Modulation of Reduction Potentials of 2Fe-2S Iron Sulfur clusters

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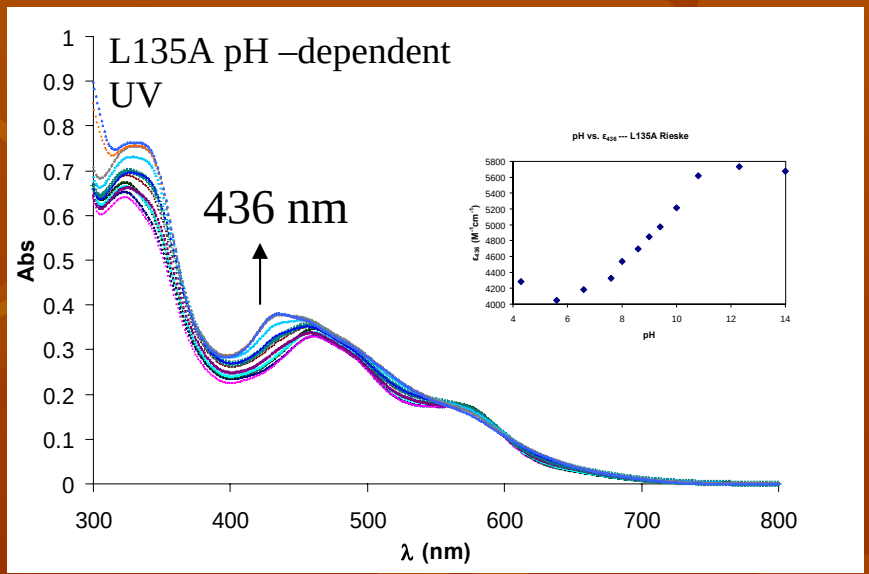
How iron sulfur proteins modulate reduction potentials is a fundamental question in biological chemistry. The factors that govern reduction potentials are thought to include the number of (NH or OH to S) hydrogen bonds to the S of the cluster or the Sγ of a ligating cysteine, the number of charged residues near the cluster, and hydrophobicity of the environment around the cluster. In our work, each of the implicated factors in the tuning of reduction potential are being tested by modifying specific residues and determining the resultant reduction potential in the Rieske protein from *Thermus thermophilus* . Any conformational or electronic changes that might accompany the changes in reduction potential are being ascertained through X-ray crystallographic and spectroscopic studies. Only with both the biochemical and structural changes accounted for in the analysis will a full understanding of how proteins tune reduction potential be possible.



Toward this end, three mutations Y158F, G156S and L135A have been produced. These mutations test the effect of removing an OH-S hydrogen bond, adding a hydrogen bond, and changing the hydrophobic nature of the area surrounding the cluster, respectively. They have been characterized through DNA sequencing, mass spectrometry and UV-Visible spectroscopy at multiple pH values. We have crystallized the wild type and the mutants. Crystals of G156S are of high enough quality to have obtained a 2.8 Å resolution data set. Refinement of the structure is in progress.

We have also constructed a pyrolytic graphite electrode and are beginning studies to determine the reduction potentials of each mutant at multiple pH values.