Movement at the Molecular Level

Diffusion: \(<r^2> = 6 D t\) (\(D \approx 6 \pi \mu a\))

Typical numbers:

10 nm protein in water \(D = 10^{-10} \text{ m}^2/\text{s}\)

....in cells \(D = 10^{-12} \text{ m}^2/\text{s}\) (\(D = 10^{-14} \text{ m}^2/\text{s}\) lipids)

\([<r^2>]^{1/2} = 1 \mu\text{m}, t \sim 0.2 \text{ sec in cells}\)

\([<r^2>]^{1/2} = 10 \mu\text{m}, t \sim 20 \text{ sec in cells}\)

Slow and isotropic.

How to generate fast vectorial motion?

Axonal transport of organelles in giant squids
Directed (Vectorial) Molecular Movement

Polymerization:
*Living* polymerization of actin/microtubules

Springs:
Conformational changes of molecules

Motor Proteins:
nucleotide (ATP) hydrolysis: chemical energy -> work

Pumps:
Hydrolysis of ATP
Create concentration gradients
Listeria monocytogenes moving in PtK2 cells

These pathogenic bacteria grow directly in the host cell cytoplasm. The phase-dense streaks behind the bacteria are the actin-rich comet tails. Actin-based motility is also used in cellular motility; this cell is using its cytoskeleton to crawl toward the lower right-hand corner. Speeded up 150X over real time.

--Julie Theriot & Dan Portnoy
Actin is Transiently Tethered to the Bacteria

Images removed due to copyright considerations.

Noireaux et al. (2000): it takes about 10 picoN to separate the actin from the comet...
Images removed due to copyright considerations.
Elastic Brownian Ratchets and Tethered Filaments

Images removed due to copyright considerations.
See Mogilner, A. and G. Oster.

Brownian:
Actin filament tips fluctuate

Some filaments are tethered
Actin Ruffles in Motile Cells
Supramolecular Springs

Energy stored in chemical bonds which act as “latches’

Regulated by Spasmin:
Calcium binding protein

Images removed due to copyright considerations.
See Mahadevan, L. and P. Matsudaira.
"Motility powered by supramolecular springs and ratchets."
Horseshoe Crab Sperm

Uncoiling of an actin spring

(unlike the echinoderm sperm - no polymerization!)

Images removed due to copyright considerations.
Molecular Motors

- Molecules that convert chemical energy into mechanical force

- Motors are specialized for specific tasks:
  - cell division
  - cell movement
  - organelle transport
  - synthesis of ATP

- Most move **unidirectionally** along polymer filaments

- Coupled **mechanical and chemical cycles** (fuel)
## Motor Types

<table>
<thead>
<tr>
<th>Motor</th>
<th>Track</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin</td>
<td>F-actin</td>
<td>Cell crawling</td>
</tr>
<tr>
<td>Myosin II</td>
<td>F-actin</td>
<td>Muscle contraction</td>
</tr>
<tr>
<td>Kinesin</td>
<td>microtubule</td>
<td>Cell division</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phagocytosis</td>
</tr>
<tr>
<td>Dynein</td>
<td>microtubule</td>
<td>Organelle transport</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitosis &amp; meiosis</td>
</tr>
<tr>
<td>Polymerases,</td>
<td>ds and ssDNA</td>
<td>Flagella &amp; cilia</td>
</tr>
<tr>
<td>Helicases</td>
<td></td>
<td>Replication, Repair</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recombination</td>
</tr>
</tbody>
</table>
Muscle Anatomy

Muscle Types:
- Skeletal: fast
- Cardiac: fast
- Smooth: slow
Myosin Heads Walk Along Actin Filaments
**Polar Biopolymer Molecules**

The Tracks Motor Proteins Walk Along

Myosin walks along Actin

G-Actin (globular) F-Actin (microfilaments)

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-2.

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**Properties:**

- **Diameter:** 6-8 nm
- **Persistence length:** 16 µm
- **Young’s modulus:** $1.3-2.5 \times 10^9$ Pa
**Myosin: the actin motor protein**

All myosins have head, neck, and tail domains with distinct functions

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-20.
Viewable online at the PubMed Bookshelf:

Figure 18-20
Myosin Types

Conventional Type II

- muscle contraction
- cytokinesis
- cell adhesion, migration

Unconventional Types I, III-…

- type I: cell migration

We will concentrate on type II, but other types display similar mechanisms…
Skeletal muscle contains a regular array of actin and myosin II: the sarcomere

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-27.
Viewable online (Fig. 18-27b only) at the PubMed Bookshelf:
Skeletal muscle contains a regular array of actin and myosin

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-27c.
Capping proteins stabilize the ends of actin thin filaments in the sarcomere

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-28.
Viewable online at the PubMed Bookshelf:
Thick and thin filaments slide past one another during contraction

During contraction the myosin head initially binds tightly the thin filament (actin) to form a cross-bridge.

Once in contact the head rapidly bends towards the center of the sarcomere during the power stroke.

The thin filament is then displaced towards the center of the sarcomere by about 10 nm.

The head releases from the thin filament, reverts back to the initial conformation and the cycle repeats.

The heads are only in contact with the filament about 5% of the time.

Continuous movement because several heads are marching along the filament.
Muscle Contraction
Conformational changes in the myosin head couple ATP hydrolysis to movement

1. **Nucleotide binding**
   - Myosin head
   - Actin
   - ATP
   - Head dissociates from filament

2. **Hydrolysis**
   - ATP
   - ADP + P_i
   - Head pivots and binds to a new actin subunit

3. **P_i release**
   - P_i
   - Head pivots and moves filament (power stroke)

4. **ADP release**
   - ADP
Sliding Filament Model
Huxley & Huxley 1954

The Myosin ‘Power Stroke’

pre-stroke

Small conformational change
In head is amplified by swinging
movement of the neck.

Light chains increase
rigidity of the neck.

post- stroke

Images removed due to copyright considerations.
See Figures 4 and 6 in Geeves and Holmes.
"Structural mechanism of muscle contraction.”
ATP: Cellular Fuel

ATP Hydrolysis:

\[ \text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i \quad K_{eq} = 4.9 \times 10^5 \]

\text{Depends on conditions}

\text{Strongly favored}

- Large activation barrier w/o a catalyst = stable fuel
- Free energy change at cellular conditions: -25 kT
Mechanochemical Coupling: Myosin II

Motor and no actin: low activity \( \sim 0.1 \text{ s}^{-1} \)

\[
\begin{align*}
M & \leftrightarrow MT \leftrightarrow MDP \leftrightarrow MD + P \\
\text{Rate limiting step}
\end{align*}
\]

Motor and with actin: increased activity \( \sim 25 \text{ s}^{-1} \)

\[
\begin{align*}
MT & \leftrightarrow MDP \leftrightarrow AMDP \rightarrow AMD \leftrightarrow AM \leftrightarrow AMT \leftrightarrow MT
\end{align*}
\]

Key ideas:
- Release of P (chemical) is catalyzed by binding to actin (mechanical)
- w/o ATP myosin bonds strongly to actin
- Release of myosin (mechanical) is catalyzed by ATP binding (chemical)
The actin-myosin ATPase cycle

Diffusion to/ from filament
ATP hydrolysis

Myosin S1
binds to filament

Pi acts as spring “latch”
ADP-Pi bound
pre-stroke
I = 10nm
ADP-bound, Pi released

“Rigor” state
No ATP available – remains attached
Actin filament

Cycle ~ 50ms

ATP hydrolysis induces a recovery stroke.
Allostery: release of P after MDP binds to actin creates a highly strained AMD state that relaxes via a change of configuration and may generate a power-stroke.

Mechanochemical Coupling: Myosin Power-Stroke
Tropomyosin and troponin regulate contraction in skeletal muscle

Ca$^{2+}$ influences the position of TP & TN on the actin filament.

Image removed due to copyright considerations.

Binding sites: closed    open
Increasing the Working Stroke Distance in Myosin

Image removed due to copyright considerations.
Diagram with caption Figure 4.

Processive

J. Howard 1997
Assays to Study Motor Proteins *in vitro*

Image removed due to copyright considerations.
Polar Biopolymer Molecules: Microtubules
The Tracks Motor Proteins Walk Along

Image removed due to copyright considerations.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>24 nm</td>
</tr>
<tr>
<td>Persistence length</td>
<td>60,000 µm</td>
</tr>
<tr>
<td>Young’s modulus</td>
<td>$1.9 \times 10^9$ Pa</td>
</tr>
</tbody>
</table>
The Role of Kinesin & Dyneins

Image removed due to copyright considerations.

MTOC: Microtubule-organizing center

**Bidirectional transport of organelles**
# Structure of Motor Proteins

<table>
<thead>
<tr>
<th>Myosin</th>
<th>Kinesin</th>
</tr>
</thead>
</table>

The head contains ATP and filament binding sites.

Image removed due to copyright considerations. See [Lodish].
Mechanochemical Coupling: Conventional Kinesin

Key Points:

- with ATP kinesin is bound tightly to microtubules

- unbinding of kinesin from microtubule requires hydrolysis of ATP

- in the absence of microtubule kinesin dissociates P quickly

- release of ADP from kinesin is catalyzed by binding to microtubule

- 2 heads coordinate movement
Hand-over-Hand Model for Conventional Kinesin

Image removed due to copyright considerations.

http://www.current-opinion.com/jcel/mov1.mov
Release of trailing head (K1) is contingent on the binding of the leading head (K2)...

...binding of ATP to K1 catalyzes attachment of K2 to microtubule...

...this catalyzes release of ADP from K2...

...which catalyzes detachment of K1 from the microtubule...

...release of P from K1...

Schief & Howard 2001
Motor Proteins: Power Strokes

Image removed due to copyright considerations.
Common Themes

• Filaments are polar and motor binding is stereospecific.

• This leads to movement in one direction (+ or -).

• Stall forces ~ few (6-10) pN
• Cyclic motors

• Nucleotides roles:

  1. regulates attachment/detachment.

  2. drives working/recovery strokes.

  3. chemical steps are contingent on the completion of mechanical steps.
### Differences

<table>
<thead>
<tr>
<th>Myosin II</th>
<th>Conv. Kinesin</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-processive</td>
<td>processive (~100 steps of more)</td>
</tr>
<tr>
<td>5-15 nm step sizes</td>
<td>8 nm</td>
</tr>
<tr>
<td>slips</td>
<td>no slipping</td>
</tr>
<tr>
<td>walks towards + end of filament</td>
<td>walks towards -end of filament</td>
</tr>
<tr>
<td>found in large assemblies</td>
<td>works well alone or low #'s</td>
</tr>
<tr>
<td>‘rower’</td>
<td>porter</td>
</tr>
</tbody>
</table>
## Motor Speeds (assemblies)

<table>
<thead>
<tr>
<th>Motor type</th>
<th>speed in vivo (nm/s)</th>
<th>in vitro (nm/s)</th>
<th>in vitro ATPase (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin II (skeletal muscle)</td>
<td>6000</td>
<td>8000</td>
<td>20</td>
</tr>
<tr>
<td>Myosin II (smooth muscle)</td>
<td>200</td>
<td>250</td>
<td>1.2</td>
</tr>
<tr>
<td>Myosin V (vesicle transport)</td>
<td>200</td>
<td>350</td>
<td>5</td>
</tr>
<tr>
<td>Conv. Kinesin (axonal transp)</td>
<td>1800</td>
<td>840</td>
<td>44</td>
</tr>
<tr>
<td>Nkin (sec. Vesicle transp.)</td>
<td>800</td>
<td>1800</td>
<td>78</td>
</tr>
<tr>
<td>BimC/Eg5 (Mitosis/meiosis)</td>
<td>18</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>Dyneins (cytoplasmic)</td>
<td>-1100</td>
<td>-1250</td>
<td>2</td>
</tr>
</tbody>
</table>

**Speed in vivo** = cell/extracts, motion of motor relative to filament w/o a load. Positive values indicate movement toward positive end of filament.

**Speed in vitro** = purified motors at high ATP concentrations.

**ATPase** = max rate of hydrolysis per head per sec, measured at high ATP, filament concentrations.
Kinesin is attached during its rate limiting step while myosin is detached during its rate limiting step - ATP hydrolysis.

While one myosin is dissociated from the actin the others can continue pushing (rowing)!

\[ \delta \sim 8 \text{ nm} \]

\[ \Delta = 8 \text{ nm} \]

\[ \delta \sim 5 \text{ nm} \]

\[ \Delta = 36 \text{ nm} \]

in skeletal muscle
Mechanical Models for Motor Proteins

Myosin

Kinesin
Common Themes for Molecular Motors

• ATP hydrolysis drives the motors.

• The motors walk along polar tracks (polymers).

• Type of motor fits the function: processive/non.