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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. The binding partners pKM378 (CRIB) shows a stable monomer in solution and the pKM417 (SH3) a mixture of monomer/dimer/trimer. The interaction of theses partners with pKM400 needs to be improved for further SEC-SAXS experiments.

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. With SEC SAXS we tried to separate the species from each other, but unfortunately the partials are so big that we reach the technical limitations of the system.

HlyD

One of the most prominent members of T1SSs is the hemolysin A (HlyA) secretion system, which occurs in some uropathogenic E. coli stains. HlyA is a protein toxin which can form pores in the membrane of host cells, which will lead to the death of the cells. In the HlyA secretion system the ABC transporter is hemolysin B (HlyB), the MFP is hemolysin D (HlyD) and the OMP is TolC. Despite extensive research, still little is known about the exact stoichiometry and mechanism of the HlyA secretion system. Especially, little is known about the crucial MFP HlyD, which is involved in substrate recognition and in recruitment and opening of TolC. By reconstituting a dimeric subcomplex of the full-length integral transmembrane protein HlyD into lipid-nanodiscs, the structure of the protein complex can be probed via SAXS in a native-like environment. We did initial SAXS experiments to determine the structure and the stoichiometry of the reconstituted HlyD in nanodiscs, as well as empty nanodiscs. The evaluation of the data is challenging and needs more time.

ETR1 (Ethylene receptor 1 ARATH)

Ethylene receptor 1 (ARATH) has been expressed in E.Coli, purified and reconstituted in lipid nanodiscs. The goal of the SAXS experiment is the validation that ETR1 has been successfully

reconstituted inside the discs and to gain a first impression of the structural shape of the assembled complex. We did initial SEC-SAXS runs with empty nanodiscs and the reconstituted ETR1 receptor, but signal strength needs to be improved for better signal to noise ratios. Data evaluations is still ongoing.