Supporting Text

Transmembrane Potential. The transmembrane potential needed to simulate ion fluxes was calculated by using a modified Poisson-Boltzmann (PB) theory, in which the intra and extracellular bulk regions are kept in equilibrium with electrodes at a potential difference of V_{mp}, yielding the PB-Voltage equation (1,2)

$$\begin{aligned}
&\boldsymbol{\nabla}\cdot[\epsilon(\mathbf{r})\boldsymbol{\nabla}\phi_{\mathrm{mp}}(\mathbf{r})]=0 && \text{Pore region}\\
&\boldsymbol{\nabla}\cdot[\epsilon(\mathbf{r})\boldsymbol{\nabla}\phi_{\mathrm{mp}}(\mathbf{r})]-\bar{\kappa}^2(\mathbf{r})[\phi_{\mathrm{mp}}(\mathbf{r})]=0 && \text{Bulk region, side I}\\
&\boldsymbol{\nabla}\cdot[\epsilon(\mathbf{r})\boldsymbol{\nabla}\phi_{\mathrm{mp}}(\mathbf{r})]-\bar{\kappa}^2(\mathbf{r})[\phi_{\mathrm{mp}}(\mathbf{r})-V_{\mathrm{mp}}]=0 && \text{Bulk region, side II,}
\end{aligned} \tag{1}$$

where $\epsilon(\mathbf{r})$ and $\bar{\kappa}(\mathbf{r})$ are the space-dependent dielectric constant and Debye-Hückel screening factor, respectively, and V_{mp} is the equilibrium electrode potential far away on side II. It should be noted that the protein and ion charges must be formally turned off in this calculation, and the ionic screening factor, $\bar{\kappa}^2(\mathbf{r})$, must be set to zero in the pore region (between $Z_{\min} = 0$ Å to $Z_{\max} = 22.5$ Å), where the K$^+$ ions are represented explicitly (1). The PBEQ module (3) implemented in the CHARMM program (4) was used with the optimized atomic Born radii for amino acids (5). The total potential of mean force calculated from Eq. **1** in the article was stored on a 3D discrete grid going from $Z_{\min} = 0$ Å to $Z_{\max} = 22.5$ Å with a spacing δZ of 0.1 Å for a total of about 11.5 million points.

Diffusion constant. The diffusion-constant profile was extracted from the friction kernel, calculated from the velocity autocorrelation function of the ions, using an analysis based on the generalized Langevin equation for non-uniform systems (6-8). In practice, the diffusion constant was calculated at different positions along the pore axis from the biased umbrella sampling simulations from the expression (8),

$$D=\lim_{s\to 0}\ \frac{-\hat{C}(s)\langle\delta Z^2\rangle\langle\dot{Z}^2\rangle}{\left(\hat{C}(s)\left[s\langle\delta Z^2\rangle+\langle\delta\dot{Z}^2\rangle/s\right]-\langle\delta Z^2\rangle\langle\delta\dot{Z}^2\rangle\right)} \tag{2}$$

where $\hat{C}(s)$ is the Laplace transform of the velocity autocorrelation function $C(t) = \langle v(t)v(0)\rangle$ (the right-hand-side of the equation is equal to $k_{\mathrm{B}}T/\hat{\gamma}(s)$, where $\hat{\gamma}(s)$ is the Laplace transform of the friction kernel). The expression was extrapolated to small values of s to avoid the numerical instabilities as $s \to 0$.

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