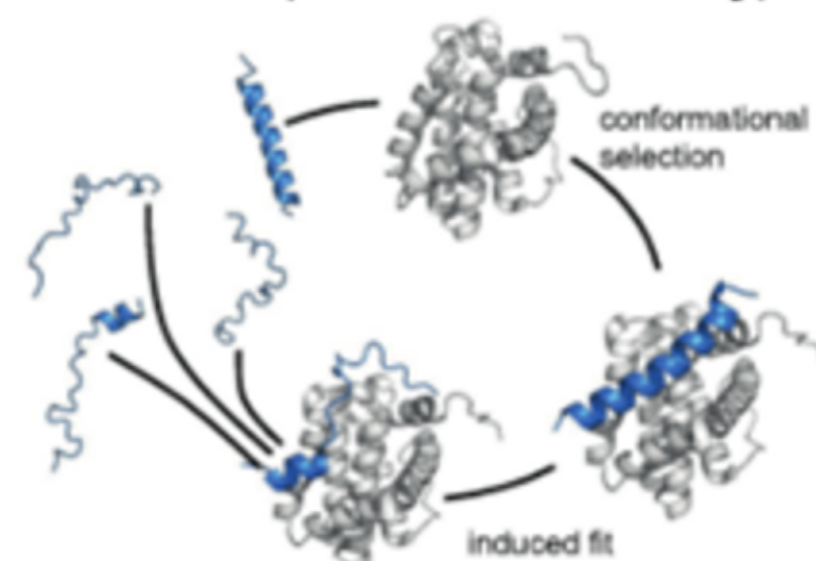


# Coupled folding and binding of intrinsically disordered proteins

Liza Dahal, Basile I M Wicky, Sarah L Shammass and Jane Clarke

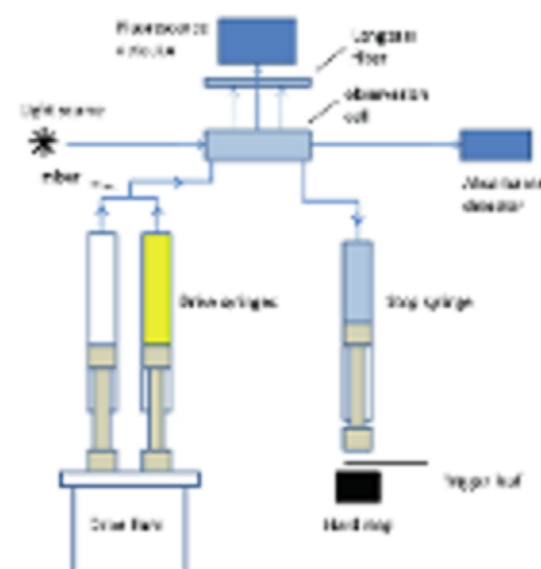
Department of Chemistry, University of Cambridge, Lensfield Rd CB2 1EW, UK

**Abstract:** The traditional structure-function paradigm of proteins – where the function of a protein is dictated by its 3D structure – has been challenged by the discovery of protein molecules that have function despite a lack of well-defined tertiary structure<sup>1,2</sup>. These so-called intrinsically disordered proteins (IDPs) have been identified as having a number of important cellular roles, such as signalling, cell differentiation and some even play a structural role. Many of these IDPs undergo coupled folding and binding reactions, where they become structured upon interaction with their binding partners.



**Mechanisms:** Two extreme mechanisms have been proposed.

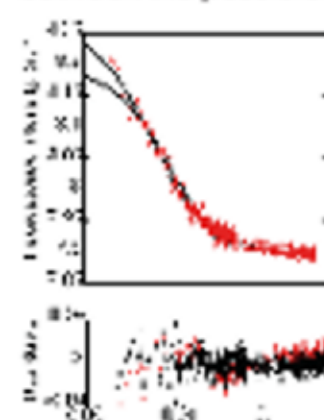
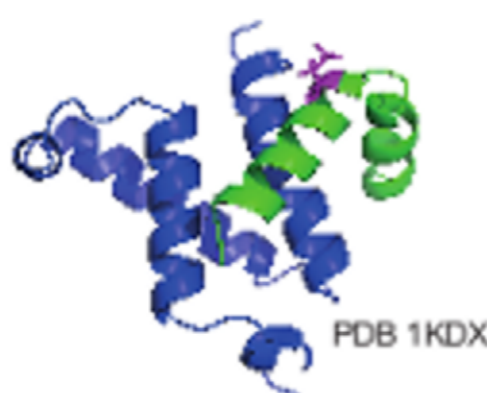
1. Conformational selection – describes a situation where only a certain conformational state of the IDP is capable of binding.
2. Induced fit – describes an initial binding event of the unstructured protein onto its binding partner, followed by its folding.



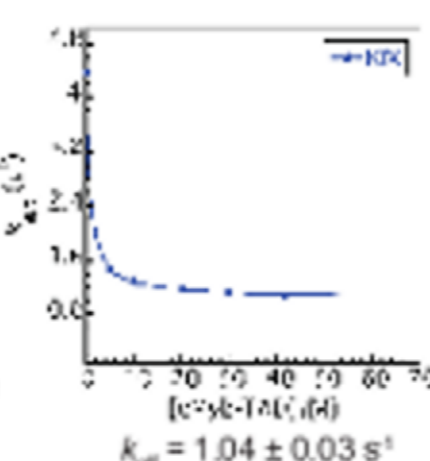
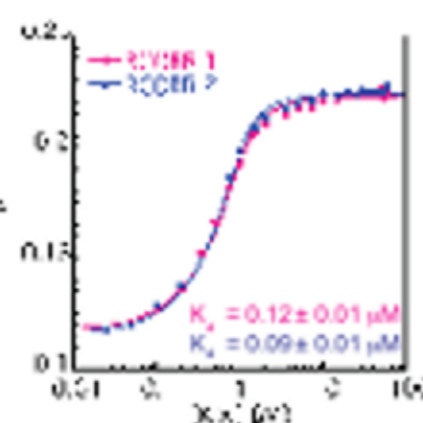
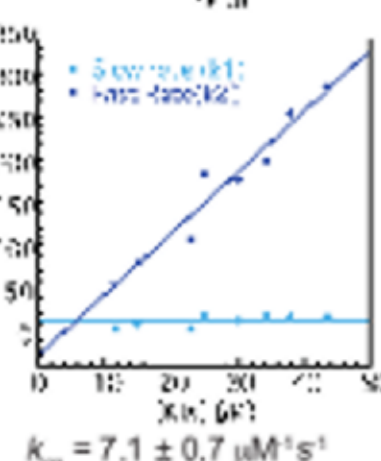
**Method of investigation:** Important mechanistic information can be gained from investigating the kinetics of IDPs coupled folding and binding reactions. In particular, the extraction of fundamental rate constants and their individual dependences on external variables (e.g. concentration, temperature, ionic strength, etc...) can be studied. Depending on the mechanism of association, the effect of the variables is likely to be different.

## Association of pKID with KIX

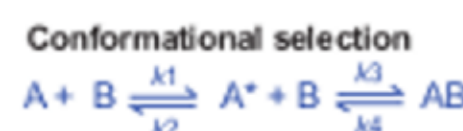
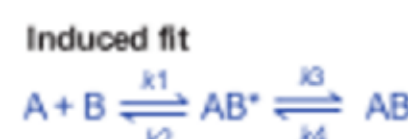
KIX is a folded domain of CREB Binding Protein (CBP) that binds different transcription factors through their transactivation domains, e.g. the Kinase Inducible Domain (KID) of CREB. When phosphorylated at S133, pKID promotes recruitment of co-activator CBP by binding to KIX. The disordered pKID folds to a helical structure upon interaction with KIX. Equilibrium and kinetics measurements were carried out to differentiate between the possible mechanisms of association.



Association kinetics experiments were performed using stopped-flow under pseudo-first order conditions. FITC labelled pKID peptides were mixed with various concentrations of KIX. Equilibrium experiments were done by studying the change in anisotropy upon mixing different concentrations of KIX and FITC-pKID. The dissociation kinetics were measured by mixing a pre-equilibrated FITC-pKID-KIX solution and out-competing with an unlabelled ligand at different concentrations.



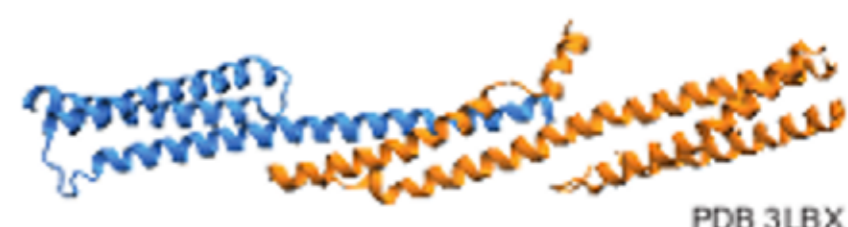
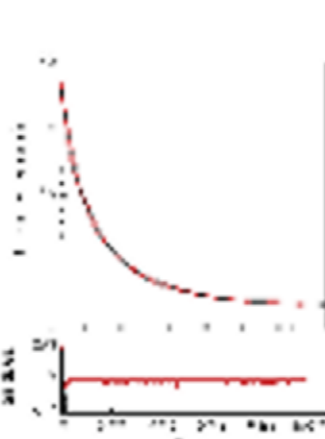
In association experiments, two phases – one concentration dependent and the other concentration independent – are observed at high KIX concentrations, which is consistent with previous reports of an induced fit mechanism<sup>3</sup>. Both these apparent rate constants are a mixture of four fundamental rates  $k_1$ ,  $k_2$ ,  $k_3$  and  $k_4$ .



	Induced fit	Conformational selection
Equilibrium constant	$k_3/k_4$	$(k_3/k_4) \cdot k_1/k_2$
Dissociation rate	$k_4$	$k_4 + k_2$
Fast rate [k]	$k_1 + k_3$	$k_1 + k_3$
Slow rate [k]	$k_2 + k_4$	$k_2$

## Ionic strength dependence of spectrin association

Spectrins are essential cytoskeletal proteins composed of multiple domains. Dimerization occurs when the disordered C-terminus of  $\beta$ -spectrin binds to the disordered N-terminus of  $\alpha$ -spectrin, folding into a new three-helix bundle. This system was used as a model to deconvolute the effect of electrostatic steering in IDP associations, which, having no structured binding interfaces and different sequence compositions, might show different behaviours from folded protein interactions.



Kinetic traces were collected by mixing both spectrin partners in an equimolar ratio. The association reactions were fitted to a reversible two-state model to extract both the association rate constant and the equilibrium constant.

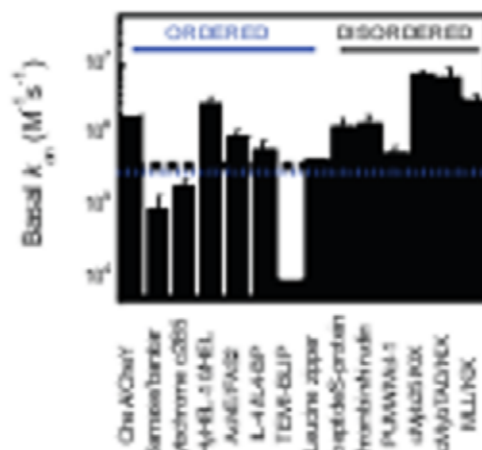
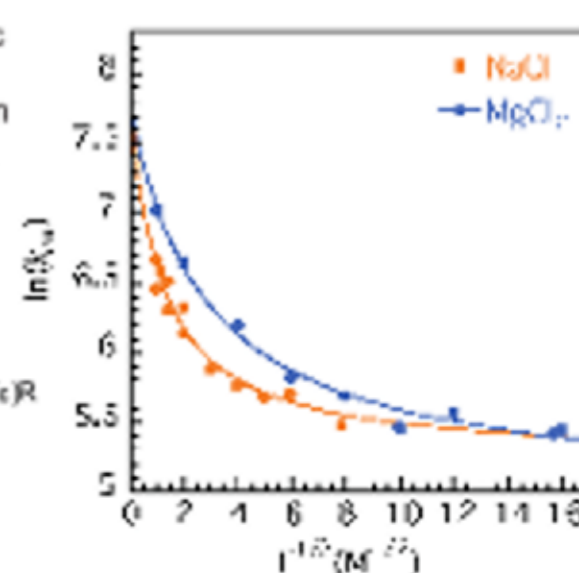
The association rate constant at infinite ionic strength is independent of the type of salt. However different salts affect the association rate differently. Basal rate constant obtained by fitting to a Debye-Hückel-like model<sup>4</sup>.

$$\ln k_{on} = \ln k_{on}^{\infty} + \frac{AB}{BR} \frac{-1/2}{BR + 1/2}$$

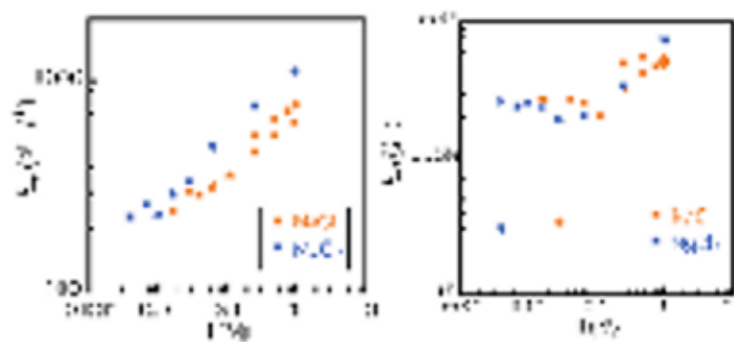
$$AB = Q_A Q_B / k T \epsilon \epsilon_0 \sqrt{2 \pi N_A / k T \epsilon}$$

$$BR = e^{-1} (2 \pi N_A / k T \epsilon) R$$

The reaction is slow compared with other IDP association reactions studied so far<sup>5</sup>.



While increasing ionic strength increases the rate of association, the dissociation rate is only moderately affected.



**Summary:** Using kinetic measurements on two different IDPs systems has allowed us to study the role of charge interactions in spectrin association and to gain insight into the mechanism of the pKID-KIX association. Further salt dependences will be investigated for the spectrin system and further data collected on the pKID-KIX system in order to determine individual rate constants. A  $\phi$ -value analysis will be performed to distinguish between the possible mechanisms of association.

1. Dyson HJ, Wright PE, Nat Rev Mol Cell Biol, 2005, 6: 197-208.
2. Wright PE, Dyson HJ, J Mol Biol, 1999, 293: 321-331.
3. Sugawara K, Dyson HJ, Wright PE, Nature, 2007, 447: 1021-1025.
4. Shammass S, Torris AJ, Clarke J, Proc Natl Acad Sci U S A, 2014, 111: 12055-12060.
5. Shammass S, Torris AJ, Clarke J, J Phys Chem B, 2013, 117: 13346-13356.