Supporting Information

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SI Text

Proton Pumping Against a pH Gradient. A pH gradient across the membrane provides a driving force for a proton. The proton motive force (PMF) $\Delta\mu$ across the membrane is then given by

$$\Delta\mu = V_{\mathrm{m}} - 2.303 k_{\mathrm{B}} T \Delta\mathrm{pH},$$

where V_m is the membrane potential while $\Delta\mathrm{pH} = \mathrm{pH_P} - \mathrm{pH_N}$ is the pH gradient between the P and N sides of the membrane. To explore the effect of the pH gradient on the pumping efficiency in the absence of the membrane potential ($V_m = 0$), ΔpH is incorporated into the rate coefficients involving the proton uptake/release. We assume, for simplicity, that the ΔpH is uniformly distributed among the rates of proton uptake/release for both sides of the membrane. Explicitly, these rates are written as

$$k_{\mathrm{N}\to 1}(\Delta\mathrm{pH}) = k^0_{\mathrm{N}\to 1}\exp(\Delta\mu/4k_{\mathrm{B}}T), \quad k_{1\to\mathrm{N}} = k^0_{1\to\mathrm{N}}\exp(-\Delta\mu/4k_{\mathrm{B}}T)$$

$$k_{\mathrm{P}\to 2}(\Delta\mathrm{pH}) = k^0_{p\to 2}\exp(-\Delta\mu/4k_{\mathrm{B}}T), \quad k_{2\to\mathrm{P}} = k^0_{2\to\mathrm{P}}\exp(\Delta\mu/4k_{\mathrm{B}}T),$$

where, for example, $k_{\mathrm{Narrow\ 1}}$ is the rate coefficient of the proton uptake from the N side to site, and superscripts 0 denote the rates at zero pH gradient. By using the optimized solutions of the three-site model (Fig. 3 in the main text), we find that protons are pumped against pH gradients $\Delta\mathrm{pH} \approx -2.5$, as shown in Fig. S2. The lower limit of ΔpH is thermodynamically equivalent to a proton motive force of $\approx$150 mV across the membrane. This value is similar to the maximum membrane potential, V_m, against which protons are pumped in the absence of a pH gradient.

Protonation Equilibria and Calculation of pK_a. Proton populations (Fig. 4 of the main text) are obtained by calculating the probability of a proton site μ to be occupied. The thermodynamic average of the proton population, $< x_\mu >$, at equilibrium (without product formation) is given by

$$\langle x_\mu \rangle = \frac{1}{Z}\sum_{i=1}^{2^N} X^{(i)}_\mu \exp(-G_i/k_{\mathrm{B}}T),$$

where the partition function is

$$Z = \sum_{i\text{-}1}^{2^N} \exp(-G_i/k_{\mathrm{B}}T).$$

Note that $X^{(i)}_\mu$ is 0 when site μ is empty in state i and 1 when it is occupied, while G_i is the free energy of state i. For a given pH, the free energy G_i can be written as

$$G_i(\mathrm{pH}) = \sum_{\mu\text{-}1}^{N} X^{(i)}_\mu 2.3 k_{\mathrm{B}}T(\mathrm{pH} - \mathrm{pK}^{\mathrm{int}}_{\mathrm{a},\mu}) + \sum_{\mu=1}^{N-1}\sum_{\upsilon=\mu+1}^{N} X^{(i)}_\mu X^{(i)}_\upsilon \epsilon_{\mu\upsilon},$$

where $\mathrm{pK}^{\mathrm{int}}_{a,\mu}$ is the intrinsic $\mathrm{pK_a}$ of the site μ, which is related to G^0_μ via $G^0_\mu = 2.3\, k_{\mathrm{B}}T(7 - \mathrm{pK}^{\mathrm{int}}_{a,\mu})$, and $\varepsilon_{\mu\nu}$ is the electrostatic interaction between sites μ and ν. The apparent $\mathrm{pK_a}$ of the proton site μ then corresponds to the pH value that yields $< x_\mu > = \frac{1}{2}$.

Midpoint Potential of the Electron Site. The midpoint potential of the electron site of the kinetic model is equivalent to the experimentally measured midpoint potential of the heme *a* relative to either cytochrome *c* or $\mathrm{Cu_A}$. In the presence of a proton in Glu-242 (i.e., the proton site 1) and the absence of a proton in the pump site, the relative midpoint potential of the electron site is given by

$$\Delta E_m = -25.6(G^0_3 + \epsilon_{13}) \qquad [\mathrm{mV}],$$

where G^0_3 is the intrinsic free energy of the site 3, and ε_{13} is the electrostatic coupling between sites 1 and 3. Results are shown in Fig. S3.

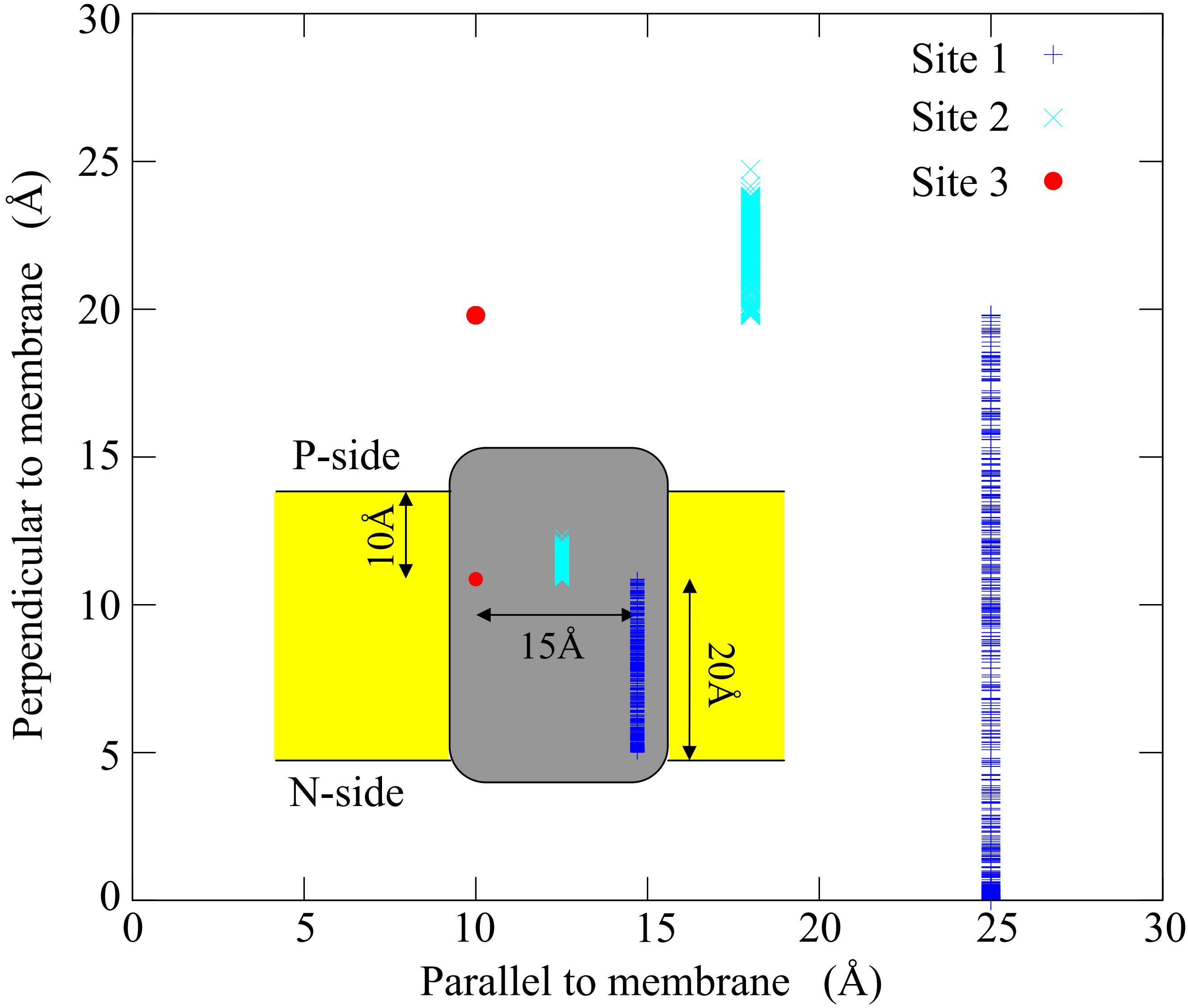

Fig. S1. Coordinates of the proton and electron sites obtained from Monte Carlo optimization of the three-site kinetic models.

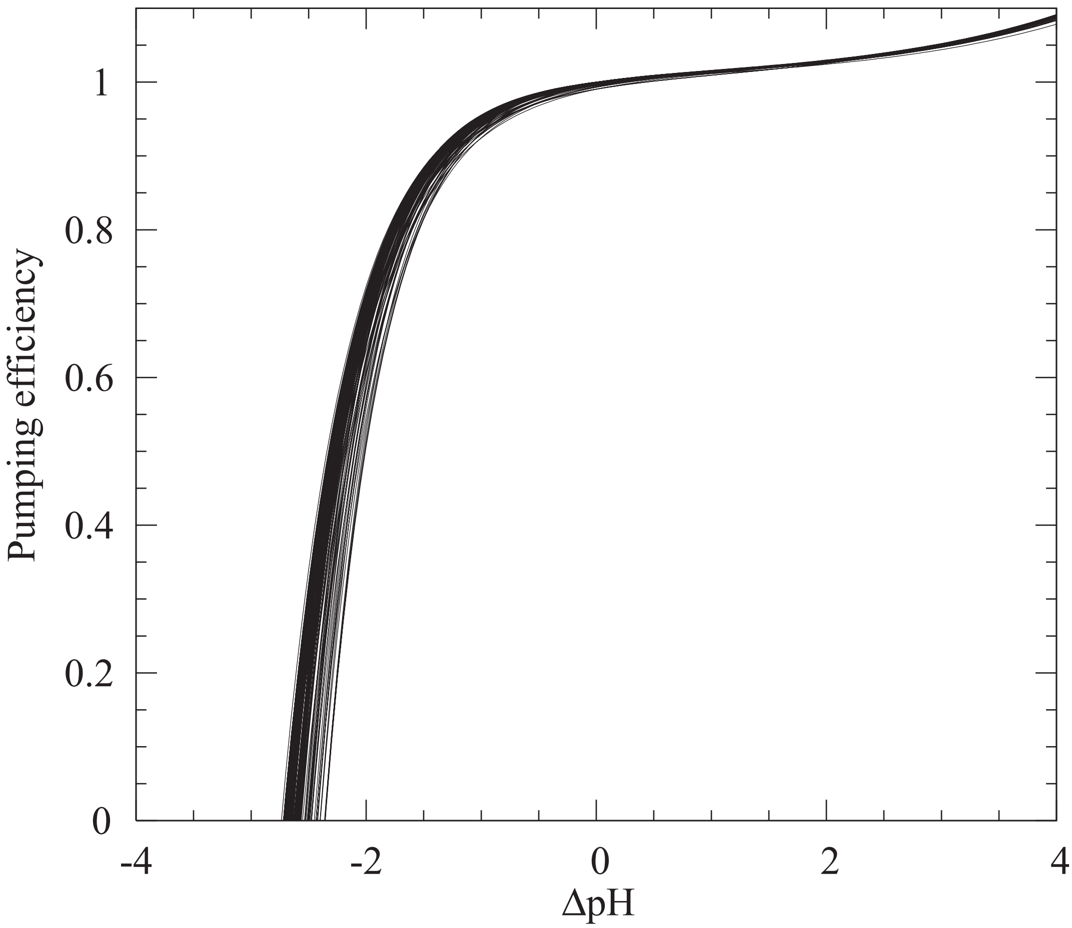

Fig. S2. Pumping efficiency as a function of a pH gradient ($\Delta pH = pH_P - pH_N$) across the membrane at zero membrane potential ($V_m = 0$) for the optimized proton pumping solutions of the three-site model.

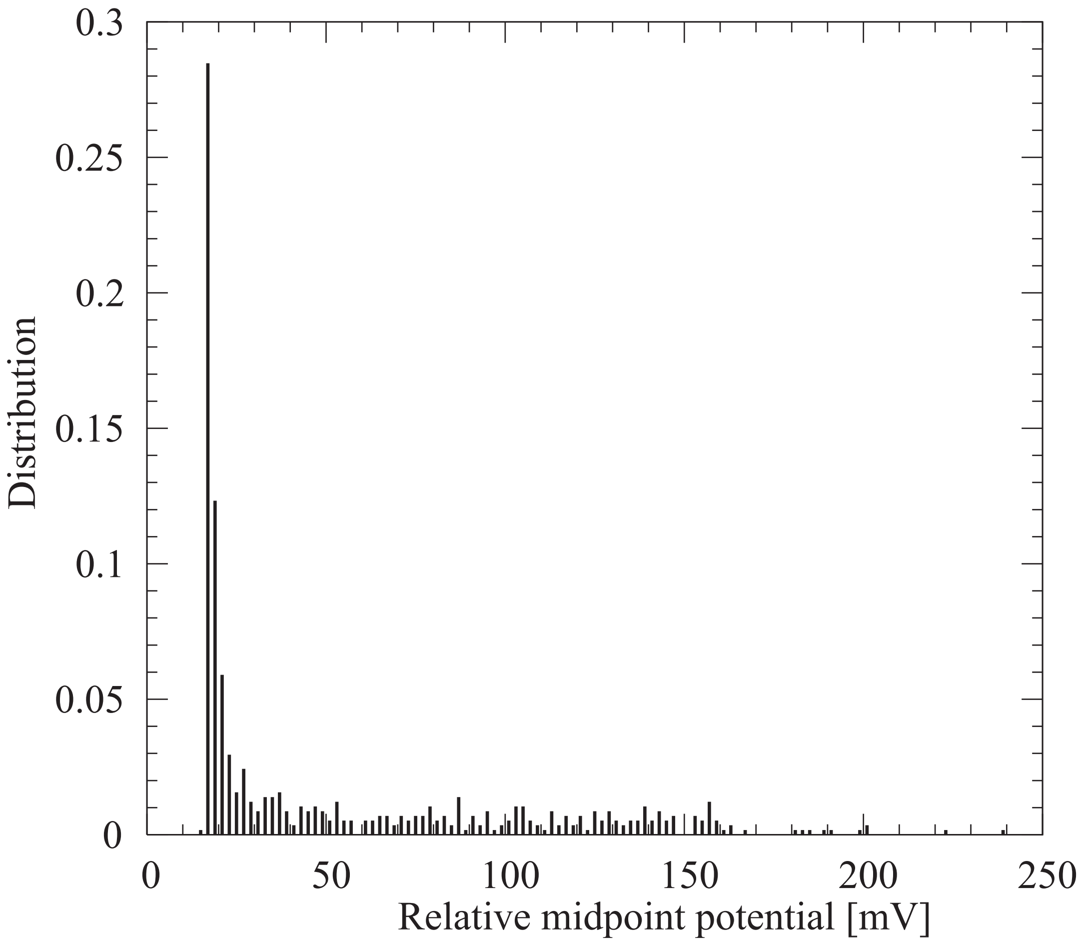

Fig. S3. Distribution of the relative midpoint potentials for the electron site 3 with site 1 (Glu-242) protonated and site 2 (pump site) empty.