Molecular motors

[Diagram of molecular motors]

[Diagram of myosin V structure]

[Diagram of free energy change and reaction coordinate]

[Diagram of translocating polymer and binding proteins]

[Diagram of molecular motor components: Axle and Stator]
**Molecular motors**

**Molecular** motors move in fits and starts, in contrast to **macroscopic** ones that appear to move continuously forward.

Ultimately, they are **stochastic**, due (as usual) to the large effect of thermal forces at molecular scales.

Biased random walkers, they use energy to **rectify** brownian motion (*driven diffusion*).

“two steps forward, one step back”
Four broad classes

Translational (ex. myosin)

Rotary (ex. ATP synthase)

Polymerization (ex. actin)  
“pushing by growing”

Translocation (ex. BiP)  
“pushing by pulling”
Translational motors

myosins move along actin polymers

kinesins and dyneins move along microtubules

all function by taking discrete steps of fixed size, coupled to ATP hydrolysis
Translational motors: dynein, kinesin

Kinesins walk toward the **plus end**, i.e., from cell center to periphery. Dyneins walk toward the **minus end**, i.e., toward the cell’s center.

Kinesins transport large cargos, e.g., vesicles, organelles along microtubules, also involved in cell division.

Kinesins are structurally similar to myosins, dyneins not.

Most kinesins walk toward **plus end**, i.e., from cell center to periphery. Dyneins walk toward the **minus end**, i.e., toward the cell’s center.
translational motor proteins moving along tracks in the cell

further evidence that motor proteins = attractive covers

Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5-nm Localization
Ahmet Yildiz, Joseph N. Forkey, Sean A. McKinney, Taekjip Ha, Yale E. Goldman, and Paul R. Selvin
how myosin moves

black dot is fluorophore label

two models for how myosin moves along actin, **hand-over-hand** or **inchworm**

let’s predict what they see…
how myosin moves

black dot is fluorophore label

two models for how myosin moves along actin, **hand-over-hand** or **inchworm**

let's predict what they see…

hand-over-hand predicts **pairs** of jumps that **sum** to 74 nm

inchworm model predicts **separate** jumps of 37 nm only

**What do the experiments show?**
how myosin moves

myosin V moves hand-over-hand!
analyzing the experiments

the actual data from the myosin V experiments

**FIONA** - Fluorescent Imaging with One-Nanometer Accuracy

fit image with a gaussian (point-spread function)

with enough photons, accuracy is **unlimited** (assuming the distribution is correct)

even for "**real**" experiments, everything is rooted in **modeling**!!!
analyzing the experiments

High-speed AFM experiments - do you see the myosin?

myosin motion involves distinct states

- Head is in rigor state (no ATP or products), bound to actin.

ATP binds, head releases and bends forward.

- ATP is hydrolyzed.

- Head contacts actin again.

ADP is released.

- Release of phosphate triggers "power stroke", head moves to original position.
How many distinct states?

Each step is itself an independent stochastic process that takes an average time $\tau$ to occur, e.g., ATP binding, ADP unbinding, etc.

For a single rate-limiting step:

$$p(t) = \frac{1}{\langle t \rangle} e^{-t/\langle t \rangle}$$

For two steps, $p(t)$ is the convolution of two independent probabilities

$$p(t) = \int_0^t p_A(\tau)p_B(t - \tau) d\tau$$
How many distinct states?

\[ p(t) = \frac{1}{\tau_B - \tau_A} \left( e^{-t/\tau_B} - e^{-t/\tau_A} \right) \]

Shape of curve and resulting fit can reveal the number of intermediate states, even if hidden from direct observation.
hidden Markov models (HMM)

An HMM is a sequence of hidden states and corresponding probabilities underlying a connected sequence of observations. The goal is to solve for the most likely hidden states that explain the observed sequence.

**Ex:** Myosin V stepping, don’t know dwell times, have only trace of position vs. time. Build a model parameterized by (1) $n$ kinetic states (hidden) and (2) $m$ step sizes. The HMM solution reveals two discrete step sizes, allows reconstruction of noise-less trace with dwell times (red).

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**muscle contraction**

(A) **myosin II hexamer**

- Coiled coil of two α-helices
- 2 nm
- C-terminus
- 150 nm

(C) **filament of left- and right-pointing myosins**

- N-terminus
- Light chains
- Neck region
- Myosin heads
muscle contraction

mediated by three proteins: myosin + actin (both acting as filaments) and titin (spring)
Ca\textsuperscript{2+} ions (the **signal**) cause tropomyosin to release from actin, exposing myosin-binding sites simultaneously!

Each myosin moves 11 nm at a rate of \(~5/s\) (55 nm/s), action of hundreds simultaneously increases rate to 8,000 nm/s

**coordinating the signal**

nerve cell releases achetylcholine-containing synaptic vesicles, causes Ca\textsuperscript{2+} channels to open
ATP is made by two coupled rotary motors known as ATP synthase, likely arose through modular evolution.

One motor, $F_0$, sits in the membrane and is driven by a proton gradient (similar to flagellar motor).

The other motor, $F_1$, is in the cytoplasm and makes (or uses!) ATP (similar to DNA helicase).

The two motors are coupled by an axle (rotates) and a stator (stationary).

Each motor is reversible, one can drive the other!
**Fo rotary motor**

**Stator** holds **rotor** in place until proton/ion enters from one side through salt bridge (+/- residues).

Proton/ion neutralizes charge, rotor then can move freely after which proton/ion leaves to other side.

Rotation is thermally driven but **rectified** by proton transport across membrane, clockwise/ccw determined by which side proton comes from.

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*Figure 16.42  Physical Biology of the Cell, 2ed. (© Garland Science 2013)*
First direct evidence $F_1$ rotates unidirectionally in discrete (3x) steps.
**F**₁** rotary motor**

catalytic cycle just like a Mazda rotary combustion engine (convergent evolution???)
difference is ATP synthase is almost 100% efficient!

ATP synthase by the numbers

phosphate (x3)

adenine

ribose

cleavage site

ATP + H₂O → ADP + Pᵢ

~ 14 kcal/mol in the cell (23-24 kT)

typical membrane potential ~ 100 mV
energy gained by moving 1 ion/proton: 0.1 eV = 4 kT
actual amount is 7 kT, due to chemical (in addition to electrical) potential

Each step of the turn (120 degrees) generates 1 ATP from the numbers above, need at least 3-4 protons to get 1 ATP and indeed, this is the case (Fₒ has ~12 subunits)
ATP synthase is practically 100% efficient!!!
Polymerization motors

Addition of monomer to filament is energetically favorable even the tracks on which translational motors move are motors themselves (e.g., actin, microtubules)

Addition of monomer to filament is energetically favorable

\[ \Delta G = kT \ln \frac{m}{m^*} \quad m^* = \frac{\Omega}{V} e^{-\beta \Delta \epsilon} \]

\( m \) is monomer concentration, \( m^* = K_d \) is concentration at equilibrium

This free energy can be harnessed to do useful mechanical work
Polymerization motors

random fluctuations of barrier or filament can allow a monomer to come in and bind

the force exerted by the barrier alters the off and on rates

\[ F_{\text{max}} = \frac{kT}{\delta} \ln \frac{m}{m^*} \]

estimates for single actin polymer around 5-7 pN

many cells move through actin polymerization forces!

keratocyte (from Julie Theriot lab)
Translocation motors

directed motion generated by ratcheting mechanism, used for, e.g., protein import/export across membranes

binding of proteins on one side prevents backsliding, energy is utilized to induce unbinding

alternatively, energy is used directly to pull protein through, tight hold in channel prevents backsliding

Figure 16.17 Physical Biology of the Cell, 2ed. © Garland Science 2013
through diffusion alone, polymer would spontaneously end up on other side in time $t_{\text{diff}}$.

\[ t_{\text{diff.}} = \frac{L^2}{D} = \frac{(nd)^2}{D} \]

with binding to prevent backsliding, time is reduced to $t_{\text{trans}}$.

\[ t_{\text{trans.}} = n \left( \frac{d^2}{D} \right) \]

\[ \frac{t_{\text{trans.}}}{t_{\text{diff.}}} = \frac{1}{n} \]