Supporting Information

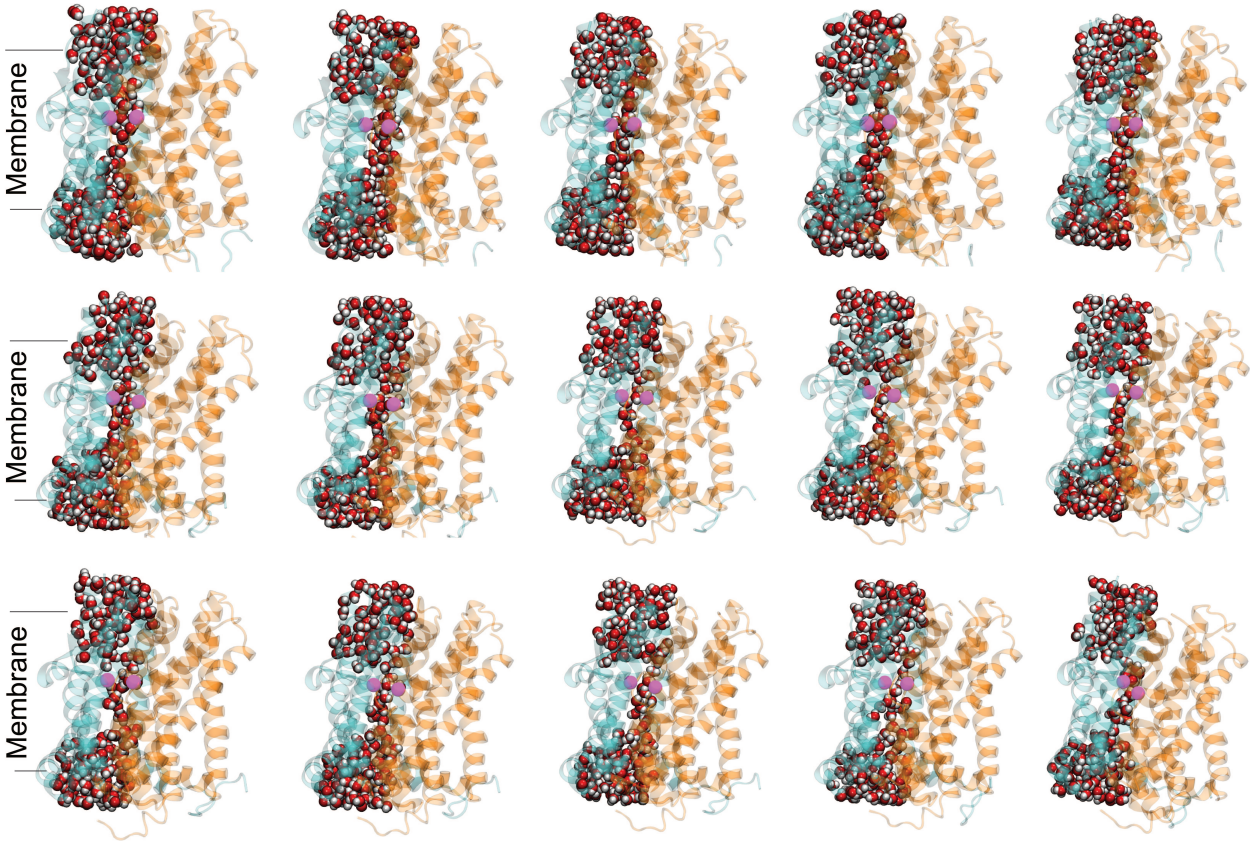

Figure S1: **Captured ClCS conformations in hEAAT1.** Using a WT-MetaD simulation, we captured 15 water-filled, and potentially Cl^- conducting, conformations. The transport and scaffold domains are shown in orange and cyan, respectively. Water molecules are shown in vDW, with oxygen atoms in red and hydrogens in white. The Cα atoms of key residues (L224/G349) crosslinked to trap the ClCS in GltPh in a previous study are shown in purple. Approximate location of upper and lower leaflet is shown in black lines.

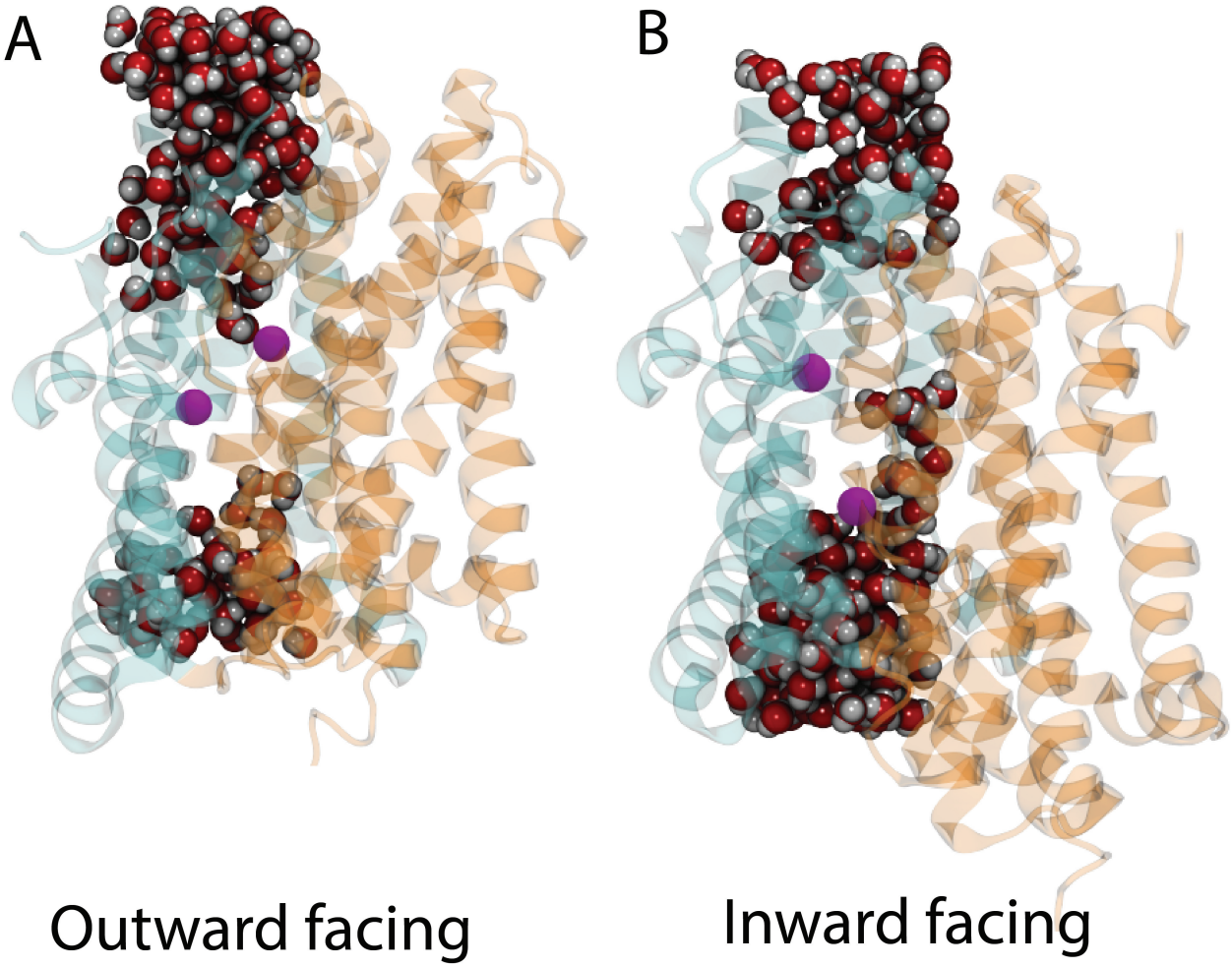

Figure S2: **Water discontinuity in OFS and IFS hEAAT1.** Snapshots highlighting luminal water occupancy of the hEAAT1 OFS (A) and IFS (B). The scaffold domain is shown in cyan and the transport domain in orange. The Cα atoms of the two residues cross-linked in an earlier study to trap the ClCS in Glt_{Ph}[11] are shown in purple.

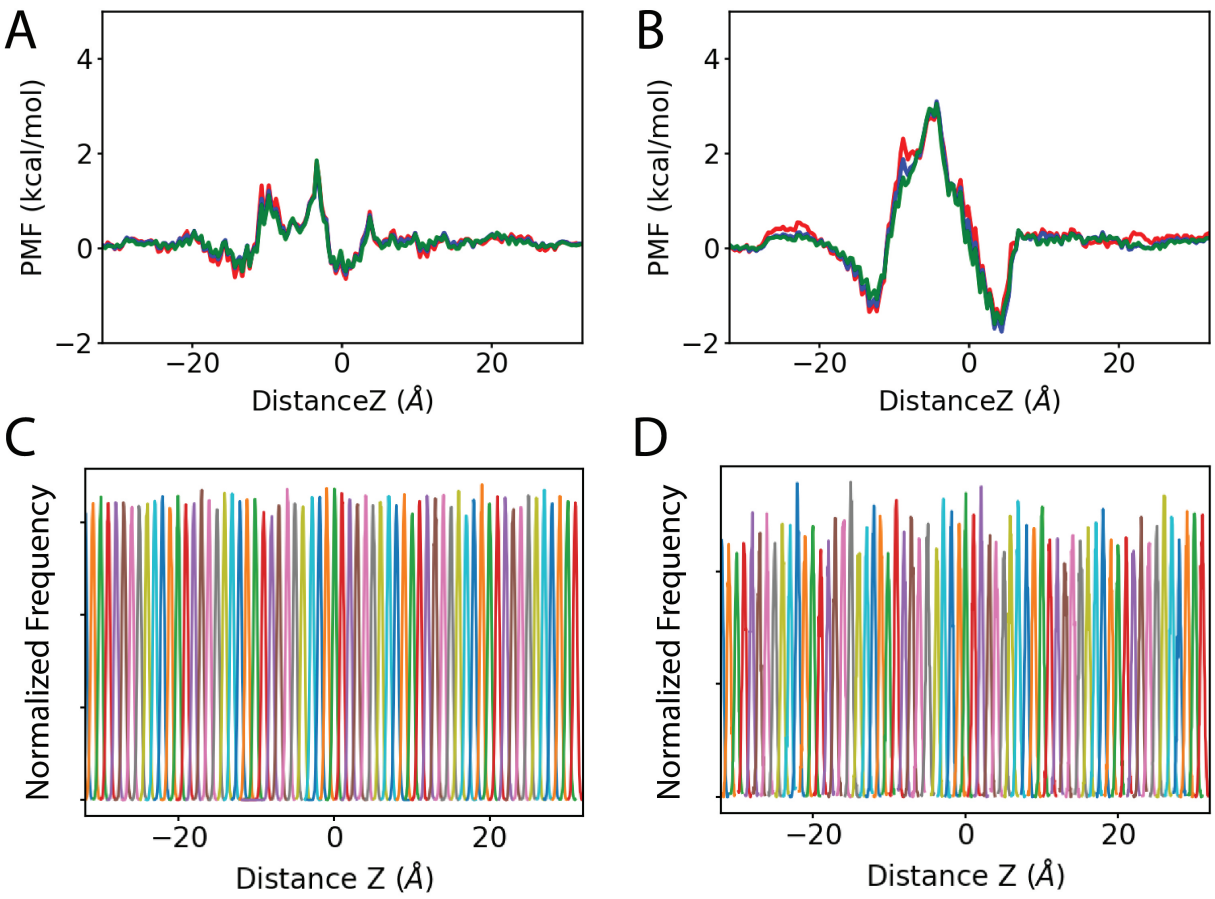

Figure S3: **Convergence of US simulations used to capture Cl^- and Na^+ movements through hEAAT1-ClCS.** Convergence of the free-energy profiles are shown for Cl^- (A) and Na^+ (B) by comparing the PMF profiles obtained after 10 ns (red), 15 ns (blue), or 20 ns (green) of sampling each window. (C-D) Overlap between the corresponding windows in the US simulations for Cl^- (C) and Na^+ (D) permeation through EAAT1-ClCS.

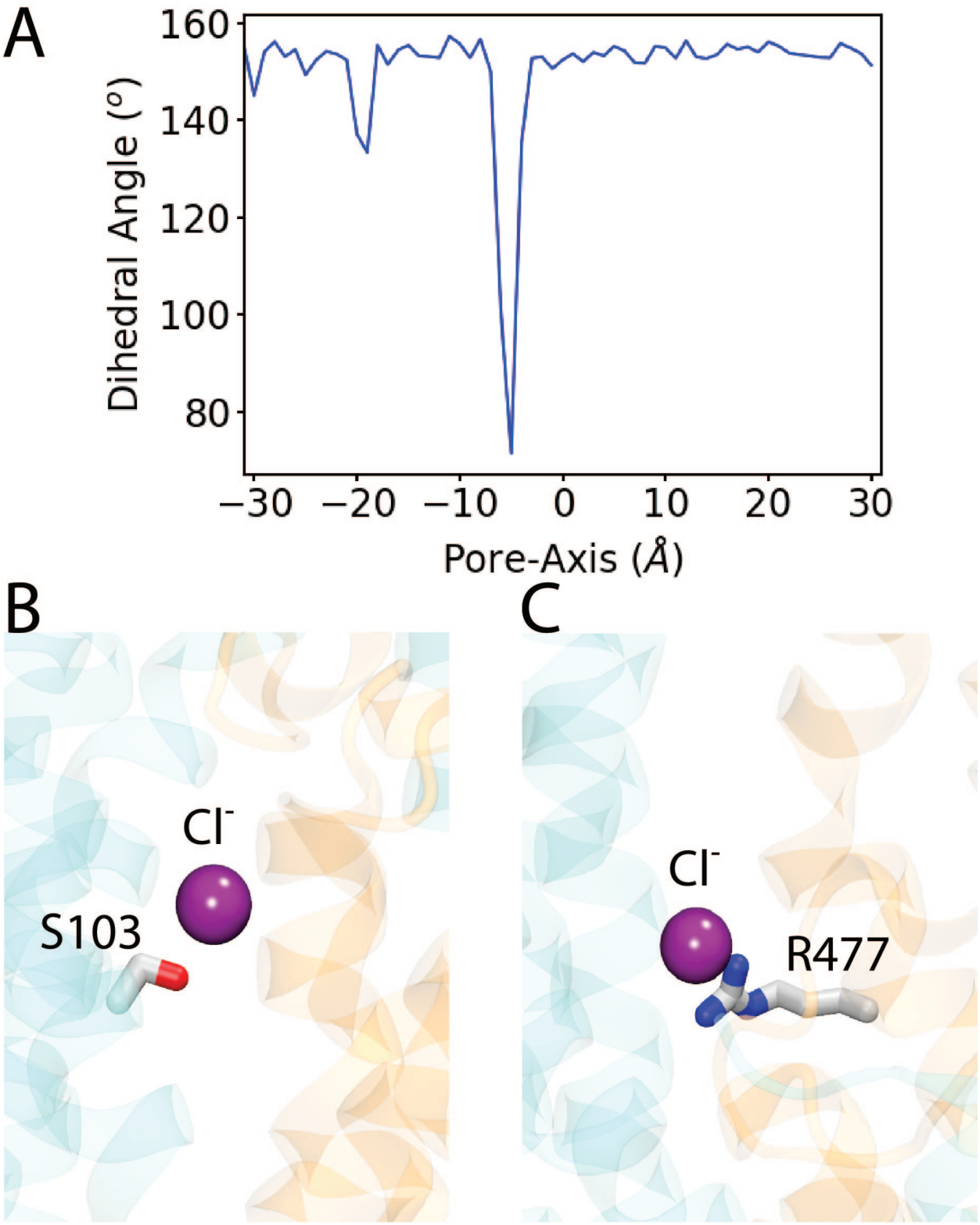

Figure S4: **Local conformational events during Cl^- permeation.** (A) The dihedral angle profile of M286 suggesting the re-orientation of this side chain accompanies movement of Cl^-. Closeup view of the interaction between Cl^- and (B) S103 and (C) R477.