Metalloproteins: Kinetic and spectroscopic characterization of [FeFe]-hydrogenases by ATR-FTIR spectroscopy & electrochemical methods

[FeFe]-hydrogenases are complex metalloproteins that catalyze the reversible reduction of protons and electrons to molecular hydrogen at unprecedented turnover rates (up to 9000 s^{-1}). Protons (H^+) and electrons are transported to the active site [6Fe6S]-cluster (H-cluster) at which catalytic conversion of H_2 takes place. The [2Fe2S]-moiety of this cluster (Fig. 1) bears three CO and two CN^- groups in which the electronic and coordination features reflect individual catalytic states of the enzyme. ATR-FTIR spectroscopy allows for the identification of these ligands as the infrared stretching frequencies are apart from water and those of the protein environment and thus serve as a fingerprint of the catalytic cofactor. This technique will be demonstrated to analyze the different catalytic states of two different Hydrogenases, CrHydA1 and CpI. The highly conserved H^+-transfer pathway involves five potential amino acids that funnel protons to the H-cluster. We will further compare wild type protein with site-directed mutagenesis variants with deficiency in this proton transfer pathway. The resulting differences will be examined by kinetic activity studies via cyclic voltammertry.

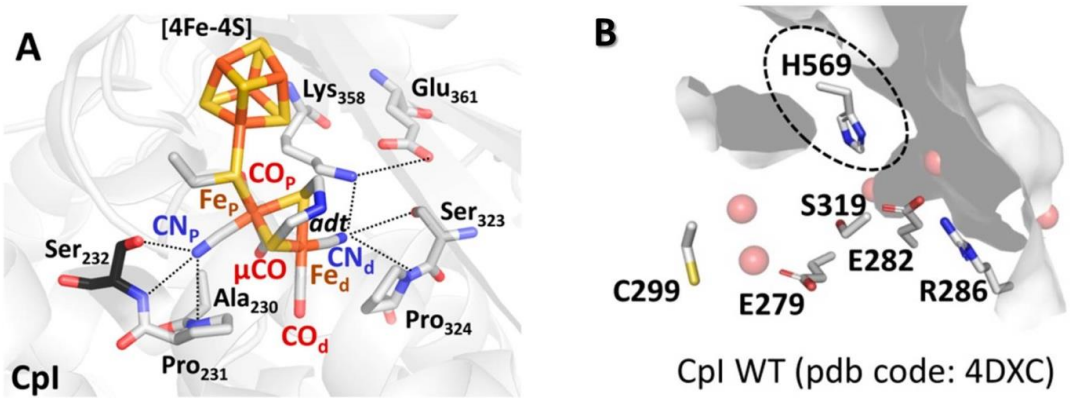

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Figure 1: (A) Stick model of the H-cluster of CpI embedded into the protein environment. (B) Stick- and surface model of the H^+-transfer pathway in CpI. Color code: gray/black, carbon; blue, nitrogen; red, oxygen; yellow, sulfur; orange, iron.