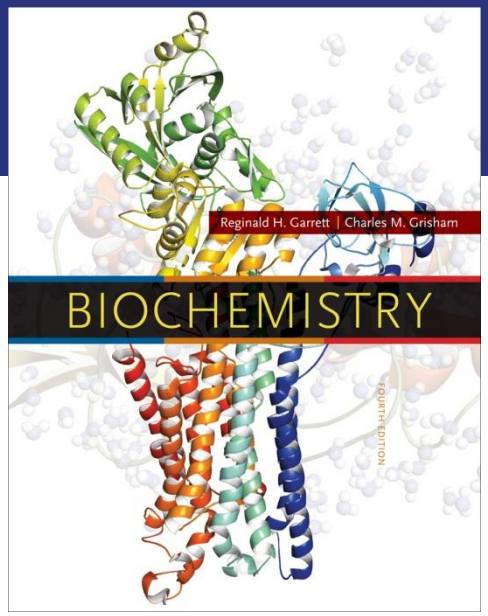
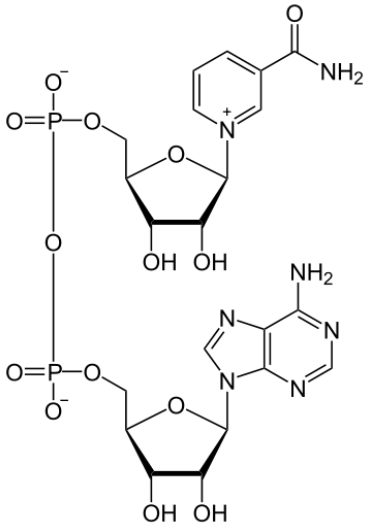


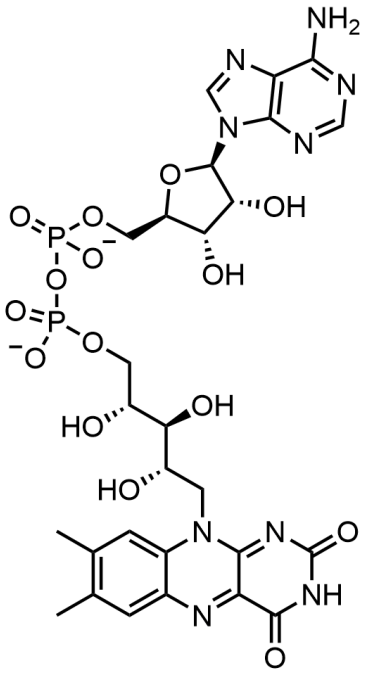
Reginald H. Garrett
Charles M. Grisham

Chapter 20
Electron Transport and Oxidative Phosphorylation

Essential Question

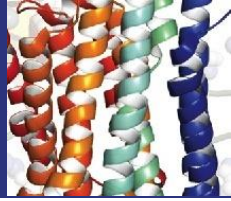
- How do cells oxidize NADH and [$FADH_2$] and convert their reducing potential into the chemical energy of ATP?





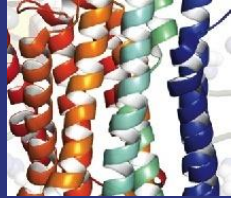
- Nicotinamide Adenine Dinucleotide
- Flavin Adenine Dinucleotide

Outline



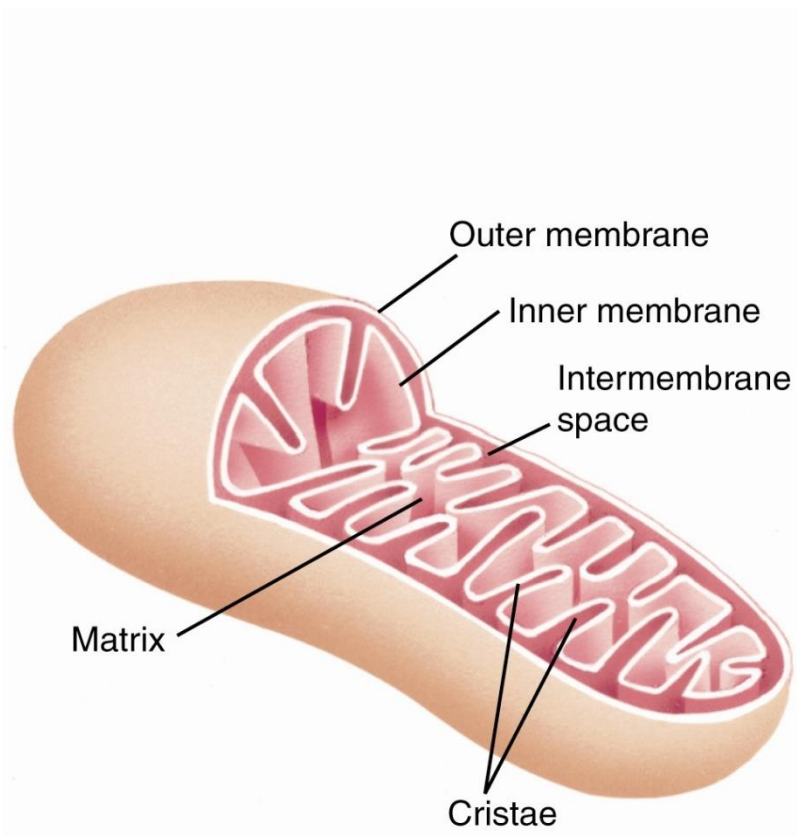
- Where in the cell do electron transport and oxidative phosphorylation occur?
- How is the electron-transport chain organized?
- What are the thermodynamic implications of chemiosmotic coupling?
- How does a proton gradient drive the synthesis of ATP?
- What is the P/O ratio for mitochondrial oxidative phosphorylation?
- How are the electrons of cytosolic NADH fed into electron tansport?
- How do mitochondria mediate apoptosis?

20.1 Where in the Cell Do Electron Transport and Oxidative Phosphorylation Occur?



- Electron Transport: Electrons carried by reduced coenzymes (NADH and [$FADH_2$] are passed through a chain of proteins and coenzymes to drive the generation of a proton gradient across the inner mitochondrial membrane
- Oxidative Phosphorylation: The proton gradient runs downhill to drive the synthesis of ATP
- It all happens in or at the inner mitochondrial membrane

20.1 Where in the Cell Do Electron Transport and Oxidative Phosphorylation Occur?



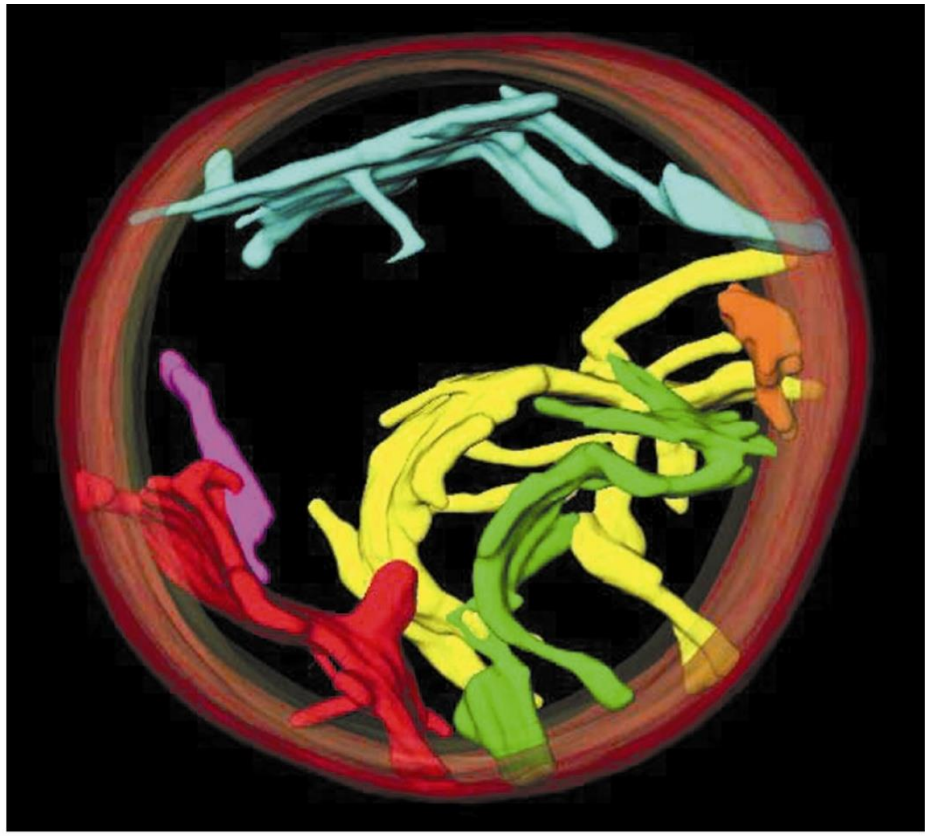
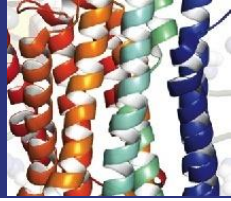


Figure 20.1 (a) A drawing of a mitochondrion. (b) Tomography of a rat liver mitochondrion. The colors represent individual cristae.

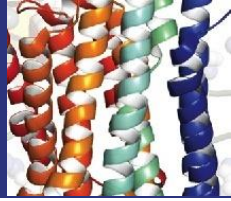
20.3 How Is the Electron Transport Chain Organized?



Figures 20.3 and 20.4 hold the secrets

- Four protein complexes in the inner mitochondrial membrane
- A lipid soluble coenzyme (UQ, CoQ) and a water soluble protein (cyt *c*) shuttle between protein complexes
- Electrons generally fall in energy through the chain - from complexes I and II to complex IV

20.3 How Is the Electron Transport Chain Organized?



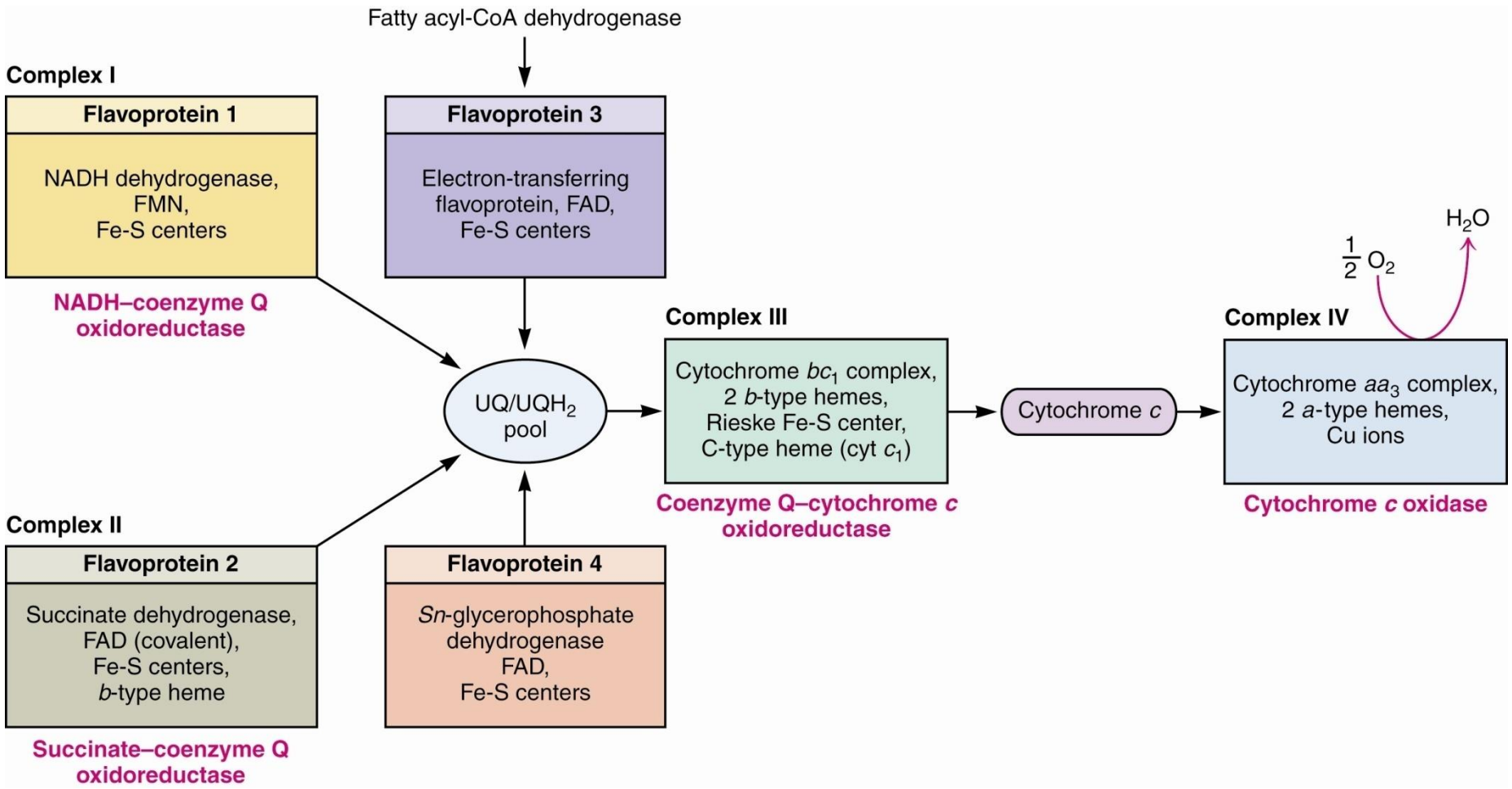
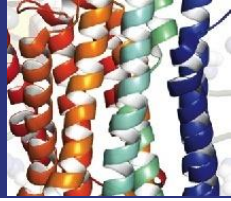


Figure 20.4 An overview of the complexes and pathways in the mitochondrial electron-transport chain.

Complex I Oxidizes NADH and Reduces Coenzyme Q



aka NADH-CoQ Reductase

aka NADH dehydrogenase

- Complex I carries out electron transfer from NADH to CoQ
- This complex contains at least 45 protein subunits – estimated mass of 980 kD
- The electron path:

  NADH $\rightarrow$ FMN $\rightarrow$ Fe-S $\rightarrow$ UQ$\rightarrow$ FeS $\rightarrow$ UQ
- Four H^+ transported out per 2 e^-

20.3 How Is the Electron Transport Chain Organized?

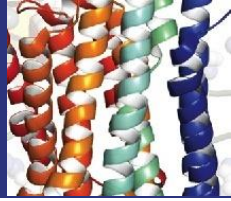
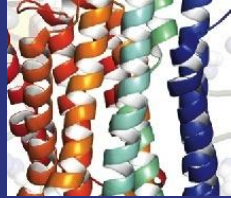


TABLE 20.2 Protein Complexes of the Mitochondrial Electron-Transport Chain

Complex	Mass (kD)	Subunits	Prosthetic Group	Binding Site for:
NADH–UQ reductase	980	≥45	FMN Fe-S	NADH (matrix side) UQ (lipid core)
Succinate–UQ reductase	140	4	FAD Fe-S	Succinate (matrix side) UQ (lipid core)
UQ–Cyt *c* reductase	250	9–10	Heme b_L Heme b_H Heme c_1 Fe-S	Cyt *c* (intermembrane space side)
Cytochrome *c*	13	1	Heme *c*	Cyt c_1 Cyt *a*
Cytochrome *c* oxidase	162	13	Heme *a* Heme a_3 Cu_A Cu_B	Cyt *c* (intermembrane space side)

Coenzyme Q is a Mobile Electron Carrier



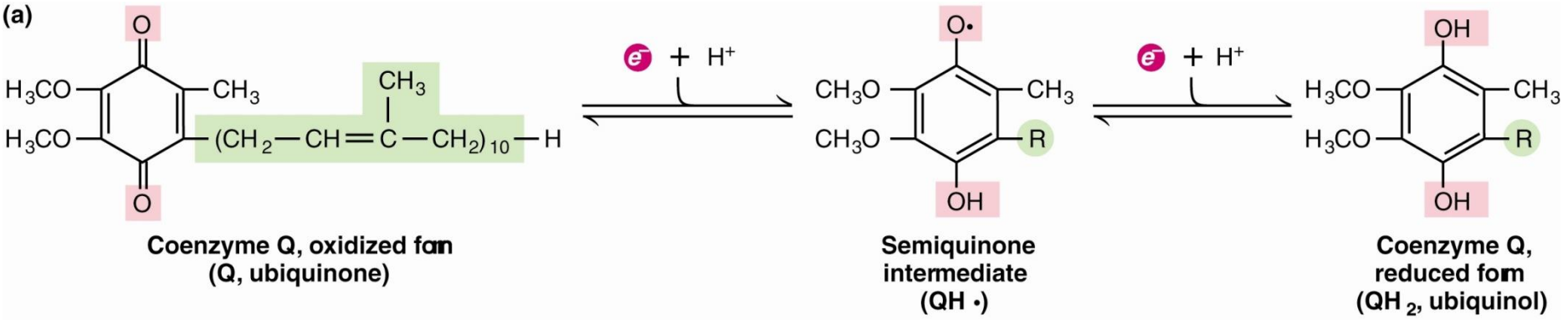
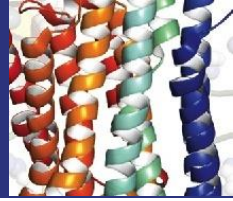


Figure 20.5 (a) The three oxidation states of coenzyme Q.

Complex I Transports Protons From the Matrix to the Cytosol



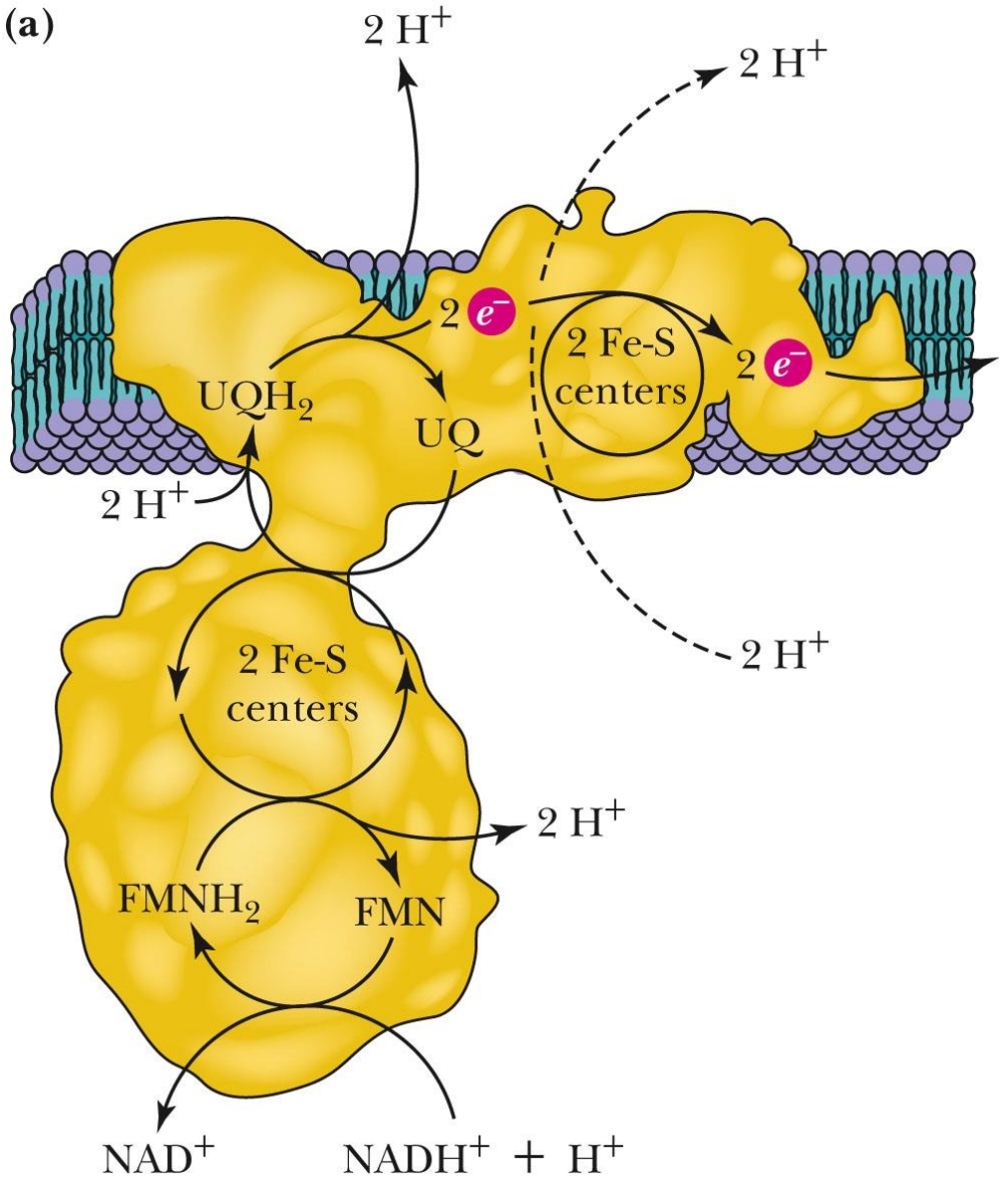


Figure 20.6 Structural organization of mammalian Complex I, based on electron microscopy, showing functional relationships within the L-shaped complex. Electron flow from NADH to UQH_2 in the membrane pool is indicated.

Complex I Transports Protons From the Matrix to the Cytosol

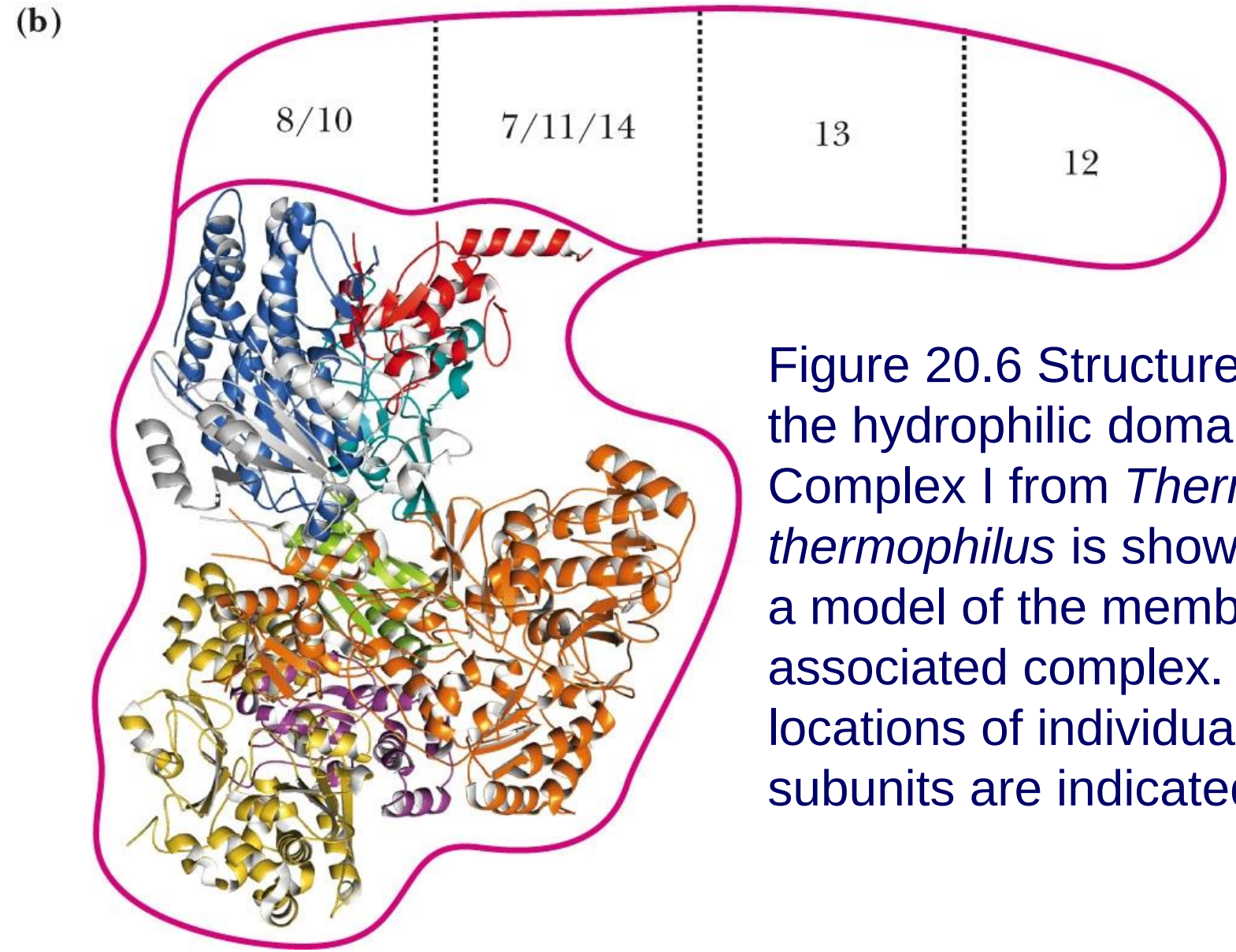
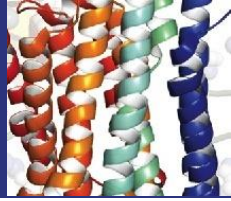


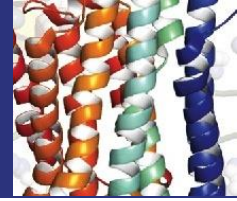
Figure 20.6 Structure of the hydrophilic domain of Complex I from *Thermus thermophilus* is shown on a model of the membrane-associated complex. The locations of individual subunits are indicated.

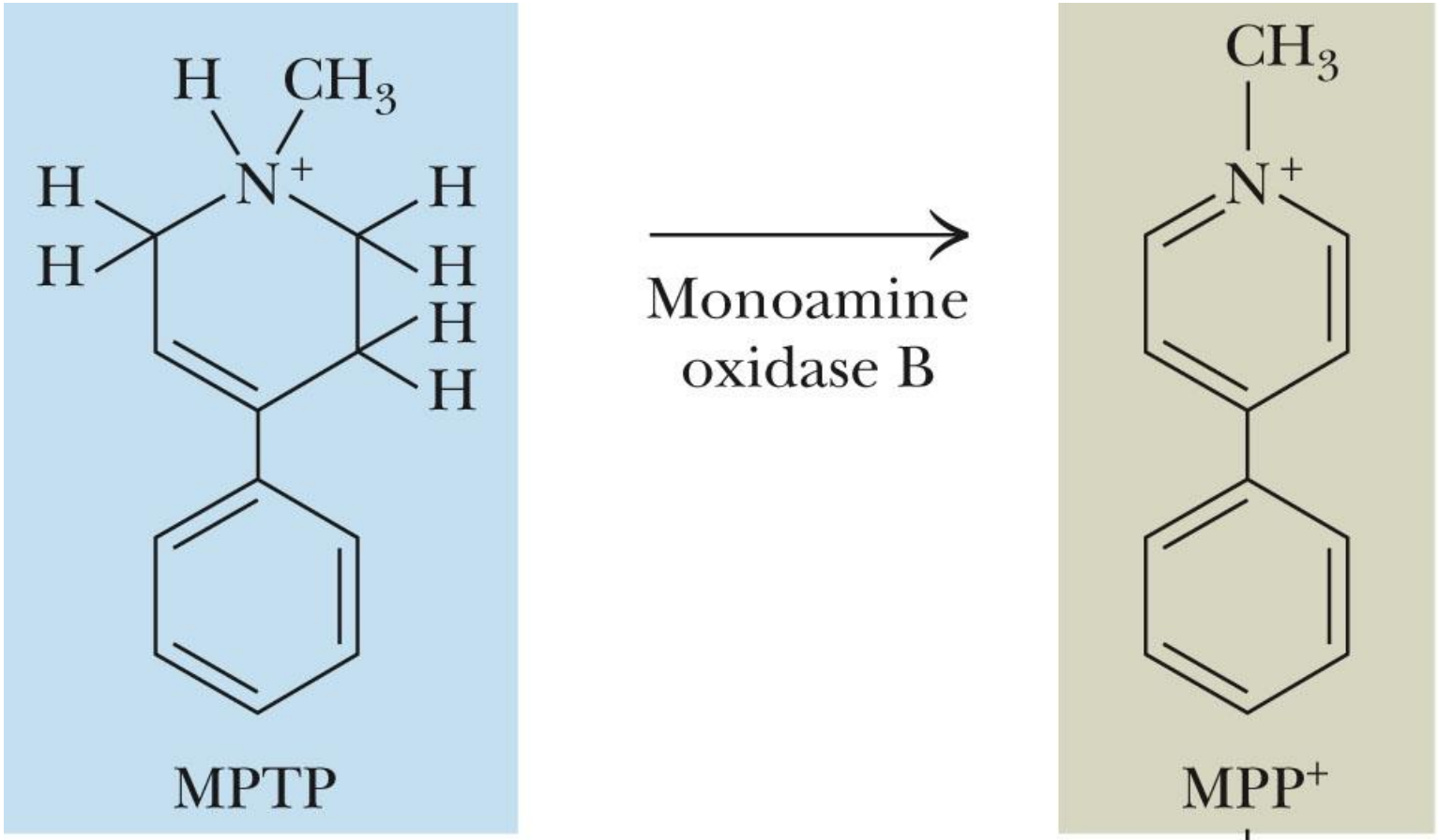
Solving a Medical Mystery Revolutionized Our Treatment of Parkinson's Disease



- Cases of paralysis among illegal drug users in 1982 was traced to synthetic heroin that contained MPTP as a contaminant-(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin
- MPTP is converted rapidly in the brain to MPP^+
- MPP^+ is a potent inhibitor of mitochondrial Complex I
- Such inhibition occurs especially in regions of the brain that deteriorate in Parkinson's disease
- Treatment of the paralysis victims with L-Dopa restored normal movement
- Implantation of fetal brain tissue also worked
- These treatments revolutionized the use of tissue implantation to treat neurodegenerative diseases

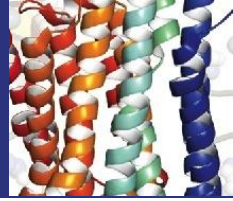
Complex I Transports Protons From the Matrix to the Cytosol





- MPTP is converted in the brain to MPP^+
- MPP^+ is a potent inhibitor of mitochondrial Complex I

Complex II Oxidizes Succinate and Reduces Coenzyme Q



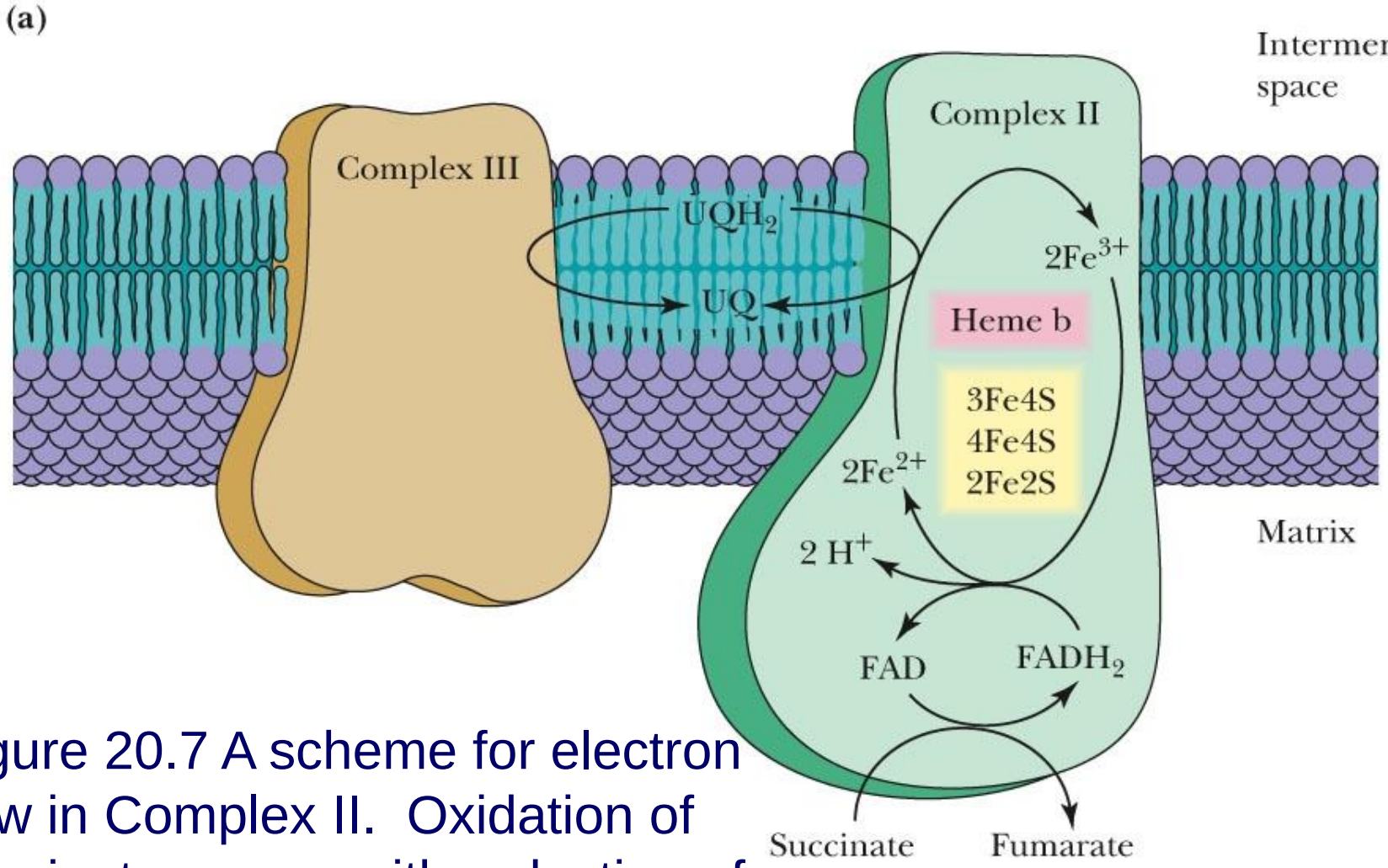


Figure 20.7 A scheme for electron flow in Complex II. Oxidation of succinate occurs with reduction of [FAD]. Electrons are then passed to Fe-S centers and then to CoQ.

Complex II Oxidizes Succinate and Reduces Coenzyme Q

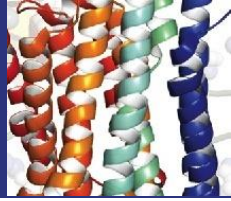
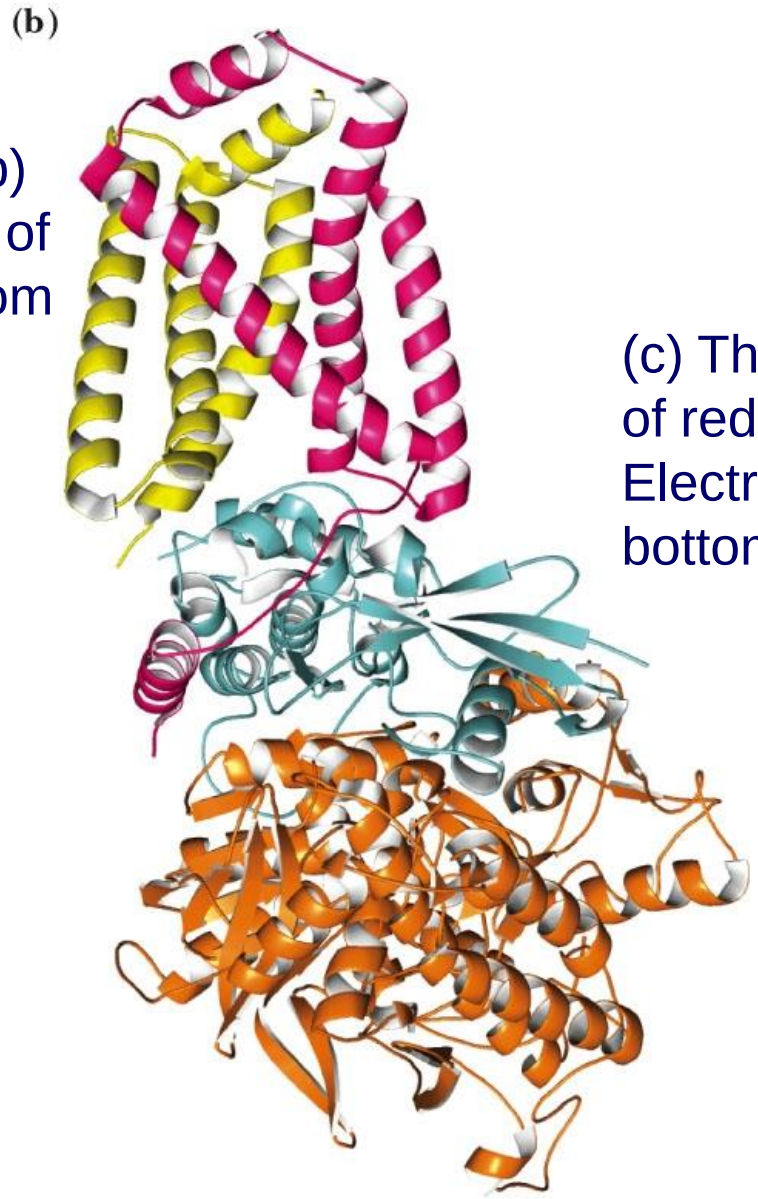
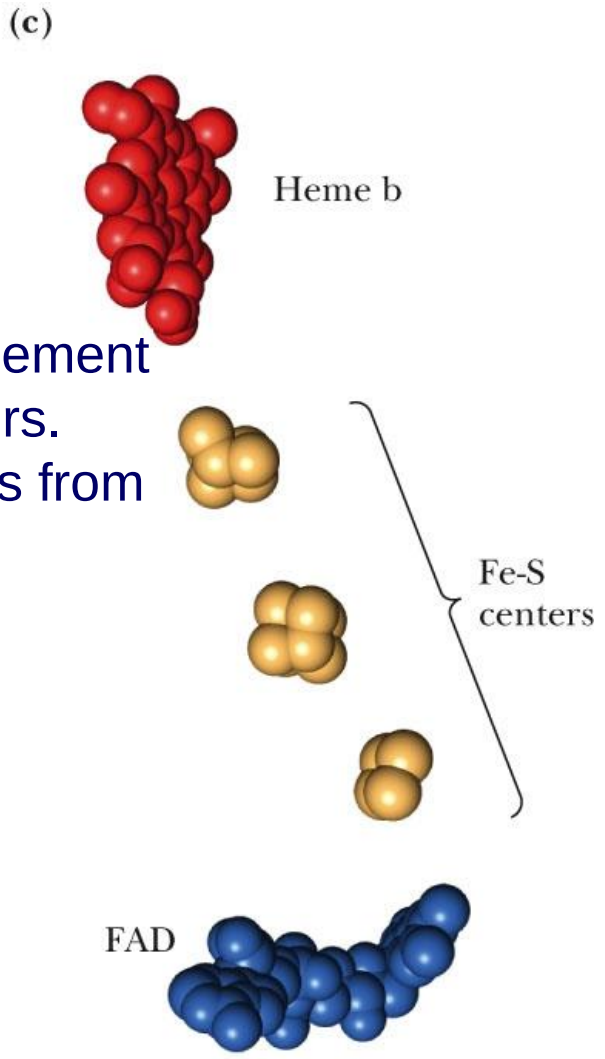


Figure 20.7 (b) The structure of Complex II from pig heart.



(c) The arrangement of redox centers. Electron flow is from bottom to top.



Fatty-Acyl-CoA Dehydrogenases Also Supply Electrons to UQ

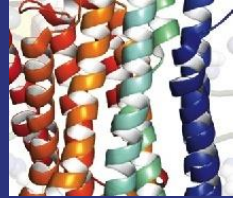
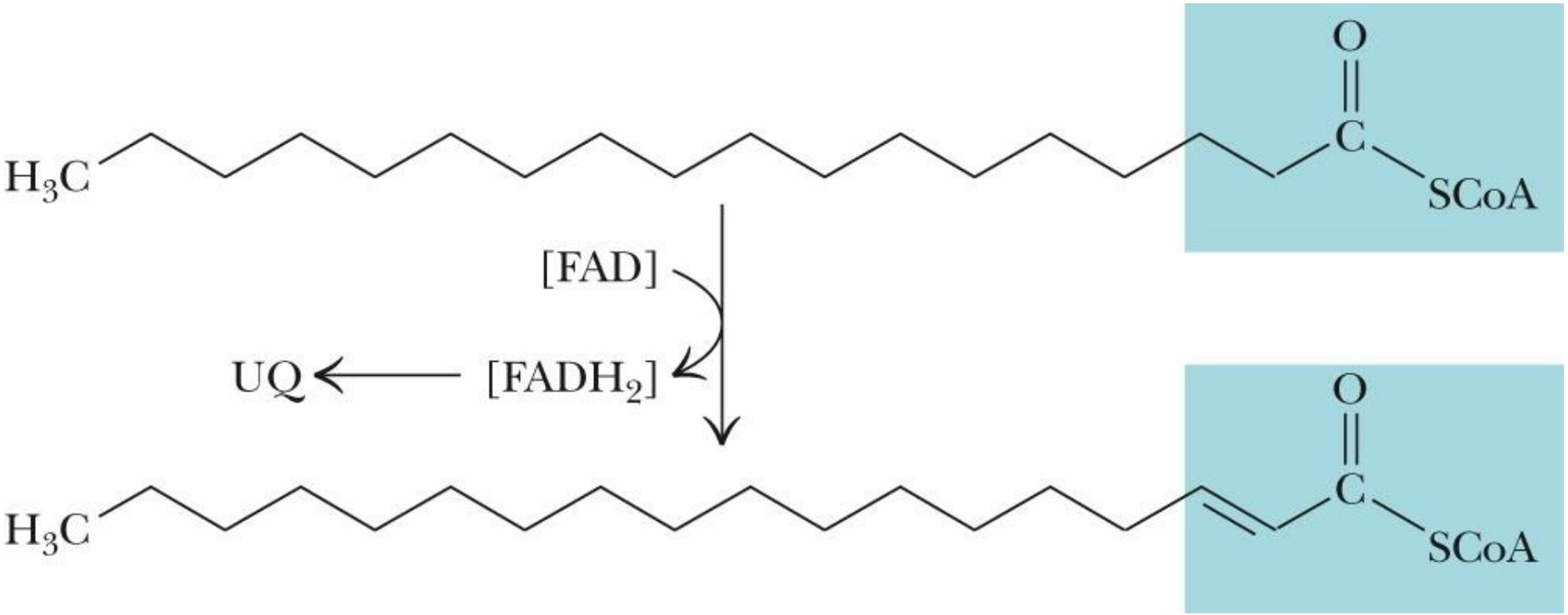
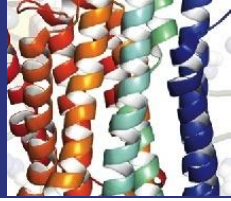


Figure 20.8 The fatty acyl-CoA dehydrogenase reaction, emphasizing that the reaction involves reduction of enzyme-bound FAD (indicated by brackets).



The fatty acyl-CoA dehydrogenases are three soluble matrix enzymes involved in fatty acid oxidation (See also Chapter 23).

Complex III Mediates Electron Transport from Coenzyme Q to Cytochrome *c*

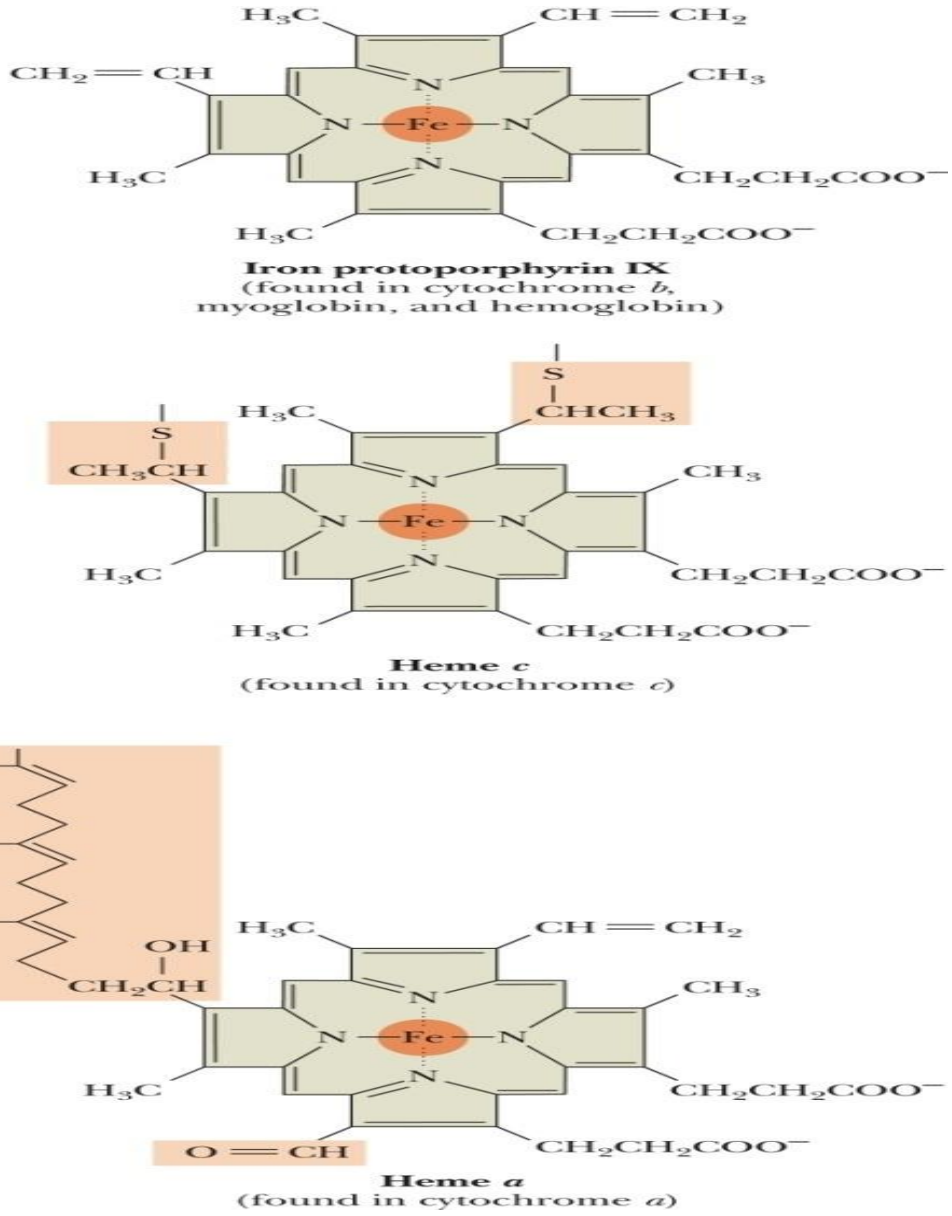


UQ-Cytochrome c Reductase

- CoQ (UQ) passes electrons to cyt *c* (and pumps H^+) in a unique redox cycle known as the Q cycle
- The principal transmembrane protein in complex III is the *b* cytochrome - with hemes b_L and b_H
- Cytochromes, like Fe in Fe-S clusters, are one-electron transfer agents
- Study Figure 20.12 - the Q cycle
- UQH_2 is a lipid-soluble electron carrier
- cyt *c* is a water-soluble electron carrier

Complex III Mediates Electron Transport from Coenzyme Q to Cytochrome *c*

Figure 20.10 The structures of iron protoporphyrin IX, heme *c*, and heme *a*.



Complex III Mediates Electron Transport from Coenzyme Q to Cytochrome *c*

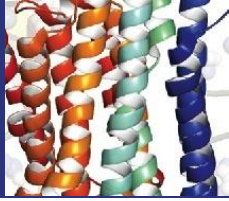
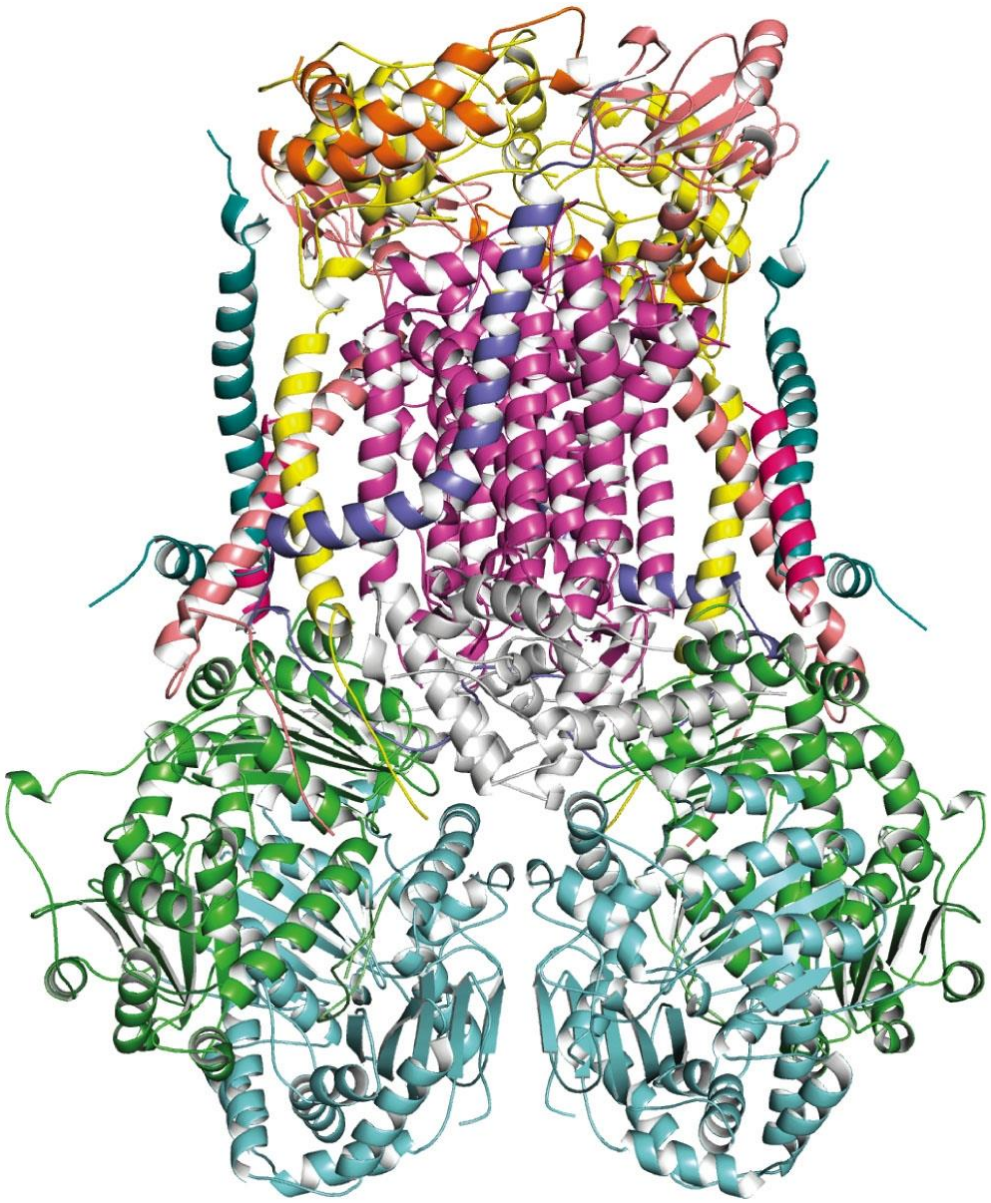
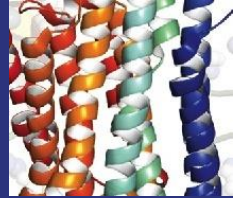


Figure 20.11 The structure of UQ-cyt *c* reductase, also known as the cytochrome bc_1 complex. The α-helical bundle near the top of the structure defines the transmembrane domain of the protein.



Complex III Mediates Electron Transport from Coenzyme Q to Cytochrome *c*



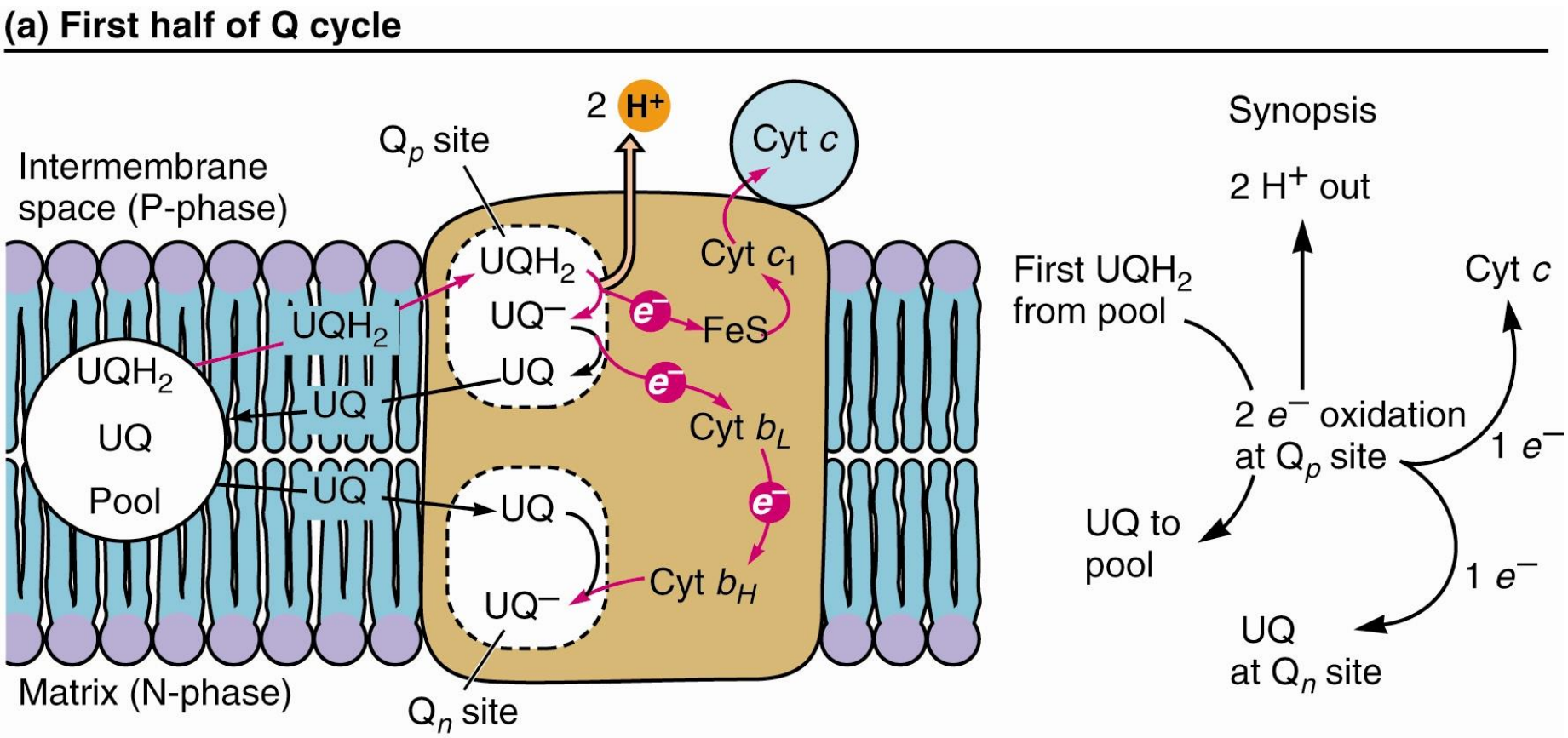
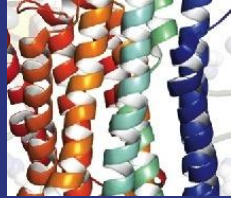


Figure 20.12 The Q cycle in mitochondria. (a) The electron-transfer pathway following oxidation of the first UQH_2 at the Q_p site near the cytosolic face of the membrane.

Complex IV Transfers Electrons from Cytochrome *c* to Reduce Oxygen on the Matrix Side



Cytochrome c Oxidase

- Electrons from cyt *c* are used in a four-electron reduction of O_2 to produce $2H_2O$
- Oxygen is thus the terminal acceptor of electrons in the electron transport pathway
- Cytochrome *c* oxidase utilizes 2 hemes (*a* and a_3) and 2 copper sites
- Complex IV also transports H^+ across the inner mitochondrial membrane
- Four H^+ participate in O_2 reduction and four H^+ are transported in each catalytic cycle

Cytochrome *c* is a mobile electron carrier

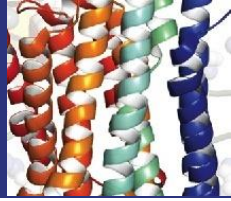
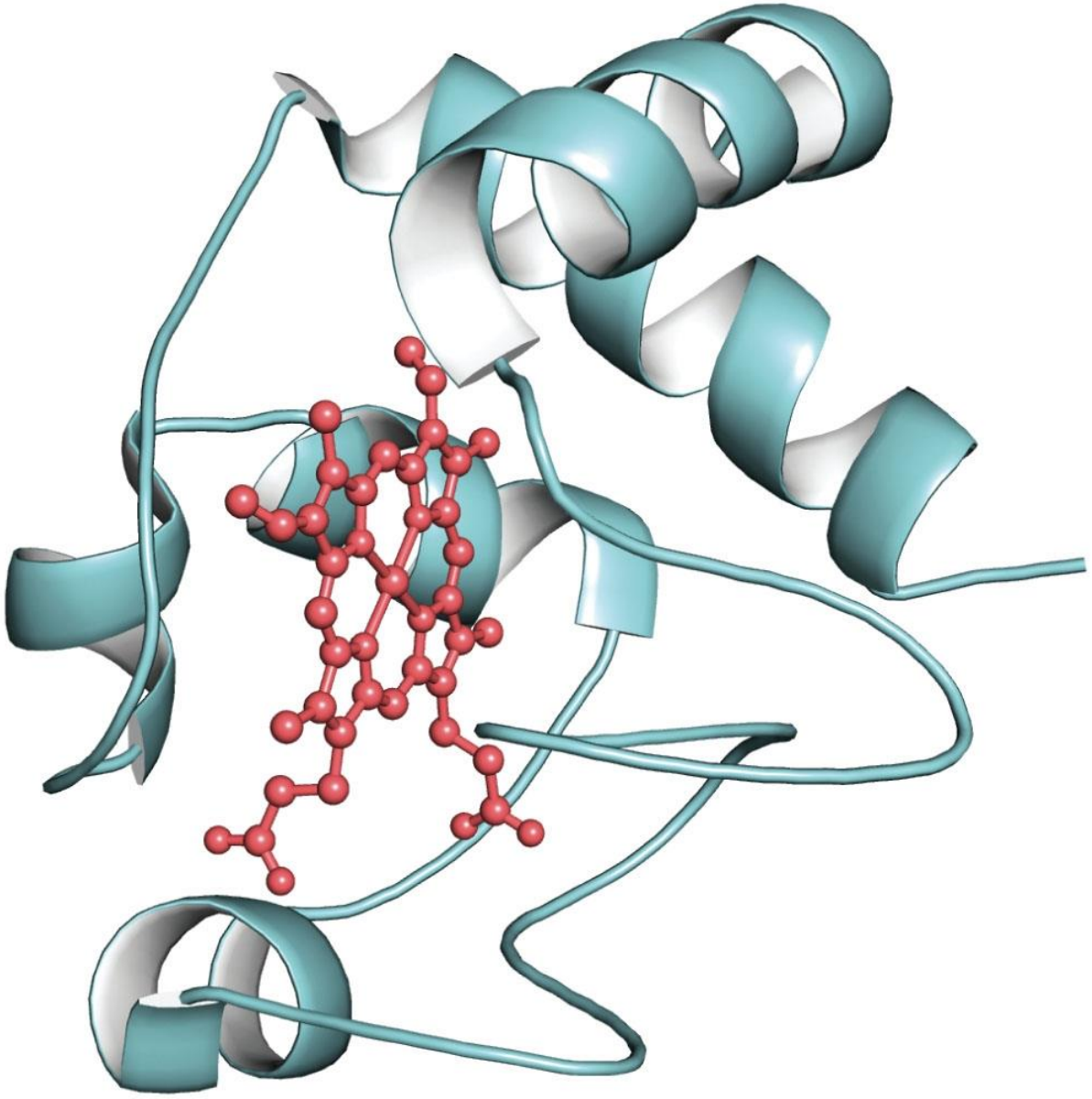


Figure 20.13 The structure of mitochondrial cytochrome *c*. The heme is shown at the center of the structure. It is covalently linked to the protein via two sulfur atoms. A third sulfur from a methionine residue coordinates the iron.



Complex IV Transfers Electrons from Cytochrome *c* to Reduce Oxygen on the Matrix Side

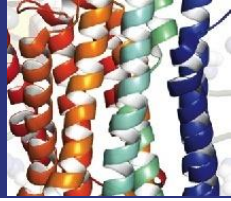
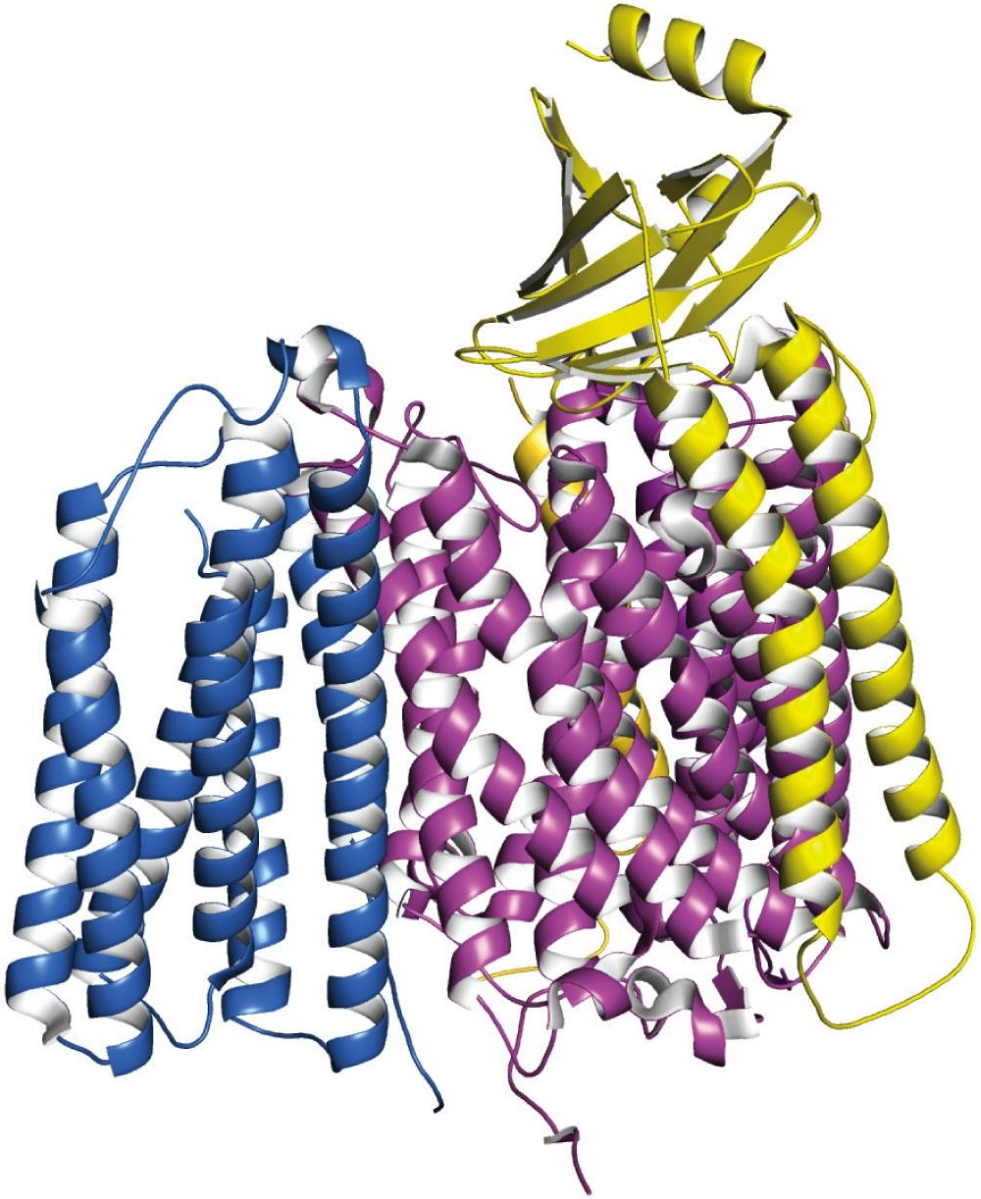


Figure 20.14 Bovine cytochrome *c* oxidase consists of 13 subunits. The 3 largest subunits – I (purple), II (yellow), and III (blue) – contain the proton channels and the redox centers.

Subunits I, II, and III are common to most organisms. This minimal complex is sufficient to carry out both oxygen reduction and proton transport.



Complex IV Transfers Electrons from Cytochrome *c* to Reduce Oxygen on the Matrix Side

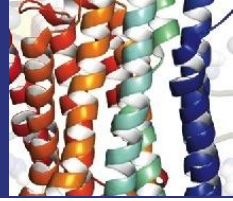
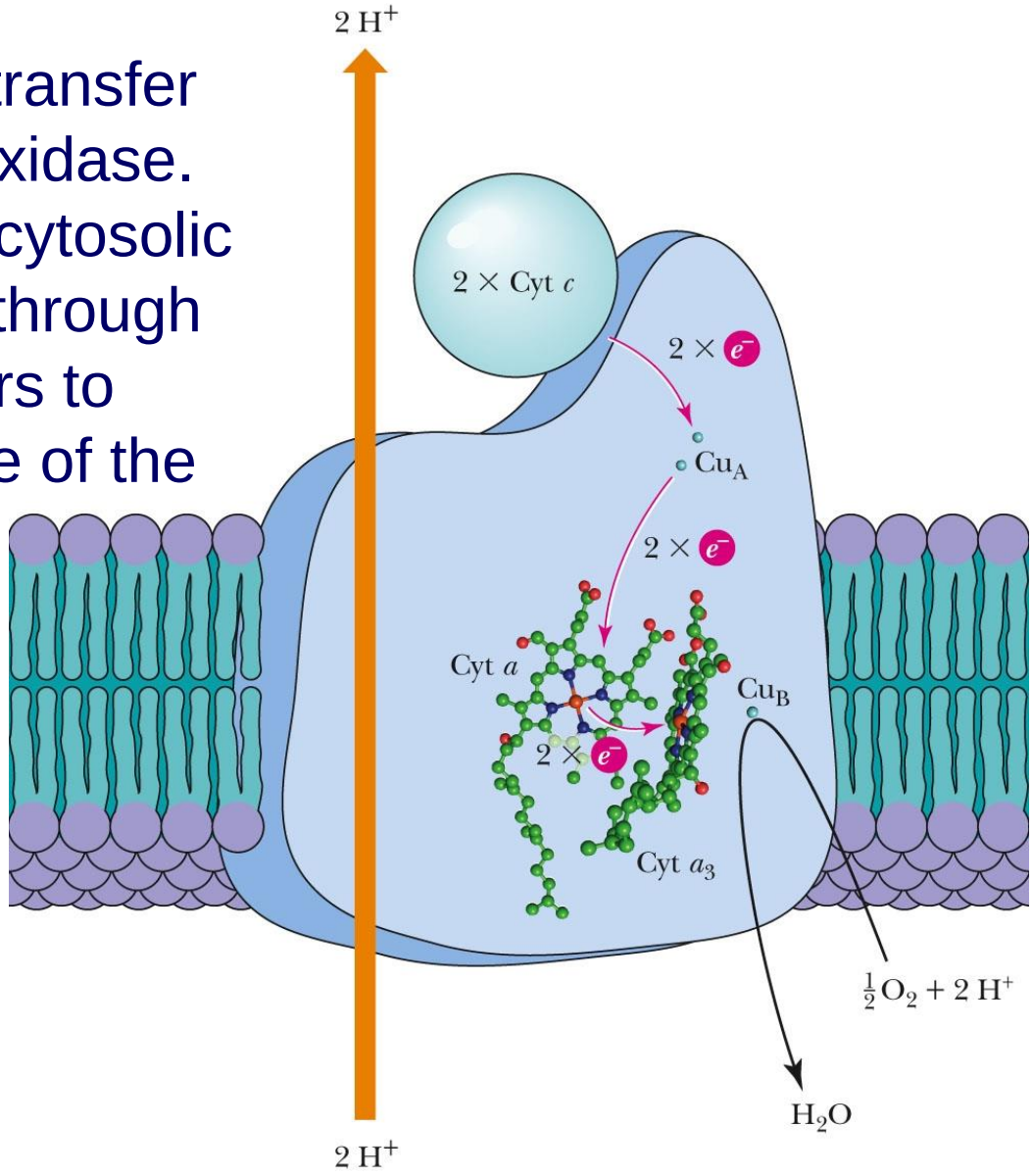
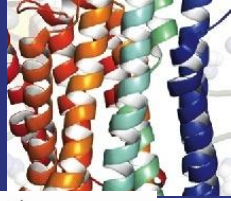


Figure 20.16 The electron-transfer pathway for cytochrome *c* oxidase. Cytochrome *c* binds on the cytosolic face, transferring electrons through the copper and heme centers to reduce O_2 on the matrix side of the membrane.



The Four Electron-Transport Complexes are Independent



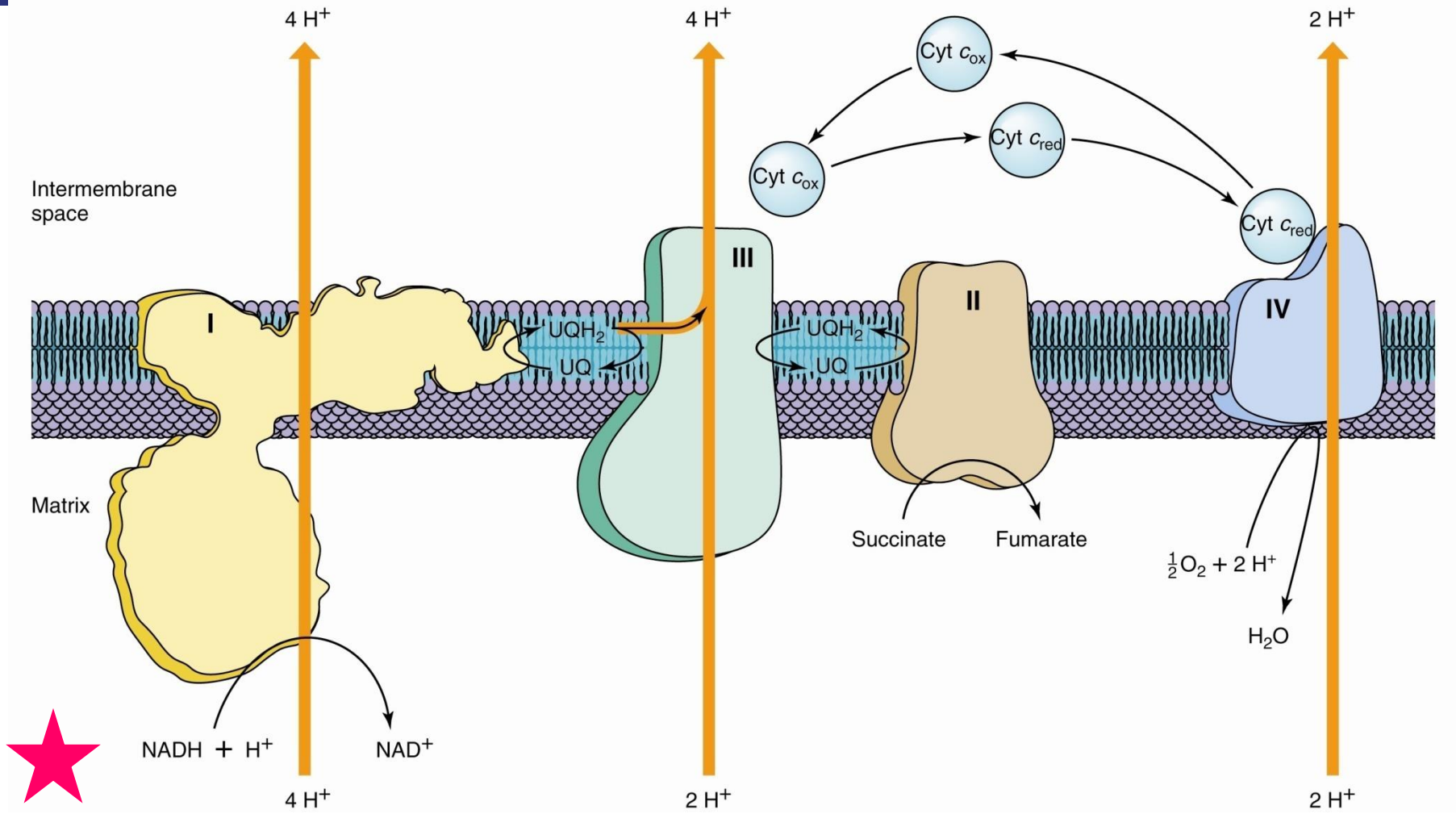
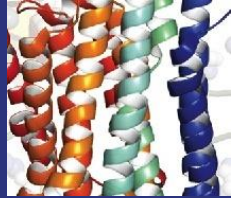


Figure 20.19 A model for the electron-transport pathway in the mitochondrial inner membrane. UQ/UQH2 and cyt c are mobile carrioers and transfer electrons between the complexes.

Electron Transfer Energy Stored in a Proton Gradient: The Mitchell Hypothesis



This coupling was a mystery for many years

- Many biochemists squandered careers searching for the elusive "high energy intermediate"
- Peter Mitchell proposed a novel idea - a proton gradient across the inner membrane could be used to drive ATP synthesis
- The proton gradient is created by the proteins of the electron-transport pathway (Figure 20.20)
- Mitchell was ridiculed, but the **chemiosmotic hypothesis** eventually won him a Nobel prize
- Be able to calculate the ΔG for a proton gradient (Equation 20.24)

Electron Transfer Energy Stored in a Proton Gradient: The Mitchell Hypothesis

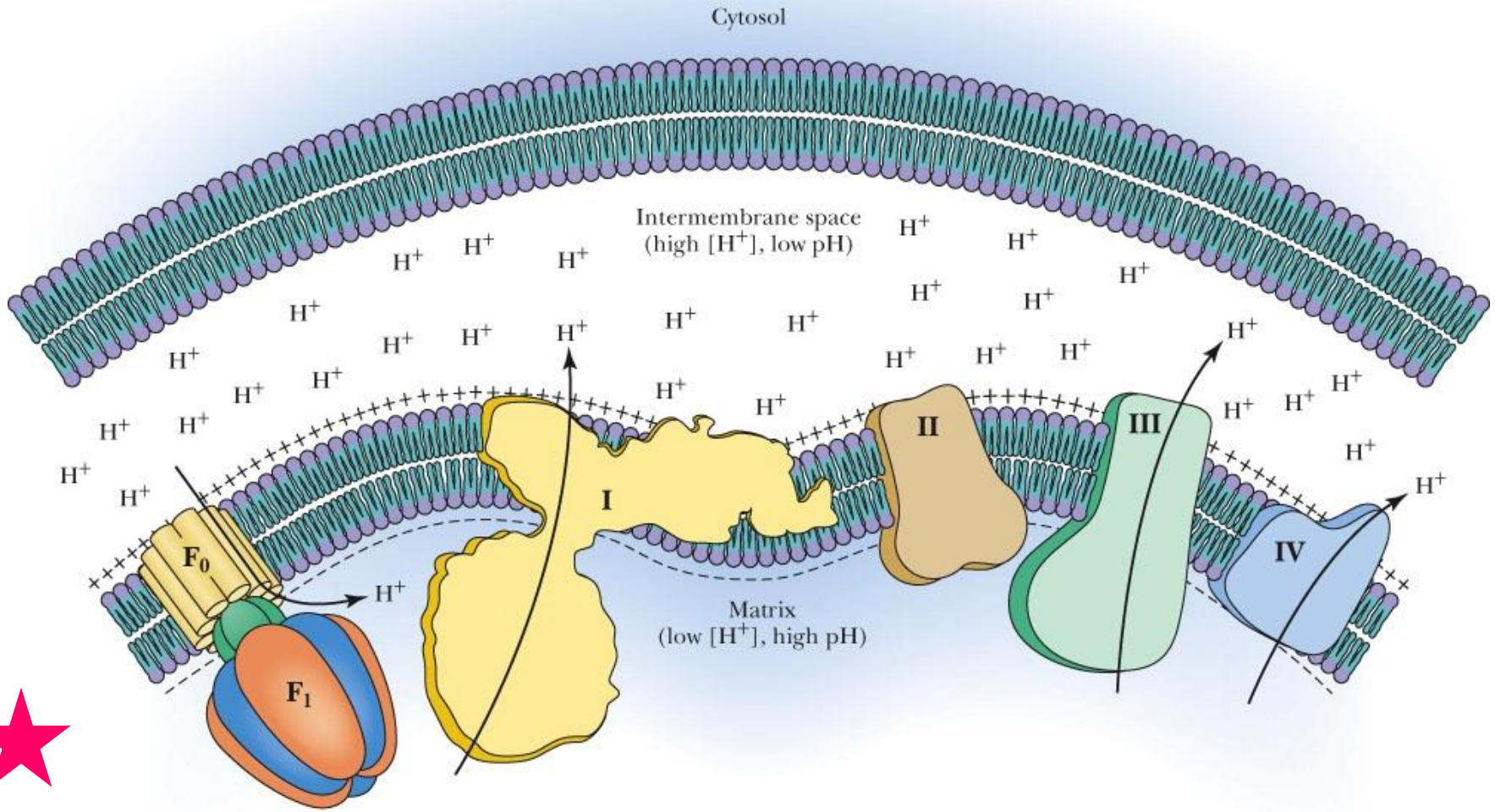
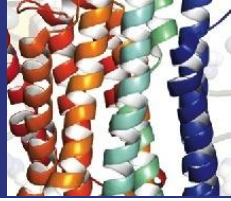


Figure 20.20 The proton and electrochemical gradients existing across the inner mitochondrial membrane.

20.5 How Does a Proton Gradient Drive the Synthesis of ATP?



*Proton diffusion through the **ATP synthase** drives ATP synthesis*

- The ATP synthase consists of two parts: F_1 and F_0 (latter was originally named "F_o" for its inhibition by oligomycin)
- See Figure 20.21 and Table 20.3 for details
- F_1 consists of five polypeptides: α, β,γ, δ, and ε
- F_0 includes three hydrophobic subunits denoted *a*, *b* and *c*
- F_0 forms the transmembrane pore or channel through which protons move to drive ATP synthesis
- The *a* and *b* subunits comprise part of the **stator** and a ring of *c* subunits forms a **rotor**

ATP Synthase is Composed of F_1 and F_0

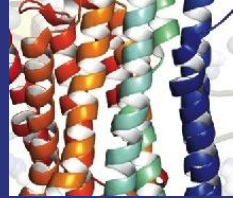
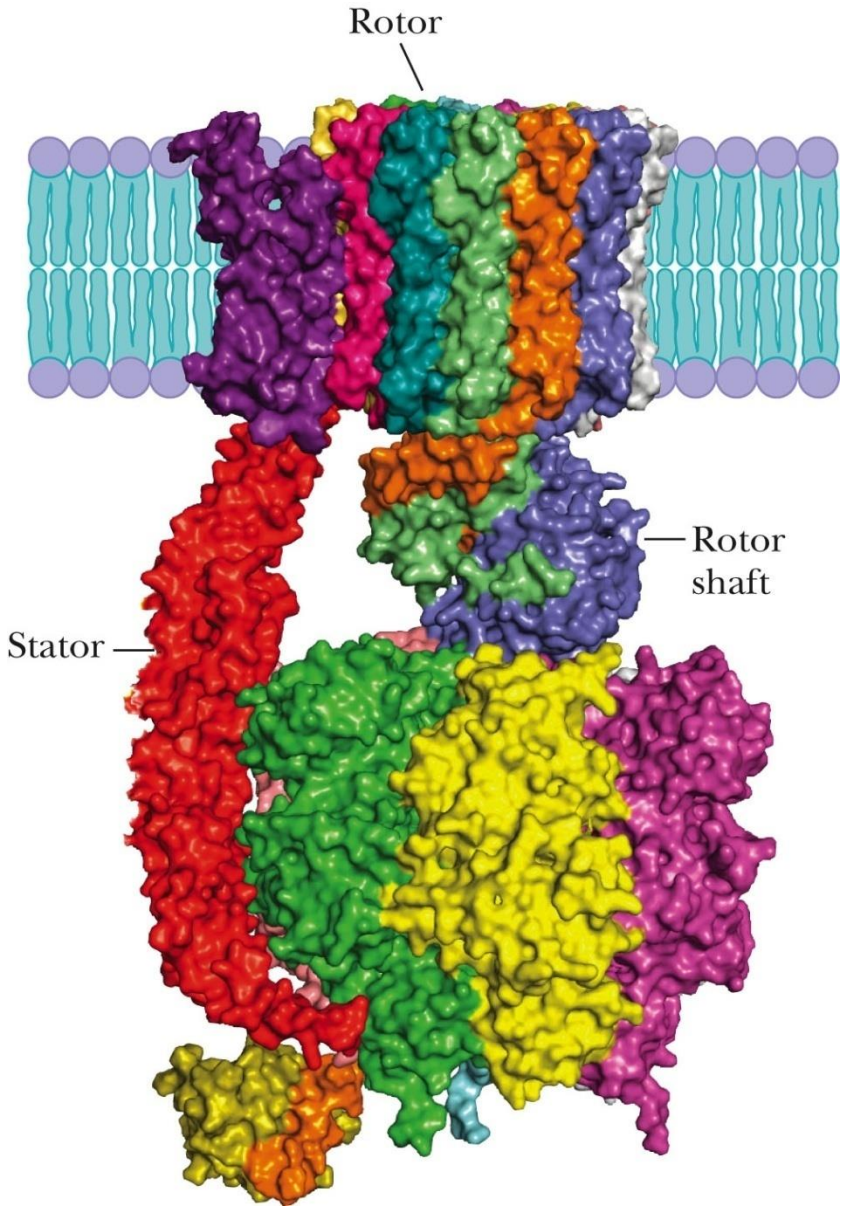


Figure 20.21 The ATP synthase, a rotating molecular motor. The *c*, γ and ε subunits constitute the rotating portion (the rotor) of the motor. The b, d and h subunits form a long, slender stalk that connects F_0 in the membrane and F_1. Flow of protons from the *a*-subunit through the *c*-subunits turns the rotor and drives the cycle of conformation changes in α and β that synthesize ATP.



ATP Synthase is Composed of F_1 and F_0

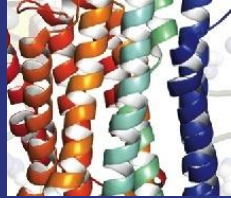
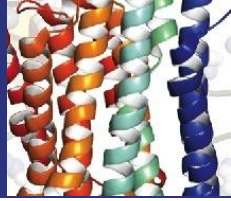


TABLE 20.3 Yeast F_1F_0–ATP Synthase Subunit Organization

Complex	Protein Subunit Function	Mass (kD)	Stoichiometry	
F_1	α	55.4	3	Stator
	β	51.3	3	Stator
	γ	30.6	1	Rotor
	δ	14.6	1	Rotor†
	ϵ	6.6	1	Rotor
F_0	a	27.9	1	Stator
	b	23.3	1	Stator
	c	7.8	10–15*	Rotor
	d	19.7	1	Stator
	h	10.4	1	Stator
	OSCP	20.9	1	Stator

oligomycin-sensitivity conferring protein (OCSP)

The Catalytic Sites of ATP Synthase Adopt Three Different Conformations



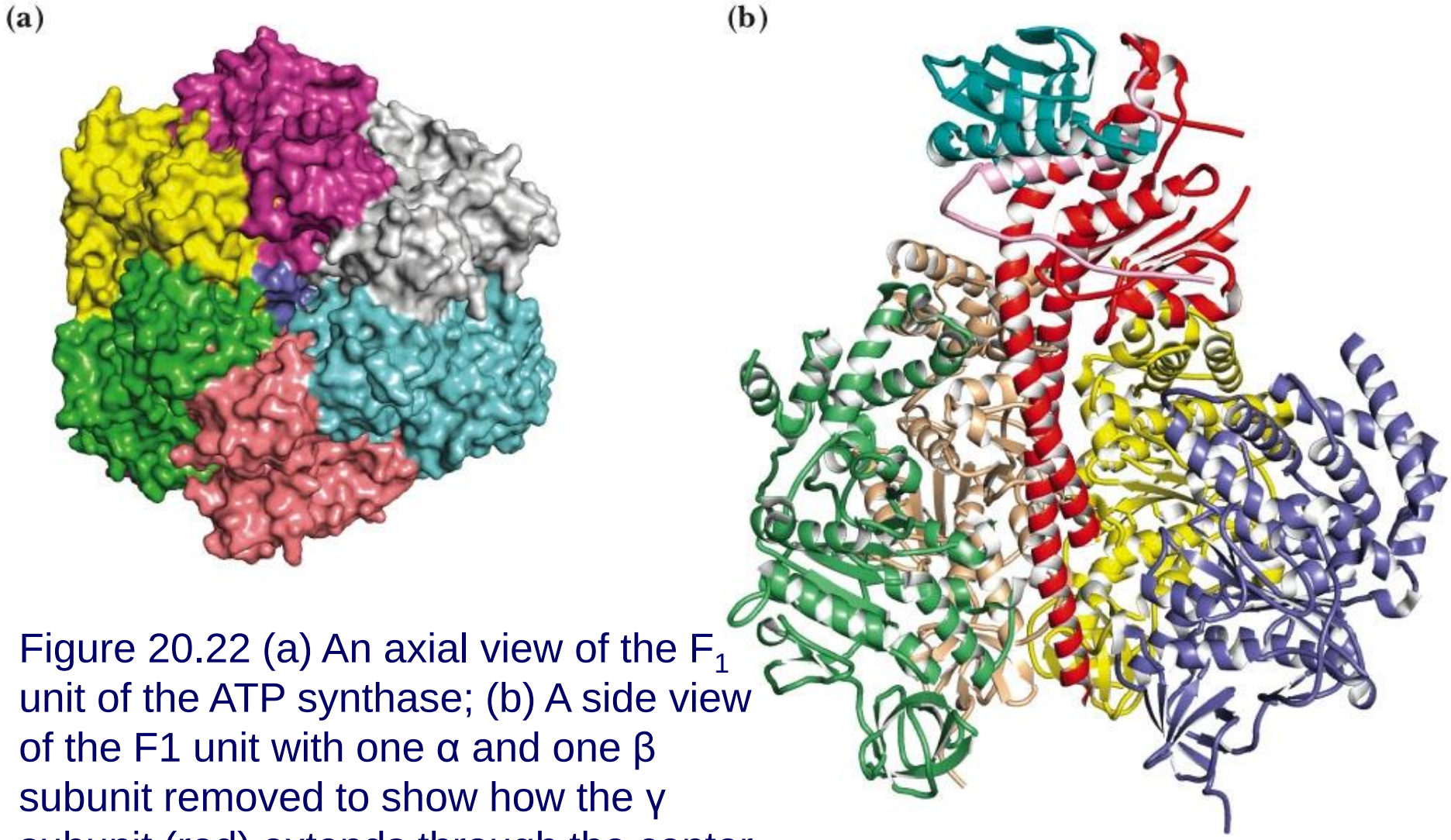
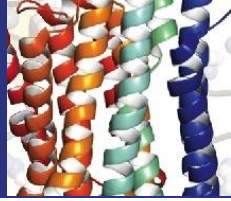


Figure 20.22 (a) An axial view of the F_1 unit of the ATP synthase; (b) A side view of the F1 unit with one α and one β subunit removed to show how the γ subunit (red) extends through the center of the hexamer.

Boyer's Binding Change Mechanism Describes Events of Rotational Catalysis



- Paul Boyer proposed that, at any instant:
  - the three β subunits of F1 exist in three different conformations
  - these different states represent the three steps of ATP synthesis
  - each site steps through the three conformations or states to make ATP
- In Boyer's **binding change mechanism**, the three catalytic sites thus cycle through the three intermediate states of ATP synthesis

The Binding Change Mechanism

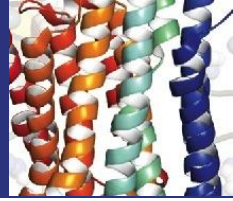
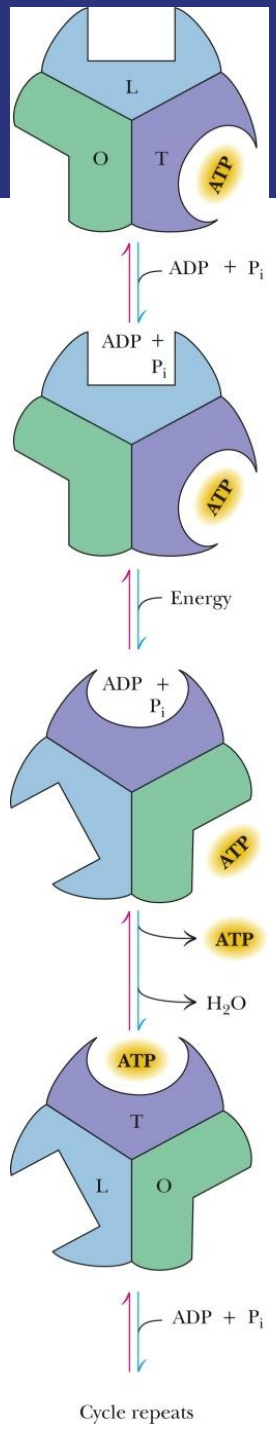


Figure 20.24 The binding change mechanism for ATP synthesis by ATP synthase. This model assumes that F1 has three interacting and conformationally distinct active sites: an open (O) conformation with almost no affinity for ligands, a loose (L) conformation with low affinity for ligands, and a tight (T) conformation with high affinity for ligands.



Proton Flow Through F_0 Drives Rotation of the Motor and Synthesis of ATP

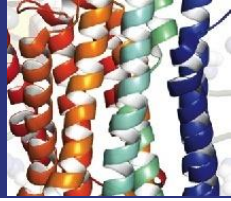
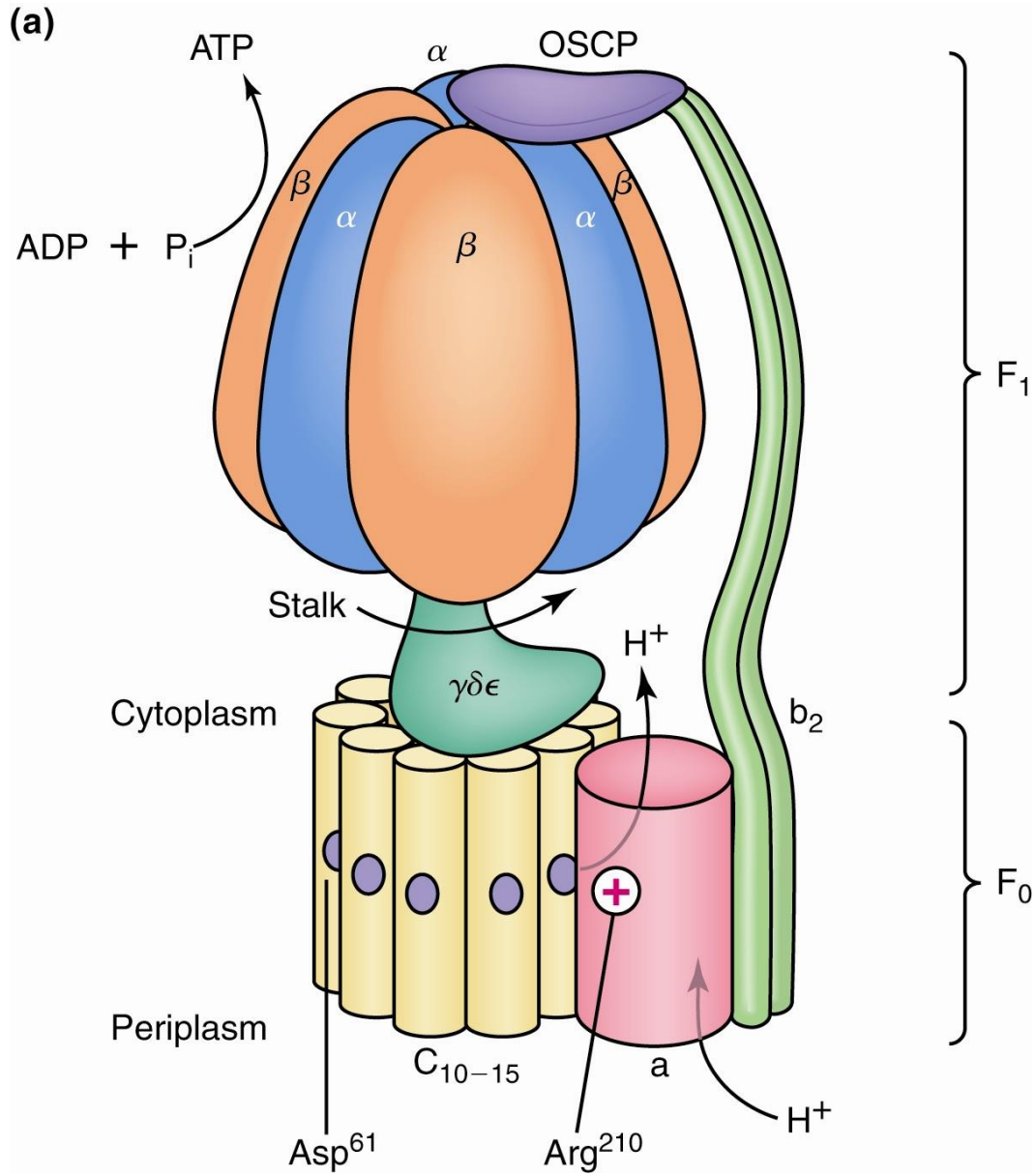


Figure 20.25 (a) Protons entering the inlet half-channel in the α-subunit are transferred to binding sites on *c*-subunits. Rotation of the *c*-ring delivers protons to the outlet half-channel in the α-subunit. Flow of protons through the structure turns the rotor and drives the cycle of conformational changes in β that synthesize ATP.



Proton Flow Through F_0 Drives Rotation of the Motor and Synthesis of ATP

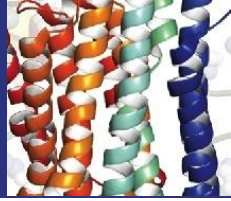
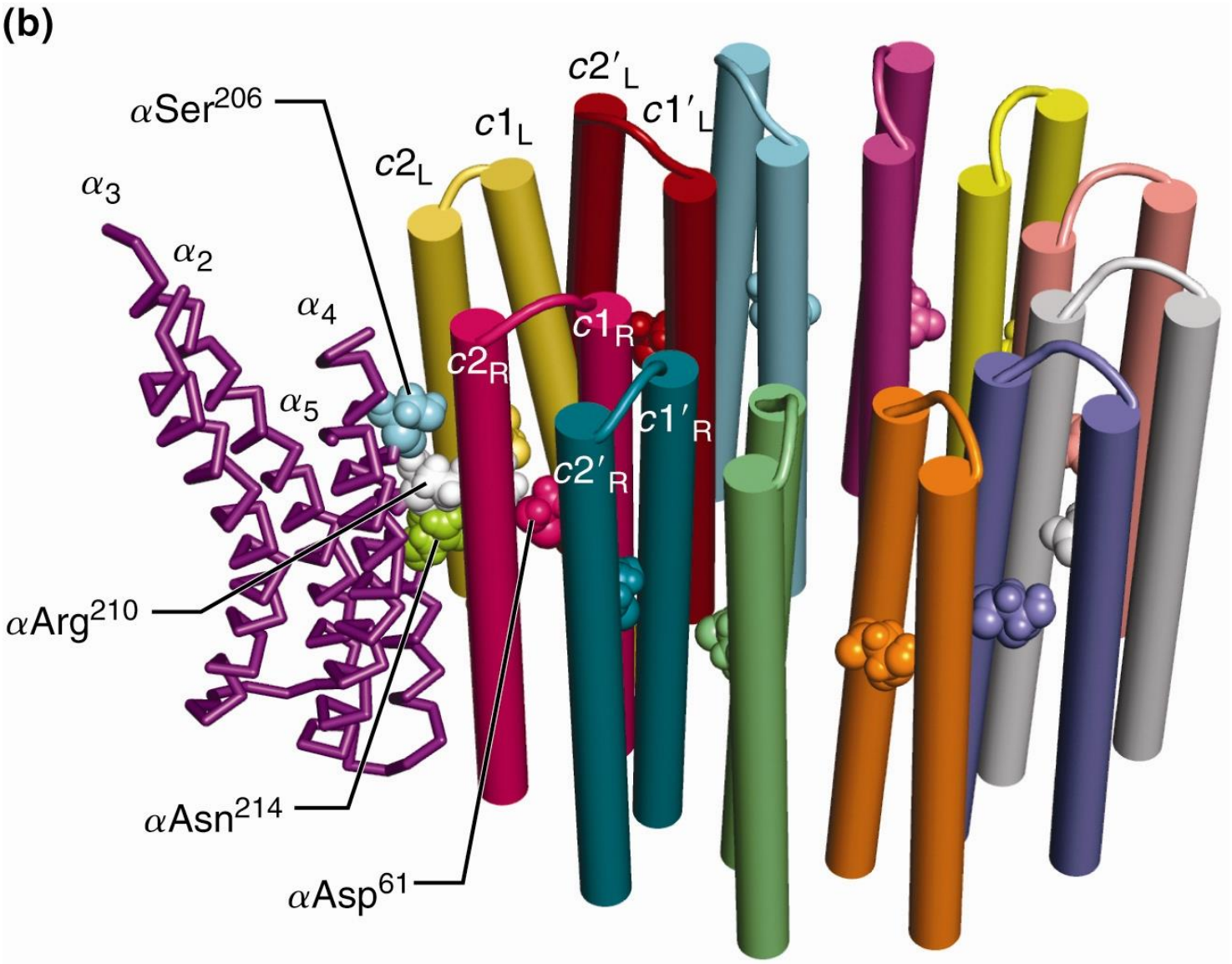


Figure 20.25 (b) Arg^{210} on the a-subunit lies between the end of the inlet half-channel (Asn^{214}) and the end of the of the outlet half-channel (Ser^{206}).



Proton Flow Through F_0 Drives Rotation of the Motor and Synthesis of ATP

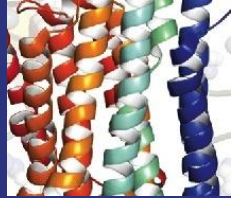
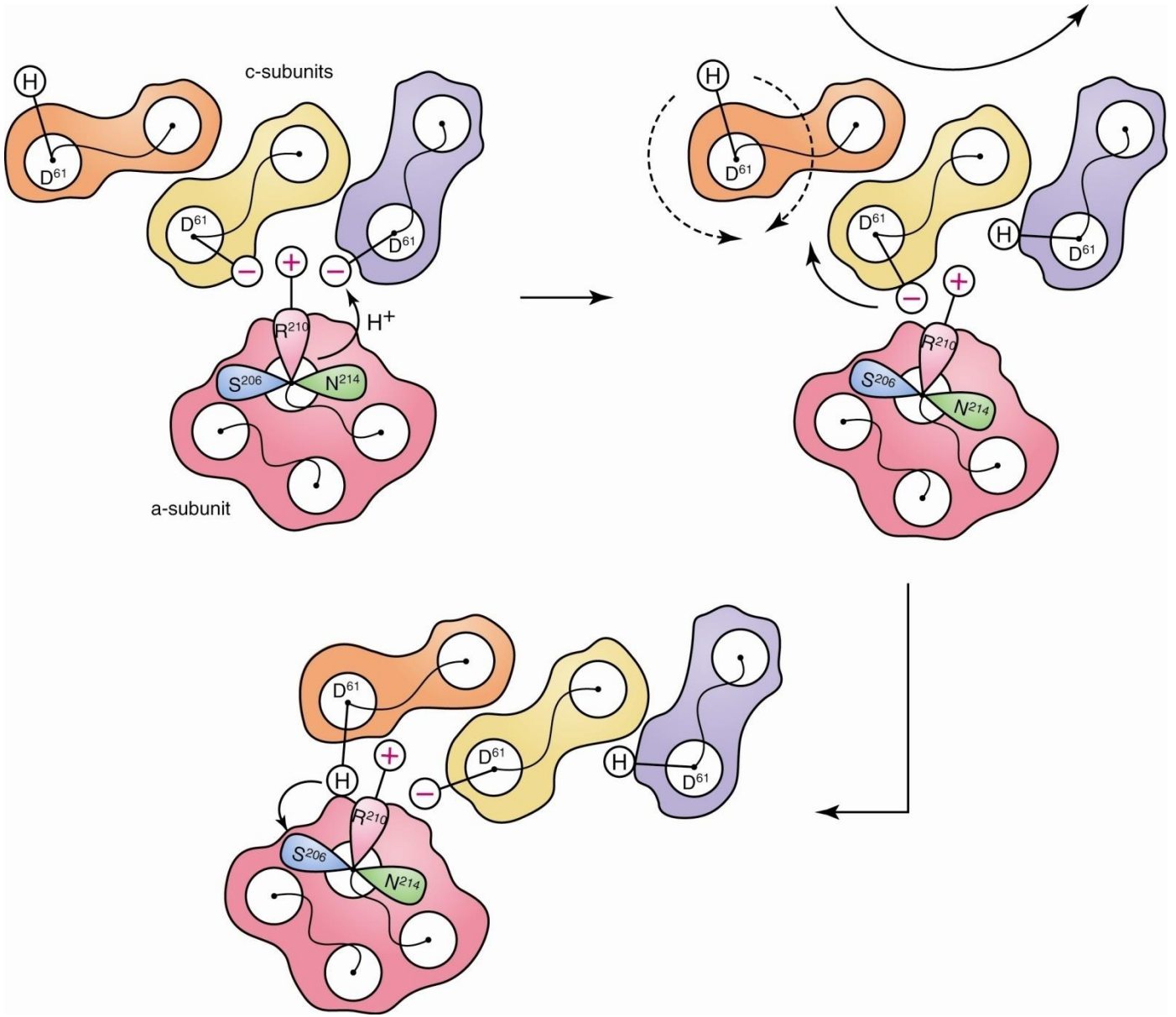


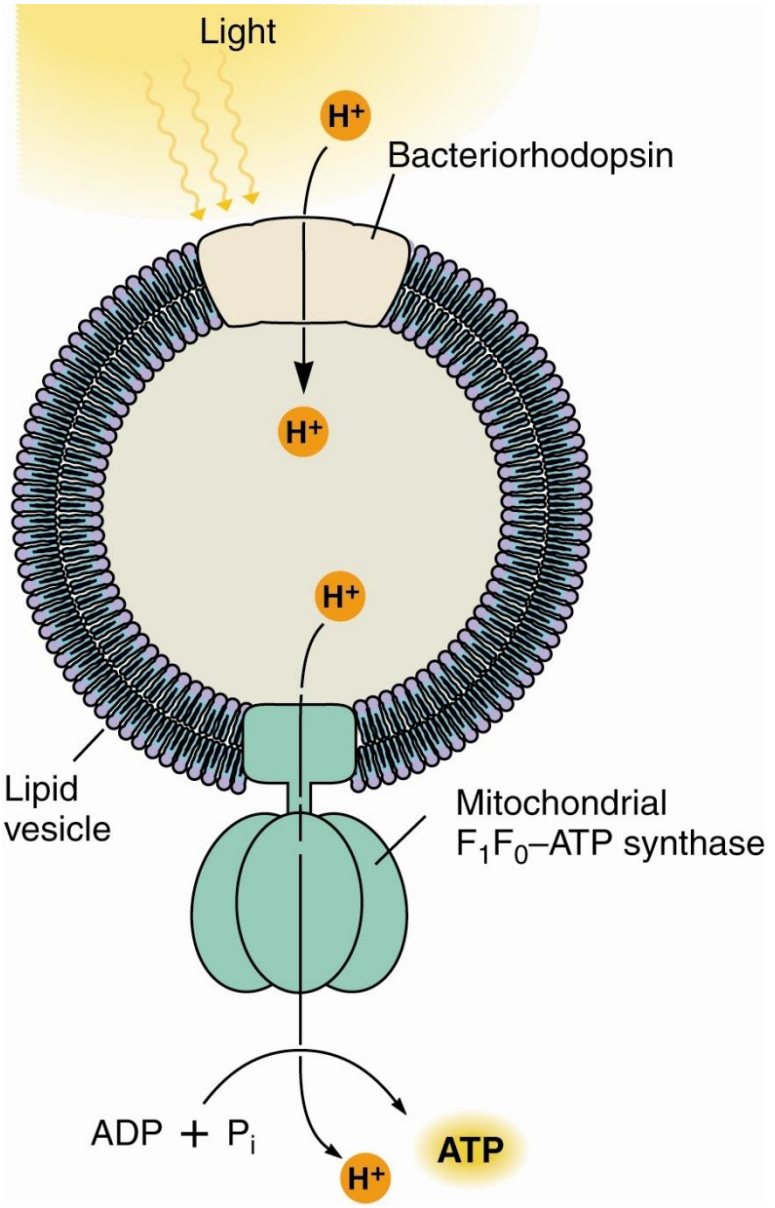
Figure 20.25 (c) A view looking down into the plane of the membrane. Transported protons flow from the inlet half-channel to Asp^{61} residues on the c-ring, around the ring, and then into the outlet half-channel.



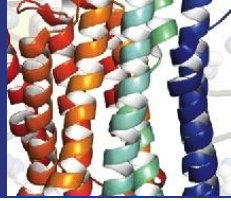
Racker and Stoeckenius Confirmed the Mitchell Model in a Reconstitution Expt

Figure 20.26 The reconstituted vesicles containing ATP synthase and bacteriorhodopsin used by Stoeckenius and Racker to confirm the Mitchell chemiosmotic hypothesis-1961.

Upon illumination, bacteriorhodopsin pumped protons into these vesicles, and the resulting proton gradient was sufficient to drive ATP synthesis by the ATP synthase.

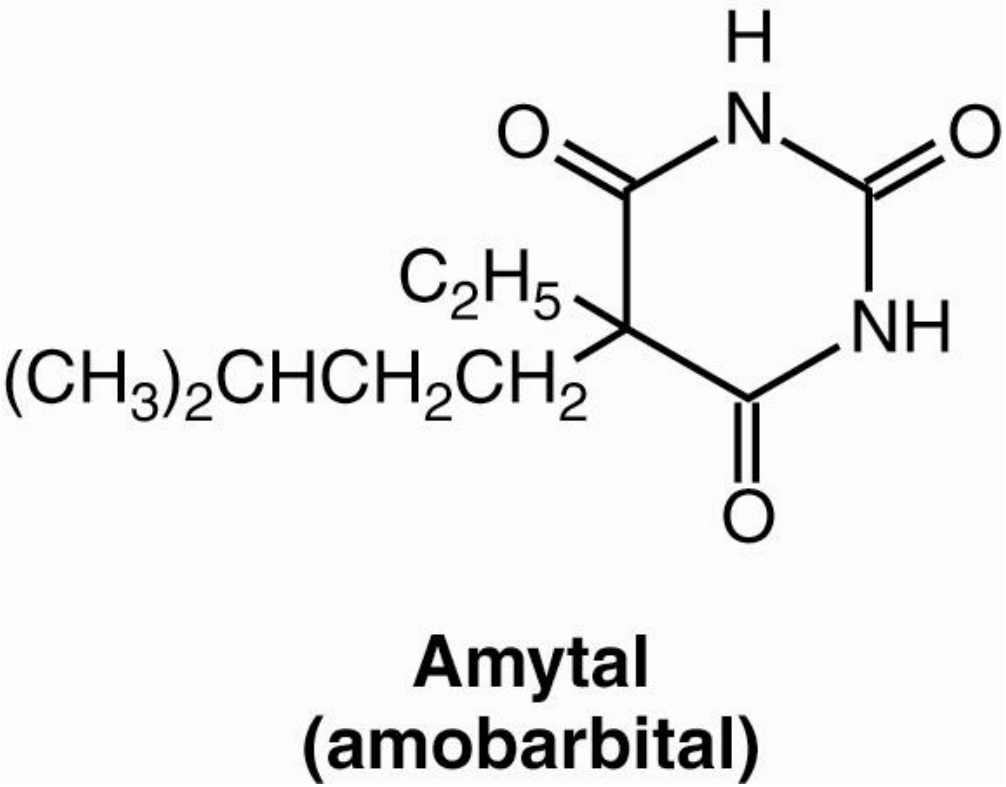


Inhibitors of Oxidative Phosphorylation Reveal Insights About the Mechanism



- Many details of electron transport and oxidative phosphorylation have been learned from studying the effects of inhibitors
- **Rotenone** inhibits Complex I - and helps natives of the Amazon rain forest catch fish
- (Natives have learned to beat the roots of certain trees along river banks, releasing rotenone, which paralyzes the fish, making them easy prey)
- **Cyanide**, **azide** and **CO** inhibit Complex IV, binding tightly to the ferric form (Fe^{3+}) of a_3
- **Oligomycin** is an ATP synthase inhibitor

Amytal, a Barbiturate, inhibits Complex I



Amytal (amobarbital)

sedative-hypnotic and analgesic

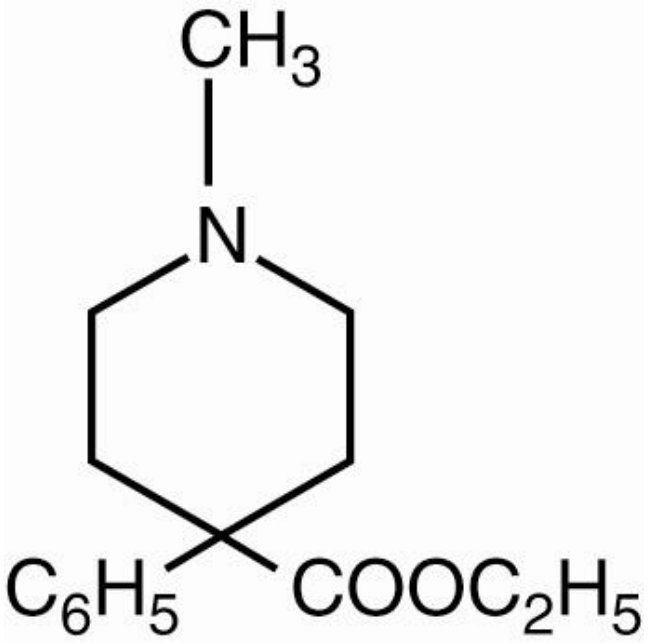
LD50 in mice of 212 mg/kg s.c.

Figure 20.27 Amytal is an inhibitor of Complex I of the electron transport system.

Inhibitors of Oxidative Phosphorylation Reveal Insights About the Mechanism

Opioid

Figure 20.27 Demerol inhibits Complex I of the electron transport system.



Demerol (meperdine)

Inhibitors of Oxidative Phosphorylation Reveal Insights About the Mechanism

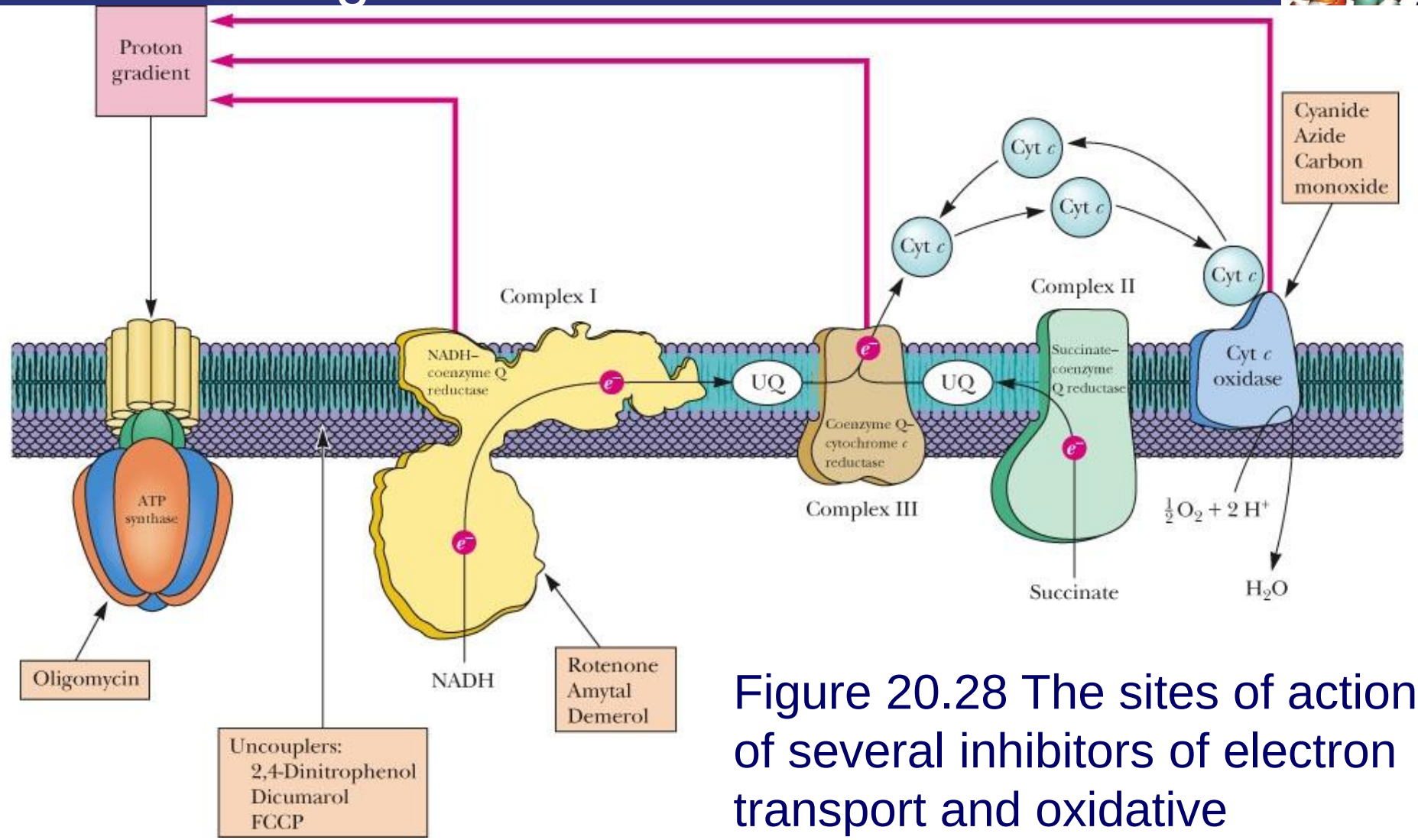
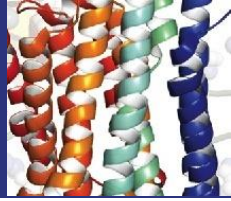


Figure 20.28 The sites of action of several inhibitors of electron transport and oxidative phosphorylation.

Uncouplers Disrupt the Coupling of Electron Transport and ATP Synthase



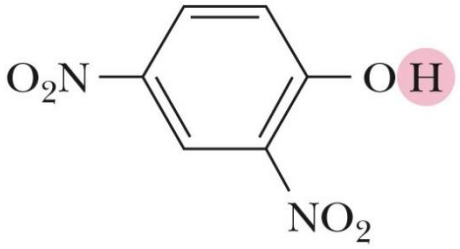
Uncoupling e^- transport and oxidative phosphorylation

- Uncouplers disrupt the tight coupling between electron transport and oxidative phosphorylation by dissipating the proton gradient
- Uncouplers are hydrophobic molecules with a dissociable proton
- They shuttle back and forth across the membrane, carrying protons to dissipate the gradient

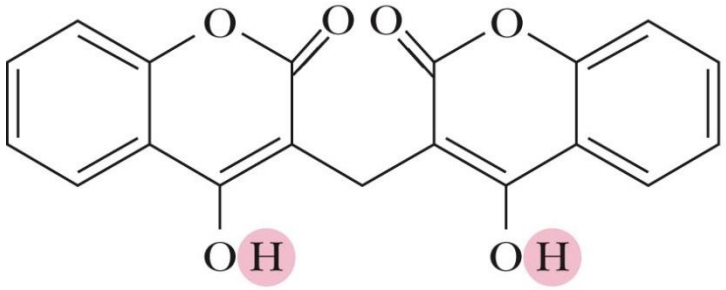
Uncouplers Disrupt the Coupling of Electron Transport and ATP Synthase

Figure 20.29 Structures of several uncouplers, molecules that dissipate the proton gradient across the inner mitochondrial membrane and thereby destroy the tight coupling between electron transport and the ATP synthase reaction.

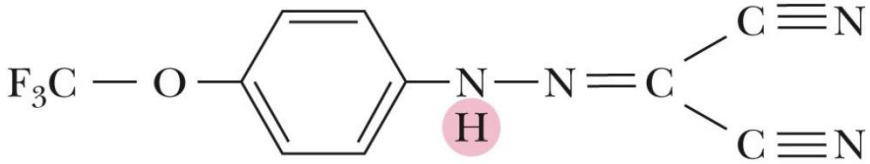
Dinitrophenol



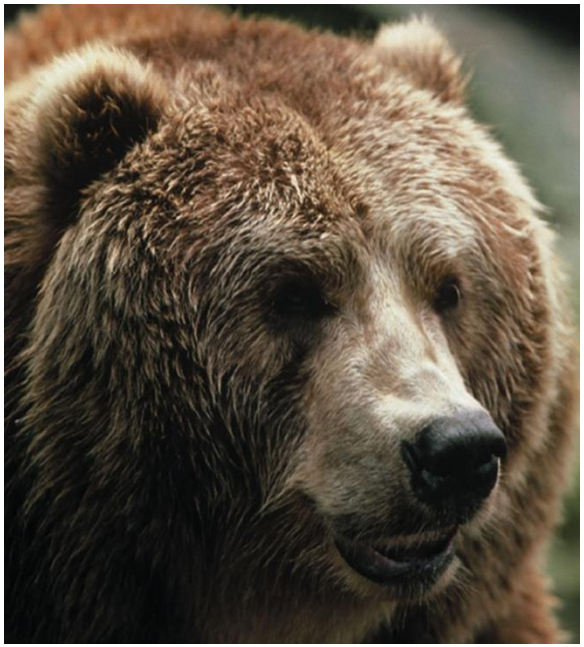
Dicumarol



Carbonyl cyanide-*p*-trifluoro-methoxyphenyl hydrazone
—best known as **FCCP**; for **F**luoro **C**arbonyl **C**yanide **P**henylhydrazone



Hibernating Animals Generate Heat by Uncoupling Oxidative Phosphorylation

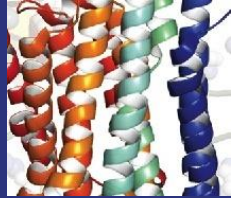


Grizzly Bear

Skunk Cabbage

Chipmunk

ATP-ADP Translocase Mediates the Movement of ATP & ADP Across the Mitochondrial Membrane



ATP must be transported out of the mitochondria

- ATP out, ADP in - through a "translocase"
- ATP movement out is favored because the cytosol is "+" relative to the "-" matrix
- But ATP out and ADP in is net movement of a negative charge out - equivalent to a H^+ going in
- So every ATP transported out costs one H^+
- One ATP synthesis costs about 3 H^+
- Thus, making and exporting 1 ATP = $4H^+$

ATP-ADP Translocase Mediates the Movement of ATP & ADP Across the Mitochondrial Membrane

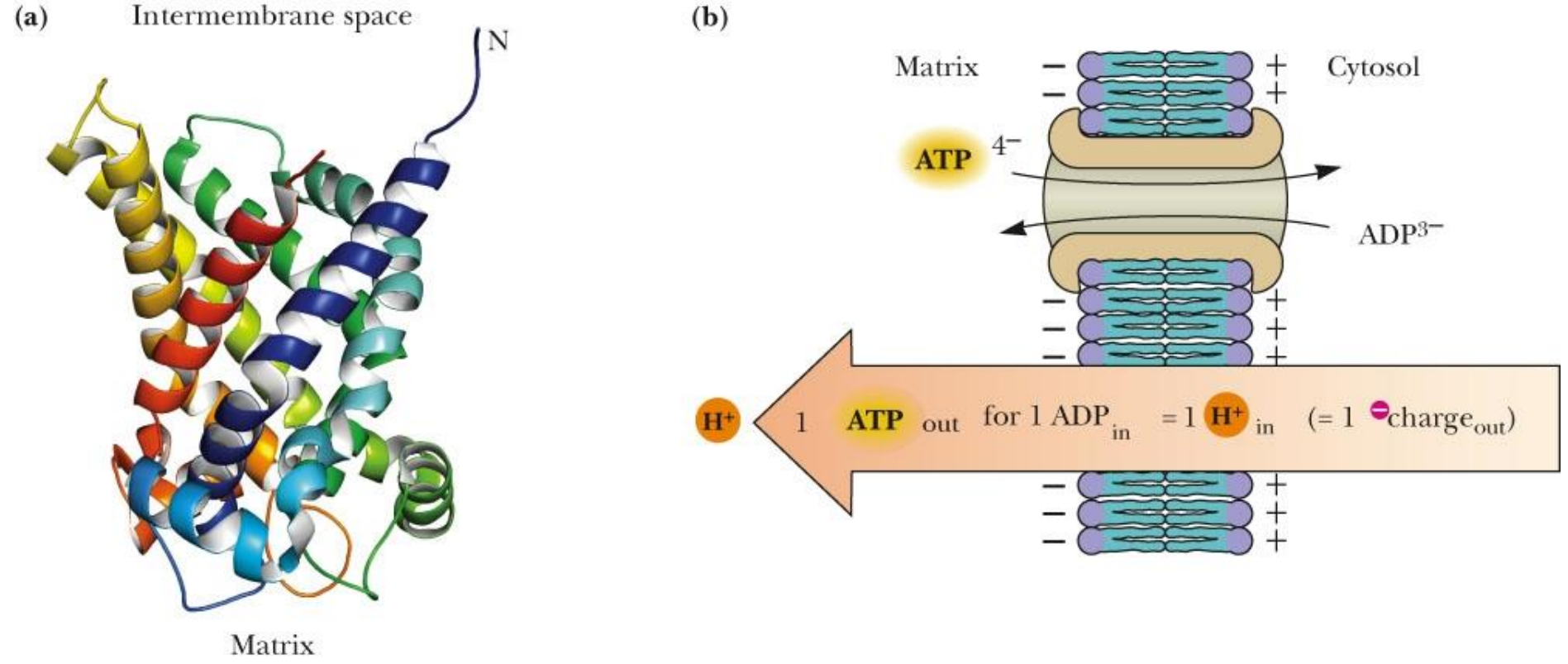
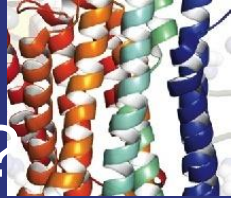


Figure 20.30 (a) The bovine ATP-ADP translocase. (b) Outward transport of ATP (via the ATP-ADP translocase is favored by the membrane electrochemical potential.

20.6 - What Is the P/O Ratio for Mitochondrial Electron Transport and Oxidative Phosphorylation?

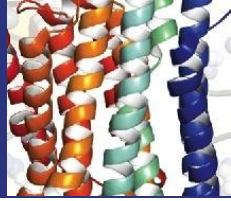


How many ATP can be made per electron pair sent through the chain?

- The e^- transport chain yields 10 H^+ pumped out per electron pair from NADH to oxygen
- 4 H^+ flow back into matrix per ATP to cytosol
- 10/4 = 2.5 for electrons entering as NADH

- For electrons entering as succinate ($FADH_2$), about 6 H^+ pumped per electron pair to oxygen
- 6/4 = 1.5 for electrons entering as succinate

20.7 – How Are the Electrons of Cytosolic NADH Fed into Electron Transport?



Most NADH used in electron transport is cytosolic and NADH doesn't cross the inner mitochondrial membrane

- What to do?
- "Shuttle systems" effect electron movement without actually carrying NADH
- Glycerophosphate shuttle stores electrons in glycerol-3-P, which transfers electrons to FAD
- Malate-aspartate shuttle uses malate to carry electrons across the membrane

The Glycerophosphate Shuttle Ensures Efficient Use of Cytosolic NADH

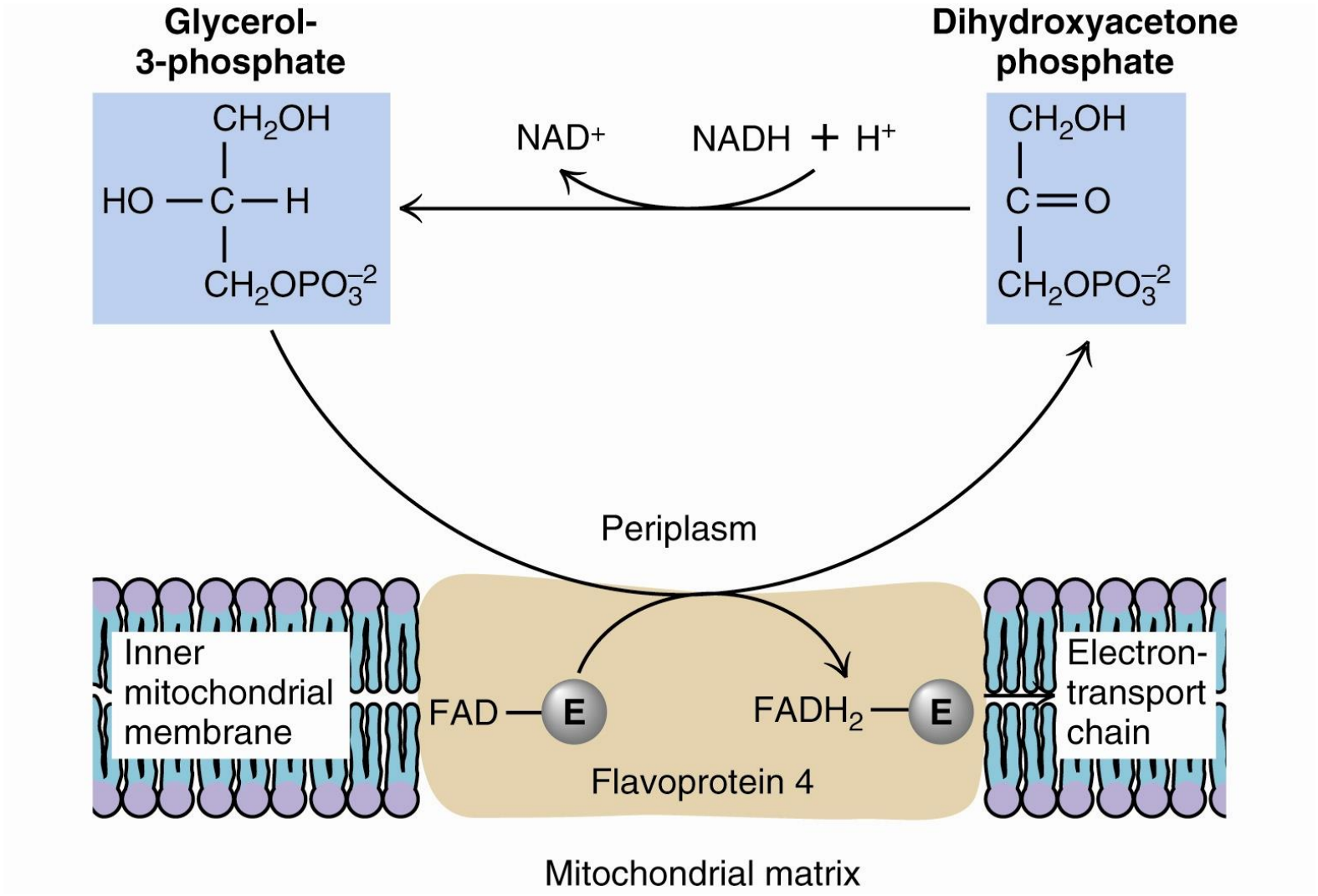
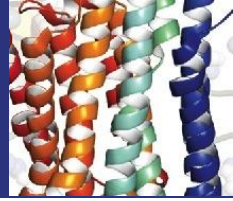


Figure 20.31 The glycerophosphate shuttle couples cytosolic oxidation of NADH with mitochondrial reduction of [FAD].

The Malate-Aspartate Shuttle is Reversible



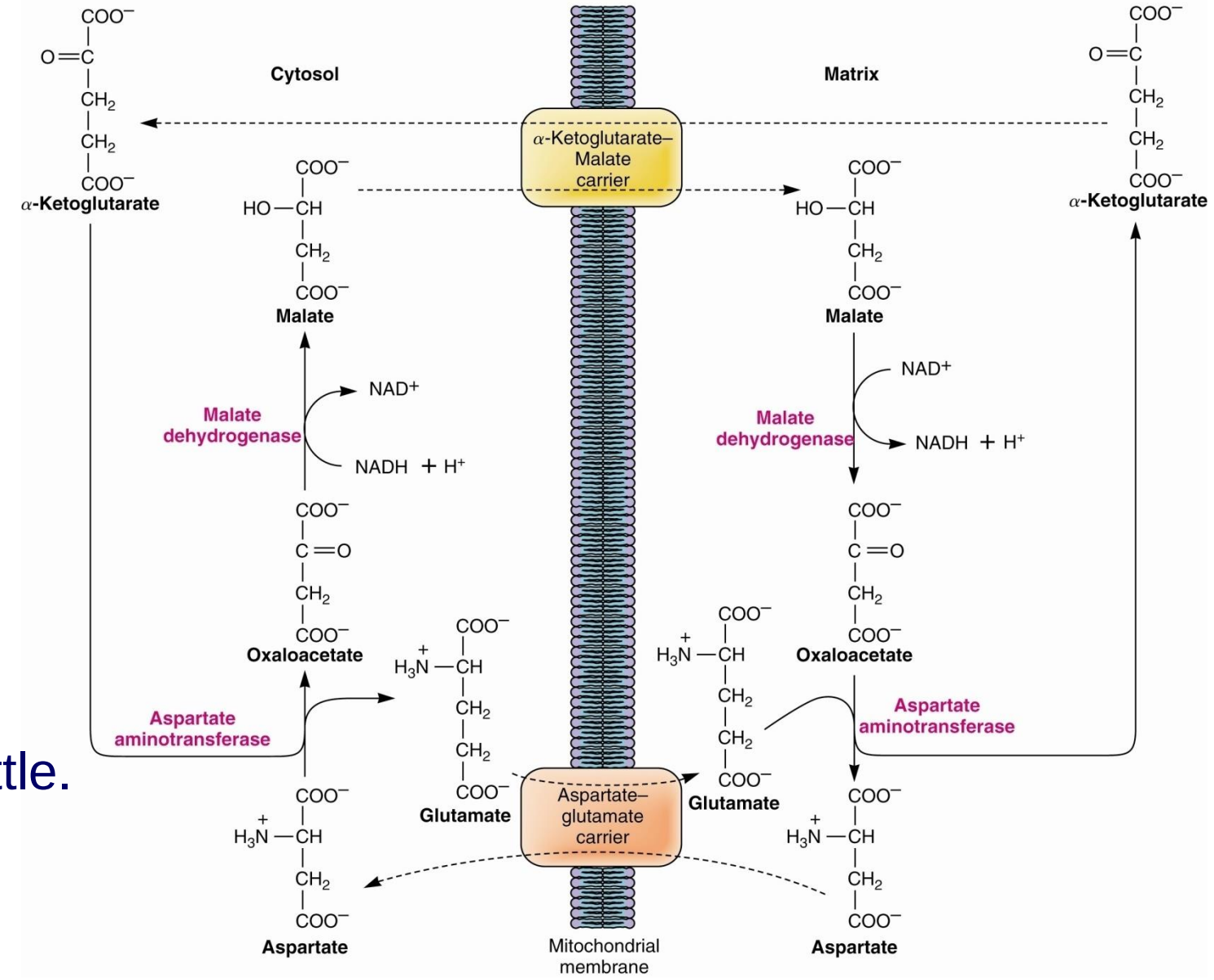
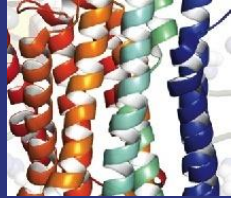


Figure 20.32
The malate-aspartate shuttle.

The Net Yield of ATP from Glucose Oxidation Depends on the Shuttle Used



- See Table 20.4
- 30 ATP per glucose if glycerol-3-P shuttle used
- 32 ATP per glucose if malate-Asp shuttle used
- In bacteria - no mitochondria - no extra H^+ used to export ATP to cytosol, so:
  - 10/3 = ~3ATP/NADH
  - 6/3 = ~ 2ATP/$FADH_2$

The Net Yield of ATP from Glucose Oxidation Depends on the Shuttle Used

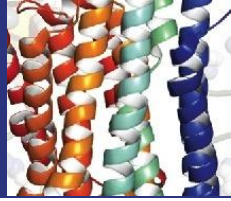
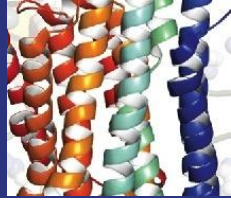


TABLE 20.4 Yield of ATP from Glucose Oxidation

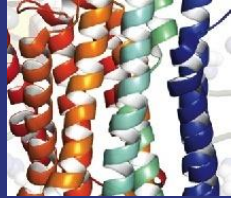
Pathway	ATP Yield per Glucose: Glycerol–Phosphate Shuttle	ATP Yield per Glucose: Malate–Aspartate Shuttle
Glycolysis: glucose to pyruvate (cytosol)		
Phosphorylation of glucose	−1	−1
Phosphorylation of fructose-6-phosphate	−1	−1
Dephosphorylation of 2 molecules of 1,3-BPG	+2	+2
Dephosphorylation of 2 molecules of PEP	+2	+2
Oxidation of 2 molecules of glyceraldehyde-3-phosphate yields 2 NADH		
Pyruvate conversion to acetyl-CoA (mitochondria)		
2 NADH		
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		
Oxidation of 2 molecules of succinate yields 2 [$FADH_2$]		
Oxidative phosphorylation (mitochondria)		
2 NADH from glycolysis yield 1.5 ATPs each if NADH is oxidized by glycerol–phosphate shuttle; 2.5 ATP by malate–aspartate shuttle	+3	+5
Oxidative decarboxylation of 2 pyruvate to 2 acetyl-CoA: 2 NADH produce 2.5 ATPs each	+5	+5
2 [$FADH_2$] from each citric acid cycle produce 1.5 ATPs each	+3	+3
6 NADH from citric acid cycle produce 2.5 ATPs each	+15	+15
Net Yield	30	32

20.8 How Do Mitochondria Mediate Apoptosis?



- Mitochondria play a significant role in **apoptosis**, the programmed death of cells
- Mitochondria do this in part, by partitioning some of the apoptotic activator molecules, e.g., cytochrome *c*
- Oxidation of bound cardiolipins releases cytochrome *c* from the inner membrane
- Opening of pores in the outer membrane releases cytochrome c from the mitochondria
- Binding of cytochrome c to Apaf-1 in the cytosol leads to assembly of apoptosomes, thus triggering the events of apoptosis

20.8 How Do Mitochondria Mediate Apoptosis?



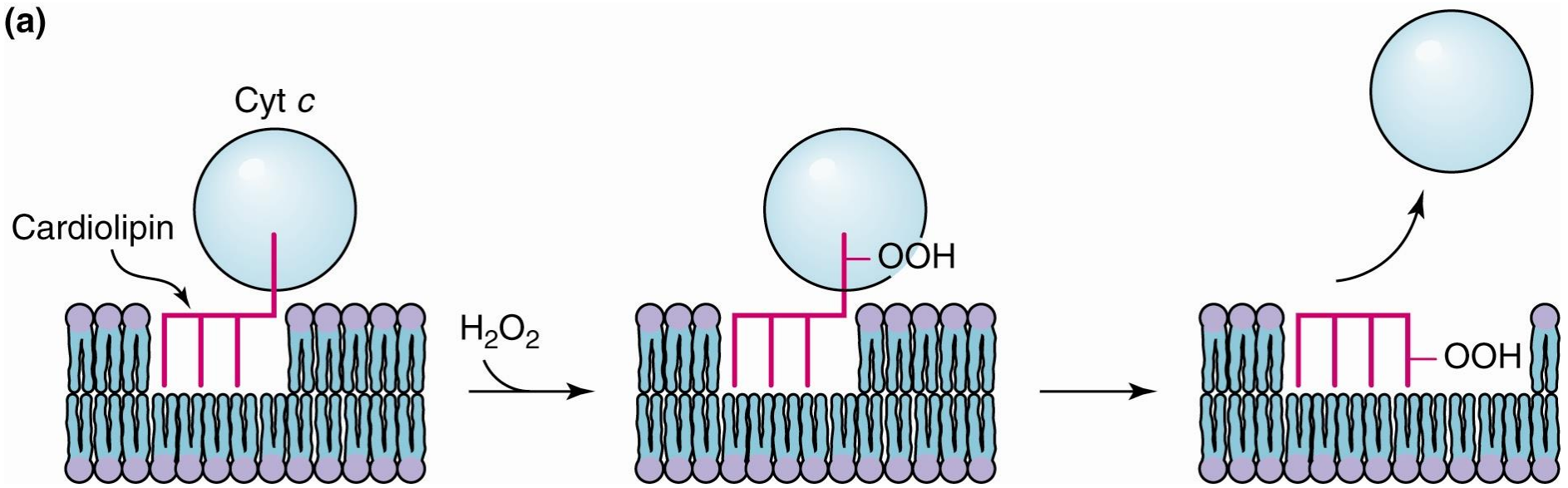


Figure 20.33 (a) Cytochrome c is anchored at the inner mitochondrial membrane by association with cardiolipin. The peroxidase activity of cytochrome c oxidizes a cardiolipin lipid chain, releasing cytochrome c from the membrane.

20.8 How Do Mitochondria Mediate Apoptosis?

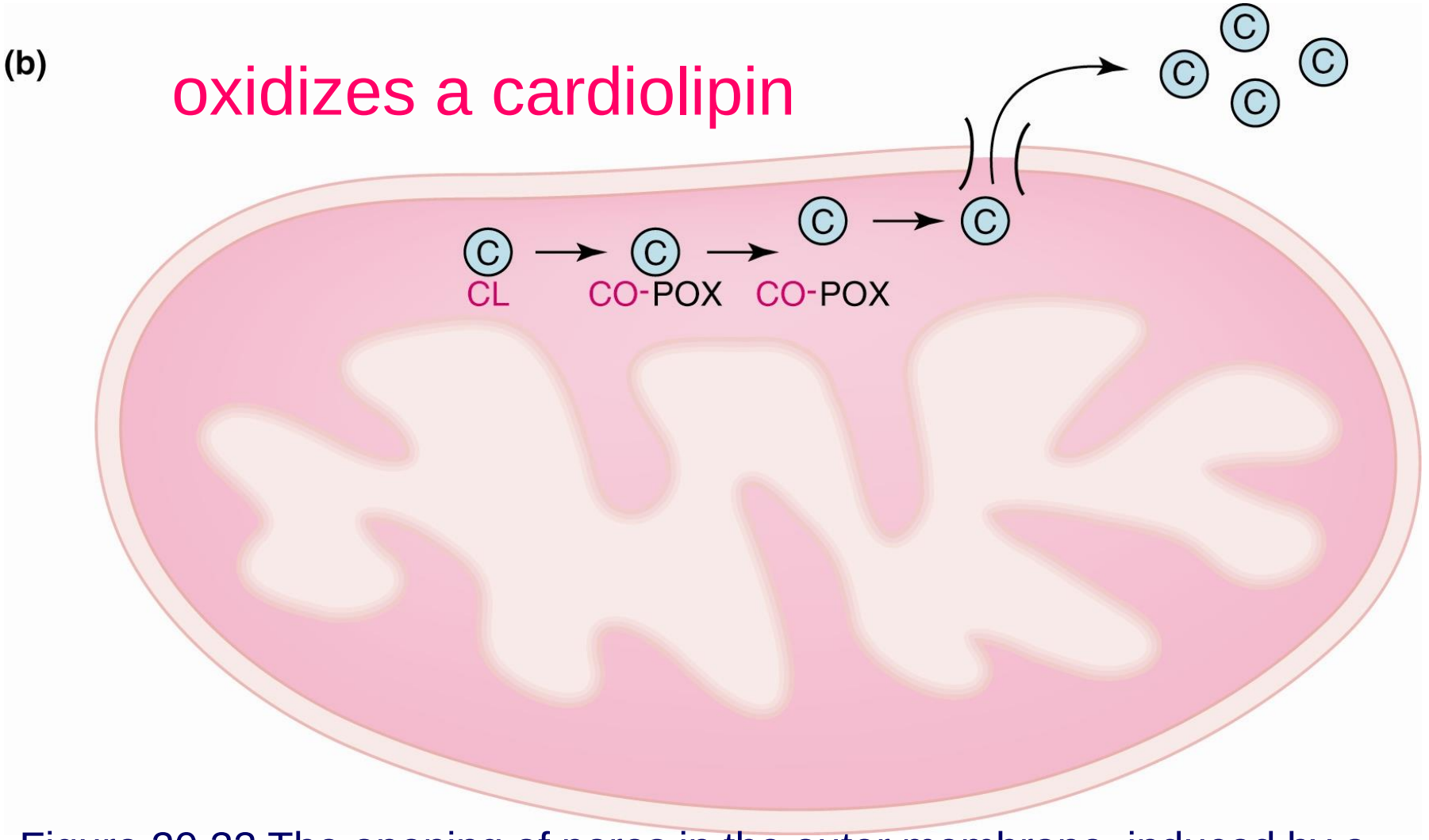
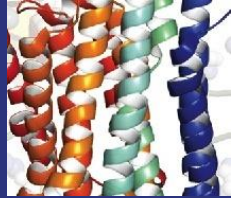


Figure 20.33 The opening of pores in the outer membrane, induced by a variety of triggering agents, releases cytochrome c to the cytosol, where it initiates the events of apoptosis.

20.8 How Do Mitochondria Mediate Apoptosis?



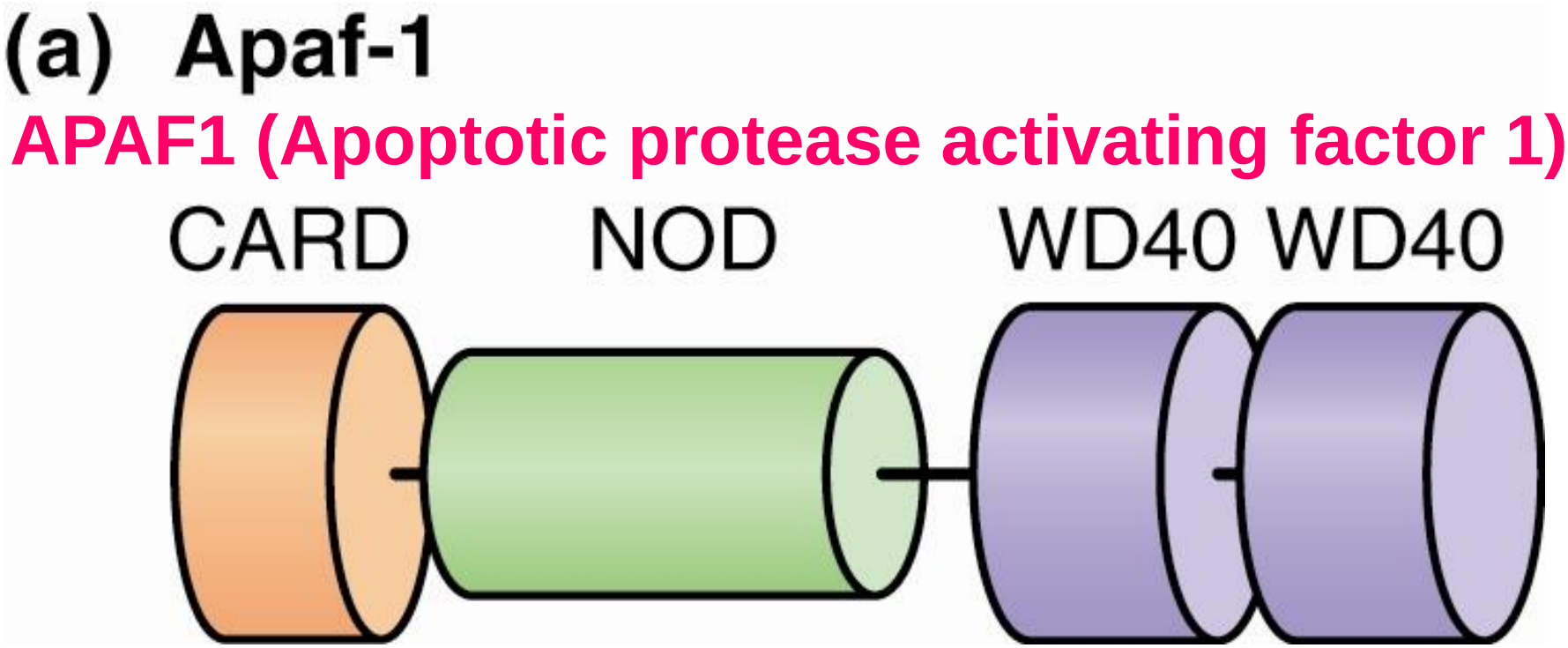
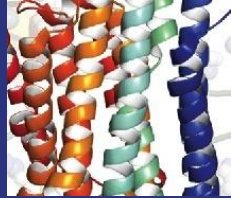


Figure 20.34 Apaf-1 is a multidomain protein, consisting of an N-terminal CARD, a nucleotide-binding and oligomerization domain (NOD), and several WD40 domains.

20.8 How Do Mitochondria Mediate Apoptosis?



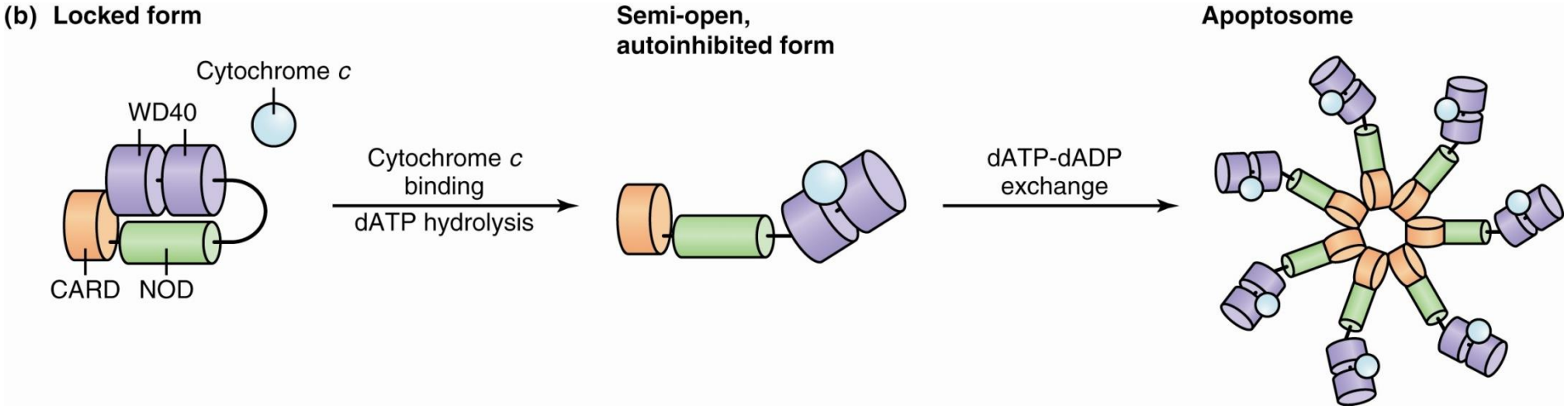


Figure 20.34 Binding of cytochrome c to the WD40 domains and ATP hydrolysis unlocks Apaf-1 to form the semi-open conformation. Nucleotide exchange leads to oligomerization and apoptosome formation.