

Supplementary figures

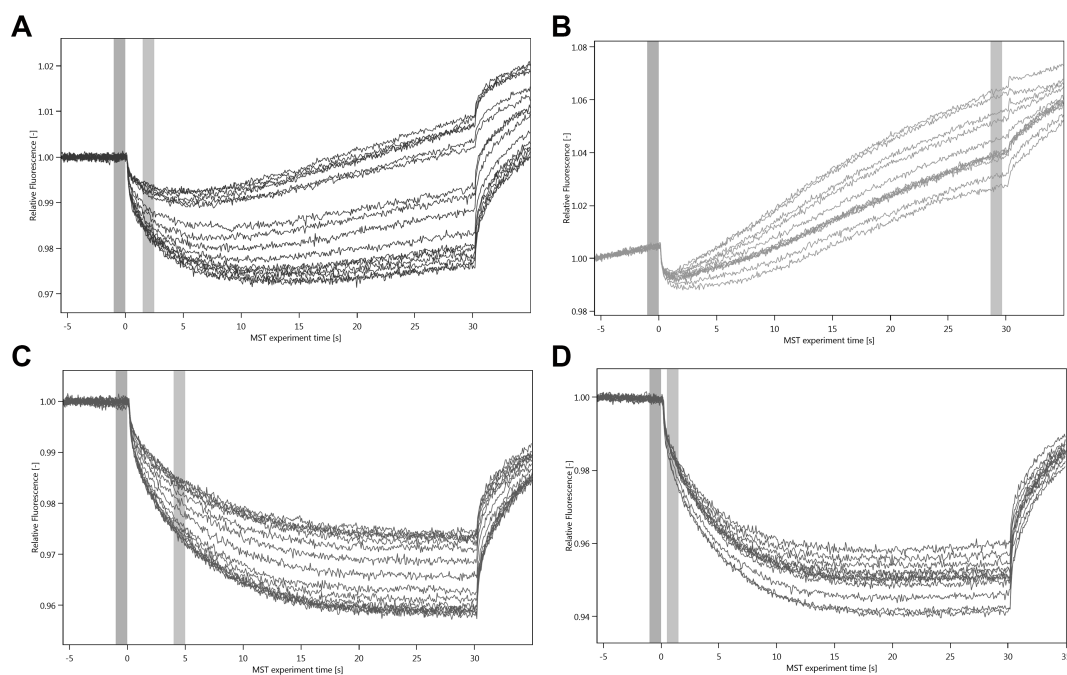


Figure S1. Typical MST traces from experiments of (A) Full-length AQP2 and LIP5 (B) AQP2 peptide and LIP5 (C) Full-length AQP0 and CaM and (D) AQP0 peptide and CaM. The grey columns represent the time-points at which F_{cold} (left column) and F_{cold} (right column) were determined.

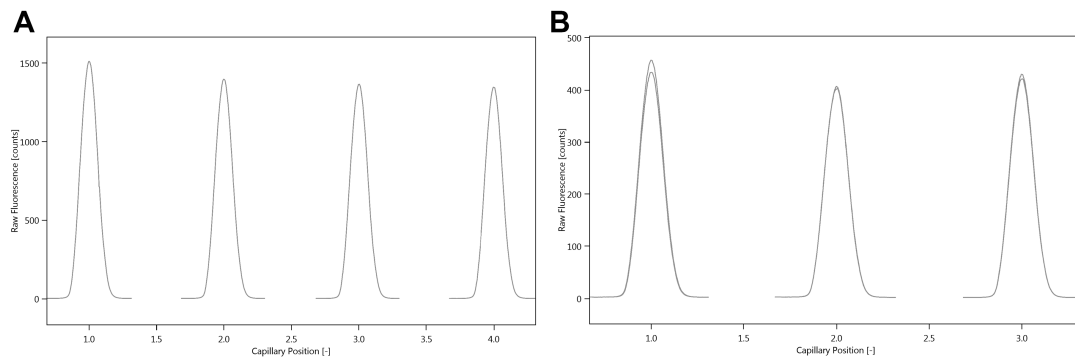


Figure S2. MST capillary scans from denaturation tests of (A) FL-AQP2 and LIP5 and (B) AQP0 peptide and CaM. The capillaries have increasing concentration of ligand from left to right, with the left peak corresponding to the fully bound state and the right peak corresponding to the unbound state. The fluorescence levels are within the error margin of the experiment ($\pm 10\%$) showing that after denaturation, the ligand concentration-dependent difference in initial fluorescence is abolished.

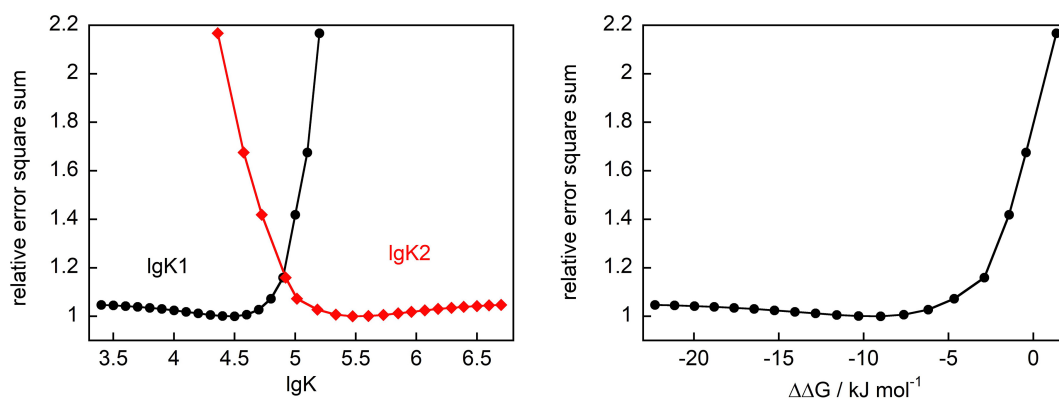


Figure S3. Error analysis of fitting to MST data from the interaction between CaM and AQP0. One parameter at a time ($\lg K_1$ or $\lg K_2$) was fixed and all other variable parameters were treated as fitted parameters to obtain the lowest possible error square sum for each value of the fixed parameter. When $\lg K_1$ was fixed, $\lg K_2$, S_0 , S_1 and S_2 were treated as variable parameters. When $\lg K_2$ was fixed, $\lg K_1$, S_0 , S_1 and S_2 were treated as variable parameters. The error square sum versus the value of the fixed parameter is shown in the left panel for $\lg K_1$ in black and $\lg K_2$ in red, and to the right versus the calculated value for $\Delta\Delta G$.