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## Voltage dependence of a stochastic model of activation of an alpha helical S4 sensor in a K channel membrane

S. R. Vaccaro<sup>a)</sup>

*Department of Physics, University of Adelaide, Adelaide, South Australia 5005, Australia*

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The voltage dependence of the ionic and gating currents of a K channel is dependent on the activation barriers of a voltage sensor with a potential function which may be derived from the principal electrostatic forces on an S4 segment in an inhomogeneous dielectric medium. By variation of the parameters of a voltage-sensing domain model, consistent with x-ray structures and biophysical data, the lowest frequency of the survival probability of each stationary state derived from a solution of the Smoluchowski equation provides a good fit to the voltage dependence of the slowest time constant of the ionic current in a depolarized membrane, and the gating current exhibits a rising phase that precedes an exponential relaxation. For each depolarizing potential, the calculated time dependence of the survival probabilities of the closed states of an alpha helical S4 sensor are in accord with an empirical model of the ionic and gating currents recorded during the activation process. © 2011 American Institute of Physics. [doi:10.1063/1.3630010]

### INTRODUCTION

Voltage-dependent K channels are composed of an ion-conducting pore with four molecular sensors that change their conformation according to the potential difference across the membrane. An early kinetic model of K activation assumed that a single transition from a closed to an activated state for each of the four independent gating particles is required to open a channel.<sup>1</sup> More accurate recordings from a cloned K channel expressed in *Xenopus* oocytes have revealed that at least six independent and identical transitions are required to reproduce the sigmoidicity observed in macroscopic ionic currents,<sup>2</sup> and therefore taking account of the tetrameric structure of the K channel, at least two transitions are required for each of the four S4 voltage sensors to attain an activated state.<sup>2,3</sup>

It has been proposed that the positively charged S4 segment of the K channel is a molecular sensor that moves in a helical path through a gating pore formed by the S1, S2, and S3 domain segments of each voltage-sensing domain (VSD) (see Fig. 1).<sup>4,5</sup> By replacing each S4 charge by a neutral residue and measuring the total gating charge per channel, the first four charges of the S4 segment were identified as having the largest contribution to the gating current.<sup>6,7</sup> Based on cysteine-accessibility experiments<sup>8,9</sup> and the conduction of protons and cations through the VSD,<sup>10,11</sup> it may be assumed that the S4 sensor traverses a focussed electric field within the VSD where positive residues interact with negative amino acids on adjacent S2 and S3 segments<sup>12</sup> (see Fig. 1).

A charged S4 residue located within the gating canal of a membrane experiences similar electrostatic forces to an ion in the pore of a channel, where the energy is dependent on the potential difference across the membrane, the fixed charge on the channel wall and the induced charge at

the dielectric boundaries.<sup>13–18</sup> By taking account of each of these forces, the calculation of the ionic current through biological channels from the Poisson Nernst-Planck equations is in good agreement with Brownian dynamics or Monte Carlo simulations.<sup>16,18</sup> A potential function for an alpha helical S4 sensor located within the gating pathway may be defined that is dependent on negatively charged amino acids on neighboring segments, and the potential difference across the membrane,<sup>19</sup> but also has contributions from the interaction energy between S4 charges and the charge at the dielectric boundaries induced by both positive and negative residues.<sup>20,21</sup> The calculated voltage dependence of the stationary distribution of the gating charge for a VSD model is in good agreement with experimental data from wild-type and charge-neutralized mutants of a K channel.<sup>20</sup> Assuming that the dielectric boundaries between the low dielectric region of the membrane and the solvent incorporate internal and external cavities formed from transmembrane helices, the variation of potential within the membrane and solvent may be determined from a numerical solution of the Poisson-Boltzmann equation.<sup>22,23</sup>

The dynamics of an ion channel voltage sensor, represented as a Brownian particle in a multibarrier energy landscape, may be described by a stochastic drift-diffusion equation which has a numerical solution that includes low frequency relaxations representing transitions between adjacent states.<sup>24</sup> An analytical solution of the Smoluchowski equation for a potential function determined by the electrostatic forces on an alpha helical S4 segment in an inhomogeneous dielectric medium may be approximated by a master equation that has a similar form to empirical models of K channel activation.<sup>21</sup> However, for the parameters adopted within the VSD model, the lowest frequency of the solution is not in accord with the voltage dependence of the activation time constant determined from a single exponential fit to the late phase of ionic or gating current for moderate depolarizing

<sup>a)</sup>Electronic mail: svaccaro@physics.adelaide.edu.au.

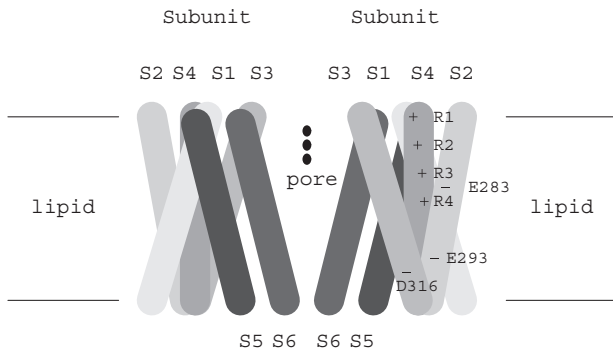


FIG. 1. A model of the segments S1, S2, S3, and S4 (light gray) of the voltage-sensing domain, and the segments S5 and S6 of the pore domain (dark gray) for each of the two subunits of a K channel in the open state, enclosing a pore with K ions. The positive residues R1, R2, R3, and R4 on the S4 segment traverse the membrane during activation and form sequential electrostatic pairs with the negative amino acids E293 and E283 on the S2 segment and D316 on the S3 segment.

potentials.<sup>2,3</sup> In this paper, it is shown that the variation of the solution frequency with voltage is dependent on the relative location of the negatively charged amino acids and their distance from the dielectric boundaries of the membrane. The potential profile between stationary states of an alpha helical sensor is determined from analytical expressions for the potential of a fixed charge within the low dielectric and aqueous regions. By modifying of the parameters of the model, consistent with the structure of the voltage sensor domain, the calculated gating current exhibits a transient increase before an exponential decay and the survival probabilities of the closed states of an S4 sensor are in good agreement with a three step kinetic description of K channel activation.<sup>3</sup>

## THE ACTIVATION OF A VOLTAGE SENSOR

The activation of a K channel is a complex physical process where the S4 sensor in each VSD absorbs energy from the thermal environment to translocate across the membrane, exerting a torque on the S5 segment which separates the intracellular end of the S6 segment of each pore domain in a concerted conformational change that opens the channel (see Fig. 1). In order to derive an approximate analytical expression for the potential function for an S4 sensor within a VSD, it is assumed that the membrane is a dielectric slab between internal and external solvents. The effect of an irregular gating pore and solvent-accessible cavities on the dynamics may be considered by adopting a finite difference method to solve the Poisson and drift-diffusion equations.<sup>22,25</sup> The potential function  $U(Z, \theta)$ , where  $Z$  is the transverse displacement and  $\theta$  is the rotation of the sensor, has contributions from  $U_V(Z)$ , the electrostatic energy of the S4 residues within the membrane field,  $U_D(Z)$ , the interaction energy between S4 residues and the charge induced at the dielectric boundaries, and  $U_R(Z, \theta) = \sum_{j=1}^4 \sum_{l=1}^7 Q_l \psi_j(\mathbf{r}_l)$ , the electrostatic energy between oppositely charged residues. The potential  $\psi_j$ ,  $j = 1-3$ , of the negative amino acids within a planar low dielectric region and associated induced charge at the dielec-

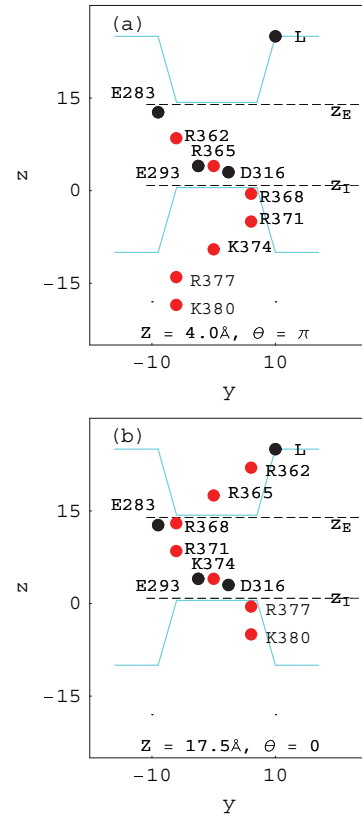


FIG. 2. A schematic representation of the location (in Å) of the negatively charged amino acids D316, E293, E283 and a lipid phosphate group L (black dot), the irregular internal and external aqueous cavities (solid line) connected through a gating pore in the lipid membrane, the internal ( $z_I$ ) and external ( $z_E$ ) boundaries of the low dielectric region of the VSD model (dotted line), and the positive S4 residue charges (red dot) for the resting state (a) and the activated state, (b) where  $Z$  is the transverse displacement and  $\theta$  is the rotation of the alpha helical S4 sensor.

tric boundaries may be derived from Poisson's equation (see Appendix A),

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

where  $\tilde{\mathbf{r}}_j = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_j)$ ,  $j = 1, 2$ , and 3 is the location of each amino acid D316, E293, and E283 with negative charge  $\tilde{Q}_j$  on a neighboring segment of the VSD,  $\mathbf{r}_l(Z, \theta)$  is the position

of the positive S4 residue charge  $Q_l$ ,  $l = 1-7$ , (see Fig. 2),  $\tilde{\mathbf{r}}_{jE,n} = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_{jE,n})$ ,  $\tilde{\mathbf{r}}_{jI,n} = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_{jI,n})$ ,

$$\begin{aligned}\tilde{z}_{jE,n} &= (-1)^n \left[ \tilde{z}_j - \frac{(z_I + z_E)}{2} \right] \\ &\quad + z_E \left( n + \frac{1}{2} \right) - z_I \left( n - \frac{1}{2} \right) > z_E, \\ \tilde{z}_{jI,n} &= (-1)^n \left[ \tilde{z}_j - \frac{(z_I + z_E)}{2} \right] \\ &\quad + z_I \left( n + \frac{1}{2} \right) - z_E \left( n - \frac{1}{2} \right) < z_I.\end{aligned}\quad (2)$$

Although low order terms give an approximate estimate for the potential  $\psi_j$ ,<sup>19-21</sup> the exact expression in Eq. (1) is a slowly converging series, in general and, therefore, terms of higher order are required to calculate the voltage dependence of the energy barriers of activation, and sufficient accuracy is obtained by including at least six terms in the expansion. The dielectric constant  $\epsilon(z)$  is assumed to have the value of  $\epsilon_w$  in the solvent regions ( $z < z_I$  and  $z > z_E$ ), an effective value of  $\epsilon_m$  ( $< \epsilon_w$ ) within the low dielectric medium ( $z_I \leq z \leq z_E$ ), representing a reduced shielding of the interaction between oppositely charged residues within the confined volume of a membrane gating pore,<sup>26</sup> and  $\lambda = (\epsilon_m - \epsilon_w)/(\epsilon_m + \epsilon_w)$ .

From molecular dynamics simulations of the S4 sensor, it may be shown that in the activated state, interactions develop between the first two residues R1 and R2 of the S4 segment and polar lipid headgroups<sup>27,28</sup> and are important for the normal function of the ion channel.<sup>29</sup> The potential  $\psi_j$ ,  $j = 4$ , of a negative lipid group of charge  $\tilde{Q}_j$  and location  $\tilde{\mathbf{r}}_j = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_j)$  within the external solvent may be expressed as (see Appendix A)

$$\begin{aligned}\psi_j(\mathbf{r}_l) &= \frac{1 - \lambda^2}{\epsilon_w} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} + \sum_{n=1}^{\infty} \frac{\lambda^{2n} \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE,n}|} \right), \quad z_l < z_I, \\ &= \frac{2}{(\epsilon_m + \epsilon_w)} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} + \sum_{n=1}^{\infty} \frac{\lambda^{2n-1} \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jI,n}|} \right. \\ &\quad \left. + \sum_{n=1}^{\infty} \frac{\lambda^{2n} \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE,n}|} \right), \quad z_I \leq z_l \leq z_E, \\ &= \frac{1}{\epsilon_w} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} - \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jI,0}|} \right. \\ &\quad \left. + (1 - \lambda^2) \sum_{n=1}^{\infty} \frac{\lambda^{2n-1} \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jI,n}|} \right), \quad z_l > z_E,\end{aligned}\quad (3)$$

where  $\tilde{z}_{jE,n} = \tilde{z}_j + 2n(z_E - z_I)$  and  $\tilde{z}_{jI,n} = -\tilde{z}_j + 2z_E - 2n(z_E - z_I)$ .

It is assumed that each positively charged S4 residue of the *Shaker* K channel (R362, R365, R368, R371, K374, R377, and K380) is located on a rigid circular alpha helix of radius  $R = 7 \text{ \AA}$  with vertical separation  $a = 4.5 \text{ \AA}$  and angular separation  $\pi/3$  (rads),<sup>30</sup> and that the axis of the helix is normal

to the membrane plane with no change of tilt. During activation there is a translocation of the helix between the resting state ( $Z = 4 \text{ \AA}$ ,  $\theta = \pi$ ), the activated state ( $Z = 17.5 \text{ \AA}$ ,  $\theta = 0$ ), and two intermediate states, and therefore the position of each S4 residue is  $\mathbf{r}_l = (x_l, y_l, z_l) = (R \cos[\theta - 5\pi(l+1)/3], R \sin[\theta - 5\pi(l+1)/3], Z + a[2-l])$  – see Fig. 2. The first five residues change their location within the electric field during the activation process and, therefore, contribute to the gating current and residues at the inner and outer membrane boundaries interact with negative lipid groups.<sup>27</sup>

For a planar dielectric membrane between internal and external solvents, the electrostatic energy of the S4 residues within the membrane field  $U_V(Z) = \sum_l Q_l \tilde{V} F_V(z_l)$ ,<sup>20-22,31</sup> 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,  $F_V(z) = (z_E - z)/(z_E - z_I)$ ,  $z_I \leq z \leq z_E$ ,  $F_V(z) = 1$  for  $z < z_I$ , and  $F_V(z) = 0$  for  $z > z_E$ . If the S4 residues are transferred across the membrane through an aqueous cylindrical pore within a lipid bilayer, the electrostatic potential across the membrane is a nonlinear function of the transverse coordinate, but may be approximated by  $F_V(z)$ .<sup>14,15</sup>

The interaction energy  $U_D(Z)$  between S4 residues and the induced charge at the plane dielectric boundaries  $z = z_I$  and  $z = z_E$  may be expressed as an infinite sum of image charge contributions.<sup>32</sup> The self-energy barrier for the passage of a charge through an aqueous cylindrical gating pore of infinite length within a dielectric slab may be derived from Poisson's equation,<sup>33</sup> and in the case of a finite gating pore, the energy barrier may be evaluated from an image method solution of Poisson's equation and Monte Carlo simulations of ion permeation.<sup>14,15,25</sup> The variation of the self-energy of the S4 segment in the transverse direction may be approximated by  $U_D(Z) = \sum_l U_{Dl} F_D(z_l - z_I) F_D(z_E - z_l)$ , where  $U_{Dl}$  is the energy barrier for each residue and is dependent on the radius and length of the gating pore,  $F_D(z) = 0.5(\sigma_D z + 1)$ ,  $-1/\sigma_D < z < 1/\sigma_D$ ,  $F_D(z) = 0$  for  $z < -1/\sigma_D$ ,  $F_D(z) = 1$  for  $z > 1/\sigma_D$ , and  $\sigma_D$  is a constant.

The dynamics of ion channel gating may be described by the Smoluchowski equation,<sup>34</sup>

$$\frac{\partial p(Z, t)}{\partial t} = \frac{\partial}{\partial Z} \left[ D(Z) \left( \frac{\partial p(Z, t)}{\partial Z} + \frac{1}{k_B T} \frac{\partial U_A(Z)}{\partial Z} p(Z, t) \right) \right], \quad (4)$$

where  $p(Z, t)$  is the probability density of the voltage sensor and  $k_B$  is Boltzmann's constant. Equation (4) may be solved numerically<sup>24,35</sup> or analytically when the potential function  $U_A(Z)$  is piecewise linear<sup>36,37</sup> or the diffusion parameter  $D(Z)$  has an exponential variation.<sup>38,39</sup> In general,  $U_A(Z)$  is dependent on a potential of mean force that, in principle, may be derived from molecular dynamics simulations based on an atomic model of the ion channel VSD.<sup>40</sup> However, in this paper, it is assumed that  $U_A(Z) = U(Z, \theta_L(Z))$  and the rotation  $\theta_L(Z)$  is a linear function of  $Z$  between adjacent energy wells. By approximating the potential function  $U_A(Z)$  by a square-well potential with minimum  $U_i = U_A(Z_i)/k_B T$  in the region  $R_i$  and maximum  $\hat{U}_i = U_A(\hat{Z}_i)/k_B T$  in the region  $\hat{R}_i$  for each  $i$ , where  $Z_i$  and  $\hat{Z}_i$  are the corresponding transverse

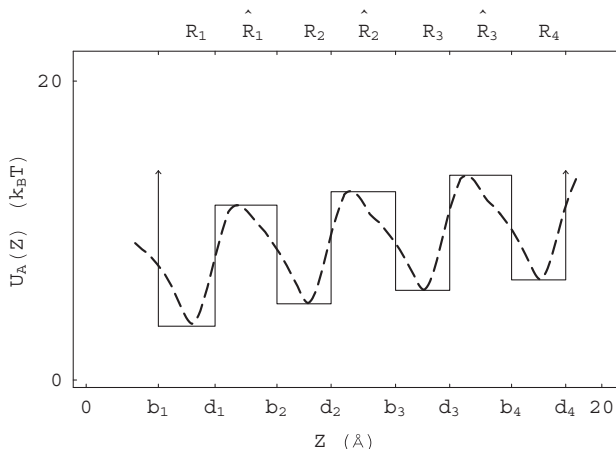


FIG. 3. The potential function  $U_A(Z)$  (dashed line), and the square-well potential approximation (solid line) for three step activation of a K channel alpha helical S4 sensor from the resting region  $R_1$  to the activated state region  $R_4$ .

displacements of the helix, the sequential translocation of an S4 sensor through a membrane may be represented by transitions between the regions  $R_i$ . The parameter values for the function  $U_A(Z)$  in Fig. 3 may be determined by comparison of the analytical solution with the empirical data, as described in “The voltage dependence of S4 activation” section –  $z_I = 1 \text{ \AA}$ ,  $z_E = 14.1 \text{ \AA}$ ,  $U_{DI} = 3.8k_B T$ ,  $\sigma_D = 0.7 \text{ \AA}^{-1}$ ,  $\epsilon_w = 80$ ,  $\epsilon_m = 15$ ,  $d_i - b_i \approx b_{i+1} - d_i \sim 3 \text{ \AA}$ ,  $\tilde{V} = -25 \text{ mV}$ ,  $V_s = 20 \text{ mV}$ , the location (in  $\text{\AA}$ ) of each negatively charged amino acid D316, E293, and E283 is  $\tilde{\mathbf{r}}_1 = (9.6, 2.4, 3)$ ,  $\tilde{\mathbf{r}}_2 = (9, -2.5, 4)$ , and  $\tilde{\mathbf{r}}_3 = (-7, -9, 12.7)$  and the lipid headgroup has position vector  $\tilde{\mathbf{r}}_4 = (2, 10, 25)$ .

In general, the numerical solution of Eq. (4) for a gating particle includes a fast redistribution of the probability density within each stationary state, and a slower component representing the transitions from energy wells of varying barrier height.<sup>24</sup> The solution includes a discrete frequency for each deep energy well, as well as a spectrum of frequencies for each well that satisfies  $\tau \sim 1/\alpha$ , where  $\tau$  is the diffusion time in the well and  $\alpha$  is the escape rate. Subsidiary wells may occur when S4 residues are transferred across the dielectric boundary,<sup>20</sup> whereas the deeper wells correspond to the formation of salt bridges between S4 residues and oppositely charged groups on neighboring segments. Assuming that the S4 sensor has four stable stationary states and that  $\tau_i = (d_i - b_i)^2/D_i \ll \alpha_i^{-1}$ ,  $\beta_i^{-1}$  for each  $i$ , where the transition rates  $\alpha_i$  and  $\beta_i$  are dependent on the energy barriers  $\tilde{U}_{\alpha_i}(V) = \hat{U}_i - U_i$  and  $\tilde{U}_{\beta_i}(V) = \hat{U}_i - U_{i+1}$  of  $U_A(Z)$ , the survival probabilities derived from the analytical solution of Eq. (4) satisfy an approximate master equation, in the millisecond range, that has the same form as empirical models of K channel gating<sup>2,3,21</sup> (see Appendix B).

### THE VOLTAGE DEPENDENCE OF S4 ACTIVATION

By variation of the coordinates of the negative amino acids and lipid headgroup, the self-energy barrier  $U_{DI}$  at the dielectric boundary for each residue, the locations of the plane

boundaries  $z = z_I$  and  $z = z_E$ , and the surface charge potential  $V_s$ , a set of parameters may be determined that provide agreement between calculations from the solution of the Smoluchowski equation and the master equation description of gating current measurements for a K channel. However, initial values of the parameters may be estimated from independent experimental studies of K activation. The negatively charged amino acids D316 and E293 are positioned close to the inner dielectric boundary and the amino acid E283 is located in the external aqueous cleft, separated by a distance of  $\sim 9 \text{ \AA}$ , and the thickness of the low dielectric medium is of the order of  $13 \text{ \AA}$ , based on x-ray structures of the K channel.<sup>41</sup> The interaction energy between S4 residues and the negatively charged amino acids is assumed to be  $\sim 8 k_B T$ , similar to the value derived from the measurement of salt-bridge energies in the gating of an OmpA ion channel<sup>42</sup> and consistent with a distance of closest approach between oppositely charged residues of  $2\text{--}3 \text{ \AA}$ , and an effective dielectric constant  $\epsilon_m$  of  $10\text{--}15$  within the VSD.

When a constant potential difference is applied to the membrane, the gating charge  $\bar{Q}_i$  associated with the transition to the  $i$ th state from the first state is the change in charge induced in the solvent,<sup>22,31</sup>

$$\bar{Q}_i = - \sum_l Q_l [F_V(z_l(Z_i)) - F_V(z_l(Z_1))]. \quad (5)$$

For each transition, each of the three residues is transferred  $4.5 \text{ \AA}$  across the membrane field, and therefore the total gating charge generated by the four sensors for the transitions between the resting and activated states is  $\sim 12e$  and consistent with that determined from experimental studies<sup>7,43</sup> and molecular dynamics simulations.<sup>28</sup> The observable gating charge  $Q_g(t)$  for each subunit is also dependent on the survival probability for each state  $P_i(t)$  (Refs. 20 and 22) and hence  $Q_g(t) = \sum_{i=2}^4 \bar{Q}_i P_i(t)$ , and the slow component of the gating current,

$$I_g(t) = \sum_{i=2}^4 \bar{Q}_i \frac{dP_i}{dt}. \quad (6)$$

From the approximate master equation solution of the Smoluchowski equation (see Appendix B), we may write

$$I_g(t) \approx \bar{Q} [\alpha_1 P_1(t) + (\alpha_2 - \beta_1) P_2(t) + (\alpha_3 - \beta_2) P_3(t) - \beta_3 P_4(t)], \quad (7)$$

if  $\bar{Q} = \bar{Q}_2 \approx \bar{Q}_3 - \bar{Q}_2 \approx \bar{Q}_4 - \bar{Q}_3$ . For a small depolarization from a hyperpolarized holding potential,  $\beta_1 > \alpha_2$  and  $\beta_2 > \alpha_3$ , and hence only the first term is positive, and the gating current does not have a rising phase. However, for a large depolarization,  $\beta_i \approx 0$ , and  $I_g(t)$  exhibits a rising phase that precedes the exponential relaxation when  $\alpha_1 < \alpha_2, \alpha_3$ . The dielectric boundary force on the S4 segment for the first forward transition (see Fig. 2) is generated at the inner dielectric boundary, whereas for subsequent transitions, a small net force is the result of opposing contributions of similar magnitude from each dielectric boundary. For each value of the self-energy barrier  $U_{DI}$ , by variation of the distance between E283 and a neighboring S4 residue in a stationary state, values of the  $x$ ,  $y$ , and  $z$  coordinates of E283 may be determined



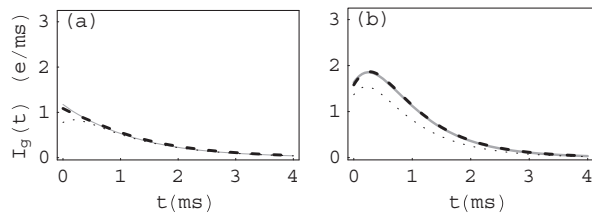


FIG. 4. The experimental gating current during the activation of a K channel voltage sensor from a hyperpolarized state to depolarizing potentials of  $-40$  mV (a) and  $-10$  mV (b) (Ref. 3) (dotted line), and the gating current calculated from an analytical solution of the Smoluchowski equation for the VSD model of Fig. 2 (gray line), and the VSD model with the self-energy barrier  $U_{DI}$  increased to  $7 k_B T$  and the amino acid E283 displaced to the position  $\tilde{\mathbf{r}}_1 = (-7, -7, 13.2)$  (dashed line).

such that the second and third activation barriers  $\tilde{U}_{\alpha_2}(V)$  and  $\tilde{U}_{\alpha_3}(V) \approx \tilde{U}_{\alpha_1}(V)$  and the gating current  $I_g(t)$  has a magnitude and shape comparable to experimental gating currents (see Fig. 4).<sup>24</sup> A three step empirical model<sup>3</sup> underestimates the gating charge of the voltage sensor compared with the value obtained from the voltage dependence of the channel open probability,<sup>2</sup> and measurements of the absolute charge movement per channel,<sup>6,7,43</sup> and, therefore, the corresponding gating current is less than calculated from Eq. (6).

If the location  $Z_i$  and  $\hat{Z}_i$  of each extremum of the potential function  $U_A(Z)$  is weakly dependent on  $V$ ,

$$k_B T \frac{\partial \tilde{U}_{\alpha_i}(V)}{\partial V} \approx \frac{\partial [U_V(\hat{Z}_i, V) - U_V(Z_i, V)]}{\partial V},$$

$$k_B T \frac{\partial \tilde{U}_{\beta_i}(V)}{\partial V} \approx \frac{\partial [U_V(\hat{Z}_i, V) - U_V(Z_{i+1}, V)]}{\partial V}, \quad (8)$$

and, therefore, the energy barriers  $\tilde{U}_{\alpha_i}(V)$  and  $\tilde{U}_{\beta_i}(V)$  are linear functions of the membrane potential  $V$ , usually assumed by kinetic models of activation<sup>2,3</sup> and supported by the experimental voltage dependence of transition rates between stationary states. Thus,  $\tilde{U}_{\alpha_i}(V) \approx \tilde{U}_{\alpha_i}(0) - q_{\alpha_i} V / k_B T$  and  $\tilde{U}_{\beta_i}(V) \approx \tilde{U}_{\beta_i}(0) + q_{\beta_i} V / k_B T$ , where  $q_{\alpha_i}$  and  $q_{\beta_i}$  are the partial charges between the  $i$ th and  $(i+1)$ th states, and  $\tilde{U}_{\alpha_i}(0)$  and  $\tilde{U}_{\beta_i}(0)$  are constants, and from Eq. (B5),

$$\alpha_i = \alpha_{i0} \exp[q_{\alpha_i} V / k_B T], \quad \beta_i = \beta_{i0} \exp[-q_{\beta_i} V / k_B T], \quad (9)$$

where  $\alpha_{i0} = \tau_{\alpha_i}^{-1} \exp \tilde{U}_{\alpha_i}(0)$ ,  $\beta_{i0} = \tau_{\beta_i}^{-1} \exp \tilde{U}_{\beta_i}(0)$ ,  $\tau_{\alpha_i} = (d_i - b_i)(b_{i+1} - d_i) / \hat{D}_i$ ,  $\tau_{\beta_i} = (d_{i+1} - b_{i+1})(b_{i+1} - d_i) / \hat{D}_i$ , and the diffusion parameter  $\hat{D}_i \sim 10^{-8}$  cm<sup>2</sup>/s is estimated from the lateral diffusion of proteins in a lipid membrane.<sup>44</sup> From Eq. (8), the partial charges  $q_{\alpha_i} \approx e[3(\hat{Z}_i - Z_1) + Z_1 - a - z_I] / (z_E - z_I)$ ,  $q_{\alpha_i} \approx e[3(\hat{Z}_i - Z_i - a) + z_E - z_I] / (z_E - z_I)$  for  $i > 1$ , and  $q_{\beta_i} \approx e[3(Z_{i+1} - \hat{Z}_i)] / (z_E - z_I)$  and hence  $q_{\alpha_i} + q_{\beta_i} \approx e$ , and  $q_{\alpha_i} < q_{\beta_i}$  when  $\hat{Z}_i < (Z_i + Z_{i+1})/2$ . An asymmetry in the potential profile may be generated by the simultaneous interaction between S4 residues and negatively charged amino acids within the membrane.

The voltage dependence of the opening rate  $\alpha_H(V)$  of the voltage sensor of a Na or K channel has been described by the empirical function  $(V - V_H) / (1 - \exp[-q_H(V - V_H) / k_B T])$ , where  $q_H$  is the gating charge and  $V_H$  is a constant.<sup>1,36,38</sup> However, from experimental studies

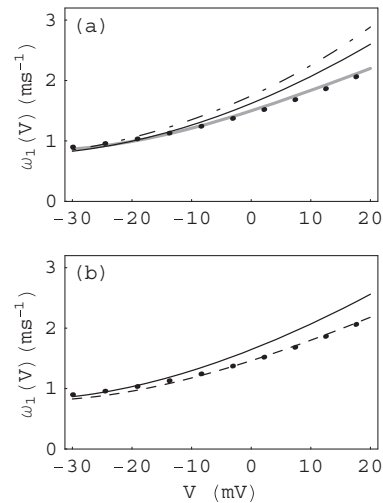


FIG. 5. The voltage dependence of the lowest frequency  $\omega_1$  of the solution of an empirical three step master equation model of activation of a K channel sensor (Ref. 3) (dotted line), and the lowest frequency of the survival probabilities  $P_i(t)$  of the closed states of an alpha helical S4 sensor for (a) the VSD model of Ref. 21 (dotted-dashed line), the VSD model of Fig. 2 (gray line), and the VSD model with the dielectric boundaries  $z_I = 1.5$  Å and  $z_E = 14.6$  Å (solid line), and (b) the VSD model of Fig. 2 with the amino acid D316 located at  $\tilde{\mathbf{r}}_1 = (9.6, 5.4, 3)$  (solid line), and the VSD model with the self-energy barrier  $U_{DI}$  increased to  $7k_B T$ , and the amino acid E283 displaced to the position  $\tilde{\mathbf{r}}_3 = (-7, -7, 13.2)$  (dashed line).

of the late phase of the ionic or gating current during a large membrane depolarization of a cloned K channel, the slowest activation time constant  $\tau \propto \exp(-q_\alpha V / k_B T)$ , where  $q_\alpha \approx 0.3e$ .<sup>2,3</sup> For a K channel VSD model with a symmetric potential profile between energy minima,<sup>21</sup> the partial charges  $q_{\alpha_i} \approx q_{\beta_i} \approx 0.5e$ , and the voltage dependence of  $\omega_1(V) \approx \alpha_1(V) \propto \exp(q_{\alpha_1} V / k_B T)$  is not in agreement with experimental data<sup>2,3</sup> (see Fig. 5(a)). By variation of the relative location of D316 and E293, values of the coordinates of D316 may be determined such that  $\hat{Z}_i < (Z_i + Z_{i+1})/2$  (see Fig. 3) and the lowest frequency  $\omega_1(V) \approx \alpha_1(V) \propto \exp(q_{\alpha_1} V / k_B T)$ ,  $q_{\alpha_1} \approx 0.3e$  is in accord with studies of the voltage dependence of the activation time constant of the late phase of the gating current, for a moderate membrane depolarization (see Fig. 5(a)).<sup>2,3</sup> For each membrane potential, the low frequencies  $\omega_1(V)$ ,  $\omega_2(V)$ , and  $\omega_3(V)$  of the solution of the Smoluchowski equation for the VSD model may be calculated from Eq. (B6) (see Fig. 6). The partial charges  $q_{\alpha_i} = (0.26, 0.32, 0.32)e$  and  $q_{\beta_i} = (0.68, 0.66, 0.66)e$  are in the range of values determined experimentally for the *Shaker IR* K channel,<sup>3</sup> and the approximate equality of  $q_{\alpha_1}$ ,  $q_{\alpha_2}$ , and  $q_{\alpha_3}$  ensures that the sigmoidicity or relative delay of the activation process is almost constant for a large range of depolarizing potentials, consistent with the measurement of macroscopic ionic currents during activation.<sup>2</sup> The voltage dependence of the backward rates  $\beta_i(V) \propto \exp(-q_{\beta_i} V / k_B T)$  may be determined from measurements of the off gating currents in the hyperpolarization range, where each partial charge  $q_{\beta_i} \approx 0.6e$ ,<sup>3</sup> and in agreement with the calculated values. If the structure of the VSD is modified by positioning the inner dielectric boundary closer to D316 and E293 (see Fig. 5(a)) or moving D316 away from E293 and the path of

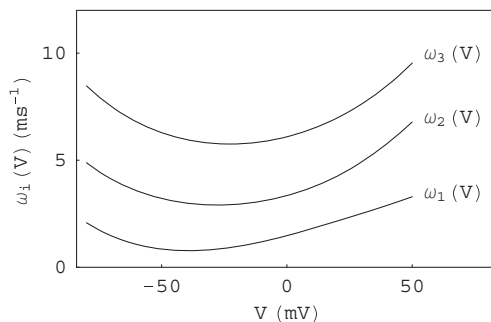


FIG. 6. The voltage dependence of the frequencies  $\omega_1$ ,  $\omega_2$ , and  $\omega_3$  of the survival probabilities of the closed states of an alpha helical S4 sensor may be calculated from Eq. (B6) and the transition rates for the energy barriers of the potential function  $U_A(Z)$  of Fig. 3.

the S4 residues (see Fig. 5(b)), the calculated value of  $q_{\alpha_1}$  is increased and the voltage dependence of the lowest frequency  $\omega_1(V)$  is displaced from the empirical curve determined from the K channel gating current.

The surface charge potential  $V_s$  is estimated from the empirical voltage dependence of the probabilities for each stationary state, and for each clamp potential, the survival probabilities of the closed states  $P_i(t)$  are in approximate agreement with a three step kinetic description of the K channel gating current during the activation process<sup>3</sup> (see Fig. 7). By assum-

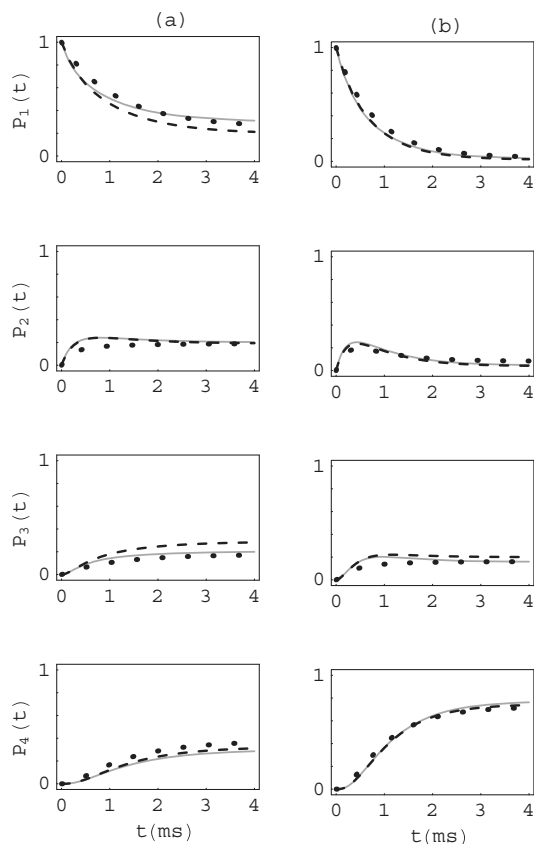


FIG. 7. The probabilities of the closed states of a K channel voltage sensor for an empirical three step master equation model of activation (Ref. 3) from a hyperpolarized state to depolarizing potentials of  $-40$  mV (a) and  $-10$  mV (b) (dotted line), and the survival probabilities calculated from an analytical solution of the Smoluchowski equation for the VSD model of Fig. 2 (gray line), and the VSD model with the lipid group neutralized (dashed line).

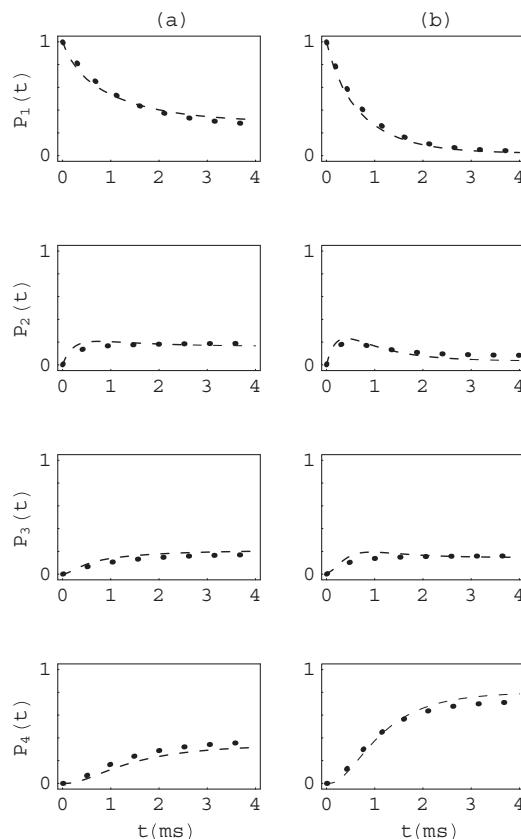


FIG. 8. The probabilities of the closed states of a K channel voltage sensor for an empirical three step master equation model of activation (Ref. 3) from a hyperpolarized state to depolarizing potentials of  $-40$  mV (a) and  $-10$  mV (b) (dotted line), and the survival probabilities calculated from the Smoluchowski equation for the VSD model of Fig. 2 with the self-energy barrier  $U_{DI}$  increased to  $7k_B T$  and the amino acid E283 displaced to the position  $\tilde{\mathbf{r}}_3 = (-7, -7, 13.2)$  (dashed line).

ing the existence of a negative lipid group located within the external solvent in the vicinity of the residue R1 in the activated state, consistent with molecular dynamics simulations (Ref. 27) the potential function  $U_A(Z)$  is modified according to Eq. (3), and the survival probabilities of the closed states  $P_i(t)$  are in better agreement with a three step kinetic description of the K channel gating current<sup>3</sup> (see Fig. 7). Increasing the self-energy barrier for each residue  $U_{DI}$  to  $7k_B T$ , the coordinates of the amino acid E283 relative to a neighboring S4 residue may be determined such that the voltage dependence of the gating current  $I_g(t)$ , the frequency  $\omega_1(V)$ , and the survival probabilities of the closed states  $P_i(t)$  are also in agreement with experimental data (see Figs. 4(b), 5(b), and 8).

## DISCUSSION AND CONCLUSION

Assuming that continuum electrostatics is applicable to the dielectric medium of a gating pore within the VSD of a K channel subunit, an expression for the energy of an S4 sensor within a planar membrane between solvent regions may be derived that is dependent on the potential difference across the membrane, the location of the negative amino acids on S2 and S3 segments and the induced charge at the dielectric boundaries.<sup>20,21</sup> The fast and slow components of the gating

charge within a channel may be derived from an analytical solution of the Smoluchowski equation, and the low frequency terms for the survival probabilities for each of the stationary states may be expressed as a master equation.<sup>21</sup> By variation of the parameters of the model, a good fit between the calculated gating current and survival probabilities of each state, and the empirical data may be derived. If it is assumed that the S4 segment has a  $3_{10}$  helical structure and moves only in the transverse direction between stationary states without a large rotation,<sup>45</sup> a set of parameters may also be determined that result in good agreement with the voltage dependence of the data (unpublished). The potential function may be further modified to incorporate an additional interaction between S4 residues and either a negative amino acid on another segment or a phosphate group within the internal lipid layer.<sup>41</sup>

The relative location of D316 and E293 and their distance from the internal dielectric boundary may be determined such that, for a moderate membrane depolarization, the lowest frequency of the solution of the Smoluchowski equation  $\omega_1(V) \propto \exp(q_\alpha V/k_B T)$ ,  $q_\alpha \approx 0.3e$ , and for reverse transitions in the hyperpolarization range,  $\omega_1(V) \propto \exp(-q_\beta V/k_B T)$ ,  $q_\beta \approx 0.6e$ , and in agreement with studies of the voltage dependence of activation and deactivation time constants of the late phase of the gating current.<sup>2,3</sup> The surface charge potential is estimated from the empirical voltage dependence of the stationary probabilities for each state, and by variation of the distance between E283 and a neighboring S4 residue for each value of the self-energy barrier  $U_{DI}$ , the gating current  $I_g(t)$ , and the survival probabilities of the closed states of the voltage sensor are also in accord with a three step master equation model of K channel activation.<sup>3</sup>

The parameters of the VSD model that are required for the correct voltage dependence are generally consistent with values that are determined from independent simulation and experimental studies of activation in K channels. The assumed resting state has been based on a structural model of the K channel compatible with a wide range of biophysical data and the Kv1.2 crystal structure,<sup>41,46,47</sup> where the first arginine R1 is located in the gating pathway between the internal and external aqueous cavities, and in the activated state, the fourth residue R4 moves to a position previously occupied by R1. The focused electric field across the low dielectric membrane of thickness 13 Å ensures that a large gating charge is possible for a moderate transverse displacement of the S4 sensor and is supported by the voltage-dependent accessibility of cysteine residues,<sup>8</sup> x-ray structures of the ion channel,<sup>41</sup> and the conduction of protons and cations through the VSD following histidine substitutions of S4 residues.<sup>10,11</sup> The ion-ion interaction barriers are assumed to be of the order of  $8k_B T$ , consistent with the measurement of salt-bridge energy between residues in the OmpA channel,<sup>42</sup> and a self-energy barrier  $U_{DI}$  between 4 and  $7k_B T$  has been estimated from solutions of the Poisson equation for a cylindrical channel and Monte Carlo simulations of ion permeation.<sup>25</sup> The effective value of the dielectric constant  $\epsilon_m$  assumed for the low dielectric medium is similar to the value derived from molecular dynamics simulations for ion-ion interactions within a channel pore.<sup>26</sup> The separation between the position of the amino acid E283 close

to the external dielectric boundary of the VSD model, and the amino acids D316 and E293 is  $\sim 9$  Å, consistent with x-ray diffraction data,<sup>41</sup> and thus permits the simultaneous interaction of a pair of S4 residues with the amino acids E293 and E283 within the gating pore, an important feature of the activation process.<sup>48</sup>

Therefore, the experimental voltage dependence of the activation of an ion channel sensor may be described qualitatively by solutions of a stochastic diffusion equation where the potential function is determined by the principal electrostatic forces on the residues of the S4 segment in an inhomogeneous dielectric medium, and the estimated values of the parameters are compatible with independent empirical studies. Although the model does not take account of the nonlinear variation of the electrostatic potential at the membrane boundary, and the expressions for the self-energy of residues of the S4 segment and the ion-ion interaction energy are only approximate, the accuracy of the voltage dependence of the gating current and the survival probabilities of the closed states may be assessed by comparison with the results from a numerical solution of the equations for an irregular dielectric boundary between the solvent and a gating pore within the membrane.

## APPENDIX A: ELECTROSTATIC POTENTIAL OF A CHARGE WITHIN AN INHOMOGENEOUS DIELECTRIC MEDIUM

An expression for the electrostatic potential of a charge within a dielectric slab between aqueous regions may be derived from Poisson's equation. If the charge  $\tilde{Q}_j$  within the dielectric medium  $\epsilon_m$  is positioned at  $\tilde{z}_j$  on the  $z$  axis of an earthed conducting cylinder of radius  $c$ , the electrostatic potential  $\psi$  at a point  $(r, \Omega, z)$ ,  $z > \tilde{z}_j$  satisfies the Laplace equation in cylindrical coordinates,

$$\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial \psi}{\partial r} \right) + \frac{1}{r^2} \frac{\partial^2 \psi}{\partial \Omega^2} + \frac{\partial^2 \psi}{\partial z^2} = 0, \quad (\text{A1})$$

with a general solution,

$$\psi(r, z) = \sum_{l=1}^{\infty} A_l e^{-k_l(z-\tilde{z}_j)} J_0(k_l r), \quad z > \tilde{z}_j, \quad (\text{A2})$$

where  $k_l$  satisfies the boundary condition  $J_0(k_l c) = 0$ ,  $A_l$  is dependent on  $k_l$ , and from Gauss's law, the upward flux through  $z = \tilde{z}_j$  is

$$\iint \frac{\partial \psi}{\partial z} \Big|_{z=\tilde{z}_j} r d\Omega dr = -\frac{\tilde{Q}_j}{2\epsilon_m}. \quad (\text{A3})$$

Multiplying the derivative of Eq. (A2) by  $r J_0(k_m r)$ , and integrating from  $r = 0$  to  $c$ , and  $\Omega = 0$  to  $2\pi$ ,

$$\begin{aligned} & \iint J_0(k_m r) \frac{\partial \psi}{\partial z} \Big|_{z=\tilde{z}_j} r d\Omega dr \\ &= 2\pi \sum_{l=1}^{\infty} A_l (-k_l) \int_0^c J_0(k_l r) J_0(k_m r) r dr, \end{aligned} \quad (\text{A4})$$

where the Bessel functions satisfy the orthonormal condition,

$$\int_0^c J_0(k_l r) J_0(k_m r) r dr = \delta_{lm} \int_0^c J_0(k_l r)^2 r dr. \quad (\text{A5})$$



Therefore,

$$A_l = \frac{\tilde{Q}_j}{4\pi\epsilon_m k_l} \frac{J_0(0)}{\int_0^c J_0(k_l r)^2 r dr}, \quad (\text{A6})$$

where  $\int_0^c J_0(k_l r)^2 r dr = (c^2/2)J_1(k_l c)^2$ ,  $J_0(0) = 1$ , and hence

$$\psi(r, z) = \frac{\tilde{Q}_j}{2\pi\epsilon_m} \sum_{l=1}^{\infty} \frac{J_0(k_l r)}{c^2 k_l J_1(k_l c)^2} e^{-k_l(z-\tilde{z}_j)}, \quad z > \tilde{z}_j. \quad (\text{A7})$$

A similar result may be derived for  $z < \tilde{z}_j$ , and for large  $c$ ,  $\pi/c = k_{l+1} - k_l = \Delta k$ , and in the limit  $c \rightarrow \infty$ ,  $J_1(k_l c) = (2/\pi k_l c)^{1/2}$  and, therefore,

$$\psi(r, z) = \frac{\tilde{Q}_j}{4\pi\epsilon_m} \int_0^{\infty} J_0(kr) e^{-k|z-\tilde{z}_j|} dk. \quad (\text{A8})$$

If the charge  $\tilde{Q}_j$  is positioned within a dielectric slab ( $z_l \leq \tilde{z}_j \leq z_E$ ) with dielectric constant  $\epsilon_m$  between internal and external solvents ( $\epsilon_w$ ), the solution is

$$\begin{aligned} \psi_{jI}(r, z) &= \frac{\tilde{Q}_j}{\epsilon_m} \int_0^{\infty} \chi_I(k) J_0(kr) e^{kz} dk, \quad z < z_l, \\ \psi_{jm}(r, z) &= \frac{\tilde{Q}_j}{\epsilon_m} \left( \int_0^{\infty} J_0(kr) e^{-k|z-\tilde{z}_j|} dk \right. \\ &\quad \left. + \int_0^{\infty} \chi_1(k) J_0(kr) e^{kz} dk \right. \\ &\quad \left. + \int_0^{\infty} \chi_2(k) J_0(kr) e^{-kz} dk \right), \quad z_l < z < z_E, \\ \psi_{jE}(r, z) &= \frac{\tilde{Q}_j}{\epsilon_m} \int_0^{\infty} \chi_E(k) J_0(kr) e^{-kz} dk, \quad z > z_E, \end{aligned} \quad (\text{A9})$$

satisfying the boundary conditions,

$$\begin{aligned} \psi_{jI}(r, z) &= \psi_{jm}(r, z), \quad z = z_l, \\ \psi_{jE}(r, z) &= \psi_{jm}(r, z), \quad z = z_E, \\ \epsilon_w \frac{\partial \psi_{jI}}{\partial z} &= \epsilon_m \frac{\partial \psi_{jm}}{\partial z}, \quad z = z_l, \\ \epsilon_w \frac{\partial \psi_{jE}}{\partial z} &= \epsilon_m \frac{\partial \psi_{jm}}{\partial z}, \quad z = z_E, \end{aligned} \quad (\text{A10})$$

and, therefore,

$$\begin{aligned} \chi_1(k) &= \frac{\lambda e^{k(\tilde{z}_j-2z_E)} + \lambda^2 e^{-k(\tilde{z}_j+2z_T)}}{1 - \lambda^2 e^{-2kz_T}}, \\ \chi_2(k) &= \frac{\lambda e^{-k(\tilde{z}_j-2z_l)} + \lambda^2 e^{k(\tilde{z}_j-2z_T)}}{1 - \lambda^2 e^{-2kz_T}}, \\ \chi_I(k) &= \frac{(\lambda+1)(e^{-k\tilde{z}_j} + \lambda e^{k(\tilde{z}_j-2z_E)})}{1 - \lambda^2 e^{-2kz_T}}, \\ \chi_E(k) &= \frac{(\lambda+1)(e^{k\tilde{z}_j} + \lambda e^{-k(\tilde{z}_j-2z_l)})}{1 - \lambda^2 e^{-2kz_T}}. \end{aligned} \quad (\text{A11})$$

where  $z_T = z_E - z_l$ . From the identities,

$$\begin{aligned} \int_0^{\infty} J_0(kr) e^{-k|z-\tilde{z}_j|} dk &= (r^2 + (z - \tilde{z}_j)^2)^{-1/2}, \\ \int_0^{\infty} J_0(kr) e^{\pm k(z+\Delta)} dk &= (r^2 + (z + \Delta)^2)^{-1/2}, \end{aligned} \quad (\text{A12})$$

it may be shown that

$$\begin{aligned} \psi_{jI}(\mathbf{r}) &= \frac{2}{(\epsilon_m + \epsilon_w)} \left( \frac{\tilde{Q}_j}{|\mathbf{r} - \tilde{\mathbf{r}}_j|} + \sum_{n=1}^{\infty} \frac{\lambda^n \tilde{Q}_j}{|\mathbf{r} - \tilde{\mathbf{r}}_{jE,n}|} \right), \quad z_l < z_l, \\ \psi_{jm}(\mathbf{r}) &= \frac{1}{\epsilon_m} \left( \frac{\tilde{Q}_j}{|\mathbf{r} - \tilde{\mathbf{r}}_j|} + \sum_{n=1}^{\infty} \frac{\lambda^n \tilde{Q}_j}{|\mathbf{r} - \tilde{\mathbf{r}}_{jI,n}|} \right. \\ &\quad \left. + \sum_{n=1}^{\infty} \frac{\lambda^n \tilde{Q}_j}{|\mathbf{r} - \tilde{\mathbf{r}}_{jE,n}|} \right), \quad z_l \leq z_l \leq z_E, \\ \psi_{jE}(\mathbf{r}) &= \frac{2}{(\epsilon_m + \epsilon_w)} \left( \frac{\tilde{Q}_j}{|\mathbf{r} - \tilde{\mathbf{r}}_j|} + \sum_{n=1}^{\infty} \frac{\lambda^n \tilde{Q}_j}{|\mathbf{r} - \tilde{\mathbf{r}}_{jI,n}|} \right), \quad z_l > z_E, \end{aligned}$$

where  $\tilde{\mathbf{r}}_j = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_j)$ ,  $j = 1, 2, 3$ ,  $\tilde{\mathbf{r}}_{jE,n} = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_{jE,n})$ ,  $\tilde{\mathbf{r}}_{jI,n} = (\tilde{x}_j, \tilde{y}_j, \tilde{z}_{jI,n})$ ,  $\lambda = (\epsilon_m - \epsilon_w)/(\epsilon_m + \epsilon_w)$ , and

$$\begin{aligned} \tilde{z}_{jE,n} &= (-1)^n \left[ \tilde{z}_j - \frac{(z_l + z_E)}{2} \right] \\ &\quad + z_E \left( n + \frac{1}{2} \right) - z_l \left( n - \frac{1}{2} \right) > z_E, \\ \tilde{z}_{jI,n} &= (-1)^n \left[ \tilde{z}_j - \frac{(z_l + z_E)}{2} \right] \\ &\quad + z_l \left( n + \frac{1}{2} \right) - z_E \left( n - \frac{1}{2} \right) < z_l. \end{aligned}$$

If the charge  $\tilde{Q}_j$  is positioned above the dielectric slab ( $\tilde{z}_j > z_E$ ), it may be shown that the solution of Poisson's equation that satisfies the boundary conditions Eq. (A10) is

$$\begin{aligned} \psi_j(\mathbf{r}) &= \frac{1 - \lambda^2}{\epsilon_w} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} + \sum_{n=1}^{\infty} \frac{\lambda^{2n} \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE,n}|} \right), \quad z_l < z_l, \\ &= \frac{2}{(\epsilon_m + \epsilon_w)} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} + \sum_{n=1}^{\infty} \frac{\lambda^{2n-1} \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jI,n}|} \right. \\ &\quad \left. + \sum_{n=1}^{\infty} \frac{\lambda^{2n} \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jE,n}|} \right), \quad z_l \leq z_l \leq z_E, \\ &= \frac{1}{\epsilon_w} \left( \frac{\tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_j|} - \frac{\lambda \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jI,0}|} \right. \\ &\quad \left. + (1 - \lambda^2) \sum_{n=1}^{\infty} \frac{\lambda^{2n-1} \tilde{Q}_j}{|\mathbf{r}_l - \tilde{\mathbf{r}}_{jI,n}|} \right), \quad z_l > z_E, \end{aligned}$$

where  $\tilde{z}_{jE,n} = \tilde{z}_j + 2n(z_E - z_l)$ , and  $\tilde{z}_{jI,n} = -\tilde{z}_j + 2z_E - 2n(z_E - z_l)$ .<sup>49</sup>

## APPENDIX B: SOLUTION OF THE SMOLUCHOWSKI EQUATION FOR AN S4 SENSOR WITH FOUR STATES

The solution of the stochastic diffusion equation, Eq. (4), for an S4 sensor in a square well potential with four stationary states may be derived by application of the Laplace transform method.<sup>21</sup> Defining  $\tilde{p}_i(Z, s) = \int_0^{\infty} p_i(Z, t) e^{st} dt$ , and  $\tilde{P}_i(s) = \int_{b_i}^{d_i} \tilde{p}_i(Z, s) dZ$ , where  $p_i(Z, t)$  is the probability density in the region  $R_i$ ,  $i = 1-4$ , the Laplace transform

$\tilde{P}_i(s)$  of the survival probabilities may be determined from the interface boundary conditions and the initial condition  $p_1(Z, 0) = \delta(Z - b_1)$ ,  $p_i(Z, 0) = 0$  for  $i > 1$ , and inverting the transform, the solution may be expressed as an infinite sum of high frequency components, describing the redistribution of the probability density within each region, and low frequency terms

$$P_1(t) = P_{1s} + \alpha_1 \sum_{i=1}^3 a_i g_1(\omega_i) \exp(-\omega_i t), \quad (\text{B1})$$

$$P_2(t) = P_{2s} - \alpha_1 \sum_{i=1}^3 a_i g_2(\omega_i) \exp(-\omega_i t), \quad (\text{B2})$$

$$P_3(t) = P_{3s} - \alpha_1 \alpha_2 \sum_{i=1}^3 a_i (\beta_3 - \omega_i) \exp(-\omega_i t), \quad (\text{B3})$$

$$P_4(t) = P_{4s} - \alpha_1 \alpha_2 \alpha_3 \sum_{i=1}^3 a_i \exp(-\omega_i t), \quad (\text{B4})$$

where the transition rates between states

$$\alpha_i = \frac{\hat{D}_i \exp[U_i(d_i) - \hat{U}_i]}{\Delta_i \int_{d_i}^{b_{i+1}} \exp[\hat{U}_i(Z) - \hat{U}_i] dZ},$$

$$\beta_i = \frac{\hat{D}_i \exp[U_{i+1}(b_{i+1}) - \hat{U}_i]}{\Delta_{i+1} \int_{d_i}^{b_{i+1}} \exp[\hat{U}_i(Z) - \hat{U}_i] dZ}, \quad (\text{B5})$$

the frequencies  $\omega_i$  are determined from the solution of the eigenvalue equation,

$$E(\omega) = \omega^3 - e_1 \omega^2 + e_2 \omega - e_3 = 0, \quad (\text{B6})$$

$\hat{D}_i(D_i)$  is the diffusion parameter in  $\hat{R}_i(R_i)$ ,  $\Delta_i = d_i - b_i$ ,  $g_1(\omega) = \omega^2 - (\alpha_2 + \alpha_3 + \beta_2 + \beta_3)\omega + \alpha_2(\alpha_3 + \beta_3) + \beta_2 \beta_3$ ,  $g_2(\omega) = \omega^2 - (\alpha_3 + \beta_2 + \beta_3)\omega + \beta_2 \beta_3$ ,  $P_{1s} = a_0 \beta_1 \beta_2 \beta_3$ ,  $P_{2s} = a_0 \alpha_1 \beta_2 \beta_3$ ,  $P_{3s} = a_0 \alpha_1 \alpha_2 \beta_3$ ,  $P_{4s} = a_0 \alpha_1 \alpha_2 \alpha_3$ ,  $a_i^{-1} = \omega_i \prod_{j \neq i} (\omega_j - \omega_i)$ ,  $a_0^{-1} = \omega_1 \omega_2 \omega_3$ ,  $\omega_1 < \omega_2 < \omega_3$ ,  $e_1 = \sum_{i=1}^3 (\alpha_i + \beta_i)$ ,  $e_2 = \alpha_1(\alpha_2 + \alpha_3 + \beta_2 + \beta_3) + \alpha_2(\alpha_3 + \beta_3) + \beta_2 \beta_3 + \beta_1(\alpha_3 + \beta_2 + \beta_3)$ , and  $e_3 = \alpha_1 \alpha_2 (\alpha_3 + \beta_3) + \beta_2 \beta_3 (\alpha_1 + \beta_1)$ .

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