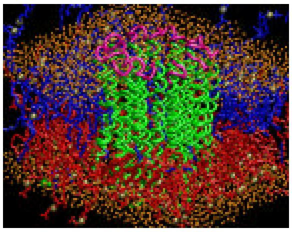
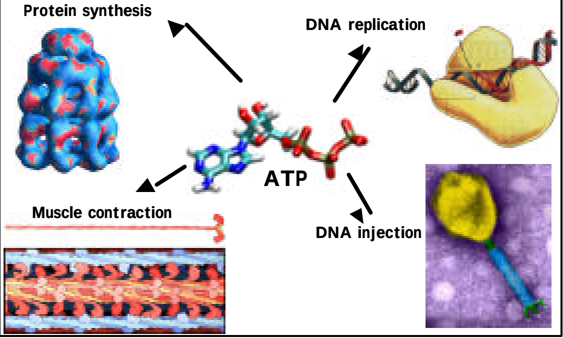
ATP synthase

Soluble F_1 ATPase



Membrane bound F_0

ATP is the main energy source for almost all chemical processes in living systems



Why do we consume so much ATP?

Many reactions in biological cells are thermodynamically **unfavorable** ($\Delta G > 0$).

A thermodynamically ***unfavorable*** reaction can be driven by a ***favorable*** one, if they are coupled.

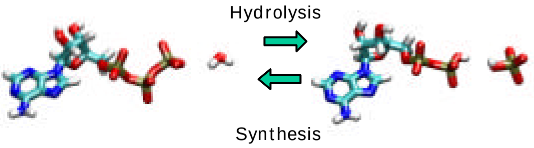
$$A \leftrightarrow B \qquad \Delta G°' = +4 \text{ kcal/mol}$$

$$K'_{eq} = [B]/[A] = 10^{(-\Delta G°'/1.36)} = 10^{(-4/1.36)} = 1.15 \times 10^{-3}$$

No spontaneous formation of B, when $[B]/[A] > 1.15 \times 10^{-3}$, so most of A remains **unconverted.**

We can make much more of B if we **couple** $A \leftrightarrow B$ with a favorable reaction.

ATP, universal energy carrier of living systems



$$\mathbf{ATP + H_2O \rightleftharpoons ADP + P_i}$$

$$\Delta \mathbf{G^0 = -12.2\ k_BT = -30.6\ kJ/mol = -7.3\ kcal/mol}$$

$$\Delta \mathbf{G} = \Delta \mathbf{G^0} + \ln \underbrace{([ADP][P_i]/[ATP])}_{\sim 1/500} = -13.7 \text{ kcal/mol}$$

ATP hydrolysis shifts the equilibria of coupled reactions

Coupled reaction:

$$A \leftrightarrow B \qquad \Delta G°' = +4.0 \text{ kcal/mol}$$

$$ATP + H_2O \leftrightarrow ADP + P_i + H^+ \qquad \Delta G°' = -7.3 \text{ kcal/mol}$$

$$A + ATP + H_2O \leftrightarrow B + ADP + P_i + H^+ \qquad \Delta G°' = \mathbf{-3.3\ kcal/mol}$$

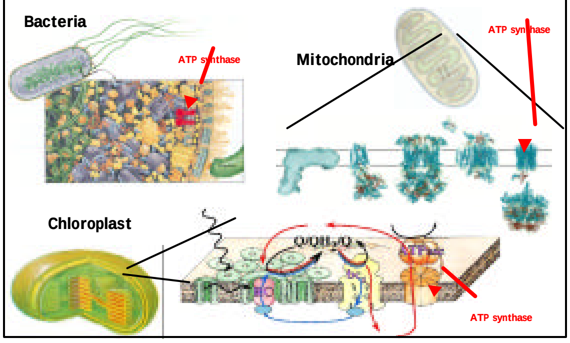
$$K'_{eq} = \frac{[B]_{eq}}{[A]_{eq}} \cdot \frac{[ADP]_{eq}[P_i]_{eq}}{[ATP]_{eq}} = 10^{(-\Delta G°'/1.36)} = 10^{(-(-3.3)/1.36)} = 2.67 \times 10^2$$

$$\frac{[B]_{eq}}{[A]_{eq}} = K'_{eq} \frac{[ATP]_{eq}}{[ADP]_{eq}[P_i]_{eq}}$$

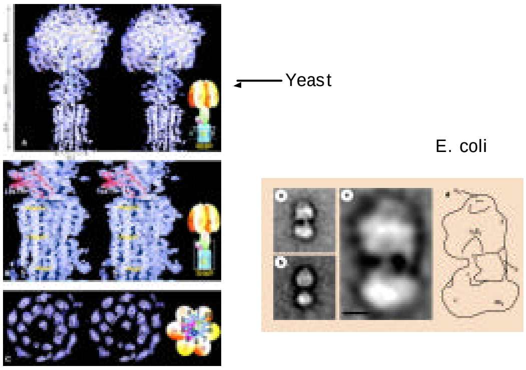
If the cell keeps it's $[ATP] / ([ADP][P_i])$ ratio at about 500, $[B]_{eq}/[A]_{eq} = 2.67 \times 10^2 \times 500 = \mathbf{1.34 \times 10^5}$, most of A has been converted!

*Without ATP hydrolysis, this was 1.15×10^{-3}, so **A ↔ B conversion has been increased by a factor of 10^8.***

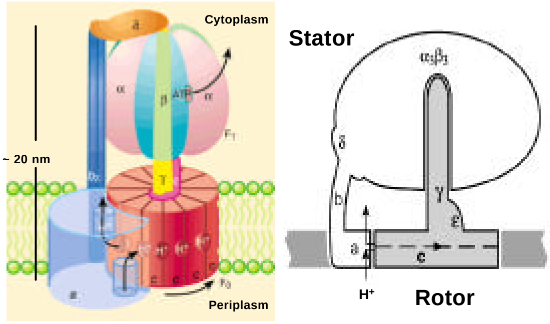
ATP synthase is the ATP factory which synthesizes most of ATP in organisms



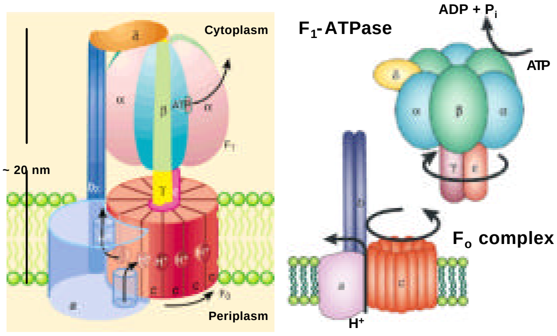
ATP synthase Structural Data



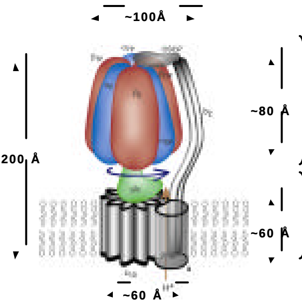
ATP synthase is a rotary motor that couples proton translocation to ATP synthesis



ATP synthase is a rotary motor that couples proton translocation to ATP synthesis



One shaft, two motors



Soluble part, F_1-ATPase

- Synthesizes ATP when torque is applied to it *(main function of this unit)*
- Produces torque when it hydrolyzes ATP
- 5 subunits: 3a ,3b, 1g, 1d and 1e

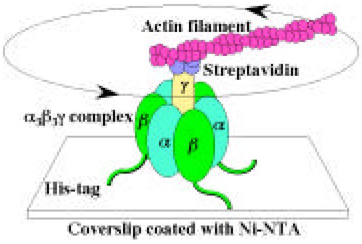
Membrane-bound part, F_0 Complex

- Produces torque when positive proton gradient across membrane *(main function of this unit)*
- Pumps protons when torque is applied
- 3 subunits: 1**a**, 2**b** and 9-12**c**).

Torque is transmitted between the motors via the central stalk.

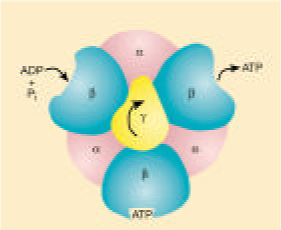
“ATP synthesis” requires **both** F_0 and F_1 parts.

Direct observation of ATP synthase rotary motion

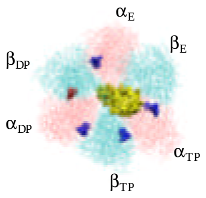


ATP synthesis mechanism

The applied torque causes rotation of the γ-subunit which causes cyclic transformation of three catalytic sites.

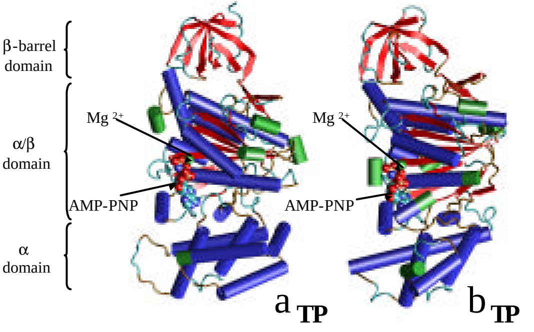


Bottom view



We have six nucleotide binding sites, 3 catalytic and 3 non-catalytic

a **and** b **subunits are similar in secondary structures**



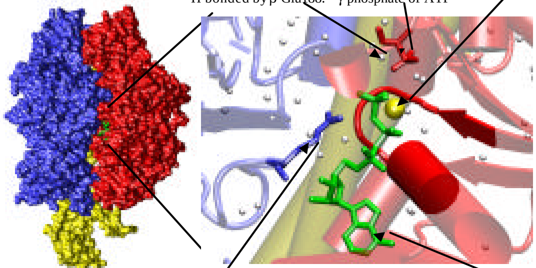
20% sequence identity.

- when isolated, hydrolyse ATP very slowly.

Catalytic Nucleotide Binding Site

b **-Glu188** makes H-bonds to a water molecule and has it ready to attack the γ-phosphate of ATP

Water molecule is H-bonded by β-Glu188.

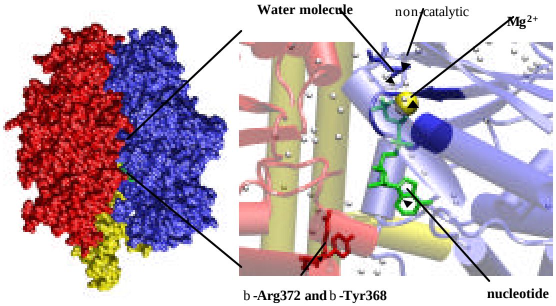


a $_{TP}$-b $_{TP}$ **interface**

a **-Arg 373** helps stabilize the negative charge

Non-Catalytic Nucleotide Binding Site

a **-Gln208** corresponds to Glu188 in b -subunit. It makes a -subunit non-catalytic



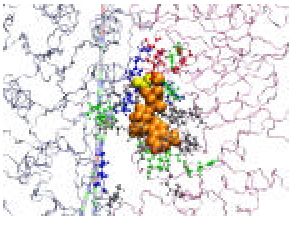
b$_E$-a$_{TP}$ **interface**

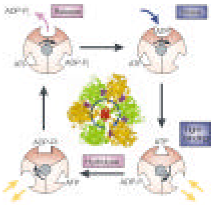
b **-Arg372 and** b **-Tyr368** side chains which help forming the binding pocket move out of the pocket.

A rough idea of central stalk's tasks

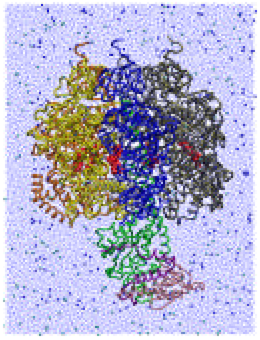
TP -> E -> DP -> TP

Interpolation of observed states



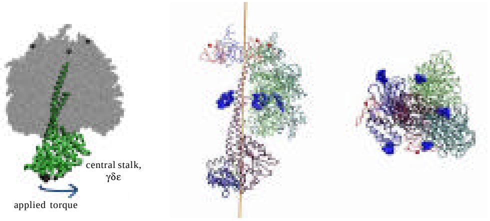


Assembling ATP Synthase F_1



Torque application to F_1

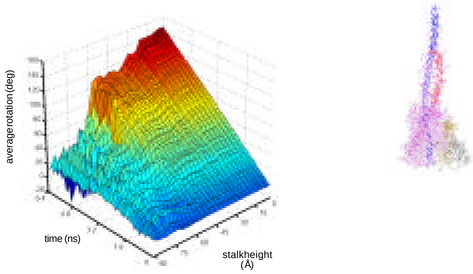
Torque is applied to the central stalk atoms at the F_1-F_o interface to constrain their rotation to constant angular velocity ω = 24 deg/ns.



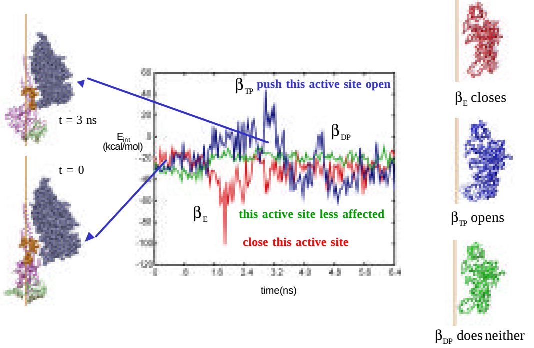
0.0 to 5.0 ns (0 to 120 deg) of torqued F_1 rotation, ω = 24 deg/ns.

Stalk analysis

Slowed torque transmission along central stalk



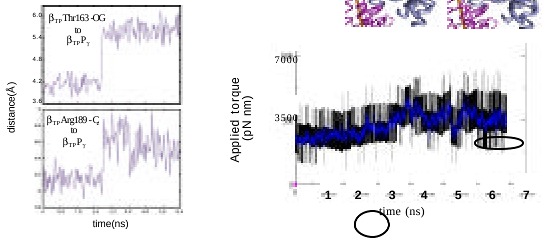
Different interactions between the central stalk and various β-subunits



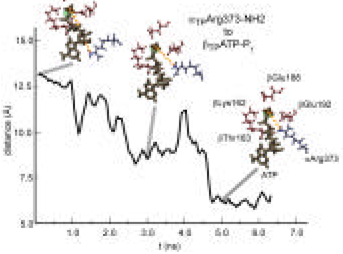
Rotation Produces Synthesis-like Events (3)

At 3.0 ns (72 deg) of rotation, we observe:

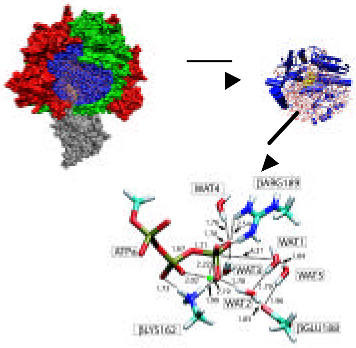
- slowed torque transmission along central stalk
- unbinding from ATP at the β_{TP} catalytic site

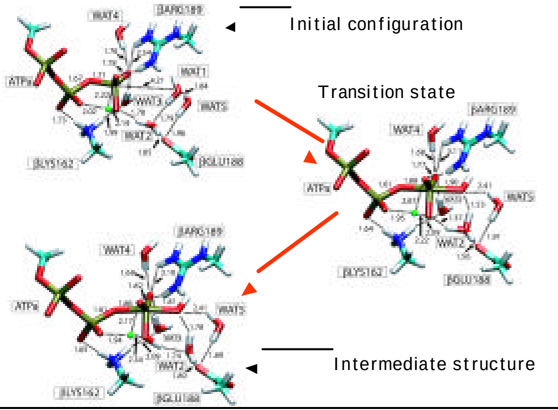


Motion of α_{TP} Arg373 towards β_{TP} phospate binding pocket



QM/MM calculation of ATP hydrolysis

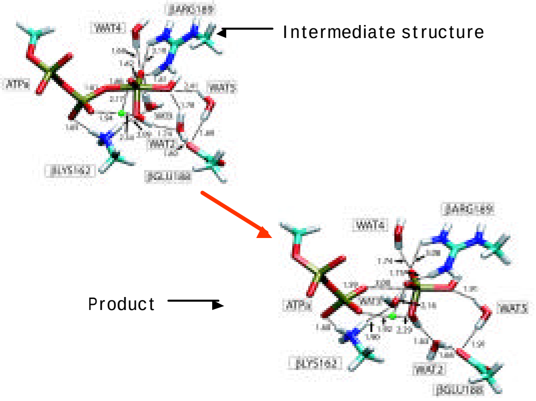




Initial configuration

Transition state

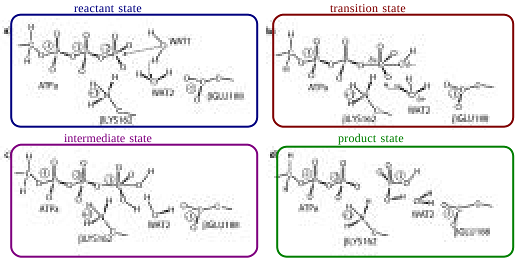
Intermediate structure



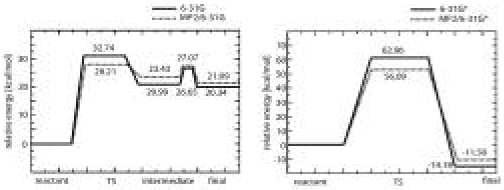
Intermediate structure

Product

Schematic view of ATP hydrolysis in b_{TP}



Energetics of ATP hydrolysis in b_{TP}

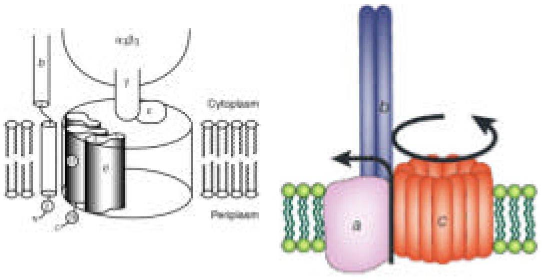


The overall ATP hydrolysis reaction is found to be endothermic. This suggests that β_{TP} favors binding of ATP over product and hence corresponds to the tight conformation.

Presence of the protein environment leads to a substantial reduction in barrier height at the TS from 56 kcal/mol (gas phase) to 28 kcal/mol (protein).

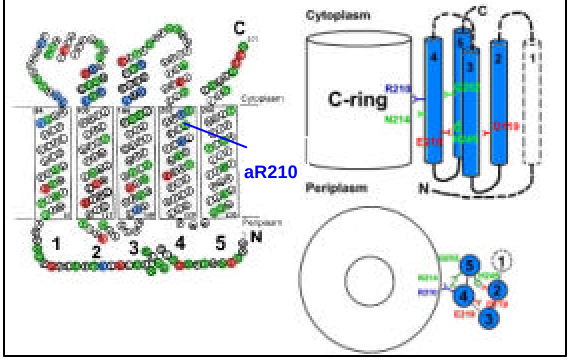
The final product conformation is separated by a "rugged" energy barrier from the final product conformation and only slightly lower in energy.

Cross-linking has been used to determine positions of a- and b-subunit relative to the c_{10}-ring

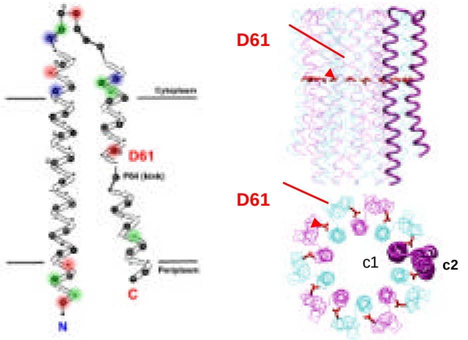


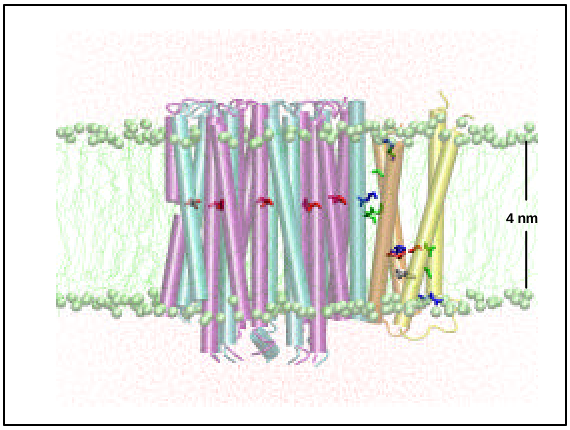
Jones et. al, *JBC* , **275**(2000)31340

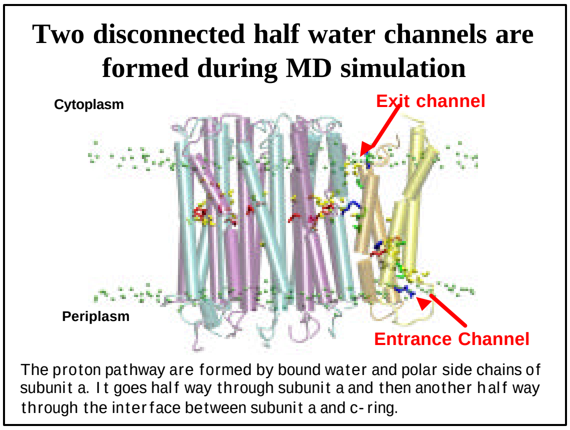
a-subunit contains a proton path and aR210 is essential for proton translocation



Ten c-subunits form c_{10} ring which is a rotor and D61 is essential for proton transfer

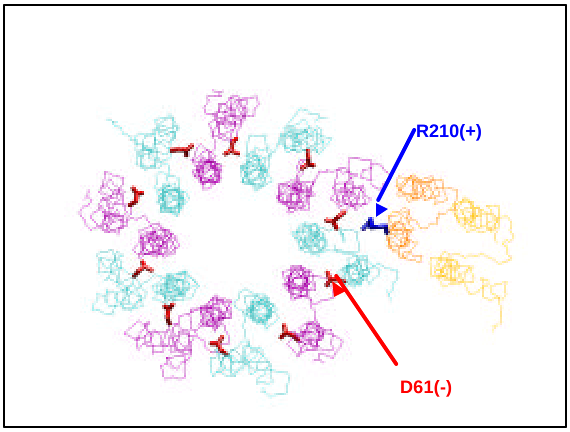


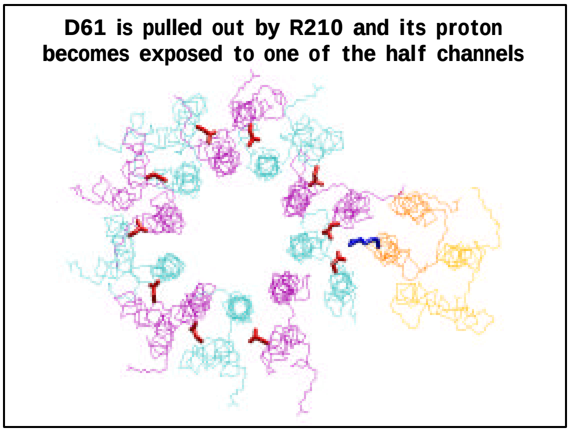




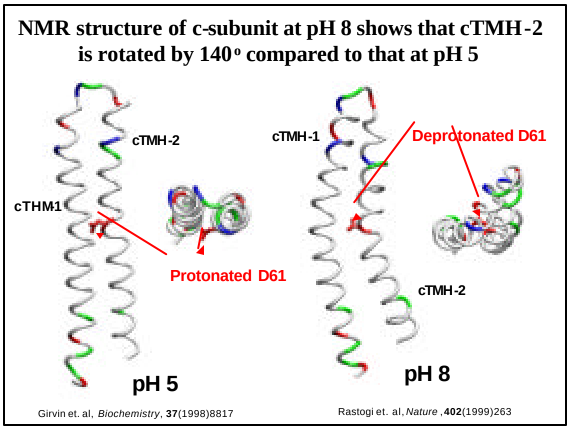
Two disconnected half water channels are formed during MD simulation

The proton pathway are formed by bound water and polar side chains of subunit a. It goes half way through subunit a and then another half way through the interface between subunit a and c-ring.





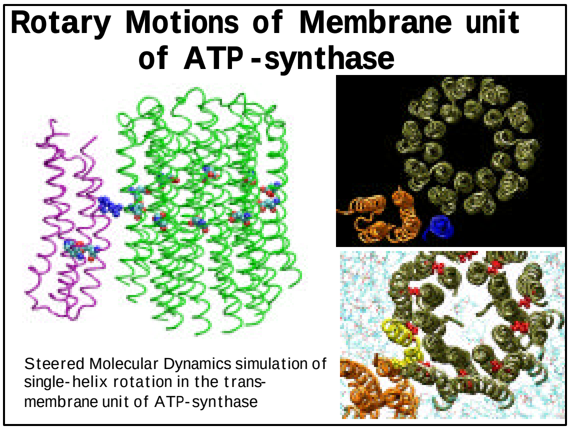
D61 is pulled out by R210 and its proton becomes exposed to one of the half channels



NMR structure of c-subunit at pH 8 shows that cTMH-2 is rotated by 140° compared to that at pH 5

Girvin et. al, *Biochemistry*, **37**(1998)8817

Rastogi et. al, *Nature* ,**402**(1999)263



Rotary Motions of Membrane unit of ATP-synthase

Steered Molecular Dynamics simulation of single-helix rotation in the trans-membrane unit of ATP-synthase