Supporting Information for

The Origin of Coupled Chloride and Proton Transport in a Cl⁻/H⁺ Antiporter

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Additional system setup details

The simulation system consists of the ClC-ec1 dimer (PDB ID: 1OTS),¹ 163 POPE lipids, 17 Cl⁻s, and ~11,000 water molecules in a 92 Å × 92 Å × 79 Å box with a periodic boundary condition. The CHARMM22 force field² was used for protein and ions, and CHARMM27³ for lipids. The excess proton and water molecules were described by the MS-EVB model⁴ ver. 3.1.⁵ Except for residues E148 and E203 in monomer A, which were treated with MS-RMD models,⁶⁻⁷ the protonation states of all other residues were determined based on previous pKa calculations on the same crystal structure.⁸ Specifically, E113 in monomer B and D417 in monomer A were protonated, while the standard protonation states were chosen for all other residues. All other initial system setup and simulation details are described in our previous work.⁷

Additional metadynamics simulation details

The initial configurations for the metadynamics (MetaD) simulation were taken from the a previous study, using the last MD snapshot of the windows at the free energy basin of the product state in the potential of mean force (PMF) for PT from E203 to E148 in both the presence and the absence of Cl⁻cen.⁷ (The PMF in Figure 3B for the case with Cl⁻cen present, and the PMF in Figure 4B with Cl⁻cen absent in ref. 7) Consistent with these PMFs, E203 is deprotonated. Four different sets of the MetaD simulations were set up, depending on whether
$\text{Cl}_{\text{cen}}$ is present or absent, and whether MS-RMD model parameters for E148 were taken from E148 up or down models shown in Table S1. The curvilinear pathway with E148 down model was sampled in the range where E148 side chain rotates from the down to the up conformation, while the PT pathway with E148 up model was sampled for both the rotation of E148 and its deprotonation to the extracellular solution.

The CV in all MetaD simulations was defined as the z coordinate of the excess proton center of excess charge (CEC, referenced in the main text and defined in Eq. (S3) below). The hill height and the width of a MetaD Gaussian potential were set to be 0.1 kcal/mol and 0.35 Å, respectively. The Gaussian potential was added every 1000 time steps in the trajectory. The half-sided harmonic potential with the force constant, 100 $\text{kcal} \cdot \text{mol}^{-1} \text{Å}^{-2}$, was added as a wall at the lower boundary of the CV, which is 2 Å below in the z axis from the position of E148 side chain in the initial configurations. This wall potential prevents the excess proton on E148 from transferring back to E203. Another wall was added at the upper boundary. In the MetaD with E148 down model, the upper wall was placed at 6 Å above from the E148 side chain in the initial configurations, which prevents the deprotonation of E148. The upper wall with E148 up model was placed at 15 Å above from E148, which is right above the position of the entrance/exit for the proton from/to the solution. Additional wall potentials were added to the x and the y directions, in order to prevent the CEC from escaping from the entrance horizontally, where the pore size of the protein channel becomes larger. The wall potentials were placed at each side of the 35 Å × 30 Å rectangular box on the xy plane, which is large enough to cover the area of the pore. The MetaD simulation was run for ~3 ns, until the CV trajectory visited between the upper and the lower boundaries ~2-3 times. The coordinates of the CEC were recorded every time step, which were used to create the “MetaD curvilinear pathway” as described below.
Creating the MetaD curvilinear path

The creation of the “MetaD curvilinear path” is schematically represented in Figure S1. The results of the MetaD simulations described above (Figure S1A) form the basis of the MetaD curvilinear path. CEC coordinates from every 100 steps during the MetaD simulations were binned based on their $z$ value, with each bin separated by 0.25 Å. Within each bin, the $x$ and $y$ coordinates were averaged, so that each bin produced a single point such as the blue dot shown in Figure S1B. The resulting set of points formed the basis of the “MetaD curvilinear path” (Figure S1C). A continuous line connecting these points (blue line in Figure S1C) was obtained by using the “Interpolation” function in Mathematica ver. 10.0 $^9$ which allowed calculation of a derivative and thus tangent at each point (such as the red line in Figure S1D). These points also serve as the window centers for umbrella sampling as detailed below.

Figure S1. Schematic representation of the construction of the curvilinear reaction pathway from the MetaD trajectories. (A) The path of the excess proton CEC during PT from E148 to the extracellular solution was explored by biasing the CEC $z$ coordinate with MetaD. (B) Selected CEC coordinates from the MetaD simulations (green dots) were binned according their $z$ coordinate. In each bin, the $x$ and the $y$ coordinates were averaged, creating one point per bin (blue dot). (C) The collection of the resulting points creates the for the curvilinear reaction pathway for PT in this study (line created by the overlapping blue spheres) as shown here for PT with Cl$^-_{\text{cen}}$ present and E148 “up.” (D) These average points also served as the window centers for umbrella sampling (one center shown here as a red circle) with a harmonic potential applied tangentially to the curvilinear pathway (tangent shown as a red line). To confine sampling to regions relevant for the PT path, a flat-bottomed cylindrical potential was applied based on the perpendicular distance from the tangent line passing through the window center (beginning of the applied cylinder potential shown as an orange circle).
Additional REUS simulation details

As described in the main text, replica-exchange umbrella sampling (REUS) was preformed to sample an ensemble of states to determine the PMF. Consistent with our previous work,\textsuperscript{7,10} points along the curvilinear path, such as the red point shown in Figure S1D, serve as window centers. A harmonic bias of 30 kcal·mol\(^{-1}·\text{Å}^{-2}\) was applied based on the deviation of the CEC distance from window center only in the direction of the tangent vector (red line in figure S1D). Thus, this harmonic bias is not applied within the plane shown in Figure S1D, which is perpendicular to the tangent line and pass through the point serving as the window center. Configurations in which the CEC lies on a plane parallel to that plane would have an applied bias based on the distance from this zero-bias plane. The direction of the harmonic umbrella potential follows the tangent vector of the path at the window center, so that the 3D coordinate of the CEC position is properly projected onto the 1D straight line. Within the selected window size of 0.25 Å, the curvilinear path is locally smooth with respect to neighboring windows and the tangent vector did not significantly deviate from the curvilinear path. The standard deviation of the CV distribution from the window center was 0.19 ± 0.03 Å, and the tilted angles of the tangent vectors between neighboring windows were 8.0 ± 5.5° across the windows.

Within narrow regions of the pore, sampling along those planes is restricted by steric interactions with the protein. As the pore widens to the opening to the bulk solution, the accessible area to the CEC increases, but is not relevant in determining the energetics of transport through the pore, would unnecessarily slow convergence of the PMF, and would allow unnecessary deviation from a uniquely define PT path. Thus, in addition to the harmonic potential used as the basis for umbrella sampling, a cylindrical potential was added to serve as a wall to confine sampling to relevant regions. The cylindrical potential is a flat-bottom harmonic
potential, where the bias is zero when the perpendicular distance from the CEC to the curvilinear pathway is smaller than 5 Å (area inside the orange circle in Figure S1D; the circle shows the intersection of the cylindrical potential through the “zero-bias” plane perpendicular to the tangent line to the curve and passing through the point that serves as the window center). At distances greater than 5 Å (area outside the orange circle on the plane shown in Figure S1D), the half-sided harmonic potential was applied to force the CEC toward the curvilinear pathway. This form of cylindrical potential has been widely applied in other computational studies to sample a one-dimensional PMF for the motion of the substrate through a transmembrane protein.\textsuperscript{11-12} Such cylindrical potentials help converge the PMF around the channel entrance and prevent the substrate from diffusing along the membrane surface. In the REUS, the initial configuration of each window was chosen from the MetaD trajectories based on smallest distance between the CEC and the window center. The conventional Metropolis Monte Carlo exchange criterion was applied every 5000 time steps to determine swapping the neighboring windows. The average acceptance ratio for the successful exchange of the neighboring windows was about 20 % over all windows. Each window was sampled for 1–2 ns. The CV values were recorded every step.

**Calculating the PMF**

The PMFs were calculated using WHAM.\textsuperscript{13} The total CV range in each PMF (0 ~ 20 Å for WT and 0 ~ 32 Å for E148A mutant) was divided into bins with 0.1 Å spacing. The tolerance of the PMF convergence was $10^{-4}$ kcal/mol. The bin size and tolerance were chosen to be small enough so that the height of the free energy barrier was changed only by 0.04 and 0.09 kcal/mol, respectively, when the bin size and the tolerance were decreased by an order of magnitude each (to 0.01 Å and $10^{-5}$ kcal/mol, respectively).
As described above, the bias in each window was applied according to the deviation of the CEC position from the window center in the direction of the tangent line. In the limit of infinitely small window spacing, this projects the three-dimensional coordinates onto a one-dimensional reaction coordinate, approximated by the MetaD pathway followed by the CEC during PT. As noted above, the change in tangent direction between adjacent windows and sampling within each window were analyzed to determine if the window spacing and force constants were chosen so that the application of the bias along the tangent was not significantly different than if the bias were applied exactly along the reaction coordinate. As a further test of accuracy of the resulting PMF calculated using WHAM, we also calculated a PMF using a force integration method formulated to determine the change in free energy along a multidimensional path via a force applied tangent to the reaction coordinate.\textsuperscript{10,14} In this method, the mean force in each window using the equation:\textsuperscript{10,14}

$$\frac{\partial F(z)}{\partial z} = k \int_{t=0}^{t=\text{total}} (z_j - \theta_j(x(t))) dt$$  

(S1)

where $k$ is the force constant used in biasing in each window; $t$ is the simulation time, which spans from $t=0$ to $t=\text{total}$; $z_j$ is the value of the collective variable $\theta_j(x(t))$ evaluated at the point $x_j$ (thus, $z_j=\theta_j(x_j)$); and $x(t)$ are Cartesian coordinates at the simulation time $t$. To create a continuous function of the mean force as a function of the pathway $z$ ($\partial F(z)/\partial z$), we used the “Interpolation” function in Mathematica ver. 10.0\textsuperscript{9}, which allowed calculation of the integral in:

$$F(z') - F(z_0) = \int_{z_0}^{z'} \frac{\partial F(z)}{\partial z} dz$$  

(S2)

where $z_0$ is the initial CV value used to determine the relative energy at CV value $z'$. Figure S2 compares a PMF calculated with WHAM (red curve) versus calculated with Eq. S1 and S2 (blue
curve). As noted in the main text, error bars show statistical error due to finite sampling, estimated using a block averaging method by dividing each trajectory into four consecutive blocks. The difference in calculated barrier height for PT is 0.6 kcal/mol between the two methods, with an average difference of 0.1 kcal/mol across all CV values. These differences are similar to the calculated statistical error due to finite sampling.

![Figure S2](image)

**Figure S2.** The calculated PMF for PT from E203 to the extracellular solution in the E148A mutant with Cl<sub>cen</sub> present, calculated either with WHAM (red curve, reproduced from Figure 6 in the main text) or with the force integration method described.

**Combining the PMFs from E148 rotation and protonation/deprotonation**

As noted in the main text, the overall PMF for PT from E148 “down” to the extracellular solution was created by combining two PMFs. The separate PMFs and their combination are shown in Figure S3: the blue curve is the PMF created with the MS-RMD model parameterized for E148 down, the red for E148 up, and the dotted black curve shows their combination. The PMFs for both E148 up and down models overlap around the free energy barrier at CV ~3 Å (i.e., the transition state between the up and down conformations). This overlapping region was used to merge the two PMFs into a single continuous PMF (dotted lines in Figure S3). Thus, the down
and up conformations of E148 were sampled with the down and up models, respectively, and the deprotonation of E148 to the extracellular solution was calculated with the E148 up model.

For the E148A simulations, MetaD simulations were initiated with E203 protonated. The MetaD potential was added to the z coordinate of the CEC to sample the configuration space covering proton migration from E203 to the extracellular solution. The wall potentials were the same as those used for WT.

**Figure S3.** PMFs for a two-step PT process from E148 to the extracellular solution with Cl\textsuperscript{−}cen present (left) or absent (right), calculated with two different MS-RMD models—E148 down (blue) and E148 up (red). The two PMFs are merged into one PMF (dotted line) by overlapping the rotation free energy barrier. E148 is protonated at the low values of the x-axis, including at the two minima labeled “E148 (down)” and “E148 (up)”. The barrier between “E148 (up)” and “Extra. Solution” corresponds to deprotonation; the excess proton is in solution (E148 deprotonated) to the right of the barrier.

**Fitting the MS-RMD model for E148 in the up conformation**

The MS-RMD model for E148 in the down orientation (interacting with water molecules in the central region) was parameterized in our previous work (included in Table S1). Because the MS-RMD model parameters depend on the surrounding environment of the protein and the solvent, the MS-RMD model for E148 was re-parameterized in this study for the up configuration (interacting with the water molecules from the extracellular region). Umbrella
sampling was used, starting from the up conformation of E148, to collect configurations during E148 deprotonation toward the extracellular bulk. The CV in the umbrella sampling was defined as the distance from the center of excess charge (CEC) to the center of mass of the carboxyl group of E148. The CEC, $r_{\text{CEC}}$, is defined as:

$$r_{\text{CEC}} = \sum_i c_i^2(r) r_i^{\text{COC}}$$

where $c_i^2(r)$ is the population of $i$th MS-RMD state, and $r_i^{\text{COC}}$ is the center of charge (COC) of the $i$th state given by:

$$r_i^{\text{COC}} = \frac{\sum_k q_k r_k}{\sum_k |q_k|}$$

where the coordinate of the $k$th atom, compromising the MS-RMD complex in the $i$th state, $r_k$, is averaged, weighted by the absolute value of its atomic partial charge, $q_k$. When E148 is protonated, the CEC is located near the center of mass of the carboxyl group of E148. The umbrella windows ranged from 2 to 4 Å with a 0.25 Å interval between neighboring windows. The force constant of the harmonic potential was 30 kcal·mol$^{-1}$·Å$^{-2}$. Approximately 500 evenly spaced configurations along the CV (~50-60 from each window) and in time (~5 ps intervals) were selected for fitting. For each configuration, a single point energy calculation was performed with the CP2K software$^{15}$ to calculate the atomistic forces in the QM region with the QM/MM method.

In the QM/MM single point energy calculations, the QM atoms were treated with density functional theory (DFT) using the BLYP-D3/TZV2P functional and basis set$^{16-18}$ including the third generation of the dispersion correction developed by Grimme et al.$^{19}$ The QM region included Cl$^{-\text{cen}}$, if present, the side chains of residues E148, Y445, and S107, the water molecules
in the central region within 3 Å of E148, and the water molecules from the extracellular region within 8 Å of E148. The total number of the QM atoms was ~130 on average. The QM box size was ~20-30 Å in each dimension to ensure it included a ~6-8 Å buffer between the QM atoms and the boundary of the QM box. The Gaussian Expansion of the Electrostatic Potential (GEEP) scheme was used to treat the QM/MM electrostatic coupling with periodic boundary conditions (PBC), and the spurious QM/QM periodic image interactions were decoupled as described in ref. 22. The chemical bonds that crossed the QM/MM boundary between the alpha carbon (MM) and the beta carbon (QM) of the residue were capped with hydrogen atoms, on which the forces were calculated following the IMOMM scheme with a scaling factor of 1.5. Otherwise, the QM setup was consistent with previous work.

The MS-RMD model parameters for E148 in the up conformation were optimized by using the FitRMD method, which minimizes the residual of differences between the forces calculated with QM/MM and those calculated with MS-RMD. The new MS-RMD parameters for E148 in the up conformation for Cl–cen present and absent are given in Table S1.
Table S1. The MS-RMD model parameters for E148 in the down/up conformations with Cl<sub>cen</sub> absent/present. The definitions of the parameters are described in our previous work.<sup>5-6</sup> The parameters for E148 (down) were taken from previous work for PT in the central region.<sup>7</sup>

|                  | Cl<sub>cen absent</sub> | Cl<sub>cen present</sub> |
|------------------|-------------------------|--------------------------|
|                  | E148 (down)             | E148 (up)                | E148 (down)             | E148 (up)                |
| B                | 0.063153                | 0.454014                 | 0.012536                | 0.413895                 |
| b                | 1.571751                | 0.075642                 | 0.232384                | 0.025128                 |
| b′               | 1.320947                | 0.010841                 | 1.469007                | 0.024147                 |
| d<sub>0o</sub>   | 2.4                     | 2.4                      | 2.4                     | 2.4                      |
| C                | 0.363648                | 0.371124                 | 0.014044                | 0.356169                 |
| c                | 1.117167                | 0.709177                 | 1.152912                | 0.665787                 |
| d<sub>0h</sub>   | 1.0                     | 1.0                      | 1.0                     | 1.0                      |
| r<sub>1</sub>    | 3.5                     | 3.5                      | 3.5                     | 3.5                      |
| r<sub>2</sub>    | 4.0                     | 4.0                      | 4.0                     | 4.0                      |
| V<sub>H</sub>    | -147.095673             | -157.105468              | -151.11473              | -154.325345              |
| c<sub>1</sub>    | -36.090543              | -42.974750               | -30.414842              | -30.319574               |
| c<sub>2</sub>    | 1.879933                | 2.552372                 | 3.331769                | 3.436706                 |
| c<sub>3</sub>    | 1.193253                | 1.556170                 | 1.422240                | 1.456306                 |
| C                | 143.003                 | 143.003                  | 143.003                 | 143.003                  |
| α                | 1.8                     | 1.8                      | 1.8                     | 1.8                      |
| r<sub>0</sub>    | 0.975                   | 0.975                    | 0.975                   | 0.975                    |

Differences between the MS-RMD models for E148 “up” and “down”

Figure S3 shows the influence of the E148 model (up versus down) on the PMFs of E148 rotation (CV = ~ 0-8 Å) in the presence and absence of Cl<sub>cen</sub>. The two models give different relative free energies for the up and the down conformations, and different barrier heights for rotation. This difference is more pronounced with Cl<sub>cen</sub> absent (~3 kcal/mol difference between the two minima), than with Cl<sub>cen</sub> present (~1 kcal/mol difference). E148 is predominantly protonated during rotation (i.e., the MS-RMD state for protonated E148 has the largest coefficient over the rotation CV range). However, deprotonated states do contribute some (i.e., they have coefficients > 0). The contribution of deprotonated states effectively describes transfer
and polarization of the charge density on E148, in response to the local electrostatic environment, which cannot be described by a classical force field.

Figure S4 shows the population (i.e., $c_i^2(\mathbf{r})$ in Eq. 1) of the state with E148 protonated calculated from the two models for the umbrella windows during E148 rotation. The difference in populations, similar to the difference in PMFs, is much more pronounced in the absence of Cl\textsuperscript{−}cen, when E148 rotates all the way from central site to the upper site. When the E148 down model is used with Cl\textsuperscript{−}cen absent, the population of protonated E148 state is decreased to $\sim$75-85 %, causing that the excess proton on E148 is be more delocalized into the surrounding water molecules. The PMF with Cl\textsuperscript{−}cen absent is decreased by $\sim$3 kcal/mol, compared to that with the up model, showing that the delocalized excess charge is energetically more favorable. However, the population of protonated E148 state with Cl\textsuperscript{−}cen present is $\sim$93-98 %, which similar for the two models, just as the PMFs are similar.

![Figure S4](image)

**Figure S4.** Average value of $c_i^2(\mathbf{r})$ of the protonated E148 state during the rotation of E148 in the PMF in Figure S3, where $c_i^2(\mathbf{r}) = 1.00$ indicates protonation with no delocalization to water molecules.
Figure S5. PMF for deprotonated E148 rotation between the down and up orientations with Cl\textsubscript{cen} absent. The CV of the PMF is defined as the center of mass of the carboxyl group of E148 along the curvilinear pathway.

Figure S6. Comparison of simulation structures (blue) and the X-ray crystal structures (red). (A) Simulation structure of E148 in the up conformation with Cl\textsubscript{cen} present compared to the crystal structure of the E148Q mutant (1OTU). (B) Simulation structure of E148 in the down conformation with Cl\textsubscript{cen} present compared to the crystal structure of WT ClC-ec1 (1OTS). (C) Simulation structure of E148 in the down conformation with Cl\textsubscript{cen} absent compared to the crystal structure of WT cmClC (3ORG).
More detailed kinetic rate constant analysis

It is assumed that the motion of the excess proton along the reaction coordinate $z$ in the umbrella sampling trajectory can be described by the generalized Langevin equation for a harmonic oscillator,

$$\mu_i \dot{C}_v(t; z_i) = -k_i \int_0^t C_v(t'; z_i) \, dt' - \int_0^t M(t - t'; z_i) C_v(t'; z_i) \, dt'$$

(S5)

where $C_v(t; z_i) = \left< \dot{z}(t) \dot{z}(0) \right>_i$ is the auto-correlation function of the velocity $\dot{z}$ at window $i$, and $M(t; z_i)$ is the friction kernel. The effective mass $\mu_i$ and the force constant $k_i$ are determined using the relations, $\mu_i = k_i T / \left< \dot{z}_i^2 \right>$ and $k_i = k_B T / \left< \delta z_i^2 \right>$, respectively, where $\delta z_i = z_i - \left< z_i \right>$ is the displacement of $z_i$ from its average.

Figure S7. The position-dependent diffusion coefficient $D(z_i)$ for the motion of the excess proton along the reaction coordinate of the PMFs in Fig. 3 with Cl$^-_{cen}$ present (blue) and absent (red). $D(z_i)$ at each window was calculated using Eq. S7.
The position-dependent diffusion constant $D(z_i)$ at $z_i$ is obtained from the Laplace transform of the friction kernel, $\hat{M}(s; z_i) = \int_0^\infty dt \ e^{-st} M(t; z_i)$, through Einstein’s relation,

$$D(z_i) = \lim_{s \to 0} \frac{k_B T}{\hat{M}(s; z_i)} \quad (S6)$$

From Eq. S5 and S6, Woolf and Roux$^{25-26}$ proposed a formulation to calculate $D(z_i)$ from the umbrella sampling trajectory at window $i$:

$$D(z_i) = \lim_{s \to 0} \frac{-\hat{C}_i(t; z_i) \langle \delta z_i^2 \rangle \langle \dot{z}_i^2 \rangle}{\hat{C}_i(t; z_i) (s \langle \delta z_i^2 \rangle + s^{-1} \langle \dot{z}_i^2 \rangle) - \langle \delta z_i^2 \rangle} \quad (S7)$$

$D(z_i)$ for each window was determined by extrapolating its value from the range $1 < s < 3$ to $s = 0$. Fig. S7 shows $D(z_i)$ calculated over all windows in the PMFs with Cl$_{\text{cen}}$ either present or absent.

The value of $D(z_i)$ is decreased near $z=5\text{Å}$, where the protonated E148 rotates through a narrow region in the surrounding protein residues, then becomes $\sim 1\text{Å}^2/\text{ps}$ at $z>12\text{Å}$, where E148 is deprotonated and the excess proton is delocalized in the water molecules in the extracellular bulk. The statistical error in $D(z_i)$ was estimated using a block averaging method by dividing each trajectory into four consecutive blocks. The uncertainty was of the same order of magnitude of its average value, which is a typical result when $D(z_i)$ is calculated using the generalized Langevin equation. Similar results have been reported in other simulation studies,$^{11,27}$ for the ion transport in protein channels. Although $D(z_i)$ in Figure S7 appears to be
about 2-fold greater in the range 6<z<8 with Cl\textsubscript{cen} present than that with Cl\textsubscript{cen} absent, the differences between two cases are comparable to the size of the uncertainty.

The effective rate constant $k\textsubscript{eff,MFPT}$ for the two steps can be calculated as the inverse of the mean first passage time $\tau\textsubscript{MFPT}^{-1}$ from the reactant to the product well in the PMF $W(z)^{28}$:

$$k\textsubscript{eff,MFPT} = \tau\textsubscript{MFPT}^{-1} = \left[ \int_{z_l}^{z_r} dz' D(z')^{-1} e^{-W(z')/k_bT} \int_{z_r}^{z} dz'' e^{-W(z'')/k_bT} \right]^{-1}$$  \hspace{1cm} (S8)

where $z_r$ is the reactant well, which is defined at the points corresponding to E148 down state in the PMF in Figure 3, where $z_r = 2.2$Å in the PMF with Cl\textsubscript{cen} present and 2.9 Å with Cl\textsubscript{cen} absent. $z_p = 20$Å is the product well, when the excess proton is transferred to the extracellular solution. $z_l = 1.0$Å is chosen as the lower boundary from the reactant well. The calculated $k\textsubscript{eff,MFPT}$ is insensitive to the choice of the value for $z_i$ in the range $0 < z_l < z_r$.

In order to validate the accuracy of the pre-factor $\omega_0 / 2\pi$ of Eq. 1 of the main text, Eq. S8 was reformulated to $k\textsubscript{eff,MFPT} = A\textsubscript{MFPT} \cdot e^{-\Delta F^\dagger / k_b T}$, where $\Delta F^\dagger$ is the free energy barrier height in the PMF, and $A\textsubscript{MFPT}$ was compared with $\omega_0 / 2\pi$ of Eq. 1. As an example, $A\textsubscript{MFPT}$ was calculated for the case of the first step in the PMF ($k_1$), 2.9 ps\textsuperscript{-1}, which was also comparable to $\omega_0 / 2\pi$ calculated in Eq. 1, 3.6 ps\textsuperscript{-1}.

**Table S2.** The effective rate constants for two steps in the PMFs with Cl\textsubscript{cen} present and absent in Figure 3. $k\textsubscript{eff}$ and $k\textsubscript{eff,MFPT}$ were calculated using either the transition state theory (Eq. 1), or the equation for the mean first passage time (Eq. S8), respectively. The unit for the rate constant is ms\textsuperscript{-1}.

|         | $k\textsubscript{eff}$ (Eq. 1) | $k\textsubscript{eff,MFPT}$ (Eq. S8) |
|---------|-------------------------------|-------------------------------------|
| Cl\textsubscript{cen} present | 0.81                          | 0.24                                |
| Cl\textsubscript{cen} absent  | $7.7 \times 10^{-3}$           | $3.6 \times 10^{-3}$                |
The value of $k_{\text{eff,MFPT}}$ was 0.24 ms$^{-1}$ with Cl$^{-}_{\text{cen}}$ present and $3.6 \times 10^{-3}$ ms$^{-1}$ with Cl$^{-}_{\text{cen}}$ absent, which slightly decreased compared to $k_{\text{eff}}$ in the manuscript (0.81 ms$^{-1}$ and $7.7 \times 10^{-3}$ ms$^{-1}$, respectively, calculated with transition state theory (Eq. 1)), but still in the same order of magnitude (Table S1). The result for $k_{\text{eff,MFPT}}$ with Cl$^{-}_{\text{cen}}$ present from Eq. S8 deviates further from the experimental value of the turnover rate for the overall PT process, 1.0 ms$^{-1}$. However, considering the uncertainty of the diffusion coefficients and other sources of the error, such as the accuracy of the force field or finite sampling time, and comparing to other computational studies estimating the rate constant from the PMF in a large biological system should be considered to be adequate if within the correct order of magnitude, certainly if within a factor of 2-3. The cumulative barrier heights in the PMF with Cl$^{-}_{\text{cen}}$ present is smaller than that with Cl$^{-}_{\text{cen}}$ absent by 2.2 kcal·mol$^{-1}$, and the Boltzmann factor of the free energy difference results in a 40-fold increase in the rate constant at 300 K, which is comparable to the difference in $k_{\text{eff,MFPT}}$ between the two PMFs (67-fold increase).

Supplemental References

(1) Dutzler, R.; Campbell, E. B.; MacKinnon, R. Science 2003, 300, 108.
(2) MacKerell, A. D., Jr.; Bashford, D.; Bellott, M.; Dunbrack, R. L., Jr.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, W. E., III; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiórkiewicz-Kuczera, J.; Yin, D.; Karplus, M. J. Phys. Chem. B 1998, 102, 3586.
(3) Feller, S. E.; MacKerell, A. D., Jr. J. Phys. Chem. B 2000, 104, 7510.
(4) Wu, Y.; Chen, H.; Wang, F.; Paesani, F.; Voth, G. A. J. Phys. Chem. B 2008, 112, 467.
(5) Nelson, J. G.; Peng, Y.; Silverstein, D. W.; Swanson, J. M. J. Chem. Theory Comput. 2014, 10, 2729.
(6) Lee, S.; Liang, R.; Voth, G. A.; Swanson, J. M. J. Chem. Theory Comput. 2016, 12, 879.
(7) Lee, S.; Swanson, J. M. J.; Voth, G. A. Biophys. J. 2016, 110, 1334.
(8) Faraldo-Gomez, J. D.; Roux, B. J. Mol. Biol. 2004, 339, 981.
(9) Wolfram Research, I. Mathematica, Version 10.0: Champaign, Illinois, 2014.
(10) Zhang, Y.; Voth, G. A. J. Chem. Theory Comput. 2011, 7, 2277.
(11) Allen, T. W.; Andersen, O. S.; Roux, B. Proc. Natl. Acad. Sci. U. S. A. 2004, 101, 117.
(12) Gordon, D.; Chen, R.; Chung, S.-H. Physiol. Rev. 2013, 93, 767.
(13) Kumar, S.; Rosenberg, J. M.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A. J. Comput. Chem. 1992, 13, 1011.
(14) Maraglioano, L.; Fischer, A.; Vanden-Eijnden, E.; Ciccotti, G. J. Chem. Phys. 2006, 125, 024106.
(15) VandeVondele, J.; Krack, M.; Mohamed, F.; Parrinello, M.; Chassaing, T.; Hutter, J. *Comput. Phys. Commun.* **2005**, *167*, 103.

(16) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098.

(17) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

(18) Schäfer, A.; Huber, C.; Ahlrichs, R. *J. Chem. Phys.* **1994**, *100*, 5829.

(19) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. *J. Chem. Phys.* **2010**, *132*, 154104.

(20) Laino, T.; Mohamed, F.; Laio, A.; Parrinello, M. *J. Chem. Theory Comput.* **2006**, *2*, 1370.

(21) Laino, T.; Mohamed, F.; Laio, A.; Parrinello, M. *J. Chem. Theory Comput.* **2005**, *1*, 1176.

(22) Blochl, P. E. *J. Chem. Phys.* **1995**, *103*, 7422.

(23) Maseras, F.; Morokuma, K. *J. Comput. Chem.* **1995**, *16*, 1170.

(24) Liang, R. B.; Li, H.; Swanson, J. M. J.; Voth, G. A. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 9396.

(25) Woolf, T. B.; Roux, B. *J. Am. Chem. Soc.* **1994**, *116*, 5916.

(26) Crouzy, S.; Woolf, T. B.; Roux, B. *Biophys. J.* **1994**, *67*, 1370.

(27) Mamonov, A. B.; Kurnikova, M. G.; Coalson, R. D. *Biophys. Chem.* **2006**, *124*, 268.

(28) Szabo, A.; Schulten, K.; Schulten, Z. *J. Chem. Phys.* **1980**, *72*, 4350.