بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Lecture 7

Oxidative Phosphorylation

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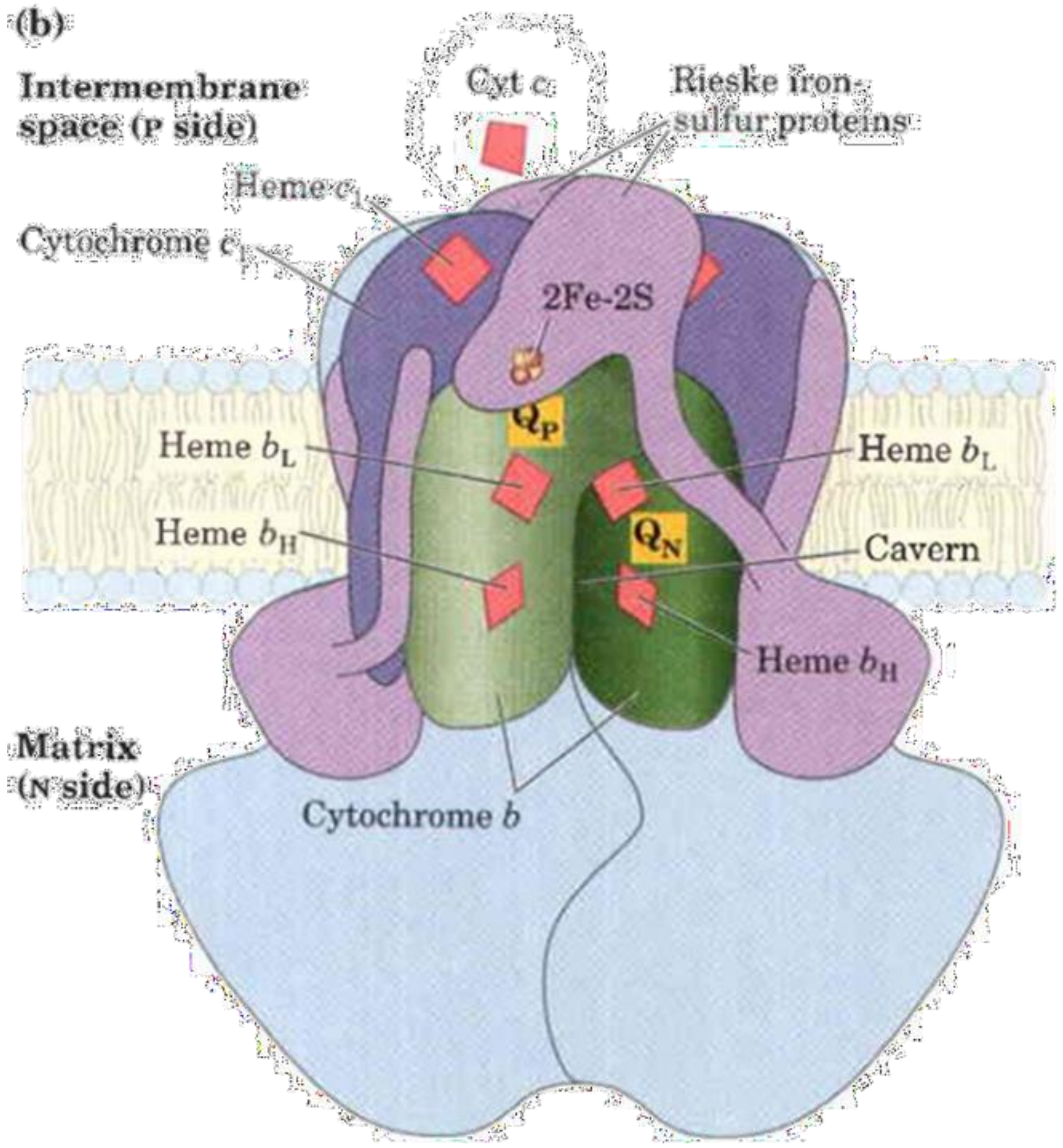
OXI–RED COMPONENTS OF THE ETC: "CYTOCHROME BC$_1$" – COMPLEX III Dimer

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



Complex III (Cytochrome bc_1 Complex)

- It contains two types of heme b groups :

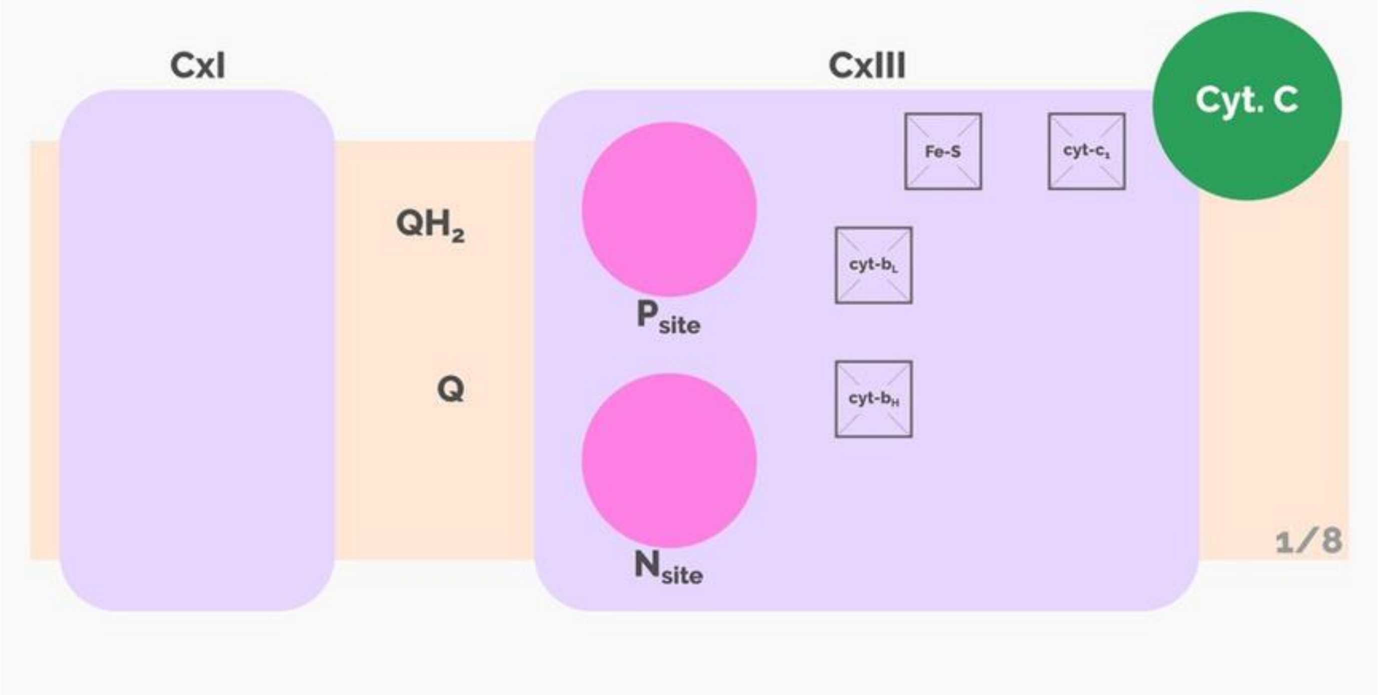
1) **Heme bL**
2) **Heme bH**

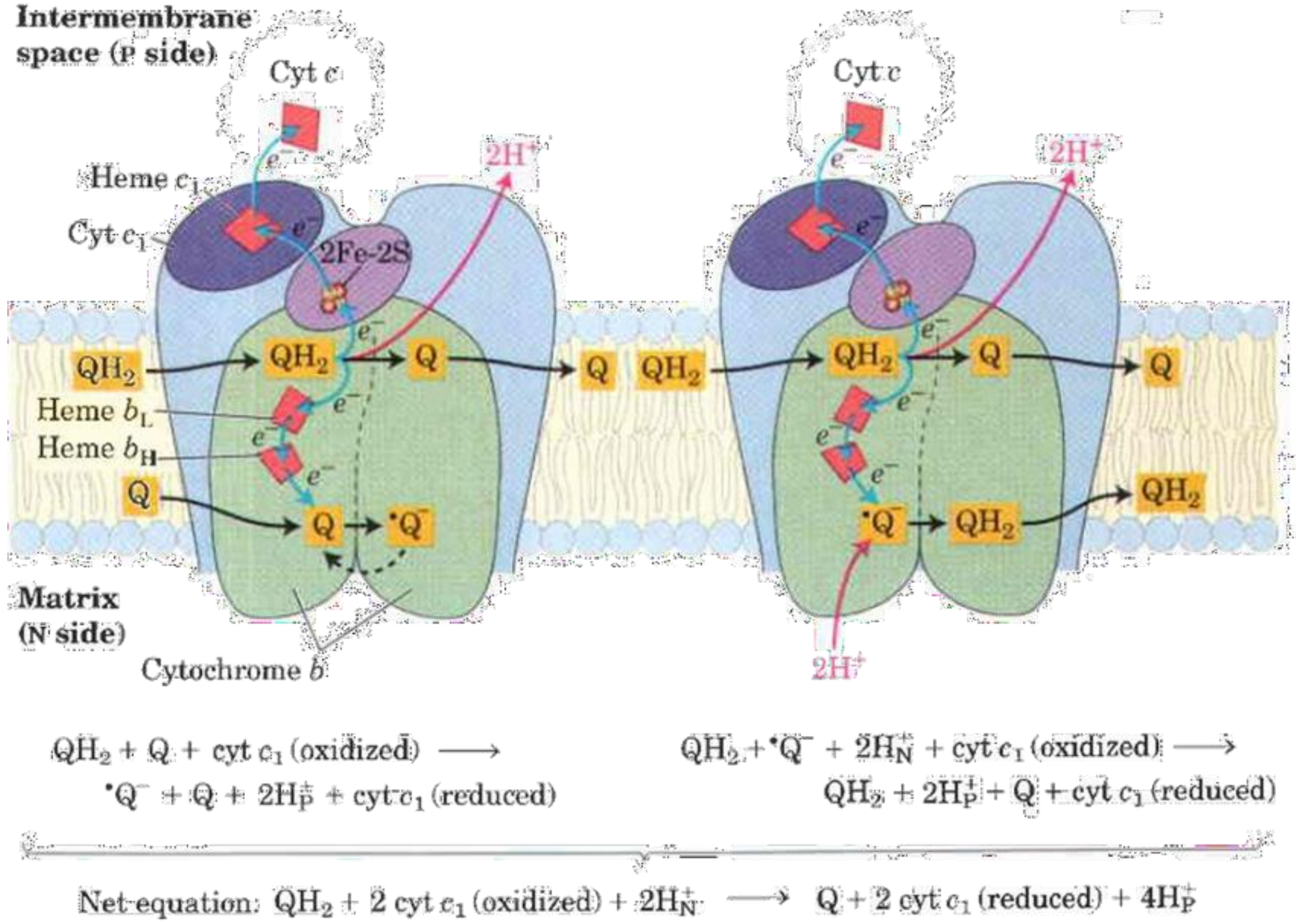
based on their **redox potential** (**bH: Higher** reduction potential**, bL lower reduction** potential).

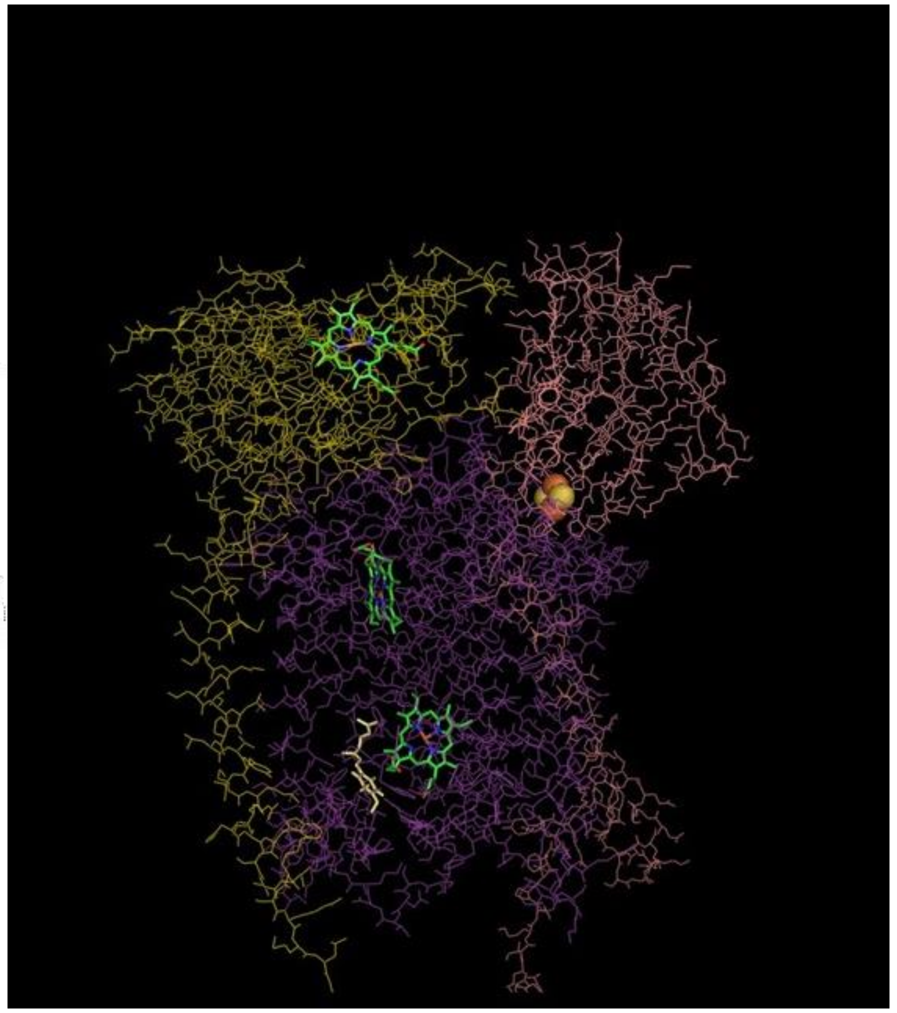
- It also contains an iron-sulfur (Fe-S) cluster and a heme c within cytochrome c_1 , which transfers electrons to cytochrome c.

How does Complex III function?

The electrons' destination is **cytochrome c**, which can carry **only one electron.** However, two electrons are delivered from **ubiquinol (QH_2)** at a time.
This issue is resolved by the **Q-cycle** mechanism, which splits the two electrons from **QH_2** and transfers them **one by one.**





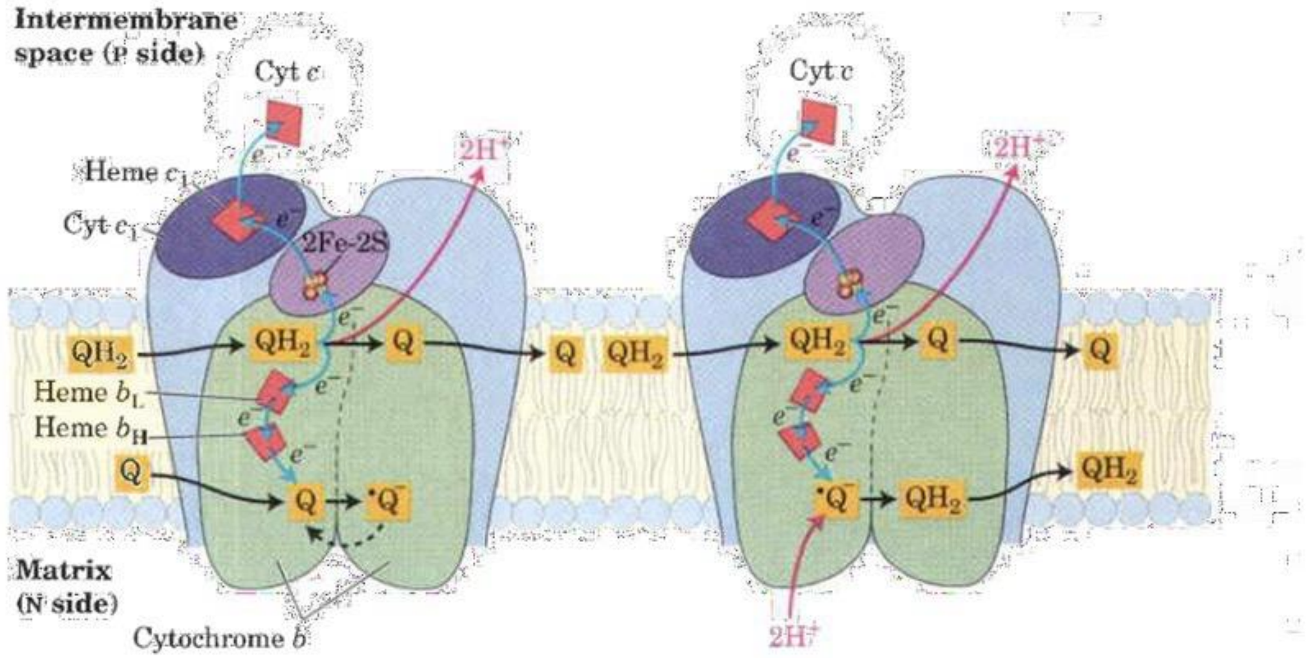


THE Q-CYCLE

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

$$QH_2 + 2 \text{ cyt } c_1 \text{ (oxidized)} + 2H^+_N \longrightarrow Q + 2 \text{ cyt } c_1 \text{ (reduced)} + 4H^+_P$$

How Complex III works (binding sites):



$$QH_2 + Q + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow {}^{\bullet}Q^- + Q + 2H^+_P + \text{cyt } c_1 \text{ (reduced)}$$

$$QH_2 + {}^{\bullet}Q^- + 2H^+_N + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow QH_2 + 2H^+_P + Q + \text{cyt } c_1 \text{ (reduced)}$$

$$\text{Net equation: } QH_2 + 2 \text{ cyt } c_1 \text{ (oxidized)} + 2H^+_N \longrightarrow Q + 2 \text{ cyt } c_1 \text{ (reduced)} + 4H^+_P$$

- Complex III has two Q-binding sites:

- One site binds reduced Q (QH_2).
- The other site binds oxidized Q (Q).

- These two sites have different affinities:

The site for reduced Q has high affinity for QH_2, which means it has low affinity for oxidized Q

The reduced molecule (QH_2) comes to Complex III and binds to the site with high affinity. It carries two electrons:

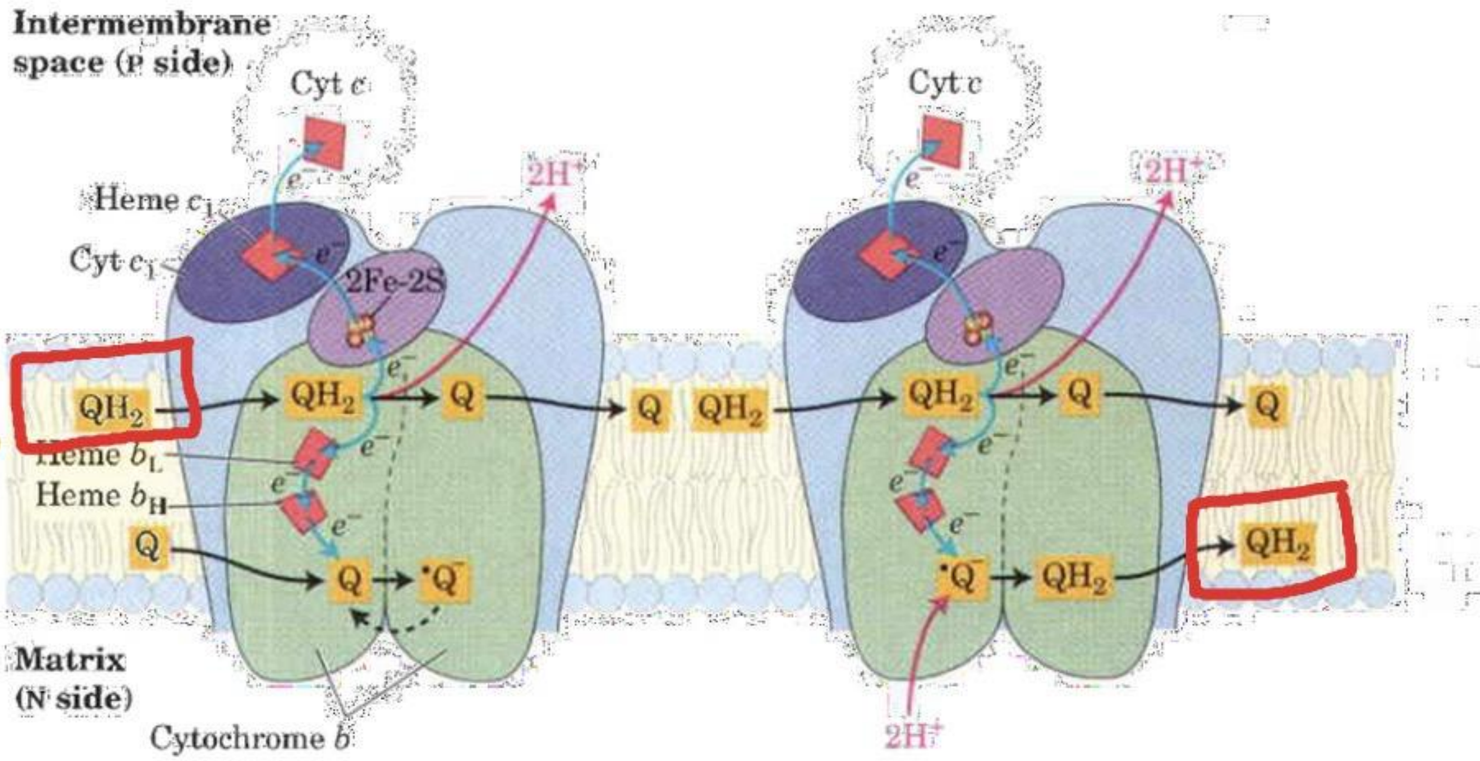
The first electron passes to the iron-sulfur cluster inside the enzyme, then to heme c, and finally to cytochrome c.

The second electron goes to heme bL, then to heme bH. This heme bH is very close to the site where the oxidized Q binds, so it transfers the electron to the oxidized Q, which becomes partially reduced

The partially reduced Q still has affinity for the site, but it is lower than that of the oxidized Q.
When the reduced molecule donates its electrons, it detaches from the site and returns to the membrane, leaving the site available for another QH_2 to bind.

From the new QH_2, one electron passes to the iron-sulfur cluster, then to heme c, and finally to cytochrome c.
The second electron travels through heme bL and heme bH to fully reduce the semireduced Q, converting it into QH_2.

This newly formed QH_2 then detaches from the site and returns to the membrane — the same form that initially started the cycle

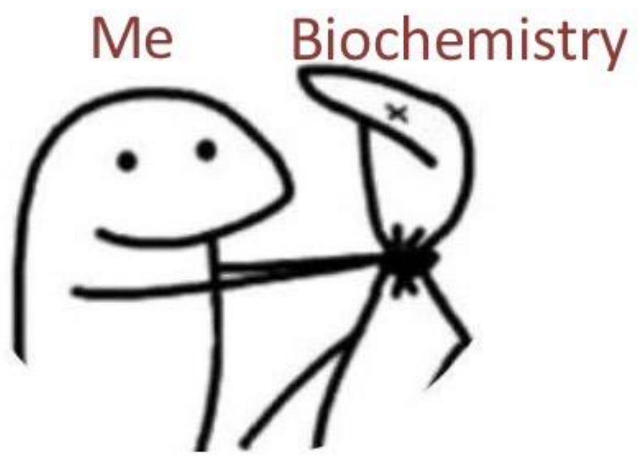


$QH_2 + Q + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow {}^{\bullet}Q^- + Q + 2H_P^+ + \text{cyt } c_1 \text{ (reduced)}$

$QH_2 + {}^{\bullet}Q^- + 2H_N^+ + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow QH_2 + 2H_P^+ + Q + \text{cyt } c_1 \text{ (reduced)}$

Net equation: $QH_2 + 2 \text{ cyt } c_1 \text{ (oxidized)} + 2H_N^+ \longrightarrow Q + 2 \text{ cyt } c_1 \text{ (reduced)} + 4H_P^+$

The difference in potential between Complex III and cytochrome c provides the energy needed to pump 2 protons across the membrane each time an electron is released.
Since the cycle repeats twice, a total of 4 protons are pumped per full Q cycle.



How many QH_2 molecules are used in the cycle?

Two QH_2 molecules are used.

How many QH_2 molecules are regenerated?

One QH_2 molecule is regenerated.

How many electrons are used in total?

Four electrons are used.

How many electrons are regenerated?

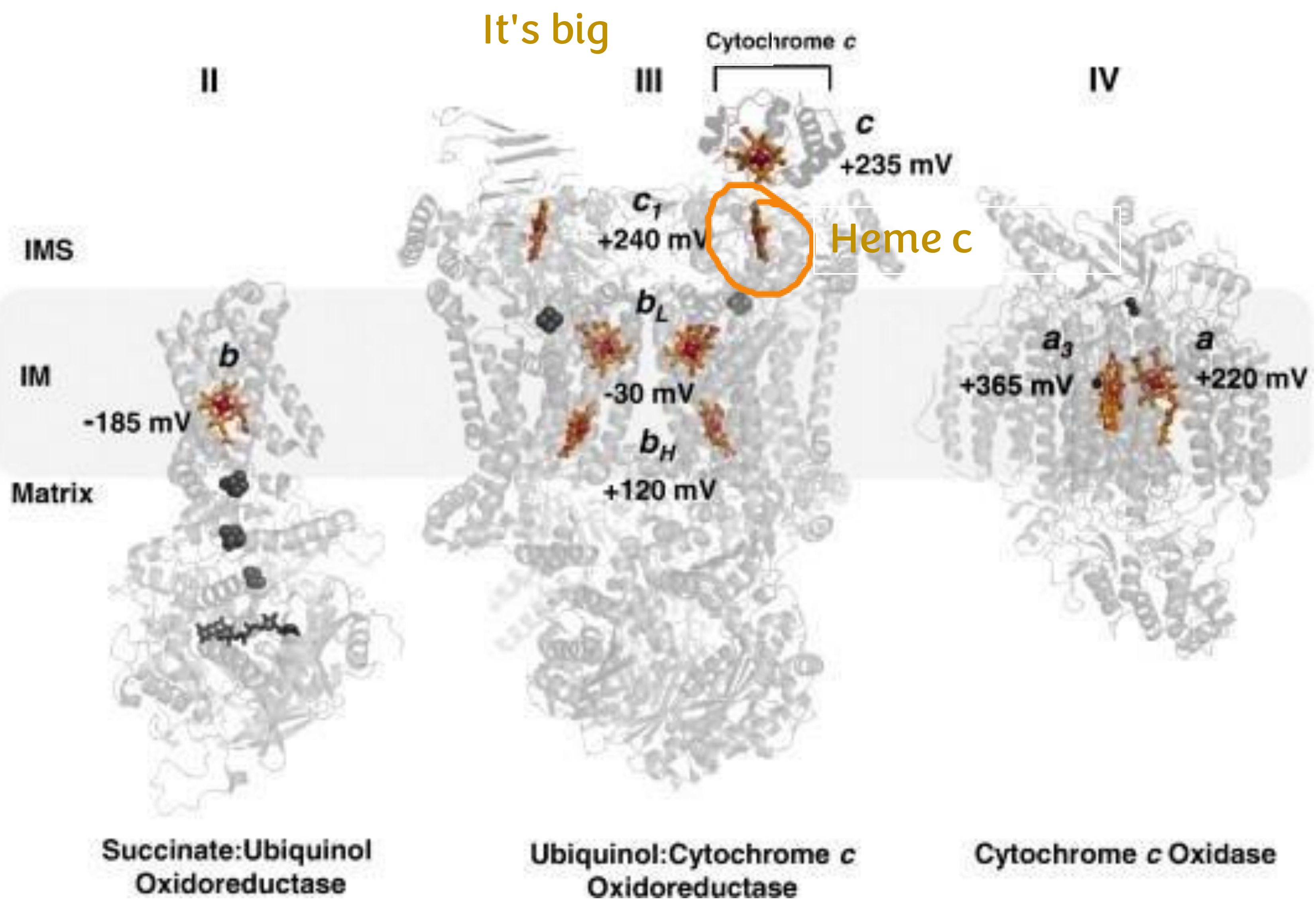
Two electrons are regenerated.

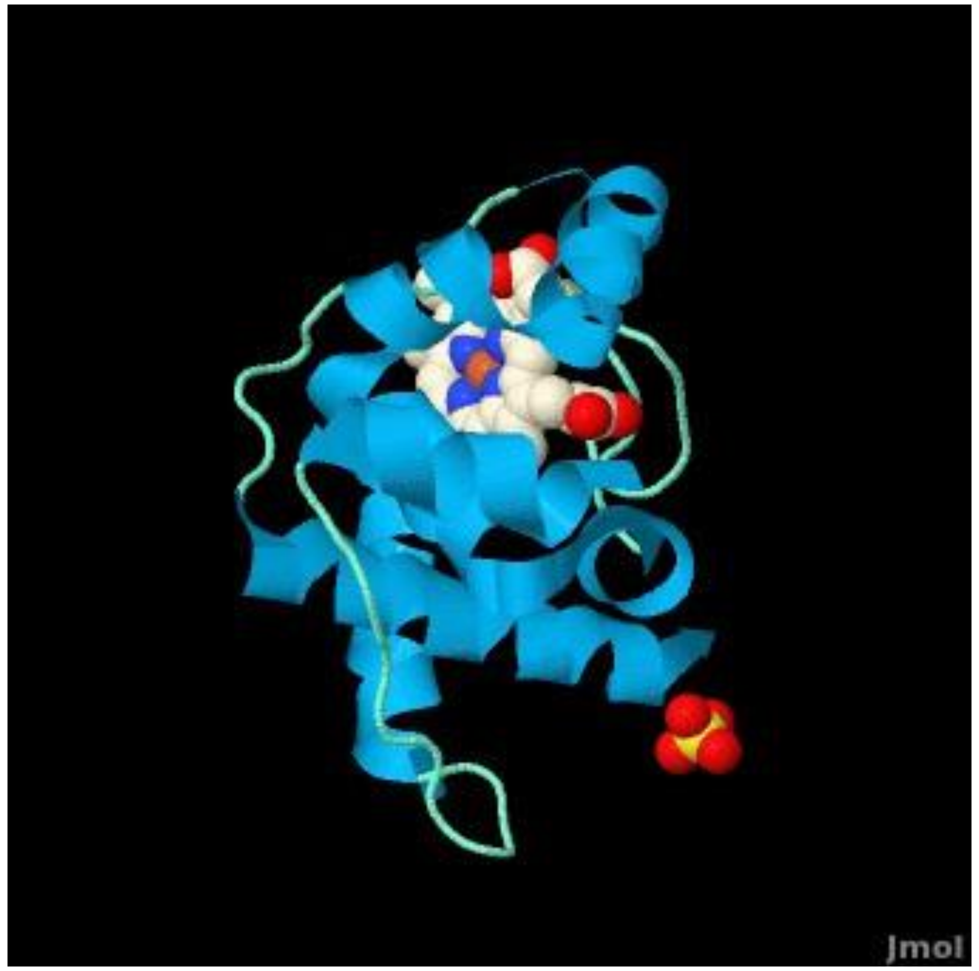
What is the net use of electrons?

Two electrons are the net consumption.

How many cytochrome c molecules receive electrons?

Two cytochrome c molecules.



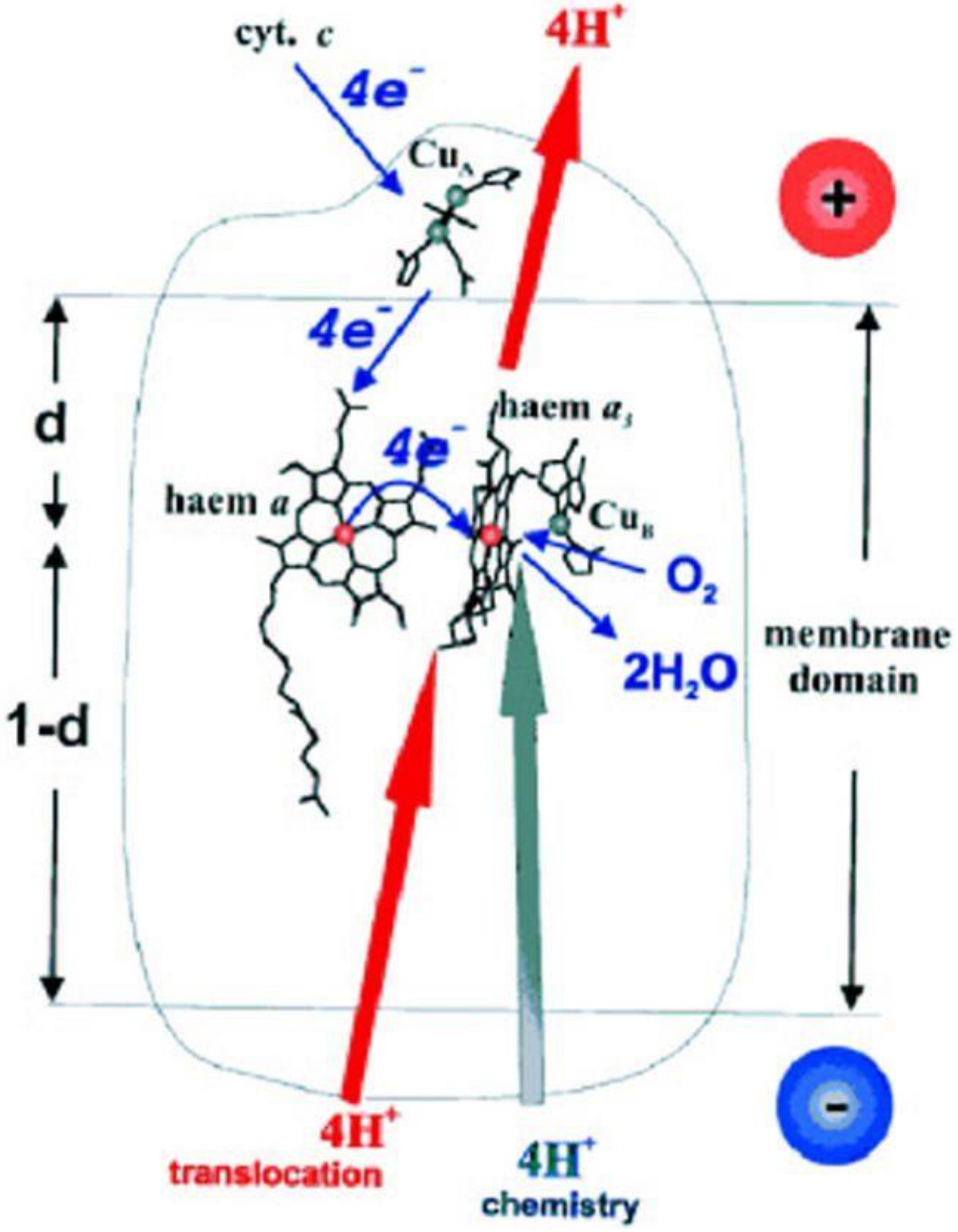


It's all about how close these structures are to each other to facilitate the electron transfer. They need to be near one another so that the transfer can happen efficiently.

CYTOCHROME C: THE SURFACE MESSENGER

OXI–RED COMPONENTS OF THE ETC:“CYTOCHROME C OXIDASE” – COMPLEX IV

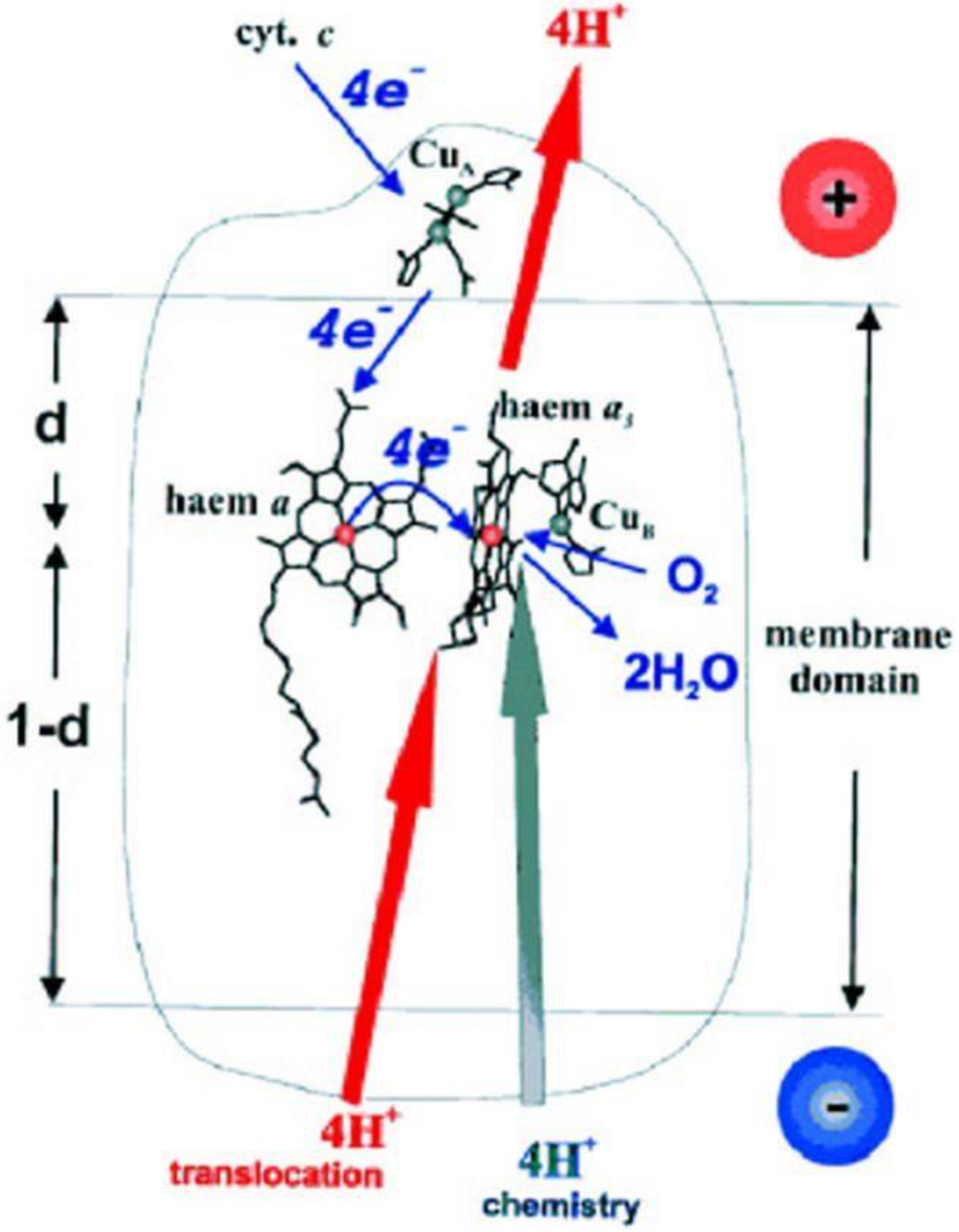
- Contains cytochrome a and a_3
- Contains two copper sites
- Contains oxygen binding sites
- O_2 must accept 4 electrons to be reduced to 2 H_2O ($2H^+/2e-$)
- Cytochrome c is one electron carrier
    - Cyt cred + $4H^+$ + O_2 → Cyt cox + $2H_2O$
- Cytochrome oxidase has a much lower Km for O_2 than myoglobin (hemoglobin, myoglobin, complex IV)
- Partial reduction of O_2 is hazardous



Complex IV (Cytochrome c oxidase):

It functions as an electron transfer complex because it contains heme and copper atoms.
There are two copper sites: CuA and CuB.
The term site does not mean a single atom – for example, CuA site contains more than one copper atom.

CuA is located close to heme a, and CuB is located close to heme a3.
Because copper atoms are near the heme groups, they can share electrons with them.
CuA can share electrons with heme a, and CuB can share electrons with heme a3.



How many redox components are there?
→ 4 components: two heme groups (a and a3) and two copper sites (CuA and CuB).

How many electrons?
→ 4 electrons.

Complex IV oxidizes cytochrome c by using molecular oxygen (O_2) as the final electron acceptor.

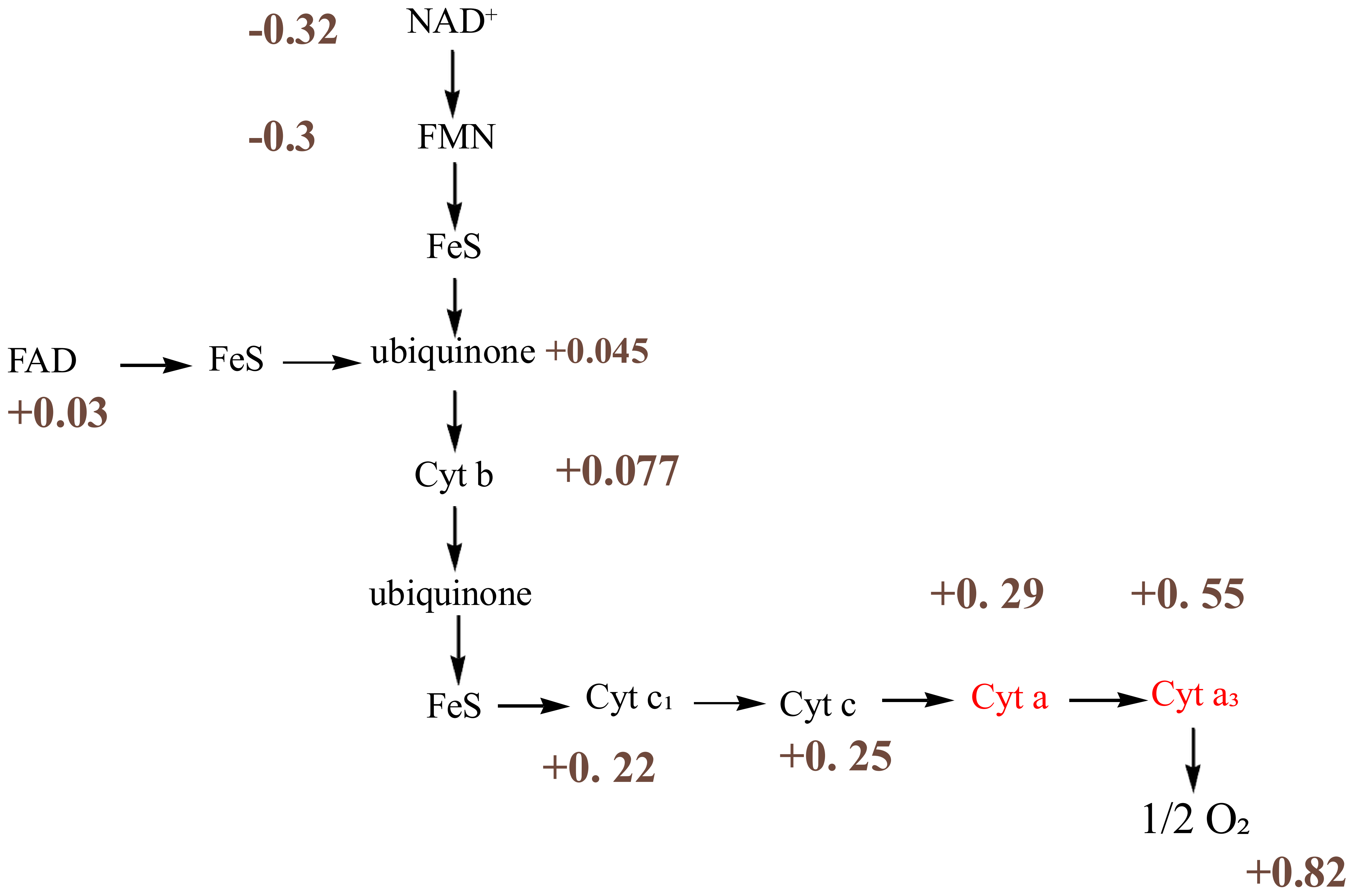
It converts oxygen into water.

Oxygen requires four electrons ($4e^-$) to be completely reduced into two water molecules ($2H_2O$).

Oxygen binds to the iron (Fe) atom in heme a3, and we need the heme a3 to be fully reduced before it can bind oxygen.

THE RIGHT ARRANGEMENT: HOW CAN WE PROVE IT?

1. MEASURING THE STANDARD REDUCTION POTENTIALS (REAL?)



-0.32 NAD^+

-0.3 FMN

FeS

FAD +0.03 → FeS → ubiquinone +0.045

Cyt b +0.077

ubiquinone

FeS → Cyt c_1 +0. 22 → Cyt c +0. 25 → Cyt a +0. 29 → Cyt a_3 +0. 55

$1/2\ O_2$ +0.82

How do we know the specific order of the components in the Electron Transport Chain (ETC)? In other words, what is the scientific evidence used to arrange the electron carriers (from NADH to Complex I, CoQ, Complex III, Cytochrome c, Complex IV, and finally O_2) in their precise sequence?

Answer

The correct sequence of the ETC components is determined by measuring their standard reduction potentials (E°).

Key Concepts:

Reduction Potential (E°): This value measures the tendency of a molecule to gain electrons (to be reduced).

Molecules with a more negative E° are better electron donors (e.g., NADH).

Molecules with a more positive E° are better electron acceptors (e.g., O_2).

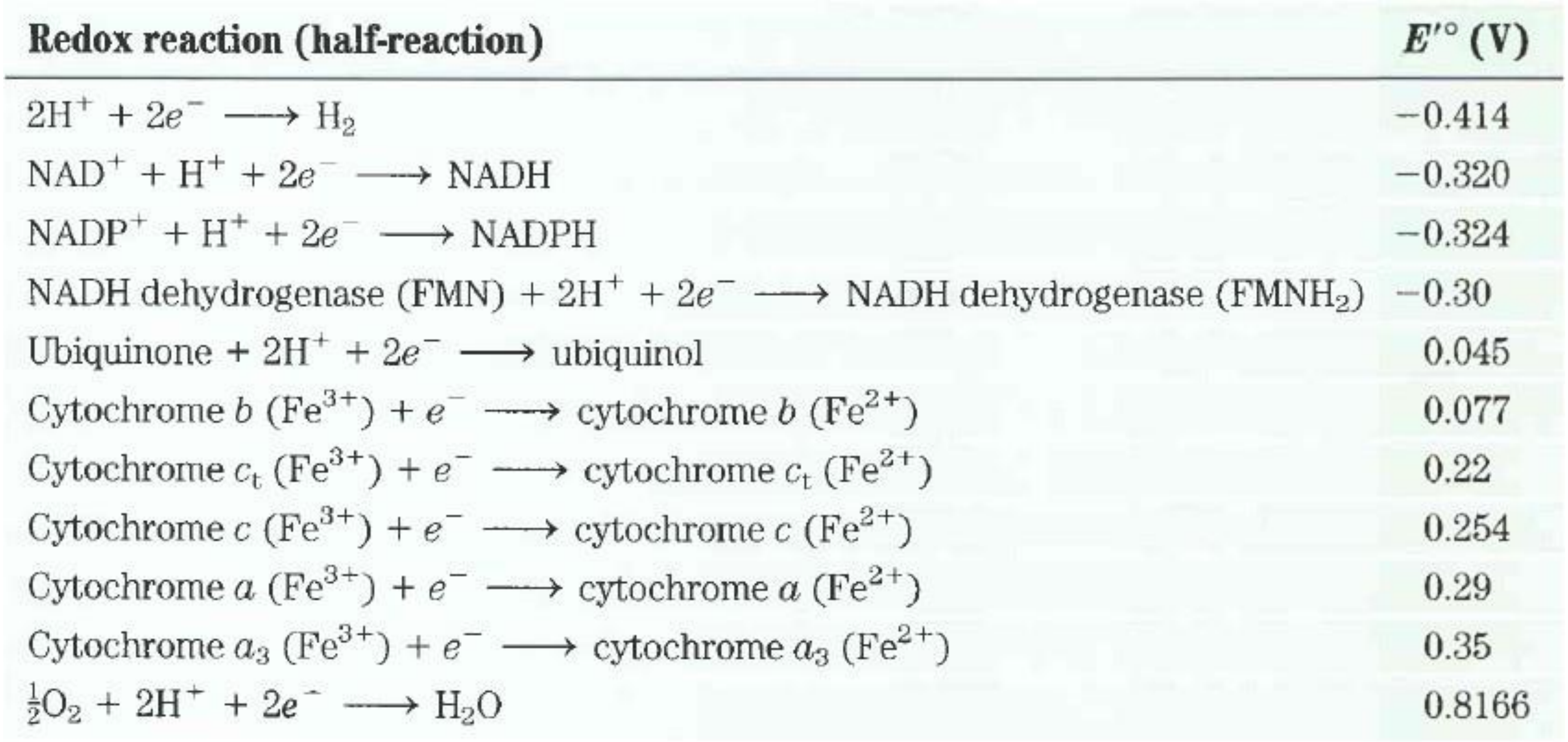
Principle of Electron Flow: Electrons are transferred spontaneously from a molecule with a lower (more negative) E° to a molecule with a higher (more positive) E°.

Arrangement:

The ETC is organized sequentially from the component with the most negative E° (NADH at approximately -0.32 V) to the component with the most positive E° (Oxygen at approximately +0.82 V). This arrangement ensures a downhill, spontaneous flow of electrons, which releases the energy necessary to pump protons and synthesize ATP.

THE RIGHT ARRANGEMENT: HOW CAN WE PROVE IT?

1. MEASURING THE STANDARD REDUCTION POTENTIALS (REAL?)



Redox reaction (half-reaction)	E'° (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

NADH → Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O2

THE RIGHT ARRANGEMENT: HOW CAN WE PROVE IT?

NADH → Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O2

Scientists have discovered this arrangement of molecules and complexes but **how to proof this arrangement ?**

Some of the students' suggestions :

1) **We take off every complex and measure its reduction potential and by this we can know their reduction potentials and the flow of electrons and the arrangement of these complexes,** but **we can't take off the complexes from the mitochondrial inner membrane,** and even if we could take off the complexes out, **we won't have the same environment as when they are embedded inside the inner mitochondrial membrane.**

2) **We can measure their potential while they are in the membrane** and this is **impossible** because, we should bring a very small electrode, also we should embed it inside the complex and we know that complexes are enzymes and enzymes are proteins and protein are **dynamic**, so it will not make sense because the complexes will be always moving which makes it impossible to have an electrode embedded inside rather than detecting it and placing the electrode inside it at the first place, **so it is impossible.**

THE RIGHT ARRANGEMENT: HOW CAN WE PROVE IT?

NADH → Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O2

How to prove the arrangement of complexes in the inner mitochondrial membrane?

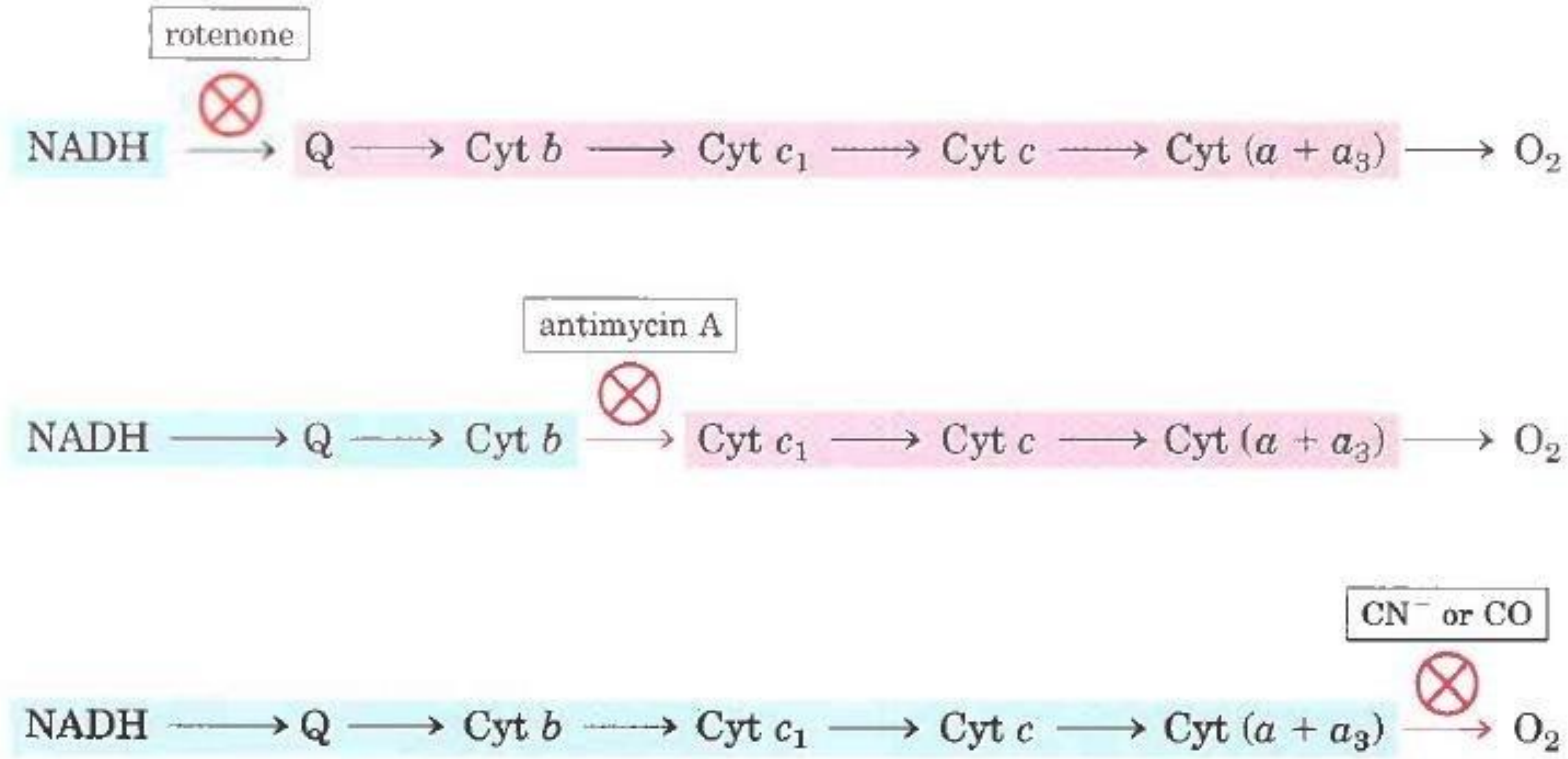
We can do that using **inhibitors**, by using an inhibitor **for one of the complexes** it will **not get reduced** and **non of the complexes that after it will be reduced (oxidized) since electrons are not reaching them,** and by knowing the complexes that are **reduced** and **oxidized** when using a specific complex inhibitor, we could know **the ones that are after (oxidized)** and **the ones that are before (reduced) the inhabited complexes**, **by doing this over and over with other complexes (inhabiting them),** we can arrange the complexes depending on **what is reduced** and **what is oxidized** when **inhabiting a specific complex.**

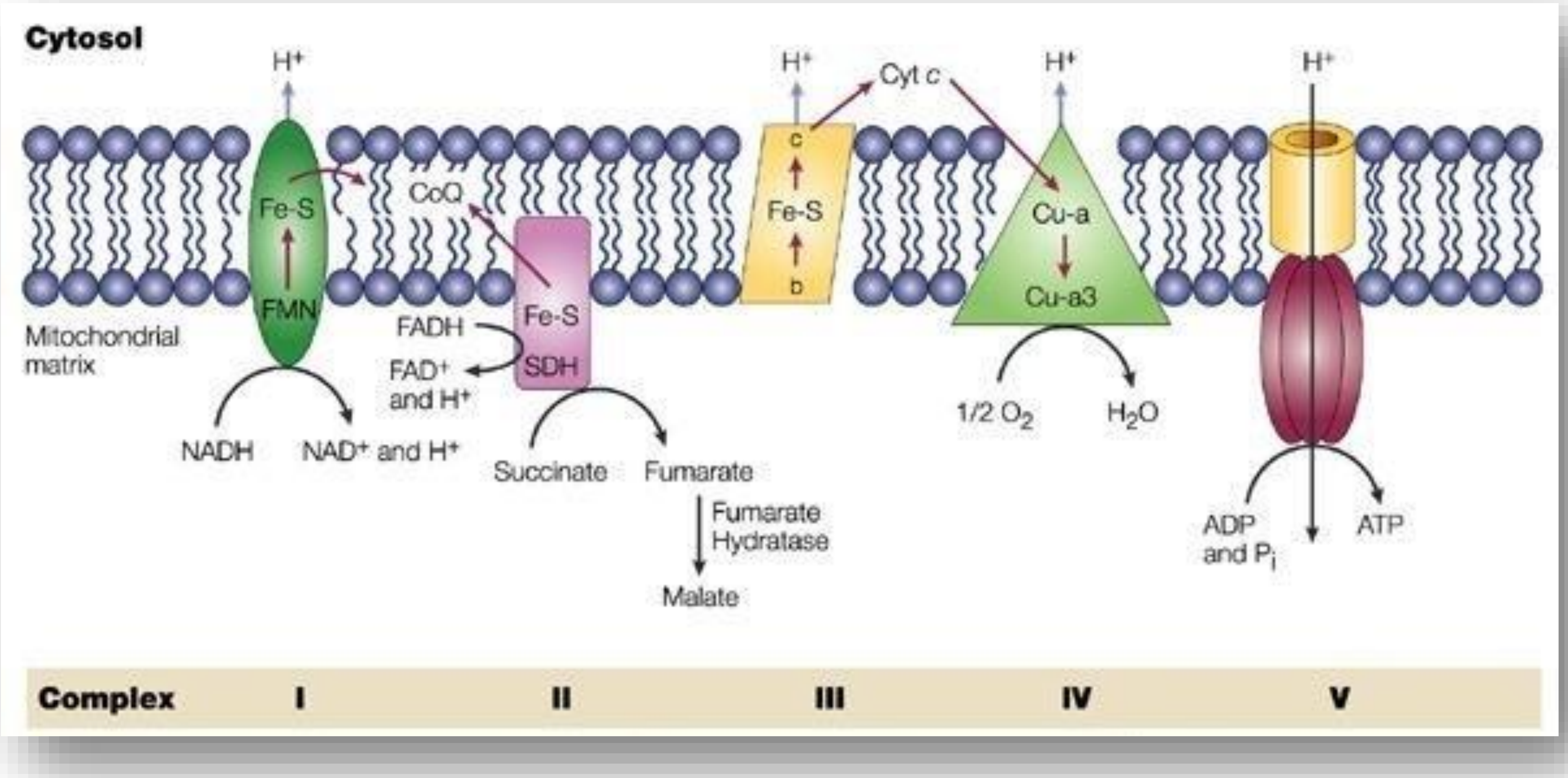
And that's how scientists discover the complexes arrangement.

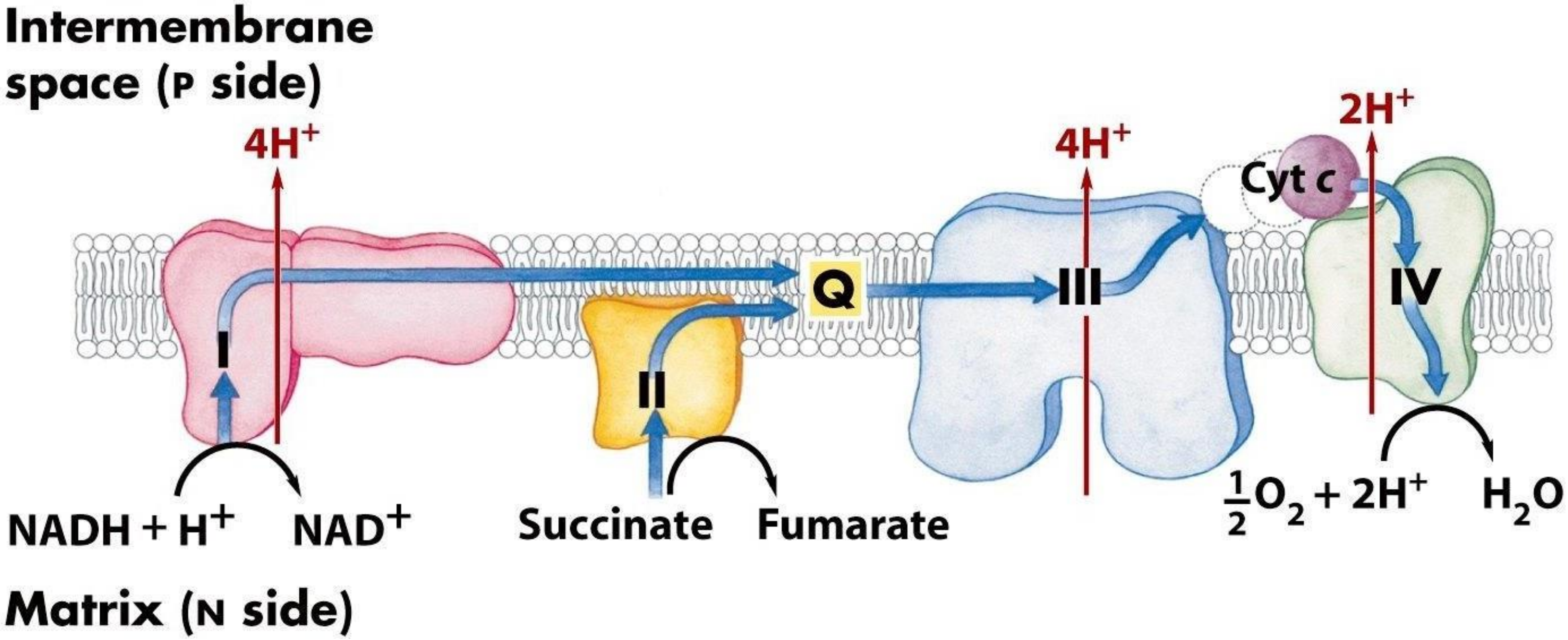
THE RIGHT ARRANGEMENT: HOW CAN WE PROVE IT?

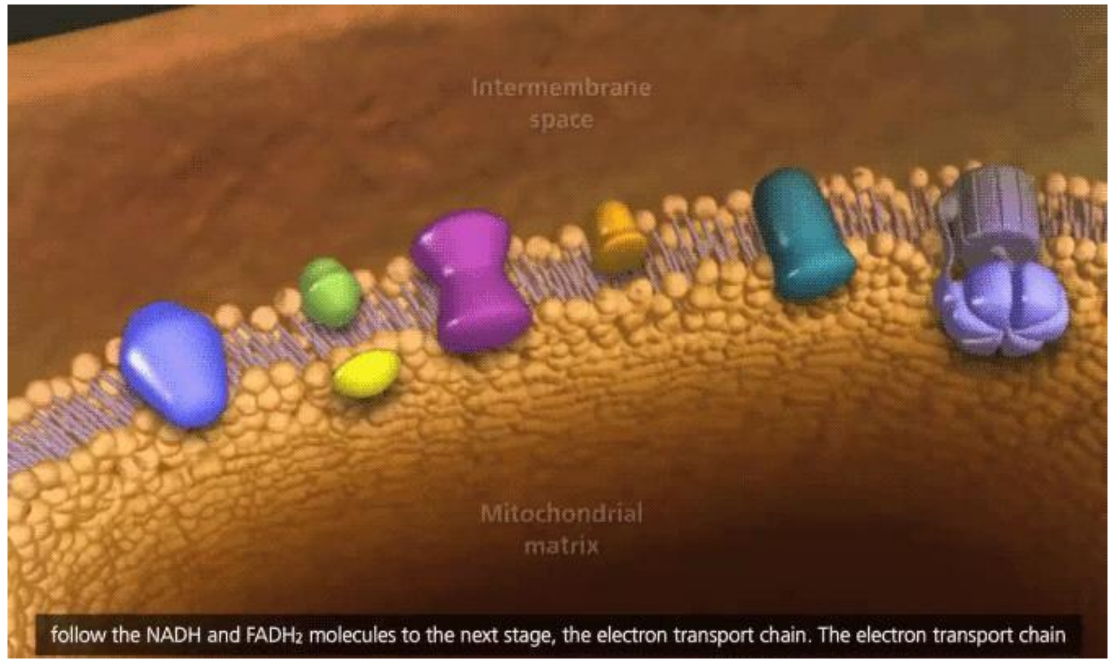
NADH → Q → cytochrome b → cytochrome c_1 → cytochrome c → cytochrome a → cytochrome a_3 → O_2

- 2. Reduction of the entire ETC with no O_2
- 3. Addition of inhibitors









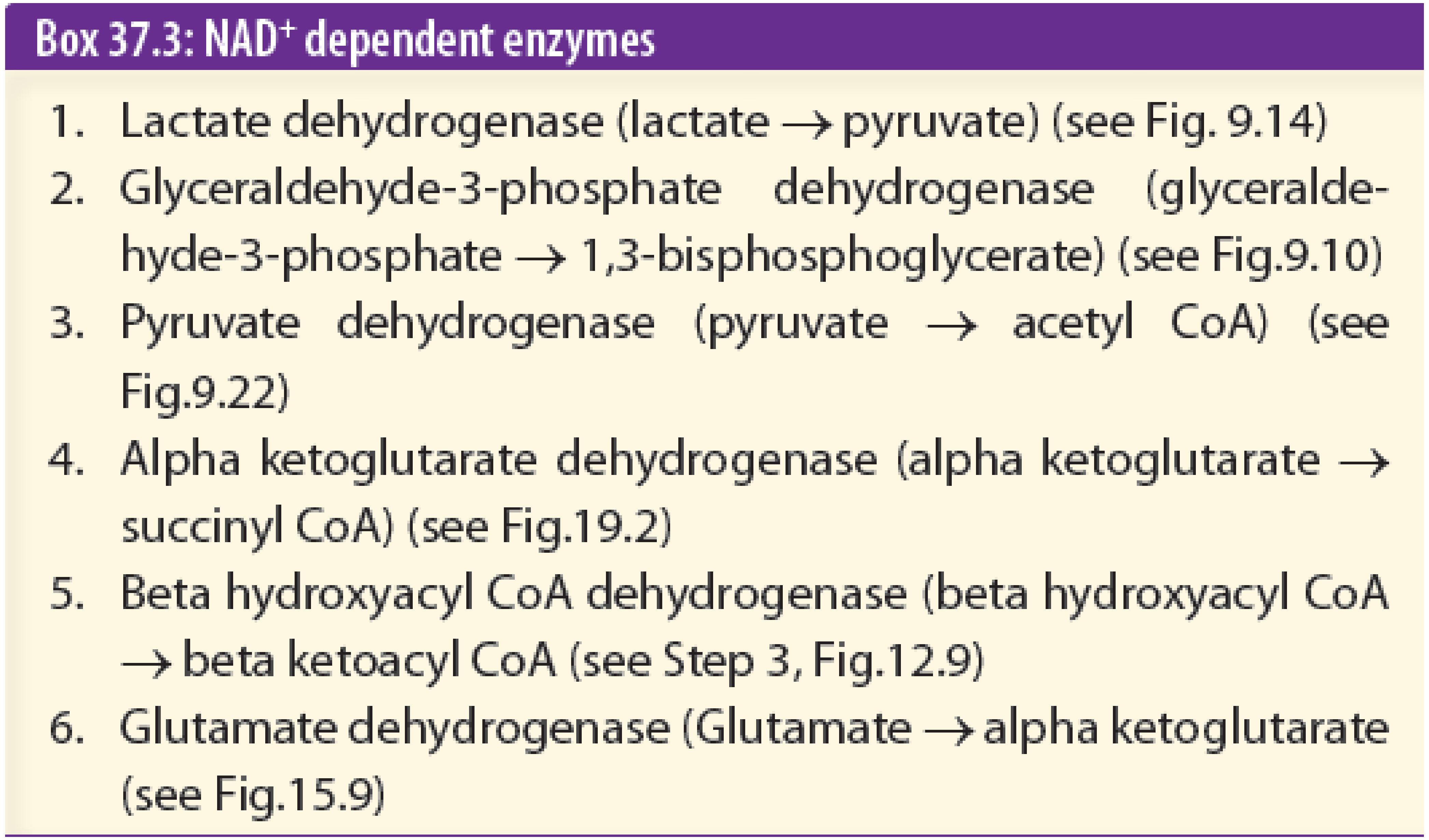
follow the NADH and FADH₂ molecules to the next stage, the electron transport chain. The electron transport chain

PUMPING OF PROTONS

- For every 2 electrons passing:
- $4H^+$ (complex I); $0H^+$ (complex II); $4H^+$ (complex III), $2H^+$ (complex IV)

If the 2 electrons starts their movement at complex 2 such as FADH2, the result is 6 protons pumped across the membrane.

SOME NADH PRODUCING ENZYMES



Box 37.3: NAD$^+$ dependent enzymes

1. Lactate dehydrogenase (lactate → pyruvate) (see Fig. 9.14)
2. Glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate → 1,3-bisphosphoglycerate) (see Fig.9.10)
3. Pyruvate dehydrogenase (pyruvate → acetyl CoA) (see Fig.9.22)
4. Alpha ketoglutarate dehydrogenase (alpha ketoglutarate → succinyl CoA) (see Fig.19.2)
5. Beta hydroxyacyl CoA dehydrogenase (beta hydroxyacyl CoA → beta ketoacyl CoA (see Step 3, Fig.12.9)
6. Glutamate dehydrogenase (Glutamate → alpha ketoglutarate (see Fig.15.9)

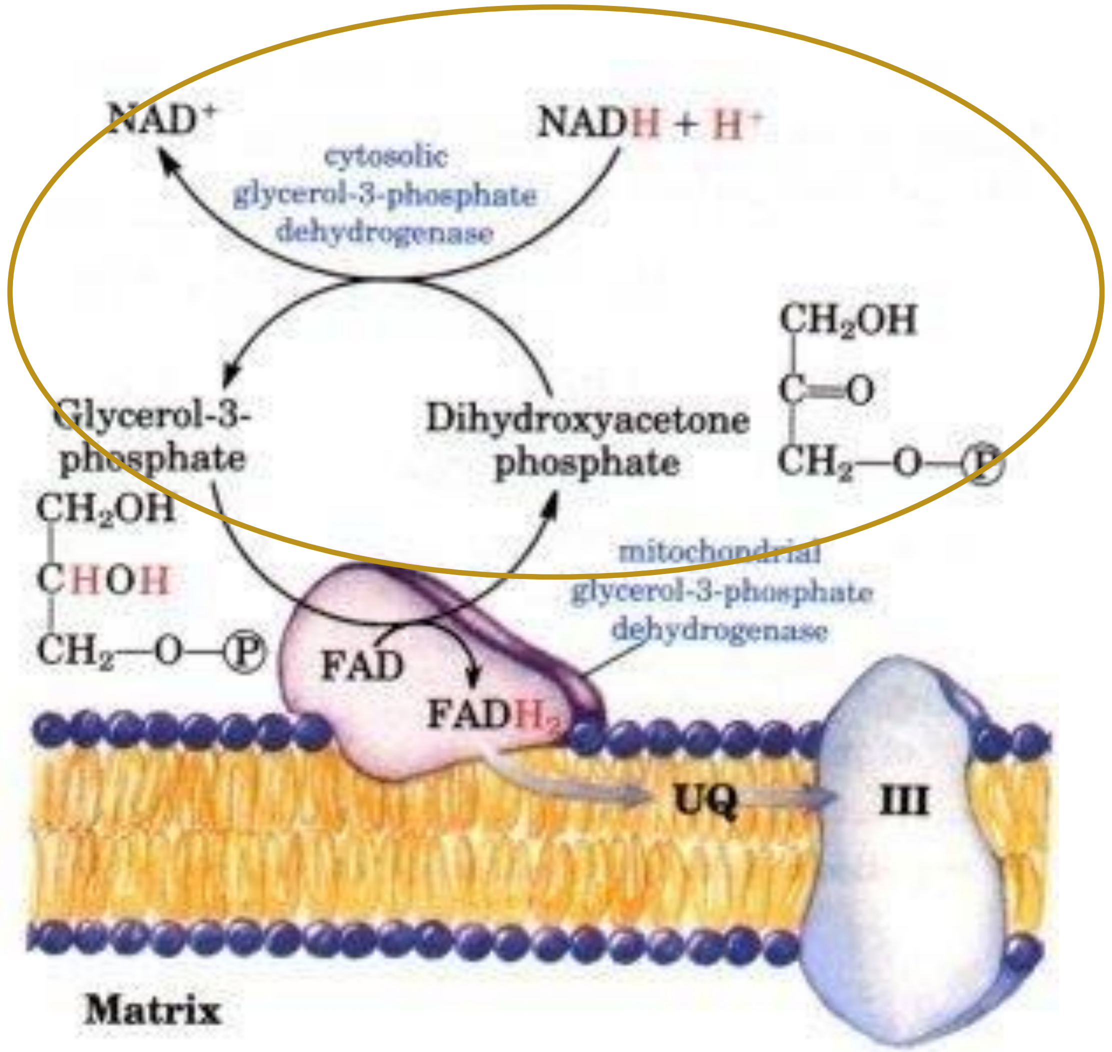
How to benefit from NADH produced outside the mitochondria (glycolysis)?

There are 2 molecules of NADH that are produced in the process of glycolysis, they should be transported to the inner mitochondrial membrane, but they **can't** be transported through the membrane since they are **large charged particles.**

There will be the formation of a **mitochondrial shuttling system.**

There is an enzyme called **glycerol-3-phosphate dehydrogenase,** this enzyme has **2 copies : cytosolic** copy & **mitochondrial** copy (has coenzyme **FAD** inside it)

The electrons along with the hydrogen ions (hydrogen atoms) will be transferred to the **Dihydroxyacetone phosphate** molecule converting it to **glycerol-3-phosphate** a reaction that is catalysed by **glycerol-3-phosphate dehydrogenase.**

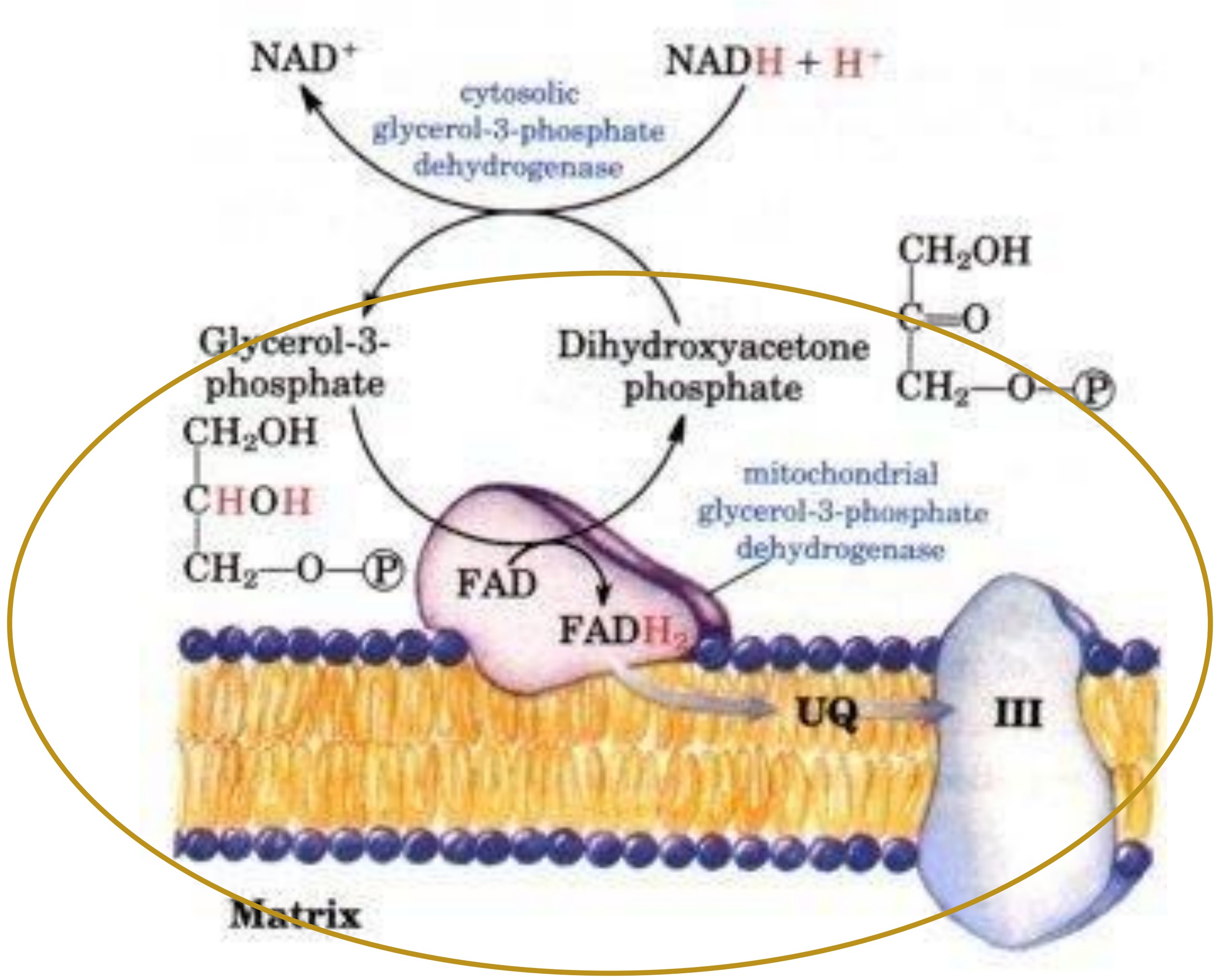


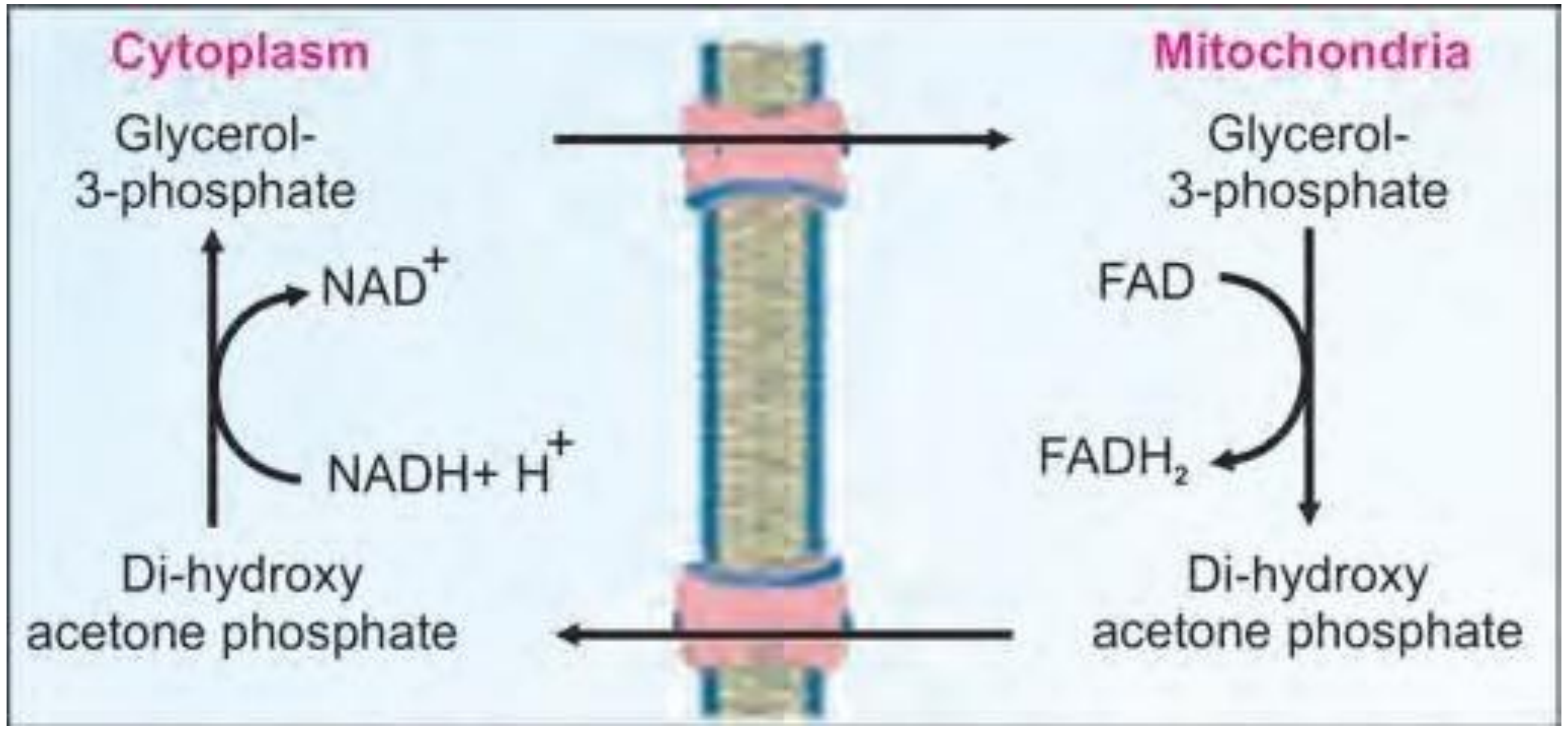
How to benefit from NADH produced outside the mitochondria (glycolysis)?

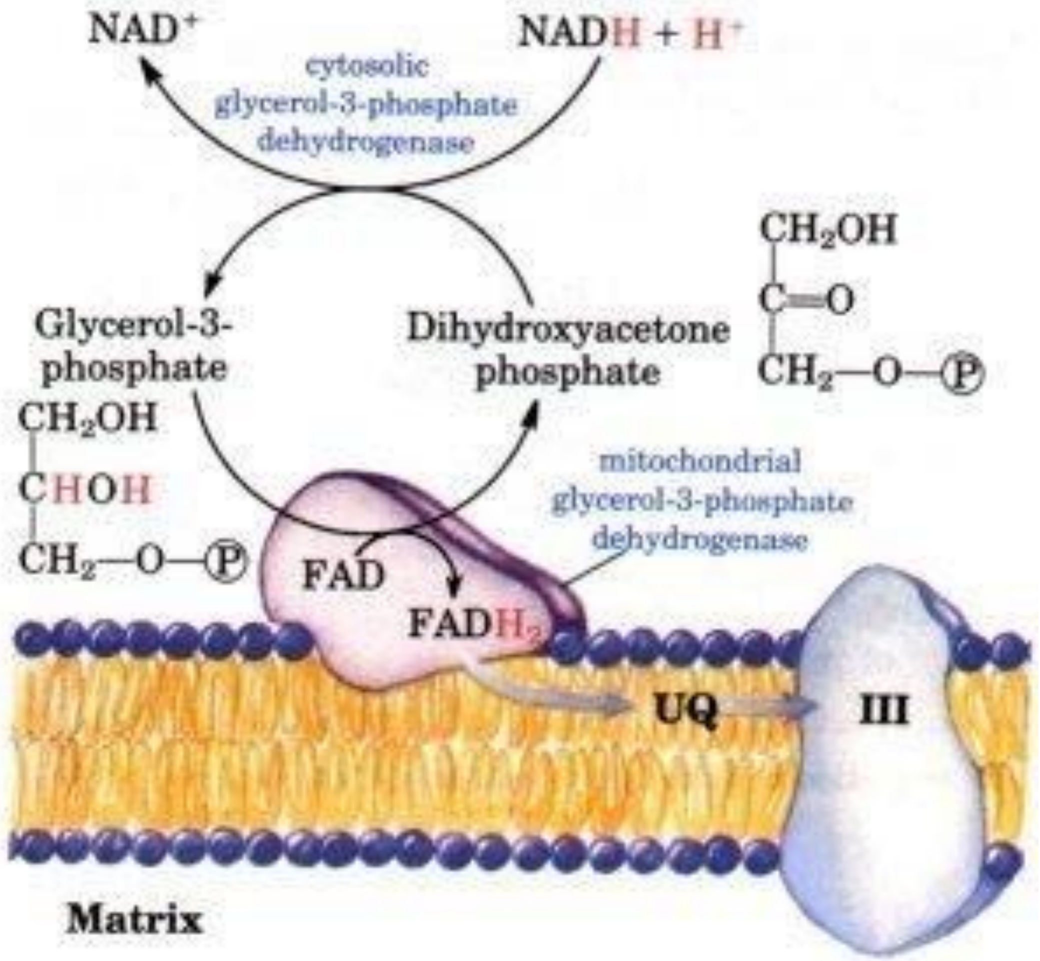
Glycerol-3-phosphate is then converted back to **dihydroxyacetone, a reaction** that is catalyzed by glycerol-3-phosphate dehydrogenase (mitochondrial or which has the Coenzyme **FAD**), FAD will accept the 2 hydrogen atoms from the **glycerol-3-phosphate** and reduced to **FADH2.**

FADH2 will give its electrons to **CoQ (ubiquinone)** and it will get reduced to **CoQH2 (Upiquinol).**

CoQ distinction is **complex III,** and then the electrons will be transferred to it and then from **complex III** to **complex IV** via **Cytochrome C**, and then to its final acceptor which is **Oxygen (O2)**.







MITOCHONDRIAL SHUTTLING SYSTEMS "CYTOSOLIC NADH"

- Glycerol 3-phosphate shuttle
- In skeletal muscle and brain
- Glycolytic pathway as an example
- How NADH passes?
- ATP yield?

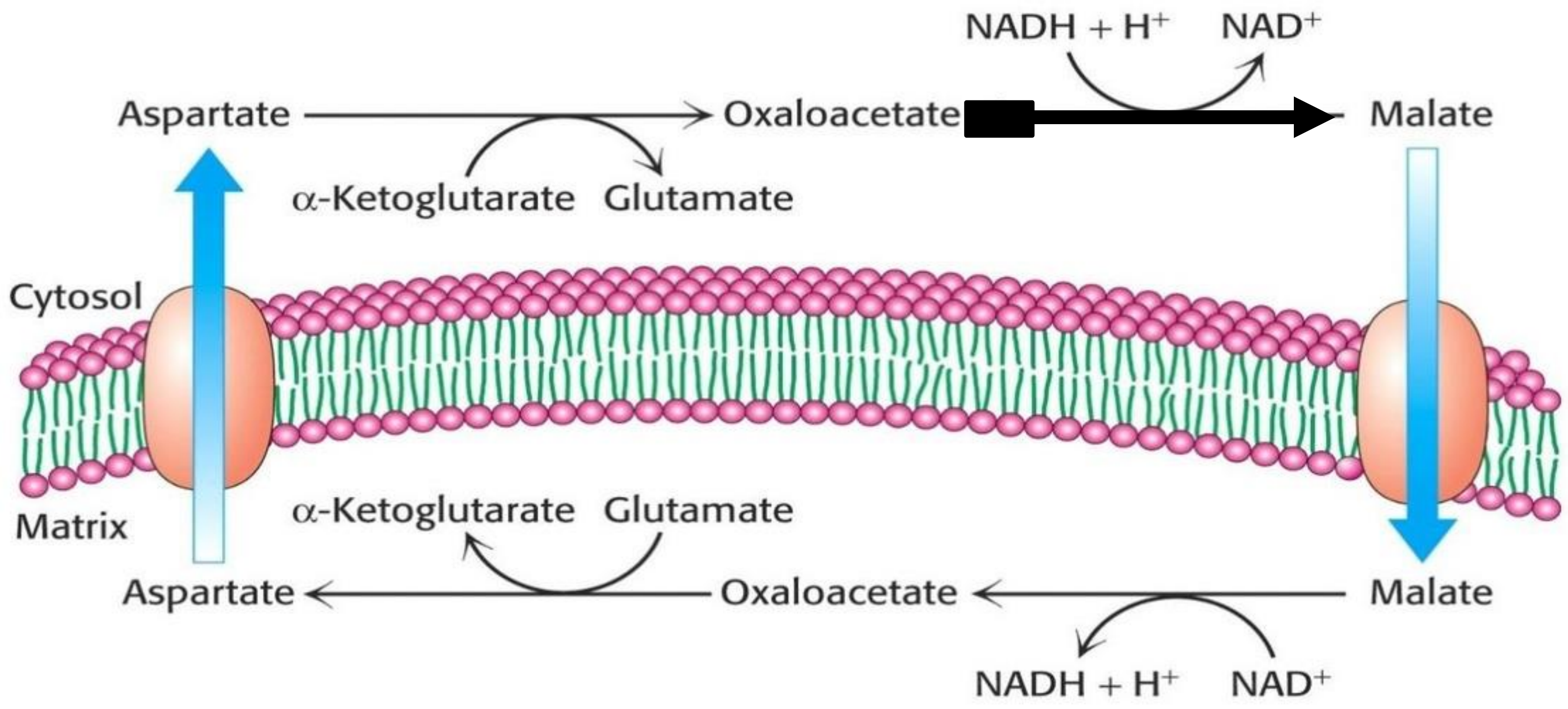
MITOCHONDRIAL SHUTTLING SYSTEMS - "CYTOSOLIC NADH"

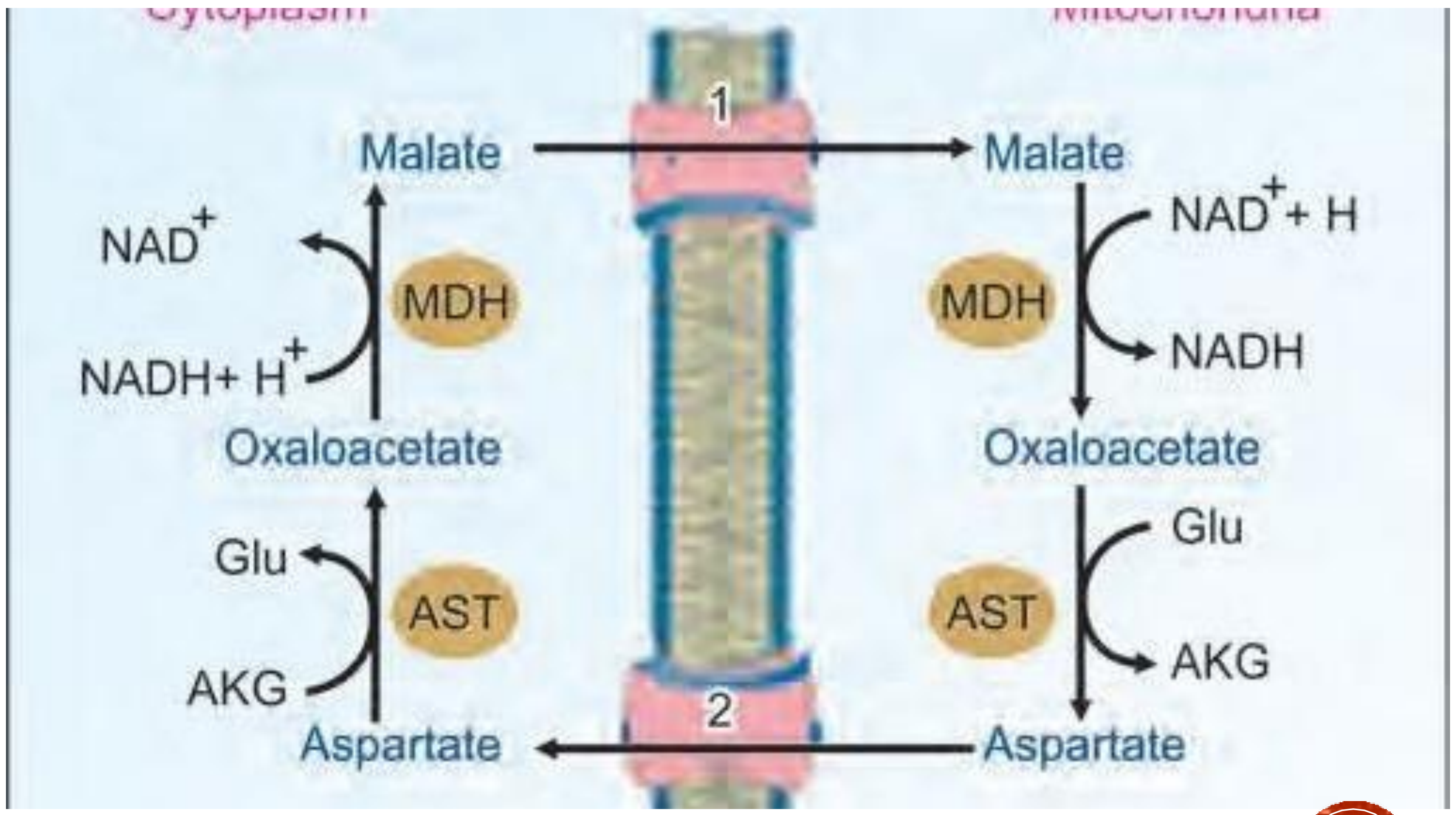
Malate is oxidized during TCA Cycle into Oxaloacetate, a reaction that is catalyzed by malate dehydrogenase

Oxaloacetate can be converted into aspartate.

Aspartate can pass through the mitochondrial inner membrane by a channel.

Aspartate formation reaction from Oxaloacetate is reversible reaction, Aspartate reform Oxaloacetate at the cytosol.

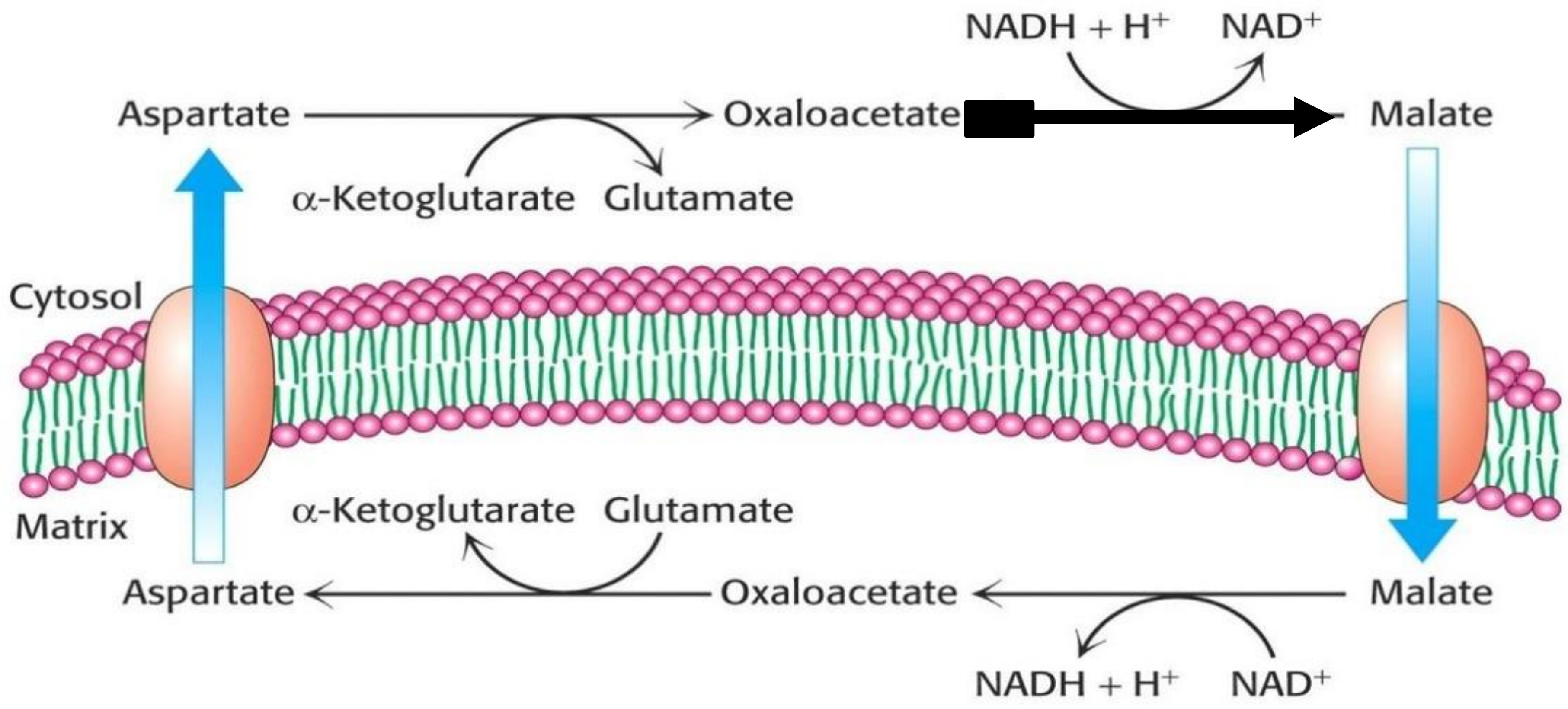


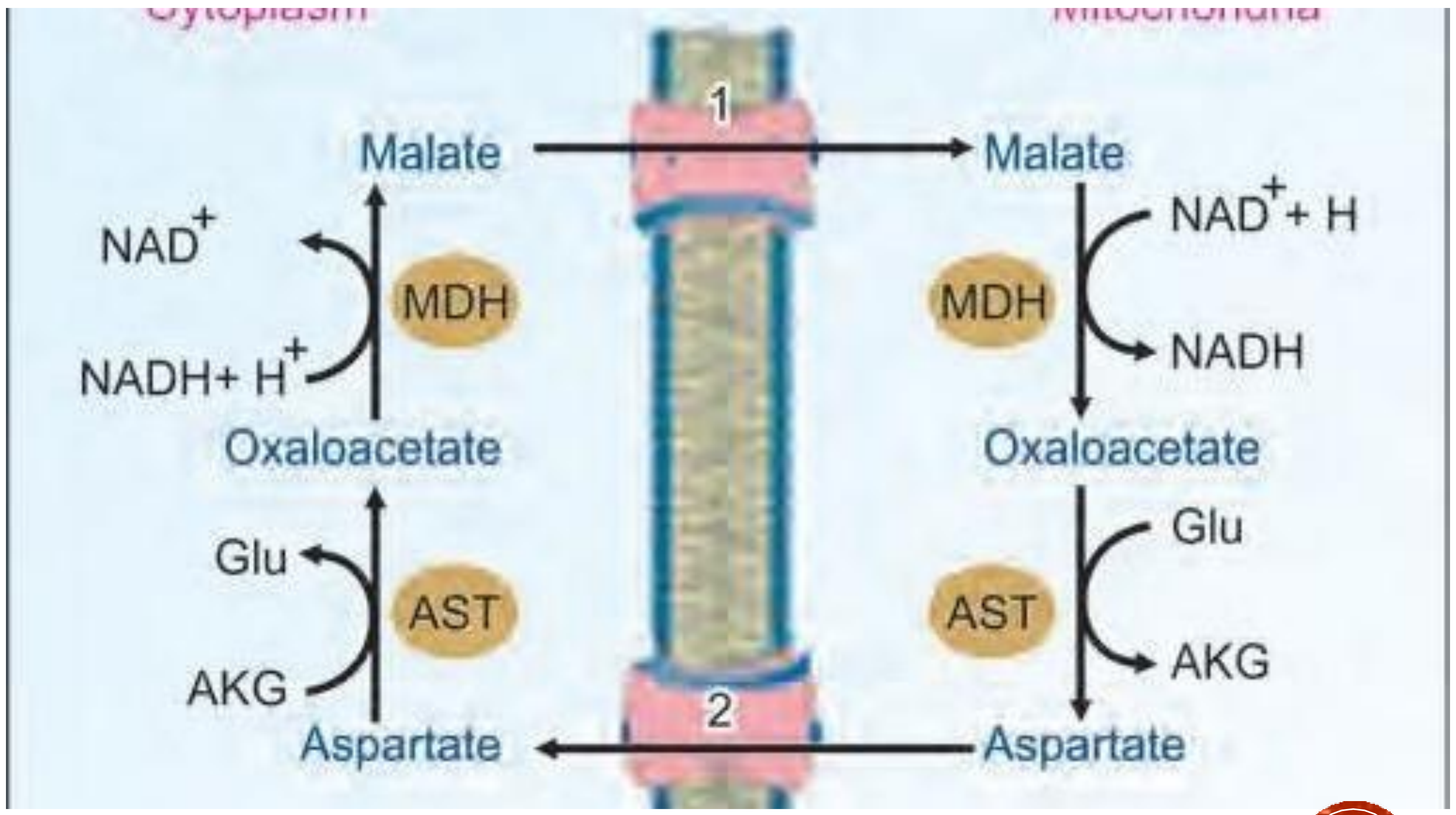


MITOCHONDRIAL SHUTTLING SYSTEMS - "CYTOSOLIC NADH"

The conversion of **malate** to **Oxaloacetate** is a **reversible reaction** reaction, so **Oxaloacetate** is converted into malate, by taking the **2 electrons from NADH (produced by glycolysis)** producing malate which can **pass** through **the mitochondrial inner** membrane and enter the matrix.

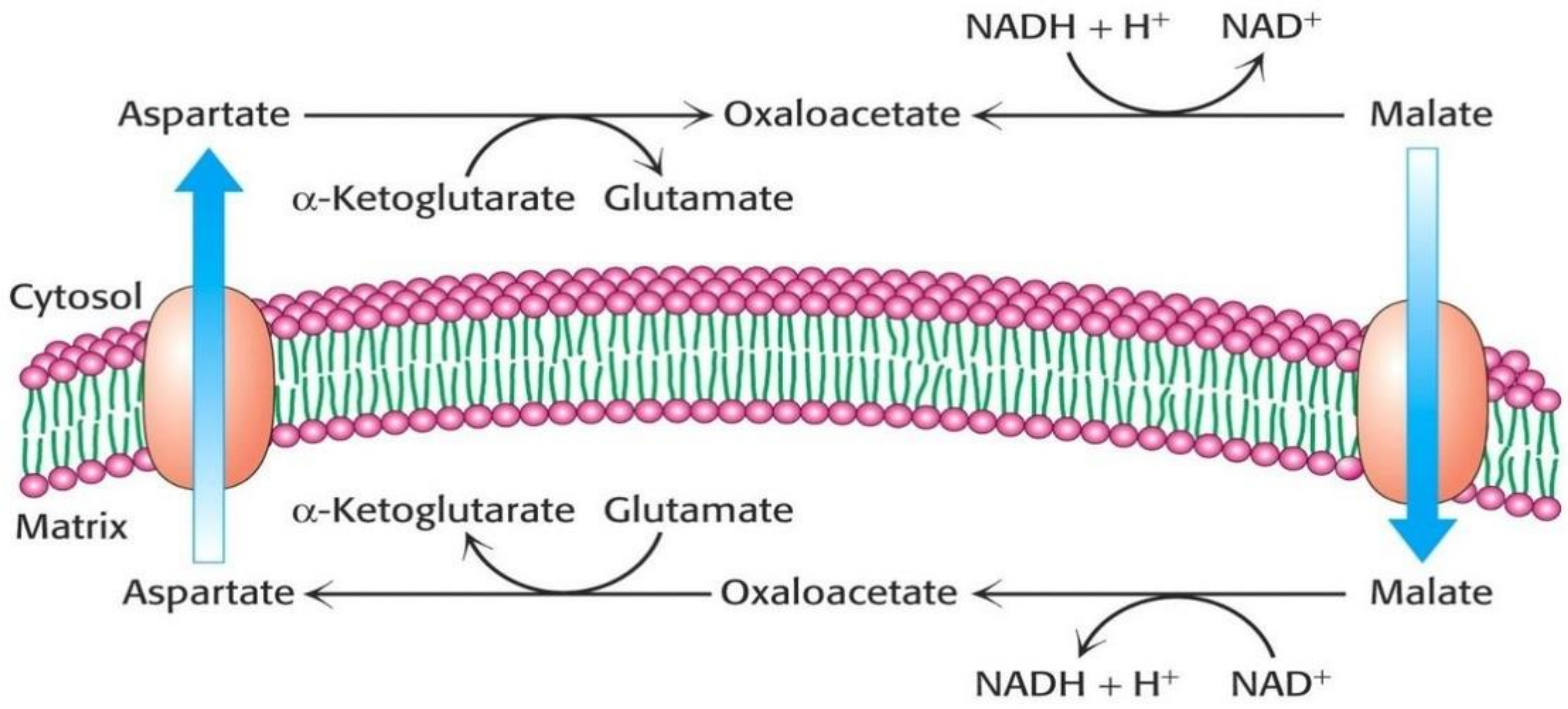
Once malate is at the mitochondrial matrix it **reforms oxaloacetate** reducing **NAD+ to NADH** which is used in the **electron transport chain.**

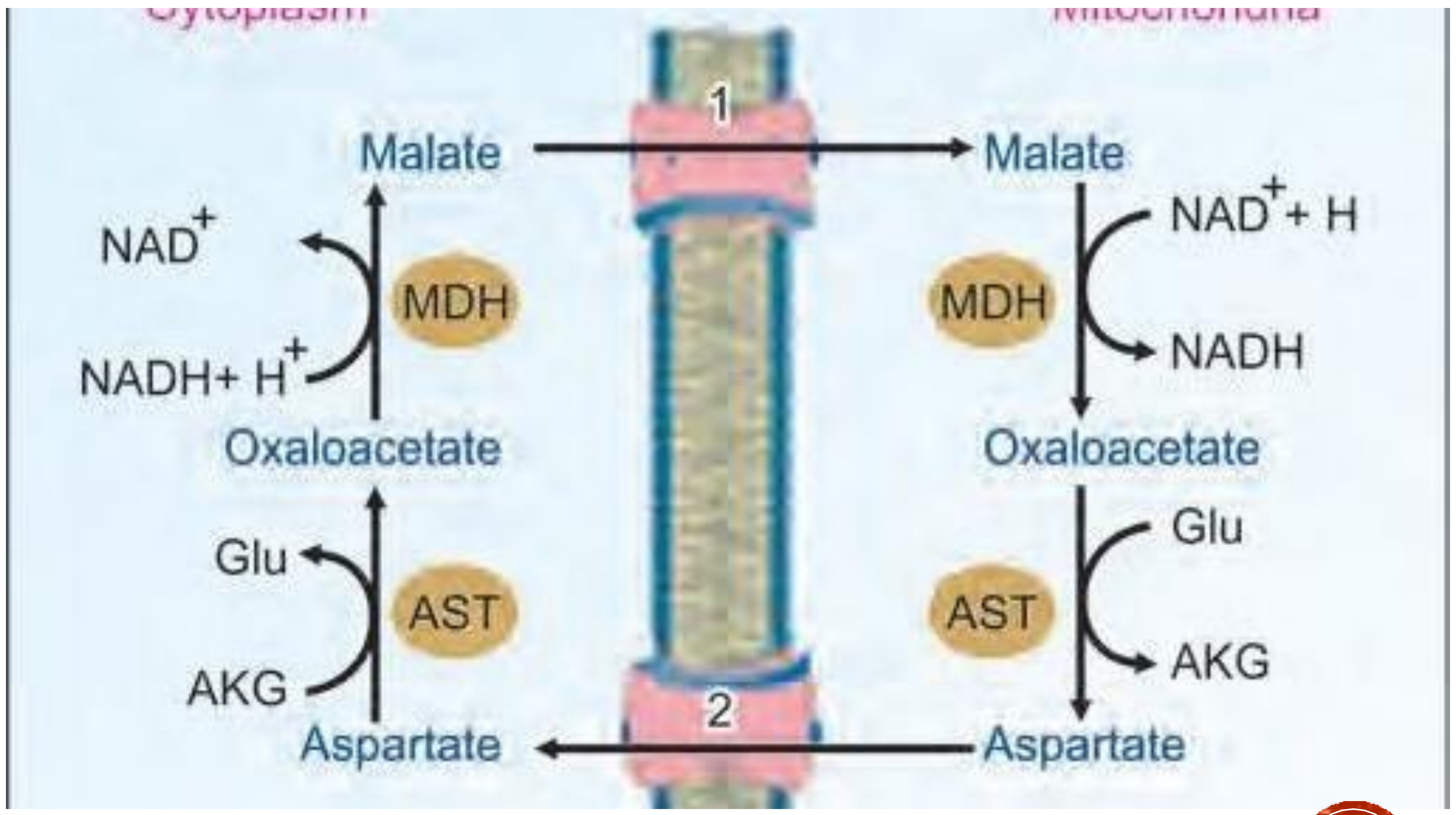




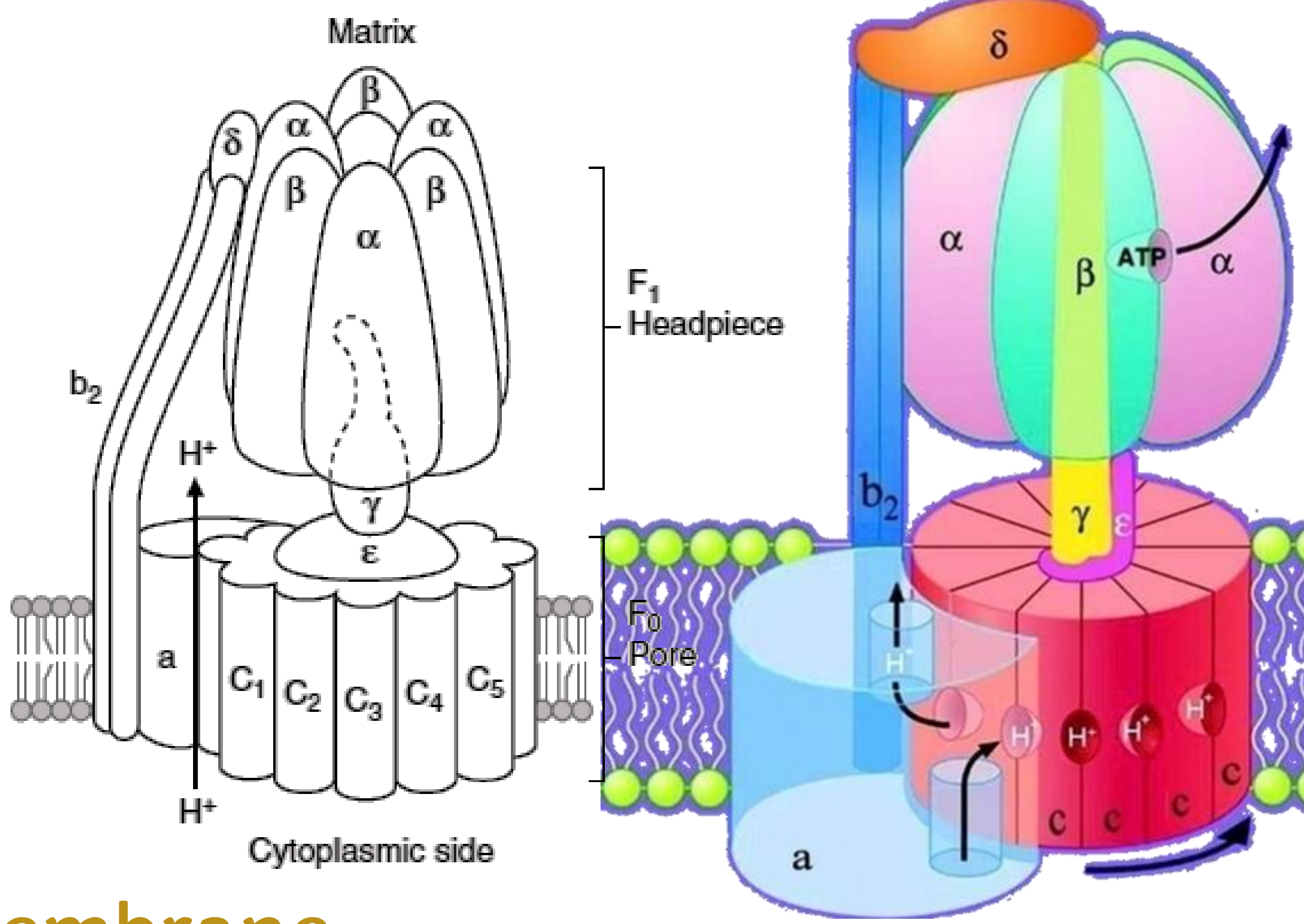
MITOCHONDRIAL SHUTTLING SYSTEMS - “CYTOSOLIC NADH”

- Malate-Aspartate shuttle
- operates mainly in liver, kidney
- 2 membrane carriers & 4 enzymes
- Readily reversible (vs. Glycerol 3- phosphate shuttle)
- NADH can be transferred only if the NADH/NAD+ ratio is higher in the cytosol than in the mitochondrial matrix
- Exchange of key intermediates between mitochondria & cytosol





ATP SYNTHASE



- **ATP Synthase** is also called **Complex V.**

- Looks like a turbine.

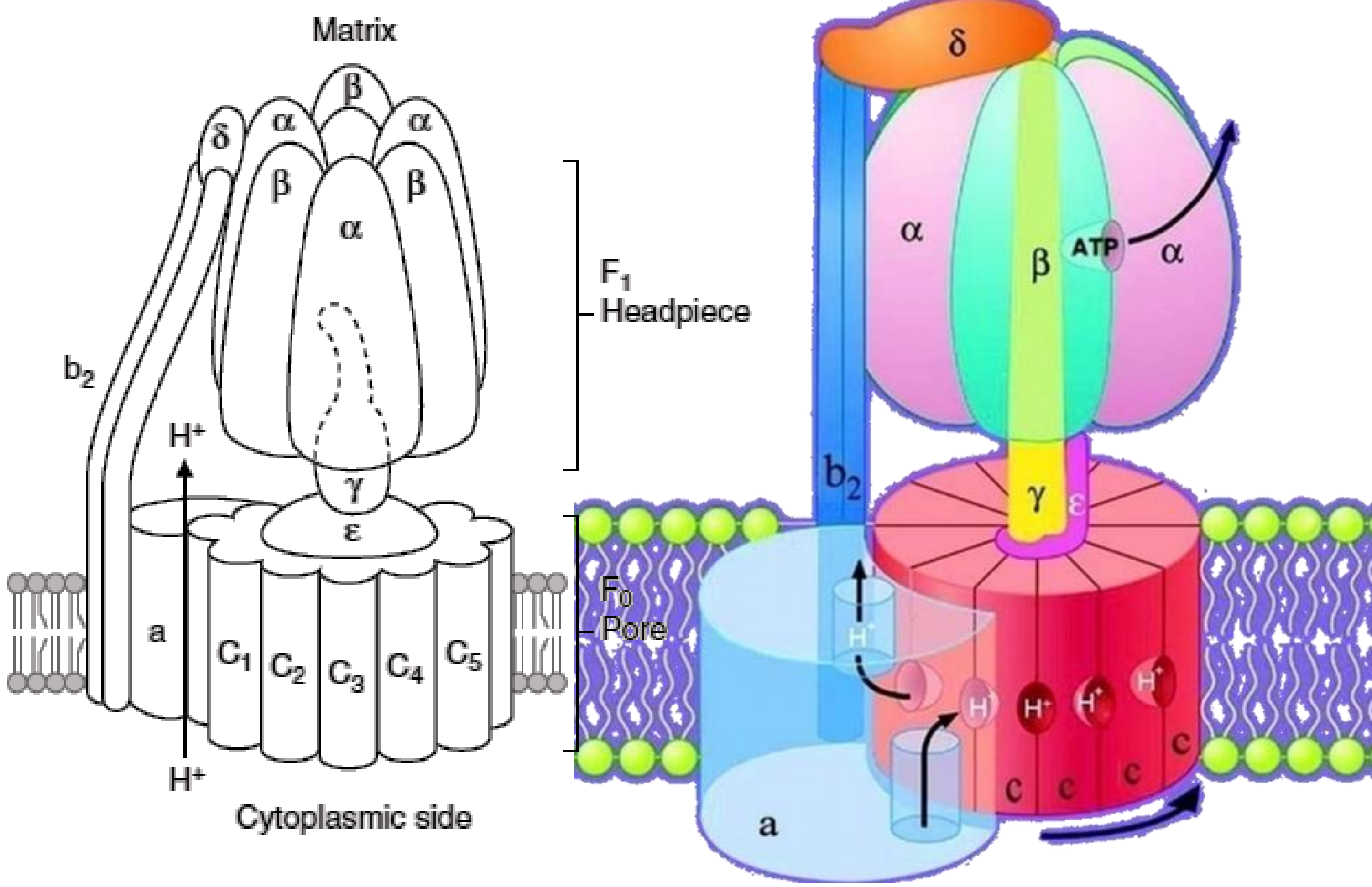
- Composed of **2** fragments :

- The O Fragment (FO), which is embedded inside the membrane.
  - Composed of 2 Subunits :
  - ❑ C subunits (cylinder shape).
    - Looks like a cylinder.
    - Composed of 12 C subunits, (differs among specifies but we consider them 12).
    - Linked with the A Subunit which is also embedded inside the inner mitochondrial membrane.

  - ❑ A Subunit (C shaped) \(Proton entry & exit way)
    - Protons enter and exit through this subunit.

- The I Fragment (FO), which is projected towards the matrix.

ATP SYNTHASE



The process of proton passing across the membrane by ATP Synthase

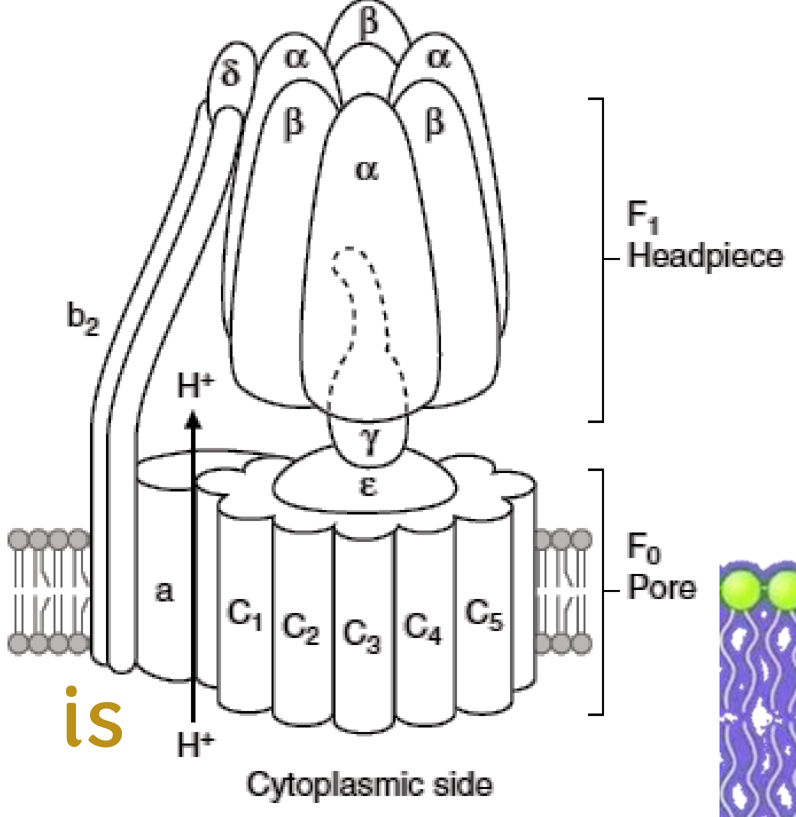
1) **Protons** enter to the **A subunit** through holes present at it

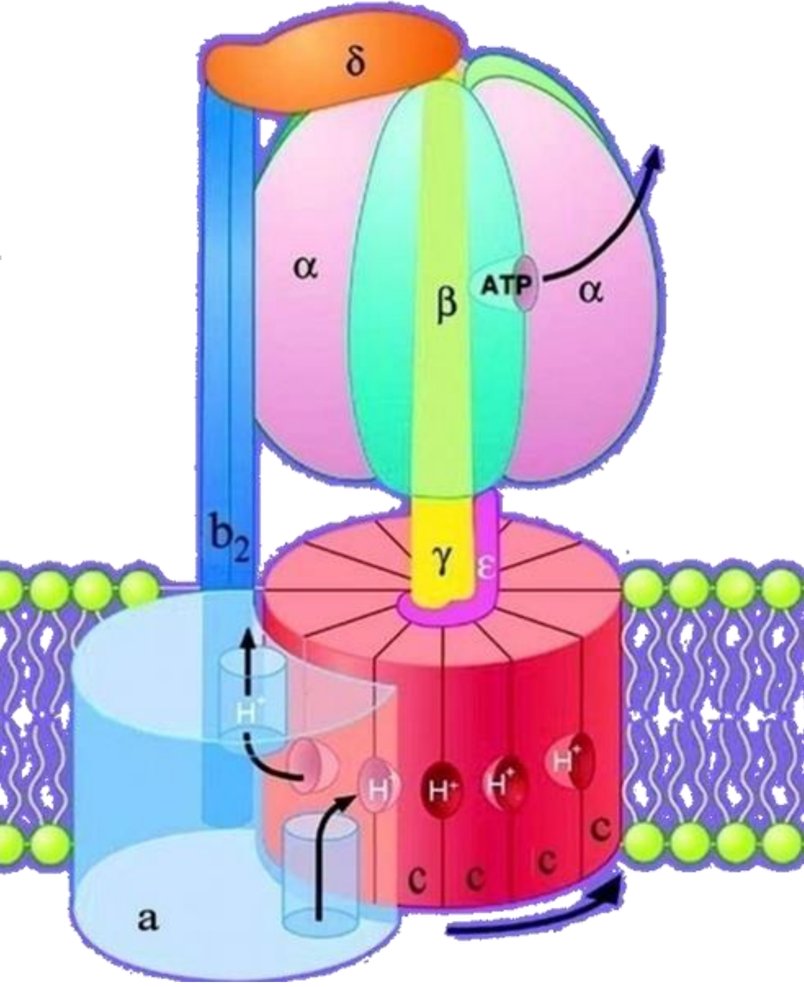
2) Then it faces the C subunit which contains the amino acid Glutamate (Negatively Charged, stabilized in its place inside the C subunit by ionic interactions)

3) Once it the hydrogen is bound to the glutamate, glutamate is neutral and ionic interactions are disrupted; this will cause a conformational change which will cause another C unit to face the opening of A subunit, where another proton will come to bind to the C subunit's Glutamate.

ATP SYNTHASE

The process of proton passing across the membrane by ATP Synthase





The ability of amino acid to be bound to H+, is determined by its PKa and the PH of the solution.

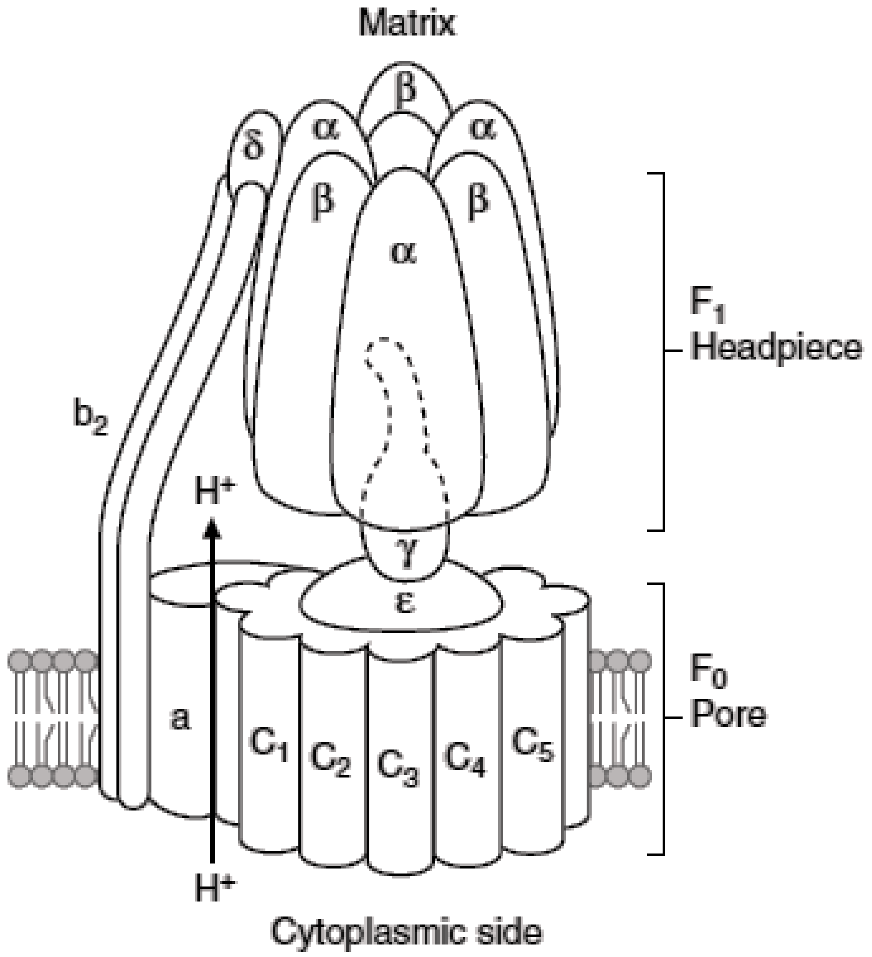
When ATP synthase had made full rotation of the C subunits, the protons that are bound to the glutamate of the first C subunit is facing the other side of the membrane where it is exposed to different PH which make the proton to be released from the glutamate to the matrix.

There is another fragment (I Fragment) that is linked to the C subunits by another sub unit called gamma subunit.

the I Fragment is composed of **6 Subunits :**

1) 3 Alpha subunits(for structural reasons) 2) 3 Beta Subunits. (catalytic)

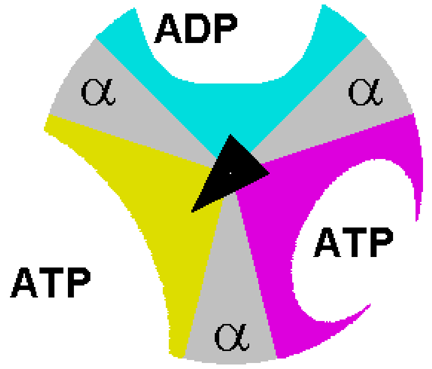
ATP SYNTHASE



The process of proton passing across the membrane by ATP Synthase

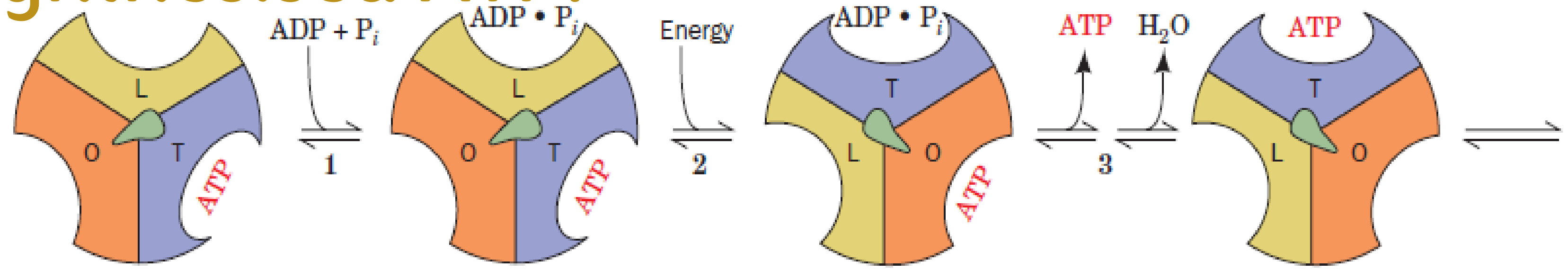
The gamma subunit isn't straight, it is angled (معقوفة / مطعوجة).

Whenever the C Subunits rotate, the gamma subunit also rotates since it is bind to them, and since it is angled (مطعوجة), it keep hitting the Beta subunits as it is rotating which cases a conformational change of the Beta subunit that it hit.



The Beta subunit active site switches between 3 states :

Open state : Low affinity for ATP, releasing the synthesised ATP.



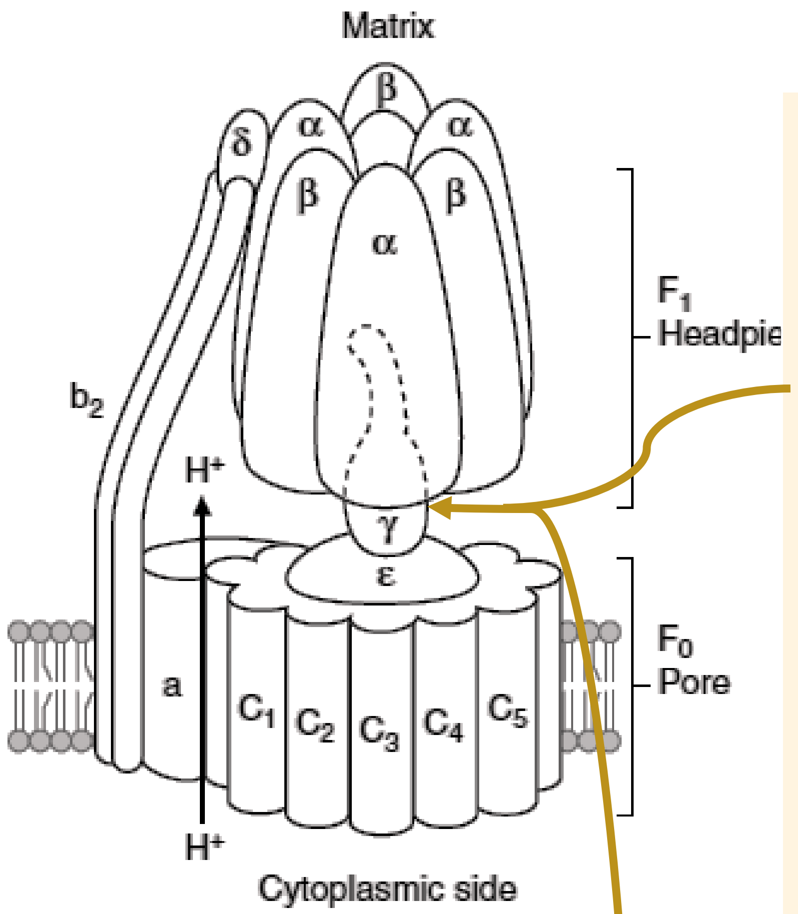
Loose state : High affinity for ADP & Pi

Tight State : close on the substrate to catalyze the phosphorylation reaction.

ATP SYNTHASE

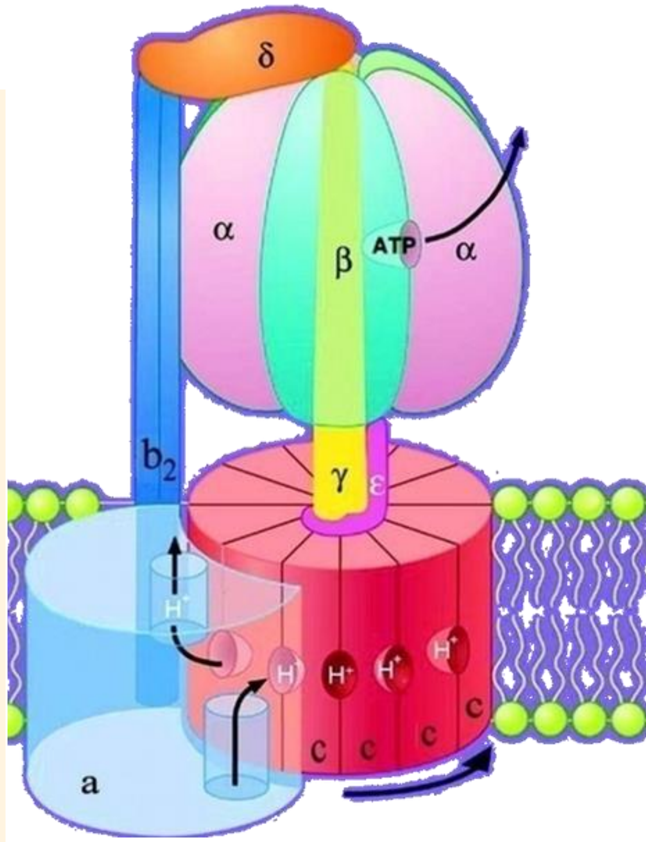
- F1:
  - "γ" subunit: rotates
  - "β" subunit: binds
  - "α" subunit: structural
  - 3 conformations: tight (T), loose (L), open (O)
- F0:
  - "a" subunit: point of entry & exit
  - "c" subunit rotates

- 4H+/ATP
- Can run backwards

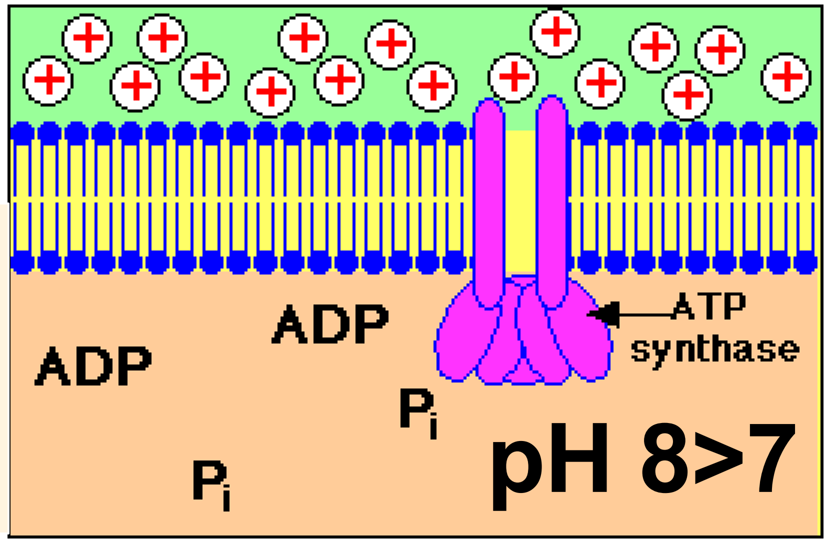


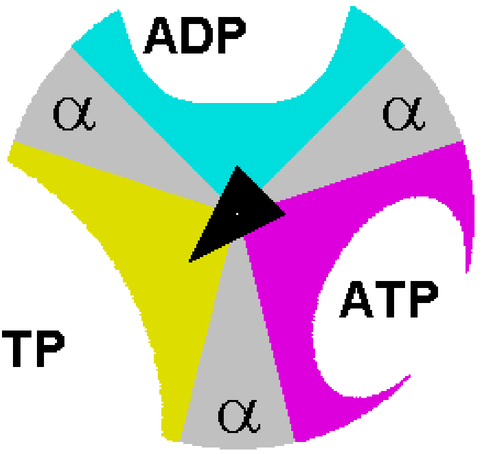
The C subunits will rotate in the other direction, and the Gamma will hit Beta subunit from opposite side, causing different conformational change.

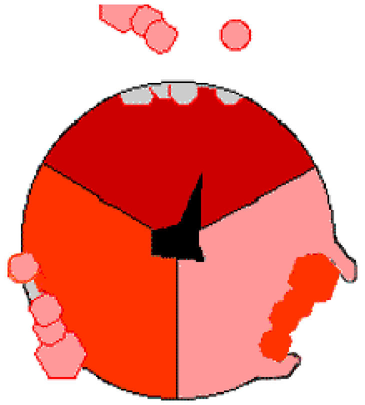
Which will make beta subunits active site hydrolyse ATP o ADP and Pi.

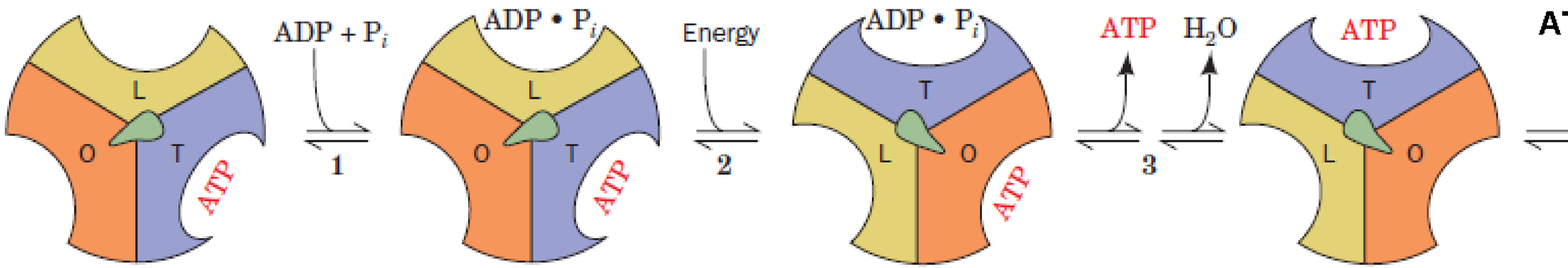


If the concentration of Protons becomes higher at the matrix, the ATP synthase can pass protons to the inter membrane space.







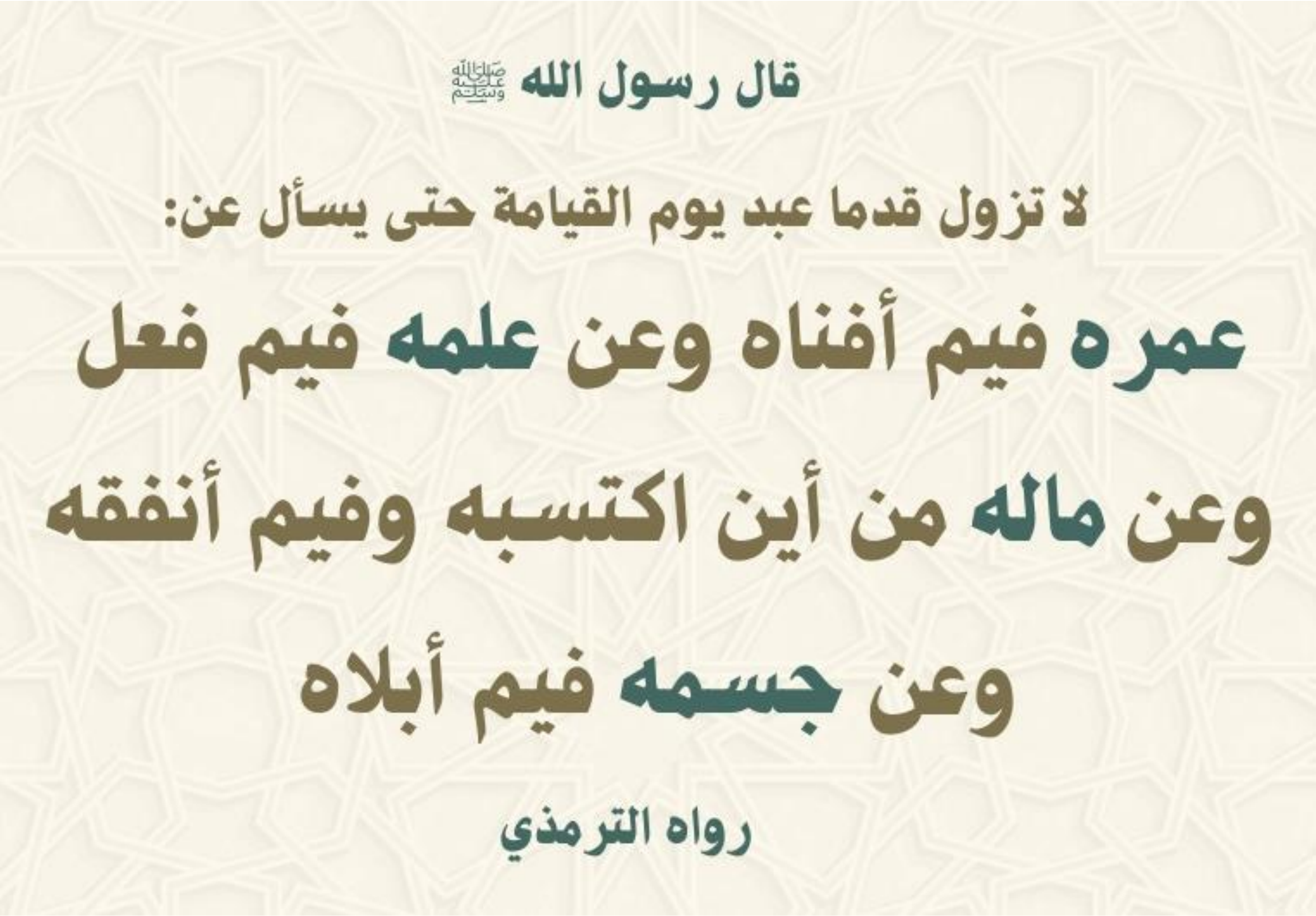


رسالة من الفريق العلمي:

قال رسول الله ﷺ:

" لا تزول قدما عبدٍ يوم القيامة حتى يُسأل عن أربع :عن عمره فيما أفناه، وعن جسده فيما أبلاه
وعن علمه ماذا عمل فيه، وعن ماله من أين اكتسبه وفيم أنفقه".

تأمل هذا الحديث العظيم ...لحظات صادقة مع النفس تذكّرنا أن الحياة أمانة، وأن كل نَفَسٍ
سنُسأل عنه، فطوبى لمن جعل عمره في طاعة الله، وعلمه نافعًا، وماله في الخير 🌸 .



قال رسول الله ﷺ

لا تزول قدما عبد يوم القيامة حتى يسأل عن:

عمره فيم أفناه وعن علمه فيم فعل

وعن ماله من أين اكتسبه وفيم أنفقه

وعن جسمه فيم أبلاه

رواه الترمذي

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			