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Abstract

Modelling The Multifarious Conformations Of The Intrinsically Disordered Protein 4e-Bp2 With Sm-Fret, Saxs & Pre Restraints

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Abstract: Hierarchical phosphorylation of the disordered 4E-BP2 protein stabilizes a binding incompatible 4-stranded beta domain while the C-terminal domain remains disordered. Ensemble descriptions of both phosphorylation states were calculated. The ensembles were restrained using Small-angle X-ray scattering (SAXS) and Paramagnetic Resonance Enhancement (PRE), while the single-molecule Förster Resonance Energy Transfer (smFRET) between residues 32 and 91 was used as validation of the ability of the restrained ensemble to agree with independent experimental evidence. Initially, conformational ensembles were calculated using ENSEMBLE[1] and SAXS-only restraints on an initial pool of 4E-BP2 conformers generated in TraDES.[2] Bimodal distributions of the radius of gyration (R_G) were obtained for both phospho forms of the protein. For non-phospho 4E-BP2, the back-calculated FRET efficiency was lower than the experimental value, while for the five-phospho 4E-BP2, the opposite happened. Adding PRE restraints for the five-phospho form, amplified the disagreement with the smFRET data. For both phospho forms, the average hydrodynamic radius (R_H) of the calculated ensemble was smaller than the experimental value determined by Fluorescence Correlation Spectroscopy (FCS). These discrepancies highlight the inability of the TraDES prior to capture the secondary structure of 4E-BP2. Alternatively, we used a new Rosetta-based method (Fast Floppy Tail, FFT)[3] to generate initial pools of conformations for the two 4E-BP2 phosphoforms. Applying SAXS, PRE, chemical shifts and hydrodynamic restraints in ENSEMBLE on these FFT-generated initial pools lead to better agreement with smFRET data and to a more accurate ensemble representation of 4E-BP2 in its two functionally-relevant forms.

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