Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Effect of ligand binding on a protein with a complex folding landscape

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Supplementary Information

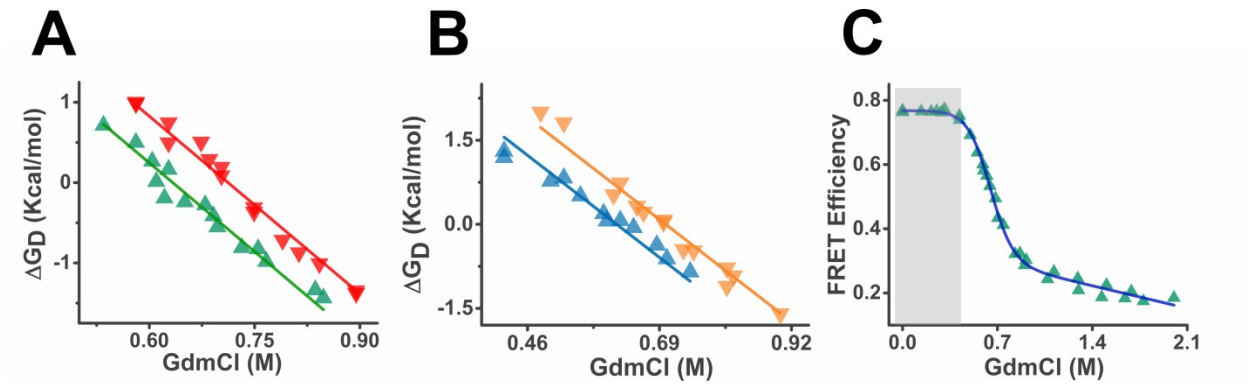

Figure S1| Comparison between bulk chemical denaturation and smFRET denaturation curves. (A) Bulk chemical denaturation of AKE at 0 M (green) and 200 μM (red) Ap_5A. (B) smFRET denaturation of AKE at 0 M (blue) and 200 μM (orange) Ap_5A. Free energies of unfolding at 0 M and 200 μM Ap_5A, obtained from a fitting to a two-state model were in a good agreement between the two methods (see Table S1). (C) Full denaturation curve of AKE at 0 M Ap_5A constructed from smFRET experiment. The shaded area indicates the range of denaturant concentrations over which the MST measurements of Fig. 4A were conducted, showing that the protein is still folded within this range.

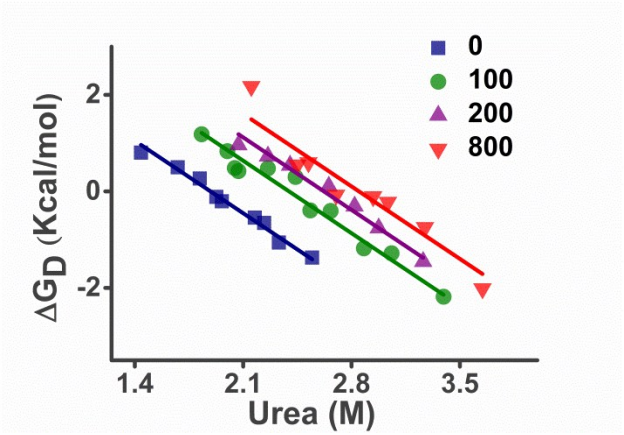

Figure S2| Urea denaturation experiments using smFRET in the presence of Ap_5A. Free energy versus denaturant concentration at different Ap_5A concentrations (given in the legend in μM).

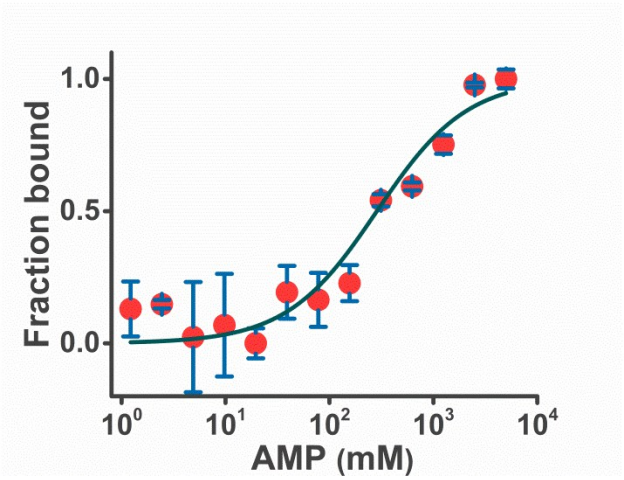

Figure S3| AMP binding to AKE measured by MST. The fraction of bound AKE as a function of AMP concentration is plotted. Data was fitted to obtain a K_d value of 286.0 ± 56.9 μM, close to the literature value of 210 μM [1]. The error values shown in the figure were calculated from three repeats of the experiment.

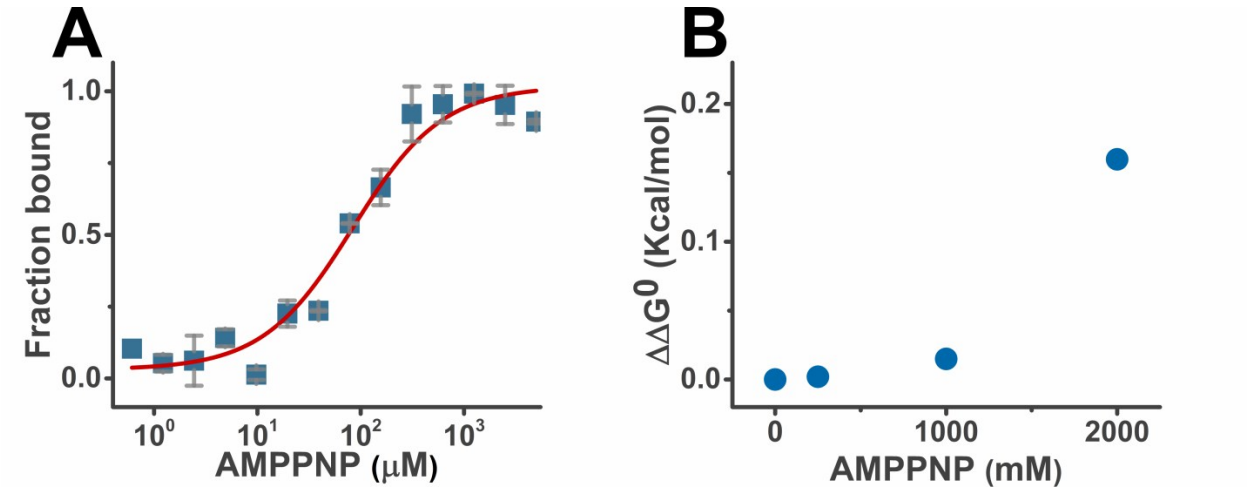

Figure S4| Effect of AMPNP binding on AKE stability measured using urea denaturation. (A) MST measurement of AMPPNP binding to native single-labeled AKE. The fraction of bound AKE as a function of AMPPNP concentration is plotted. From a fit to the data we obtained a K_d of 83.7 ± 21.2 μM, close to the literature value of 50 μM [1]. (B) The change in AKE stability with AMPPNP concentration was measured. No significant stabilization was registered. The error values shown A were calculated from three repeats of the experiment.

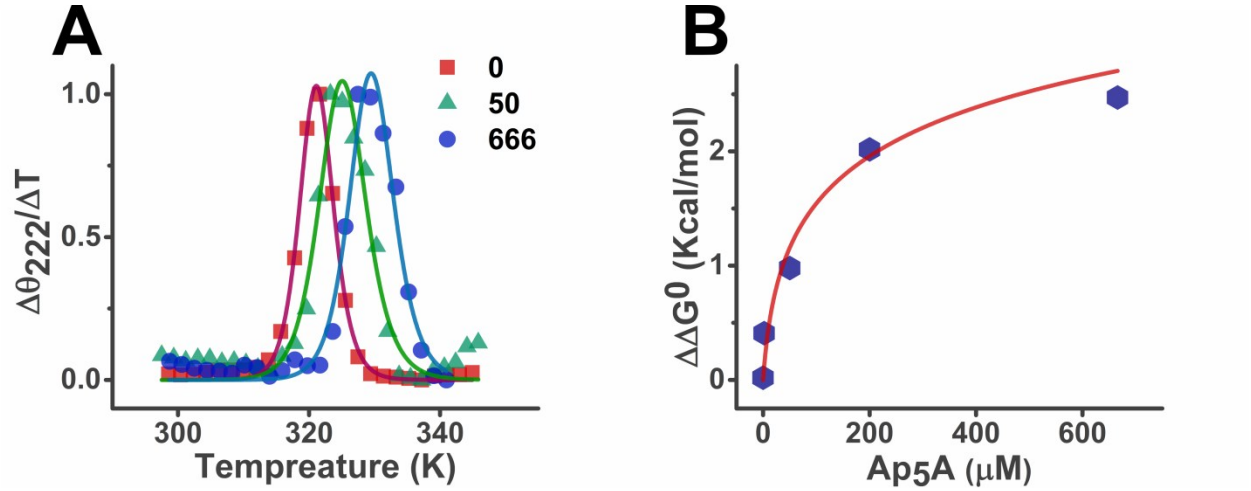

Figure S5| Bulk thermal denaturation of AKE in the presence of Ap_5A. (A) Thermal unfolding of AKE at different substrate concentrations (given in the legend in μM) was monitored by CD at 222 nm (θ_{222}). The graph shown was obtained by using the "Van't Hoff with no baseline" method [2], and shows the derivative of the CD signal with temperature. Curves were fitted to a two-state denaturation model to obtain the transition enthalpy and the melting temperature, from which the free energy of binding was calculated (equation 2 of the main text). (B) Effect of Ap_5A concentration on the free energy of binding. A K_d value of 10.9 ± 1.2 μM was obtained from a fit of these results, which is much higher than the value measured by MST, as well as literature value [1], but in good agreement with the result obtained from the smFRET chemical denaturation curves. The error values for the data in (B) are very small and are not shown. They were calculated from two repeats of the experiment.

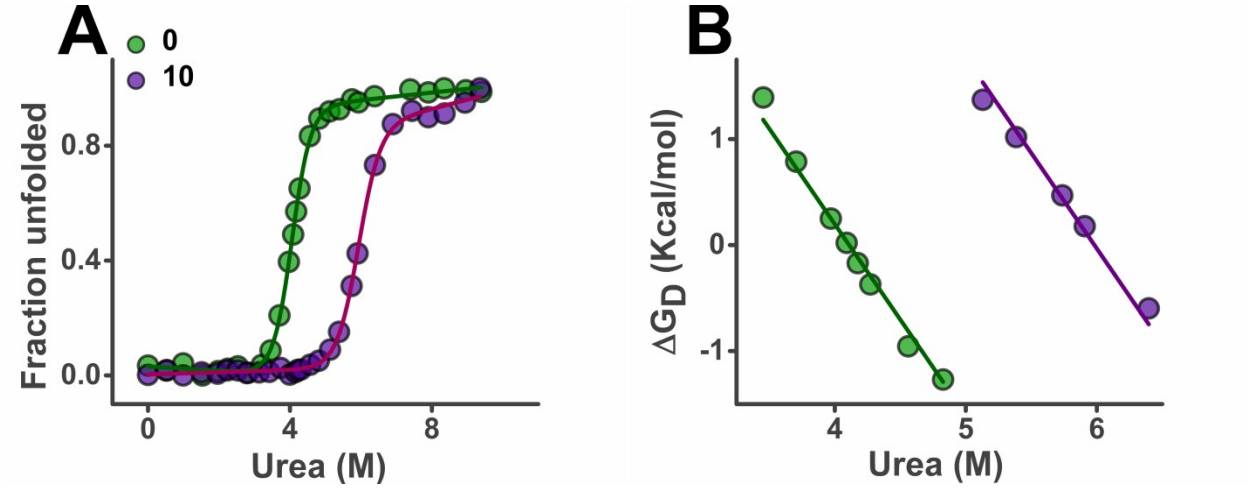

Figure S6| Human carbonic anhydrase stability in the presence of the inhibitor ligand trifluoromethane sulfonamide (TFMSA). (A) Bulk chemical denaturation at 0 and 10 μM of the inhibitor. (B) Free energy versus denaturant concentration calculated from the results in A. Ligand binding increases protein stability significantly. The calculated K_d obtained using equation 1 of the main text is 33.7 ± 6.9 nM, close to the literature value of 163 nM [3].

Table 1. Comparison of between the free energies of unfolding of AKE obtained from bulk and smFRET experiments.

Ap_5A **concentration [**μ**M]**	**Bulk [Kcal/mol]**	**smFRET [Kcal/mol]**
0	4.6 ± 0.3	4.8 ± 0.4
200	5.2 ± 0.4	5.5 ± 0.4

References

1 J. Adén and M. Wolf-Watz, NMR identification of transient complexes critical to adenylate kinase catalysis., J. Am. Chem. Soc., 2007, **129**, 14003–14012.

2 D. M. John and K. M. Weeks, Van't Hoff enthalpies without baselines., Protein Sci., 2000, **9**, 1416–1419.

3. A. Schön, R. K. Brown, B. M. Hutchins and E. Freire, Ligand binding analysis and screening by chemical denaturation shift, Anal. Biochem., 2013, **443**, 52–57.