

Supporting Information

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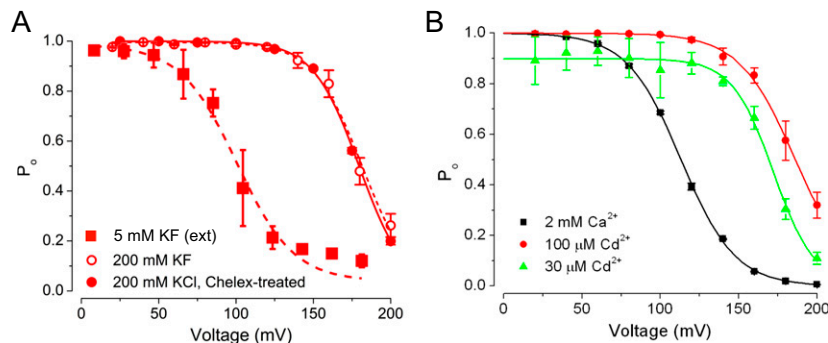


Fig. S1. Intrinsic inactivation of MthK channels at depolarized voltages. (A) P_o vs. voltage for MthK channels recorded with either KF-based solutions or KCl-based solutions from which Ca^{2+} was sequestered using Chelex-100 resin. In each case, solutions at the cytoplasmic side of the channel contained 100 μM Cd^{2+} and 200 mM K^+ (as either chloride or fluoride salt) as well as 10 mM Hepes (pH 8.1). Solutions at the extracellular side contained 5 mM KF, 195 mM *N*-methyl glucamine (red squares), 200 mM KF (red open circles), or 200 mM KCl (red filled circles). All extracellular solutions additionally contained 10 mM Hepes at pH 7.0. These results illustrate that gating into the inactivated state persists even after sequestration or buffering of free Ca^{2+} to low levels ($<1 \mu M$) and that inactivation was enhanced by lowering extracellular $[K^+]$. (B) P_o vs. voltage for single MthK channels in the presence of 2 mM Ca^{2+} (black), 30 μM Cd^{2+} (green), and 100 μM Cd^{2+} (red). Gating into the nonconducting, inactivated state shifts $V_{1/2}$ values to less positive voltages (i.e., to the left) with 2 mM Ca^{2+} . If 100 μM Cd^{2+} also promoted gating into the inactivated state, then one would predict that lowering $[Cd^{2+}]$ to 30 μM would shift the $V_{1/2}$ to more positive values, leading to higher P_o at 200 mV compared with that observed with 100 μM Cd^{2+} . Instead, lowering the $[Cd^{2+}]$ leads to overall lower levels of maximal activation but does not preferentially decrease entry to the inactivated state. Together, these results suggest that gating into the inactivated state does not arise from 100 μM Cd^{2+} or low levels of contaminant Ca^{2+} .

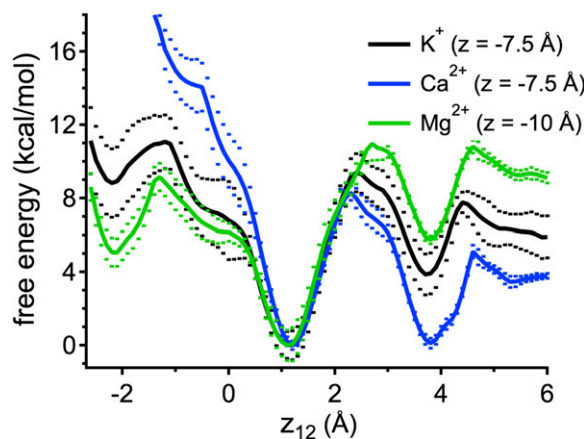


Fig. S2. Free energies of K^+ ions in the selectivity filter with K^+ , Mg^{2+} , or Ca^{2+} at their respective energy wells in the pore; z_{12} corresponds to the center of mass of the K^+ ions in the filter relative to the center of mass of the selectivity filter atoms. With a Ca^{2+} ion at S5 ($z = -7.5 \text{\AA}$), K^+ ions are driven to an energy well at the extracellular end of the selectivity filter compared with the effect of K^+ at S5 ($z = -7.5 \text{\AA}$) or Mg^{2+} at its energy well in S_{cav} ($z = -10 \text{\AA}$). Dotted lines represent \pm SD.

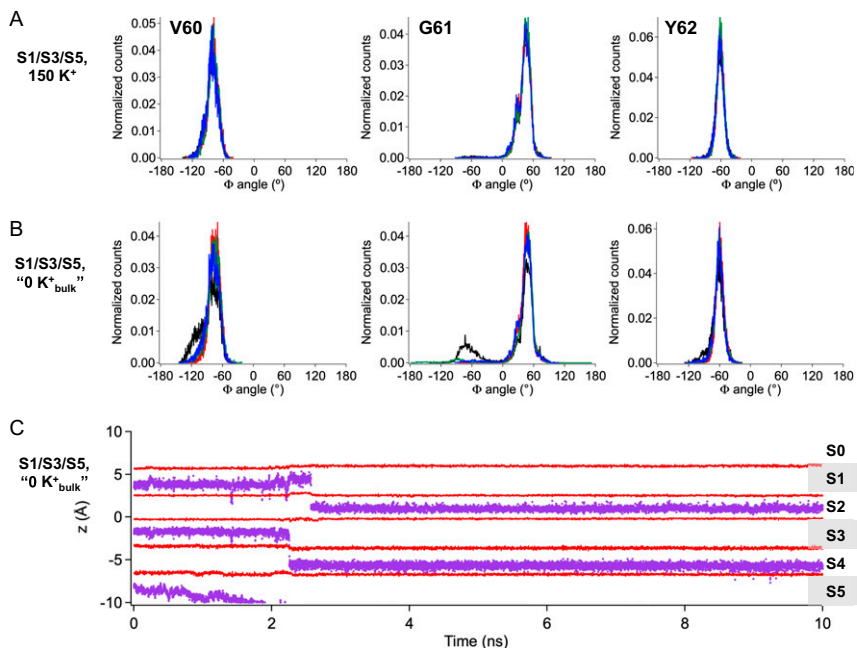


Fig. S3. K^+ dissociation facilitates a conformational change in the MthK selectivity filter. (A) Histogram of dihedral (Φ) angles for the indicated residue sampled during molecular simulations; each of four subunits is represented by a different color (black, red, green, and blue). With K^+ occupying the S1/S3/S5 sites, all carbonyl oxygen atoms face the central axis of the pore (in canonical positions) during the course of simulation; thus, the Φ -angles for each residue are centered around single peaks. (B) With K^+ ions initially occupying the S1/S3/S5 sites but with a force applied to repel binding of K^+ ions coming from the bulk ($0 K_{\text{bulk}}^+$) (*Methods*), the S1/S3 ions redistribute to the S2/S4 sites, leading principally to a rotation of the V60 carbonyl oxygen (illustrated in Fig. 7). (C) K^+ ion (purple) and protein oxygen positions (red) as a function of time during a representative molecular simulation illustrating movement of K^+ ions from their initial positions in the S1/S3/S5 sites. After the K^+ ion diffuses from its initial position in the S5 site, the S1 and S3 K^+ ions move rapidly into the S2 and S4 sites.

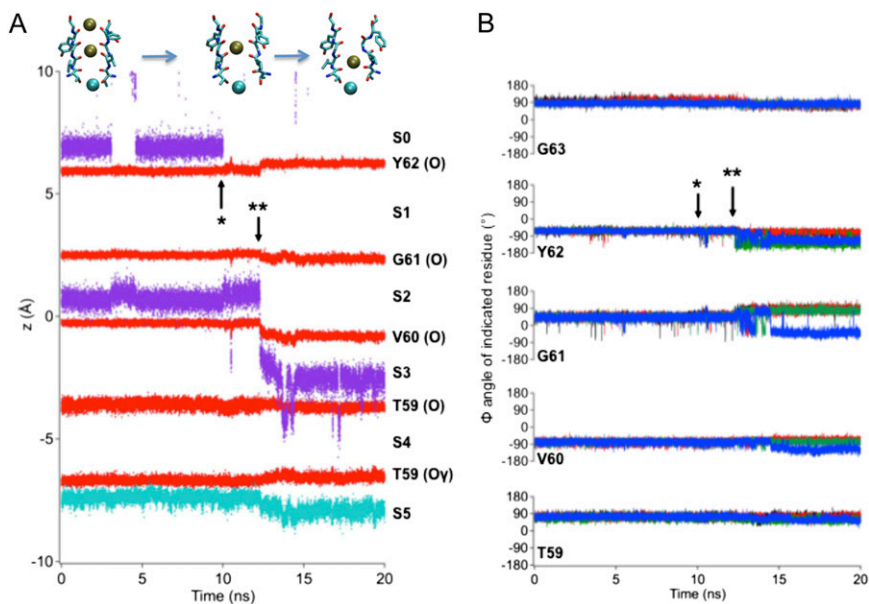


Fig. S4. Ion dissociation from S0 can facilitate a conformational change in the selectivity filter. (A) Axial positions of ions and protein oxygen as a function of time during a representative molecular simulation. (O) indicates the carbonyl oxygen of the indicated residue, whereas (O_y) indicates the sidechain oxygen of residue T59. (B) Φ -Angles for selectivity filter residues during the same simulation plotted as a function of time. Initially, with Ca^{2+} at S5 (cyan) and K^+ ions at S2 and S0 (purple), carbonyl oxygen atoms forming the selectivity filter (red) are in their canonical positions. *Dissociation of K^+ from S0. **Movement of the remaining K^+ ion from S2 to S3 leads to transitions between the canonical and rotated conformations.