Bioenergetics (342 بف)

Lecture 5

Prepared by:
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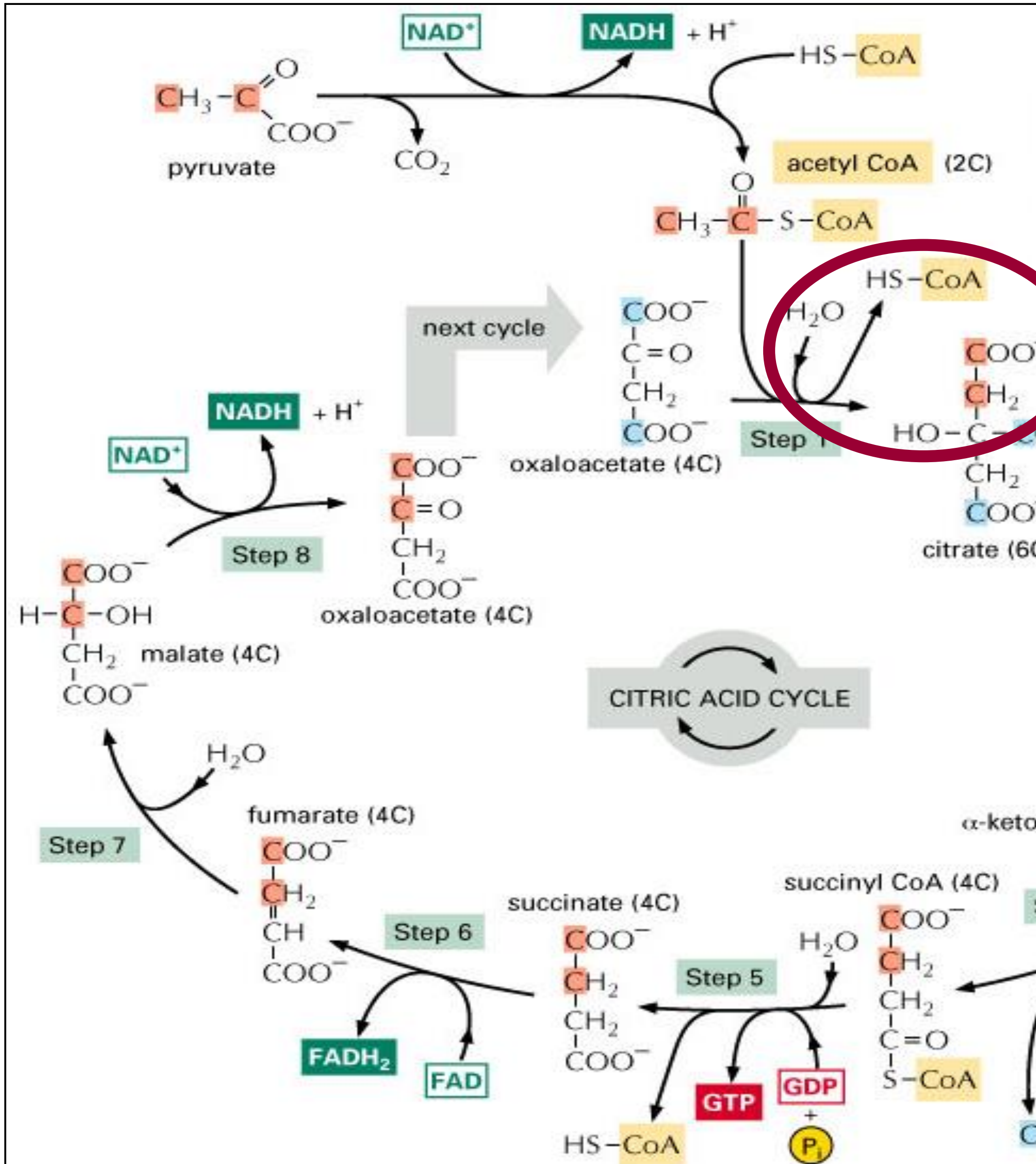
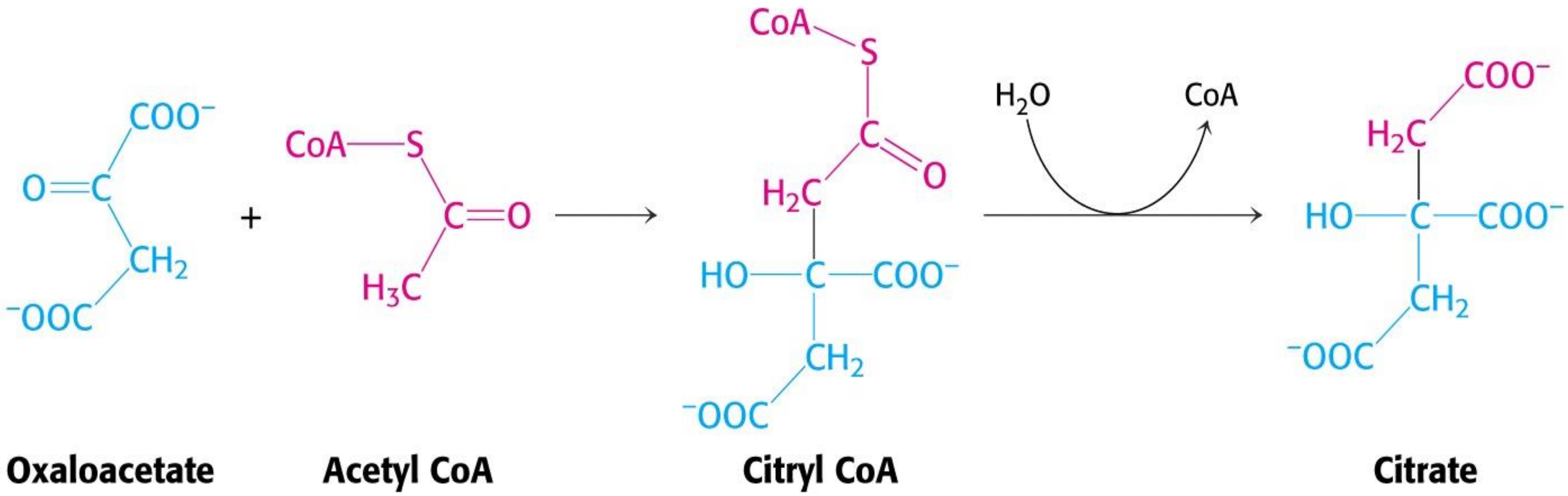
Citric acid cycle

Aerobic Respiration



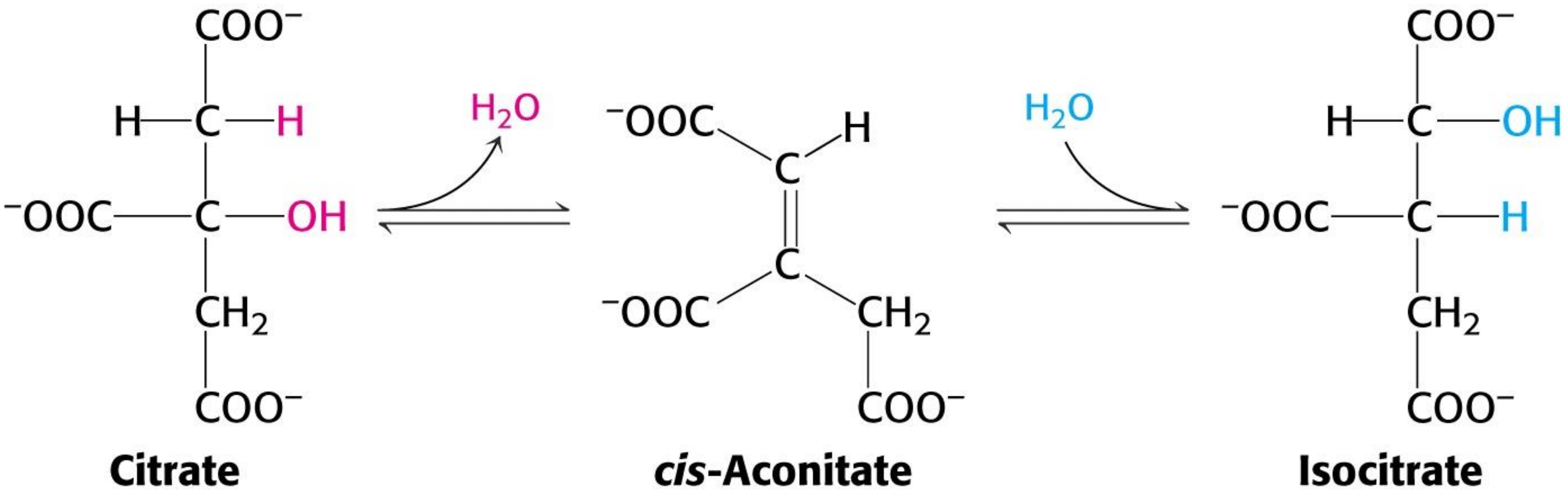
The complete citric acid cycle. The two carbons from acetyl CoA that enter this turn of the cycle (shadowed in red) will be converted to CO_2 in subsequent turns of the cycle: it is the two carbons shadowed in blue that are converted to CO_2 in this cycle.

step 1 (Citrate synthase)



Condensation reaction Hydrolysis reaction

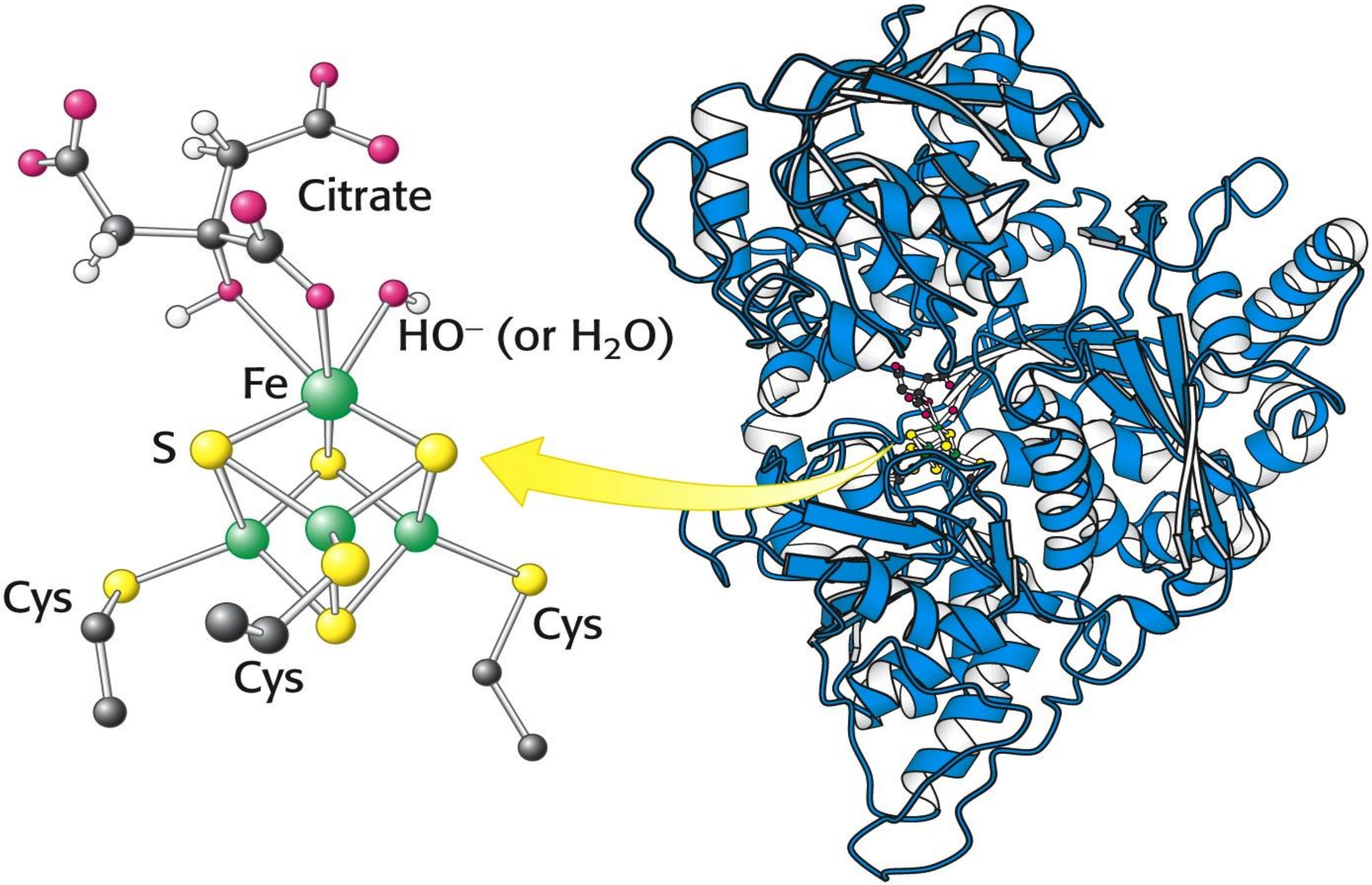
Step 2 (aconitase)



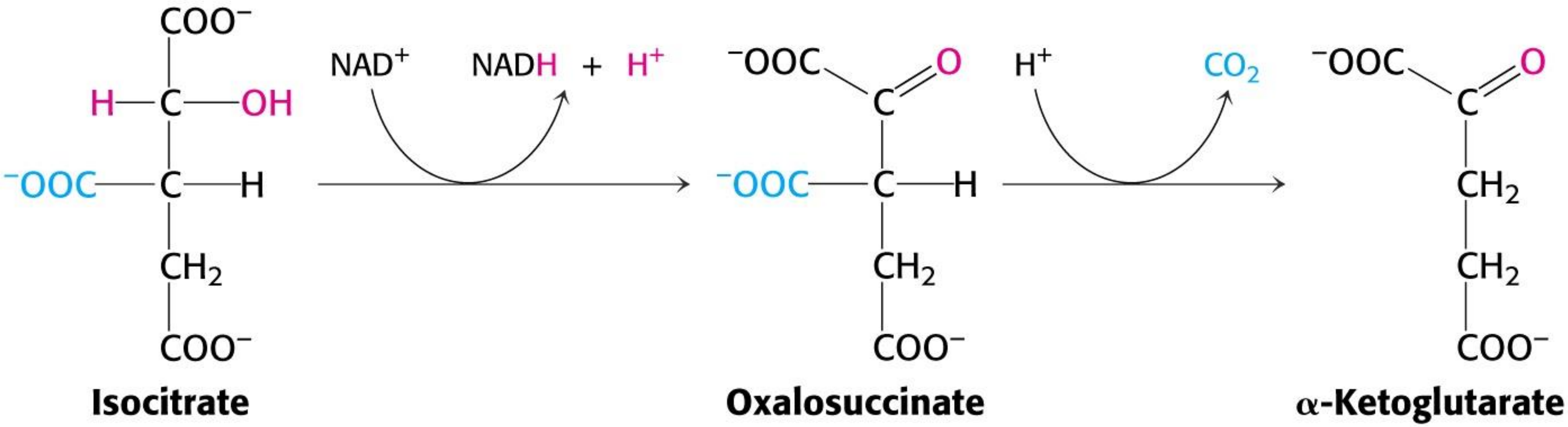
Dehydration

Hydration

Aconitase: citrate binding to iron-sulfur cluster



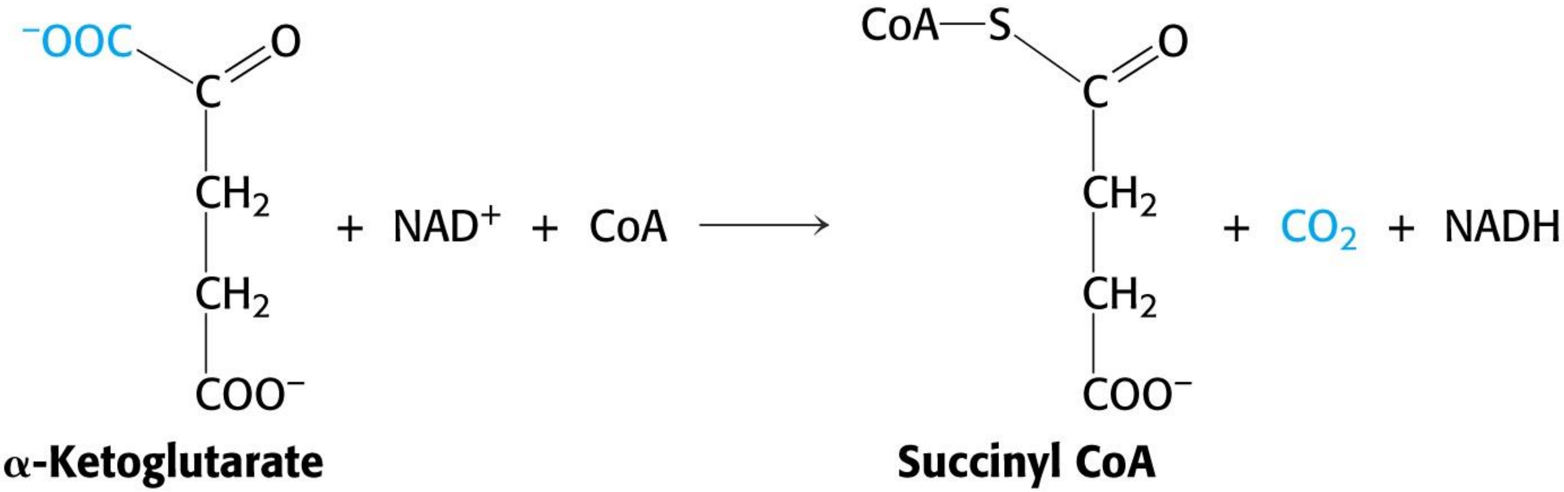
Step 3 (isocitrate dehydrogenase)



1st NADH produced

1st CO_2 removed

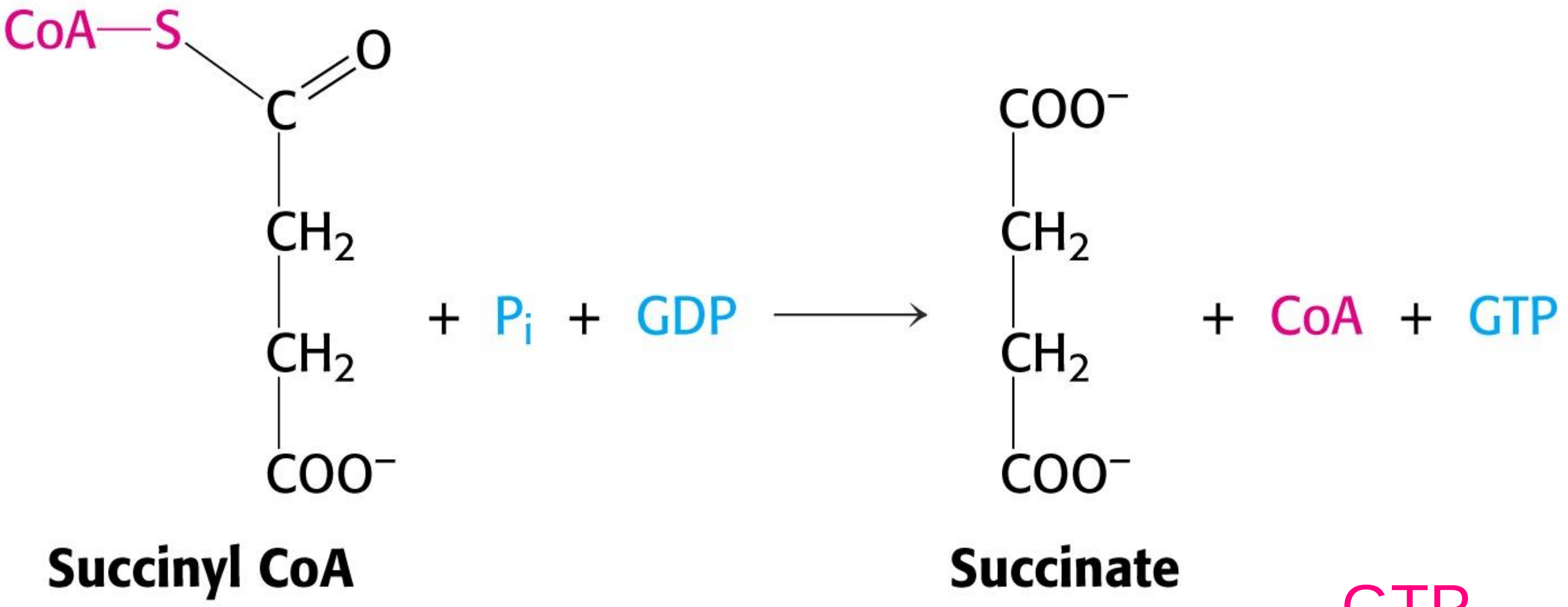
Step 4 (a-ketoglutarate dehydrogenase)



2nd NADH produced

2nd CO_2 removed

Step 5 (succinyl CoA synthetase)



GTP produced

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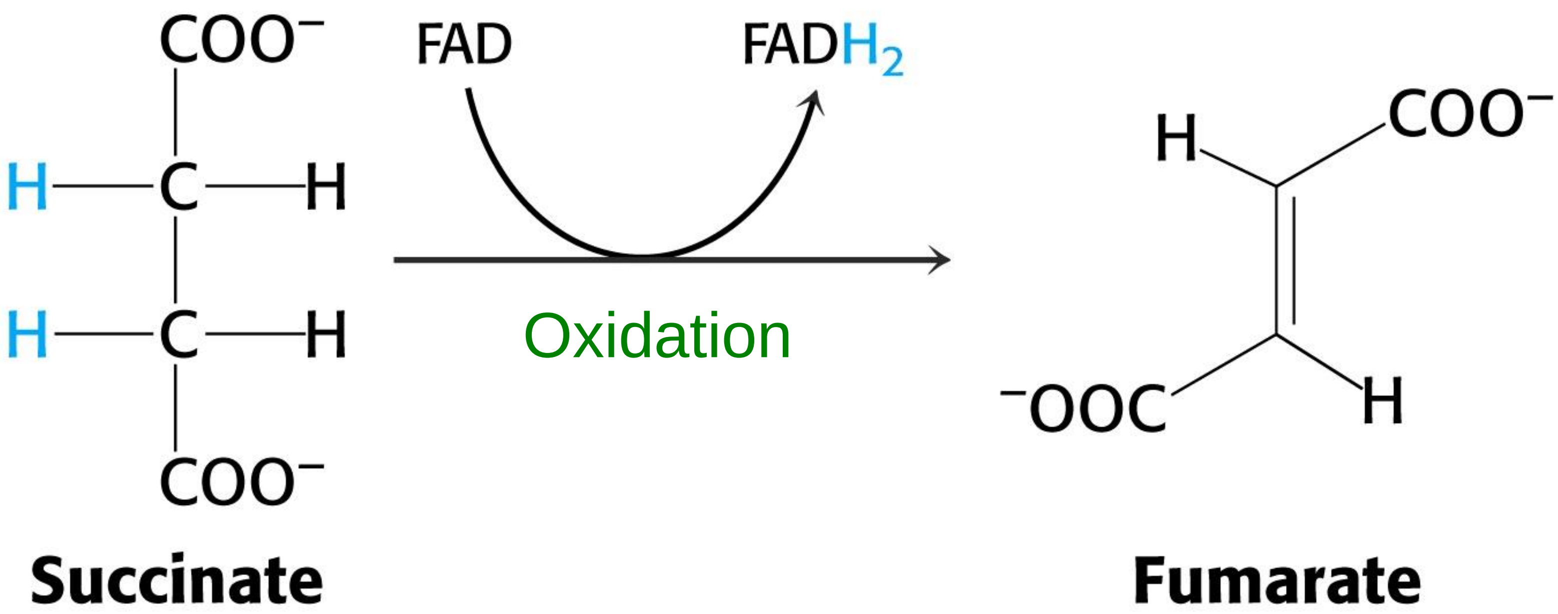
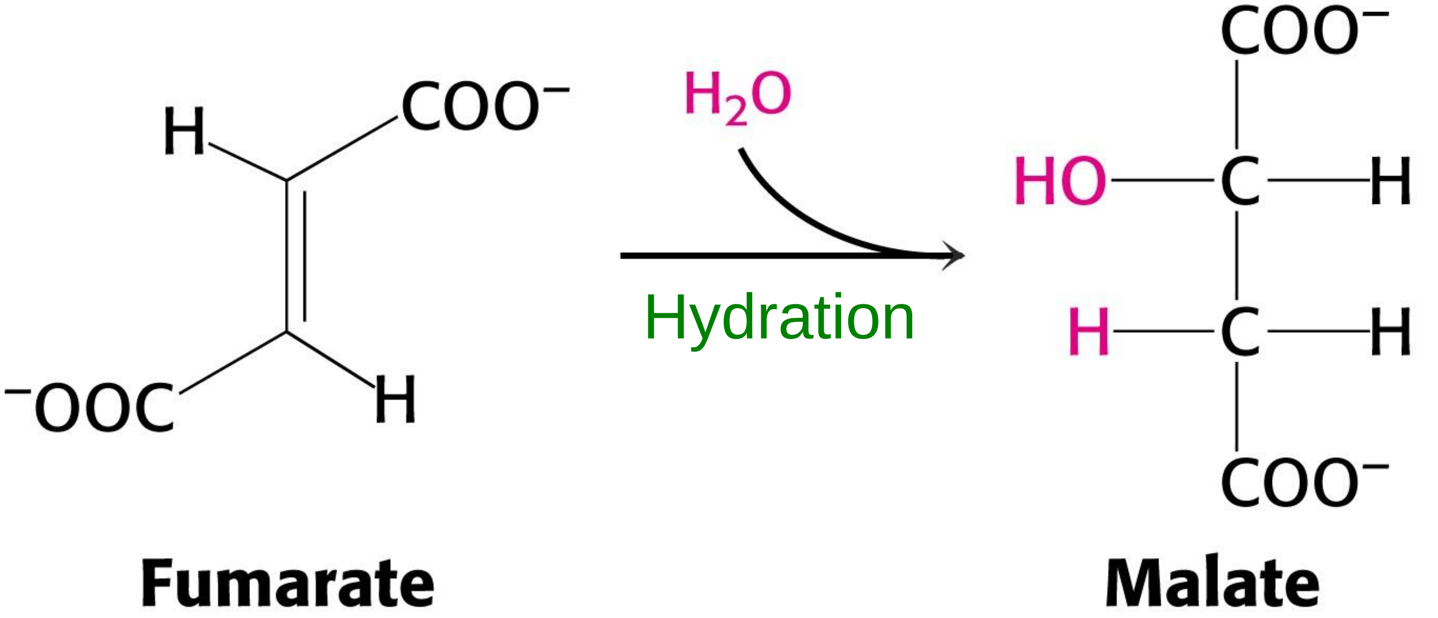
GTP + ADP ⇒ GDP + ATP (NPTase)

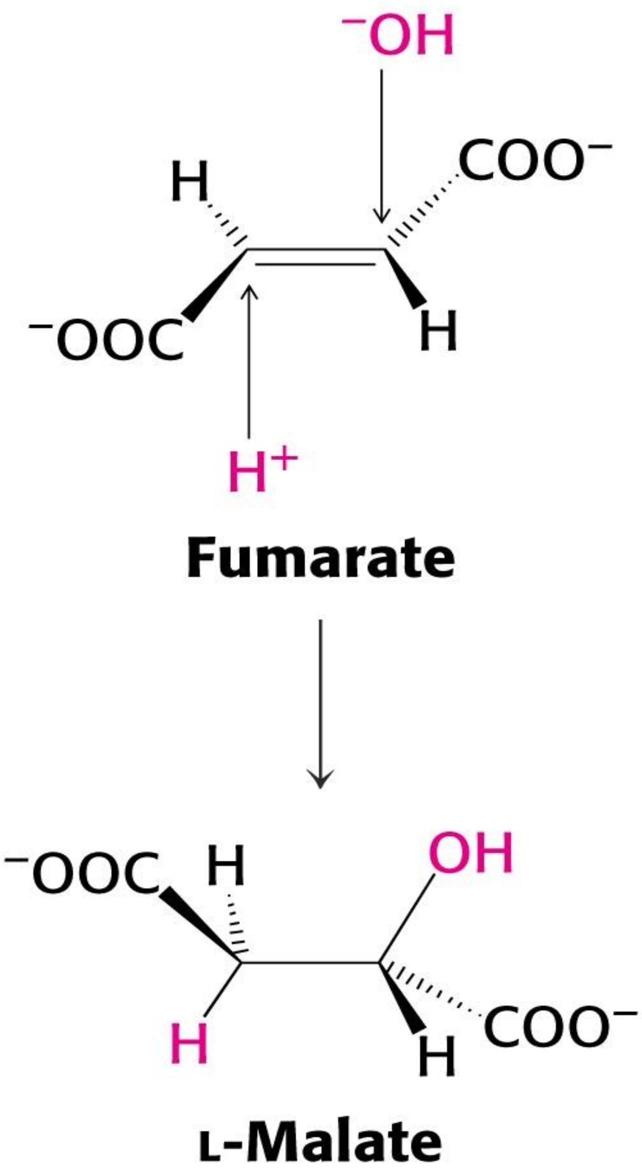
Step 6 (succinate dehydrogenase)



$FADH_2$ produced

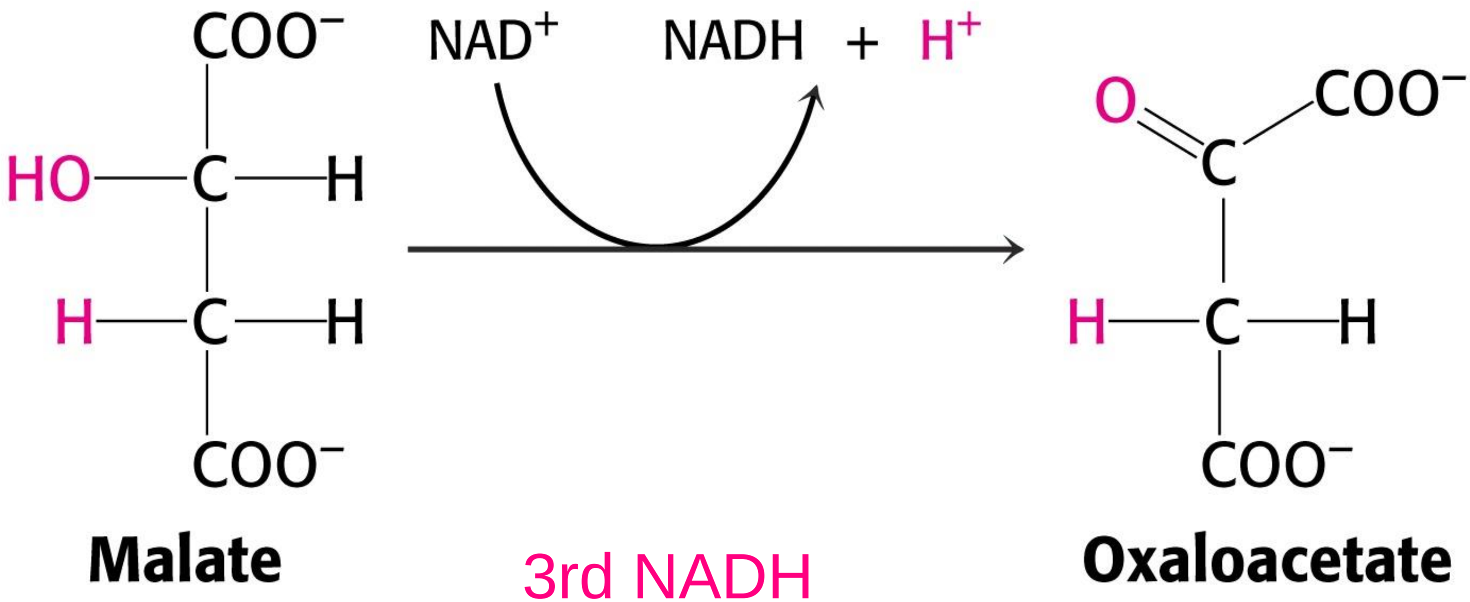
Step 7 (fumarase)





Hydroxyl group to one side only of fumarate double bond; hence, only L isomer of malate formed

Step 8 (malate dehydrogenase)



3rd NADH produced

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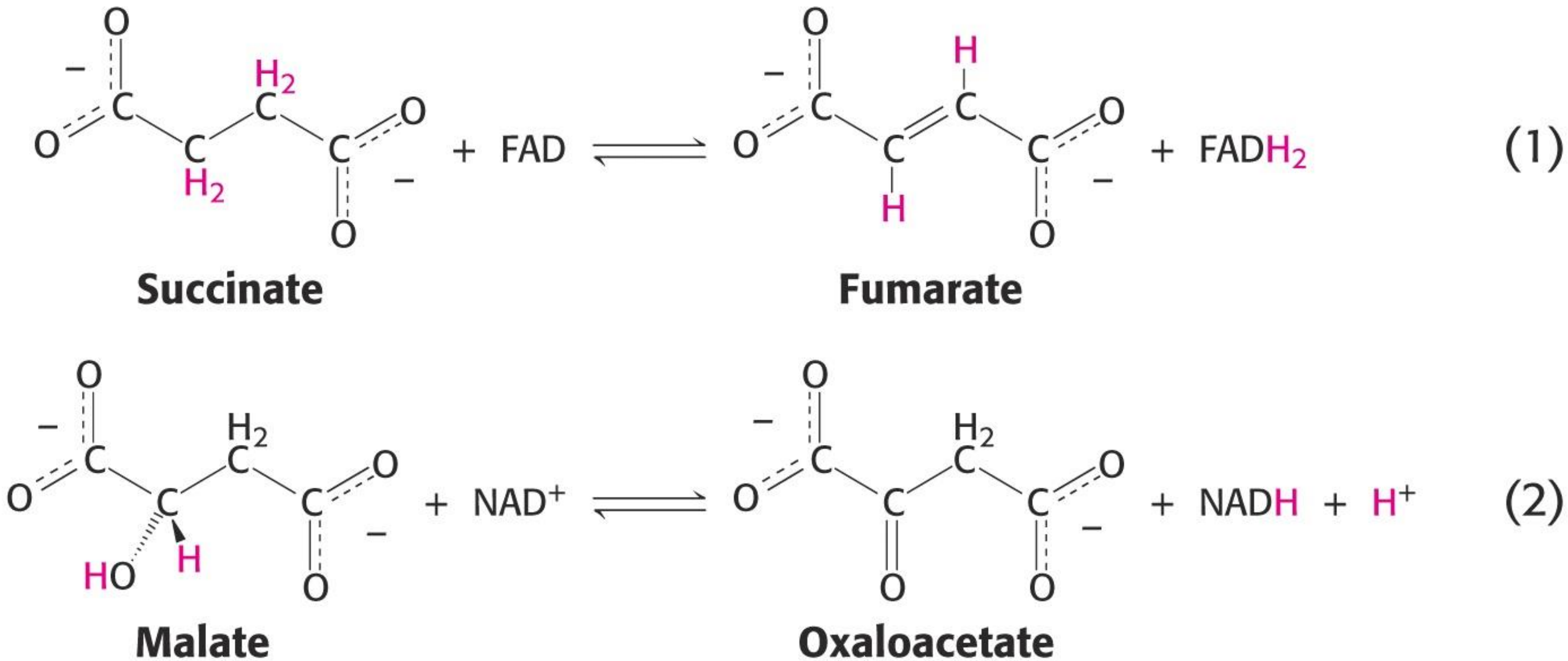
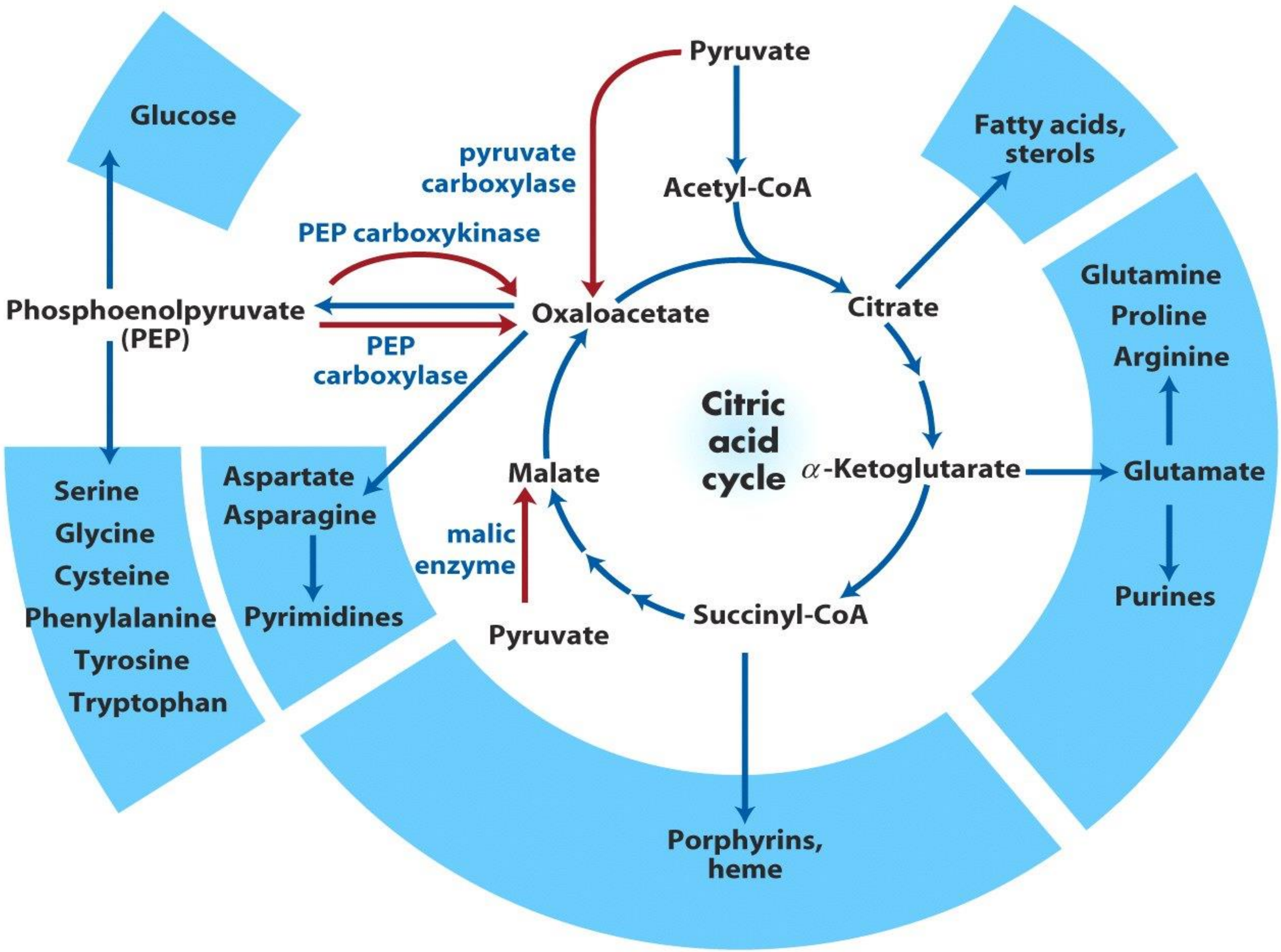

+ FAD
+ FADH2
(1)
Succinate
Fumarate
+ NAD+
+ NADH + H+
(2)
Malate
Oxaloacetate



Pyruvate
Glucose
pyruvate carboxylase
Acetyl-CoA
Fatty acids, sterols
PEP carboxykinase
Phosphoenolpyruvate (PEP)
Oxaloacetate
Citrate
Glutamine
Proline
Arginine
PEP carboxylase
Citric acid cycle
Malate
α-Ketoglutarate
Glutamate
Serine
Glycine
Cysteine
Phenylalanine
Tyrosine
Tryptophan
Aspartate
Asparagine
Pyrimidines
malic enzyme
Pyruvate
Succinyl-CoA
Purines
Porphyrins, heme

Citric acid cycle regulation

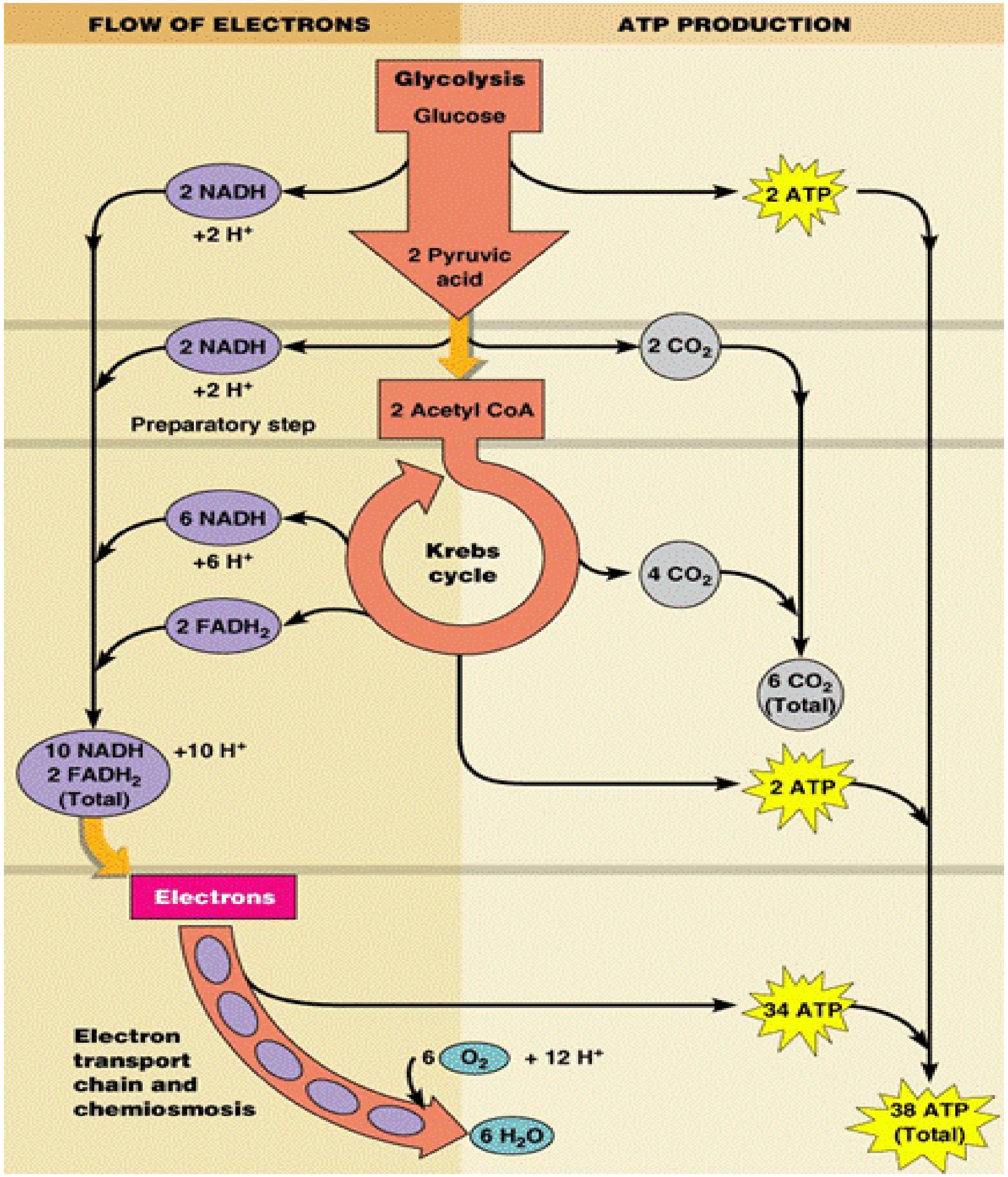
- Because the TCA cycle is central to many pathways of metabolism, there must always be a large supply of the intermediates. For example, oxaloacetate is the direct precursor of the amino acid aspartate, with the alpha-keto group being replaced by an amino group. Likewise, alpha-ketoglutarate is the direct precursor to glutamate. These two amino acids are important, not only for protein synthesis, but even more so for maintaining nitrogen balance and eliminating toxic ammonia.

Citric acid cycle regulation (continued)

- Therefore, there are a variety of pathways that serve to regenerate TCA cycle intermediates if the supply falls low. For example, the breakdown of amino acids leads to TCA cycle intermediates.
- If the supply of intermediates falls low, for example, during even short periods of starvation, muscle can be broken down and the carbon skeletons of the amino acids used to build up the supply of 4-carbon dicarboxylic acids. Oxaloacetate and malate can be synthesized from pyruvate by carboxylation using bicarbonate.

Electron transport chain

Aerobic Respiration



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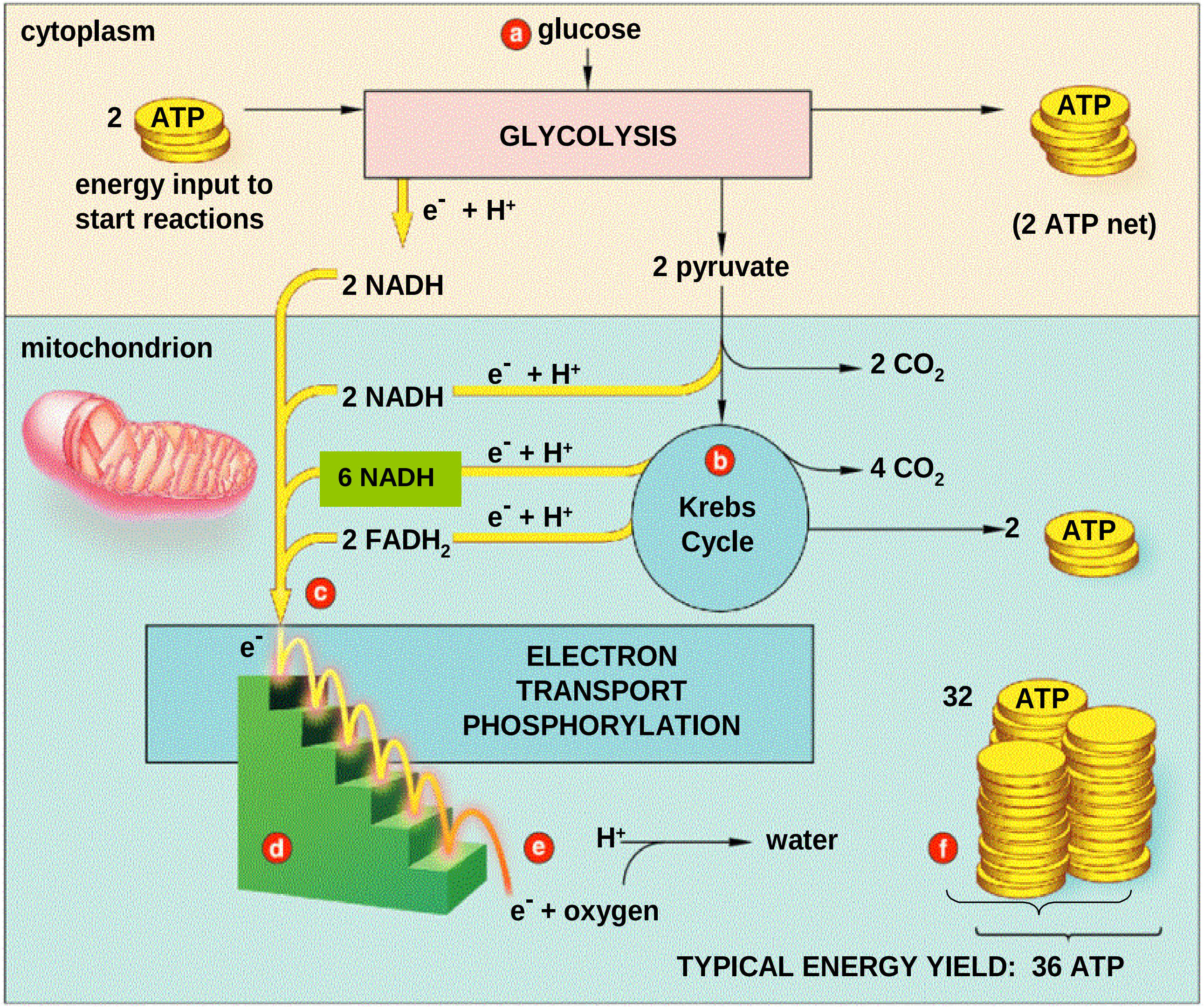


cytoplasm
a glucose
2 ATP
GLYCOLYSIS
ATP
energy input to start reactions
e- + H+
(2 ATP net)
2 pyruvate
2 NADH
mitochondrion
2 CO2
2 NADH
e- + H+
6 NADH
e- + H+
b
4 CO2
Krebs Cycle
2 FADH2
e- + H+
2 ATP
c
e-
ELECTRON TRANSPORT PHOSPHORYLATION
32 ATP
d
e
H+
water
f
e- + oxygen
TYPICAL ENERGY YIELD: 36 ATP

Electron transport chain

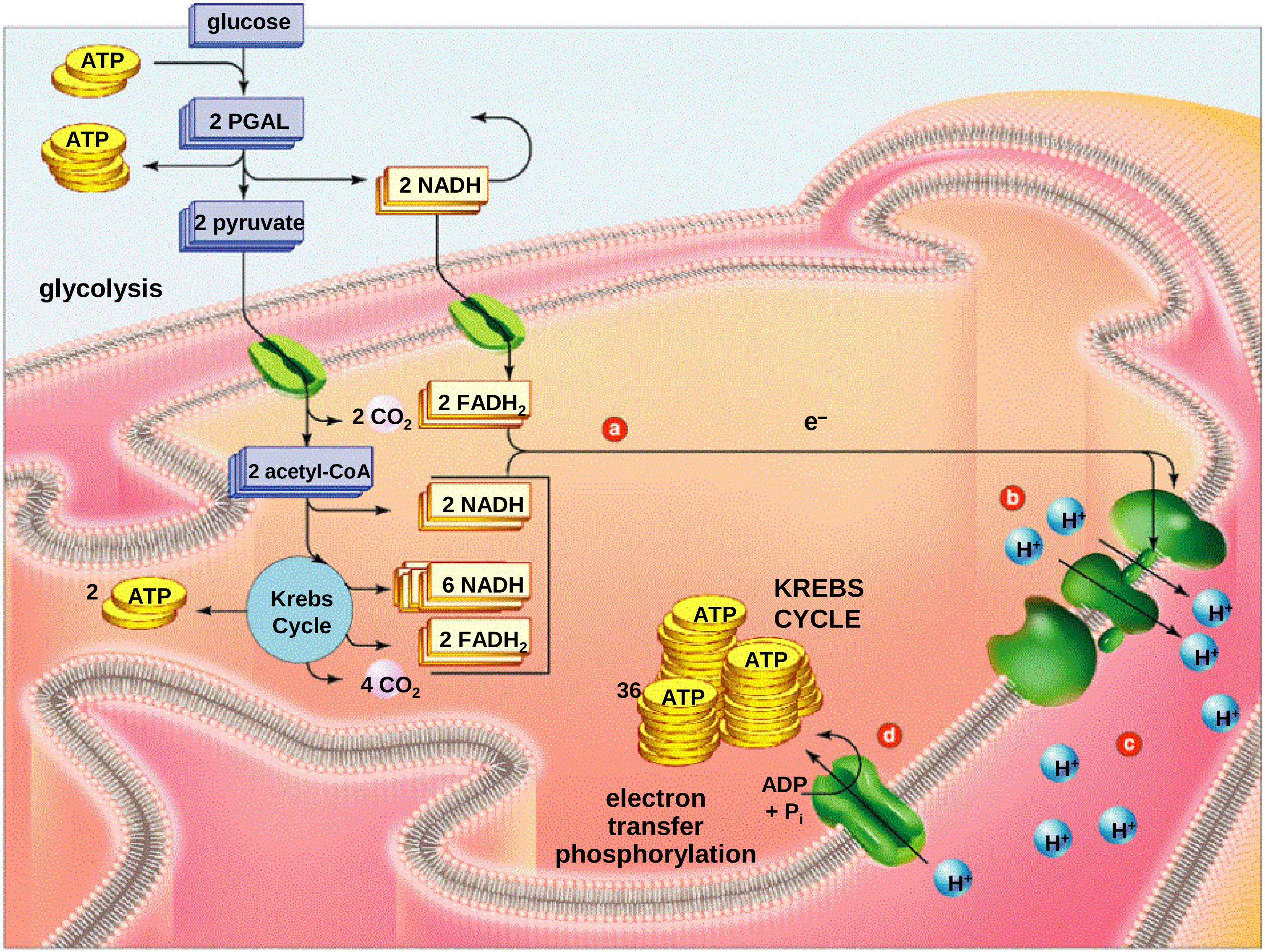
- Resides in the inner mitochondrial membrane – also called respiratory chain

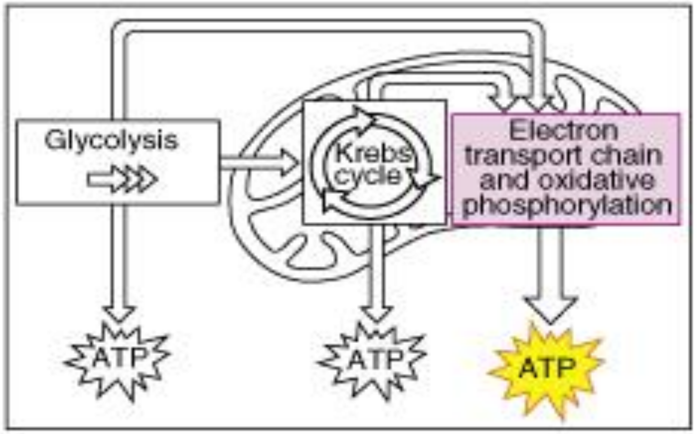
- 15 proteins involved in the chain – grouped in 3 large respiratory enzyme complexes
- NADH dehydrogenase complex
- Cytochrome b-c1 complex
- Cytochrome oxidase complex

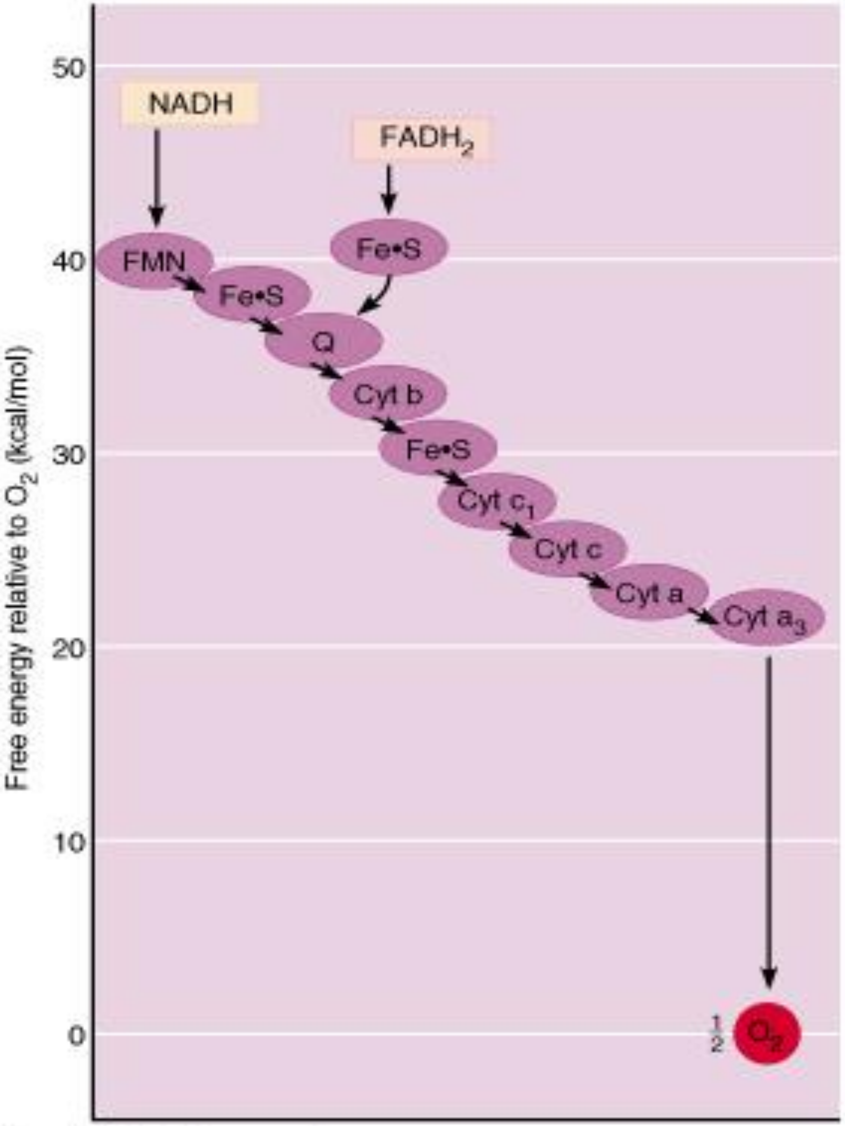
- Pumps protons across the membrane as e- are transferred thru them

Chemiosmotic mechanism

- The electron transport chain, consisting of several molecules (primarily proteins), is built into the inner membrane of a mitochondrion.
- NADH shuttles electrons from food to the "top" of the chain.
- At the "bottom", oxygen captures the electrons and H+ to form water.
- Electrons are passed by increasingly electronegative molecules in the chain until they are caught by oxygen, the most electronegative.
- This model for ATP synthesis is called the ***chemiosmotic mechanism***


glucose
ATP
2 PGAL
ATP
2 NADH
2 pyruvate
glycolysis
2 CO2
2 FADH2
a
e⁻
2 acetyl-CoA
2 NADH
b
H+
6 NADH
2
ATP
Krebs Cycle
KREBS CYCLE
ATP
ATP
2 FADH2
36
ATP
4 CO2
d
c
electron transfer phosphorylation
ADP + Pi


Glycolysis
Krebs cycle
Electron transport chain and oxidative phosphorylation
ATP
ATP
ATP


Free energy relative to O_2 (kcal/mol)
50
40
30
20
10
0
NADH
$FADH_2$
FMN
Fe•S
Fe•S
Q
Cyt b
Fe•S
Cyt c_1
Cyt c
Cyt a
Cyt a_3
$\frac{1}{2}$ O_2

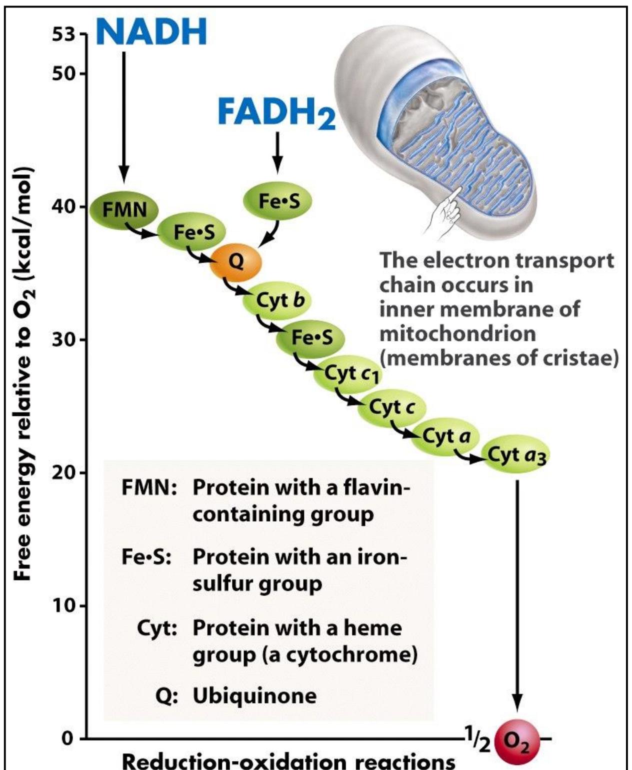
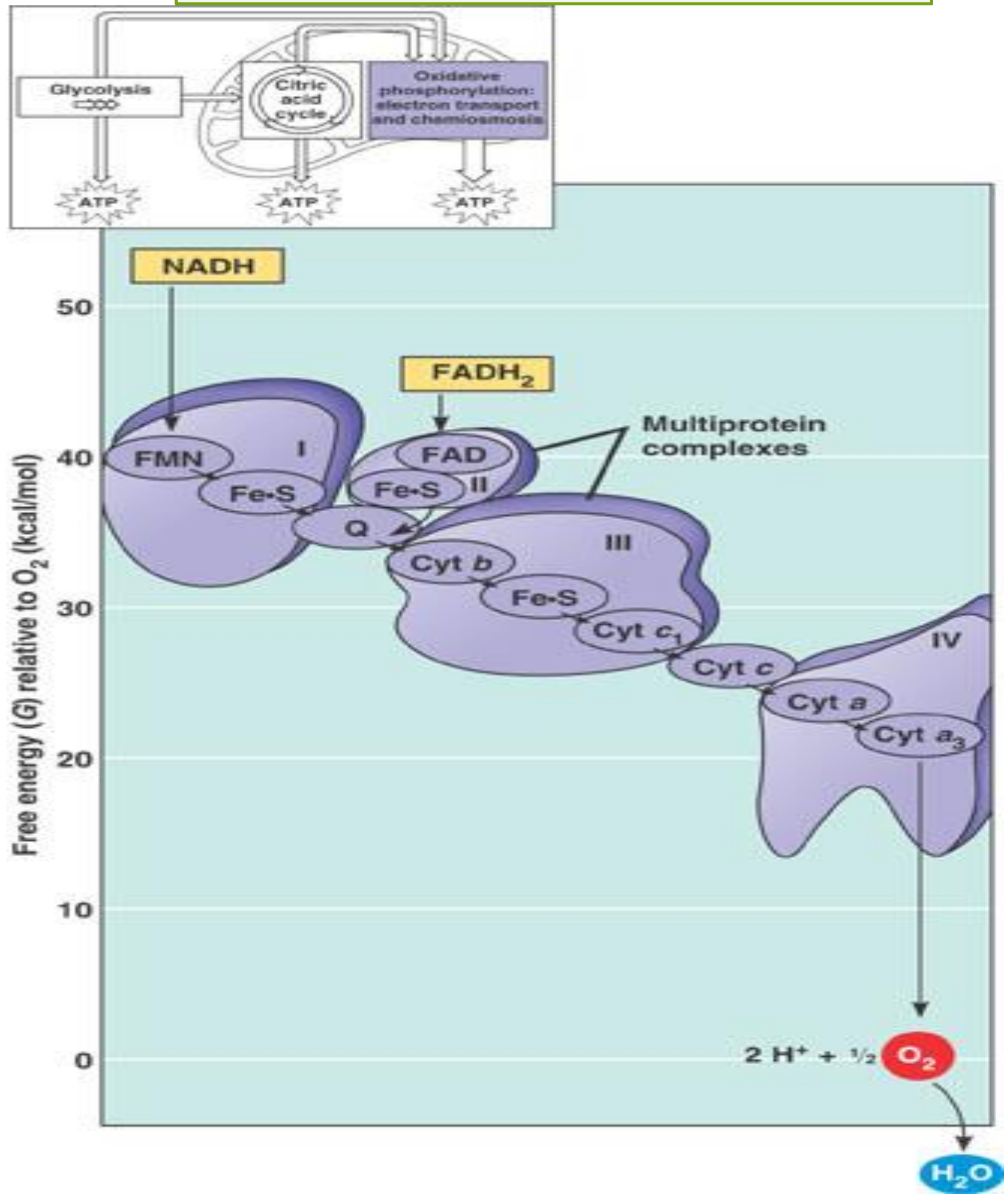
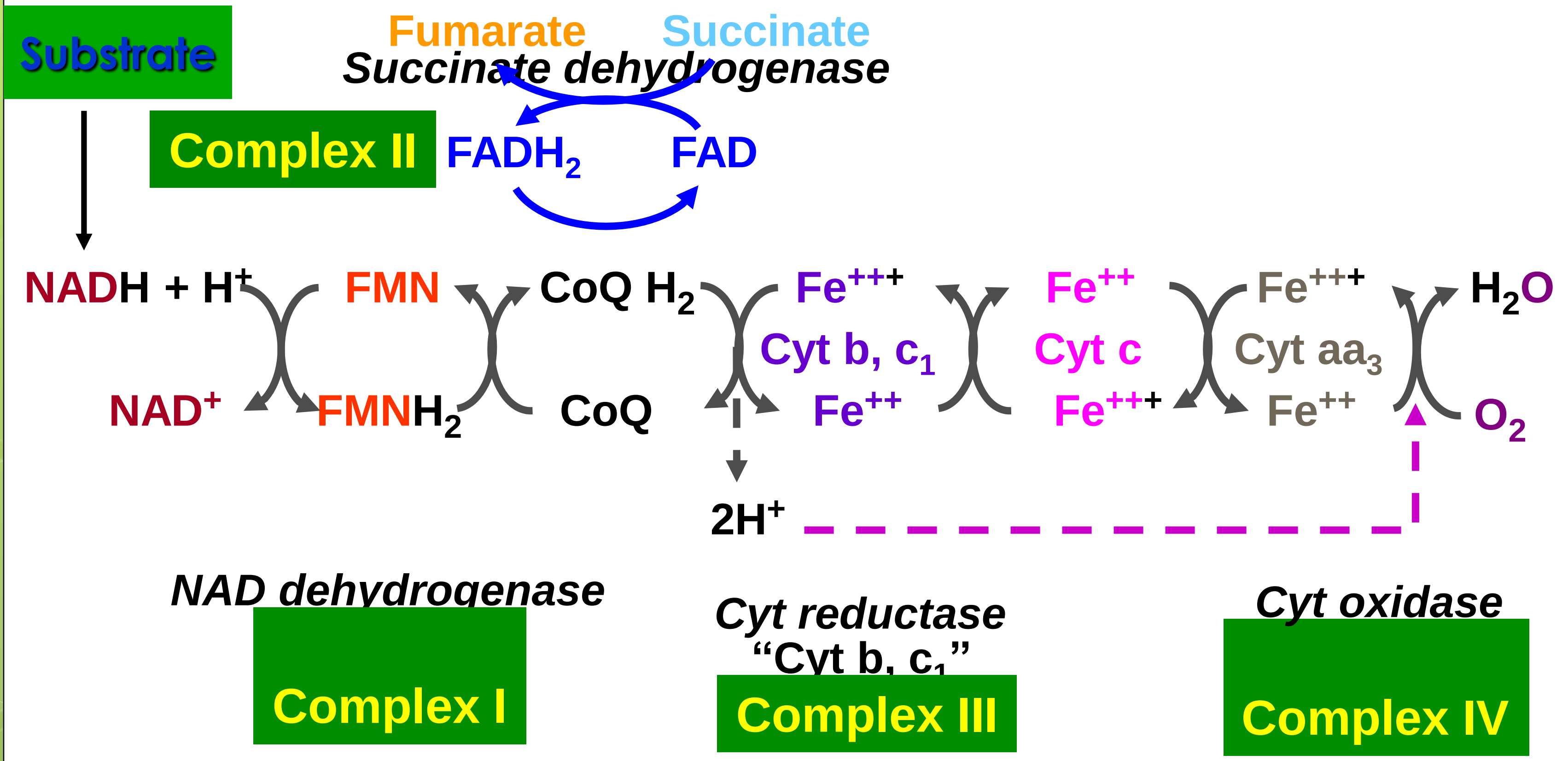


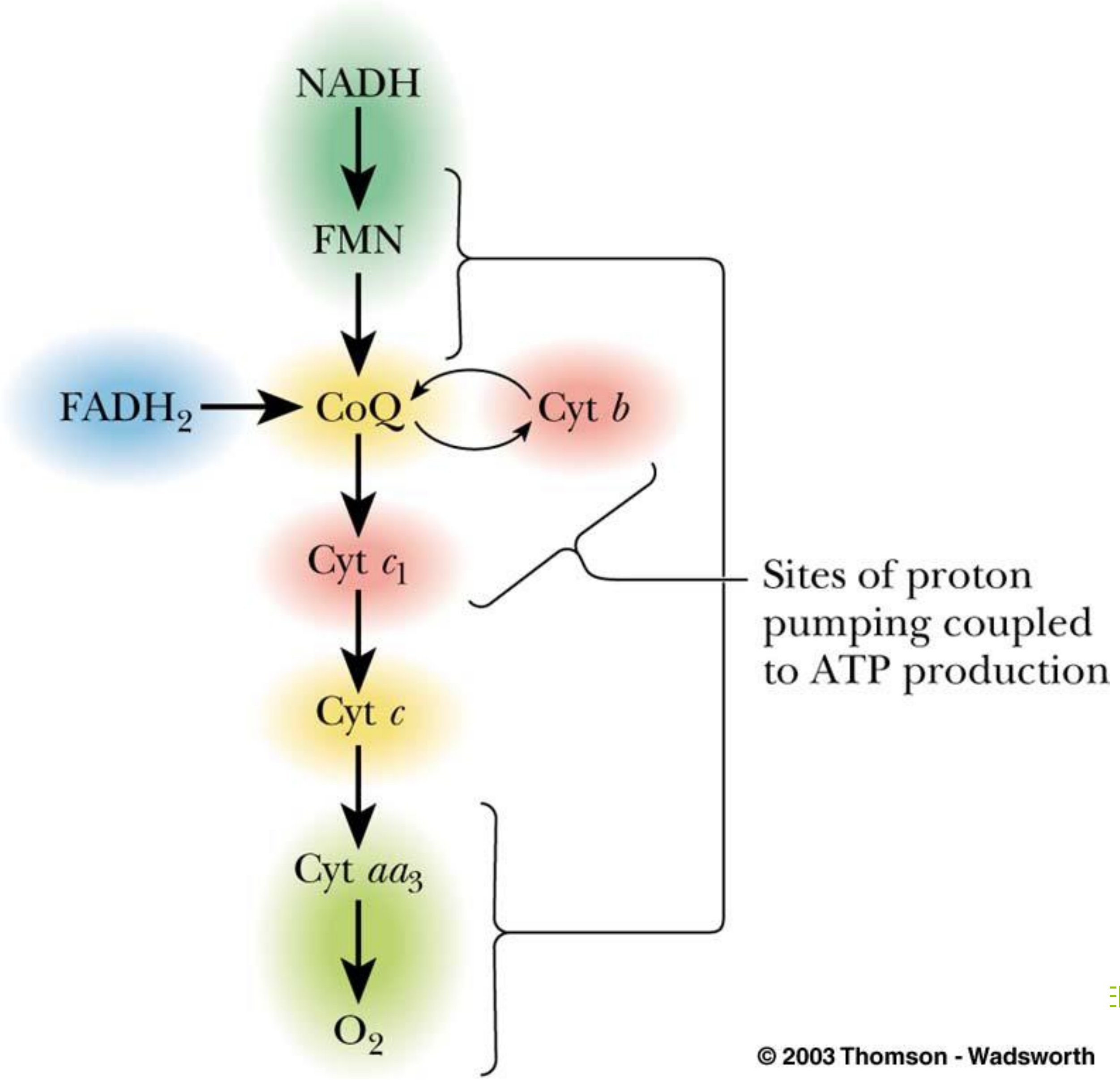
Figure 9-17 Biological Science, 2/e



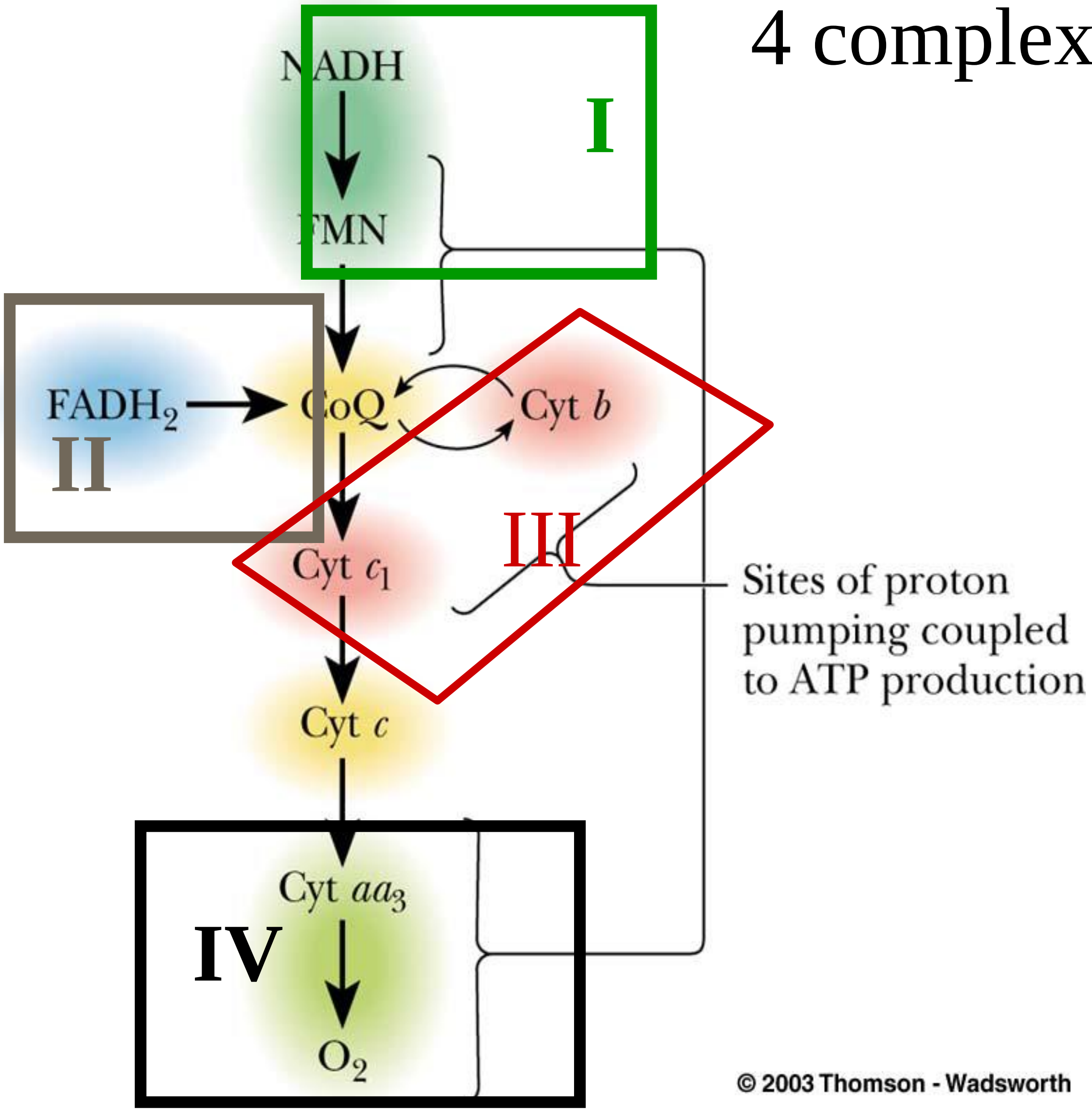
Composition of Respiratory Chain Complexes

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

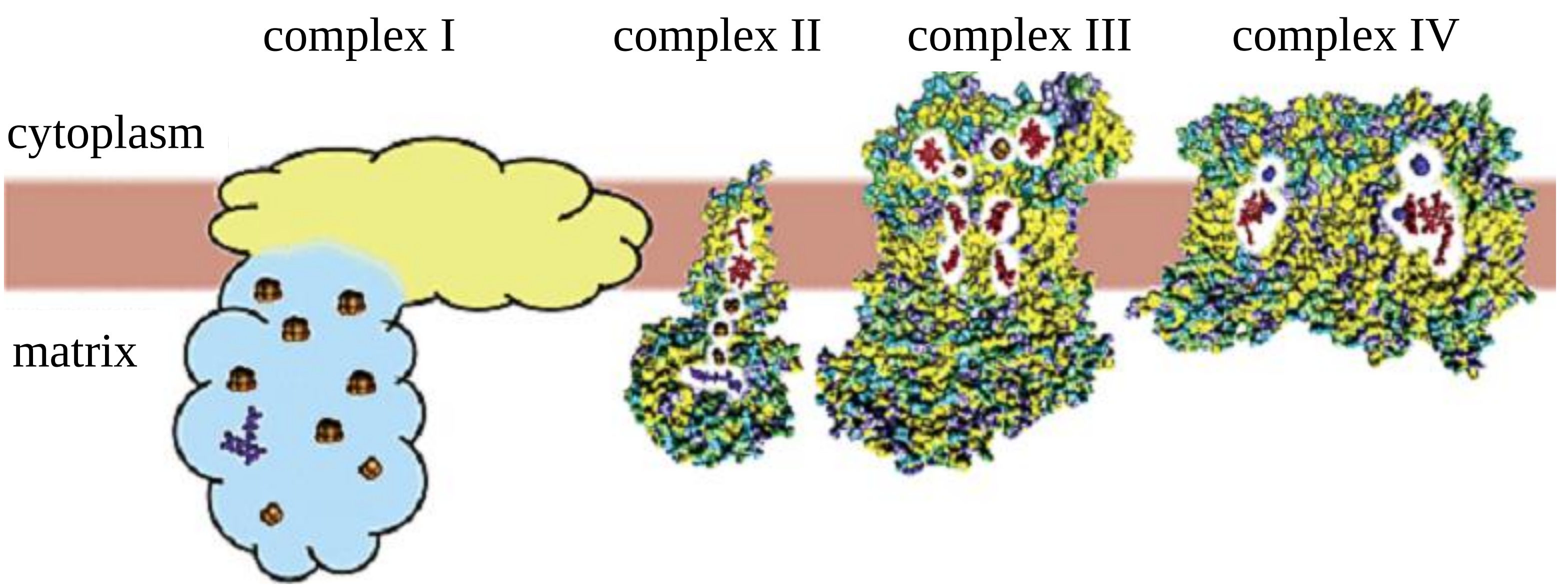

Substrate
Fumarate
Succinate
Succinate dehydrogenase
Complex II
FADH2
FAD
NADH + H+
NAD+
FMN
FMNH2
CoQ H2
CoQ
Fe+++
Cyt b, c1
Fe++
Fe++
Cyt c
Fe+++
Fe+++
Cyt aa3
Fe++
H2O
O2
2H+
NAD dehydrogenase
Complex I
Cyt reductase
“Cyt b, c1”
Complex III
Cyt oxidase
Complex IV


NADH
FMN
FADH2
CoQ
Cyt b
Cyt c1
Cyt c
Cyt aa3
O2
Sites of proton pumping coupled to ATP production

4 complexes

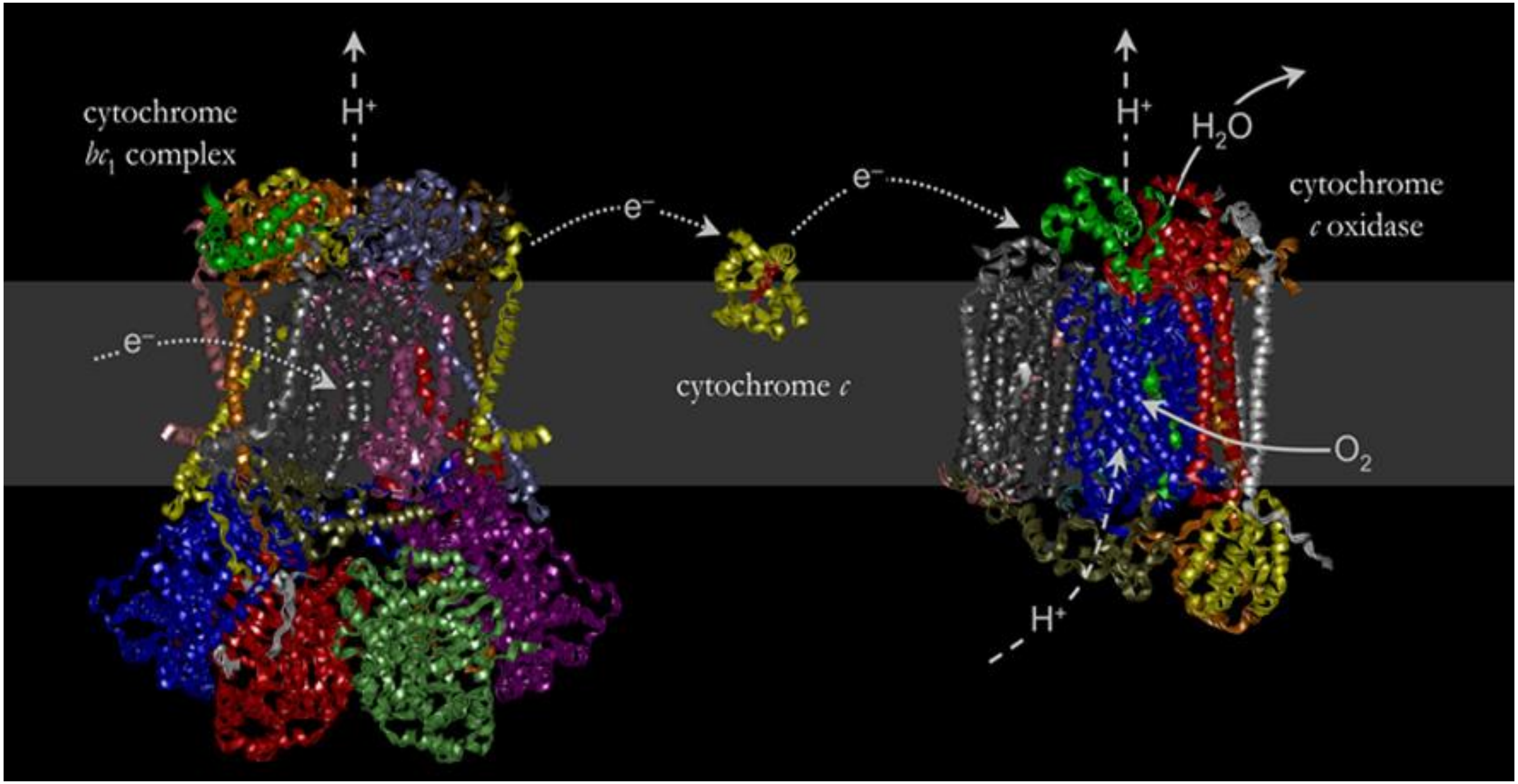

NADH
I
FMN
FADH2
II
CoQ
Cyt b
III
Cyt c1
Cyt c
Cyt aa3
IV
O2
Sites of proton pumping coupled to ATP production

Each of the complexes possesses multiple bound redox cofactors



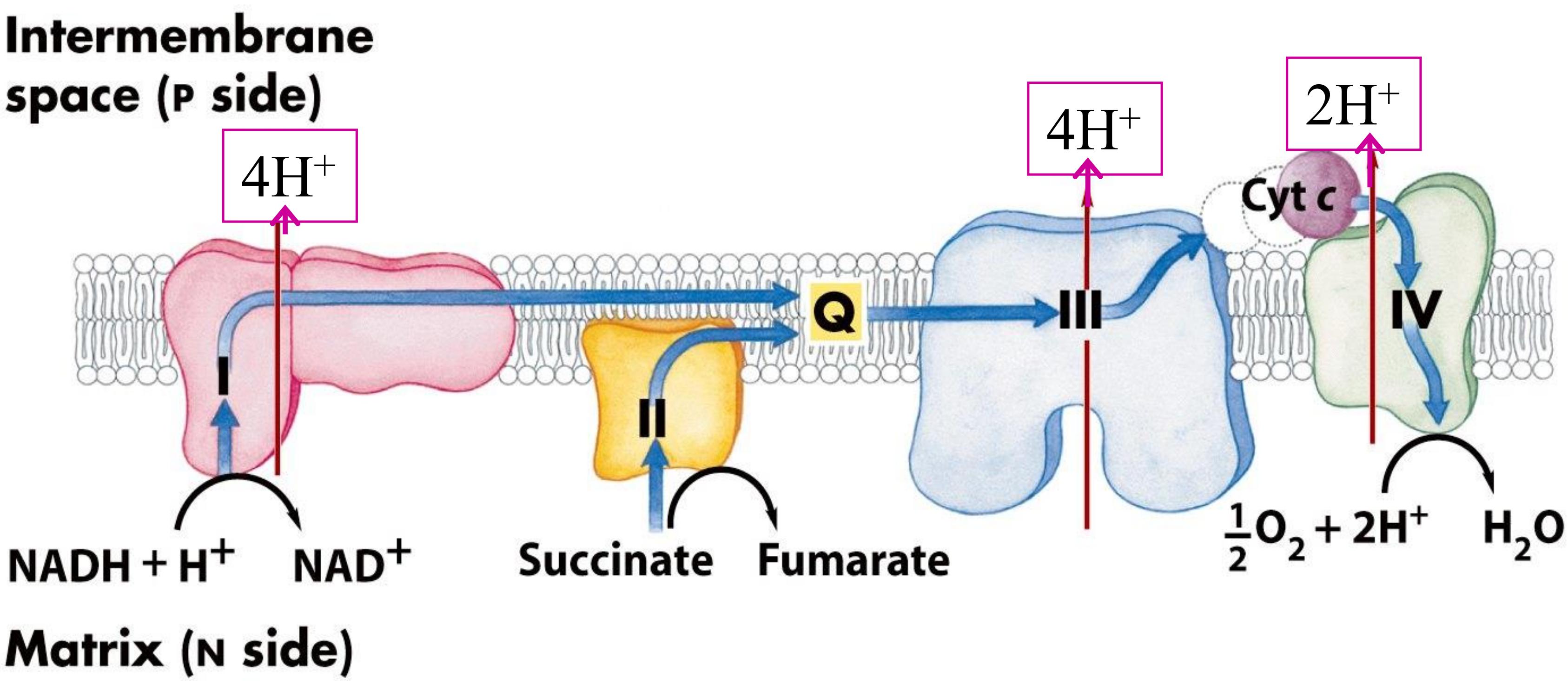
from: Biochemistry, **42** (8), 2266 -2274, 2003

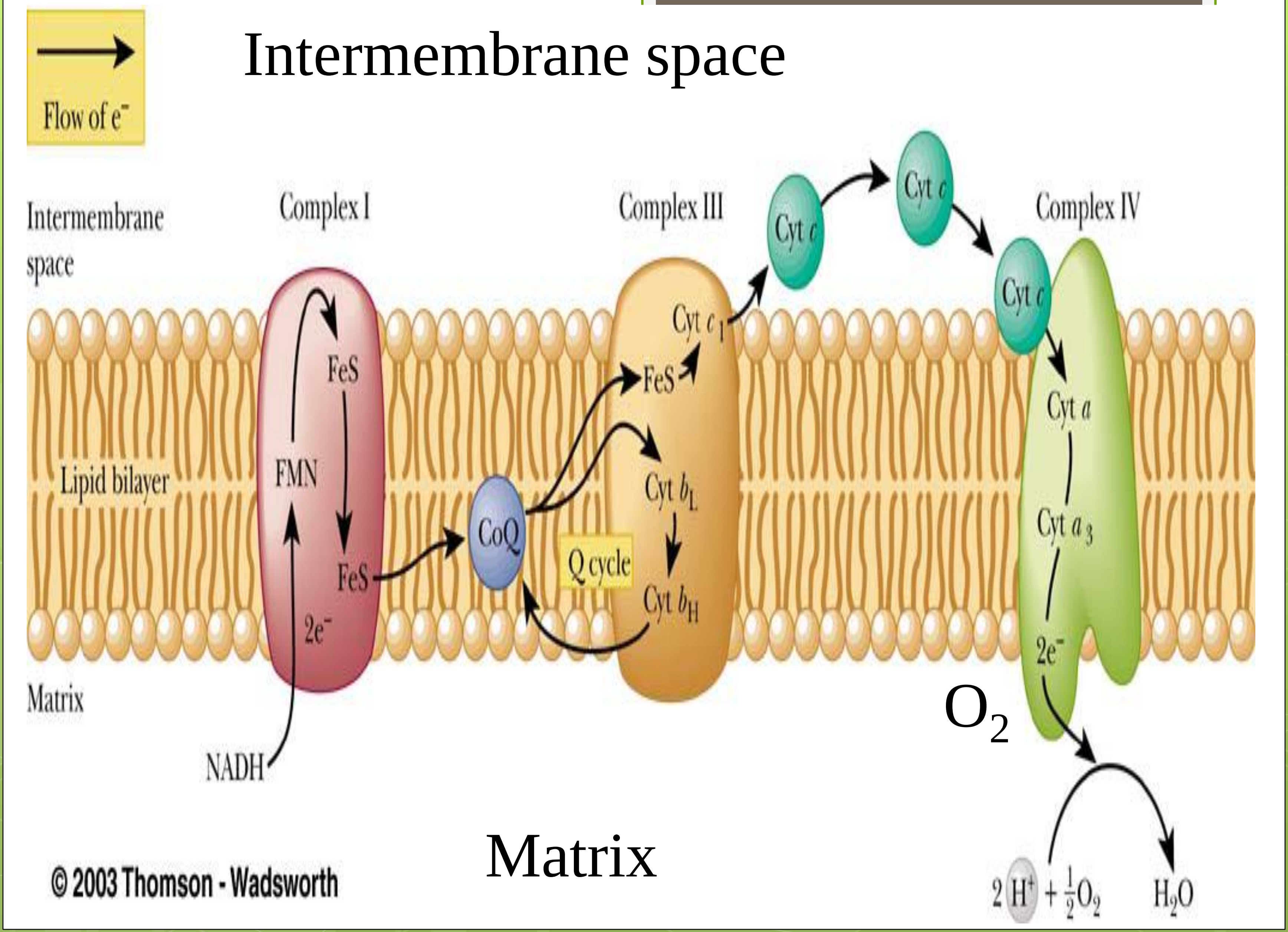
3D structure of cytochromes



How is energy captured?

- As a proton gradient:




Flow of e⁻
Intermembrane space
Intermembrane space
Complex I
Complex III
Complex IV
Cyt c
Cyt c
Cyt c
Cyt c1
FeS
FeS
FeS
FMN
2e⁻
CoQ
Q cycle
Cyt bL
Cyt bH
Cyt a
Cyt a3
2e⁻
Lipid bilayer
Matrix
NADH
O2
2 H⁺ + ½O2
H2O
Matrix
© 2003 Thomson - Wadsworth

Complex I: NADH dehydrogenase

- It has a tightly bound molecule of flavin mono- nucleotide (FMN) that accepts 2 hydrogen atoms(2 e^- +2 H^+) becoming FMNH2.
- It also contains iron-sulfur centers (composed of several iron atoms paired with sulfur atoms) that are necessary for the transfer of hydrogen atoms to the next member of the chain, coenzyme Q.

Complex II: $FADH_2$ dehydrogenase

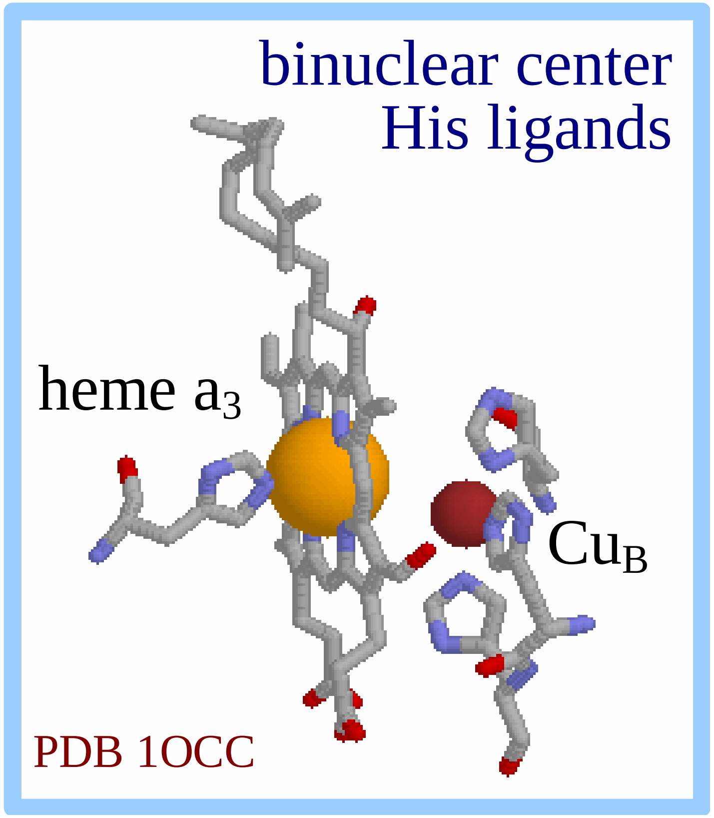
- It is produced by succinate dehydrogenase and acyl COA dehydrogenase.

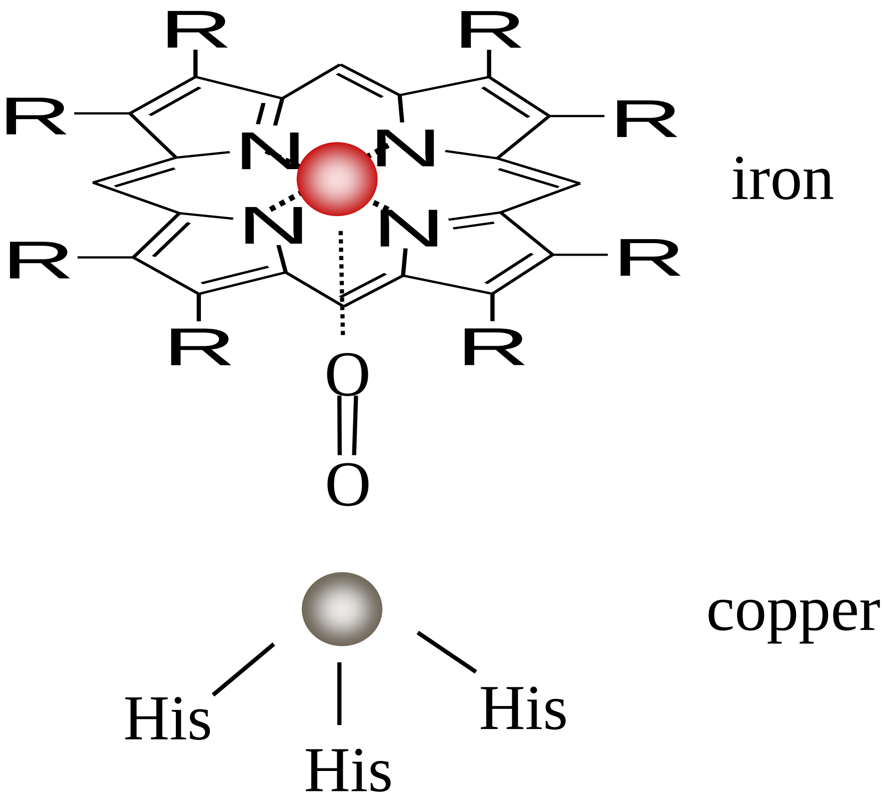
Complex III: Cytochromes b and c (cytochrome reductase)

- Each contains a heme group made of a porphyrin ring containing an iron atom.
- The iron atom acts as a reversible carrier of electrons since it can be reversibly converted from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) form
- It accepts electrons from coenzyme Q.

Complex IV: Cytochrome a+a3 (Cytochrome oxidase)

- Metal centers of cytochrome oxidase are heme a & heme a_3, Cu_A (2 adjacent Cu atoms) & Cu_B.
- It is the only electron carrier in which the heme iron has a free ligand that can react with molecular oxygen. It also contains a copper atoms that are required for this complex reaction to occur.





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

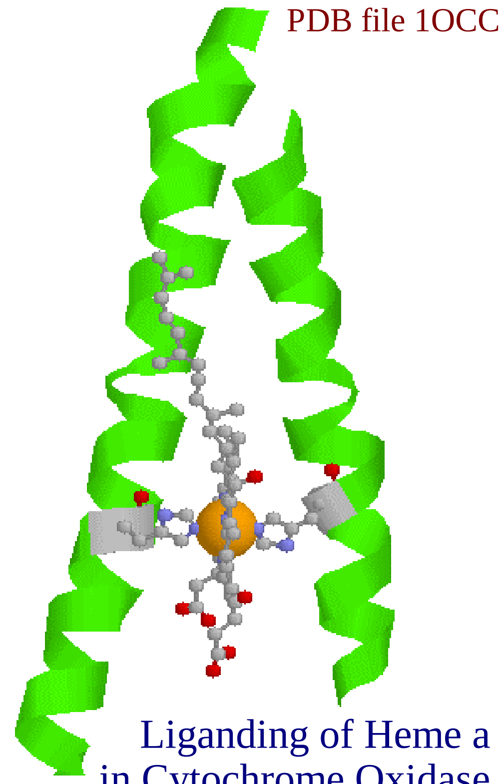
Metal center ligands in complex IV

Heme a in which heme axial ligands are His N atoms.

Heme a is held in place between 2 transmembrane α-helices by its axial His ligands.

Heme a_3, which sits adjacent to Cu_B, has only one axial ligand.

Cu ligands consist of His N, & in the case of Cu_A also Cys S, Met S, & a Glu backbone O.



Liganding of Heme a in Cytochrome Oxidase

Metal center ligands in complex IV (Continued)

Electrons enter complex IV one at a time from cyt c to **Cu_A**. They then pass via **heme a** to the **binuclear center** (heme a_3/Cu_B) where the chemical reaction takes place.

$$\text{cyt c} \rightarrow Cu_A \rightarrow \text{heme a} \rightarrow \text{heme } a_3/Cu_B$$

O_2 binds at the open axial ligand position of **heme a_3**, adjacent to **Cu_B**.

Complex V: (ATP Synthase Complex)

- It synthesizes ATP, using the energy of the proton gradient generated by the ETC.
- It is also called ATPase, because it also catalyzes the hydrolysis of ATP to ADP and inorganic phosphate (Pi).

Respiratory chain inhibitors

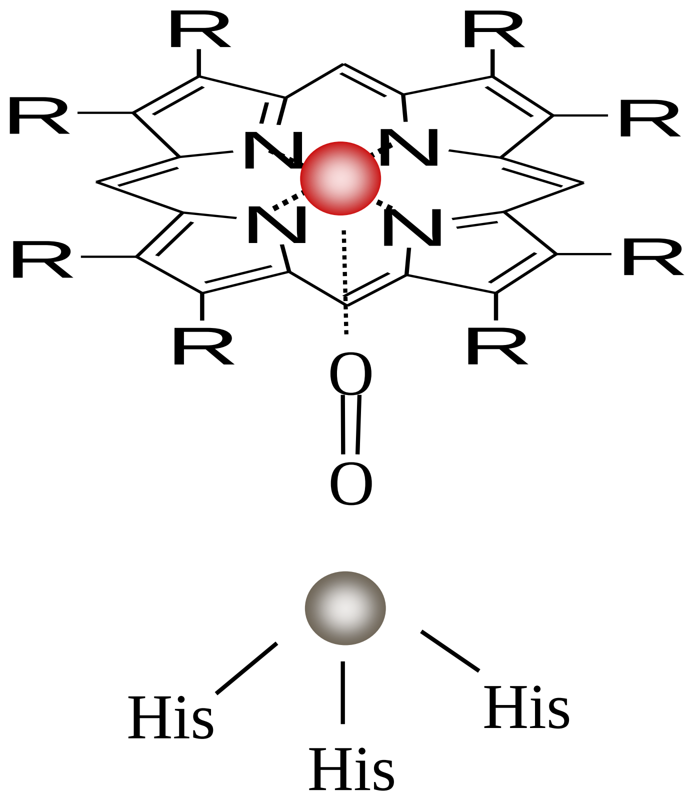
- ***Rotenone*** (a rat poison) blocks complex I.
- ***Antimycin A*** blocks electron transfer in complex III.
- ***CN-*** & ***CO*** inhibit complex IV.

- The open axial ligand position makes heme a_3 susceptible to binding each of the following inhibitors:

  CN^-, **CO**, and the radical signal molecule **·NO**

- Inhibition at any of these sites will block e- transfer from NADH to O2.

Respiratory chain inhibitors (continued)



iron

copper