Bioenergetics (634 فج ج)

Lecture 3

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Evolution of Bioenergetic Mechanisms

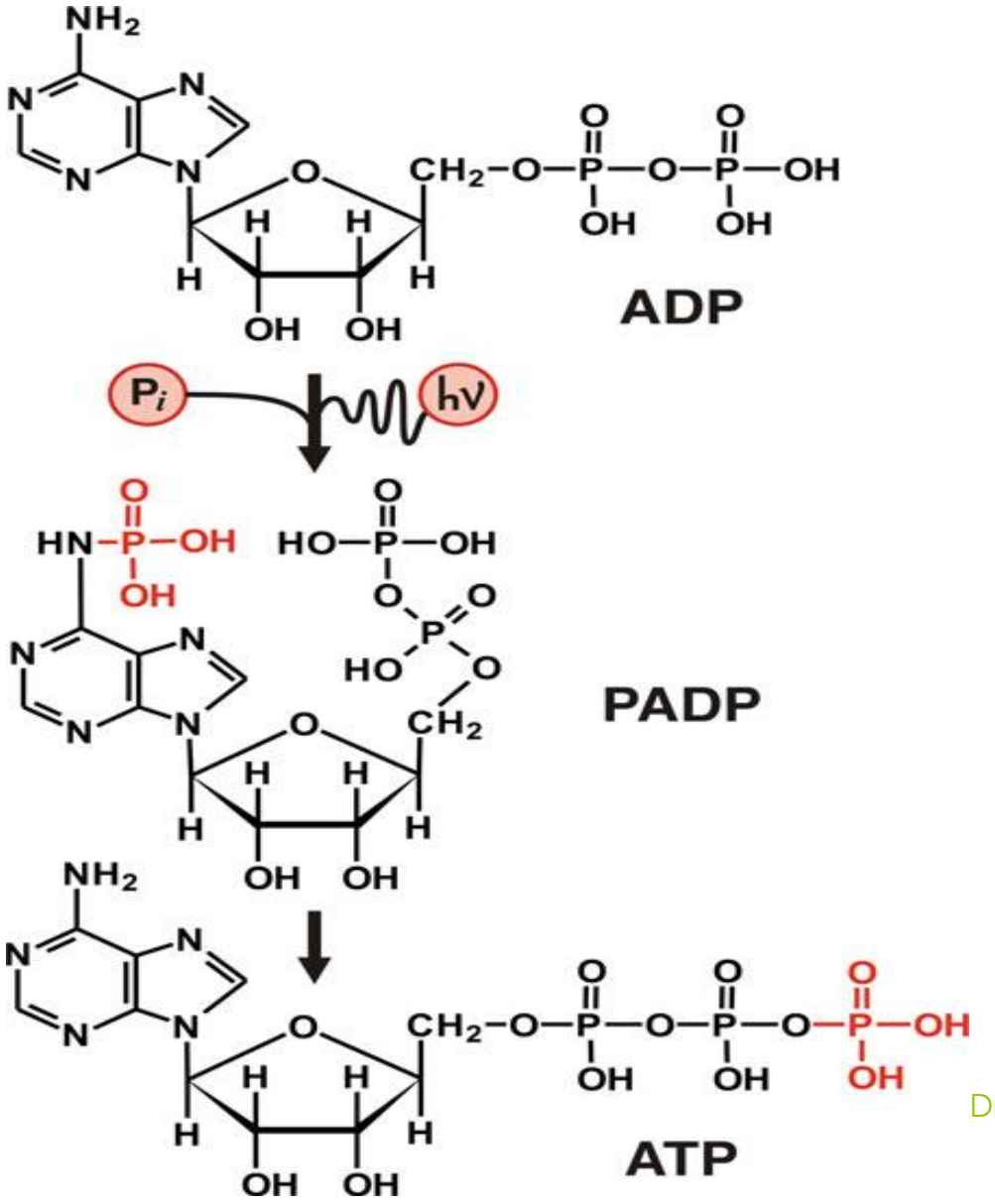
- Mechanism of ATP formation.
- Light-Dependent Cyclic Redox Chain.
- Electron Carriers:
  - Pyridine nucleotides
  - Quinone redox sensors
  - Flavin cofactor-based redox sensors
  - Heme-based sensors
  - Protein-coordinated metal ions -based sensors
  - Thiol-based redox sensors

Mechanism of ATP formation

1. The adenine part of ATP absorbs an ultraviolet quantum, which leads to an excited state with a disturbed system of double bonds. At the same time, the amino group of adenine, which normally corresponds to an aromatic amine, adopts the properties of an aliphatic group, which makes it easier for a phosphorus atom of inorganic phosphate to attack it.
2. Excited adenine of ADP is phosphorylated, thus producing a PADP, the ATP isomer with the third phosphoryl group at the adenine amino group.

Mechanism of ATP formation

3. A phosphoryl group is transferred from adenine to the terminal (second) ADP phosphate. This transfer is enhanced by the fact that the distance between the adenine and the second phosphate ADP is exactly equal to the size of another (the third) phosphate residue. The transfer of the phosphoryl group from the adenine ‘‘head’’ of the nucleotide to the phosphate ‘‘tail’’ is coupled with its stabilization, because a very labile phosphoamide is being replaced with a less labile phosphoanhydride.


ADP
Pi
hν
PADP
ATP

Light-Dependent Cyclic Redox Chain

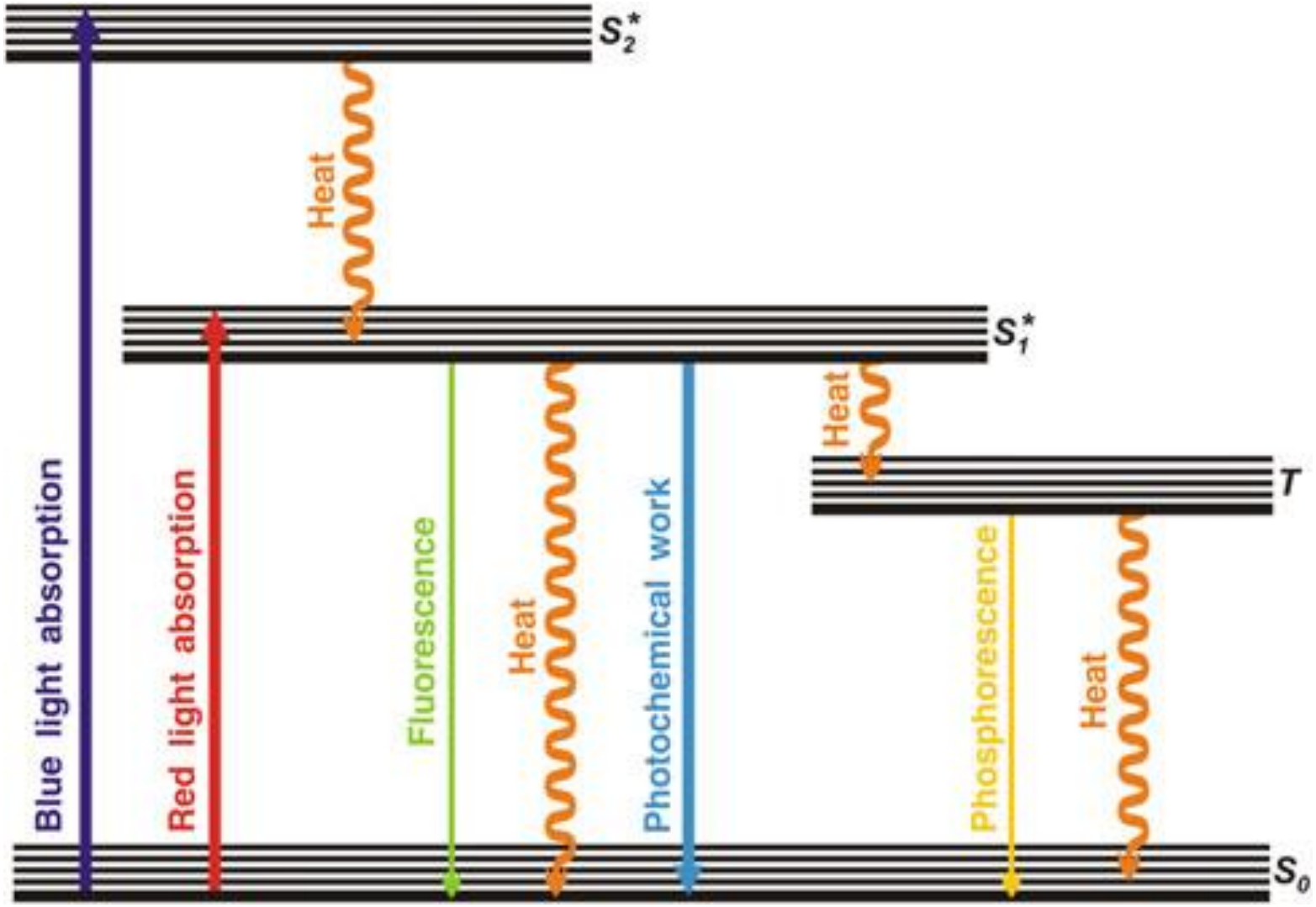
- Adenine and less often other purines and pyrimidines are known to be part of key coenzyme and enzyme prosthetic groups, such as nicotinamide adenine dinucleotide (NAD+), nicotinamide adenine dinucleotide phosphate (NADP+), flavin adenine dinucleotide (FAD), coenzyme A (CoA), thiamine pyrophosphate (vitamin B1 derivative), and vitamin B12.
- The structures of all these compounds are based on the same principle; They include:
  1. A functional group directly involved in catalysis
  2. Purine (or sometimes pyrimidine); and
  3. A flexible linker that allows two other parts of the molecule to interact.

Light-Dependent Cyclic Redox Chain (continued)

- Absorption of visible light by a chromophore molecule is known to induce transfer of an electron from the main orbital (S0) to one of the singlet (S1 * , S2 * …) or triplet (T) excited orbitals.
- Photon energy is spent during this process to transfer of the electron to an orbital that is further away from the nucleus. When in the excited orbital, the electron has a relatively weak connection to the nucleus, and thus it can be easily torn away from the chromophore molecule. So, this molecule in an excited state becomes a good reductant.

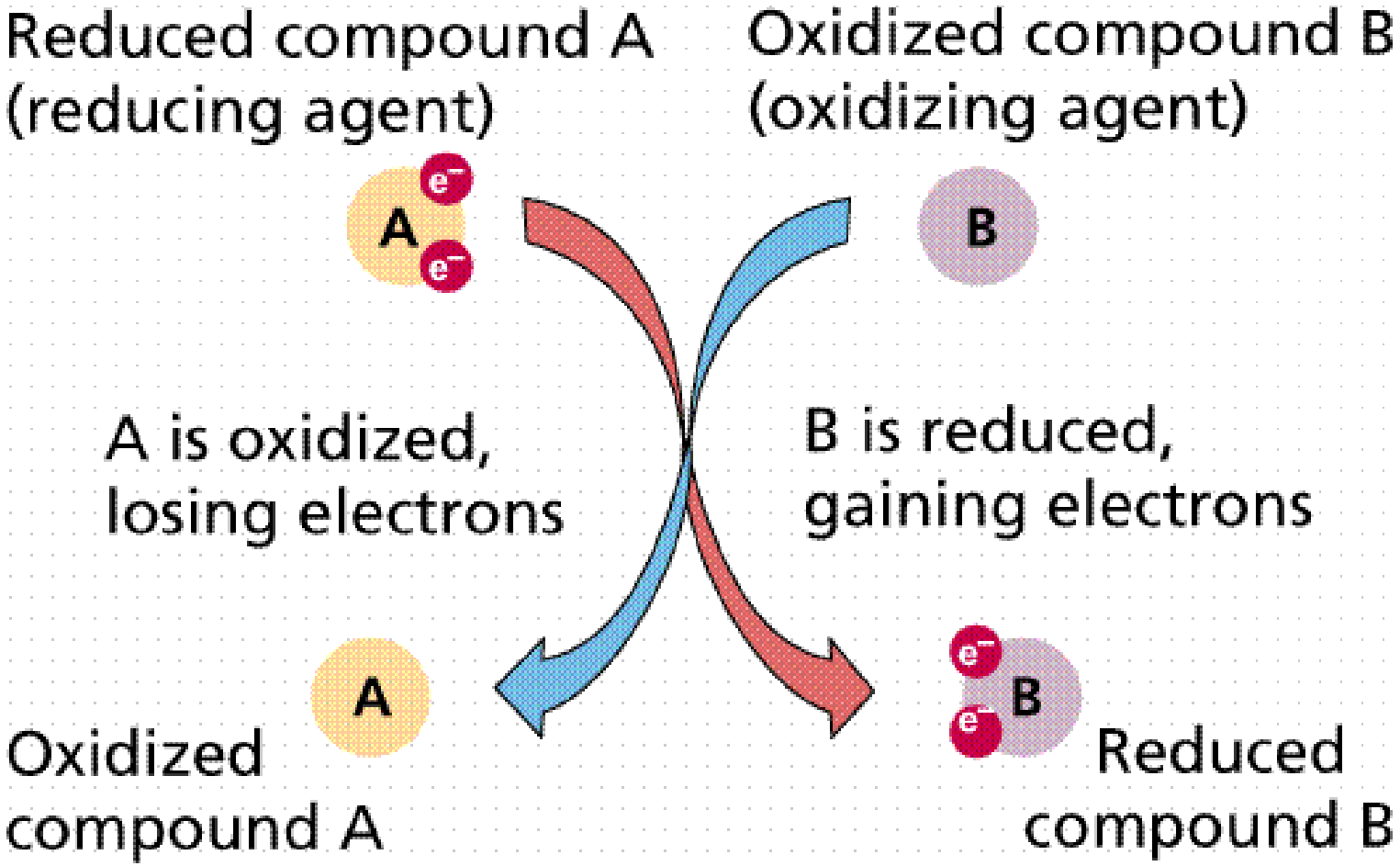
Light-Dependent Cyclic Redox Chain (continued)

- Absorption of a light quantum produces at the same time a vacancy for an electron in the main orbital (hole). This hole has substantial affinity for an electron, i.e. it is a good oxidant.
- The lifetime of a chromophore molecule in an excited state (especially in a singlet state) is extremely short. The electron is inclined to return from an excited to the main orbital. Such a return is accompanied with dissipation of the absorbed photon energy in the form of heat or in the form of a light of longer wavelength (fluorescence or phosphorescence).


S_2^*
Heat
S_1^*
Heat
T
Blue light absorption
Red light absorption
Fluorescence
Heat
Photochemical work
Phosphorescence
Heat
S_0

Light-Dependent Cyclic Redox Chain (continued)

- photosynthesizing organisms have learned to use the effect of chromophore transition into an excited state for the storage of the energy of the quantum (hv) in a form which is useful for a cell.
- For instance, if during the excited state lifetime an electron is transferred from the excited orbital to some acceptor X, and the vacancy on the main chromophore orbital is filled from some donor Y, then part of the energy of the photon can be stored as a difference of redox potentials of substances X and Y. If this redox reaction is organized in such a way that an electron is transferred from Y to X across the membrane, then a certain part of the energy of the light quantum can also be stored as $\Delta\bar{\mu}_{H^+}$. Just in this way the most important photosynthetic process, i.e. oxygenic photosynthesis, is organized.


Reduced compound A
(reducing agent)
Oxidized compound B
(oxidizing agent)
A
e⁻
e⁻
B
A is oxidized,
losing electrons
B is reduced,
gaining electrons
A
Oxidized
compound A
e⁻
e⁻
B
Reduced
compound B

Light-Dependent Cyclic Redox Chain (continued)

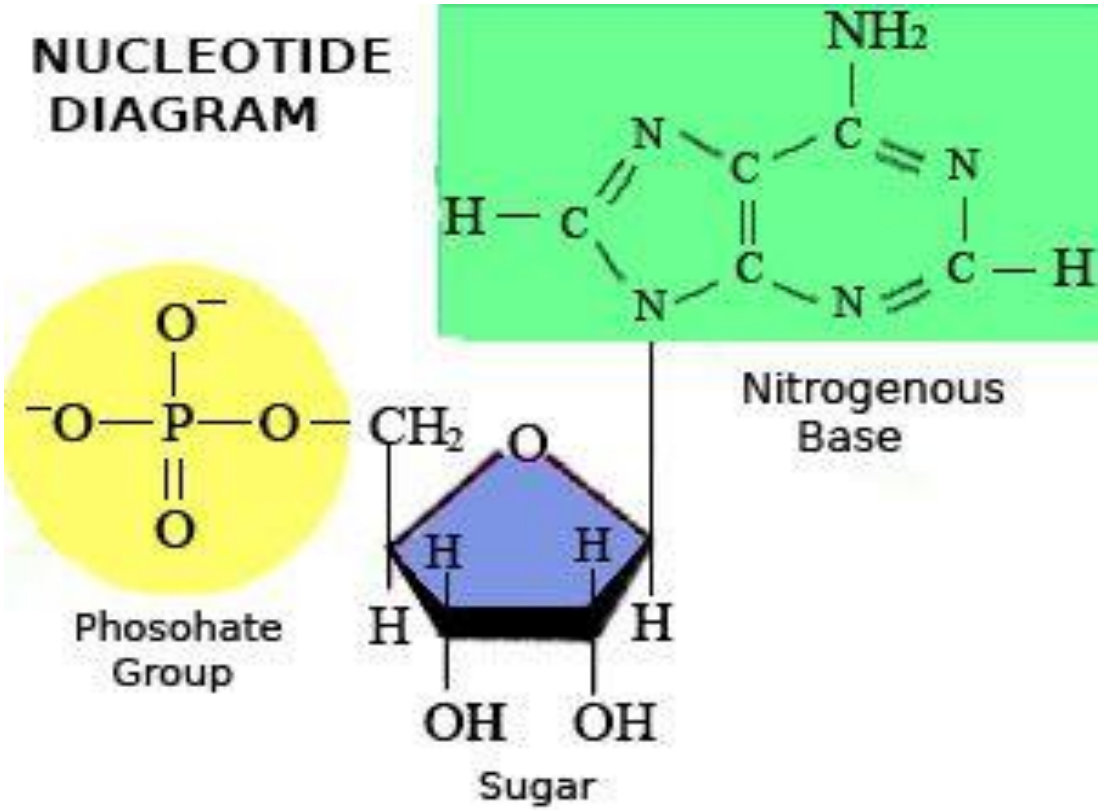
- In an alternative and simplified version of photosynthesis, an electron from an excited orbital is returned to the main orbital, but not directly. It has to cross the membrane first, which leads to generation of $\Delta\bar{\mu}_{H^+}$ This type of photosynthesis is found in purple bacteria.

Chemical classes of electron carriers

- Purely organic rings:
  - NAD (Nicotine-amide Adenine Dinucleotide)
  - FAD and FMN (Flavine Adenine Dinucleotide and Flavine Mono-Nucleotide
  - Ubiquinone (coenzyme Q)
- Organic rings carrying iron: heme
- Iron sulfur clusters
- Protein-coordinated metal ions

Electron Carriers

Pyridine nucleotides



- Nitrogenous base:
  - Purines (adenine- guanine)
  - Pyrimidiene (cytosine- thymine – uracil)

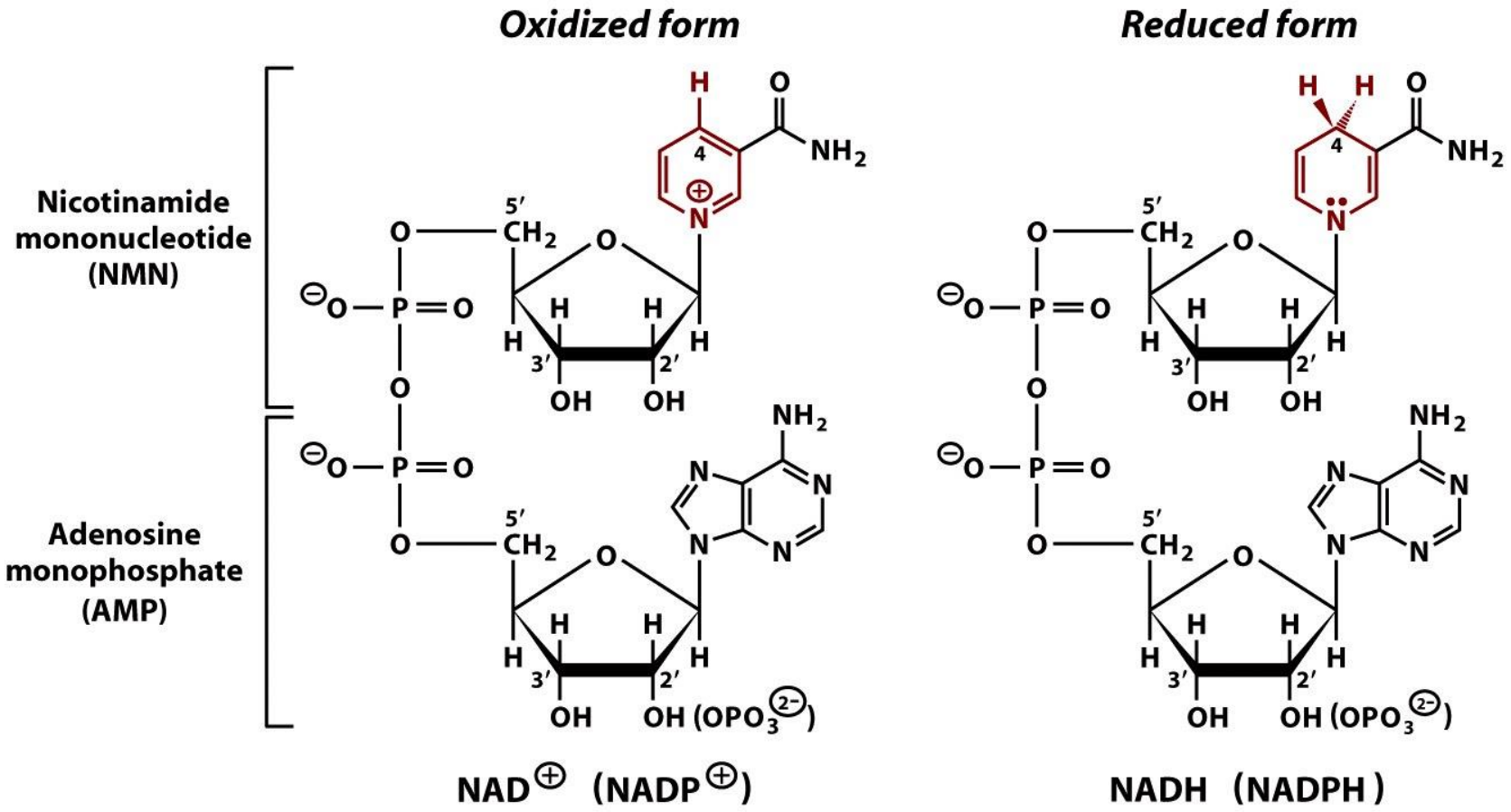
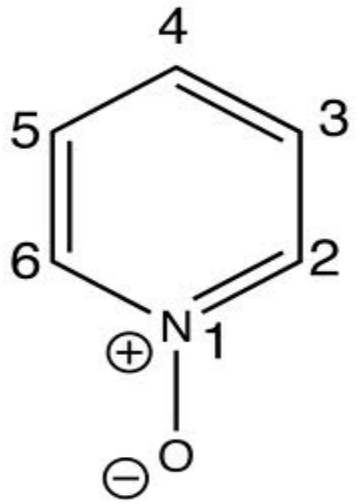


Figure 7-8 Principles of Biochemistry, 4/e

- NADP+/NAD + is similar, except for an additional phosphate esterified to a hydroxyl group on the adenosine ribose. It is oxidizing agent can accept electron.

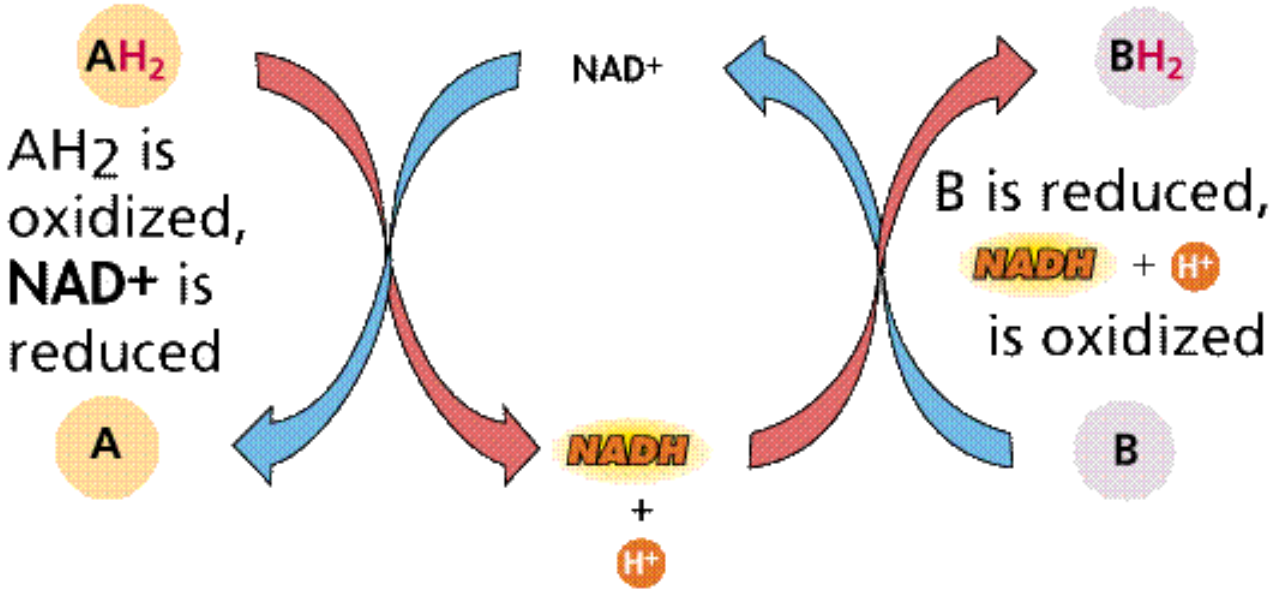
NAD+/NADP+

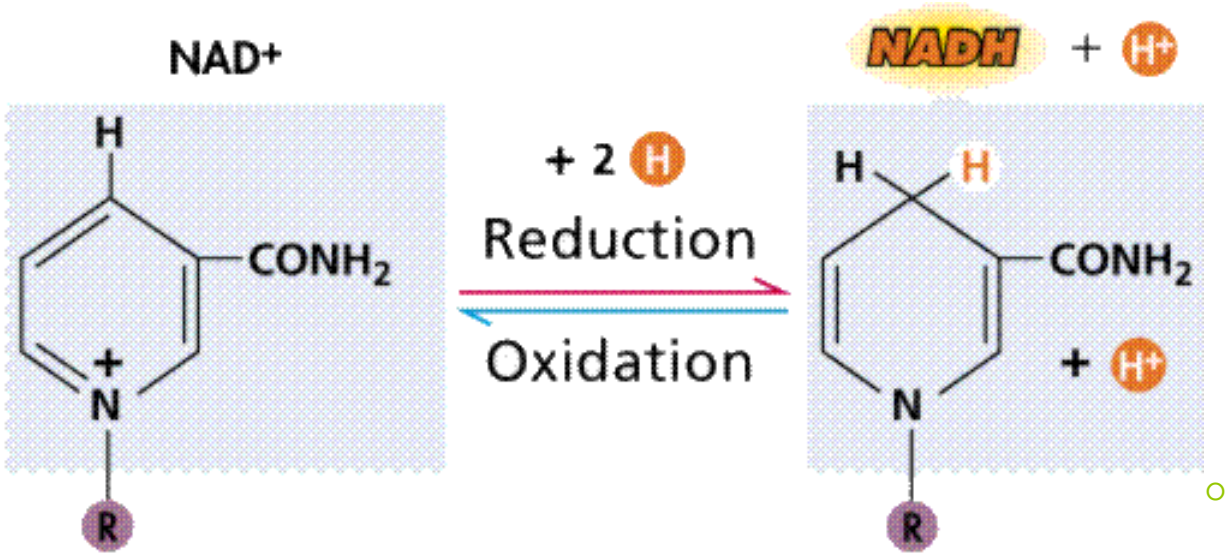


- Pyridine:

Heterocyclic compound C5H5N

- It is structurally related to benzene with one C-H group replaced by nitrogen.
- *How NAD+ carry the proton?*
- Electrophilic substitutions at pyridine or resonance system which allow delocalization of electrons to create site on the ring where carbon (4) can bound to H+.


AH2
AH2 is oxidized, NAD+ is reduced
A
NAD+
NADH
+
H+
BH2
B is reduced,
NADH + H+
is oxidized
B


NAD+
H
CONH2
+
N
R
+ 2 H
Reduction
Oxidation
NADH + H+
H
H
CONH2
+ H+
N
R

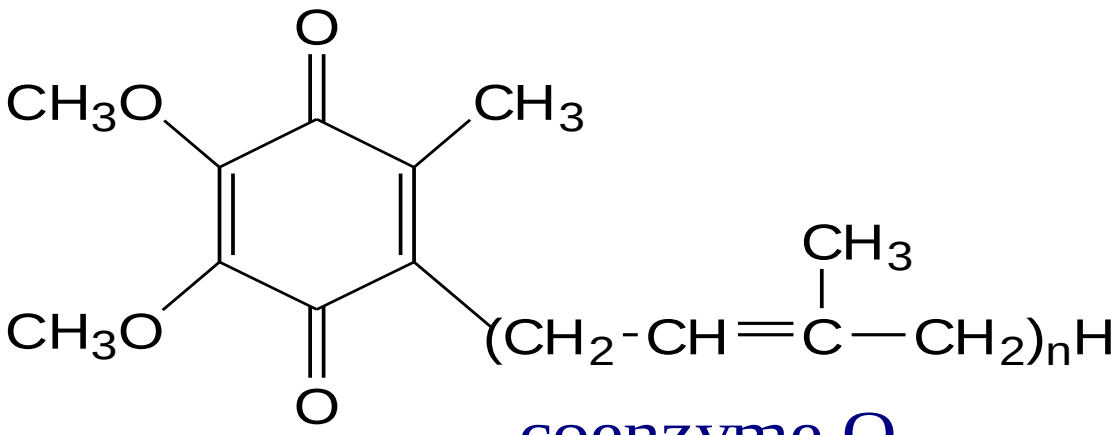
Electron Carriers

Quinone redox sensors

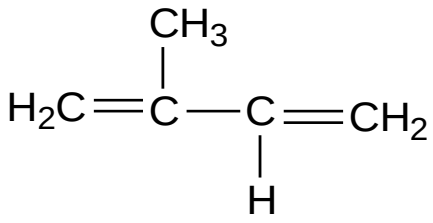
Coenzyme Q (Co Q)

- The quinone ring of coenzyme Q can be reduced to the quinol in a 2e- reaction:

$$Q + 2\,e^- + 2\,H^+ \leftrightarrow QH_2$$



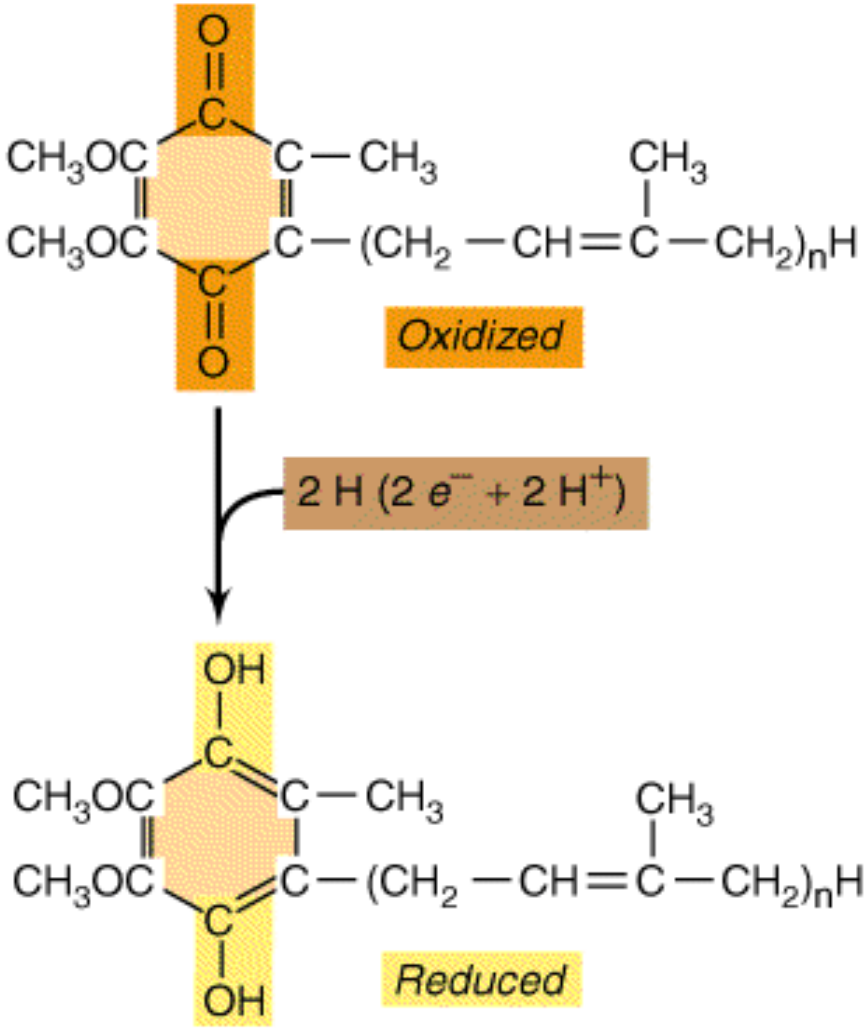
coenzyme Q



isoprene

Coenzyme Q (Co Q) (continued)

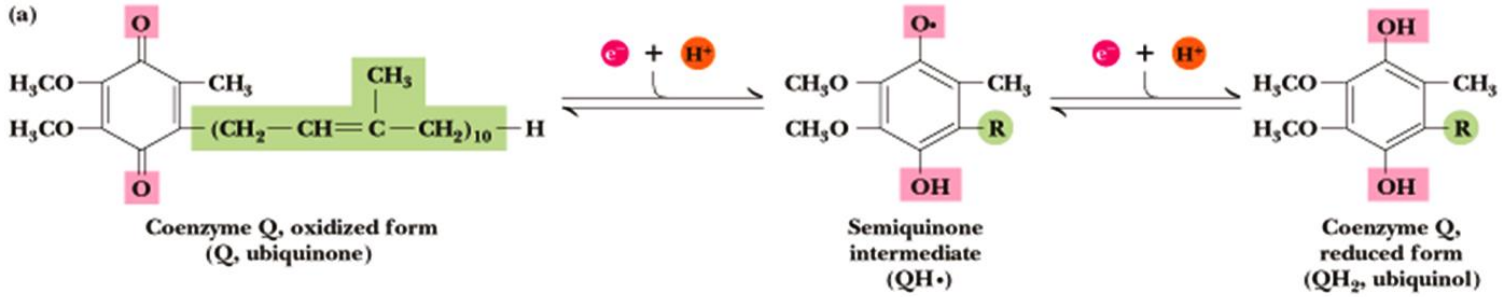
- Coenzyme Q (Co Q, Q, ubiquinone) is very hydrophobic. It dissolves in the hydrocarbon core of a membrane.
- It includes a long isoprenoid tail, with multiple units having a carbon skeleton comparable to that of isoprene. In human cells, most often n = 10.
- Q10's isoprenoid tail is longer than the width of a bilayer. It may be folded to yield a more compact structure, & is postulated to reside in the central domain of a membrane, between the 2 lipid monolayers.


Oxidized
2 H (2 e⁻ + 2 H⁺)
Reduced

Coenzyme Q (Co Q)
(continued)

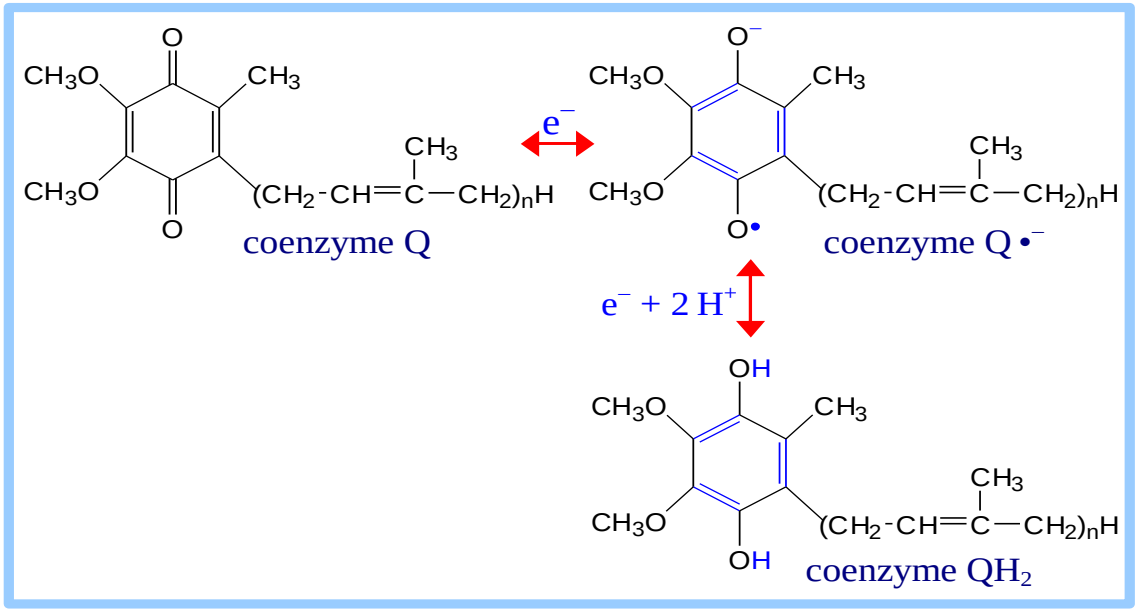
- The "Q cycle" depends on mobility of coenzyme Q within the lipid bilayer.
- The reaction cycle may involve one-electron transfers, with an intermediate semiquinone radical.

Garrett & Grisham: Biochemistry, 2/e
Figure 21.5



Coenzyme Q (Co Q)
(continued)

- When bound to special sites in respiratory complexes, CoQ can accept 1 e- to form a semiquinone radical (Q·-). Thus Co Q, like FMN, can mediate between 1 e- & 2 e- donors/acceptors

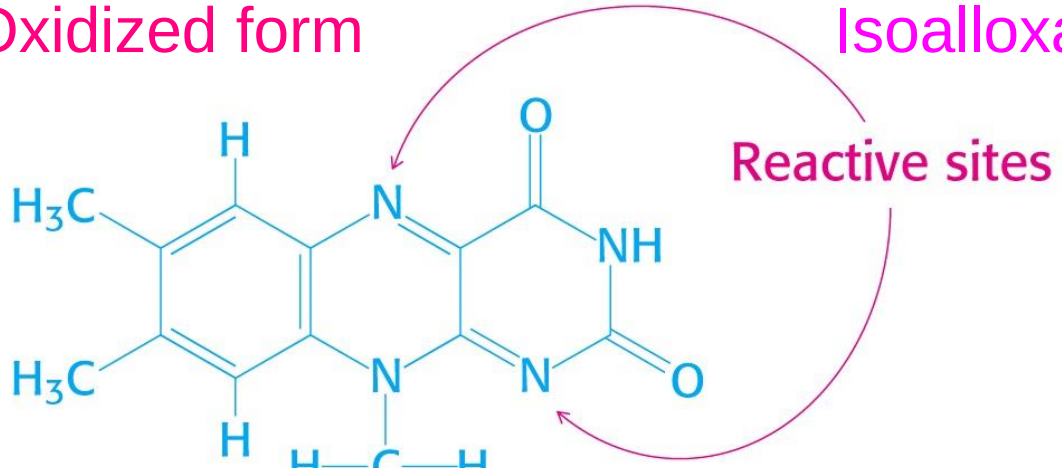


Electron Carriers

Flavin cofactor-based redox sensors

Oxidized form

Isoalloxazine ring of riboflavin

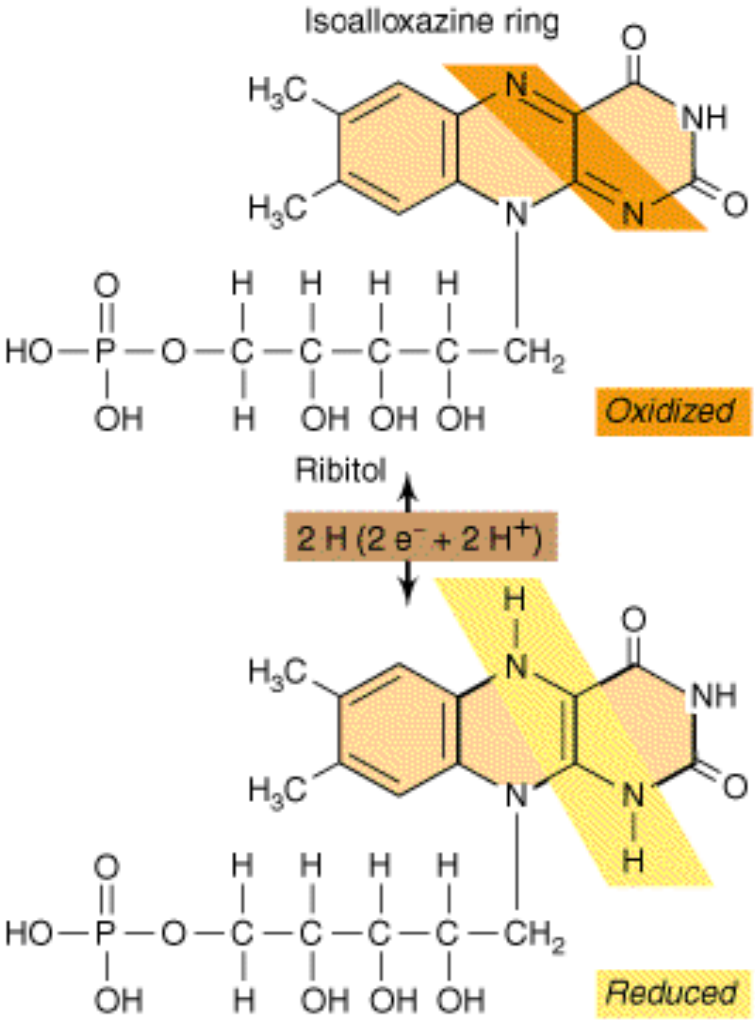


Electron carrier, accepts 2 electrons, & 2 protons

FMN

AMP

Ifiky


Isoalloxazine ring
Ribitol
2 H (2 e⁻ + 2 H⁺)
Oxidized
Reduced

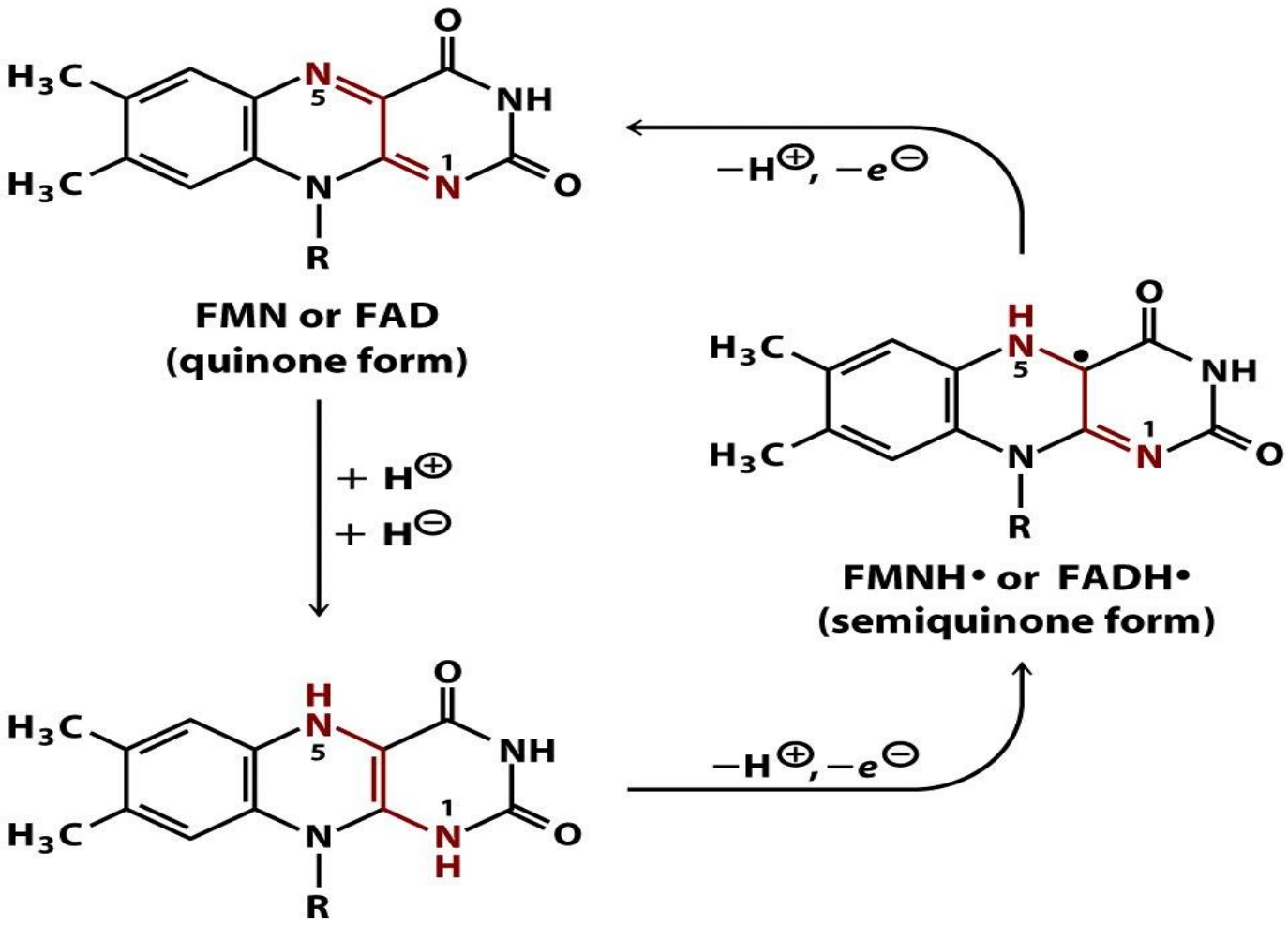


Figure 7-11 Principles of Biochemistry, 4/e

Flavin cofactor-based redox sensors

- FMN, when bound at the active site of some enzymes, can accept 1 e- to form the half-reduced semiquinone radical. The semiquinone can accept a 2nd e- to yield FMNH2.

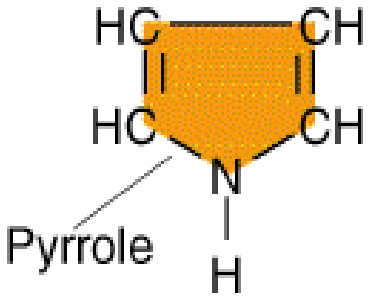
- Since it can accept/donate 1 or 2 e-, FMN has an important role mediating e- transfer between carriers that transfer 2e- (e.g., NADH) & those that can accept only 1e- (e.g., Fe+++)

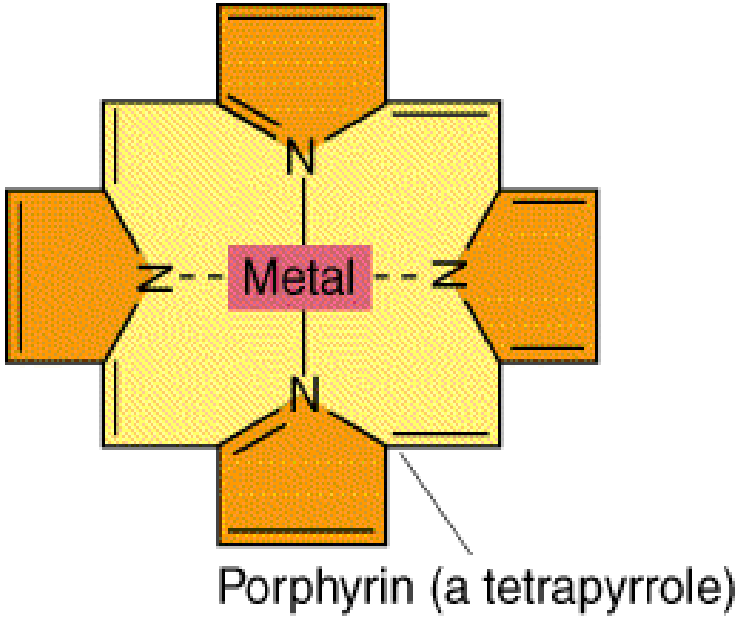
Electron Carriers

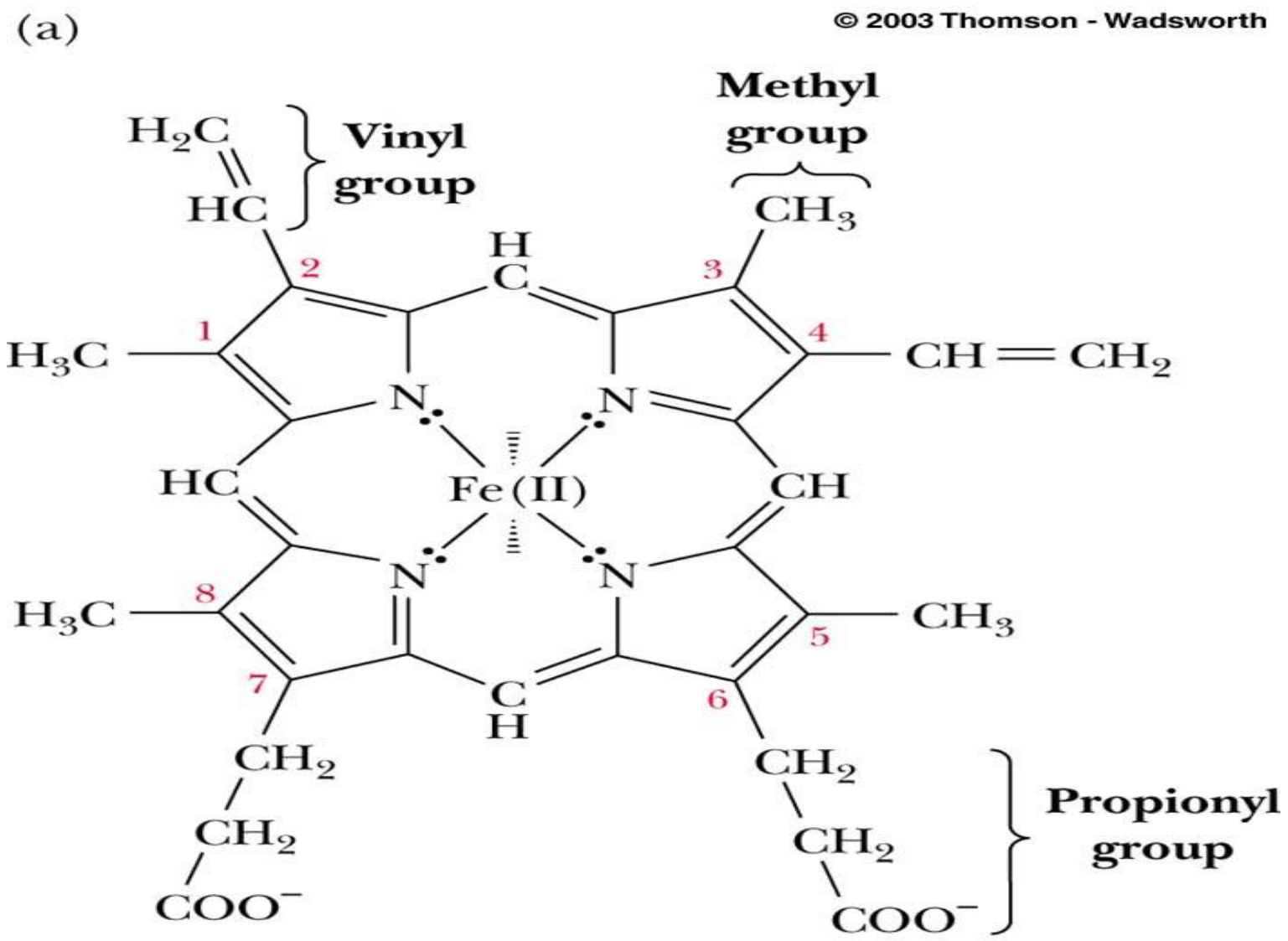
Heme-based sensors

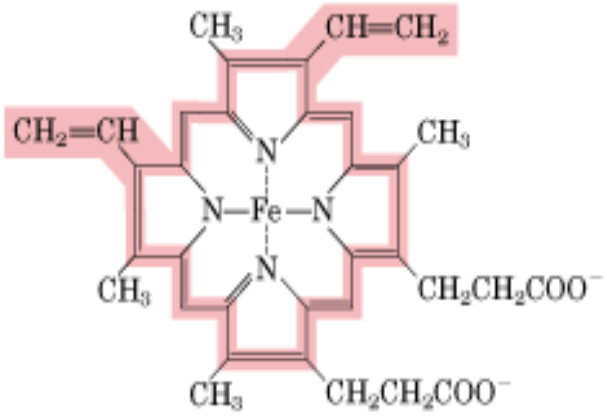
Heme-based sensors

- Heme is a *prosthetic group* of cytochromes.
- Heme contains an iron atom in a porphyrin ring system. The Fe is bonded to 4 N atoms of the porphyrin ring.
- Hemes in the 3 classes of cytochrome (a, b, c) differ slightly in substituents on the porphyrin ring system.
- A common feature is 2 *propionate side-chains*. Only heme c is covalently linked to the protein via thioether bonds to cysteine residues.

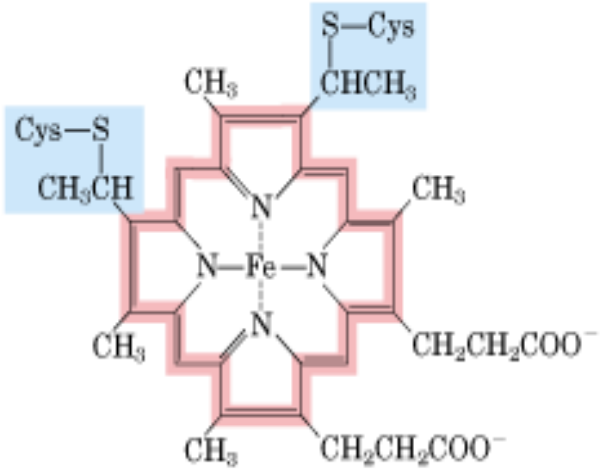

HC
CH
HC
CH
N
H
Pyrrole


N
N
Metal
N
N
Porphyrin (a tetrapyrrole)

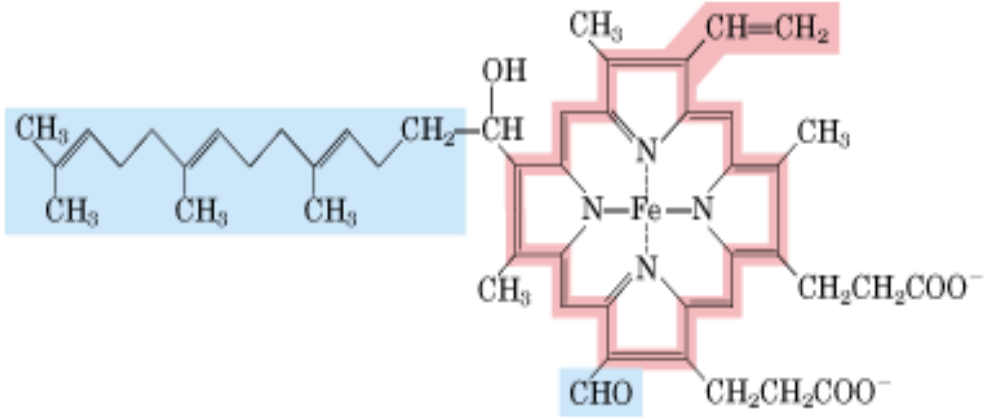

(a)
© 2003 Thomson - Wadsworth
Vinyl group
Methyl group
Propionyl group
Fe(II)
H2C
HC
CH3
H3C
CH=CH2
HC
CH
H3C
CH3
CH2
CH2
COO−
CH2
CH2
COO−
1
2
3
4
5
6
7
8
N
N
N
N



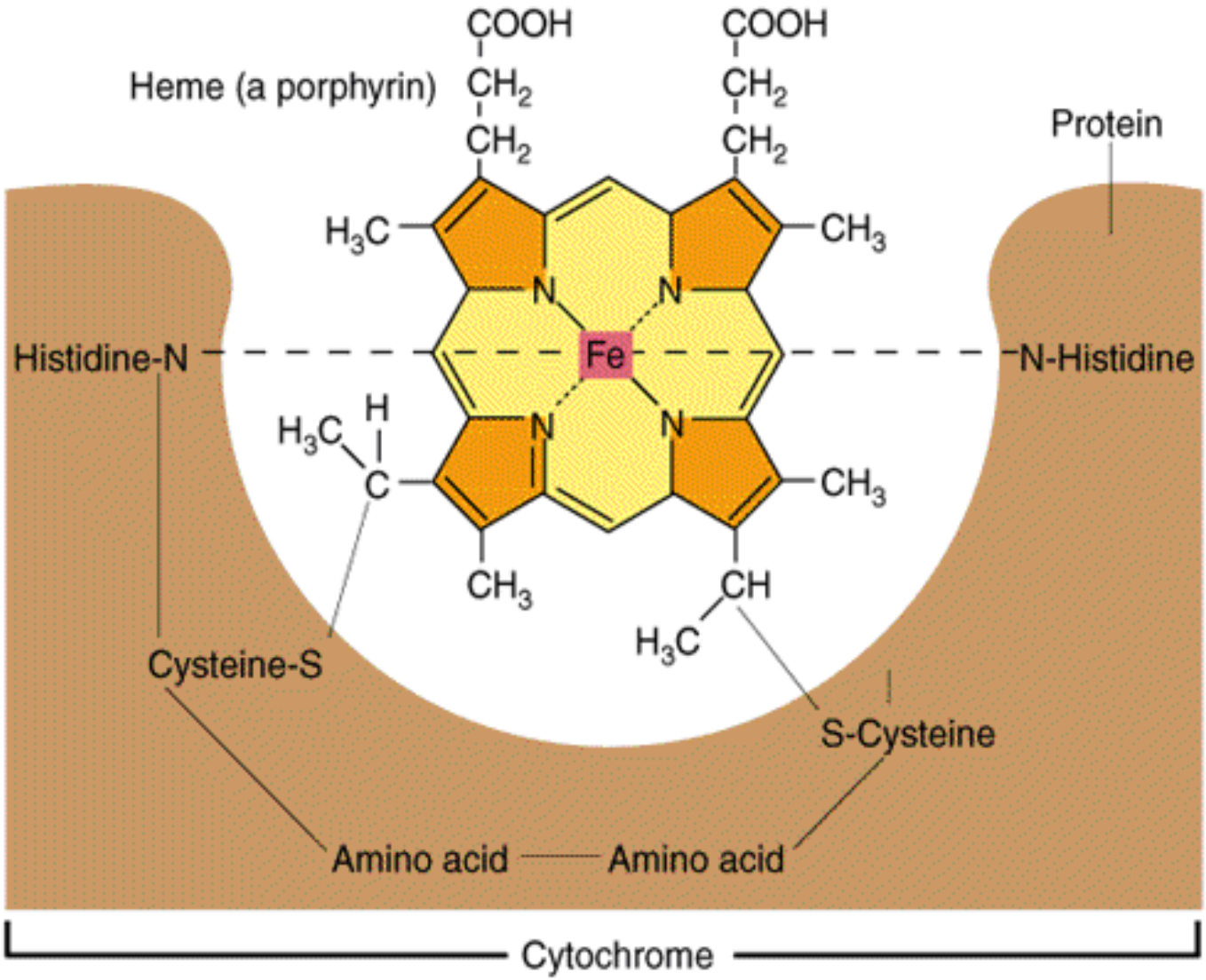
Iron protoporphyrin IX
(in *b*-type cytochromes)



Heme C
(in *c*-type cytochromes)

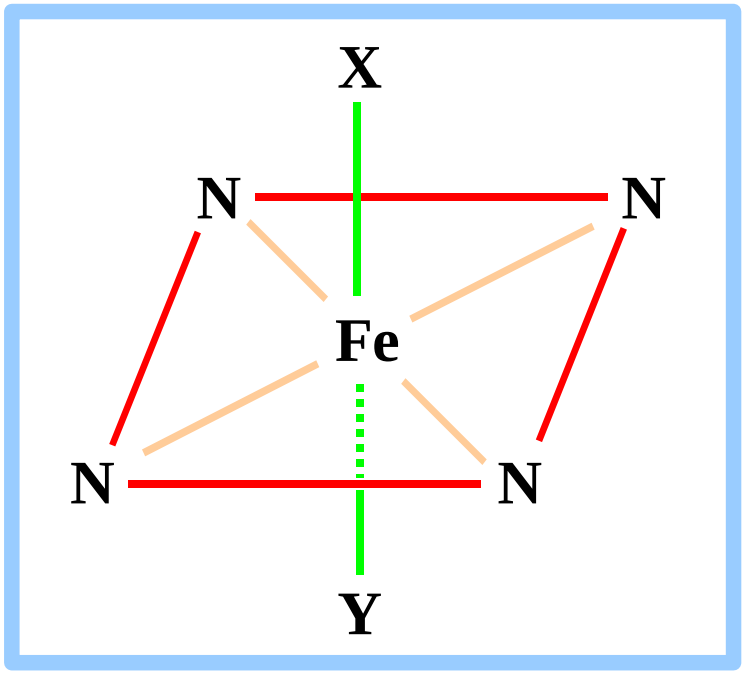


Heme A
(in *a*-type cytochromes)


Heme (a porphyrin)
COOH
CH2
CH2
COOH
CH2
CH2
Protein
H3C
CH3
N
N
Histidine-N
Fe
N-Histidine
H3C
H
C
N
N
CH3
CH3
CH
H3C
Cysteine-S
S-Cysteine
Amino acid
Amino acid
Cytochrome

Heme-based sensors (continued)

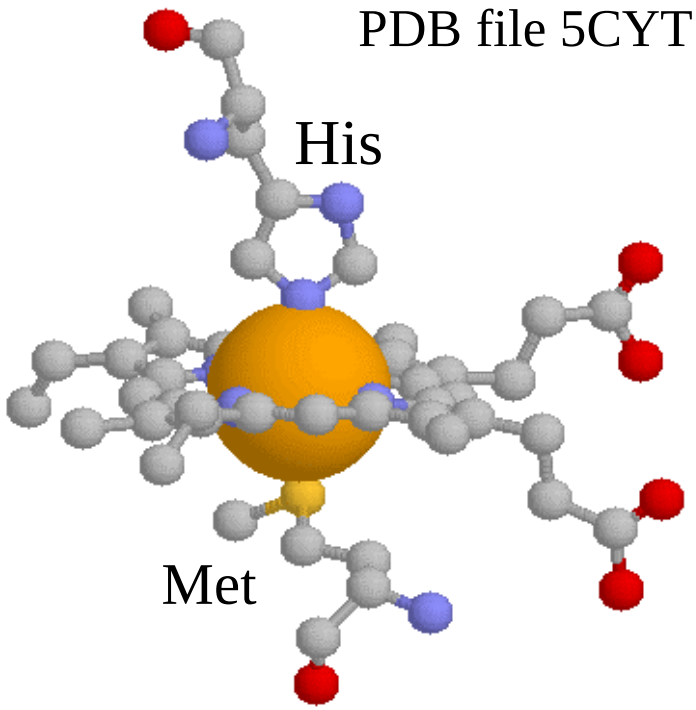
- The porphyrin ring is planar.
- The heme Fe is usually bonded to 2 axial ligands, above & below the heme plane (X,Y) in addition to 4 N of porphyrin.



Heme-based sensors

(continued)

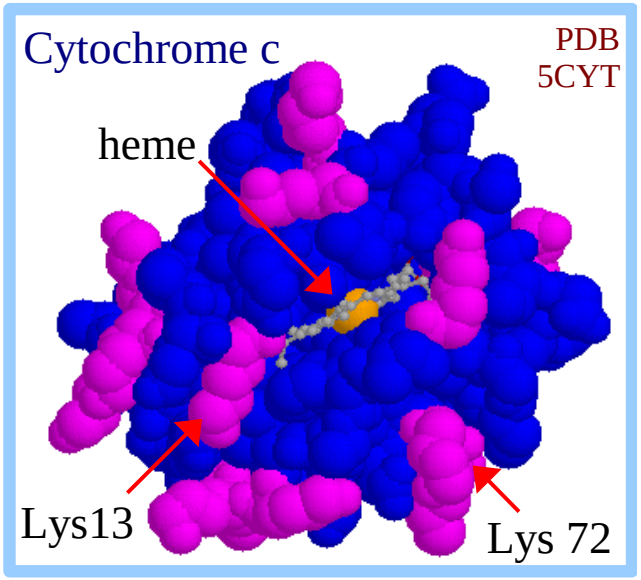
- Axial ligands may be S or N atoms of amino acid side-chains.
- Axial ligands in cyt c are Met S (yellow) and His N (blue).
- A heme that binds O2 may have an open (empty) axial ligand position.

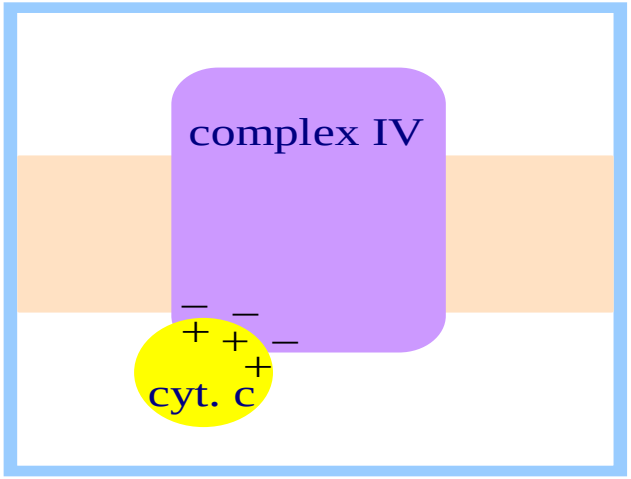


Heme in cytochrome c

Heme-based sensors (continued)

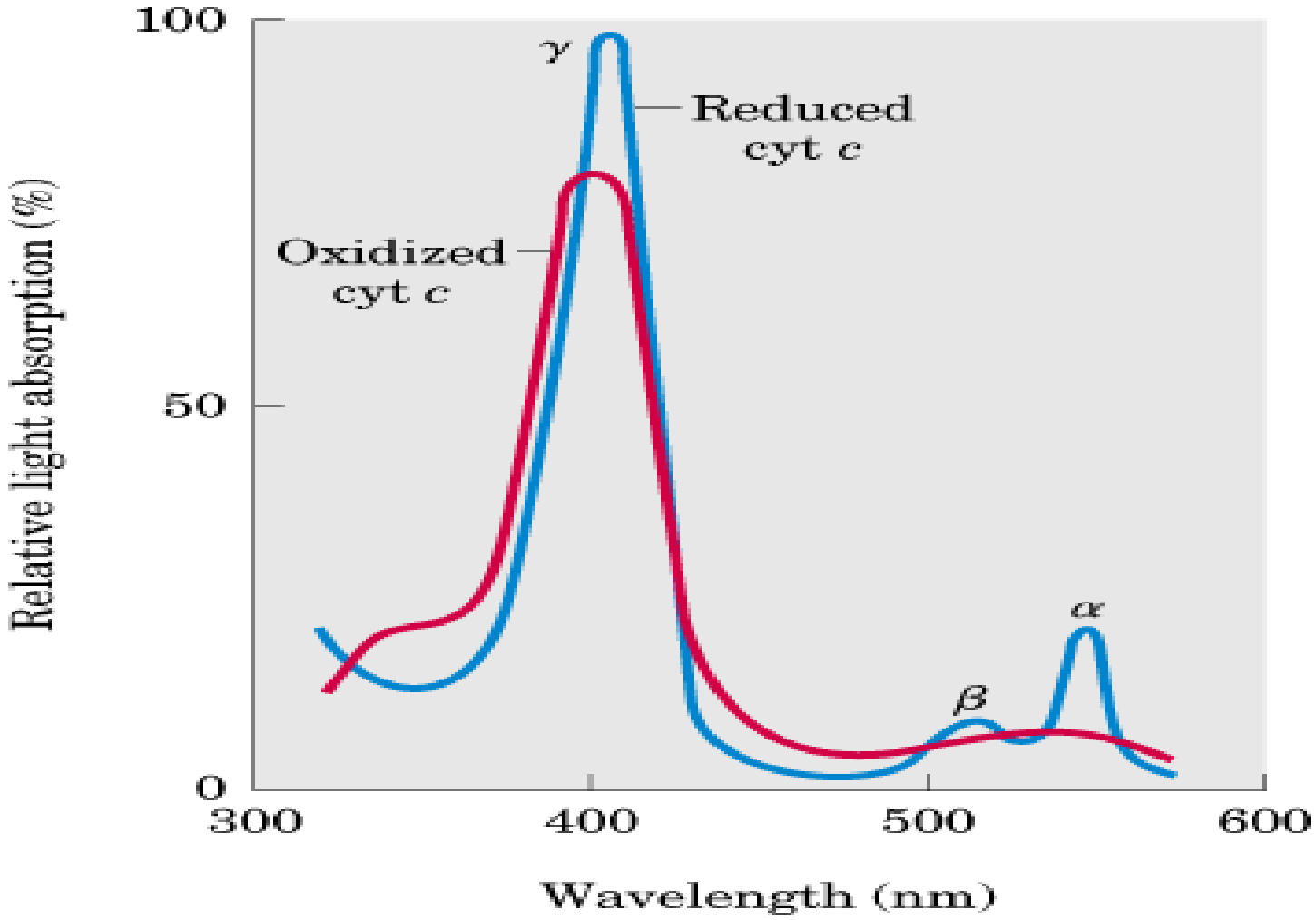
- Positively charged lysine residues (in magenta) surround the heme crevice on the surface of cytochrome c.
- These may interact with anionic residues on membrane complexes to which cyt c binds, when receiving or donating an e-.





Heme-based sensors (continued)

- Cytochromes are proteins with heme prosthetic groups. They absorb light at characteristic wavelengths.
- Absorbance changes upon oxidation/reduction of the heme iron provide a basis for monitoring heme redox state.


100
50
0
Relative light absorption (%)
γ
Reduced
cyt c
Oxidized
cyt c
α
β
300
400
500
600
Wavelength (nm)

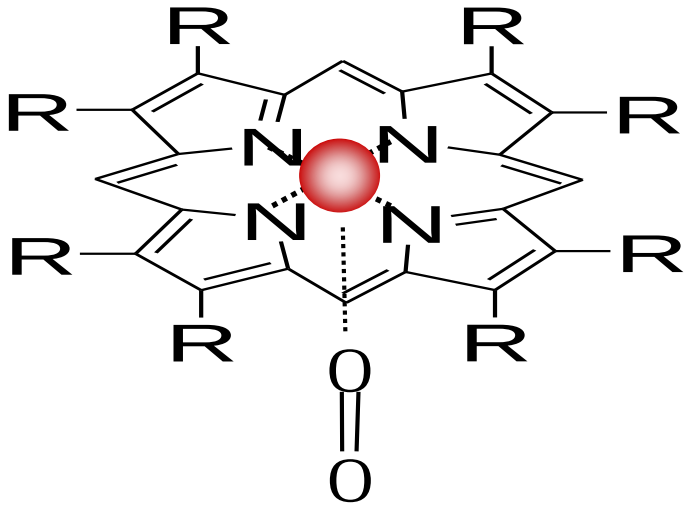
Heme-based sensors (continued)

- Some cytochromes are part of large integral membrane complexes, each consisting of several polypeptides & including multiple electron carriers. Individual heme prosthetic groups may be separately designated as cytochromes, even if in the same protein. E.g., hemes a & a3 that are part of the respiratory chain complex IV are often referred to as cytochromes a & a3.
- Cytochrome c is instead a small, water-soluble protein with a single heme group.

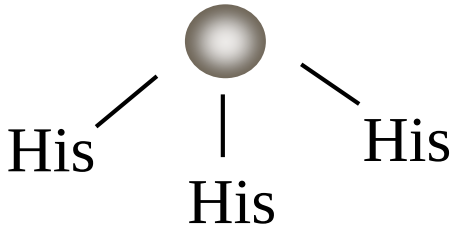
Electron Carriers

Protein-coordinated metal ions -based sensors

The active site of cytochrome C oxidase



iron

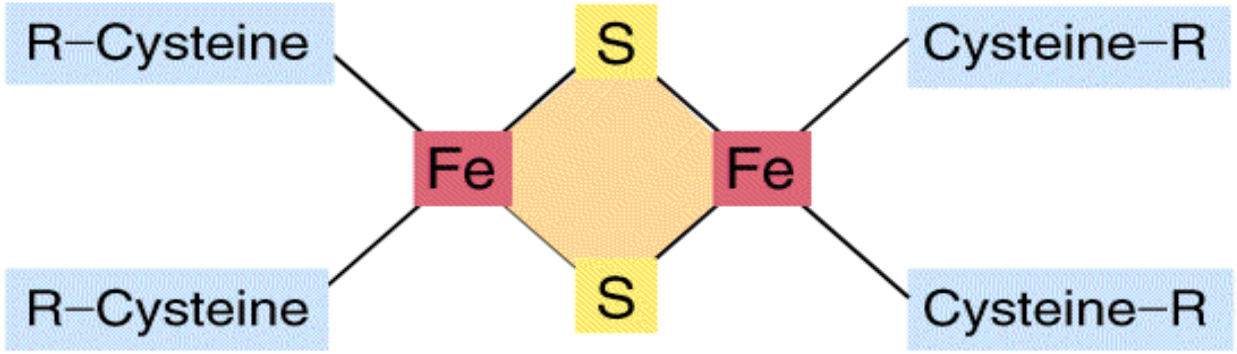


copper

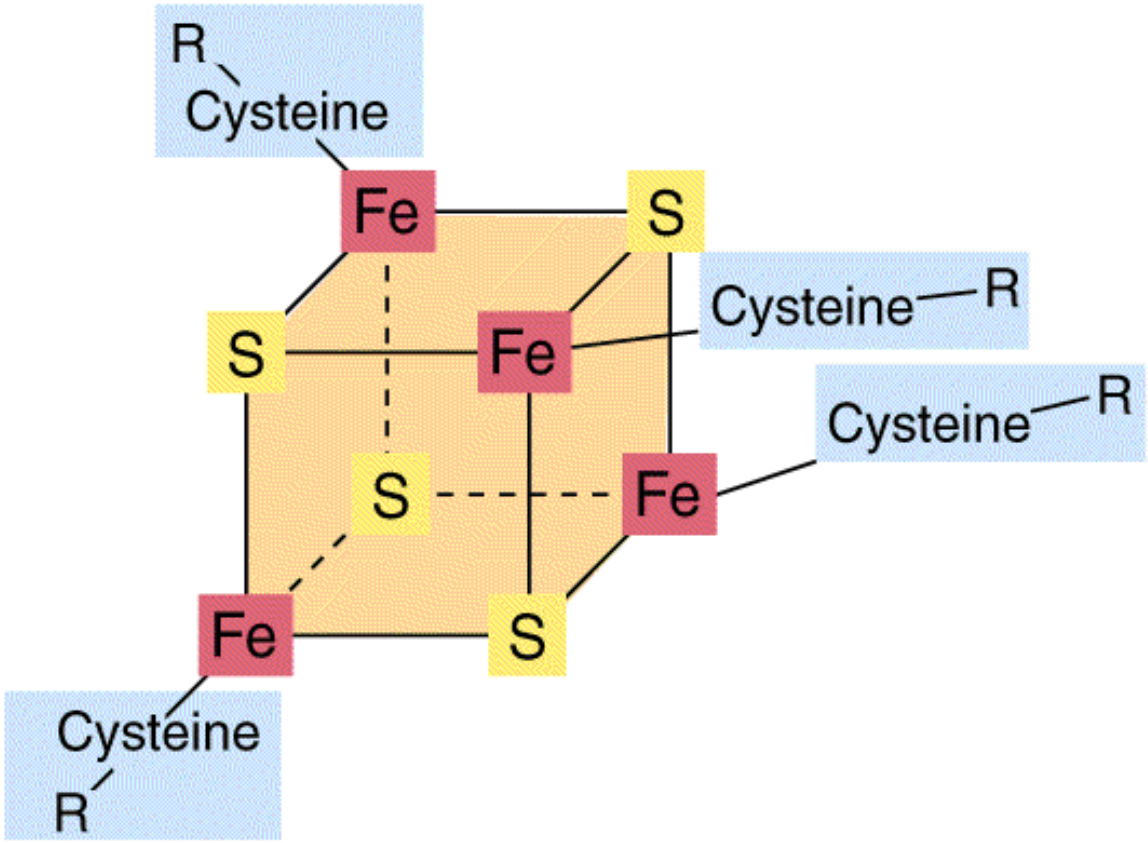
Fe–S cluster-based sensors

- Iron-sulfur centers (Fe-S) are prosthetic groups containing 2, 3 , 4 or 8 iron atoms complexed to elemental & cysteine S.
- 4-Fe centers have a tetrahedral structure, with Fe & S atoms alternating as vertices of a cube.
- Cysteine residues provide S ligands to the iron, while also holding these prosthetic groups in place within the protein.

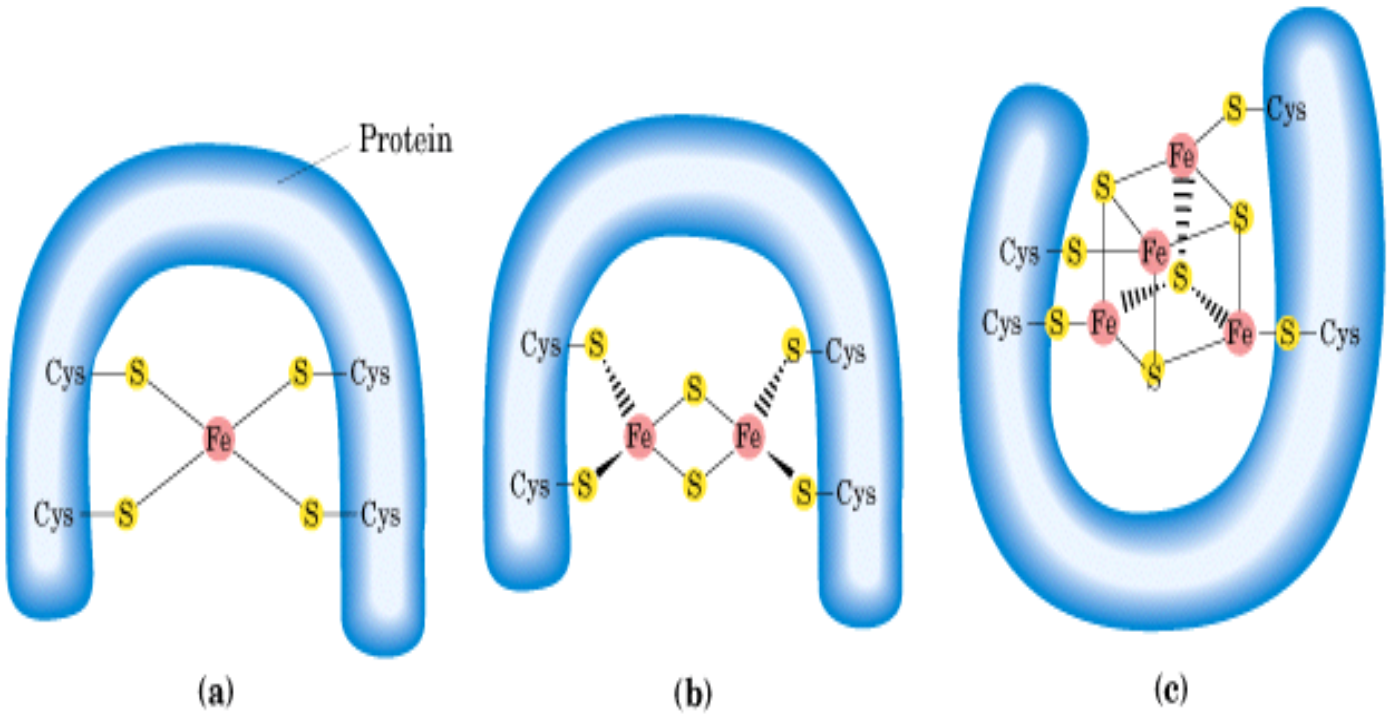
Iron-sulfur proteins (Fe_2S_2)



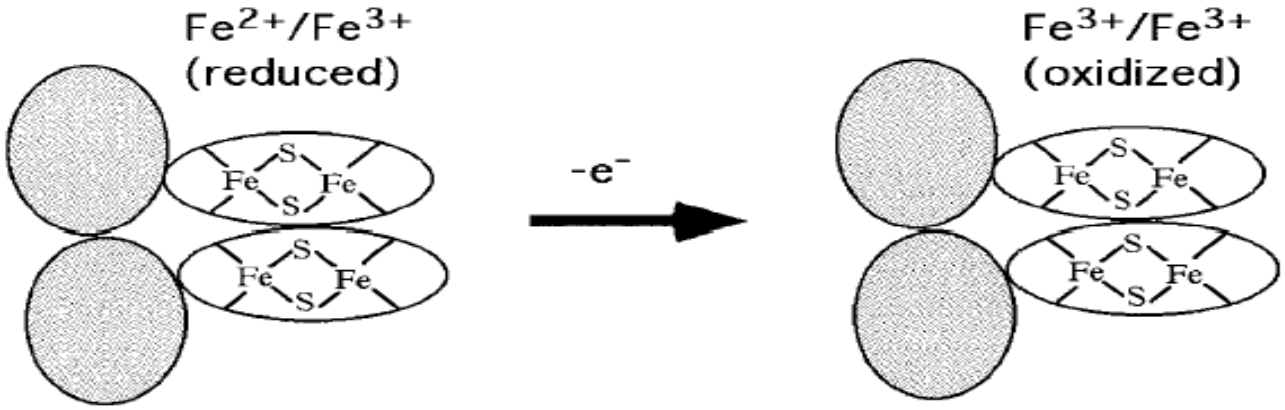
Iron-sulfur protein (Fe_4S_4)



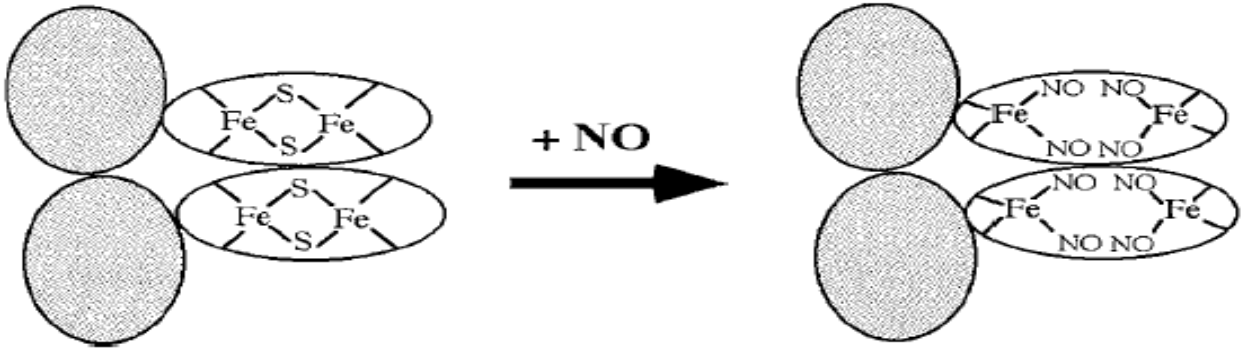
Fe–S cluster-based sensors (continued)



A. *Oxidation*

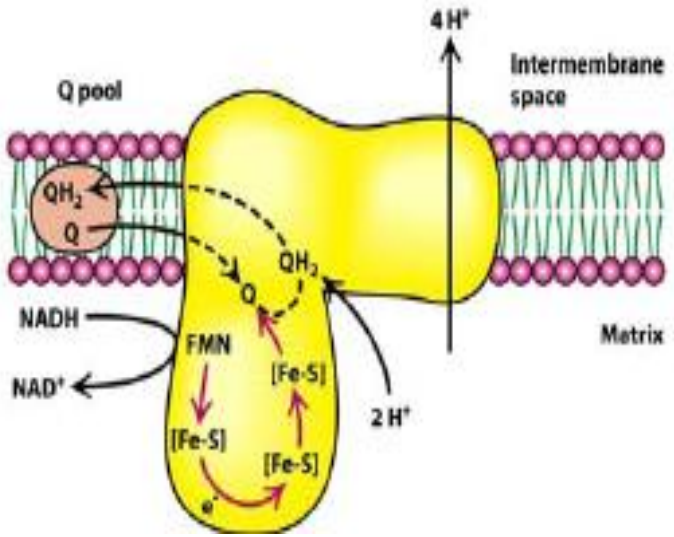


B. *Nitrosylation*



Fe–S cluster-based sensors (continued)

- Electron transfer proteins may contain multiple Fe-S centers.
- Iron-sulfur centers transfer only one electron, even if they contain two or more iron atoms, because of the close proximity of the iron atoms.



Electron Carriers

Thiol-based redox sensors

Coenzyme A – high energy compound

- Activating molecules Agent for transfer of acetyl groups CoA is the acetyl-transporting molecules linked via a thioester bond (high energy bond) 7.51 kcal/mol CoA contain ADP linked to pantothenic acid and mercaptoethylamine active part.

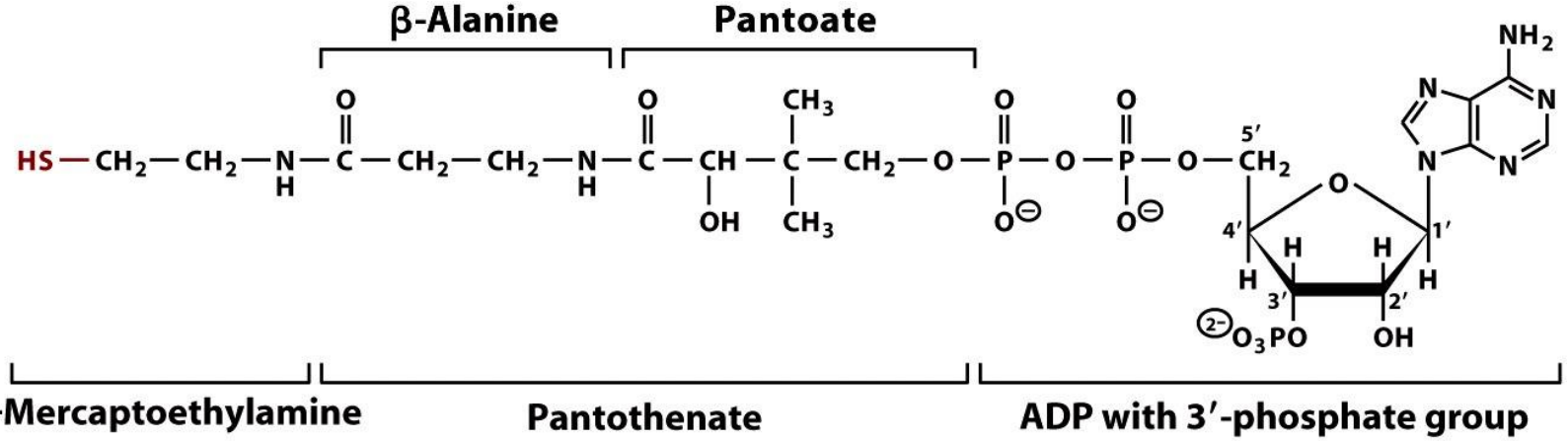
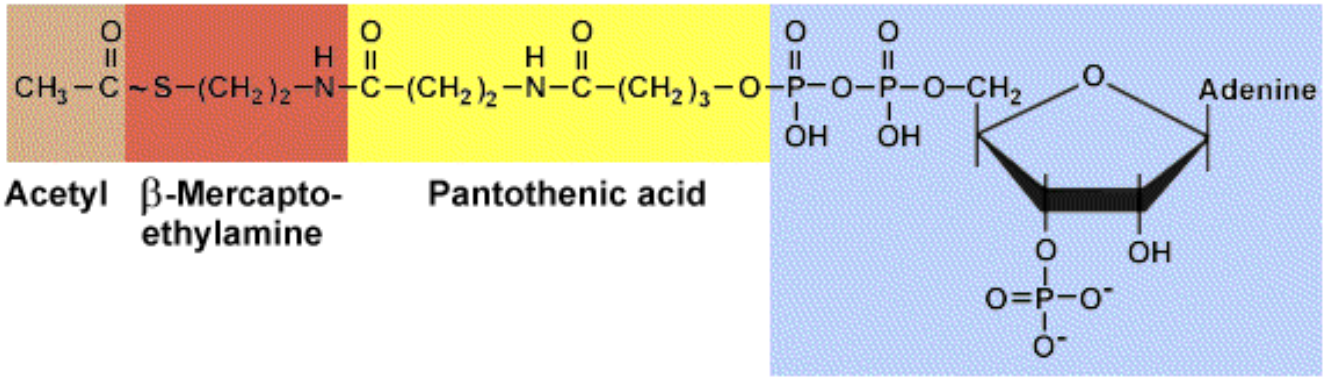
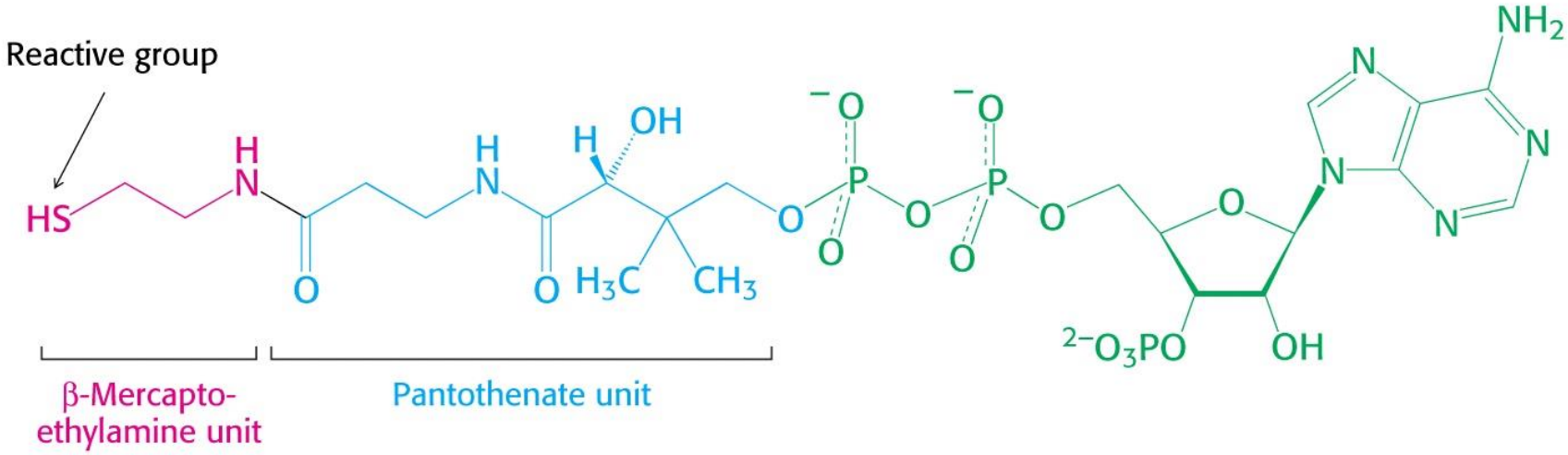


Figure 7-12a Principles of Biochemistry, 4/e

Acetyl coenzyme A



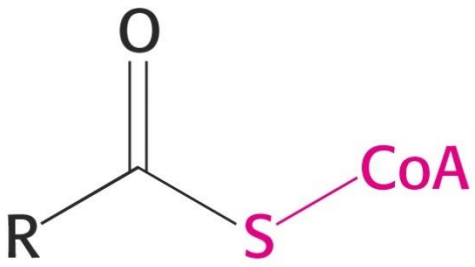
Structure of Coenzyme A



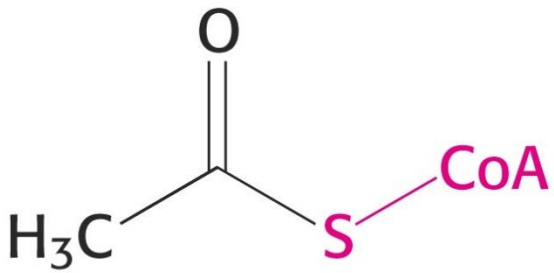
B vitamin

Activated carrier of two-carbon fragments

- Acyl groups linked to CoA by thioester bonds: high acyl group transfer potential (transfer is exergonic)
- Acetyl CoA carries an activated acetyl group just like ATP carries an activated phosphoryl group



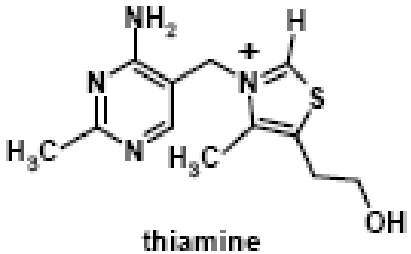
Acyl CoA



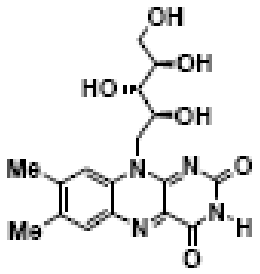
Acetyl CoA

Structure of water soluble vitamins

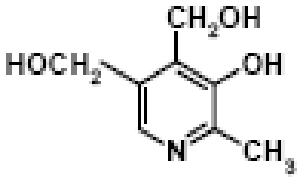
BO/BBM



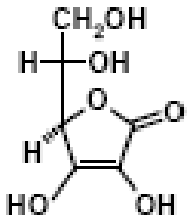
thiamine
vitamin B_1



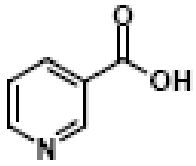
riboflavin
vitamin B_2



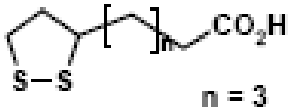
pyridoxine
vitamin B_6



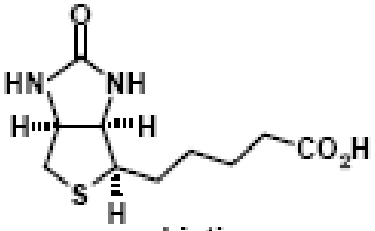
vitamin C
ascorbic acid



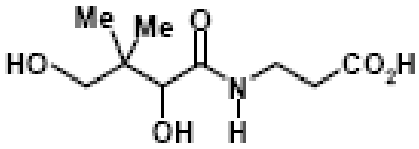
niacin
nicotinic acid



lipoate

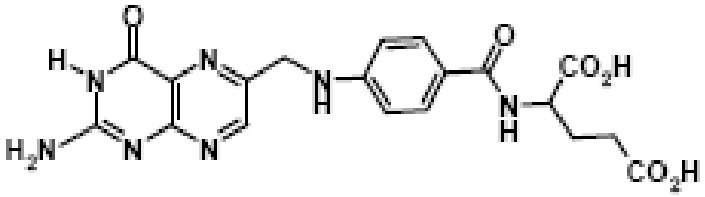


biotin
vitamin H



pantothenate

subunit of CoA-SH



folate

vitamin B_{12}