NMR Observation of Hysteretic Behaviour in Solvent-Induced Protein Unfolding/Refolding Processes via Encapsulation in a Coordination Cage

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Previously, our group demonstrated encapsulation of individual protein molecules in a self-assembled $M_{12}L_{24}$ cage (Fig 1a).[1] Isolation in the cage's inner space stabilised a protein against organic solvent by preventing aggregation of partially unfolded intermediates. We thought to use this system to study protein unfolding/refolding processes by NMR analysis of otherwise invisible transient protein folding intermediates.

In this work, we report NMR observation of transient intermediate structures in the solvent-induced unfolding/refolding of a caged protein, revealing hysteretic refolding behaviour (Fig.1). A model protein Cutinase-Like Enzyme (CLE) was encapsulated in the metallo-cage, and by increasing or decreasing the organic solvent ratio in an acetonitrile/water mixture we investigated the transient intermediate structures occurring upon unfolding and refolding respectively. The spatial isolation in the cage prevented precipitation of unstable intermediates, enabling observation of their structures via ^{1}H–^{15}N HSQC NMR. For the unfolding, we saw a sharp transition point at 83% acetonitrile, indicated by sudden peak intensity attenuation (Fig. 1c). For the reverse process, however, there was no structural change yet at 83%, and the refolding transition started at around 80% acetonitrile, indicating hysteresis behaviour for the refolding. Moreover, structural mapping visualised the regions of structural change (Fig. 1b) and showed that the unfolding and refolding are not exactly reversible.

In summary, we have shown that protein encapsulation in the $M_{12}L_{24}$ cage can be used to visualise transient protein folding structures with great potential for detailed studies of folding pathways and mechanisms.

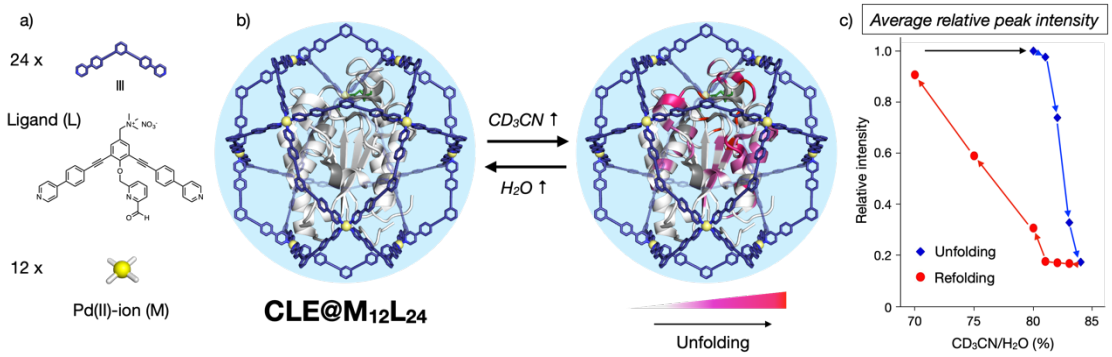

Figure 1: a) Structure of $M_{12}L_{24}$ cage building blocks (M) and (L). b) Solvent-induced unfolding/refolding of CLE protein encapsulated in the cage. c) Refolding hysteresis of average relative HSQC peak intensity of assigned CLE residues.

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