النادي الطبي

Biochemistry

Title : Electron transport chain

Lec no : 5

Done By : Baraa Safi

وقل رب زدني علما

Electron transport and oxidative phosphorylation

Ahmed Salem, MD, MSc, PhD, FRCR

*كيف نصنع الـ (ATP) وأين ؟

نقل الالكترونات من مادة إلى أخرى بس ما بصير فيها نفسها صناعة (ATP) وبصير الصناعة في (oxidative phosphorylation)

Electron Transport Chain

- Energy-rich molecules, such as glucose, are metabolized by a series of oxidation reactions ultimately yielding carbon dioxide and water (H2O).

  في نهاية المطاف

  CO_2 / H_2O

- The metabolic intermediates of these reactions **donate electrons to specific coenzymes**, nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide (FAD), to form the **energy-rich** reduced forms, **NADH** and **FADH2**.

  Coenzymes

*عشانه أنقل (e) لازم يصير (تأكسد واختزال) للـ(NAD و FAD)

ETC & OXPHOS

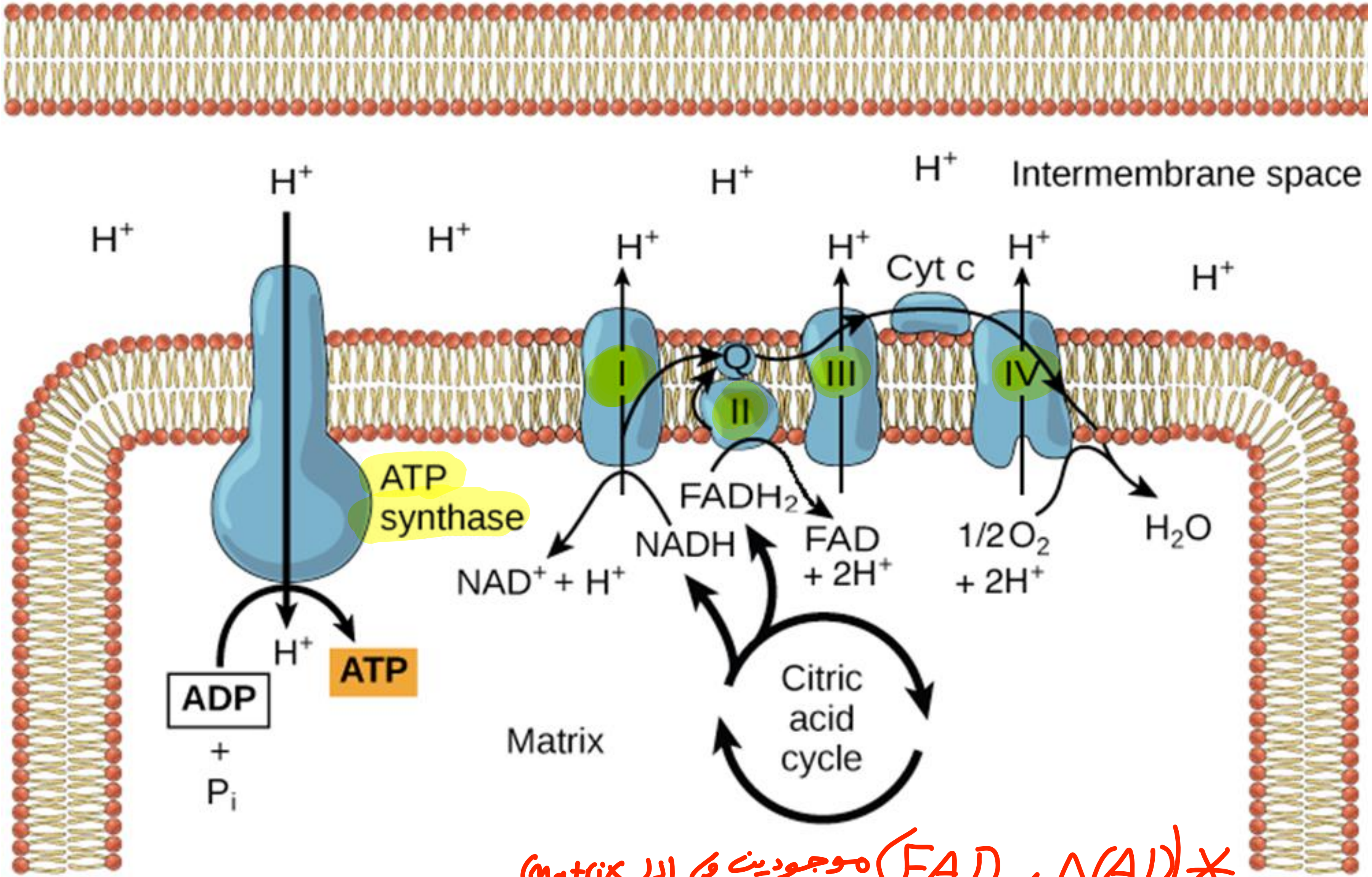
*لما أنقل (e) بصير في عندي طاقة وبتوديني للـ(oxidative phosphorylation)

- These **reduced coenzymes** can, in turn, each **donate a pair of electrons** to a specialized set of **electron carriers**, collectively called the electron transport chain (ETC).
- As electrons are passed down the ETC, they **lose much of their free energy.**
- This energy is used to move H+ across the inner mitochondrial membrane, creating a **H+ gradient** that **drives the production of ATP** from ADP and inorganic phosphate (Pi).
- The coupling of electron transport with ATP synthesis is called **oxidative phosphorylation**. It proceeds continuously in all tissues that contain mitochondria.

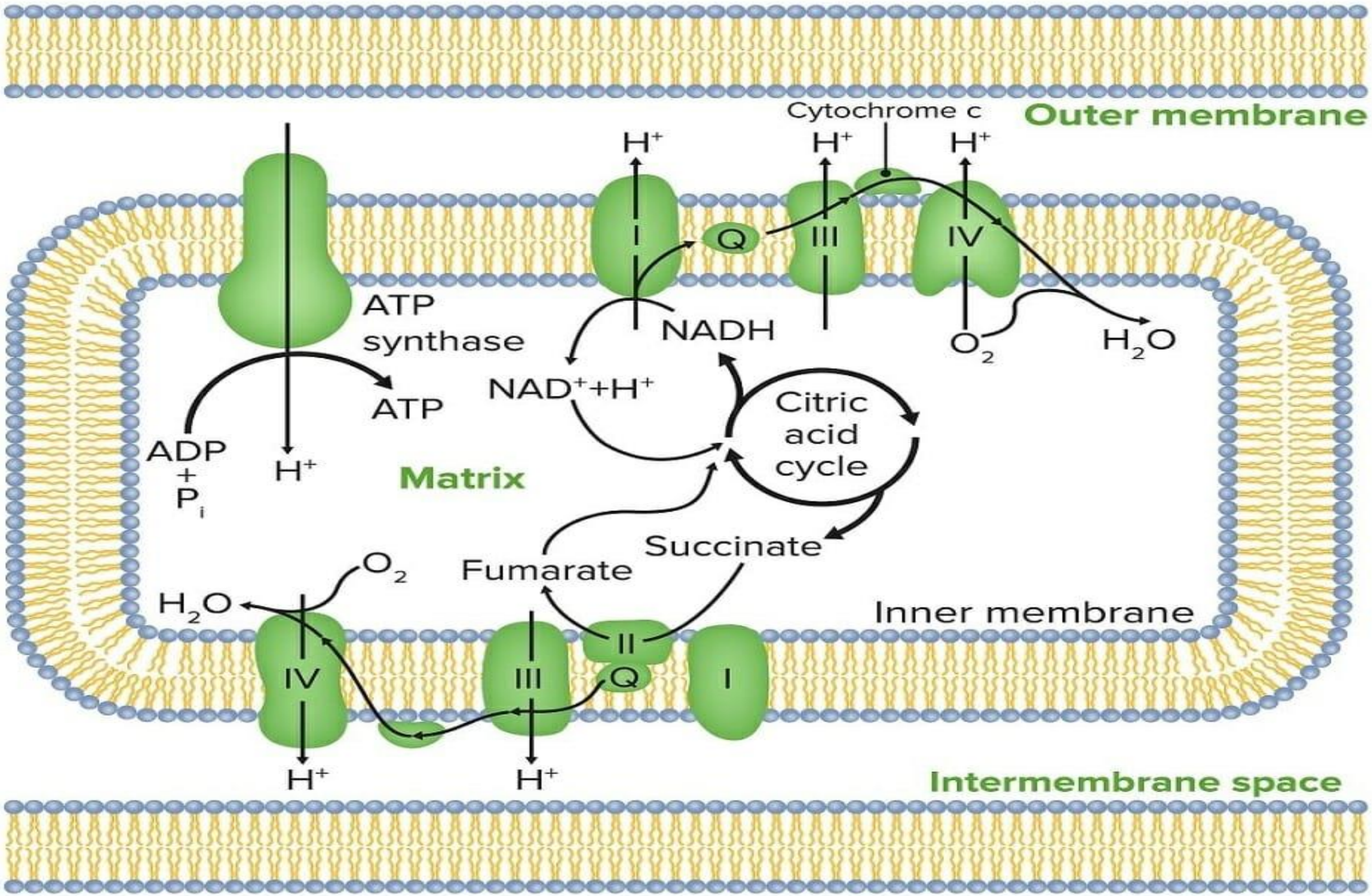
Oxidative phosphorylation

- **Definition: coupling of oxidation (loss of electrons) & phosphorylation**

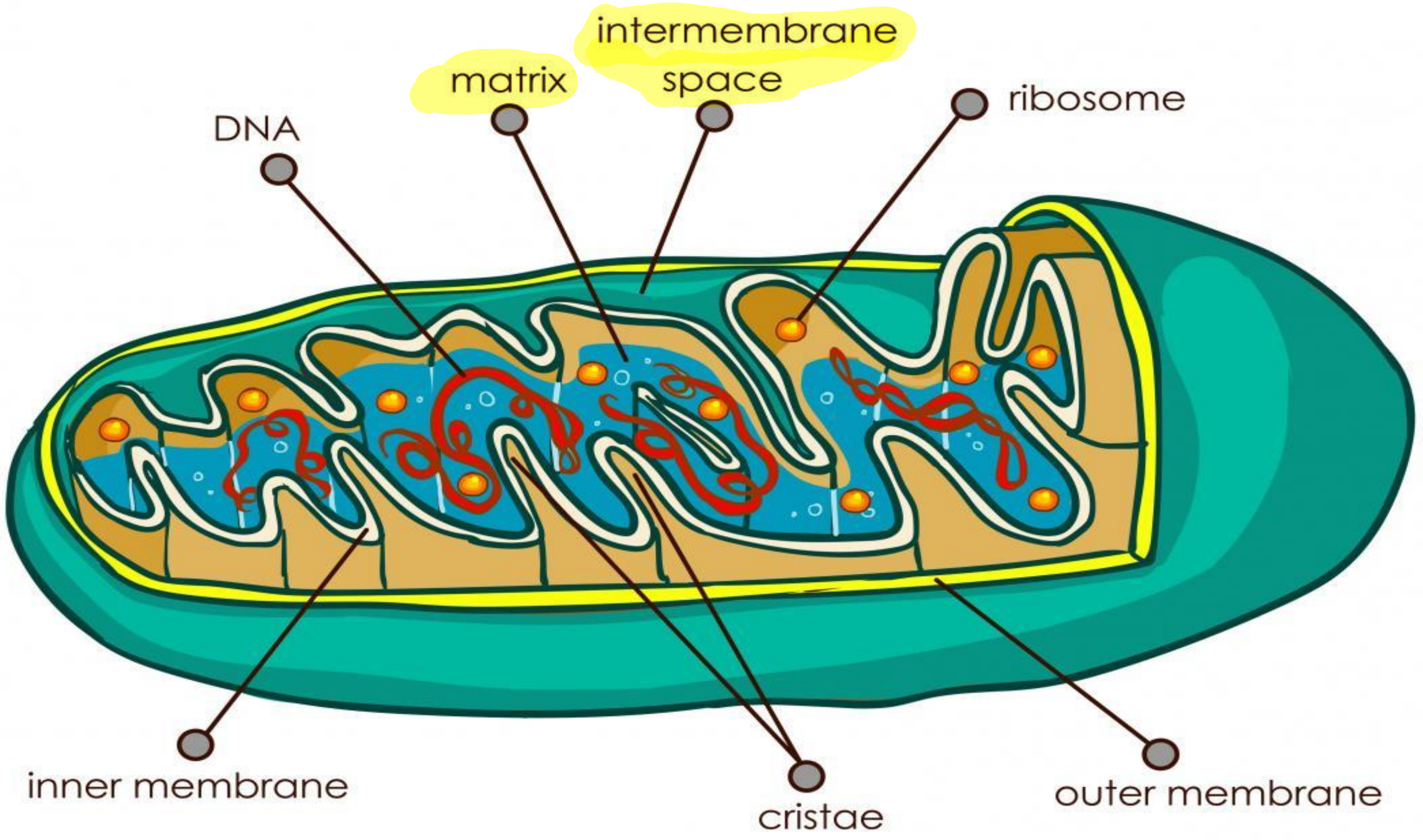
- **Electron transport (respiratory) chain:**
  - Oxidizes reduced cofactors by **transferring electrons in series of steps** to O2 (terminal electron acceptor)
  - Free energy released by these oxidation reactions **is used to derive synthesis of ATP**
  - During removal of electrons, protons are also removed and pumped from matrix across inner membrane → forms electrochemical gradient → **provides energy for synthesis of ATP**
  - Consists of **4 multistep enzyme complexes** with series of electron carriers

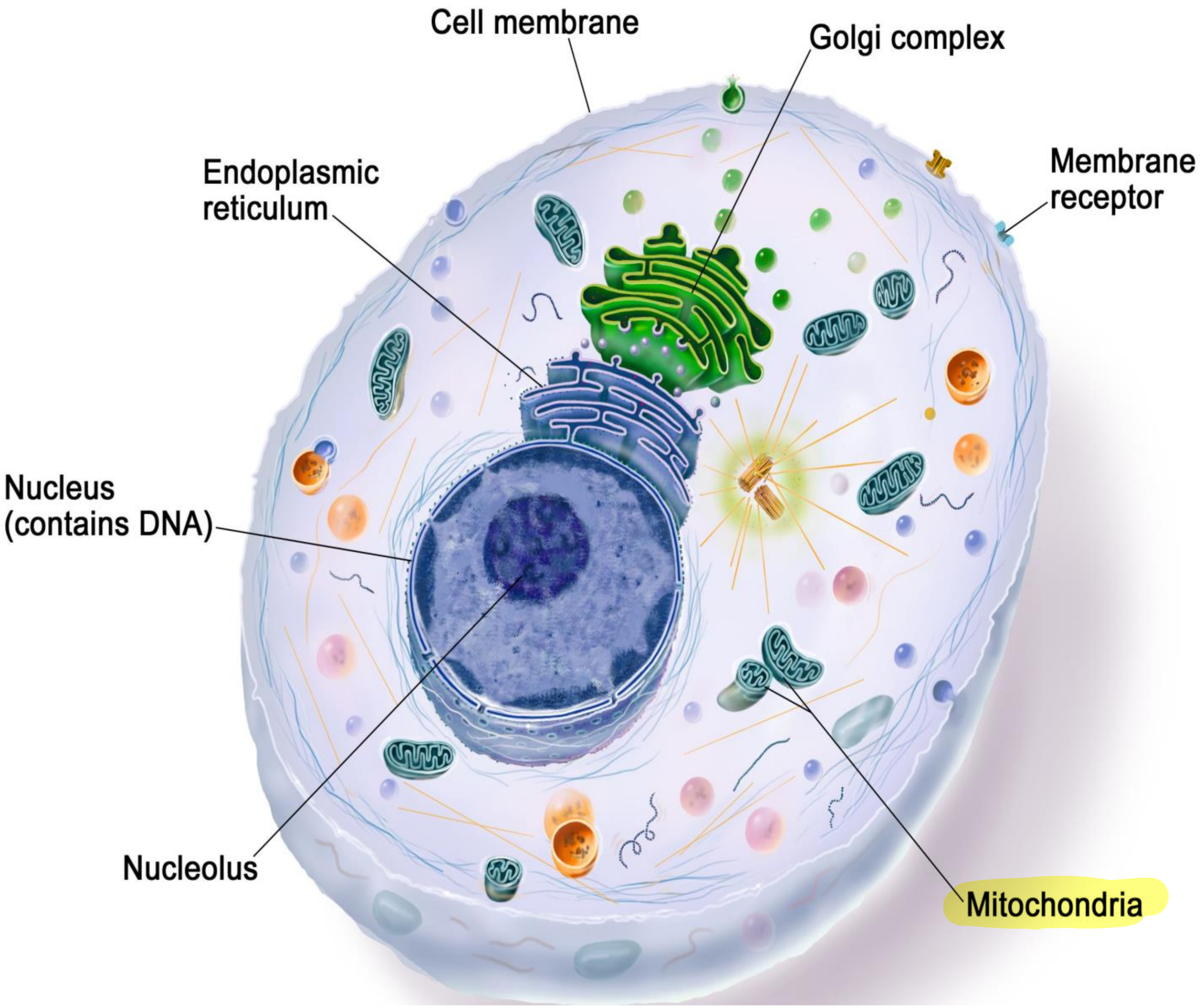


*(FAD , NAD) موجودين في الـ matrix)



Cytochrome c
Outer membrane
H+
H+
H+
I
Q
III
IV
ATP synthase
NADH
O2
H2O
NAD+ +H+
ATP
Citric acid cycle
ADP + Pi
H+
Matrix
Succinate
O2
Fumarate
H2O
Inner membrane
II
IV
III
Q
I
H+
H+
Intermembrane space


intermembrane space
matrix
DNA
ribosome
inner membrane
cristae
outer membrane


Cell membrane
Golgi complex
Endoplasmic reticulum
Membrane receptor
Nucleus (contains DNA)
Nucleolus
Mitochondria

Electron transfer

Electrons are transferred across molecules in 4 different ways

- Directly as electrons (e.g. Fe2+ / Fe 3+ redox pair: oxidases) $Fe^{2+} \rightarrow Fe^{3+} + e^-$
- Incorporated in hydrogen atoms (e.g. FAD) $FADH_2 \longrightarrow FAD + 2H^+ + 2e^-$
- Transferred as hydride ion (H+) $NADH + H^+ \longrightarrow NAD^+ + 2H^+ + 2e^-$
- When there is direct combination of an organic reductant with oxygen (oxygenases) → (ETC آخر خطوة بال)

- **All 4 types could occur in cells**
  - **→ term "reducing equivalent" is used to designate any of these types** لتعيين

Electrochemical gradient

- **4 multi-subunit enzyme complexes** have groups capable of accepting or donating either one or two electrons

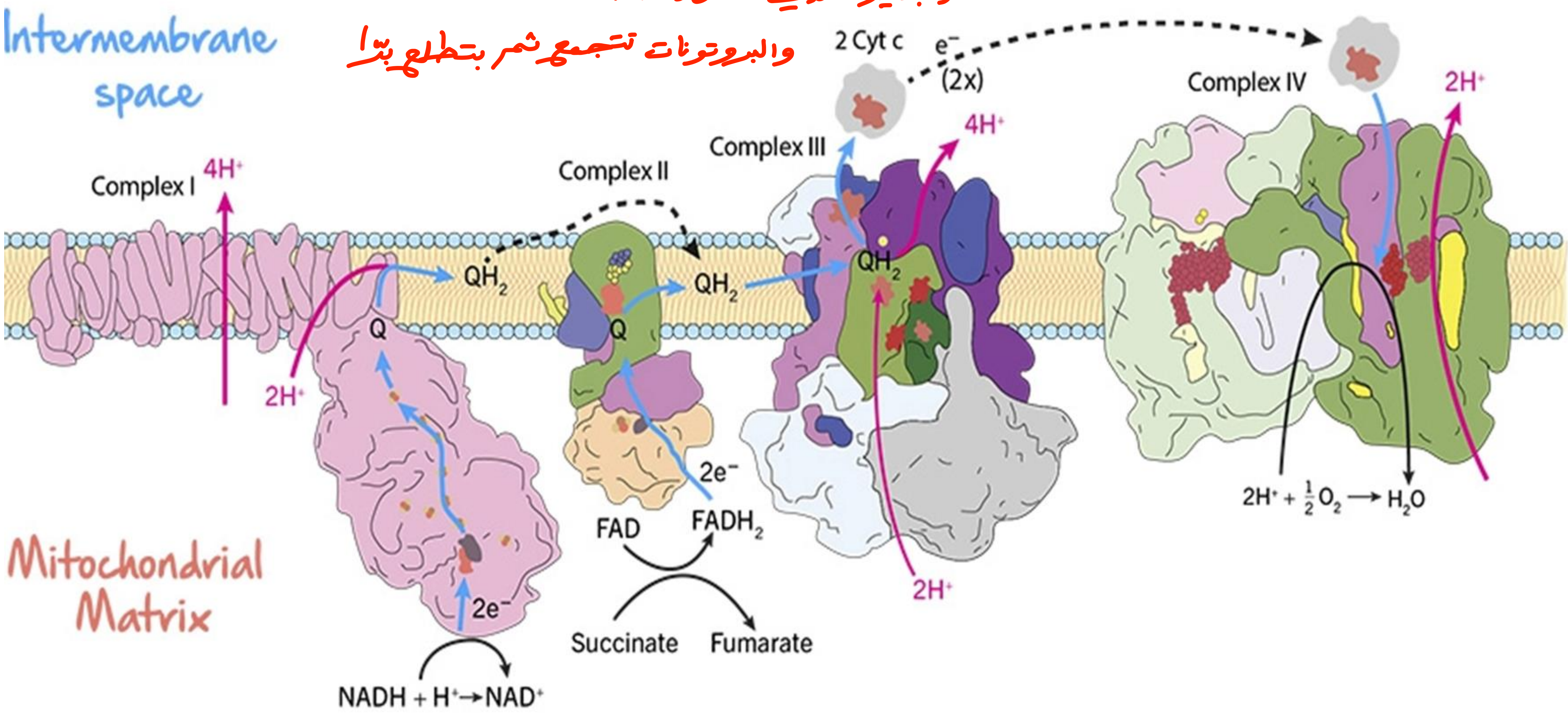
- Electron carriers have standard redox potential ranging from:
  - Most electronegative electron donor (NADH) → - 0.32 volt to
  - Most electropositive electron acceptor (O2) → + 0.82 volt
  - → 1.14 volt difference

- Each component of the chain will accept electrons from proceeding carrier & transfer them to following carrier

كل (Complix) بصيرفيه (oxidation and reduction)

و بصير عندي انتقال (e)

والبروتونات تتجمع ثم بتطلع برا



Electrochemical gradient

- Most of the electrons arise by action of dehydrogenases that collect electrons from catabolic pathways and funnel them into electron acceptors NAD^+ and FAD

- The **driving force** of the chain is the electron transfer potential of NADH or $FADH_2$

Three other types of electron carriers in ETC

1. Coenzyme Q

- Can accept 2 electrons (& 2 protons) to become reduced CoQ
- Lipid soluble → diffusible between lipid bilayer of inner mitochondrial membrane
- Plays a central role in compelling electron flow to proton movement as it carries both

2. Cytochromes

- Are a class of proteins that have iron-containing heme group tightly bound to protein
- Iron can be alternatively oxidized (Fe^{3+}) or reduced (Fe^{2+}) as it functions in ETC
- 3 types participate in ETC (a (cytochrome c oxidase), b & c)
  → all integral membrane proteins except Cyt C which is a mobile electron carrier

Three other types of electron carriers in ETC

ركزوا هون

3. Iron-sulphur proteins

- Iron is present in association with **inorganic sulphur** or sulphur atoms of cysteine residues

بكونوا موجودين بشكل (complix)

- These iron-sulfur (Fe-S) centers range from simple structures with a single Fe atom coordinated to four Cys OSH groups to more complex Fe-S centers with two or four Fe atoms

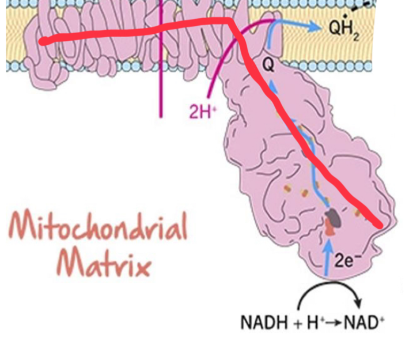
- Rieske iron-sulfur proteins are a variation on this theme, in which one Fe atom is coordinated to two His residues rather than two Cys residues.

- At least eight (8) Fe-S proteins function in mitochondrial electron transfer.

Respiratory (ETC) chain

- **Consists of 4 enzymatic complexes:**
  - **Complex I**: NADH-Q dehydrogenase complex
  - **Complex II**: Succinate-Ubiquinone Oxidoreductase (Succinate Dehydrogenase)
  - **Complex III**: Cytochrome reductase complex
  - **Complex IV**: Cytochrome C oxidase complex

Complex I: NADH to Ubiquinone



- **Complex I** is called, **NADH:ubiquinone oxidoreductase** or **NADH dehydrogenase**

- L-shaped, with one arm embedded in the inner membrane and the other extending into the matrix.

- Large enzyme composed of 45 different polypeptide chains, including an **FMN-containing flavoprotein** and at least 8 **iron-sulfur centers**.

Hydride ion: H^+

Complex I: NADH to Ubiquinone

NADH → FMN → Fe-S → Q → QH_2

1. Complex I catalyzes the transfer of a hydride ion from **NADH to** flavin mononucleotide (**FMN**). The FMN is reduced to the form **$FMNH_2$**.

2. $FMNH_2$ is then oxidized, and two electrons pass through a series of **iron-sulfur groups** and are transferred to the associated coenzyme Q (ubiquinone).

3. **Coenzyme Q** also extracts two protons from the matrix to form the **fully reduced ubiquinol (QH_2)**.

4. As the electrons are moving through the series of FeS clusters, they use the provided electrical energy (12 kcal/mol) **to pump 4 H^+ ions out of the mitochondrial matrix and into the intermembrane space.**
   - To provide them for ATP production in oxidative phosphorylation.

Complex I: NADH to Ubiquinone

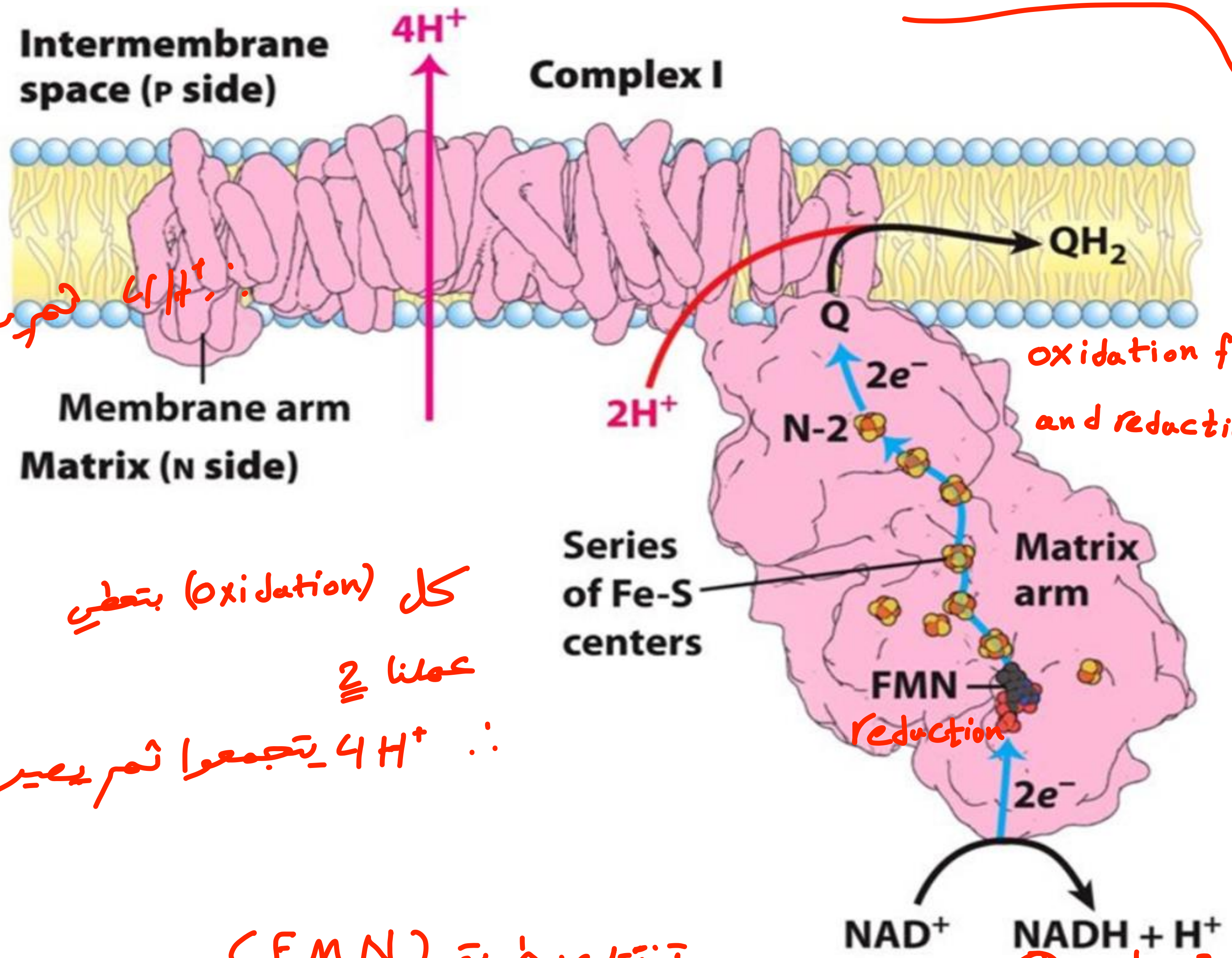
Complex I catalyzes two simultaneous **INDIRECT** coupled processes:

1. The exergonic transfer to ubiquinone of a hydride ion from NADH and a proton from the matrix, expressed by
   - $NADH + H^+ + Q \rightarrow NAD^+ + QH_2$

   بصير عندي (release) للـ(energy) اللي بتضخ البروتونات من ال (matrix) إلى (intermembrane space)

2. The endergonic transfer of **four** protons from the matrix to the intermembrane space (protons are moved against a transmembrane proton gradient in this process.)
   - It moves protons in a from the matrix, which becomes negatively charged with the departure of protons to the intermembrane space, which becomes positively charged. (مغادرة)
     - $NADH + 5H^+_N + Q \rightarrow NAD^+ + QH_2 + 4H^+_P$
   - Complex I is therefore a proton pump driven by the energy of electron transfer

* قبل نقل ال(H+) يكون الداخل سالب والخارج موجب وبعد النقل يصبح العكس



(L shaped)

oxidation for FMN
and reduction for Q

reduction

release لهم تم يصير 4H+ ∴

كل (oxidation) بتعطي 2H+

عملنا 2

∴ 4H+ يتجمعوا ثم يصير لهم release

(FMN) تنتقل عن طريق ← لما تعطي 2 e

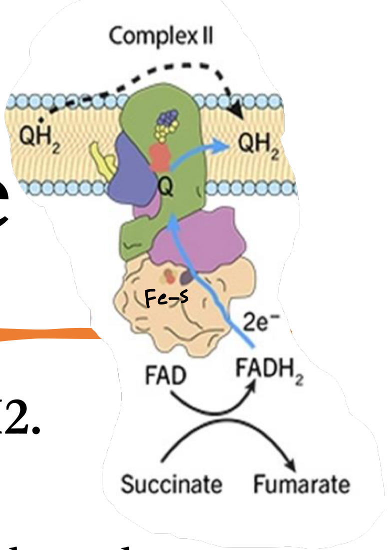
Inhibitors of Complex I

Inhibit electron flow from the **Fe-S centers** of Complex I to **ubiquinone** and therefore block the overall process of oxidative phosphorylation.

1. **Amytal** (a barbiturate drug)
2. **Rotenone** (a plant product commonly used as an insecticide),
3. **Piericidin A** (an antibiotic)

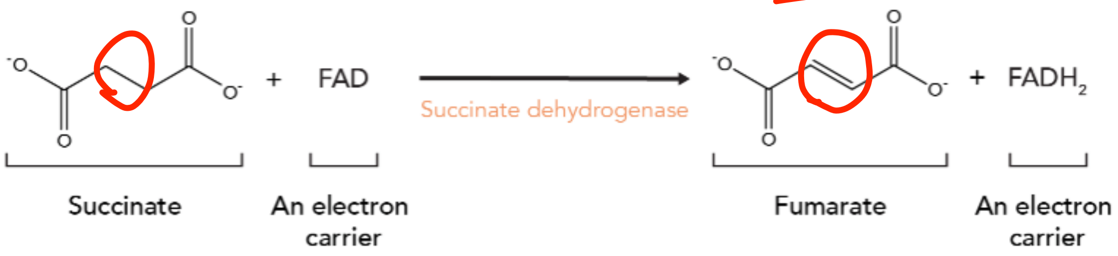
succinate → fumarate
FAD → $FADH_2$ } → Fe-S → CoQ → QH_2

Complex II: Succinate to Ubiquinone



- **This protein complex (succinate dehydrogenase) provides the entry point for FADH2.**

1. In Complex II the enzyme **succinate dehydrogenase** produces fumarate from succinate and produces **FADH2**.



2. FADH2 gives off two energetic electrons to a chain of FeS clusters, ultimately transferring them to coenzyme-Q (to contribute to the flow of electrons in the electron transport chain).

   FADH2 + Ubiquinone (Q) ⟶ FAD + Ubiquinol (QH2)

3. Electron transfer through Complex II is not accompanied by proton pumping across the inner membrane, although the QH2 will be used by Complex III to drive proton transfer.

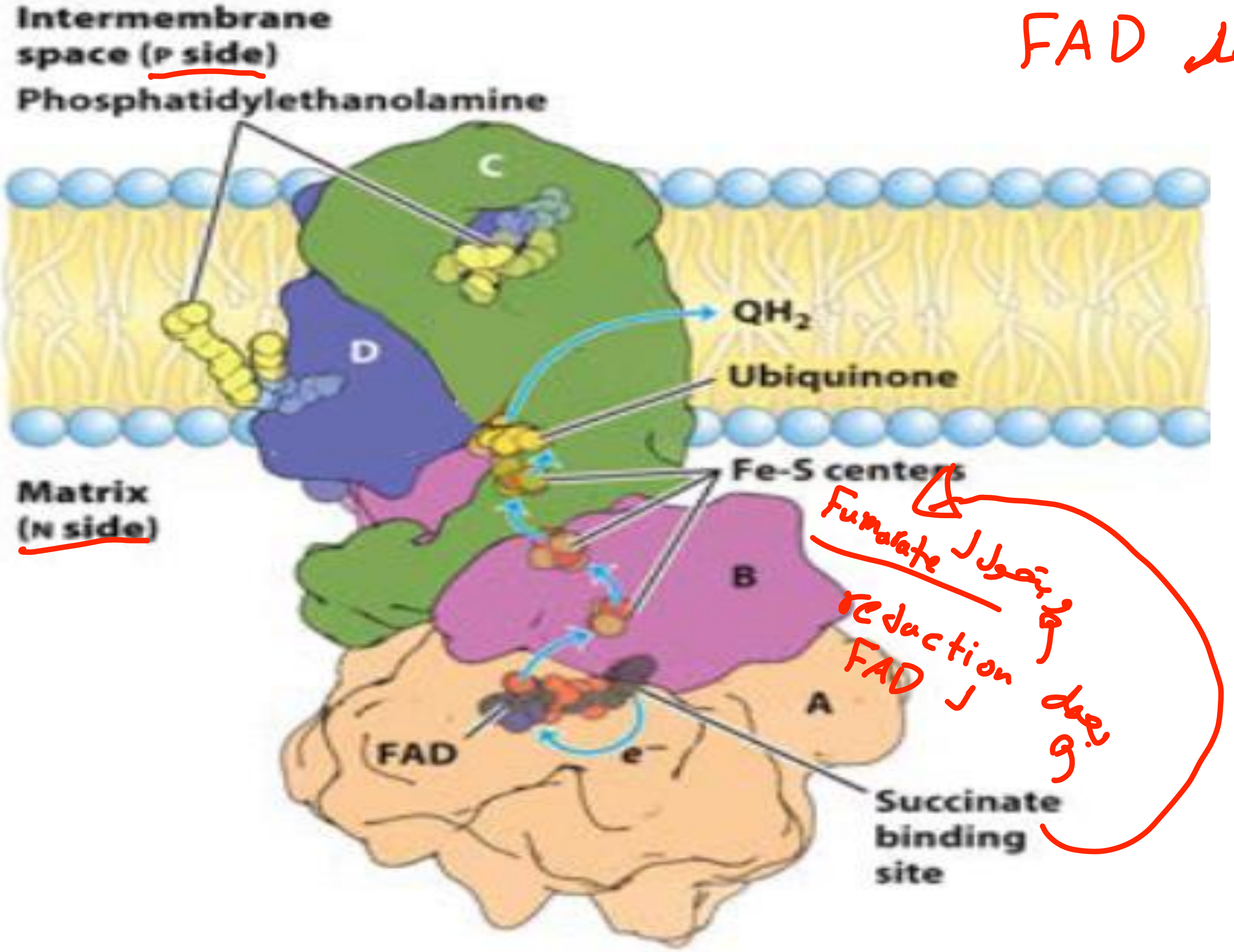
ليش ؟
واجبه

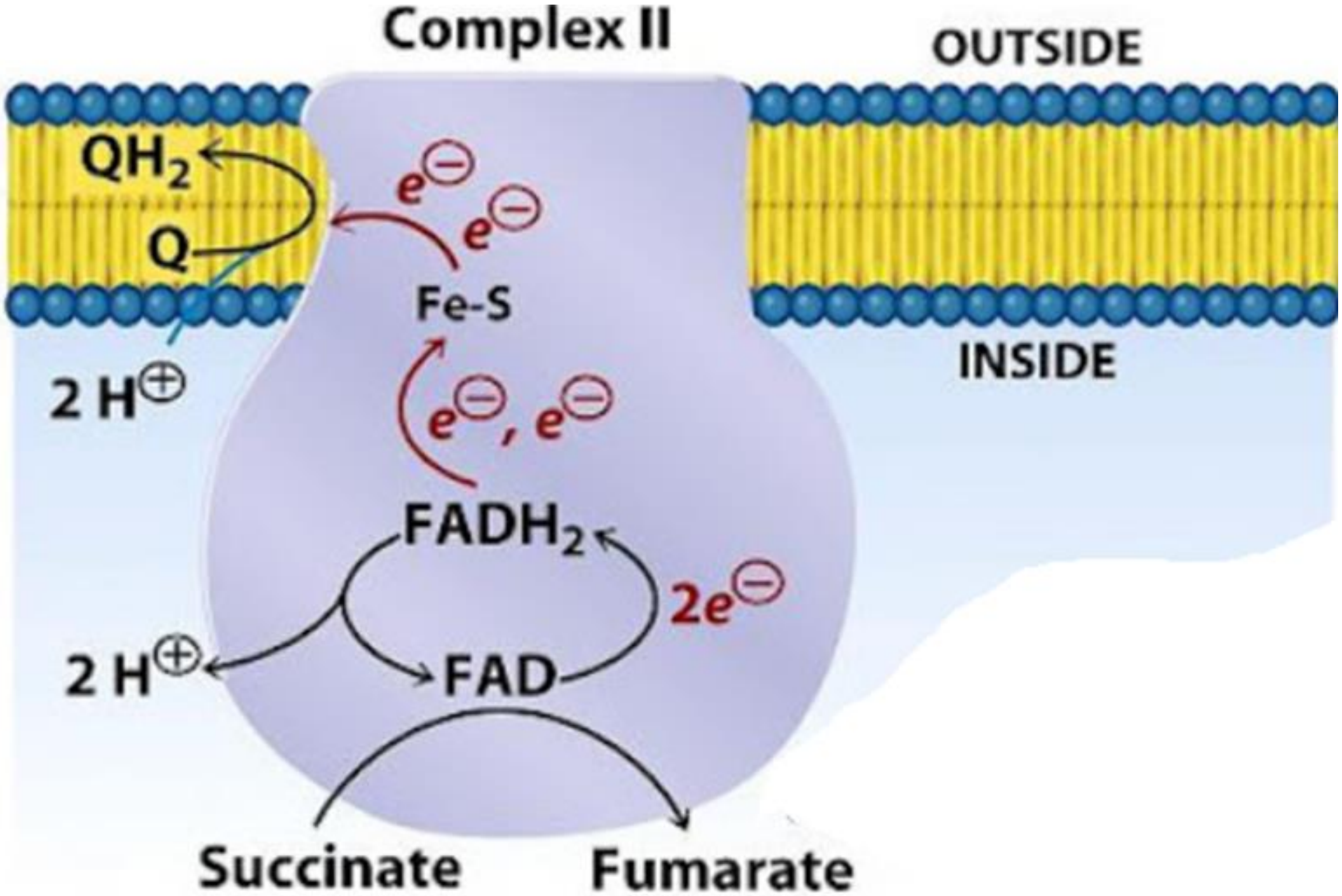
Complex II (2) succinate: ubiquinone oxidoreductase also called succinate dehydrogenase complex, convert coenzyme Q oxidized to reduced coenzyme QH2 with electrons accepted by succinate.

This reactions is usually characterized with very little free energy. For this reason, complex II cannot directly contribute to proton concentration gradient across the membrane.

أول complex بدخل NAD

ثاني complex بدخل FAD




Complex II
OUTSIDE
INSIDE
QH_2
Q
e^{-}
e^{-}
Fe-S
$2H^{+}$
e^{-}, e^{-}
$FADH_2$
$2e^{-}$
$2H^{+}$
FAD
Succinate
Fumarate

1) (succinate) بعمل (reduction) لـ (FAD) ويتحول لـ (Fumarate)

2) FAD صار $FADH_2$ بصير له (oxidation) ويتم نقل ال (e) عبر (Fe-S)

3) تصل بعدها إلى Q ليحدث له (reduction) ويتحول إلى QH_2

Inhibitors of Complex II

- **Malonate**: acts as **competitive inhibitor** for succinate
- Mutations that affect the succinate-binding region in Complex II may lead to **degenerative changes in the central nervous system**, and some mutations are associated with **tumors of the adrenal medulla**.

Dimer: so → two subunits

Complex III: Ubiquinone to Cytochrome c

- Called **ubiquinone:cytochrome c oxidoreductase**
- The functional unit of Complex III is a dimer.
- Each monomer consists of **three proteins central to the action of the complex**: **cytochrome b**, **cytochrome c1**, and the **Rieske iron-sulfur protein**.
  - The Rieske cluster allows these proteins to efficiently transfer electrons during redox reactions.

Complex III: Ubiquinone to Cytochrome c

1. Complex III couples the transfer of electrons from **ubiquinol** to **cytochrome c** with the transport of protons from the matrix to the intermembrane space.

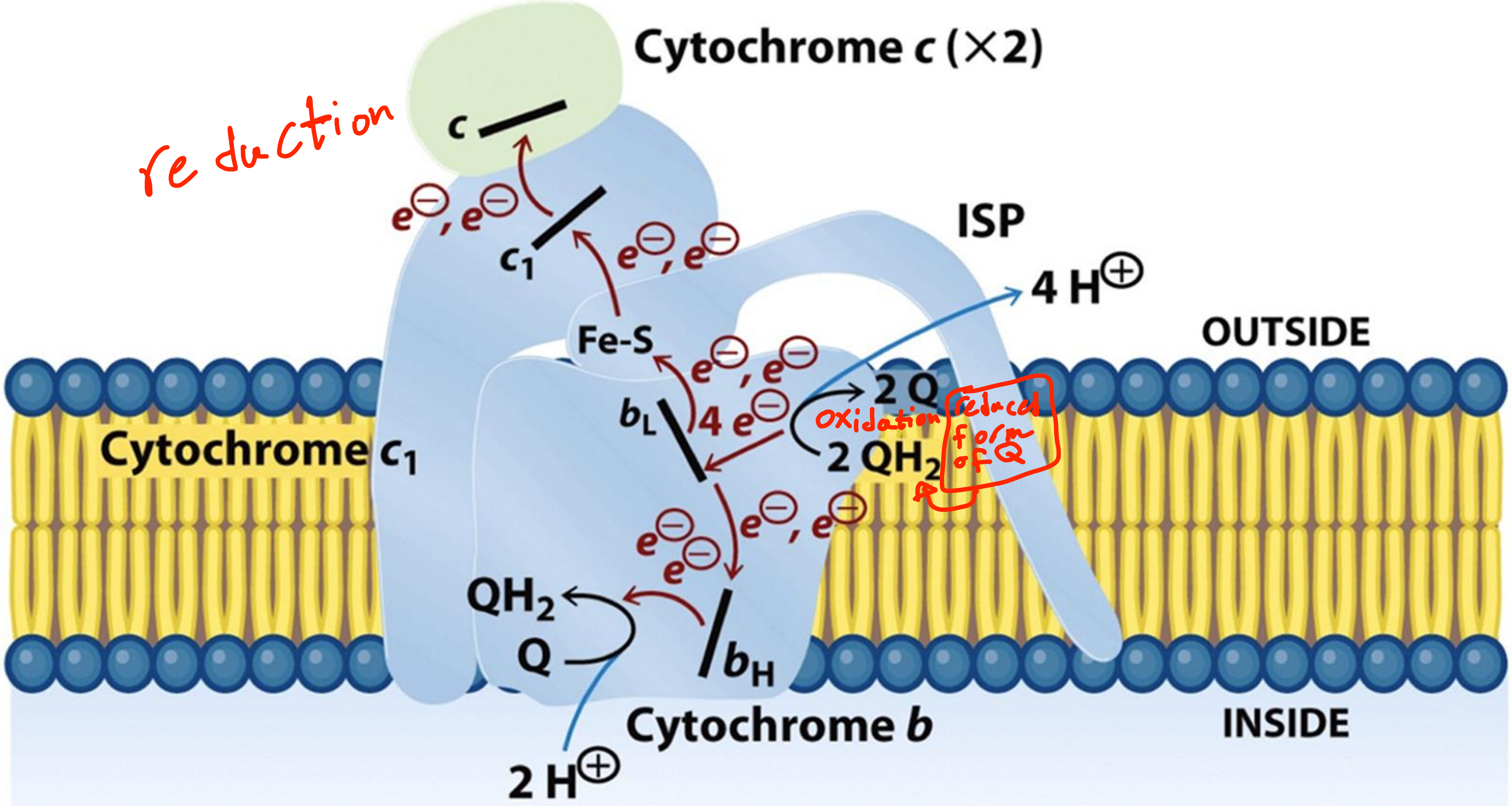
2. Complex III catalyzes the transfer of electrons from the **reduced coenzyme Q** (ubiquinol) to **cytochrome c.**

3. QH_2 is oxidized to Q, two molecules of cytochrome c are reduced, and two protons are moved from the N side to the P side of the inner mitochondrial membrane.

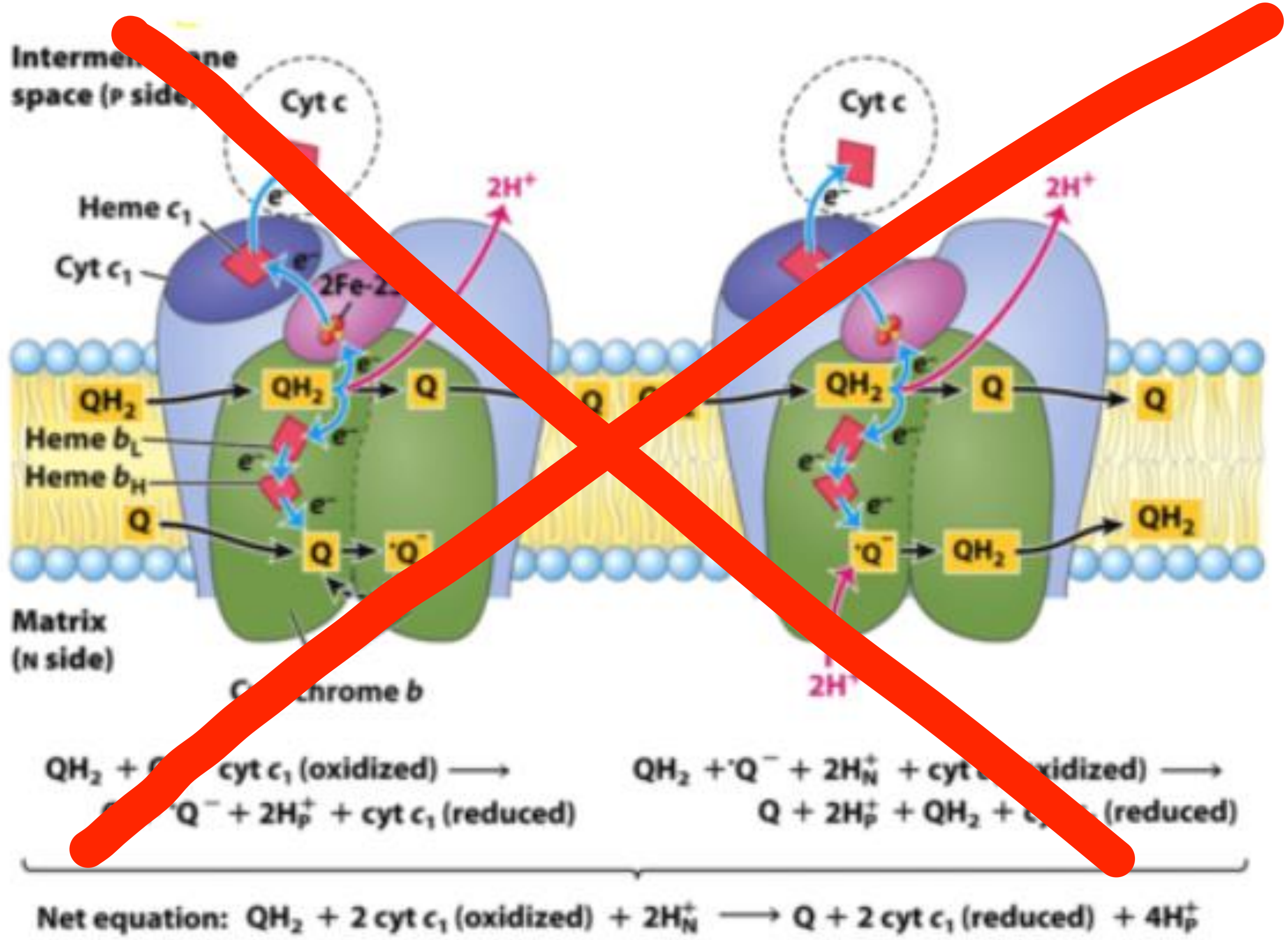
   $$QH_2 + 2\ \text{cyt c (oxidized)} + 2H^+_N \rightarrow Q + 2\ \text{cyt c (reduced)} + 4H^+_P$$

   Dimer [illegible]

4. **4 protons are pumped out**



Cytochrome c (×2)
c
reduction
e⊖, e⊖
c1
e⊖, e⊖
ISP
4 H⊕
OUTSIDE
Fe-S
e⊖, e⊖
bL
4 e⊖
2 Q
Oxidation
2 QH2
reduced form of Q
Cytochrome c1
e⊖
e⊖
e⊖, e⊖
QH2
Q
bH
Cytochrome b
INSIDE
2 H⊕


Cyt c
Cyt c
2H⁺
2H⁺
Heme c₁
Cyt c₁
QH₂
QH₂
Q
Q
Heme b_L
Heme b_H
Q
Q
·Q⁻
·Q⁻
QH₂
QH₂
Matrix
(N side)
2H⁺
Net equation: QH₂ + 2 cyt c₁ (oxidized) + 2H⁺_N ⟶ Q + 2 cyt c₁ (reduced) + 4H⁺_P

Inhibitors of Complex III

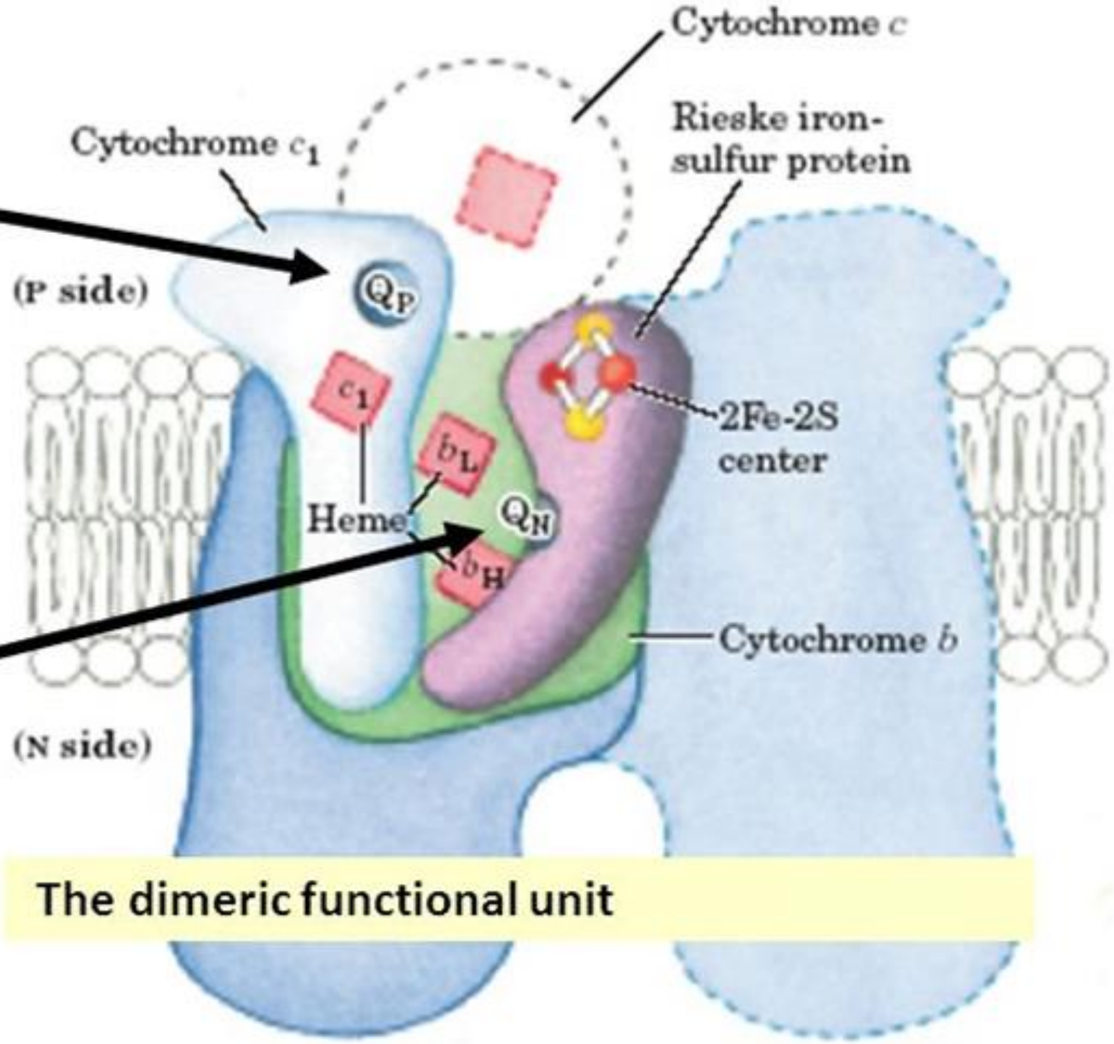
- **Antimycin A**, binds at ubiquinol oxidation site, which blocks electron flow from cytochrome b to cytochrome c1. This binding prevents the transfer of electrons from ubiquinol (QH2) to cytochrome c.

- **Myxothiazol**, which prevents electron flow from QH_2 to the Rieske iron-sulfur protein, binds at Q_P.

توضيحية فقط وبنه شغالة (I)

Myxothiazol, which prevents electron flow from QH2 to the Rieske iron-sulfur protein, binds at QP,

Antimycin A, which blocks electron flow from heme *b*H to Q, binds at QN,



Complex IV: Cytochrome c to O_2

Terminal acceptor

- **Complex IV (Cytochrome Oxidase), which reduces an oxygen molecule to a water molecule and providing 4 hydrogens (2 protons per pair of electrons) to the intermembrane space:**

(H_2O) (4H+)

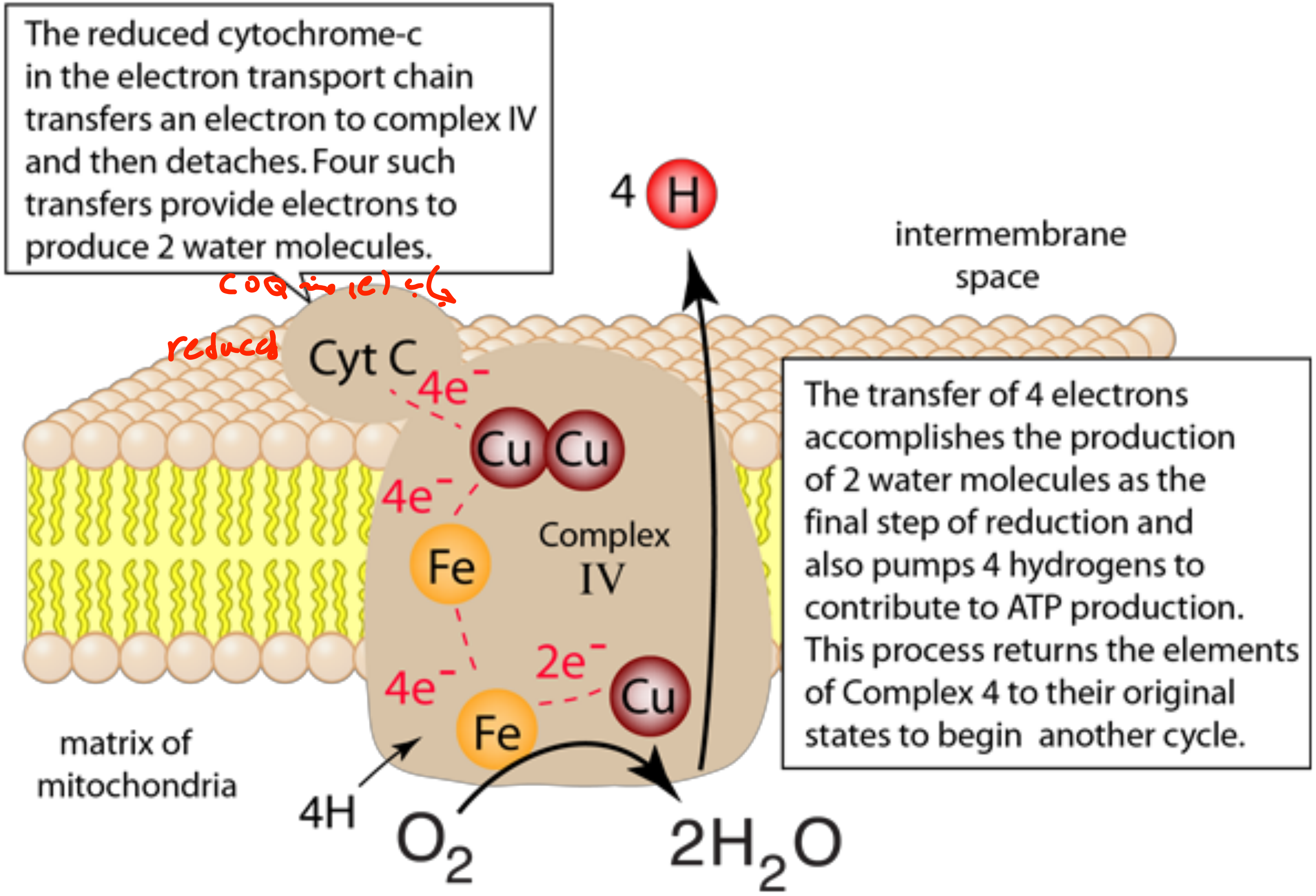
1. Electron transfer through Complex IV is from **cytochrome c** to the **CuA center**, to **heme a**, to the **heme a3–CuB center**, and finally to **O2**.

2. For every four electrons passing through this complex, the enzyme consumes four "substrate" H+ from the matrix ($_N$ side) in converting O_2 to two H_2O.

3. It also uses the energy of this redox reaction to pump four protons outward into the intermembrane space ($_P$ side) for each four electrons that pass through.

$$4 \text{ cyt c(reduced)} + 8H^+_N + O_2 \rightarrow 4 \text{ cyt c (oxidized)} + 4H^+_P + 2H_2O$$

Exergonic is oxidation


The reduced cytochrome-c in the electron transport chain transfers an electron to complex IV and then detaches. Four such transfers provide electrons to produce 2 water molecules.
reduced
Cyt C
4e⁻
Cu Cu
4e⁻
Fe
Complex IV
2e⁻
4e⁻
Fe
Cu
4 H
intermembrane space
matrix of mitochondria
4H
O_2
$2H_2O$
The transfer of 4 electrons accomplishes the production of 2 water molecules as the final step of reduction and also pumps 4 hydrogens to contribute to ATP production. This process returns the elements of Complex 4 to their original states to begin another cycle.

* (Cytochrome c) ينقل (e) إلى (CuA center) بعدها إلى (heme a)

بعدها إلى (heme a3-CuB center) وآخر شيء إلى (O_2) عشان يصير له (reduction)

ويتحول إلى ماء ويتم ضخ ($4H^+$) إلى الخارج وبعد كل هاد بالآخر برجع كل شيء طبيعي

عشان يتم استقبال (ETC) جديدة

بصير عندي (concentration) بتبدأ أعلى فبتقل H^+ جوّا ثم برجع عشان يساعد في (Oxidetive phosphorilase)

Inhibitors of Complex IV

Cyanide

- One of most potent (قوية) & rapidly acting poisons
- Bind to cytochrome a & a3 (oxidised form of heme) → **inhibit oxidative phosphorylation**
- Energy produced by cells will be blocked → **asphyxia (الإختناق) especially of CNS → death**

Carbon monoxide

- Bind to reduced form of heme **competitively with O2**
- **Prevents electron transfer to O2**
- Inhibition of mitochondrial electron transport → **impairment (ضعف) of energy generating function of oxidative phosphorylation** → death

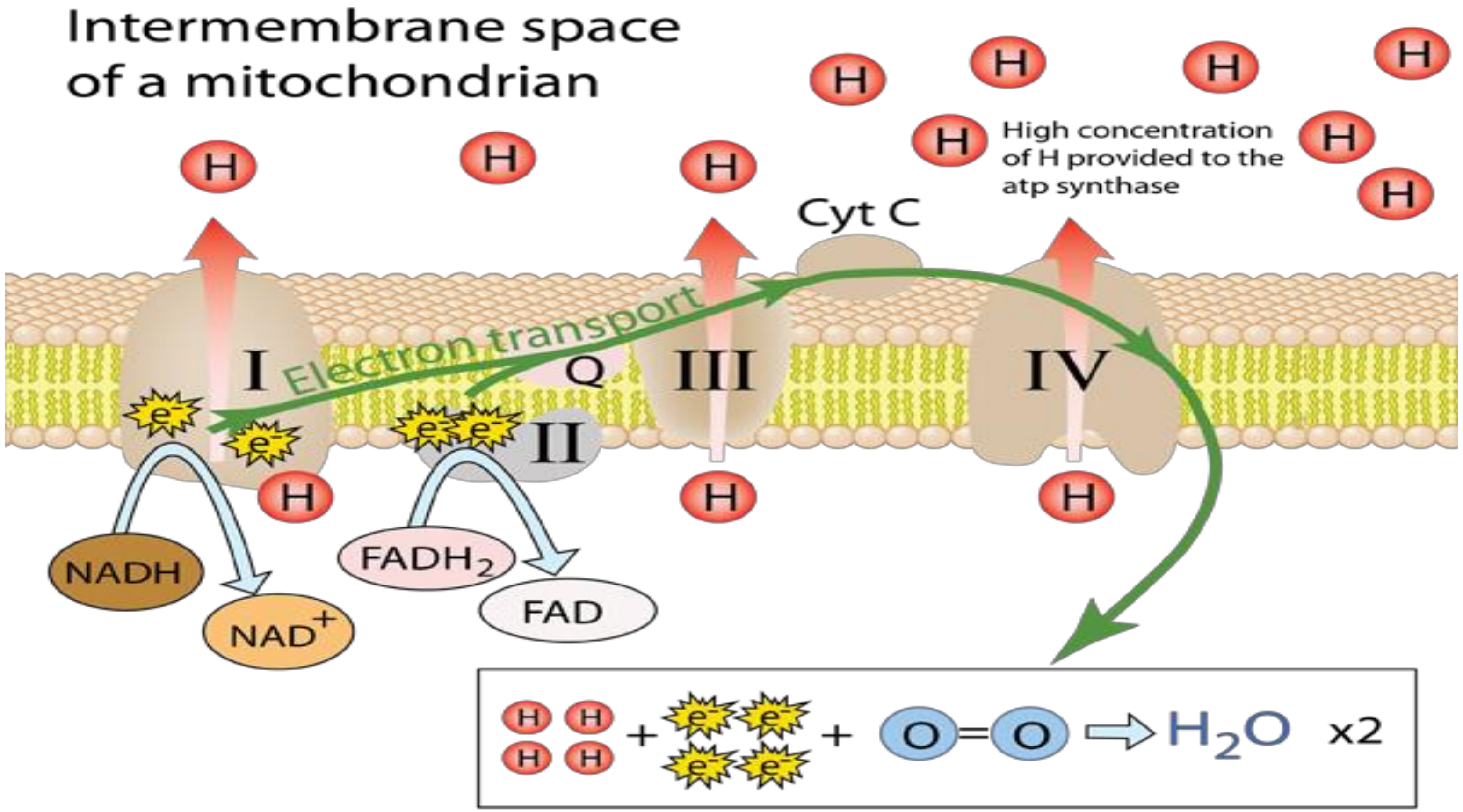
Summary

- **Complexes I and II** catalyze electron transfer to ubiquinone from two different electron donors: **NADH** (Complex I) and **succinate** (Complex II).

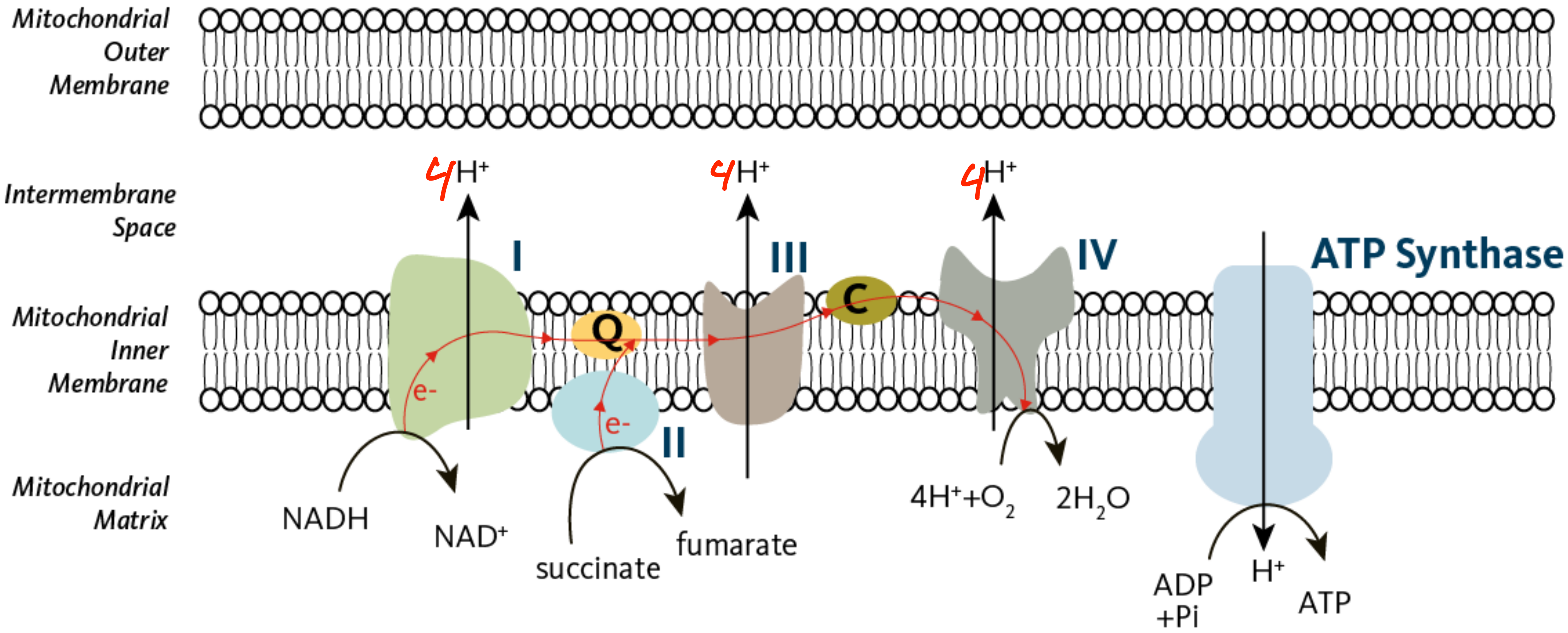
  FADH2

- **Complex III** carries electrons **from** reduced ubiquinone to cytochrome c.

- **Complex IV** completes the sequence by transferring electrons from cytochrome c to O2.



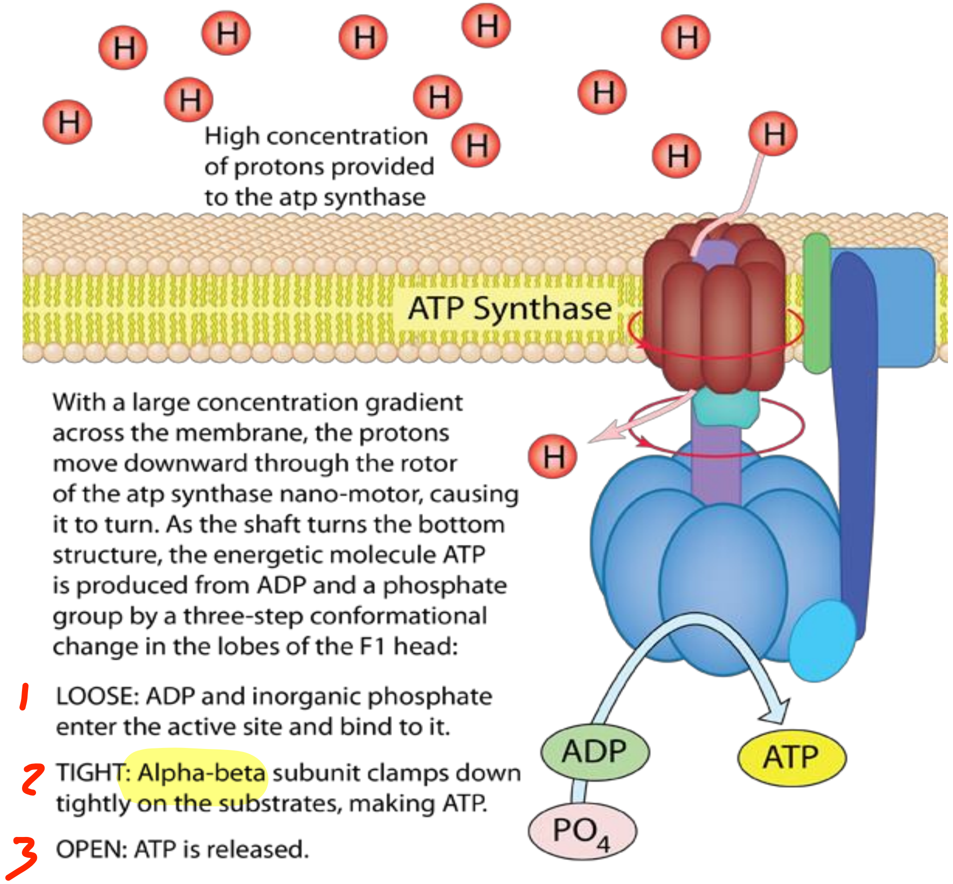
Inside the mitochondrial matrix, the electron transport chain and the atpsynthase nano-machine are tightly coupled systems to provide energy for metabolism.


Mitochondrial Outer Membrane
Intermembrane Space
Mitochondrial Inner Membrane
Mitochondrial Matrix
4H+
4H+
4H+
I
III
IV
ATP Synthase
Q
C
e-
e-
II
NADH
NAD+
succinate
fumarate
4H+ +O2
2H2O
ADP +Pi
H+
ATP

ATP synthesis

<u>Chemiosmotic theory</u>: Transfer of electrons along ETC is accompanied by outward pumping of protons.

- Protons accumulate outside inner membrane
- External surface becomes more positively charged, matrix negatively charged → **<u>gradient</u>**
- ***This electrochemical gradient drives ATP synthesis by movement of protons down gradient using ATP synthase***



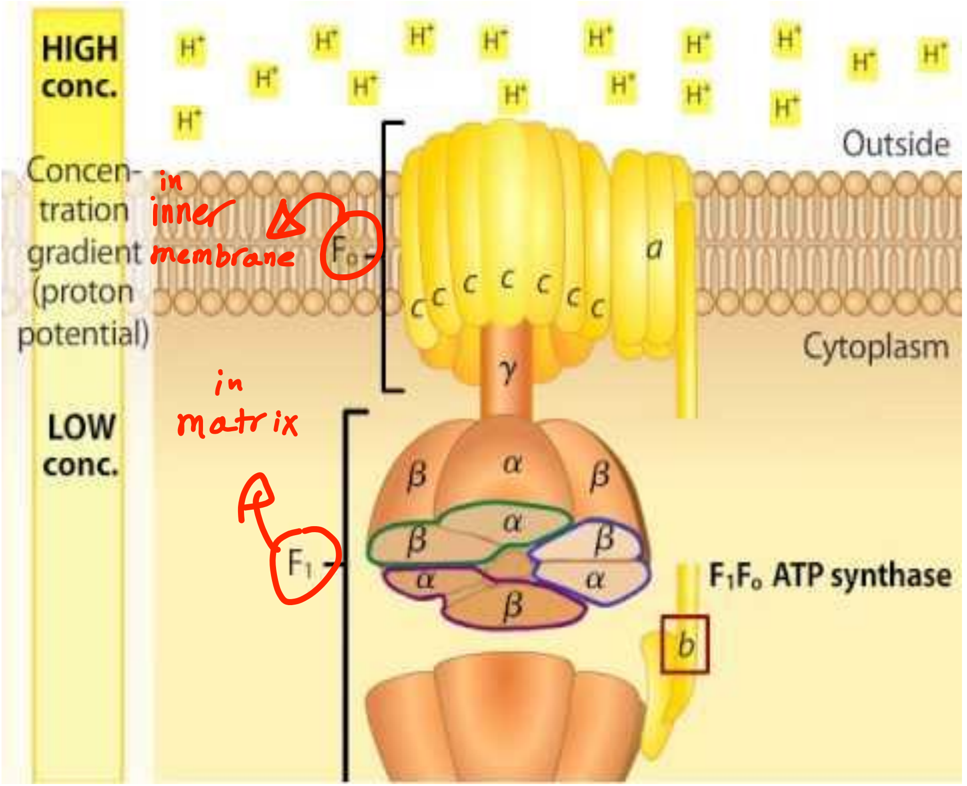
https://www.youtube.com/watch?v=zJNx1DDqIVo

شرح الطريقة

ATP synthesis

ATP synthase enzyme:

- Composed of **2 major components**: F0 (oligomycin sensitive portion) and F1
- Present in **inner mitochondrial membrane**
- Uses **proton-motive force for ATP synthesis**



Protons passage leads to:

→configurational changes → activation of catalytic F1 subunit

- **Inhibition of F0 subunit by oligomycin** → blocks electron movement
  [explains coupling between electron movement & ATP synthesis)

Findings that support chemiosmotic theory

1. Addition of protons (acid) to external medium of mitochondria → **stimulates ATP production**

2. Oxidative phosphorylation **does not occur** in case of **solubilizing mitochondrial membranes**:
   - If **leak of H+ across membrane is induced** → proton gradient would be discharged → energy coupling would fail

3. Uncouplers

Inhibitors/ uncouplers of OXPHOS

1. Inhibitors of **ETC** proper

2. Inhibitors of **phosphorylation** → oligomycin (antibiotic): completely blocks F0 (ATP synthase) so it inhibits ATP synthesis

3. **ATP/ ADP transporter inhibitor** → atractyloside [natural, toxic glycoside present in numerous plant species]

4. Uncouplers of oxidative phosphorylation

(O Ph) بتمنع

Uncouplers of oxidative phosphorylation

بتوخذه من برا

- **Interrupt/ uncouple oxidation & phosphorylation** (carry H+ across inner mitochondrial membrane without passing through complex V)
  - i.e. oxidation will proceed building proton gradients but **will not result in ATP synthesis**
  - Energy that would have been used for ATP synthesis is **dissipated as heat**

- **In presence of uncouplers**, oxidative process becomes **uncontrolled** as concentration of **ADP no longer a limiting rate**
  - Proton gradient will give heat

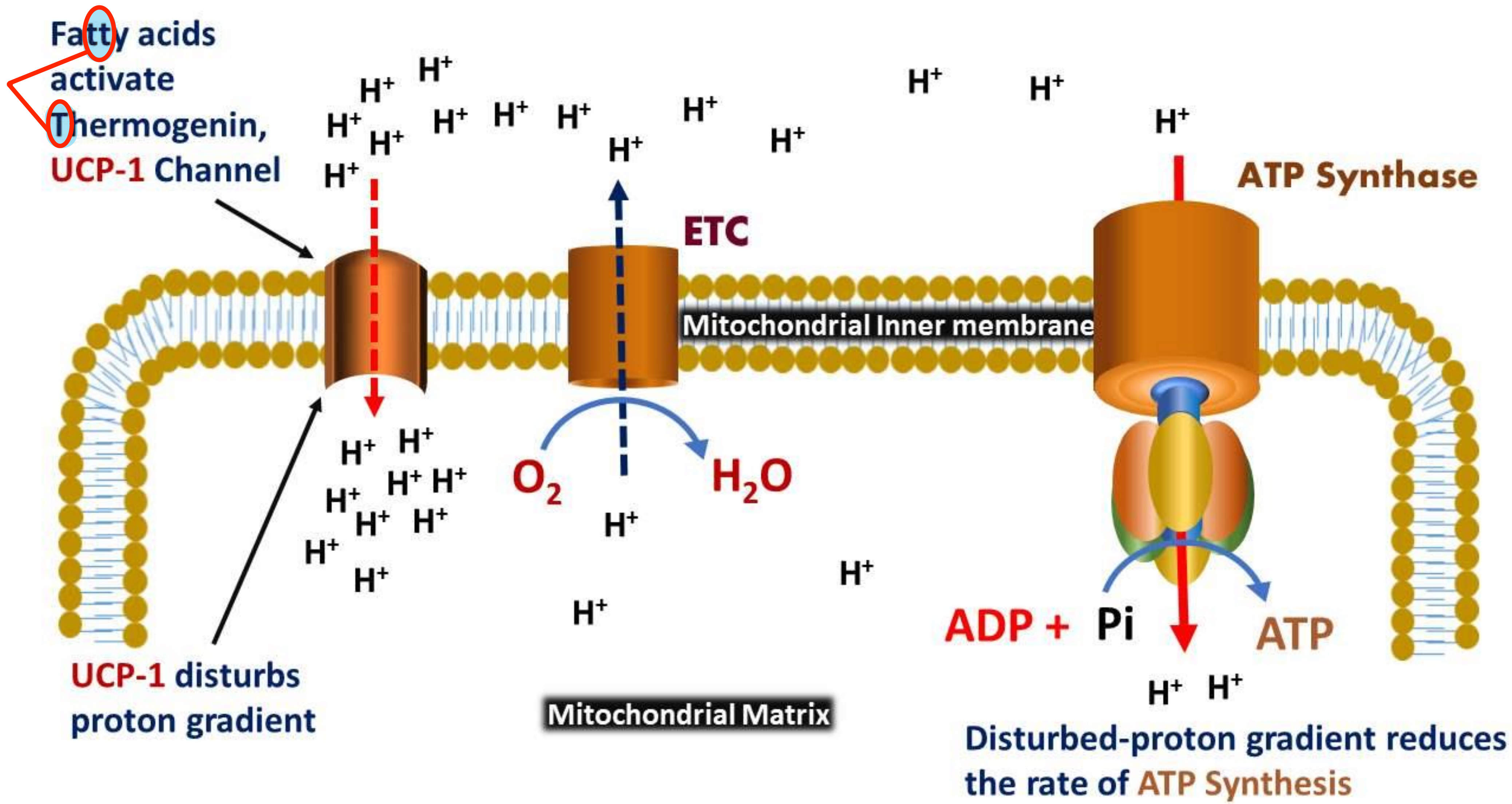
Examples of uncouplers of oxidative phosphorylation

بوجود (uncouplers)
هل في انتقال للـ (e)؟ يس
هل بصير (oph)؟ نو
هل ينتج ATP؟ نو

- High level of <u>bilirubin</u>
- High level of <u>thyroxin</u>
- <u>Snake venoms</u> (their phospholipases)
- Halothane intoxication

هيك مخلوقة

- **<u>Thermogenin (physiological uncoupler present in brown fat)</u>**
  - **Brown fat**: high content of mitochondria, rich blood supply → characteristic brown colour
  - **Uses oxidation of fuel not to produce ATP** but **heat** to keep **new-born warm**
  - A specialised protein called <u>**thermogenin**</u> is present in inner mitochondrial membrane → provides a path for protons to return to the matrix without passing through the F0/F1 complex


Fatty acids
activate
Thermogenin,
UCP-1 Channel
ETC
ATP Synthase
Mitochondrial Inner membrane
O_2
H_2O
ADP + Pi
ATP
UCP-1 disturbs
proton gradient
Mitochondrial Matrix
Disturbed-proton gradient reduces
the rate of ATP Synthesis

كل ما زاد ADP / NADH / FADH2 / O2 / Pi رح تزيد (ATP Synthesis)
الأعظم هو ADP

Respiratory control of ETC

- **Rate of oxidative phosphorylation is determined by the need for ATP**
  - When ADP levels increase in the cell, it reflects a higher demand for ATP.
  - This elevated ADP concentration acts as a signal to the ETC to accelerate the flow of electrons and enhance the proton pumping, **resulting in increased ATP synthesis** to meet the cellular energy demands.

- **The most important determining factor of oxidative phosphorylation is:**
  - ADP level
- Other important regulatory factors include:
  - NADH, FADH2, O2, Pi

P: O ratio

- **It is a measure of how many moles of ATP are formed per gram atom of oxygen for a given substrate**

  - It is 3 for NADH-linked substrates **(old system)**

  - It is 2 for FADH2 linked substrates (as succinate) → **old system**

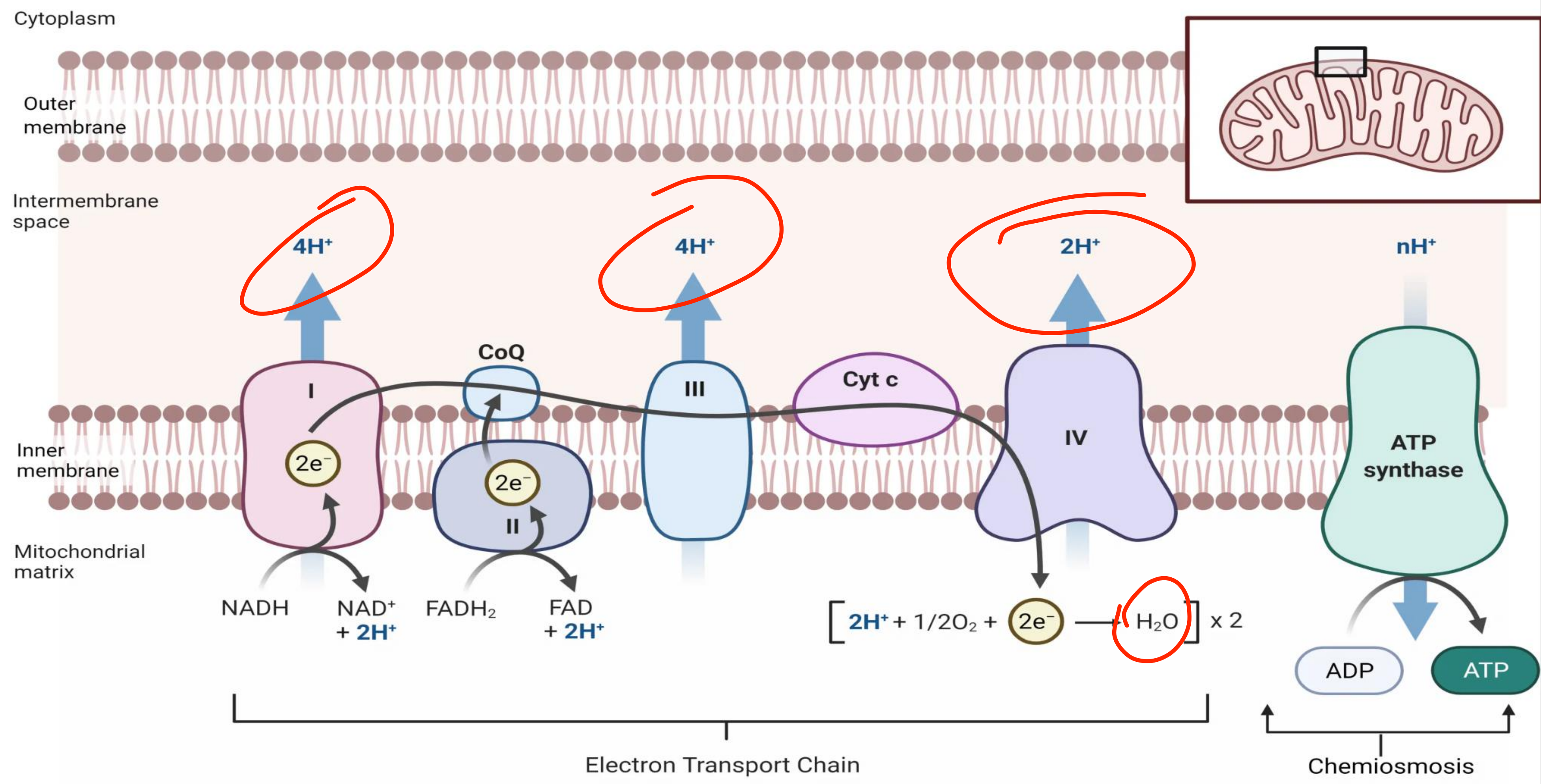
  - It is equal to **0 in the presence of uncouplers**

ما تركز عليهم

Table 19.4. ATP generation, old and new values

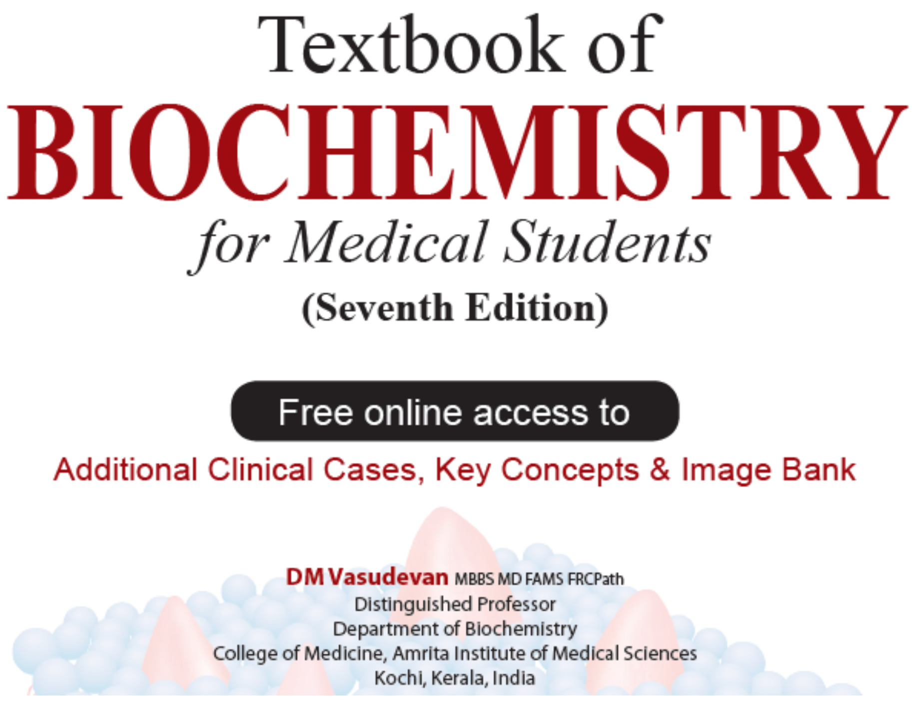
ATP generation by oxidation of	Old value	Presently accepted
NADH	3	2.5
FADH	2	1.5
Glucose	38	32
Acetyl CoA	12	10
Palmitate	129	106

Oxidative Phosphorylation



Read from book

- Paragraph on "Diseases associated with mitochondria"



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