Oxidative Phosphorylation

In eukaryotes, oxidative phosphorylation occurs in mitochondria, photophosphorylation in chloroplasts.

Oxidative phosphorylation involves the reduction of O2 to H2O with electrons donated by NADH and FADH2; it occurs equally well in light or darkness.

Photophosphorylation involves the oxidation of H2O to O2, with NADP as ultimate electron acceptor; it is absolutely dependent on the energy of light. Despite their differences, these two highly efficient energy-converting processes have fundamentally similar mechanisms.

Our current understanding of ATP synthesis in mitochondria and chloroplasts is based on the hypothesis, introduced by Peter Mitchell in 1961, that transmembrane differences in proton concentration are the reservoir for the energy extracted from biological oxidation reactions.

This **chemiosmotic theory has been accepted** as one of the great unifying principles of twentieth century biology. It provides insight into the processes of oxidative phosphorylation and photophosphorylation, and into such apparently disparate energy transductions as active transport across membranes and the motion of bacterial flagella.

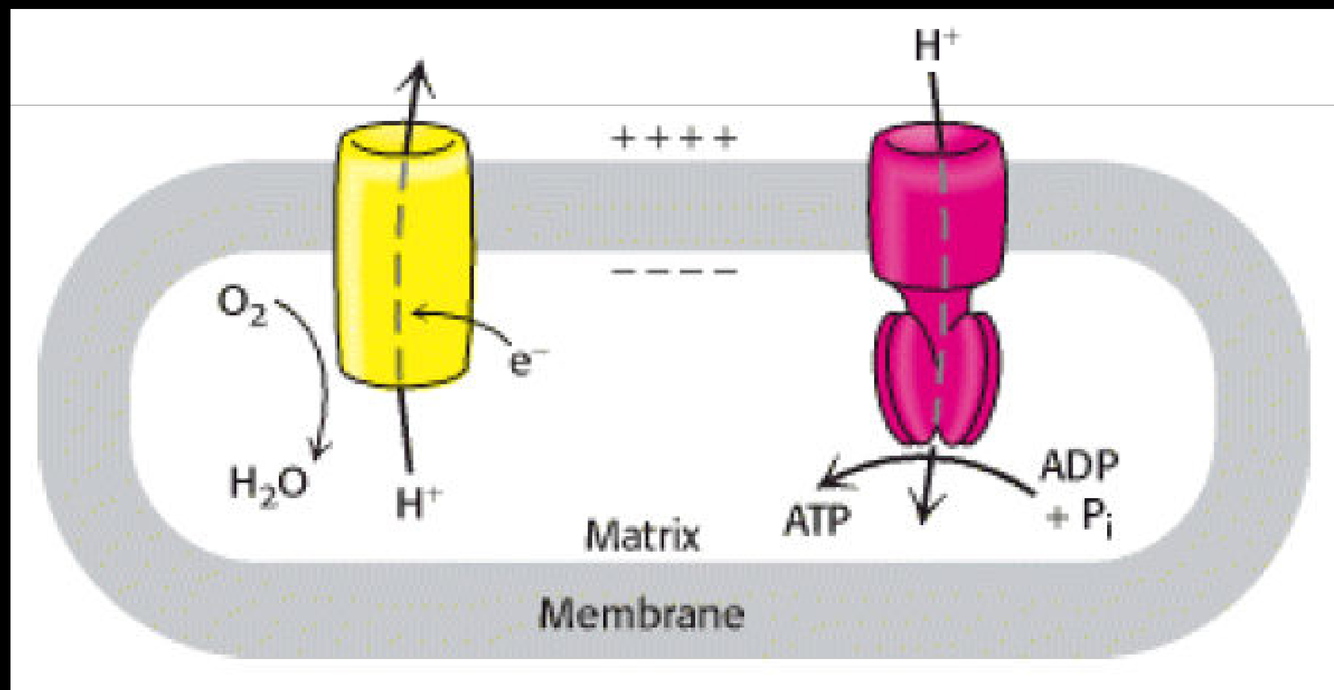
The NADH and $FADH_2$ formed in glycolysis, fatty acid oxidation, and the citric acid cycle are energy-rich molecules because each contains a pair of electrons having a high transfer potential.

When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP.

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. This process, which takes place in mitochondria, is the major source of ATP in aerobic organisms.

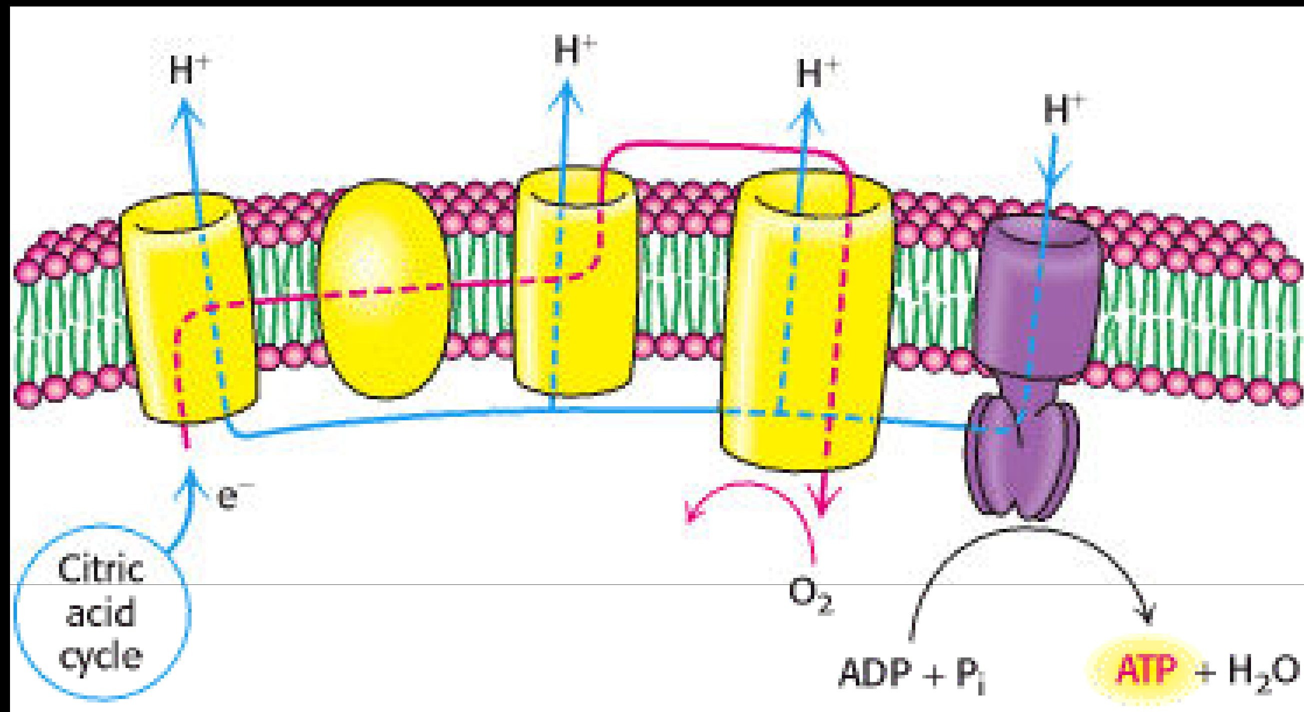
For example, oxidative phosphorylation generates 26 of the 30 molecules of ATP that are formed when glucose is completely oxidized to CO_2 and H_2O.

The flow of electrons from NADH or FADH2 to O2 through protein complexes located in the mitochondrial inner membrane leads to the pumping of protons out of the mitochondrial matrix. The resulting uneven distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a *proton-motive force. ATP is synthesized when protons* flow back to the mitochondrial matrix through an enzyme complex. Thus, *the oxidation of fuels and the phosphorylation of ADP are coupled by a proton gradient across the inner mitochondrial membrane.*



Essence of Oxidative Phosphorylation. Oxidation and ATP synthesis are coupled by transmembrane proton fluxes.

- *Oxidative phosphorylation is the culmination of a series of energy transformations that are called cellular respiration or* simply *respiration in their entirety.*
- *First, carbon fuels are oxidized in the citric acid cycle to yield electrons with high* transfer potential. Then, this electron-motive force is converted into a proton-motive force and, finally, the proton-motive force is converted into phosphoryl transfer potential.
- The conversion of electron-motive force into proton-motive force is arried out by three electron-driven proton pumps NADH-Q oxidoreductase, Q-cytochrome *c oxidoreductase, and* cytochrome *c oxidase. These large transmembrane complexes contain multiple oxidation-reduction centers, including* quinones, flavins, iron-sulfur clusters, hemes, and copper ions.
- The final phase of oxidative phosphorylation is carried out by *ATP synthase, an ATP-synthesizing assembly that is driven by the flow of protons back into the mitochondrial* matrix. Components of this remarkable enzyme rotate as part of its catalytic mechanism. Oxidative phosphorylation vividly shows that *proton gradients are an interconvertible currency of free energy in biological systems.*



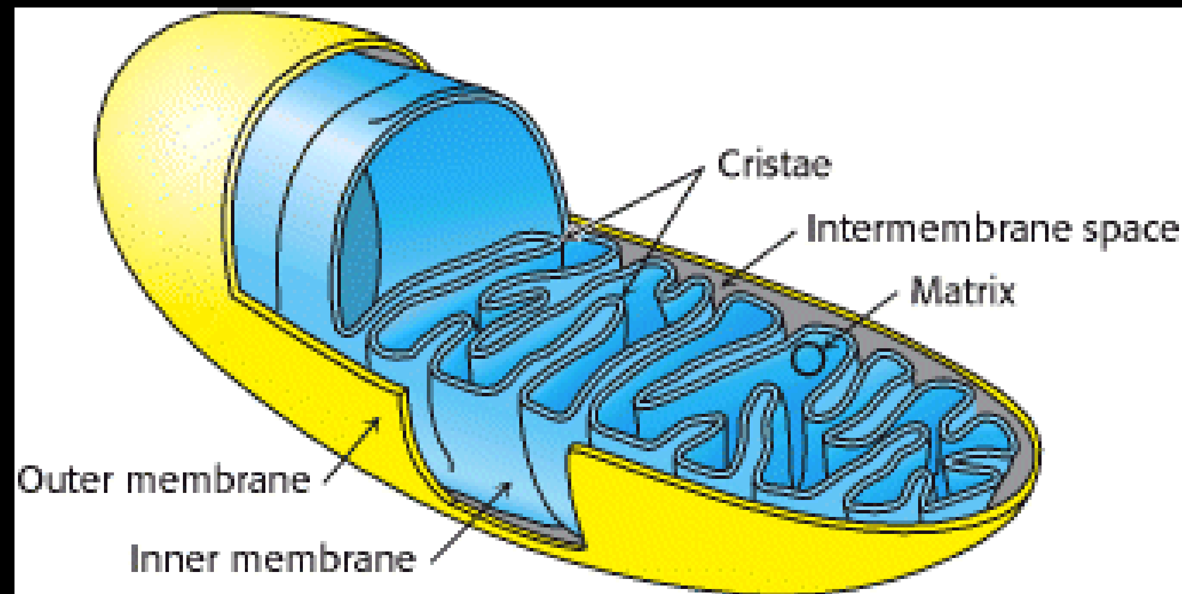
Mitochondria oxidize carbon fuels to form cellular energy. This transformation requires electron transfer through several large protein complexes (above), some of which pump protons, forming a proton gradient that powers the synthesis of ATP.

Oxidative phosphorylation and photophosphorylation are mechanistically similar in three respects.

(1) Both processes involve the flow of electrons through a chain of membrane-bound carriers.

(2) The free energy made available by this “downhill” (exergonic) electron flow is coupled to the “uphill” transport of protons across a proton-impermeable membrane, conserving the free energy of fuel oxidation as a transmembrane electrochemical potential.

(3) The transmembrane flow of protons down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP, catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.



High-Energy Electrons: Redox Potentials and Free-Energy Changes

High-energy electrons and redox potentials are of fundamental importance in oxidative phosphorylation. In oxidative phosphorylation, the electron transfer potential of NADH or FADH2 is converted into the phosphoryl transfer potential of ATP.

We need quantitative expressions for these forms of free energy. The measure of phosphoryl transfer potential is already familiar to us: it is given by $\Delta G°'$ *for the hydrolysis of the activated phosphate compound.*

The corresponding expression for the electron transfer potential is E'_0*, the reduction potential (also called the redox potential or oxidation-reduction potential).*

The reduction potential is an electrochemical concept.

Consider a substance that can exist in an oxidized form X and a reduced form X^-. Such a pair is called a *redox couple*.

The reduction potential of this couple can be determined by measuring the electromotive force generated by a sample half-cell connected to a standard reference half-cell .

The sample half-cell consists of an electrode immersed in a solution of 1 M oxidant (X) and 1 M reductant (X^-).
The standard reference half-cell consists of an electrode immersed in a 1 M H^+ solution that is in equilibrium with H_2 gas at 1 atmosphere pressure. The electrodes are connected to a voltmeter, and an agar bridge establishes electrical continuity between the half-cells. Electrons then flow from one half-cell to the other. If the reaction proceeds in the direction

$$X^- + H^+ \longrightarrow X + \frac{1}{2}H_2$$

the reactions in the half-cells (referred to as *half-reactions or couples) must be*

$$X^- \longrightarrow X + e^- \qquad H^+ + e^- \longrightarrow \frac{1}{2}H_2$$

Electrons Are Funneled to Universal Electron Acceptors

Oxidative phosphorylation begins with the entry of electrons into the respiratory chain. Most of these electrons arise from the action of dehydrogenases that collect electrons from catabolic pathways and funnel them into universal electron acceptors—nicotinamide nucleotides (NAD or NADP) or flavin nucleotides (FMN or FAD).

Nicotinamide nucleotide–linked dehydrogenases : catalyze reversible reactions of the following general types:

in the cytosol, others are in mitochondria, and still others have mitochondrial and cytosolic isozymes.

NAD-linked dehydrogenases remove two hydrogen atoms from their substrates. One of these is transferred as a hydride ion ($:H^-$) to NAD^+; the other is released as H^+ in the medium.

NADH and NADPH are water-soluble electron carriers that associate reversibly with dehydrogenases. NADH carries electrons from catabolic reactions to their point of entry into the respiratory chain, the NADH dehydrogenase complex described below.

NADPH generally supplies electrons to anabolic reactions. Cells maintain separate pools of NADPH and NADH, with different redox potentials.

This is accomplished by holding the ratios of [reduced form]/[oxidized form] relatively high for NADPH and relatively low for NADH.

Neither NADH nor NADPH can cross the inner mitochondrial membrane, but the electrons they carry can be shuttled across indirectly,

Flavoproteins contain a very tightly, sometimes covalently, bound flavin nucleotide, either FMN or FAD .

The oxidized flavin nucleotide can accept either one electron (yielding the semiquinone form) or two (yielding $FADH_2$ or $FMNH_2$).

Electron transfer occurs because the flavoprotein has a higher reduction potential than the compound oxidized. The standard reduction potential of a flavin nucleotide, unlike that of NAD or NADP, depends on the protein with which it is associated.

Local interactions with functional groups in the protein distort the electron orbitals in the flavin ring, changing the relative stabilities of oxidized and reduced forms. The relevant standard reduction potential is therefore that of the particular flavoprotein, not that of isolated FAD or FMN.

Electrons Pass through a Series of Membrane-Bound Carriers

The mitochondrial respiratory chain consists of a series of sequentially acting electron carriers, most of which are integral proteins with prosthetic groups capable of accepting and donating either one or two electrons.

Three types of electron transfers occur in oxidative phosphorylation:

(1) Direct transfer of electrons, as in the reduction of $Fe3^{+}$ to $Fe2^{+}$;

(2) Transfer as a hydrogen atom ($H^{+} + e^{+}$); and

(3) Transfer as a hydride ion ($:H^{+}$), which bears two electrons.

The term *reducing equivalent* is used to designate a single electron equivalent transferred in an oxidation-reduction reaction.

In addition to *NAD and flavoproteins*, three other types of electron-carrying molecules function in the respiratory chain: a hydrophobic quinone (ubiquinone) and two different types of iron-containing proteins (cytochromes and iron-sulfur proteins).

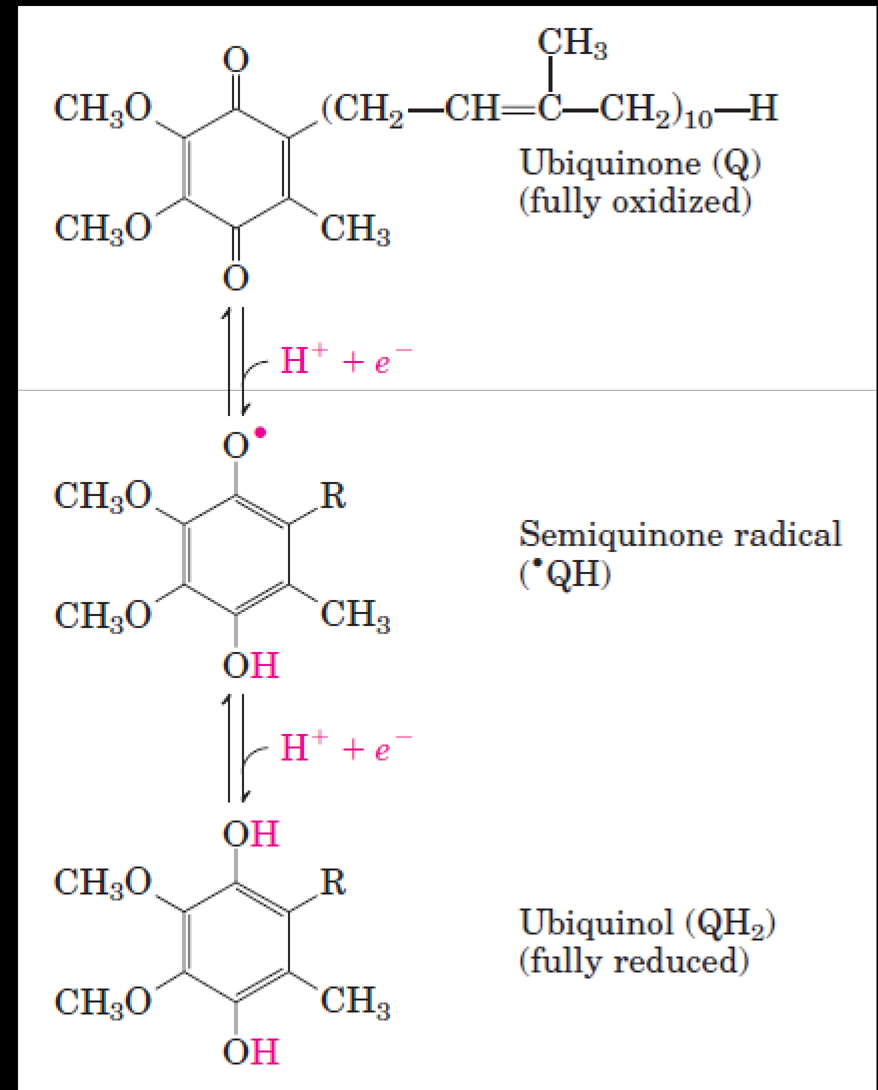
Ubiquinone (also called coenzyme Q, or simply Q) is a lipid-soluble benzoquinone with a long isoprenoid side chain.

The closely related compounds plastoquinone (of plant chloroplasts) and menaquinone (of bacteria) play roles analogous to that of ubiquinone, carrying electrons in membrane-associated electron-transfer chains.

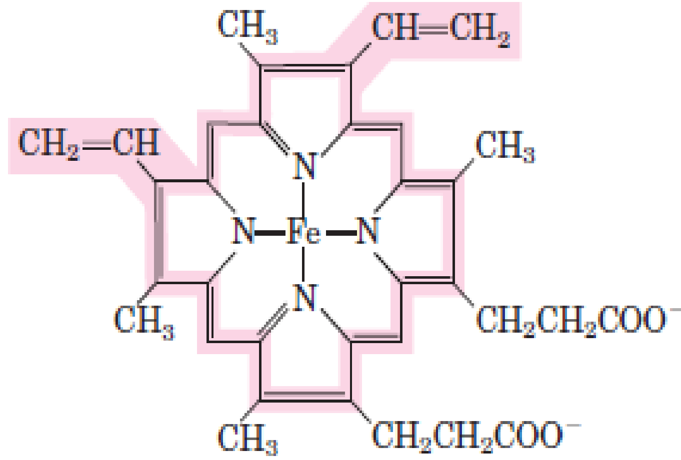
Ubiquinone can accept one electron to become the semiquinone radical (·QH) or two electrons to form ubiquinol (QH_2) and, like flavoprotein carriers, it can act at the junction between a two-electron donor and a one-electron acceptor.

Because ubiquinone is both small and hydrophobic, it is freely diffusible within the lipid bilayer of the inner mitochondrial membrane and can shuttle reducing equivalents between other, less mobile electron carriers in the membrane. And because it carries both electrons and protons, it plays a central role in coupling electron flow to proton movement.

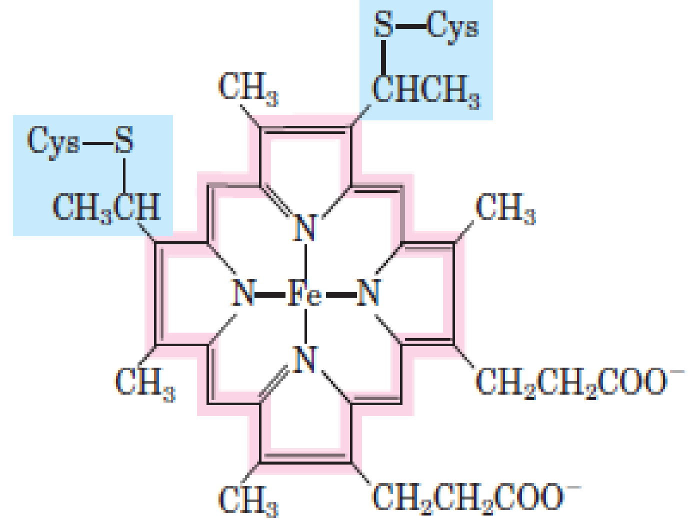
Ubiquinone (Q, or coenzyme Q). Complete reduction of ubiquinone requires two electrons and two protons, and occurs in two steps through the semiquinone radical intermediate.



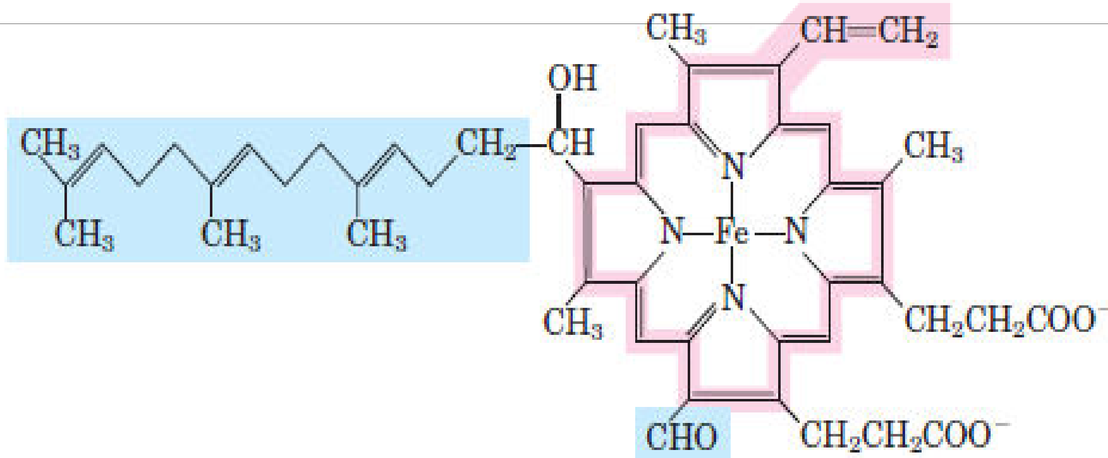
- The cytochromes are proteins with characteristic strong absorption of visible light, due to their iron containing heme prosthetic groups.
- Mitochondria contain three classes of cytochromes, designated a, b, and c, which are distinguished by differences in their light-absorption spectra.
- Each type of cytochrome in its reduced ($Fe2^{+}$) state has three absorption bands in the visible range.
- The longest wavelength band is near 600 nm in type a cytochromes, near 560 nm in type b, and near 550 nm in type c.
- To distinguish among closely related cytochromes of one type, the exact absorption maximum is sometimes used in the names, as in cytochrome b_{562}.
- The heme cofactors of a and b cytochromes are tightly, but not covalently, bound to their associated proteins; the hemes of c-type cytochromes are covalently attached through Cys residues.
- As with the flavoproteins, the standard reduction potential of the heme iron atom of a cytochrome depends on its interaction with protein side chains and is therefore different for each cytochrome.
- The cytochromes of type a and b and some of type c are integral proteins of the inner mitochondrial membrane.
- One striking exception is the cytochrome c of mitochondria, a soluble protein that associates through electrostatic interactions with the outer surface of the inner membrane.



Iron protoporphyrin IX
(in *b*-type cytochromes)



Heme C
(in *c*-type cytochromes)



Heme A
(in *a*-type cytochromes)

FIGURE 19–3 Prosthetic groups of cytochromes. Each group consists of four five-membered, nitrogen-containing rings in a cyclic structure called a porphyrin. The four nitrogen atoms are coordinated with a central Fe ion, either Fe^{2+} or Fe^{3+}. Iron protoporphyrin IX is found in *b*-type cytochromes and in hemoglobin and myoglobin (see Fig. 4–17). Heme *c* is covalently bound to the protein of cytochrome *c* through thioether bonds to two Cys residues. Heme *a*, found in the *a*-type cytochromes, has a long isoprenoid tail attached to one of the five-membered rings. The conjugated double-bond system (shaded pink) of the porphyrin ring accounts for the absorption of visible light by these hemes.

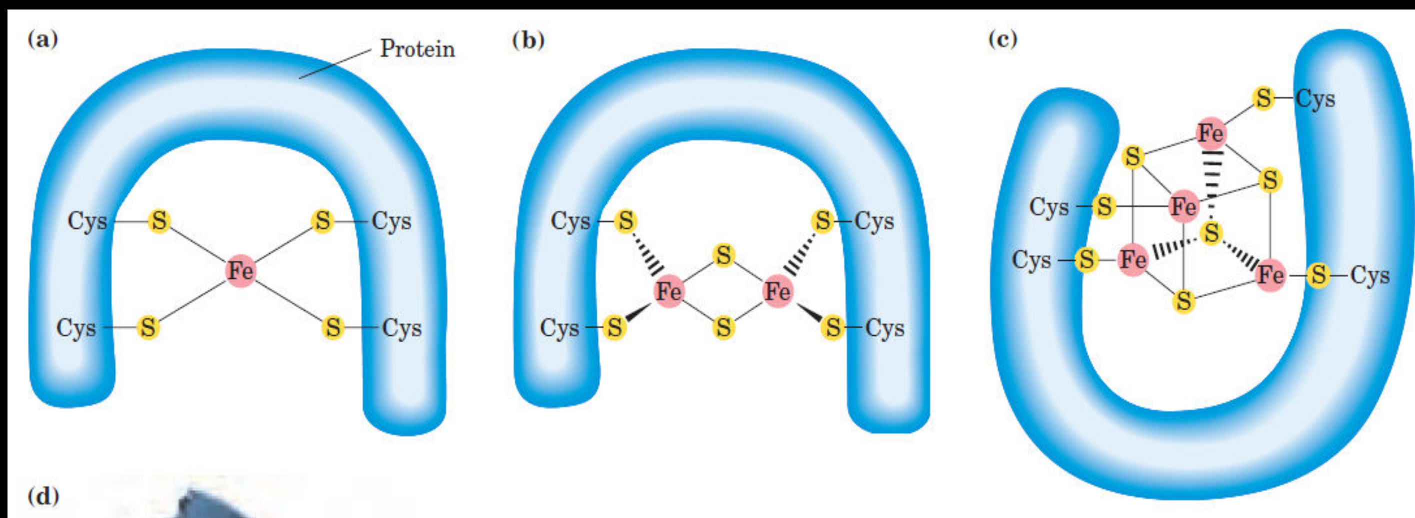
In ***iron-sulfur proteins*****,** first discovered by Helmut Beinert, the iron is present not in heme but in association with inorganic sulfur atoms or with the sulfur atoms of Cys residues in the protein, or both.

These iron-sulfur (Fe-S) centers range from simple structures with a single Fe atom coordinated to four Cys--SH groups to more complex Fe-S centers with two or four Fe atoms.

Rieske iron-sulfur proteins **(named after their discoverer,** John S. Rieske) are a variation on this theme, in which one Fe atom is coordinated to two His residues rather than two Cys residues.

All iron-sulfur proteins participate in one-electron transfers in which one iron atom of the iron-sulfur cluster is oxidized or reduced.

At least eight Fe-S proteins function in mitochondrial electron transfer. The reduction potential of Fe-S proteins varies from -0.65 V to +0.45 V, depending on the microenvironment of the iron within the protein.



Iron-sulfur centers. The Fe-S centers of iron-sulfur *proteins may be as simple as* ***(a), with a single Fe ion surrounded*** *by the S atoms of four Cys residues. Other centers include both inorganic and Cys S atoms, as in* ***(b) 2Fe-2S*** *or* ***(c) 4Fe-4S centers.***

In the overall reaction catalyzed by the mitochondrial respiratory chain, electrons move from NADH, succinate, or some other primary electron donor through flavoproteins, ubiquinone, iron-sulfur proteins, and cytochromes, and finally to O_2.

We would expect the carriers to function in order of increasing reduction potential, because electrons tend to flow spontaneously from carriers of lower *E to carriers of higher E. The* order of carriers deduced by this method is

NADH →Q → cytochrome b → cytochrome c1 → cytochrome c → cytochrome a → cytochrome a3 → O_2.

Method measures the effects of inhibitors of electron transfer on the oxidation state of each carrier. In the presence of an electron donor and O_2, each inhibitor causes a characteristic pattern of oxidized/reduced carriers.

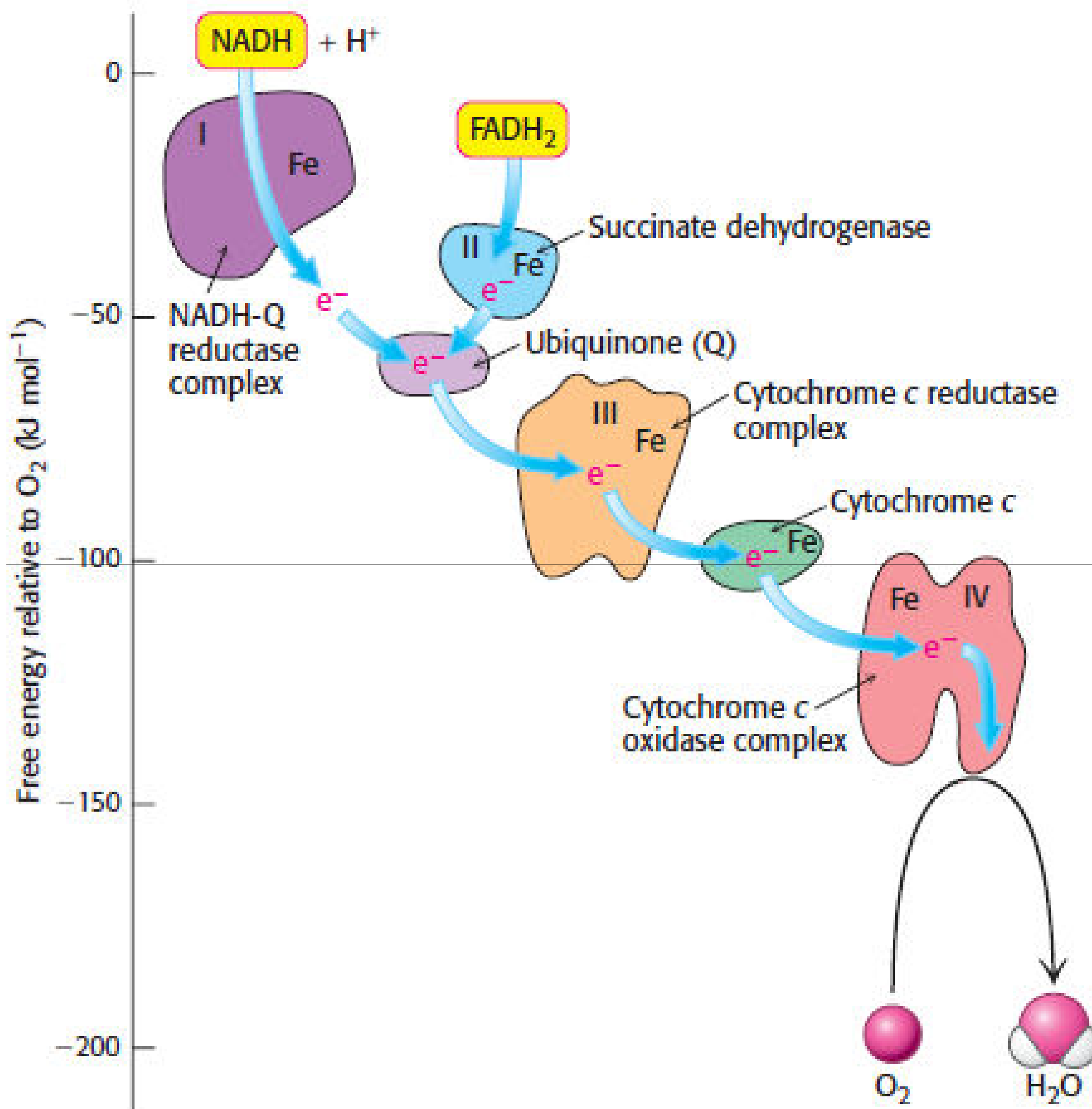
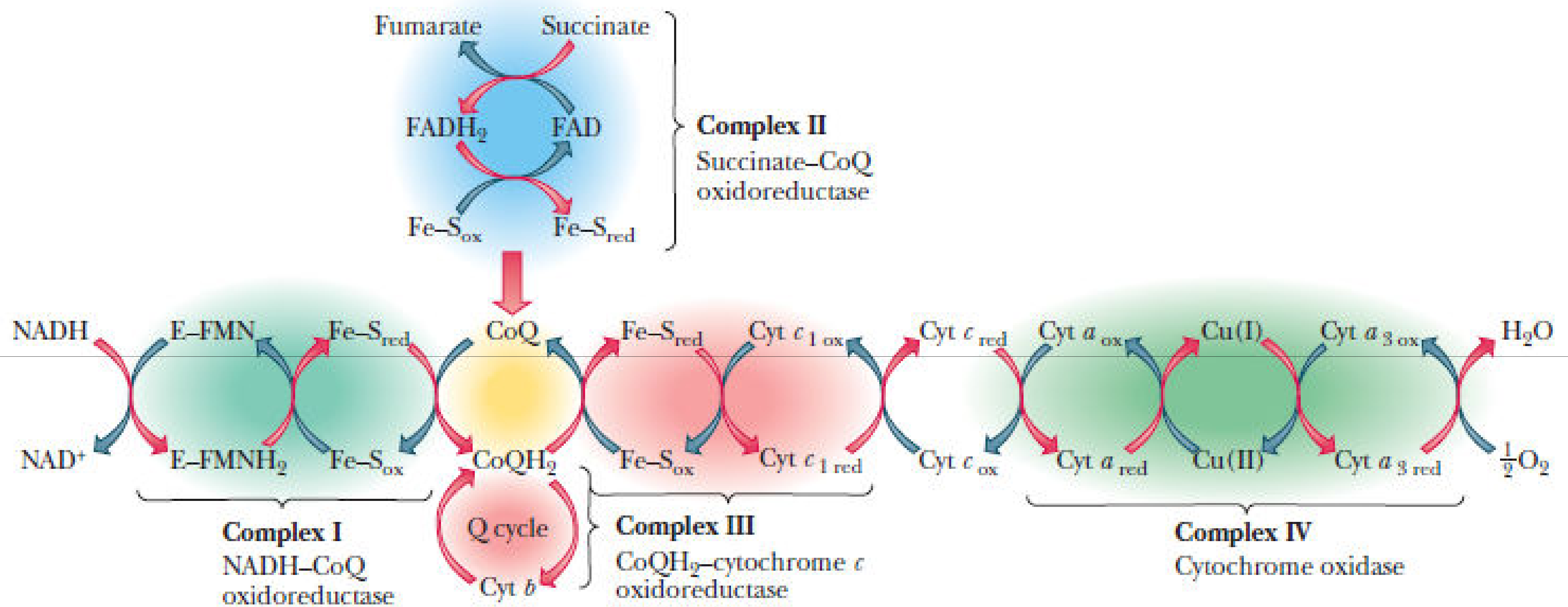


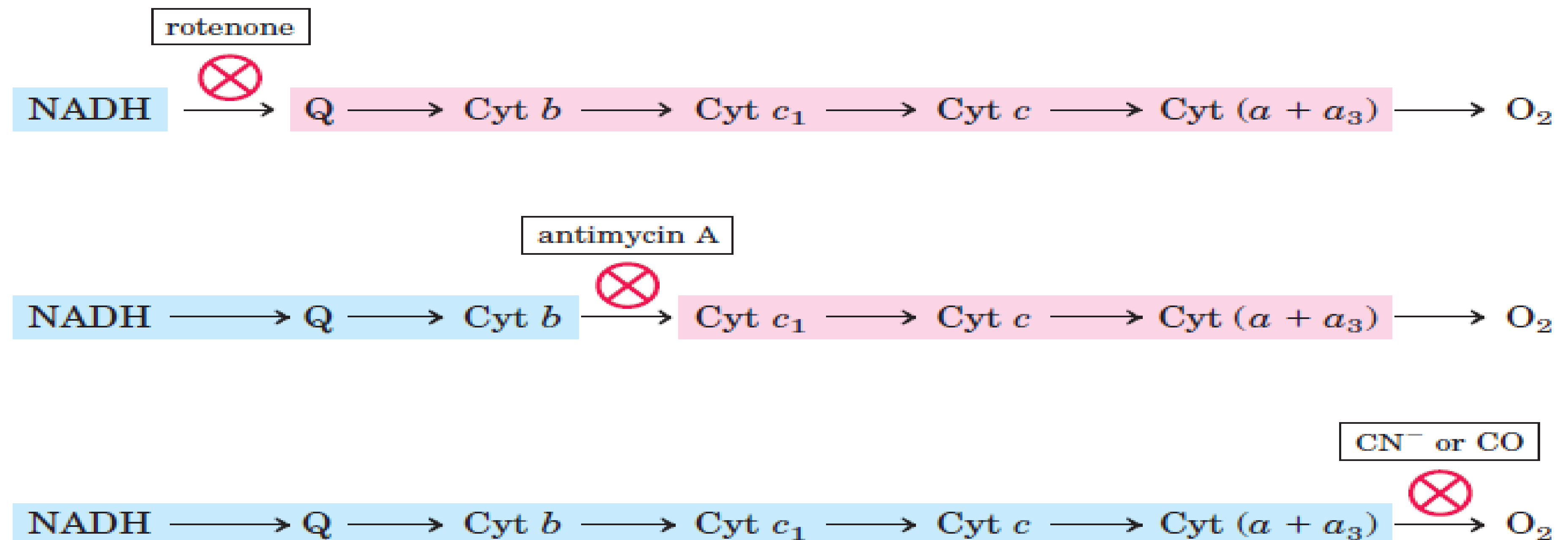
Figure 18.6 Components of the electron-transport chain. Electrons flow down an energy gradient from NADH to O_2. The flow is catalyzed by four protein complexes. Iron is a component of Complexes I, III, IV and cytochrome c. [After D. Sadava et al., *Life*, 8th ed. (Sinauer, 2008), p. 150.]



■ FIGURE 20.6 **The electron transport chain, showing the respiratory complexes.** In the reduced cytochromes, the iron is in the Fe(II) oxidation state; in the oxidized cytochromes, the oxygen is in the Fe(III) oxidation state.

TABLE 19–2 Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

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



Electron Carriers Function in Multienzyme Complexes

Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II).

Complex III arries electrons from reduced ubiquinone to cytochrome c, and Complex IV completes the sequence by transferring electrons from cytochrome c to O2.

TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

Enzyme complex/protein	Mass (kDa)	Number of subunits*	Prosthetic group(s)
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone:cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome *c*†	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

*Numbers of subunits in the bacterial equivalents in parentheses.

†Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

Complex I: NADH to Ubiquinone

Illustrates the relationship between Complexes I and II and ubiquinone.

Complex I, also called NADH:ubiquinone oxidoreductase or **NADH dehydrogenase, is a large enzyme** composed of 42 different polypeptide chains, including an FMN-containing flavoprotein and at least six ironsulfur centers.

Complex I catalyzes two simultaneous and obligately coupled processes:

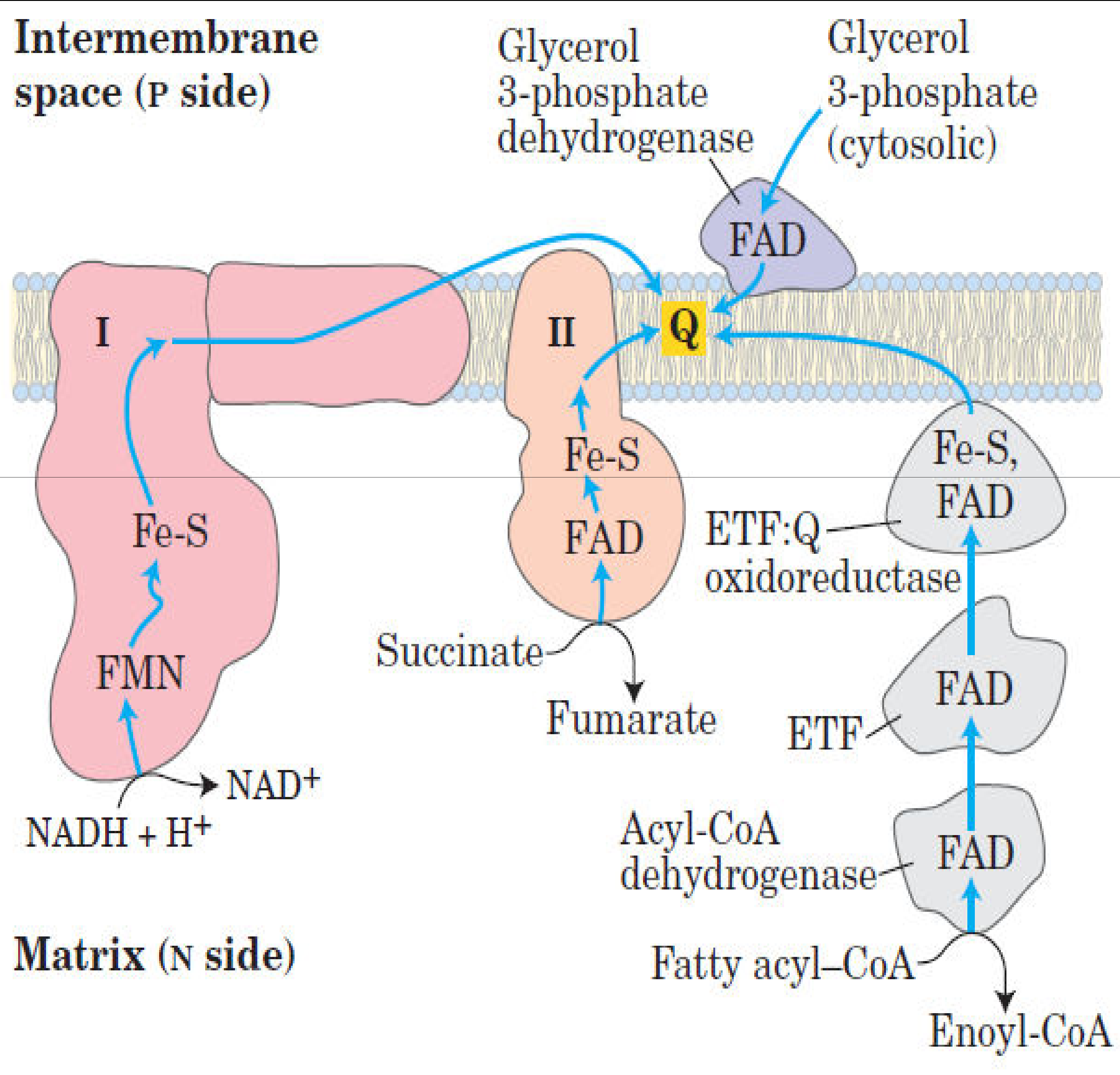
(1) the exergonic transfer to ubiquinone of a hydride ion from NADH and a proton from the matrix, expressed by

$$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$$

(2) the endergonic transfer of four protons from the matrix to the intermembrane space.

Complex I is therefore a proton pump driven by the energy of electron transfer, and the reaction it catalyzes is **vectorial: it** moves protons in a specific direction from one location (the matrix, which becomes negatively charged with the departure of protons) to another (the intermembrane space, which becomes positively charged).

Path of electrons from NADH, succinate, fatty acyl–CoA, and glycerol 3-phosphate to ubiquinone.



Electrons from NADH pass through a flavoprotein to a series of iron-sulfur proteins (in Complex I) and then to Q. Electrons from succinate pass through a flavoprotein and several Fe-S centers (in Complex II) on the way to Q. Glycerol 3-phosphate donates electrons to a flavoprotein (glycerol 3-phosphate dehydrogenase) on the outer face of the inner mitochondrial membrane, from which they pass to Q. Acyl-CoA dehydrogenase (the first enzyme of *oxidation) transfers electrons to electrontransferring* flavoprotein (ETF), from which they pass to Q via ETF:ubiquinone oxidoreductase.

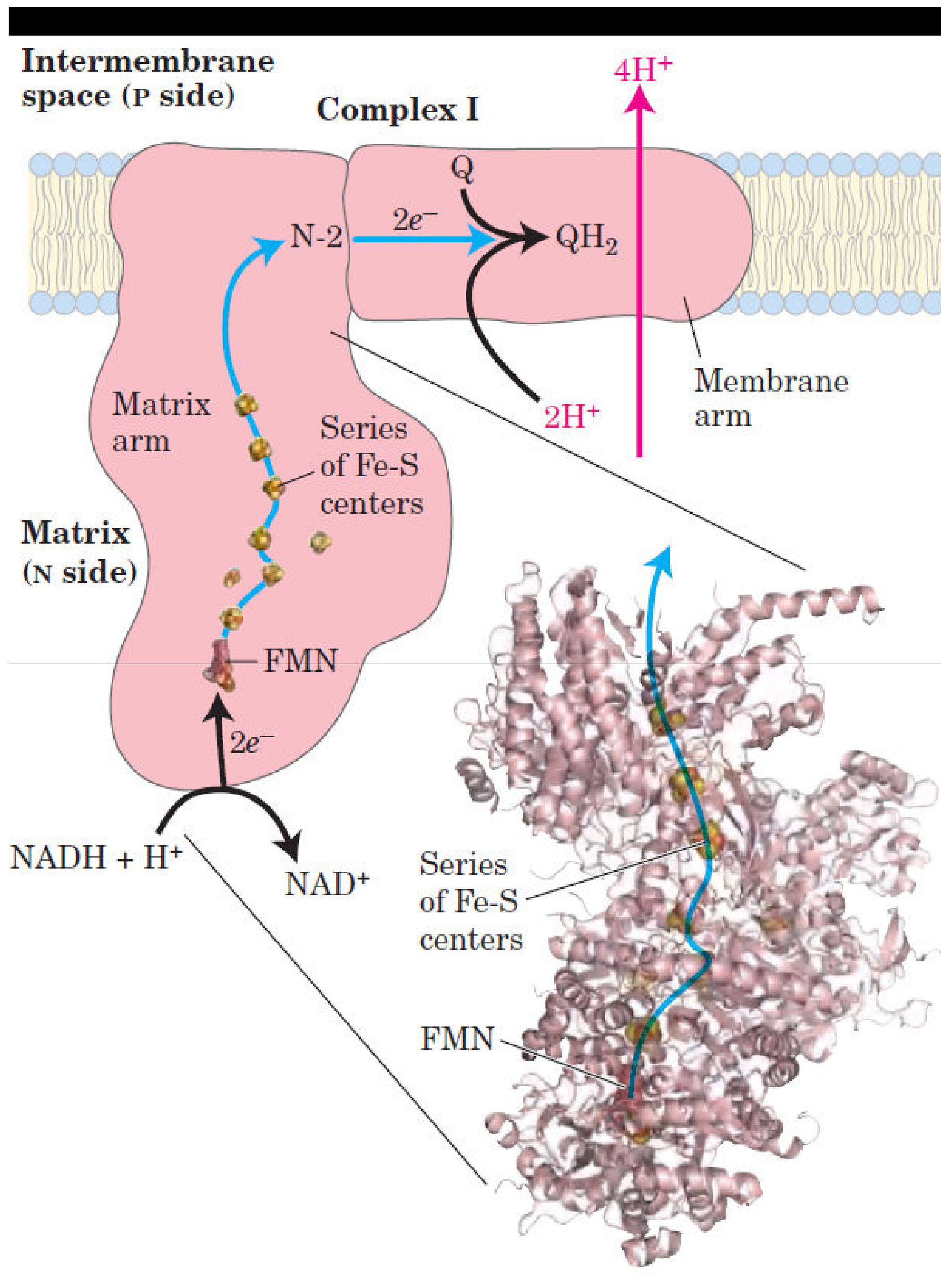


FIGURE 19–9 NADH:ubiquinone oxidoreductase (Complex I). Complex I catalyzes the transfer of a hydride ion from NADH to FMN, from which two electrons pass through a series of Fe-S centers to the iron-sulfur protein N-2 in the matrix arm of the complex. The domain that extends into the matrix has been crystallized and its structure solved (PDB ID 2FUG); the structure of the membrane domain of Complex I is not yet known. Electron transfer from N-2 to ubiquinone on the membrane arm forms QH_2, which diffuses into the lipid bilayer. This electron transfer also drives the expulsion from the matrix of four protons per pair of electrons. The detailed mechanism that couples electron and proton transfer in Complex I is not yet known, but probably involves a Q cycle similar to that in Complex III in which QH_2 participates twice per electron pair (see Fig. 19–12). Proton flux produces an electrochemical potential across the inner mitochondrial membrane (N side negative, P side positive), which conserves some of the energy released by the electron-transfer reactions. This electrochemical potential drives ATP synthesis.

Complex II: Succinate to Ubiquinone

- Complex II is succinate dehydrogenase, the only membrane-bound enzyme in the citric acid cycle. Although smaller and simpler than Complex I, it contains five prosthetic groups of two types and four different protein subunits.

- Subunits C and D are integral membrane proteins, each with three transmembrane helices. They contain a heme group, heme b, and a binding site for ubiquinone, the final electron acceptor in the reaction catalyzed by Complex II. Subunits A and B extend into the matrix (or the cytosol of a bacterium); they contain three 2Fe-2S centers, bound FAD, and a binding site for the substrate, succinate.

- The path of electron transfer from the succinate-binding site to FAD, then through the Fe-S centers to the Q-binding site, is more than 40 A° long, but none of the individual electron transfer distances exceeds about 11 A°—a reasonable distance for rapid electron transfer.

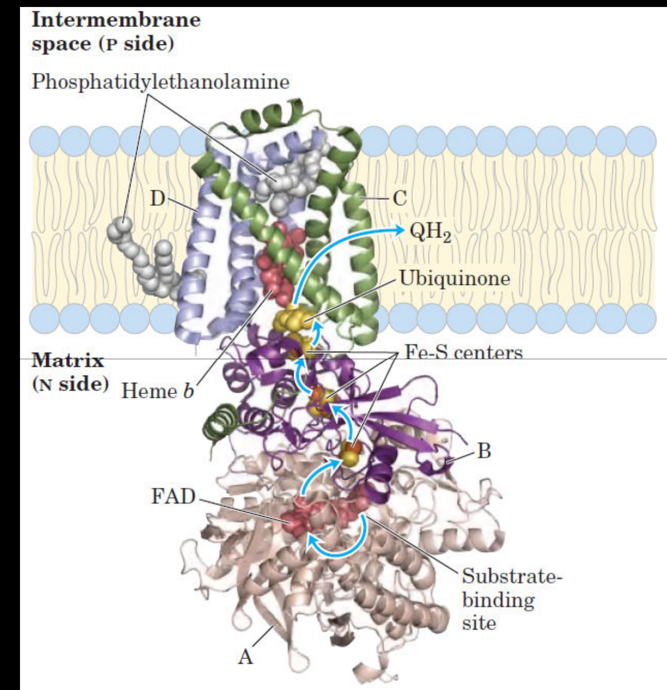


TABLE 19–4 Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

Type of interference	Compound*	Target/mode of action
Inhibition of electron transfer	Cyanide Carbon monoxide	Inhibit cytochrome oxidase
	Antimycin A	Blocks electron transfer from cytochrome *b* to cytochrome c_1
	Myxothiazol Rotenone Amytal Piericidin A	Prevent electron transfer from Fe-S center to ubiquinone
	DCMU	Competes with Q_B for binding site in PSII
Inhibition of ATP synthase	Aurovertin	Inhibits F_1
	Oligomycin Venturicidin	Inhibit F_o and CF_o
	DCCD	Blocks proton flow through F_o and CF_o
Uncoupling of phosphorylation from electron transfer	FCCP DNP	Hydrophobic proton carriers
	Valinomycin	K^+ ionophore
	Thermogenin	In brown adipose tissue, forms proton-conducting pores in inner mitochondrial membrane
Inhibition of ATP-ADP exchange	Atractyloside	Inhibits adenine nucleotide translocase

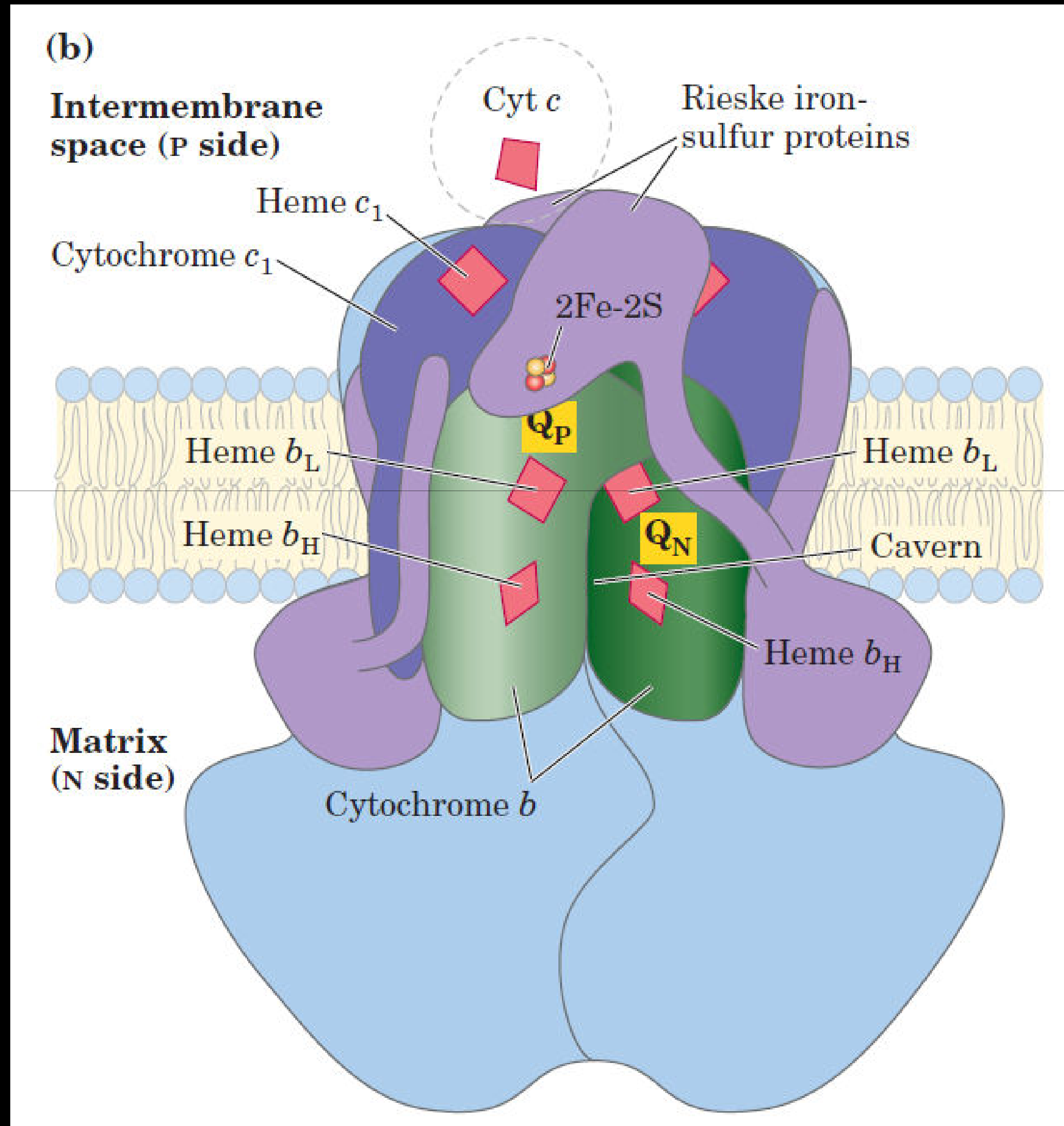
*DCMU is 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCCD, dicyclohexylcarbodiimide; FCCP, cyanide-*p*-trifluoromethoxyphenylhydrazone; DNP, 2,4-dinitrophenol.

Other substrates for mitochondrial dehydrogenases pass electrons into the respiratory chain at the level of ubiquinone, but not through Complex II.

The first step in the β oxidation of fatty acyl–CoA, catalyzed by the flavoprotein acyl-CoA dehydrogenase, involves transfer of electrons from the substrate to the FAD of the dehydrogenase, then to electron-transferring flavoprotein (ETF), which in turn passes its electrons to ETF: ubiquinone oxidoreductase.

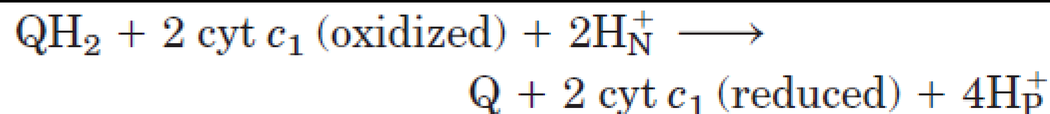
Other substrates for mitochondrial dehydrogenases pass electrons into the respiratory chain at the level of ubiquinone, but not through Complex II. The first step in the β oxidation of fatty acyl–CoA, catalyzed by the flavoprotein **acyl-CoA dehydrogenase** (see Fig. 17–8), involves transfer of electrons from the substrate to the FAD of the dehydrogenase, then to electron-transferring flavoprotein (ETF), which in turn passes its electrons to **ETF:ubiquinone oxidoreductase** (Fig. 19–8). This enzyme transfers electrons into the respiratory chain by reducing ubiquinone. Glycerol 3-phosphate, formed either from glycerol released by triacylglycerol breakdown or by the reduction of dihydroxyacetone phosphate from glycolysis, is oxidized by **glycerol 3-phosphate dehydrogenase** (see Fig. 17–4). This enzyme is a flavoprotein located on the outer face of the inner mitochondrial membrane, and like succinate dehydrogenase and acyl-CoA dehydrogenase it channels electrons into the respiratory chain by reducing ubiquinone (Fig. 19–8). The important role of glycerol 3-phosphate dehydrogenase in shuttling reducing equivalents from cytosolic NADH into the mitochondrial matrix is described in Section 19.2 (see Fig. 19–30). The effect of each of these electron-transferring enzymes is to contribute to the pool of reduced ubiquinone. QH_2 from all these reactions is reoxidized by Complex III.

Cytochrome *bc1 complex (Complex III)*



Complex III: Ubiquinone to Cytochrome c: Complex III, also called cytochrome *bc1 complex* *or* *ubiquinone:cytochrome c* oxidoreductase, couples the transfer of electrons from ubiquinol (QH2) to cytochrome *c with the vectorial* transport of protons from the matrix to the intermembrane space.

- The functional unit of Complex III is a dimer, with the two monomeric units of cytochrome *b surrounding a* “cavern” in the middle of the membrane, in which ubiquinone is free to move from the matrix side of the membrane (site QN on one monomer) to the intermembrane space (site QP of the other monomer) as it shuttles electrons and protons across the inner mitochondrial membrane.
- Based on the structure of Complex III and detailed biochemical studies of the redox reactions, a reasonable model, the **Q cycle**, has been proposed for the passage of electrons and protons through the complex. The net equation for the redox reactions of the Q cycle **is**



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

The Q cycle accommodates the switch between the two electron carrier ubiquinone and the one-electron carriers—cytochromes *b562, b566, c1, and c*—and explains the measured stoichiometry of four protons translocated per pair of electrons passing through Complex III to cytochrome *c*. Although the path of electrons through this segment of the respiratory chain is complicated, the net effect of the transfer is simple: QH2 is oxidized to Q and two molecules of cytochrome *c* are reduced.

Cytochrome *c is a soluble protein of the intermembrane* space. After its single heme accepts an electron from Complex III, cytochrome *c moves to Complex IV to* donate the electron to a binuclear copper center.

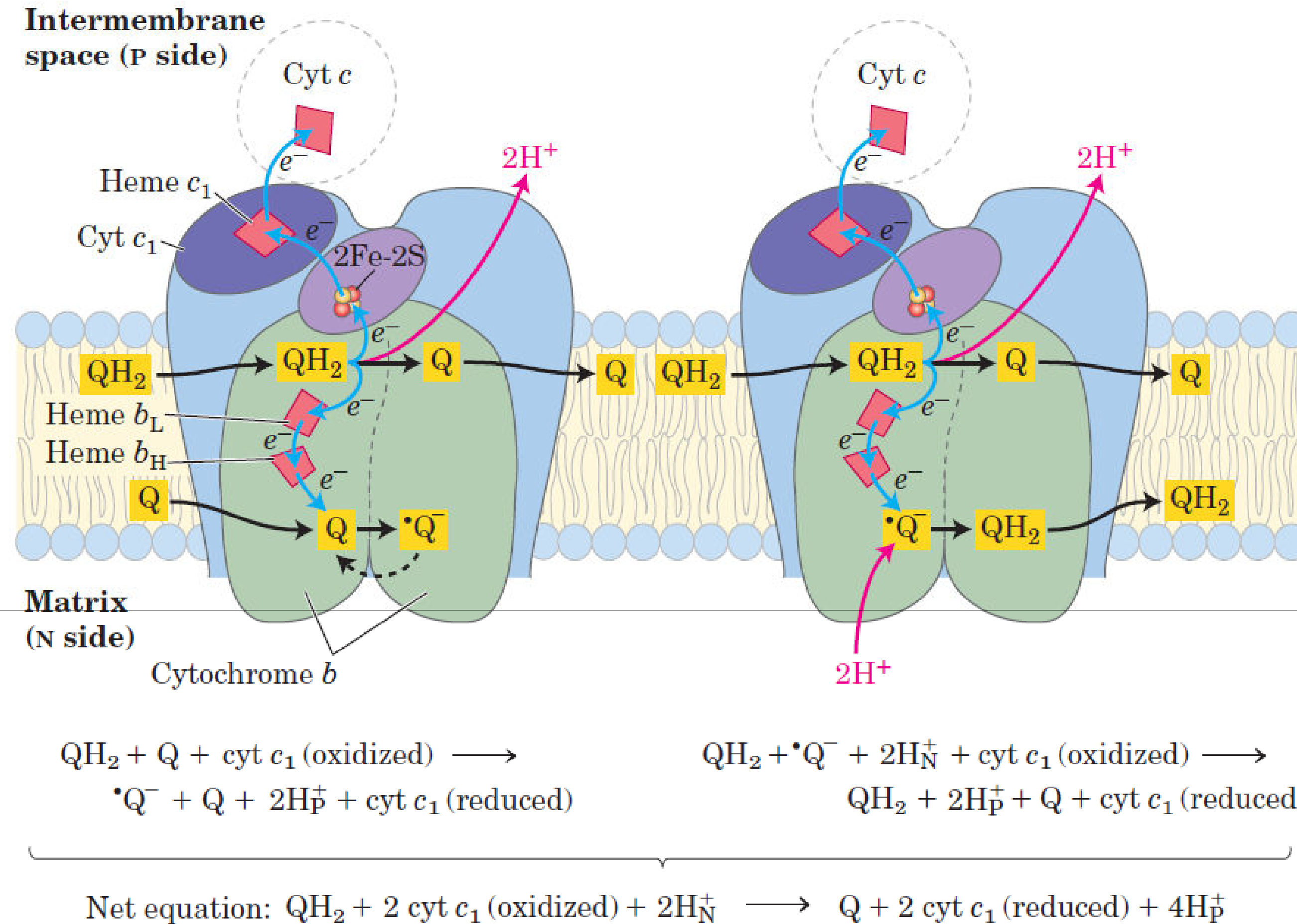


FIGURE 19–12 The Q cycle, shown in two stages. The path of electrons through Complex III is shown by blue arrows. In the first stage (left), Q on the N side is reduced to the semiquinone radical, which in the second stage (right) is converted to QH_2. Meanwhile, on the P side of the membrane, two molecules of QH_2 are oxidized to Q, releasing two protons per Q molecule (four protons in all) into the intermembrane space. Each QH_2 donates one electron (via the Rieske Fe-S center) to cytochrome c_1, and one electron (via cytochrome *b*) to a molecule of Q near the N side, reducing it in two steps to QH_2. This reduction also uses two protons per Q, which are taken up from the matrix.

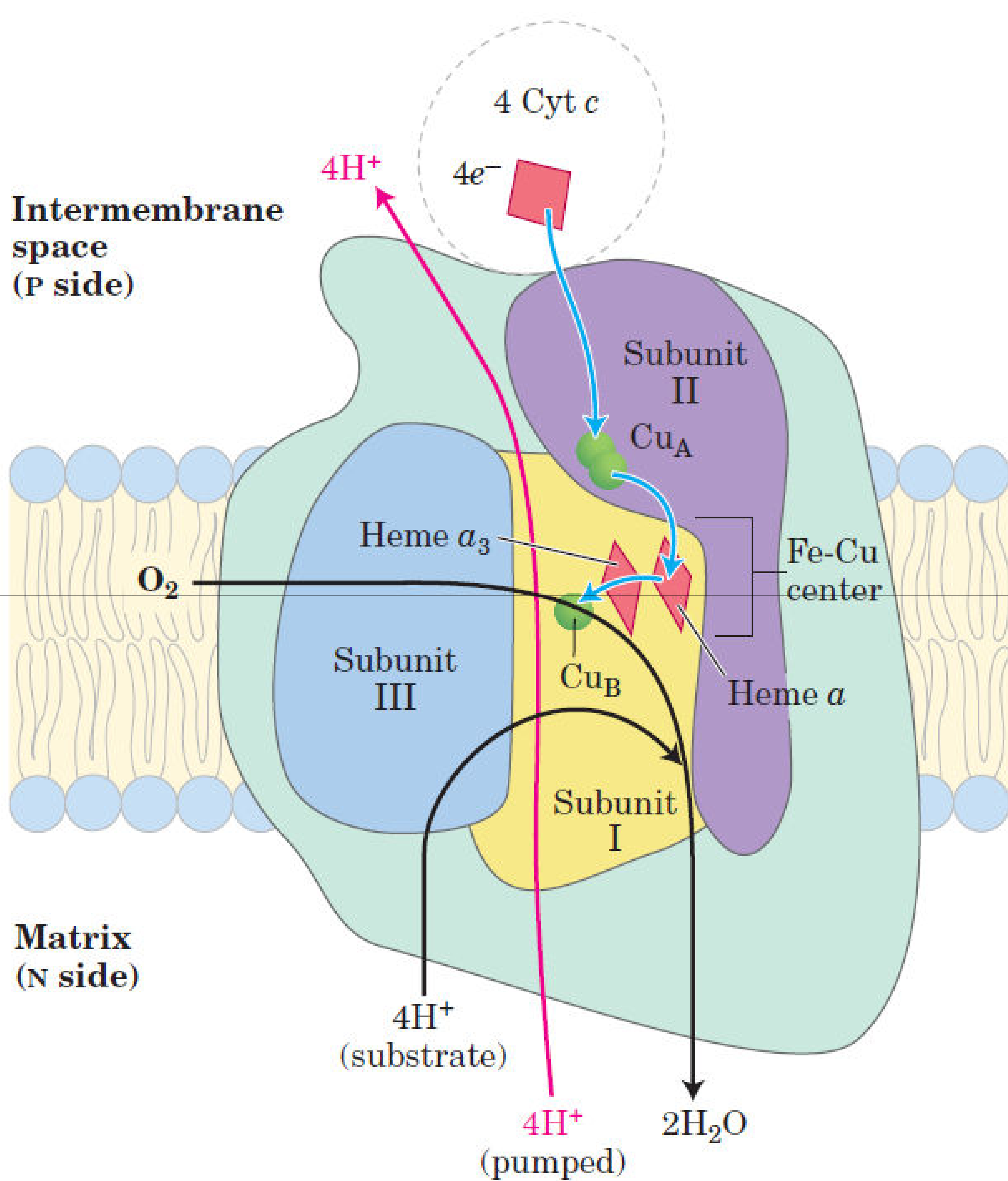


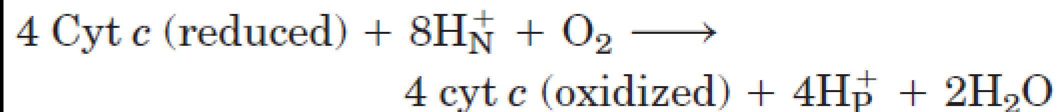
FIGURE 19–14 Path of electrons through Complex IV. The three proteins critical to electron flow are subunits I, II, and III. The larger green structure includes the other 10 proteins in the complex. Electron transfer through Complex IV begins with cytochrome *c* (top). Two molecules of reduced cytochrome *c* each donate an electron to the binuclear center Cu_A. From here electrons pass through heme *a* to the Fe-Cu center (cytochrome a_3 and Cu_B). Oxygen now binds to heme a_3 and is reduced to its peroxy derivative (O_2^{2-}; not shown here) by two electrons from the Fe-Cu center. Delivery of two more electrons from cytochrome *c* (top, making four electrons in all) converts the O_2^{2-} to two molecules of water, with consumption of four "substrate" protons from the matrix. At the same time, four protons are pumped from the matrix by an as yet unknown mechanism.

Complex IV: Cytochrome c to O_2 In the final step of the respiratory chain, **Complex IV,** also called cytochrome oxidase, carries electrons from cytochrome *c* to molecular oxygen, reducing it to H_2O.

- ✓ Complex IV is a large enzyme (13 subunits; *Mr 204,000) of the inner* mitochondrial membrane. Bacteria contain a form that is much simpler, with only three or four subunits, but still capable of catalyzing both electron transfer and proton pumping. Comparison of the mitochondrial and bacterial complexes suggests that three subunits are critical to the function.
- ✓ Mitochondrial subunit II contains two Cu ions complexed with the —SH groups of two Cys residues in a binuclear center (Cu_A) that resembles the 2Fe-2S centers of iron-sulfur proteins.
- ✓ Subunit I contains two heme groups, designated a and a_3, and another copper ion (Cu_B). Heme a_3 and Cu_B form a second binuclear center that accepts electrons from heme *a* and transfers them to O_2 bound to heme *a_3*.

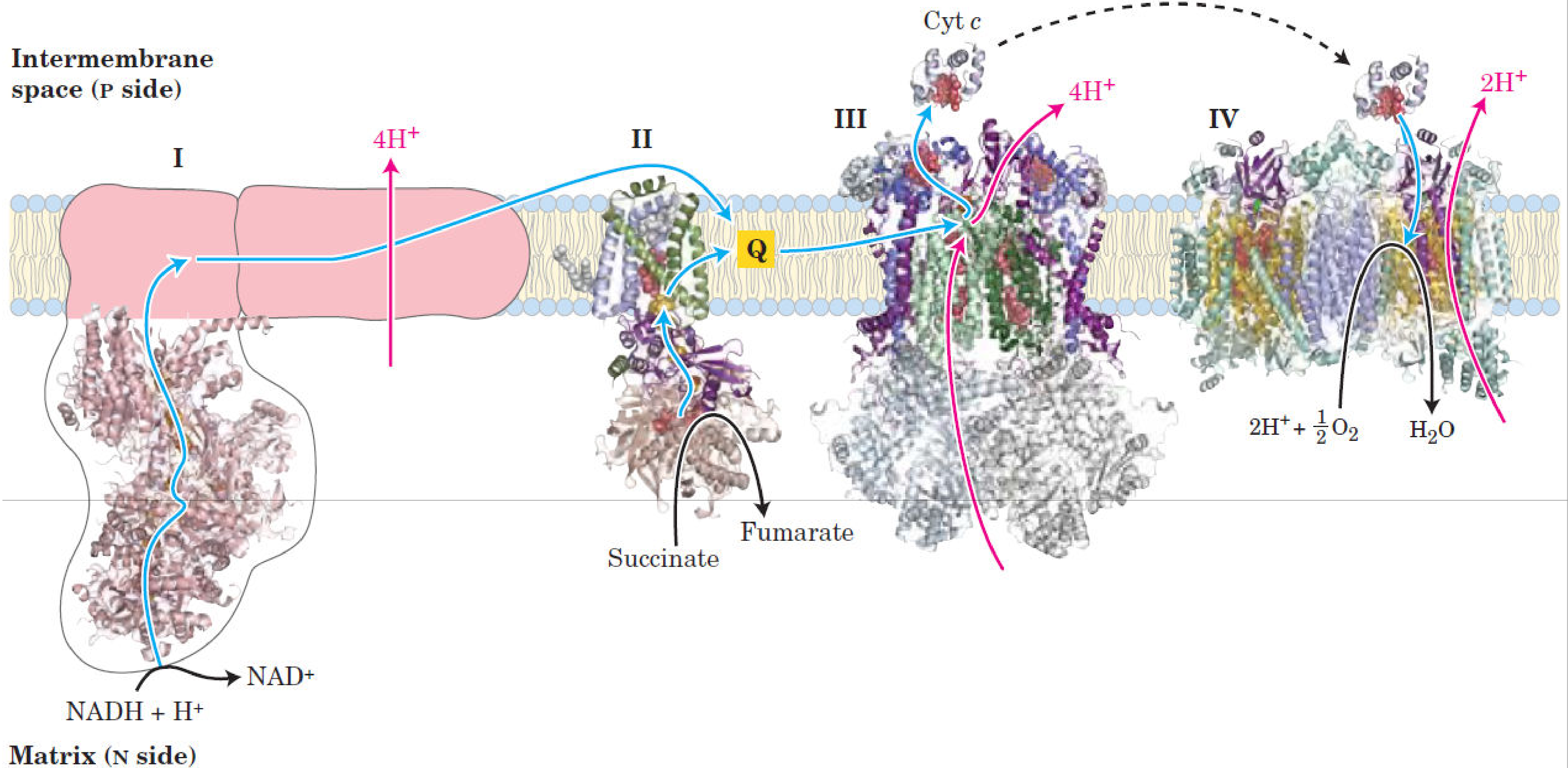
Electron transfer through Complex IV

- Electron transfer through Complex IV is from cytochrome c to the CuA center, to heme a, to the heme a3–CuB center, and finally to O2. For every four electrons passing through this complex, the enzyme consumes four “substrate” H from the matrix (N side) in converting O2 to $2H_2O$.
- It also uses the energy of this redox reaction to pump one proton outward into the intermembrane space (P side) for each electron that passes through, adding to the electrochemical potential produced by redox-driven proton transport through Complexes I and III.
- The overall reaction catalyzed by Complex IV is:



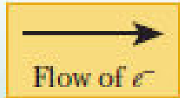
- This four-electron reduction of O2 involves redox centers that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates such as hydrogen peroxide or hydroxyl free radicals—very reactive species that would damage cellular components. The intermediates remain tightly bound to the complex until completely converted to water.

Summary of the flow of electrons and protons through the four complexes of the respiratory chain.

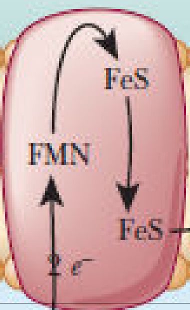


Electrons reach Q through Complexes I and II. The reduced Q (QH2) serves as a mobile carrier of electrons and protons. It passes electrons to Complex III, which passes them to another mobile connecting link, cytochrome *c.*

Complex IV then transfers electrons from reduced cytochrome *c to O2.* Electron flow through Complexes I, III, and IV is accompanied by proton flow from the matrix to the intermembrane space. Recall that electrons from *oxidation of fatty acids can also enter the respiratory* chain through Q



Flow of e^-
Intermembrane space
Lipid bilayer
Matrix



Complex I
FeS
FMN
FeS
2 e^-
NADH
CoQ

Complex III
Cyt c_1
FeS
Cyt b_L
Cyt b_H
Q cycle
Cyt c
Cyt c

Complex IV
Cyt c
Cyt a
Cyt a_3
2 e^-
2 H^+ + $\frac{1}{2}$ O_2
H_2O