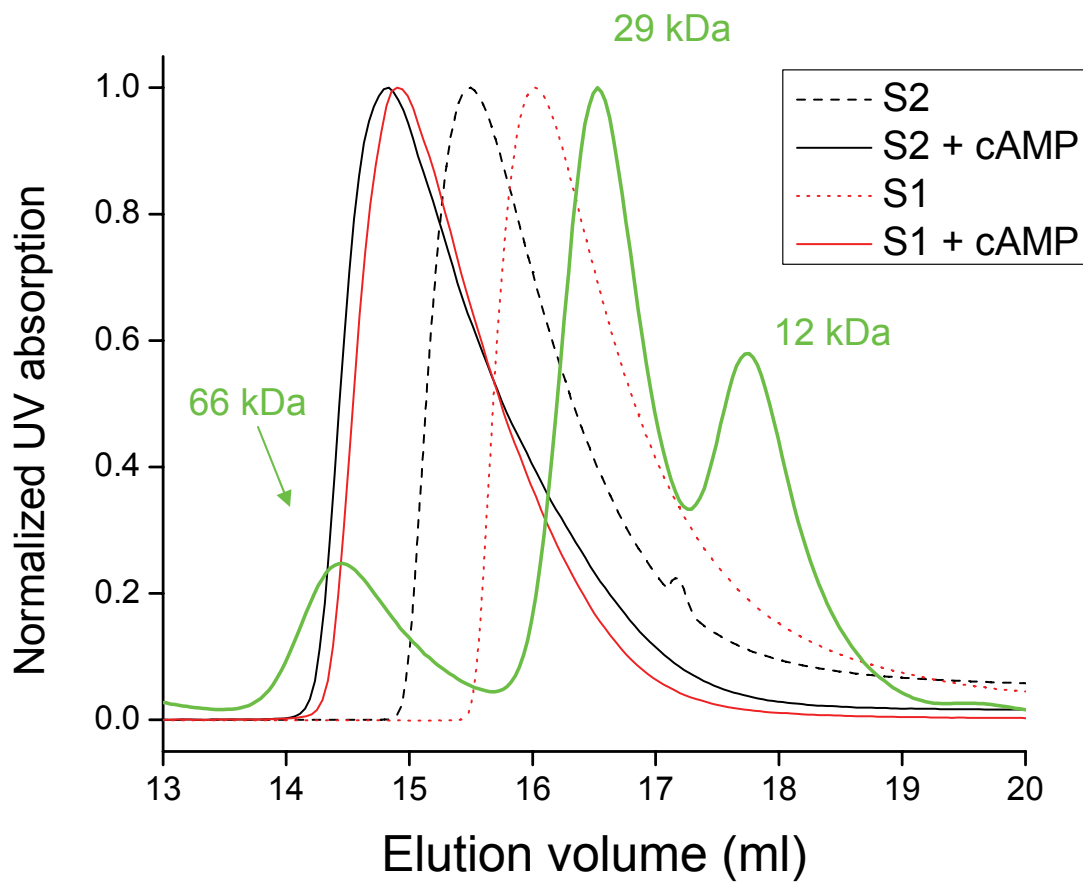


Supplemental Figures:

Supplemental Figure 1: Elution profile of the three control samples running together (shown in green). The molecular weights of control samples are 66 kDa (BSA), 29 kDa (Carbonic Anhydrase), and 12.4 kDa (Cytochrome C), respectively.

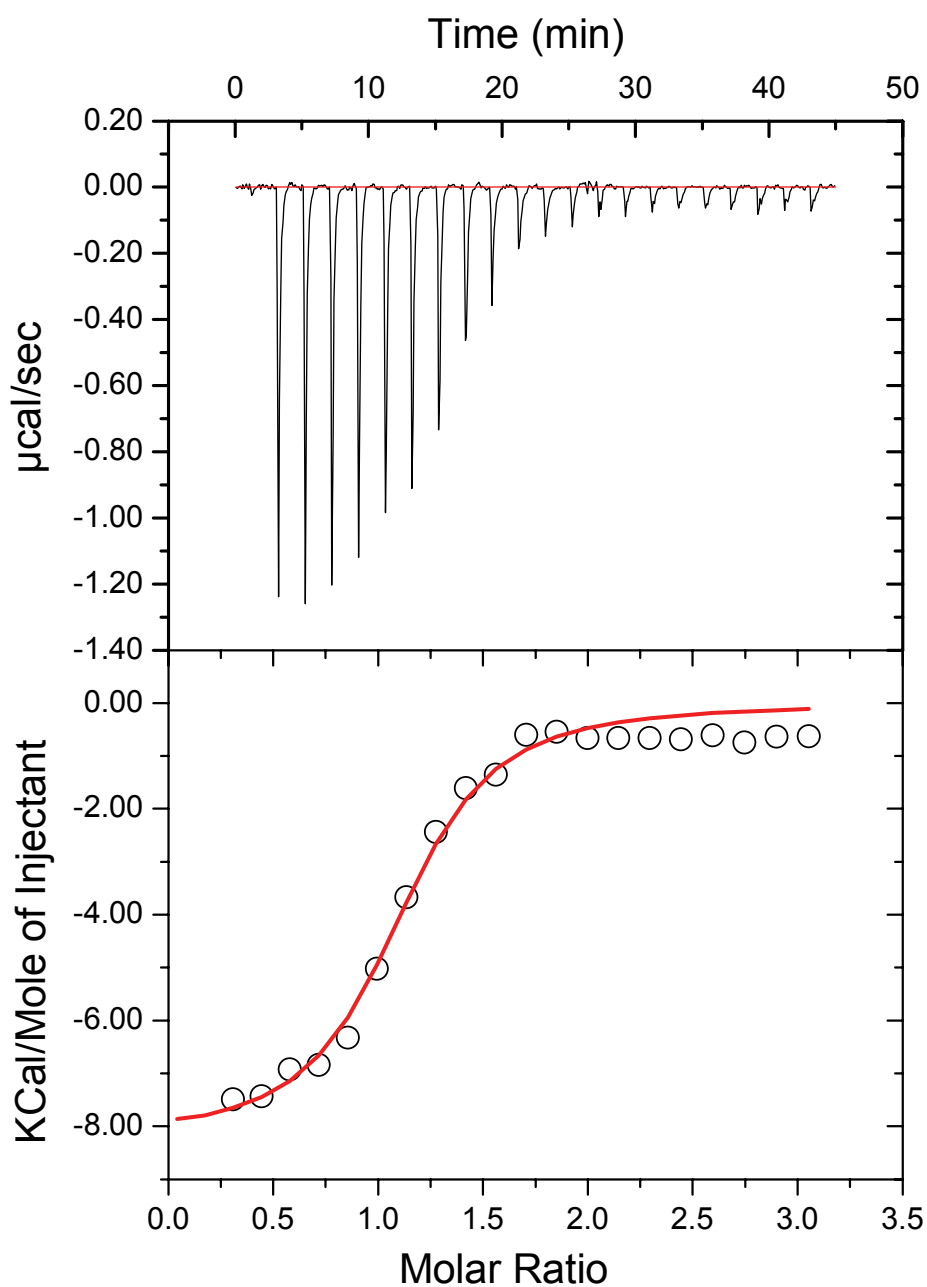
Supplemental Figure 2: Isothermal Titration Calorimetry (ITC) measurement of the binding of 8-Fluo-cAMP, used in the fluorescence anisotropy (FA) assays, to WT mHCN2 protein. The K_d value obtained, $3.75 \pm 0.84 \mu\text{M}$, was close to that of cAMP binding ($2.58 \pm 0.44 \mu\text{M}$).

Supplemental Fig. 1



Elution profile of three control samples (shown in green) from the same sizing column used in purifying hHCN4 protein samples.

Supplemental Fig. 2



Isothermal Titration Calorimetry (ITC) result showing the binding of 8-Fluo-cAMP to WT mHCN2 protein with the K_d value of $3.75 \pm 0.84 \mu\text{M}$ (K_d for cAMP, $2.58 \pm 0.44 \mu\text{M}$).