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

The [FeFe]-hydrogenase HydA1 is a complex metalloprotein that catalyzes the reversible reduction of protons and electrons to molecular hydrogen. Electrons are transported via a [4Fe4S]-subcluster to a unique [2Fe2S]-subcluster where catalytic conversion of H_2 takes place. On the other hand protons are transported via a potential proton transport pathway involving five potential amino acids which direct them to the catalytic center. The [2Fe2S]-cluster of the catalytic center 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 HydA1. We will compare HydA1 wild type (WT) protein to HydA1 protein variants with deficiency in the proton transfer pathway. The resulting differences will be examined by kinetic activity studies via gas chromatography followed by ATR-FTIR spectroscopy.