

Seminar

Mapping the Folding Landscape of the FF Domain from Human HYPA/FBP11 Using CEST NMR Spectroscopy

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Due to their unique three-dimensional structures, proteins can perform a remarkably wide range of cellular functions. Although machine-learning approaches enable accurate prediction of protein structures, the mechanisms by which they fold from an unfolded (U) ensemble into their native (F) conformations remains incompletely understood. A detailed description of protein folding therefore requires characterisation of transient folding intermediates at atomic resolution. However, these states are often difficult to detect because of their low populations and short lifetimes. Over the past two decades, NMR relaxation-dispersion methods have provided powerful approaches for characterising such sparsely populated conformational states. In particular, recent developments in chemical exchange saturation transfer (CEST) NMR have extended the ability to characterise conformational exchange over timescales ranging from approximately 100 μ s to 100 ms and to detect minor states populated at levels as low as $\sim 0.1\%$. Using ^{15}N CEST NMR experiments it was recently shown that the FF domain from Human HYPA/FBP11 folds via two intermediates I1 and I2. In this talk, I will discuss how CEST NMR experiments were used to characterise the folding landscape of the FF domain. First, using CEST experiments we have determined the structure of the newly detected folding intermediate I2. CEST was also used to determine the urea m -values for all the conformational states and the transition states to resolve the extent of compaction as the folding proceeds. We then investigated the interaction of the FF domain with 8-anilino-naphthalene-1-sulfonic acid (ANS), a widely used extrinsic fluorescent probe for partially folded protein states. Combination of ANS fluorescence with amide ^{15}N CEST NMR demonstrated that ANS interacts with folding intermediate I1 and not the F, I2, or U states. Finally, I will discuss the effects of different macromolecular crowders on the conformational equilibria of the A39G FF domain. We established that the extent to which a conformational state is perturbed by crowding depends on both the identity of the crowder and the structural properties of the state.

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14:30 Hrs (Tea / Coffee 14:15 Hrs)

Seminar Hall, TIFRH