Torque in stretched supercoiled DNA

- Twisting and pulling DNA – “state coexistence” of plectonemic supercoiled DNA and extended DNA
- In mixed state torque depends on force alone, not on linking number

What is the torque as a function of force?

- Use in studying relaxation of supercoiling by type I topoisomerases and other supercoil-relaxing enzymes
- Torque-force “phase” diagram (low forces)
- What about directly measuring torque?
Magnetic tweezer manipulation of DNA

Rotation of the magnet introduces supercoils, which translates into a reduction of the length of the DNA due to the formation of plectonemic supercoils.

Method originated by Allemand, Strick, Croquette, Bensimon (Science 1995).

Key point is that molecule is held under
• constant tension (force) via fixed position of magnet
• constant linking number via fixed angle of magnet

If magnet is not moved, then changes of \( \sigma \) (e.g., mediated by topoisomerases) occur at fixed force
Classical model of pure twist for elastic rod (or DNA)

Twist elasticity described by "twist persistence length"
\[ C = 75 \text{ to } 115 \text{ nm} \]

Why such a big range of values? (95 nm)
\[ \sigma = -0.033 \]

\[ \sigma = 0.000 \text{ (relaxed)} \]

\[ \sigma = -0.062 \text{ (in vivo)} \]

\[ \sigma = -0.016 \]

Boles, White, Cozzarelli  JMB 1991
Plectonemic Supercoiling ( |σ| > 0.01 )

Separation of helix repeat (3.5 nm) and self-crossing distance (~ A = 50 nm) allows separation of local (twisting) and nonlocal (writhing) contributions to ΔLk

\[ Lk = Tw + Wr \]

\( Wr \approx -1 \quad -1 \quad -1 \quad -1 \quad -1 \quad -1 \quad \text{RH} \)

DNA crossings can soak up ΔLk, reducing ΔTw and therefore “screening” the twisting energy
Free energy of plectonemic supercoils:

Simulation data:
Klenin, Vologodskii, Cozzarelli, JMB 1991
Vologodskii, Levene, Klenin, F-K and Cozzarelli, JMB 1992

For experiment data see:
Rybenkov, Vologodskii, Cozzarelli, NAR 1997
good survey of Na, Mg conditions

\[
\frac{F}{L} = \frac{kT \, P \, \omega_0^2}{2} \sigma^2 + \cdots
\]

\[
P = 24 \text{ nm} \quad \text{ok for DNA at physiol salt, range of } \sigma \text{ we need}
\]

\[
\omega_0 = 2\pi / 3.4 \text{ nm} = 1.85 \text{ nm}^{-1}
\]

Figure 14. Gibbs free energy and enthalpy of supercoiling as a function of superhelix density. The free energy of supercoiling (---) for the 3/3 kb molecules was calculated numerically (Klenin et al., 1991). The value of \(\Delta H_{\text{el}}(\bullet)\) is the difference between the elastic energies of supercoiled and relaxed molecules obtained from the Monte Carlo analysis. The continuous curve is the least-squares fit of a polynomial to the enthalpy data.

Figure 5. The dependence of the superhelix free energy on the linking difference for different values of the effective diameter of the duplex, \(d\), for chains with \(n = 9\). The broken line corresponds to quadratic approximation (see eqn (15)).
Fully extended DNA under torsional stress

Apply enough force to stretch out DNA even though it is twisted

\[
\frac{F}{L} = -g(f) + \frac{c_s(f)}{2} \sigma^2 + \ldots \quad \text{A} = 50 \text{ nm}
\]
\[
g'(f) = \frac{x}{L}
\]

\[
g(f) = f - \sqrt{\frac{kT f}{A}} + \ldots \quad c_s(f) = kT C \omega_0^2 \left[ 1 - \frac{C}{4A} \sqrt{\frac{kT}{Af}} \right] + \ldots
\]

Chiral fluctuations generate a bit of Wr, slightly screening twisting energy

Intermediate forces – plectoneme/extended mixed state, const $f$ and $\sigma$

$$F(\sigma) = x_s F_s(\sigma_s) + x_p F_p(\sigma_p)$$  force same in $F, F_s, F_p$

$$x_p = 1 - x_s$$

$$x_s \sigma_s + x_p \sigma_p = \sigma$$  linking number may be transferred

but must add up to fixed total

$$\tau = \frac{1}{\omega_o} \frac{\partial}{\partial \sigma} \frac{F(\sigma)}{L}$$  torque at fixed $f, \sigma$

Determine $x_s, \sigma_s$ by minimizing total free energy, const $f$ and $\sigma$
Minimized $F(\sigma)$: Common Tangent Construction

Phase separation in $\sigma$ between limiting values of the two “pure states”, $\sigma_s$ and $\sigma_p$

$F(\sigma)$ linear in $\sigma$ between these limits

\[ \frac{F_s(\sigma)}{L} = -g + \frac{c_s}{2} \sigma^2 \]

\[ \frac{F_p(\sigma)}{L} = \frac{p}{2} \sigma^2 \]

- $\sigma < \sigma_s$ pure stretched
- $\sigma > \sigma_p$ pure plectoneme
- $\sigma_s < \sigma < \sigma_p$ mixed state

Constant torque as $\sigma$ is varied at fixed force

\[ \tau = \frac{1}{\omega_0} \frac{\partial}{\partial \sigma} \frac{F(\sigma)}{L} \]
Coexistence region properties

\[ F_s(\sigma) = -g + \frac{c_s}{2} \sigma^2 \]

\[ F_p(\sigma) = \frac{p}{2} \sigma^2 \]

\[ |\sigma_s| = \frac{1}{c_s} \left( \frac{2pg}{1 - p/c_s} \right)^{1/2} \]

\[ |\sigma_p| = \frac{1}{p} \left( \frac{2pg}{1 - p/c_s} \right)^{1/2} \]

\[ \tau = \sqrt{\frac{2k_BT P g}{1 - P/C_s}} \]

We know \( g(f) \) so measuring the coexistence linking numbers (easy) gives us \( p \) and \( c_s(f) \) [can also be done for nonlinear terms] independent of detailed theoretical models

Torque is fully determined and is therefore **predicted**
Torque in Coexistence Region

\[ \tau = \sqrt{\frac{2k_BTPg}{1 - P/C_s}} \]

We more or less know everything in this formula

\[ P = 24 \text{ nm} \quad A = 50 \text{ nm} \quad C = 95 \text{ nm} \]

\[ g(f) \approx f - (kT f/A)^{1/2} \]

\[ C_s \approx C [ 1 - (C/4A)(kT/A f)^{1/2} ] \]

Compare to Previous Work

\[ \tau = \sqrt{2k_B TAf} \]

Strick, Allemand, Croquette, Bensimon, 2001  energy crossing, T=0

\[ \tau = \sqrt{4k_B TAf} \]

Love, 1944  linear buckling instability

![Graph showing torque as a function of force](image)
Comparison of Theory to Data from MC Simulation of Stretched Supercoiled DNA (no fitting)
Back to Analytical Model:
Extension by force

\[
\frac{x}{L} = - \frac{\partial}{\partial f} \frac{F(\sigma, f)}{L}
\]

Extension vs linking, fixed force

Critical force
to start opening plectoneme

\[
g(f_p) = \frac{p(1 - p/c_s)}{2} \sigma^2
\]

This is also the DNA tension inside “free” supercoiled plasmid or domain, about 0.5 pN for \( \sigma = 0.05 \)

Force at which plectonemes are entirely eliminated

\[
g(f_s) = \frac{c_s - p}{2} \sigma^2
\]

less exciting

Extension is derivative of free energy so it is continuous but not necessarily smooth
Comparison with experimental extension versus linking number data (C=95 nm, P=24 nm, A=50 nm)

Super-simple quadratic twist stiffness model describes data well in B-DNA regime…
For $f > 0.5$ pN, $s < 0$ data show a different behavior, due to DNA helix unwinding ($C=95$ nm, $P=24$ nm, $A=50$ nm).

Unwinding of dsDNA breaks L/R symmetry of extension vs $\sigma$.
Add denatured DNA state to extended and plectoneme states

\[
\frac{F_d(\sigma)}{L} = -g_d + \frac{c_d}{2}(\sigma - \sigma_d)^2 + \epsilon_d
\]

\[
\frac{F_p(\sigma)}{L} = \frac{p}{2}\sigma^2
\]

\[
\frac{F_s(\sigma)}{L} = -g + \frac{c_s}{2}\sigma^2
\]

Free energies at 5 pN

Predicted low force vs torque phase diagram – note triple points
Discussion with Vincent Croquette
Untwist DNA with long complementary-palindromic region

(neglecting initiation barrier energy)

\[
\frac{F_s(\sigma)}{L} = -g + \frac{c_s}{2} \sigma^2
\]

\[
\frac{F_{pal}}{L} = 0 \quad \text{(or } \varepsilon_{pal}/L) ; \quad \sigma_{pal} = -1
\]

\[
F(\sigma) = x_s F(\sigma_s) \quad x_s \sigma_s - (1 - x_s) = \sigma
\]

minimize \( F \) wrt \( x_s \)

\[
\sigma_c = -\left(1 - \sqrt{1 - 2g/c_s}\right) \approx -\frac{g}{c_s} \quad \tau_c = -\frac{c_s}{\omega_0} \sigma_c \approx -\frac{g}{\omega_0}
\]
Letters to Nature

Structural transitions and elasticity from torque measurements on DNA

Zev Bryant*, Michael D. Stone*, Jeff Gore†, Steven B. Smith‡†, Nicholas R. Cozzarelli* & Carlos Bustamante*†‡§

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Ingenious technique
Not thermal equilibrium
DNA under >15 pN force
Intrinsically dynamic measurement
Finite time window = big error
Experimental uncertainty of roughly 5 pN nm
Direct measurement of torque in DNA using rotated-polarization light laser tweezer and custom-made optically anisotropic tethered cylinders (Nature Methods 2007)

Nanofabricated quartz cylinders for angular trapping: DNA supercoiling torque detection

Christopher Deufel¹, Scott Forth¹, Chad R Simmons¹,², Siavash Deigosha¹ & Michelle D Wang¹

We designed and created nanofabricated quartz cylinders well suited for torque application and detection in an angular optical trap. We made the cylinder axis perpendicular to the extraordinary axis of the quartz crystal and chemically functionalized only one end of each cylinder for attachment to a DNA molecule. We directly measured the torque on a single DNA molecule as it underwent a phase transition from B-form to supercoiled P-form.

Ingenious technique
Static measurement possible
DNA under >10 pN force
Experimental uncertainty of perhaps 3 pN nm
Friction and torque govern the relaxation of DNA supercoils by eukaryotic topoisomerase IB

Daniel A. Koster, Vincent Croquette, Coes Dekker, Stewart Shuman, and Myntke H. Dekker

Topoisomerases relieve the torsional strain in DNA that is built up during replication and transcription. They are vital for cell proliferation and are a target for poisoning by anti-cancer drugs. Type IB topoisomerase (TopIB) forms a protein clamp around the DNA duplex and creates a transient nick that permits removal of supercoils. Using real-time single-molecule observation, we show that TopIB releases supercoils by a swivel mechanism that involves friction between the rotating DNA and the enzyme cavity: that is, the DNA does not freely rotate. Unlike a nicking enzyme, TopIB does not release all the supercoils at once, but it typically does so in multiple steps. The number of supercoils removed per step follows an exponential distribution. The enzyme is found to be torque-sensitive, as the mean number of supercoils per step increases with the torque stored in the DNA. We propose a model for topoisomerization in
Single molecule experiments for topoisomerase V DNA supercoiling is relaxed in multiturn steps; the step size distribution is exponential

Taneja, Schnurr, Mondragon, Slesarev

Topo V relaxes scDNA in multiple-turn events (PNAS 2007) in a fashion similar to type IB topos (Koster et al 2005)
Dependence of the relaxation rate on applied force... ...and on inferred torque

This indicates that the main source of dissipation in this experiment is rotational and not translational.

Measured rotational friction constant (energy barrier) for Topo V

\[
\omega = 2\pi \left[ r_+ - r_- \right] = \frac{2\pi k_B T}{\eta \ell^3} e^{-\beta E_B} \left[ e^{\beta \theta_+ \tau} - e^{-\beta \theta_- \tau} \right] = \frac{\tau}{\zeta} + \cdots \quad \zeta = 0.071(2) \text{ pN nm sec}
\]
Following the initial cleavage during rotation there is some angular range $\delta$ under which cleavage and religation occur at rates $k$ and $k'$.

The probability per turn of religation is

$$P_1 = \frac{(k+k'e^{-(k+k')\delta/\omega})}{(k+k')}$$

The distribution of relaxation events follows an exponential distribution

$$\langle \Delta Lk \rangle = 1/(1 - P_1)$$

Since both ends of the broken strand have to be close to religate, as the angular velocity increases, the mean step size also increases

$$k\delta = 380 \pm 130 \text{ sec}^{-1} \quad k'\delta = 33 \pm 7 \text{ sec}^{-1}$$
Summary and Conclusions

We still can’t measure small torques in DNA (< 5 pN nm)

We still don’t have a complete consensus regarding the torsional stiffness of DNA

We have a pretty good idea of how torques should vary with force in plectoneme-extended DNA coexistence

Many effects can be added to the simple coexistence model
  nonlinearities in plectoneme free energy
  sequence effects in plectoneme (and in denaturation)
  many small plectoneme domains instead of MFT

We really never have observed plectoneme domains coexisting with extended DNA – dynamics, sequence localization effects

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