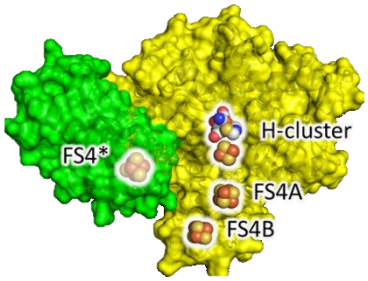
Project Thomas Happe

Identifying structure-function relations in oxygen tolerant hydrogenases

Background and preliminary work: [FeFe]-hydrogenases are efficient H_2 catalysts. Equipped with a transition metal cofactor, they operate under mild conditions and turn over H_2 with remarkably high rates. Their unique cofactor, termed the "H-cluster", is irreversibly destroyed in the presence of only trace amounts of oxygen. Very recently, a novel [FeFe]-hydrogenase was discovered: Cba5H, a dimeric enzyme from *Clostridium beijerinckii,* reversibly switches between the oxygen stable, but inactive state H_{inact} and the oxygen sensitive and active H_{ox} state. Aiming to understand the mechanism of H_{inact} on a molecular level, we solved the crystal structure of Cba5H in the presence of oxygen. Our efforts revealed that the unique property of Cba5H is attributed to a cysteine close to the H-cluster, which functions as a "safety cap", shielding the active site from O_2. Surprisingly, the cysteine itself is very conserved among all [FeFe]-hydrogenases. Site directed mutagenesis showed that the special "switching" function of the cysteine in Cba5H can be ascribed to a flexible protein loop region, allowing the cysteine to reversibly bind to the active site.



Work planned: While the active site architecture of the O_2 protected state of Cba5H is now well understood, many questions remain. The most urgent question is how the presence of oxygen is "sensed" by the enzyme. Results with other oxidants showed that the movement of the flexible loop region, which initiates the O_2 stable, "hibernating" form, is initiated not only in the presence of oxygen, but also under several other oxidative conditions. Apart from being very similar to normal, O_2 sensible hydrogenases in the H-cluster binding domain, Cba5H shows many differences in other protein regions. An additional soluble ligand binding β-grasp (SLBB) domain, binding an iron sulfur cluster of, till now, unknown structure and function, could be of importance during the sensing of oxygen. To find out more about the potential role of the SLBB domain, this project aims to draw knowledge from the biological diversity of hydrogenases. Making use of the emerging information of genomic data, we screened for novel hydrogenases, which possess a flexible loop region surrounding the cysteine to allow the thiol mediated protection mechanism. The most interesting forms showed a surprising structural diversity in terms of the presence or absence of the SLBB domain as well as of the constitution of the flexible loop. Exploiting EPR-, ATR-FTIR-spectroscopy, protein-film electrochemistry and crystallography, this project aims to understand the correlation between certain structural traits of "Cba5H-like" hydrogenases (SLBB domain, flexible loop properties) and the ability to form H_{inact}.

Interactions within MiCon: Eckhard Hofmann, Anja Hemschemeier, Dirk Tischler, Marc Nowaczyk

Selected references

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