Supporting Information

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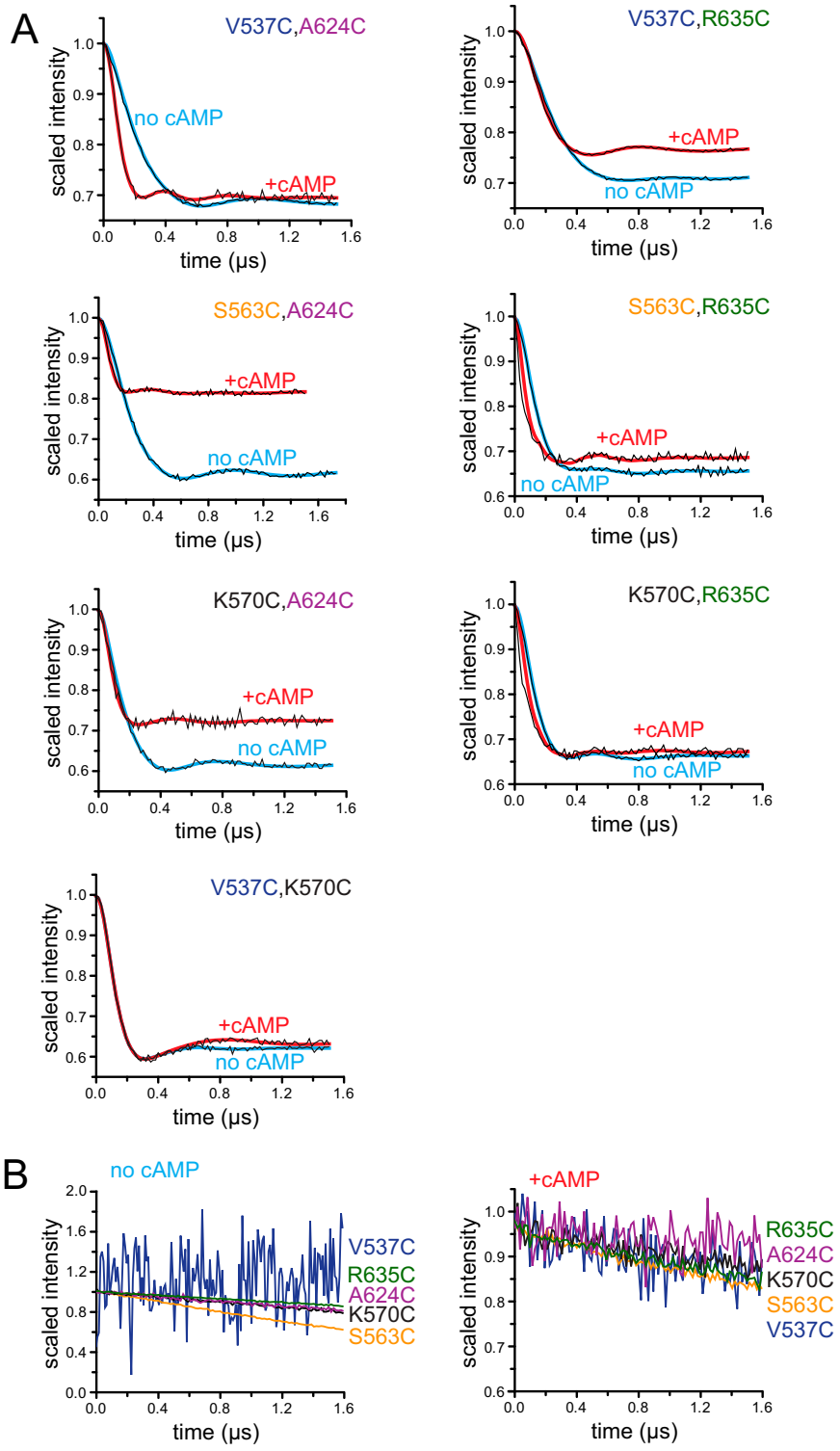

Fig. S1. Raw double electron–electron resonance (DEER) time traces. (*A*) Raw DEER time traces for $HCN2_{cys\text{-}free}$ double-cysteine mutants labeled with *S*-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate are shown in black, in the absence or presence of cAMP, 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.

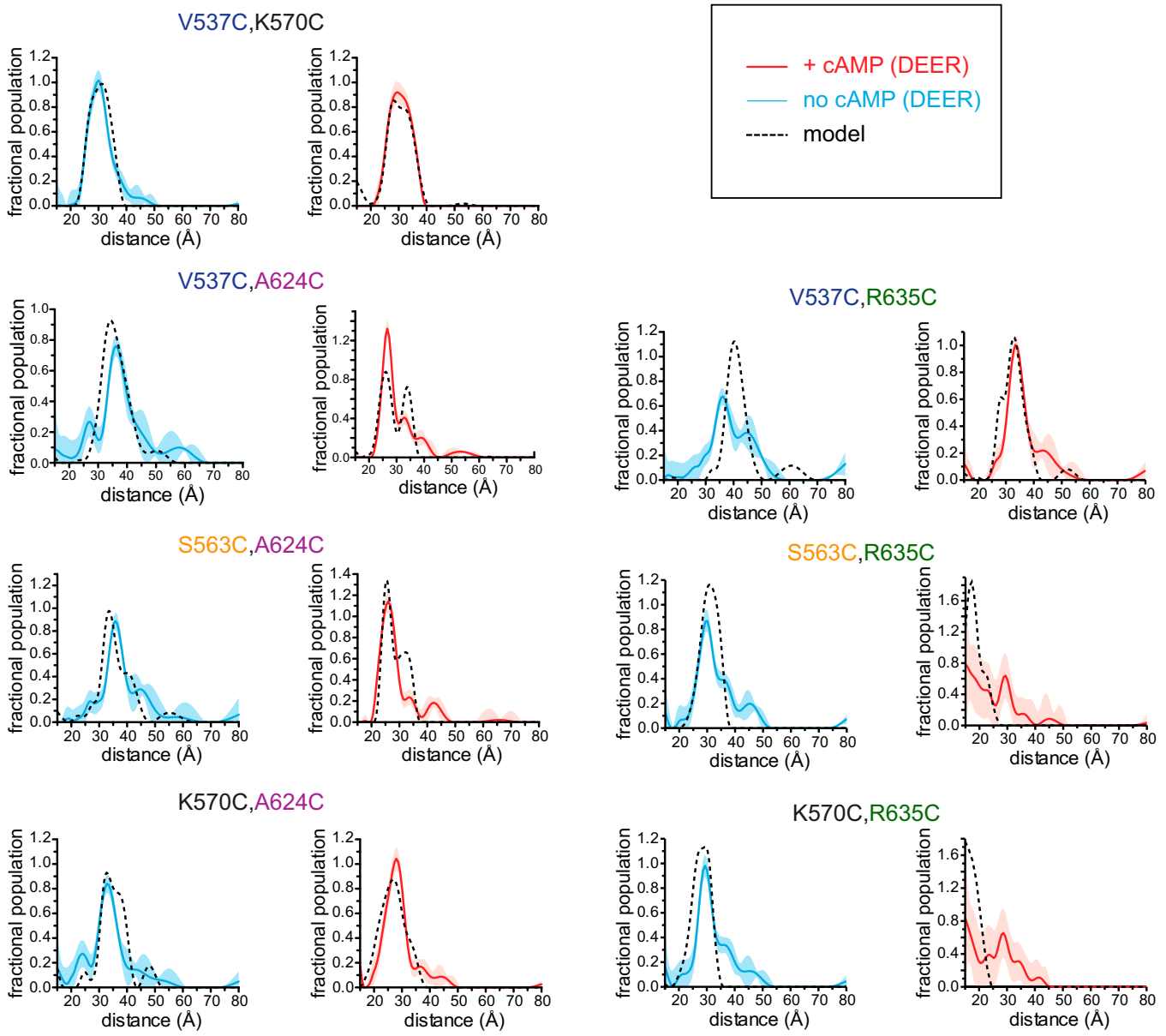

Fig. S2. Experimental distance distributions compared with those predicted from elastic network models. Distance distributions obtained from DEER for $HCN2_{cys\text{-}free}$ double-cysteine mutants in the absence and presence of cAMP compared with predicted DEER traces (dashed lines) calculated from 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.

Movie S1