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A localized transition in the size variation of circular DNA in nanofluidic slitlike confinement

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We report strong evidence for a localized transition in the size variation of circular DNA between strong and moderate regimes of slitlike confinement. A novel and rigorous statistical analysis was applied to our recent experimental measurements of DNA size for linear and circular topologies in nanofluidic slits with depths around $\approx 2p$, where p is the persistence length. This empirical approach revealed a localized transition between confinement regimes for circular DNA at a slit depth of $\approx 3p$ but neither detected nor ruled out the possibility for such a transition for linear DNA. These unexpected results provide the first indication of the localized influence of polymer topology on size variation in slitlike confinement. Improved understanding of differences in polymer behavior related to topology in this controversial and relevant system is of fundamental importance in polymer science and will inform nanofluidic methods for biopolymer analysis. [http://dx.doi.org/10.1063/1.4802594]

I. INTRODUCTION

The size and topology of a polymer are two of its most fundamental characteristics. In confinement, size is determined largely by the interaction between the confining geometry and the polymer. This interaction is of particular interest as the confinement is varied around length scales that correspond to the physical properties of the polymer. Topology describes the structure of the polymer backbone, imposing constraints on and potentially influencing the response of the polymer to confinement. Here, DNA molecules confined to nanofluidic slits are considered as a model experimental system to examine the effects of topology on the variation of polymer size in slitlike confinement for depths around twice the persistence length.

For linear DNA, size variation in slitlike confinement remains an active and controversial topic. Experimental measurements, made by fluorescence microscopy of single DNA molecules confined to nanofluidic slits,[1–5] have not been consistent with one another or with recent predictions from scaling arguments,[6] mean field approaches,[7] and simulations.[8,9] One of the most provocative aspects of this system has been competing characterizations of the transition between strong[6,10] and moderate[11,12] regimes of confinement as either gradual or abrupt.

For circular DNA, the few predictions that exist for size variation between strong and moderate regimes of slitlike confinement indicate a gradual transition.[13] We recently published the first of only two experimental studies that have measured the size variation of circular DNA confined to nanofluidic slits, characterized by the radius of gyration projected parallel to the slit surfaces,

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TABLE I. Parameters obtained from fitting the power law model (Eqn. (1)) to experimental measurements[2] of $R_{\|}(d)/R_{\|,Bulk}$. Values are reported as mean ± standard deviation.

DNA sample	k	n	Reduced χ^2	AIC[a]	BIC[a]
Linear 5:1	3.0 ± 0.2	−0.16 ± 0.01	0.0034	−65	−63
Linear 20:1	2.9 ± 0.2	−0.17 ± 0.01[b]	0.0039	−61	−59
Circular 5:1	2.6 ± 0.1	−0.15 ± 0.01	0.0013	−93	−90
Circular 20:1	2.6 ± 0.1	−0.16 ± 0.01	0.0015	−89	−86

[a]A larger negative value for the Akaike Information Criterion (AIC) or Bayesian Information Criterion (BIC) indicates a better model.
[b]The uncertainty for this value reported in Ref. 2 is corrected here.

$R_{||}$.[2,5] Nonlinear polymer topologies are of fundamental interest in polymer science, and circular polymers represent a simple and technologically relevant example.[14] Practically, differences in polymer behavior related to topology remain a largely unexplored but promising[15] approach to the nanofluidic control, differentiation, and analysis of biopolymers.

In our recent study, we measured $R_{||}$ by imaging DNA size directly using widefield fluorescence microscopy for slit depths, d, ranging from 35 nm to 342 nm.[2,16] These slit depths span the transition between traditional regimes of strong and moderate slitlike confinement expected around $d \approx 2p$, where p is the native persistence length of ≈51 nm.[17,18] Linear (bacteriophage lambda, 48.5 kbp) and circular (charomid 9-42, 42.2 kbp) DNA of comparable contour length were prepared in 5X TBE buffer and labeled with the fluorescent dye YOYO-1 at initial stoichiometric ratios of 5 or 20 base pairs per dye molecule, giving four DNA samples: linear 5:1, linear 20:1, circular 5:1, and circular 20:1. Small differences in the final experimental labeling ratios near equilibrium[2] are not expected to influence p significantly,[19] leaving topology as the salient characteristic of each DNA sample. These measurements are the most comprehensive and quantitative to date, with unprecedented resolution in the confining slit depth, approximation and correction of systematic imaging errors, and observation of both linear and circular DNA topologies, which are all essential to the analysis presented here.

In an initial analysis of our experimental measurements, the relation between $R_{||}$ and d was modeled by a power law:[2]

$$R_{||}(d) = kd^n \tag{1}$$

where k is the scaling coefficient, and n is the scaling exponent. Table I gives n values and previously unreported k values. The power law model represents an idealized description of a gradual transition between strong and moderate regimes of slitlike confinement. While the power law modeled the experimental measurements reasonably well over the range of slit depths investigated,[2] its suitability was not tested rigorously, the broadly expected scaling exponent of −0.25 was not validated, and the fits appear unconvincing around $d \approx (2\ to\ 3)p$, where the measurements suggest a localized change in size variation (Figure 1). Moreover, scaling laws are defined only in certain limits,[11] which are $d < p$ and $p \ll d < R_G$ for strong and moderate slitlike confinement, respectively, where R_G is the unconfined radius of gyration.[1] The experimental measurements analyzed here generally violate these conditions. Therefore, a power law model derived from scaling arguments for the limiting regimes of strong and moderate slitlike confinement cannot be assumed to accurately describe DNA behavior across the transition between these regimes.

Here, we present a novel statistical analysis of our experimental measurements to: (i) introduce the utility of a rigorous empirical approach towards an improved understanding of DNA behavior confined to nanofluidic slits; (ii) determine whether an abrupt variation in the relation between $R_{||}$ and d can be identified to locate the controversial transition between regimes of strong and moderate slitlike confinement; and, (iii) investigate the effect of topology on the size variation of DNA molecules between these regimes. If $R_{\|}(d)$ varies gradually, then the power law model predicted by theory should suffice to accurately describe the measurements, and the transition can be considered gradual. If, however, $R_{\|}(d)$ varies abruptly across these slit depths, then the power law model should less accurately represent the measurements, and a localized transition between different trends in

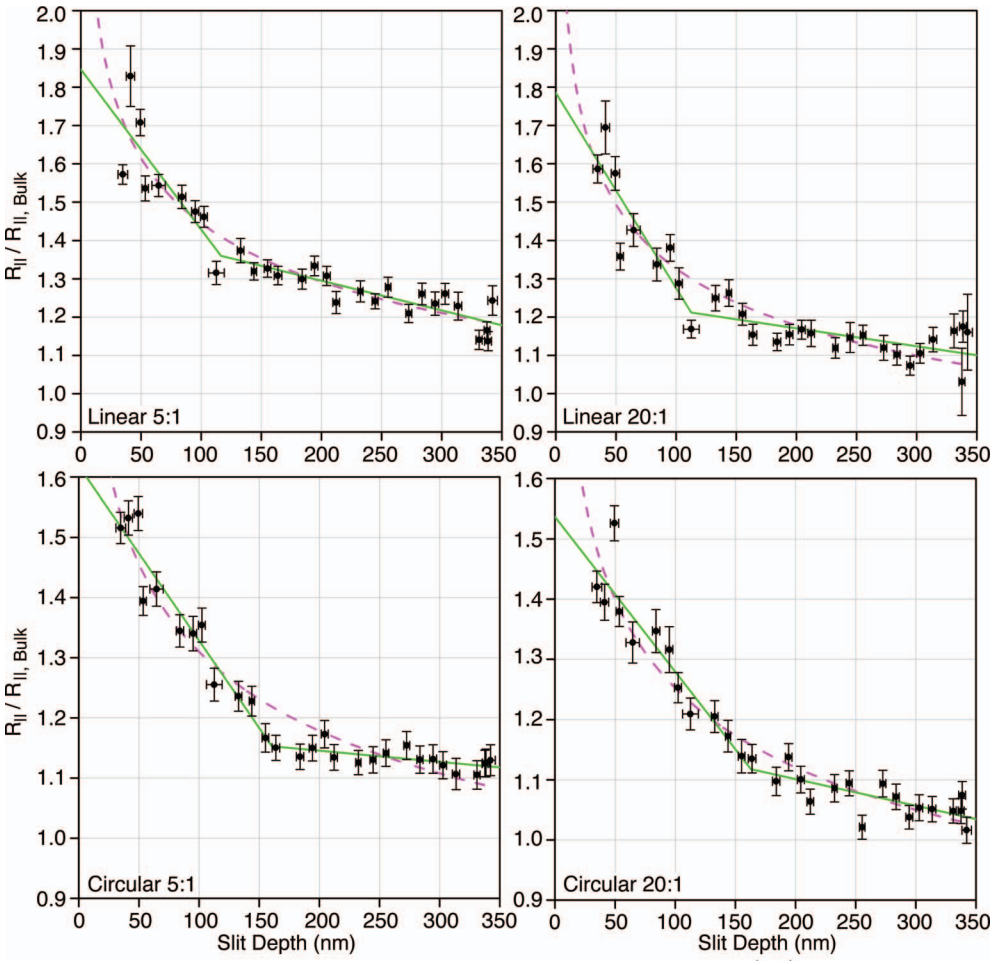

FIG. 1. Effects of topology on the size variation of DNA molecules between strong and moderate regimes of slitlike confinement. Data from Figure 8 in Ref. 2 shows $R_{\parallel}$ normalized by the corresponding bulk values for unconfined molecules, $R_{\parallel,Bulk}$, of (430 ± 5) nm, (485 ± 7) nm, (366 ± 6) nm, and (377 ± 6) nm (mean ± standard deviation) for linear 5:1, linear 20:1, circular 5:1, and circular 20:1 DNA samples, respectively. The x-axis and y-axis bars represent one standard deviation. Magenta dash lines and green solid lines show fits of the power law (Eqn. (1)) and piecewise linear (Eqn. (2)) models, respectively, to the experimental measurements. Statistical analysis of these fits to the 29 data points for each DNA sample provided strong evidence for a localized transition between strong and moderate regimes of slitlike confinement for circular DNA, while a similar analysis neither detected nor ruled out a localized transition for linear DNA.

$R_{\parallel}(d)$ should be evident in the experimental measurements. The empirical analysis presented here is necessary to validate and refine related theories and simulations of DNA size variation in nanofluidic slitlike confinement.

II. ANALYSIS

To locate the slit depth corresponding to an abrupt transition in size variation, we fit our measurements of $R_{\parallel}(d)$ to the piecewise linear model:

$$R_{||}(d) = H\,(d_o - d)\,[A + Bd] + H\,(d - d_o)\,[A + Bd_o + C\,(d - d_o)] \tag{2}$$

where A is the $R_{||}$-axis intercept, B is the slope for $d < d_0$, C is the slope for $d > d_0$, and $H\,(u)$ is the Heaviside function, which was approximated as:

$$H\,(u) = \frac{|u| + u}{2\,|u| + \varepsilon}$$

TABLE II. Parameters obtained from fitting the piecewise linear model (Eqn. (2)) to experimental measurements[2] of $R_{\parallel}(d)/R_{\parallel,Bulk}$ for DNA samples and for simulated data following a noisy power law. Values for the DNA samples are reported as mean ± standard deviation. For simulated data, d_0 is reported as mean ± standard deviation, and the reduced χ^2, AIC, and BIC values are the mean, for fits to each of 10^4 independent simulated data sets.

Sample	d_0 [nm]	Reduced χ^2	AIC[a]	BIC[a]
Linear 5:1	116 ± 14	0.0032	−65	−60
Linear 20:1	113 ± 10	0.0034	−63	−58
Circular 5:1	161 ± 7	0.0007	−109	−104
Circular 20:1	163 ± 13	0.0012	−94	−88
Simulated circular 5:1	120 ± 14	0.0015	−87	−82
Simulated circular 20:1	120 ± 14	0.0015	−87	−82

[a]A larger negative value for the Akaike Information Criterion (AIC) or Bayesian Information Criterion (BIC) indicates a better model.

where $|u|$ is the absolute value of u, and ε is a negligibly small positive number (10^{-37} for this work) relative to d to ensure that the denominator was never zero during the fitting procedure (Figure 1, Table II). In contrast to the power law model, the piecewise linear model represents an idealized description of an abrupt transition and is purely empirical. This model is the simplest mathematical function that enables quantitative localization of an abrupt variation in $R_{\parallel}(d)$. Determined as a fitted parameter, d_0 identifies the slit depth at which the curvature of $R_{\parallel}(d)$ is best concentrated by the piecewise linear model to approximate the measurements as different linear trends in size variation for $d < d_0$ and $d > d_0$. This gives the most probable slit depth for an abrupt variation in the relation between $R_{\parallel}$ and d and, consequently, for a localized transition between strong and moderate regimes of slitlike confinement. Although the piecewise linear model yields a single value for d_0, the experimental measurements have finite resolution in d. Therefore, d_0 indicates a range of slit depths, at most comparable to the average experimental confinement resolution of ≈11 nm,[20] over which more abrupt variation in $R_{\parallel}(d)$ is localized. The piecewise linear function enabled straightforward identification and interpretation of d_0 at the transition between confinement regimes, which is our primary concern here. Improved fit results may be obtained using other piecewise mathematical functions, but evaluating these models rigorously would require experimental measurements at numerous slit depths across a larger range in d.

Figure 1 compares fits of the power law and piecewise linear models to the experimental measurements. Reduced χ^2 values were calculated as $RSS/(m - j)$, where RSS is the residual sum of squares, m represents the number of data points, and j is the number of fit parameters. Although reduced χ^2 values for the piecewise linear model are similar to, but generally less than, those for the power law model, meaningful conclusions cannot be drawn from this simple analysis for several reasons (Table I, Table II). First, these small differences in reduced χ^2 are dominated by a few apparent outlying measurements. Second, the power law model has two adjustable parameters, while the piecewise linear model has four. This may be treated generally by the Akaike Information Criterion, $AIC = 2j + m\ln(RSS)$, or Bayesian Information Criterion, $BIC = j\ln(m) + m\ln(RSS)$, which compare models with different numbers of adjustable parameters, rewarding goodness of fit and penalizing adjustable parameters. The AIC and BIC do not clearly favor either model for the linear DNA samples, while these information criteria suggest a better fit using the piecewise linear model for the circular DNA samples, especially for the circular 5:1 sample, as is apparent in Figure 1. Third, these general statistical metrics are inconclusive, because the piecewise linear model may spuriously identify a value and standard deviation for d_0, even when fit to simulated data derived from a power law. These three points motivate a more sensitive statistical analysis optimized specifically for our experimental measurements, and the last point forms the basis for the following analysis of the probability for an abrupt transition between confinement regimes for the different DNA topologies. Analyses of the circular DNA samples, representative of that followed for the linear DNA samples, are presented below.

We tested the possibility for the piecewise linear model to find a spurious abrupt transition for simulated data following the power law model. Eqn. (1) was first evaluated using k and n values

from the fits to measurements of the circular DNA samples (Table I) and d values at 0.1 nm intervals across the experimental range of d. When fitted to the piecewise linear model in Eqn. (2), these data gave d_0 values of ≈120 nm, to which d_0 was initialized for all subsequent fits. Eqn. (1) was then evaluated using k and n values from the fits to measurements of the circular DNA samples (Table I) at the experimental d values. Fits of Eqn. (2) to this simulated data yielded d_0 values of (119 ± 4) nm (all quantities are reported here as mean ± standard deviation) for the k and n values for both circular DNA samples. To determine the effect of varying the exponent on d_0, this procedure was repeated for $n = -0.145$ and $n = -0.159$ for the circular 5:1 sample and $n = -0.135$ and $n = -0.185$ for the circular 20:1 sample, calculated by taking three standard deviations from the experimental fitted values. The corresponding d_0 values were unaffected.

Changing the range of d over which the piecewise linear model was fit, however, significantly affected the resulting values of d_0. To demonstrate this effect, the shallowest d value at 35 nm was discarded and replaced with an additional d value at 349 nm, which shifted the range of the simulated data toward deeper slits. A fit of the piecewise linear model to the shifted simulated data gave d_0 values of (122 ± 4) nm for both simulated data sets representing the circular DNA samples. This relatively large change shows that the fitted value of d_0 for a power law simulating our experimental measurements is influenced strongly by the fitted range of d. Therefore, were the measurements in Figure 1 accurately represented by the power law model, the values of d_0 for all DNA samples should have clustered near $d_0 \approx 120$ nm. Although fits to the measurements of the linear DNA samples gave d_0 values slightly below 120 nm, fits to the measurements of the circular DNA samples gave $d_0 \approx 160$ nm.

We then fit the piecewise linear model to simulated data following a noisy power law, to more accurately represent our experimental measurements. Simulated noisy data were calculated as the sum of two terms evaluated at d values matching the experimental measurements. The first term was calculated using Eqn. (1) and k and n values from fits to measurements of the circular DNA. The second term was normally distributed noise of zero mean and a standard deviation of 0.0375, which was chosen to produce a mean reduced χ^2 value that matched or just exceeded the value obtained from fits of the piecewise linear model to the experimental measurements. Eqn. (2) was then fit to the results of each of 10^4 independent simulations of data following the noisy power law, giving d_0 values of (120 ± 14) nm (Table II) for both circular DNA samples. By a count of the number of simulated data sets resulting in fitted d_0 values > 160 nm, the probability that both random data sets give $d_0 > 160$ nm is just 0.0015. Because the reduced χ^2 value and standard deviation of d_0 for fits of Eqn. (2) to measurements of both circular DNA samples were less than or equal to the corresponding values for the simulated data following a noisy power law, 0.0015 represents a conservative upper bound on the probability that the power law model accurately describes our measurements and that the two values of $d_0 \approx 160$ nm from fits of the piecewise linear model to the experimental measurements of circular DNA occurred by chance.

III. RESULTS AND DISCUSSION

This analysis strongly supports the piecewise linear model over the power law model for measurements of $R_{\|}(d)$ for circular DNA. Therefore, the transition is better characterized as abrupt rather than gradual, with localized variation in $R_{\|}(d)$ at $d \approx 3p$. It is unlikely that a systematic error produced this result, considering the extensive uncertainty analyses of our previous measurements.[2] These included identification of the sources of systematic error, with corrections and estimated uncertainties where the errors were not negligible. Measurements of both DNA topologies used the same experimental procedure and apparatus and resulted here in different d_0 values, greatly reducing the possibility that an unrecognized systematic error caused a localized transition for circular DNA at $d_0 \approx 160$ nm. Fits of the piecewise linear model to the uncorrected measurements for circular DNA[2] gave d_0 values of (160 ± 8) nm and (163 ± 13) nm for the circular 5:1 and 20:1 samples, respectively, showing that the imaging correction algorithm did not significantly affect the location of d_0 for this topology (Table II). No systematic influence of the YOYO-1 labeling ratio on p^2 is evident in the fitted values of d_0.

Following a similar analysis, we find that neither the power law model nor the piecewise linear model is significantly better at describing measurements of both linear DNA samples. Values of d_0 for linear DNA were below the value of ≈120 nm expected had these measurements been well-described by the power law model but were too close to this value to allow clear identification of a localized transition (Table II). Given that the range of d significantly influenced d_0 for simulated data following a power law, additional experimental measurements across a larger range of slit depths are required to confidently ascertain the nature of the transition for linear DNA. Furthermore, the analysis was complicated by larger uncertainties in our experimental measurements of size for linear DNA than for circular DNA.

Several interesting conclusions follow from this analysis. If $R_{\|}(d)$ for linear DNA is better characterized by the power law model over the range of slit depths studied, then topology imposes a gradual transition for linear DNA and a localized transition for circular DNA. If, however, $R_{\|}(d)$ for linear DNA is better characterized by the piecewise linear model, then topology shifts the likely slit depth for a localized transition from $d \approx 2p$ for linear DNA to $d \approx 3p$ for circular DNA. In either case, topology clearly influences polymer behavior across strong and moderate regimes of slitlike confinement. This is in contrast to the conclusions drawn from fits of the power law model to the experimental measurements, which yield statistically indistinguishable results for linear and circular DNA (Table I) and, therefore, incorrectly suggest that topology has little or no influence on polymer behavior between these regimes.

While unexpected, the results from the piecewise linear model are not inconsistent with the experimental study of Lin *et. al*.[5] When our empirical analysis is applied to their measurements of both circular and linear DNA, as extracted directly from Figure 3(a) of Ref. 5, fits of the power law model yield n values comparable to ours, while fits of the piecewise linear model do not preclude the possibility of a localized transition. These inconclusive results follow from the lack of sufficient resolution in d across the transition between confinement regimes in the measurements of Lin *et. al*.,[5] with only 6 measurements reported across the range of d for which we present 29. Quantitative measurements at numerous slit depths across the transition between confinement regimes, such as ours,[2] are needed to resolve localized differences in DNA size variation related to topology.

IV. CONCLUSION

We applied a novel and rigorous statistical analysis to the most comprehensive and quantitative experimental measurements of the size variation of linear and circular DNA confined to nanofluidic slits between strong and moderate regimes of slitlike confinement.[2] We identified a localized influence of topology on the size variation of circular DNA at a slit depth of ≈$3p$ but neither clearly detected nor ruled out the possibility for a similar transition for linear DNA. These results reflect actual differences in the behavior of circular and linear DNA, rather than artifacts of the experiment or analysis. Importantly, our empirical analysis does not presuppose physical knowledge of the system, which precludes a physical explanation here for these unexpected results. Considering the controversy surrounding predictions and measurements of the size variation of linear DNA confined to nanofluidic slits and the scarcity of similar predictions and measurements for circular DNA, further work is required to discern the underlying physical mechanisms and potential practical utility of the topological differences in behavior identified here. From a fundamental perspective, topological differences in the behavior of confined polymers are important in polymer science, and related theory and simulation must be validated and refined by new empirical evidence. The results described here present both a challenge and an opportunity to polymer theory in this regard, by calling for physical explanation and offering a test of competing models. From a practical perspective, topological differences could be exploited for the nanofluidic manipulation of biopolymers, as seemingly small changes in slit depth around a critical transition would have unexpectedly large effects on polymer behavior.

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