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Supplemental Information

Anticooperative Binding Governs the Mechanics of Ethidium-Complexed DNA

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Supporting theory

Linear approximation of force-extension curves with force-induced intercalation

In our analysis we assume that we can reliably extract the zero-force extension, the persistence length and the apparent stretch modulus by fitting an extensible worm-like-chain model to measured force extension data. This approach requires that: (i) the persistence length is not changing significantly within the applied force range and (ii) the contour length change from the force induced intercalation is about proportional to the applied force, such that it can be reasonably well described by a constant stretch modulus. In the following we will provide evidence that these conditions are full-filled for the force extension data taken at low force (< 8 pN).

The apparent stretch modulus is mainly governed by force induced intercalation and was determined to be as low as ~200 pN (Figure 1c, main text). The change in fractional extension due to force induced intercalation compared to zero force is then given as:

$$\Delta\gamma = \gamma(F) - \gamma(0) \approx F / S_{app} \approx 0.04$$

where the numerical value was calculated for F = 8 pN. Using the experimentally determined change of the persistence length with the fractional extension, a change of the fractional extension $\Delta\gamma \approx 0.04$ corresponds only to a change in persistence length of ~1.3 nm, which is smaller than the measurement error and practically negligible when modelling force extension data.

For sufficiently low forces with $F\delta_{Eth} < k_B T$ (valid for forces from 0 to 8 pN), the force-dependent equilibrium constant can be approximated by a Taylor expansion as:

$$K_d(F) = K_{d,0} e^{-F\delta_{Eth} \cdot z_r(F)/k_B T} \approx K_{d,0}\left(1 - \frac{F\delta_{Eth} \cdot z_r(F)}{k_B T}\right) \approx K_{d,0} + \Delta K_d(F)$$

with

$$\Delta K_d(F) \approx -K_{d,0} \frac{F\delta_{Eth} \cdot z_r(F)}{k_B T}$$

Using for simplicity a simple Langmuir adsorption isotherm, in which only every n^{th} site of a linear lattice can be occupied (to include the binding site size n), the fractional occupancy is given as:

$$\nu(F) \approx \frac{1}{n} \frac{1}{1 + K_d(F)/c_{dye}}$$

For sufficiently small changes of the equilibrium constant due to the applied force we can write:

$$\nu(F) \approx \frac{1}{n} \frac{1}{1 + K_{d,0}/c_{dye}} - \frac{1}{n} \frac{1}{\left(1 + K_{d,0}/c_{dye}\right)^2} \frac{\Delta K_d}{c_{dye}} \approx \frac{\nu_0}{n} + \frac{{\nu_0}^2 (1/\nu_0 - 1)}{n} \frac{F\delta_{Eth} \cdot z_r(F)}{k_B T}$$

Due to $S_{app} \approx$ 200 pN in the relevant regime for force dependent intercalation, marked changes of the contour length occur only for F > ~1pN. At 1pN the DNA reached already ~90% of the

relative extension, such that $z_r(F) \approx$ const. With this we get that the fractional elongation increases approximately linear with force:

$$\gamma(F) = \underbrace{\frac{\delta_{Eth}}{\delta_{bp}}}_{\approx 1} \nu(F) \approx \frac{\nu_0}{n} + \underbrace{\frac{\nu_0(1-\nu_0)}{n}\frac{\delta_{Eth}}{k_B T}}_{1/S_{app}} F$$

And thus an apparent stretch rigidity of:

$$S_{app} \approx \frac{n}{\nu_0(1-\nu_0)}\frac{k_B T}{\delta_{Eth}}$$

when neglecting the influence of the much higher stretch rigidity of bare DNA. The minimum apparent stretch rigidity is reached at $\nu_0 = 0.5$ for which the approximate formula provides $S_{app}(0.5) \approx 100$ pN being in good agreement with the minimum found for the non-cooperative value in Figure 2c, main text. Thus, an approximation of the force-extension data in presence of force-induced intercalation seems to be justified in the low force limit (F < 8 pN).

In addition to our approximate considerations, we also tested directly how well the extensible WLC model can describe the modeled force extension curves (see Figure S4). When fitting modeled curves with the extWLC model, we find (force) deviations that are <1% throughout the applied force and concentration range (Figures S4a,b). The error of the zero-force fractional extension estimated from the fit was smaller than $5 \cdot 10^{-3}$ and always lower than the error of the experimental fractional elongation (Figure S4c). Due to a practically constant persistence length throughout the applied force range the extWLC fit returned also correct values for the (zero-force) persistence length of the complexed DNA (Figure S4d)

We therefore conclude that **fitting with the extensible WLC fit allows a faithful extraction of the fractional elongation at zero force** (up to forces of 8 pN) and highly recommend the usage of this model rather than a conventional WLC model.

Stretch rigidity from a simple serial combination of bare and ethidium-complexed segments

The stretch rigidity of intercalator-complexed DNA (without considering force induced intercalation) can in first approximation be described as a serial combination of bare DNA segments with a stretch rigidity of S_{DNA} ~1200 pN and of intercalator-complexed DNA segments with reduced stretch rigidity of S_{Eth}. Let l_{Eth} and l_{DNA} be the the combined length of all ethidium-complexed and all bare DNA segments, respectively. The elastic extension of the molecule at an applied force F is then given as:

$$\Delta L_{Eth}(F) = \frac{F}{S_{DNA}} l_{DNA} + \frac{F}{S_{Eth}} l_{Eth} \qquad (1)$$

The combined lengths of the bare and ethidium-complexed DNA segments are determined by the fractional occupancy:

$$l_{DNA} = L_0(1-\nu\cdot n)$$
$$l_{Eth} = L_0\nu\cdot n + L_0\nu\frac{\delta_{Eth}}{\delta_{DNA}} \quad (2)$$

Hereby we assume that one ethidium molecule covers its own length as well as the length of *n* DNA base pairs, i.e. the excluded neighbor length is part of an ethidium-complexed segment. With this the combined tensile strain becomes:

$$\frac{\Delta L_{Eth}}{l_{DNA}+l_{Eth}} = \frac{F}{L_0(1+\nu\delta_{Eth}/\delta_{DNA})}\left(\frac{L_0(1-\nu n)}{S_{DNA}} + \frac{L_0\nu(n+\delta_{Eth}/\delta_{DNA})}{S_{Eth}}\right)$$

With

$$\frac{\Delta L_{Eth}}{l_{DNA}+l_{Eth}} = \frac{F}{S_{comb}},$$

the combined stretch rigidity S_{comb} of the serial combination of bare and ethidium-complexed DNA segments with different stretch rigidities becomes (cyan line in Figures. 2c and S1b):

$$S_{comb} = \frac{S_{DNA}S_{Eth}(1+\nu\delta_{Eth}/\delta_{DNA})}{S_{Eth}(1-\nu n)+S_{DNA}\nu(n+\delta_{Eth}/\delta_{DNA})} \quad (3)$$

The same expression is obtained for the DNA twist rigidity of such a serial combination by replacing the stretch rigidities with C_{DNA} and C_{Eth} being the twist rigidities of bare and ethidium-complexed DNA. From the twist rigidity of the serial combination one obtains the plateau width for a given buckling torque Γ_B according to:

$$2\pi N_{plateau} = 2\frac{\Gamma_B}{C_{comb}}(l_{DNA}+l_{Eth}) \quad (4)$$

Inserting then provides for the plateau width of the rotation curves assuming a serial combination of bare and ethidium-complexed DNA segments with different twist rigidities (cyan line in Figs. 3c and S:

$$N_{plateau} = \frac{\Gamma_B L_0}{\pi}\frac{C_{Eth}(1-\nu n)+C_{DNA}\nu(n+\delta_{Eth}/\delta_{DNA})}{C_{DNA}C_{Eth}} \quad (5)$$

Zero-torque DNA length as function of the ethidium induced untwisting

In the following we will derive a relation between the ethidium-induced length increase and the DNA untwisting at zero torque that provides the blue dashed line in Figure 3a. Experimentally this corresponds to the curve connecting the centers of the supercoiling curves, for which we assume zero torque. The increase of the DNA contour length is related to the fractional occupancy according to:

$$L_{Eth} - L_0 = \delta_{Eth}\cdot\upsilon\cdot\frac{L_0}{\delta_{bp}} \quad (6)$$

where the ratio on the right hand side provides the number of base pairs in the molecule. The corresponding untwisting at zero torque is similarly given by:

$$\Delta N_{Eth} = \Delta N(N_{tw} = 0) = -\frac{\varphi_{Eth}}{2\pi}\upsilon \cdot \frac{L_0}{\delta_{bp}} \tag{7}$$

Dividing both equations provides:

$$\frac{L_{Eth} - L_0}{\Delta N} = -\frac{\delta_{Eth}}{\varphi_{Eth}/2\pi} \tag{8}$$

in agreement with a stringent coupling between contour length elongation and untwisting for intercalation. To describe the DNA extension at the center of the supercoiling curve, the relative extension at the given force and fractional occupancy needs to be considered:

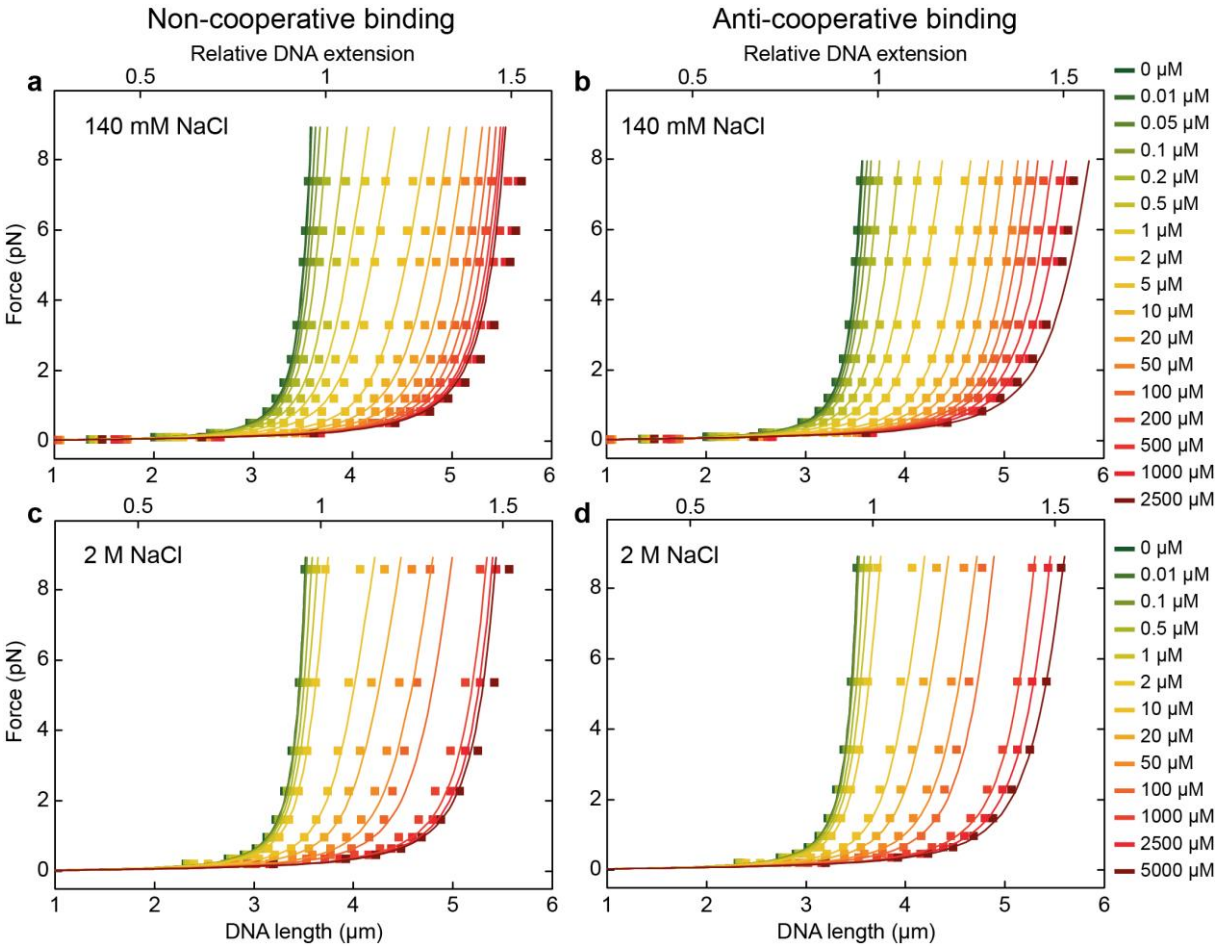
$$h(F, \Gamma = 0, \upsilon, \Delta N) = L_{Eth} z_r(F, p(\upsilon)) \tag{9}$$

where z_r is the relative DNA extension as provided from the extensible WLC model for the given force and persistence length

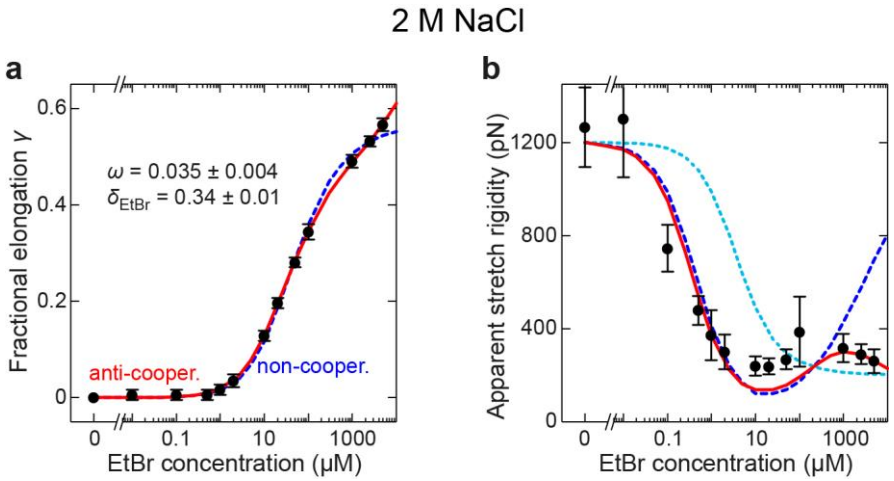
Inserting Equation 8 into Equation 9 provides for the DNA extension at zero torque:

$$h(F, \Gamma = 0, \upsilon, \Delta N) = \left(\frac{\delta_{Eth}}{\varphi_{Eth}/2\pi} \Delta N + L_0 \right) z_r(F, p(\upsilon)) \tag{10}$$

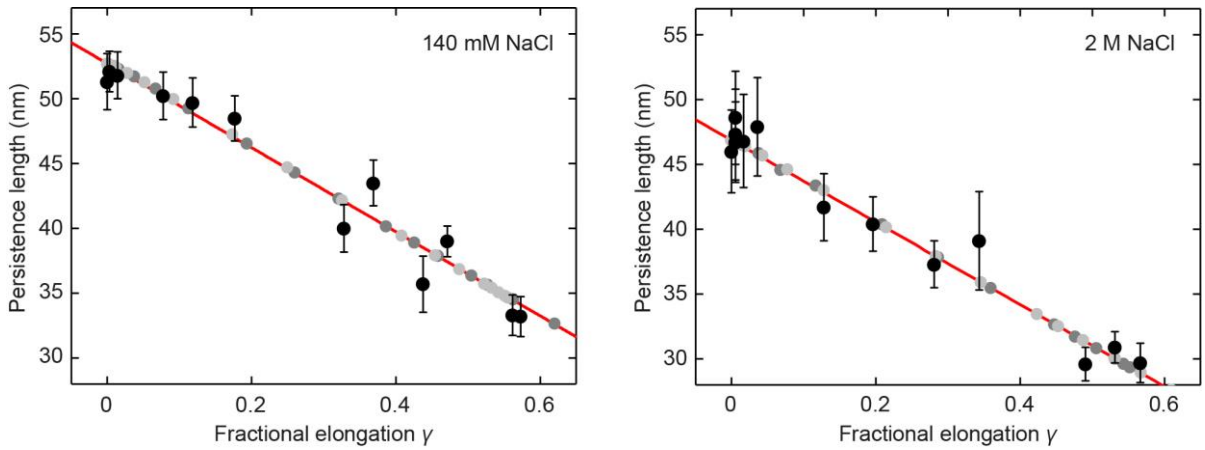
The fractional occupancy υ is obtained from Equation 7 for a given ΔN. The corresponding persistence length is provided from the measured linear dependence on the fractional elongation/occupancy (see Figure S2).



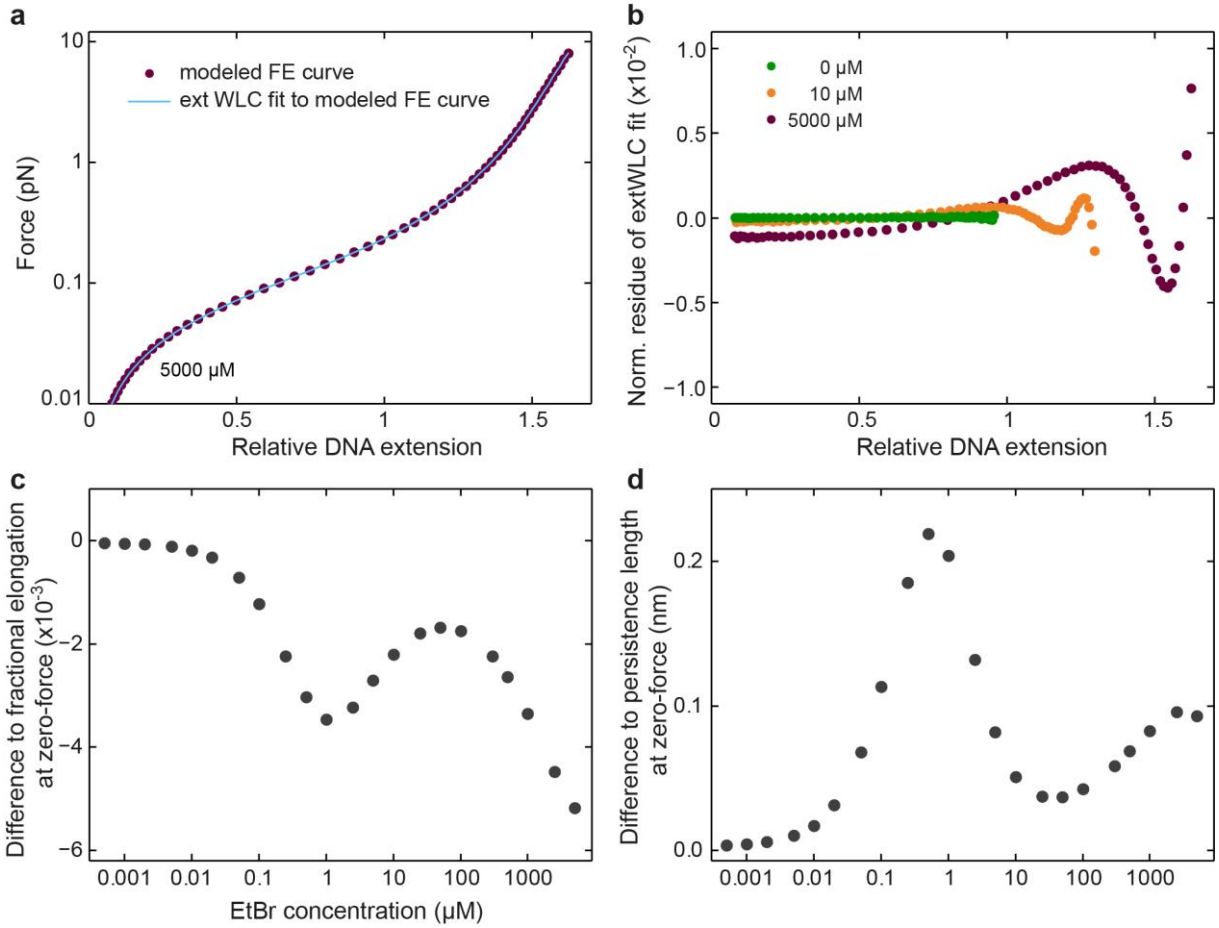
Supplementary Figure S1. DNA force extension curves in presence of EtBr compared to model predictions. (**a,c**) Measured force-extension data in presence of 140 and 2 M NaCl (filled squares) compared to the predictions (solid lines) using the non-cooperative binding model (see main text). (**b,d**) Measured force-extension data in presence of 140 and 2 M NaCl compared to the prediction using the anti-cooperative binding model (see main text). For modeling the force extension data, the best fit parameters from Table 1 (main text) were taken. Throughout a stretch rigidity of 1200 pN was used. For the persistence length a linear dependence on the fractional elongation according to Supplementary Fig. S2 was applied.



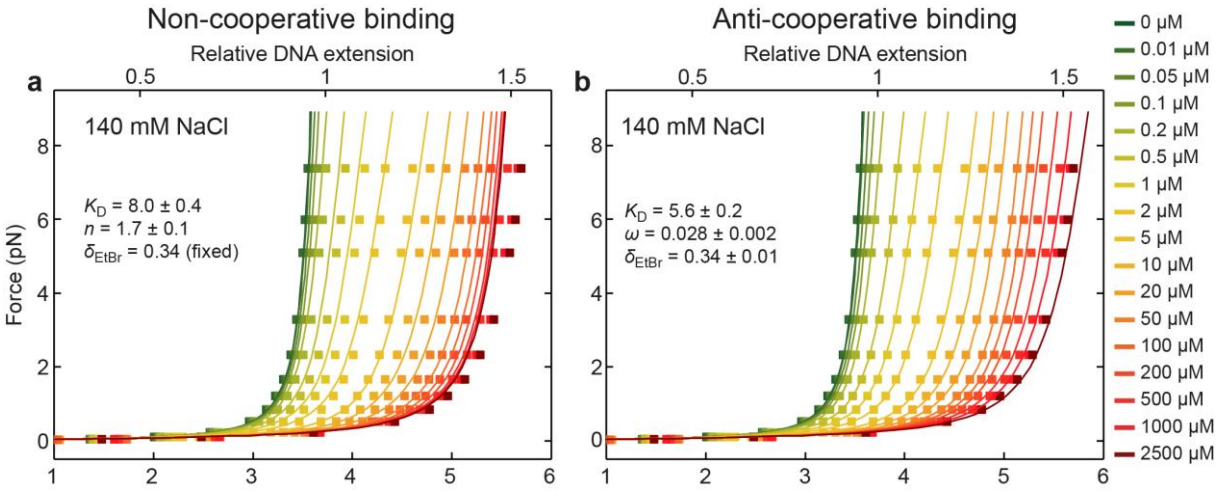
Supplementary Figure S2. DNA elongation and decreased stretch rigidity due to ethidium intercalation measured in 2M NaCl. (**a**) Fractional elongation of the DNA contour length at zero-force as function of the EtBr concentration (filled circles) obtained from force extension data recorded in 2M NaCl. Fits to the data using a non-cooperative and an anti-cooperative binding model are shown as dashed blue and solid red lines, respectively. Best fit parameters are given for the anti-cooperative model. (b) Apparent DNA stretch rigidity as function of the EtBr concentration (filled circles) obtained from the force extension data. Predictions from modelling force-induced intercalation using the non-cooperative and the anti-cooperative binding model are shown as dashed blue and solid red lines, respectively. The prediction of a serial combination of rigid bare and soft ethidium-complexed DNA segments is shown as a dotted cyan line.



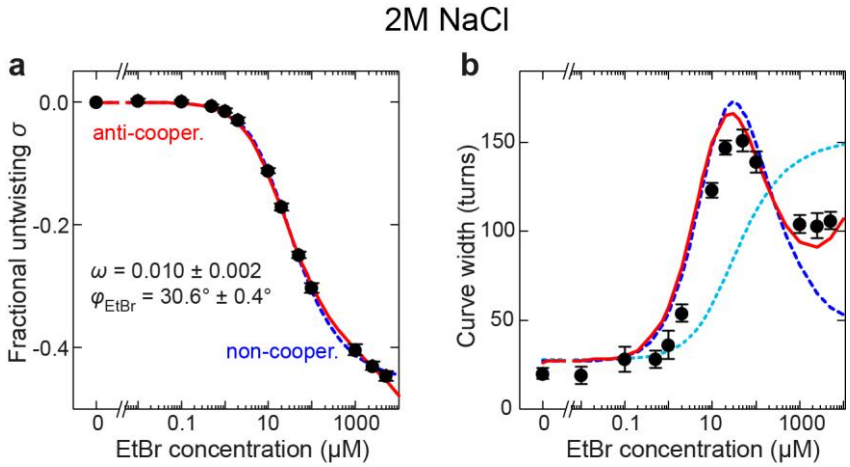
Supplementary Figure S3. DNA persistence length as function of the fractional DNA elongation. Persistence lengths (filled black circles) and fractional elongation at zero force were obtained from fitting force extensions data for varying EtBr concentrations (see Fig. 2, main text and Fig. S1). The changes in DNA persistence length are mostly originating from an altered net charge of the ethidium-bound DNA (incl. intercalation and possible external binding). The persistence length decreased approximately linearly with the fractional elongation, i.e. with the occupancy of the DNA by ethidium. Linear fits to the data are shown as red lines, which were taken in the simulations of force extension data. Persistence lengths from fitting simulated force extension data using the non-cooperative and the anti-cooperative binding model are shown as filled gray and light gray circles, respectively. These persistence lengths reproduce the input values of the simulations.



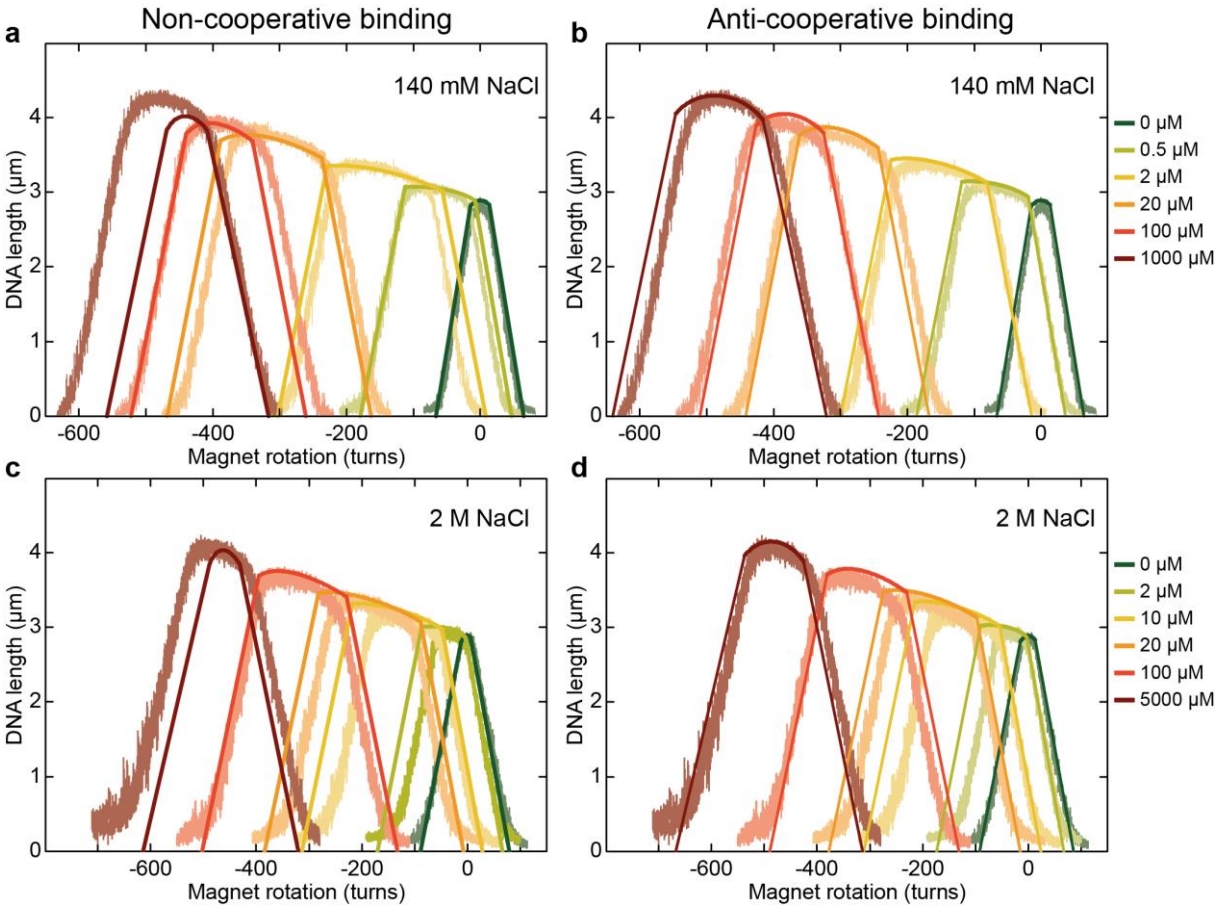
Supplementary Figure S4. Fit of modeled force extension curves including force-induced intercalation with the extensible WLC model (extWLC). (**a**) Fit of the extWLC model to a force-extension curve which was modeled for anti-cooperative binding at 5000 µM EtBr using the parameters given in Table 1, main text. (**b**) Normalized residues between extWLC fit and modeled force extension curve for EtBr concentrations of 0, 10 and 5000 µM EtBr. Even at the highest concentration with the highest fractional extensions the (force) difference between both curves was always <1%. (**c**) Difference of the (zero-force) fractional elongation from the extWLC fit to the actual zero-force fractional elongation of the modeled curve. Throughout the concentration range the difference is smaller than $5 \cdot 10^{-3}$ and always lower than the error of the fractional elongation at zero force as given in Fig. 2b, main text. This shows that an extWLC fit allows a faithful extraction of the fractional-elongation at zero force. (**d**) Difference between the persistence lengths obtained from the extWLC fit and the zero-force persistence lengths used in curve modeling. Since the persistence length changes only slightly throughout the considered force range the observed difference is rather small (<0.3 nm).



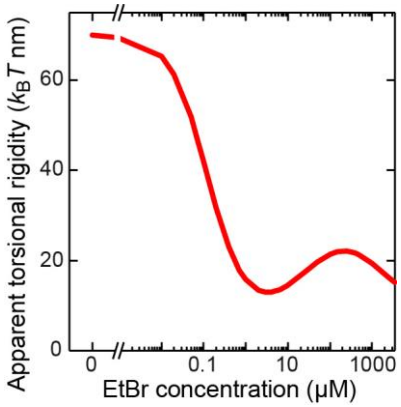
Supplementary Figure S5. Direct fit of the force extension curves in presence of EtBr considering force-dependent intercalation. Measured force-extension data in presence of 140 mM are shown as filled squares. (**a**) Experimental data was globally fit with modeled force-extension curves for non-cooperative binding (see main text). (**b**) Experimental data was globally fit with modeled force-extension curves for anti-cooperative binding (see main text). Best fit parameters (given in each plot) equal within error the parameters from fitting the fractional elongation at zero-force (see Table 1, main text). For generating model curves a DNA stretch rigidity of 1200 pN was used. For the persistence length a linear dependence on the fractional elongation according to Supplementary Fig. S2 was applied.



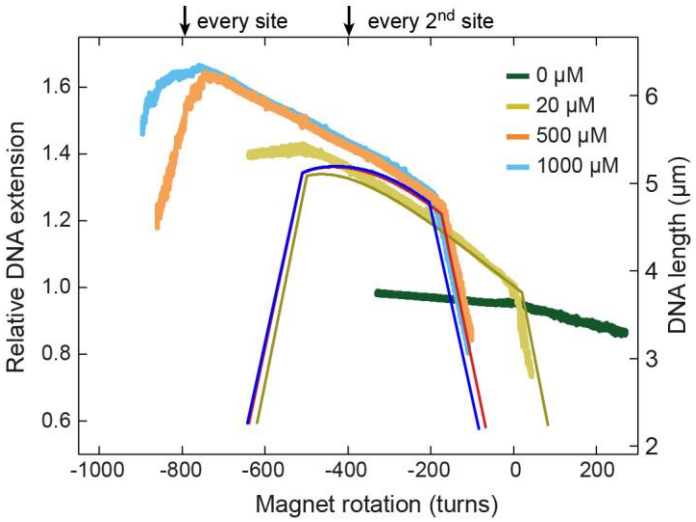
Supplementary Fig. S6. DNA untwisting and torsional softening due to ethidium intercalation measured in 2M NaCl. (**a**) Fractional untwisting of the DNA at zero-torque as function of the EtBr concentration (filled circles) from the centers of the supercoiling curves. Fits to the data using a non-cooperative and an anti- cooperative binding model are shown as dashed blue and solid red lines, respectively. Best fit parameters are given for the anti-cooperative model. The expected untwisting for binding at every second base pair stack is shown as a gray dashed line. (**b**) Plateau width of the measured supercoiling curves as function of the EtBr concentration (filled circles). Predictions from modelling torque-induced intercalation using the non-cooperative and the anti- cooperative binding model are shown as dashed blue and solid red lines, respectively. The prediction for a serial combination of torsionally rigid bare and soft ethidium-complexed DNA segments is shown as a dotted cyan line.



Supplementary Figure S7. DNA supercoiling curves in presence of EtBr compared to model predictions. (**a,c**) Measured supercoiling curves in presence of 140 and 2 M NaCl (lighter colors) compared to the predictions (darker colors) using the non-cooperative binding model (see main text). (**b,d**) Measured supercoiling curves in presence of 140 and 2 M NaCl compared to the prediction using the anti-cooperative binding model (see main text). For modeling the force extension data, the best fit parameters from Table 1 (main text) were taken. Throughout a twist rigidity of 70 k_BT nm was used. For the persistence length a linear relationships according to Supplementary Fig. S2 was applied. At both ionic strengths a similar behavior is seen. At 2 M NaCl the measured DNA length is slightly smaller compared to 140 mM due to a lower persistence length throughout the applied concentration range of EtBr (see Supplementary Figure S2).



Supplementary Figure S8. Predicted apparent torsional rigidity of the DNA at 0.4 pN as function of the EtBr concentration. The prediction is made for the cooperative binding model using the best fit parameters for 140 mM NaCl (see Table 1).



Supplementary Figure S9. DNA untwisting in presence of EtBr at an elevated force of 6.4 pN compared to the non-cooperative model prediction. Measured supercoiling curves (from Figure 5, main text) are shown in lighter colors compared to predictions from the non-cooperative model (binding site size of 1.8) that are shown in darker colors of the same tone. Arrows on top of the plot indicate the expected DNA untwisting if intercalation would occur into every second or every base-pair stack.