Motivation for high-resolution mechanical studies in biology: “SINGLE MOLECULE STUDIES”

What are optical tweezers and how do they work?

Mechanical properties of optical tweezers and energy change in biological reactions:
- picoNewton forces and nanometre movements
  - energy \( < 10k_B T \)

Motor proteins (kinesin & myosin) as model systems for single molecule studies.

Other laser-based single molecule methods e.g. TIRF microscopy.
Why work with individual molecules?

• Single molecule experiments can give unequivocal information about how enzymes work and can provide new insights into enzyme mechanism.

• Sequential steps that make up biochemical pathways can be observed directly. The chemical trajectory of an individual enzyme can be followed in space and time.

• There is no need to synchronise a population in order to study the biochemical kinetics

• Single molecule data sets can be treated in a wide variety of ways – e.g. can specifically look for heterogeneity in behaviour (i.e. strain dependence of rate constants, effects of membrane structure, etc).
Energy levels in biology:

1 Photon = 400 pN.nm
1 ATP = 100 pN.nm
1 Ion moving across a membrane = 10 pN.nm
Thermal energy \((k_bT)\) = 4 pN.nm
\((k_bT = RT/A)\)

\{ 1 pN.nm = 1 \times 10^{-21} \text{Joules} \}
SINGLE MOLECULE TECHNOLOGIES:

PATCH CLAMP:
A single ion channel admits >5000 ions when it opens and the resulting current flow (picoAmps) can be measured relatively easily using a high input-impedance transistor.

(Energy \equiv 50,000 \text{ pN.nm} = 12,000 \text{ k}_b\text{T})

FLUORESCENCE MICROSCOPY:
A single fluorophore emits >10,000 photons per second and can be imaged quite easily using modern cameras (and seen by the naked eye).

(Energy \equiv 1 \times 10^6 \text{ pN.nm/视频帧} = 250,000 \text{ k}_b\text{T})
Mechanical Studies have no “built-in” gain

**SINGLE MOLECULE MECHANICS:**
Molecule motors consume 1 ATP molecule for each step taken.  
(Energy $\equiv 50 \text{ pN.nm} = 10 k_B T$ (i.e. very challenging))

**Optical Tweezers**
- Protein-Protein
- Protein-Ligand interactions

**Magnetic Tweezers**
- DNA topology
- DNA-protein interactions

**AFM**
- Protein (un)folding
- Protein-Protein & Protein-Ligand interactions
Optical Force:

\[ E = mC^2 \]
Momentum, \( mC = E/C \)

\[ \text{Force} = mC/t = P/C \]
(\( P = \) optical power)

….calculate the force produced by a 3mW laser pointer…..
Laser beam has Gaussian intensity profile. Restoring force is proportional to displacement.

\[ F = \kappa x \]

Stiffness, \( \kappa = 0.02 \text{ pN.nm}^{-1} \)

\( \sim 10 \times \) softer than protein.
3-D trap using counter-propagating laser beams

Ashkin & Dziedzic, 1971
Single beam “gradient trap”
Ashkin et al. 1986

Optical “Trap” when: \( \sum F_{\text{grad}} = \sum F_{\text{scat}} \)
travelling acoustic wave
acoustic velocity (TeO₂) = 800 m.s⁻¹
freq. ~ 50 MHz (spacing, d ~ 15 μm)

First order:
\[ \sin(\phi) = \frac{\lambda}{d} = 1.064 \, \mu m / 15 \, \mu m \]
\[ \phi = 75 \, mRads \]
\[ \Delta \phi ( +/- 10\%) \sim 15 \, mRads \]

Zeroth order
Realistically, it's a bit more complicated
In order to measure forces we need to measure the position of the optically trapped particle with very good accuracy (<1 nm) and good time resolution (>1 kHz).

Most instruments use 4-quadrant photodiodes.

However, modern (inexpensive) CMOS cameras operate at 5kHz (over a limited field of view) and are now commonly used to “centroid” the bead image.
Super-resolution optical imaging requires statistical analysis of the spatial distribution of photons arising from a diffraction-limited source. We need to characterise the spatial autocorrelation function (the “Airey disc”)

Super-resolution mechanics requires statistical analysis of the temporal distribution of forces and movements arising from a thermal-noise-limited source. We need to understand the temporal autocorrelation function (the “power density spectrum”)

Thermal motion of an optically trapped particle

\[ \frac{1}{2} \kappa \langle x^2 \rangle = \frac{1}{2} k_B T. \]

Thermal noise is \( \sim 14 \text{ nm r.m.s.} \)
Dynamic response

\[ m \frac{\delta^2 x}{\delta t^2} + \beta \frac{\delta x}{\delta t} + \kappa \delta x = 0 \]

Stoke’s drag

\[ \beta = 6\pi \eta r \]

Typical values:

\[ m = 5 \times 10^{-16} \text{ kg} \]
\[ \beta = 1 \times 10^{-8} \text{ N.s.m}^{-1} \]
\[ K \sim 1 \times 10^{-5} \text{ N.m}^{-1} \]
Calibration of optical trap stiffness

1) Brownian motion

- Records at different laser powers
- Power Spectra

2) Bead deflection caused by viscous drag

- Step Response
- Rising Phases
- Falling Phases
Optical tweezers can be used to measure forces in biology

A. Force and Transcription
B. Molecular motors
C. Rotates Flagella Bacteria
D. Protein unfolding

Molecular motors
Microtubule
Kinesin
Titin
Stretch
SINGLE MOLECULE DATA SETS
Transition state theory describes the kinetic properties of the system.

\[ k_{AB} \propto e^{\frac{-e_A}{k_BT}} \quad k_{BA} \propto e^{\frac{-e_B}{k_BT}} \]

\[ K = \frac{k_{AB}}{k_{BA}} = e^{\frac{-(e_B-e_A)}{k_BT}} = e^{\frac{-\Delta E}{k_BT}} \]
Monte Carlo simulation

\[ k_{\text{obs}} = k_{\text{AB}} + k_{\text{BA}} \]

\[ K_{\text{eq}} = \frac{k_{\text{BA}}}{k_{\text{AB}}} \]
1 molecule

\[ k_{BA} = \frac{1}{t_1} \]

\[ k_{AB} = \frac{1}{t_2} \]
• We want to measure the duration and amplitude of single molecule “Events”.

• “Events” have different names and forms:
  “open” and “closed”
  “on” and “off”
  “bound” and “free”
  etc..

• “Events” can be single chemical states or comprise “sub-states” which can only be revealed by careful kinetic or amplitude analysis.
How can we use optical tweezers to understand how molecular motors produce force and movement from ATP?
Filament sliding causes muscle to shorten:

myofibril

Light micrograph

Electron micrograph

sarcomere
Acto-myosin ATPase pathway

Strong binding states
POWER-STROKE

Weak binding states
RECOVERY-STROKE
How do myosin motors actually produce force and movement?

Thermal Ratchet

or

Power-stroke conformational change
Acto-myosin *in vitro* motility assay:

![Diagram of acto-myosin motility assay](image)
High-resolution mechanical studies
Actin Filament Held Between Two Latex Beads

Coated with:
Monomeric NEM-Myosin & BSA-TRITC

Interacting with:
1.7μm glass bead

Coated with:
HMM @ 50μg/ml

[ATP] = 2mM
Displacement (nm) vs. Time (s) graph showing a trend from 0 to 200 nm displacement over time. The text reads, "~20->50 molecules," indicating a change in the number of molecules.

A smaller graph illustrates the variance, with a note, "1 molecule," and an annotation, "Variance tells us about the system STIFFNESS."
Single molecule optical trapping

Basic Analysis (I)

Lifetime distribution gives rate constants

\[ \frac{1}{k_{\text{cat}}} \]

\[ t_{\text{on}} \quad t_{\text{off}} \]

\[ N_{\text{obs}} \]

Binding \[ \frac{1}{t_{\text{off}}} \]

\[ k_{\text{cat}} \]

Detachment \[ \frac{1}{t_{\text{on}}} \]

Time
Detachment rate

Ampl = 27.3 cts
rate = 1.30 s^{-1}
(n) = 196
Basic Analysis (II)

Start point is uncertain

Amplitude distribution gives $d_{\text{uni}}$

$N_{\text{obs}}$

$d_{\text{uni}}$

Start point is uncertain

Amplitude

$N_{\text{obs}}$

$d_{\text{uni}}$

amplitude
Size of the power-stroke

Mean = 6.72 nm
S.D. = 17.7 nm
(n) = 196

Step size (nm)
What have we learnt by studying single myosin molecules?

**Muscle myosin:**

**Kinetics:** Binding event terminated by ATP binding
1 Powerstroke requires 1 ATP
Event lifetimes are stochastic
<10% of time bound to actin >90% unbound

**Amplitudes:** Powerstroke produces 5->10 nm movement & 2->5 pN force
Myosin stiffness is ~ 1pN.nm⁻¹

**Efficiency:** 30 pN.nm mechanical work ~ 30% ΔG ATP

*Inter alia:* One myosin head is sufficient for force and movement
Myosin binds preferentially at actin helical repeat distance
Event lifetime is load-dependent
Some myosins produce movement in two discrete phases i.e. Evidence for “different bound states”
Structural and functional diversity
The myosin family:

“Conventional” Muscle myosins

(Tony Hodge, LMB Cambridge)
Super-resolution mechanics
myosin class 1 (a “tension sensor”)

50 nm

0.5 sec
Ensemble averaging reveals “sub-states”

Veigel et al. (1999)
Nature 398, 530-533
DUTY-CYCLE RATIO

Attached time

Detached time

Intermittent Motor e.g. myosin-2

Processive Motor e.g. myosin-5
“Processive” and “Intermittent” motors

• “Intermittent” motors must work in teams to produce large movements and forces.

• “Processive” motors can take many steps before detaching from their track and they can work alone as single molecules.
Myosin-5

Veigel & Molloy

Conventional kinesin

Carter & Cross
Myosin-5 takes 36nm steps
How does myosin-5 walk??.......
How does myosin-10 localise to the tip of the filopodium?

Can we observe single molecules inside a living cell?
Myosin-10 can be both an intermittent, single headed motor and a processive dimeric motor.

**Monomeric form:**

- 15 nm
- 2 nm
- SAH domain
- 3 light chains

**Dimeric form:**

Takagi, Y., et al. (2014) PNAS 111, E1833-E1842
Using TIRF microscopy we can see individual molecules of myosin-10 moving inside a living cell.

Lecture Overview:

- Optical Tweezers are relatively simple to build and are compatible with standard laboratory microscopes.

- They have a sensitivity and time-resolution suitable for studying biological macromolecules and cells.

- They have contributed to our understanding of the mechanism and function of molecular motors (like kinesin, dynein, and myosin) and also of DNA processing enzymes.

THE FUTURE………

- The advent of fast cameras, fast parallel processing, and more powerful lasers mean that time-resolution is now in the microsecond regime; and forces of ~100pN are possible - opening the possibility to study molecular dynamics and cellular mechanics.
THANK YOU FOR YOUR ATTENTION!