

# Equivalence of two approaches for modeling ion permeation through a transmembrane channel with an internal binding site

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Ion permeation through transmembrane channels has traditionally been modeled using two different approaches. In one approach, the translocation of the permeant ion through the channel pore is modeled as continuous diffusion and the rate of ion transport is obtained from solving the steady-state diffusion equation. In the other approach, the translocation of the permeant ion through the pore is modeled as hopping along a discrete set of internal binding sites and the rate of ion transport is obtained from solving a set of steady-state rate equations. In a recent work [Zhou, *J. Phys. Chem. Lett.* **1**, 1973 (2010)], the rate constants for binding to an internal site were further calculated by modeling binding as diffusion-influenced reactions. That work provided the foundation for bridging the two approaches. Here we show that, by representing a binding site as an energy well, the two approaches indeed give the same result for the rate of ion transport. © 2011 American Institute of Physics. [doi:10.1063/1.3575585]

## I. INTRODUCTION

The modeling of ion permeation through transmembrane channels is a constant theme of molecular biophysics. Ion permeation through transmembrane channels has traditionally been modeled using two different approaches.<sup>1–4</sup> In one approach, the translocation of the permeant ion through the channel pore is modeled as continuous diffusion and the rate of ion transport is obtained from solving the steady-state diffusion equation.<sup>2,4,5</sup> In the other approach, the translocation of the permeant ion through the pore is modeled as hopping along a discrete set of internal binding sites and the rate of ion transport is obtained from solving a set of steady-state rate equations.<sup>2,3,6,7</sup> As Levitt<sup>4</sup> pointed out, “there has been a long-standing debate centered on the issue of reaction-rate ‘versus’ continuum theory.” In the continuous-diffusion approach, quantities such as the diffusion constant and the potential of mean force can be calculated from a microscopic representation of the ion-permeation system. On the other hand, in the rate-equation approach, the rate constants involved have been treated as adjustable parameters; this limitation has been a main source of criticism of the rate-equation approach.<sup>2,4</sup> Nevertheless, the rate-equation approach has been defended.<sup>3</sup>

A recent study<sup>8</sup> has injected new life into the rate-equation approach, in which the rate constants for binding to an internal site were further calculated by modeling binding as diffusion-influenced reactions. This allows the rate-equation approach to represent the ion-permeation system at the same level as the continuous-diffusion approach. An interesting question then arises: will the two approaches yield the same result for the rate of ion transport? The purpose of this paper is to show that this is indeed the case.

## II. PROOF OF EQUIVALENCE OF TWO APPROACHES

We first note a related paper by Bezrukov *et al.*,<sup>9</sup> who showed that the rate of ion transport through a singly occupied channel obtained from the continuous-diffusion approach can be mapped to that of a model consisting of two fictitious sites, located at the entrance and exit of the channel pore. The transition rates between the two sites are given by the reciprocals of the mean first passage times to reach the exit from the entrance and *vice versa*. The mapping holds for an arbitrary potential of mean force for the permeant ion inside the pore. (A somewhat related mapping between the two approaches when double occupancy is allowed in the channel was presented by Levitt.<sup>10</sup>) Bezrukov *et al.* also showed that the rate of ion transport for a model consisting of three or more internal sites can also be mapped to that of a model consisting of just the two terminal sites. The mapping to a two-site model does not constitute an independent calculation of the rate of ion transport, but a recast of the result from a more fundamental approach (based on either a continuous-diffusion or a multisite description).

Our focus is a transmembrane channel with a single internal binding site (Fig. 1). Below we first summarize the results for the rate of ion transport from two *independent* calculations. One calculation<sup>8</sup> is based on the rate-equation approach, but with the rate constants explicitly derived from modeling the binding to the internal site as diffusion-influenced reactions. The other calculation<sup>5</sup> is from the continuous-diffusion approach. We then recognize that, when the latter is specialized to a potential of mean force representing a single internal binding site, the two calculations give the same result for the rate of ion transport. This proof of the equivalence of the two approaches is thus totally different from the mapping of Bezrukov *et al.*<sup>9</sup>

We start with a summary of the main results in our recent work.<sup>8</sup> Let the rate constants for binding to the internal site

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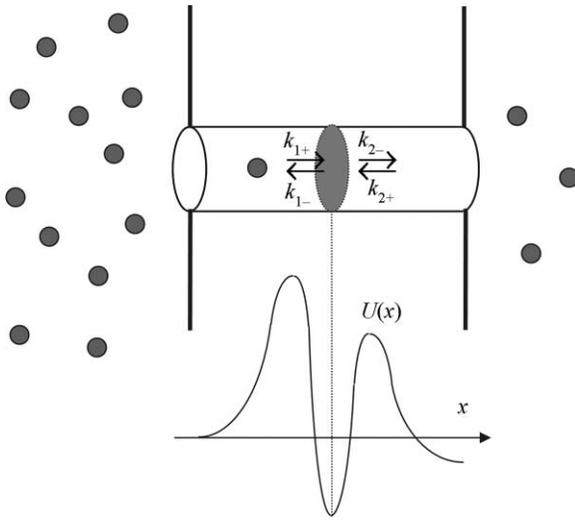


FIG. 1. A transmembrane ion channel with an internal binding site. The binding site can be represented as an energy well. The dashed vertical line indicates the correspondence of the two representations.

from the bulk solutions on the two sides of the membrane be  $k_{1+}$  and  $k_{2+}$ , and the corresponding unbinding rate constants be  $k_{1-}$  and  $k_{2-}$ . If the ion concentrations in the bulk solutions on the two sides of the membrane are  $C_i$ ,  $i = 1$  and  $2$ , the rate of ion transport is<sup>6</sup>

$$I = \frac{k_{1+}k_{2-}C_1 - k_{2+}k_{1-}C_2}{k_{1-} + k_{2-} + k_{1+}C_1 + k_{2+}C_2}. \quad (1)$$

The internal site can have at most one ion bound, but there is no restriction on the number of ions in the rest of the pore. Throughout the paper, symbols with indices 1 and 2 refer quantities relevant for the two opposite ends of the channel pore; for concreteness, the end with index 1 will be referred the pore entrance and the end with index 2 will be referred to as the pore exit.

Let the potential of mean force of a permeant ion inside the pore be  $U(x)$ , where  $x$  denotes the coordinate along the pore axis. Let the coordinates of the pore entrance, exit, and the internal binding site be  $0$ ,  $L$ , and  $L_i$ , respectively. We choose the reference of the potential of mean force such that  $U(0) = 0$ , and denote as  $\Delta U$  the change in  $U(x)$  across the membrane:  $\Delta U = U(L) - U(0)$ . A nonzero  $\Delta U$  can be easily set up by a transmembrane voltage. The four rate constants satisfy the detailed balance condition:

$$\frac{k_{1+}/k_{1-}}{k_{2+}/k_{2-}} = e^{-\Delta U/k_B T}, \quad (2)$$

where  $k_B$  is the Boltzmann constant and  $T$  the absolute temperature. Hereafter we use  $\beta$  to denote  $(k_B T)^{-1}$ .

A theoretical framework for calculating the rate constants of binding to an internal site has been developed previously.<sup>11</sup> By applying the so-called constant-flux approximation<sup>12</sup> on the channel entrance or exit,  $k_{i+}$  can be calculated as

$$\frac{1}{k_{i+}} = \frac{1}{k_{bi}} + \frac{1}{k_{pi}} \quad (3)$$

by solving two simpler problems. [A more rigorous derivation of Eq. (3) is to be published by Berezhkovskii, Szabo, and

Zhou.] The first is the “exterior” problem, for the diffusion-controlled binding of ions from the bulk solution on either side to the pore entrance or exit. This binding rate constant is denoted as  $k_{bi}$ . For a circular pore entrance or exit with radius  $a$ , one has  $k_{bi} = 4Da$ ,<sup>13</sup> where  $D$  is the diffusion constant of the ion. The second is the “interior” problem, for the diffusion-influenced binding of ions in the pore to the internal site. For a pore with uniform cross section  $\sigma$ , the binding rate constants for the interior problem are<sup>8</sup>

$$\frac{1}{k_{p1}} = \frac{1}{D\sigma} \int_0^{L_i} dx e^{\beta U(x)}, \quad (4a)$$

$$\frac{1}{k_{p2}} = \frac{e^{-\beta \Delta U}}{D\sigma} \int_{L_i}^L dx e^{\beta U(x)}. \quad (4b)$$

We now explicitly represent the internal binding site as an energy well (Fig. 1);<sup>14</sup> now  $L_i$  is at the bottom of the well. To be concrete, let the internal site be confined to the region  $[L_-, L_+]$ , with  $L_- < L_i < L_+$ .<sup>15</sup> We can then write the equilibrium constants for binding to the internal site in terms of the potential of mean force:

$$\frac{k_{1+}}{k_{1-}} = \sigma \int_{L_-}^{L_+} dx e^{-\beta U(x)}, \quad (5a)$$

$$\frac{k_{2+}}{k_{2-}} = \sigma e^{\beta \Delta U} \int_{L_-}^{L_+} dx e^{-\beta U(x)}. \quad (5b)$$

Note that, by taking the ratio of Eqs. (5a) and (5b), Eq. (2) is obtained.

With Eqs. (3)–(5b), we can now write the rate of ion transport as

$$I = \frac{p_{1 \rightarrow 2} k_{b1} C_1 - p_{2 \rightarrow 1} k_{b2} C_2}{1 + k_{b1} \tau_1 C_1 + k_{b2} \tau_2 C_2}, \quad (6)$$

where

$$p_{1 \rightarrow 2} = \frac{\kappa_{b2} e^{-\beta \Delta U}}{\kappa_{b1} + \kappa_{b2} e^{-\beta \Delta U} + (\kappa_{b1} \kappa_{b2} / D) e^{-\beta \Delta U} \int_0^L dx e^{\beta U(x)}}, \quad (7a)$$

$$p_{2 \rightarrow 1} = \frac{\kappa_{b1}}{\kappa_{b1} + \kappa_{b2} e^{-\beta \Delta U} + (\kappa_{b1} \kappa_{b2} / D) e^{-\beta \Delta U} \int_0^L dx e^{\beta U(x)}}, \quad (7b)$$

$$\tau_1 = \frac{\int_{L_-}^{L_+} dx e^{-\beta U(x)} \left[ 1 + (\kappa_{b2} / D) e^{-\beta \Delta U} \int_{L_i}^L dy e^{\beta U(y)} \right]}{\kappa_{b1} + \kappa_{b2} e^{-\beta \Delta U} + (\kappa_{b1} \kappa_{b2} / D) e^{-\beta \Delta U} \int_0^L dx e^{\beta U(x)}}, \quad (7c)$$

$$\tau_2 = \frac{\int_{L_-}^{L_+} dx e^{-\beta U(x)} \left[ 1 + (\kappa_{b1} / D) \int_0^{L_i} dy e^{\beta U(y)} \right]}{\kappa_{b1} + \kappa_{b2} e^{-\beta \Delta U} + (\kappa_{b1} \kappa_{b2} / D) e^{-\beta \Delta U} \int_0^L dx e^{\beta U(x)}}. \quad (7d)$$

In Eqs. (7a)–(7d), we have further defined

$$\kappa_{bi} = \frac{k_{bi}}{\sigma}, \quad (8)$$

which are the rate constants for escaping to the bulk solution from the pore entrance or exit.<sup>16</sup>

Equation (6) is nearly identical to the result of Berezhkovskii and Bezrukov<sup>5</sup> obtained from the continuous-diffusion approach, with the restriction that at most one ion is found in the entire pore. Their result is (see Ref. 9)

$$I = \frac{p_{1 \rightarrow 2} k_{b1} C_1 - p_{2 \rightarrow 1} k_{b2} C_2}{1 + k_{b1} \bar{t}_1 C_1 + k_{b2} \bar{t}_2 C_2}, \quad (9)$$

where  $p_{1 \rightarrow 2}$  and  $p_{2 \rightarrow 1}$ , given by Eqs. (7a) and (7b), were interpreted as the probabilities that an ion entering the pore through the entrance leaves the pore through the exit and *vice versa*, and  $\bar{t}_i$  are the average times in the pore for an ion entering it through the entrance and exit, respectively. The latter are given by

$$\bar{t}_1 = \frac{\int_0^L dx e^{-\beta U(x)} \left[ 1 + (\kappa_{b2}/D) e^{-\beta \Delta U} \int_x^L dy e^{\beta U(y)} \right]}{\kappa_{b1} + \kappa_{b2} e^{-\beta \Delta U} + (\kappa_{b1} \kappa_{b2}/D) e^{-\beta \Delta U} \int_0^L dx e^{\beta U(x)}}, \quad (10a)$$

$$\bar{t}_2 = \frac{\int_0^L dx e^{-\beta U(x)} \left[ 1 + (\kappa_{b1}/D) \int_0^x dy e^{\beta U(y)} \right]}{\kappa_{b1} + \kappa_{b2} e^{-\beta \Delta U} + (\kappa_{b1} \kappa_{b2}/D) e^{-\beta \Delta U} \int_0^L dx e^{\beta U(x)}}. \quad (10b)$$

In general,  $\bar{t}_i$  differ from  $\tau_i$ . However, for a deep well in the potential of mean force that represents the single internal binding site, they are essentially identical. To see this, we note that the integral in the numerator of Eq. (10a) over  $x$  is dominated by the deep well. Therefore, the lower limit,  $x$ , of the integral over  $y$  can be replaced by  $L_i$ , the location of the well bottom; and the lower and upper limits of the integral over  $x$  can be replaced by  $L_-$  and  $L_+$ , the lower and upper bounds of the internal site, respectively. Consequently  $\bar{t}_1 \approx \tau_1$ . Similarly,  $\bar{t}_2 \approx \tau_2$ . We have thus shown that, for a binding site represented as a deep energy well, the rate-equation approach and the continuous-diffusion approach give the same results for the average times in the pore and hence for the rate of ion transport.

### III. ILLUSTRATIVE CALCULATIONS

We now give some explicit results for the two approaches of modeling ion permeation through a channel. Here the potential of mean force of the permeant ion in the pore has the form:

$$U(x) = Fx + U_i(x), \quad (11)$$

where the first term models the effect of a transmembrane voltage, and  $U_i(x)$  features a square well that is possibly bordered by square barriers on the two sides (Fig. 2):

$$U_i(x) = \begin{cases} 0 & \text{for } 0 < x < L_- - \Delta_1 \\ U_b & \text{for } L_- - \Delta_1 < x < L_- \\ -U_w & \text{for } L_- < x < L_+ \\ U_b & \text{for } L_+ < x < L_+ + \Delta_2 \\ 0 & \text{for } L_+ + \Delta_2 < x < L \end{cases}.$$

We assume that  $L_i$  is midway between  $L_-$  and  $L_+$ :  $L_i = (L_- + L_+)/2$ . For notational simplicity we denote  $L_+ - L_-$  as  $\Delta$ . The integrals of Eqs. (7a)–(7d) and (10a) and (10b) can be

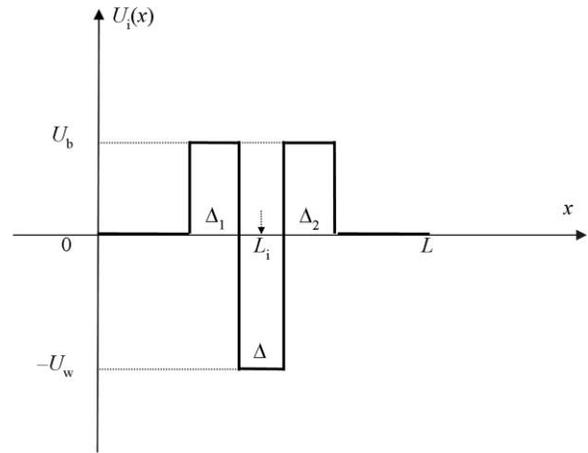


FIG. 2. The “intrinsic” part of the potential of mean force used for illustrative calculations. The barriers are absent when  $U_b = 0$ .

obtained analytically. The results for  $F = 0$  and  $U_b = 0$  are

$$\int_0^L dx e^{\beta U(x)} = L - \Delta + e^{-\beta U_w} \Delta,$$

$$\int_{L_-}^{L_+} dx e^{-\beta U(x)} = e^{\beta U_w} \Delta,$$

$$\int_{L_-}^{L_+} dx e^{-\beta U(x)} \int_{L_i}^L dy e^{\beta U(y)} = e^{\beta U_w} (L - L_+) \Delta + \frac{\Delta^2}{2},$$

$$\int_0^L dx e^{-\beta U(x)} = e^{\beta U_w} \Delta + (L - \Delta),$$

$$\int_0^L dy e^{-\beta U(x)} \int_x^L dy e^{\beta U(y)} = e^{\beta U_w} (L - L_+) \Delta + \frac{\Delta^2}{2} + \frac{(L - \Delta)^2}{2} + e^{-\beta U_w} L_- \Delta.$$

The corresponding results for  $F \neq 0$  and  $U_b \neq 0$  are not given here.

In Fig. 3 we compare  $\tau_1$  and  $\bar{t}_1$ . When the well depth  $U_w$  is small,  $\tau_1$  underestimates  $\bar{t}_1$ , but when  $\beta U_w$  reaches  $\sim 5$ , there is very good agreement between  $\tau_1$  and  $\bar{t}_1$ . For example, at  $F = 0$  and  $U_b = 0$ ,  $\tau_1$  is smaller than  $\bar{t}_1$  only by 6%. Energy barriers bordering the energy well slightly improve the agreement between  $\tau_1$  and  $\bar{t}_1$  [Fig. 3(a)], while an uphill membrane voltage (i.e.,  $F > 0$ ) magnifies the discrepancy between  $\tau_1$  and  $\bar{t}_1$  [Fig. 3(b)] (a downhill membrane voltage has essentially no effect on  $\tau_1/\bar{t}_1$ ).

While there is significant discrepancy between  $\tau_i$  and  $\bar{t}_i$  when  $\beta U_w < 5$ , the discrepancy has essentially no effect on the predicted rate of ion transport. This is because  $\tau_i$  and  $\bar{t}_i$  enter the expression for the rate of ion transport in the form of  $1 + k_{b1} \bar{t}_1 C_1 + k_{b2} \bar{t}_2 C_2$  [see Eqs. (6) and (9)]. When  $\beta U_w < 5$ , the average times that the permeant ion spent in the pore become very short; hence  $k_{b1} \bar{t}_1 C_1 + k_{b2} \bar{t}_2 C_2 \ll 1$ . Consequently, despite the discrepancy between  $\tau_i$  and  $\bar{t}_i$ , one would have  $1 + k_{b1} \tau_1 C_1 + k_{b2} \tau_2 C_2 \approx 1 + k_{b1} \bar{t}_1 C_1 + k_{b2} \bar{t}_2 C_2 \approx 1$ . The close agreement between  $1 + k_{b1} \tau_1 C_1 + k_{b2} \tau_2 C_2$  and

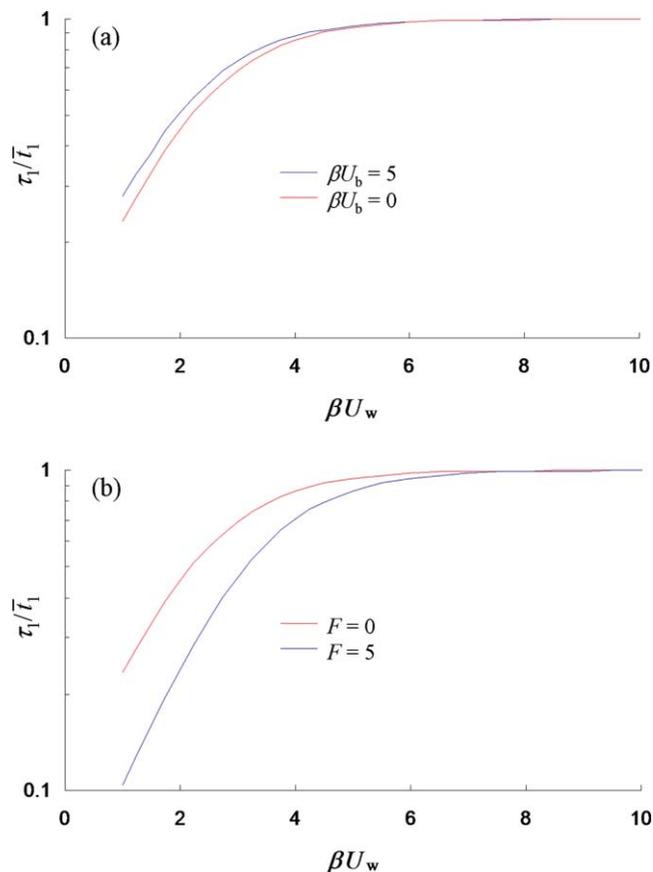


FIG. 3. Ratio of the rate-equation result  $\tau_1$  and the continuous-diffusion result  $\bar{\tau}_1$ . (a) Effect of  $U_b$  when  $F = 0$ . (b) Effect of  $F$  when  $U_b = 0$ . The other parameters are:  $L_i/L = 0.5$ ;  $\Delta_1/L = \Delta_2/L = \Delta/L = 0.1$ ;  $\kappa_{b1} = \kappa_{b2}$ ; and  $\kappa_{b2}L/D = 10$ .

$1 + k_{b1}\bar{\tau}_1C_1 + k_{b2}\bar{\tau}_2C_2$  over the full range of the well depth is shown in Fig. 4 for  $C_1 \sim 1$  mM and  $C_2 \rightarrow 0$ .

In short, the average times in the pore calculated by the rate-equation approach and the continuous-diffusion approach differ for a shallow energy well but agree well for a deep energy well. However, regardless of the depth of the energy well, our rate-equation approach predicts essentially

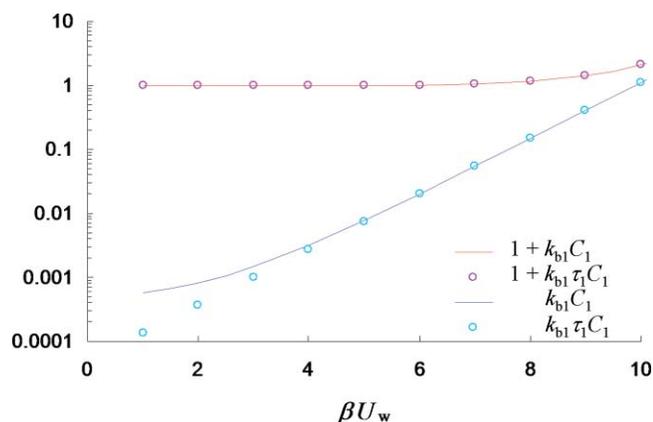


FIG. 4. Negligible effect of the discrepancy between  $\tau_1$  and  $\bar{\tau}_1$  on the rate of ion transport. Parameters are:  $F = 0$ ;  $U_b = 0$ ;  $\sigma LC_1 = 10^{-3}$ , corresponding to  $C_1$  at  $\sim 1$  mM. Very similar results are obtained for other model parameters.

the same rate of ion transport as the continuous-diffusion approach.

#### IV. CONCLUDING REMARKS

We have shown that the two different approaches for modeling ion permeation through a transmembrane channel with an internal binding site, one based on continuous diffusion and the other based on rate equations, give the same result for the rate of ion transport. In contrast to previous uses of the rate-equation approach, in which the rate constants were treated as adjustable parameters, we calculate the rate constants by modeling binding to the internal site as diffusion-influenced reactions. In our rate-equation approach the ion-permeation system is thus represented at the same level as the continuous-diffusion approach.

The ion channel studied here is restricted to single ion occupancy and has a pore cross section and an ion diffusion constant that are independent of  $x$ . Similar results for both approaches can also be obtained when  $\sigma$  and  $D$  vary with  $x$ . Again, the two independent calculations lead to the same rate of ion transport. The equivalence of the rate-equation and continuous-diffusion approaches is thus a general conclusion for channels with single ion occupancy. Thereby in this case we have settled the long-standing debate over the two approaches with a draw.

While we have proven the mathematical equivalence of the continuous-diffusion approach and the rate-equation approach, for channels where internal binding sites have been unequivocally identified the rate-equation approach may be uniquely able to provide physical insight into the mechanisms of ion permeation.<sup>3</sup> A case in point is the influenza M2 proton channel, in which a histidine tetrad is the obligatory binding site for the permeant proton.<sup>8,17-20</sup> Moreover, in contrast to the continuous-diffusion approach, where the channel is assumed to be static, our rate-equation approach has allowed for the account of channel dynamics in modeling ion permeation, by including gating dynamics in the calculation of the rate constants for binding to the internal site.<sup>8,20</sup> Consequently the proton conductance activity of the M2 channel has been quantitatively rationalized by the structure and dynamics of the channel protein.<sup>20,21</sup>

The focus of the present study is the rate of ion transport, which is determined by  $\bar{\tau}_i$ , the average times in the pore for an ion entering it through the entrance and exit. A full description of the ion translocation dynamics requires not just these average times but the distributions of the times in the pore for four kinds of ions: entering through the entrance and leaving through the entrance, entering through the entrance and leaving through the exit, entering through the exit and leaving through the entrance, and entering through the exit and leaving through the exit.<sup>22</sup> Effectively, we have shown that our rate-equation approach predicts well the first moments,  $\bar{\tau}_i$ , of these distribution functions. It will be interesting to see how well higher order moments are predicted.

The two approaches for modeling ion permeation considered here are an example of the dichotomy between continuous and discrete models that manifests in other problems of molecular biophysics.<sup>23</sup> In particular, motor proteins

have been modeled as either stepping through a series of discrete chemical states or as continuous diffusion along a periodic track.<sup>24</sup> Similarly, in ligand binding coupled to conformational change, the motion in conformational space can be either modeled as continuous diffusion or as transition between discrete conformational states.<sup>25</sup> The general conclusion seems to be that, when the energy surface in the continuous space contains deep wells such that the particle (ion, motor protein, receptor, etc.) spends most of its time in these wells, a discrete model will be appropriate. We can thus anticipate that, for channels with multiple ion occupancy, the rate-equation approach will be equivalent to the continuous-diffusion approach when the maximum number of allowed ions is the same as the number of deep wells in the potential of mean force of the permeant ion inside the pore. However, the case of multiple ion occupancy is complicated by ion-ion interactions,<sup>10</sup> and possible connection there between the continuous-diffusion and rate-equation approaches deserves close scrutiny.

## ACKNOWLEDGMENTS

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<sup>1</sup>K. Cooper, E. Jakobsson, and P. Wolynes, *Prog. Biophys. Mol. Biol.* **46**, 51 (1985).

- <sup>2</sup>D. G. Levitt, *Annu. Rev. Biophys. Biophys. Chem.* **15**, 29 (1986).  
<sup>3</sup>C. Miller, *J. Gen. Physiol.* **113**, 783 (1999).  
<sup>4</sup>D. G. Levitt, *J. Gen. Physiol.* **113**, 789 (1999).  
<sup>5</sup>A. M. Berezhkovskii and S. M. Bezrukov, *Chem. Phys.* **319**, 342 (2005).  
<sup>6</sup>P. Lauger, *Biophys. J.* **47**, 581 (1985).  
<sup>7</sup>A. B. Kolomeisky, *Phys. Rev. Lett.* **98**, 048105 (2007).  
<sup>8</sup>H.-X. Zhou, *J. Phys. Chem. Lett.* **1**, 1973 (2010).  
<sup>9</sup>S. M. Bezrukov, A. M. Berezhkovskii, and A. Szabo, *J. Chem. Phys.* **127**, 115101 (2007).  
<sup>10</sup>D. G. Levitt, *Biophys. J.* **37**, 575 (1982).  
<sup>11</sup>H.-X. Zhou, *J. Chem. Phys.* **108**, 8146 (1998).  
<sup>12</sup>D. Shoup, G. Lipari, and A. Szabo, *Biophys. J.* **36**, 697 (1981).  
<sup>13</sup>T. L. Hill, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4918 (1975).  
<sup>14</sup>The permeant ion may enter the well either through a downhill energy landscape or after crossing an energy barrier.  
<sup>15</sup>Even with the new details in representing the internal binding site, Eq. (4) continues to be valid for the binding rate constants of the interior problem. See H.-X. Zhou, *Q. Rev. Biophys.* **43**, 219 (2010).  
<sup>16</sup>S. M. Bezrukov, A. M. Berezhkovskii, M. A. Pustovoit, and A. Szabo, *J. Chem. Phys.* **113**, 8206 (2000).  
<sup>17</sup>J. D. Lear, *FEBS Lett.* **552**, 17 (2003).  
<sup>18</sup>M. Yi, T. A. Cross, and H. X. Zhou, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13311 (2009).  
<sup>19</sup>A. L. Polishchuk, J. D. Lear, C. Ma, R. A. Lamb, L. H. Pinto, and W. F. Degrad, *Biochemistry* **49**, 10061 (2010).  
<sup>20</sup>H.-X. Zhou, *Biophys. J.* **100**, 912 (2011).  
<sup>21</sup>H.-X. Zhou, "Mechanistic insight into the H<sub>2</sub>O/D<sub>2</sub>O isotope effect in the proton transport of the influenza virus M2 protein," *BMC Biophys* (submitted).  
<sup>22</sup>A. M. Berezhkovskii and S. M. Bezrukov, *Phys. Rev. Lett.* **100**, 038104 (2008).  
<sup>23</sup>H. Qian, *Protein Sci.* **11**, 1 (2002).  
<sup>24</sup>H. Qian, *J Phys.: Condens. Matter* **17**, S3783 (2005).  
<sup>25</sup>L. Cai and H.-X. Zhou, *J. Chem. Phys.* **134**, 105101 (2011).