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Topoisomerase I Equilibrates Metastable Secondary Structure in Relaxed Supercoiled DNA

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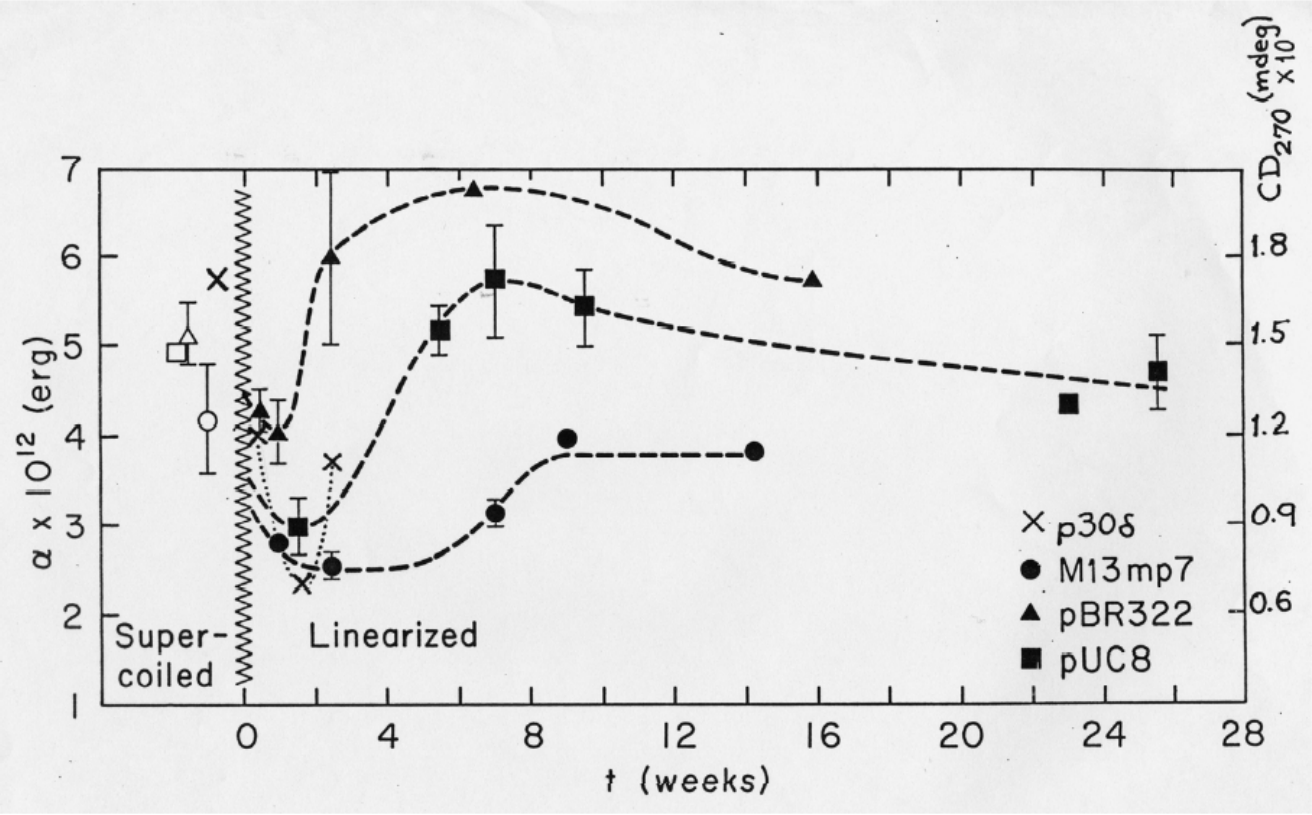
Agenda

1. Long-lived metastable secondary structure in duplex DNAs: a short tale of lengthy frustration.
2. DNA supercoiling, topology, and relaxation of superhelical stress.
3. Experimental protocols and results.
4. Analysis and conclusions.
5. Effects of ethylene glycol.

All measurements made in 0.01 M ionic strength



The Problem

Long-lived metastable states with low torsion elastic constant, α, are often observed when native supercoiled DNAs are relaxed in ~0.01 M ionic strength by:

(1) Linearization

(2) Intercalation of unwinding dyes

(3) Binding of single-strand binding protein from *E. coli*

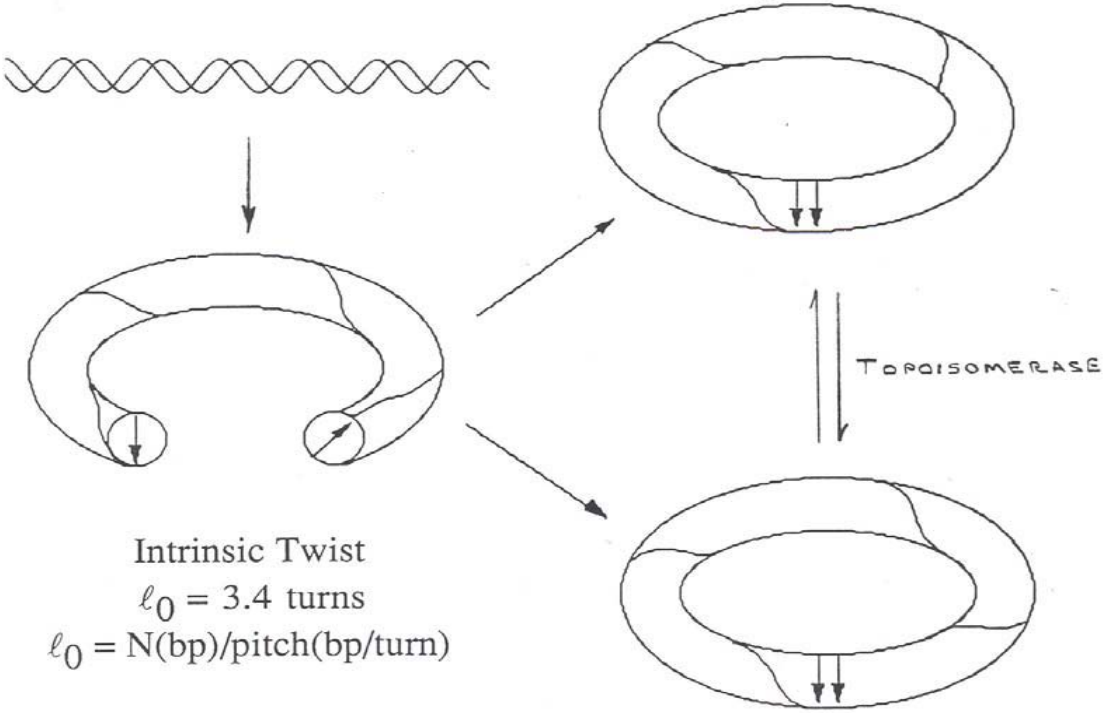
Q1: What happens when native supercoiled DNA is relaxed by a eukaryotic type b Topoisomerase I (Topo I)?

Negri, Della Seta, Camilloni, and di Mauro (Roma, ca. 1988) showed that a relaxed topoisomer created in the presence of ethidium migrated faster, the longer it was exposed to calf-thymus Topo I.

DNA TOPOLOGY

Linking Number, $\ell = 3$ turns

Linking Difference, $\Delta\ell = \ell - \ell_0 = 3 - 3.4 = -0.4$



Intrinsic Twist

$\ell_0 = 3.4$ turns

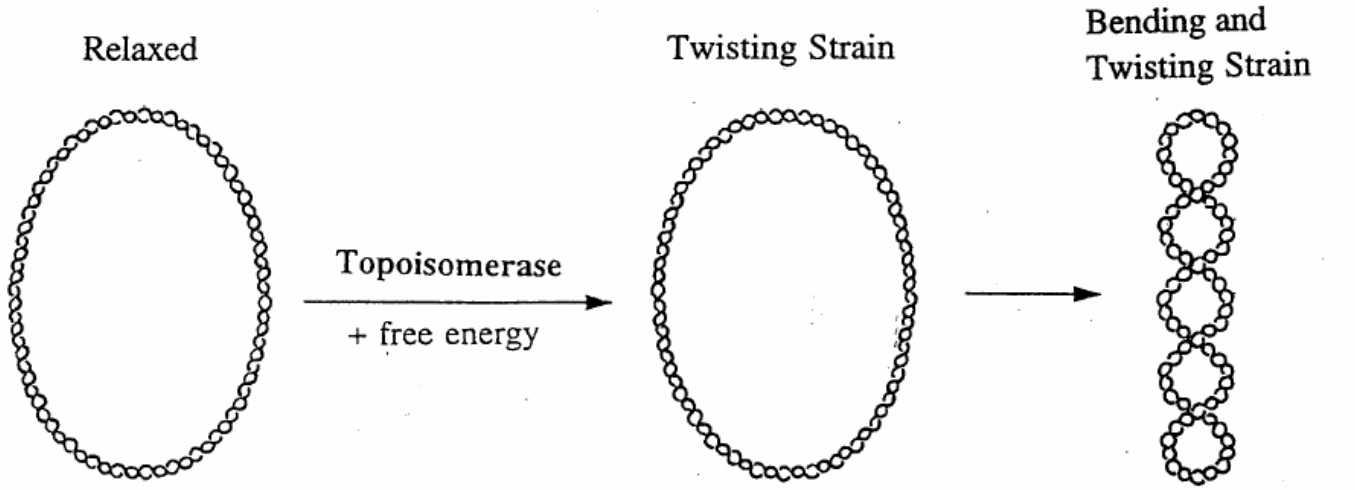
$\ell_0 = \text{N(bp)/pitch(bp/turn)}$

$\ell' = 4$ turns

$\Delta\ell' = 4 - 3.4 = +0.6$

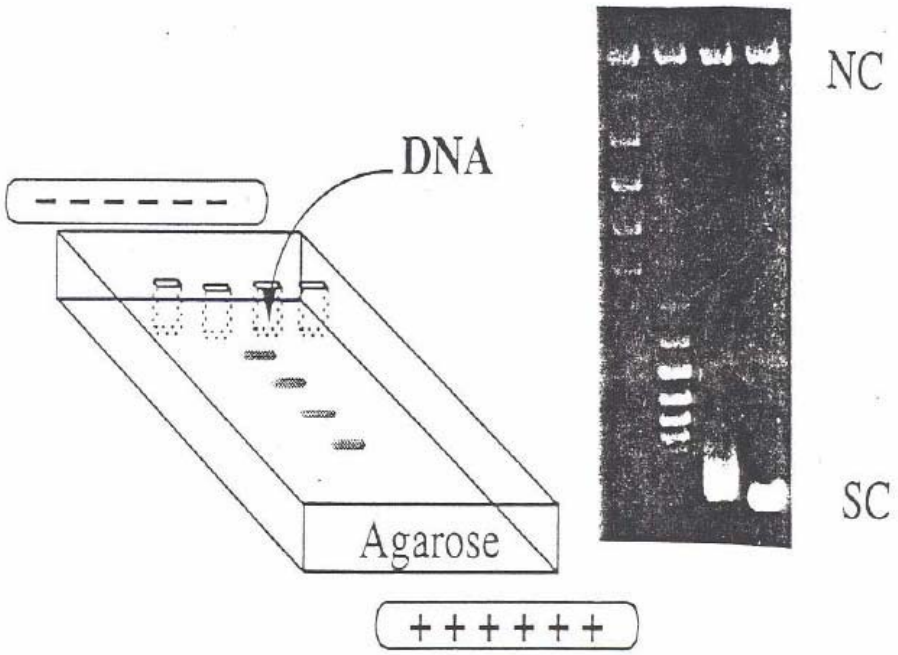
Superhelix density, $\sigma = \Delta l / l_o$

DNA Supercoiling



1. Topological constraint: $l = t + w$
2. Linking difference: $\Delta l = l - l_o$
3. Superhelix density $\sigma = \Delta l / l_o$

Separation of Weakly Supercoiled DNAs



Experimental protocols (~0.11 M ionic strength, 37 °C)

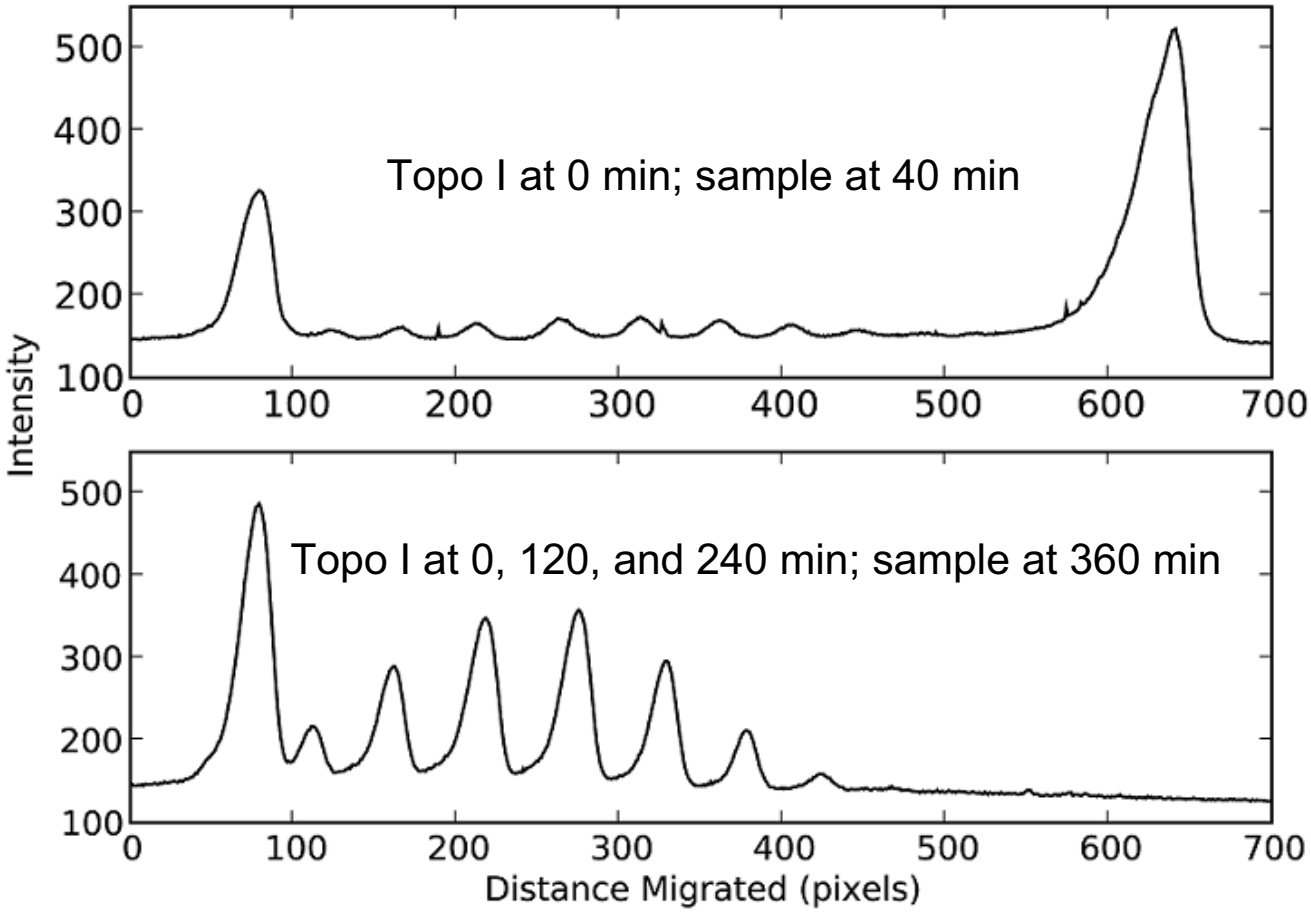
(1) Add Topoisomerase I to p30δ DNA (4932 bp) at 0,1 or 2, and 4 hours.

(2) Withdraw an aliquot every 10 or 20 minutes and halt the reaction by extracting Topo I with phenol. The "equilibrium" distribution of topoisomers is now "frozen".

(3) Separate the topoisomers by gel electrophoresis.

(4) Stain gels under conditions where amount of bound dye is independent of ΔI.

(5) Measure band (peak) intensities and positions using a gel scanner and associated software.


Topo I at 0 min; sample at 40 min
Topo I at 0, 120, and 240 min; sample at 360 min
Intensity
Distance Migrated (pixels)
100
200
300
400
500
0
100
200
300
400
500
600
700

Supercoiling free energy

Experiments and MC simulations of DNAs in 0.1 M salt yield:

$$\Delta G^{sc}_{\ell \to m} \Big/ RT = (E_T / N)\left((m - \ell_0)^2 - (\ell - \ell_0)^2 \right)$$

E_T is twist energy parameter; N is number of base-pairs

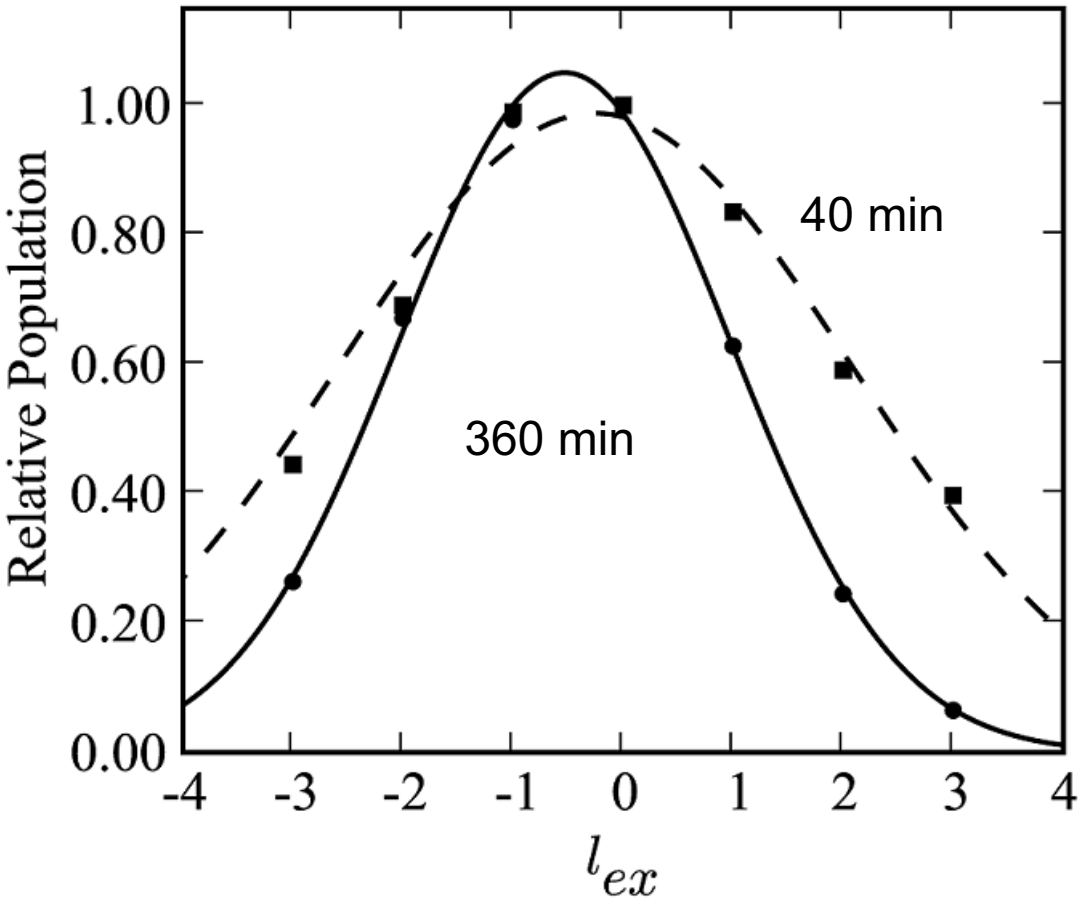
$\Delta \ell_0 = \ell_{mp} - \ell_0$ is the linking difference of the most populous topoisomer.

$\ell_{ex} = m - \ell_{mp}$ is excess linking number of mth topoisomer.

$$= \ldots + 3, +2, +1, 0, -1, -2, -3 \ldots$$

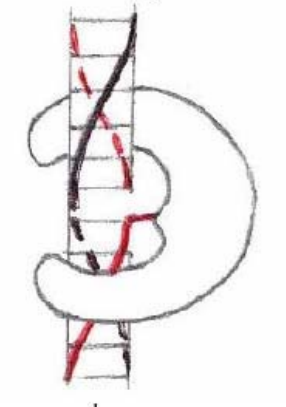
$$c_{\ell_{ex}} \Big/ c_{mp} = \exp\left[-(E_T / N)\left((\ell_{ex} + \Delta \ell_0)^2 - \Delta \ell_0{}^2 \right) \right]$$

Fitting this equation to the observed ratios of intensities yields E_T and $\Delta \ell_0$.


Relative Population
1.00
0.80
0.60
0.40
0.20
0.00
-4
-3
-2
-1
0
1
2
3
4
l_{ex}
40 min
360 min

Conclusion 1: Calf-thymus Topo I operates in a highly processive manner to produce an equilibrium population of relaxed topoisomers during a single binding event before dissociating from the DNA. (Champoux and coworkers; di Mauro and coworkers).

$$E + D(\Delta l) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \cdot D(\Delta l) \xrightarrow{k_c}$$



$$\xrightarrow{k_r}$$

$$E + D(\Delta l_0 \pm n) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} D(\Delta l_0 \pm n) \xrightarrow{k_c}$$

Brownian motion in a harmonic torsion potential well

The probability density for angle ϕ at time t, given that the angle was ϕ_o at t = 0:

$$P(\phi,t\,|\,\phi_o,0)=\frac{e^{-\left(\frac{\left(\phi-\phi_o e^{-t/\tau}\right)^2}{2(kT/g)\left(1-e^{-2t/\tau}\right)}\right)}}{\left(2\pi(kT/g)\left(1-e^{-2t/\tau}\right)\right)^{1/2}}\quad ;$$

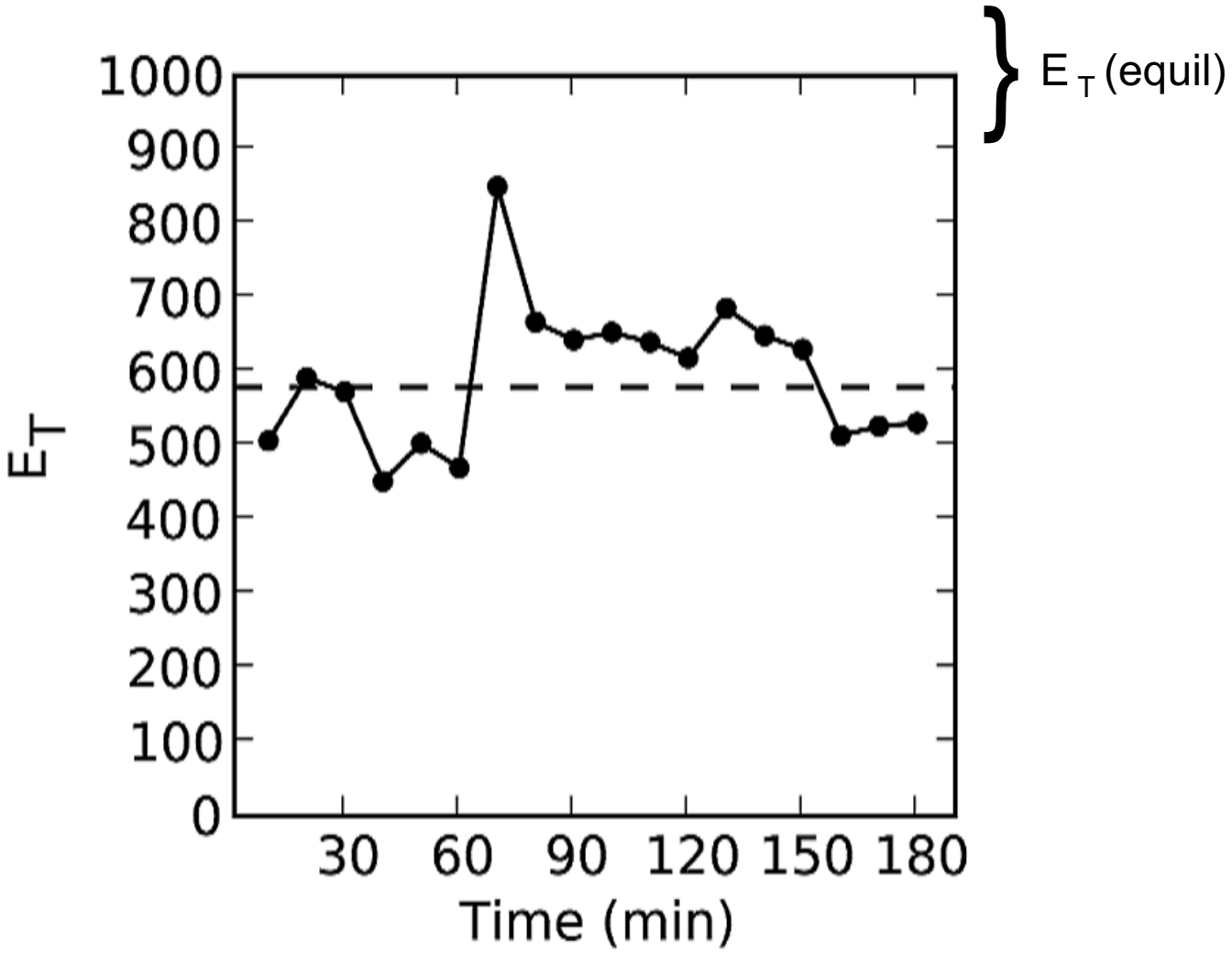
$g \equiv$ torque constant; $1/\tau \equiv g/f$ is center relaxation rate; $f \equiv$ friction factor.

For $t \gg \tau$, the probability density becomes the equilibrium distribution:

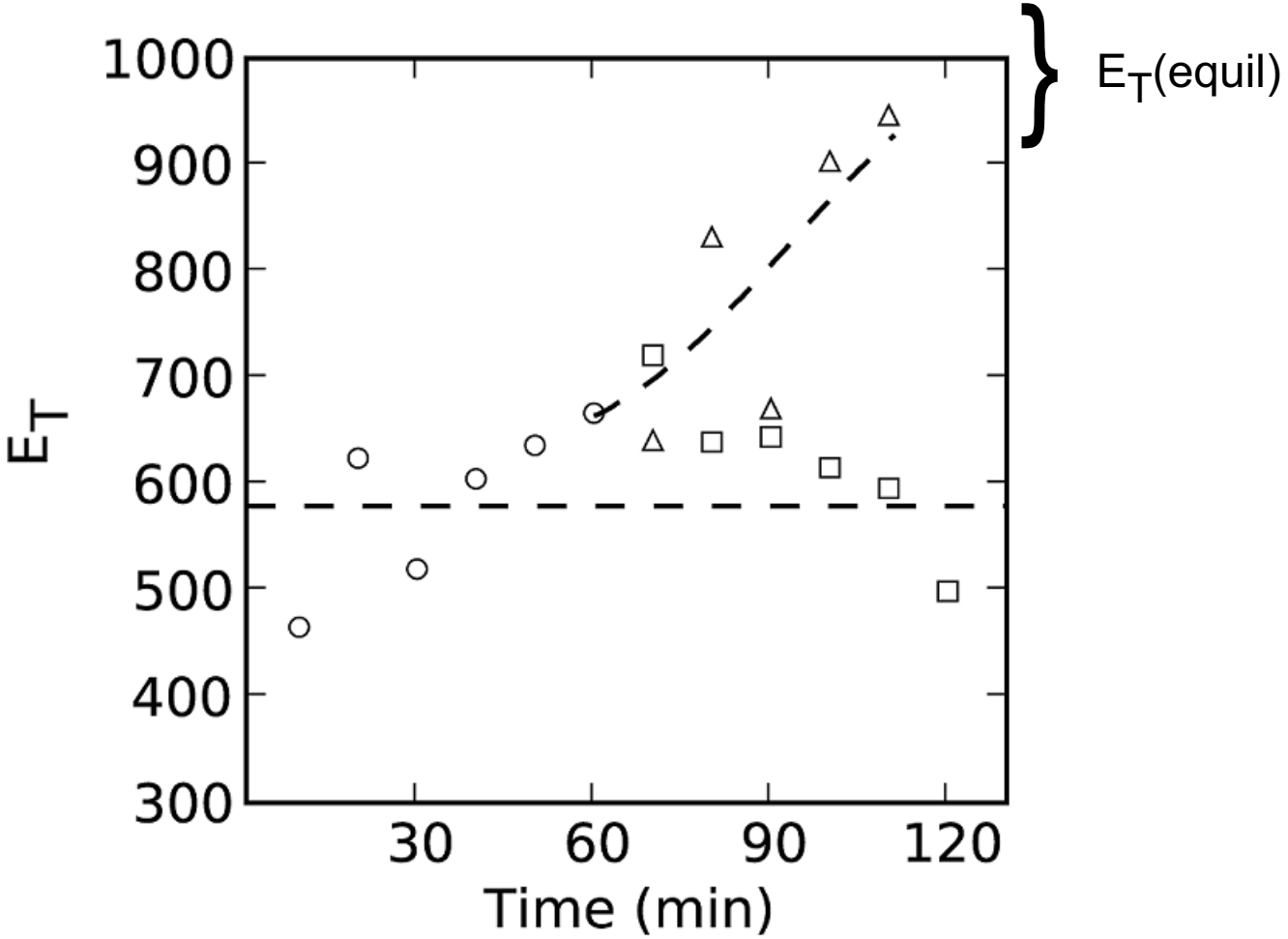
$$P(\phi)=\frac{e^{-\frac{\phi^2}{2kT/g}}}{(2\pi kT/g)^{1/2}}$$

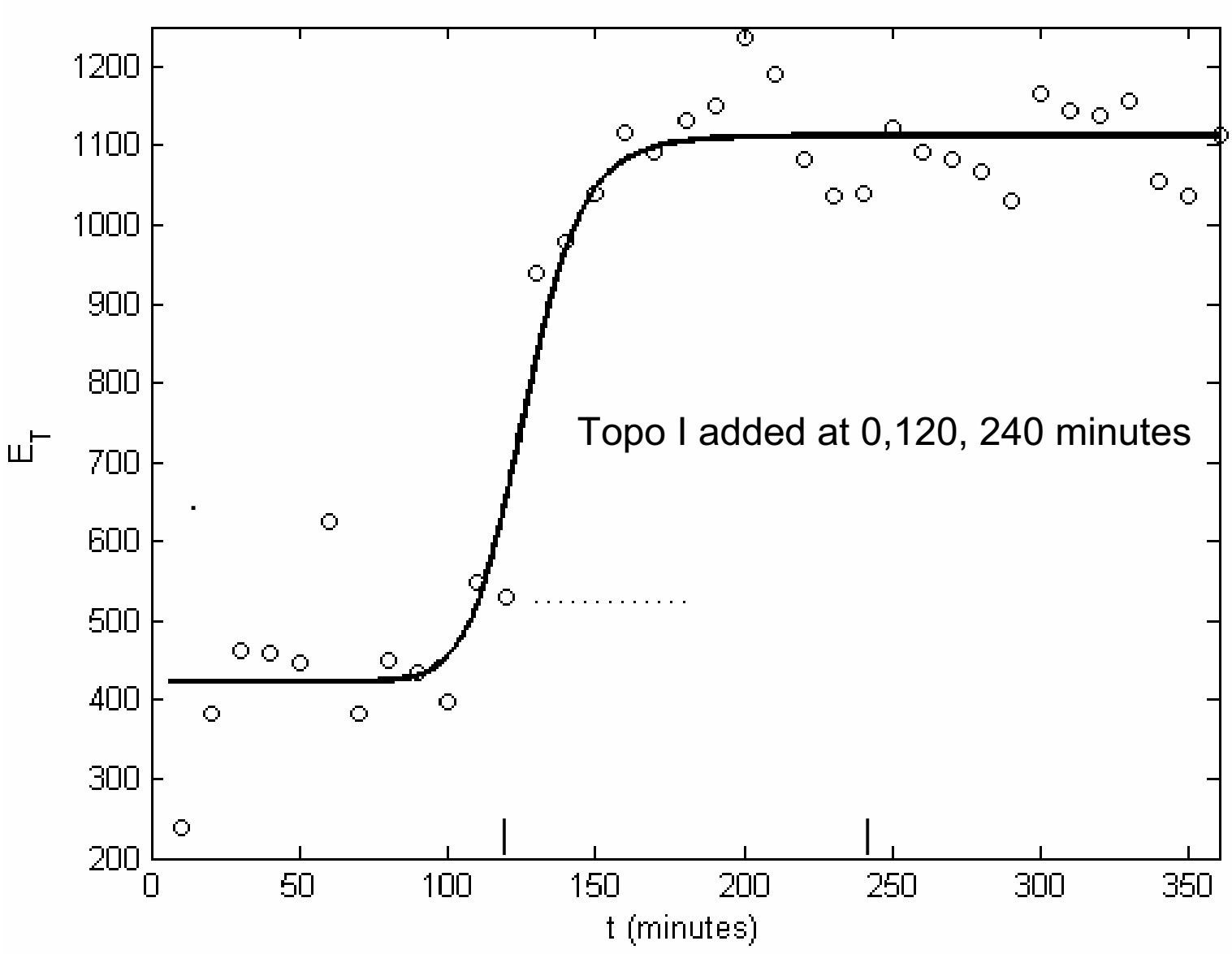
which is sampled at $\phi = 2\pi(\Delta l_0 \pm n)$, $n = 0,\pm1,\pm2,\pm3,\ldots$.

One addition of Topo I at t = 0 minutes:



Topo I added at t=0. At t=60 sample is split and one half receives 2nd Topo.




1200
1100
1000
900
800
700
600
500
400
300
200
E_T
0
50
100
150
200
250
300
350
t (minutes)
Topo I added at 0,120, 240 minutes

Invariance of the linking difference

The 360 minute exposure of DNA to active Topo I does *not* alter ΔI_0 within the experimental error, ± 0.2 turn, despite the large increase in E_T. Therefore, the initial metastable DNA must contain no more than 2 melted base-pairs, which by themselves could lower E_T by less than 2 %. Hence, the metastable low value of E_T *cannot* be attributed primarily to melted base-pairs.

The metastable low value of E_T also *cannot* be ascribed to complexes of the DNA with Topo I, since adding more Topo I actually causes an increase in E_T.

Estimated persistence lengths: Analytical theory plus simulations yield,

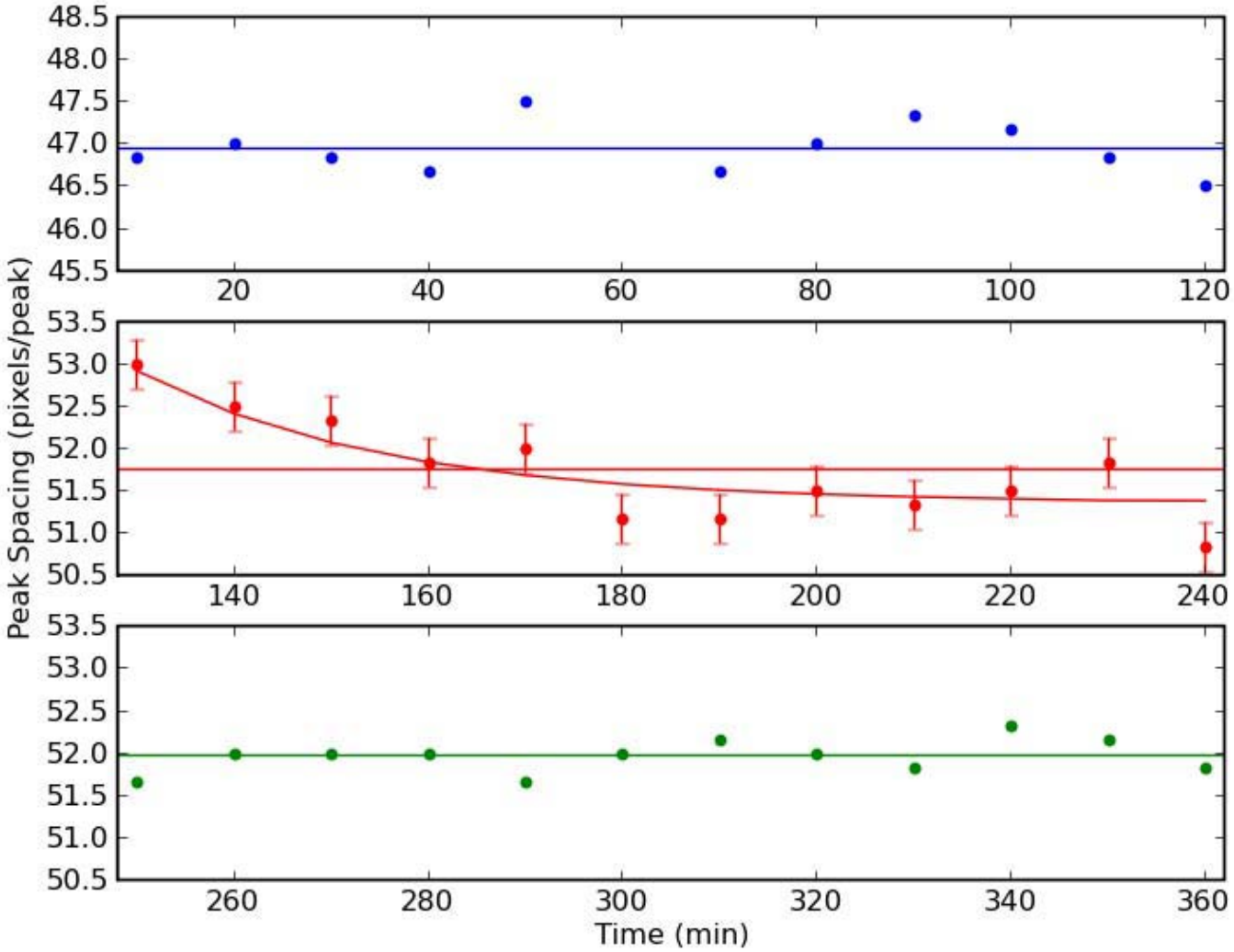
$$E_T = \left((2\pi)^2 / 2kT\right)\left(\frac{\alpha B \kappa_\beta}{\alpha + B\kappa_\beta}\right) \qquad \text{and} \qquad B = 0.594$$

α = torsion elastic constant of the effective springs between bp. (The torsional rigidity is $C = \alpha h$, where h is the rise per bp.)

κ_β = bending elastic constant of the effective springs between bp. (The persistence length is $P = \kappa_\beta h / kT$.)

$(E_T)eq \simeq 1100$ and $\alpha_{eq} = 5.9 \times 10^{-12}$ erg (observed for p30δ) imply $P_{\text{eq}} = 52$ nm.

$(E_T)_0 \simeq 500$ and $\alpha_0 = (4.2 \text{ to } 4.8) \times 10^{-12}$ erg (observed for pUC8, and M13mp7) imply $P_0 = 18.5$ to 19.1 nm.


Peak Spacing (pixels/peak)
48.5
48.0
47.5
47.0
46.5
46.0
45.5
20
40
60
80
100
120
53.5
53.0
52.5
52.0
51.5
51.0
50.5
140
160
180
200
220
240
53.5
53.0
52.5
52.0
51.5
51.0
50.5
260
280
300
320
340
360
Time (min)

Gel Spacings

The average gel spacing between the seven most populous topoisomers remains constant from 10 to 120 min. After the second addition of Topo I at 120 min, it declines with increasing time from 130 to ~180 min, then remains constant. E_T rises up to reach its plateau in precisely the same time frame.

The decline in gel spacing over time most likely reflects a decline in the $\langle w_j \rangle / \Delta \ell_j$ ratio for each topoisomer. This in turn probably stems from a decline in the α/κ_β ratio with time. Because the ratio, $(\kappa_\beta)_{eq} / (\kappa_\beta)_0 = 2.7$, exceeds the typical ratio, $(\alpha)_{eq} / (\alpha)_0 = 1.3$ to 1.5, it is expected that $((\alpha/\kappa_\beta)_{eq} < (\alpha/\kappa_\beta)_0$, which implies a decline in α/κ_β over time.

Conclusion 2 (The Bad News): Initially relaxed topoisomers produced by Topo I exhibit long-lived metastable secondary structure with substantially reduced values of E_T, α, and κ_β, and modestly enhanced average gel spacings.

Conclusion 3 (the Good News): Active calf-thymus Topo I equilibrates this metastable secondary structure. Hopefully, it will also equilibrate metastable secondary structure(s) arising from other perturbations.

Proposed kinetic diagram:

$$
\begin{array}{lcll}
 & \overset{k_1}{} & & \\
E + D(\Delta l, S_1) & \rightleftharpoons & E \cdot D(\Delta l, S_1) & \text{non-covalent complex} \\
 & \underset{k_{-1}}{} & \downarrow k_c & \\
 & & E\text{-}D(\Delta l, S_1) & \text{covalent complex} \\
 & k_1 & \downarrow k_r & \text{(winding/unwinding and religation steps)} \\
E + D(\Delta l_0 \pm n, S_2) & \rightleftharpoons & E \cdot D(\Delta l_0 \pm n, S_2) & \text{relaxed metastable DNA complex} \\
 & k_{-1} & \downarrow k_c & \\
 & \vdots & \vdots & \\
 & k_1 & & \\
E + D(\Delta l_0 \pm n, S_{eq}) & \rightleftharpoons & E \cdot D(\Delta l_0 \pm n, S_{eq}) & \text{relaxed equilibrated DNA complex} \\
 & k_{-1} & \downarrow k_c & \\
 & \vdots & \vdots &
\end{array}
$$

Unusual kinetic aspects of the $S_2 \rightarrow S_{eq}$ conversion

The *equilibration of metastable secondary structure* by Topo I proceeds initially much more slowly and less processively than does *supercoil relaxation*, with no progress visible during the first 120 minutes, when 70 % of the supercoiled DNA is topologically relaxed. However, the rate of appearance of equilibrated secondary structure appears to accelerate in the later stages of the reaction. It is completed within ~ 180 minutes, not long after the last native supercoiled DNAs are relaxed.

A detailed analysis indicates that Topo I always spends most of its time bound to a topologically relaxed molecule, even during its first binding event with an initially supercoiled DNA. The evident lag in appearance of equilibrated products may arise from a requirement for Topo I action at multiple ($m \geq 2$) sites on the DNA, where release of local mechanical stress (e.g. backbone tension) might facilitate equilibration.

Does multiple nicking facilitate equilibration of metastable secondary structure?

Circular dichroism studies on different topologically relaxed forms of the Col E1 amp plasmid yielded (Thumm, Seidl, Hinz (1988)):

(1) $[\theta]_{270}$ = 5800 for the *linearized* (by Eco RI) species;

(2) $[\theta]_{270}$ = 7300 for the *multiply nicked* (by DNAse I) circular species.

Typical equilibrium values in ~5.0 mM ionic strength lie in the range, $[\theta]_{270}$ = 7585 to 7750.

These results suggest that multiple nicks may facilitate equilibration of metastable secondary structure.

Relevant Experimental Facts

The secondary structure over most or all of a DNA sequence fluctuates among at least three and probably more different sub-conformations *within the B-family*, which may prevail over large and variable domains. Prevailing fractions of the sequence have been ascertained over a range of conditions for: (1) the normal 10.4 bp/turn helix; (2) the 10.2 bp/turn helix favored by increasing salt ; and (3) a 10.42 bp/turn helix favored by increasing ethylene glycol. Evidence for additional sub-conformations comes from *long-range* structure switching induced in the flanking DNA by *local* perturbations, e.g. (1) insertion of a (CG)n tract (n>2); (2) a change in structure of the (CG)n tract; and (3) site-specific binding of CAP or Sp1 transcriptional enhancers. The different sub-conformers exhibit different average elastic constants and intrinsic curvatures, and interconvert only extremely slowly in some cases. Consequently, the dynamics of bending is complex, and the bending rigidity at short times ($t \leq 10\ \mu s$) is 3- to 4-fold greater than its equilibrium value.

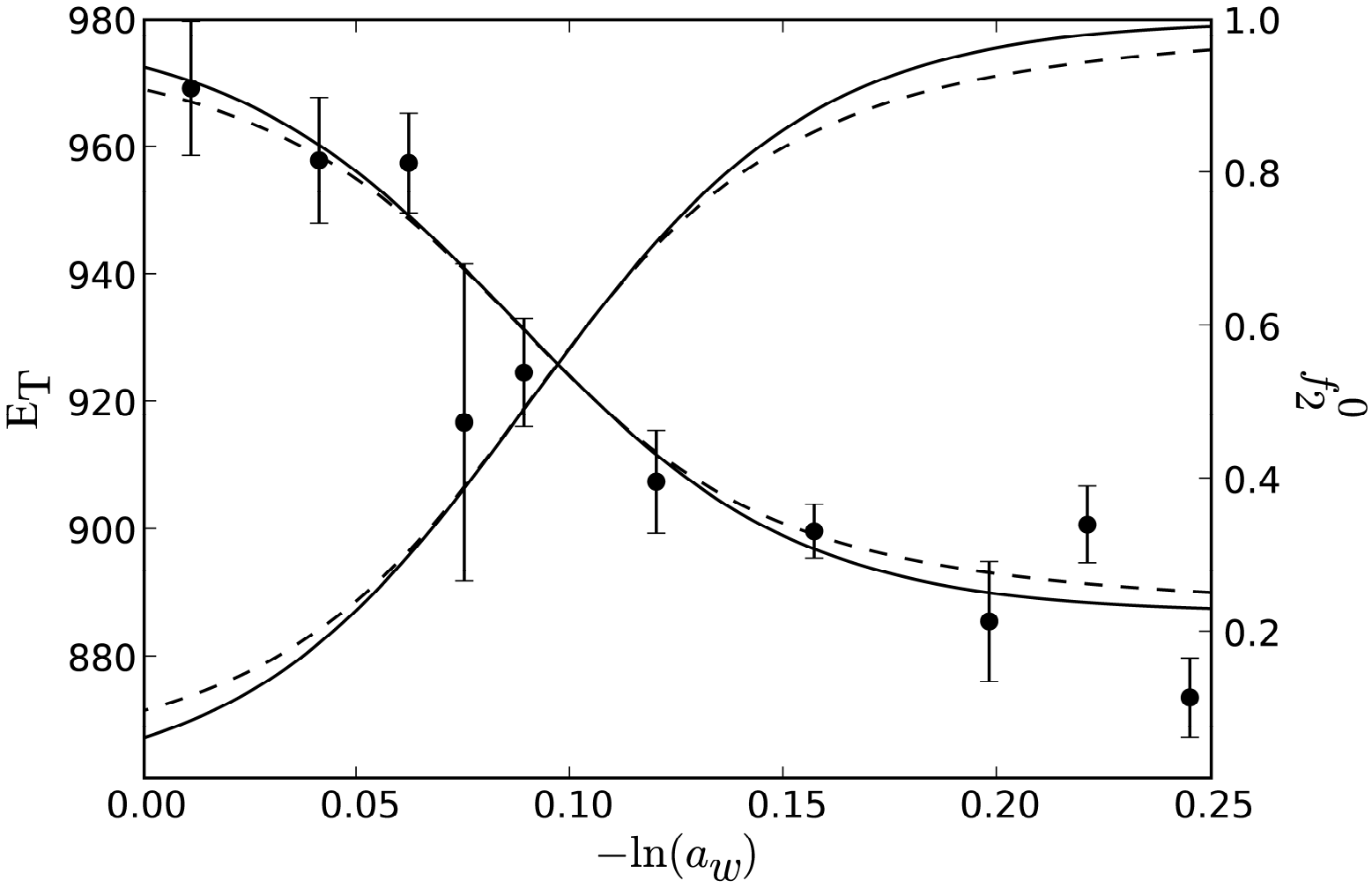
Possible interpretation of the multiple nicking requirement

The rate-limiting step in equilibration of metastable secondary structure could well be the migration of (high free energy) junctions between domains of different secondary structure, which may become trapped at particular sequence positions (where their free energy is somewhat reduced). This is analogous to pinning of migrating domain walls in magnetic materials at positions associated with impurities or dislocations. A nicking and winding/unwinding event near a trapped junction might temporarily lift a constraint, and thereby liberate a junction from its trap.

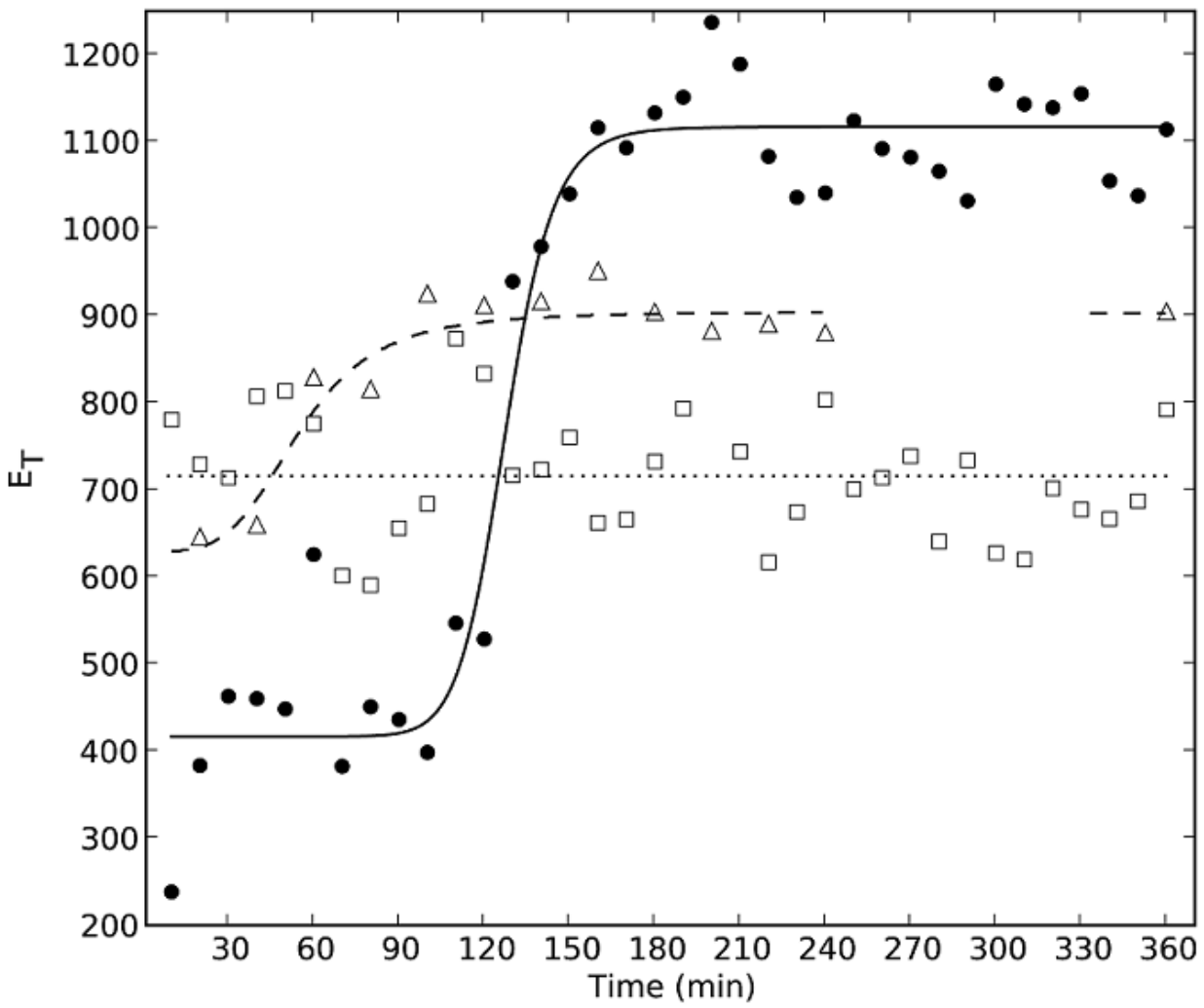
Suggestion

It would probably be wise for everyone to occasionally perform experiments on their DNAs both before and after treatment with calf-thymus Topo I to make sure that metastable states are not contributing to their results. In view of the ultra-long lifetimes of certain metastable states, this should be done even when no obvious signs of metastabililty or hysteresis have been observed.

Equilibrium E_T values in ethylene glycol-water solutions



Topo I is added at t=0, 120, and 240 minutes.



0 w/v% EG

20 w/v% EG

40 w/v% EG

Conclusion 4: Ethylene glycol (EG) reduces, but does not eliminate the processivity of Topo I, and this effect is greater at 40 than at 20 w/v% EG. In 20 w/v% EG, the metastable value of E_T is higher and shorter lived than is the case in 0 w/v% EG, and in 40 w/v% EG there is no evidence of metastable secondary structure at all. The equilibrated state in 40 w/v% EG clearly differs from that in 0 w/v% EG, and the 40 w/v% EG may propel the DNA into that state entirely on its own, perhaps even before the initial superhelical stress is removed by the enzyme.