Chapter 5-II:

Electron Transport and Oxidative Phosphorylation

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 motive force

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

$= 2.3RT\,[pH(in) – pH(out)] + Z_A F\Delta\Psi = 21.5$ kJ/mol

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

$\Delta\Psi$ (Ψ_{in} -Ψ_{out}) > 0 (from negative to positive) (electrical, 0.168 V)

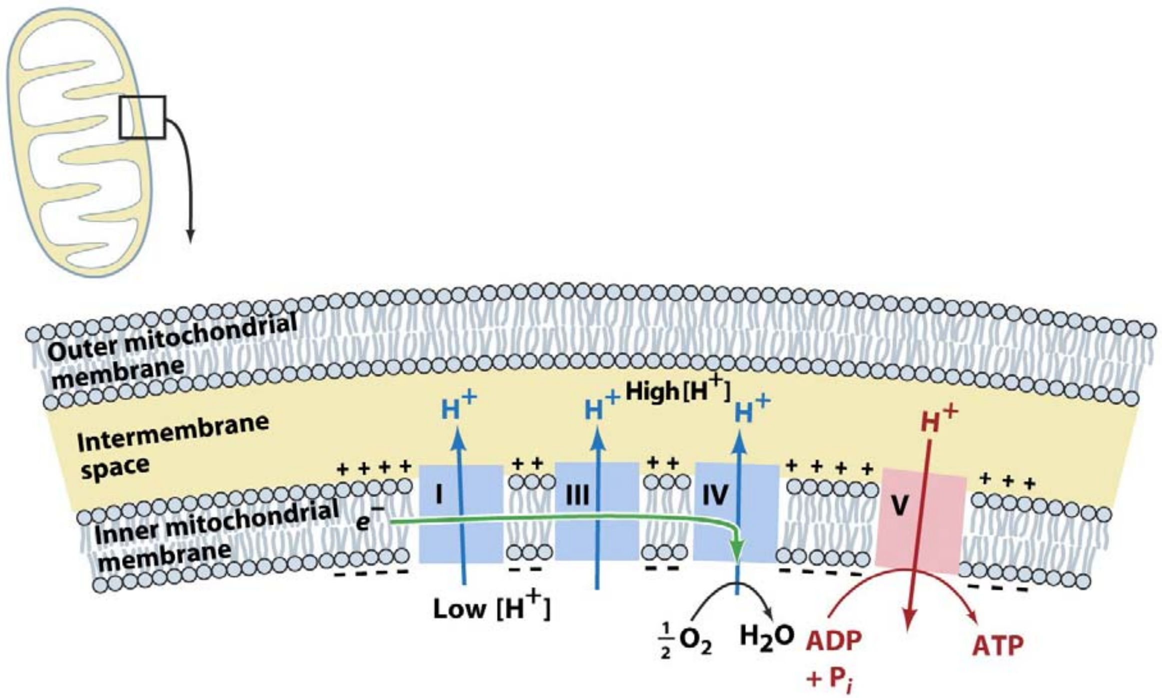
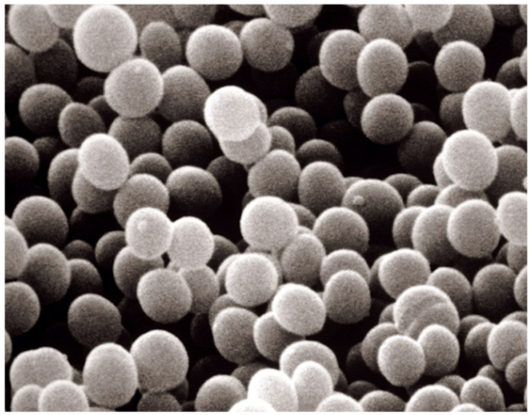
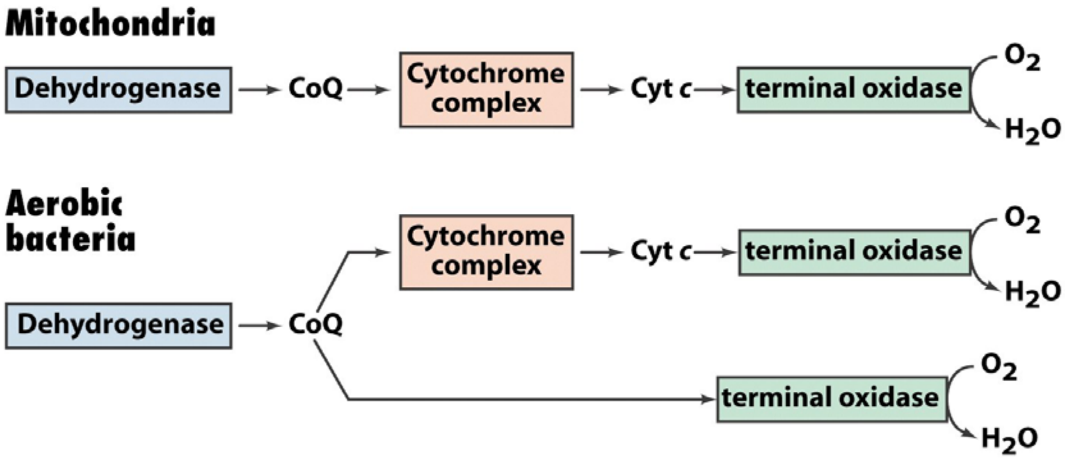


Figure 17-20 Fundamentals of Biochemistry, 2/e

Bacterial ETC and oxidative phosphorylation



Box 17-3 figure 1 Fundamentals of Biochemistry, 2/e



Box 17-3 figure 2 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

α3β3γδε, ATPase activity

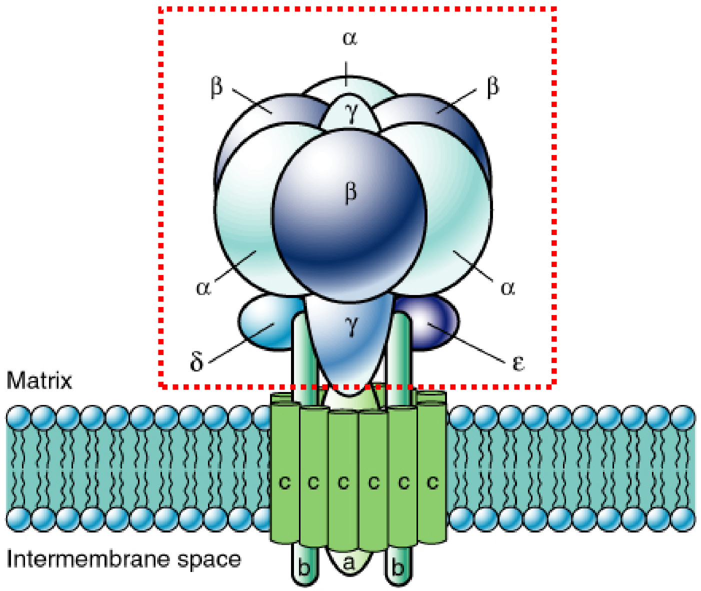
Animation of ATP synthase

http://www.dnatube.com/video/104/ATP-synthase-structure-and-mechanism

http://www.youtube.com/watch?v=3y1dO4nNaKY

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

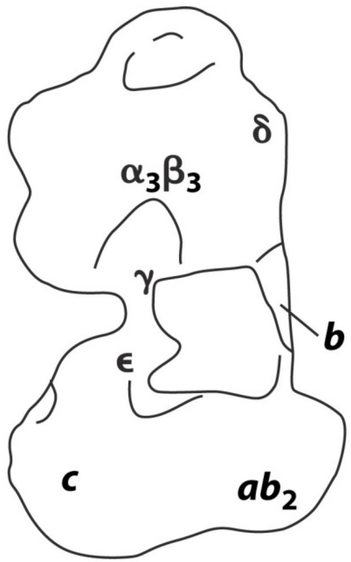


Figure 17-21c Fundamentals of Biochemistry, 2/e

F_1-ATPase from bovine heart mitochondria

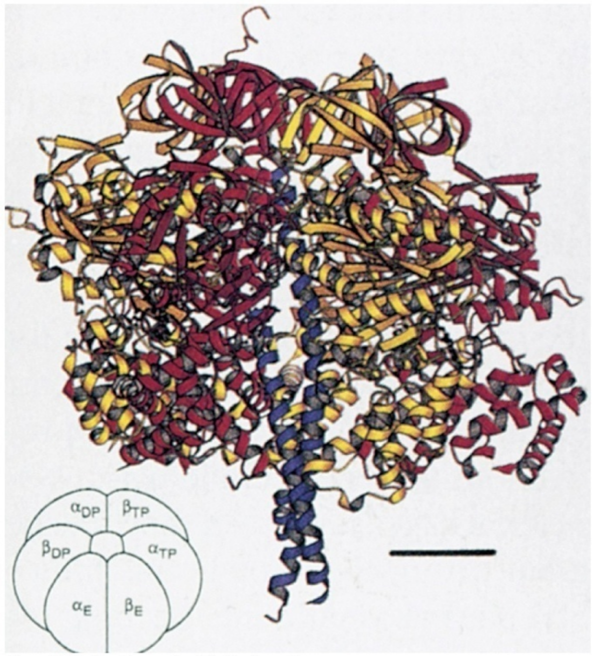


Figure 17-22a Fundamentals of Biochemistry, 2/e

γ

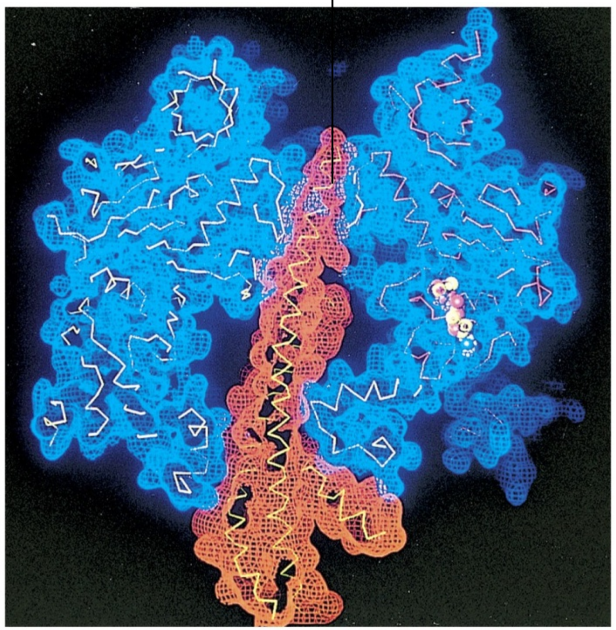


Figure 17-22b Fundamentals of Biochemistry, 2/e

Top view: αβ

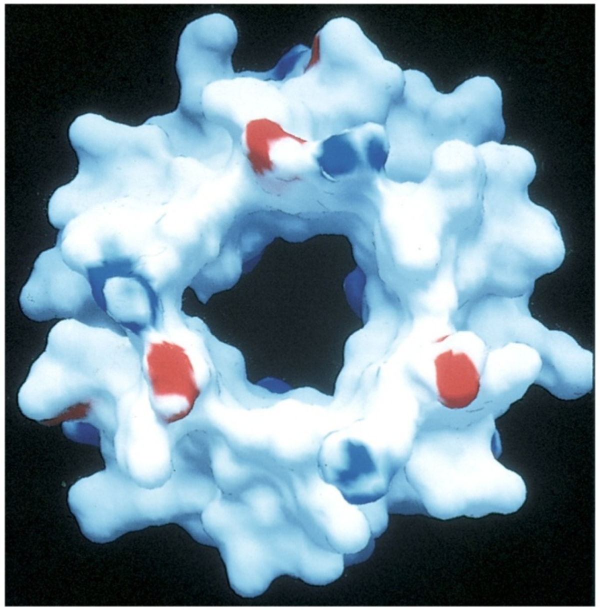
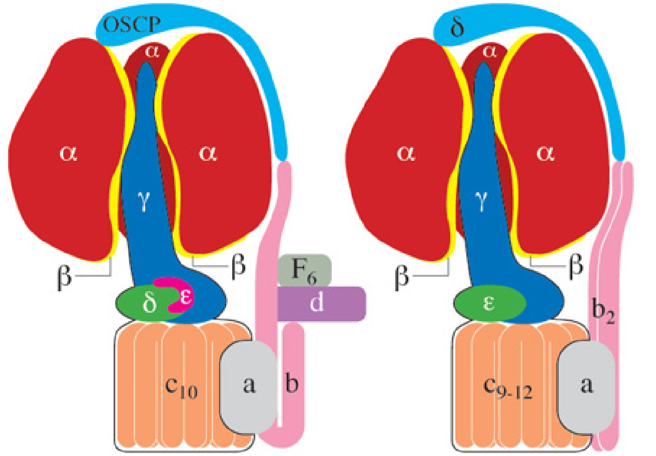


Figure 17-22c Fundamentals of Biochemistry, 2/e



Mito *E. coli*

Yeast mito F1-c10 complex

C subunit of *E. coli* F1F0-ATPase

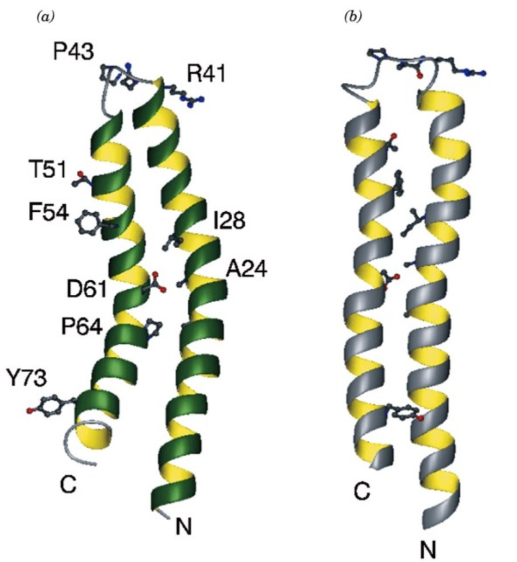


Figure 17-23 Fundamentals of Biochemistry, 2/e

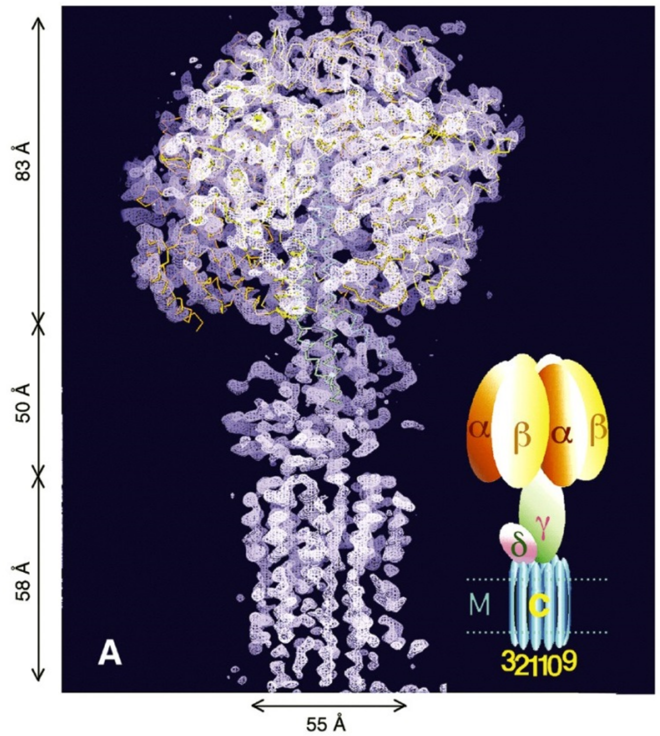


Figure 17-24 Fundamentals of Biochemistry, 2/e

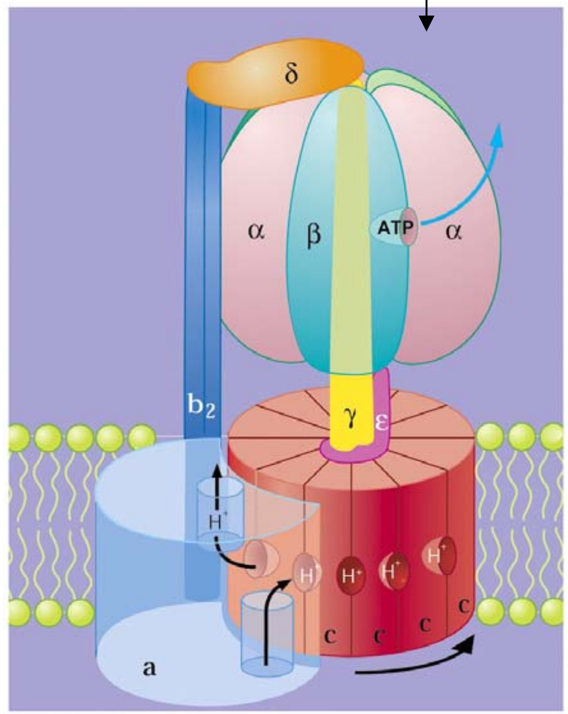


Figure 17-25 Fundamentals of Biochemistry, 2/e

The binding change mechanism

The mechanism of ATP synthesis by proton-locating ATP synthase
F0: carry out translocation of protons
F1: ATP synthesis
F0 & F1 interaction: coupling of the dissipation of the proton gradient with ATP synthesis

<u>3 binding sites, cyclic conformational changes, ATP release by E (by Paul Boyer)</u>

Binding changes driven by the rotation of catalytic subunits

Binding of ADP and Pi to L
Conformational change of L to T: ATP synthesis
Release of ATP by the free E from proton flow

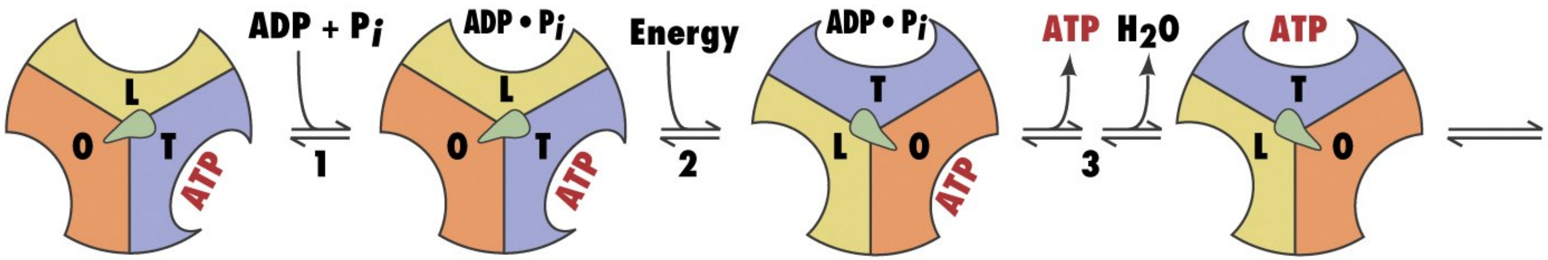


Figure 17-26 Fundamentals of Biochemistry, 2/e

http://rsb.info.nih.gov/NeuroChem/biomach/ATPsyn.html

<u>Cylindrical bearing & Lubricated free rotation</u>

γ Subunit is a molecular cam shaft in linking the proton gradient-driven rotational motor to the conformational changes in the catalytic sites of F1

Rotation of the C-ring

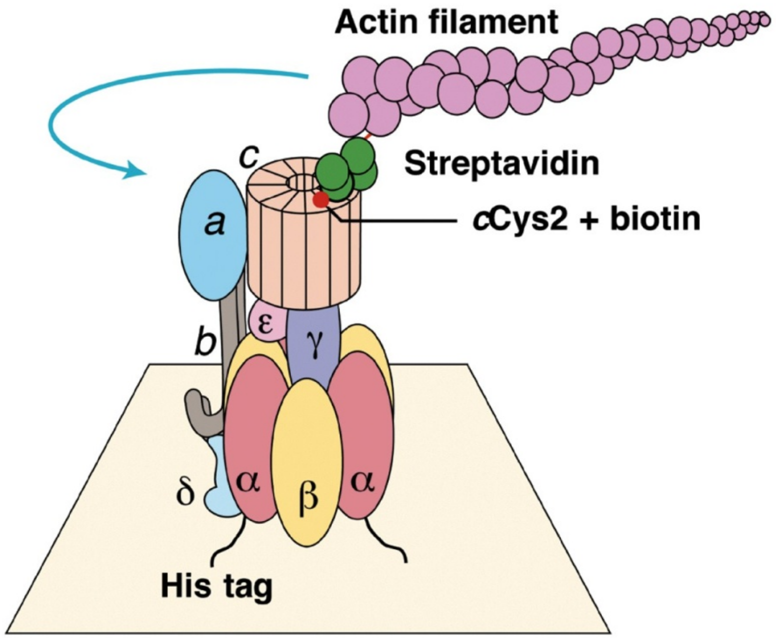


Figure 17-27a Fundamentals of Biochemistry, 2/e

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

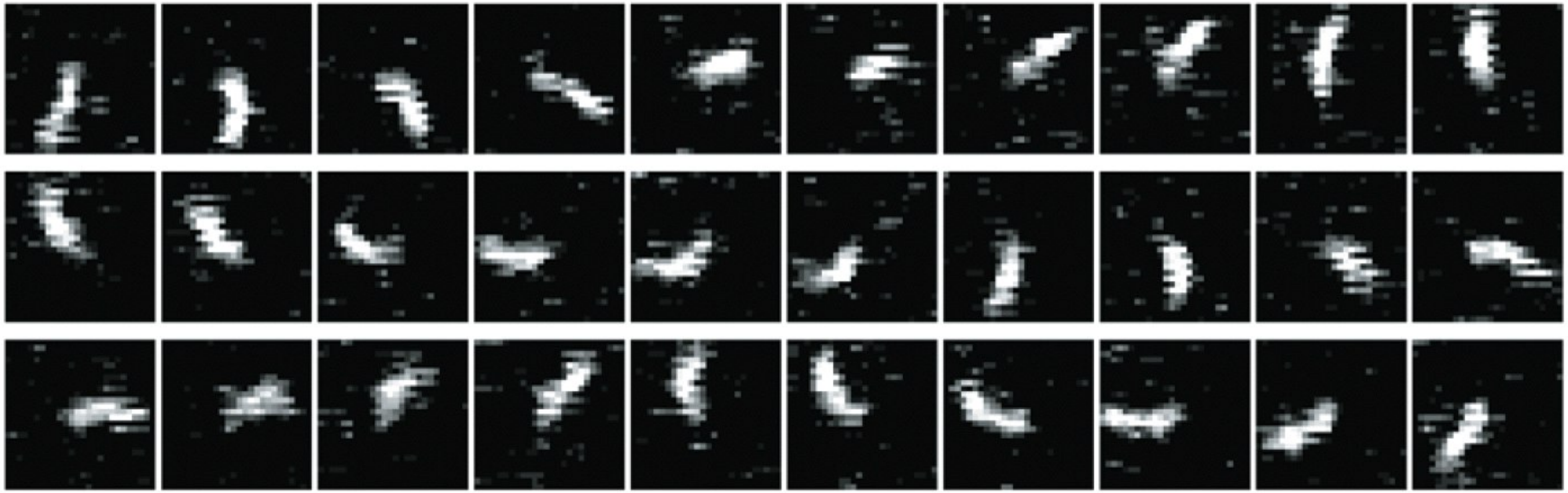


Figure 17-27b Fundamentals of Biochemistry, 2/e

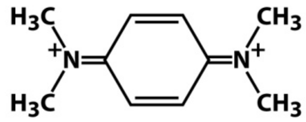
The P/O ratio

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

Complex IV ← e-

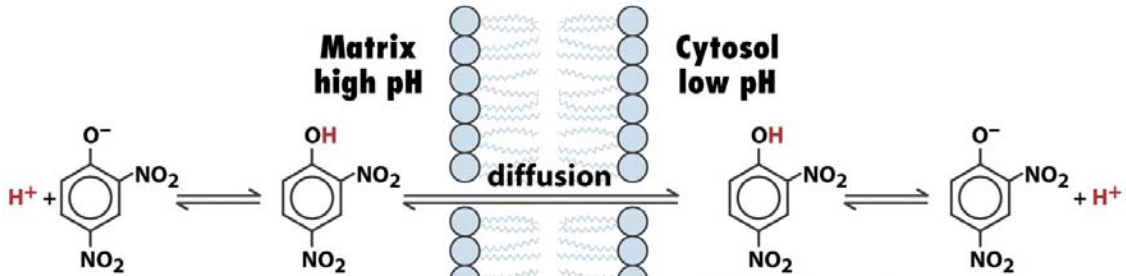


Tetramethyl-*p*-phenylenediamine

Unnumbered figure pg 577 Fundamentals of Biochemistry, 2/e

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



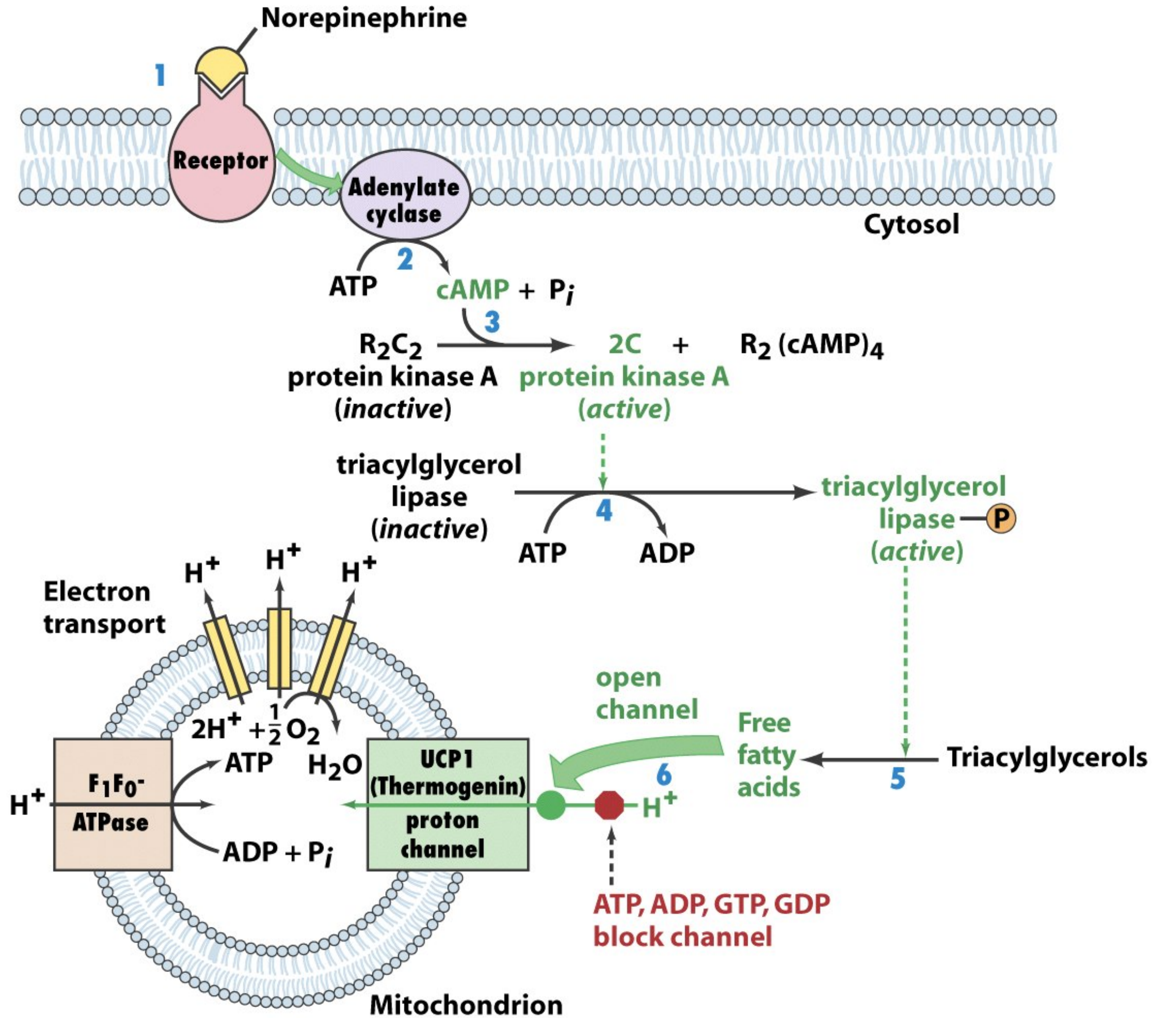
2,4-Dinitrophenol (DNP)

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 1 Fundamentals of Biochemistry, 2/e



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

Control of oxidative metabolism

ATP is never produced more rapidly than necessary
100-fold change in the rate of ATP consumption between sleep & exercise
ATP concentration in the body at any one time: <0.1 mol

Adult woman: 1500~1800 kcal (6300-7500 kJ)
Corresponding to > 200 mol of ATP hydrolysis

NADH to cytochrome c functions at near equilibrium
Cytochrome c oxidase reaction is irreversible
and depends on [reduced cytochrome c (c^{2+})]

$$\frac{1}{2}NADH + cyt.c(+3) + ADP + Pi \leftrightarrow \frac{1}{2}NAD^{+} + cyt.c(+2) + ATP$$

$$K_{eq} = \left(\frac{[NAD]}{[NADH]}\right)^{1/2} \frac{[c2+]}{[c3+]} \frac{[ATP]}{[ADP][Pi]}$$

$$\frac{[c2+]}{[c3+]} = \left(\frac{[NADH]}{[NAD]}\right)^{1/2} \frac{[ADP][Pi]}{[ATP]} K_{eq}$$

ATP mass action ratio = [ATP]/[ADP][Pi]
The higher [NADH]/[NAD] and the lower the ATP mass action ratio
the higher [c^{2+}] and the higher cyt.c oxidase activity

During resting, ATP mass action ratio is high

Coordinated control

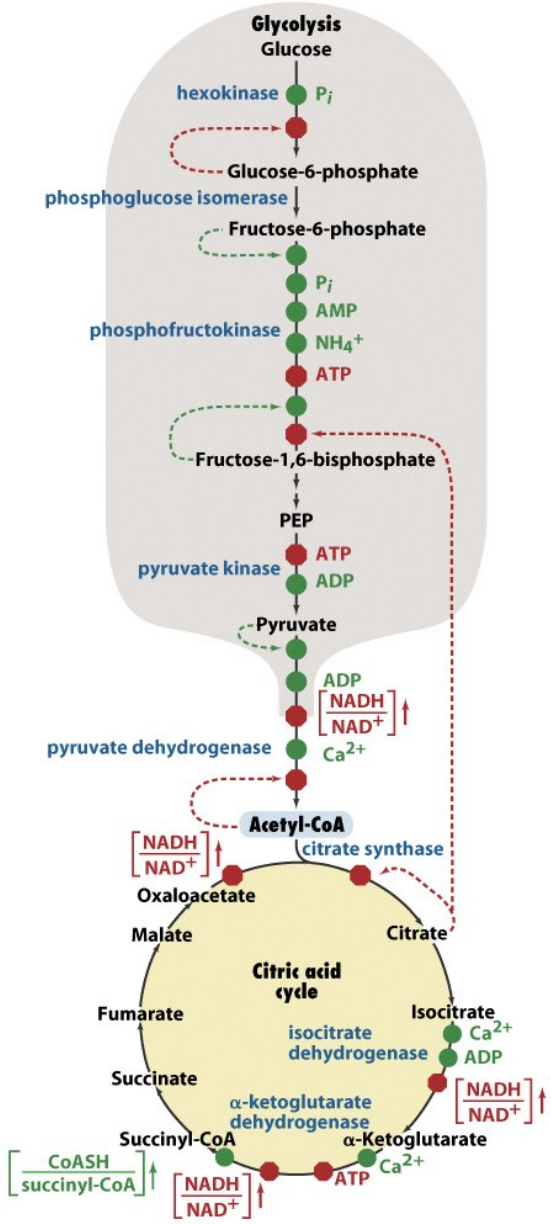


Figure 17-29 Fundamentals of Biochemistry, 2/e

Physiological implication of aerobic metabolism

High efficiency: 19 times more than anaerobic

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

Disadvantages

Oxygen deprivation

Generation of reactive oxygen species (ROS)

Electrostatic effects in human Cu,Zn-SOD

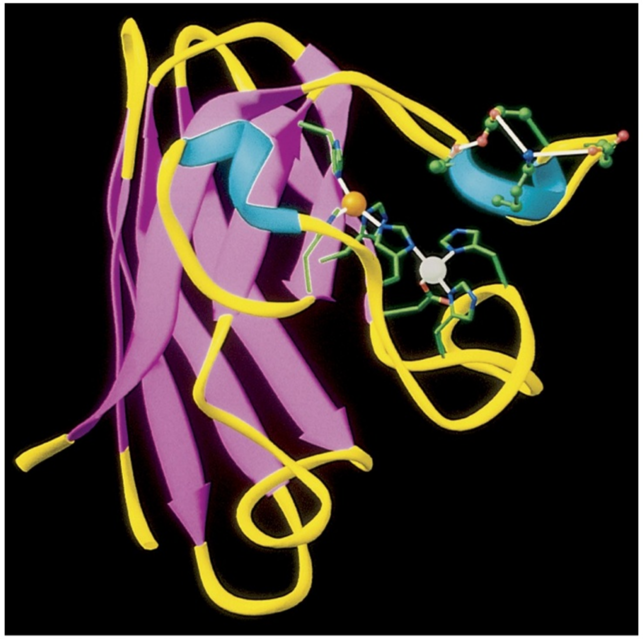


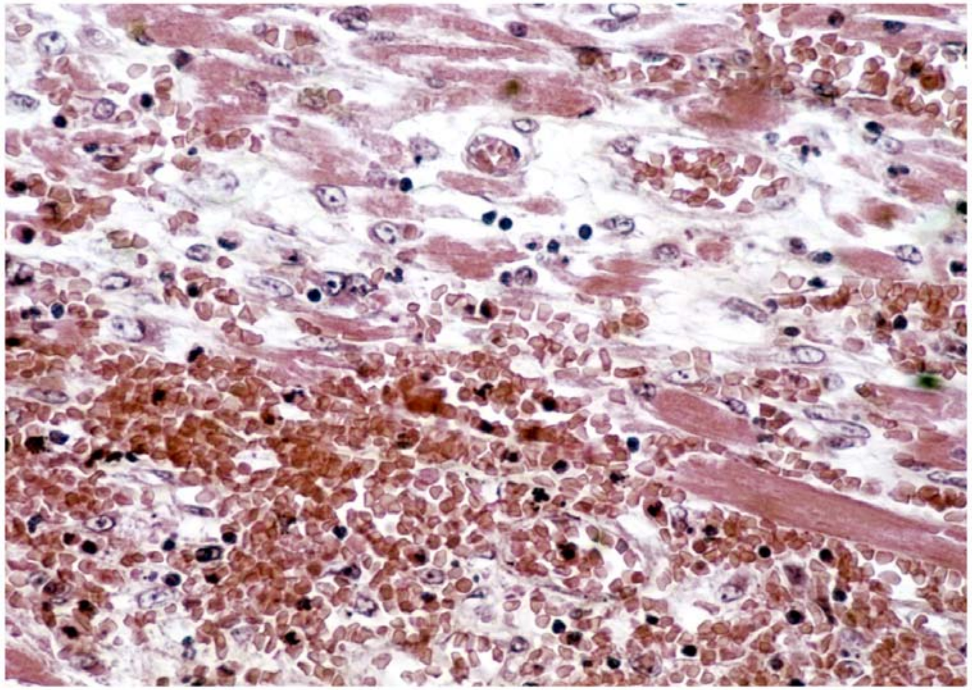
Figure 17-30a Fundamentals of Biochemistry, 2/e

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 $O_2^-\bullet$	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

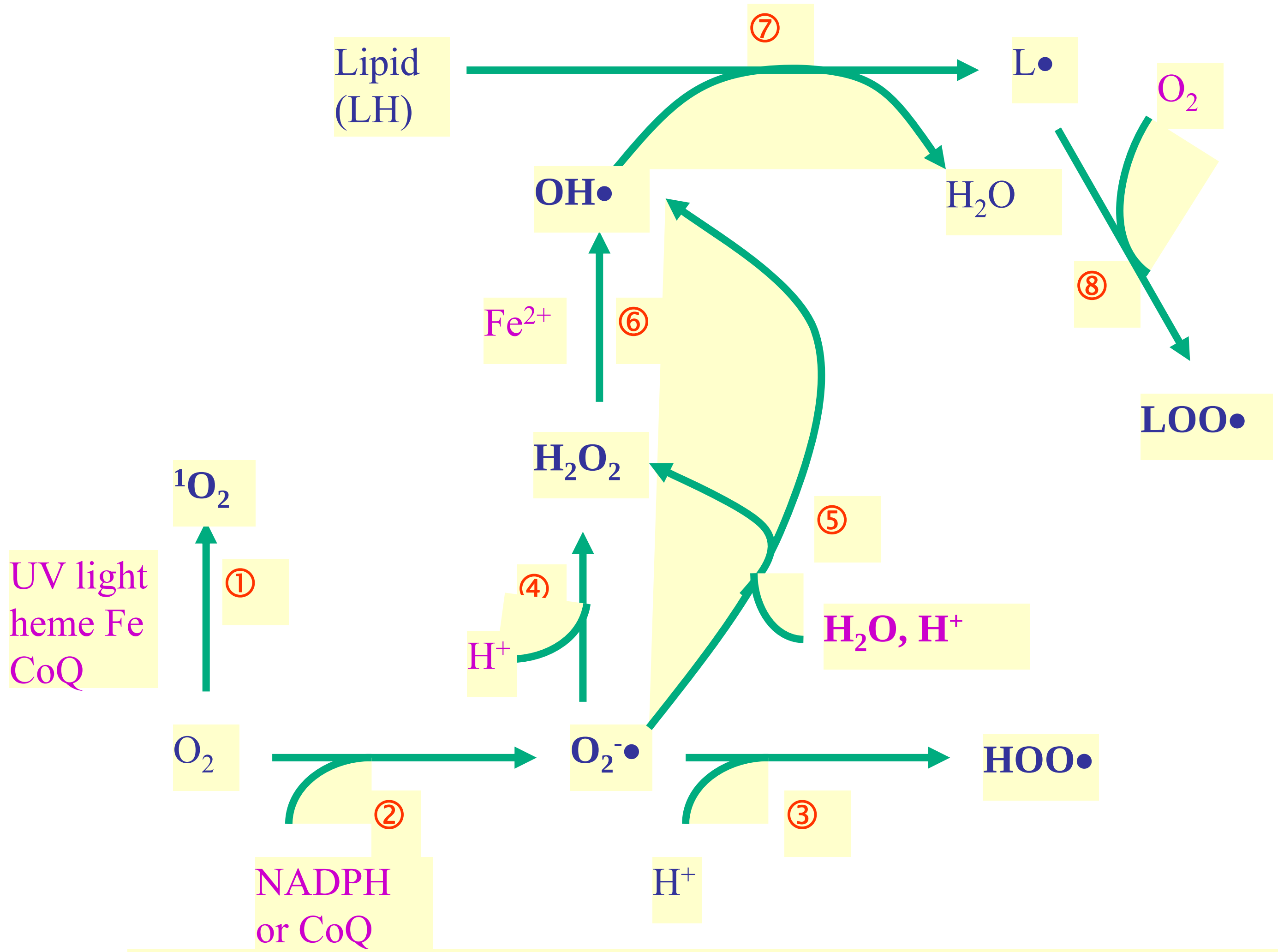


Figure 4. Pathways for the formation of reactive oxygen species

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

ANTI-OXIDANT ENZYMES

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

Mitochondrial & bacterial: Mn^{2+} (or Fe^{2+}) cofactor

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

In human: SOD1, SOD2, SOD3 (extracellular)

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)

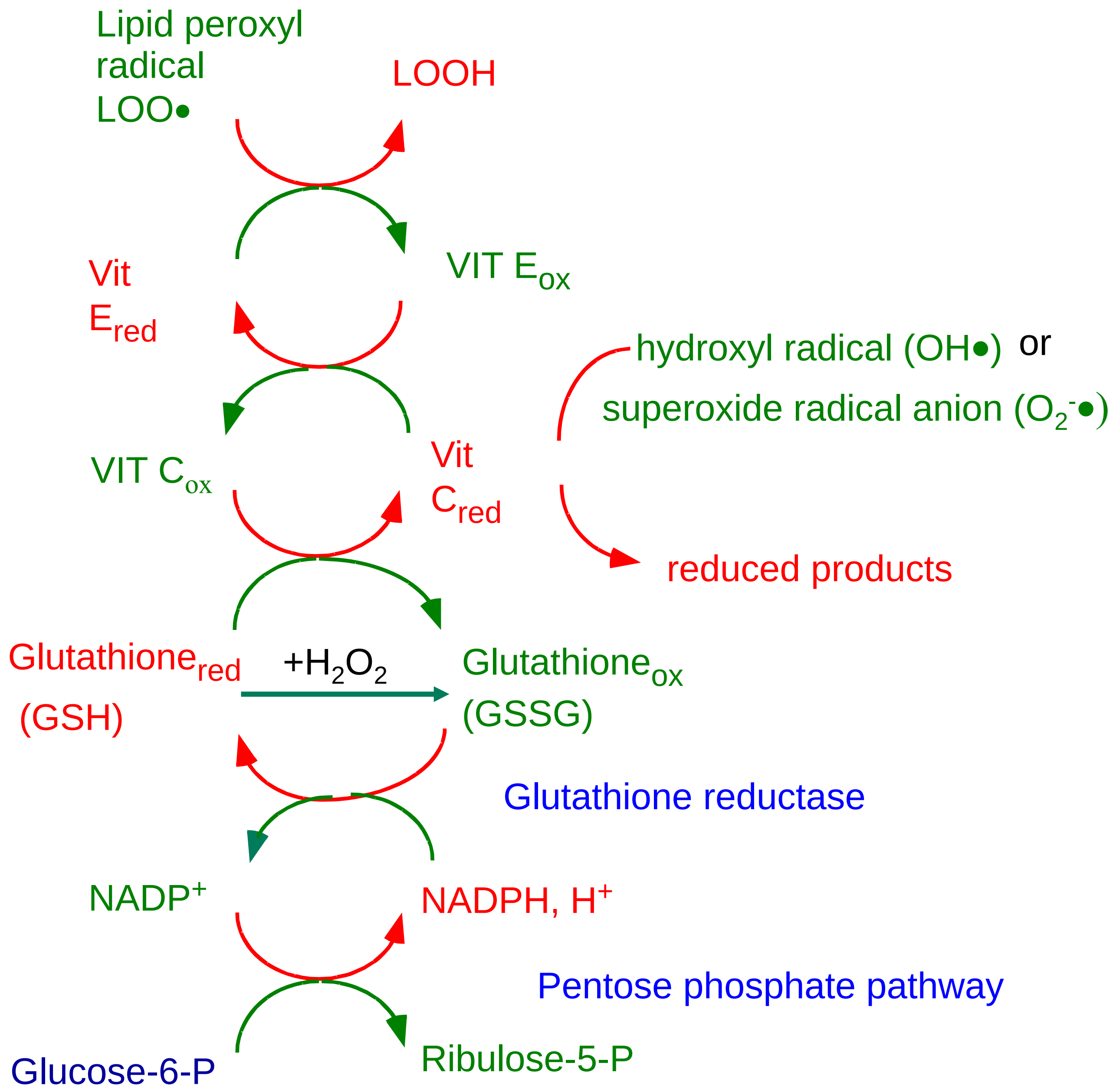


Figure 6. Antioxidant cascade