



IIT Guwahati

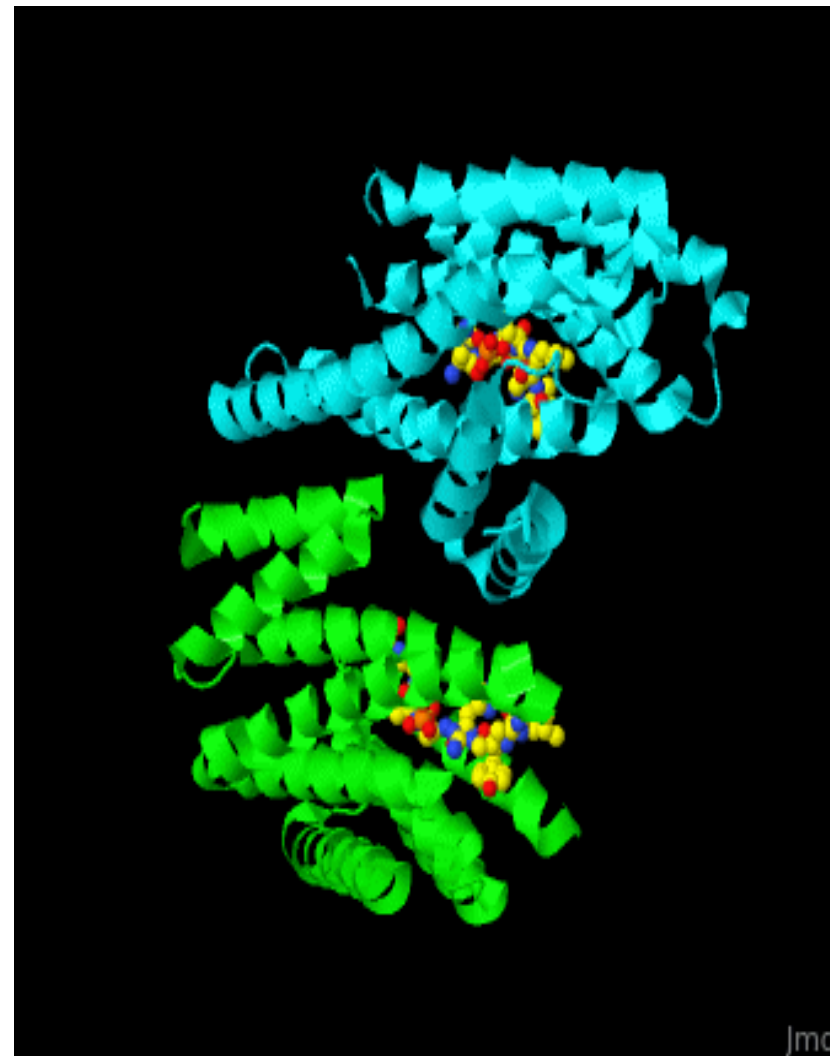
Lecture 38

Course BT 631

Protein Structure, Function and Crystallography

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Membrane Proteins

- The second large class of proteins distinct from globular proteins are the membrane proteins.
- Members of this group of proteins probably make up the vast majority of all proteins found in cells.
- For many years the purification of these proteins remained very difficult and limited our knowledge of their structure and function.

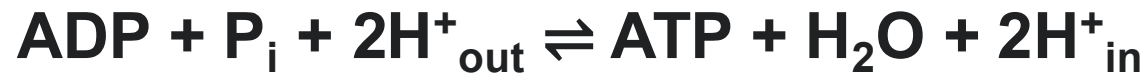
Membrane Protein (ATP synthetase)

- Even most inactive of humans metabolizes **kilogram quantities of ATP** in a normal day and this occurs by the repeated phosphorylation with an average of each molecule of **ADP/ATP being 'turned over' about 1000 times per day**.
- The enzyme responsible for the synthesis of ATP is the F_1F_0 ATPase, also called ATP synthase or ATP synthetase.
- Dissection of enzyme complex and genetic studies show that ATP synthase contains 8 different subunits in *E. coli*, whilst a greater number of subunits in mammalian enzymes.
- Despite variations in subunit number similarity exists in their ratios and primary sequences with most enzymes having a combined molecular mass between 550 and 650 kDa.

The Reaction of ATP synthetase

ATP synthase catalyzes the formation of adenosine triphosphate (ATP) using adenosine diphosphate (ADP) and inorganic phosphate (P_i).

It is classified under ligases as it changes ADP by the formation of P-O bond (phosphodiester bond). The overall reaction catalyzed by ATP synthase is:



The formation of ATP from ADP and P_i is energetically unfavorable and would normally proceed in the reverse direction.

In order to drive this reaction forward, ATP synthase couples ATP synthesis during cellular respiration to an electrochemical gradient created by the difference in proton (H^+) concentration across the inner mitochondrial membrane in eukaryotes or the plasma membrane in bacteria.

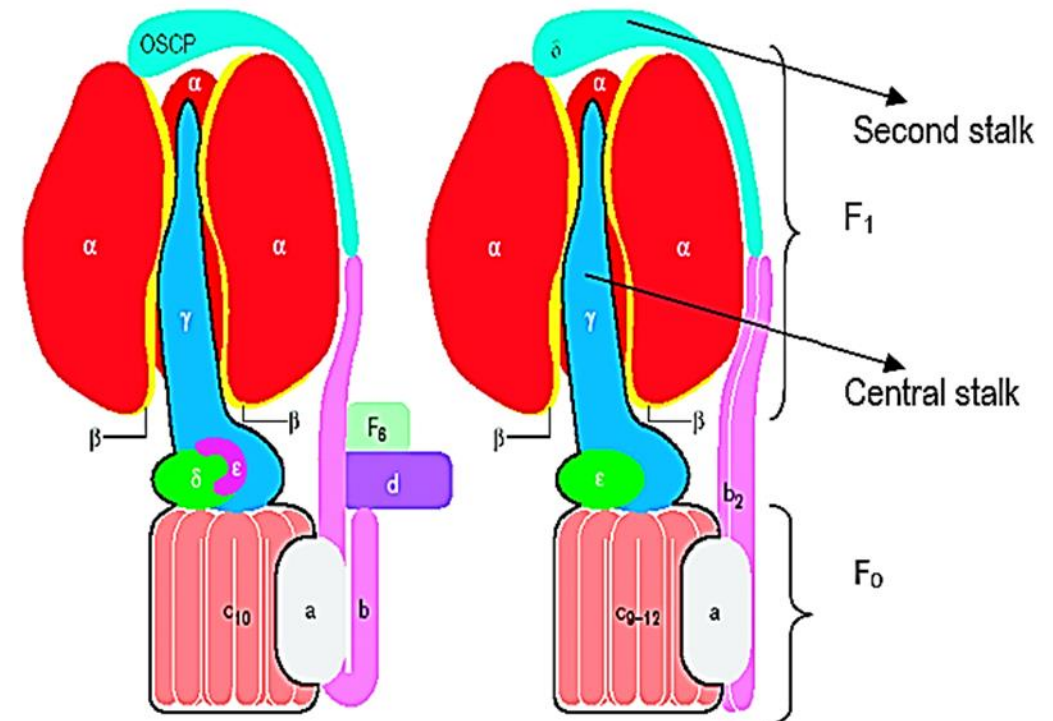
In Plants during photosynthesis, ATP is synthesized by ATP synthase using a proton gradient created in the thylakoid lumen through the thylakoid membrane.

The Structure of ATP synthetase

- ATP synthases contain two distinct functional units each with different biological and chemical properties.
- A hydrophobic, membrane-binding, domain called F_o , that interacts with a larger globular domain peripherally associated with the membrane.
- F_o stands for 'factor oligomycin' referring to the binding of this antibiotic to the hydrophobic portion, while the peripheral protein, F_1 is simply "factor one".
- In an isolated state the F_1 unit hydrolyses ATP but cannot catalyse ATP synthesis.
- For this reason F_1 domain is described as an ATPase.
- F_o forms a 'channel' that allows a flux of protons and their use in ATP synthesis.

The Structure of ATP synthetase

- The much larger F_1 unit, contains 5 different subunits (α – ϵ) having a stoichiometry $\alpha_3\beta_3\gamma_1\delta_1\epsilon_1$ (Table)
- The total mass of the F_1F_0 ATPase from bovine mitochondria is ~450 kDa with the F_1 unit having a mass of ~370 kDa and arranged as a spheroid of dimensions 8×10 nm wide supported on a stem or stalk 3 nm in length.
- ATP synthetase structure has shown a **second peripheral stalk** connecting the F_1 and F_0 domains in the enzyme (Fig.)
- The **second stalk visible** using cryoelectron microscopy of F_1F_0 from *E. coli*, chloroplast and mitochondrial ATPases is located around the outside of the globular F_1 domain and may act as a stator (stationary part) to counter the tendency of the $(\alpha\beta)_3$ domain to follow the rotation of the central stalk.



Organization of ATP synthases. Bovine F1ATPase (left) and ATP synthase of *E. coli* (right).

The Structure of ATP synthetase

- In *E. coli* F_o is extremely hydrophobic and consists of 3 subunits designated **a**, **b** and **c** (Table).
- In *E. coli* a ratio of a1, b2, c9–14 exists, with **a** and **c** subunits making to form a proton channel.

Table. The composition of ATP synthetases.

Subunits in	F1 region	Eukaryotic (bovine) $\alpha 3\beta 3\gamma\delta\epsilon$
α	509 residues, Mr~55,164	
β	480	51,595
γ	272	30,141
δ	190	20,967
ϵ	146	15,652
Subunits in <u>Fo</u> region	Prokaryotic (<i>E. coli</i>) a, b2, c9–14	
a	271	30,285
b	156	17,202
c	79	8,264

The mechanism of ATP synthesis

A mechanism for ATP synthesis was proposed by Paul Boyer before the structural data were available.

G.B. Cox independently suggested a rotary motor involved the **c** subunit ring turning, relative to the **a** and **b** subunits.

At the time (1984) the concept of enzymes using **rotary motion** to catalyse reactions was unusual.

However, the idea of a rotary switch was so novel that there were few serious attempts to test it until over a decade later when in 1995 the first structures for **bovine F_1 ATPase produced by John E. Walker and co-workers supported this model**. It was established that all species contain a, b and c subunits within the F_o part of the enzyme.

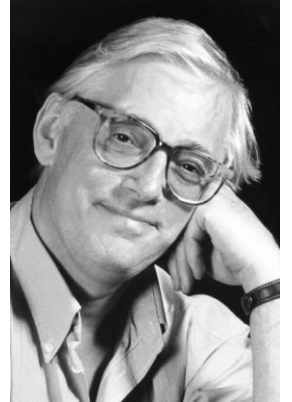
The mechanism of ATP synthesis

The Nobel Prize in Chemistry 1997

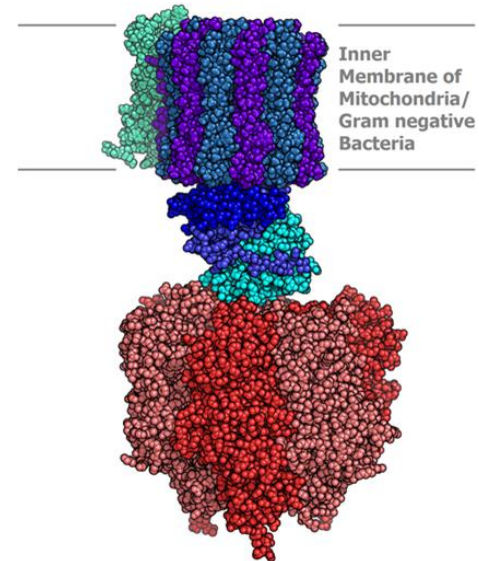
Born: 7 January 1941, Halifax, United Kingdom

Affiliation at the time of the award: **MRC Laboratory of Molecular Biology, Cambridge, United Kingdom**

Prize motivation: "for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)."



John E. Walker

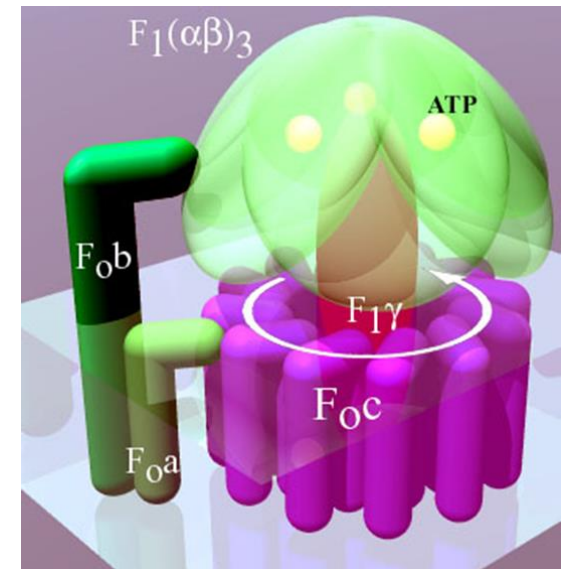
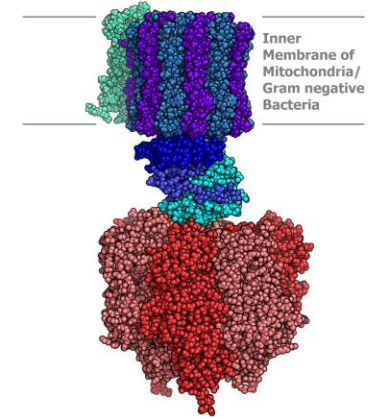


- All life requires energy. In both plants and animals, energy is stored and transported by a special molecule, adenosine triphosphate (ATP).
- Photosynthesis and respiration generate a flow of hydrogen ions, which are used to build up ATP molecules with the help of ATP synthase.
- In 1974 Paul Boyer presented a theory explaining how ATP synthase works.
- The theory was substantiated in 1994, when John Walker used **X-ray crystallography** to determine the structure of ATP synthase.

The Structure of ATP synthetase

F₁ region

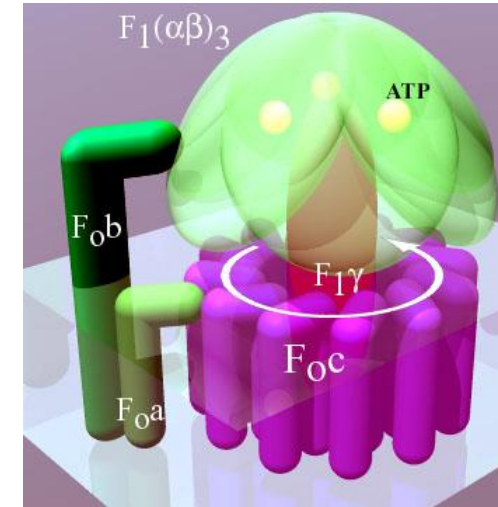
- The F₁ portion of ATP synthase is **hydrophilic** and responsible for hydrolyzing ATP. The F₁ unit protrudes into the mitochondrial matrix space. Subunits α and β make a hexamer with 6 binding sites.
- Three of them are catalytically inactive and they bind ADP.
- Three other subunits catalyze the ATP synthesis. **The other F₁ subunits γ , δ , and ϵ are a part of a rotational motor mechanism (rotor/axle).**
- The γ subunit allows β to go through conformational changes (i.e., closed, half open, and open states) that allow for ATP to be bound and released once synthesized.
- The F₁ particle is large and can be seen in the transmission electron microscope by negative staining.
- These are particles of 9 nm diameter that pepper the inner mitochondrial membrane.



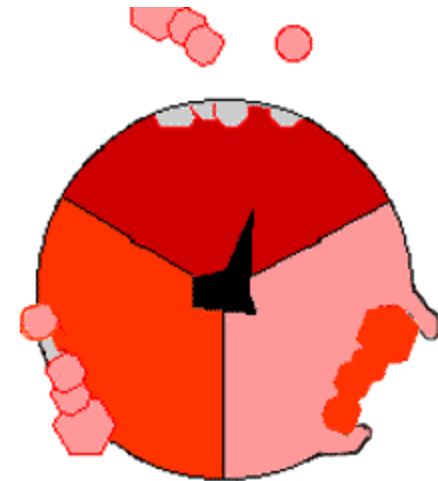
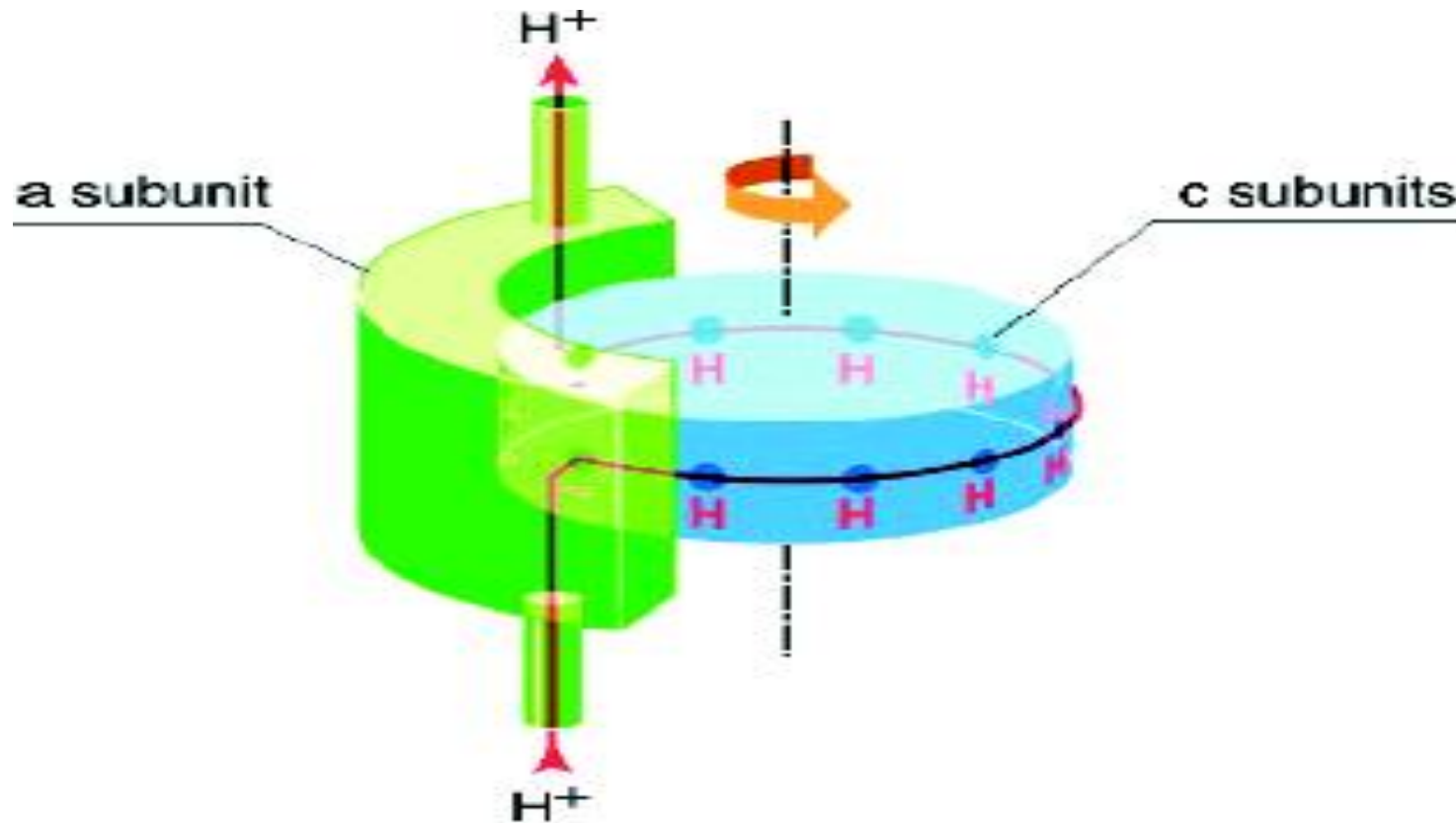
The Structure of ATP synthetase

F_o region

- F_o is a water insoluble protein with 8 subunits and a transmembrane ring.
- The ring has a tetramer shape with a helix protein that goes through conformational changes when protonated and deprotonated, pushing neighboring subunits to rotate, causing the spinning of F_o which then also affects conformation of F_1 , resulting in switching of states of alpha and beta subunits.
- The F_o region of ATP synthase is a proton pore that is embedded in the mitochondrial membrane. It consists of three main subunits, a, b, and c. Six c subunits make up the rotor ring, and subunit b makes up a stalk connecting to F_1 OSCP that prevents the $\alpha\beta$ hexamer from rotating.
- Subunit **a** connects **b** to the **c** ring. This part of the enzyme is located in the mitochondrial inner membrane and couples proton translocation to the rotation the causes ATP synthesis in the F_1 region.



The mechanism of ATP synthesis



Hypothetical model for generation of rotation by proton transfer through the F_o domain of ATP synthase. The central cylinder (blue) consists of c subunits; the external part (green) corresponds to single a subunit. Red line indicates the proton path.

The mechanism of ATP synthesis

A scheme for ATP synthesis envisaged three discrete stages

1. **Translocation of protons by the F_o .**
2. **Catalysis of ATP synthesis *via* the formation of a phospho-anhydride bond between ADP and P_i by F_1 .**
3. **A coupling between synthesis of ATP and the controlled dissipation of the proton gradient *via* the coordinated action and interaction of F_1 and F_o .**