Supplementary Materials for

Dynamics of DNA Supercoils

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- Movies S1 and S2
Materials and Methods

DNA constructs:
A 20678 bp pSupercos1 plasmid was made by removal of the MluI fragment from pSupercos1 (Stratagene) and insertion of two lambda fragments. Plasmid DNA was isolated by midiprep (Qiagen) and restricted with XhoI and NotI resulting in a 20666 bp fragment. Biotin and digoxigenin labeled handles were prepared by PCR on a pBluescriptIIISK+ template with a taq polymerase (PCR core system I, Promega) and the addition of 2 µl of Biotin-16-2’deoxy-uridine-5’- triphosphate (bio-dUTP, Roche), or 2 µl digoxigenin-11-2’-deoxy-uridine-5’- triphosphate (dig-dUTP, Roche) to the nucleotide mixture respectively. The biotin PCR product was digested with XhoI resulting in 554 bp and 684 bp fragments. The digoxigenin product was digested with NotI resulting in 624 bp and 614 bp fragments. Labeled handles were purified with a nucleospin extractII kit (Machery Nagel), combined with the restricted plasmid DNA and ligated with T4 DNA ligase overnight at 4 °C and subsequently purified by phenol extraction.

DNA Labeling:
Purified DNA constructs were Cy3 labeled with label-it reagent (Mirus Bio) (26). Typical reaction conditions used 10 µl DNA construct (250 ng/µl) and 5 µl Label-it reagent in a total volume of 50 µl for 30 min. at 37 °C. Excess dye was removed by spin column filtration (illastra ProbeQuant G-50). Labeling density was measured to be 25 ± 5 bp/Cy3 as determined from the DNA and dye adsorption ratio at 260 nm and 550 nm respectively with a spectrophotometer (NanoDrop). For spectrophotometric detection DNA was purified with a nucleospin extractII kit (Machery Nagel) to avoid increased UV-adsorption due to G50 spin columns residues.

Magnetic tweezers assay:
Experiments were performed in square glass capillaries 0.7 × 0.7 × 50 mm (VitroCom) coated with a 0.1 % polystyrene in toluene solution and functionalized with anti-digoxigenin Fab fragments (27). DNA was incubated with 1 µm streptavidin magnetic beads (New England Biolabs) for 10 minutes and flushed into the capillary. The imaging buffer contained 20, 150 or 300 mM NaCl, depending on indicated salt concentrations, 20 mM TrisHCl pH 7.5, 0.01 % Tween and an oxygen scavenging system (13) consisting of 2.5 mM protocatechuic acid, 50 nM protocatechuate-3,4-dioxygenase, 1 mM Trolox and 100 µM N-Propyl gallate (all from Sigma-Aldrich). A modified microscope (Zeiss Axiovert 200M) equipped with motorized stages was used as magnetic tweezers. Each experiment was started by locating coilable DNA tethers and measuring their rotation curves. Subsequently the molecule was pulled sideways and the position of the side magnet was set to achieve the desired force by measuring the power spectrum of the bead’s fluctuations. Next the molecule was pulled upwards again and the desired number of turns were applied (table S1). Finally the molecule was pulled sideways again and the length and force were verified before starting to record images under epi-fluorescent illumination by a 532 nm laser. Images were acquired with 20-ms time resolution using an Andor Neo camera. While the side-pulling geometry brings the DNA molecule within close distance of the surface, we observed no sticking interactions and the DNA molecules were able to move freely (Fig. S10). Plectonemes also did not show any
significant preference for locating at the DNA attachment points to either the surface or the magnetic bead.

**Image analysis:**
Images were analyzed in custom software (Fig. S3-S5, Matlab Mathworks). A fitting routine was developed to count and extract their position over time (Fig. S3–S5). The fitting routine involved background subtraction and peak fitting of the kymograph and achieved a minimum detectable plectoneme size of ~0.5 kb (Fig. S11).
Supplementary Text

Supplementary Text S1: Determination of the diffusion constant.
The plectoneme diffusion constant $D$ was determined from the \( \text{MSD} \) given by,

$$\text{MSD}(n,N) = \frac{\sum_{i=1}^{N-n} (x_{i+n} - x_i)^2}{N-n},$$

(S1)

where \( N \) is the total number of frames for a single diffusional tract, \( n \) the frame interval (time) over which the \( \text{MSD} \) is calculated, and where the fitted positions of the plectoneme are represented by \( x_i \). The observed diffusion coefficient \( D \) was obtained by a weighted-linear-least-squares fit of the \( \text{MSD} \) versus time. The slope of the \( \text{MSD} \) was fit up to 0.3 s (1 to 15 frames) and weighted by the expected uncertainty (28)

$$\delta \text{MSD}(n) = \pm \left[ \frac{2n}{3(N-n)} \right]^{1/2}$$

at frame number \( n \). In order to reduce the inherent statistical variance in the determination of \( D \) from individual diffusion trajectories, we averaged the \( \text{MSDs} \) from all plectonemes recorded under the same experimental conditions before fitting.

Plectonemes do not necessarily have a fixed size as the writhe may distribute among several plectonemes in a single DNA molecule in time. As the plectoneme size changes, the diffusion constant of the plectoneme will change accordingly. We therefore only analyzed the diffusional tracts of plectonemes that contained at least 70% of the total amount of DNA that was in a plectonemic state. The main error in the determination of \( D \) now stems from the uncertainty in the size of the plectoneme. We assume an error of 30% in the plectoneme size, matching the 70% size criterion used to obtain the diffusional tracts (Fig. S6), resulting in an error of \( \delta D \approx 0.3 D \).

The observed \( \text{MSD} \) represents the displacement of the plectoneme in linear space. This is not equal to the displacement of the plectoneme along the contour length of the DNA, as the extension of the DNA, \( z=l/l_c \), under an applied stretching force \( f \) and torque \( \Gamma \) is shorter than its contour length, \( l_c \). The diffusion constant obtained from the fit of the \( \text{MSD} \) was converted to a diffusion constant along the contour length of the DNA by correcting for this incomplete stretching with a model derived by Moroz and Nelson (29):

$$z(f,\Gamma) = 1 - \frac{1}{2} \left[ \frac{l_p f}{k_B T} \left( \frac{\Gamma}{2k_B T} \right)^2 - \frac{1}{32} \right]^{-1/2},$$

(S2)

where \( l_p \) denotes the persistence length, \( k_B \) is the Boltzmann constant, and \( T \) the temperature. We use \( l_p = 50 \) nm and \( T=297 K \). Since the torque only represents a small correction to the extension at forces between 0.8 and 3.2 pN, we neglect it here and set \( \Gamma=0 \). The diffusion constant along the contour length of the DNA is now given by:

$$D = D_{\text{obs}} / z(f,0).$$

(S3)
Supplementary Text S2: Effects of thermal fluctuation in the DNA tether on the observed diffusion of a plectoneme.

Changes in the position of a plectoneme are not only due to its diffusion along the DNA, but also result from thermal fluctuations of the DNA tether itself. These fluctuations could potentially result in an overestimate of the observed diffusion constant. Here, however we assess this effect both theoretically and experimentally and we show that its contribution to the observed diffusion rates is minimal.

Both the bead and DNA experience a frequency-independent thermal force from the aqueous environment with noise spectral density \( S_f(f) = 4\gamma k_B T \), where \( \gamma \) is the hydrodynamic drag coefficient, \( T \) is the temperature, and \( k_B \) is the Boltzmann constant. The micron-sized bead has a much larger viscous drag than the DNA and will therefore dominate the low frequency noise displacements that we observe in our 50 Hz bandwidth. By calculating bead displacements following De Vlaminck et al. (30) we can thus provide an upper bound for the DNA fluctuations. DNA close to the bead will experience positional fluctuations that are almost equal to those of the bead while these fluctuations will reduce to zero at the flow cell attachment point. The thermal force gives rise to the following power spectral density of the bead position in \( x \), the direction of the applied magnetic force (31)

\[
S_x(f) = \frac{k_B T}{\gamma^2 (f_c^2 + f^2)},
\]

where \( f_c = \kappa_{tether} / 2\pi \gamma \) is the mechanical response frequency of the DNA-bead tether, and \( \kappa_{tether} \) the force- and length-dependent stiffness of the DNA molecule calculated from the worm like chain model. The standard deviation of the bead fluctuations, \( \delta x_{thermal} \), along the contour of the DNA molecule then reads (30)

\[
\delta x_{thermal} = \left[ \int_0^{B_{eq}} S_x(f) df \right]^{\frac{1}{2}} = \left[ \frac{2k_B T}{\pi \kappa_{tether}} \arctan \left( \frac{B_{eq}}{f_c} \right) \right]^{\frac{1}{2}},
\]

where \( B_{eq} \) is defined as the equivalent noise bandwidth, which accounts for the low-pass filter effect due to the finite frame rate of the camera. We can now calculate the bead fluctuations for our 21 kb DNA tether at the experimental forces of 0.8, 1.6, and 3.2 pN, which result in a \( \delta x_{thermal} \) of 45, 20, and 7.6 nm, respectively. The mean squared displacement (MSD) is then simply given by the variance of the thermal fluctuations which are \( 2.0 \times 10^{-3} \) \( \mu m^2 \), \( 4.0 \times 10^{-4} \) \( \mu m^2 \), \( 5.7 \times 10^{-5} \) \( \mu m^2 \), respectively. The MSDs of the bead, which set an upper bound for the DNA fluctuations, are thus much smaller than the experimentally observed MSDs for diffusing plectonemes, as is evident from comparison to Fig. 3E in the main text.

We can also verify these small thermal DNA fluctuations experimentally. Panel A of the figure below shows a torsionally unconstrained molecule at 1.6 pN applied stretching force, which contains an anomalous bright spot, i.e. this molecule did not contain any plectonemes but did have a (rare) locally bright region. We can track this position
following the method outlined in Supplementary Fig. S6. Parameters of the tracking routine were relaxed to allow the tracking of this spot which had a lower intensity compared to that of plectonemes in the diffusion analysis. The kymograph and tracking shown in panel B and C below confirm that the bright spot remains stationary with only small positional fluctuations. The tracked positions of the bright spot contain fluctuations due to DNA thermal motion, but also due to tracking errors. The MSD calculated for the tracked positions (panel D) is virtually constant in time confirming that the spot does not diffuse but fluctuates around a stationary position. The observed MSD of $1.5 \cdot 10^{-3} \, \mu m^2$ is higher than the theoretical estimate at 1.6 pN (blue dashed line in panel D), likely due to the additional contribution of tracking errors to the experimentally determined MSD.

**Thermal DNA fluctuations in a 21 kb DNA molecule at 1.6 pN.** (A) Image of an torsionally unconstrained DNA molecule containing a bright spot due to a (rare) inhomogeneous dye distribution. (B) Kymograph of the molecule shown in panel A. The blue line shows the tracked position of the bright spot. (C) Tracked position of the bright spot versus time. Fluctuations are observed to be on the order of 50 nm. (D) MSD of the tracked spot (red line). A near-constant behavior is seen, in contrast to the linear increase that is expected and observed for diffusion (cf. Fig. 3E in the Main text). The experimental fluctuations, which are due to both intrinsic thermal fluctuations and experimental accuracy in the determination of the spot position, are observed to be higher than the theoretical estimate for thermal fluctuations at 1.6 pN (blue dashed line).
Supplementary Text S3: A simple model for the hydrodynamic drag of a plectoneme.

The hydrodynamic drag of a plectoneme can be simplified to consist of two components: First the slithering motion of the DNA within the plectoneme, and second, the perpendicular motion of the whole plectoneme in the direction of the diffusion.

We express the drag coefficient $\mu = k_BT/D$ for these components below in equations S6 and S7. The first contribution reads (22):

$$\mu_{\text{slithering}} = \frac{2\pi \eta L}{\log(R/r_h)},$$  \hspace{1cm} (S6)

Here $\mu$ is calculated for the motion of two parallel cylinders of hydrodynamic radius $r_h = 1$ nm, spacing $2R$, and length $L/2$, where $\eta$ is the viscosity of water and $L$ the contour length of the DNA within the plectoneme. We take $R$ to be the plectoneme radius, see equation S7 below. The second contribution reads (32):

$$\mu_\perp = \frac{4\pi \eta L}{\log(l_{\text{seg}}/r_h) - 0.1},$$  \hspace{1cm} (S7)

which gives the ratio of drag force to velocity for sideways movement of a thin cylinder through water, where $l_{\text{seg}}$ represents the length of the cylinder over which it can be considered straight. We set this segment length to the experimentally determined length of 59 nm in a single turn of the plectoneme at 150 mM and 0.8 pN. The result is, however, not very sensitive to the exact choice of $l_{\text{seg}}$, as it appears in the logarithm.

To address the salt and force dependence of $D$, we calculate the plectoneme radius $R$, which depends on the effective charge $\nu$ of the DNA. Following the theory developed by Neukirch and Marko (20):

$$\nu = \frac{1}{b} \frac{1}{\gamma(L_B, b, \kappa_D r_h K_1(\kappa_D r_h))},$$  \hspace{1cm} (S8)

where $b = 0.17$ nm is half of the 0.34 nm spacing of successive base pairs along DNA, $L_B$ is the Bjerrum length in water, $\kappa_D^{-1}$ is the Debye length, $K_n(x)$ the $n^{th}$ modified Bessel
function of the second kind, and parameter \( \gamma \) was interpolated from Table III of Stigter (33) for 20, 150 and 300 mM NaCl and \( T = 297 K \) and \( L_B = 0.7 \text{ nm} \). To lowest order, the plectoneme radius \( R \) is now given by:

\[
R = (2\kappa_D)^{-1} \log(\sqrt{9\pi/8\nu^2}L_Bk_BT/g(f)),
\]

where \( g(f) \) is, to first approximation, the free energy per unit length of the untwisted stretched DNA molecule (34):

\[
g(f) = f - k_BT\sqrt{f/A},
\]

with \( A \) the bending stiffness of DNA. The hydrodynamic diffusion constant \( D_h \) a function of force and ionic strength was finally calculated as:

\[
D_h = \left( \frac{\mu_{\text{slithering}}}{k_BT} + \frac{\mu_\perp}{k_BT} \right)^{-1}
\]

**Hydrodynamic diffusion constant of a plectoneme as a function of force.** The hydrodynamic diffusion constant \( D_h \) was calculated from Eq. S11, for 150 mM NaCl (red line) and 300 mM NaCl (blue line). The plectoneme size was held constant at 1.76 \( \mu \text{m} \) and a writhe of 30 turns. The diffusion constant decreases for increasing ionic strength and increasing force. Both of these effects reduce the plectoneme radius \( R \) and thereby increase the drag of the slithering motion.
Supplementary Text S4: Surface effects only result in a small increases in the viscous drag experienced by a moving supercoil.

The logarithmically decaying flow field of a diffusing plectoneme interacts with the surface, resulting in an increase in viscous drag. The distance of the 21 kb DNA molecule varies from 0 μm at its attachment point to the flow cell to 0.5 μm at its attachment to the 1 μm diameter magnetic bead. The viscous drag coefficient of an infinite cylinder moving parallel to a surface is given by \( \mu_{\text{surface}} = 4\pi\eta L/\log(2b/r_L) \), where \( b \) is the distance to the surface. We can compare this drag coefficient to the hydrodynamic drag of the plectoneme in the absence of a surface. If we evaluate the drag at \( b = 50 \text{nm} \), i.e. for a distance corresponding to 10% of the contour length of the DNA from the attachment point, then 90% of the DNA molecule will be further away from the surface and experience less surface-induced drag. The total drag coefficient for the plectoneme in free solution is given by \( \mu_{\text{plectoneme}} = \mu_{\text{slithering}} + \mu_{\perp} \). Taking a plectoneme radius of \( R = 2.6 \text{ nm} \), representative for 0.8 pN and 150 mM NaCl, we find that the surface-induced drag coefficient \( \mu_{\text{surface}} \) at 50 nm from the surface only represents 10% of the drag of the plectoneme in free solution. For distances further away from the surface, i.e. for 90% of the molecule the effect will be even smaller. This was confirmed experimentally as there was no obvious difference in diffusion speed between plectonemes located close to the flow cell attachment point and those located midway or even close to the bead.

Surface effects may however play a role in localization of plectonemes along the DNA molecule. In our experiments we only rarely observed plectonemes at the DNA ends. This may be due to the fact that the surfaces (bead and flow cell) restrict the available space for plectoneme movements and create an excluded volume. Such an exclude volume will exert an entropic pressure which forces the plectoneme inward, away from the DNA ends. Surfaces could thus act as soft reflecting boundaries to diffusing plectonemes, which is in line with our observations.
Supplementary Text S5: Hopping of plectonemes over long distances cannot be explained by diffusion.

Fast diffusing plectonemes could lead to false identification of nucleation events. Here we estimate this quantitatively. The probability of such large diffusional steps can be calculated from their observed diffusion constants. The mean length $l_{\text{dif}}$ of a Brownian motion step during time $\Delta t = \delta t$ is given by $l_{\text{dif}}^2 = 2D\Delta t$. If we assume a maximum diffusion constant of 0.5 $\mu$m$^2$/s, well above both the predicted theoretical diffusion constants and more than a factor of three above those observed experimentally (Fig. S7), and given our time resolution $\Delta t = 20$ ms, the mean displacement is calculated to be $l_{\text{dif}} = 0.14$ $\mu$m. This length is also equal to the standard deviation $\sigma$ of the diffusion distances for $\Delta t = 20$ ms. The probability of finding a diffusional step larger than 8 pixels, i.e. 520 nm = 3.7 $\sigma$, in a single frame is now given by $\text{erfc}(3.7/\sqrt{2}) = 2.4 \times 10^{-4}$, where $\text{erfc}$ is the complementary error function. This probability is equivalent to a rate of 0.01 falsely detected nucleation events per second. The experimentally obtained nucleation rates were found to be in the range of 0.4–22 s$^{-1}$, well above the rate for large diffusional steps. Hopping events therefore cannot be attributed to fast diffusion events but are real nucleation events of a plectoneme at a different position.
**Supplementary Text S6: Hopping of plectonemes.**

To quantify the nucleation rate of plectonemes, and related to that the hopping rate, we used the plectoneme detection method outlined in Fig. S3–S5. A nucleation event was counted as such if it occurred a distance of at least 8 pixel (520 nm) away from a plectoneme in the previous frame. Plectonemes appearing at distances shorter than 8 pixels were counted as a diffusion event, i.e. a continuation of the nearest preexisting plectoneme.
Supplementary Text S7: Experimental factors influence the observed distribution of plectoneme lifetimes.

The observed distribution of plectoneme lifetimes shows a power law decay $P(t) \sim t^\alpha$ with an exponent close to -1.5 (see main text Fig. 4F). Such behavior may be described by the first return to the origin for a random walk. The probability, $P(t)$, for this process reads:

$$P(t) = \left(\frac{t}{t/2}\right)(t-1)2^{-t}. \quad (S12)$$

It has been shown (37) that this first-return-to-the-origin random-walk probability results in a power-law scaling $P(t) \sim t^\alpha$ with an exponent of $\alpha = -1.5$. Experimentally, power law scaling is challenging to identify because it requires a data set spanning several orders of magnitude (38, 39). Although this random walk model visually agrees well with the histogram of plectoneme lifetimes (Fig. 4F main text), a more sensitive analysis using the cumulative distribution of plectoneme lifetimes shows that the experimental data considerably deviates from a true power law, see figure below.

There are several experimental factors that may cause the experimentally observed lifetimes to deviate from the theoretical random walk model even if this is the underlying mechanism. Three main differences from the theoretical model are:

1. Experiments have a finite observation time, due to photo-induced nicking of the DNA
2. The plectoneme has a finite maximum size, in contrast to the infinite size of the random walk model.
3. Experimental data include errors in plectoneme detection.

To test the effects of these experimental effects, a Monte Carlo (MC) simulation-based approach was employed to determine the first return to the origin of a random walk on a one dimensional lattice for time points $t = 2n$ where $n$ is a MC step. All three deviations of the theoretical model could be accounted for by the MC simulation as follows:

1. The actual measured experimental observation times were used in the MC simulation.
2. The finite plectoneme size was accounted for by including a reflecting boundary condition for the random walk model. The size of the simulation lattice was set equal to the number of turns in the plectoneme, i.e. 30 steps, matching the maximal 30 turns in the plectoneme at 150 mM NaCl 0.8 pN.
3. A reduction in plectoneme lifetime due to errors in the plectoneme detection method was accounted for by including an error of 3 % for the identification of plectonemes, i.e. a plectoneme had a 97 % chance of being detected in a single frame. In most cases, this is a reasonable number considering the noise in the experimental data and the involved fitting procedure (supplementary Fig. S3–S5). If the plectoneme size is large, however, such an error seems excessive, the error rate was therefore set to 0 % if the size of the plectoneme was more than 50 % of the total DNA that was in a plectonemic state.
The results of the MC simulation incorporating the above effects are shown in the figure below. By incorporating all the expected experimental differences from the random-walk model, an excellent agreement between the MC simulation and the experimental data is obtained.

A modified random walk model accounting for experimental conditions accurately captures the observed scaling of plectoneme lifetimes. A, The histogram of experimentally observed plectoneme lifetimes at 150 mM NaCl 0.8 pN (red line) shows an approximately linear behavior on a loglog plot with slope of -1.5 indicating near-power-law scaling and matching a model describing the first return to the origin for a random walk (black dashed line). A MC simulation of the random-walk model for the experimental number of events and accounting for the actual finite observation times due to nicking of DNA molecule (blue line, Supplementary text S7) deviates considerably from the experimental data. B, Cumulative distribution of plectoneme lifetimes, created by summation starting at the longest observed lifetime. While the histogram of plectoneme lifetimes (panel A) is rather insensitive to small deviations from a true power
law scaling behavior, a cumulative distribution offers a far more sensitive way to represent the data (38, 39). The cumulative distribution shows that the experimental data (red line) deviates considerably from the expected scaling of the ideal random walk model (black dashed line). The MC simulation using the actual experimental measurement times (blue line) differs from both the theoretical model and the experimental observed distribution. **C**, Histogram comparing the experimentally observed plectoneme lifetimes with a MC simulation including all experimental effects. The MC simulation including all experimental effects (green line, Supplementary text S7) provides a close match to the experimental data (red line). **D**, Cumulative distribution comparing the observed plectoneme lifetimes with MC simulation results. Both the random walk model (dashed black line) and the MC simulation using experimental measurement times (blue line) differ considerably from the experimental data (red line). The MC simulation including all experimental effects (green line) closely matches the experimental data, red line. These results show that experimental conditions significantly affect the distribution of plectoneme lifetimes and that the experimentally observed distribution is closely reproduced when these effects are taken into account. The proposed random walk model therefore is a good candidate to predict the observed scaling as it presents both a valid physical model for growing and shrinking plectonemes, and, when accounting for experimental factors, accurately describes the observed distribution of lifetimes.
Supplementary Text S8: The theoretically expected hop distance for plectonemes scales, to first order, linearly with time in contrast to the expected diffusion distance which scales as \( \sqrt{t} \).

Hopping involves the corkscrew motion of the growing and shrinking plectonemes around their axes as well as the rotation and sideways movement of the DNA in between the old and new plectoneme. We now quantify the drag associated with hopping by taking these three drag coefficients into account.

The corkscrew motion results in a rotation of the end loop of the plectoneme. We approximate the drag coefficient for this motion by the drag coefficient for perpendicular movement of the DNA through water, \( \mu_p \), as given in equation S7. We set the length of moving DNA equal to twice the contour length of DNA in a single turn of the plectoneme to account for the rotation of both the shrinking and growing plectoneme.

The second contribution to the drag for hopping results from the sideways movement of the intermediate DNA and this drag coefficient is given by

\[
\mu_p = \frac{2\pi \eta L_{hop}}{\log(l_{ps} / r_h) - 1.2},
\]

where \( L_{hop} \) is the hopping distance and \( l_{ps} \) represents the length over which the DNA may be approximated by a straight cylinder. Following Nelson (40) we set \( l_{ps} \) equal to the structural persistence length of DNA, i.e. \( l_{ps} = 130 \text{ nm} \). An applied stretching force will tend to increase the length over which the DNA can be considered to be straight, this effect will, however, be minor as \( l_{ps} \) appears in the logarithm.

The third contribution to the hopping drag results from the rotation of the DNA in between the growing and shrinking plectoneme around its axis. The drag coefficient for axial rotation is given by

\[
\mu_{rot} = 4\pi \eta L_{hop} r_h^2.
\]

To compare the hopping distance with the diffusion distance, we evaluate the typical energy involved in diffusion and calculate the hopping distance for this typical energy.

The diffusion distance is given by:

\[
L_{dif} = \sqrt{\langle x_{dif}^2 \rangle} = \sqrt{2Dl} = \sqrt{\frac{k_B T}{\mu} t},
\]

which can be
rearranged to \( L_{\text{dif}} \mu v_{\text{dif}} = 2k_B T \), where \( v_{\text{dif}} \) is the typical diffusion velocity \( v_{\text{dif}} = L_{\text{dif}} / t \).

We now calculate the typical hop distance for this \( 2k_B T \) energy scale from the energy balance,

\[
2k_B T = \mu_{\perp} (2q) R^2 \omega^2 t + \mu_{\text{rot}} (L_{\text{hop}}) \omega^2 t + \mu_{\parallel} (L_{\text{hop}}) v_{\text{hop}}^2 t
\]

where \( q \) is the contour length of DNA in a single turn of the plectoneme, \( N \) the number of turns in the plectoneme, \( \omega=2\pi N/t \) the angular frequency of the spinning DNA, and \( v_{\text{hop}}=L_{\text{hop}}/t \) the hop velocity. Solving equation S15 for \( L_{\text{hop}} \) we find:

\[
L_{\text{hop}} = \left( 2k_B T \left( \frac{(2\pi R N)^2}{t} \frac{8\pi \eta q}{\log(l_{\text{seg}}/r_s) - 0.1} \right) \right) ^{-1} \left( \frac{t}{N^2 2\pi \eta} \left( 8\pi^2 r_s^2 + \frac{q^2}{\log(l_{\mu} / r_s) - 1.2} \right) \right)
\]

Remarkably, equation S16 shows that the typical hop distance depends, to first order, linearly on time, in contrast to the expected diffusion distance which scales as \( \sqrt{t} \). The linear time dependence of the hop distance allows for much larger displacements of plectonemes by a hopping compared to diffusion. The drag due to the corkscrew motion results in an energy penalty, making diffusion the preferred mode of transport for writhe over short distances. Also noteworthy is the fact that the hop distance scales as the inverse square of the number of turns in the plectoneme, \( N \), thereby allowing small plectonemes to hop over even larger distances in the same amount of time.

![Graph](image.png)

The theoretically expected hop distance for plectonemes scales, to first order, linearly with time in contrast to the expected diffusion distance which scales as \( \sqrt{t} \). Diffusion distance \( l_{\text{dif}} \) (blue line) versus time was calculated as \( l_{\text{dif}} = \sqrt{2Dt} \) for a diffusion constant of \( D = 0.28 \, \mu \text{m}^2 \text{s}^{-1} \) representative for a plectoneme of \( L = 1.76 \, \mu \text{m} \) at 0.8 pN and 150 mM NaCl. The distance over which a plectoneme can typically hop within a certain time period (red line) scales linear with time making it the preferred mode of movement for large distances. The typical hopping distance was calculated for identical parameters as the diffusion i.e. for a plectoneme size of \( L = 1.76 \, \mu \text{m} \) at 0.8 pN and 150 mM NaCl.
Supplementary Figure S1: Rotation curves of cy3-labeled 21 kb DNA molecules closely match those of unlabeled molecules. A, Rotation curves of cy3-labeled DNA molecules in a buffer containing 20 mM NaCl for an applied stretching force of 0.4, 0.8, 1.6, and 3.2 pN (blue, green, red, and black points). The data of the labeled molecules closely match those of unlabeled molecules (grey dots). All plectoneme visualization experiments were performed at conditions where 25 % of the contour length of the DNA molecule was in a plectonemic state, indicated by the open circles. B, Rotation curves similar to panel A at 150 mM NaCl. C, Rotation curves similar to panel A at 300 mM NaCl.
Supplementary Figure S2: Plectonemes are only present in supercoiled DNA molecules. **A**, Kymograph of a 21 kb DNA molecule at 0.8 pN 150 mM NaCl in the absence of supercoiling shows a homogeneous intensity along the molecule. **B**, The same DNA molecule as in panel A after applying 61 positive turns, equivalent to a supercoiling density of $\sigma = 0.031$. The end-to-end distance of the DNA tether is reduced and plectonemes appear along the molecule as dynamic bright spots. At $t = 2.9$ s the molecule nicks due to photo damage, and it immediately extends and regains its pre-coiling end-to-end distance (compare to panel A). At the same time, the plectonemes disappear as is evident from the absence of bright spots along the molecule. **C**, Image series showing the nicking event in detail; the time interval between the images shown is 20 ms.
**Supplementary Figure S3: Background subtraction method for localizing plectonemes.**

**A,** Kymograph of a supercoiled DNA molecule where each line is constructed by summation of the pixel intensity perpendicular to the DNA molecule. **B,** In a first step the kymograph is down sampled by a factor of two by averaging pixel pairs along the x-axis, thereby increasing the signal-to-noise ratio. **C,** For each x-position the intensity of the kymograph is reordered from low to high along the time-axis. This procedure separates the high intensity spots, when a plectoneme is present at a given location, from the intensity in the absence of a plectoneme at that x-position. **D,** Mean intensity of the 10% lowest values of the kymograph, i.e. the mean of the top 10% rows of panel C. This provides the profile of the background intensity of the fluorescence along the DNA molecule. If plectonemes are very immobile, i.e. when they are present for more than 90% of the time at a given x-position, peaks will still be present in this profile. To remove these peaks, a single manual threshold level was applied where appropriate, thereby limiting the maximum value of the profile. This profile will function now as a background intensity curve in the next step. **E,** Kymograph created by subtracting the curve from panel D from the kymograph of panel B. This results in a kymograph which highlights the position of the plectonemes and which has a value close to zero for all positions where no plectonemes are present.
**Supplementary Figure S4: Plectoneme identification by peak fitting.**

**A,** Individual identified plectonemes shown as different colored dots overlaid on the kymograph of Fig. S3E. Plectonemes were connected between consecutive frames if their position changed less than 8 pixels from frame-to-frame, (equivalent to 520 nm), which is roughly equal to the ~500 nm observed spot size of the plectonemes. Plectonemes were identified by a peak-identification algorithm (findpeaks, Matlab Mathworks) applied to the intensity along the x-direction in the background-corrected kymographs (e.g. Supplementary Fig. S3E). The peak threshold was typically set to 130 % of the fluorescence intensity level of the DNA in the absence of plectonemes and was manually optimized for each molecule. For comparison, the standard deviation of intensity fluctuations in the absence of plectonemes was normally around 5–7 % of the total intensity. The threshold level of 30 % above the background intensity therefore corresponds to a 5σ confidence level resulting in a low number of falsely identified plectonemes (cf. Fig. S5B). **B,** The number of plectonemes present as a function of frame number obtained by the peak-fitting procedure from the kymograph of panel A. **C,** Histogram of the number of plectonemes obtained from the data in panel B. **D,** Cumulative counts of nucleation events as a function of frame number. **E,** Histogram of the detected plectoneme lifetimes.
Supplementary Figure S5: Plectonemes are only detected in supercoiled DNA molecules. A, Kymograph of a supercoiled DNA molecule that nicks due to photo damage at time t = 2.9 (data of Fig. S2B). B, Individual plectonemes identified in the kymograph of panel A, shown as colored lines. Plectonemes are readily identified in the supercoiled molecule, but not in the relaxed molecule: after nicking, no plectonemes are detected anymore, confirming the accuracy of the plectoneme detection method.
Supplementary Figure S6: Fitting the position of a diffusing plectoneme. A, Plectoneme sub-pixel positions were determined by a 9-point parabolic fit (green line) of a single line in a kymograph (blue points). We fit only plectonemes with an integrated intensity greater than 70 % of the total intensity contained in the plectonemes of the molecule, i.e. the plectoneme had to contain at least 70 % of the DNA that was in a plectonemic state. B, Fitting the plectoneme position for each line in the kymograph results in a diffusional tract showing the center position of the plectoneme in time (blue line).
Supplementary Figure S7: The effect of a rugged energy landscape on the diffusion of a plectoneme. The experimentally observed diffusion constant decreases rapidly with applied stretching force (red squares 150 mM; blue squares 300 mM NaCl; error bars denote the estimated error in the plectoneme size). The experimentally observed diffusion constants are considerably below those predicted for a simple hydrodynamic model (red and blue lines for 150 and 300 mM NaCl respectively, see Supplementary text S3). A model incorporating a rugged energy landscape for diffusion along the DNA (orange and green line for 150 and 300 mM NaCl, respectively, for description see main text) reduces the diffusion constants significantly and describes the observed data much better than the hydrodynamic model.
Supplementary Figure S8: The distribution of plectoneme lifetimes shows the same scaling behavior for all experimental conditions. The probability of plectoneme lifetimes in buffers containing 20, 150 and 300 mM NaCl (black, red and blue lines respectively) under applied stretching forces of 0.4, 0.8, 1.6 and 3.2 pN (solid, dashed, dotted and dash-dotted lines respectively) shows identical scaling behavior for all conditions. The predicted power-law scaling is indicated by the green line.
Supplementary Figure S9: Distribution of hop distances for plectonemes. Plectonemes are able to hop over large distances along a DNA molecule, in the range of 0.5 μm to 5 μm. Hop distances are shown for salt concentrations of 0.2, 150, and 300 mM NaCl, (black, red, and blue bars), and forces 0.4, 0.8, 1.6, and 3.2 pN (from left to right for each salt concentration), gray and white areas indicate bins. Hopping occurs more frequently for shorter distances. This can be attributed to the fact that multiple plectonemes are present at any given time for most conditions and the fact that the maximum distance is limited by the end-to-end distance, (± 5 μm), of the supercoiled DNA molecule.
Supplementary Figure S10: Tethered DNA molecules move freely and show no interactions with the surface of the flow cell. 

A, Image of a supercoiled DNA molecule. The center position of the DNA molecule along its length (black line) was determined by a Gaussian fit of the intensities in the \( y \)-direction. No spatial digression were observed in the fitted positions, the plectonemes merely appear as near-diffraction-limited spots of higher intensity. Experimental conditions for the molecule shown are 300 mM NaCl at 3.2 pN. 

B, Fitted DNA positions for a time series of images. Each colored line represents the fit to an single image such as in panel A. The DNA molecule freely swivels around its attachment point on the surface (see also Movies S1–S2), here located at position (12,14) in panel A. The DNA is stretched to a virtually straight line and has a 5.3 \( \mu \text{m} \) end-to-end distance between its attachment point and the fluctuating bead. 

C, The standard deviation (std) of the detected DNA positions in the \( y \)-direction increases linearly along the molecule, confirming the absence of surface interactions at specific locations. If present, surface interactions would impede the movement of molecule and this would lead to regions with a reduced standard deviation.
Supplementary Figure S11: Size distribution of detected plectonemes. Plectoneme sizes were determined from the ratio of the intensity for identified plectonemes to the total intensity of the fluorescently labeled DNA molecule. The plectoneme intensity was calculated as the integrated intensity of 9 pixels centered on the detected plectoneme position. The histogram shows that the minimum detectable plectoneme size for the 130 % threshold level used in the peak-fitting procedure is approximately 0.5 kb.
Table S1.

<table>
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<tr>
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<td>124</td>
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<tr>
<td>300 mM</td>
<td>43</td>
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<td>129</td>
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</tbody>
</table>

Supplementary Table S1: Measurements were performed under conditions where 25 % of the molecule was in a positively supercoiled plectonemic state. The table shows the number of applied turns for the respective applied stretching force and ionic strength at which experiments were performed.
**Movie S1**

**Movie showing plectoneme dynamics within a fluorescently labeled 21 kb DNA molecule.** The movie corresponds to the molecule shown in Fig. 1 C and D in the main text at 0.8 pN applied stretching force and 150 mM NaCl. Plectonemes are clearly visible as bright spots which appear and disappear and move along the DNA molecule. Experimental images were recorded at 50 Hz, the movie is slowed down to 0.5X real-time i.e. 25 Hz, scale bar in the lower left hand corner corresponds to 1 µm.

**Movie S2**

**Movie showing plectoneme dynamics for all probed experimental conditions.** The movie corresponds to the kymographs shown in Fig. 2A in the main text. Rows correspond to ionic strength, from top to bottom 300, 150 and 20 mM NaCl; columns correspond to force, from left to right 0.4, 0.8, 1.6 and 3.2 pN. Plectonemes are clearly visible as bright spots which appear and disappear and move along the DNA molecules. The number and dynamics of the plectonemes change with applied stretching force and salt concentration. Experimental images were recorded at 50 Hz, the movie is slowed down to 0.5X real-time i.e. 25 Hz, Scale bar in the lower left hand corner corresponds to 1 µm.
References and Notes


14. Materials and methods are available as supplementary materials on Science Online.


