Lecture 7: Molecular Motors

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Molecular Motors

Energy Sources:
Protonmotive Force, ATP

Single Molecule Biophysical Techniques:
Optical Tweezers, Atomic Force Microscopy, Single Molecule Fluorescence Microscopy

Physical Models:
Brownian Ratchets, Smoluchowski Equation
Molecular Motors

Molecular motors are proteins that are able to convert energy stored in ATP or ion gradients into mechanical work

- **Cytoskeletal motor proteins**: myosin, kinesin, dynein
- **Polymerization motors**: actin, microtubules, RecA
- **Ion pumps**: Na-K pump
- **Rotary motors**: ATP-synthase, bacterial flagellar motor
- **DNA motors**: RNA polymerase, helicases
Molecular Motors Functions

- Contraction of stress fibres (actin - myosin)
- Muscle contraction (actin - myosin)
- Cell motility (actin - myosin)
- Cell division (actin - myosin)
- Separation of chromosomes (MT - dynein)
- Transport of cargo (MT - kinesin/dynein)

Bacterial chemotaxis (flagellar motor)

Energy storage in the form of ATP (ATP synthase motor)
Motor Protein Structures

Myosin: moves towards +end of actin

Kinesin: moves towards +end of microtubule

Dynein: moves towards -end of microtubule

100 nm
Molecular Motors in action

“The Inner Life of a Cell” by Cellular Visions and Harvard
MECHANISM OF MUSCLE CONTRACTION

Myosin binding site

Actin
Molecular Motors

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Energy Flow In Living Systems

Life processes including:

- Reproduction
- Growth
- Transport
- Movement

Food e.g. glucose

Photons

NADH pmf ATP

Movement

Transport

Reproduction

Growth

Life processes including:
Protonmotive Force

\[ \text{pmf} = V_m + \frac{\Delta \mu}{e} = V_m + \frac{k_B T}{e} \ln \left( \frac{c_i}{c_o} \right) \]

pmf is the extra free energy per unit charge inside the cell versus outside
- Sign convention inside minus outside
- Units of volts
- \( V_m \) is the electrical potential, \( \Delta \mu \) is the chemical potential
- Typically pmf is in the range -150 mV to -200 mV
- Usually both components negative: inside has lower voltage and lower \([H^+]\)
- Powers FLAGELLAR MOTOR and ATP SYNTHASE
Hydrolysis of ATP: \( \Delta G = -20 \, k_B T \) to \(-30 \, k_B T \)

**Hydration free energy** is larger for the products. In part due to entropy, in part due to better hydrogen bonding and electrostatic screening.

**Charge repulsion** between phosphate groups

**Resonance**: partially delocalized electrons free to move between oxygens. The shared oxygen bond reduces the number of arrangements of electrons (entropy) compared to hydrolysis products
**Glucose**

Food e.g. glucose → NADH → ATP

Life processes including:
- Reproduction
- Growth
- Transport
- Movement

1 glucose → 38 ATP

8 ATP → ~30 ATP

10 NADH plus other high-energy molecules → ~100 H+ (pmf)

<table>
<thead>
<tr>
<th></th>
<th>ΔG(each)</th>
<th>ΔG(total)</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>1160</td>
<td>1160</td>
</tr>
<tr>
<td>NADH</td>
<td>90</td>
<td>900</td>
</tr>
<tr>
<td>H+(pmf)</td>
<td>8</td>
<td>800</td>
</tr>
<tr>
<td>ATP</td>
<td>25</td>
<td>950</td>
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Actin-Myosin Gliding Assay (In Vitro)

➢ Myosin Gliding Motility Assay (in-vitro)
➢ Individual motors attached to surface/Actin Fluorescently Labeled
➢ Addition of ATP induces gliding (30s interval)
Optical Tweezers

- 10s – 100s pN
- Can be used to control beads specifically attached to proteins and cells
- Infrared light (low absorbance, cheap lasers)
- Acousto-optic deflectors enable beam
- Multiple traps made possible by time sharing
- Micro-Tetris with 1um glass beads
Two Bead Actin-Myosin Expts

➢ Actin filaments stretched between two trapped beads and brought close to myosin heads
➢ ATP: actin filament is pulled towards one end by myosin

➢ **Powerstroke**: Single myosin head binding to actin filament held loose in optical traps reduces Brownian noise.
➢ **Force**: Optical traps moved to keep the beads in the same position (feedback). Distance between the trap and the (fixed) beads is proportional to the force.

Atomic Force Microscopy

**laser diode**
- spring which deflects as probe tip
- scans sample surface

**mirror**

**cantilever**
- positions sample (x, y, z) with Å accuracy

**piezoelectric scanner**
- comes in contact with sample surface

**position sensitive photodetector**
- measures deflection of cantilever

**sensor output, δc, Fc**

**feedback loop**
- controls z-sample position

**ERROR**
- actual signal - set point

**computer**
- controls system
- performs data acquisition, display, and analysis

http://web.mit.edu/cortiz/www/afm.gif
AFM - Biolmolecules

HS- AFM : Myosin V walking on an actin filament
Ref Annual Review of Biophysics Vol. 42: 393-414

ATP Synthase – F0 rotors

Protein force-extension curves
Ref: Bustamante et al Nature reviews Molecular Cell Biology 1 131-6
Single Molecule Fluorescence Imaging: TIRF

http://www.microscopyu.com/articles/fluorescence/tirf/tirfintro.html

➢ TIRF, imaged single molecules for s, myosin tracked and ATP turnover monitored, real bio systems closer
➢ Photobleaching, camera noise can be an issue, EMCCD


\[
I(z) = I_0 e^{-z/d}
\]

penetration depth:
\[
d = \frac{\lambda}{4\pi \sqrt{n_1^2 \sin^2 \theta - n_2^2}}
\]

\( n_1 \) ...index of refraction of glass slide
\( n_2 \) ...index of refraction of water
\( \theta \) ...incident angle
\( \lambda \) ...wavelength of light
Single Molecule Fluorescence Imaging: FRET

FRET a molecular ruler: Electronic excitation energy can be transferred between two chromophores in close proximity.

Key Use:
Protein-Protein interactions & Protein conformation changes at high spatial precision < 10 nm

Practical Limitations: Control of chromophore concentrations, Photobleaching, Signal Separation and Detection.

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Consider an idealised nanoscale device operating in a thermal environment subject to random fluctuations: shaft with series of bevelled bolts mounted on springs. Thermal push $\epsilon + fL$ will push shaft to right. Prevented from moving to left by tapered sliding bolts. Violates 2$^{nd}$ law (loop version). If the energy for bolt retraction $\epsilon$ comparable to $kT$ the machine can step to the left.
Thermal Ratchet with stored potential energy

- Consider a modification in which a latch keeps bolt down when it’s to left of wall but releases the bolt on right side
- Movement to right doesn’t violate 2nd Law (Why?)
- Rectified Brownian Motion
- Stall force \( f = \frac{\varepsilon}{L} \)
Rectified Brownian Motion

This rectified Brownian ratchet is equivalent to a random walk with absorbing and reflecting boundary conditions.

The ratchet diffuses back and forth until it happens to move a distance L to the right, when the next spring engages. At this time, the ratchet starts at position $x = 0$.

If the free energy drop is very high, the ratchet cannot move to the left i.e. leftward flux is not allowed, so the LH boundary condition (at $x = 0$) is perfectly reflecting. If the free energy drop $\epsilon$ is finite, there is a probability of moving leftward, but it’s small if $\epsilon / k_B T$ is large.

If the ratchet ever reaches position $x = L$, the next spring is triggered and if the free energy drop $\epsilon$ is very high the position $x = L$ acts like a perfectly absorbing boundary. If the free energy drop $\epsilon$ is finite, the walker simply falls off a very large cliff and is unlikely to be able to return.

This type of problem involves calculating the time it takes for a random walker to reach a specified target and is called a first passage time problem.
Diffusion in Force Field

- Consider particles moving under the influence of a constant external force $F$
- Diffusive motion of the particles modelled by Fick’s law:
- External force $F = -\partial \phi / \partial x$ which in the absence of any diffusive motion would impart drift velocity $v = F/\zeta$
- The motion of the particle is the sum of the contributions of diffusion and field-driven drift

$$J_x = \underbrace{-D \frac{\partial c}{\partial x}}_{\text{Diffusion flux}} - \underbrace{\left( \frac{D}{k_B T} \cdot \frac{\partial \phi}{\partial x} \right)}_{\text{Drift flux}} c = -D \left( \frac{\partial c}{\partial x} + \frac{\partial (\phi/k_B T)}{\partial x} \cdot c \right)$$  \[1\]

- At equilibrium the flux vanishes : $J_x = 0$. Integrating with respect to $x$ gives the equilibrium concentration of particles in external field $\phi(x)$: $c_{eq} = c_0 e^{-\phi/k_B T}$. (Boltzmann Distribution)
Smoluchowski Equation

• Reframe the problem in terms of the probability of finding a single particle at (x,t).
• Normalize the concentration in the previous slide: \( p(x, t) \equiv c(x, t)/(\int_0^L c(x, t)dx) \) [2]
• Conservation of particles implies: \( \frac{\partial c}{\partial t} = -\frac{\partial J_x}{\partial x} \) [3]
• Combining [1],[2] & [3] gives the Smoluchowski equation. At steady state LHS is 0 (not necessarily equilibrium)

\[
\frac{\partial p}{\partial t} = D \left[ \frac{\partial}{\partial x} \left( p \frac{\partial (\phi/k_B T)}{\partial x} \right) \right] + \frac{\partial^2 p}{\partial x^2}
\]

\underbrace{\text{Drift}}_{D}\quad \underbrace{\text{Diffusion}}_{p'}
Molecular Ratchet : Smoluchowski Equation

• Consider circular ratchet with bolts reset at (x = x+4L). Look at motion of ensemble M.

• To find the speed of the ratchet we need to find the flux of particles $J_x$ from which we can calculate the average time it takes for the ratchet to cross a ‘step’ in the potential energy landscape. The speed is the step length divided by this time.

• The steady state probability distribution $p(x)$ for the ‘perfect ratchet’ case where $\varepsilon>K_BT$
Speed of the Ratchet

[Question Sheet Problem]

• Verify $P(x) = C(b e^{-(x-L)F/k_B T} - 1)$ solves Smoluchowski equation for a constant force $F$. You can assume steady state.

• Find the current flux: Expression in terms of $p$ sub equation [2] in [1]

• Show average speed of the loaded ratchet perfect ratchet is

$$v = \frac{2D}{L}$$

$$v = \left( \frac{F L}{k_B T} \right)^2 \frac{D}{L} \left( e^{F L / k_B T} - 1 - F L / K_B T \right)^{-1}$$

$F = 0$ $\quad$ $F < 0$ and $FL/kT >> 1$

Forward stepping contains exponential activation barrier
Polymerization Ratchet

- Addition of monomers generates a force
- When filament reaches the barrier (e.g. cell wall) fluctuations in position of cell wall or filament will allow another monomer to squeeze through and bind
Polymerization Ratchet contd

- How do we find the velocity (filament polymerization rate)?
  Driven diffusion equation
- Want to find $p(x)$ of finding a particle at $x$ in width $\delta$
- Force $p(x)$ to be 1 in $(0, \delta)$ interval
- Mean rate at which particle reaches boundary at $x = \delta$ starting at $x = 0$ is

$$j_0 = -D \frac{\partial p}{\partial x} - \frac{F}{\zeta} p$$

- We want to solve for $p(x)$ in terms of $j_0$

Figure 16.46 Physical Biology of the Cell (© Garland Science 2009)
Velocity of Polymerization Ratchet

The solution is the sum of the general solution of homogeneous Equation (absence of force) and a particular solution to inhomogeneous equation:

\[ p(x) = A \exp\left(-\frac{Fx}{k_B T}\right) - j_0 \frac{\zeta}{F}, \text{ } BC \rightarrow p(\delta) = 0; \int_0^\delta p(x)dx = 1 \]

Find \( j_0, A \Rightarrow j_0 = \frac{1}{k_B T \frac{\zeta}{F^2} \left(\exp\left(\frac{F \delta}{k_B T}\right) - 1\right) - \frac{\zeta \delta}{F}} \)

\[ v = \frac{\delta j_0}{\delta} = \frac{D \left(\frac{F \delta}{k_B T}\right)^2}{\exp\left(\frac{F \delta}{k_B T}\right) - 1 - \frac{F \delta}{k_B T}} \]

Low Force: \( v = \frac{2D}{\delta} \) Same as no force limit – diffusion – first passage problem

High Force:

\[ v = \frac{F^2 D \delta}{(k_B T)^2} \exp\left(-\frac{F \delta}{k_B T}\right); \]

\[ FPT \rightarrow \tau = \frac{\delta}{v} = \left[\frac{(k_B T / F)^2}{D}\right] \exp\left(\frac{F \delta}{k_B T}\right) \]

Probability particle will find itself with energy \( F\delta \)
Summary

• A Brownian ratchet can use stored internal energy into rectify thermal motion. To do this there must be a structural asymmetry.
• Corresponds to sliding down an energy landscape: out of equilibrium but steady-state
• A ratchet’s speed does not increase without bound with increasing internal energy. Saturation kinetics.
• Good starting point for models of some molecular motors e.g. polymerisation ratchet, translocation of proteins through a pore in a cell membrane