

Supplementary Data

Derivation of kinetic equations

Competition on (+) supercoiled DNA

From the branched kinetic pathway in Fig. 7B, the burst velocity for (+) supercoil relaxation $V_+(F, \tau)$ is given by

$$V_+(F, \tau) = \frac{k_\alpha(F, \tau) + k_\chi}{1 + \frac{k_\alpha(F, \tau)}{k_{\alpha,RL}(\tau)} + \frac{k_\chi}{k_{\chi,RL}}}. \quad (1)$$

By using transition state theory the rate constants can be expressed as a function of force and torque (Wang, 1998), so that

$$\ln\left(\frac{k_\alpha(F, \tau)}{k_{\alpha,0}}\right) = \frac{F \cdot \Delta x_\alpha + \tau \cdot \Delta \theta_\alpha}{k_B T}, \quad (2)$$

and

$$\ln\left(\frac{k_{\alpha,RL}(\tau)}{k_{\alpha,RL,0}}\right) = \frac{\tau \cdot \Delta \theta_{\alpha,RL}}{k_B T}, \quad (3)$$

where $k_{\alpha,0}$ and $k_{\alpha,RL,0}$ are the rates in the absence of mechanochemical stresses, k_B is Boltzman's constant, T the temperature in degrees Kelvin, and $\Delta x_\alpha, \Delta\theta_{\alpha,RL}$, and $\Delta\theta_\alpha$ are the distance and angles to the transition states. For plectonemic DNA under tension, the torque can be expressed in terms of force and bending rigidity of DNA (B) as $\tau(F) = (2BF)^{1/2}$. The ratio between wrapping and dissociation rates at zero force and torque $k_{\alpha,0}/k_{off}=250$ was deduced from parameters measured in our previous study¹ using $\frac{k_{\alpha,0}}{k_{off}} = \frac{k_\alpha(F=0, \tau)}{k_{off}} e^{\frac{-\tau \cdot \Delta\theta_\alpha}{k_B T}}$. We had previously obtained $\frac{k_\alpha(F=0, \tau)}{k_{off}} = 85$ under conditions of small negative torques ($\tau = -3$ pN nm), and found that wrapping was mildly torque sensitive (corresponding to an angle to the transition state $\Delta\theta = 1.5$ rad).

Competition on (-) supercoiled DNA

From the branched kinetic pathway in Fig. 7C, the burst velocity for (-) supercoil introduction on (-) supercoiled DNA $V_-(F, \tau)$ is given by

$$V_-(F, \tau) = \frac{k_\alpha(F, \tau) - k_p(\tau)}{1 + \frac{k_\alpha(F, \tau)}{k_{\alpha,RL}(\tau)}} \quad (4)$$

where k_p is the overall rate for (-) supercoiling relaxation. In this case, we allow for the possibility of k_p being torque dependent by expressing it as $\ln\left(\frac{k_p(\tau)}{k_{p,0}}\right) = \frac{\tau \cdot \Delta\theta_p}{k_B T}$, where $k_{p,0}$ is the rate at zero torque and $\Delta\theta_p$ is the angle to the transition state.

Equations 1 and 4 were used to fit the experimental data, assuming that $k_{off} \sim 10$ Hz (Fig. 7A). This assumption was based on our finding that k_{off} was larger than the cycling rate in our previous study ($k_{\alpha,RL} \sim 0.4$ Hz)¹ and by analogy to yeast topoisomerase II, which dissociates from a 40 bp DNA segment at ~ 10 -100 Hz (F. Mueller-Planitz and D. Herschlag, personal communication). Larger values of k_{off} (e.g. ~ 100 Hz) would not affect our basic result that $k_{\alpha,0}/k_\chi \sim 22$, and produce a fit to the data as good as that for $k_{off} = 10$ Hz. In this case, the best-fit ($\chi^2 = 1.02$) is obtained for $k_{\chi,RL} = 0.6$ Hz, $k_{\alpha,RL,0} = 0.6$

Hz, $\Delta\theta_{\alpha, RL} = 0.2$ rad, and $k_{p,0} \sim 5$ Hz. The same conclusions drawn from the fits presented in the text can be made here.

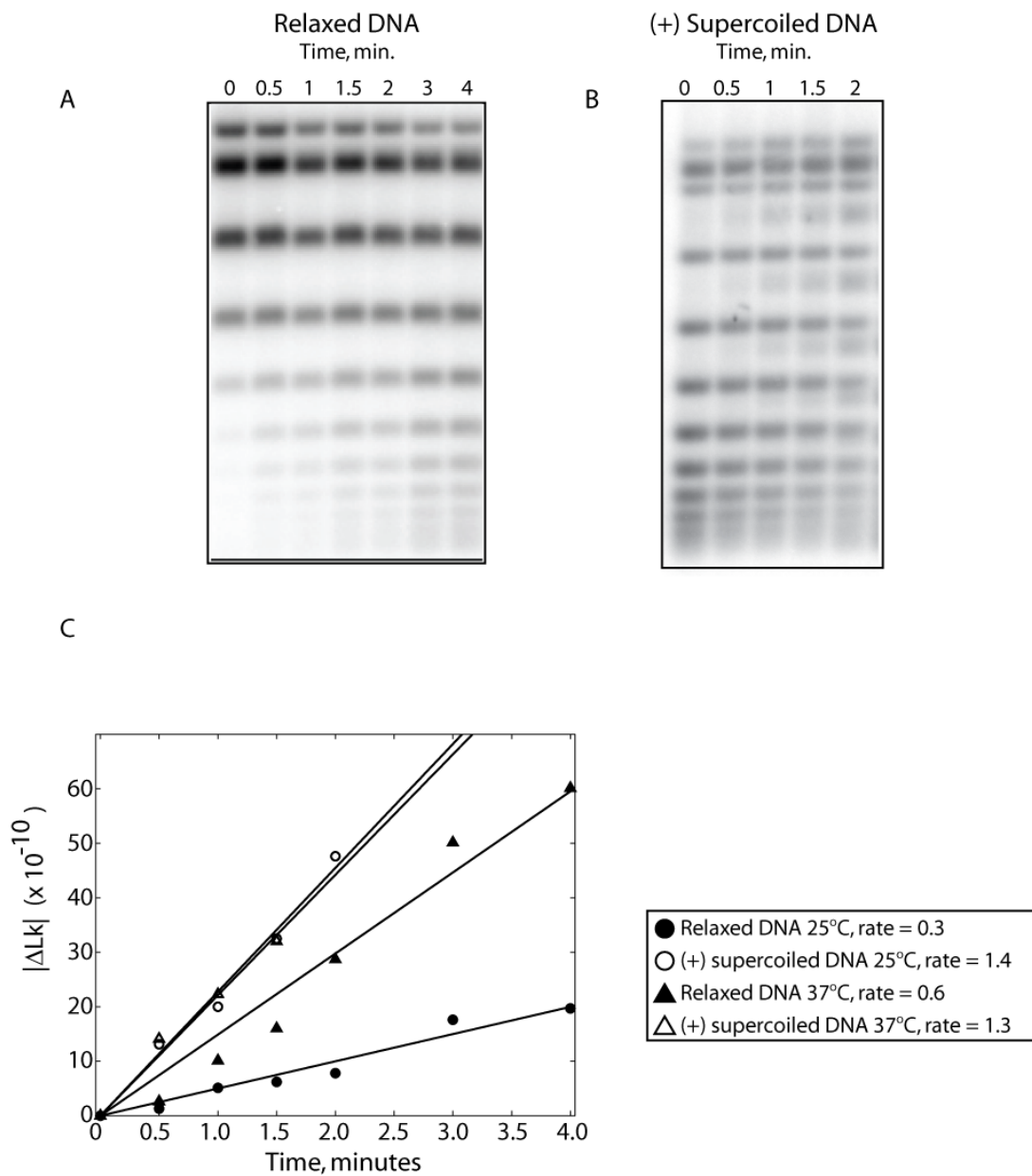
References

1. Gore, J. et al. Mechanochemical analysis of DNA gyrase using rotor bead tracking. *Nature* **439**, 100-4 (2006).

Supplementary Figure 1

Gyrase activity on (+) and (−) supercoiled substrates in bulk

We tested whether our model was also consistent with kinetic experiments performed in bulk. The small value for $\Delta\theta_{RL}$ suggests that for moderate σ there should be only a modest dependence of the ensemble enzymatic rate on the sign of substrate supercoiling. To test this prediction, we performed ensemble kinetic experiments where we measured the introduction of (−) supercoils on relaxed closed circular DNA and the removal of (+) supercoils on a (+) supercoiled DNA substrate under the same conditions as the single-molecule experiments (Supplementary Fig. 1). This experiment showed about a three-fold difference in rates with (+) supercoiled compared with relaxed DNA, significantly lower than the 20-fold rate difference for (+) over (−) supercoil relaxation reported for topo IV^{1,2}. Ensemble measurements taken in standard gyrase reaction conditions (1.8 mM spermidine, 37°C) yielded similar results to experiments performed in conditions optimized for single molecule analysis (0.2 mM spermidine, 25°C) (Supplementary Fig. 1).



Supplementary Figure 1

Kinetics of (-) supercoil introduction by gyrase into relaxed and (+) supercoiled DNA in ensemble experiments.

Relaxed (A) and (+) supercoiled (B) DNAs were incubated with gyrase at 25 °C, and the reactions were terminated at the times indicated (top). The reacted DNA was analyzed by agarose gel electrophoresis in TAE buffer (A) or TAE buffer containing 10 μM netropsin (B). Images of Southern blots of the gels are shown.

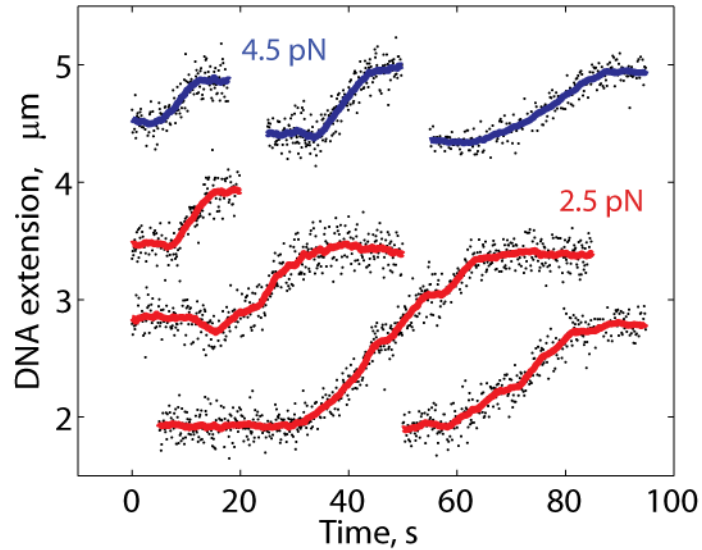
(A) As gyrase introduces (–) supercoils into the relaxed DNA, the topoisomers migrate faster. (B) As gyrase introduces (–) supercoils into the (+) supercoiled substrate, the more relaxed topoisomers migrate as bands interdigitated between the substrate topoisomers. The σ of the (+) supercoiled DNA was +0.03.

References

1. Crisona, N.J., Strick, T.R., Bensimon, D., Croquette, V. & Cozzarelli, N.R. Preferential relaxation of positively supercoiled DNA by E. coli topoisomerase IV in single-molecule and ensemble measurements. *Genes Dev* **14**, 2881-92 (2000).
2. Stone, M.D. et al. Chirality sensing by Escherichia coli topoisomerase IV and the mechanism of type II topoisomerases. *Proc Natl Acad Sci U S A* **100**, 8654-9 (2003).

Supplementary Figure 2

The rate of (+) supercoiling relaxation at high forces is force-independent..

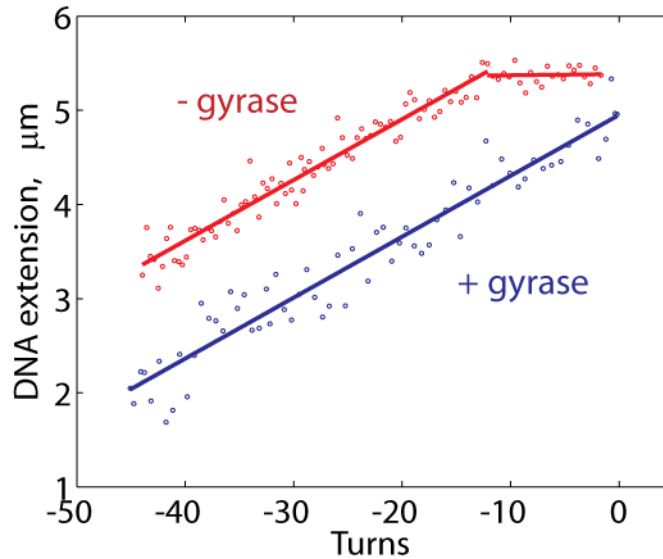


Supplementary Figure 2:

Activity of gyrase at high DNA tensions. Processive relaxation of (+) supercoiled DNA at high forces. Curves show representative single gyrase relaxation traces of (+) supercoiled DNA molecules at 4.5 (blue) and 2.5 pN (red). Raw data are plotted as dots and 2-sec moving averages are shown as solid curves. Curves were shifted in both axes for clarity.

Supplementary Figure 3

The passive mode requires the presence of (-) supercoils.



Supplementary Figure 3: Relaxation of (-) supercoils by the passive mode requires (-) crossings. In the absence of enzyme (red curve), the introduction of (-) supercoils from $\sigma = 0$ leaves the DNA extension unchanged up to the buckling transition and decreases its extension linearly thereafter. In the presence of 20 nM gyrase, 45 (-) supercoils were introduced in a topologically constrained DNA molecule. After plectonemic DNA relaxation ceased, 45 new (-) supercoils were mechanically introduced while the DNA extension was monitored in real-time (blue curve). The absence of a plateau demonstrates that gyrase was unable to relax the DNA beyond the critical buckling torque. Raw data are plotted as dots and piece-wise linear fits are shown as solid curves. Curves were vertically shifted for clarity.