Supplementary material to: Internal mechanical response of a polymer in solution

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Below we give details of materials and methods, and algorithms for analyzing and interpreting DNA conformational fluctuations. Throughout, we index quantities both by their time of measurement, t, and their measurement number, k.

I. SAMPLE PREPARATION

Double-stranded λ-DNA was dissolved in a buffer of 10 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA, pH 8.0. The fluorescent dye YOYO-1 (Molecular Probes) was added at a concentration of 1 dye:10 base pairs of DNA. Imaging was performed in the presence of an anti-adsorption polymer (POP-6, without denaturant, Applied Biosystems) and a molecular oxygen scavenger.[1] The ABEL trap cell was placed on an inverted optical microscope (TE300, Nikon), and epifluorescence images were acquired on a fast EMCCD camera (Cascade 512B, Roper Scientific) using an oil-immersion objective with a numerical aperture of 1.3.

II. IMAGE ACQUISTION AND PRE-PROCESSING

The video images were formatted for data analysis as follows. Each frame was 32 x 32 pixels, with a pixel width corresponding to 118 nm in the sample plane. The small image size was chosen to allow a fast video frame-rate. A background image (acquired under identical conditions to the data, except with no DNA in the field of view) was subtracted from each frame. In a small fraction of the frames ($\sim 5\%$) a second DNA molecule was seen floating through the field of view. In these frames, the pixels affected by the second molecule were manually set to the background level. When the images were shifted to align the center of mass between frames, shifts by a fraction of a pixel were accomplished with a bicubic interpolation. The total intensity of each frame was normalized to account for the slow rate of photobleaching of the YOYO-1 during the trapping period. For the present analysis, the data from all 21 molecules was aggregated.

A. Determination of center of mass

The CCD registered roughly 1000 photons per frame (calibrated by comparing the CCD signal with the counts from an APD subject to the same illumination). The localization accuracy of each photon is limited by the point-spread function of the microscope to 250 nm. Collecting N photons improves the accuracy of localization of the center of mass by a factor of $N^{1/2}$, so the shot-noise limit is 8 nm. In addition to the shot noise, the center of mass diffuses an r.m.s. distance of 55 nm during the 4.5 ms exposure time. Both of these uncertainties are much less than the pixel size (118 nm) or the optical resolution (250 nm). Since we do not consider features smaller than 250 nm, the errors introduced by uncertainty in the position of the center of mass are small. Furthermore, localization errors are expected to be uncorrelated between successive video frames. Thus these errors do not affect correlation functions between quantities measured at unequal times

B. Correspondence between fluorescence intensity and molecular density

There are two sources of error in mapping fluorescence intensity into molecular density: a) the stochastic incorporation of dye molecules in the DNA backbone and b) the stochastic generation of photons from each dye molecule. Process (b) is uncorrelated from frame to frame, so all correlations between quantities taken from different frames are insensitive to photon shot-noise. We labeled the DNA with 1 dye:10 base pairs, or ~ 5000 dyes/molecule. The DNA images had an average area of 2 μm^2, corresponding to 32 diffraction-limited spots. Thus on average there were ~ 156 dye molecules/diffraction-limited spot, corresponding to an error in determining the density of $156^{-1/2} \sim 8\%$.

III. ROUSE SIMULATION OF DYNAMICS

Our minimal model of the DNA consisted of a chain of 150 beads joined by linear springs. Each bead experienced the same thermal and viscous forces it would have experienced in isolation from the rest of the chain (i.e. no hydrodynamic interactions). The only other force was from the springs joining nearest neighbors. The motion of the chain was simulated using Brownian dynamics. At each time-step the entire chain was translated to keep the center of mass fixed at the origin. The conformation of the chain was then projected as an intensity

distribution on a 2-D array. We simulated the dynamics of this chain for 10^4 relaxation times. Simulations were performed in MATLAB. The simulated data was analyzed in exactly the same way as the real data. For comparison to experiment, the overall chain relaxation time and the radius of gyration were matched to the values for λ-DNA.

IV. PSEUDO-FREE TRAJECTORIES

The technique of pseudo-free trajectories mathematically undoes the effect of the feedback, to recreate a center of mass trajectory statistically similar to the one the particle would have followed had it not been trapped. One extracts the mobility μ from a least-squares fit of $d\mathbf{x}/dt$ vs. $\mathbf{E}$. The residuals from this fit are the Brownian displacements $\xi(t)$. Two facts complicate this task:

1. The electric field $\mathbf{E}$ is related to the voltage $\mathbf{V}$ by a 2×2 matrix, $\mathbf{C}$, which is a function of the location of the target position for the molecule. The off-diagonal elements of $\mathbf{C}$ are typically small and arise when the target position is not exactly in the geometrical center of the trap: due to fringing fields, $\mathbf{E}$ may not be parallel to $\mathbf{V}$. While $\mathbf{C}$ may be estimated from finite-element simulations of the electric field, these simulations do not take into account imperfections in the sample cell and other experiment-specific factors. Thus it is best to treat the matrix $\mathbf{C}$ as an a priori unknown quantity.

2. The feedback voltage $\mathbf{V}[k]$ switches to $\mathbf{V}[k+1]$ at a time intermediate between t_k and t_{k+1}. Thus $\mathbf{x}[k+1]-\mathbf{x}[k]$ depends on both $\mathbf{V}[k]$ and $\mathbf{V}[k+1]$.

The above considerations lead to a multivariate linear model with 8 free parameters, $\alpha_{i,j}^{(0,1)}$:

$$\begin{pmatrix} x[k+1]-x[k] \\ y[k+1]-y[k] \end{pmatrix} = \begin{pmatrix} \alpha_{xx}^{(0)} & \alpha_{xy}^{(0)} & \alpha_{xx}^{(1)} & \alpha_{xy}^{(1)} \\ \alpha_{yx}^{(0)} & \alpha_{yy}^{(0)} & \alpha_{yx}^{(1)} & \alpha_{yy}^{(1)} \end{pmatrix} \begin{pmatrix} V_x[k] \\ V_y[k] \\ V_x[k+1] \\ V_y[k+1] \end{pmatrix} + \begin{pmatrix} \xi_x[k] \\ \xi_y[k] \end{pmatrix}. \qquad (1)$$

For a trapped particle, one knows $\mathbf{V}[k]$ and $\mathbf{x}[k]$. One can use multivariate least squares fitting to extract the 8 unknown coefficients. The cumulative sum of the residuals, $(\xi_x[k], \xi_y[k])$, yields the pseudo-free trajectory.

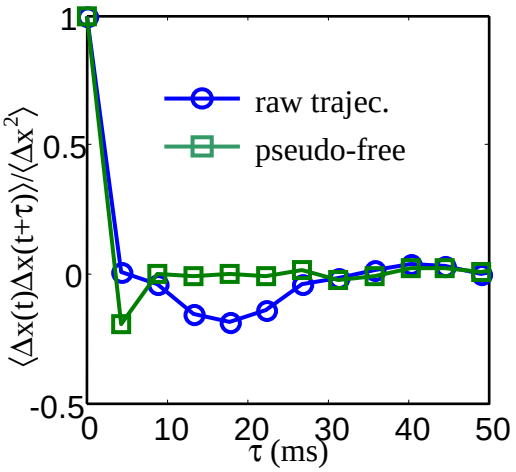

FIG. 1: Two-time autocorrelation of the displacements in the measured and pseudo-free trajectories. The slow overshoot in the measured trajectories is due to the finite response time of the feedback: at the settings used in this experiment, the feedback was slightly underdamped. Calculating the pseudo-free trajectory undoes the effects of the feedback.

Pseudo-free trajectories for trapped DNA molecules were calculated as outlined above. Fig. 1 shows the autocorrelation functions of the measured x-displacements and of the reconstructed pseudo-free trajectory. The negative autocorrelation in the pseudo-free displacements at lag = 1 is due to localization noise.[2]

V. FLUCTUATING TRANSPORT COEFFICIENTS

In the simplest model of a rigid, non-interacting particle in the ABEL trap, the parameters $\alpha_{i,j}^{(0,1)}$ are independent of time and the Brownian displacements $\xi[\mathbf{k}]$ are statistically independent and identically distributed. These conditions may be violated if the particle undergoes conformational fluctuations, ionization events, or binding and unbinding with other objects in the solution. Fluctuations in $\alpha_{i,j}^{(0,1)}$ or in the variance of ξ lead to heteroskedasticity in the measured trajectories, which can be detected in the residuals from the fit to Eq. 1.

A. Fluctuating mobility

First we consider fluctuations in the mobility. Theory predicts that the free-solution mobility of DNA should be independent of conformation,[3] but it is worth checking whether this prediction is supported by the data. A simplified 1-D version of Eq. 1 is:

$$\Delta x[k] = (\alpha + \delta\alpha[k])V[k] + \xi[k], \tag{2}$$

where α is related to the time-average mobility, $\delta\alpha[k]$ is related to the fluctuations in mobility, and ξ is Gaussian white noise with variance σ_ξ^2. A least-squares fit of Δx against V yields α, with residuals (i.e. pseudo-free hops) $Q[k] = \delta\alpha[k]V[k] + \xi[k]$. Assuming the fluctuations $\delta\alpha$ are uncorrelated with the voltage V, the autocovariance of the residuals is

$$\begin{aligned} \left\langle Q[k]^2 \right\rangle &= \left\langle V[k]^2 \right\rangle \left\langle \delta\alpha[k]^2 \right\rangle + \sigma_\xi^2 \\ \langle Q[k]Q[k+h] \rangle &= \langle V[k]V[k+h] \rangle \langle \delta\alpha[k]\delta\alpha[k+h] \rangle \end{aligned} \tag{3}$$

Thus fluctuations in the mobility show up in the autocorrelation of the steps of the pseudo-free trajectory. Fig. 1 shows that in our data the autocovariance is a δ-function, except for the noise-induced bounce at lag = 1. Thus we detect no fluctuations in the electrokinetic mobility of λ-DNA.

B. Fluctuating diffusion coefficient

Now we consider a fluctuating diffusion coefficient. Assume that the mobility is constant. Then the pseudo-free trajectory obeys

$$\Delta x[k] = \sqrt{2D[k]\delta t}N(0,1) \tag{4}$$

where $N(0,1)$ is Gaussian white noise with mean 0 and variance 1 and $D[k]$ is the fluctuating diffusion coefficient. Now consider the 4^{th} order correlation,

$$\begin{aligned} C^{(4)}[h] &= \operatorname{corr}\left(\Delta x[k+h]^2, \Delta x[k]^2\right) \\ &= \frac{\langle \Delta x[k+h]^2 \Delta x[k]^2 \rangle - \langle \Delta x[k]^2 \rangle^2}{\langle \Delta x[k]^4 \rangle - \langle \Delta x[k]^2 \rangle^2}. \end{aligned} \tag{5}$$

The normalization is chosen so that $C^{(4)}[0] = 1$. Each of the ingredients of Eq. 5 can be expressed in terms of correlation functions of D:

$$\begin{aligned} \left\langle \Delta x[k+h]^2 \Delta x[k]^2 \right\rangle &= 4\delta t^2 (1 + 2\delta[h]) \left\langle D[k+h] D[k] \right\rangle \\ \left\langle \Delta x[k]^2 \right\rangle &= 2\delta t \left\langle D[k] \right\rangle \\ \left\langle \Delta x[k]^4 \right\rangle &= 12\delta t^2 \left\langle D[k]^2 \right\rangle, \end{aligned} \tag{6}$$

where we used the fact that $\langle N(0,1)^2 \rangle = 1$ and $\langle N(0,1)^4 \rangle = 3$. Making the above substitutions into Eq. 5 yields

$$C^{(4)}[h] = \frac{(1 + 2\delta[h]) \left\langle D[k+h] D[k] \right\rangle - \left\langle D[k] \right\rangle^2}{3 \left\langle D[k]^2 \right\rangle - \left\langle D[k] \right\rangle^2}. \tag{7}$$

Thus the 4^{th} order correlation $C^{(4)}[h]$ is sensitive to fluctuations in the diffusion coefficient of the particle which might arise, for instance, from conformational fluctuations that change its hydrodynamic radius.

C. Spherical cow model of fluctuating D

Here we describe the "spherical cow" model (black line in Fig. 5), which models the diffusion as a sphere with fluctuating radius $R_G[k]$. The 2-D radius of gyration, $R_G[k]$, at video frame k, is:

$$R_G[k] = \left(\sum_i (\mathbf{r}_i - \mathbf{r}_{CM})^2 I_i[k] \right)^{1/2}, \tag{8}$$

where the index i runs over all pixels in the image, $\mathbf{r}_i$ is the position of pixel i, $\mathbf{r}_{CM}$ is the position of the center of mass, and I_i is the intensity at pixel i (recall that the images are normalized so $\sum_i I_i[k] = 1$).

In the Zimm model, the translational diffusion coefficient is:

$$D = 0.196 \frac{k_B T}{\sqrt{6} \pi \eta R_G} \tag{9}$$

where η is the viscosity of the medium. We calculated a time-dependent diffusion coefficient, $D[k]$, by substituting $R_G[k]$ of Eq. 8 for R_G in Eq. 9.

The 1-D diffusion of a particle with changing radius, sampled at discrete intervals δt, obeys:

$$x[k+1] = x[k] + \sqrt{2D[k]\delta t} N(0,1), \tag{10}$$

where $N(0,1)$ is Gaussian white noise with mean 0 and variance 1. Multiplication of the simulated trajectory by a constant factor does not affect the value of $C^{(4)}[k]$. Thus we simulated trajectories according to the rule:

$$x[k+1] = x[k] + N(0,1)/\sqrt{R_G[k]}, \tag{11}$$

which uses the instantaneous radius of gyration to set the diffusion coefficient and hence the next displacement at each time step. From the simulated trajectories we extracted $C^{(4)}[k]$ in the same manner as for the pseudo-free trajectories. We simulated 1000 trajectories, each of length 58,421 (the same length as the data). This approach avoids the problem of performing a full simulation of the polymer dynamics, which would have required us to assume a model for the internal dynamics. It is remarkable that this very crude approximation is in such good agreement with the data.

It is also possible to calculate $C^{(4)}[k]$ directly from the time-varying radius of gyration. Inserting the displacements of Eq. 11 into Eq. 5 and assuming the fluctuations in R_G are small, yields:

$$C^{(4)}[k] = \frac{\mathrm{cov}(R_G[k+h], R_G[k])}{2\left\langle R_G \right\rangle^2}, \tag{12}$$

i.e. $C^{(4)}[k]$ is a direct probe of the conformational fluctuations. The value of $\lim_{k\to 0} C^{(4)}[k]$ can be estimated from a random walk model of the polymer conformation, without making assumptions about the internal dynamics. We simulated 10^6 polymer conformations, where each conformation was a Gaussian 3-D random walk of $N = 100$ steps, and then extracted R_G from each conformation. From Eq. 12 we obtained $\lim_{k\to 0} C^{(4)}[k] \approx 0.0278$. This result is independent of N for $N > 50$ and is somewhat larger than our experimental result, implying that the molecule exists in between the non-draining and freely draining limits. Based on a calculation using only the dominant conformational mode, Dubois-Violette and de Gennes estimated $\lim_{k\to 0} C^{(4)}[k] \approx 0.017$.

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[1] A. E. Cohen and W. E. Moerner, Proc. Natl. Acad. Sci. USA **103**, 4362 (2006).

[2] A. E. Cohen, Ph.D. thesis, Stanford University (2006).

[3] J.-L. Viovy, Rev. Mod. Phys. **72**, 813 (2000).