Supporting Information

Kazmier et al. 10.1073/pnas.1410431111

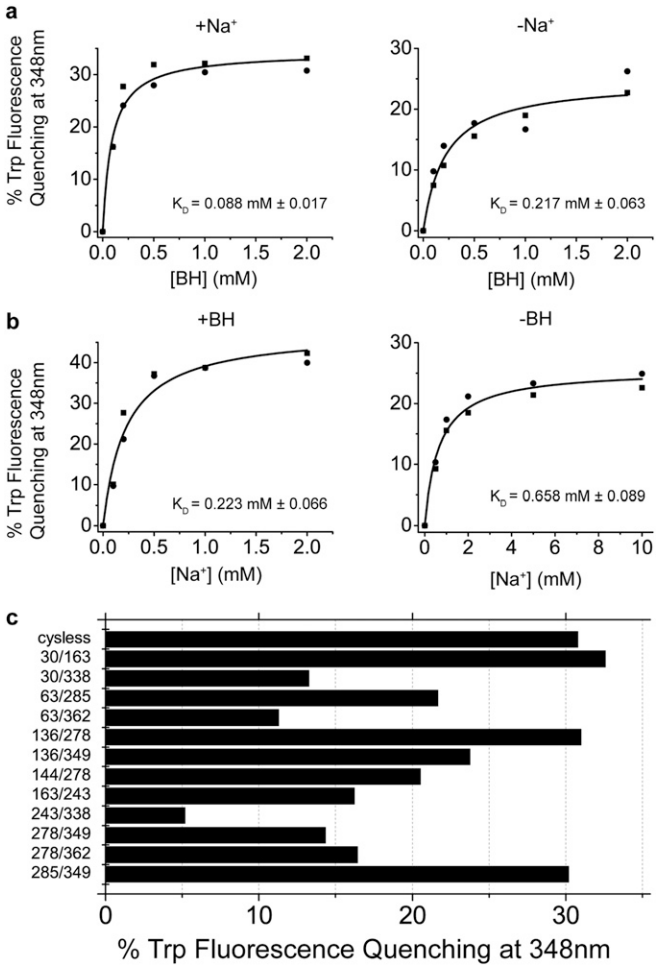

Fig. S1. Na^+ and benzyl-hydantoin (BH) bind to cysless Mhp1 and its spin-labeled mutants. Trp fluorescence emission was measured at 348 nm. Na^+ and BH binding leads to reduction in signal intensity, which is reported as a percentage quenching of the signal in the absence of ligands. (*A*) BH-binding isotherms determined from Trp fluorescence quenching were generated for cysless Mhp1 in the presence and absence of saturating concentrations of Na^+ for increasing concentrations of BH. The results demonstrate that the substitution of the native cysteines does not compromise substrate binding or abrogate the increase in substrate affinity in the presence of Na^+. (*B*) Na^+ binding isotherms were generated for cysless Mhp1 in the presence and absence of saturating concentrations of BH for increasing concentrations of Na^+. The results show that cysless Mhp1 binds Na^+ in the presence and absence of BH. The data in *A* and *B* represent two distinct measurements. The solid line is a nonlinear least-squares fit to the average. (*C*) Trp fluorescence quenching for spin-labeled pairs demonstrates BH binding in the presence of saturating levels of Na^+ and BH.

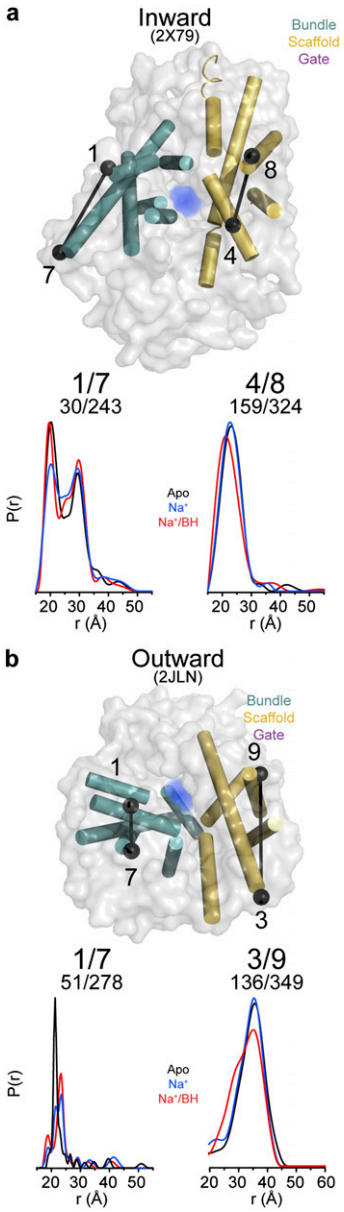

Fig. S2. Rigidity of the bundle and scaffold motifs of Mhp1. Intracellular (*A*) and extracellular (*B*) Mhp1 mutants are shown on the Mhp1 structures as black dots connected by a black line. Approximate location of permeation pathways are shown in blue. Distance distributions between transmembrane (TM) helices in the bundle and scaffold show changes in average distance and width upon Na^+/BH binding on the extracellular side. In contrast, intrabundle and intrascaffold distance distributions are relatively invariant on the intracellular side.

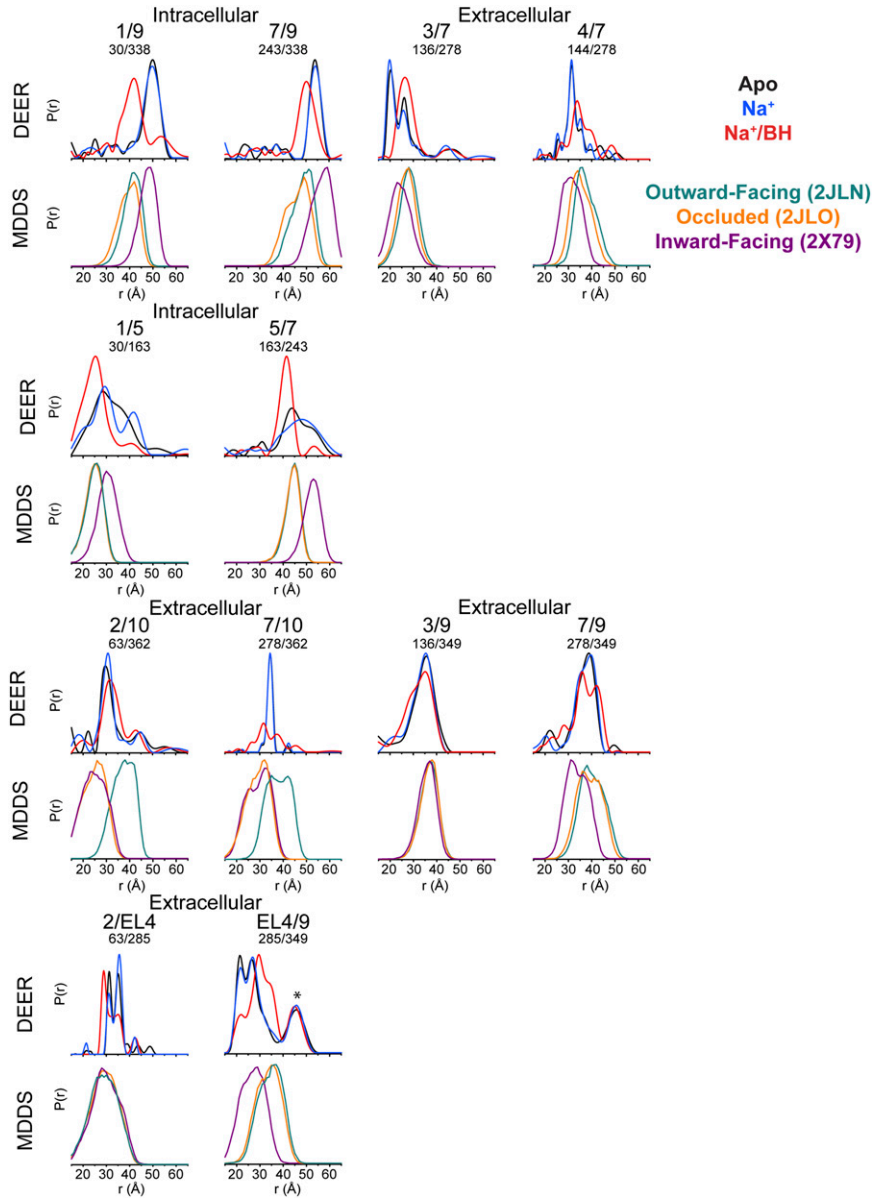

Fig. S3. Comparison between experimental distance distributions and distributions calculated by molecular dynamics of dummy spin label (MDDS) from the crystal structures. Model spin labels were introduced at the spin-labeled positions in each of the three crystal structures representing the outward-facing (2JLN), occluded (2JLO), and inward-facing conformations (2X79), and distance distributions were calculated as described in *Methods*. Both double electron-electron resonance (DEER) distributions and MDDS simulations are shown as a probability, P(r), relative to distance, r.

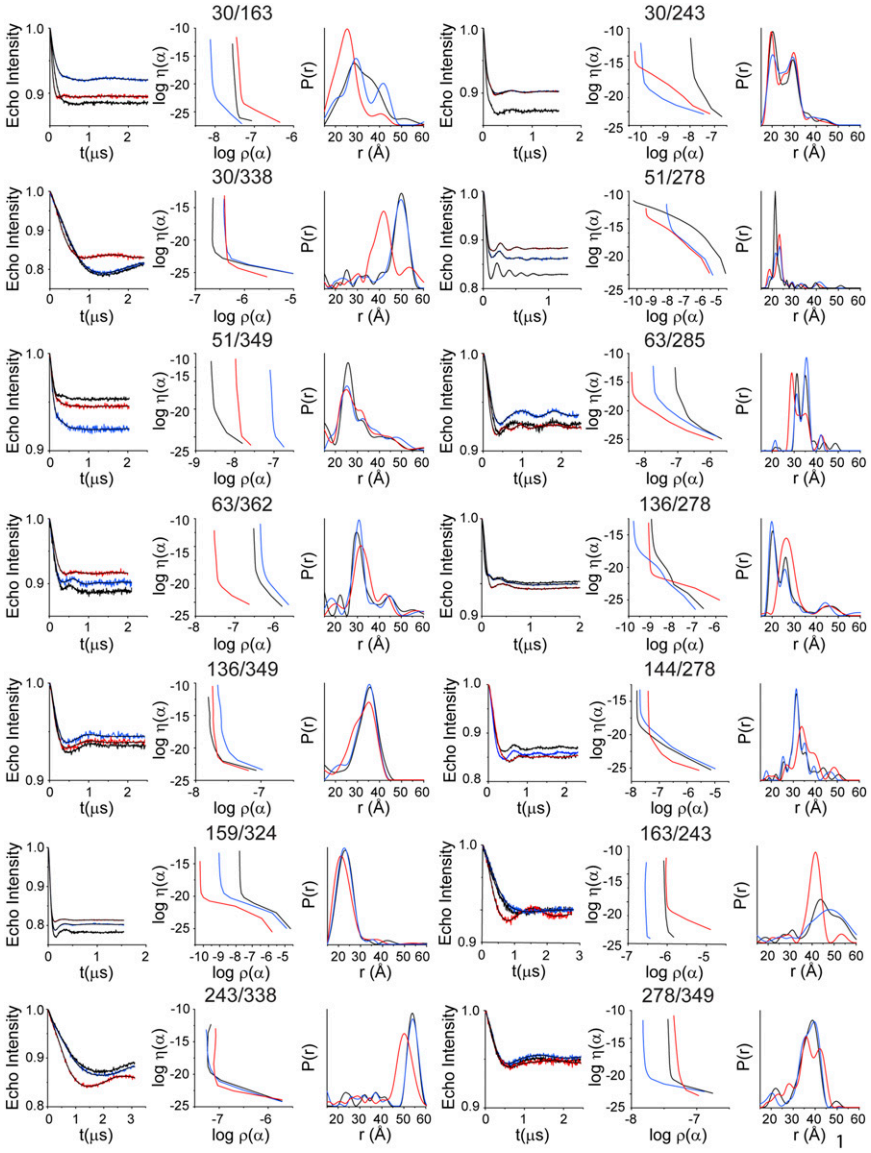

Fig. S4. DEER data analysis. For each mutant, normalized echo decays along with the corresponding fits (*Left*), L curves (*Center*), and distance distributions (*Right*) are shown.