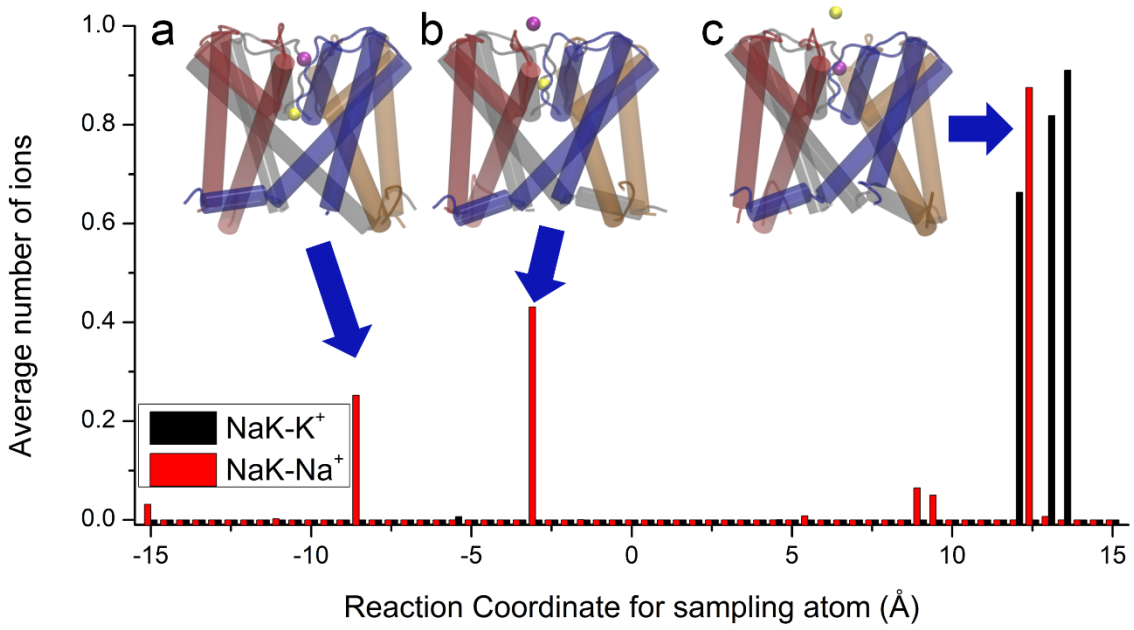
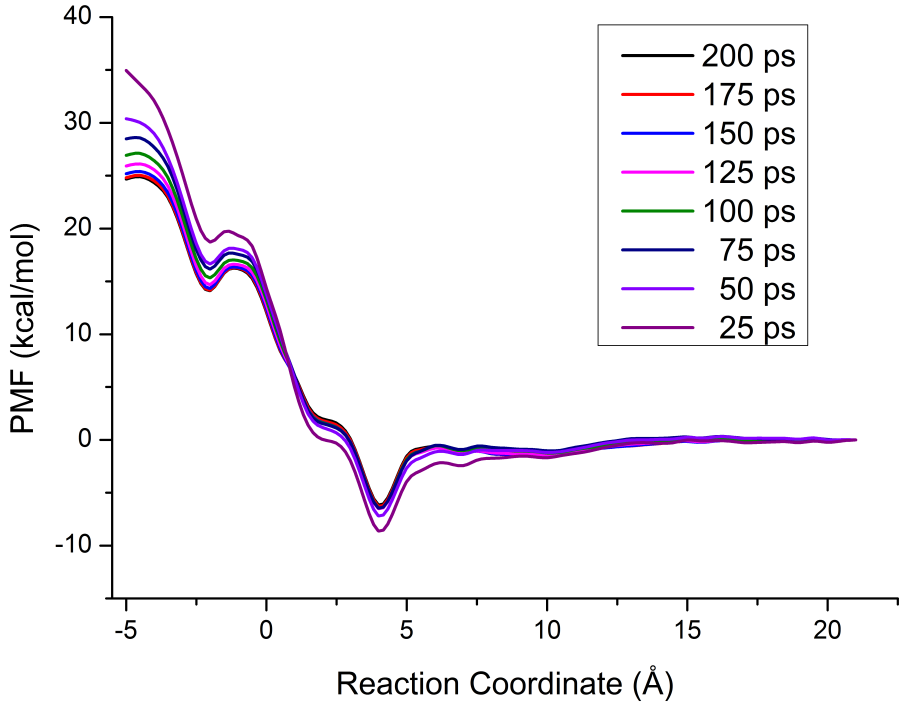
Supplementary Figure S1. The average number of ions within a confinement radius of 5 Å around the carbonyl oxygen of V63 (except for the sampling ion, shown in yellow) against the reaction coordinate of the sampling ion. Each window in the histograph below is averaged over a 1 ns trajectory (20,000 frames). The conformation of last frame for several windows were displayed. As shown in the figure, only 1 ion (purple) was found near the mouth of the filter when the sampling ion was either in the bulk solution or deep in the filter. The most likely place that we would see perturbation of the 1D PMF would be the conformation of **b** below when the ion is ~-3 Å along the reaction coordinate, and even there the 2 ions are fairly distant, ~5 Å, and the extracellular ion is present in less than half the windows.



Supplementary Figure S2. Convergence of the 1D multi-ion PMF profiles integrated from a set of 2D PMF maps. The PMFs were plotted every 25 ps from 25 ps to 200 ps. Note that relatively little change occurs in the PMFs for simulations of 125 ps and longer.



Supplementary Table S1. Kramer's rate for the K^+ and Na^+ ions in the 1D PMFs integrated from the 2D PMF maps of NaK2K channel (unit: ns^{-1}).

Moving from the cavity to the bulk water		
K from K-K-K	S2→S0	0.78(±0.0003)
	S0→water	0.002(±0.0001)
Na from Na-Na-Na	S2→S1	2.02(±0.0235)
	S1→S0	6.39(±0.3862)
	S0→water	0.13 (±0.0062)
Na from Na-K-K	S0→water	0.0005 (±0.0001)