Structure and dynamics of chaperone-preprotein recognition in the secretory pathway.

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Over one third of a cell's proteome is non cytoplasmic. As a consequence, protein secretion constitutes a major biosynthetic process. Here we aim to decipher targeting of Sec-dependent substrates using a combination of complementary tools. This targeting event has been proposed to be achieved and/or facilitated by molecular chaperones, such as Trigger Factor, SecB as well as the SecA ATPase, which is the final receptor of the substrates. SecA shuttles between a cytoplasmic and a membrane-bound state. Up to now we sought to characterize interactions of a model substrate with all these chaperones.

The main substrate used for in vitro studies so far was proOmpA, that aggregates in aqueous solutions. Development of a new substrate (proPhoA) and a methodology to keep it water-soluble allowed us to apply various biophysical tools.

We first characterized the translocation-incompetent and competent states of proPhoA. We used Size Exclusion Chromatography coupled online to Multi Angle Light Scattering and Quasi Elastic Light scattering (SEC-MALLS-QELS) and by Gas phase Electrophoretic Mobility Molecule Analysis (GEMMA). Hydrogen Deuterium exchange experiments coupled with Mass Spectrometry allowed us to map, at a residue level resolution, solvent accessible areas of the two states of proPhoA.

The interaction of proPhoA with the the first potential interactor residing at the ribosomes, Trigger Factor, was also characterized using Isothermal Titration Calorimetry (ITC) and SEC-MALLS.

Afterwards, we characterized the interaction of proPhoA with the chaperone of the Sec system, SecB , using SEC-MALS and ITC. Using HDX, crosslinking MS and native MS we are trying to obtain information about the interacting surfaces.

The final destination of a preprotein before its secretion is SecA. It has been proposed that SecA oligomerization may affect recognition and/or secretion events. Using advanced bioinformatics tools to analyze existing crystallographic data, X-Ray crystallography and SEC-MALS we identified a SecA dimerization interface .This permitted us to examine monomeric and dimeric derivatives and characterize their interaction with SecB, proPhoA and SecB-proPhoA (SEC-MALLS, HDX).

Here we show that specific interactions of a preprotein with molecular chaperones maintain it in a translocation-competent state and guide it to its final membrane-bound receptor, SecA. For these interactions the oligomeric state of the chaperones and the non-native state of the preprotein is crucial for the recognition. Finally, the preprotein is delivered to an asymmetric SecA, where one SecA protomer is necessary and sufficient for recognition but not for secretion.