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# Stochastic diffusion model of multistep activation in a voltage-dependent K channel

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The energy barrier to the activated state for the S4 voltage sensor of a K channel is dependent on the electrostatic force between positively charged S4 residues and negatively charged groups on neighboring segments, the potential difference across the membrane, and the dielectric boundary force on the charged residues near the interface between the solvent and the low dielectric region of the membrane gating pore. The variation of the potential function with transverse displacement and rotation of the S4 sensor across the membrane may be derived from a solution of Poisson's equation for the electrostatic potential. By approximating the energy of an S4 sensor along a path between stationary states by a piecewise linear function of the transverse displacement, the dynamics of slow activation, in the millisecond range, may be described by the lowest frequency component of an analytical solution of interacting diffusion equations of Fokker–Planck type for resting and barrier regions. The solution of the Smoluchowski equations for an S4 sensor in an energy landscape with several barriers is in accord with an empirical master equation for multistep activation in a voltage-dependent K channel. © 2010 American Institute of Physics. [doi:10.1063/1.3368602]

## I. INTRODUCTION

The increase in the conductance of voltage-dependent K and Na channels with membrane depolarization is fundamental to the propagation of the nervous impulse. The opening of a voltage-dependent ion channel is dependent on the conformation of a sensor which may undergo transitions between resting and activated states with a rate that is dependent on the membrane potential.<sup>1</sup> The experimental data from the recording of macroscopic ionic current as well as single channel and gating current from a *Shaker IR* K channel have been described by kinetic models where the opening of the channel requires two or three transitions for each of the four subunits as well as one or more concerted transitions.<sup>2,3</sup>

Each subunit is comprised of the transmembrane segments S1-S4, the voltage-sensing domain (VSD), and segments S5-S6, which form the ion-conducting pore domain.<sup>4</sup> The external four positively charged arginine residues (R1-R4) that occur at every third position of the S4 segment are the principal gating charges that move in a helical path across the membrane in response to a change in voltage, and are stabilized by interaction with negatively charged amino acids in S1-S3 and lipid phosphate groups. The arginine and lysine residues on S4 move within aqueous regions that are formed in the VSD from internal and external crevices between other protein segments, separated by an energy barrier and a focused electric field of approximately 10 Å thickness, referred to as a gating or omega pore<sup>5</sup> (see Fig. 1). Mutating specific arginines on the voltage sensor to smaller uncharged residues permits a proton or cation flux through the VSD when the channel is in an activated or deactivated state.<sup>6,7</sup>

Transmembrane movement of the S4 segment requires the attached residues to sequentially translocate through the gating pore, and therefore the process is compatible with the transporter and helical screw models<sup>7-9</sup> as well as the crystal structure of a K channel.<sup>10</sup> The transition of each S4 residue through the membrane requires sufficient energy to overcome a barrier that is dependent on the Coulomb force between positively charged residues on S4 and the negatively charged residues on neighboring helices,<sup>8,9,11,12</sup> the dielectric boundary force,<sup>13,14</sup> and the electric field between the internal and external aqueous crevices.<sup>15</sup> The energy barrier to the activated state is described by a potential function which may be derived from a solution of Poisson's equation for the electrostatic potential in the membrane and its environment (see Appendix).

Although the activation of a K channel is often described in terms of discrete transitions between a sequence of states, it may also be considered to be a continuous process determined by drift-diffusion equations with a linear potential, followed by a discrete opening transition.<sup>16</sup> Single channel behavior can be simulated by Brownian dynamics where the trajectories of the charged molecules are determined by the Langevin equation supplemented by continuum electrostatics.<sup>16</sup> Molecular dynamics simulations have been applied to the description of permeation within an ion channel, but generally are limited to durations which are small by comparison to the timescale of voltage-dependent activation.<sup>17</sup>

If the energy landscape has several large voltage dependent barriers, a numerical solution of the Smoluchowski equation for a Brownian gating particle includes relaxations that approximate those of a discrete state model.<sup>18</sup> An analytical solution of the stochastic diffusion equation also has an exponential relaxation for a large ramp potential, but as

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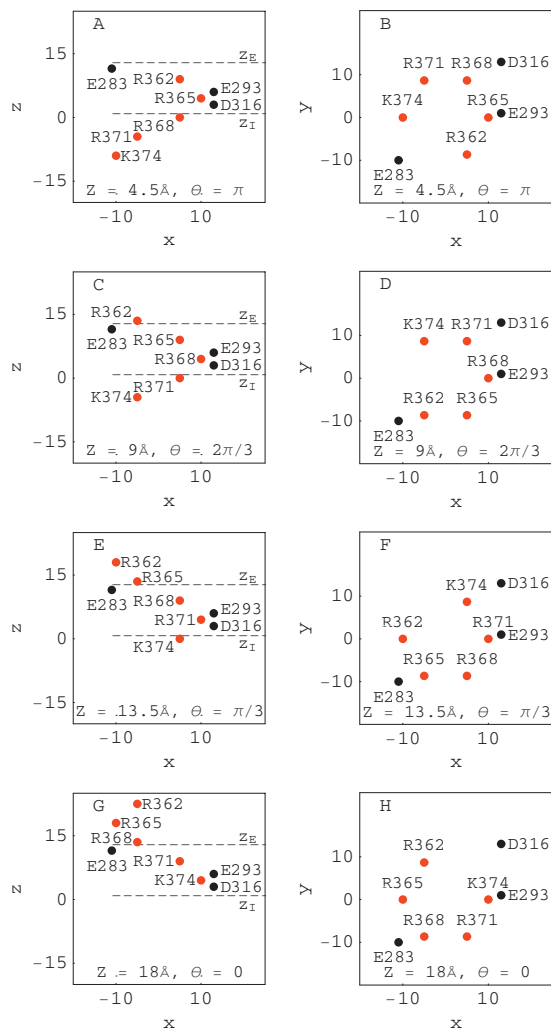


FIG. 1. Schematic diagram of the positive residue charges  $Q_l, l=1,5$  (red dot) on the S4 helix and the fixed negative charges (black dot), and the projection of their position in the  $x$ - $y$  and  $x$ - $z$  planes for the sequential translocation from the resting state at  $Z=4.5$  Å,  $\theta=\pi$  (A and B) to the intermediate states at  $Z=9$  Å,  $\theta=2\pi/3$  (C and D) and  $Z=13.5$  Å,  $\theta=\pi/3$  (E and F), and the activated state at  $Z=18$  Å,  $\theta=0$  (G and H), where  $z_I$  and  $z_E$  are the internal and external boundaries of the low dielectric region and the focused electric field, and  $Z$  and  $\theta$  represent the transverse displacement and rotation of the S4 sensor.

the voltage dependent barrier is attenuated, the response to a membrane depolarization is characterized by a power law for intermediate times, and therefore is in accord with the distribution of closed times recorded during a patch clamp of an ion channel.<sup>19–24</sup> If it is assumed that the diffusion barrier is sufficiently large at the entrance to the protein region of the gating pore, the activation process may be described by a rate equation for each membrane depolarization.<sup>25</sup>

In this paper, an expression for the energy of an S4 helix is derived which is dependent on the transverse displacement and rotation of the sensor within a gating pore. An analytical solution of the interacting Smoluchowski equations is obtained for the resting and potential barrier regions of an S4 segment, where the lowest frequency component represents the transition across the barrier and the higher frequencies are associated with the redistribution of the probability density within each region. The analysis is then applied to the activation of an S4 sensor in an energy landscape with sev-

eral potential barriers, where the analytical solution of the diffusion equations is in accord with an empirical transition-state model that describes multistep activation in a voltage-dependent K channel.

## II. SINGLE BARRIER ACTIVATION OF AN S4 SENSOR

The activation of a K channel is characterized by a sequential translocation through a gating pore of specific arginines of each of the four helical S4 segments, followed by a concerted conformational change in the VSDs and the S5 and S6 segments which opens the K gate and permits the conduction of ions through the central pore. The low dielectric region of the gating pore provides a barrier to the transfer of positively charged residues across the membrane that is dependent on the location of the negative fixed charge, the dielectric boundary force and the focused electric field (see Fig. 1). It is shown in the Appendix that the energy of an S4 sensor within a low dielectric slab between internal and external solvent has contributions from:

(1) The electrostatic energy between oppositely charged residues which may be expressed as a function of the translocation  $Z$  and rotation  $\theta$  of the S4 segment

$$U_R(Z, \theta) = \sum_{j=1}^3 \sum_{l=1}^7 Q_l \psi_j(\mathbf{r}_l), \quad (1)$$

where the potential of the negative fixed charge and the induced charge at the dielectric boundary may be expressed as

$$\begin{aligned} \psi_j(\mathbf{r}_l) &= \frac{2}{\epsilon_m + \epsilon_w} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} \right) + \frac{2}{\epsilon_m + \epsilon_w} \left( \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE}|} \right) + \psi_{jI} \\ &\text{if } z_l < z_I, \\ &= \frac{1}{\epsilon_m} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} \right) + \frac{1}{\epsilon_m} \left( \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jI}|} \right) \\ &\quad + \frac{1}{\epsilon_m} \left( \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE}|} \right) \\ &\text{if } z_I \leq z_l \leq z_E, \\ &= \frac{2}{\epsilon_m + \epsilon_w} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} \right) + \frac{2}{\epsilon_m + \epsilon_w} \left( \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jI}|} \right) + \psi_{jE} \\ &\text{if } z_l > z_E, \end{aligned} \quad (2)$$

$z_I$  and  $z_E$  are the locations of the internal and external boundaries of the low dielectric medium, the position  $\mathbf{r}_l = (x_l(\theta), y_l(\theta), z_l(Z))$  of the positive S4 residue charge  $Q_l$  is dependent on the conformation of the helix, the fixed negative charge  $\tilde{Q}_j$  on a neighboring segment is located at  $\tilde{\mathbf{r}}_j = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_j)$ ,  $j=1,2,3$ , representing the amino acids D316, E293, and E283,  $\tilde{\mathbf{r}}_{jI} = (\tilde{x}_j, \tilde{y}_j, 2z_I - \tilde{z}_j)$ ,  $\tilde{\mathbf{r}}_{jE} = (\tilde{x}_j, \tilde{y}_j, 2z_E - \tilde{z}_j)$ ,  $\lambda = (\epsilon_m - \epsilon_w)/(\epsilon_m + \epsilon_w)$  and  $\psi_{jI}$  and  $\psi_{jE}$  are constants. It may be noted that the expression for the potential  $\psi_j(\mathbf{r}_l)$  of the negative fixed charge in Eq. (2) reduces to a Coulomb potential when  $\epsilon_m = \epsilon_w$ .<sup>11,12</sup>

The S4 segment of the voltage-dependent *Shaker* K channel has positively charged residues R362, R365, R368,

R371, K374, R377, and K380 ( $l=1$  to 7), located on a rigid circular helix of radius  $R$ , with vertical separation  $a=4.5$  Å and angular separation  $\pi/3$  rad. The position of the S4 residues is also dependent on the translation  $Z$  and rotation  $\theta$  of the helix, and therefore

$$\begin{aligned} x_l(\theta) &= R \cos[\theta - 5\pi(l+1)/3], \\ y_l(\theta) &= R \sin[\theta - 5\pi(l+1)/3], \\ z_l(Z) &= Z + a(2-l). \end{aligned} \quad (3)$$

The translocation of the S4 segment across the membrane during activation generally includes a translation of 13.5 Å and a rotation of 180°. The dielectric constant  $\epsilon(z)$  is dependent on the position within the membrane gating pore, and is assumed to have the value of  $\epsilon_m$  within the low dielectric medium ( $z_I \leq z \leq z_E$ ), and  $\epsilon_w$  ( $>\epsilon_m$ ) in the solvent regions ( $z < z_I$  and  $z > z_E$ ).

(2) The electrostatic energy of the S4 residues within the membrane field, with potential function

$$U_V(Z) = \sum_l Q_l \tilde{V} F_V(z_l), \quad (4)$$

where  $\tilde{V} = V + V_s$  is the potential difference across the gating pore,  $V$  is the potential difference across the membrane,  $V_s$  is the surface charge potential and

$$F_V(z) = \frac{z_E - z}{z_E - z_I}, \quad z_I \leq z \leq z_E,$$

$F_V(z) = 1$  for  $z < z_I$ ,  $F_V(z) = 0$  for  $z > z_E$ .

(3) The dielectric boundary force on S4 residues which are positioned close to the interface between the low dielectric protein region of the membrane and the solvent in the internal and external crevices, and may be described by the approximate potential function

$$U_D(Z) = \sum_l U_{Bl} F_D(z_l - z_I) F_D(z_E - z_l), \quad (5)$$

where the Born energy for each residue of radius  $b_l$  is<sup>13</sup>

$$U_{Bl} = \frac{Q_l^2}{2b_l} \left( \frac{1}{\epsilon_m} - \frac{1}{\epsilon_w} \right),$$

$$F_D(z) = \frac{1}{2} (\sigma_D z + 1), \quad -1/\sigma_D \leq z \leq 1/\sigma_D,$$

$F_D(z) = 0$  for  $z < -1/\sigma_D$ ,  $F_D(z) = 1$  for  $z > 1/\sigma_D$ , and  $\sigma_D$  is a constant.

The potential function for the activation process

$$U(Z, \theta) = U_R(Z, \theta) + U_V(Z) + U_D(Z), \quad (6)$$

exhibits energy minima  $U_i$  at distinct values of the displacement  $Z_i$  and rotation  $\theta_i$  of the S4 helix, separated by a displacement of  $\approx 4.5$  Å and a rotation of  $\approx \pi/3$  radians, where  $i=0$  represents the fully deactivated state, and  $i=3$  is the activated state (see Fig. 1). These minima generally correspond to positions of positive residues of the helix which are in close proximity to the negative fixed charge, but are also dependent on the membrane potential and the locations of

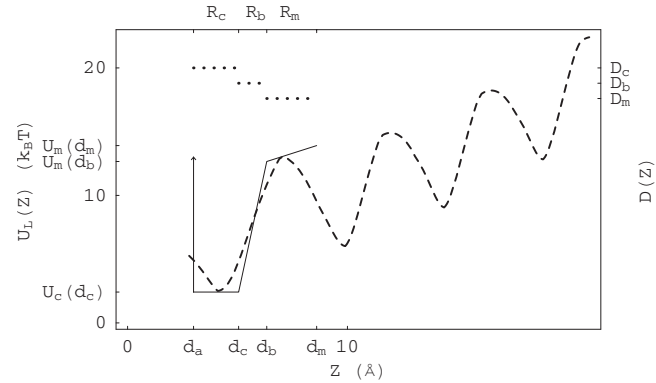


FIG. 2. The potential function  $U_L(Z) = U_R(Z, \theta_L(Z)) + U_V(Z) + U_D(Z)$  (dashed line) generated by a superposition of the force between S4 residues and oppositely charged particles on neighboring segments and their image charges, the interaction between S4 residues and the electrostatic field from the membrane potential, and the dielectric boundary force on S4 residues in the vicinity of the interface between the solvent and the low dielectric region, the diffusion coefficient  $D(Z)$  (dotted line) and the approximation to  $U_L(Z)$  by a piecewise linear function (solid line) in the regions  $R_c$ ,  $R_b$ , and  $R_m$ , where the energy barriers  $U_{bc} = U_m(d_b) - U_c(d_c)$ ,  $U_{mb} = U_m(d_m) - U_m(d_b)$ , and the potential difference across the gating pore  $\tilde{V} = -100$  mV. The quasistationary states with energy minima  $U_i$ ,  $i=0-3$ , are separated by a transverse displacement of  $\approx 4.5$  Å.

the dielectric boundaries. Assuming that there are two or three transitions between the resting and activated states, and that the rotation  $\theta_L$  has a linear variation with the translation  $Z$  between successive energy wells, the function  $U_L(Z) = U(Z, \theta_L(Z))$  describes the energy of the S4 sensor along a one-dimensional path between quasistationary states (see Fig. 2). The parameters of the model are based on structural information derived from a range of experiments, including cysteine accessibility data,<sup>5</sup> histidine substitution studies,<sup>7</sup> and the crystal structure of a K channel:<sup>10</sup>  $R=10$  Å,  $z_I=1$  Å,  $z_E=13$  Å,  $\sigma_D=0.7$  Å<sup>-1</sup>,  $b_l=3.1$  Å,  $\epsilon_w=80$ ,  $\epsilon_m=15$ ,  $\tilde{V}=-100$  mV, and the position vectors of the negative fixed charge are  $\vec{r}_1=(13,13,3)$ ,  $\vec{r}_2=(13,1,6)$ , and  $\vec{r}_3=(-11,-10,11.5)$ .

The energy of the S4 sensor  $U_L(Z)/k_B T$  may be approximated by a piecewise linear function  $U_c(Z)$ ,  $U_b(Z)$ , and  $U_m(Z)$  in the regions  $R_c(d_a \leq Z \leq d_c)$ , where the S4 segment initially resides, and transition regions  $R_b(d_c \leq Z \leq d_b)$  and  $R_m(d_b \leq Z \leq d_m)$ . The energy required for the translocation of the S4 segment to a position between stationary states is approximated by  $U_{bc} = U_m(d_b) - U_c(d_c)$ , and if sufficient energy is acquired from the thermal environment to overcome the electrostatic forces, a transition occurs from the initial state to an adjacent energy well (see Fig. 1). In each region, it is assumed that the diffusion coefficient  $D(Z)$  is constant, but there may be a discontinuity in  $D(Z)$  at the interfaces between  $R_c$  and  $R_b$ , and between  $R_b$  and  $R_m$ .

The dynamics of the S4 sensor within the regions  $R_c$ ,  $R_b$ , and  $R_m$  may be described by the probability densities  $p_c(Z, t)$ ,  $p_b(Z, t)$ , and  $p_m(Z, t)$  which satisfy the Smoluchowski equations<sup>26,27</sup>

$$\frac{\partial p_c(Z, t)}{\partial t} = D_c \frac{\partial}{\partial Z} \left( \frac{\partial p_c(Z, t)}{\partial Z} + \frac{\partial U_c(Z)}{\partial Z} p_c(Z, t) \right), \quad (7)$$

$$\frac{\partial p_b(Z,t)}{\partial t} = D_b \frac{\partial}{\partial Z} \left( \frac{\partial p_b(Z,t)}{\partial Z} + \frac{\partial U_b(Z)}{\partial Z} p_b(Z,t) \right), \quad (8)$$

$$\frac{\partial p_m(Z,t)}{\partial t} = D_m \frac{\partial}{\partial Z} \left( \frac{\partial p_m(Z,t)}{\partial Z} + \frac{\partial U_m(Z)}{\partial Z} p_m(Z,t) \right). \quad (9)$$

The probability density  $p(Z,t)$  and the probability current  $j(Z,t)$  for each region are continuous at the interfaces between  $R_c$ ,  $R_b$ , and  $R_m$

$$j_c(d_c,t) = j_b(d_c,t), p_c(d_c,t) = p_b(d_c,t), \quad (10)$$

$$j_b(d_b,t) = j_m(d_b,t), p_b(d_b,t) = p_m(d_b,t), \quad (11)$$

a reflecting boundary is imposed at  $Z=d_a$ ,

$$\frac{\partial p_c(Z,t)}{\partial Z} + \frac{\partial U_c(Z)}{\partial Z} p_c(Z,t) = 0, \quad (12)$$

and for unidirectional activation, an absorbing boundary exists at  $Z=d_m$ ,  $p_m(d_m,t)=0$ . The ion channel is initially in a hyperpolarized state before it is depolarized to a membrane potential  $V$ , and maintained at a constant value by an electronic feedback circuit, and hence the initial condition may be specified as  $p_c(Z,0)=\delta(Z-d_c)$  and  $p_b(Z,0)=p_m(Z,0)=0$ , and the potential profile across the gating pore is stationary (see Appendix).

Equations (7)–(9) may be solved analytically when the width of the region  $R_b$  is small relative to the other regions, and therefore the probability current within  $R_b$  is quasistationary and independent of  $Z$ ,

$$j_b(t) = - \frac{D_b [p_b(d_b,t) \exp U_b(d_b) - p_b(d_c,t) \exp U_b(d_c)]}{\int_{d_c}^{d_b} \exp U_b(Z) dZ}. \quad (13)$$

From the solution of Eqs. (7), (9), and (13), with the initial and boundary conditions using the method of Laplace transforms, the eigenvalue equation is

$$\begin{aligned} & \mu_i(d_m - d_b) \tan \mu_i(d_c - d_a) \\ &= \left( \frac{D_m}{D_c} \exp(-U_{bc}) - \frac{D_m}{D_b} \mu_i(d_b - d_c) \tan \mu_i(d_c - d_a) \right) \\ & \quad \times \left( -U_{mb}/2 + \frac{\Delta_i}{\tanh \Delta_i} \right), \end{aligned} \quad (14)$$

where  $\tau_m = (d_m - d_b)^2 / D_m$ ,  $\Delta_i = \sqrt{U_{mb}^2/4 - \omega_i \tau_m}$ ,  $\mu_i = \sqrt{\omega_i / D_c}$ , and  $\mu_i < \mu_{i+1}$  for  $i \geq 1$ . It may be shown that the dwell times

$$T_c = (d_c - d_a)(d_m - d_b) \exp U_{bc} \left( \frac{d_b - d_c}{D_b(d_m - d_b)} + \frac{\exp U_{mb} - 1}{D_m U_{mb}} \right), \quad (15)$$

$$T_m = \tau_m \frac{\exp U_{mb} - U_{mb} - 1}{U_{mb}^2}, \quad (16)$$

where  $T_c = \int_0^\infty P_c(t) dt$ ,  $T_m = \int_0^\infty P_m(t) dt$  and the survival probabilities  $P_c(t) = \int_{d_a}^{d_c} p_c(Z,t) dZ$  and  $P_m(t) = \int_{d_b}^{d_m} p_m(Z,t) dZ$ .

In the limiting case  $d_b \rightarrow d_c$ , from Eqs. (7), (9), and (13) it may be shown that the survival probability for the regions  $R_c$  and  $R_m$  is

$$P_{cm}(t) = \int_{d_a}^{d_m} p(Z,t) dZ = \sum_{i=1}^{\infty} a_i \exp(-\omega_i t), \quad (17)$$

where

$$a_i = \frac{2 \exp(-U_{mb}/2)}{h_1(\omega_i) + h_2(\omega_i)}, \quad (18)$$

$$h_1(\omega_i) = \omega_i \tau_m \left( \eta_i + [\nu \mu_i(d_c - d_a) \tan \mu_i(d_c - d_a) + U_{mb}/2] \right)$$

$$\times \frac{\eta_i - \cosh \Delta_i}{\Delta_i^2},$$

$$\begin{aligned} h_2(\omega_i) &= \omega_i \tau_c \left( \frac{\tan \mu_i(d_c - d_a)}{\mu_i(d_c - d_a)} \right. \\ & \quad \left. \times [\eta_i(\nu - U_{mb}/2) + \cosh \Delta_i] + \eta_i \nu \right), \end{aligned}$$

$$\eta_i = \frac{\sinh \Delta_i}{\Delta_i},$$

$\tau_c = (d_c - d_a)^2 / D_c$ , and  $\nu = [D_c(d_m - d_c) / D_m(d_c - d_a)] \exp U_{bc}$ . If  $U_{mb} = U_{bc} = 0$  and  $d_m - d_c \ll d_c - d_a$ , from Eqs. (14) and (17) the survival probability  $P_{cm}(t)$  is dependent on multiple relaxations if  $D_m = D_c$ ,<sup>20</sup> but if there is a diffusion barrier with  $D_m / D_c \ll (d_m - d_c) / (d_c - d_a) \ll 1$ ,  $P_{cm}(t) \approx \exp(-t/T_c)$ , where  $T_c$  is in the millisecond range.<sup>25</sup>

The effect of a large potential barrier between the resting and transition regions is to significantly decrease the lowest frequency  $\omega_1$  relative to the higher frequencies, and it may be shown from Eq. (18) that

$$\frac{1}{a_1} \approx \frac{1}{2} + \frac{T_m}{T_c} + \frac{\tan \sqrt{\tau_c/T_c}}{2\sqrt{\tau_c/T_c}} \left( 1 + \frac{\tau_c}{T_c} \right), \quad (19)$$

and from the initial condition,  $\sum_i a_i = 1$ . Therefore, when  $\tau_c \ll T_c$  and  $T_m \ll T_c$  or from Eqs. (15) and (16),

$$\begin{aligned} \exp(-U_{bc}) \frac{D_m U_{mb}}{D_c \exp U_{mb} - 1} &\ll \frac{d_m - d_c}{d_c - d_a} \\ &\ll \exp U_{bc} \frac{U_{mb}(\exp U_{mb} - 1)}{\exp U_{mb} - U_{mb} - 1}, \end{aligned} \quad (20)$$

$P_{cm}(t)$  may be approximated by an exponential relaxation which is in agreement with the survival probability of the slow closed state for a delayed rectifier K channel<sup>28</sup> (see Fig. 3). The conditions  $\tau_c \ll T_c$  and  $T_m \ll T_c$  derived from the analytical solution of Eqs. (7), (9), and (13) are a more general form of the assumptions previously considered in the derivation of a rate equation ( $T_m \approx \tau_m/2$  when  $U_{mb}$  is small).<sup>24</sup> Relation (20) is satisfied if  $U_{bc}$  is sufficiently large for each membrane depolarization, and from Eqs. (14) and (15), the lowest frequency

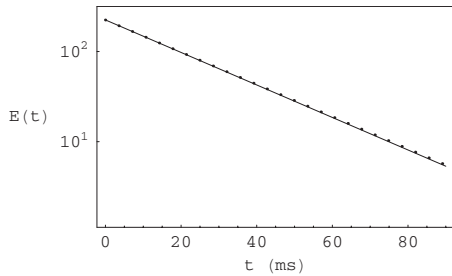


FIG. 3. The cumulative distribution  $E(t)$  of closed times greater than  $t$  for the slow closed state of a delayed rectifier K channel (Ref. 28) (dotted line) and  $E(t) = E(0)P_{cm}(t)$  (solid line), where  $P_{cm}(t)$  is the survival probability from the analytical solution, Eq. (17),  $d_c - d_a = d_m - d_c$ ,  $\tau_c = \tau_m = 2.2 \mu\text{s}$ ,  $U_{bc} = 9.3$ ,  $U_{mb} = 0$ ,  $a_i = (1.0, 0.0033, \dots)$ , and  $\omega_i = (0.041, 4459, \dots) \text{ms}^{-1}$ .

$$\omega_1 \approx \frac{D_m \exp[-U_{bc}]}{(d_c - d_a) \int_{d_c}^{d_m} \exp[U_m(Z) - U_m(d_c)] dZ} = \frac{1}{T_c}, \quad (21)$$

where it has been assumed that  $U_m(Z) = U_m(d_m) - U_{mb}(d_m - Z)/(d_m - d_c)$ . The expression in Eq. (21) is of the same form as the rate of escape over a potential barrier derived by assuming a slow diffusion process from a bound state.<sup>27</sup> A brief time after the membrane is depolarized, the probability current  $j(Z, t)$  is independent of position in the region  $R_m$  (see Fig. 4), and this approximation may be applied to the derivation of an analytical solution for multistep activation of a K channel (see Sec. III).

From Eq. (4), the energy barrier  $U_{bc}$  has a linear dependence on the membrane potential  $V$  when  $U_{mb}$  is small and therefore from Eq. (21), the opening rate  $\alpha = 1/T_c$  is an exponential function of  $V$ , and this is supported by the voltage-dependence of the burst frequency determined from single K channel data.<sup>28</sup> The higher frequency components of Eq. (17) are associated with the redistribution of the probability density within the regions  $R_c$  and  $R_m$  and from Eq. (14),  $1/\omega_2 \approx \tau_c/\pi^2 \approx 0.2 \mu\text{s}$  if  $\tau_m = \tau_c = 2.2 \mu\text{s}$ . It has been proposed that the experimental fast gating current relaxation time of  $12 \mu\text{s}$ , at least an order of magnitude greater than  $1/\omega_2$ , is associated with a state of the ion channel sensor that precedes the slow gating process.<sup>29</sup>

### III. MULTISTEP ACTIVATION OF A K CHANNEL

The sequential translocation of an S4 sensor through a membrane may be represented by a multistep transition from a resting region  $R_0$  to the activated regions  $R_1$  to  $R_3$  through the barrier regions  $\hat{R}_0$  to  $\hat{R}_2$  of the potential function  $U_L(Z)$  (see Fig. 5). For each of the regions  $R_0$  to  $R_3$ , two S4 residues are located within the low dielectric medium of the gating pore and the remaining residues are within an internal or external aqueous phase (see Fig. 1). From the analysis of Sec. II, the probability current within each transition region is independent of position when the diffusion times  $\hat{\tau}_0$  to  $\hat{\tau}_2$  are sufficiently small relative to the dwell times within the potential wells (see Fig. 4).

In this section, we consider the transitions between the regions  $R_1$ ,  $R_2$ , and  $R_3$ , where the potential function  $U_L(Z)$  is

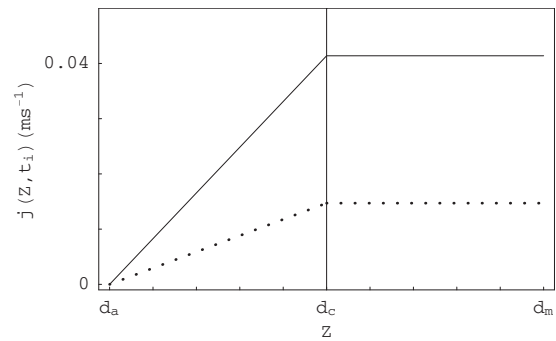


FIG. 4. The probability current  $j(Z, t)$  in the regions  $R_c(d_a \leq Z \leq d_c)$  and  $R_m(d_c \leq Z \leq d_m)$  for  $t_1 = 0.07 \text{ms}$  (solid line) and  $t_2 = 25 \text{ms}$  (dotted line) where  $d_c - d_a = d_m - d_c$ ,  $\tau_c = \tau_m = 2.2 \mu\text{s}$ ,  $U_{bc} = 9.3$ ,  $U_{mb} = 0$ ,  $a_i = (1.0, 0.0033, \dots)$ , and  $\omega_i = (0.041, 4459, \dots) \text{ms}^{-1}$ .

approximated by a square-well potential with minima  $U_i$  and maxima  $\hat{U}_i$  for each  $i$ , and the probability densities  $p_i(Z, t)$  satisfy the equations

$$\begin{aligned} \frac{\partial p_1(Z, t)}{\partial t} &= D_1 \frac{\partial^2 p_1(Z, t)}{\partial Z^2}, \\ \frac{\partial p_2(Z, t)}{\partial t} &= D_2 \frac{\partial^2 p_2(Z, t)}{\partial Z^2}, \\ \frac{\partial p_3(Z, t)}{\partial t} &= D_3 \frac{\partial^2 p_3(Z, t)}{\partial Z^2}, \end{aligned} \quad (22)$$

for each region  $R_i$  with diffusion parameter  $D_i$ . A brief time after membrane depolarization, the probability current within the regions  $\hat{R}_1$  and  $\hat{R}_2$  is given by

$$\hat{j}_1(t) = -\hat{D}_1 \frac{[\hat{p}_1(b_2, t) \exp \hat{U}_1(b_2) - \hat{p}_1(d_1, t) \exp \hat{U}_1(d_1)]}{\int_{d_1}^{b_2} \exp \hat{U}_1(Z) dZ}, \quad (23)$$

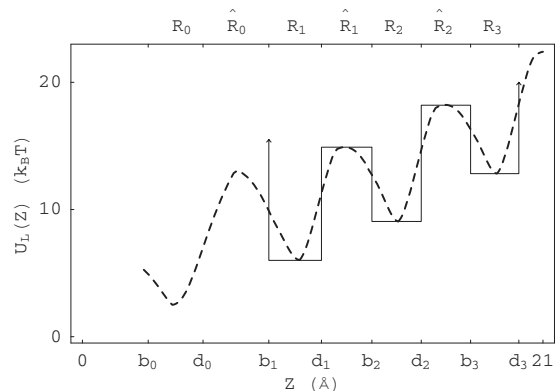


FIG. 5. The potential function  $U_L(Z)$  (dashed line), and the approximation to  $U_L(Z)$  by a square-well potential (solid line) for multistep activation of a K channel S4 sensor from the region  $R_1$  to the activated state regions  $R_2$  and  $R_3$  through the transition regions  $\hat{R}_1$  and  $\hat{R}_2$ . Each of the regions  $R_1$  to  $R_3$  corresponds to two of the principal voltage sensing residues of the S4 segment located within the low dielectric region of the gating pore.

$$\hat{j}_2(t) = -\hat{D}_2 \frac{[\hat{p}_2(b_3, t) \exp \hat{U}_2(b_3) - \hat{p}_2(d_2, t) \exp \hat{U}_2(d_2)]}{\int_{d_2}^{b_3} \exp \hat{U}_2(Z) dZ}, \quad (24)$$

where  $\hat{p}_i(Z, t)$  is the probability density and  $\hat{D}_i$  is the diffusion parameter in  $\hat{R}_i$ , and the probability current is continuous at the boundary between regions

$$j_i(d_i, t) = \hat{j}_i(t) = j_{i+1}(b_{i+1}, t),$$

where

$$j_i(Z, t) = -D_i \frac{\partial p_i(Z, t)}{\partial Z}.$$

For each interface between regions, jump conditions may be obtained from the expression for the probability current<sup>26</sup>

$$\exp[U_i(d_i)] p_i(d_i, t) = \exp[\hat{U}_i(d_i)] \hat{p}_i(d_i, t),$$

$$\exp[U_{i+1}(b_{i+1})] p_{i+1}(b_{i+1}, t) = \exp[\hat{U}_i(b_{i+1})] \hat{p}_i(b_{i+1}, t),$$

and therefore from Eqs. (23) and (24), we may write

$$\hat{j}_1(t) = \alpha(d_1 - b_1) p_1(d_1, t) - \beta(d_2 - b_2) p_2(b_2, t),$$

$$\hat{j}_2(t) = \gamma(d_2 - b_2) p_2(d_2, t) - \delta(d_3 - b_3) p_3(b_3, t),$$

where

$$\alpha = \frac{\hat{D}_1 \exp[U_1(d_1) - \hat{U}_1]}{(d_1 - b_1) \int_{d_1}^{b_1} \exp[\hat{U}_1(Z) - \hat{U}_1] dZ}, \quad (25)$$

$$\beta = \frac{\hat{D}_1 \exp[U_2(b_2) - \hat{U}_1]}{(d_2 - b_2) \int_{d_1}^{b_1} \exp[\hat{U}_1(Z) - \hat{U}_1] dZ}, \quad (26)$$

$$\gamma = \frac{\hat{D}_2 \exp[U_2(d_2) - \hat{U}_2]}{(d_2 - b_2) \int_{d_2}^{b_2} \exp[\hat{U}_2(Z) - \hat{U}_2] dZ}, \quad (27)$$

$$\delta = \frac{\hat{D}_2 \exp[U_3(b_3) - \hat{U}_2]}{(d_3 - b_3) \int_{d_2}^{b_2} \exp[\hat{U}_2(Z) - \hat{U}_2] dZ}. \quad (28)$$

An analytical solution of Eq. (22) may be obtained by imposing reflecting boundaries at  $Z=b_1$ , and  $Z=d_3$ ,

$$\frac{\partial p_1}{\partial Z}(b_1, t) = 0, \quad \frac{\partial p_3}{\partial Z}(d_3, t) = 0,$$

as well as the interface boundary conditions and the localized initial condition  $p_1(Z, 0) = \delta(Z - b_1)$  and  $p_2(Z, 0) = p_3(Z, 0) = 0$ . It may be shown that the survival probabilities in an energy landscape with two available states are  $P_1(t)$  and  $P_2(t)$  where  $P_1(t) + P_2(t) = 1$  and

$$P_2(t) = P_{2s} - \sum_{i=1}^{\infty} a_i \exp(-\omega_i t), \quad (29)$$

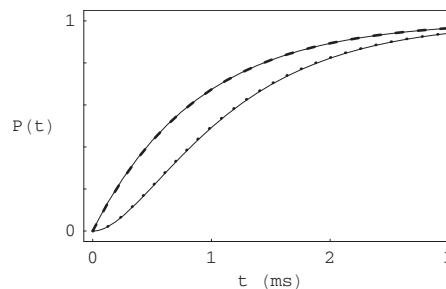


FIG. 6. The probability of the final activated state for a single step (dashed line) and two step (dotted line) master equation model of the K channel, and the lowest frequency component of the analytical solution of the stochastic diffusion equations for an energy landscape with one or two barriers [ $P_2(t)$  and  $P_3(t)$ , solid lines] with  $\alpha=1.12 \text{ ms}^{-1}$ ,  $\gamma=2.8 \text{ ms}^{-1}$ ,  $\beta=\delta=0$ , and  $\tau_1=\tau_2=0.01 \text{ ms}$ .

$$a_i = \frac{2\alpha\sqrt{\tau_1} \tan\sqrt{\omega_i\tau_2}}{\cos\sqrt{\omega_i\tau_1}[h_1(\omega_i) + h_2(\omega_i) + h_3(\omega_i)]},$$

$$h_1(\omega) = \alpha\sqrt{\tau_1} \tan\sqrt{\omega\tau_2}(1 + \tau_1\alpha + \tau_2\beta + \omega/\alpha),$$

$$h_2(\omega) = \beta\sqrt{\tau_2} \tan\sqrt{\omega\tau_1}(1 + \tau_1\alpha + \tau_2\beta + \omega/\beta),$$

$$h_3(\omega) = -(\alpha + \beta)\sqrt{\tau_1\tau_2\omega}, \quad (30)$$

$P_{2s} = \alpha/(\alpha + \beta)$ ,  $\tau_i = (d_i - b_i)^2/D_i$ ,  $\omega_i$  satisfies the eigenvalue equation

$$\sqrt{\omega_i} \tan\sqrt{\omega_i\tau_1} \tan\sqrt{\omega_i\tau_2} = \alpha\sqrt{\tau_1} \tan\sqrt{\omega_i\tau_2} + \beta\sqrt{\tau_2} \tan\sqrt{\omega_i\tau_1}, \quad (31)$$

and the lowest frequency  $\omega_1 \approx \alpha + \beta$  and  $a_1 \approx \alpha/(\alpha + \beta)$  if  $\tau_1$  and  $\tau_2$  are sufficiently small (see Fig. 6).<sup>1</sup> The higher frequency components of the solution describe the redistribution of the probability density in the regions  $R_1$  and  $R_2$ , and if  $\tau_1 = \tau_2 = \tau$ ,  $\omega_i \approx (i-1)^2\pi^2/\tau$ , where the integer  $i \geq 2$ .

In general, the low frequency components of the solution of Eq. (22) are in agreement with a transition-state model of two step activation in a K channel<sup>2,3</sup> when the diffusion times  $\tau_i \ll \alpha^{-1}, \beta^{-1}, \gamma^{-1}, \delta^{-1}$  for each  $i$ , and the delay in the rise of the probability of the final state increases with the number of transitions for each sensor (see Fig. 6). For a Brownian particle in an energy landscape with several large potential barriers, the low frequency components determined from a numerical solution of the Smoluchowski equation also approximate the solution of a discrete state model.<sup>18</sup>

The Laplace transform of the survival probabilities for the regions  $R_1$ ,  $R_2$ , and  $R_3$  may be expressed as, to lowest order,

$$\tilde{P}_1(s) = \frac{s^2 + (\beta + \gamma + \delta)s + \beta\delta}{s(s + \omega_1)(s + \omega_2)},$$

$$\tilde{P}_2(s) = \frac{\alpha(s + \delta)}{s(s + \omega_1)(s + \omega_2)},$$

$$\tilde{P}_3(s) = \frac{\alpha\gamma}{s(s + \omega_1)(s + \omega_2)},$$

where  $\omega_1$  and  $\omega_2$  are solutions of  $\omega^2 - \omega(\alpha + \beta + \gamma + \delta) + \alpha(\gamma + \delta) + \beta\delta = 0$ , and inverting the Laplace transform, we obtain

$$\begin{aligned} P_1(t) &= \frac{\beta\delta}{\omega_1\omega_2} - \frac{\alpha(\gamma + \delta - \omega_1)}{\omega_1(\omega_1 - \omega_2)}\exp(-\omega_1 t) \\ &\quad + \frac{\alpha(\gamma + \delta - \omega_2)}{\omega_2(\omega_1 - \omega_2)}\exp(-\omega_2 t), \\ P_2(t) &= \frac{\alpha\delta}{\omega_1\omega_2} - \frac{\alpha(\omega_1 - \delta)}{\omega_1(\omega_1 - \omega_2)}\exp(-\omega_1 t) \\ &\quad + \frac{\alpha(\omega_2 - \delta)}{\omega_2(\omega_1 - \omega_2)}\exp(-\omega_2 t), \\ P_3(t) &= \frac{\alpha\gamma}{\omega_1\omega_2} + \frac{\alpha\gamma}{\omega_1(\omega_1 - \omega_2)}\exp(-\omega_1 t) \\ &\quad - \frac{\alpha\gamma}{\omega_2(\omega_1 - \omega_2)}\exp(-\omega_2 t), \end{aligned} \quad (32)$$

where  $P_i(t) = \int p_i(Z, t) dZ$ , for each  $i$ . From the Boltzmann distribution for the energy levels of sequential states of the S4 sensor,<sup>12</sup> or from Eq. (32), the stationary probabilities  $P_{is}$  are given by

$$P_{is} = \frac{\exp(-U_i)}{\exp(-U_1) + \exp(-U_2) + \exp(-U_3)},$$

where  $U_i = U_i(Z)$ ,  $Z \in R_i$ .

The survival probabilities in Eq. (32) that have been derived from the solution of Eq. (22), also satisfy the master equation

$$\frac{dP_1(t)}{dt} = -\alpha P_1(t) + \beta P_2(t),$$

$$\frac{dP_2(t)}{dt} = \alpha P_1(t) - (\gamma + \beta)P_2(t) + \delta P_3(t),$$

$$\frac{dP_3(t)}{dt} = \gamma P_2(t) - \delta P_3(t),$$

which describes activation in a multistep single subunit K channel, where the transition rates  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  are determined from the measurement of ionic and gating currents.<sup>2,3</sup> From Eq. (6), the activation energy barriers for the S4 sensor are estimated to be  $8 - 11k_B T$ , depending on the value of the dielectric constant  $\epsilon_m$  within the gating pore and the distance of closest approach in charge-charge interactions. Therefore, assuming that  $d_i - b_i \approx b_{i+1} - d_i \sim 3 \text{ \AA}$  and the diffusion pa-

rameter  $\hat{D}_i$  is in the range of values determined for the diffusion of proteins in a lipid bilayer ( $\sim 10^{-8} \text{ cm}^2/\text{s}$ ),<sup>30</sup> from Eqs. (25)–(28) the transition rates are of the same order of magnitude as experimental values ( $0.1 - 1 \text{ ms}^{-1}$ ).

#### IV. DISCUSSION

The activation barriers for the gating sensor of a voltage-dependent channel, and therefore the transition rates between closed states, are dependent on the potential difference across the membrane.<sup>1-3</sup> However, the other contributions to each activation barrier are also important in determining the properties of the gating process. During the transition of the S4 sensor across the membrane, it is also subjected to a dielectric boundary force<sup>13</sup> when residues are near the solvent-protein interface, as well as a Coulomb force from neighboring segments.<sup>8,9,11</sup> By taking account of each of these forces within the channel, the voltage dependence of the stationary distribution of the gating charge may be calculated from an expression for the energy of the S4 segment, and is in agreement with experimental results from wild-type and charge-neutralized mutants of a K channel.<sup>12</sup>

In this paper, the potential function for an S4 sensor has been derived from Poisson's equation for a low dielectric slab between solvent regions, and exhibits a sequence of energy wells that correspond to S4 residues in close proximity to negative amino acids on neighboring segments, and located near each dielectric interface. By approximating one of the potential wells by a piecewise linear function, the dynamics of single step activation has been determined from the solution of interacting Smoluchowski equations for an S4 sensor of a voltage-dependent K channel. A large energy barrier for the transition between stationary states may be estimated from the structure of an S4 sensor, and therefore the survival probability for a closed state of the ion channel may be approximated by the lowest frequency component of the solution.

For each stable configuration of the S4 sensor, it has been assumed that two of the four principal voltage sensing residues are located within the low dielectric region of the gating pore, and therefore approximately 3 e per subunit is transferred across the focused membrane electric field for the three transitions of the S4 sensor between the internal and external ion channel cavity. When the diffusion times in each region are at least an order of magnitude smaller than the mean residence times for each stationary state of the S4 sensor, the low frequency components of the analytical solution of the Smoluchowski equations for a system with several barriers are in accord with an empirical master equation solution for multistep activation of a single sensor in a voltage-dependent K channel, and the calculated transition rates are of the same order of magnitude as experimental values.<sup>2,3</sup> If it is assumed that the opening of the ion pore occurs when each of the four independent S4 sensors attains an activated state in  $i$  steps with probability  $P_i$ , the open probability  $\propto P_i^4$ . However, the opening of the K channel gate is considered to be a concerted conformational change, and therefore a physi-



cal model of the opening process requires a description of the interaction between the S4 sensors and the S5 and S6 segments in the pore domain.

## APPENDIX: ELECTROSTATIC ENERGY OF AN S4 SENSOR

The potential function for an S4 sensor within a gating pore may be derived from a solution of Poisson's equation for the electrostatic potential  $\Phi(\mathbf{r})$  in a region comprised of internal solvent ( $z \leq z_I$ ), a low dielectric medium ( $z_I \leq z \leq z_E$ ) and external solvent ( $z \geq z_E$ ), where  $z_I$  and  $z_E$  are the locations of the internal and external boundaries of the dielectric media,

$$\nabla \cdot [\epsilon(\mathbf{r}) \nabla \Phi(\mathbf{r})] = -4\pi\rho_{\text{pr}} - 4\pi\rho_{\text{ion}}.$$

The charge densities of the protein and ionic components are

$$\rho_{\text{pr}} = \sum_l Q_l \delta(\mathbf{r} - \mathbf{r}_l) + \sum_j \tilde{Q}_j \delta(\mathbf{r} - \tilde{\mathbf{r}}_j),$$

$$\rho_{\text{ion}} = \sum_{\alpha} q_{\alpha} n_{\alpha} \exp[-q_{\alpha}(\phi - \phi_0)/k_B T],$$

where  $Q_l$  is the positive S4 residue charge located at  $\mathbf{r}_l = (x_l(\theta), y_l(\theta), z_l(Z))$ ,  $\theta$  and  $Z$  are the rotation and displacement of the S4 segment,  $\tilde{Q}_j$  is the negative charge on a neighboring segment with position vector  $\tilde{\mathbf{r}}_j = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_j)$ ,  $\mathbf{r} = (x, y, z)$ ,  $q_{\alpha}$  is the ionic charge,  $n_{\alpha}$  is the ionic number density within the solvent ( $\rho_{\text{ion}} = 0$  in the low dielectric medium),  $\phi_0$  is a constant, the position-dependent dielectric constant  $\epsilon(\mathbf{r}) = \epsilon_w$  in the solvent regions, and  $\epsilon(\mathbf{r}) = \epsilon_m$  in the low dielectric medium.

Expressing  $\Phi = \phi + \psi$ , where  $\phi$  is the potential from the ionic separation across the membrane and  $\psi$  is the potential generated by the residue charges, we may write

$$\nabla \cdot [\epsilon(\mathbf{r}) \nabla \phi(\mathbf{r})] = -4\pi\rho_{\text{ion}},$$

$$\nabla \cdot [\epsilon(\mathbf{r}) \nabla \psi(\mathbf{r})] = -4\pi\rho_{\text{pr}}.$$

Therefore, denoting the electrostatic potential  $\phi$  in the three regions  $z \leq z_I$ ,  $z_I \leq z \leq z_E$  and  $z \geq z_E$  by  $\phi_j$ ,  $j=1, 2, 3$ , and assuming that the argument of the exponential is small, we may write<sup>31,32</sup>

$$\nabla^2 \phi_1 = \kappa^2(\phi_1 - \tilde{V}),$$

$$\nabla^2 \phi_2 = 0,$$

$$\nabla^2 \phi_3 = \kappa^2 \phi_3,$$

where  $\tilde{V}$  is the potential difference across the gating pore, and the Debye length  $\kappa^{-1}$  is given by

$$\kappa^2 = \frac{4\pi}{\epsilon_w k_B T} \sum_{\alpha} q_{\alpha}^2 n_{\alpha}.$$

Applying the boundary conditions

$$\phi_1(z_I) = \phi_2(z_I),$$

$$\epsilon_w \nabla \phi_1(z_I) = \epsilon_m \nabla \phi_2(z_I),$$

$$\phi_2(z_E) = \phi_3(z_E),$$

$$\epsilon_m \nabla \phi_2(z_E) = \epsilon_w \nabla \phi_3(z_E),$$

the solution is

$$\phi_1(z) = \tilde{V} - V_1 \exp[\kappa(-z_I + z)],$$

$$\phi_2(z) = \frac{\tilde{V}}{V_2} \left( \frac{z_E - z}{z_E - z_I} + \frac{2\epsilon_m}{\epsilon_w \kappa(z_E - z_I)} \right),$$

$$\phi_3(z) = V_1 \exp[\kappa(z_E - z)],$$

where

$$V_1 = - \frac{\epsilon_m \tilde{V}}{\epsilon_w \kappa(z_E - z_I) + 2\epsilon_m},$$

$$V_2 = 1 + \frac{2\epsilon_m}{\epsilon_w \kappa(z_E - z_I)}.$$

The interaction energy of the S4 segment with the membrane field is given by

$$U_V(Z) = \int \phi(\hat{\mathbf{r}}) \sum_l Q_l \delta(\hat{\mathbf{r}} - \mathbf{r}_l) d\hat{\mathbf{r}},$$

and if  $\epsilon_w \gg \epsilon_m$ , we may write  $V_1 \ll \tilde{V}$ ,  $V_2 \approx 1$ , and

$$U_V(Z) \approx \sum_l Q_l \tilde{V} F_V(z_l),$$

where

$$F_V(z) = \frac{z_E - z}{z_E - z_I}, z_I \leq z \leq z_E,$$

$F_V(z) = 1$  for  $z < z_I$ ,  $F_V(z) = 0$  for  $z > z_E$ . Although the net effect of each translocation step during depolarization is the transfer of approximately a single positive charge from the internal to external solvent, depending on the location of the stationary states of the S4 helix (see Fig. 1), the electronic feedback circuit supplies an equal transfer of charge in the reverse direction, and therefore the potential profile within the low dielectric region may be assumed to be stationary.

An additional contribution to the potential function for the S4 segment may be derived when an S4 residue is located near the interface between the solvent phase and the low dielectric medium within the gating pore.<sup>33,34</sup> Assuming that a residue charge  $Q_l$  within a dielectric  $\epsilon_w$  is located at  $(x_l, y_l, z_l)$ , where  $z_l$  is dependent on the displacement  $Z$  of the S4 helix, and that a plane interface with the hydrophobic region with dielectric constant  $\epsilon_m < \epsilon_w$  occurs at  $z = z_I$ , the electrostatic potential  $\psi_l$  may be determined from the equations

$$\epsilon_w \nabla^2 \psi_l = -4\pi Q_l \delta(\mathbf{r} - \mathbf{r}_l), z < z_l,$$

$$\epsilon_m \nabla^2 \psi_l = 0, z > z_l,$$

with the boundary conditions

$$\epsilon_w \frac{d\psi_l}{dz}(z_l) = \epsilon_m \frac{d\psi_l}{dz}(z_l),$$

$$\frac{d\psi_l}{dx}(z_l) = \frac{d\psi_l}{dx}(z_l),$$

$$\frac{d\psi_l}{dy}(z_l) = \frac{d\psi_l}{dy}(z_l).$$

Assuming an image charge  $Q_{II}$  is located at a point  $\mathbf{r}_{II} = (x_l, y_l, 2z_l - z_l)$  and a charge  $Q'_l$  has position  $\mathbf{r}_l$ , the potential  $\psi_l$  at the point  $(x, y, z)$ ,  $z < z_l$  is given by

$$\psi_l(\mathbf{r}) = \frac{1}{\epsilon_w} \left( \frac{Q_l}{|\mathbf{r} - \mathbf{r}_l|} + \frac{Q_{II}}{|\mathbf{r} - \mathbf{r}_{II}|} \right),$$

and if  $z > z_l$ ,

$$\psi_l(\mathbf{r}) = \frac{1}{\epsilon_m} \left( \frac{Q'_l}{|\mathbf{r} - \mathbf{r}_l|} \right).$$

Applying the boundary conditions, it may be shown that

$$Q_l - Q_{II} = Q'_l,$$

$$\frac{1}{\epsilon_w} (Q_l + Q_{II}) = \frac{1}{\epsilon_m} Q'_l,$$

and hence

$$Q_{II} = -\frac{\epsilon_m - \epsilon_w}{\epsilon_m + \epsilon_w} Q_l,$$

$$Q'_l = \frac{2\epsilon_m}{\epsilon_m + \epsilon_w} Q_l.$$

Therefore, the potential function of the interaction between each residue charge  $Q_l$  and its image may be approximated by

$$U_D(Z) = \int \sum_l \frac{Q_l Q_{II}}{\epsilon(z_l) |\mathbf{r}_{II} - \mathbf{r}_l|^2} dz_l,$$

and hence

$$U_D(Z) = \sum_l U_{Bl} + \sum_l \frac{Q_l^2}{4(z_l - z_l)} \frac{\epsilon_m - \epsilon_w}{\epsilon_m(\epsilon_m + \epsilon_w)}, \quad z_l > b_l + z_l,$$

$$U_D(Z) = \sum_l \frac{Q_l^2}{4(z_l - z_l)} \frac{\epsilon_m - \epsilon_w}{\epsilon_w(\epsilon_m + \epsilon_w)}, \quad z_l < -b_l + z_l,$$

where for each charged residue,  $b_l$  is the radius and the Born energy required to cross the dielectric boundary is<sup>13</sup>

$$U_{Bl} = \frac{Q_l^2}{2b_l} \left( \frac{1}{\epsilon_m} - \frac{1}{\epsilon_w} \right).$$

A similar expression may be derived for the external interface at  $z = z_E$ , and assuming a linear variation for  $U_D(Z)$  at each boundary,

$$U_D(Z) = \sum_l U_{Bl} F_D(z_l - z_l) F_D(z_E - z_l),$$

where

$$F_D(z) = \frac{1}{2}(\sigma_D z + 1), \quad -1/\sigma_D \leq z \leq 1/\sigma_D,$$

$F_D(z) = 0$  for  $z < -1/\sigma_D$ ,  $F_D(z) = 1$  for  $z > 1/\sigma_D$ , and  $\sigma_D$  is a constant.

The interaction energy of the S4 segment and the neighboring negative charges is given by

$$U_R(Z, \theta) = \int \sum_j \psi_j(\mathbf{r}) \sum_l Q_l \delta(\mathbf{r} - \mathbf{r}_l) d\mathbf{r} = \sum_j \sum_l Q_l \psi_j(\mathbf{r}_l),$$

where  $\psi_j(\mathbf{r}_l)$ , in general, may be expressed as an infinite sum of image charge potentials,<sup>33</sup> but may be approximated by

$$\begin{aligned} \psi_j(\mathbf{r}_l) &= \frac{2}{\epsilon_m + \epsilon_w} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} \right) + \frac{2}{\epsilon_m + \epsilon_w} \left( \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE}|} \right) \\ &\quad + \psi_{jl} \quad \text{if } z_l < z_l, \\ &= \frac{1}{\epsilon_m} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} \right) + \frac{1}{\epsilon_m} \left( \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE}|} \right) + \frac{1}{\epsilon_m} \left( \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE}|} \right), \end{aligned}$$

if  $z_l \leq z_l \leq z_E$ ,

$$\begin{aligned} &= \frac{2}{\epsilon_m + \epsilon_w} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} \right) + \frac{2}{\epsilon_m + \epsilon_w} \left( \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE}|} \right) \\ &\quad + \psi_{jE} \quad \text{if } z_l > z_E, \end{aligned}$$

$\lambda = (\epsilon_m - \epsilon_w)/(\epsilon_m + \epsilon_w)$ ,  $\tilde{\mathbf{r}}_{jl} = (\tilde{x}_j, \tilde{y}_j, 2z_l - \tilde{z}_j)$ ,  $\tilde{\mathbf{r}}_{jE} = (\tilde{x}_j, \tilde{y}_j, 2z_E - \tilde{z}_j)$ , and  $\psi_{jl}$  and  $\psi_{jE}$  are constants determined by the boundary conditions. Therefore, the total potential function  $U(Z, \theta)$  for the S4 segment is given by

$$U(Z, \theta) = U_R(Z, \theta) + U_V(Z) + U_D(Z).$$

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