بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Lecture 6

Oxidative Phosphorylation

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OXIDATIVE PHOSPHORYLATION

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Revision

(Stages of energy metabolism)

1

Ingestion, **digestion** and **absorption** then **distribution** (delivering micro nutrients into the cells

2

Dealing with nutrients inside the cells (breaking them down and forming **Acetyl Co-A**)

3

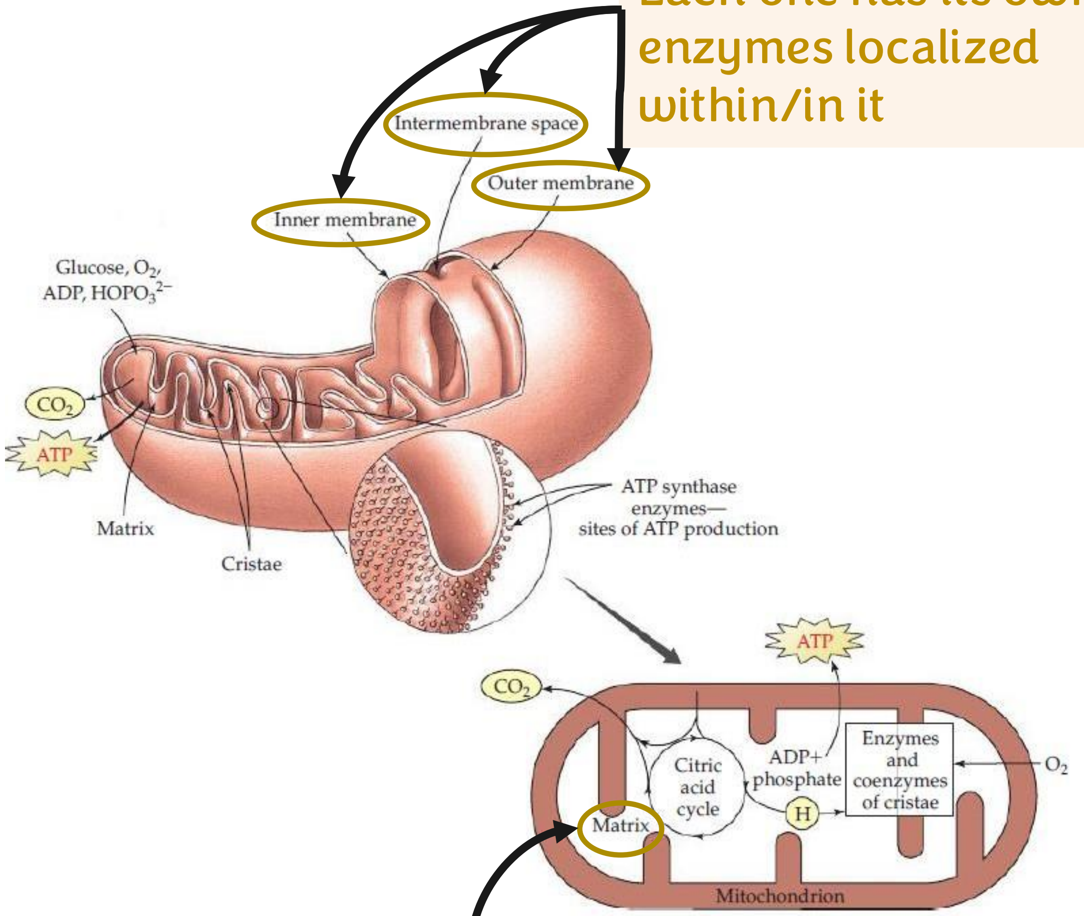
Krebs cycle (production of NADH/FADH2/ATP)

4

Oxidative phosphorylation

MITOCHONDRIAL ARCHITECTURE

Each one has its own enzymes localized within/in it



The matrix has the **enzymes of Krebs cycle except succinate dehydrogenase** (that is localized in the inner membrane for step **no.6 in TCA**), and also **the enzymes of degeradation the carbon skeleton of certain amino acids.**

- OMM: permeable (MW<5,000); (transmembrane channels)
- IMM: impermeable (even to protons H^+); specific transporters
- IMM: components of respiratory chain and ATP synthase
- Matrix: pyruvate dehydrogenase complex; TCA cycle enzymes, fatty acid β-oxidation pathway, and the pathways of amino acid oxidation
- Matrix contains all pathways of fuel oxidation except glycolysis

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

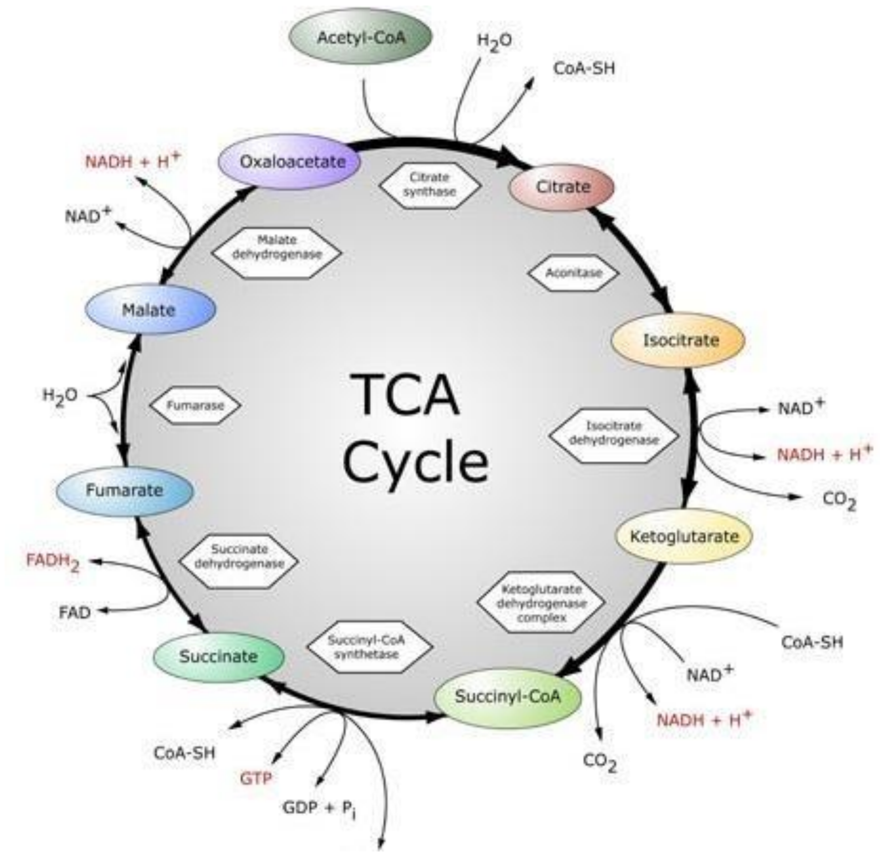
Why the **inner** mitochondrial membrane is **impermeable**?

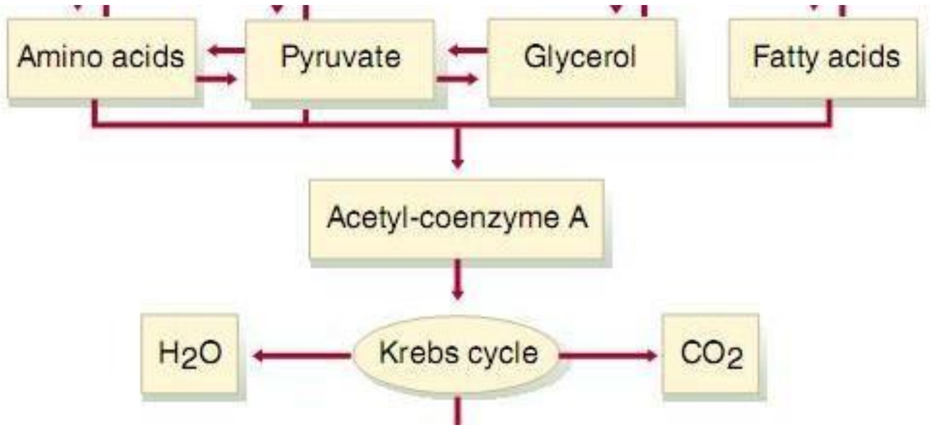
1) The inner membrane has **high** concentration of **proteins** (**channels/ transporters**) and **cardiolipins**, materials that enter or exit the matrix **should pass through transporters or channels that are specific.**

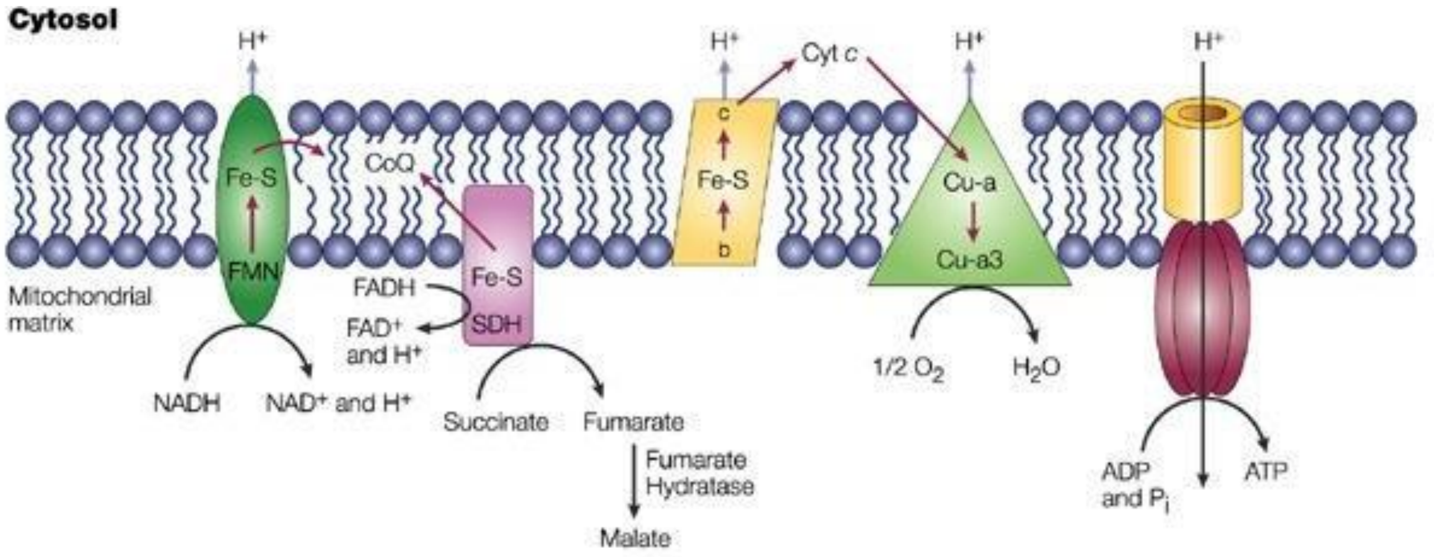
2) The inner membrane has **NO cholesterol** at all , which makes it **very dense** (**NO buffering**) so fatty acids will be **packed** -> **impermeable**.

MITOCHONDRIAL MEMBRANES

- **Inner membrane:**
  - **Impermeable**
  - 22% cardiolipin
  - No cholesterol
- **Outer membrane:**
  - Similar to cell membrane **(porous).**
  - Less than 3% cardiolipin
  - 45% cholesterol







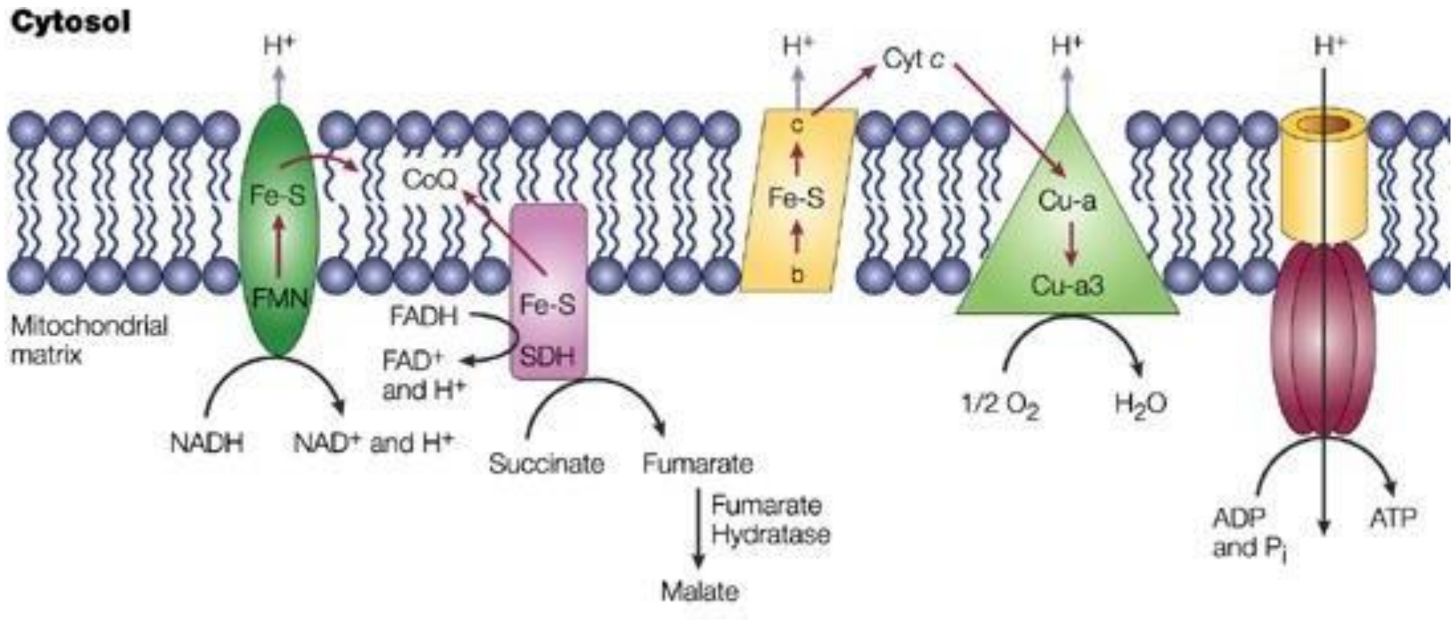
Complex	I	II	III	IV	V
Nuclear gene mutations	*NDU FS1, 2, 4, 7,8* *NDU FV1*	*SDHA, B, C, D*	*BCS1L*	*SURF1, SCO1, SCO2, COX10*	
Phenotype	Leigh syndrome Leukodystrophy	Leigh syndrome Paraganglioma	Hepatopathy Encephalopathy	Leigh syndrome Cardioencephalo-	Cardioencephalo-myopathy

THE OXIDATIVE PHOSPHORYLATION, WHERE ARE WE?

The **last** step of energy metabolism,

It happens in the **inner** mitochondrial membrane.

Stages: Digestion; Acetyl-CoA; TCA; OxPhos



(OXPHOS) OXIDATIVE PHOSPHORYLATION

- Generation of ATP aided by O_2 reduction
- Peter Mitchell (1961): the chemiosmotic theory
- 3 major aspects:
  - ✓ (1) Flow of electrons
  - ✓ (2) The free energy available (exergonic) is coupled to transport of protons
  - ✓ (3) Flow of protons provides the free energy for synthesis of ATP (ATP synthase)

OXPHOS (Oxidation Phosphorylation)

- **Oxphos** is two different processes :

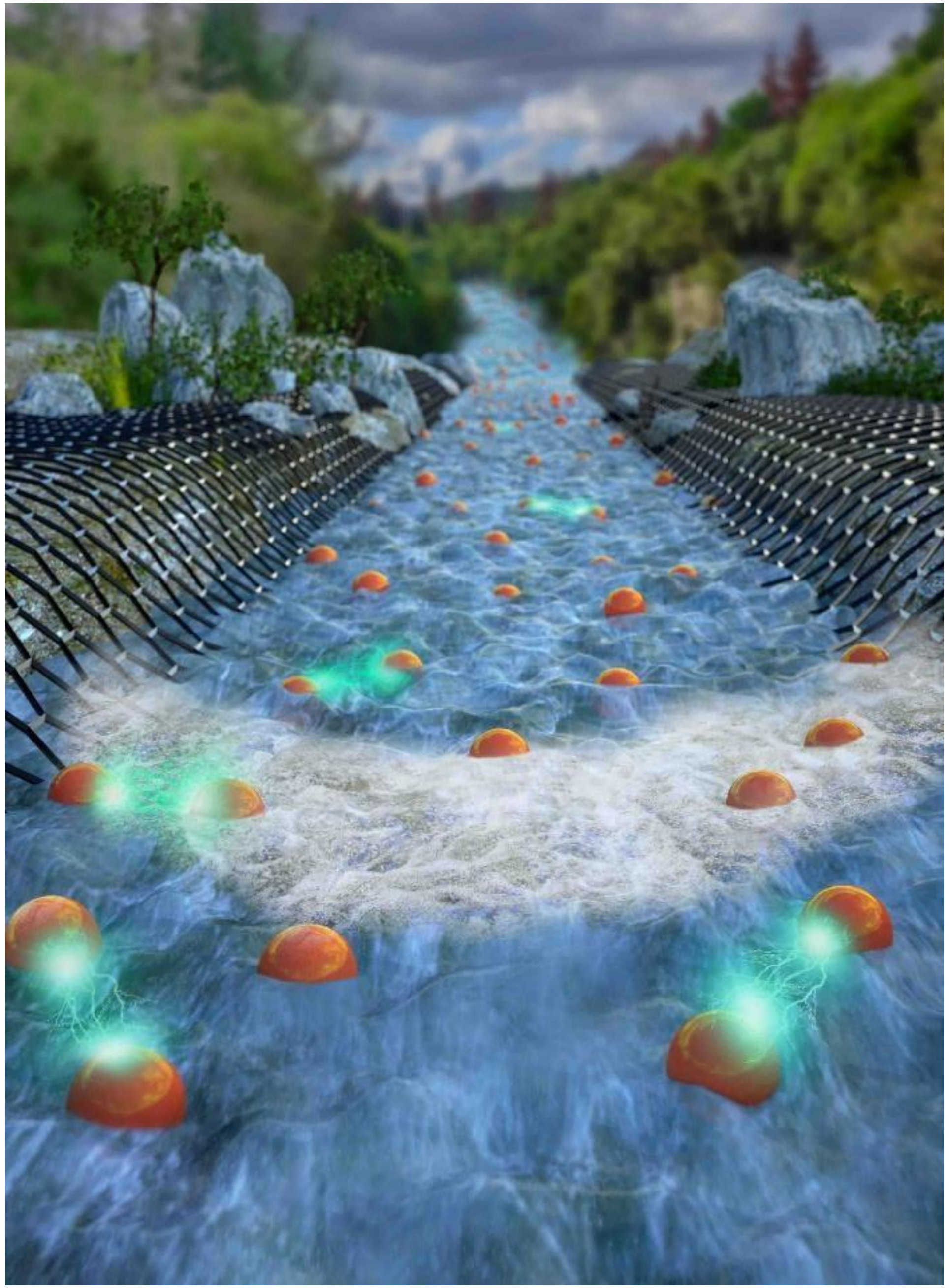
1) **Oxidative** → A series of oxidation reduction reactions that involves the transfer of electrons from one molecule to another (e.g. : Complex I → Complex III)

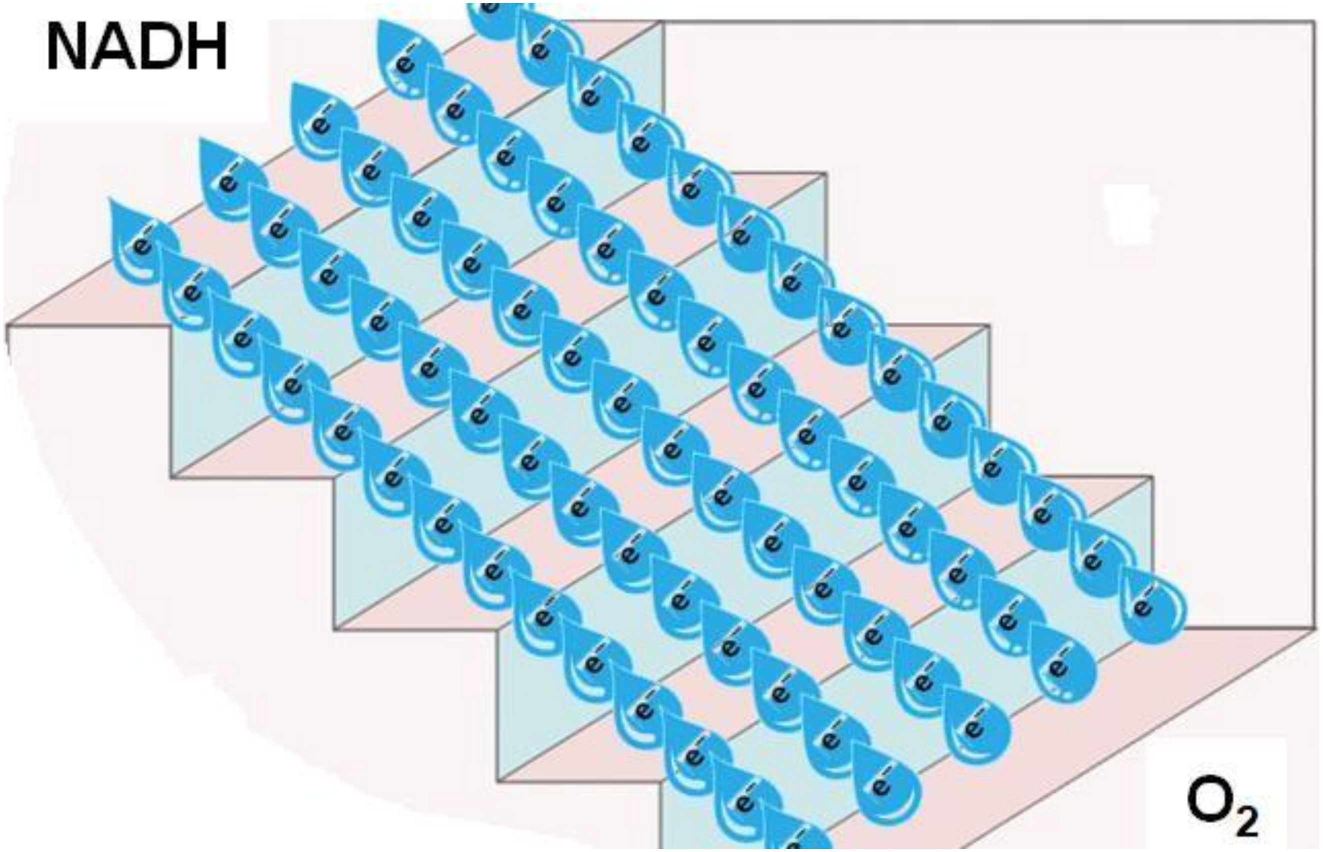
2) **Phosphorylation** →

- Those two reactions are **COUPLED** ; if there is **no oxidation- reduction** there will **be no phosphorylation** and vice versa.

- After Krebs cycle there are electron carrying molecules, electrons are going to pass through different complexes → electron transport chain.

Note: All energy production processes happens inside the mitochondrial matrix except for glycolysis which happens in the cytoplasm.





THE ELECTRON TRANSPORT CHAIN: THE RIVER OF ELECTRONS THAT PROVIDES A STEPWISE ENERGY RELEASE

Because of **potential difference**. Electrons moves from **higher potential** molecule to lower **potential molecule**.

Electron Transport Chain

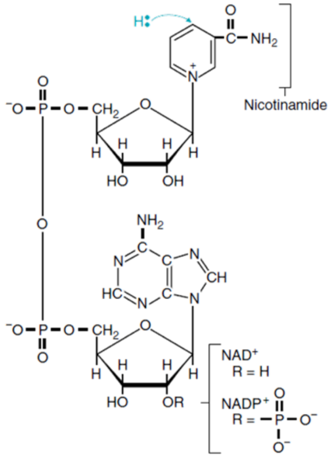
- Electron transport chain occurs because of the **potential difference → difference in energy (ΔG)** **(Also difference in E).**

- There is excess energy provided that is **used to pump (active transport against concentration gradient)** protons from **the mitochondrial matrix** to the **inter membrane space.**

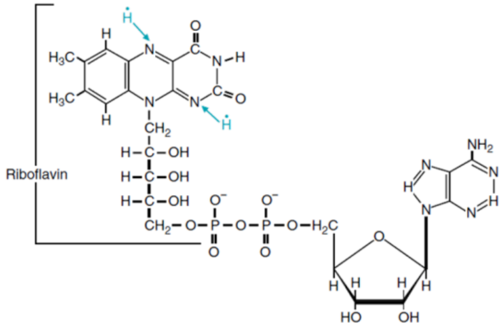
- Protons pumped outside can **only** come back through **ATP synthase** (it is the 3rd arm of the equation)

- This is a passive mechanism which uses energy flow in **protons** to **run ATP synthase enzyme to phosphorylate ADP to ATP.**

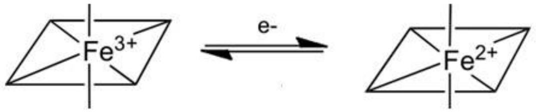
- What links **oxidation- reduction processes** to **phosphorylation** processes is **protons gradient.**



NAD+



FAD



Heme group

THE CAST OF CHARACTERS: ELECTRON CARRIERS

NAD+* , *NADP can carry **2 electrons at a time** in the form of **hydride ion** and it **don't** produce a **free radical** so it can be freely movable inside solutions.

In ***FAD or FMN*** it can carry 2 electrons that are being loaded in a **sequential manner (one electron after the other)**; and it **can** generate a **free radical**; that's why it is always contained within enzymes and **can't** be find **alone** within solutions.

Heme Proteins

- We have **3 types of hemp proteins:**

1) **binding** hemoprotein.

2) **catalytic**, (catalyses a specific reaction, binds to a molecule and releases another, ex. : bind to **hydrogen peroxide (H2O2)** and release **water (H2O).**

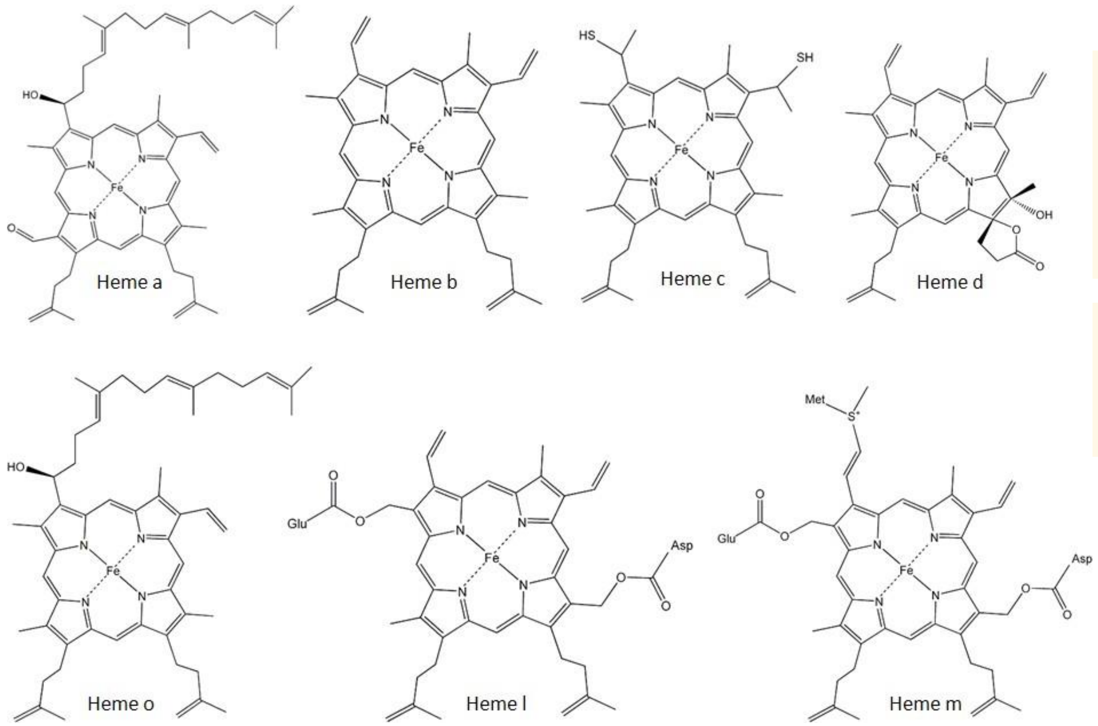
3) **Electron transfer :** hemeproteins that function in electron transport are called : Cytochrome C.

- **Heme** consists of :

1) **Porphyrin ring :**

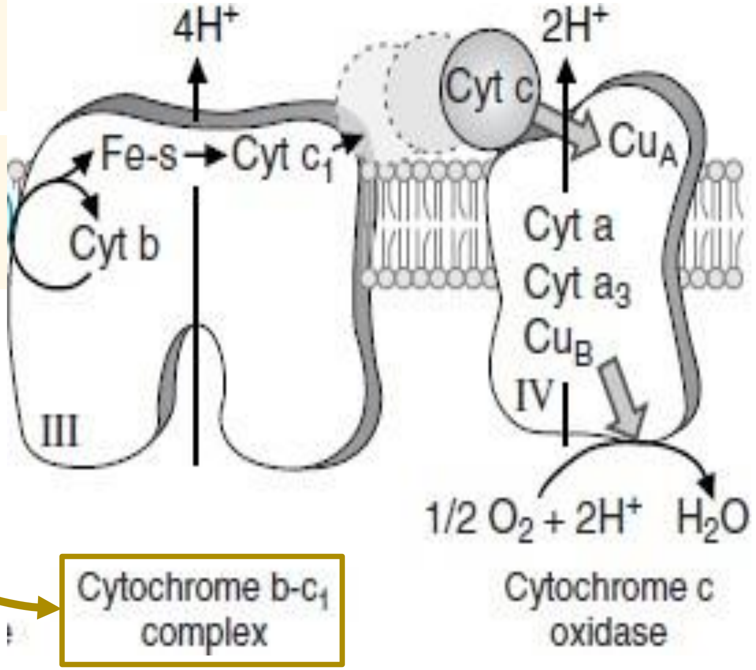
- depending on the **porphyrin ring's attachments** we have different types of hemes.

2) **Iron (Fe+).**



You refer to the cytochrome by the heme group it has.

It has both heme b and heme c.



We can differentiate between different heme rings stucturally, or... (read next 4 slides to know).

OTHER ELECTRON-CARRYING MOLECULES "CYTOCHROMES"

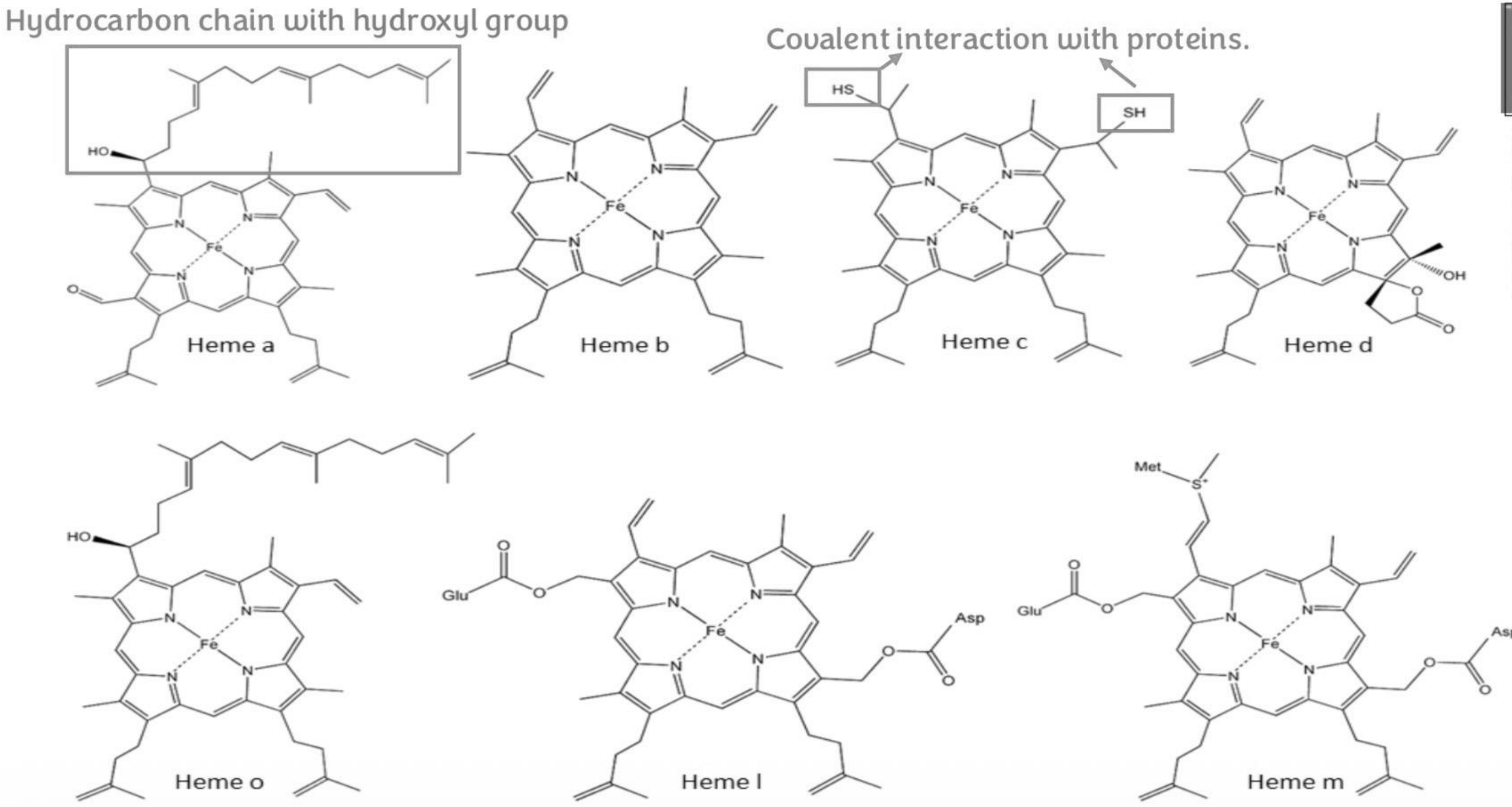
Mitochondria contain three classes o f cytochromes (a , b, & c)

Into To Biochemistry Slides

The next 4 slides are from the summer course in order to remember how to differentiate between different heme groups structurally & spectroscopiclly.

STRUCTURES OF PORPHYRIN & HEME

There are so many types of heme groups depending **on the side chains binding to the Heme**



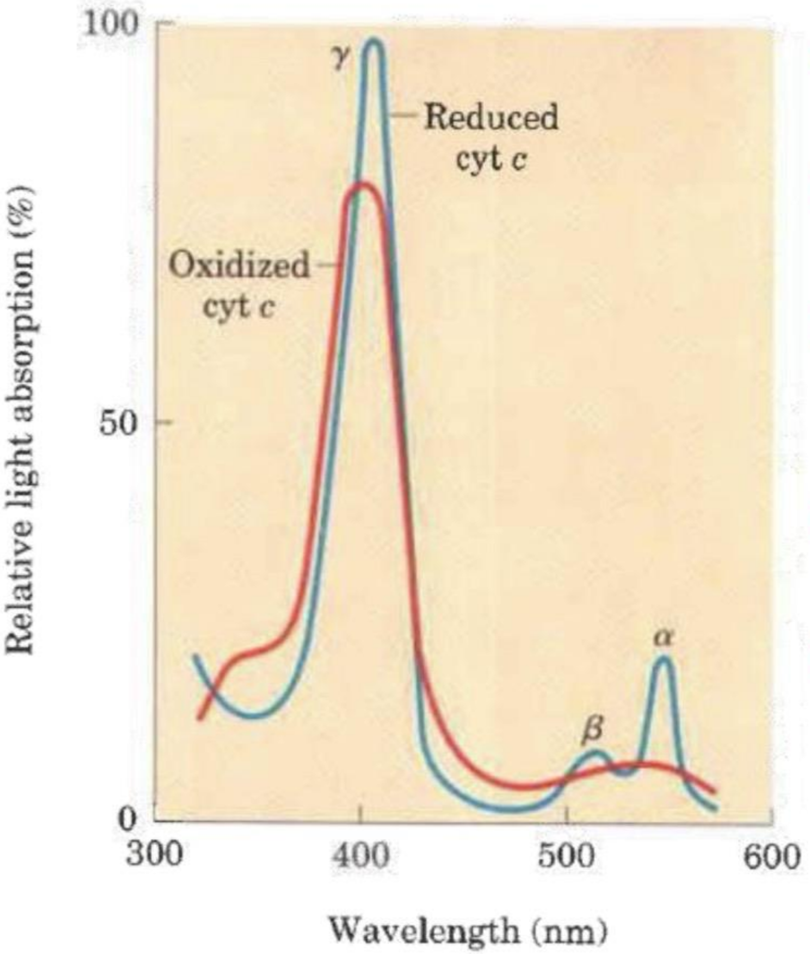
Pyrrole, Porphyrin, Fe, Heme, Ligation

(Cytochrome B contains Heme B And Cytochrome C contains Heme C
Cytochrome aa3 contains two types of heme a and like this).

Most abundant Heme protein in our bodies → Hemoglobin → Heme B → which means Heme b is the most abundant hemoprotein in our bodies.

Simply we can recognise these types depending on structure, but if we don't know the structure can we differentiate between them in other way ? Read next slide ☺

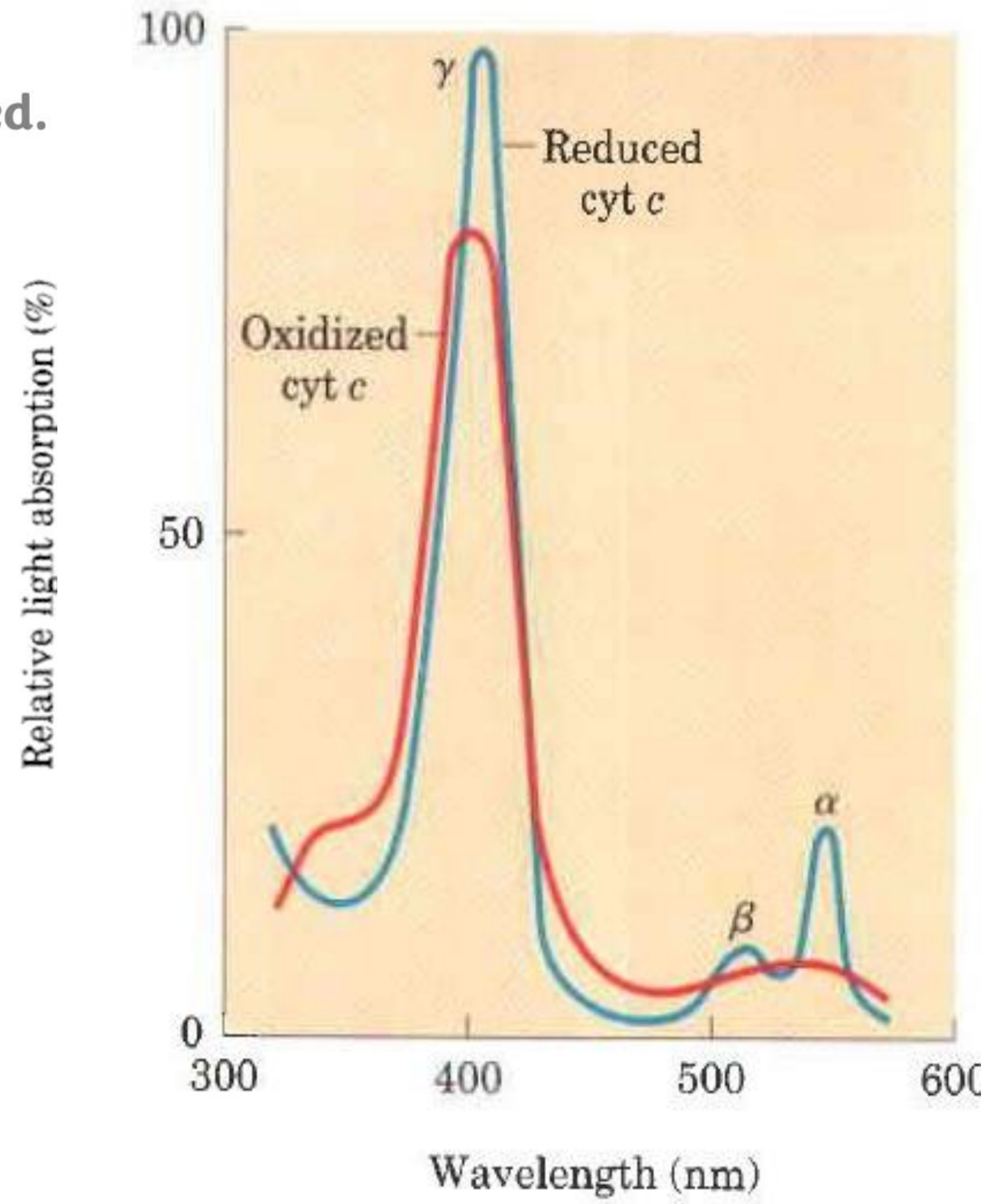
HEME – SPECTROSCOPIC FEATURES



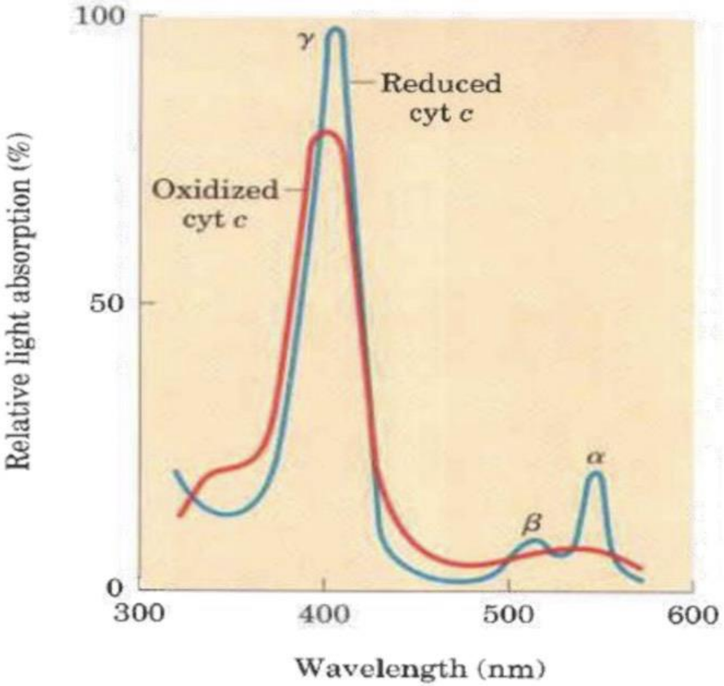
- **Characteristic strong absorption of visible light (Fe-containing heme prosthetic groups).**
- **Classification based on light absorption & Mode of binding (a, b, c).**
- **Light absorption: Each cytochrome in its reduced (F^{+2}) state has 3 absorption bands in the visible range.**
- **α band : near 600 nm in type a; near 560 nm in type b, & near 550 nm in type c.**
- **Heme can carry one electron.**

- Heme group could be **oxidized** and could be **reduced.**
- **Oxidized heme group** has only one peak around **400nm**
- **Reduced heme group** will give us 3 peaks each peak around a specific wavelength representing different heme type.
  Named from higher wave length to shortest wave length **α, β** and **γ.**

- We differentiate between them spectroscopically (the percentage of the light absorbed at different wave length by heme group depending on its type).
- Every type has its own distinct peak at which it absorbs light with specific length most effectively and by this characteristic we can recognise what type of heme does the protein has.



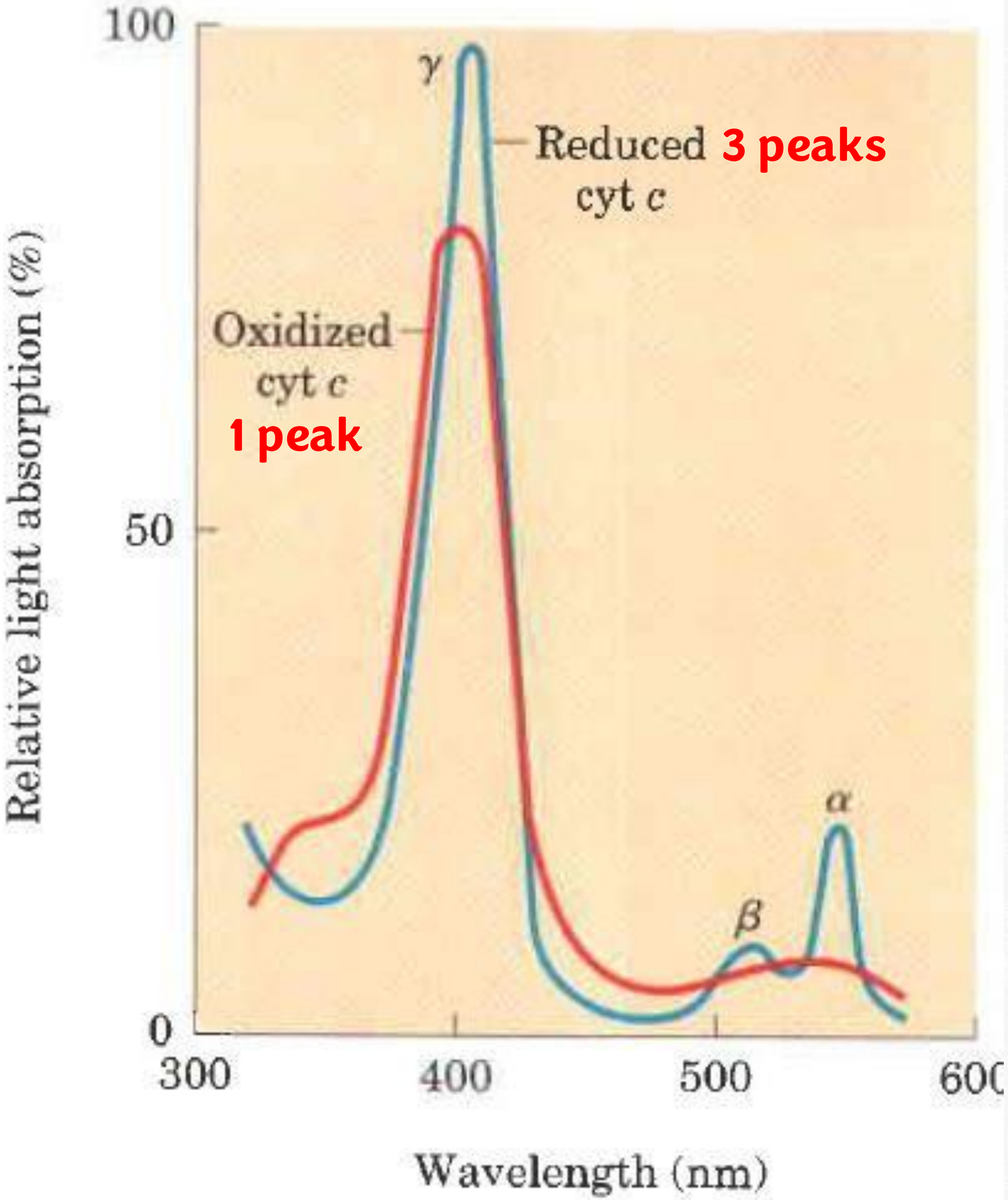
- Imagine you have a solution containing proteins with different types of heme groups. If you shine light starting from 300 nm onto the solution and measure the percentage of light absorbed by the heme groups, you would observe that at 400 nm, 98% of the light is absorbed, forming a peak (meaning absorption is lower on both sides of this wavelength). This indicates the presence of a specific type of heme that absorbed this light. Another peak appears at 530 nm, showing that light at this wavelength is absorbed by another protein containing a different type of heme group.



- If all the heme groups are in the oxidized state, it becomes harder to distinguish their types spectroscopically, because the characteristic α and β bands are less pronounced or even shifted compared to the reduced state.
- Spectroscopic classification of heme types mainly relies on the reduced form, where the α band position is clearly visible and type-specific.

- In the oxidized form, peaks can overlap or have lower intensity, making it difficult to identify the type accurately without additional techniques (like redox cycling the sample to reduce the heme first).

Back To 2^{nd} Year

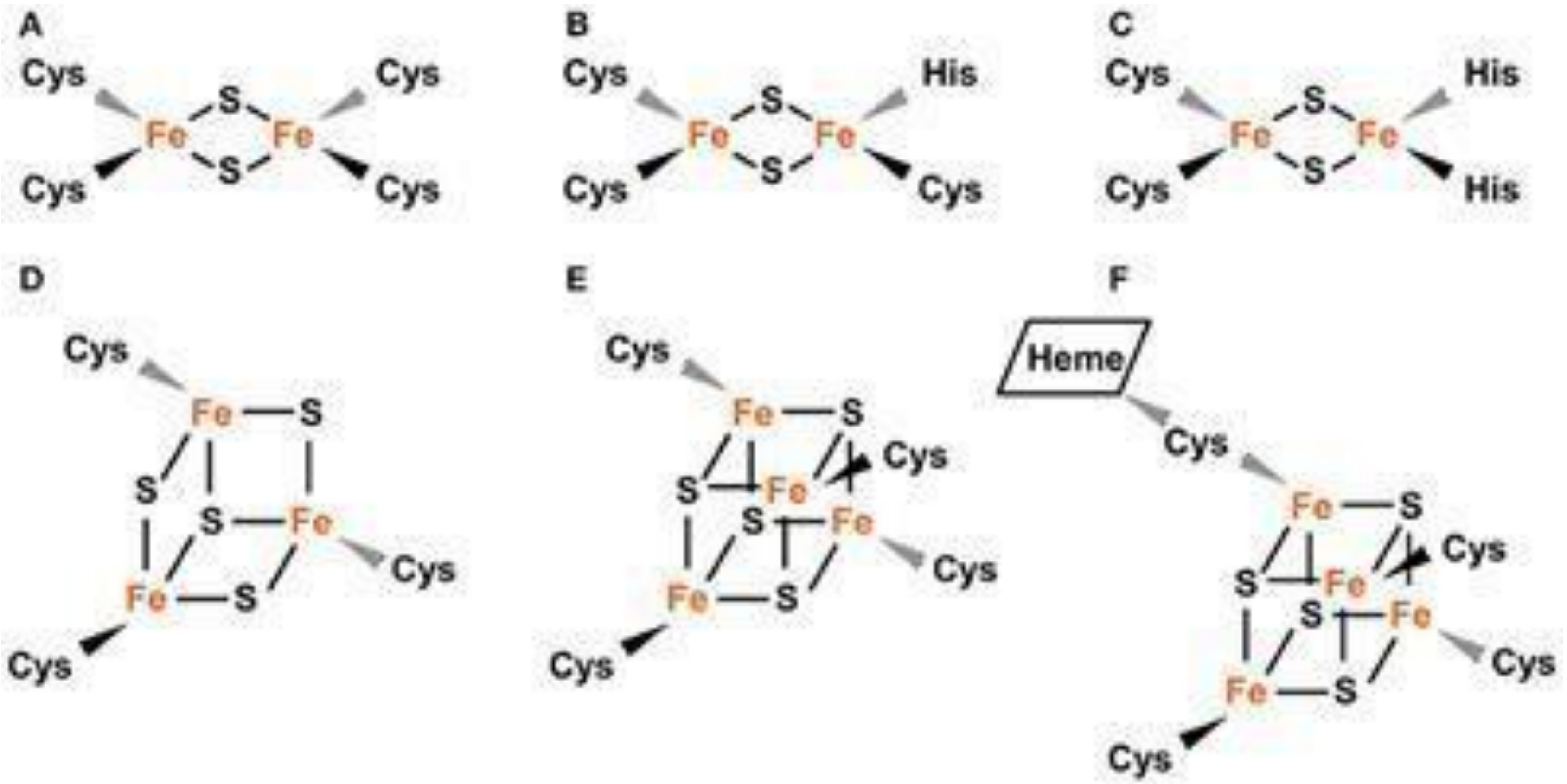


OTHER ELECTRON-CARRYING MOLECULES: "CYTOCHROMES"

- Light absorption: α band (~600 nm in type a; ~ 560 nm in type b; ~550 nm in type c)
- Some cytochromes are named by the exact α band wavelength:
  - Cytochrome b_{562}; Cytochrome c_{550}; Cytochrome c_{551}
- Heme can carry one electron
- ΔE^o depends on the protein

Cytochrome b 562 has heme B and had the center of alpha band on 562

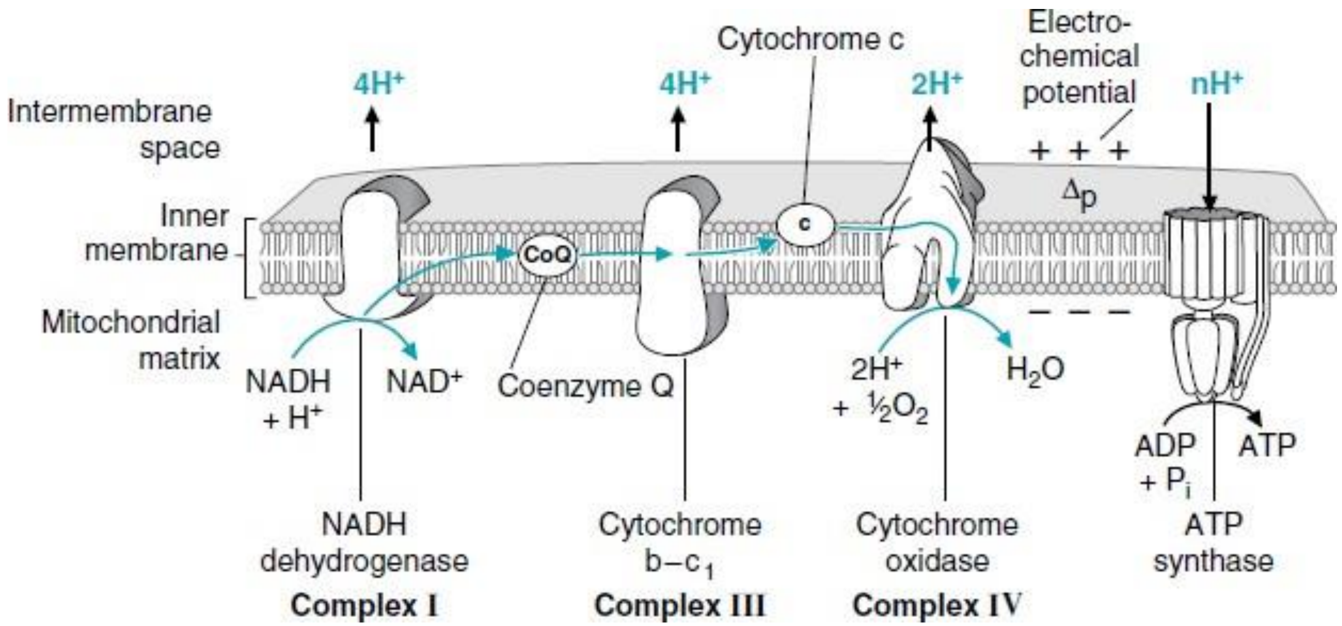
Different types of hemes differ in the center of the heme depending on the center of the alpha band → specific for the type of heme (either 600, 560,550…).



OTHER ELECTRON-CARRYING MOLECULES: IRON-SULFUR CLUSTERS

Named depending on how many **irons** and how many **sulfurs** they have. These electrons carriers can carry and pass electrons through iron-sulfar cluster.

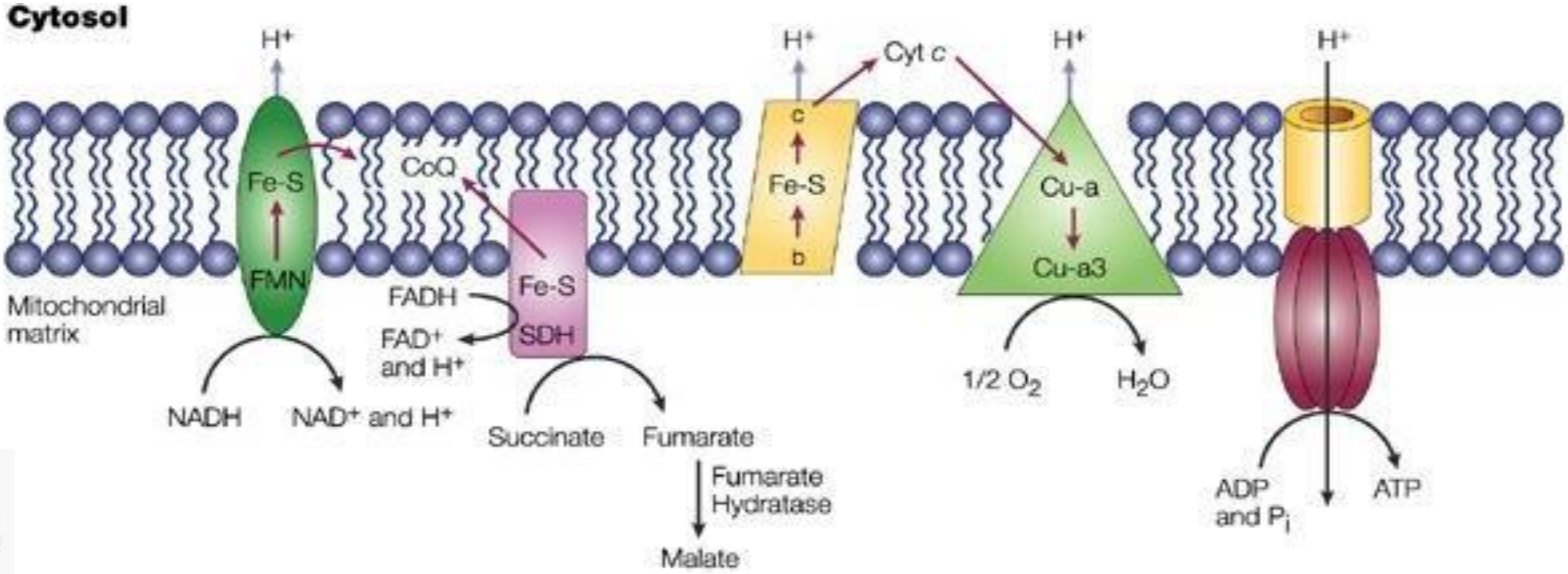
Note: Amino acids can't carry, stabilize and pass electrons through them; as there should be an electron carrier.
→ If a protein **lacks** molecules that can be **oxidized and reduced** then it won't function in electron transport chain.



REQUIREMENTS OF OXPHOS

- Electrons' donor (NADH or FADH2) & acceptor (O_2)
- ETC of proteins
- ATP synthase
- An intact IMM

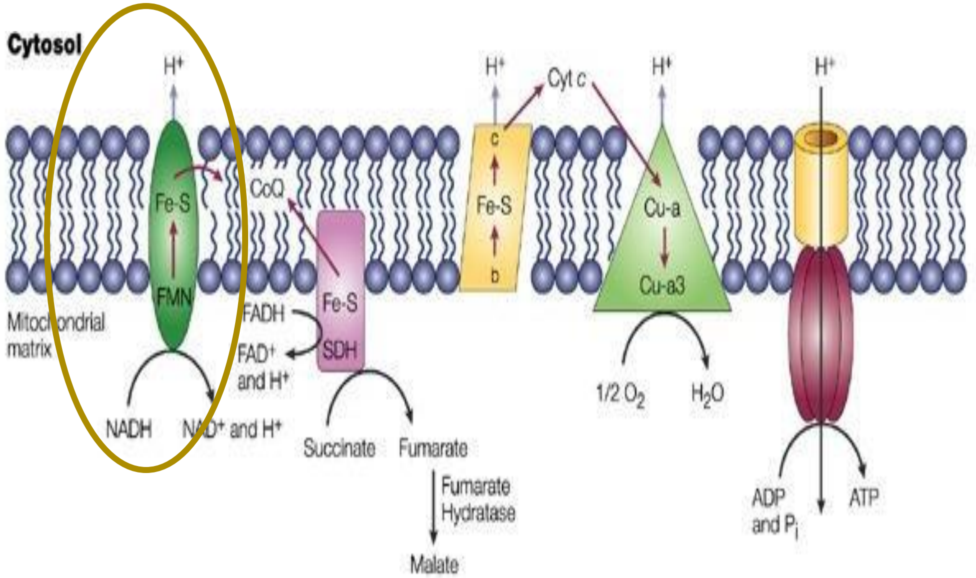
➢ And we also need a **proton gradient** (proton motive force).



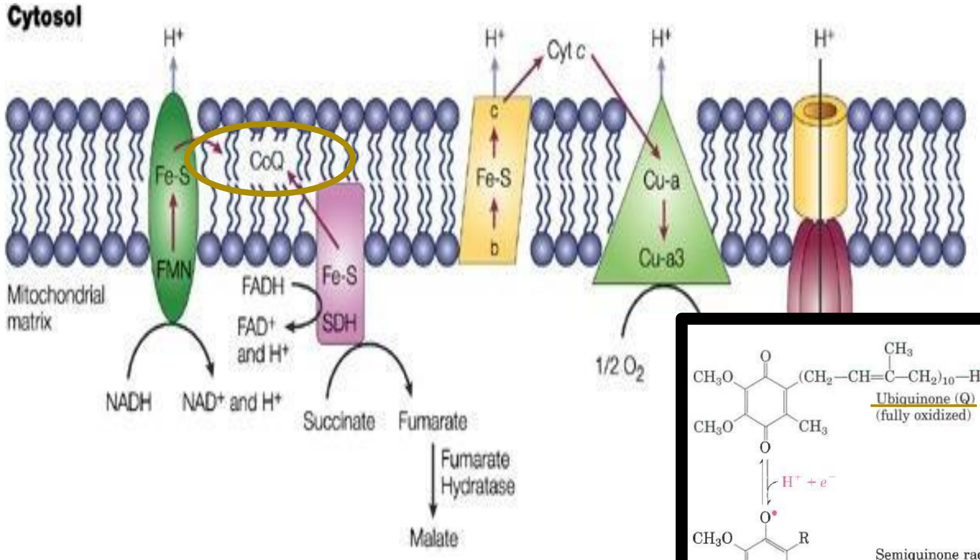
OXIDATIVE PHOSPHORYLATION (OXPHOS)

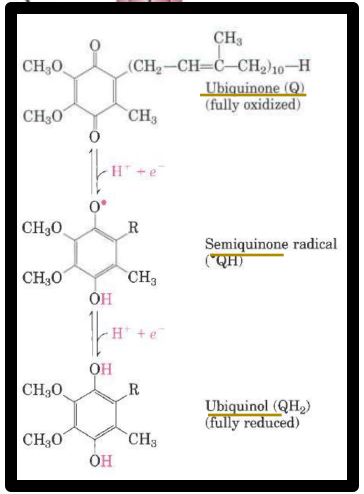
The next slides are gonna be the doctor explanation and then I’m gonna put the doctor’s slides.

One of the products of the TCA cycle is **NADH**. It donates **two electrons (and a proton)** to **Complex I,** also known as **NADH dehydrogenase**, where **NADH** is **oxidized to NAD$^+$**, and the released electrons enter the electron transport chain.



Complex I will pass the electrons that were from NADH to **CoQ**.
That's why we call it (complex 1) **NADH-CoQ oxidoreductase**, since it performs both **oxidation and reduction**.





CoQ, as the name implies, is a **quinone→ketone**, which means it contains a **carbonyl group (C=O).** When **CoQ is reduced**, it becomes **quinol(alcohol).** It can accept **one or two electrons**, forming either a **partially reduced semiquinone** or a **fully reduced ubiquinol.**

So we have **oxidized CoQ (ubiquinone)** and **reduced CoQ (ubiquinol).**

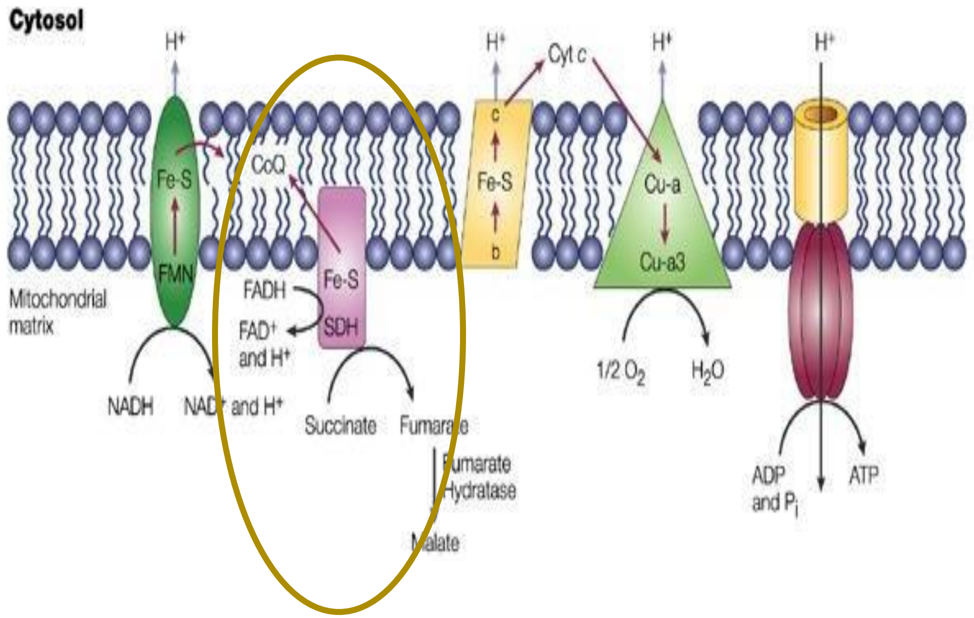
Complex II takes electrons **from $FADH_2$** and delivers them to **coenzyme Q (CoQ).**

Complex II has several names, such as **Succinate ubiquinone oxidoreductase** or **succinate dehydrogenase.**

When **$FADH_2$ is released**, it remains **bound** to an enzyme called **succinate dehydrogenase.**
Complex II is actually the same enzyme – **succinate dehydrogenase.**

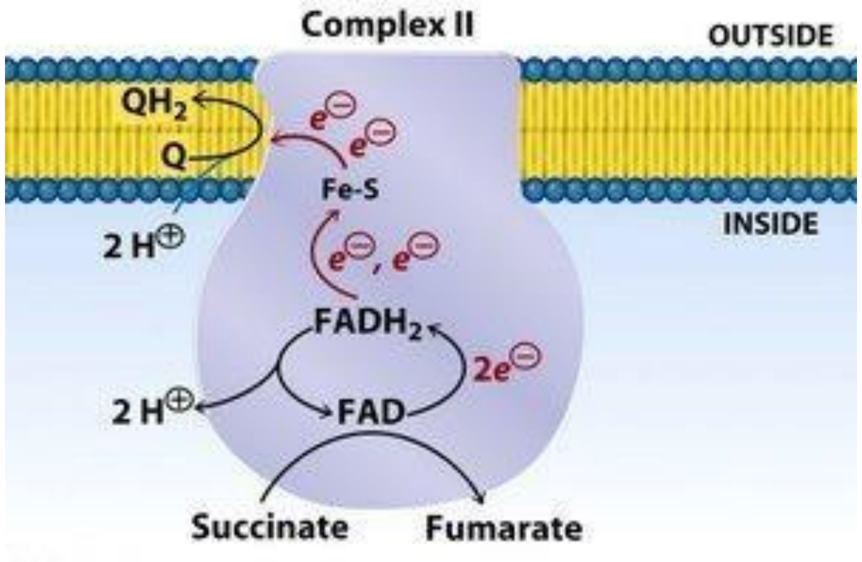
This is very important because it represents the only **direct link between the Krebs cycle and the electron transport chain.**

In other words, **succinate dehydrogenase** is the connecting **bridge between the two pathways.**



Succinate dehydrogenase is the enzyme involved in step number 6 of the citric acid cycle.

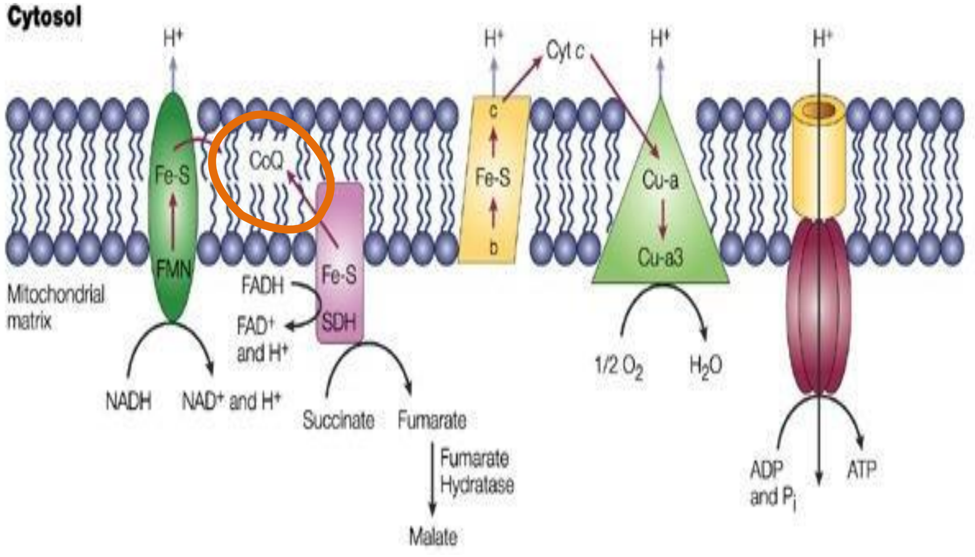
So imagine the citric acid cycle happening in the mitochondrial matrix – steps 1, 2, 3, and so on, until step 6, at this step, **succinate is converted to fumarate, and electrons are released.**
These electrons are **loaded onto FAD**, forming **$FADH_2$.**



From there, the **electrons move to the iron-sulfur (Fe-S) clusters within this complex (complex 3).** (There is also one heme group present there).

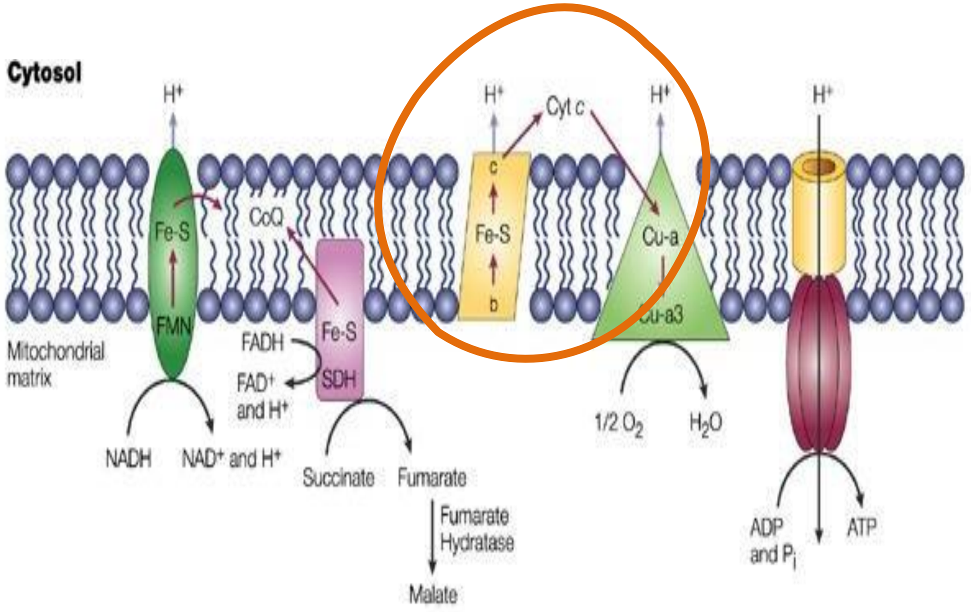
Then the electrons **continue to Coenzyme Q (CoQ), which carries them further down the electron transport chain.**

CoQ (ubiquinone), when reduced to ubiquinol ($CoQH_2$), becomes the common carrier molecule. It collects electrons from both $FADH_2$ (through Complex II) and NADH (through Complex I) and then delivers them to Complex III in the electron transport chain.

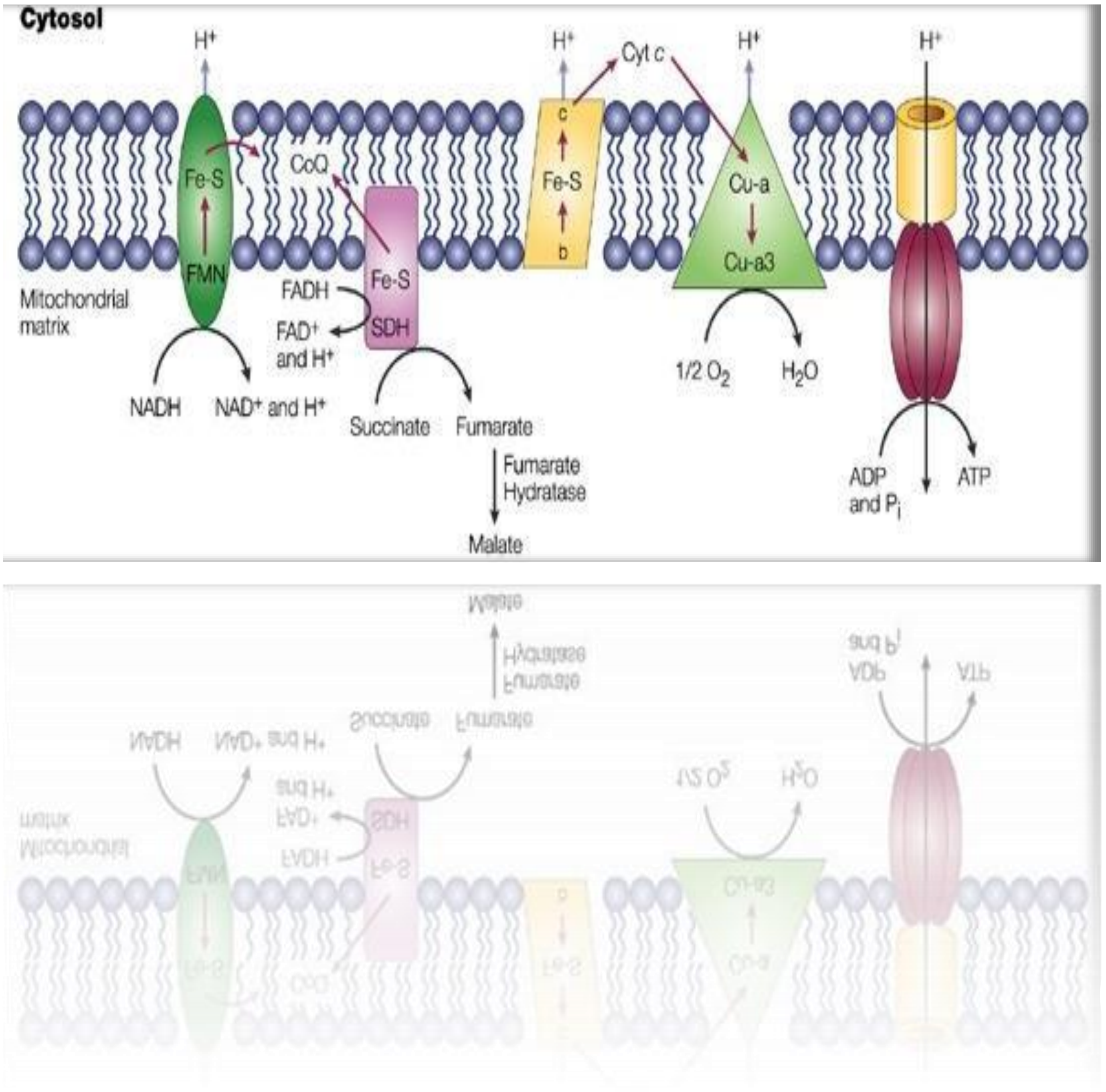


Complex III, also called the cytochrome bc_1 complex, contains heme b, heme c_1, and an iron-sulfur cluster.

Electrons are then transferred from Complex III to Complex IV through the carrier cytochrome c.



Cytochrome c is a protein that contains heme c, **capable of carrying one electron at a time.** Since the protein is soluble, it cannot be embedded within the inner membrane.
Accordingly, it is located on the outer surface of the inner mitochondrial membrane, where it picks up electrons from Complex III and delivers them to Complex IV.



Complex I works as an entry point for electrons coming from NADH.
The electrons are **first delivered** to a **structure inside it called FMN (flavin mononucleotide).**

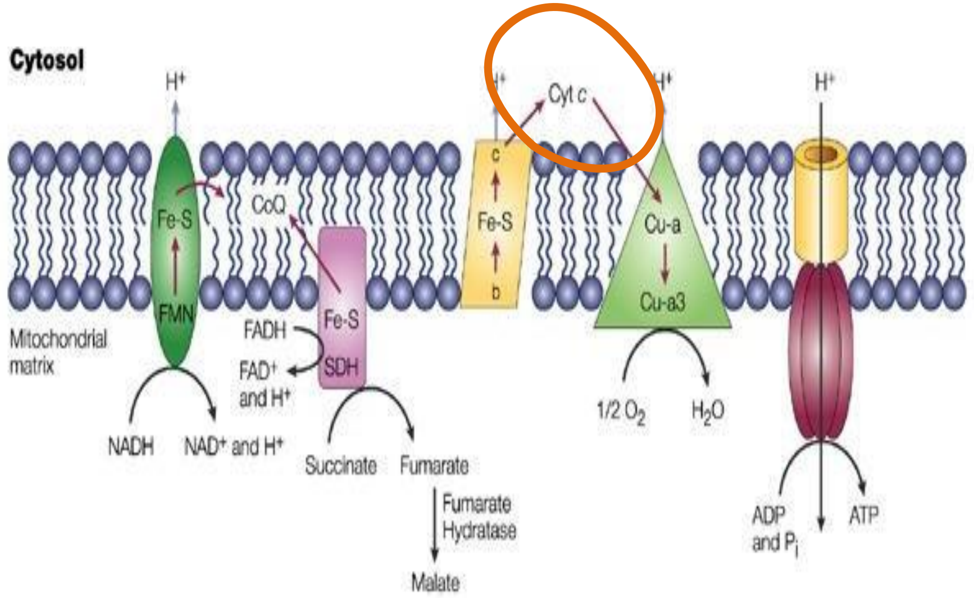
From FMN, they pass through **a series of iron-sulfur (Fe-S) clusters inside the complex – about 7 to 9 clusters,** depending on the species.

Electrons travel one at a time through these clusters until they reach the surface, where two electrons are finally used to **reduce CoQ (coenzyme Q) to $CoQH_2$.**

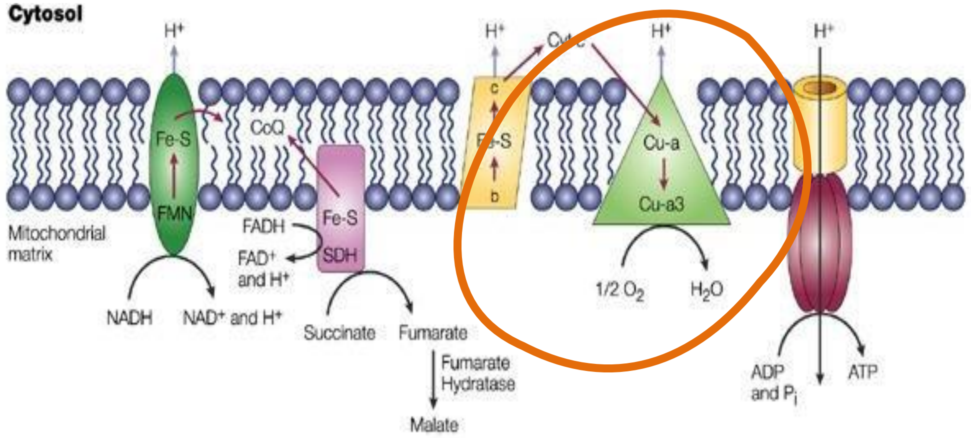
In Complex II, the main groups involved are **FAD, iron-sulfur clusters, and heme b.**

Complex III, also called the **cytochrome bc_1 complex, contains heme b, heme c_1, and an iron-sulfur cluster.**

Electrons are then transferred from Complex III to Complex IV through the carrier cytochrome c. Cytochrome c is a protein that contains heme c, capable of carrying one electron at a time.
Since the protein is soluble, it cannot be embedded within the inner membrane. Accordingly, it is located on the outer surface of the inner mitochondrial membrane, where it picks up electrons from Complex III and delivers them to Complex IV.



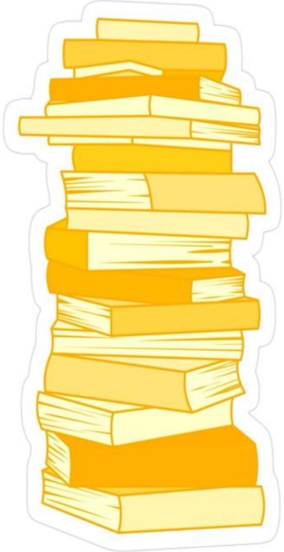
What makes Complex IV work as **oxidoreductase** is the presence of **copper and heme groups**. It has two types of heme, A and A3, and three copper atoms located in two sites: the A site (with two copper atoms) and the B site (with one copper atom)



If we **compare cytochrome c to CoQ as electron carriers:**

•***CoQ*** is located within the **inner mitochondrial membrane**, so it is lipid-soluble (**hydrophobic**). This allows it to move freely through the membrane, but it also has a **hydrophilic part to carry the charged electrons.**

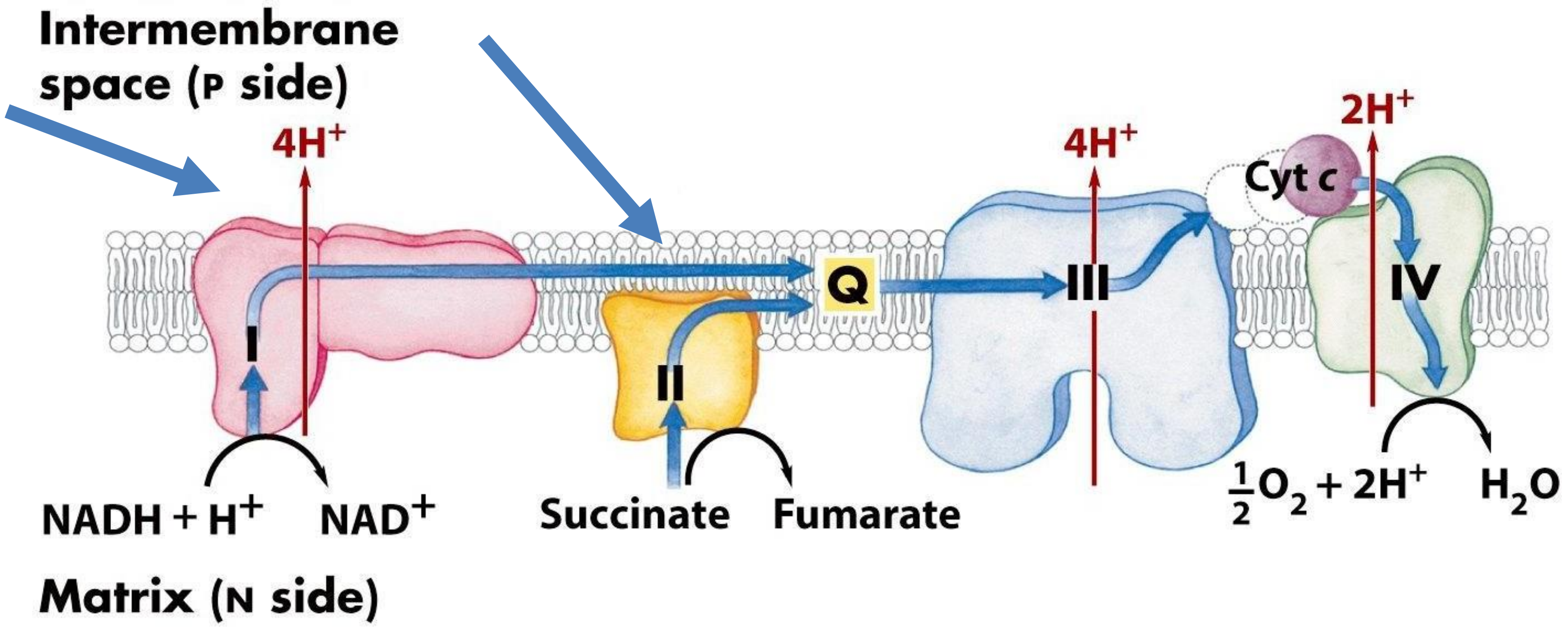
•***Cytochrome c,*** on the other hand, is **hydrophilic.**



Electrons move through the chain because of differences in energy potential (ΔG).

For example, when electrons pass from NADH → Complex I → CoQ, there is an energy drop of about 13–14 kcal/mol.
This energy is not wasted; it is **used to pump protons from the mitochondrial matrix to the intermembrane space(inside to outside).**

So, the energy from the **2 electrons passing through Complex I to CoQ is used to move 4 protons across the membrane.**



When electrons are transferred from **FADH2 to Coenzyme Q (CoQ)**, the ΔG is **minimal, very low, close to zero.** The potential difference is very small – **enough for the electrons to move, but not enough to pump any protons.**

This is why the succinate dehydrogenase (Complex II) is embedded in the inner mitochondrial membrane. It is **not** a transmembrane enzyme, **because it does not pump protons across the membrane.**

The **electrons on CoQ have the same energy,** regardless of whether they came from Complex I or Complex II. **At CoQ, they have a fixed reduction potential.**

When electrons move from **Complex III to cytochrome c**, there is a gap in energy sufficient to **pump 4 protons (H^+) out.**

In **Complex IV,** there is also an energy gap when electrons are transferred from the complex to one oxygen atom, converting it into water, **which allows 2 protons to be pumped out.**

As a result:

- For **2 electrons** from **NADH**, a total of **10 protons are pumped out across the inner mitochondrial membrane.**
- For **2 electrons** from **$FADH_2$,** a total of **6 protons are pumped out.**

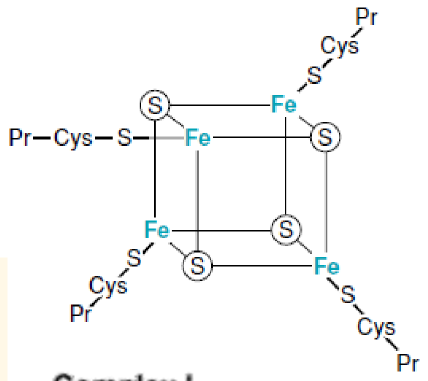
We generate ATP from the proton motive force, which is created by the proton gradient. By default, **NADH is more efficient in generating ATP <u>because approximately 4 protons are needed to produce 1 ATP.</u>**

- **So NADH produces ~ 2.5 ATP.**
- **$FADH_2$ produces ~ 1.5 ATP.**

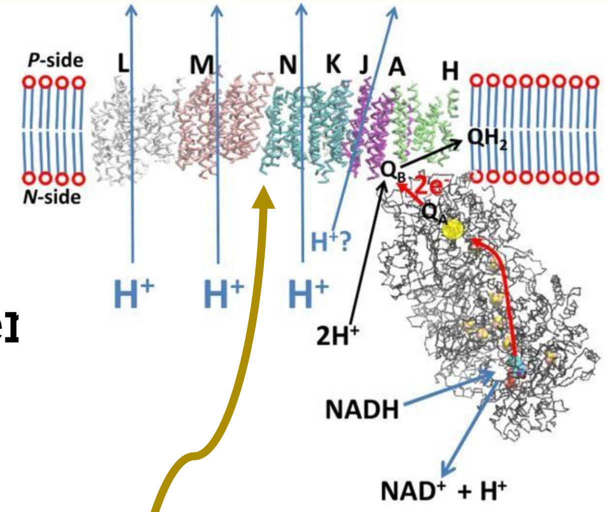
OXI–RED COMPONENTS OF THE ETC:

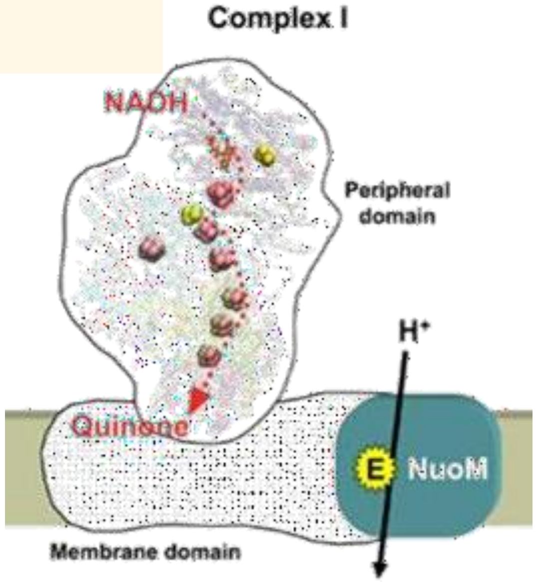
THE NADH DEHYDROGENASE GIANT COMPLEX I

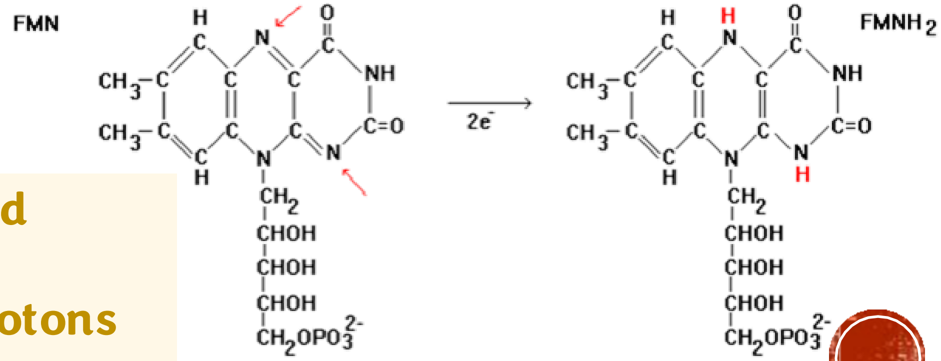
- NADH-Q oxidoreductase
- More than 25 polypeptide chain
- A huge flavoprotein membrane-spanning complex
- FMN is tightly bound
- 7-9 Fe-S centers of at least two differen types
- Drop in energy ≈ 13 to 14 kcal
- Binds NADH & CoQ
- 4 H^+

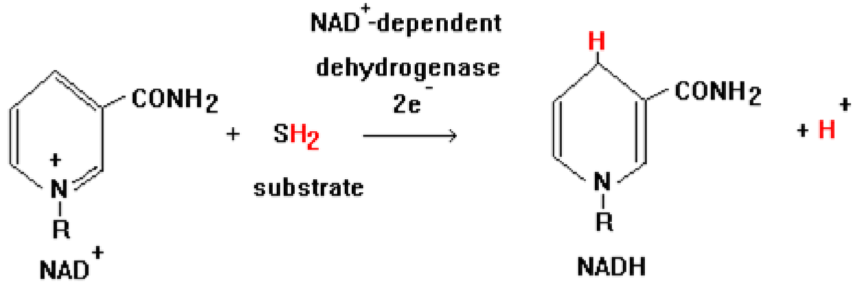


L-shaped molecule embedded inside the membrane projecting towards the matrix of the mitochondria it is very large







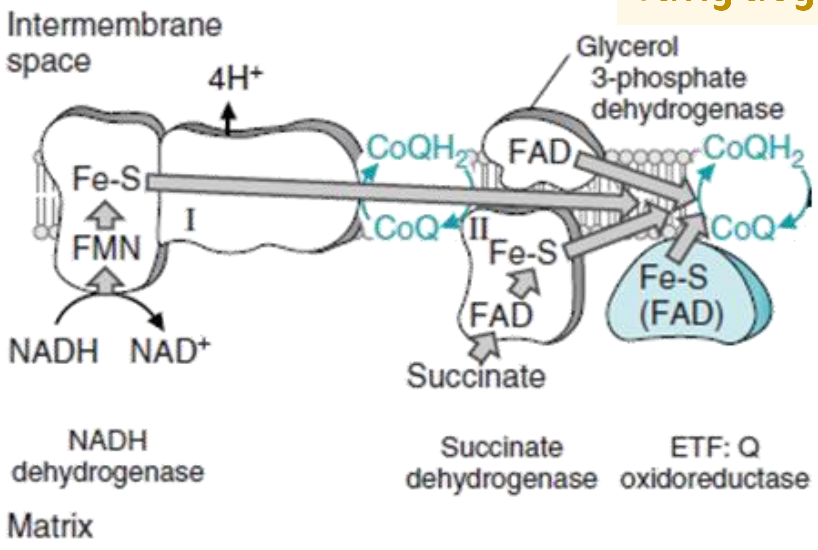


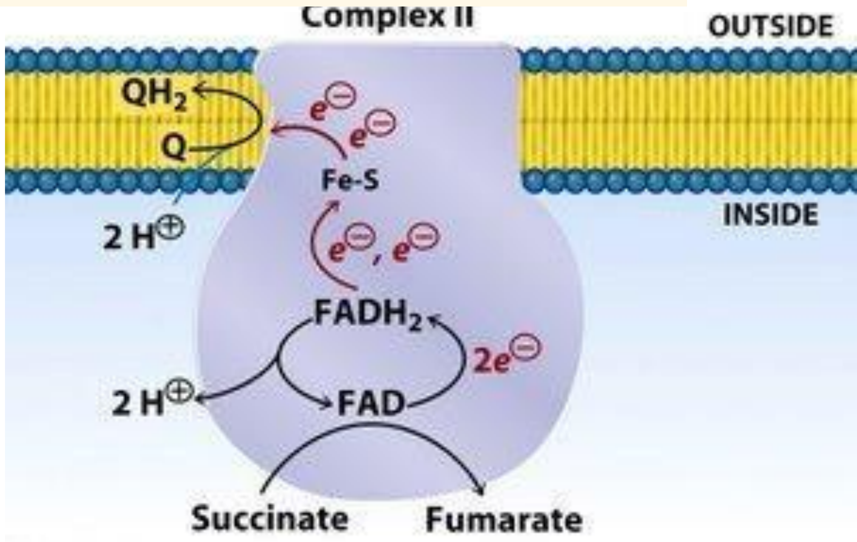
This region that is embedded inside the membrane is responsible for pumping protons to the inter membrane space.

OXI–RED COMPONENTS OF THE ETC:"SUCCINATE DEHYDROGENASE" COMPLEX II; THE CITRIC ACID CYCLE LINK

- Succinate Dehydrogenase & other flavoproteins
- TCA cycle
  - ✓ETF-CoQ oxidoreductase (ex. fatty acid oxidation); Fatty acyl CoA dehydrogenase; Mitochondrial glycerol phosphate dehydrogenase
  - ≈ 0 Kcal, H^+?

There are other proteins and enzymes that contain flavin structures and can also donate their electrons to CoQ (ubiquinone), not only Complex II. Examples include fatty acyl-CoA dehydrogenase and glycerol-3-phosphate dehydrogenase.



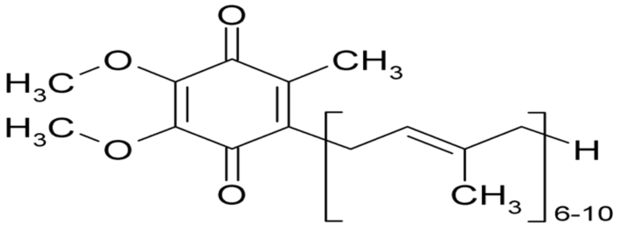


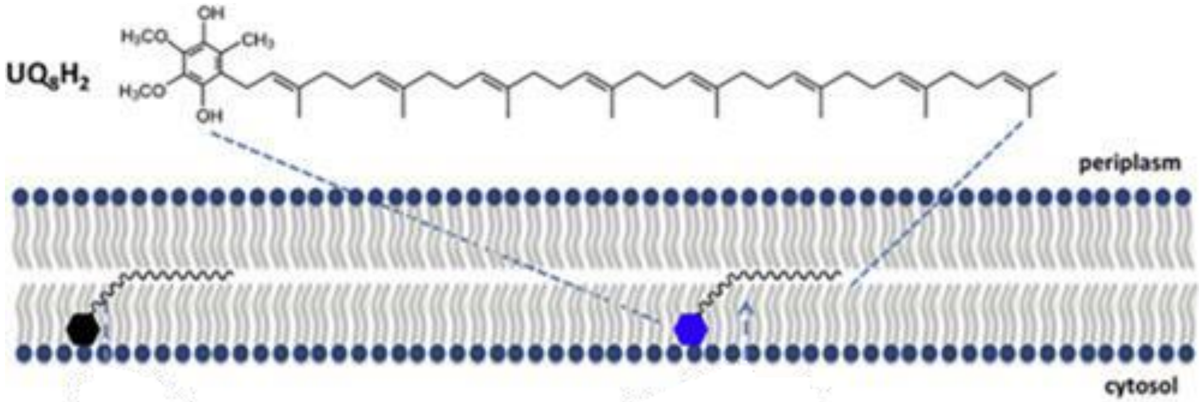
OTHER ELECTRON-CARRYING MOLECULES: “UBIQUINONE” (COENZYME Q): THE MOBILE SHUTTLE

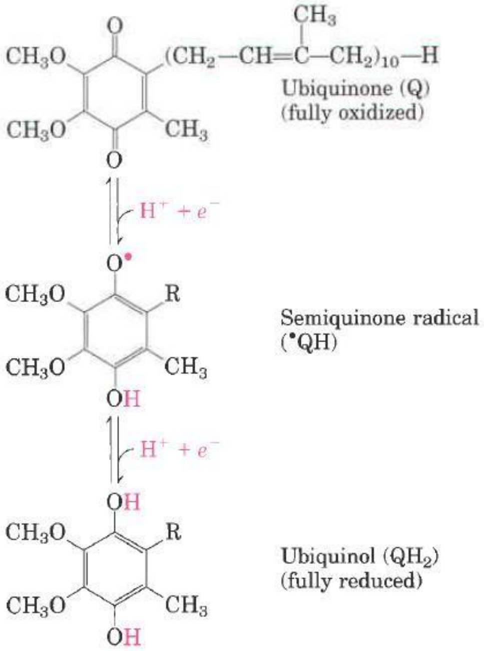
- Lipid-soluble benzoquinone with a long isoprenoid side chain
- Small & hydrophobic (freely diffusible)
- Act at the junction between a 2(e^-) donor and a 1 (e^-) acceptor
- Sometimes prescribed for recovering MI patients

quinone can be described as a cyclic diene with two ketone groups (C=O).

In MI (myocardial infarction) patients, some heart tissues are dead. This reduces the efficiency of ATP production in the affected area. To help, one approach could be giving CoQ as a supplement or drug, which may enhance ATP production in the surviving, non-dead tissues and improve the overall energy supply to the heart.

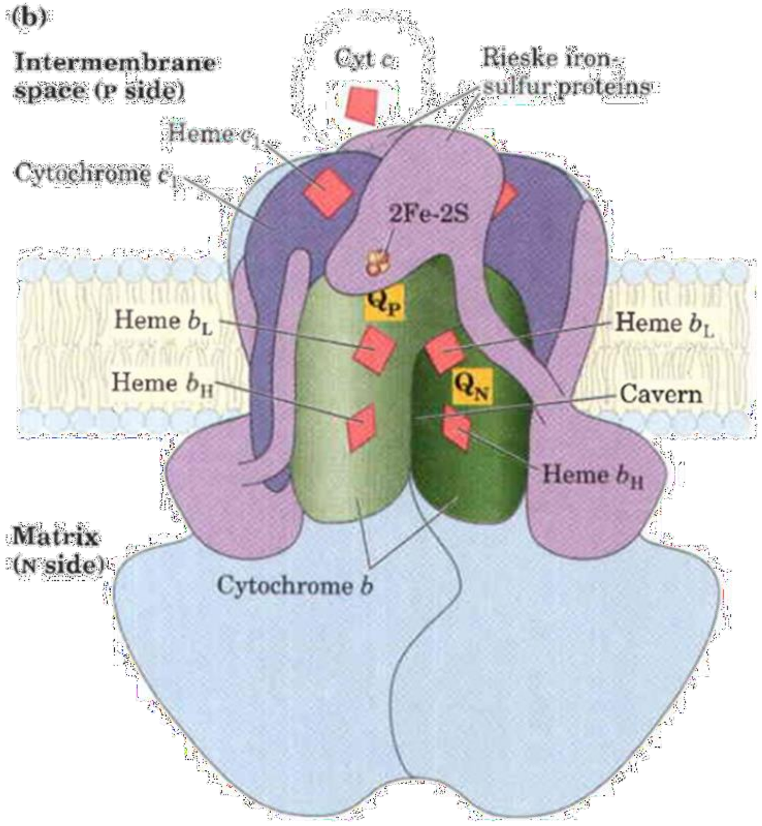




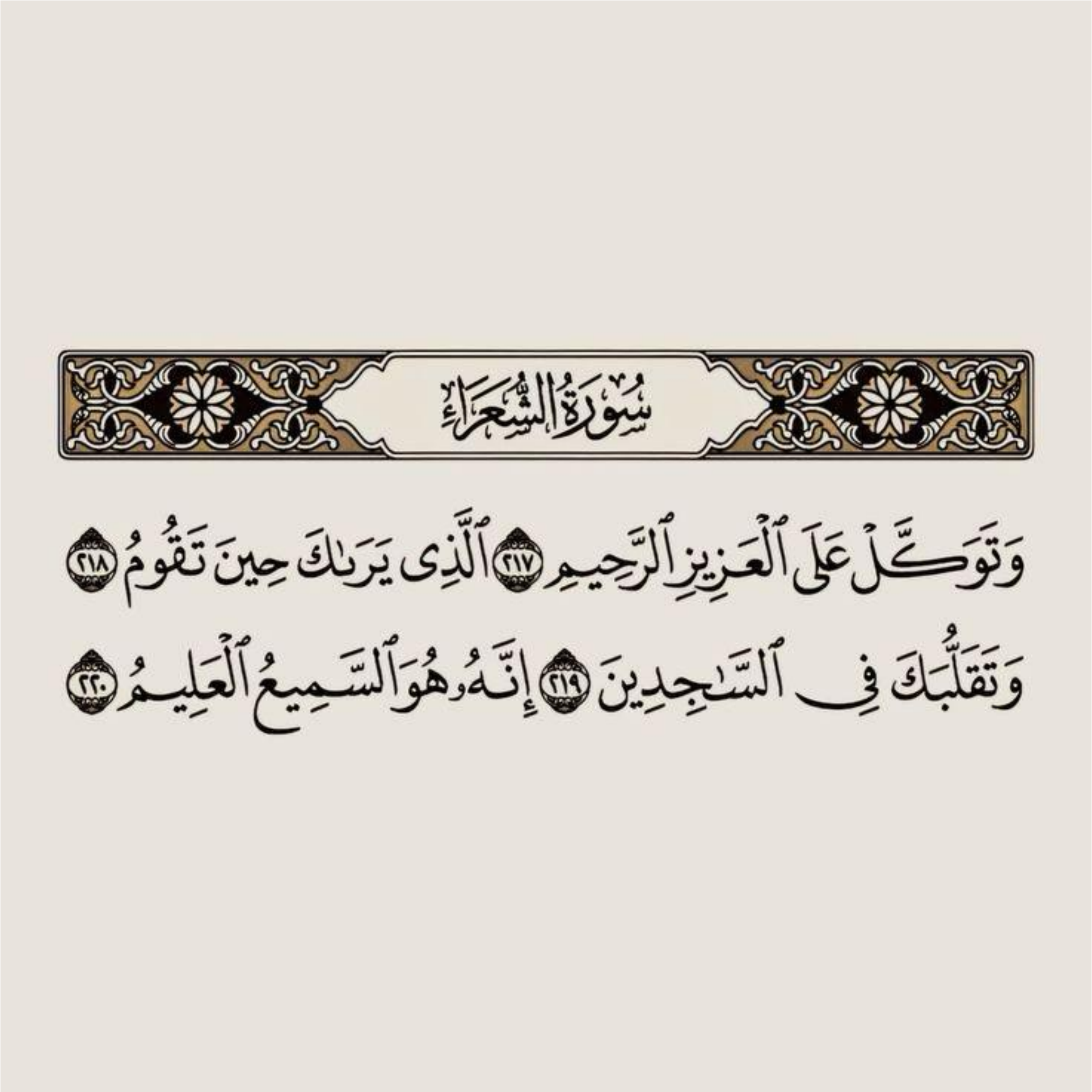


OXI–RED COMPONENTS OF THE ETC: "CYTOCHROME BC$_1$" – COMPLEX III

- Q-cytochrome c Oxidoreductase
- 11 subunits including two cytochrome subunits
- Contains iron sulfur center
- Contain three heme groups in two cytochrome subunits
- b_L and b_H in cytochrome b; c type in cytochrome c_1
- Contain two CoQ binding sites
- 4H+



رسالة من الفريق العلمي:



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			