ATPase Synthase - A Molecular Double Motor
Photosynthetic Unit of Purple Bacteria
Module that converts sun light into chemical energy (ATP)

Light in
hν

H+

Q/QH₂/Q

bc₁

ATPase

ADP

ATP out

Light in

Light in

RC

cytochrome c₂

e⁻

H⁺

H⁺

module that converts sun light into chemical energy (ATP)

Photosynthetic Unit of Purple Bacteria
Module that converts sun light into chemical energy (ATP)
Mechanism of the bc1 Complex in the Photosynthetic Unit

two pathways for oxidation of $Q_0$ site

Iron Sulfur Protein (ISP) head rotation can redirect 2nd electron
Enforcing domain rotation in the bc$_1$ complex
Events during torque application to ISP head


Torque applied to 126 C$_{\square}$ atoms
$K = 70$ pN/Å
$\square = 0.0561$ rad/s

206,720 atoms

average rotation angle (deg)
torque (pNÅ)
time (ps)
Mechanisms of Rotatory Molecular Motor that Converts Voltage (proton gradient) into ATP Synthesis
Animation of the ATP Synthase
Adenosine Triphosphate (ATP) Synthase

**Rotary catalysis:** Two protein motors coupled via common central stalk

Solvent exposed $F_1$ unit ($\alpha_3\beta_3\gamma\delta\epsilon$): central stalk rotation causes conformational changes in catalytic sites, driving ATP synthesis

Transmembrane $F_0$ unit ($ab_2c_{10}$): converts proton motive force into mechanical rotation of central stalk
Reaction Mechanism of ATP Hydrolysis

[Diagram showing the reaction mechanism with labels for reactant, transition state, and product, along with energy diagrams for different steps.]
Mechanism of ATP Hydrolysis in F1 ATPase
Reaction Mechanism of ATP Hydrolysis

**Reaction Site**

- **Reactant**
  - ATPα
  - αLYS162
  - βGLU188

- **Transition State**
  - WAT1
  - WAT3
  - WAT5

- **Product**
  - ATP α
  - αLYS162
  - βGLU188

**Energies**

- Relative energy (kcal/mol)

- **Reactant**
  - T5
  - IM
  - B1
  - IM2
  - B2

- **Product**
  - T5
  - IM
  - B1
  - IM2
  - B2

**Alpha, Beta, Gamma**
Reaction Mechanism of ATP Hydrolysis

Role of protein side groups

Interaction with reactant  Control of TS barrier

Reaction energies

Product
One shaft, two motors: Let’s look at F1

Soluble part, F₁-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, F₀ 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.
To observe rotation, the three beta subunits were fixed on a glass surface through histidine tags engineered at the N terminus. To the putative rotor subunit gamma, a micrometer-sized actin filament was attached through streptavidin. When ATP was added, the actin filament rotated continuously clockwise (movie). Note that, in this movie, the rotation occurs around the middle of the filament. If you hold an end of a long rod, you could make a fake rotation by twisting your wrist. If you hold the middle, however, you have to rotate yourself to keep the rod rotating. Thus, the propeller rotation in this movie shows that the __ subunit really slides against the surrounding alpha₃beta₃ subunits over finite angles.

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At low ATP concentrations, $F_1$ rotates in discrete 120° steps. The stepping rate is proportional to the ATP concentration, indicating that each step is driven by one and only one ATP molecule. In the movie at 20 nM ATP, there is an instant where the $F_1$ motor makes a mistake and steps backward (clockwise). A molecular machine occasionally makes mistakes, and its operation is always stochastic as seen in the figure at left. Because of the stochastic nature, one can never synchronize multiple molecular machines. To analyze their mechanism, therefore, one needs to observe individual molecules closely.

At speeds below the maximal, we were able to resolve substeps with an amplitude of 90° and 30° in the 120° step powered by the hydrolysis of one ATP molecule (see figure at left). If you have very good eyes, you may be able to detect some of the substeps in the actual images on the right. The 90° substep turned out to be driven by binding of ATP to a catalytic site on F$_1$, and the 30° substep by the release of a hydrolysis product(s). The hydrolysis reaction per se appeared to be mechanically almost silent.

Let’s look at F1

Torque is transmitted between the motors via the central stalk.
A rough idea of central stalk’s tasks:

TP -> E -> DP -> TP (1994 Walker, 1BMF)

Interpolation of observed states

(phosphate / orthophosphate is fixed at TP position)
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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Torque application to $F_1$

Torque is applied to the central stalk atoms at the $F_1$-$F_0$ interface to constrain their rotation to constant angular velocity $\omega = 24$ deg/ns.

0.0 to 5.0 ns (0 to 120 deg) of torqued $F_1$ rotation, $\omega = 24$ deg/ns.
Stalk analysis

Using best RMSD rotation fit for stalk sections binned along axis direction, at 3.0 ns (72 deg) of rotation, we observe:

- slowed torque transmission along central stalk
Winding of coiled-coil

t = 3.0 ns
θ = 72°

Different coupling for the two helices:
1—50, partially via subunit
197—272, directly to $F_o$
Rotation Produces Synthesis-like Events (1)

Around 3 ns (72 deg) of rotation, we observe:

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

![Graph showing average rotation, stalk height, and time](image)

![Graph showing energy change over time](image)
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)…

△\text{E} closes

△\text{TP} opens

△\text{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 $\square_{TP}$ catalytic site

0 ns: active site closed

3 ns: active site open

ATP separates from active site residues
Fo ATP synthase

Transmembrane $F_0$ unit ($ab_2c_{10}$): converts proton motive force into mechanical rotation of central stalk

Asp 61 (D61) side groups take protons
Suggested Mechanism of Proton Translocation

(R.H. Fillingame, 2002)
Key Amino Acids Participating in Electro-Mechanical Motor

- Serine (Ser = S)
- Arginine (Arg = R)
- Asparagine (Asn = N)
- Aspartate (Asp = D)

H+ from S206, R210, N214, D61
Structural Model of \textit{E. coli} F_o

\[ a_1c_{12} \text{ (Rastogi & Girvin, 1999, NMR)} + c_{10} \text{ (Fillingame et al, 1999, NMR)} = a_1c_{10} \text{ (2001-2002, modeling)} \]
Forced Rotation of the c10 Subunit

Forces were applied to all backbone atoms of c\textsubscript{10}.

Estimated friction coefficient $\approx 10^5$ kcal/(M sec)
Salt Bridge Arg$_{210}$-Asp$_{61}$ is Formed

With only one Asp$_{61}$ residue deprotonated, SMD rotation of $c_{10}$ breaks the structure apart.

No restraints     Subunit $a$ is restrained
Single Helix Rotation

To minimize steric hindrance (critical on nanosecond time scale), helix was forced to rotate in a reptation tube (local pivot points and directors).
Salt Bridge Can Be Transferred

The salt bridge can be transferred by the concerted rotation of the $c_{10}$ complex and the outer TMH of subunit $c$. 
6 degrees of freedom: $\theta_0, \theta_1, \theta_2, \theta_3, \theta_4$ are TMH rotation angles; $q_A$ - position of the $a$ subunit

Each Asp61 can be in either of two chemical states (protonated or deprotonated).

$$ \frac{d\square_i}{dt} = \square \frac{d}{d\square_i} \left[ U_{\text{group}} + U_{\text{hydroph.}} + U_{\text{internal}} \right] + \square_i(t) $$
Stochastic Simulations of $F_o$ Operation

Time evolution of rotation angles $\theta_1$ (black), $\theta_2$ (red), $\theta_4$ (green), and $\theta_A$ (blue). Motor rotation speed is close to physiological.

The c10 complex rotates in steps

Load torque 41pN nm