SUPPORTING MATERIAL

Coexistence of twisted, plectonemic, and melted DNA in small topological domains

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Parameters used in the 3 state model

The free energy per base pair in each state follows a parabolic function in σ [1, 2]:

$$G_i(F, \sigma_i) = -g_i(F) + \frac{c_i(F)}{2}(\sigma_i - \sigma_{0,i})^2 + \varepsilon_i,$$

where i denotes the twisted (t), plectonemic (p), or melted (m) state. The values of the individual parameters are shown in Table S1:

Table S1: The force and torque dependent descriptions for the free energy of the 3 state model

	$g_i(F)$	$c_i(F)$	$\sigma_{0,i}$	$\varepsilon_i(k_BT/bp)$	$A_i\,(nm)$	$C_i\,(nm)$
t	$F - \sqrt{k_BT \cdot F/A_t}$	$k_BT\omega_0^2 \cdot C_t\left[1 - \frac{C_t}{4A}\left(\frac{k_BT}{AF}\right)\right]$	0	0	50	100
p	0	$k_BT\omega_0^2 \cdot C_p$	0	0	0	24
m	$1.2(F - \sqrt{k_BT \cdot F/A_m})$	$k_BT\omega_0^2 \cdot C_m$	-1	1.6	4	28

A_i and C_i are the persistence length and twist modulus. $\omega_0 = 2\pi/3.6\,nm = 1.75\,nm^{-1}$, is the inverted pitch of the double helix. All parameters used are the same as [1, 2], except for the twist modulus of melted DNA.

Torque calculation

The torque was calculated separately for each state. The torque is defined as

$$\Gamma_i = \frac{1}{\omega_0}\frac{\partial G_i}{\partial \sigma_i} = k_BT\omega_0 \cdot C_i(\sigma_i - \sigma_{0,i}). \tag{1}$$

The mean torque is calculated as:

$$< \Gamma >= \frac{\sum\limits_{i=t,\,p,\,m} n_i\Gamma_i}{N}. \tag{2}$$

Fluctuations in extension

In Fig. S6 A, we plot the calculated standard deviations (SD) in the extension of a $7.0\,kbps$ DNA molecule from experimental and modeled data (colored and dashed lines, respectively) as a function of the force applied at different linking number densities. Transitions between states are not the only cause of fluctuations in the experimental twist-extension curves. Thermal fluctuations due to the low stiffness of the tether also contribute. Such thermal noise was not included in Fig. 5 A. The magnitude of these fluctuations can be calculated using the equipartition theorem:

$$SD_{thermal} = \sqrt{k_B T / k_z}, \tag{3}$$

where k_z equals $\frac{\partial F}{\partial z}$. In the case of a worm-like chain[3]:

$$k_z = \frac{k_B T}{AL}(1 + \frac{1}{2(1 - \frac{z}{L})^3}). \tag{4}$$

In Fig. S6 A the SD in the extension caused by thermal fluctuations is shown as gray dashed lines. The SD by our 3-state model is obtained by

$$SD_{statistical} = \sqrt{< (\frac{z}{L})^2 > - < \frac{z}{L} >^2}. \tag{5}$$

The black dashed line in Fig. S6 A shows the SD at $\sigma_{tot} = -0.045$. For $F > 1.2\,pN$, where only two states coexist, the experimental data largely follow the thermal fluctuations of the molecule. Between 0.6 and 1.0 pN, we observe increased fluctuations due to the coexistence of the three states. We observe the same trends in the experimental and calculated data, though the amplitude of the fluctuations in the experimental data sometimes exceeds the predicted amplitude.

Some reduction of the fluctuations in extension can be attributed to the slow response time of the bead or to the limited frame rate of the camera. The temporal resolution in the extension is calculated from the corner frequency f_c[4].

$$f_c = \frac{k_z}{12\pi^2 \eta R}. \tag{6}$$

The results calculated for a 7.0 *kbps* DNA molecule are shown in Fig. S7 (black line) using a viscosity $\eta = 1.0 \times 10^{-3}\,Pa\,s$, and a radius of the magnetic bead $R = 0.5\,\mu m$.Imperfect alignment of the magnetic field with the optical axis of the microscope results in small oscillations in extension when the external magnets of the tweezers are rotated[5]. This artifact can be omitted by following the extension of the tether at a fixed linking number.

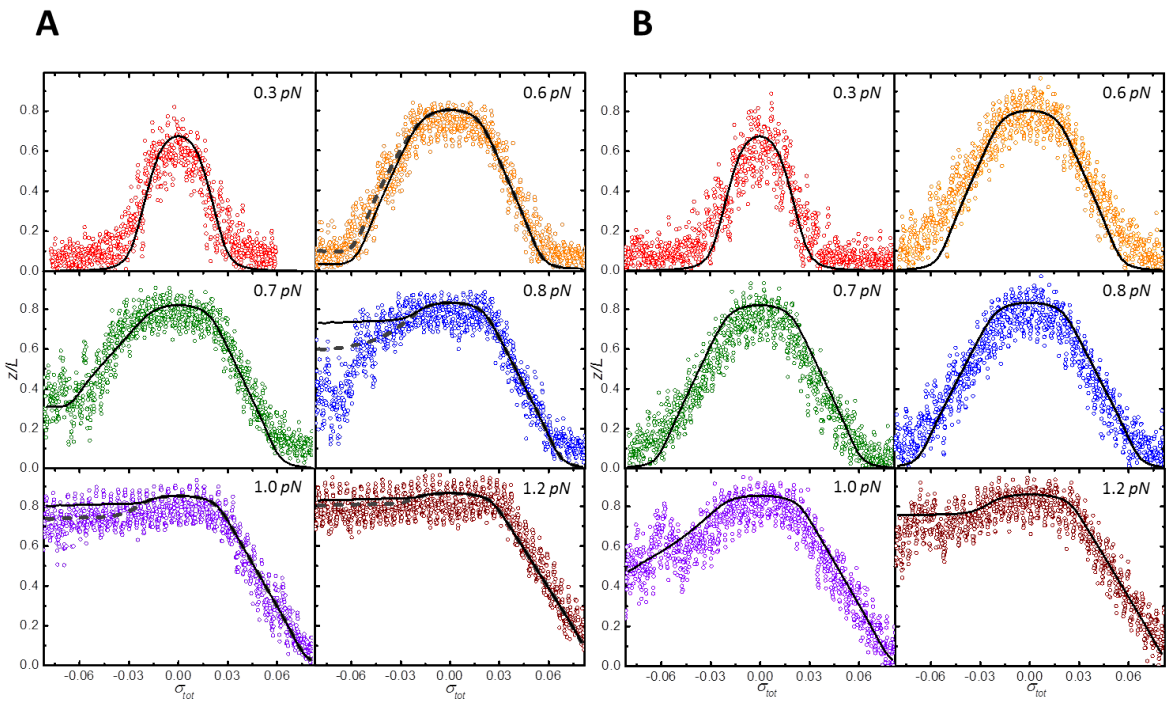

Figure S1

The relative extension as a function of the linking number density of a 2.4 *kbps* DNA molecule in (A) 100 mM KAc at various forces (colored circles). The relative extension as calculated by the numerical model is shown as black solid lines. The dashed lines are the theoretical results for a melting energy that increases linearly with force between $\varepsilon_m = 1.5\,k_BT$ at 0.6 pN to $\varepsilon_m = 2.1\,k_BT$ at 1.2 pN. Such a force dependent melting energy results in a better overlap with the experimental data. (B) Same experiments at 300 mM KAc (colored circles). The relative extension as calculated by the numerical model is shown as black solid lines using a melting energy of $2.0\,k_BT/bp$.

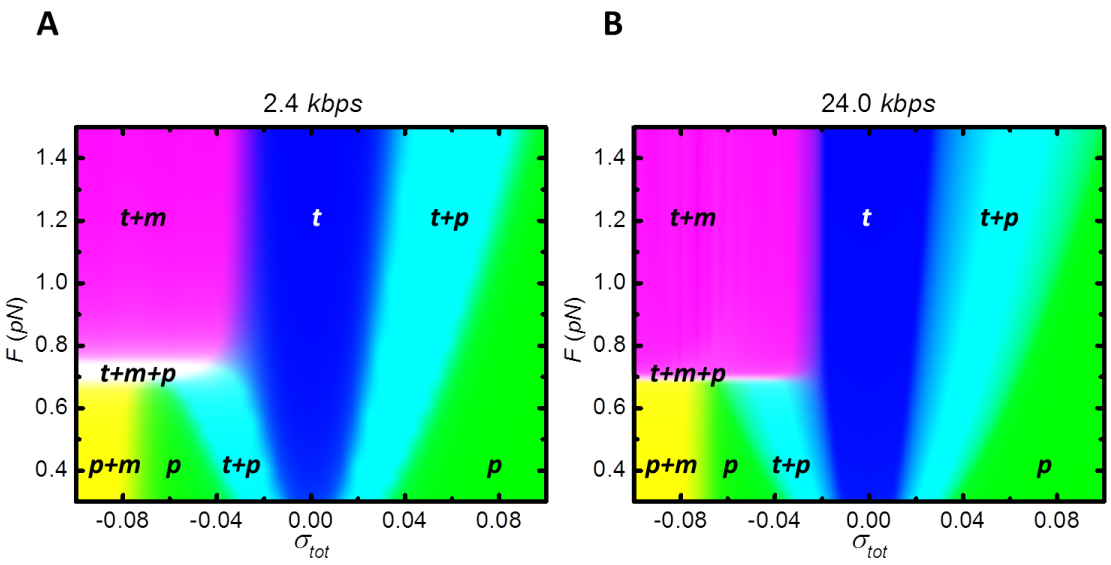

Figure S2

Force-linking number density phase diagrams. (A) A 2.4 *kbps* DNA molecule shows transitions between extended twist-extended (t), plectonemic (p), and melted (m) DNA results, (B) A 24.0 *kbps* DNA molecule shows the same trend as 2.4 *kbps* DNA but a highly reduced 3-state coexistence region.

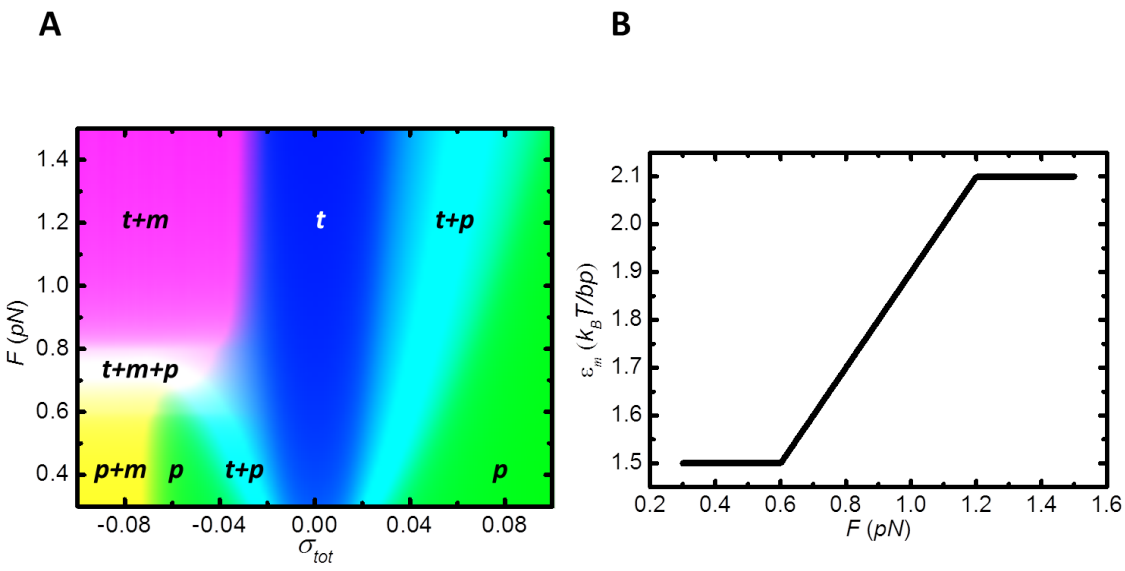

Figure S3

(A) The force linking number density phase diagram of a 7.0 *kbps* DNA with a force dependent free melting energy. (B) The melting energy as a function of the applied stretching force as used for computing the density phase diagram in (A).

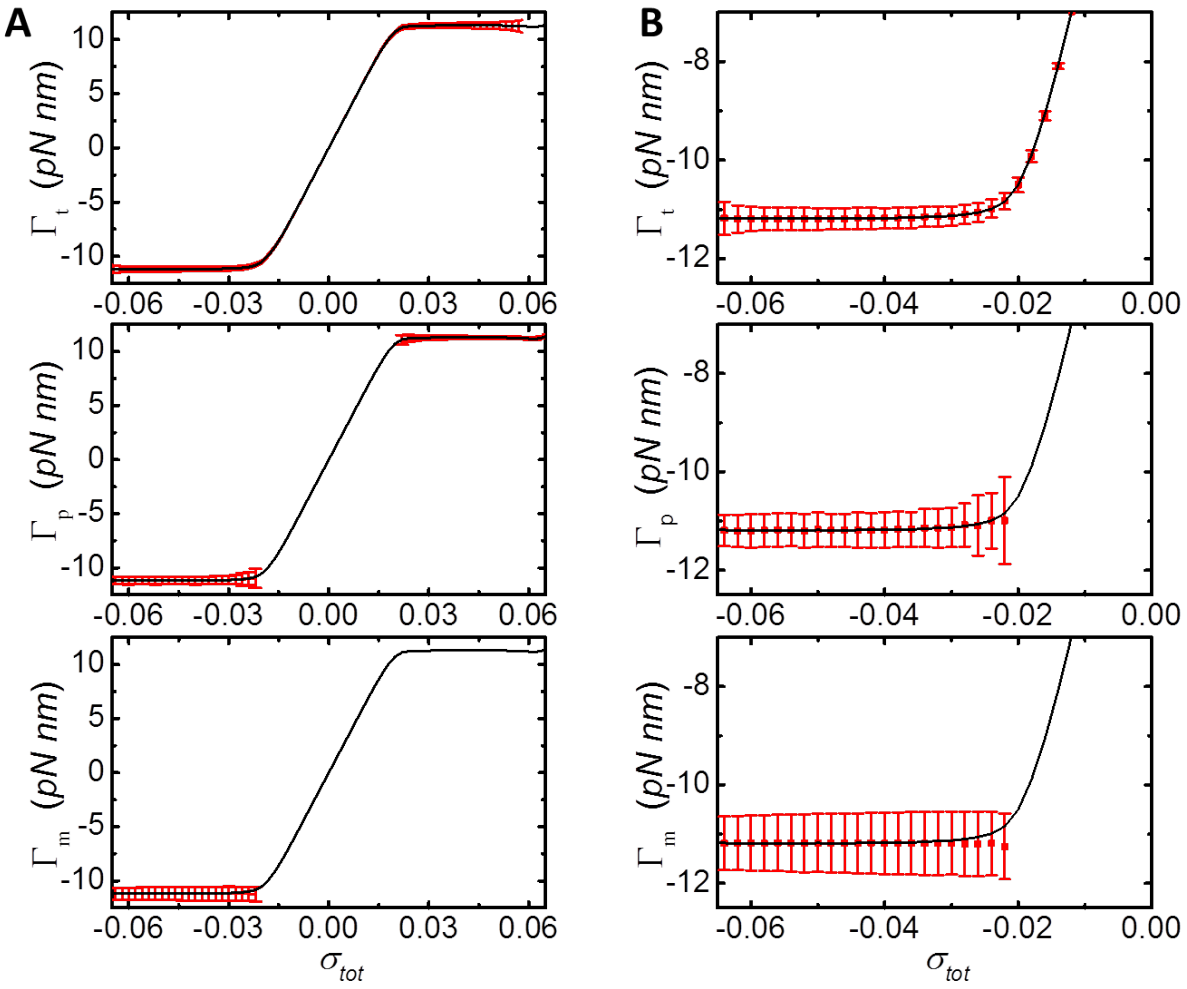

Figure S4

The torque distribution in each state of a 7.0 *kbps* DNA molecule at 0.7 pN. (A) The black line is the average torque in the molecule, whereas the red dots with their respective error bars represent the torque and its standard deviation in each of the occupied states. When the corresponding state is not occupied, the red dots are not shown. (B) Zoom in for a negative linking number density showing significant variations in the torque while the 3-state coexistence region is occupied.

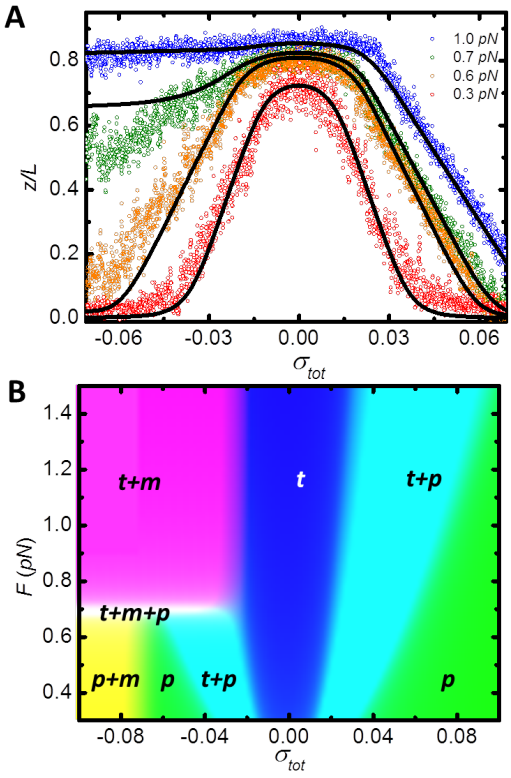

Figure S5

Torque-fixed calculation results of a 7.0 *kbps* DNA molecule. (A) The relative extension as calculated by the numerical model with a constant torque throughout the molecule as black solid lines, compared with experimental data. (B) Force-linking number density phase diagram.

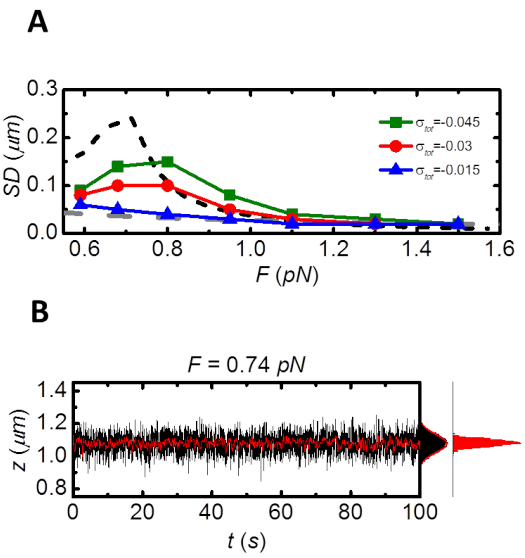

Figure S6

(A) Experimental data of the standard deviation of the extension of a 7.0 *kbps* DNA molecule at different linking number densities with respect to the force applied. Data show increased fluctuations in extension at 0.7 pN. Fluctuations in the extension calculated based on thermal fluctuations only (gray dashed line) cannot capture this effect. SD calculated with the 3-state model (black dashed line) at $\sigma_{tot} = -0.045$ shows however a similar trend. (B) Constant force measurement of a 7.0 *kbps* DNA molecule at $\sigma_{tot} = +0.03$. The red data is the 20 points median filtered data which do not reveal discrete steps as observed for negative linking number densities (Fig. 6). Histograms of the extension are shown on the right side of time traces (black and red bars). The raw data histogram is well fit by a Gaussian (red solid lines).

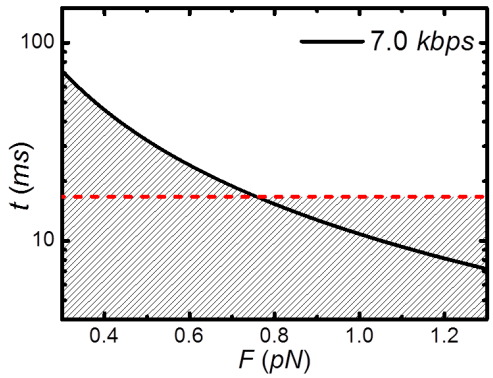

Figure S7

The temporal resolution of the experimental setup at different stretching forces. The black solid line represents the response time of a 7.0 *kbps* DNA molecule as calculated by Equation 6. The red solid line represents the frame rate of the CCD camera used. The shadowed region denotes the dynamics which cannot be resolved in this experiment.

Supporting References

[1] Marko, J. 2007. Torque and dynamics of linking number relaxation in stretched supercoiled DNA. *Physical Review E.* 76:021926.

[2] Sheinin, M. Y., S. Forth, J. F. Marko, and M. D. Wang. 2011. Underwound DNA under Tension: Structure, Elasticity, and Sequence-Dependent Behaviors. *Physical Review Letters.* 107:108102.

[3] Marko, J. F., and E. D. Siggia. 1995. Stretching DNA. *Macromolecules.* 28:8759–8770.

[4] Neuman, K. C., and A. Nagy. 2008. Single-molecule force spectroscopy : optical tweezers , magnetic tweezers and atomic force microscopy. *Nature methods.* 5:491–505.

[5] De Vlaminck, I., T. Henighan, M. T. J. van Loenhout, D. R. Burnham, and C. Dekker. 2012. Magnetic forces and DNA mechanics in multiplexed magnetic tweezers. *PloS one.* 7:e41432.