ATP Synthase F_1 : simulated rotation of central stalk

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All enzymes are beautiful,
but "**ATP synthase**" is one of the most:

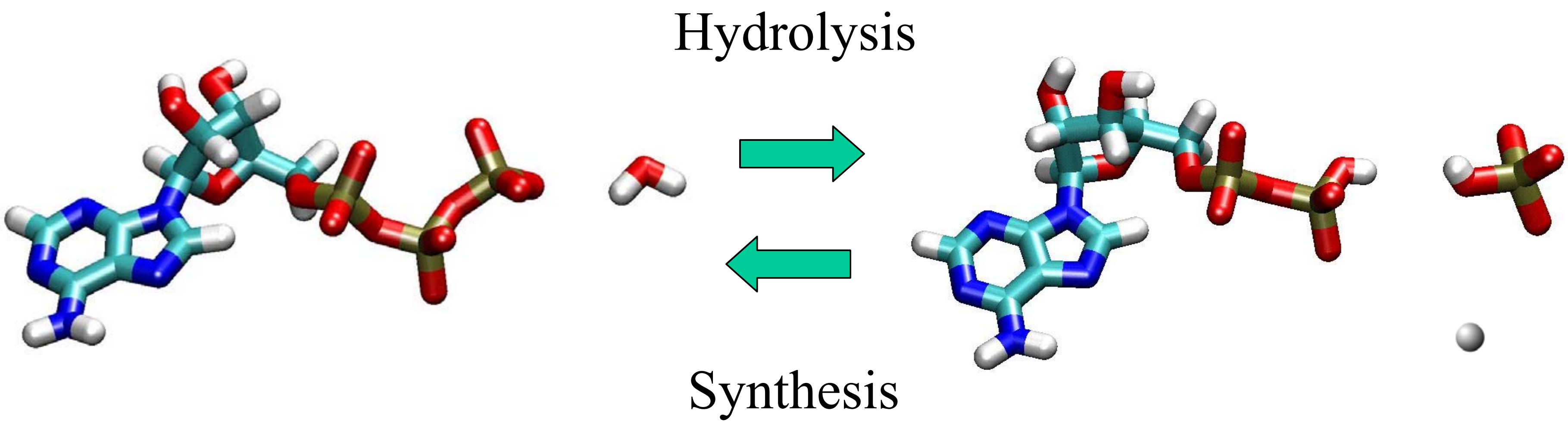
- **Beautiful** because of its "3D structure",

- **Unusual** because of its "structural complexity and reaction mechanism",

- **Important** because "everyday an active graduate student makes more than his/her body weight of ATP".

1. Introduction

1.1 ATP, universal energy carrier of living systems



$$\mathbf{ATP} \quad + \quad \mathbf{H_2O} \quad \rightleftharpoons \quad \mathbf{ADP + H^+ + PO_4^{2-} + H^+}$$

-12.2 k_BT = -30.6 kJ/mol = -7.3 kcal/mol

(ATP = Adenosine **tri**phosphate, ADP = Adenosine **di**phosphate)

Why do we consume so much ATP?

Many reactions of cell function and growth are thermodynamically unfavorable.

A thermodynamically *unfavorable* reaction can be driven by a *favorable* one.

Suppose a cell needs to perform the unfavorable conversion of A into B:

$A \leftrightarrow B, \quad \Delta G°' = +4$ kcal/mol

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

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,

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

Couple hydrolysis of one ATP molecule to every conversion of A into B…

ATP hydrolysis shifts the equilibria of coupled reactions

Coupled reaction:

$A \leftrightarrow B$ $\Delta G°' = +4$ kcal/mol

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

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

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

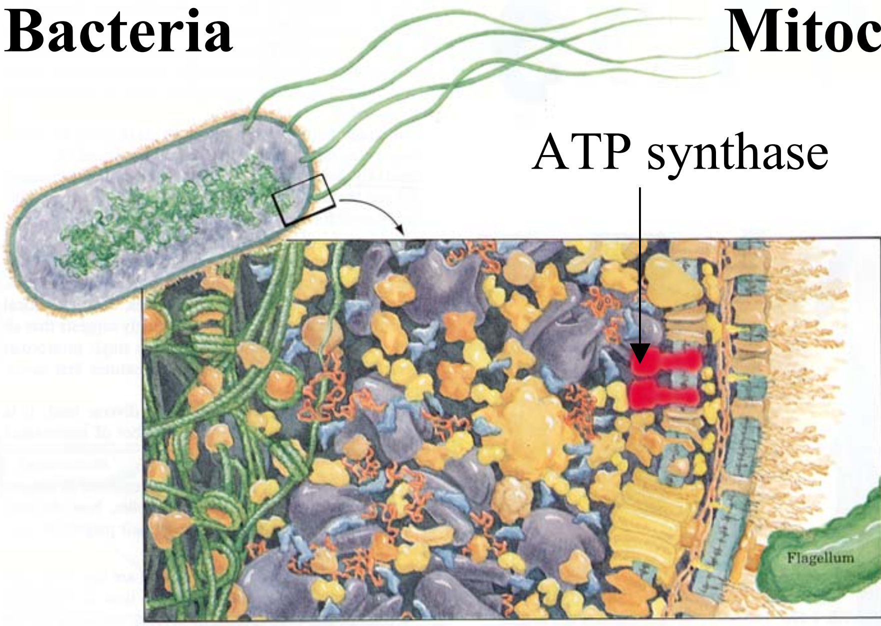
$$\frac{[B]_{eq}}{[A]_{eq}} = K'_{eq} \frac{[ATP]_{eq}}{[ADP]_{eq}[P_i]_{eq}}$$

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

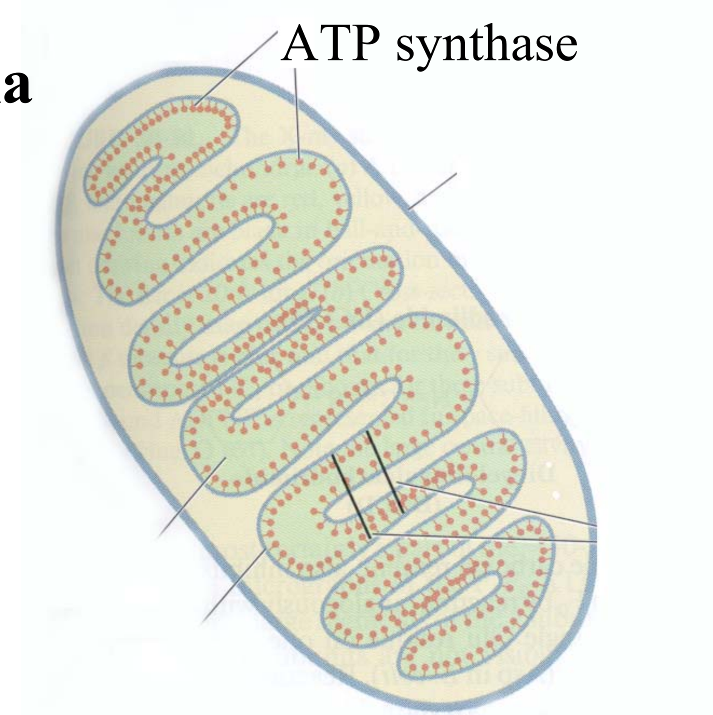
Without ATP hydrolysis, this was 1.15×10^{-3}, so ***$A \leftrightarrow B$ conversion has been increased by a factor of 10^8.***

ATP synthase is the ATP factory of cells

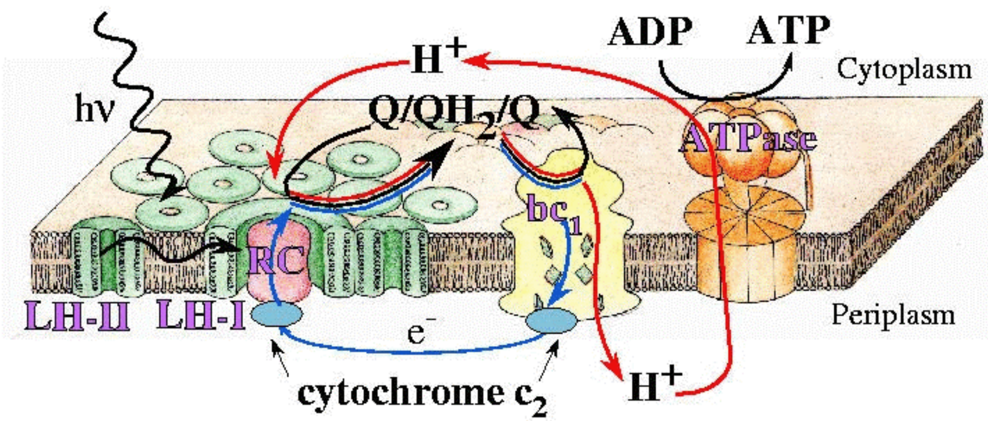
Bacteria



Mitochondria

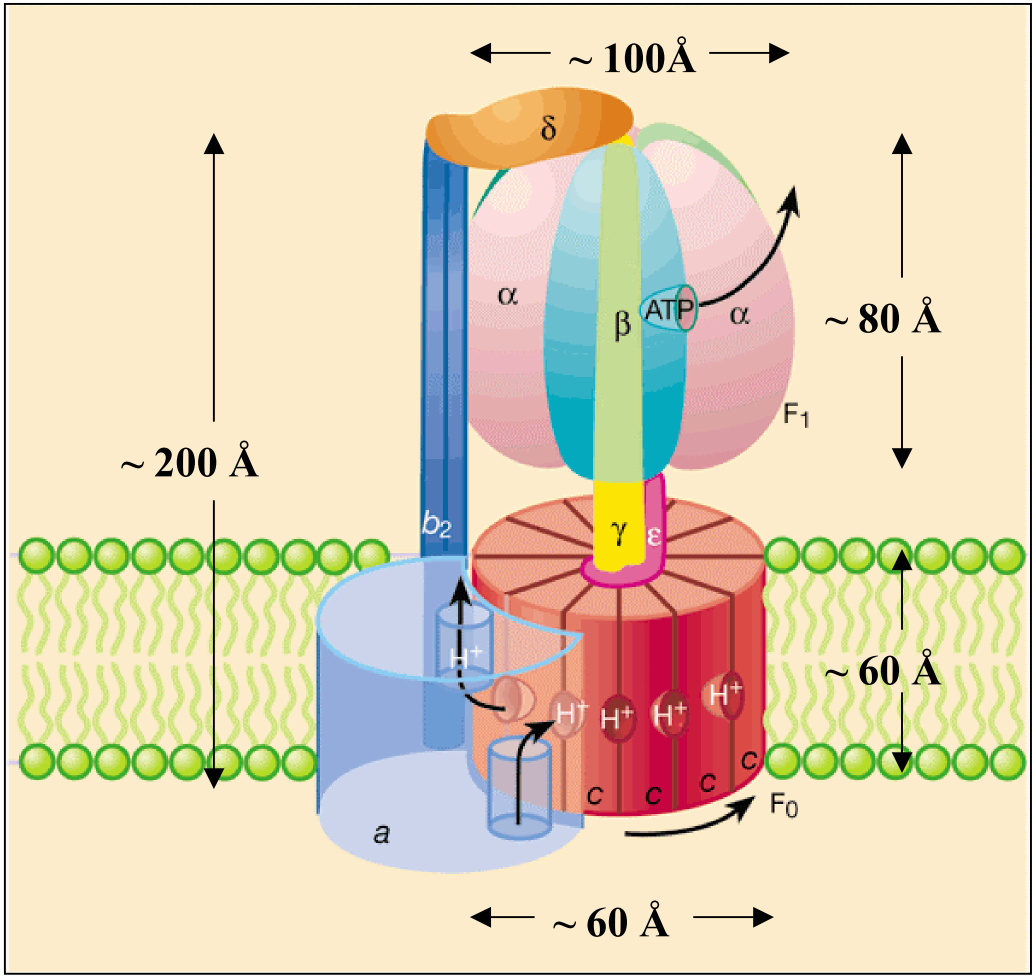


Chloroplast



Theoretical Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign

Structure of ATP synthase



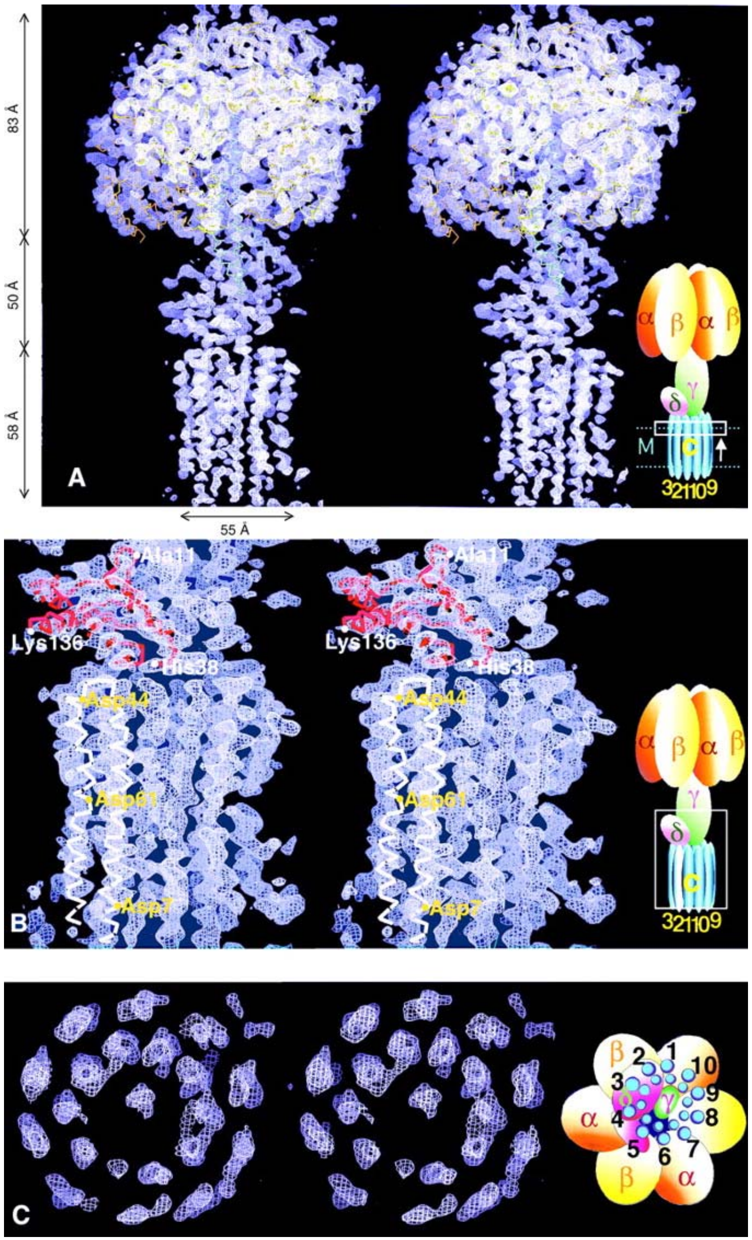
Soluble part, F_1-ATPase

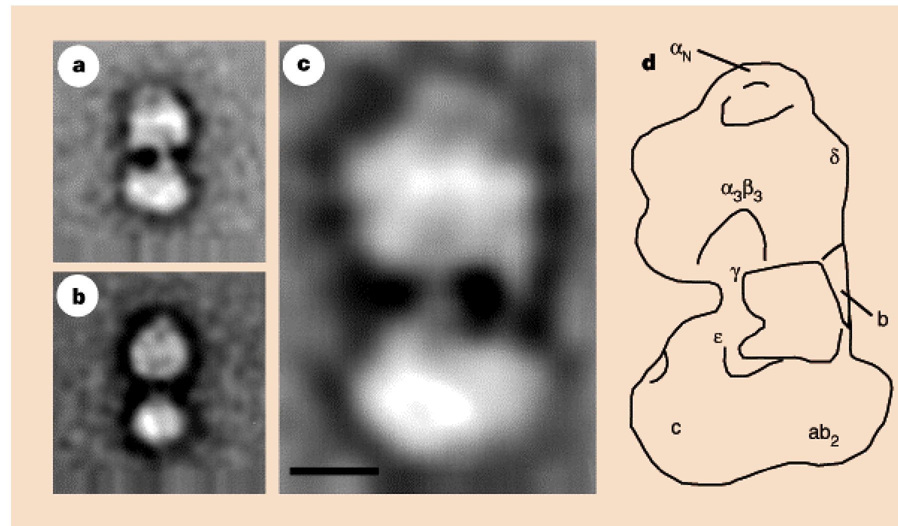
- consists of 5 subunits (3 α, 3 β, 1 γ, 1 δ and 1 ε)
- catalyzes/hydrolyses ATP

Membrane-bound part, F_0 Complex

- consists of 3 subunits (1 **a**, 2 **b** and 9-12 **c**)
- involved in proton translocation.

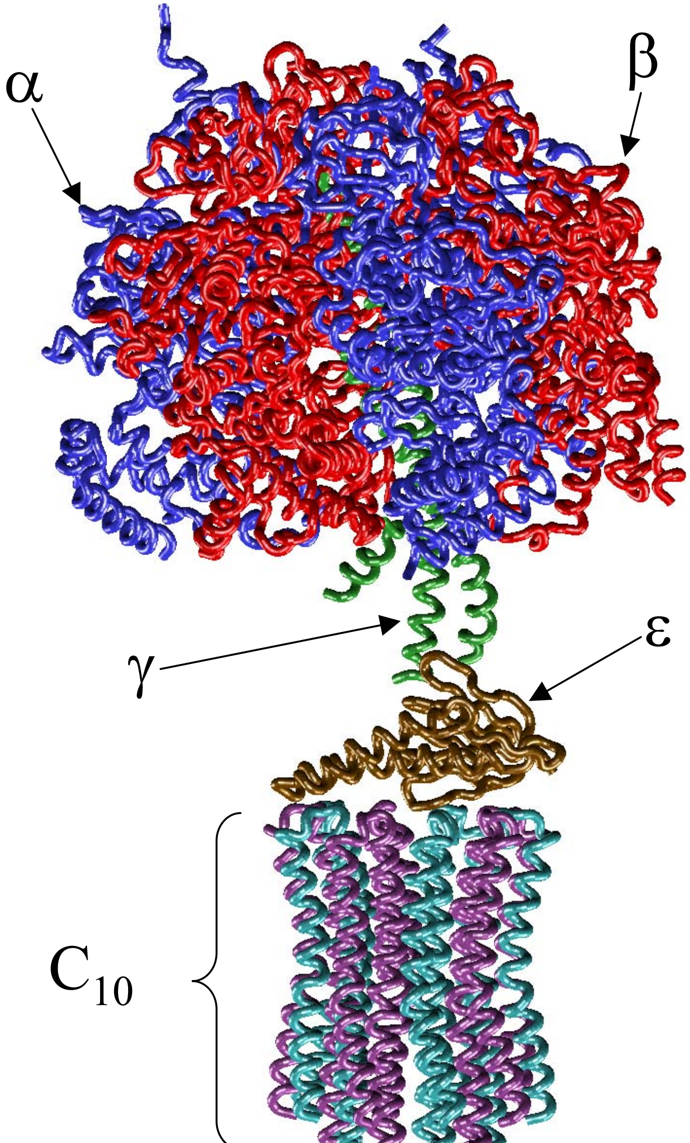
ATPase Structure Data

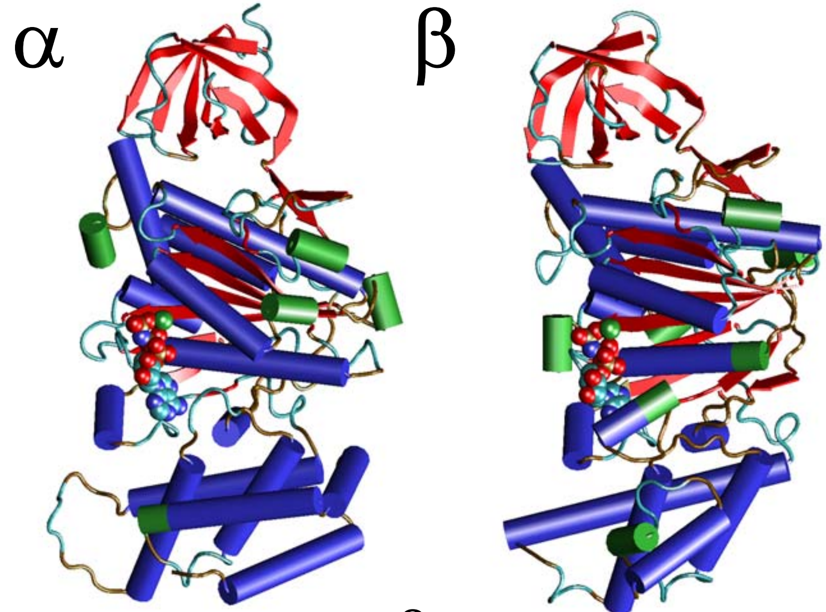




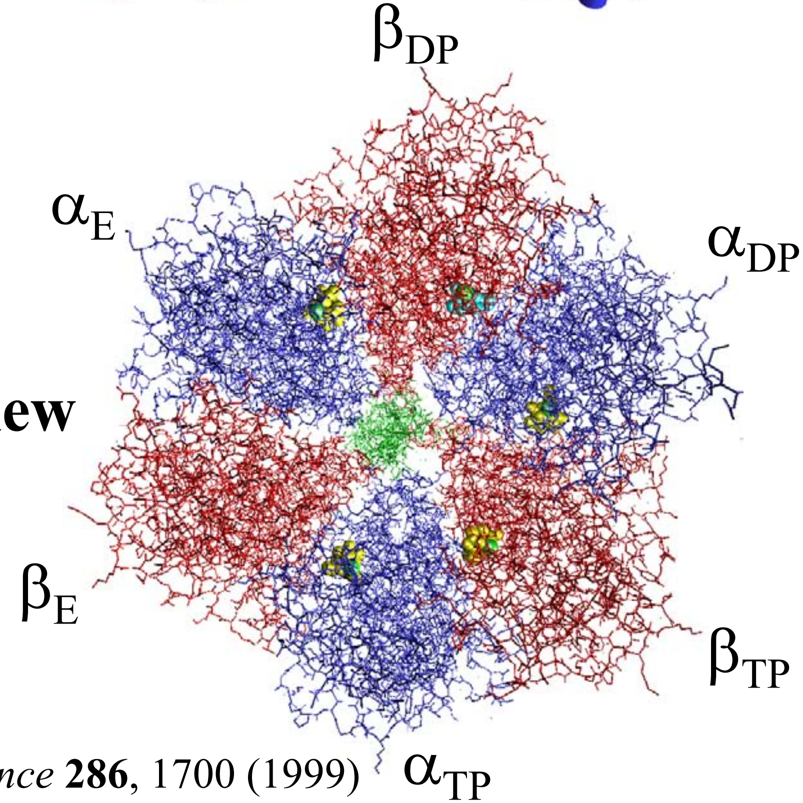
Stock et al., Science (1999)

2.1 ATP synthase X-ray structure (Walker'94)



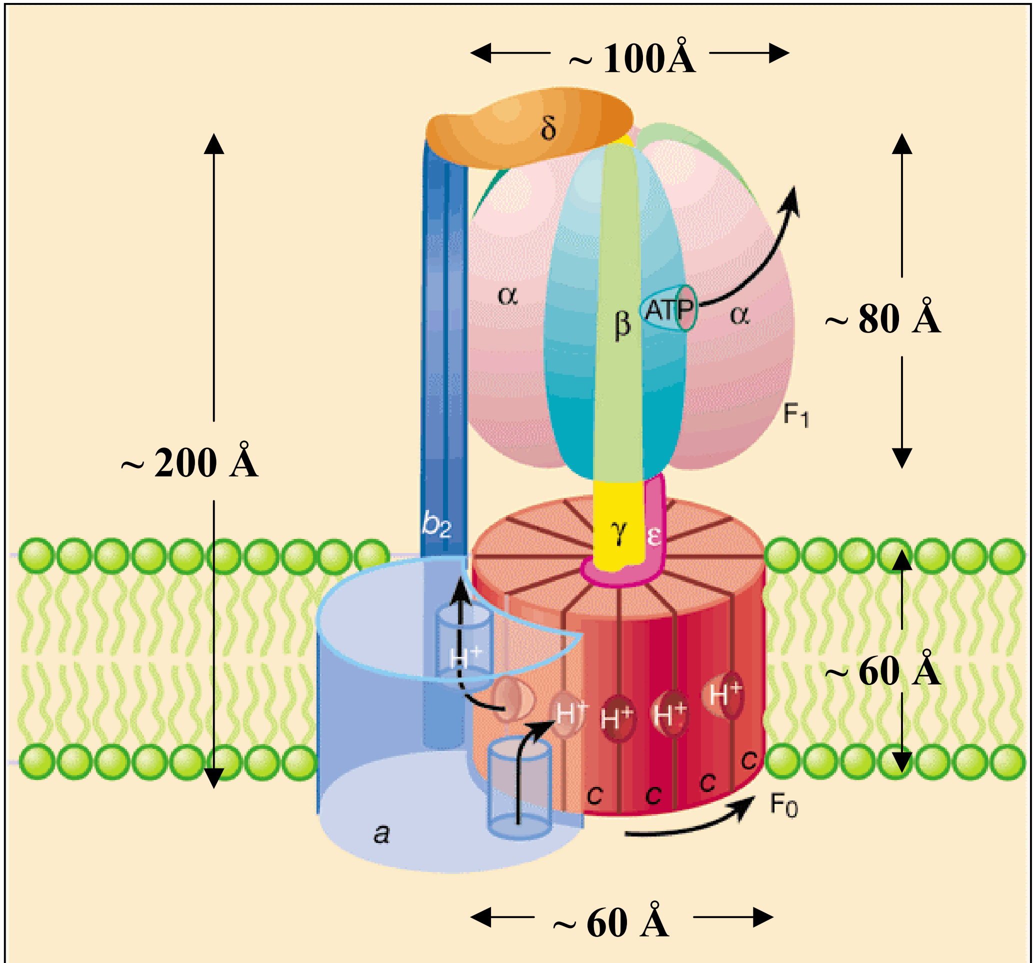


Top view



Abrahams et al., *Nature* **370**, 621 (1994); Stock et al., *Science* **286**, 1700 (1999)

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 *(not main function)*

Membrane-bound part, F_0 Complex

- Produces torque when positive proton gradient across membrane*(main function of this unit)*

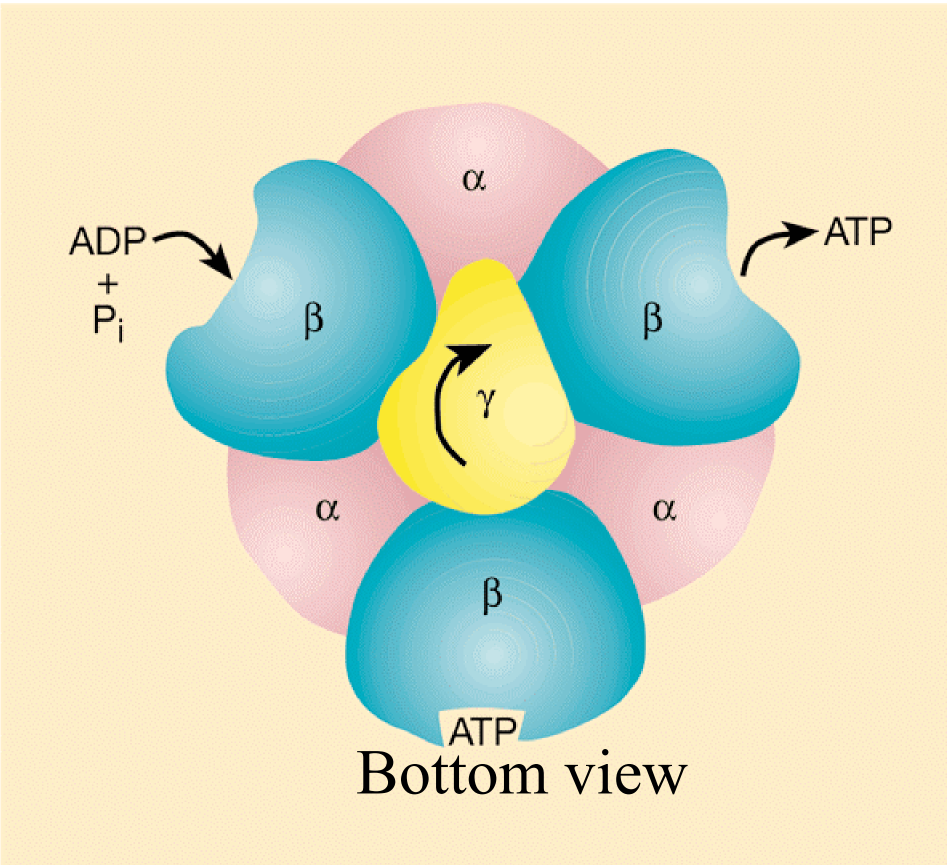
- Pumps protons when torque is applied *(not main function)*

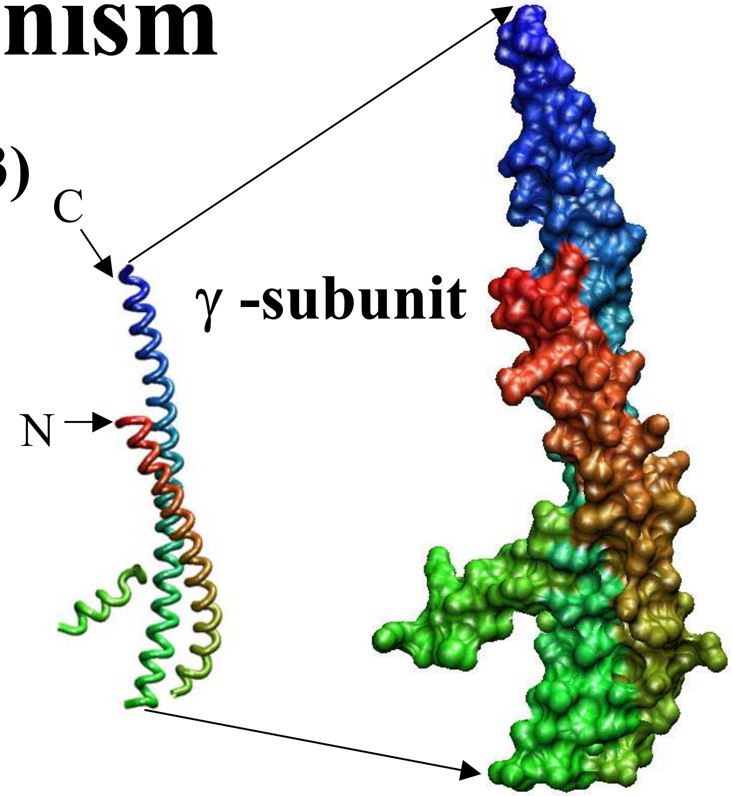
Torque is transmitted between the motors via the central stalk.

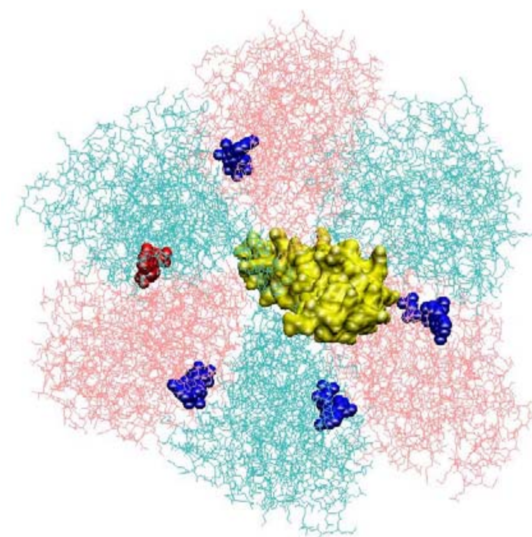
ATP synthesis mechanism

3.1 Binding Change Mechanism (Boyer'93)

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

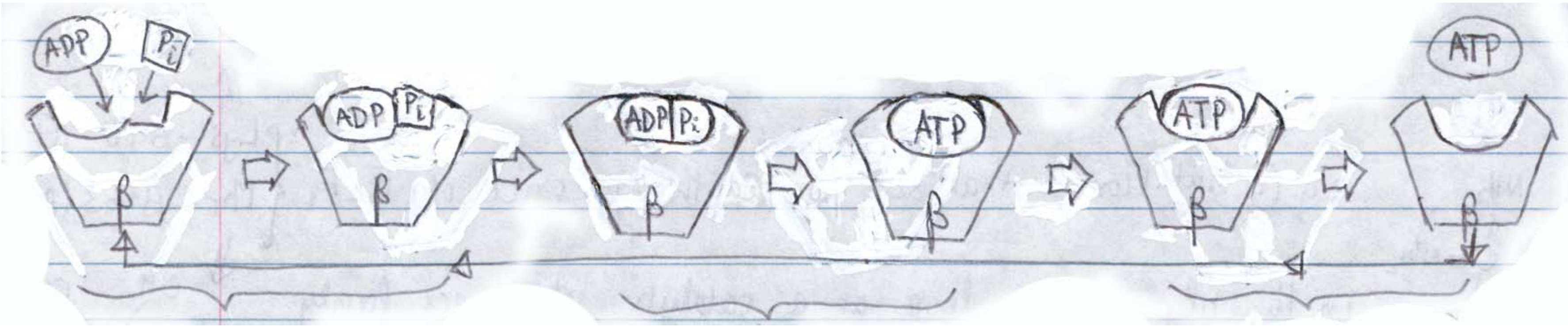






Boyer, P. D. *Biochim. biophys. Acta* **1140**, 215 (1993)

Synthesis: Binding Change Mechanism

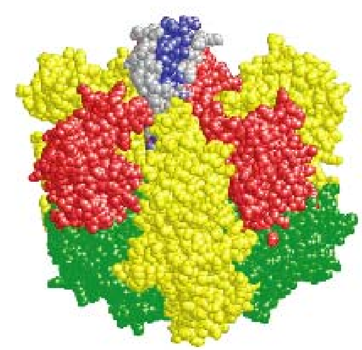


β_{DP} **(Loose)**
- Loosely binding ligands.
- Catalytically "inactive".

β_{TP} **(Tight)**
- Tightly binding ligands.
- Catalytically "active".

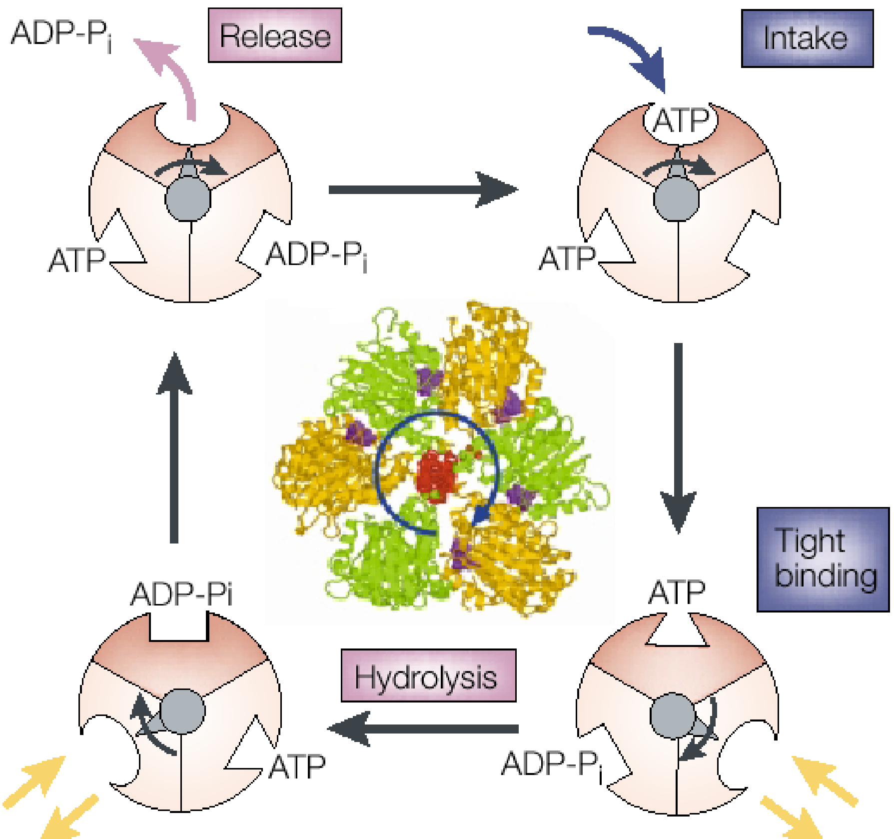
β_E **(Open)**
- Low affinity for ligands
- Catalytically "inactive"

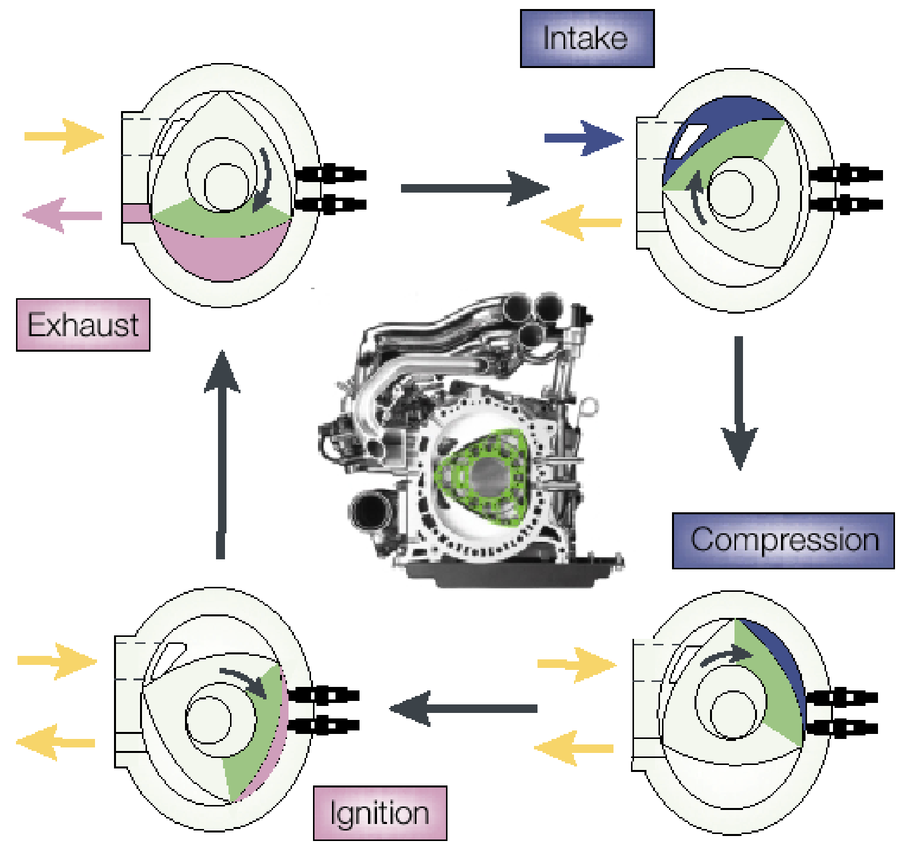
Energy of proton gradient is used to
- **BIND** ADP and Phosphate
- **RELEASE** tightly bound ATP



G. Oster and H Wang (1998)

Hydrolysis may be synthesis changes in reverse



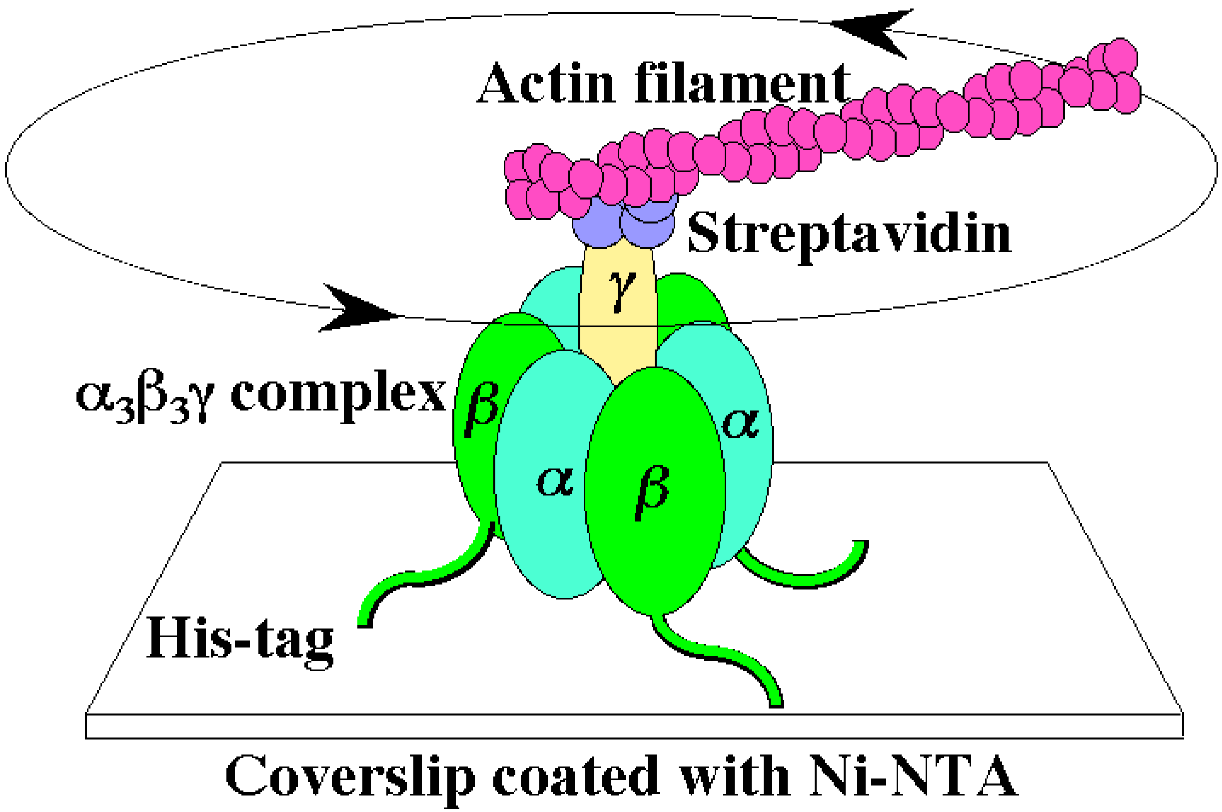


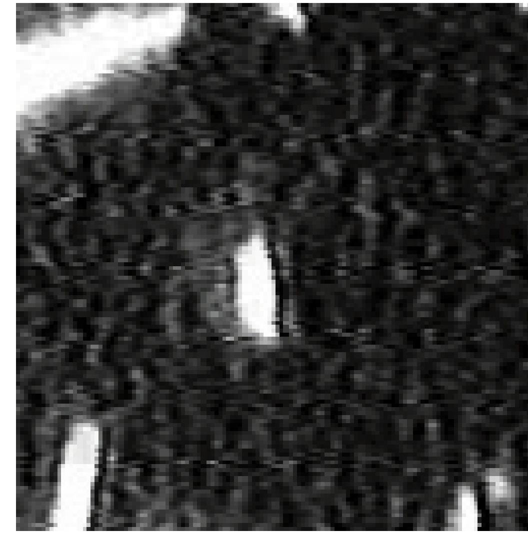
ATP hydrolysis can be thought of like a rotary engine.

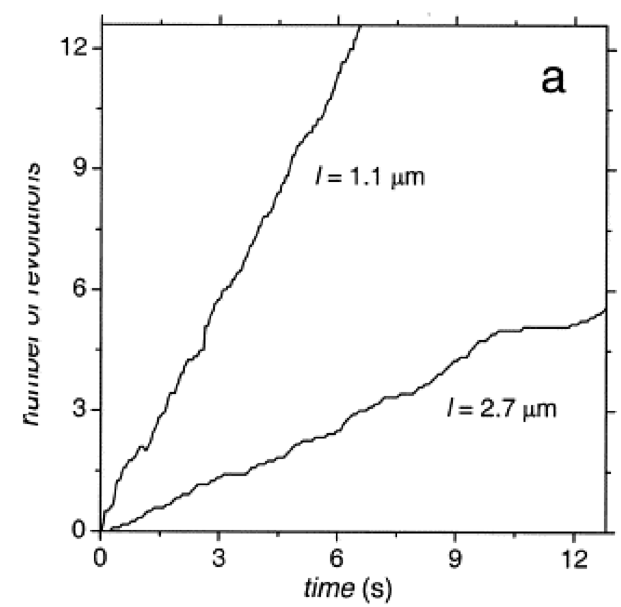
Boyer, P. D. *Biochim. biophys. Acta* **1140**, 215 (1993)

Direct Observation of ATPase Rotary motion

Yoshida'97

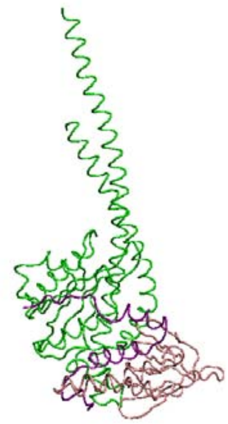






Noji et al., *Nature* **386**, 299 (1997); Pänke et al. *FEBS Lett.* **472**, 34 (2000)

Assembling ATP Synthase F_1



• Start with DCCD-inhibited structure, has near-complete stalk. (Gibbons 2000, PDB code 1E79)
•Total 327,000 atoms (3325 residues, 92,000 water molecules, nucleotides, and ions).
• The 1.2 ns equilibration + 10.5 ns torque application were performed on NCSA Platinum and PSC Lemieux as parallel NAMD jobs using up to 512 processors.

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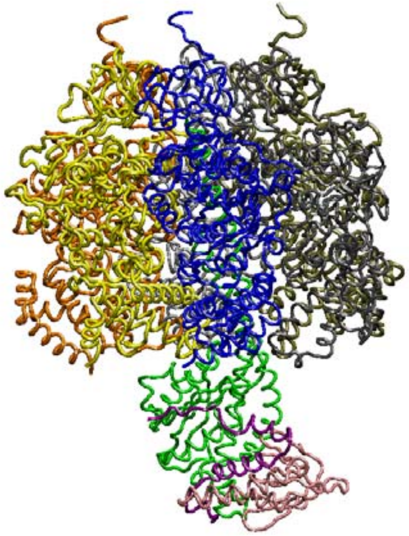

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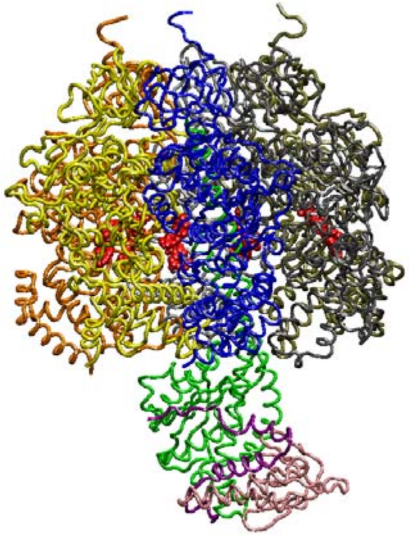

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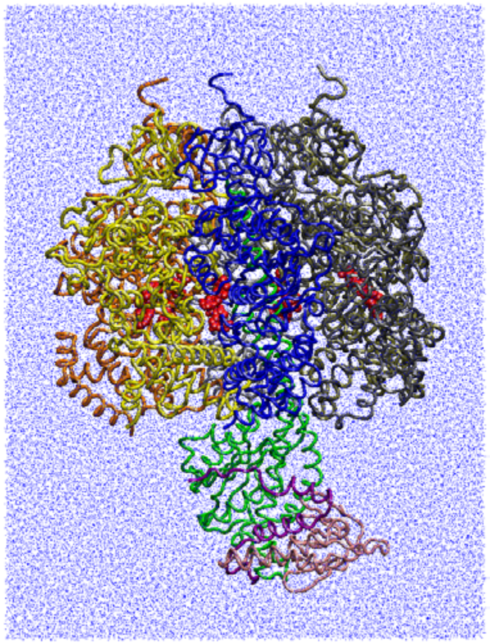

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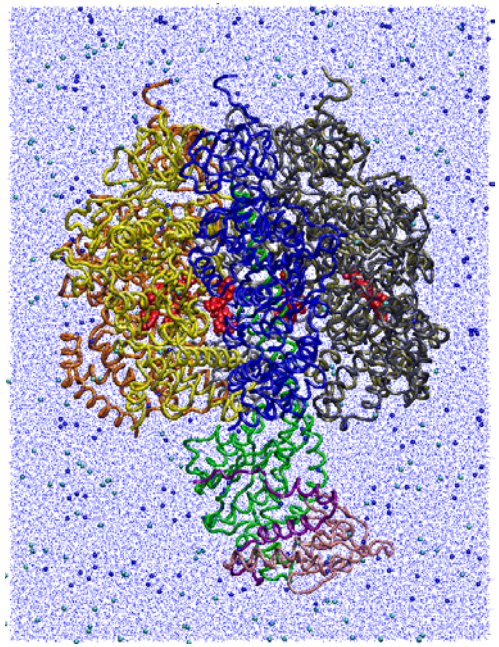

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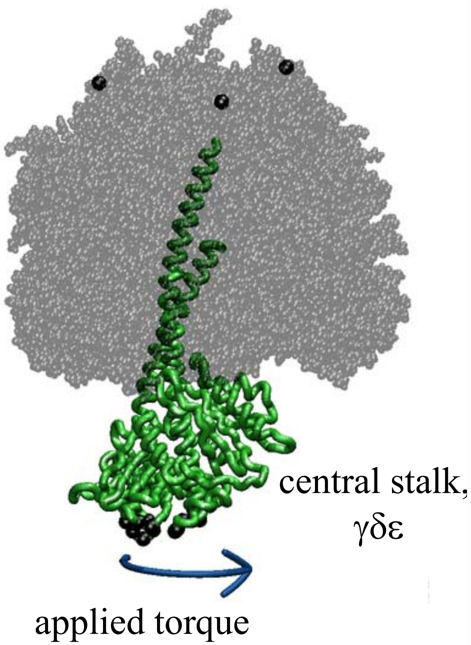
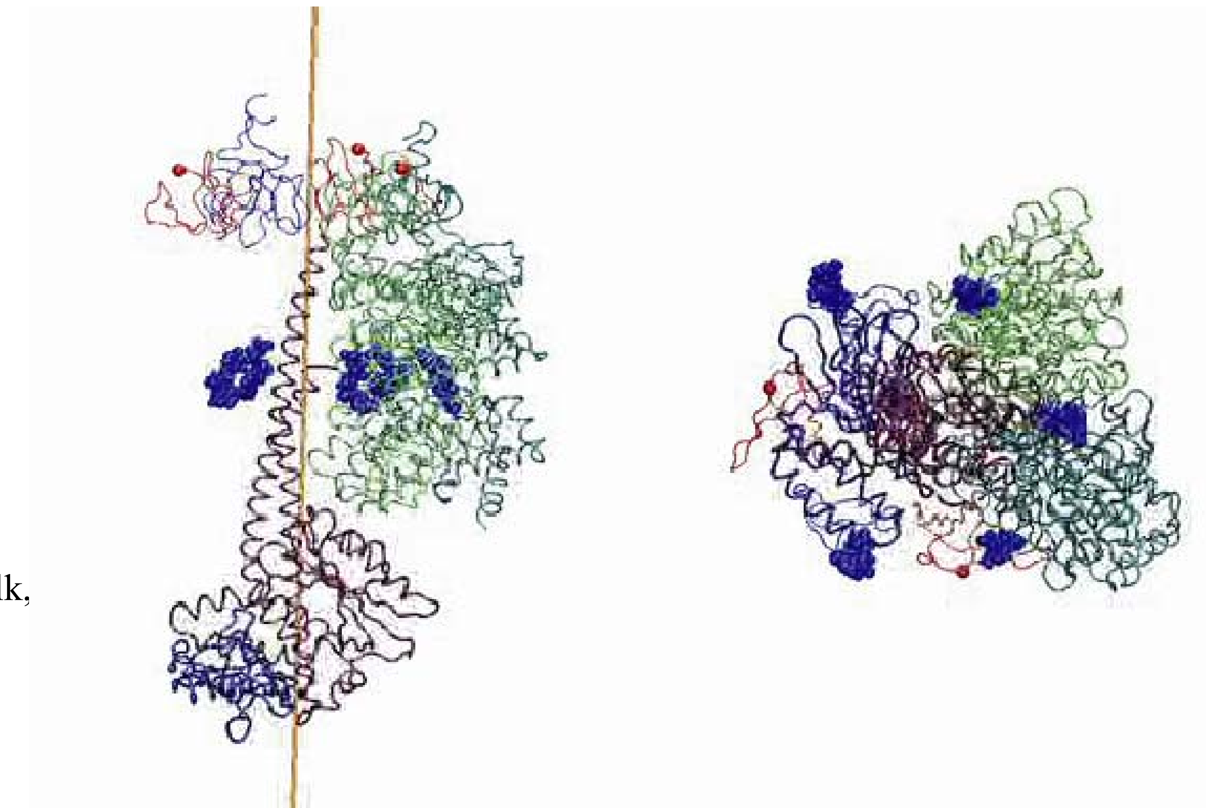
Torque application to F_1

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.

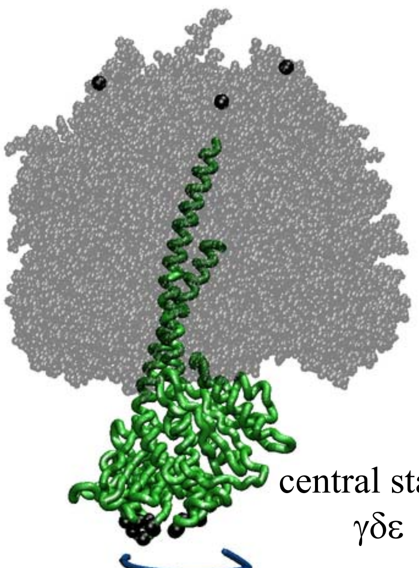


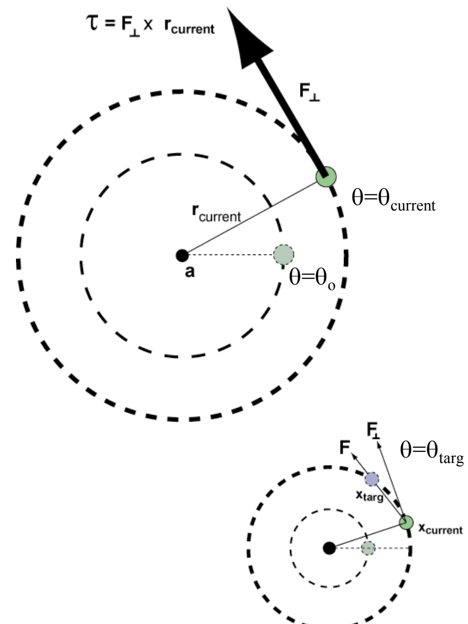


0.0 to 5.0 ns (0 to 120 deg) of torqued F_1 rotation, ω = 24 deg/ns.

Torque application method

Only angular velocity is restrained, no restraints placed on radial or longitudinal motion.





Applied force=

$\mathbf{F}_{\perp}$=

$\mathbf{F} \bullet (\hat{\mathbf{a}} \times \hat{\mathbf{r}}_{current})$=

$(k\,(\mathbf{x}_{targ} - \mathbf{x}_{curr})) \bullet (\hat{\mathbf{a}} \times \hat{\mathbf{r}}_{current})$

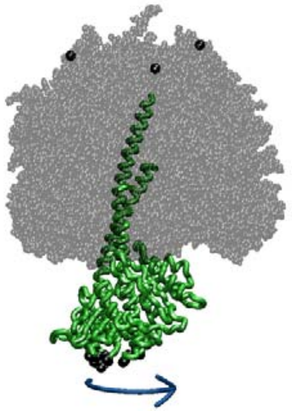
$\theta_{targ} = \theta_o + \omega t$

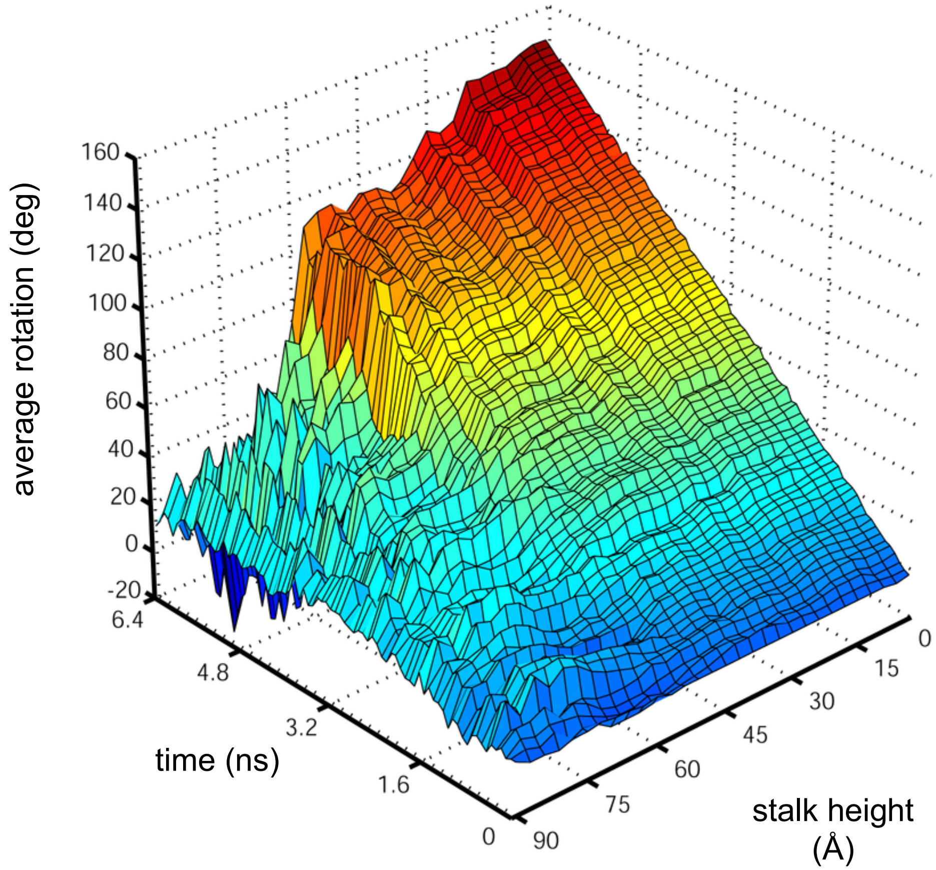
Stalk analysis

Using best RMSD rotation fit for stalk sections binned along axis direc

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

- slowed torque transmission along central stalk

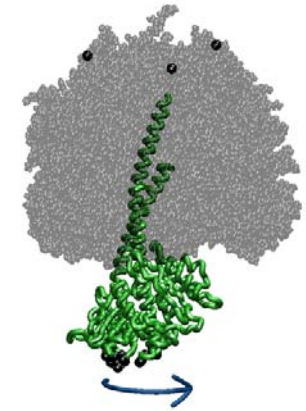


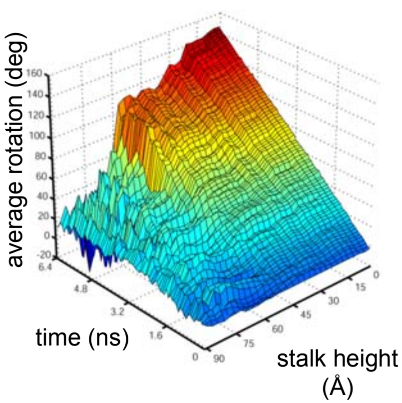


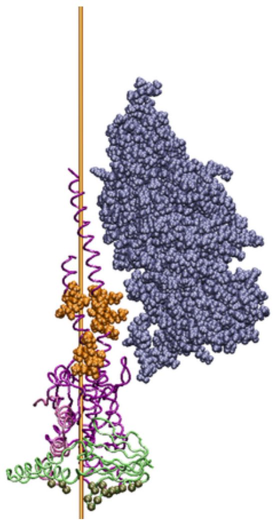
Rotation Produces Synthesis-like Events (1)

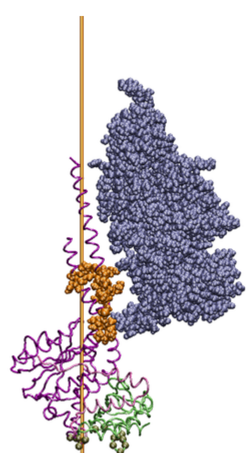
Arounf 3.0 ns (72 deg) of rotation, we observe:

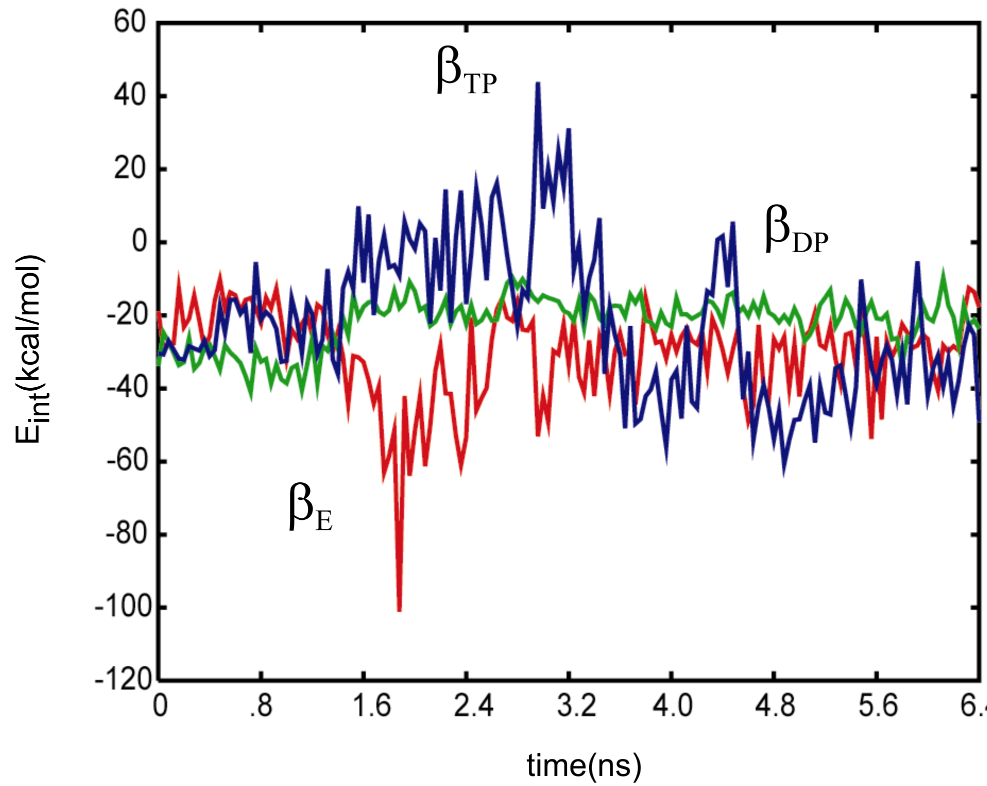
- slowed torque transmission along central stalk
- cooperative interactions at stalk - β subunit interfaces







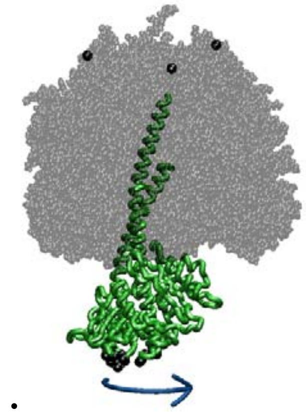




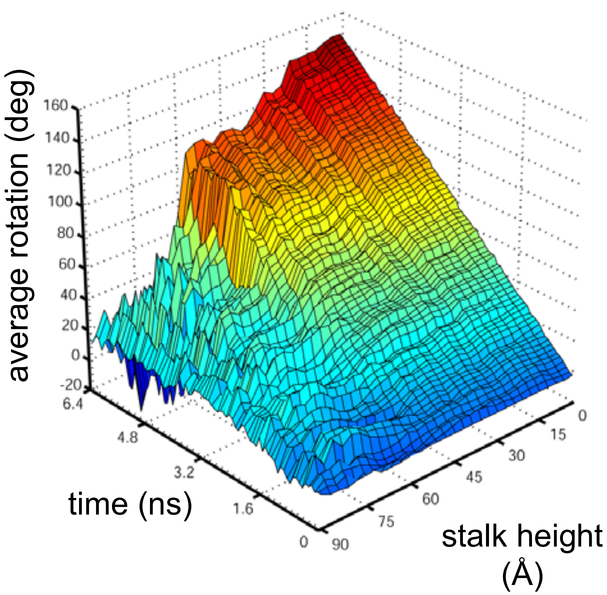
Rotation Produces Synthesis-like events (2)

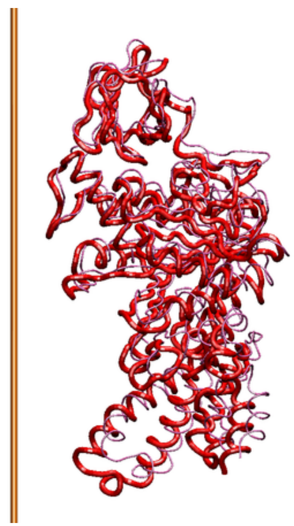
Around 3.0 – 3.5 ns (72 – 84 deg) of rotation, we observe:

- slowed torque transmission along central stalk
- opening and closing motions as expected

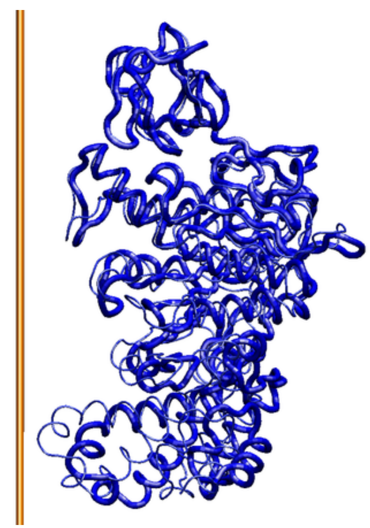


At 3.5 ns (84° rotation)…





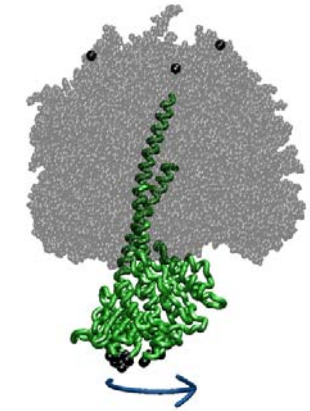
β_E closes



β_{TP} opens

β_{DP} does neither

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