SUPPLEMENTARY DATA

Direct force measurements and determination of DNA regime during the micro-manipulation experiments

The stretching force exerted on the DNA by the magnetic wrench was directly measured with the technique first introduced by Strick et al. (1,2). In this approach, the DNA-bead tether system is described as an inverted pendulum undergoing Brownian fluctuations, whose amplitude depends on the vertical stretching force. The force can be expressed as (1):

\[ F = k_B T \cdot l / <\delta x^2>, \]  

(S1)

where \( l \) is the vertical (along \( z \)) extension of the molecule (i.e., the DNA end-to-end distance) and \( <\delta x^2> \) is the mean square value of the bead fluctuations along the \( x \)-direction.

This method requires three-dimensional tracking of the bead position, in order to simultaneously measure both \( <\delta x^2> \) (as done normally in TPM) and the vertical extension \( l \) of the DNA molecule. To measure the latter, we developed a \( z \)-tracking method, which has been coupled with the \( x-y \) Single-Particle Tracking used for TPM measurements. The \( z \)-tracking is based on the analysis of the diffraction pattern in the micro-sphere images (see Supplementary Figure S1a-top panels). The radius of the diffraction ring in the bead image (\( R_D \)) depends on the relative position between the micro-sphere and the objective focal plane. When the bead moves away from the focal plane \( R_D \) increases, allowing the measurement of the vertical position \( z \) of the bead.
Supplementary Figure S1. Three-dimensional tracking. (a) The top panels show images ($M_{ij}$ matrix) of a 1µm diameter bead at different heights (from left to right: focal plane, +1µm, +2µm, +3µm). The diffraction ring appears as a white corona around the black core in the micro-sphere images. The middle panels show the bead core images ($T_{ij}$ matrix), once processed (i.e. inverted and background subtracted) for the x-y tracking algorithm. The bottom panels show the images ($I_{ij}$ matrix), once elaborated (see eqn. S2) for the vertical tracking algorithm. White bar represents 1µm. (b) Calibration curve of the vertical tracking algorithm. The diffraction ring radius $R_D$ is measured while the piezoelectric actuator moves the objective along the z-axis with nanometer precision. $R_D$ behaves linearly in a range of 2µm in the case of the 1µm diameter tethered micro-sphere shown in panel (a). The calibration parameter $\rho$ is obtained by the reciprocal value of the slope of the linear fit (red line in figure). In the example reported in figure, $\rho = 195$nm/pixel. The red arrow indicates the working point used during measurements. (c) x-y tracking performed simultaneously with the vertical calibration reported in (b). The x and y signals are clearly decoupled from the vertical tracking.

The images are acquired at video rate (25Hz) and stored in a matrix ($M_{ij}$). A second matrix $I_{ij}$ is obtained as:

$$I_{ij} = \frac{(M_{ij} - t) + |M_{ij} - t|}{2},$$  

(S2)

where $t$ is the value of the threshold used to highlight only the diffraction ring in the bead images (see Supplementary Figure S1a-bottom panels). The radius $R_D$ of the diffraction ring is then calculated as:

$$R_D = \frac{\sum_{i,j=1}^{N} \left[ (i - X_C)^2 + (j - Y_C)^2 \right] I_{ij}}{\sum_{i,j=1}^{N} I_{ij}},$$  

(S3)

where $X_C$ and $Y_C$ are the coordinates of the micro-sphere centroid in the sample plane (3).

$R_D$ depends linearly on the bead vertical position (see Supplementary Figure S1b) and does not correlate with the x-y tracking (see Supplementary Figure S1c). In order to relate the $R_D$ signal (measured in pixel) to a vertical displacement expressed in nanometers, each bead requires a calibration curve. The principle of the calibration is to relate a change in the optical
The image of the micro-sphere (i.e. the radius of first (white) diffraction ring) to a position along the vertical axis. Calibrations were obtained by measuring $R_D$ while the objective was slowly translated, at a constant velocity, along the $z$-axis by a piezoelectric actuator (PZ 127E, Physik Instrumente, Germany) in steps of 1nm. An example of calibration curve is shown in Supplementary Figure S1b. The range of measurable vertical displacements is roughly twice the micro-sphere diameter. In the case of the 1μm-diameter bead shown in Supplementary Figure S1a, the linear range is about 2μm (see Supplementary Figure S1b), which is appropriate for our purpose of measuring vertical displacements of the order of few hundred nanometers (for a DNA of 1000bp the vertical excursion is about 340nm). Due to heterogeneities in the size of super-paramagnetic beads, each tether was calibrated before the measurement session. Then the sample was placed in its working point, i.e. the middle of the $z$ linear range (indicated by the red arrow in Supplementary Figure S1b). In this way, we were able to distinguish displacements above and below the reference plane chosen for the tracking. This, in turn, implied to track the micro-sphere motion slightly out of focus. Nevertheless, as it is shown in Supplementary Figure S1c, the $x$-$y$ tracking did not result affected by this constraint.

Our vertical tracking method does not allow an absolute determination of the bead position (thus of the DNA extension) with respect to the microscope cover slip: without a reference point, we can only measure relative fluctuations of the micro-sphere along $z$. In actuality, the micro-sphere fluctuations are limited between a minimum value (when the bead clashes with the cover slip) and a maximum value (when the DNA is extended), as shown in Supplementary Figure S2. We consider the average value of the micro-sphere vertical fluctuations as the DNA extension $l$. $l = 170$nm in the case of Supplementary Figure S2 when no stretching force was applied (upper panel - magnetic wrench off). For best performance, this approach requires stabilization of the sample in the vertical direction, so that the relative fluctuations measured are always referred to the same surface position. The stabilization is achieved via a position feedback routine (see Supplementary Figure S3a), based again on the measurement of the diffraction ring radius. The vertical stabilization relies on measuring $R_D$ at time 0 and keeping it constant by correcting the sample position with respect to possible vertical drifts of the microscope stage by moving the objective with the piezoelectric actuator. The feedback routine was set to measure $R_D$ at a frequency of 10Hz and to correct the sample position at the same frequency. The difference in the $R_D$ values at time $t$ and $t + 100$ms was multiplied for the calibration parameter $r$ (see Supplementary Figure S1b) and for another parameter $g$ (i.e. the feedback gain) always set between 0.2 and 0.4, before being used as (error) signal to drive the feedback via the piezoelectric actuator. With this routine we obtained an RMS noise on the bead position of the order of $5 \div 10$nm (see Supplementary Figure S3b), allowing reliable measurements of $l$ not affected by drifts. We assume the precision in the measurement of the position $z$ of the micro-sphere to be the same as the stabilization of the sample achieved, i.e. $\Delta z = 10$nm.

The 3D tracking technique, with the stabilization routine, has been applied to infer the stretching force exerted during the DNA manipulation. In our measurements of the LacI-DNA interaction kinetics under torsional constraints beads with 400÷500nm diameter were used. The measurement method described above works best for larger beads; thus, it was applied to first obtain a careful measurement of the force on larger beads and then estimate, from these measurements, the force exerted by the apparatus on beads of the size used in LacI experiments, as discussed below.
Supplementary Figure S2. Direct force measurements. From left to right: tracking signal of the $z$ position of the micro-sphere; sketch (not to scale) of the system (the $z$ signal varies between a minimum value, when the bead clashes with the cover slip, and a maximum value, when the DNA is extended); histogram of the $z$ signal (from the total extension of the distribution we infer the DNA contour length $L_C = 430\text{nm}$ or $1250\text{bp}$, in the case reported in the figure); $x$-$z$ diffusion cloud (used to measure $\langle \delta x \rangle$). The upper panels report measurements performed in the absence of magnetic field, while the bottom panels show measurements done on the same tether in the presence of magnetic field ($B = 48\text{Gauss}$).

An example of these measurements, for a 1$\mu$m-diameter bead, is reported in Supplementary Figure S2. The figure shows, from left to right, the measured vertical position $z$ of the micro-sphere, a schematic drawing of the system (not to scale), the distribution of the $z$ positions measured, and the $x$-$z$ diffusion cloud with magnetic wrench off (top panels) and with magnetic wrench on ($B = 48\text{Gauss}$, bottom panels). We infer $l$ from the difference between the mean and the minimum value of the $z$ signal. For the tether considered in Supplementary Figure S2, we measured $l = (170\pm20)\text{nm}$, with wrench off, and $l = (225\pm20)\text{nm}$ with wrench on (the error $\Delta l$ is considered equal to twice the stabilization achieved along $z$: $\Delta l = 2\Delta z = 20\text{nm}$). From the total extension of the $z$ histogram we estimate the DNA contour length as $L_C = 430\text{nm}$ (corresponding to about $1250\text{bp}$). From the $x$-$z$ diffusion clouds in the right panels of Supplementary Figure S2, we measure $\langle \delta x \rangle$ as the standard deviation of the horizontal fluctuations of the micro-sphere. $\langle \delta x \rangle = (170\pm8)\text{nm}$ with wrench off, and $\langle \delta x \rangle = (90\pm5)\text{nm}$ with wrench on. Thus, using equation S1, we estimate a stretching force $F = (115\pm20)\text{fN}$, due to the application of the magnetic field during the manipulation. Measurements on 12 tethered micro-spheres (with diameters in the range $0.8\div1.4\mu$m), never exceeded the value of $300\text{fN}$. This value is actually an overestimation of the stretching force effectively exerted by the magnetic wrench during the LacI-DNA manipulation experiments. In fact, the stretching force depends on the interaction between the gradient of the magnetic field and the induced magnetic dipole of the bead.
Supplementary Figure S3. Behavior of the tether length vs. magnetic field intensity and vertical position feedback. (a) Drift and stabilization along $z$ of a 1μm-diameter bead stuck to the surface. The rate of the drift, due to thermal effects and to the magnetic manipulator ($B = 48$Gauss) is about 0.15nm/s (linear fit shown by the red curve in the figure). The red arrow shows when the feedback routine is switched on, the cyan dotted line indicates the initial position which is kept stable by the feedback. (b) Distribution of the $z$ signal with the feedback routine running. The width of the Gaussian fit (red curve) is 8nm and indicates the stabilization level achieved with this method. (c) The behavior of the tether length $<R>$ is reported versus the intensity of the magnetic field applied by the magnetic wrench. The quadratic behavior exhibited by $<R>$ is due to the interaction between the magnetic field gradient and the bead induced magnetic dipole, as discussed in section S1 (see also (4)). The red curve is the best fit to the data with the fit function $y = R + a / [(x + B_{min})^2]$; where $R$ is the asymptotic value of $<R>$, $a$ is a proportional coefficient, and $B_{min}$ is the minimum intensity value of the magnetic field capable of biasing the tether behavior. The fit parameters found from the fit were: $R = (168\pm5)$nm, $a = (6\pm2)$nm/Gauss$^2$, and $B_{min} = (2.0\pm0.5)$Gauss. The magnetic field value used for the LacI-DNA experiments was always 48Gauss. In the example shown in the figure, a magnetic field of 48Gauss reduced the tether length by about 15%. (d) Tether relative extension $(<R>/<R>_{Boff})$ vs. number of rotations ($r$) imposed to the micro-sphere. Data show curves for 10 different tethers (each coded with a different color). A single curve of this kind of measurements is also shown and described in Figure 3b of the main text. Dotted vertical lines indicate the symmetry point of the two outermost curves and, thus, report on the maximum tether-to-tether variability.

The bead induced dipole, in turn, depends on the field intensity (4), leading to a quadratic dependence of the force vs. the magnetic field intensity. Such dependence is reflected by the quadratic behaviour of the tether length $<R>$ vs. the magnetic field intensity shown in Supplementary Figure S3c). The induced magnetic dipole of the bead depends also on the
quantity of magnetic pigment inside the micro-sphere. Assuming the magnetic pigment
density constant for all the beads of the same batch (as from manufacturer's specifications),
for a given magnetic field intensity, force scales with the micro-sphere volume.

Based on the measurements of $F$ reported above for 0.8÷1.4µm diameter micro-
spheres, we estimate a stretching force lower than 50÷100fN for the 400÷500nm diameter
micro-spheres employed in the LacI-DNA measurements (5). These measurements of the
stretching force show that our DNA manipulation experiments are carried out in the DNA
entropic regime (which applies when the force acting on the DNA is below 0.5pN (6)). This
result was also confirmed by the (symmetrical) behaviour of the DNA in the presence of
supercoiling (see Supplementary Figure S3d and Figure 3 in the main text).
Supplementary Data S2

Independence of the observed effects of supercoiling on method of TPM data analysis

Due to the limited time resolution of TPM measurements, and filtering of the data, a number of concerns may rise during data analysis (7). Recently, we have described a method to address this issue and obtain robust estimates of the kinetic parameters measurable in a TPM experiment (8). This method requires accurate measurements of the standard $<R(t)>$ distributions displayed by the system in a well-defined and constant looped and unlooped state, independently. The measurements presented in this work did not lend themselves to the corrections method illustrated in (8), mainly due to the difficulty of obtaining robust estimates of the above mentioned standard distributions. In fact, each looped and unlooped state standard would have to be measured with high precision for each value of $\sigma$. Unfortunately, limitations in the total duration of recordings (due to intrinsic limits of the experimental system, for example recording terminated by the sudden non-specific sticking of the micro-sphere to the cover slip surface) and, more importantly, heterogeneities in the sizes of the super-paramagnetic beads would not allow such measurements. Thus, a simpler approach was undertaken, as described in the Materials and Methods. It is important to notice, however, that while the absolute values of $t_L$ and $t_U$ are affected by data filtering (7,8), the supercoiling-dependent modulation of these kinetic parameters was not affected (data not shown).

Additionally, the $t_U/t_L$ ratio proved to be totally insensitive to the extent of filtering operated on the data, as demonstrated in Supplementary Figure S4a. Also, when corrections following the methods described in (8) were attempted, a robust estimate of the intramolecular equilibrium constant ($K^*$, (9)) could be obtained, as shown in Supplementary Figure S4b.

**Supplementary Figure S4. Robustness of data analysis.** (a) $t_U/t_L$ ratio from dwell-time analysis performed on the data at different DNA supercoiling filtered with $T_d = 4.05s$ (blue circles), 5.40s (red circles), and 6.75 (green circles). (b) Intramolecular equilibrium constant $K^*$ (8,9) versus supercoiling.
Supplementary Table S1

Interaction kinetics in the presence of supercoiling

| Turns applied | $\sigma$ | $n$ | $\tau_L$ | $\tau_U$ | $\#_{3\text{States}}$ | $\#_{\text{Total}}$ | Time |
|---------------|---------|-----|---------|---------|----------------|----------------|------|
| Wrench Off    | 0       | 0   | 63 (3)  | 79 (3)  | 8              | 27             | 25.5 |
| Wrench On     | 0       | 0   | 61 (3)  | 81 (4)  | 2              | 16             | 15.0 |
| 3             | 0.026   | 0.76| 40 (3)  | 73 (3)  | 1              | 4              | 4.0  |
| 2             | 0.017   | 0.51| 25 (2)  | 52 (3)  | 2              | 6              | 5.5  |
| 1             | 0.0087  | 0.25| 40 (3)  | 78 (3)  | 1              | 5              | 4.5  |
| -1            | -0.0087 | -0.25| 69 (4) | 52 (4)  | 0              | 3              | 2.5  |
| -2            | -0.017  | -0.51| 42 (3) | 47 (3)  | 1              | 5              | 5.0  |
| -3            | -0.026  | -0.76| 38 (3) | 76 (3)  | 2              | 7              | 5.5  |

**Supplementary Table 1.** The Table summarizes the experimental observations reported in the text. Turns applied indicates the number of rotations imposed to the micro-sphere, $\sigma$ and $n$ indicate, respectively, the corresponding degree of supercoiling and the relative phase between the $lac$ operators, expressed as inter-operators turns (see text for the definitions of $\sigma$ and $n$). $\tau_L$ and $\tau_U$ are the looped and unlooped state lifetimes obtained analyzing the data according to the method described in the Materials and Methods section. $\#_{3\text{States}}$ is the number of tethers reporting the "three-state" behavior, $\#_{\text{Total}}$ is the total number of tethers observed during the experiments. The last column reports the total recording times (in hours).
References

1. Strick, T.R., Allemand, J.F., Bensimon, D., Bensimon, A. and Croquette, V. (1996) The elasticity of a single supercoiled DNA molecule. *Science, 271*, 1835-1837.
2. Strick, T.R., Allemand, J.F., Croquette, V. and Bensimon, D. (1998) Physical approaches to the study of DNA. *Journal of Statistical Physics, 93*, 647-672.
3. Capitanio, M., Cicchi, R. and Pavone, F.S. (2005) Position control and optical manipulation for nanotechnology applications. *European Physical Journal B, 46*, 1-8.
4. Normanno, D., Capitanio, M. and Pavone, F.S. (2004) Spin absorption, windmill, and magneto-optic effects in optical angular momentum transfer. *Physical Review A, 70*, 053829.
5. Normanno, D. (2007) PhD Thesis, LENS – University of Florence.
6. Strick, T.R., Allemand, J.F., Bensimon, D. and Croquette, V. (1998) Behavior of supercoiled DNA. *Biophys J, 74*, 2016-2028.
7. Vanzi, F., Broggio, C., Sacconi, L. and Pavone, F.S. (2006) Lac repressor hinge flexibility and DNA looping: single molecule kinetics by tethered particle motion. *Nucleic Acids Res, 34*, 3409-3420.
8. Vanzi, F., Sacconi, L. and Pavone, F.S. (2007) Analysis of kinetics in noisy systems: application to single molecule tethered particle motion. *Biophys J, 93*, 21-36.
9. Hsieh, W.T., Whitson, P.A., Matthews, K.S. and Wells, R.D. (1987) Influence of sequence and distance between two operators on interaction with the lac repressor. *J. Biol. Chem., 262*, 14583-14591.