Single Molecule Methods
and their applications in protein/nucleic acid interactions
Advantages of single-molecule approaches

• dissect un-synchronized *kinetics*

• measure parameter *distributions*

• determine *kinetic paths*

• apply *force*

• measure molecular *distances*
dissect un-synchronized kinetic pathways
dissect un-synchronized kinetic pathways

* measure individual dwell times *
measure the distribution instead of the average
measure the distribution instead of the average
measure the distribution instead of the average

poisson processes have exponential dwell times (Δt) (k = rate, p = probability)

\[ p(Δt) = k \cdot \exp(-kΔt) \]
OPTICAL TRAPPING

- apply **force**
- measure **distances**
OPTICAL TRAPPING

manipulate the position of micron-sized polystyrene beads
OPTICAL TRAPPING

light momentum
change in light momentum
change in bead momentum

\[ \vec{F} = \frac{d\vec{p}}{dt} \]
OPTICAL TRAPPING

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OPTICAL TRAPPING

the result is an effective spring constant, k
OPTICAL TRAPPING

the result is an effective spring constant, $k$

$$F = kx$$

Fluctuation analysis can be used to calibrate the spring constant for a given laser power. The corner frequency ($f_c$) is proportional to the trap stiffness ($k$).
OPTICAL TRAPPING

the result is an effective spring constant, $k$

$$k = 2\pi f_c \gamma$$  \hspace{1cm} trap stiffness

$$\gamma = 6\pi \eta r$$  \hspace{1cm} drag coefficient of bead

$$\eta = \text{viscosity}$$

$$r = \text{bead radius}$$
OPTICAL TRAPPING
material properties of DNA and other biopolymers
MANUAL CONTROL
FORCE-CLAMP CONTROL
OPTICAL TRAPPING
material properties of DNA and other biopolymers

\[ F = kx \]

Hookean spring

dsDNA

ssDNA

F

X
The worm-like-chain (WLC) describes the elasticity of DNA.
The persistence length ($L_p$) describes tangent vector de-correlation

\[
f = \left( \frac{k_B T}{L_p} \right) \left[ \frac{1}{4(1 - z/L)^2} - \frac{1}{4} + \frac{z}{L} \right]
\]

\[
\langle \vec{t}(0) \cdot \vec{t}(s) \rangle = e^{-\frac{L}{L_p}}
\]

$L_p$(dsDNA) = 150 bp

$L_p$(ssDNA) = 3 bp

(smaller $L_p$ = more flexible)
The persistence length ($L_p$) describes tangent vector de-correlation.

\[
\langle \vec{t}(0) \cdot \vec{t}(s) \rangle = e^{-\frac{L}{L_p}}
\]
What is the average end-to-end length ($z$) of an unconstrained ($f = 0$) flexible polymer of length $L$?

What do you have to do to make $z = L$?

Why?
Entropic Elasticity (inextensible)

OPTICAL TRAPPING

decreasing configurational entropy
**Enthalpic Stretching**
*(the extensible WLC)*

\[
x = L_0 \left(1 - \frac{1}{2} \left(\frac{k_BT}{FP}\right)^{1/2} + \frac{F}{K_0}\right)
\]

\[K_0 = 1000 \text{ pN}\]

Structural changes in DNA structure in this regime.

Can you convince yourself that at a high force, this is \( F = K_0 \Delta x \)?
The overstretch transition

Phase transition where the DNA length increases nearly two-fold at constant force. Must involve a dramatic change in DNA structure.

DNA Unwinding?
An unwound double-helix (ladder-like structure)?
OPTICAL TRAPPING
specific folding/unfolding transitions
OPTICAL TRAPPING
specific folding/unfolding transitions

constant pulling rate

constant force

$t_{\text{unfold}}$

$t_{\text{fold}}$

$F_{\text{unfold}}$
FORCE-DEPENDENT RATES

\[ W = Fx \]

\[ A(F) - A(0) = B(0) - A(0) - F(x_B - x_A) \]

\[ dG(F) = dG(0) - F(x_B - x_A) \]

\[ TS(F) - A(F) = TS(0) - A(0) - F(x_{TS} - x_A) \]

\[ dG_{TS}(F) = dG_{TS}(0) - F(x_{TS} - x_A) \]

\[ k_F = k_0 \exp(-F(x_{TS} - x_A)) \]
FORCE-DEPENDENT RATES

Measure “dwell-times” for folding and unfolding

For a single step, we expect an exponential distribution of times (Poisson process)

A fit of this exponential gives us a rate-constant
Measurements of rate-constant at different forces can reveal the distance to the transition state of molecular transitions.
OPTICAL TRAPPING
specific folding/unfolding transitions

Liphardt and Bustamante (Science, 2001)
OPTICAL TRAPPING
specific folding/unfolding transitions

Woodside and Block (Science, 2008)
OPTICAL TRAPPING
tracking single molecular motors

• molecular motor
OPTICAL TRAPPING
tracking single molecular motors
OPTICAL TRAPPING
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Laboratory of Steven Block, Stanford
8 nm steps of kinesin and 0.34 nm steps of RNA polymerase
OPTICAL TRAPPING
tracking single molecular motors

Greenberg and Ostap (MethMolBio, 2017)
OPTICAL FLEEZEERS

Lohman and Chemla (Science, 2015)