

اللهم لا سهل إلا ما جعلته سهلا  
وأنت تجعل الصعب إذا شئت سهلا

# Mitochondria

The mitochondrion has 2 membranes:

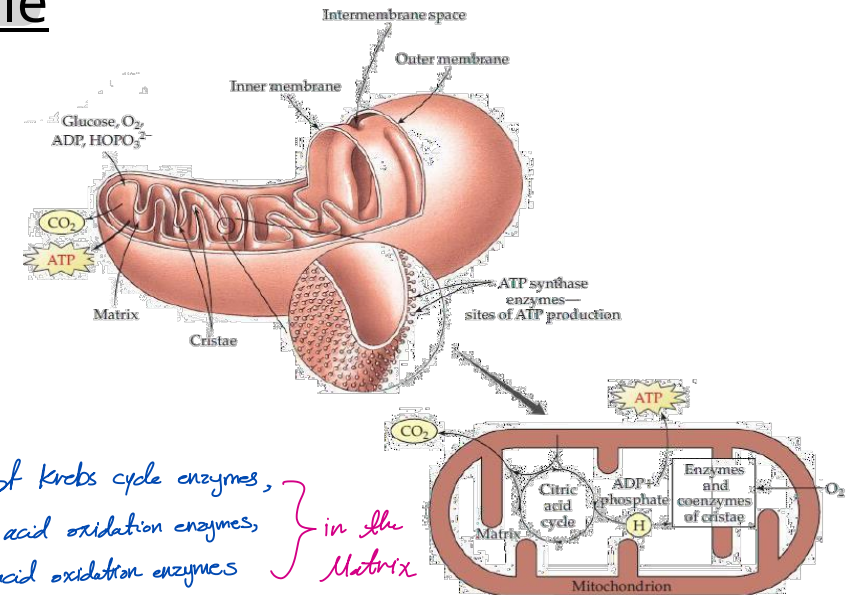
1. **OMM (outer mitochondrial membrane)** is permeable to:
  - A. Small molecules (MW < 5,000 daltons)
  - B. Ions
  - C. It also has porins (transmembrane channels, pores)
2. **IMM (inner mitochondrial membrane)** is impermeable even to  $H^+$ ; and it has specific transporters.

- **IMM bears the components of the respiratory chain and the ATP synthase**

We expect a high concentration of proteins in the IMM because it lacks general pores and is composed of about **75% proteins**, which include various transport proteins. The outer mitochondrial membrane (OMM), on the other hand, has protein channels and pores typical of other biological membranes.

Remember, the mitochondrion is:

1. A very dynamic organelle
2. The energy factory of cells
3. Present in varying numbers in different cell types depending on the activity of the cell.
4. Capable of dividing and fusing
5. Possesses mitochondrial DNA, RNA & ribosomes



Step 6 of Krebs cycle, ETC.

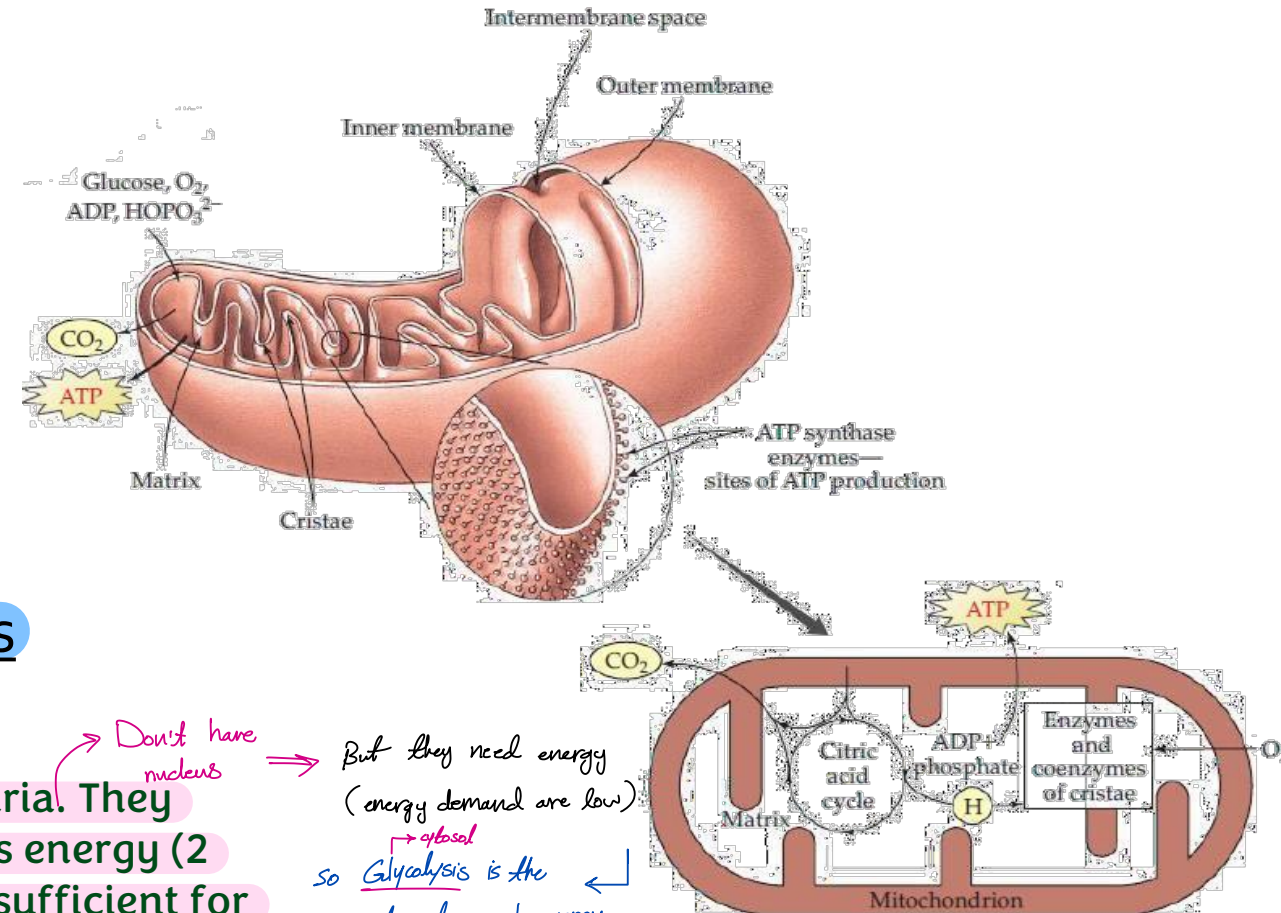
Most of Krebs cycle enzymes,  
Amino acid oxidation enzymes,  
fatty acid oxidation enzymes } in the Matrix  
most pathways that oxidize the main substrate to extract energy  
Except Glycolysis

# Mitochondria

- **Matrix** (the innermost compartment of the mitochondria): gel-like solution 50% proteins, it contains pyruvate dehydrogenase complex & TCA cycle enzymes, fatty acid  $\beta$ -oxidation pathway, and the pathways of amino acid oxidation. mtDNA, mtRNA, mt-ribosomes

- In other words: matrix contains all pathways of fuel oxidation Except Glycolysis

For example, red blood cells (RBCs) do not have mitochondria. They obtain all their energy from glycolysis, which produces less energy (2 ATP per glucose) compared to aerobic respiration, but it is sufficient for their energy requirements.



Don't have nucleus  $\Rightarrow$  But they need energy (energy demand are low)  $\leftarrow$  so Glycolysis is the way to get enough energy for RBCs

# Mitochondrial Membranes

- **Outer membrane:**

- **Similar to cell membrane** Around 50% of it is protein
- **45% cholesterol**
- **Less than 3% cardiolipin** → 4 fatty acids  
Bulky phospholipid, with glycerol group

Cardiolipin distinguishes the IMM because of its presence there in a large percentage

- **Inner membrane:**

- **22% Cardiolipin**
- **No cholesterol**
- Around 75% proteins
- The remanent (13%) is lipid.

**TABLE 20.3: Location of enzymes in mitochondria**

**Mitochondria, outer membrane:**

Monoamino oxidase

Acyl CoA synthetase

Phospholipase A2

**In between outer and inner membrane:**

Adenylate kinase

Creatine kinase

**Inner membrane, outer surface:**

Glycerol-3-phosphate dehydrogenase

**Inner membrane, inner surface:**

Succinate dehydrogenase

Enzymes of respiratory chain

**Soluble matrix:**

Enzymes of citric acid cycle

Enzymes of beta oxidation of fatty acid

# Mitochondrial Membranes

Proteins that are present in the IMM or OMM are referred to as **membrane proteins**.

There are also proteins present in the **intermembrane space** and in the **matrix**.

Most enzymes that catalyze metabolic reactions are located in the matrix, including enzymes involved in the **TCA cycle** and  **$\beta$ -oxidation** (the process of fatty acid oxidation).

Proteins in the IMM, particularly enzymes, may have their catalytic sites oriented either toward the matrix or the intermembrane space, depending on their **function**.

Glycerol-3-phosphate dehydrogenase, for example, is an enzyme which faces the intermembrane space.

Additionally, succinate dehydrogenase, which functions in the Krebs cycle, faces the matrix because its product, fumarate, remains in the matrix.

Similarly, enzymes involved in the **electron transport chain** also face the matrix.

**TABLE 20.3: Location of enzymes in mitochondria**

## Mitochondria, outer membrane:

**Monoamino oxidase** → involved in the Metabolism of Epinephrine, norepinephrine, Dopamine, Serotonine.

**Acyl CoA synthetase**

**Phospholipase A2** Degradation of phospholipids

## In between outer and inner membrane:

**Adenylate kinase** NOT adenyl kinase

**Creatine kinase** **Phosphorylate creatine** → that acts as a source of energy in muscle cells

## Inner membrane, outer surface:

**Glycerol-3-phosphate dehydrogenase**

## Inner membrane, inner surface:

**Succinate dehydrogenase**

**Enzymes of respiratory chain**

} facing the matrix

## Soluble matrix:

**Enzymes of citric acid cycle**

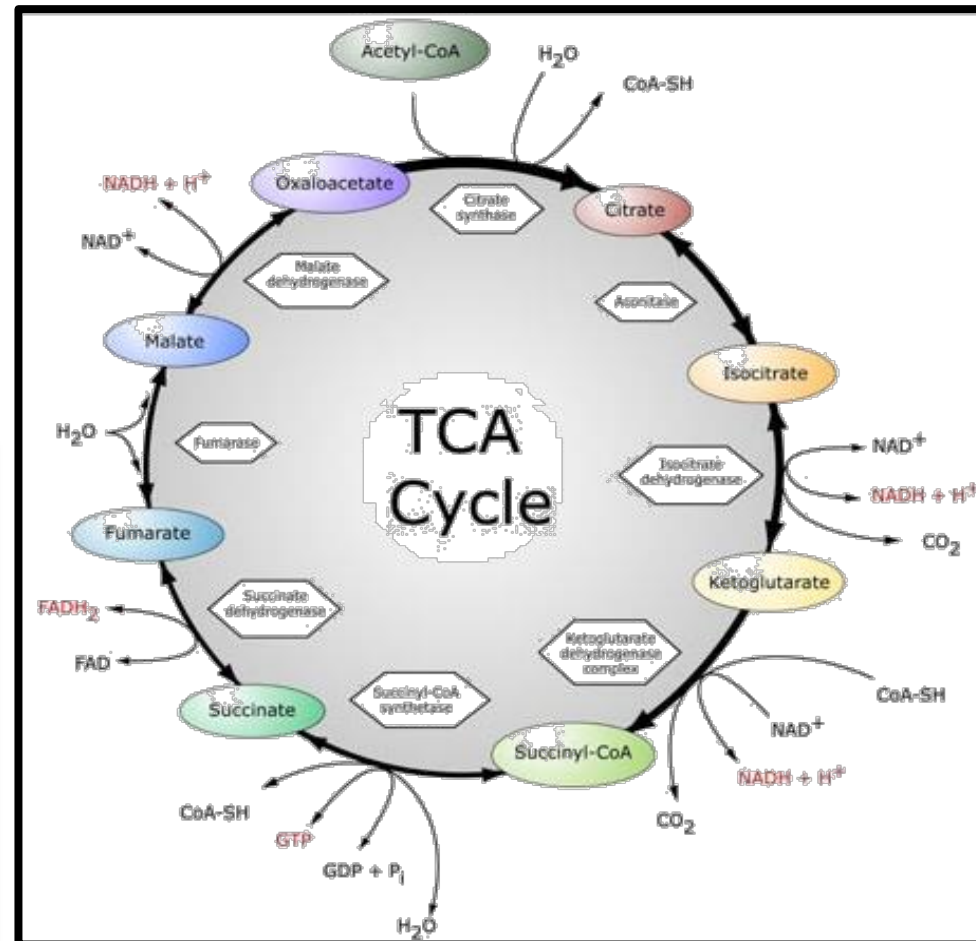
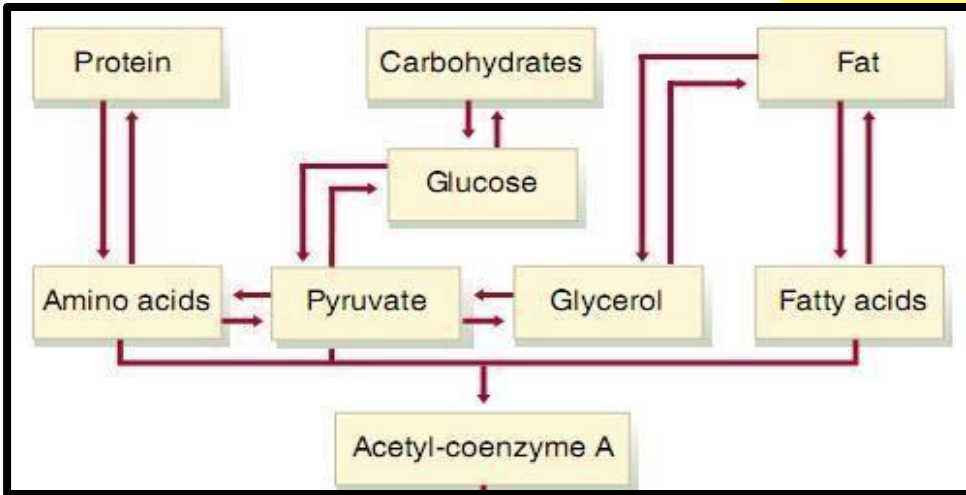
**Enzymes of beta oxidation of fatty acid**

# Where does Oxidative Phosphorylation occur?

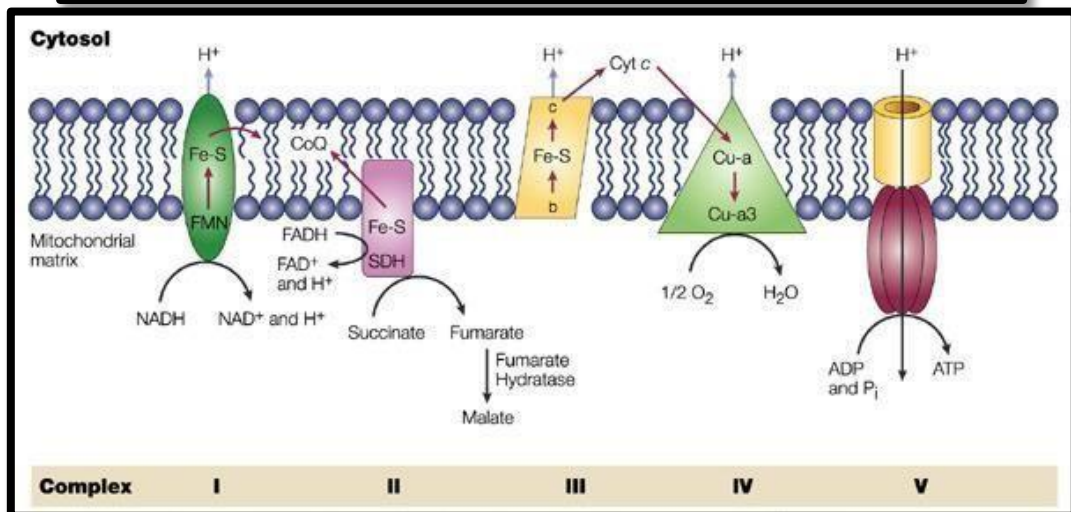
- **Stages: Digestion; Acetyl-CoA, TCA, OxPhos**  
ETC takes place after Krebs cycle, and after it, Oxidative Phosphorylation (OxPhos) occurs, producing ATP.

Why is it called oxidative phosphorylation?

1. **Phosphorylation:** Because ADP is phosphorylated to form ATP.
2. **Oxidative:** Because this phosphorylation is driven by a series of redox (oxidation-reduction) reactions that occur in the electron transport chain. This process differs from substrate-level phosphorylation, such as in the conversion of GDP to GTP. → Chemical reaction



\* ETC is going to transport the electrons along the chain between different complexes (some of these complexes are enzymes) → 5

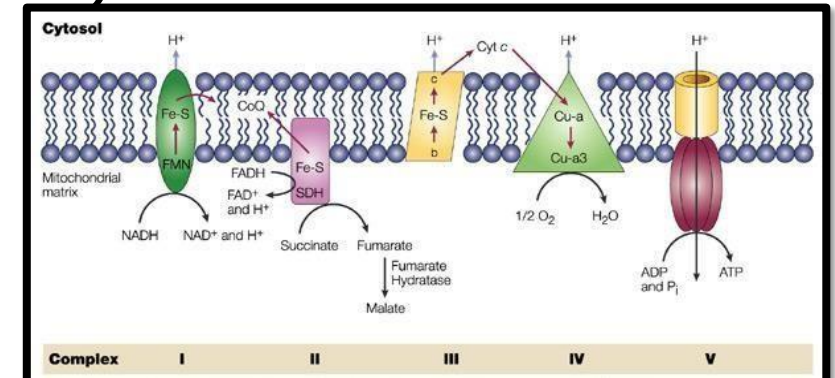


# Oxidative Phosphorylation (OxPhos)

- **Generation of ATP aided by the reduction of  $O_2$**
- **Peter Mitchell (1961): the chemiosmotic theory**
- **Oxidative phosphorylation has 3 major aspects:**
  - **(1) It involves the flow of electrons through a chain of membrane-bound carriers (prosthetic groups)** *like Heme* This movement of electrons is energetically favorable. (exergonic)
  - **(2) The free energy available (exergonic) is coupled to transport protons across a proton-impermeable membrane** From the matrix to intermembrane space, which creates an electrical gradient (+ out, - in) and a pH gradient (lower out, higher in).
  - **(3) The transmembrane flow of protons down their concentration gradient provides the free energy for synthesis of ATP (ATP synthase)**

*it means that  $E^\circ$  is increasing so the molecules at the end of the chain are tending to be reduced which contributes to the flow of  $e^-$  along ETC*

- **Five separate protein complexes I, II, III, IV, and V.**
- **Complexes I-IV each contain part of the electron transport chain.** Complexes 1-4 are responsible for electron transport while complex 5 is ATP synthase enzyme that synthesizes ATP by OxPhos
- **Each complex accepts or donates electrons to relatively mobile electron carriers, such as coenzyme Q and cytochrome c.**



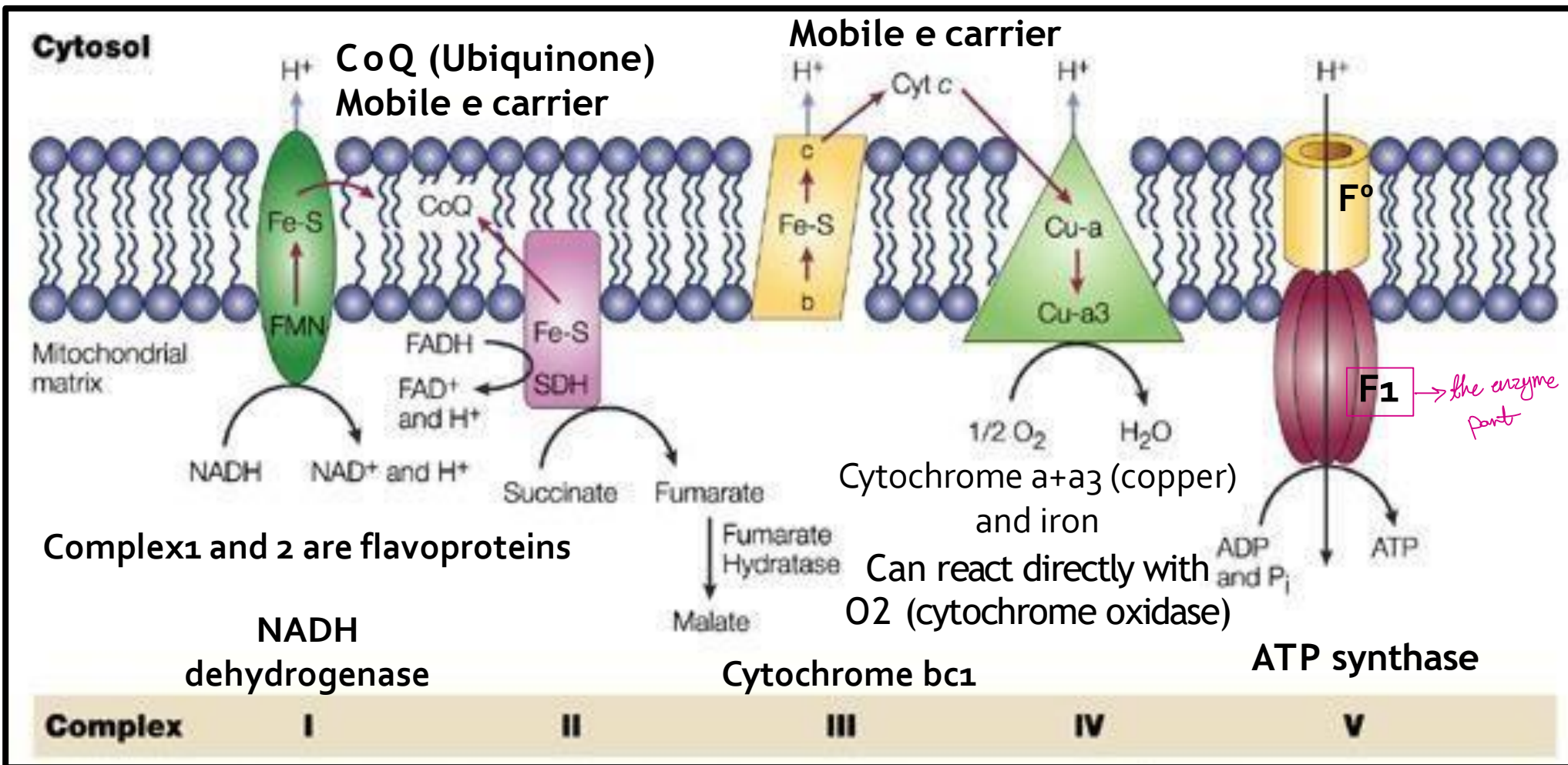
ETC is composed of:

1. **proteins (Complexes I-IV)**  $\Rightarrow$  The majority of the components of ETC
2. **coenzyme Q and cytochrome C (these 2 are mobile)**

$\hookrightarrow$  They may contribute to other rxns in the mitochondria

# Oxidative Phosphorylation (OxPhos)

As electrons are passed down the electron transport chain, they lose much of their free energy. Part of this energy can be captured and stored by the production of ATP



**NADH:** carries electrons in the form of hydride ions (H<sup>-</sup>).

**FADH<sub>2</sub>:** carries electrons in the form of hydrogen atoms (H + e<sup>-</sup>).

**Cytochromes:**

1. Complex 3 (cytochrome bc<sub>1</sub>)
2. Complex 4 (Cytochrome a+a<sub>3</sub>)
3. Cytochrome C

# Electrons' pathway through Electron transport chain to oxidative phosphorylation:

- Complex I is an enzyme complex (NADH dehydrogenase) that <sup>Accept</sup> receive electrons from NADH, and it has a tightly bound molecule of (Flavin Mononucleotide), it also contains peptide subunits with Fe-S centers.

↳ FMN

In Complex I electrons move this way:

**NADH → FMN (becomes FMNH<sub>2</sub>) → Fe (in Fe-S) → CoQ**

\* To obtain the electrons of FADH<sub>2</sub>. We have to have another complex, which is complex II :-

- Complex II is an enzyme complex (succinate dehydrogenase, the same enzyme used in the 6th step of the Krebs cycle) that receives electrons from FADH<sub>2</sub>. It contains iron-sulfur (Fe-S) clusters.

Becomes FAD ←

just like complex I

At Complex II, the electrons move in the following path:

**FADH<sub>2</sub> → Fe (in Fe-S centers) → coenzyme Q (CoQ).**

- <sup>Not a protein</sup> Coenzyme Q (CoQ or <sup>ubiquitous</sup> ubiquinone/<sup>ketone</sup>), is a mobile carrier and can accept hydrogen atoms both: from FMNH<sub>2</sub>, produced on NADH dehydrogenase (Complex I), and from FADH<sub>2</sub>, produced on succinate dehydrogenase (Complex II).

\* CoQ can deal with complex I & II that can receive e<sup>-</sup>s in different format

↳ Hydride (NADH)  
↳ Hydrogen (FADH<sub>2</sub>)

↳ so it can act as a junction between the carriers of 2e<sup>-</sup> & the carriers of 1e<sup>-</sup>

**FMNH<sub>2</sub> → CoQ → Cytochromes**

**FADH<sub>2</sub>**

!! Note: CoQ, links the flavoproteins to the cytochromes.



# Electrons' pathway through Electron transport chain to oxidative phosphorylation:

*Hemoproteins*

*have Fe & other elements*

➤ **Cytochromes:** Each contains a heme group (a porphyrin ring plus iron).

Electrons are passed along the chain from:

CoQ → Cytochromes **bc<sub>1</sub>** (Complex III) → Cytochrome C → Cytochrome a + a<sub>3</sub> (Complex IV)

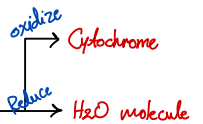
*↳ Depending on the type of Heme group.*

*↳ Mobile component of the ETC*

*↳ Fe & Cu*

➤ **At complex IV the transported electrons, O<sub>2</sub>, and free protons are brought together, and O<sub>2</sub> is reduced to water.** (This complex is able to combined the electrons & protons to O<sub>2</sub> to make H<sub>2</sub>O)

- This cytochrome complex is the only electron carrier in which the heme iron has an available coordination site that can react directly with O<sub>2</sub>, and so also is called **cytochrome oxidase**.
- **Cytochrome oxidase contains copper atoms that are required for this complex reaction to occur.**



➤ **The enzyme complex ATP synthase (Complex V) synthesizes ATP using the energy of the proton gradient generated by the electron transport chain.** *↳ inserted within the membrane (responsible for proton movement)*

- It contains a membrane domain (**F<sub>0</sub>**) that spans the IMM, and extramembranous domain (**F<sub>1</sub>**) that appears as a sphere that protrudes into the matrix.

*responsible for synthesizing  
ATP by phosphorylation of ADP*

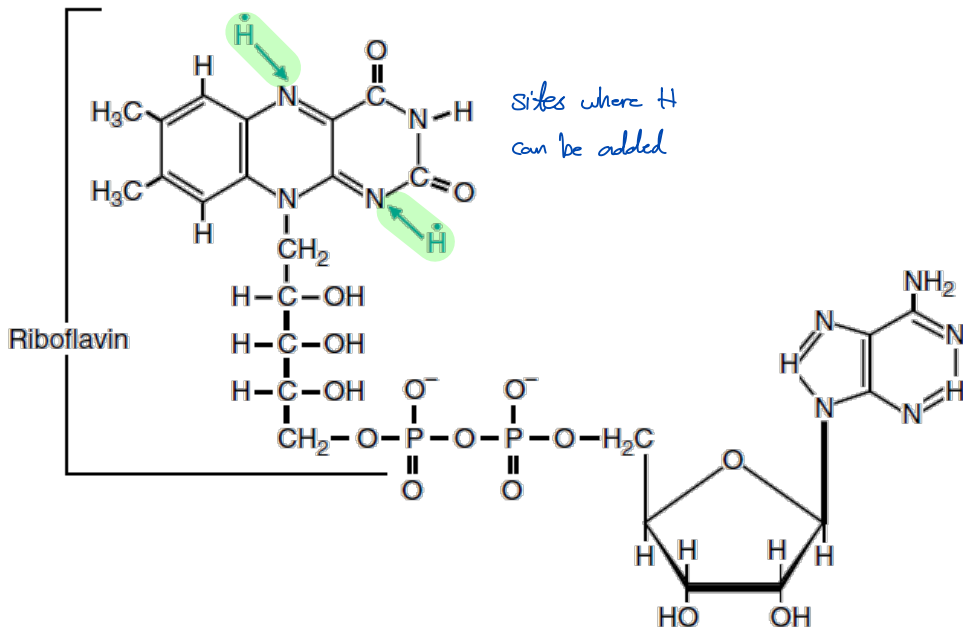
*الأكسجين قد لا يتقبل بشكل كامل :-*

*it may become superoxide ion → Free radical (ROS)  
\* it's not always a bad thing, it may have positive sides, it's used as a mechanism by the immune sys. to fight bacteria & viruses that infect ourselves, by producing Anti-oxidants like: Vit C, Vit E, ...*

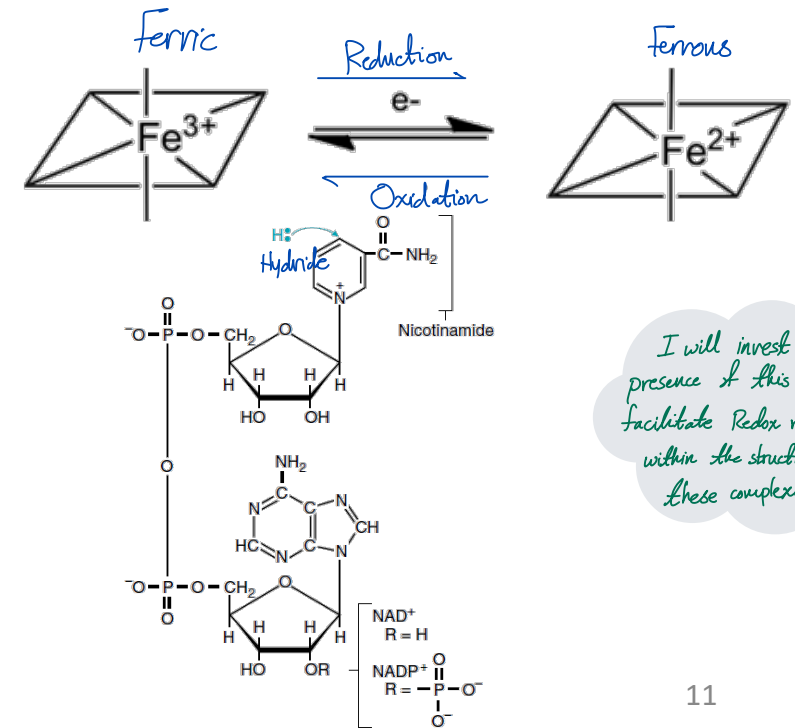


# Types of electron transfer (ET) through the electron transport chain (ETC)

- **3 types of ET occur in OxPhos:**
  - **Direct ET, as in the reduction of  $\text{Fe}^{+3}$  to  $\text{Fe}^{+2}$**  In heme group of hemoproteins
  - **Transfer as a hydrogen atom  $\{(\text{H}^+) + (\text{e}^-)\}$**  In  $\text{FADH}_2$
  - **Transfer as a hydride ion  $(:\text{H}^-)$**  In  $\text{NADH}$



Cytochromes are a specific type of hemoproteins. Unlike hemoglobin where iron remains in the ferrous ( $\text{Fe}^{2+}$ ) state to bind oxygen, cytochromes' iron is reversibly converted between ferrous ( $\text{Fe}^{2+}$ ) and ferric ( $\text{Fe}^{3+}$ ) states. This redox cycling enables cytochromes to carry out their primary function of electron transfer. (slide 12)

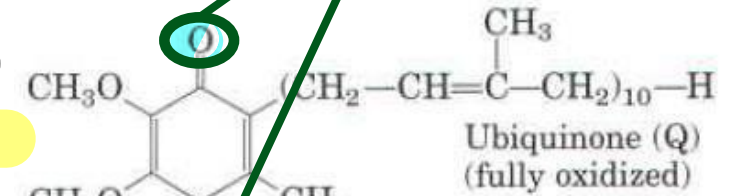


*I will invest the presence of this iron to facilitate Redox reactions within the structure of these complexes.*

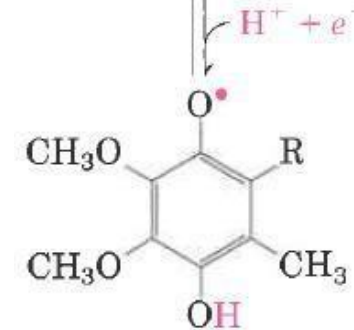
# Other electron-carrying molecules

## “Ubiquinone”

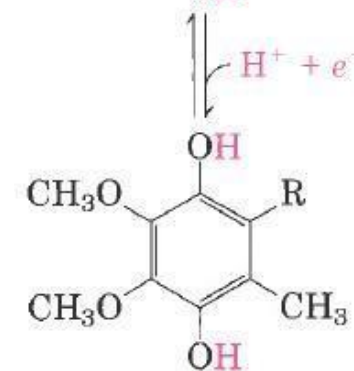
The place where oxidation & reduction happens (ketones)



Ubiquinone is Nonpolar, freely movable, and contributes to different reactions



Semiquinone radical (\*QH)



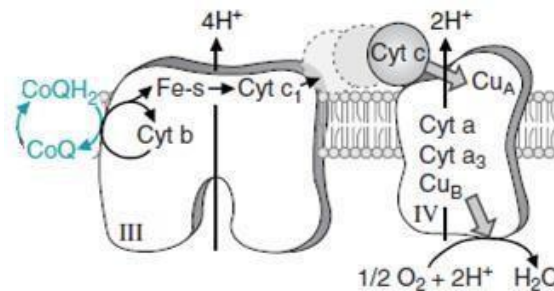
Alcohol  
Ubiquinol (QH<sub>2</sub>) (fully reduced)

- Also called coenzyme Q, or Q (ubiquitous in biologic systems)
- Lipid-soluble benzoquinone with a long isoprenoid side chain
- Small & hydrophobic (freely diffusible)
- Carries electrons through the IMM
- Can accept either 1 e<sup>-</sup> or 2 e<sup>-</sup>
- Act at the junction between a 2-electron donor and a 1-electron acceptor
- Sometimes prescribed for recovering MI patients

Myocardial infarction

Less blood supply  $\rightarrow$  less nutrients & O<sub>2</sub> to cells  $\rightarrow$  Ischemia  $\rightarrow$  cell death.  
Prescribing CoQ to MI patients enhances ETC.

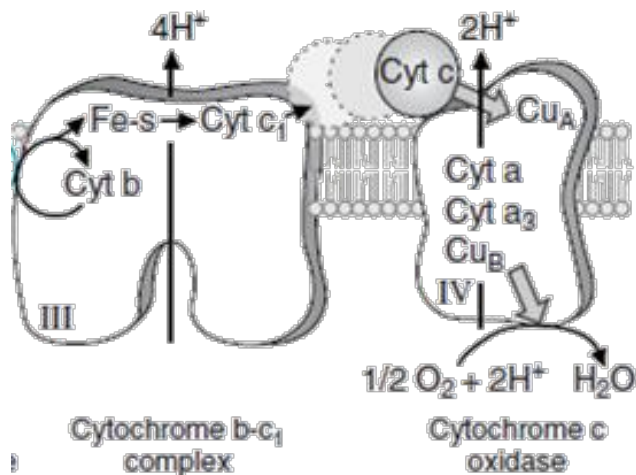
CoQ enhances mitochondrial energy production, reduces oxidative stress, improves endothelial function, and helps prevent adverse cardiac remodeling, leading to better clinical outcomes.



# Other electron-carrying molecules

## “Cytochromes”

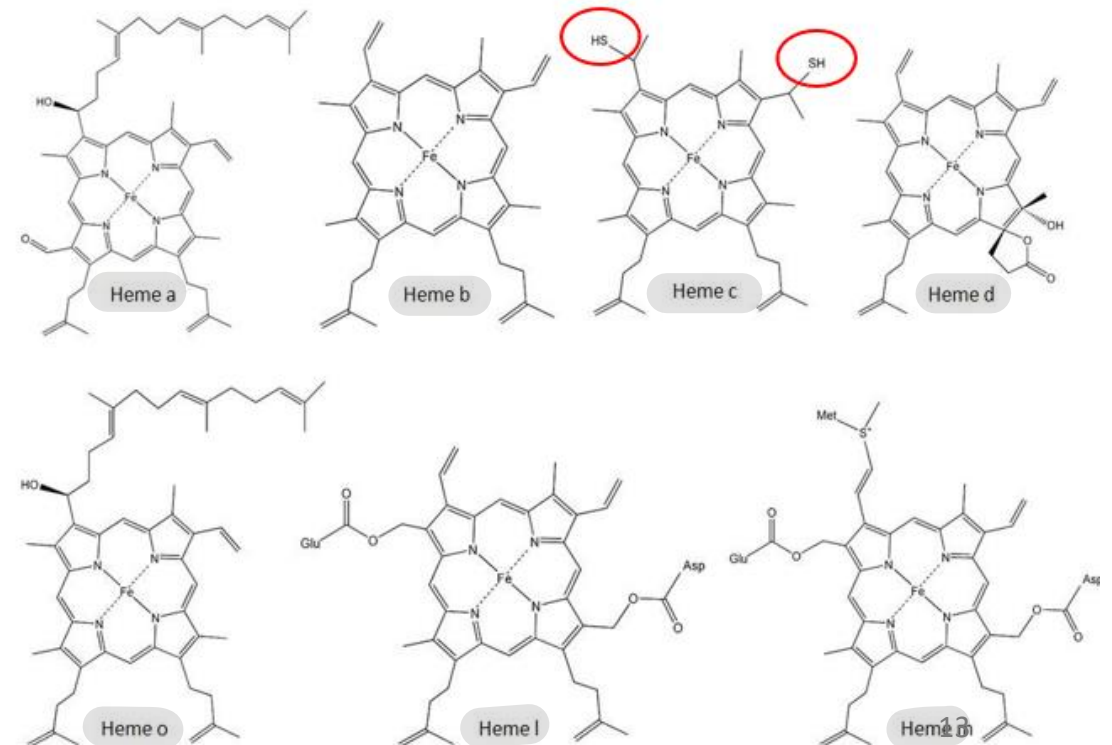
- **Proteins with Fe-containing heme prosthetic groups**
- **Mode of binding (a, b, c)**
- **Mitochondria contain three classes of cytochromes (a, b & c)**



The heme which is a **porphyrin ring (a type of tetrapyrrole) + iron atom in its center.**

**The way the heme group binds to proteins can vary, leading to different types of heme.**

**For example: Heme B is attached to proteins through a single coordination bond between the heme iron and an amino acid sidechain, while Heme C binds to proteins by thioether bonds between cysteine's (SH) groups in it.**



# Other electron-carrying molecules

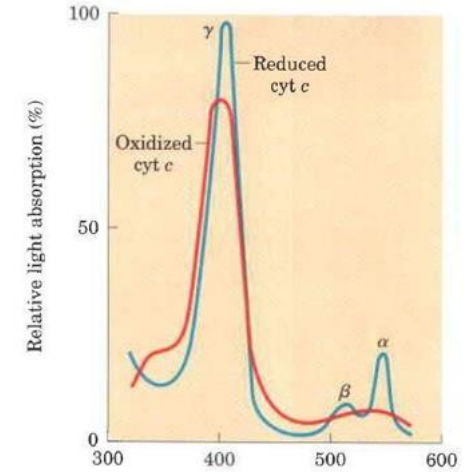
*if we exposed these different types of cytochromes, they can absorb light at different wavelengths.*

## “Cytochromes”

Don't worry about numbers, just understand the concept

- **Light absorption:** Each cytochrome in its reduced ( $\text{Fe}^{+2}$ ) state has 3 absorption bands in the visible range
- **$\alpha$  band:** near 600 nm in type a; near 560 nm in type b, & near 550 nm in type c
- Some cytochromes are named by the exact  $\alpha$  band wavelength:
  - Cytochrome  $b_{562}$ ; Cytochrome  $c_{550}$ ; Cytochrome  $c_{551}$
- Heme can carry one electron
- $\Delta E^{\circ'}$  depends on the protein
- Cytochromes a, b & c are transmembrane (c is the exception)

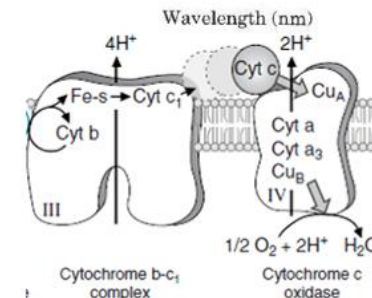
Take home message: They vary in their light absorption patterns according to their heme type, and their oxidation state (oxidized/reduced).



In the electron transport chain (ETC), the standard reduction potential ( $E^{\circ'}$ ) increases progressively, indicating that each subsequent component has a higher reduction potential than the previous one. This higher  $E^{\circ'}$  value allows for efficient electron acceptance as electrons are transferred through the chain. The exergonic process of ATP synthesis relies on these favorable reactions, where the flow of electrons to components with increasingly positive  $E^{\circ'}$  values creates a gradient essential for maximizing energy yield in cellular respiration.

Cyto. c is associated with the outer face of the IMM, and is a mobile carrier of electrons

*(it's in the outer leaflet of the inner mitochondrial membrane)*



# Requirements of OxPhos

- Redox reaction: electron donor (NADH or FADH<sub>2</sub>) & electron acceptor (O<sub>2</sub>)
- An intact IMM
- ETC of proteins
- ATP synthase

