**Motivation**

Optical Tweezers

Molecular Machines

Summary

**Bionanomechanics with Optical Tweezers: Molecular Machines under Tension**

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**Mechanical Processes Fulfill Essential Cellular Functions**

Cell division  (Ted Salmon 2006)

⇒ Single-molecule force measurements
Optical Tweezers: Ideal Tool for Motor Protein Mechanics

Kinesin-1 (BioVisions, Harvard University)

Outline

- Optical Tweezers — A Sensitive Position & Force Transducer
- Bionanomechanics
  * Transport & Friction of Molecular Machines
  * DNA repair: Molecular Clamping
- Outlook
Motivation

Optical Tweezers

Molecular Machines

Summary

Invisible, High-Power, Infrared Lasers for Trapping

Biological matter absorbs little in the near infrared

Microsphere Remains Trapped Against Hydrodynamic Flow
Contact-Free, Live Cell Mechanical Manipulation

trapped bacteria, Block laboratory 1989

Optical Tweezers for High-Resolution 3D Measurements

Molecular Position & Force Resolution

\[ \text{Å, fN, } \mu \text{s resolution} \]

with 0.001 °C stability

Opt. Express 2009

Antireflection-Coated Microspheres Improve Optical Trapping

Stabilizing gradient force ⇔ Destabilizing scattering force

⇒ Reduce scattering force by coating

partially antireflection-coated glass

Science 1999
Nanonewton Optical Forces with Coated Microspheres

Antireflection coating reduces scattering via destructive interference

High-refractive index titania particles:
• 1 nN force with optimized tweezers
• Novel experiments feasible


Kinesin Motors Take 8 nm Steps

LED-DIC
J. Microsc. 2007
Kinesin-1 Steps Efficiently & Transports Piconewton Loads

efficiency = 
\[
\frac{\text{(max. load } \times \text{ step size)}}{\text{ATP energy}}
\]
\[
\approx \frac{5 \text{ pN } \times 8 \text{ nm}}{100 \text{ pN nm}} = 40\%
\]
macroscopic engines: typically 15–35%

Kinesin-8 Is a Weak Motor That Depolymerizes Microtubules & Slips

Slippage for \(F > 1\) pN
(Biophys. J. 2013)

Friction?
(Science 2009)
**Friction Is Related to Diffusion**

Friction resists the relative motion of two bodies in contact.

- Contact mediated by adhesive bonds between molecules
- Friction: force necessary to deform & break these bonds

Einstein relation:

\[
\gamma = \frac{k_B T}{D}
\]

Kinesin-8 diffuses in ADP

**Kinesin-8: Model System for Protein Friction**

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**Friction Limits Diffusive & Directed Motor Movement**

Diffusion coefficient \(D\) from single molecule tracking

Frictional drag coefficient \(\gamma\) from optical tweezers dragging

Einstein relation holds for *single* molecular machines

(Science 2009)
Mechanics of DNA Repair: Homologous Recombination

Redβ forms complexes with DNA

- Redβ catalyzes DNA strand annealing without ATP
- Redβ forms helical superstructures with DNA
- Redβ is related to Rad52

Redβ - DNA complex

only Redβ

Redβ-DNA complex

Passy et al., PNAS 96, 4279 (1999)

Redβ Strongly Clamps DNA Strands Together

Mechanical Insight into Chromosome Segregation

Diffusive contacts:

1. Rapid microtubule end targeting (MCAK, XMAP215)
2. Chromosome attachment to shrinking microtubule tips (Dam1, Ndc80)
3. Cross-linking of microtubules while allowing for relative sliding (Eg-5, Ase1p, Ncd)
4. Processivity enhancement via slip state (load sharing?) (Kinesin-8, Eg-5)
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