# ATP-Making Strategies in Mitochondria

We need energy to do our daily activities e.g. walking requires energy (from ATP) to make our muscle (or proteins in muscle) contract. We get the energy from the food we eat by breaking it into small energy-rich molecules like fat and sugar. However, these molecules cannot readily deliver energy that can be used conveniently by any cells in our body. So they have to be processed further to a more stable universal energy carrier molecule known as "ATP (Adenosine Tri-Phosphate)".

ATP is made in mitochondria within which the fat and sugar are transported in and broken further into a high-energy electron carrier called NADH (Reduced Nicotinamide Adenine Dinucleotide) and a waste product as $CO_2$ (Carbon dioxide). The energy is stored in the two high-energy electrons within NADH. These two high-energy electrons are then transported through a series of 4 enzyme complexes (within the so called "Respiratory Electron Transport Chain") which extract the electrons' energy and use it to pump protons across the inner membrane of mitochondria creating a proton gradient. At the end of the chain, the electrons are then combined to $O_2$ (Oxygen molecules that we breathe in) and some protons to form water molecules as waste products. Finally an enzyme called "ATP synthase" translocates these protons back along the proton gradient and uses the energy released to synthesize ATP from ADP (Adenosine Di-Phosphate) and an Inorganic Phosphate $P_i$. The Respiratory Electron Transport Chain and ATP Synthesis are together very well known as "Oxidative Phosphorylation (OXPHOS)" since it involves the consumption of $O_2$ and the addition of a phosphate group $P_i$ to ADP.

In this paper, I would like to provide a review on how energy is transported and converted into the universal energy currency "ATP" by the five enzyme complexes which function together in Oxidative Phosphorylation (See Figure 1) i.e.

Complex I: NADH:Ubiquinone Oxidoreductase: Proton Pump with Unknown Mechanism,
Complex II: Succinate:Ubiquinone Oxidoreductase: Respiratory Enzyme that doesn't pump Protons,
Complex III: Cytochrome $bc_1$: Redox-linked Proton Pump using small molecule as Proton Carrier,
Complex IV: Cytochrome c Oxidase: Redox-linked Proton Pump using Transmembrane Ion Channnel,
Complex V: ATP Synthase: Revesible Rotary Stepping Motor.

The focus will be on the last 3 enzymes complexes (Complex III, IV and V) as recently their structures have been solved.

## Respiration Electron Transport Chain: Multiple Proton Pumping Strategies

The energy from food is stored in the high-energy electrons in NADH but it is not so stable to be transported and use within cell since NADH is a strong electron donor. This energy has to be converted further into a better form. Respiratory Electron Transport Chain does this job by converting the chemical bonding energy of the high-energy electrons into electrochemical energy of protons stored across the membrane. This process is done by a passing of the electrons through a series of 4 enzyme complexes (and a small protein and chemical compound i.e. cytochrome c and ubiquinone). Energy is extracted during each step to pump protons across the membrane from the matrix side (colored in Orange in Figure 1) to the cytoplasmic side (Green).

The first step of the electron transport chain involves the **Complex I: NADH:Ubiquinone Oxidoreductase** which is a proton pump. It is the largest enzyme in OXPHOS process. Its structure has not yet been solved. However its pictures from electron microscope show that it has an L-shaped structure (See Figure 1.) and consists of 2 major domains. In bovine, the enzyme consists of at least 43 subunits, 1 flavin mononucleotide, as many as 9 different FeS (Iron-Sulfur) clusters, covalently bound lipids, and at least 2 ubiquinol ($QH_2$) binding sites. Its molecular weight is about 900,000.

In Complex I, the hydride ion $H^-$ (a hydrogen atom with an extra electron) is removed from NADH and then it is separated into two high-energy electrons and a proton. These electrons are passed from the flavin group to a set of Iron-Sulfur clusters of increasing redox potentials (each FeS clusters can carry one electron at a time.) Then these electrons are donated to ubiquinone (Q) which is a lipid soluble molecule that can move in the lipid bilayer. A ubiquinone can pick up or donate a proton for each electron it carries. During this electron transfer, another proton is also pumped and transported to the ubiquinone (Q).

$$NADH + H^+ \qquad NAD^+ + H^- + H^+ \qquad NAD^+ + H^+ + 2e^- + H^+ \qquad (1)$$

$$NAD^+ + H^+ + 2e^- + H^+ + Q \qquad NAD^+ + QH_2 \qquad (2)$$

**Complex II: Succinate:Ubiquinone Oxidoreductase** is a component of both the electron transport chain and the citric cycle (it is a process which breaks fat and sugar into NADH and $CO_2$.) It has a covalently bound flavin adenine dinucleotide (FAD) and several FeS clusters. It takes part in transporting electrons (or removing 2 hydrogens) from Succinate to the ubiquinone (Q) and produces Fumarate (it has 2 hydrogens less than Succinate) as the end product i.e.

$$Succinate + Q \qquad Fumarate + QH_2 \qquad (3)$$

However, Complex II does not pump protons! (net charge is zero on both sides of the chemical equation (3) )

**Complex III: Cytochrome $bc_1$** exists functionally as a dimer (See Figure 2A). Each monomer can pump protons. In mammals, each monomer consists of 11 subunits but only three of these (cytochrome b [colored in Green in Figure 2B], cytochrome $c_1$ [Blue] and Rieske protein or Iron-Sulfur protein [Purple]) have three redox centers (heme c, $b_L$ and $b_H$ [Red]) and are used to pump protons. Only these three have bacterial homologs.

There are 2 binding sites (See Figure 2C & 2B) in cytochrome $bc_1$. One is called "$Q_o$ site" is at the outer surface near FeS cluster and heme $b_L$. It binds a ubiquinol ($QH_2$). The other is called "$Q_i$ site" is at the inner surface near heme heme $b_H$ and it binds the ubiquinone (Q). The ubiquinols ($QH_2$) from both Complex I and II then delivers the high-energy electrons to the cytochrome $bc_1$. The release of two electrons from the ubiquinol ($QH_2$) on the $Q_o$ site is coupled to the adding of an electrons into ubiquinone (Q) on the $Q_i$ site accoding to "the Q-Cycle Mechanism" (See Figure 2C). First an electron is transferred from the ubiquinol ($QH_2$) to the FeS cluster and then to Cytochrome $c_1$ and finally to cytochrome c (a water soluble protein which transport an electron to Complex IV: Cytochrome c Oxidase.) The second electron is then transferred along a different path to the heme $b_L$, then continue to the heme $b_H$ and finally it is passed to the ubiquinone (Q) at the $Q_i$ site. During this electron transfer,

2 protons are released from the ubiquinone ($QH_2$) to the cytoplasmic site. A proton is pumped to combine with the ubisemiquinone ($Q^-$) at the $Q_i$ site. This process is repeated with the binding of the second ubiquinol ($QH_2$) to the $Q_o$ site to complete the Q-Cycle. Crystal structure shows that the Reiske protein is mobile depending the occupation of inhibitor at the $Q_o$ site.

**Complex IV: Cytochrome c Oxidase** (see Figure 3A) is another proton pump which functions differently from cytochrome $bc_1$. It is the last complex which extracts energy from the high-energy electrons to pump protons. Bovine cytochrome c oxidase has 13 subunits. Three of these (subunit I, II and III) form the functional core of the enzyme. Unlike the other 10 subunits, these three subunits are coded by mitochondrial DNA possibly to conserve their function. Subunit I (colored in Green in Figure 3B) contains the active site consisting of the heme a (with an iron) and the heme $a_3$ with bimetal Fe-$Cu_B$ surrounded by 6 histidines. Subunit II (Purple) has dicopper cluster $Cu_A$. Subunit III (Blue) contains bound phospholipids but its functional role has not yet been known.

The soluble cytochrome c which carries a high-energy electron from cytochrome $bc_1$ can donate two electrons (one for each trip) to the dicopper cluster $Cu_A$ in the subunit II of the cytochrome c oxidase (See Figure 3C & 3B). These two electrons are transferred (twice to get 4 electron into the active site) to the "heme a" (which has an iron) and then to the "heme $a_3$" (which also has an iron) with metal $Cu_B$ in the active site of subunit I to break oxygen $O_2$ (oxygen that we breathe in) into 2 water molecules.

$$4\text{cyt } c^{2+} + O_2 + 4H^+ + 4H^+ \qquad 4 \text{ cyt } c^{3+} + 2H_2O + 4H^+ \qquad (4)$$

During this reaction, 8 protons are pumped along two chains of water molecules in the D- and K-channels (4 protons for each channel) in subunit I to the active site. (The name of each channel indicates the conserved functional residue that may participate in pumping protons within the channel i.e. D91, K319.)

## ATP synthesis: Rotary Mechanism for Making ATP

With many protons pumped into the cytoplasmic side (the inner region of mitochondria) during respiratory electron transport chain, a proton gradient is generated i.e. the energy from the food we eat has been converted and stored in the form of the electrochemical energy of the protons between the 2 sides of the membrane. Even though the proton gradient is stable but it is not very easy to be transported to other parts of cells and be used by other enzyme complexes in the cell. The last enzyme complexes "Complex V: ATP synthase" is capable of converting this proton energy into an energy-rich chemical bond connecting ADP and inorganic phosphate $P_i$ and make the universal energy currency molecule "ATP" which is very stable (with a lifetime of approximately a few days) and can easily be transported to other parts of cells and be used by other enzymes.

**Complex V: ATP synthase** (See Figure 4) is the smallest rotary motor known today (a size of ~2x1x1 $nm^3$). It consists of 8 subunits in bacteria (and 16 subunits in bovine). The enzyme complex can be considered as composed of two parts i.e. the water-soluble $F_1$ part and the transmembrane $F_o$ part (they can be separated by changing the $Mg^{2+}$ ion concentration of the solution.)

The water soluble $F_1$ part (See Figure 4) consists of 5 different subunits (3 $\alpha$-, 3 $\beta$-, 1 $\gamma$-, 1 $\delta$, and 1 $\varepsilon$-subunits). Each $\beta$-subunit has a catalytic binding site for binding ADP and $P_i$ (or ATP). So ATP is made at this subunit. Each $\alpha$-subunit also contains a non-catalytic binding site which binds an ATP but its function is still not known. The $\gamma$- and $\varepsilon$-subunits form an asymmetric rotating central stalk which interacts directly with the 3 $\beta$-subunits causing cyclic structural changes in their three catalytic binding sites. For each turn of the central stalk, each catalytic binding site undergoes 3 main states according to the "Binding Change Mechanism" i.e.

1) Open state (the binding site is empty and is ready to bind ADP and inorganic phosphate $P_i$)
2) Loose state (the binding site contains ADP and $P_i$ and is ready to form ATP)
3) Tight state (the binding site contains ATP and is ready to release the ATP)

This structural changes transform ADP and $P_i$ into ATP. So 3 ATPs are produced every cycle.

The Transmembrane $F_o$ part consists of 3 different subunits (1 a-, 2 b- and 10 to 12 c-subunits). The a-subunit forms a proton channel which allows protons to pass through (from the cytoplasmic side to the matrix side) and release energy to drive the rotation of a cylindrical barrel (formed by 10 to 12 c-subunits) which is connected to the central stalk of the $F_1$ part. (The mechanism that couples the proton flow to the rotation c-subunit barrel is still under investigation.) The rotational speed is about 100 revolutions per sec (~ 1 msec. per turn). This requires 10 (to 12) protons for each revolution. The two b-subunits form a second stalk linked with the $\delta$ subunit of the $F_1$ part. This second stalk holds the $\alpha_3\beta_3$ of $F_1$ part such that it does not rotate with the central stalk.

The full picture of the complex is that we have a proton flow through the $F_o$ part causing the c-subunit cylindrical barrel and the central stalk to rotate. This stalk rotation in turn makes the three binding sites in $F_1$ part undergo a three step cycle to make ATP from ADP and inorganic phosphate $P_i$. The rotating part (rotor) is $\gamma\varepsilon c_{10\text{-}12}$ and the non-moving part (stator) is $\alpha_3\beta_3\delta ab_2$. So in each rotation 3 ATPs are produces from the flow of 10-12 protons i.e. 3.3-4 protons per ATP.

More interestingly, this enzyme complex can also function reversibly! It can break an ATP into an ADP and an organic phosphate $P_i$ in its binding sites in $F_1$ part and use the energy to rotate the central stalk in the opposite direction and pump protons back to the cytoplasmic side. So effectively there are 2 motors which functions differently in this single enzyme complex. The $F_o$ motors functions by the proton gradient and the $F_1$ motor functions by breaking ATP into ADP and $P_i$.

## Summary

Almost all the structures of enzyme complexes involving in the making of ATP in Oxidative Phosphorylation are known. This leads us to understand how the high-energy electrons are transferred among these enzymes and how this electron transfer process is coupled to the proton pump mechanism. The mechanism of proton transfer within these complexes is still unclear. It presents a very challenge problem awaiting to be solved. ATP is made by the rotary mechanism of the ATP synthase which is a reversible motor. The present understanding these enzyme complexes has given us some insight and many surprises into the fascinating world of proteins at atomic level. More are expected to come.

## References


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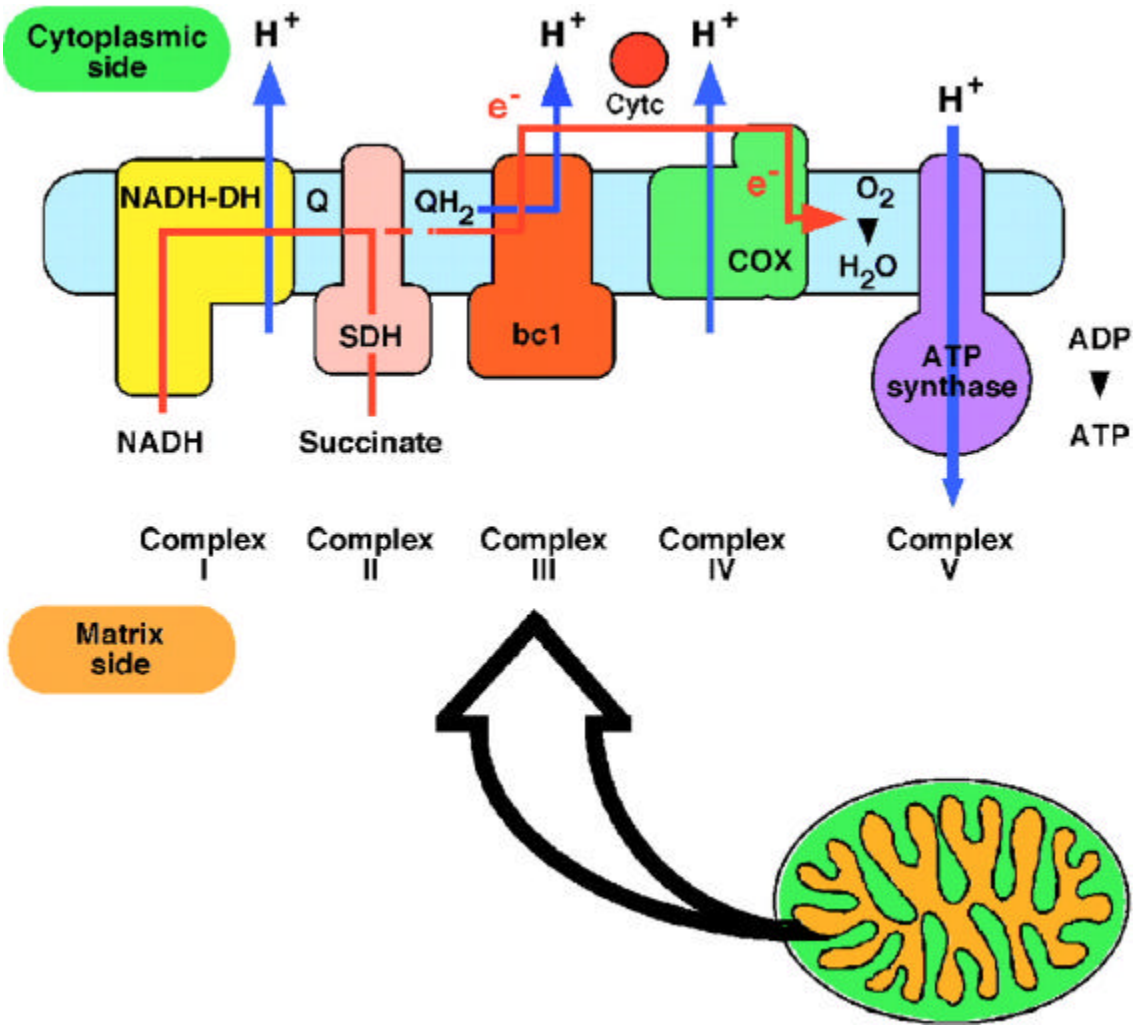


**Figure 1** shows 5 enzyme complexes which performs Oxidative Phosphorylation in the inner membrane of mitochondria. Complex I: NADH (Yellow), Complex II: SDH (Pink), Complex III: $bc_1$ (Orange), Cytochrome c (Red sphere between $bc_1$ and COX), Complex IV: COX (Green) and Complex V: ATP synthase (Magenta). The proton paths are colored in dark blue, electron paths in red, Lipid bilayer membrane in light blue. (Q = ubiquinone)

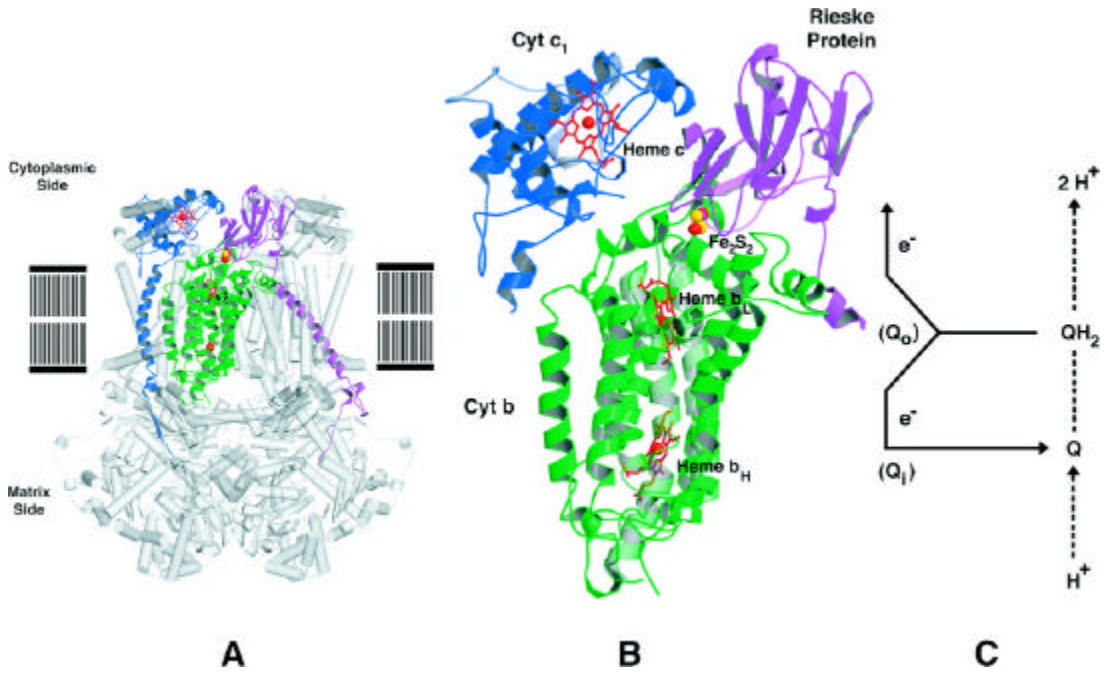


**Figure 2** shows A) Complex III: Cytochrome $bc_1$ as a functional dimer. The 3 subunits that form functional core are colored and also shown in B). Cytochrome b (Green), Cytochrome $c_1$ (Blue) and Rieske Protein (Purple). C) The Q-cycle.

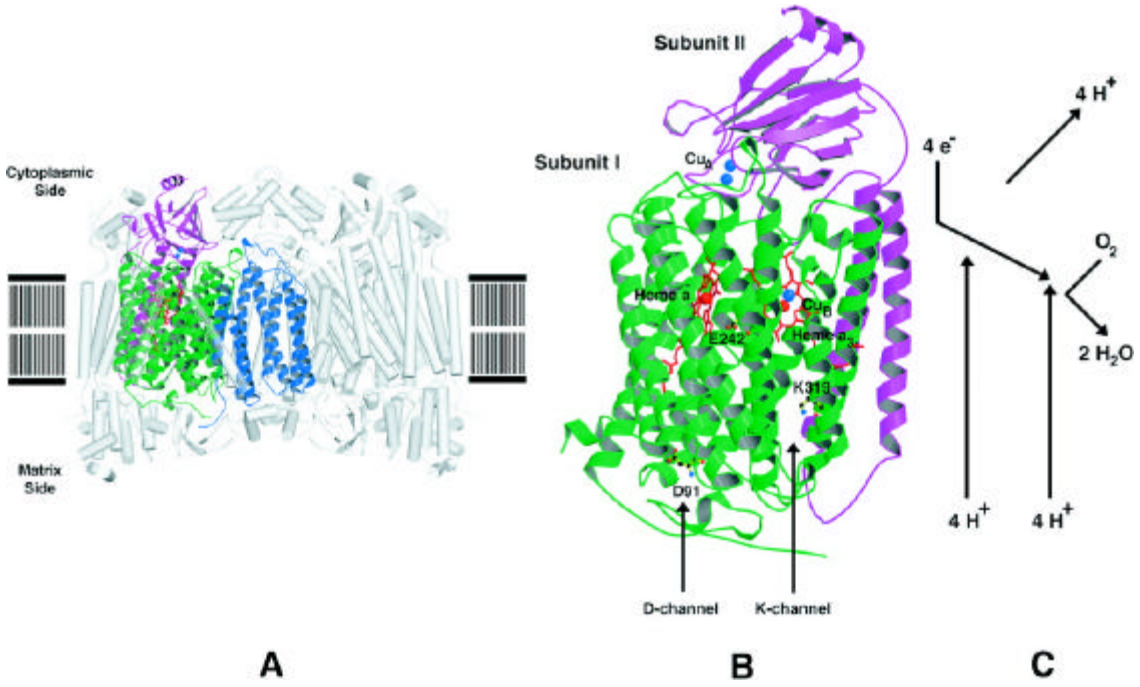


**Figure 3** shows A) Comple IV: Cytochrome c Oxidase. 3 subunits that form functional core are colored. Subunit I in green, subunit II in purple and subunit III in blue. B) shows active site $Cu_A$ (blue) in subunit II and Heme a, $a_3$ (red) and $Cu_B$ (blue) and the D- and K- channels in subunit I.

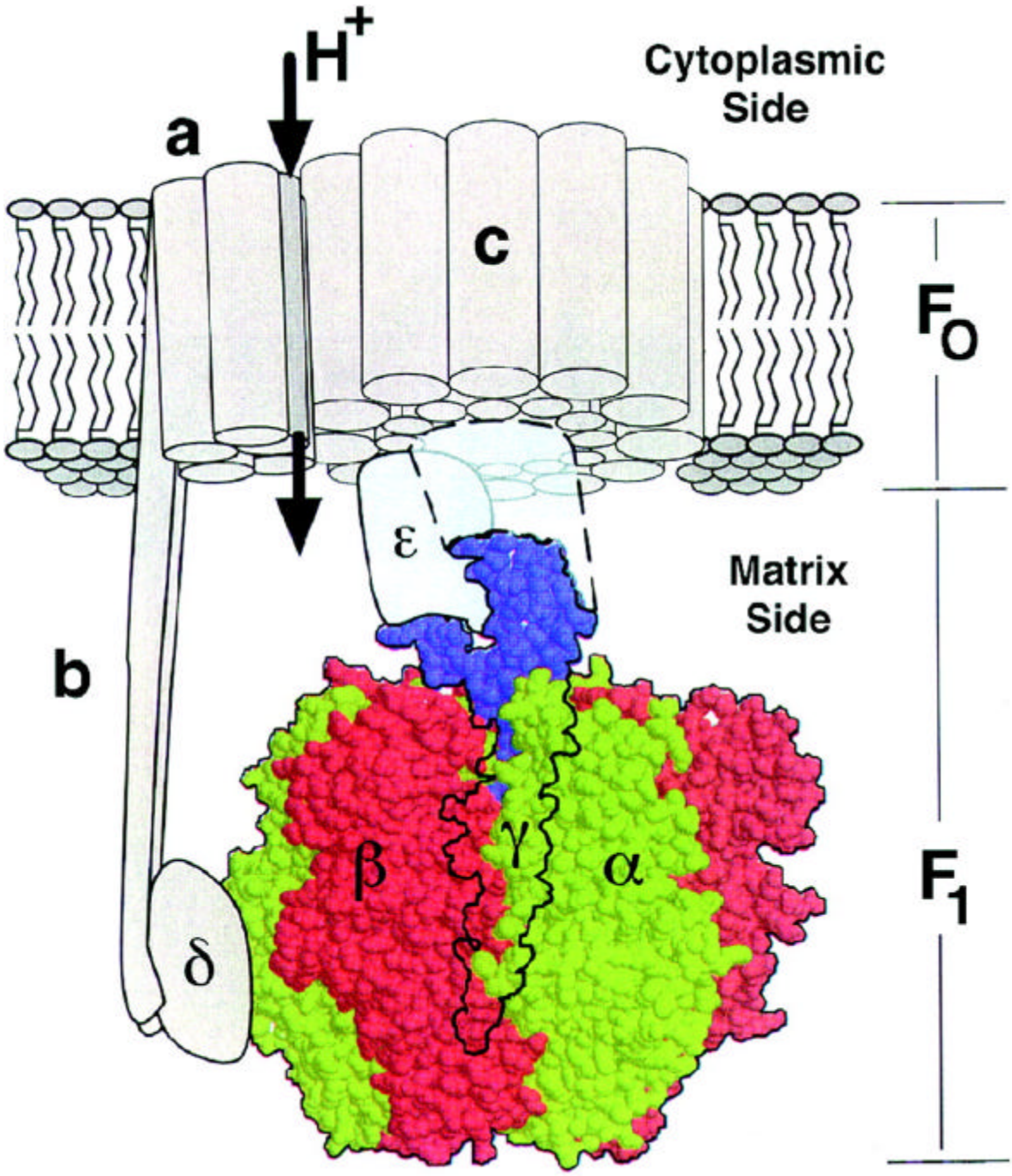


**Figure 4** shows Complex V: ATP synthase. Its consists of the water soluble part $F_1$ ($\alpha_3\beta_3\gamma\delta\varepsilon$) that contains three catalytic sites for binding ADP and $P_i$ and the transmembrane part Fo ($ab_2c_{10\text{-}12}$) that contains a proton channel. The rotating part (rotor) is $\gamma\varepsilon c_{10\text{-}12}$ and the non-moving part (stator) is $\alpha_3\beta_3\delta ab_2$.