Mechanics:
from macromolecules to cells

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BP204
March 9 2015
Today’s goal


Molecules  Mechanics

structure, chemistry
What biological activities are associated with force? Examples...

- Protein/RNA folding
- Replication, transcription, translation
- Viral infection
- Cytoskeletal organization
- Intracellular transport
- Cell signaling and adhesion
- Muscle movement
- Tissue development and morphology

What biological activities are not associated with force?
What kinds of forces? (piconewtons)

1. Spring: \[ F = k \cdot x \] 
   \((k \text{ is spring constant})\)

2. Drag: \[ F = \gamma \cdot v \]
   \[= \gamma \cdot \frac{dx}{dt} \] 
   \((\gamma \text{ is drag coefficient})\)

3. Inertia: 
   \[ F = m \cdot a \] 
   \[= m \cdot \frac{d^2x}{dt^2} \] 
   \((m \text{ is mass})\)

\[
v(t) = v_0 e^{-t/\tau}, \quad \tau = m/\gamma \quad \rightarrow \quad 10^{-12} \text{ sec for ribosome}
\]

**Process** | **Force**
---|---
Single motors | Myosin 3-5 pN, Kinesin 5-7 pN, Dynein 7 pN
Cell regulation | Transcription 15 pN, Translation 1-20 pN, Replication 20-30 pN
Cells and tissue | Muscle contraction 1-1000 N, Blood pressure 0.01 pN/nm²

\[
\text{energy} = \text{force} \times \text{distance}
\]

\[
1 \, k_B T = 4 \, \text{pN-nm}
\]

\[
1 \, \text{ATP} = 80-100 \, \text{pN-nm at 37C}
\]

mass normally irrelevant
### What kinds of timescales?

<table>
<thead>
<tr>
<th>Types of processes</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elementary processes</strong></td>
<td></td>
</tr>
<tr>
<td>Electronic transition</td>
<td>$10^{-14}$ s</td>
</tr>
<tr>
<td>Bond vibration</td>
<td>$10^{-12}$ s</td>
</tr>
<tr>
<td>Conformational change</td>
<td>$10^{-9}$ to $10^{-6}$ s</td>
</tr>
<tr>
<td><strong>Intermediate processes</strong></td>
<td></td>
</tr>
<tr>
<td>Bimolecular dissociation</td>
<td>$10^{-6}$ to $10^{-3}$ s</td>
</tr>
<tr>
<td>Bond formation or breaking</td>
<td>$10^{-6}$ to $10^{-3}$ s</td>
</tr>
<tr>
<td><strong>Motor movements</strong></td>
<td></td>
</tr>
<tr>
<td>Kinesin stepping at Vmax</td>
<td>10 ms</td>
</tr>
<tr>
<td>Myosin stepping at Vmax</td>
<td>5 ms</td>
</tr>
<tr>
<td>Bacteriophage stepping rate</td>
<td>10 ms/bp</td>
</tr>
<tr>
<td><strong>Enzyme turnover</strong></td>
<td></td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>10 ms</td>
</tr>
<tr>
<td>ATP synthase</td>
<td>5 ms</td>
</tr>
<tr>
<td><strong>Gene expression</strong></td>
<td></td>
</tr>
<tr>
<td>Prokaryotic replication (E. coli)</td>
<td>2 ms/bp</td>
</tr>
<tr>
<td>Eukaryotic replication (X. laevis)</td>
<td>2 ms/bp</td>
</tr>
<tr>
<td>RNA transcription</td>
<td>40 ms/bp</td>
</tr>
<tr>
<td>Translation</td>
<td>50-100 ms/aa</td>
</tr>
</tbody>
</table>

Average out elementary processes can generally probe mechanics of molecules at ms to min range.
Outline

Measuring & exerting forces

Generating force (active forces)

Responding to force (passive forces)

Integrating forces (molecules to cells, self-organization)

Linear forces

Torque
Measuring and perturbing forces on single molecules

Goal: measure how forces affect the dynamics of molecules under force.
## Comparing different techniques

**Table 1** | Comparison of single-molecule force spectroscopy techniques

<table>
<thead>
<tr>
<th></th>
<th>Optical tweezers</th>
<th>Magnetic (electromagnetic) tweezers</th>
<th>AFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spatial resolution (nm)</td>
<td>0.1–2</td>
<td>5–10 (2–10)</td>
<td>0.5–1</td>
</tr>
<tr>
<td>Temporal resolution (s)</td>
<td>$10^{-4}$</td>
<td>$10^{-1}$–$10^{-2}$ (10$^{-4}$)</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>Stiffness (pN nm$^{-1}$)</td>
<td>0.005–1</td>
<td>$10^{-3}$–$10^{-6}$ (10$^{-4}$)</td>
<td>$10^{-10^{5}}$</td>
</tr>
<tr>
<td>Force range (pN)</td>
<td>0.1–100</td>
<td>$10^{-3}$–$10^{2}$ (0.01–10$^{-4}$)</td>
<td>$10^{10^{4}}$</td>
</tr>
<tr>
<td>Displacement range (nm)</td>
<td>0.1–10$^{5}$</td>
<td>5–10$^{-4}$ (5–10$^{5}$)</td>
<td>0.5–10$^{4}$</td>
</tr>
<tr>
<td>Probe size (μm)</td>
<td>0.25–5</td>
<td>0.5–5</td>
<td>100–250</td>
</tr>
<tr>
<td>Typical applications</td>
<td>3D manipulation Tethered assay Interaction assay</td>
<td>Tethered assay DNA topology (3D manipulation)</td>
<td>High-force pulling and interaction assays</td>
</tr>
<tr>
<td>Features</td>
<td>Low-noise and low-drift dumbbell geometry</td>
<td>Force clamp Bead rotation Specific interactions</td>
<td>High-resolution imaging</td>
</tr>
<tr>
<td>Limitations</td>
<td>Photodamage Sample heating Nonspecific</td>
<td>No manipulation (Force hysteresis)</td>
<td>Large high-stiffness probe Large minimal force Nonspecific</td>
</tr>
</tbody>
</table>
Why look at single molecules?

Distributions → Information!

- Mechanics:
  - force output, step sizes, energy efficiency
  - force-dependent kinetic step (coupling of ATP with force generation)
- Different reaction pathways
- Different folding & unfolding trajectories
Optical tweezers: what can we measure?

Examples of biomolecules probed with optical tweezers:
Optical tweezers: how do they work?

Microscope objective focuses laser light to diffraction-limited spot

Conservation of momentum

As force $\uparrow$, trapped bead moves linearly out of equilibrium position:

$$F = k \cdot x$$
Optical tweezers: how to measure (F=\(kx\)) force?

Measuring bead position \(x\)

\[
V_x = \frac{(B + D) - (A + C)}{A + B + C + D}
\]

Measuring trap stiffness \(k\)

\[
V_y = \frac{(A + B) - (C + D)}{A + B + C + D}
\]

\[
p(x) = \frac{1}{\sigma \sqrt{2\pi}} \exp\left(-\frac{x^2}{2\sigma^2}\right)
\]

\[
\sigma = \sqrt{\frac{k_B T}{k}}
\]

thermal fluctuations on spring →
Gaussian noise

fluctuations report
on trap stiffness \(k\)

Quadrant PhotoDiode (QPD)
Optical tweezers: what limits spatiotemporal resolution?

Signal-to-noise ratio (SNR) limited by thermal fluctuations (and experimental noise):

\[ SNR \leq \frac{k_{tether} \cdot \Delta x}{\sqrt{4 \cdot k_B \cdot T \cdot B \cdot \gamma}} \]

- \( k_{tether} \): stiffness of biomolecule
- \( \Delta x \): size of physical displacement (e.g. step size)
- \( B \): rate of data collection/averaging (lower limit imposed by speed of reaction!)
- \( \gamma \): drag coefficient of bead

Inverse relationship between spatial and temporal resolution
Magnetic tweezers: can make parallel measurements on a field of molecules ... and can measure and apply torque!

Need to know distance to measure force: measure distance with bead imaging

Dekker and coworkers
Biosystems with torque: single molecule torque measurements

Transcription:
- 
  - Supercoiling
  + Supercoiling
  RNA polymerase
  Topo type I
  Topo type II

Replication:
- 
  + Supercoiling
  RNA polymerase
  Topo type I
  Topo type II

ATP synthase:
- 
  ADP + P_i
  ATP
  H^+

Viscous drag

Optical tweezers + twisting pipette

Magnetic tweezers

Forth et al., Annual Reviews 2013
Calculate frictional torque:

\[(\pi/3)\omega \eta L^3/[\ln(L/2r) - 0.447]\]

Angular velocity (\(\omega\))
Medium viscosity (\(\eta\))
Filament length (\(L\)) and radius (\(r\))

\(F_1\)-ATPase: nearly 100% efficient motor that rotates with discrete 120° steps
Looking ahead:

- Combining mechanical measurements with other observables (fluorescence, etc.)
- Purified macromolecular complexes from cells
- Measurements inside cells

Comstock et al., Nat Meth 2011
Outline

Measuring & exerting forces

Generating force (active forces)

Responding to force (passive forces)

Integrating forces (molecules to cells, self-organization)

(energy consumed)

Translocation motor

Polymerization motor
Example #1: step size (kinesin, the original)

Direct observation of kinesin stepping by optical trapping interferometry

Karel Svoboda†, Christoph F. Schmidt†‡, Bruce J. Schnapp§ & Steven M. Block*†

* Rowland Institute for Science, 100 Edwin Land Boulevard, Cambridge, Massachusetts 02142, USA
† Committee on Biophysics, Harvard University, Cambridge, Massachusetts 02138, USA
‡ Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA

Do biological motors move with regular steps? To address this question, we constructed instrumentation with the spatial and temporal sensitivity to resolve movement on a molecular scale. We deposited silica beads carrying single molecules of the motor protein kinesin on microtubules using optical tweezers and analysed their motion under controlled loads by interferometry. We find that kinesin moves with 8-nm steps.

NATURE · VOL 365 · 21 OCTOBER 1993
Example #2: force-dependent enzymatic step

How is chemical potential converted to mechanical force?

\[ v = \frac{V_{\text{max}}[S]}{K_M + [S]} \]

**Substrate binding**

- Force affects \( V_{\text{max}}/K_M \),
- but not \( V_{\text{max}} \)

**Product release**

- Force affects \( V_{\text{max}} \),
- but not \( V_{\text{max}}/K_M \)

**Hydrolysis**

- Force affects both \( V_{\text{max}} \)
- and \( V_{\text{max}}/K_M \)
Example #2: force-dependent enzymatic step (viral packaging)

\[ v = \frac{V_{\text{max}}[ATP]}{K_M + [ATP]} \]

\( \varphi 29 \) viral packaging motor:

Capsid pressure:

Force affects \( V_{\text{max}} \)

but not \( V_{\text{max}}/K_M \):

force generation at product release

Minimal mechanochemical cycle proposed:

Chemla et al., Cell 2005
Example #3: force-dependent attachment

Purified yeast kinetochores:

Catch bond:
Detaches less under higher force!

Question: How do you know you’re holding on to a single molecule?

Akiyoshi et al., Nature 2010
Controlling the # and position of motors with DNA origami

Number of motors affects run length and time, but not velocity
Active force: polymerization motors

Dynamic polymers can move objects, without needing translocation motors

Dogterom et al., Current Opinion Cell Biology 2005
Polymerization motors: microtubule example

What’s coupling cellular objects to depolymerizing microtubules?

≈ 0.5pN
≈ 5pN if consider lever arm
≈ 30-65pN if consider all protofilaments

Grishchuk et al., Nature 2005
Generating force
(active forces)

Responding to force
(passive forces)

Integrating forces
(molecules to cells, self-organization)

Molecular friction

Elasticity, folding/unfolding

Measuring & exerting forces

(no energy consumed)
Polymer elasticity at different scales

- **Short length scale**: deform elastic beam

\[ \langle \cos \theta \rangle = \exp\left( -\frac{L}{P} \right) \]

\[ P = \frac{\pi \cdot E \cdot a^4}{4 \cdot k_B \cdot T} \]

- \( L \) = distance
- \( P \) = Persistence length
- \( a \) = radius
- \( E \) = Young’s modulus

(1 GPa for most proteins = plexiglass)

\[ a \approx 2 \text{ nm for actin} \Rightarrow P \approx 3 \mu\text{m (unbundled)} \]

\[ a \approx 10 \text{ nm for microtubules} \Rightarrow P \approx 2 \text{ mm} \]

(compared to \( P \approx 50 \text{ nm for DNA!} \))

Reasonable agreement with experiment

Gittes et al. JCB 1993

- **Longer length scale**: extend random coil to overcome entropy (next slide)
Why do we care about the elasticity biomolecules? For DNA, for example ...

- Predicting DNA conformations *in vivo*
- Thermodynamics of protein:DNA interactions with DNA distortion
- Quantitative design when using DNA as a building material
- DNA as a model system to test polymer physics theories
Passive force: folding and unfolding a RNA hairpin

Can get:
- Position of mechanical barriers
- Position of transition state ($\Delta x^*$): small = brittle, large = compliant
- $\Delta G$ if equilibrium pulling
- Dependence on sequence, Mg$^{2+}$ buffer, etc.

Liphardt et al., Science 2002
Effect of force on chemical reactions

**Thermodynamics:**

\[ \Delta G = \Delta G^0 - F\Delta x \]

\[ K_{eq}(F) = \frac{k_{forward}(F)}{k_{reverse}(F)} = \frac{k_{forward}^0}{k_{reverse}^0} e^{(F\Delta x)/k_BT} \]

\[ K_{eq}(F) = K_{eq}^0 e^{(F\Delta x)/k_BT} \]

**Kinetics:**

\[ k_i = A_i e^{-\Delta G^{0*}/k_BT} \]

\[ k_{forward} = A_{forward} e^{-(\Delta G^{0*} - F\Delta x_{forward})/k_BT} \]

\[ k_{reverse} = A_{reverse} e^{-(\Delta G^{0*} - F\Delta x_{reverse})/k_BT} \]

\[ k_{forward} = k_{forward}^0 e^{F\Delta x_{forward}/k_BT} \]

\[ k_{reverse} = k_{reverse}^0 e^{F\Delta x_{reverse}/k_BT} \]

Force has a larger effect on reactions with large \( \Delta x \)'s (and \( \Delta x^* \)) \rightarrow discussion paper #1!
Funny things you see at small energy scales

Jarzynski’s equality:
2nd law of thermodynamics in small systems

$$\frac{-\Delta G}{e^{\frac{W_i}{k_B T}}} = <e^{\frac{-W_i}{k_B T}}>_N \rightarrow \infty$$

Equilibrium info  Non-equilibrium data

The secret: left tail!

Liphardt et al., Science 2001
Passive force: secondary and tertiary structure of complex RNA

*T. thermophila* ribozyme self-splicing intron:

How to assign mechanical “rips” to molecular features?
- truncation mutants
- positional mutants
- oligos to prevent postulated interactions

### Secondary and tertiary structure assignment

<table>
<thead>
<tr>
<th>Barrier</th>
<th>Length of rip (nt)</th>
<th>Mean rip force (pN)</th>
<th>Domain unfolded</th>
<th>Length of domain (nt)</th>
<th>Nature of barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>38</td>
<td>12 ± 2</td>
<td>P9.2</td>
<td>39</td>
<td>Base of hairpin</td>
</tr>
<tr>
<td>b*</td>
<td>25</td>
<td>18 ± 4</td>
<td>P9.1</td>
<td>22</td>
<td>G-C rich hairpin</td>
</tr>
<tr>
<td>c*</td>
<td>35</td>
<td>21 ± 4</td>
<td>P9.1a &amp; P9</td>
<td>14 + 19</td>
<td>P9.1a/P2.1 kissing loop (P13) P9/P5 contact (T4)</td>
</tr>
<tr>
<td>d</td>
<td>60</td>
<td>19 ± 5</td>
<td>P2 &amp; P2.1</td>
<td>27 + 36</td>
<td>P2/P5c kissing loop (P14) P2.1/P3 contact (T5)</td>
</tr>
<tr>
<td>e</td>
<td>60</td>
<td>21 ± 3</td>
<td>P3-P7-P8</td>
<td>65</td>
<td>Multiple catalytic core contacts (Tcc)</td>
</tr>
<tr>
<td>f</td>
<td>50</td>
<td>16 ± 2</td>
<td>P4-P6</td>
<td>50</td>
<td>P5b tetraloop/P6 tetraloop receptor (T3)</td>
</tr>
<tr>
<td>g</td>
<td>35</td>
<td>15 ± 2</td>
<td>P4-P5</td>
<td>33</td>
<td>P5a A-rich bulge/P4 (T2)</td>
</tr>
<tr>
<td>h</td>
<td>73</td>
<td>19 ± 2</td>
<td>P5abc</td>
<td>71</td>
<td>P5a A-rich bulge/P5c (T1)</td>
</tr>
</tbody>
</table>
Passive force: molecular friction
(discussion paper #1)

Microtubule-binding protein called NuMA: lower friction to minus-ends than plus-ends!

Forth et al., Cell 2014
Generating force (active forces)

Responding to force (passive forces)

Measuring & exerting forces

Integrating forces (molecules to cells, self-organization)
Connecting mechanics across scales: an example

<table>
<thead>
<tr>
<th>molecular</th>
<th>macromolecular</th>
<th>cellular</th>
<th>organismal</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP → ADP + Pi</td>
<td>$\rightarrow$ F</td>
<td>microtubule</td>
<td>tissue</td>
</tr>
<tr>
<td>enzyme</td>
<td>kinetochore</td>
<td>spindle</td>
<td></td>
</tr>
<tr>
<td>1 nm</td>
<td>20 nm</td>
<td>4 μm</td>
<td>20 μm</td>
</tr>
</tbody>
</table>

Need tools to:
① measure ...
② perturb ...
③ control ..
... forces in cellular context
An intermediate: reconstituted networks

Actin networks stiffen then soften under load:

\[ \sigma = 0 \quad \sigma < \sigma_c \quad \sigma \geq \sigma_c \quad \sigma > \sigma_c \]

Stiffening

Softening

Chaudhuri et al., Nature 2007
Feedback between motor activity and network architecture

Use micropatterning to assemble geometrically controlled and polarized actin networks:

Myosin VI contracts antiparallel, but not parallel, actin network

Motor activity

Network architecture

Reymann et al., Science 2012
Cell mechanics: solid & liquid

How do molecular properties give rise to these cell-scale properties?

Quantities involved in mechanics measurements

- Change in length, $\Delta L$
- After time, $\Delta \tau$
- Stress: $\sigma = \frac{F}{A}$
- Strain: $\gamma = \frac{\Delta L}{L_0}$
- Strain rate: $\dot{\gamma} = \frac{\Delta \gamma}{\Delta \tau}$

Elastic response

- $\sigma = G' \gamma$

Viscous response

- $\sigma = [G''(\Delta \tau) \Delta \tau] \dot{\gamma}$

Solid-like ($\approx$ stiffness)

Liquid-like ($\approx$ viscosity)
Physical methods to probe mechanical properties of cells

Summary: Poke and/or track!

**Bulk rheology**
A material is sheared between two plates using an oscillatory stress to probe the shear elastic, $G'$, (in-phase) and viscous, $G''$, (out-of-phase) moduli.

**Magnetic bead cytometry**
An external magnetic field applies a stress to a magnetic bead. The bead is position tracked to determine the response.

**Traction force microscopy**
Cell contractions deform a flexible substrate. Forces are estimated from bead displacements.

**Atomic force microscopy**
A cantilever applies stress to the cell. The cantilever deflection is measured by laser reflection.

**Microrheology**
The motion of probe particles is measured using either video or laser tracking techniques. Particle motion is either driven externally or thermally induced and is interpreted to yield the viscoelastic modulus.

**Whole cell stretching**
A cell is attached to two surfaces. A force is applied to one surface and the plate separation is measured.
Measuring molecular forces inside cells: FRET sensor

FRET-based force sensor: molecular measurements inside cells

- **In vivo FRET sensor**
- **In vitro force-FRET calibration**

---

Grashoff et al., Nature 2010
Measuring tension across vinculin reveals regulation of focal adhesion dynamics

Forces in new focal adhesions are higher than at old focal adhesions (probably)

Grashoff et al., Nature 2010

Where to insert FRET sensor?

How to interpret FRET sensor data?
Forces required outside cells to activate signaling: “tension gauge tether”

Other conclusions:
① Formation of focal adhesions requires a higher tension tolerance than adhesion
② Notch signaling does not have a detectable minimum tension tolerance

Adhesion via integrin has a sharp threshold (33-43 pN)
Mechanotransduction: beyond measuring forces

Fuel (ATP, GTP)

Mechanotransduction

Enzyme-substrate displacement
Unfolding of protein
New binding site exposed

Mechanical signals:
- fast signal propagation
- orientation specific signal
- global info from local cues
Emergent mechanics of biological structures

How to integrate forces over length scales and time scales?

Hierarchical thread ...
From macro world mechanics to emergent mechanics of biological structures

**Biological structures:**

1. Material properties of individual building blocks (e.g. titin stiffness → muscle stiffness)
2. Basic architecture: orientation of blocks, etc. (e.g. microtubule orientation in spindle)
3. Density of building blocks and crosslinks (e.g. cytoskeletal networks)
4. Shape of structure and internal geometry (e.g. shape of tissue → where cells proliferate)
5. Affinity of interactions (e.g. cell-cell junction affinity → tissue integrity)

**Genentech Hall:**

1. Elastic moduli of materials
2. Support beam orientations
3. Support beam densities
4. Anchor point geometrical distributions
5. Anchor point strengths

What mechanics emerge? Why is this hard?
**Self-assembly:** Order arises from local interactions between “passive” parts. No energy is consumed, and structures typically reach a steady-state.

**Self-organization:** Order arises from self-driven “active” parts that consume energy (from metabolism), bringing biological processes out of equilibrium.

Biological structures:
1. Change their own structure over time
2. Building blocks come on and off
3. Building blocks actively generate force (i.e. consume energy)

Emergent structures:
Tornados and sand dunes exhibit properties that their smallest parts do not, arising from collective behavior of parts.

Biological emergent structures can be even more complex: spatially heterogeneous, and made from great diversity of parts and interactions (e.g. cytoskeleton, active membranes, repairing tissue).
Emergent mechanics: ways forward

How do forces generated by individual molecules affect larger scale structures?

How do stresses across the whole structure flow through individual molecules?

Need to be able to externally **control** and **tune**

1. architecture
2. on-and-off kinetics
3. active force-generation ability of the structure’s building blocks in real-time!
Design of cytoskeletal motors that reversibly change gears (speed up, slow down, change direction) when exposed to blue light ...
Looking forward

- Measuring & exerting forces
- Generating force (active forces)
- Responding to force (passive forces)
- Integrating forces (molecules to cells, self-organization)

Challenges:
1. How to measure and perturb forces inside cells?
2. How do mechanics of cellular structures emerge from those of molecules?
Asymmetric Friction of Nonmotor MAPs Can Lead to Their Directional Motion in Active Microtubule Networks

What is the basis of friction asymmetry? How could it be tuned? Implications on cellular structures?

Remote control of myosin and kinesin motors using light-activated gearshifting

What do these motors test about our understanding? What would you do with these motors? How to control other motors?