Supporting Text

Here we supply some additional material concerning the implementation of the numerical aspects of the manuscript.

**Boundary Conditions for $\Phi^m$ and $\Phi^p$.** In a typical cell, electrogenic transporters create a difference in the electrical potential inside the cell versus outside the cell. Typically the cytoplasmic potential, $V_{in}$, is $-60$ to $-80$ mV while the extracellular potential, $V_{out}$, is zero. We will always choose $V_{out} = 0$. A small violation in electroneutrality near the membrane gives rise to this potential difference; however, more than a Debye length from the membrane electroneutrality is restored. It is possible to model this behaviour with the Poisson-Boltzmann equation. For simplicity, we start with the linearized Poisson-Boltzmann equation:

$$-\nabla \cdot [\varepsilon(\vec{r})\nabla \phi(\vec{r})] + \kappa^2(\vec{r})\phi(\vec{r}) = \frac{e}{k_B T} 4\pi \rho(\vec{r}).$$  \(1\)

However, it is easy to see that this equation does not satisfy the asymptotic boundary conditions: $\Phi(x, y, z \to -\infty) \to V_{in}$. This oversight can easily be fixed by adding the appropriate constant term to the equation for positions in the inner solution space:

$$-\nabla \cdot [\varepsilon(\vec{r})\nabla \phi(\vec{r})] + \kappa^2(\vec{r})\left(\phi(\vec{r}) - f(\vec{r})\frac{e}{k_B T} V_{in}\right) = \frac{e}{k_B T} 4\pi \rho(\vec{r}),$$  \(2\)

where $f(\vec{r})$ is 1 for all points in the inner solution space and zero otherwise. Now far from the protein charge density where the variation of $\phi$ goes to zero, $\Phi(x, y, z \to -\infty) \to V_{in}$ as desired. We can rewrite Eq. 2 as

$$-\nabla \cdot [\varepsilon(\vec{r})\nabla \phi(\vec{r})] + \kappa^2(\vec{r})\phi(\vec{r}) = \frac{e}{k_B T} 4\pi \left(\rho(\vec{r}) + \frac{\kappa^2 V_{in}}{4\pi} f(\vec{r})\right).$$  \(3\)

So now we see that the modified Poisson-Boltzmann equation takes the same form as Eq. 1 with the membrane potential arising from a term that enters like a uniform source charge. On the right hand side, the spatial dependence of $\kappa$ is carried by $f(\vec{r})$. The linearity of Eq. 3 permits us to write $\phi = \phi^p + \phi^m$:

$$-\nabla \cdot [\varepsilon(\vec{r})\nabla \phi^p(\vec{r})] + \kappa^2(\vec{r})\phi^p(\vec{r}) = \frac{e}{k_B T} 4\pi \rho(\vec{r}),$$

$$-\nabla \cdot [\varepsilon(\vec{r})\nabla \phi^m(\vec{r})] + \kappa^2(\vec{r})\phi^m(\vec{r}) = \frac{e}{k_B T} 4\pi \frac{\kappa^2 V_{in}}{4\pi} f(\vec{r}),$$  \(4\)

where $p$ indicates the potential arising from the protein charges and $m$ is the potential arising from the slight charge separation across the membrane. Far away from the protein, $\phi^p$ is zero. This condition is included in all Poisson-Boltzmann solvers; however, the boundary conditions for $\phi^m$ are not standard, and they must be determined and implemented.
We will solve for \( \phi^m \) assuming a planar slab of low dielectric material with symmetric electrolyte solution in the half-spaces above and below the slab. This presentation follows that of Roux (2) with a slight change in reference geometry. By symmetry \( \phi^m(\vec{r}) = \phi(z) \), and we assign \( z = 0 \) to the center of the membrane slab. The slab is of length \( L \) and there are three distinct regions: \( z > L/2 \) (out); \( L/2 \geq z \geq -L/2 \) (membrane); and \( z < -L/2 \) (in). The dielectric constant of water is \( \epsilon_w \), and the dielectric constant of the membrane is assigned \( \epsilon_m \). The membrane region is assigned zero ion accessibility, \( \kappa = 0 \), whereas the inner and outer spaces have the same value of the screening parameter. According to Eq. 4 the appropriate differential equations in each region become

\[
\begin{align*}
-\epsilon_w \frac{\partial^2 \phi^m_1(z)}{\partial z^2} + \kappa^2 \phi^m_1(z) &= 0 \quad \text{(region 1, out)} \\
-\epsilon_m \frac{\partial^2 \phi^m_2(z)}{\partial z^2} &= 0 \quad \text{(region 2, membrane)} \\
-\epsilon_w \frac{\partial^2 \phi^m_3(z)}{\partial z^2} + \kappa^2 (\phi^m_3(z) - \frac{e}{k_B T} V_{in}) &= 0 \quad \text{(region 3, in).}
\end{align*}
\]

From elementary electrostatics, we know that the potential is continuous at the membrane boundaries but the \( z \) component of the electric field is discontinuous because of the jump in dielectric value:

\[
\begin{align*}
\phi^m_3(-L/2) &= \phi^m_2(-L/2); \quad \epsilon_w \partial_z \phi^m_3|_{-L/2} = \epsilon_m \partial_z \phi^m_2|_{-L/2} \\
\phi^m_2(L/2) &= \phi^m_1(L/2); \quad \epsilon_m \partial_z \phi^m_2|_{L/2} = \epsilon_w \partial_z \phi^m_1|_{L/2}.
\end{align*}
\]

With some algebra Eqs. 5 and 6 can be solved for the potential profile in the absence of an inclusion protein:

\[
\begin{align*}
\phi^m_1(z) &= \frac{e}{k_B T} V_{in} \frac{1 - e^\kappa (L/2 - z)}{e^{\kappa L} + e^{\kappa z}} \quad \text{(region 1, out)} \\
\phi^m_2(z) &= \frac{e}{k_B T} V_{in} \left( \frac{1}{2} - \frac{1}{e^{\kappa L} + e^{\kappa z}} \right) \quad \text{(region 2, membrane)} \\
\phi^m_3(z) &= \frac{e}{k_B T} V_{in} \left( 1 - \frac{1}{e^{\kappa L} + e^{\kappa z}} \right) \quad \text{(region 3, in)}
\end{align*}
\]

where \( \kappa^2 \equiv \epsilon_w \kappa^2 \). When calculations are performed using APBS in the presence of an electric field, the analytic solution, Eq. 7, is used to assign \( \phi^m \) on the domain boundary. In order to specify this solution, the vertical values of the top and bottom of the membrane and the dielectric constant of the membrane must be provided at the solve step.

**Rewriting Dielectric, Charge, and Ion-Accessibility Maps.** Whether solving for \( \phi^m \) or \( \phi^p \), the influence of the membrane and implicit protein must be included in the calculation. Initially, the S4 helix was placed in the desired spatial configuration. This orientation was then used to generate dielectric, charge, and ion-accessibility maps of the molecule in solution using APBS 0.3.1 (1). Maps were generated at a coarse level and then at a finer level. We refer to this set of maps as the positive set, and a second set of maps was generated in the same manner, but with all of the molecule charges set to zero. We call this second set of maps the neutral set. The dielectric value of the S4 segment was assigned \( \epsilon = 2.0 \), and the solvent boundary was created by using the molecular surface definition.
Both sets were then modified to add the presence of a generalized protein embedded in a low-dielectric slab acting as a surrogate membrane. The APBS 0.3.1 solver uses a finite differencing scheme; therefore, all map points are associated with a regular grid in 3-space. Code was written to read in the initial text maps, and then the numeric value of points on the grid were modified based on the spatial position. Every value of the grid was looped over and checked in the following order:

(i) Determine if the point is inside the S4 helix. If the initial dielectric map value equals $\epsilon_p = 2.0$, the point is within the helix. Dielectric map values are not changed for these points.

(ii) Determine if the point is inside the implicit protein. A series of conditional statements are checked in order to determine if the point falls within the implicit protein. These conditions depend on the particular hypothetical geometries posited in the main text. Points in the implicit protein have the ion-accessibility map value set to zero. If the point falls within the implicit protein and the initial value of the dielectric map is not $\epsilon_p = 2.0$, indicating that the point is NOT inside the S4 helix, then the dielectric map value is assigned $\epsilon = 10.0$. Charge maps are not changed.

(iii) Determine if the point is inside the membrane. If the point does not fall within the S4 helix or the implicit protein and it falls within the $z$ extent of the membrane, $-15 \text{ Å} \leq z \leq 15 \text{ Å}$, the value of the dielectric map is set to 2.0, the ion-accessibility is set to zero, and the charge map is not changed.

(iv) Determine if the point is in the inner solution space. If the point falls below all implicit protein and the membrane and the initial value of the ion-accessibility is NOT zero, then the neutral charge map is modified for the calculations of $\phi^m$. The value given to the charge map position is determined from the right hand side of Eq. 4 (bottom equation). The effective charge density, $\rho_{\text{eff}}$, follows from the right hand sides of the upper and lower equations:

$$\frac{e}{k_B T} 4\pi \rho_{\text{eff}} = \frac{e}{k_B T} 4\pi \kappa^2 V_{\text{in}}.$$

The text maps are written in terms of the number density, $n_{\text{eff}} = \rho_{\text{eff}}/e$, rather than the charge density. This consideration of the number density along with the definition of the Debye length gives the modified value for the charge map:

$$n_{\text{eff}} = \epsilon_\text{w} \frac{\kappa^2 V_{\text{in}}}{4\pi e} = \frac{\epsilon_\text{w} V_{\text{in}}}{4\pi e} \left( \frac{8\pi e^2 N_a I}{\epsilon_\text{w} k_B T} \right),$$

where $I$ is the molar concentration of one of the salt species (assumed balanced) and $N_a$ is Avogadro’s number. The Debye constant above is twice the value that you will find on page 497 of ref. 3, because here it is assumed that there are mobile cationic and anionic species, not just one mobil species. Simplifying this equation, we arrive at

$$n_{\text{eff}} = 0.001204428 I u_{\text{in}},$$

3
where \( u_{in} = e V_{in}/k_B T \) is the reduced inner potential and the counter-ion concentration is given in moles per liter. The effective number density is now in inverse Ångstroms cubed, which is consistent with the APBS solver.

**The Solution Step for Electrostatic Calculations.** Electrostatic solvation energies were calculated with the nonlinear equation, and the membrane potential calculations were solved in a separate step using the linearized version. In both cases, two levels of focusing were used. In the first level, Dirichlet boundary conditions were assigned to the edge of a 300-Å cubed box centered on the helix. Zero value was assigned to \( \phi^p \) on the boundary, while the analytic solution to the Poisson-Boltzmann equation for a planar geometry developed in the first section was used to assign \( \phi^m \). The numeric grid then focused to a cube with a 60-Å side length. The final spatial discretization at the finest level was 0.6 Å per grid point. Dummy runs for each of the two levels were first run in APBS to create dielectric, charge, and ion-accessibility maps for the S4 helix in solution using the molecular surface definition. These maps were then modified as described in the last section.

All calculations included a symmetric 100 mM salt concentration and were carried out at 298.15 K. Dielectric smoothing near the membrane and protein surfaces was not used. The solvent probe radius was set to 2 Å for the atomistic S4 segment; however, we ignored the probe radius with respect to the low-resolution implicit protein and bilayer.

Each configuration of the S4 molecule involved three calculations: (i) The total electrostatic energy of the S4 helix in solution, corresponding to \( \Delta G^1 \) in Fig. 1d; (ii) the total electrostatic energy of the S4 helix in the membrane-protein complex, corresponding to \( \Phi^p \); and (iii) the electrostatic energy of the S4 helix in the presence of the membrane potential, corresponding to \( \Phi^m \). The rewritten maps from the last section are read into APBS in order to calculate \( \Phi^p \) and \( \Phi^m \) for steps ii and iii. Step ii uses the positive set of maps and Step iii uses the neutral set of maps. The charged protein was subsequently used to calculate \( E^m \) from \( \Phi^m \) as in Eq. 3 from the main text. In principle, Step i need only be computed once; however, for numerical accuracy, one must always calculate steps i and iii with the S4 helix in the same configuration. This redundancy is due to the singular behavior of \( \phi^p \) at the positions of the point charges in the protein.

**Estimate of Nonpolar Energies.** The nonpolar component of the free energy for transfer from water to lipid for a solute molecule is empirically proportional to solvent exposed surface area

\[
\Delta G^{np} = \gamma \cdot A + b,
\]

where \( \gamma = 0.0469 \ k_B T/\AA^2 \) and \( b = -2.89 \ k_B T \) (4). This empirical expression describes the transfer of solute from water to lipid. Two of our models involve the transfer of solute from water to protein; however, we apply the formula uniformly to all three models while realizing that the van der Waals component of \( \Delta G^{np} \) is different for these two cases.

In solution, the total solvent accessibly surface area of the S4 helix is 2,600 Å². We have estimated the percentage of buried surface area in the three different models based on visual inspection of the models as in Fig. 2, the corresponding energy with respect to the fully solvated state is in parenthesis:
<table>
<thead>
<tr>
<th>model</th>
<th>down state</th>
<th>middle</th>
<th>up state</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipid-exposed</td>
<td>85% (-100 $k_BT$)</td>
<td>100% (-120 $k_BT$)</td>
<td>70% (-85 $k_BT$)</td>
</tr>
<tr>
<td>translation</td>
<td>25% (-30 $k_BT$)</td>
<td>25% (-30 $k_BT$)</td>
<td>25% (-30 $k_BT$)</td>
</tr>
<tr>
<td>rotation</td>
<td>50% (-60 $k_BT$)</td>
<td>50% (-60 $k_BT$)</td>
<td>50% (-60 $k_BT$)</td>
</tr>
</tbody>
</table>

The amount of buried surface area for both the translation and rotation models is nearly constant throughout the reaction pathway.

In the main text, we discuss the difference in total solvation energy between the down and up states under zero applied field. From our estimates, the nonpolar energy is nearly the same in both of these states for the translation and rotation models. Therefore, only the electrostatic component of the free energy, $E^p$, contributes to the profile shape; and from Fig. 3 b and c, we see that the up state is 10 $k_BT$ lower than the down state. From the table above and Fig. 3a of the main text, the up state of the lipid-exposed model is $\Delta E^p + \Delta G^{np} = ((284)_{up} - (318)_{down}) + ((-85)_{up} + (-100)_{down}) = -20 k_BT$ lower than the down state.

**Estimating Activation Times.** From Kramers’ reaction rate theory, the mean first passage time, $\tau$, for a process which must first surmount a sizable energy barrier is given by the inverse of the escape rate:

$$
\tau = \frac{2\pi}{D\sqrt{\phi''_{max}|\phi''_{min}|}} e^{\phi_{max}-\phi_{min}},
$$

(8)

where $\phi_{min,max}$ are the values of the reduced energy at the initial minimum and the barrier crossing, respectively (5). In our case, min refers to the down state. The reduced activation energy is $\Delta G = \phi_{max} - \phi_{min}$, and generally, this term dominates the kinetics. However, the shape of the energy profile influences the reaction time through the curvature of the profile both at the barrier crossing, $\phi''_{max}$, and at the initial resting position, $\phi''_{min}$.

The activation barrier, $\Delta G$, is a combination of the nonpolar and electrostatic energies. From the curves in Fig. 3 a and b (redrawn here in Fig. 6) and the nonpolar energies from the last section, we arrive at barrier heights. For both models we have

$$
\Delta G = \frac{(440)_{max} - (320)_{min} + (-120)_{max} - (-100)_{min}}{2} = 100 k_BT \quad \text{(lipid-exposed)}
$$

$$
\Delta G = \frac{(52)_{max} - (40)_{min} + (-30)_{max} - (-30)_{min}}{2} = 12 k_BT \quad \text{(translation)}.
$$

(9)

Secondly, we must estimate the curvature of the solvation energy profiles at the maximum and minimum values. We have estimated the curvatures in Fig. 6, where we fit quadratic approximations to the electrostatic free energy profiles at the relevant positions. We ignored the influence of the nonpolar component to the solvation free energy on the curvature for the lipid-exposed model in doing this. As we will see, the characteristic change in energy with distance in these models means $|\phi''|$ is usually bound between 0.01 and 10. From the fits presented in the caption of Fig. 6, we see that the lipid-exposed model has $|\phi''|_{max} = 3$ and that the translation model has $|\phi''|_{max} = 0.6$. 


Despite energies that are separated by hundreds of $k_B T$, these profiles have curvatures that are within a factor of 5. A shortcoming of this analysis is that, over the given range, the lipid-exposed model lacks an energy minimum, and the minimum of the translation model is a very weak metastable state. In fact, we see that the green quadratic curve in Fig 6b, which gives $|\phi''|_{\text{min}} = 2$, is not a very good fit to the energy profile. The closed, or minimum, energy states were identified from our interpretation of models from the literature; it is most likely that the linker regions between S4-S3 and S4-S5 act as springs to restrain the S4 segment from such extreme motions. Because of the low-resolution approach of our analysis, we have not included the mechanical effects of other helices on the free energy of the S4 segment; however, it is unlikely that the S4 segment could move 20 Å without inducing such stresses in the nearby portions of the channel protein. Therefore, we assume that there exists an inner free energy minimum for S4 near $z = -10$ in Fig. 6a for the lipid-exposed model. Based on the three fits presented here, it is reasonable to assume that the curvature of this minimum will be on the order of 1.

Finally, there is no indication of what the diffusion coefficient for the S4 segment should be along the respective reaction coordinates. We have taken the lateral diffusion coefficient for gramicidin C in lipid bilayer as an estimate, $D = 3 \times 10^8 \, \text{Å}^2/s$ (6); however, this is an open question as discussed in the main text. By using Eq. 8, the estimates of the activation times are then

\[
\tau = \frac{2\pi}{3 \times 10^8 \sqrt{1.3}} e^{100} \approx 3 \times 10^{38} \, \text{ms} \quad \text{(lipid - exposed)}
\]

\[
\tau = \frac{2\pi}{3 \times 10^8 \sqrt{2.06}} e^{12} \approx 3 \, \text{ms} \quad \text{(translation).}
\]

(10)

This final calculation highlights the disparity between the two models. The errors created from our assumptions of the curvatures of the two energy profiles are eclipsed by the large energy barrier presented to the S4 helix by the lipid-exposed model, which makes activation nearly impossible. Meanwhile, this back of the envelope calculation gives very reasonable results for the translation model.


