# Announcements & Homework

## Today

## ATPase

How it produces ATP from ADP & $P_i$
Amazingly efficient enzyme
How to measure this.

Homework, Due next Wednesday, Feb 15

(I'll try to have HW due on Wednesday, due to popular appeal.)

1) HW #3

2) Reading: a) Chpt 6-6.5 of Campbell's Biology

b) Mark J. Schnitzer, Doing a rotary two-step
Nature **410,** 19 April 2001, 878 - 881 (PDF 633K)

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

**Next Monday, first quiz:**

**Covering general knowledge about biology:**

Know this weeks homework—qualitative Essay questions;
Go over lectures, especially main highlighted points

# Archea

The Archaea are *a group of single-celled microorganisms. A single individual or species from this domain is called an archaeon* (sometimes spelled "archeon"). They have no cell nucleus or any other membrane-bound organelles within their cells.

Archaea are divided into four recognized phyla, but many more phyla may exist.

**Finding Archaea :** The hot springs of Yellowstone National Park, USA, were among the first places Archaea were discovered. At left is Octopus Spring, and at right is Obsidian Pool. Each pool has slightly different mineral content, temperature, salinity, etc., so different pools may contain different communities of archaeans and other microbes. The biologists pictured above are immersing microscope slides in the boiling pool onto which some archaeans might be captured for study.

http://www.ucmp.berkeley.edu/archaea/archaea.html

# Archeans

<u>Archaeans include inhabitants of some of the most extreme environments on the planet</u>. Some live near rift vents in the deep sea at temperatures well over 100 degrees Centigrade. Others live in hot springs (such as the ones pictured above), or in extremely alkaline or acid waters. They have been found thriving inside the digestive tracts of cows, termites, and marine life where they produce methane. They live in the anoxic muds of marshes and at the bottom of the ocean, and even thrive in petroleum deposits deep underground.

Archaeans may be the only organisms that can live in extreme habitats such as thermal vents or hypersaline water. They may be extremely abundant in environments that are hostile to all other life forms. <u>However, archaeans are not restricted to extreme environments;</u> new research is showing that archaeans are also quite abundant in the plankton of the open sea. Much is still to be learned about these microbes, but it is clear that the Archaea is a remarkably diverse and successful clade of organisms.

http://www.ucmp.berkeley.edu/archaea/archaea.html

# You are what you eat

You get virtually all of the energy and the stuff

which makes you, from the food that you eat.

(You get a tiny bit from sunlight.)

Energy (food)→ Energy (body can use)

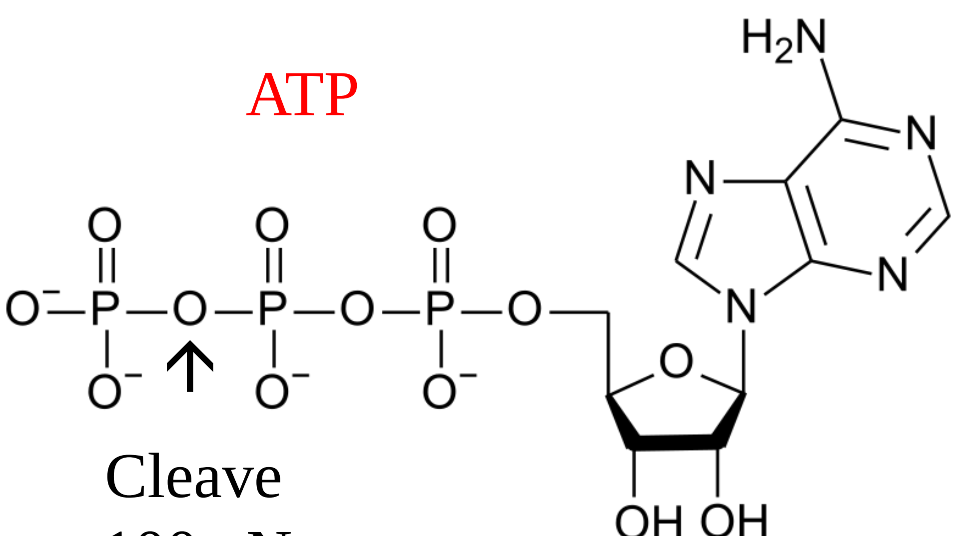
= Adenosine triphosphate, ATP (most important)

+ NADH (More later)

+ glucose (sugar) [42 kT→ glycolysis]

ADP+ Pi → ATP

ATP



Cleave

get 100 pN-nm

= 25 kT

http://tuberose.com/Dighttp://tuberose.com/Digestion.html

# ATP, your "just-in-time" energy source

## Amazing facts about ATP

¼ ounce!

- Only about 100g of ATP in body, yet it's main source of immediate energy for most chemical reactions.

How possible?

- Because ATP is turned over (used and restored) very quickly.
- Once ATP made, stays around in cell about 1 minute.
- Typically use about 40kg (90 lbs!) of ATP in 24 hrs.
- In 2 hr run can use 60kg (132 lbs!).

How can use 90 lbs?

Net weight = $W_{ATP}$-$W_{ADP}$

# Energetics of ATP

1 ATP= 80-100 pN-nm of energy at 37 ºC

= 20-25 kT of energy

(much more than kT = 4 pN-nm)

A lot of energy

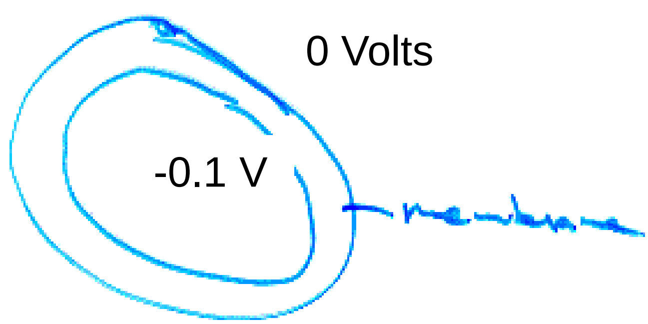
Why do I say 80 to 100 pN-nm? Why not an exact amount?

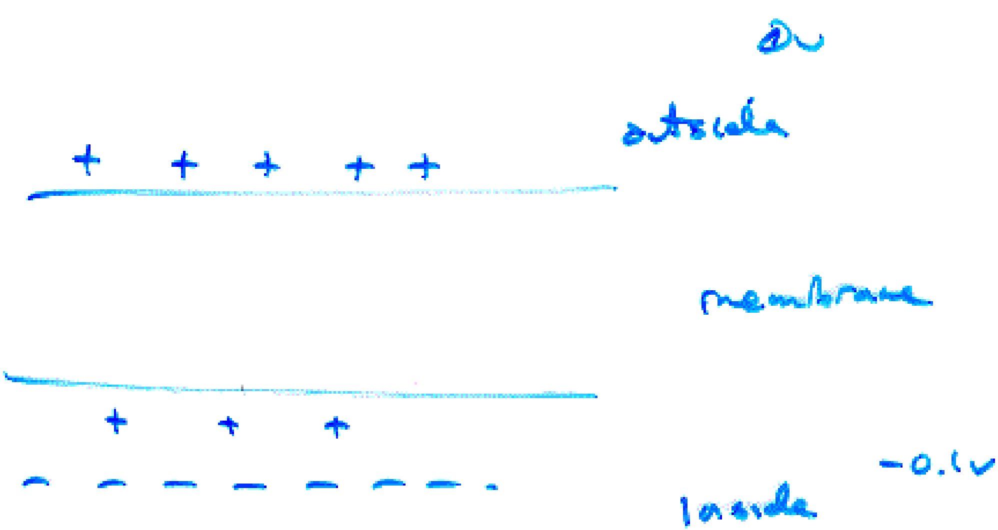
What counts is $\Delta G$, not $\Delta E$, where $\Delta G = \Delta E - T\Delta S$

ATP → ADP + $P_i$ : depends on [ADP] & also [$P_i$] concentration

(Homework problem)

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.



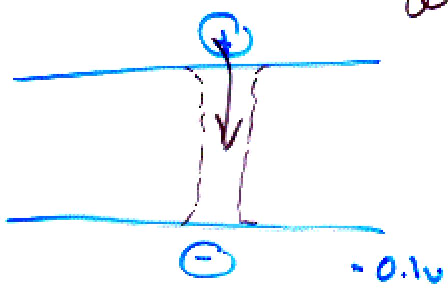




Negative electrical potential across from more negative charges (or fewer ⊕ charges

# 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

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

ATP = 20 – 25 kT of energy.

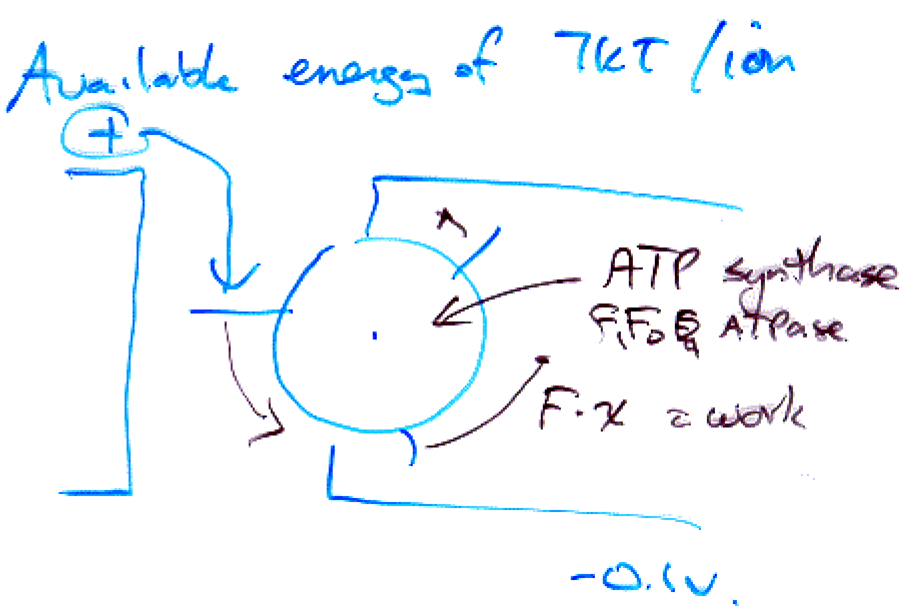
If 100% efficient, need 3 (x 7 pN-nm) charges to cross membrane.

Amazingly:

$F_1F_o$ ATPase operates at 100% efficiency!

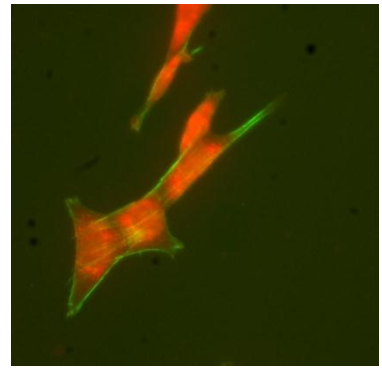
Takes 3 protons and converts that energy into 1 ATP (from ADP+ $P_i$) !!

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




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

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

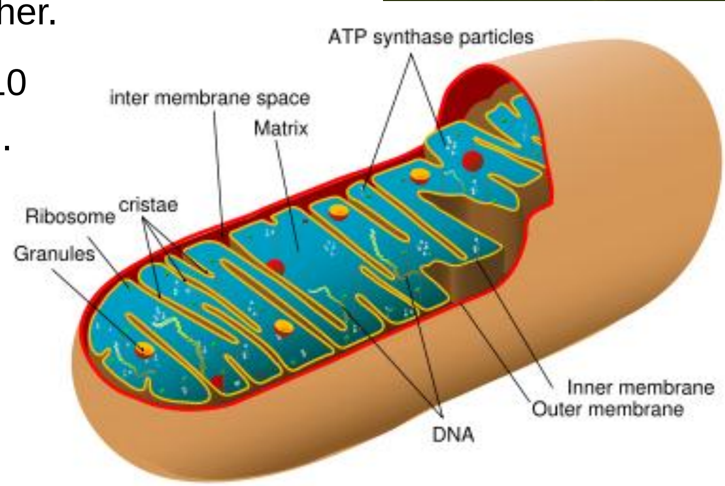


## Mitochondria is where ATP is generated from ADP

**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 + $P_i \rightarrow$ ATP happens spontaneously.

Once have ATP, have usable energy for biology.

# Mitochondrial Cartoons

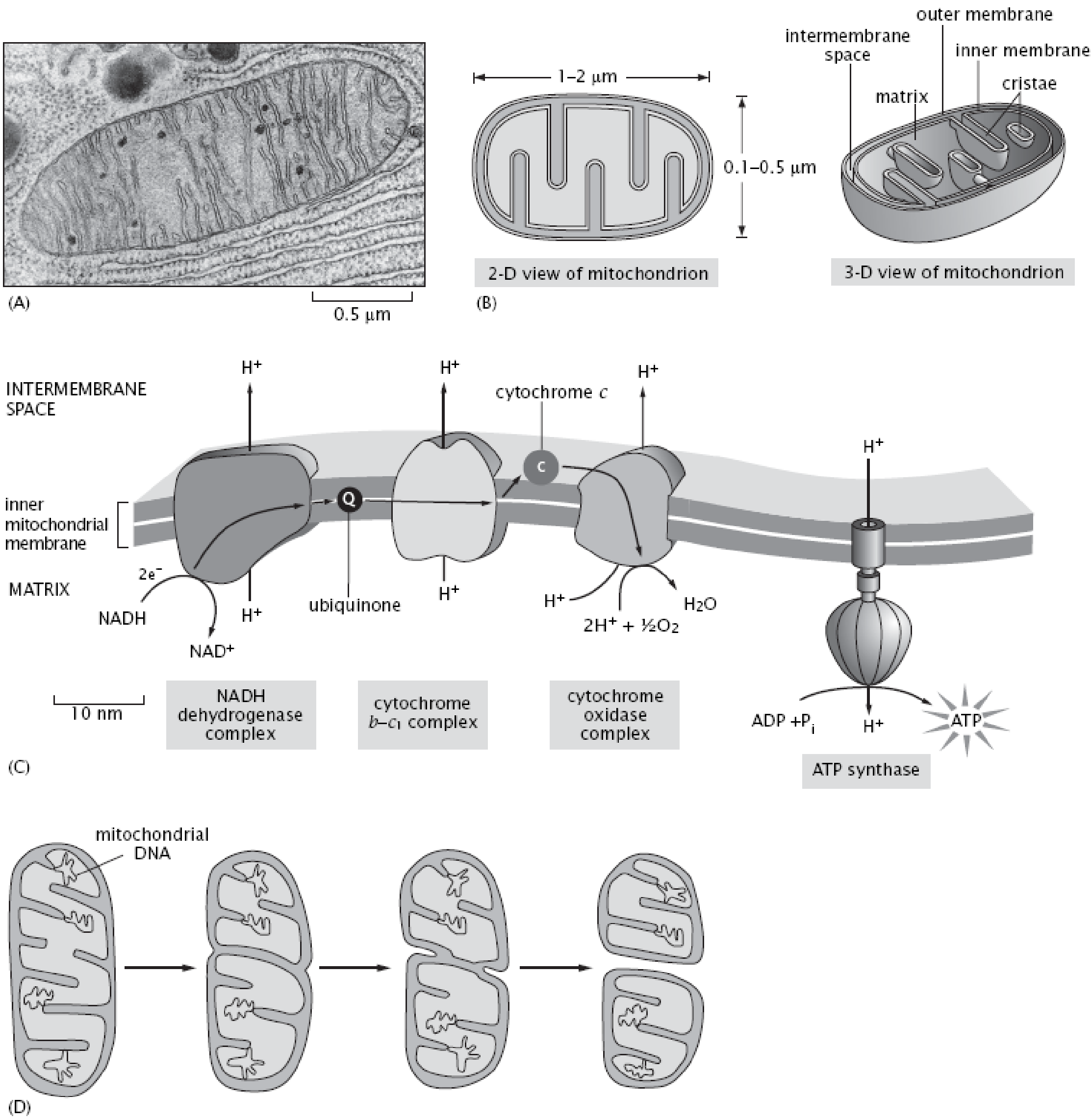
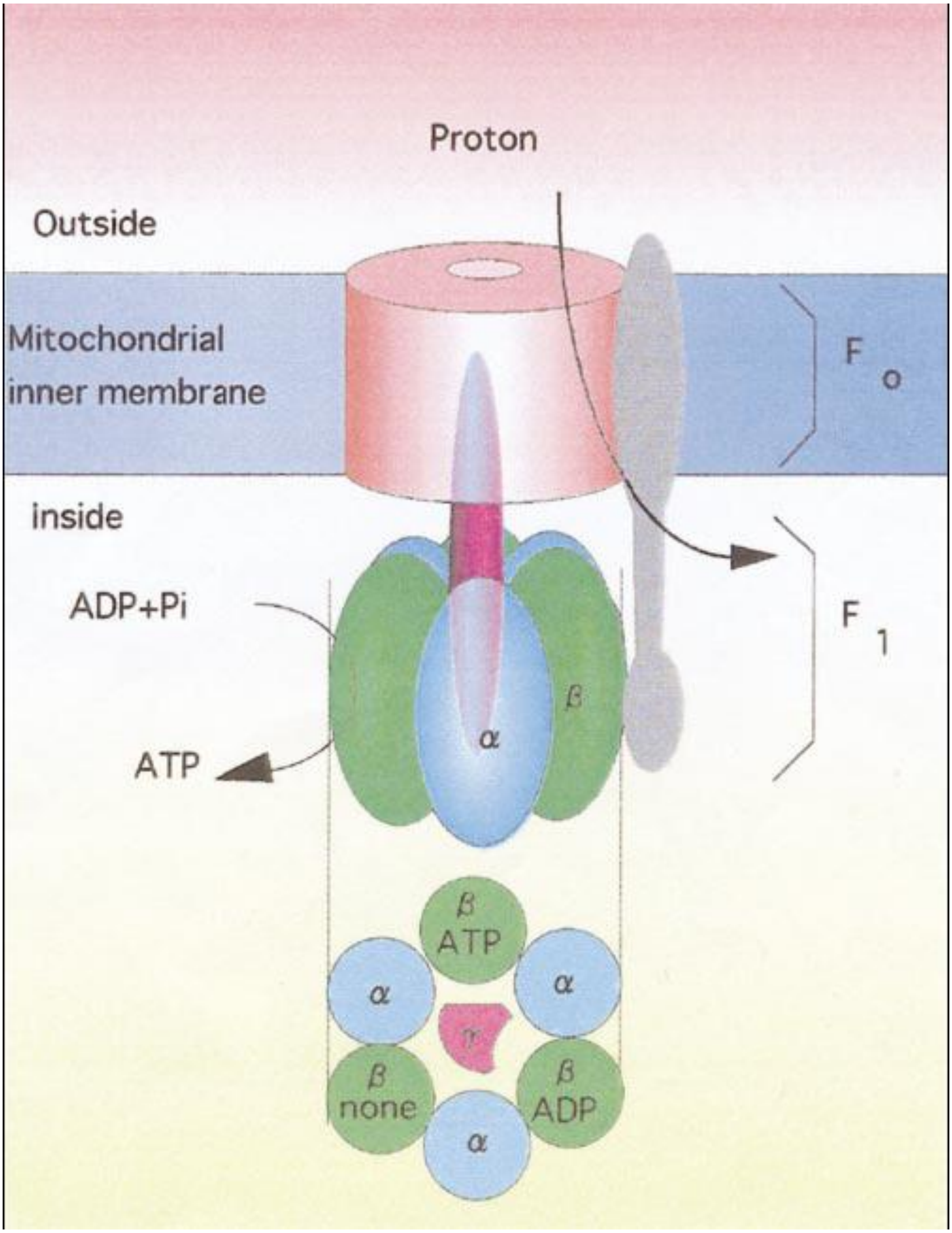



**Figure 1.10** Several different ways of illustrating mitochondria. (A) Thin-section electron micrograph showing a mitochondrion found in a cell within the pancreas of a bat. (B) Diagrams showing the arrangement of membranes dividing the mitochondrion into distinct compartments. While the outer membrane forms a smooth capsule, the inner membrane is convoluted to form a series of cristae. Distinct sets of proteins are found in the matrix (inside the inner membrane) and in the intermembrane space (between the inner and outer membranes). (C) Schematic illustration of the major proteins involved in the electron transport chain and in ATP synthesis in the inner membrane of the mitochondrion. The overall purpose of the complex chemical reactions carried out by this series of proteins is to catalyze the creation of the important energy carrier molecule ATP. (D) Illustration of the distribution and partitioning of mitochondrial DNA during the process of mitochondrial division (fission). (A, from D. W. Fawcett, The Cell, Its Organelles and Inclusions: An Atlas of Fine Structure, Philadelphia, W. B. Saunders & Co., 1966; C, from B. Alberts et al., Molecular Biology of

From Phillips, 2009, Physical Biology of the Cell

# $F_1F_0$ ATPase

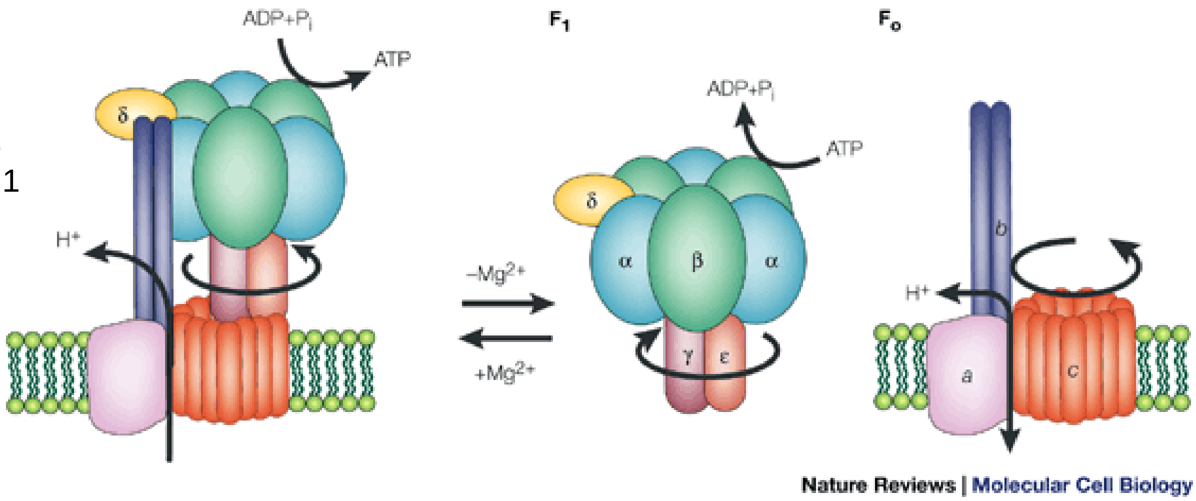



Paul Boyer (UCLA) had predicted that some subunits in the ATP synthase rotated during catalysis to produce ATP from ADP+ Pi. John Walker (MRC, Britain) crystallized the ATP. They won Nobel Prize in 1997.

# $F_1$ and $F_0$ can be separated

$F_1$

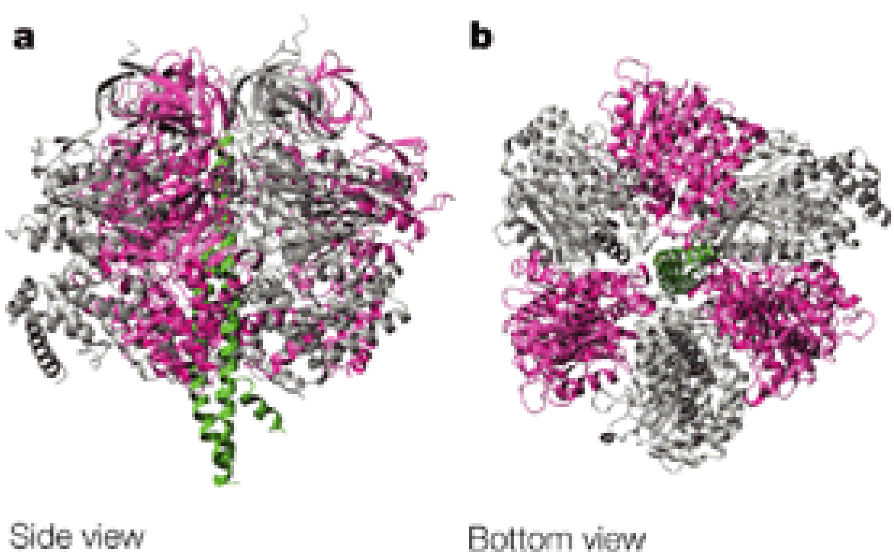
$F_0$




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.

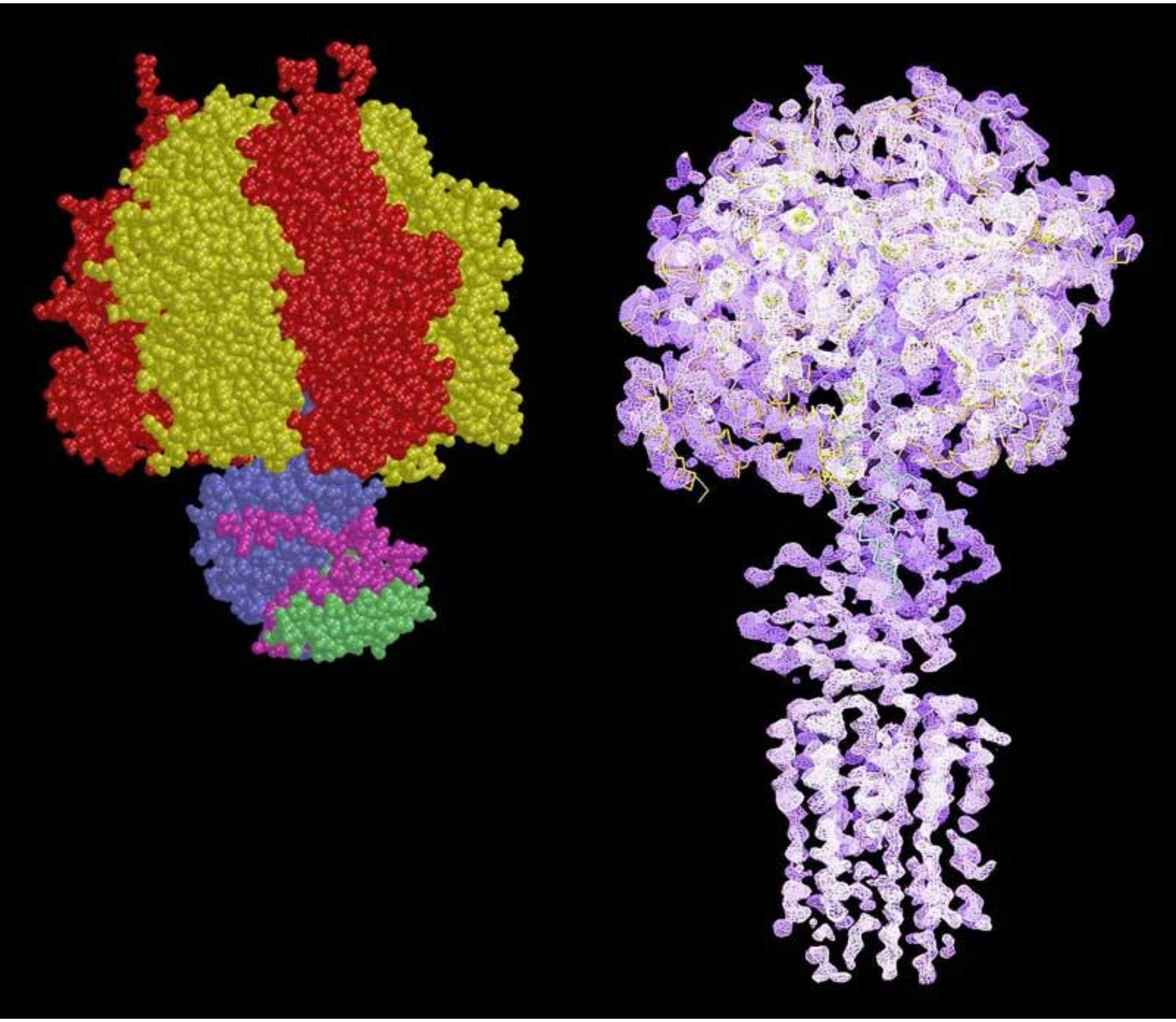
**F1 has been crystallized and extensively studied.**

$F_1$




$F_1$

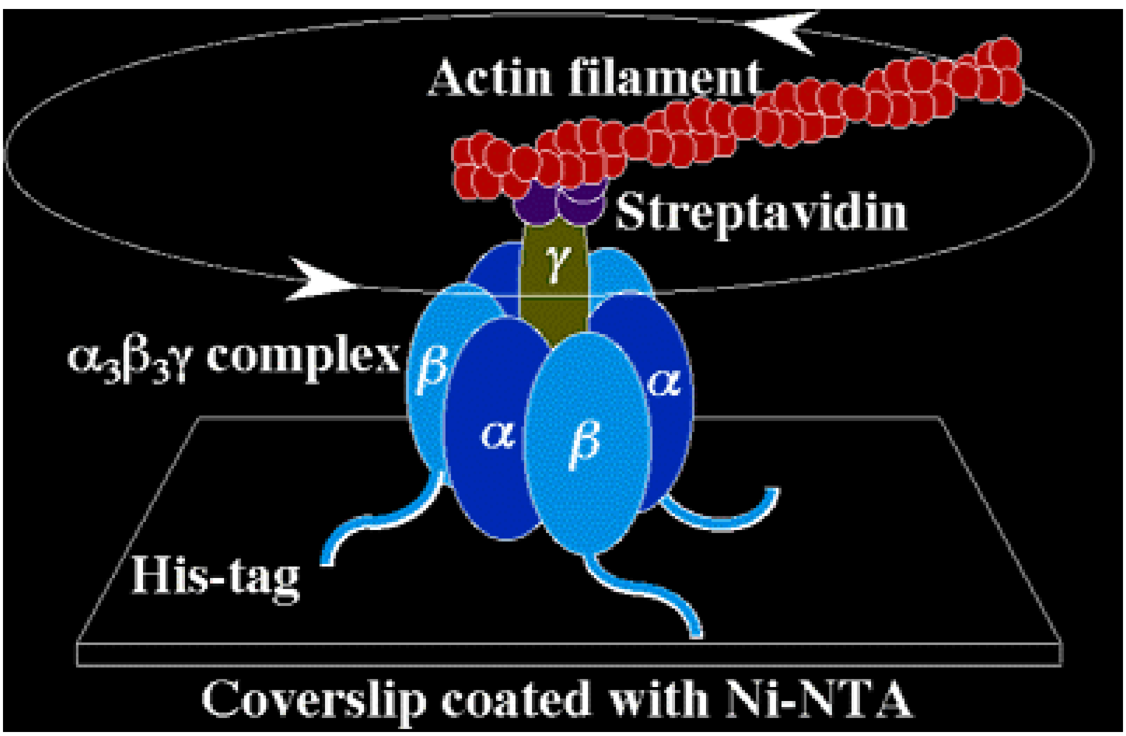
# Atomic Structure of $F_1F_o$ ATPase



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.

# F1-ATPase

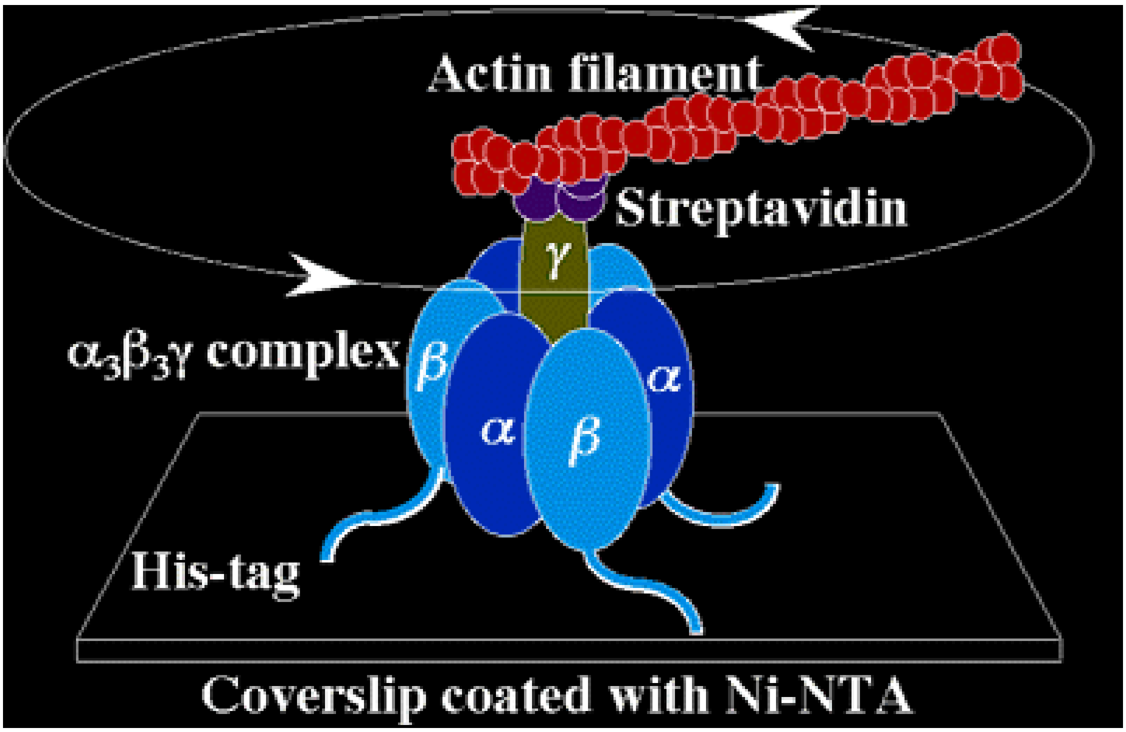
**Enzyme which makes**
**ATP from ADP and $P_i$**
**by using electrochemical gradient**
**Energy + ADP+ $P_i \leftrightarrow$ ATP**




Amazingly efficient enzyme!

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

# 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 micron-sized actin filament was attached through streptavidin."

## 1. What is His-tag?

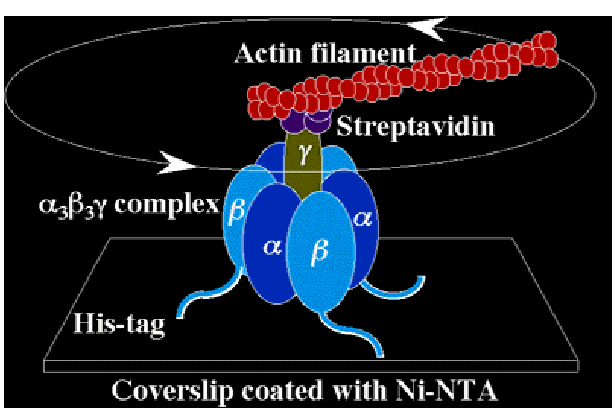
-- 6 histidines which you can attach to a Ni-NTA on glass.
His-tag binds Ni, which is held by NTA: it will be bind and be stable.

## 2. What is (Fluorescent) Actin?

a) A stiff "highway"/polymer used by the cell: used by myosin
Here it's basically a stiff rod.

b) Somethings are fluorescent, i.e. absorb light at one color and emit light as a red-shifted color.

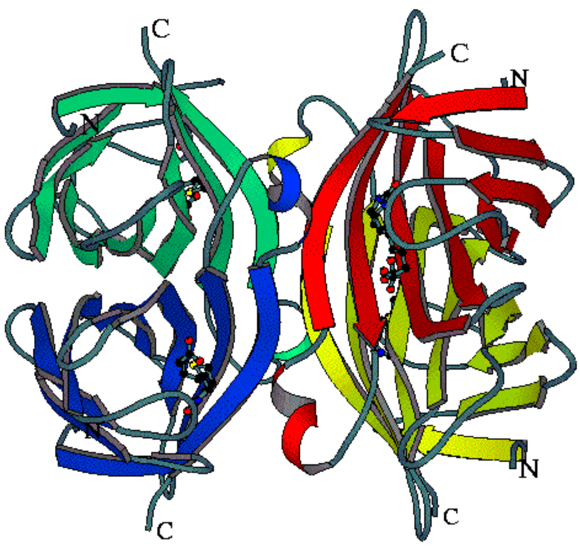
Important thing: can see very little of it,
e.g. a single molecule!
or here, a single actin filament.

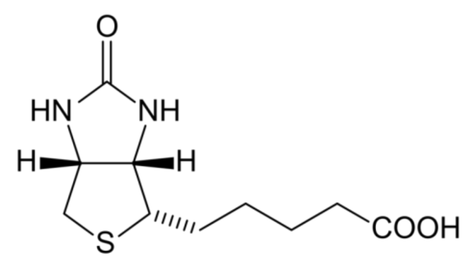



Why can you see just one with fluorescence?
Why not with absorption?
(Will discuss more, later.)

# 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.

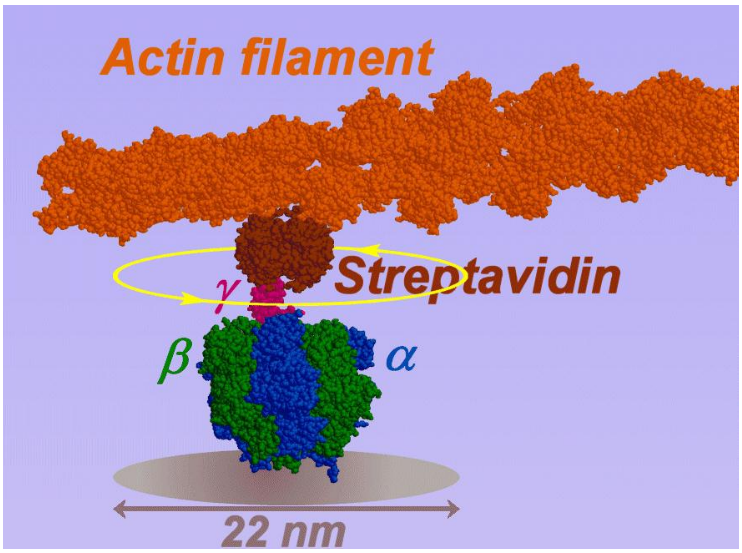



Biotin: also called Vitamin H, $B_7$

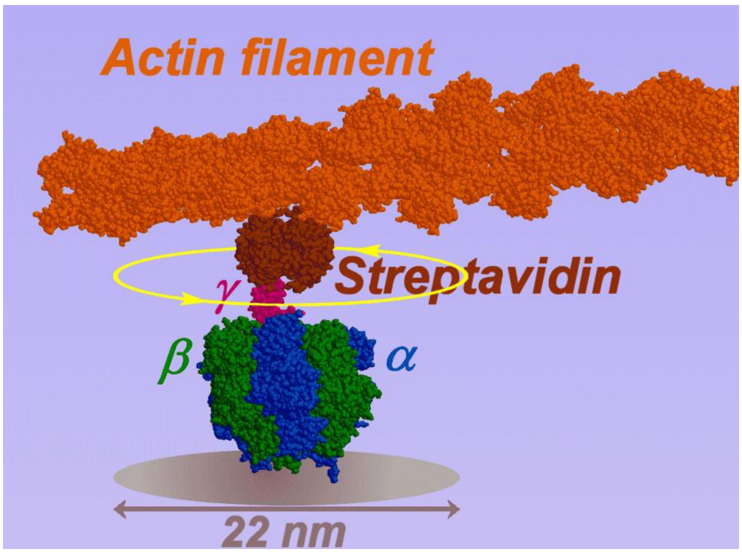
- hydrophobic and van der Waals interactions of mainly four streptavidin tryptophan side chains,
- - an effective hydrogen bonding network
- - a binding surface loop, which folds over the ligand.

# Yes, a Rotary Engine!




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

# 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 $\Delta G$)

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

(Homework)

$\Delta G_0$ = -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:

$\Delta G$ = -100 pN-nm for [ADP] = 10 $\mu$m

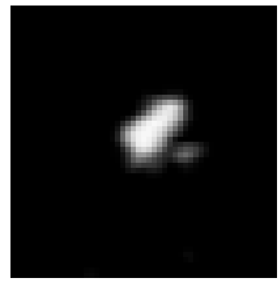
$\Delta G$ = -90 pN-nm for [ADP] = 100 $\mu$m

Practically 100% Efficient!

# Load Dependence of ATPase

## Vary the length of Actin lever Arm– moving under friction





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

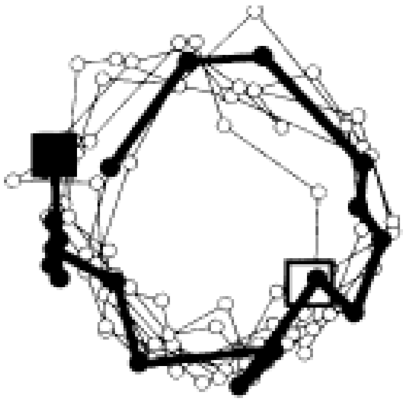
Rotation of F1 is slower when a longer rod (actin filament) is attached to the rotor subunit. This is because the rod is rotating in water and the viscous friction imposed on the rod is proportional to the cube of the rod length. Precise analysis indicates that F1 produces a constant torque (rotary force)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

http://www.k2.phys.waseda.ac.jp/Movies.html

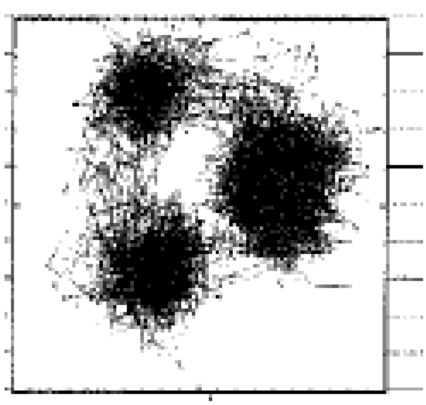
# Stepping rotation: 1 ATP per 120º



## 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.**

# How Efficient is ATPase?

## (2nd: 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)

$\omega$ = rotational rate (radians/sec)
$\tau$ = Torque
$\xi$ = 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 $\mu$m at 6 rev/sec (≈ 40 rad/sec)

$\tau$ = 40 pN-nm

Energy = $\tau\ \omega$

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

Therefore (again) operates at nearly 100% efficiency.

# Amazing Animation!

$F_1F_0$ ATPase

http://www.grahamj.com/fivth2.html

# 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.