Studying Self-Entangled DNA at the Single Molecule Level

by

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Submitted to the Department of Chemical Engineering
on August 7, 2015, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemical Engineering

Abstract

Knots seem to be found every time one encounters long, stringy objects. At the macroscopic scale, knots are seen every day in shoelaces, tangled hair, or woven clothing, yet they also present themselves at the microscopic scale in long polymer molecules. Knots can be found often in DNA packaged within the viral capsid, occasionally in proteins, and during the transcription and replication of genomic DNA. Biological knots are similarly thought to change the dynamics of viral ejection, protein digestion, and translocation of biomolecules through nanopores. Despite the prevalence of knots in important biological polymers, to date, the physics of knots is only partially understood.

DNA has become a well-accepted model system for investigating the physics of single polymer molecules due to its tremendous biological significance and useful experimental properties. Recent advances in microscopy and nanofabrication have enabled the real-time manipulation and imaging of single DNA molecules, facilitating fundamental studies concerning the physics of individual polymers. Leveraging these experimental techniques, this thesis aims to explore the changes knots can impart on the static and dynamic properties of single DNA molecules.

We first demonstrate a mechanism for the previously observed phenomenon of the compression and self-knotting of a single DNA molecule in the presence of an electric field. We then use this mechanism to study the process of stretching complex DNA knots in an extensional field. These knots dramatically alter the way DNA stretches in two ways: an initially arrested state and a subsequently slowed stretching phase. Our work consists of the first experimental support of these phenomena, originally predicted by simulation and theory.

We then develop theoretical arguments, shown to agree with simulation results, for the physics that govern the distribution of sizes of knots that stochastically occur on DNA molecules, and more broadly, all semiflexible polymers. We then extend our theory to the case where the entire DNA molecule is confined and elongated within a channel. Here, the complex non-monotonic behavior of the sizes of knots agrees with our modified theory.
We finally present the results of dynamical simulations where knots on polymers interact with flows or forces. We first examine the behavior of a knot along a polymer extended by extensional flow. The flow may cause a knot to be swept off a polymer molecule, and the motion of a knot is consistent with a model. Different families of knots display different rates of motion, and we explain this difference with a simple topological mechanism. We then turn to examine the case of knots jamming on a polymer molecule extended with high tensile forces. A simple energy barrier hopping argument qualitatively explains the observed slowdown in dynamics of knots. We use these results to reexamine the problem of DNA knots jamming during nanopore translocation, and our results establish the potential for using knots to slow and control the rate of translocation by a ratcheting mechanism.

The impact of this thesis is threefold. First, we have demonstrated a novel experimental platform capable of interrogating DNA knots, likely the most efficient of its kind. Second, we have established a theoretical framework for the size and probability of knotting in single molecules capable of directing experiments where these properties need to be controlled. Finally, we have shown how knotted topologies can be manipulated by external flows or forces, which have applications involving preconditioning molecules to unknotted states or the jamming of knotted molecules in nanopores.

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