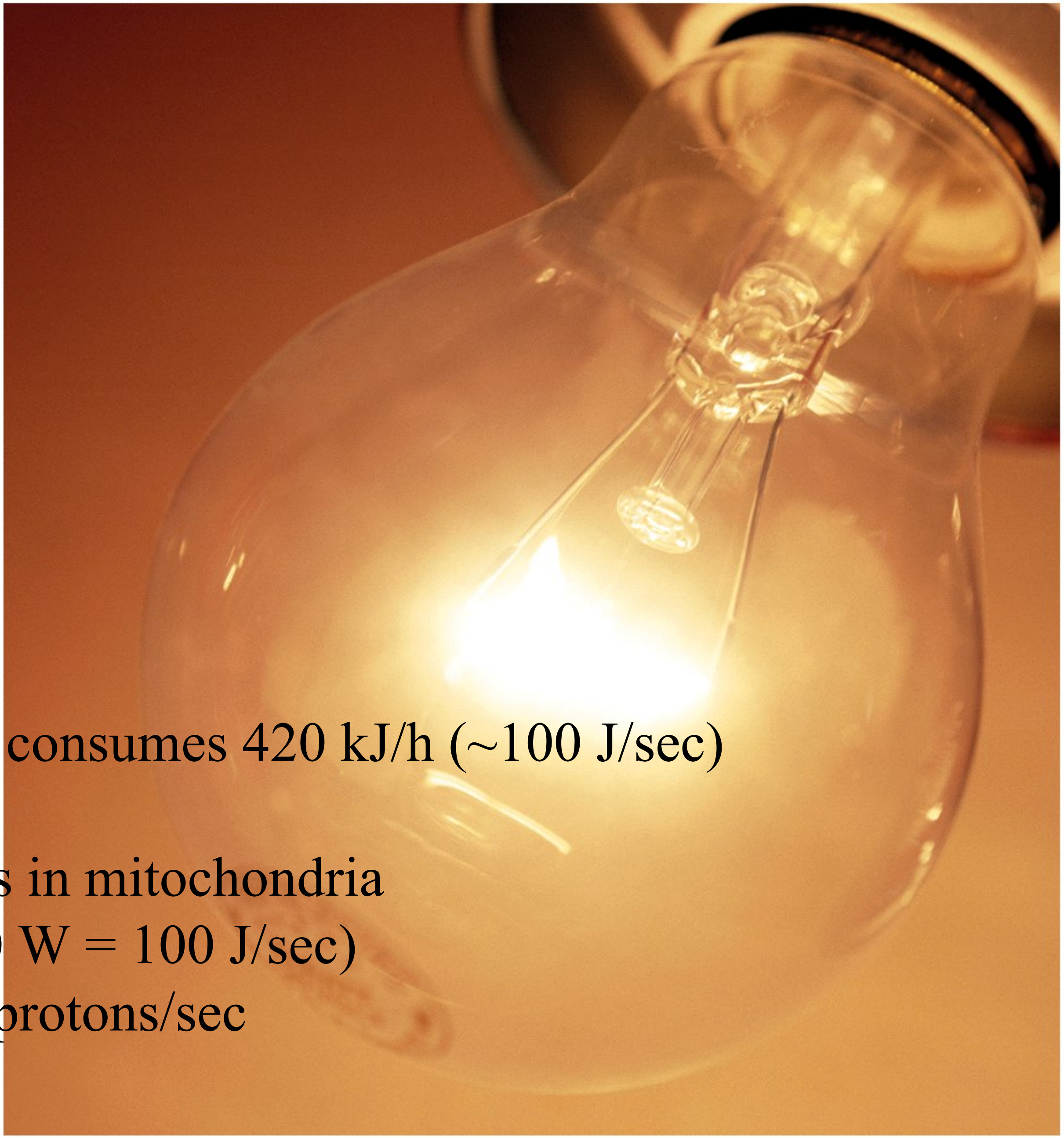
Electron Transport and Oxidative Phosphorylation



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

Chapter 17 Opener Fundamentals of Biochemistry, 2/e

Mitochondrial electron-transport chain

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

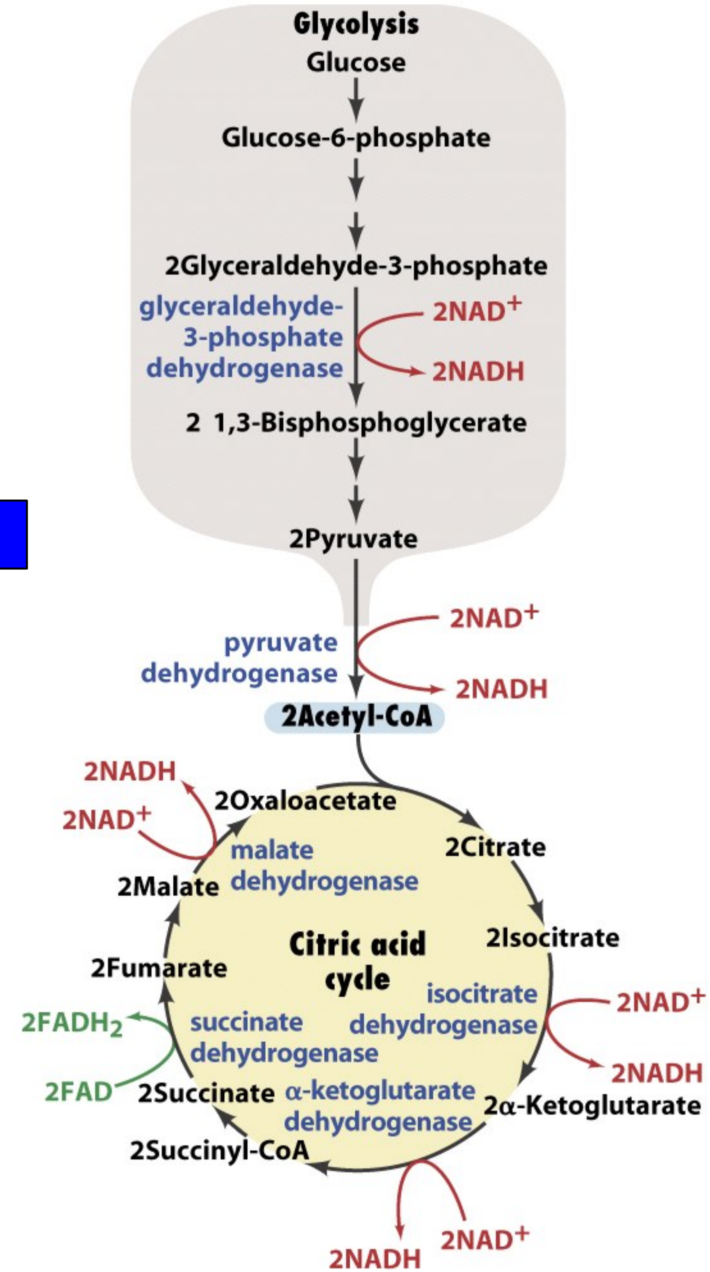


Figure 17-1 Fundamentals of Biochemistry, 2/e

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

Anatomy

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

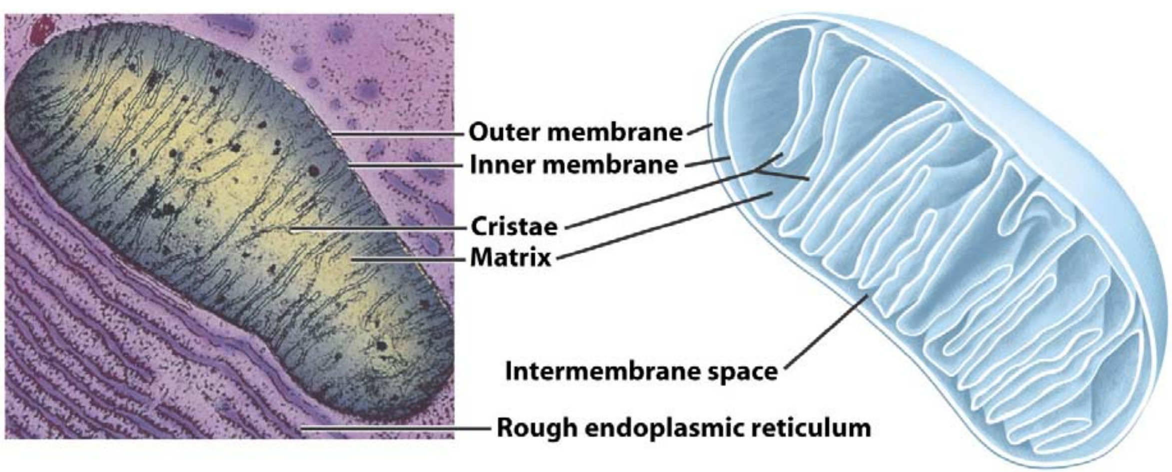


Figure 17-2 Fundamentals of Biochemistry, 2/e

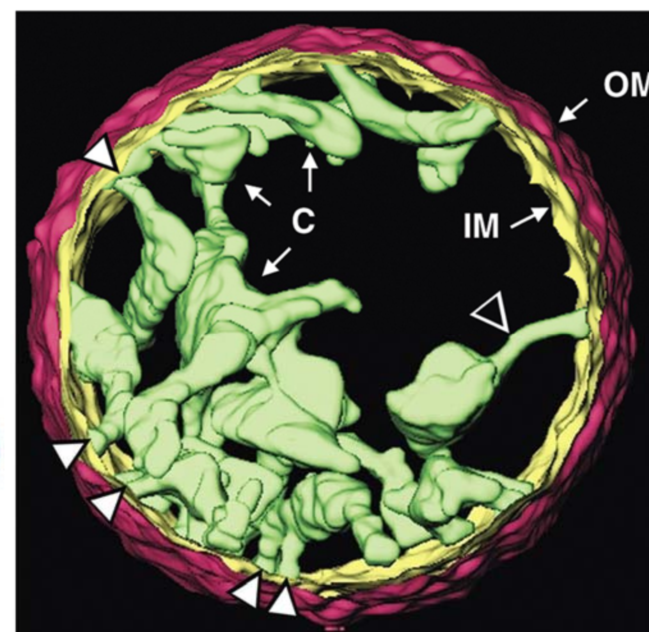


Figure 17-3 Fundamentals of Biochemistry, 2/e

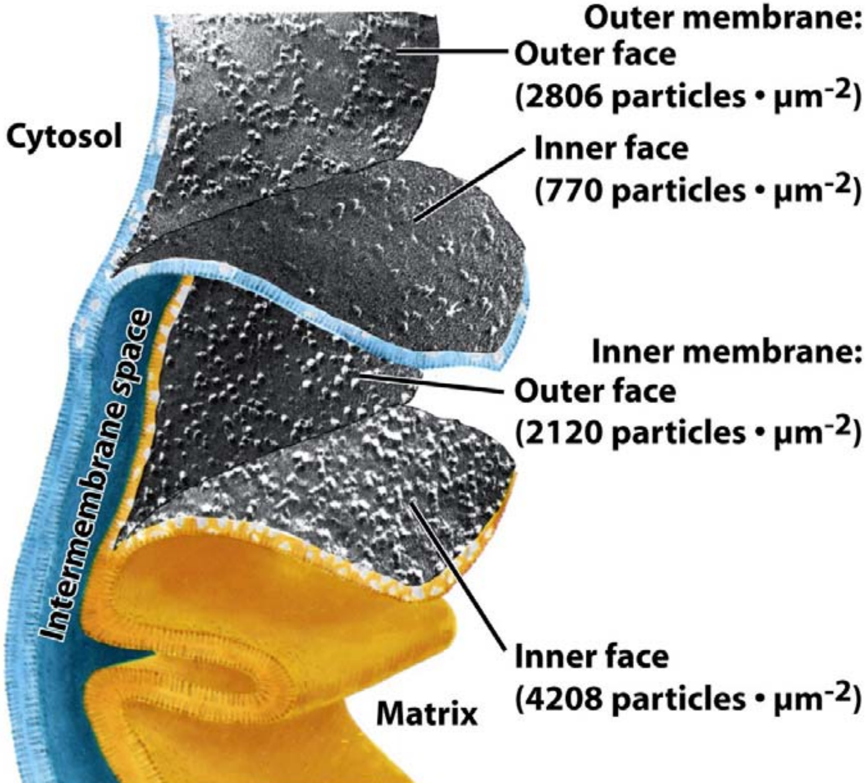
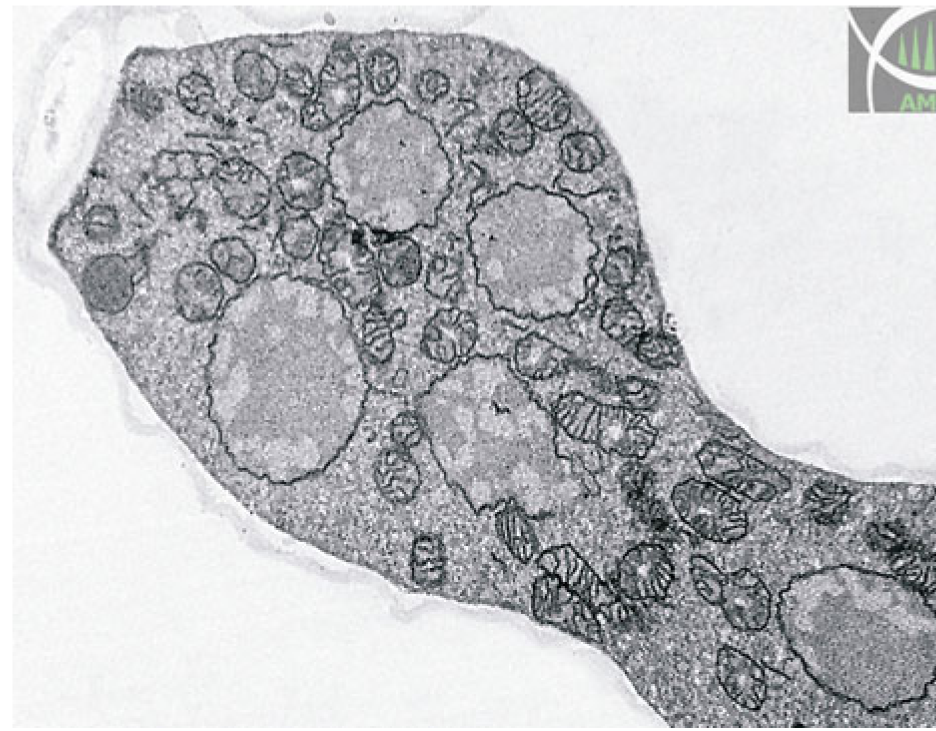


Figure 17-4 Fundamentals of Biochemistry, 2/e



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

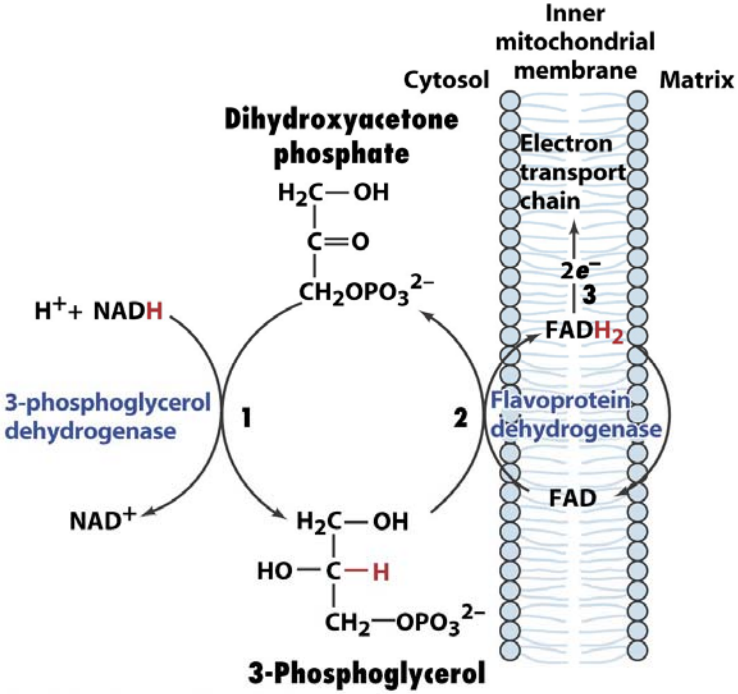


Figure 17-5 Fundamentals of Biochemistry, 2/e

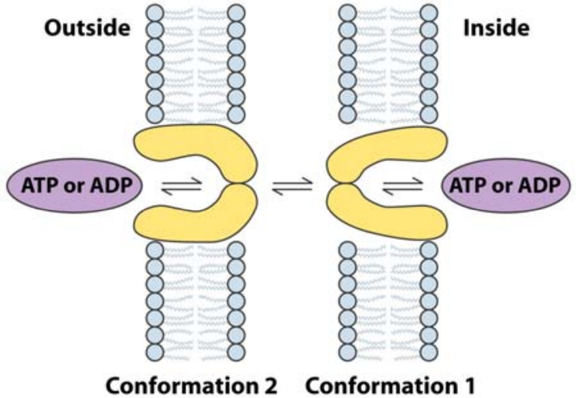


Figure 17-6 Fundamentals of Biochemistry, 2/e

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

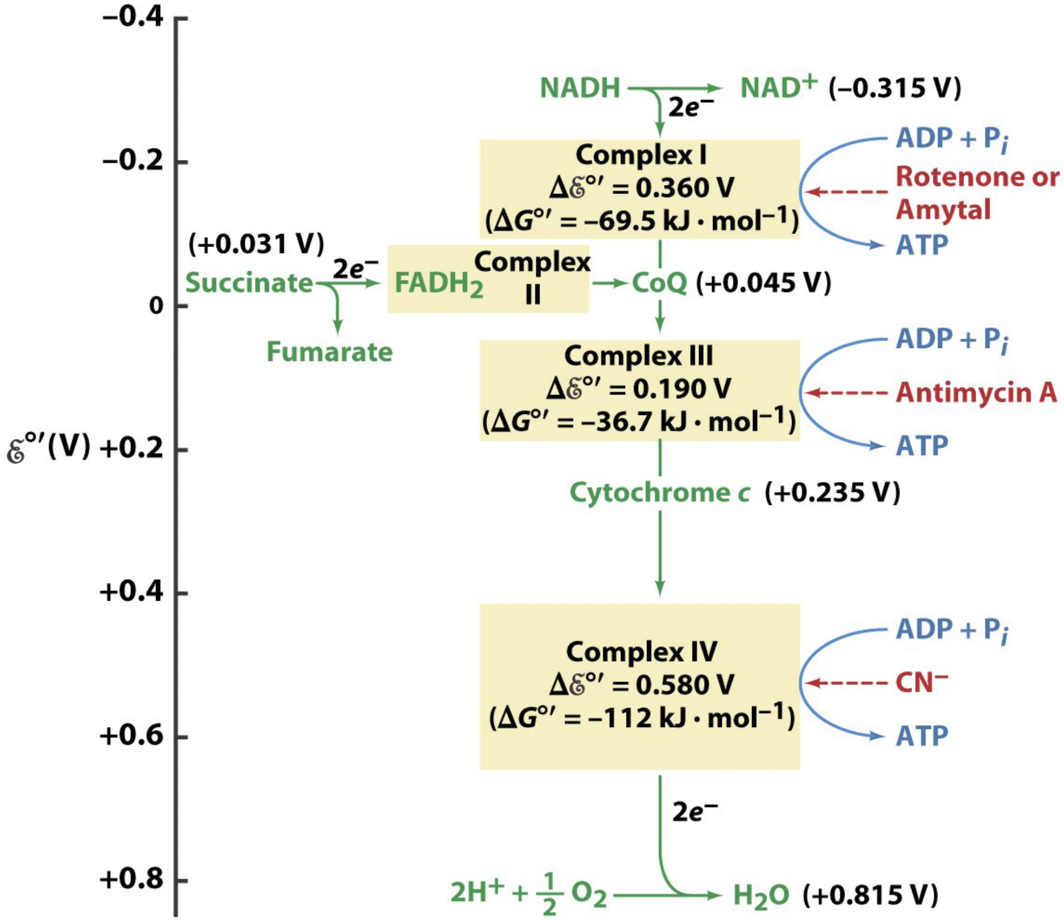


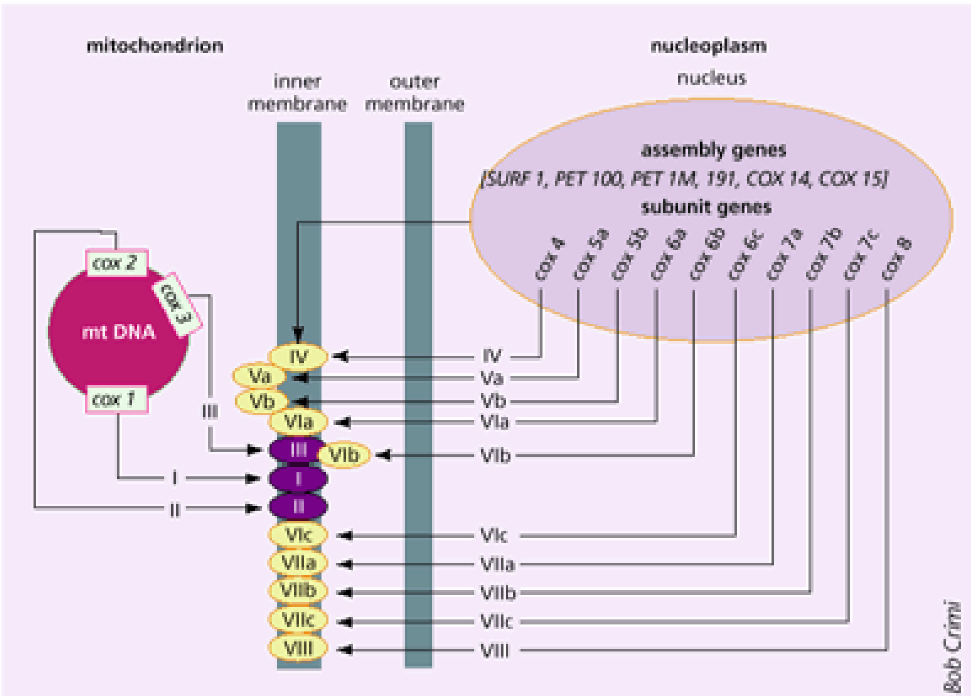
Figure 17-7 Fundamentals of Biochemistry, 2/e

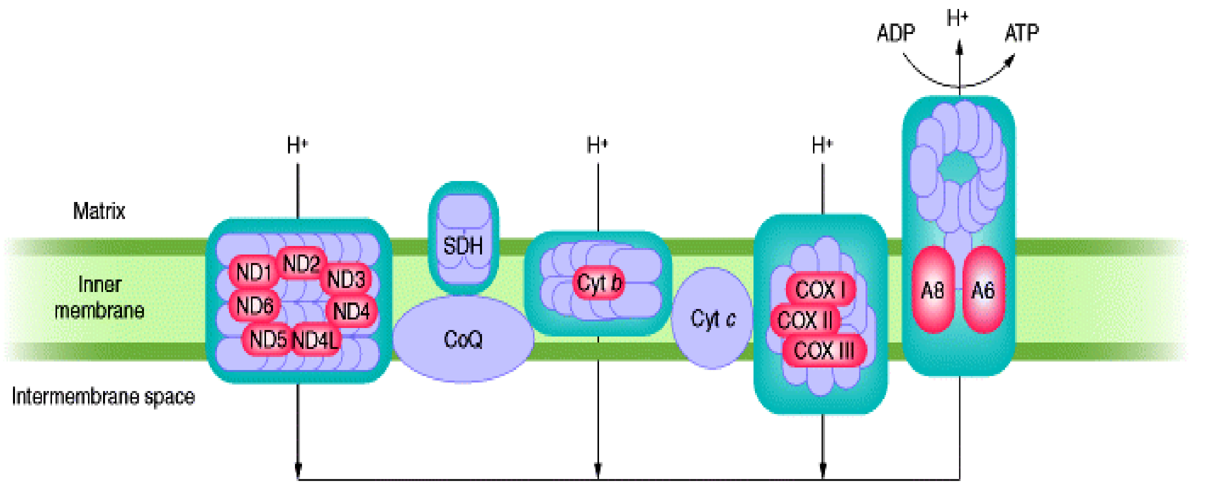
Table 17-1 Reduction Potentials of Electron-Transport Chain Components in Resting Mitochondria

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





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

Oxidative phosphorylation

Energy coupling: electron transport & ATP synthesis

The chemiosmotic theory: 1961, Peter Mitchell

key observation: page 568

Electron transport generates a proton gradient: electrochemical H^+ gradient in IMS

proton motif force

$\Delta G = RT \ln([A]_{in}/[A]_{out}) + Z_A F\Delta\Psi$ (page 285)

pH (out) < pH (in) (chemical, 0.75 units higher)

$\Delta\Psi > 0$ (from negative to positive) (electrical, 0.168 V)

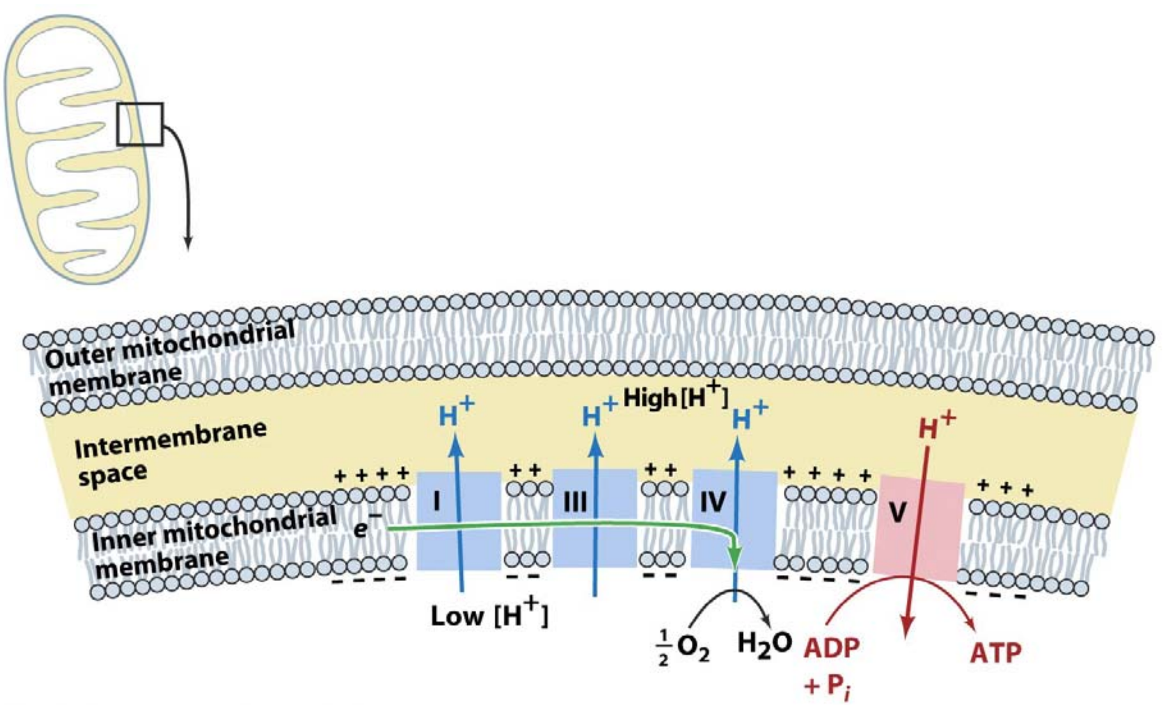


Figure 17-20 Fundamentals of Biochemistry, 2/e

ATP synthase

Proton-pumping ATP synthase, F_1F_0-ATPase

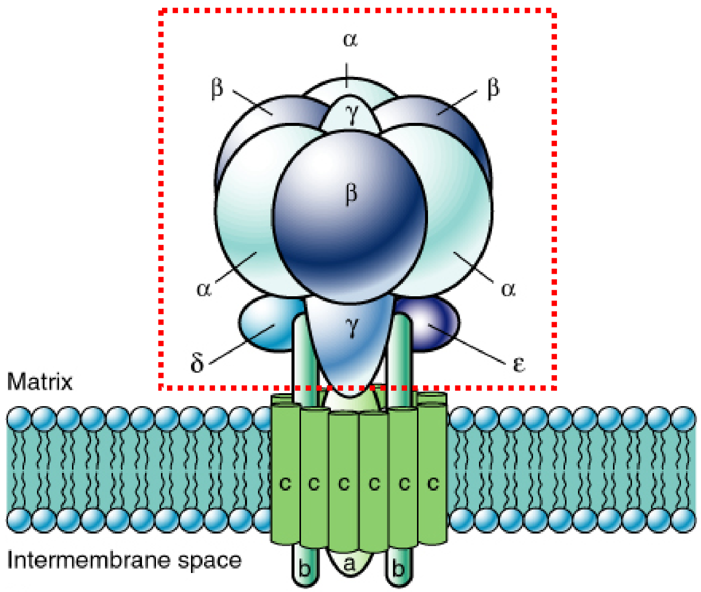
Composed of two functional units: F0, F1

F0: a water insoluble transmembrane protein containing as many as 8 subunits
    a:b:c=1:2:9~12

F1: a water soluble peripheral membrane protein composed of 5 types of subunits
    $\alpha 3\beta 3\gamma\delta\varepsilon$, ATPase activity

EM of cristae

Figure 17-21a Fundamentals of Biochemistry, 2/e



(b)

EM of F_1F_0-ATPase

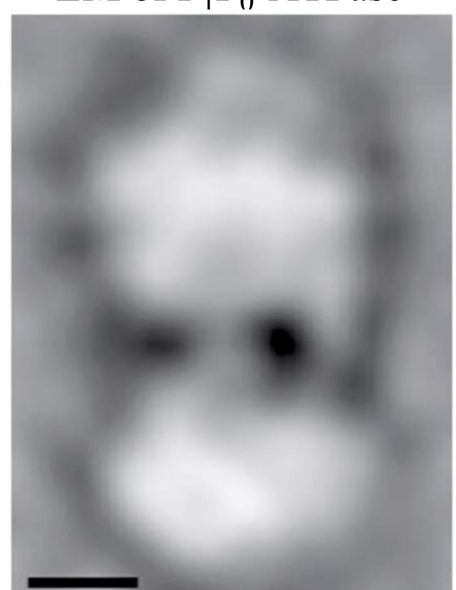


Figure 17-21b Fundamentals of Biochemistry, 2/e

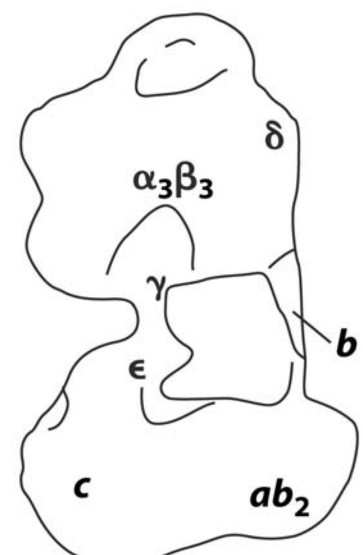


Figure 17-21c Fundamentals of Biochemistry, 2/e

F_1-ATPase from bovine heart mitochondria

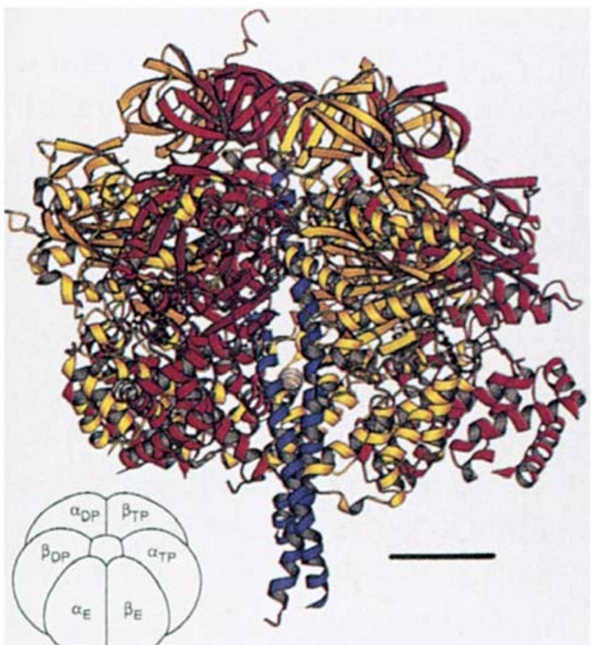


Figure 17-22a Fundamentals of Biochemistry, 2/e

γ

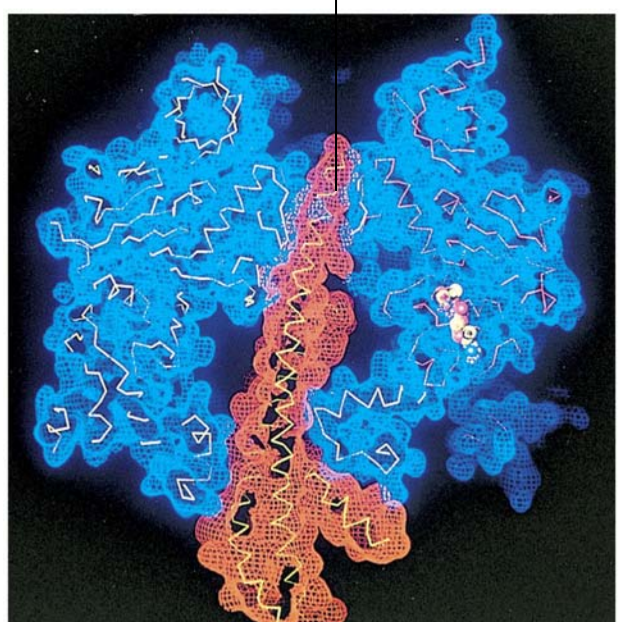


Figure 17-22b Fundamentals of Biochemistry, 2/e

Top view: αβ

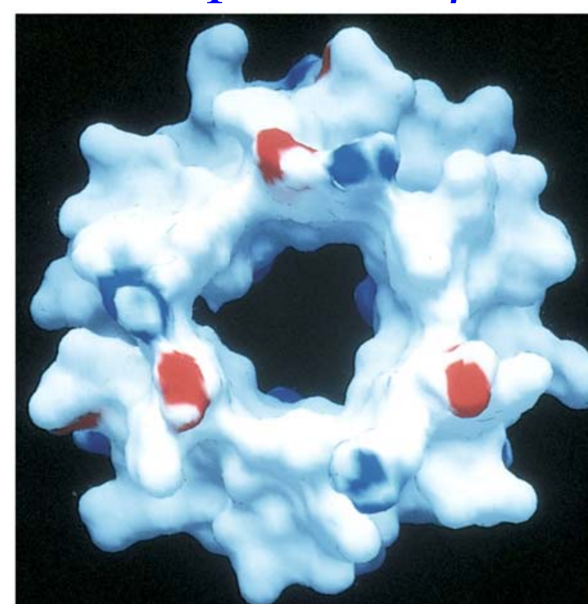
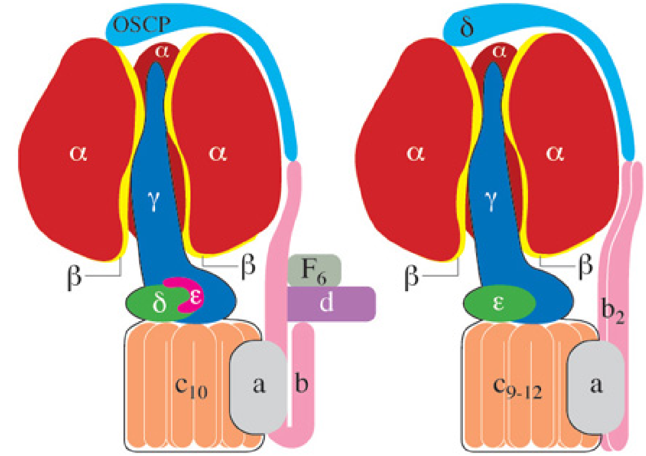


Figure 17-22c Fundamentals of Biochemistry, 2/e



Mito

E. coli

Yeast mito F1-c10 complex

C subunit of *E. coli* F1F0-ATPase

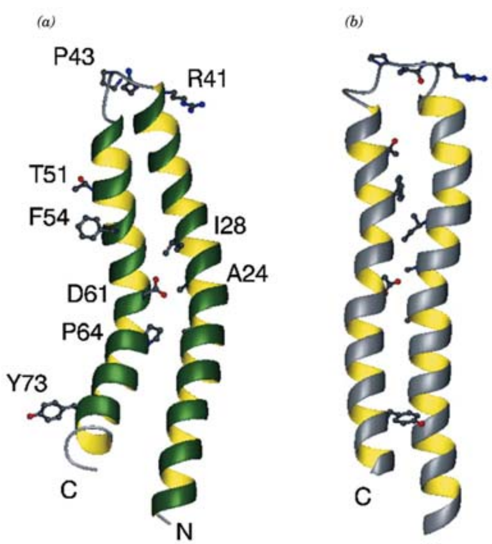


Figure 17-23 Fundamentals of Biochemistry, 2/e

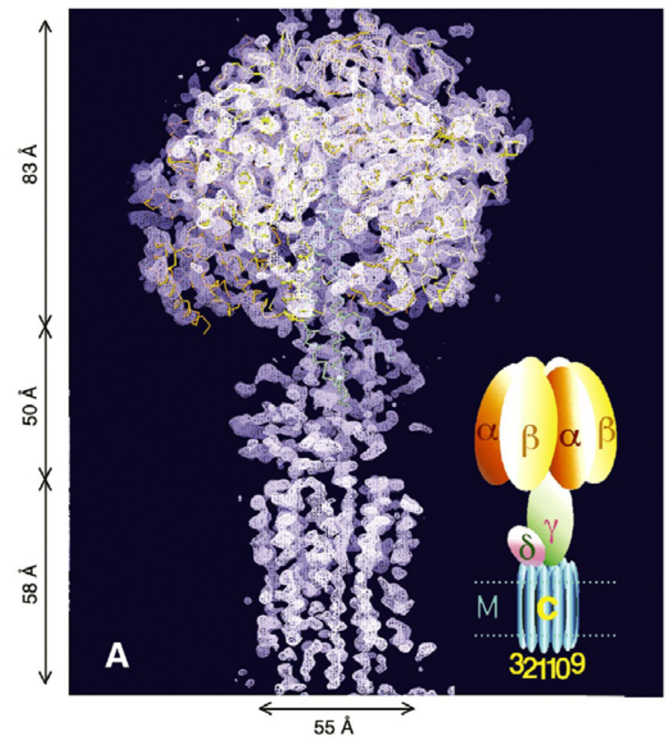


Figure 17-24 Fundamentals of Biochemistry, 2/e

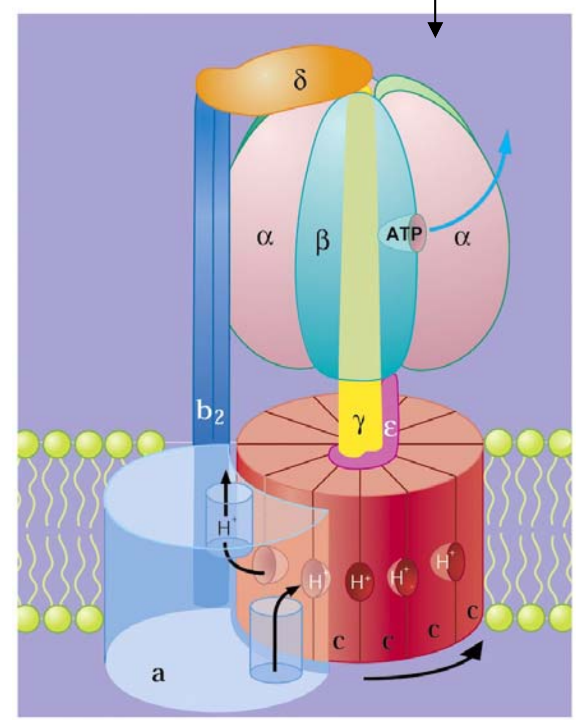


Figure 17-25 Fundamentals of Biochemistry, 2/e

Rotation of the C-ring

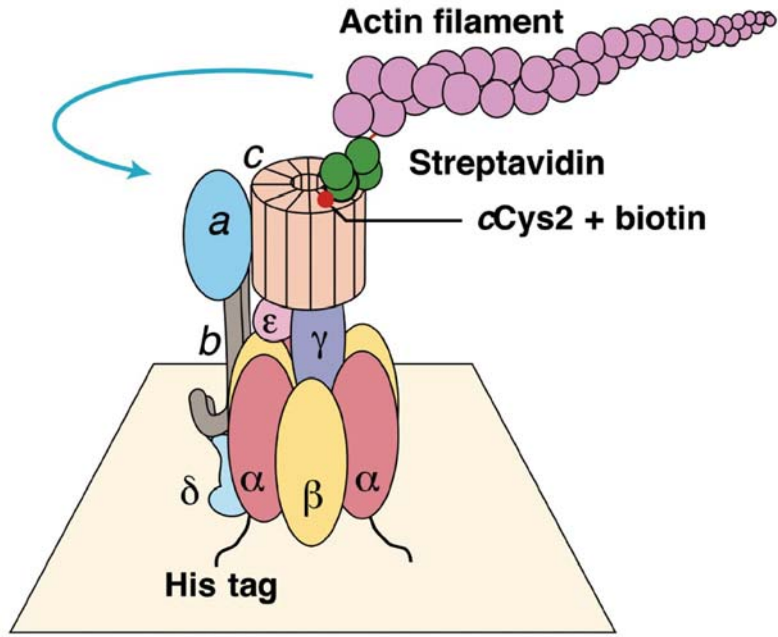


Figure 17-27a Fundamentals of Biochemistry, 2/e

http://www.res.titech.ac.jp/~seibutu/main.html?right/~seibutu/projects/f1_e.html

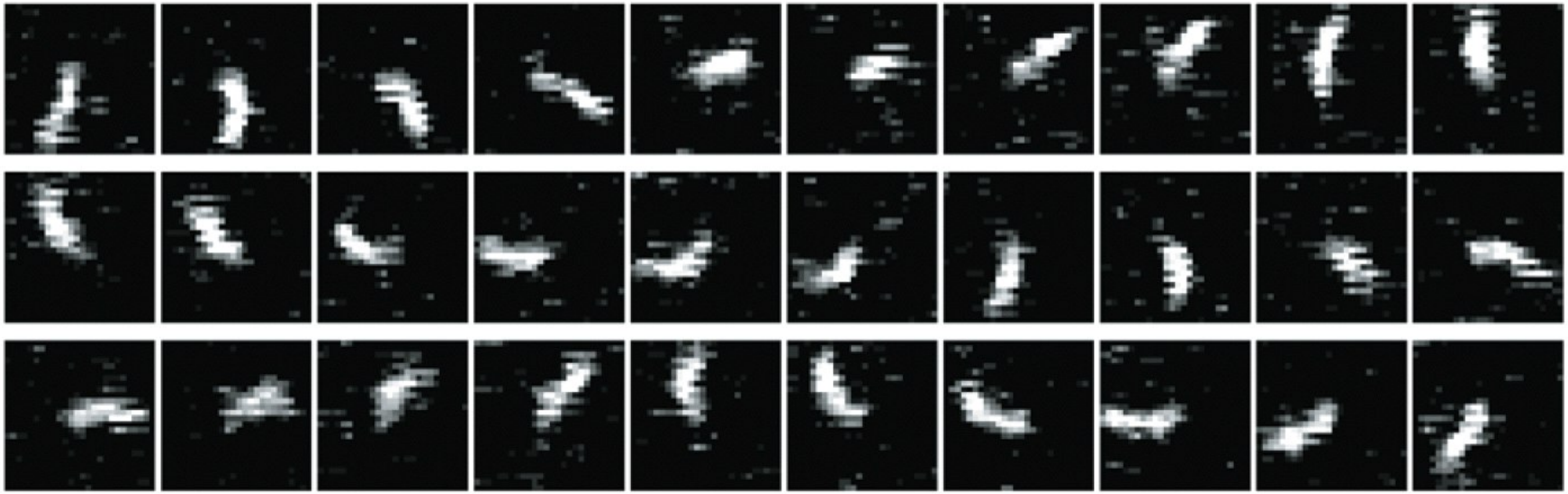


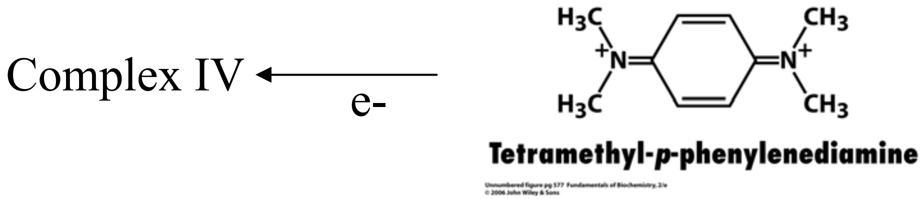
Figure 17-27b Fundamentals of Biochemistry, 2/e

The P/O ratio

The amount of ATP synthesized to the amount of oxygen reduced

- 3 ATP from NADH
- 2 ATP from FADH2
- 1 ATP from tetramethyl-p-phenylenediamine
- *** the actual P/O ratios may not be integral numbers

Transfer of two electrons
10 proton transport
One complete turn of c subunits



Uncoupling oxidative phosphorylation

Protons are permeable only through F0 portion of ATP synthase
Increased permeability via another route: dissipation of electrochemical gradient:
Artificial uncoupler: 2,4-dinitrophenol (DNP), once used as a diet pill

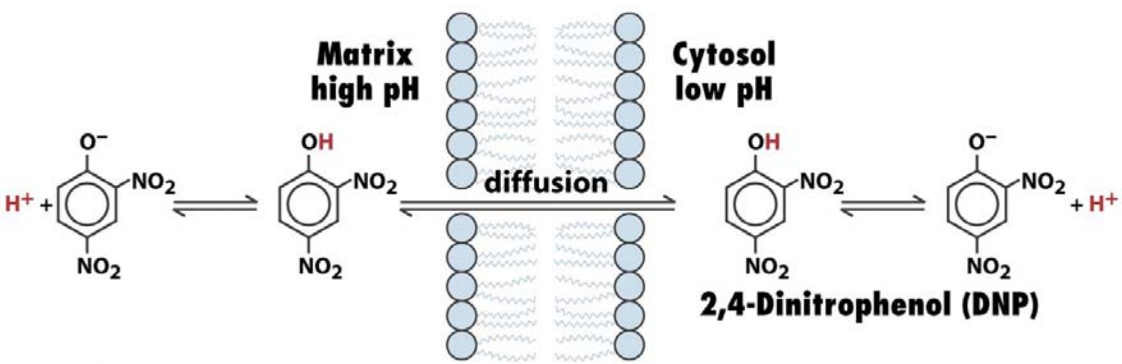
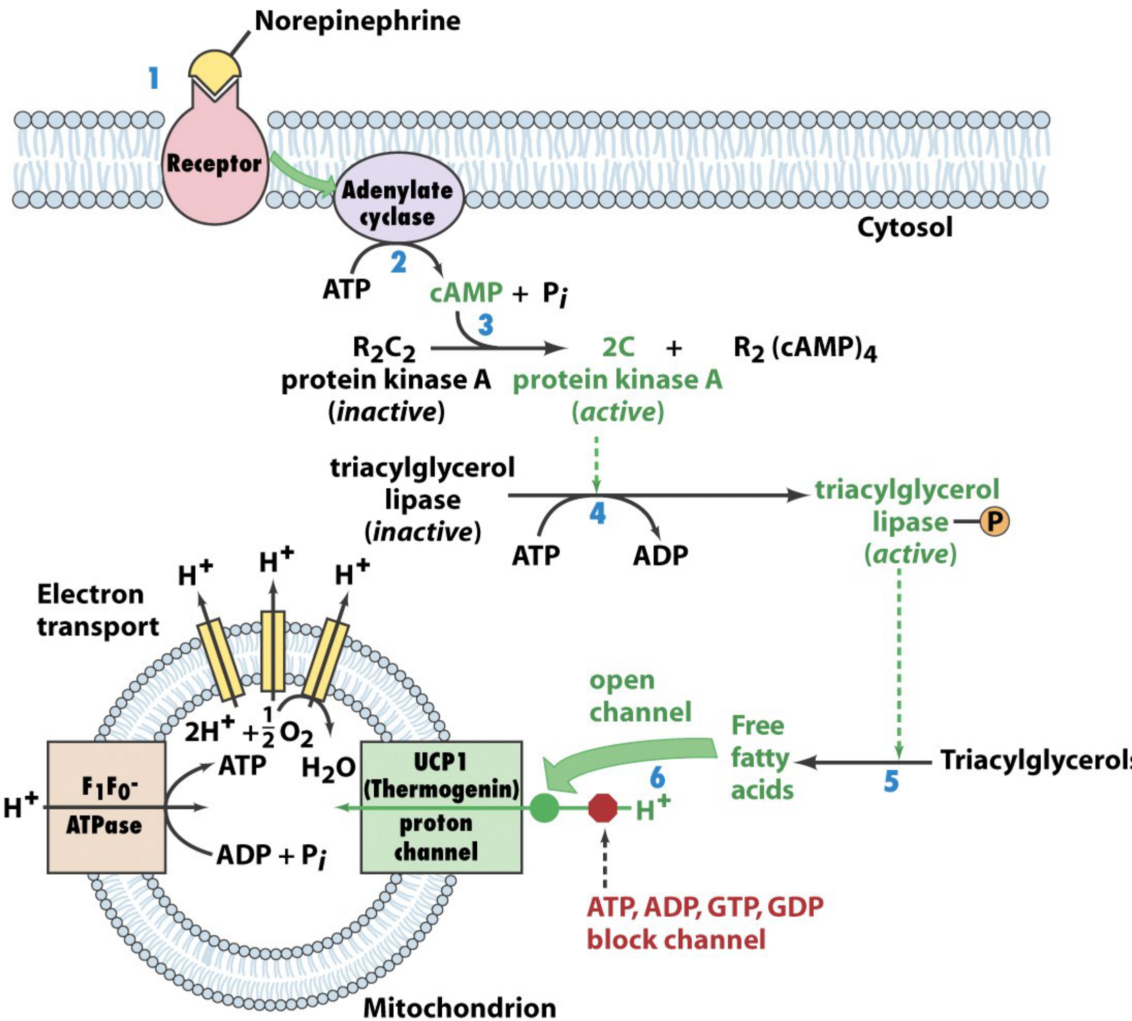


Figure 17-28 Fundamentals of Biochemistry, 2/e

Uncoupling in brown adipose tissue: heat generation

Nonshivering thermogenesis
Uncoupling protein: UCP1, UCP2, UCP3
Affects metabolism rate & body temp



Box 17-4 figure 2 Fundamentals of Biochemistry, 2/e

Box 17-4 figure 1 Fundamentals of Biochemistry, 2/e

Physiological implication of aerobic metabolism

High efficiency: 19 times more than anaerobic

When anaerobically growing yeast are exposed to oxygen, glucose consumption drops

<u>Disadvantages</u>

Oxygen deprivation

Generation of reactive oxygen species (ROS)

Electrostatic effects in human Cu,Zn-SOD

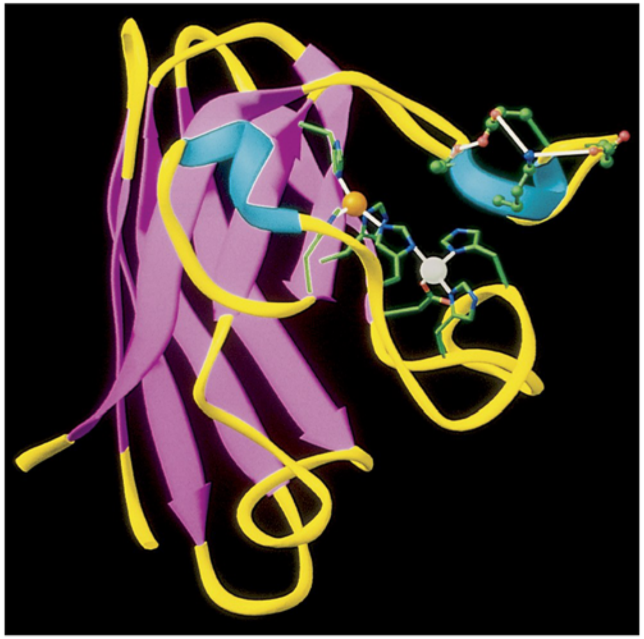


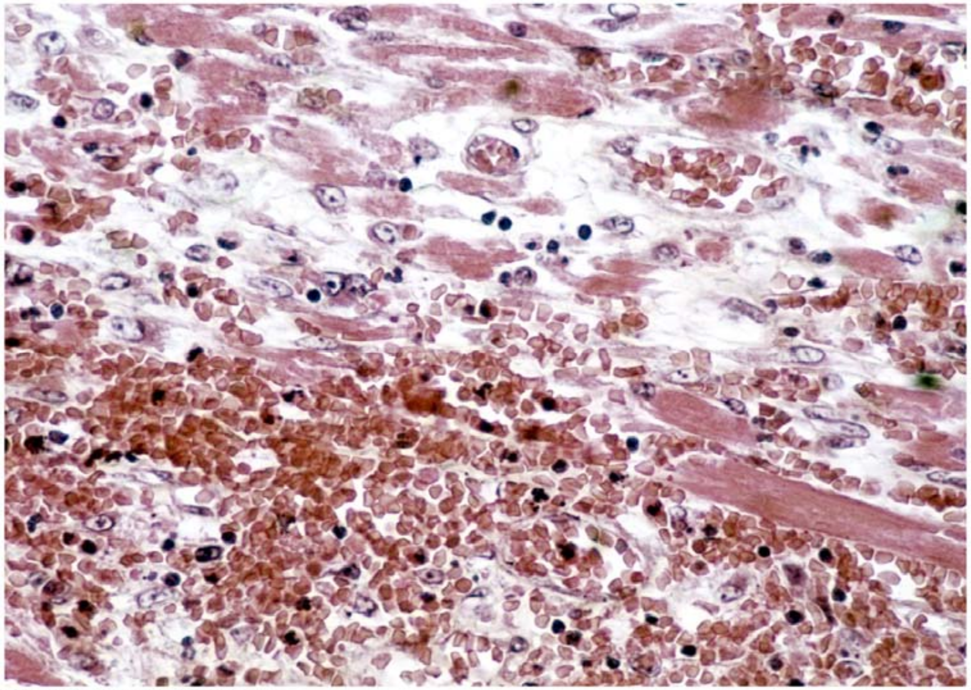
Figure 17-30a Fundamentals of Biochemistry, 2/e

Figure 17-30b Fundamentals of Biochemistry, 2/e

Oxygen deprivation in heart attack and stroke

Myocardial infarction (heart attack)
Stroke (brain)
Failure in maintaining intracellular ion conc.
Increased membrane permeability
Increased anaerobic glycolysis: decreased pH to allow lysosomal enzyme active
Cell death

Necrotic tissue after a heart attack



Box 17-5 Fundamentals of Biochemistry, 2/e

Reactive oxygen species (ROS) resulting from partial reduction of oxygen

Extremely short-lived but readily extract electrons from other molecules, converting them to free radicals and thereby initiating a chain reaction

Responsible for neurodegenerative diseases & aging process: oxidative damage

Reactive species	Antioxidant
Single oxygen $^1O_2$	vitamin A, vitamin E
Superoxide radical O_2^-•	superoxide dismutase, vitamin C
Hydrogen peroxide H_2O_2	catalase, glutathione peroxidase
Peroxyl radical ROO•	vitamin C, vitamin E
Lipid peroxyl radical LOO•	vitamin E
Hydroxyl radical OH•	vitamin C

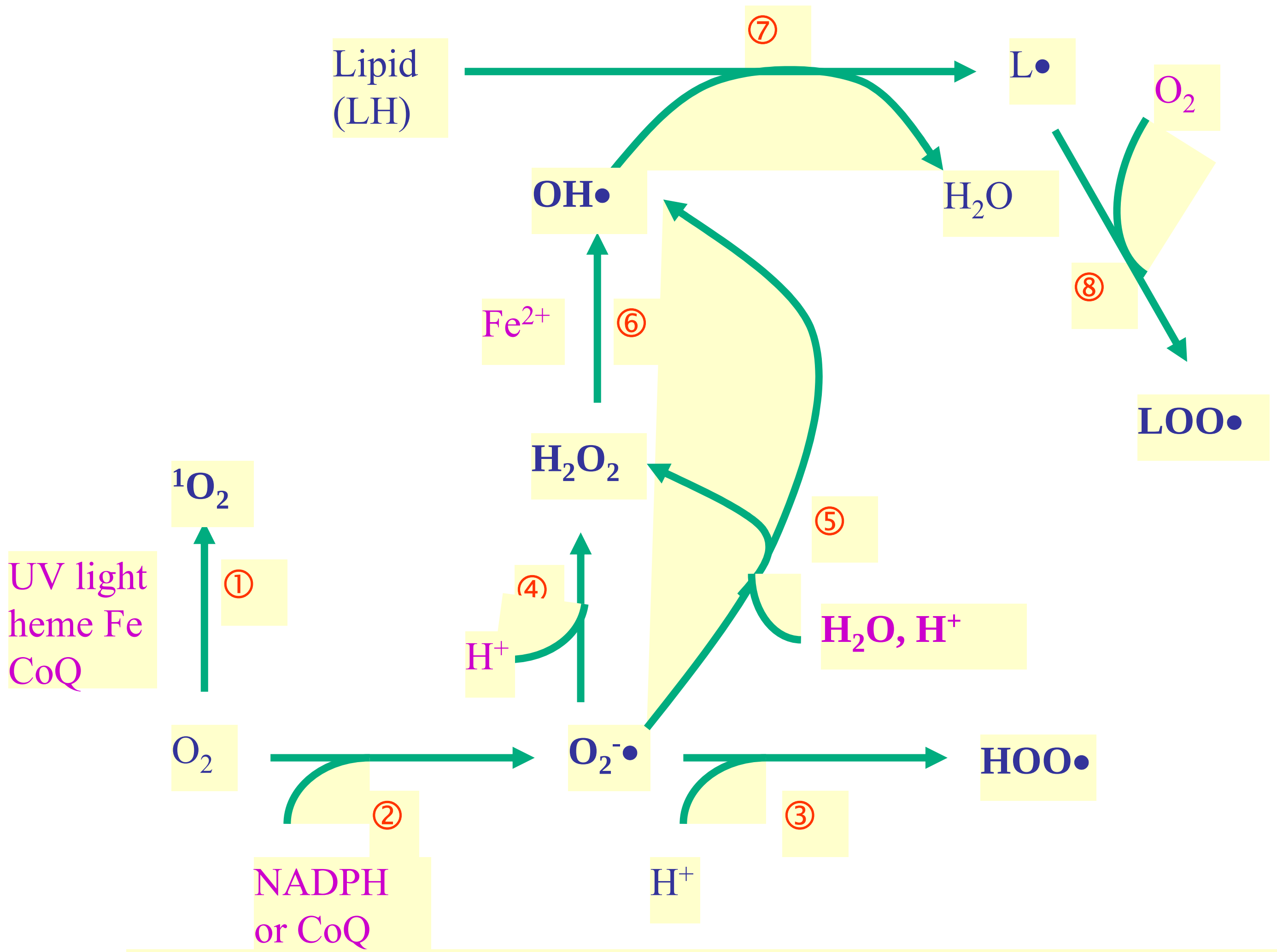


Figure 4. Pathways for the formation of reactive oxygen species

① Singlet oxygen

② Superoxide radical anion

③ Peroxyl radical

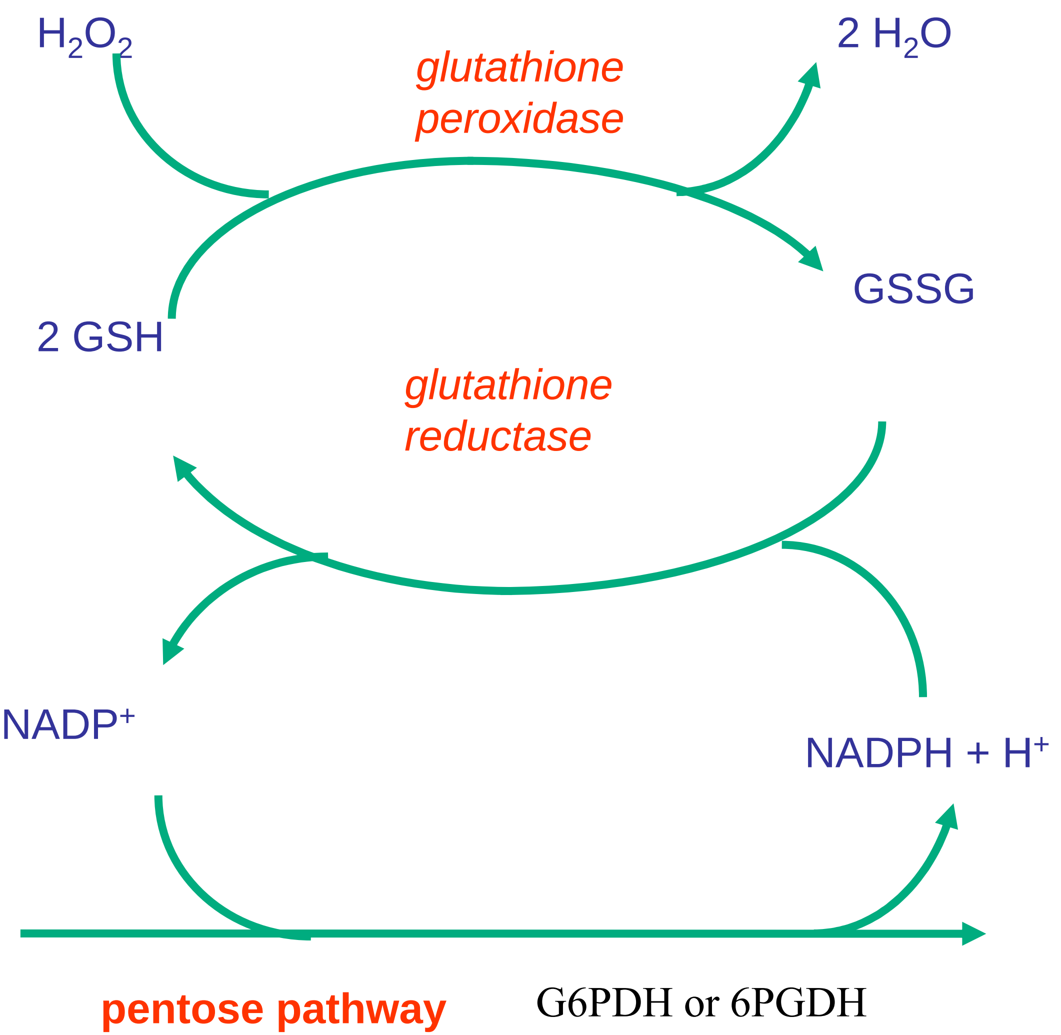
④ Superoxide dismutase

⑤ Haber-Weiss reaction;

⑥ Fenton reaction

⑦ lipid radical

⑧ lipid peroxyl radical



Metabolism of glutathione and its relationship to the pentose phosphate pathway

ANTI-OXIDANT ENZYMES

Superoxide dismutase (SOD): $2\ O_2^{-}\bullet + 2H^+ \rightarrow H_2O_2 + O_2$

Mitochondrial & bacterial: Mn^{2+} cofactor

Cytoplasmic – Cu^{2+}-Zn^{2+} cofactors; mutations associated with familial amyotrophic lateral sclerosis (FALS)

Catalase : $2\ H_2O_2 \rightarrow H_2O + O_2$

Glutathione peroxidase: $2\ GSH + H_2O_2 \rightarrow GSSG + 2\ H_2O$

(Uses selenium as a cofactor)

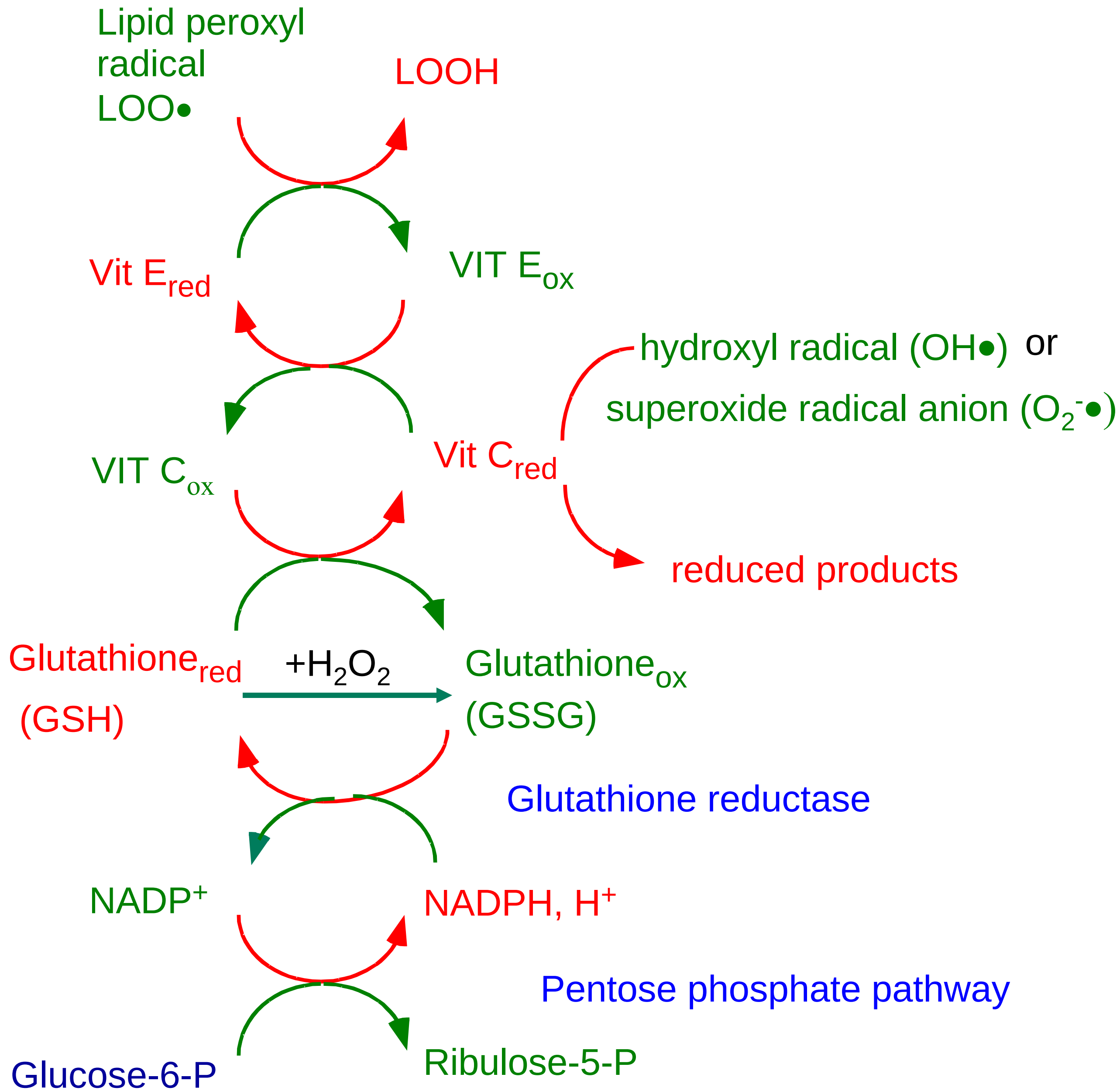


Figure 6. Antioxidant cascade