**Supplementary Materials**

**Torque Spectroscopy**

In a simple two-state Boltzmann model in which torque stabilizes an otherwise unfavorable unwinding reaction, the torque-dependence of the forward and backward transition rates is a function of the extent of DNA unwinding in the transition state, $k_f(\Gamma) = k_f(0) \exp(2\pi n_f \Gamma / k_B T)$ and $k_r(\Gamma) = k_r(0) \exp(-2\pi n_r \Gamma / k_B T)$ (see Fig. 3B and 5G). Here $k_f(0)$ and $k_r(0)$ are related to the torque-free transition rates, and $n_f$ and $n_r$ are the amounts of B-DNA unwinding that separate the B-DNA state from the transition state, and then the transition state from the cruciform state, respectively [1] (Fig. 5G); their sum, $n_f + n_r = n_{cruciform}$, is well-determined experimentally.

Thus over an amount of unwinding $n_f$ the torque assists in cruciform extrusion by working with the unwinding reaction to lower the energy barrier corresponding to the transition state. However over the remaining unwinding $n_r$ the torque assists in cruciform extrusion by working to increase the energy barrier to rewinding. The mechanical constraint applied to the DNA – torque – works to activate the unwinding reaction only until the transition state is reached; thereafter it acts to reduce the rewinding reaction rate. The van ‘t Hoff relations for the torque-dependence of the rates are $d \ln k_f / d \Gamma = 2\pi n_f / k_B T$ and $d \ln k_r / d \Gamma = 2\pi n_r / k_B T$. Since torque varies linearly with supercoiling in this “tuning” regime, we can instead simply determine the slope of $\ln k_f$ and $\ln k_r$ with respect to supercoiling and use this to obtain $d \ln k_f / d \ln k_r = n_f / n_r$.

**Supplementary Figures and Legends**

*Supplementary Figure S1.* Abrupt changes in DNA extension occur only for negatively supercoiled Charomid 9-11 DNA extended by at least a ~0.4 pN extending force. (A) Time-averaged extension vs. supercoiling curves and (B) real-time traces of the extension of positively (n=+25 turns) or negatively supercoiled (n=-25 turns) 11 kb DNA extended at low (F ~ 0.2 pN) or high (F ~ 0.45 pN) force. The buckling transition at which DNA begins to form writhe occurs at $|n_b|$ turns and is indicated on the time-averaged trace obtained at low force by vertical dashed lines. The change in extension per turn $\delta$ is obtained by taking the slope of the extension vs. supercoiling curve in the linear regime for positive and negative supercoiling; in experiments with Charomid DNA the slope is typically ~75 nm/turn. Thus by detecting a 75 nm change in extension one observes a unit change in DNA topology $\Delta Wr = (+$ or $-) 1$.

*Supplementary Figure S2.* The CharomidX sequence does not require a specific sequence context to undergo cruciform extrusion. The DNA sequence presented in Figure 2 was inserted into a Charomid-S3 construct lacking the cruciform sequence and flanking regions. Cruciform extrusion was observed for negatively supercoiled DNA as with the wt Charomid 9-5 construct.
Supplementary Figure S3. The amplitude of the unwinding transition observed on Charomid 9-5 kb does not change significantly with the amount of unwinding.

Supplementary Figure S4. Force-dependence of cruciform “tuning” for Charomid 9-11 kb. Experiments were performed at F = 0.5 pN (magenta), F = 0.6 pN (blue) and F = 0.7 pN (red). (□) Free energy barrier to cruciform extrusion; (○) free energy barrier to cruciform rewinding. When the two lines intersect the transition rates are equal and the cruciform state is in equilibrium with the B-DNA state (ΔG=0). As the extending force increases so does the buckling torque $J_B$: $J_B \sim F^{0.4}$ [2, 3]; at higher torque less twisting of the DNA is required to stabilize the cruciform, explaining the enhanced sensitivity of transition rates to supercoiling as the force increases. For F = 0.5 pN we obtain $n_f/n_r = 1.8$, for F = 0.6 pN we find $n_f/n_r = 1.8$ and for F = 0.7 pN we obtain $n_f/n_r = 1.6$. Thus the position of the transition state along the reaction coordinate ($n_r \sim 1.8 n_f$) is essentially independent of the extending force in the range of forces for which these measurements can be made. Note that the free energies are not absolute values.

Supplementary Figure S5. Histogram of the lifetime of the B-DNA state prior to formation of wtColE1 cruciform (n=-21 turns). The mean time elapsed prior to formation of the wtColE1 cruciform is 410 +/- 100 seconds (27 events).

Supplementary Figure S6. Repeated cycles of extrusion and rewinding of the wtColE1 perfect cruciform. The cruciform is observed to rapidly extrude (uparrows) when the DNA is unwound by n = -19 turns (dark blue shading). Then, the DNA is rewound to n = -8 turns (light blue shading), after which the cruciform will rewind, but only after a few tens of seconds (downarrows). In the last rewinding event (beginning at 12950 seconds) the DNA is rewound only to n = -9 turns, conditions under which the cruciform now takes several hundred seconds to rewind. This indicates that the perfect cruciform is kinetically trapped and therefore not a good thermodynamic system.

Supplementary Figure S7. Histogram of the amplitude of the unwinding transition on the mutated ColE1 inverted repeat bearing (A) a 5-base loop and (B) an 8-base loop. Dashed lines represent mean amplitudes. For the 5-base loop the mean amplitude of the change in DNA extension is 213 nm +/- 4 nm (76 events), and for the 8-base loop the mean amplitude of the change in DNA extension is 233 +/- 3 nm (39 events). With a slope $\delta = 75$ nm/turn in these experiments, this corresponds to unwinding of 30 +/- 1 bp for the construct in (A) and 33 bp +/- 1 for the construct in (B), in agreement with values expected based on the cruciform sequence.

Supplementary Figure S8. Role of flanking A+T-rich sequence in extrusion of the ColE1 inverted repeat (A) Construct containing both the Inverted Repeat and the A+T-rich flanking region. Cruciform extrusion is apparent. (B) Construct containing the Inverted Repeat but not the A+T rich flanking region. No cruciform extrusion is observed. (C) Construct containing no Inverted Repeat but with the A+T rich flanking region. No cruciform extrusion is observed, but transient unwinding fluctuations generated by the A+T rich region are apparent. (D) Construct containing neither the Inverted Repeat nor
the A+T rich flanking region. Neither cruciform extrusion nor transient unwinding fluctuations are observed.

**Supplementary Figure S9.** Analysis of a second cruciform with reversible kinetics derived from the wtColE1 inverted repeat. (A) The cruciform tested here was obtained from the wtColE1 inverted repeat by changing a G-C basepair to an A-T base pair and by inserting an unpaired adenine a few bases from the base of the loop. (B) Time-trace shows reversible cruciform extrusion. (C) Torque spectroscopic analysis of cruciform extrusion. The ratio of the dependency of the forward and backwards rates on unwinding yields \( \frac{\text{nr}}{\text{nf}} = 4.3 \pm 0.5 \), in agreement with results obtained using the reversible cruciform described in the main text.

**Supplementary References**

[1] M. Rief, J.M. Fernandez, and H.E. Gaub. Elastically coupled two-level system as a model for biopolymer extensibility. *Phys. Rev. Lett.*, 81:4764–4767, 1998.
[2] G. Charvin, J.-F. Allemand, T.R. Strick, D. Bensimon, and V. Croquette. Twisting DNA: single molecule studies. *Contemp. Phys.*, 45:385–403, 2004.
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Supplementary Figure S3
Supplementary Figure S4
Supplementary Figure S6
Supplementary Figure S8
Supplementary Figure S9