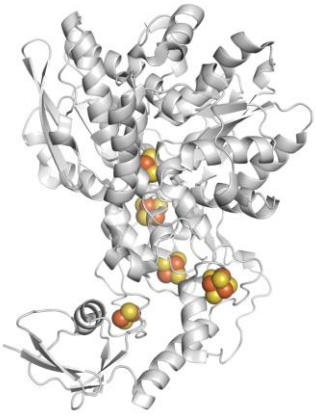
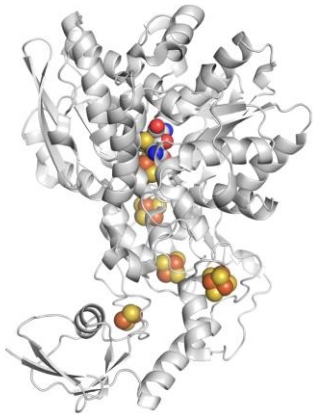
Project Eckhard Hofmann

Fine-tuning catalytic activity of FeS clusters by protein coordination in [FeFe] hydrogenases

Background and preliminary work: Activity of FeS cluster containing enzymes is crucially dependent on the correct integration of the cofactor into the apoprotein. Based on a reconstitution system for incomplete [FeFe]-hydrogenases with chemically synthesized [2Fe2S]-clusters established in the group of Thomas Happe, the semisynthetic bacterial [FeFe]-hydrogenase CpI was shown to be structurally not discernible from the native enzyme. Additionally, the structure of the [2Fe2S]-cluster-free precursor form of CpI was solved and helped to understand the process of [FeFe]-hydrogenase maturation. Together with the Happe and Gerwert groups, the structure of HydA1 from *Chlamydomonas reinhardtii* reconstituted with a modified [4Fe4Se] cluster was investigated.



CpI-apo (4XDD)



CpI-holo (4XDC)

Work planned: In the future, several semisynthetic variants will be studied from a structural point of view. While the enzyme cavity harbouring the complex appears to be very rigid, the [2Fe2S]-complex itself has only one covalent attachment point and is thus prone to structural changes. The complex alone in solution has no catalytic activity, and attempts to mimic the active site of [FeFe]-hydrogenases with a smaller scaffold failed so far. Thus investigations into the structure/function relationship of the inorganic core of these enzymes and its interplay with the protein hull are expected to help in the understanding of the mechanism of catalysis of [FeFe]-hydrogenases. We will also investigate unnatural enzymes reconstituted with derivatives of the [4Fe4S]-subcluster. This cluster seems to be a major structural anchor for the overall fold of the protein besides its role as electron relay during turnover.

The reconstitution system established by the Happe group offers the unique opportunity to study the cofactor-protein interaction both by modification of the cofactors and by site-directed mutagenesis of the binding pocket. Primarily X-ray crystallography will be used to determine structures of several altered complexes. Together with biochemical and biophysical data on these systems obtained both in this project and in the projects of Happe and Gerwert, this will provide new insights into the fine-tuning of cofactor chemistry by the protein environment in hydrogenases.

Selected references:

Esselborn J, Muraki N, Klein K, Engelbrecht V, Metzler-Nolte N, Apfel U-P, Hofmann E, Kurisu G, Happe T. 2016. A structural view of synthetic cofactor integration into [FeFe]-hydrogenases. Chem. Sci. 7:959–968.

Noth J, Esselborn J, Güldenhaupt J, Brünje A, Sawyer A, Apfel U-P, Gerwert K, Hofmann E, Winkler M, Happe T. 2016. [FeFe]-Hydrogenase with Chalcogenide Substitutions at the H-Cluster Maintains Full H2 Evolution Activity. Angew. Chem. 128:8536–8540.

Winkler M, Senger M, Duan J, Esselborn J, Wittkamp F, Hofmann E, Apfel U-P, Stripp ST, Happe T. 2017. Accumulating the hydride state in the catalytic cycle of [FeFe]-hydrogenases. Nature Communications 8:16115.