

Synthetic cofactor integration into metalloproteins

Harboring a unique metallo-cofactor termed the “H-cluster”, [FeFe]-hydrogenases catalyze the reversible reduction of protons to molecular hydrogen with unprecedented turnover rates (up to 9000 s^{-1}). The H-cluster comprises a “standard” cubane [4Fe-4S]-cluster ([4FeH]) coupled via the thiol group of a bridging cysteine to a unique binuclear iron moiety ([2FeH]). Within the [2FeH] subunit, the two Fe ions are bridged by an azadithiolate (adt) and are coordinated to CN- and CO ligands. The electronic and coordination features of the latter two can be identified by ATR-FTIR spectroscopy. Thereby, individual states of the catalytic cycle of the enzyme can be monitored.

The process of cofactor assembly starts with a pre-form of the [FeFe]-hydrogenase (apo-hydrogenase), containing only the 4Fe_H subsite. Providing either chemically or enzymatically synthesized precursors of the [2FeH] subsite, the H-cluster is formed. In comparison to the co-expression of the natural maturase enzymes to enzymatically assemble the catalytic cofactor and subsequently investigate catalytic features of [FeFe]-hydrogenases, the chemical synthesis of [2FeH]-mimics and the so-called *in vitro* maturation of apo-enzymes bears the advantage of control over all parts of the enzyme. Either synthetic mimics of the [2FeH]-subsite can be provided and/or protein scaffolds with point-mutations can be used to understand and improve the enzymatic function of semi-synthetic [FeFe]-hydrogenases.

During this module the participants will learn how to monitor the integration of an artificially synthesized cofactor mimic into the apo [FeFe]-hydrogenases via ATR-FTIR spectroscopy. Furthermore, kinetic activity studies will be conducted via gas-chromatography to verify the successful reconstitution of the proteins.

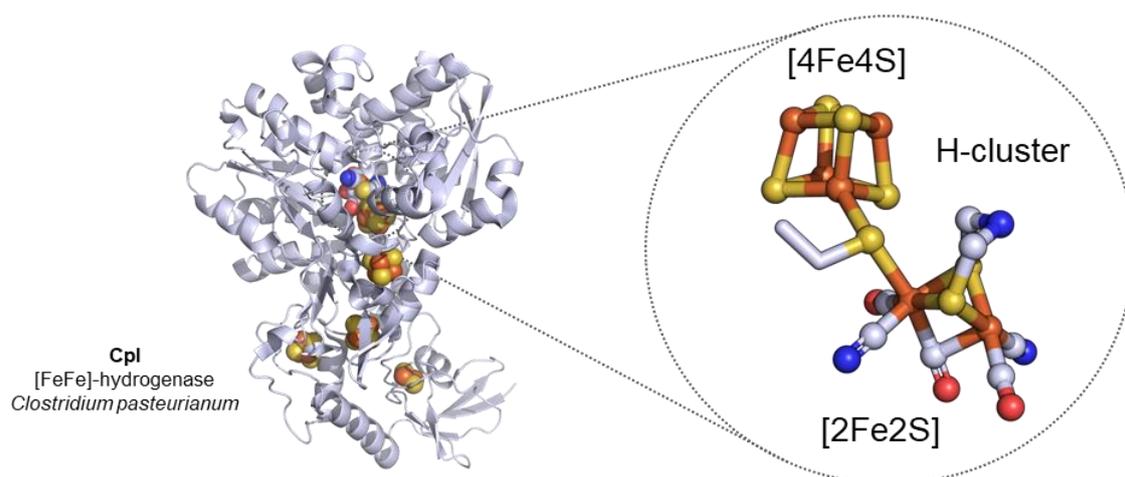


Figure 1: Schematic depiction of a [FeFe]-hydrogenase and its unique cofactor, the H-cluster.