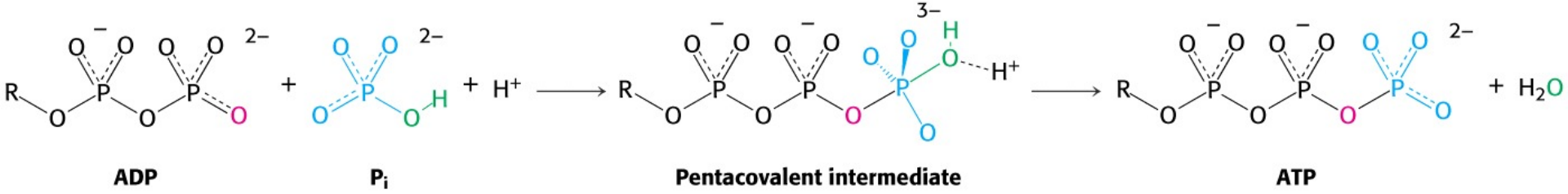
"It is easier to make a theory of everything, than a theory of something"

Katchalsky

ATP synthesis



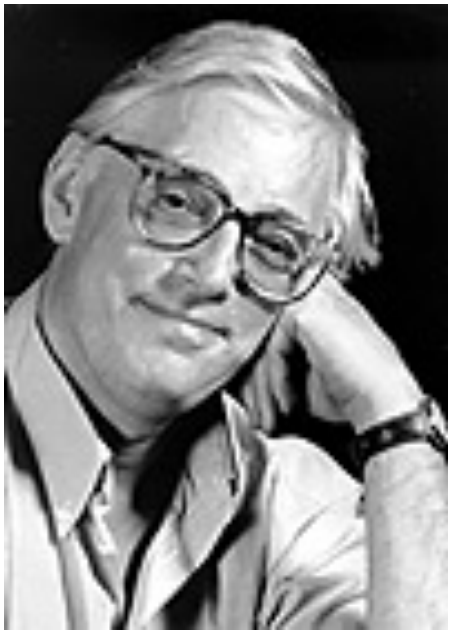
$$ADP + P_i + nH_P^+ \Leftrightarrow ATP + nH_N^+$$

Nobel Prize in Chemistry, 1997

"for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)"

Paul D. Boyer

Binding change mechanism.

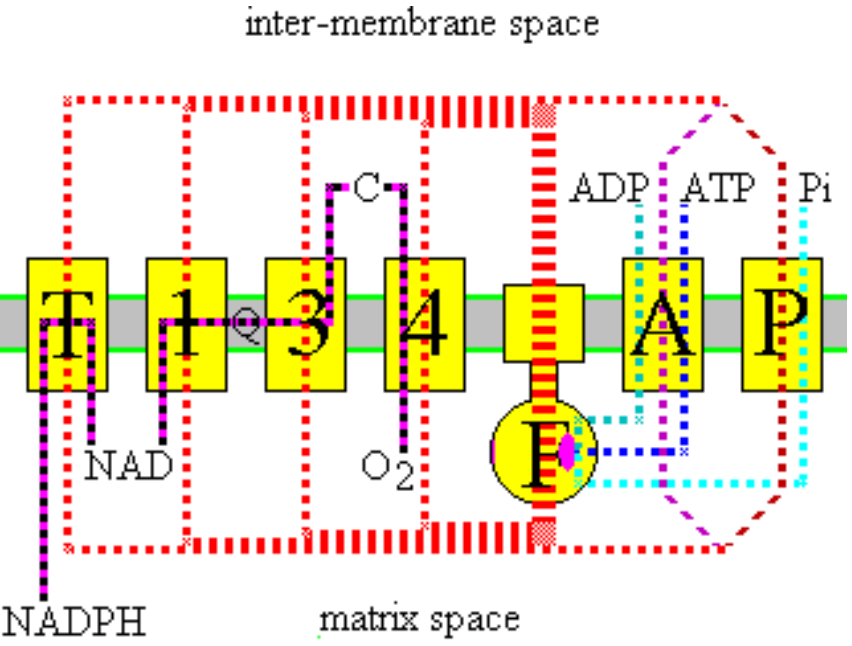


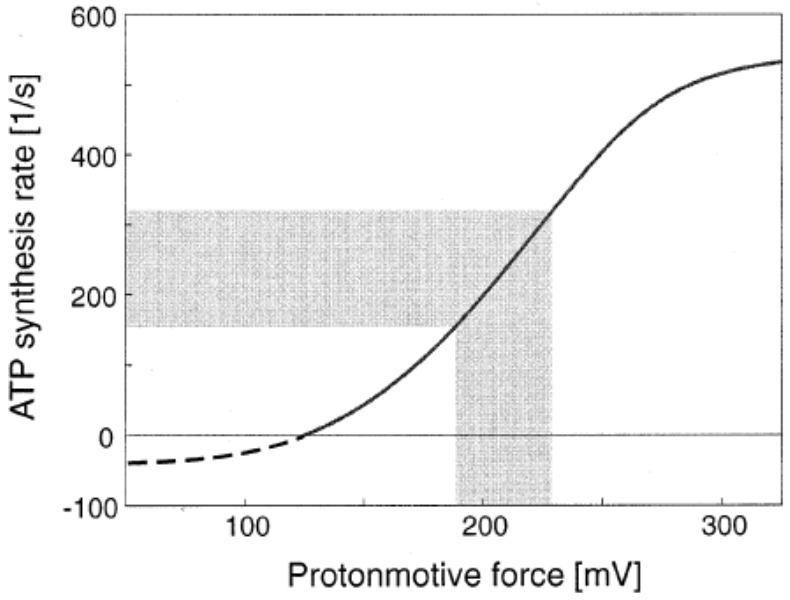
John E. Walker

Determination of the tertiary structure of ATP synthase by X-ray crystallography.

Michell's chemiosmotic theory

Energy stored in a transmembrane electrochemical gradient is converted into the chemical bond energy of ATP.





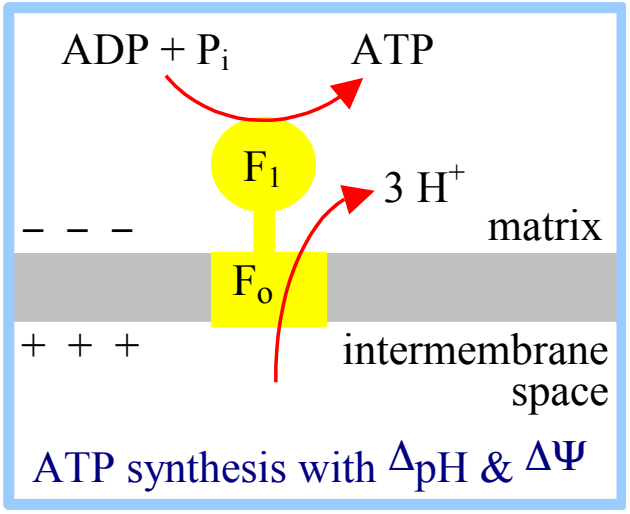
$$protomotive \quad force = \Delta\mu_{Na^+(H^+)} = 2{,}3\left(\frac{k_B T}{e}\right)\Delta pNa^+(H^+) + \Delta\psi$$

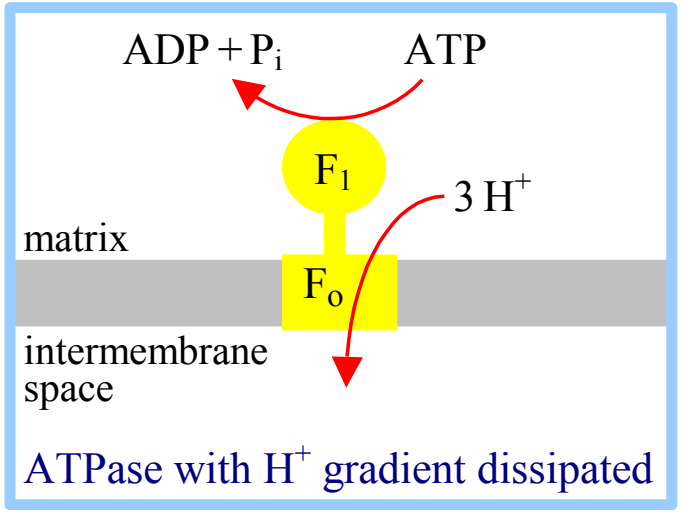
F_1F_0 ATP Synthase

"The Worlds Smallest Motor"

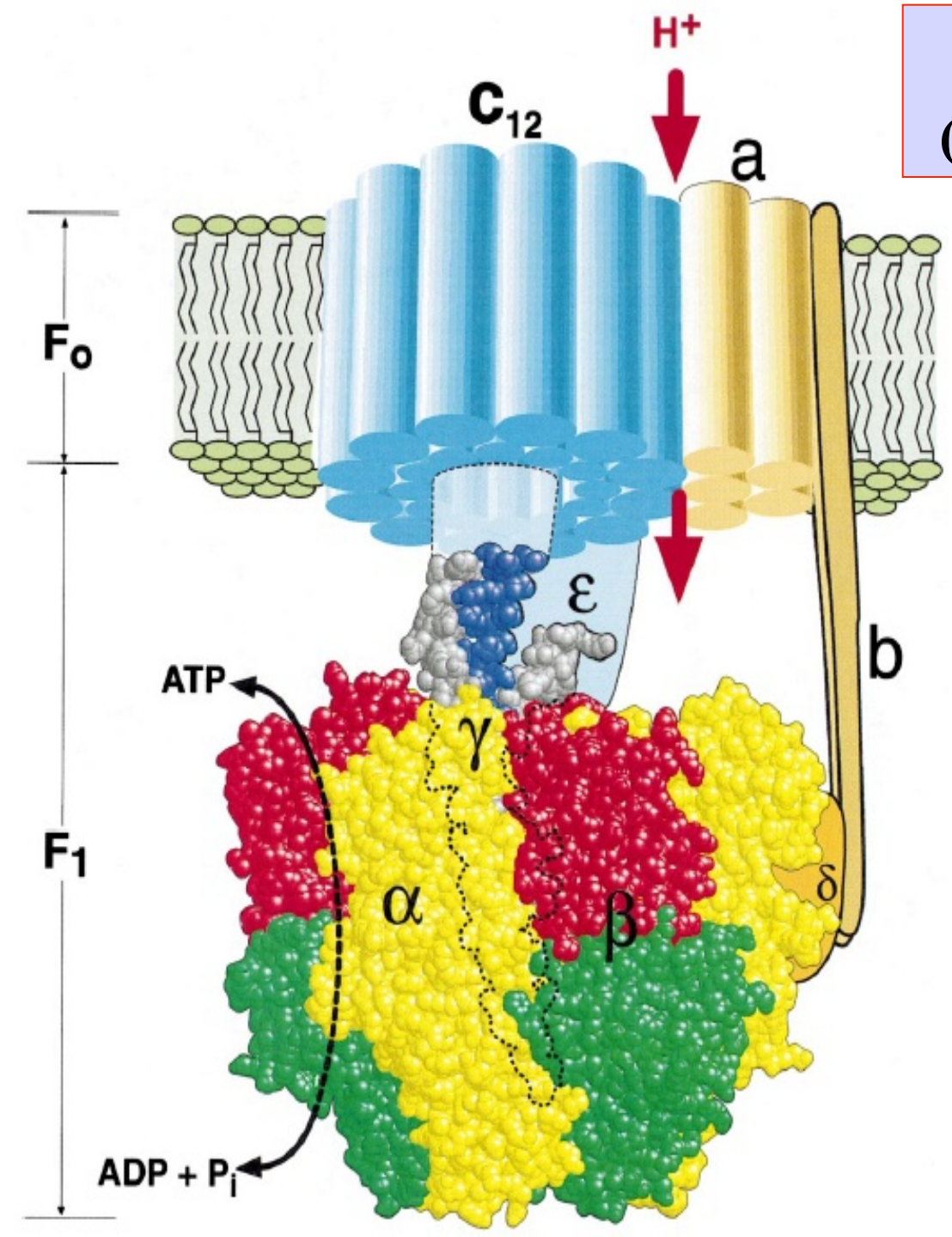
It is the most active enzyme in our Universe.

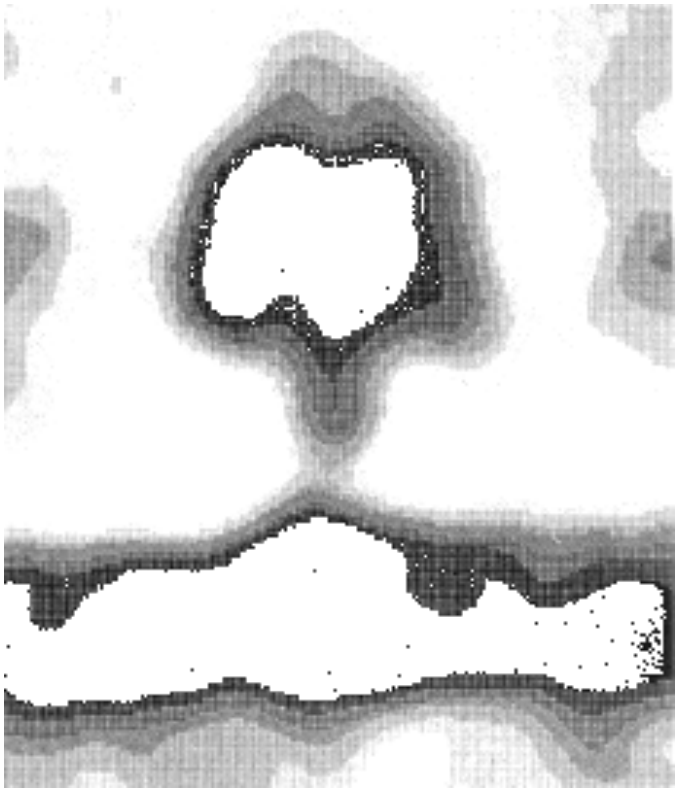
The reaction catalyzed by ATP synthase is fully reversible



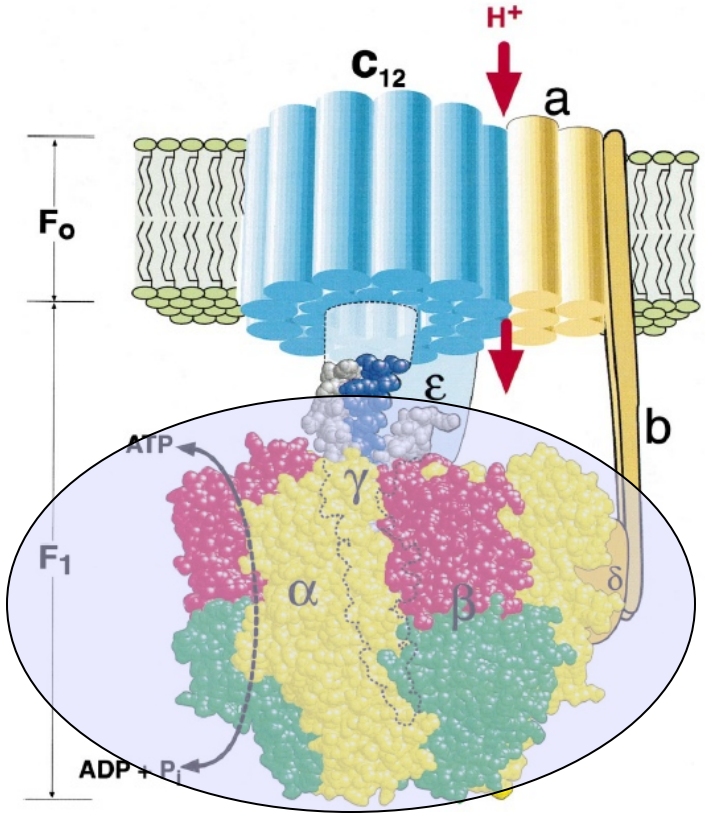


E. coli ATPase
(cryo-electron microscopy).

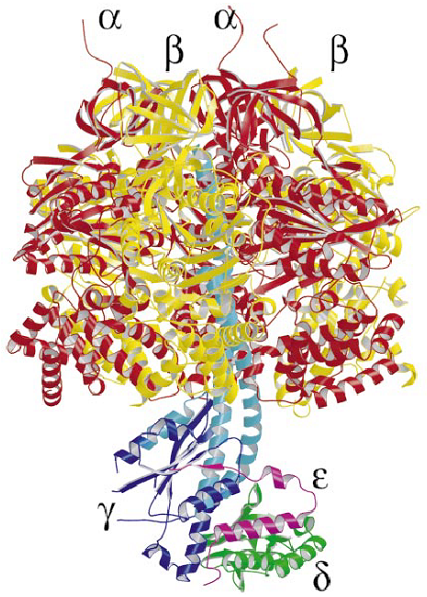


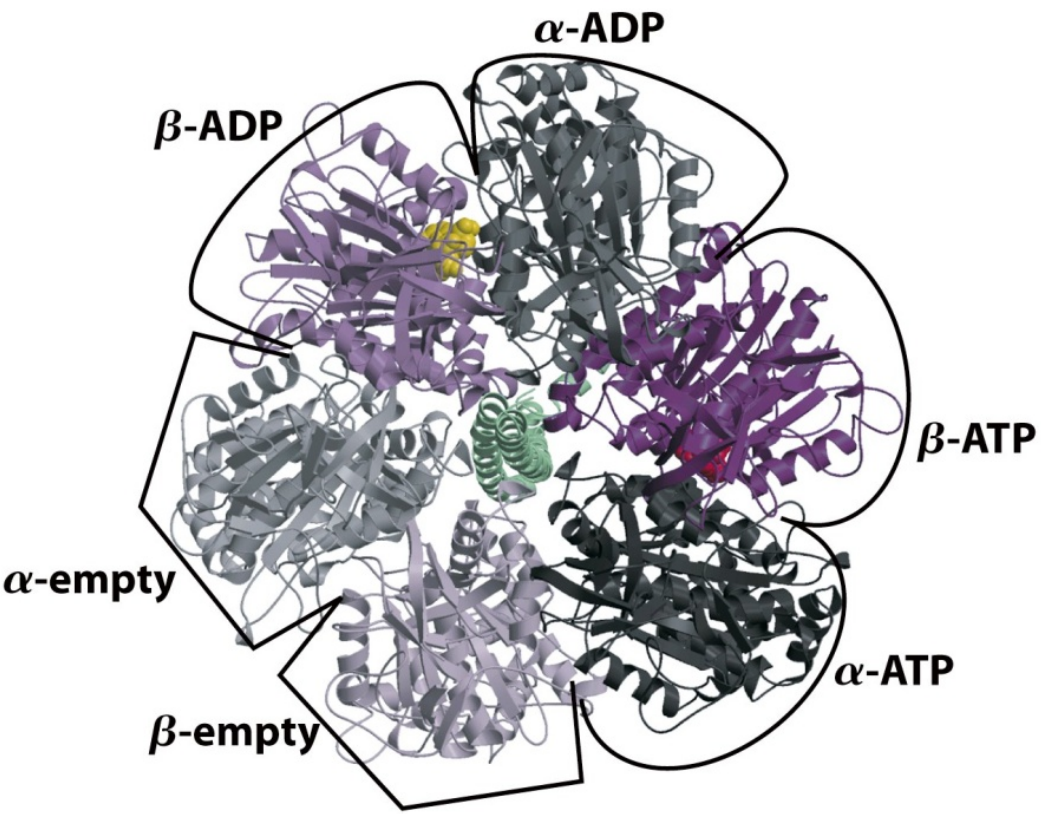


The γ subunit rotates about 100 times per second.



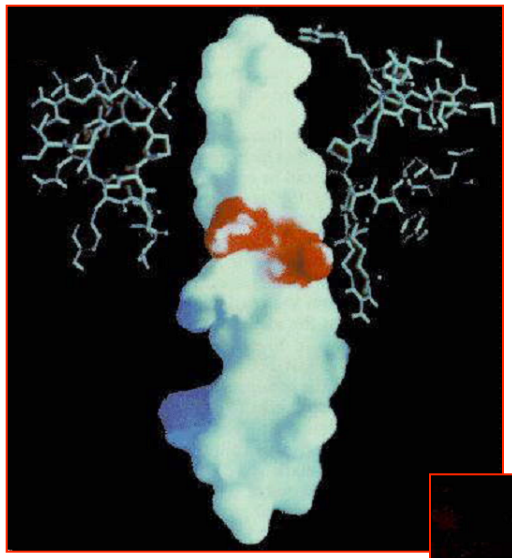
- *The F_1 alone catalyzes ATP hydrolysis, but not ATP-synthesis.*
- *The time scale of the ATP release or binding – milliseconds.*
- *F_1 in E. coli consists of 5 polypeptides with stoichiometry α_3, β_3, γ, δ, ε (named in order of decreasing mol. weights).*

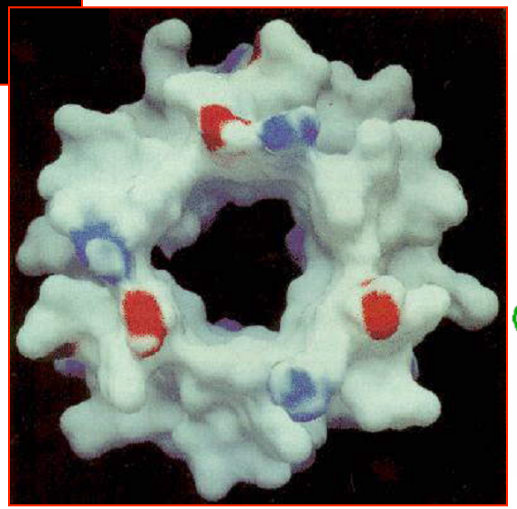


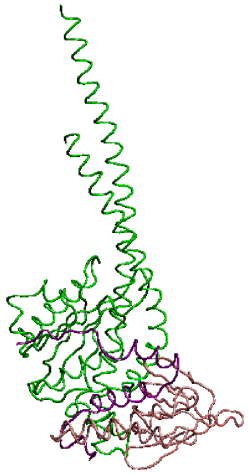


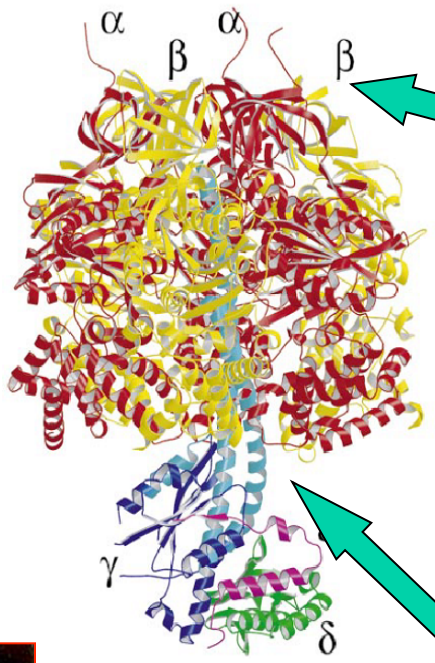
- α & β are arranged as a ring of alternating subunits.
- There are **three nucleotide-binding catalytic sites**, located at αβ interfaces but predominantly involving residues of the **β** subunits.

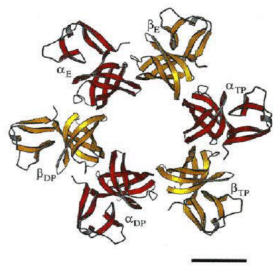
- Each of the three **α** subunits contains a tightly bound ATP, but is inactive in catalysis.
- Adenine nucleotides bind to α & β subunits with **Mg^{++}**.

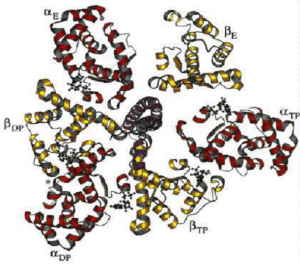


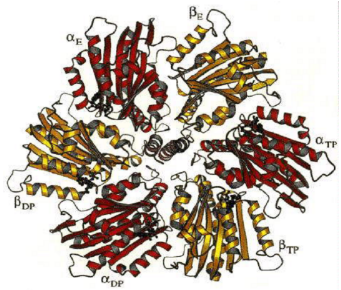






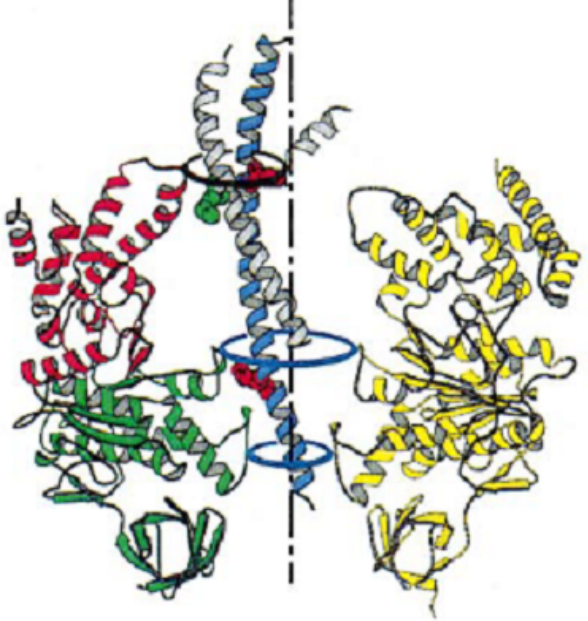




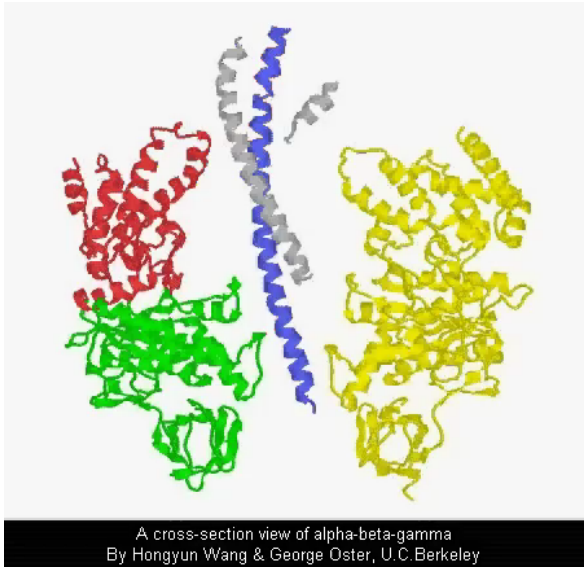


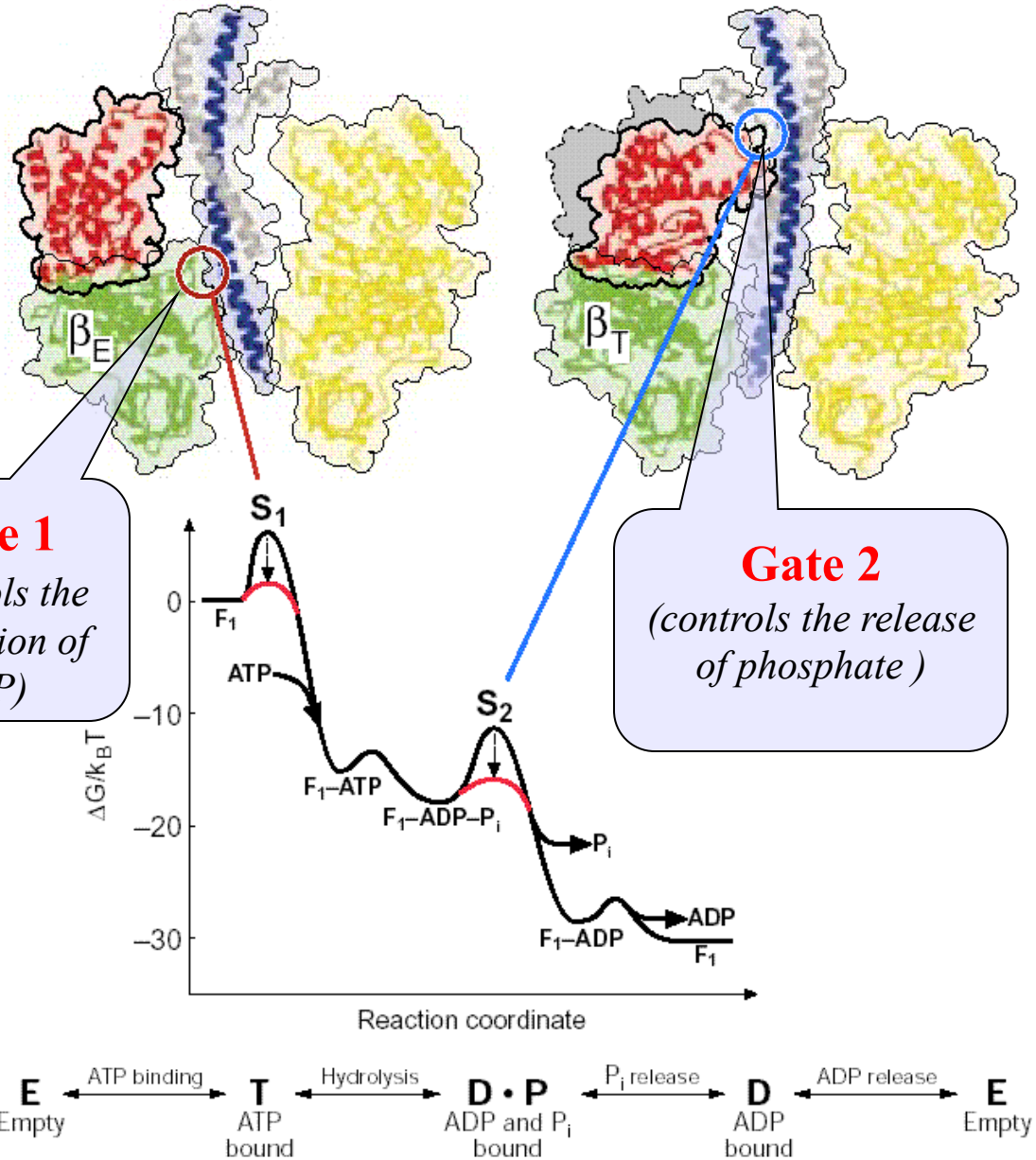
Mechanical dissipation is minimal ***– the bending of β is tightly coupled mechanically to the rotation of γ and the hydrophobic sleeve holding the γ shaft is nearly frictionless.***

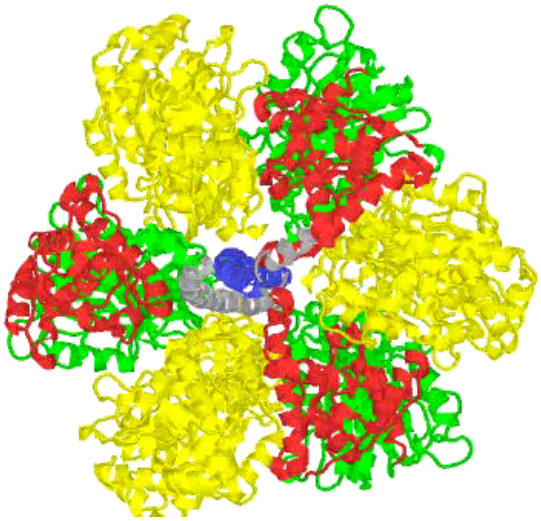
Conformational changes during the rotation of the γ shaft.



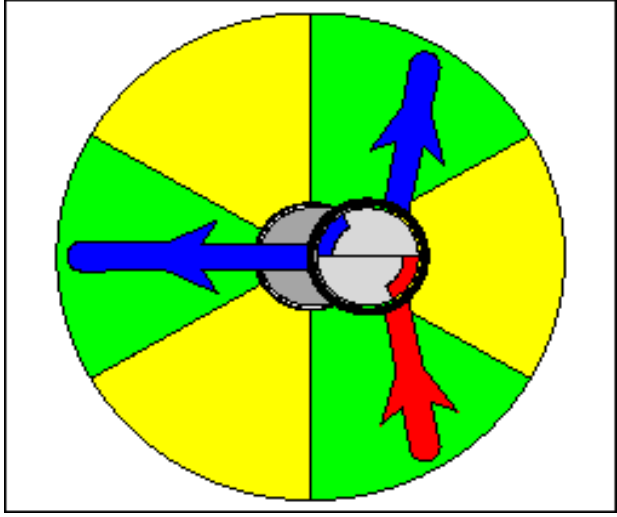
The three contact levels of γ with the $\alpha_3\beta_3$ hexamer.

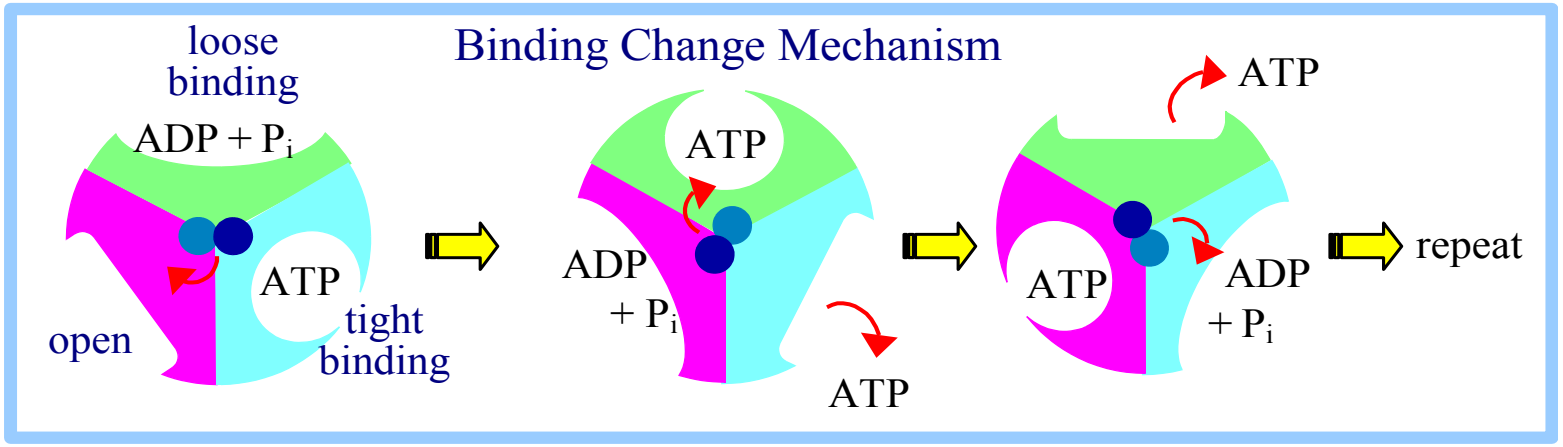




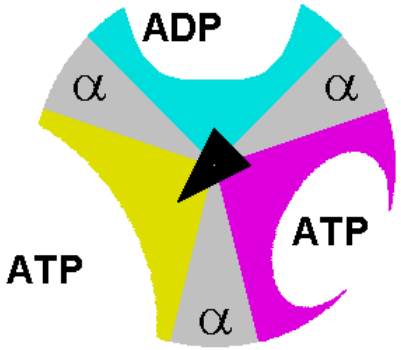


Three units are working together

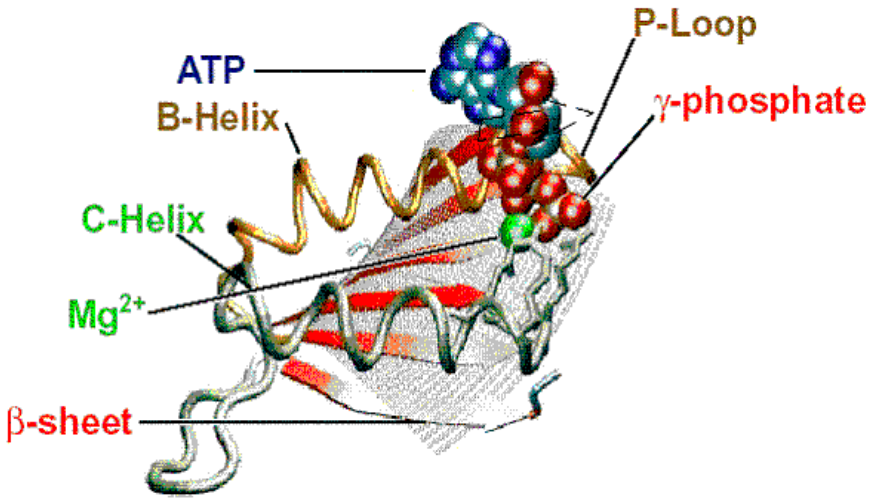


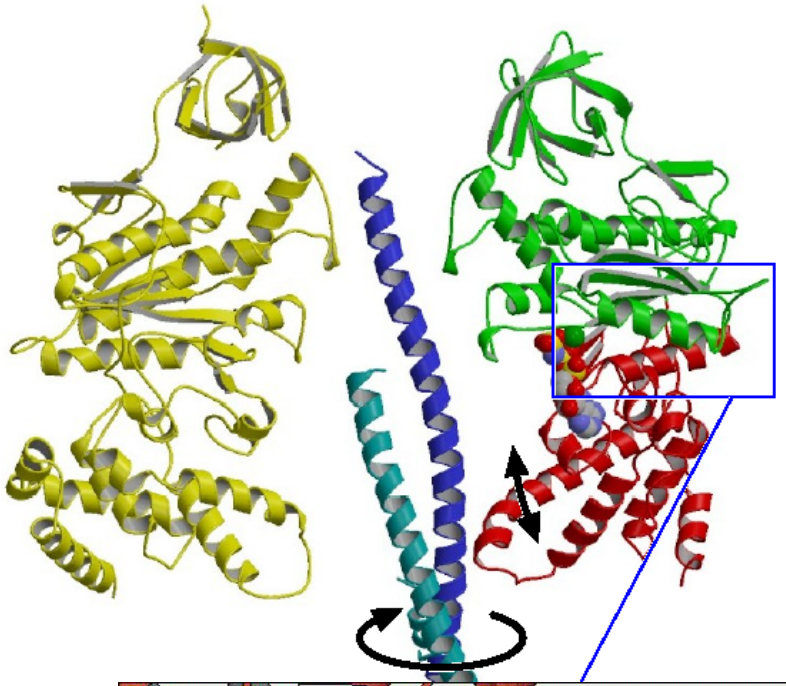


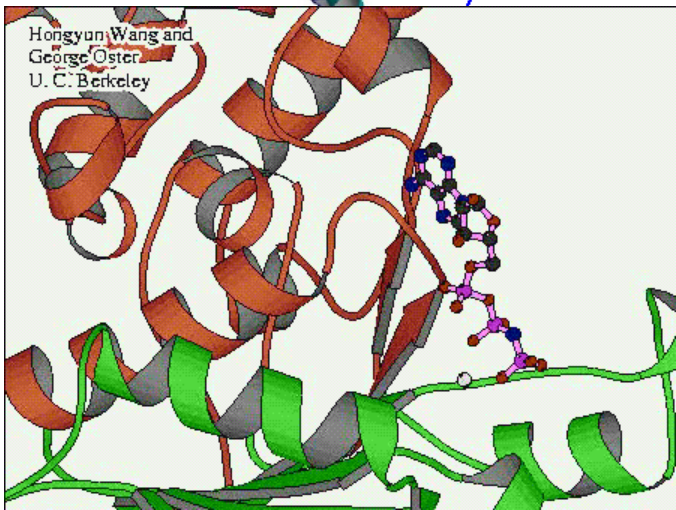
A complete revolution took place in 3 steps, and consumed a single ATP per step. ***Each hydrolized ATP liberates about 12 kcal/mol.***



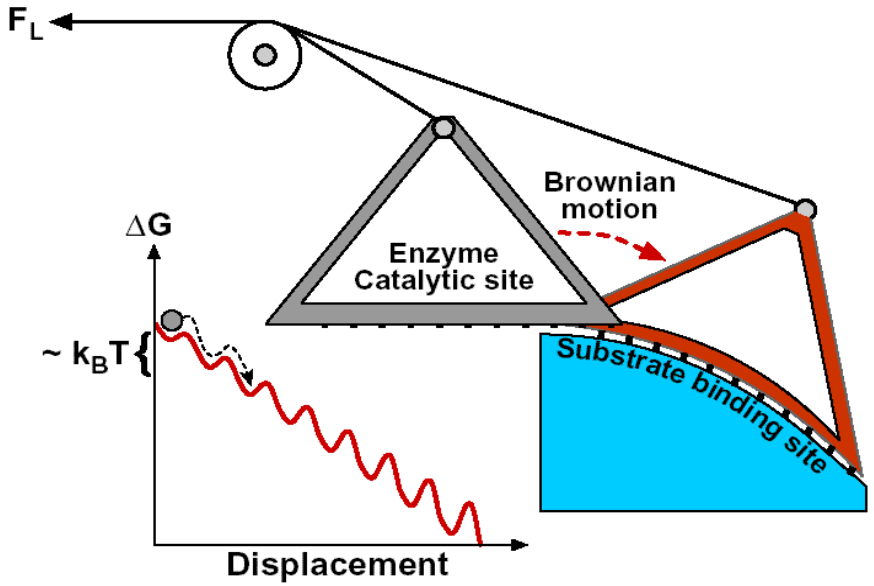
The ATP binding site







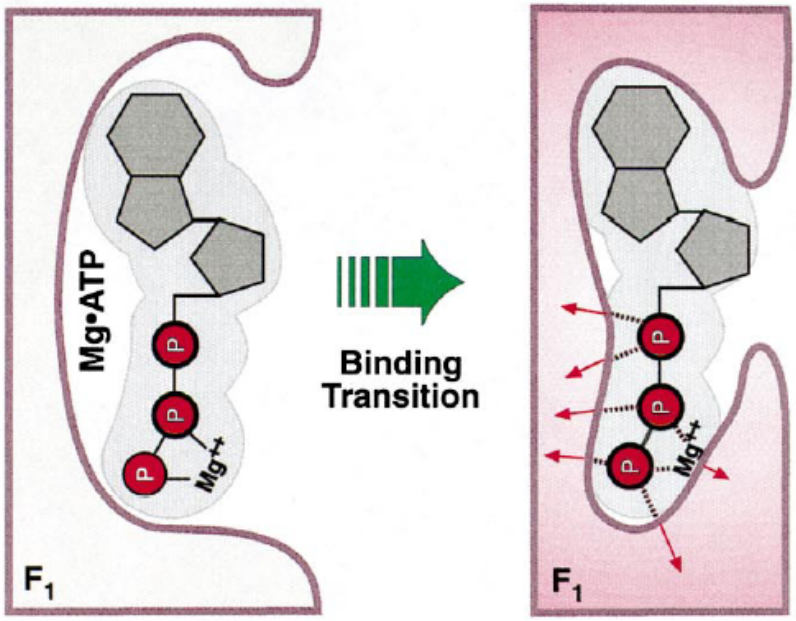
- The nucleotide is held by two α-helix/loops emanating from the β-sheet that forms the floor of the catalytic site.
- The P-loop connecting the B-helix with the β-sheet is the force-generating element.
- Mg^{2+} increases the ATP affnity for the tight binding site up to five orders of magnitude.



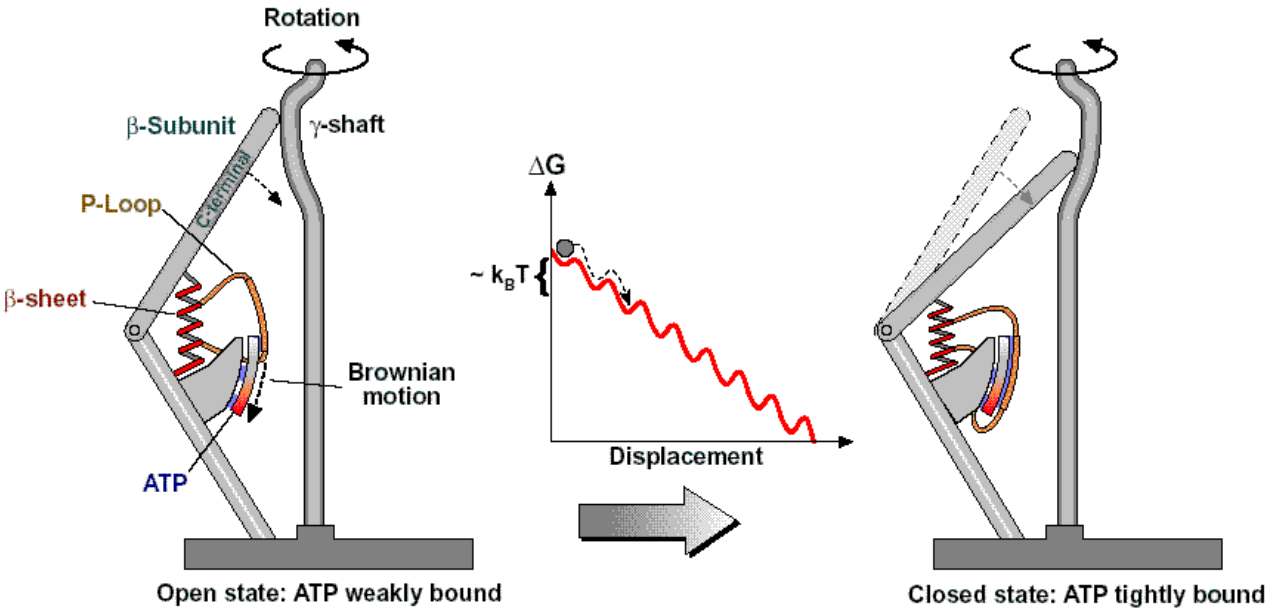
The power stroke

The top part of β rotates about 30° toward the bottom part. This rotation closes the angle between helices B and C.

ATP binds to the catalytic site by a rapid thermal `zippering' of hydrogen bonds.

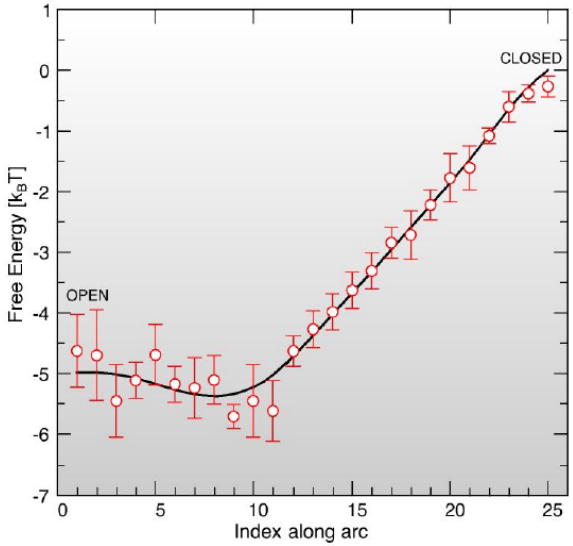


During hydrolysis, the Binding Zipper utilizes the binding free energy of ATP to generate a nearly constant primary power stroke.

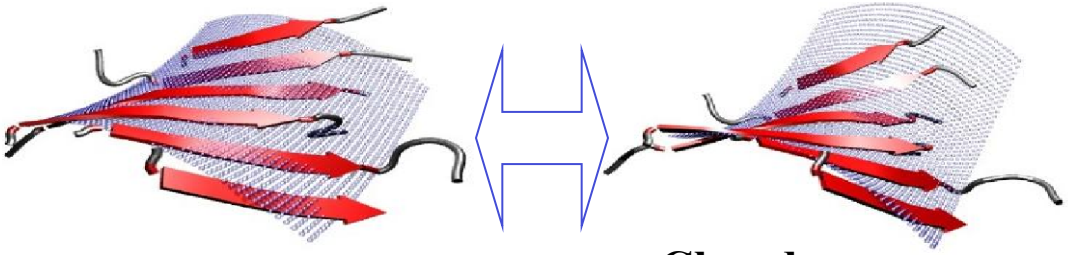


The power stroke

- *A flexible binding site on the enzyme slides over the binding surface of a fixed ligand.*
- *Its stochastic motion is driven by biased Brownian fluctuations.*
- *During the binding process the free energy decrease encounters only small energy barriers of order k_BT*
- *The binding energy is converted directly into mechanical work.*



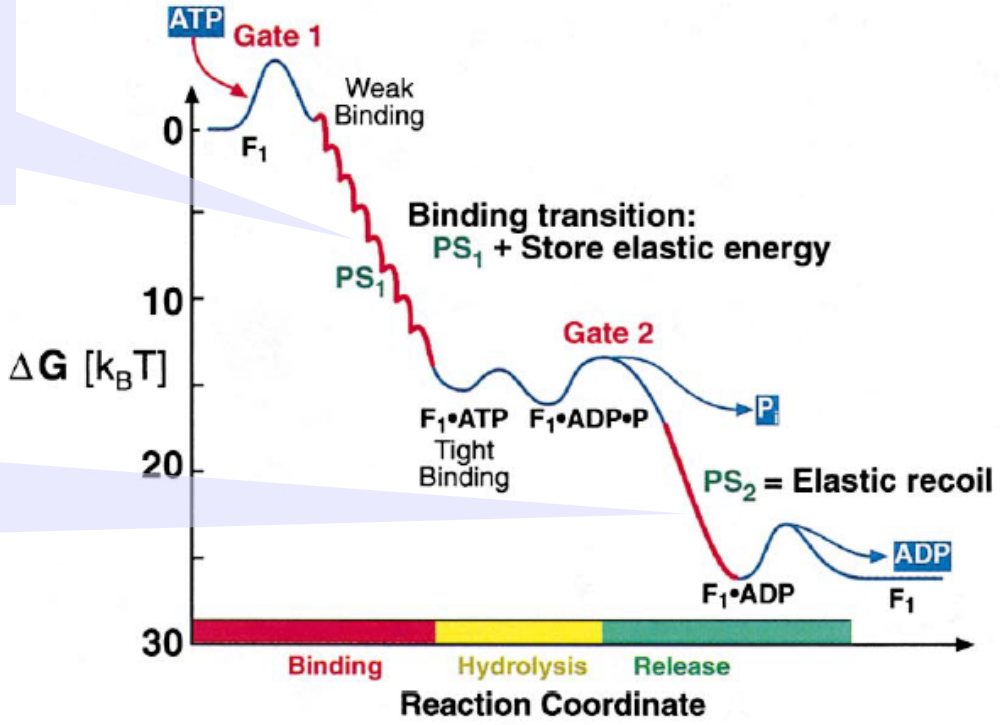
Internal energy of the β subunit



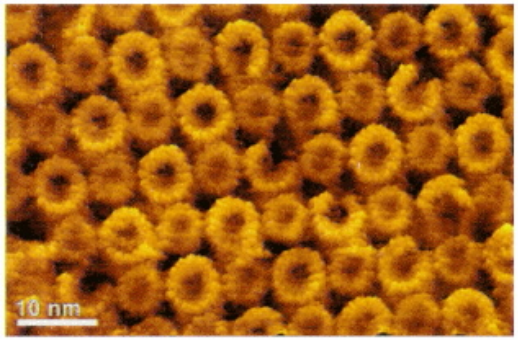
Open **Closed**

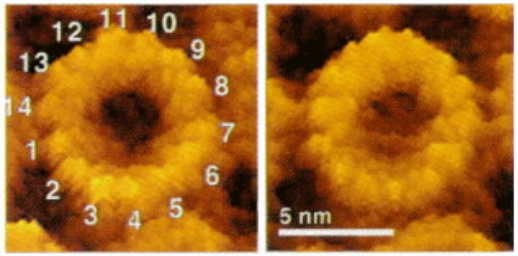
The binding transition from weak to tight generates the primary power stroke (PS_1).

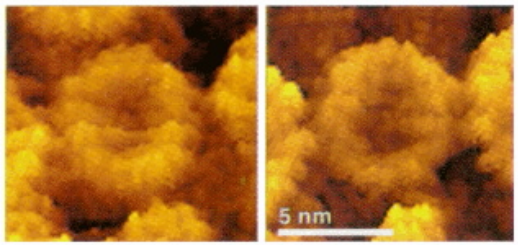
Upon release of phosphate, the over-compression of the passive spring drives the secondary power stroke (PS_2) by elastic recoil.

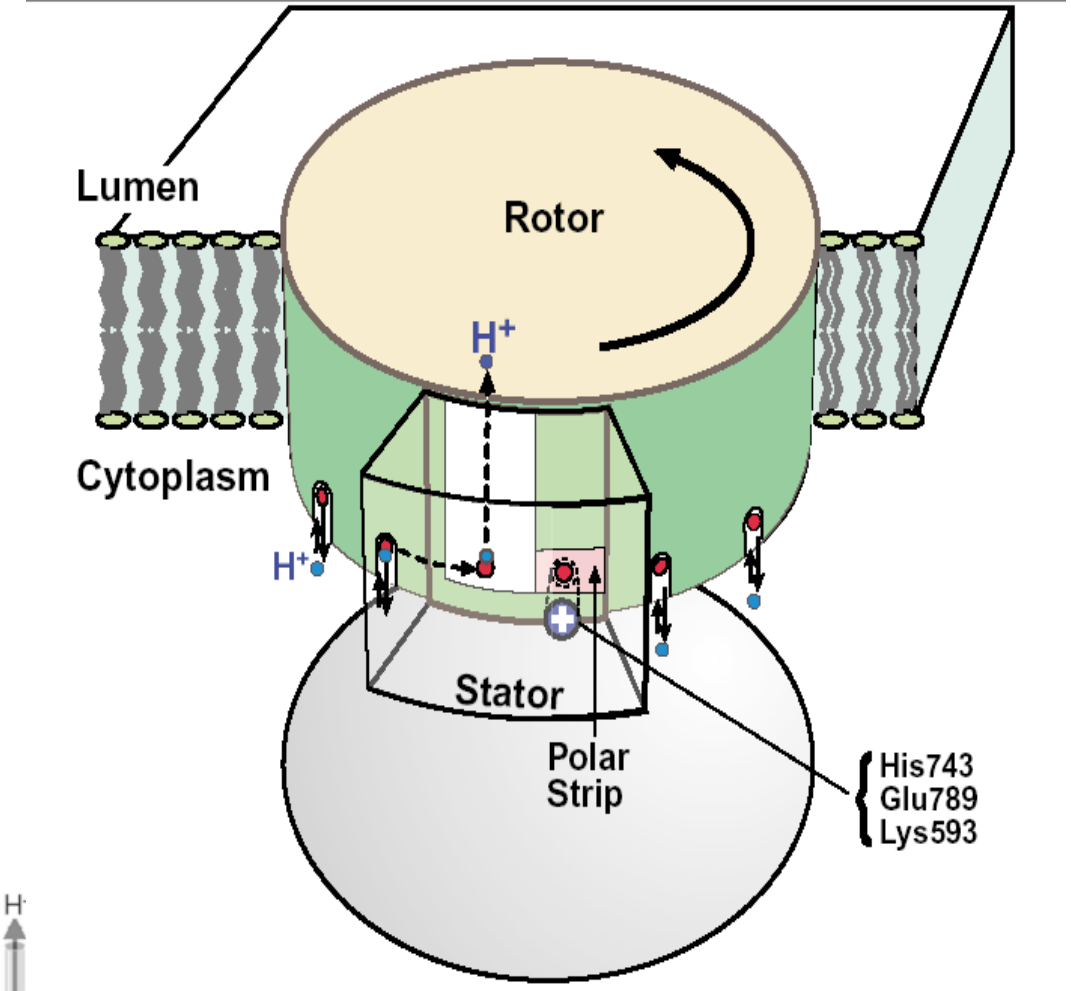


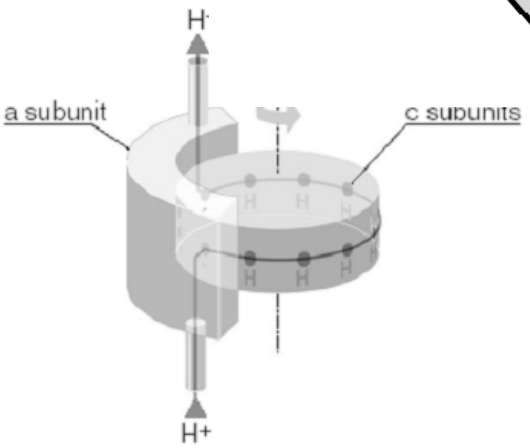
F_0 ATPase



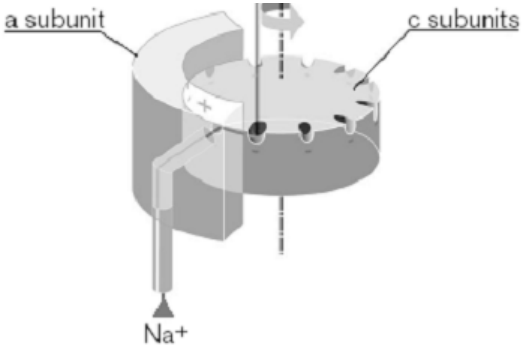






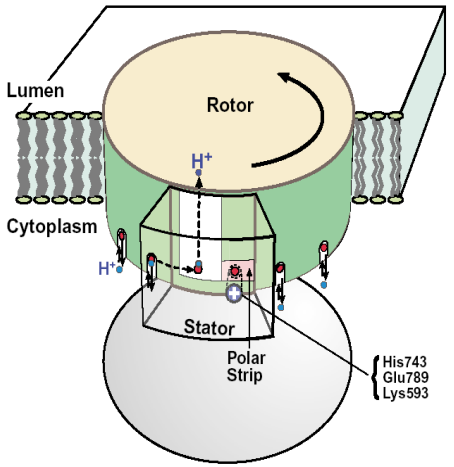


The H^+ ATP-ase.



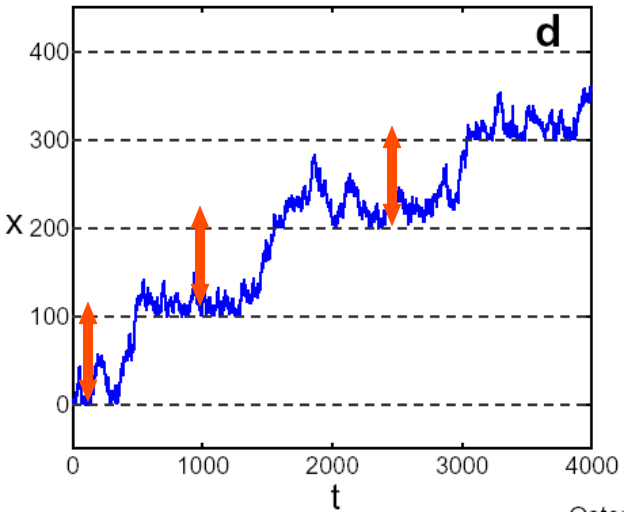
The Na^+-motive ATP-ase.

- **F_o** is a complex of integral membrane proteins.
- If F_1 is removed from the **membrane** containing F_o becomes **leaky to H^+**.
- Adding back F_1 restores normal low permeability to H^+.



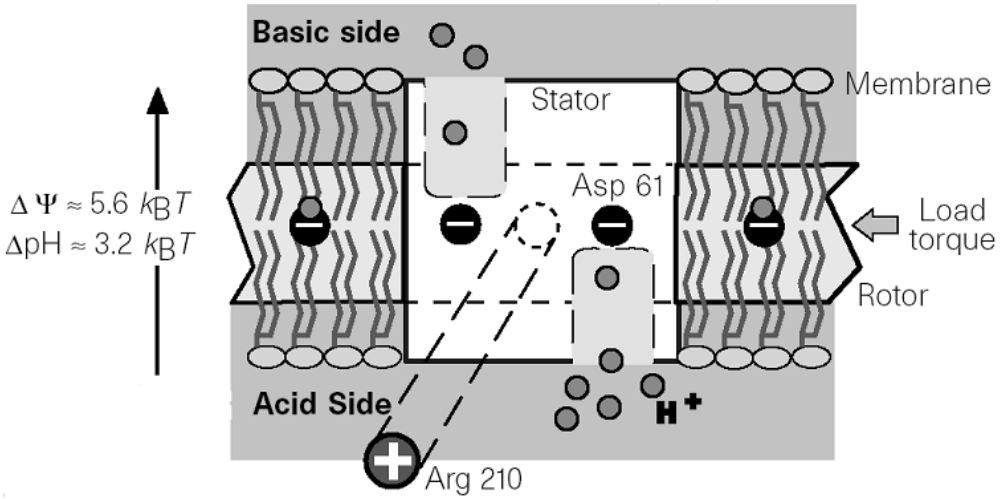
F_o includes a "proton channel."

- Ion movements across the membrane drive rotation of the c subunit ring in steps.
- Each step represents the movement of one c subunit into, and other c subunit out of, an interaction with the „a" subunit.



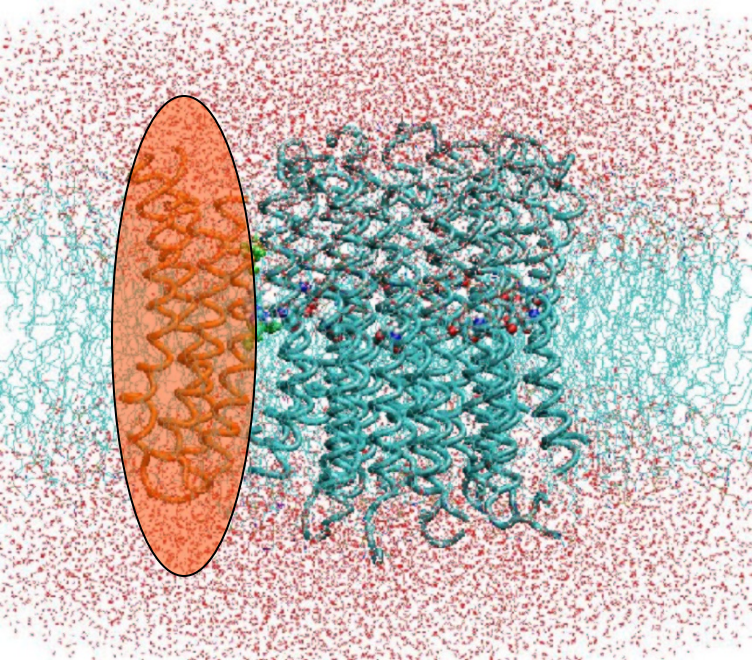
A transmembrane electrochemical gradient provides the energy that the motor converts into a rotary torque.

The thermodynamic measure of this energy gradient is the chemical potential difference between the periplasm (high ion concentration) and the cytoplasm (low ion concentration)



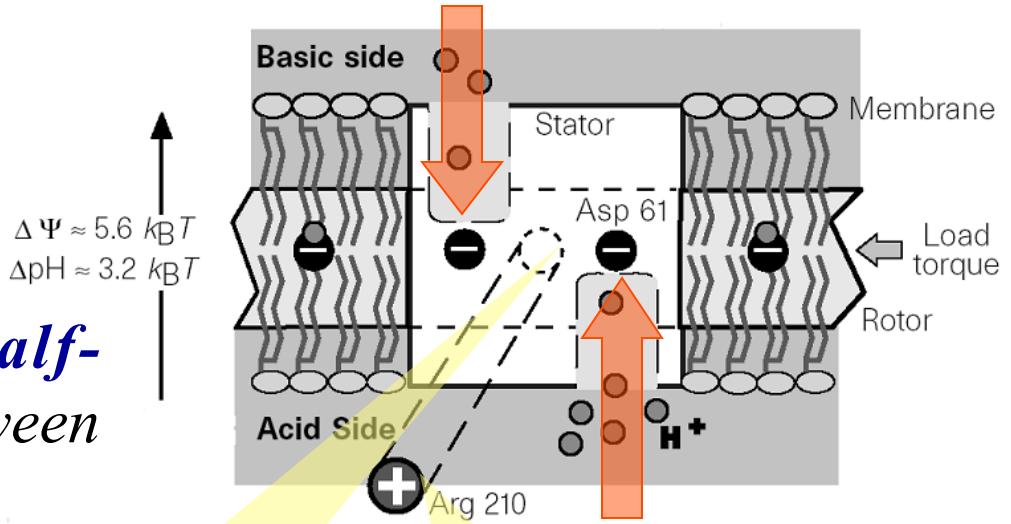
$$\Delta\mu_{Na^+(H^+)} = 2{,}3\left(\frac{k_B T}{e}\right)\Delta pNa^+(H^+) + \Delta\psi$$

pNa⁺ = -log [Na⁺] (the sodium analogue of pH).
Δψ - the transmembrane electrical potential.

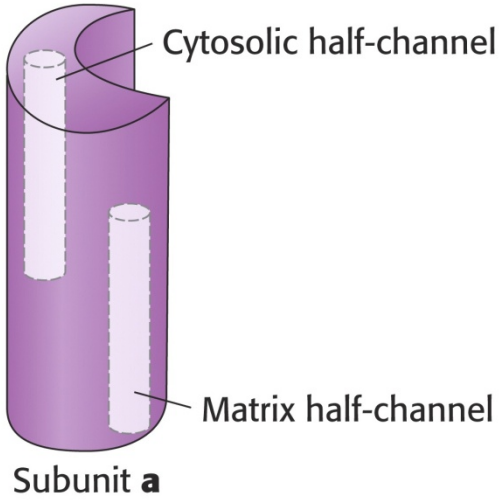


The "stator"

*The **a subunit of F_o** includes several transmembrane α-helices.*

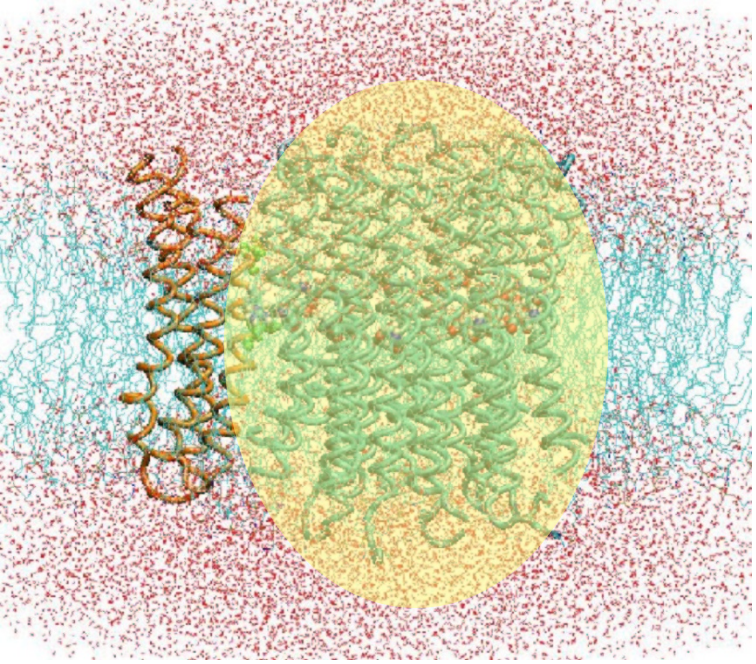


*The **a-subunit** forms **2 half-channels** that let H^+ pass between the two membrane surfaces.*



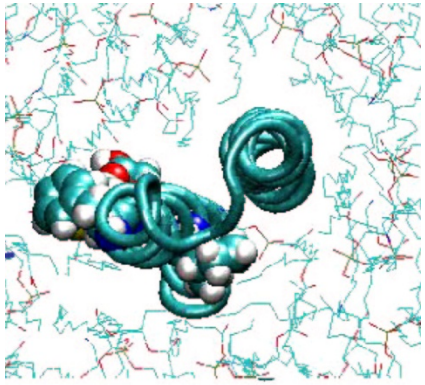
A hydrophobic seal prevents ions from leaking across the membrane.

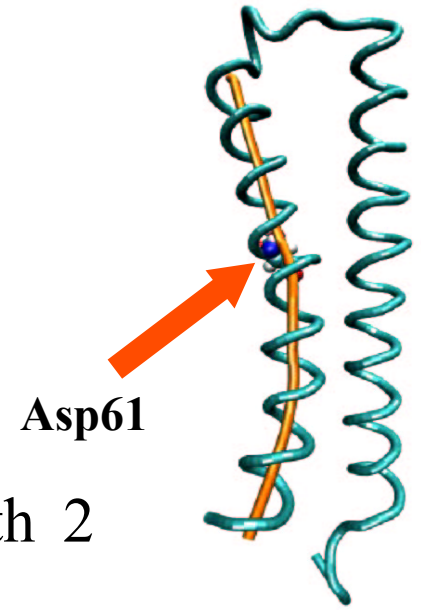
A single positive charge on the stator located close to the strip repels the bound ions.



The "rotor"

- 10-12 c subunit.

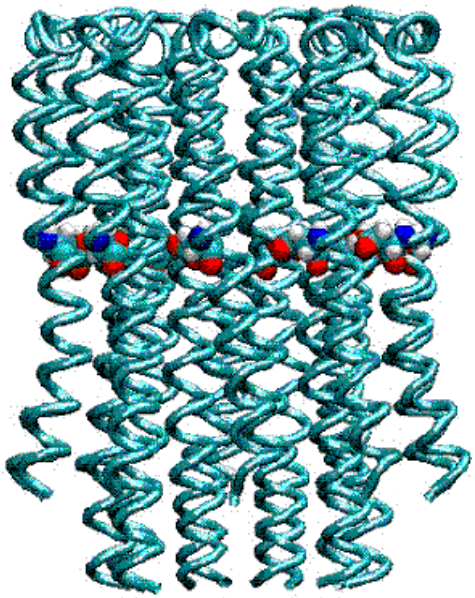


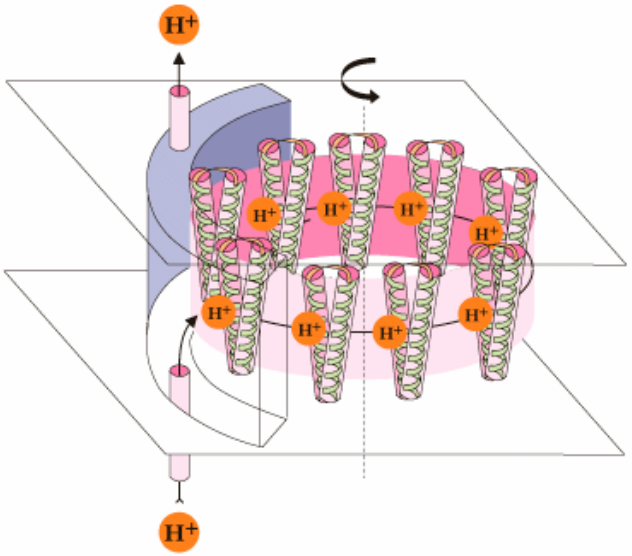


- The **c subunit** of F_o has a hairpin structure with 2 transmembrane α-helices & a short connecting loop.

- One α-helix includes an Asp or Glu residue.

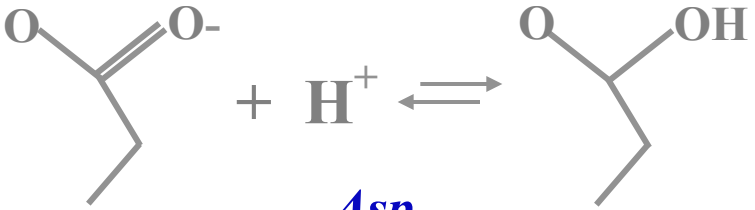
- 10-12 negatively charged ion binding sites are equally spaced around the periphery, and lying below the level of the membrane.





H*$^+$** *may be relayed from one half-channel or* H^+ *wire to the other only via the* ***carboxyl *group of a* ***c-subunit****.*

- Asp residues in the c subunits pick up a proton from one side of the membrane.

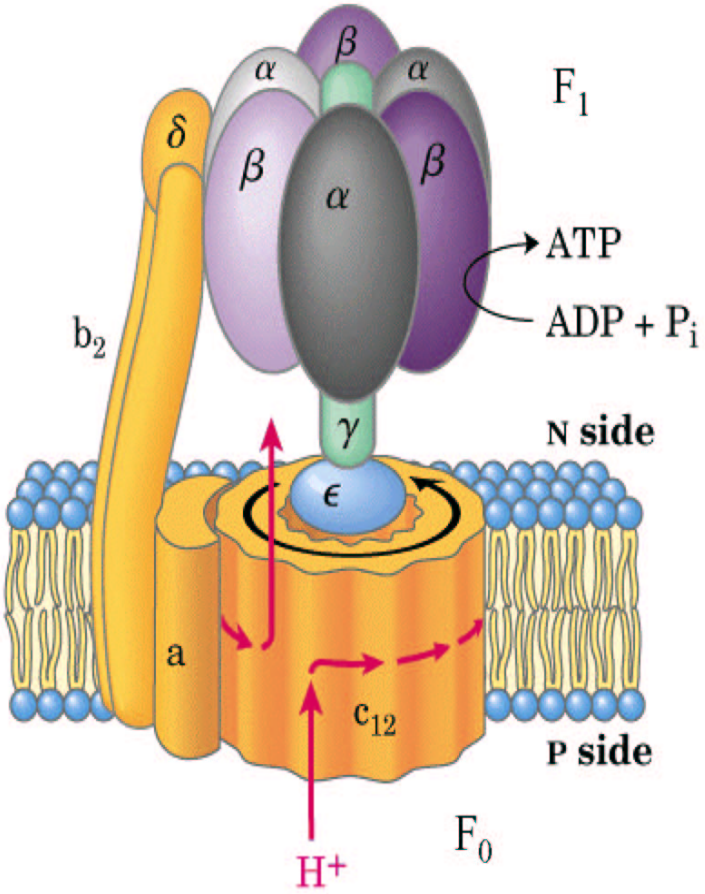


Asp

- They become **uncharged** and can then contact the lipid membrane.

- As the **ring of 10 c subunits rotates**, the c-subunit carboxyls relay protons between the 2 α-subunit half-channels.

This allows H^+ ***gradient-driven*** H^+ ***flux across the membrane to drive the rotation.***



Spec Sheet

Size: **8 x 14 nm**
Weight: **~350,000 Daltons**
Fuel Type: **Adenosine triphosphate (ATP)**
Alternative Fuel: **Guanosine triphosphate (GTP)**
Fuel Efficiency: **50 – 100%**
Catalytic sites: **3 (α/β junction)**
Average speed: **~1 – 5 rps**
Top speed: **~ 120 rps (unloaded)**
Rotary torque: **~240 pN•nm per revolution**
Function: **Synthesize ATP in living systems**

The F_0 Motor of ATP Synthase
A Brownian Ratchet with a Power Stroke

- The F_1 motor uses the free energy of ATP hydrolysis to rotate in one direction,
- The F_0 motor uses the energy stored in a transmembrane electrochemical gradient to turn in the opposite direction

Which motor "wins" that is, develops more torques depends on cellular conditions.

- Vesicles acidified by bacteriorhodopsin could drive the V-ATPase in reverse to synthesize ATP. The region of synthesis is shown as shaded.