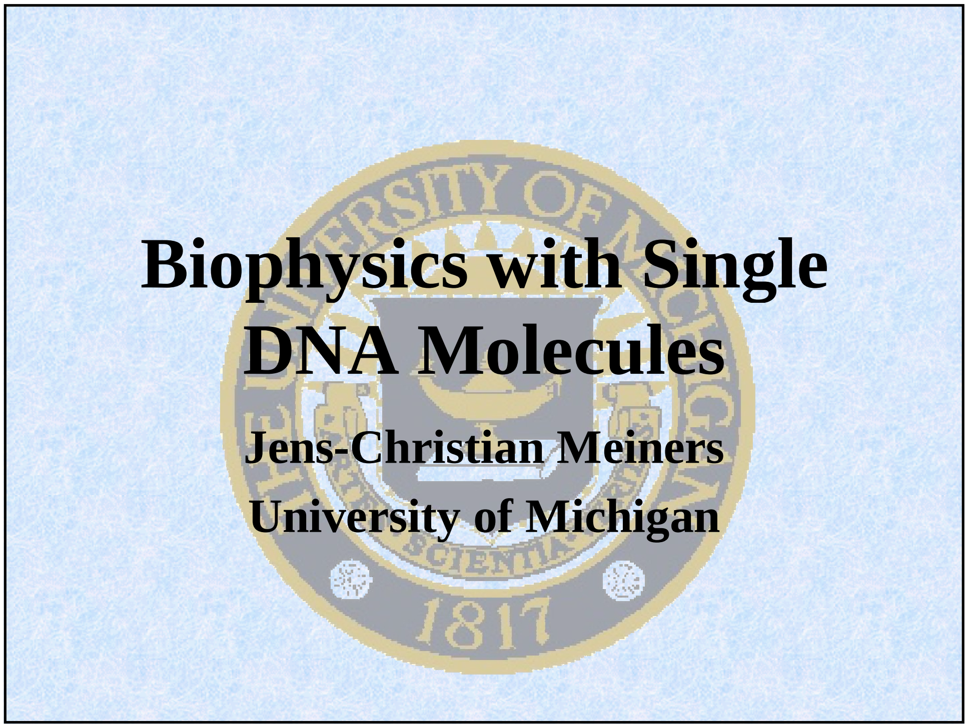
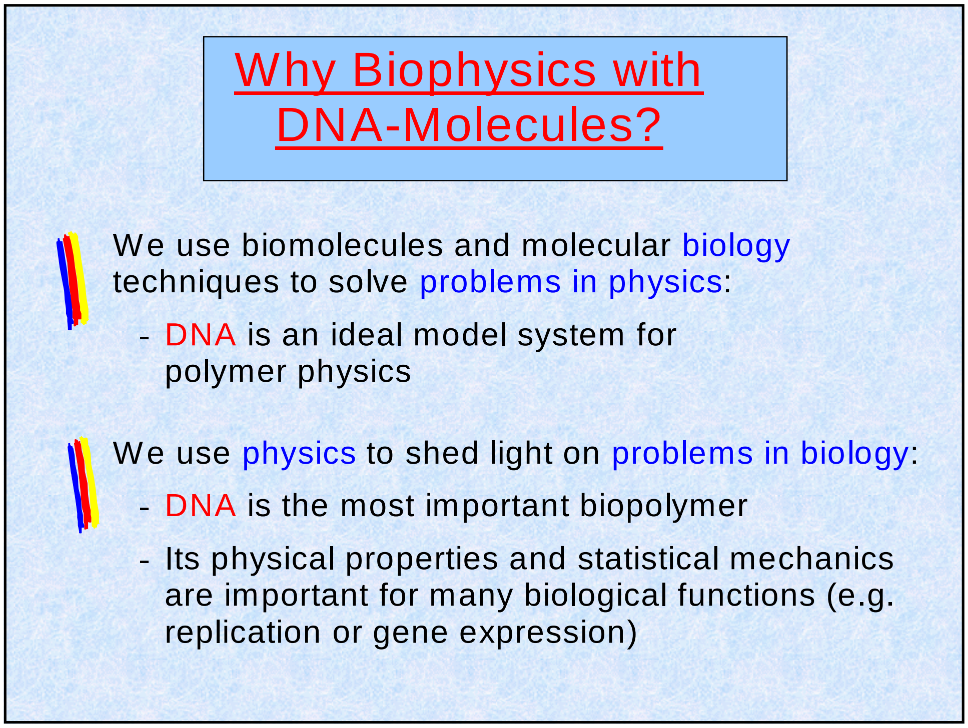


Biophysics with Single DNA Molecules

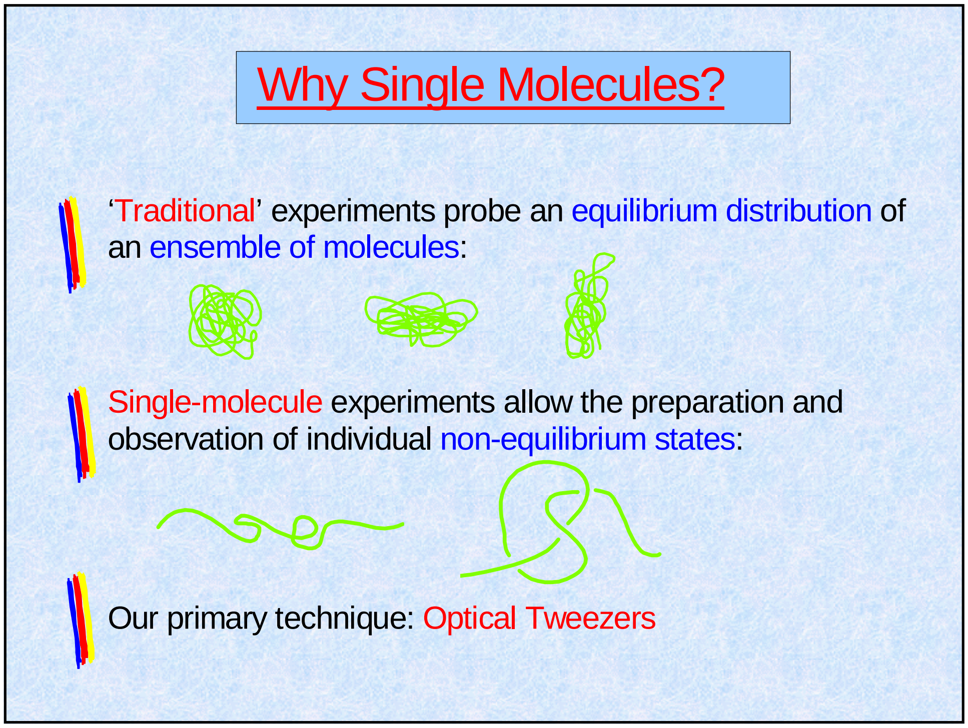
Jens-Christian Meiners

University of Michigan



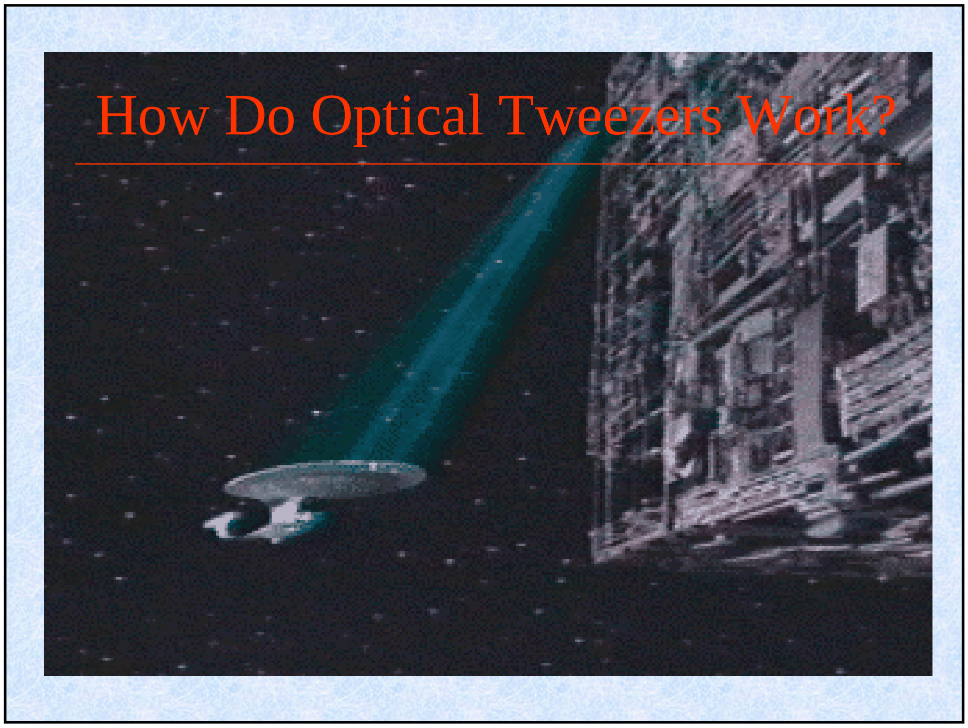
Why Biophysics with DNA-Molecules?

- We use biomolecules and molecular biology techniques to solve problems in physics:
  - DNA is an ideal model system for polymer physics
- We use physics to shed light on problems in biology:
  - DNA is the most important biopolymer
  - Its physical properties and statistical mechanics are important for many biological functions (e.g. replication or gene expression)

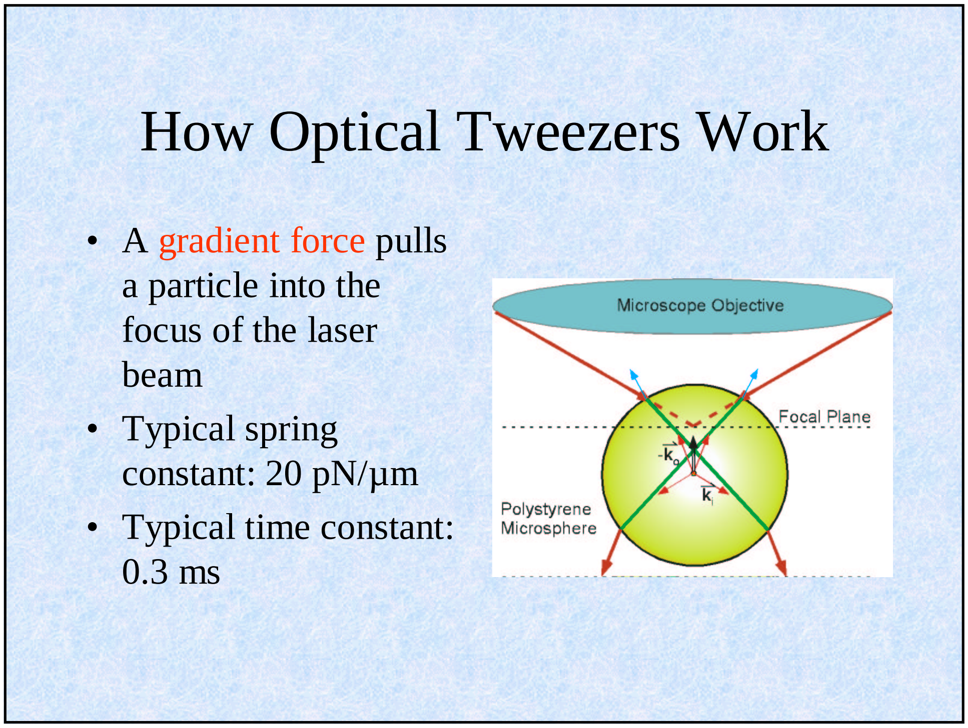


Why Single Molecules?

- 'Traditional' experiments probe an equilibrium distribution of an ensemble of molecules:
- Single-molecule experiments allow the preparation and observation of individual non-equilibrium states:
- Our primary technique: Optical Tweezers

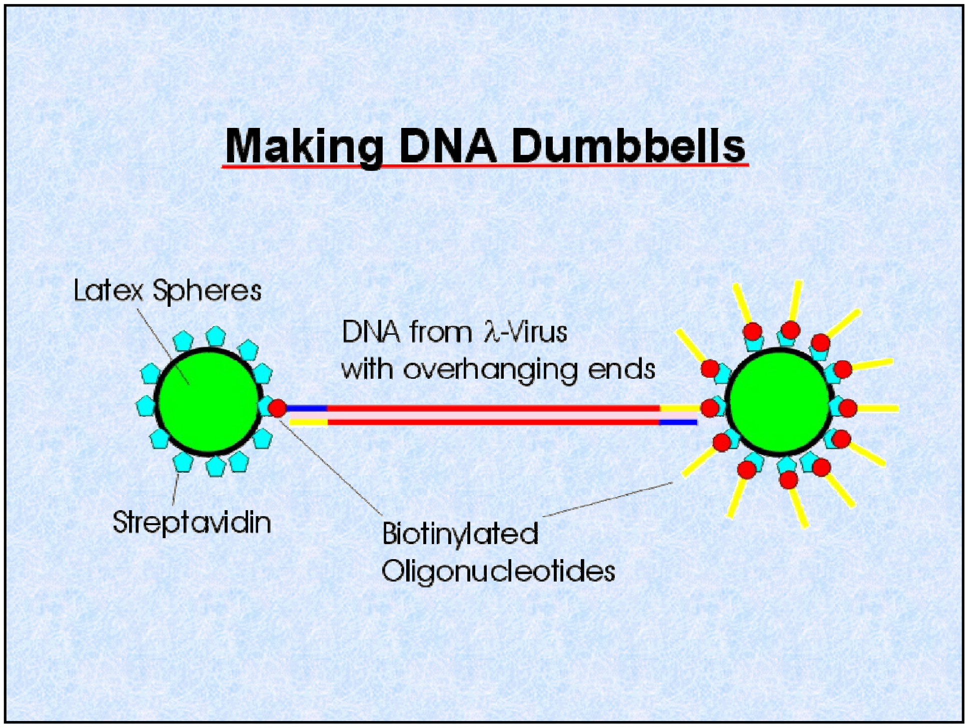


How Do Optical Tweezers Work?

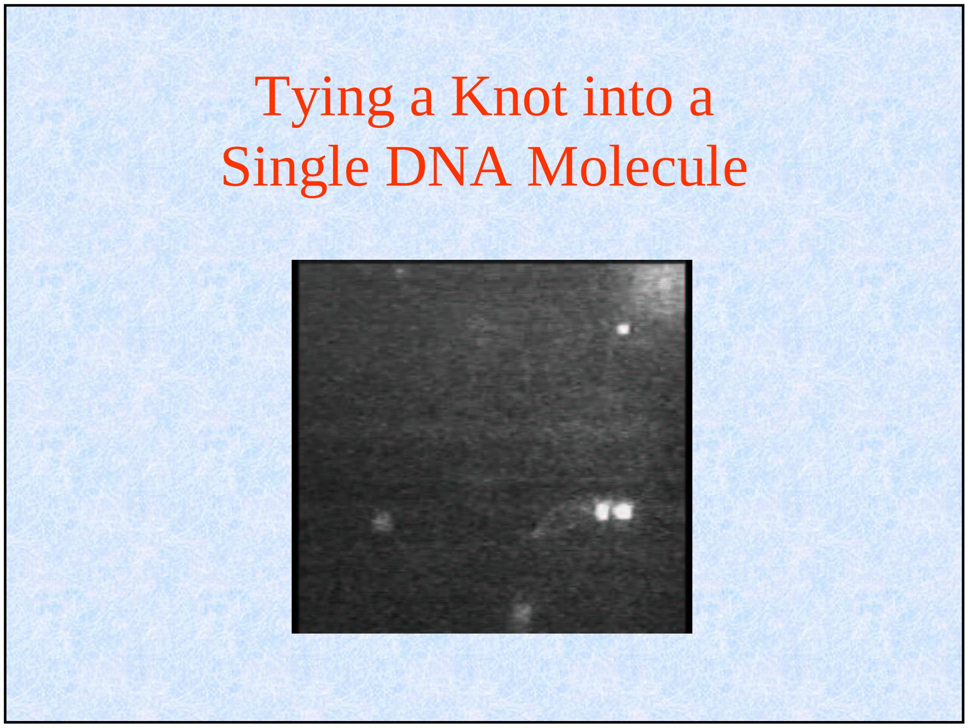


How Optical Tweezers Work

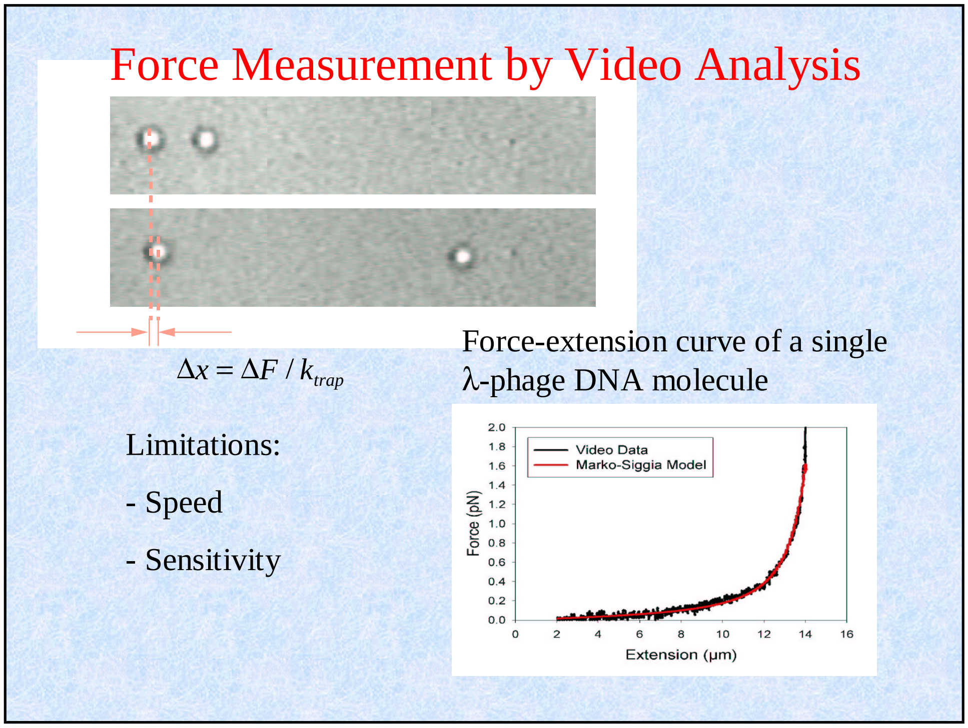
- A gradient force pulls a particle into the focus of the laser beam
- Typical spring constant: 20 pN/µm
- Typical time constant: 0.3 ms



Making DNA Dumbbells



Tying a Knot into a Single DNA Molecule



Force Measurement by Video Analysis

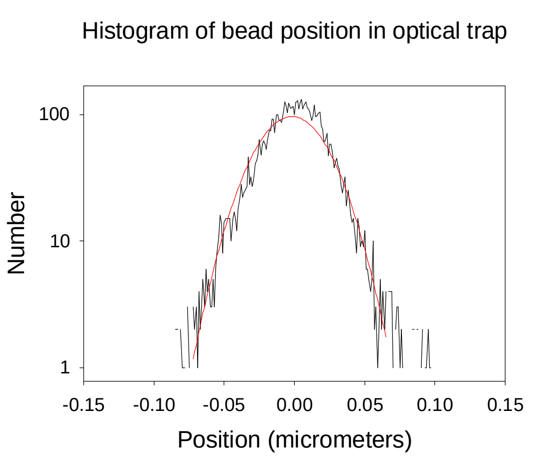
$\Delta x = \Delta F / k_{trap}$

Limitations:

- Speed
- Sensitivity

Force-extension curve of a single λ-phage DNA molecule

Optical Tweezer Calibration



$$P(x) \propto \exp\left(\frac{-\frac{1}{2}k_{trap}x^2}{k_B T}\right)$$

Spring constant
k_{trap} = 7.37 pN/μm

What is the Force Sensitivity?

Limited by Brownian motion of bead in trap; time constant $\tau = \frac{\zeta}{k}$

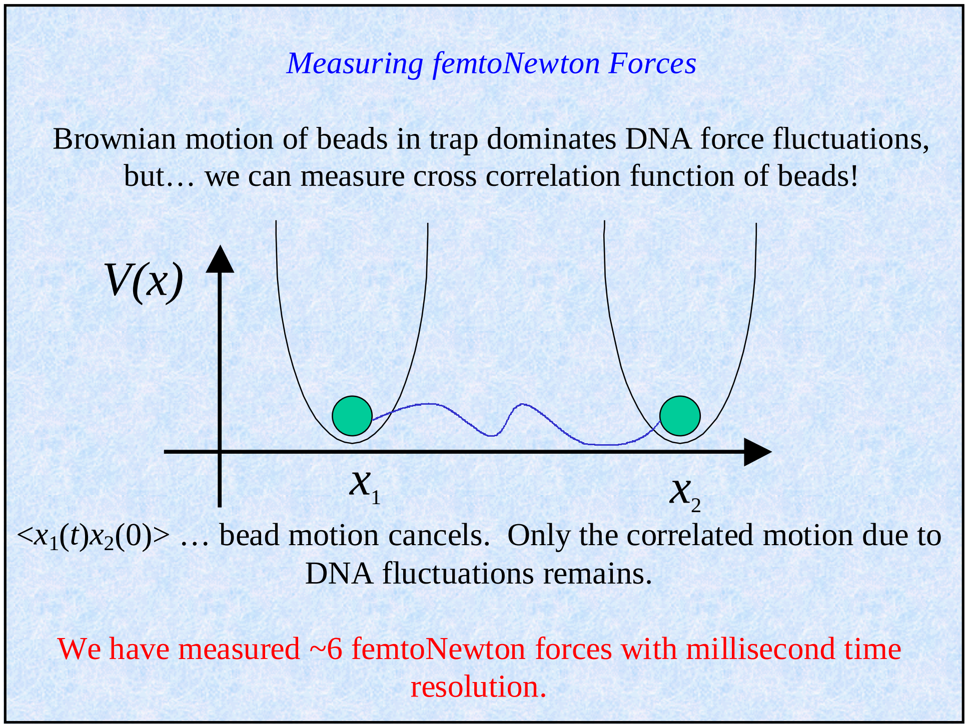
Equipartition theorem: $< x^2 >= \frac{k_B T}{k}$; $f = -kx \quad \Rightarrow \quad < f^2 >= k k_B T$

Averaging force for time δt, the error is $\sqrt{\frac{< f^2 >}{\delta t / 2\tau}} = \frac{\sqrt{2 k_B T \zeta}}{\sqrt{\delta t}}$.

For a 1 μm bead, sensitivity is $12\sqrt{\frac{fN}{Hz}}$.
In 1 kHz bandwidth, error is ~0.4pN rms.

Molecular motors exert ~2 pN in 1-10 msec.

DNA force fluctuations are ~10 fN in 1 msec

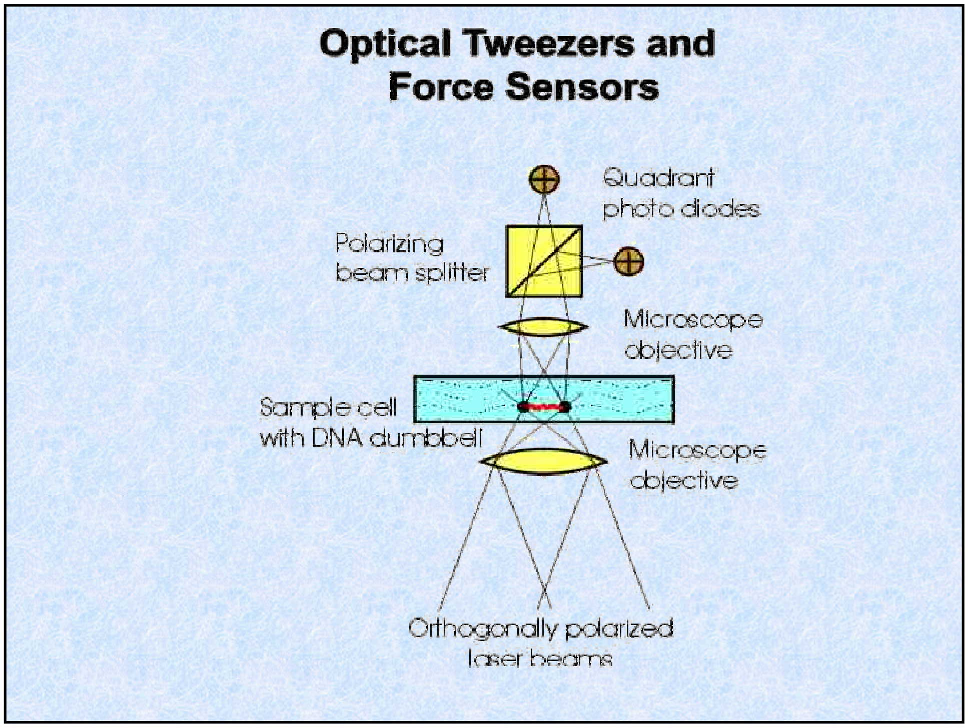


Measuring femtoNewton Forces

Brownian motion of beads in trap dominates DNA force fluctuations, but… we can measure cross correlation function of beads!

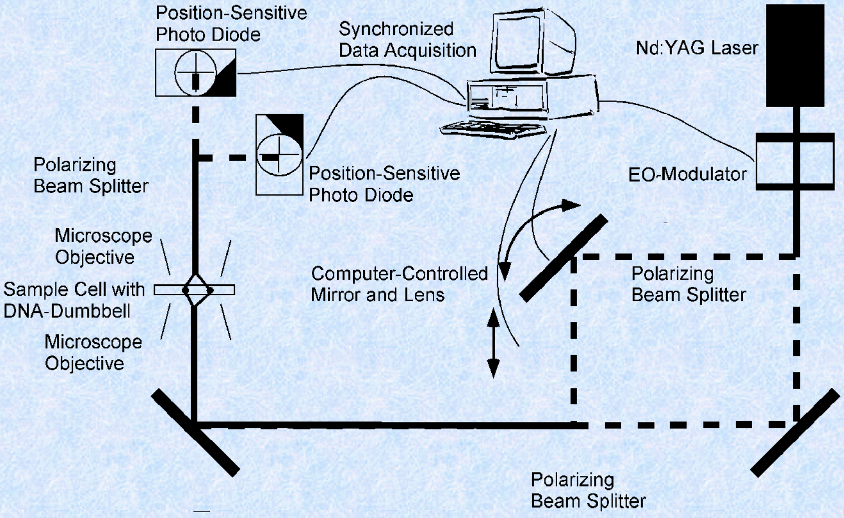
$<x_1(t)x_2(0)>$ … bead motion cancels. Only the correlated motion due to DNA fluctuations remains.

We have measured ~6 femtoNewton forces with millisecond time resolution.

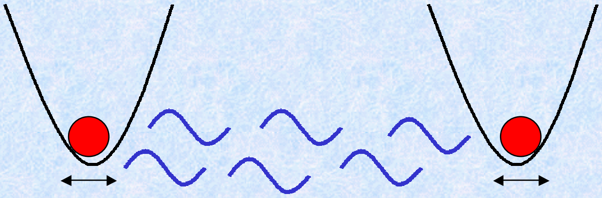


Optical Tweezers and Force Sensors

Experimental Apparatus



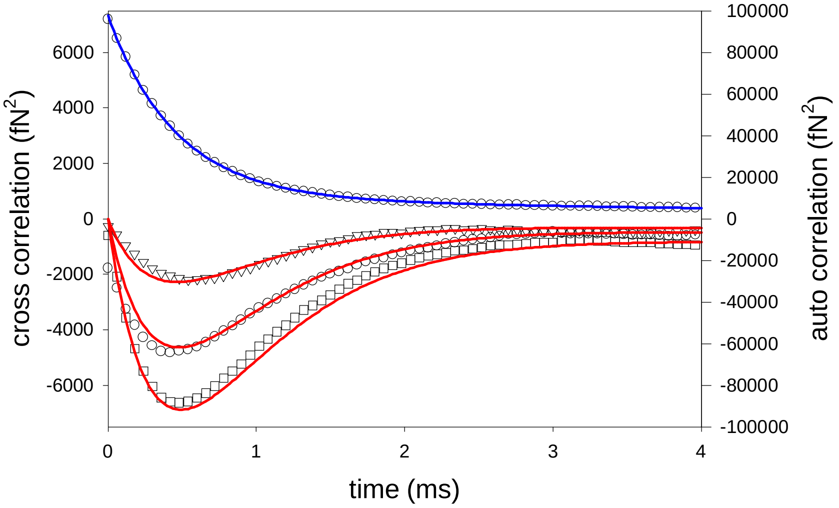
Hydrodynamic Coupling Between Two Brownian Particles



The Brownian motion of the two trapped spheres is coupled through hydrodynamic interaction.

This is a toy model for the motion of peptide fragments on a large protein complex.

Hydrodynamic Interaction between Two Trapped Spheres



Phys. Rev. Lett. **82**, 2211 (1999).

The Quantitative Picture

Time scale from the Brownian motion of the beads in their traps:

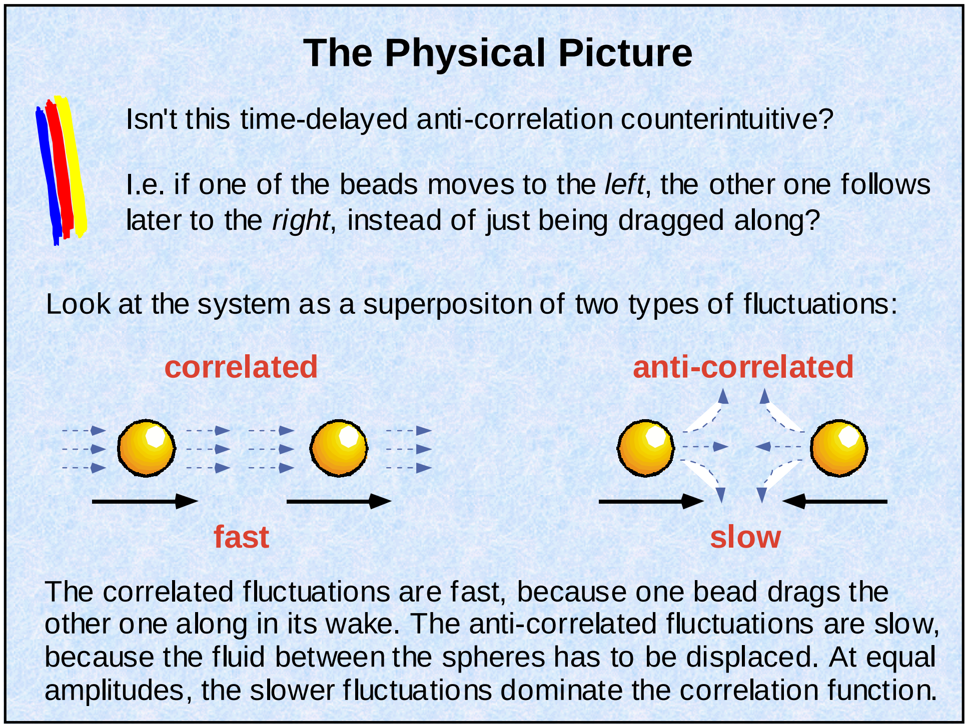
$$\tau = \frac{\zeta_{bead}}{k_{trap}} \approx 0.5\,ms$$

Using Smoluchowski’s equation and the Oseen tensor to describe the hydrodynamic interaction, we find:

$$\langle x_1(0)x_1(t)\rangle = \frac{1}{2}\frac{k_BT}{k}\left(e^{-t(1+\delta)/\tau} + e^{-t(1-\delta)/\tau}\right)$$ auto-correlation

$$\langle x_1(0)x_2(t)\rangle = \frac{1}{2}\frac{k_BT}{k}\left(e^{-t(1+\delta)/\tau} - e^{-t(1-\delta)/\tau}\right)$$ cross-correlation

with $\delta = 3r/E$



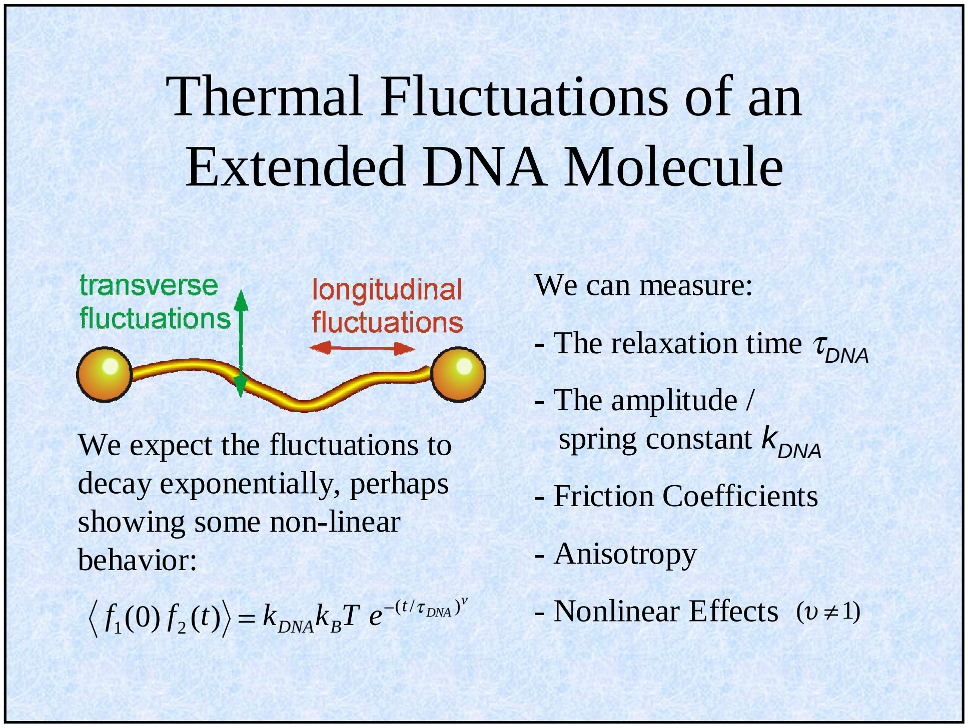
The Physical Picture

Isn't this time-delayed anti-correlation counterintuitive?

I.e. if one of the beads moves to the *left*, the other one follows later to the *right*, instead of just being dragged along?

Look at the system as a superpositon of two types of fluctuations:

The correlated fluctuations are fast, because one bead drags the other one along in its wake. The anti-correlated fluctuations are slow, because the fluid between the spheres has to be displaced. At equal amplitudes, the slower fluctuations dominate the correlation function.



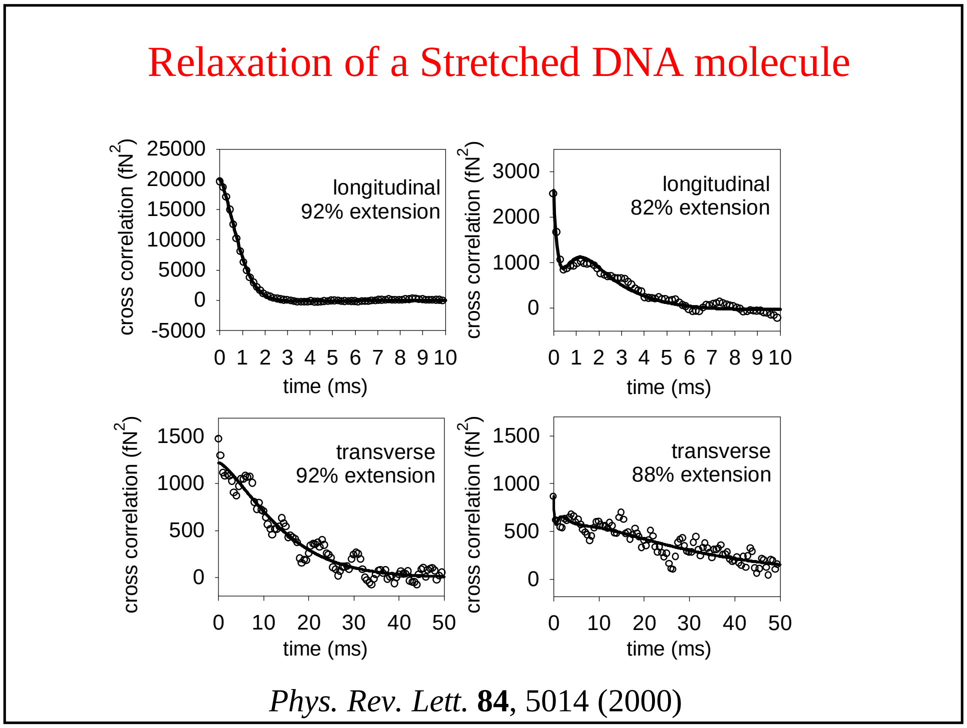
Thermal Fluctuations of an Extended DNA Molecule

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

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

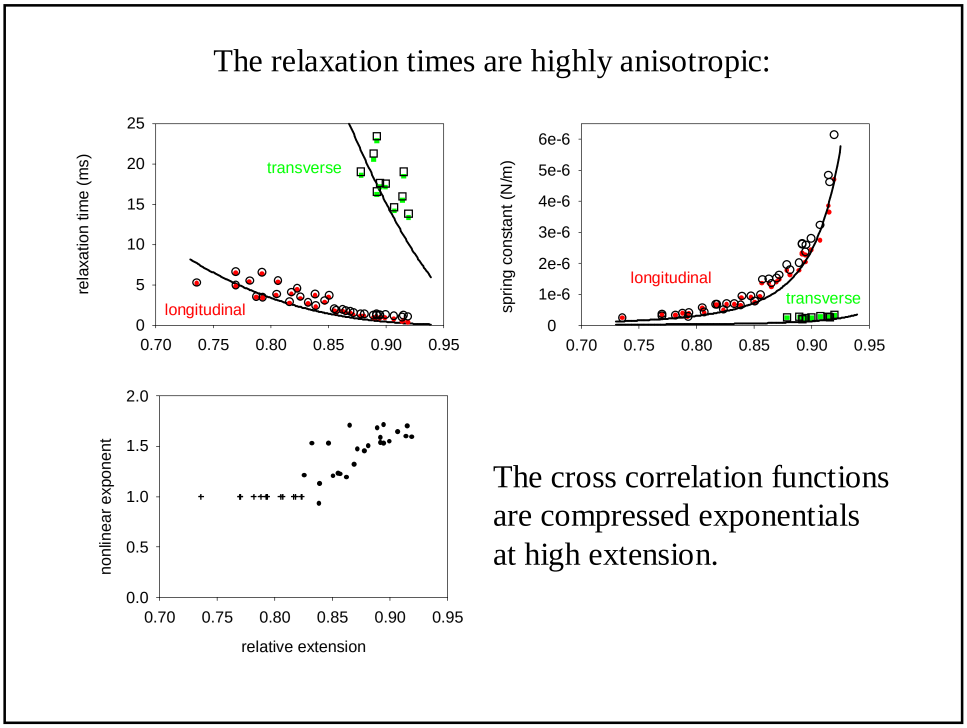
We can measure:

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



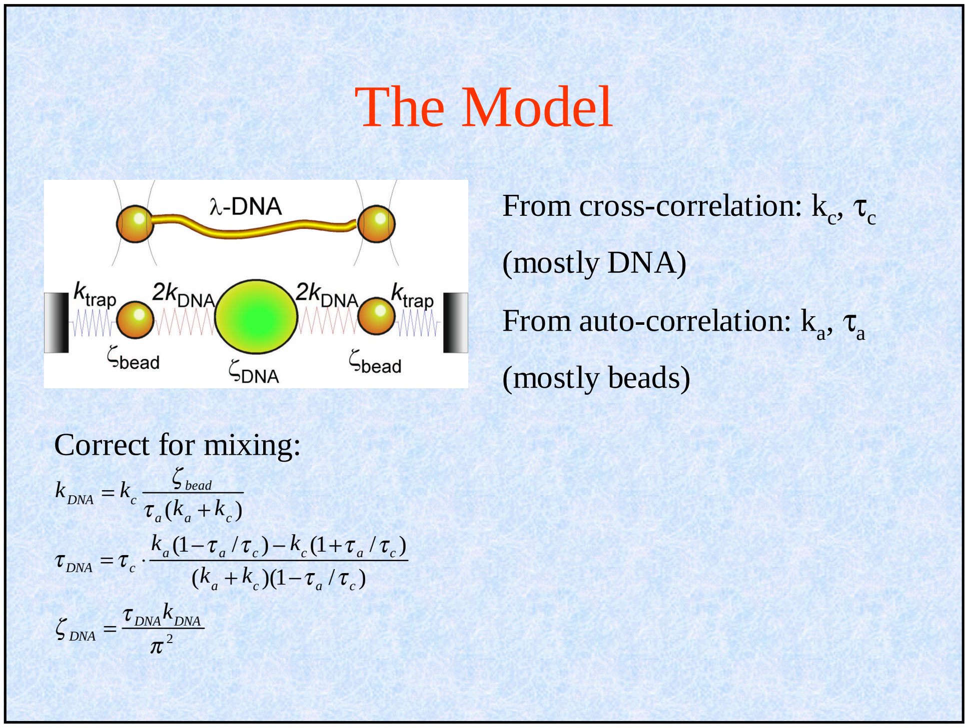
Relaxation of a Stretched DNA molecule

Phys. Rev. Lett. **84**, 5014 (2000)



The relaxation times are highly anisotropic:

The cross correlation functions are compressed exponentials at high extension.



The Model

From cross-correlation: k_c, τ_c

(mostly DNA)

From auto-correlation: k_a, τ_a

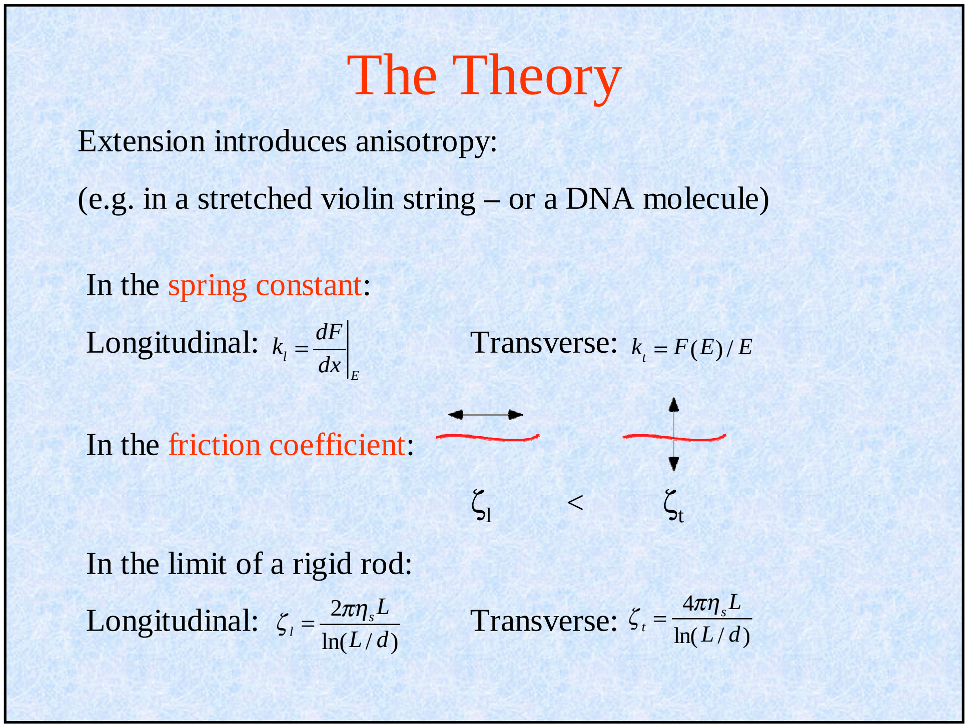
(mostly beads)

Correct for mixing:

$$k_{DNA} = k_c \frac{\zeta_{bead}}{\tau_a (k_a + k_c)}$$

$$\tau_{DNA} = \tau_c \cdot \frac{k_a (1 - \tau_a / \tau_c) - k_c (1 + \tau_a / \tau_c)}{(k_a + k_c)(1 - \tau_a / \tau_c)}$$

$$\zeta_{DNA} = \frac{\tau_{DNA} k_{DNA}}{\pi^2}$$



The Theory

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:

ζ_l < ζ_t

In the limit of a rigid rod:

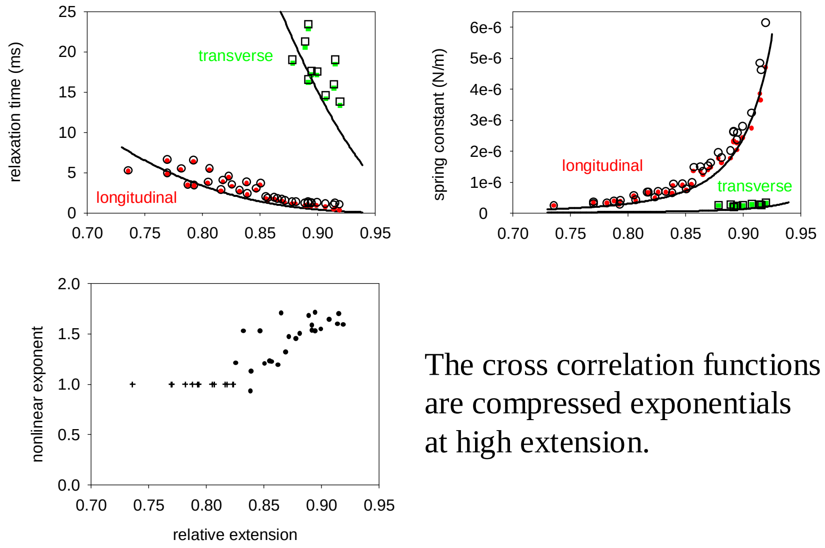
Longitudinal: $\zeta_l = \frac{2\pi\eta_s L}{\ln(L/d)}$ Transverse: $\zeta_t = \frac{4\pi\eta_s L}{\ln(L/d)}$

Using the Marko-Siggia approximation for the wormlike chain, we can predict spring constants and relaxation time for the extended DNA molecule:

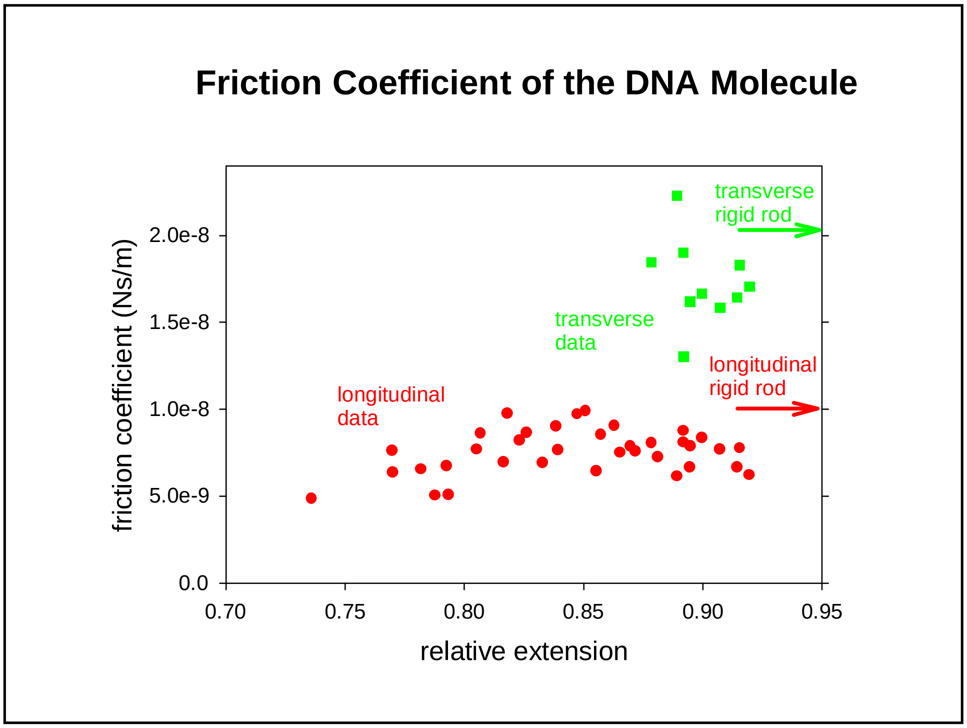
$$k_l = \frac{k_B T}{2b}\left(1-\frac{E}{L}\right)^{-3}+1 \qquad k_t = \frac{k_B T}{b}\left(\frac{1}{4}\left(1-\frac{E}{L}\right)^{-2}-\frac{1}{4}+\frac{E}{L}\right)$$

$$\tau_l = \frac{2\eta_s L b}{\pi\, k_B T \ln(L/d)}\cdot\frac{1}{\frac{1}{2}\left(1-\frac{E}{L}\right)^{-3}+1} \qquad \tau_t = \frac{4\eta_s L b}{\pi\, k_B T \ln(L/d)}\frac{E}{\frac{1}{4}\left(1-\frac{E}{L}\right)^{-2}-\frac{1}{4}+\frac{E}{L}}$$

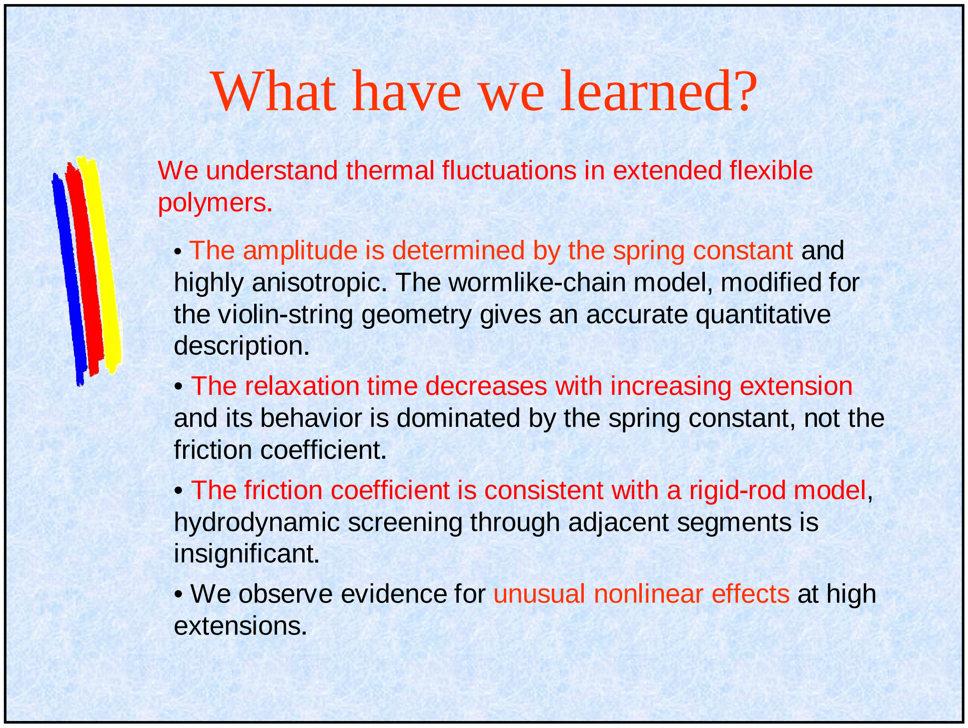
The relaxation times are highly anisotropic:



The cross correlation functions are compressed exponentials at high extension.



Friction Coefficient of the DNA Molecule



What have we learned?

We understand thermal fluctuations in extended flexible polymers.

- The amplitude is determined by the spring constant and highly anisotropic. The wormlike-chain model, modified for the violin-string geometry gives an accurate quantitative description.
- The relaxation time decreases with increasing extension and its behavior is dominated by the spring constant, not the friction coefficient.
- The friction coefficient is consistent with a rigid-rod model, hydrodynamic screening through adjacent segments is insignificant.
- We observe evidence for unusual nonlinear effects at high extensions.

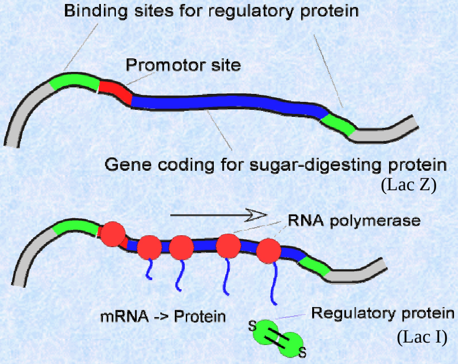
Biological Relevance?

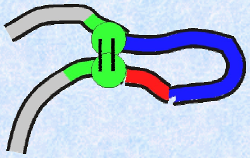
Thermal fluctuations bring distant sites on a DNA molecule temporarily in close contact.

Important for

- Regulation of Gene Expression
- Control of Replication
- Site-specific Recombination

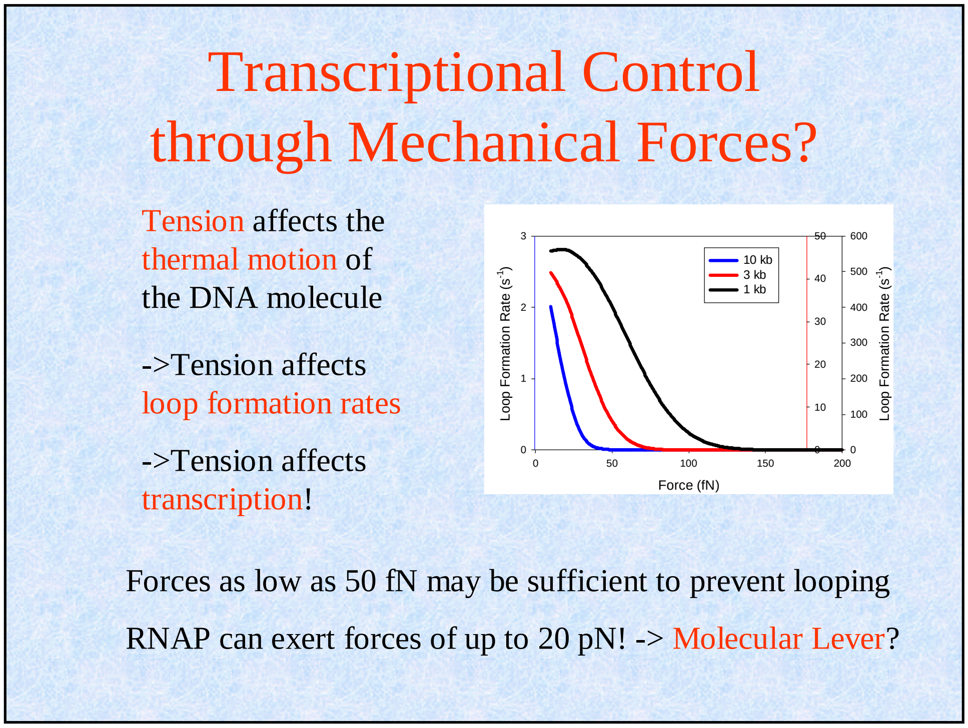
Transcriptional Control through DNA Looping





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



Transcriptional Control through Mechanical Forces?

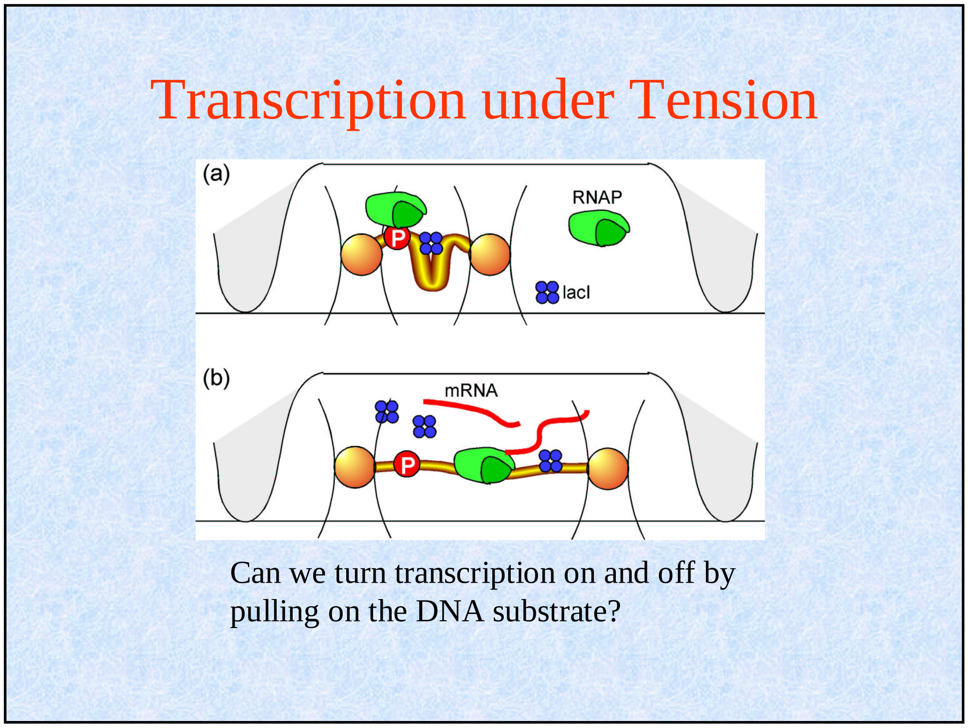
Tension affects the thermal motion of the DNA molecule

->Tension affects loop formation rates

->Tension affects transcription!

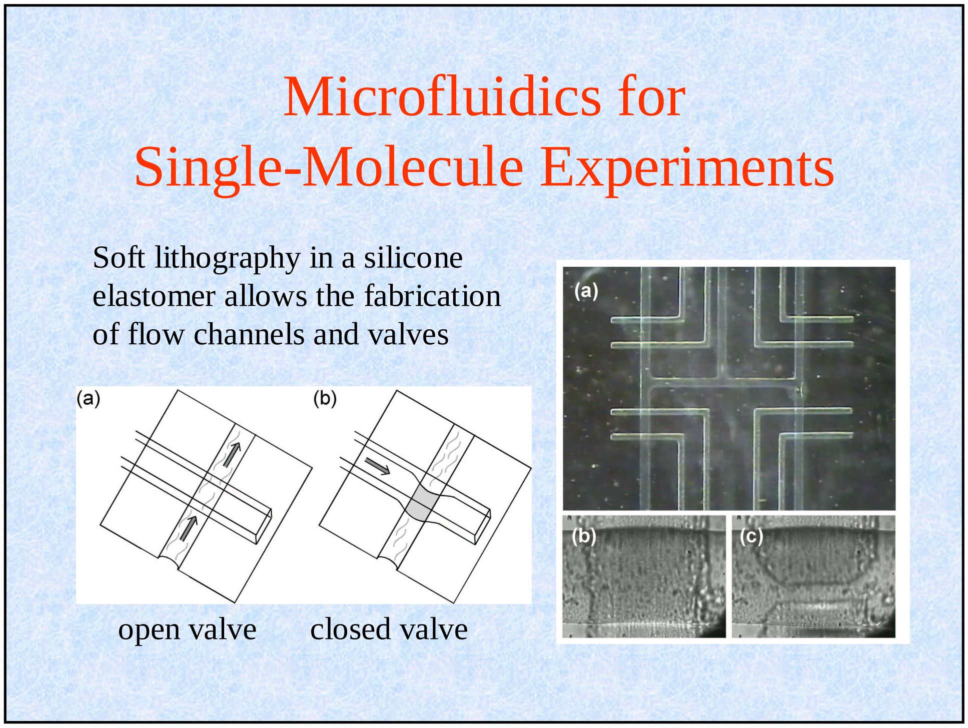
Forces as low as 50 fN may be sufficient to prevent looping

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



Transcription under Tension

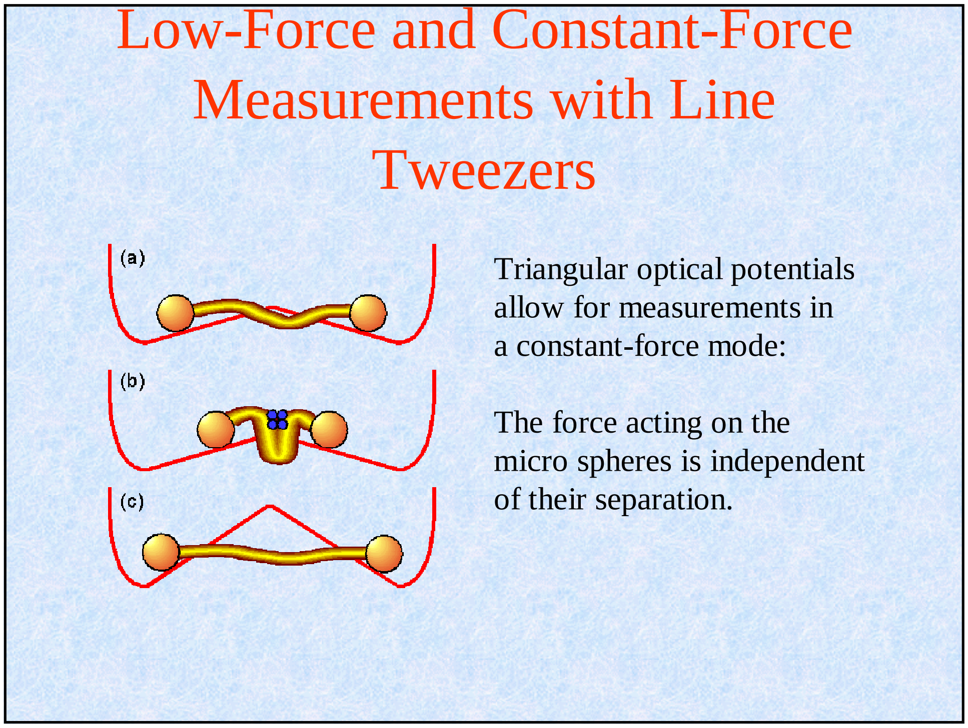
Can we turn transcription on and off by pulling on the DNA substrate?



Microfluidics for Single-Molecule Experiments

Soft lithography in a silicone elastomer allows the fabrication of flow channels and valves

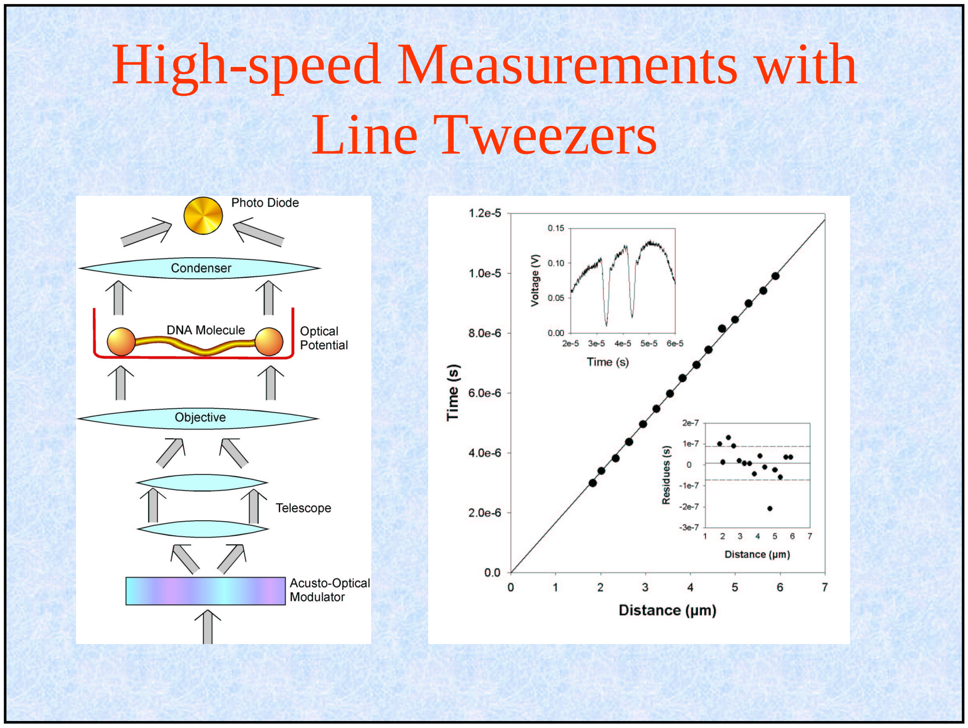
open valve closed valve



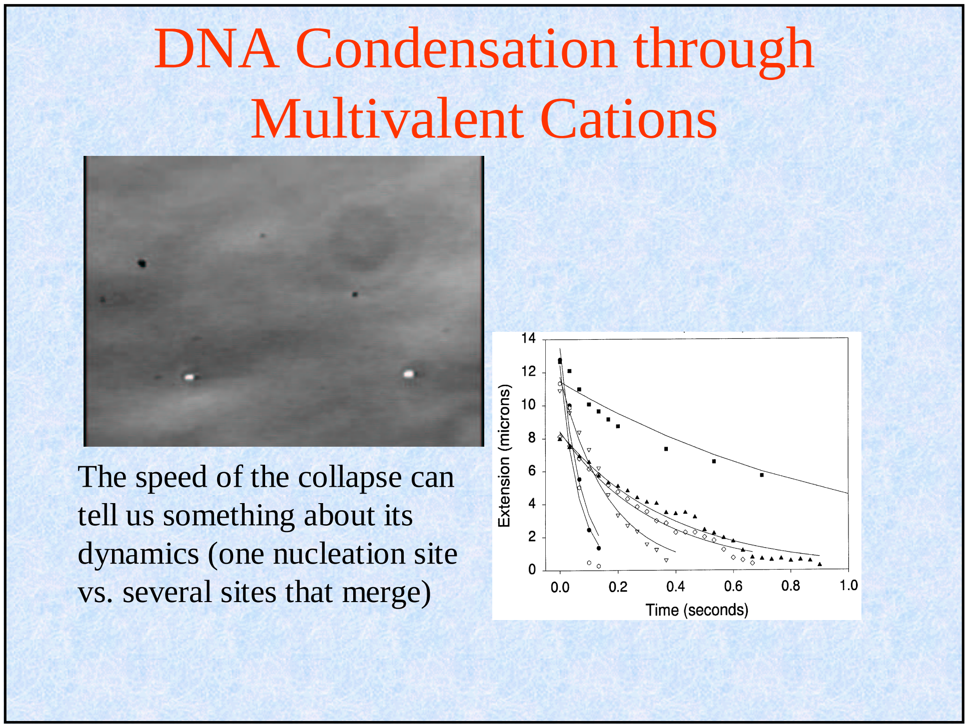
Low-Force and Constant-Force Measurements with Line Tweezers

Triangular optical potentials allow for measurements in a constant-force mode:

The force acting on the micro spheres is independent of their separation.

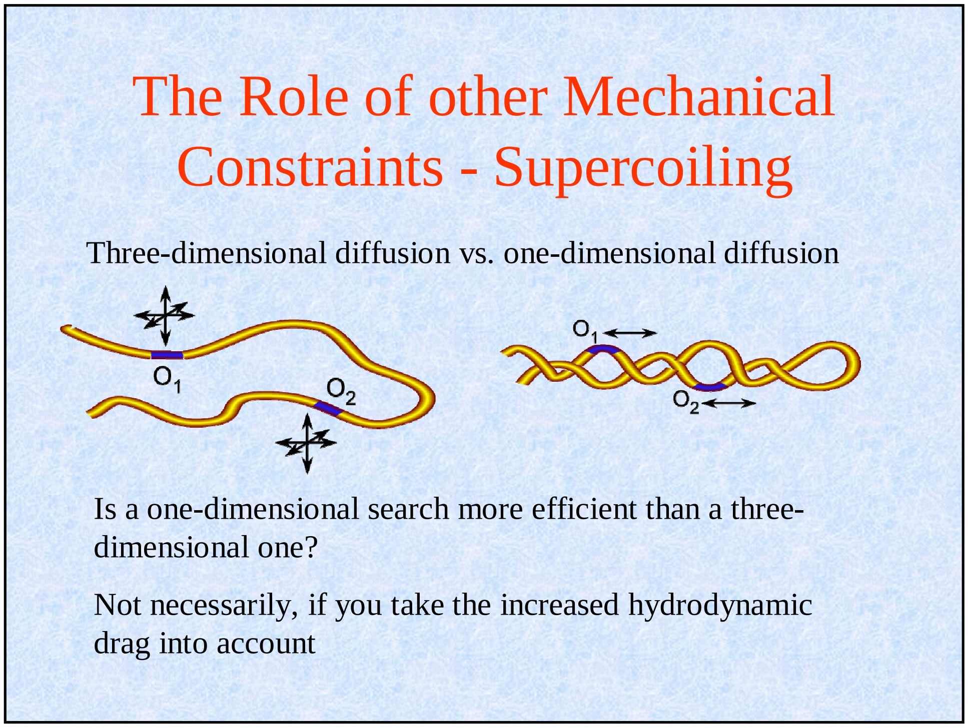


High-speed Measurements with Line Tweezers



DNA Condensation through Multivalent Cations

The speed of the collapse can tell us something about its dynamics (one nucleation site vs. several sites that merge)

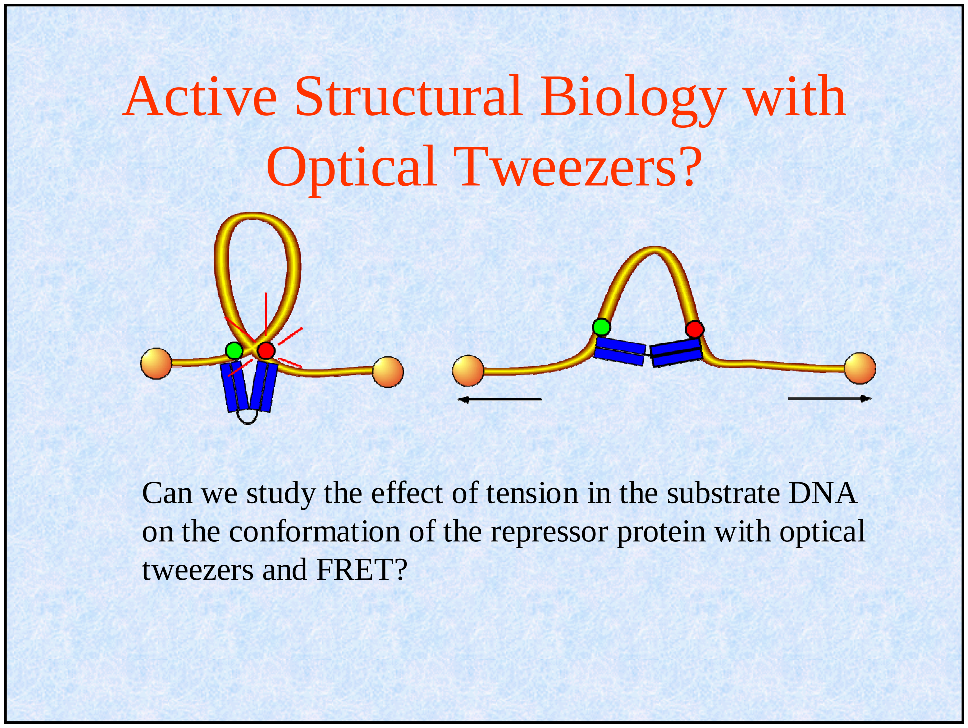


The Role of other Mechanical Constraints - Supercoiling

Three-dimensional diffusion vs. one-dimensional diffusion

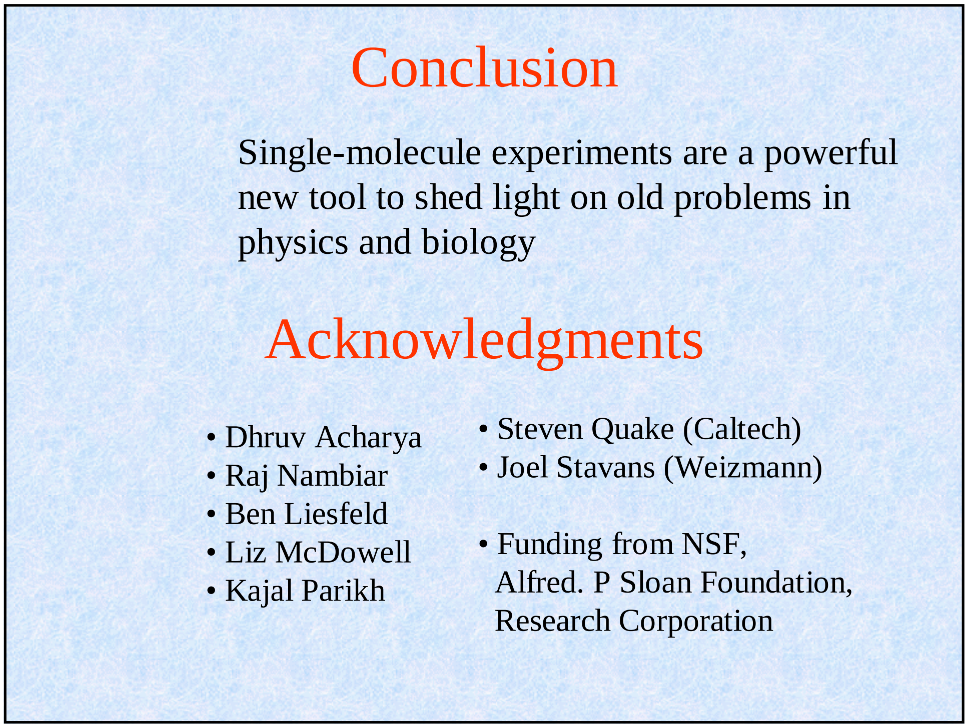
Is a one-dimensional search more efficient than a three-dimensional one?

Not necessarily, if you take the increased hydrodynamic drag into account



Active Structural Biology with Optical Tweezers?

Can we study the effect of tension in the substrate DNA on the conformation of the repressor protein with optical tweezers and FRET?



Conclusion

Single-molecule experiments are a powerful new tool to shed light on old problems in physics and biology

Acknowledgments

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