Lecture 9 Motors (ch16)

Diffusion is a highly active process inside cells, but it is unable to mix/transport small molecule over distances as big as 10 μm in reasonable time

Active, energy consuming processes are needed instead.

But we are still firmly in the $Re \ll 1$ limit:

$\Rightarrow$ noninertial; no flywheels

$\Rightarrow$ k_BT is an important energy scale, so

slide. Brownian Ratchet mechanisms are good.

Energy efficiency is important, so reversible processes prevail, driven by gradients..

Four basic kinds:

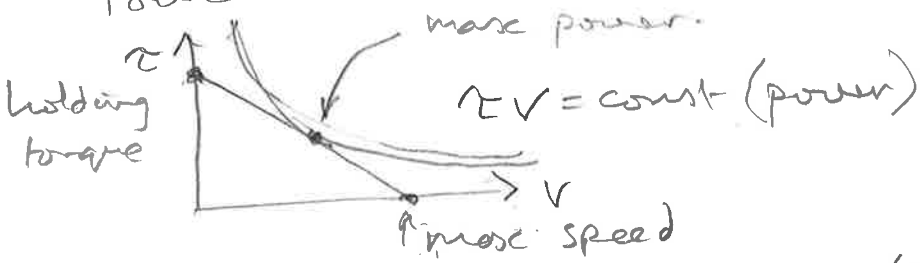
i) Linear motors – track of binding sites
ii) Rotary motors – many examples
iii) Polymerization motors – actin, μ tubules
iv) translocation motors – moving in/out of cells or compartments.

Performance specification:

slides Force – Velocity curve (as in Engineering)

"Torque – speed" eg for stepper motor

Power = torque × speed.



Also in muscle physiology (body-building)

1. Translational motor

myosin V — walks along actin, carrying cargo.

Kinesin I — similar, but microtubule } all ATP binding.

slide dyenin — also microtubules.

RNA polymerase. — highly "processive"

<u>Processivity</u> = number of steps before falling off.

— cargo transporters can reattach

— RNAP. needs to complete message or wasted.

achieved with strong binding const, but wastes energy.

Muscle actin-myosin (below) only a few steps.

— highly parallel, so never loses contact.

i) ATP fuel : $20\,k_BT$ per step.

ATP is hydrolysed as part of step.

ii) Force estimate ← 10x smaller than myosin V

Kinesin-microtubule 8 nm step / ATP.

$$\text{Force} = \frac{\text{Work}}{\text{Distance}} = \frac{20\,k_BT}{8\,\text{nm}} = \frac{20 \times 4\ \text{pN.nm}}{8\,\text{nm}}$$

$= 10\,\text{pN}$ typical upper limit

iii) ATP binding site

conformation change of "head" domain

highly conserved in evolution

iv) Typical velocity?

axon-microtubules $0.2-0.4$ m/day.

(neurons) fast example $\sim 3\,\mu\text{m/sec}$

~ 500 steps / sec.

Estimate size of cargo : [3x more cytoplasm]

$F = \gamma v \quad \gamma = 6\pi\eta R \quad \eta = 10^{-3}\,\text{Pa·s}.$

$R = (F/v)/6\pi\eta = (10\,\text{pN}/3\,\mu\text{m/s})/6\pi \times 10^{-3}$

$= 0.2 \times 10^{-3}\,\text{m} = 200\,\mu\text{m}$

plenty of capacity!

2. Cell Division + Cilia.

Microtubule spindle
Kinesin motor, CENP-E
pulls chromosomes apart within one hour
Kim et.al. measured 8nm/s } 2500 sec
20 μm cell diameter

slide movies.

Eukaryotic Cilia are present on surface of tissues for moving contamination from the lungs "mucociliary escalator"
also motor of sperm cells
+ flagellum of paramecium (Eukaryote)
[not to be confused with E.Coli flagellum, which has an entirely different motor].
"Axoneme": μtubules connected by dyenin motor protein.

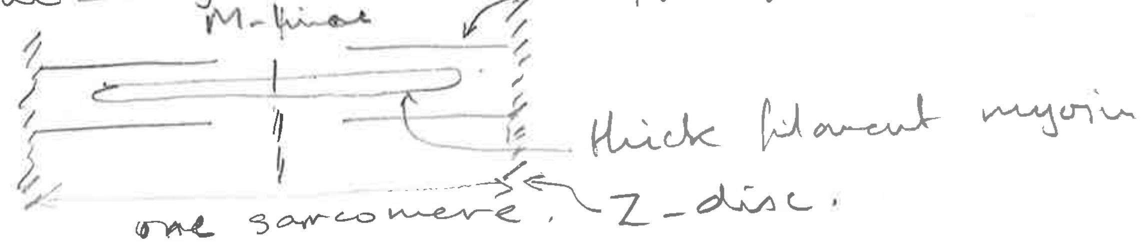
slide movie?

Crosslinks causes sliding ↔ flexing
Synchronization unknown?
10 μtubule pair structure conserved across evolution

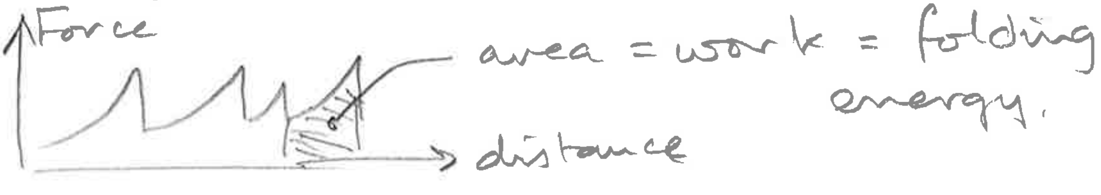
3. Mammalian Muscle. p631

Actin-Myosin motor.

movie



titin shock absorber.
many domains in sequence which unfold, costing energy when overextended.
energy is recovered on refolding.



Coordination of muscle
is by tropomyosin blocking the actin sites.
- unbound by infusion of Ca^{2+} ions
- diffuse very fast through muscle.

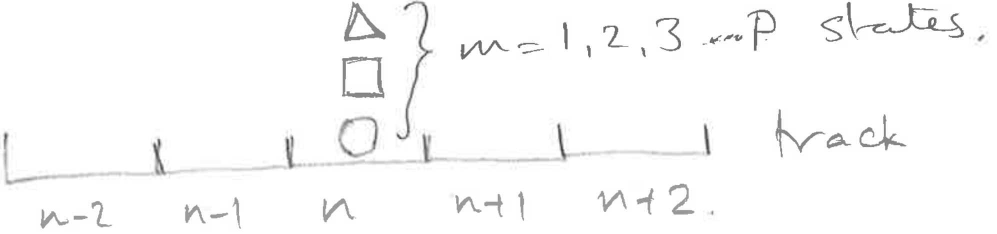
slide Muscle myosin is mostly unbound and
only binds during its ATP power stroke.

4. Myosin V >50% bound so as not to lose cargo

slide inchworm × vs hand-over-hand ✓
slide movie models. Single molecule imaging expts
show hand-over-hand. Selvin – Ha (2003)
Step size = 74 nm.
TEM microscopy sees motion as animation
Peter Knight (Leeds).
Vary Myosin V arm length (change number
of "IQ-repeats" genetically.
slide Step size seems to scale.
16.3.15

5. Position - State Models.

Following the asymmetric polymerization ideas from the last lecture, we model ATP-dependent myosin as a multi-state motor.



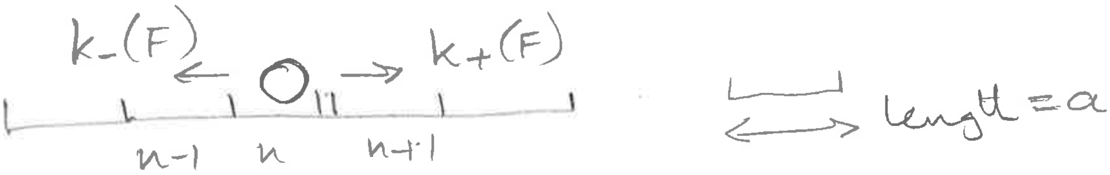
"Rectification" or "ratchet" idea can be included in the kon/koff rates for the transitions between states.

Expect 'random walk' behaviour obeying the diffusion equation.

6. One-state model.

Cannot really apply to real motors because it cannot have a direction.

Like with rate equations, introduce an external driving force: (notes 7.12)



$$p(n, t+\Delta t) = k_+ \Delta t \; p(n-1, t) + k_- \Delta t \; p(n+1, t) + (1 - k_+ \Delta t - k_- \Delta t) \; p(n, t)$$

as before, Taylor expand $p(n,t)$: (p643)

$$\frac{\partial p}{\partial t} = -V \frac{\partial p}{\partial x} + D \frac{\partial^2 p}{\partial x^2}$$ Smoluchowski equation

$V = a(k_+(F) - k_-(F))$ drift

$D = \frac{a^2}{2}(k_+(F) + k_-(F))$ diffusion

9.6

Change of variables to remove drift:

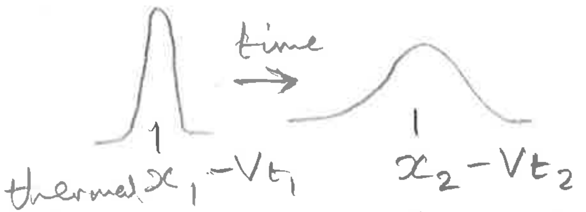
$$\bar{t} = t \qquad \bar{x} = x - Vt$$

$$\frac{\partial p}{\partial t} = \frac{\partial p}{\partial \bar{t}}\frac{\partial \bar{t}}{\partial t} + \frac{\partial p}{\partial \bar{x}}\frac{\partial \bar{x}}{\partial t} = \frac{\partial p}{\partial \bar{t}} - V\frac{\partial p}{\partial \bar{x}}$$

$$\frac{\partial p}{\partial x} = \frac{\partial p}{\partial \bar{t}}\cancel{\frac{\partial \bar{t}}{\partial x}} + \frac{\partial p}{\partial \bar{x}}\frac{\partial \bar{x}}{\partial x} = \frac{\partial p}{\partial \bar{x}} \quad \text{etc.}$$

$$\Rightarrow \frac{\partial p}{\partial \bar{t}} = D\frac{\partial^2 p}{\partial \bar{x}^2} \quad \text{Standard diffusion equation}$$

So construct all solutions from Green's funct.



Gaussian form:
i) spreads in time
ii) shifts along axis.

i) In equilibrium (no Force)

$$k_+ p_n = k_- p_{n+1} \quad \text{no time dependence}$$

$$p_n = \frac{1}{Z}e^{-\beta G_n}$$

$$\Rightarrow \frac{k_+}{k_-} = e^{-\beta \Delta G} \qquad \Delta G = G_{n+1} - G_n$$

ii) In equilibrum with Force

When motor moves from n to $n+1$
the force does work Fa:

$$\frac{k_+(F)}{k_-(F)} = e^{-\beta(\Delta G + Fa)}$$

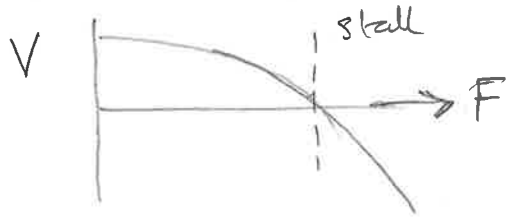
Consider F-depence in <u>either</u> k_+ or k_-

$$V(F) = ak_-\left(e^{-\beta(\Delta G + Fa)} - 1\right) \quad \text{if } k_+(F)$$

<u>or</u> $$V(F) = ak_+\left(1 - e^{+\beta(\Delta G + Fa)}\right) \quad \text{if } k_-(F).$$

Second one agrees better with data

slides.



"rectification"

7. Two-State Model.

Asymmetry can now be introduced between the forward and backward directions:

$p_0(n,t)$ $p_1(n,t)$

Coupled rate equations now:

$$\frac{dp_0(n,t)}{dt} = k_A^+ p_1(n-1,t) + k_B^- p_1(n,t) - k_A^- p_0(n,t) - k_B^+ p_0(n,t)$$

$$\frac{dp_1(n,t)}{dt} = k_A^- p_0(n+1,t) + k_B^+ p_0(n,t) - k_A^+ p_1(n,t) - k_B^- p_1(n,t)$$

Quite a lot of work to solve, left to "reader" of text book (p678).

Note this is a wave equation similar to the phonon dispersion in solid state physics where the rates are given by masses and springs.

So assume a complex wave:

$$\begin{pmatrix} p_0(n,t) \\ p_1(n,t) \end{pmatrix} = \begin{pmatrix} A \\ B \end{pmatrix} e^{i(Kn-\omega t)}$$

$$-i\omega A = (k_A^+ e^{-iK} + k_B^-) B - (k_A^- + k_B^+) A.$$

$$-i\omega B = (k_A^- e^{iK} + k_B^+) A - (k_A^+ + k_B^-) B.$$

Standard eigenvalue equation; omit steps:

$$\omega^2 + 2ik_4\omega + k_A^- k_B^- (e^{iK} - 1) + k_A^+ k_B^+ (e^{-iK} - 1) = 0$$

where $2k_4 = k_A^- + k_A^+ + k_B^- + k_B^+$.

Consider limit $K \ll 1$:

$$e^{iK} - 1 \simeq iK + \frac{K^2}{2}$$

$$e^{-iK} - 1 \simeq -iK + \frac{K^2}{2}$$

Two roots of quadratic, again omitting steps:

$$\omega_1 \simeq -2ik_4$$

$$\omega_2 \simeq + \left(\frac{k_A^+ k_B^+ - k_A^- k_B^-}{2k_4} \right) K + i \left(\frac{k_A^- k_B^- + k_A^+ k_B^+}{4k_4} \right) K^2$$

This is the "dispersion relation" of the wave showing how K and ω are related.

Identity of these terms can be seen from the wave solution of Smoluchowski equation

$$\frac{\partial p(x,t)}{\partial t} = -V \frac{\partial p}{\partial x} + D \frac{\partial^2 p}{\partial x^2} \qquad p = p_0 e^{i(Kx/a - \omega t)}$$

$$-i\omega = -V \frac{iK}{a} + D \left(\frac{-K^2}{a^2} \right)$$

$$\Rightarrow \omega = \frac{V}{a} K + i \frac{D}{a^2} K^2$$

Hence $V = a \left(\frac{k_A^+ k_B^+ - k_A^- k_B^-}{2k_4} \right)$ drift velocity

$D = a^2 \left(\frac{k_A^+ k_B^+ + k_A^- k_B^-}{4k_4} \right)$ diffusion const.

Can now see that V can be controlled by the rate constants similar to Michaelis-Menten.

eg $k_A^+ \gg$ others $\Rightarrow V = a k_B^+$

$k_B^+ \gg$ others $\Rightarrow V = a k_A^+$

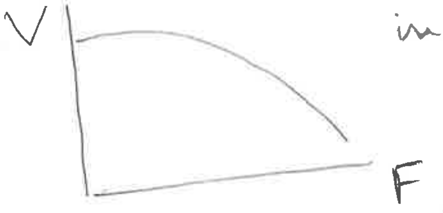
ATP-concentration dependence is built into the k's as 2nd-order rates $k_A^+ = k_A' [ATP]$

Then expect saturating MM kinetics depending on the concentration.

Similarly Force → $k_A^+(F)$ as before:

as seen in expts.

slides.



Similar to mechanical motors

8. Waiting Time Distribution

Method of detecting multistate motors.

Random-walk motion resembles Brownian motion in that the moves can occur at any time

No inertia or memory of previous moves.

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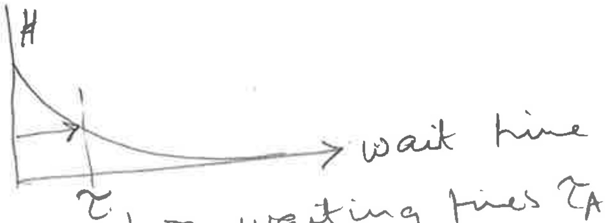
We saw before (8.2) exponential dist:

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



2-state motor will have two waiting times τ_A & τ_B.

Combined wait time:

$$p(t) = \int_0^t p_A(t')\, p_B(t-t')\, dt'$$

convolution of probabilities

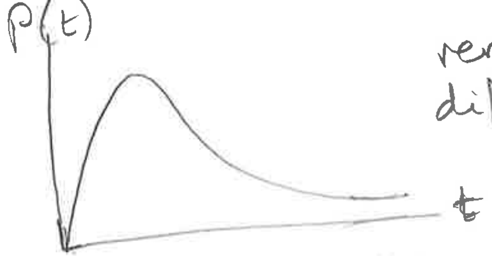
To get a short wait, <u>both</u> events have to be short.

$$p(t) = \frac{1}{\tau_A \tau_B} \int_0^t e^{-t'/\tau_A} e^{-(t-t')/\tau_B}\, dt'$$

$$= \frac{1}{\tau_A \tau_B} e^{-t/\tau_B} \left[e^{-t'(1/\tau_A - 1/\tau_B)} \frac{1}{1/\tau_A - 1/\tau_B} \right]_0^t$$

$$= \frac{1}{\tau_B - \tau_A} e^{-t/\tau_B} \left(1 - e^{-t/\tau_A} e^{+t/\tau_B}\right)$$

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



very different from

This characteristic is seen in real data

Multistep motors all have the probability vanishing at t = 0.

9. Rotary Motors.

Bacterial "flagellum" - seen before

slide Coupled to trans-membrane proton pump.

ATP synthase.

movies x2 Very beautiful bi-directional enzyme.

12 × protons transmitted / turn

3 × ATP synthesized / turn.

Stator / rotor paths for protons to link motion.

\+ shaft connects F_1 to F_0

Energetics:

1 × ATP synthesis ~ 20 k_BT (F_1)

4 × protons across 90mV potential drop. (F_0)

25mV ~ k_BT, hence 16 k_BT total.

Can express as torque as function of conc:

Units of torque = units of energy (N-m).

$$\text{In } F_0: \quad \tau_{F_0} = \frac{12}{2\pi}\Big(4k_BT + k_BT \underbrace{\ln\frac{[H]_{out}}{[H]_{in}}}_{\propto \Delta pH \sim 0.75 \text{ typ.}}\Big)$$

$$\simeq 30 + 20\,\Delta pH$$

$$\text{In } F_1: \quad \tau_{F_1} = \frac{3}{2\pi}\Big(20k_BT + k_BT\ln\frac{[ADP][P_i]}{[ATP]}\Big)$$

$$\simeq 40 \text{ pN-nm.}$$

Torques are balanced when $\Delta pH = 0.5$

Healthy E. Coli maintain $\Delta pH \sim 0.75$, which drives ATP synthesis.

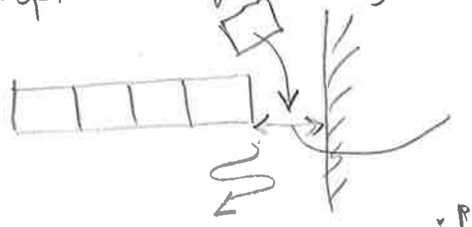
But if ΔpH drops, motor rotatates the other way and consumes ATP to restore the proton gradient.

Same enzyme found in E. Coli to mitochondria with only small evolutionary differences.

[John Walker + Paul Boyer 1997 Nobel]

10. Polymerization Ratchet

Actin polymerization drives force against wall. Brownian motion of polymer occasionally leaves a gap. → quickly filled by monomer.



gap = x < monomer length, δ.

Trajectory simpler if we assume k_{on} is much faster than fluctuation time.

i) First Passage Time. (no Force)

1D Brownian motion for $0 < x < \delta$ when $x = \delta$, polymerisation takes place and system resets : $x \to 0$ start again $t = 0$

With no force:

$$\frac{\partial^2 p(x)}{\partial x^2} = 0 \quad \Rightarrow p(x) = Ax + B.$$

B.C. 1 : normalization

$$\int_0^\delta p(x)\,dx = \left[A\frac{x^2}{2} + Bx\right]_0^\delta = \frac{A\delta^2}{2} + B\delta = 1$$

B.C. 2 : $p(\delta) = 0$ for reset condition

$$A\delta + B = 0 \quad \Rightarrow \quad B = -A\delta.$$

$$A\frac{\delta^2}{2} - A\delta^2 = 1 \quad \Rightarrow A = \frac{-2}{\delta^2}$$

Flux at $x = \delta$ determines rate of passage:

$$j_0 = -D\frac{\partial p(x)}{\partial x} \quad \text{which is Fick's law.}$$

$$= -DA = +2D/\delta^2$$

Filament polymerization velocity V = rate × δ.

$$V = \delta \times 2D/\delta^2 = 2D/\delta$$

ii) Repeat in presence of force F: [HW problem]

slide

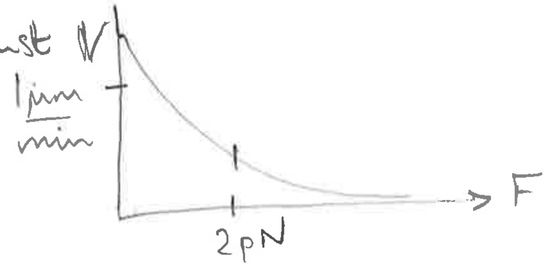
$$V = \frac{D}{\delta} \cdot \frac{(F\delta/k_BT)^2}{e^{F\delta/k_BT} - 1 - F\delta/k_BT}$$

9.12

11. Experiments on microtubules

i) Microtubules pushing against barrier. Similar shape to derived equation.

slide.



ii) Bead in optical trap. Bundle of microtubules pressing against barrier. Force builds up then collapses in "catastrophe"

slide

Timescale = minutes

Force scale = pN

Velocity (above) = μm/min = 10^{-8} m/s.

estimate $D \sim V\delta \sim 10^{-8}\, m/s \times 10^{-9}\, m = 10^{-5}\, \mu m^2/s$

compare proteins in solution $\sim 10\, \mu m^2/s$.

Estimate decay of Force $\sim e^{-F\delta/k_BT} = e^{-F/F_0}$

$F_0 = k_BT/\delta \sim 2pN = 4pN.nm/\delta$

$\delta = 2nm$ about right for microtubules.

iii) Atomic force microscope (AFM)

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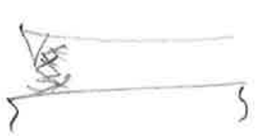
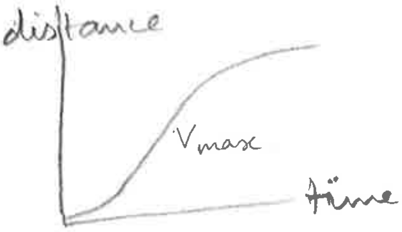


cantilever forced up by actin polymerization



$V_{max} \sim 4\mu m / 60 min \simeq 1nm/sec$

even lower than single molecule values.

12. Translocation Ratchet (p638, 674)

i) Thermal ratchet. Polymer passing through pore. Binding sites, eg RNA-Polymerase on inside diffuses by spacing of binding sites. direction of flow follows concentration gradient

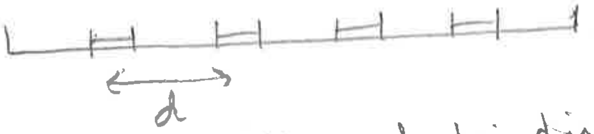
eg Virus (T4, T7) discharging DNA by force of compaction, helped by RNA-P

9-13

ii) Cross bridge Ratchet.

Import + Export of proteins in mitochondria.
Membrane bound channel protein (TIM)
Helper protein hsp70 moves target by ATP-dependent conformation change.

iii) Ratchet vs Diffusion (p674)



$L = nd$

separation of binding sites (RNA-P)

By normal diffusion,

$$t_1 \sim \frac{L^2}{D} = n^2 \frac{d^2}{D}.$$

By local diffusion, repeated n times:

$$t_2 \sim \frac{d^2}{D} \times n = n \frac{d^2}{D}. \quad = n\text{-times faster.}$$

Bacterial virus (phage) $\left.\begin{array}{l} d \sim 1\mu m \\ L \sim 10\mu m \end{array}\right\} n \sim 10.$

Same model, based on Smoluchowski equation,
as for ratchet polymerisation:
calculate "first transit time"