Outline of the thesis

This thesis describes a study on the structural changes and flexibility of the SecYEG channel during protein translocation and insertion.

Chapter 1 provides an introduction and describes the components of the major protein secretion pathway in *Escherichia coli*. The focus lies on the three main protein (complexes) that fulfill key roles in the post-translational pathway of protein export, i.e., SecA, the motor protein which drives translocation using ATP as energy source; SecYEG, the membrane complex functioning as the translocation channel, and SecDF, an accessory membrane complex that assists in protein translocation and that utilizes the proton motive force as energy source. The review summarizes current insights and research and integrates this knowledge into a proposed mechanism of action.

Chapter 2 describes a study on the structural plasticity of the SecYEG translocation channel using a combination of a biochemical analysis and molecular dynamics modeling. The plug domain of the SecY subunit that localizes at the channel exit site was immobilized inside the channel with different sized chemical crosslinkers. Subsequently the translocation activity was measured to analyze the effect of the introduced crosslinkers on protein translocation.

In **Chapter 3**, the plug domain was labeled with an environment sensitive fluorophore to examine its conformational dynamics using a spectroscopic approach. A protein translocation intermediate was introduced to analyze its effect on the solvation state of the plug domain. Similarly, thez insertion of a membrane protein segment provided insight in the structural rearrangements in the plug domain region during the initial stages of membrane protein insertion.

Chapter 4 investigates the flexibility of interaction between the translocation channel SecY and the supporting subunit SecE. Interaction points were immobilized by means of cysteine-based crosslinking after which the translocation activity was measured. Subsequently, the SecY and SecE subunits were immobilized at multiple interaction points that were simultaneously crosslinked and its effect on protein translocation was analyzed. Furthermore, the SecE subunit was cleaved at

different sites at the hinge region to analyze the minimal requirements for the translocation function.

Chapter 5 provides a summary of the work presented and gives a short outlook on future research.