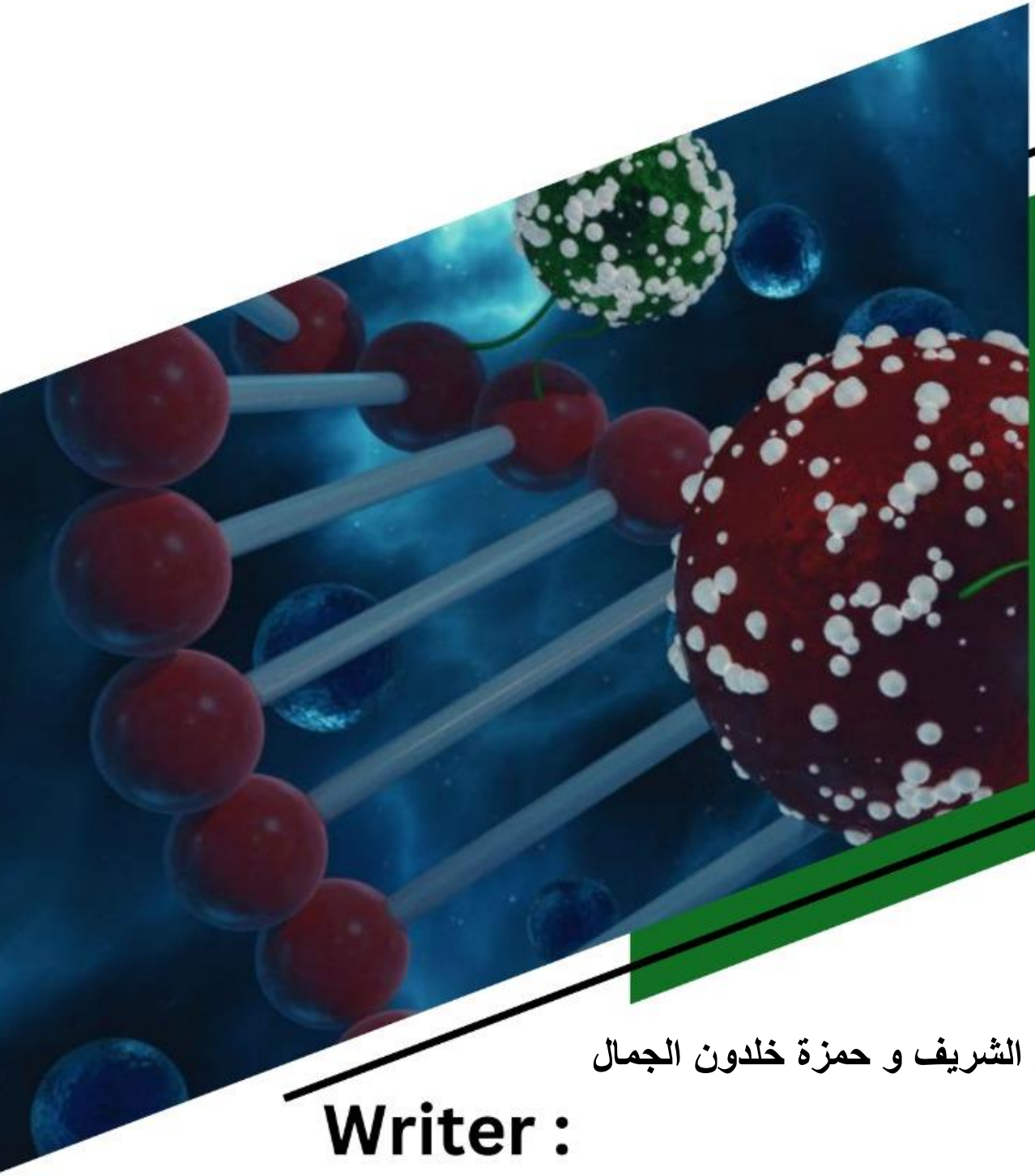
Doctor 021

# METABOLISM

## Sheet no. 7

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# MITOCHONDRIAL MEMBRANES

Quick revision:

**Inner membrane: 22% cardiolipin, No cholesterol.**

**Outer membrane: Similar to cell membrane, less than 3% cardiolipin and 45% cholesterol.**

The difference in potential energy drives the transfer of electrons.

Protons will move from inside IMM to outside against its concentration gradient so this will put more and more pressure on the membrane causing the influx of protons through ATP Synthase.

The absence of cholesterol, cardiolipin(22%) and having a high amount of protein will lock the IMM and make it impermeable to anything in this life even to H+ accordingly anything wants to move through this membrane needs a carrier.

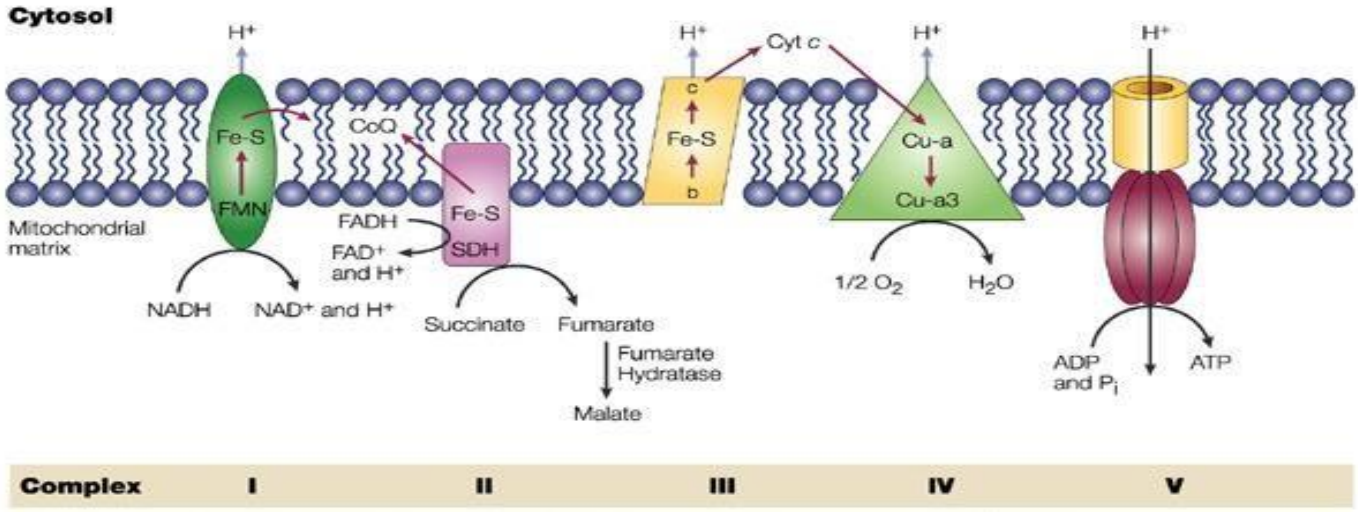
The sources of electrons in ETC that we got from Krebs cycle are NADH and FADH2.

Electron transport chain is composed of complexes (enzymes) that have the ability of passing electrons due to their structure (this will cause multiple redox reactions).

So why these enzymes can act as oxidoreductases? because they have non-proteinous components (these components could be specific structures like Metals, Heme, FMN and FAD).

Vitamins are required to make Co enzymes.

***Now lets start talking about ETC in details:***




ETC starts from complex one that takes up electrons from NADH oxidizing it and converting it to NAD+ that's why we call it NADH dehydrogenase.

Complex one is an oxidoreductase because it contains structures that enable it to function as oxidoreductase for example FMN (flavin mononucleotide) which is a structure resembling FAD but without the adenosine part, it has the capacity of carrying two electrons (it takes up electrons from NADH and get reduced ).

As you know complex one is large so FMN passes those electrons through beautiful structures called Iron-Sulfur clusters (Fe-S in the figure) here in complex one  we have 7 Iron sulfur clusters that pass one electron through them at a time reaching complex one surface and getting ready to leave complex one (WHAT A SAD STORY!!!) after one electron passes the other one enters.

The Iron-Sulfur cluster can only carry one electron at a time because iron has only two states of ionization Fe+2 and Fe+3.

As we all know proteins movement in membranes is nearly restricted so we need a special carrier to transport those electrons to their second destination (complex three) so we call the man of difficulties…….Co-Q (which will deliver those 2e- to complex three).

There is not any direct relation between complex one and complex two (both send their electrons to complex three).

There is a way of naming enzymes special for oxidoreductases: Substrate-product oxidoreductase.

In our case complex one takes up the electrons from NADH (so NADH is the substrate) and gives them to Co-Q ( so Co-Q is the product) accordingly complex one is called NADH-CoQ Oxidoreductase.

As mentioned before the first source of electrons is NADH (complex 1) and the second source is FADH2 as we know from Krebs cycle, FADH2 is not free it is embedded inside succinate dehydrogenase.

Succinate dehydrogenase is the enzyme that catalyzes step 6 in Krebs cycle and it is the same as complex 2 in ETC, it is the ONLY DIRECT link between Krebs cycle and ETC.

It is the only enzyme of Krebs cycle that is embedded in IMM others are free in the mitochondrial matrix.

In complex 2 FADH2 provides 2e- to Iron-Sulfur clusters which deliver electrons to the surface of the protein to be taken by Co-Q reaching their next destination (complex 3) .

Iron-Sulfur clusters in complex 1 and 2 work in the same mechanism.

There are two other names for complex 2 (succinate dehydrogenase AND succinate-CoQ oxidoreductase).

Is succinate dehydrogenase the only source of electrons loaded on FADH2? NO there are two other sources (fatty acid Co-A Dehydrogenase, mitochondrial glycerol phosphate dehydrogenase ) we will cross over them later.

Electrons reach complex 3 directly through Co-Q and indirectly through complexes 1 or 2.(**It seems to be a Good exam question**).

Complex3 gives those electrons gained by Co-Q to cytochrome-C so it is called CoQ-Cytochrome C Oxidoreductase.

Enzymes that have heme in their structure and this heme has a role in electron transfer are called Cytochromes.

Complex 3 has two kinds of heme (C1 and B ) so in this case we have Cytochrome BC1 complex.

Complex 3 takes up the two electrons from CoQ and transfer them to Cytochrome C (which contains one heme) so it can carry only one electron at a time but because we have huge amount of Cytochrome C ,the two electrons are delivered.

Complex 4 works as oxidoreductase because it contains two copper ions (that switch between two states Cu+1 and Cu+2), heme A and heme A3.

There are other two names for complex 4: Cytochrome-AA3 and Cytochrome-C oxidase.

In complex 4 we have two coppers and two hemes, so we need 4 electrons to fully reduce complex 4.

Note: Copper can carry one electron and heme contains an iron that can carry one electron too.

The carrier that we've used to carry electrons from one complex to another should have special characteristics to function:

1) mobile.

2)lipid soluble.

But those two characteristics are opposite to each other as lipid soluble substances cannot transport electrons (charges) because they are non-polar so how does this happen???!!

Ans:The carrier itself has structure that can carry electrons and another structure that controls the movement through the membrane.

When electrons are moving from complex 3 to complex 4 they need another carrier(Cytochrome C ),however this carrier is not present in the membrane (as you can see in the figure in page 2) ,it is not embedded in the membrane , this is because Cytochrome -C is a protein which is not lipid soluble.

When electrons are being passed from complex one to Co-Q there is a difference in potential this will lead to difference in energy (about 12 Kcal)which is used to pump protons.

If two electrons are passed from NADH through complex 1 to Co-Q the difference in energy that comes from their transfer is capable of pumping four protons outside (if the difference of potential between the two sides of the membrane is more than 12 Kcal there will be no protons pumping).

When these two electrons pass from Co-Q to Cytochrome C through complex 3, there will be a difference in free energy also capable of pumping another 4 protons.

After they reach complex 4 the difference of free energy is capable of pumping only two protons.

The total protons pumped due to the transport of NADH's two electrons to oxygen is 10 protons.

Let's talk about another source of electrons (FADH2) when the electrons come from FADH2 inside succinate dehydrogenase reaching Co-Q, the difference in energy is very minimal and not enough to pump protons

فبالتاااااالي .....

There is no need for complex 2 (succinate dehydrogenase) to span the membrane as it won't pump any protons.

Side note complex2 is the only enzyme taking place in the inner side of the IMM.

The energy produced from transporting electrons taken from FADH2 is used to pump protons as the following:

No protons are pumped when electrons arrive to complex 2 from FADH2.

4H+ are pumped when electrons arrive to complex 3.

2H+ are pumped when electrons arrive to complex 4. ***In total 6 protons are pumped***.

Recap: we generate ATP when protons turn back to the matrix through ATP Synthase, so the more protons pumped out, the more protons turn back through ATP Synthase as a result more ATP is generated.

We need 4 protons (H+) to generate one ATP.

As NADH pumps 10 protons it generates 2.5 ATP, on the other hand FADH2 generates 1.5 ATP because it pumps 6 protons.

# TYPES OF ELECTRON TRANSFER(ET) THROUGH THE ELECTRON TRANSPORT CHAIN (ETC)

**3 types of ET occur in OxPhos:**

**1- Direct ET, as in the reduction of Fe+3 to Fe+2 (heme and copper)**

**2- Transfer as a hydrogen atom {(H+ ) + (e- )} (FAD becomes FADH2 )**

**3-Transfer as a hydride ion (:H- )**

# OTHER ELECTRON-CARRYING MOLECULES "UBIQUINONE"

Quinones as a family are defined as cyclic-diene structures.

## Ubiquinone and Co-Q are the same.

As the name implies Ubiquinone is a ketone it has the capacity to take 2 e- (after taking those electrons it will be fully reduced to an alcohol, it will be called Ubiquinol).

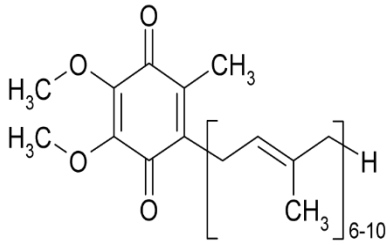
When it is not fully reduced (when it takes only one electron it forms a free radical called semi-quinone or semi-quinol).

The more amount of ubiquinone we have the more:

1- Electrons will be transferred from complex 1 to complex 3.
2- Protons will be pumped.
3- ATP will be generated.
   بسرع انتاج الطاقة
   It is a natural supplement that is given to patients with myocardial

Infarction (heart attack, جلطة) where we have dead tissue not capable of generating ATP, so we give patients ubiquinone to make the live tissue generate more ATP to compensate the lack of ATP.

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




# OXI-RED COMPONENTS OF THE ETC "NADH DEHYDROGENASE -COMPLEX 1 "

**-NADH-Q oxidoreductase**

**-More than 25 polypeptide chain**

**-A huge flavoprotein membrane-spanning complex**

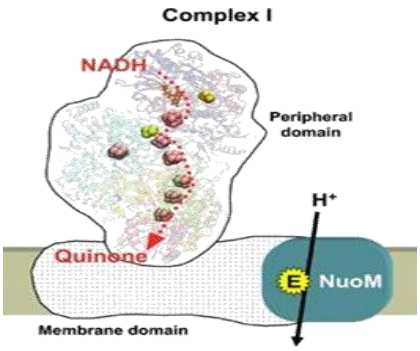
**-The FMN is tightly bound**

**-Seven Fe-S centers of at least two different types**

**-Drop in energy ≈ 13 to 14 kcal**

**-Binds NADH & CoQ**

**-4 $H^+$**



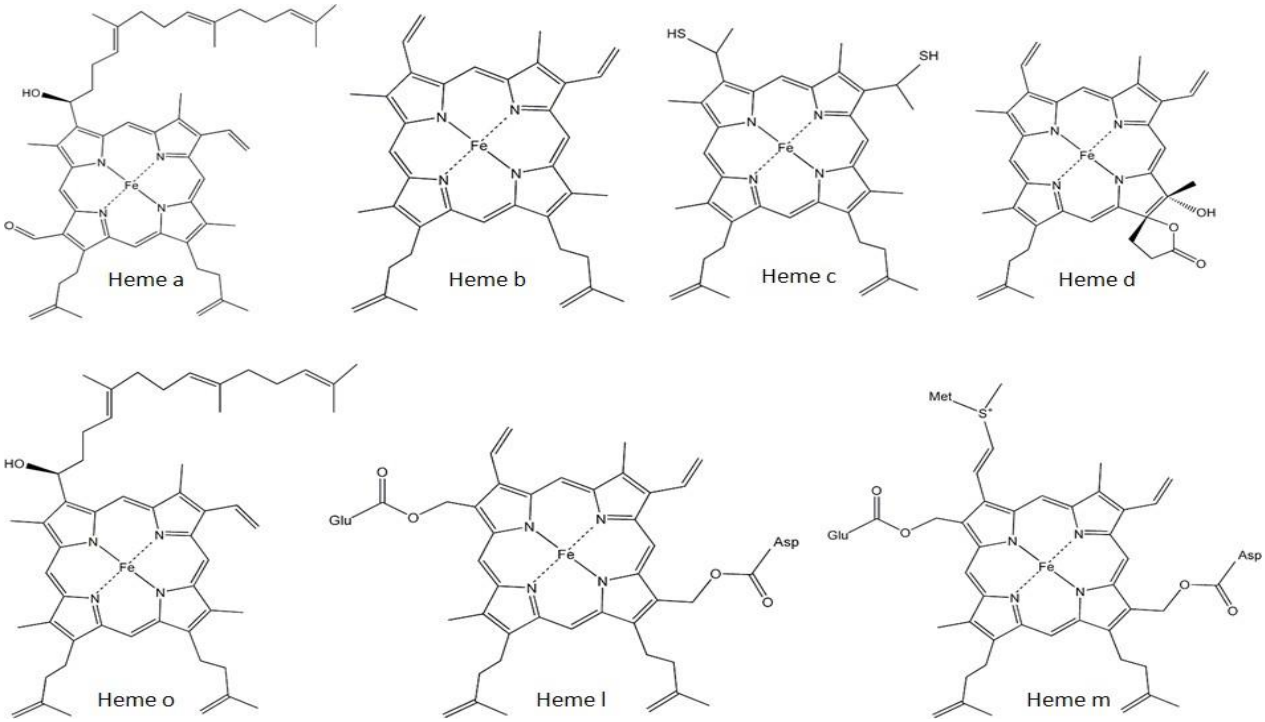

The structure that stabilizes electrons and make Ubiquinone lipophilic is The hydrophobic hydrocarbon chain (called polyisoprene).

The isoprene itself is short but in the Ubiquinone it is repeated (6-10 times).

# OTHER ELECTRON-CARRYING MOLECULES "CYTOCHROME"

Cytochromes have heme and this heme functions in electron transfer and the Cytochrome is named according to the heme kind it contains.

Heme molecules have a lot of types all of them share a macro-cyclic ring structure (Porphyrin ring).




- **Proteins with characteristic strong absorption of visible light (Fe-containing heme prosthetic groups)**
- **Classification based on light absorption**
- **Mode of binding (a, b, c)**
- **Mitochondria contain three classes o f cytochromes (a , b, & c)**
- **Light absorption: Each cytochrome in its reduced (F+2) state has 3 absorption bands in the visible range .**

**In solution we can differentiate between different types of heme …. The oxidized heme has a peak when you expose it to a light it gives you spectroscopically a peak around 400 nm when we reduce it, it will show 3 peaks (alpha, beta and gamma) the center of the alpha peak is what differentiate from one heme and other.**

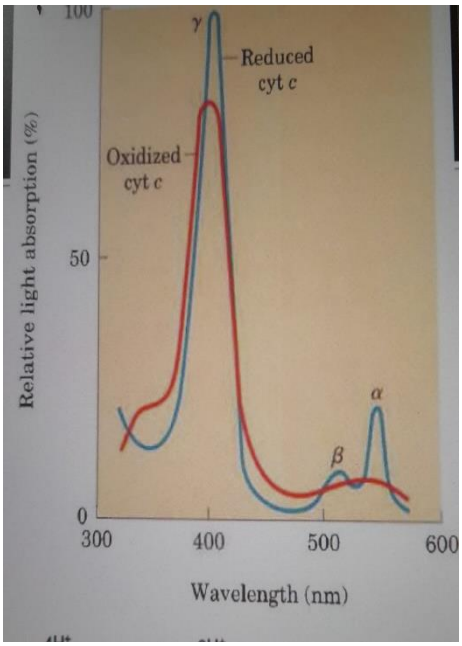
¬ α band : near 600 nm in type a; near 560 nm in type b, & near 550 nm in type c

¬ Some cytochromes are named by the exact α band wavelength: ⎫ Cytochrome b562; Cytochrome c550; Cytochrome c551

¬ Heme can carry one electron

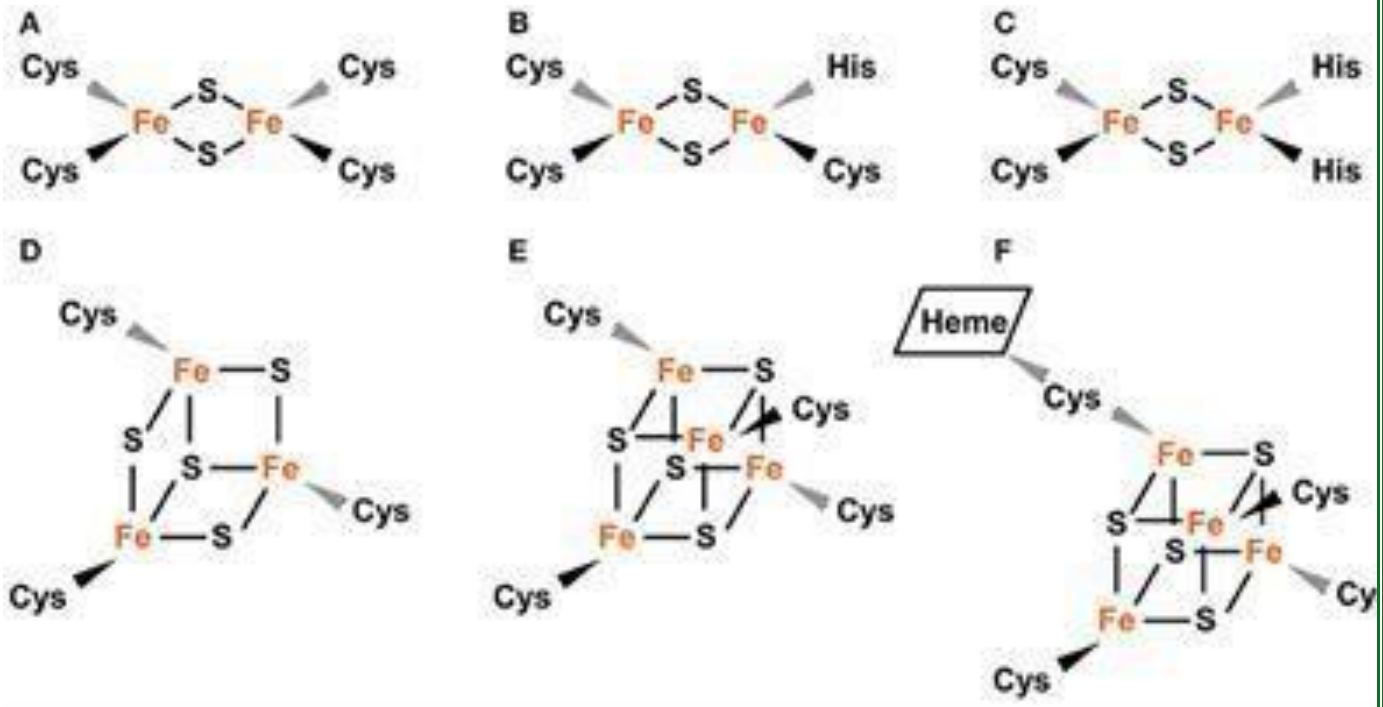
¬ ΔE o ’ depends on the protein

¬ Cytochromes a, b & c are transmembrane (c is the exception)




# OTHER ELECTRON-CARRYING MOLECULES IRON-SULFUR CLUSTERS

Just have a look




What do we need to make a complete oxidative phosphorylation:

1- Source of electrons
2- Complexes
3- Final e- acceptor (O2)
4- Intact IMM

5- Pumps

6- ATP synthase

# OTHER ELECTRON-CARRYING MOLECULES IRON-SULFUR CLUSTERS

**-Redox reaction: electron donor (NADH or FADH2) & electron acceptor (O2).**

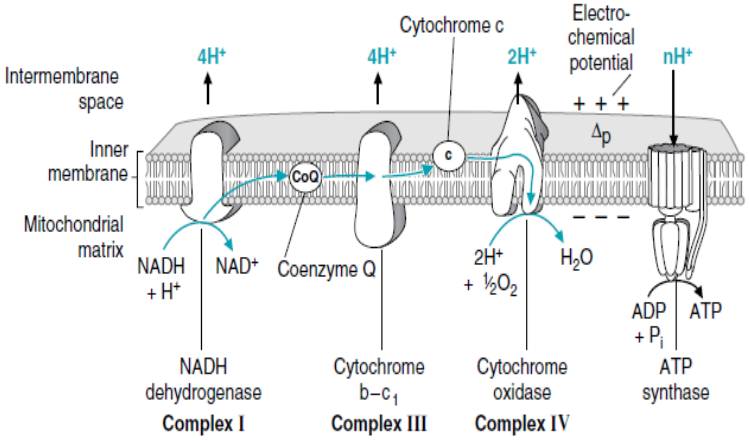
**-An intact IMM.**

**-ETC of proteins.**

**-ATP synthase.**

**-Membrane is impermeable to protons.**

**-≈ - 12 kcal with each step.**



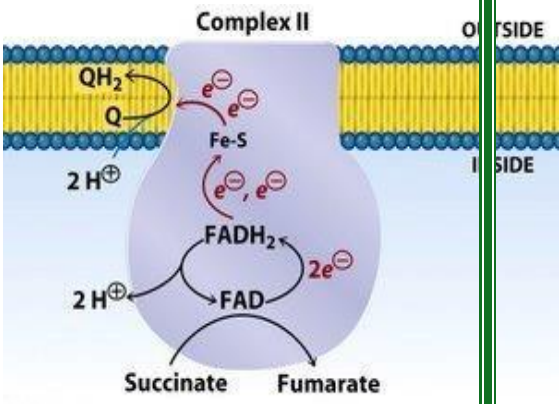

# Oxi-red component of the ETC "SUCCINATE DEHYDROGENASE" complex-2

**-Succinate Dehydrogenase & other flavoproteins**

**-TCA cycle**

**-ETF-CoQ oxidoreductase (ex. fatty acid oxidation)**

**-≈ 0 kcal, H+?**




**-substrates oxidized by FAD linked enzymes by pass complex 1**

**- Three major enzyme systems :**

**1- Succinate dehydrogenase.**

**2- fatty acyl Co-A dehydrogenase.**

**3-Mitochondrial glycerol phosphate dehydrogenase.**

# Oxi-red component of the ETC " cytochrome bc1" complex 3

-Also called Q-cytochrome c oxidoreductase.

- catalyzes the transfer of electrons from QH2 to cytochrome c.

-11 subunits including 2 cytochrome subunits.

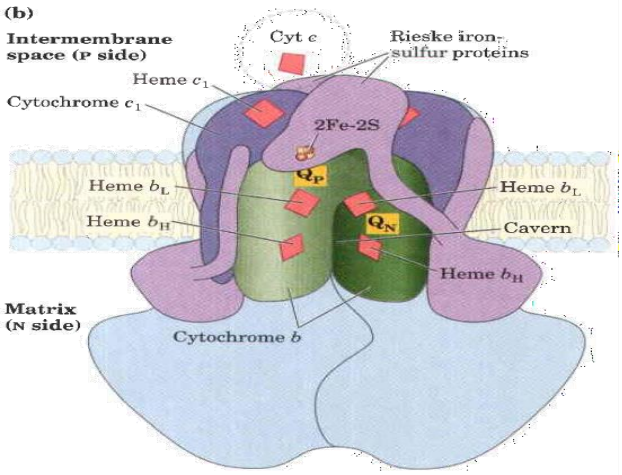
- Contains iron-sulfur center.

-contain three heme groups in two cytochrome subunits.

-bL and bH in cytochrome b ; c type in cytochrome c1.

-Contain two Co-Q binding sites.

-4H+.




# Oxi-red component of the ETC "Cytochrome c oxidase" complex 4:

- Passes electrons from Cytocrome c to O2
- Contains cytochrome a and a3
- Contains two copper sites
- Contains oxygen binding sites
- O2 must accept 4 electrons to be reduced to 2H2O (2H+/2e-)
- Cytochrome c is one electron carrier

$$\text{Cyt } c_{red} + 4H^+ + O_2 \rightarrow \text{Cyt } c_{ox} + 2H_2O$$

- Cytochrome oxidase has a much lower

Km for O2 than myoglobin (hemoglobin, myoglobin,complex IV)

- Partial reduction of O2 is hazardous

What are the structures that allow complex 4 to work as an oxidoreductase ?

1) Heme A and Heme A3

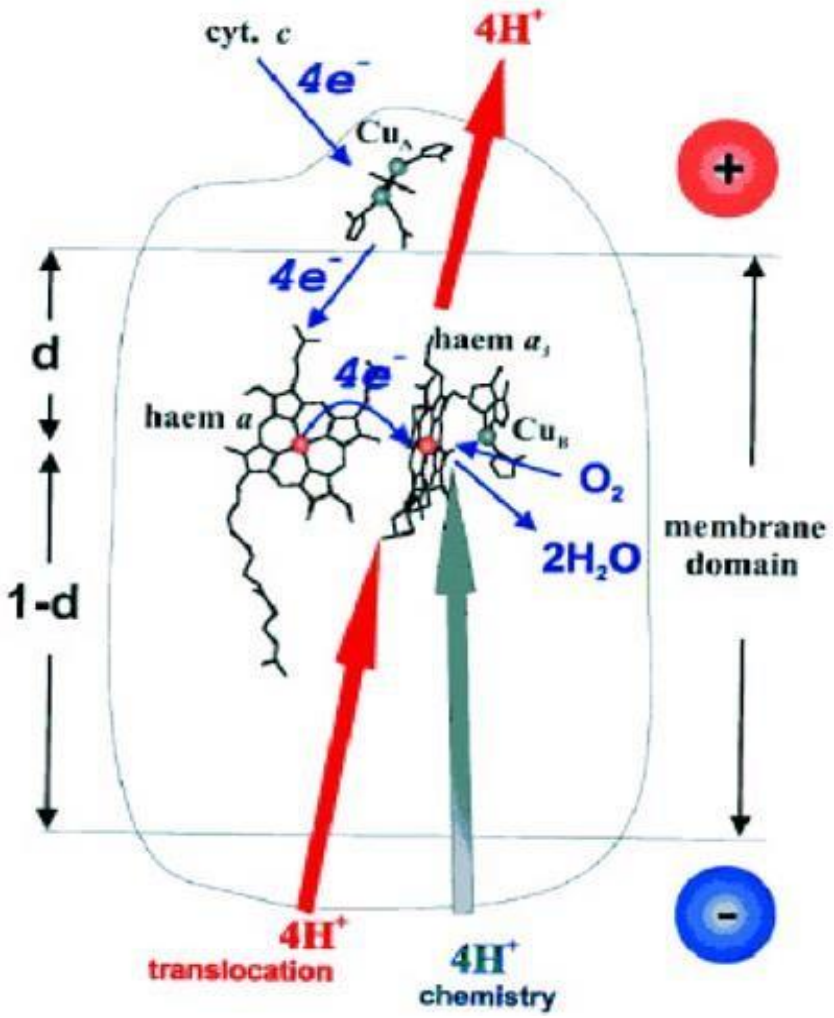
2)Copper A and Copper B

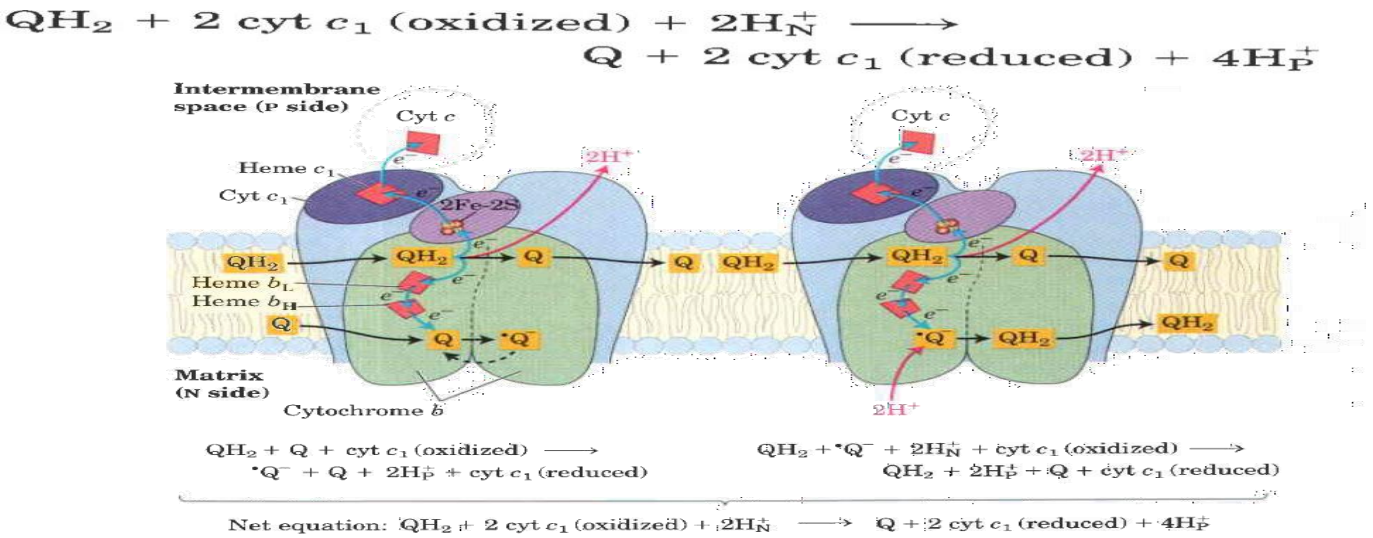
Because Copper A and Heme A are very close to each other the share the electron between them (which is very unique in science).

Second electron then arrives ,accordingly, first electron goes to Heme A3 and Copper B ( also they are very close to each other so the first electron is shared between them ).

The second electron now is shared between Copper A and Heme A.

We need 4 electrons to reduce the molecular oxygen into water.






# THE Q-CYCLE

- **Partial reduction is hazardous**
- **Accommodates the switch between 2e-/1e-**
- **Explains the measured stoichiometry of 4 H+/2e-**

Q cycle explains the stoichiometry of Quinone cycle with respect to ETC ( how com 2 electrons are coming and how come one electron is getting out ??!! )

Complex 3 has two binding sites, one is close to the outer surface of the IMM and the other is close to the inner surface of the IMM.

The one which is close to the outer surface has high affinity for Ubiquinol ( the reduced Co-Q which comes with 2 electrons )

The other one which is close to the inner surface of the IMM has high affinity for Ubiquinone ( the oxidized form of Co-Q)

The first molecule of the reduced Co-Q arrives complex 3 ( loaded with two electrons ) , the first electron will be donated to the Iron-Sulfer clusters , heme C1 then to Cytochrome C

The second electron will pass down the complex by being donated to the first heme B, to the second heme B reaching the inner surface of IMM and then ( because the oxidized form has high affinity to this binding site ) Ubiquinone gets partially ruduced, so its name will be Semi-Reduced Upiquinone (or Semi-Oxidized Ubiquinol) .

The partially Reduced form still has affinity for this binding site ( it's still gonna bind to the inner surface of the IMM).

Once the reduced Co-Q donates its two electrons, it becomes oxidized so there is no affinity for its Binding site ( it's gonna release from the outer surface of the IMM ).

After that another reduced Co-Q comes.....donating the first electron to Cytochrome C through Iron-Sulfer clusters and Cytochrome C1 " as mentioned before ".

And the second electron is transferred to heme B and to the other heme B meeting the partially reduced Co-Q then this Co-Q become fully reduced ( becomes Ubiquinol)يعني رجع زي ما كان .

So we generated one molecule out of two being reduced.

SOME STATISTICS

We used 2 reduced Ubiquinols ( two reduced Co-Q), we regenerated one.

We used 4 electrons, we regenerated 2 ( so the net use of electrons is 2 electrons).

These 2 electrons are coupled to how many Cytochrome C molecules ???

Ans: 2 Cytochrome C

The stoichiometry now is clear.

# THE RIGHT ARRANGEMENT…HOW CAN WE PROVE IT?

## 1. Measuring the standard reduction potentials (real?)

How did we know the right sequence of ETC?… in other words how did we know the right arrangement of steps?

Potential is the answer but how to know it?

In the lab by measuring the standard reduction potential of all molecules in ETC and know the arrangement.

| Redox reaction (half-reaction) | $E'^{\circ}$ (V) |
|---|---|
| $2H^+ + 2e^- \longrightarrow H_2$ | −0.414 |
| $NAD^+ + H^+ + 2e^- \longrightarrow NADH$ | −0.320 |
| $NADP^+ + H^+ + 2e^- \longrightarrow NADPH$ | −0.324 |
| NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$) | −0.30 |
| Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol | 0.045 |
| Cytochrome $b$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $b$ ($Fe^{2+}$) | 0.077 |
| Cytochrome $c_1$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $c_1$ ($Fe^{2+}$) | 0.22 |
| Cytochrome $c$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $c$ ($Fe^{2+}$) | 0.254 |
| Cytochrome $a$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $a$ ($Fe^{2+}$) | 0.29 |
| Cytochrome $a_3$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $a_3$ ($Fe^{2+}$) | 0.35 |
| $\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$ | 0.8166 |

But is this real? Is it what happens in the mitochondria?... we don't know so another method is used.

## 2. Reduction of the entire ETC with no O2 (doing the experiment anaerobically).

We put electrons and look at the mitochondria then we see which molecule is reduced before the other.

## 3. Addition of inhibitors.

Put inhibitor for complex 2 then everything before it will be reduced and everything after it will be oxidized.

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وإنّما الأُمَمُ الأخلاقُ ما بَقِيَتْ
فَإِنْ هُمُ ذَهَبَتْ أَخلاقُهُمْ ذَهَبُوا