Fig. S1. Raw double electron–electron resonance (DEER) time traces. (A) Raw DEER time traces for HCN2_cys-free double-cysteine mutants labeled with S-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate are shown in black, in the absence or presence of cAMP, as indicated. The smooth curves are distance-distribution fits to the data. (B) DEER time traces for single-cysteine mutants in the absence (Left) and presence (Right) of cAMP show only slow, quasilinear decays.
**Fig. S2.** Experimental distance distributions compared with those predicted from elastic network models. Distance distributions obtained from DEER for HCN2_cys-free double-cysteine mutants in the absence and presence of cAMP compared with predicted DEER traces (dashed lines) calculated from the elastic-network model structures in Fig. 4.
Movie S1. Conformational transition in hyperpolarization-activated cyclic nucleotide-gated channel upon cAMP binding. A morph of two structural models derived from DEER data based on the elastic-network model is shown.