Supporting Information for
Molecular Dynamics Investigation of the Interaction Between Central-Site Chloride Binding and Proton Transport in *Ec*CLC

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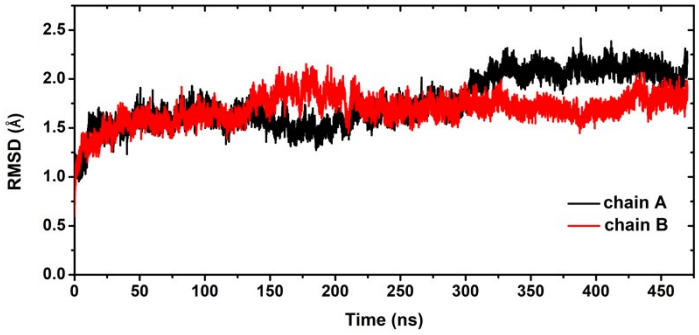

Figure S1. Root-mean-square deviation (RMSD) of Cα atoms in the protein backbone. The black line represents Chain A, and the red line represents Chain B.

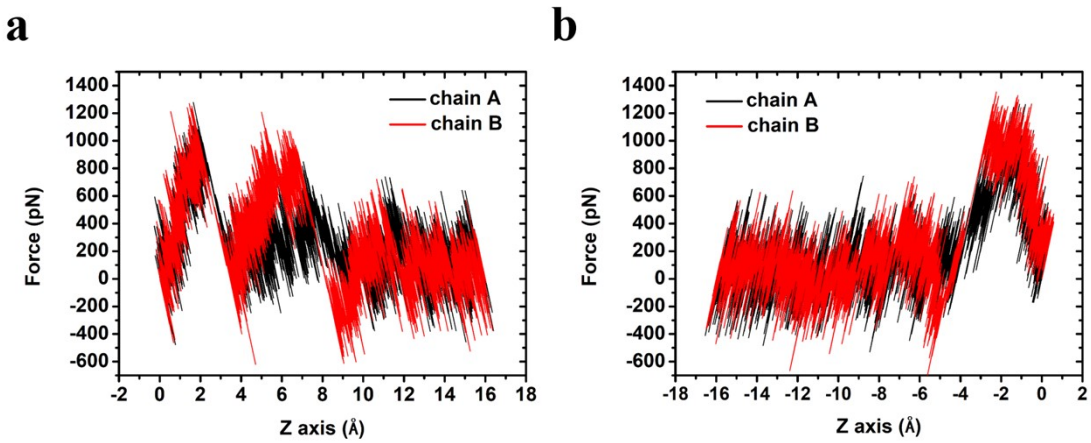

Figure S2. Variation in the stretching force applied to the chloride ion at the central site of Chain A along the z-axis during the SMD simulation. (a) Force fluctuations experienced by the chloride ion at the central binding site in the negative z-direction (toward the intracellular side). (b) Force fluctuations experienced by the chloride ion at the central binding site in the positive z-direction (toward the extracellular side). In both panels, the black line represents the system with water molecules present in the proton channel (chain A), while the red line represents the system without water molecules in the proton channel (chain B).

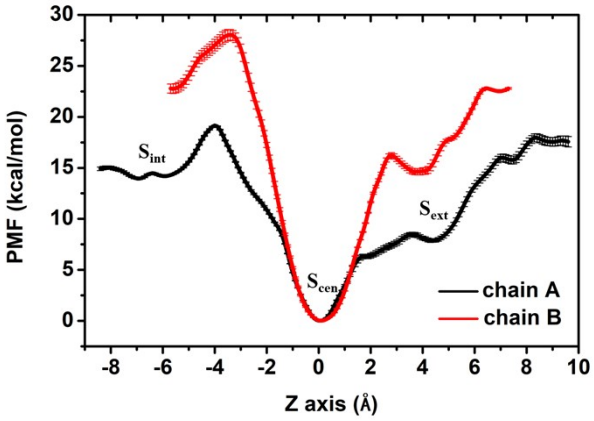

Figure S3. Potential of mean force (PMF) and associated error bars for chloride ion translocation along the Z-axis of the chloride channel. The black line represents the system with water molecules present in the channel (chain A), whereas the red line represents the system without water molecules (chain B).

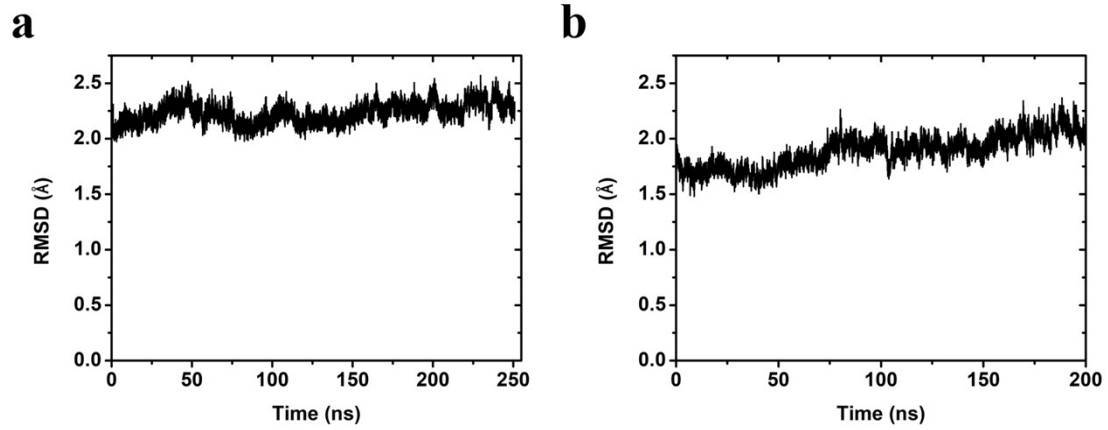

Figure S4. Backbone Cα atom RMSD following SMD removal of the chloride ion. The RMSD is shown for the two independent control simulations initiated from different conformations of E148 in Chain A. (a) Simulation starting from the "out" conformation of E148. (b) Simulation starting from the "up" conformation of E148.

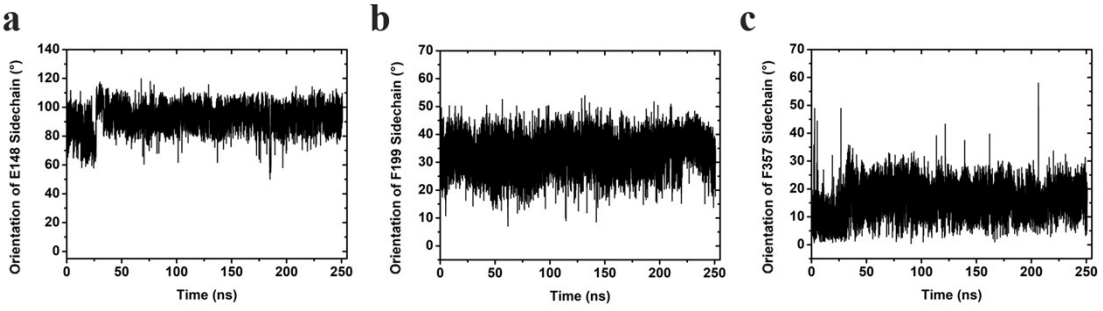

Figure S5. (a) Time-dependent orientation of the E148 side chain in the system where Cl^- departs from the S_{cen} site of chain A. (b) Time-dependent orientation of the F199 side chain in the same system. (c) Time-dependent orientation of the F357 side chain in the same system. In the initial structure, residue E148 in Chain A adopts the “out” conformation.

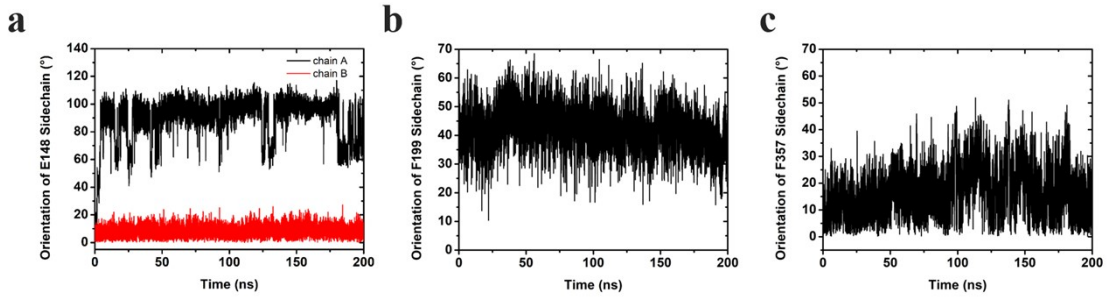

Figure S6. (a) Time-dependent orientation of the E148 side chain in the system where Cl^- departs from the S_{cen} site of chain A (black) and chain B (red). (b) Time-dependent orientation of the F199 side chain in chain A. (c) Time-dependent orientation of the F357 side chain in chain A. In the initial structure, residue E148 in Chain A adopts the “up” conformation.