Summary

Bacteria are amongst the smallest known living organisms and yet they carry out an incredible variety of different functions and are able to respond to a large variety of different external stimuli. Growing evidence is suggesting that specific proteins and sub-cellular components (e.g. the nucleoid) need to be highly organized both on the membrane and inside the cellular volume in order to carry out vital tasks like cell replication or chemotaxis and to respond to external changes in the environment. In this thesis I investigated experimentally the spatial organization of different sub-cellular components (nucleoid) and protein species (NAPs and chemoreceptors) and its importance for physiological processes inside living bacterial cells. Most of the experiments presented here are carried out in living cells, and our results thus provide insights also into the spatio-temporal organization and the dynamics of the response to controlled perturbations.

The work introduced in Chapter 4 presents novel results on the dynamic organization of membrane chemoreceptor clusters in response ligand stimulation. Opposing results are found in the literature about cluster stability upon stimulation: experiments employing fluorescent fusion and cryo-EM reported no change in the global and local arrangement of chemoreceptors upon sensory stimulation while independent results exploiting immunofluorescent labeling showed a diffuse staining of the plasma membrane upon ligand stimulation, interpreted to mean chemoreceptor clusters are destabilized by attractant binding. In this section we utilized two orthogonal approaches (PALM imaging and particle tracking) to show that the stability of chemoreceptor clusters can actually be altered by cognate ligand stimulation (e.g. Tar clusters are destabilized by methyl-aspartate stimulation and Tsr clusters by serine) without changing the local arrangement of the receptor lattice. I discuss the implications of such destabilization in relation to signal amplification, chemotactic adaptation, membrane diffusion and crowding: we found that both the cluster size and the mobility of chemoreceptor clusters are modulated shortly after and upon prolonged ligand stimulation in an adaptation dependent fashion.

In Chapter 5 I focused on nucleoid morphology and size in relation to intracellular crowding, investigating the dynamic response of the nucleoid under intracellular crowding

perturbations. Crowding modulations have been shown to modulate in turn the size of isolated nucleoids and to compress nucleoid inside cells imaged after fixation but the importance of crowding in the dynamic spatio-temporal organization of the nucleoid remain elusive. I present a set of pioneering experiments where thousands of fluorescently labeled nucleoids were imaged in living *E. coli* under osmotic perturbations of different magnitudes (which have been shown to perturb intracellular crowding), showing that nucleoid size anticorrelates with crowding levels measured by a FRET crowding sensor expressed in living cells. I discuss the importance of intracellular crowding in nucleoid spatio-temporal organization suggesting that nucleoid size and morphology is determined by the concerted action of passive (e.g. crowding) and active processes.

To produce the data discussed in chapter 4, I developed a novel tracking technique that we named "Localized-Photo-Activation Single-Particle Tracking" (LPA-SPT). LPA-SPT makes use of photoswitchable fluorophores and a focused activation beam to allow for tracking of chemoreceptor clusters in the crowded membrane environment. This technique together with the extended PALM setup and protocol for chemoreceptor imaging at high spatial resolution, are described in Chapter 2. The data presented in Chapter 5 was acquired via an adapted "mother machine" setup, allowing 4-channel imaging and rapid medium switching. Our mother machine setup is described in Chapter 3, together with a novel DNA label (H-NS-dbd) based on the nucleoid-associated protein H-NS, that can be expressed inside living cell. Chapter 3 also presents a set of pioneering nucleoid imaging experiments utilizing a non-perturbative Fluorescent-Repressor-Operator-System (FROS) and PALM (both 3D and 2D). Appendix A is the result of a collaboration with Eric Garnett's group at AMOLF where I designed and executed the experimental part (extended PALM protocol by means of caged fluorophores) together with E. Johlin.

Taken together, the results presented in this thesis resolve a long-standing controversy in the chemotaxis field about stability of clusters of chemoreceptors: ligand binding can destabilize clusters not at the level of the receptor lattice structure but more at the cluster size level. Also, this work gives new insights into the role of intracellular crowding inside living cells, suggesting that crowding is an important but not the sole determinant in shaping nucleoid morphology, size and dynamics. We thus provide more experimental evidences for the importance of spatio-temporal organization of proteins and sub-cellular components inside living bacteria. In addition, a set of novel techniques and extensions/additions to state of the art microscopy techniques are presented. This imaging toolkit will be used in the future to investigate further the dynamics of receptor clusters under ligand stimulation as well as the relationship between crowding, nucleoid morphology, growth conditions and gene expression patterns.