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How the Circular and Linear Conformational Fluctuations of Giant DNA Molecules Change with the Viscosity of the Solvent

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We are studying how structural changes in microscopic regions of DNA affect large structures such as genomic structures. As the first step, we were investigating the variation of DNA structure in solution using BAC DNA that can be observed by a single molecule with a total internal reflection fluorescence microscope (TIRF). We found that the long DNA strands in glycerol solution can vary between the Rouse and Zimm models depending on conditions. In this presentation we shall provide more details on the results of these researches.

In order to minimize the damage to BAC DNA caused by laser light, the experimental method was improved. By using this improved TIRF method, a single molecule of DNA can be measured continuously for longer than 100 seconds with high resolution. We continued this measurement and observe how the length of the long axis changes. The changes in the length of the long axis were compared at different glycerol concentrations (0, 8, 16, and 40%). In the log-log graph of the time interval and the change in the length of the long axis, the slope should be 1/2 in the Rouse model and 2/3 in the Zimm model. Our experimental results showed that in the region with a short time interval, the slope of BAC DNA at glycerol concentrations of 0% and 8% is about 2/3, while the slope 1/2 for BAC DNA at 16% and 40% glycerol concentrations. In addition, we shall report the relaxation time of each DNA from the intersection of the zero-slope line in the long time interval region and the lines of 1/2 or 2/3 slope in the region in which the Rouse model or Zimm model is applicable, respectively.

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Restricted Mobility and Jamming of Densely Packed DNA Pulled Out from Phage Phi29 Virus Capsids

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Many viruses, such as dsDNA bacteriophages, contain DNA packed to such high density that the mobility of the DNA inside the capsid is severely restricted, which can significantly affect the processes of motor-driven DNA packaging and ejection. We use single-molecule optical tweezers measurements to probe the dynamics and mobility of the tightly confined DNA by rapidly removing ATP near the end of packaging, which releases the motor's grip, and applying constant force to pull out the DNA. We find that, on average, the speed of DNA exit is significantly slowed when the DNA is packed at a density greater than ~0.3 g/ml, consistent with decreased molecular mobility. Significant heterogeneity in the dynamics and long pauses are observed, which we attribute to nonequilibrium DNA conformations arising during DNA packaging and/or exit. Addition of Mg 2+ ions, which electrostatically screen DNA interactions, decrease exit velocity and increase pause durations, consistent with lower internal pressure and increased DNA intrastrand friction. Preliminary measurements with varying applied and internal forces suggest that, contrary to a prior hypothesis, exit velocity may not be linearly proportional to driving force as expected for a process governed only by simple viscous friction.

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Shape and Sedimentation Coefficients of Supercoiled DNA Minicircles

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Studies of DNA minicircle structures show a strong dependence of the shape on the linking number. With increasing supercoiling, DNA minicircles can buckle and writhe to relieve the torsional stress, which results in complex structures that deviate greatly from existing models. Using linear elasticity theory, we can successfully predict the spectrum of shapes of minicircles of a given length and defined linking number.

We formulate analytical predictions for regime boundaries where the circular configuration is stable and where multiple solutions can coexist. We find an agreement with earlier cryo- electron micrographs showing supercoiling-driven transitions between ''open circular'' twisted and writhed 3-D shapes. Our new model captures the influence of the length-to-width ratio of small DNA minicircles on the threshold values.

Using the theoretical expressions, we compare the characteristic time scales of sedimentation, Brownian motion, bending and twist in the experiments. We conclude that the effects of bending are negligible and shapes of DNA minicircles can be approximated as rigid.

The 3-D supercoiled minicircle shape data were used to predict the hydrodynamic properties of the molecules using a bead-model, which allows us to calculate sedimentation coefficients, friction ratios, and diffusion coefficients that can be compared to analytical ultracentrifugation (AU) measurements. Our results for sedimentation coefficients compare favourably with preliminary AU data for 336 bp minicircles that are nicked or supercoiled.

Our theoretical framework can be applied to the analysis of the hydrodynamic properties and shapes of arbitrary relatively stiff looped biofilaments with lengths comparable to their persistence length.

A better understanding of how DNA supercoiling affects DNA structure and how DNA minicircles behave in solution have important ramifications to the fields of gene expression, DNA replication, gene therapy, and virtually every aspect of DNA structure and function.

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Influence of DNA Length on Supercoiling-Dependent 3-D Shape

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Gene therapy has emerged as a promising treatment for many human diseases. Recently, minimized non-viral DNA vectors have been the subject of increased interest. However, they have limited effectiveness due, in part, to problems associated with cellular transfection. To improve this shortcoming, DNA minivectors, small DNA nanocircles with as few as 250 base pairs (bp), have been developed. As previous research has demonstrated a size and shape influence on cellular transfection efficiency and intranuclear accumulation, with rod-shaped polystyrene nanoparticles displaying the best performance in several clinically relevant cell types, it is hypothesized that modulating the 3-D conformation of DNA minivectors may both improve transfection and provide a method for cell or tissue specificity. The 3-D conformation of DNA minivectors, which can range from ovals to rods, is governed by sequence length, sequence identity, and supercoiling in complex ways that are only now beginning to be unraveled. We previously found that the majority of highly supercoiled 336 bp DNA minivectors adopted a rod-shaped conformation (Irobalieva *et al*. 2015 *Nature Comm*. 6,8440). To determine how long the DNA minivector sequence can be lengthened before the advantageous rod-shaped conformation disappears, we used oxDNA2 to perform coarse-grained molecular dynamics simulations of highly negatively supercoiled DNA minivectors. We found that highly negatively supercoiled DNA minivectors were increasingly less likely to adopt a rod-shaped conformation as the sequence length increased from 336 bp to 672 bp. The biophysical insights from this study reveal the contribution of curvature (loop length) to supercoiling-induced 3-D conformations and may facilitate the development of DNA minivector gene therapies.

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Supercoiling and Looping Promote DNA Base Accessibility and Coordination among Distant Sites

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Despite the importance and ubiquity of DNA supercoiling, relatively little is known about how supercoiling modulates DNA structure and its interactions with proteins and other biological molecules. To begin to address this shortfall, we studied the 3-D structures of individual 336 bp DNA minicircles over a wide range of supercoiling—from positive to hyper-negative—using electron cryotomography. Supercoiled minicircles contort into a surprisingly wide variety of conformations, many of them containing sharp bends, even though DNA is rigid and should be resistant to bending. Sharp bends and small loops of DNA are common in biology with many essential roles, including genome packaging and gene regulation. We found that negative supercoiling transmits mechanical stress along the DNA backbone to disrupt base pairing at specific distant DNA sites. Cooperativity among distant sites localizes certain