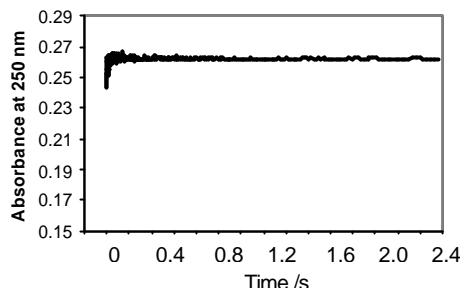


# Metal-dependent protein folding kinetics of metallothionein

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Protein folding is an area of great interest in biological chemistry. A precise structural conformation is often necessary for proper protein function, emphasizing the need for complete understanding of the driving forces and mechanisms involved in folding. The metals located within metalloproteins are essential due to their common roles in catalysis or structural stability and thus are crucial for protein function. Significant fractions of metalloproteins require the coordination of metal ions to facilitate the formation of the secondary or tertiary protein structure, however, little is known about the mechanisms that govern metal-dependent protein folding. Metallothionein is used in the present study as a model metal-binding protein as it is a low molecular weight protein, which coordinates seven divalent metal ions into two clusters,  $\alpha$  and  $\beta$ , in stoichiometries of  $M_4S_{11}$  and  $M_3S_9$ , respectively. The folding of the  $\alpha$  and  $\beta$  domains relies entirely on the formation of the metal-thiolate clusters. The mechanism and rate of the metallation process is unknown but is of importance for understanding the nature of the reaction *in vivo*. In the present study, stopped-flow spectroscopy was utilized to measure the metallation reaction of the individual domains of apo-MT. Under all conditions, Cd binding to apo-MT occurred within the 2 ms dead time of the instrument, which is faster than previous reports indicate. Molecular dynamics calculations were also carried out, using a modified MM3 force field, on the individual domains of metallothionein. The data suggest that the metal-free peptide is stabilized by H-bonds, which are not present in the metallated structure.<sup>1</sup>



1. Kelly E. Rigby and Martin J. Stillman, *Biochem. Biophys. Res. Commun.*, 325, 1271-1278 (2004)