Supplementary Information for Knots Modify the Coil-Stretch Transition in Linear DNA Polymers

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Knot size measurements

In this work, we use as a measure of fractional knot size the integrated intensity of the knotted region divided by the integrated intensity of the molecule, where the knotted region is defined as a 9-pixel (1.6 μm) window centered around the brightest pixel of the knot. To determine the appropriate pixel window size to use, we looked at the intensity contained within odd-sized windows (3, 5, 7, 9, 11 and 13 pixels) centered around the knot peak for a range of knots at different chain extensions, as well as the knot intensity offset by the intensity of an unknotted section of the chain contained within the same-sized window. As an example, Fig. S1 shows the intensity plots for knots at ∼30% chain extension corresponding to those reported in Fig. 4. In Fig. S1b, we observe that the knot intensity minus unknot intensity increases with increasing pixel window size across all knots, but does not change appreciably after a window size of 9 pixels. This suggests that increasing the window size above 9 pixels for knots at ∼30% chain extension does not capture more of the knot, and hence we chose to use a 9-pixel window size.

We can visualize the chosen pixel size windows using surface plots of the knots. Figs. S2–S4 display surface plots of knots at ∼30% chain extension with a range of knot sizes. Based on Figs. S2–S4, it can be seen that a 9-pixel window is sufficient to capture the entirety of the knot intensity for different knot sizes. For some knots, the 9-pixel window might lead to an overestimate of the knot contour (Fig. S2), but for others, the window size is apparently suitable (Fig. S3). Due to the heterogeneity of knots, it is not possible to define a window size that will suit all knots. We believe this to be the most systematic way of characterizing knot sizes for the purpose of this work.

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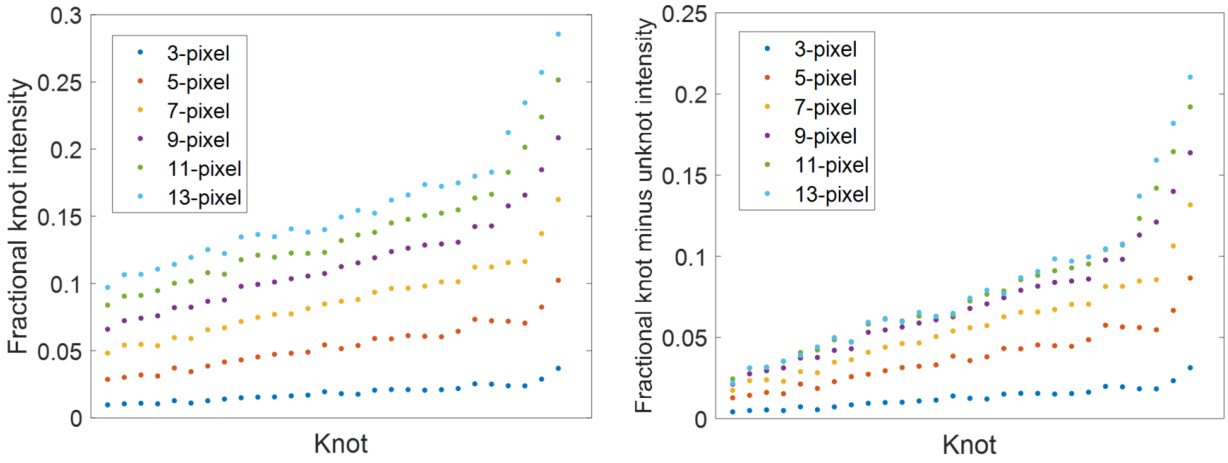

Figure S1: (a) Fractional knot intensity contained in windows of 3, 5, 7, 9, 11 and 13 pixels centered around the knot peak for a range of knots at ∼30% chain extension. (b) Fractional knot intensity contained in different sized pixel windows minus fractional intensity of an unknotted section of the chain for a range of knots at ∼30% chain extension. The x-axis represents the different knots interrogated in Fig. 4 of the main manuscript.

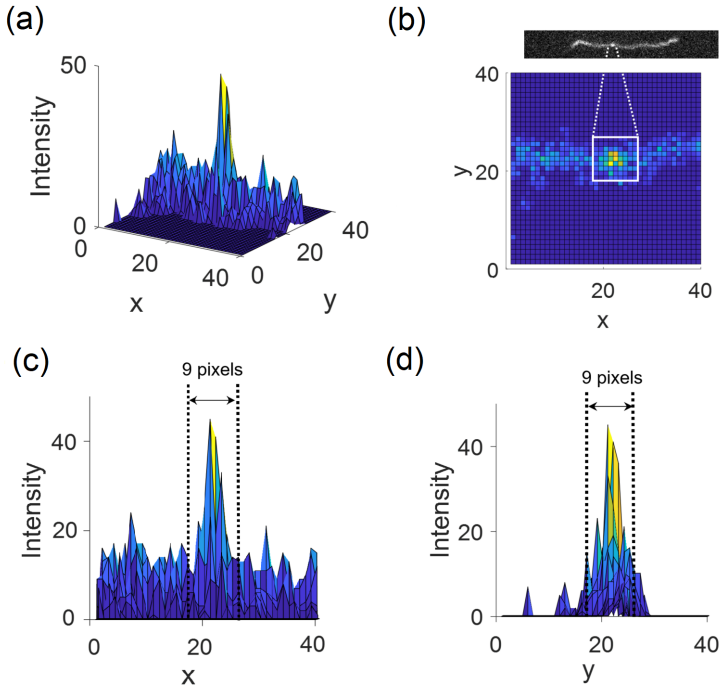

Figure S2: (a) Surface plot of a knot at ∼30% chain extension (fractional knot size = 0.074, 9-pixel window). (b–d) 2D projections of surface plot.

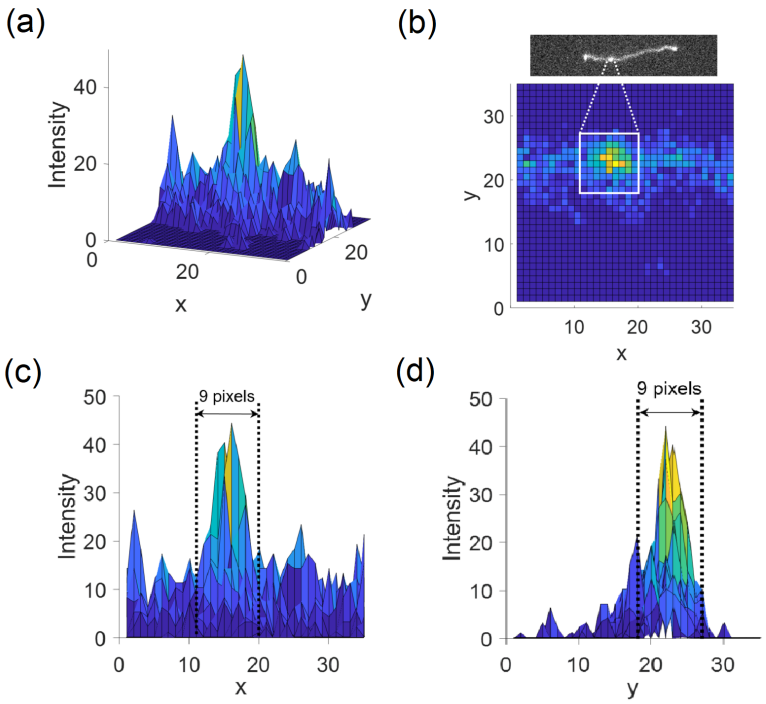

Figure S3: (a) Surface plot of a knot at ∼30% chain extension (fractional knot size = 0.11, 9-pixel window). (b–d) 2D projections of surface plot.

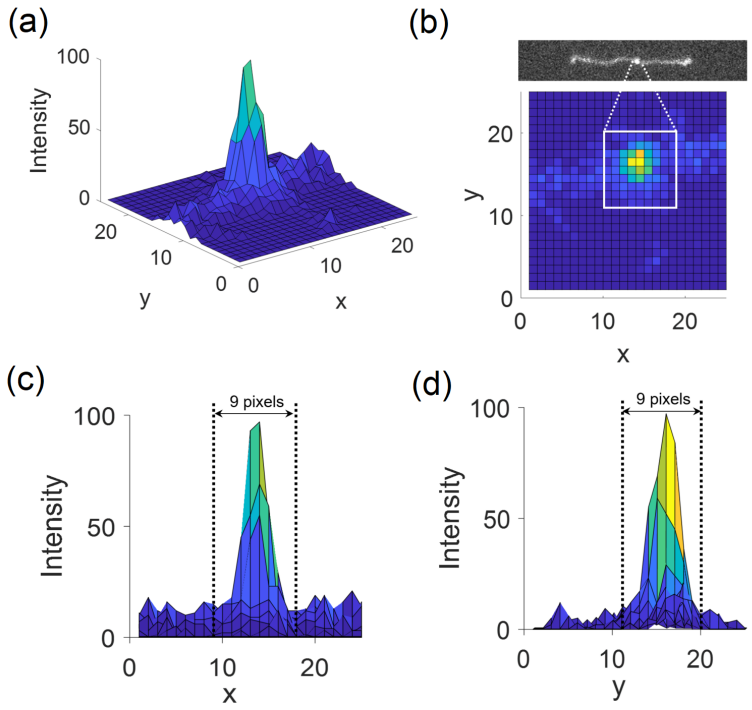

Figure S4: (a) Surface plot of a knot at ∼30% chain extension (fractional knot size = 0.15, 9-pixel window). (b–d) 2D projections of surface plot.

We performed the same analysis for knots on highly extended and almost coiled chains. Fig. S5 shows the intensity plots for knots on highly extended chains ($> 65\%$ chain extension) corresponding to those reported in Fig. 7. In Fig. S5b, we observe that the fractional knot intensity minus unknot intensity increases with increasing pixel window size across the three knots, and stays approximately constant upon using a 9-pixel window size or larger. Surface plots of the knots at $> 65\%$ chain extension (Figs. S6–S8) show that a 9-pixel window can sufficiently capture the entirety of the knot intensity.

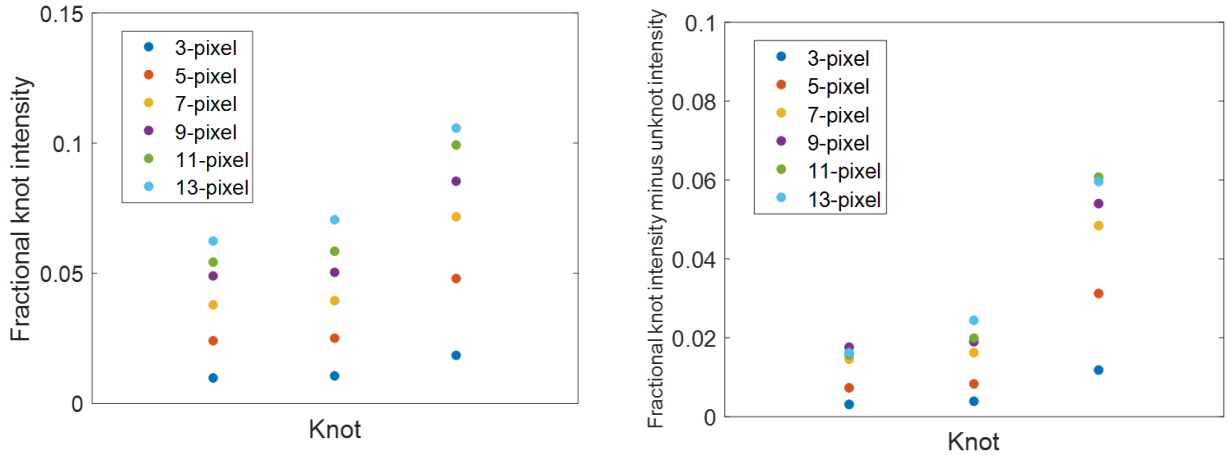

Figure S5: (a) Fractional knot intensity contained in windows of 3, 5, 7, 9, 11 and 13 pixels centered around the knot peak for three knots at $> 65\%$ chain extension. (b) Fractional knot intensity contained in different sized pixel windows minus fractional intensity of an unknotted section of the chain for three knots at $> 65\%$ chain extension. The x-axis represents the different knots interrogated in Fig. 7 of the main manuscript.

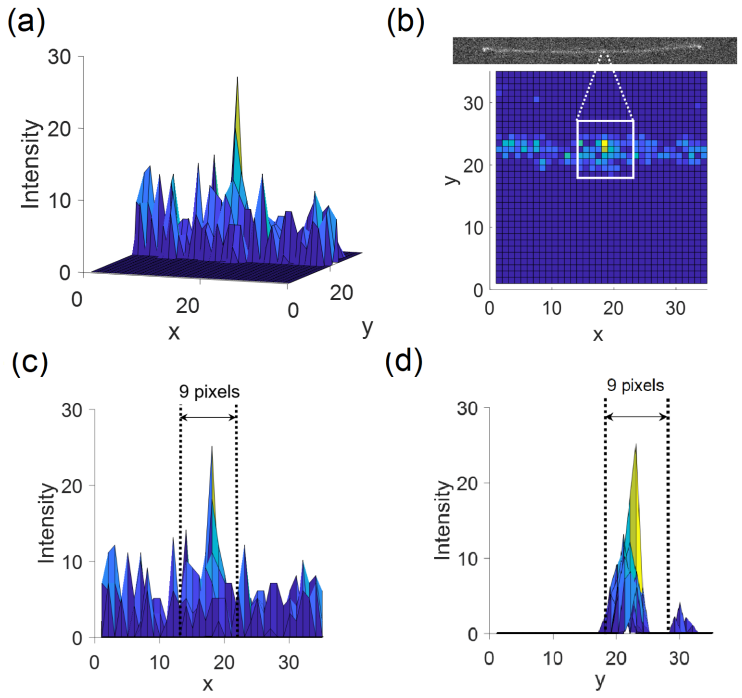

Figure S6: (a) Surface plot of a knot at 70% chain extension (knotted molecule 1, fractional knot size = 0.047, 9-pixel window). (b–d) 2D projections of surface plot.

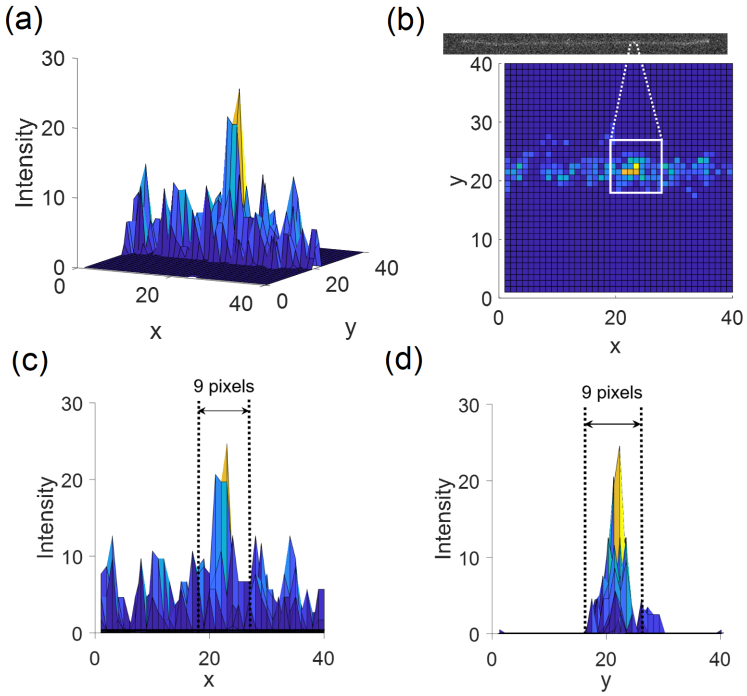

Figure S7: (a) Surface plot of a knot at 72% chain extension (knotted molecule 2, fractional knot size = 0.051, 9-pixel window). (b–d) 2D projections of surface plot.

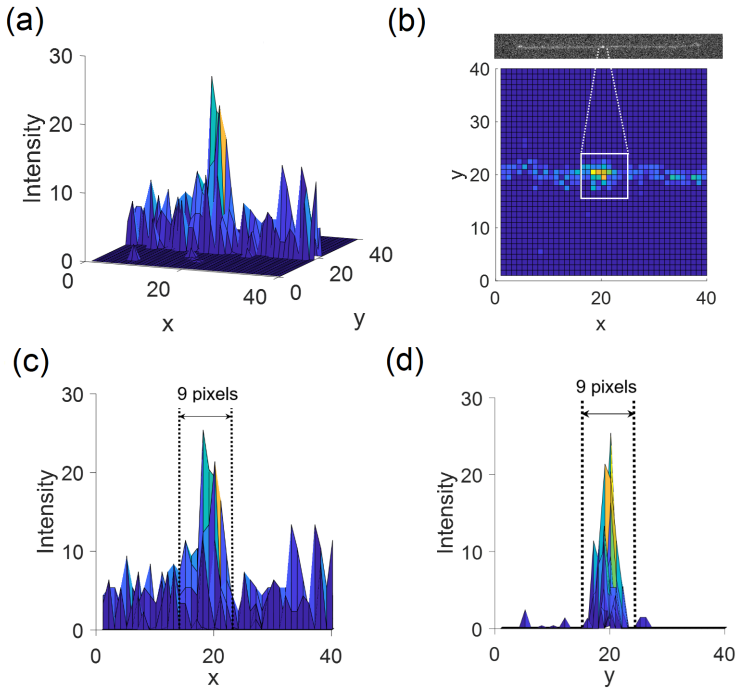

Figure S8: (a) Surface plot of a knot at 67% chain extension (knotted molecule 3, fractional knot size = 0.079, 9-pixel window). (b–d) 2D projections of surface plot.

Fig. S9 displays the intensity plots for knots on almost coiled chains (∼7% chain extension) corresponding to those reported in Fig. 7. In Fig. S9b, we see that the fractional knot intensity minus unknot intensity increases with increasing pixel window size across the three knots, and while it does not plateau as seen previously for more extended chains, we observe less significant increases upon using a 9-pixel window size. We believe the absence of a consistent knot intensity offset by the unknot intensity is because of the difficulty in measuring a knot size when the molecule is close to the coiled state. Nevertheless, surface plots of the knots at ∼7% chain extension (Figs. S10–S12) show that a 9-pixel window appears to be able to capture the intensity peak associated with the knot.

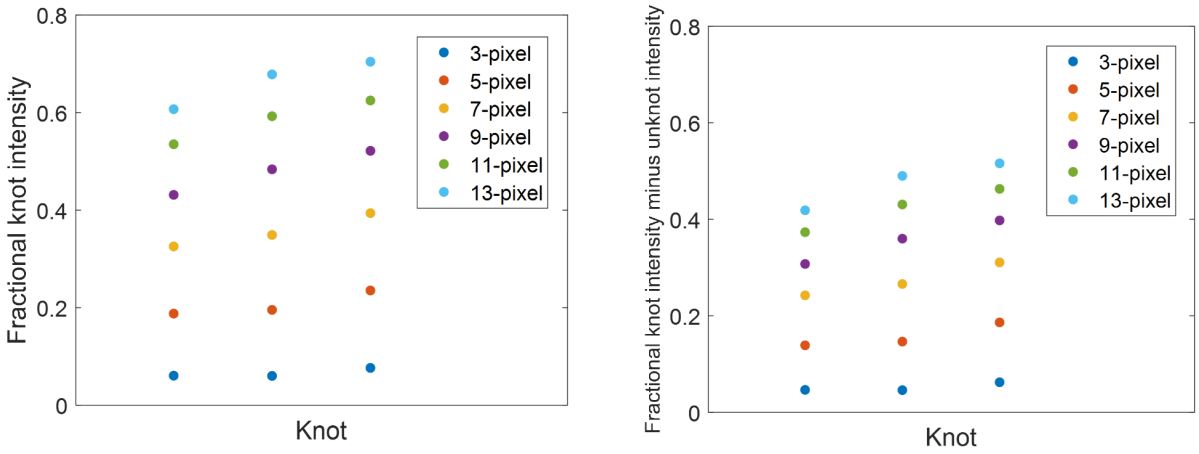

Figure S9: (a) Fractional knot intensity contained in windows of 3, 5, 7, 9, 11 and 13 pixels centered around the knot peak for three knots at ∼7% chain extension. (b) Fractional knot intensity contained in different sized pixel windows minus fractional intensity of an unknotted section of the chain for three knots at ∼7% chain extension. The x-axis represents the different knots interrogated in Fig. 7 of the main manuscript.

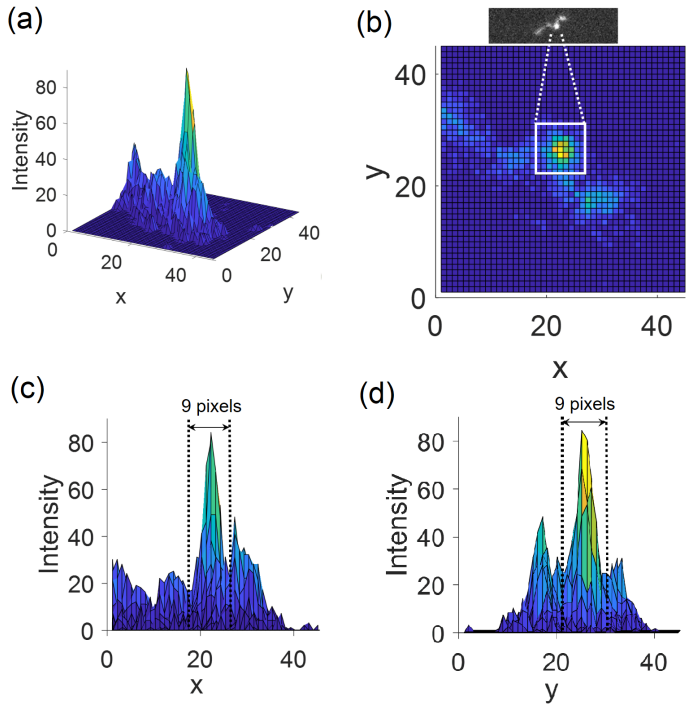

Figure S10: (a) Surface plot of a knot at 6.3% chain extension (knotted molecule 1, fractional knot size = 0.48 using 9-pixel window). (b–d) 2D projections of surface plot.

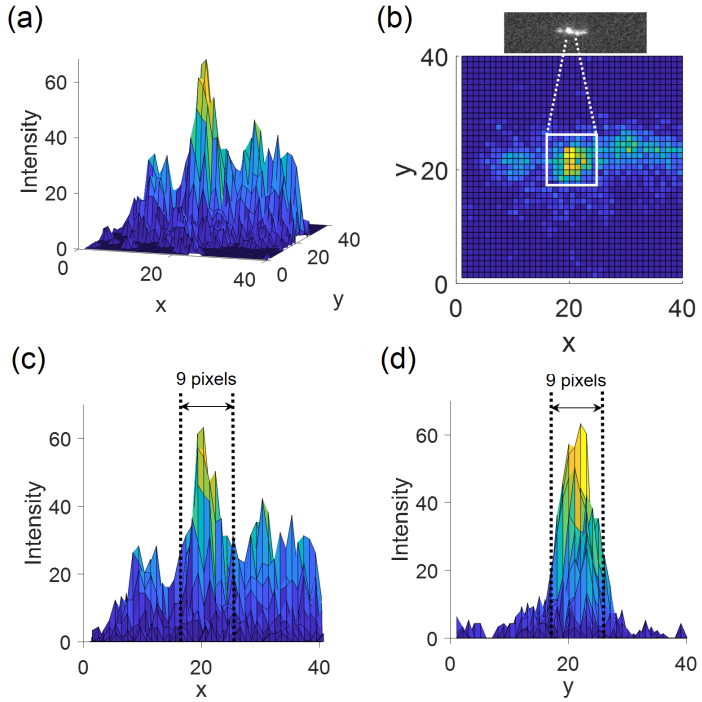

Figure S11: (a) Surface plot of a knot at 7.4% chain extension (knotted molecule 2, fractional knot size = 0.43 using 9-pixel window). (b–d) 2D projections of surface plot.

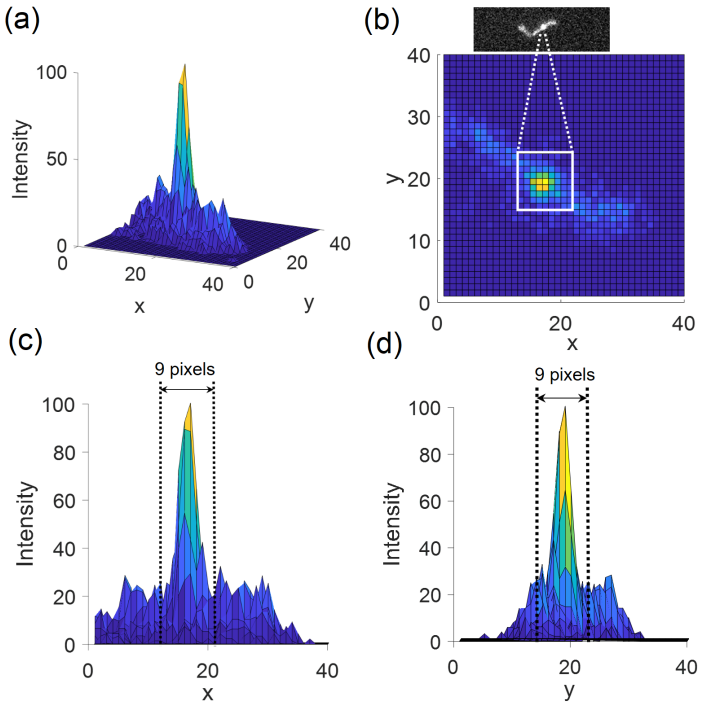

Figure S12: (a) Surface plot of a knot at 6.9% chain extension (knotted molecule 3, fractional knot size = 0.52 using 9-pixel window). (b–d) 2D projections of surface plot.

Knot size over multiple relaxations

The relaxation time of knotted molecules is obtained from the relaxation trajectory averaged over at least four relaxations. While the knot in a molecule during the first relaxation process might not have been the same knot in the molecule during the last relaxation, we do not observe any discernible trend in knot size over > 10 relaxations (Fig. S13).

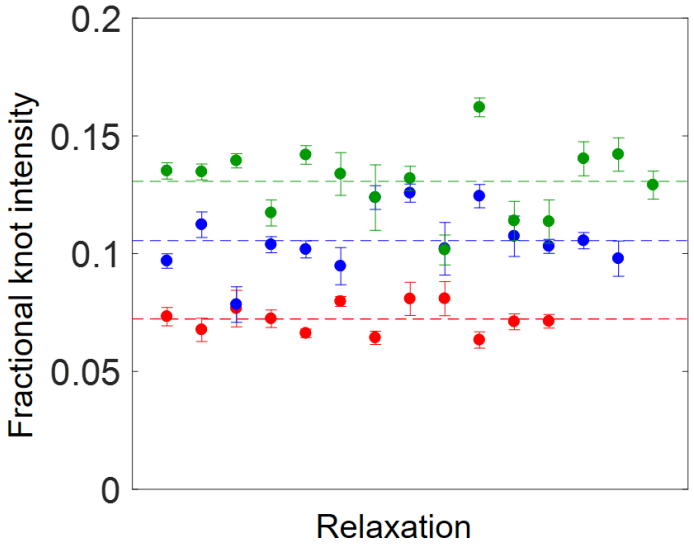

Figure S13: Knot size of three different molecules (measured at ∼30% chain extension) over >10 relaxations. Each color represents a different molecule and the dotted lines are the average knot size over all relaxations. Note that there is no apparent trend in knot size with relaxation.

Absence of hysteresis in extension-Wi curves

We note that no hysteresis was observed in the extension-Wi curves. As shown in Fig. S14, the fractional extension of a knotted molecule was the same as measured in the direction of increasing and decreasing strain rates.

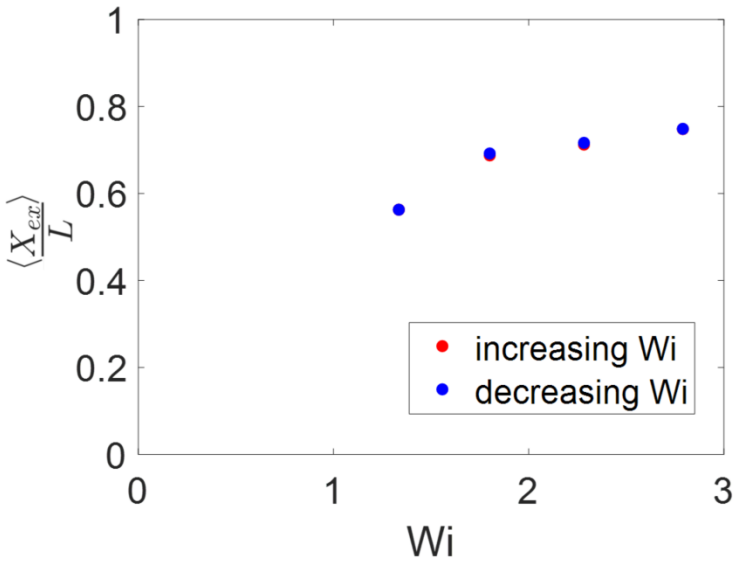

Figure S14: Steady-state fractional extension as a function of Wi for a knotted molecule measured in direction of increasing and decreasing strain rate.

Relaxation times and knot sizes of molecules in extension-*Wi* plots

The fractional knot sizes measured at ∼30% chain extension and relaxation times of the knotted molecules presented in Fig. 7 are reported in Table S1.

Knot	L_{knot}/L_{unknot}	$\tau_{knot}/\tau_{unknot}$
1	0.11 ± 0.01	0.90 ± 0.03
2	0.10 ± 0.01	0.81 ± 0.02
3	0.12 ± 0.04	0.81 ± 0.02
4	0.16 ± 0.06	0.63 ± 0.02
5	0.16 ± 0.03	0.57 ± 0.02

Table S1: Fractional knot sizes at ∼30% extension and relaxation times of knotted molecules in Fig. 7.

The fractional knot sizes of the knotted molecules presented in Fig. 7 measured at each strain rate are reported in Table S2.

Wi	Knot 1	Knot 2	Knot 3	Knot 4	Knot 5
0.47	0.48	–	0.46	–	–
0.70	0.13	0.13	0.43	0.73	–
1.0	0.062	0.078	0.081	0.16	0.52
1.3	0.058	0.072	0.073	0.10	0.14
1.5	0.049	0.061	0.065	0.081	0.11
2.1	0.048	0.045	0.055	0.062	0.098
2.7	0.047	0.046	0.051	0.068	0.079

Table S2: Fractional knot size of knotted molecules in Fig. 7 at each strain rate.

Estimate of $L_{\max}$

To rescale the extension of knotted molecules in Fig. 7, we recognize that knotted molecules are unable to attain the full chain contour length because of stored contour in the knot. We estimate the maximum extension $L_{\max}$ that the knotted molecule can achieve first by measuring the fractional knot size at the highest strain rate applied to the molecule ($Wi = 2.7$), so as to determine the amount of contour stored in the tight knot. We then subtract the knot contour from the chain contour length (77 μm), and add the window size used to measure the knot size (L_{window} =1.6 μm) to account for the span of the knot (see Fig. S15). The estimated $L_{\max}$ for the knotted molecules in Fig. 7 are 74.9 μm, 74.6 μm and 72.5 μm respectively. Note that this method likely leads to an overestimate of the tight knot size – and consequently an underestimate of $L_{\max}$ – because the knot might be pulled tighter at higher strain rates that are difficult to realize with the current experimental setup. Nevertheless, we believe that this method of estimating $L_{\max}$ is sufficient for the purpose of rescaling data in this work.

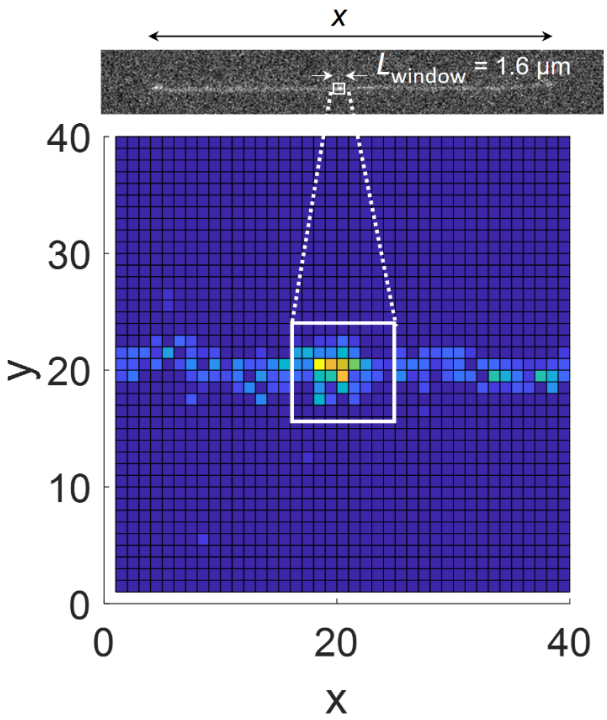

Figure S15: Schematic demonstrating estimation of $L_{\max}$.

Calculation of confidence intervals

In this section, we describe the calculation of confidence intervals for all data reported in the main text. Note that all error bars reported in the main text represent 95% confidence intervals.

Relaxation time

The longest relaxation time is determined by fitting the last 30% chain extension to

$$\langle X_{\mathrm{ex}}^2 \rangle = \langle X_{\mathrm{ex,eq}}^2 \rangle + \left(\langle X_{\mathrm{ex},0}^2 \rangle - \langle X_{\mathrm{ex,eq}}^2 \rangle \right) \exp\left(\frac{-t}{\tau} \right)$$

using the method of least squares, with $X_{\mathrm{ex,eq}}$ and τ being estimated parameters. We can write the above equation as a regression function f:

$$f(X_{\mathrm{ex,eq}}, \tau) = \langle X_{\mathrm{ex,eq}}^2 \rangle + \left(\langle X_{\mathrm{ex},0}^2 \rangle - \langle X_{\mathrm{ex,eq}}^2 \rangle \right) \exp\left(\frac{-t}{\tau} \right)$$

To estimate the confidence interval for the relaxation time, we obtain an estimator of the covariance matrix of the estimated parameters, given by

$$\sigma_r (\mathbf{J}_f^T \mathbf{J}_f)^{-1}$$

where $\mathbf{J}_f$ is the Jacobian matrix of f and σ_r is the variance of the noise. We can calculate σ_r as

$$\sigma_r = \frac{1}{N-k} \sum_{i=1}^{N} r_i^2$$

where N is the number of data points, k is the number of estimated parameters and r_i^2 are the residuals. The confidence interval for the relaxation time is then determined to be

$$\tau \pm c \sqrt{(\sigma_r (\mathbf{J}_f^T \mathbf{J}_f)^{-1})}$$

where c is chosen from the t-distribution table for a 95% confidence interval and $N - k$ degrees of freedom. A detailed method for constructing confidence intervals in least squares estimation can be found elsewhere, such as in van de Geer (2005).

Ratio of relaxation time as a function of knot size (Fig. 4)

The confidence intervals for knot size in Fig. 4 of the main text are calculated based on the standard error of the mean. The confidence intervals for ratio of relaxation time are estimated as

$$\frac{\Delta\left(\frac{\tau_{\text{knot}}}{\tau_{\text{unknot}}}\right)}{\left|\frac{\tau_{\text{knot}}}{\text{unknot}}\right|} = \frac{\Delta\tau_{\text{knot}}}{|\tau_{\text{knot}}|} + \frac{\Delta\tau_{\text{unknot}}}{|\tau_{\text{unknot}}|}.$$

Steady-state fractional extension as a function of Wi (Fig. 7)

The confidence intervals for Wi_{eff}, Wi_{rouse} and Wi_{zimm} in Fig. 7 of the main text are calculated

$$\begin{aligned}
\frac{\Delta Wi_{\text{eff}}}{|Wi_{\text{eff}}|} &= \frac{\Delta\tau_{\text{knot}}}{|\tau_{\text{knot}}|} \\
\frac{\Delta Wi_{\text{rouse}}}{|Wi_{\text{rouse}}|} &= (1+2\nu)\frac{\Delta\text{knot size}}{|\text{knot size}|} \\
\frac{\Delta Wi_{\text{zimm}}}{|Wi_{\text{zimm}}|} &= (3\nu)\frac{\Delta\text{knot size}}{|\text{knot size}|}
\end{aligned}$$

where the confidence interval for knot size is obtained based on the standard error of the mean. The confidence intervals for fractional extension are estimated as

$$\frac{\Delta\left(\frac{X_{\text{ex}}}{L_{\text{max}}}\right)}{\left|\frac{X_{\text{ex}}}{L_{\text{max}}}\right|} = \frac{\Delta X_{\text{ex}}}{|X_{\text{ex}}|} + \frac{\Delta L_{\text{max}}}{|L_{\text{max}}|}$$

where

$$\frac{\Delta L_{\text{max}}}{|L_{\text{max}}|} = \frac{\Delta\text{knot size}}{|\text{knot size}|}.$$

References

S. A. van de Geer, *Least Squares Estimation*, John Wiley & Sons, Chichester, England, 2005, vol. 2, pp. 1041–1045.