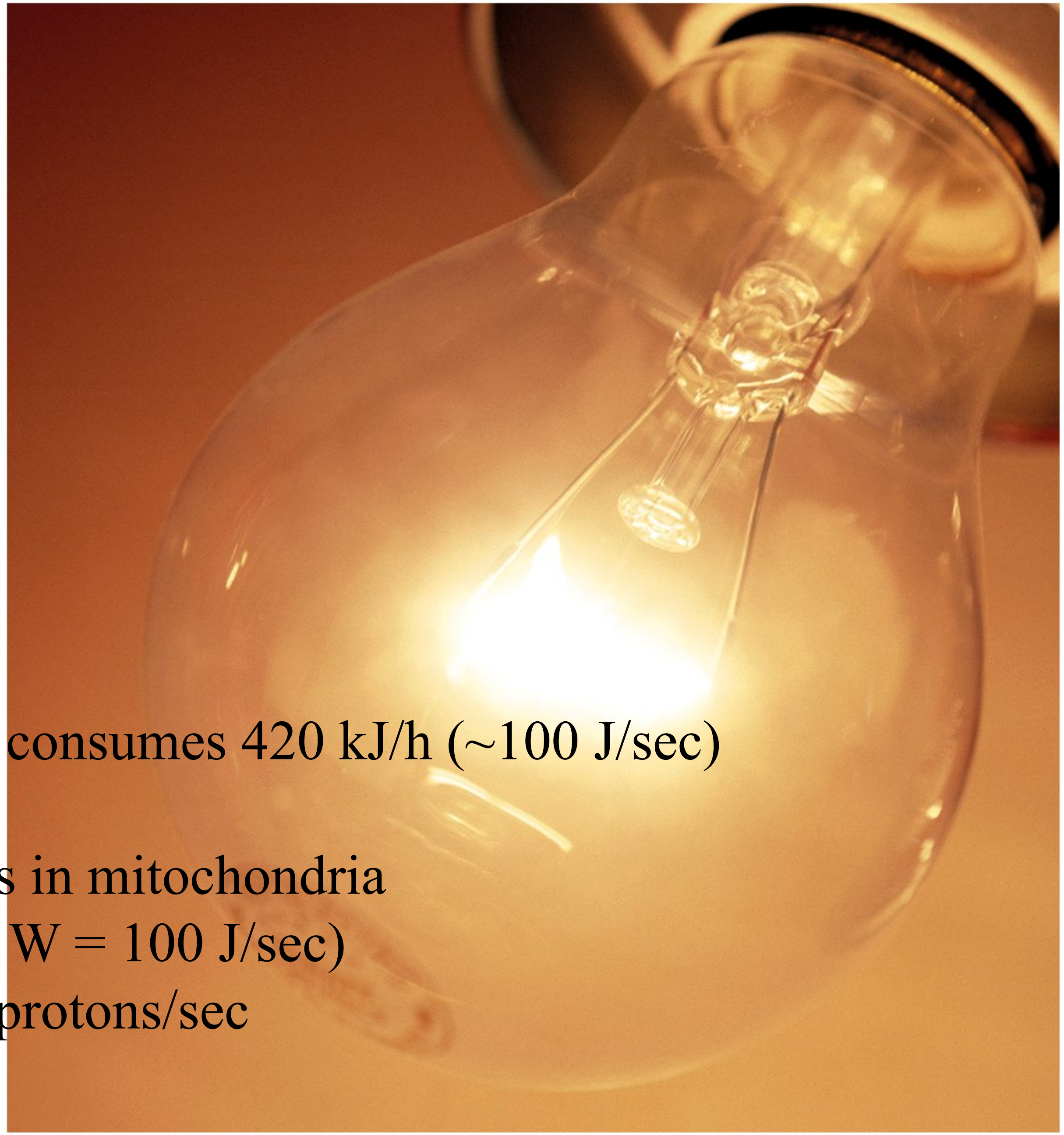
Chapter 5-I:

Electron Transport and Oxidative Phosphorylation



Chapter 17 Opener Fundamentals of Biochemistry, 2/e

A resting human body consumes 420 kJ/h (~100 J/sec)

Electrochemical events in mitochondria
0.2 V, 500 Amp (=100 W = 100 J/sec)
equivalent to ~3×10^{21} protons/sec

Mitochondrial electron-transport chain

- Electron transfer to O_2
- Regeneration of NAD^+ & FAD
- Proton transfer
- Electrochemical gradient
- Oxidative phosphorylation

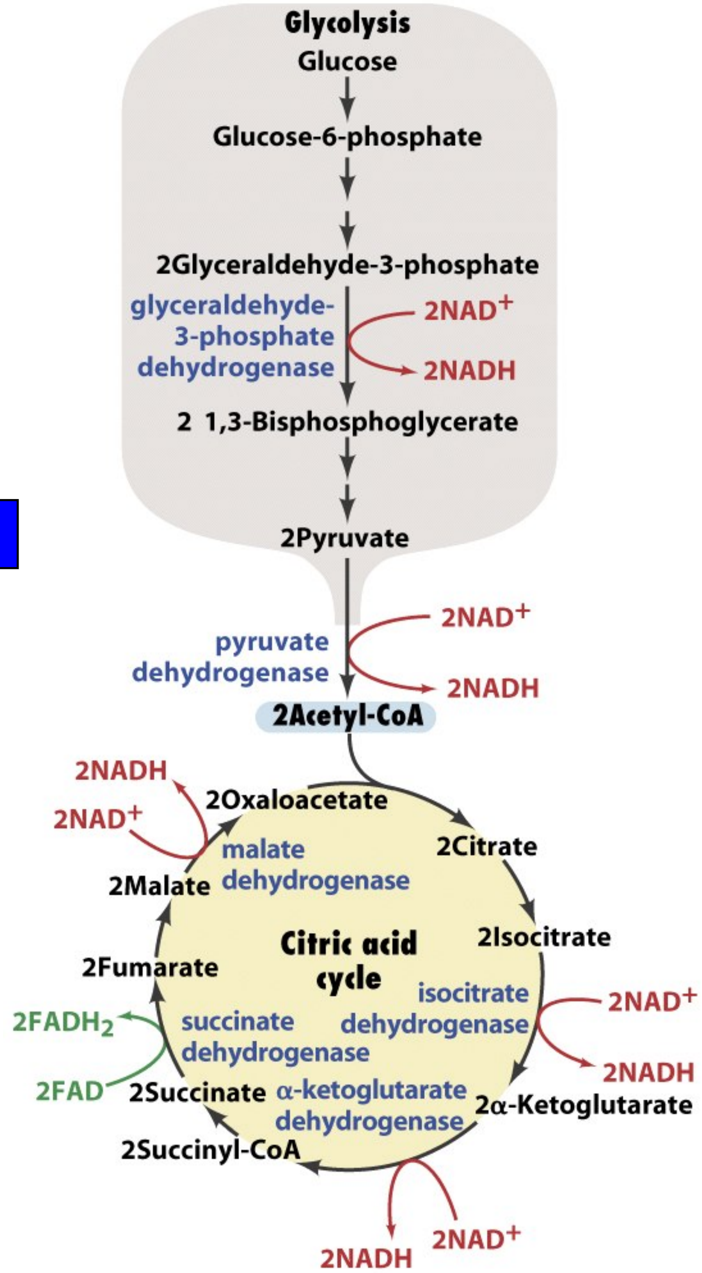


Figure 17-1 Fundamentals of Biochemistry, 2/e

The mitochondrion: bacterial size, variable shapes, ~2000/cell

<u>Anatomy</u>

Outer membrane: porins (free diffusion of molecules up to 10 kD),

Inner membrane: ~75% protein by mass, freely permeable only to O_2, CO_2, H_2O

numerous transport proteins (ATP, ADP, pyruvate, Ca^{2+}, phosphate)

Intermembrane space: equivalent to cytosol

Matrix: enzymes, DNA, RNA, ribosomes

Cristae: form microenvironments, local concentration of chemicals

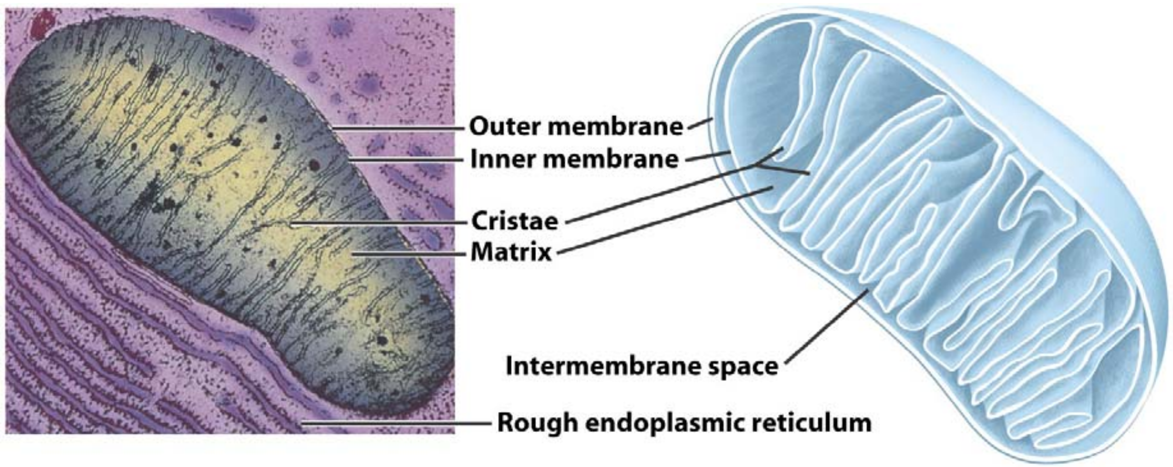


Figure 17-2 Fundamentals of Biochemistry, 2/e

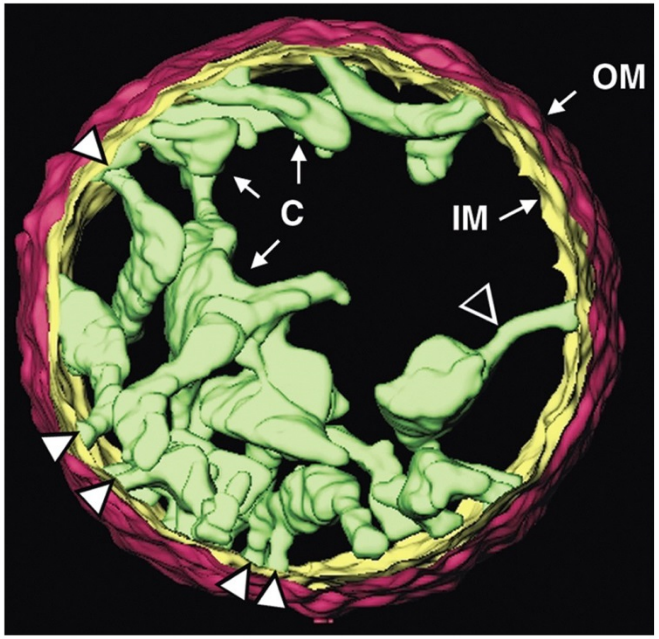


Figure 17-3 Fundamentals of Biochemistry, 2/e

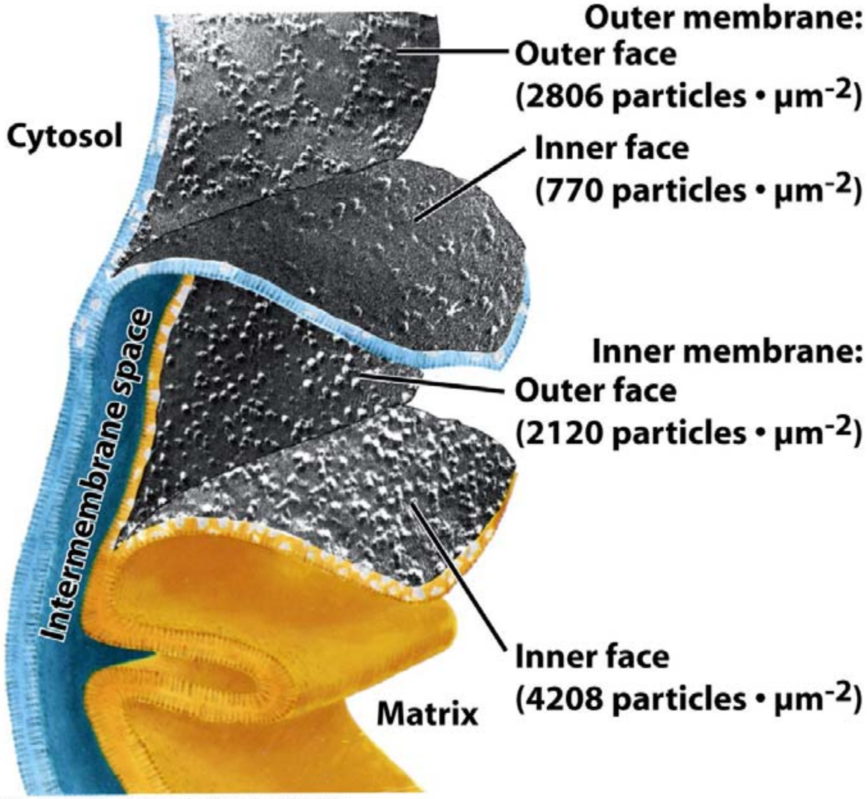


Figure 17-4 Fundamentals of Biochemistry, 2/e

***Neurospora* sp. mitochondrial structure**

Mitochondrial transport system

Inner membrane proteins

1. Transport of cytosolic reducing equivalents
    - Malate-aspartate shuttle (page 504)
    - Glycerophosphate shuttle: in insect flight muscle

2. ADP-ATP transport
    - Translocator (adenine nucleotide translocase)
    - Electrogenic antiport: export of ATP & import of ADP
        - resulting in export of one negative charge
        - driven by membrane potential difference

3. Phosphate transport
    - Phosphate carrier: Pi return to mitochondria
    - ATP synthesis in mitochondria, utilization in cytosol
    - Electroneutral Pi-H symport driven by ΔpH

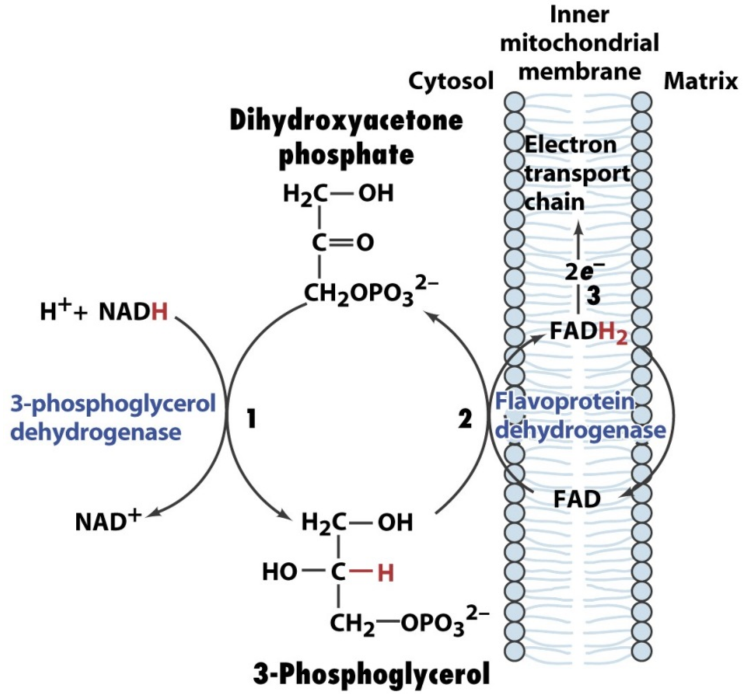


Figure 17-5 Fundamentals of Biochemistry, 2/e

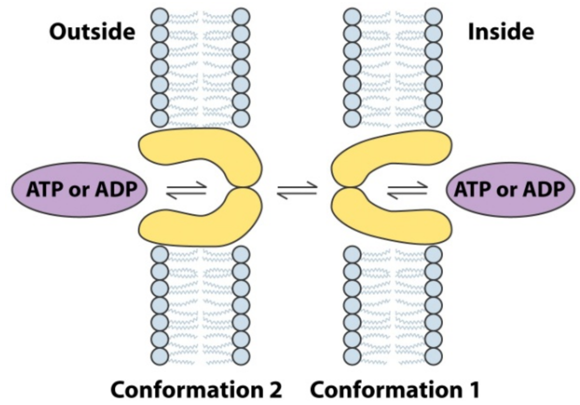


Figure 17-6 Fundamentals of Biochemistry, 2/e

Electron transport

Electron transport chain (ETC) depending on reduction potentials

$\Delta G^{o\prime} = -nF\Delta E^{o\prime}$

1 mol of NADH oxidation: -218 kJ/mol

1 mol of ATP synthesis: 30.5 kJ/mol

Thermodynamic efficiency: 30.5 x 3 x 100 / 218 = 42% under standard conditions

It is ~70% under physiological conditions

The sequence of ETC

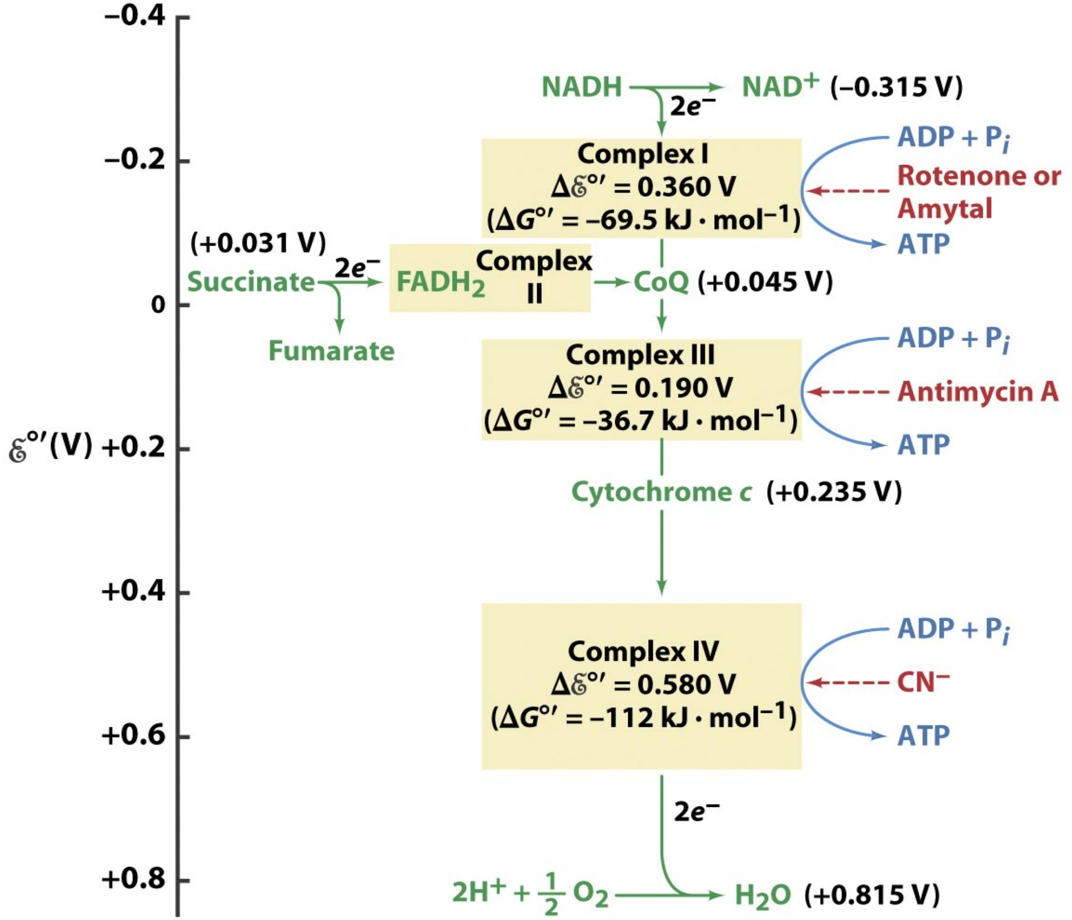


Figure 17-7 Fundamentals of Biochemistry, 2/e

Table 13-3 Standard Reduction Potentials of Some Biochemically Important Half-Reactions

Half-Reaction	$\mathscr{E}°'$ (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O$	0.815
$SO_4^{2-} + 2H^+ + 2e^- \rightleftharpoons SO_3^{2-} + H_2O$	0.48
$NO_3^- + 2H^+ + 2e^- \rightleftharpoons NO_2^- + H_2O$	0.42
Cytochrome a_3 (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome a_3 (Fe^{2+})	0.385
$O_2(g) + 2H^+ + 2e^- \rightleftharpoons H_2O_2$	0.295
Cytochrome *a* (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome *c* (Fe^{2+})	0.235
Cytochrome c_1 (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome *b* (Fe^{2+}) (*mitochondrial*)	0.077
Ubiquinone + $2H^+ + 2e^-$ $\rightleftharpoons$ ubiquinol	0.045
Fumarate$^-$ + $2H^+ + 2e^-$ $\rightleftharpoons$ succinate$^-$	0.031
FAD + $2H^+ + 2e^-$ $\rightleftharpoons$ $FADH_2$ (*in flavoproteins*)	~0.
Oxaloacetate$^-$ + $2H^+ + 2e^-$ $\rightleftharpoons$ malate$^-$	−0.166
Pyruvate$^-$ + $2H^+ + 2e^-$ $\rightleftharpoons$ lactate$^-$	−0.185
Acetaldehyde + $2H^+ + 2e^-$ $\rightleftharpoons$ ethanol	−0.197
FAD + $2H^+ + 2e^-$ $\rightleftharpoons$ $FADH_2$ (*free coenzyme*)	−0.219
$S + 2H^+ + 2e^- \rightleftharpoons H_2S$	−0.23
Lipoic acid + $2H^+ + 2e^-$ $\rightleftharpoons$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \rightleftharpoons NADH$	−0.315
$NADP^+ + H^+ + 2e^- \rightleftharpoons NADPH$	−0.320
Cystine + $2H^+ + 2e^-$ $\rightleftharpoons$ 2 cysteine	−0.340
Acetoacetate$^-$ + $2H^+ + 2e^-$ $\rightleftharpoons$ β-hydroxybutyrate$^-$	−0.346
$H^+ + e^- \rightleftharpoons \frac{1}{2}H_2$	−0.421
Acetate$^-$ + $3H^+ + 2e^-$ $\rightleftharpoons$ acetaldehyde + H_2O	−0.581

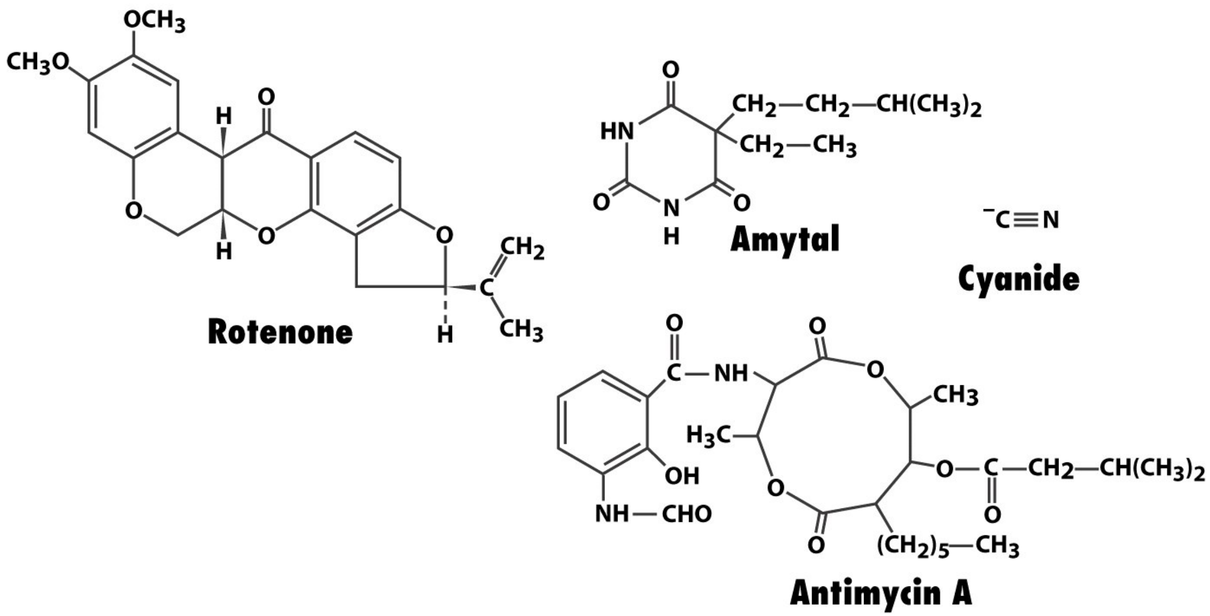
Source: Mostly from Loach, P.A., *In* Fasman, G.D. (Ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), Physical and Chemical Data, Vol. I, pp. 123–130, CRC Press (1976).

Table 13-3 Fundamentals of Biochemistry, 2/e

ETC inhibitors

Measuring the effect on O_2 consumption

Restoration of ETC by addition of intermediates



Unnumbered figure pg 552 Fundamentals of Biochemistry, 2/e

Table 17-1 Reduction Potentials of Electron-Transport Chain Components in Resting Mitochondria

Component	$\mathscr{E}^{\circ\prime}$ (V)
NADH	−0.315
Complex I (NADH–CoQ oxidoreductase; ~900 kD, 43 subunits):	
FMN	?
(Fe–S)N-1a	−0.380
(Fe–S)N-1b	−0.250
(Fe–S)N-2	−0.030
(Fe–S)N-3,4	−0.245
(Fe–S)N-5,6	−0.270
Succinate	0.031
Complex II (succinate–CoQ oxidoreductase; ~120 kD, 4 subunits):	
FAD	−0.040
[2Fe–2S]	−0.030
[4Fe–4S]	−0.245
[3Fe–4S]	0.060
Heme b_{560}	−0.080
Coenzyme Q	0.045
Complex III (CoQ–cytochrome *c* oxidoreductase; ~240 kD, 9–11 subunits):	
Heme b_H (b_{562})	0.030
Heme b_L (b_{566})	−0.030
[2Fe–2S]	0.280
Heme c_1	0.215
Cytochrome *c*	0.235
Complex IV (cytochrome *c* oxidase; ~205 kD, 8–13 subunits):	
Heme *a*	0.210
Cu_A	0.245
Cu_B	0.340
Heme a_3	0.385
O_2	0.815

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

Table 17-1 Fundamentals of Biochemistry, 2/e

ETC proteins

Not associated
Not in equimolar amounts

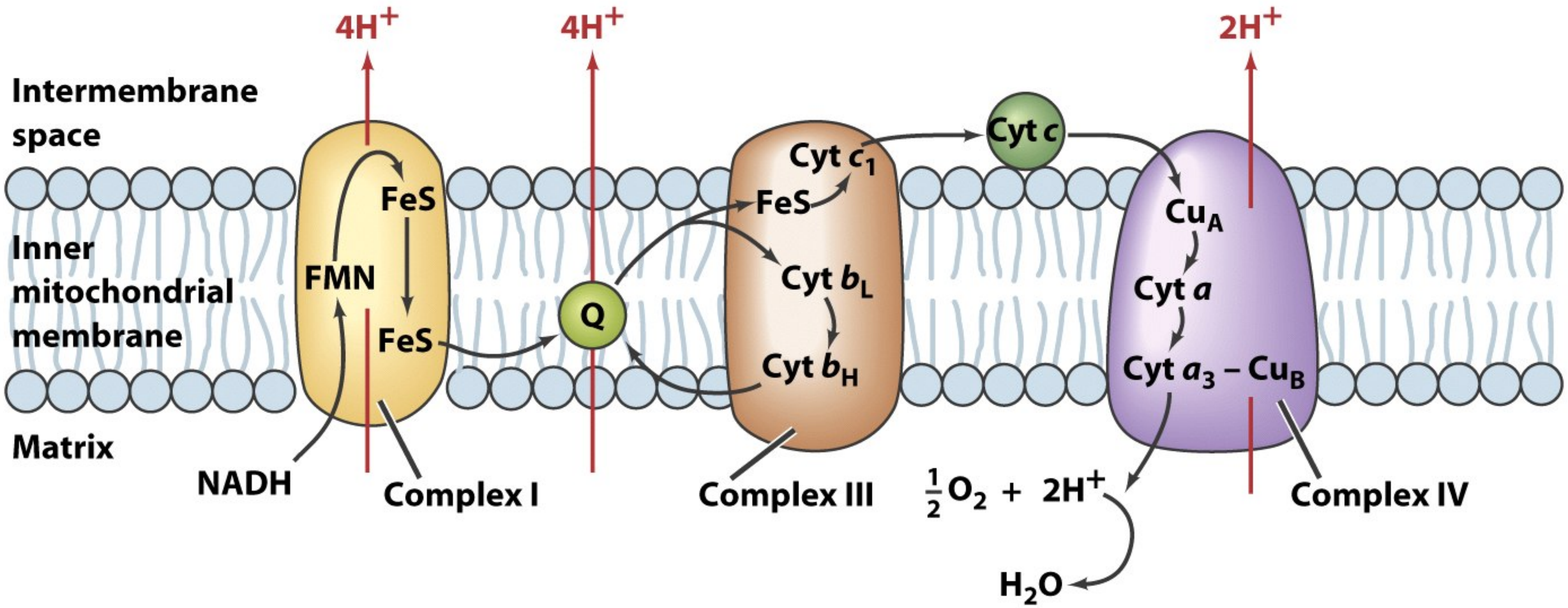


Figure 17-8 Fundamentals of Biochemistry, 2/e

Complex I

NADH-CoQ oxidoreductase
43 subunits in mammals
1 FMN
6~7 iron-sulfur clusters

EM at 22 Å

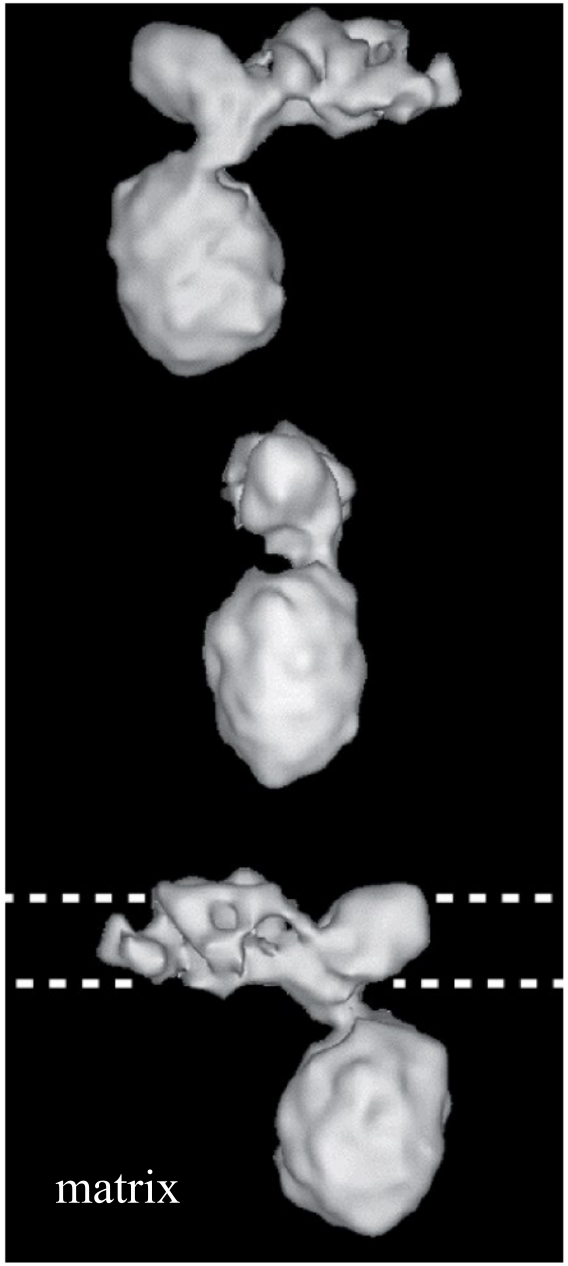
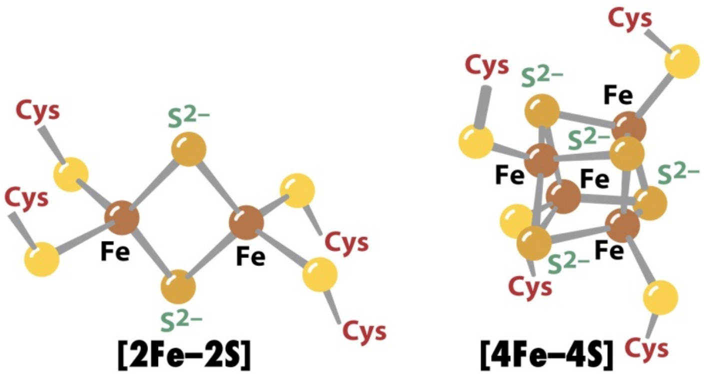


Figure 17-9 Fundamentals of Biochemistry, 2/e



Unnumbered figure pg 554 Fundamentals of Biochemistry, 2/e

Iron-sulfur clusters
Reduction & oxidation of one-electron

Bacterial ferredoxin

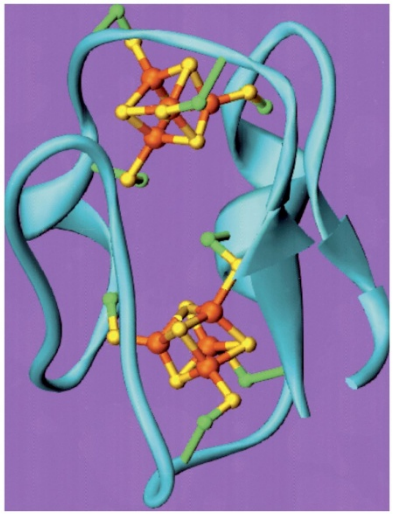
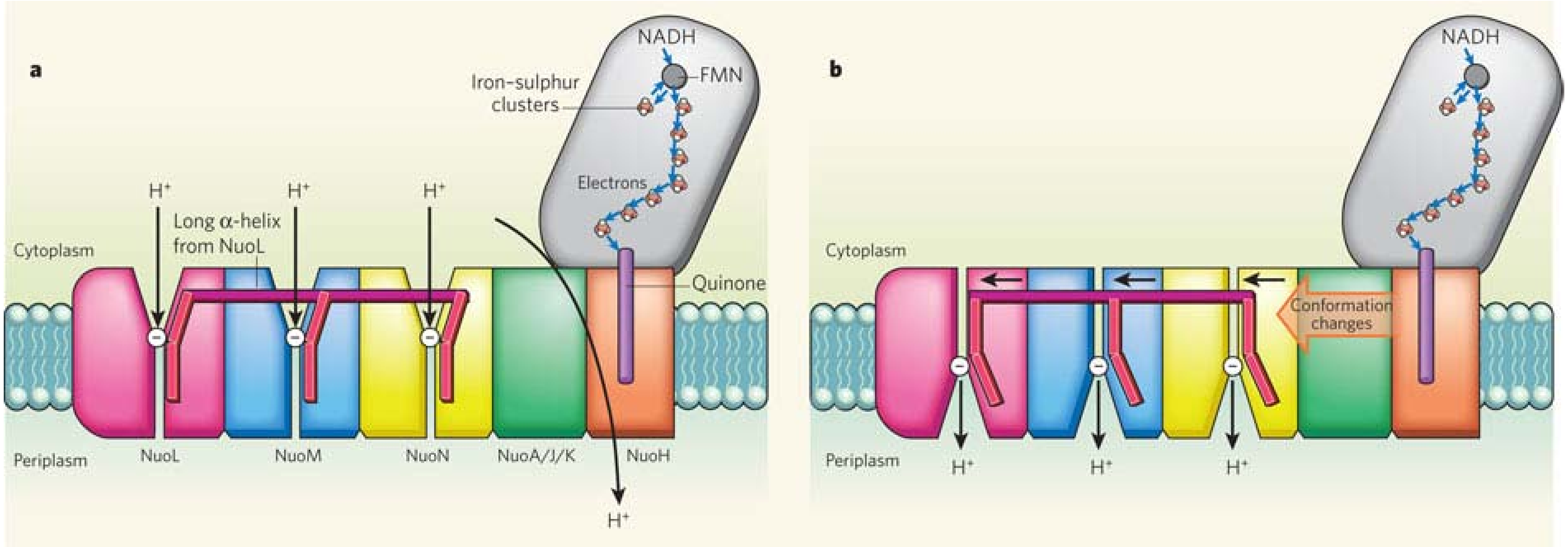


Figure 17-10 Fundamentals of Biochemistry, 2/e


a
NADH
FMN
Iron–sulphur
clusters
Electrons
H⁺
H⁺
H⁺
Long α-helix
from NuoL
Cytoplasm
Quinone
Periplasm
NuoL
NuoM
NuoN
NuoA/J/K
NuoH
H⁺
b
NADH
Cytoplasm
Conformation
changes
Periplasm
H⁺
H⁺
H⁺

Mediate one-electron transfer

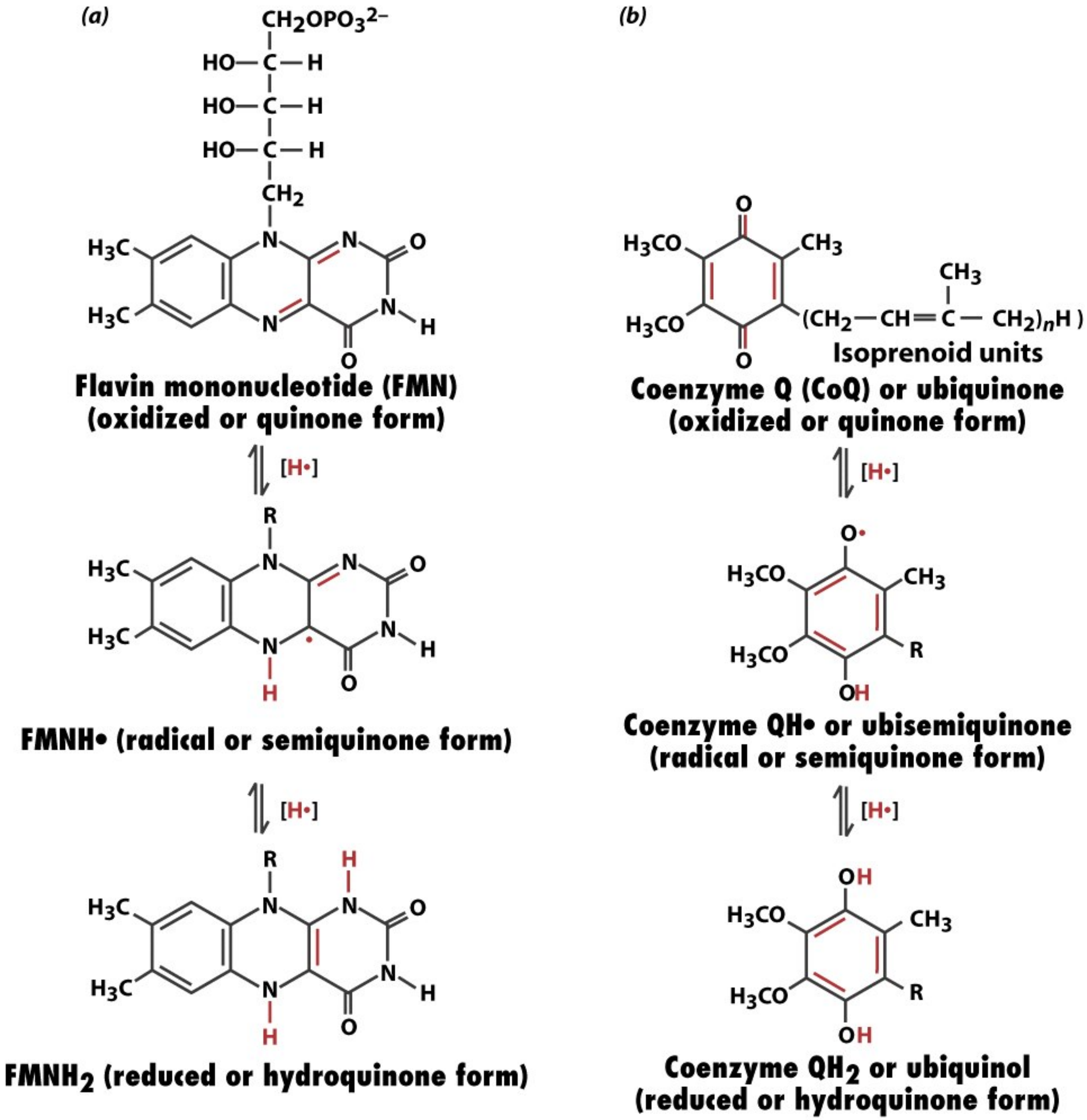


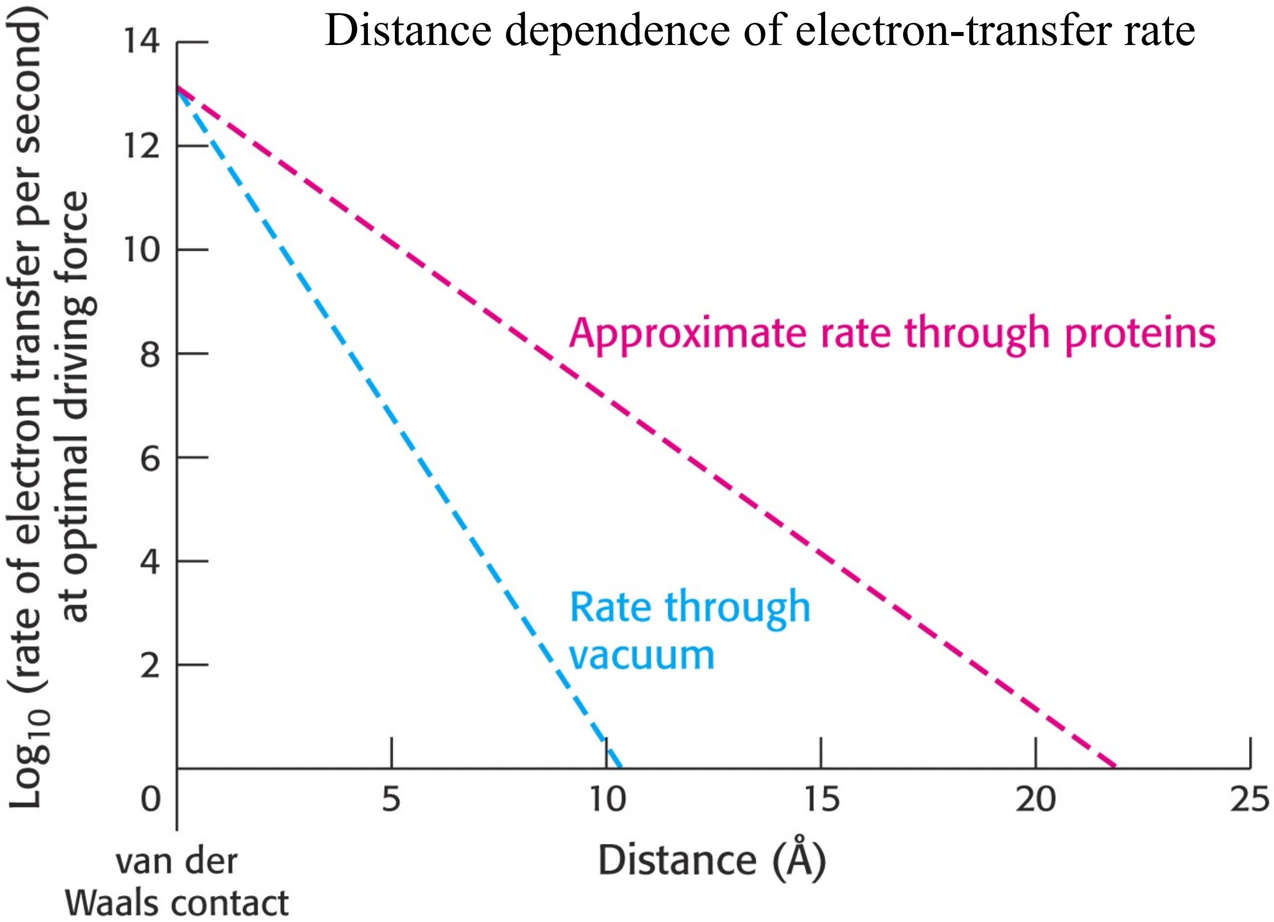
Figure 17-11 Fundamentals of Biochemistry, 2/e

Electron transfer

Jumping between protein-embedded redox groups separated by <14 Å

~10 fold decrease in ET rates for each 1.7 Å increase in distance

Distance dependence of electron-transfer rate



Proton translocation

Transfer of 4 protons

Driven by **<u>conformational changes</u>** induced by redox state,

which **<u>alter pK values</u>** of ionizable side chains

probably via proton jumping (proton wire) including water

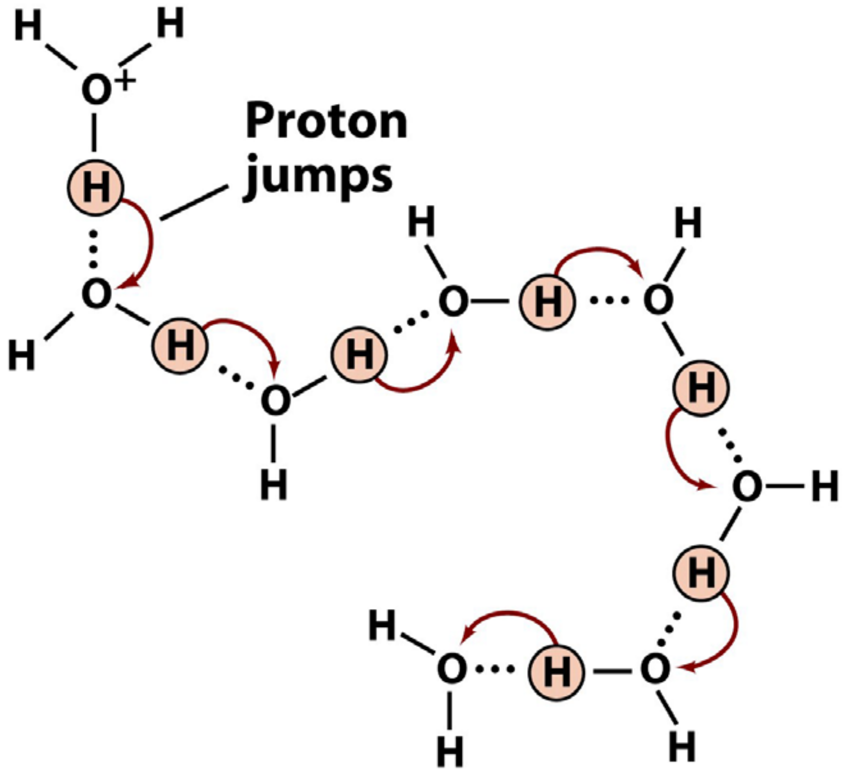
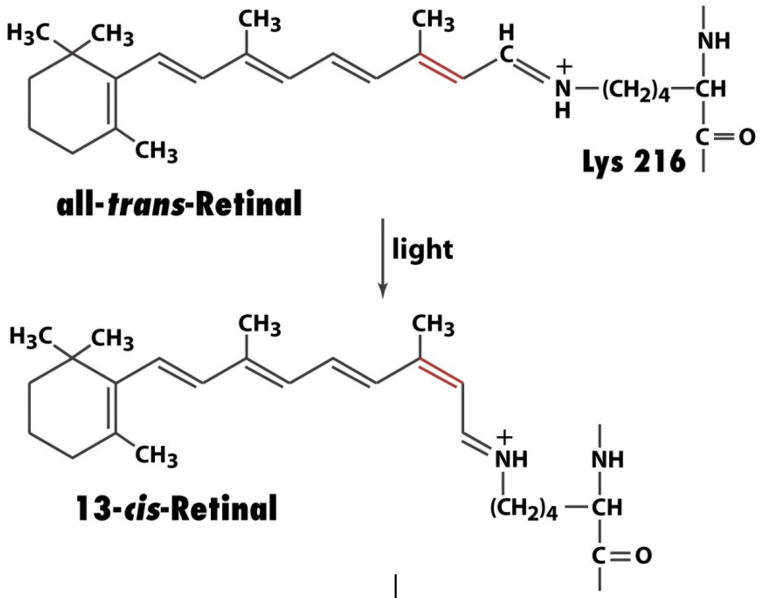
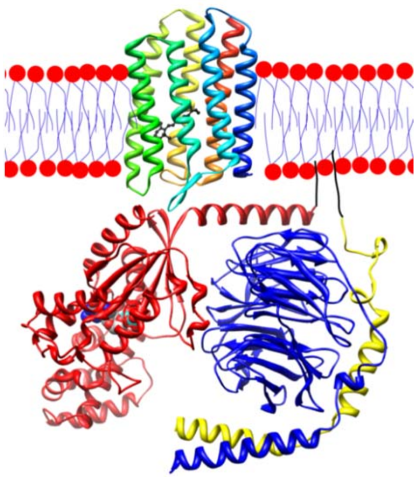


Figure 2-15 Fundamentals of Biochemistry, 2/e

Bacteriorhodopsin: a model proton pump
Light driven proton pump



A sequence of protein conformational adjustments (~1 Å) that restore the system to its ground state over a period of ~10 ms
Change pKs of several a.a. side chains



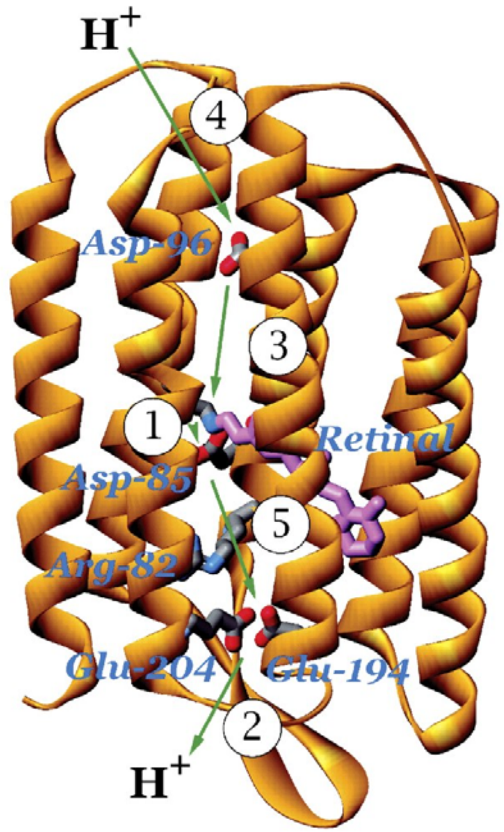
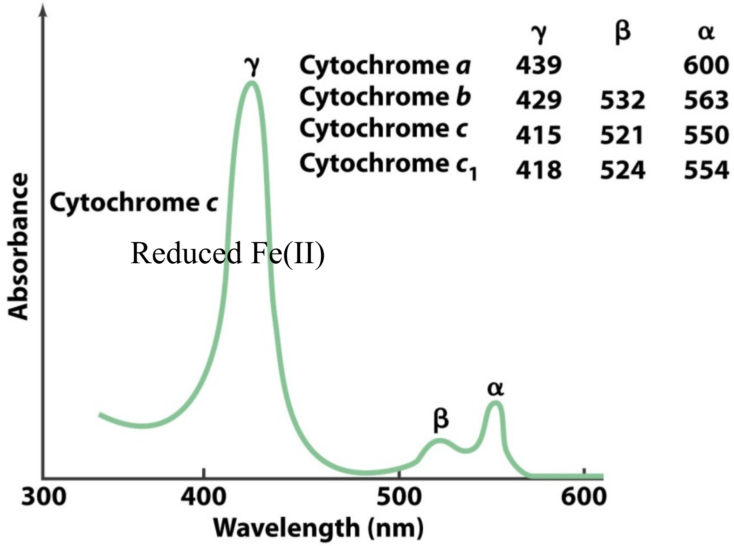


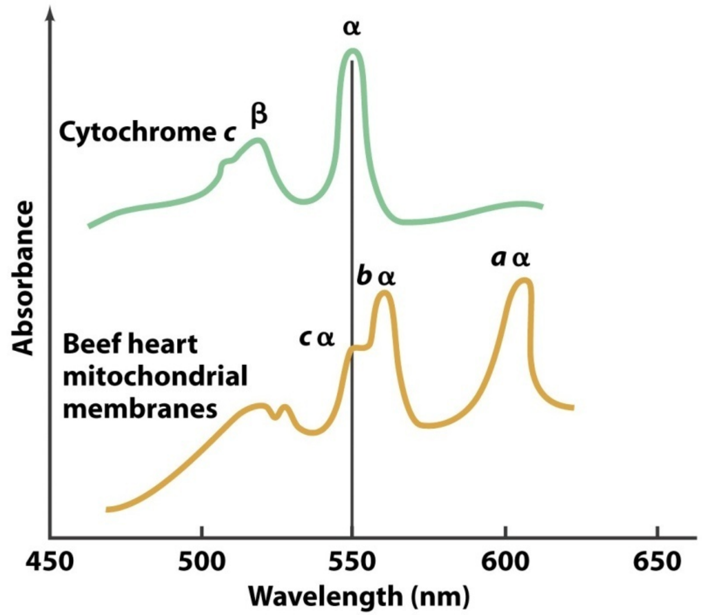
Figure 17-12 Fundamentals of Biochemistry, 2/e

Cytochromes: electron transfer heme proteins



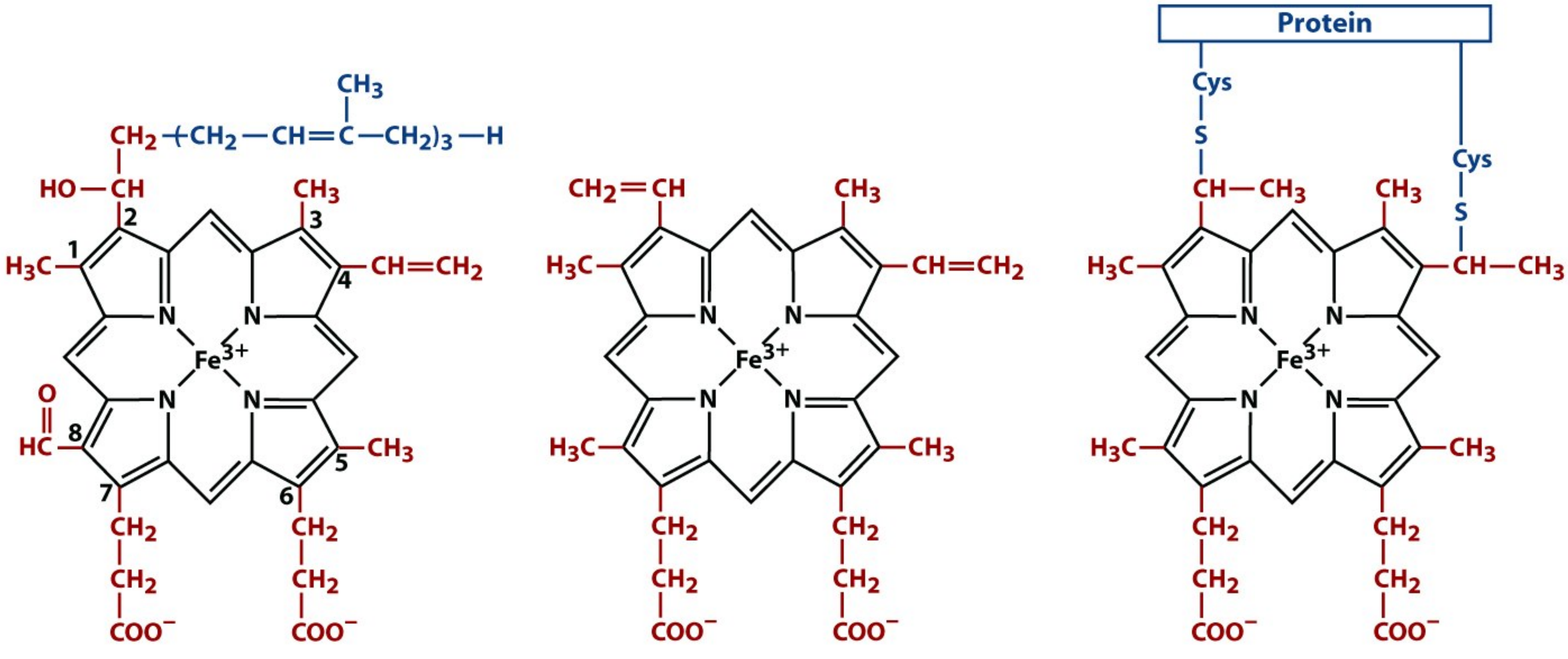
Box 17-1 figure 1a Fundamentals of Biochemistry, 2/e

Variable wavelength of α peak
b560: heme b with α band absorbance at 560 nm



Box 17-1 figure 1b Fundamentals of Biochemistry, 2/e

Different cytochromes with differently substituted heme group
Different heme environment causes different α peak wavelengths



Heme *a* **Heme *b*** **(iron–protoporphyrin IX)** **Heme *c***

Box 17-1 figure 2 Fundamentals of Biochemistry, 2/e

Complex II

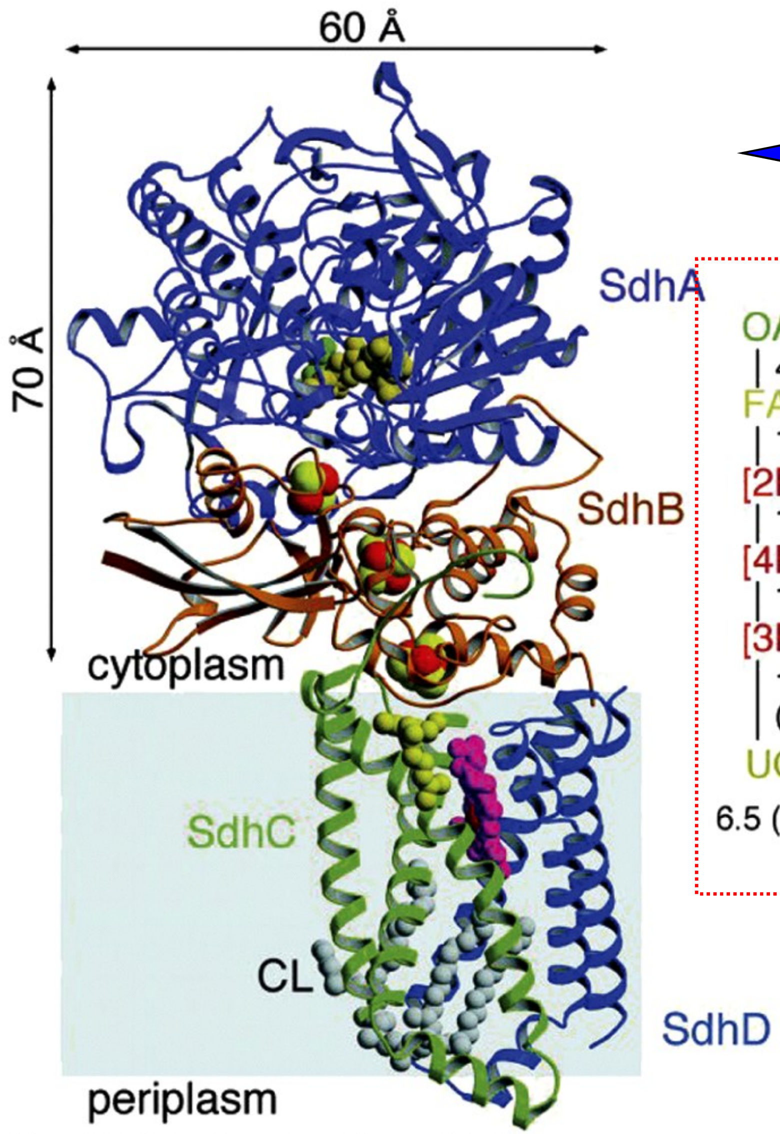
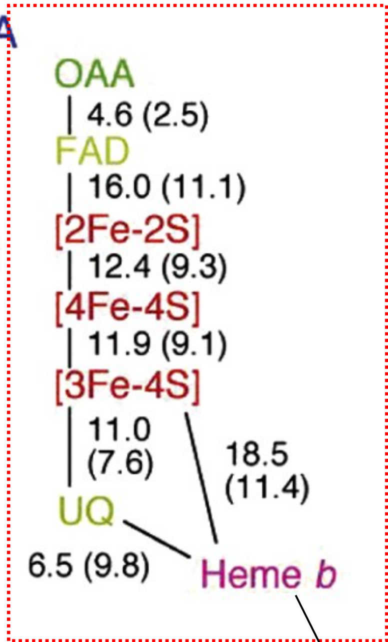


Figure 17-13b Fundamentals of Biochemistry, 2/e



4 subunits

E. coli Complex II

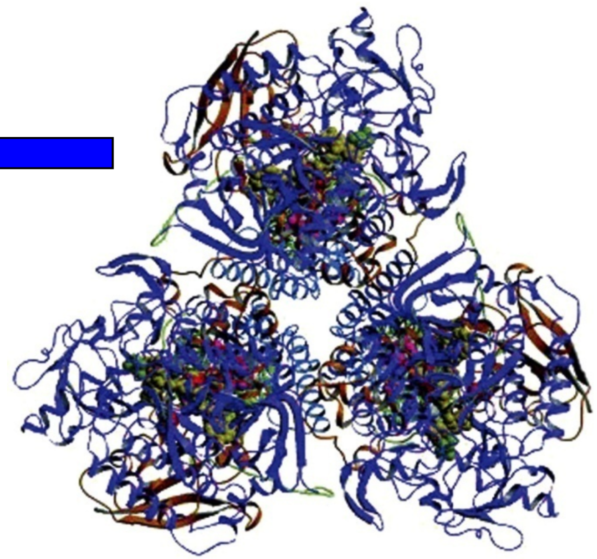


Figure 17-13a Fundamentals of Biochemistry, 2/e

Fine tuning the system's electronic properties to suppress side reaction: prevent ROS

Complex III (cytochrome bc1)

Two b type cytochrome: Heme b_L & b_H.

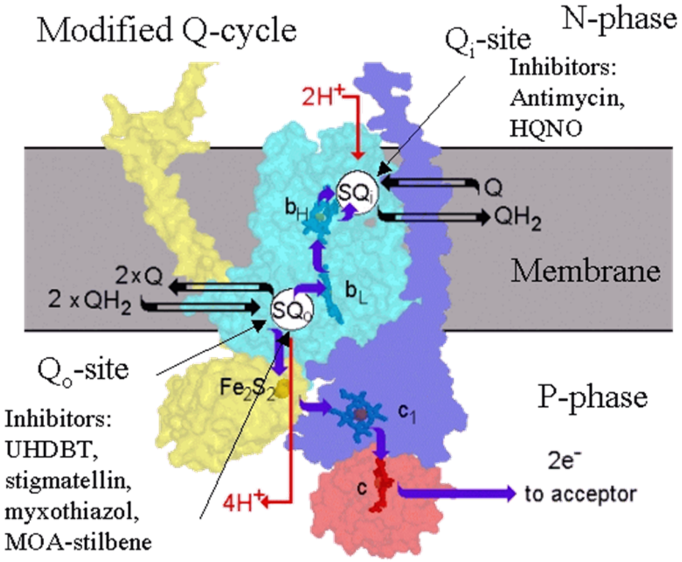
b562 (High potential)

b566 (Low potential)

One c1 cyt.

One [2Fe-2S] cluster: Rieske center (ISP)

His instead of Cys



Yeast

Homodimer with 9 subunits

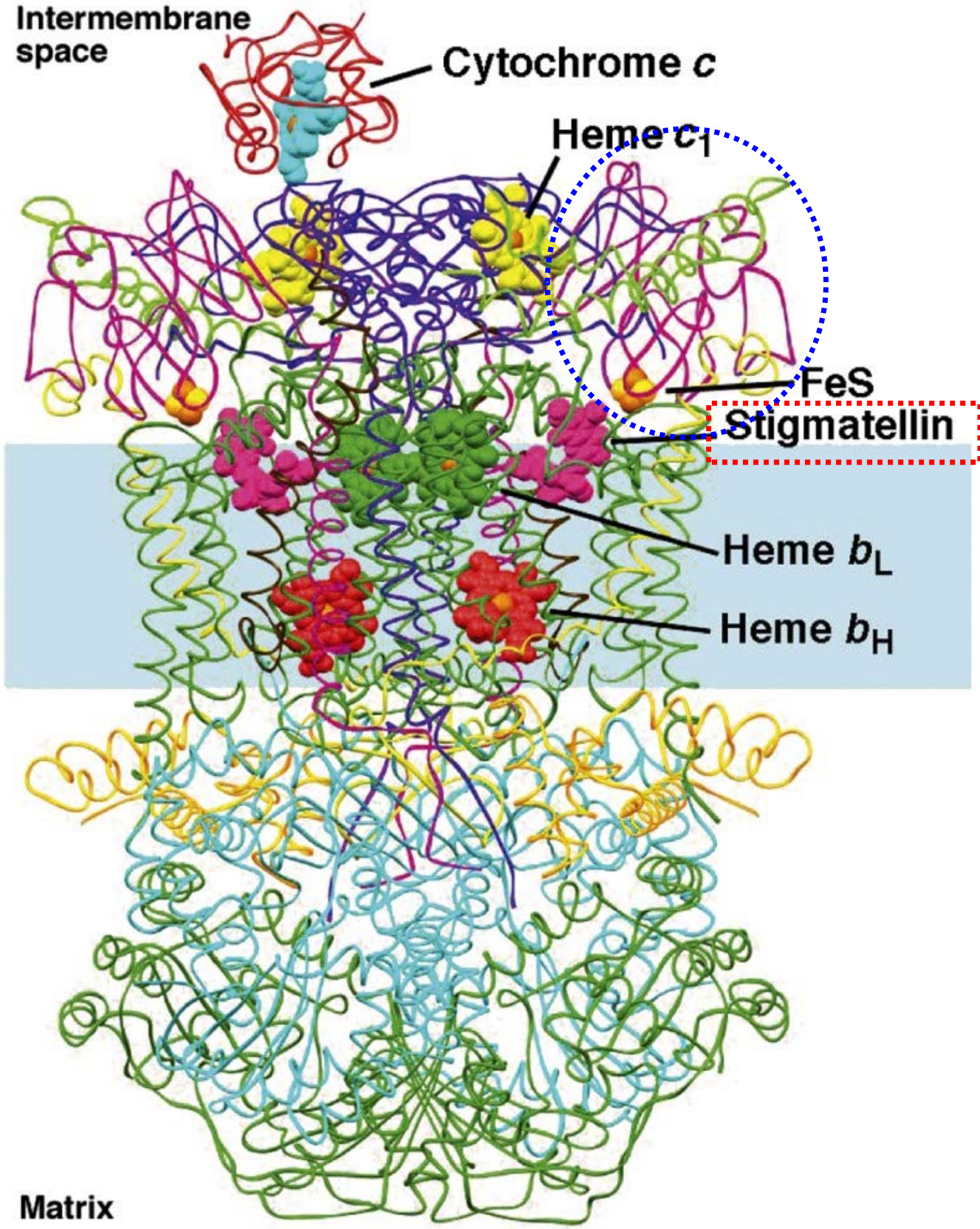
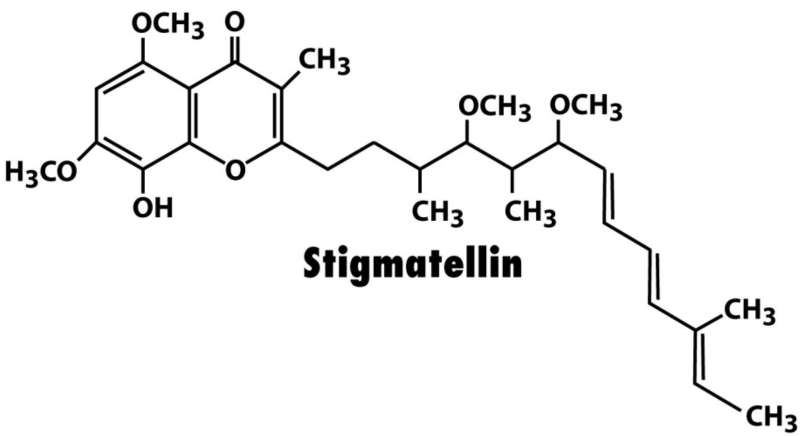


Figure 17-14 Fundamentals of Biochemistry, 2/e

The Q cycle

Transport of proton
driven by electron transport

Independent presence of Q_o & Q_i sites
Stigmatellin binds Q_o site, blocking steps 3&4
Antimycin A binds Q_i site, blocking steps 7&8



Stigmatellin

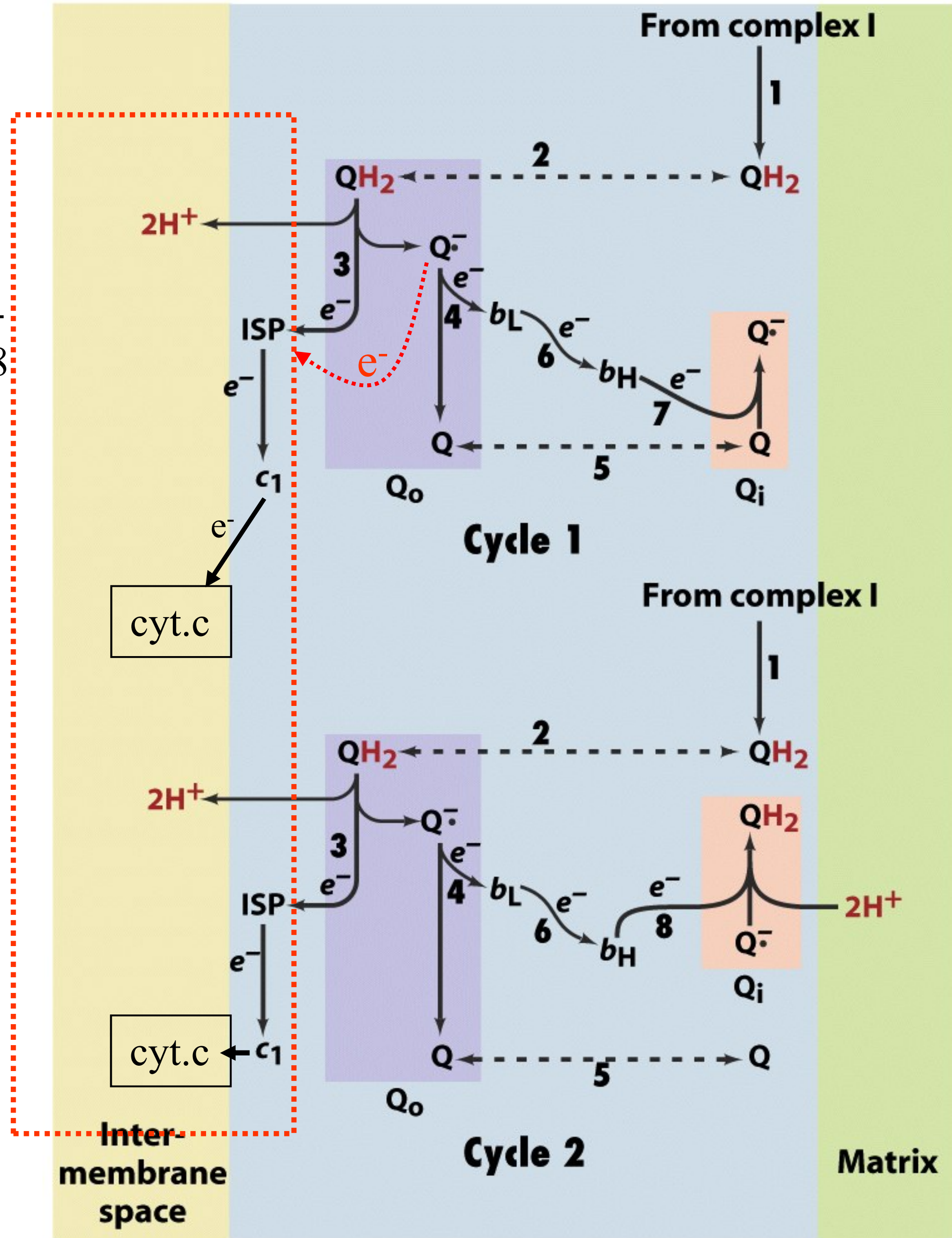


Figure 17-15 Fundamentals of Biochemistry, 2/e

Cyt.c: a soluble electron carrier

A peripheral membrane protein
Shuttle electrons between Complex III and IV

Conserved structure and invariant Lys residues
- Differential labeling of ε-amino by acetylation
- Complementary interaction with – charges

Binding to bc1 complex: Interfacial area: 880 Å^2
- Less tenuous than protein-protein interaction (<1600)
- Fast binding & release

Lys protected from acetylation when bound to bc1 or oxidase

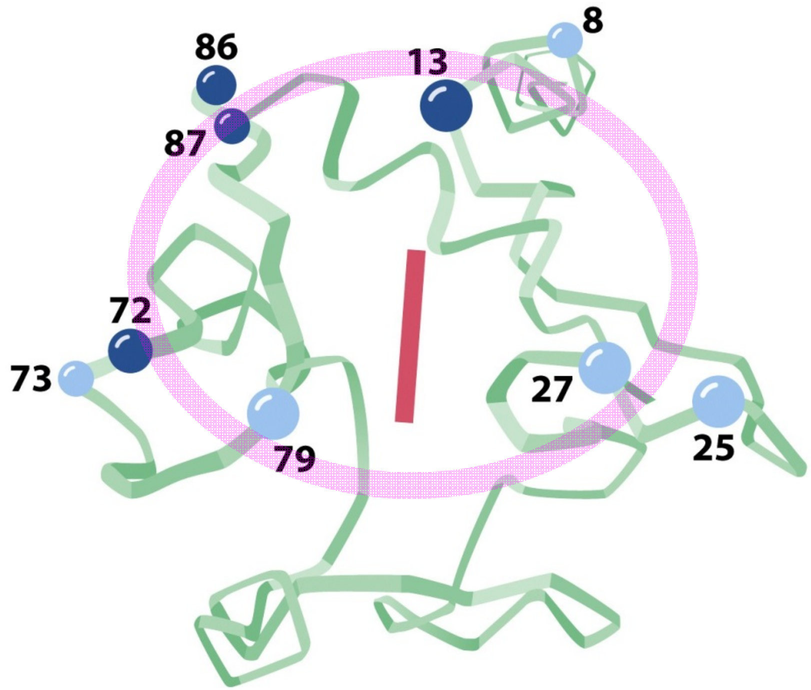
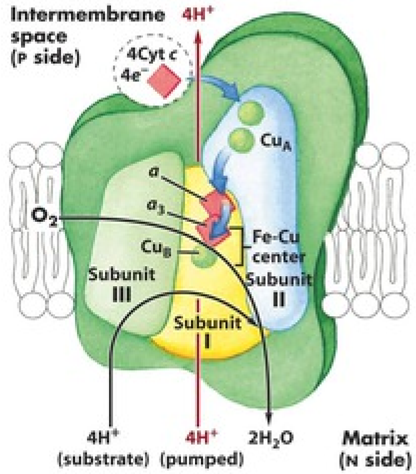


Figure 17-16 Fundamentals of Biochemistry, 2/e

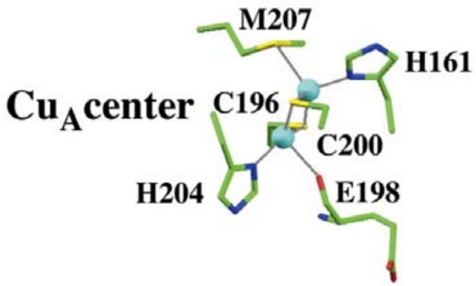
Complex IV (cyt.c oxidase)



4 cytochrome c (Fe^{2+}) + 4 H^+ + O_2 → 4 cytochrome c (Fe^{3+}) + 2 H_2O
Encoded by mitochondrial (Subunit I, II, III) & nuclear DNAs

Four redox centers
Cytochrome a: Subunit I
Cytochrome a3: Subunit I
CuB: Subunit I
CuA center: a pair of copper atoms, bridged by the sulfur atoms of Cys in Subunit II

Electron flow
Cytochrome c
CuA center
Heme a
Heme a3
CuB



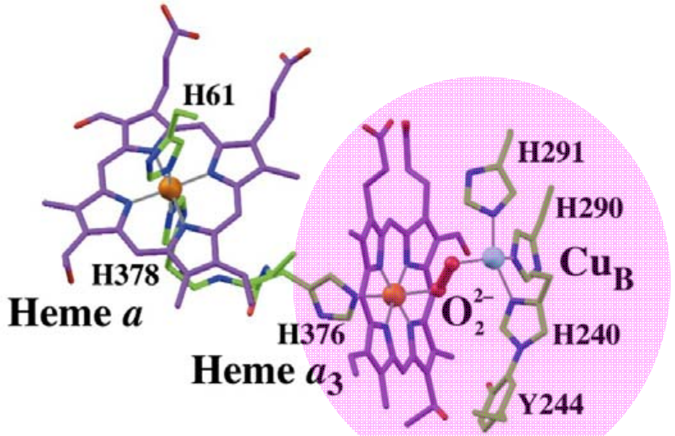


Figure 17-18 Fundamentals of Biochemistry, 2/e

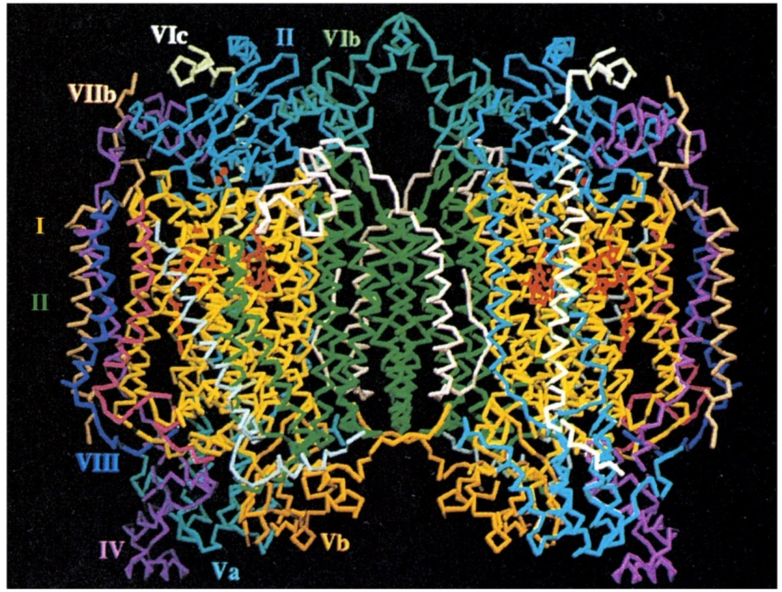


Figure 17-17 Fundamentals of Biochemistry, 2/e

Bovine heart complex (homodimer of 26 subunits)

Reduction of O_2 by complex IV

Take place at cytochrome a3-CuB binuclear complex

Simultaneous input of 4 electrons

Fully reduced Fe(II)-Cu(I) provide only 3 electrons to O2

Where comes the 4th? Tyr244 by forming a transient Tyr radical (TyrO$^\bullet$)

(Tyr244: electron and proton donor)

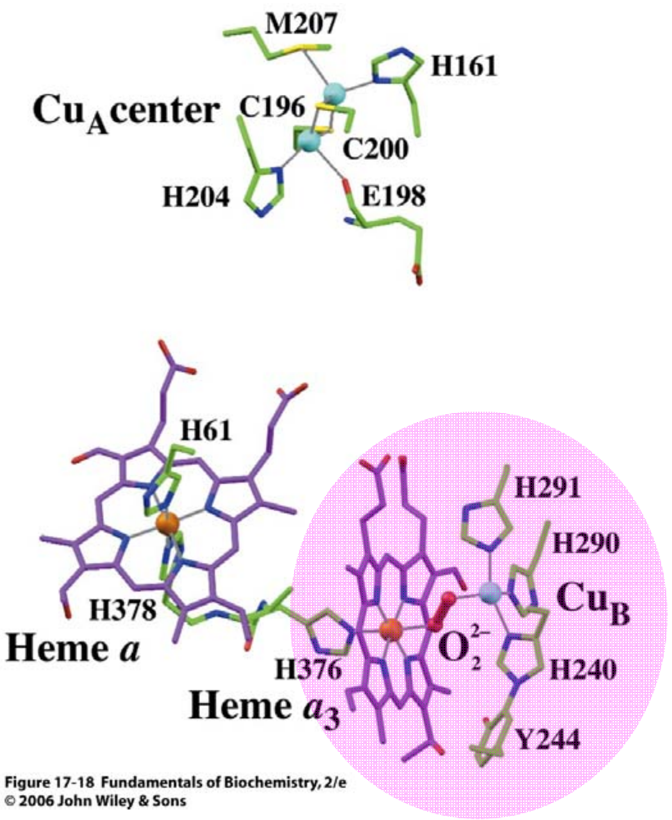


Figure 17-18 Fundamentals of Biochemistry, 2/e
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Proposed reaction sequence

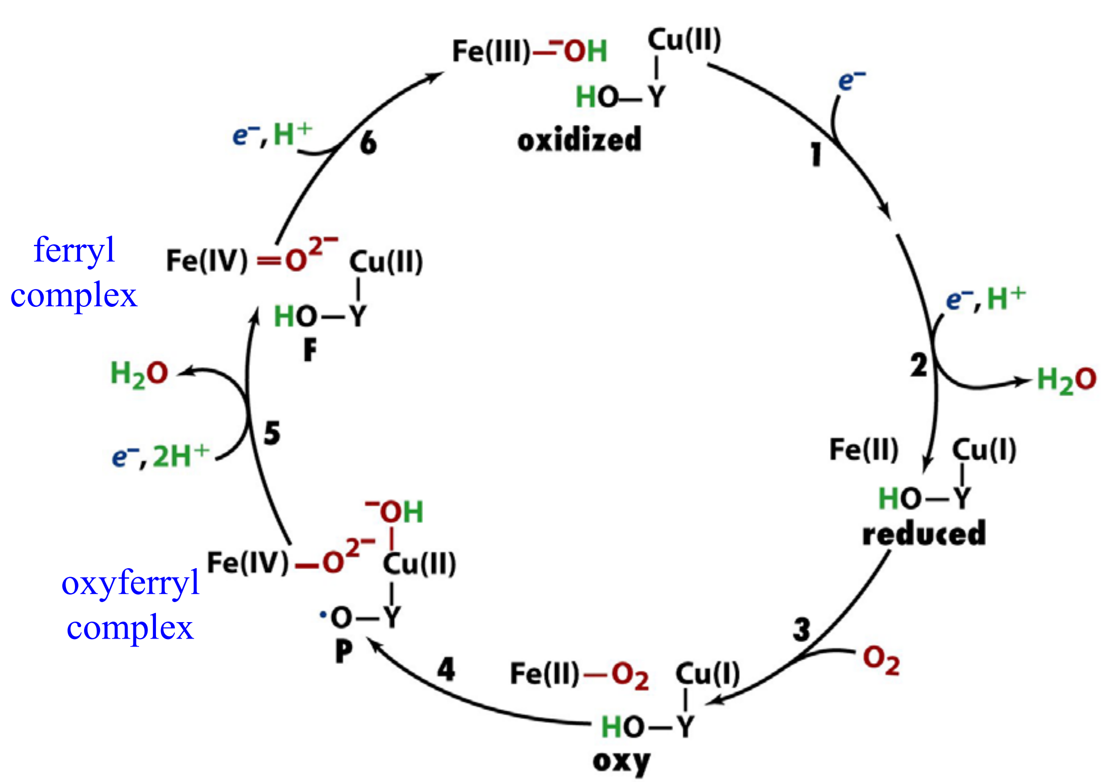

Figure 17-19 Fundamentals of Biochemistry, 2/e
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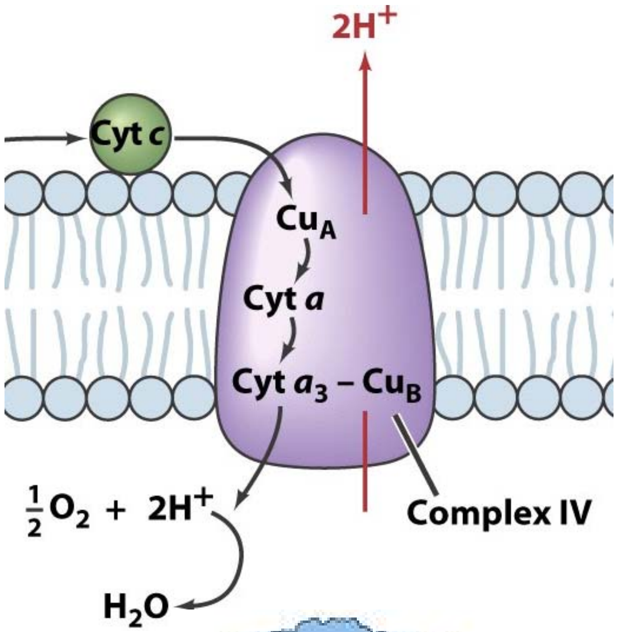
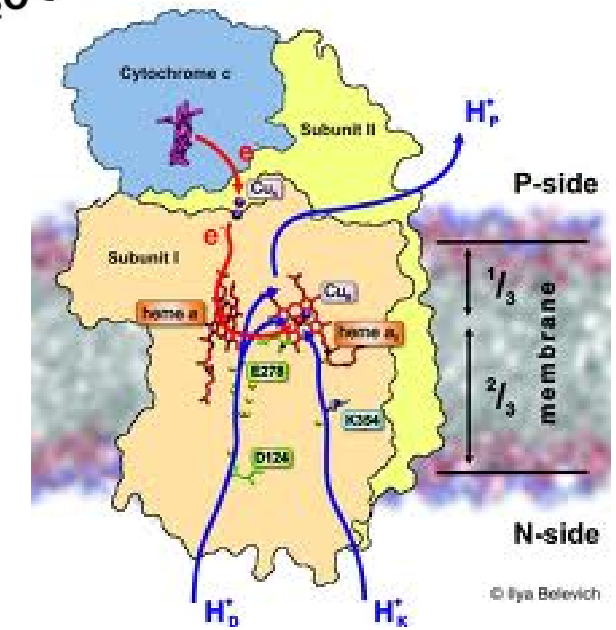
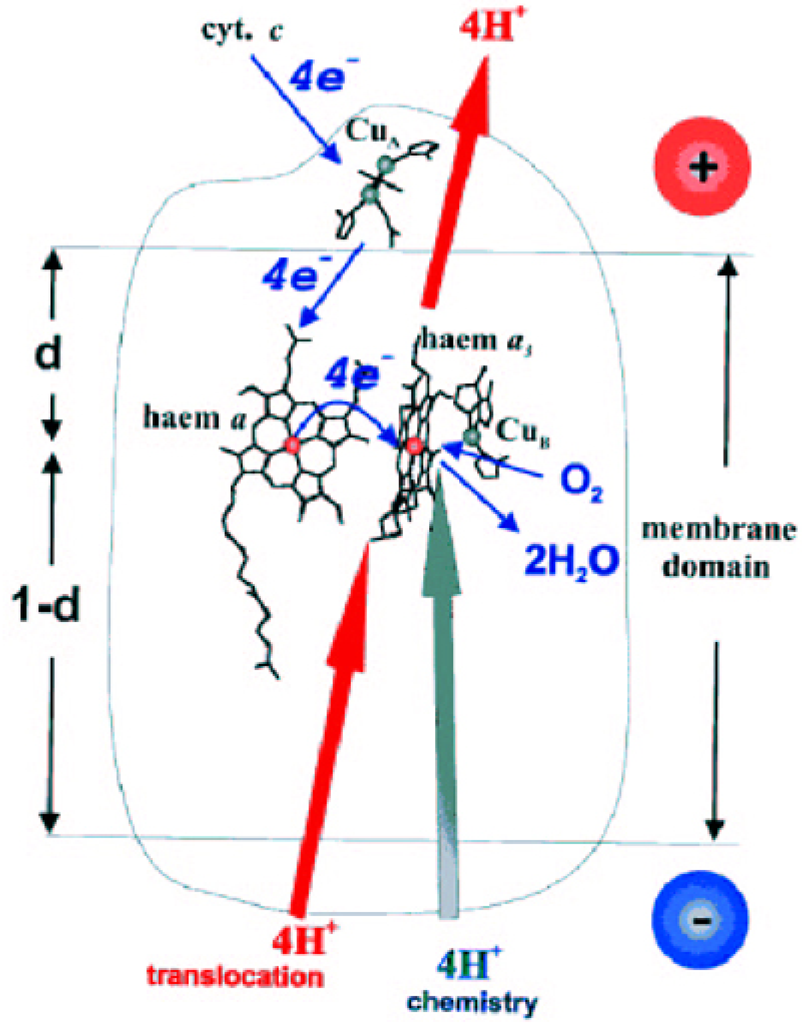
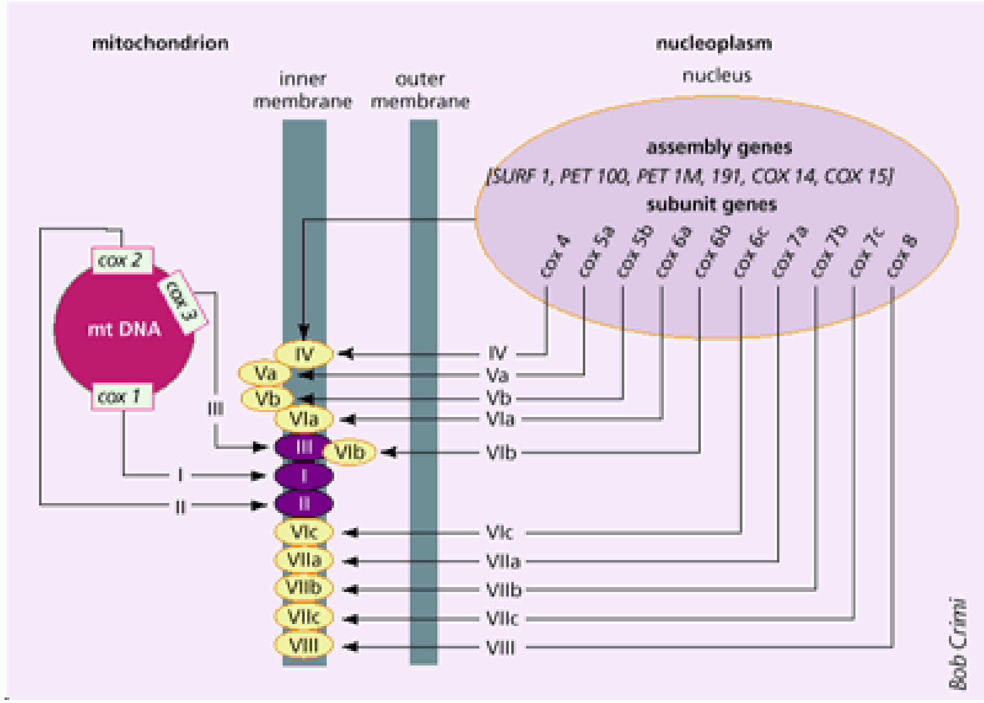
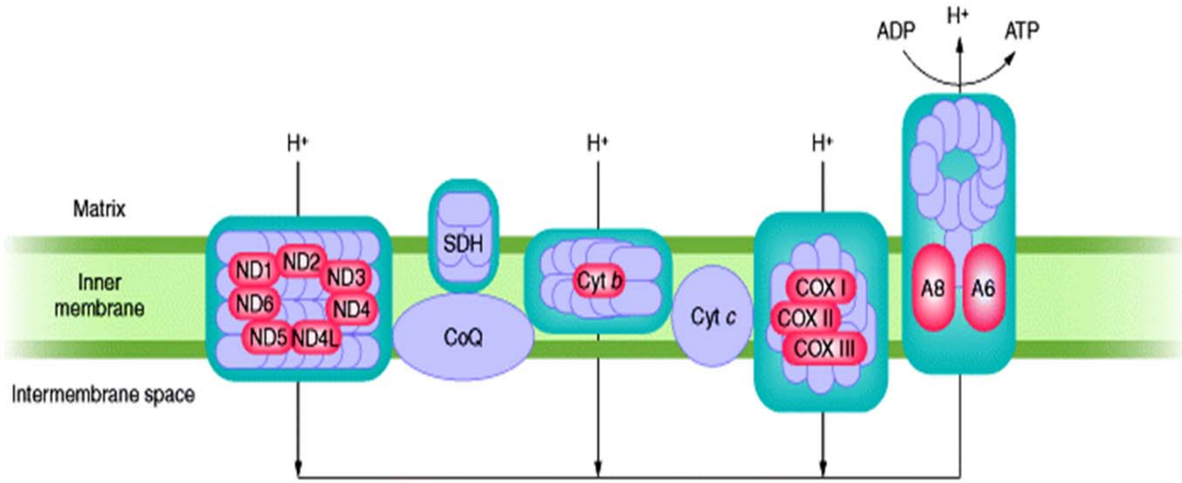
Cytochrome c oxidase has two proton-translocating channels











Subunits	Complex I	Complex II	Complex III	Complex IV	Complex V
nDNA-encoded	35	4	10	10	12
mtDNA-encoded	7	0	1	3	2