Summary

Intracellular transport studied with dynamic single-molecule super-resolution microscopy

What characterizes cells, the basic unit of life, is their inimitable ability to organize their interior into dynamically maintained microscopic to nanoscopic patterns. On one hand, intracellular transport is the basis for the spatiotemporal control of the cell's internal organization, on the other hand, it takes place in the highly compartmentalized and heterogeneous intracellular environment it assists to create.

In this work, we studied the two main transport mechanism cells utilize - passive diffusion of trans-membrane proteins and lipids in the inner membrane of *Escherichia coli* (*E. coli*) and active motor-driven transport in the chemosensory cilia of *Caenorhabditis elegans* (*C. elegans*). In both cases, we used live cell single-molecule super-resolution microscopy which was essential to probe the dynamics of individual biomolecules with respect to the diverse physical environment they occur in.

Although membrane protein diffusion essentially taking place in the two dimensions of a thin sheet - the cell membrane -, the small size of bacterial cells entails a highly curved membrane that practically renders it into a three-dimensional problem. Chapter 2 describes a newly developed framework for three-dimensional diffusion analysis to obtain unbiased diffusion data, which allows for reliable detection of anomalous diffusion modes. This enabled us to study the spatio-temporal organization of the inner membrane of *E.coli* by probing the lipid and transmembrane protein diffusion in a comprehensive fashion summarized in Chapter 3. Our results show that *E.coli*, similar to eukaryotic cells, employs its cortical cytoskeleton to control transmembrane protein and lipid diffusion, inducing physically distinct lipid nano-domains that resemble the so-called lipid rafts observed in eukaryotic membranes.

What sets intracellular transport in eukaryotic cells apart from that of prokaryotic cells is the employment of molecular motors in active transport along cytoskeletal filaments. In the second half of this thesis, motor-driven intra-flagellar transport (IFT) occurring in chemosensory cilia in *C. elegans* is studied. This bidirectional transport system driven by two different types of kinesin motors in the anterograde direction and by dynein in the retrograde direction builds and maintains the cilium's core structure the axoneme. In chapter 4, a functional differentiation of the two types of anterograde kinesin motors is elucidated that optimizes cargo transport. Also here, employing dynamic single-molecule super-resolution yielded key insights that enabled understanding the basic principles of how IFT organizes this intriguing motor cooperation. In chapter 5, single-molecule traces of IFT components could be used for highlighting the underlying microtubule tracks, allowing for direct visualization of the complex ciliary ultra-structure. This enabled to directly correlate the intriguing IFT single-molecule dynamics to the ultra-structurally distinct ciliary subdomains. Subdomain-specific IFT dynamics revealed a strong control of IFT by means physical interaction with its environment.