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Ancestral IMPDH & E. coli IMPDH

Prokaryotic IMPDH proteins are arranged in tetramers and octamers. Some of them can switch the equilibrium between the two oligomerization states in presence of the natural substrates. We wanted to test if other ligands can affect the oligomeric equilibrium of the IMPDH protein. We measured the IMPDH in APO state and with two ligands: Ap4A and ATP via SAXS. The measured ancestral IMPDH SAXS data showed no difference on the oligomeric mixture state when incubated with Ap4A or ATP, but the sample quality needs to be improved for a better data evaluation. The E. coli version shows a stable octamer in apo form and only small changes when incubated with Ap4A and ATP.

Bre5

in Saccharomyces cerevisiae, Bre5 together with ubiquitin protease Ubp3 forms an active deubiquitination complex that cleaves ubiquitin from specific substrates. Bre5 is a cofactor and an essential positive regulator for Ubp3-mediated biological function. We used size exclusion chromatography-coupled SAXS (SECSAXS) to separate aggregates from the sample. We calculated ab-intio envelopes of the Bre5 monomer. Until now no structure of full length Bre5 is available and AlphaFold could only predict two domains, so we used EOM in this approach to “model” the amino acids which are random, to visualize the protentional position in the structure. The EOM results show that the Bre5 protein is compact and not really flexible. Further experiments are planned with protentional binding partners.

pKM400

CPn0677 is a type III secreted protein, secreted early during invasion of the pathogen into the host cell. The protein binds and deforms the plasma membrane of the host and recruits several host proteins to the bacterial entry site. Among them BAR domain containing proteins, which are bound by CPn0677 and further deform the invaginating membrane. In addition, CPn0677 recruits and activates the actin modulator N-WASP. Together with N-WASP CPn0677 promotes local actin polymerization at the bacterial entry site. Initial SAXS experiments revealed that the protein form concentration dependent oligomers. The ab initio model showed a core domain with a long flexible part. This flexible part is the region for a protentional binding partner and further experiments are planned to test this interaction.

CC2D1B

CC2D1B belongs to highly conserved CC2D1 protein family. In mammals, there are two orthologs called CC2D1A and CC2D1B. Several functions such as involvement in innate immunity response and centrosomal cleavage processes, but also regulation of signaling pathways have been shown for CC2D1A. Both CC2D1A and CC2D1B are able to interact with CHMP4B, a core component of the ESCRT-III complex. The biochemical background of this interaction is not fully understood. All structural analyses are based on the fragments of this proteins. Initial SAXS experiments shows that the protein

forms a high oligomer, which need further improvements e.g. SEC SAXS to separate the species from each other.

murine guanylate binding protein (mGBP9)

Members of the murine guanylate binding protein (mGBP) family localize around the parasitophorous vacuole (PV) leading to its disruption, but the underlying molecular mechanisms are poorly understood. Getting to know its biochemical features and its three dimensional structure, ideally from X-ray data combined with SAXS, will help to understand how the protein interacts with other molecules, how it undergoes conformational changes and how it enables GTP hydrolysis. All in all these data will help to get more insights into mGBP functions in host resistance against the protozoan parasite Toxoplasma gondii.
Initial SAXS experiment so far showed differences in the oligomeric state of the mGBP7 (dimer) and mGBP9 (monomer) proteins. mGBP9 is a long-stretched protein in solution, visualized in the SAXS envelope. Further analysis was done with the substrate GTP to see conformational changes on the overall structure upon binding. We could see a shift to dimer species upon GTP binding and visualized a dimerization of the GTPase domains.

VanW

VanW is part of the Vancomycin resistance cluster, but with unknown function. We performed SAXS to determine the overall shape and the oligomeric state of the protein. VanW is a long-stretched monomer in solution. No crystal structure is available so for and only parts of the protein could be modelled. Saturation experiments with potential substrates showed no different in the overall shape so far.