Abstract

Rapid genome characterization is one of the grand challenges of genome science today. Although the complete sequences of certain representative human genomes have been determined, genomes from a much larger number of individuals are yet to be studied in order to fully understand genome diversity and genetic diseases. While current state-of-the-art sequencing technologies are limited by the large timescale and cost required to analyze a single sample, an alternative strategy termed DNA mapping has recently received considerable attention. Unlike sequencing which produces single-base resolution, DNA mapping resolves coarse-scale (~kbp) information of the sequence, which is much faster and cheaper to obtain, but still sufficient to discern genomic differences among individuals within a given species.

Advances in fluorescence microscopy have allowed the possibility to directly map a single DNA molecule. This concept, though straightforward, faces a major challenge that the entropic tendency of polymeric DNA to adopt a coiled conformation must be overcome so as to optically determine the position of specific sequences of interest on the DNA backbone. The ability to control and manipulate the conformation of single DNA molecules, especially, to stretch them into a linear format in a consistent and uniform manner, is thus crucial to the performance of such mapping devices. The focus of this thesis is to develop a reliable single DNA stretching device that can be used in single molecule DNA mapping, and to experimentally probe the fundamental physics that govern DNA deformation.

In the aspect of device design, the strategy we pursue is the use of an elongational electric field with a stagnation point generated in the center of a cross-slot or T channel to stretch DNA molecules. The good compatibility of electric field with small channel dimensions allows us to use micro- or nano-fabricated channels with height on the order of or smaller than the natural size of DNA to keep the molecule always in focus, a feature desirable for any mapping applications. The presence of the stagnation point allows the possibility to dynamically trap and stretch single DNA molecules. This trapping capability ensures uniform stretching within a sample ensemble, and also allows prolonged imaging time to obtain accurate detection results.

We primarily investigate the effects of channel height on the stretching process, specifically, we seek the possibility of utilizing slit-like nanoconfinement to aid DNA stretching. Although extensive previous studies have demonstrated that geometric confinement of DNA can substantially alter the conformation and dynamics of these molecules at equilibrium, no direct studies of this non-equilibrium stretching process in confinement exist prior to the work presented in this thesis. We find that slit-like confinement indeed facilitates DNA stretching by reducing the deformation rate required to achieve a certain extension. However, due to the fact that the steric interactions between the DNA and the confining walls vanish at large extensions, highly stretched DNA under confinement behaves qualitatively similar to unconfined DNA except with screened hydrodynamic interactions, and a new time scale arises that should be used to describe the large change in extension with applied deformation rate. In a consecutive study, we examine the low-extension stretching
process and observe a strongly modified coil-stretch transition characterized by two distinct critical
deformation rates for DNA in confinement, different from the unconfined case where a single critical
deformation rate exists. With kinetic theory modeling, we demonstrate that the two-stage coil-
stretch transition in confinement is induced by a modified spring force law, which is essentially
related to the extension-dependent steric interactions between DNA and the confining walls.

We also study aspects of the equilibrium conformation and dynamics of DNA in slit-like con-
finement in order to provide insight into regimes where existing studies show inconsistent results.
We use both experiments and simulations to demonstrate that the in-plane radius of gyration and
the 3D radius of gyration of DNA behaves differently in weak confinement. In strong confinement,
we do not identify any evident change in the scalings of equilibrium size, diffusivity, and longest
relaxation time of the DNA with channel height from the de Gennes regime to the Odijk regime.
Although the transition between the de Gennes and Odijk regimes in slit-like confinement still
remains an open question, our finding adds more experimental evidence to the side of a continuous
transition between these two regimes.

The impact of this thesis will be two-fold. We design a DNA stretching device that is readily
applicable to single molecule DNA mapping and establish guidelines for the effective operation of
the device. Our fundamental results regarding both the equilibrium and non-equilibrium dynamics
of DNA molecules in slit-like confinement will serve as a solid basis for both the design of future
devices aiming to exploit confinement to manipulate biopolymers, and more complicated studies of
confined polymer physics.

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