Supporting Material

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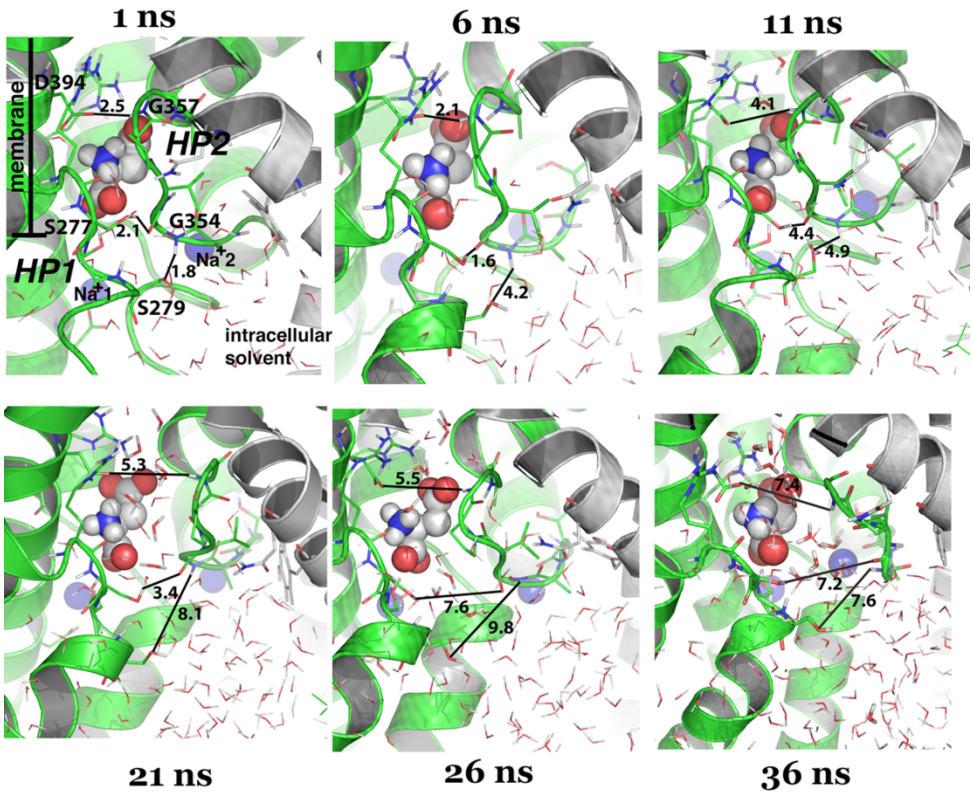

Fig. S1. Succession of events leading to HP2 opening and solvation of the binding site, MD3. Snapshots at different times illustrate the time-resolved events associated with the opening of the HP2 loop. Distances are reported in Ångstroms. At t = 0, the bound substrate is occluded from the IC solvent by the closed loop conformations of HP2 and HP1. At t = 11 ns, water molecules have diffused deeper into the binding cavity and wedged between D394 and G357 thereby disrupting the hydrogen bond that initially existed. Overall, snapshots 21 to 36 ns illustrate that the HP2 loop undergoes a large opening as demonstrated by the significantly increased distance between S277/S279 (HP1) and G354 (HP2), and D394 (TM8) and G357 (HP2), and the solvation of the binding site. The distinguishing characteristic compared to that of MD1 (See Fig 3) and MD2 (See Fig 6) is that the HP2 loop can open while $Na^{+}_{(2)}$ remains bound to the transporter.

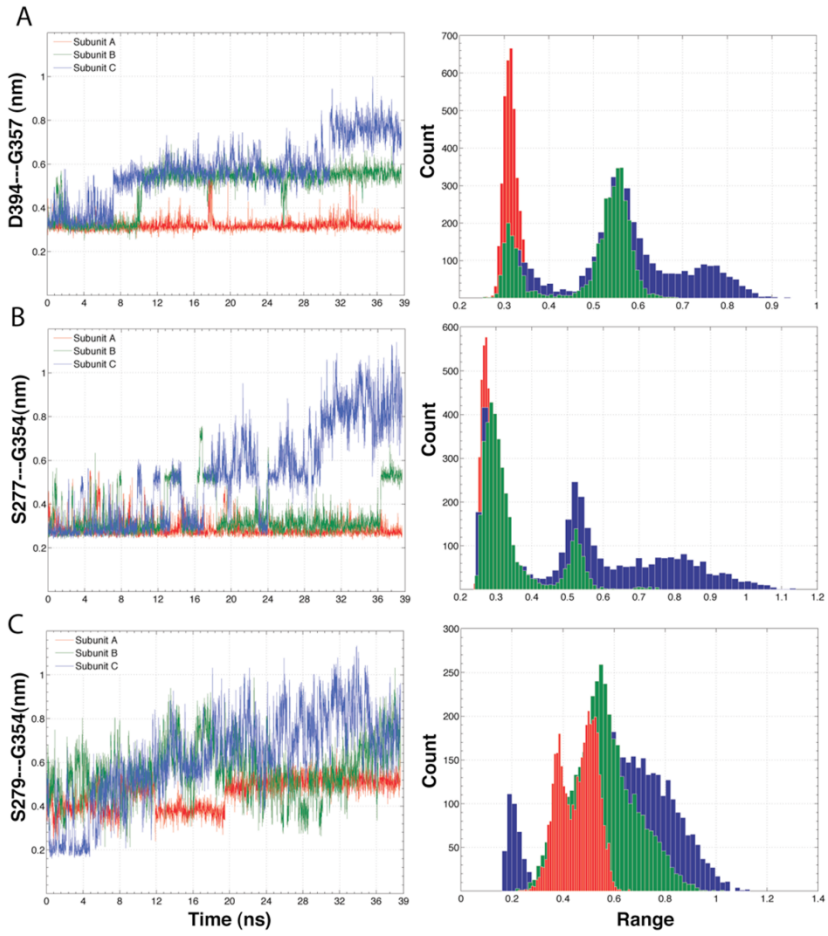

Fig. S2. Time evolution of events associated with the IC gate opening in MD3. Results for subunits, A, B, C are colored red, green, and blue, respectively. Panels A-D (*left*) refer to specific distances between residue atoms, and (*right*) show histograms for the particular distances. (A) D394 (side-chain carboxylate carbon) and G357 (amide hydrogen). (B) S277 (side-chain oxygen) and G354 (backbone carbonyl oxygen). (C) S279 (side-chain oxygen) and G354 (backbone amide hydrogen).

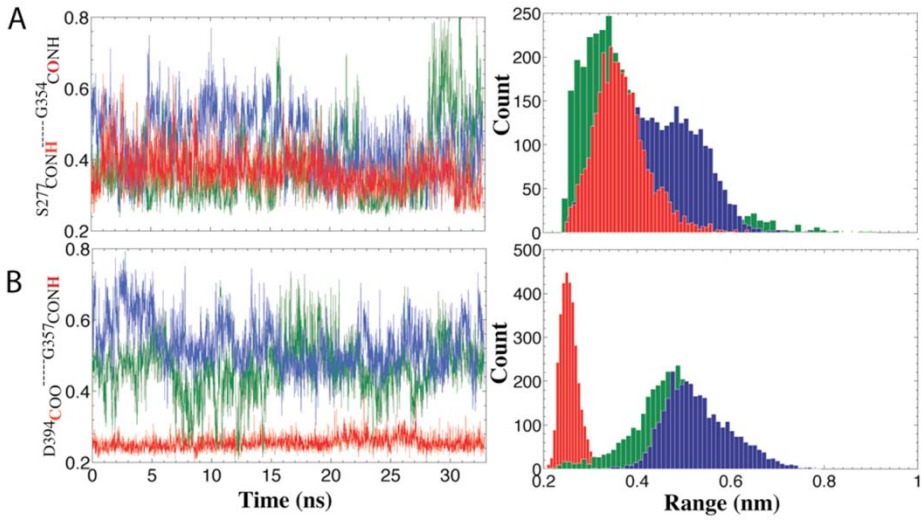

Fig. S3. Time evolution of events associated with the IC gate, MD4, substrate-free transporter. Results for subunits, A, B, C are colored red, green, and blue, respectively. The snapshot at t = 16ns from MD3 (See Fig S2) provided the starting coordinates for the simulations; however, the substrate in each subunit was removed prior to the simulation. Panels A-B, (left) refer to specific distances between residue atoms, and (right) show histograms of the distribution for the particular distance. (A) S277 (backbone amide hydrogen) and G354 (backbone carbonyl oxygen). (B) D394 (side-chain carboxylate carbon) and G357 (amide hydrogen).

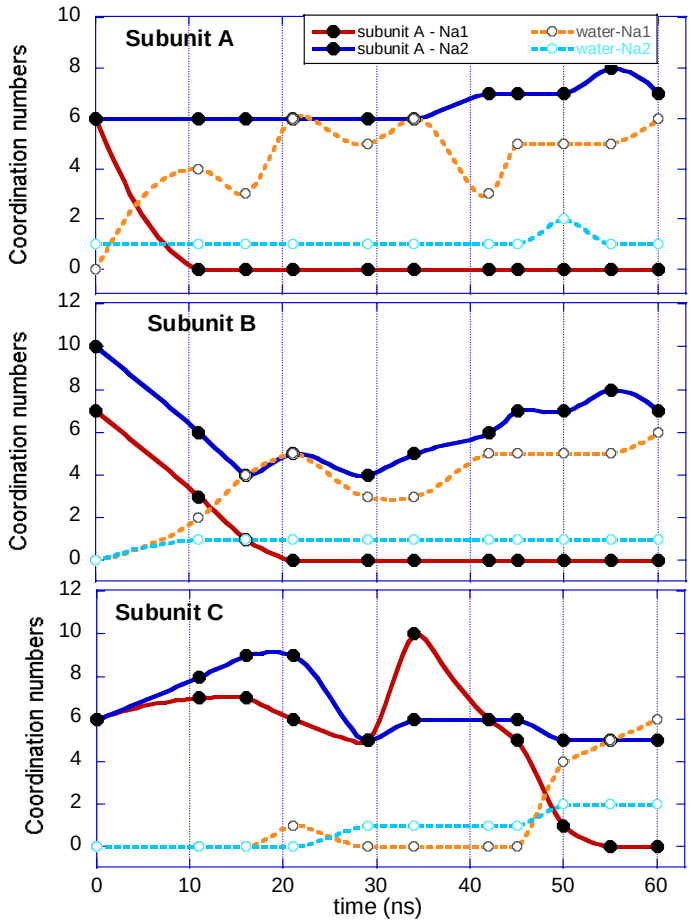

Figure S4 Coordination of sodium ions in the respective subunits. The solid curves, in each panel, correspond to the coordination number of the outer sodium ($Na^{+}_{(1)}$; *red*) and inner sodium ($Na^{+}_{(2)}$; *blue*) with protein atoms. The dashed curves correspond to the coordination number of $Na^{+}_{(1)}$ (*orange*) and $Na^{+}_{(2)}$ (*light blue*) with water molecule. We note that the inner sodium, $Na^{+}_{(2)}$ in all the three subunits remains coordinated with at least six or more protein atoms, while the outer sodium, $Na^{+}_{(1)}$, tends to have lower coordination and exits the protein in all the three subunits, while it gets hydrated with water molecules.

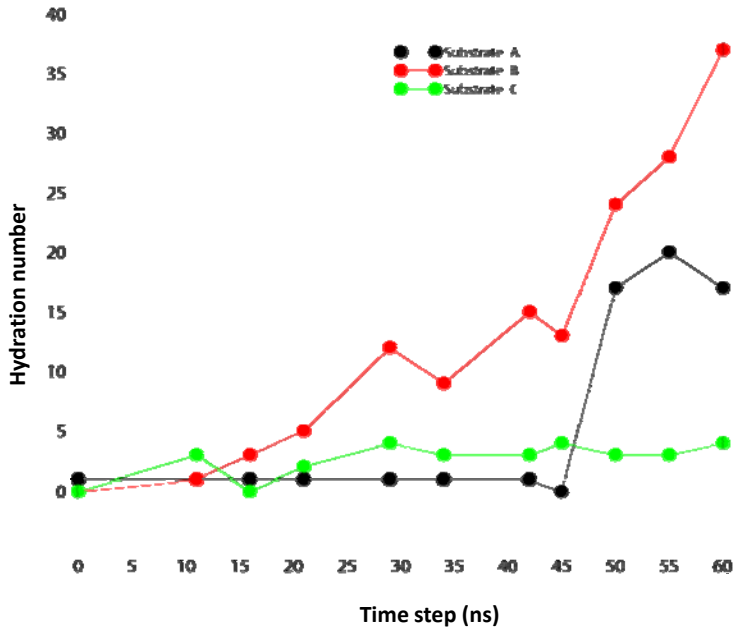

Figure S5. The hydration of the substrate in the three subunits, plotted as a function of time. The hydration number is defined as the number of water molecules within 5Å of any atom of the substrate. Results refer to MD2.