Supporting Information

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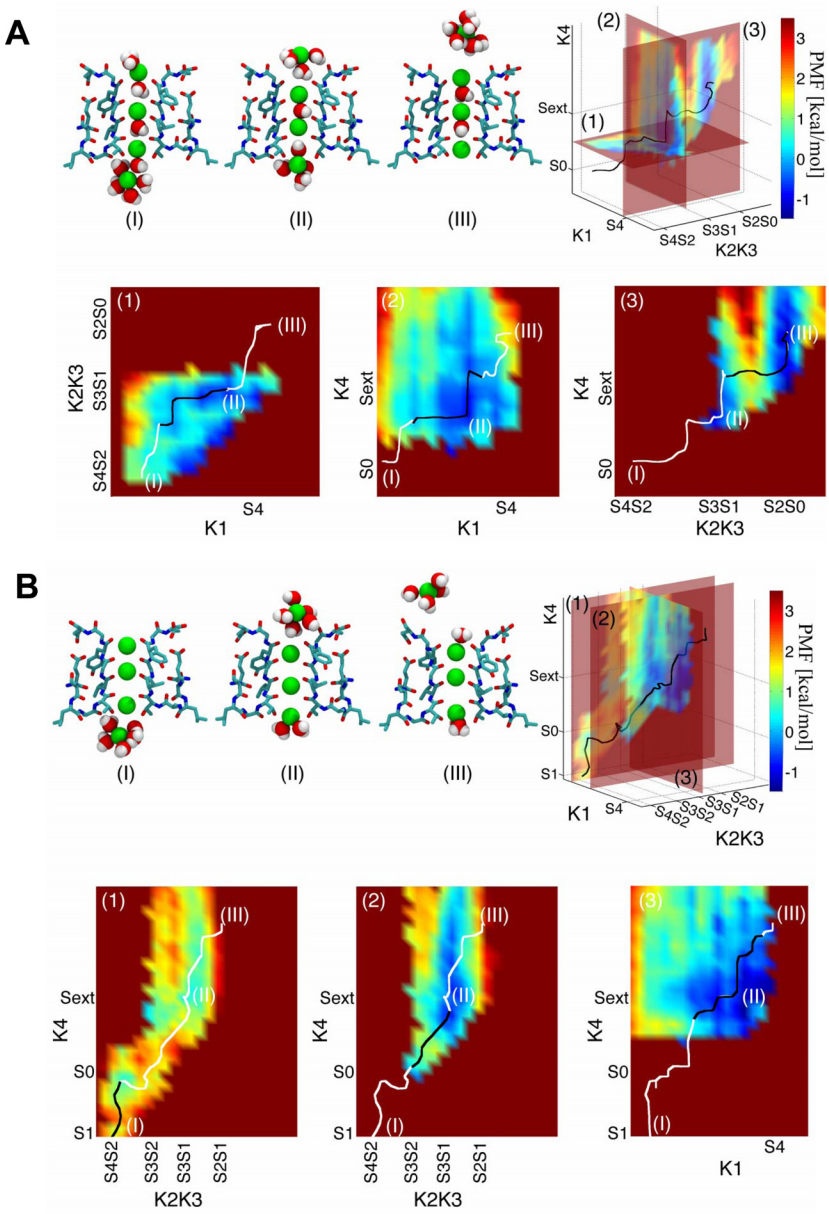

Fig. S1. (*A*) KWK conduction mechanism: different snapshots of the SF of KirBac along the KWK conduction pathway. Two opposite subunits of the SF region are shown in licorice representation, colored by atom type (Glu-106-Asp-115); K^+ ions (green) and water molecules are depicted in VDW representation. The potential of mean force and the minimum energy path (MEP) are shown in the 3D plot and reported in 2D maps (1–3). In the 2D maps a black line is used for the fraction of the MEP lying on the plane, while a white line is used for the remaining part of the path. Labels I–III highlight the position along the MEP of the SF structures shown in the snapshots. Outward conduction is initiated by the ions in S4 and S2 moving toward S3 and S1 sites; the cavity ion is attracted toward S4 (map 1, snapshots I–II). This movement in turn destabilizes the ion in S0, which moves toward the extracellular milieu (map 2). Finally, ions in S3 and S1 move to S2 and S0, causing the outermost ion to travel/advance toward the lumen (map 3, snapshot III). The reverse mechanism describes the inward permeation process. (*B*) KK conduction mechanism. Snapshots I–III show the SF of KirBac in different states along the KK conduction pathway. The same notation as in Fig. 1 is adopted. Outward conduction is initiated by the ion in S1 moving toward S0 (map 1). After that, ions in S4 and S2 move to S3 and S1 (map 2). Configuration II is the most stable one along the minimum energy path in the KK conduction mechanism, being ≈2 kcal/mol more stable than configurations I and III. When the outermost ion reaches the lumen, upward movement of the ion in S3 to S2 concludes the conduction event (map 3, snapshot III). The reverse mechanism describes the inward permeation process.

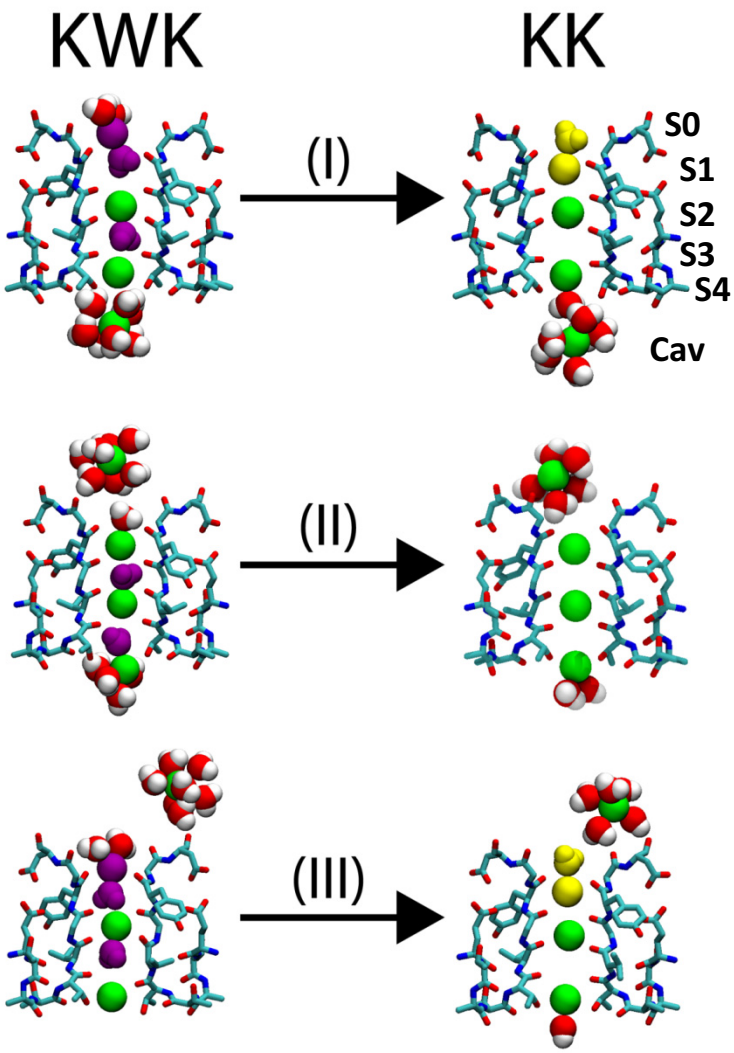

Fig. S2. Initial and final configurations of ions and water molecules in the SF in the FEP simulations. Representative structures of the KcsA SF at the beginning and at the end of the FEP simulations are shown; analogous alchemical transformations were performed for the KirBac channel. Only 2 opposite subunits of the SF region are shown in licorice representation, colored by atom type (Glu-71-Asp-80) for simplicity. K^+ ions and water molecules are depicted in VDW representation. Atoms disappearing are in purple in contrast to those appearing, which are in yellow. Transformation I links configuration I of the KWK mechanism to configuration I of the KK mechanism. It involves the annihilation of the water molecule in site S3, the creation of a water molecule in bulk solution (not shown), the transformation of the water molecule in site S1 into a K^+ ion, and the transformation of the K^+ ion in site S0 into a water molecule. Analogous changes took place in alchemical transformation III, which links alike configurations of the KWK and KK mechanisms. In transformation II, the water molecules in sites S4 and S2 are annihilated, and 2 water molecules are created in bulk solution (not shown). FEP simulations of these alchemical transformations directly estimate the free energy difference between the 2 conduction mechanisms.