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Speed dating with KIX: a single domain with many partners

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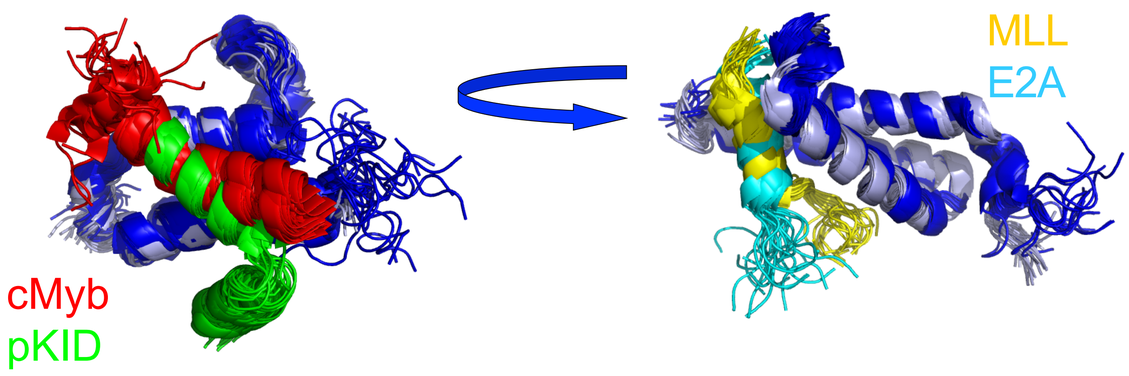
Abstract

IDPs are overrepresented in processes such as signalling and transcription, where proteins often interact with a range of partners. One much-studied key hub protein is the coactivator CBP/p300, whose folded KIX domain binds a number of different intrinsically disordered transcription factors at two separate sites on its surface. The interaction of KIX with several of its ligands has been well studied by equilibrium methods, and structural information is available for many of the complexes. By careful control and consideration of experimental conditions such as temperature and ionic strength we have been able to perform kinetic studies that reveal the mechanism of the association reaction of KIX with cMyb; the fastest protein-protein interaction yet reported. We further describe the mechanistic basis for the positive allostery between the two binding sites of KIX. Through comparative studies with several different binding partners we shed light on an important outstanding question in the IDP field: what is the advantage of disorder to a protein?

KIX: a single domain with many partners

CBP, and its paralogue p300, are general coactivators that act as a bridge between the basal transcriptional machinery and DNA-bound transcription factors (3).

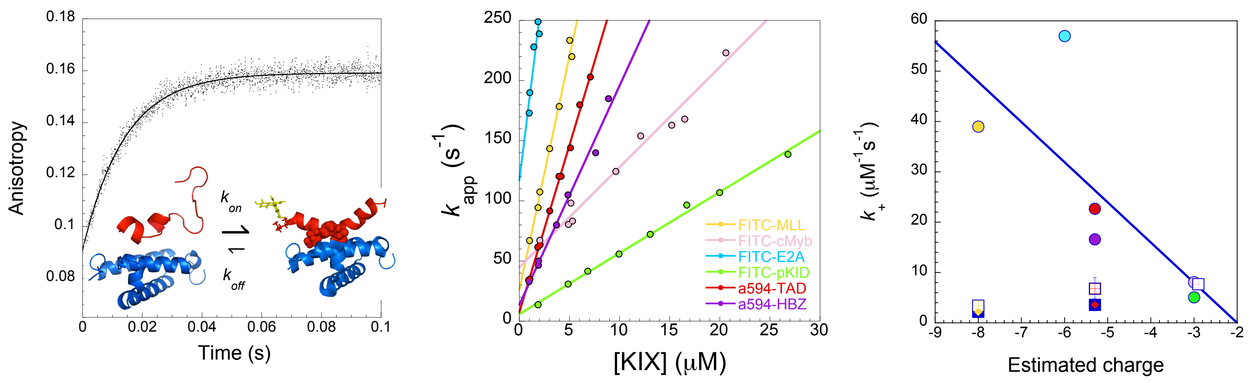
The transactivation domains of various transcription factors, which are disordered in isolation, fold into a single α-helix on binding to two separate sites on the KIX domain of CBP/p300 (4)



Mechanism of coupled folding and binding

We have performed kinetic studies to determine the binding parameters of several ligands to KIX, that differ in size, charge, helical propensity and sequence.

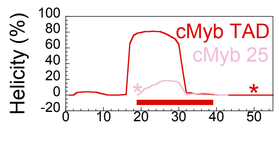
Fluorophores were incorporated into several KIX binding peptides. The association process was followed by fluorescence stopped-flow mixing under pseudo-first order conditions.

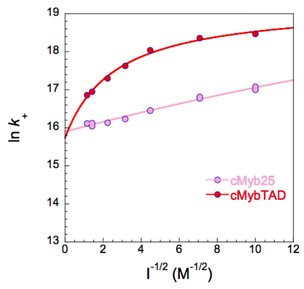


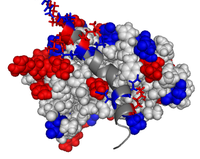
Association rate constants vary over one order of magnitude between the different peptides. There is no observable correlation between intrinsic helicity (overall or in the binding region) and association rate. All peptides associate fast (even when long-range electrostatic attraction is screened by high ionic strengths). These are hallmarks of an ***induced fit*** mechanism of association.

cMybTAD GSPAAAAIQRHYNDEDPEKEKRIKE**L**EL**LL**MSTENELKGQQVLPTQNHTCSYPGW

cMyb25 KEKRIKE**L**EL**LL**MSTENELKGQQV



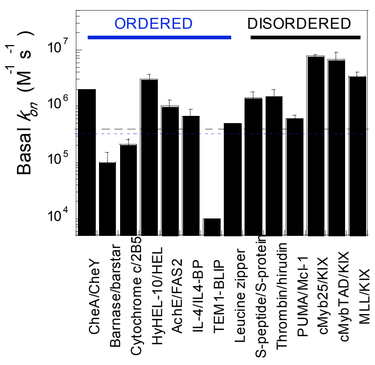


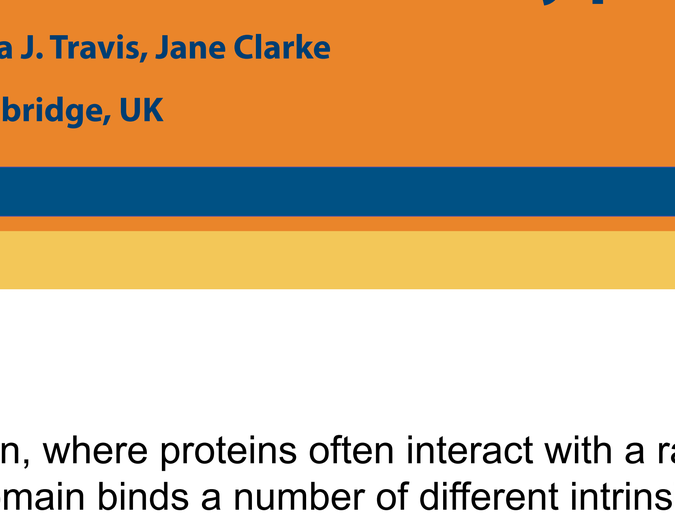


Fly-casting?

One stated advantage to disorder for a protein is that it could accelerate the association process, through a phenomenon known as 'fly casting', whereby the unstructured protein has a greater capture radius for its partner than a folded counterpart would (5). This effect is likely to give only a modest rate enhancement, perhaps a factor of 1.6, which would be masked by the effect of different long-range electrostatic effects that can alter association rates by several orders of magnitude.

In order to compare ordered and disordered proteins in this respect it is therefore necessary to account for these differences by extrapolating rates to infinite ionic strength before comparison.

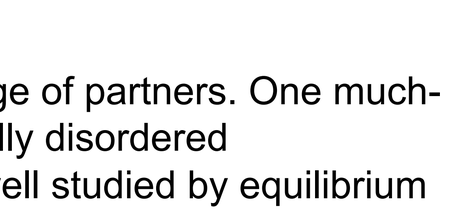




Allostery in the KIX system

Allostery has been reported in the KIX system i.e. ligand binding at one site is made more favourable when ligand is also bound at the alternative site on KIX (3).

There are structures available for two ternary complexes: cMyb-KIX-MLL, and pKID-KIX-MLL (4).

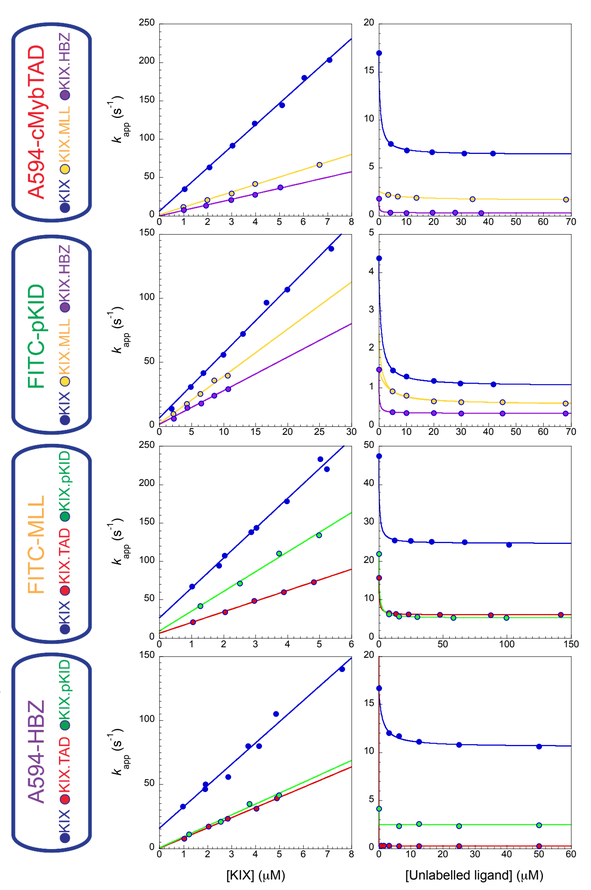


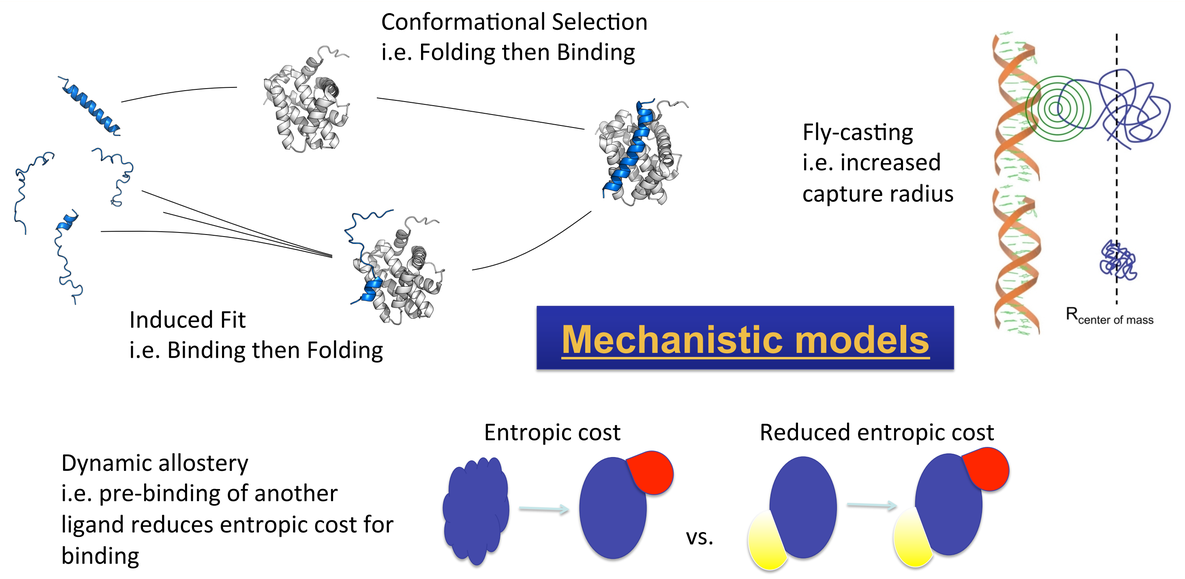
Mechanism of allostery

Previous NMR experiments and simulations have demonstrated the existence of an excited state of KIX when MLL binds that has a higher affinity for cMyb. It has been suggested that the allostery is mediated through enhanced conformational selection of the folded domain KIX by cMyb (6).

We tested for an increase in k+ for cMyb when MLL was pre-bound to KIX, but actually observed a decrease. Instead allostery is mediated through more significant decreases in dissociation rate constants. Dissociation was followed by mixing pre-formed complex with a large excess of unlabelled peptide.

We observed the same behaviour for all the pairs of peptides that we tested. Since the backbone of KIX has been shown to stiffen upon ligand binding (by NMR and simulation) we are able to explain these results through a mostly ***dynamic allosteric*** phenomenon, whereby an entropic cost must be paid upon binding a first ligand and not a second (7). This entropic (rather than structural) argument has since gained support from molecular dynamics simulations (Law et al., accepted for back-to-back publication in PNAS).





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