Intrinsically Disordered Proteins: the Importance of Helix-Flanking Prolines

Michael D. Crabtree[1]*, Wade Borcherds[2], Gary W. Daughdrill[2], Sarah L. Shammas[1] and Jane Clarke[1]
[1] University of Cambridge, UK, [2] University of South Florida, US
*mdc52@cam.ac.uk

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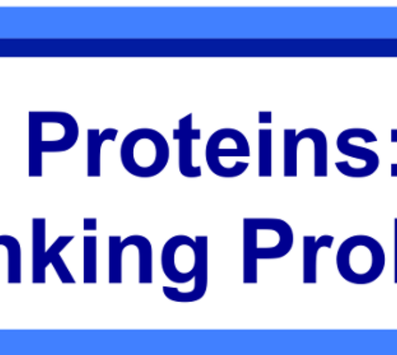
Background and Aims

Intrinsically disordered proteins (IDPs) are proteins that are functional, but have no defined structure in isolation. A subset of IDPs can undergo coupled folding and binding, and become structured upon binding to a folded partner protein. In the case of IDPs that form α-helicies, conserved proline residues commonly flank the regions that become helical. It has been proposed that these prolines control levels of residual structure and binding mechanism. However this has not been fully examined experimentally; kinetic information is required to justify the mechanistic comments. We have investigated the role of helix-flanking prolines in the coupled folding and binding of two IDPs, MLL and c-Myb, to their respective binding sites on the KIX domain of CREB binding protein (CBP KIX). Helix-flanking prolines were mutated to alanine, and resultant changes in the kinetics of the reactions were determined using fluorescence stopped-flow measurements.

Prolines Flank the α-Helical Region of c-Myb and MLL TAD when Bound to CBP KIX

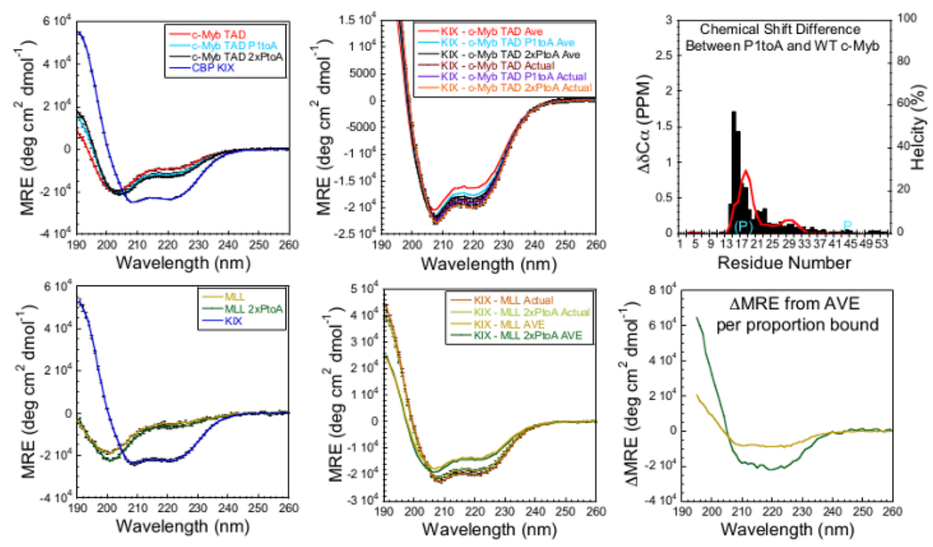
- The transactivation domains (TAD) of the intrinsically disordered transcription factors c-Myb (red) and MLL (beige) both form α-helicies upon binding to the folded transcriptional co-activator CBP KIX (blue). Prolines flank the helicies of both MLL (spheres) and c-Myb (P).
- It is not uncommon for prolines to be found at the ends of α-helices[1], however, the propensity and conservation of helix-flanking prolines is higher than at other regions within IDPs[2], suggesting that they may have an important role.
- Prolines have a rigid backbone and sample limited regions of the Ramachandran plot without forming hydrogen bonds. As a result they generally disrupt/terminate α-helices[3].

✧ **It has been suggested that helix-flanking prolines are conserved in order to control levels of residual structure (and hence binding affinities) for IDPs that undergo coupled folding and binding. Mutation of helix-flanking prolines to alanines would then be predicted to increase residual helical structure, and increase the affinity of the interaction due to an enhancement in the rate of association.**



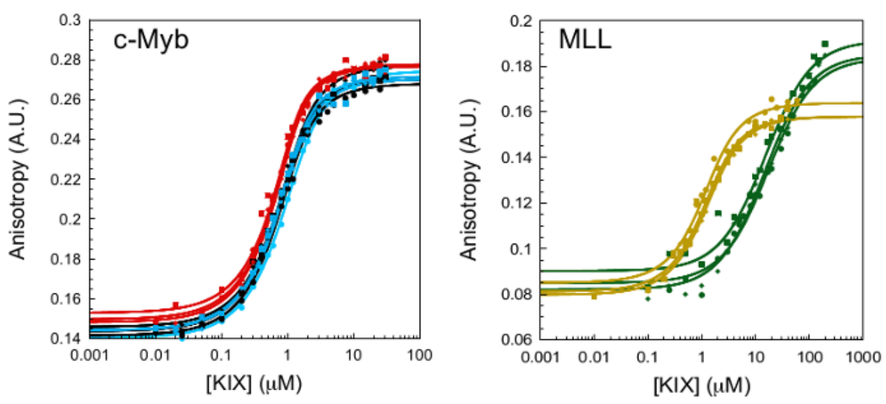
c-Myb: (GS)PAAAAIQRHYNDED**P**EKEKRIKELELLLMSTENELKGQQVL**P**TQNHT**C**SYPGW
MLL: **FITC**-SDDGNIL**P**SDIMDFVLKNT**P**SMQALGESPES
Underlined residues are helical in the bound structure

Residual Helical Structure



An increase in residual and bound (complex) helicity was observed by CD for both c-Myb PtoA mutants. For c-Myb P1toA, NMR chemical shifts showed that the increased residual structure was within and towards the N-terminus of the helix. An increase in helical structure was observed for MLL 2xPtoA in its bound context.

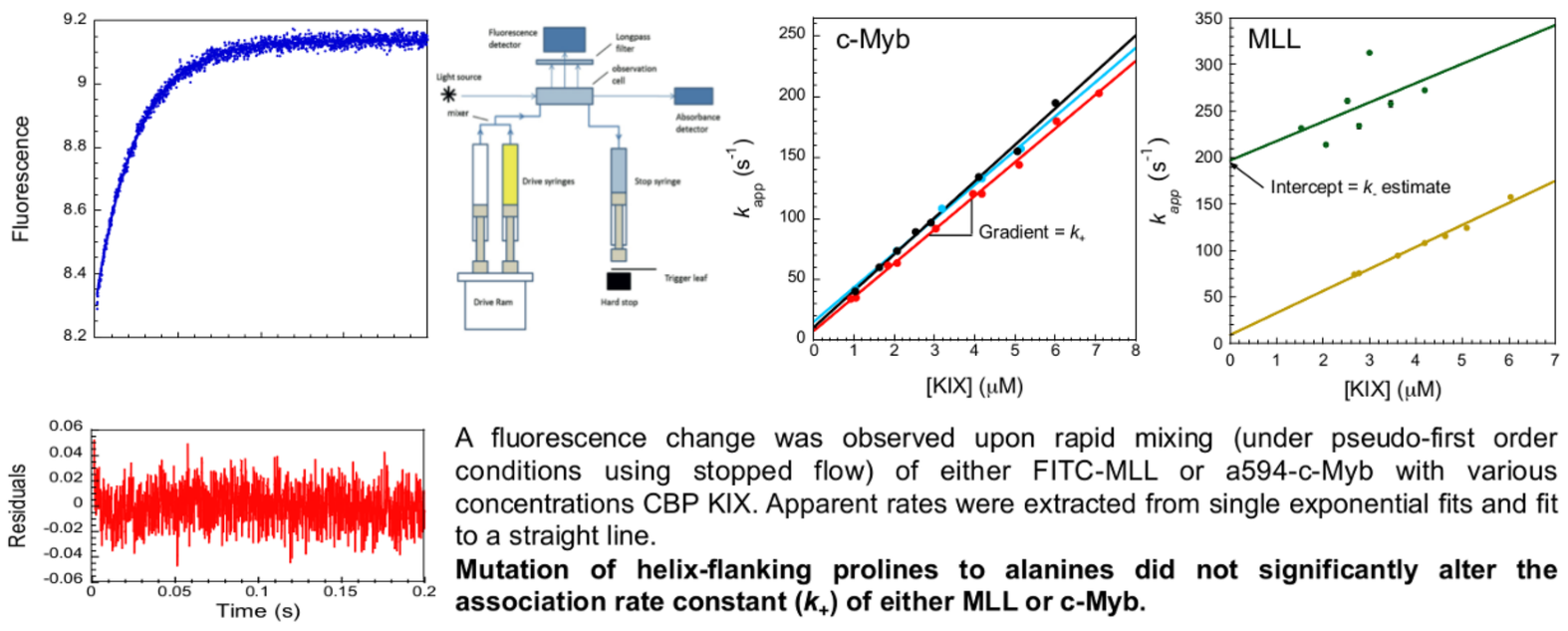
Affinity of the Interaction



FITC-MLL and a594-c-Myb peptides were incubated with various concentrations of CBP KIX and the fluorescence anisotropy recorded. Equilibrium constants (K_d) were obtained by fitting to a one site binding model.

Mutation of helix-flanking prolines to alanines increased the K_d by less than 1.9 ± 0.4 fold for c-Myb, whereas an increase of 20 ± 2 fold was observed for MLL.

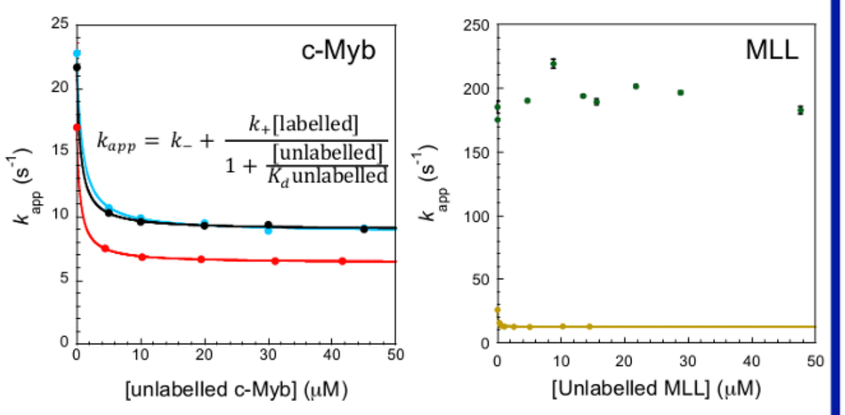
Rate of Association



A fluorescence change was observed upon rapid mixing (under pseudo-first order conditions using stopped flow) of either FITC-MLL or a594-c-Myb with various concentrations CBP KIX. Apparent rates were extracted from single exponential fits and fit to a straight line.

Mutation of helix-flanking prolines to alanines did not significantly alter the association rate constant (k_+) of either MLL or c-Myb.

Rate of Dissociation



A fluorescence change was observed upon mixing a pre-formed complex of fluorescently labelled peptide and CBP KIX with unlabelled competing peptide. Dissociation rate constants (k_-) would be reached with infinite concentrations of competing peptide.

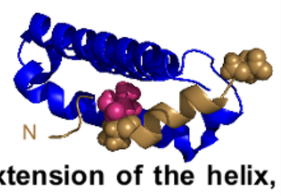
Mutation of helix-flanking prolines to alanines increased the k_- by less than 1.5 ± 0.02 fold for c-Myb, whereas an increase of 15 ± 0.5 fold was observed for MLL.

Results Summary

Peptide	Residual Helicity (%)[4]	Complex helicity (%)[4]	k_+ ($\mu M^{-1} s^{-1}$)	k_- estimate (s^{-1})	k_- (s^{-1})	Kinetic K_d (μM)	Equilibrium K_d (μM)
WT-c-Myb	31	55	27.7 ± 0.5	8 ± 2	6.38 ± 0.03	0.23 ± 0.004	0.17 ± 0.01
c-Myb-P1toA	37 (3)	56	28.1 ± 0.9	15 ± 3	8.8 ± 0.1	0.31 ± 0.01	0.32 ±0.06
c-Myb-2xPtoA	41	58	30.0 ± 0.8	10 ± 3	8.97 ± 0.08	0.30 ± 0.008	0.27 ±0.02
WT-MLL	18	56	23 ± 1	9 ± 5	12.4 ± 0.3	0.54 ± 0.03	0.70 ± 0.04
MLL-2xPtoA	21	56	21 ± 14	200 ± 40	196 ± 5	9 ± 6	15 ± 1

Mutation of helix-flanking prolines to alanines resulted in:

- **Increased residual structure for c-Myb.**
- **Increased bound (complex) structure for both c-Myb and MLL.**
- **No change in k_+ for either MLL or c-Myb.**
- **Small increase in k_- for c-Myb.**
- **Relatively large increase in k_- for MLL.**



Could the increase in k_- for MLL be due to an extension of the helix, preventing the leucine N-terminal to P1 from interacting with CBP KIX?

Conclusions

By mutating helix-flanking prolines to alanines in two IDPs, we found that:

a. **Helix-flanking prolines are not just switches that control the level of residual IDP structure, they may also influence the bound structure of IDPs.**

b. **An increase in IDP residual structure does not always lead to an increase in affinity for the interaction with a folded partner. Instead, for MLL and c-Myb, mutation of helix-flanking prolines led to an increased dissociation rate (and consequently decreased affinity) for the interaction with KIX.**

c. **The association rate of c-Myb binding to KIX is not dependent upon the amount of free structured c-Myb.**

Methods

P1toA refers to mutation of the proline at the N-terminus of the helix, 2xPtoA refers to mutation of both helix-flanking prolines.
All experiments were performed at 10 °C for c-Myb and 5 °C for MLL.
Unlabelled c-Myb, with a CtoA mutation to prevent dimerisation, was used for CD and NMR experiments.
Biophysical buffer was 100mM sodium phosphate pH 7.4 buffer.
The k_- for MLL 2xPtoA was calculated as the average of the apparent rates obtained when mixing with unlabelled competitor.

References

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