ENSEMBLE MODELLING THE DISORDER-TO-ORDER TRANSITION OF 4E-BP2

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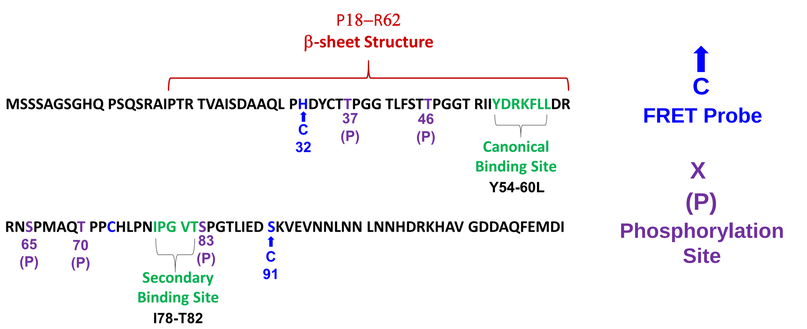
Abstract

Hierarchical phosphorylation of the disordered 4E-BP2 protein stabilizes a binding incompatible 4-stranded beta domain. Ensemble descriptions of both phosphoforms were calculated, restrained using Small-angle X-ray scattering (SAXS), single-molecule Förster Resonance Energy Transfer (smFRET) between residues 32 and 91 was used as validation. SAXS-only restrained conformational ensembles were calculated using ENSEMBLE [1] with a TraDES[2] generated initial pool. The back-calculated FRET efficiencies of these ensembles were not in agreement with experimental values. A new Rosetta-based method (Fast Floppy Tail, FFT)[3] was used to generate initial pools of conformations for 4E-BP2. SAXS, Paramagnetic Relaxation Enhancement (PRE), chemical shifts and hydrodynamic restraints in ENSEMBLE on these FFT-generated initial pools should improve agreement with smFRET data.

Key points:

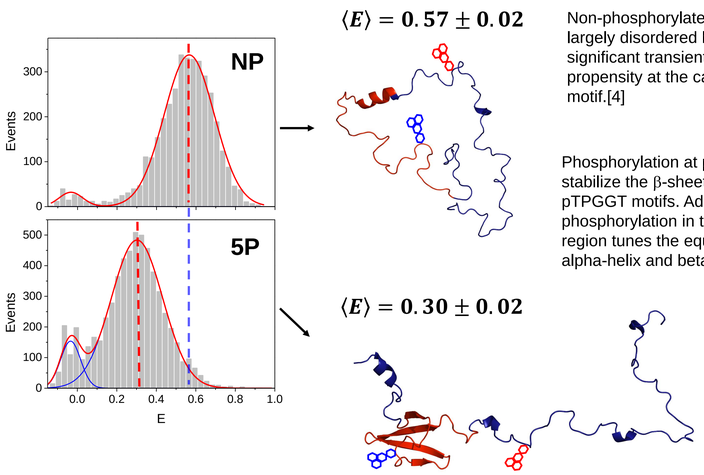
- **Phosphorylation of 4E-BP2 stabilizes a 4-stranded beta domain, and leads to a (surprising) decrease of smFRET efficiency, from 0.57±0.02 to 0.30±0.02.**
- **FRET efficiencies estimated from SAXS-restrained conformational ensembles for both phosphorylation states are not consistent with the experiments.**
- **Starting conformational pools of phosphorylated 4E-BP2 were generated by concatenating the NMR derived folded structure with N- and C-terminal 'floppy tails'.**

Sequence Motifs of 4E-BP2



4E-BP2 forms a beta-sheet structure from residues P18-R62 when phosphorylated at five different sites (purple)
The labelling positions for FRET studies are shown in blue (donor: Alexa 488, acceptor: Alexa 647).
The canonical and secondary sites where 4E-BP2 binds to eIF4E are shown in green.

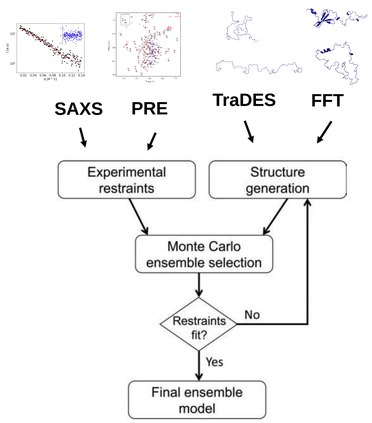
Disorder-to-Order Transition Decreases FRET Efficiency



Non-phosphorylated 4E-BP2 is largely disordered but it contains significant transient helical propensity at the canonical binding motif.[4]

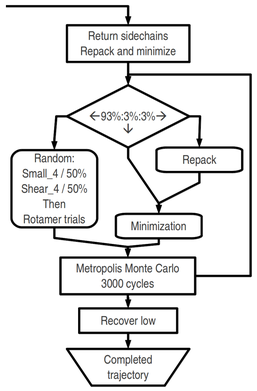
Phosphorylation at pT37 and pT46 stabilize the β-sheet structure via a pTPGGT motifs. Additional phosphorylation in the C-terminal region tunes the equilibrium between alpha-helix and beta-fold. [5]

ENSEMBLE



- A Monte Carlo algorithm is used to select a set of structures from an initial pool of conformers generated by TraDES or FFT
- Experimental properties are back-calculated from a selected set of structures to check for consistency with experimental restraints
- If the pool is not consistent a new pool based on the current is selected, the process is repeated until the set of structures satisfies all experimental restraints

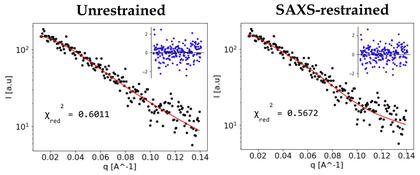
Fast Floppy Tail (FFT)



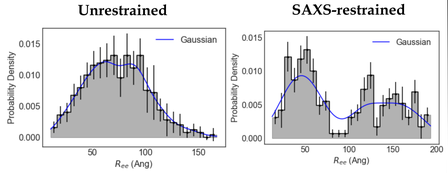
Conformation refinement using the FFT method. [6]

- Metropolis Monte Carlo cycles
- 3-residue fragments from the Protein Databank are inserted to sample secondary structural preferences
- Several tools to perturb structure in order to sample a wide range of orientations
- The scoring function contains physical terms such as van der Waals repulsion, hydrogen bonding and electrostatics

Non-Phosphorylated 4E-BP2: SAXS-restrained ENSEMBLE



Fits of the back-calculated SAXS curves to experimental data for 400-conformer ensembles, unrestrained TraDES generated 100% coil sampling (left) and 200 SAXS-restraints (right).



Distributions of end-to-end distance for the SAXS-restrained (right) and unrestrained (left) ensembles. The restrained ensemble shows clear bimodality.

	Mean FRET Efficiency	Hydrodynamic Radius (Å)
SAXS Restrained ENSEMBLE	0.32 +/- 0.01	25.83 +/- 2.30
SAXS Restrained ENSEMBLE Compact Conformers	0.57 +/- 0.01	22.85 +/- 0.11
SAXS Restrained ENSEMBLE Extended Conformers	0.054 +/- 0.005	30.21 +/- 0.14
smFRET	0.57 +/- 0.02	N/A
FCS	N/A	29.0 +/- 0.2

Back-calculated and experimental smFRET efficiencies and hydrodynamic radii. Compact (215-conformers) and extended (182-conformers) from the SAXS-restrained populations.

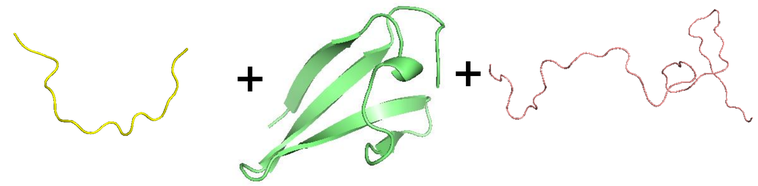
Future directions

- PRE and CS data are currently being collected to provide local constraints complementary to SAXS and smFRET
- FFT-constructed conformers will provide a more realistic starting pool for ensemble refinement

ENSEMBLE Modelling of the Phosphorylated 4E-BP2

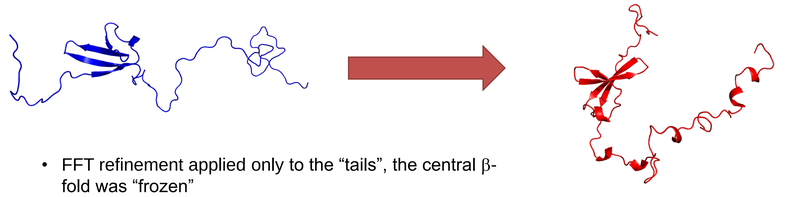
Having applied the ENSEMBLE method to the phosphorylated state using a TraDES initial pool to sample from, fits to experimental values (smFRET, FCS, CS, PRE) were quite poor, despite utilizing SAXS, PRE and CS data as restraints.

Solution: Apply FFT and Maintain the Folded Region



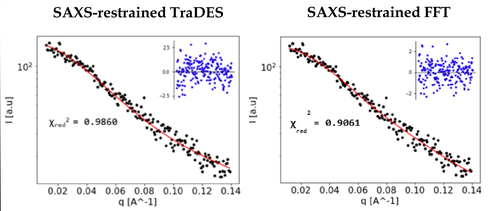
Residues: 1-17 18-62 63-120

Concatenated Structure → **FFT Algorithm** → **Resulting Structure**



- FFT refinement applied only to the "tails", the central β-fold was "frozen"
- 3mer sampling produced important secondary structure

SAXS-restrained ENSEMBLE: FFT vs. TraDES priors



Method	FRET Efficiency	SAXS Reduced Chi^2
SAXS Restrained (TraDES prior)	0.43 +/- 0.01	0.9860
PRE + SAXS Restrained (TraDES prior)	0.50 +/- 0.01	0.8845
SAXS Restrained (FFT prior)	0.40 +/- 0.01	0.8784
smFRET	0.30 +/- 0.02	N/A

- Better fit of the SAXS curve by FFT ensemble, which includes more extended conformers.
- FFT back-calculated smFRET efficiency is closer to the experimental value

Future directions

- Additional restraints: PRE, CS
- Identify critical residue contacts in the fully-restrained ensembles

Conclusions

- **Non-Phosphorylated 4E-BP2:** The SAXS-restrained ensemble was unable to predict the FRET efficiency and the hydrodynamic radius. Additional experimental restraints (PREs) will provide the internal distance information to capture both local and global dimensions of the protein.
- **Phosphorylated 4E-BP2:** TraDES serves as a poor prior as it is unable to generate the folded-structure of phosphorylated 4E-BP2. FFT was used append N and C-terminal disordered segments to the PDB structure of the P18-R62 folded region. The resulting SAXS-restrained ENSEMBLE provided a better fit to the data then the TraDES generated initial pool.

References

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