Fluctuation modes of nanoconfined DNA

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(Received 20 July 2011; accepted 22 November 2011; published online 17 January 2012)

We report an experimental investigation of the magnitude of length and density fluctuations in DNA that has been stretched in nanofluidic channels. We find that the experimental data can be described using a one-dimensional overdamped oscillator chain with nonzero equilibrium spring length and that a chain of discrete oscillators yields a better description than a continuous chain. We speculate that the scale of these discrete oscillators coincides with the scale at which the finite extensibility of the polymer manifests itself. We discuss how the measurement process influences the apparent measured dynamic properties, and outline requirements for the recovery of true physical quantities. © 2012 American Institute of Physics. [doi:10.1063/1.3675207]

I. INTRODUCTION

DNA is a model system for the physical properties of polymers and polyelectrolytes, and DNA stained using fluorescent dyes has reached particular prominence because it allows the direct observation of molecular conformations.^{1–8} It has been extensively used in the exploration of the influence of confinement on polymers, and has been used to map the dynamics of polymer fluctuations.

Here we consider DNA in nanochannels that restrict the configuration of the chain along two dimensions, but leave the polymer free along the third axis. Under such conditions, DNA stretches out along the nonconfined axis.⁹ The same chain configuration underlies the reptation model of polymer-polymer diffusion, where each mobile chain is thought to move along a tube formed by the matrix of other chains.¹⁰

The dynamics of the chain fluctuations are an important parameter in that model, as it enables the sampling of different paths.¹¹ The basic description of DNA in such nanochannels is that of an overdamped harmonic oscillator chain (Fig. 1). The description is highly analogous to the Rouse model of a free polymer,¹² with the difference that each spring of the nanochannel-confined polymer has a nonzero equilibrium length. The precise physical mechanism of the elongation inside nanochannels is not critical for justifying this harmonic model because of the central limit theorem, as long as the molecule is long enough. However, for short segments that fluctuate strongly, we do expect a deviation from the harmonic case.

Previous authors have mainly concentrated on the fundamental relaxation mode of end-to-end fluctuations of nanochannel-confined polymers, and quantified it using the magnitude of fluctuations and fundamental relaxation time.^{13,14} Other authors used fluorescent beacons that attached to points within the molecule to map the amplitude of fluctuations as a function of separation between points.¹⁵ However, the Rouse-like harmonic model makes predictions beyond point-point distances, and demands that the displacement fluctuations can be expressed in cosine-shaped modes. In a recent publication we have explored the relationship between wave-

number and relaxation time for density fluctuations, and found a slightly lower scaling than expected, which was nevertheless consistent with the harmonic oscillator chain.¹⁶

In this publication we investigate the amplitude of fluctuation modes within the polymer, and whether the prediction of discrete modes can be verified. We will explore the questions of whether an analytical model can predict the experimental data, how well a numerical model predicts parameters that are cumbersome to obtain analytically, and whether data analysis can identify individual modes without prior knowledge of the fundamental modes of relaxation.

We find that a mode picture can yield a satisfactory description of the experimental data. However, we also find that modes with decreasing wavelength carry less amplitude than expected from an infinite chain, but rather that the chain behaves as if it consists of a finite number of oscillators. We attribute this finding to the limiting action of finite extensibility and self-avoidance of the polymer in our system.

II. EXPERIMENTAL METHODS

λ -DNA and its concatemers were stained with YOYO-1 at a ratio of 1 dye for each 5 basepairs. The contour length of unstained λ -DNA (New England Biolabs), which has 48.5 kbp, is $\approx 16 \mu\text{m}$. After staining, that length can increase by about 30%, but we do not believe that our system was sufficiently thermalized to obtain that value. DNA was suspended in $0.5\times$ TBE buffer, pH 7.8 at room temperature. 0.2% by weight PVP (360 kDa, Sigma Aldrich) was added to prevent electroendosmosis, and 1% v/v β -mercapto ethanol (Sigma Aldrich) prevented rapid bleaching.

All experiments used mixed micro- and nanofluidic devices made from fused silica, which were prepared by methods discussed elsewhere.¹⁷ Nanofluidic channels with a $160 \times 80 \text{ nm}^2$ cross-section and a length of $200 \mu\text{m}$ were placed between microchannels that carried solution from the injection ports of the device to the active zone. DNA was moved through microchannels by application of pressure and injected into nanochannels through application of voltages at the microfluidic ports or application of pressure. We observed that DNA after electrophoretic injection had a

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FIG. 1. (Color) Rouse-like model of nanoconfined DNA. Under confinement to a channel much smaller than the radius of gyration, DNA assumes an extended geometry. If the extension is large, the polymer can be modeled as a chain of oscillators with finite equilibrium spring lengths.

tendency toward drifting after the voltage was removed, while pressure-injected molecules were generally more stable.

Fluorescence microscopy was performed on an inverted fluorescence microscope (Nikon, TE-2000) with an EM-CCD camera (iXon, Andor). A 100 $\times$ oil immersion objective (NA1.35) was used. Illumination was provided by a 50 mW, 473 nm DPSS laser that was controlled through a manufacturer-provided gating input (Shanghai Dream Lasers). The intensity of the laser was adjusted using a neutral density filter. The fluorescence was filtered through a FITC-specific filter set (Semrock). Image frames were taken using strobed illumination, with 5 to 45 ms flash lengths. Frame rates were 19.5 to 30 frames per second. Speckle patterns were blurred by a scanning mirror that is confocal with the back-focal plane of the microscope objective. The angle of incidence is scanned in a 20-fold zigzag pattern during each frame. Illumination, exposure, and scanning are synchronized through an in-house FPGA solution.

For calculation of molecules extensions and their fluctuations we fitted the brightness along the molecule backbone to¹⁸

$$I(x, t) = \frac{I_0}{2} \left[\operatorname{erf} \left(\frac{x - x_c + \ell/2}{\sigma} \right) - \operatorname{erf} \left(\frac{x - x_c - \ell/2}{\sigma} \right) \right] - I_{\text{off}}, \quad (1)$$

$I(x, t)$ is the intensity along the channel, I_0 is the brightness, x_c is the center of the molecule, ℓ is the extension, σ is the width of the molecule end, and I_{off} an offset.

III. THEORY

A. Length fluctuations

The theoretical description of nanoconfined DNA is a topic of considerable controversy. That is particularly the case for channels with a cross-section of about two persistence length, since analytical models rely on simplifications that are not fully applicable here. The primary models are the de Gennes model for wide channels, which partitions the polymer into noninterpenetrating blobs, and the Odijk model which considers narrow channels and a polymer “bouncing” between deflections from the wall.²³ For the channels in our

experiments we are more likely governed by the De Gennes model. In the application of the de Gennes model, more questions arise. The polymer stored within one blob of channel width in diameter is conventionally thought to act ideal, that is without volume interactions, in free solution. The scaling of polymer extension with channel width and other parameters apparently show that the polymer within blobs cannot be ideal as such, or that ideal blobs can partially interpenetrate.^{13,19} However, the self-excluding regime^{20,21} also appears not to be fully applicable. Interestingly, circular DNA appeared to be well-described by the self-excluding model.²² Odijk outlines some of the challenges in finding a complete description of the field.²³

Instead of considering describing recent progress in the field in detail,^{24–38} we note that on large scales, all models should yield similar statistical characteristics. That is because the one-dimensional and finite-extensible nature of the polymer demands that above a certain length scale the central limit theorem imposes a Gaussian distribution of states, and the apparent potential around the equilibrium point must approximately follow a harmonic potential. Extended DNA in this manuscript fluctuates by about 3% to 7% of its contour length, and arguably should be harmonic-like. Previous studies^{13,15} considered a single figure of merit—the distance between two points along the contour—as the measure of polymer fluctuations. We attempt here to look at the parametrization in terms of collective variables, or modes. Such studies have been undertaken for free and microconfined DNA.^{2,39}

The polymer is approximated as an overdamped oscillator chain governed by the Langevin equation (N beads, L equilibrium extension, elastic modulus κ , friction coefficient per unit length ξ)

$$-\frac{\xi L \dot{x}_i}{N} + \frac{\kappa N}{L} (x_{i+1} + x_{i-1} - 2x_i) = F_{\text{Random}}. \quad (2)$$

The functional relationship of κ and ξ with respect to channel width, persistence length and effective width were discussed elsewhere,¹³ but are not essential in this setting. The solutions are the modes

$$x_m = \frac{Lm}{N} + \sum_{\nu=1}^N c_{\nu}(t) \cos \left(\frac{\pi \nu m}{N} \right), \quad (3)$$

where c_{ν} is a Gaussian distributed weight with

$$\langle c_{\nu}(t) c_{\mu}(t + \Delta t) \rangle_t = \frac{\gamma^2}{\nu^2} \exp \left(\frac{-t \nu^2}{\tau_0} \right) \delta(\mu, \nu). \quad (4)$$

In this expression we have $\tau_0 = \xi L^2 / \kappa \pi^2$ and $\gamma^2 = 2k_B T L / \kappa \pi^2$. The description is in essence that of the Rouse model, with the exception that the springs between oscillators have an equilibrium length larger than zero.

In this model, the friction per unit length of polymer was taken as constant. Brochard and de Gennes have argued that for a molecule under intermediate stretching this is incorrect, and that the friction should instead be taken constant per occupied channel length.⁴⁰ However, at our molecule extension the fully screened regime might be approached,⁴ in which

case the constant friction per unit length may not present a large error.

The two quantities that are easily measured on nanoconfined DNA are the extension of the molecule, and the density profile along the nanochannel. However, we previously have not considered the effect of the observation conditions on the measured data. As we will argue on the basis of our experimental findings, neglecting those experimental conditions leads to errors in the estimation of fluctuation amplitudes. In particular, any quantity is always measured over a finite interval. For instance, the measured length of the molecule will be determined by

$$\bar{L}(t) = \frac{1}{t_f} \int_{t'}^{t+t_f} dt' L(t'), \quad (5)$$

where t_f is the exposure time for one frame. We quantify the relaxation dynamics of the extension by forming the time-autocorrelation of the deviation from the mean. Since Rouse modes are statistically independent, and only odd modes contribute to the extension fluctuations, the expected result is given by

$$\begin{aligned} \langle \Delta \bar{L}(t) \Delta \bar{L}(t + \Delta t) \rangle_t &= \left\langle 4 \sum_{\nu \dots \text{odd}}^N \int_{t'=t}^{t+t_f} dt' \int_{t''=t+\Delta t}^{t+\Delta t+t_f} dt'' c_\nu(t') c_\nu(t'') \right\rangle_t. \end{aligned} \quad (6)$$

The result for the time averaged results emerging from the integral is similar to the one for instantaneous lengths $\langle \Delta L(t) \Delta L(t + \Delta t) \rangle_t$, but contains a factor $\theta(\nu, t_f, \tau_0)$ that accounts for the finite exposure

$$\langle \Delta \bar{L}(t) \Delta \bar{L}(t + \Delta t) \rangle_t = 4 \sum_{\nu \dots \text{odd}}^N \theta(\nu, t_f, \tau_0) \frac{\gamma^2}{\nu^2} e^{-\frac{\Delta t \nu^2}{\tau_0}}. \quad (7)$$

The correction factor θ has a different functional form for Δt greater and smaller than t_f . The most insightful representation.

$$\theta(\nu, t_f, \tau_0) = \begin{cases} 2 \sum_{\mu=0}^{\infty} \frac{1}{(\mu+2)!} \left(-\frac{\nu^2 t_f}{\tau_0} \right)^\mu & \Delta t = 0 \\ 2 \sum_{\mu=0}^{\infty} \frac{1}{(2\mu+2)!} \left(\frac{\nu^2 t_f}{\tau_0} \right)^{2\mu} & \Delta t > t_f. \end{cases} \quad (8)$$

Naturally, for $t_f \ll \tau_0$, this reduces to unity. However, experimentally we are not able to access this point while ensuring speckle-free illumination. At larger t_f we come to the peculiar conclusion that for rising t_f the $\Delta t = 0$ value drops with rising t_f , while it rises at $\Delta t > t_f$. It is also apparent that the correction factor is small for $\Delta t > t_f$.

B. Density fluctuations

We have previously shown that for density fluctuations along the molecule the mixing of displacement modes leads to nonideal behavior in the sense that density modes follow displacement modes only approximately.¹⁶ However, for long molecules modes are approximately decoupled. In that

case a similar influence of the exposure time on the observed magnitude of fluctuations can be established. In the following we also include a Gaussian approximation for the point spread function with width σ into the consideration. Since we extract an interior space from the molecule that does not coincide with the strict Rouse modes, we now parametrize the length scale of fluctuations with the circular wavenumber $k = 2\pi\nu/L$ (L equilibrium extension). We arrive at

$$\begin{aligned} \Re(\langle \Delta \mathcal{I}(k, t) \Delta \mathcal{I}^*(k, t + \Delta t) \rangle_t) &\propto I_0^2 e^{-\frac{1}{2} k^2 \sigma^2} \theta(kL/2\pi, t_f, \tau_0) \frac{\gamma^2}{L} e^{-\frac{\Delta t}{\tau_0} \left(\frac{kL}{2\pi} \right)^2}, \end{aligned} \quad (9)$$

that is we anticipate the fluctuations spectrum at low k to be flat or “white,” until either optical resolution or camera exposure time impose an upper cutoff.

We anticipate that the actual spectrum $I(k)$ will not follow the ideal white prediction, but rather will have some k dependence. $I(k)$ can be extracted if the relaxation dynamics characterized by the relaxation time $\tau(k)$ are determined first, and the measurement artifacts are then removed using a simple exposure model. As noted above, the factor θ that describes the influence of the illumination is different at $\Delta t = 0$ and $\Delta t > 0$. However, the $\Delta t = 0$ point is contaminated with Gaussian and Poisson noise of the camera system. Hence we will attempt to extract the physical fluctuation amplitude $I(k)$ from comparing the experimental curves at $\Delta t > 0$ to

$$\begin{aligned} \Re(\langle \Delta \mathcal{I}(k, t) \Delta \mathcal{I}^*(k, t + \Delta t) \rangle_t) &\propto I^2(k) e^{-\frac{1}{2} k^2 \sigma^2} \theta(kL/2\pi, t_f, \tau_0) \frac{\gamma^2}{L} e^{-\frac{\Delta t}{\tau(k)}} \\ &\times \sum_{\mu=0}^{\infty} \frac{2}{(2\mu+2)!} \left(\frac{t_f}{\tau(k)} \right)^{2\mu}. \end{aligned} \quad (10)$$

C. An alternative measure of dynamics

While both the earlier introduced Fourier transform approach that considers the interior of the molecule and the fitting of the end to end distance yield dynamic data, one could object to their use on the grounds that both contain analysis steps which involve some human intervention, such as the choice of the transformation frame or the judgment whether a fit represents the true optimum, or simply is a local minimum. Thus, a fully hands-off approach would be desirable. We can find such a fully hands-off approach by forming the Fourier transform of the entire molecule including a stretch of channel that is not occupied before and after the molecule.

We can predict the form of such a transform by using a chain of thought similar to that used in the derivation of the dynamic structure factor.¹¹ Starting with a discrete set of beads, we note that the density of beads $\rho(x, t)$ can be given by

$$\rho(x, t) = \sum_{m=1}^N \delta[x - x_m(t)], \quad (11)$$

which yields the Fourier transform

$$\rho(k, t) = \sum_{m=1}^N \exp[ikx_m(t)]. \quad (12)$$

We neglect the lowest mode that corresponds to center of mass motion, which we can remove from data prior to transformation. The density thus becomes

$$\rho(k, t) = \sum_{m=1}^N \exp\left(ik \left[\frac{Lm}{N} + \sum_{\nu=1}^{\text{inf}} c_{\nu}(t) \cos\left(\frac{\pi\nu m}{N}\right) \right]\right). \quad (13)$$

We wish to measure the real part of $\mathcal{C}(k, \Delta t) = \langle \rho(k, t) \rho^*(k, t + \Delta t) \rangle_t$. Before taking the expectation value, we note that for arbitrary constants a and b Gaussian random variables $c_{\alpha}(t)$ and $c_{\beta}(t)$

$$\langle e^{ac_{\alpha}(t)} e^{b^*c_{\beta}(t+\Delta t)} \rangle = e^{a^2\langle c_{\alpha}^2 \rangle + 2\langle c_{\alpha}(t)c_{\beta}(t+\Delta t) \rangle ab^* + (b^2)^* \langle c_{\beta}^2 \rangle}. \quad (14)$$

Using the appropriate terms for c_{ν} , a , and b the resulting quantity becomes

$$\begin{aligned} \mathcal{C}(k, \Delta t) &= \sum_{m,n=1}^N \exp\left\{i \frac{kL(m-n)}{N}\right\} \\ &\exp\left\{-\sum_{\nu=1}^{\infty} \frac{(k\gamma)^2}{2\nu^2} \left(\cos^2\left(\frac{\pi m\nu}{N}\right) + \cos^2\left(\frac{\pi n\nu}{N}\right)\right)\right\} \\ &\exp\left\{\sum_{\nu=1}^{\infty} \frac{(k\gamma)^2}{\nu^2} e^{-\frac{\Delta t\nu^2}{\tau_0}} \cos\left(\frac{\pi m\nu}{N}\right) \cos\left(\frac{\pi n\nu}{N}\right)\right\} \end{aligned} \quad (15)$$

We were not able to find a closed form for the expression containing the exponential decay, although it may well exist. The form above does not take the effect of finite exposure time into account, but it will obviously simply introduce an additional factor of θ . However, we will not test this with our experimental data.

In Fig. 2 we present typical $\mathcal{C}(k, \Delta t)$ maps. We see that at $\Delta t \rightarrow \infty$ the autocorrelation is dominated by the $\text{sinc}^2(k)$ envelope that is the power spectrum of a boxcar function. Interestingly, we can utilize the location of maxima and minima at $\Delta t \rightarrow \infty$, or the sum over the autocorrelation over all Δt , to determine the average length of the molecule. There are two appealing features about this approach. Firstly, since the $\text{sinc}^2(k)$ profile has a multitude of nodes and peaks, it provides more critical features for a numerical fitting approach than the two edges which are the effective features in the fitting to the position-space data. The second appealing feature is that the averaging of power spectra removes the center of mass location, and thus averaging over a moving molecule does not widen the mean as is done when the averaging is done over the position-space data. In the fitting of real-space data we deal with this widening by fitting every frame individually. Because peculiar conformations lead to a breakdown of fitting in position space, statistical measures have to be used to detect such failure to find a meaningful molecule length. Naturally, the global autocorrelation method breaks down

FIG. 2. (Color) Autocorrelation of global Fourier transform. The color map is logarithmic, and its scale is truncated at the bottom to reveal the structure of interest clearly. In the left column we show the raw correlations, and in the right column the offsets are removed from all constant k lines and their amplitudes are normalized.

once molecules become too short and the convolution of boxcar function and Gaussian fluctuation becomes dominated by the Gaussian. The transform then yields a Gaussian itself—without distinct valleys and peaks.

We now turn to the dynamics of the global transform. For that, we estimate the equilibrium spectrum from the $\Delta t \gg 0$ region of the time-correlation surface. We then subtract this equilibrium spectrum from the time-dependent surface, and normalize the curve for each constant k such that the $\hat{C}(k, \Delta t = 0) = 1$. The second column of Fig. 2 shows such a normalized surface. In general, the time dependence at constant k represents a mixture of modes rather than the pure relaxation modes. However, at selected k we can either recover a very good sampling of the end to end fluctuations, or for low γ/L even recover a multiexponential decay of distinct modes. In Fig. 3 we show that normalized correlation decays at k close to minima of the $\text{sinc}^2(k)$ envelope of the raw correlation follow at most times the fundamental mode of the fluctuating polymer. However, correlation decays at k close to the maxima of the $\text{sinc}^2(k)$ envelope of the raw correlation appear to be mostly decoupled from end to end fluctuations since they decay like the first even ν mode, which does not couple into end to end length fluctuations. At very large Δt , some coupling into the fundamental mode is observed. Thus the global transform method has the potential to detect the signature of individual modes since clearly separated regions are present within the multiexponential decay.

FIG. 3. (Color) Autocorrelation of global Fourier transform for $\gamma/L = 2.5 \times 10^{-3}$. (a) Raw correlation, (b) normalized correlation, (c) profiles of normalized correlation at select k . The color maps are logarithmic, and the time ranges in all panels are identical.

IV. BROWNIAN DYNAMICS SIMULATION

We simulated the dynamics of the oscillator chain using a one-dimensional Brownian dynamics simulation of an overdamped bead-spring chain employing a 4th-order Runge-Kutta integrator. The general update equation was

$$\Delta x_i = \frac{\Delta t}{\eta} \left(F_i^{\text{rnd}} + F_{i,i-1}^{\text{el}} - F_{i+1,i}^{\text{el}} \right). \quad (16)$$

Here x_i is the position of the i th bead, Δt is the time step, F^{rnd} is the random force, and F^{el} is the elastic force. η is the friction coefficient of a single bead, which is on the order of the friction of a bead of diameter equal to the channel width.

We utilized two different force laws. The first one is a harmonic force law, where the spring stiffness of the harmonic potential was chosen as directed by Reisner *et al.*¹³ We further utilized a potential that is the combination of a Marko-Siggia finite-extensible⁴¹ chain with an additional self-avoiding core that follows Flory.⁴² The finite-extensible chain force law thus is in essence one proposed by De Gennes²⁰ augmented by finite extensibility. Work by Jun *et al.* suggests that such a model could hold over a large range of extensions of nanoconfined DNA, while a conventional treatment without the finite extensibility fails for stretching states well out of equilibrium.²⁷ The force here was given by the equation

$$\frac{F_{\text{el},i,j}L}{k_B T} = \left(\alpha \frac{\Delta x_{i,j}}{r_0} + \frac{1/4}{1 - \left(\frac{\Delta x_{i,j}}{r_0} \right)^2} - \frac{1}{4} - \frac{\gamma}{(\Delta x_{i,j})^2} \right). \quad (17)$$

The parameters γ and α can be adjusted, and r_0 is the equilibrium length of a link. We chose α as unity, and adjusted γ to yield the same equilibrium spring stiffness as the harmonic solution. However, in the limit of many beads or large links no significant difference between harmonic and anharmonic potential was found, and thus we compared experimental results only to harmonic potential calculations.

For the simulation of the density data that we obtain in experiments, we convolved with a Gaussian response function of the microscope. Poisson noise was added to simulate the photon counting statistics of the camera. The simulated data then was processed like the experimental data.

V. RESULTS AND DISCUSSION

A. Length fluctuations

The mean autocorrelation of length fluctuations for different illumination times are shown in Fig. 4. Experimental curves for different illumination times follow each other closely, except at the $\Delta t = 0$ point, where the signal drops with increasing illumination time. That is the effect that we expect for a molecule with multiple modes in which the slowest mode carries the highest amplitude. A single-exponential decay provides an unsatisfactory description of the physical system. Note that the number of photons per frame was held constant regardless of illumination time in order to make the contribution of stochastic camera noise equivalent for all curves.

We next explore whether the Rouse-like model of Eq. 7 describes our experimental result for the extension fluctuation autocorrelations. In Fig. 4, we clearly see that the prediction of the continuous chain model consistently overfits the experimental result at short time scales. This overfitting cannot be caused by noise from the measurement or fitting procedures, since in all cases we would always expect the

FIG. 4. (Color) Autocorrelation of end-to-end fluctuations of λ -DNA at varying duty cycles. Solid lines are the experimental data, circles are simulated data of a 64-bead harmonic oscillator chain, and crosses are analytical predictions. The frame rate was 20 Hz, and the duty cycle was 10% (blue), 30% (green), 90% (red). The black dashed line is a single-exponential fit.

experimental data to exceed the prediction at the $\Delta t = 0$ point for stochastic errors.

For the simulated data from Brownian dynamics, we can obtain lengths both exactly (from bead coordinates), and with the artifacts introduced through camera noise and data processing. In general, we find a good agreement of experiment and simulation within the experimental errors (Fig. 4). Note that the chain used for this numerical model contained as many beads as there are channel width sized blobs in our experimental data.

Since we found agreement between experiment and simulation, but not between experiment and analytical model prediction, we proceeded to test the source of the divergence of the analytical model. The conceivable sources would be a fundamental difference between the finite chain and the infinite one, or the experimental sampling of physical system. In Fig. 5 we compare extension fluctuation correlations for the exact numerical end positions in the simulated data of the 64-bead chain with the decay resulting from the analytical eigenvalues and eigenmodes of Eq. 2 if all eigenmodes are assigned equal energies. We find excellent agreement. However, for the same fundamental relaxation, the continuous chain has considerably higher amplitudes at shorter times. Hence the overfitting of Eq. 7 appears chiefly due to a breakdown of the continuous assumption. We should not be at all surprised by that fact. Indeed, the ratio of fluctuation amplitude versus contour length diverges as the chain is made smaller and smaller, and so we should expect such a breakdown of the continuous model. A chain with finite extensibility, or discrete beads must thus experience a reduction in mode amplitudes.

We next investigate the influence of the sampling scheme using the simulated 64-bead chain (Fig. 6). We find that the combination of optical blurring, Poisson noise, and numerical fitting leads to a systematic underrepresentation of the actual end-to-end distance. However, that underrepresentation is not sufficient to explain the large difference between experiment and analytical continuous model.

We now turn our attention to the question whether a method can be found that could identify discrete relaxation modes of the end to end fluctuation autocorrelation in the absence of a known eigenbasis of such modes. Perkins *et al.*

FIG. 5. Applicability of discrete vs continuous mode. Differences of autocorrelation of extension fluctuations between experiment and eigenmodes of discrete model (solid) or continuous model (dashed), respectively. Both axes are normalized to the amplitude and relaxation time of the fundamental mode, which is chosen equal for both models.

FIG. 6. (Color) Influence of the measurement process on the autocorrelation of fluctuations in the end-to-end distances from Brownian dynamics simulation. The solid black curve is the instantaneous, exact curve. The exact simulated autocorrelation under experimental time sampling (solid lines), the fitted simulated autocorrelation with time sampling, optical blurring, and fitting (circles), and the latter with addition of Poisson noise (crosses) are shown for $t_f/\Delta t = 0.1, 0.3, 0.9$ in blue, green, and red, respectively. The frame to frame time Δt is about 10% of the relaxation time of the fundamental mode. Both axes are normalized to the amplitude and relaxation time of the fundamental mode, respectively.

have used an inverse Laplace transform in their study of the relaxation of a polymer strand released from tension,² and Cohen *et al.* have used principal component analysis of density modes.³⁹ The potential problem is that multiple mathematical descriptions could be of equivalent quality within the experimental error.

We show the result of Laplace transforms of the end to end distance of λ -DNA in Fig. 7. We find a broad distribution of modes, with a shape consistent to a stretched exponential with $\beta \approx 0.7$. Inverse Laplace transforms are not exactly solvable on a finite frame of data, and thus making assumptions is often used to find narrow modes.⁴³ If we force our transform to narrow the distribution, we do obtain modes which are mutually inconsistent between different illumination times, and thus appear not physical. Utilizing the stretched exponential assumption, we do indeed find reasonable agreement between experiment and fitted curve, as well as good agreement between harmonic Brownian dynamics simulation (with 64 beads). That is remarkable since the numerical model does not contain any heterogeneities of elastic properties or hydrodynamic effects that are commonly attributed to the nonobservance of discrete modes in

FIG. 7. Inverse Laplace transform of end to end fluctuation autocorrelation. Curves for 10% duty cycle (dashed), 30% duty cycle (dash-dot), and 90% duty cycle (solid) are offset for easy readability, and the relaxation time of the fundamental mode is about 9 frames. Broadened distributions reminiscent of stretched exponentials are found, but no discrete modes at short Δt . The maximum is the maximum of the fundamental mode.

FIG. 8. Amplitude of density fluctuations $I(k)$ as a function of k . The error bars are the one-sigma confidence intervals of the decay curve fits.

disordered polymer systems, and the harmonic chain certainly does contain discrete decay modes. We conclude that the inverse Laplace transform is not of high utility in this case.

B. Amplitudes of density modes in the interior

In the consideration of end-to-end fluctuations we were limited to remarking that short wavelength modes appear not to carry as much amplitude as expected from the continuous harmonic chain model. The spectrum of density fluctuations should however be able to quantify how much amplitude is present in each mode. We had argued above that the density fluctuation spectrum should be white for a continuous harmonic chain once the response function of the experiment is accounted for according to Eq. 10. While we could use the $\Delta t = 0$ point of the k -dependent density autocorrelation to estimate the mode amplitude, we have previously argued that that point is susceptible to contamination by stochastic noise. Thus we will use amplitude of exponential fits to decay curves. This data set is similar to the one published by us,¹⁶ and is thus not discussed at length here.

The resulting graph of apparent amplitude $I(k)$ as a function of k for λ -DNA quadromers after removal of diffraction limit and illumination time artifacts is shown in Fig. 8. The upper k limit was imposed by our need to be able to determine all dynamic parameters of the time correlation for each k , which naturally restricts the relaxation time of a mode roughly to the inverse frame rate of the video. We find an apparent reduction in the amplitude at high k , which is consistent with the previous observation that the contributions of high- k

FIG. 9. Amplitude of density fluctuations $I(k)$ as a function of k from discrete harmonic Brownian dynamics simulation, and after undergoing the same analysis routine as the data in Fig. 8.

FIG. 10. (Color) Mean normalized autocorrelation of global Fourier transform of a set of ten λ -DNA molecules observed using 20 Hz frame rate and a 30% duty cycle. The color map is logarithmic. $\sqrt{\langle \Delta L^2 \rangle}/L$ is in the vicinity of 7% for these molecules.

modes to the end-to-end distance are far below the anticipated magnitude. A simulated discrete chain also showed a drop-off at high k (Fig. 9), although not as pronounced as the experimental data did. We currently do not know whether the lower amplitude in the lowest k in the simulated data is a sampling artifact, or an actual feature.

C. Global transform method

The normalized autocorrelation relaxation landscape of the global Fourier transform for a set of λ -DNA molecules is shown in Fig. 10. We find that the mean autocorrelation of the measured λ -DNA ($\sqrt{\langle \Delta L^2 \rangle}/L \approx 7\%$) resembles the analytically calculated surface for $\gamma/L = 0.075$ (Fig. 2). At such high relative fluctuation amplitudes, we are not able to identify the signature of discrete modes, should they exist. We note that experimental curves such as Fig. 10 contain a transformation artifact due to the implied periodicity of the

FIG. 11. (Color) Normalized autocorrelation of global Fourier transform for a set of λ quadromers. The color map is logarithmic.

FIG. 12. Sections of the autocorrelation of global Fourier transform for long molecule along k indicated in Fig. 11.

Fourier transform over a finite interval. We can choose the location of the artifact by choosing the ratio between length of transformation frame and molecule length. If a long transformation frame is chosen, the artifact is located at low k , while a short frame will locate the artifact at high k . However, since a longer transformation frame results in a higher k resolution, we typically pad the array to make the molecule a fraction of the entire transformation interval. We have not included this effect into the analytical form in the theory section.

Since λ -DNA monomers have a high relative fluctuation amplitude, and thus strong density mode mixing, we turn to λ -DNA quadromers to see if we can identify discrete odd and even ν modes from the normalized autocorrelation of the Fourier transform. In Fig. 11 we can clearly identify fast and slow modes in a landscape that shows the expected pattern for a long γ/L molecule. In order to reduce noise, we averaged over the first 3 true valleys and hills, and obtained relaxation curves similar to Fig. 3 (shown in Fig. 12). We can see a clear decoupling of the fast modes from the end to end fluctuations at short times, while at long times the fundamental mode dominates. The almost single exponential nature between 0.5 and 1.7 s suggests that modes may be discrete and resolvable.

D. Conclusion

We have demonstrated that the fluctuations of nanoconfined DNA on large length scales can be understood in terms of normal modes of an oscillator chain. We have also shown that the description breaks down beyond a length scale at which finite extensibility and volume interactions become dominant. Strong indication was found that a careful examination of the fluctuation properties of nanoconfined DNA necessarily has to include the effects of illumination time and relaxation dynamics.

ACKNOWLEDGMENTS

We acknowledge funding from the National Institutes of Health (1R21CA132075). This work was performed in part at the Cornell NanoScale Facility, a member of the National

Nanotechnology Infrastructure Network, which is supported by the National Science Foundation (Grant ECS-0335765).

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