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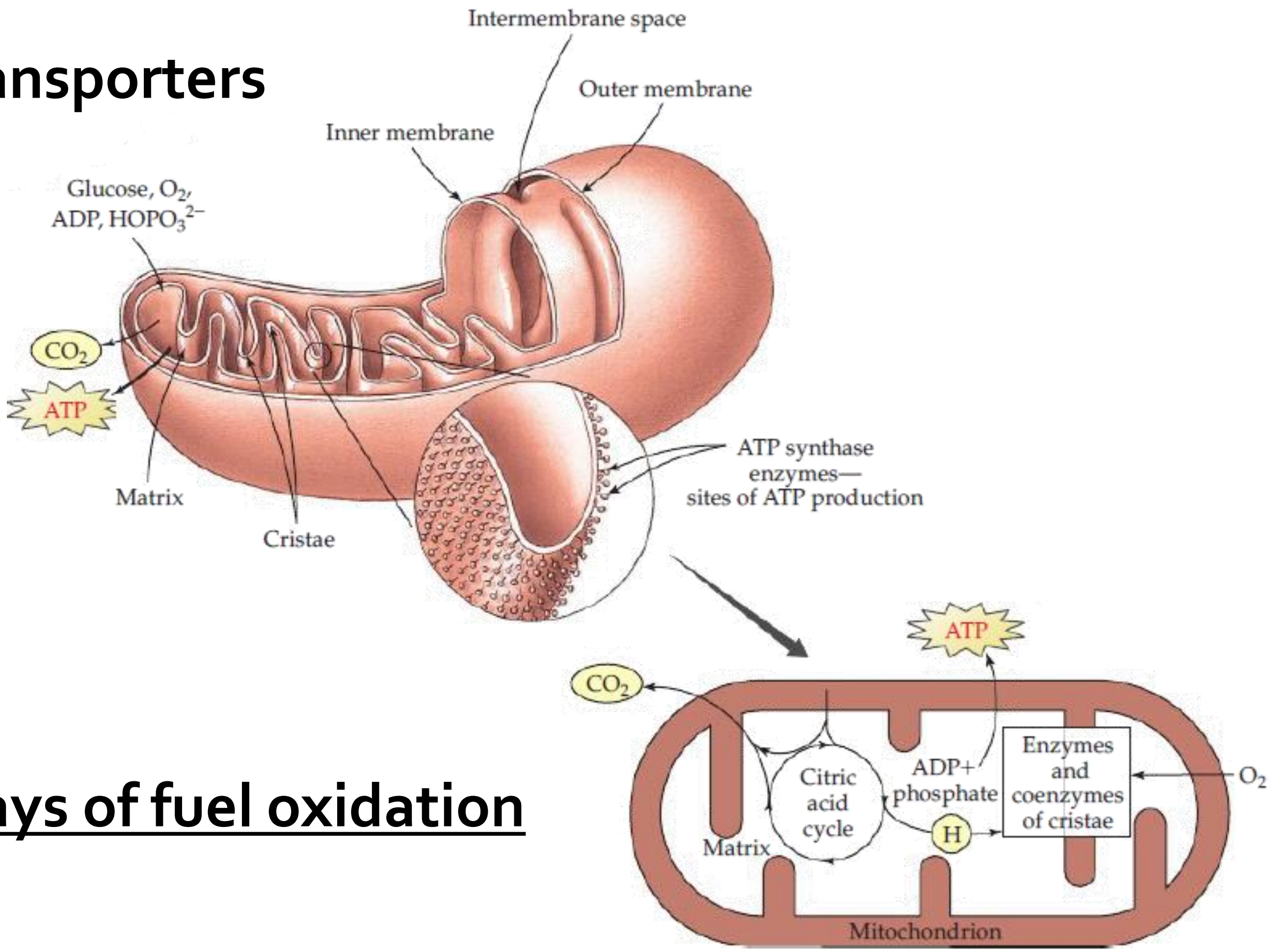
Oxidative Phosphorylation

The Mitochondria

- OMM: permeable to small molecules (MW<5,000) & ions, porins (transmembrane channels)
- IMM: impermeable even to H+; specific transporters

- IMM bears the components of the respiratory chain and the ATP synthase
- Matrix: contains pyruvate dehydrogenase complex & TCA cycle enzymes, fatty acid β-oxidation pathway, and the pathways of amino acid oxidation

- In other words: matrix contains all pathways of fuel oxidation except glycolysis (cytosol)



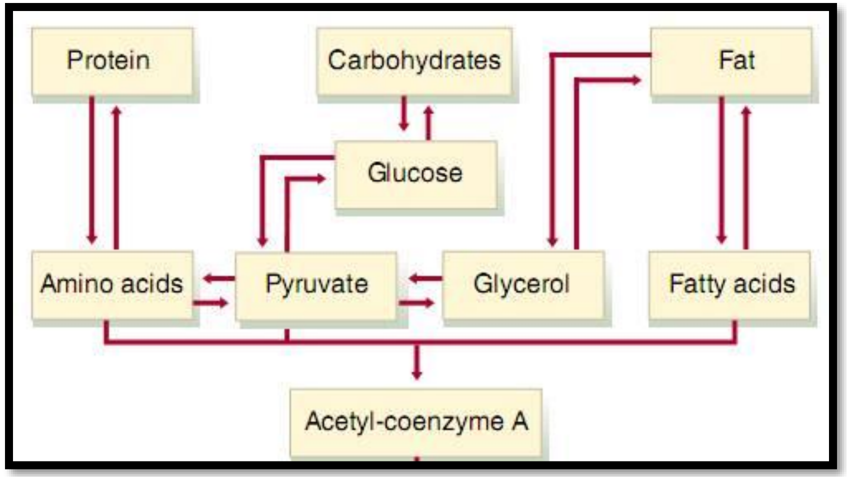
Mitochondrial Membranes

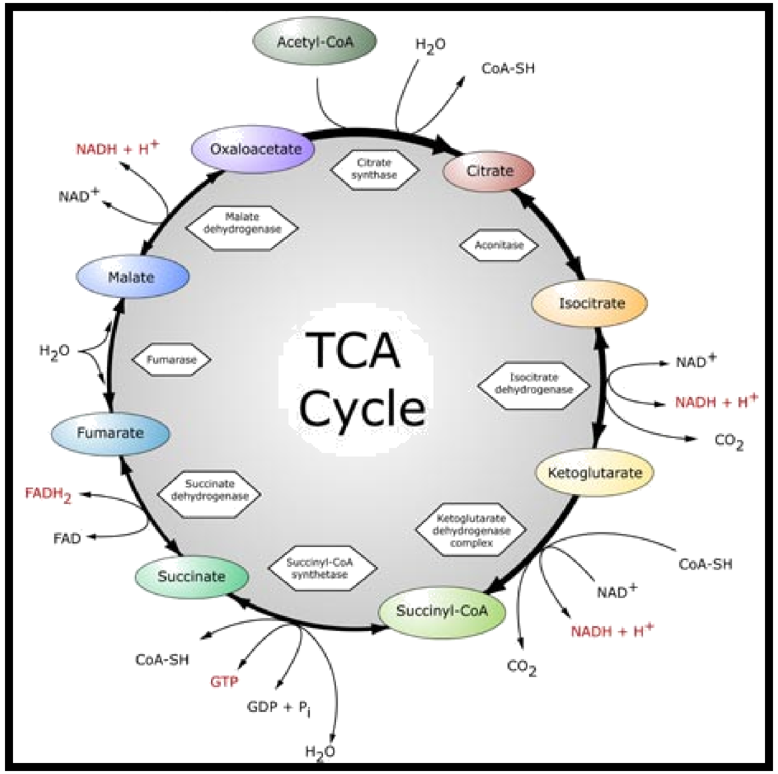
- ✓ Inner membrane:
  - ✓22% cardiolipin
  - ✓No cholesterol
- ✓ Outer membrane:
  - ✓Similar to cell membrane
  - ✓Less than 3% cardiolipin
  - ✓45% cholesterol

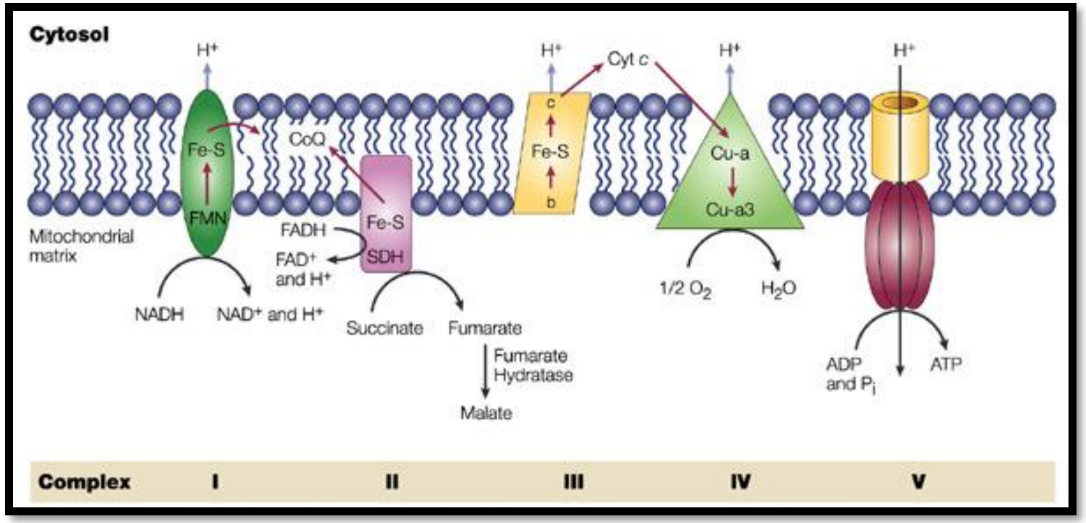
TABLE 20.3: Location of enzymes in mitochondria
Mitochondria, outer membrane:
Monoamino oxidase
Acyl CoA synthetase
Phospholipase A2
In between outer and inner membrane:
Adenylate kinase
Creatine kinase
Inner membrane, outer surface:
Glycerol-3-phosphate dehydrogenase
Inner membrane, inner surface:
Succinate dehydrogenase
Enzymes of respiratory chain
Soluble matrix:
Enzymes of citric acid cycle
Enzymes of beta oxidation of fatty acid

The oxidative phosphorylation, Where are we?

- Stages: Digestion; Acetyl-CoA, TCA, OxPhos

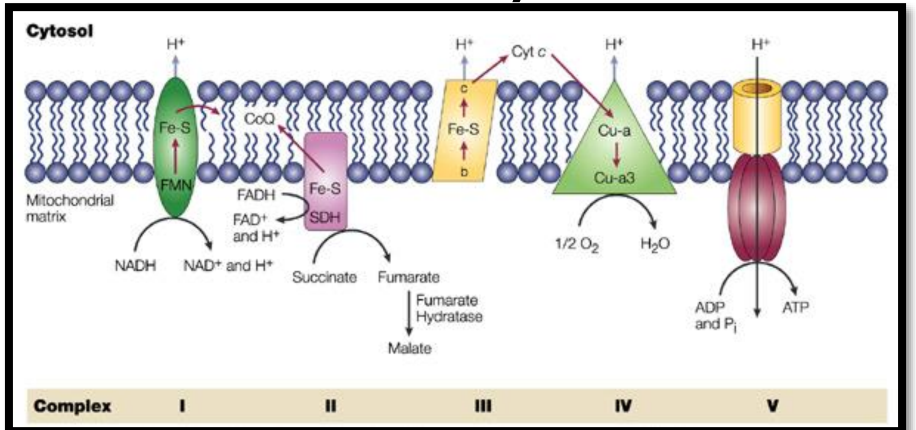




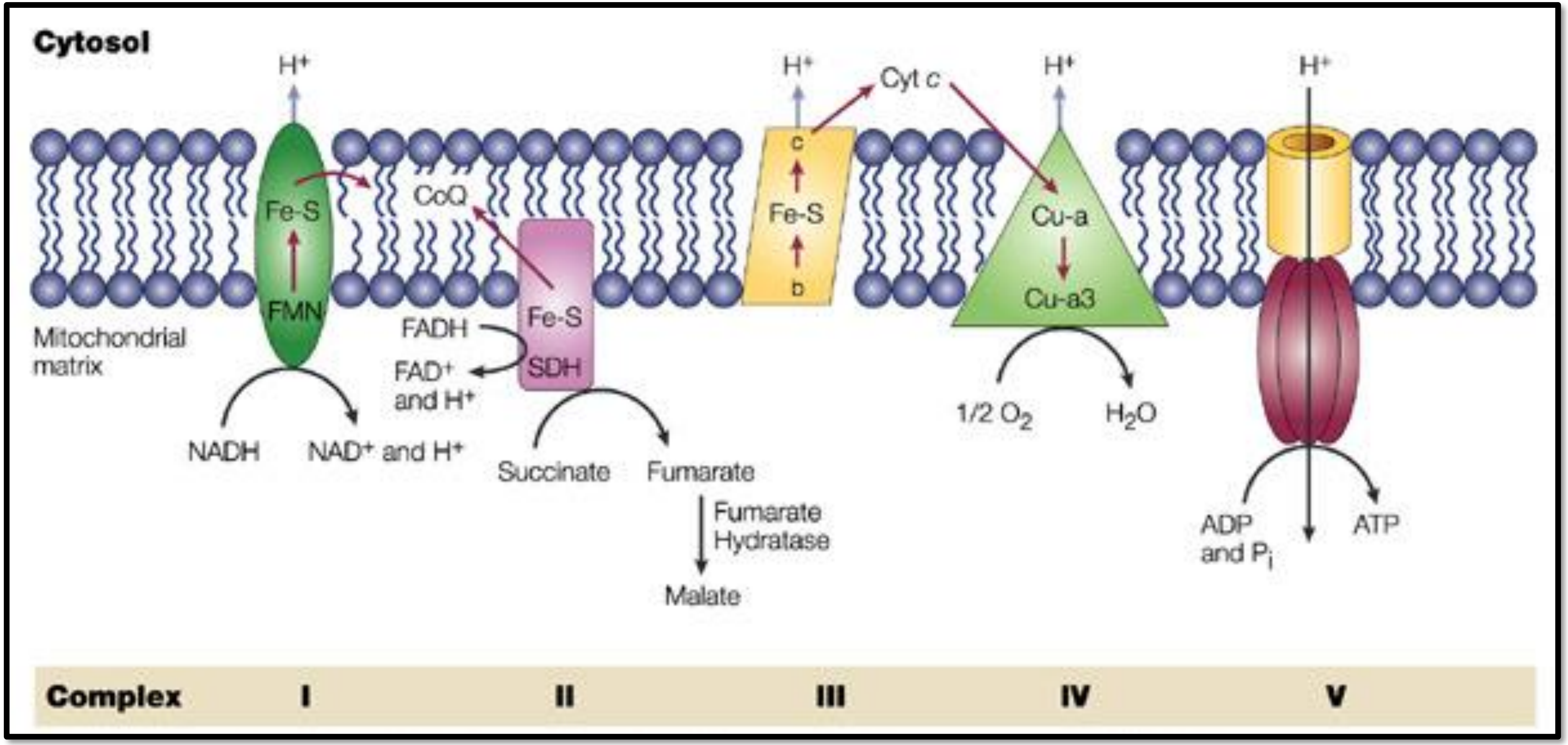


Oxidative phosphorylation (OxPhos)

- Generation of ATP aided by the reduction of O_2
- Peter Mitchell (1961): the chemiosmotic theory
- Oxidative phosphorylation have 3 major aspects:
  - ✓ (1) It involves flow of electrons through a chain of membrane-bound carriers (prosthetic groups)
  - ✓ (2) The free energy available (exergonic) is coupled to transport protons across a proton-impermeable membrane
  - ✓ (3) The transmembrane flow of protons down their concentration gradient provides the free energy for synthesis of ATP (ATP synthase)

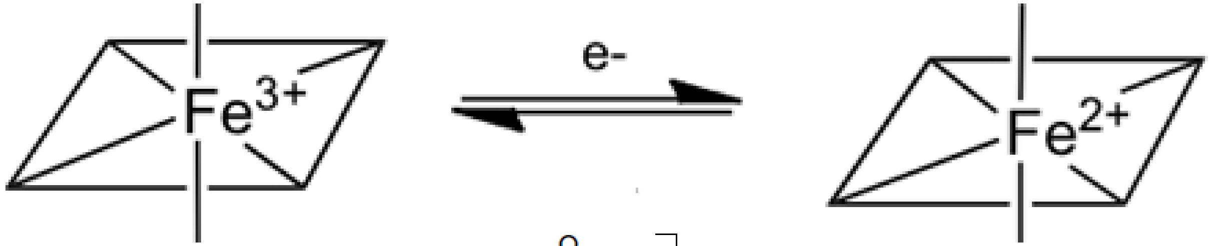


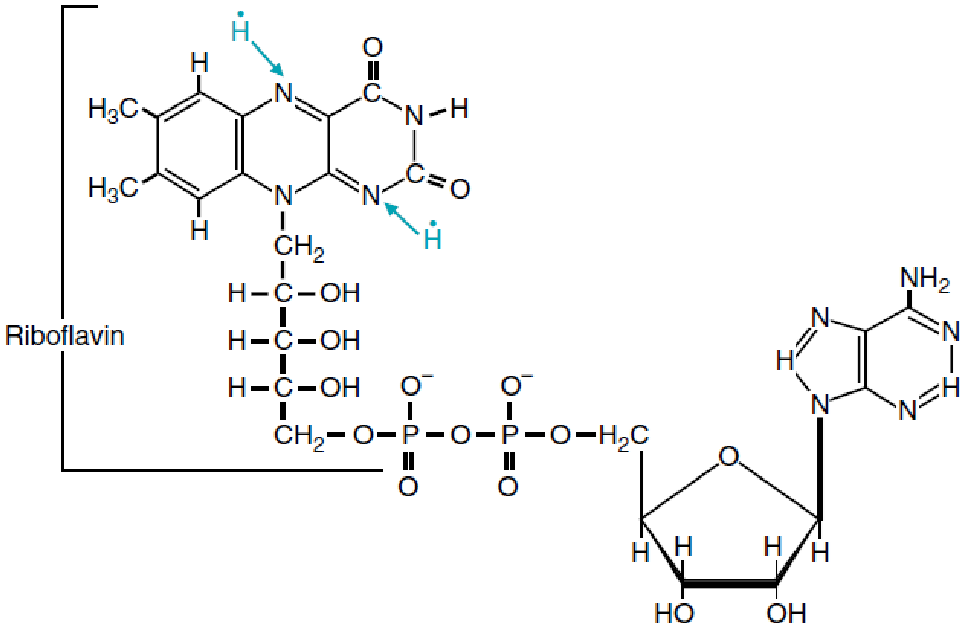
Oxidative phosphorylation (OxPhos)

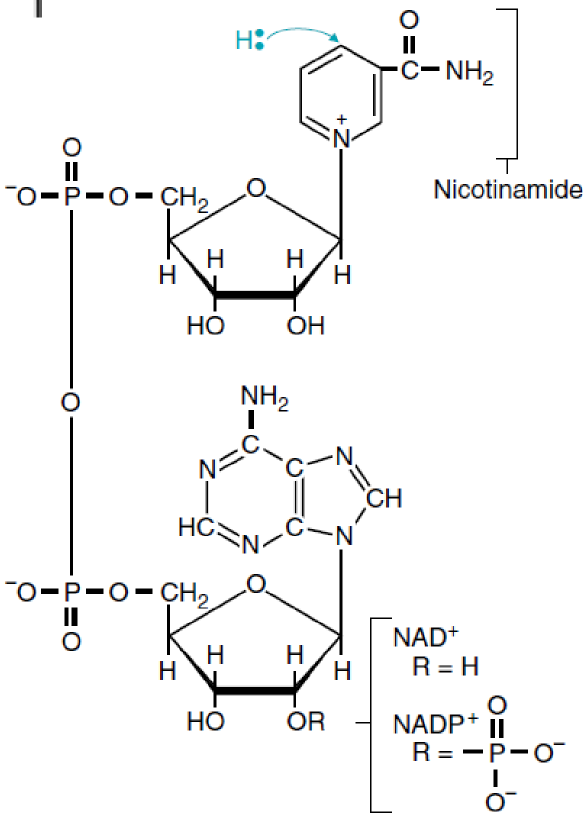


Types of electron transfer (ET) through the electron transport chain (ETC)

- 3 types of ET occur in OxPhos:
  - ✓ Direct ET, as in the reduction of Fe^{+3} to Fe^{+2}
  - ✓ Transfer as a hydrogen atom $\{(H^+) + (e^-)\}$
  - ✓ Transfer as a hydride ion $(:H^-)$

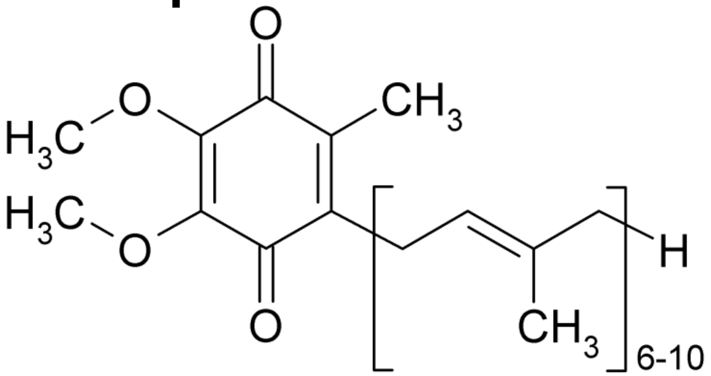


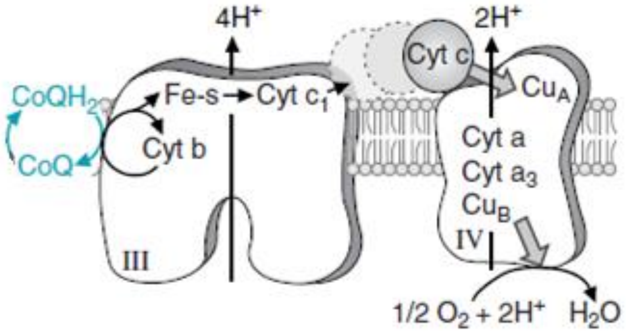


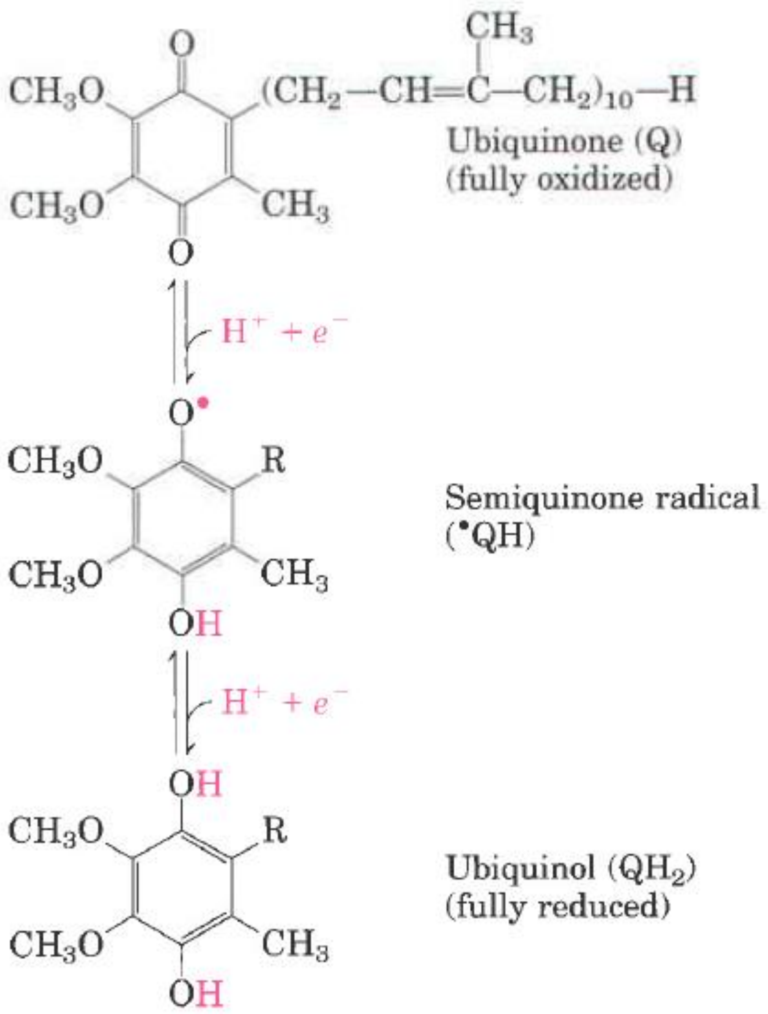


Other electron-carrying molecules "Ubiquinone"

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

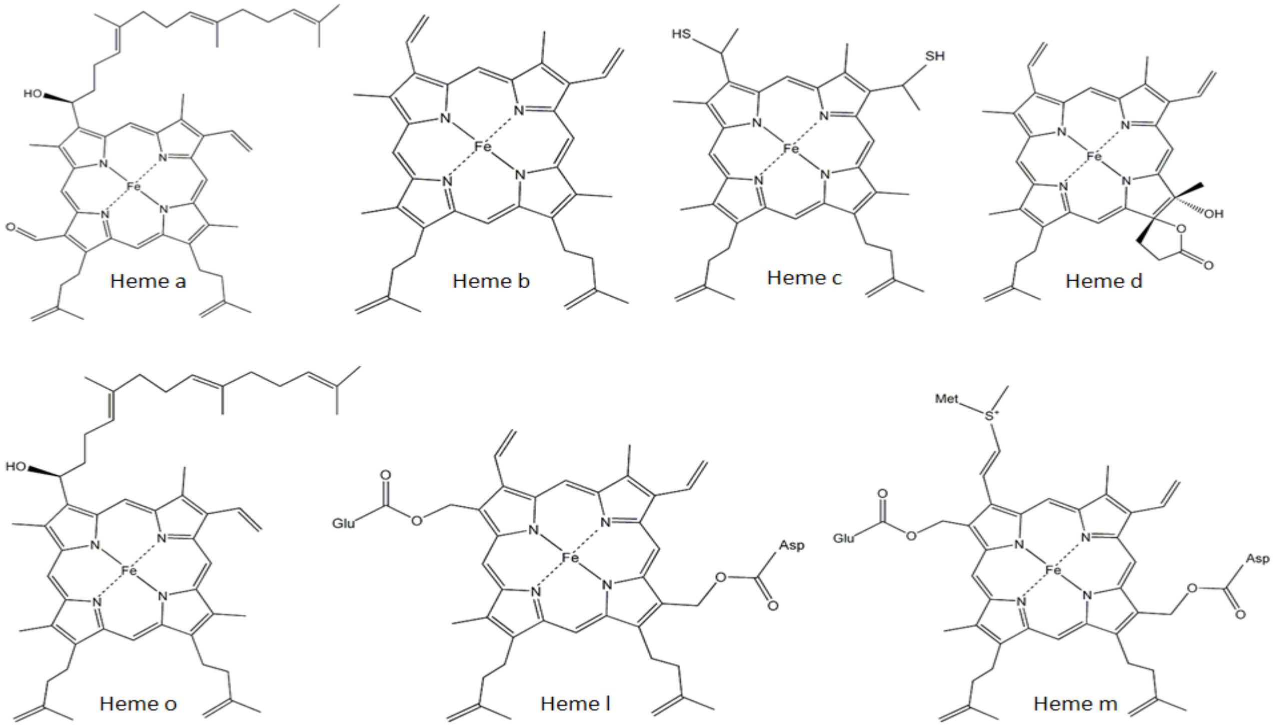


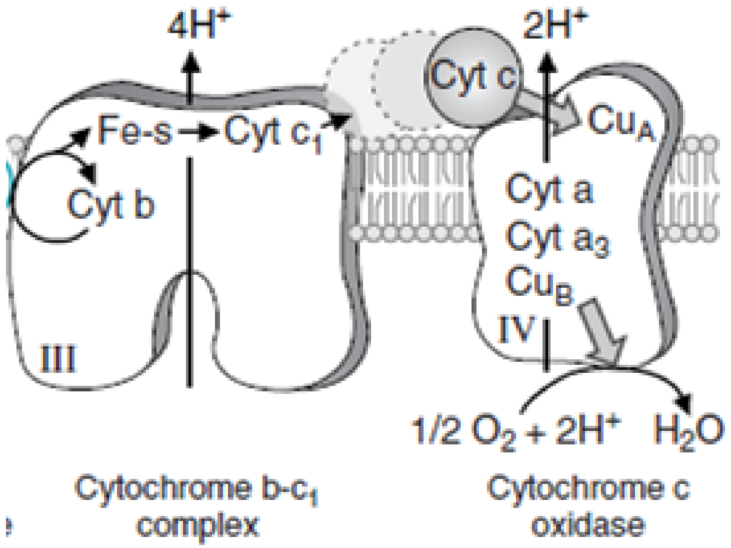




Other electron-carrying molecules "Cytochromes"

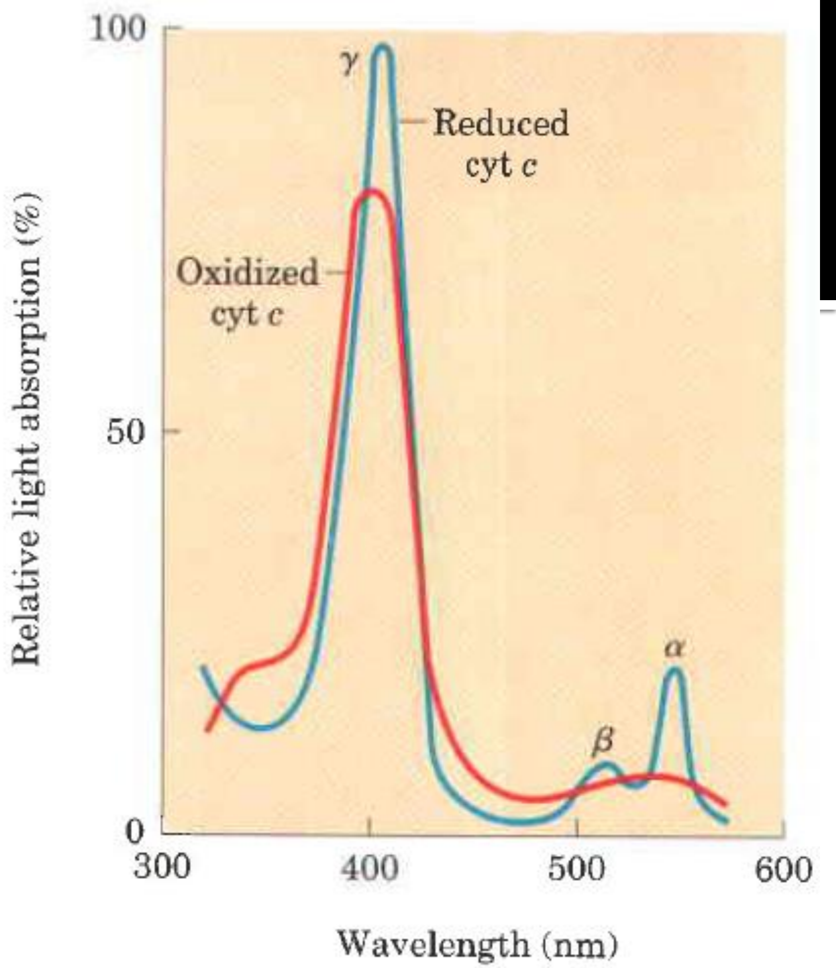
- Proteins with characteristic strong absorption of visible light (Fe-containing heme prosthetic groups)
- Classification based on light absorption
- Mode of binding (a, b, c)
- Mitochondria contain three classes o f cytochromes (a , b, & c)



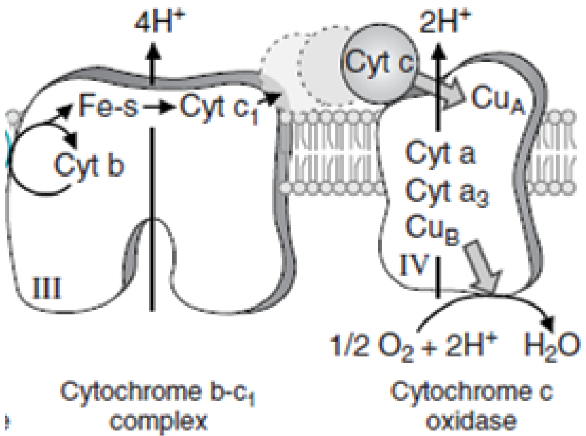


Other electron-carrying molecules "Cytochromes"

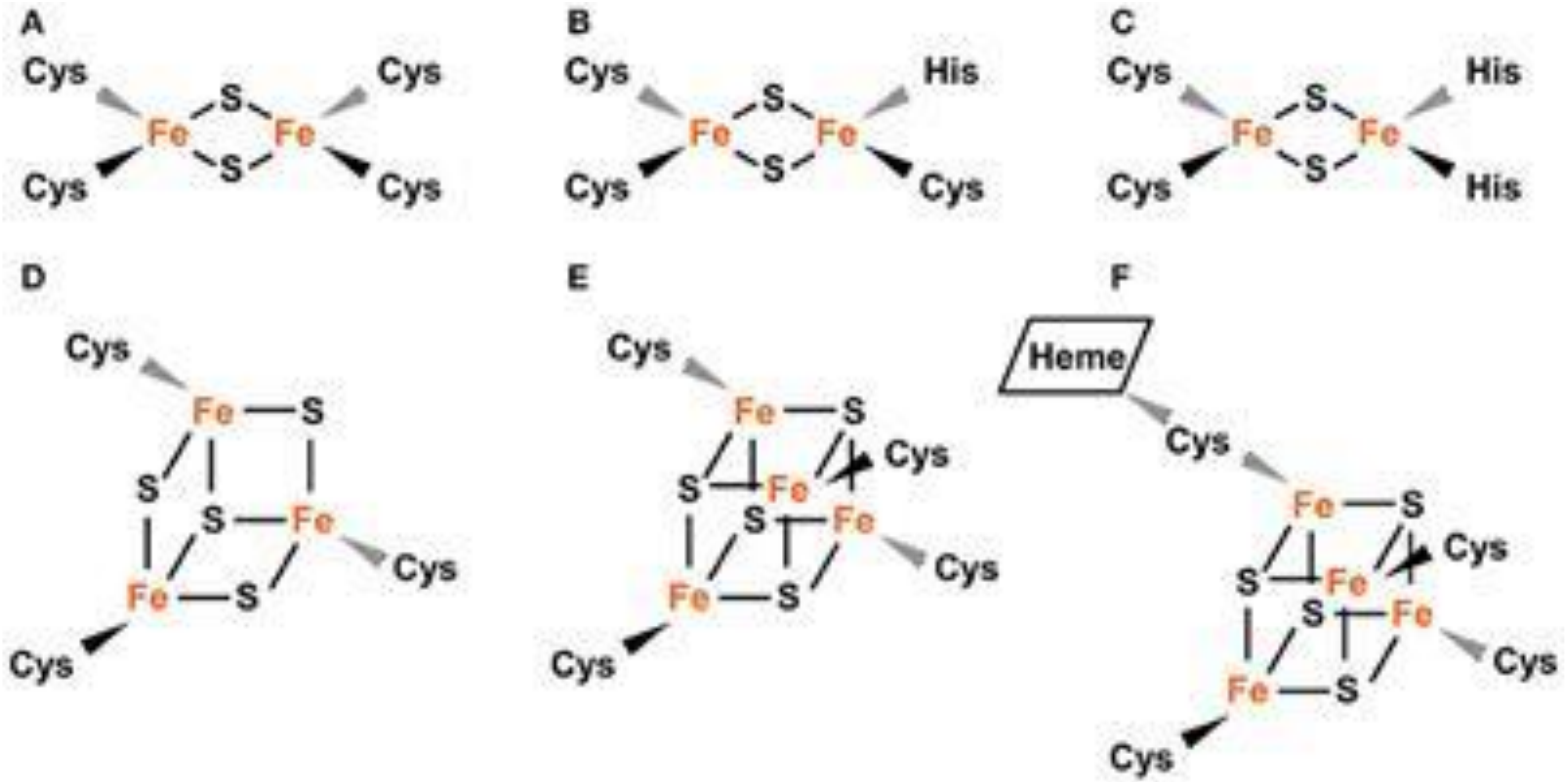
- Light absorption: Each cytochrome in its reduced (F^{+2}) state has 3 absorption bands in the visible range
- α band : near 600 nm in type a; near 560 nm in type b, & near 550 nm in type c
- Some cytochromes are named by the exact α band wavelength:
  - ✓ Cytochrome b_{562}; Cytochrome c_{550}; Cytochrome c_{551}



- Heme can carry one electron
- *ΔE°′* depends on the protein
- Cytochromes a, b & c are transmembrane (c is the exception)

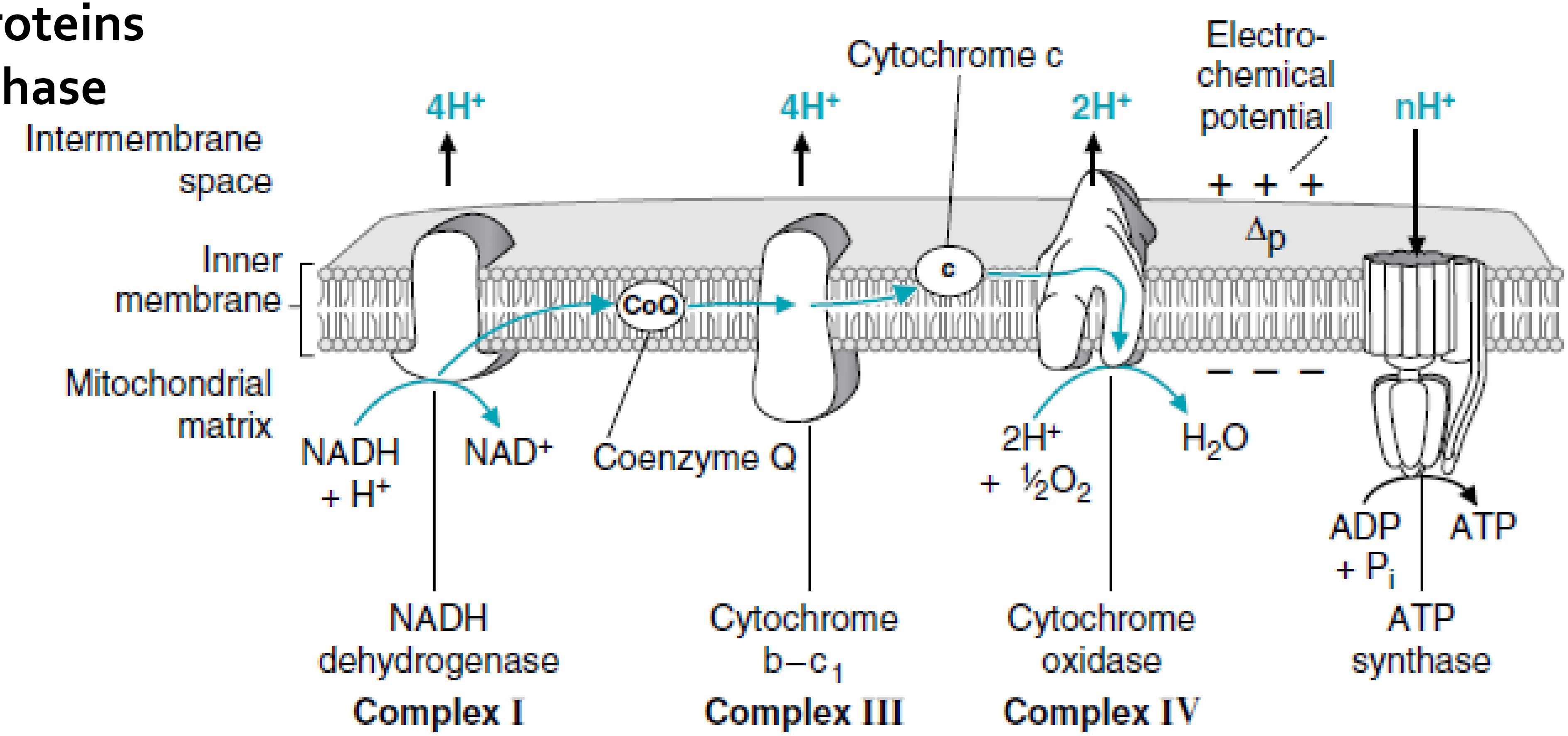


Other electron-carrying molecules
Iron-Sulfur Clusters

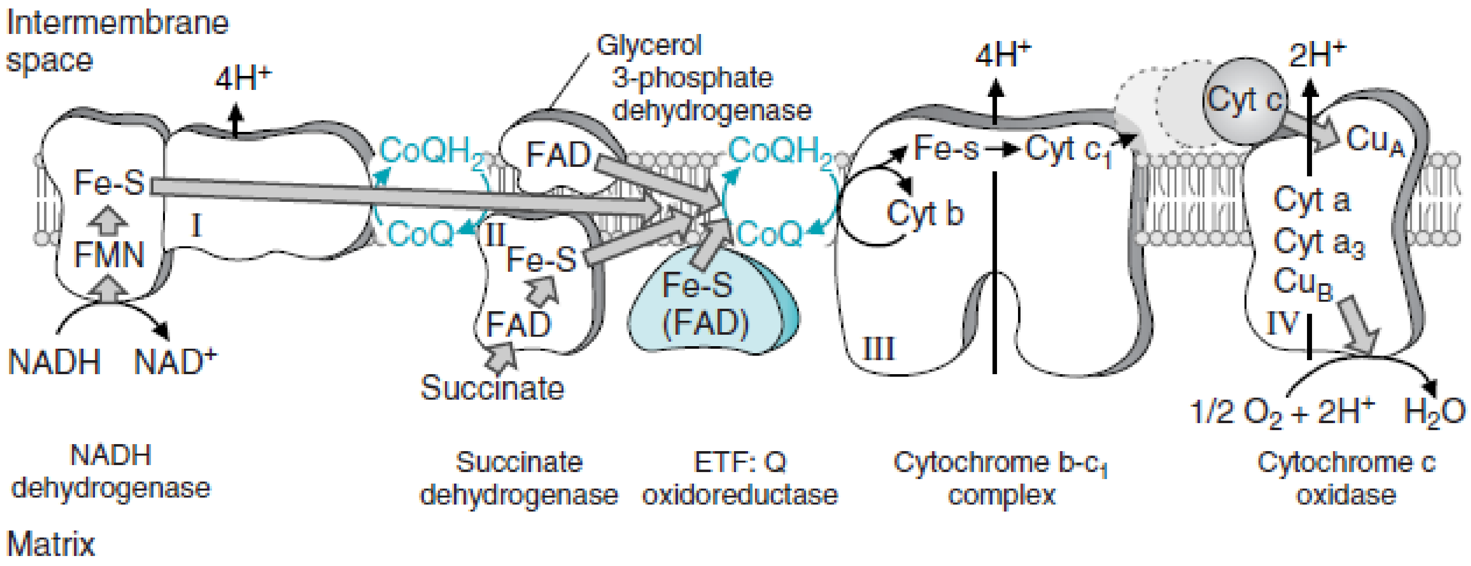


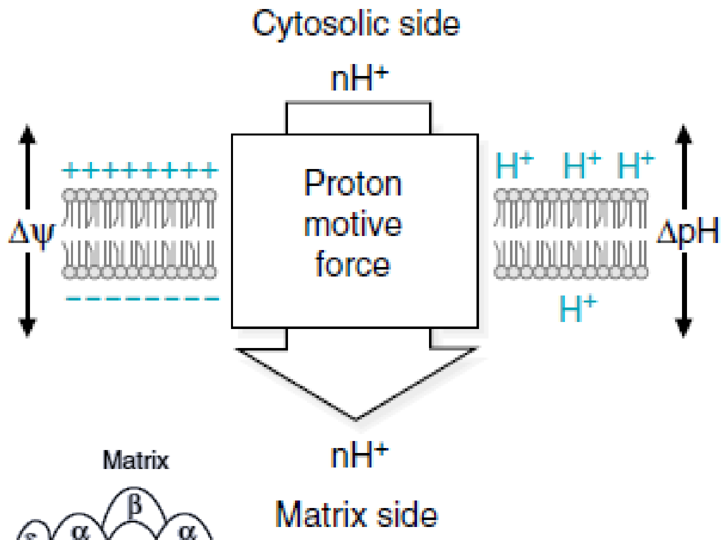
Requirements of OxPhos

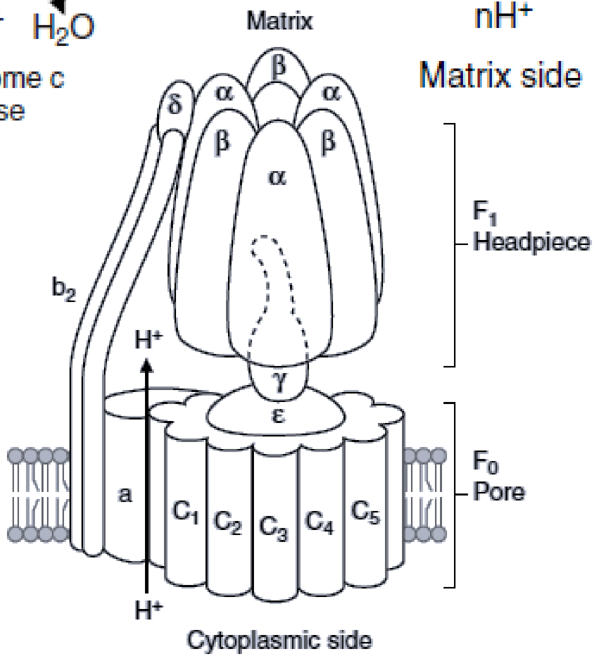
- Redox reaction: electron donor (NADH or FADH2) & electron acceptor (O2)
- An intact IMM
- ETC of proteins
- ATP synthase



ET to O_2, how does the process occurs?
"The chemi-osmotic theory"

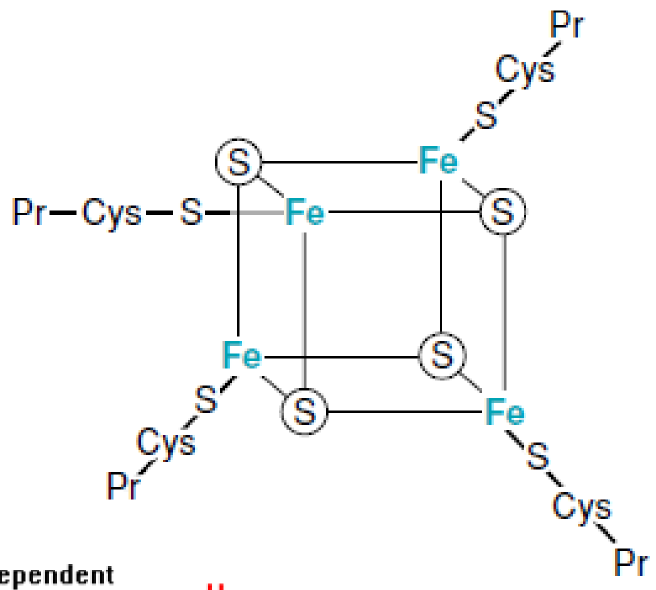




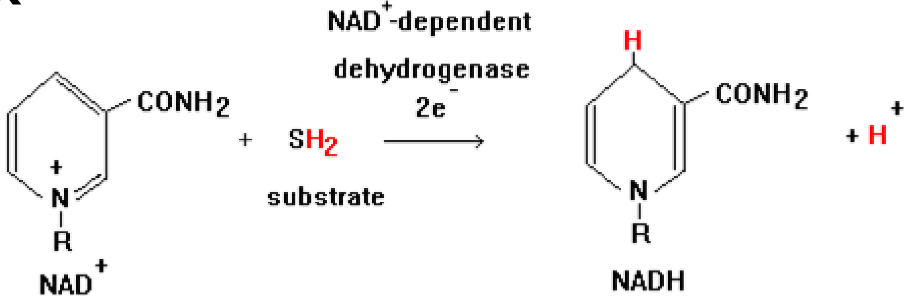


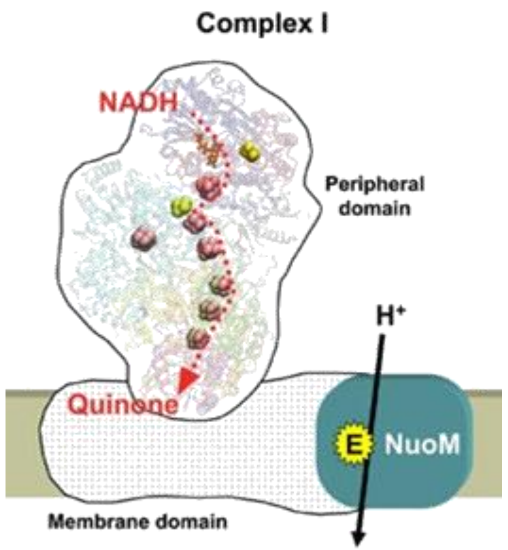
- Membrane is impermeable to protons
- ≈ - 12 kcal with each step

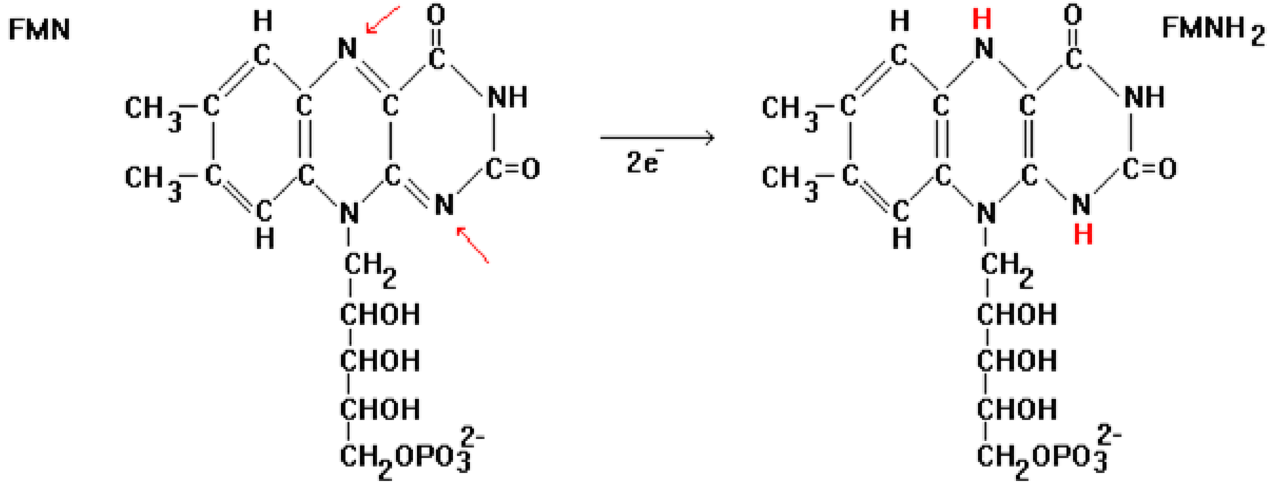
Oxi–Red Components of the ETC
"NADH Dehydrogenase" – Complex I



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

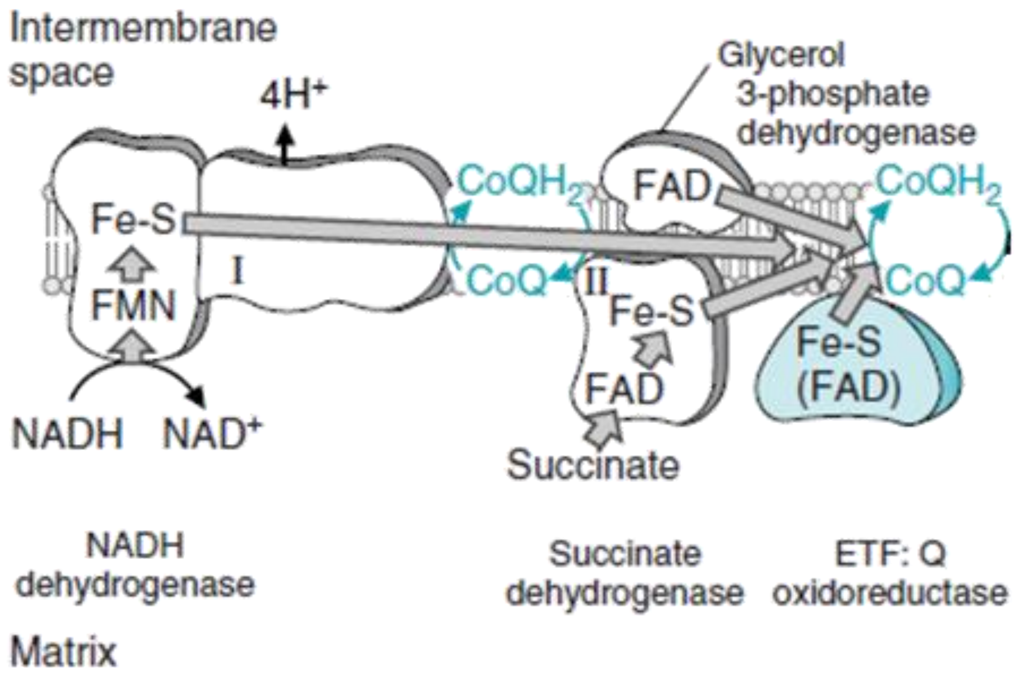


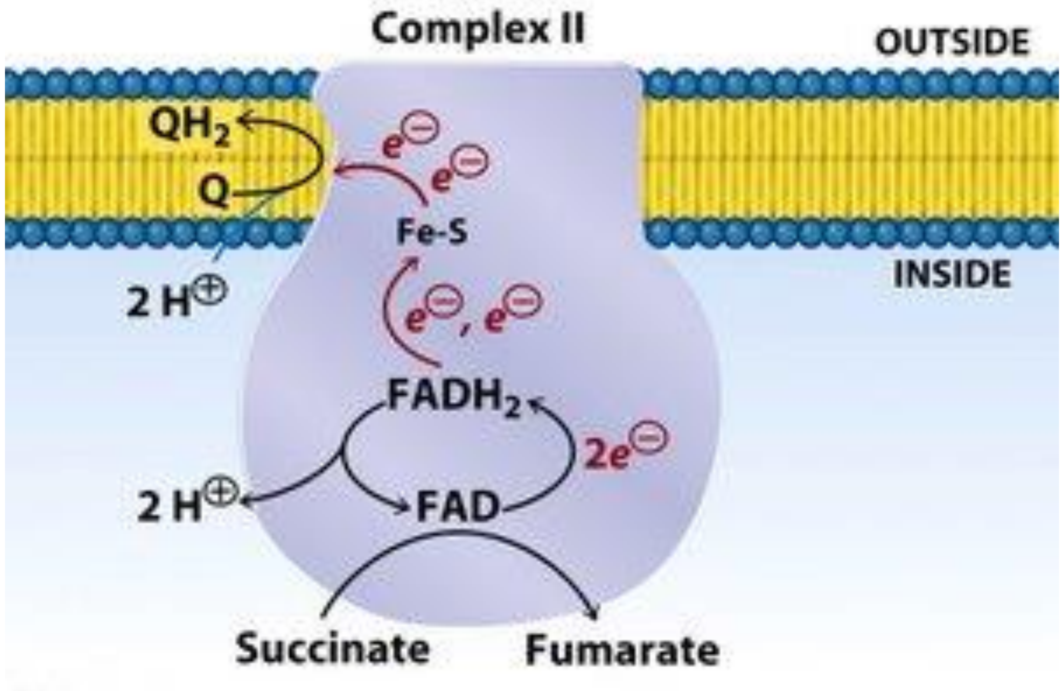




Oxi–Red Components of the ETC
"Succinate Dehydrogenase" – Complex II

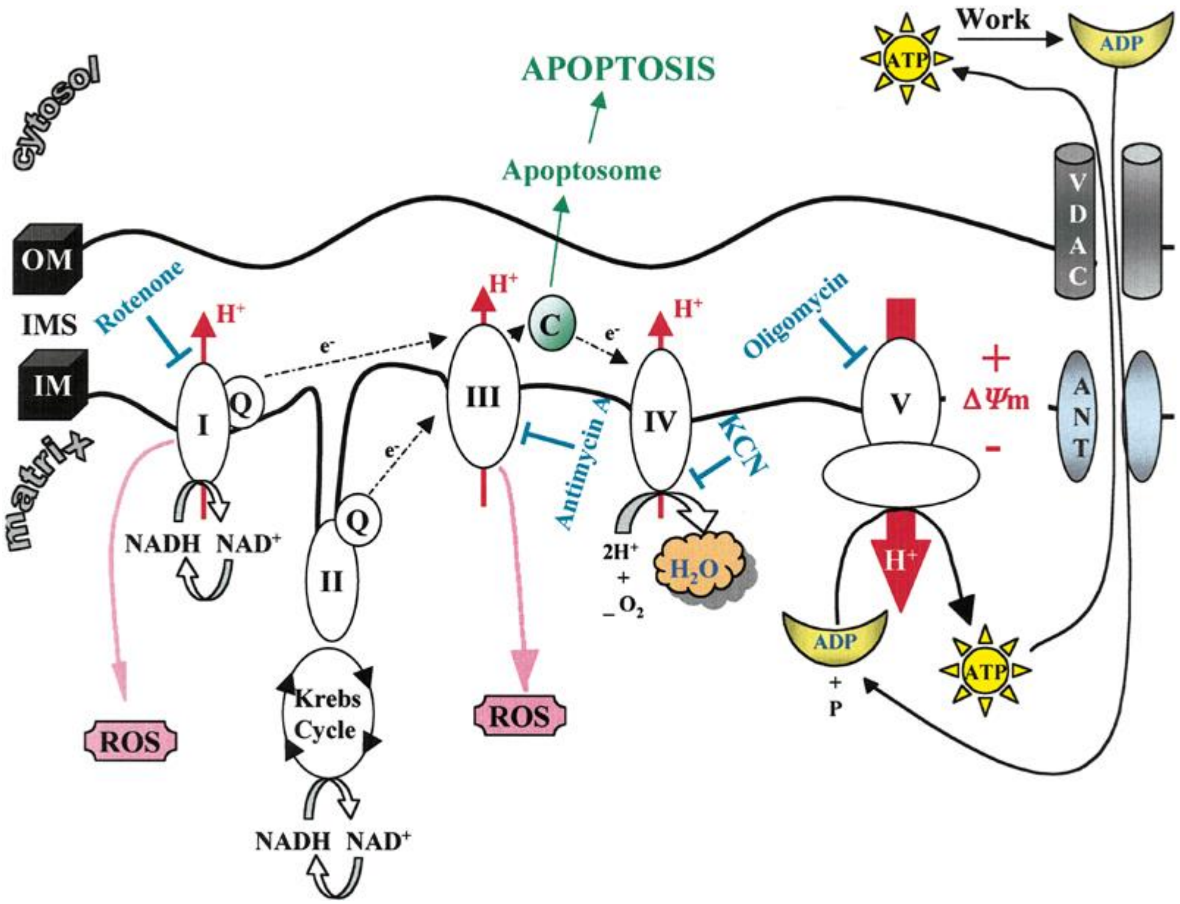
- Succinate Dehydrogenase & other flavoproteins
- TCA cycle
  - ✓ ETF-CoQ oxidoreductase (ex. fatty acid oxidation)
  - ✓ ≈ 0 kcal, H+?





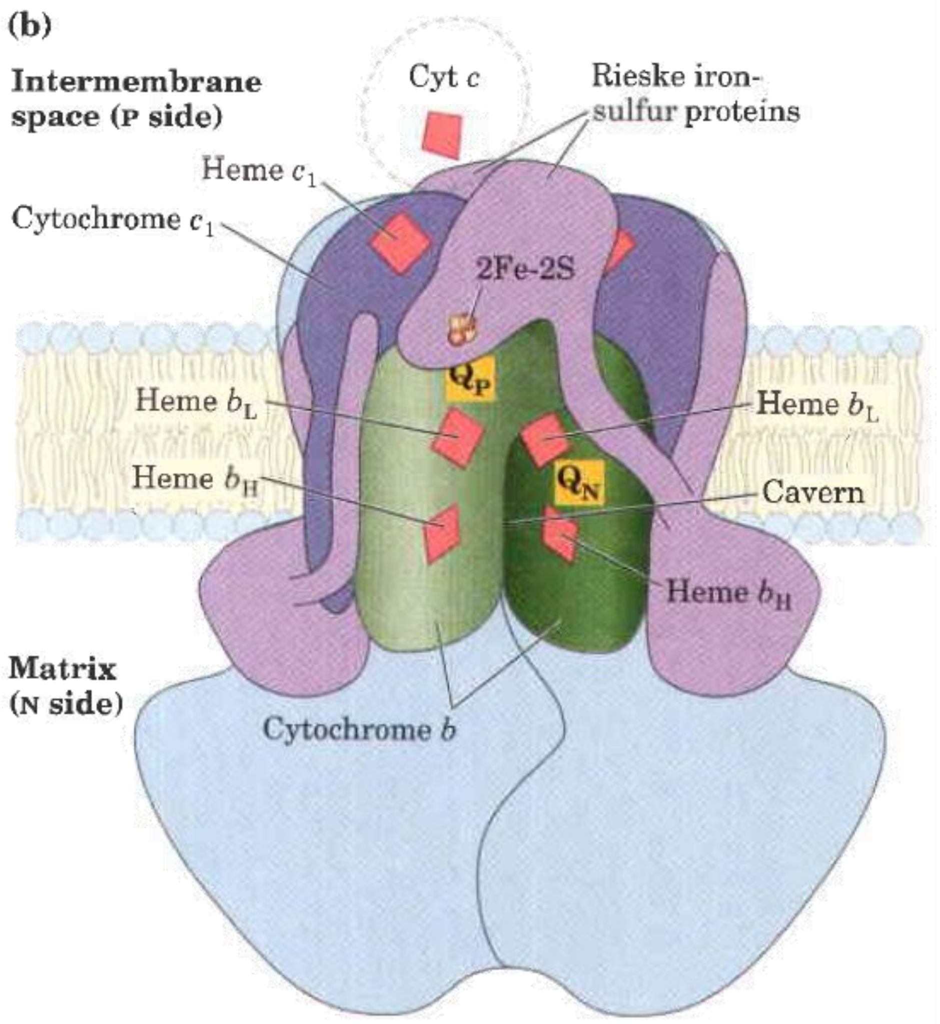
Oxi–Red Components of the ETC
"Succinate Dehydrogenase" – Complex II

- ✓ Substrates oxidized by FAD-linked enzymes bypass complex-I
- ✓ Three major enzyme systems:
  - ✓ Succinate dehydrogenase
  - ✓ Fatty acyl CoA dehydrogenase
  - ✓ Mitochondrial glycerol phosphate dehydrogenase



Oxi–Red Components of the ETC
"Cytochrome *bc1*" – Complex III

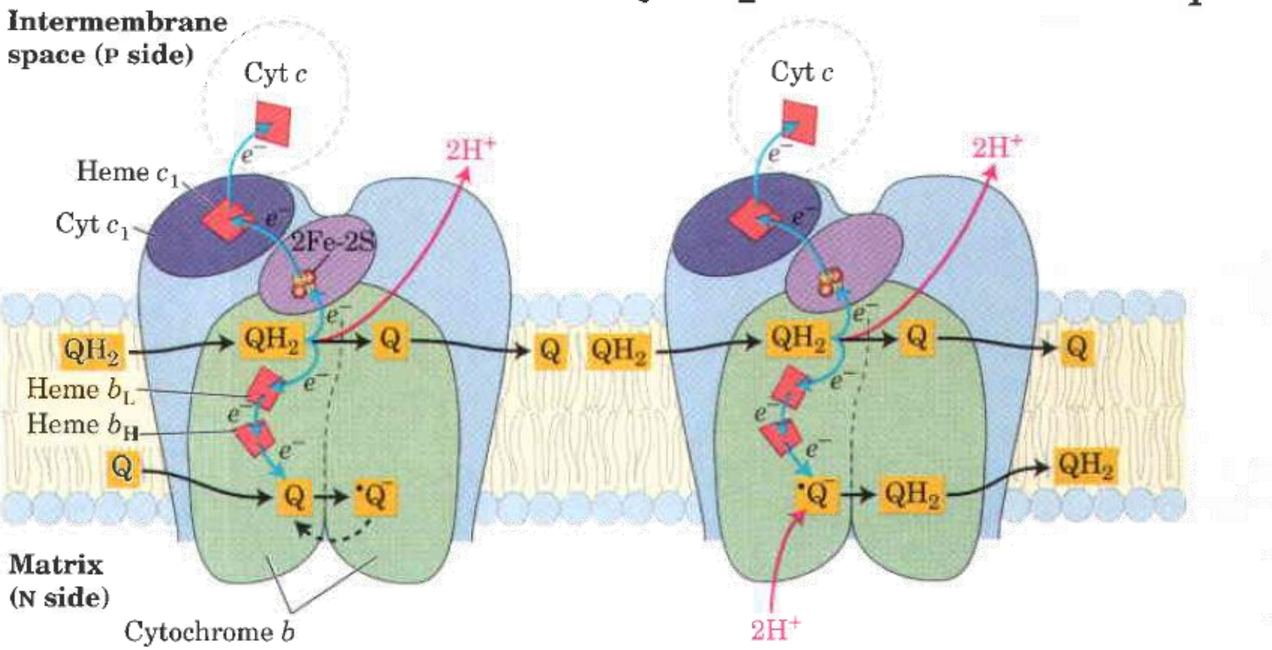
- Also called: Q-cytochrome c Oxidoreductase
- Catalyzes the transfer of electrons from QH2 to cytochrome c
- 11 subunits including two cytochrome subunits
- Contains iron sulfur center
- Contain three heme groups in two cytochrome subunits
- b_L and b_H in cytochrome b; c type in cytochrome c1
- Contain two CoQ binding sites
- 4H+



The Q-cycle

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

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



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

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

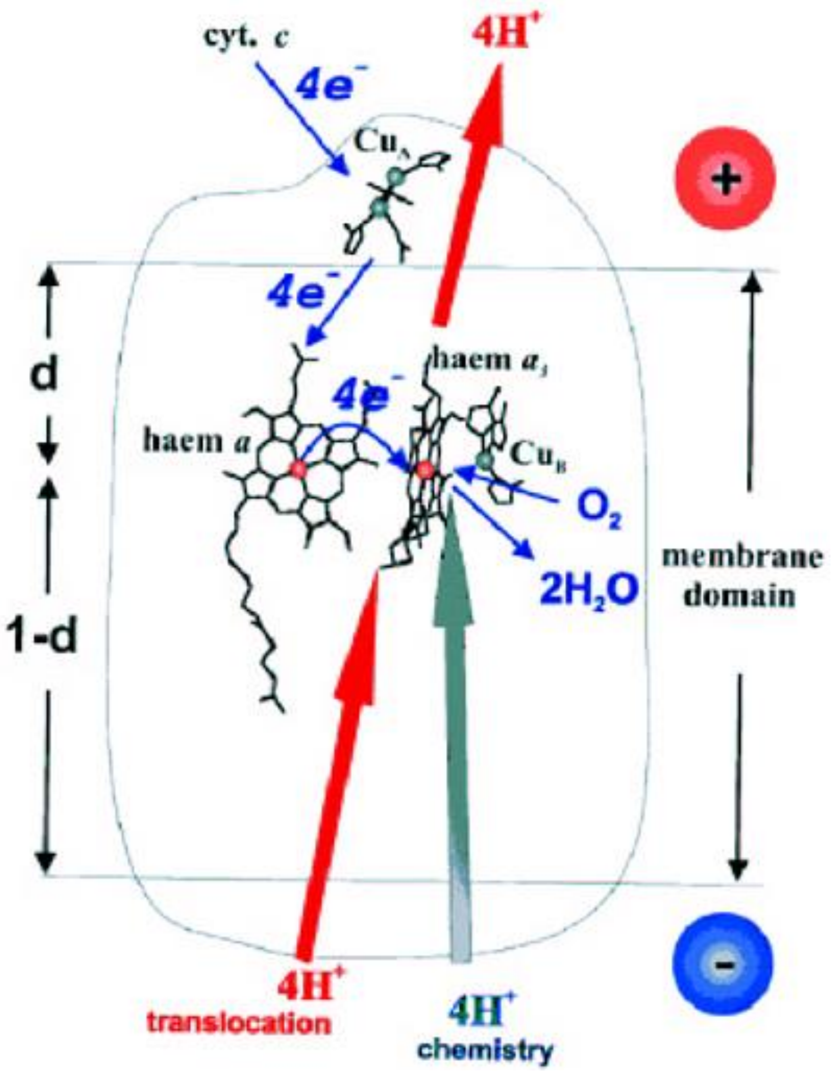
Net equation: $QH_2 + 2\ \text{cyt}\ c_1\ (\text{oxidized}) + 2H^+_N \longrightarrow Q + 2\ \text{cyt}\ c_1\ (\text{reduced}) + 4H^+_P$

Oxi–Red Components of the ETC "Cytochrome c oxidase" – Complex IV

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

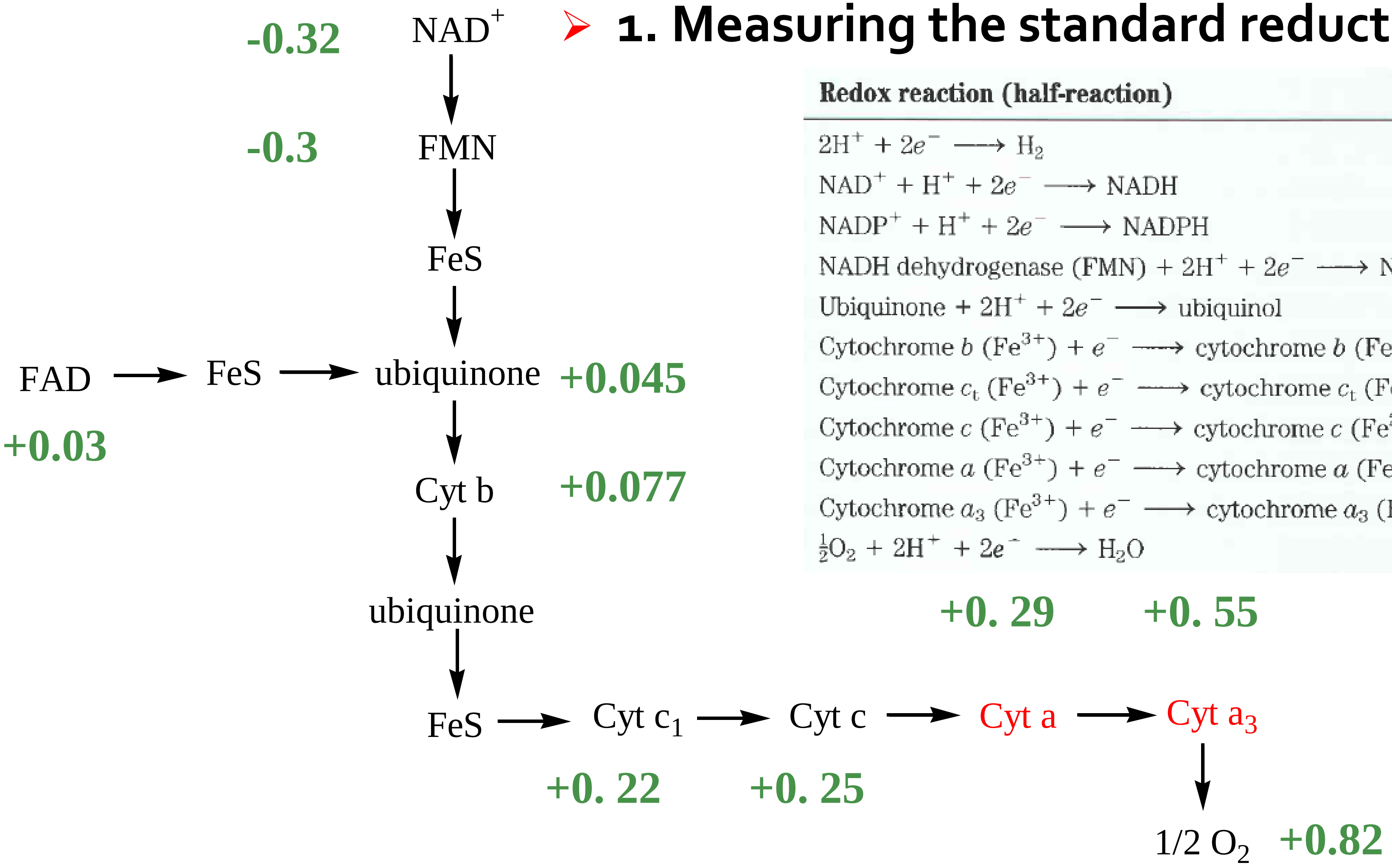
$$\text{Cyt } c_{red} + 4H^+ + O_2 \rightarrow \text{Cyt } c_{ox} + 2H_2O$$

- Cytochrome oxidase has a much lower Km for O2 than myoglobin (hemoglobin, myoglobin, complex IV)
- Partial reduction of O2 is hazardous



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

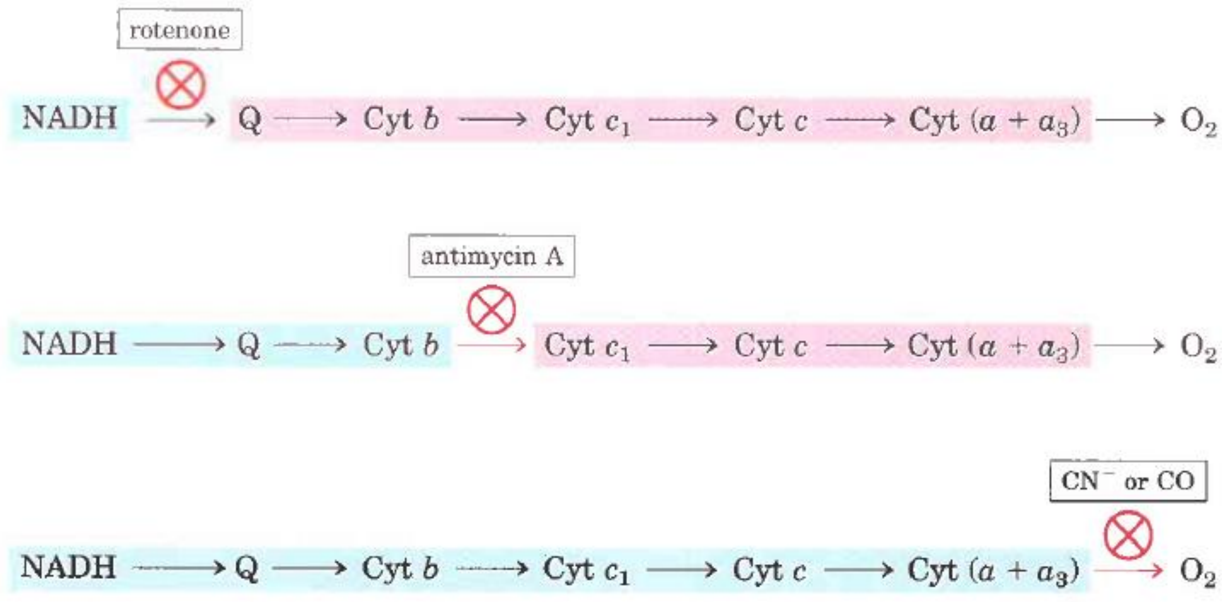
NADH → Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O2

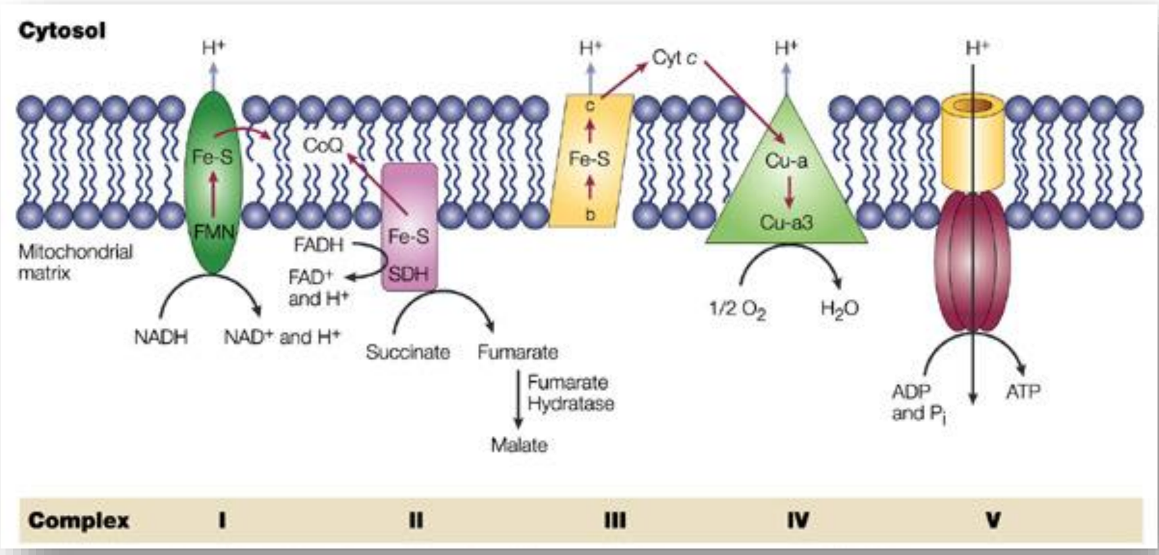
The right arrangement
How can we prove it?

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

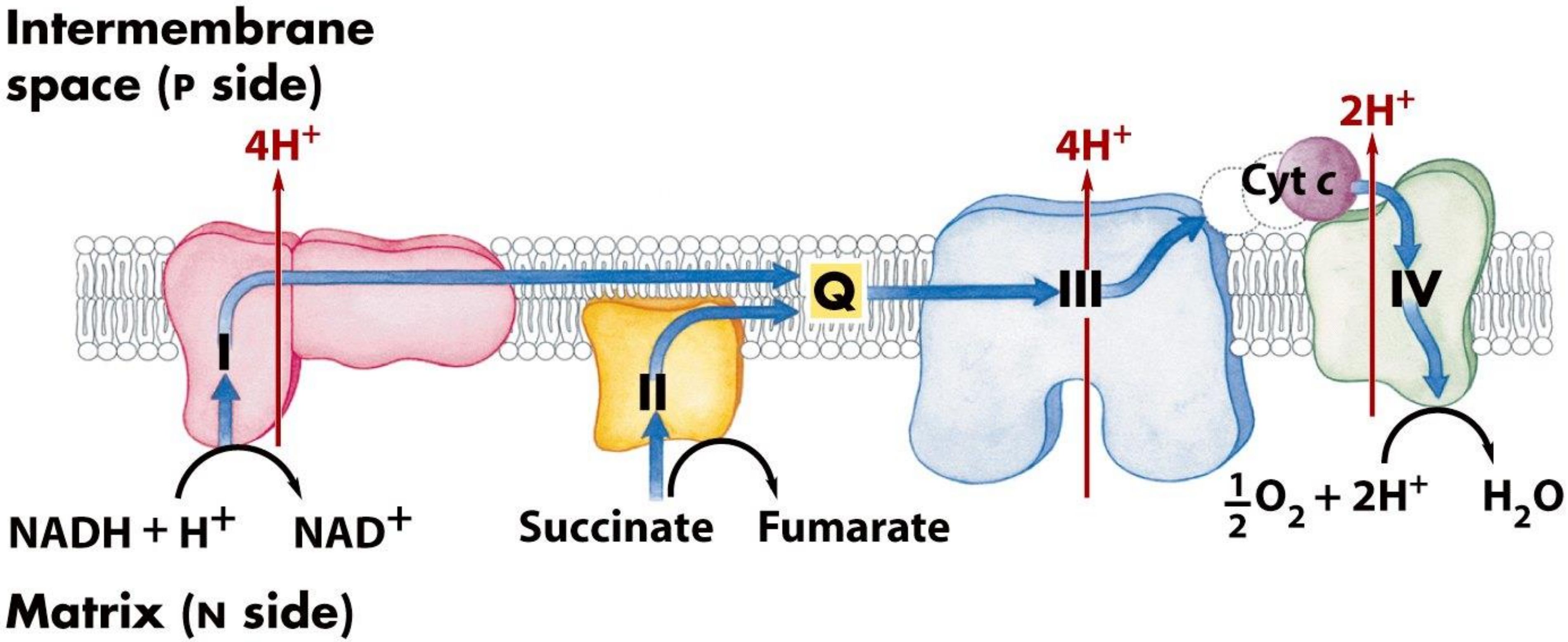
- 2. Reduction of the entire ETC with no O2

- 3. Addition of inhibitors





Pumping of Protons



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