

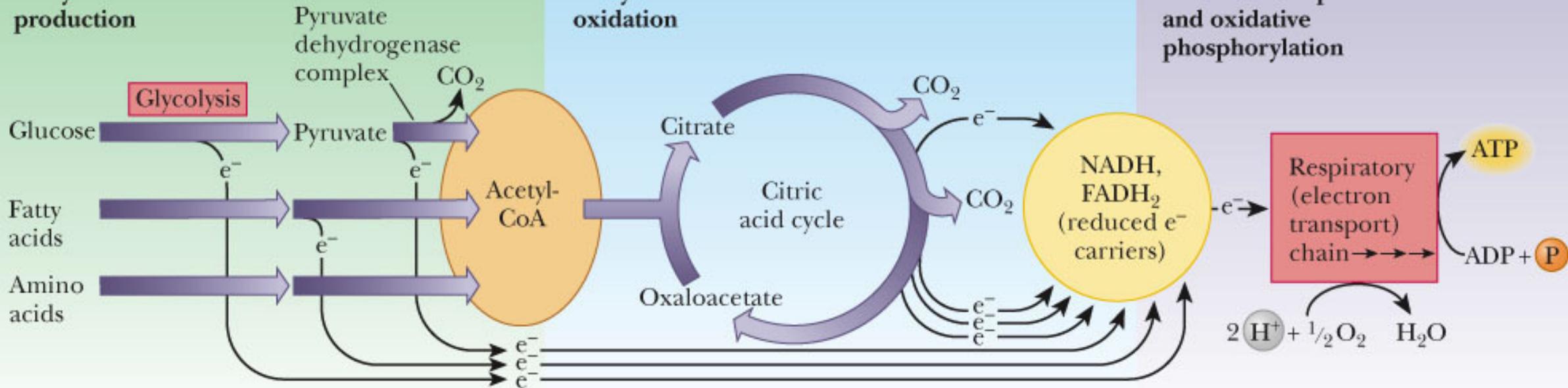
Electron Transport and Oxidative Phosphorylation

Chapter 20

Introduction

- Up to this point, we have dealt with:
 - Oxidation of substrates.
 - Collection of electrons by cofactors.
- Then:
 - Energy from the cofactors is recovered using O_2 as the final electron acceptor
 - This is accomplished using a series of carriers in the inner mitochondrial membrane
- Since NAD^+ and FAD are in limited supply, they must be recycled.

Stage 1
Acetyl-CoA production



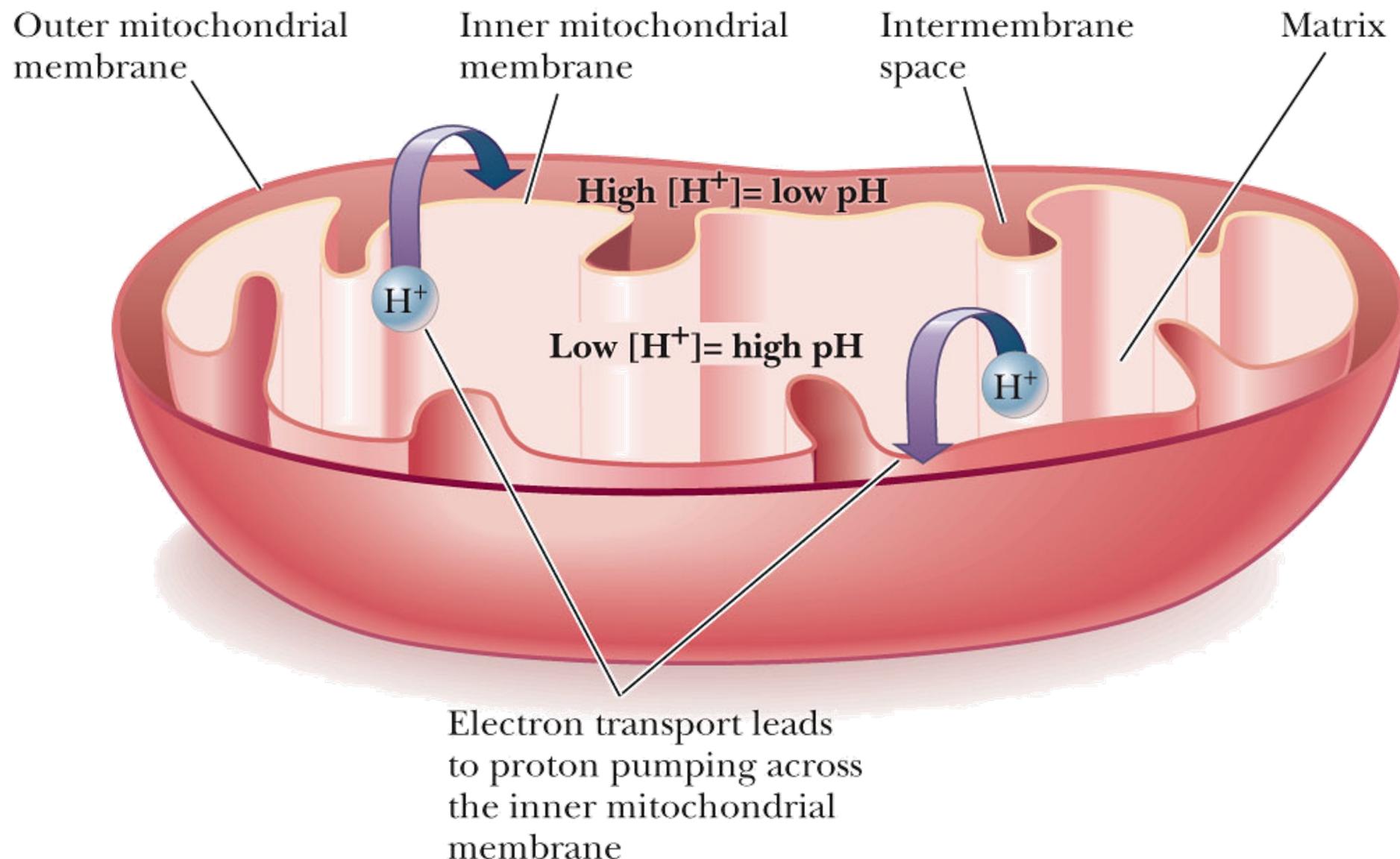
The Role of Electron Transport in Metabolism

- Electron transport is carried out by four closely related multi-subunit membrane-bound complexes and two electron carriers, **coenzyme Q** and **cytochrome c**
 - (mobile carriers – not part of the respiratory complex)
- In a series of oxidation-reduction reactions, electrons from FADH_2 and NADH are transferred from one complex to the next until they reach oxygen
 - O_2 is reduced to H_2O

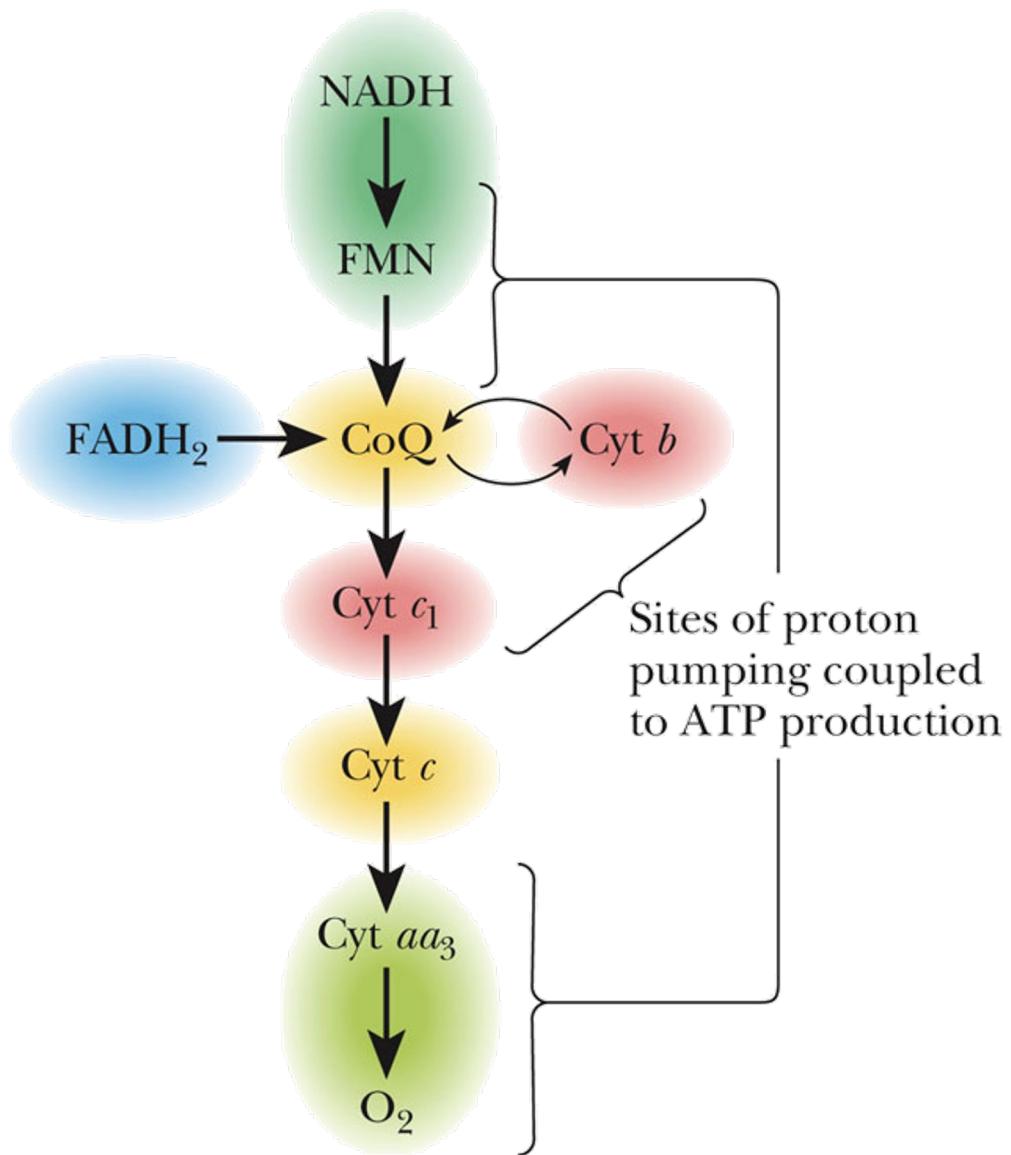
ATP Production in the Mitochondrion

- As a result of electron transport, hydrogen are pumped across the inner membrane to the intermembrane space, creating a pH gradient
- The production of ATP in the mitochondria is the result of oxidative phosphorylation
- The proton gradient establishes a voltage gradient
- The proton and voltage gradients together provide the mechanism to couple electron transport with Oxidative phosphorylation

Establishment of the Proton Gradient



Summary



Reduction Potentials

- A useful way to look at electron transport is to consider the change in free energy associated with the movement of electrons from one carrier to another
 - If we have two electron carriers, for example NADH and coenzyme Q, **Are** electrons more likely to be transferred from NADH to coenzyme Q, or vice versa?
 - What we need to know is the **Reduction Potentials** for each carrier
 - A carrier of high reduction potential will tend to be **reduced** if it is paired with a carrier of **low** reduction potential

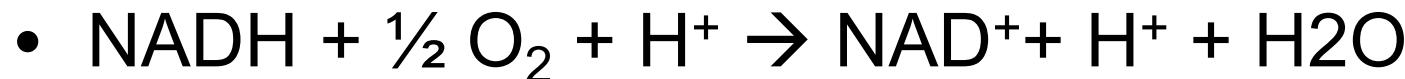
Reduction Potentials

- High $E^{\circ'}$ indicates a strong tendency to be reduced
- **Crucial equation:**

$$\Delta G^{\circ'} = - nF \Delta E^{\circ'}$$

- $\Delta E^{\circ'} = E^{\circ'}(\text{acceptor}) - E^{\circ'}(\text{donor})$

Reduction Potentials



$$\Delta G^\circ' = -nF(E^\circ'(\text{O}_2) - E^\circ'(\text{NADH}))$$

$$\Delta G^\circ' = -nF(0.82 - (-0.32)) = -nF(1.14)$$

$$\Delta G^\circ' = -2(96.5 \text{ kJ mol}^{-1}\text{V}^{-1})(1.136) = -220 \text{ kJ mol}^{-1}$$

Reduction Potentials

- Oxygen has the highest reduction potential, which means it gets reduced to water; and also mean it is the ultimate electron acceptor

Table 20.1

Standard Reduction Potentials for Several Biological Reduction Half Reactions

Reduction Half Reaction	$E^{\circ'} \text{ (V)}$
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}$	0.816
$\text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+}$	0.771
Cytochrome $a_3(\text{Fe}^{3+}) + e^- \rightarrow$ Cytochrome $a_3(\text{Fe}^{2+})$	0.350
Cytochrome $a(\text{Fe}^{3+}) + e^- \rightarrow$ Cytochrome $a(\text{Fe}^{2+})$	0.290
Cytochrome $c(\text{Fe}^{3+}) + e^- \rightarrow$ Cytochrome $c(\text{Fe}^{2+})$	0.254
Cytochrome $c_1(\text{Fe}^{3+}) + e^- \rightarrow$ Cytochrome $c_1(\text{Fe}^{2+})$	0.220
$\text{CoQH}^\bullet + \text{H}^+ + e^- \rightarrow \text{CoQH}_2$ (coenzyme Q)	0.190
$\text{CoQ} + 2\text{H}^+ + 2e^- \rightarrow \text{CoQH}_2$	0.060
Cytochrome $b_H(\text{Fe}^{3+}) + e^- \rightarrow$ Cytochrome $b_H(\text{Fe}^{2+})$	0.050
Fumarate + $2\text{H}^+ + 2e^- \rightarrow$ Succinate	0.031
$\text{CoQ} + \text{H}^+ + e^- \rightarrow \text{CoQH}^\bullet$	0.030
$[\text{FAD}] + 2\text{H}^+ + 2e^- \rightarrow [\text{FADH}_2]$	0.003–0.091*
Cytochrome $b_L(\text{Fe}^{3+}) + e^- \rightarrow$ Cytochrome $b_L(\text{Fe}^{2+})$	-0.100
Oxaloacetate + $2\text{H}^+ + 2e^- \rightarrow$ Malate	-0.166
Pyruvate + $2\text{H}^+ + 2e^- \rightarrow$ Lactate	-0.185
Acetaldehyde + $2\text{H}^+ + 2e^- \rightarrow$ Ethanol	-0.197
$\text{FMN} + 2\text{H}^+ + 2e^- \rightarrow \text{FMNH}_2$	-0.219
$\text{FAD} + 2\text{H}^+ + 2e^- \rightarrow \text{FADH}_2$	-0.219
1,3-bisphosphoglycerate + $2\text{H}^+ + 2e^- \rightarrow$	
Glyceraldehyde-3-phosphate + P_i	-0.290
$\text{NAD}^+ + 2\text{H}^+ + 2e^- \rightarrow \text{NADH} + \text{H}^+$	-0.320
$\text{NADP}^+ + 2\text{H}^+ + 2e^- \rightarrow \text{NADPH} + \text{H}^+$	-0.320
$\alpha\text{-Ketoglutarate} + \text{CO}_2 + 2\text{H}^+ + 2e^- \rightarrow$ Isocitrate	-0.380
$\text{Succinate} + \text{CO}_2 + 2\text{H}^+ + 2e^- \rightarrow \alpha\text{-Ketoglutarate} + \text{H}_2\text{O}$	-0.670

*Typical values for reduction of bound FAD in flavoproteins such as succinate dehydrogenase.

Note that we have shown a number of components of the electron transport chain individually. We are going to see them again as part of complexes. We have also included values for a number of reactions we saw in earlier chapters.

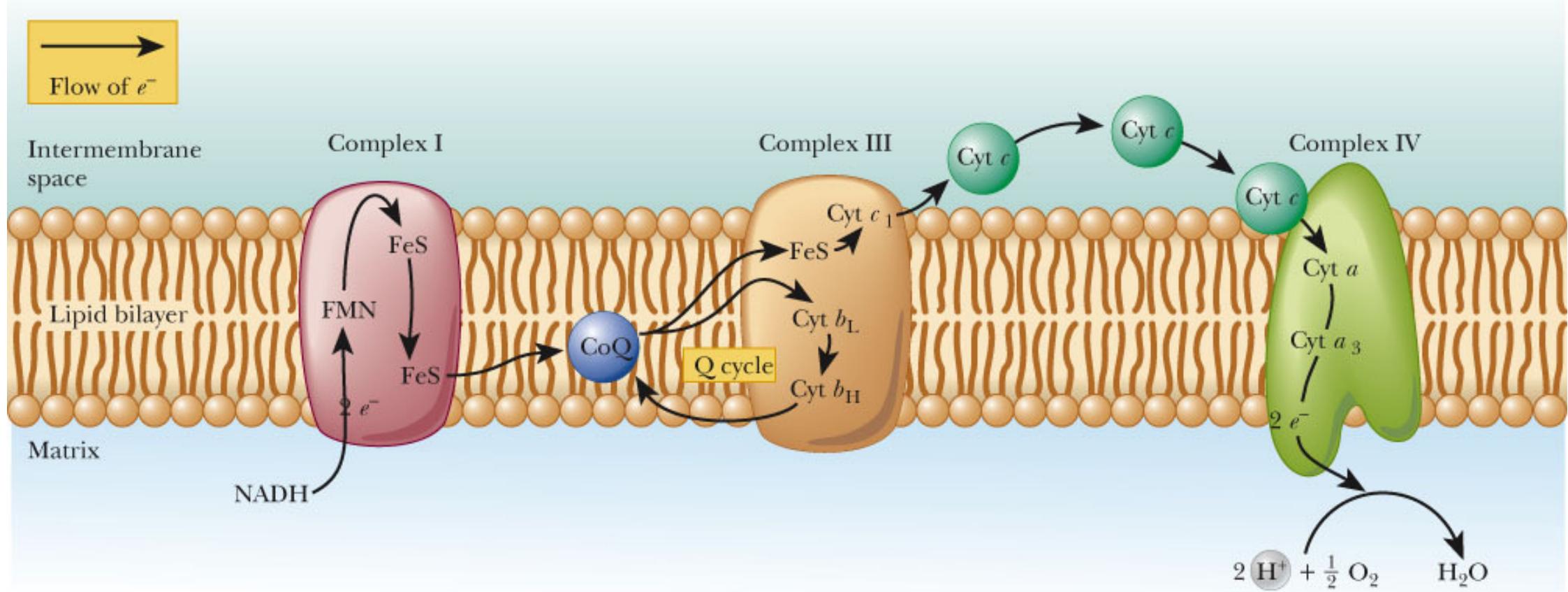
Electron-transport chain

- Composed of four large protein complexes:
 - 1. Complex I** - NADH-Coenzyme Q oxidoreductase
 - 2. Complex II** - Succinate-Coenzyme Q oxidoreductase
 - 3. Complex III** - Coenzyme Q-Cytochrome c oxidoreductase
 - 4. Complex IV** - Cytochrome c oxidase

Electron-transport chain

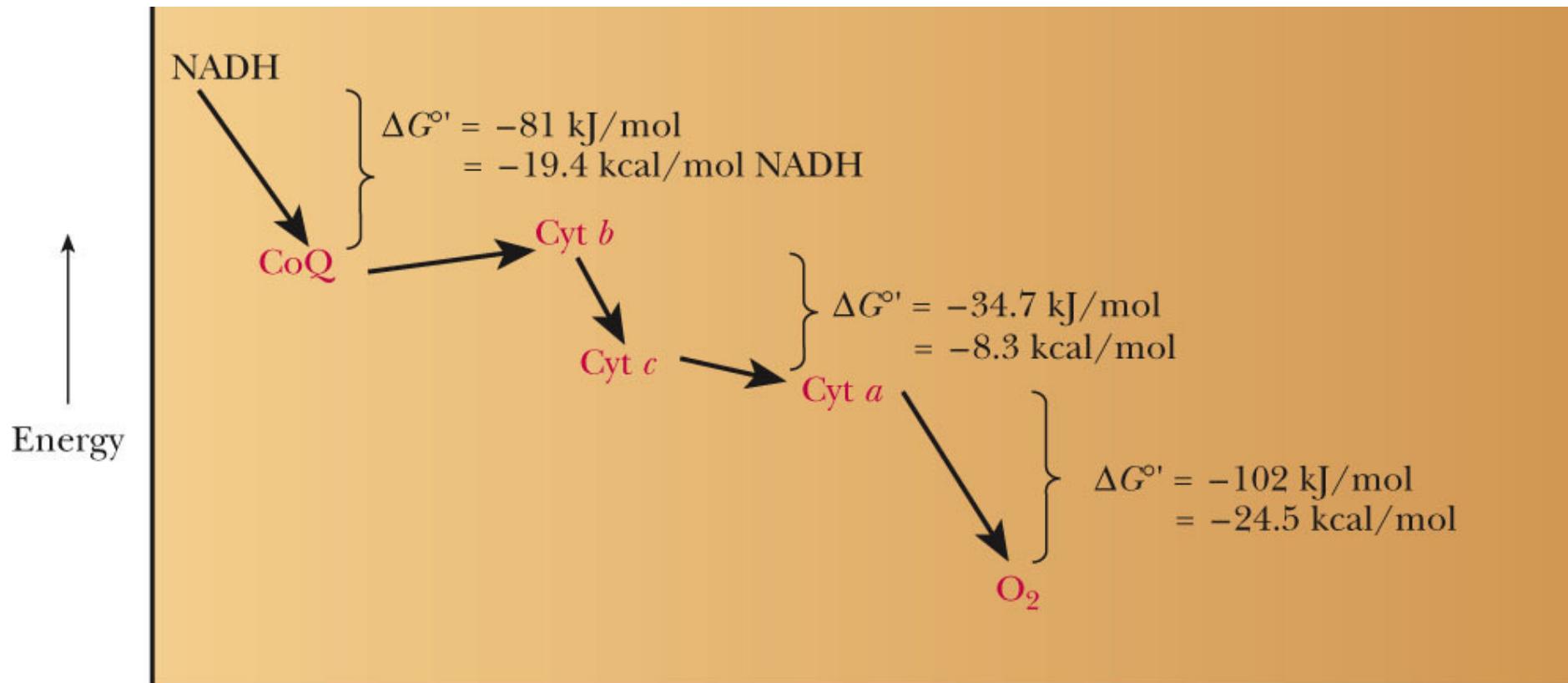
- Two important characteristics of the electron-transport chain
 - Order of electron carriers
 - Quantity of energy produced
- Electron carriers are arranged in order of increasing electron affinity.
 - Reduction potential
- This results in the spontaneous flow of electrons from carrier to carrier.

Electron Flow

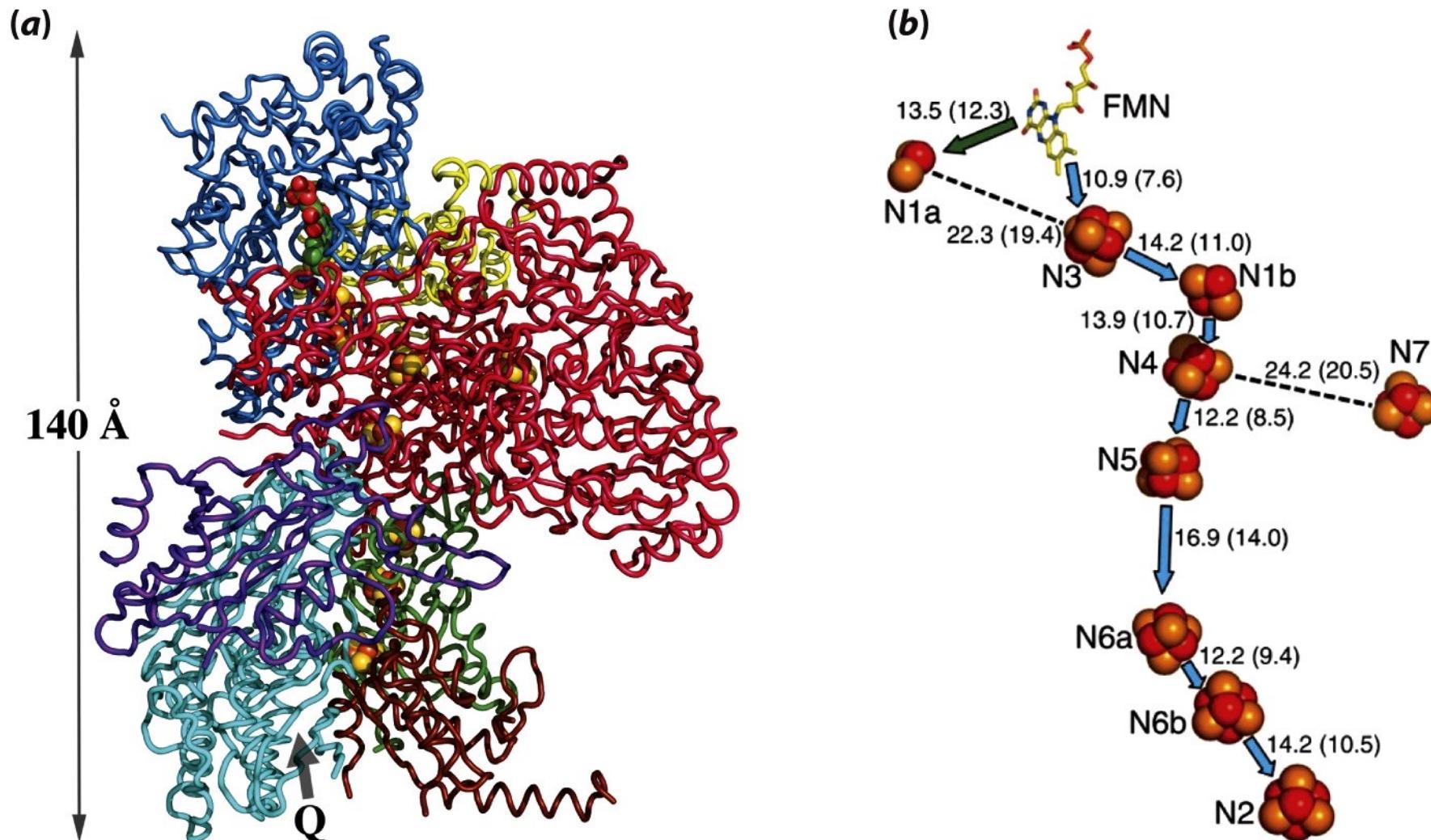


Energetics of Electron Transport

- The transfer of electrons is strongly exergonic and sufficient to drive the phosphorylation of ADP



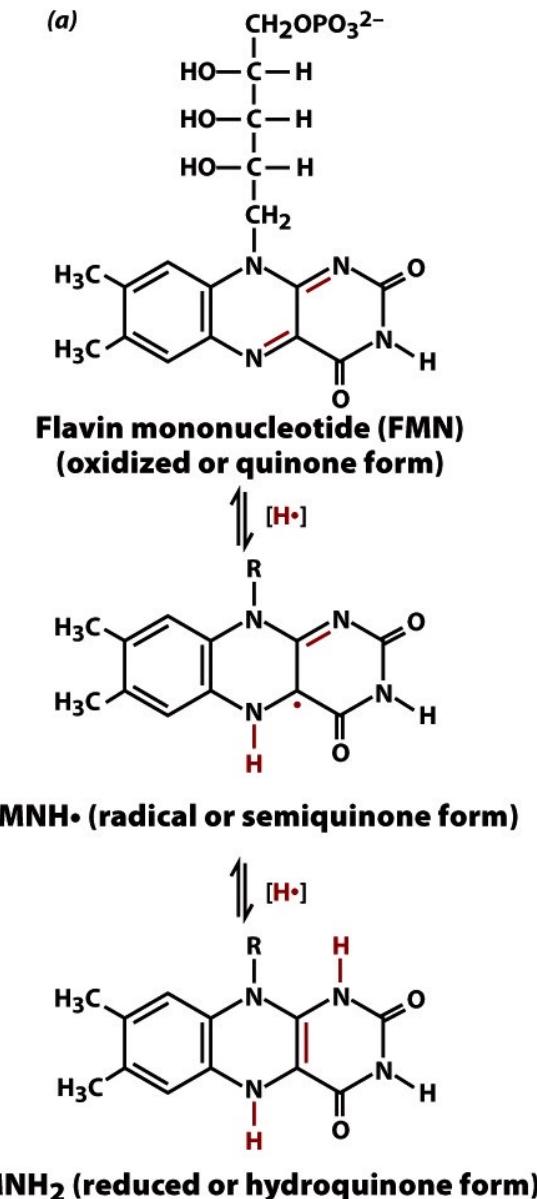
Complex I



Part a based on an X-ray structure by and Part b courtesy of Leonid Sazanov, Medical Research Council, Cambridge, U.K.

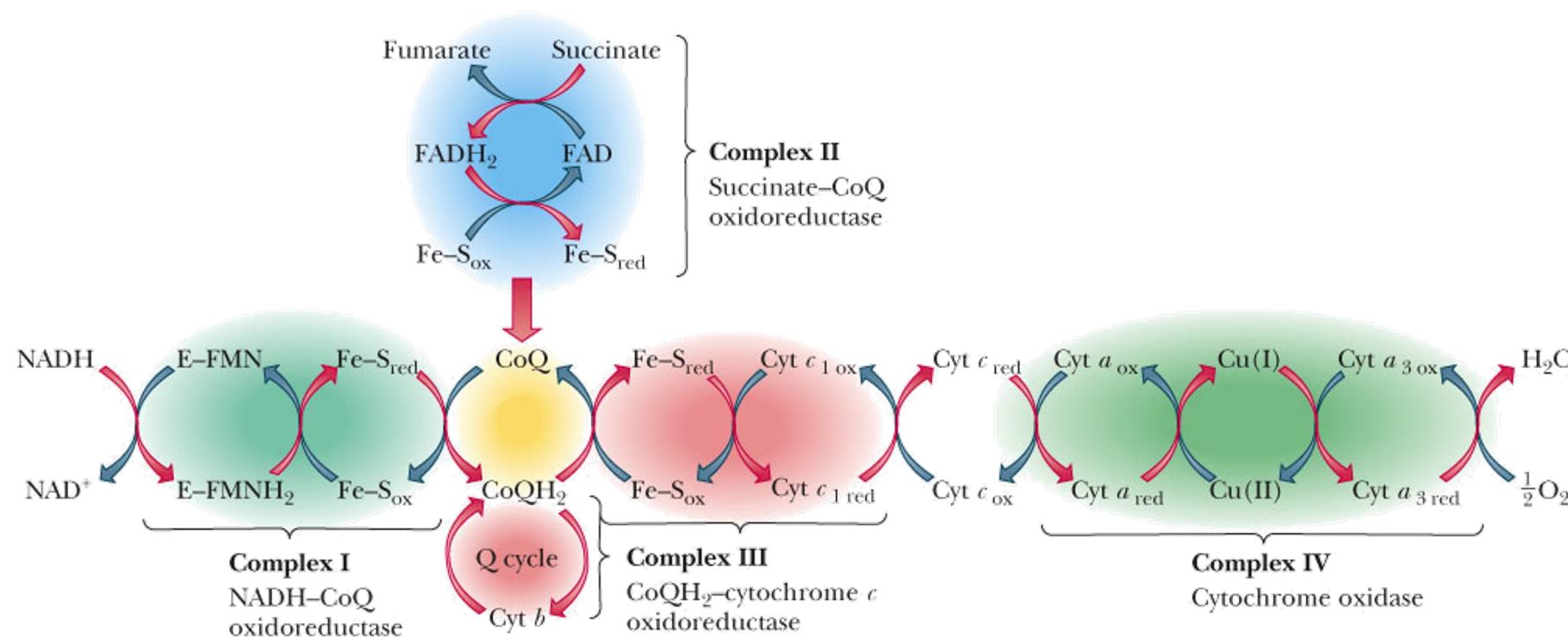
Electron Transport Complexes

- **Complex I:** NADH-CoQ oxidoreductase
- Electrons are passed from NADH to FMN
- Flavin is covalently bond to the enzyme

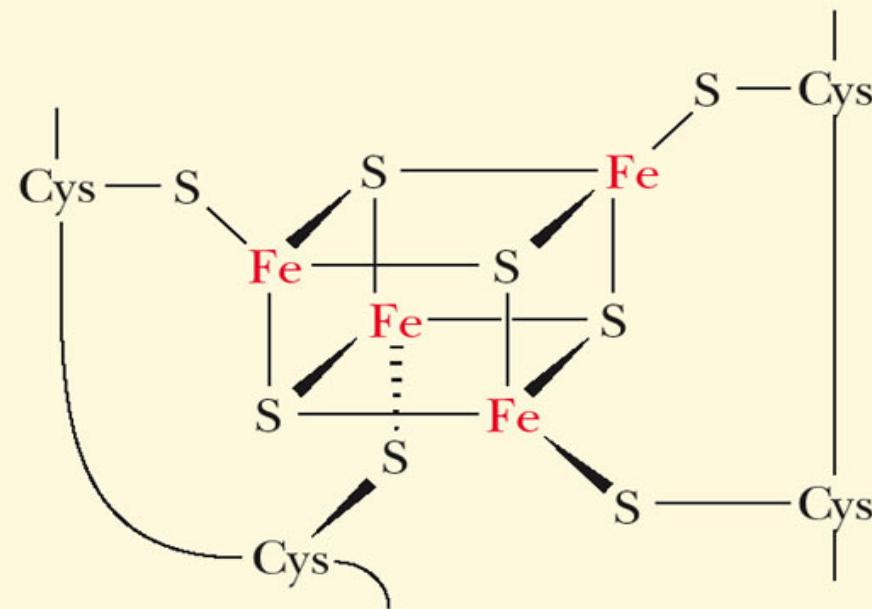
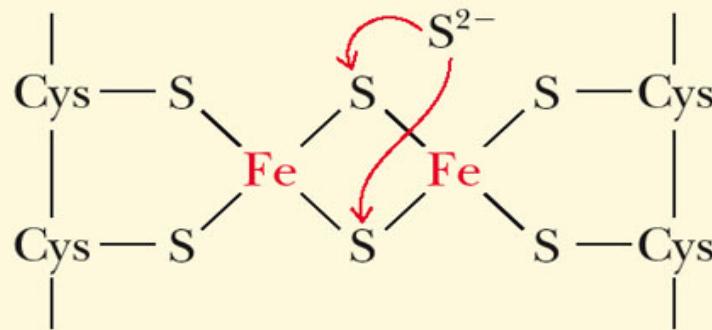


Electron Transport Complexes

- Electrons are then passed to the iron-sulfur clusters
 - Iron cycles between 3+ and 2+ states.
- The last step of Complex I involves electrons being passed to coenzyme Q (also called ubiquinone)



Iron-sulfur bonding in nonheme iron proteins



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Noneheme Iron containing Protein: do not contain a heme group, they contain sulfur complex called Iron-Sulfur Cluster complex.

Electron Transport Complexes

Complex II: Succinate-coenzyme Q oxidoreductase

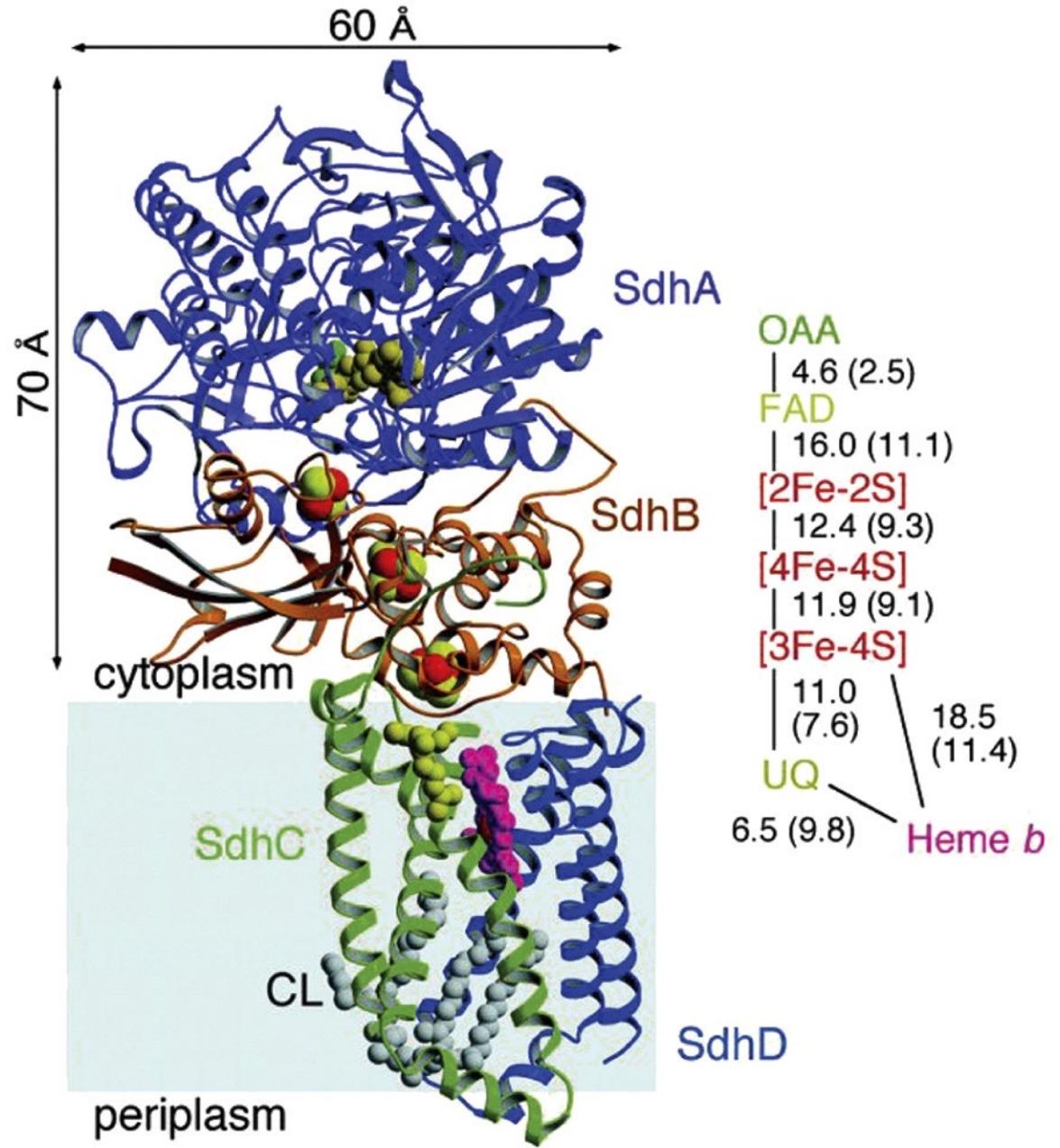


- The overall reaction is exergonic (-13.5 kJ/mol), but not enough to drive ATP production
 - No H⁺ is pumped out of the matrix during this step

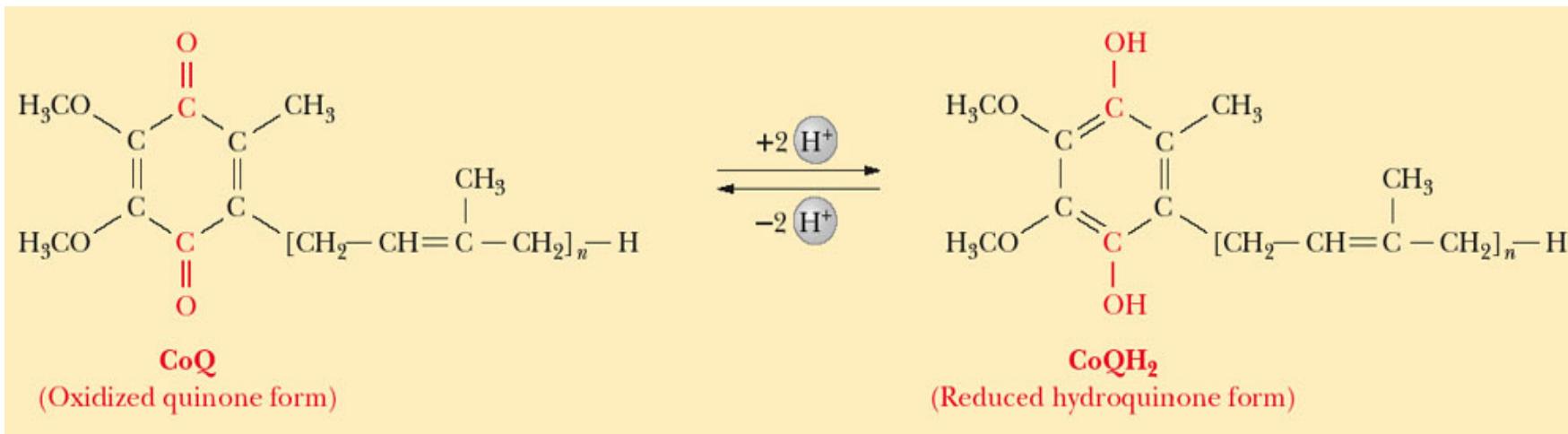
Complex II

Complex I and Complex II are not sequential, but accept electrons from different sources.

Complex I – NADH
Complex II - succinate



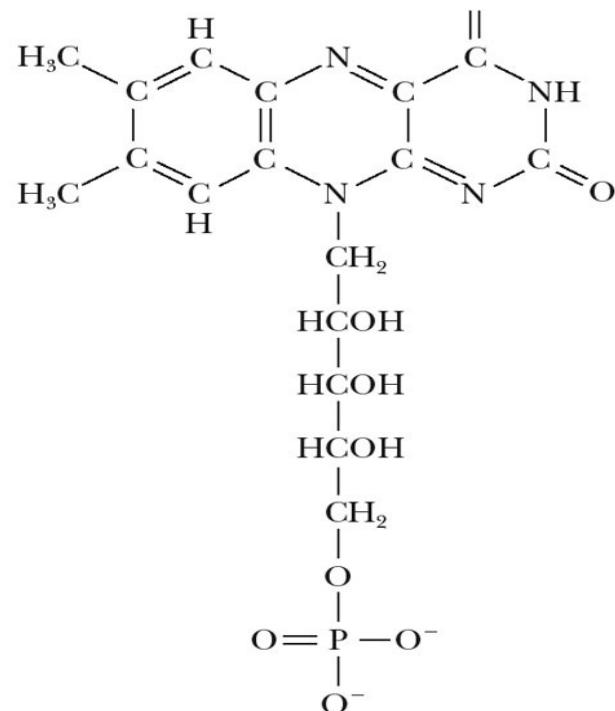
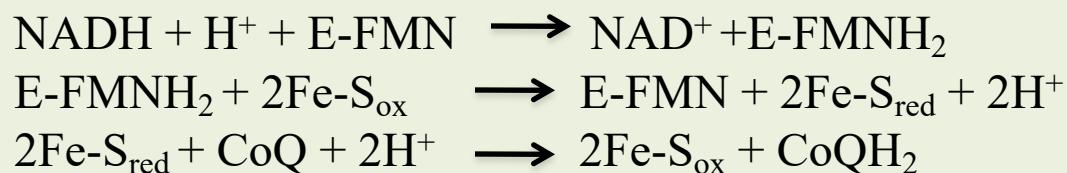
Courtesy of So Iwata, Imperial College London, U.K.



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The oxidized and reduced forms of coenzyme Q.
 Coenzyme Q is also called ubiquinone.

Complex I: NADH-CoQ oxidoreductase



The structure of FMN
 (Flavin mononucleotide)

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Electron Transport Complexes

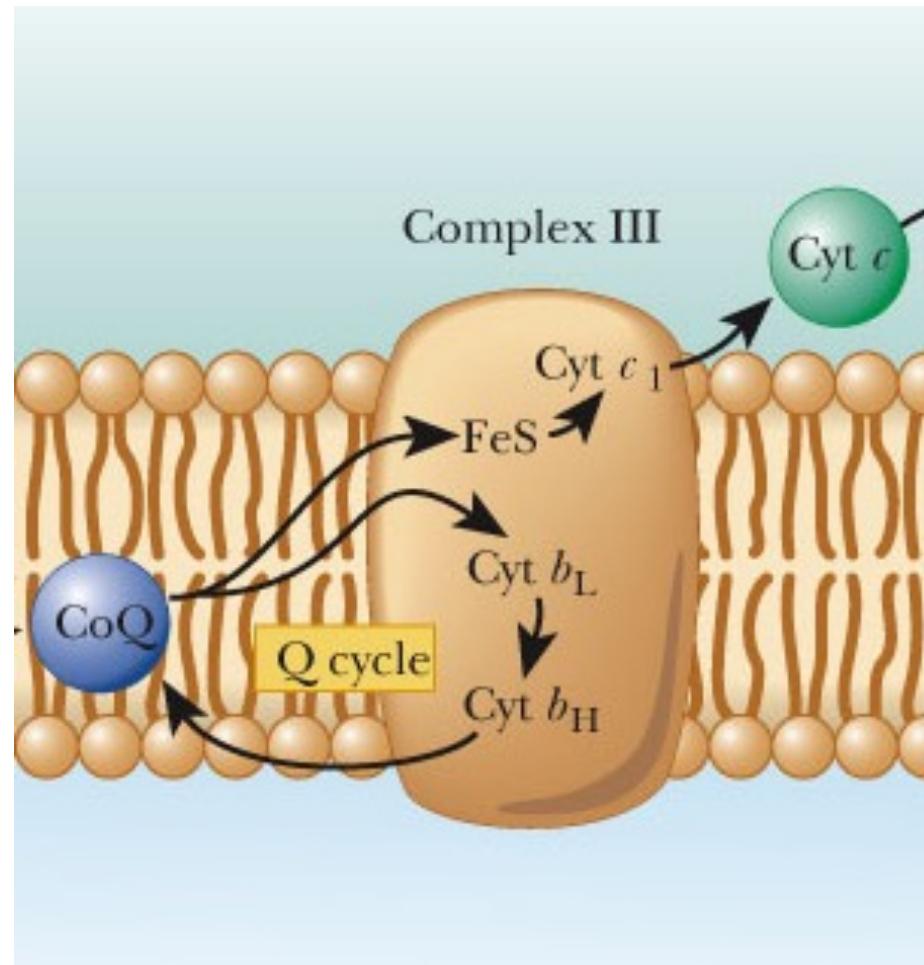
Complex III: CoQH₂-cytochrome c oxidoreductase

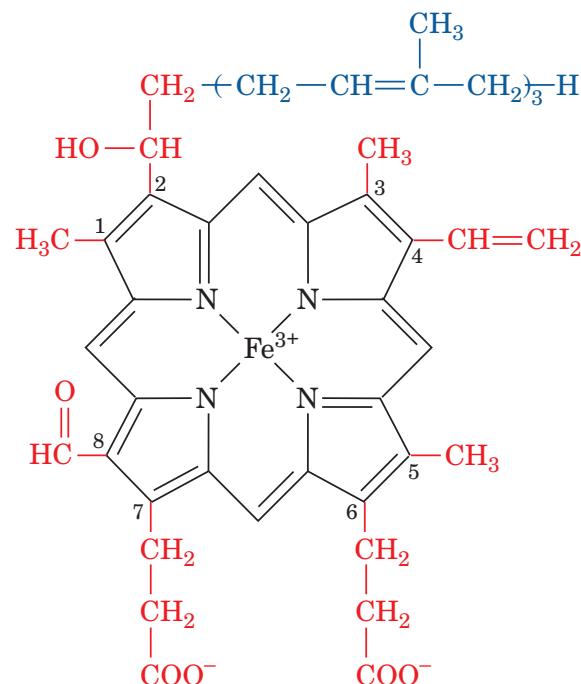


- This reaction of this complex results in a decrease in free energy that is sufficient to drive the phosphorylation of ADP to ATP
 - Cytochromes carry e- but not H
 - This is the second place where H⁺ get released and pumped outside to the intermediate space
- The flow of electrons from reduced CoQ, a quinone that can exist in 3 forms, is known as the Q cycle

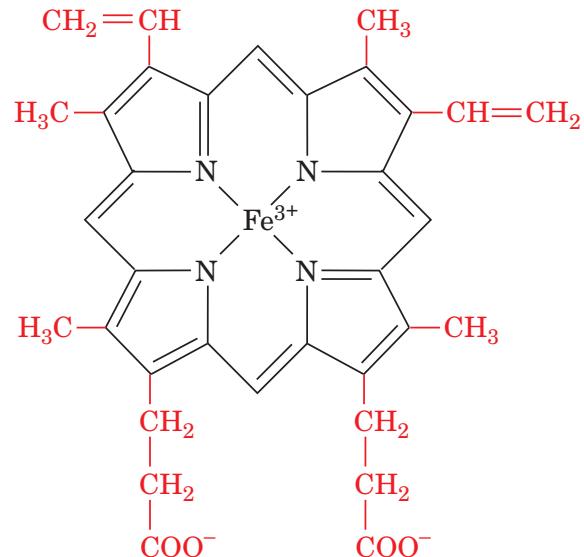
Complex III

- One electron thrown on FeS the other is catched by cyt b_L which get recycled back to CoQ (semiquinone)

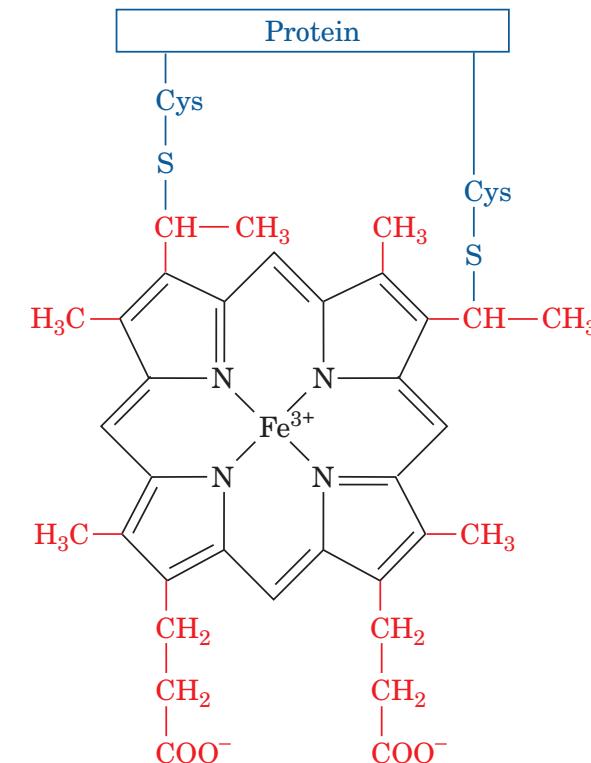




Heme a



Heme b
(iron-protoporphyrin IX)



Heme c

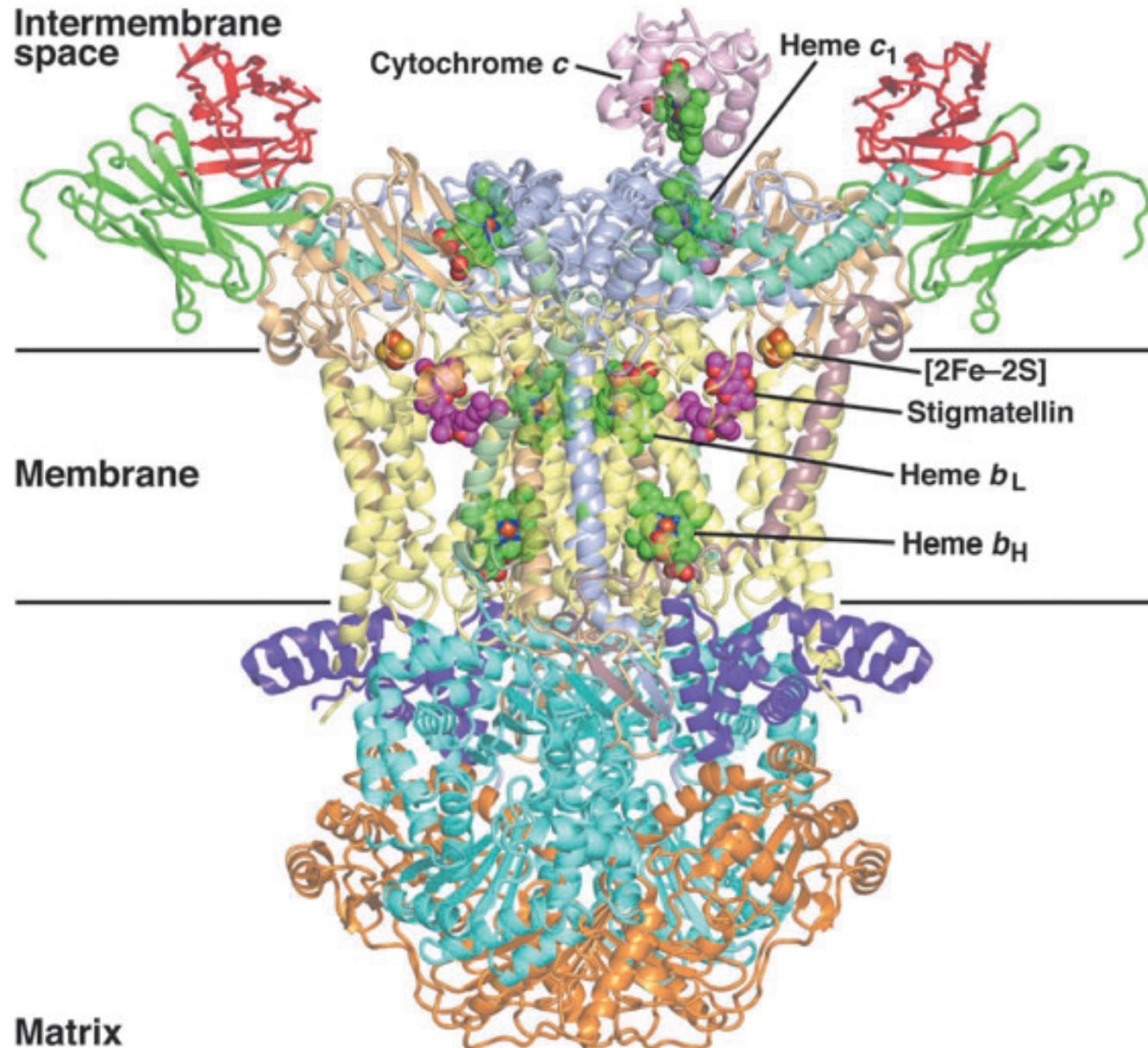
The heme group of cytochromes (heme-iron containing protein):

(a) Structures of the heme of all *b* cytochromes and of hemoglobin and myoglobin. The wedge bonds show the fifth and sixth coordination sites of the iron atom.

Complex III

Pass electrons from CoQ to cytochrome c

Electrons in Complex III must go from a 2 e⁻ carrier (CoQH₂) to a 1 e⁻ carrier, cytochrome c.



Electron Transport Complexes

Complex IV: Cytochrome c oxidase

- Catalyzes the final step in electron transport



- Complex IV contains cytochrome a, cytochrome a₃, and Cu(II), which are also involved in the electron transport
- Complex IV is the link to proton pump

The Energetics of Electron Transport Reactions

Table 20.2

The Energetics of Electron Transport Reactions

Reaction	ΔG° kJ (mol NADH) ⁻¹	kcal (mol NADH) ⁻¹
$\text{NADH} + \text{H}^+ + \text{E-FMN} \rightarrow \text{NAD}^+ + \text{E-FMNH}_2$	-38.6	-9.2
$\text{E-FMNH}_2 + \text{CoQ} \rightarrow \text{E-FMN} + \text{CoQH}_2$	-42.5	-10.2
$\text{CoQH}_2 + 2 \text{ Cyt } b[\text{Fe(III)}] \rightarrow \text{CoQ} + 2\text{H}^+ + 2 \text{ Cyt } b[\text{Fe(II)}]$	+11.6	+2.8
$2 \text{ Cyt } b[\text{Fe(II)}] + 2 \text{ Cyt } c_1[\text{Fe(III)}] \rightarrow 2 \text{ Cyt } c_1[\text{Fe(II)}] + 2 \text{ Cyt } b[\text{Fe(III)}]$	-34.7	-8.3
$2 \text{ Cyt } c_1[\text{Fe(II)}] + 2 \text{ Cyt } c[\text{Fe(III)}] \rightarrow 2 \text{ Cyt } c[\text{Fe(II)}] + 2 \text{ Cyt } c_1[\text{Fe(III)}]$	-5.8	-1.4
$2 \text{ Cyt } c[\text{Fe(II)}] + 2 \text{ Cyt } (aa_3)[\text{Fe(III)}] \rightarrow 2 \text{ Cyt } (aa_3)[\text{Fe(II)}] + 2 \text{ Cyt } c[\text{Fe(III)}]$	-7.7	-1.8
$2 \text{ Cyt } (aa_3)[\text{Fe(II)}] + \frac{1}{2}\text{O}_2 + 2\text{H}^+ \rightarrow 2 \text{ Cyt } (aa_3)[\text{Fe(III)}] + \text{H}_2\text{O}$	-102.3	-24.5
Overall reaction: $\text{NADH} + \text{H}^+ + \frac{1}{2}\text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O}$	-220	-52.6

Connection between Electron Transport & Phosphorylation

- The energy-releasing oxidations give rise to proton pumping and a pH gradient across the **inner mitochondrial** membrane
- Differences in the concentration of ions across the membrane generates a **pH gradient**
- A coupling process converts the electrochemical potential to the chemical energy of ATP
- The coupling factor is **ATP synthase**, a complex protein oligomer, separate from the electron transport complexes
 - Is needed to link oxidation and phosphorylation
 - **Uncouplers** inhibit the phosphorylation of ADP without affecting electron transport; examples are 2,4 dinitrophenol, valinomycin & gramicidin A

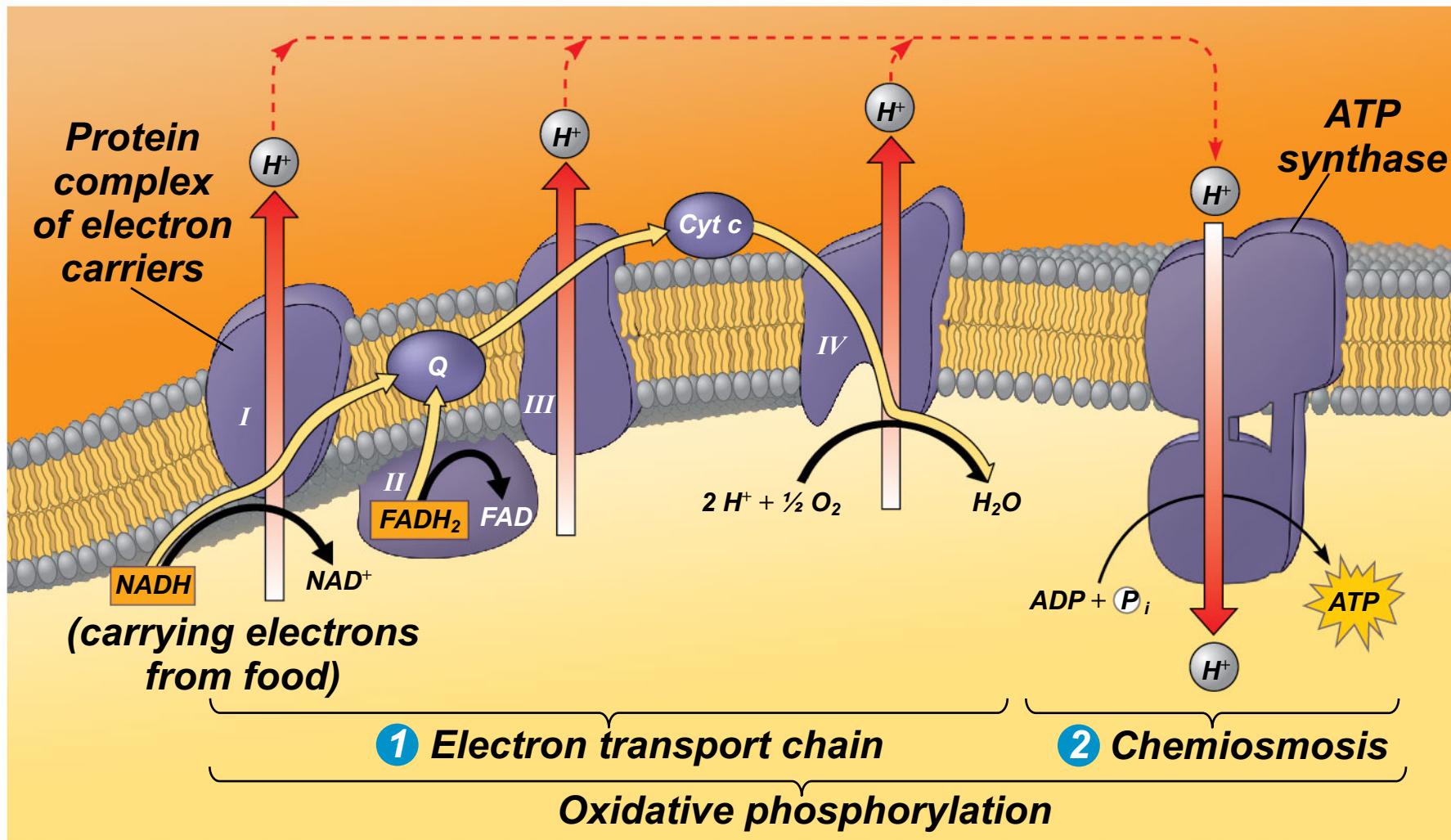
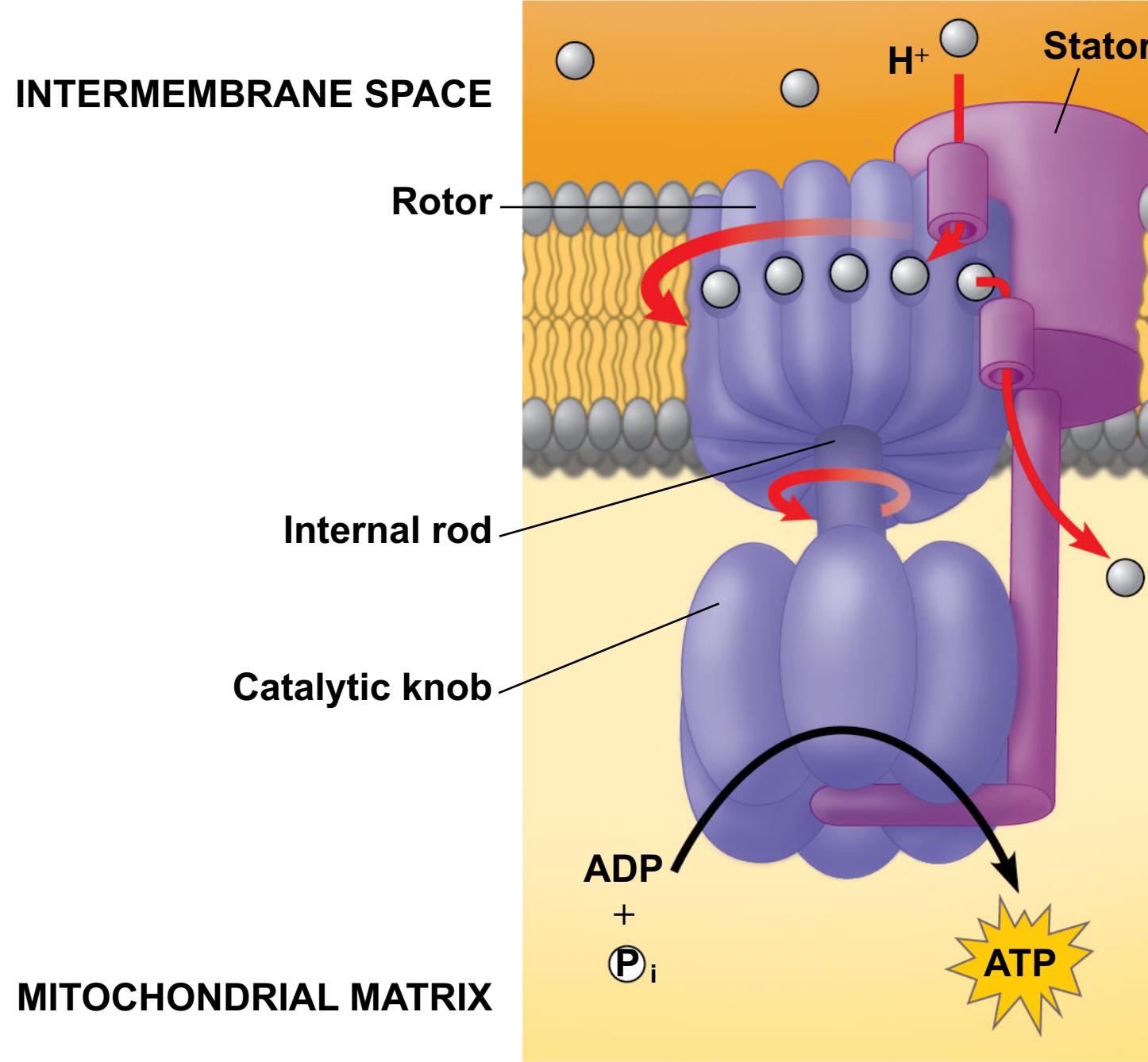


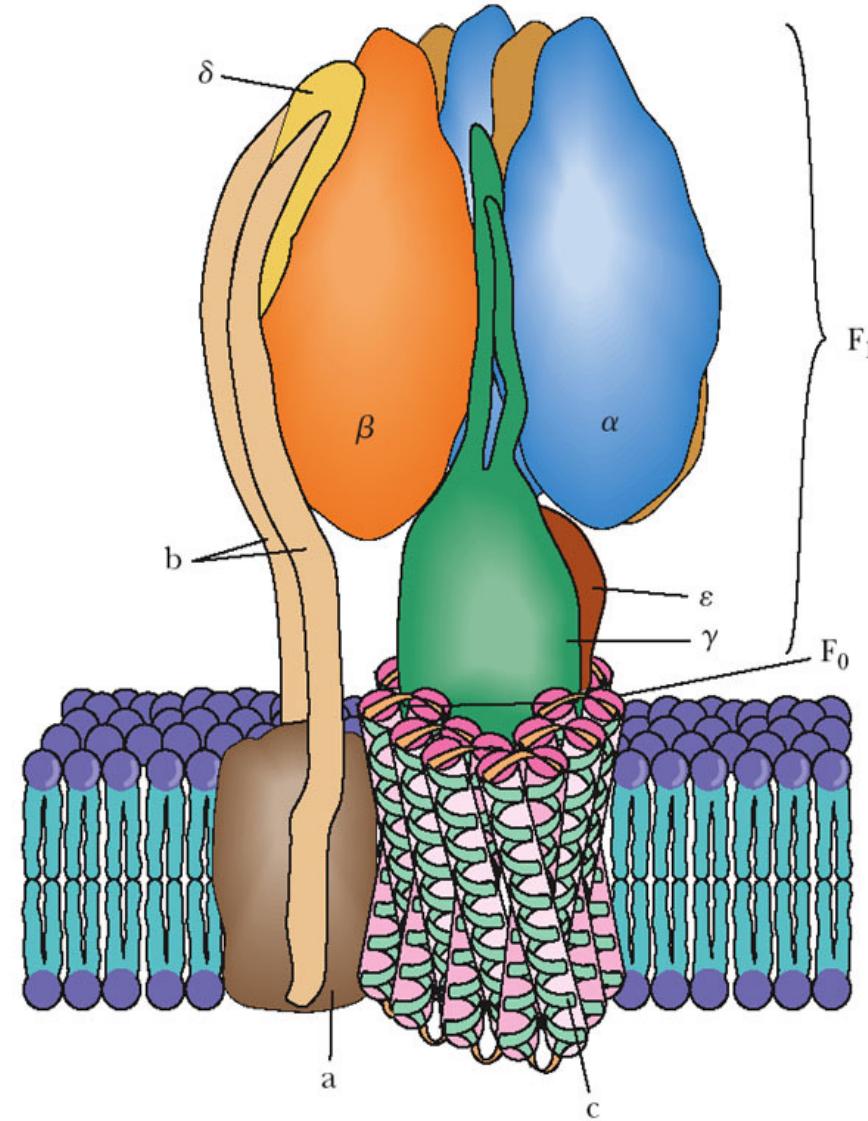
Figure 10.14

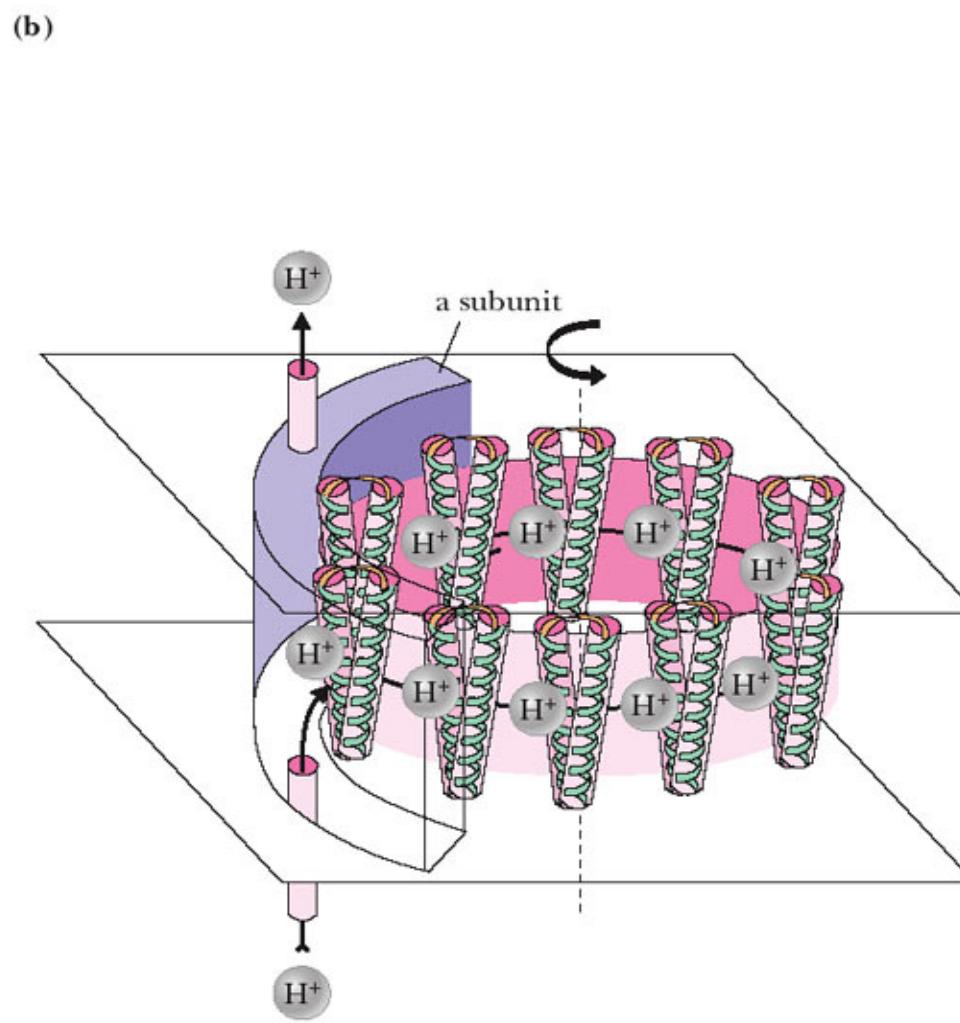
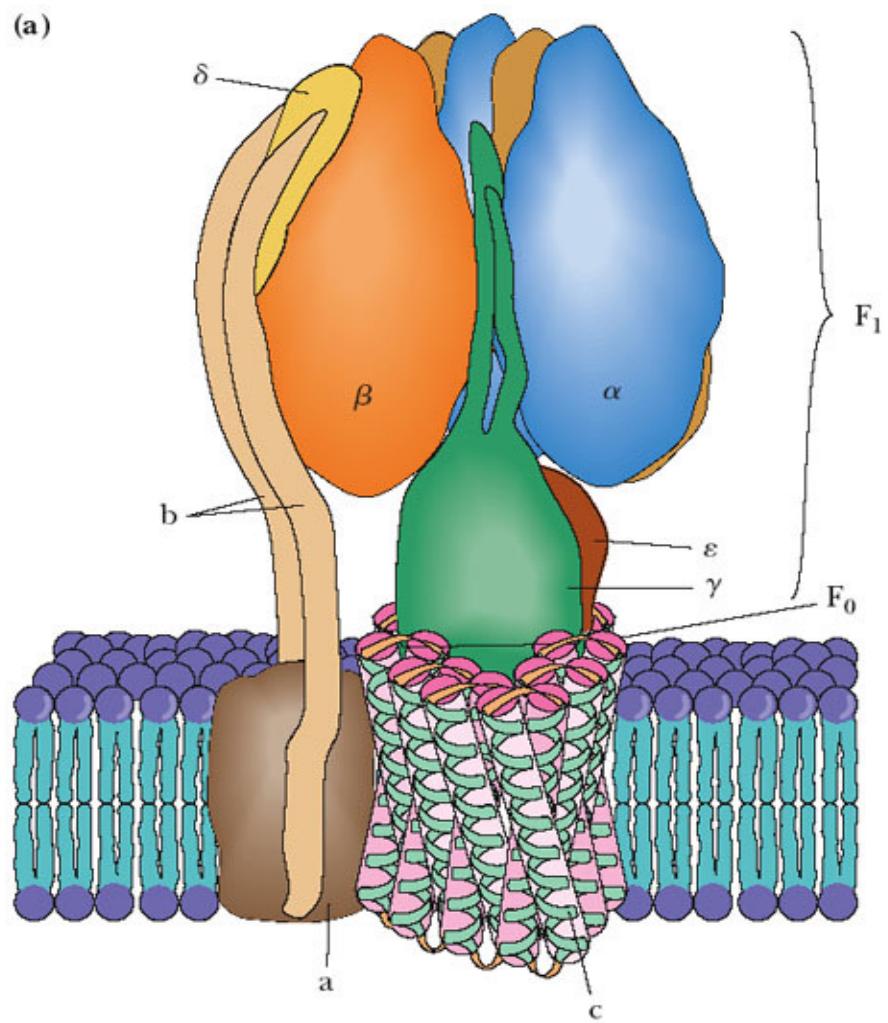


- Certain electron carriers in the electron transport chain accept and release H^+ along with the electrons
- In this way, the energy stored in a H^+ gradient across a membrane couples the redox reactions of the electron transport chain to ATP synthesis
- The H^+ gradient is referred to as a **proton-motive force**, emphasizing its capacity to do work

Components of ATP synthase

- These are knob-like projections into the matrix side of the inner membrane.
- Two units
 - F₁ contains the catalytic site for ATP synthesis.
 - F₀ serves as a transmembrane channel for H⁺ flow.
- F₁-F₀ complex serves as the molecular apparatus for coupling H⁺ movement to ATP synthase.





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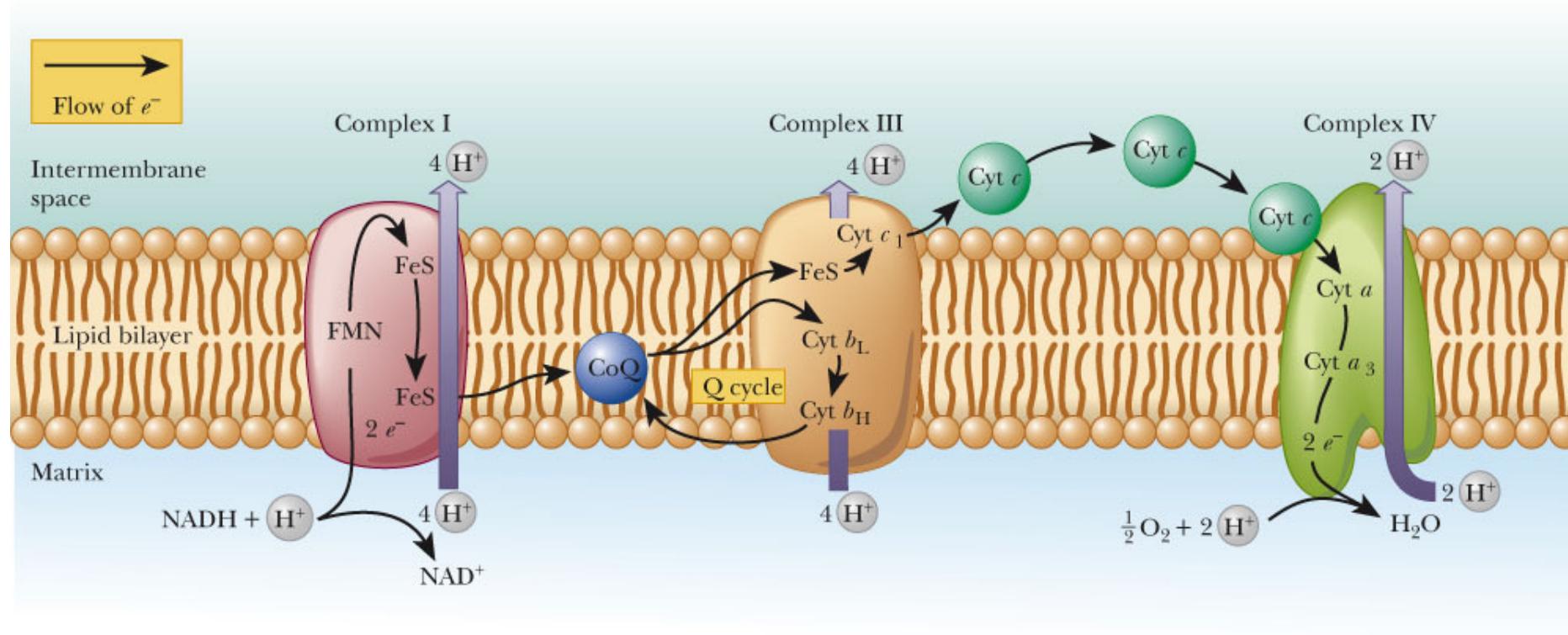
A model of the F1 and F0 components of the ATP synthase:

a rotating molecular motor. The **a**, **b**, α , β , and δ subunits constitute the stator of the motor, and the **c**, γ , and ϵ subunits form the rotor. Flow of protons through the structure turns the rotor and drives the cycle of conformational changes in α and β that synthesize ATP.

Chemiosmotic Coupling

- Based on a H⁺ concentration gradient between the intermembrane space and the matrix
- A proton gradient exists because the various proteins that serve as electron carriers are not symmetrically oriented with respect to the two sides of the inner mitochondrial membrane
- These proteins take up protons from the matrix when they are reduced and release them to the intermembrane space when they are reoxidized
- The reactions of NADH, coq, and O₂ all lead to proton pumping to the intermediate space

Chemiosmotic Coupling



Chemiosmotic Coupling

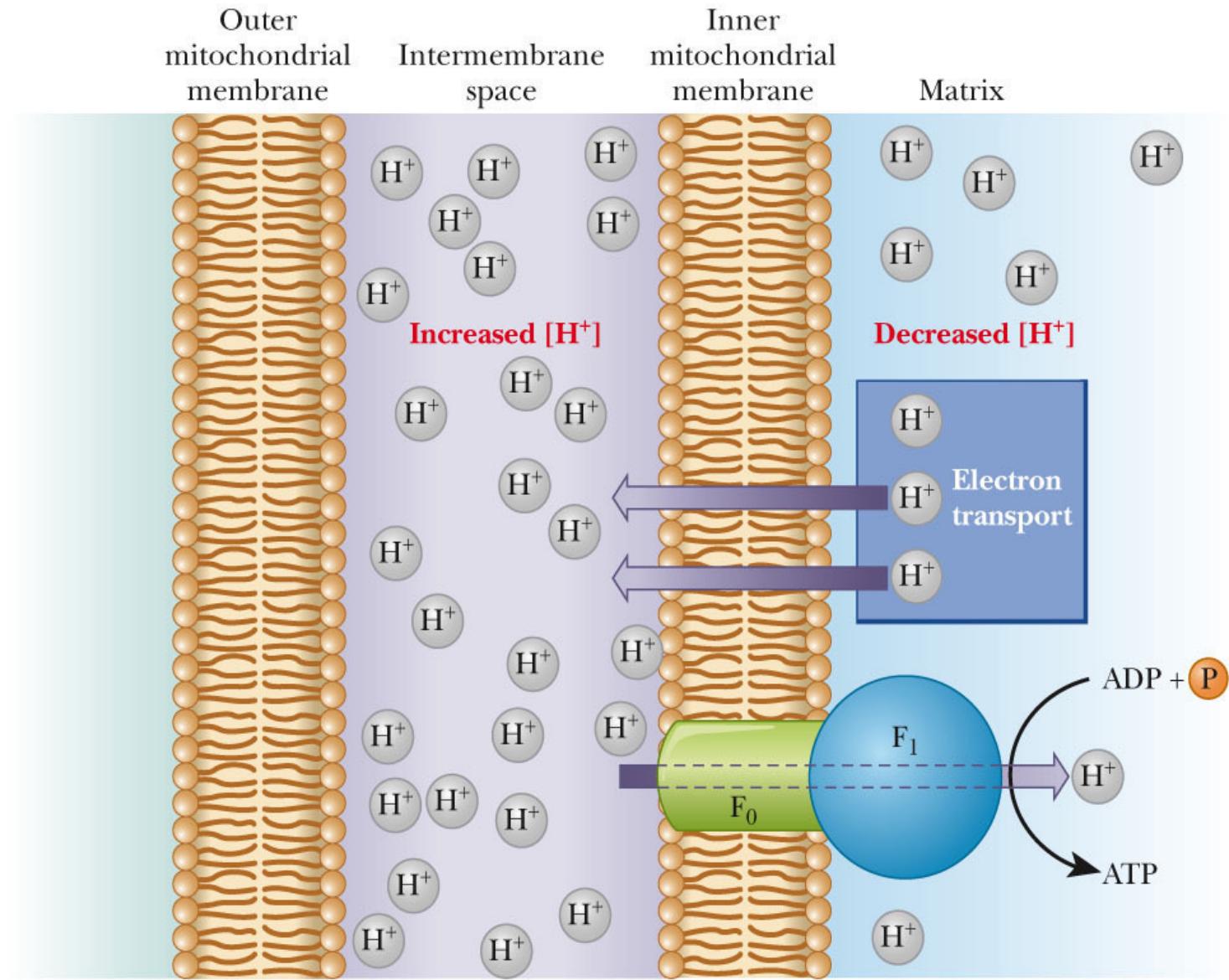
Evidence for chemiosmotic coupling suggested (Mitchell 1961):

- A system with definite inside and outside compartments (closed vesicles) is essential.
- Submitochondrial vesicles can be prepared, which carry out oxidative phosphorylation and have an asymmetric orientation of respiratory complexes.
- A model system for oxidative phosphorylation can be constructed with proton pumping in the absence of electron transport; the model system consists of reconstituted membrane vesicles, mitochondrial ATP synthase, and a proton pump.
- The existence of the pH gradient has been demonstrated and confirmed experimentally

Chemiosmotic Coupling

- The mechanism by which the proton gradient leads to the production of ATP depends on ion channels through the inner mitochondrial membrane
 - Protons flow back into the matrix through channels in the F_0 unit of ATP synthase
 - The flow of protons is accompanied by formation of ATP in the F_1 unit of ATP synthase

Chemiosmotic Coupling

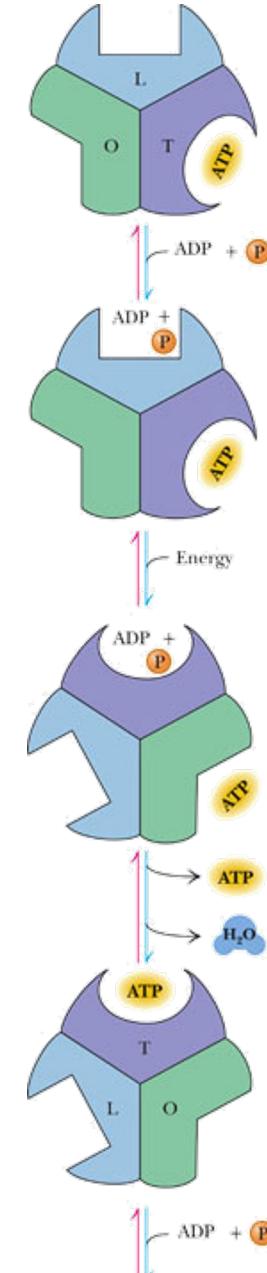
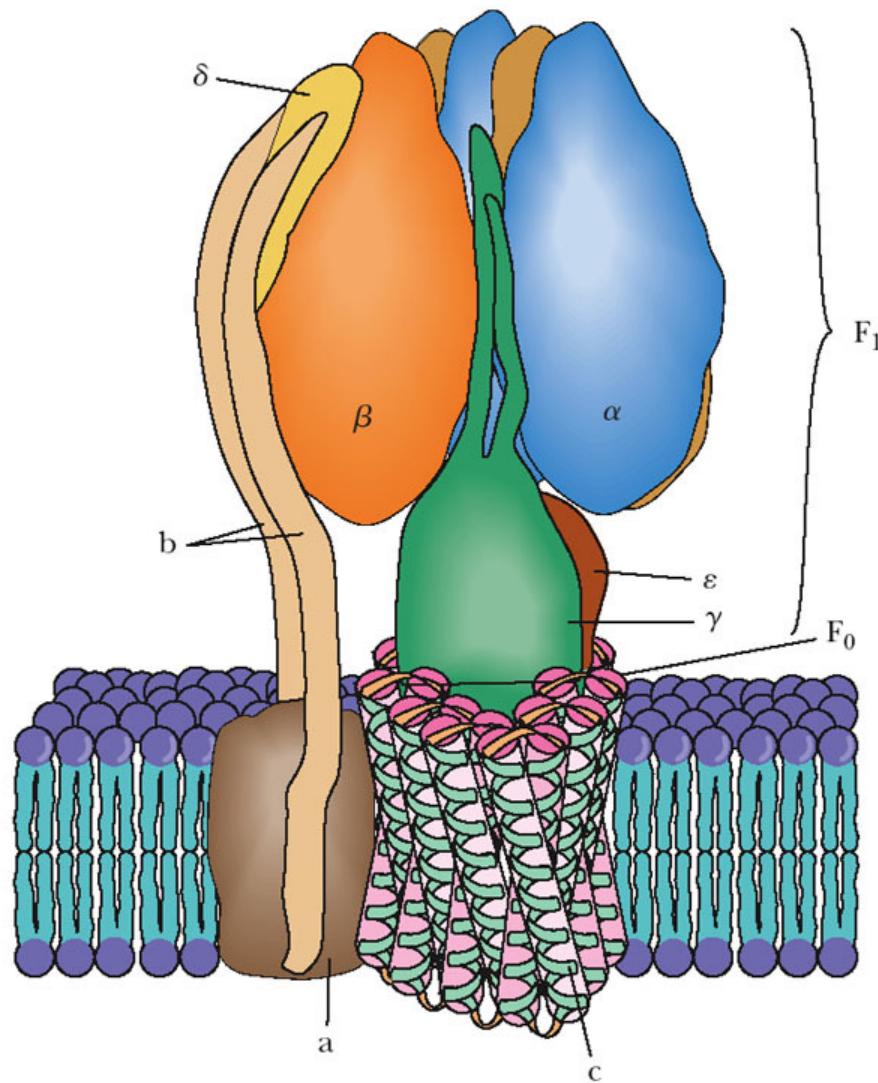


Conformational Coupling

- The proton gradient leads to changes in conformation in a number of proteins, including ATP synthase
- There are 3 sites for substrate on ATP synthase and 3 possible conformations:
 - **Open (O); a low affinity for substrate**
 - **Loose-binding (L); not catalytically active, binds ADP & P_i**
 - **Tight-binding (T); catalytically active, binds ATP**
- These sites interconvert as a result of proton flux through ATP synthase
- Proton flux converts L to T, which produces ATP
- Proton flux converts T to O, releasing ATP

Release of ATP from ATP Synthase

Chemical--mechanical --- chemical



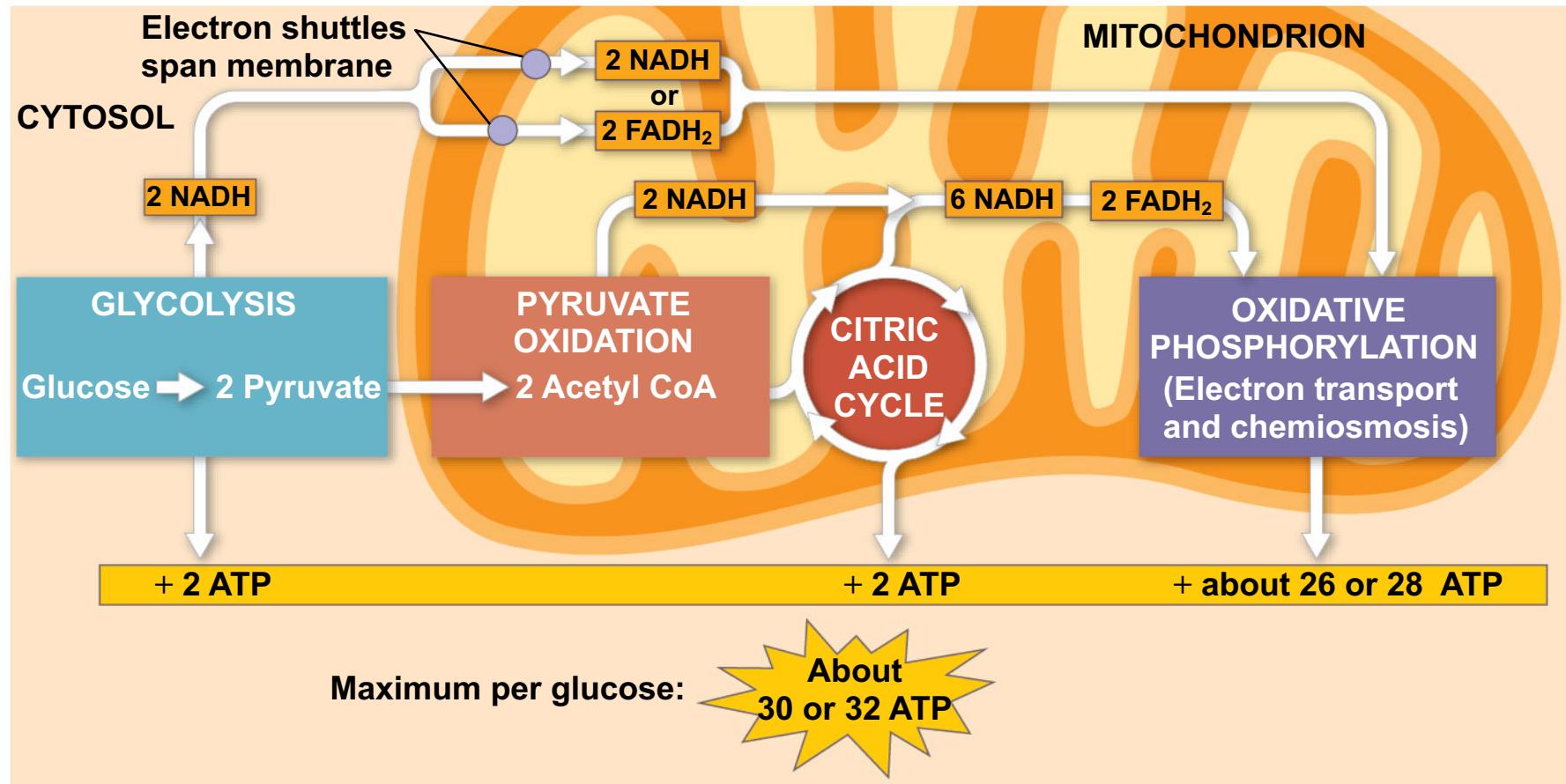
Cycle repeats

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An Accounting of ATP Production by Cellular Respiration

- During cellular respiration, most energy flows in this sequence:
glucose → NADH → electron transport chain → proton-motive force → ATP
- About 34% of the energy in a glucose molecule is transferred to ATP during cellular respiration, making about 32 ATP
- The rest of the energy is lost as heat

Figure 10.16



P/O Ratio

- **P/O ratio:** the number of moles of P_i consumed in **Phosphorylation** to the number of moles of oxygen atoms consumed in **Oxidation**
- **Phosphorylation:** $ADP + P_i \rightarrow ATP + H_2O$
- **Oxidation:** $1/2O_2 + 2H^+ + 2e^- \rightarrow H_2O$
 - $P/O = 2.5$ when NADH is oxidized
 - $P/O = 1.5$ when $FADH_2$ is oxidized

ATP Yield from Complete Oxidation of Glucose

- In the complete oxidation of glucose, a total of 30 or 32 molecules of ATP are produced for each molecule of glucose, depending on the shuttle mechanism

The ATP Yield from Complete Oxidation of Glucose

Table 20.3

Yield of ATP from Glucose Oxidation

Pathway	ATP Yield per Glucose			
	Glycerol–Phosphate Shuttle	Malate–Aspartate Shuttle	NADH	FADH ₂
Glycolysis: glucose to pyruvate (cytosol)				
Phosphorylation of glucose	-1	-1		
Phosphorylation of fructose-6-phosphate	-1	-1		
Dephosphorylation of 2 molecules of 1,3-BPG	+2	+2		
Dephosphorylation of 2 molecules of PEP	+2	+2		
Oxidation of 2 molecules of glyceraldehyde-3-phosphate yields 2 NADH			+2	
Pyruvate conversion to acetyl-CoA (mitochondria)				
2 NADH produced			+2	
Citric acid cycle (mitochondria)				
2 molecules of GTP from 2 molecules of succinyl-CoA	+2	+2		
Oxidation of 2 molecules each of isocitrate, α -ketoglutarate, and malate yields 6 NADH			+6	
Oxidation of 2 molecules of succinate yields 2 FADH ₂				+2
Oxidative phosphorylation (mitochondria)				
2 NADH from glycolysis yield 1.5 ATP each if NADH is oxidized by glycerol–phosphate shuttle; 2.5 ATP by malate–aspartate shuttle	+3	+5	-2	
Oxidative decarboxylation of 2 pyruvate to 2 acetyl-CoA: 2 NADH produce 2.5 ATP each	+5	+5	-2	
2 FADH ₂ from each citric acid cycle produce 1.5 ATP each	+3	+3		-2
6 NADH from citric acid cycle produce 2.5 ATP each	+15	+15	-6	
Net Yield	+30	+32	0	0

(Note: These P/O ratios of 2.5 and 1.5 for mitochondrial oxidation of NADH and FADH₂ are “consensus values.” Since they may not reflect actual values and since these ratios may change depending on metabolic conditions, these estimates of ATP yield from glucose oxidation are approximate.)