Density fluctuations dispersion relationship for a polymer confined to a nanotube

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DNA confined to rigid nanotubes shows density fluctuations around its stretched equilibrium conformation. We report an experimental investigation of the length-scale dependent dynamics of these density fluctuations. We find that for highly elongated molecules a Rouse description is consistent with observations at sufficiently large length scales. We further find that for strongly fluctuating molecules, or short length scales, such Rouse modes cannot be detected due to strong mixing of fluctuation modes. © 2011 American Institute of Physics. [doi:10.1063/1.3602922]

DNA stretching in nanochannels is an emerging technique for genetic and epigenetic analysis of DNA.¹ Apart from this practical motivation for investigating the dynamics of the stretching process, the DNA-nanochannel system is also a perfect test bed for the verification of models in polymer physics. That is in particular the case since the crossover between the major confinement regimes is possible within a single set of experiments.² It has further importance in testing the basic dynamics that underly polymer-polymer diffusion processes, since the well-defined geometry is a controllable implementation of reptation tubes.^{3,4} We are here addressing the question of fluctuations of a polymer within such tubes as a function of length scale. This letter was inspired by recent progress in the study of fluctuations in unconfined or weakly confined polymers.^{5,6} In particular, for principal components (PC) of fluctuation of free DNA good agreement of experiment and theory was obtained while it was also established that the dynamics of these PC are not those of Zimm modes.⁷⁻⁹ The work in nanochannels builds on a growing set of dynamic experimental studies of DNA in nanochannels,^{10,11} and recent insights gained through simulation and theory.^{12,13} We will report the observation of Rouse modes in the nanochannel system.

DNA in one-dimensional nanochannels a few persistence lengths wide is described by noninterpenetrating blobs that line up to extend the molecule along the channel.³ The lateral size of blobs is given by the channel dimensions while their longitudinal extent or nature is a subject of some discussion. When viewed at a sufficiently large length scales and at equilibrium, the details of the elongation mechanism are not observable due to the central limit theorem. While nanoconfined DNA has negligible long-range hydrodynamic self-interaction, strong hydrodynamic interaction with the side-walls is predicted to lead to an extension-dependent drag on the molecule.¹⁴ However, as long as the extension fluctuations are small, that is as long as the considered length-scales are large, we again expect that a constant friction per contour length can be assumed, and thus Rouse dynamics are expected.⁴

The eigenmodes of the autocorrelation of the monomer displacement from the equilibrium are the Rouse modes with circular wave number (or wave vector) $k = \pi\nu/L$ for mode

number ν . The corresponding relaxation times are $\tau(k) = \xi/\kappa k^2$, and the mode amplitudes are $a(k) = k^{-1}\sqrt{2k_B T/\kappa L}$. Here κ is the elastic modulus and ξ the friction coefficient per unit length. The functional relationship of κ and ξ with respect to channel width, persistence length, and effective width have been discussed elsewhere, and are not essential here.¹¹

The core challenge in verifying this model is that it describes positions, and not the density that is observable. The only position information that is easily accessible is the location of molecule ends. Because of the relative strength of modes within the molecule, it is not possible to observe any but the lowest mode as an identifiable line in a semilogarithmic plot of the extension fluctuation autocorrelation. The obvious alternative, to obtain position information from densities through integration of the intensity along the channel, is challenged by noisy signals. While it is possible to recover Rouse modes from simulated data, we were not able to do so as long as a noise term replicating the experimental procedure was applied. A PC analysis leads to Rouse-like modes, which however display irregular relaxation times and non-monotonic mode amplitudes when viewed as a function of mode number.

A common strategy in obtaining length-scale dependent dynamical data is to use the Fourier transform of the polymer density. However, the transform along the channel axis is dominated by the transform of the average fluorescence intensity, which is a box-car function. The resulting sinc^2 envelope is strongly modulated by the end-to-end distance for most k , and thus it contains little useful information about the internal relaxation dynamics of higher modes. We overcome this effect by excluding the edges, and only transforming the interior of the molecule.

All experiments used mixed micro- and nanofluidic devices made from fused silica, which were prepared by methods discussed elsewhere.¹⁵ Nanofluidic channels with 160×80 or 260×150 nm² cross-sections and a length of 200 μm were placed between microchannels that carried solution from the injection ports of the device to the active zone. λ -DNA and λ -DNA concatemers were suspended in 0.5x Tris-Borate EDTA (TBE) buffer and fluorescently stained using an intercalating dye (YOYO-1) at a ratio of one dye per five basepairs. The local DNA mass density was determined from the fluorescence intensity collected by a

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FIG. 1. Fluorescence intensity along a λ -DNA molecule in a $160 \times 80 \text{ nm}^2$ channel. (a) Full molecule with fixed center of mass, (b) sub-frame used for dynamic mode analysis.

microscope. DNA was injected into nanochannels through application of voltages at the microfluidic ports. Image frames were taken under strobed illumination, with 10 or 20 ms flash lengths. Fluctuations with a relaxation time below the flash length were not measurable. More details on experimental setup and materials are provided in the online supplement.¹⁶

Figure 1 shows the intensity profile of a λ -monomer in a $160 \times 80 \text{ nm}^2$ nanochannel as it assumes an extension that is roughly 50% of its contour length. For each frame we then Fourier transformed the fluctuations around the normalized fluorescence intensity along molecules. The length of the transformed window was chosen such that ends did not intrude into the transformed frame, or about 80% of the equilibrium extension. The center of the transformed window was the center of mass, as shown in Fig. 1(b).

The real part of the time autocorrelation of the transformed intensity was calculated for each molecule [Fig. 2(a)]. Time autocorrelations are not purely exponential, chiefly due to inhomogeneities in the staining density along the polymer, and mixing of modes. The former lead to finite correlations at infinite time lags Δt , as seen in Fig. 2(a). For quantification of the correlation time we assume that at short times a dominant time scale exists, and thus fitted correlations to $\rho_k e^{-\Delta t / \tau(k)} + \rho_{k,0}$. Correlations after removal of the correlation at infinite Δt are shown in Fig. 2(b). At low photon fluxes the $\Delta t=0 \text{ s}$ point also contains the square of the approximately k -independent noise amplitude due to Poisson photon counting statistics. If such a correlation was detected, the $\Delta t=0 \text{ s}$ point was excluded from fitting the relaxation dynamics. The expected mixing of modes stems both from the fact that position fluctuations of blobs couple into density fluctuations of modes with different k , and that our chosen k potentially are not the normal modes of the Rouse description. Not having chosen the normal modes does nevertheless not modify the expectation that the short-time relaxation dynamics follow a $\tau(k) \propto k^{-2}$ scaling.

For λ -DNA quadromers in $160 \times 80 \text{ nm}^2$ channels we observed a $\tau(k) \propto k^\gamma$ relationship with $\gamma = -1.8 \pm 0.2$, where the error is 95% confidence of the fit (Fig. 3). That is con-

FIG. 2. Fluctuation autocorrelations for λ -DNA monomers in $160 \times 80 \text{ nm}^2$ channels. (a) Raw correlations for single molecule. (b) Mean correlation for ten molecules at $k=1.4 \mu\text{m}^{-1}$ (solid line) and $k=2.8 \mu\text{m}^{-1}$ (broken line) after removal of infinite-time offset. Markers show average data, error bars are the standard deviations, and curves are single-exponential fits.

sistent with the power of -2 for the pure Rouse modes, as well as the -1.75 obtained by Arnold *et al.* from molecular dynamics. However, for λ -DNA monomers an exponent of -1.13 ± 0.20 was observed. The apparent discontinuity between the curves for short and long molecules also needs to be explained. The nonideal behavior of short molecules could be either due to a breakdown of the harmonic oscillator assumption, or an artifact of the sampling approach.

While we cannot exclude the former, we have clear indication that the latter becomes significant for molecules whose extension fluctuations become a significant fraction of the equilibrium extension. In particular, λ -DNA monomers

FIG. 3. Relaxation times as a function of k in $160 \times 80 \text{ nm}^2$ channels. The solid and broken lines show λ -DNA quadromers and monomers, respectively. Bars represent the 95% confidence interval from fitting correlation vs time lag curves.

confined to $260 \times 150 \text{ nm}^2$ channels are shorter and softer,¹¹ and thus have an increased relative extension fluctuation of 13%. The expected mode auto-correlation times are thus faster than our frame-to-frame time, and hence any correlation observed at frames with $\Delta t \neq 0 \text{ s}$ must be linked to the dynamics with lower k vector than the basis of the transform. We find that relaxation times at 2.4 and $4.8 \mu\text{m}^{-1}$ are both 0.1 s . Since the extension correlation is dominated by a mode with a wavelength twice the molecular extension, we can estimate $\tau = 0.49 \text{ s}$ for the fundamental mode from the relaxation time of the extension autocorrelation. However, since this mode is symmetric, it couples only weakly into our analysis. The first mode to do so is the asymmetric mode with a wavelength identical to the extension, and thus a relaxation time of 0.12 s . The approximate agreement of the relaxation time of this mode with the correlation time observed at all higher k vectors indicates that a transformation artifact of this mode is at the source of the apparent correlation dynamics.

This mixing of desired mode and lower-order asymmetric modes should lead to a reduction in the scaling exponent between τ and k and increase the relaxation time for all molecules that have a high relative extension fluctuation amplitude. Since the magnitude of extension fluctuations scale roughly as the square root of the extension, this is only the case for the shorter molecules in Fig. 3. The magnitude of fluctuations for λ -monomers in narrow channels was $\sim 7\%$, and for λ -quadromers was $\sim 3\%$. We have tested that hypothesis by comparing the experimental results to a Brownian dynamics simulation of an overdamped oscillator chain. The model was coarse-grained to well beyond the channel diameter (the size of self-avoiding blobs in De Gennes' model) since unphysically large local fluctuation amplitudes were required to generate the observed macroscopic fluctuations. The model was robust within our experimental uncertainty under changes that replaced perfectly harmonic blobs with ones that have a more realistic force-extension law and possible hydrodynamic screening. A relationship of $\tau \propto k^{-1.8}$ was found for long molecules, similar to our experimental results. The simulation showed that a reduced slope for shorter molecules and a brake in the relaxation times between short and long molecules can be caused through sampling effects of shorter molecules. We thus believe that our interpretation of the experimental results is valid, and that a

normal mode picture is correct as long as fluctuations are small.

In summary, we have introduced an analysis that allows the observation of the dispersion relationship of density fluctuations in a nanochannel. We have found that the measurements for long molecules with small extension fluctuations are consistent with a $\tau \propto 1/k^2$ relationship. However, for molecules with large extension fluctuations the relationship is masked by the mixing of modes imposed through our analysis technique. The mixing is dominated by the lowest asymmetric mode of the polymer, which directly leads to a transformation artifact.

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¹⁶See supplementary material at <http://dx.doi.org/10.1063/1.3602922> for experimental details, additional data confirming assertions made in the manuscript, and a description of the Brownian dynamics simulation.