Supplementary Information for Remarkably Fast Coupled Folding and Binding of the Intrinsically Disordered Transactivation Domain of cMyb to CBP KIX

Sarah L. Shammas, Alexandra J. Travis, and Jane Clarke
Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW

Supplementary Figures

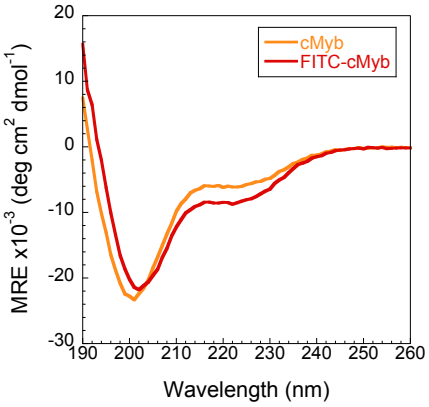

Figure S1. CD spectrum for FITC-cMyb and unlabeled cMyb peptide in pH 7.4 100 mM sodium phosphate at 25 ºC. Various methods for estimation of helical content of peptides (see Methods in main text) suggest that the labeled version is around 3% more helical.

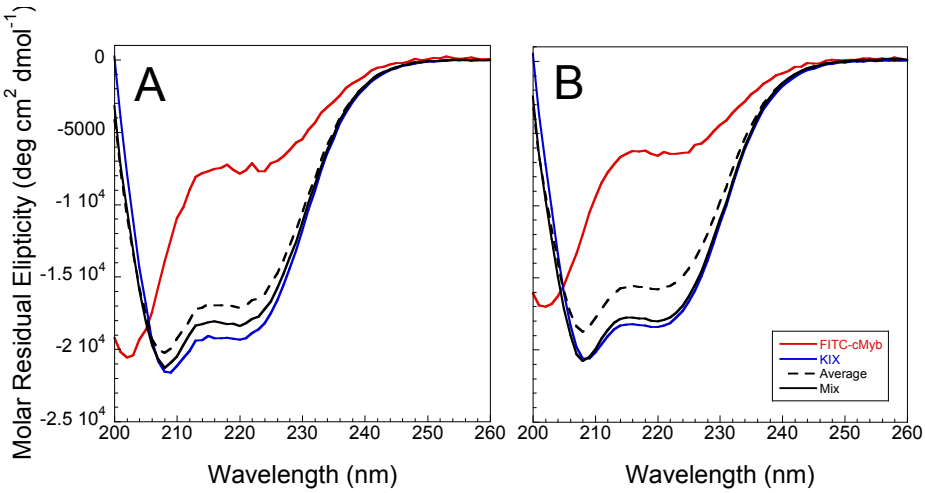

Figure S2. Dependence of the apparent increase in helicity on mixing FITC-cMyb with KIX on protein concentration. Solutions of KIX and FITC-cMyb in pH 7.4 100 mM sodium phosphate were mixed in a 1:1 ratio. Protein concentrations for the individual protein solutions were (A) 20 μM and (B) 100 μM. An equilibrium binding curve (Figure 2B) performed under the same conditions suggests that 38% (A) and 64% (B) of the protein will be in complex, which is consistent with these results.

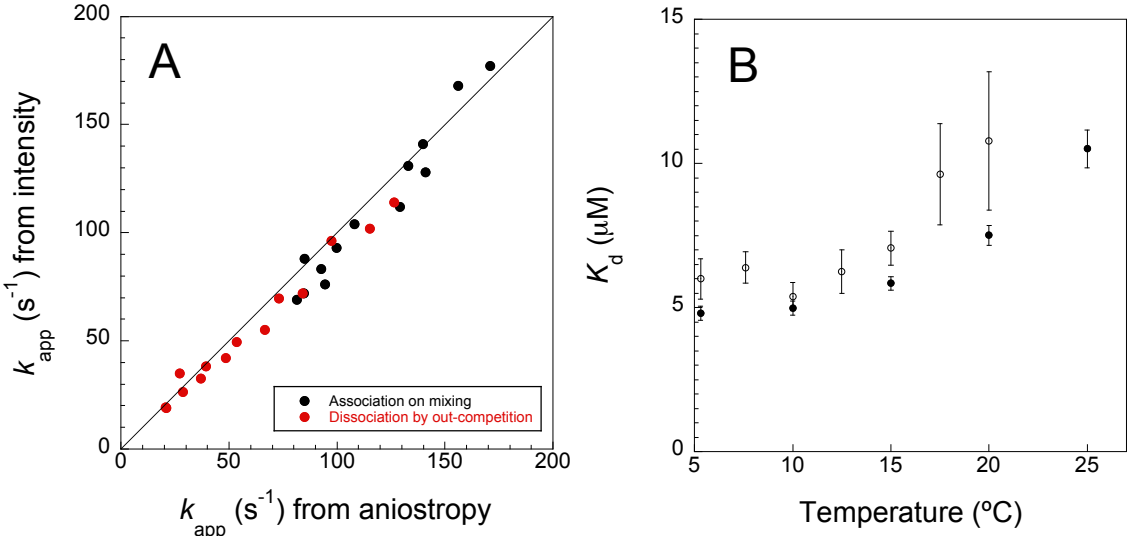

Figure S3. (A) Apparent rates obtained from fitting fluorescence anisotropy and intensity kinetic traces are similar, with no apparent additional kinetic phase. (B) Equilibrium dissociation constants estimated from equilibrium (closed circles) and kinetic (open circles) anisotropy measurements give similar values. Kinetic estimates for K_d were determined from the ratio $k_{app,0M}/k_{on}$, where the rate constants are extracted from the intercept and gradient of straight line fits in Figure 3B. Consistency of equilibrium constants between the two approaches implies there are no populated intermediates in the association process.

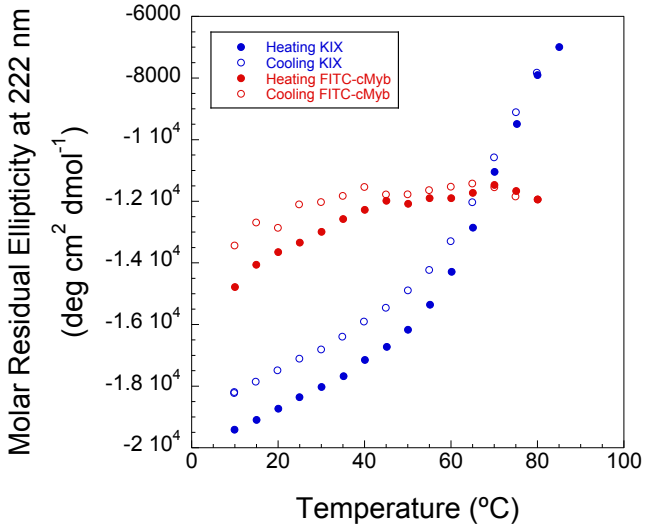

Figure S4. Thermal denaturation curves for KIX and FITC-cMyb in pH 7.4 100 mM sodium phosphate buffer from CD[. Helical content was reduced on increasing temperature for both proteins. The curve for KIX shows probable helix fraying, followed by a cooperative unfolding transition. Curves were not fit to determine melting temperature because of apparent non-reversibility.

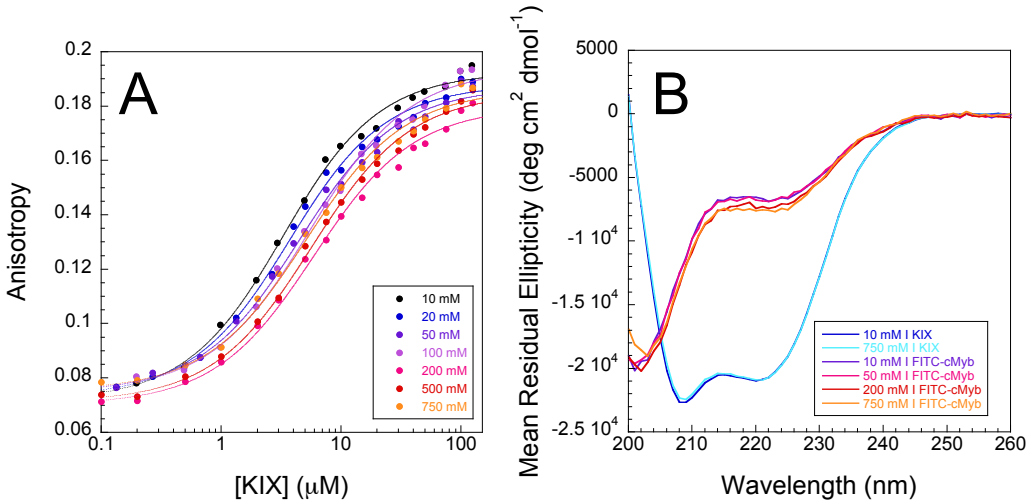

Figure S5. (A) Binding curves for FITC-cMyb with KIX in pH 7.3 MOPS buffers of various ionic strengths at 10 ºC. Solid lines represent fits to Equation 2. Equilibrium constants obtained from these curves are shown in Figure 4A. (B) Circular dichroism spectra for FITC-cMyb and KIX in MOPS buffers of selected ionic strengths at 10 ºC.

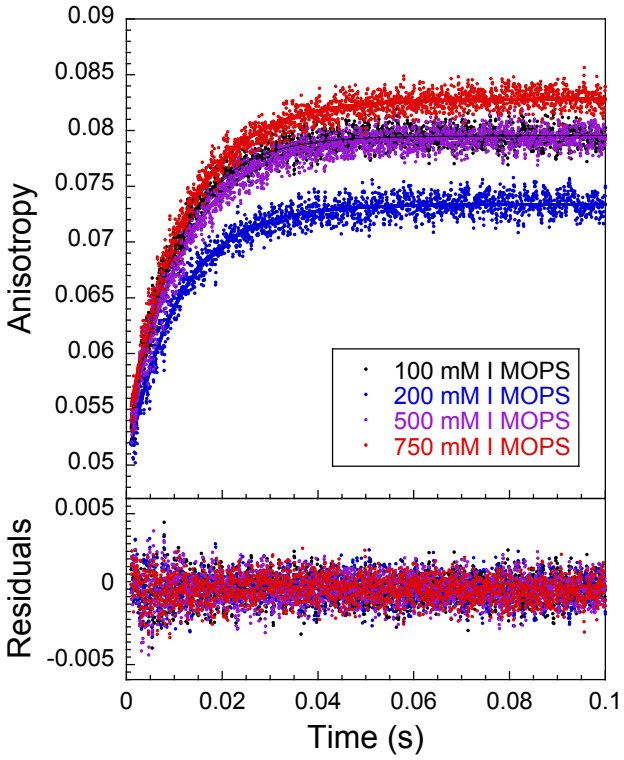

Figure S6. Example stopped-flow anisotropy traces following roughly equimolar mixing of FITC-cMyb and KIX in pH 7.30 MOPS buffers of various ionic strengths. Solid lines represent fits to Equation 3.

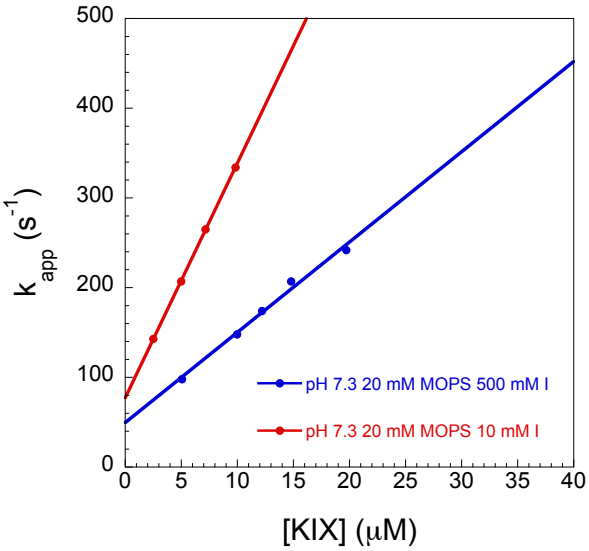

Figure S7. Plots used for extraction of association rate constants using a pseudo-first order approach in MOPS buffers of two different ionic strengths at 10 ºC. The association rate constant was obtained from the gradient of the straight line fits, and is shown in Figure 4B (open circles).

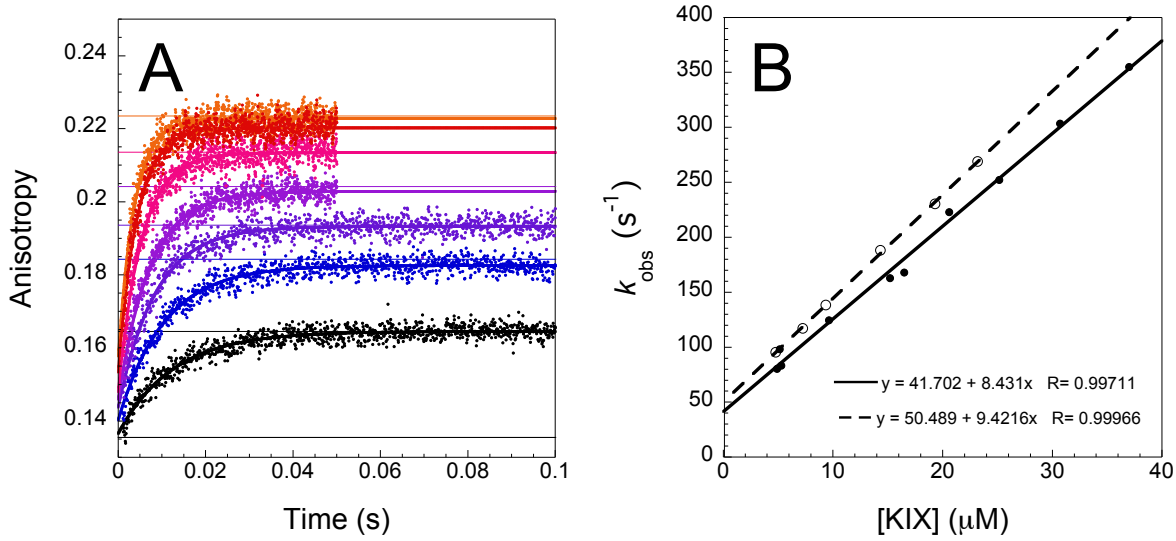

Figure S8. KIX and his-KIX proteins have very similar association and dissociation rates with FITC-cMyb at 10.0 ºC in pH 7.4 100 mM sodium phosphate. (A) Stopped-flow anisotropy traces for mixing of FITC-cMyb with KIX at final concentrations of 5 μM (black), 10 μM (blue), 15 μM (purple), 20 μM (violet), 30 μM (pink), 40 μM (red) and 50 μM (orange). Thick solid lines show single exponential fits to obtain apparent rate constants. Thin solid lines show final anisotropy values (after five minutes) for each concentration of KIX. (B) Dependence of observed rate constants on KIX concentration for KIX (solid circles) and his-KIX (open circles). The gradient and intercept of the straight-line fits provide estimates for the association and dissociation rate constants respectively.

Summary of previous ionic strength dependence studies of protein association

The most extensive study of ionic strength dependence on association rate constants was reported by Schrieber and Fersht for the proteins barnase and barstar[1]. Second order rate constants are reported as a function of ionic strength for a large selection of single point mutants. The data, which showed a very strong dependence on I, were fitted with a Debye-Huckel like model to account for long-range electrostatic effects (Equation 4). The various mutants displayed large variations in association rate at low ionic strengths, but converged at high ionic strengths to give very similar estimates for the basal k_{on} of around 10^5 $M^{-1}s^{-1}$. Similar behavior has since been observed in mutational studies for several different protein complexes, suggesting that changes in long-range electrostatic effects that originate from changes in charge are well accounted for[2-4].

The best fit to the barnase/barstar data was obtained with a value for *a* of 6 Å, which was identified in the initial paper as the minimum distance of approach between an anion and cation, but amended in a later paper after theoretical considerations to be the sum of the radii for the two associating proteins[5]. The relatively small value of *a* was rationalized by Vijayakumar *et al.* by noting that the residues which were found to affect the association rate significantly were those along the (oppositely charged) interacting surfaces between barnase and barstar in the complex, and that the distance between these two groups of charges is around 7 Å.

Three of the reported studies have applied the same approach as that of Schreiber et al., including fixing the distance *a* of 6 Å. These are for the CheA/CheY[6], PUMA/Mcl-1[7] and TEM1/BLIP[3] protein complexes reported in Figure 5. We found our data to be better described by a larger value of *a* (of the order of 30 Å), so we allowed this to be a freely varying parameter[8] when fitting our data as suggested by Schreiber. We also used this approach when analyzing the data for other protein complexes, where ionic strength dependence of association rate was investigated, but a basal k_{on} not reported[9-10]. For the interactions between IL4/IL4-BP[11-12], ColE9/Im9[12] and HEL/HyHEL-5[10] the errors in estimated k_{on} were larger than the values themselves. In the case of IL4-IL4-BP we were able to obtain an estimate of k_{on} after fixing *a* to 6 Å, and this estimate is included in Figure 5.

Interactions between S-protein/S-peptide[13] , thrombin/hirudin[4], heterodimeric leucine zipper[14] and AchE/FAS2[2] were modeled with alternative Debye-Huckel like equations to estimate basal k_{on} values. These reported values are shown without alteration in Figure 4.

We found a further two systems where ionic strength dependence of association rates had been investigated, but were unfortunately not able to include the data in Figure 4. Firstly, in the association of intrinsically disordered HPV E7 protein and RbAB[15] there appeared no plateau effect of increasing concentration to give a basal association rate, which makes it difficult to compare their results with those presented here and by other groups. It does seem likely that electrostatics play an important role since there was an approximate 15-fold reduction in the association rate between 0.1 M and 1 M sodium chloride to 4×10^6 $M^{-1}s^{-1}$ (in a 20 mM phosphate solution). Secondly, apparent rate constants for the association of Cdc42 and G-protein at several concentrations of sodium chloride have been reported[16]. We used this data to estimate an upper limit of the basal k_{on} of 2×10^6 $M^{-1}s^{-1}$, by assuming that k_{off} was negligible.

Other candidates for fast associating protein complexes

Extremely fast association between two intrinsically disordered proteins, the activation domain of p160 and nuclear co-activator binding domain of CBP, has recently been observed[17]. The reported k_{on} at 1 M ionic strength is 1.6×10^7 $M^{-1}s^{-1}$ (at 5 ºC), however at around 200 mM ionic strength this rate is significantly increased to 2.8×10^7 $M^{-1}s^{-1}$, which suggests electrostatic steering plays a role, and indeed mutation of a conserved salt-bridge between the two proteins caused a 20-fold reduction in the association rate constant. It is thus unclear how this rate relates to the one we have obtained for the cMyb/KIX interaction.

A basal k_{on} of $(3.6 \pm 0.5) \times 10^6$ $M^{-1}s^{-1}$ (at 25 ºC) was reported for 250 mM ionic strength for the interaction between HLE and elafin[18]. This interaction is a good candidate for a fast association reaction because on increasing the ionic strength to 1 M there was only a marginal decrease in rate to $(3.2 \pm 0.7) \times 10^6$ $M^{-1}s^{-1}$. This value is in good agreement with the basal rate of 2.9×10^6 $M^{-1}s^{-1}$ predicted for the system by the TransComp[19]. As is the case in our system, the two components are relatively small, with HLE being 30, 000 Da and elafin only 6000 Da. The NMR solution structure of r-elafin shows that is has a flexible N-terminus and a flexible loop[20].

Finally, a basal k_{on} of 2×10^6 $M^{-1}s^{-1}$ was reported for the interaction between the two folded proteins CheA and CheY[6]. (Stewart2004). CheA rapidly phosphorylates CheY *in vivo*, and the authors point out that since the complex does not need to be long-lived it is possible that few contact points are required, and thus the orientation of the two proteins may not need to be too specific.

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