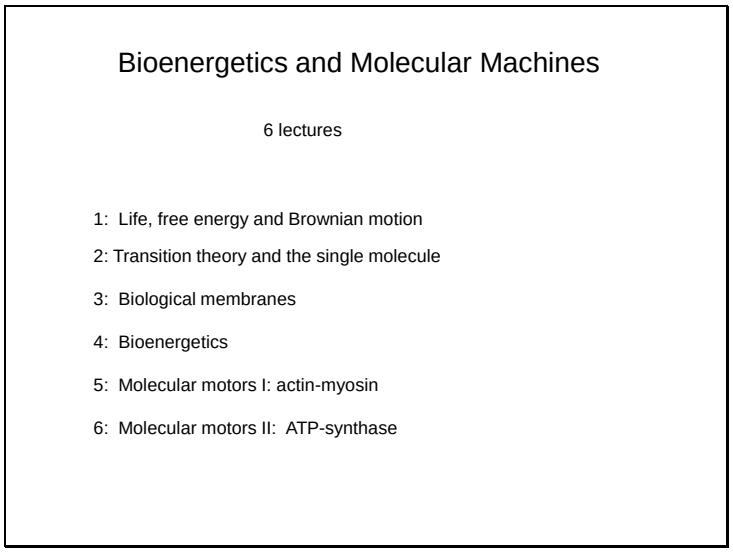
Slide 1



[Section overview] (1)

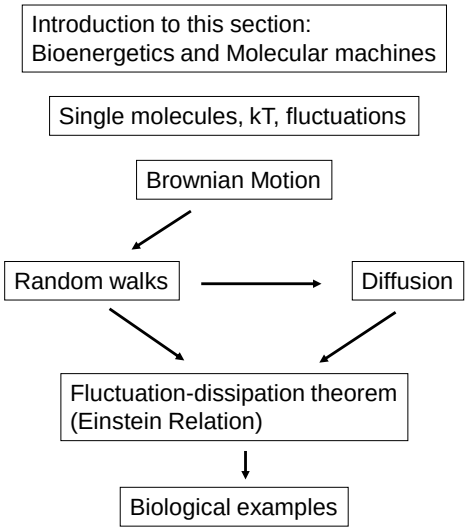
- 6 lectures
  - Lecturer: Richard Berry
  - 1-2: the physics of small things at room temperature, single molecules, biological examples.
  - 3,4: Biological membranes and bio-energetics.
  - 5-6: Molecular motors in muscle and ATP-synthase as examples of biological machines

- Problem set: classes middle of Michaelmas term

Hand-outs – most of the lecture slides plus notes

Slide 2

Lecture 1: Life, Free Energy and Brownian Motion

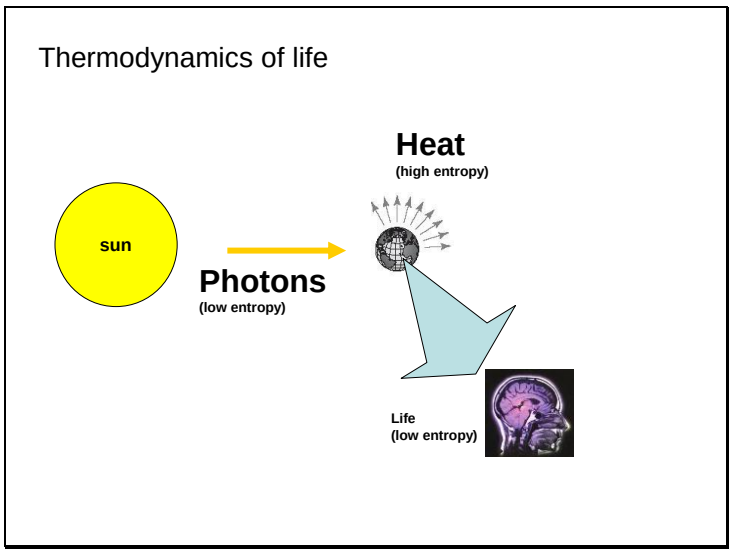


Slide 3

Reading for Lecture 1

- Nelson, Chapter 4
- Howard, Chapter 4
- Berg, H.C.
  "Random Walks in Biology" 152pp Princeton University Press,

Slide 4



[Thermodynamics of life] (4)

•2nd law of thermodynamics: Entropy Increases

•Life **Decreases** it's own entropy – at the expense of the rest of the universe

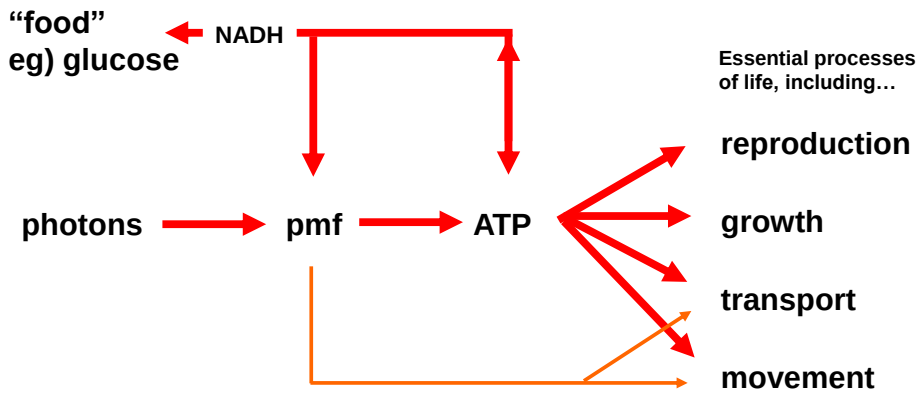
- •Most globally – takes photons (low entropy – straight line !) and converts them ultimately into heat (high entropy), with all of life in-between.

•To do this, life needs to gather, store and manipulate sources of **Free-Energy**

- •Free energy can be thought of as the "**currency**" of life. Any reaction that requires free energy input (eg. making DNA from nucleic acids, doing mechanical work, building a protonmotive force) must be "paid for" by coupling to a reaction that releases free energy.

Slide 5

An overview of bioenergetics:
Free Energy flow in living things



Main topics in this section of the course:

Biological Free energy:	Protonmotive force, (biological membranes), ATP
Movement, transport:	Molecular Motors

[Biological Free Energy] (5)

•This section of the course will look at some of the molecular machines that carry out the free energy transactions that constitute life, and at their mechanisms.

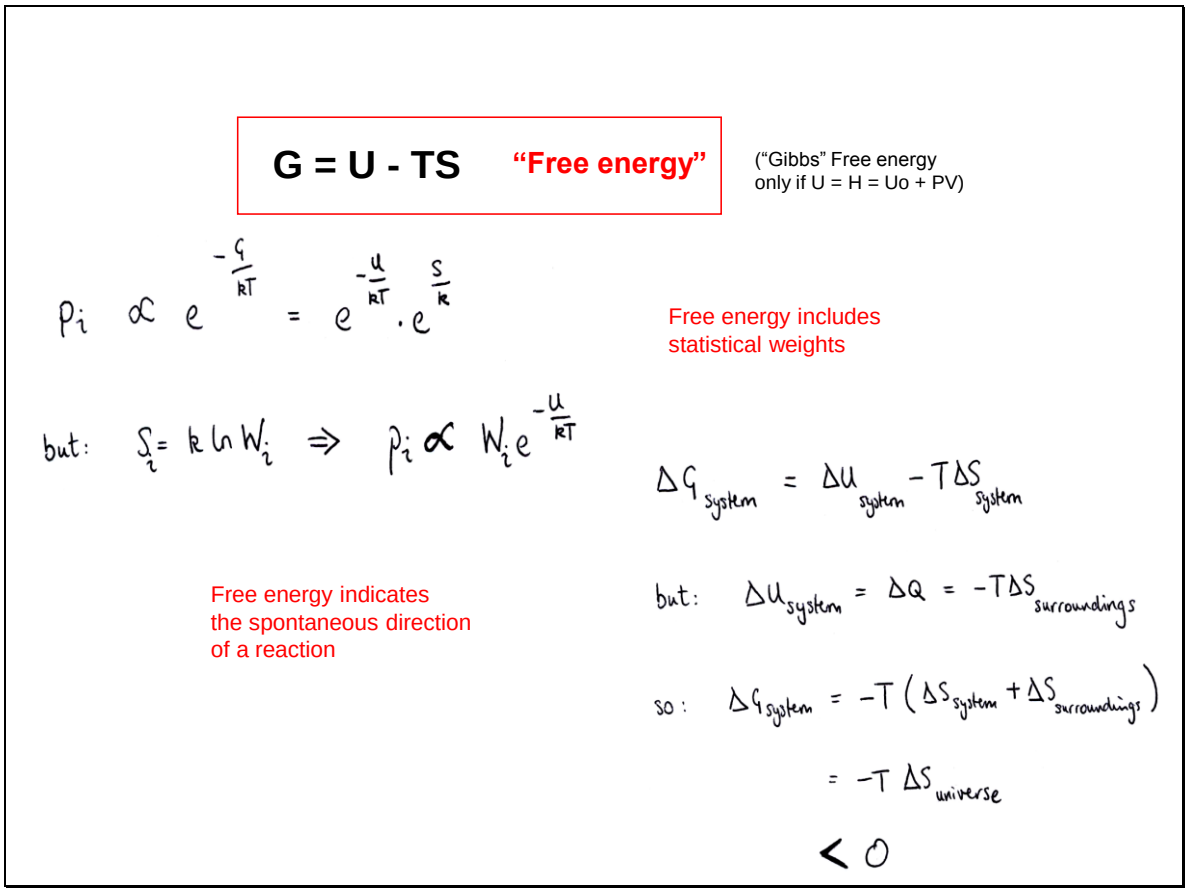
•**Free Energy**

•Each state is really an ensemble of **microstates**

- •eg. Quantum mechanical energy levels
- •eg. Brownian motion – various positions of a protein domain within one "state"

•The more microstates, the more probable the macro-state – **Entropy Increases**

Slide 6

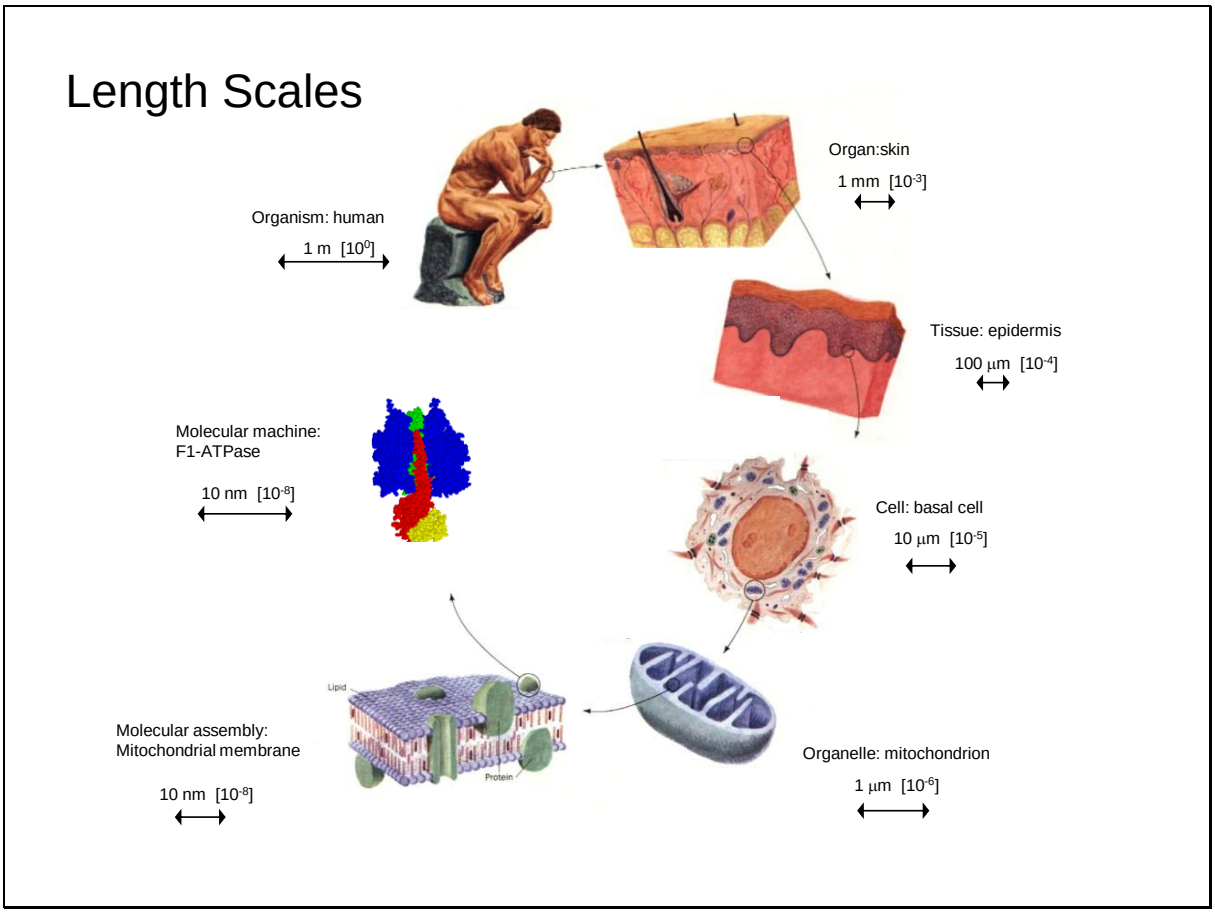


[Free Energy, a reminder] (6)

•"free" in the sense of being available to do work without violating the 2nd law of thermodynamics.

•Use G instead of U for all rate calculations where each state is really an ensemble of states.

Slide 7



[Length scales] (7)

•This course will focus mostly on the Molecular Level.
•Small molecules, macromolecules, molecular machines
•Experiments on the mechanism of single molecular machines.

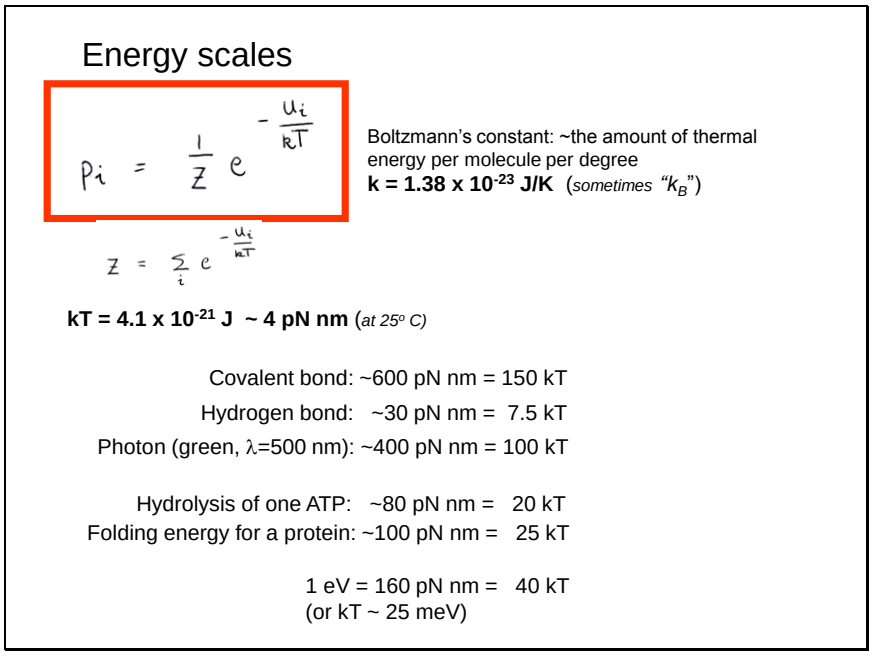
Slide 8

[Examples of molecular machines] (8,9)
•Scale bars in slide 8 are 50 nm (flagellar motor), 36 nm (myosin V)

Slide 9

•An organism is a "society" of cells, each of which is a "society" of macromolecules and molecular machines. Each has its particular set of functions, all are regulated, it can go wrong.
•Level of complexity is very high – human brain contains 100 billion cells, each of which can connect to up to 10,000 others.
•The small size of molecular machines means that their physics is dominated by **thermal fluctuations** – macroscopic intuition is of limited use.

Slide 10



[Energy scales] (10)

•**Boltzmann's Law**

•Several ways to derive/justify this (Statistical Mechanics)

•Based on the postulate that all micro-states are equally probable

•Boltzmann distribution maximises W with the constraints $\Sigma(N_i) = N$ and $\Sigma(N_i E_i) = E$

•Combining two systems into a larger system: probabilities multiply, energies add.

•Z ("partition function") is a normalizing factor

•For most biological systems each state is defined such that it is really an ensemble of microstates : so the energy *U* should be replaced by the free energy, *G*.

•**kT is a natural unit of energy**

•The amount of thermal energy per molecule

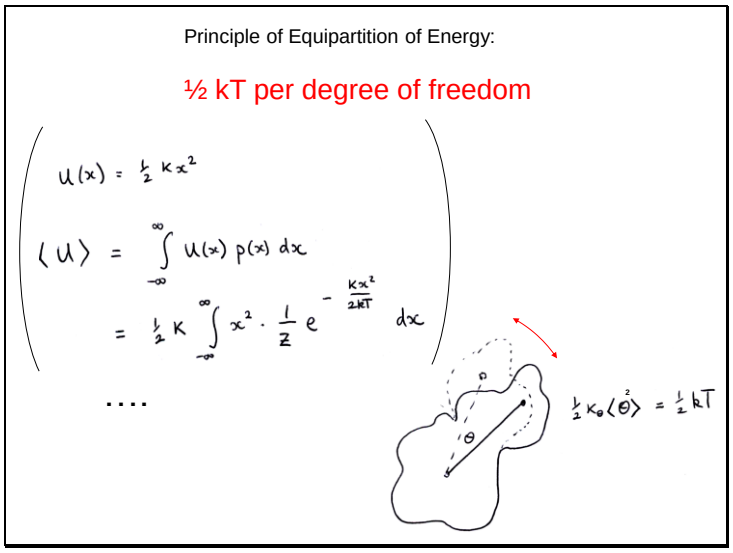
•Note: the gas constant, $R = k \times N_A$, where $N_A = 6.02 \times 10^{23}$ is Avogadro's number, the number of molecules in a "Mole". This is a HUGE number – kT is a very small energy.

•Covalent bonds are unlikely to break (exp(150) = 1064.5)

•**Hydrogen bonds (and protein folds) are thermally labile (exp(7.5) = 1800)**

•**Biological processes take place on energy scales close to kT**

Slide 11



[Equipartition of Energy] (11)
•Familiar from molecular velocity distributions in a gas for Kinetic Theory
•Factor of half only if energy is quadratic function of degree of freedom (eg ~k_x^2 or mv_x^2). But always <U> ~ kT . (see Howard for derivations)
•This is what it means to say the kT is the "amount of thermal energy per molecule". Note: **size doesn't matter**.
•For proteins or other particles in water, this energy is due to constant collisions with water molecules…

Brownian motion, the theory of fluctuations
Before we can understand the processes of life, we need tools to deal with systems dominated by thermal fluctuations.

Slide 12

[Demonstration: Brownian motion, beads] (12)
• Brownian Motion: 1828, Robert Brown, a botanist, saw it in pollen grains in water, then later in (dead) soot particles.
• It led to the demonstration of the reality of molecules, and a measurement of how small they are: see below.
• The question: how can something so small as a water molecule move something so big as a pollen grain? It's all about AVERAGING…

Random walks and Diffusion

• A simple model:
    • consider each step a particle takes between collisions as independent of all others: no memory.
    • Let all steps be equal in size, to start with

[computer demo: 2-D random walk] (12)
• The particles spread into what looks like a Gaussian distribution.
• The mean-SQUARE displacement is proportional to time
• The approximation of equal steps is not a problem:
    • if each "real" collision is modelled as several equal steps, collisions have a distribution of step-sizes.
    • the results are independent of scale except at the scale of individual collisions.
    • (Almost) all biological processes in this course are much slower than this.
• The first two features are the key characteristics of DIFFUSIVE transport or motion. We will demonstrate this with two different types of derivation, then discuss its importance.

Slide 13

1-D random walk

k_n gives the direction of the last step, Δx is the step size

$$\langle x_n^2 \rangle = \langle (x_{n-1} + k_n \Delta x)^2 \rangle \qquad (k_n = \pm 1 \cdot \Delta x = \text{constant})$$

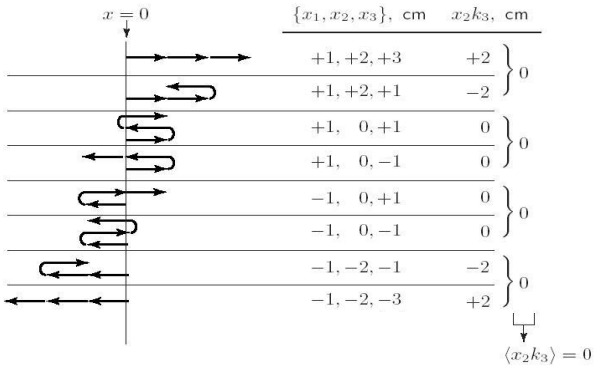
$$= \langle x_{n-1}^2 \rangle + 2\Delta x \langle x_{n-1} k_n \rangle + \Delta x^2 \langle k_n^2 \rangle \quad (\langle k_n^2 \rangle = 1)$$

$$= \langle x_{n-1}^2 \rangle + \Delta x^2$$

When the average is taken over all possible walks, they sum to zero in pairs differing only in the direction of the last step

This is a recurrence relation: combined with $\langle x^2_0 \rangle = 0$ we get…

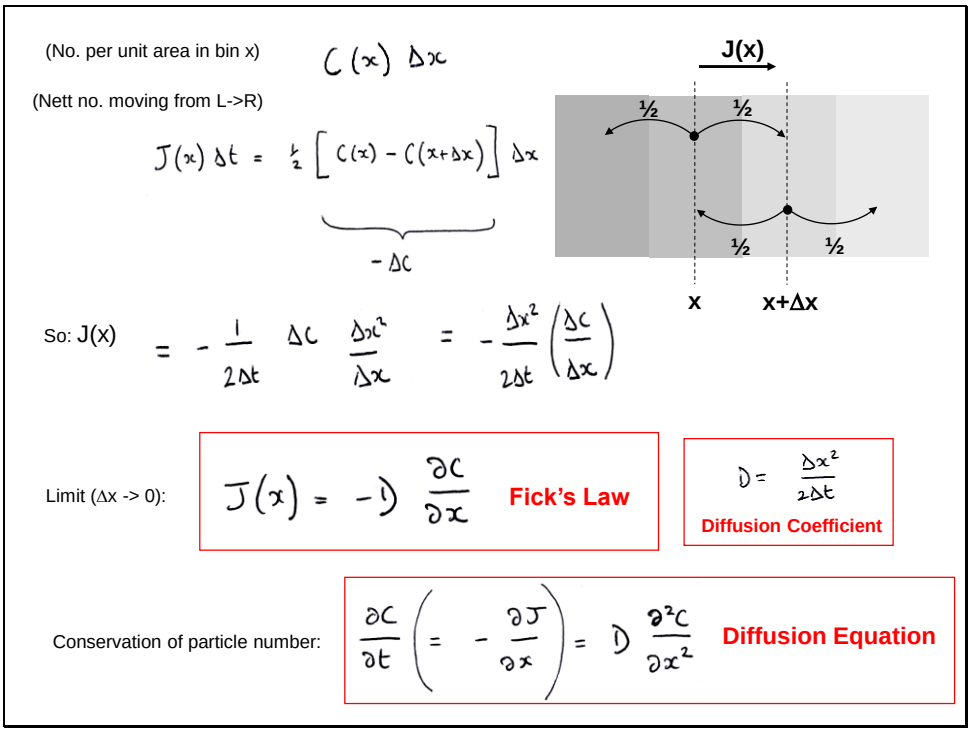
$$\langle x_n^2 \rangle = n \Delta x^2$$



[diffusion, discrete approximation] (13)

- Processes like this, with no memory, are called **Markov Processes**.
- For a random walk, the **mean square displacement (MSD) is proportional to the number of steps.**
- Once more, it is assumed that all possible walks are equally likely.
- (For more spatial dimensions, probability distributions multiply and MSDs add.)

Slide 14



[diffusion equation] (14)

A discrete random walk can be described by the **diffusion equation**.

• Assumption is that in some characteristic time Δt, each particle will move either way at random into the next "bin" of width Δx.

•Particles flow from where there are many to where there are few.
Δx (~mean free path?) and Δt (~ m.f.p. / mean velocity?) are not well defined, but D can be measured by comparing observed concentration and flux to solutions of the Diffusion Equation.

• How far and how fast do molecules diffuse – can we demonstrate MSD proportional to time?

Slide 15

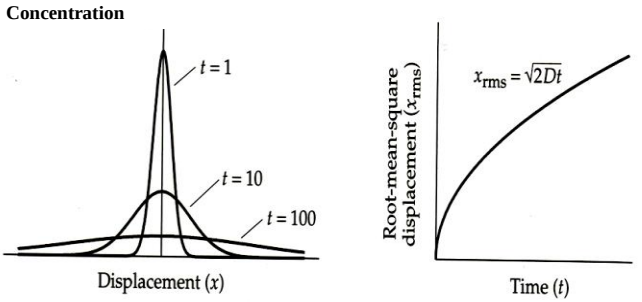
$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$ **Diffusion Equation**

One solution: $C(x,t) = \frac{C_0}{\sqrt{4\pi D t}} \exp\left\{ \frac{-x^2}{4Dt} \right\} \quad t > 0$

(Test it by substitution)

At time t : $C \propto \exp\left\{ \frac{-x^2}{2\sigma^2} \right\}$, $\sigma^2 = 2Dt = \langle x^2 \rangle$

Gaussian Distribution



$c(x,0) = C_0 \, \delta(x)$

(b.c. – molecule[s] released at x=0 at t=0)

[distance travelled, using diffusion equation] (15)

•Once more, $(\langle x^2 \rangle)^{1/2} = x_{rms} = (2Dt)^{1/2}$ is a measure of how far the particles get, on avarage, in time *t*.

•**Note that the dimensions of *D* are (length^2 / time)**

• Note: compare the two treatments …

- Discrete treatment: $\langle x^2 \rangle = n \, \Delta x^2, \; t = n \, \Delta t$
- Diffusion equation: $\langle x^2 \rangle = 2 \, D \, t,$
  - *So* $D = \Delta x^2 / (2 \, \Delta t)$, …

• For short times and distances, diffusion is very fast

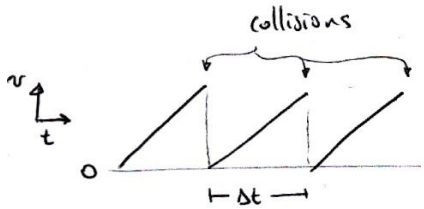
- K^+ ion in water goes 1 micron in 0.25 ms, 0.1mm in 2.5s

• For long times and distances, diffusion is very slow.

- K^+ ion goes 1 m in 8 years
- Lord Kelvin's demonstration in Glasgow is still going after more than a century

Slide 16

A random walk under a constant force F…



$$a = \frac{F}{m}$$

$$\langle \delta x \rangle = \tfrac{1}{2} \frac{F}{m} \Delta t^2$$

$$\langle v \rangle = \frac{\langle \delta x \rangle}{\Delta t} = \tfrac{1}{2} \frac{F}{m} \Delta t$$

$$\gamma = \frac{F}{\langle v \rangle} = \frac{2m}{\Delta t}$$

This is the definition of γ

Previously we had…

$$D = \frac{\Delta x^2}{2\Delta t}$$

Diffusion Coefficient

Multiplying the two…

$$D\gamma = \frac{\Delta x^2}{2\Delta t} \frac{2m}{\Delta t} = m\left(\frac{\Delta x}{\Delta t}\right)^2$$

Here $\langle \delta x \rangle$ is the average distance travelled, due to the force, on top of the thermal motion, and $\langle v \rangle$ is the average or "drift" velocity

This is a constant in the random walk model. It's dimensions ($m\ v^2$) are the same as KINETIC ENERGY

Equipartition of Energy gives…

$$K.E. = \tfrac{1}{2} m v_T^2 = \tfrac{1}{2} kT$$

Einstein Relation $D\gamma = kT$

The Einstein relation, an example of a "Fluctuation-Dissipation theorem"

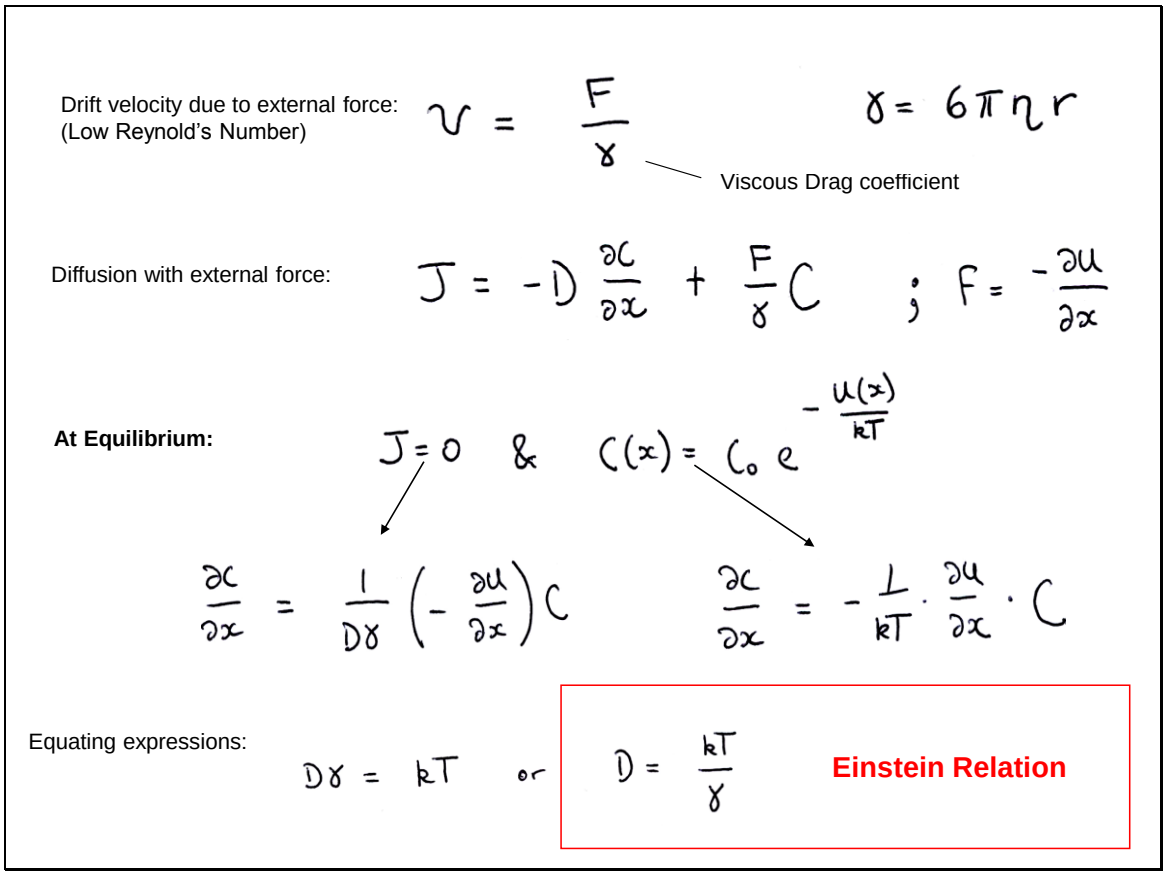
• The diffusion coefficient can be related to viscous drag coefficient using the Einstein Relation.

• Einstein did this in 1905, the same time as the photoelectric effect and special relativity. Smoluchowski and Sutherland also derived it, both independently, at about the same time…

[Einstein Relation – via discrete approximation] (16)

• The key result here is that the product $D\gamma$ is a constant, and that that constant is equal to kT.

Slide 17



[Einstein Relation – via diffusion equation] (17)

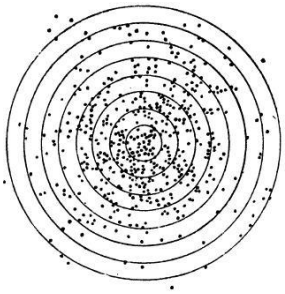
• *D* is constant, so the expression applies even out of equilibrium (when $J \neq 0$ and concentration does not obey Boltzmann distribution).

• Brownian Motion and friction are essentially the same thing.

• They are the average effect of many collisions and can be quantified by the application of Boltzmann's law, or equivalently the principle of Equipartition of Energy.

Slide 18

Molecules are real!



Measurements of the displacements in successive 30s
Intervals of a 0.37 μm particle in water. Rings of radius n x 1.96 μm.
Jean Perrin 1948

$D\gamma = kT$ Einstein Relation

$N_A = \frac{RT}{kT} = \frac{RT}{D\gamma}$ R is the Gas Constant

$\gamma = 6\pi\eta a$ Stokes' Law – from hydrodynamics

$D = \frac{\langle r^2 \rangle}{4t}$ $\left(\langle r^2 \rangle = \langle x^2 \rangle + \langle y^2 \rangle = 4Dt\right)$ Diffusion in 2-D

<r^2> and a measured, the rest are constants

$R = 8.3\ J\ K^{-1}\ mole^{-1}$

$T = 295\ K$

$t = 30s$

$\langle r^2 \rangle = 61.5 \mu m^2$

$\eta = 10^{-3}\ N m^{-2} s$

$a = 0.37 \mu m$

$$N_A = \frac{RT\ 4t}{\langle r^2 \rangle\ 6\pi\eta a}$$

$$N_A = \frac{8.3\ Nm\ K^{-1}\ mole^{-1} \times 295K \times 4 \times 30s}{61.5 \times (10^{-6}m)^2 \times 6\pi \times 10^{-3}\ Nm^{-2}s \times 0.37 \times 10^{-6}m}$$

$= 6.85 \times 10^{23}\ mole^{-1}$ Pretty close, dimensions check out

[molecules are real!] (18)

• These data are from Perrin, 1948 via Nelson's book. But we could just as well have got them live in the lecture from the microscope: this measurement is part of the practical BP01.

  • Note: one particle doing an extended walk (as here) is equivalent to many particles (as in the simulation) if the walk is broken into equal-sized segments and each treated as if it were a separate walk. This is allowed by the **ergodic hypothesis**.

• The **theory was confirmed by measurements** with different particle sizes, viscosities and temperatures – all gave the same answer for kT and N_A.

• Before this calculation, no-one knew how big a molecule was.

Slide 19

Diffusive transport: examples So

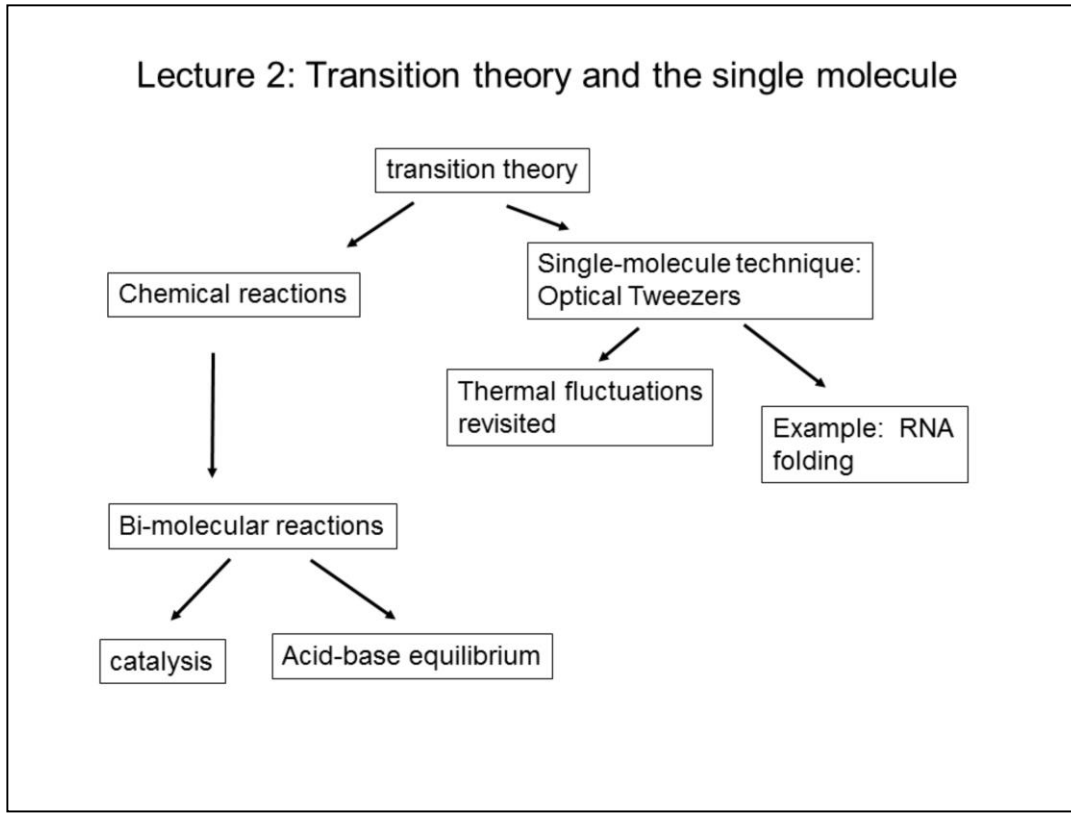
Particle	radius (nm)	D ($\mu m^2/s$)	time to go… ($t = x_{rms}^2 / 2D$) 1μm	1mm	1m
glucose molecule	0.5	700	0.71 ms	12 min	23 yrs
Haemo-globin	3.1	70	7.1 ms	2 hrs	230 yrs
bacterium	500	0.4	1.2 s	15 days	40000 yrs
goldfish	10^8	$2x10^{-6}$	2.8 days	8200 yrs	8 bn yrs

[examples of diffusive transport in biology] (19)

- (not shown) a protein diffuses it's own radius in ~100 ns.
- A small molecule like glucose will travel throughout a bacterium (~1μm) in ~1 ms : bacteria do not need transport systems for nutrients. But a large cell (1mm) might, and a nerve cell certainly will!
- A bacterium will need days to diffuse 1 mm: they may need to swim to get places.
- Diffusion is irrelevant to the motion of macroscopic organisms.

• Note: by stokes' law, D varies as $1/r$. But this is for spheres, and in addition the effective radius of ions and molecules may be larger than their van der Waals radius if they drag a layer of water molecules with them.

• Diffusion is adequate for some biological processes, but **active transport** is necessary for others – molecular motors.



[Reading for lecture 2]

Reaction Rates

•So far we've talked about particles (which could be molecules) and how they are constantly in motion due to thermal agitation.

•Thermal energy can also make them change into something else – "uni-molecular" reaction.

•Or make them collide and react with each other – "bi-molecular" reaction.

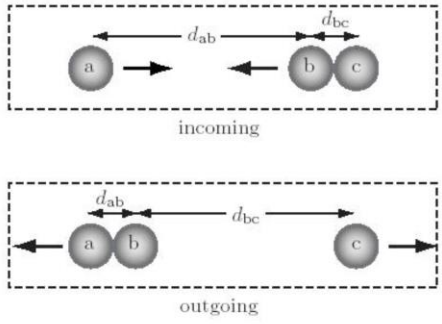
•What can we say about these reactions?

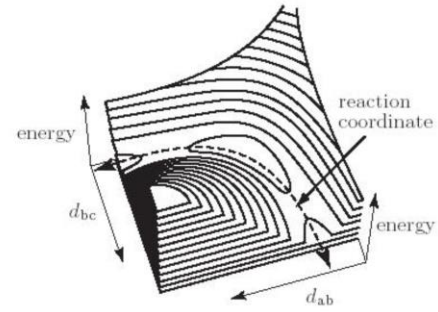
Reading for Lecture 2

- Howard, Chapters 4,5
- Nelson, Chapters 8,10.3,10.4
- (Research papers)

Reaction co-ordinates

Hydrogen atoms swapping places in a molecule,
in a straight line

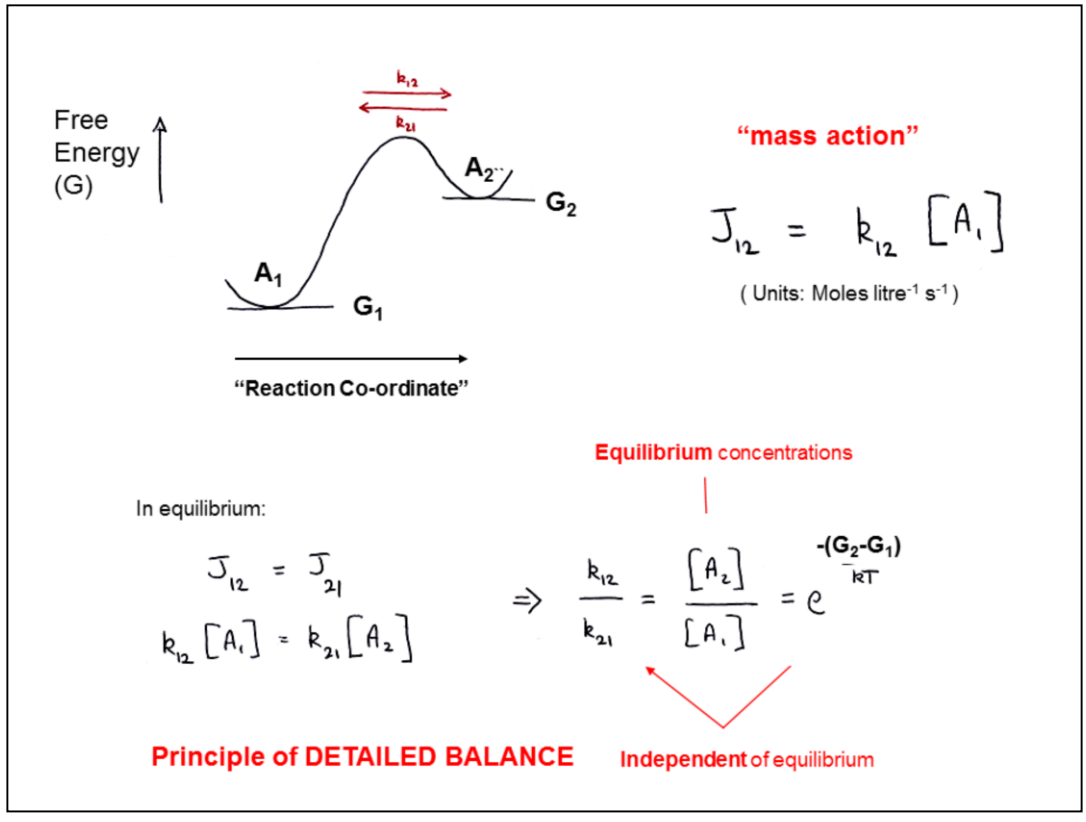




[Reaction co-ordinate] (3)

• This example, three hydrogen atoms in a straight line, has a 2-D reaction space. Most reactions, especially biological ones, have a (very-) many-dimensional space.

• BUT: the path of lowest free-energy between two local minima defines a 1-D **"reaction co-ordinate"** between two **"states"**



• Treating it as **instantaneous** transition between states works if transitions are fast compared to typical lifetimes in each state.

• Law of **Mass action**: Number of molecules that make a transition is proportional to the number that are available. (Sort of obvious).

  • Rate constants in units of s^{-1} so that $k\,dt$ is the **fraction** of molecules in a given state that make a transition in a given time.

  • **Notation**: *[A]* is the concentration of *A* in units **"Molar" = moles per litre**

  • 1 mole is Avogadro's number of molecules

  • Avogadro's number is HUGE and is chosen so that one mole weighs the same number of grams as the sum of atomic numbers for the molecule ("formula weight")

• The law of mass action can be derived from statistical mechanics (Nelson chapter 8), however here we use it as a self-evident starting point.

• Again, the ratio of rate constants is **always** $exp(\Delta U / kT)$, even out of equilibrium.

• The **principle of Detailed Balance** (a.k.a. "Microscopic Reversibility") states that *if a system is in equilibrium then each of its degrees of freedom is separately in equilibrium* (Howard chapter 5). This means that the expression for the ratio of rate-constants holds true for **any** transition between **any** two states, regardless of how the states are defined and regardless of any other transitions that might be possible from each state.

Mass action and Chemical Potential

In general:

$$\frac{J_{12}}{J_{21}} = \frac{k_{12}[A_1]}{k_{21}[A_2]}$$

Mass action, definition of rate constants

$$= \frac{[A_1]}{[A_2]} e^{\frac{-(G_2 - G_1)}{kT}}$$

Using detailed balance for the rate constants

$$= e^{-\left(\frac{G_2 + kT \ln[A_2] - G_1 - kT \ln[A_1]}{kT}\right)}$$

Take concentration terms into the exponent

$$= e^{-\left(\frac{\mu_2 - \mu_1}{kT}\right)}$$

Where we define the "Chemical Potential" : $\mu = G_o + kT\, lnC$

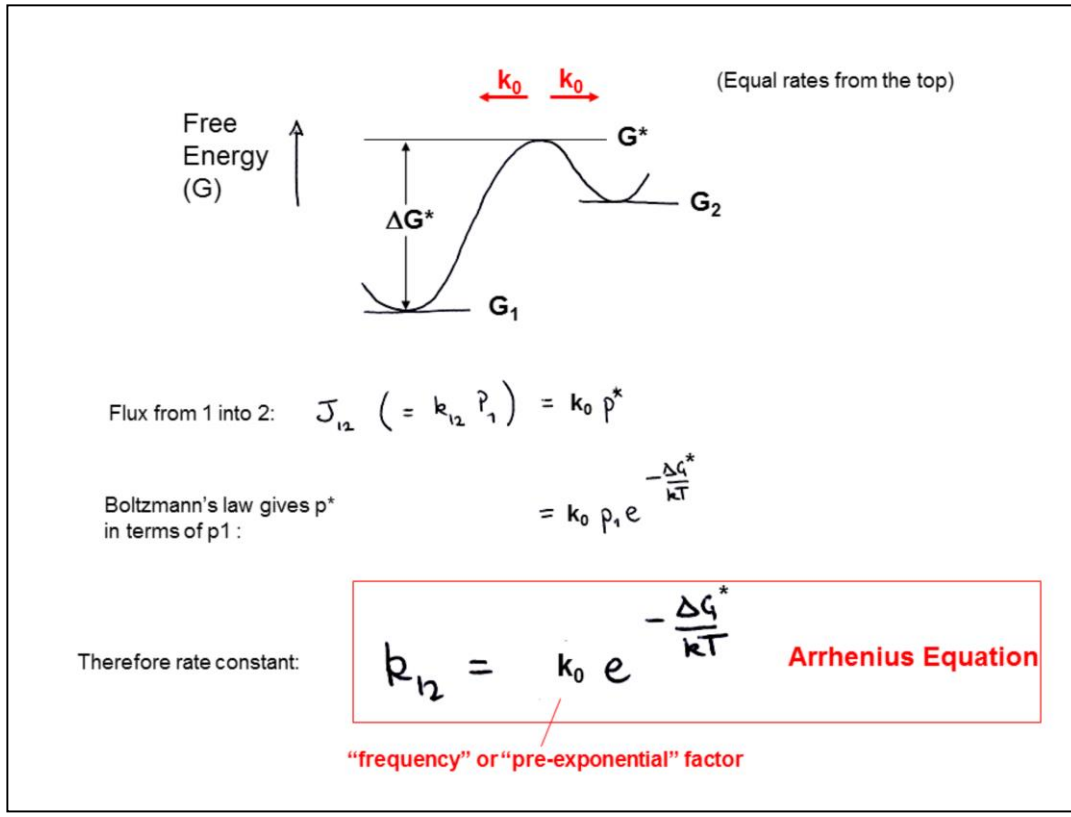
(I've written "C" instead of "[A]" as it's more conventional)

• This is the simplest way to understand chemical potential: it is the equivalent energy that would account for the effect of mass action on the direction and rate of a chemical reaction.

• NOTE: the term *kT lnC* in the chemical potential is the free energy for EACH molecule that makes any transition. Thus *the existence of many other molecules of the same type affects the free energy of each one*. This might sound a bit counter-intuitive, but it illustrates that the chemical potential is really an entropic phenomenon. Nelson (chapter 8) gives a more rigorous treatment.

• As with any energy, it is only **changes** in chemical potential that have any meaning. Thus $\Delta\mu = kT\,(\ln C - \ln C_0) = kT \ln (C/C_0)$ is the quantity that matters, where C_0 is a **standard concentration** at which the chemical potential, by definition, is zero.

• Note that the expression *kT lnC* technically speaking includes the log of a quantity with dimensions, which is not allowed. Using dimensionless (scaled) concentrations, $\Delta\mu = kT \ln (C/C_0)$, corrects things.



Transition state has high energy and low probability => transitions can be treated as instantaneous.

Arrhenius equation immediately satisfies detailed balance:

$k_{12}/k_{21} = exp(\Delta G / kT)$,

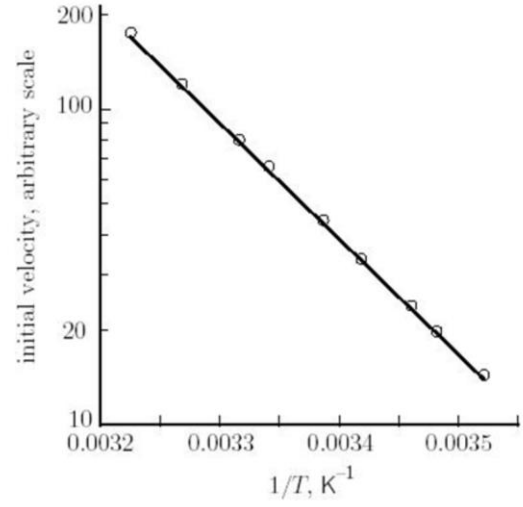
Arrhenius plot

$$k_{12} = k_0 e^{-\frac{\Delta G^*}{kT}}$$ **Arrhenius Equation**

$$ln(k_{12}) = ln\, k_0 - (\Delta G^*/k)\,(1/T)$$

Data for a biochemical reaction, taken from Nelson chapter 10:

The slope gives an activation barrier of $\Delta G^* = 29$ kT



Predicted temperature dependence is verified experimentally

Arrhenius Plot: ln(rate) vs 1/*T*

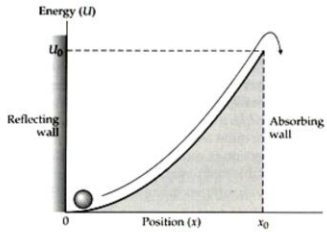
•**What is the frequency factor ?**

Eyring Theory: Quantum mechanical oscillations

$$k_o \approx \frac{kT}{h} = 6 \times 10^{12}\ s^{-1}$$

Appropriate for single-bond transitions, electronic transitions, light absorption

Kramers Theory: Diffusion out of energy well



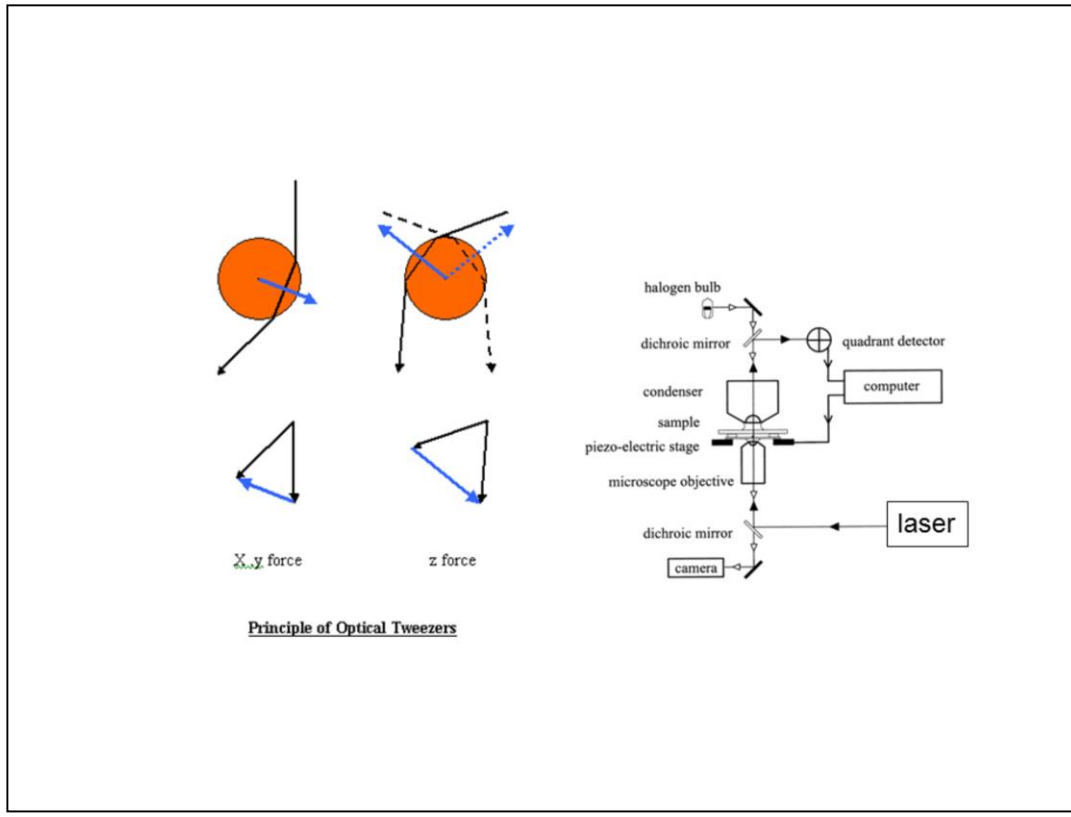
•Solve Diffusion Equation for this potential, particle starting at x=0.

t_0 = mean time to the top
ε = probability to go over rather than come back

$$k_o = \frac{\varepsilon}{2t_o} \quad ; \quad t_o = \frac{\gamma}{\kappa}\left(\frac{\pi kT}{4\Delta G}\right)^{1/2}$$

Appropriate for protein shape changes – except that potentials are not really quadratic.

- Eyring and Kramers theories are both approximate
- Protein rate constants are typically ~ 10^3 s^{-1} , which gives $\Delta G \sim 22\ kT$. (~4 H-bonds)
- In practice, rate constants are measured empirically rather than calculated



Single Molecules

Modern techniques allow individual transitions of single molecules to be observed. One of these techniques is called Optical Tweezers…

• Optical tweezers use a focussed laser beam to trap transparent spheres with radiation pressure.

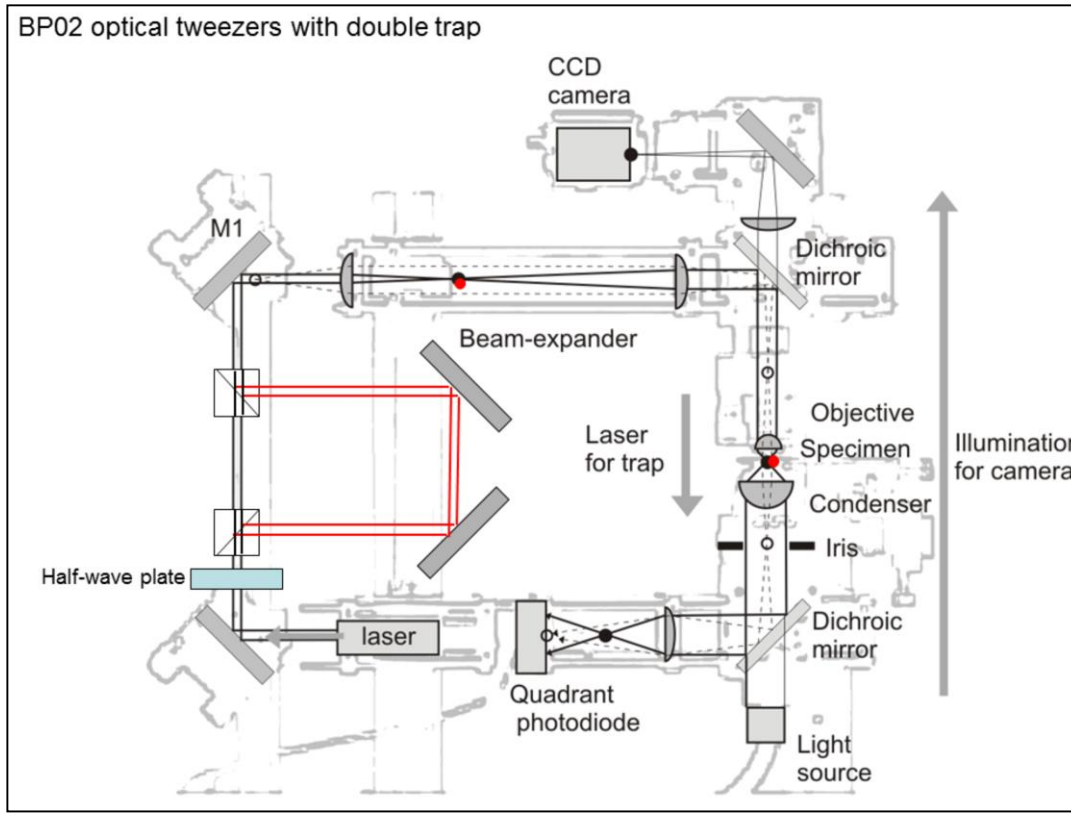
• *Ashkin A. (1997) "Optical trapping and manipulation of neutral particles using lasers". Proc Natl Acad Sci U S A. 94(10):4853-60.*

By the inventor of optical tweezers.

• The technique has revolutionized the study of molecular motors

• *Mehta AD, Rief M, Spudich JA, Smith DA, Simmons RM. (1999) "Single-molecule biomechanics with optical methods." Science 283(5408):1689-95.*

One of many reviews of molecular motors experiments using optical tweezers.

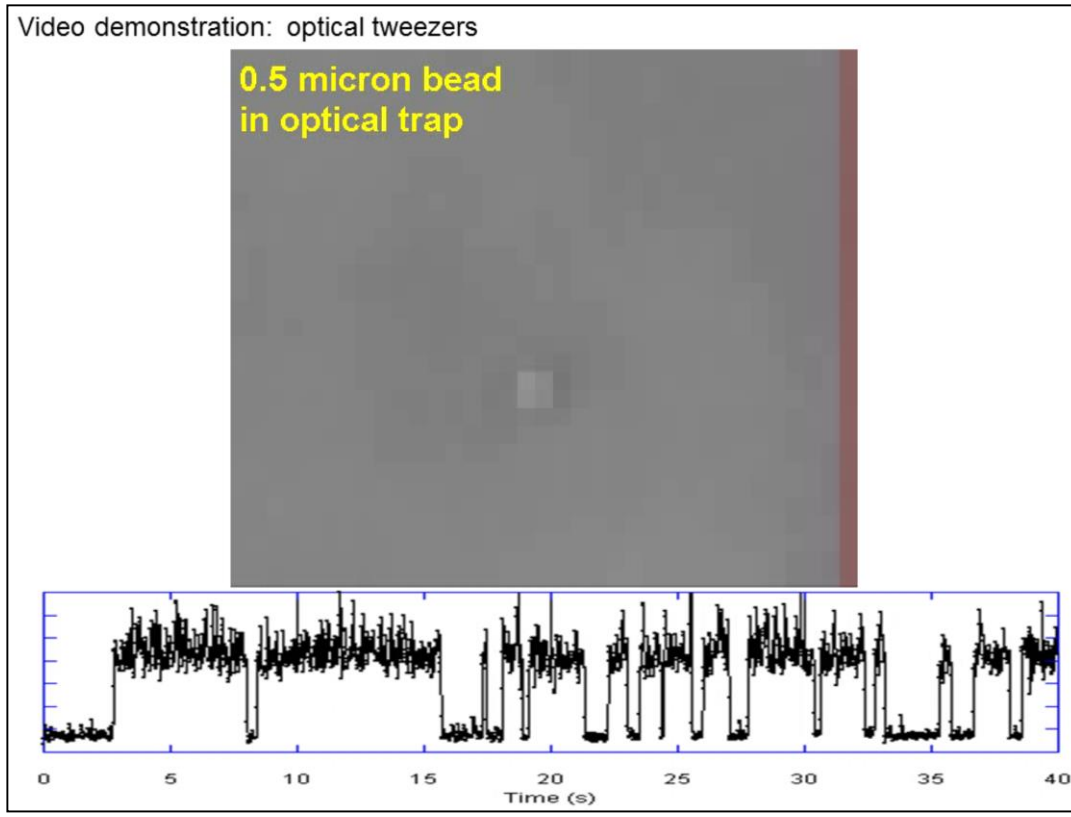


• Forces are in the range of several piconewtons (pN, 10^{-12} N), typical of biological molecular motors.

• Tweezers can also measure nanometre displacements of the trapped particle, typical of the distances that molecular motors move.

• For small displacements, force is proportional to displacement – the trap behaves like a linear spring.

• The lateral deflection of the laser beam by the trapped particle is a measure of the momentum exchanged, and thus of the force and the displacement.



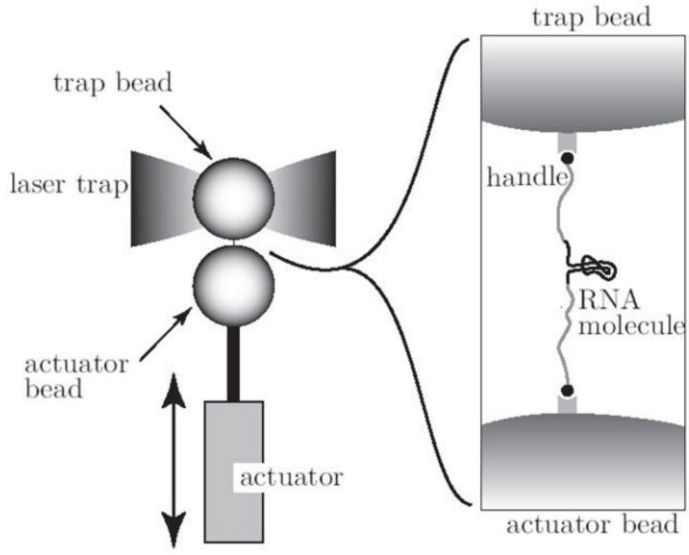
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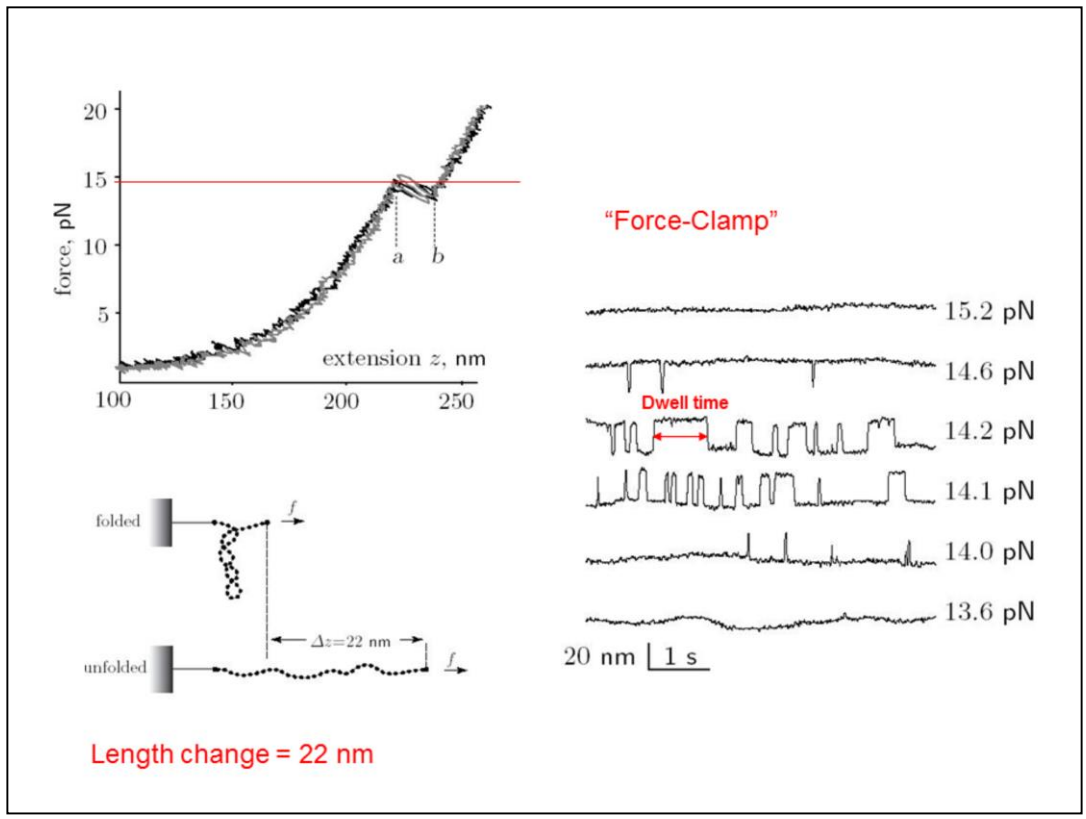
• The lateral deflection of the laser beam by the trapped particle is a measure of the momentum exchanged, and thus of the force and the displacement.

Using Optical tweezers to measure transitions in Single Molecules



Reference: Liphardt J et al, 2001, Reversible unfolding of single RNA molecules by mechanical force. ***Science*** *292:733-737*

- The beads are 3 microns in diameter, the RNA is only about 20nm across
- The handles are double-stranded DNA
- The “actuator” is a suction pipette on a piezo-electric mount.
- In a variant of this method in 2006, the Block lab in Stanford has resolved steps of ONE BASE PAIR taken by RNA-polymerase on a DNA template…

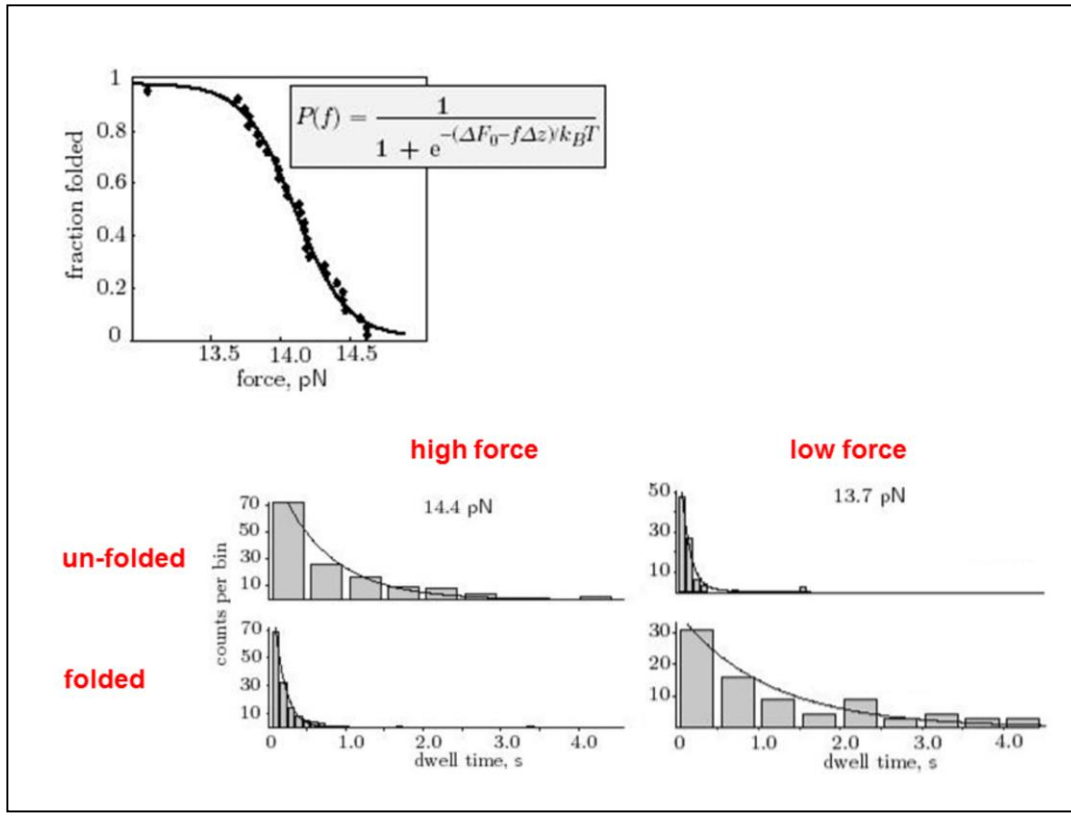


[Single-Molecule data]

• The force-extension curve can be described by the "wormlike chain" model, BUT with a transition at about 14 pN

• The length change is 22 nm, consistent with the end-to-end distance change between folded and unfolded RNA of this length.

• If the force is kept constant using electronic feedback (there are other ways – Block and Wang's passive force clamp), repeated folding and unfolding events can be observed. These are classic barrier-hopping events.



[Free energy, probability and interval distributions]

• The probability of folded vs un-folded obeys Boltzmann's Law, with

$\Delta G = \Delta G^0 - F\Delta z$. That is, the work done against the external force appears as an extra, variable, term in the free energy.

• The time taken before each transition is exponentially distributed, which is the signature of a single transition (see next slide).

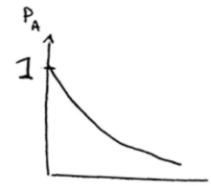
Transient kinetics:

$$A \xrightarrow{k} B$$

Probability of particle still being in state A at time t :

$$\frac{dP_A}{dt} = -kP_A \quad \Rightarrow \quad P_A = A e^{-kt}$$

(b.c. : P(0) = , so A=1)

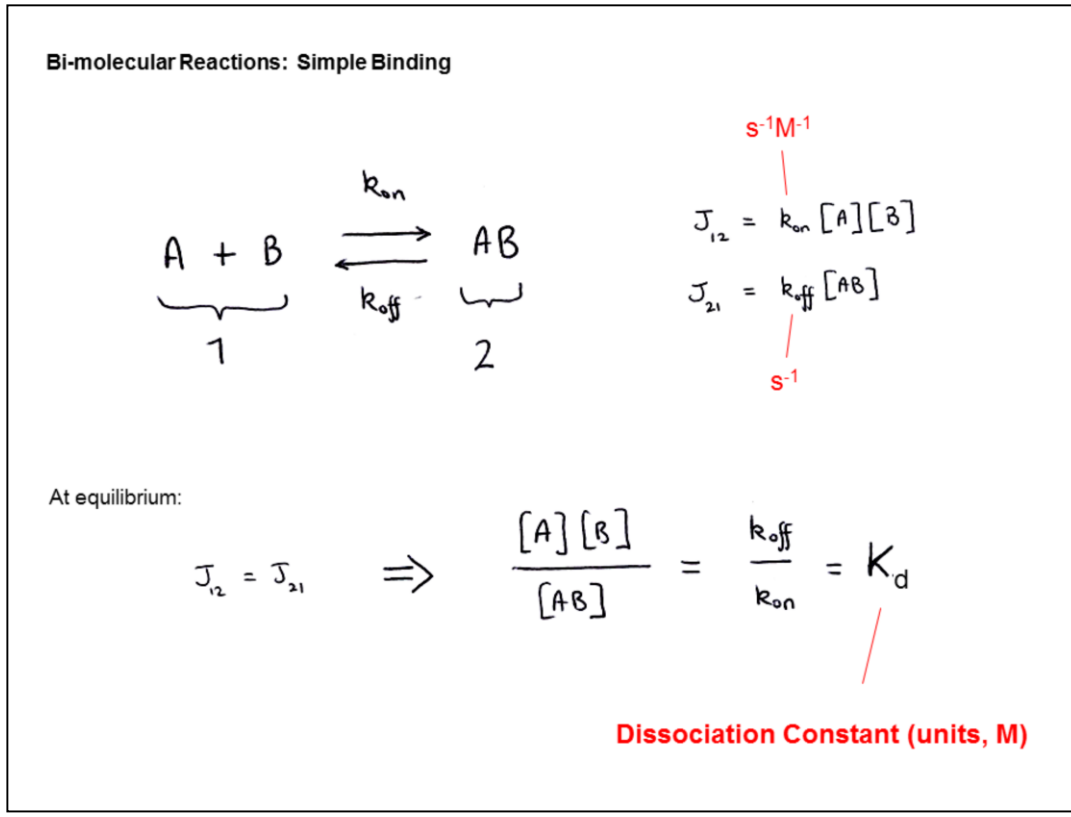


Probability of particle Making a transition between time t and t+dt :

$$P_t(t) = k\,P_A(t)$$

(mean lifetime = $\int t.P_t\,dt = 1/k$)

• A process like this, where there is a constant probability per unit time of a single event occurring, is called a **Poisson Process**.



• (k_{on} has units of $s^{-1}M^{-1}$: "Bi-molecular" rate constant)

• K_d is an example of an "equilibrium constant", which is equal to the ratio of [the product of concentrations on one side of the reaction] / [the product of concentrations on the other side of the reaction] at equilibrium.

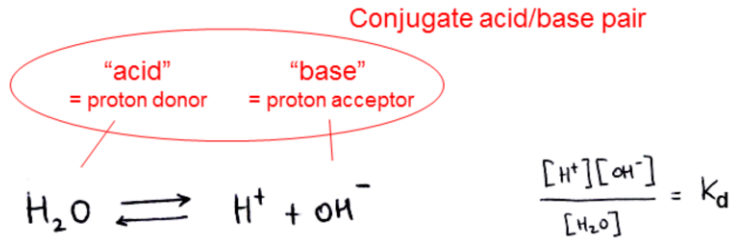
• Diffusion-limited collision rates can be calculated by solving the diffusion equation (see Berg Ch 3, Howard Chapter 5.11): $k_{on,max}=(2kT/3\nu)\,(r_1+r_2)^2/r_1r_2$

  • for equal-sized particles the collision rate is ~8 x 10^9 $M^{-1}s^{-1}$ **independent of particle size**.

• Actual reaction rates are 100-10,000x slower, as molecules still have to surmount the activation barrier after they find each other.

  • Note: part of the "activation barrier" is entropic – the molecules may well have to be aligned properly before they can react.

Water acid-base equilibrium:



In fact, pure water dissociates very little (K_d= 1.8 x 10^{-16} M), and:

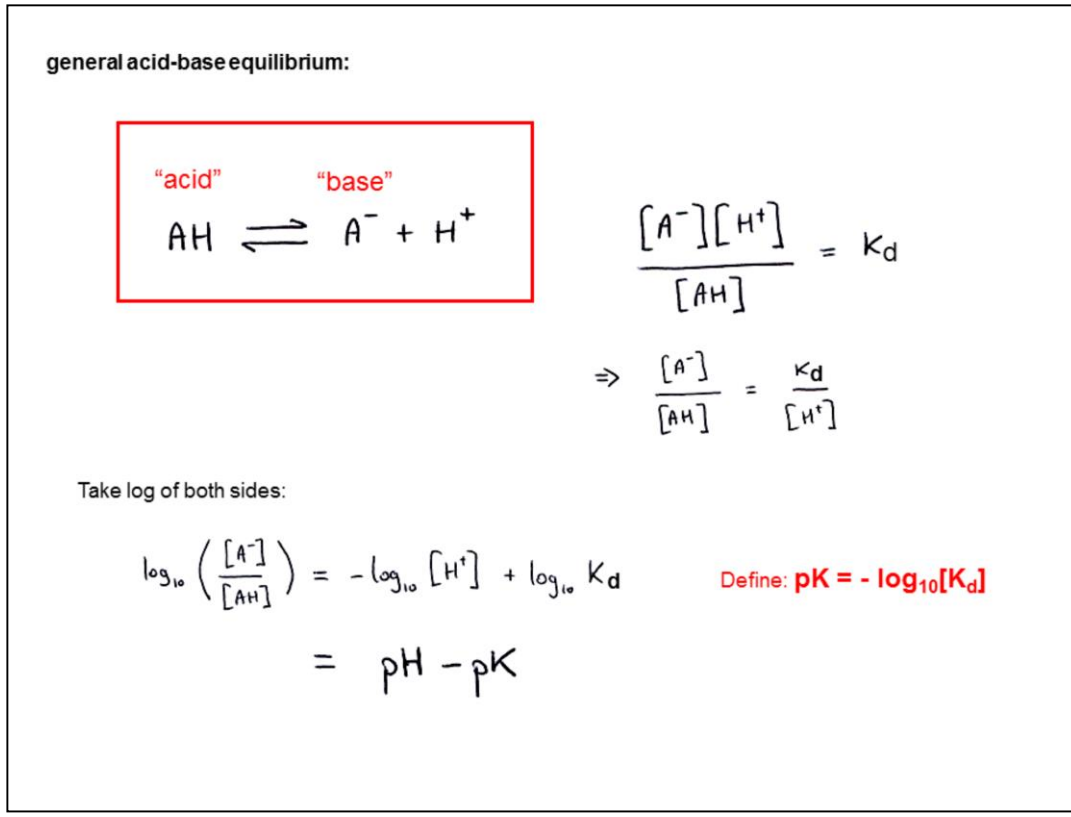
$[H^+][OH^-] = 10^{-14}$ M^2

$[H_2O]$ = 55.5 M

Define: **$pH = -\log_{10}[H^+]$**

(In neutral (pure) water, pH = 7 as there are equal concentrations of H+ and OH-)

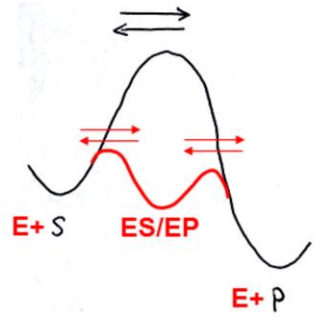
- Neutral water has very low concentrations of H+ and OH-
- However, pH is very important as it dictates whether "charged" molecules really are charged…



- pK is the pH at which a site has equal probability of being charged or neutral.
- High pH = low [H^+] = sites tend to lack protons unless they have high pK
    - High pK sites are more likely to be protonated – eg NH_3^+ not NH_2
    - Low pK sites are more likely to be de-protonated – eg COO^- not COOH
- "Strong acids" like HCl have low pK – nearly all H become H^+, so pH goes low.
- "Strong alkalis" (Na)OH have high pK (~13 for the "base" OH^-, compare slide 13) – nearly all OH^- mop up a proton, so pH goes high.
- "Weak acids" like acetic acid (pK close to 5) act as **"buffers"** at pH values close to their pK.
    - Small amounts of extra H^+ added (as "strong acid" eg HCl) are "mopped up" by small shifts in the fraction of dissociated weak acid.
- Buffering is extremely important in biology: keeps pH steady (usually close to 7) and therefore charged groups on proteins etc in their correct state.

Catalysed Exothermic ("downhill") reaction:

Catalysed reaction:



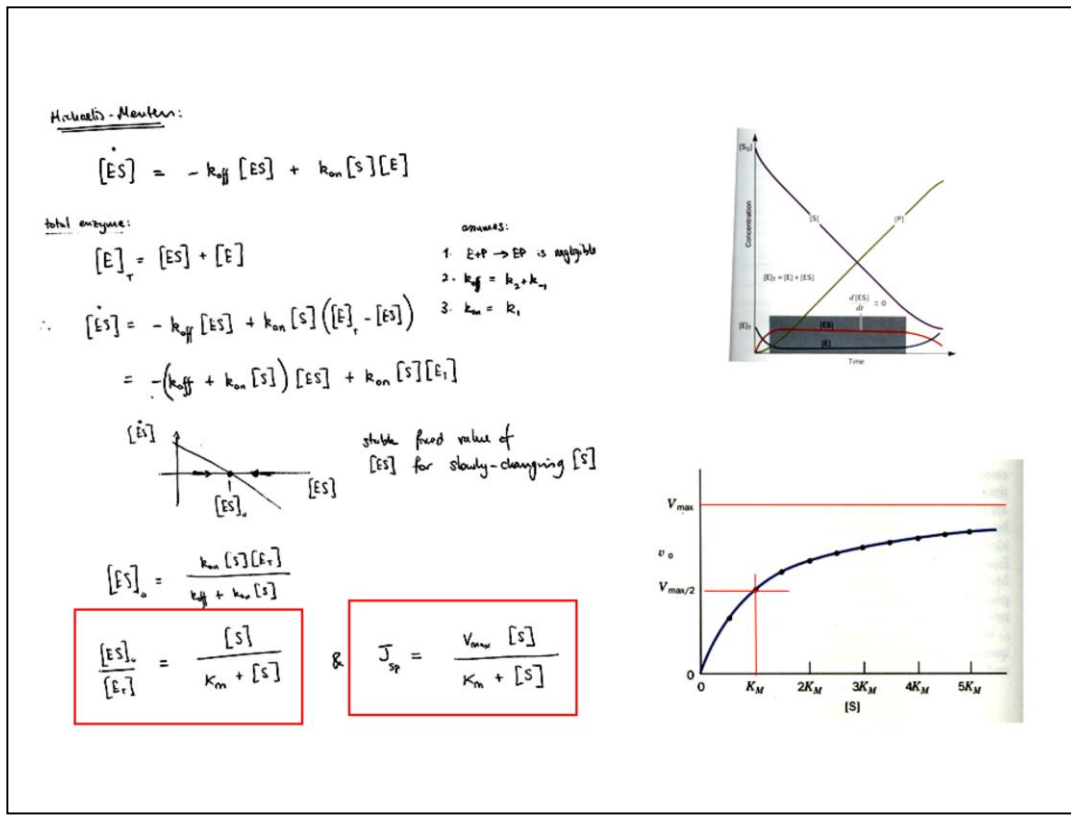
Catalysed reaction: $S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightleftharpoons} P + E$

Assuming negligible rate for reverse reaction : $S + E \rightleftharpoons ES \longrightarrow P + E$

$J_{SP} = k_2 [ES]$

[Catalysis]

- Nearly all reactions in biology are catalysed
- Catalysts stabilize the transition state.
- Usually extremely specific – only the right enzyme catalyses the right reaction.
- Protein machines have complicated mechanisms – can be thought of as a series of catalysed reactions.

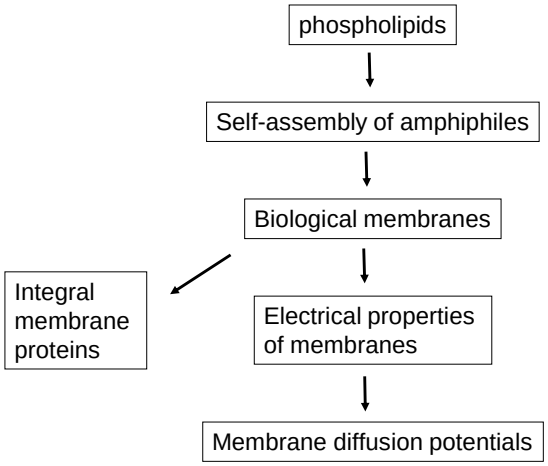


Michaelis-Menten Kinetics (Derivation in problem set)

- This is a typical **saturation** curve
- Same relation for bound fraction in simple binding/unbinding reactions
- As $S \rightarrow \infty$, $J \rightarrow$ Const = V_{max}, proportional to E_t : saturating substrate concentration, all enzyme has substrate.
- As $S \rightarrow 0$, $J \rightarrow (V_{max}/K_m)S$: proprotional to S
- When $S = K_m$, rate is half-maximal, and half of enzyme has substrate.
- Much more complex steady-state kinetics can be done.
    - Many reactants and products
    - Sequences of reactions
- These calculations give overall reaction rates, but DO NOT say anything about details of the mechanism
- HOWEVER, they do allow possible mechanisms to be tested by comparing predicted rates to measured rates.

Slide 1

Lecture 3: Biological Membranes

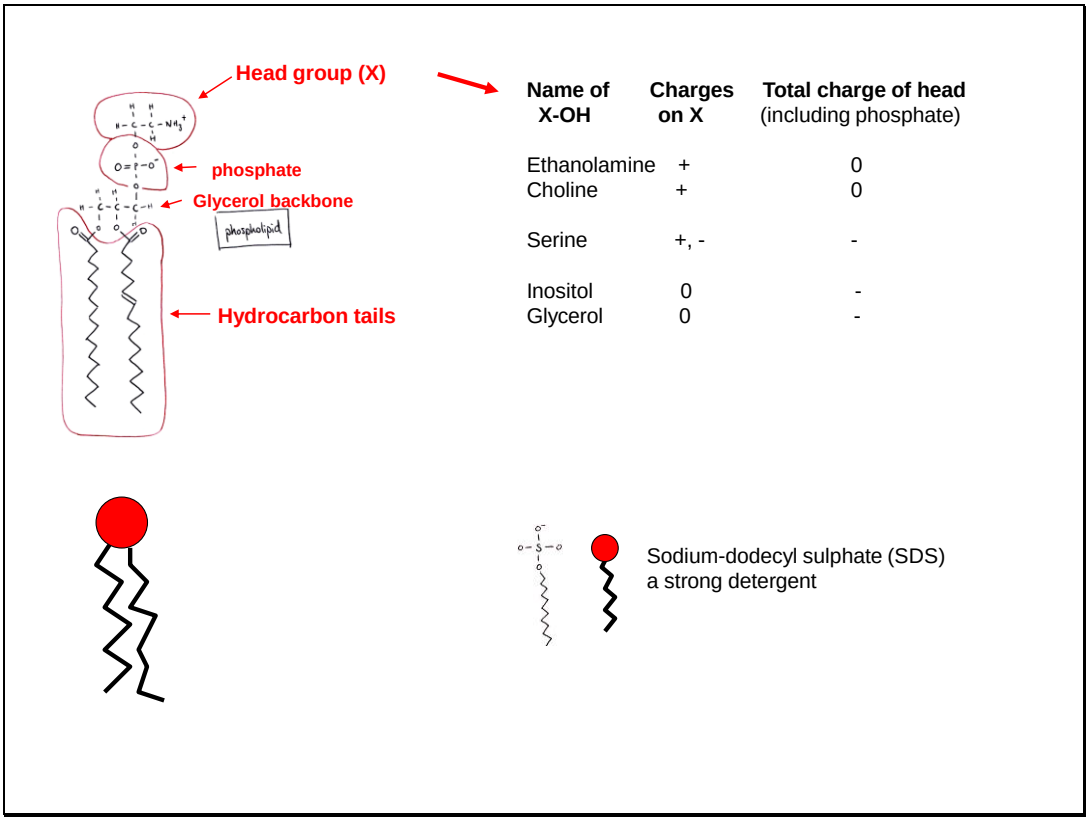


•Biological Membranes are predominantly **phospholipid bilayers**
• Bilayers are self-organized structures
• Purposes of the membrane…
  • Physical barrier – separates inside cell from outside, compartments.
  • Electrical barrier – separation of charges across membrane is primary form of free-energy storage for living cells. (Lecture 4)
  • Large surface area for various reactions and processes.
  • Specialized, for example for transmission of nerve impulses.

Reading for Lecture 3

- Alberts Chapter 10
- Nelson Chapters 7.1,7.2, 8.4,8.6, 11.1
- Jones, R.A.L, "Soft Condensed Matter" 195pp Oxford University Press, ISBN 0-19-850590-6

Slide 3



Self-organization of amphiphilic molecules

• Heads are negative or neutral ("Zwitterionic", one each of positive and negative charges) therefore biological membranes are negatively charged.

- • Tails have even number of Carbons due to synthesis from C_2 units. Range is 12-24, 16-18 are most common.
- • About half of tails contain double bonds, 0-5 in total. Double bonds introduce a 30-degree bend in the tail.
- • Shape and size of head and tail determine how lipids will self-organize when mixed with water.

• Charge of head also matters.

- • Charge-charge repulsion increases effective head size
- • Negative surface charge means that membranes have a negative surface potential.

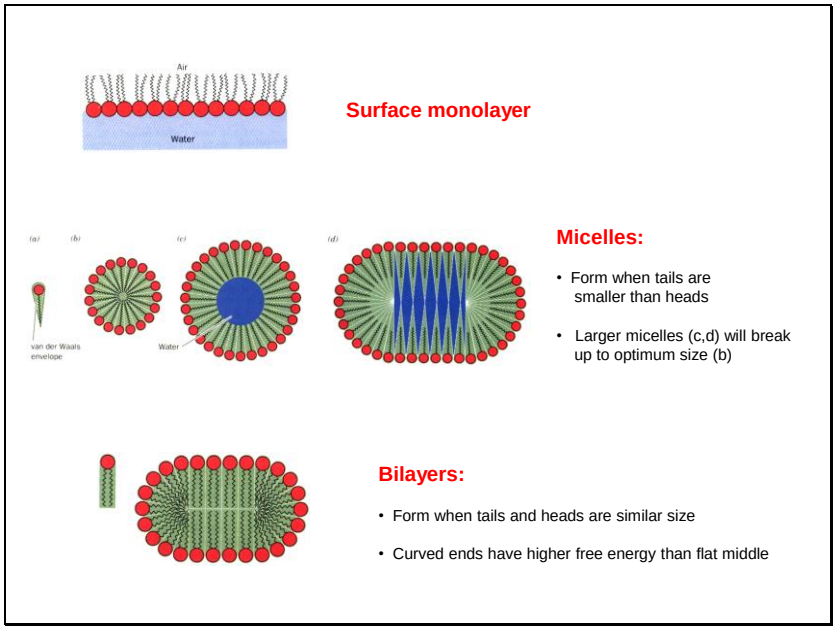
• SDS is another example of an amphiphile – a strong detergent used to disrupt biological membranes and proteins which are held together by the hydrophobic force.

Biological membranes contain a lot of different amphiphiles, not all of which are phospholipids.

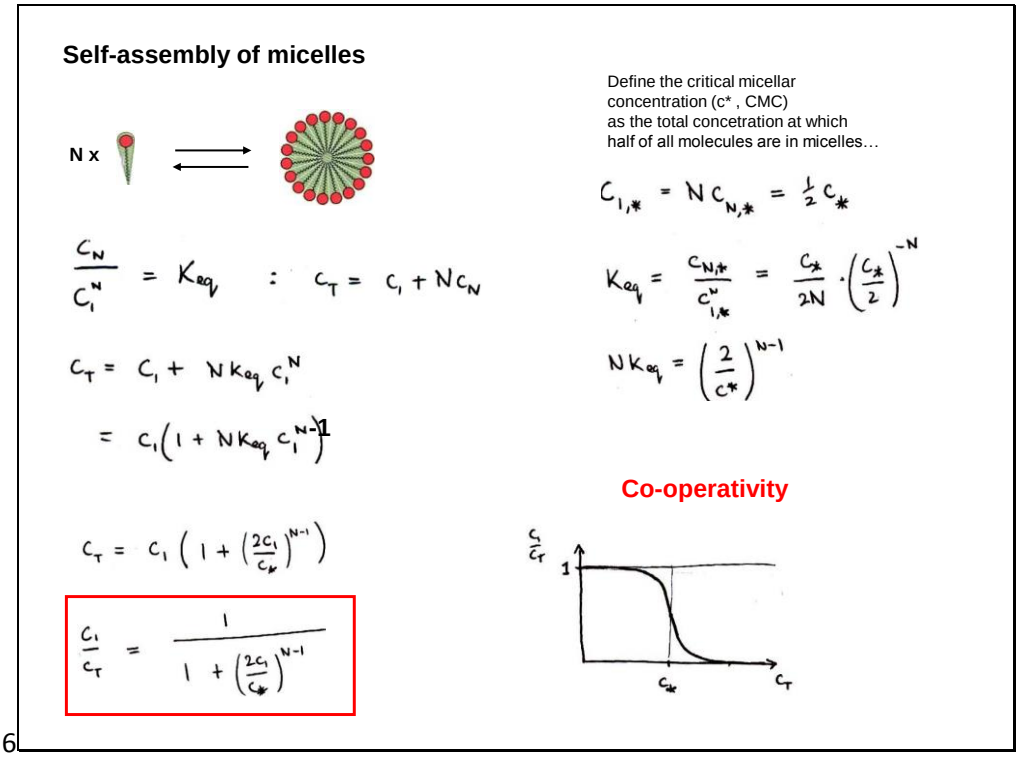
These affect fluidity, curvature and some may be specifically associated with membrane proteins.

The bilayer is spatially organized, with "lipid rafts" that segregate different components probably by self-organization (still slightly new and controversial).

Slide 5



[Some common phases of amphiphilic molecules in water]
• Increased free energy at the edge means small bilayers (as shown here) will aggregate to form extended sheets, minimizing free energy.



Slide 6

•This is an example of a co-operative transition.
- The transition from lone molecules to micelles is steeper the larger N.
- The exponent is often called a “Hill coefficient” in biochemistry.

Slide 7

A simple 3-parameter model

a_o optimum headgroup area

volume v  l_c critical (extended) chain length

Spherical micelle with N molecules:



Volume: $\frac{4}{3}\pi r^3 = Nv$

Surface area: $4\pi r^2 = Na_o$

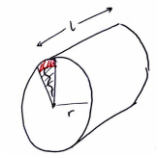
No hole in middle: $\Rightarrow r = \frac{3v}{a_o} \leq l_c$

Condition for Spheres: $\frac{v}{l_c a_o} \leq \frac{1}{3}$

•"optimal head-group area" depends on electrostatic repulsion as well as actual size.

Slide 8

Cylindrical micelle:



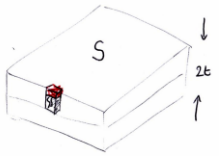
Volume: $\pi r^2 l = Nv$

Surface area: $2\pi r\, l = Na_o$

No hole in middle: $\Rightarrow r = \frac{2v}{a_o} \leq l_c$

Condition for Cylinders: $\frac{v}{l_c a_o} \leq \frac{1}{2}$

Planar bilayer:



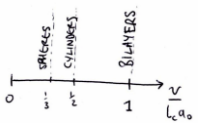
Volume: $2St = Nv$

Surface area: $2S = Na_o$

$\Rightarrow t = \frac{v}{a_o} \leq l_c$

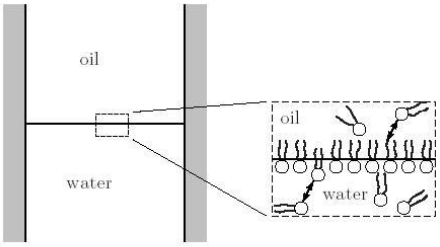
$\frac{v}{l_c a_o} \leq 1$

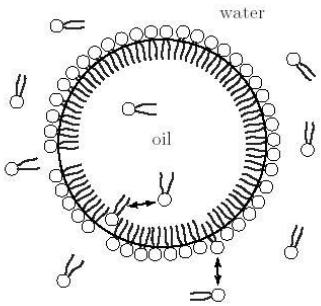
Condition for bilayers:



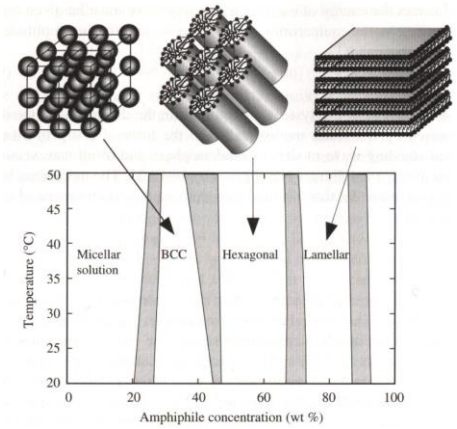
"Phase diagram"

Slide 9





(For more on self-organized amphiphile phases see Jones, Chapter 9.)



[Soap]

• Detergents work by stabilizing the surfaces between oil and water – creating an **emulsion** that allows the oil to be dispersed in water.

• As the fraction of amphiphile increases, new phases are seen

- •Eg. The lamellar phase is found in bars of soap.

•Biological membranes are basically planar bilayers.

• The edges of a bilayer can seal to form a closed "vesicle" or "liposome".

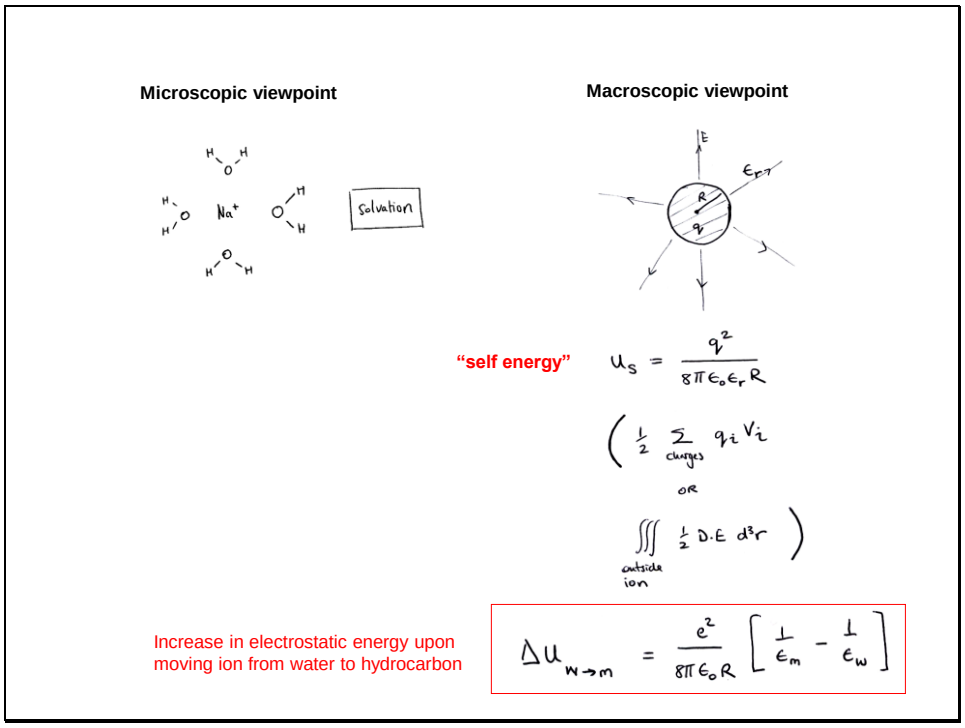
- • A bit like a cell membrane
- • Free energy is reduced by eliminating edges, but may increased by adding membrane curvature
- • For large vesicles, curvature is negligible. For small vesicles, it limits their size.
- • Vesicles have many (potential) uses, eg delivery of drugs.

• Lipid heads do not cross the bilayer, and biological membranes are asymmetrical w.r.t. lipid composition (control of curvature to form desired structures may be one of the reasons for this)

Slide 11

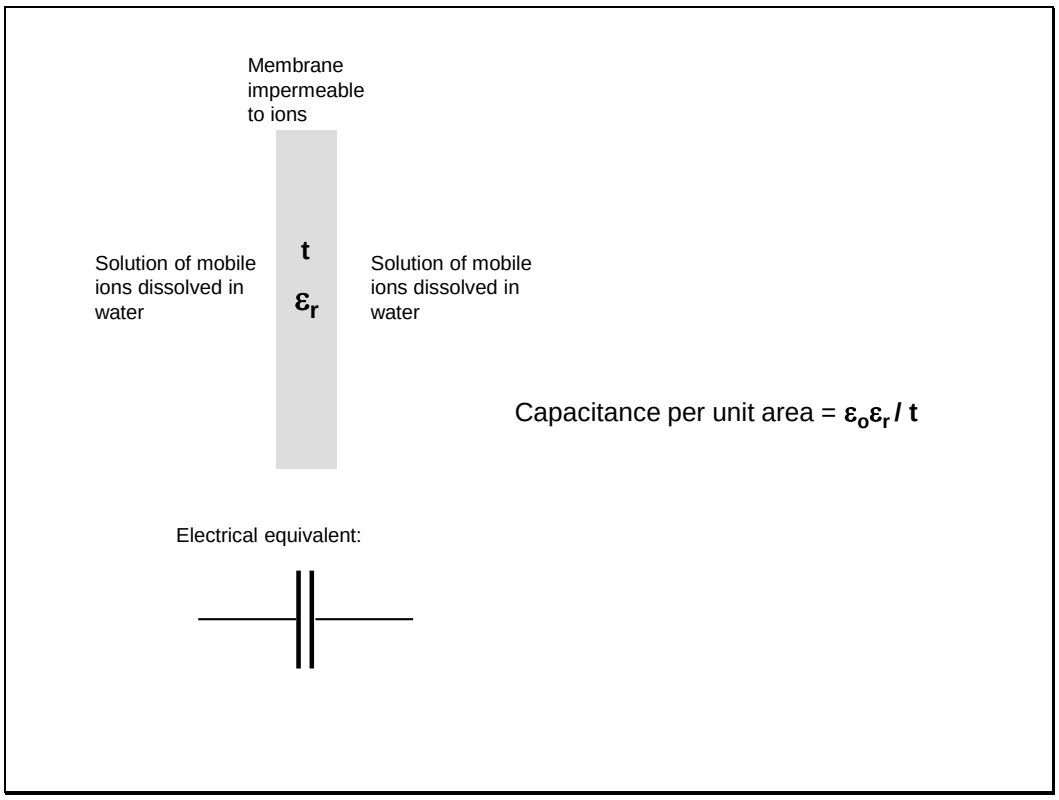
[Biological phospholipid bilayers]
• At “physiological” temperatures (ie. when things are alive and molecules are functional), bilayers form a 2-D fluid.
    • Membrane fluidity is increased by double-bonds in hydrocarbon tails, also by cholesterol.
    • Lipid mobility within each half of the bilayer is high.
    • Proteins etc embedded in the bilayer are also mobile
    •

Slide 12



Electrical Properties of lipid bilayers: Lipid bilayers are EXTREMELY effective barriers to charged molecules, in particular, small ions
• “Self energy” can be thought of as the work required to assemble the charge distribution by gradual charging (integrate *V dq* with fixed geometry) – OR as stored throughout space in the electric field due to the charge (integrate ½ ***D.E***).
• For K^+, *R* ~= 0.2 nm. Dielectric constant of hydrocarbon lipid tails ε_m ~= 2. Therefore hydration energy is ~260 x 10^{-21} J = 65 kT
    •exp(-65) ~ 10^{-28}, so there is essentially no chance of spontaneous partition of the ion into the membrane.
• In both microscopic and macroscopic views, it is re-orientation of water molecules that is responsible for the hydration energy of ions and therefore for the high energy for transfer to the membrane
    • In fact, the first “hydration shell” of water molecules is the most important, and thus the microscopic view is necessary for accuracy.

Slide 13



[Membrane Capacitance]

• The membrane is an insulating plane between two conducting solutions, ie) a capacitor.

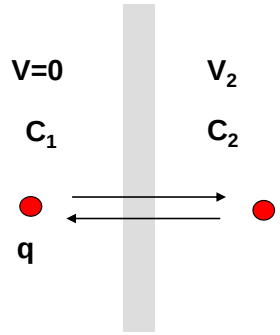
• The specific capacitance of the membrane is relatively high because it is only two molecules thick.

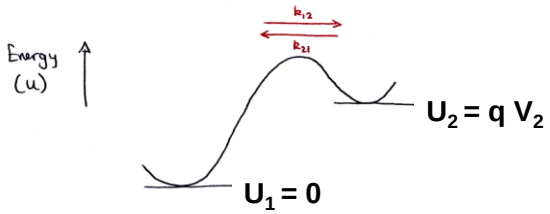
• (For membrane calculations, it's useful to remember that an infinite plane of charge produces a uniform electric field, from Gauss Law).

Slide 14

"Nernst" or "Diffusion" Potential

If ions are allowed to cross the membrane:





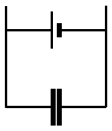
At equilibrium: $C_1 k_{12} = C_2 k_{21}$

Nernst Equation

$$\frac{C_2}{C_1} = \frac{k_{12}}{k_{21}} = e^{-\frac{qV_2}{kT}}$$

(Boltzmann)

Electrical equivalent:



$V_2 = (-kT/q)\ ln\ (C_2 / C_1)$

•Despite the relatively high membrane capacitance, the number of charges that need to move to establish a typical membrane potential is VERY SMALL compared to the number present in a typical solution.

•

• Biological membranes usually have a negative surface potential due to a negative surface charge. The balance between electrostatic and entropic forces on dissolved ions near charged surfaces is treated by Nelson, chapter 7.4, 11.1. You should be aware of the concepts of the **Poisson-Boltzmann equation**, **charge screening** and the **Debye length**.

Slide 15

Nernst Equation

$$\frac{C_2}{C_1} = \frac{k_{12}}{k_{21}} = e^{-\frac{qV_2}{kT}}$$

We defined chemical potential as $\mu = G_0 + kT\ lnC$

For ions crossing a membrane, the ions stay the same so G_0 is a constant which we can set to zero…

thus $\Delta\mu = kT\ ln\ (C_2 / C_1) = -q\ V_2$

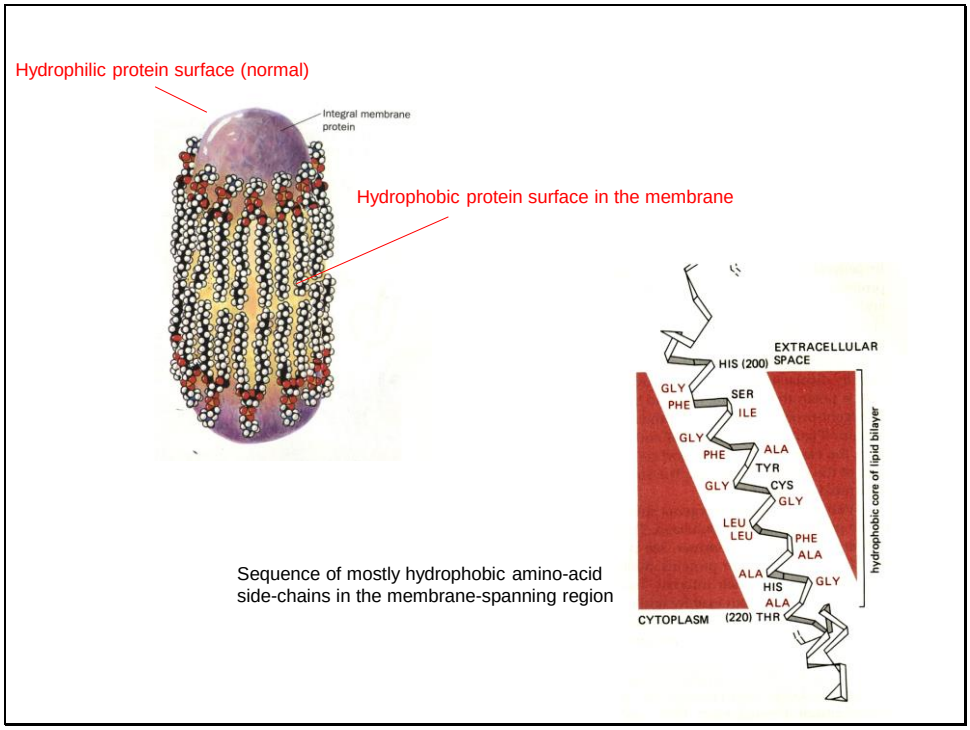
The Nernst equation represents equilibrium between electrical and chemical potentials

•"chemical potential" is essentially a way to formulate mass action as an equivalent free energy, via Boltzmann's law.

•

• Chemical and electrical potentials contribute (add) to the free energy of a "state"
    - The sum of electrical and chemical potentials is called the "electrochemical potential".
    - The Nernst potential is simply the *electrical* potential when the *electrochemical* potential is zero
    - Electrical and chemical potentials are the two forces that can drive ions across the membrane. The first is electrostatic, the second is entropic

Slide 16



Membrane Proteins

• Total isolation of a cell is not an option – the membrane must be made permeable.

• Hydrophobic amino acids at the surface of proteins will allow them to insert into the membrane (ie. proteins can be amphiphilic too).

[Integral Membrane Proteins] (19)

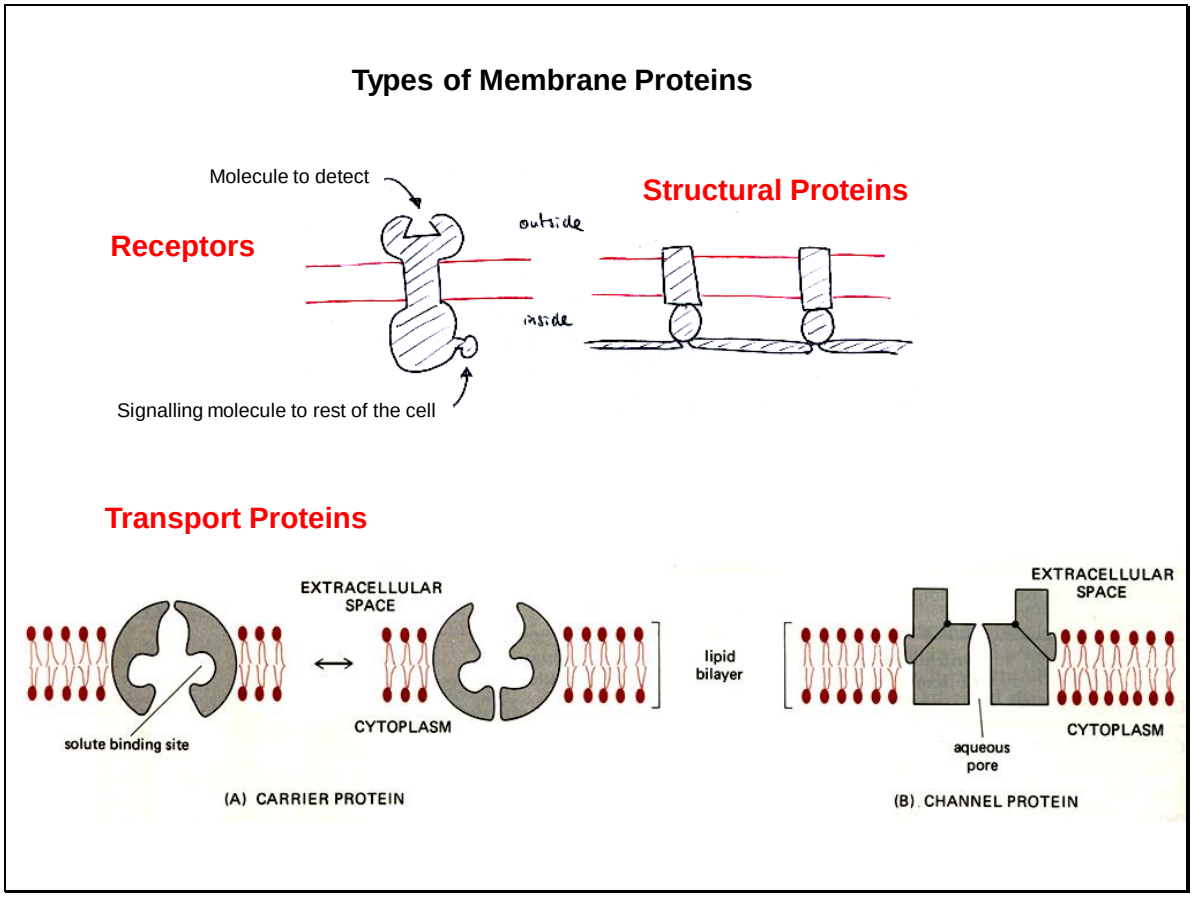
• How do they get there? (The hydrophilic parts on the outside have to cross the membrane)

- Actually inserted into membranes while being synthesized, before folding
- A "Signal Sequence" of ~20 amino acids at the N-terminus identifies membrane proteins to the machinery that does this, and is later chopped off.

• Membrane spanning segments are often alpha-helices or beta-barrels

- Backbone H-bonds are all satisfied within the secondary structure – no loss to put them in the membrane
- Side-chains all stick out – makes a hydrophobic rod or tube.

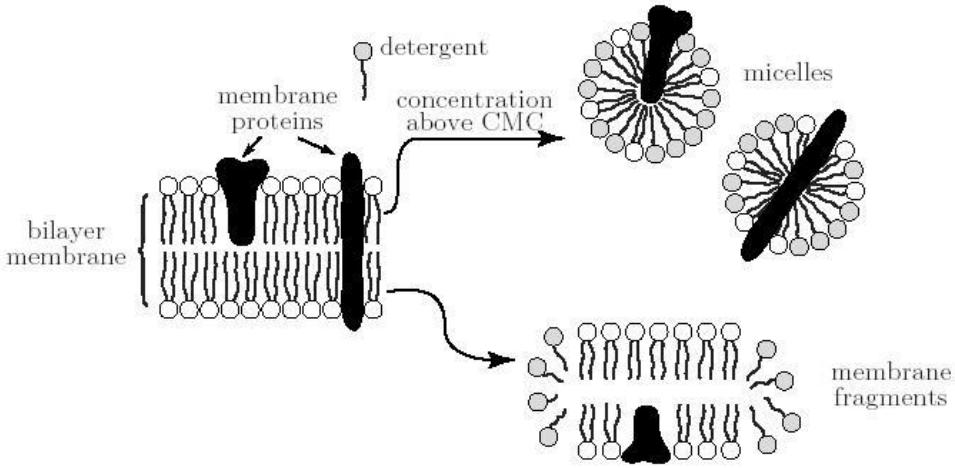
Slide 17



- Much more in section 4 of the course, a bit more in this section.

Slide 18

Detergents are used to purify membrane proteins…

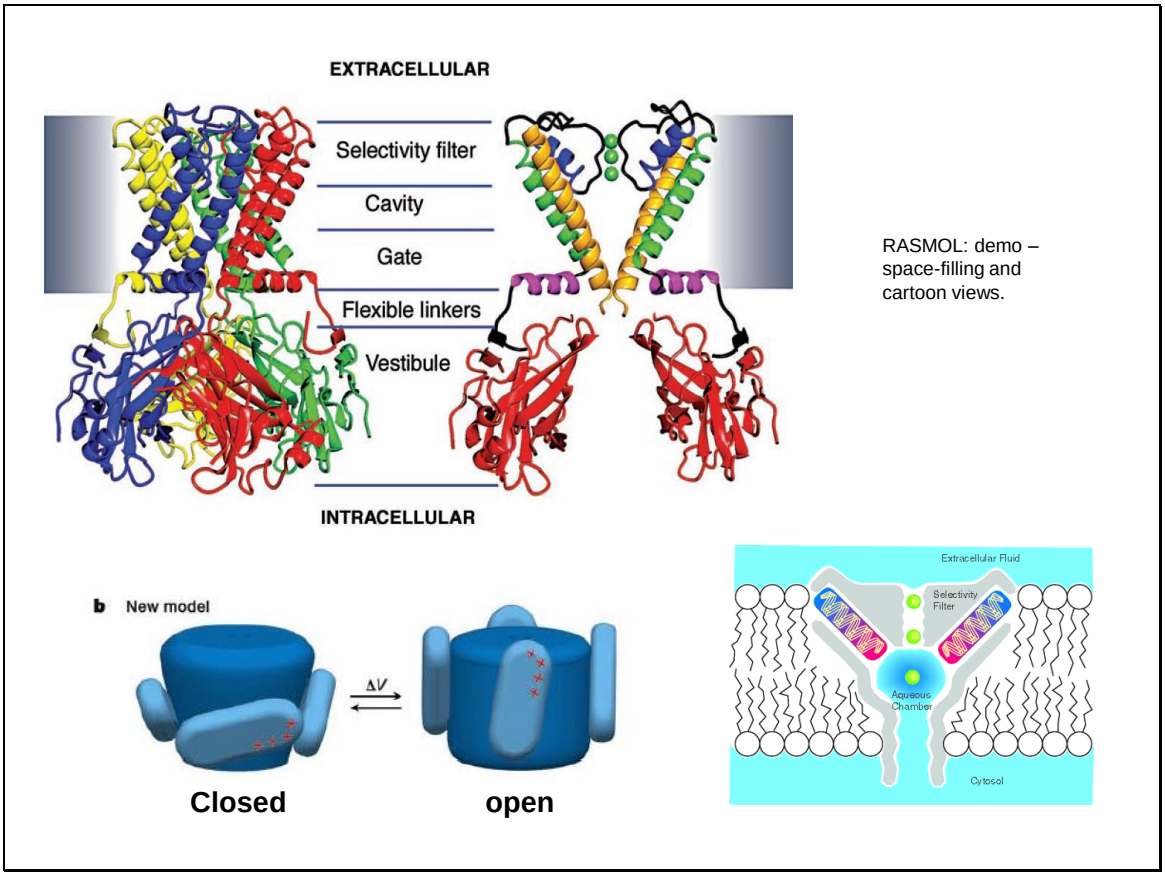


•Detergents allow the hydrophobic membrane-spanning part of a membrane protein to stay folded out of a membrane.
• Despite this, there are relatively few crystal structures for membrane proteins. This is because they depend upon the membrane for their correct structure.

number of different types of protein x-ray crystal structure hits in protein databank…

Date	search term integral membrane protein	membrane protein"	protein
26/8/06	132		30,626
22/10/13	252	2262	59,213

Slide 20

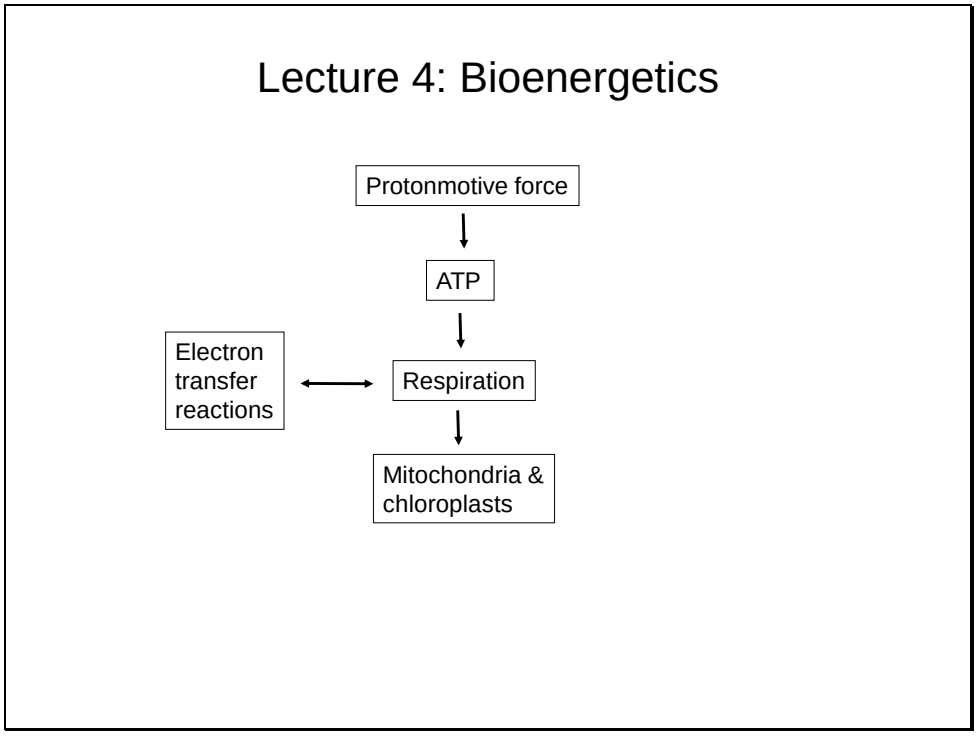


RASMOL: demo – space-filling and cartoon views.

[Potassium channel crystal structure]

- *KcsA – a small, simple bacterial K^+ channel. Typical of K^+ and Na^+ channels.*
- *water-filled cavity with short "selectivity filter" – to minimize energy barrier of membrane.*
    - *Also, alpha-helix dipoles and*
    - *H-bonds from water and protein backbone in selectivity filter.*
    - *Nb: This is probably the closed structure – opening by twisting the helices.*
- *Multiple ion occupancy in single-file with water molecules*
- *"Molecular Dynamics" –Giant computer simulations to try and work out the details of the conduction mechanism (rates ~10^7 ions per second).*

Slide 1



Reminder from the introduction…

•2nd law of thermodynamics: Entropy Increases

•Life **Decreases** it's own entropy – at the expense of the rest of the universe

- •Most globally – takes photons (low entropy – straight line !) and converts them ultimately into heat (high entropy), with all of life in-between.

•To do this, life needs to gather, store and manipulate sources of **Free-Energy**

- •Free energy can be thought of as the "**currency**" of life. Any reaction that requires free energy input (eg. making DNA from nucleic acids, doing mechanical work, building a protonmotive force) must be "paid for" by coupling to a reaction that releases free energy.

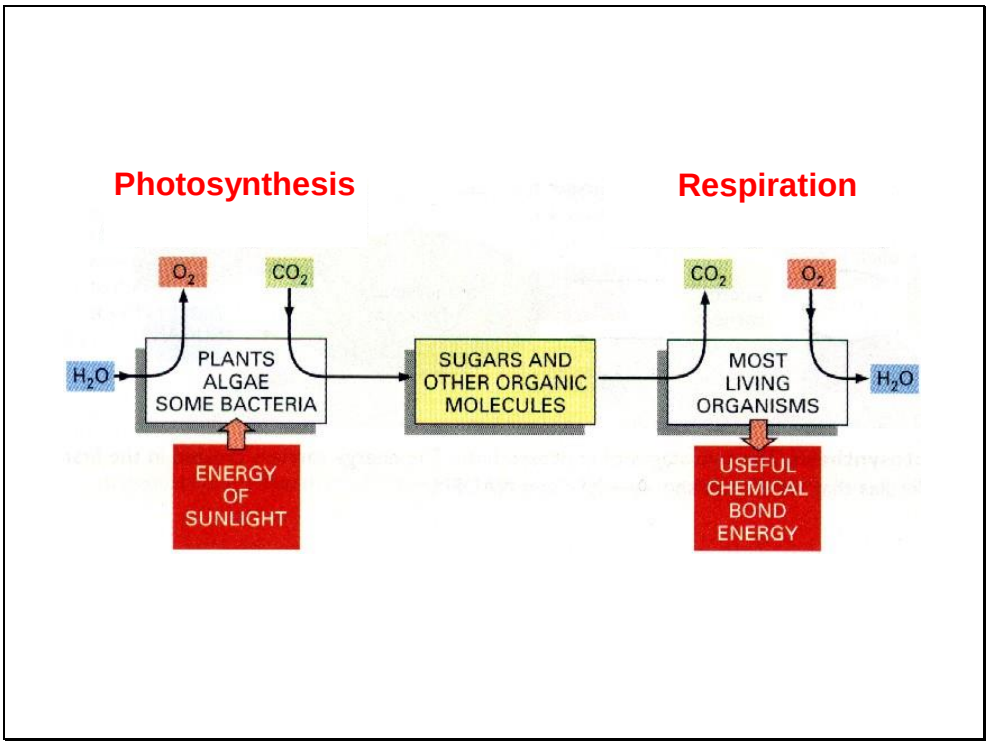
•**This topic**: Types of **biological free-energy**, ways and mechanisms in which they are interconverted. These processes are essentially what life is.

•Bioenergetics is a whole lecture course in itself. Here we will only give a very brief overview.

Reading for Lecture 4

- Alberts Chapters 2, 11,14
- Nelson Chapters 11

Slide 3



[life: the overall chemistry]

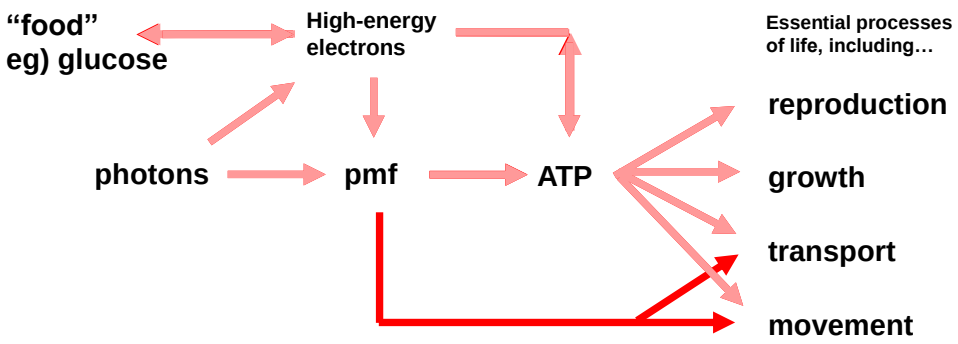
•Free energy is handled by chains of coupled chemical reactions or processes, each of which passes free energy from one form to another.

•Many of the reactions are called “Electron Transport” reactions, because they can be described in terms of the transfer of electrons between molecules with different electron affinity.

- •Respiration: overall, uses conversion of food + oxygen to CO_2 and H_2O to transfer electrons from molecules where they have High free-energy (low affinity) onto molecules where they have low free energy.
- •Photosynthesis: overall, uses light energy to transfer electrons from molecules where they have low free-energy (high affinity) onto molecules where they have high fee energy.

Slide 4

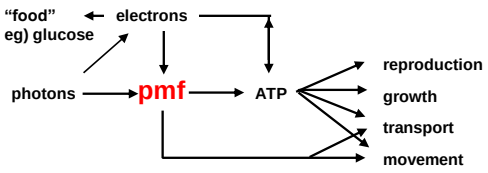
An overview of bioenergetics: Free Energy flow in living things



But some processes are driven directly
by the protonmotive force

Slide 6

Protonmotive Force (pmf)



Electro-chemical potential (gradient) of H^+:

Pmf = $\Delta\mu^*$ = $V_m + \Delta\mu / e$ = $V_m + (kT/e) \ln (C_i / C_o)$

Sign convention: inside minus outside

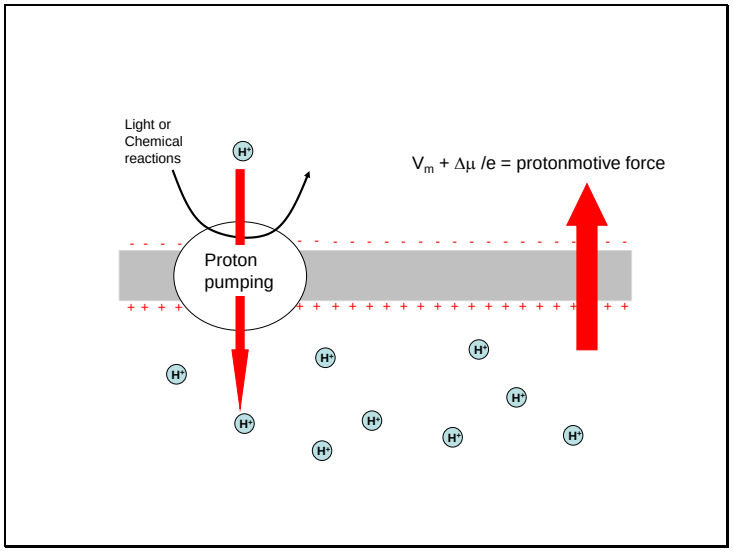
Units are Volts:
Pmf is the extra free energy per unit charge inside the cell versus outside

V_m is the electrical potential (defined as free energy per unit charge)
$\Delta\mu$ is the chemical potential (defined as free energy – see lecture 2)

Typically, pmf is in the range -150 mV to -200 mV
Usually **both** components are negative: Inside has lower voltage and lower $[H^+]$

- Electrical potential plus concentration gradient, H^+ or "protons".
  - nb. Nernst potential is the voltage when pmf is zero, at equilibrium.
  - Pmf is a measure of how far from equilibrium the membrane is –the "driving force" for proton transport across the membrane.
- Generated by active transport of protons across the membrane
  - Free-energy sources: absorption of photons, break-down of food.
- pH gradient (chemical potential) is necessary if the pmf is to do significant work
  - Very few protons need to be pumped to establish the membrane voltage, BUT…
  - Just like charging a battery, you need to provide current as well as voltage.
  - pH gradient also increases the free energy per proton –diffusion as well as voltage drives protons.

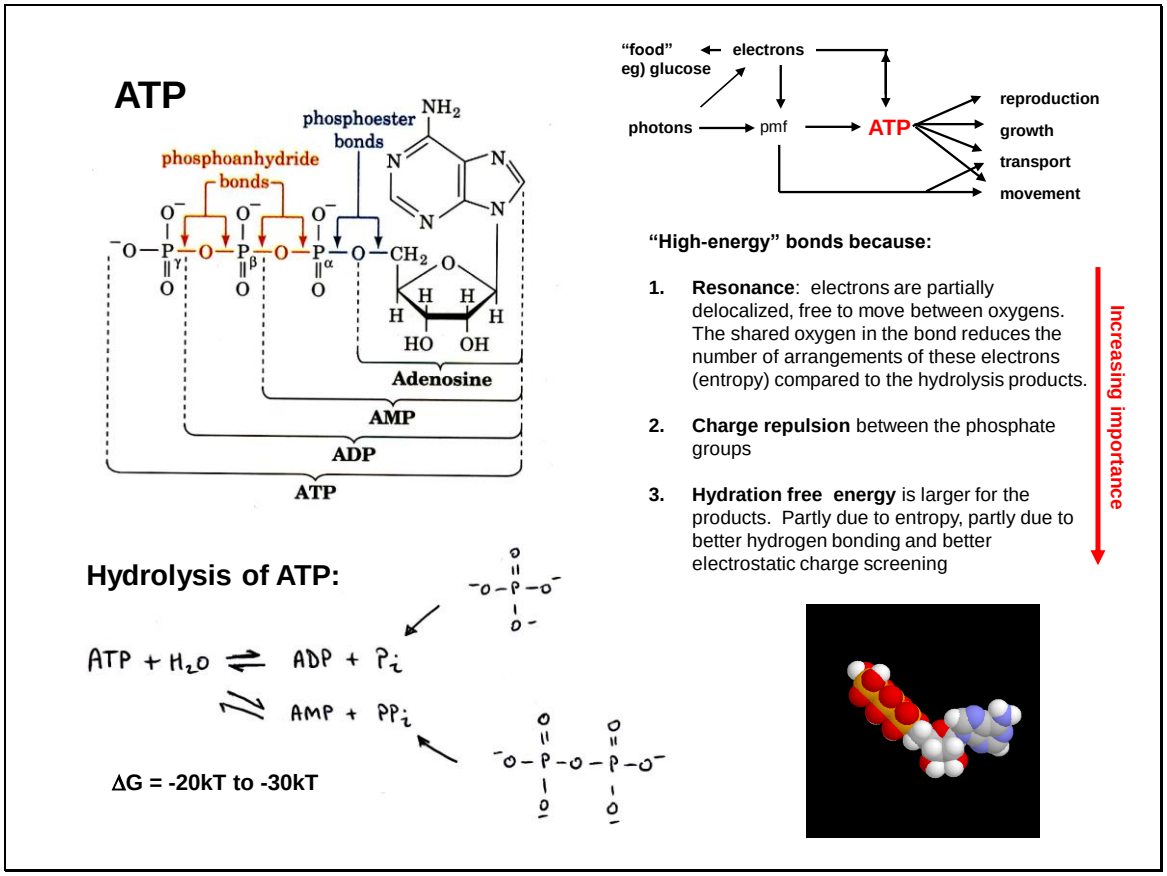
Slide 7



•Numbers:
- •kT ~ 25 meV: so 150 mV ~ 6 kT per proton.
- •“e”-fold concentration ratio (~2.7-fold) ~ 25 mV ~ 1 kT per proton.

•Pmf is used directly to drive some processes
•Mainly, it is used to generate ATP, which drives most processes.
•The central role of pmf in bioenergetics is known as the **Chemi-osmotic Hypothesis.**

Slide 8



ATP / ATP hydrolysis

•The **free-energy currency** of the cell. Each day a human will synthesize and use ~100 kg of ATP: each molecule turns over ~2500 times per day.

•Biochemical standard free energy of ATP hydrolysis:

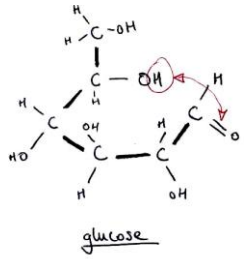
- $\Delta G_0' =$ 30 kcal / mole ~ 18 kT /molecule
- •Standard conditions: 25°C, in water at pH 7, 1 atmosphere pressure, all chemical species at 1 M concentration.
- •Actual free energy in living cells is nearer 20-30 kT, but it depends upon the concentrations of ATP, ADP, Pi etc.

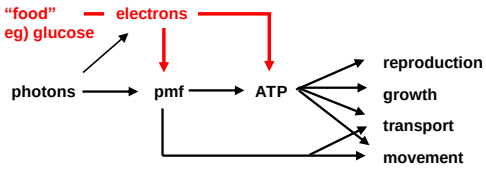
•$[Mg^{2+}]$ is also important. ATP has charge 4^-, ADP has charge 2^-, usually they are electrostatically bound to one magnesium ion (Mg^{2+}).

•Some processes use **GTP** instead of **ATP.** (**G** instead of **A** as the base.)

Slide 9

Respiration

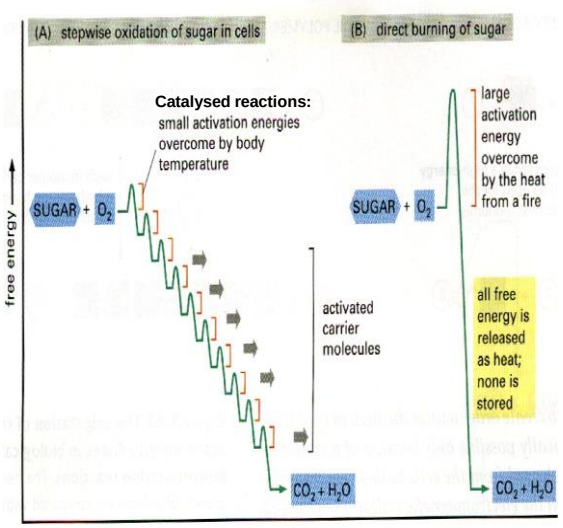




Burning Glucose:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

$\Delta G^{0'}$ = -1160 kT



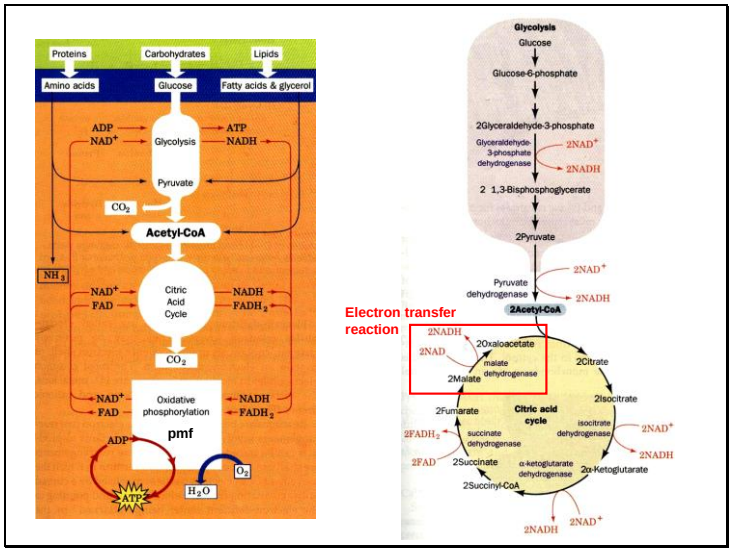
Respiration

- This is the process of generating ATP by combining food with oxygen. It procceds in three stages...
  - Glycolysis
  - Proton pumping
  - ATP synthesis
- 1 and 2 are **Electron-transport reactions**
- 2 and 3 together are called "oxidative phosphorylation" because phosphate is added to ADP and oxygen is used up.
- 3 is performed by a rotary molecular machine, ATP-synthase.
- [Glucose, burning and glycolysis] (9)
- Glucose is the free energy currency of the body in the same way as ATP for the cell…
  - Circulates in the bloodstream, organs like the brain require a constant supply from elsewhere in the body.
- Other types of energy-storage molecule.
  - Poly-saccharides: eg glycogen in the liver, stocks last about half a day
  - Fat: Long-term energy storage
  - Protein: In extreme conditions (starvation)
- **Gradual release** of the free energy of glucose in **many steps** allows it to be used for many processes before eventual release as heat.
- Isothermal process, NOT a "heat engine"

Slide 10

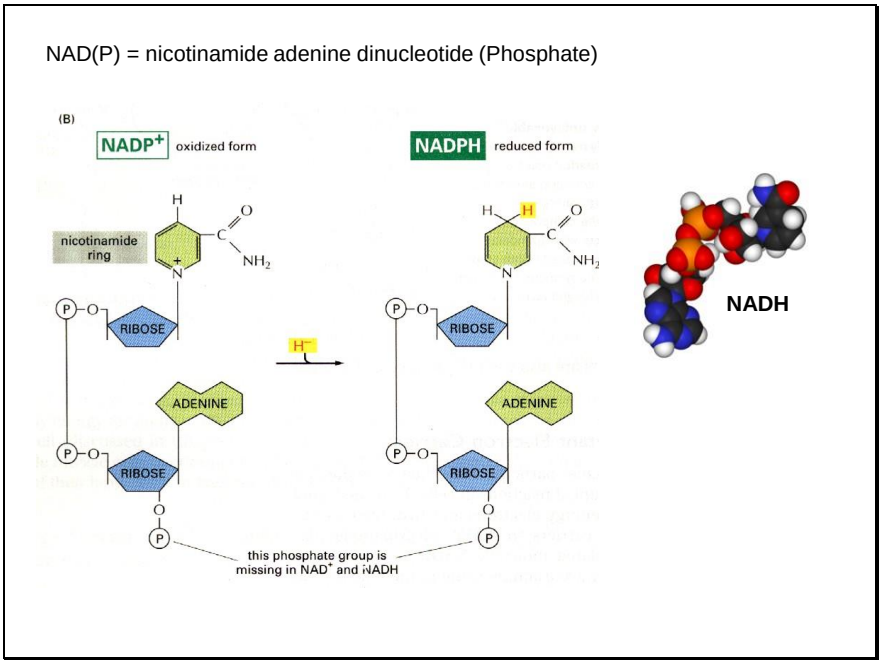
How is this done?...
•Biochemists have worked out most of the reactions that are common to most or all organisms, and many of the enzymes or molecular machines that catalyse them.
•*We will focus on one reaction: to make sure we have an idea what the "fabric" of a metabolic network is. Note: the details of the chemistry are off-syllabus.*

Slide 11

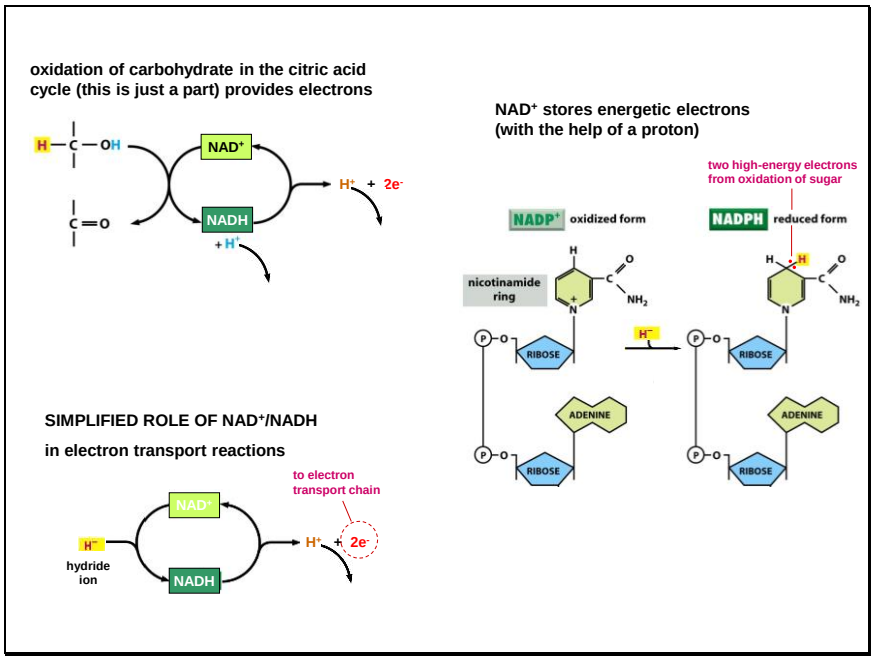


Glycolysis , [glucose metabolism]
•The "citric acid cycle" is also known as the "Krebs cycle" or the "tricarboxylic acid (TCA) cycle".
•***NAD+→NADH*** is half of an electron transfer reaction...

Slide 12



•NAD^+ / NADH is used in respiration, $NADP^+$ / NADPH is used in photosynthesis.
•The phosphate means that they can be recognized by different enzymes, and used in different reactions and maintained in a different chemical balance.



NAD^+ can carry two high-energy electrons together with a proton – being converted to NADH or *reduced* nicotinamide adenine dinucleotide. This is an example of a redox reaction – the substrate is oxidized (loses electrons) and NAD^+ is reduced (gains electrons) [*oil rig – oxidation is loss, reduction is gain*]. NAD^+ has a more stable electronic configuration (the π electrons in the nicotinamide ring are delocalized) so NADH stores free energy and releases it when it gives up its electrons again (is oxidized). In the mitochondrion, NADH gives up its electrons to the beginning of the electron transport chain in the membrane.

Slide 14

Electron-transfer or "REDOX" reactions

Half reactions: **Reduction (gain electrons)** Standard reduction potential (V)

1. $NAD^+ + H^+ + 2e^- \longrightarrow NADH$ -0.315
2. $(\text{oxalo-acetate})^- + 2H^+ + 2e^- \longrightarrow \text{malate}^-$ -0.166

Oxidation (lose electrons)

Full reaction, (1) – (2):

$NAD^+ + \text{malate}^- \longrightarrow NADH + H^+ + (\text{oxalo-acetate})^-$ -0.149

Nb. Negative reduction potential means that the free energy change for this reaction is positive, and it will not occur spontaneously **under standard conditions**.

However, in the cell **concentrations are not standard** (not all 1M). Free energy input of acetyl-CoA depletes oxaloacetate and builds up citrate, and the resulting concentration differences (== chemical potentials) drives each reaction in the cycle in the direction shown.

•Reactions involving electron transfer from one molecule to another are also called **REDOX** reactions.

- •The molecule losing the electron(s) is "Oxidized"
- •The molecule gaining the electron(s) is "Reduced"
- •Mnemonic "OIL RIG" – "**O**xidation **I**s **L**oss, **R**eduction **I**s **G**ain"

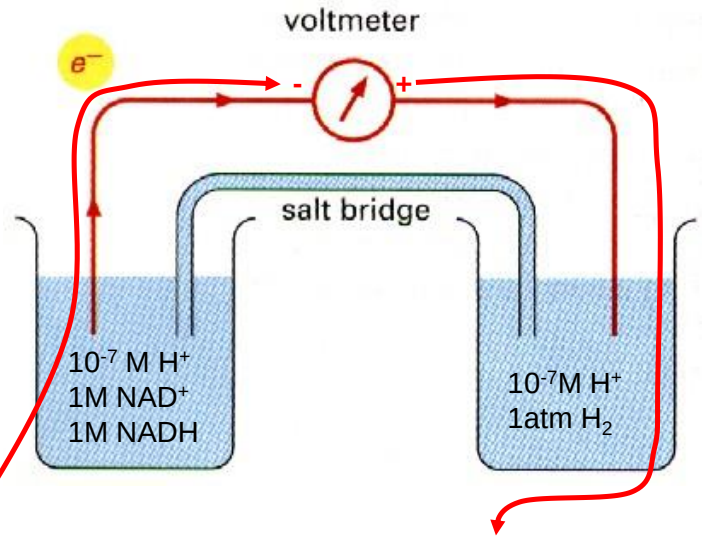
•Free energy changes in Redox reactions can be attributed to "half-reactions", in which each species either loses or gains an electron.

- •"Reduction Potential" is the free energy per unit charge in a half-reaction
- •(Note: since electrons are negatively charged, a positive reduction potential means negative ΔG : ie) positive reduction potential means the reaction is downhill.)
- •Free energy change ΔG for any given reaction is the difference in reduction potentials of the half-reactions that make it up, times the charge transferred.

•Standard Conditions are defined by chemists as 1M concentration of all reactants in water, at 25° C, with all gases at one atmosphere pressure . Biochemists define a different standard where $[H^+] = 10^{-7}$ M - since pH7 is typical for most biological reactions.

Slide 15

Measuring (biochemists) standard redox potentials



$NAD^+ + 2e^- + 2H^+ \rightleftharpoons NADH$

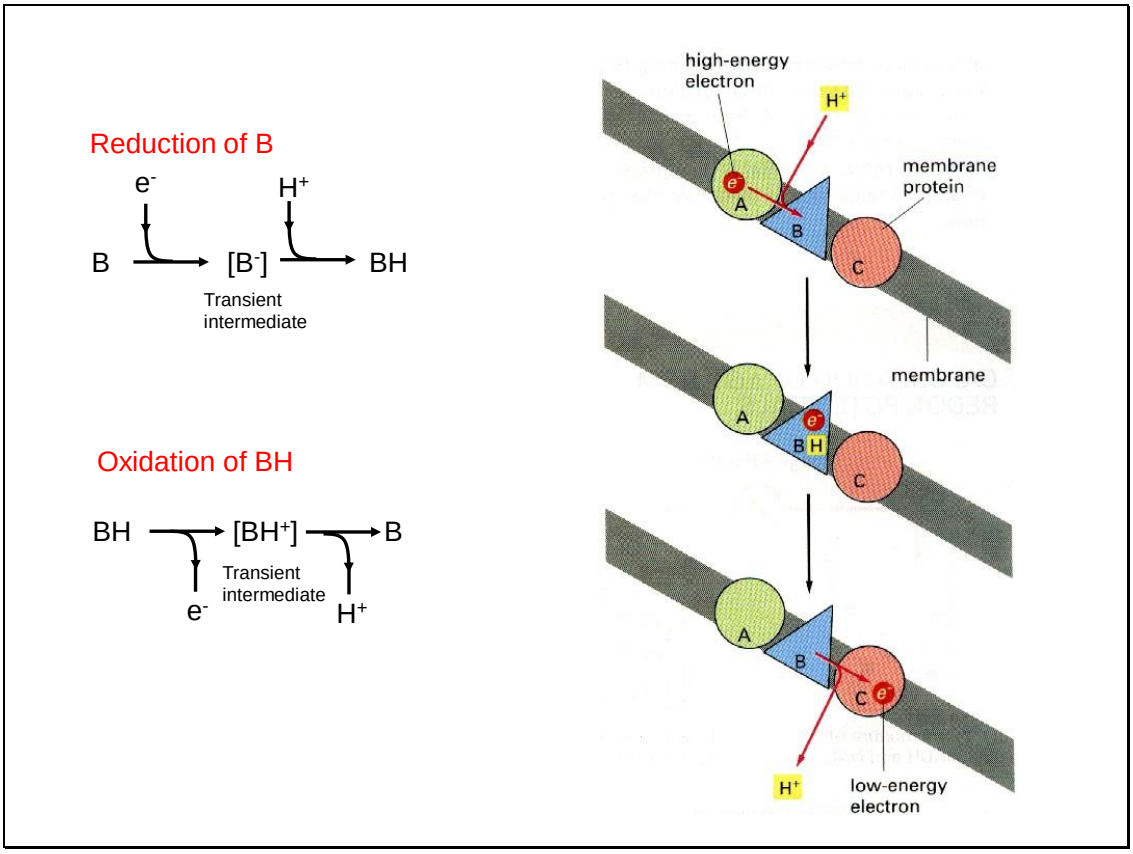
Standard potential = -0.315V: reaction proceeds in reverse

$2e^- + 2H^+ \rightleftharpoons H_2$

Standard potential = 0V (By definition, this is the reference reaction)

•Electrons will pile onto the wire on the left and off from the wire on the right, until the voltmeter reads -0.315 V. At this point, the chemical free energy reduction for an electron leaving the “high-energy” NADH is balanced by the electrostatic energy gain for the same electron jumping onto the wire at a negative potential. The salt bridge makes sure the two solutions are at the same potential.

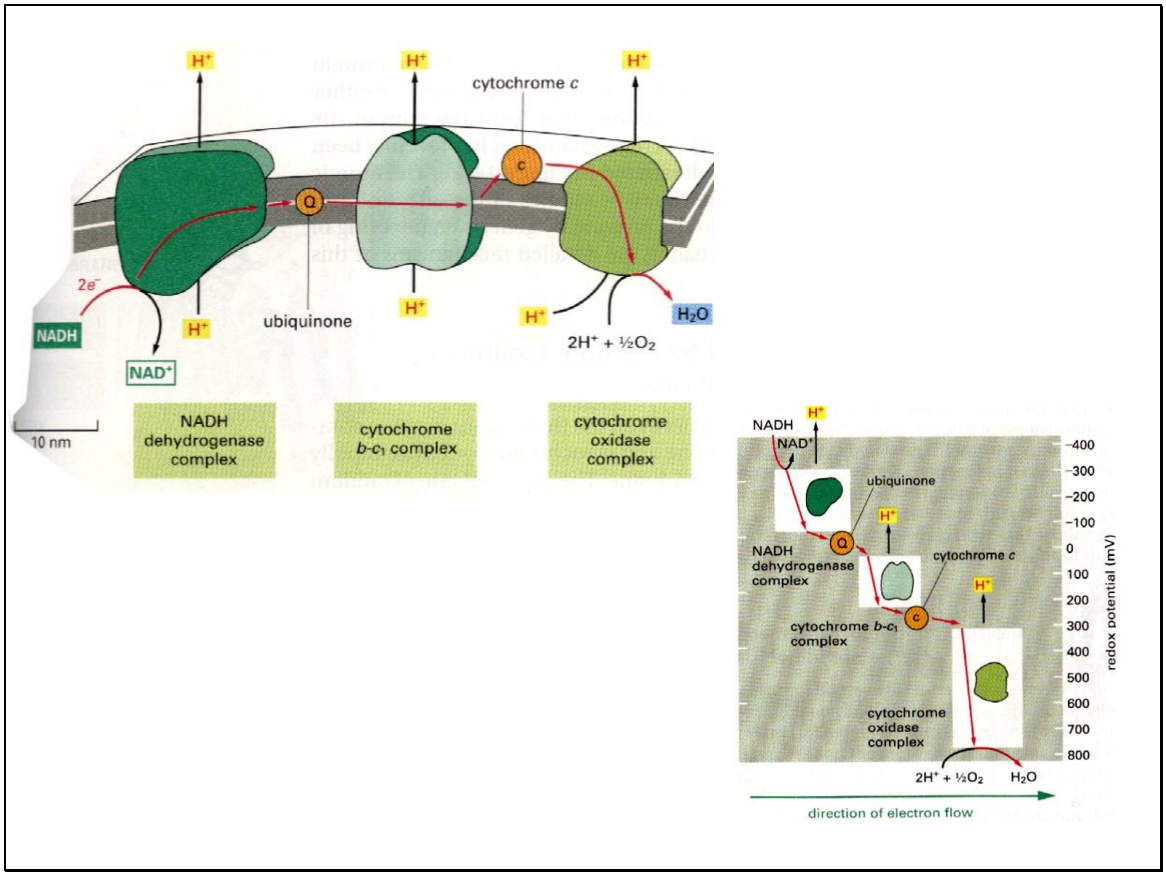
Slide 16



Proton pumping by electron transfer

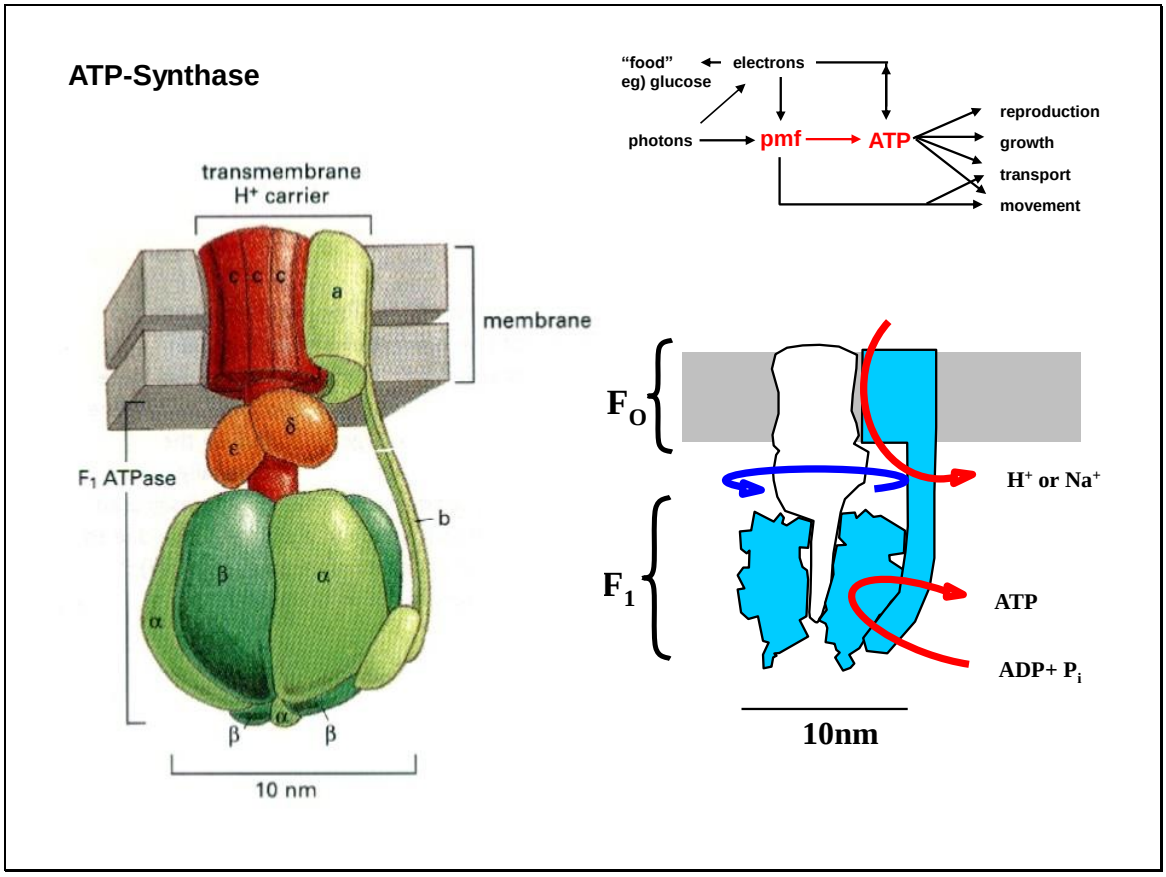
•These are just redox reactions, but with a spatial organization that couples them to proton pumping.

Slide 17



[Electron Transport and proton pumping in respiration]
•Electron transfer from NADH to O2, in a series of reactions.
•Free energy released is coupled to pumping protons out of the cell against the pmf, thus generating and maintaining the pmf.

Slide 18



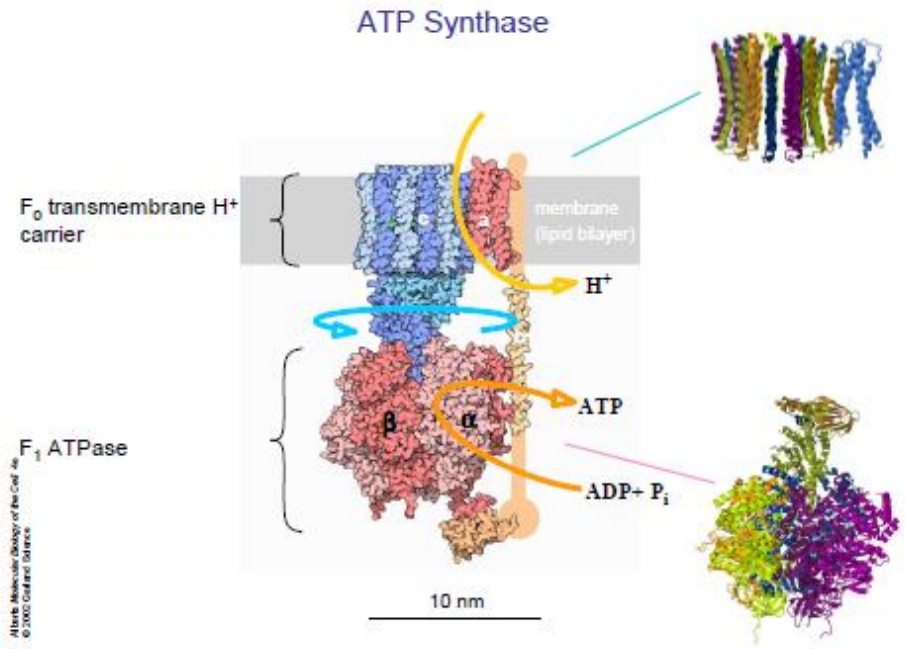
ATP synthesis

•This machine generates ATP from the pmf by using two rotary motors back-to-back, one running on each free energy source. Under normal conditions the pmf motor (F_O) gnererates more torque, so the ATP (F_1) motor runs backwards as a generator.

•We will learn more about ATP synthase in lecture 5.

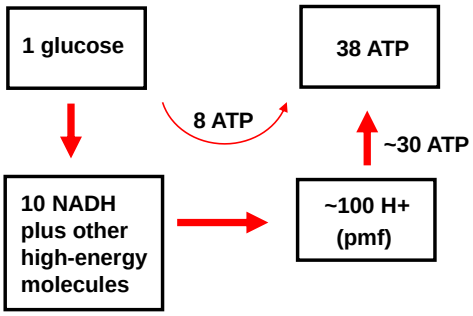
Slide 19

ATP-synthase – prettier pictures



Slide 20

Free Energy Accountancy

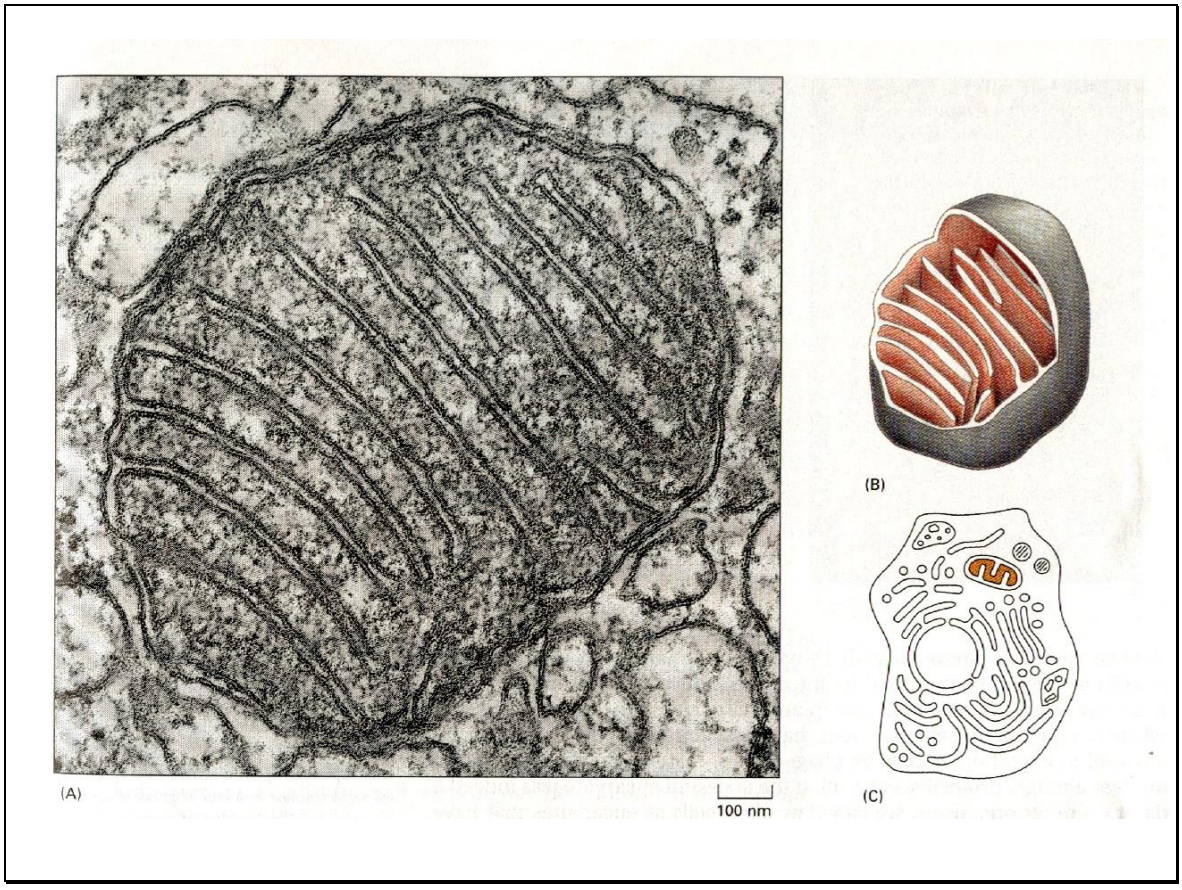


Approximate free energies:

	ΔG(each)	**ΔG**(total)
Glucose	1160	1160
NADH	90	900
H+(pmf)	8	800
ATP	25	950

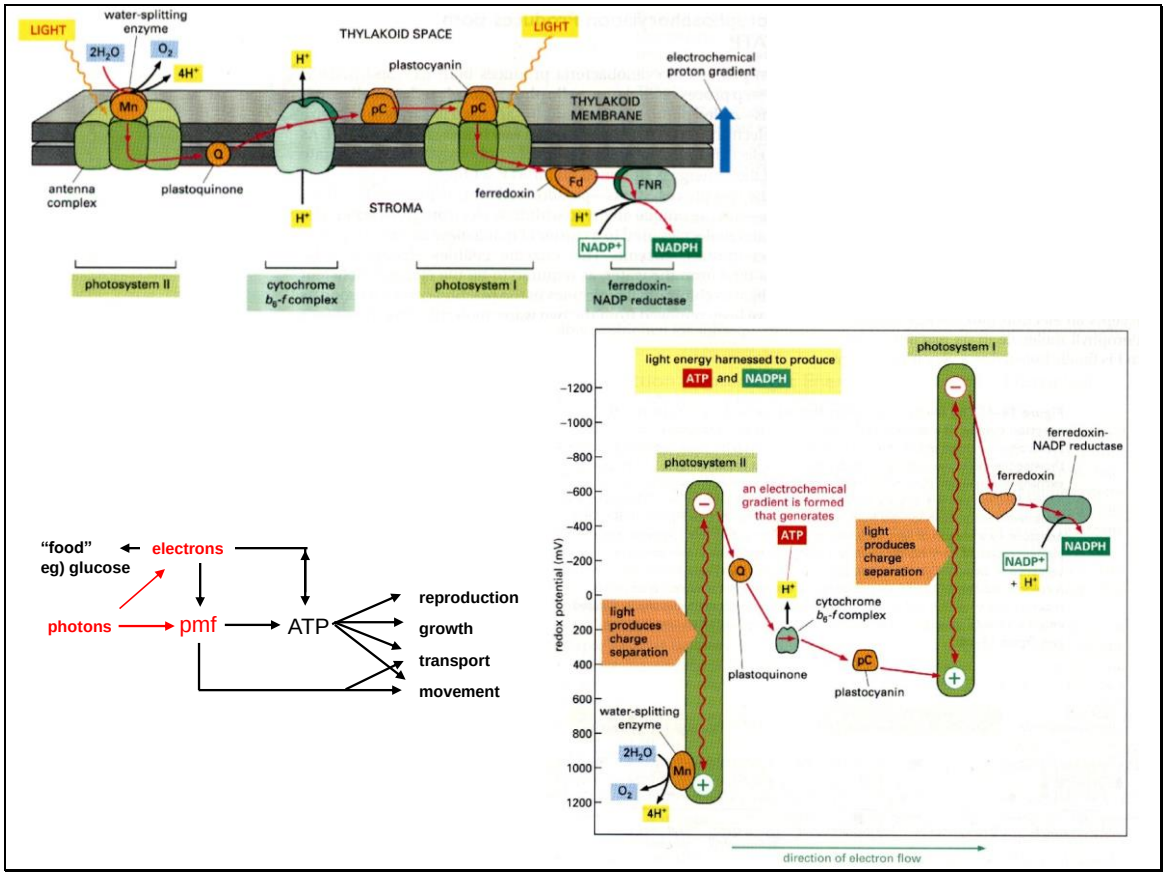
•The extraction of free energy from Glucose has high efficiency because it is gradual, lots of small steps.
•Note: Non-integer number of protons pumped per ATP molecule– this is possible because of the mechanism of **mechanical coupling** in ATP-synthase.

Slide 21



Respiration happens in the cell membrane of bacteria
[mitochondria]
•In eukaryotes it is mostly in special organelles called **mitochondria**
mitochondria are almost certainly captured prokaryotes.

Slide 22



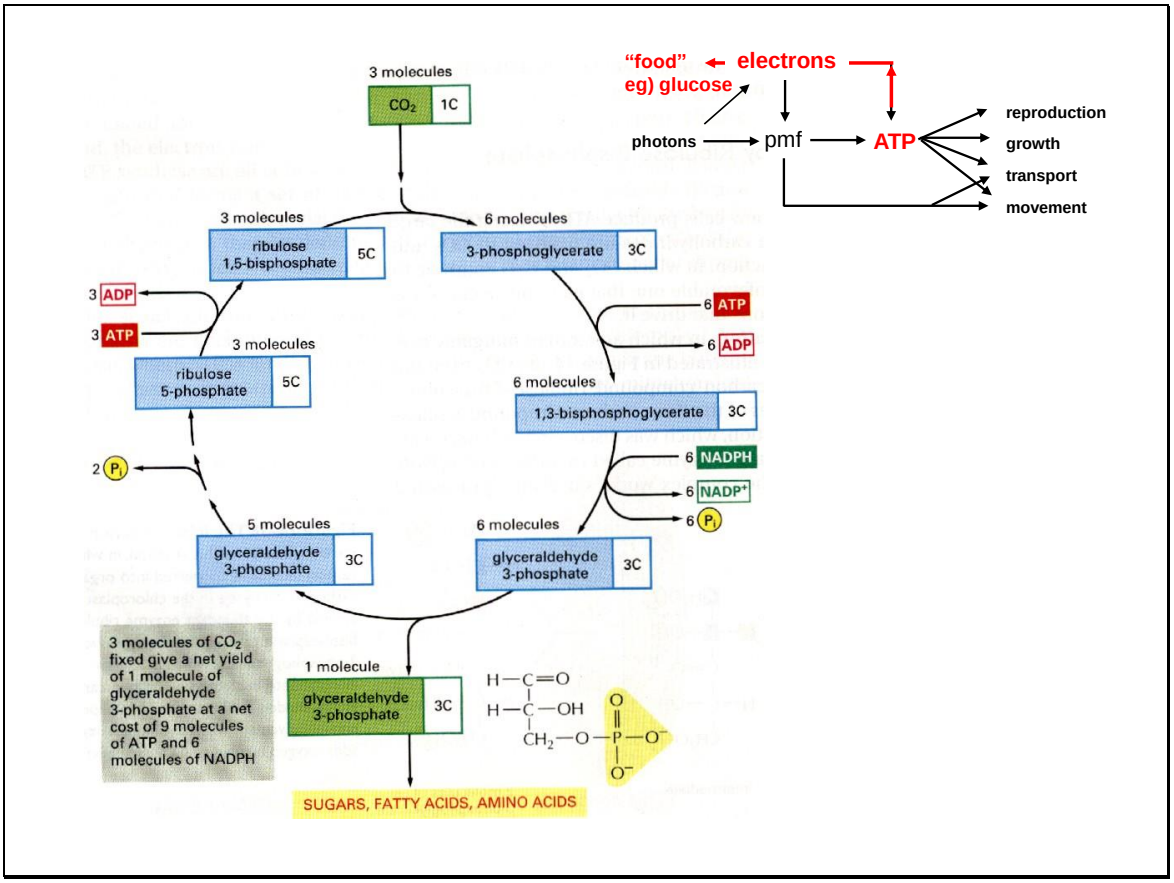
Photosynthesis is the process of generating food (organic carbon) from water, CO_2 and light. It procceds in two stages...

- Light harvesting**:** light is absorbed and used to make a pmf, ATP and NADPH.
- Carbon fixation: ATP (and NADPH) are used to make organic carbon (eg glucose) from CO_2 and H_2O.

Light harvesting

- This is the system used by plants and certain types of bacteria.
- Other, simpler but less efficient systems are also found in bacteria.
- The basic principles are the same as in slide 16, but now with the addition of two major "leg-ups" in energy when photons are absorbed.
- The machinery for light-harvesting works using quantum-mechanical tunnelling of electrons, and is amazingly sophisticated.

Slide 23



Carbon Fixation

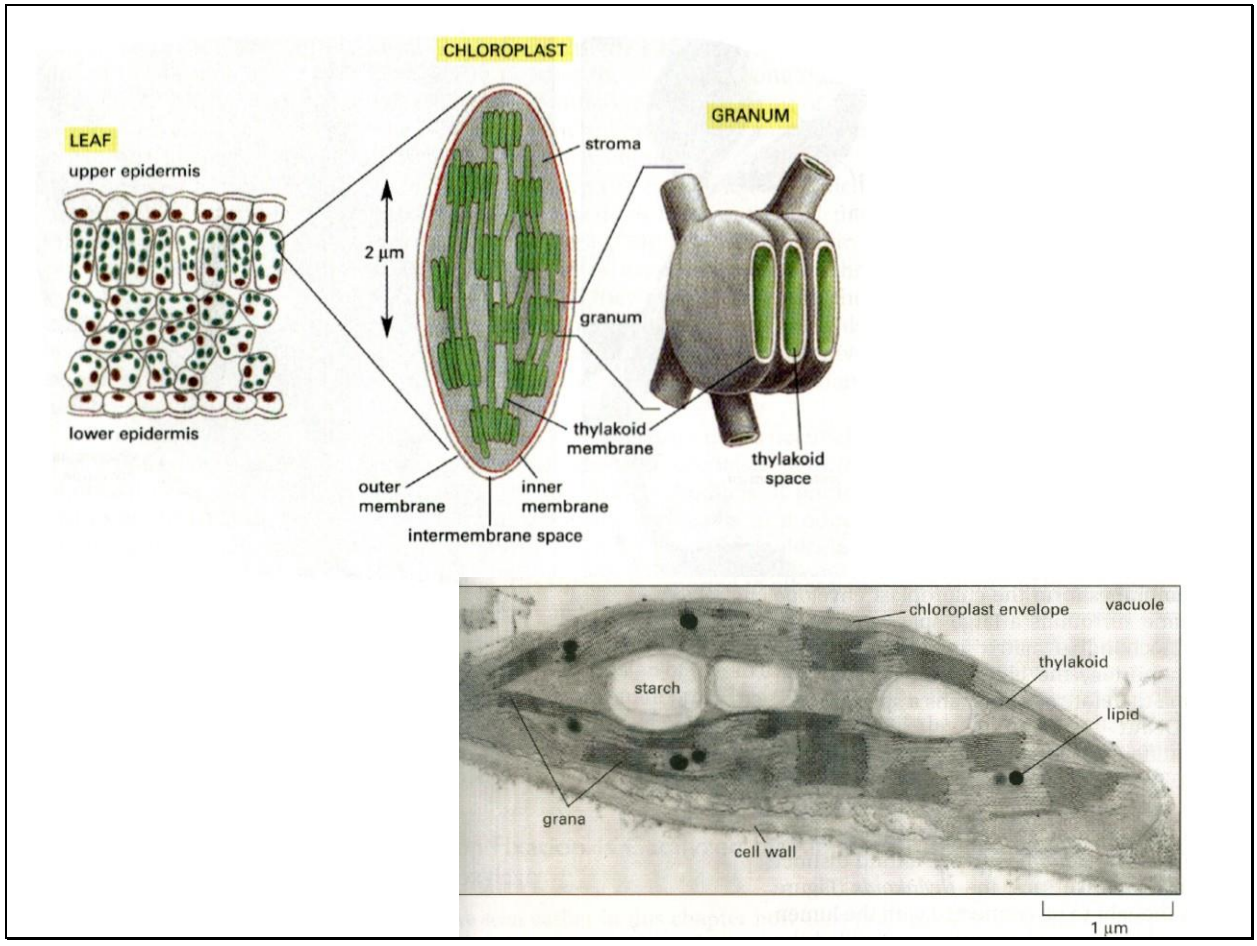
[Calvin Cycle]

•In essence, this is the reverse of the citric acid cycle (slide 11), although the details are different.

•It consumes high-energy electrons (generated by light harvesting) and makes organic carbon which can be used, for example, to make glucose.

•It is called the "carbon-fixation cycle" or "Calvin Cycle".

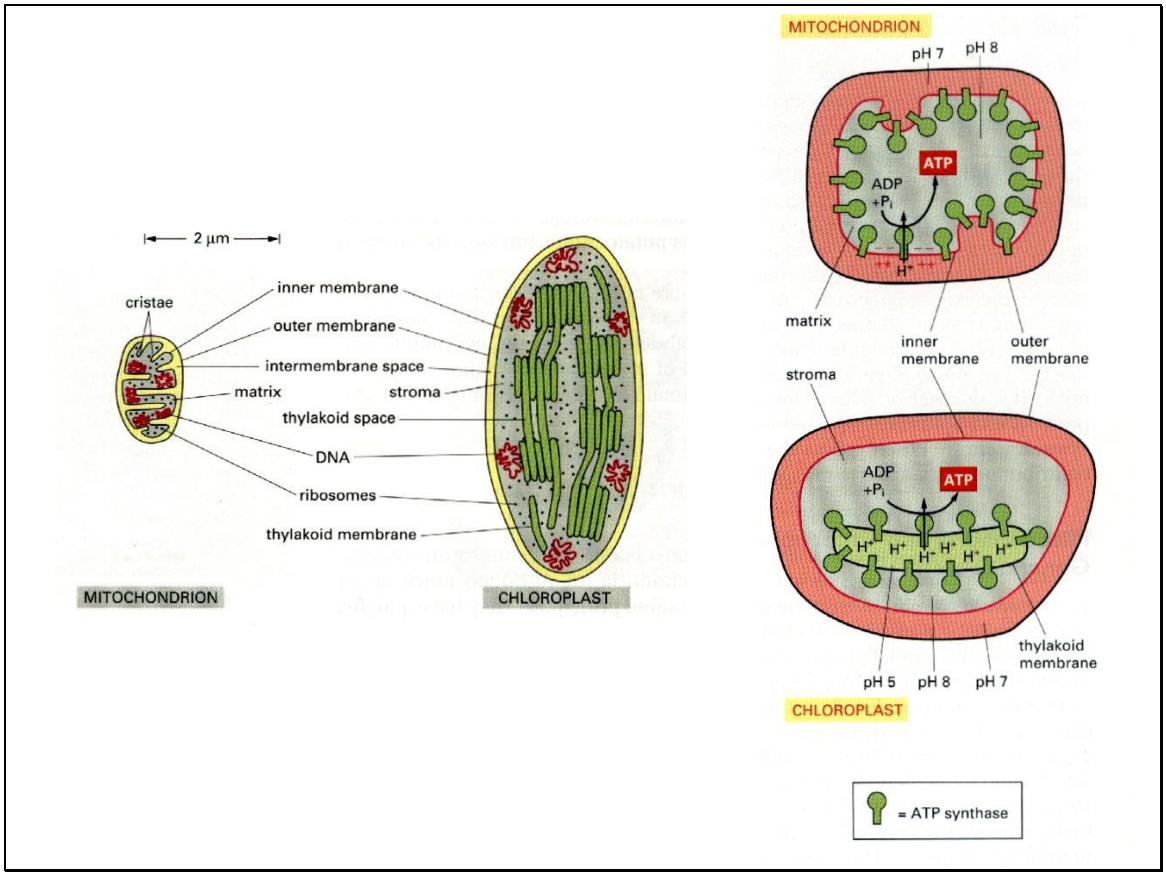
Slide 24



•In eukaryotes photosynthesis happens in special organelles called **chloroplasts**

•chloroplasts, like mitochondria, are almost certainly captured prokaryotes.

Slide 25



[comparison between mitochondria and chloroplasts]

•in common

- •the matrix/stroma is where ATP is accumulated and used to make other things
  (This is equivalent to the cytoplasm in a simple bacterium)
- •ATP synthase is very abundant
- •Protons are pumped out of the matrix/stroma by electron transport
- •Both are prokaryotes captured long ago in evolutionary time

•Different

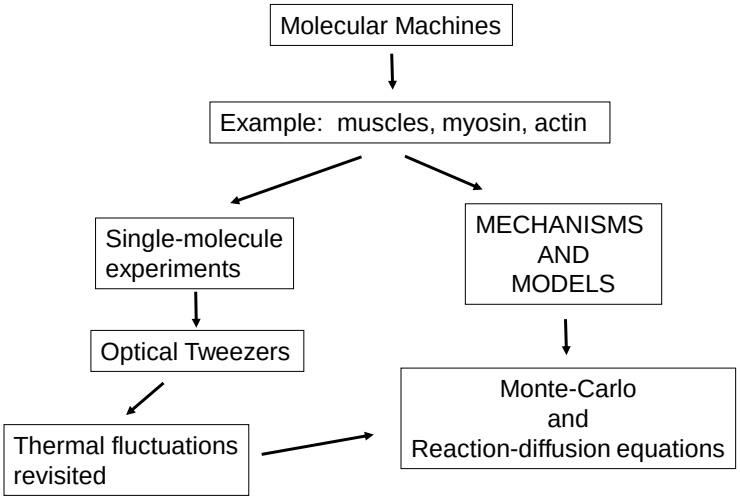
- •Chloroplasts have an extra compartment, the thylakoid membranes.
- •Proton pumping in chloroplasts is into the thylakoids driven by light, in mitochondria it is into the inter-membrane space driven by respiration.

•Almost all of the useful free energy on earth originates from sunlight and was captured by plant chloroplasts or their prokaryotic equivalents the cyanobacteria, or their evolutionary ancestors. Much of this is now oil, but it will run out soon. Perhaps we will go back to harvesting plants or cyanobacteria for our energy needs…

•Photosynthesis also made the (highly reactive) oxygen that now forms 20% of our atmosphere.

Slide 1

Lecture 5: Molecular Machines I
Linear motors

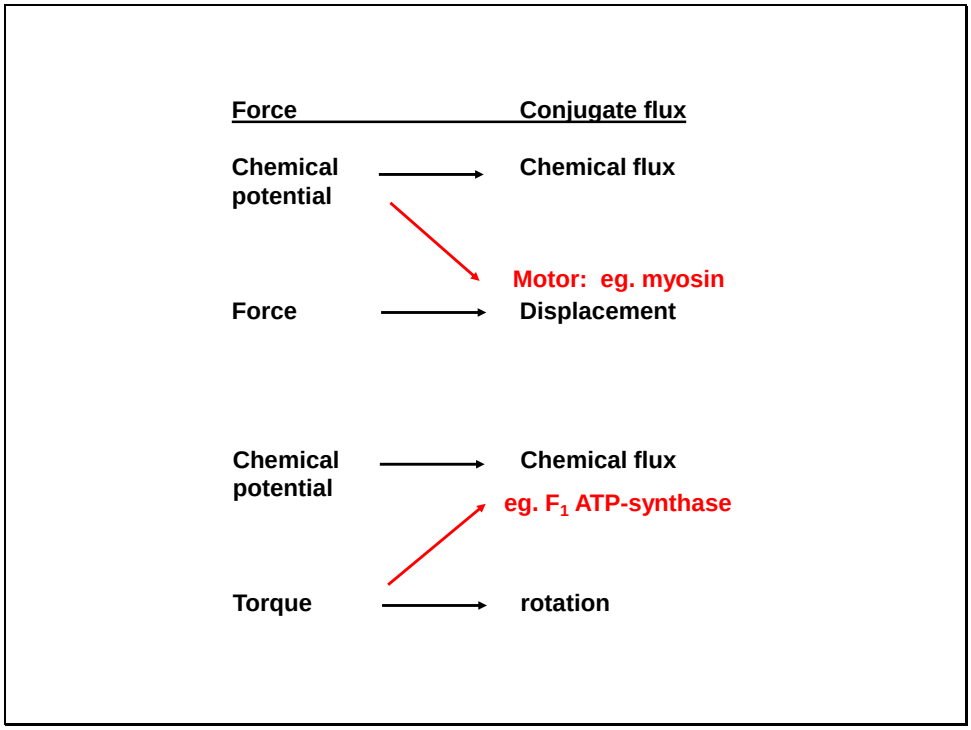


Slide 2

Reading for Lecture 5

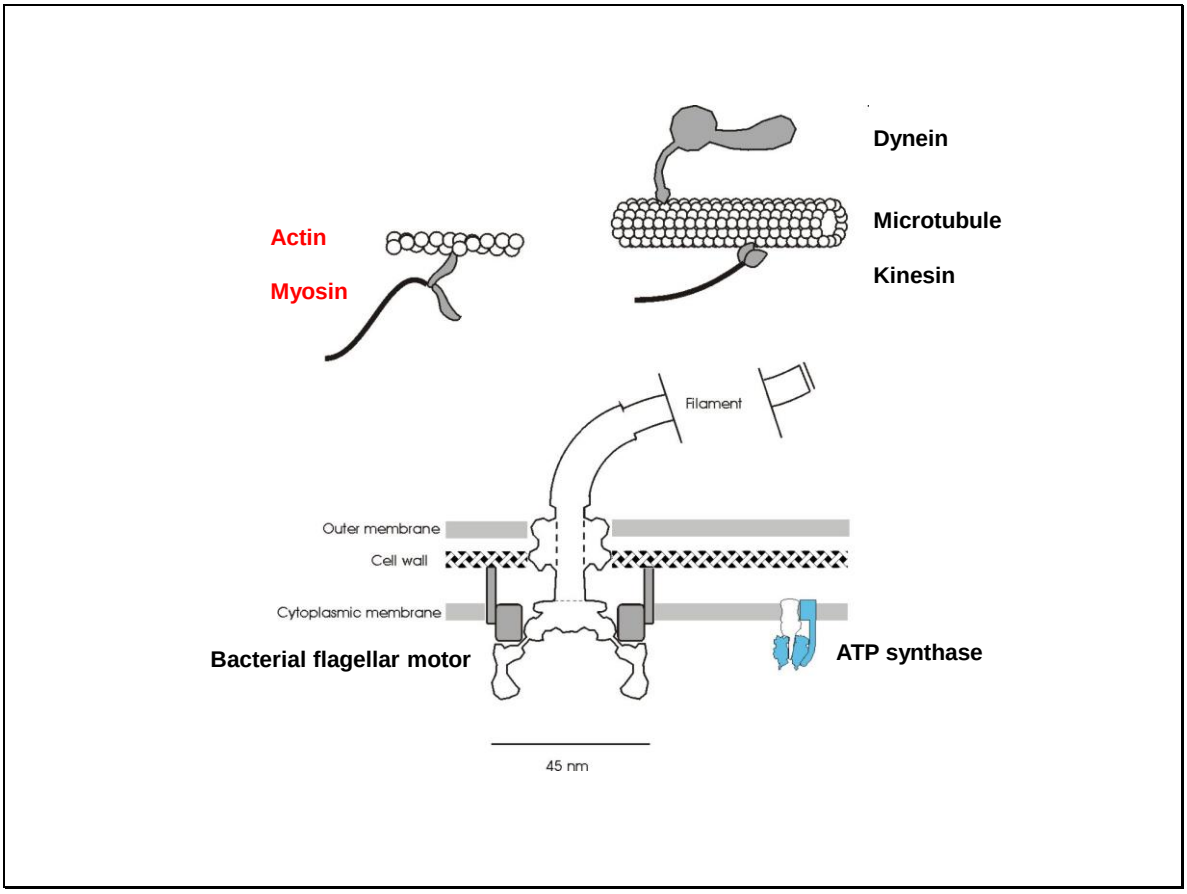
- Howard Chapters (5), 14,16
- (Nelson Chapter 10)
- (Alberts Chapter 16)
- Journal Articles

Slide 3



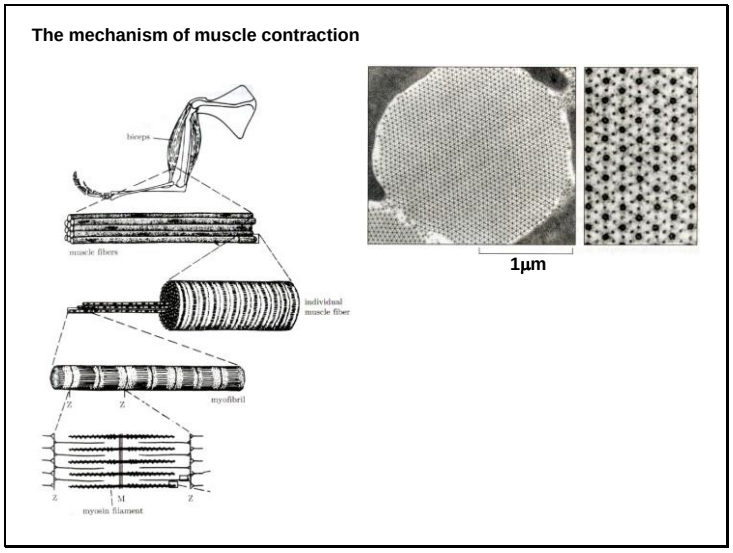
[Free-Energy Transduction]
• The product of a force and its conjugate flux is Energy.
• Coupling between non-conjugate forces and fluxes is called Free Energy Transduction
• Motors couple non-mechanical forces and mechanical fluxes
• Generators do the reverse

Slide 4



[Gallery of Molecular Motors]
• This slide is not exhaustive –there are many biological molecular motors
• In particular, enzymes that move DNA and move on DNA are often considered as motors – more in section 3 of the course.

Slide 5



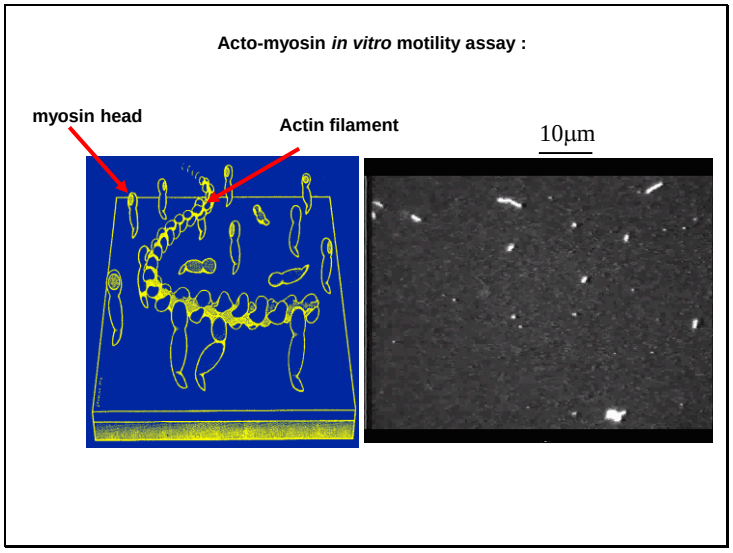
[Muscles]
• Muscles can only shorten actively – opposing muscles are used to lengthen them.
• The cross-section shown is insect flight muscle – vertebrate skeletal muscle is slightly different.

Slide 6

[Actin, myosin]
• Muscles have a repeating unit called the “sarcomere”
• In each sarcomere, thin (actin) filaments slide past think (myosin) filaments, making the sarcomere contract.
    • High force by multiple sarcomeres in parallel.
    • Fast contraction by multiple sarcomeres in series.
• The sliding filament mechanism, along with nerve action potential and patch clamp, is one of the classic successes of modern biophysics –
A.F. and H.E. Huxley (no relation), experiments on muscles fibres in the 1950s.

Single- molecule experiments with myosin date back to the 1990s.
Myosin and F1–ATPase, along with kinesin, are probably the best understood molecular motors.
Knight AE, Molloy JE. (2000) “Muscle, myosin and single molecules.” Essays Biochem 35:43-59
    A review of single-molecule experiments on myosin.

Slide 7



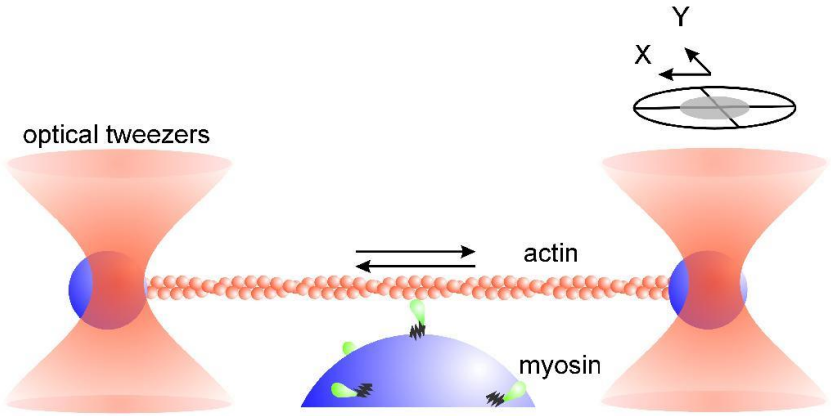
[Sliding filament assay]
• Actin filaments are visible because they have “fluorescent label” molecules attached.
• Not really “single-molecule” – several myosin heads propel each actin filament
• Direction is determined by the polarity of the actin filament – myosin heads are randomly oriented on the surface.
• Each myosin molecule only binds actin intermittently – so the actin and myosin need to be held close together (as in muscle) if single interactions are to be seen “*in vitro*” (“in glass”, as opposed to “*in vivo*”, “in life”)
• Fancy optical tweezers…

Slide 8

[Optical Tweezers –two bead assay]
• The position of the optical trap can be controlled by computer, using “acousto-optic deflectors” to steer the trap laser beam.
• Actin filaments are stretched between two trapped beads and brought up against immobilized myosin heads.
• When ATP is present, the actin filament is pulled towards one end by the myosin.

Slide 9

Single-molecule experiment on acto-myosin with optical tweezer dumbbell



(view make dumbbell.mov with quicktime)

[Optical Tweezers –two bead assay]
• The position of the optical trap can be controlled by computer, using “acousto-optic deflectors” to steer the trap laser beam.
• Actin filaments are stretched between two trapped beads and brought up against immobilized myosin heads.
• When ATP is present, the actin filament is pulled towards one end by the myosin.

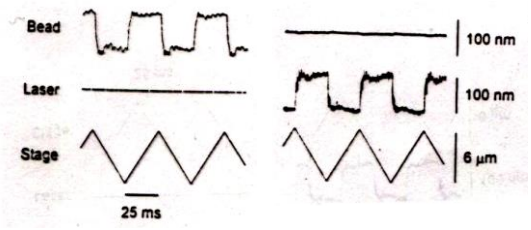
Slide 11

Single-molecule experiments on Myosin II

Individual myosin heads binding and powerstroke:

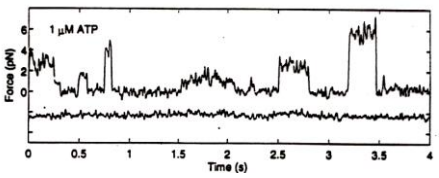


Feedback – measuring force at fixed displacement:



fixed trap

Moving trap with feedback



Binding and powerstrokes

[Myosin, single steps]

• "Myosin II" is skeletal muscle myosin, to distinguish it from many other types of myosin that are found in eukaryotic cells.

• Events are seen corresponding to single myosin heads making single "pulls"

• Originally, the average distance moved per event was estimated at about 12 nm – but see next slide for subsequent re-interpretation of the data…

• The **force** generated in the powerstroke can also be measured…

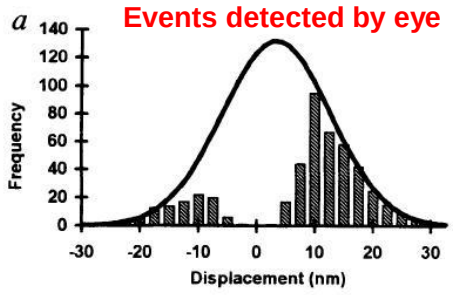
  • Move the optical traps so as to keep the beads in the same position (feedback). The distance between the trap and the (fixed) beads is then proportional to the force.

  • Powerstrokes generate about 6 pN under these conditions.

  Finer JT, Simmons RM, Spudich JA. (1994) "Single myosin molecule mechanics: piconewton forces and nanometre steps". Nature.368(6467):113-9
  The first paper using optical tweezers with myosin. Feedback force measurements.

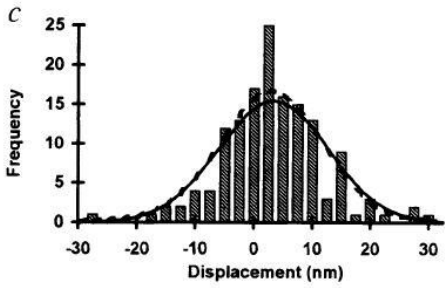
Slide 12

Single-molecule experiments on Myosin: Revisited



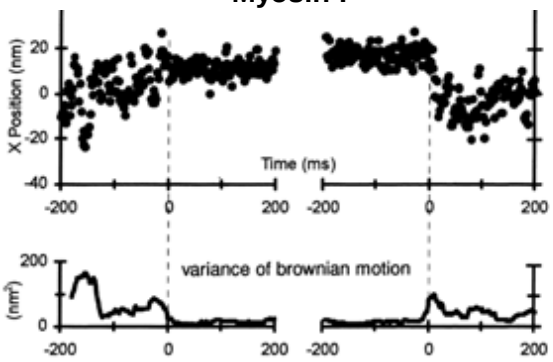
Events detected by eye

(view Myosin-Vale1.mov with quicktime)



Events detected by reduced Brownian Motion

Myosin I



[Myosin, steps re-visited]

• When a single myosin head binds an actin filament held loose in the optical traps, the Brownian noise is reduced.

• The average position while bound is shifted about 5 nm from the average position when not bound. This is an estimate of the size of the powerstroke.

• Molloy JE, Burns JE, Kendrick-Jones J, Tregear RT, White DC. (1995) "Movement and force produced by a single myosin head." Nature. 378(6553):209-12.

Detection of binding events and estimate of step-size.

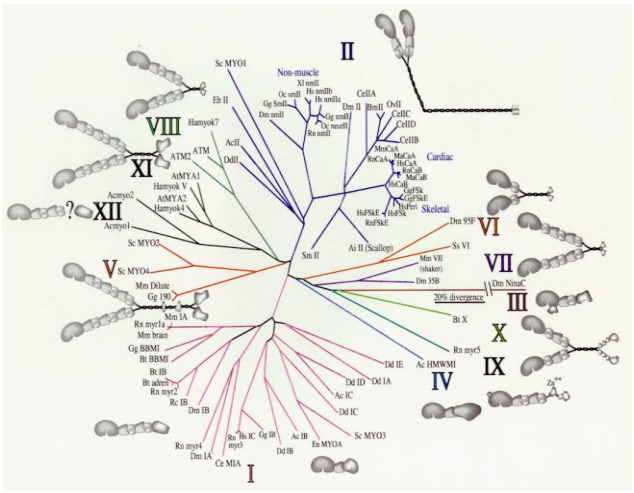
• Note: the 1994 paper didn't realize the importance of the effect of Brownian motion of the free dumb-bell considered in the 1995 paper.

• A **second** shift of 5-6 nm can be observed **during** the binding event in a different sort of Myosin, Myosin I.

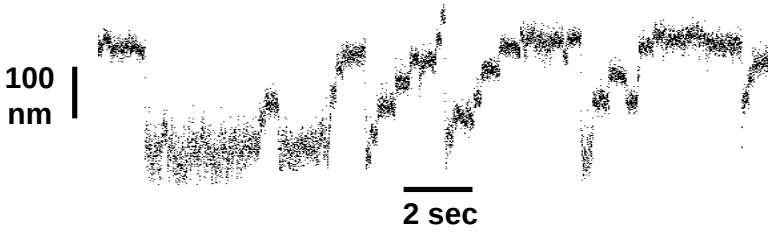
Veigel C, Coluccio LM, Jontes JD, Sparrow JC, Milligan RA, Molloy JE. (1999) The motor protein myosin-I produces its working stroke in two steps. Nature. 398:530-3.

Slide 13

The myosin family :



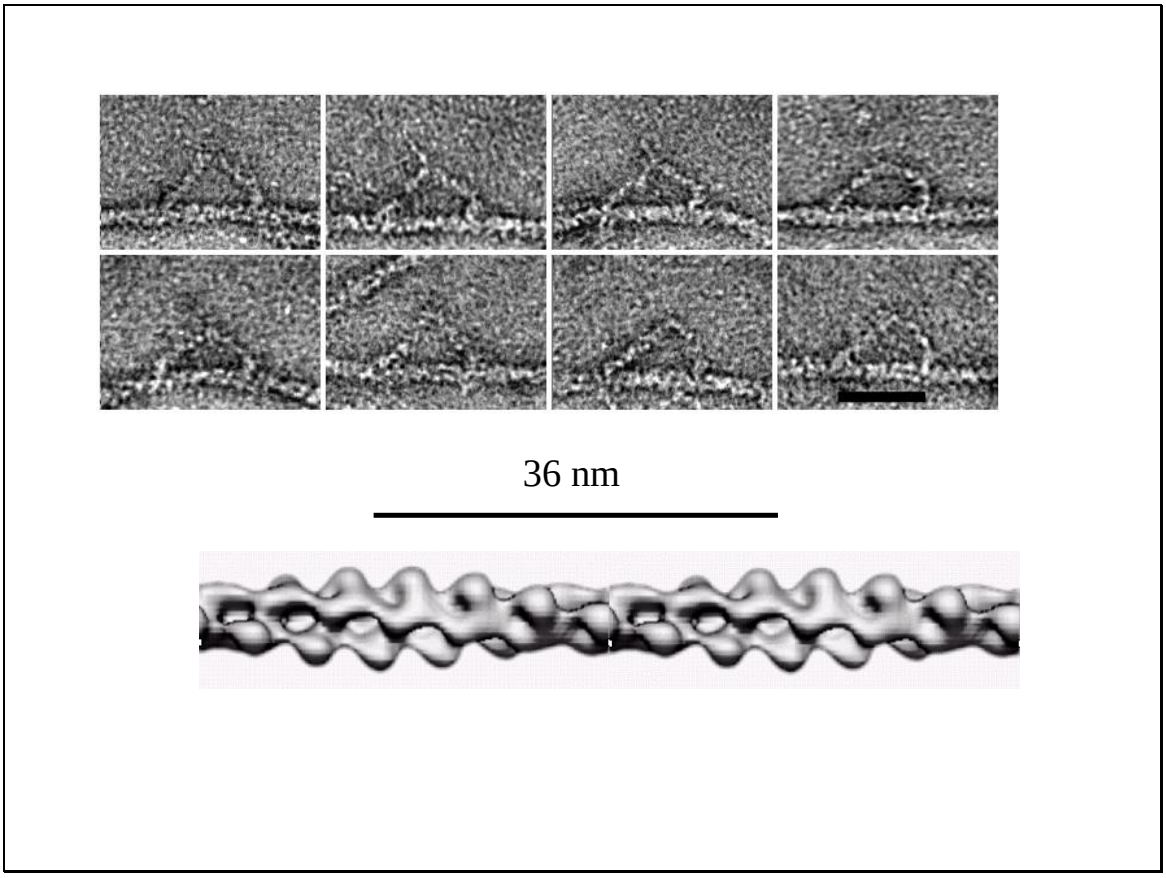
Myosin V takes several big steps before letting go:



[Other myosins]

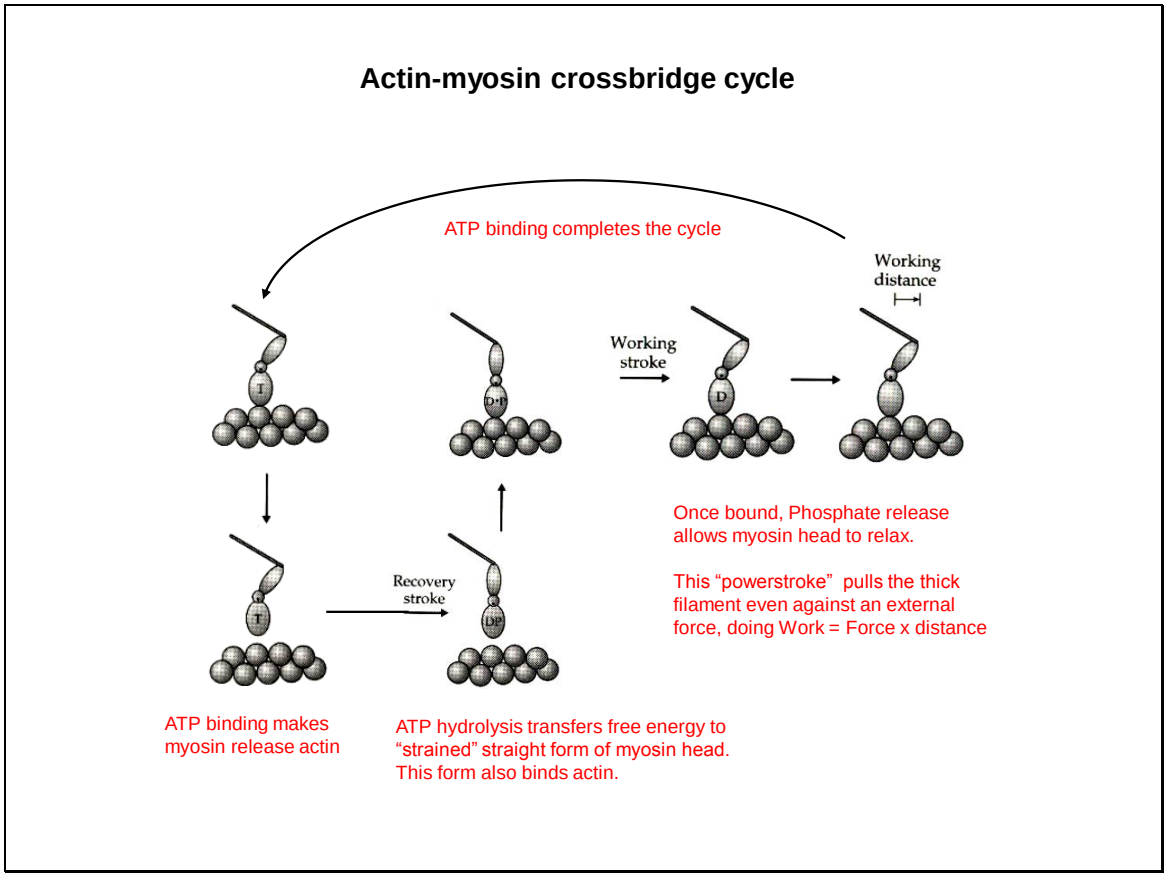
- Muscle myosin is atypical – most linear ATP-driven motors work alone or in smaller groups, not in a muscle.
  - "Processivity" is the ability of a single motor to take several steps while remaining attached to the track.
  - More and more processive motors (myosins, kinesins, dyneins) are being discovered and studied.
- Kinesins are more processive than myosin V. A single kinesin molecule takes hundreds of steps without falling off the microtubule track.
- Processive motors usually have two heads – "hand-over-hand" mechanism?

Slide 14



[Myosin V steps –electron microscopy]
• The actin filament helical pitch is 36 nm. Myosin V binds once per turn.
• Electron microscope pictures are “snapshots” of the motor mechanism.
• *Note: many more excellent single-molecule experiments have been published on step sizes and patterns in different myosins. In particular, the “walking” gait of Myosin V is now fairly well established…*

Slide 15



Models of Molecular Motor Mechanisms

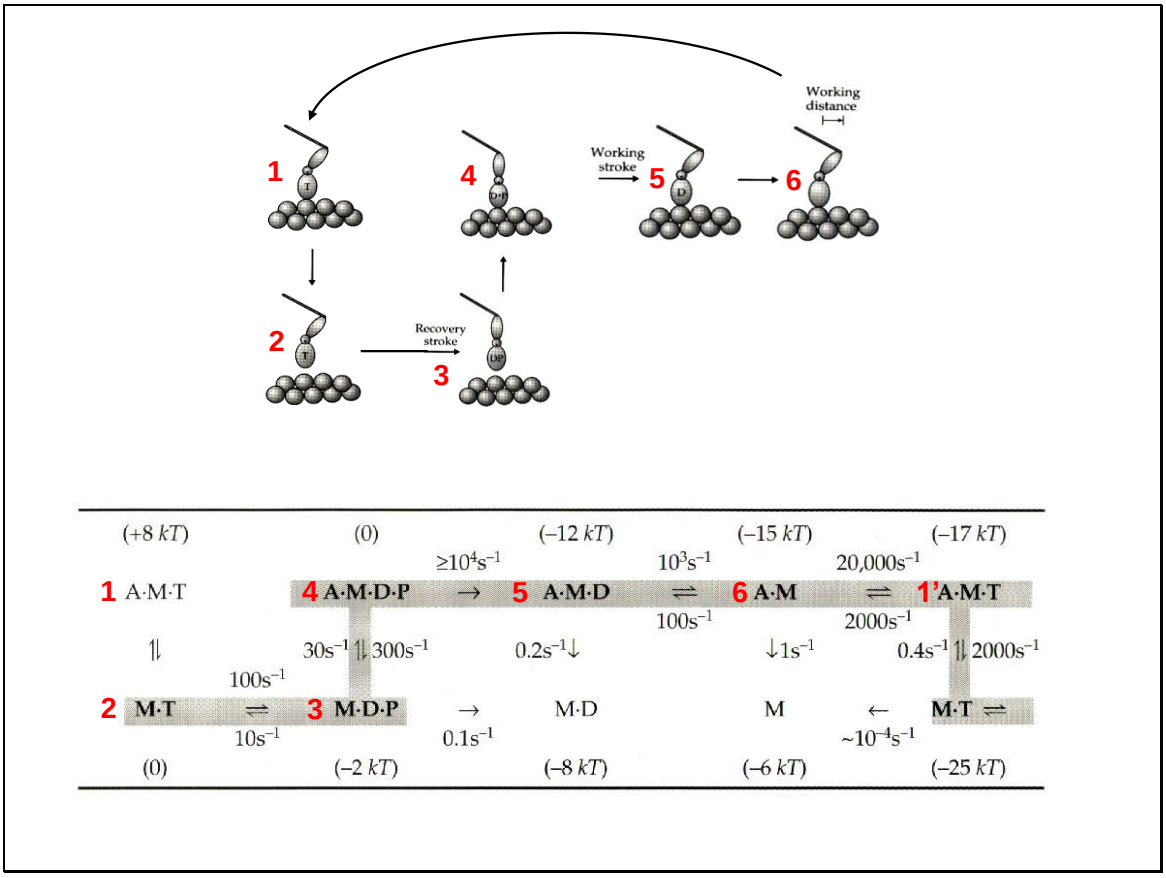
[Crossbridge cycle]

• Transitions between states which differ **both** in their chemistry (eg the state of ATP) **and** in the mechanical output variable (here, the relative position of actin and myosin, determined by whether the head is bound and which shape it has) are the essential core of the way molecular motors work.

- This idea is the basis of models of all sorts of molecular motor
- Transitions between two states that depend simultaneously on two different types of free energy are the basis of **ANY** free energy coupling mechanism.

• Remember, each "state" is a collection of microstates which have been grouped together because they share some global property, such as having ATP bound or having the lever arm at a particular angle.

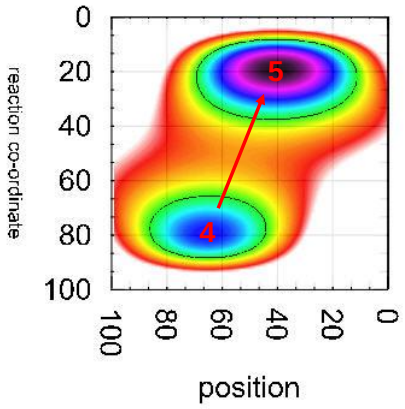
Slide 16

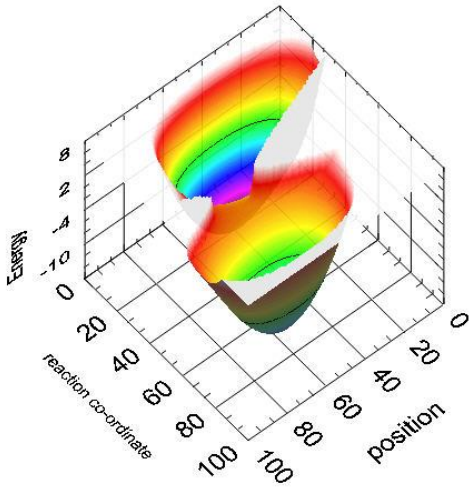


[Rates and free energies in crossbridge cycle]

• These rates and free energies are taken from Howard Chapter 14, and are assembled from a great deal of experimental data plus a certain amount of educated guesswork – see Howard for details…

Phosphate release with powerstroke: free energy landscape





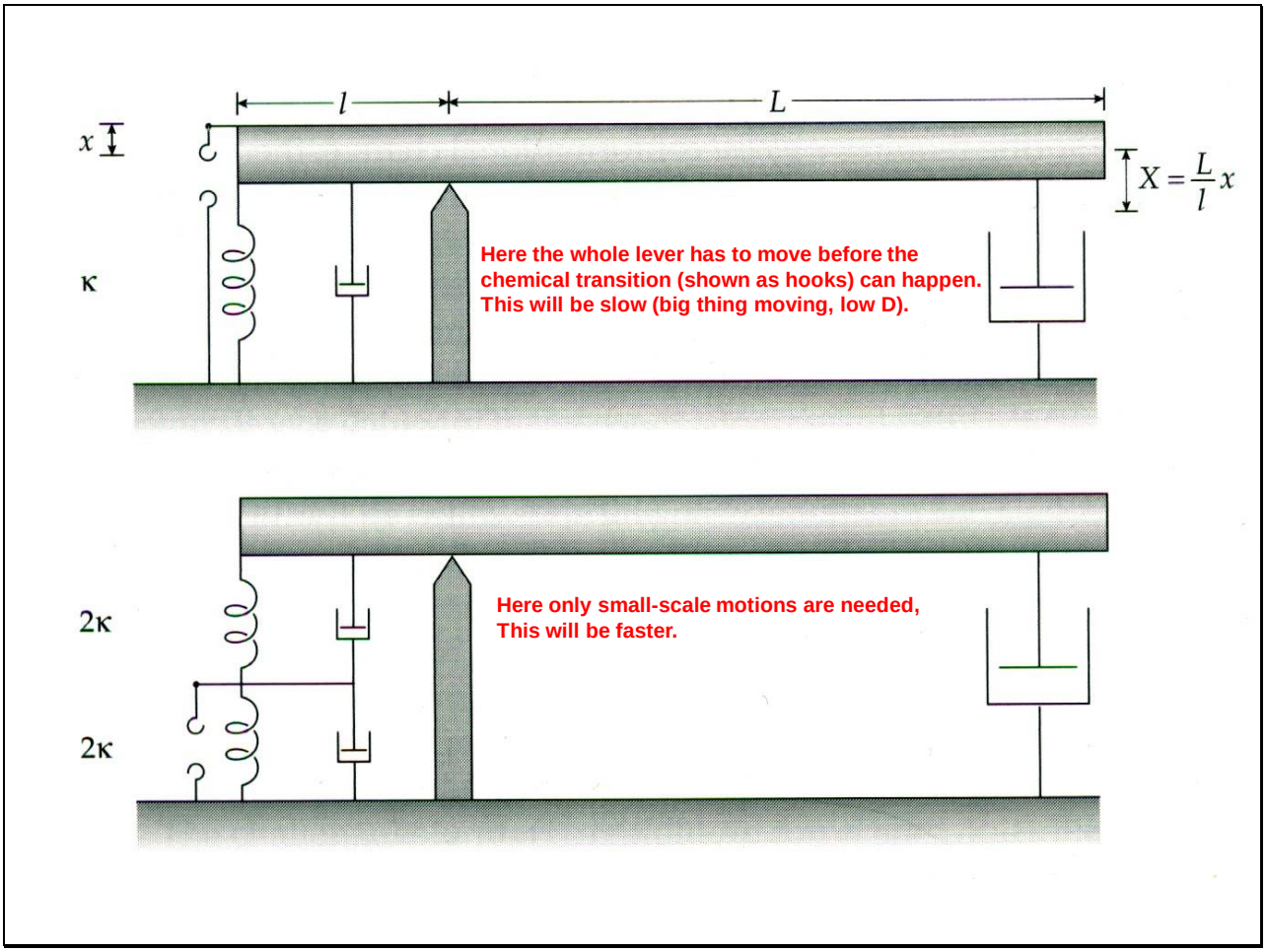
Cartoons and animations are all very well, but how can we turn them into quantitative, predictive, models? Myosin II will serve as an example… [coupled mechanical and chemical transitions]

• The chemical transition (phosphate release, 4 → 5) shifts the position of the energy minimum: this is the POWERSTROKE.

• There may be a gap or "gate" in the free-energy barrier between the chemcial states (lower right picture), which means that the reaction can only happen at certain positions where everything is lined up correctly.

• *Bustamante C, Keller D, Oster G (2001) The physics of molecular motors. Acc Chem Res. 34(6):412-20.*
Available plus a lot of related stuff from
http://nature.berkeley.edu/~goster/pubs.html

Slide 18



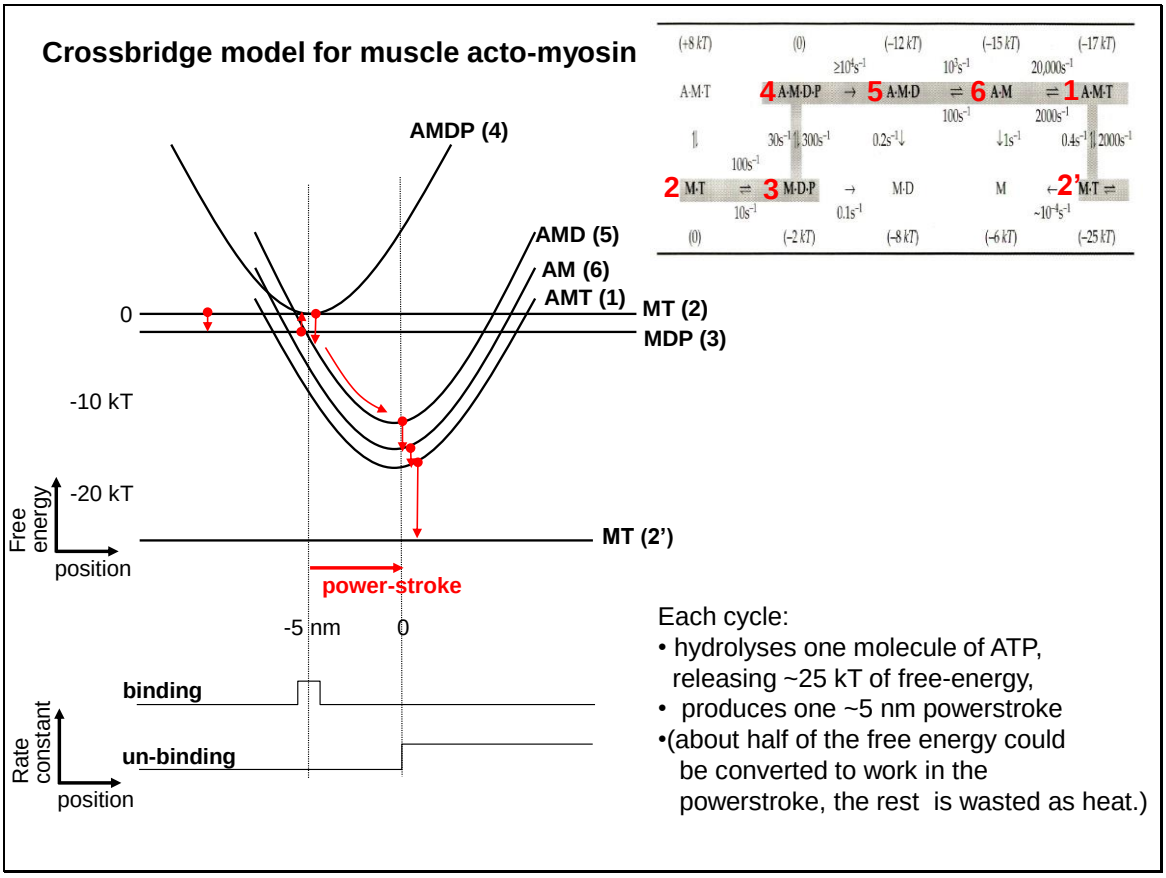
•[Separation of time-scales]
• In both cases, the activation energy barrier is represented by stretching the springs a distance *x*. Even though the bottom springs are stiffer, giving a higher activation barrier, the bottom case will still be faster because the diffusion coefficient of the small part of the molecule is bigger.

- Chemical transitions (eg. ion movements, ATP hydrolysis, lower diagram) are local and fast. Typically nanoseconds.
- Mechanical transitions (eg. movements of whole protein domains, upper diagram) are global and slow. Typically microseconds to milliseconds.

• Motions of the whole motor (eg the actin/myosin filament, or rotation of the whole bacterial flagellar rotor) are EVEN slower. Typically milliseconds.

• To predict motor behaviour on the timescale of motor movements (milliseconds), we can model chemical transitions as instantaneous jumps between discrete states while treating mechanical changes as a continuous variable …

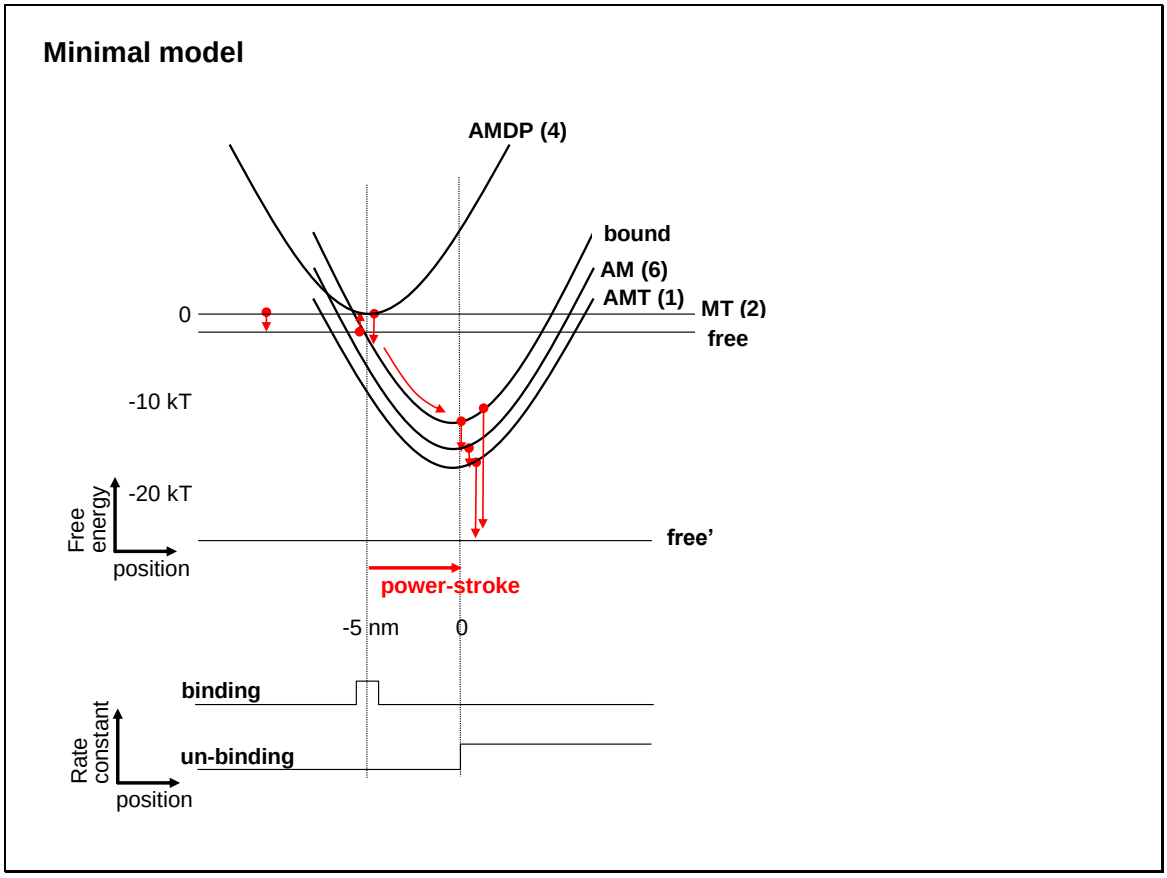
Slide 19



Mathematical treatment of models [energy-profiles]

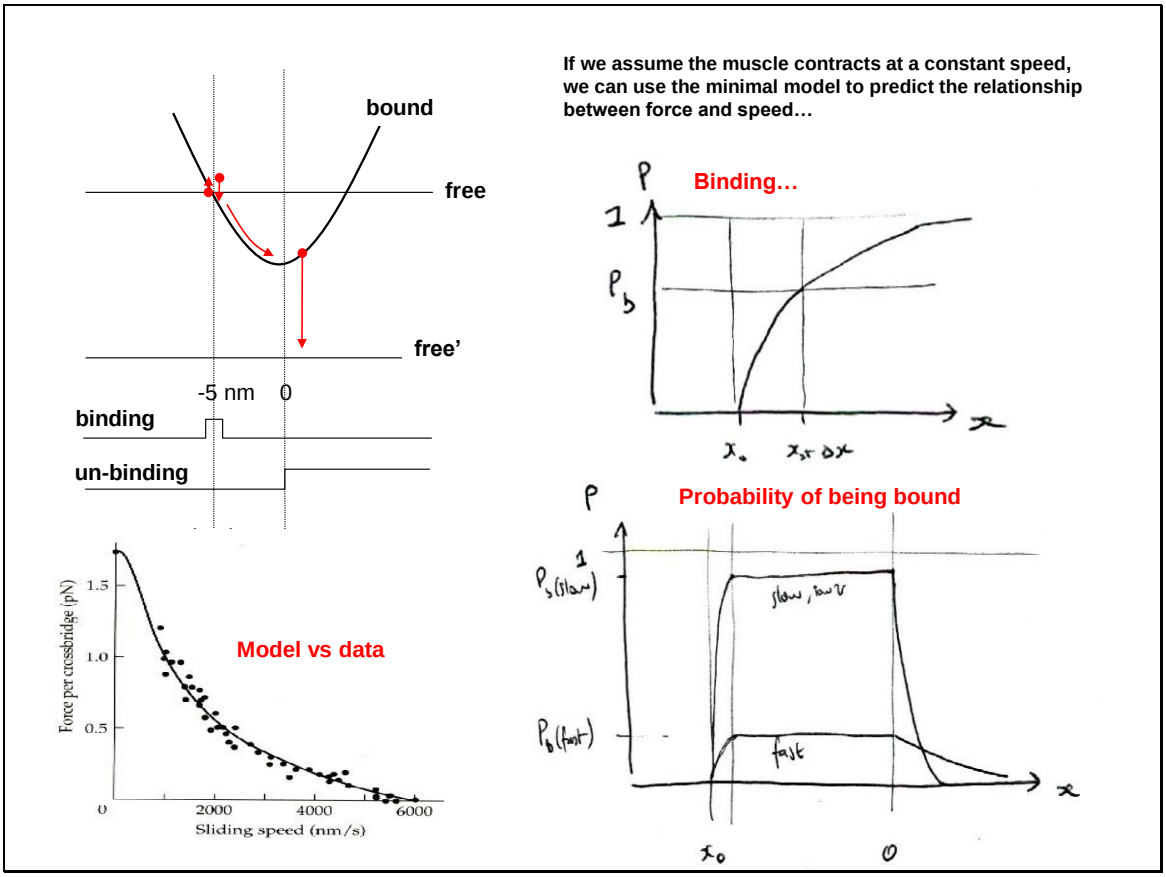
• Energy profiles may be due to electrostatic forces or protein elasticity.
• For small displacements from energy minima, profiles are quadratic, like linear spring.
    • Note 1: the powerstroke is represented as a shift in the equilibrium position between states.
    • Note 2: External Force will simply tilt the energy profiles by adding a work term ***F.x*** to the free energies of all bound states.
• The essence of the mechanism now becomes visible:
    • binding (and the following power-stroke tranition, 4 → 5) is only allowed at positions corresponding to the beginning of the powerstroke
    • release is only allowed after the powerstroke is completed
    • small free energy drops correspond to "wasting" free energy as heat, but they ensure the cycle proceeds forwards
    • External force will prevent the powerstroke completing by tilting the profiles in bound states 4,5,6, 1 to the left.

Slide 20



[minimal model]
• To model how the motor will work, we can ignore all transitions except those that involve mechanical motions…

Slide 21



[solving the minimal model]

- There are 2 reasons why the average force goes down as speed goes up…
    - The probability of binding each time you go past the "target site" scales exponentially with speed – the slower you go, the more chance of binding before the target site is past.
    - The "drag stroke" where the crossbridge has gone past the energy minimum but has not yet released is shorter the slower you go, for the same reason.
- Even this extremely simplified model give a reasonable prediction of the measured Force-Velocity relationship of muscle.
- *The full calculation is in the problem sets, also in Howard's book.*

Reaction-Diffusion Equation

Diffusion with an external force: (As in topic 1, slide 17, but ***P(x),*** the probability of finding a motor at position ***x***, replaces the concentration ***C(x)***)

"Probability flux":

$$J = -D\frac{dP}{dx} + \frac{F}{\gamma}P \qquad F = \frac{-dU}{dx}$$

Continuity equation:

$$\frac{\partial P}{\partial t} = -\frac{\partial J}{\partial x} = D\frac{\partial^2 P}{\partial x^2} - \frac{\partial}{\partial x}\left[\frac{F}{\gamma}P\right]$$

Fokker-Planck Equation

With allowed transitions between states:

$$\frac{\partial P_i}{\partial t} = D\frac{\partial^2 P_i}{\partial x^2} - \frac{\partial}{\partial x}\left[\frac{F}{\gamma}P_i\right] + \sum_j\left[k_{ji}P_j - k_{ij}P_i\right]$$

Mechanical:
Motor movement while in state ***i***

Chemical:
Transitions into and out of state ***i*** at position ***x***

Reaction-diffusion Equation

The solution in slide 19 was for a very simple 2-state model with the assumption of constant speed. Generally, we need to solve a set of **reaction-diffusion equations…**

[Reaction-Diffusion Equation]

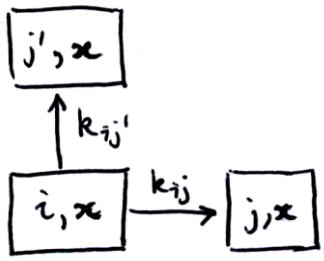
• Coupled Partial Differential Equations nearly always have to be solved numerically to obtain probability functions. Average speeds, ion fluxes etc can be derived from probability functions.

Advantage. Average properties obtained directly.

Disadvantage. No microscopic information.

Monte-carlo, chemical transitions

At time ***t***, motor is in state ***i*** at position ***x***.
State ***i*** has chemical transitions to states ***j*** (***j'***)



The probability of being in state ***j*** at time ***t+Δt*** (ie. making the transition to j) is:

$$P_j = k_{ij}\,\Delta t$$

and with sufficiently small ***Δt***…

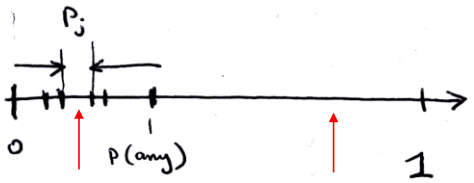
$$P(\text{any transition}) = \sum_j k_{ij}\,\Delta t \ll 1$$

Rate constants for these transitions obey detailed balance:

$$\frac{k_{ij}(x)}{k_{ji}(x)} = e^{\frac{U_i(x) - U_j(x)}{kT}}$$

(Non-zero rate constants define positions where the motor can switch states.)

A random number between 0 and 1 decides whether any transition should occur in time ***Δt***, and if so which…



Random number here, transition to ***j***

Random number here, no transition

[Monte-Carlo Simulation]

• At each time step, random numbers are used to determine the state of the motor at the next step **based on probabilities calculated from the current state of the motor**.

  • Note, the time step must be small enough that the motor does not move a significant distance, and thus it is ok to calculate rate constants and force *(-dU/dx*) based on the position before the time step. Similarly, the probability of a transition in a time step must be small, so that it is okay to assume that the motor is in one particular state during the time step.

  • Such a semi-random process is called “stochastic”.

Monte-carlo, movement

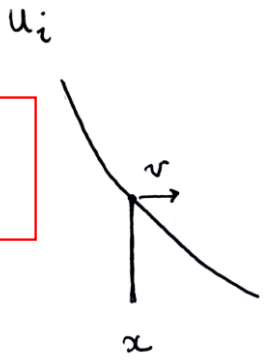
At time ***t***, motor is in state ***i*** at position ***x,*** moving with instantaneous velocity ***v***.

$F = ma$:

"Thermal force" due to collisions with water molecules

$$m\ddot{x} = -\frac{dU_i}{dx} + F_t - \gamma\dot{x}$$

"Langevin Equation"



Inertial forces are negligible:

$$\frac{dx}{dt} = -\frac{1}{\gamma}\left(\frac{dU_i}{dx}\right) + \frac{1}{\gamma}F_t$$

The distance moved in time Δt is…

$$\Delta x = -\frac{1}{\gamma}\left(\frac{dU_i}{dx}\right)\int_0^{\Delta t} dt + \frac{1}{\gamma}\int_0^{\Delta t} F_t\, dt$$

$$= -\frac{1}{\gamma}\left(\frac{dU_i}{dx}\right)\Delta t + RND\left(\sqrt{2D\Delta t}\right)$$

"forced" motion at velocity v (deterministic)

Brownian motion (stochastic)

The second term is the distance the particle would have travelled by free diffusion in time Δt.

Thus choose a random number drawn from a Gaussian distribution with mean-square $2D\Delta t$…

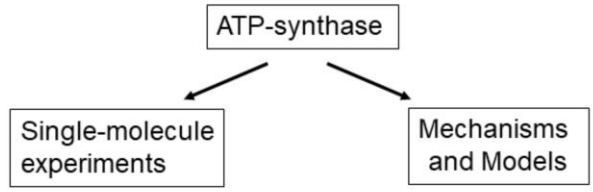
•The output of a Monte-Carlo simulation is a trajectory of the position and chemical state of the motor as functions of time.

- **Advantage.** Individual runs reveal the microscopic behaviour of the motor. Step sizes, distribution times for intervals between steps etc.
- **Disadvantage**. Many or long runs are needed to determine average properties like motor speed, ion flux, dependence upon pmf or external load etc. This can be computer intensive (but still far less so than "molecular dynamics" simulations).
- Sometimes called "Brownian dynamics" simulation.

• Monte-Carlo and reaction-diffusion approaches are mathematically complicated, sometimes a simpler treatment can still give useful predictions of motor behaviour and insight into motor mechanisms.

• More examples of molecular motors and models of their mechanisms in Topic 6…

Lecture 6: Molecular Motors, ATP-synthase

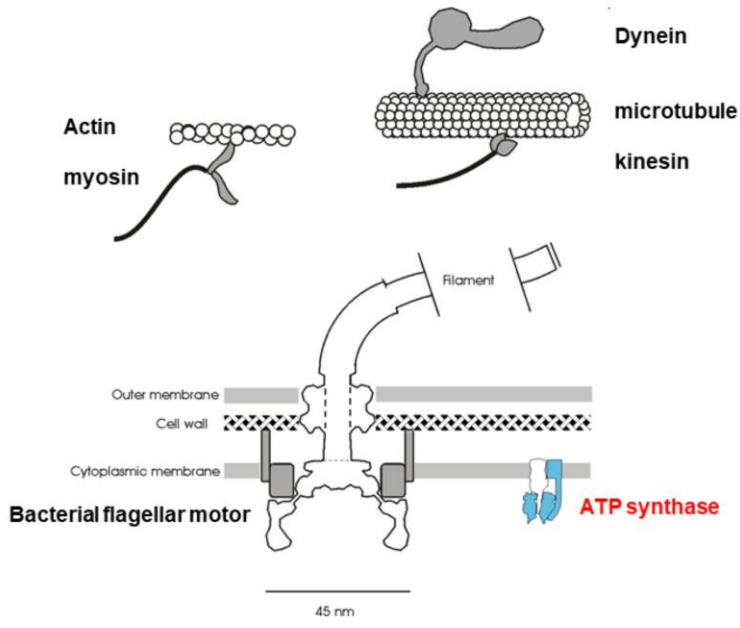


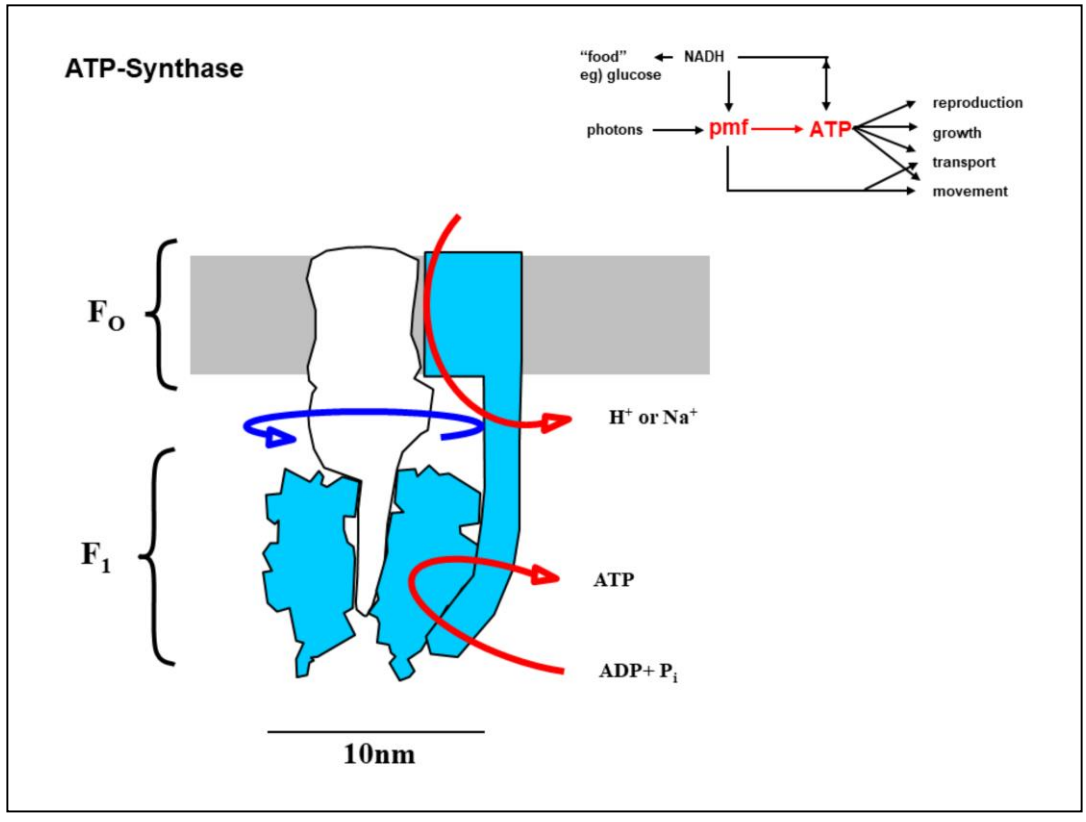
Reading for Lecture 6

- (Nelson Chapter 5.3, 11.3, 11.44)
- Journal Articles

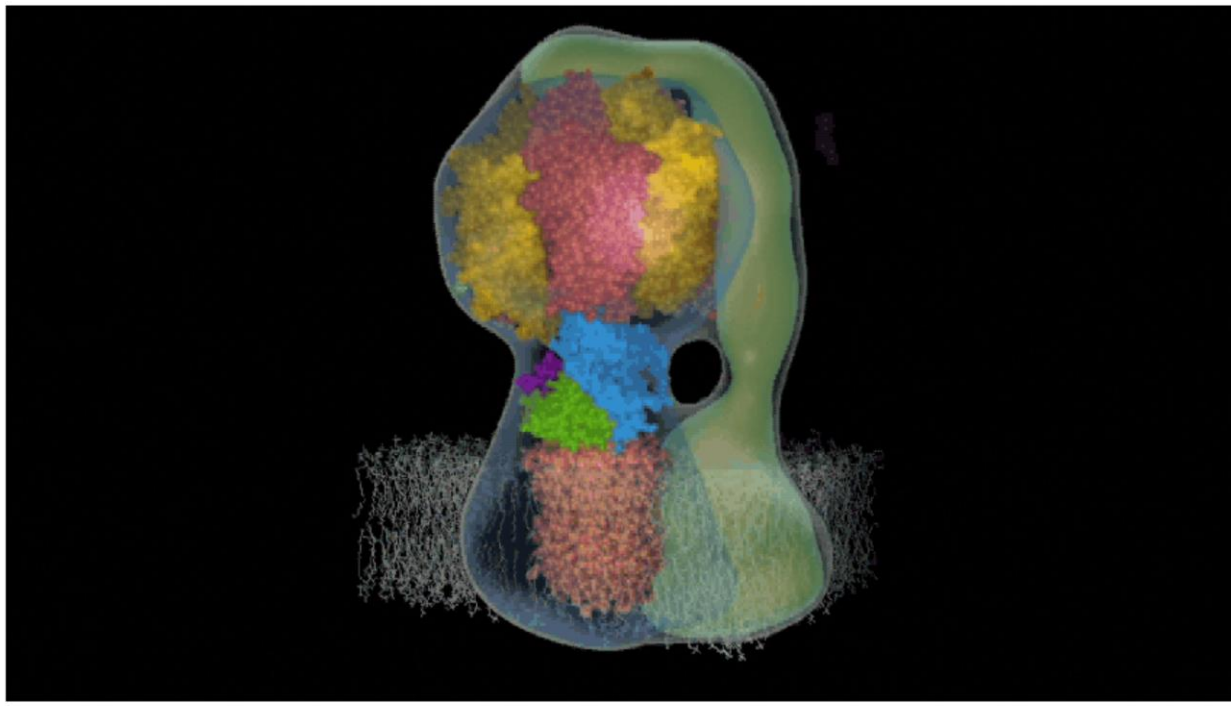
Reviews of (rotary) molecular motors in Journals:

- Yoshida M, Muneyuki E, Hisabori T. (2001). "ATP synthase--a marvellous rotary engine of the cell". *Nat Rev Mol Cell Biol Sep;2(9):669-77*
- Schliwa M, Woehlke G. (2003) "Molecular motors" *Nature 17;422(6933):759-65*


Dynein
microtubule
kinesin
Actin
myosin
Filament
Outer membrane
Cell wall
Cytoplasmic membrane
Bacterial flagellar motor
ATP synthase
45 nm



- Two rotary motors connected "back-to-back".
  - Normally F_O is stronger, it rotates forwards driven by pmf. F_1 is driven backwards, making ATP.
- Rotary mechanical coupling with different axial symmetries allows non-integer number of ATP per proton.
- Sodium-driven versions also exist.



Animation of the mechanism of ATP-synthase

Cryo-EM structures of E. coli F_1F_O – 2018 onwards

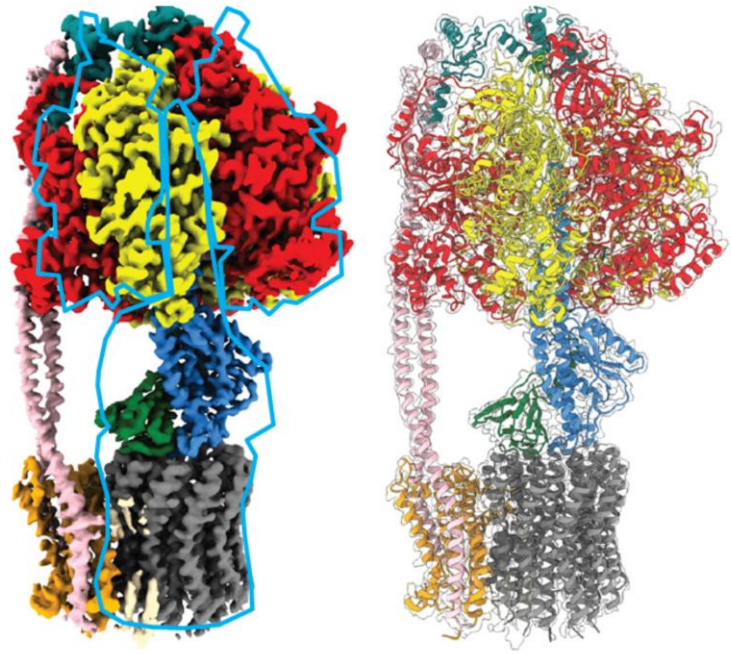


Fig. 1 Cryo-EM map and atomic model of *E. coli* F_1F_o ATP synthase.

Because ATP-synthase is partly a membrane protein, full crystal structures are not available.

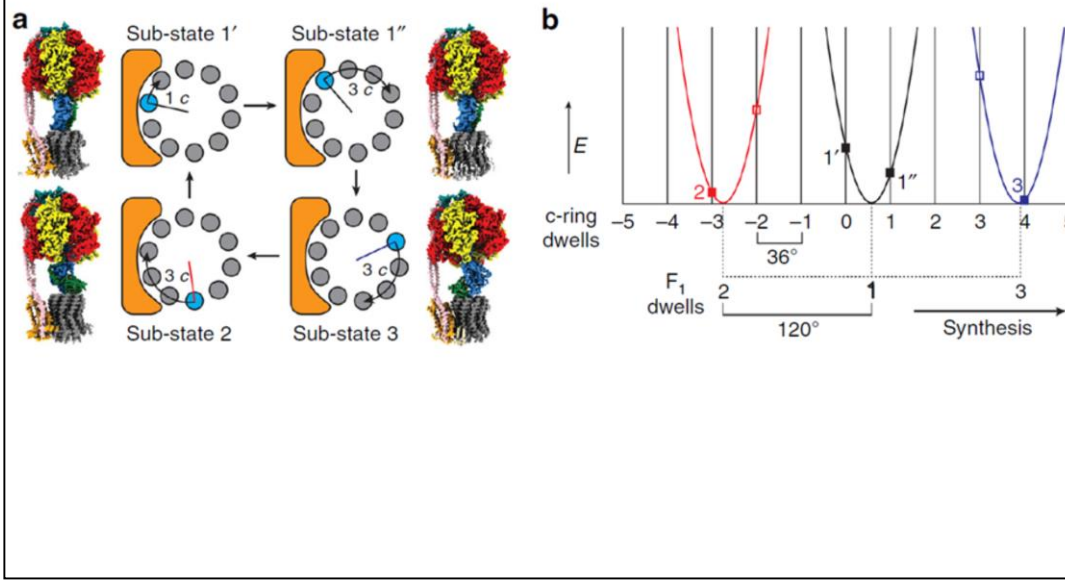
However, since ~2018 new "direct electro detectors" have allowed near-atomic resolution of many large, and membrane, protein complexes by Cryo Electron Microscopy.

This example is from

Sobti M, Walshe JL, Wu D, Ishmukhametov R, Zeng YC, Robinson CV, Berry RM, Stewart AG. (2020). Cryo-EM structures provide insight into how E. coli F_1F_o ATP synthase accommodates symmetry mismatch. Nature Communications. ***26;11(1):2615***.

(The blue outline is from an earlier, inclmplete structure, which was used for the schematic in earlier slides.)

Elastic coupling between F1 and FO – energy storage, smooth running.



This paper classifies each EM images of each individual molecules into one of several categories. Then each category is averaged to get one particular "sub-state" structure.

In this paper, there were 4 main sub-states, differing in the rotation angle about the F1 and FO rotating interfaces.

SIGNIFICANCE ?

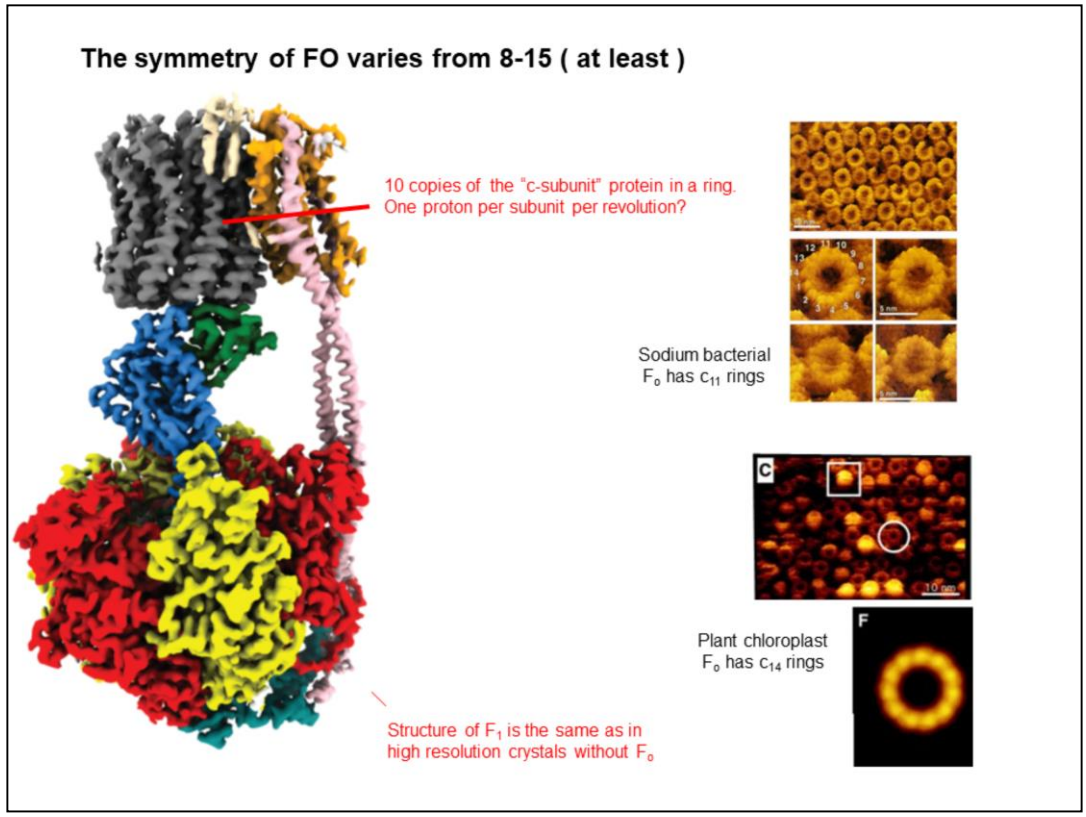
On average, for each ATP produced (3 per rev), FO needs to take 10/3 = 3.333 steps.

Energy from the FO steps is stored in elastic deformation of the entire machine – somewhere between the rotating interfaces in each of F1 and FO.

Different recent structural papers see deformations in different parts of the machine – it probably varies between organisms.

These structures come from de-energized F1FO – there is no membrane so no membrane potential. It's just measuring the symmetry mismatch that is accommodated by the elastic linkage between F1 and FO

Sobti M, Walshe JL, Wu D, Ishmukhametov R, Zeng YC, Robinson CV, Berry RM, Stewart AG. (2020). Cryo-EM structures provide insight into how E. coli F_1F_o ATP synthase accommodates symmetry mismatch. Nature Communications. ***26;11(1):2615***.



Because ATP-synthase is partly a membrane protein, full crystal structures are not available.

• Variable F_O rotor symmetry (***n***-fold, where ***n*** is the number of c-subunits) in different species.

  • 9 - 15 seen so far (2011). +8, (2012)

  • Assume this is the number of protons per revolution. Different "gear ratios", ***n*** protons per 3 ATPs.

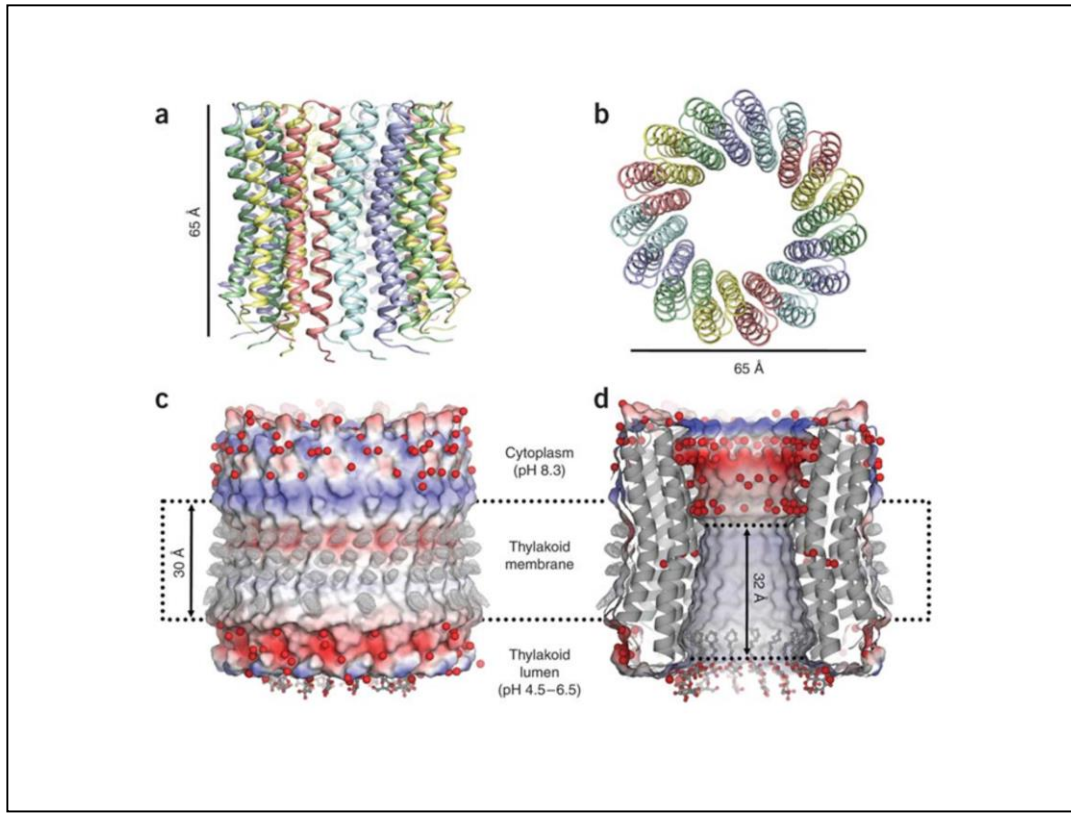
• c_{11} and c_{14} images by Atomic Force Microscopy (AFM).

• Models for the mechanism of F_O are adapted from early models of the bacterial flagellar motor…

T. Elston, H. Wang and G. Oster. (1998) Energy transduction in ATP synthase. Nature 391:510-513.

•This paper plus lots more available at…

http://nature.berkeley.edu/~goster/pubs.html



•Since ~2009 there have been a few atomic structures of c-rings from F_O of ATP-synthases.

- •The slide shows an x-ray crystal structure of a 15-fold c-ring from a photosynthetic cyanobacterium.

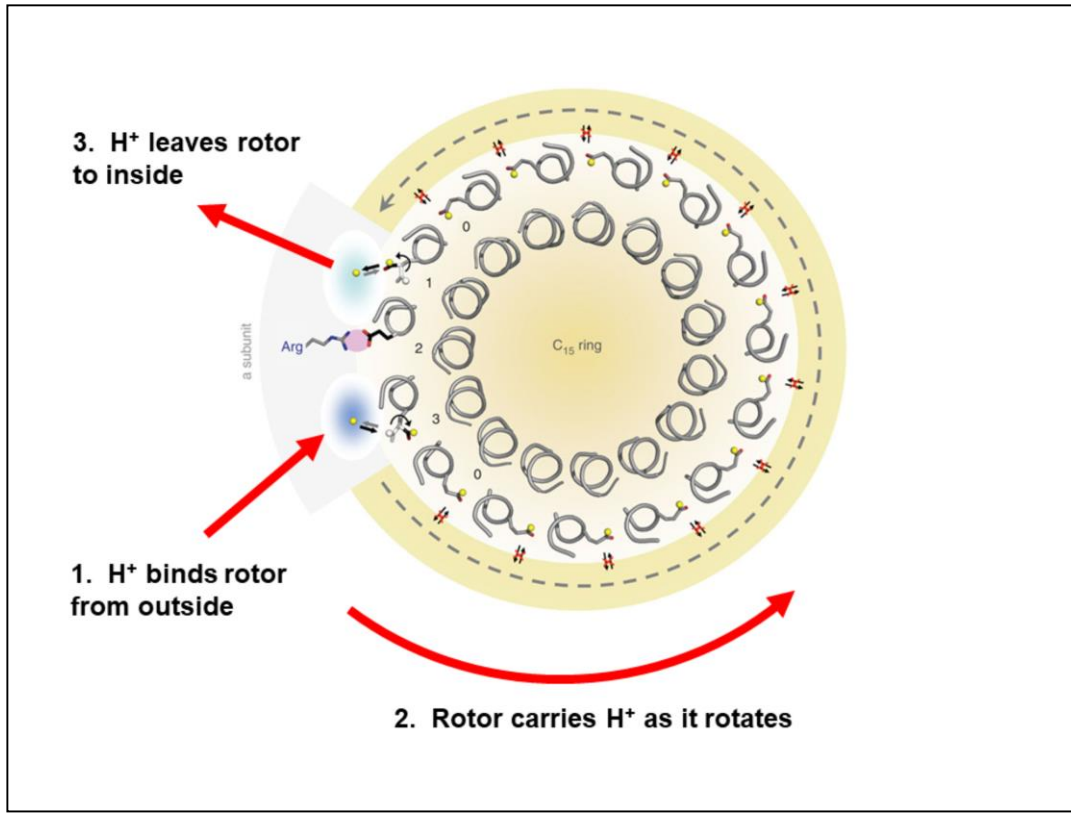
•The middle belt is hydrophobic as expected for a membrane protein.

•Detergent molecules are bound where the lipid tails would be in a natural setting.

•A binding-site for H^+ ions is present at the “waist”

Pogoryelov D, Yildiz O, Faraldo-Gómez JD, Meier T. High-resolution structure of the rotor ring of a proton-dependent ATP synthase. Nat Struct Mol Biol. 2009 16:1068-73.

The second c-ring structure at atomic resolution. The first was an 11-fold ring from a Na^+ ATP-synthase.



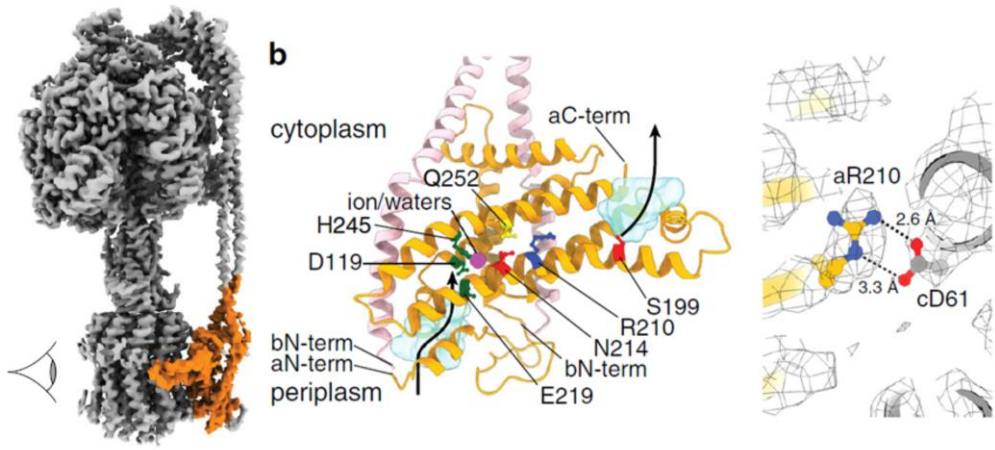
"Turnstile" mechanism for F_O

•Ingredients:

- •A-subunit contains half-channels that link the binding sites on the rotor to EITHER inside or outside, but don't go all the way through
- •Ions on the rotor cannot pass the centre of the a-subunit
- •(in some versions: binding sites WITHOUT ions cannot leave the a-subunit, because that exposes net charge of the binding site to the membrane interior)
- •Thus the only path for ions across the whole membrane, driven by the PMF, includes being carried around by rotation of the ring. This couples ion transit to rotation, making a rotary electric motor.

•This mechanism was first proposed for the bacterial flagellar motor in 1983, but is no longer believed to be likely there as no binding site for ions has been found on the rotor of that motor.

Structures support the (predicted) turnstile mechanism



Cryo-EM structures show the details of how the turnstile works.

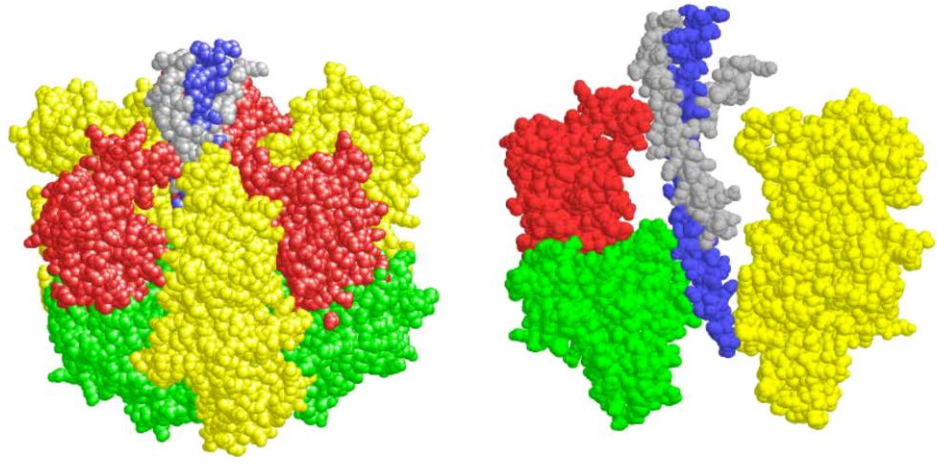
Alpha-helices in the plane of the membrane in the middle of the membrane had (have still ?) never been seen anywhere else.

These provide the barrier to direct ion transits required by the turnstile model

Details of the ionic “half-channels” can be seen, and are being worked out still as of 2021.

Sobti M, Walshe JL, Wu D, Ishmukhametov R, Zeng YC, Robinson CV, Berry RM, Stewart AG. (2020). Cryo-EM structures provide insight into how E. coli F_1F_o ATP synthase accommodates symmetry mismatch. Nature Communications. ***26;11(1):2615***.

Crystal Structure of F_1 – Leslie and Walker 1994

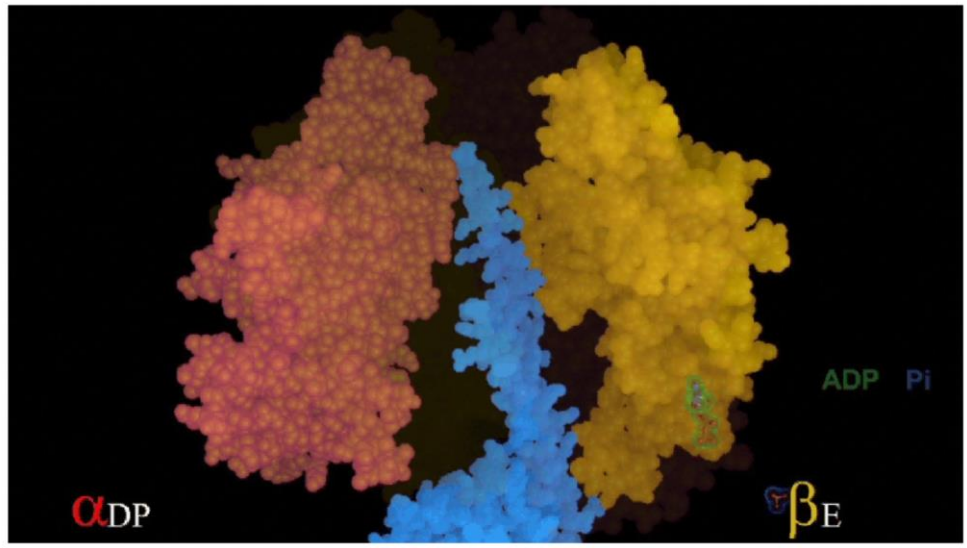


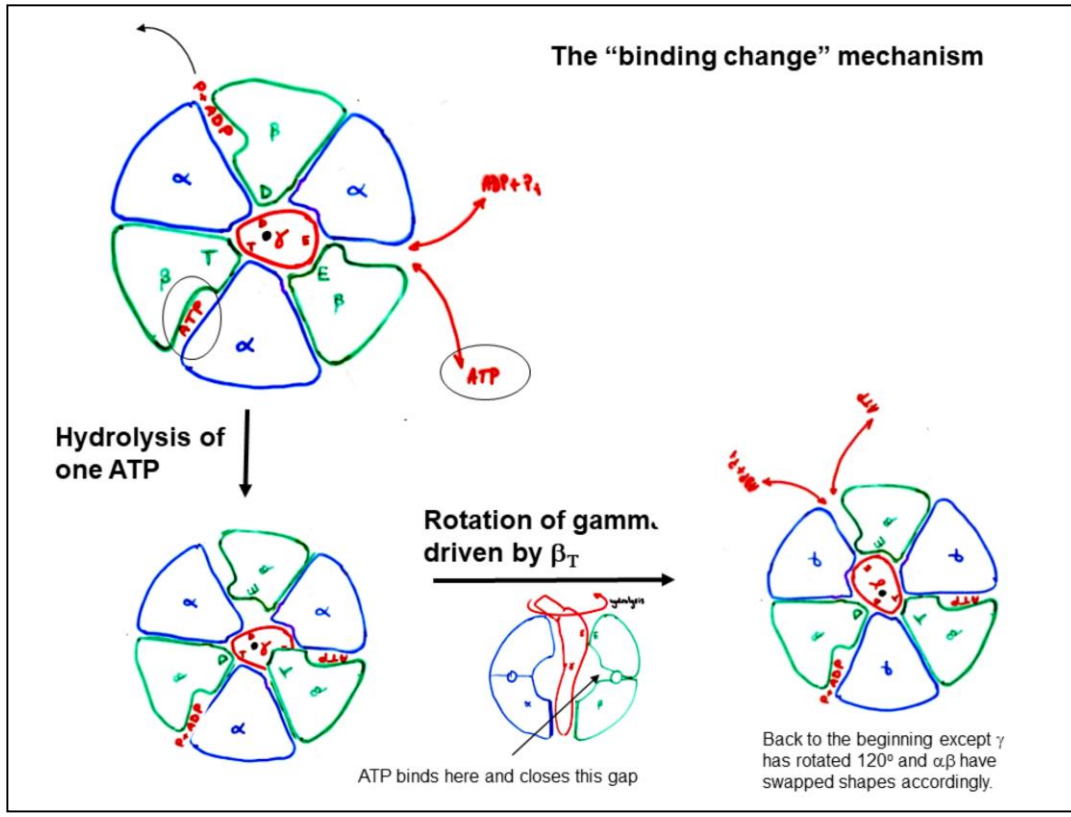
http://nature.berkeley.edu/%7Ehongwang/Project/ATP_synthase/

• The 3-fold symmetry of the outer "orange" part of the structure ($\alpha_3\beta_3$ subunits) was strong evidence for the "binding change" mechanism (next slide, this movie).

> • *Abrahams JP, Leslie AG, Lutter R, Walker JE. Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria. Nature. 1994 Aug 25;370(6491):621-8.*

• The movie was made by linear interpolation of the position of each atom between it's positions before and after a 120-degree rotation of the whole structure. The key, however, was to interpolate atoms in $\alpha_3\beta_3$ radially, but atoms in the central γ subunit by rotation of the whole subunit.


αDP
ADP
Pi
βE

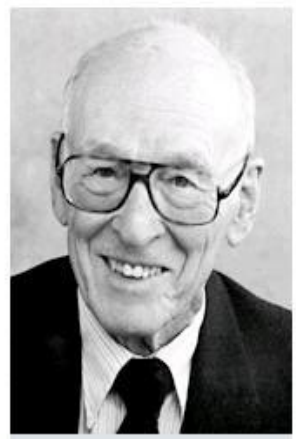


• Postulated long before rotation was demonstrated, based on indirect evidence for rotation and quasi-3-fold symmetry.

• Crystal structure and recent rotation experiments (next) provide strong support.

• 3 ATP hydrolysed per revolution.

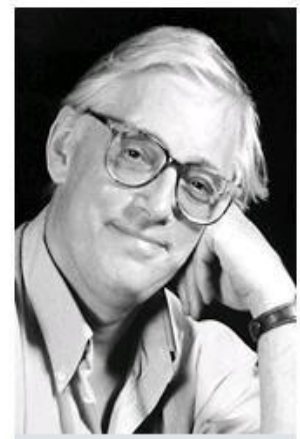
Walker and Boyer got the Nobel Prize in Chemistry (1997) for their work on F_1.



Paul D. Boyer

1/4 of the prize

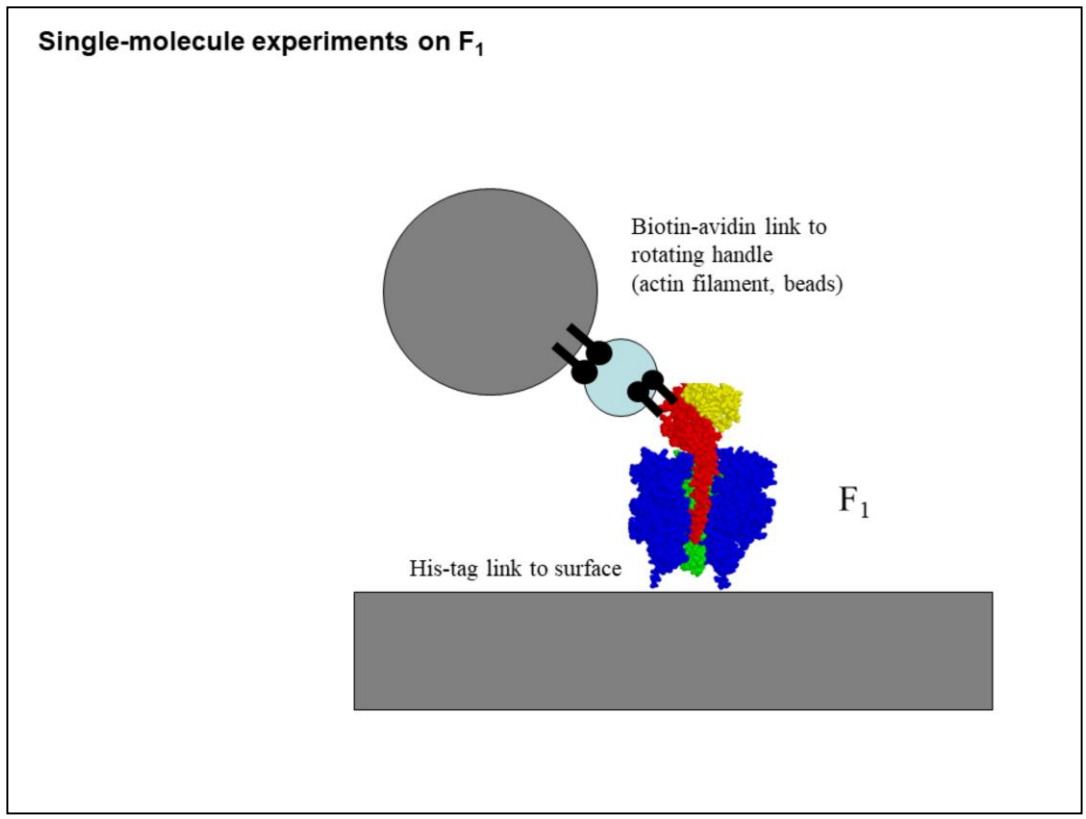
USA



John E. Walker

1/4 of the prize

United Kingdom



• A great deal of biochemistry and bulk reaction kinetics was done on F_1 over the years, but in 1997 the incontrovertible direct proof of rotation was obtained…

• A visible handle must be attached to the rotating γ-subunit if rotation is to be detected.

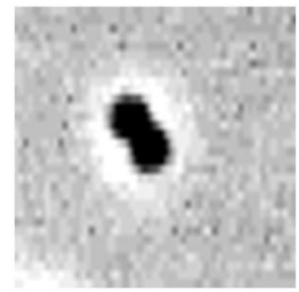
• Numerous techniques are available for making specific attachments to the right parts.

- Biotin is a small vitamin that is tightly bound by streptavidin, both are available commercially.
- A derivative of biotin was used that make covalent bonds with only the –SH groups of genetically engineered cysteine residues into the γ-subunit. All other surface cysteines are removed, again genetically.
- Multiple histidines were added to the end of the β-subunit by genetic engineering. This “his-tag” binds Nickel ions that are linked to the surface by a molecule called “NTA”.
- (His-tags are commonly used to purify the protein that is encoded by a selected gene.)

Fluorescent actin filament
Rotated by F1



0.5 micron beads
Rotated by F1



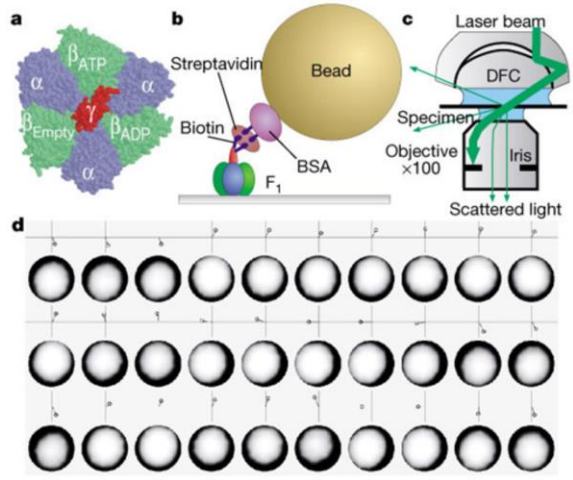
(movie: Wolfgang Junge)

- There can be no doubt that F_1 has a rotary mechanism !
- Many groups all over the world are now using this assay
- It's still very difficult!

•Noji H, Yasuda R, Yoshida M, Kinosita K Jr. Direct observation of the rotation of F1-ATPase. Nature. 1997 Mar 20;386(6622):299-302

The first direct observation of rotation of F1, using actin filaments

Rotation with 40 nm gold particles

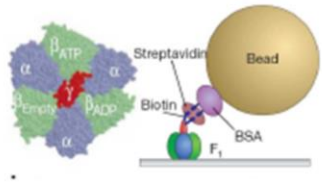


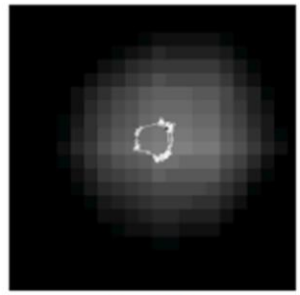
• The link between the handle and the γαμμα-subunit has torsional stiffness *k*, and therefore the handle filters rotation of the γαμμα-subunit with a time constant ϕ/k, where ϕ is the rotational drag coefficient of the handle.

- ϕ can be calculated for a sphere, and depends on radius cubed. Therefore smaller handles reveal faster events, larger handles smooth them out.
- 40 nm gold particles can be seen using laser dark-field microscopy.

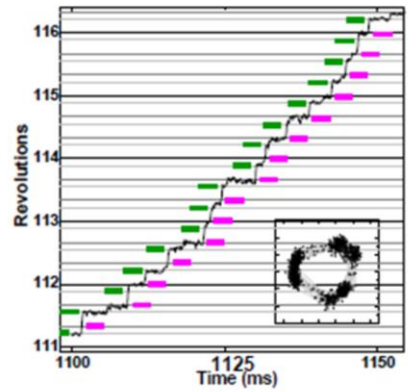
•The position of a single point scatterer of light can be fitted to arbitrary accuracy, depending only on the amount of light collected, not limited by the diffraction-limited size of the particle's image.

Direct observation of 120° steps and sub-steps



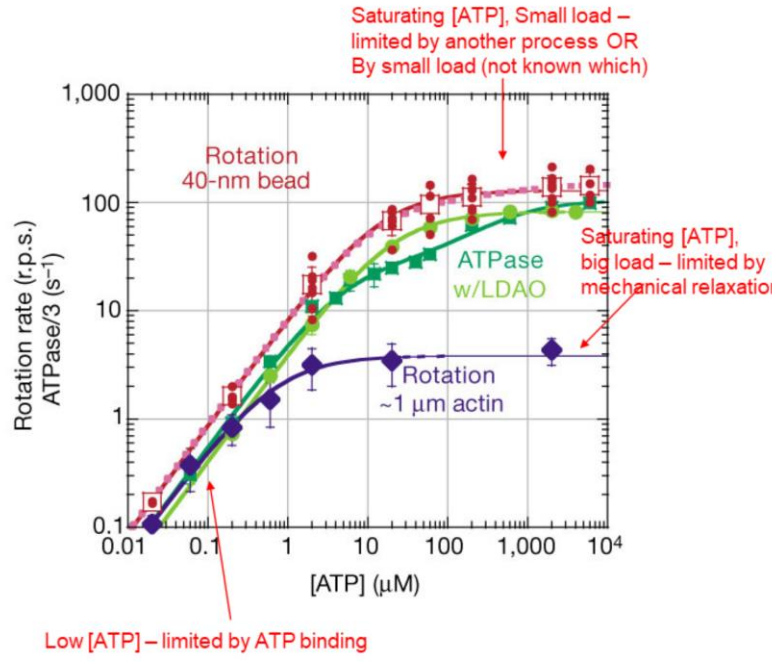


slowed ×400



R. Yasuda *et al.*, *Nature* 2001

Speed vs [ATP] for different sized handles…

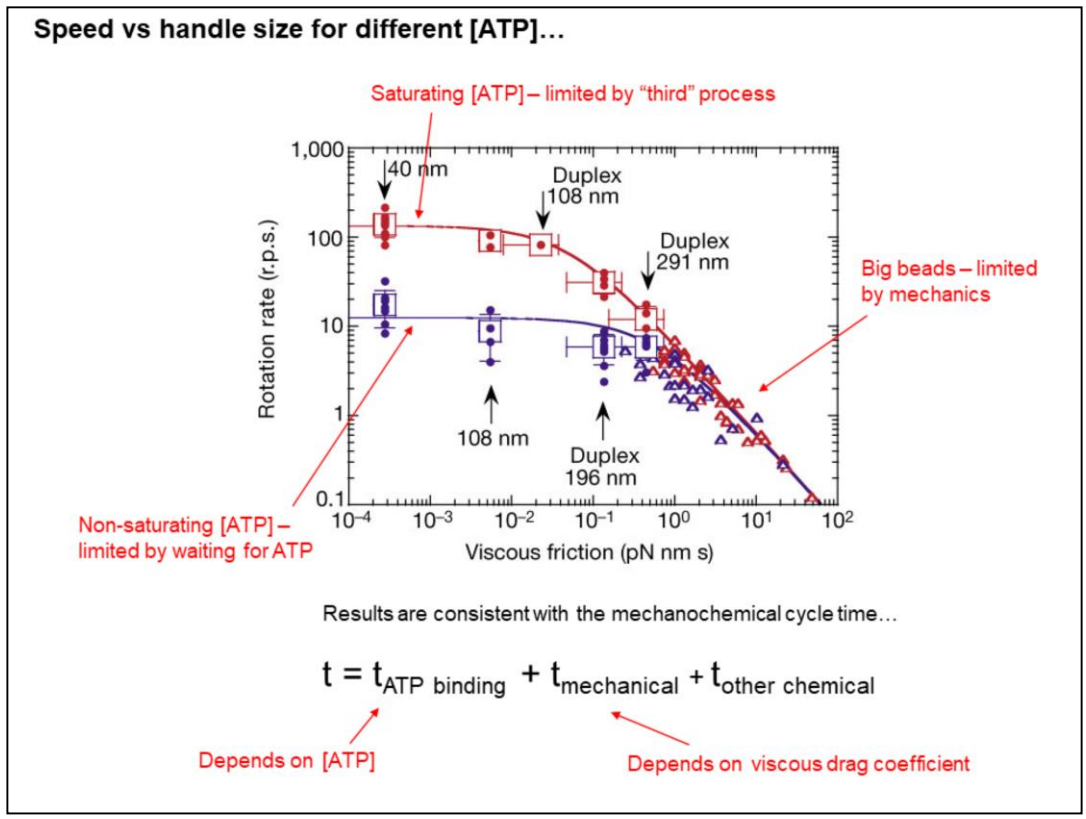


• Rotation rates (top and bottom curves for small and larger handles respectively) obey Michaelis Menten kinetics (see topic 3)…

  • Maximum speed, at saturating [ATP], is faster for smaller handles. Thus for the larger handles at least, mechanical relaxation of the motor is rate-limiting at high [ATP].

  • At low [ATP], the speed is determined by waiting for ATP to bind, and speed is proportional to [ATP].

• Plotting the data this way allows us to infer the existence of steps that depend on ATP binding and those that don't.



• Plotting the data this way allows us to distinguish between steps that depend on viscous drag (ie. mechanical relaxation to the energy minimum of the new state) and those that don't.

• At saturating [ATP], making handles smaller than 100 nm does not make the motor go faster.

  • In this case, mechanical relaxation, is no-longer rate-limiting, even at saturating [ATP]. Something else must be rate-limiting here…

  • Thus a 3rd type of step is identified, that depends on neither viscous drag nor ATP binding.

• All becomes clear when individual steps in the mechanochemical cycle can be seen…

Another derivation of the Michaelis-Menten curve

$$t = t_{ATP\ binding} + t_{mechanical} + t_{other\ chemical}$$

Rate V, vs [ATP]

$$V = 1/t = 1/(t_{ATP} + t_o)$$

Define k_A, V_o …

$$V = \frac{1}{1/(k_A[ATP]) + 1/V_o} = \frac{V_o[ATP]}{V_o/k_A + [ATP]}$$

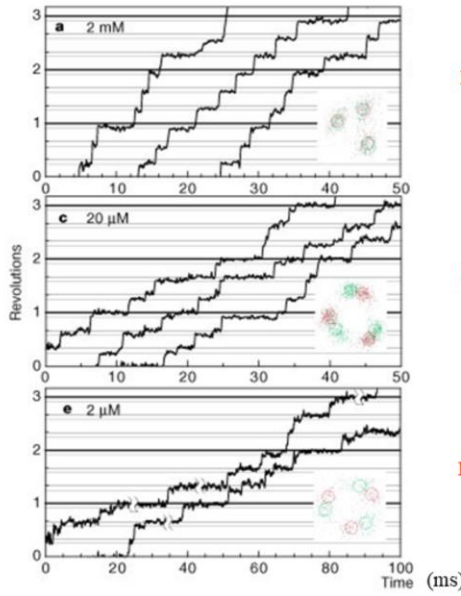
This has the same form as the Michaelis-Menten relationship (topic 3)

• Here the rate is in units of reaction cycles per second. The rotation rate is 3x slower as each cycle corresponds to a 120-degree rotation (below).

• The Michaelis-Menten form is not surprising – after all, this IS a catalysed reaction. F_1 catalyses the hydrolysis of ATP.

- At low [ATP] the reaction is limited by waiting for ATP, and the rate is proportional to [ATP]
- At high [ATP] the ATP arrival time is negligible, and the reaction proceeds at a maximum rate $V_o = 1/t_o$
- This is the “essence” of M-M kinetics, the same arguments apply to the derivation in topic 3.

Stepping rotation of F_1



High [ATP] : no wait before 90° step

Medium [ATP] : ~ms before 90° step

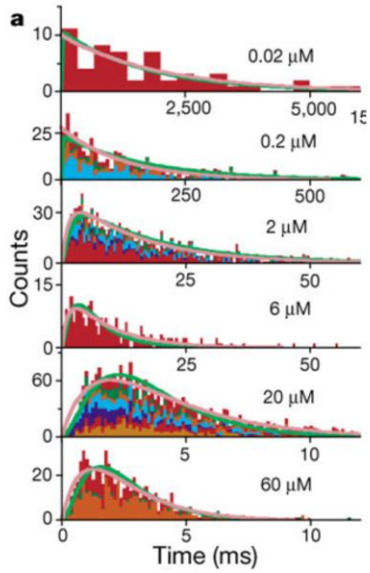
Low [ATP] : long wait before 90° step

All [ATP] : ~ms before 30° step

- The motor rotates in 120° steps, each corresponding to hydrolysis of 1 ATP.
- Sub-steps of 90° and 30° are resolved when 40 nm gold handles are used to give high time-resolution.
- Key results:
  - The time interval before a 90° step is inversely proportional to [ATP] – this is the ATP waiting step. ("t_{ATP}" from slide 35)
  - The time interval before a 30° step is about 2 ms, independent of [ATP] – this is ATP hydrolysis and product release steps. ("t_{other}" from slide 35)
  - The time interval during any step is very short, ~0.25 ms, close to the time-resolution limit of the technique – this is the mechanical relaxation, and is consistent with a torque of about 40 pN nm, equal to the torque at stall, during the powerstroke. ("$t_{mechanical}$" from slide 35)

- Note: chunks of the trace where nothing happens have been removed from the low [ATP] trace.

Interval length distributions



Low [ATP] :
- 90° step rate-limiting
- exponential distribution
- single step

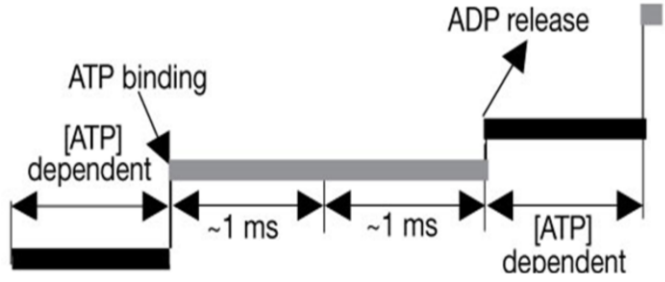
High [ATP] :
- 30° step rate-limiting
- peaked distribution
- double (or more) step

• When the ATP waiting step is rate-limiting, distributions are single-exponential – ATP binding is a single transition.

• When the 30° step is rate-limiting, distributions have a peak – this step is actually at least two sub-steps with similar rates.

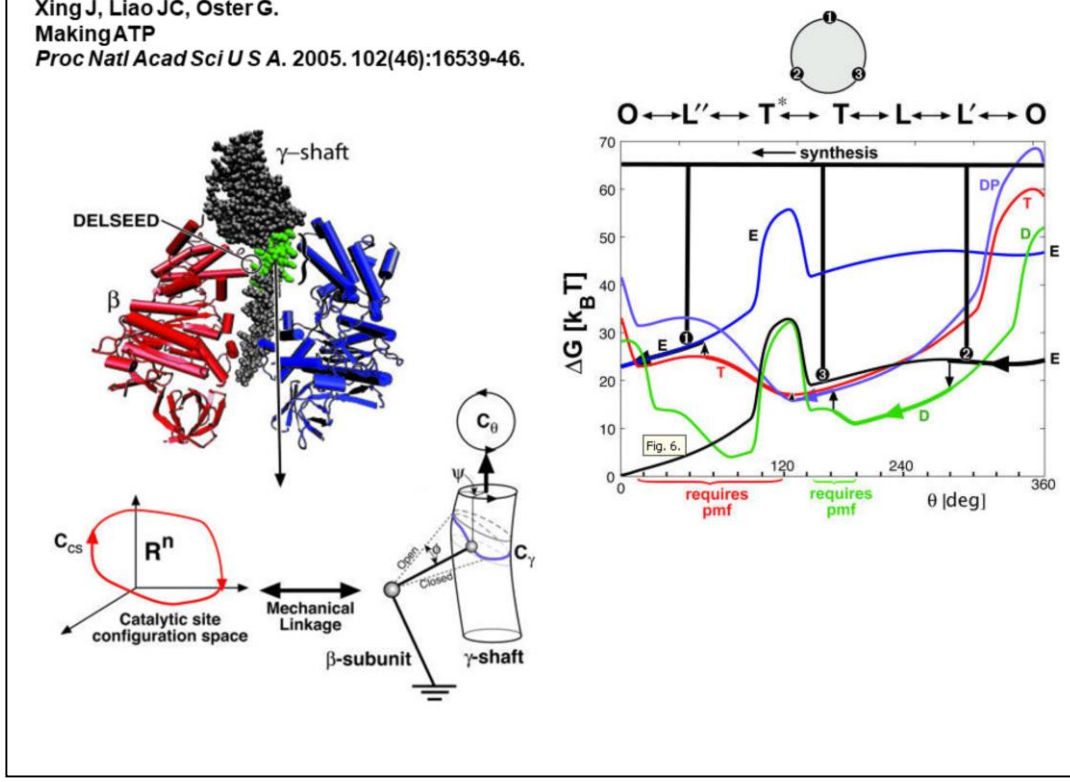
•See topic 2 and problem 4 for the interpretation of these distributions.

Summary of ATP step results



If you want to know more about ATP-synthase…

Xing J, Liao JC, Oster G.
Making ATP
Proc Natl Acad Sci U S A. 2005. 102(46):16539-46.

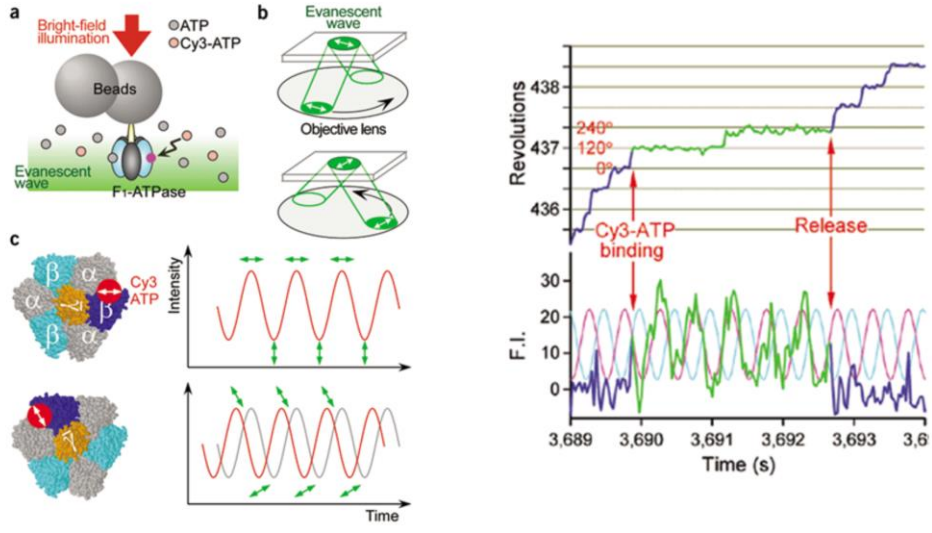


• This paper is part of an evolving series of reaction-diffusion models of F_1 and F_O.

• It attempts to justify in detail…

- the use of a single reaction co-ordinate for the coupled bending motions of the β-subunit and rotation of the γ-subunit (left of slide)
- the shape of the free energy profiles (right of slide)

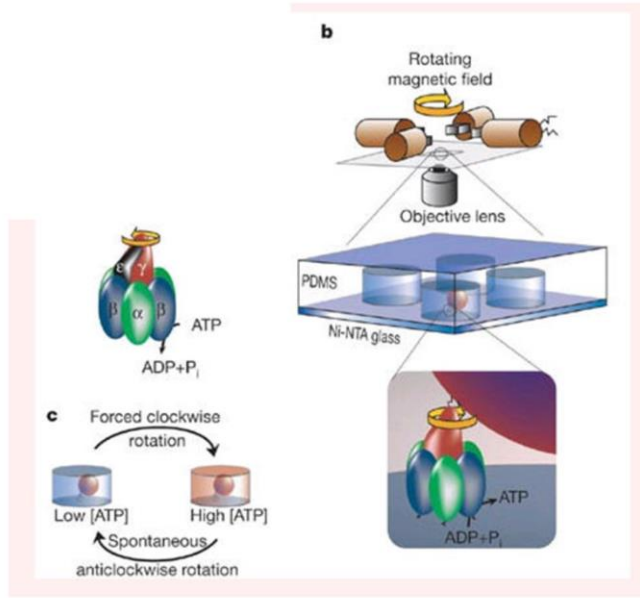
Nishizaka T, Oiwa K, Noji H, Kimura S, Muneyuki E, Yoshida M, Kinosita K Jr
Chemomechanical coupling in F1-ATPase revealed by simultaneous observation of nucleotide kinetics and rotation.
Nat Struct Mol Biol. 2004. 11:142-8



•This work is great! They get simultaneous single-molecule information about the mechanics AND the chemistry.

• Technically very difficult.

Rondelez Y, Tresset G, Nakashima T, Kato-Yamada Y, Fujita H, Takeuchi S, Noji H.
Highly coupled ATP synthesis by F1-ATPase single molecules.
Nature. 2005. 433(7027):773-7.

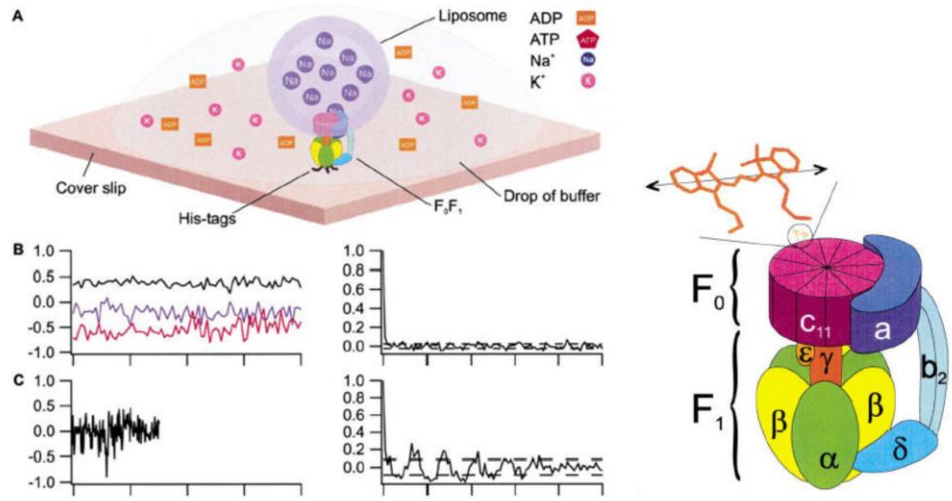


•They counted them out, and they counted them in…

•More great stuff from Noji :

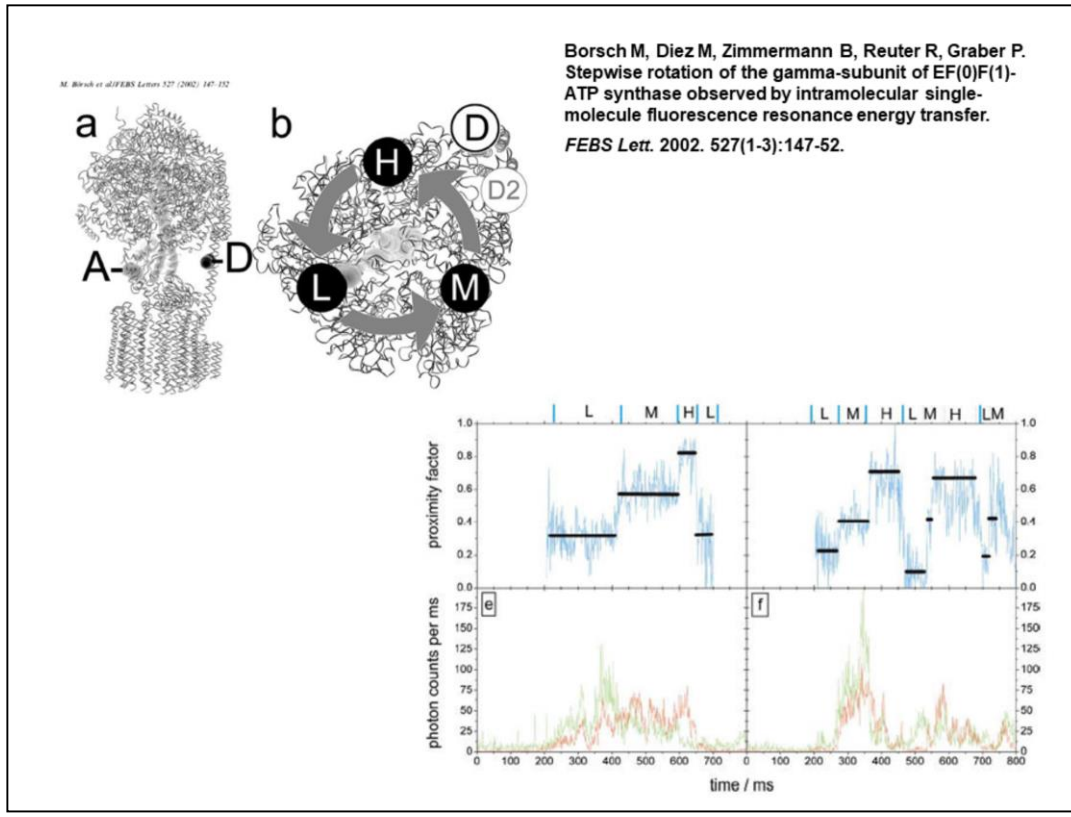
•Using rotation of a single F1 molecule to detect the same ~1000 molecules of ATP that it made when rotated using a magnet.

Kaim G, Prummer M, Sick B, Zumofen G, Renn A, Wild UP, Dimroth P.
Coupled rotation within single F0F1 enzyme complexes during ATP synthesis or hydrolysis.
FEBS Lett. 2002 Aug 14;525(1-3):156-63.



•By using a single-molecule label, they could observe step-wise rotation in intact ATP-synthase in vesicle, without having to attach a bead.

•But the signal quality and lifetime is MUCH lower than with a bead…



Borsch M, Diez M, Zimmermann B, Reuter R, Graber P. Stepwise rotation of the gamma-subunit of EF(0)F(1)-ATP synthase observed by intramolecular single-molecule fluorescence resonance energy transfer. *FEBS Lett.* 2002. 527(1-3):147-52.

•Another way to measure rotation of F_O with single-molecule fluorescent labels. Also very noisy.

•Achillefs Kapanidis will talk a lot more about “FRET”

•More from the same lab…

Diez M, Zimmermann B, Borsch M, Konig M, Schweinberger E, Steigmiller S, Reuter R, Felekyan S, Kudryavtsev V, Seidel CA, Graber P. Proton-powered subunit rotation in single membrane-bound F0F1-ATP synthase. Nat Struct Mol Biol. 2004 11(2):135-41

•The latest papers from this group offer evidence for 10-fold stepping of *E. coli* F_O. But the resolution and quantity of data are very far short of the equivalent 3-fold stepping data on F_1.