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

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

ATP hydrolysis shifts the equilibria of coupled reactions.

ATP, universal energy carrier of living systems.

Why do we consume so much ATP?
Many reactions in biological cells are thermodynamically unfavorable (ΔG > 0).

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

\[
A \leftrightarrow B \quad \Delta G^\circ = +4 \text{ kcal/mol}
\]

\[
K_{eq} = \frac{[B]}{[A]} = 10^\frac{\Delta G^\circ}{RT} = 10^\frac{+4}{1.36} = 115 \times 10^{-3}
\]

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.

\[
\Delta G = \Delta G^\circ + \ln \left(\frac{[ADP][P_i]}{[ATP]}\right) = -13.7 \text{ kcal/mol}
\]

\[
\Delta G = -12.2 \text{ kcal/mol} - 30.6 \text{ kcal/mol} = -7.3 \text{ kcal/mol}
\]

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

ATP synthase

Soluble F₁ ATPase

Membrane bound F₀

ATP, universal energy carrier of living systems.

Hydrolysis

Synthesis

ATP + H₂O \rightarrow ADP + Pᵢ

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\]

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\[
[\text{B}]_{eq} = \frac{[\text{ADP}][P_i]}{[\text{ATP}]} = 10^{\frac{-13.7}{1.36}} = 10^{-10}
\]

Without ATP hydrolysis, this was 1.15 \times 10^{-3}, so \(A \leftrightarrow B\) conversion has been increased by a factor of 10^8.
ATP synthase Structural Data

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

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One shaft, two motors

Direct observation of ATP synthase rotary motion

ATP synthesis mechanism

Torque is transmitted between the motors via the central stalk.

"ATP synthesis" requires both F0 and F1 parts.

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

We have six nucleotide binding sites, 3 catalytic and 3 non-catalytic.
α and β subunits are similar in secondary structures

β-barrel domain

αβ domain

α domain

AMP-PNP

Mg²⁺

AMP-PNP

Mg²⁺

20% sequence identity.

- When isolated, hydrolyse ATP very slowly.

Catalytic Nucleotide Binding Site

Water molecule is H-bonded by β-Glu188.

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

Mg²⁺

α⁺TP β⁻TP interface

α⁻Arg 373 helps stabilize the negative charge

Non-Catalytic Nucleotide Binding Site

βα⁻TP interface

β⁻Arg372 and β⁻Tyr368 side chains which help forming the binding pocket move out of the pocket.

Mg²⁺

Water molecule

A rough idea of central stalk's tasks

TP → E → DP → TP

Interpolation of observed states

Assembling ATP Synthase F₁

Torque application to F₁

Torque is applied to the central stalk atoms at the F₁-F₀ interface to constrain their rotation to constant angular velocity \( \omega = 24 \text{ deg/ns} \).
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 βTP catalytic site

Motion of αTPArg373 towards βTPphosphate binding pocket

QM/MM calculation of ATP hydrolysis

Initial configuration

Transition state

Intermediate structure
Energetics of ATP hydrolysis in $\beta_{TP}$

The overall ATP hydrolysis reaction is found to be endothermic. This suggests that $\beta_{TP}$ 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 reactant 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

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

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

Rotary Motions of Membrane unit of ATP-synthase.

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