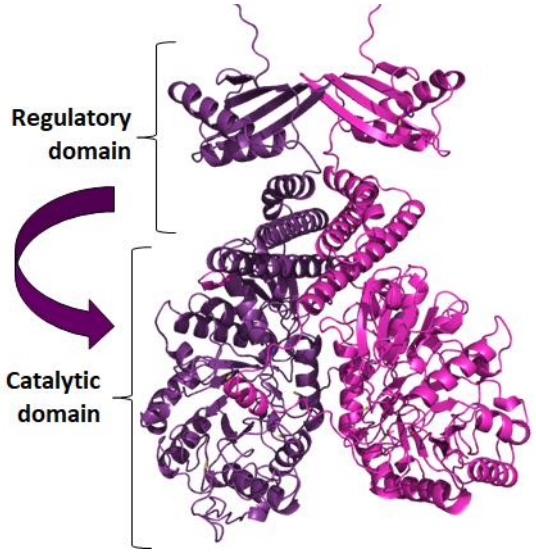
Project Eckhard Hofmann

Structural role of non-catalytic protein domains involved in the cross-talk of metabolic pathways

Background and preliminary work: In several projects within MiCon, folded domains were identified in target proteins that do not seem to be directly involved in catalysis. Nevertheless, these domains are strictly conserved and were shown to control the catalytic efficiencies of enzymatic reactions of the target proteins, either through intra- or inter-protein interactions. Notably, the effectors that result in different enzymatic activities suggest that the target proteins and their modulating domains, respectively, act at important points of metabolic routes that allow the integration of signals from other pathways. In this project we will focus on the structural and, in particular, structurally dynamic aspects of such interactions for two model systems, namely the lipopolysaccharide (LPS) biosynthesis pathway in bacteria and the starch metabolism in green algae.



AlphaFold2 model of the dimeric form of one of the target proteins. Biochemical analyses have shown that its activity is influenced by the regulatory domain, but real structures and their dynamics upon effector binding are required for a mechanistic understanding.

Work planned: For both target proteins, domain movement and/or protein-protein interaction will be in the focus, so that we will apply CryoEM as the method of choice. Starting from already established protein purification protocols, we will establish the single particle analysis of the proteins and protein complexes. Grid preparation and data collection will be done in cooperation with CryoEM facilities off-campus, while data processing and structure analysis will be carried out at RUB. Once the structural pipeline is established, the interaction of the target proteins with ligands and potential partners will be investigated both by structural and biophysical means. The aim of these studies is to gain mechanistic insight into how the non-catalytic domains dictate the enzymatic activities of the target proteins in response to external or internal stimuli.

The work will be a very close cooperation with the Hemschemeier and Narberhaus groups within MiCon.

In addition, the candidate will be involved in collaborative structural investigations with other groups within the MiCon consortium. These direct interactions of the PhD candidates were an essential asset for the high number of joint publications in the previous funding period.

Selected references:

Duan, J., Veliju, A., Lampret, O., Liu, L., Yadav, S., Apfel, U.-P., Armstrong, F.A., Hemschemeier, A., Hofmann, E., 2023. Insights into the Molecular Mechanism of Formaldehyde Inhibition of [FeFe]-Hydrogenases. J. Am. Chem. Soc. 145, 26068–26074. https://doi.org/10.1021/jacs.3c07800

Möller, A.-M., Brückner, S., Tilg, L.-J., Kutscher, B., Nowaczyk, M.M., Narberhaus, F., 2023. LapB (YciM) orchestrates protein-protein interactions at the interface of lipopolysaccharide and phospholipid biosynthesis. Mol Microbiol 119, 29–43. https://doi.org/10.1111/mmi.15005

Düner, M., Lambertz, J., Mügge, C., Hemschemeier, A., 2018. The soluble guanylate cyclase CYG12 is required for the acclimation to hypoxia and trophic regimes *in Chlamydomonas reinhardtii*. Plant J 93: 311-337 https://doi.org/10.1111/tpj.13779