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Summary

Imaging membrane-protein diffusion in living bacteria

In living cells, essential processes such as transcription, translation, intracellular transport and protein secretion are driven by proteins that interact transiently in a highly dynamic manner. Single-molecule fluorescence microscopy has been used successfully to study the mobility of proteins in eukaryotic and prokaryotic cells. With this technique, it is possible to probe the behaviour of single proteins in millisecond time resolution and nanometer spatial resolution.

Using single-molecule fluorescence microscopy, I have investigated the lateral mobility of 10 different *E. coli* inner membrane proteins (WALP-KcsA, YedZ, YidC, CstA, GlpT, MscL, MscS, MreB, TatA and YqiK) to understand how heterogeneity and macromolecular crowding influence the mobility and function of proteins in the bacterial cytoplasmic membrane. To achieve this, I, first of all, fluorescently labeled all ten proteins with fluorescent proteins and expressed them from plasmids in *E. coli* bacteria. Then, using wide-field fluorescence microscopy, I visualized individual fluorescent protein molecules within bacterial cells. Finally, I tracked their mobility using single-particle tracking software.

In Chapter 3 of this thesis, I have shown the influence of MreB cytoskeletal network on the mobility of trans-membrane proteins in *E. coli* bacteria. In

this chapter, I have highlighted the existence of membrane micro-domains in the inner membrane of *E. coli*. These domains appear to be stabilized by the cytoskeletal network formed by MreB just below the inner membrane. In addition to stabilizing micro-domains, the MreB cytoskeletal network also confines the mobility of trans-membrane proteins.

In Chapter 4 of this thesis, I have studied the mobility of the TatA component of the twin-arginine protein transport system in *E. coli*. This chapter highlights the heterogeneity in diffusion properties of the TatA complex, which is caused by complexes that move faster and others that move slower. Based on this observation I proposed a new model, the so called "substrate-induced flip-flop model" for the dynamics of the Tat system. In this model, the Tat translocation cycle begins with TatA oligomers and monomers attached to the membrane and TatB in complex with TatC. Then, substrate protein binds to this TatBC complex, followed by the PMF-dependent recruitment of TatA oligomers. Subsequently, the TatBC-bound TatA oligomers flip into the membrane, forming a trans-membrane substrate-TatABC complex that diffuses relatively slowly. Now, a functional pore is formed that allows the substrate to pass from the cytoplasmic to the periplasmic side of the membrane. Finally, after translocation, the C-terminus of the substrate is cleaved off and the TatABC complex disassembles, resetting the translocation cycle.

In Chapter 5, I have studied the mobility of membrane-organizing proteins YqiK and MreB in *E. coli* bacteria. In this chapter, I have shown that YqiK harbours flotillin-like properties and might have a similar function as eukaryotic flotillins. Visualizing fluorescently labeled MreB and YqiK revealed that regions of increased fluidity (RIFs) and regions of high order

(rafts) are intrinsic structural features of the *E. coli* cytoplasmic membrane that are spatially separated from each other.

In conclusion, my findings show that the *E. coli* cytoplasmic membrane is heterogeneous and is organized by the MreB cytoskeletal network. By studying the diffusion properties of various *E. coli* cytoplasmic membrane proteins, I find membrane heterogeneity and macromolecular crowding as two key parameters that govern trans-membrane protein mobility and function.