Supporting information for:
Nanoconfinement greatly speeds up the nucleation and the annealing in single-DNA collapse

Liang Dai¹, Jeremy J. Jones², Alexander R. Klotz², Stephen Levy³, and Patrick S. Doyle¹,²*

¹BioSystems and Micromechanics IRG, Singapore-MIT Alliance for Research and Technology Centre, Singapore 117543
²Department of Chemical Engineering, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139
³Department of Physics, Binghamton University, Binghamton, NY 13902
*Email: pdoyle@mit.edu

S1. Bulk experiments

Experimental chambers for bulk experiments were made using two coverslips. The coverslips were first cleaned by immersing them in a 1M NaOH bath for at least 1 hour. The chambers were sealed on two edges by sandwiching strips of parafilm in between the glass slips and then subsequently melting the film. This created a channel of approximately 1cm x 1cm x 100µm tall channels. Experimental samples were immediately loaded into the chamber using a pipette and capillarity. The chambers were filled with the experimental buffers and the opened ends were sealed using vacuum grease. The experimental buffer consisted of 1.5 TBE (134 mM Tris base, 134 mM boric acid, and 3 mM EDTA), 4 vol% β-mercaptoethanol (BME, Cabiochem) and ethanol. The chambers, filled with experimental solutions, were allowed to sit for variable amounts of times before experimental observation.

Single DNA molecules were observed using a Zeiss Axiovert 200 microscope with a 100 × NA 1.4 oil-immersed objective. An X-Cite 120 light source filtered using a XF100-2 (Omega) filter set was used to excite the single molecules. In-plane images of single molecules were acquired using a Hamamatsu EB-CCD camera (model 7190-20) and NIH image software at 30 frames per second. A typical experiment involved first adjusting the objective such that the focus was sufficiently away from side walls. Subsequently, 15s of video was acquired. Each movie contained anywhere between 5-10 molecules that stayed in-focus during video acquisition. At least 5 movies per chamber were collected before photobleaching rendered samples unusable. This procedure was repeated for a variety of ethanol concentrations and incubation times. At least 3 chambers were used for each set of conditions.
S2. Slit experiments

Figure S1 shows an image of the slit channel. The experimental buffer for the slit channel experiments contained 35% ethanol and was held constant for all channel depths. This concentration was tuned during the bulk experiments. The channels were filled by capillarity using a 50/50 ethanol/water solution. Subsequently this solution was replaced with deionized water and the channels were rinsed using an electrical potential. To insure channels were clear of particulates, a solution of 1 M NaOH was electroosmotically driven into the channel for 3-4 min using a high electric field (200V/cm) immediately before experiments. Channels were subsequently rinsed with deionized water and then the experimental buffer was flushed into the channels for approximately 1 hour prior to each experiment. DNA was electrophoresed into the channel using an electric field around 20 V/cm. Depending on channel height, the first appearance of DNA in the larger side arm of channel required 5-30 min of applied field.

![Figure S1](image)

**Figure S1.** The device consisted of one 150 μm wide, 1 mm long nanoslit in the center and two 1 cm long deep regions on the sides of the slit.

S3. Data analysis

The in-plane images of single molecules of DNA were analyzed using a combination of Interactive Data Language (IDL) and MATLAB software. We characterize the effective molecule
size with the in-plane radius of gyration $R_{\parallel}$. First, we calculate the center-of-mass vector $\vec{C} = \{C_x, C_y\}$ and the gyration tensor $G$ of the DNA in each frame:

$$C_x(t) = \frac{\sum r_x(t)I(r,t)}{\sum I(r,t)}$$  \hspace{1cm} \text{(S1)}

$$C_y(t) = \frac{\sum r_y(t)I(r,t)}{\sum I(r,t)}$$

$$G_{ij}(t) = \frac{\sum [r_i(t) - C_i(t)][r_j(t) - C_j(t)]I(r,t)}{\sum I(r,t)}, \hspace{0.5cm} i = x \ or \ y; \ j = x \ or \ y$$  \hspace{1cm} \text{(S2)}

where the sum was taken over all pixels spanned by the molecule, $I(r, t)$ is the fluorescence intensity at position $r$ and time $t$. We calculated the two eigenvalues of tensor $G$: $\lambda_1$ and $\lambda_2$, with $\lambda_1 \geq \lambda_2$. We define the major and minor principal axes for a conformation:

$$R_M(t) = 2\sqrt{\lambda_1(t)}, \hspace{0.5cm} R_m = 2\sqrt{\lambda_2(t)}$$  \hspace{1cm} \text{(S3)}

The radius of gyration is calculated as

$$R_{\parallel}(t) = \sqrt{\lambda_1 + \lambda_2}$$  \hspace{1cm} \text{(S4)}

To obtain the rotational relaxation time, we calculated the angle between the major principal axis of the DNA and the x-axis:

$$\theta(t) = \arctan\left(\frac{\lambda_1(t) - G_{xx}(t)}{G_{xy}(t)}\right), \hspace{0.5cm} -\frac{\pi}{2} < \theta < \frac{\pi}{2}$$  \hspace{1cm} \text{(S5)}

The rotational relaxation time $\tau_{\text{relax}}$ can be extracted by fitting a single-exponential function to the time autocorrelation function of $\theta(t)$.

Direct calculation of $R_{\parallel}$ is limited in resolution and accuracy. This problem is more profound when the DNA takes on a highly condensed state in bulk experiments. For condensed DNA conformations, DNA sizes can be more accurately determined through the diffusion coefficient of DNA. Thus, when creating the bulk phase diagram the diffusivity $D$ is used to extract a hydrodynamic radius $R_h$. The in-plane diffusivity is obtained from the in-plane mean-squared displacement (MSD):

$$MSD(\delta t) = MSD_x(\delta t) + MSD_y(\delta t) = 4D\delta t$$  \hspace{1cm} \text{(S6)}

where $MSD_x = \langle [r_{cm,x}(t + \delta t) - r_{cm,x}(t)]^2 \rangle, \hspace{0.5cm} MSD_y = \langle [r_{cm,y}(t + \delta t) - r_{cm,y}(t)]^2 \rangle$, and $\delta t$ is the lag time.
S4. T4-DNA collapse in bulk

Figure S2 shows the images of T4-DNA in bulk with various ethanol concentrations.

<table>
<thead>
<tr>
<th>Incubation Time</th>
<th>0%</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
<th>80%</th>
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<tr>
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<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
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<tr>
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<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure S2.** T4-DNA collapse in bulk with various ethanol concentrations. These images are the original microscopy images in black/white before converted to color for better visualization.
S5. T4-DNA collapse in slits

Figure S3 shows images along T4-DNA collapse in slits. Some videos are also attached in supporting information.

<table>
<thead>
<tr>
<th>Metastable coil</th>
<th>Nucleation and annealing</th>
<th>Collapsed</th>
</tr>
</thead>
<tbody>
<tr>
<td>h=2000 nm</td>
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<td>220 min</td>
</tr>
<tr>
<td>h=549 nm</td>
<td><img src="image2.png" alt="Image" /></td>
<td>80 min</td>
</tr>
<tr>
<td>h=31 nm</td>
<td><img src="image3.png" alt="Image" /></td>
<td>10 min</td>
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</tbody>
</table>

**Figure S3.** Images along T4-DNA collapse in slits.
S6. Comparison of T4-DNA and λ-DNA

Figure S4 compares the collapse and annealing times between T4-DNA and λ-DNA.

**Figure S4.** (Top) The ratio of the collapse time of T4-DNA to the collapse time of λ-DNA. (Bottom) The ratio of the annealing time of T4-DNA to the annealing time of λ-DNA.
S6. Determination of the nucleation time

We approximate that the evolution of $R_\parallel$ in stage 1 and stage 2 can be fit by two straight lines, respectively, and the intersection of these two straight lines corresponds to $\tau_{nucl}$. We also approximate that the intersection is located on one of our data points as shown in Figure S4. For every data point with $t < \tau_{collapse}$, we generate two straight lines from a given intermediate data point to the first data point and the last point with $t \leq \tau_{collapse}$. Three examples are included in Figure S5. We generate two yellow lines from the third data point ($t=30$ min) to the first data point ($t=10$ min) and the 22nd data point ($t=220$ min). We also generate two green lines for the seventh data point ($t=70$ min) and two cyan lines for the fifteenth data point ($t=150$ min). Then, we pick the data point which can minimize the deviation between two straight lines and the actual data. We calculate the root-mean-square deviation between the first line with the corresponding experimental data, which is referred as $\sigma_{line 1}$. Similarly, we calculate $\sigma_{line 2}$. The mean value of $\sigma_{line 1}$ and $\sigma_{line 2}$ is plotted against the index of the data point selected as the intersection in Figure S6. The deviation is minimized at the seventh data point, corresponding to the nucleation time $\tau_{nucl} = 70$ min. The same method has been applied to T4-DNA with other slit heights and also $\lambda$-DNA.

![Figure S5](image-url) Evolution of $\langle R_\parallel \rangle$ for T4-DNA with h= 2000 nm.
Figure S6. The deviation between the two straight lines and the actual data as a function of the index of data point selected as the line intersection.

Figure S7. Evolution of $\langle R_\parallel \rangle$ for T4-DNA with h= 549 nm. The two green lines are the best fit.
Figure S8. Evolution of $\langle R_{\|} \rangle$ for $\lambda$-DNA with $h=2000$, 549 and 250 nm. The green lines are the best fit.