Cleaving, Condensing and Manipulating Single DNA Molecules

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1.5 Outline of this thesis

This thesis consists of a collection of published and (to be) submitted research articles. Chapters 2 to 5 have a common theme: the interactions between restriction enzymes and DNA. In these chapters, various aspects of restriction enzymes are examined using the single-molecule techniques described above.

Chapter 2 describes for the first time the effect of DNA tension on type II restriction enzymes, one of the best-known model systems for DNA recognition. Although these enzymes are widely used and studied, the relation between mechanical and chemical interactions in DNA recognition and cleavage are largely unknown. By using optical tweezers to catch and stretch single DNA molecules, reaction rates of EcoRV and BamHI as a function of DNA tension could be determined. The presented results elucidate mechanochemical aspects of the reaction pathway and link existing biochemical and structural knowledge of type II restriction enzymes.

In the experiments, we found that the binding of these enzymes to the recognition site on the stretched DNA molecules was slowed down significantly. These observations led us to investigate the influence of DNA conformation on the association rate of EcoRV. The results are presented in **Chapter 3**. We show that target finding in a coiled DNA molecule is accelerated by jumping of the protein between non-specific DNA segments, while in a stretched DNA configuration this 3D searching pathway is switched off. Although the effect is not very large, we anticipate that *in vivo*, where DNA densities are much higher, this pathway plays an important role in the rapid localization of target sequences.

In **Chapter 4**, we report a new technique, based on four independent optical traps, that allows the simultaneous manipulation of two DNA molecules with unprecedented control. We elaborate on the developed setup and flow system. The capabilities of the instrument are demonstrated in a novel experiment, in which we use one DNA molecule as scanning probe to detect restriction enzymes specifically bound to a second DNA molecule. These experiments also provide a unique method of investigating the strength of 'roadblocks' that can be encountered by proteins moving along DNA tracks. The developed instrument can be used to assess many key problems concerning interactions between multiple distant DNA segments, e.g. DNA recombination, gene regulation and DNA organization.

In **Chapter 5** I present results of tethered particle motion experiments performed at the European Laboratory for Non-linear Spectroscopy (LENS) in Florence, Italy. The chapter describes real-time measurements of DNA looping by two members of the Type IIE restriction enzyme family. Unexpected large differences in site recognition, looping specificity and binding energy are revealed. Among the results is a remarkable compaction of DNA, driven by non-specific looping by NarI. We also show that loop formation by NaeI and NarI is rate-limited by the association of a protein-

DNA complex to the second site. This result might be generic for proteins capable of bridging two recognition sites. Similar real-time measurements had so far only been carried out on specific gene regulators such as Lac repressor and Gal repressor. Therefore, the here presented data provides an opportunity to compare mechanisms of DNA looping by proteins that are very different in structure and function.

In the final part of this thesis, **Chapter 6**, we employ our setup to study the phenomenon of DNA condensation. We utilize a combination of optical tweezers and fluorescence imaging techniques to study the dynamics of condensation of individual DNA molecules by the polyamine *spermine*. We visualize the formation and disruption of single DNA condensates in stretched DNA molecules. By using high-resolution force spectroscopy we provide evidence for a torus-shaped condensate, yet that the size of these toroids under tension is much smaller than previously assumed in single-molecule assays [14, 135]. Furthermore, we show that the condensation process is rate-limited by the nucleation of the first DNA loop and that a minimum of two loops is required for a stable toroid.