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FRET based Sucrose Sensor (Flip & Matryoshka)

Metabolite networks within microbial communities are essential for metabolic processes, signaling and cell communication. In aim to track metabolites genetically encoded biosensors represent a generous tool. Here the characterization of a ratiometric sucrose biosensor is aimed. Additional structural information is required to obtain an comprehensive overview and complete characterization of these biosensors. Initial SEC SAXS experiments showed that both sensors types (Flip & Matryoshka) are a monomer in solution. Predicated AlphaFold models did not described the in solution structure and were modified until they fit to the experimental data. We could observe changes within the scattering pattern when sucrose was bound to the sensor.

HlyA

Hemolysin A (HlyA) is a Repeats In Toxins (RTX) toxin secreted by uropathogenic E. coli and strongly influences the course of urinary tract infections. HlyA lysis several cell types like erythrocytes, epithelial cells and leukocytes but the exact virulence mechanism is still not understood. Initial SAXS data with the shorter constructs of HlyA showed a different folding state, depending on the chosen buffer, but further optimizations are needed.

The Src-homology 3 domain of phosphatidyl-inositol-3-kinase (SH3)

Structural information on amyloid fibrils is crucial for the understanding of their formation mechanisms and for the rational design of amyloid inhibitors in the context of protein misfolding diseases. The Src-homology 3 domain of phosphatidyl-inositol-3-kinase (PI3K-SH3) is a model amyloid system that plays a pivotal role in our basic understanding of protein misfolding and aggregation. SH3 is a stable protein in neutral pH and unfolds under acid pH (~2.5). After heating up the fibrillation starts. SAXS experiments showed that SH3 is a stable monomer at neutral pH and starts unfolding under acetic pH. This unfolding was initiated with different buffer compositions. Initial SAXS data showed a strong effect depending on the buffer strength on the unfolding speed. Further investigations of these process are needed.

Hemolysin Type I Secretion System

Type I secretion systems (T1SSs) in gram-negative bacteria can transport various substances, for instance toxins, in one step from the cytosol to the extracellular space and are crucial for the survival and pathogenicity of different bacteria. T1SSs are tripartite systems, which are composed of an ABC transporter and a membrane fusion protein (MFP) in the inner membrane and of an outer membrane tunnel-protein (OMP). One of the most prominent

members of T1SSs is the hemolysin A (HlyA) secretion system, which occurs in some uropathogenic E. coli stains. Initial SAXS experiments showed an elongated particle with an approximated molecular weight of around 1.2 MDa. This would fit the theoretical model of the stalled T1SS system, but further evaluation is needed.

SecA

The Sec-pathway consists of the most prominent SecYEG heterotrimeric protein translocating channel, the ATPase SecA and the Foldase SecB. SecA is important because it contacts the translocon and supports the necessary powerstrokes required for protein translocation through SecYEG by ATP hydrolysis. With SEC SAXS and batch measurements we were able to determine the envelope of the SecA Dimer model. Further experiments with lower concentrations are needed to deconvolute the monomer of SecA.