Investigation of the Factors Affecting Reactivity of Thiolate-rich Zn(II)-sites in Proteins

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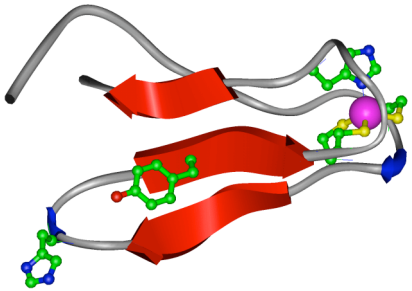
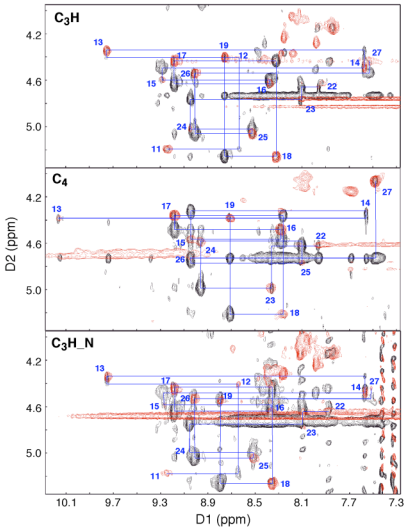
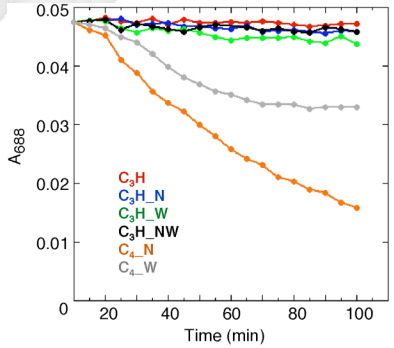
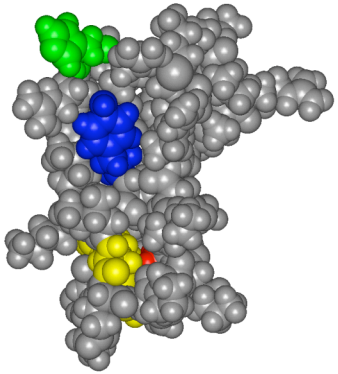
Cysteine-rich Zn(II)-binding sites in proteins serve two distinct functions: to template or stabilize protein folds, and to facilitate chemical reactions such as alkyl transfers. We are interested in how the protein environment controls metal site properties, specifically how naturally occurring tetrahedral Zn(II) sites are affected by the surrounding protein.



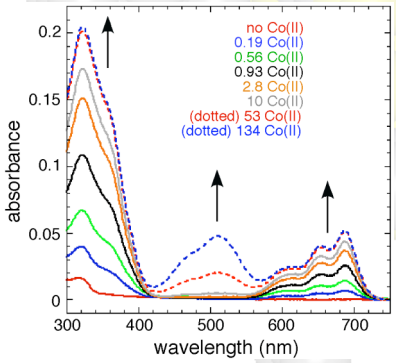


We are studying derivatives of L36, a small 37-residue zinc ribbon protein containing a Cys3His metal coordination site, as a model system. Through strategic changes to the protein sequence, including one located at the opposite end of the protein from the metal-binding site, we have demonstrated modulation of the Co(II)- and Zn(II)-binding affinities without affecting the overall protein fold.





Co(II) titration



Recent work has focused on correlating differences in reactivity with methyl transfer reagents to sequence changes we introduce into L36. We have monitored the methylation reactions using several techniques, including changes in the optical spectra upon addition of methylation agents to Co(II)-bound derivatives. For Cys4 species, we see a rapid release of Co(II) (reflected in a loss of the Co(II) d-d transitions), whereas for the Cys3His derivatives the absorbance does not change. We are currently using chemically-prepared methyl-Cys derivatives of L36 to understand if the Co(II) is due to methylation of a Cys in the metal-binding site, or if the anion generated from the methylation reaction extracts the Co(II) from the protein.