NMR analysis of solvent-induced protein unfolding via encapsulation in a giant self-assembled cage

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Precise knowledge of transient protein structures is vital for the advancement of biochemical science but methods to isolate and observe them are scarce. Our group has synthesised giant hollow $M_{12}L_{24}$ complexes through the self-assembly of bis(pyridine) ligands (L) with Pd^{II} ions (M). We have recently reported the encapsulation of a protein in such a complex, which stabilized the protein against organic solvents and heat by isolation into the nano-space of the cage.[1,2] We thought to use this stabilisation to observe transient protein structures under destabilising conditions. Here, we used NMR spectroscopy to analyse protein unfolding in water-organic solvent mixtures via encapsulation in a self-assembled $M_{12}L_{24}$ cage (Fig.1).

A model protein, cutinase-like enzyme (CLE), was encapsulated by the N-terminus-selective conjugation with the 2-formylpyridinyl group of the ligand and the subsequent self-assembly with Pd^{II} ions.[2] The caged CLE was subjected to increasing ratios of acetonitrile to water. In more than 50% acetonitrile the free protein precipitated out of solution (Fig. 1A). The caged protein, in contrast, partially unfolded but aggregation and precipitation were prevented through the encapsulation effect even in 80% acetonitrile (Fig. 1B). Through ^{1}H–^{15}N HSQC analysis, unfolding was analysed by mapping of the chemical shift at increasing acetonitrile ratios. Thus, the transient protein structure was successfully visualised by encapsulation in the $M_{12}L_{24}$ cage. This method could be used for a wide range of other proteins to analyse gradual structural changes under otherwise unstable conditions.

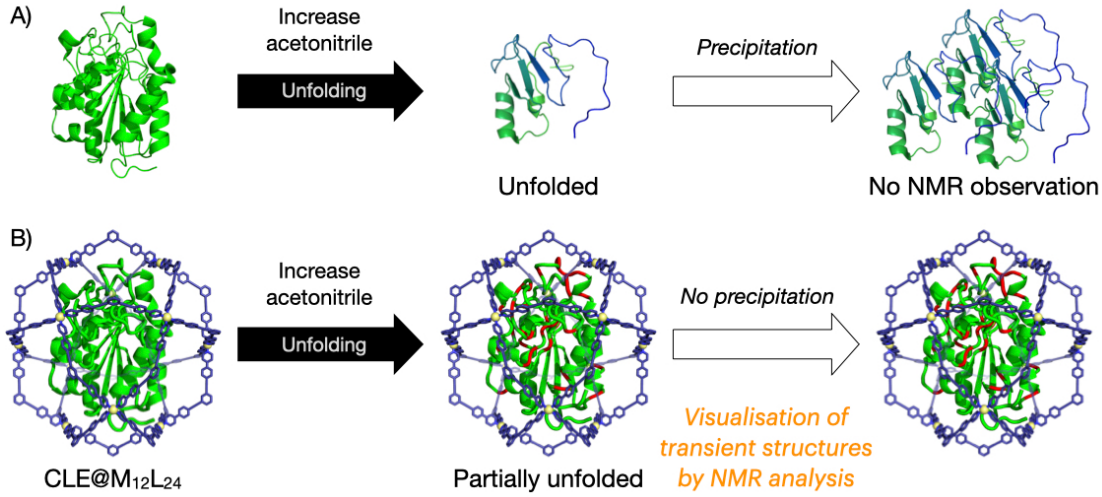

Figure 1: NMR analysis of unfolding of A) free CLE and B) CLE encapsulated in an $M_{12}L_{24}$ cage at increasing acetonitrile ratio.

1) D. Fujita, *et al.*, *Nat. Commun.* **2012**, *3*, 1093. 2) D. Fujita, *et al.*, *Chem.* **2021**, *7*, 2672–2683.