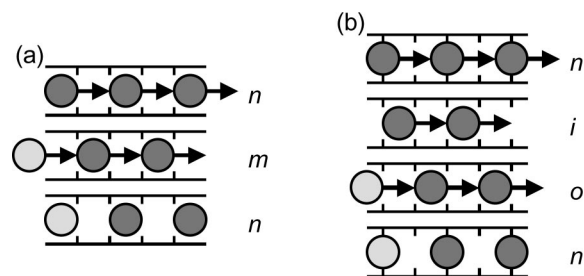


FIG. 1. Permeation mechanisms including concerted-motion association/dissociation steps in an ion channel selectivity filter. Arrows indicate ionic movement in a sequence of steps that result in the net transfer of a single ion (all steps are reversible). (a) Simplified mechanism motivated by the MD simulations of Bernèche and Roux (Ref. 4). Dissociation ( $n \rightarrow m$ ), two ions help push out the third ion. Association ( $m \rightarrow n$ ), the entering ion displaces the helper ions toward the far end of the filter. (b) Mechanism proposed by Morais-Cabral *et al.*—Ref. 9. ( $n \rightarrow i$ ), helper ions aid in concerted dissociation. ( $i \rightarrow o$ ), concerted translocation of the helper ions. ( $o \rightarrow n$ ), entering ion displaces the helper ions during concerted association.

show that the presence of this additional state modifies the voltage dependence of  $K_M$ , but that the theory retains “universal”  $j^* - c^*$  behavior, and that it appears to fit the experimental data equally well. Further comparison of this model with experiment is planned in future work.

The shaking stack model of Schumaker,<sup>12</sup> included a concerted translocation step similar to that of the recent model,<sup>1</sup> and association of the  $n$ th ion required a vacant ion binding site,<sup>12</sup> analogous to the (single ion)–(water molecule) exchanges of the previous model.<sup>1</sup> In both these prior models, the helper ions did not move during dissociation,

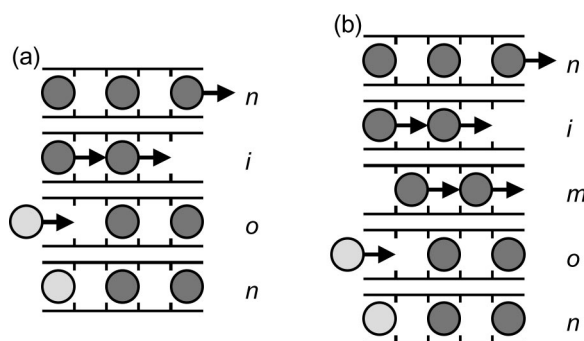


FIG. 2. Permeation mechanisms including (single ion)–(water molecule) exchange association/dissociation steps, motivated by the MD simulations of Bernèche and Roux (Ref. 4). Arrows indicate ionic movement in a sequence of steps that result in the transmembrane transfer of a single ion (all steps are reversible). (a) Mechanism similar to that proposed in the previous article—Ref. 1. ( $n \rightarrow i$ ), dissociation occurs via (single ion)–(water molecule) exchange at the filter end. ( $i \rightarrow o$ ), concerted translocation of the helper ions. ( $o \rightarrow n$ ), association occurs via (water molecule)–(single ion) exchange at the filter end. (b) Mechanism with an intermediate translocation state. Helper ion translocation proceeds via two concerted steps ( $i \rightarrow m$ ) and ( $m \rightarrow o$ ).

and hence the electrical translocation distance  $\ell$  was related to the physical distance separating the outer ions in the  $n$ -occupied state.<sup>1,12</sup> As a result, reducing  $\ell$  to zero was an unphysical limit. However, once the concerted association/dissociation hypothesis is adopted, the electrical translocation distance  $\ell$  is no longer constrained by the physical distance between the outer ions [see Eq. (2)], and the physically meaningful range of  $\ell$  is extended down to zero ( $\delta$  up to  $\frac{1}{2}$ ).

The generalization proposed herein thus makes the original mathematical model consistent with many microscopically distinguishable permeation mechanisms.<sup>13</sup> As a result, model parameters fitted to experimental data should be considered as *ensemble average* values (including all possible permeation mechanisms). Clearly, the microscopic details of permeation are more complex than any of the simplified schematic representations of Figs. 1 and 2. Nevertheless, it appears that the proposed kinetic model provides a useful “lumped” representation of the real kinetic processes. The model requires just three physically meaningful parameters, which can be determined in a straightforward manner from permeation experiments.<sup>1,11</sup>

In light of the concerted association/dissociation generalization proposed in this letter, it is suggested that the electrical dissociation distance  $\delta$  should be interpreted as the *ensemble average* sum of the electrical distances traveled by all  $n$  ions during association/dissociation. Further validation of the model will require asymmetric-solution and tracer-counter permeation experiments, and detailed comparison with atomistic simulations.<sup>1</sup>

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<sup>3</sup>However, details of the concerted motion appear to depend on the interatomic potential functions and the boundary conditions used in the simulations.

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<sup>5</sup>The factor of 1/2 arises because of the lumping of the two original states.

<sup>6</sup>Extension of these models to asymmetric selectivity filters is in progress.

<sup>7</sup>There is no translocation step, and hence  $\ell = 0$  by definition.

<sup>8</sup>This assumption implies that the channel selectivity filter is nonconductive while it remains in any of the discrete states of the kinetic model, and that the voltage drop occurs entirely across the selectivity filter.

<sup>9</sup>J. H. Morais-Cabral, Y. Zhou, and R. MacKinnon, Nature (London) **414**, 37 (2001).

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<sup>13</sup>For example, the mechanisms of Figs. 1 and 2 differ in both the resting positions of the ions and the concertedness of association/dissociation.