Many eukaryotic proteins do not adopt folded structures under native conditions. These “intrinsically disordered proteins” (IDPs) are frequently involved in cellular regulatory processes.

We use single-molecule FRET to directly observe the conformational heterogeneity and binding dynamics of NCDB, a flexible protein domain involved in transcriptional coactivation. We find that NCDB populates two distinguishable conformations which are both capable of binding the same IDP ligand.

Furthermore, we study the influence of macromolecular crowding on the binding reaction between NCDB and its IDP ligand. With increasing size and concentration of the crowder, we observe a decrease of $k_{\text{off}}$ concomitant with an increase of $k_{\text{on}}$, resulting in a six-fold affinity enhancement of the protein complex. This effect can be described by depletion interactions induced by the crowders.

Our findings provide an explanation for the remarkable binding promiscuity of NCDB and suggest a general mechanism for the favorable effect of crowding on protein-protein interactions.