BIOLOGICAL OXIDATION

Synopsis

- What is Biological Oxidation?
- Enzymes and Coenzymes of Biological Oxidation Reactions.
- Electron Transport Chain(ETC)
- Oxidative Phosphorylation Mechanisms
- Inhibitors of ETC and Oxidative Phosphorylation
- Uncouplers
- Shuttle Systems
- High Energy Compounds
- Substrate Level Phosphorylation.

What Is Biological Oxidation?

- Biological oxidation are **oxidation reactions** taking place in **living systems.**

Importance Of Biological Oxidation

- Biological Oxidation reactions are associated with **metabolism**.
- **Vital for functioning of cells, survival and existence of human body.**

Definition Of Oxidation Reactions

- **Oxidation reactions** are biochemical reactions where there is either:
  - **Removal / Loss of Hydrogen (Dehydrogenation)**
  - **Removal or Loss of Electrons**
  - **Addition of Oxygen (Oxygenation)**

- Most **predominant type** of **Oxidation reaction in body** is:
- **Dehydrogenation Reaction**
- Catalyzed by **Dehydrogenases**

- **Dehydrogenases** **remove Hydrogen** from substrates.
- Which are temporarily accepted by **Coenzymes.**

- **Coenzymes involved in Oxidation/Dehydrogenation reactions.**
  - **NAD^+**
  - **$NADP^+$**
  - **FAD**
  - **FMN**

- Coenzymes temporarily accept the hydrogen from substrates and get transformed to reduced coenzymes.
  - **$NADH+H^+$**
  - **$FADH_2$**
  - **$NADPH+H^+$**
  - **$FMNH_2$**

Enzymes and Coenzymes of Biological Oxidation Reactions

5 Enzymes of Biological Oxidation

1. AEROBIC DEHYDROGENASES
2. ANAEROBIC DEHYDROGENASES
3. OXYGENASES
4. OXIDASES
5. HYDROPEROXIDASES

- All 5 Enzymes of Biological Oxidation reactions are classified in

Class I

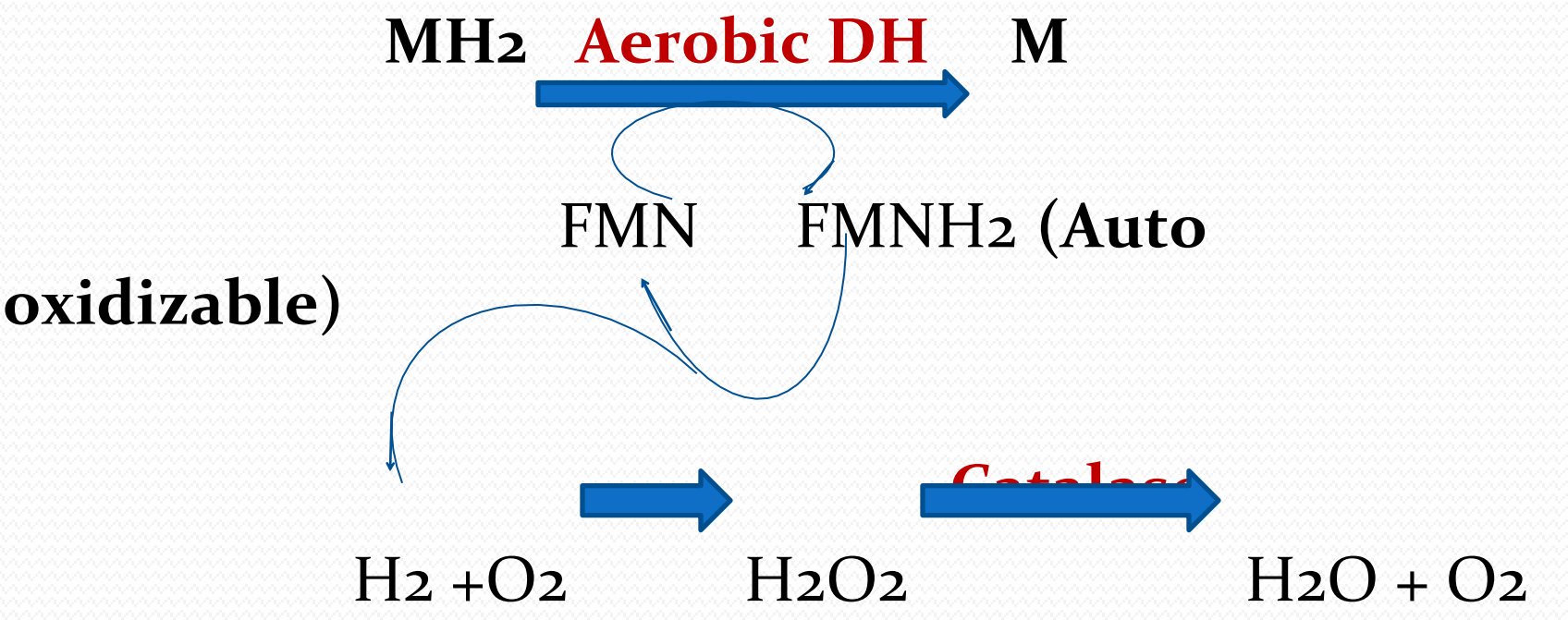
Oxido Reductases

Coenzymes and Inorganic Cofactors Of Biological Oxidation Reactions

- FMN
- FAD
- NAD^{+}
- $NADP^{+}$
- THBP (Tetra Hydro Biopterin)
- Cu^{++}
- Fe^{+++}

AEROBIC DEHYDROGENASES

- **Aerobic Dehydrogenases** are **Flavoproteins.**
- Enzymes covalently bound to **coenzymes FMN or FAD**



- FMN/FAD are acceptors of removed Hydrogen
- Reduced Coenzymes ($FMNH_2/FADH_2$) formed are auto oxidizable

- Reduced coenzymes get **reoxidized at reaction level.**
- **Oxygen** gets **directly involved** at reaction level to **reoxidize the reduced coenzymes.**

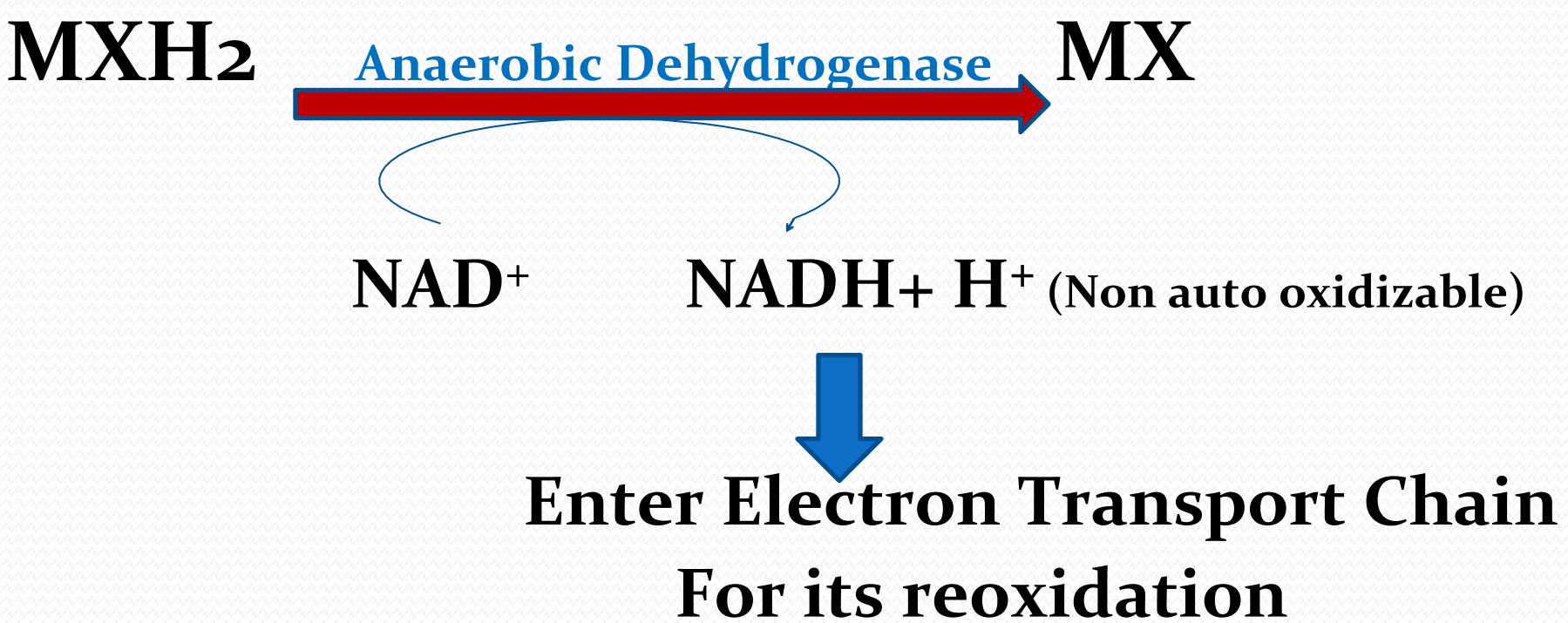
- **H_2O_2** is a byproduct of **Aerobic Dehyrogenase** activity.
- **Catalase** then detoxify the H_2O_2 to H_2O and O_2.

Specific Examples Of Aerobic Dehydrogenases

- **L Amino acid Oxidase**
  (Oxidative Deamination of A.A)
- **Xanthine Oxidase**
  (Purine Catabolism)
- **Glucose Oxidase**
  (Glucose oxidation to Gluconic acid)
- **Aldehyde Dehydrogenase**
  (Alcohol Metabolism)

ANAEROBIC DEHYDROGENASES

- Anaerobic Dehydrogenases catalyzes to **remove hydrogen from substrates.**
- With the help of coenzymes **NAD^+/$NADP^+$/FAD.**



- Coenzymes temporarily accept the hydrogen from substrates and get reduced to
  - $NADH+ H^+$
  - $FADH_2$
  - $NADPH+H^+$
  - $FMNH_2$

- Reduced coenzymes formed in Anaerobic Dehydrogenase reactions are :
  - Non autoxidizable/not reoxidized at reaction level.

- **Reduced coenzymes $NADH+H^+$ and $FADH_2$** formed at Anaerobic Dehydrogenase reaction **enter ETC for its reoxidation.**

- **Oxygen** is **involved indirectly** at the **end of ETC** as **electron and proton acceptor** .
- **Metabolic water** is end product of ETC.

- Reduced coenzyme **NADPH+H$^+$ do not enter ETC**
- **NADPH+H$^+$** is utilized as **reducing equivalent** for **reduction reactions** catalyzed by **Reductases.**

NAD^+ Dependent Anaerobic Dehydrogenases

- Glyceraldehyde -3-PO_4 Dehydrogenase
- Pyruvate Dehydrogenase
- Isocitrate Dehydrogenase
- α Ketoglutarate Dehydrogenase
- Malate Dehydrogenase
- Lactate Dehydrogenase
- Glutamate Dehydrogenase
- β Hydroxy Acyl Dehydrogenase

$NADP^+$ Dependent Dehydrogenases

- Glucose -6-Phosphate Dehydrogenase (HMP Shunt)
- Phospho Gluconate Dehydrogenase (HMP Shunt)
- Note **$NADPH+H^+$ does not enter ETC** for its reoxidation instead they are involved in reduction reactions.

FAD Dependent Anaerobic Dehydrogenases

- **Succinate Dehydrogenase**
  (TCA Cycle)
- **Acyl CoA Dehydrogenase**
  (β Oxidation Of Fatty Acids)

FMN Dependent Anaerobic Dehydrogenase

- **NADH Dehydrogenase (Warburg's Yellow Enzyme)**
  First Component of ETC/ Complex I of ETC

OXYGENASES

- Oxygenases **add Oxygen** atom from molecular oxygen (O_2) **to** the **substrate**.
- Form Oxidized Product.

Mono Oxygenases

- Mono Oxygenases add **one oxygen atom** from molecular oxygen to the substrate.
- Forms Hydroxyl group (-OH)
- **Monoxygenases are** also termed as **Hydroxylases** or **Mixed Function Oxidase.**

$$AH + O_2 + BH_2 \xrightarrow{\text{Mono Oxygenase}} AOH + B + H_2O$$

$$\text{Tyrosine} + O_2 + THBP \xrightarrow[\text{Hydroxylase}]{\text{Tyrosine}} DOPA + DHBP + H_2O$$

Examples Of Mono Oxygenases

- **Phenylalanine Hydroxylase**
  (Phenylalanine to Tyrosine)
- **Tryptophan Hydroxylase**
  (Tryptophan to 5HydroxyTryptophan)
- **25 Hydroxylase**
  (Vitamin D - Cholecalciferol activation)
- **1 α Hydroxylase**
  (Vitamin D - Cholecalciferol activation)

Di Oxygenases

- Dioxygenases are **true Oxygenases**
- Incorporates **two Oxygen atoms from O2.**

A+ O2 → (Dioxygenase) AO2

Examples Of Dioxygenases

- **Tryptophan Di Oxygenase/ Tryptophan Pyrrolase**
  (Tryptophan NFormyl Kynurenine)
- **PHPP Dioxygenase**
- **Cysteine Dioxygenase**
- **Homogentisate Oxidase**
  (Homogentisate to 4 Maleyl Acetoacetate)

Oxidases

- Oxidases involve **molecular Oxygen** as **Hydrogen** (electron and proton) **acceptor.**
- Oxidases **reduces molecular** Oxygen to Water (H_2O)

$$AH_2 + \tfrac{1}{2} O_2 \xrightarrow{\text{Oxidase}} A + H_2O$$

$$\text{Tyrosine} + O_2 \xrightarrow{\text{Tyrosinase -}Cu^{++}} \text{DOPA} + H_2O$$

Examples Of Oxidases

- **Cytochrome Oxidase**
  (ETC enzyme) Classic Example
- **Ascorbate Oxidase**
- **Mono Amine Oxidase**
- **Catechol Oxidase**

Hydroperoxidases

- Hydroperoxidases detoxify **Hydrogen Peroxide** in body.
- **H_2O_2** is a **substrate/reactant** for **Hydroperoxidases.**

- Hydroperoxidases are Hemoproteins.
- Contains loosely bound Heme as prosthetic group.

- Hydroperoxidases prevent accumulation of H_2O_2 in cells.
- H_2O_2 if accumulated in cells is toxic
  - Leads to disruption of membranes(Hemolysis).
  - Increases risk of cancer and atherosclerosis.

Specific Examples Of Hydroperoxidases

- Peroxidases
- Catalase

- **Peroxidases**
- **Indirectly react with H_2O_2.**
  - **Glutathione Peroxidase**
    (In R.B.C's)
  - **Leukocyte Peroxidase**
    (In W.B.C's)

H_2O_2 + 2 GSH —(Glutathione Peroxidase)→ $2H_2O$ + GSSG

(Reduced active Form) → (Oxidized inactive Form)

- **Catalase**
- **Directly reacts with H_2O_2.**
- Associated with Aerobic Dehydrogenase catalyzed reaction.

$2H_2O_2$ —(Catalase)→ $2H_2O + O_2$

Biological Oxidation Process Electron Transport Chain (ETC)

Synonyms

- Electron Transport Chain (ETC)
- Electron Transport System (ETS)
- Respiratory Chain
- Internal/Cellular Respiration
- Tertiary metabolism
- Fate of reduced Coenzymes $NADH+H^+/FADH_2$
- Final Oxidative Pathway
- Oxidative Phosphorylation

What is Electron Transport Chain?

- **Electron Transport chain**
- **Vital biological oxidation process.**
- **Carried** out in **aerobic condition.**
- **Located** at **inner membrane** of **Mitochondria.**

- Fate of ETC is to **reoxidize** the **reduced coenzymes $NADH+H^+/FADH_2$.**
- Formed during **Anaerobic Dehydrogenase** reactions.

- **Electron Transport Chain**
- **Transports Electrons and Protons**
- Through a **series of ETC components** and
- **Finally** to **activated molecular oxygen.**
- **Generates ATP and metabolic water.**

What is Oxidative Phosphorylation?

- **Oxidative process** (ETC) is tightly **coupled with Phosphorylation** of ADP with pi to **generate ATP.**

- Oxidative Phosphorylation is a major mode of ATP generation.

Location Of ETC

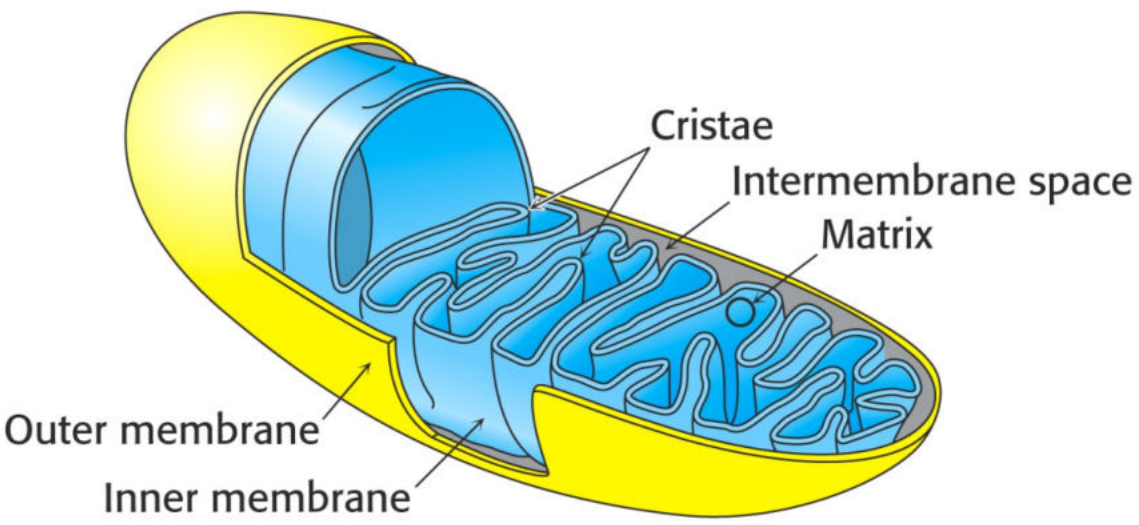
- **ETC is carried out in all cells which contain mitochondria (Power house of Cell).**

(Except mature Erythrocytes which are devoid of mitochondria)

- Components and Enzymes of **ETC are arranged** towards inner surface of **inner membrane of mitochondria.**
- In **vectorial conformation**
- In **increased order of positive redox potential**

Location of Mitochondrial ETC Complexes

- **Inner membrane of mitochondria**



Condition Of ETC Operation

- ETC operates in **truly aerobic condition.**
- Oxygen unloaded at cellular level by HbO_2 gets **utilized at** the end of **ETC** process. **(Respiratory Chain)**

- **ETC depends on**

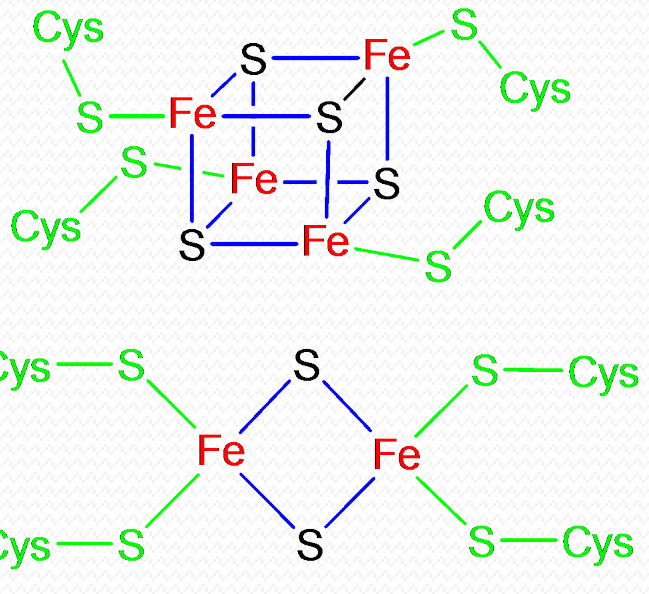
- **Respiration Process**
- **Oxygen Concentration**
- **Hemoglobin Structure and Function**
- **Metabolic Status**

Components Of ETC

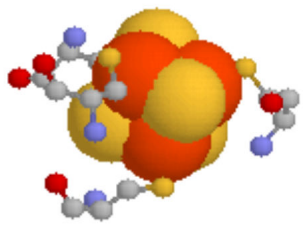
- **Flavo Protein- (First Component)**

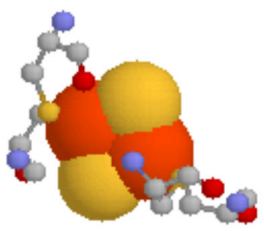
  NADH Dehydrogenase-FMN and FeS centers(Warburg's Yellow Enzyme)
- **Coenzyme Q/ Ubiquinone**
- **Series of Cytochromes-**

  Cytochrome b-Cytochrome c1-Cytochrome c- Cytochrome aa3



Iron-Sulfur Centers


4-iron Fe-S


2-iron Fe-S

Two iron-sulfur centers from complex I

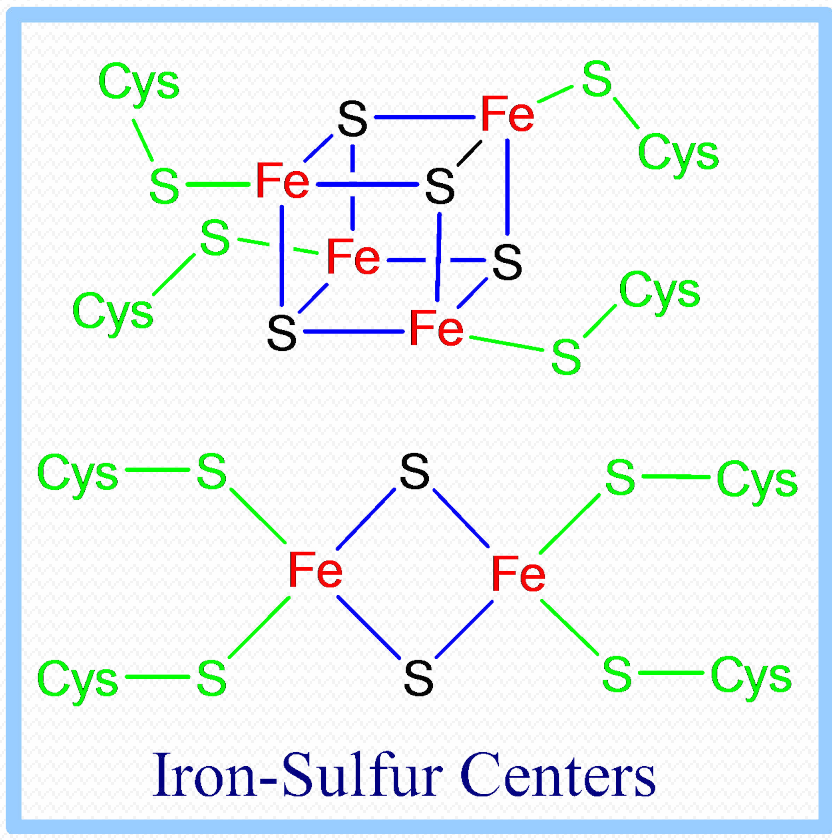
Iron-sulfur centers (Fe-S) are prosthetic groups containing **2, 3, 4 or 8 iron atoms** complexed to elemental & cysteine **S**.

4-Fe centers have a tetrahedral structure, with **Fe** & **S** atoms alternating as vertices of a cube.

Cysteine residues provide **S** ligands to the iron, while also holding these prosthetic groups in place within the protein

Electron transfer proteins may contain multiple Fe-S centers.

Iron-sulfur centers transfer only **one electron**, even if they contain two or more iron atoms, because of the close proximity of the iron atoms.



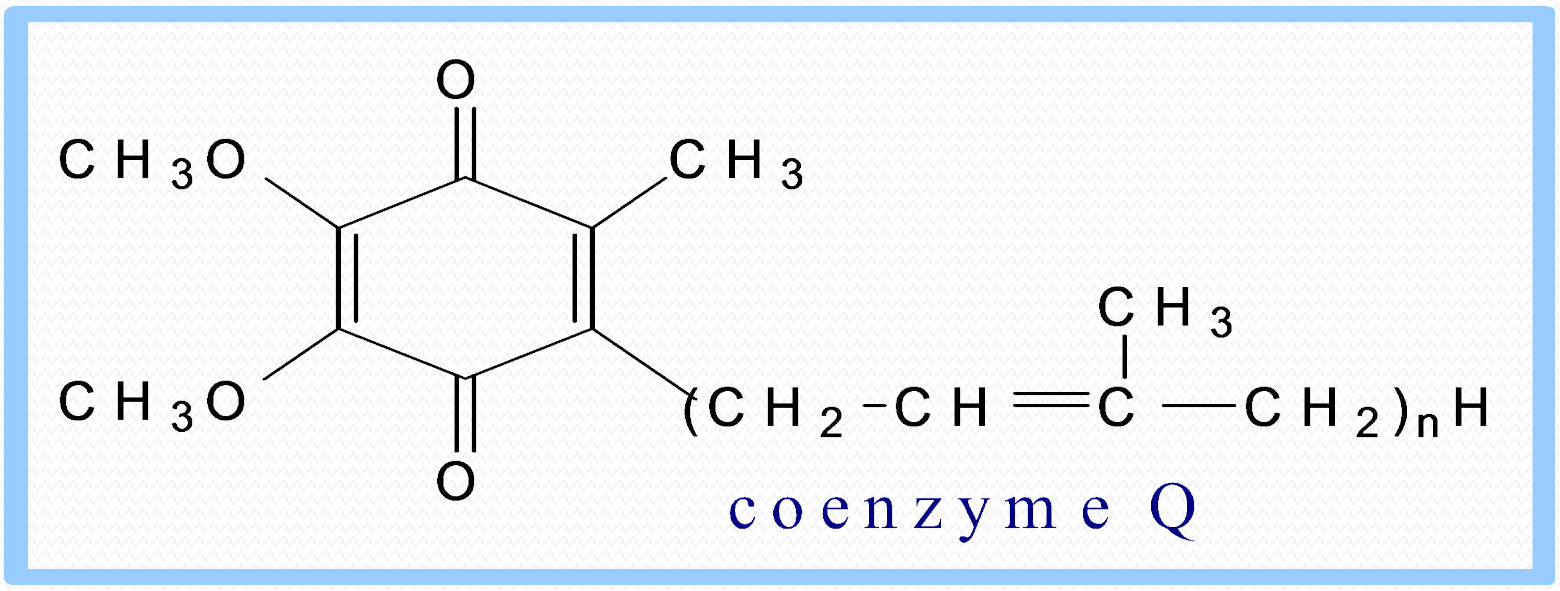
Iron-Sulfur Centers

Fe^{+++} (oxidized) + 1 e^- ←→ Fe^{++} (reduced)

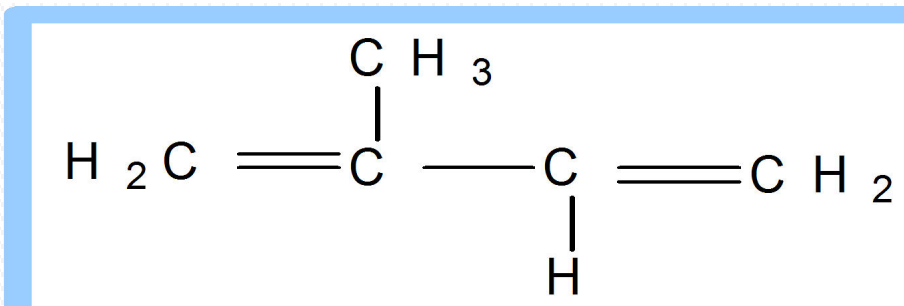
Coenzyme Q / Ubiquinone

- **Coenzyme Q** (CoQ)/ **Ubiquinone**) is **located in the lipid core** of the mitochondrial membrane.
- It is a **Quinone derivative**
- **Lipophilic** dissolves in the hydrocarbon core of a membrane.

- Cytochrome has a long **Poly isoprenoid tail**, with multiple units of isoprene.
- In human cells, most often n = 10.
- **Q_{10} isoprenoid tail** is longer than the width of a bilayer.



coenzyme Q



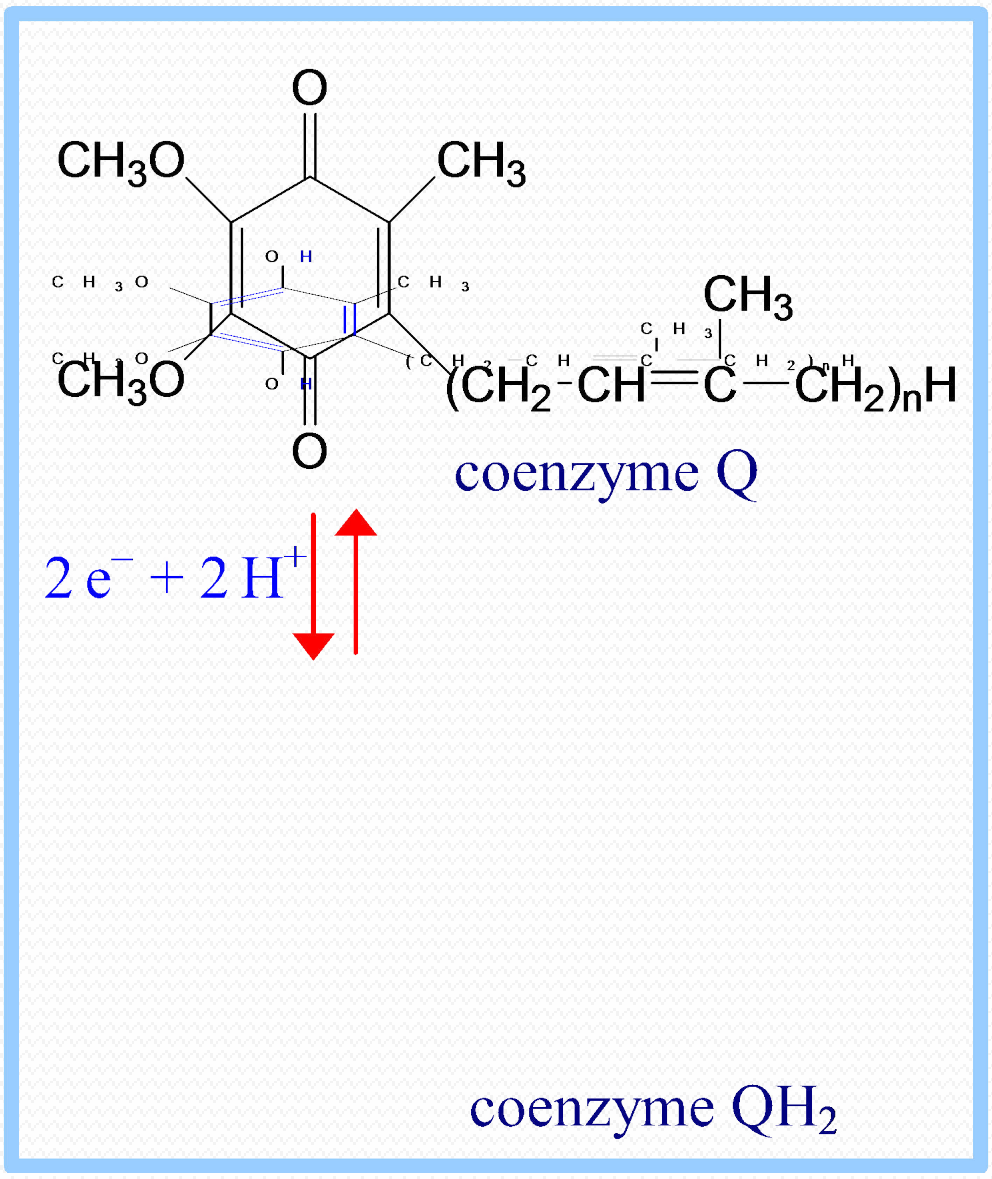
isoprene

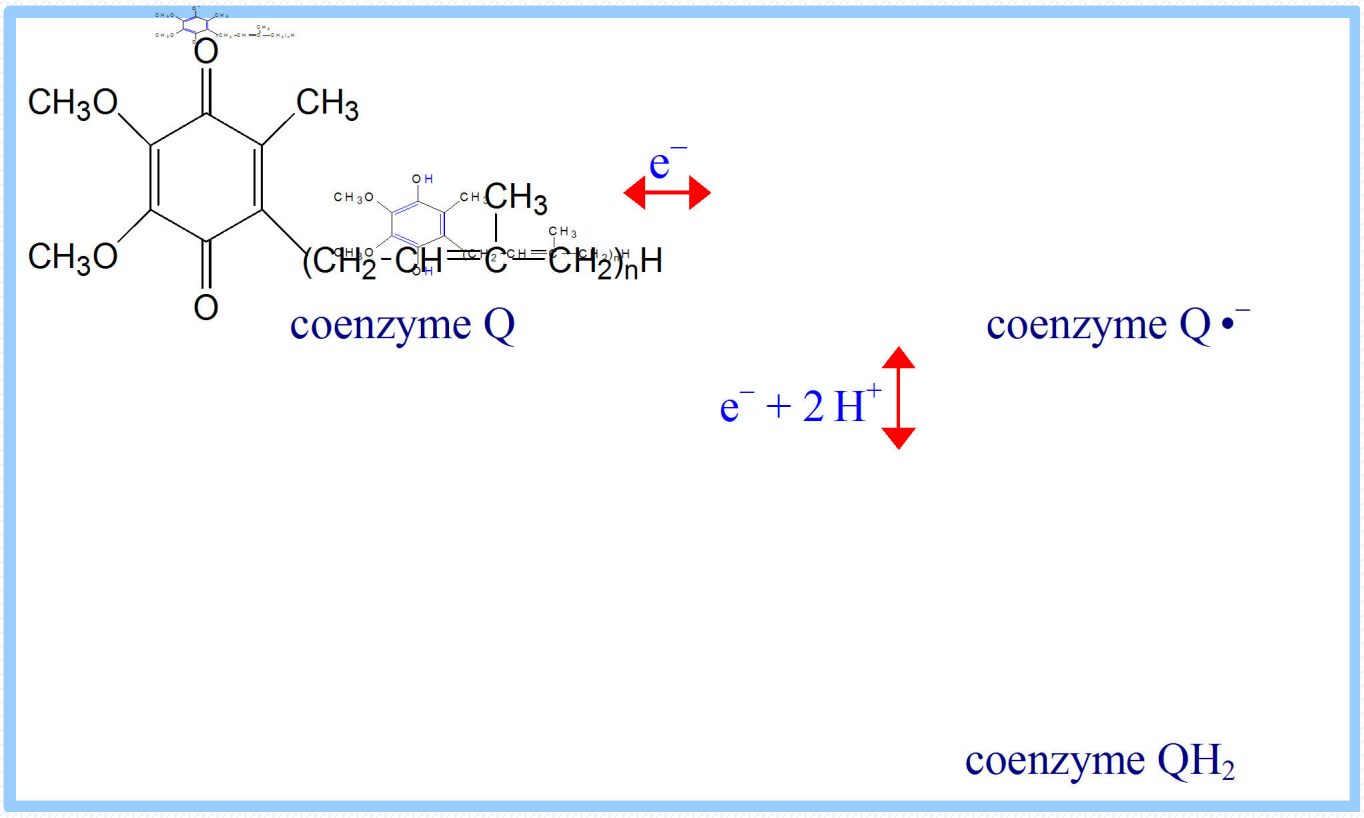
- **Coenzyme Q** is very **hydrophobic**.

- **Coenzyme Q** functions as a **mobile e^- carrier** within the mitochondrial inner membrane.
- Its role in trans-membrane **H^+ transport** coupled to e^- transfer (**Q Cycle**).

The **Quinone** ring of coenzyme Q can be reduced to the **Quinol** in a $2e^-$ reaction:

$Q + 2\ e^- + 2\ H^+ \leftrightarrow QH_2$.





When bound to special sites in respiratory complexes, **CoQ** can accept **1 e⁻** to form a **semiquinone radical** (**Q·⁻**).

Thus CoQ, like FMN, can mediate between 1 e⁻ & 2 e⁻ donors/acceptors.

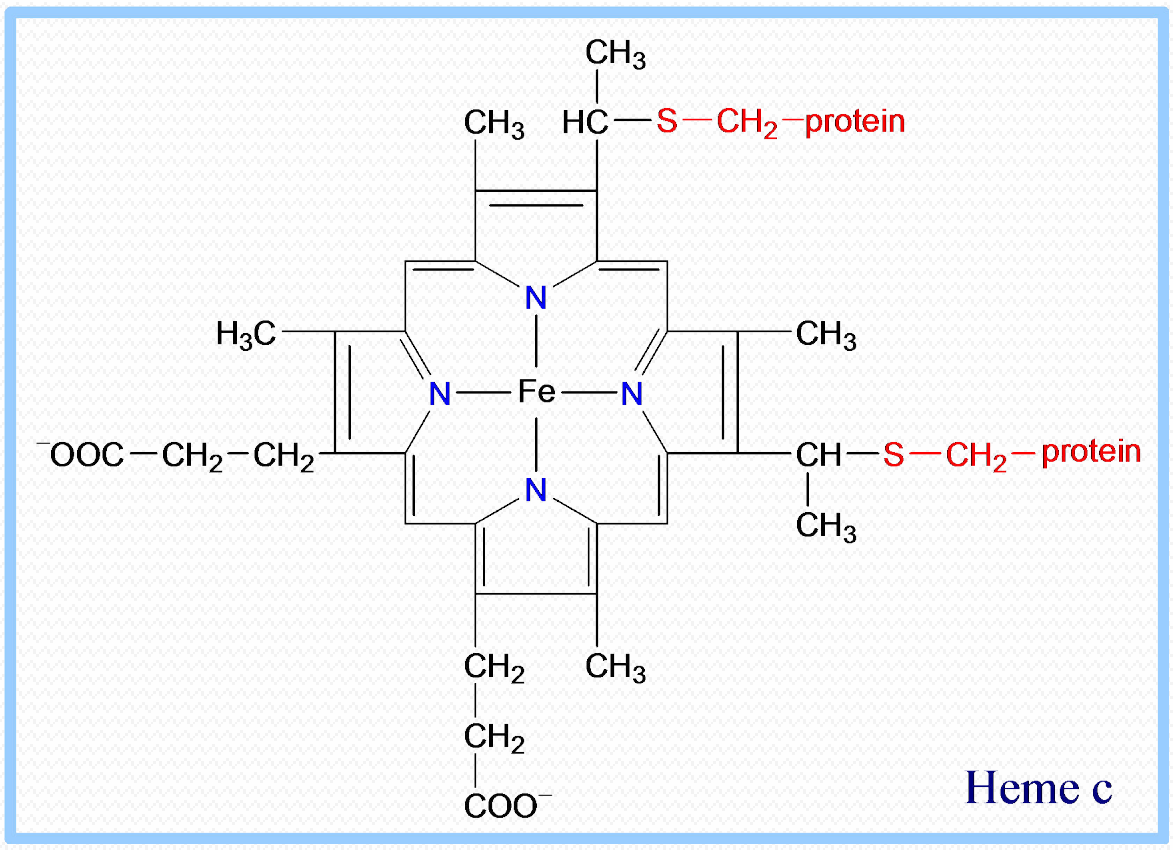
Cytochromes

- **Cytochromes** are Hemoproteins **conjugated proteins in ETC**
- Carrier of electrons
- Contain **heme as prosthetic group.**

- Cytochromes absorb light at characteristic wavelengths.
- **Absorbance changes** upon oxidation/reduction of the Heme Iron

Cytochrome Heme

- Cytochrome heme Iron is in transitional state
- Carries electrons only:
- $Fe(III) + e^- \leftrightarrows Fe(II)$
- Only *one* electron is transferred at a time.



- Cytochrome heme iron can undergo a **1 e⁻** transition between ferric and ferrous states:
- **$Fe^{+++} + e^-$ ←→ Fe^{++}**

(oxidized) (reduced)

- ☛ **Series of *Cytochromes* *b*, c_1, *c*, aa_3** relay electrons (one at a time, in this order ☚

- **Cytochrome c** is a small, water soluble protein with a single heme group.

- Cytochromes a & a_3 are often referred to as **Cytochrome Oxidase /complex IV.**
- Cytochrome aa3 has Fe and Cu.

- All **Cytochromes** except Cytochrome Oxidase are **Anaerobic Dehydrogenases**.

ETC Complexes

- ETC complexes are combination of one or two components of ETC.
- There are **5 ETC complexes.**

- **Complex I- NADH CoQ Reductase**

  NADH Dehydrogenase FMN and FeS centre
- **Complex II – Succinate CoQ Reductase**

  Succinate Dehydrogenase FAD and FeS centre
- **Complex III–CoQ Cytochrome C Reductase**

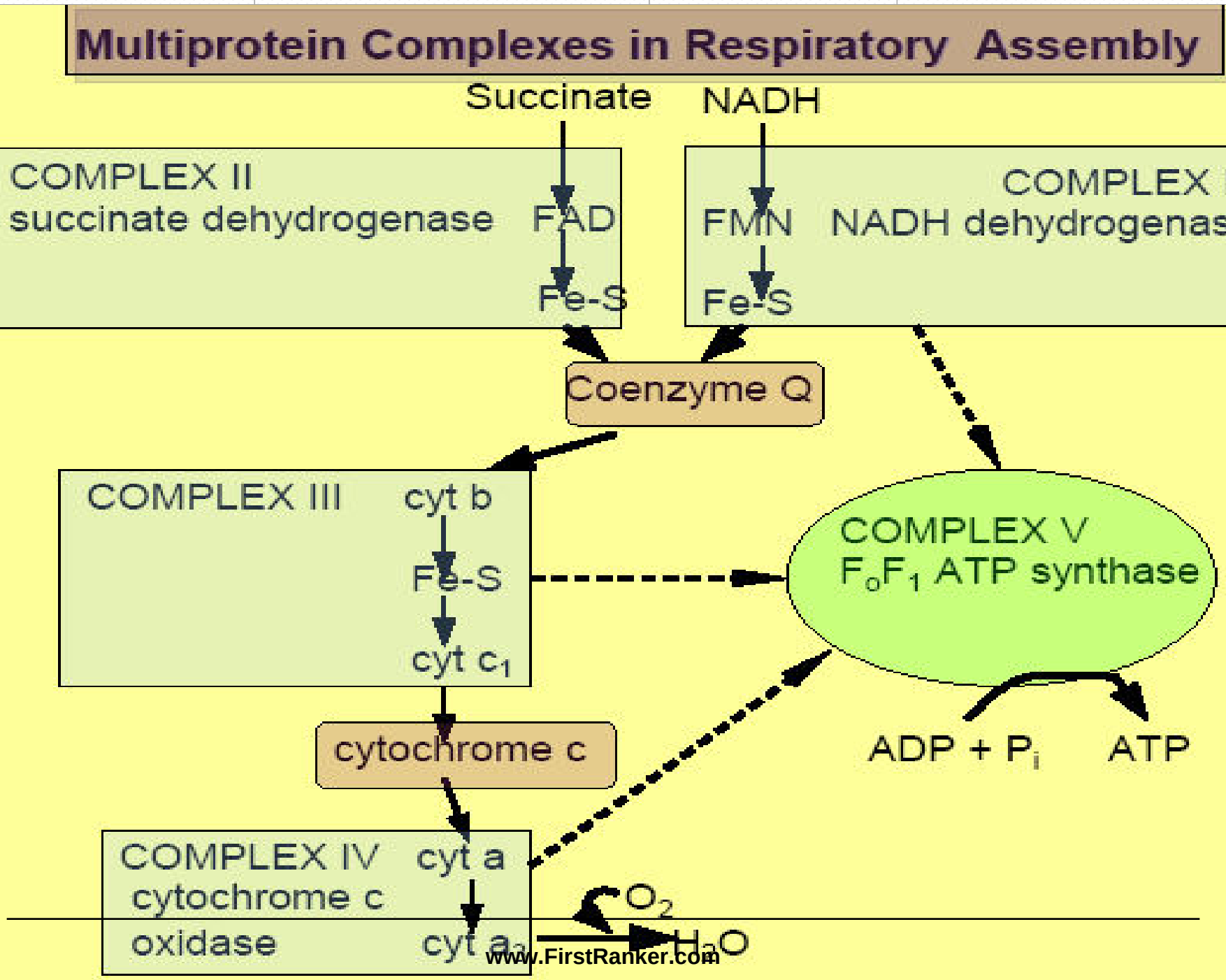
  Cytochrome b – Cytochrome c1
- **Complex IV- Cytochrome Oxidase**

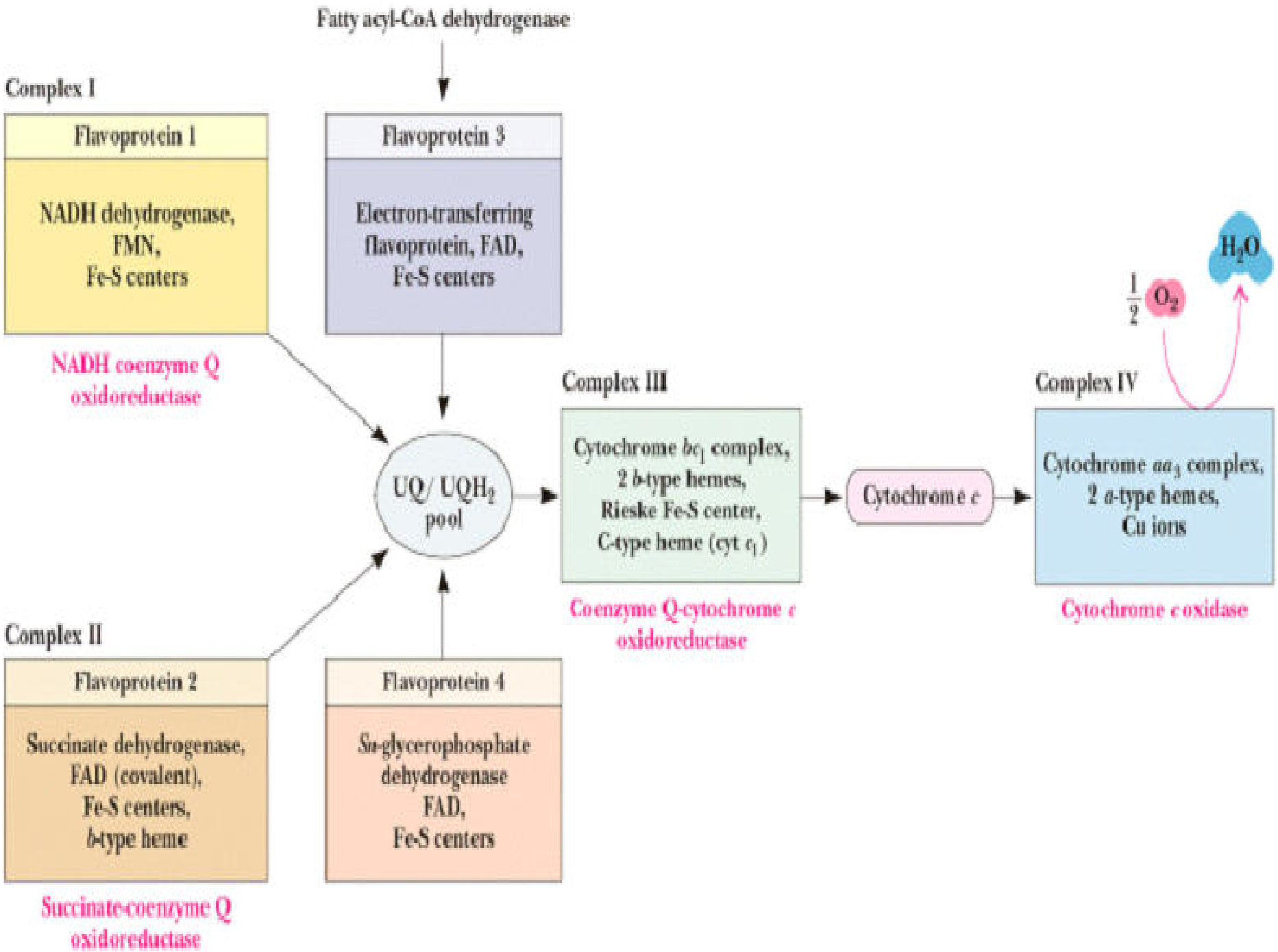
  Cytochrome aa3
- **Complex V – ATP Synthetase**

  Fo and F1 of ATP Synthase

Composition of Respiratory Chain Complexes

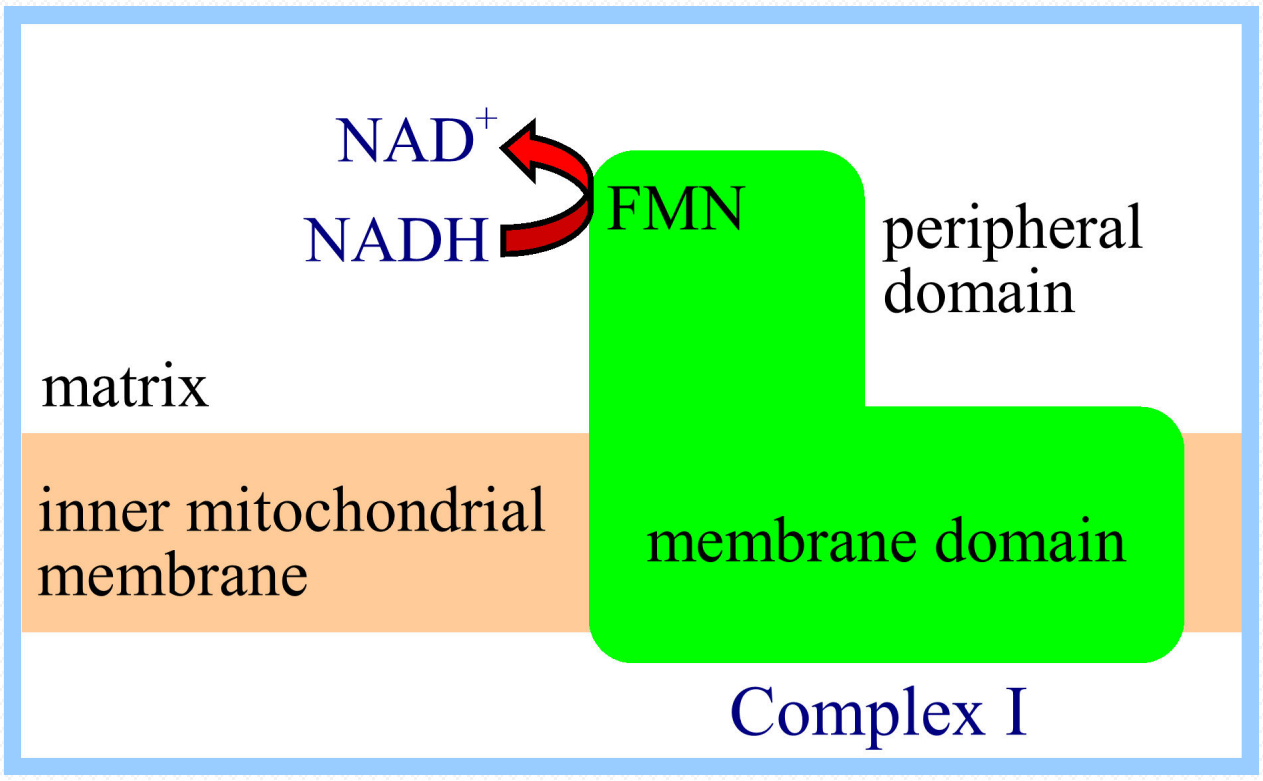
Complex	Name	No. of Proteins	Prosthetic Groups
Complex I	NADH Dehydrogenase	46	FMN, 9 Fe-S centers
Complex II	Succinate-CoQ Reductase	5	FAD, cyt b_{560}, 3 Fe-S centrs.
Complex III	CoQ-cyt c Reductase	11	cyt b_H, cyt b_L, cyt c_1, Fe-S_{Rieske}
Complex IV	Cytochrome Oxidase	13	cyt a, cyt a_3, Cu_A, Cu_B





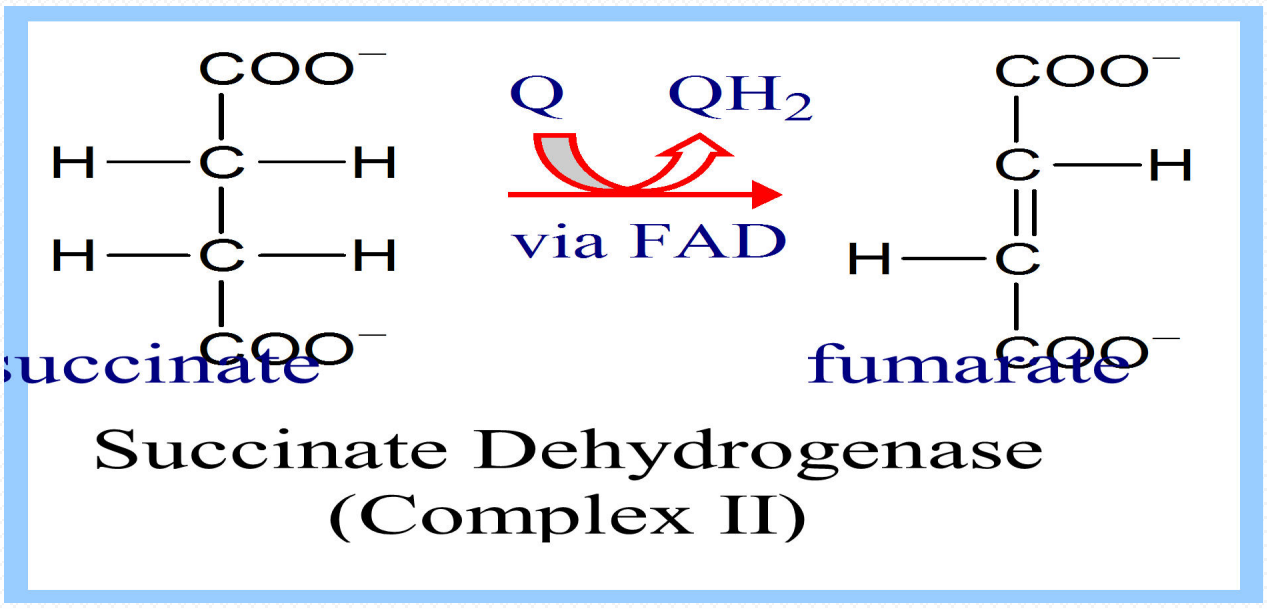
- Electrons are transferred from **NADH → O_2** via multisubunit inner membrane **complexes I, III & IV**, plus **CoQ** & **Cytochrome c**.
- Within each complex, electrons pass sequentially through a series of electron carriers.

Complex I catalyzes oxidation of NADH+H$^+$ with reduction of coenzyme Q:



NADH + H$^+$ + FP → NAD$^+$ + FPH2

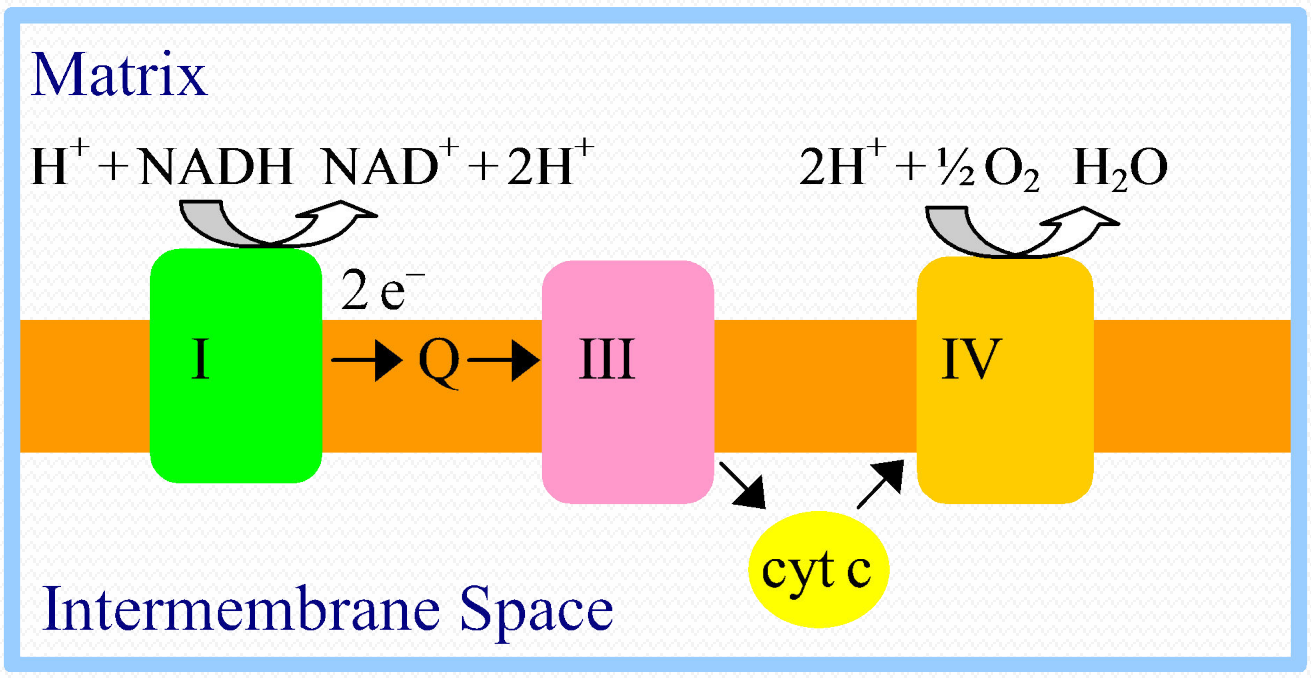
Coenzyme Q accepts 2 e$^-$ and picks up 2 H$^+$ from FPH2 to yield the fully reduced **QH_2**.



- **Succinate Dehydrogenase** of the Krebs Cycle is also called **complex II** or **Succinate-CoQ Reductase.**
- **FAD** is the initial e^- acceptor.
- FAD is reduced to **$FADH_2$** during oxidation of Succinate to Fumarate.

- **$FADH_2$** is then **reoxidized** by **transfer** of **electrons** through a series of 3 iron-sulfur centers **to CoQ**, yielding **QH_2**.
- The **QH_2** product may be reoxidized via **complex III.**
- Providing a pathway for transfer of electrons from Succinate into the respiratory chain.

- **CoQ accepts** electrons via ETC **complexes I and II.**

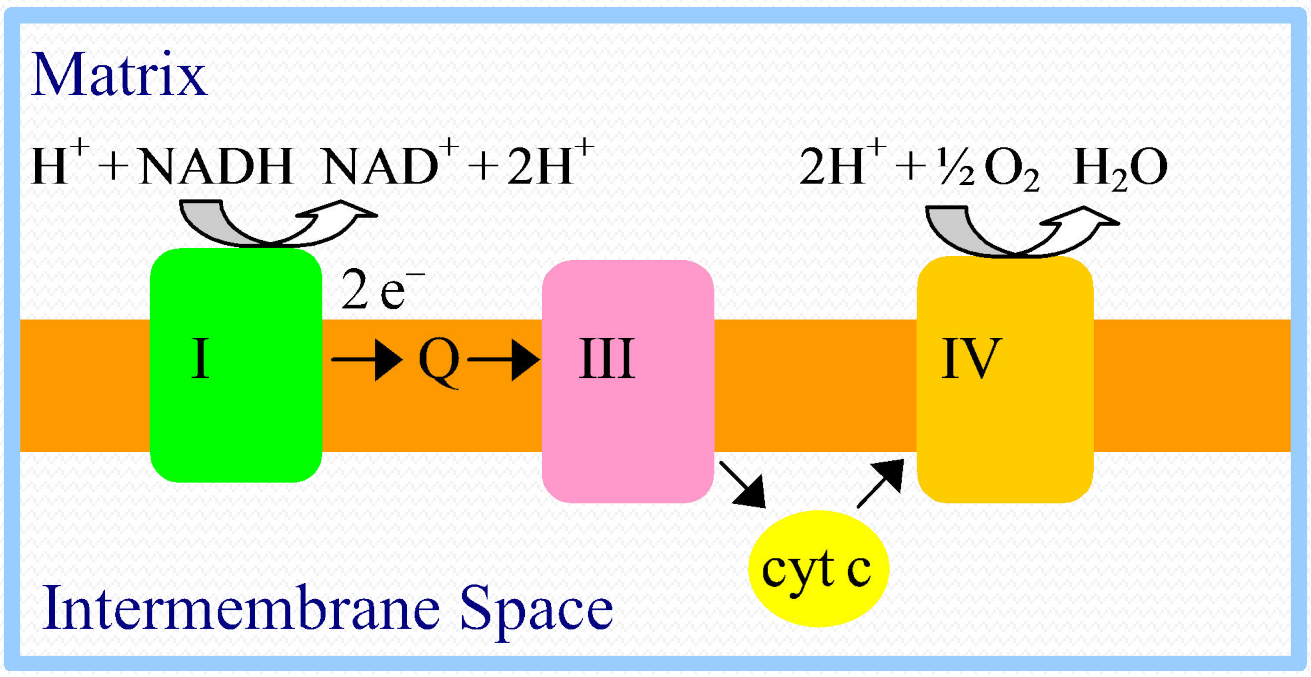


Complex III/ Cytochrome b-c1 complex accepts electrons from coenzyme **QH_2** that is generated by electron transfer in complexes I & II.

- **Cytochrome c** resides in the intermembrane space.
- It alternately binds to complex III or IV during e^- transfer.

COMPLEX IV

- **Cytochrome a-a3/ Cytochrome Oxidase**
  large protein
- Both a and a_3 contain heme and Cu
- a_3 Cu binds to oxygen and donates electrons to oxygen
- **Cytochrome a3** - only component of ETC that can interact with O_2



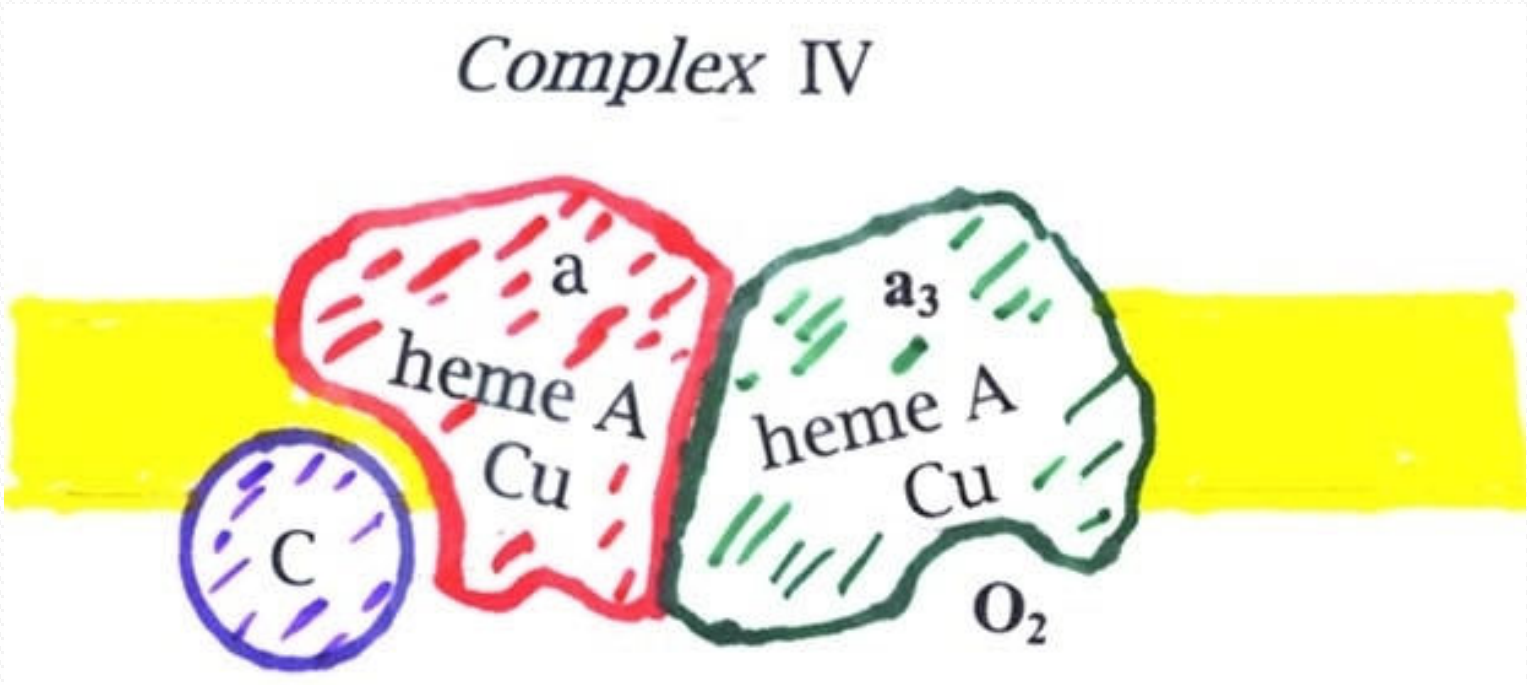
Cytochrome oxidase (complex IV) carries out the following irreversible reaction:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

The four electrons are transferred into the complex one at a time from Cytochrome c.

- Complex IV/Cytochrome Oxidase reduces molecular Oxygen to water.

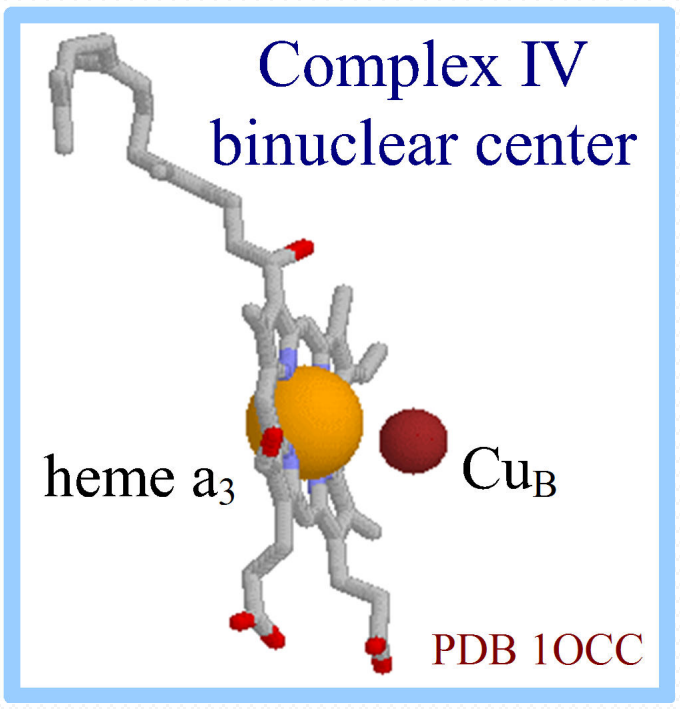
Cytochrome Oxidase



e- from cyt c to a

Cu(II) ⇆ Cu(I)

Heme A and Cu act together to transfer electrons to oxygen



Metal centers of cytochrome oxidase (complex IV):
heme a & heme a_3,
Cu_A (2 adjacent Cu atoms) & Cu_B.

O_2 reacts at a **binuclear center** consisting of heme a_3 and Cu_B.

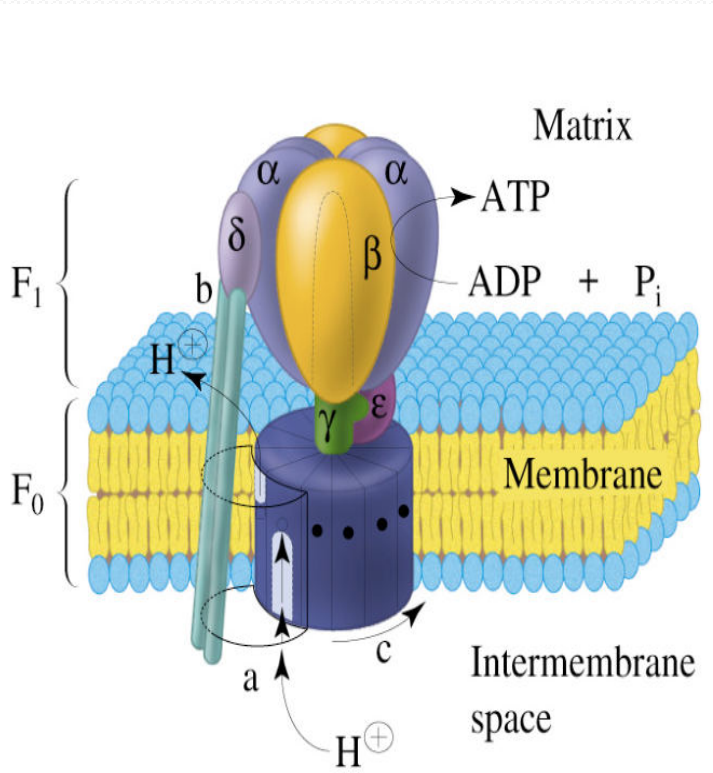
- An Iron-Copper Center in Cytochrome Oxidase Catalyzes Efficient O2 Reduction

Complex V
ATP Synthase

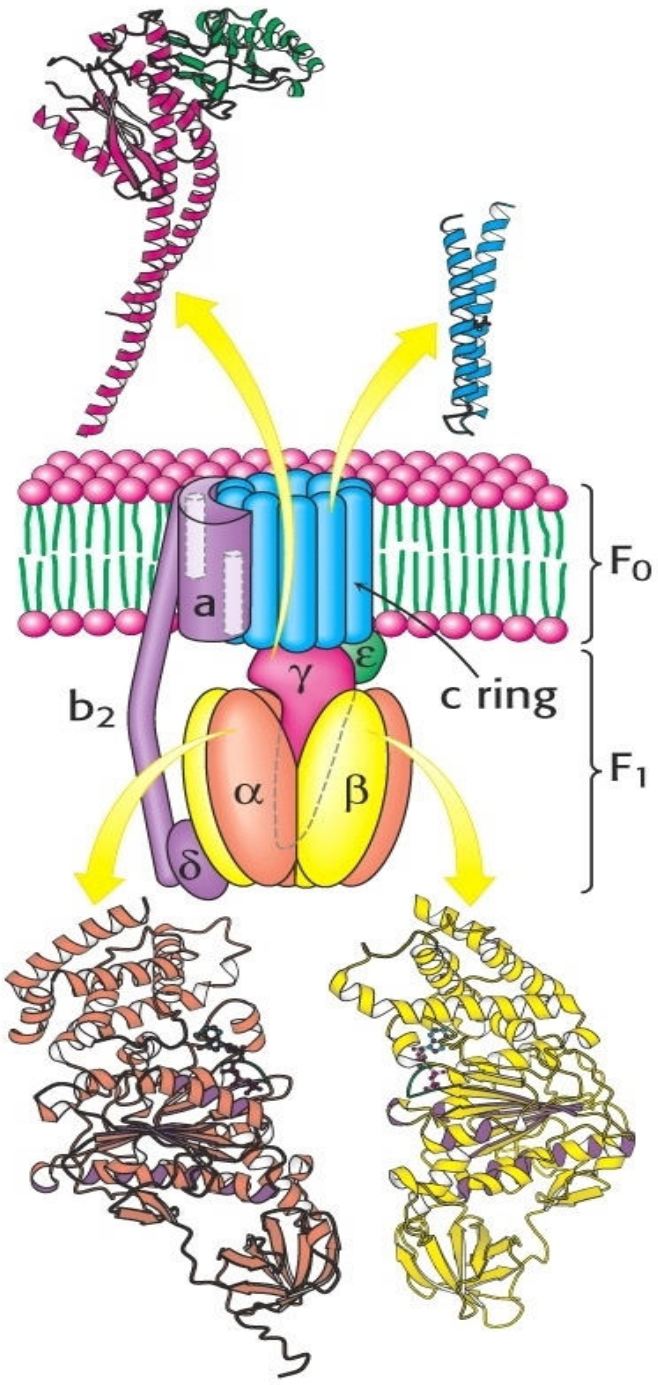
Two units, F_o and F_1
("knob-and-stalk"; "ball on a stick")

F_1 contains the catalytic subunits where ADP and P_i are brought together for combination.

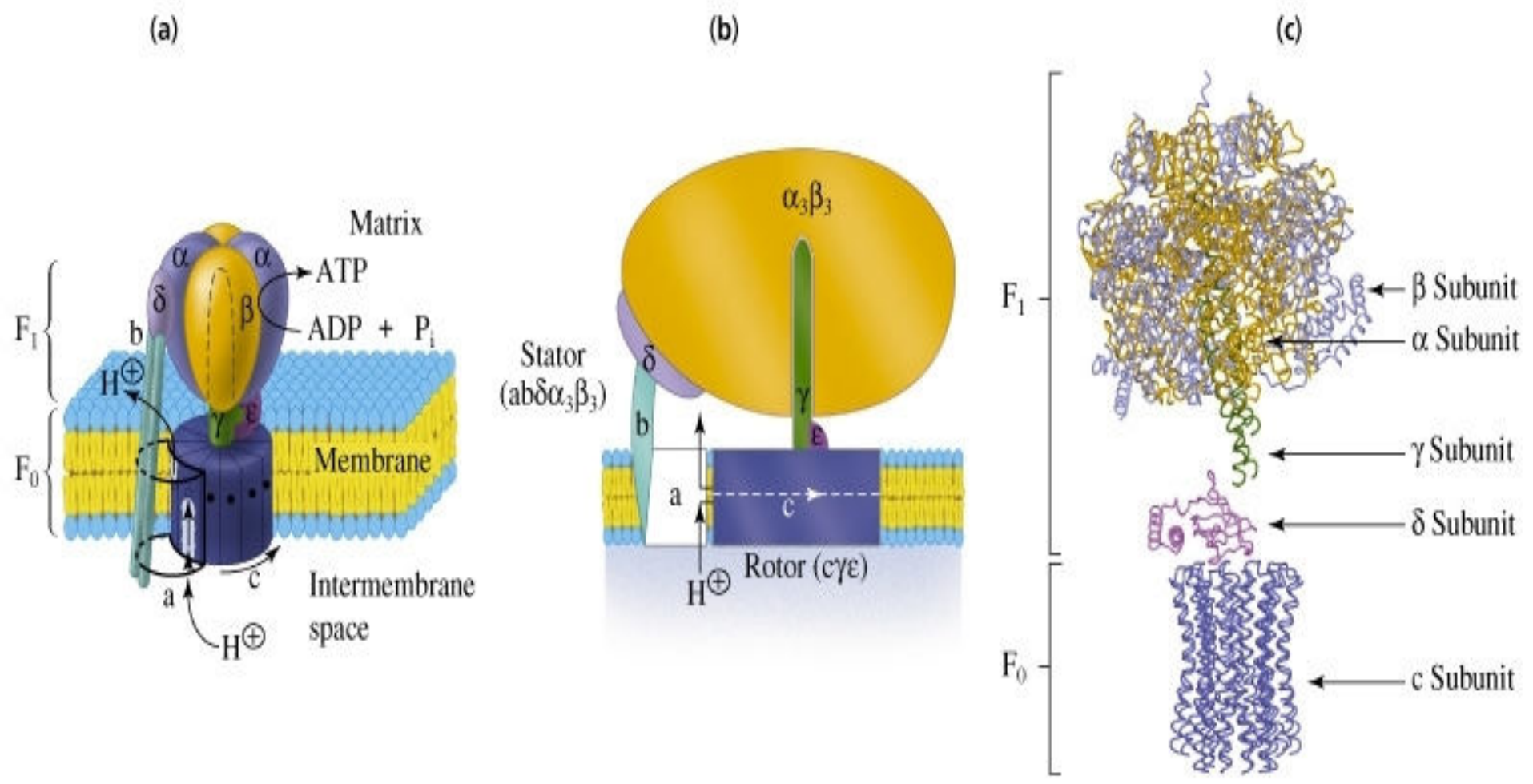
F_0 spans the membrane and serves as a proton channel.



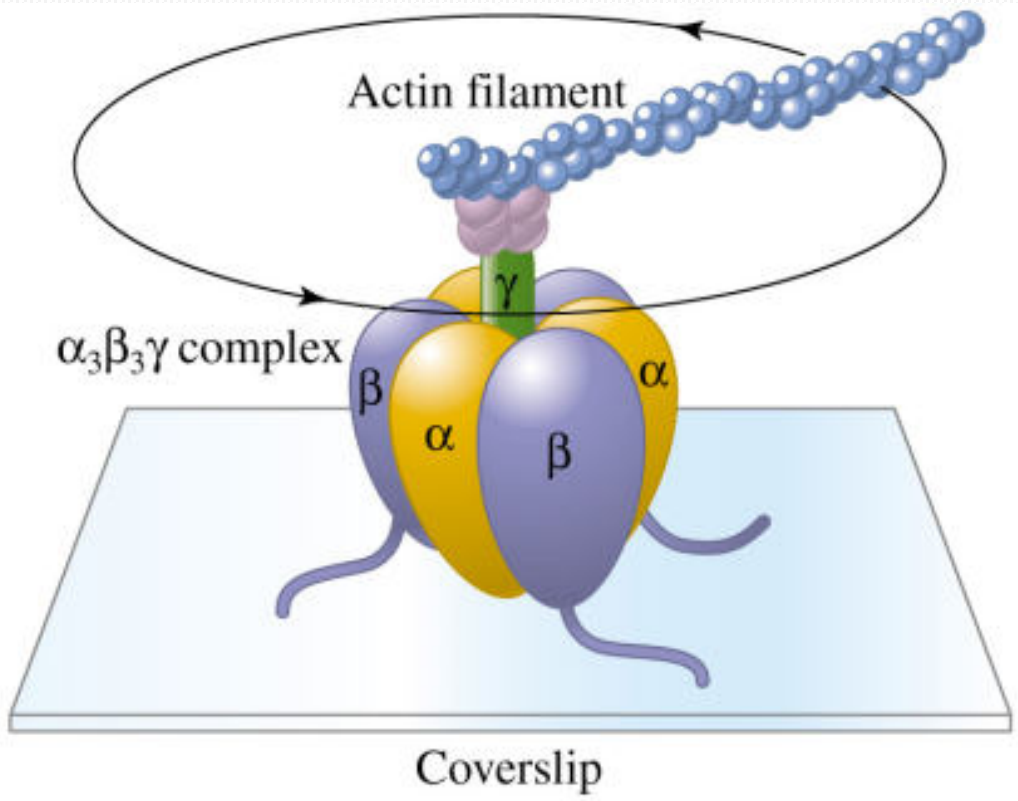
- F_1 contains 5 types of polypeptide chains - $\alpha_3\beta_3\gamma\delta\varepsilon$
- F_o - $a_1b_2c_{10\text{-}14}$ (c subunits form cylindrical, membrane-bound base)
- F_o and F_1 are connected by a $\gamma\varepsilon$ stalk and by exterior column (a_1b_2 and δ)
- The proton channel - between c ring and a subunit.



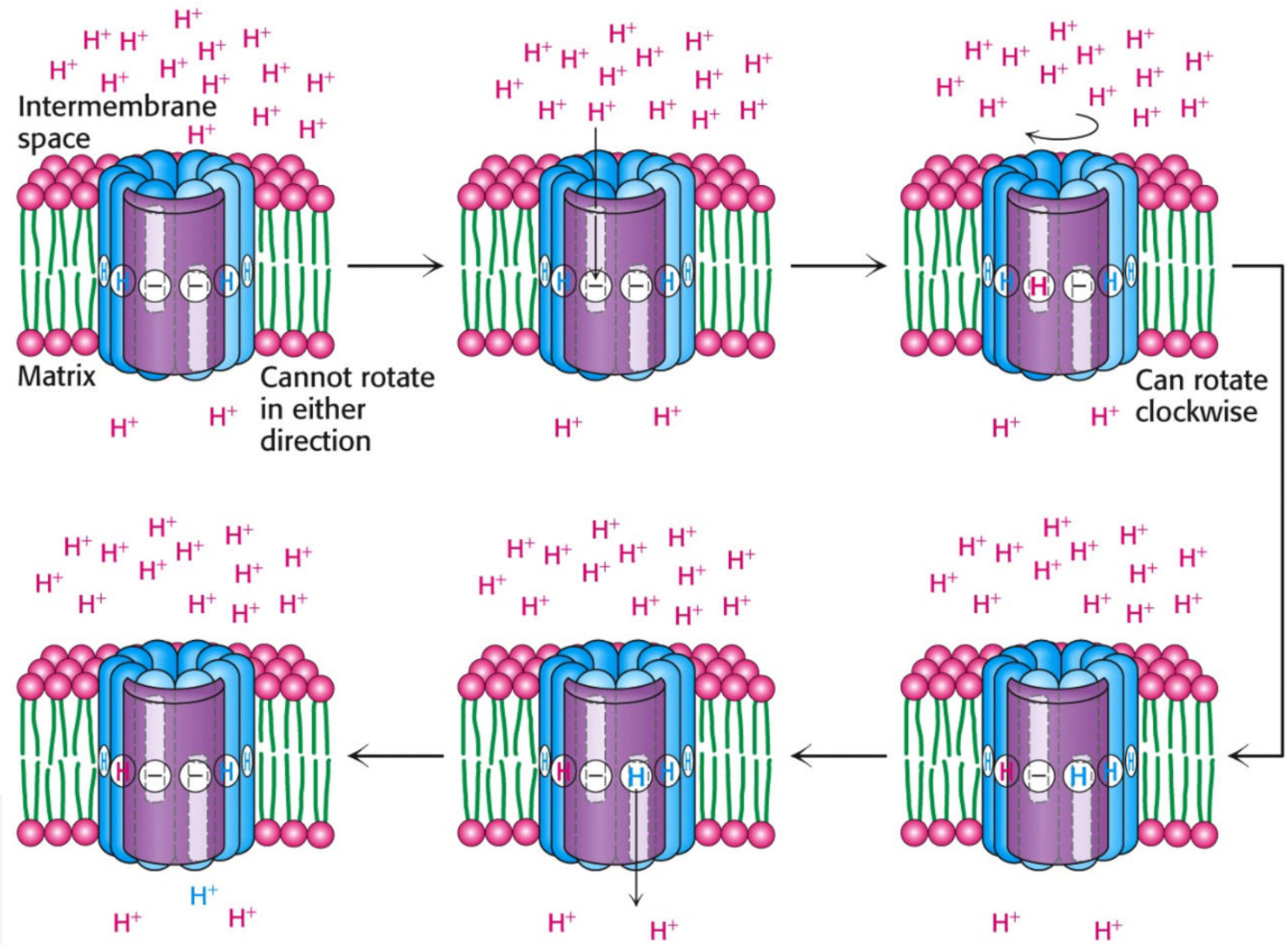
Complex V ATP Synthase

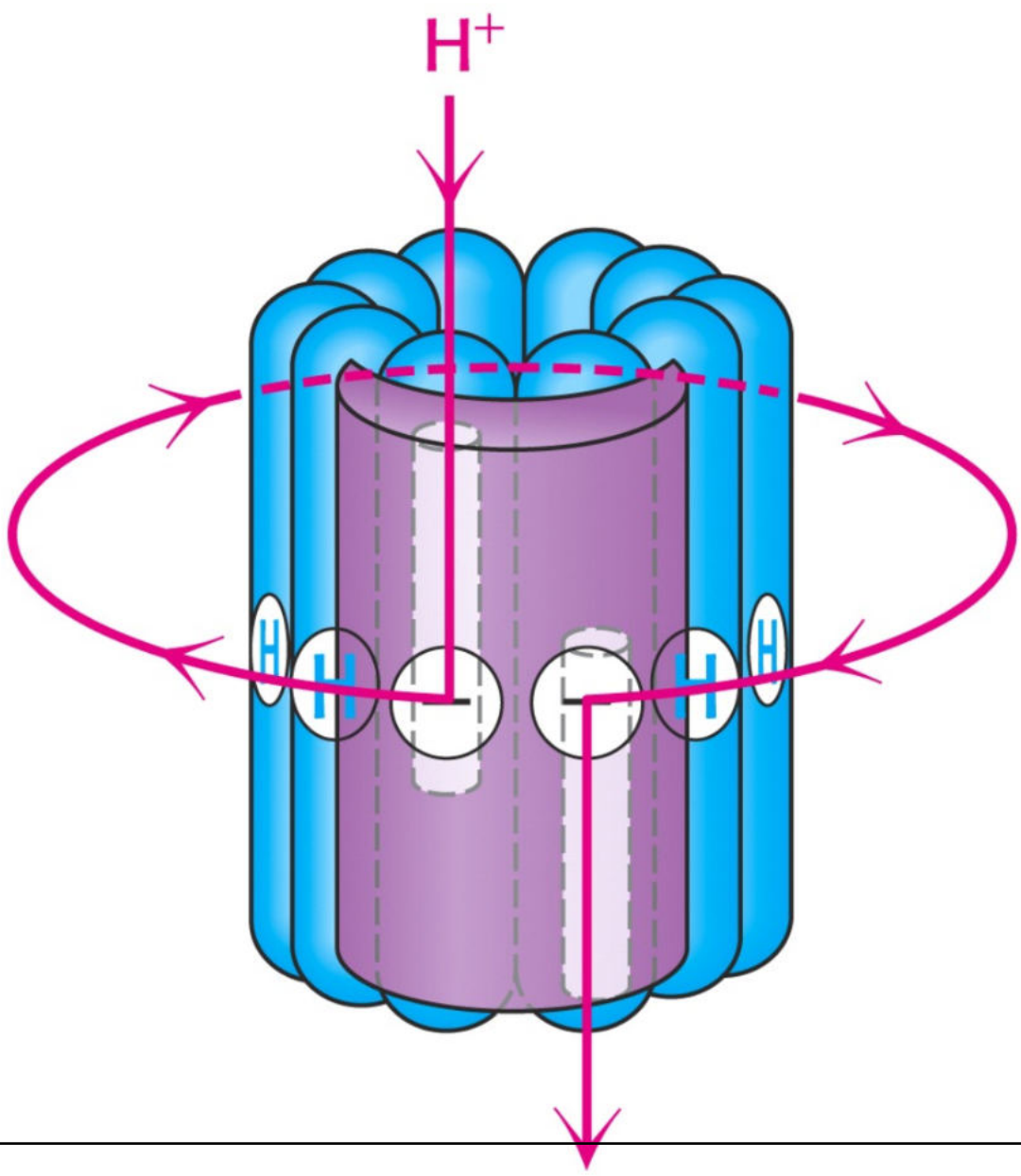


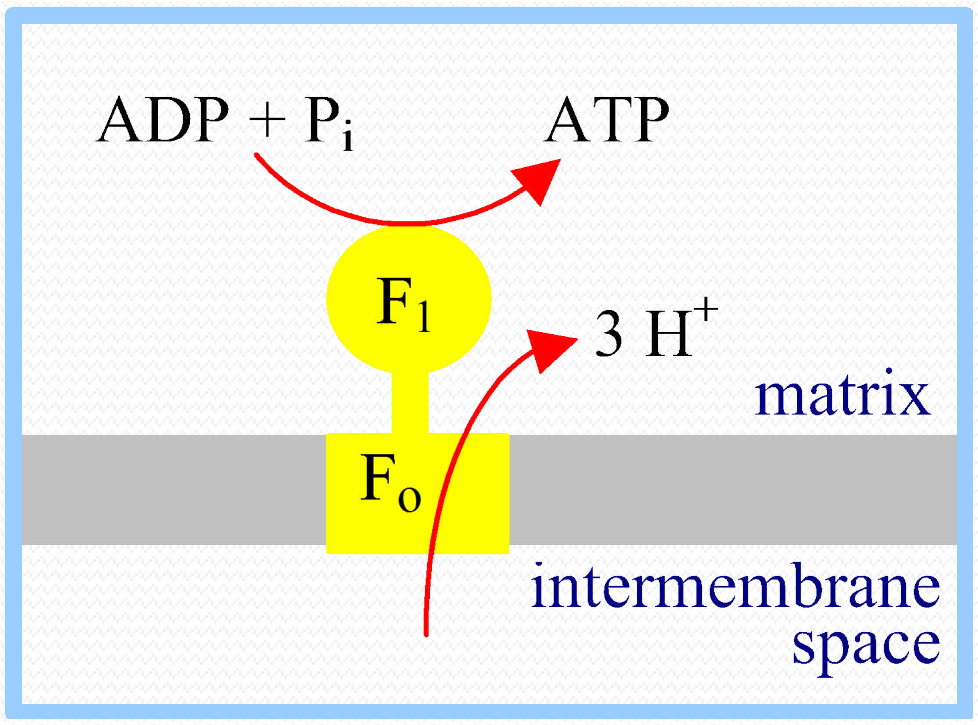
ATPase is a Rotating Motor



- Complex **I ,III and IV** act as a **Proton Pump.**
- Pump out the protons from matrix side to **inter membrane space** of mitochondria.
- Develop a **proton gradient** in inter membrane space.
- This supports the mechanism of Oxidative Phosphorylation.



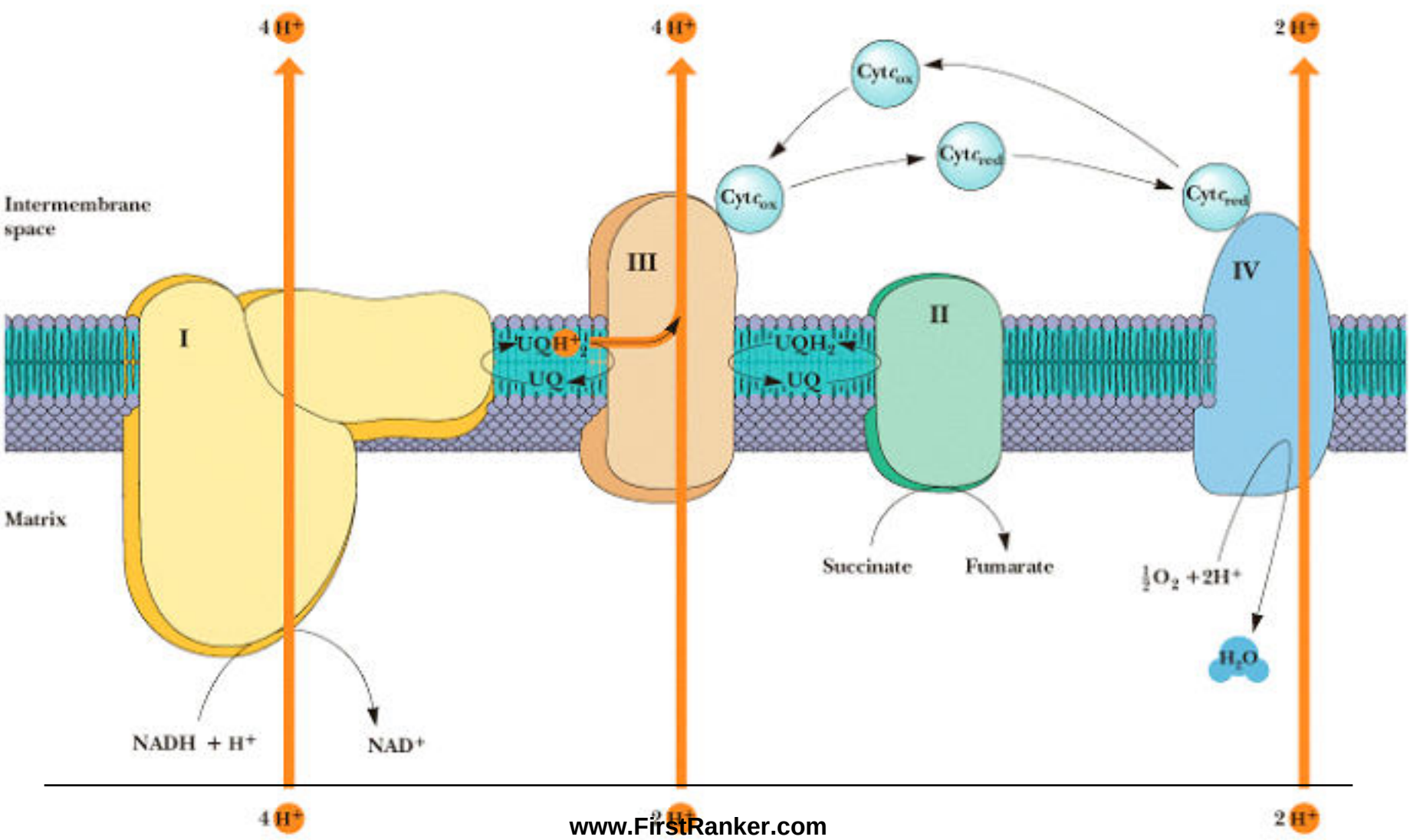




F_1F_o couples ATP synthesis to H^+ transport into the mitochondrial matrix.

Transport of at least **3 H^+** per ATP is required.

Electron Transport is coupled to Oxidative Phosphorylation

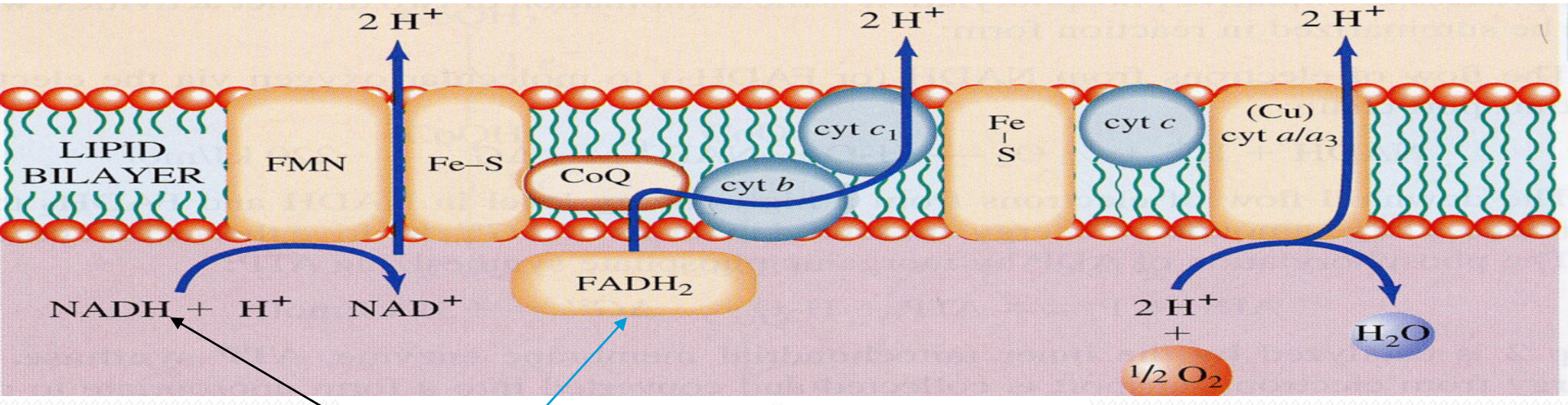


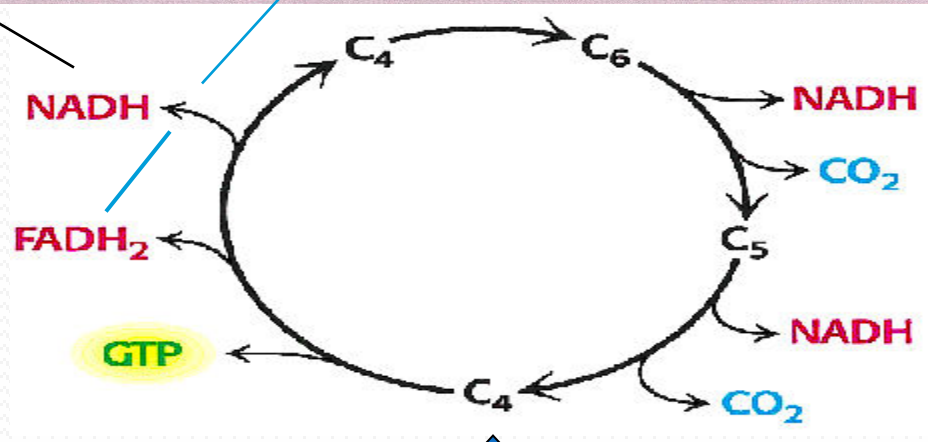
Salient Features Of ETC/ETS/Oxidative Phosphorylation

- **Reduced coenzymes NADH and $FADH_2$ are formed in matrix from**
  - Oxidative Decarboxylation of Pyruvate to Acetyl CoA by **PDH complex**.
  - Oxidation of acetyl CoA by the **citric acid cycle**
  - Beta Oxidation of fatty acids
  - Amino acids metabolism

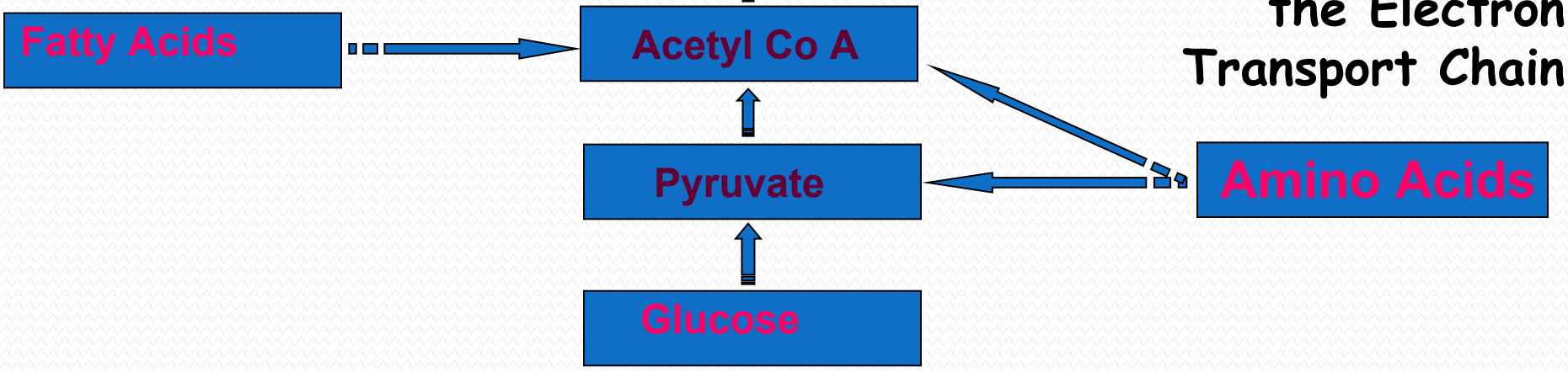
- Reduced coenzymes **$NADH+H^+/FADH_2$**
- **Generated** during **Anaerobic Dehydrogenase reactions** of Carbohydrates, Lipids metabolic pathways.
- **Get reoxidized on entering E.T.C**

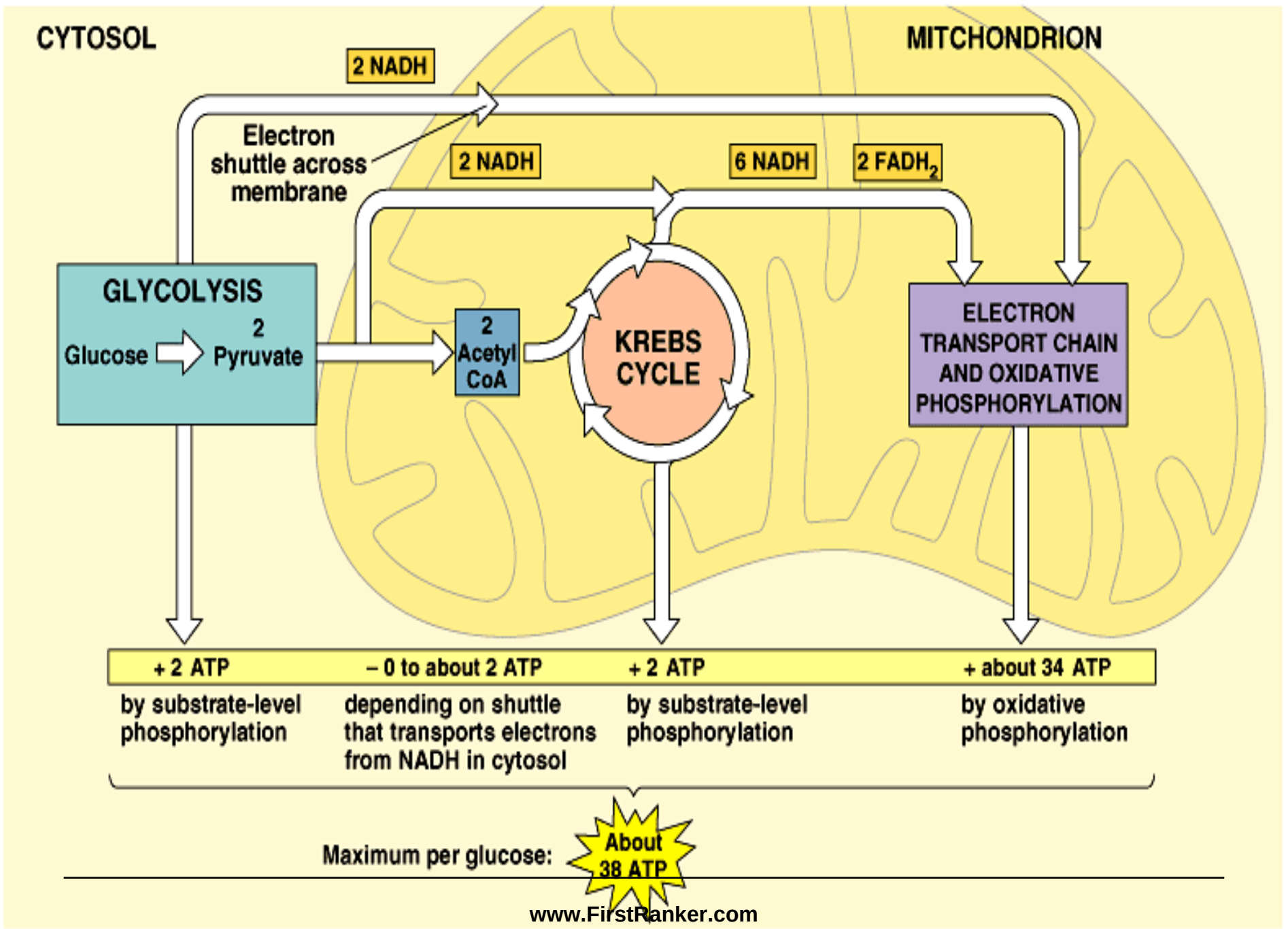
- The $NADH+H^+$ and $FADH_2$ are energy rich molecules
- Each contains a pair of electrons having a high transfer potential.

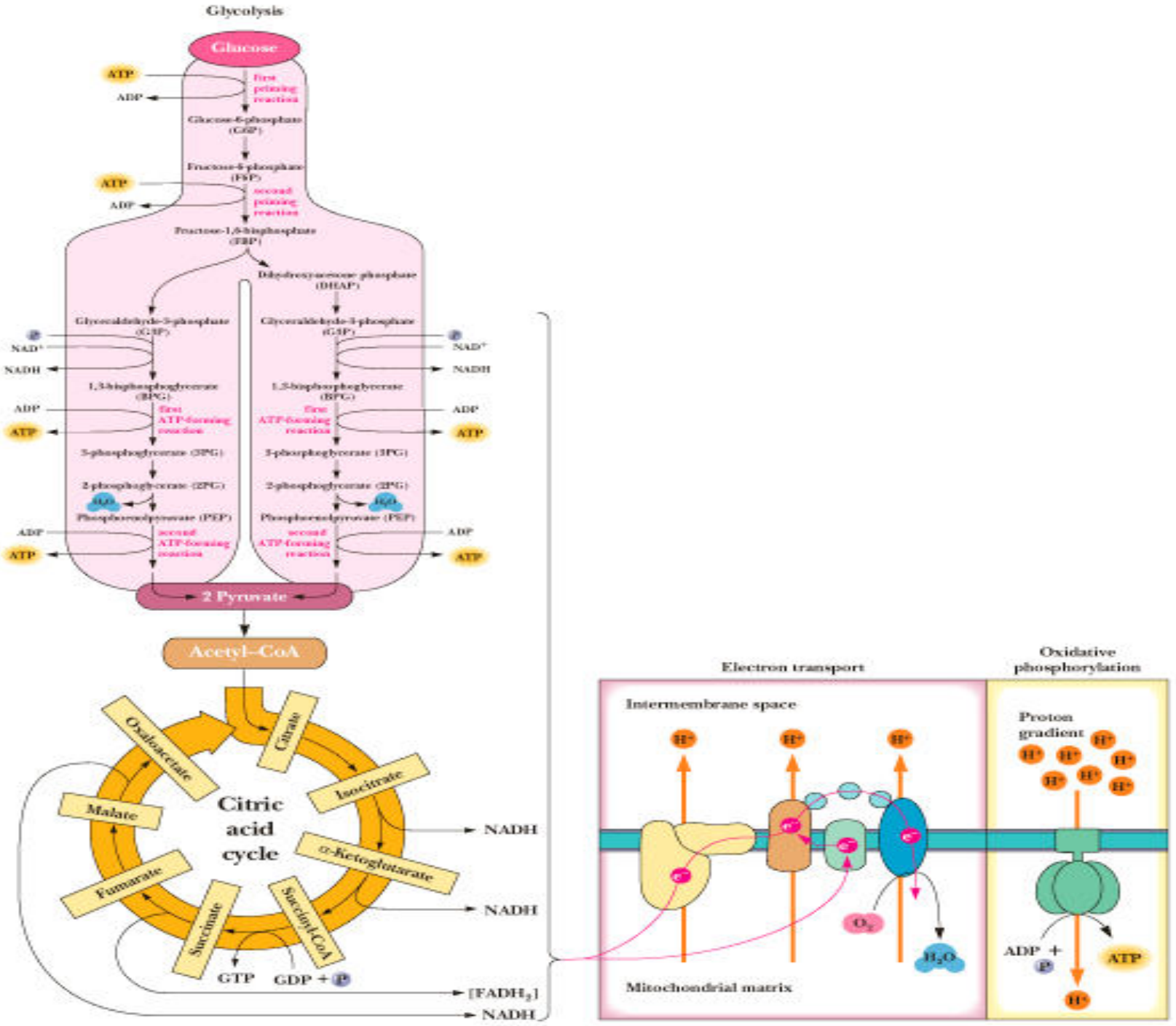


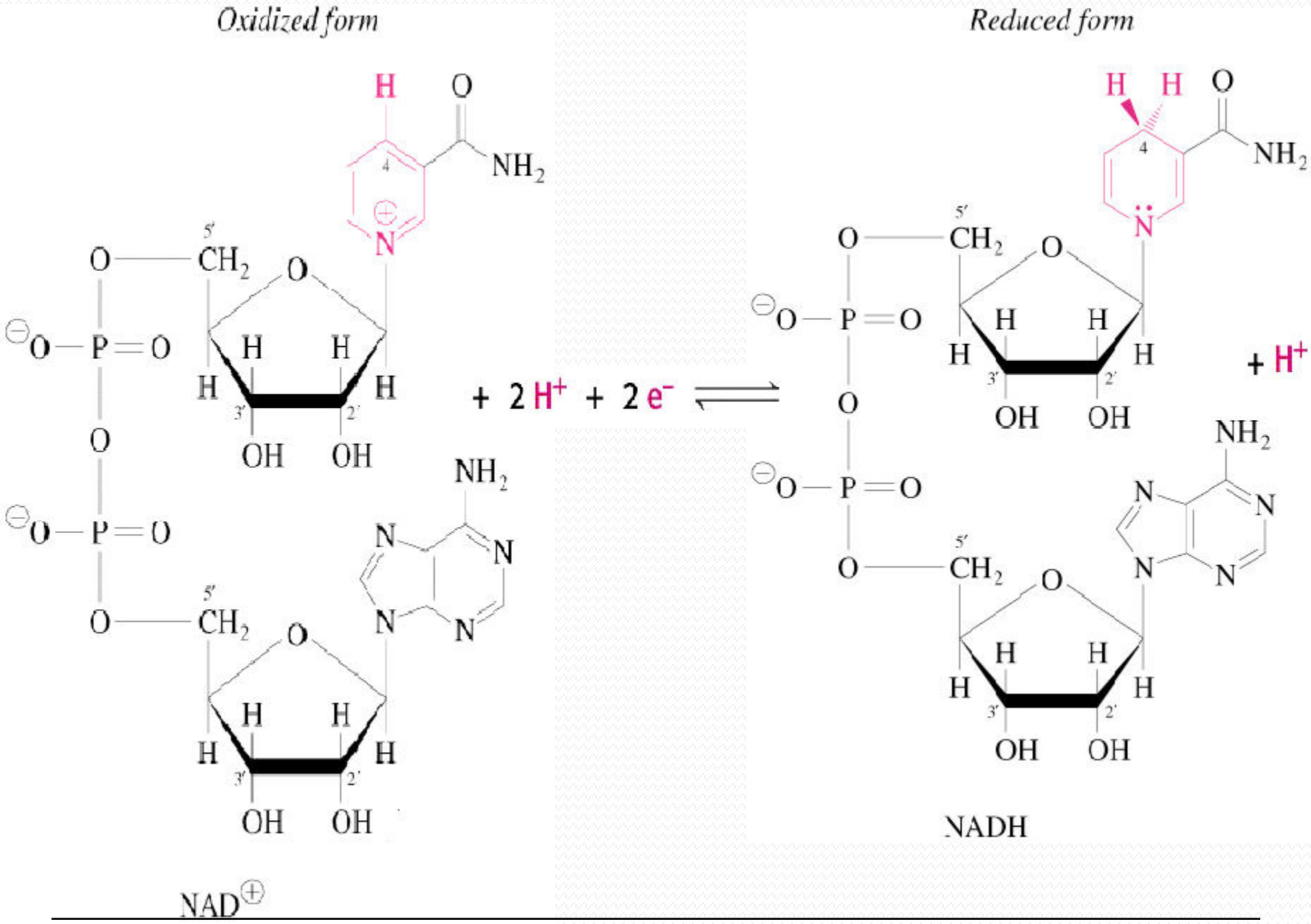


Glycolysis, Fatty acid oxidation TCA cycle supplies NADH and $FADH_2$ to the Electron Transport Chain

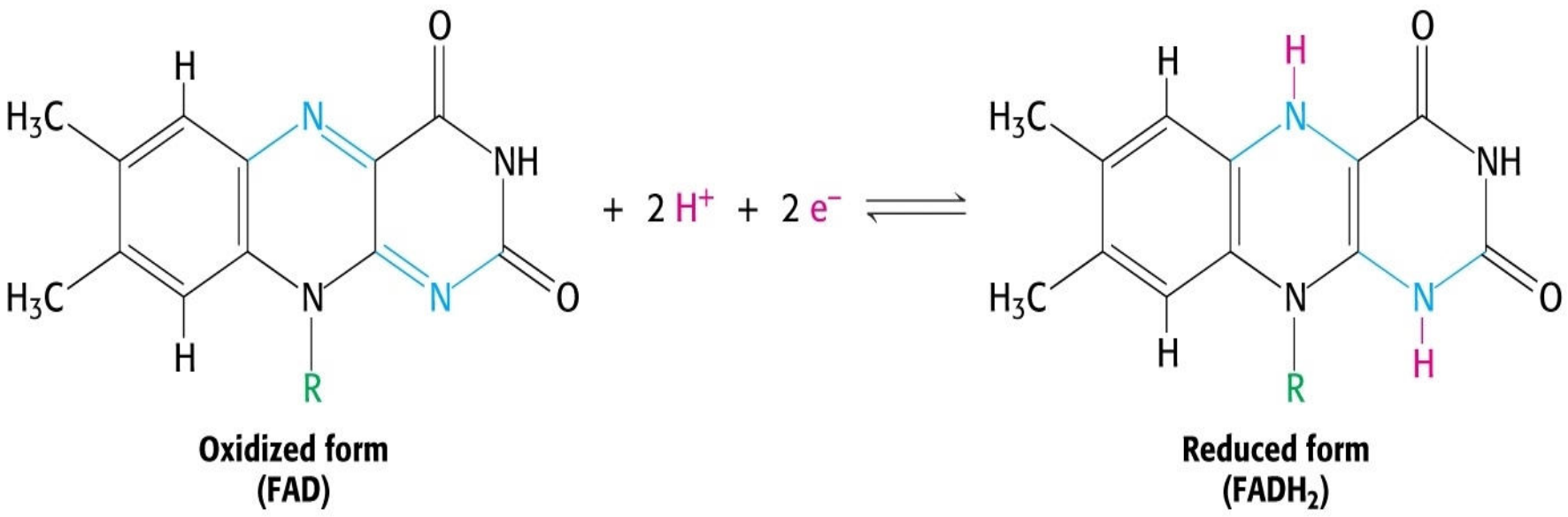








The reduced and oxidized forms of NAD



The reduced and oxidized forms of FAD

Redox Couples and Redox Potentials

- The components of ETC has capacity to exist in **oxidant and reductant forms.**
- This pair is known as **redox couple.**
  - CoQ/CoQH2
  - Cyt b Fe^{+++}/Cyt b Fe^{++}

- Redox Potential is a measure of the **tendency** of a **redox couple** to **accept or donate electrons** under standard condition.

- Components that have the **most negative redox potentials** have the **weakest affinity for electrons.**

- Redox couple with **most positive redox potentials** have
- The **strongest affinity for electrons** therefore
- Possess strongest tendency to **accept electrons.**

- In ETC electrons flow from **most electro negative potential** $NADH+H^+$ (-0.32) to most **electro positive potential** (+0.82) ½ O2 .

- In E.T.C **both Protons and Electrons** are **transferred up to Coenzyme Q** level.
- At coenzyme Q level protons ($2H^+$)are released in the medium.
- From **Coenzyme Q onwards only electrons are transferred** through a series of Cytochromes in E.T.C.

- Electrons get transfer through series of Cytochromes
- Cytochrome Fe is in transitional state (Ferric/Ferrous).

- In E.T.C there are **alternate reduction** and **oxidation** reactions.

- During E.T.C there is transfer of reducing equivalents from **low redox potential** to **high redox potential.**
- This **exhibit free energy change** there by **liberating heat energy.**

- Electrons move spontaneously from one component of ETC to another with a
- **low redox potential** (a low affinity for electrons) to a component with a
- **high redox potential** (a high affinity for electrons)

- In ETC electrons move from a carrier with
- **low redox potential** (high tendency to donate electrons) toward carriers with
- **higher redox potential** (high tendency to accept electrons).

- The proton gradient runs downhill to drive the synthesis of ATP

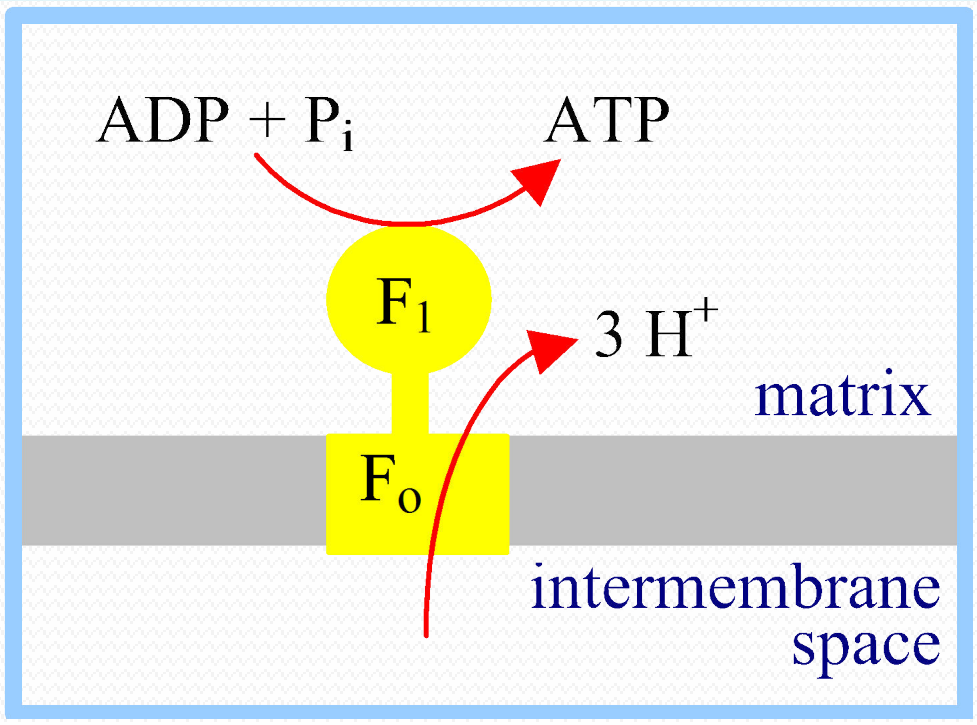
- **Oxygen** is the **terminal acceptor of electrons** in the electron transport chain.

- At the end of E.T.C by the catalytic activity of **Cytochrome Oxidase**
- **Protons** released at Coenzyme Q and **electrons** transported by Cytochromes are
- **Accepted** by activated molecular **oxygen (1/2 O2)** to form metabolic water.

- **Oxygen** has the **highest** (most positive) **standard redox potential .**
- Most likely to accept electrons from other carriers.

- Electrons ultimately reduce Oxygen to water (metabolic water)
  - $2\ H^+ + 2\ e^- + ½\ O_2 \rightarrow\ H_2O$

- Cytochrome oxidase controls the rate of O_2 uptake which
- Means this enzyme determines how rapidly we breathe.



F_1F_o couples ATP synthesis to H^+ transport into the mitochondrial matrix.

Transport of at least **3 H^+** per ATP is required.

- The respired Oxygen transported by Hb unloaded at tissue/ cellular level is utilized during E.T.C.

WHY ETC OPERATES ?

- During E.T.C operation the **total energy change is released in small increments**
- So that **energy can be trapped** as chemical bond energy to **form ATP.**

- When **two redox couples** of ETC **differ from each other** by **0.22 volts** in standard redox potential.
- The **free energy in the form of heat** released is more than 7.3 Kcal.
- This **free heat energy** is **conserved** to **chemical form of energy-ATP**.

- The sites in E.T.C at which energy liberated is **less than 7.3 Kcal** is simply **dissipiated** in the **form of heat.**

- Certain sites in E.T.C where the heat energy liberated **more than 7.3 Kcal** is **utilized for phosphorylation reaction** of ADP with pi to **form ATP.**

- Thus heat energy is transformed to chemical form of energy (ATP) in E.T.C.

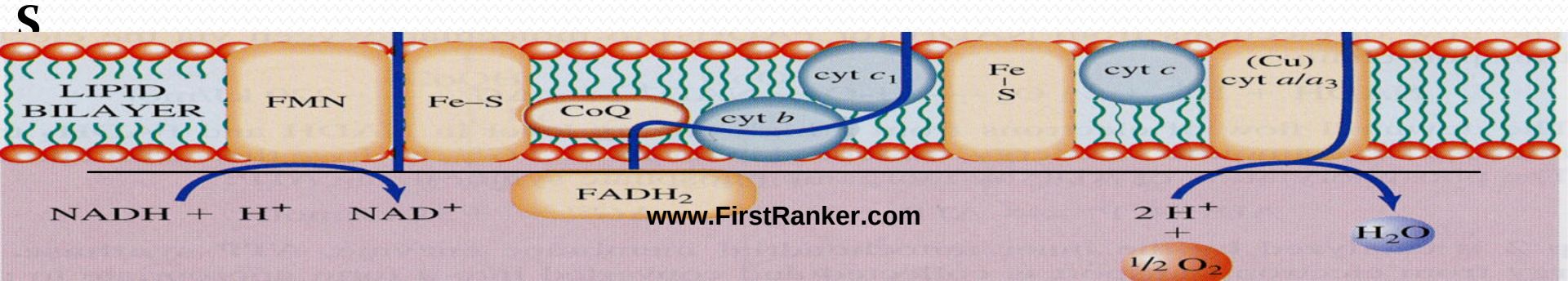
THE ELECTRON TRANSPORT CHAIN

Series of enzyme complexes (electron carriers) embedded in the inner mitochondrial membrane, which oxidize NADH+H$^+$ and $FADH_2$ and transport electrons to oxygen is called Respiratory Electron-Transport Chain (ETC).

The sequence of electron carriers in ETC

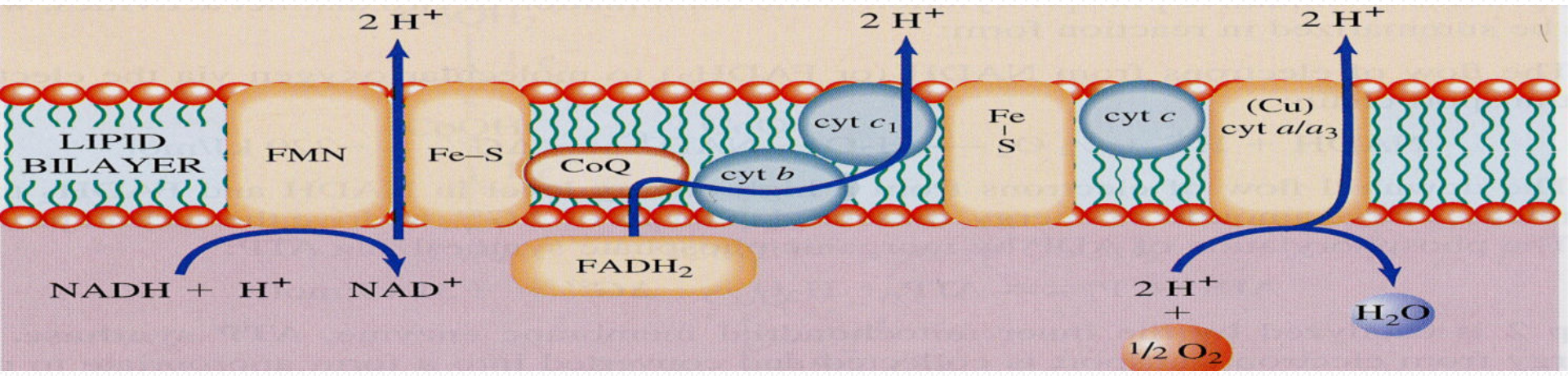
NADH → FMN-Fe-S → Co-Q Fe-S cyt b → cyt c_1 → cyt c → cyt a → cyt a_3 → O

Succinate → FAD → Fe-S



Electrons of NADH or $FADH_2$ are used to **reduce molecular oxygen to water.**

A large amount of free energy is liberated.



The electrons from NADH+H⁺ and $FADH_2$ **are not transported directly to O_2** but **are transferred through series of electron carriers** that undergo reversible reduction and oxidation.

A PROTON GRADIENT POWERS THE SYNTHESIS OF ATP

The transport of electrons from NADH or $FADH_2$ to O_2 via the electron-transport chain is exergonic process:

$$NADH + \tfrac{1}{2}O_2 + H^+ \Leftrightarrow H_2O + NAD^+$$

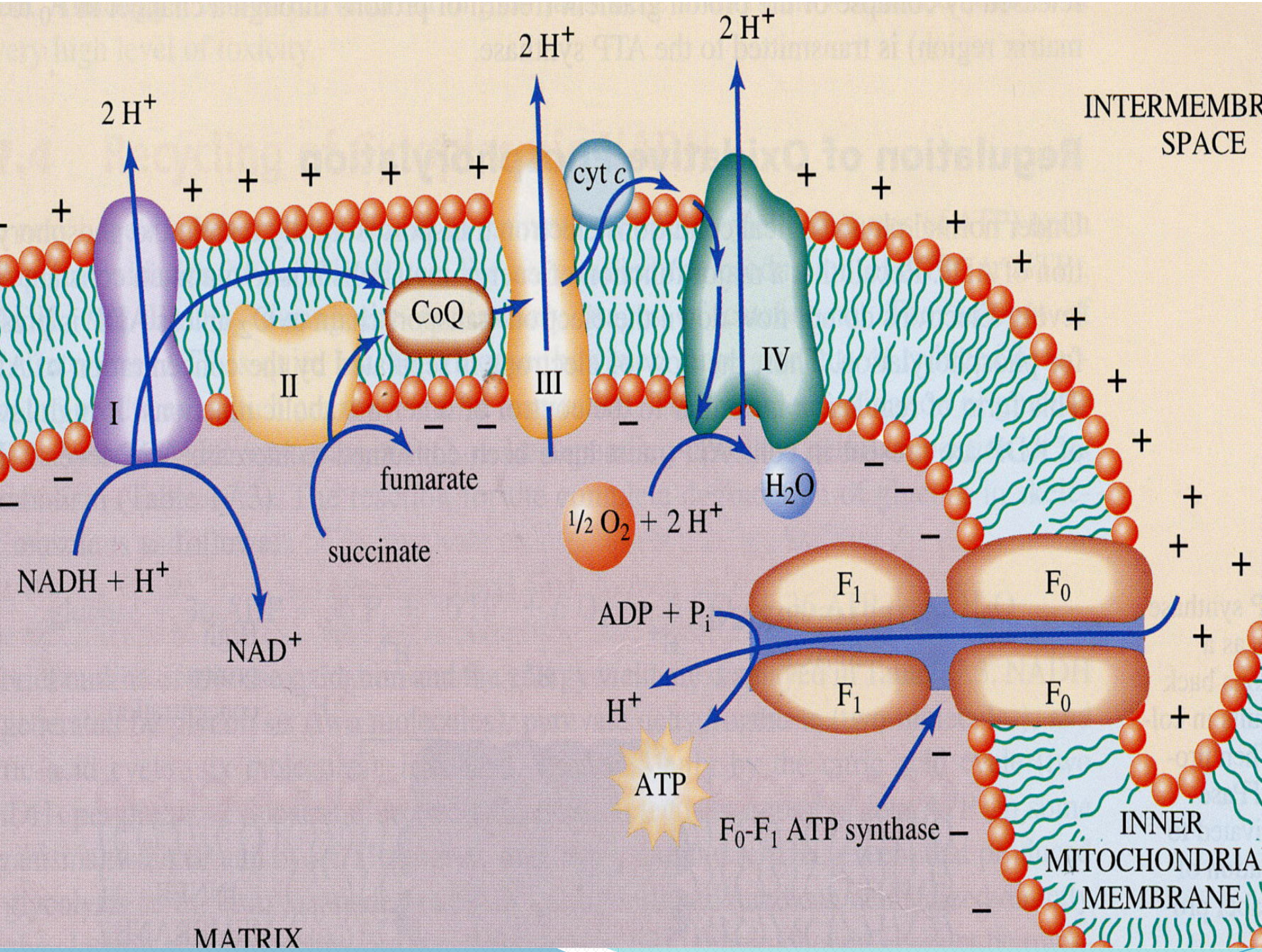
$$FADH_2 + \tfrac{1}{2}O_2 \Leftrightarrow H_2O + FAD^+$$

$\Delta G^{o\prime}$ = -52.6 kcal/mol for NADH

-36.3 kcal/mol for $FADH_2$

This process is coupled to the synthesis of ATP (endergonic process)

$$ADP + P_i \Leftrightarrow ATP + H_2O \qquad \Delta G^{o\prime} = +7.3 \text{ kcal/mol}$$



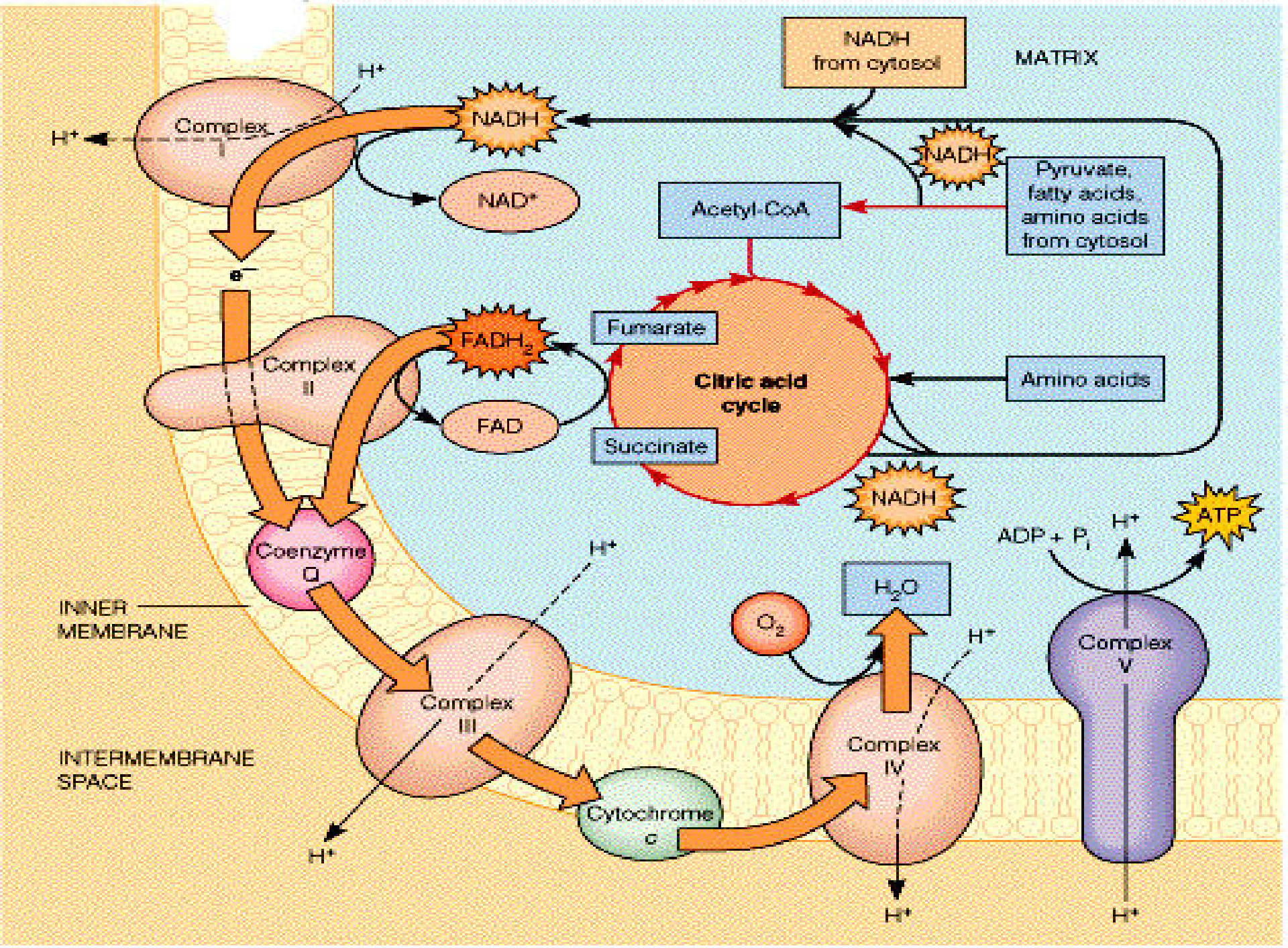
- The flow of electrons through **ETC complexes leads to the pumping** of **protons** out of the mitochondrial matrix in **intermembrane space**.
- The resulting distribution of protons generates a **pH/Proton gradient** and a transmembrane electrical potential that creates a **proton motive force.**

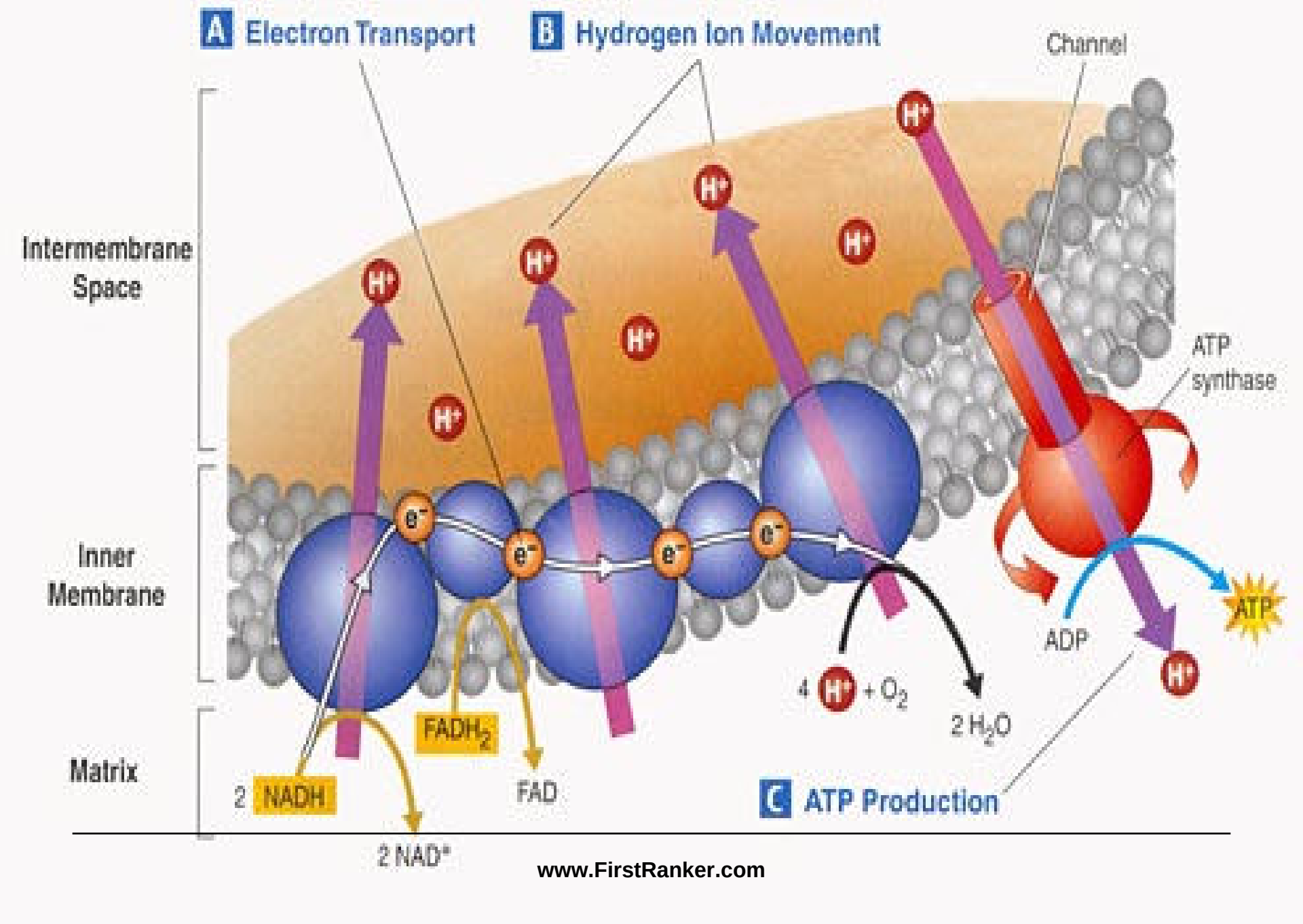
- **A Large Drop in Redox Potential across each of the three Respiratory Enzyme Complexes (I,III,IV).**
- Provides the **Energy for H^+ Pumping**

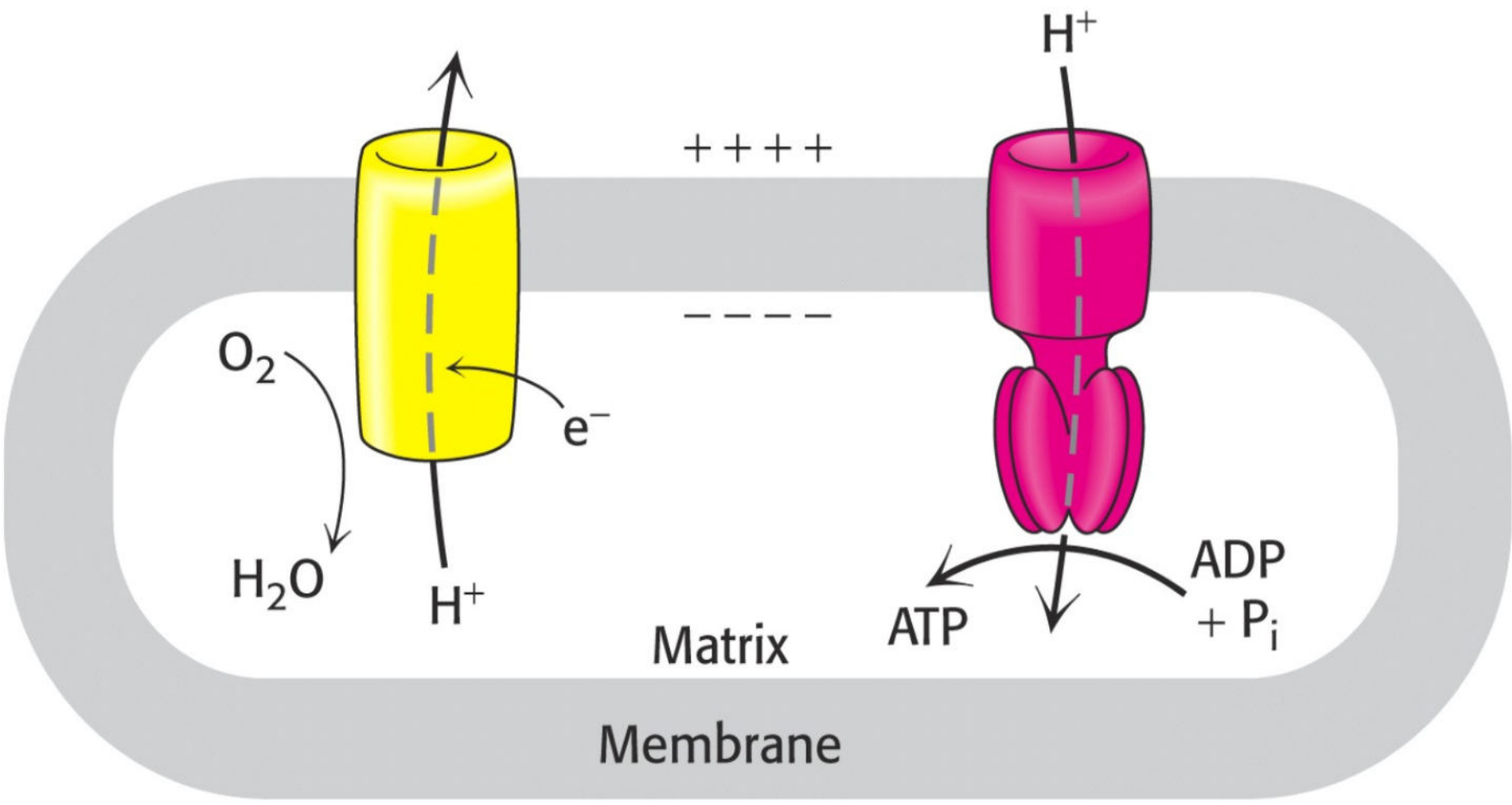
- **ATP is synthesized** when **protons flow back from intermembrane space of mitochondria to the mitochondrial matrix** through an **enzyme complex ATP synthase.**

- The oxidation of fuels and the phosphorylation of ADP are coupled by a proton gradient across the inner mitochondrial membrane.

- Oxidative phosphorylation is the process in which **ATP is formed**
- As a result of the **transfer of electrons from NADH or $FADH_2$ to O_2** by a **series of electron carriers.**







E.T.C is a Mode For Free Radical Generation

- During E.T.C operation there occurs leakage of small amounts of electrons
- Which are transferred directly to oxygen to form super oxide ion (Free radicals).

What is a Free Radical ?

- Any chemical species with one of more unpaired electrons.
- Unstable/Highly Reactive
- Powerful Oxidant
- Short half life (nanoseconds)
- Can exist freely in the environment

Significance Of ETC

- **Reduced coenzymes gets reoxidized** to NAD^+ /FAD in ETC for its reutilization in metabolic oxidation reactions.
- Reduced coenzymes NADH+ H^+/$FADH_2$ give its reducing equivalents to E.T.C components and get reoxidized.
- **E.T.C generates chemical form of energy ATP** as a valuable by product.

P/O Ratio

- The ratio of ATPs formed per oxygen reduced

OR

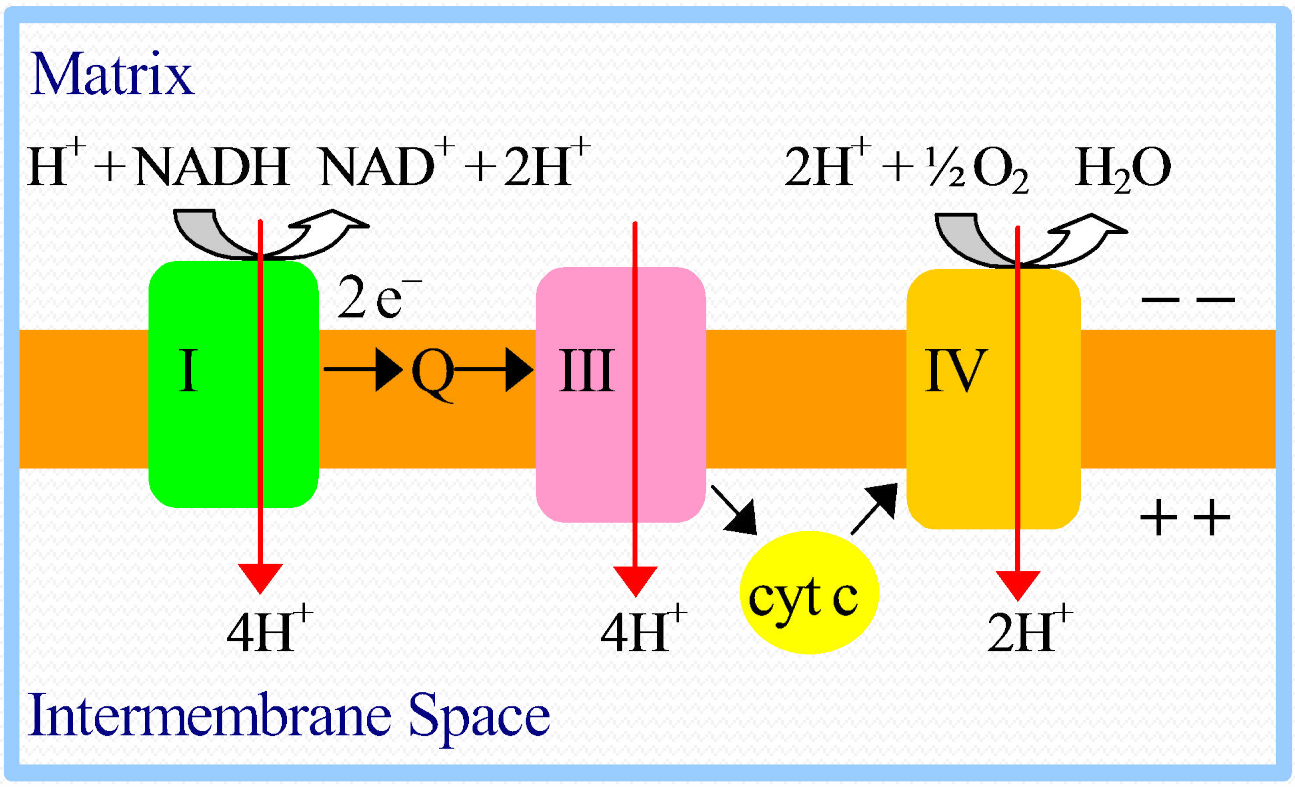
- Number of ATPs generated per Oxygen atom used in ETC process.

- To make 1 ATP need 30 kJ/mole.
- There needs more than one proton to translocate during ETC process to generate 1 ATP.

- **Ten protons** are pumped out of the matrix during the two electrons flowing **from NADH+H$^+$ to O2 (Complex I, III and IV).**
- **Six protons** are pumped out of the matrix during the two electrons flowing from **FADH2 to O2 (Complex III and IV).**

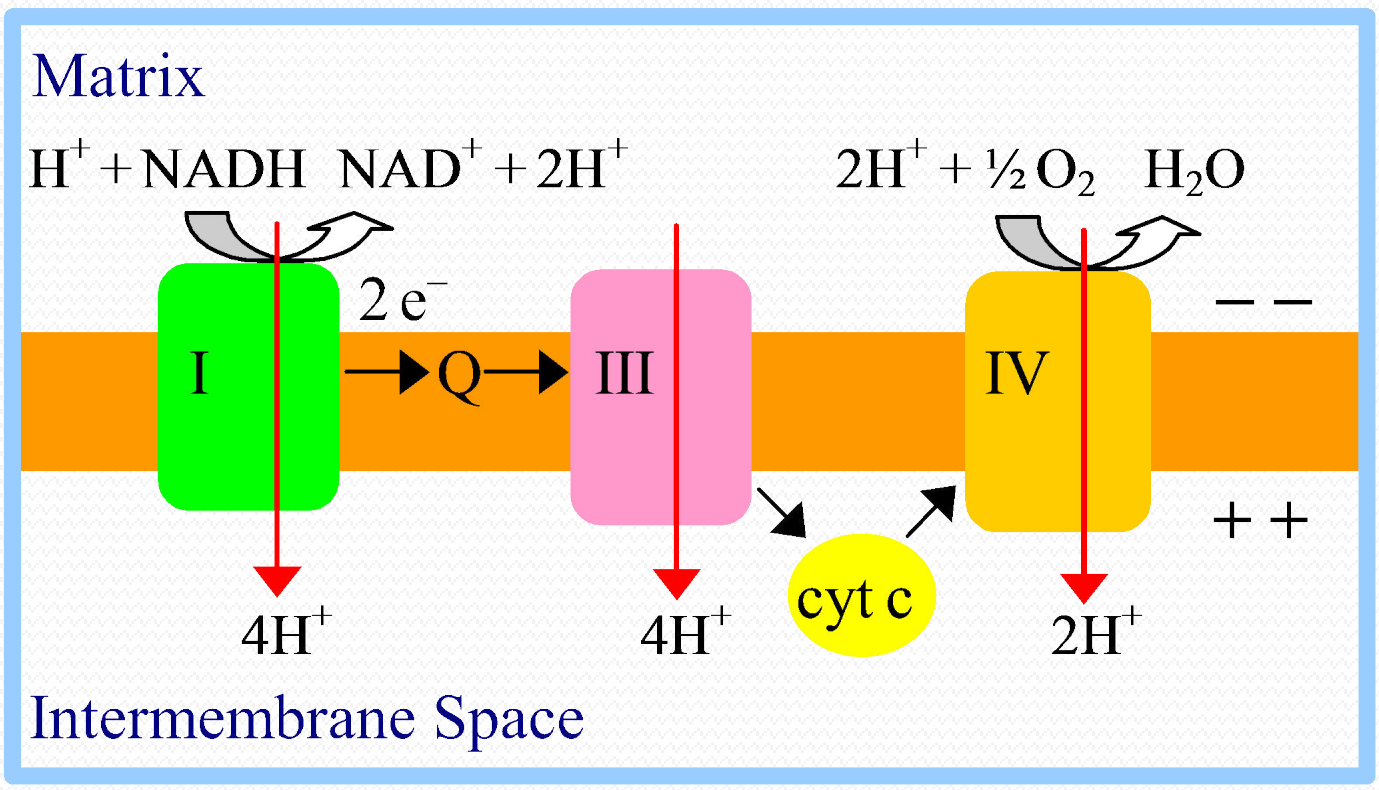
Spontaneous **electron flow** through each of complexes I, III, & IV

is coupled to **H^+ ejection** from the matrix.

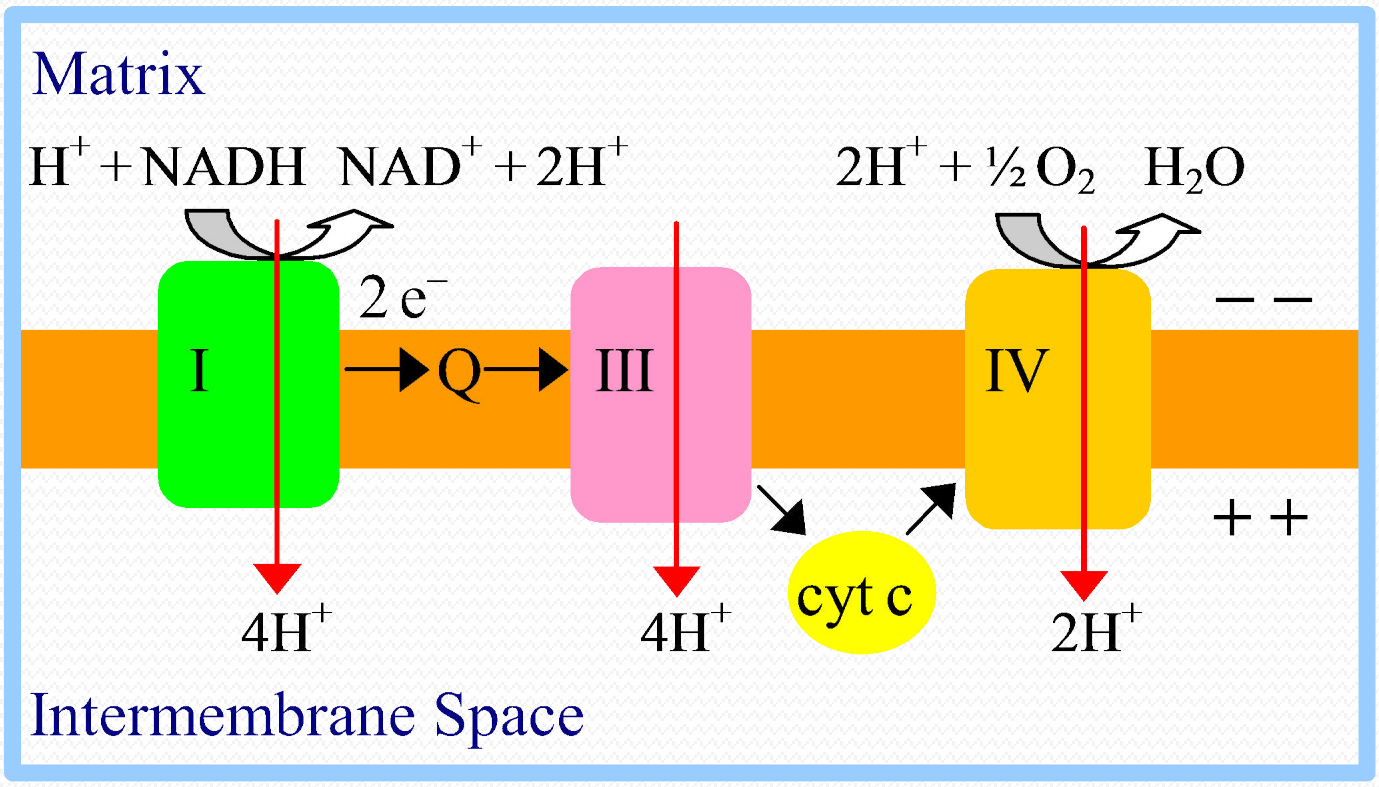


A total of **10 H^+** are ejected from the mitochondrial matrix **per 2 e^-** transferred **from NADH** to oxygen via the respiratory chain.

A total of **6 H^+** are ejected from the mitochondrial matrix **per 2 e^-** transferred **from FADH2** to oxygen via the respiratory chain.

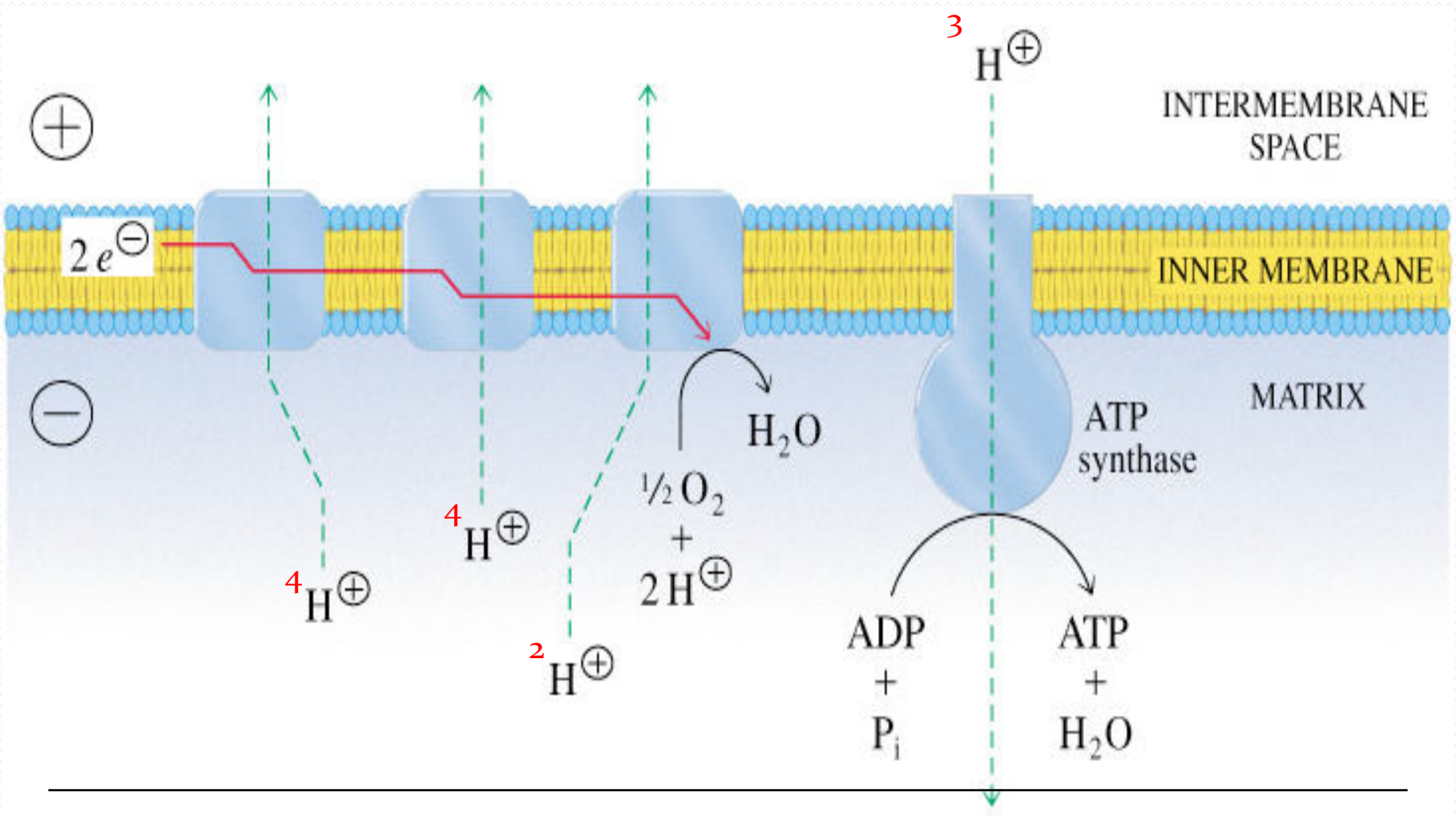


Complex I and **Complex III** transports **$4H^+$** out of the mitochondrial matrix per **$2e^-$** transferred from NADH.



Thus there are **2H⁺** per 2e⁻ that are effectively transported by **complex IV.**

ATP Yield



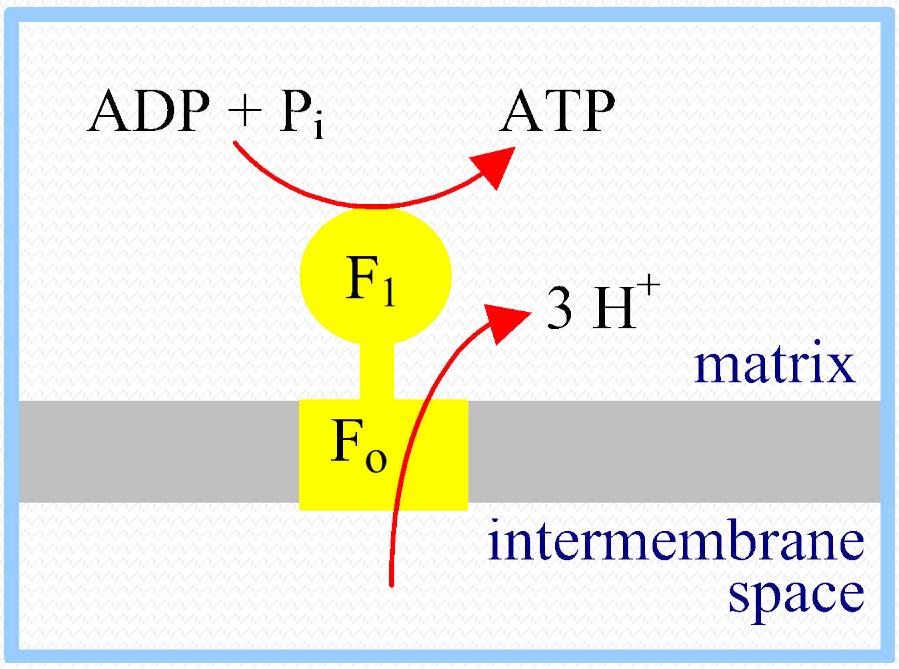
- **ETC** translocates **10 protons** per **NADH+H$^+$**
- **ETC** translocates **6 protons** per **FADH2**

- Proton gradient created as electrons transferred to oxygen forming water

  10 H^+ / $NADH+H^+$

  6 H^+ / $FADH_2$

- Translocation of $3H^+$ required by ATP Synthase for each ATP produced
- 1 H^+ needed for transport of Pi.
- **Net: 4 H^+ transported for each ATP synthesized through ATP Synthase.**



F_1F_o couples ATP synthesis to H^+ transport into the mitochondrial matrix.

Transport of least **3 H^+** per ATP is required.

P:O Ratio for $NADH+H^+$

$$10\ H^+ \times \frac{1\ ATP}{4\ H^+} = 2.5\ ATP$$

P.O Ratio for $FADH_2$

$$6\ H^+ \times \frac{1\ ATP}{4\ H^+} = 1.5\ ATP$$

- **P:O ratio for NADH: 10 H^+/4H^+ = 2.5 ATP**
- **P:O ratio for FADH2: 6 H^+/ 4H^+ = 1.5 ATP**

ATP Is A Valuable Byproduct Of E.T.C/ Oxidative Phosphorylation.

- ATP is a high energy phosphate compound
- Biologically important free nucleotide
- ATP has 2 high energy Phospho anhydride bonds.

- ATP is energy currency of cell.
- Predominantly generated through Oxidative Phosphorylation.

Sites Of ATP Production In ETC

- **3 sites Of ATP Generation in ETC**

- **Site I/Complex I-**

Electrons transferred from Complex I to CoQ

- **Site II/Complex III-**

Electrons transferred from Cyt b to Cyt c1.

- **Site III/Complex IV-**

Electrons transferred from Cytochrome aa3/Complex IV/Cytochrome Oxidase to ½ O2

- **Uses of ATP generated in Oxidative Phosphorylation**

- **Synthetic/Anabolic reactions**
- **Active transport mechanism.**
- **Muscular contraction**
- **Nerve impulse conduction.**

- ATP allows the coupling of thermodynamically unfavorable reactions to favorable reactions.

- ATP is continually being hydrolyzed and regenerated
- A person at rest consumes and regenerate 3 ATP/ sec

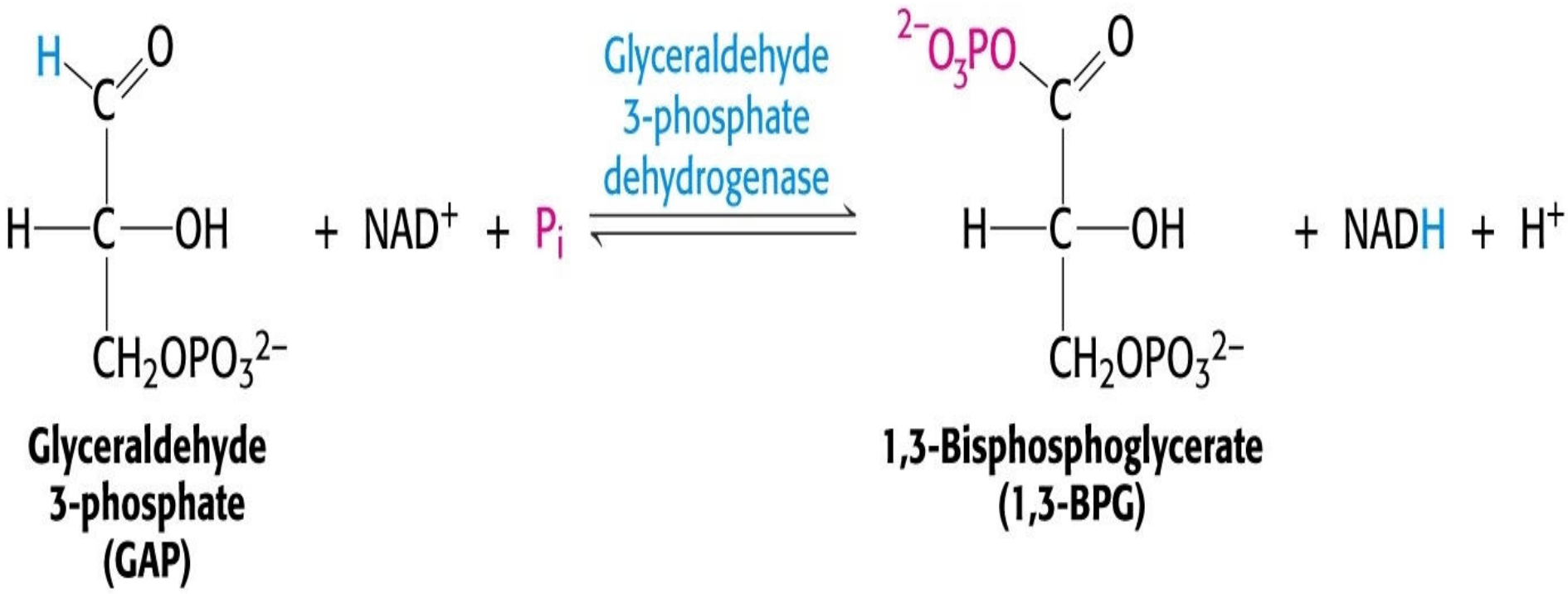
Staying Alive Energy Wise

- We need 2000 Cal/day or 8,360 kJ of energy per day
- Each ATP gives 30.5 kJ/mole of energy on hydrolysis
- **We need 246 moles of ATP**
- Body has less than 0.1 moles of ATP at any one time
- We need to make 245.9 moles of ATP
- Each mole of Glucose yields 38 ATPs or 1160 kJ
- We need **7.2 moles of Glucose** (1.3 kg or 2.86 pounds)
- Each mole of Stearic acid yields 147 ATPs or 4,484 kJ
- We need **1.86 moles of stearic acid** (0.48 kg or 1.0 pound of fat)

Shuttling Electron Carriers from Cytosol into the Mitochondrion

- NADH+H$^+$ carrier of reducing equivalents generated in the cytosol via Glycolysis should make its entry into mitochondrial ETC.
- Since the inner mitochondrial membrane is **impermeable to NAD$^+$ and NADH**.
- This reducing equivalents **must be shuttled** into the mitochondrial matrix before they can enter the ETC.

NADH+H$^+$ is generated in the cytosol in Glycolysis

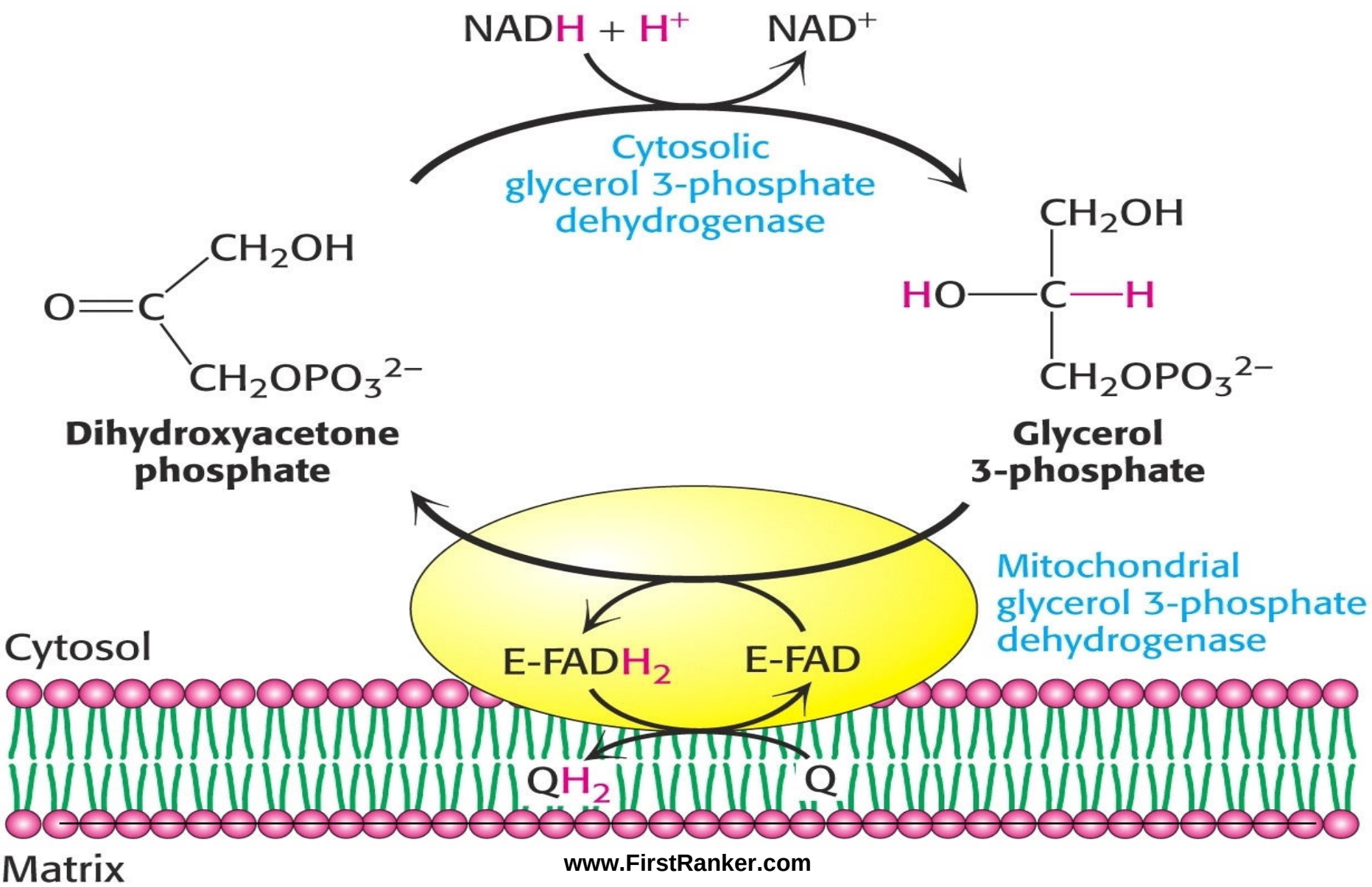


Reducing Equivalents from Cytosolic $NADH+H^+$ Enter Mitochondria via Shuttle Systems

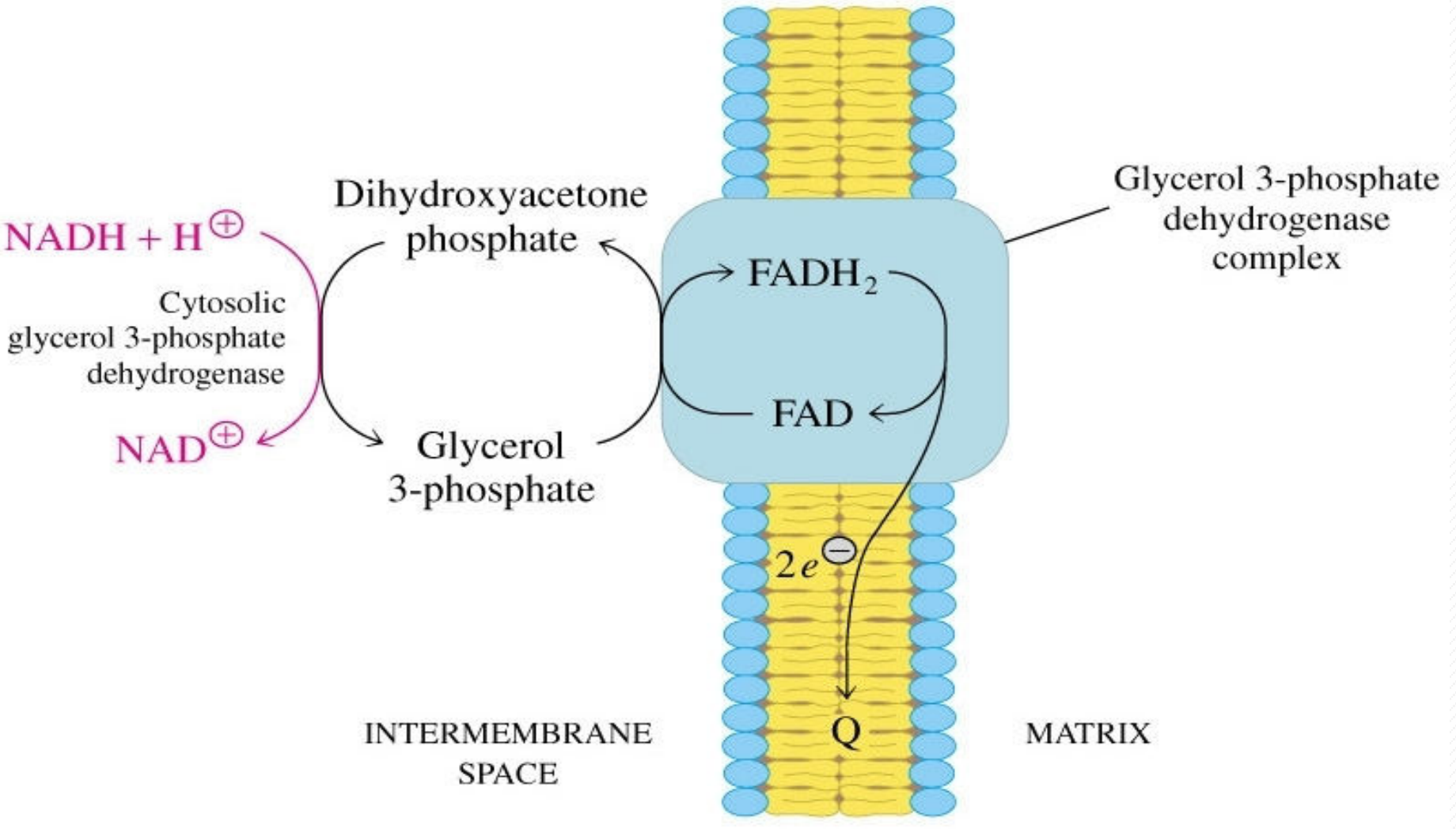
- NADH itself is not carried across the mitochondrial membrane.
- Protons and Electrons of Cytosolic $NADH+H^+$ are carried through shuttle systems.

- **Two shuttles Involved:**
- Glycerol 3-phosphate Shuttle
- Malate-Aspartate Shuttle

Glycerol-3-Phosphate Shuttle

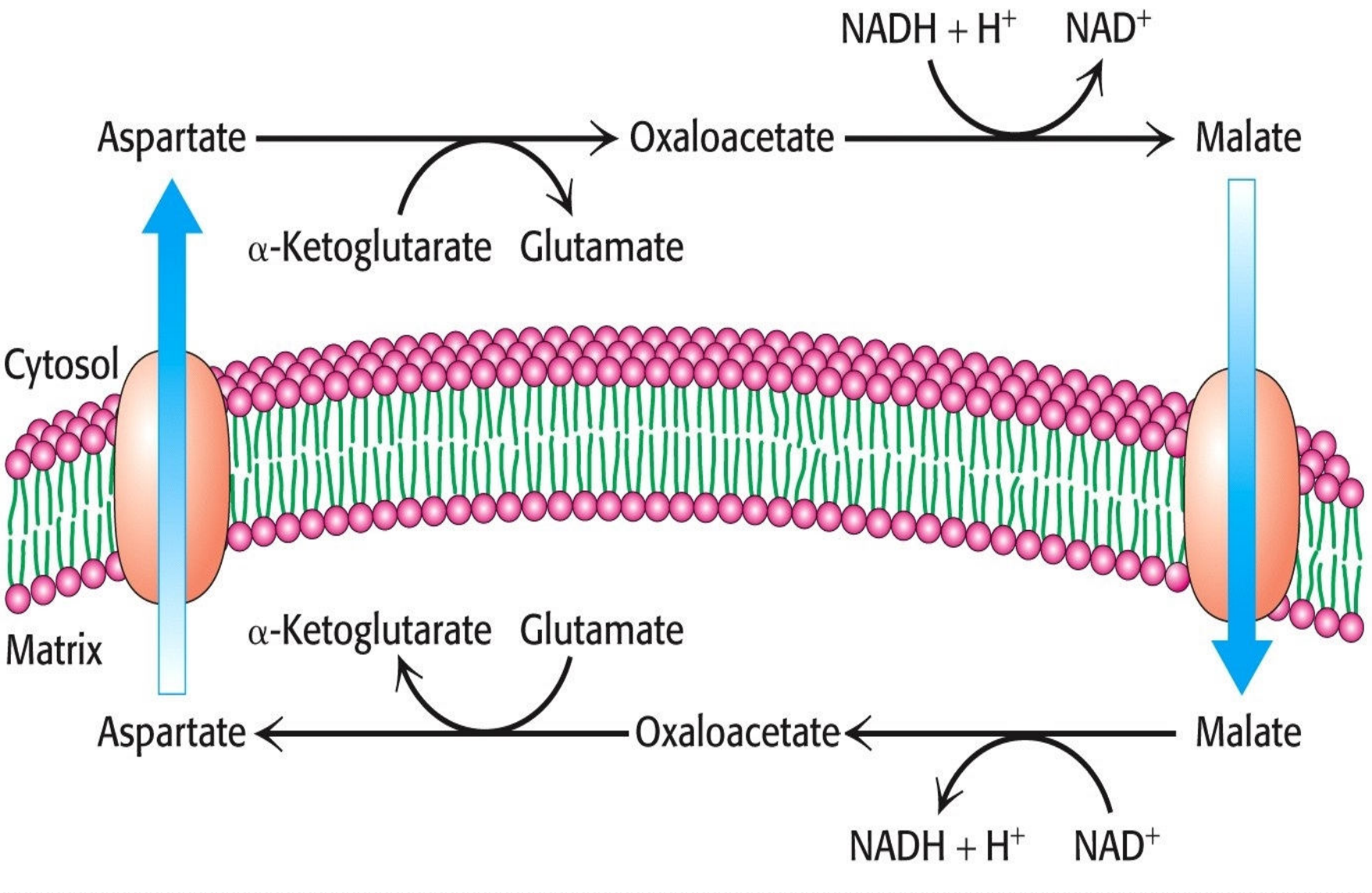


Glycerol Phosphate Shuttle

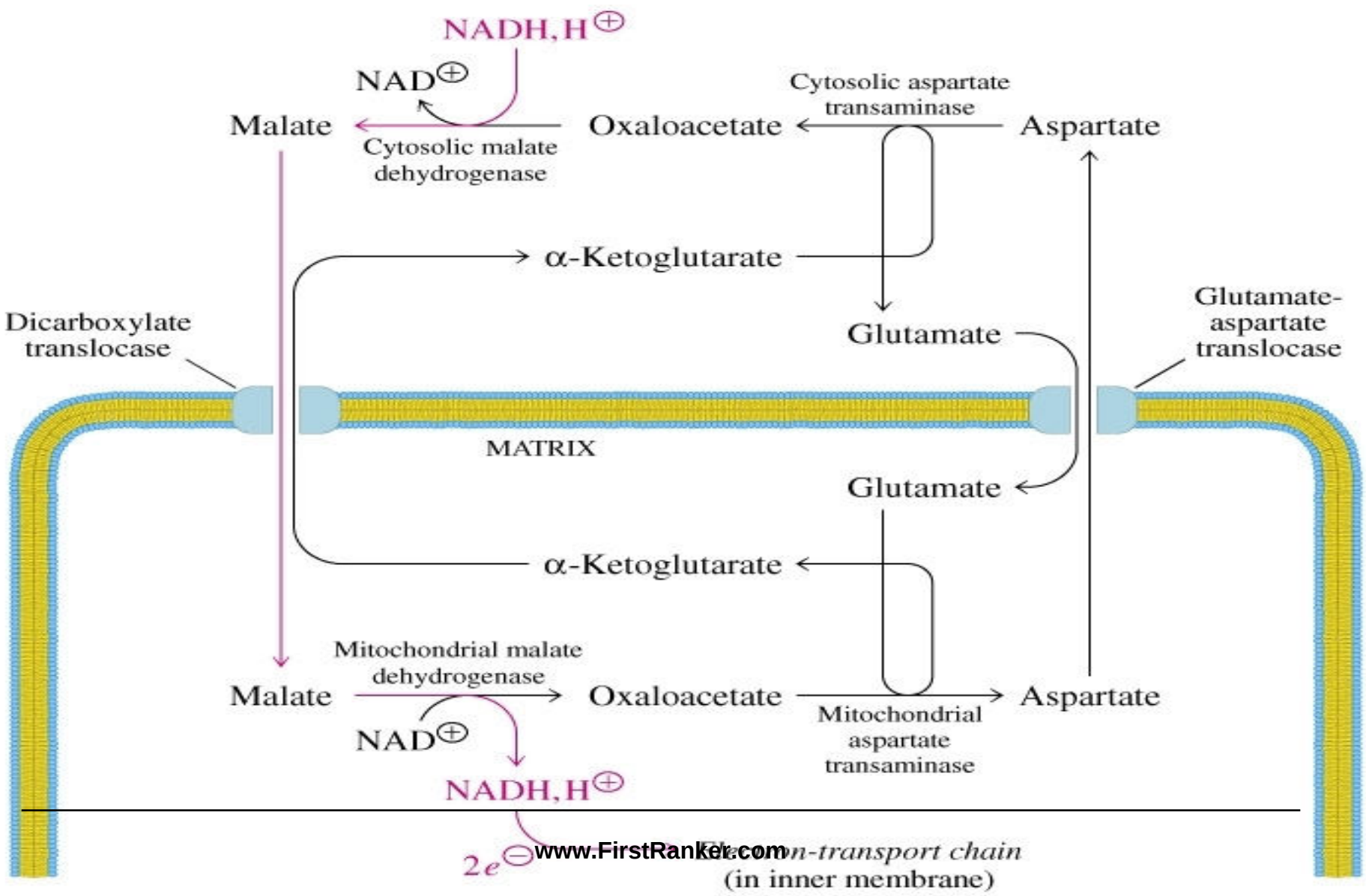


- Glycerol Phosphate Shuttle is active in **Skeletal muscles** and **Brain**.
- FADH2 formed enter the electron-transport chain through CoQ.
- Therefore only 1.5 molecules of ATP are produced.

Malate-Aspartate Shuttle



Malate/Aspartate Shuttle System



- **Malate Aspartate Shuttle**

- Active in Heart and Liver.
- 2.5 molecules of ATP are produced.

Summary

- **Total ATP Generated / 1 Glucose Oxidation**

- **Muscle and Brain** **30.0 ATP**
  - **Uses Glycerol phosphate Shuttle**
- **Heart and Liver** **32.0 ATP**
  - **Uses Malate Aspartate Shuttle**

E.T.C Inhibitors

- Chemical substances **having affinity for ETC components.**

- Chemically **interact with ETC complexes** and **functionally inactivate them.**

- **ETC Inhibitors**

- **Enemies of ETC components who stop its normal** operation.

- **No ETC** and No **ATP generation.**

- **Site I/Complex I- E.T.C Inhibitors.**
  - **Amobarbital /Amytal**
  - **Rotenone (Fish/Rat Poison)**
  - **Mercurials**
  - **Piercidin -A**

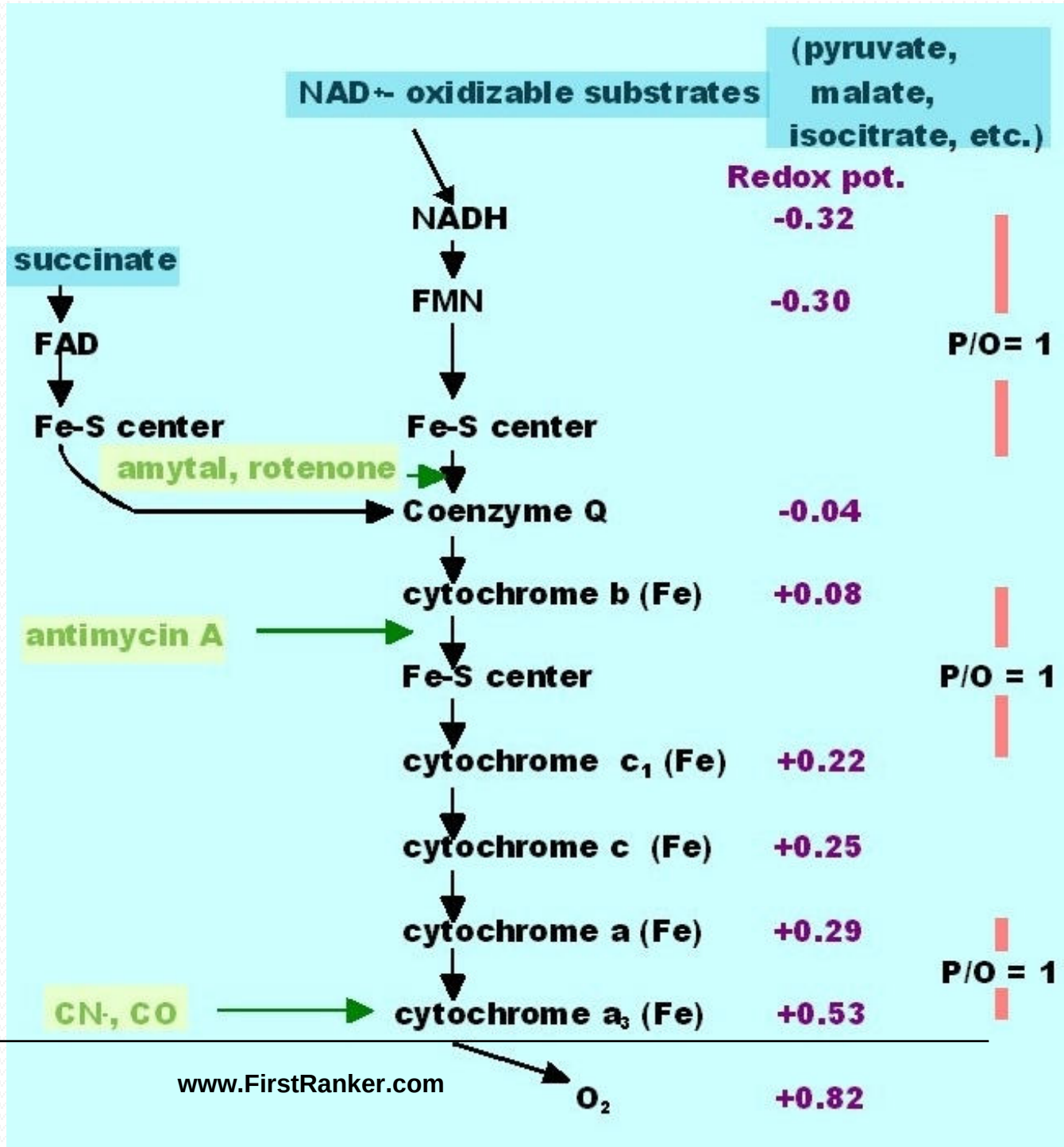
- **Site II/Complex III- E.T.C Inhibitors.**
  - **British Anti Lewisite (BAL)**
  - **Antimycin –A**
  - **Dimercaprol**

- **Site III/Complex IV- E.T.C Inhibitors/Cytochrome Oxidase Inhibitors.**
  - **Cyanide**
  - **Carbon Monoxide**
  - **H_2S**
  - **Azide**

- These chemicals arrest respiration by inhibition of the ETC.

Sequence of Respiratory Electron Carriers

Inhibitors in green



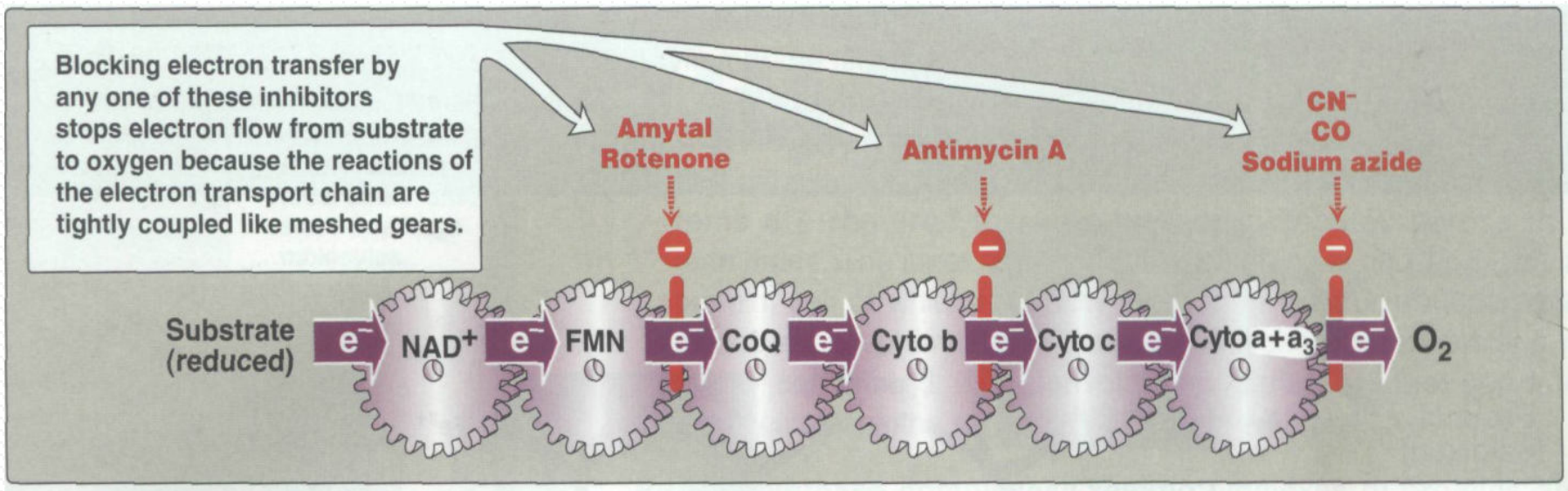
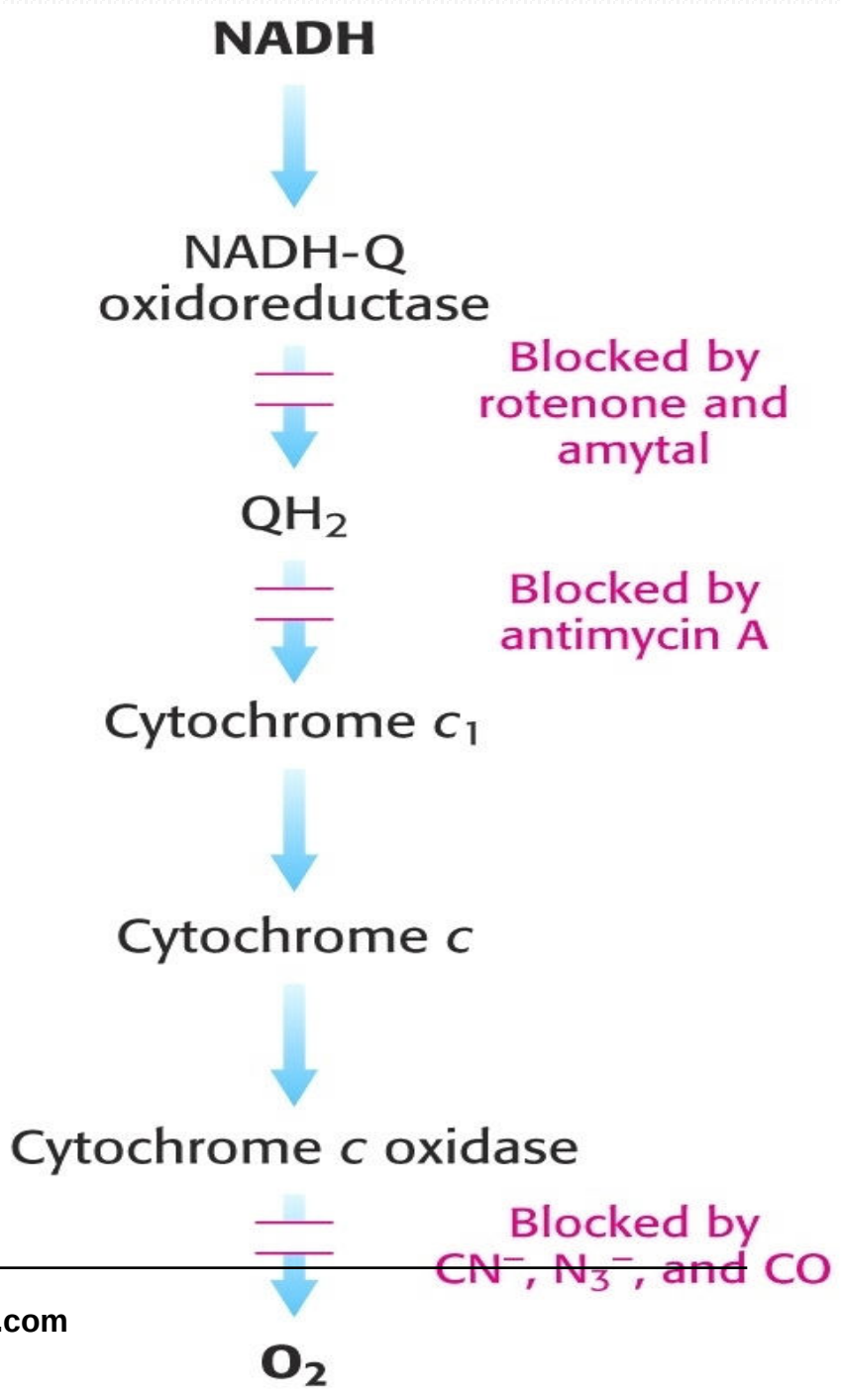
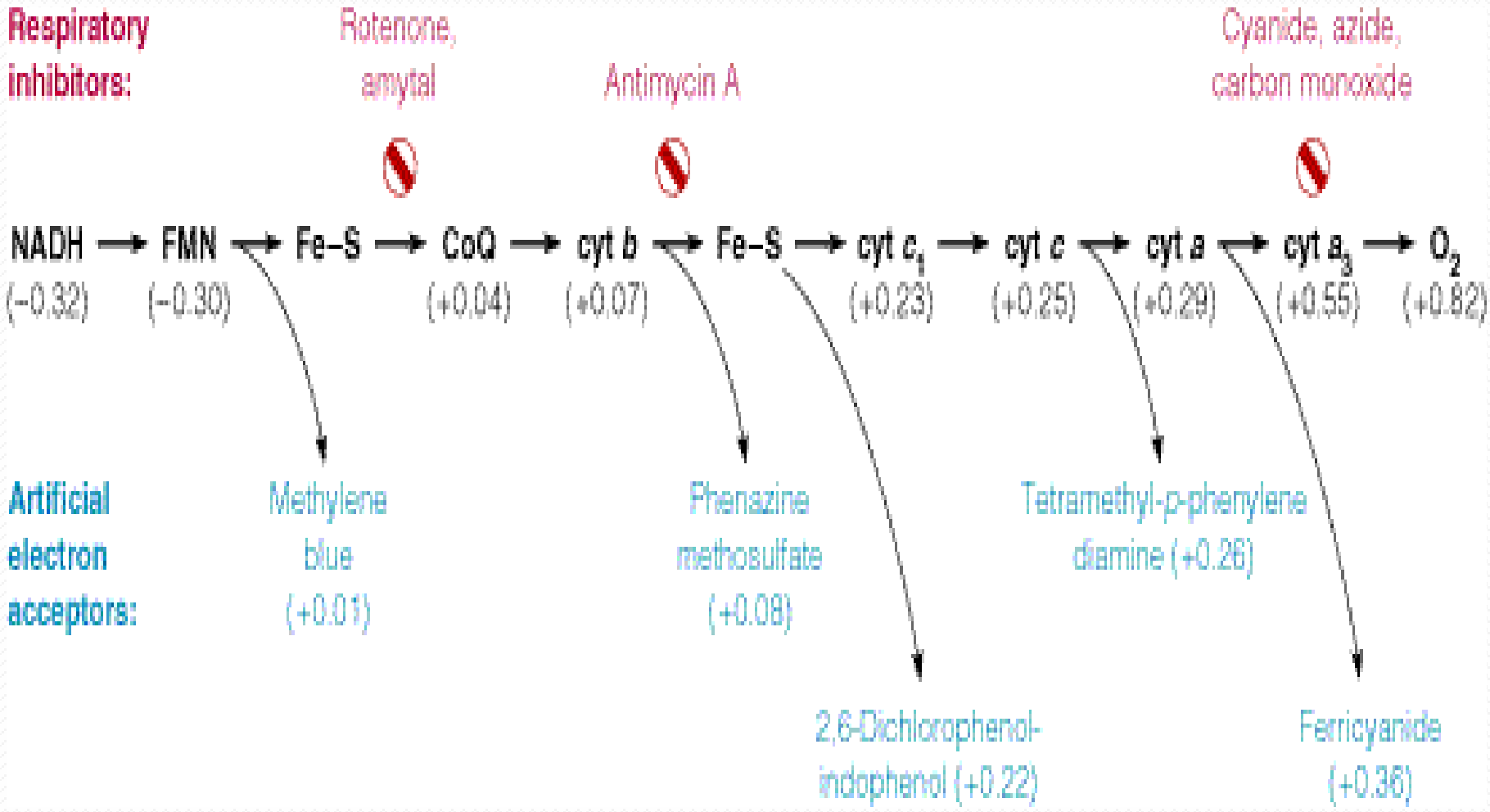


Figure 6.10
Site-specific inhibitors of electron transport shown using a mechanical model for the coupling of oxidation-reduction reactions. [Note: Figure illustrates normal direction of electron flow.]

Specific inhibitors of Electron Transport Chain and ATP-Synthase





Mechanism Of Oxidative Phosphorylation

- Oxidation tightly coupled with Phosphorylation
- E.T.C Process coupled with phosphorylation of ADP+pi to generate ATP.

- **Chemical Coupling Hypothesis**
- **Conformational Coupling Hypothesis**
- **Chemiosmotic Theory**

Chemical Coupling Hypothesis:

- Put forward by **Edward Slater (1953)**
- Proposed **series of high energy phosphorylated intermediates** are produced during E.T.C operation.
- Which are **used to produce ATP.**

Conformational Coupling Hypothesis

- **Paul Boyer 1964**
- Mitochondrial Cristae undergo **conformational change in the components of E.T.C.**
- E.T.C **components attain high energy state** which are responsible for the ATP production.

Chemiosmotic Theory

- Put forward by **Peter Mitchell (1961)** (Nobel Prize, 1978)
- E.T.C process and ATP synthesis are coupled by a proton gradient developed in intermembrane space of mitochondria.

Mitchell's Postulates for Chemiosmotic Theory

- Intact inner mitochondrial membrane is required
- Electrons are pumped through ETC **complex I,III and IV.**
- Generates a **proton gradient in intermembrane space** of mitochondria.

- **Proton gradient** in inter membrane space creates **Proton Motive Force.**
- Due to **electrochemical potential difference**.

- The proton gradient have a thermodynamic tendency.

- Proton Motive Force drives the Protons from mitochondrial intermembrane space back to matrix side
- Through a specific site of Fo and F1 particle of ATP Synthase.

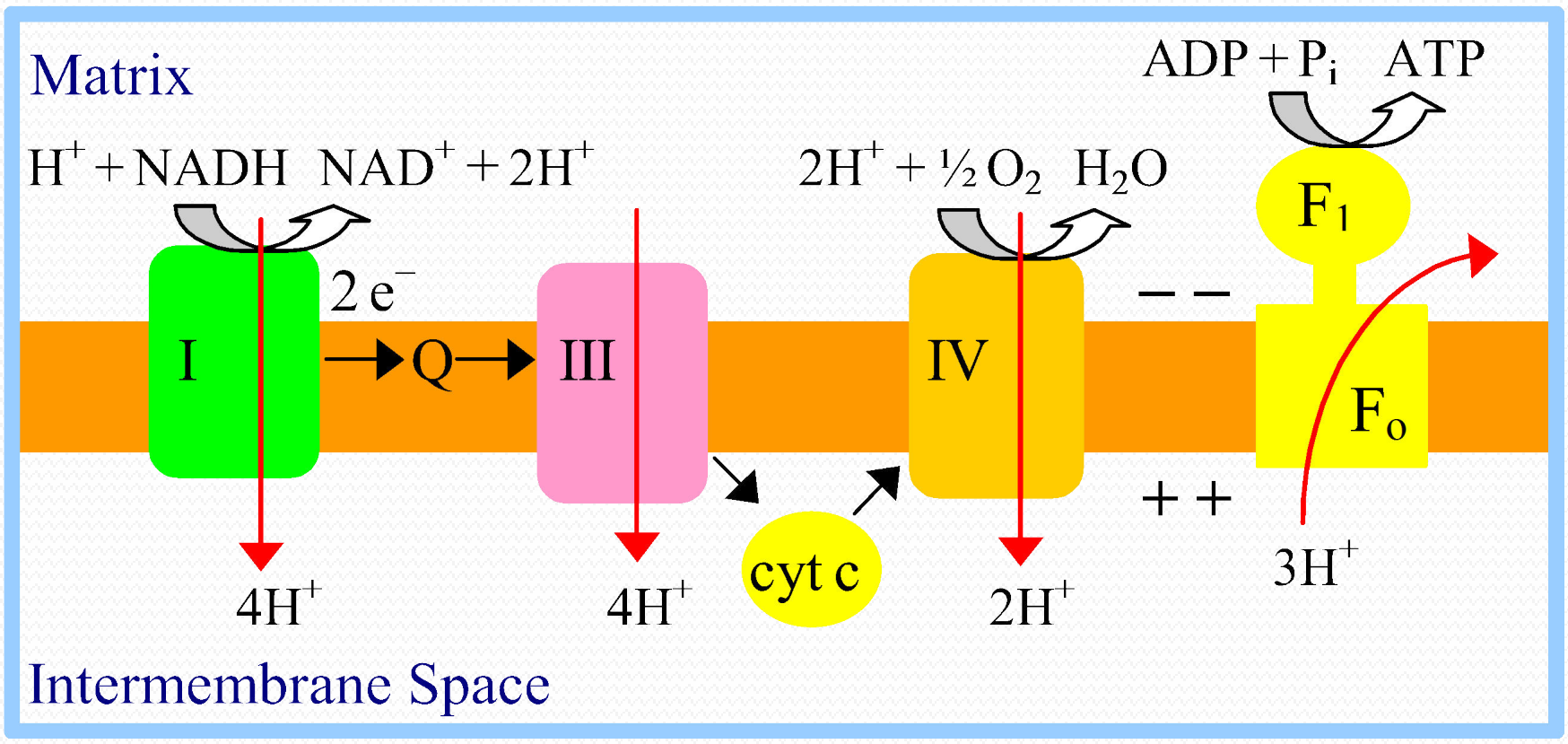
- ATP Synthase catalyzes the phosphorylation of ADP with pi
- In a reaction driven by movement of H^+ across the inner membrane back into the matrix.

- **Translocation of protons through ATP Synthase**
- **Stimulates and activates ATP Synthase**
- For the catalytic action of phosphorylation- ADP with pi to form ATP.
- Supports the mechanism of Oxidative Phosphorylation.

- The flow of three H^+ through an ATP Synthase complex
- Causes a conformational change, which causes the ATP synthase to synthesize ATP from ADP + Pi.

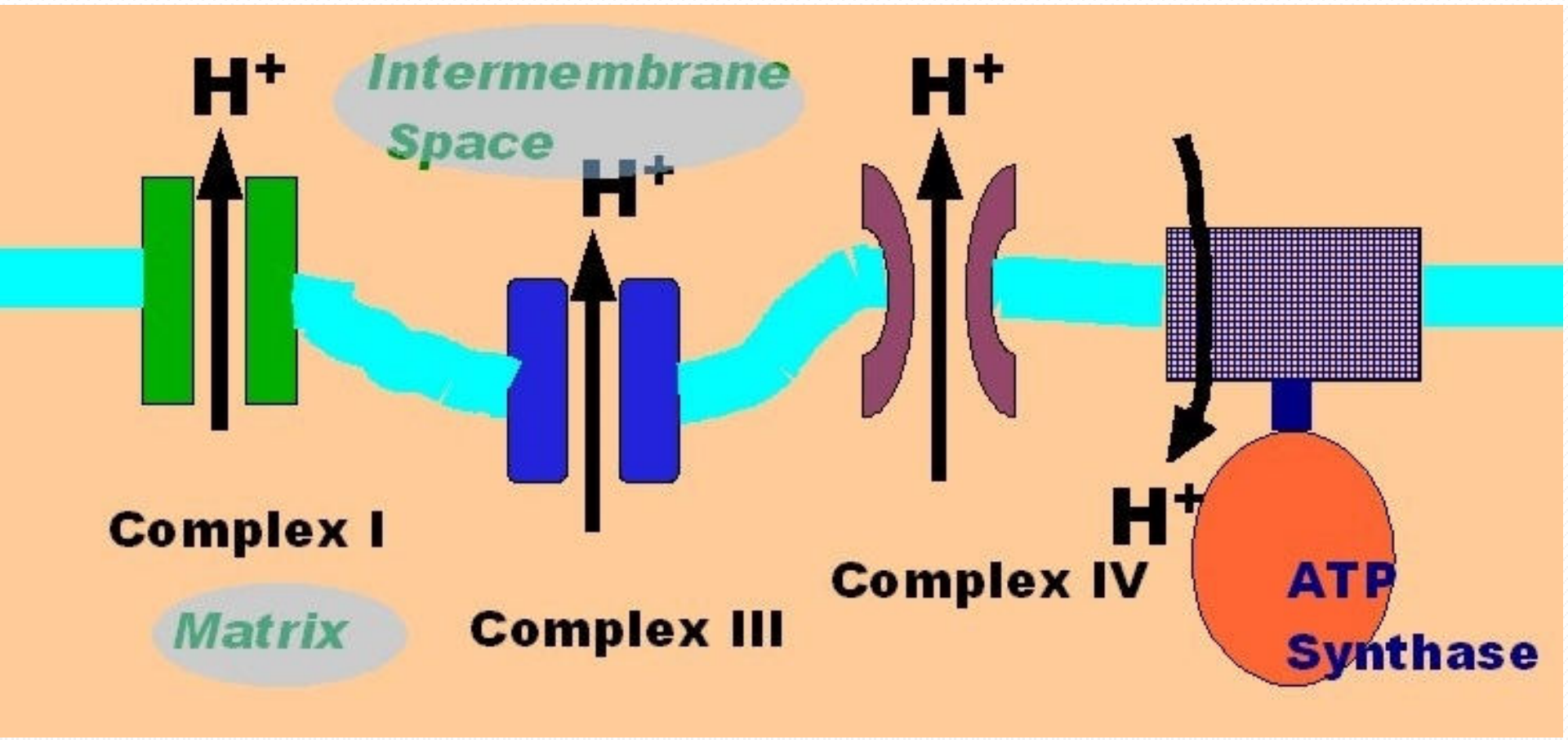
- This process of producing ATP is known as oxidative phosphorylation.
- The entire process of using the proton motive force to make ATP is called **Chemiosmosis.**

- During oxidative phosphorylation **the total energy change is released in small increments.**
- So that energy can be trapped as chemical bond energy and form ATP.

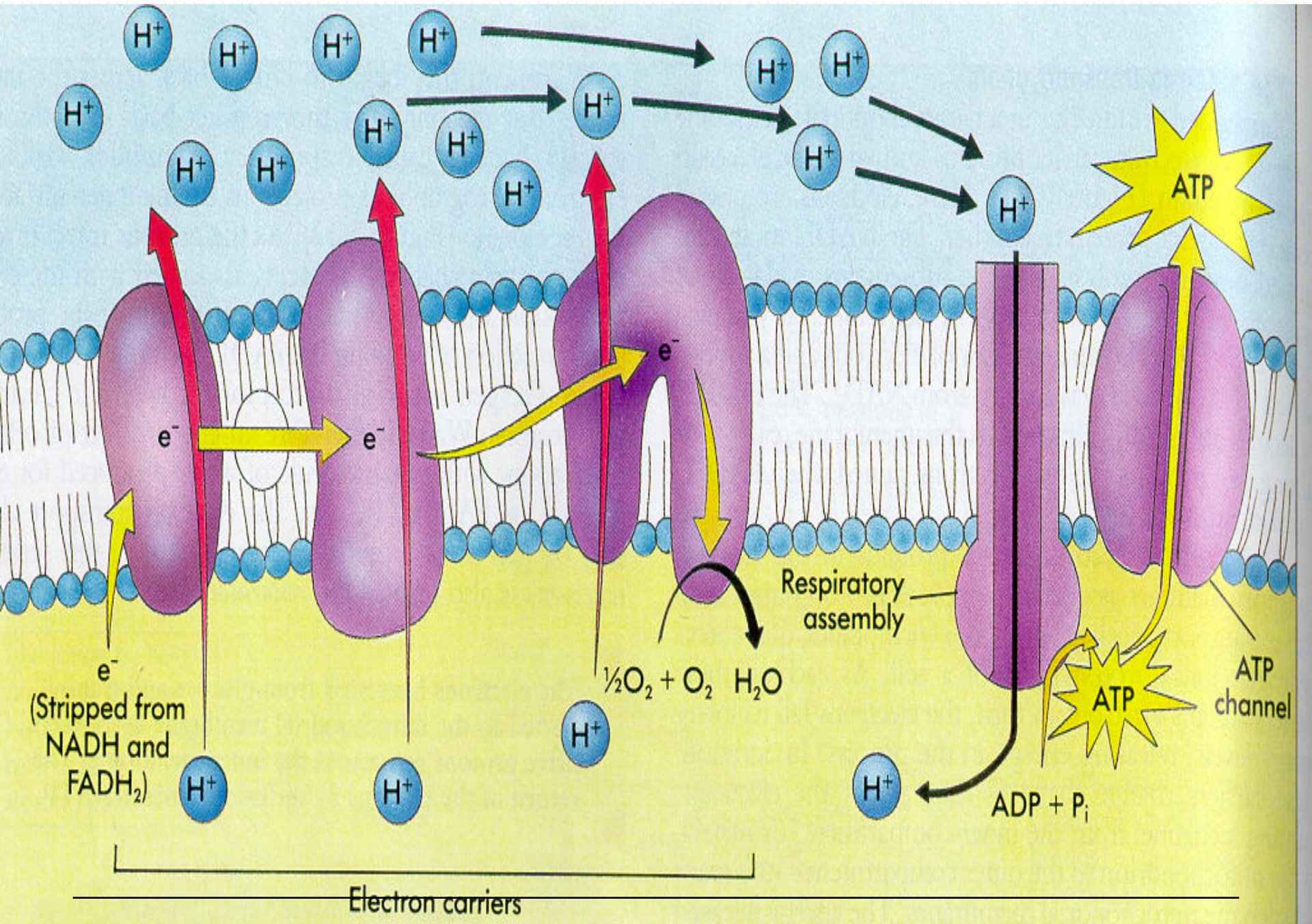


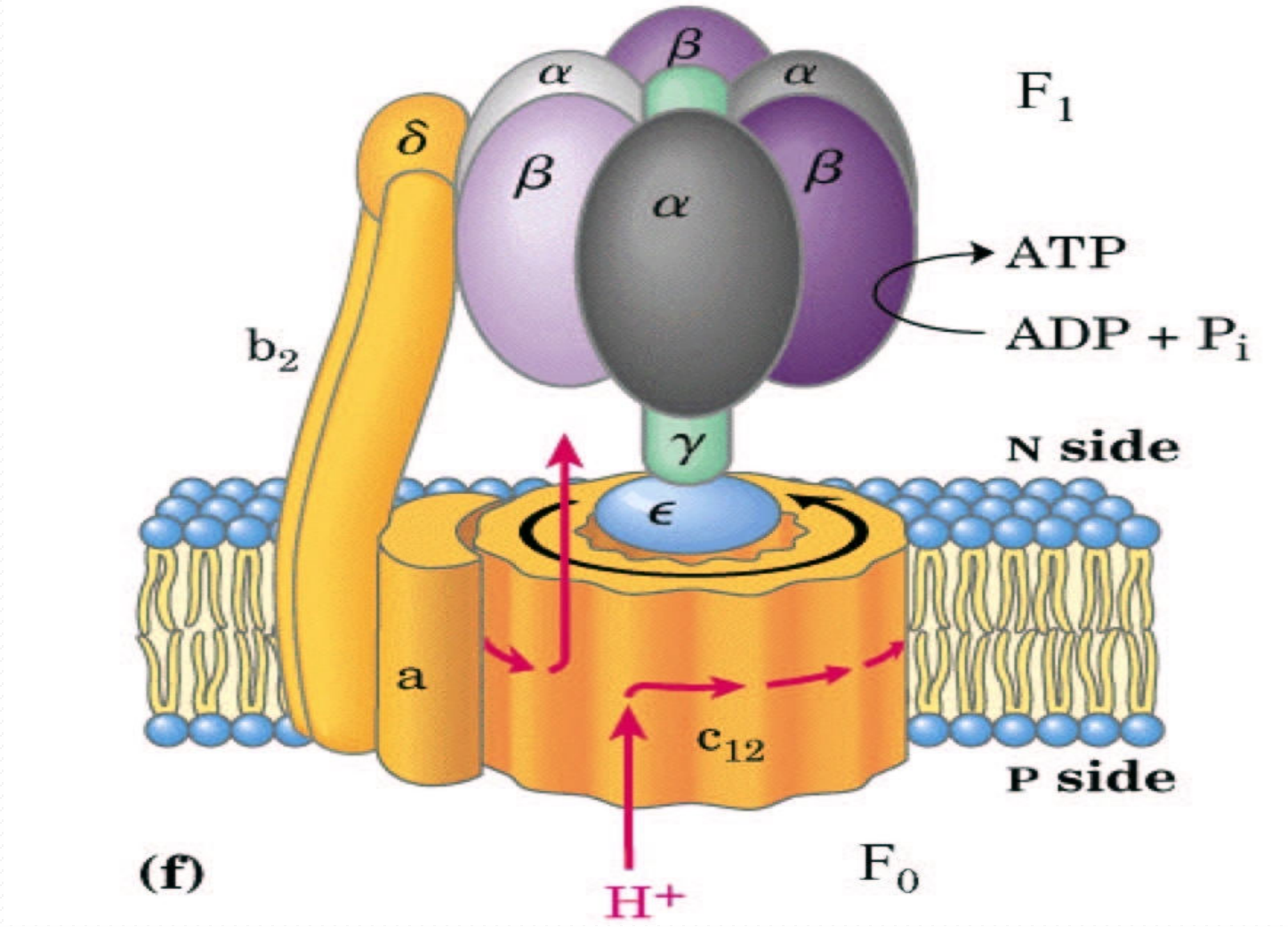
Coupling of ATP synthesis to respiration is **indirect**, via a H^+ electrochemical gradient.

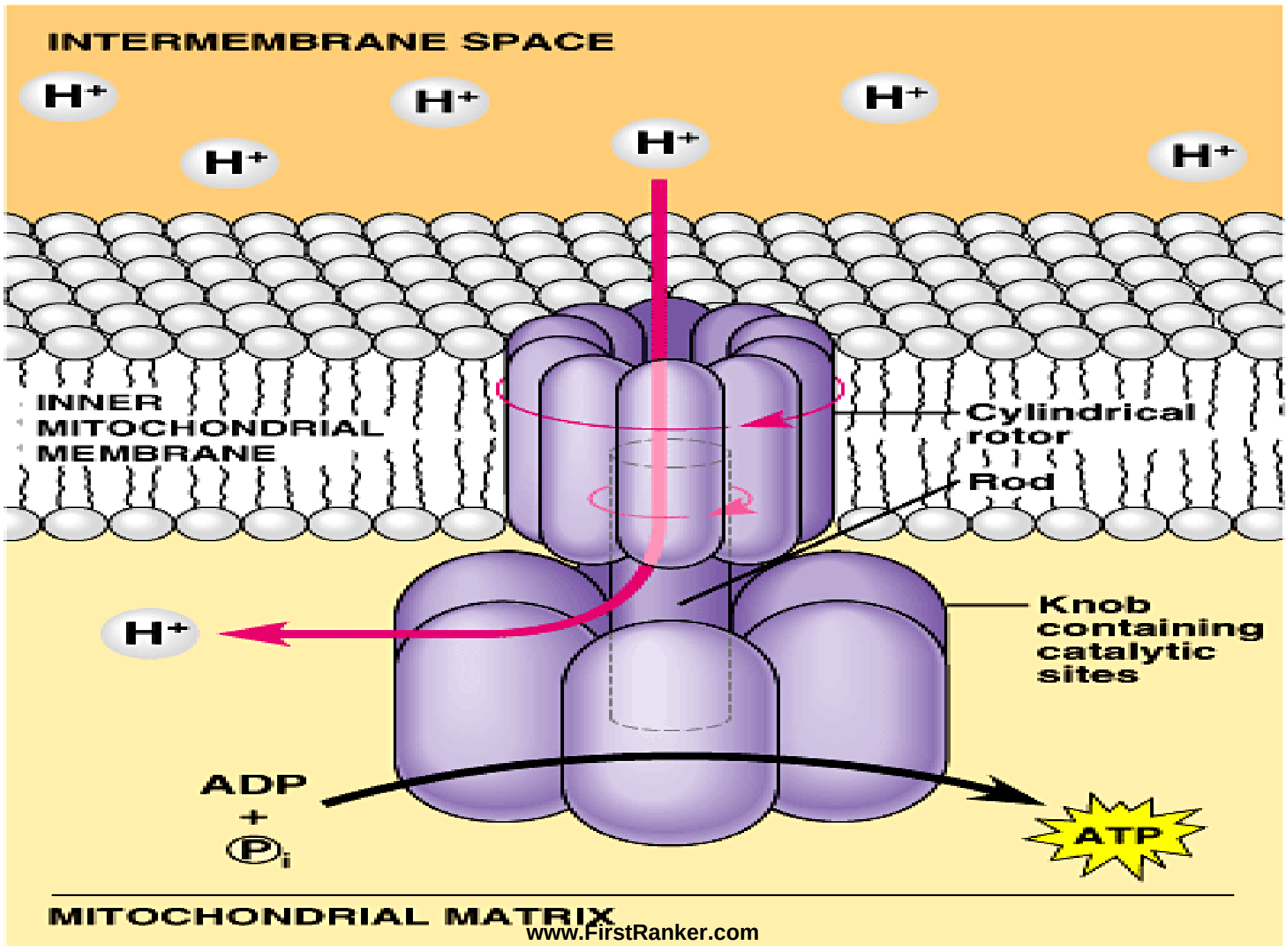
The proton pumps are *Complexes* I, III and IV.



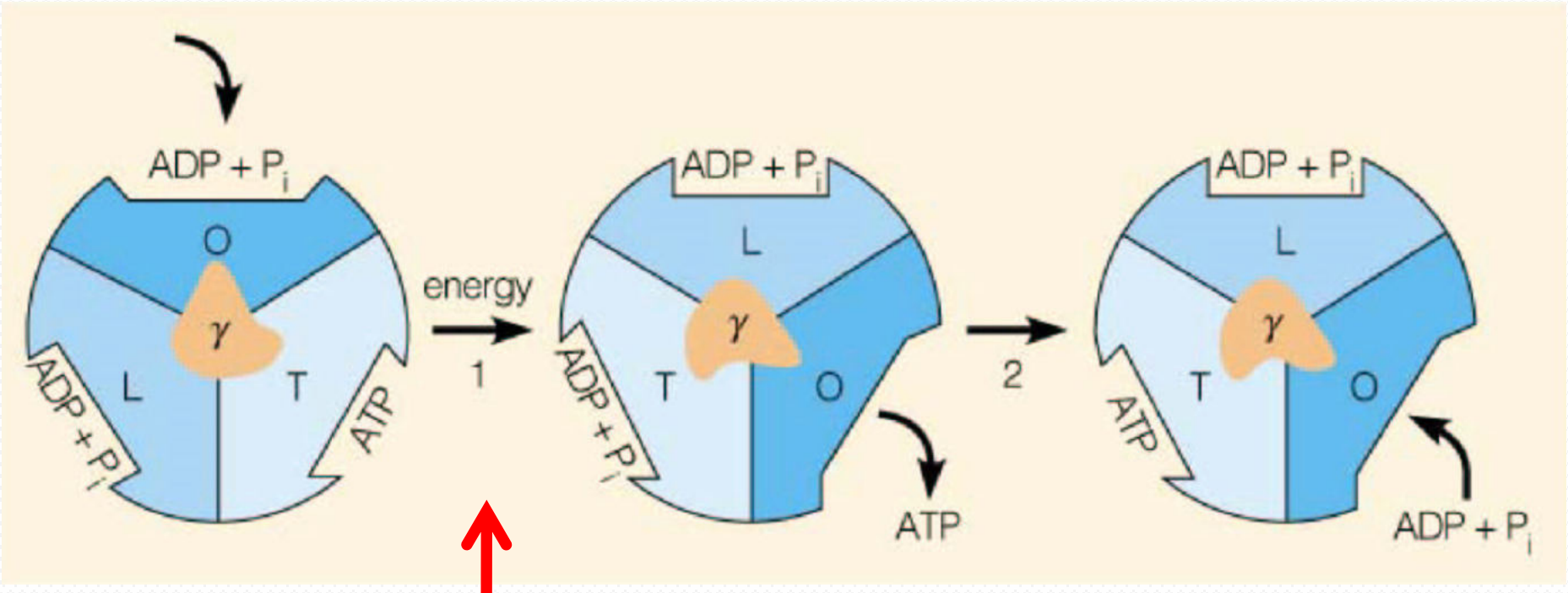
Protons return through ATP synthase







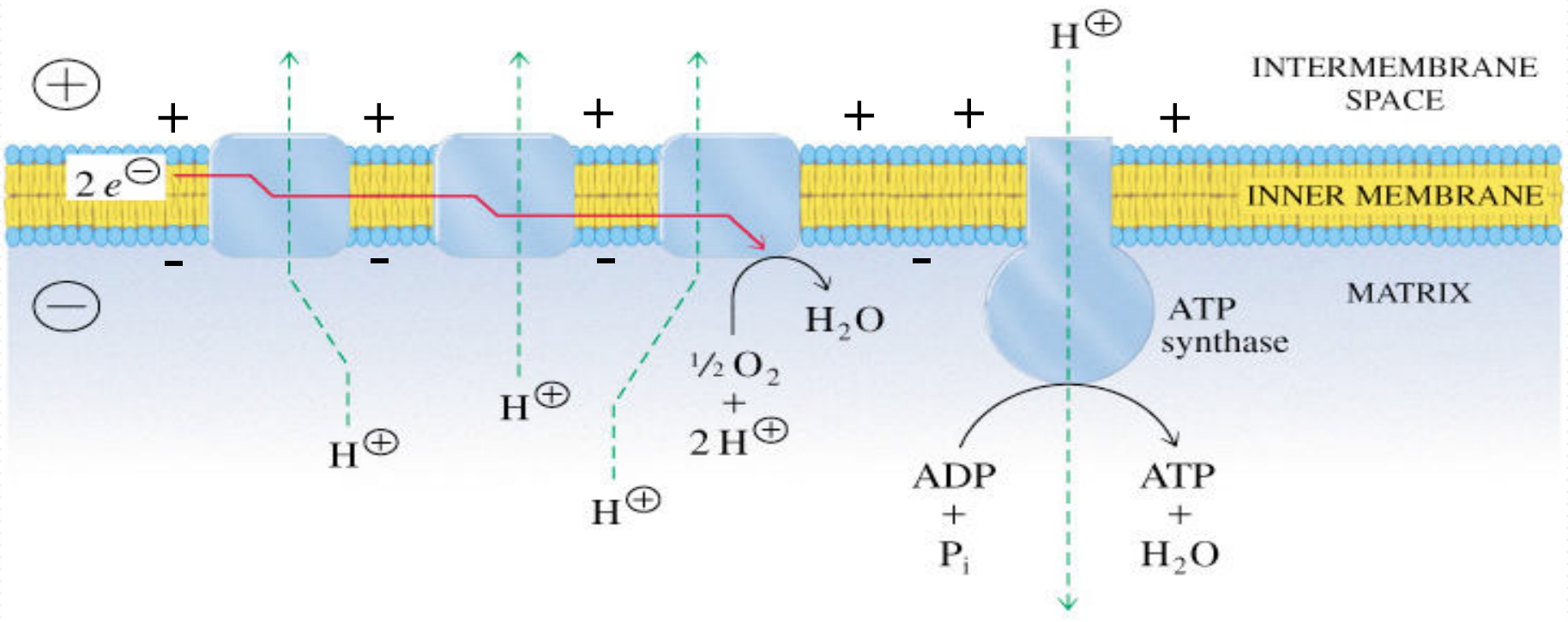
ATP synthesis at F_1 results from repetitive comformational changes as γ rotates



γ rotates 1/3 turn-
energy for ATP *release*

- ATP Synthase, a molecular mill.

Overview of Oxidative Phosphorylation



As **electrons flow** through complexes of ETC, **protons are translocated** from matrix into the intermembrane space.

The free energy stored in the **proton concentration gradient** is tapped as protons reenter the matrix via **ATP synthase**.

As result **ATP is formed from ADP and P_i.**

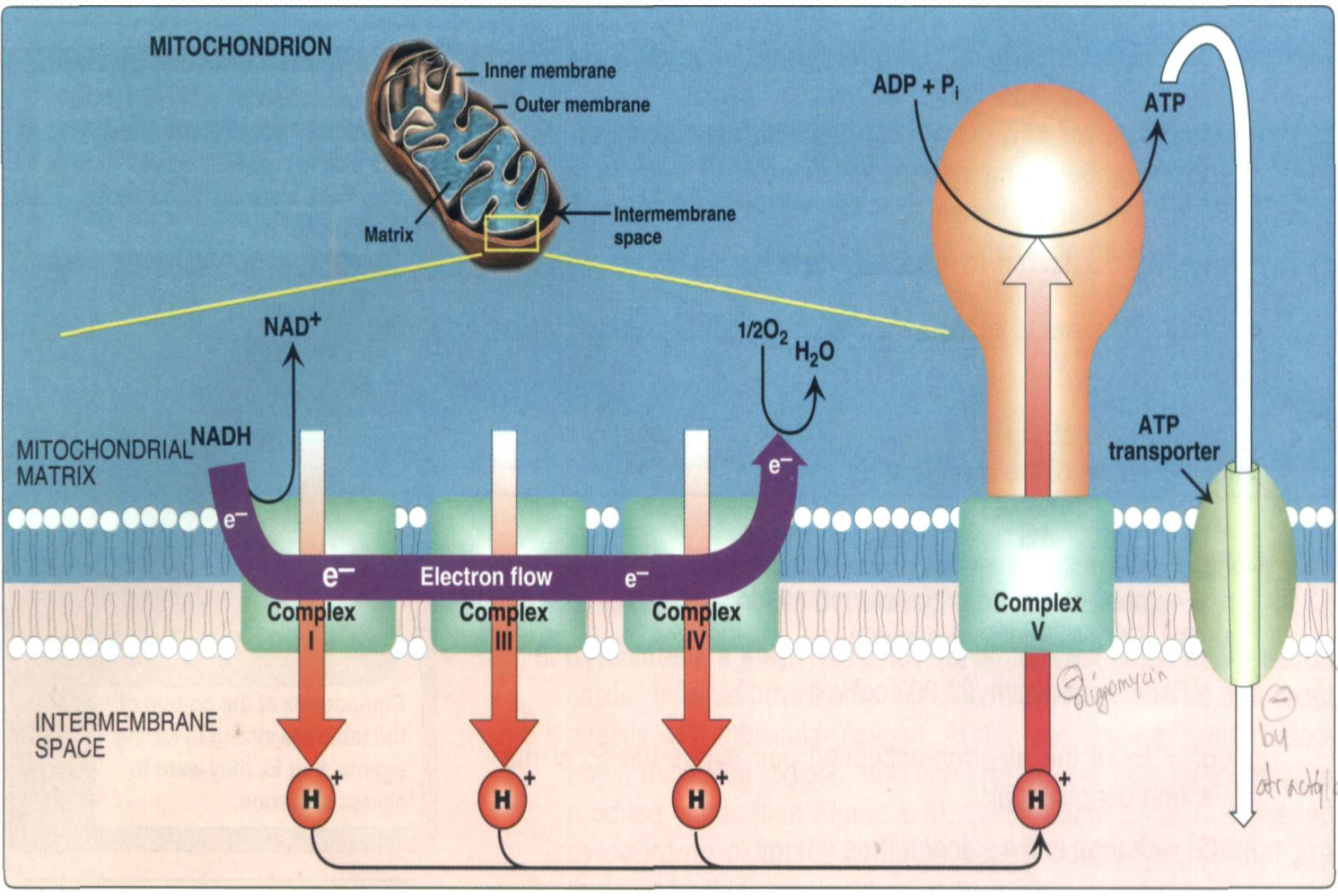


Figure 6.13

Electron transport chain shown coupled to the transport of protons. [Note: Complex II is not shown.]

- ATP molecules are transported out of the mitochondrial matrix through specific transporters.

REGULATION OF OXIDATIVE PHOSPHORYLATION

- Important substrates:
  - $NADH/FADH_2$
  - O_2
  - ADP

- Electron transport is tightly coupled to phosphorylation.

- ATP cannot be synthesized by oxidative phosphorylation unless there is energy from electron transport.

- Electrons do not flow through the electron-transport chain to O2 unless ADP is phosphorylated to ATP.

- ADP and pi is required for ETC process.
- **Intramitochondrial ratio ATP/ADP is a control mechanism**

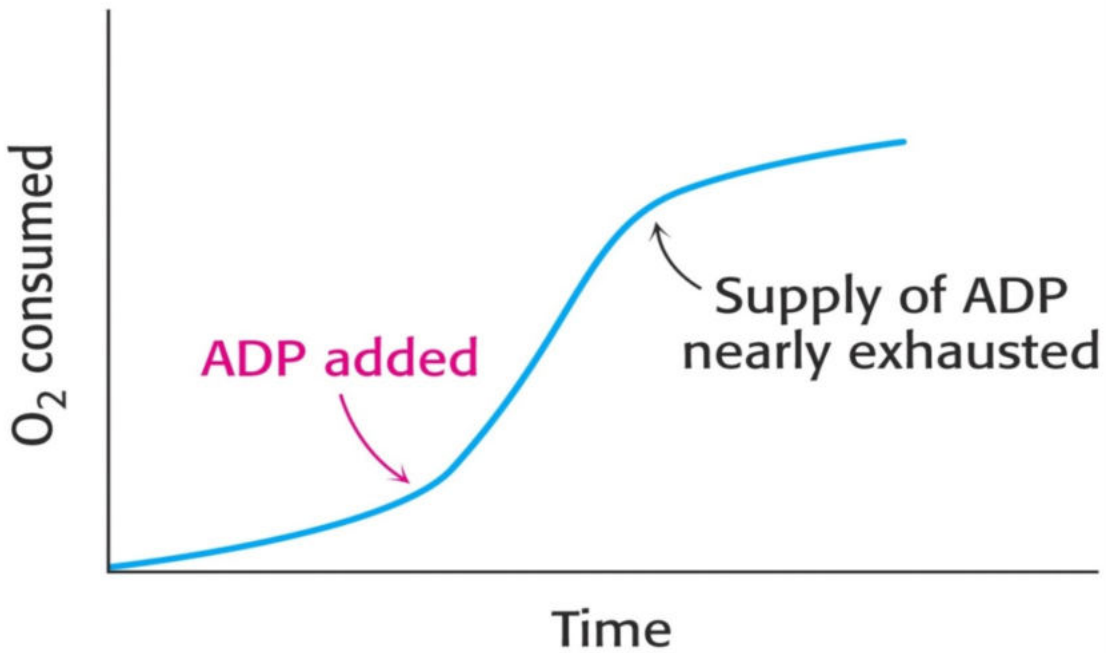
- At low ADP levels oxidative phosphorylation low.
- ADP levels reflect rate of ATP consumption and energy state of the cell.

- At high ATP/ADP
- **ATP acts as an allosteric inhibitor** for **Complex IV** (Cytochrome Oxidase)
- Inhibition is reversed by increasing ADP levels.

Respiratory Control

The most important factor in determining the rate of oxidative phosphorylation is the *level of ADP.*

The regulation of the rate of oxidative phosphorylation by the ADP level is called *respiratory control*



Inhibitors Of Oxidative Phosphorylation

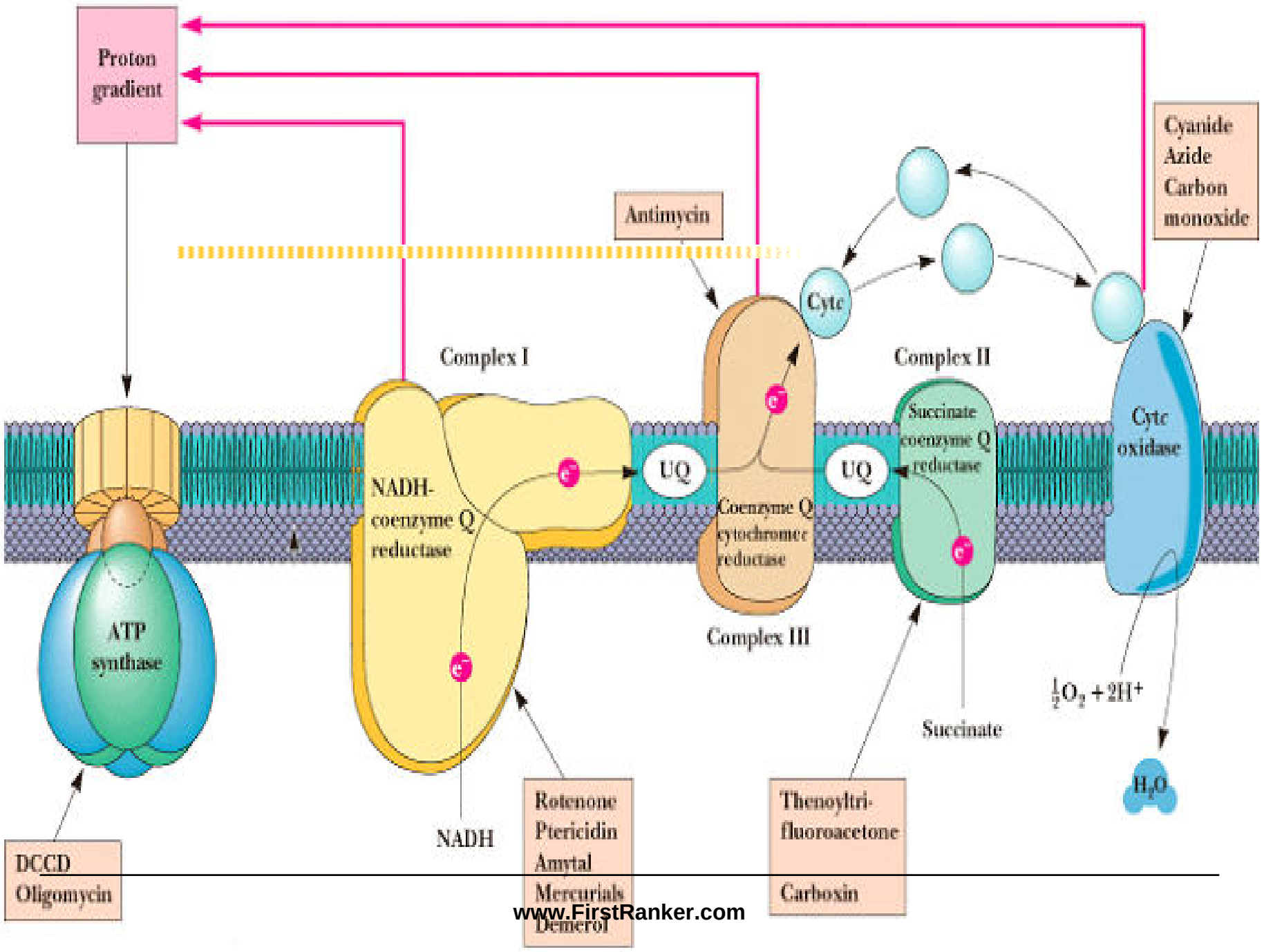
- **Oligomycin**

- An antibiotic binds with Fo particle of ATP Synthase
- Fo particle serve as proton channel.
- Inhibits phosphorylation of ADP to ATP.

- **Atractyloside**

- A **Glycoside prevents** the **translocation of ADP** across mitochondrial membrane.
- Make it unavailable for phosphorylation reaction.

- **Bongregate**
- Pseudomonas toxin has inhibitory action **similar like Atractyloside.**



Uncouplers Of Oxidative Phosphorylation

What are Uncouplers?

- Uncouplers are chemical agents

- That **uncouple the two tightly coupled processes** E.T.C (Oxidation) from Phosphorylation.

Uncouplers break the connection between Electron Transport Chain and Phosphorylation

Electron transport is a motor
Phosphorylation is the transmission

~~**Uncouplers put the car in NEUTRAL**~~

- Uncouplers are mostly **lipid soluble** aromatic weak acids.

- Uncouplers **deplete proton gradient of intermembrane space during ETC operation.**

- Uncouplers just bring oxidation (E.T.C) without phosphorylation.

- **Uncouplers inhibit generation of ATP.**

Physiological Uncouplers

- Thermogenin /UCP-1
- Excess of Thyroxine
- Long Chain Fatty acids
- Unconjugated Hyperbilirubinemia

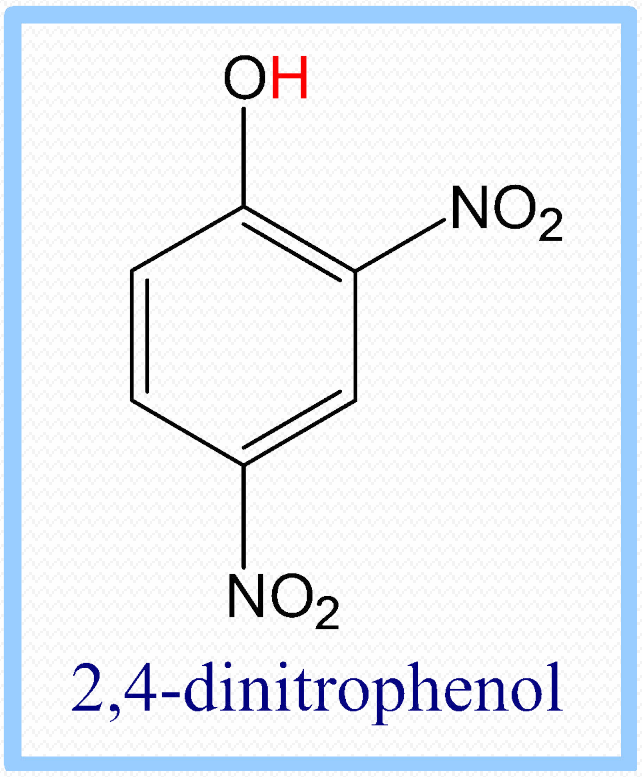
Chemical Uncouplers

- 2,4 Di Nitro Phenol
- Di Nitro Cresol
- Dicumarol
- Aspirin
- FCCP
- Valinomycin

Mode Of Action Of Uncouplers

- Certain Uncouplers are **ionophores, lipophilic substances.**
- They **carry protons from intermembrane space across mitochondrial membrane to matrix**
- From the **site other than specific site**. (i.e through Fo and F1 particles of ATP Synthase).

- Certain **Uncouplers changes the permeability** of the mitochondrial membrane to protons.
- **Translocate protons easily through mitochondrial membrane.**

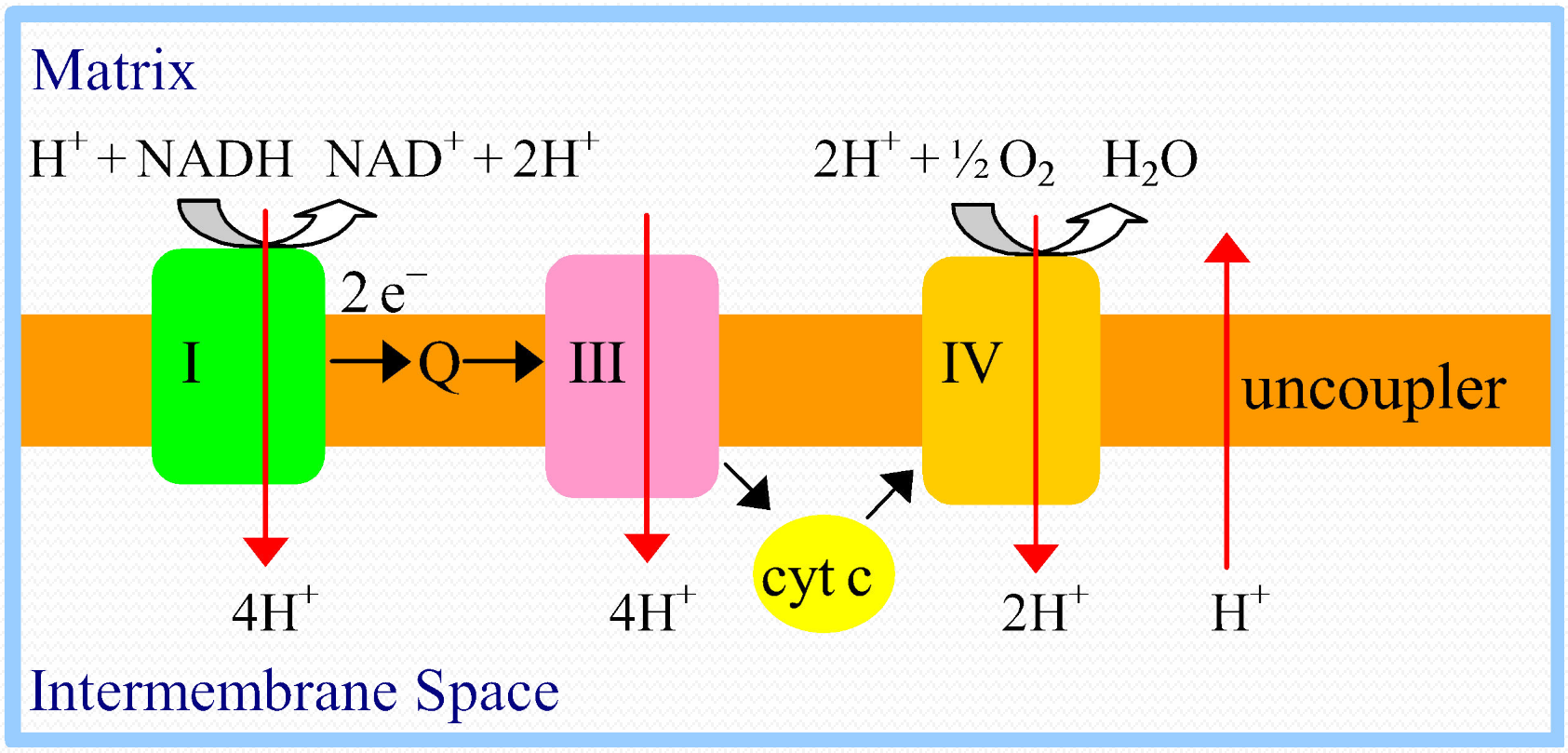


2,4-dinitrophenol

2,4 DNP dissolve in the membrane and function as **carriers for H^+**.

- **Do not** allow to **develop proton gradient** and **proton motive force** in the **intermembrane space** of mitochondria.
- Causes **no stimulation or activation of ATP Synthase**
- **No catalysis of Phosphorylation** of ADP with pi to generate ATP.

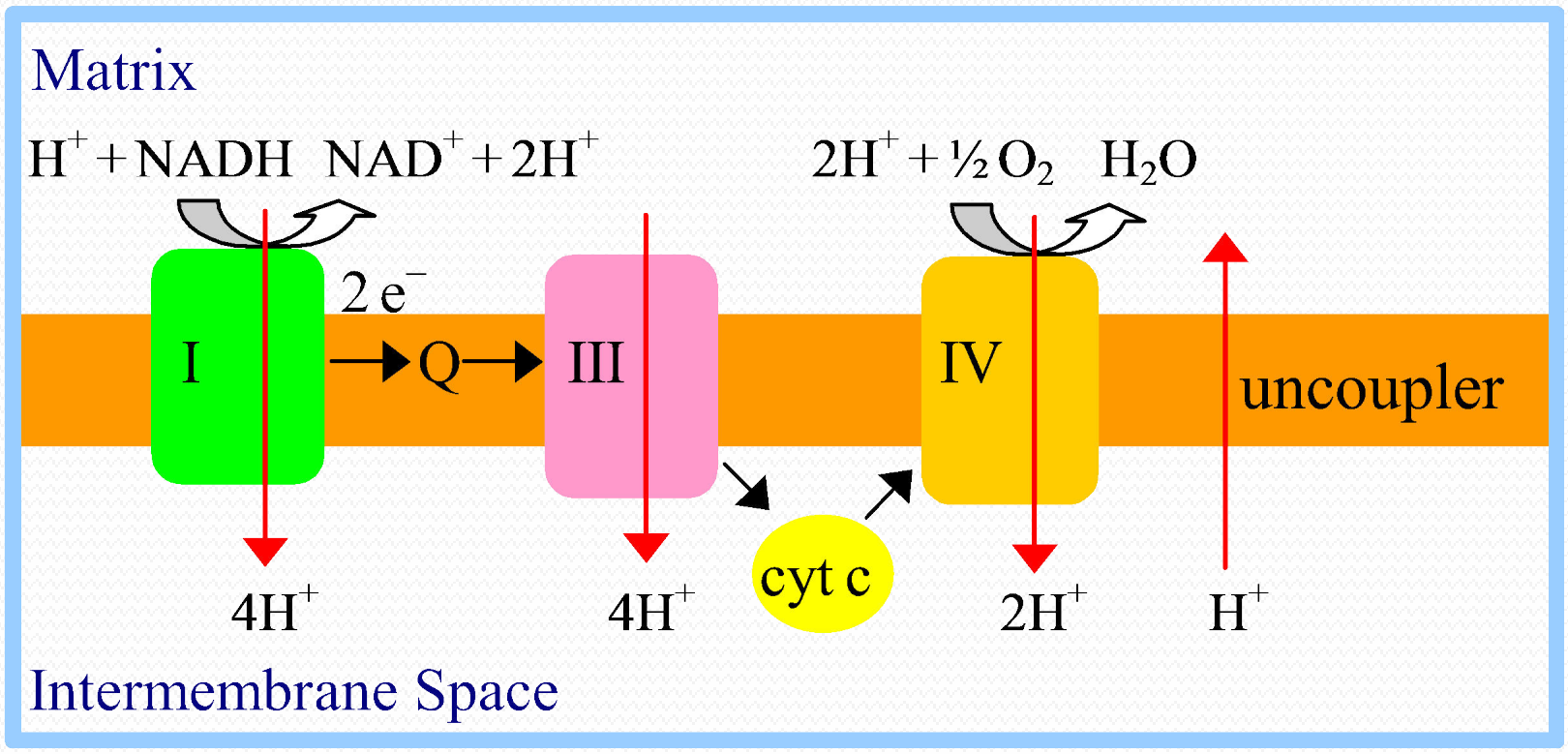
- **During uncoupling phenomena**
- Heat energy dissipiated as it is and causes **swelling of mitochondria**.



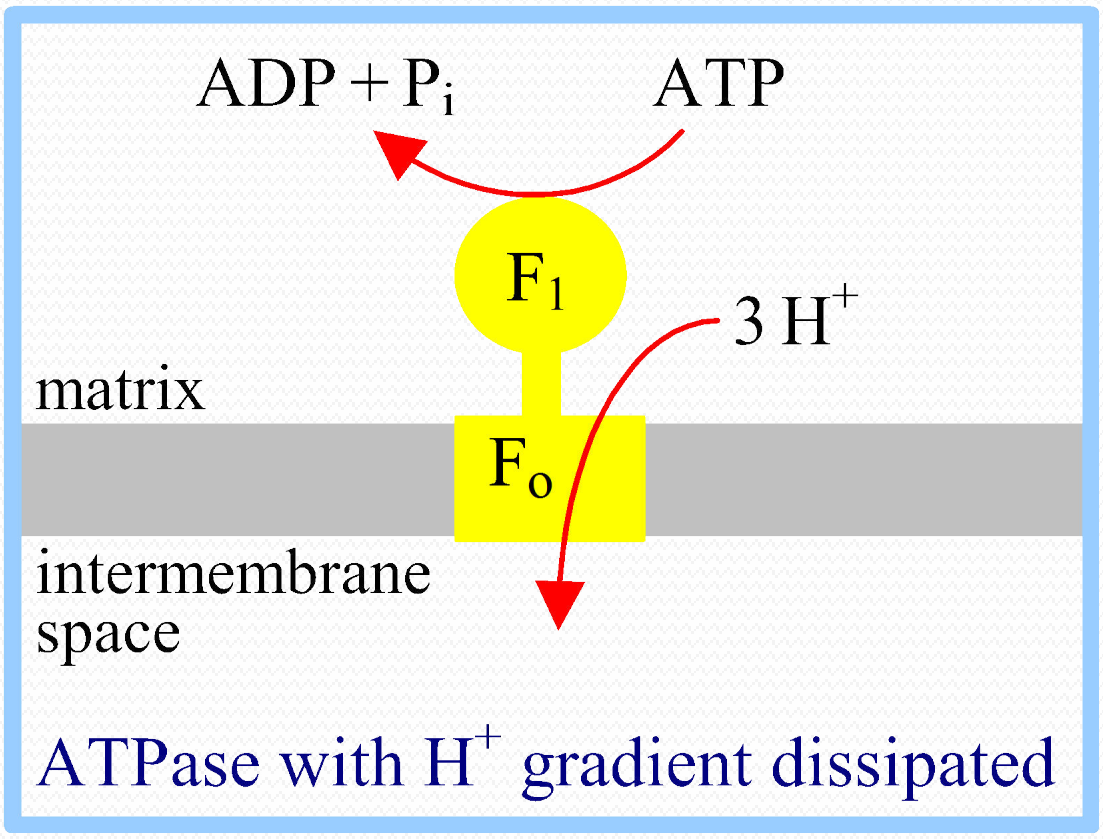
Uncouplers block oxidative phosphorylation by **dissipating the H^+ electrochemical gradient**.

Protons pumped out **leak back into the mitochondrial matrix,**

preventing development of proton gradient and proton motive force.



Respiration proceeds in the presence of an uncoupler, whether or not ADP is present.



The **ATP Synthase** reaction runs **backward** in presence of an **uncoupler**.

- Hydrolysis of ATP is spontaneous.

Physiological Uncoupling By Uncoupling Protein (UCP-1)

- An **Uncoupling Protein** (**UCP-1**)/ **Thermogenin** is produced in **brown adipose tissue** of newborn mammals and hibernating mammals.

- This protein of the inner mitochondrial membrane functions as a **H^+carrier**.

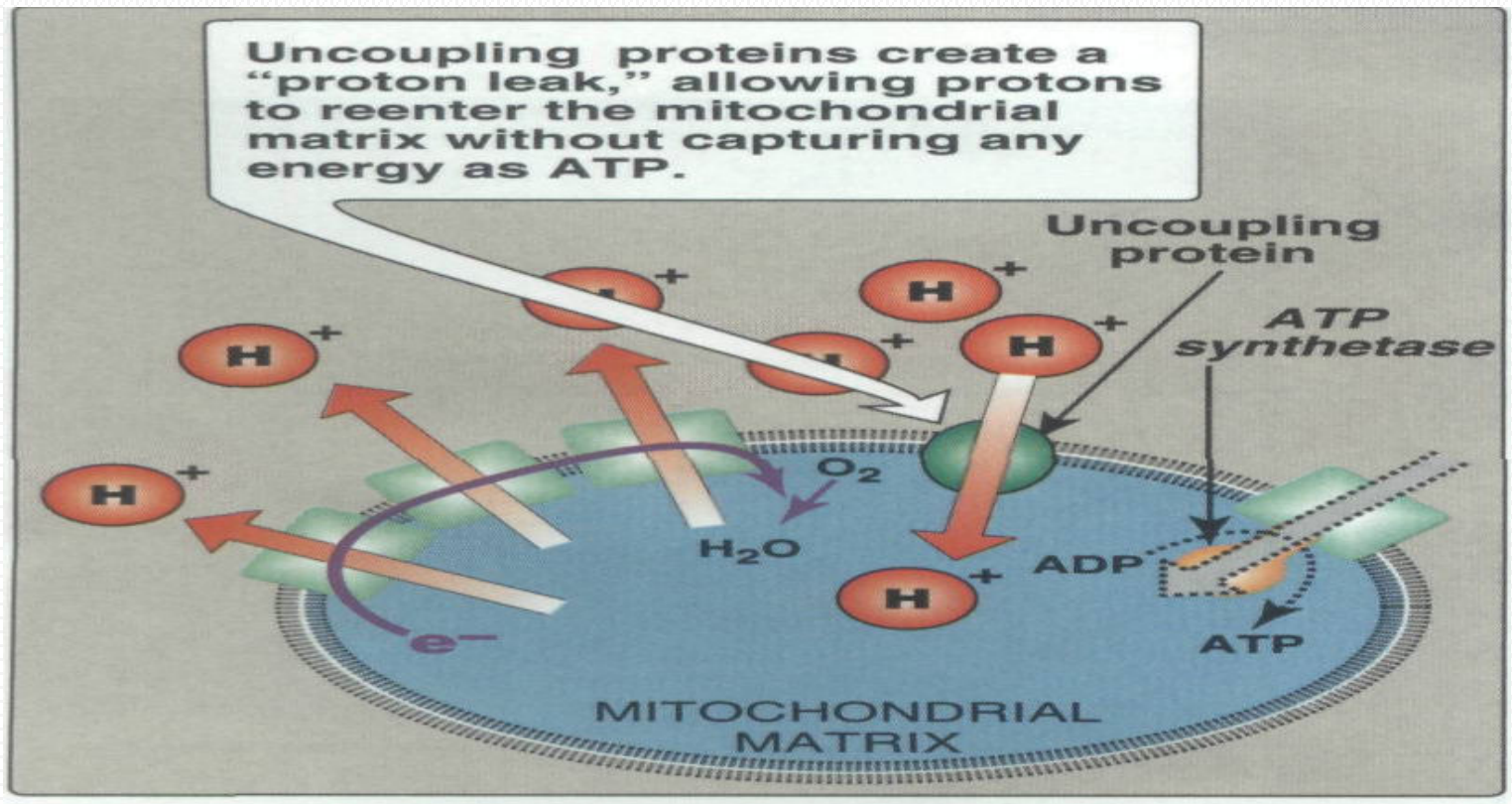


Figure 6.14
Transport of H^+ across mitochondrial membrane by 2,4-dinitrophenol.

- The uncoupling protein blocks development of a H^+ electrochemical gradient, thereby stimulating respiration.
- Free energy of ETC is dissipated as heat.

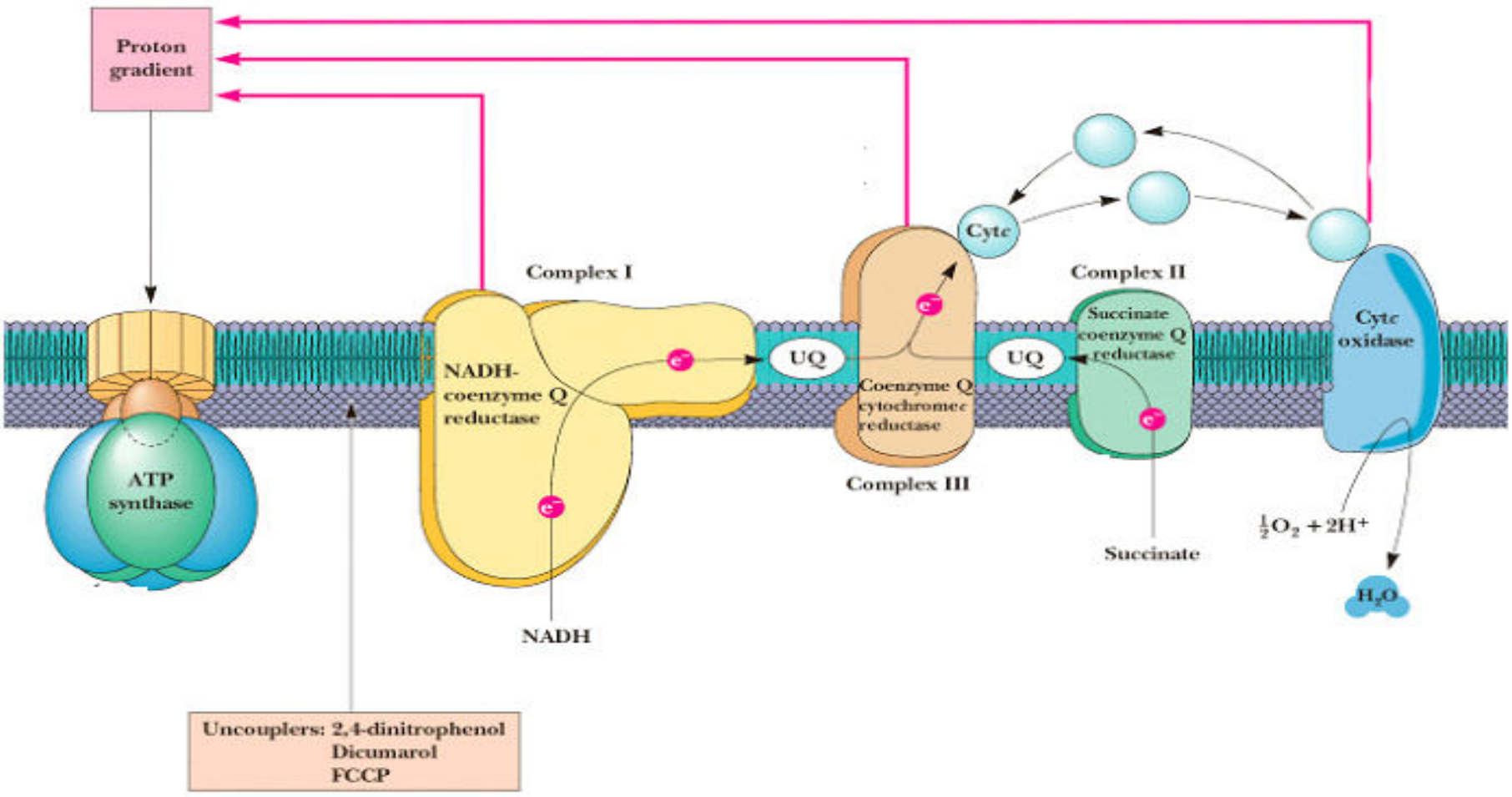
- Uncoupling of ETC and phosphorylation occurs in animals as a means to produce heat
- **Non shivering thermogenesis.**
- Occurs in brown adipose tissues (rich in mitochondria)

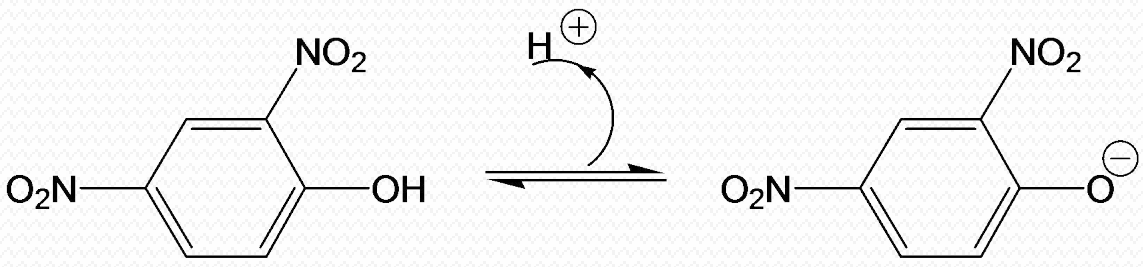
- This "**non-shivering thermogenesis**" is costly in terms of respiratory energy,
- Heat energy unavailable for ATP synthesis, but provides valuable warming of the organism.

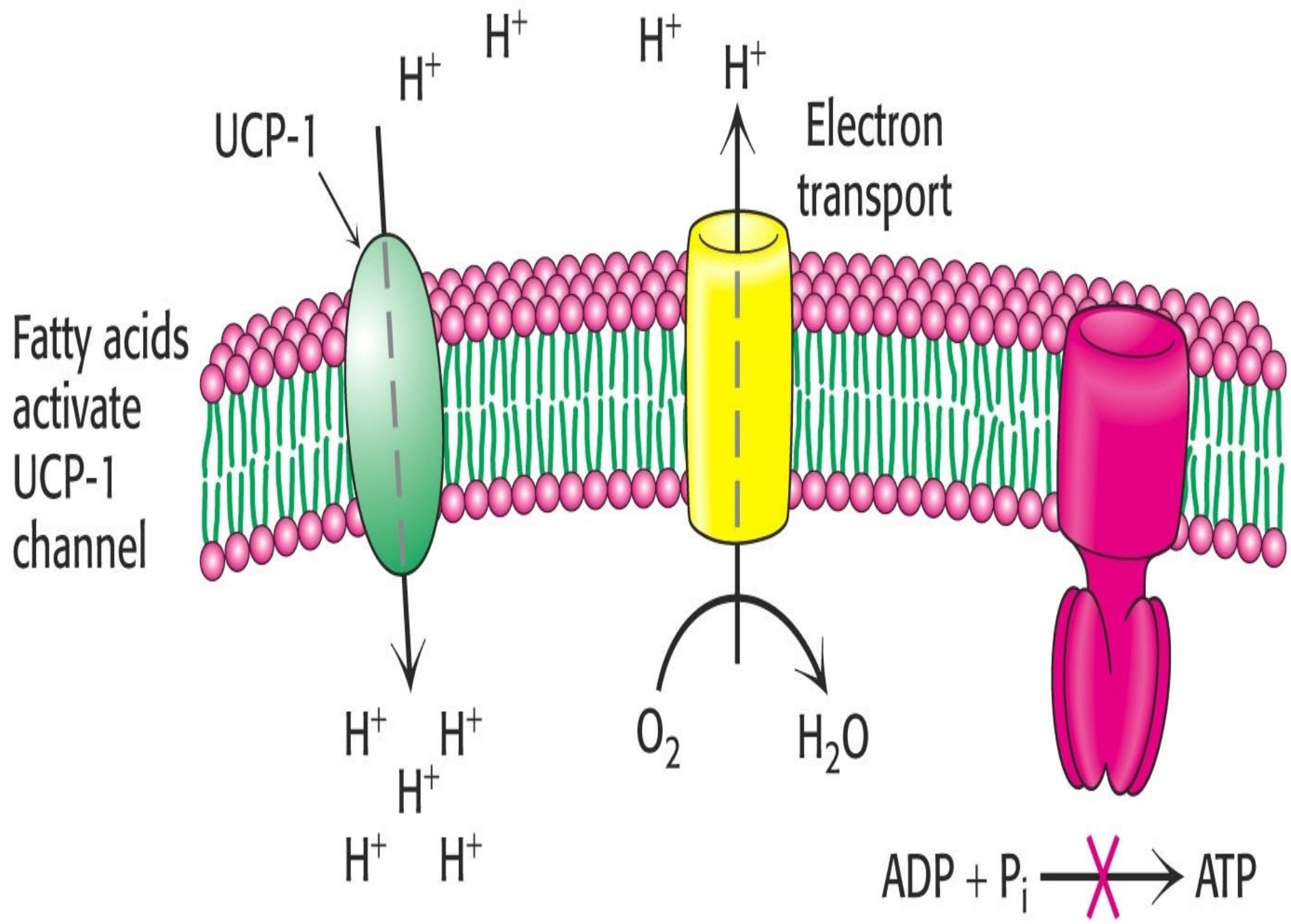
Significance Of Physiological Uncouplers

- In extreme cold conditions and in hibernating animals
- Physiological Uncouplers bring uncoupling phenomena
- The heat liberated inside the body helps to **restore and maintain the body temperature.**

Brown adipose (fat) cells contain natural Uncouplers to warm animals cold adaptation and hibernation.







ETC Inhibitors and Uncouplers

Table 1. Inhibitors of Respiration and Oxidative Phosphorylation

Site-Specific	Target Complex
Carbon monoxide	IV
Cyanide	IV
Sodium Azide	IV
Rotenone	I
Antimycin A	III
Amytal	I
Phosphorylation	
Oligomycin	F_o
Uncouplers	
2,4-Dinitrophenol (DNP)	Proton gradient
Trifluorocarbonylcyanide Phenylhydrazone (FCCP)	Proton gradient

Any compound that stops electron transport will stop respiration…this means you stop breathing

Electron transport can be stopped by inhibiting ATP synthesis

An uncoupler breaks the connection between ATP synthesis and electron transport

Inherited Disorders Related To E.T.C

Infantile Mitochondrial Myopathy

- **Defect in E.T.C components located in mitochondria.**
- Associated with renal dysfunction.
- Mostly fatal in early age.

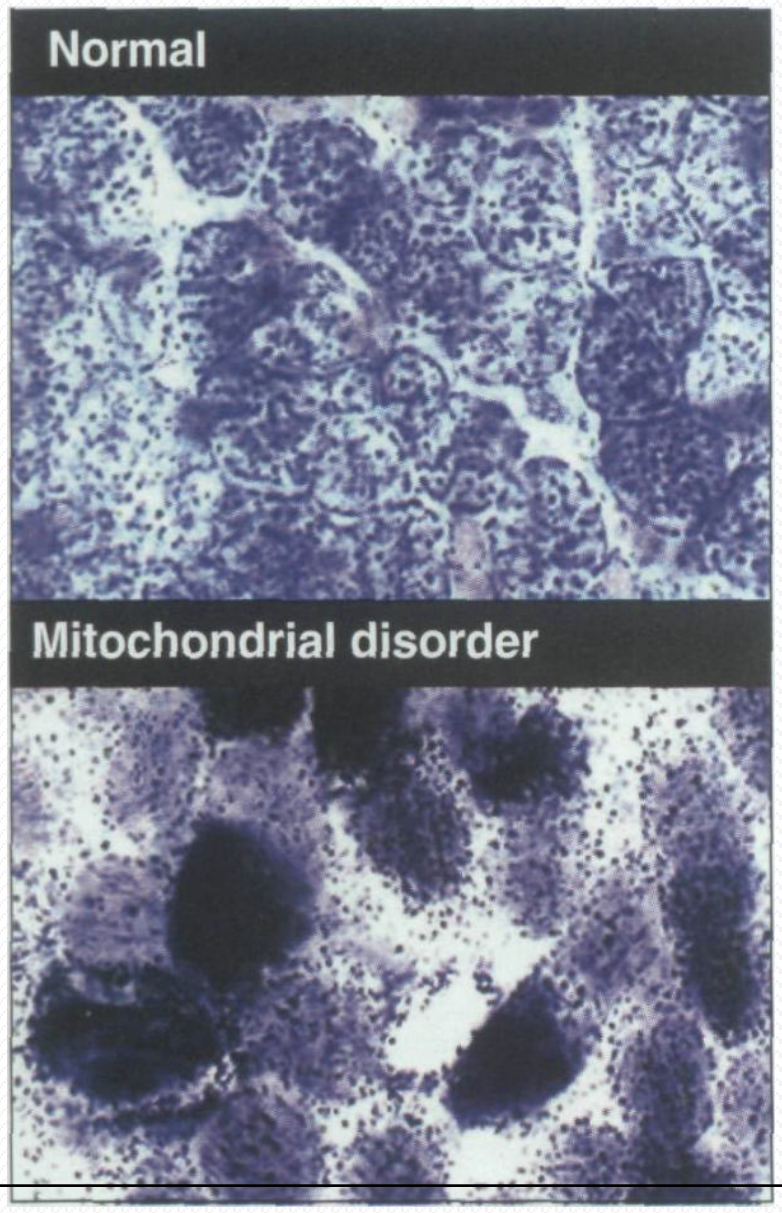
MELAS

- An inherited disorder caused due to **deficiency of complex I or IV** of E.T.C
- Associated with
  - **Mitochondrial Myopathy**
  - **Encephalopathy**
  - **Lactic Acidosis**
  - **Stroke**

Inherited Disorders of Oxidative Phosphorylation

Leber's Hereditary Optic Neuropathy (LHON)

- Caused due to **mutations in mitochondrial DNA**
- Affects oxidative phosphorylation.
- Loss of bilateral vision due to neuroretinal degeneration.



Factors Affecting ETC Process

- Oxygen supply to cells.
- Hemoglobin structure and function
- Respiratory system and its function
- Mitochondrial structure and ETC components.
- Availability of reduced coenzymes.
- Adequate amount of ADP and pi.
- Presence of ETC inhibitors.

Conditions Affecting ETC and ATP

- Low/slow operation of ETC and less production of ATPs is noted in conditions of:

- Hypoxia
- Anemia
- Ischemia
- Hemoglobinopathies
- Emphysema
- Respiratory Distress Syndrome
- Asthma
- Inherited Disorders of Mitochondria
- ETC inhibition by chemicals/drugs.

High Energy Compounds Of Human Body.

- High energy compounds are energy rich compounds.
- Possess **high energy bonds** in its structures.
- **Cleavage** of these high energy bonds **liberate more energy** than that of ATP hydrolysis.

S.No	Examples Of High Energy Compounds	Free Energy Released On Hydrolysis. Cal/mol
1	Phospho Enol Pyruvate	-14.8
2	Carbamoyl Phosphate	- 12.3
3	Cyclic AMP	-12.0
4	1,3 Bis Phospho Glycerate	-11.8

S.No	Examples Of High Energy Compounds	Free Energy Released On Hydrolysis. Cal/mol

5	Creatine Phosphate	-10.3
6	S Adenosine Methionine (SAM)	-10.0
7	Succinyl CoA	-7.7
8	Acetyl CoA	-7.7
9	ATP	-7.3

Significance Of High Energy Compounds.

- **In catabolic pathways/reaction**
- High energy compounds follow **substrate level phosphorylation reaction.**
- High energy compounds cleave to generate energy used for phosphorylation of ADP with pi at reaction level.
- **Generate ATP at substrate level**.

Substrate Level Phosphorylation

- **Mode of generation of ATP at substrate level.**
- Involves **cleavage of high energy bond present in high energy compound**.
- Bond energy released is used for Phosphorylation reaction.
- Generates ATP directly and instantly without involvement of ETC process.

Examples Of High Energy Compounds Undergoing Substrate Level Phosphorylation.

S.No	High Energy Compound	Enzyme Catalyzing	Product Obtained	High energy Phosphate Compound Generated	Metabolic Pathway Involved
1	1,3 Bis Phospho Glycerate	Phospho Glycerate Kinase	3 Phospho Glycerate	ATP	Glycolysis
2	Phospho Enol Pyruvate	Pyruvate Kinase	Enol Pyruvate	ATP	Glycolysis
3	Succinyl CoA	Succinate Thio Kinase	Succinate	GTP	Krebs/TCA Cycle

- **In anabolic pathways/reaction**
- High energy compounds follow **condensation** or **bond building reactions.**
- High energy compound cleave to generate energy
- Energy used for building C-C bonds.

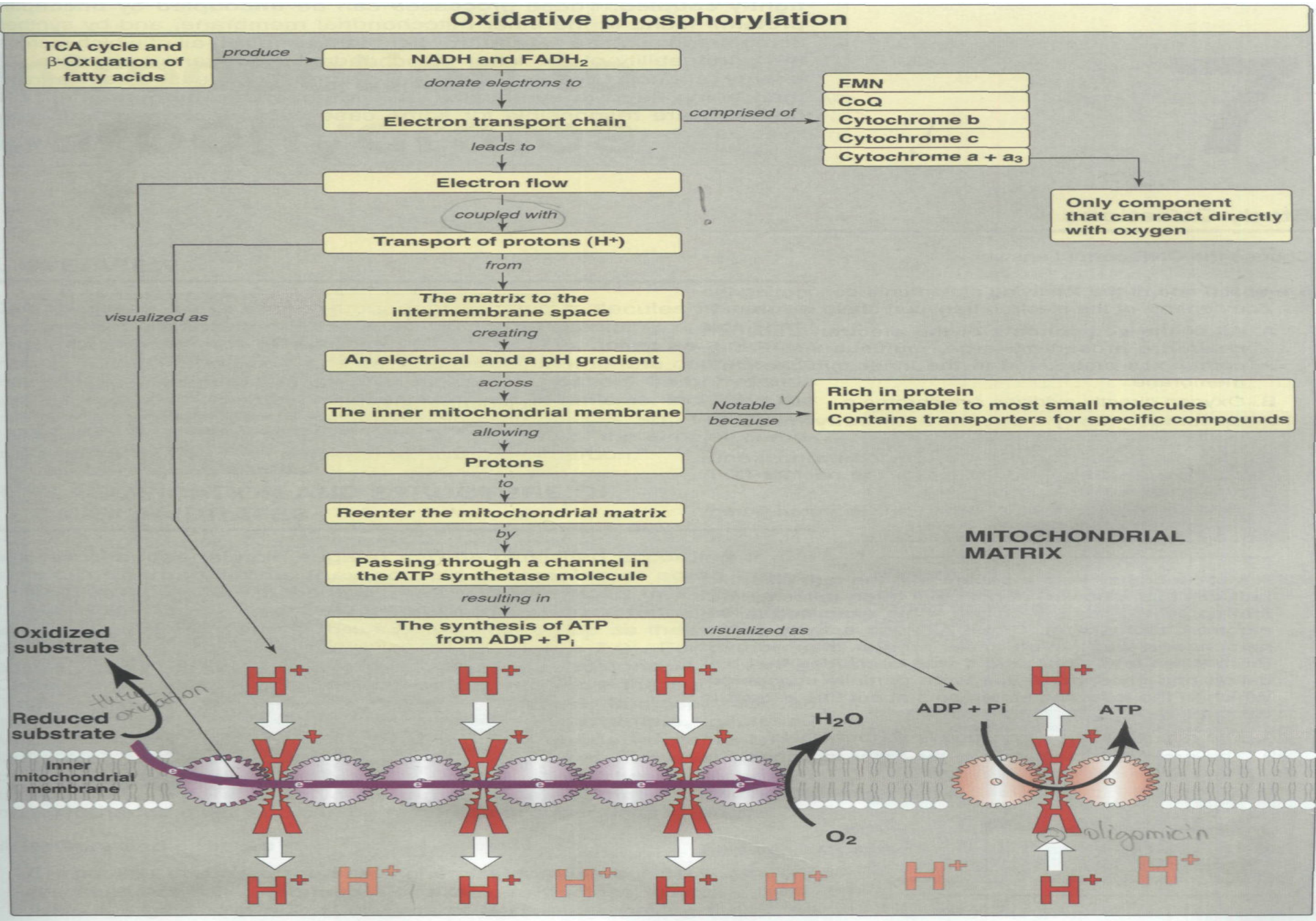


Figure 6.17
Summary of key concepts for oxidative phosphorylation. [Note: Electron flow and ATP synthesis are are envisioned as sets of interlocking gears to emphase the idea of coupling.]

Questions

- **Long Essays.**

- Q.1 Define Biological oxidation. Enumerate and Describe various enzymes carrying out biological oxidation reactions with suitable examples.

- Q.2 Describe Respiratory chain and Give its significance.

OR

- Explain the Electron. Transport chain (E.T.C.) and its significance.

OR

- How the reduced equivalents generated in anaerobic dehydrogenase reactions are reoxidized.

- Q.3 What is oxidative phosphorylation? Explain the mechanism with respect to various theories and hypothesis.

- **Short Notes**
- Cytochromes
- Inhibitors of E.T.C
- Shuttle systems and its significance
- Inhibitors and Uncouplers of oxidative phosphorylation
- Complexes of E.T.C.
- Redox potential and free energy changes.
- Inherited Disorders related to E.T.C. abnormality.
- ATP – Mode of its formation and it's role in the Body.

Short Answer Questions

- Give the sites for ATP generation of in E.T.C.
- Enumerate the High energy compounds of our body
- Substrate level phosphorylation and it's importance.
- Enumerate the Enzymes catalyzing Biological oxidation reactions. Write the class to which these enzymes classified.

- Define P.O ratio. What is the P:O ratio for reduced $NADH+H^+$ & $FADH_2$ respectively.
- List the components of E.T.C. and their location.
- Redox couple & Redox potential.

- FlavoProteins
- Product of Aerobic and Anaerobic dehydrogenation reactions.
- Write enzymes catalyzing Aerobic and Anaerobic dehydrogenation reaction's during metabolism.

THANK YOU