Summary

Homeostasis is the ability of an organism to actively regulate intracellular conditions and to keep metabolite concentrations, pH, ionic strength, macromolecular crowding and other parameters within certain limits. In their natural habitat, cells can experience a changeful environment, e.g. variations in nutrient availability, temperature, pH or osmolarity, all of which pose serious stress onto the cell. Organisms have evolved mechanisms to adapt to such variations in order to ensure steady growth over a wide range of conditions. In this thesis, we tackle some questions related to cellular homeostasis using Escherichia coli as a model organism. A range of fluorescence-based techniques was used, especially photo-activated localization microscopy (PALM) and single-molecule tracking (SMT), which allows analyzing the E. coli ultrastructure and dynamics at high resolution.

In the first chapter, we discuss the phenomenon of macromolecular crowding, which reflects the volume occupied by large molecules, or in other words, the ‘fullness’ of the cell. We review how macromolecular crowding affects biochemical processes and how it may promote the structuring of the cytoplasm. Because of the diverse effects of crowding on cell functioning, we propose that cells have mechanisms to maintain crowding within a narrow limit. Cells can likely tune such mechanisms to optimize biochemical reactions, for instance, reactants are closer together at high crowding but their diffusion will be slowed down making it more difficult for molecules to meet each other and react. The underlying mechanisms of ‘homeocrowding’ are poorly understood, but we suggest possibilities of how cells could regulate macromolecular crowding.

For microscopy studies on live cells, fluorescent proteins are the key to visualizing subcellular structures. Every fluorescent protein has a specific maturation time, which is defined as the time required for the post-translational steps to form the chromophore. Long maturation times can be a hurdle when the kinetics of cellular processes needs to be determined. In Chapter 2, we present strategies to determine the maturation of fluorescent proteins in vivo. We find that the in vivo maturation time can be significantly longer than inferred from in vitro measurements.

In Chapter 3, we study the abundance, location and dynamics of mechanosensitive (MS) channels. These membrane proteins are important to survive hypoosmotic stress conditions, as they open in response to increased membrane tension and release solutes to lower the internal osmotic pressure. Without MS channels, cells would lyse if the medium osmolarity is rapidly
decreased. We also established a protocol based on super-resolution microscopy to count individual channel proteins and determine how many are needed to confer protection to an osmotic downshock. Additionally, we found that fluorescent proteins can cause artificial aggregation of the mechanosensitive channel of large conductance (MscL). We highlight that caution is urged when working with fluorescent proteins, as most have a (weak) tendency to dimerize. This behavior is augmented if the freedom of motion is restricted, as in the case of the fluorescent-tagged oligomeric channel MscL.

In Chapter 4, we investigate how the cellular volume of *E. coli* is affected in different growth conditions, using dual-color PALM. We distinguish two compartments, the cytoplasm and the periplasm, and discovered that the fractional volume of the periplasm is increased in slower growing cells relative to fast growing cells. The growth rate-dependent changes in periplasmic and cytoplasmic volume seem to parallel the amount of proteins residing in each compartment and, consequently, the actual protein concentrations are nearly constant. Since protein concentration is a major component of macromolecular crowding, the ‘fullness’ of the compartments appears to remain steady.

We wanted to experimentally test whether or not macromolecular crowding is constant in the cytoplasm and periplasm, independent of growth conditions, by directing a genetically encoded crowding sensor to the periplasm. This approach is described in Chapter 5; however, the export of the sensor to the periplasm was unsuccessful, which we attribute to the complex domain structure of the sensor. The lateral diffusion of proteins is related to the crowding and viscosity of a compartment, and this property was subsequently used to characterize the cytoplasm and periplasm. By taking advantage of published data and our diffusion measurements, we could show that the lateral diffusion of fluorescent proteins in the cytoplasm and periplasm are very similar and comparable over a range of growth conditions, which supports the proposal of homeocrowding, as discussed in Chapter 1.

In conclusion, in this thesis I present quantitative data on the volume and crowding of the *E. coli* cytoplasm and periplasm, protein mobility in these compartments, and the number of channel proteins required to control the cell volume under osmotic stress conditions. The research questions covered in this work are related by one common trait: how do cells maintain steady state conditions in a changing environment and how do cells react to stress. The work contributes to a better understanding of the complex functioning of a cell, with focus on cellular homeostasis.