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# (U*) Protein Conformational Ensembles for a Disordered Protein Restricted by Separate Biophysical Experiments

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We combined information from Fluorescence Correlation Spectroscopy (FCS), Nuclear Magnetic Resonance (NMR), Small-Angle X-ray Scattering (SAXS) and Single-Molecule Förster Energy Transfer (smFRET) to calculate conformational ensembles for the neuronal transcription factor initiation protein 4E-BP2. This is a 120-residue intrinsically disordered protein which toggles between an active, non-phosphorylated (NP) state and an inactive multi-phosphorylated (5P) state. An initial pool of 4E-BP2 structures was generated using Trajectory Directed Ensemble Sampling (TraDES) (Feldman & Hogue, Proteins: Structure, Function, and Bioinformatics, 46, 8-23, 2002). The ENSEMBLE method (Krzeminski et al, Bioinformatics, 29, 398-399, 2013) was used to search and select conformations within the initial pool that are consistent with the experimental data. NMR and SAXS data were used as restraints for the conformational search and FCS and smFRET data were used as validation.

The NP 4E-BP2 ensemble calculated with SAXS-only restraints showed a bimodal distribution of protein global dimensions such as the radius of gyration and the end-to-end distance. The back-calculated FRET efficiency and the hydrodynamic radius for this ensemble were lower than the respective experimental values. These inconsistencies are likely due to lack of short-range (5-20 Å) distance restraints, which are currently addressed by acquiring Paramagnetic Resonance Enhancement (PRE) data. Such data is available for the phosphorylated state (5P 4E-BP2), however this is partly folded, with a 4-stranded beta domain spanning residues 18-62. To address this challenge, we used the FastFloppyTail (FFT) program (Ferrie & Petersson, The Journal of Physical Chemistry B, 124, 5538-5548, 2020) to generate structures in the initial pool. As such, the folded region was modelled using the 5P 4E-BP2 folds from the Protein Databank, while the disordered tails were generated through the FFT algorithm. By incorporating the more realistic FFT-built prior in ENSEMBLE calculations, more accurate conformational ensembles of both phospho- and non-phospho- variants of 4E-BP2 were obtained. Analysis of these ensembles in terms of polymer physics and contact maps reveal new molecular level details lead to new insights into the biological function of 4E-BP2.

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