Summary

Mechanisms of DNA Organization Unraveled with Novel Single-Molecule Methods

Outline of this thesis

This thesis describes the scientific work I did during my PhD-period at the Vrije Universiteit on the subjects described in the first half of this chapter using techniques described in the second half of this chapter. Chapters 2, 3, 4, and 5 are published in scientific journals and chapters 6 and 7 are manuscripts in preparation.

Chapter two is a description of the apparatus I have designed and built to do the experiments described in the other chapters. The apparatus allows for the simultaneous but independent manipulation of multiple DNA molecules. In this chapter we demonstrate the full control over the orientation it provides in the manipulation of two DNA molecules. As if it were micro-ropes, we used one of the DNA molecules to make a tight loop around another. Next, we pulled this loop carefully along this DNA molecule. Any obstacle this loop finds on its way along the DNA causes the required pulling force to increase briefly. In this way we can detect at which locations specific proteins bind to DNA. Moreover, when the loop is pulled tighter we can even wipe bound proteins off.

Chapter three reviews the proteins that play an important role in the organization of the prokaryotic genome. Moreover, it introduces a new classification of the major nucleoid associated proteins according to their structural effect on DNA (e.g. bridging or bending), which we believe is key to understanding the interplay between these proteins.
Chapter four utilizes the setup described in chapter two to study a key player in prokaryotic DNA organization: the protein H-NS. The instrumental advance described in chapter two opens up the possibility of investigating proteins that interact with multiple DNA duplexes. By applying this new experimental approach we describe numerous important aspects of H-NS-DNA interactions such as the energy landscape, interaction kinetics, the nature of the cooperative binding and organization of H-NS bridged regions. With these results we can for the first time appreciate the interplay between DNA compaction in vivo and DNA tracking enzymes such as RNA polymerase. Moreover, our findings reveal how bacterial chromatin can be effectively organized and compacted, but at the same time remain dynamic in nature.

In chapter five we combine a new analysis of the results obtained by another group, William Navarre and co-workers, and the results obtained in chapter four. By combining the data from these studies we demonstrate that the nucleoid associated protein H-NS is responsible for looped domain formation. With this approach we can show for the first time that the large majority of loops in vivo are protein-induced. The fact that H-NS binds to defined sites implies that the bacterial genome is much more ordered than generally believed and that there is limited cell-to-cell variation in basal genome organization. The observations in this chapter also have direct implications for how chromosomes are temporarily remodeled and how such remodeling is exploited for global gene regulation.

In chapter six we present the results obtained with experiments on Alba 1, an archaeal DNA architectural protein. Alba has been suggested to play an important role in DNA organization and regulation in Archaea. However, very little is known about the structural role of Alba in DNA-organization. Based on previous work it was suggested that Alba might be capable of dsDNA bridging as part of its organizational role. Using optical tweezers we show that under saturating conditions nucleoprotein filaments form when Alba interacts with DNA, stiffening the DNA up to three-fold with respect to bare DNA. Upon lowering the concentration, the persistence length quickly reverses to that of bare DNA in an apparently cooperative process. In addition, dual DNA experiments using quadruple optical tweezers, SFM experiments and tethered-particle-motion experiments provide the first experimental evidence in the DNA cross-linking ability of this protein.

Chapter seven describes the results of an experiment in which for the first time the formation of DNA condensates is directly imaged with high resolution, integrating optical manipulation techniques with fluorescence. Using this combination, we describe the formation and unraveling of DNA condensates with very high temporal, spatial and force resolution. These data provide important new insights in the understanding of the formation of DNA condensates. In this manuscript we demonstrate these insights and put forward a new mechanism for the formation of DNA condensates.