

# Studying Topologically Complex DNA at the Single-Molecule Level

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Over two decades ago, with advances in microfabrication techniques and fluorescence microscopy, single-molecule studies emerged as a powerful approach to investigate polymer dynamics at the molecular level. By providing a platform for the direct observation and precise manipulation of individual polymer molecules, single-molecule studies allow for the probing of microscopic interactions that give rise to the macroscopic properties of the polymer system. Single molecule studies have been widely used to investigate the static and dynamic properties of double-stranded deoxyribonucleic acid (DNA) as a model polymer. Such studies not only help to develop a fundamental understanding of key topics in polymer physics that cannot be easily accessed via traditional bulk experimental methods, but also facilitate the development of emerging DNA mapping and sequencing techniques.

The majority of single-molecule studies to date have involved linear DNA molecules. It is known that topological constraints on the molecular level have a significant influence on polymer dynamics. A nascent area in the field of polymer physics is the study of polymers with complex topologies. In this thesis, we present a series of single-molecule experiments and Brownian dynamics simulations used to investigate the polymer physics of topologically complex DNA. Specifically, we focus on knotted polymers, ring polymers and catenated polymer networks.

To investigate the impact of a knot on polymer dynamics, we employ a combined approach of single-molecule experiments and Brownian dynamics simulations. We study experimentally the steady-state behavior of knotted polymers in planar elongational fields and find that the presence of a knot leads to a faster relaxation time and, accordingly, a shift in the coil-stretch transition for the molecule. In consequence, the untying of a knot near the coil-stretch transition can give rise to dramatic changes in chain conformation. We use Brownian dynamics simulations to study in detail the impact of the knot untying process on polymer dynamics in planar elongational fields and complement the simulations with experimental results. As a knot moves off the chain in an elongational field, the knot size changes due to the non-uniform tension profile along the chain and causes a change in the effective Weissenberg number, which in turn leads to a change in chain extension. With the use of simulations, we further investigate the knot untying process by probing the topological pathway of an untying knot. We study the distributions of knot conformational states and knot untying pathways on uniformly tensioned chains and chains subjected to elongational fields, and demonstrate that external fields can be used to influence how a knot unties from a chain.

Next, we shift focus to ring polymers. We use single-molecule experiments to study the dynamics of self-entangled circular DNA. Our results demonstrate that ring polymers can self-entangle by forming self-threadings, and that such threadings can lead to a significant slowdown in polymer dynamics. It seems counterintuitive that self-entanglements can arise in ring polymers, which lack chain ends. To delve into the physics of self-entanglements on circular chains, we implement a macroscopic system that allows for the direct visualization of chain conformation. We investigate the formation of self-entanglements on granular chains subjected to a tumbling motion, and use the well-studied self-entanglements on linear chains as a framework for interpreting self-entanglements on circular chains. We develop a method to characterize the self-entanglements on circular chains with known topological descriptors from knot theory and propose a general mechanism for the self-entanglement of circular chains.

Finally, we consider the deformation dynamics of catenated DNA networks. A kinetoplast is a complex network of catenated DNA rings that resembles a two-dimensional polymeric system. We perform single-molecule experiments to study the deformation response of kinetoplasts in a planar elongational field. Our results demonstrate that kinetoplasts deform in a stagewise fashion and undergo transient deformation at large strains, as a result of conformational rearrangements from a metastable state. In contrast to linear polymers that display a coil-stretch transition, kinetoplasts do not exhibit an abrupt transition between the non-deformed and deformed states.

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