Announcements & Homework

Quiz today, Chpt 2 of ECB!

Today: ATPase:
How it produces ATP?

HW #4: On web, due following Wednesday, Feb. 13.

Read, K. Kinosita, "**Real time imaging of rotating molecular machines"** 1999. *Faseb J* **13 Suppl 2**: S201-208. on-web.

Will get to DNA polymerases and efficiency– next time?

Quiz #3: ECB Chpt #2

1. Steroid molecules are a type of large multi-ringed ____**fatty acids**____ involved in cell signaling.

2. In order to make long polymers of sugar, two monomers can be brought together by enzymes such that their hydroxyl groups (-OH) through couple together. This catalysis is an example of a ____**condensation**____reaction.

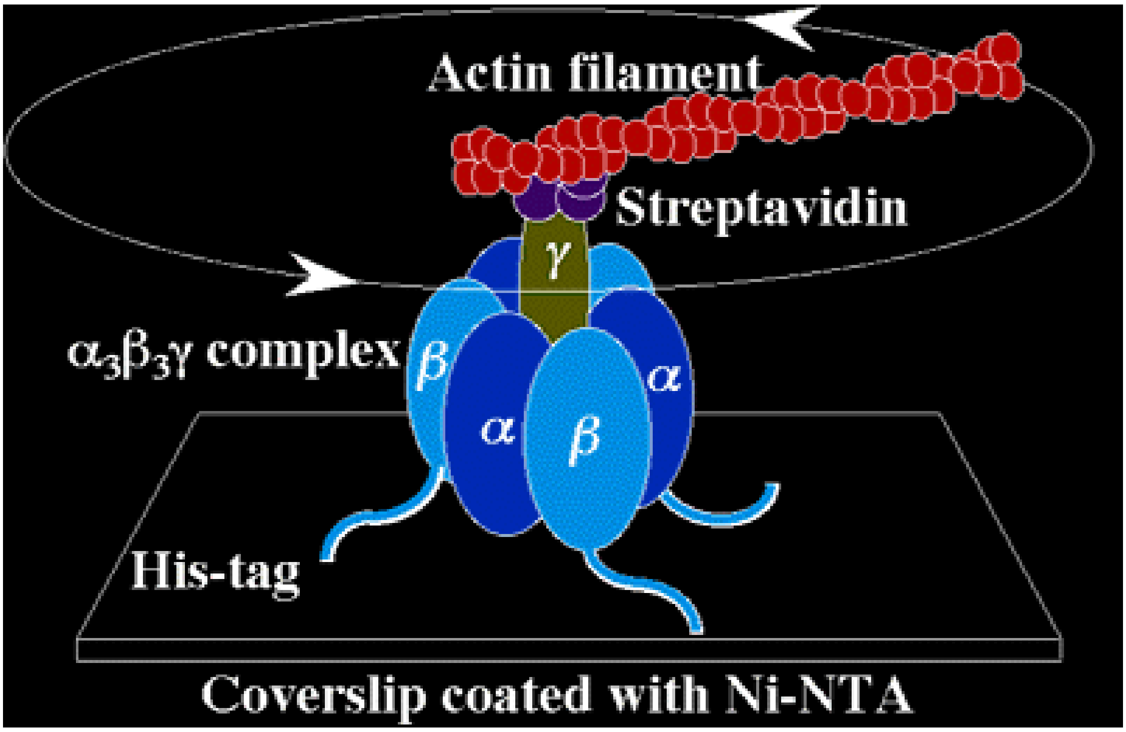
3. What is the most prolific type of macromolecule in a cell?
   proteins

4. The ____**lipid**____ bilayer forms the structural basis for all cell membranes.

5. What are the four major families of small organic molecules in a cell?
   sugars, nucleotides, fatty acids, amino acids
   (Each can be used for:
   storage of information, signalling, and an energy sourc

ATPase

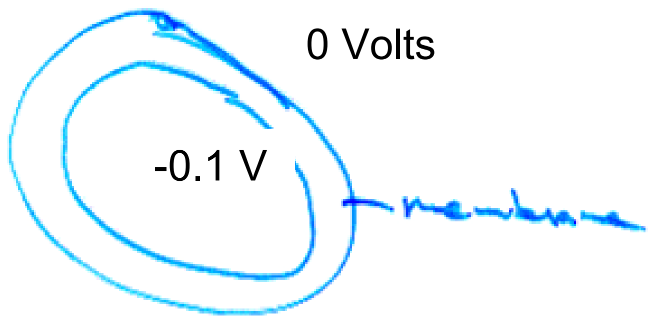
Enzyme which makes
ATP from ADP and P_i
by using electrochemical gradient
Energy + ADP+ $P_i \leftrightarrow$ ATP

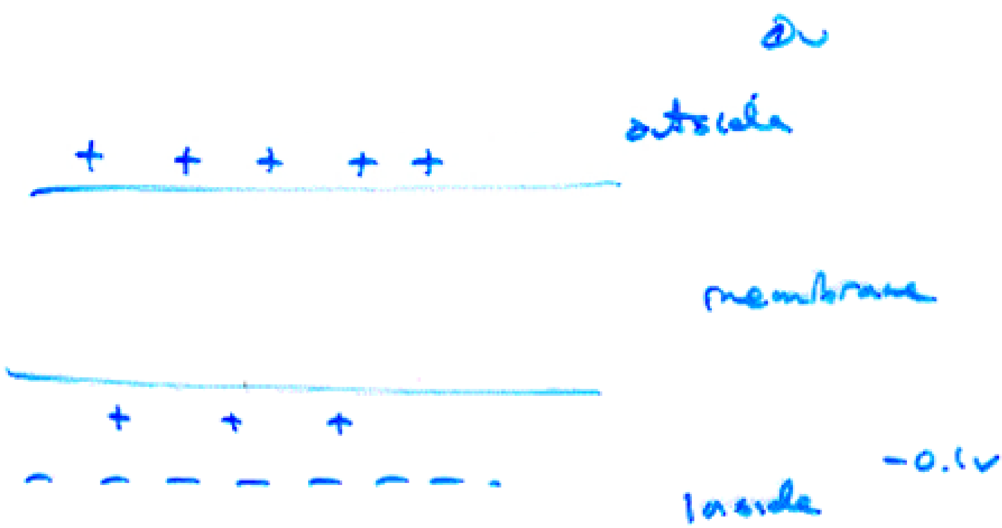


Amazingly efficient enzyme!

Many pictures taken from:
Real time imaging of rotating molecular machines,
By Kinosita

Life is powered by proton (ion) batteries.
In units of 4kT of electrical energy
(7kT of total electrochemical or “free” energy)
Energy to make ATP is from electrical potential across cell membrane.



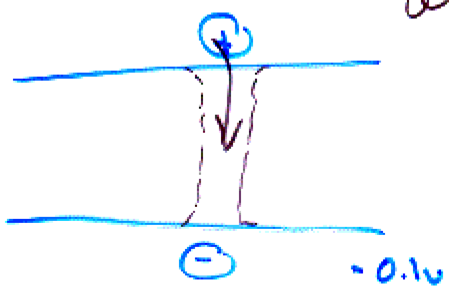


Negative electrical potential arises from more negative charges (or fewer ⊕ charges

Electrical P.E. across our Cells

Move a positive ion from outside to inside, get 7kT of Potential Energy.

We have electrical P.E.



membrane insulator

How much K.E. is gained from loss of Potential Energy?

For each + charge that goes across -0.1V, P.E. is converted into K.E.you get:

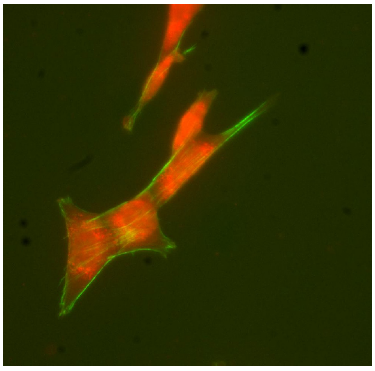
$$|e|0.1V = 0.1\ eV$$

$K_BT = \frac{1}{40}\,eV = 0.025\,eV$

→ 4kT

Actually you get ~7kT (entropic)
electrochemical potential
4kT ↳3kT

Many of our cells have a chemical gradient, where "chemical" happens to be charge (Na^+, K^+, H^+)

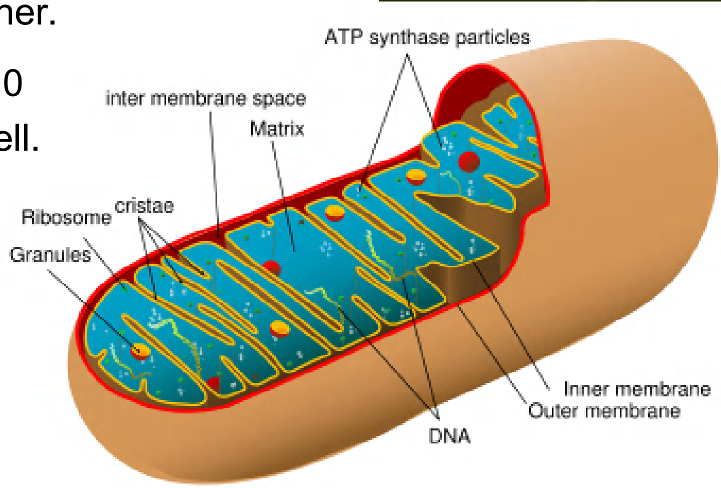


Mitochondria is where ATP is generated from ADP

Mitochondria have their own DNA and may be descended from free-living prokaryotes. DNA comes from mother.

Mitochondria vary in size (0.5 μm-10 μm) and number (1 - >1000) per cell.

Chloroplasts are larger than mitochondria, have there own DNA, and convert solar energy into a chemical energy via photosynthesis. Chloroplasts are found only in photosynthetic eukaryotes, like plants and algae.



http://en.citizendium.org/wiki/Cell_(biology)

A gigantic enzyme called **ATP synthase** whose molecular weight is over 500 kg/mole (made of many proteins), synthesizes ATP in the mitochondria [in eukaryotes]. Very similar enzymes are working in plant chloroplasts and bacterial cell membranes.

By coupling the cells P.E. to the formation of ATP, the reaction ADP + P_i → ATP happens spontaneously.

Once have ATP, have usable energy for biology.

At minimum, how many charges need to be used up to generate 1 ATP?

ATP = 20 – 25 kT of energy.

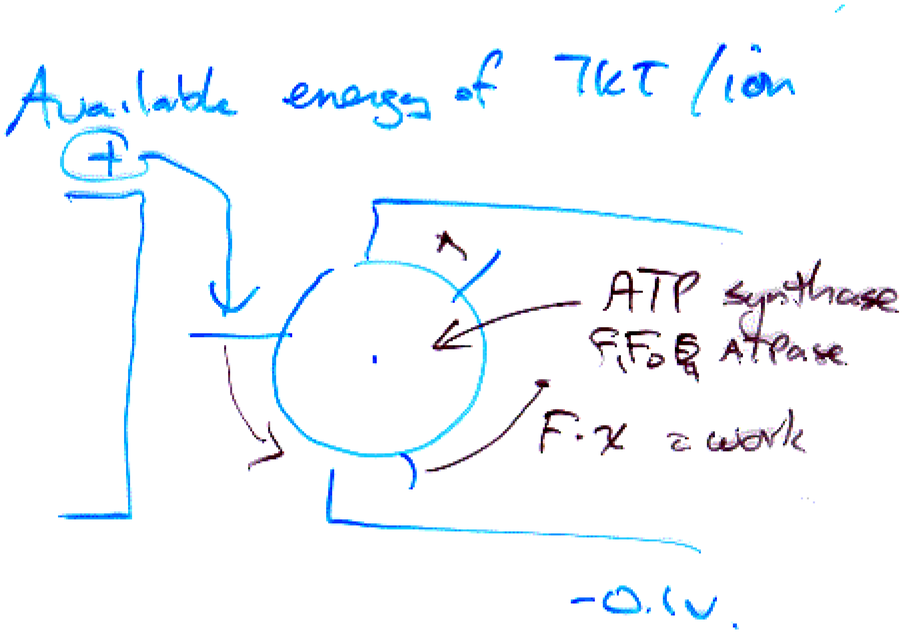
If 100% efficient, need 3 (x 7 pN-nm) charges to cross membrane.

Amazingly:

F_1F_o ATPase operates at 100% efficiency!

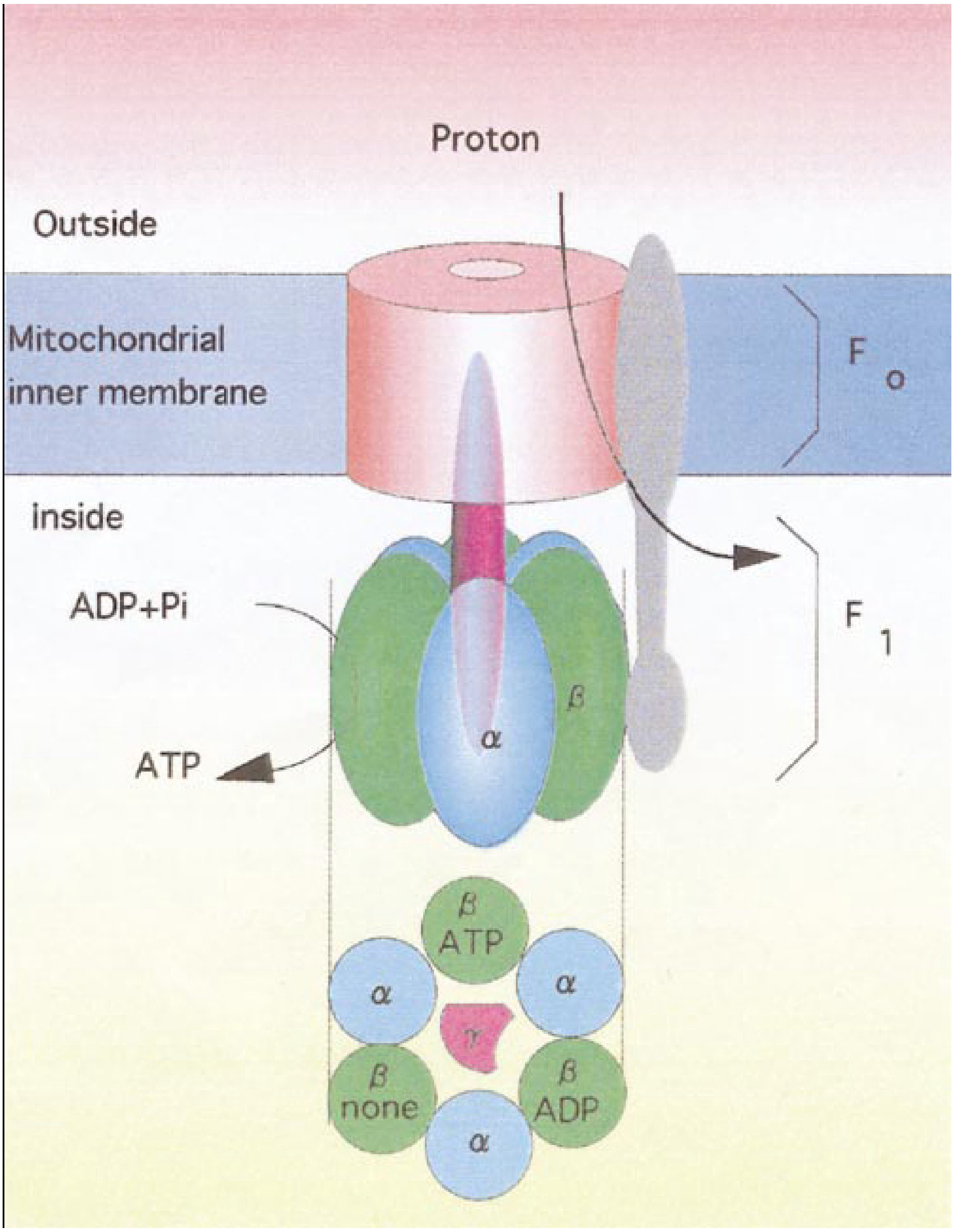
Takes 3 protons and converts that energy into 1 ATP (from ADP+ P_i) !!

Does it by “turning a wheel”, 3 x 120º.



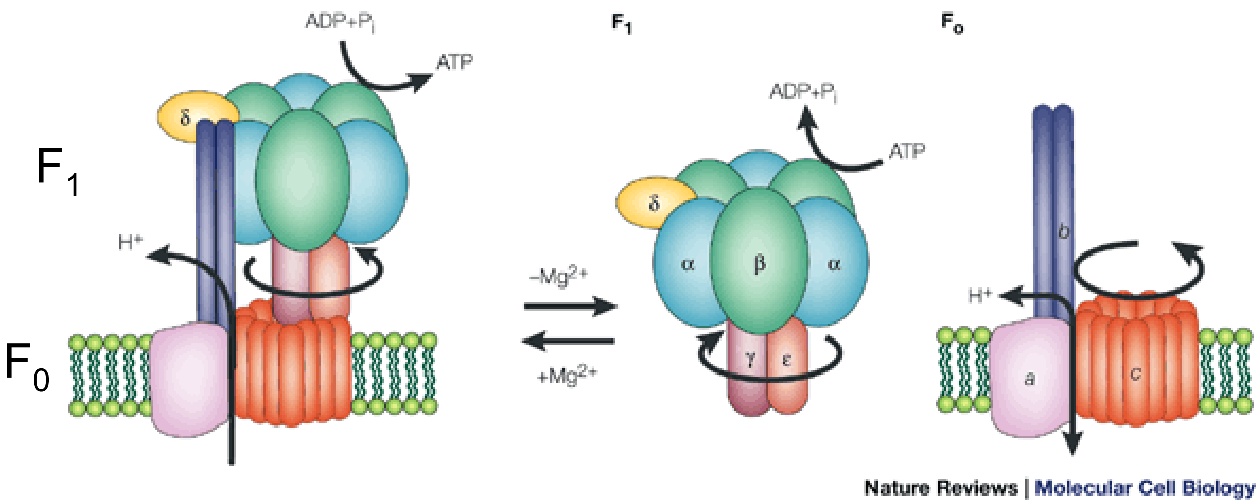
ATP Synthase: A rotary engine in the cell that drives you!

F_1F_0 ATPase

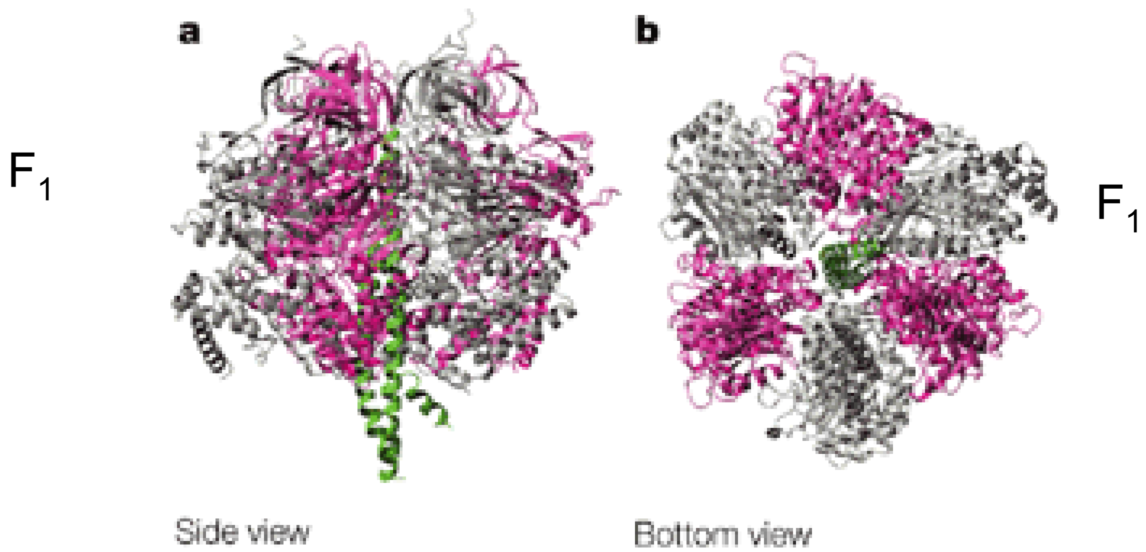


Paul Boyer (UCLA) had predicted that some subunits in the ATP synthase rotated during catalysis to produce ATP from ADP+ Pi. John Walker (MRC, Britain) crystallized the ATP. They won Nobel Prize in 1997.

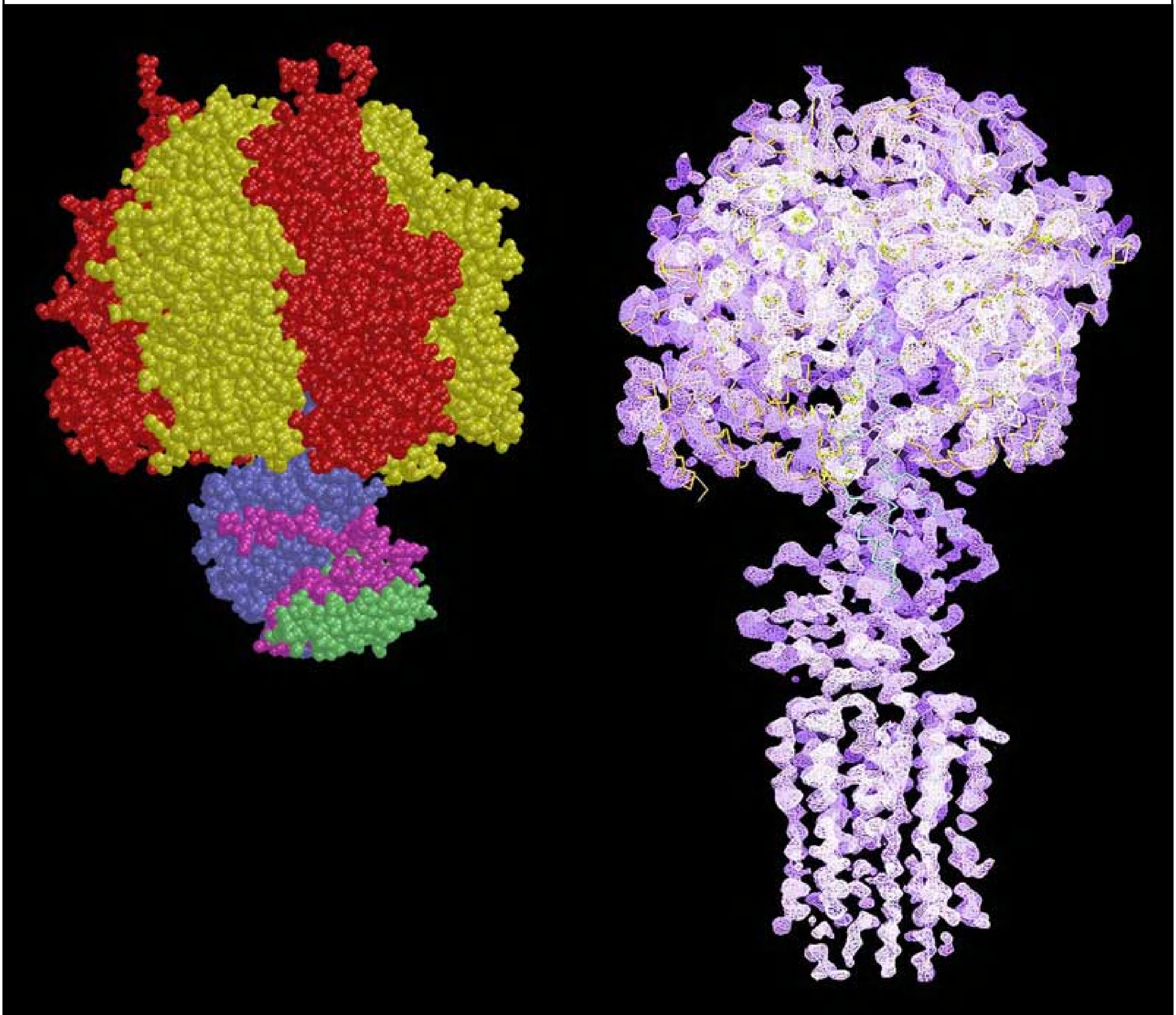
F_1 and F_0 can be separated



It is composed of a water-soluble protein complex, F1, of 380 kDa, and a hydrophobic transmembrane portion, F_o. Removal of Mg^{2+} at low concentrations of salt allows the F_1 part to be extracted in water, leaving the F_o portion in the membrane.

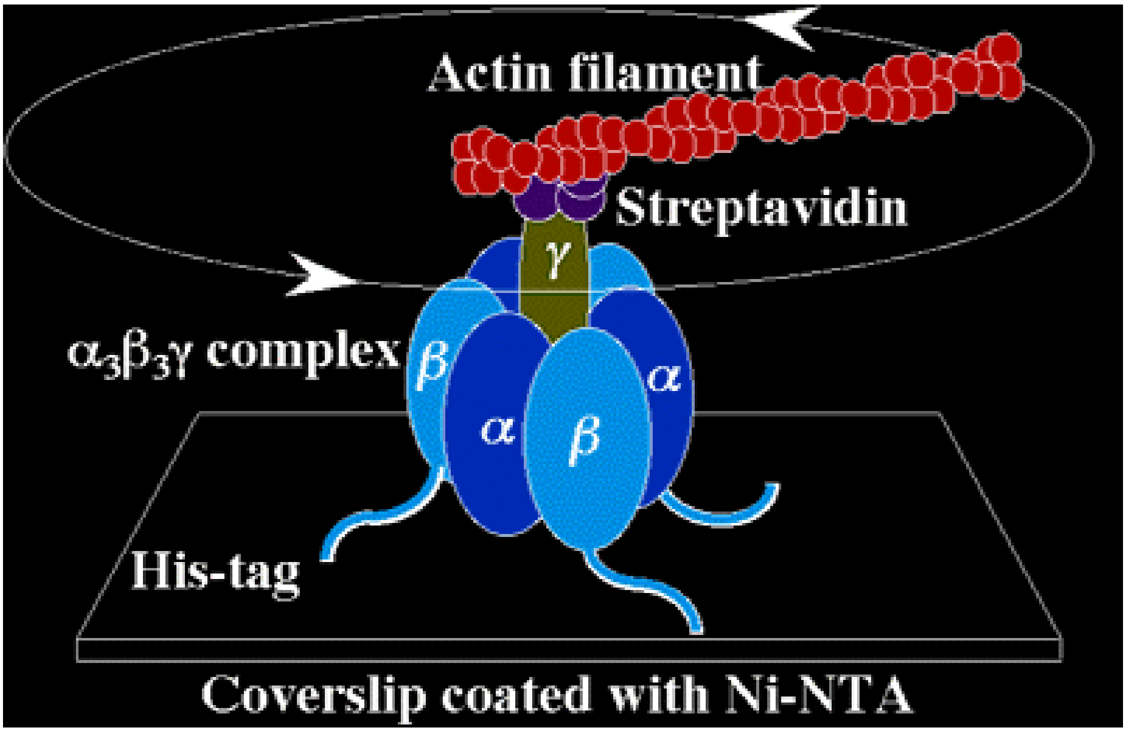


Atomic Structure of F_1F_o ATPase



The X-ray structure of the catalytic F1 domain has been completed (on the left– Nobel Prize, 1997 in Chemistry) and an electron density map of the F1-ATPase associated with a ring of ten c-subunits from the F_o domain (on the right) has provided a first glimpse of part of the motor.

Does ATPase really go around in a circle?



(Noji et al. *Nature* **386** 299-302 1997)

Rotation of the gamma subunit of thermophilic F1-ATPase was observed directly with an epifluorescent microscope. The enzyme was immobilized on a coverslip through His-tag introduced at the N-termini of the β subunit. Fluorescently labeled actin filament was attached to the γ subunit for the observation. Images of the rotating particles were taken with a CCD camera attached to an image intensifier, recorded on an 8-mm video tape and now can be viewed by just clicking on the figures below.

--http://www.res.titech.ac.jp/~seibutu/

Year of Nobel Prize for ATPase.

How to make such a complex?

"To observe rotation, the three β subunits were fixed on a glass surface through histidine tags engineered at the N terminus. To the γ -subunit, a μm-sized actin filament was attached through streptavidin."

What is His-tag?

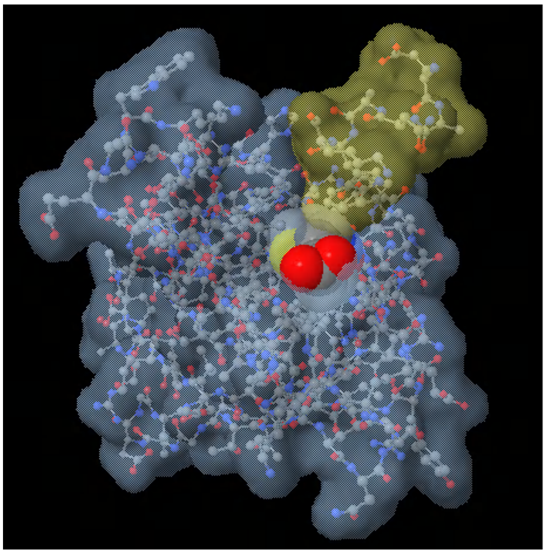
-- 6 histidines which you can attach to a Ni-NTA on glass. His-tag binds Ni, which is held by NTA: it will be bind and be stable.

What is Actin?

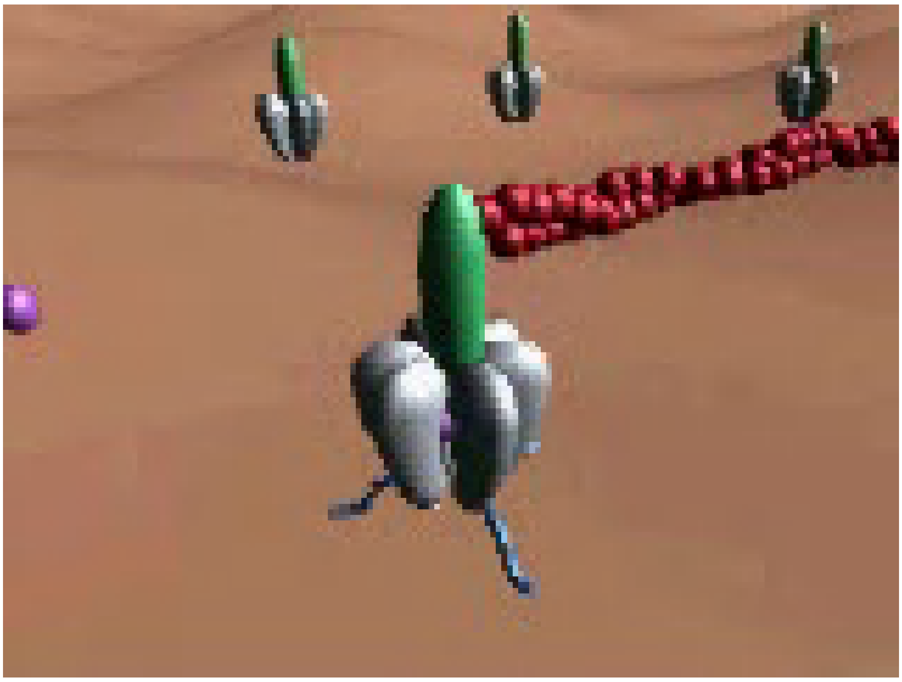
A stiff "highway"/polymer used by the cell: used by myosin.

What is Biotin-Streptavidin?

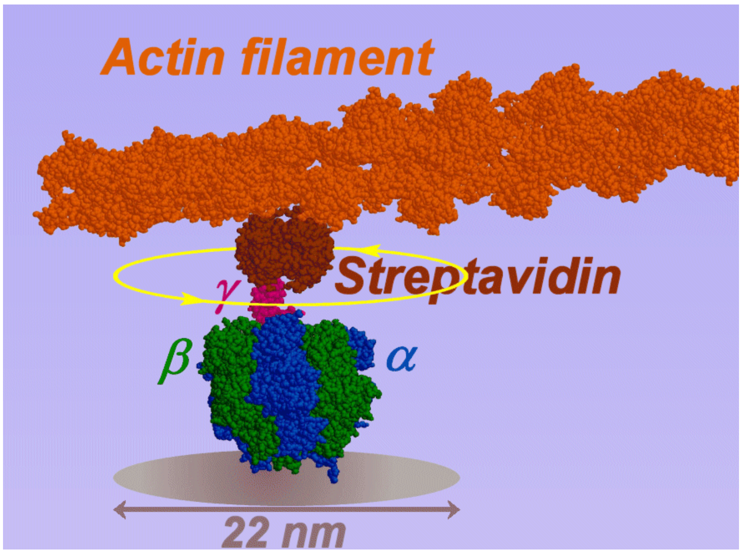
The streptavidin/biotin system is of special interest because it has one of the largest free energies of association of yet observed for noncovalent binding of a protein and small ligand in aqueous solution ($K_{assoc} = 10^{14}$). The complexes are also extremely stable over a wide range of temperature and pH.



Model of how ATPase synthase works.



Yes, a Rotary Engine!



Noji, H. et al., *Nature* **386,** 299-302 (1997).
http://www.k2.phys.waseda.ac.jp/F1movies/F1Prop.htm

Scaling F1 to Size of a Person

Each step is made in a time of the order of 0.1 s. If F_1 is scaled to the size of a person, the person standing at the bottom of a swimming pool would be rotating a rod some 300 m long at the speed of 120° per 0.1 s! The hydrodynamic friction against rotation is enormous. F_1 is really a powerful motor. Naturally, though, the stepping velocity is slower for a longer filament that is subject to a greater friction (**Fig. 5**).

Kinosita, FASEB

How Efficient is ATPase?

1^{st}: Free energy in ATP

(Calculate #pN-nm for ΔG)

$$\Delta G = \Delta G_0 + k_b T \cdot \ln \{[ADP][P_i]/[ATP]\}$$

ΔG_0 = -50 pN nm (standard free-energy change per molecule for ATP hydrolysis at pH 7,

$k_b T$ = 4.1 pN nm

$[ATP] = [P_i] = 10^{-3}$ M

Therefore:

ΔG = -100 pN-nm for [ADP] = 10 μm

ΔG = -90 pN-nm for [ADP] = 100 μm

How Efficient is ATPase?

(Calculate Work done through Rotation)

$$\tau = \omega\xi$$

Torque = rotation rate x friction

All energy (torque) is used in moving water:
(kinetic energy is ~ zero)

ω = rotational rate (radians/sec)
τ = Torque
ξ = frictional drag coefficient

$\xi = (4\pi/3\eta)\, L^3/[\ln(L/2r) - 0.447]$ (complicated formula)

$\eta = 10^{-3}\ Nm^{-2}s$ is the viscosity of medium.
r = 5 nm is radius of the filament.

Actin filament of 1 μm at 6 rev/sec ($\approx$ 40 rad/sec)

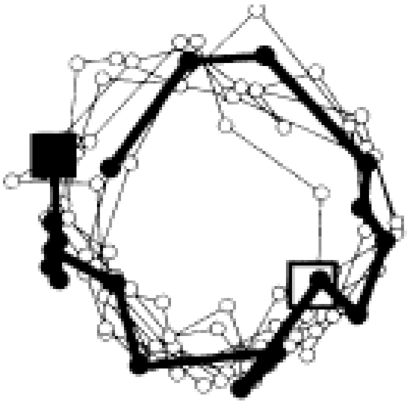
τ = 40 pN-nm

Energy = $\tau\ \omega$

40 pN-nm x $2\pi/3$ (= 120º) $\approx$ **80 pN nm !!**
Close to free energy in ATP!

Therefore operates at nearly 100% efficiency.

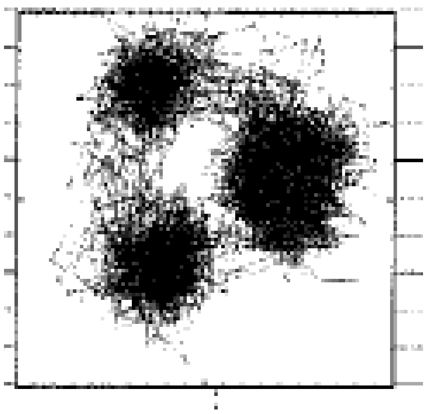
Stepping rotation: 1 ATP per 120º



High ATP (2 mM)

A trace of the centroid of the actin going around (2.6um actin, 0.5 rps). Start: solid square; end: empty square.

Low ATP (20 nM)

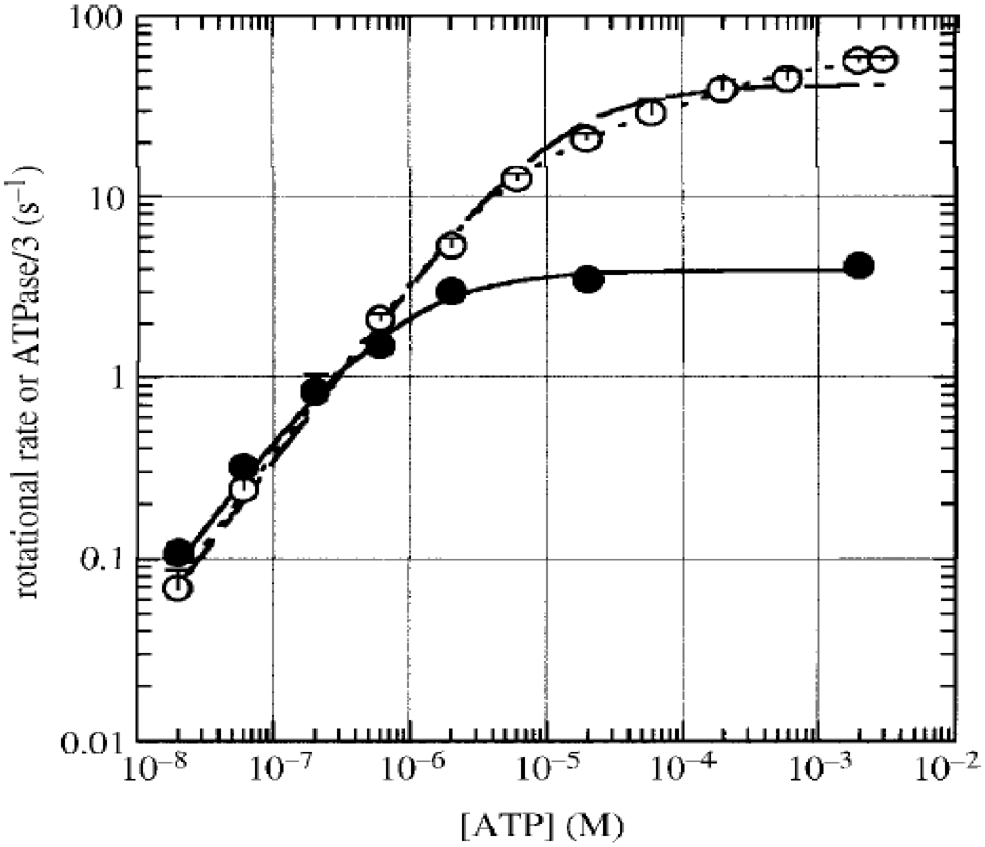


As shown at left, the back steps are as fast as the forward steps, characterized by short stepping times, $\tau_{120°}$, that would require a constant work per step, *W*, as large as 90 pN·nm ($\tau_{120°} = (2\pi/3)^2\xi/W$). **Because the work, *W*, amounts to 20 times the thermal energy, the steps, should be powered by ATP.**

F1 uses 1 ATP/step

Rotational (speed) and Hydrolytic rate α [ATP]

Both processes fuelled by individual ATP (over this range).



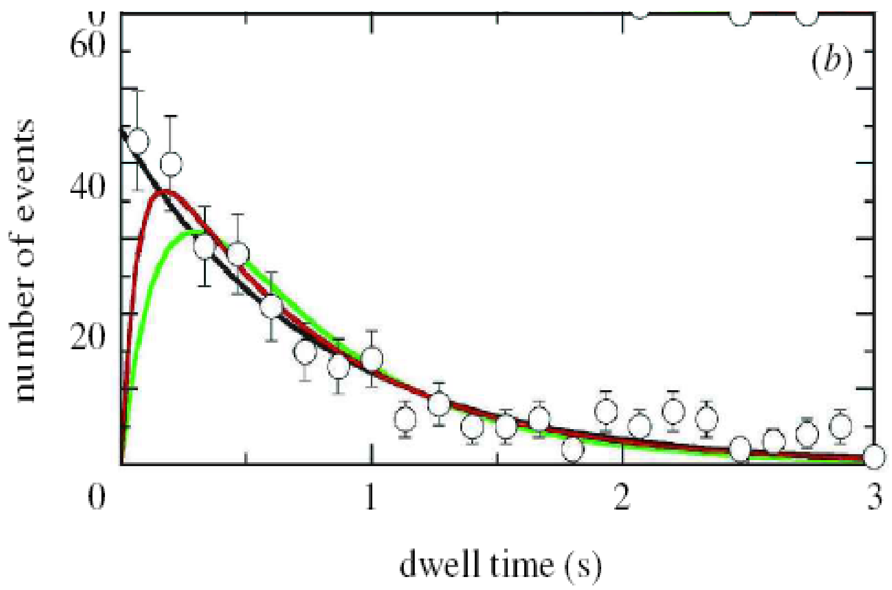
Hydrolysis (open circles) and Rotational rate (closed circles) was measured by attaching a short (0.8 -1.2 μm) actin filament (closed circle). Hydrolysis was measured in solution. (Kinosita, Royal Trans, 2000)

(d) *One ATP molecule per step*

In figure 6, the rotational and hydrolysis rates are proportional to the ATP concentration in the sub-micromolar range. This suggests that, at least in this range, both processes are fuelled by individual ATP molecules, not by the combination of two or more ATP molecules.

F_1 uses 1 ATP/step (confirmed)

Figure 7 shows histograms of the dwell times (Yasuda *et al.* 1998). If each 120° step is driven by one ATP molecule, the histogram should be exponential, as was indeed the case (black lines in figure 7). If, on the other hand, two or more ATP molecules were required for each step, the histogram would have started from the origin at the lower left, as in the green and red lines, because simultaneous arrivals of two or more ATP molecules should be a rare event. The data point to one ATP molecule per step.

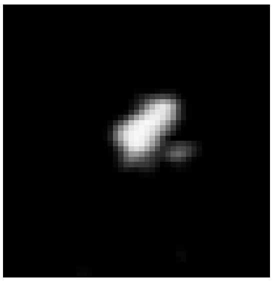


How do you get an exponential in time from a random arrival time? (homework?)

How do you get a te^{-kt} from two steps? [Will derive when talk about linear motors…. Or in homework!]

Load Dependence of ATPase

Vary the length of Actin lever Arm– moving under friction

http://www.k2.phys.waseda.ac.jp/F1movies/F1long.htm

Rotation of F1 is slower when a longer rod (actin filament) is attached to the rotor subunit. This is because the rod is rotating in water and the viscous friction imposed on the rod is proportional to the cube of the rod length. Precise analysis indicates that F1 produces a constant torque (rotary force) of about 40 pN nm, irrespective of the viscous load.

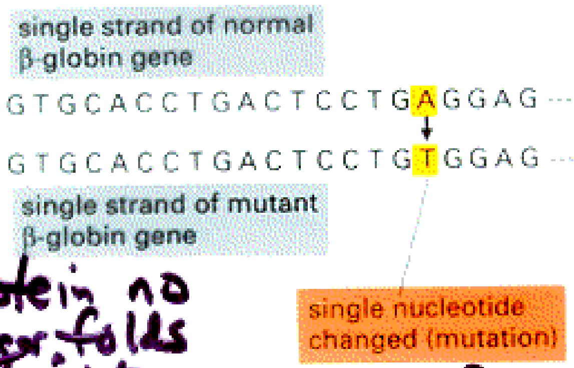
At 2 mM (and 20 μM) ATP, the rotational rates were consistent with a constant frictional torque (the drag coefficient × the rotational rate) of 40 pN·nm, indicating that the sub-complex produced this much of torque irrespective of the frictional load.

Always operating at ~100% efficiency!

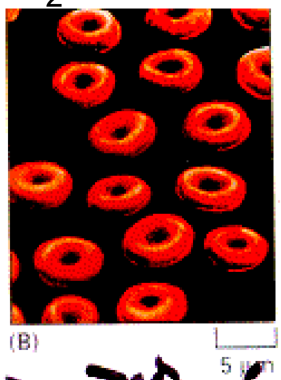
DNA replication must be made with very high fidelity

A single base pair change can be deadly

Sickle Cell Anemia caused by single base change.



Normal cell with or w/o O_2



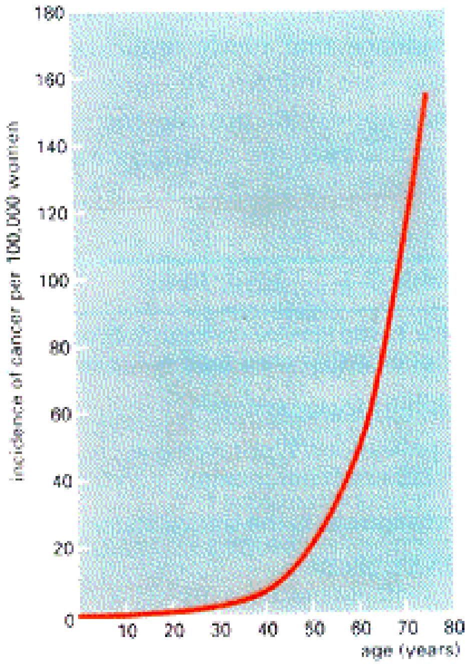
Sickle cell w/o O_2

Protein no longer folds quite right

cystic fibrosis - 70% of cases are due to a single pt mutation

Cancer Incidence vs age:

P(cancer) α Age^6



Age

Question: What no.of mutations on average causes cancer?

Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.