Reading for lecture 7

1. **Biological Free Energy**
2. **Bioenergetics and Electron Transport**
3. **Molecular Motors**

- Alberts et al Chapter 7
- Voet and Voet, Chapters 15,22
- Howard, Chapters 12,14
- Nicholls DG and Ferguson SJ. "Bioenergetics 3" 297pp Academic Press

Reviews of 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*
- Berry, R.M. and Armitage, J.P. (1999). "The bacterial flagella motor". *Adv. Microb. Physiol. 41:291-337.*
- Schliwa M, Woehlke G. (2003) "Molecular motors" *Nature Apr 17;422(6933):759-65*

[Reading for lecture 7] (1)

2nd law of thermodynamics: Entropy Increases

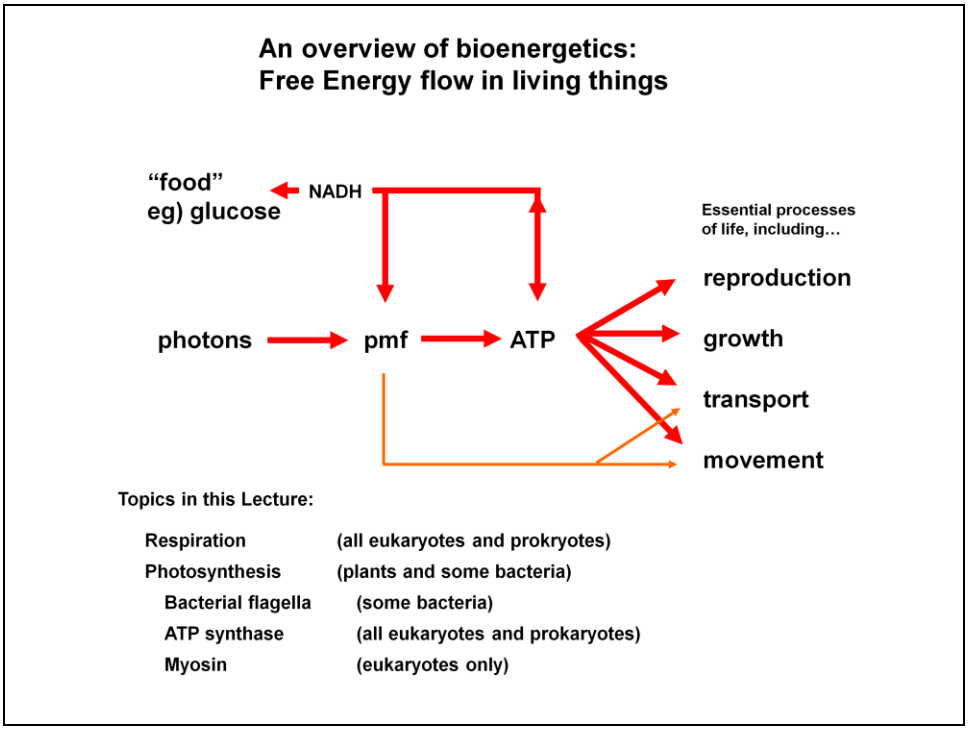
Life **Decreases** it's own entropy – at the expense of the rest of the universe

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

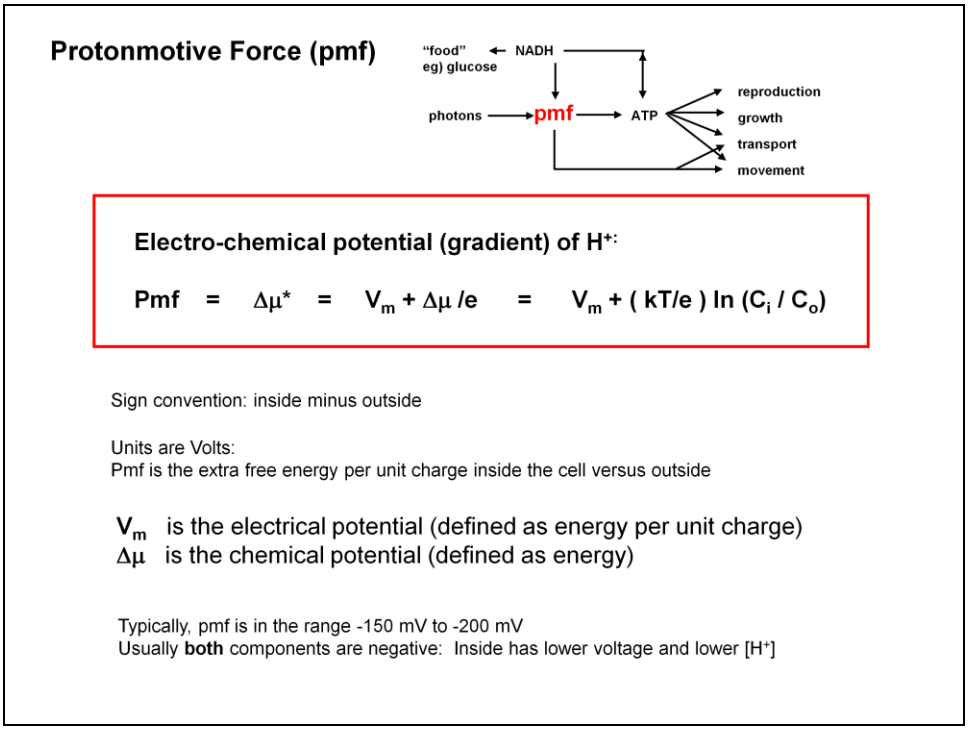
To do this, life needs to gather, store and manipulate sources of **Free-Energy**

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



Types of biological free-energy



Protonmotive force (pmf)

[Protonmotive Force] (3)

Electrical potential plus concentration gradient, H+ or "protons". (see lecture 6)

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.

Numbers:

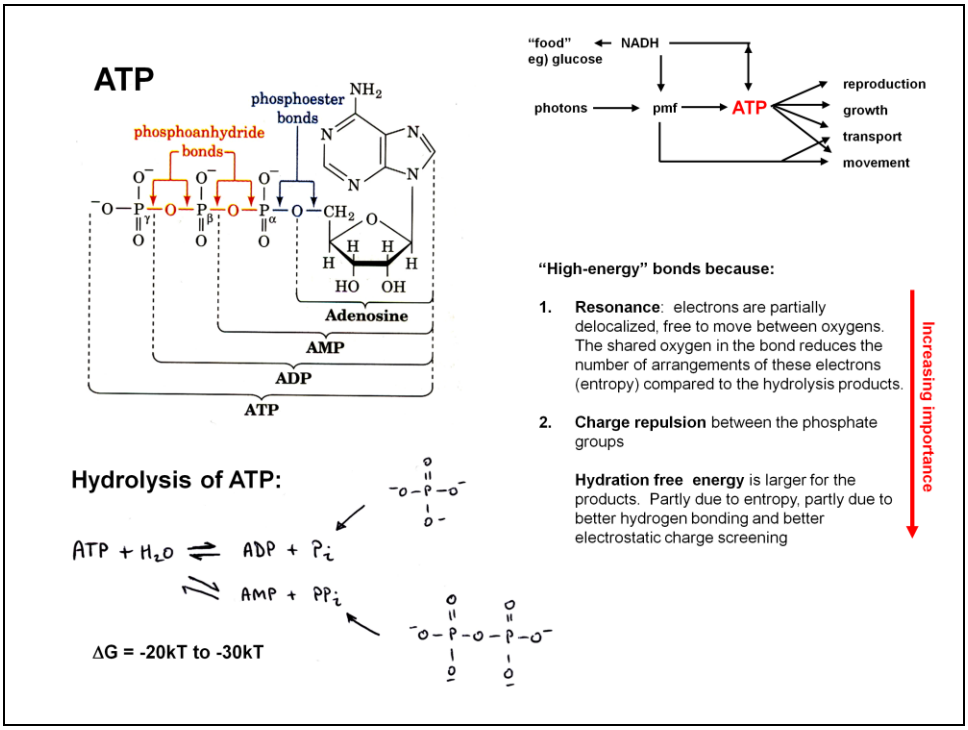
kT ~ 25 meV: so 150 mV ~ 6 kT per proton.

"e"-fold concentration ratio (~2.7-fold) ~ 25 mV ~ 1 kT per proton.

Pmf is used directly to drive some processes (especially in bacteria)

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.**



ATP

The **free-energy currency** of the cell.

[ATP / ATP hydrolysis] (4)

Biochemical standard free energy of ATP hydrolysis:

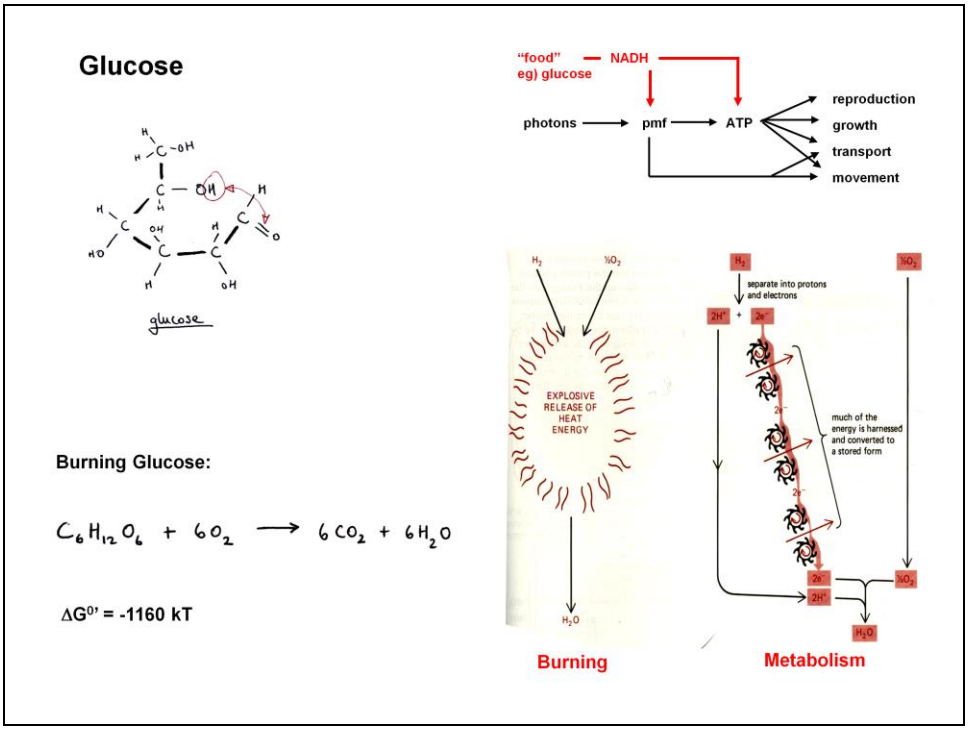
DG0' = 30 kcal / mole ~ 18 kT

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.

[Mg2+] is also important. ATP has charge 4-, usually it is electrostatically bound to one magnesium ion.

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



Glucose

[Glucose, burning and glycolysis] (5)

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.

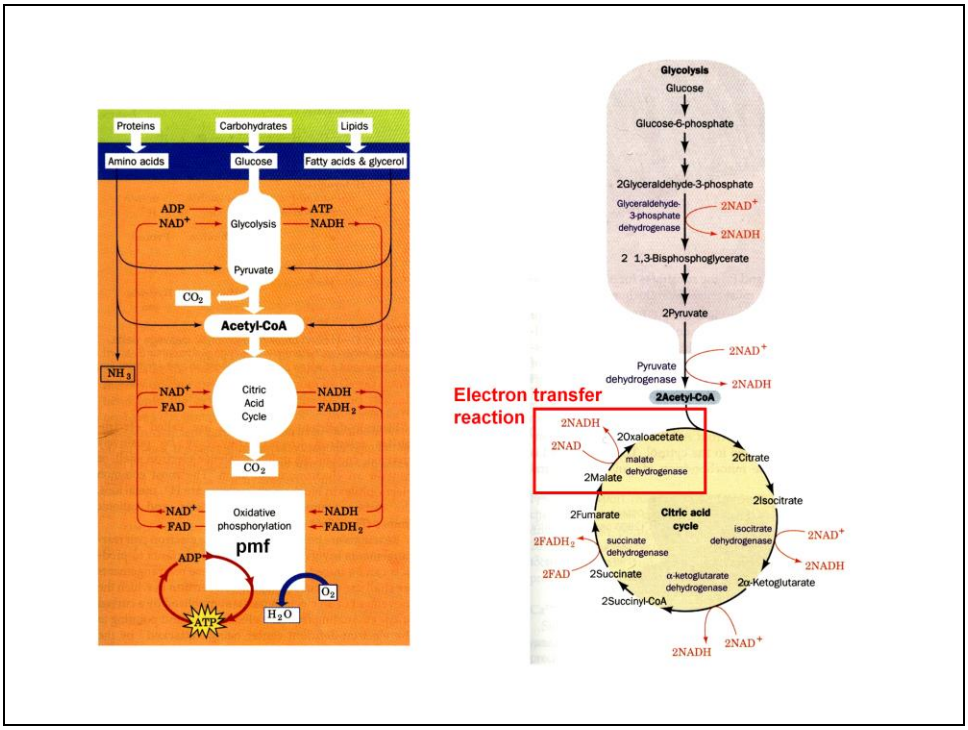
Poly-saccharides: eg glycogen in the liver, stocks last about half a day

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. (nb. The slide shows a simpler example, burning hydrogen, to illustrate the point)

Isothermal process, NOT a “heat engine”



How is this done?...

Bioenergetics and electron-transport

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

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

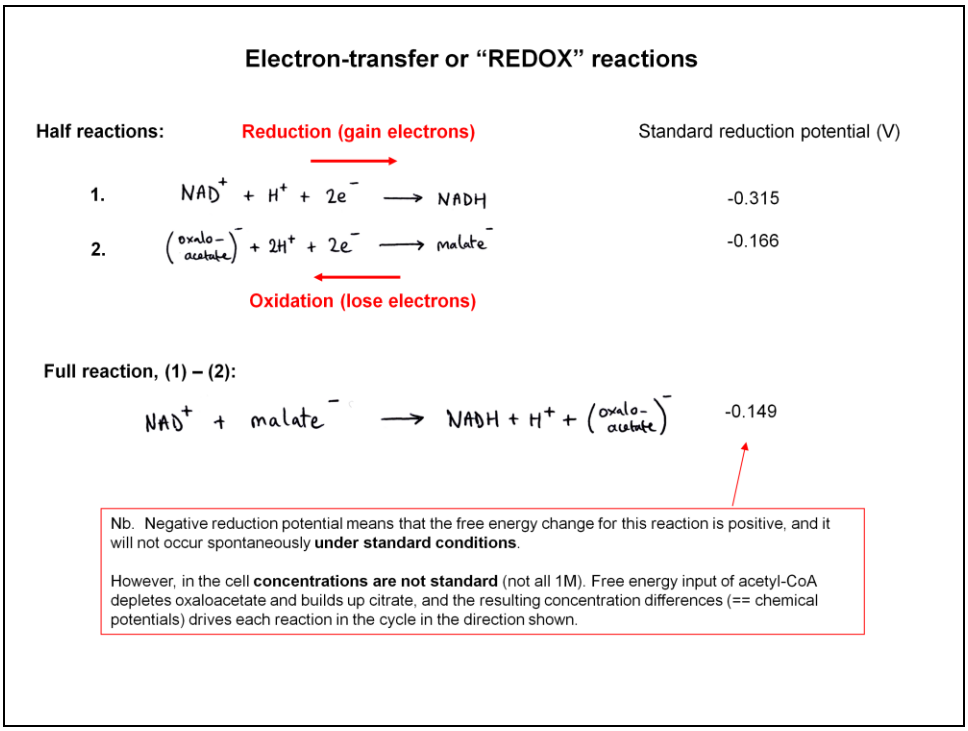
> Respiration: overall, uses conversion of food + oxygen to CO2 and H2O to transfer electrons from molecules where they have low free-energy (high affinity) onto molecules where they have high fee energy.
>
> Photosynthesis: overall, uses light energy to transfer electrons from molecules where they have low free-energy (high affinity) onto molecules where they have high fee energy.

Respiration

[glucose metabolism] (6)

The reverse pathway – synthesis of glucose from CO2 and H2O by consuming free energy – is similar but not identical. Less well understood.

NAD+→NADH is half of an electron transfer reaction...



[Electron Transport or REDOX reactions] (7)

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"

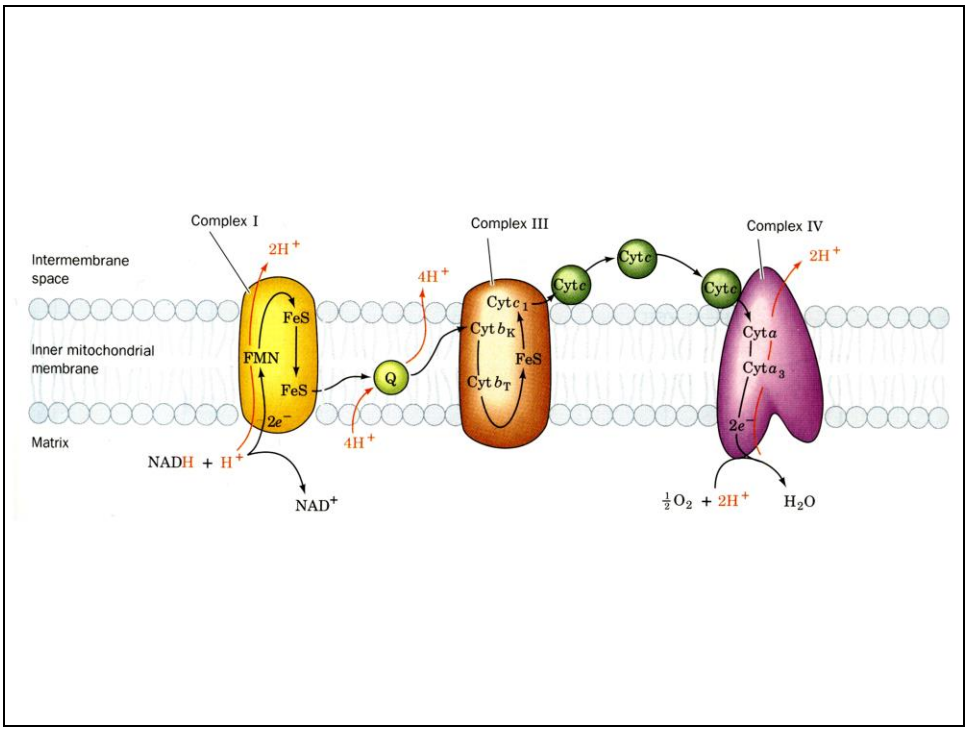
Mnemonic "OIL RIG" – "**O**xidation **I**s **L**oss, **R**eduction **I**s **G**ain"

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

Free energy change DG for any given reaction is the difference in reduction potentials of the half-reactions that make it up, times the charge transferred.

(Note: since electrons are negatively charged, a positive reduction potential means negative DG : ie) positive reduction potential means the reaction is downhill.)



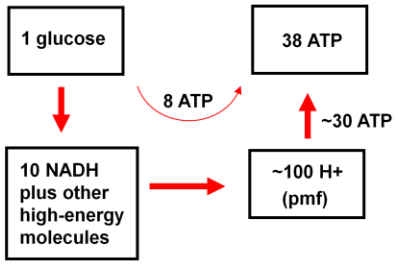
[Electron Transport and proton pumping in respiration] (8)

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.

The details of this process are not known.

Free Energy Accountancy

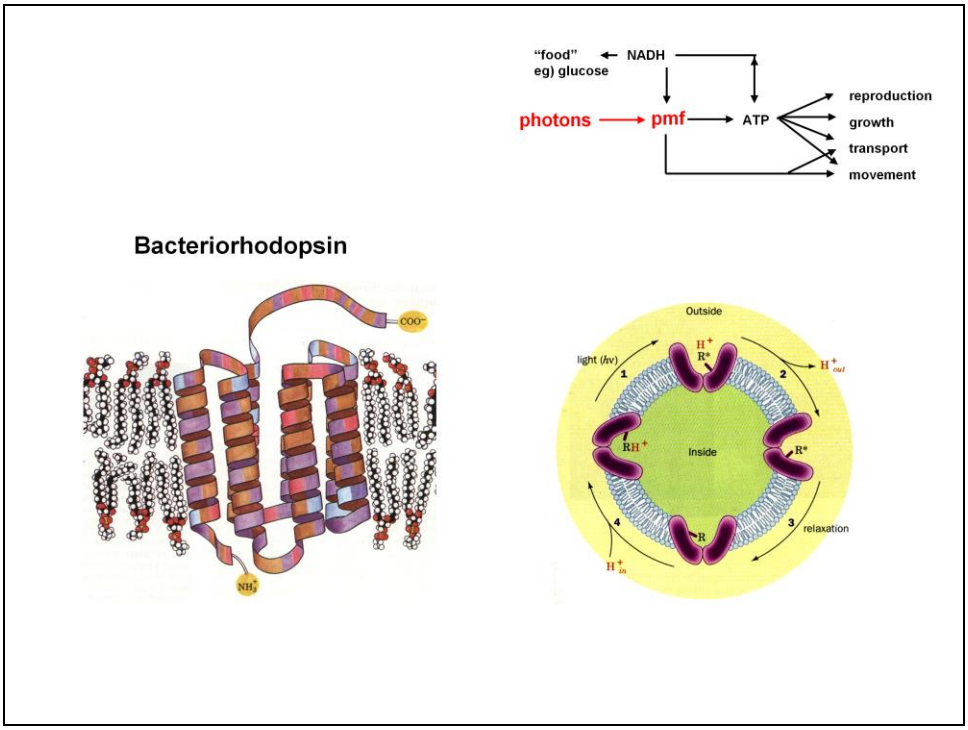


Approximate free energies:

	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.



Photosynthesis

Photosynthesis in plants comes in 2 parts:

Light energy is absorbed and used to make a pmf (and NADH). Pmf is used to make ATP

ATP (and NADH) are used to make organic carbon (eg glucose) from CO2 and H2O

Here we'll cover only the first part, specifically, the generation of pmf by light.

[Bacteriorhodopsin] (10)

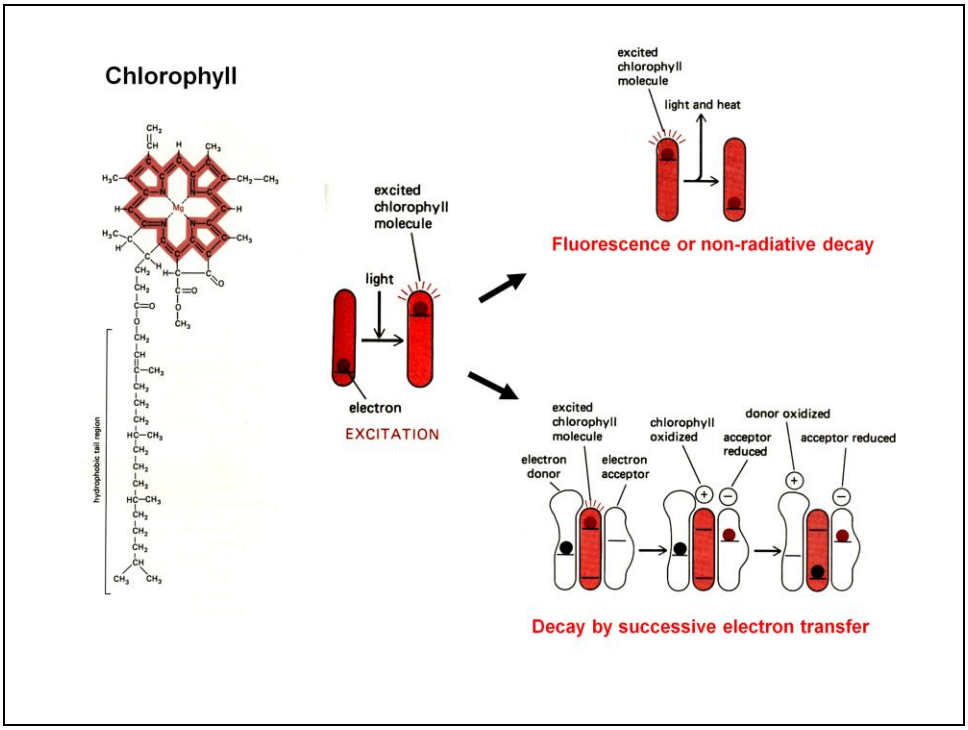
Crude but simple proton pump in halobacteria (archaic bacteria which live in high salt) – robust and lots of it in the bacterial membrane, so relatively easy to study.

Simple active carrier mechanism?

Low efficiency – one or two protons per photon

Similar to rhodopsin that detects light in our eyes.

Other bacteria have a better system…

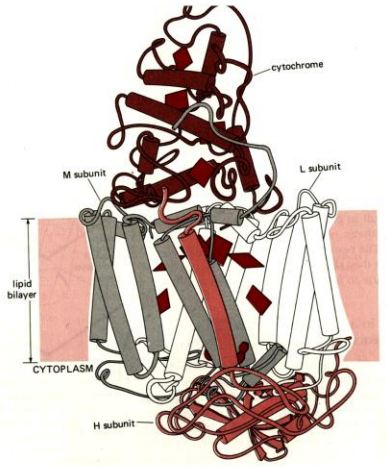


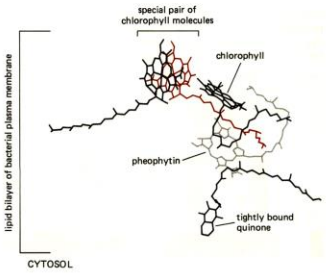
[Chlorophyll] (11)

Absorbed photon puts an electron into high-energy level or “excited state”. This high energy electron may be transferred to other molecules.

Chlorophyll is one of many “pigments” in biology - molecules that absorb light.

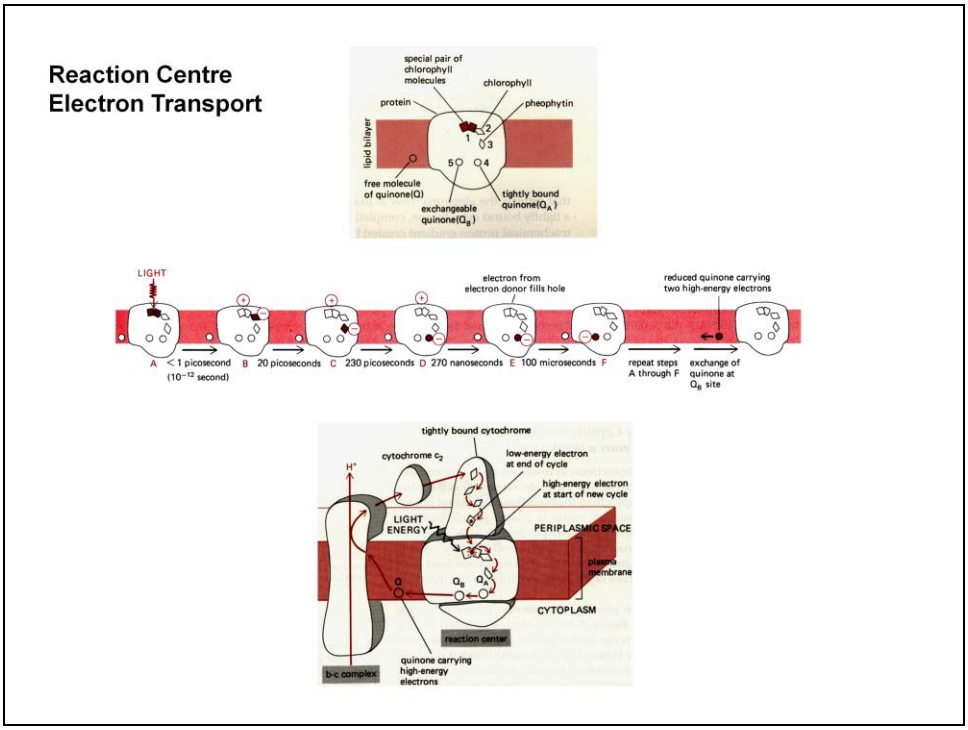
Bacterial Reaction Centre





[Bacterial Reaction centre] (12)

Pigments and other electron-transport molecules are held in a protein "scaffold" in the membrane. (molecular machines are not necessarily pure protein.)



[Electron transport in photosynthesis] (13)

The reaction centre transfers the high-energy electron to quinone, a molecule shared with respiratory electron transport.

The path(s) of electrons from the reaction centre is/are similar to those in respiratory electron transport (slide 7). (Only they are even less well known!)

The main path is a closed cycle – electrons go round and round.

Electron transport within the reaction centre is measured by ultra-fast laser spectroscopy.

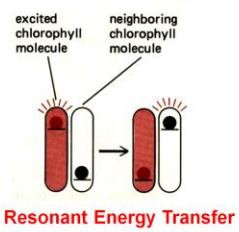
> Pico-second (*ps*) laser pulse to excite chlorophyll (Question, how long is this pulse in space?)
>
> Second pulse to measure absorption spectrum after a set delay time.
>
> Spectrum tells where the excited electron has got to as a function of time.

Electrons move by quantum mechanical tunnelling. Distances between groups can be varied by making mutant proteins.

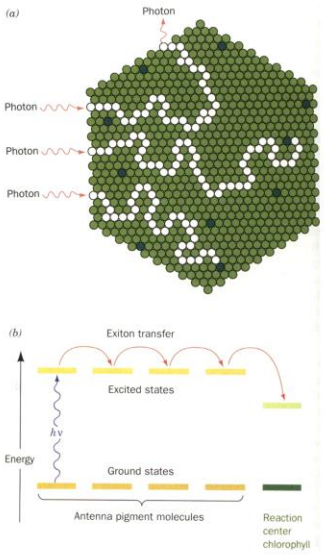
This is probably the best understood membrane protein. Crystal structure dates back to 1985.

Photosynthesis in plants and cyanobacteria is more complicated

> The cycle has two steps where photons are absorbed
>
> Electron transport is NOT a cycle, the end result of electron transfer is to split water into oxygen (O2) and H+
>
> Oxygen is very reactive – that's why things burn and why glucose can release so much free energy.
>
> The oxygen on earth was made by photosynthesis, life has altered geology.



Resonant Energy Transfer



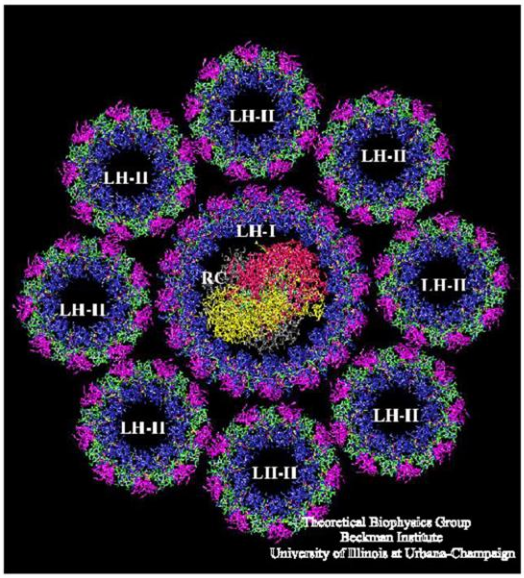
[Light-Harvesting or antenna complex] (14, 15)

Huge increase in the area for light capture.

Excitation is stored in a ring of molecules in each LHC unit and passed towards the reaction centre.

Crystal structures are now available, eg) search “light harvesting complex” on the web

Resonant energy transfer depends very steeply on the distance and angle between the donor and acceptor molecules, and the basis of a new technique (“FRET”) for measuring the distance between biological molecules.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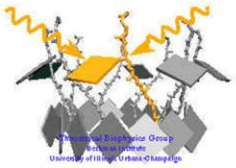
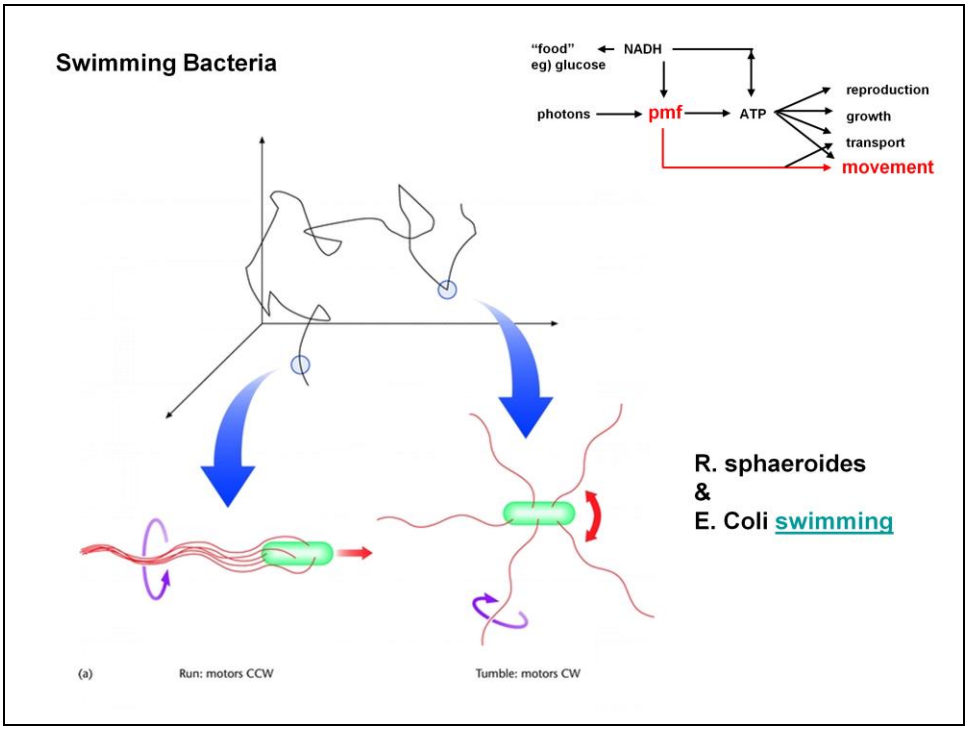
Beckman Institute
University of Illinois at Urbana-Champaign





Molecular Motors

Self-propelled motion is one of the hallmarks of life

Molecules also need to move around WITHIN living cells

- Active transport of nutrients etc.
- Translocation along DNA

Three molecular motors in this lecture: Bacterial flagellar motor, ATP-synthase, Myosin.

- Lecture 9: Models of how they *might* work
- Lecture 10: Single molecule experiments on how they *do* work.

There are many more, this research area is only just beginning.

Much of what is in the textbooks is out of date.

Bacterial flagella

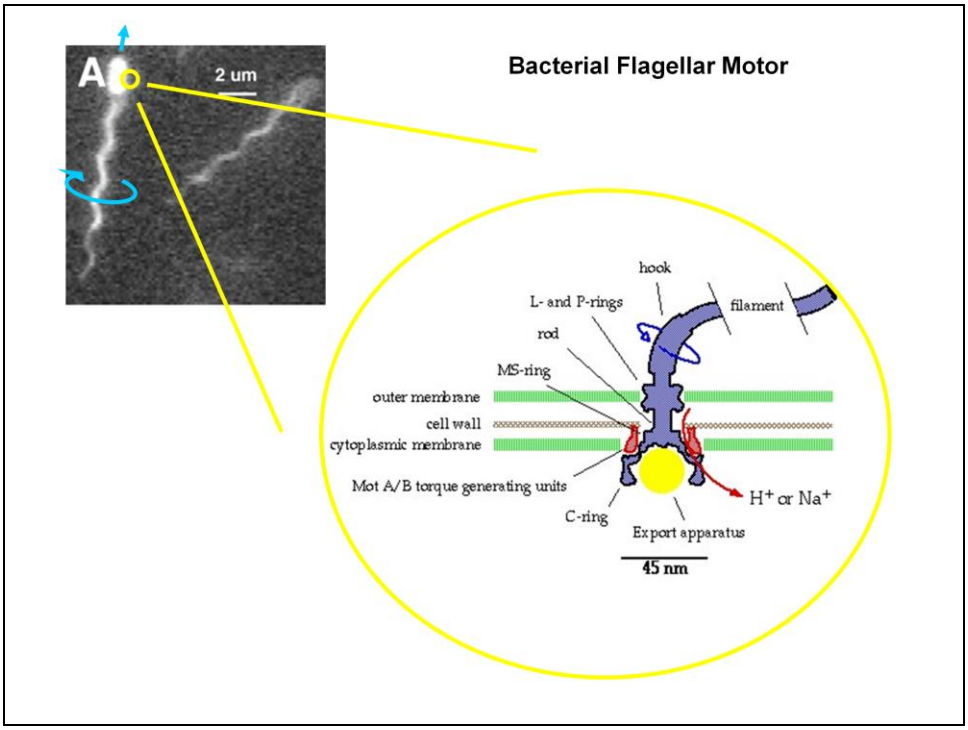
[Swimming Bacteria] (16)

Many types of bacterial swim – to reach places where life is better.

- The navigate using a biased random walk - if things are getting better, they suppress changes of direction.

They can swim fast, several body lengths per second.

Flagellar filaments are helical crystals of a single protein

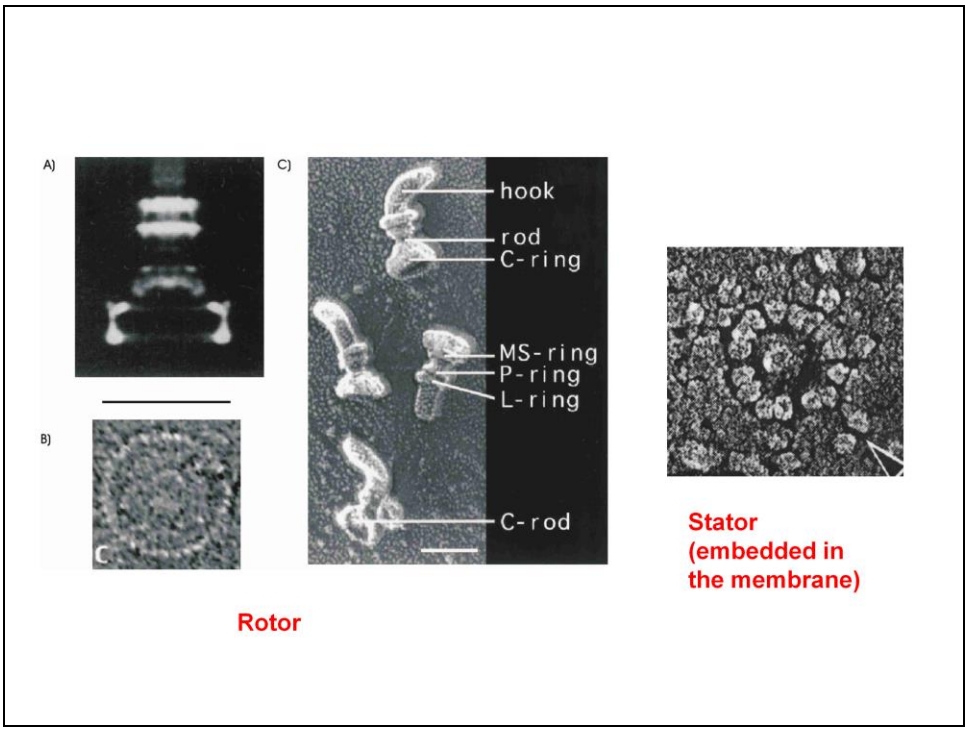


[Bacterial Flagellar Motor] (17)

The motor rotates, driven directly by the pmf. In some species it is an electrochemical gradient of Sodium (sodium motive force or smf) that drives the motor.

Speeds of several 100 revs per second (up to 1700 in sodium-driven motors)

Torque generation between the C-ring and the MotA/MotB units. Details unknown.



[Motor structure] (18)

Structures are obtained by electron-microscopy – too big for x-ray structures. Scale bar is 50 nm.

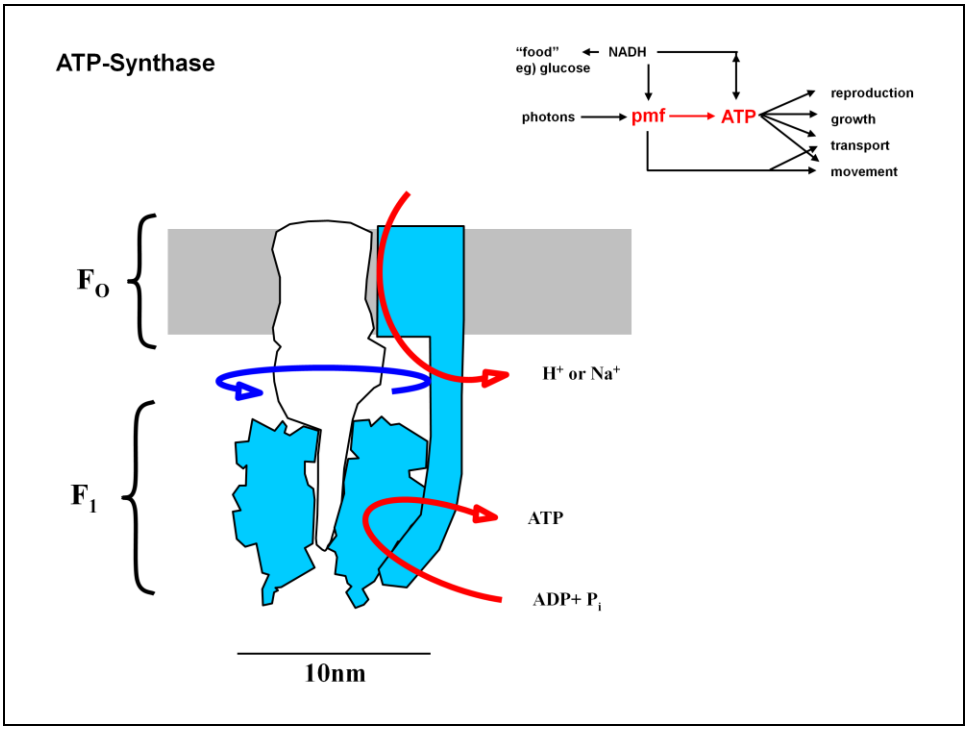
About 40 genes needed to make the motor, about 17 code for proteins in the structure.

Stator ring of particles is seen by “freeze-fracture” – the lipid bilayer is frozen and then split in two. Integral membrane proteins stay in one half and appear either as lumps or holes, depending on which half you look at.

More on the mechanism of the flagellar motor in lectures 9 and 10.

For many years, the flagellar motor was the only wheel known to biology.

In 1997 it was joined by ATP-Synthase…



ATP-synthase

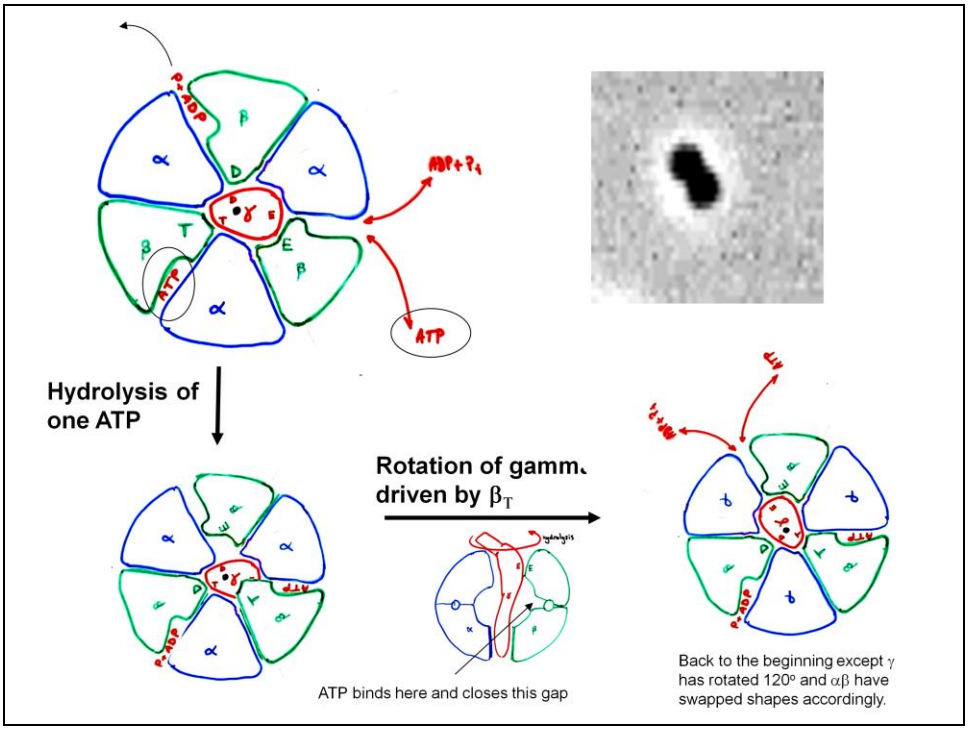
[ATP-synthase] (19)

Two rotary motors connected "back-to-back".

> Normally Fo is stronger, it rotates forwards driven by pmf. F1 is driven backwards, making ATP.

Rotary mechanical coupling with different axial symmetries allows non-integer number of ATP per proton.

Smf versions also exist.



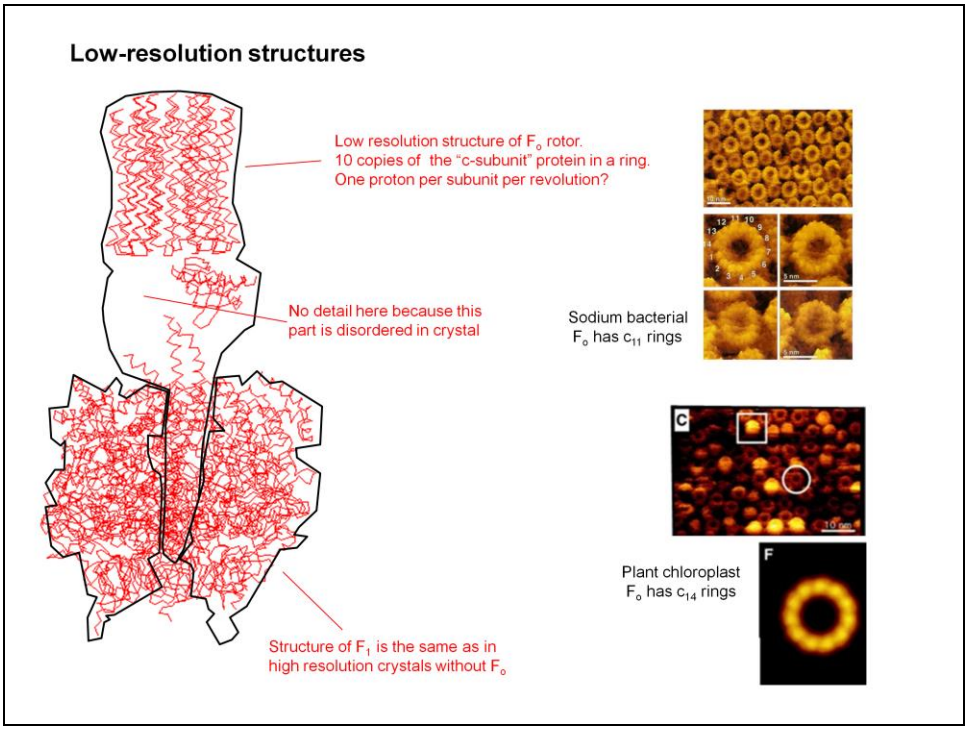
[Binding-change mechanism for F1] (20)

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

Crystal structure and recent rotation experiments (Lecture 10) provide strong support.

3 ATP hydrolysed per revolution.

(Remember animation from lecture 4)



[Symmetry of Fo rotor] (21)

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

> As of 2013, everything from 8-15 has been found.
>
> Assume this is the number of protons per revolution. Different "gear ratios", ***n*** protons per 3 ATPs.

c11 and c14 images by Atomic Force Microscopy (AFM). More in Lecture 11.

Also a few x-ray structures of c-rings (at 2013)

Rotation of Fo has been observed only at low resolution and very seldom due to technical difficulty (2013).

Models for the mechanism were first proposed for the bacterial flagellar motor.

Next lecture:

Myosin, models of motor mechanisms…