Dynamics of Single DNA Molecules

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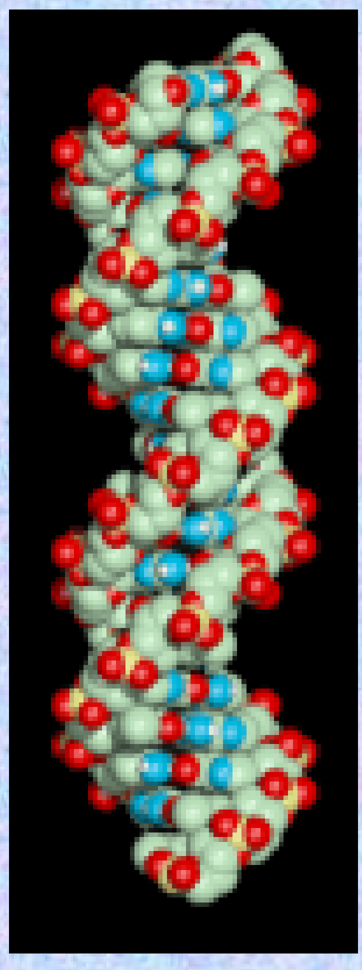
What is Biophysics?

- We use biological molecules, systems, and techniques to solve problems in physics.
- We apply fundamental physical principles to biological systems to understand their function.
- We use physics to develop new methods and instrumentation for the life sciences.

The Physics of DNA

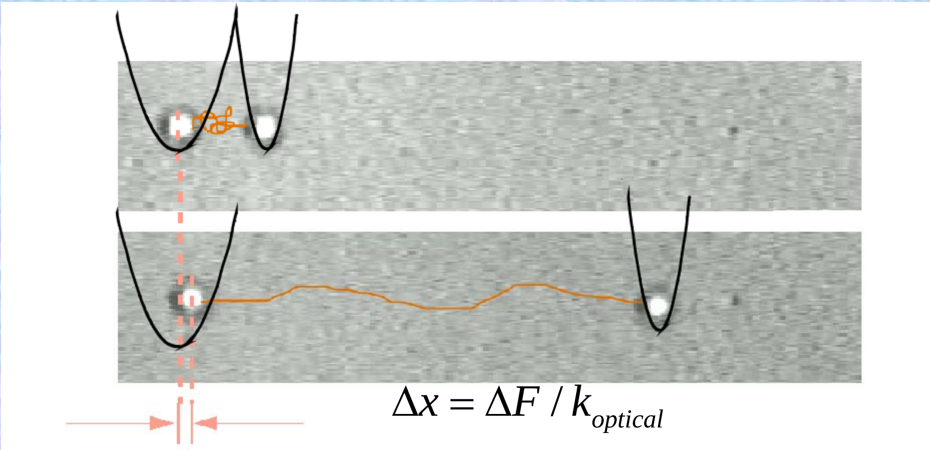


ds-DNA as a biopolymer:

- 2 nm diameter
- Length from microns to meters
- Monodisperse samples
- 50 nm persistence length
- 100 µs smallest Rouse time constant
- Entropic force scale: $k_B T/l_p$=80 fN

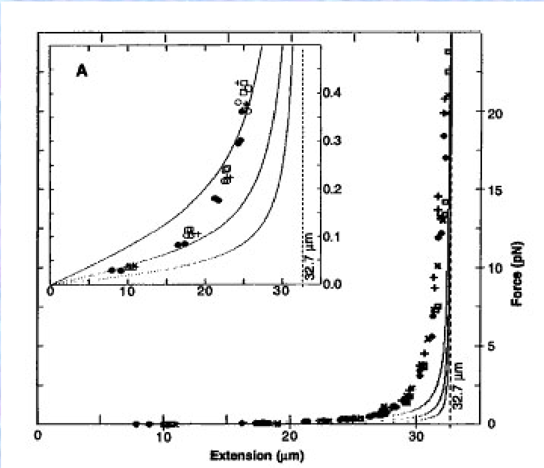
Compare to other intracellular forces:
H-bond breaking: ~50 pN
Molecular motors ~20 pN

Polymer Physics with DNA Molecules



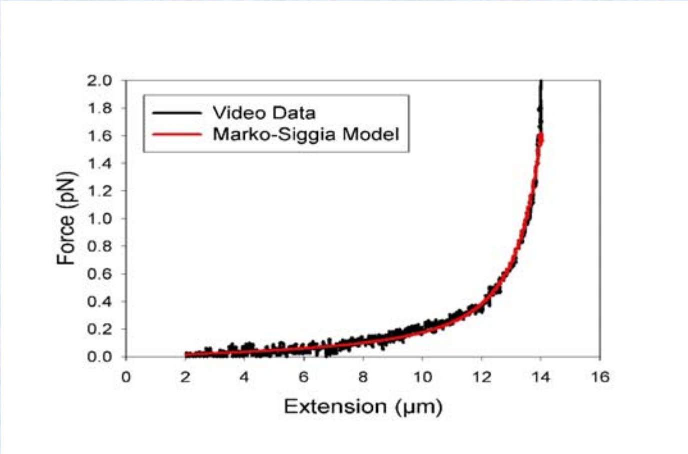
Measuring the elasticity of a single DNA molecule

Purely entropic force!



Smith et al., Science **258**, 1122 (1994)

Measurements do not fit the finite FJC model, but the WLC (Marko –Siggia) model.



Polymer Physics with Single DNA Molecules

We can study:

- Dynamical properties at and far off equilibrium

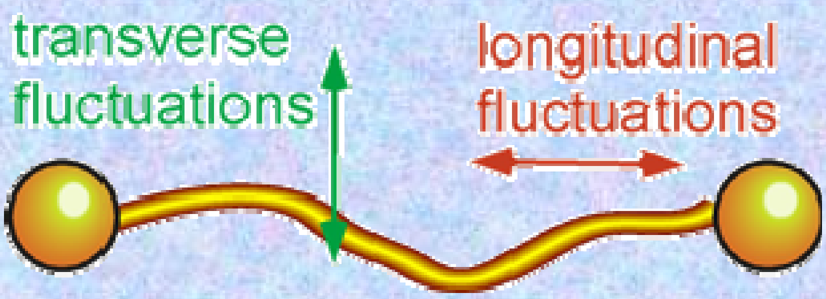
- Hydrodynamic Interactions

- Mechanical Constraints

- Nonlinear effects

- Inhomogeneous polymers (sequence-dependent mechanical properties)

Measuring Thermal Fluctuations of an Extended DNA Molecule



We expect the fluctuations to decay exponentially, perhaps showing some non-linear behavior:

$$\left\langle f_1(0)\, f_2(t) \right\rangle = k_{DNA} k_B T \; e^{-(t/\tau_{DNA})^{\nu}}$$

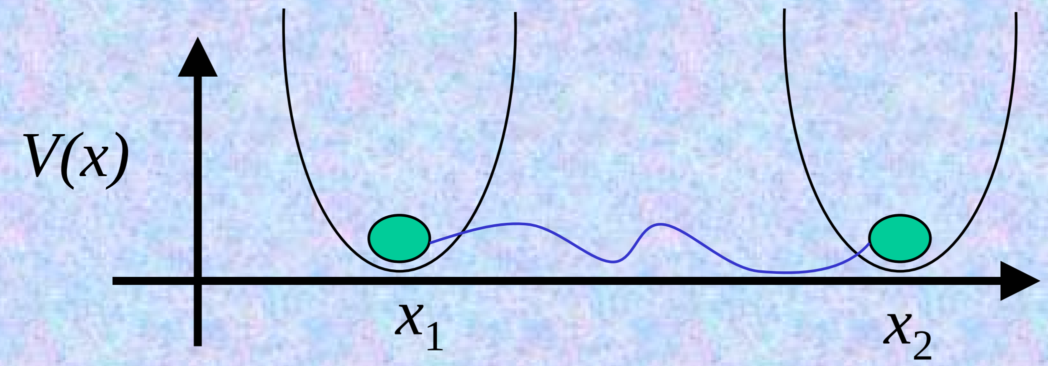
We can measure:

- The relaxation time τ_{DNA}
- The amplitude / spring constant k_{DNA}
- Friction Coefficients
- Anisotropy
- Nonlinear Effects $(\upsilon \neq 1)$

Measuring Femtonewton Force Fluctuations

Force measurements with optical tweezers typically limited by thermal motion of the beads in their traps.

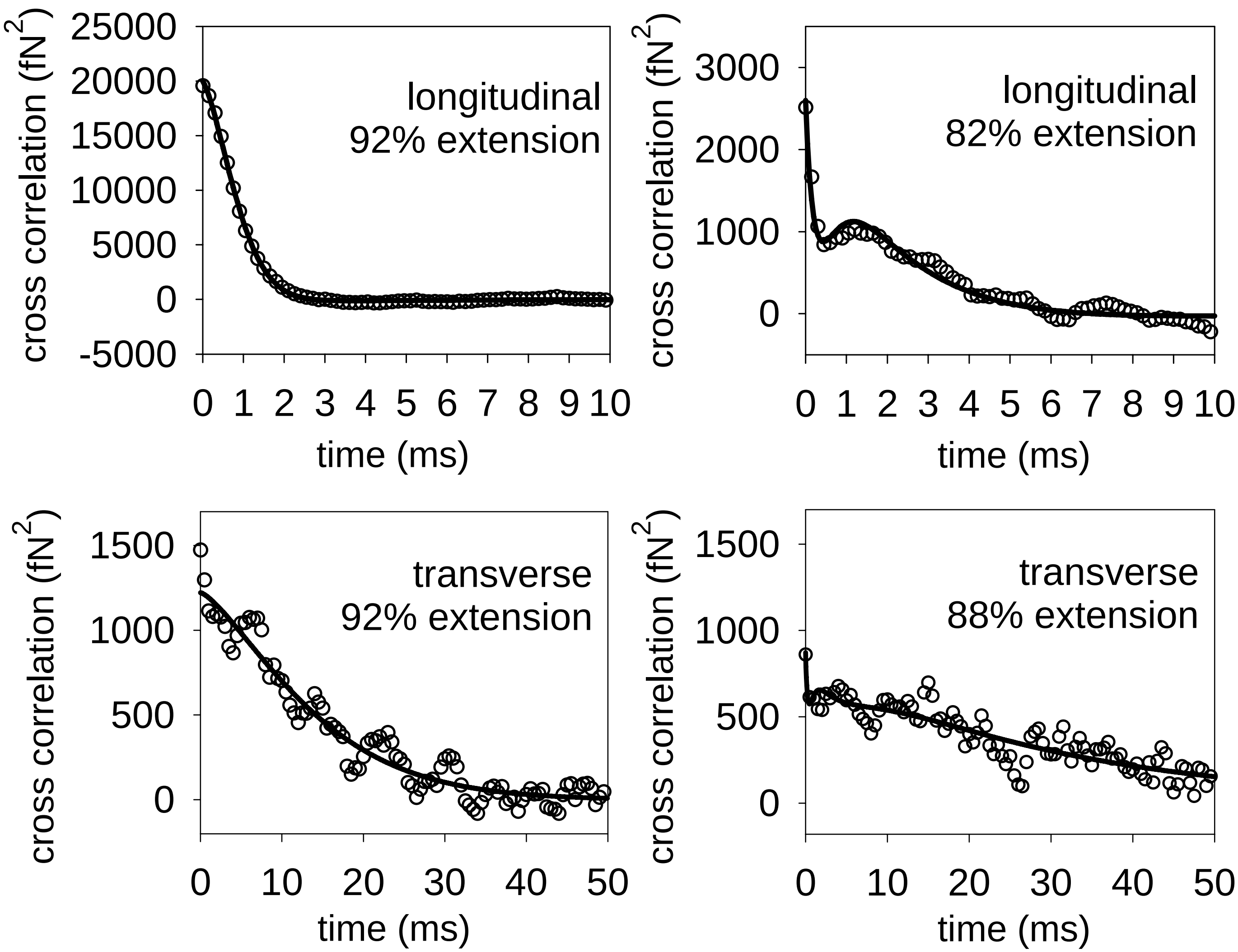
Fundamental limit: ~ 0.5 pN at 1 kHz bandwidth



$\langle x_1(t)x_2(0)\rangle$ … bead motion cancels. Only the correlated motion due to DNA fluctuations remains.

We have measured~6 femtoNewton forcesfluctuations with sub-millisecond time resolution.

Relaxation of a Stretched DNA molecule



The fluctuations decay superexponentially at high extensions

The Model

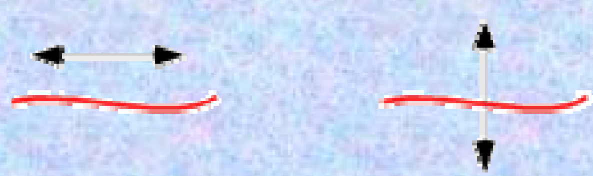
Extension introduces anisotropy:

(e.g. in a stretched violin string – or a DNA molecule)

In the spring constant:

Longitudinal: $k_l = \left.\frac{dF}{dx}\right|_E$ Transverse: $k_t = F(E)/E$

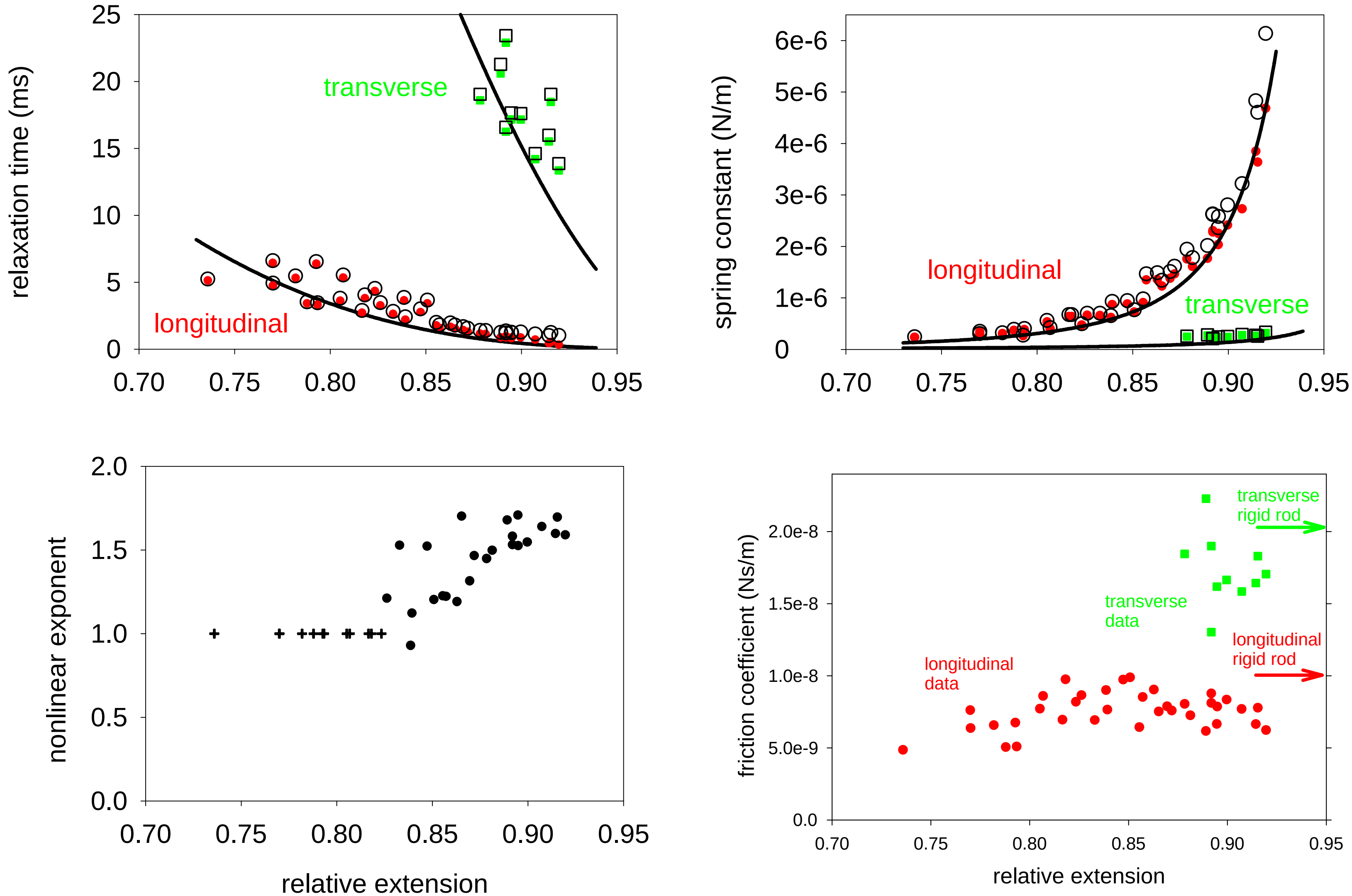
In the friction coefficient:



$\zeta_l \quad < \quad \zeta_t$

In the limit of a rigid rod:

Longitudinal: $\zeta_l = \frac{2\pi\eta_s L}{\ln(L/d)}$ Transverse: $\zeta_t = \frac{4\pi\eta_s L}{\ln(L/d)}$



The agreement between theory and experiment is remarkable!

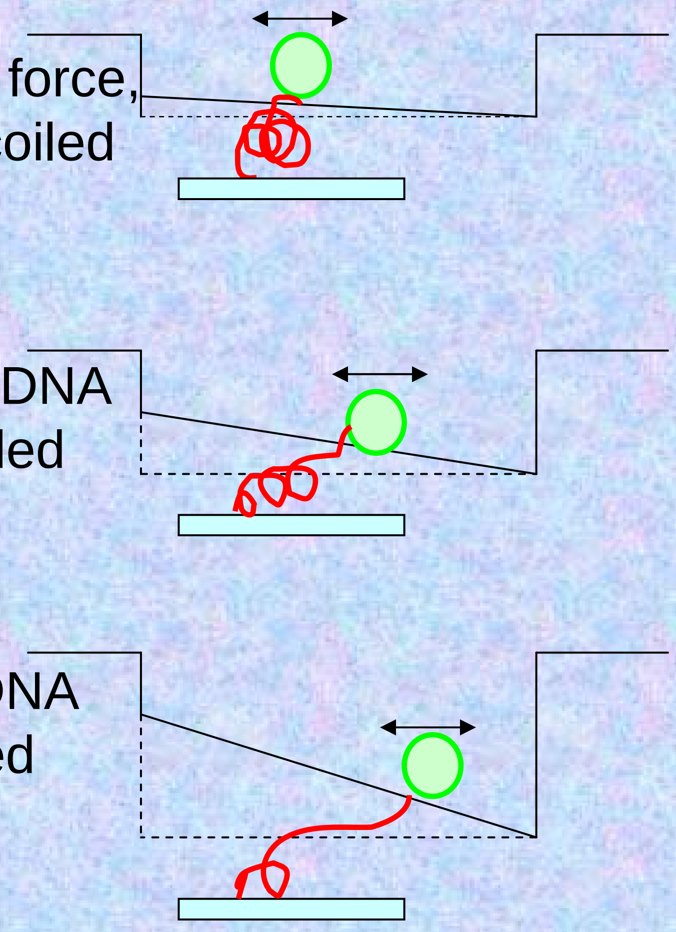
But DNA in vivo is hardly ever stretched this hard…

Dynamics of DNA under Femtonewton Tension

Close to zero force, DNA mostly coiled

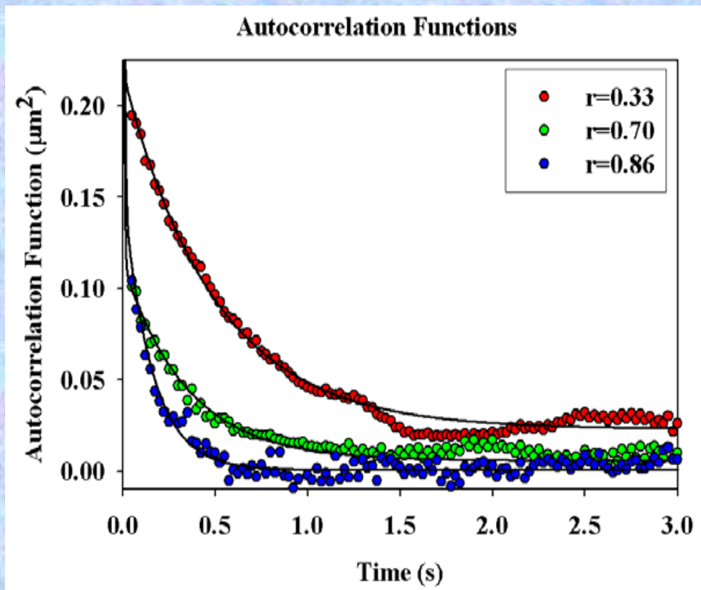
Medium force, DNA partially extended

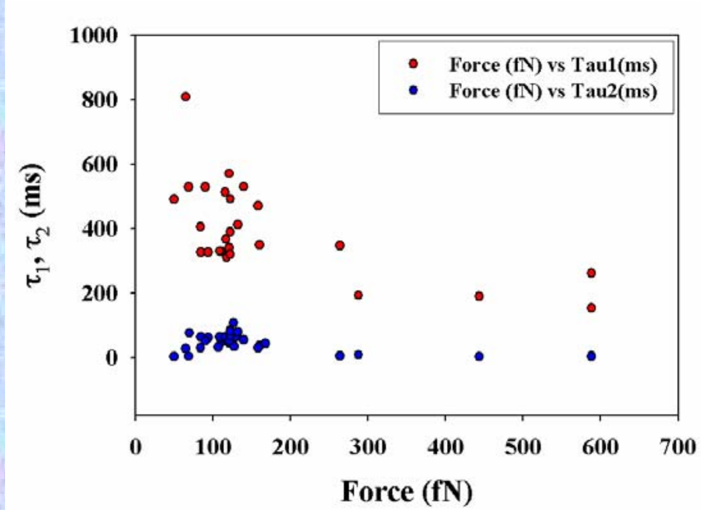
Higher force, DNA mostly extended



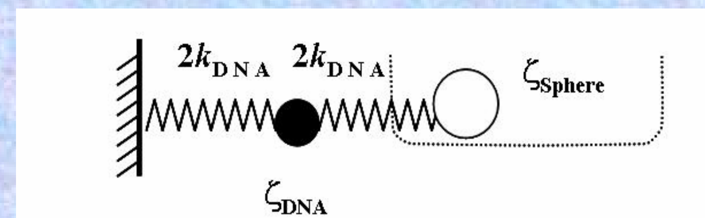
- The DNA molecule is partially extended by the optical potential
- The position of the microsphere is measured with high spatial and temporal resolution
- Time-correlation functions are computed from the position data

Thermal Fluctuations of DNA Molecules under Femtonewton Forces





- Double-exponential decay of the time correlation functions, as predicted by simple bead-and-spring model.

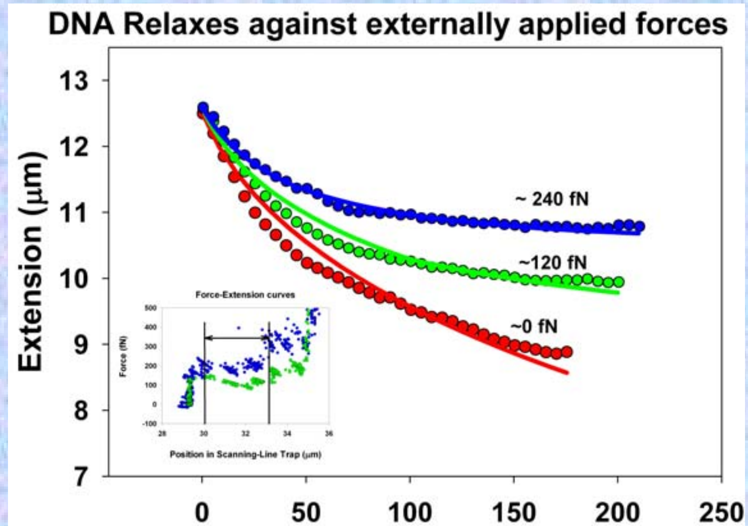


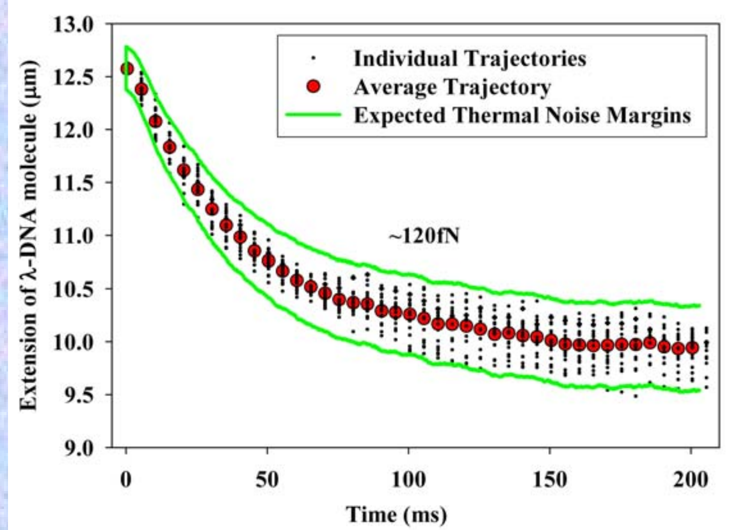
$$\zeta_{DNA}\frac{dx_1}{dt} = -4k_{DNA}x_1 + 2k_{DNA}x_2 + f_1(t)$$

$$\zeta_{Sphere}\frac{dx_2}{dt} = -2k_{DNA}x_2 + 2k_{DNA}x_1 + f_2(t)$$

- Femtonewton forces affect the time constants and amplitudes of fluctuations.

Relaxation of an Extended DNA Molecule against a Constant Force





- The relaxation of extended DN molecules against an applied force can be studied with high spatial and temporal resolution.
- The trajectories match *a-priori* predictions from the static wormlike-chain model reasonably well, no significant dynamic effects.
- The fluctuations around the predicted trajectories appear smaller than expected from the WLC model.

What have we learnt?

We understand thermal fluctuations in extended polymers and answer some long-standing questions in polymer physics:

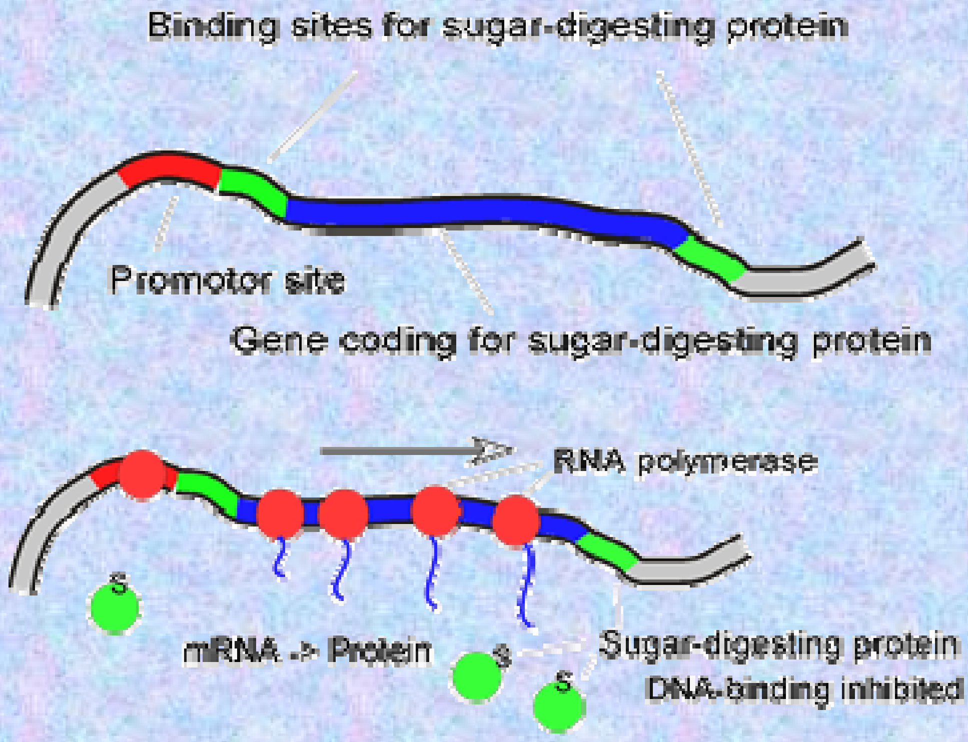
• The amplitudes and relaxation times of the thermal fluctuations of extended DNA molecules can be understood with relatively simple wormlike-chain-baed models.

• The friction coefficient is consistent with a rigid-rod model, intramolecular hydrodynamic screening is insignificant.

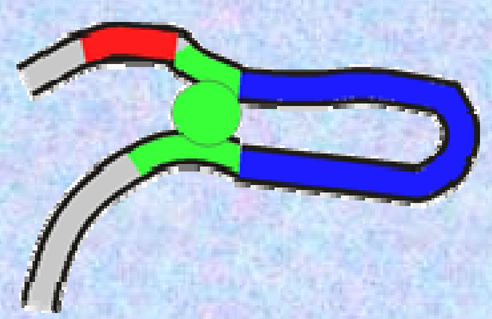
• The relevant force scale is ~100 fN.

Biological Relevance ???

Transcriptional Control through Protein-mediated DNA Looping

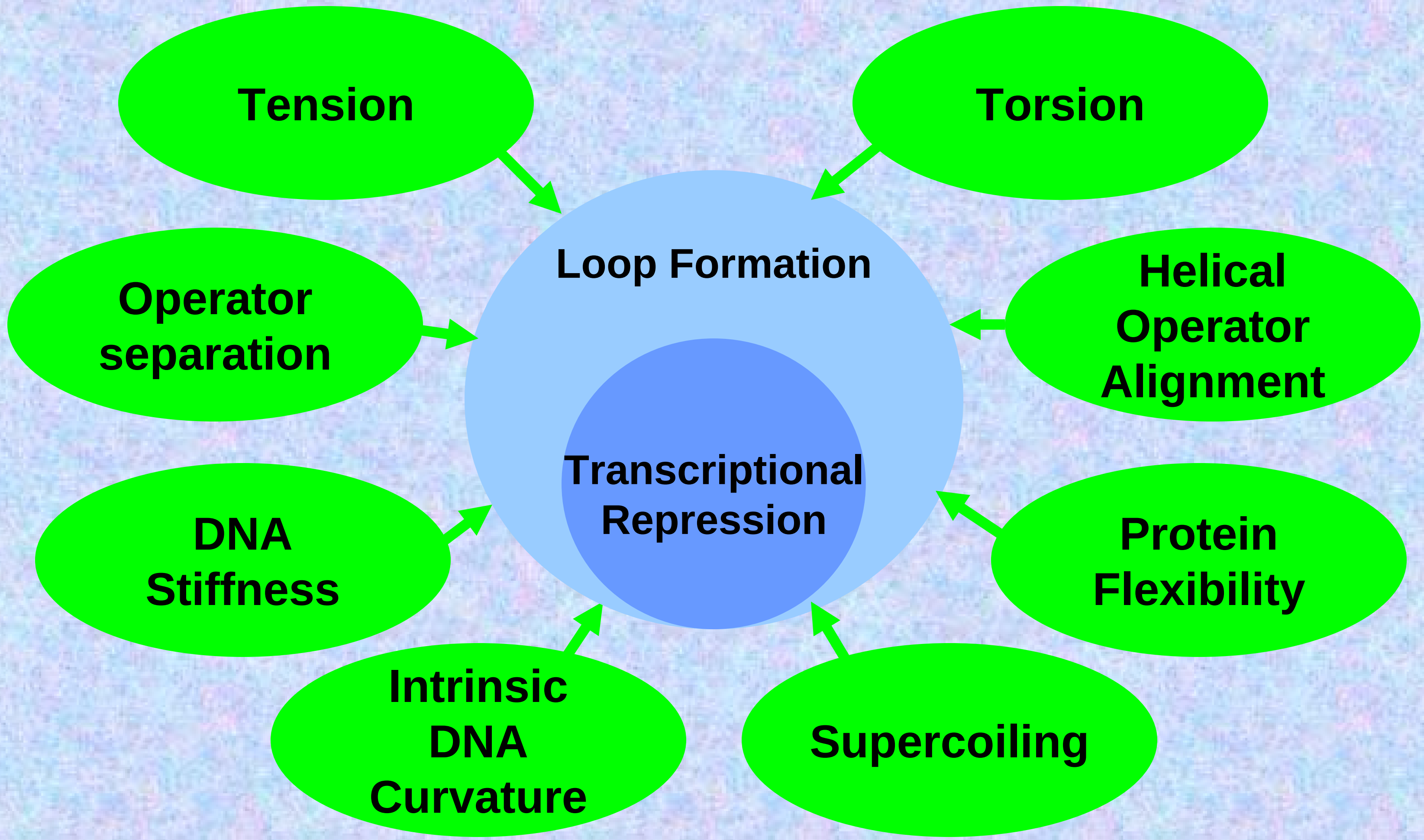


In the presence of sugar, binding of the regulatory protein is inhibited, the gene is tuned on



In the absence of sugar, the regulatory protein bends the DNA into a loop, the gene is tuned off

Mechanical Constraints that Affect Loop Formation



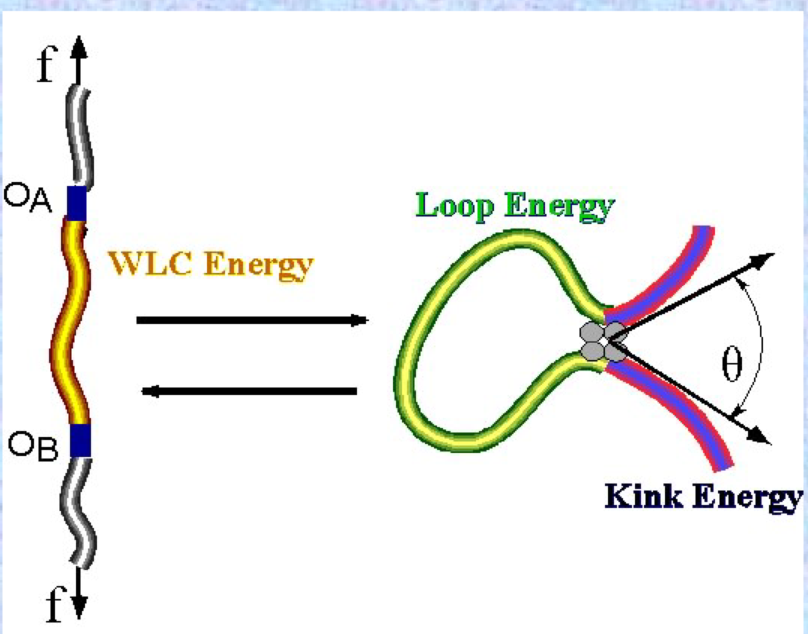
Statistical Mechanics of DNA Looping under Tension

Principle of Detailed Balance:

$$R_l = R_u \exp\left[\frac{-\Delta F}{k_B T}\right]$$

Free Energy calculation:

$$\Delta F = F_{loop} - F_{DNA}(l, f) + F_{kink}(f, \theta)$$



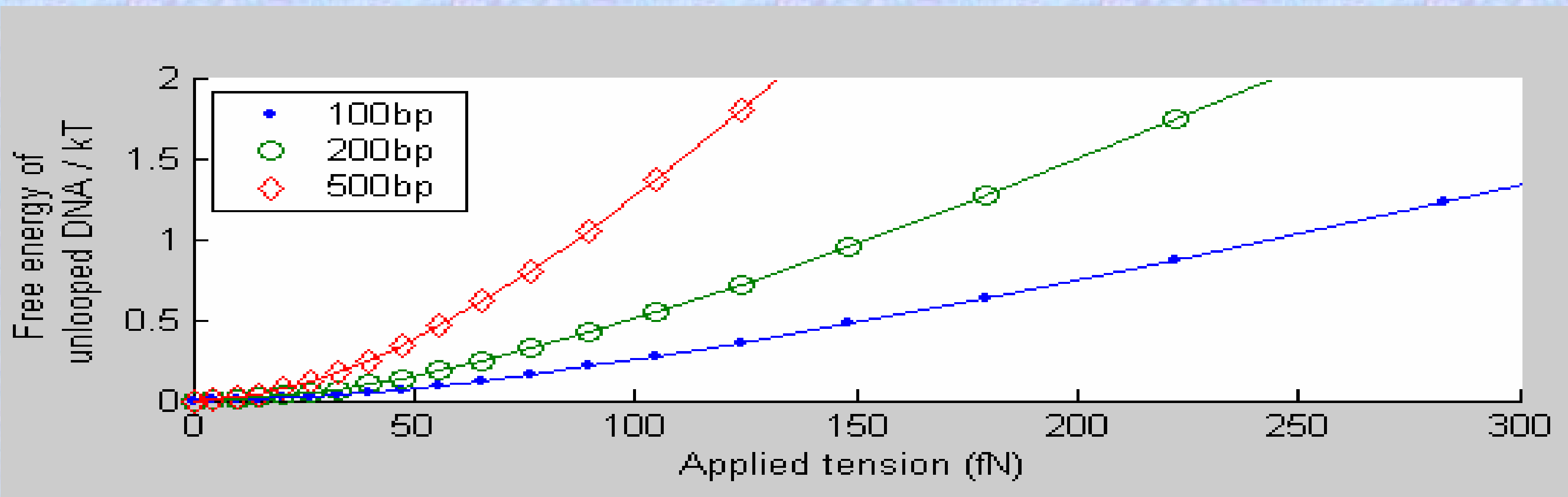
Free energy of Unlooped DNA

Force-Extension for Wormlike Chain
(J. Marko & E. Siggia)

$$\frac{f l_p}{kT} = \frac{1}{4}\left[\frac{1}{(1-x)^2} - 1\right] + x$$

Free energy
of wormlike chain:

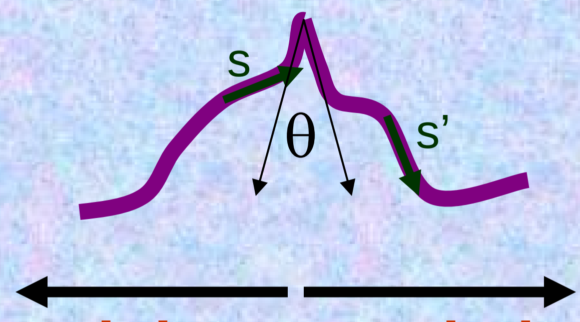
$$\frac{F_{free}(x)}{L} = \int_0^x f(x')\mathrm{d}x' - fx = -\frac{kTx^2}{4l_p}\left[\frac{1}{(1-x)^2} + 2\right]$$



Kink Energy

Low Force Limit:

DNA acts as an ideal spring and entropy effects dominate:



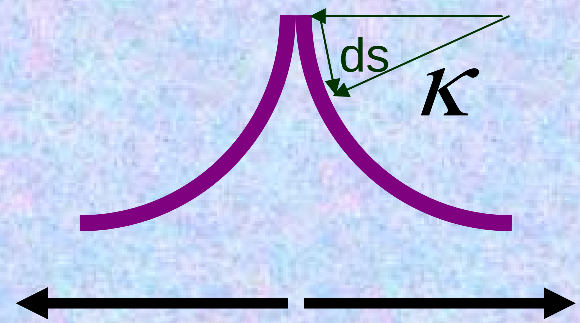
$$F_{kink}^{l} = \frac{\delta\left\langle \vec{R}^2 \right\rangle}{6k_BT} f^2$$

$$\left\langle \vec{R}^2 \right\rangle = \int_0^L\int_0^L \varsigma(s,s') e^{-|s-s'|/l_p} ds ds'$$

$$F_{kink}^{l} = \frac{l_p^2(\cos\theta + 1)}{3k_BT} f^2$$

High Force Limit:

DNA acts a semiflexible rod and bending energy dominates:

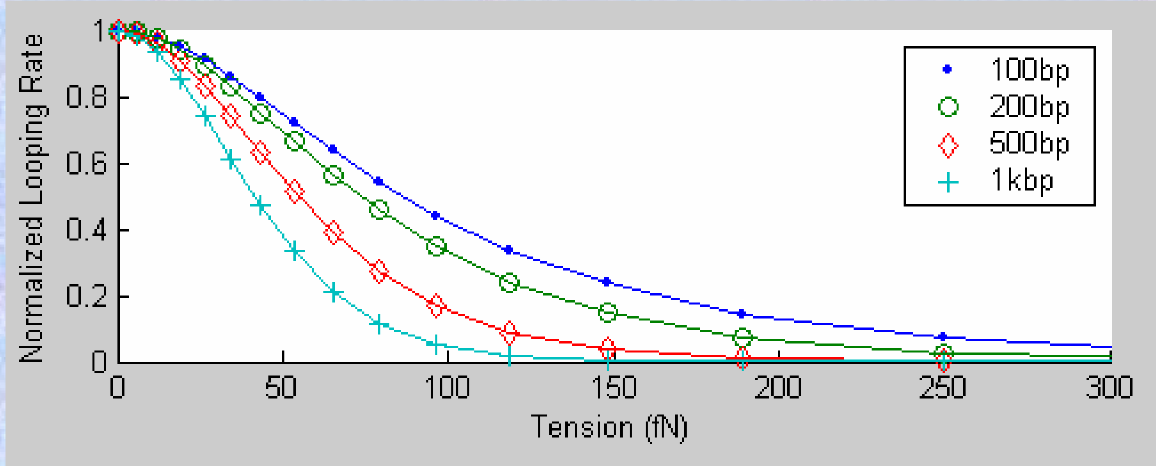


$$dF_{kink}^{h} = \frac{1}{2}\frac{k_BTl_p}{\kappa^2} ds$$

$$F_{kink}^{h} = 4\sqrt{k_BTl_p f}\left[1 - \cos\left(\left(\pi - \theta\right)/4\right)\right]$$

Transcriptional Control through Mechanical Forces?

Tension affects loop formation rates:



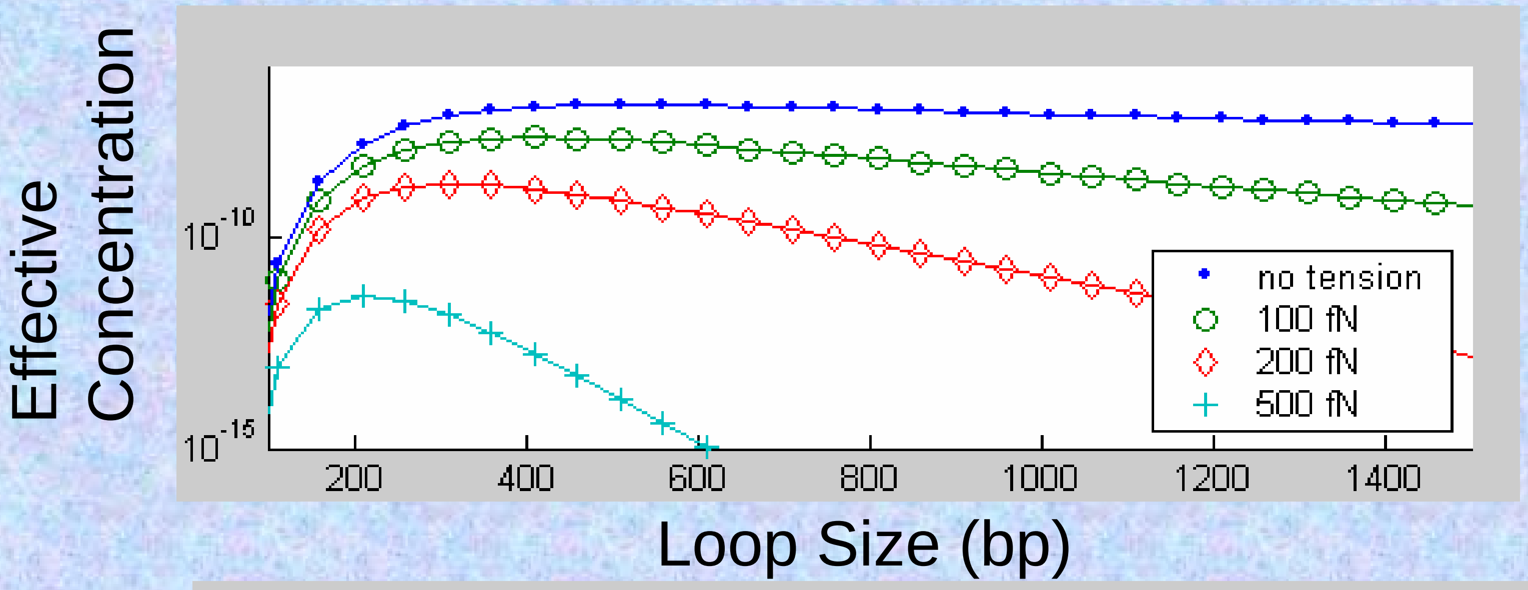
Forces as low as 100 fN may be sufficient to prevent looping

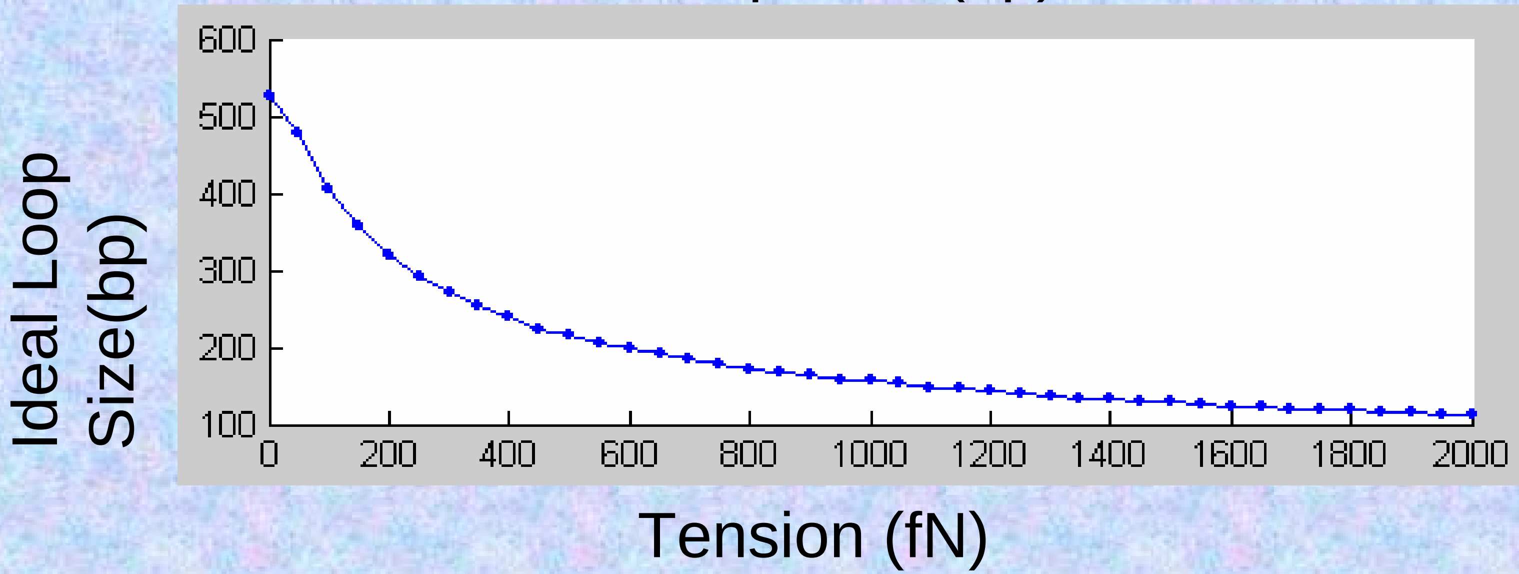
Tension affects transcription!

RNAP can exert forces of up to 20 pN! -> Molecular Switch?

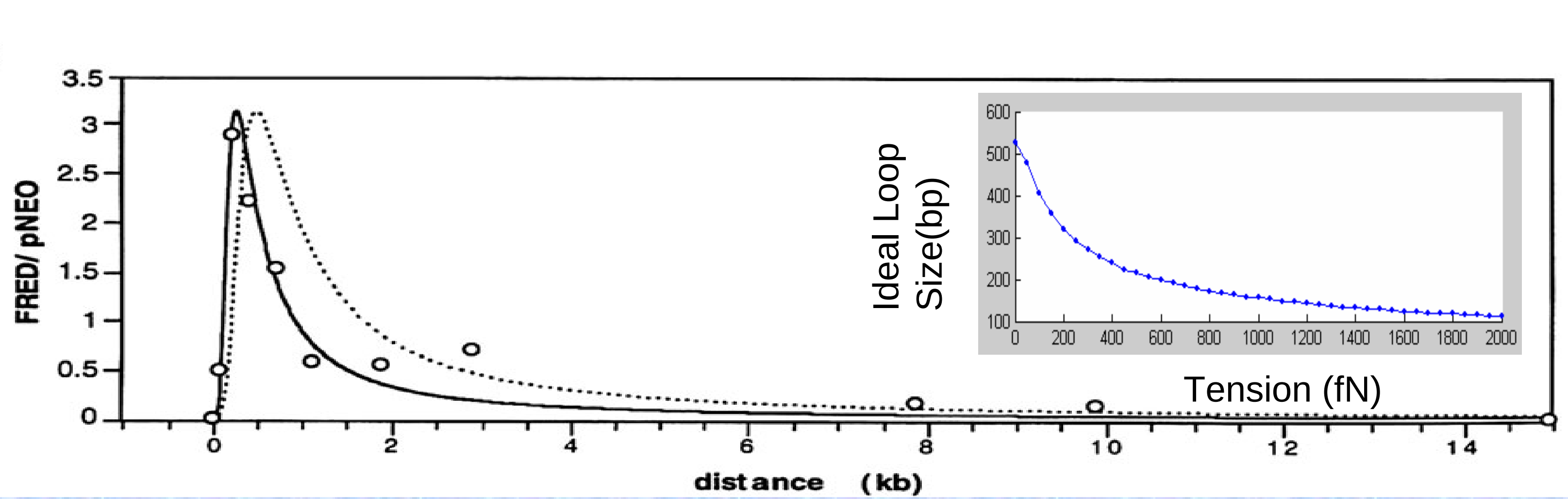
Mechanical mechanism for controlling gene expression?

Effect of Tension on Ideal Loop Size





<u>Reduced ideal loop size *in vivo*: Evidence for constitutive tension?</u>



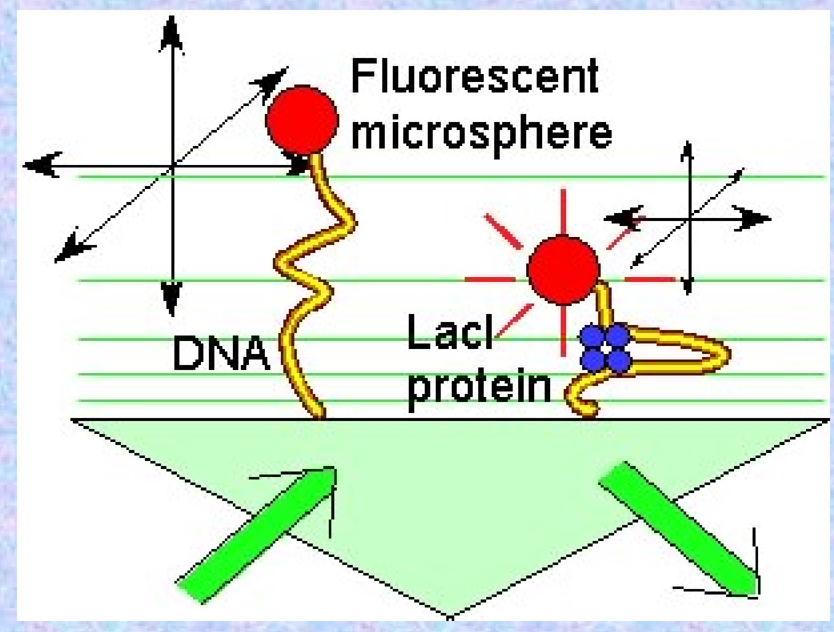
Ringrose, L., Chabanis, S., Angrand, P. O., Woodroofe, C. & Stewart, A. F. (1999) *Embo Journal* **18,** 6630-6641.

In-vivo data for the loop-forming FLP recombinase shows an optimal loop size of ~200 bp.

This could be explained by a constitutive tension of ~600 fN.

Single-Molecule Model for DNA Loop Formation and Breakdown

- A fluorescently labeled microsphere is tethered to a glass surface via a DNA construct
- Evanescent wave excites fluorophores
- Tracking the image location and intensity of the fluorescent emission provides three-dimensional position information, yielding information about the tehter length

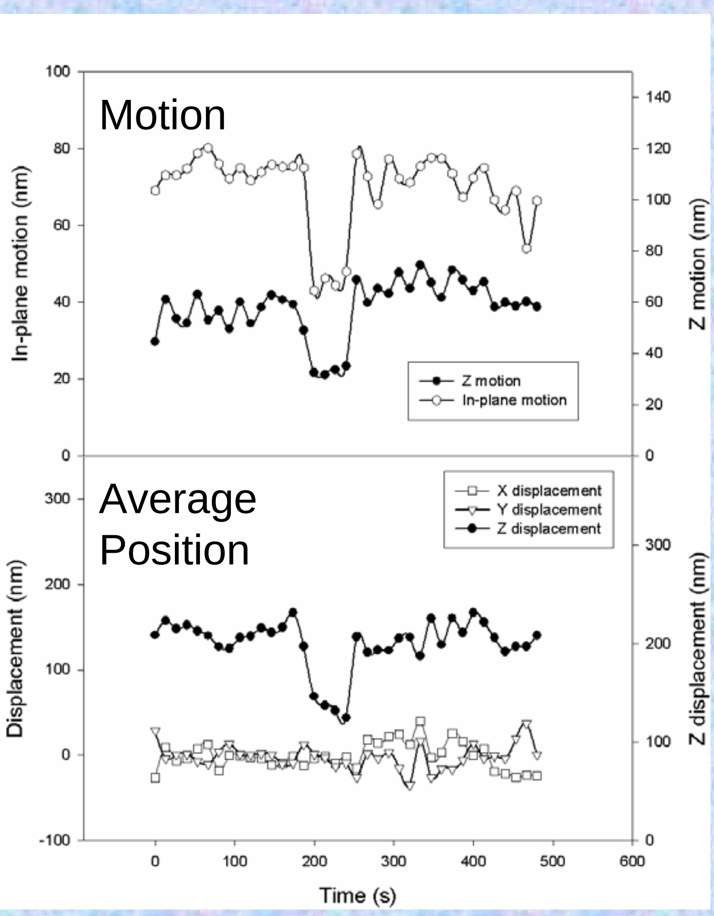


„You can observe a lot by just watching“

Berra

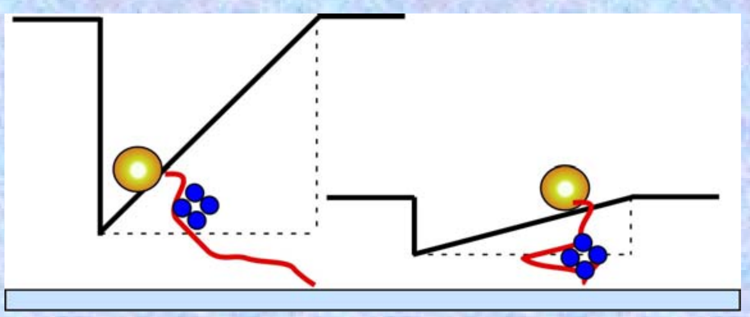
Observing Protein-Mediated DNA Loop Formation and Breakdown

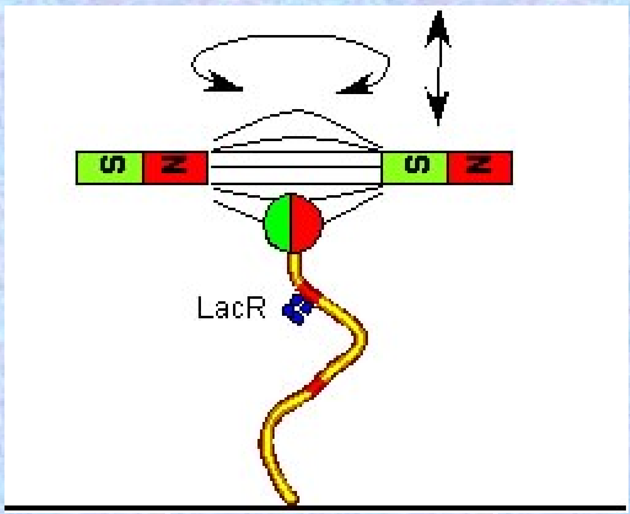
- Loop formation is seen as a decrease of the motion of the microsphere, as well as a reduced average distance from the cover glass.
- Monitoring the average lateral position provides an important control to identify non-specific DNA-glass binding events.
- Can measure loop formation and breakdown rates.



Next Step: Measuring Looping Rates under Tension and Torque

- Constant-force optical tweezers are used to study loop formation rates under mechanical tension in the femonewton range.
- Magnetic tweezers will be used to twist the DNA.
- Long-term: turning on genes by pulling at them, direct study of loop formation *in vivo*.

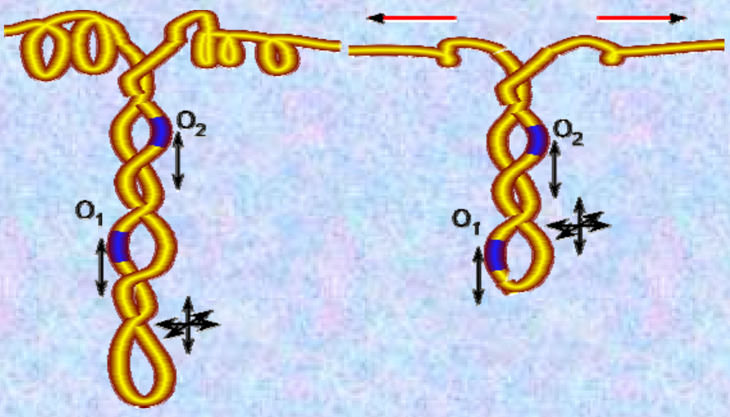




The Role of Supercoiling in DNA Looping

Supercoiling is known from bulk experiments with plasmids to make protein-mediated DNA looping more effective.

Increased local operator concentration, or stabilization against disruptive mechanical forces?

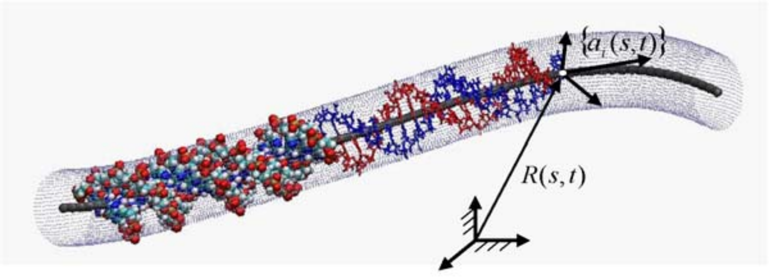


Tension shifts the balance between plectonemic and solinoidal domains.

Supercoiling insulates the operators in the pectonemic domain from tension in the DNA.

Can sequence-induced bends determine the location of the apex?

Computational Rod Mechanics Model



Constitutive Law (Linear)

$$q(s,t) = B(s)(\kappa(s,t) - \kappa_0(s))$$

Intrinsic curvature κ_0 from consensus trinucleotide model

$$B(s) = \begin{bmatrix} A_1(s) & 0 & 0 \\ 0 & A_2(s) & 0 \\ 0 & 0 & C(s) \end{bmatrix}$$

Collaboration with N.Perkins, UM Mech. Eng.

Linear Momentum Equation

$$\frac{\partial f}{\partial s} + \kappa \times f = \rho_c A_c \left\{ \frac{\partial v}{\partial t} + \omega \times v \right\} - B$$

Angular Momentum Equation

$$\frac{\partial q}{\partial s} + \kappa \times q = I \frac{\partial \omega}{\partial t} + \omega \times (I\omega) + f \times a_3$$

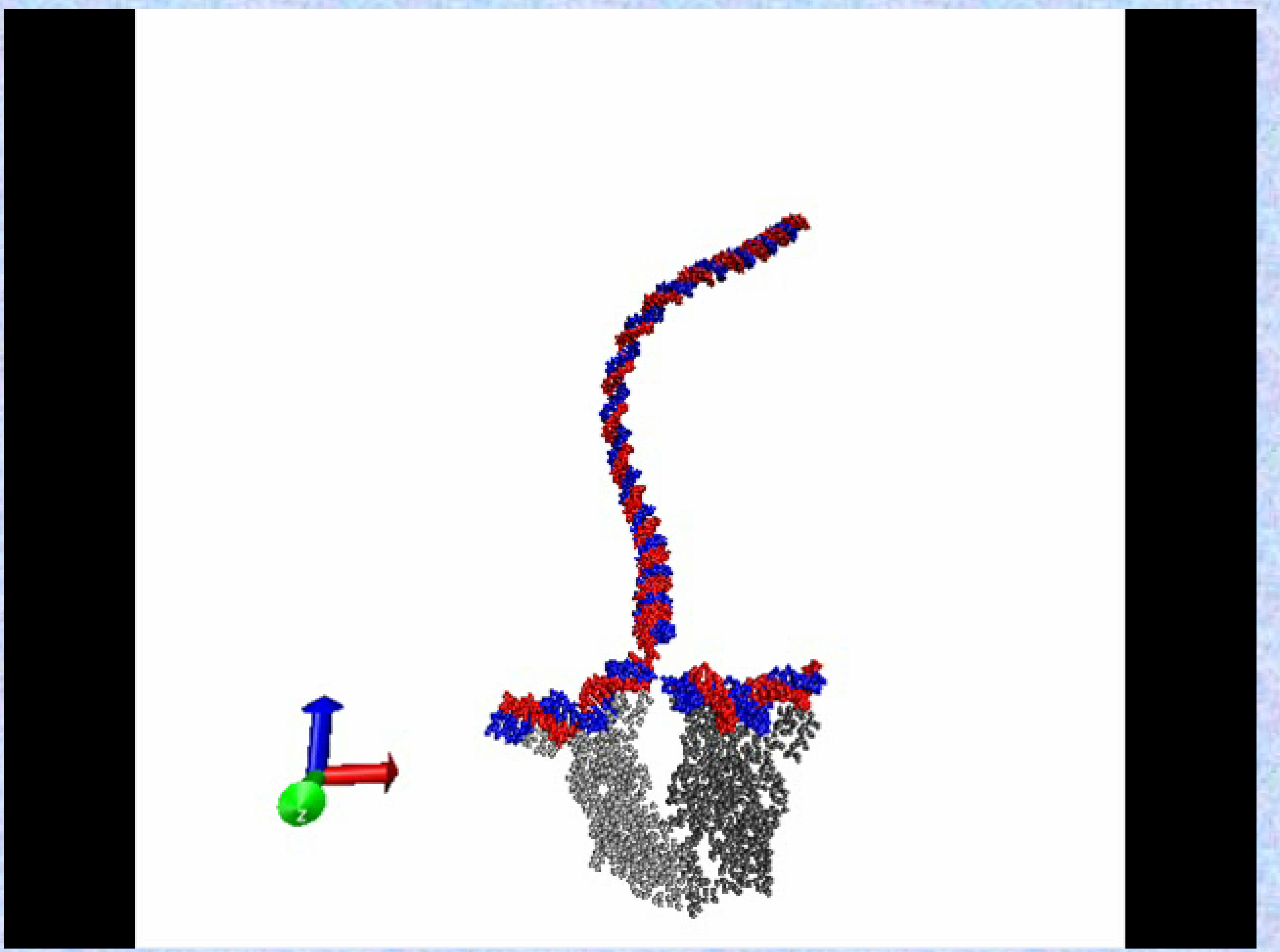
Compatibility Condition

$$\frac{\partial \omega}{\partial s} + \kappa \times \omega = \frac{\partial \kappa}{\partial t}$$

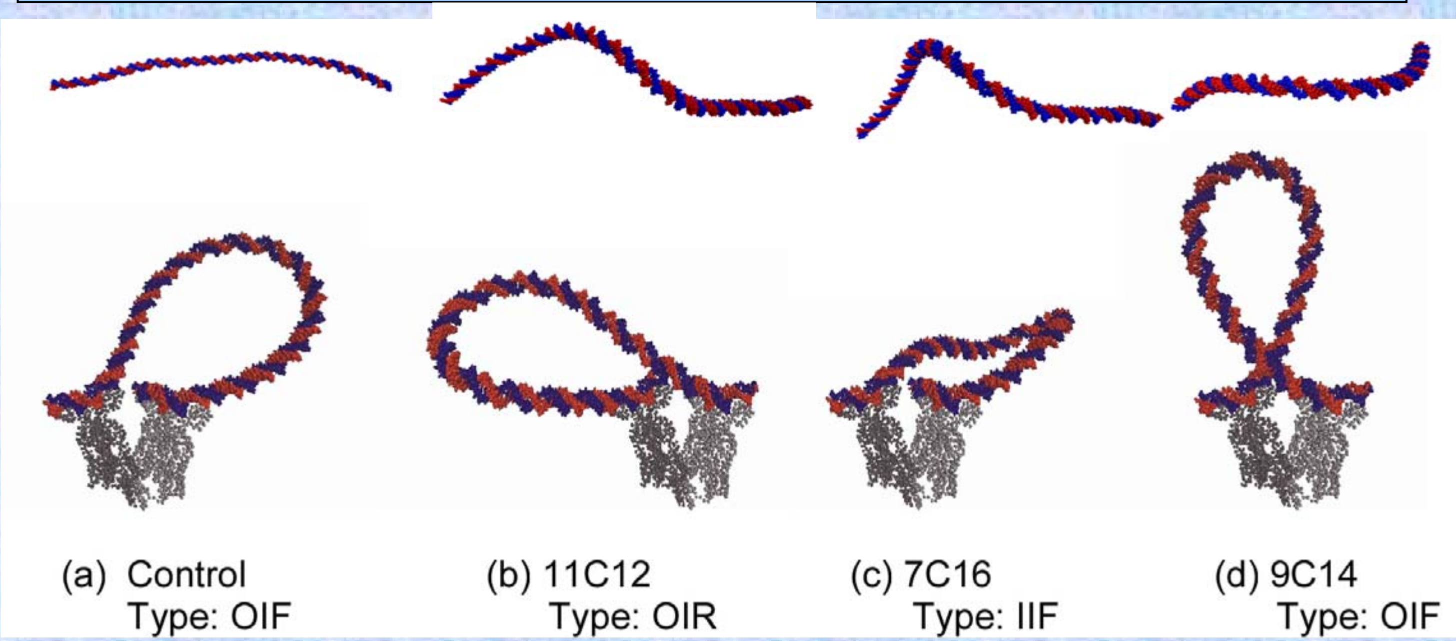
Inextensibility Constraint

$$\frac{\partial v}{\partial s} + \kappa \times v = \omega \times a_3$$

Computational Rod Mechanics Model



Modeling the Effects of Sequence Specific Curvature



Loop Energy (k_BT)	11.93	7.80	8.65	10.81

(3.8%)	(63.3%)	(10.7%)	(46.5%)

% of looped complex remaining in a 24-hr competition assay [J. Mol. Biol. (1999) 294, 67-77]

Intrinsic curvature always facilitates loop formation!

Entropy of Thermal Fluctuations

Potential Energy Functional

$$\Pi_V = \frac{1}{2}\int_0^L ds \left\{ Y\varepsilon^2 + A(\mathrm{K}^f - \kappa^o)^2 + C(\Theta^f - h^o)^2 \right\}$$

Symbol	Definition
Y	Axial Stiffness
A	Bending Stiffness
C	Torsional Stiffness
ε	Strain of deformed state
Κ,κ	Curvature
Θ,h	Twist

We look at small deformations of the equilibrium shape about the U_t U_n U_b U_θ directions.

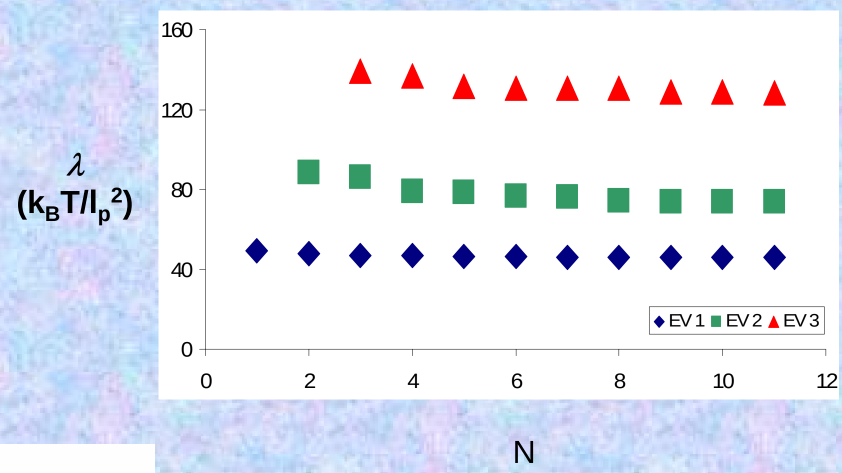
Using a suitably chosen basis set of comparison functions, we can determine a stiffness tensor *Q* for fluctuations around the equilibrium shape.

Free energy of loop:

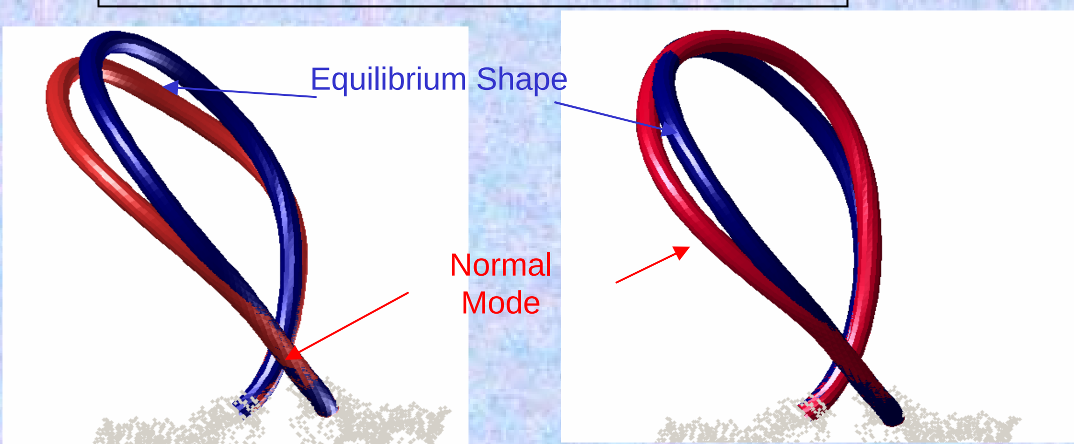
$$F_L = E_L + \frac{1}{2}k_B T \log\left(\frac{\det Q}{(4\pi k_B T)^N}\right)$$

Normal Mode Analysis

- The eigenvalues and eigenvectors of the stiffness tensor *Q* correspond to the eignemodes of the fluctuating loop.
- The lowest eigenvalues converge when a sufficiently large basis set is chosen.



Kahn-Mehta Sequence: 11C12 Parallel



1st normal mode,

$$\lambda_1 = 46\ k_B T / l_p^2$$

2nd normal mode,

$$\lambda_2 = 74\ k_B T / l_p^2$$

- The loops are surprisingly stiff: rms thermal motion ~7nm.

Conclusions

• Single DNA molecules exhibit a wealth of new physical phenomena, that can be understood quantitatively using statistical mechanics and polymer physics.

•Understanding the mechanics of single DNA molecules through experiment, theory and simulation gives us insight in their biological function.

• Mechanics plays a key role in fundamental life processes on the nanoscale – a cell is more than just a small test tube!

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