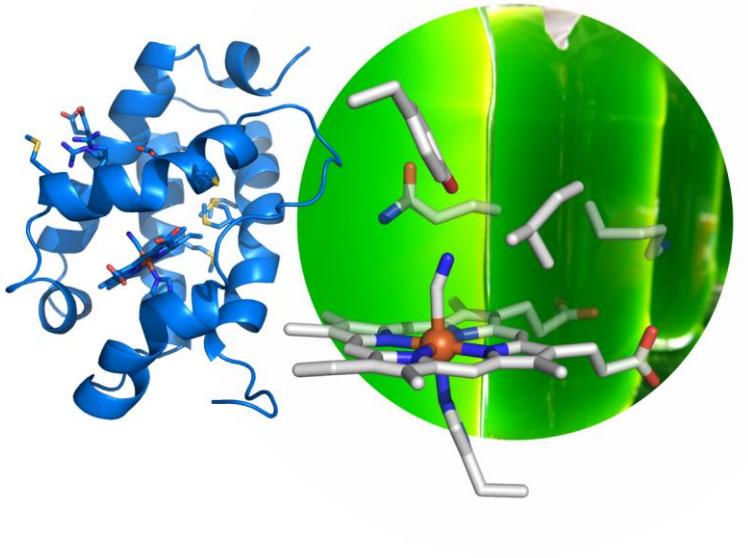
Project Anja Hemschemeier

Analysis of NO-producing hemoglobins from microalgae

Background and preliminary work: The acclimation of photosynthetic organisms to adverse conditions is pivotal for Ecosystem Earth. Our previous research indicates that the eukaryotic microalga *Chlamydomonas reinhardtii* acclimates to energy crises caused by darkness or hypoxia by employing a nitric oxide (NO-) dependent signaling cascade based on an NO-sensitive guanylate cyclase homologous to those found in humans. While reports on further NO signaling pathways in *C. reinhardtii* exist, the cellular source of NO is unknown in most cases. We could show recently that certain hemoglobins (Hbs) found in *Chlamydomonas* are capable of nitrite-dependent NO production *in vitro*. Hbs exist in all kingdoms of life, and, in contrast to the perception of red blood cell hemoglobin, they often function as enzymes that convert substrates such as NO and nitrite. Sophisticated interactions between the polypeptide and the heme group tune the protein towards a certain function, but even one and the same Hb can have different reactivities *in vivo* depending on the cellular environment. For example, animal Hbs function as NO-forming nitrite reductases in hypoxia. Most organisms possess only few Hbs, but the genome of *Chlamydomonas* contains twelve genes that encode so-called Truncated Hemoglobins (THBs). Here, we aim to analyze if these THBs function as physiological NO producers and how the amino acid environment steers their catalytic activity.



Work planned: We will try to answer our research questions on the two levels of protein biochemistry and living cells. From the crystal structure of one of the algal THBs we hypothesize possible structure-function relationships that might regulate the enzyme's activity. Protein variants created by site-directed mutagenesis will be characterized regarding their catalytic performance. One focus will be on NO generating reactions under envisioned physiological conditions. On the cellular level, interacting proteins will be identified by pull-down approaches and the NO production capability of *C. reinhardtii thb* mutants, created by the CRISPR/Cas technology, will be tested. The results obtained here will contribute to unraveling principles that govern the manifold substrate conversion activities of Hbs.

Selected References

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