Electron Transport Chain

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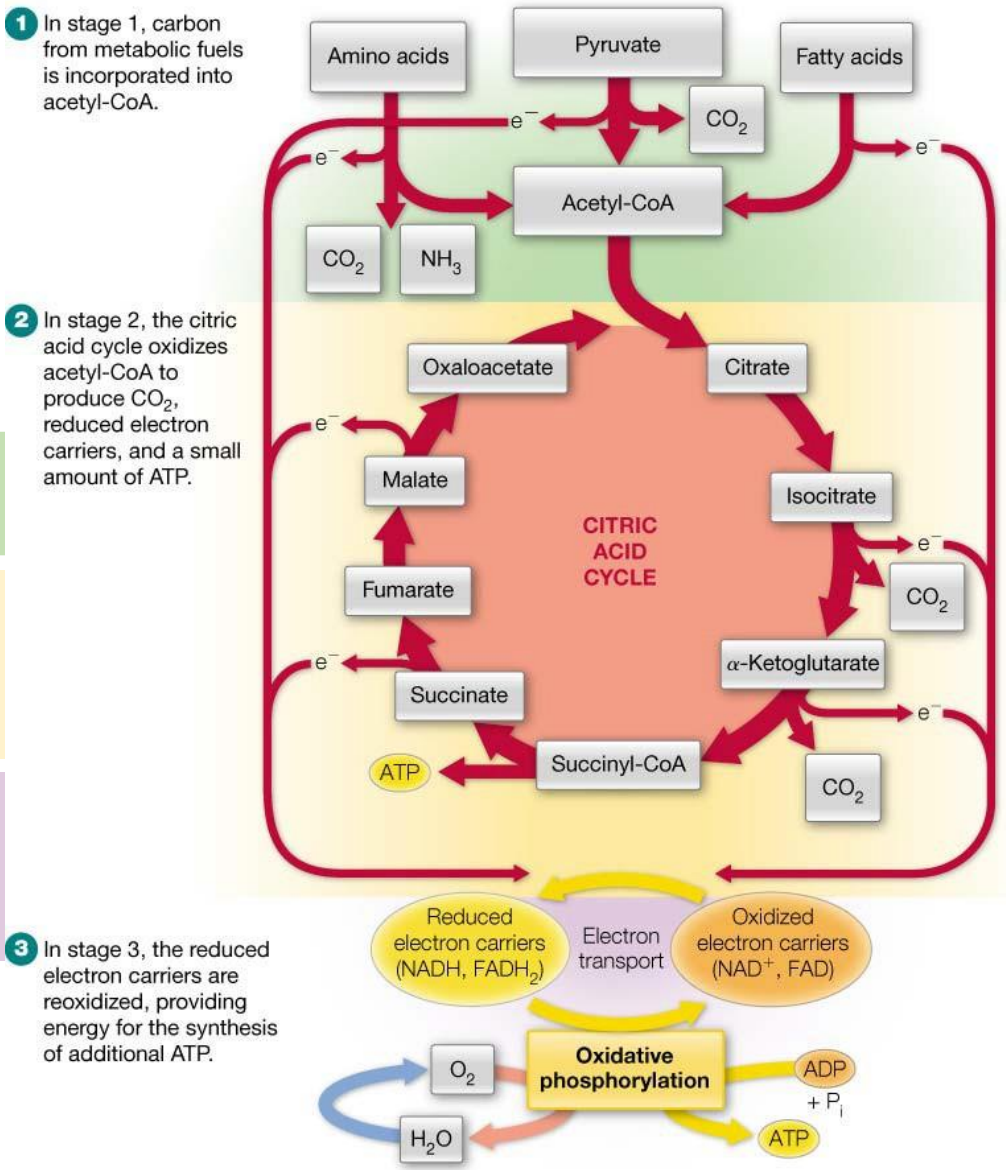
Objectives

- The Mitochondrion: Scene of the Action
- Free Energy Changes in Biological Oxidations (intro from Chap. 3)
- Electron Transport

- Textbook Chap. 14

Stages of Cellular Respiration

- Metabolic oxidation of organic substrates (cellular respiration) occurs in three stages:
  - In stage 1, carbon from metabolic fuels is incorporated into acetyl-CoA
  - In stage 2, the citric acid cycle oxidizes acetyl-CoA to produce CO_2, reduced electron carriers, and a small amount of ATP
  - In stage 3, the reduced electron carriers are reoxidized, providing energy for the synthesis of additional ATP
- In eukaryotic organisms, **these three stages are located in the mitochondria**.



Energy from Different Stages of Cellular Respiration

- Relatively little ATP is generated per mole of glucose in stages 1 and 2 (including glycolysis and citric acid cycle). Stages 1 and 2 produce 10 moles NADH and 2 moles of FAD to FADH2 per mole of glucose
- Reoxidation of NADH and FADH2 in stage 3 (cellular respiration, oxidative phosphorylation) provides most of the energy used for ATP synthesis

• In eukaryotic organisms, **stages 2 and 3 as well as part of stage 1 in the mitochondria**.

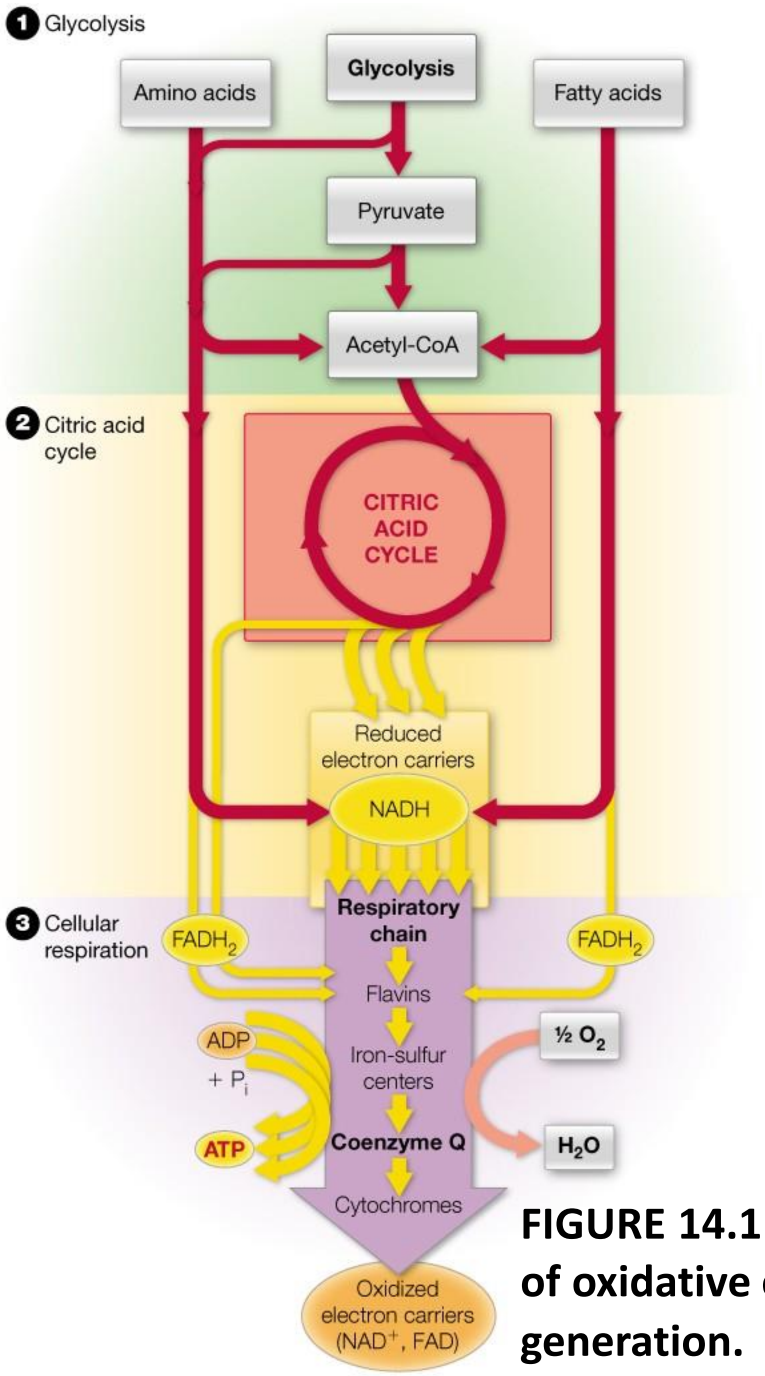
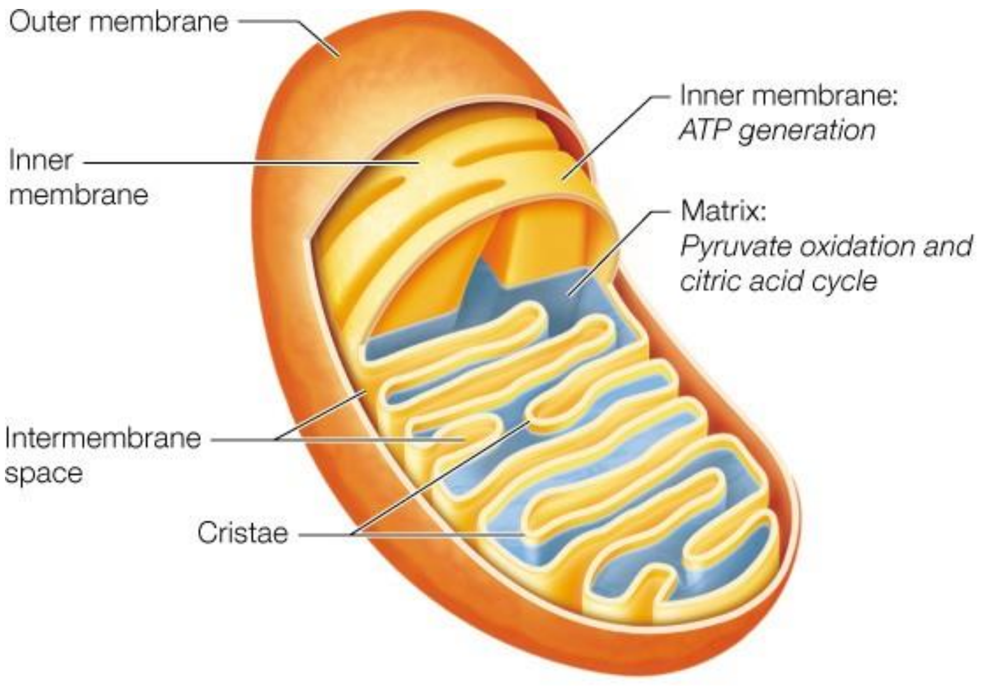
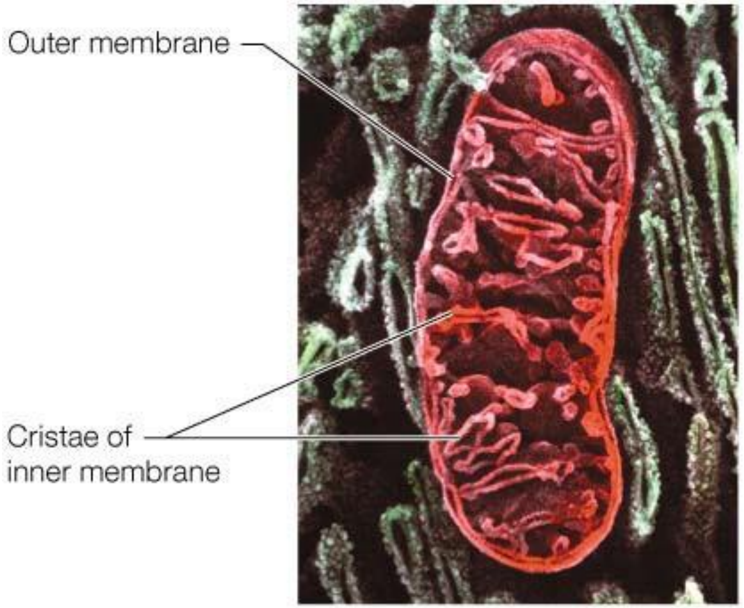


FIGURE 14.1 Overview of oxidative energy generation.

Structure of Mitochondria - recap



(a) Schematic of a mitochondrion.



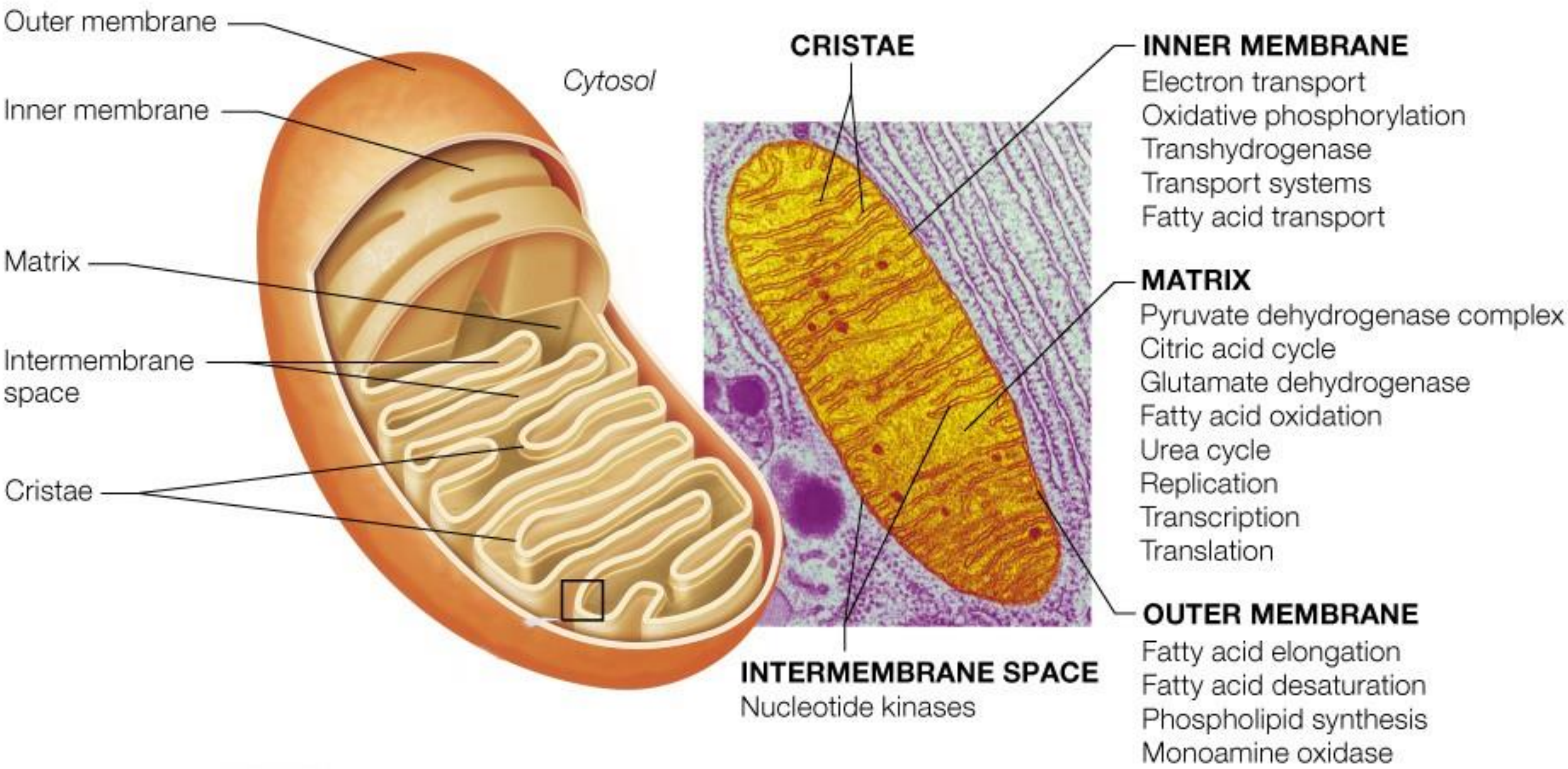
(b) Colored scanning electron micrograph of a single mitochondrion in the cytoplasm of an intestinal epithelial cell. The stacked and folded cristae are clearly extensions of the inner membrane.

FIGURE 13.3 Structure of the mitochondrion.

The reactions of stages 1 and 2 of respiration occur in the mitochondrial matrix. Reactions of stage 3 are catalyzed by membrane-bound enzymes in the inner mitochondrial membrane

Mitochondrial Location of Citric Acid Cycle and Oxidative Phosphorylation

(a) A mitochondrion from a pancreatic cell, shown as a thin section in a color-enhanced transmission electron micrograph. The major mitochondrial compartments are shown, along with principal enzymes and pathways localized to each compartment.



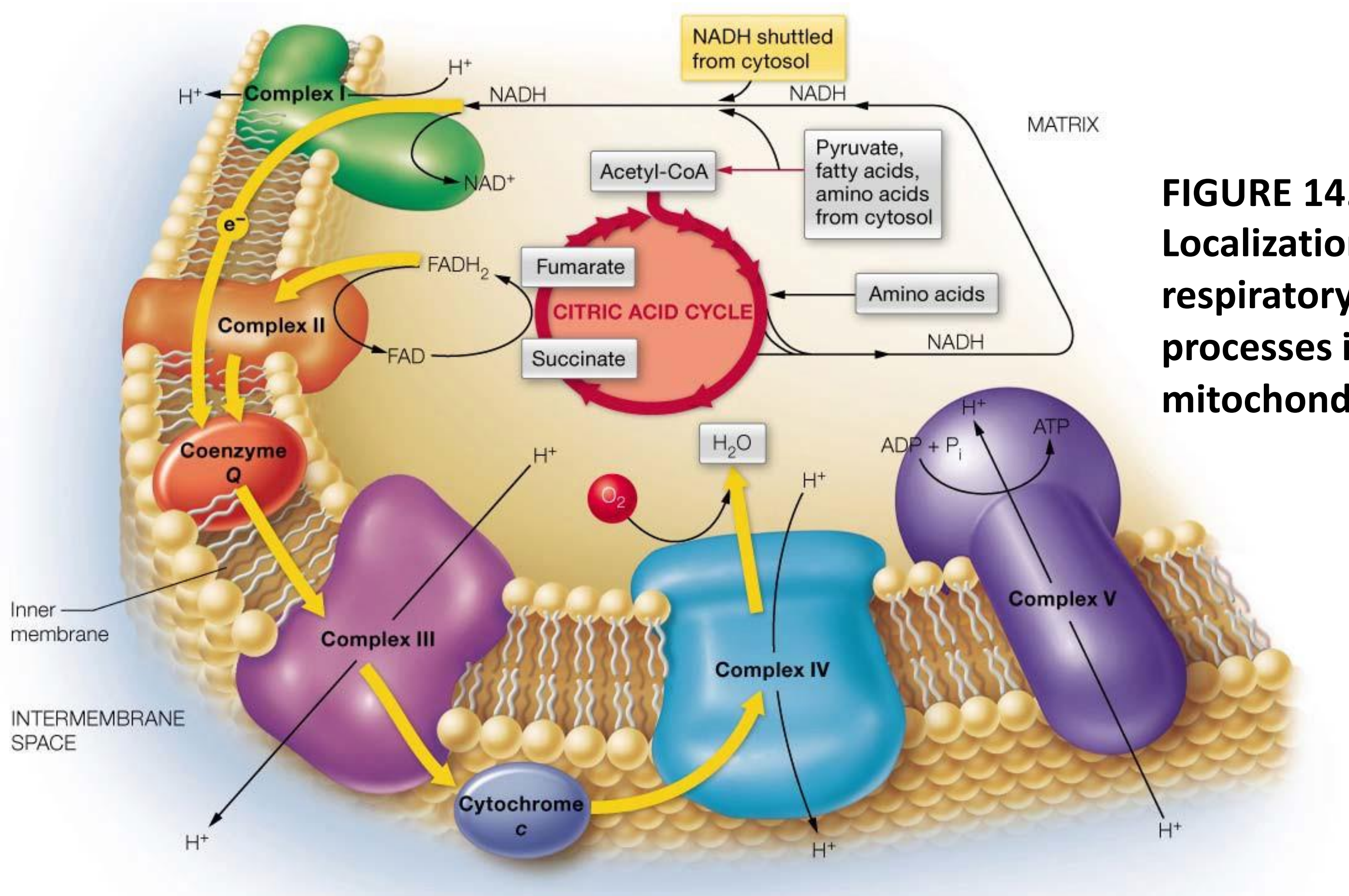
Electron Transport Chain and Oxidation Phosphorylation are located in the inner mitochondrial membrane

ETC:

- 4 integral membrane complexes (I to IV) and two mobile electron carriers
- 3 chemical reactions.
- 1-electron and 2-electron transfers depending on the cofactor.

OxPhos

- 1 integral membrane complex (V) coupled to ETC



(b) Overview of oxidative phosphorylation. Reduced electron carriers, produced by cytosolic dehydrogenases and mitochondrial oxidative pathways, become reoxidized by enzyme complexes bound in the inner mitochondrial membrane. These complexes actively pump protons outward from the matrix into the intermembrane space, creating an energy gradient whose discharge through complex V drives ATP synthesis.

FIGURE 14.2 Localization of respiratory processes in the mitochondrion.

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Free Energy changes for Redox reactions

- A complete redox reaction has one reactant **as an electron acceptor**, which becomes ***reduced*** by ***gaining electrons***, and another reactant **as an electron donor**, which becomes ***oxidized*** by ***losing electrons***.
- "OILRIG": Oxidation Is Loss (of electrons); Reduction Is Gain (of electrons).
- The general form of a redox reaction is then:

$$\text{reductant} + \text{oxidant} \rightleftarrows \text{oxidized reductant} + \text{reduced oxidant}$$

or:

$$A_{(red)} + B_{(ox)} \rightleftarrows A_{(ox)} + B_{(red)}$$

- $E°$ = Standard Reduction Potential, which describes the tendency of some species to lose electrons under standard conditions
  - ➢ The greater the standard reduction potential, the greater the tendency for a given electron carrier to become reduced

Standard Free Energy Changes in Oxidation–Reduction Reactions

$$\Delta G^{\circ\prime} = -nF\,\Delta E^{\circ\prime} = -nF\left[E^{\circ\prime}_{(e^{-}\text{acceptor})} - E^{\circ\prime}_{(e^{-}\text{donor})}\right]$$

- n = number of electrons transferred (typically 1 or 2 for most biochemical reactions)
- F = Faraday's constant (96.5 kJ/mol · V)
- $\Delta E^{\circ\prime}$ = difference in standard reduction potential between the two redox half reactions

- Once again, ΔG determines if the redox reaction is spontaneous.

Extracting the remaining energy in reduced cofactors and generating ATP (recap)

- Aerobic organisms consume oxygen and generate CO_2 from metabolic activity. E.g. glucose can be fully oxidised:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$

- In glycolysis and CAC, half of this is carried out:

$$C_6H_{12}O_6 + 6\,H_2O \rightarrow 6\,CO_2 + 24\,H^+ + 24\,e^-$$

- The other half occurs in the mitochondria:

$$6\,O_2 + 24\,H^+ + 24\,e^- \rightarrow 12\,H_2O$$

using the electron-transport chain (ECR) and oxidative phosphorylation (OxPhos).

Oxidative Fuel Metabolism

- Each –H in the reduced cofactor = 2 e^-
- Glycolysis: 2 NADH = 4 e^-
- PDC: 2 NADH = 4 e^-
- CAC: 6 NADH = 12 e^-
- CAC: 1 $FADH_2$ = 4 e^-
- *Total:* *= 24 e^-*

Most e^-'s generated in the mitochondria: hence ECR/OxPhos are also co-localized here!

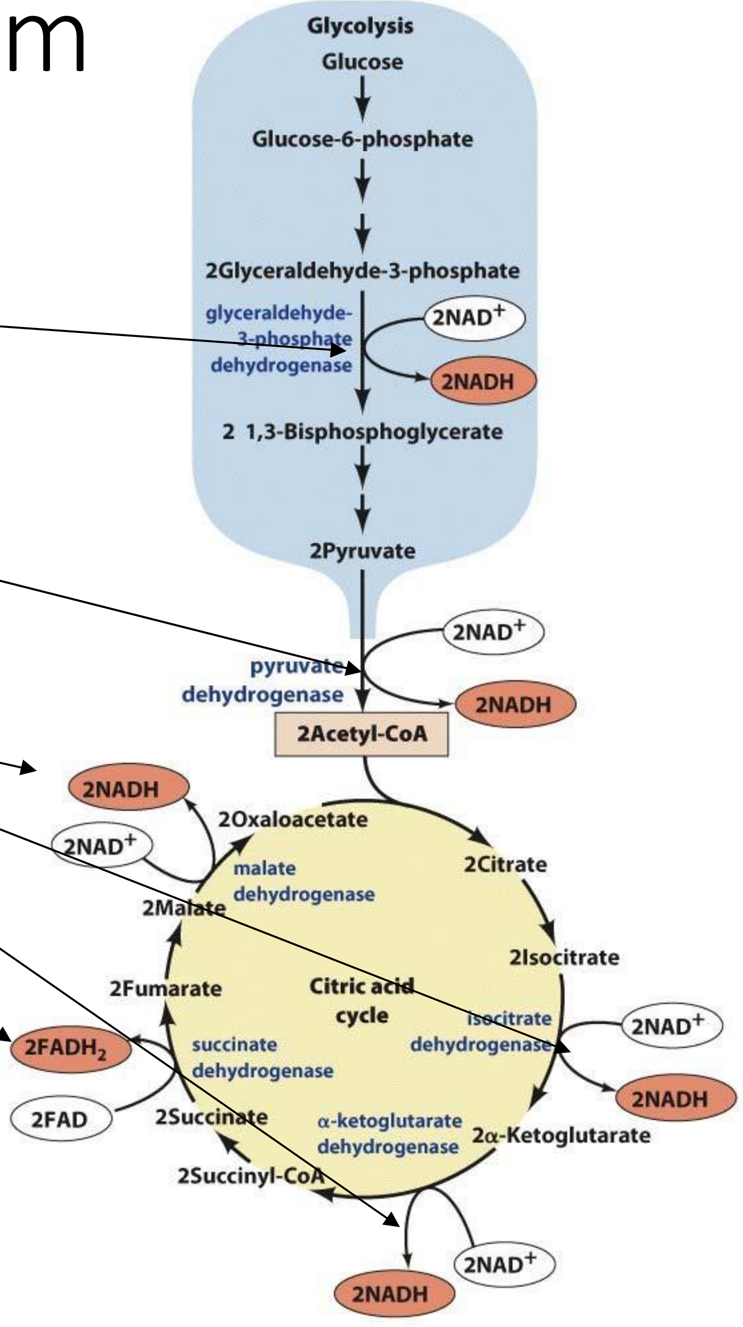
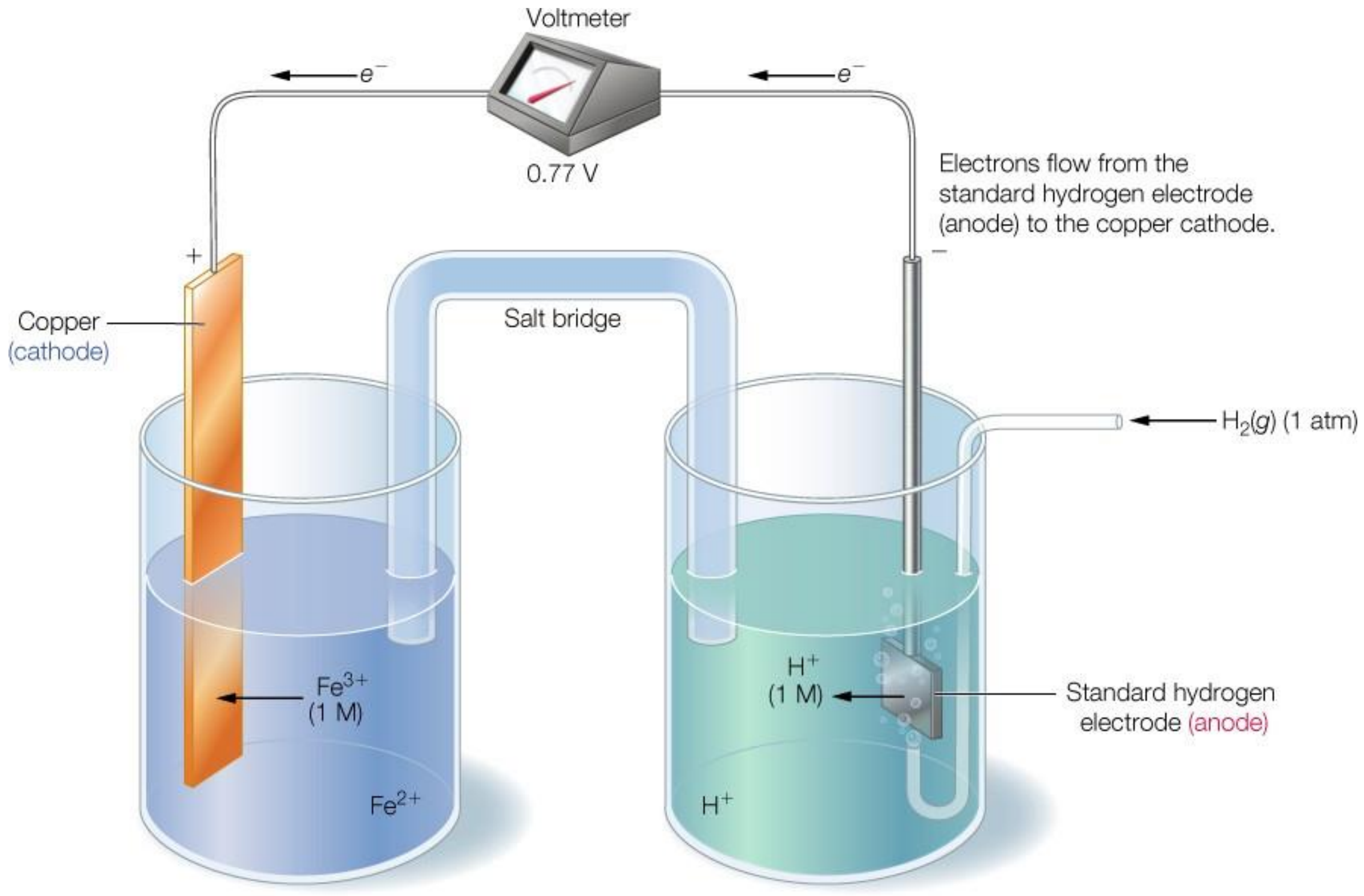


Figure 18-1

$E°$ in a Galvanic Cell provides an idea of the order of oxidations in the ETC/OxPhos



The Biochemical Standard Reduction Potential

TABLE 3.6 A few standard reduction potentials ($E°'$) of interest in biochemistry

Oxidant (e^- acceptor)		Reductant (e^- donor)	n	$E°'$ (V)
$H^+ + e^-$	$\rightleftharpoons$	$\frac{1}{2}H_2$	1	−0.421
$NAD^+ + H^+ + 2e^-$	$\rightleftharpoons$	NADH	2	−0.315
1,3-Bisphosphoglycerate + $2H^+ + 2e^-$	$\rightleftharpoons$	Glyceraldehyde-3-phosphate + P_i	2	−0.290
$FAD + 2H^+ + 2e^-$	$\rightleftharpoons$	$FADH_2$	2	−0.219
Acetaldehyde + $2H^+ + 2e^-$	$\rightleftharpoons$	Ethanol	2	−0.197
Pyruvate + $2H^+ + 2e^-$	$\rightleftharpoons$	Lactate	2	−0.185
$Fe^{3+} + e^-$	$\rightleftharpoons$	Fe^{2+}	1	+0.769
$\frac{1}{2}O_2 + 2H^+ + 2e^-$	$\rightleftharpoons$	H_2O	2	+0.815

More reducing power

More oxidising power

Note: $E°'$ is the standard reduction potential at pH 7 and 25 °C, n is the number of electrons transferred, and each potential is for the partial reaction written as follows:

$$\text{Oxidant} + ne^- \rightarrow \text{reductant}$$

The entry for the H^+/H_2 couple $E°' = -0.421$ V is not zero because it is measured with $[H^+] = 1$ M in the reference cell (i.e., the standard hydrogen electrode) and $[H^+] = 10^{-7}$ M in the test cell.

- ***E°'*** **is the biochemical standard reduction potential (measured at pH = 7)**
- **The greater the standard reduction potential, the greater the tendency of the oxidized form of a redox couple to attract electrons.**

Reduction Potentials of ETC Components

- Complex I to CoQ
- Complex II to CoQ
- Then in order

TABLE 18-1 Reduction Potentials of Electron-Transport Chain Components in Resting Mitochondria

Component	$\mathscr{E}^{\circ\prime}$ (V)
NADH	−0.315
Complex I (NADH–CoQ oxidoreductase; ~900 kD monomer, 45 unique subunits):	
FMN	−0.380
[2Fe–2S]N1a	−0.370
[2Fe–2S]N1b	−0.250
[4Fe–4S]N3, 4, 5, 6a, 6b, 7	−0.250
[4Fe–4S]N2	−0.150
Succinate	0.031
Complex II (succinate–CoQ oxidoreductase; ~420 kD trimer, 4 unique subunits):	
FAD	−0.040
[2Fe–2S]	−0.030
[4Fe–4S]	−0.245
[3Fe–4S]	−0.060
Heme b_{560}	−0.080
Coenzyme Q	0.045
Complex III (CoQ–cytochrome *c* oxidoreductase; ~450 kD dimer, 9–11 unique subunits):	
Heme b_H (b_{562})	0.030
Heme b_L (b_{566})	−0.030
[2Fe–2S]	0.280
Heme c_1	0.215
Cytochrome *c*	0.235
Complex IV (cytochrome *c* oxidase; ~410 kD dimer, 8–13 unique subunits):	
Heme *a*	0.210
Cu_A	0.245
Cu_B	0.340
Heme a_3	0.385
O_2	0.815

Source: Mainly Wilson, D.F., Erecinska, M., and Dutton, P.L., *Annu. Rev. Biophys. Bioeng.* 3, 205 and 208 (1974); *and* Wilson, D.F., *in* Bittar, E.E. (Ed.), *Membrane Structure and Function,* Vol. 1, p. 160, Wiley (1980).

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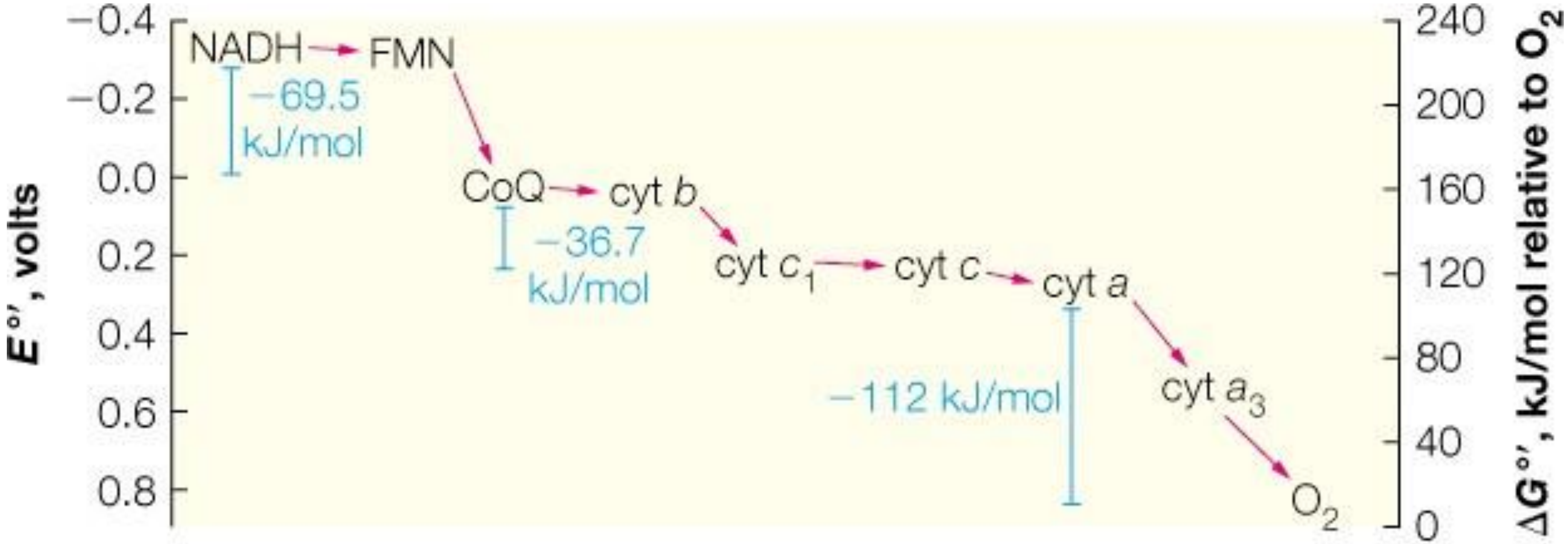
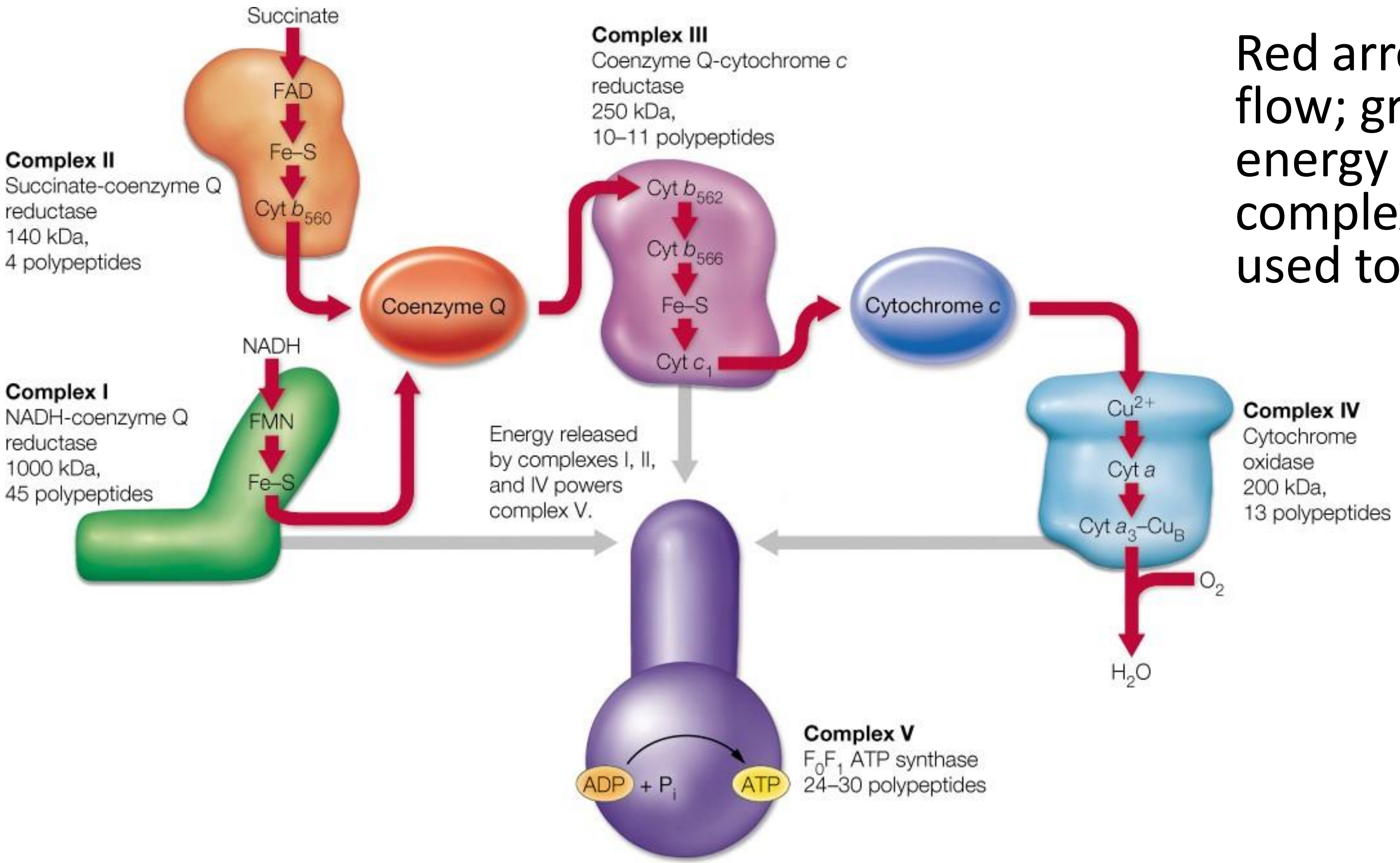
Standard Reduction Potentials of Major Electron Carriers in the Respiratory Chain



- Series of coupled reactions
- Three steps are sufficiently exergonic to drive ATP synthesis
- Free energy available from the oxidation of NADH by O_2 is converted into a **proton gradient** that powers the synthesis of ATP from ADP and P_i

Multienzyme Complexes Involved in the Mitochondrial Respiratory Chain



Red arrows show electron flow; gray arrows denote energy released from complexes I, III, and IV used to drive ATP synthesis

Free Energy Relevant to Cellular Processes

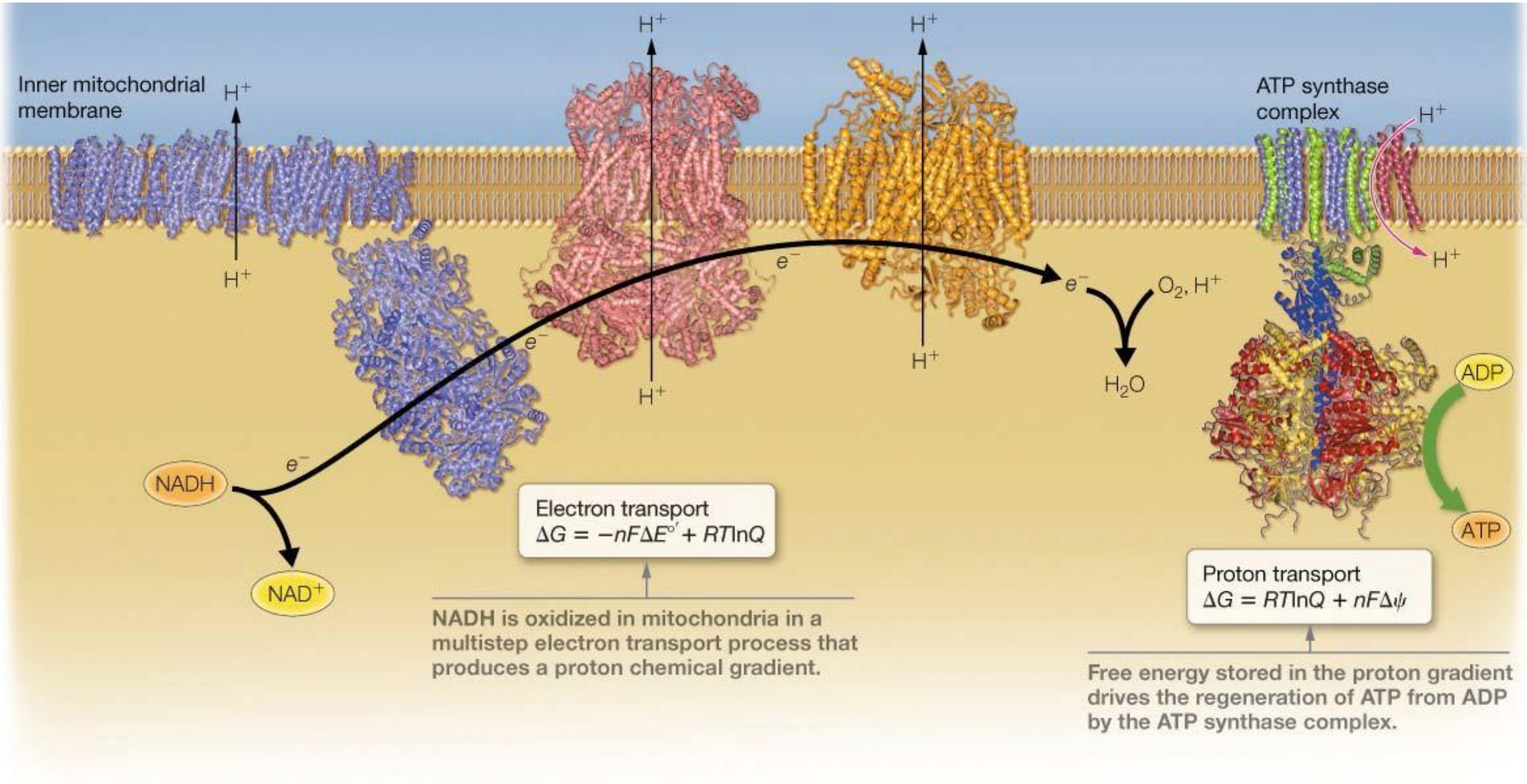
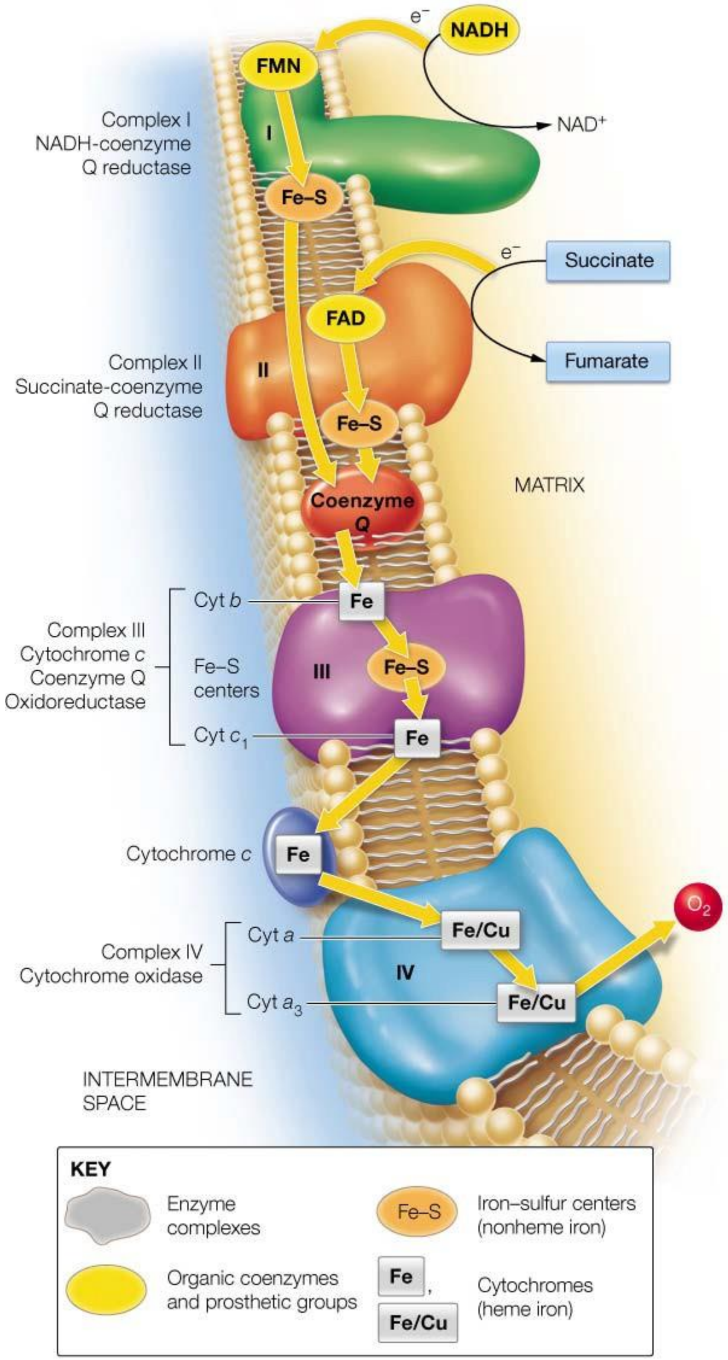


FIGURE 3.10 Examples of bioenergetic calculations applied to cellular processes.

Electron Carriers in the Respiratory Chain

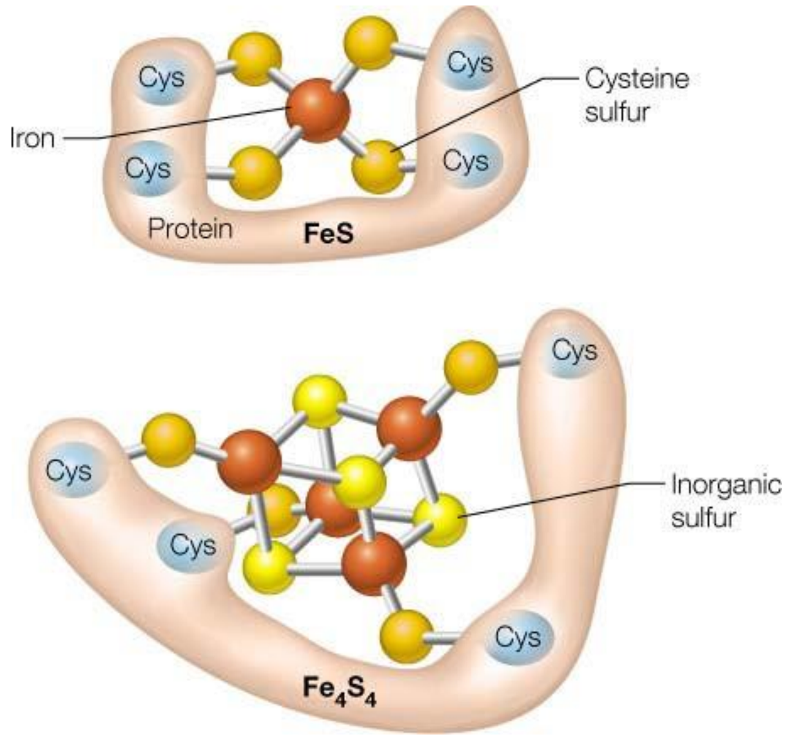
- The respiratory chain catalyzes the flow of electrons from low reduction potential carriers to high reduction potential carriers (exergonic)
- The respiratory chain uses a variety of electron carriers:
  1) Flavoproteins contain **FMN** (I) or **FAD** (II)
  2) Iron–sulfur proteins contain nonheme iron clusters such as FeS and Fe_4S_4 (I, II, III)
  3) **Coenzyme Q** (ubiquinone, Q)
  4) Cytochromes contain hemes (*b*, *c*, or *a*) (II and III – three *b*-type cytochromes; III – cytochrome c_1; IV – *a* and a_3; **cytochrome *c*** is membrane associated protein)

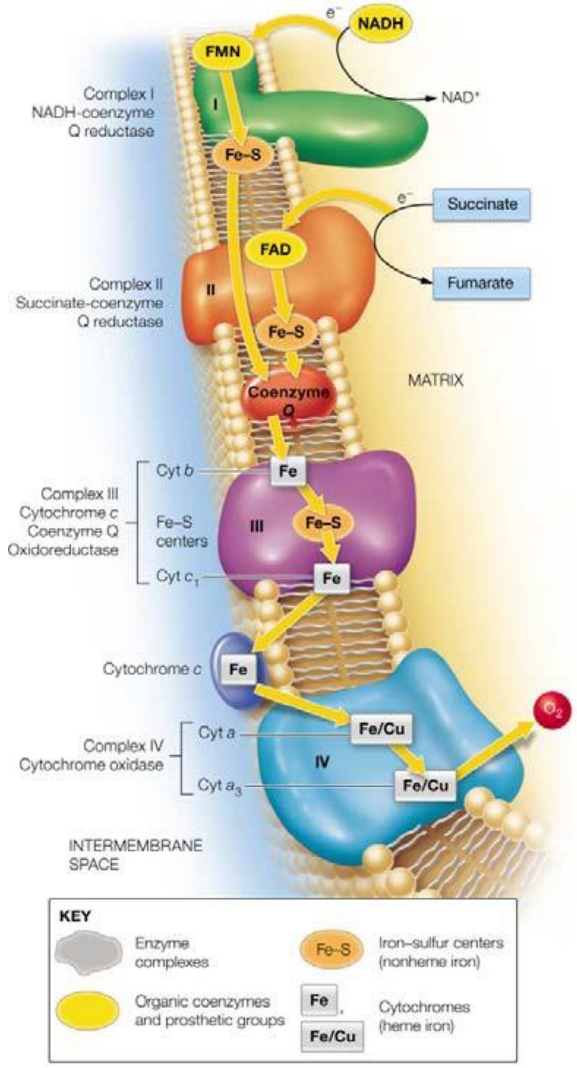


Iron-Sulfur clusters in the Respiratory Chain

Iron–sulfur proteins contain nonheme iron clusters such as FeS and Fe_4S_4 (I, II, III)

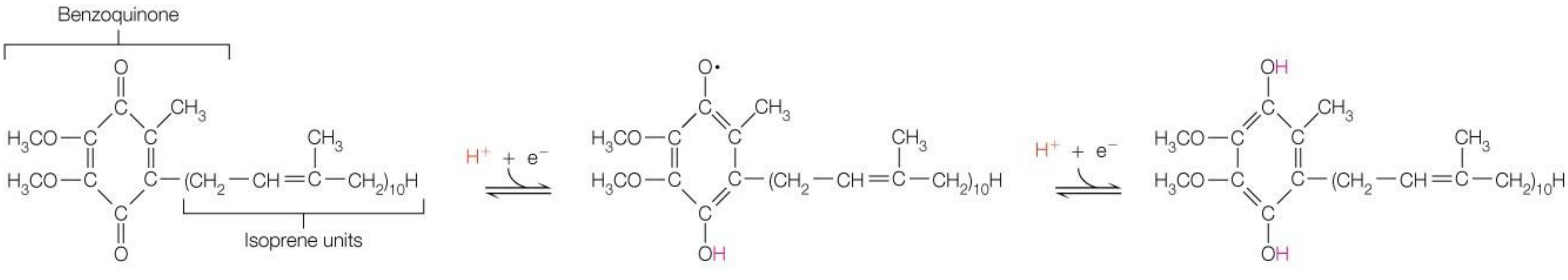
- Non-heme iron complexed with thiol sulfurs of cysteine residues within a protein
- Single electron carriers





Coenzyme Q (CoQ10)

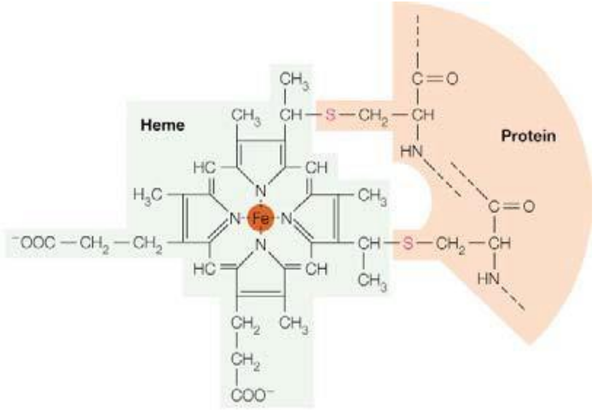
- is a lipophilic electron carrier with a benzoquinone linked to an isoprene-containing tail
- can transfer ***two electrons*** in **one electron steps** (via a stable semiquinone intermediate)
- provides a link between two-electron carriers and one-electron carriers



Oxidized coenzyme Q_{10} (CoQ) **Semiquinone form of coenzyme Q** **Reduced coenzyme Q_{10} ($CoQH_2$)**

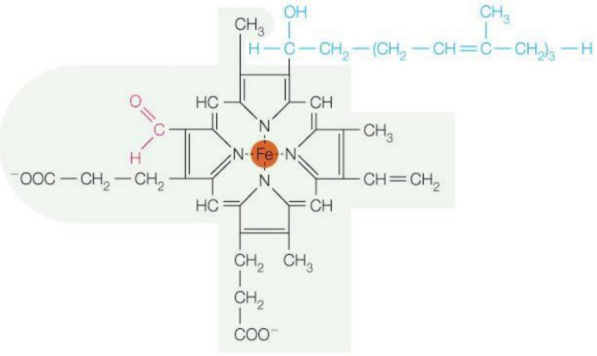
Cytochromes

- Cytochrome c and c_1 hemes are covalently bound to the protein component
- Cytochrome a and a_3 hemes are noncovalently attached to protein and contain formyl group (red) and isoprenoid (blue) modifications



(a) General structure of cytochromes c and c_1.
Covalent bonds join the heme and the protein component in cytochromes c and c_1. Two vinyl groups on heme are linked to the thiol groups of two cysteine residues (red).

Cytochrome c and c_1



(b) Heme A in cytochromes a and a_3.
Heme A, the form found in cytochromes a and a_3, has two modified side chains–a formyl group (red) and an isoprenoid side chain (blue).

Cytochrome a and a_3 heme

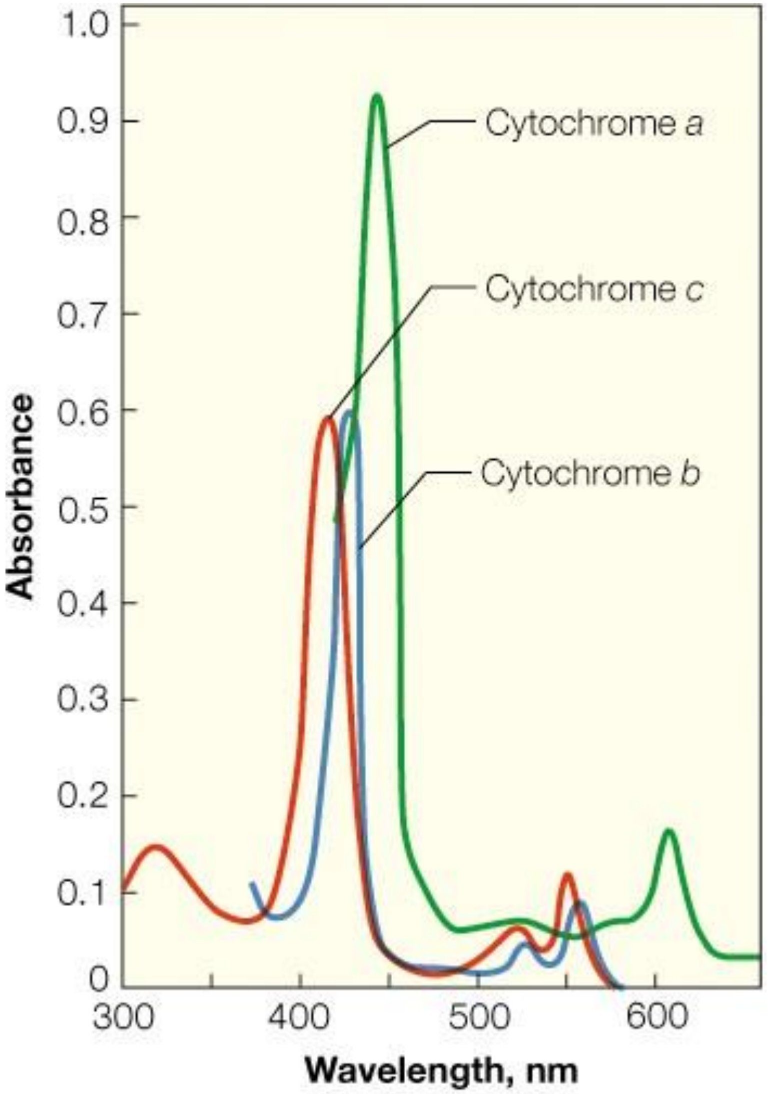


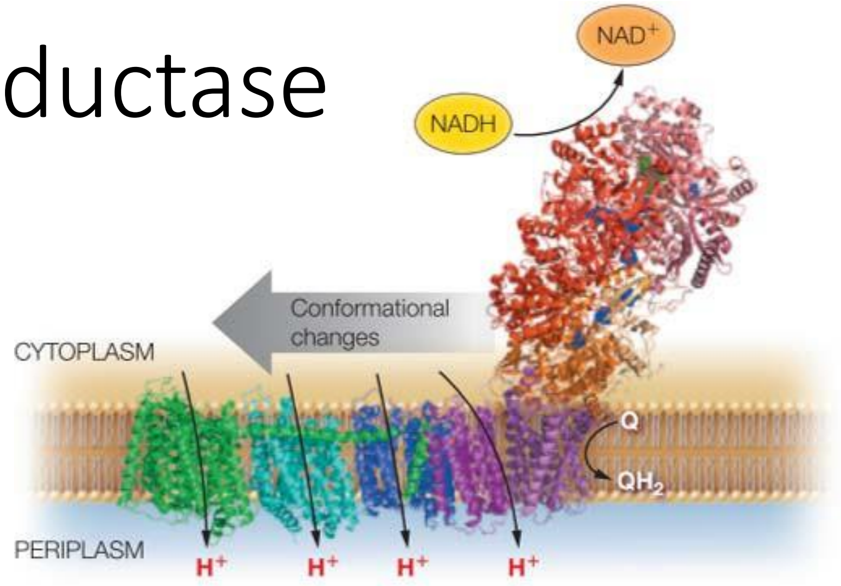
FIGURE 14.6 Absorption spectra of cytochromes.

Complex I: NADH–Coenzyme Q Reductase

- is a multisubunit complex of about 1000 kDa containing approximately 45 polypeptide chains
  - ➢The bacterial complex shown contains only 14 “core” subunits
- harbors one bound FMN (flavin mononucleotide) and eight iron–sulfur clusters
- Electrons are transferred in multiple steps from NADH to coenzyme Q (CoQ) via FMN and iron–sulfur clusters

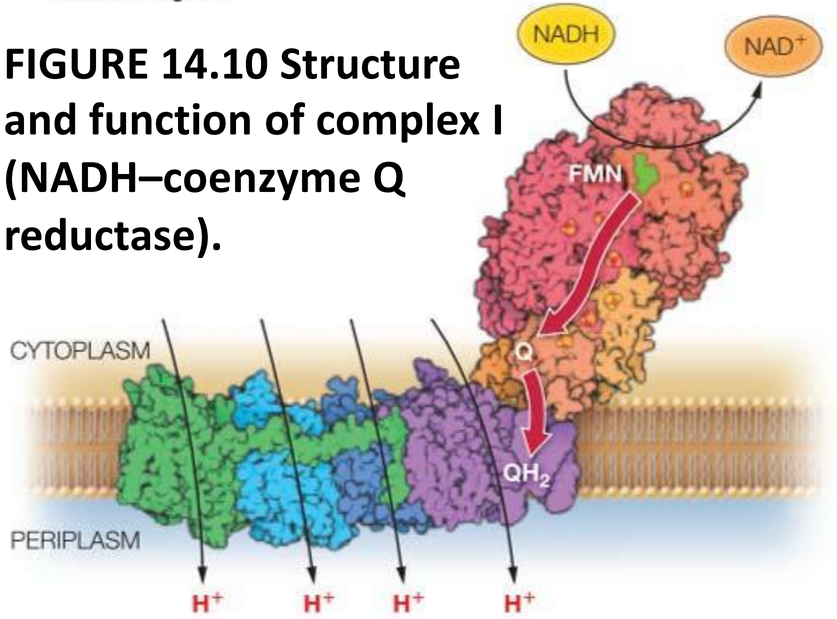
$$NADH + 5H^+_{\text{matrix}} + Q \rightleftharpoons NAD^+ + QH_2 + 4H^+_{\text{intermembrane space}}$$

- The flow of **two electrons** from **a single molecule of NADH to CoQ** causes conformational changes in complex I, which pumps **four protons from the mitochondrial matrix to the IMS**



(a) Structure of the entire complex I from the archaea *Thermus thermophilus*, derived from X-ray analysis (PDB ID: 4hea). In the hydrophilic peripheral arm, iron–sulfur clusters are shown in blue, and the FMN is green.

FIGURE 14.10 Structure and function of complex I (NADH–coenzyme Q reductase).



(b) The path of electron transport from NADH to CoQ and the direction of H^+ pumping are shown schematically in this cartoon.

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Complex II: Succinate–Coenzyme Q Reductase

- In addition to accepting electrons from NADH, CoQ can also accept electrons from intermediates in fatty acid oxidation and from succinate
- Complex II (= succinate dehydrogenase) is an inner membrane multisubunit protein complex, which is also part of the citric acid cycle, transferring electrons from succinate through FAD and a series of iron–sulfur clusters to CoQ

$$\text{succinate} + \text{CoQ} \rightleftharpoons \text{fumarate} + \text{CoQH}_2$$

- **Complex II transfers two electrons** from **succinate through FAD and a series of iron–sulfur clusters to CoQ**, but ***complex II does not pump protons into the IMS***

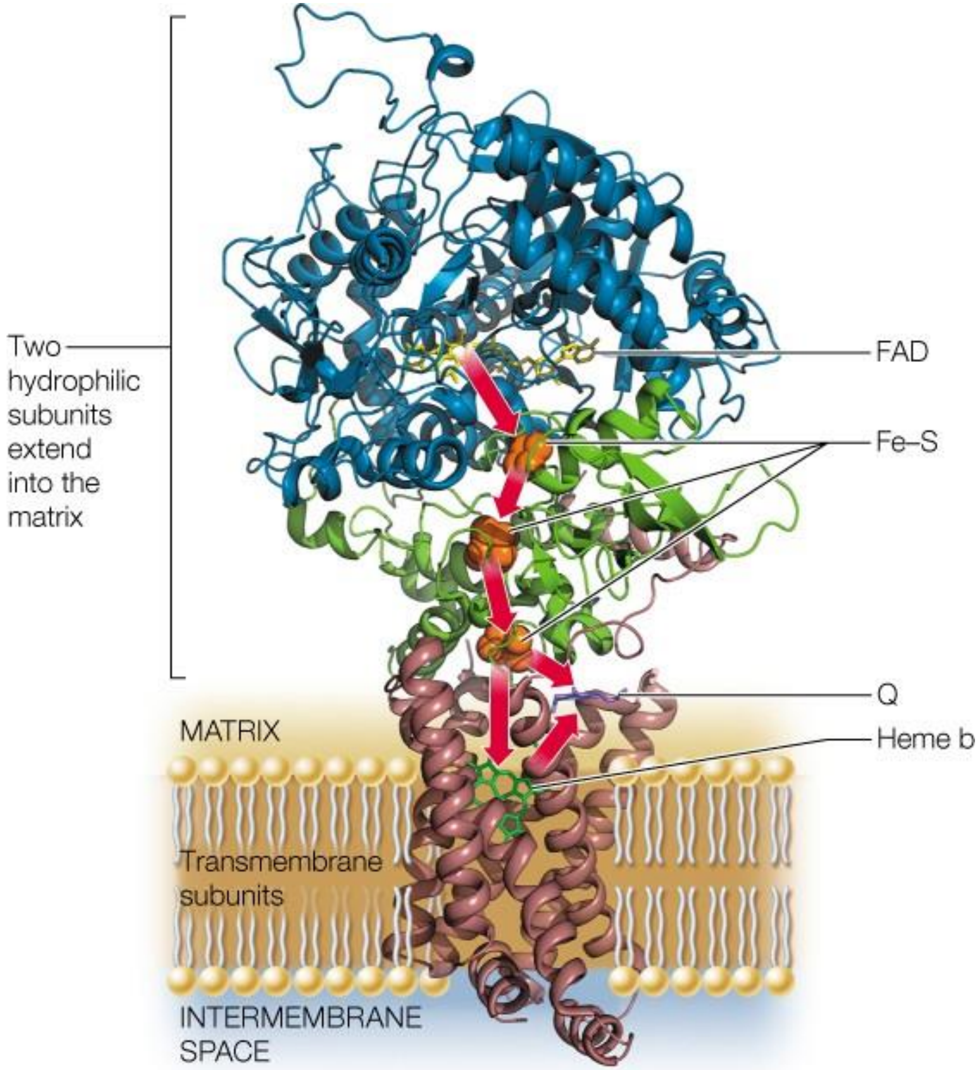


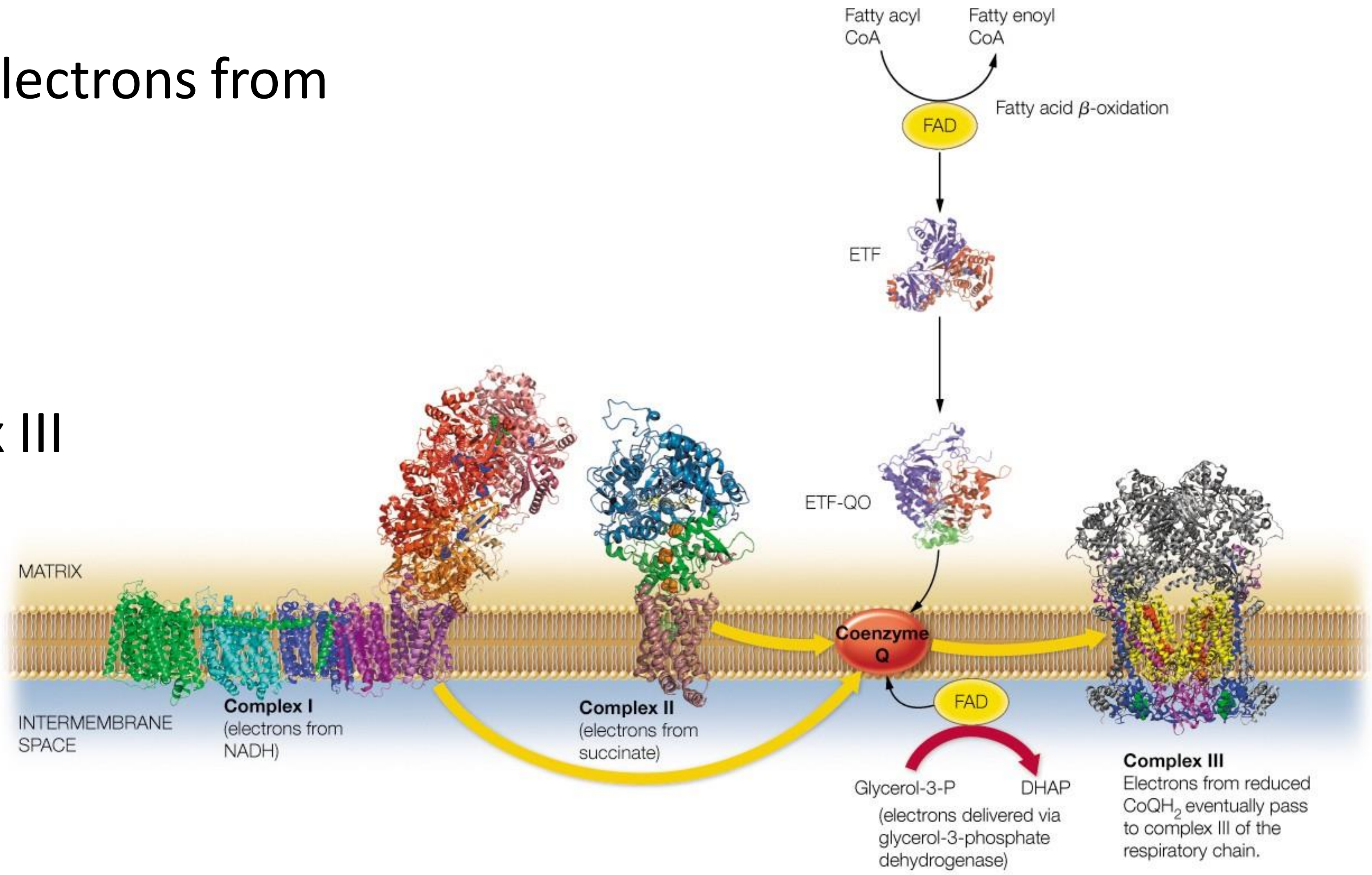
FIGURE 14.11 Structure of complex II (succinate–coenzyme Q reductase) from pig heart mitochondria (PDB ID: 1zoy).

Role of Coenzyme Q in Electron Transport

Coenzyme Q collects electrons from

- complex I,
- complex II and
- other flavoproteins

for transfer to complex III



Complex III: Coenzyme Q: Cytochrome *c* Oxidoreductase

- Complex III catalyzes the transfer of electrons from $CoQH_2$ (reduced coenzyme Q) to **cytochrome *c*** in the intermembrane space
- Mammalian complex III is about 250 kDa and functions as a dimer with each monomer composed of 10 or 11 protein chains (bovine complex III shown)

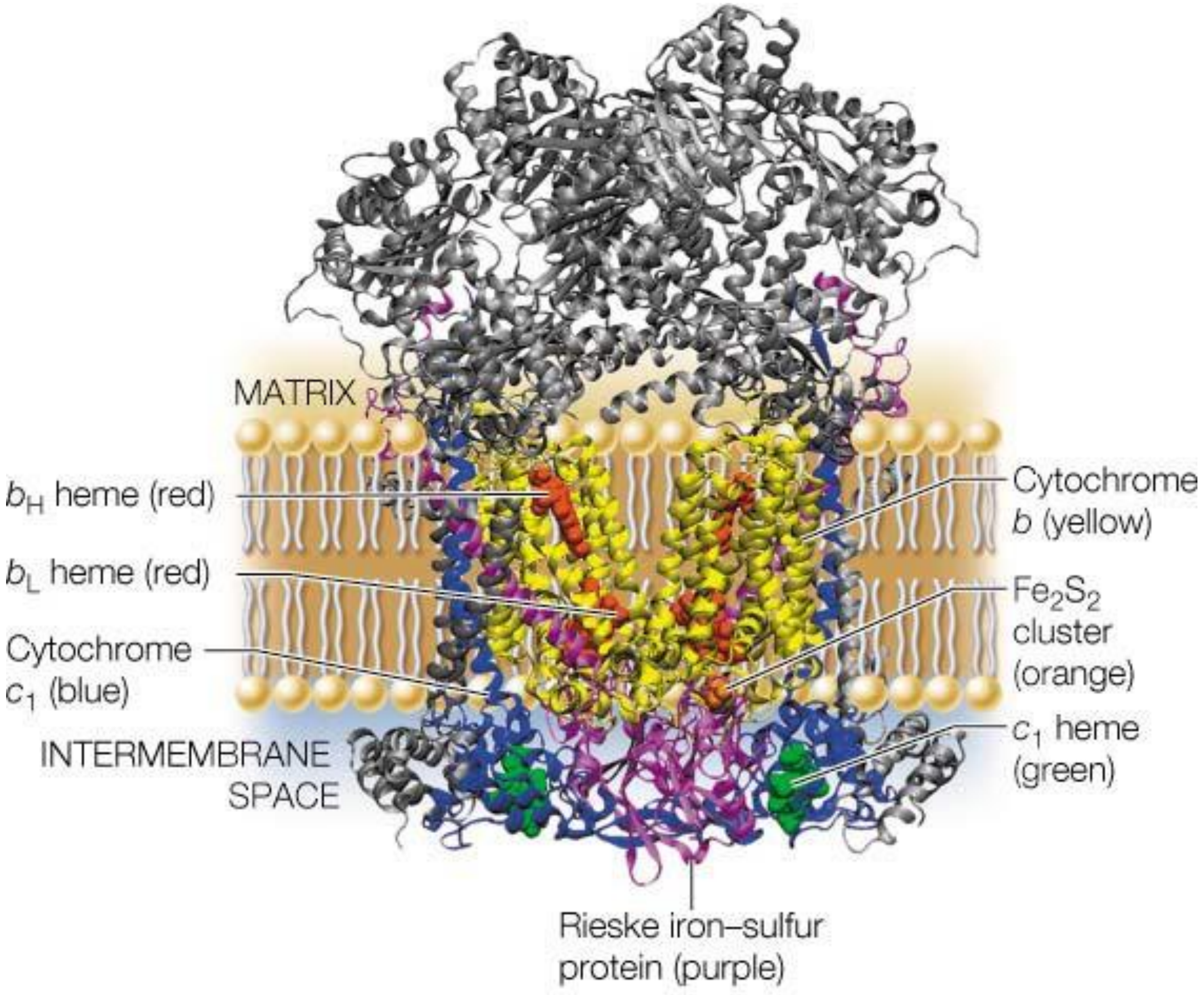


FIGURE 14.13 Structure of complex III (coenzyme Q:cytochrome *c* oxidoreductase).

Path of Electrons from $CoQH_2$ to Cytochrome *c* – the Q Cycle

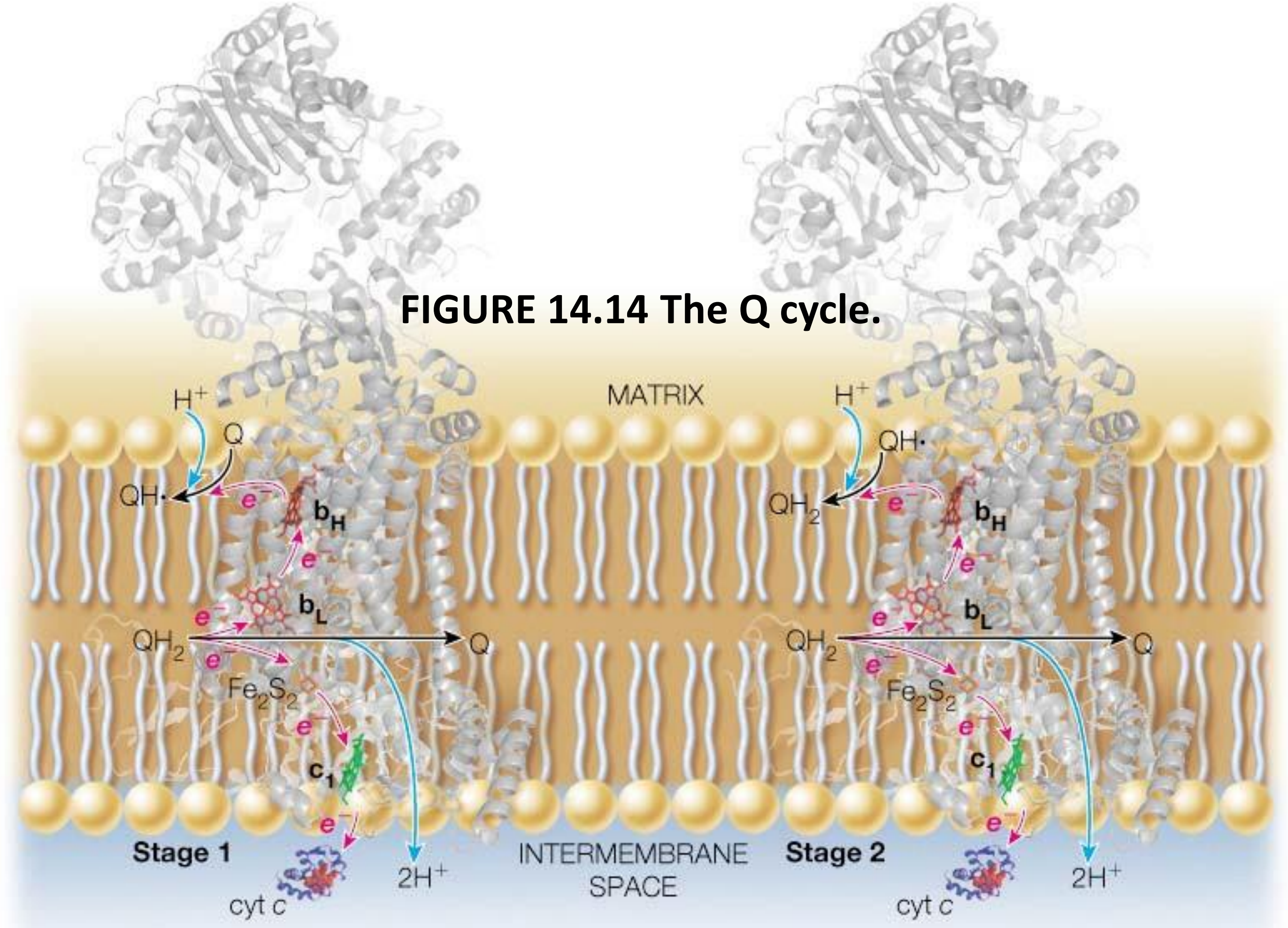


FIGURE 14.14 The Q cycle.

Stage 1: $QH_2 + Q + H^+_{Mat} + \text{cyt } c_{ox} \longrightarrow QH\cdot + Q + 2H^+_{IMS} + \text{cyt } c_{red}$

Stage 2: $QH_2 + QH\cdot + H^+_{Mat} + \text{cyt } c_{ox} \longrightarrow QH_2 + Q + 2H^+_{IMS} + \text{cyt } c_{red}$

Net: $QH_2 + 2\text{cyt } c_{ox} + 2H^+_{Mat} \longrightarrow Q + 2\text{cyt } c_{red} + 4H^+_{IMS}$

- During the Q cycle a two-electron donor, $CoQH_2$, is transferring •electrons to one-electron acceptors
- Each complex III monomer has two Q binding sites
- Thus, the electrons transferred from $CoQH_2$ take different paths, and occur in two stages
- The net process **pumps four protons from the matrix into the IMS**

Complex IV: Cytochrome *c* Oxidase

- exists as a homodimer with each monomer consisting of 13 subunits
- catalyzes the transfer of electrons from **reduced cytochrome *c*** to **oxygen**, and pumps two protons into the IMS for every two electrons transferred
- The net reaction (for the transfer of four electrons):

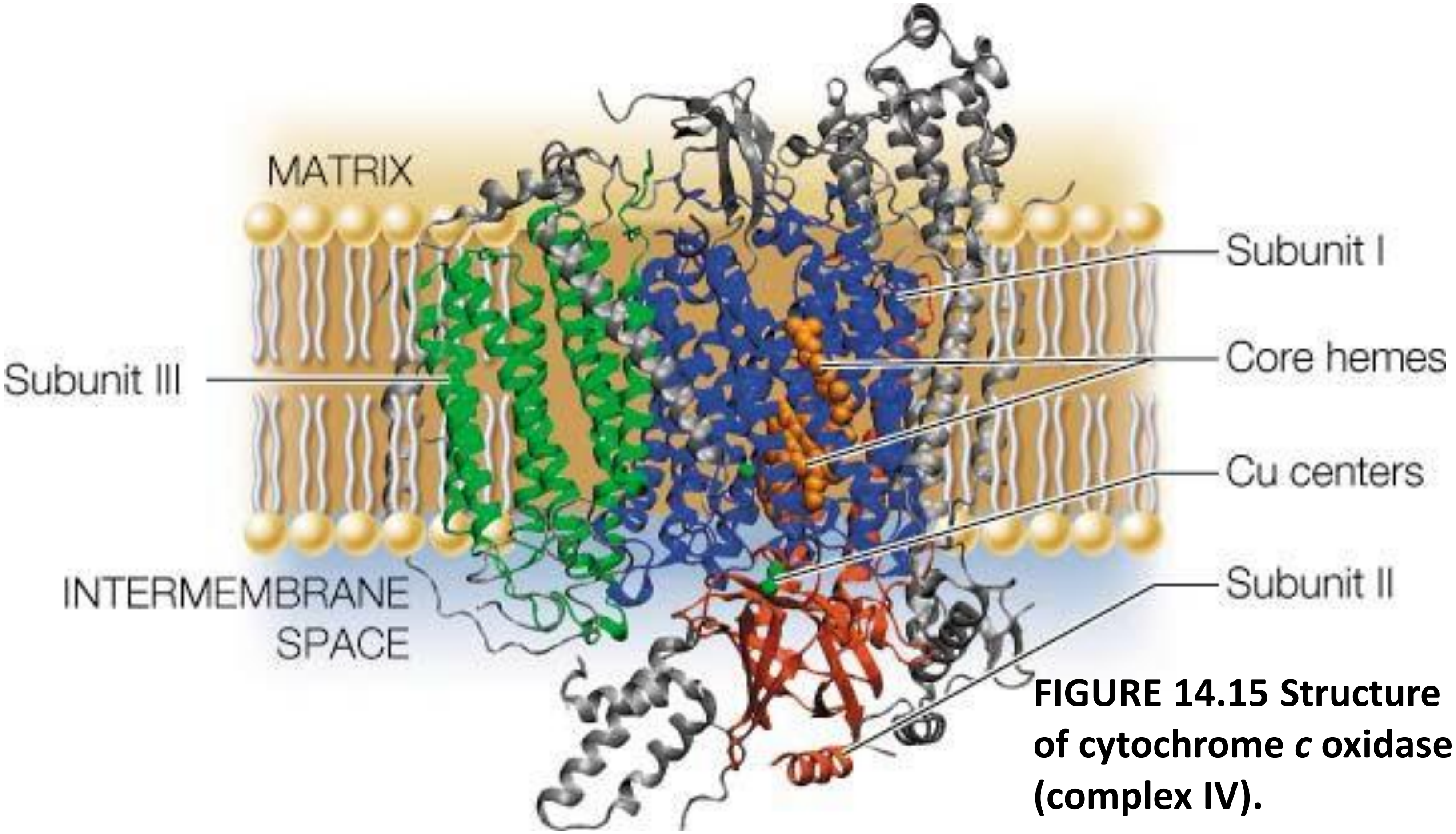
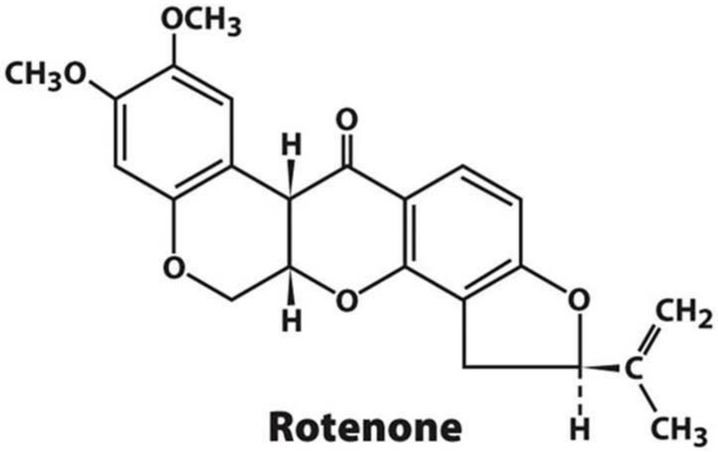


FIGURE 14.15 Structure of cytochrome *c* oxidase (complex IV).

$$4\ \text{cyt}\ c_{\text{red}}\,(Fe^{2+}) + O_2 + 8H^{+}_{\text{matrix}} \rightleftharpoons 4\ \text{cyt}\ c_{\text{ox}}(Fe^{3+}) + 2H_2O + 4H^{+}_{\text{intermembrane space}}$$

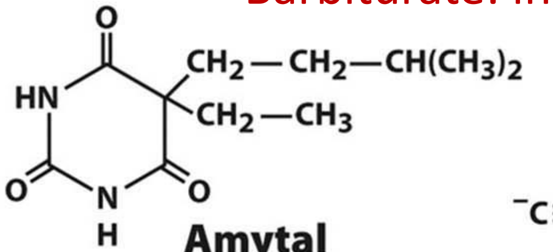
Inhibitors Reveal Electron-Transport Chain Sequence of Events



Rotenone

Fish poison used by Amazonian Indians: inhibits Complex I

Barbiturate: inhibits Complex I

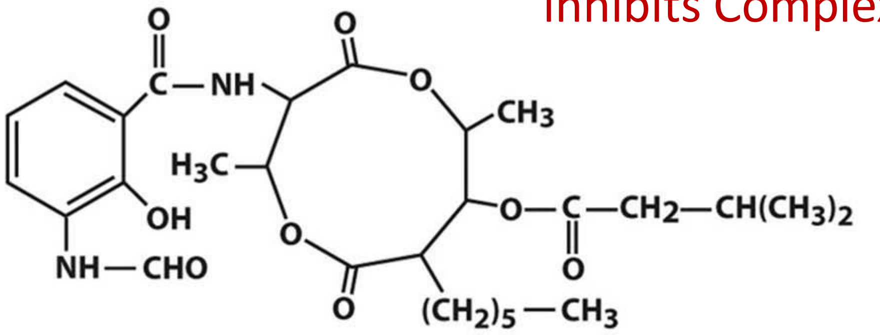


Amytal

$^-C\equiv N$

Cyanide

inhibits Complex IV



Antimycin A

Antibiotic: inhibits Complex III

Experimental O_2 consumption with and without inhibitors indicate where the ETC was interrupted

Electron carriers in ETC

- Flow of electrons from **most negative** to **most positive** half-reactions
- Mobile/soluble components in **green**
- 3 steps (in **blue**) release sufficient energy by **pumping protons out of the mitochondrial matrix** for ***ATP synthesis***

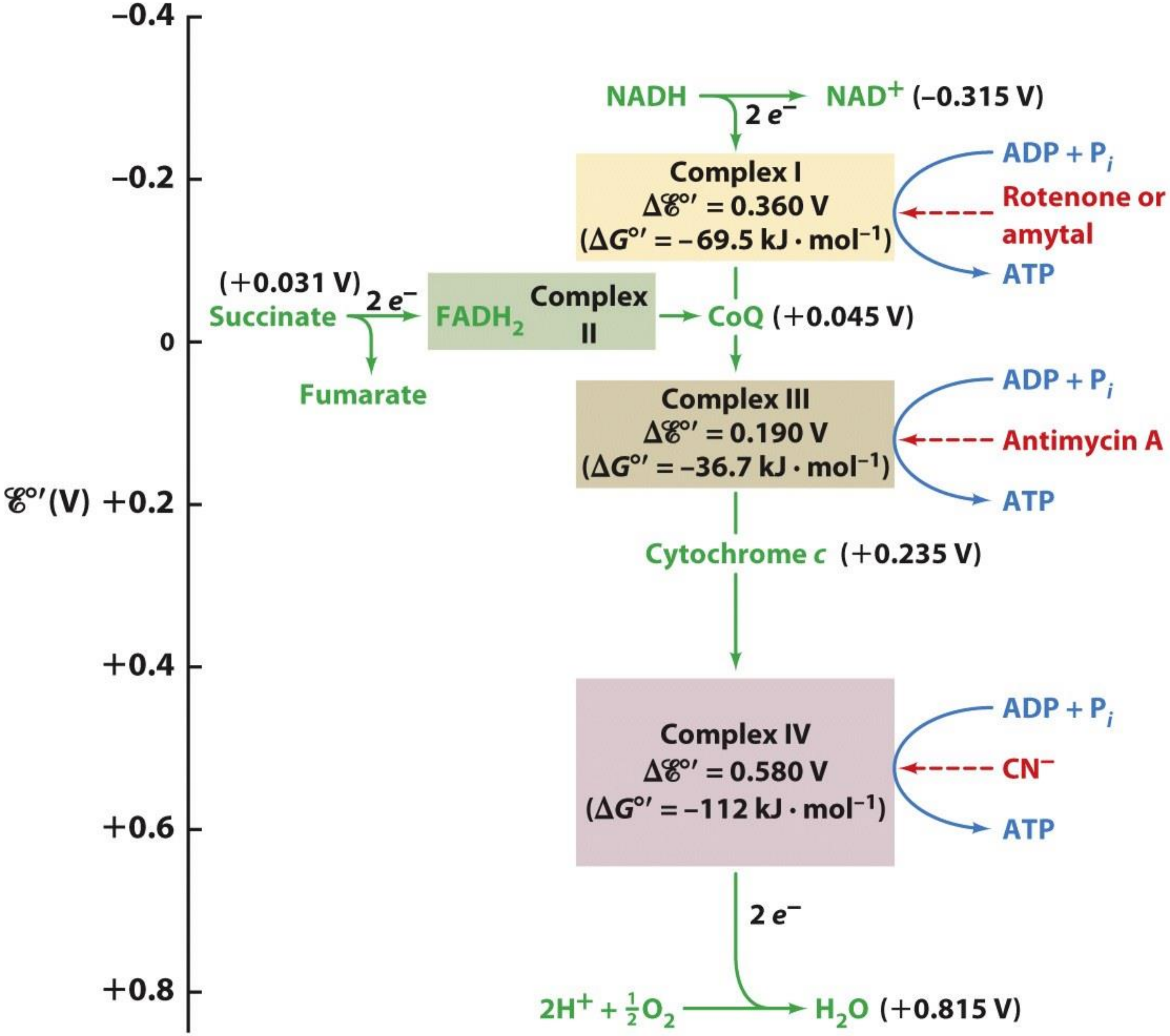
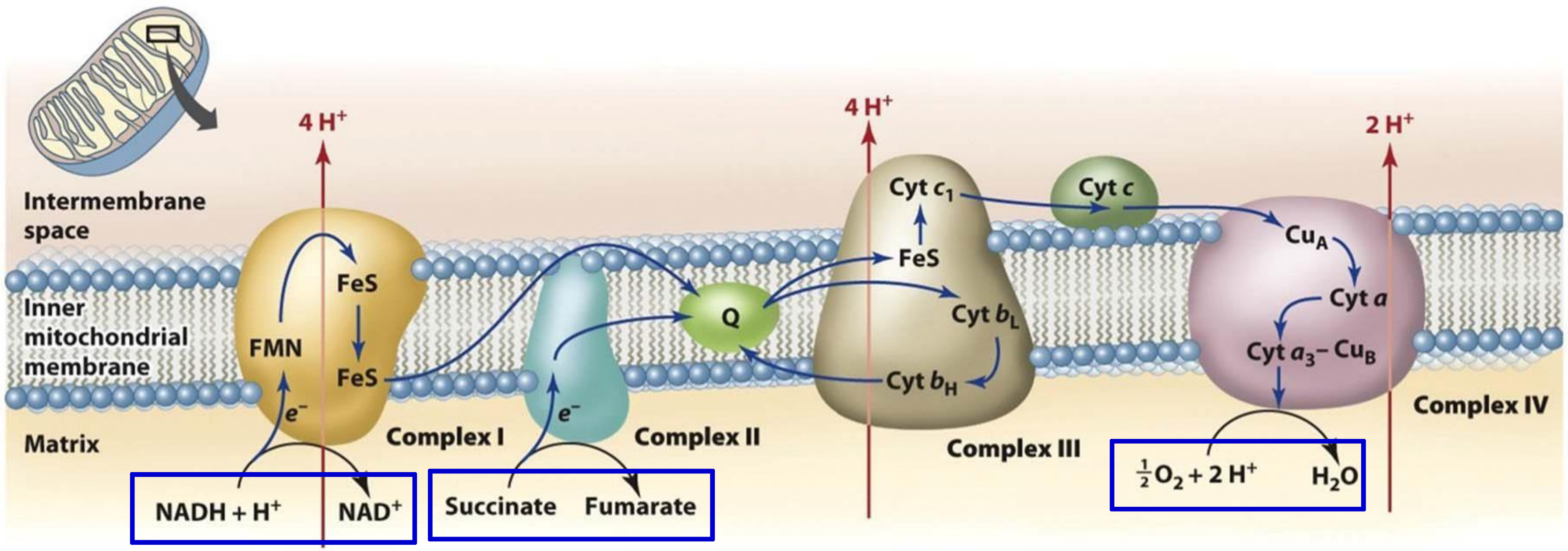


Figure 18-7

Mitochondrial Electron-Transport Chain

- ETC has
  - 4 membrane-embedded redox proteins: Complexes I, II, III and IV and
  - 2 mobile electron carriers: lipophilic coenzyme Q (CoQ or Q for ubiquinone) and the peripheral membrane protein cytochrome c (Cyt C)
  - 3 chemical reactions occur here



Summary

- Complexes I and II transfer electrons to CoQ
- Complex III transfers electrons from reduced CoQ to Cyt *c*
- Complex IV transfers electrons from reduced Cyt c to oxygen
- For 2 electrons transferred (= one chemical bond), 4 (Complex I) + 4 (Complex III) + 2 (Complex IV) = 10 protons pumped from matrix to intermembrane space. *Complex II is not a proton pump.*