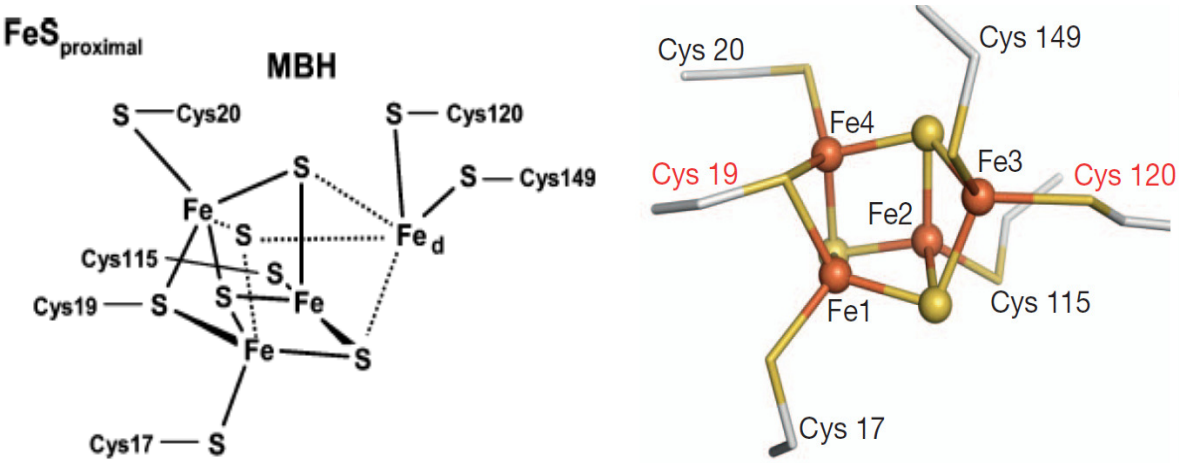
Spectroscopy on catalysis, assembly, and activation of NiFe hydrogenases



Model of the proximal FeS cluster in the O_2-tolerant membrane-bound NiFe hydrogenase (MBH) based on XAS results (left) and respective crystal structure (right).

Summary:

NiFe hydrogenases are metalloenzymes catalyzing the reversible cleavage of molecular hydrogen. Particularly interesting are the three different NiFe hydrogenases ("regulatory" and "energy-converting" enzymes) from the β-proteobacterium *Ralstonia eutropha* which are all not inhibited by dioxygen. To deduce the unusual structural and functional features of their metal cofactors (NiFe, FeS) during multistep redox chemistry, in relation to the specific protein-cofactor interactions, was the primary goal of this project. A broad spectroscopic approach with a focus on X-ray absorption spectroscopy (XAS) techniques at nickel and iron energies has been applied.

In the first funding period, models of the atomic structures of the Ni-Fe active site have been developed for the hydrogen sensor (regulatory hydrogenase, RH) and for the NAD-reducing enzyme (soluble hydrogenase, SH) and structural and electronic changes during activation and catalysis were unraveled. Information on the reasons for the O_2-insensitive behaviour was obtained. Both enzymes reveal features of their metal centers not commonly observed in the standard-type NiFe hydrogenases.

In the second funding period we have concentrated on investigations of the membrane-bound Ni-Fe hydrogenase (MBH) by XAS to deduce the structures and redox states of its cofactors during activation and catalysis; on the pure population and study of key intermediates during catalysis of the three enzymes; on isolated (sub-)complexes and maturation intermediates to follow the assembly of the metal centers, and on specific mutants to unravel the different strategies and molecular basis of their O_2-tolerance. A unique proximal FeS cluster in the MBH was characterized.

The goal was to obtain a comprehensive picture of the structures and dynamics of the metal centers in the three hydrogenases in all functionally relevant states of the protein-cofactor complexes and their analysis in atomic detail.