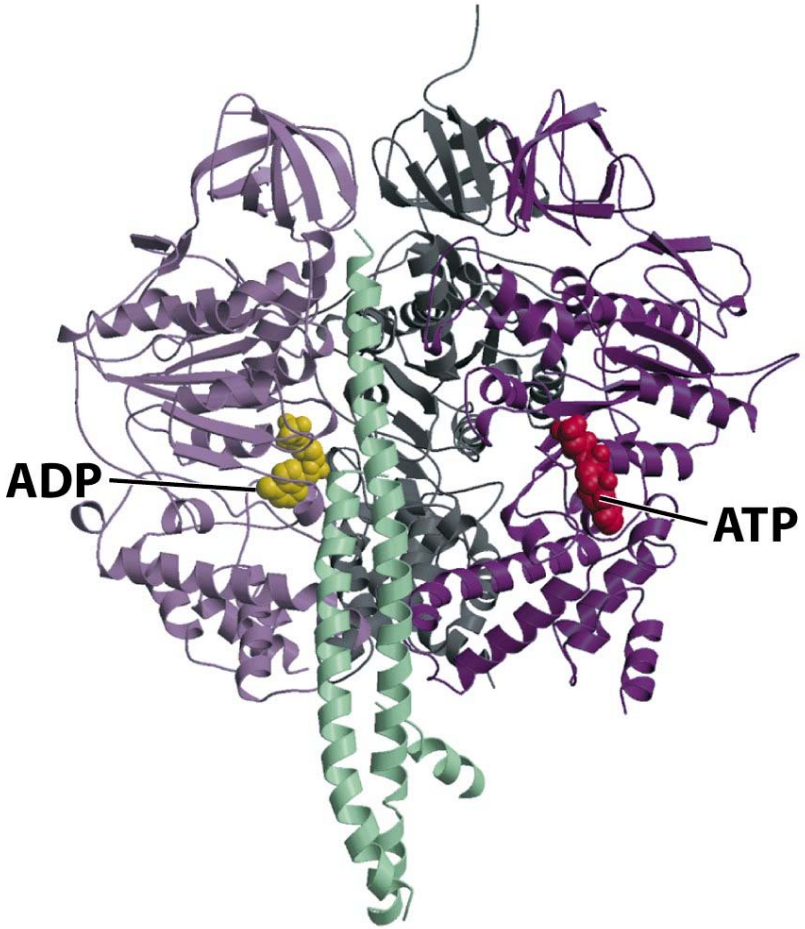
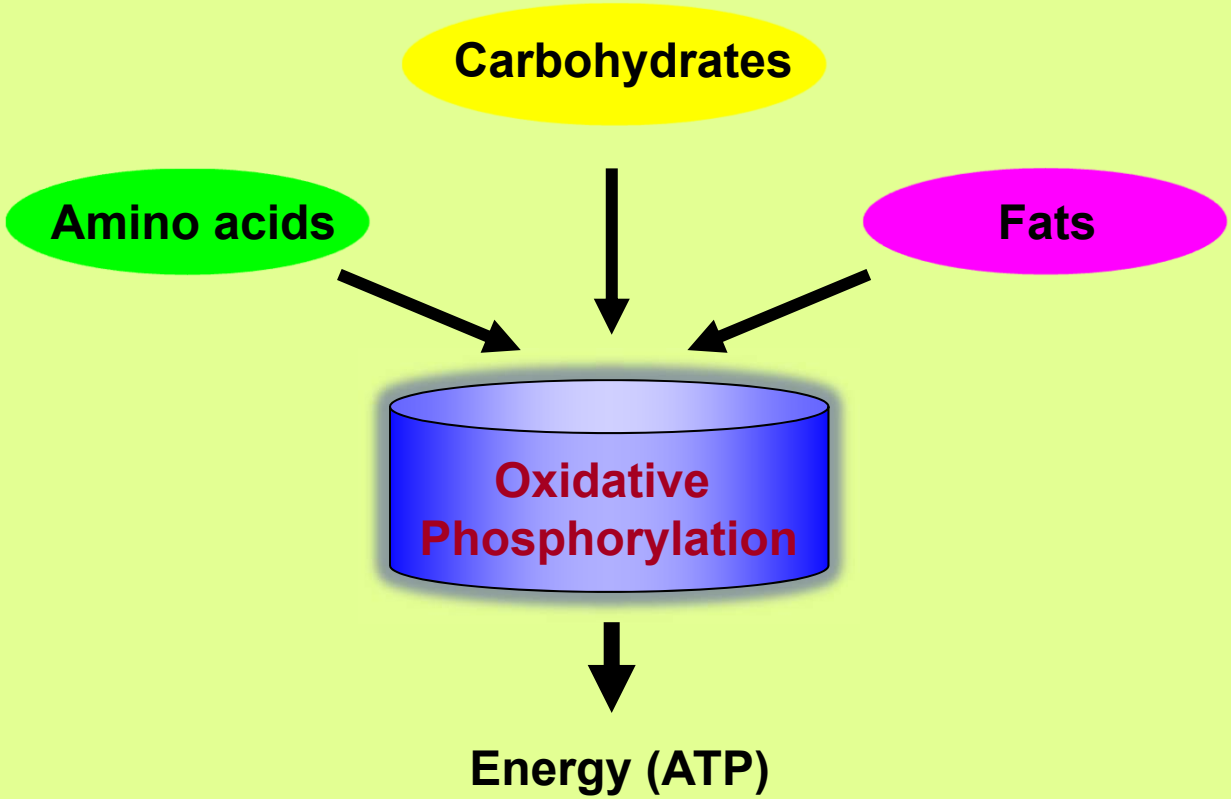
Chpt. 19

Oxidative Phosphorylation

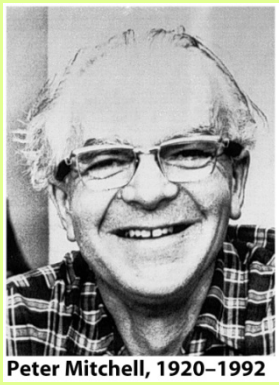


1. Electron-transfer reaction in mitochondria
2. ATP synthesis
3. Regulation of oxidative phosphorylation
4. Mitochondrial genes (origin, mutation effects)
5. The role of mitochondria in apoptosis and oxidative stress



ATP synthesis

Chemiosmotic theory (Peter Mitchell, 1961)


Peter Mitchell, 1920–1992

1. The flow of electrons through a chain of membrane-bound carriers.

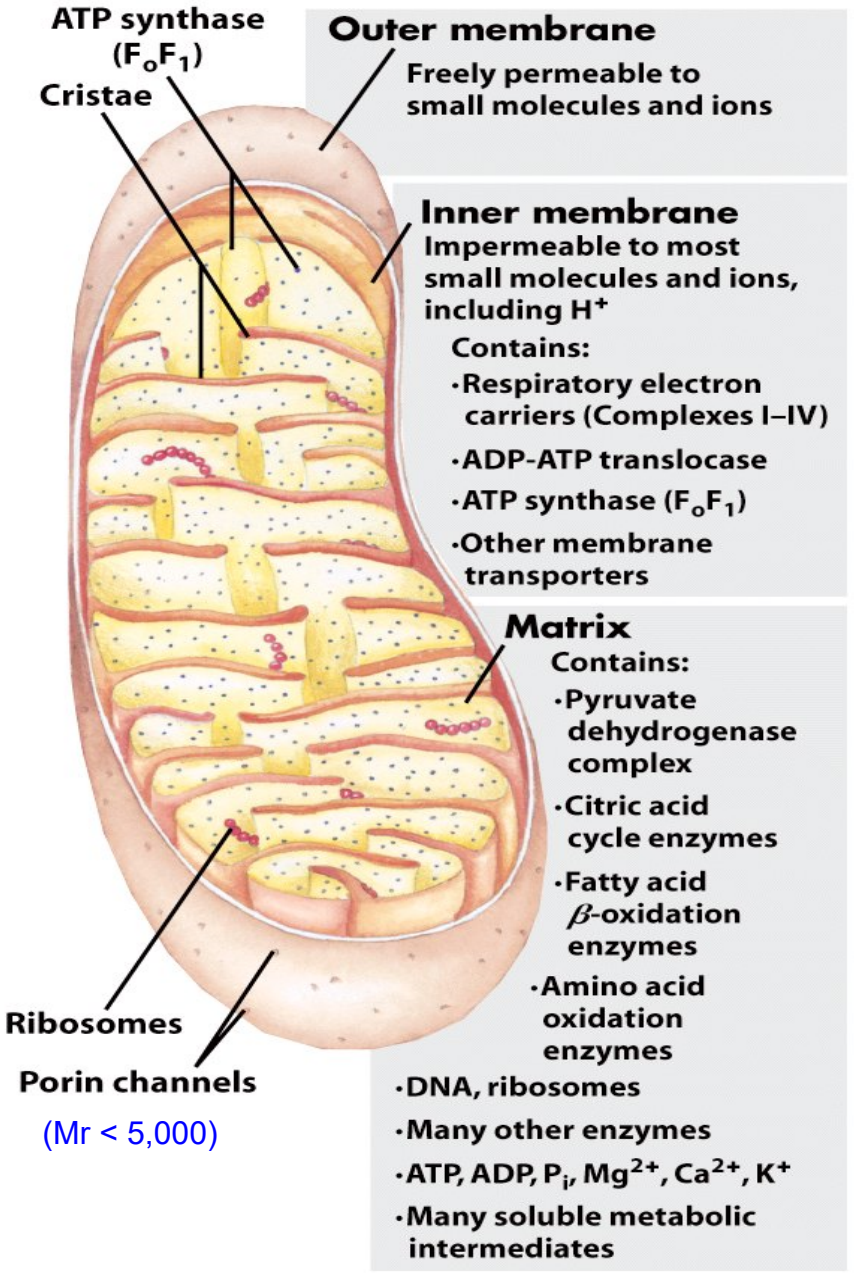
2. The free E made available by this “downhill” (exergonic) electron flow is coupled to the “uphill” transport of protons across a proton-impermeable membrane, conserving the free E of fuel oxidation as a transmembrane electrochemical potential.

3. The transmembrane flow of protons down their concentration gradient through specific protein channels provides the free E for synthesis of ATP, catalyzed by a membrane protein complex (ATP synthase).

Biochemical anatomy of a mitochondrion

Mitochondria:
the site of oxidative phosphorylation in eukaryotes
(E. Kennedy & A. Lehninger, 1948)

Albert L. Lehninger
1917–1986

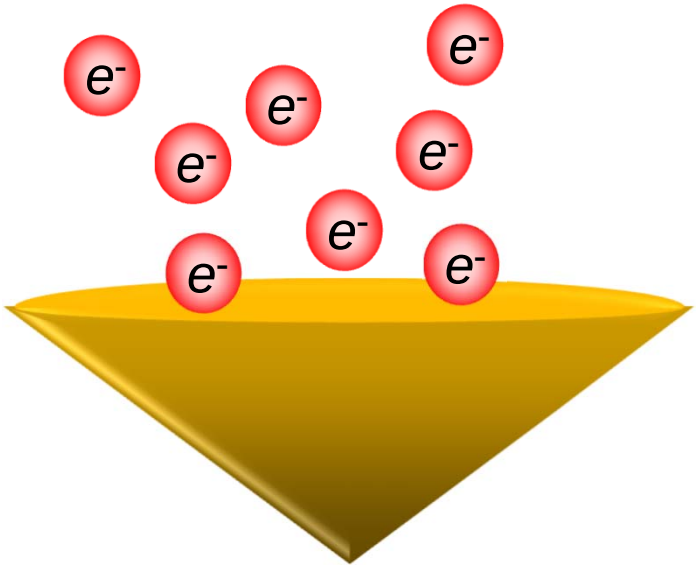


> 10,000 sets

Electrons are funneled to universal electron acceptor

-Oxidative phosphorylation begins with
the entry of electrons into the respiratory chain.

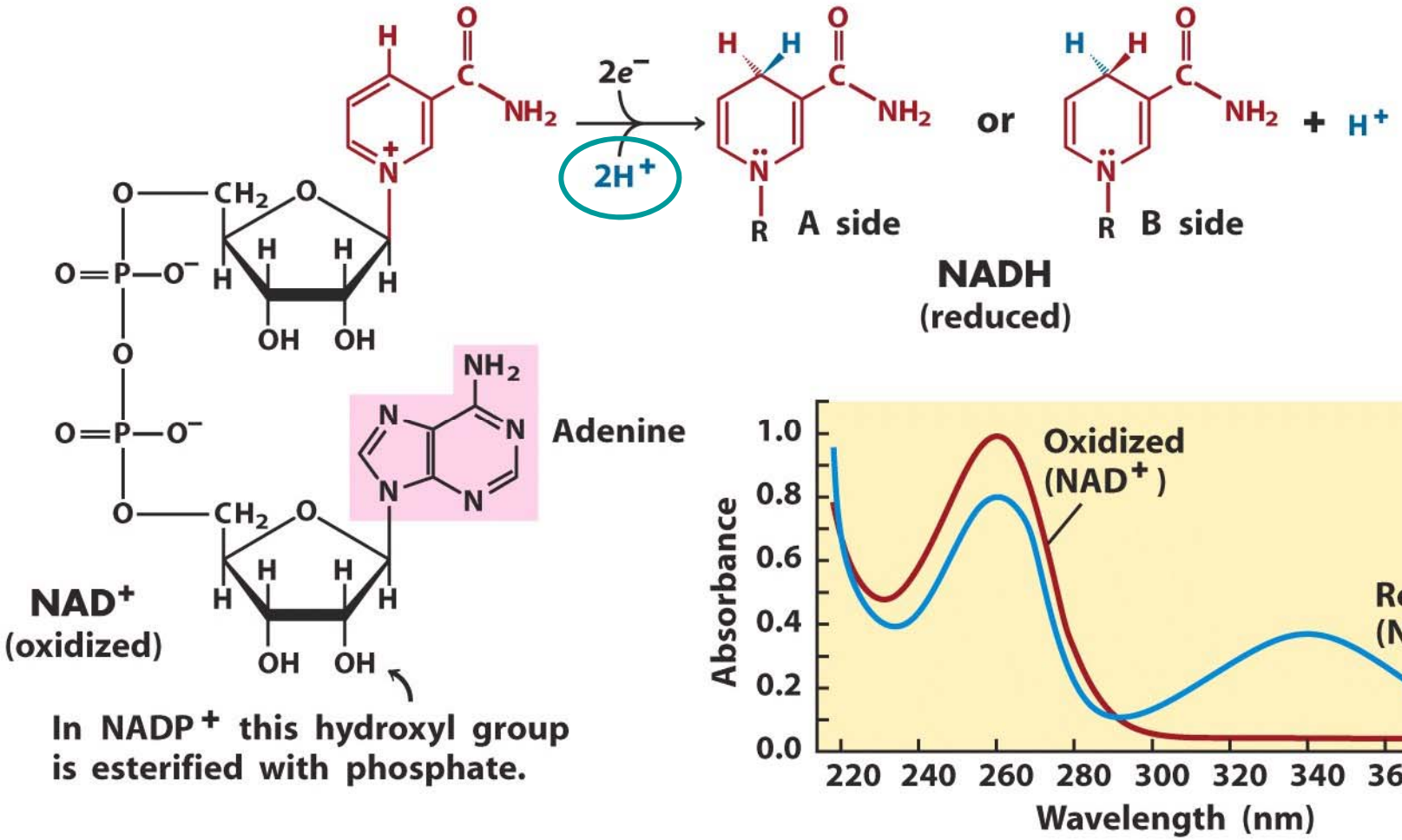
[Dehydrogenase]



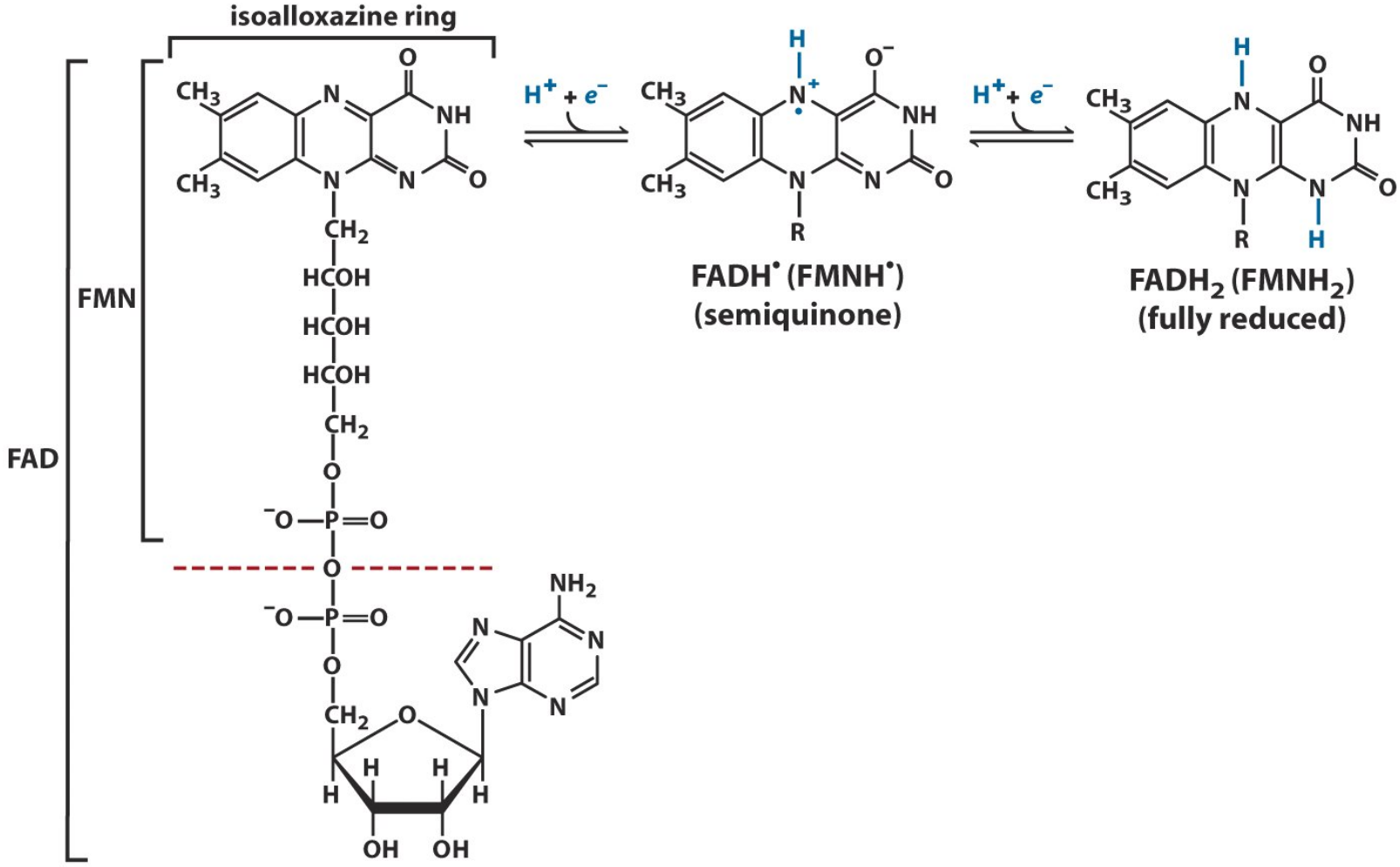
e- **acceptor**

NAD^+ / $NADP^+$
FMN / FAD

NAD and NADP


2e⁻
2H⁺
or
+ H⁺
A side
B side
NADH
(reduced)
Adenine
NAD⁺
(oxidized)
In NADP⁺ this hydroxyl group is esterified with phosphate.
Oxidized (NAD⁺)
Reduced (NADH)
Absorbance
1.0
0.8
0.6
0.4
0.2
0.0
220 240 260 280 300 320 340 360 380
Wavelength (nm)

Structures of oxidized and reduced FAD and FMN



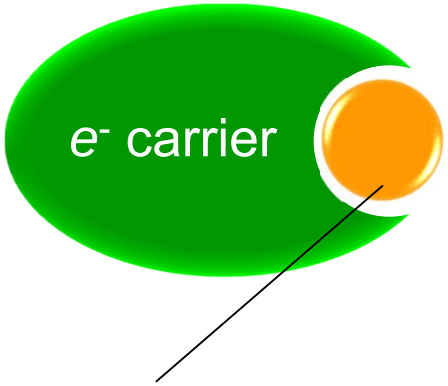
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

Electron pass through a series of memb.-bound carrier

Electron transfer (1) direct transfer of electrons as in the reduction of Fe^{3+} to Fe^{2+}

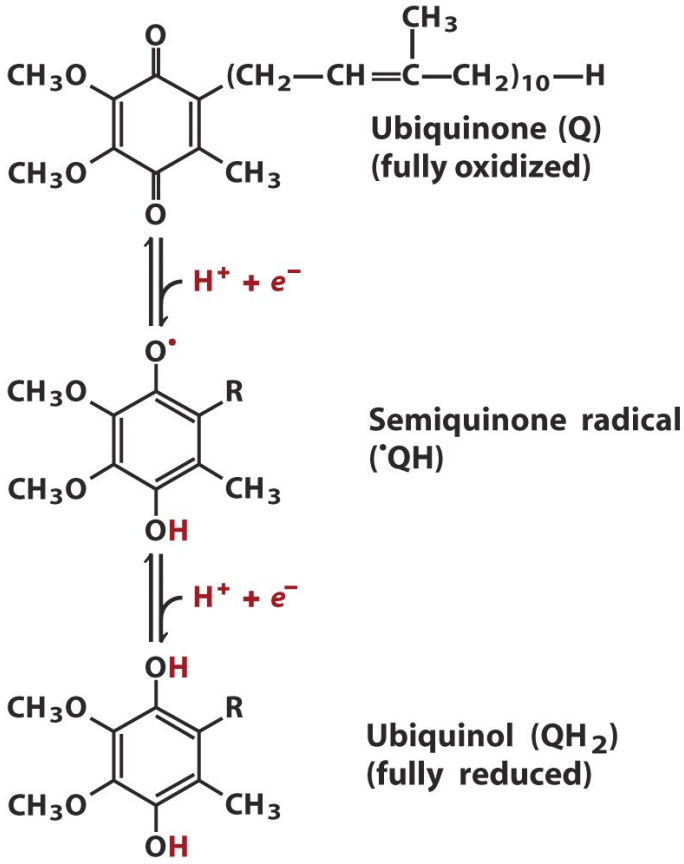
(2) transfer as a hydrogen atom ($H^+ + e^-$)

(3) transfer as a hydride ion ($:H^-$)



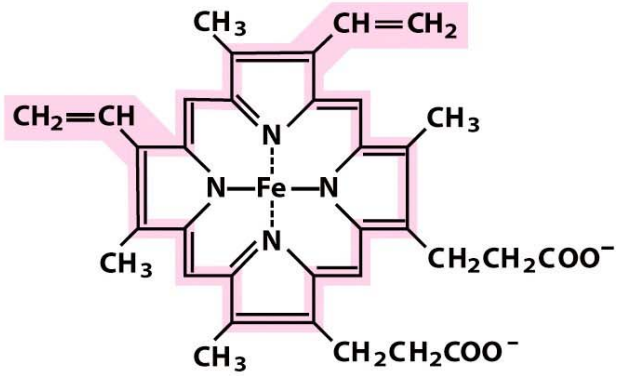
prothetic group

(1) Ubiquinone (Q, a hydrophobic quinone)
(2) iron-containing proteins
(cytochromes & iron-sulfur proteins)

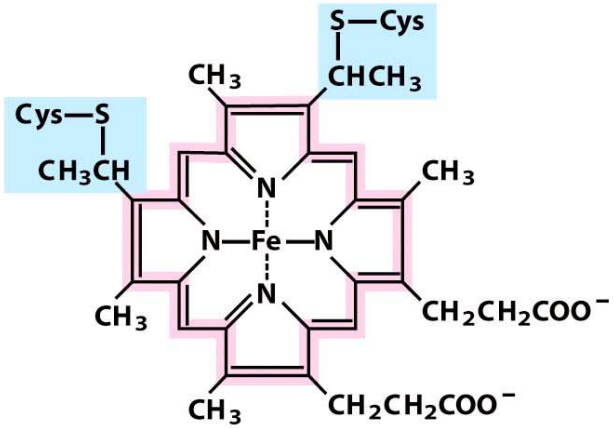


Prosthetic groups of cytochromes

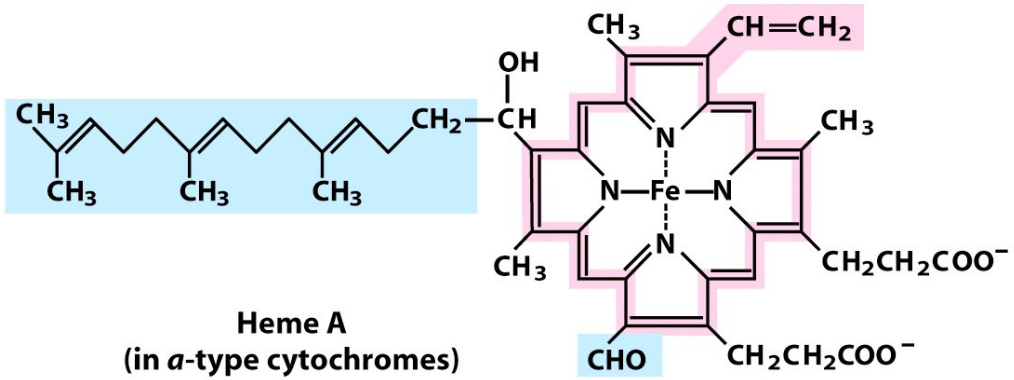
cyt *a* (600 nm), *b* (560 nm), *c* (550 nm) in mitochondria



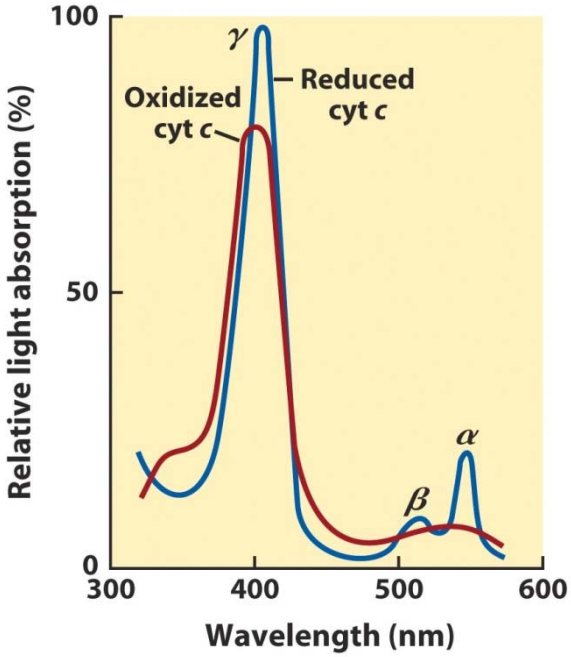
Iron protoporphyrin IX
(in *b*-type cytochromes)



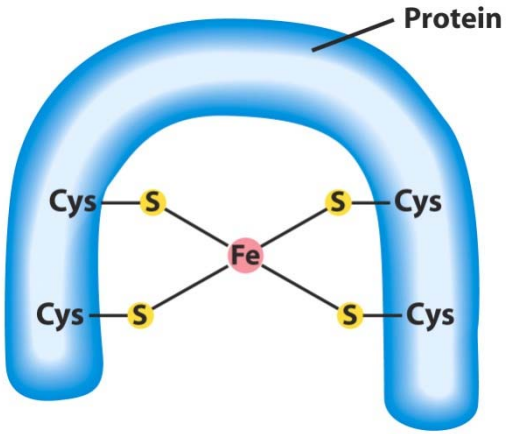
Heme C
(in *c*-type cytochromes)

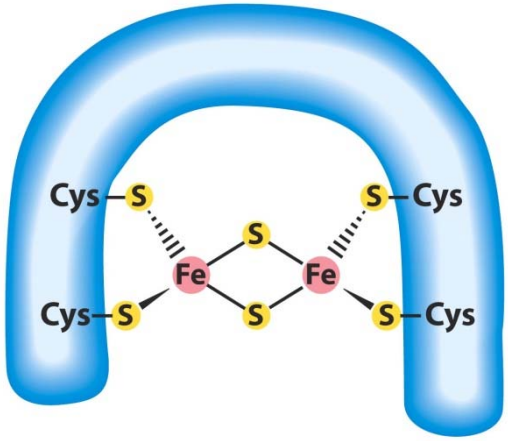


Heme A
(in *a*-type cytochromes)

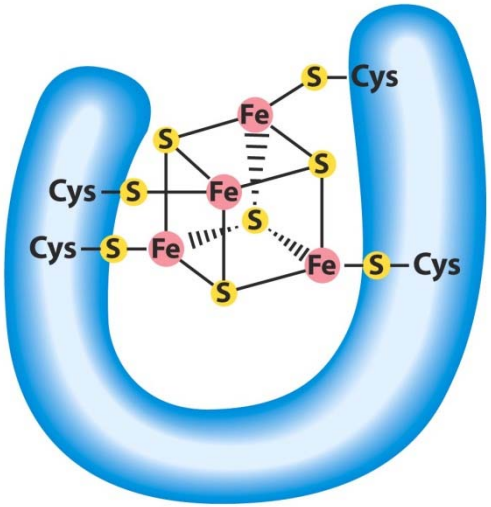


Fe-S centers in iron-sulfur proteins





2Fe-2S



4Fe-4S

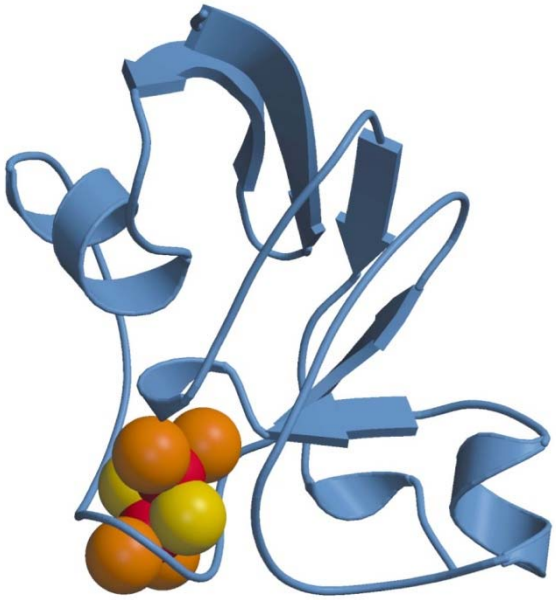


TABLE 19–2 Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

Redox reaction (half-reaction)	*E′° (V)*
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) $+ 2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone $+ 2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) $+ e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.8166

* Depend on the concentration of reduced and oxidized forms under actual cellular conditions

Method for determining the sequence of electron carriers

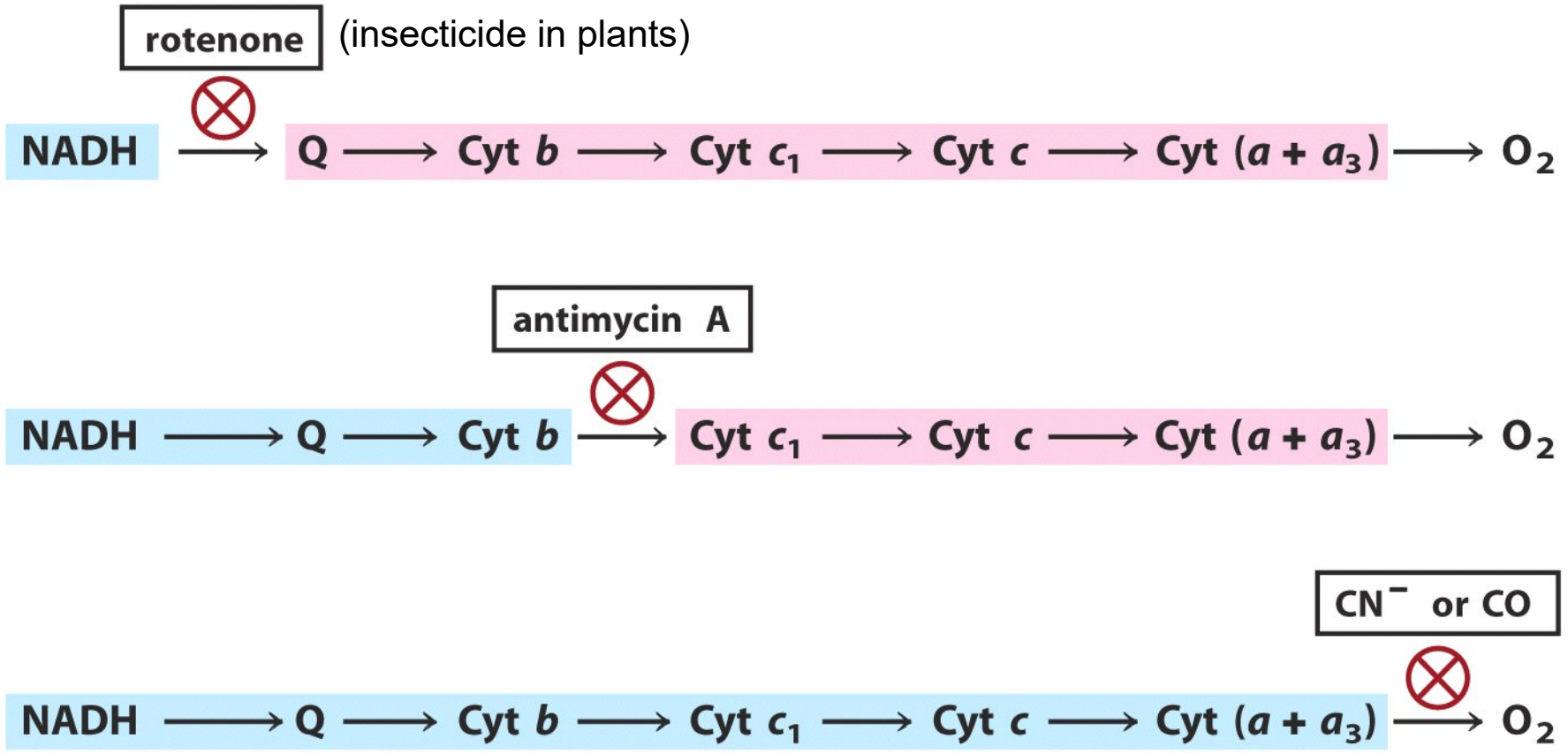


TABLE 19–4 Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

Type of interference	*Compound*[*]	*Target/mode of action*
Inhibition of electron transfer	Cyanide	Inhibit cytochrome oxidase
	Carbon monoxide	
	Antimycin A	Blocks electron transfer from cytochrome *b* to cytochrome c_1
	Myxothiazol	Prevent electron transfer from Fe-S center to ubiquinone
	Rotenone	
	Amytal	
	Piericidin A	
	DCMU	Competes with Q_B for binding site in PSII
Inhibition of ATP synthase	Aurovertin	Inhibits F_1
	Oligomycin	
	Venturicidin	
	DCCD	Blocks proton flow through F_o and CF_o
Uncoupling of phosphorylation from electron transfer	FCCP	Hydrophobic proton carriers
	DNP	
	Valinomycin	K^+ ionophore
	Thermogenin	In brown fat, forms proton-conducting pores in inner mitochondrial membrane
Inhibition of ATP-ADP exchange	Atractyloside	Inhibits adenine nucleotide translocase

[*]DCMU is 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCCD, dicyclohexylcarbodiimide; FCCP, cyanide-*p*-trifluoromethoxyphenylhydrazone; DNP, 2,4-dinitrophenol.

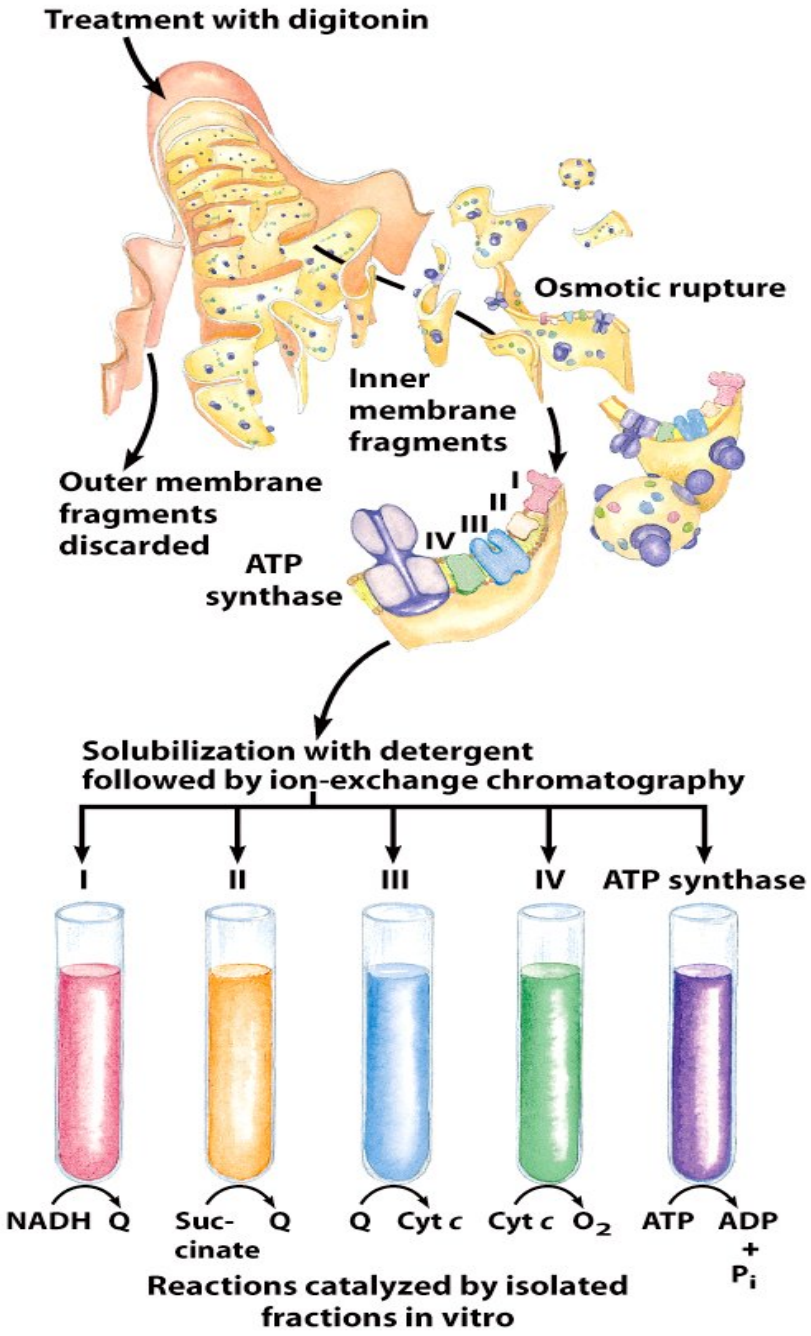
Electron carriers function in multienzyme complexes

TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

	Enzyme complex/protein	*Mass (kDa)*	*Number of subunits**	*Prosthetic group(s)*
I	NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II	Succinate dehydrogenase	140	4	FAD, Fe-S
III	Ubiquinone cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
	Cytochrome *c*†	13	1	Heme
IV	Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

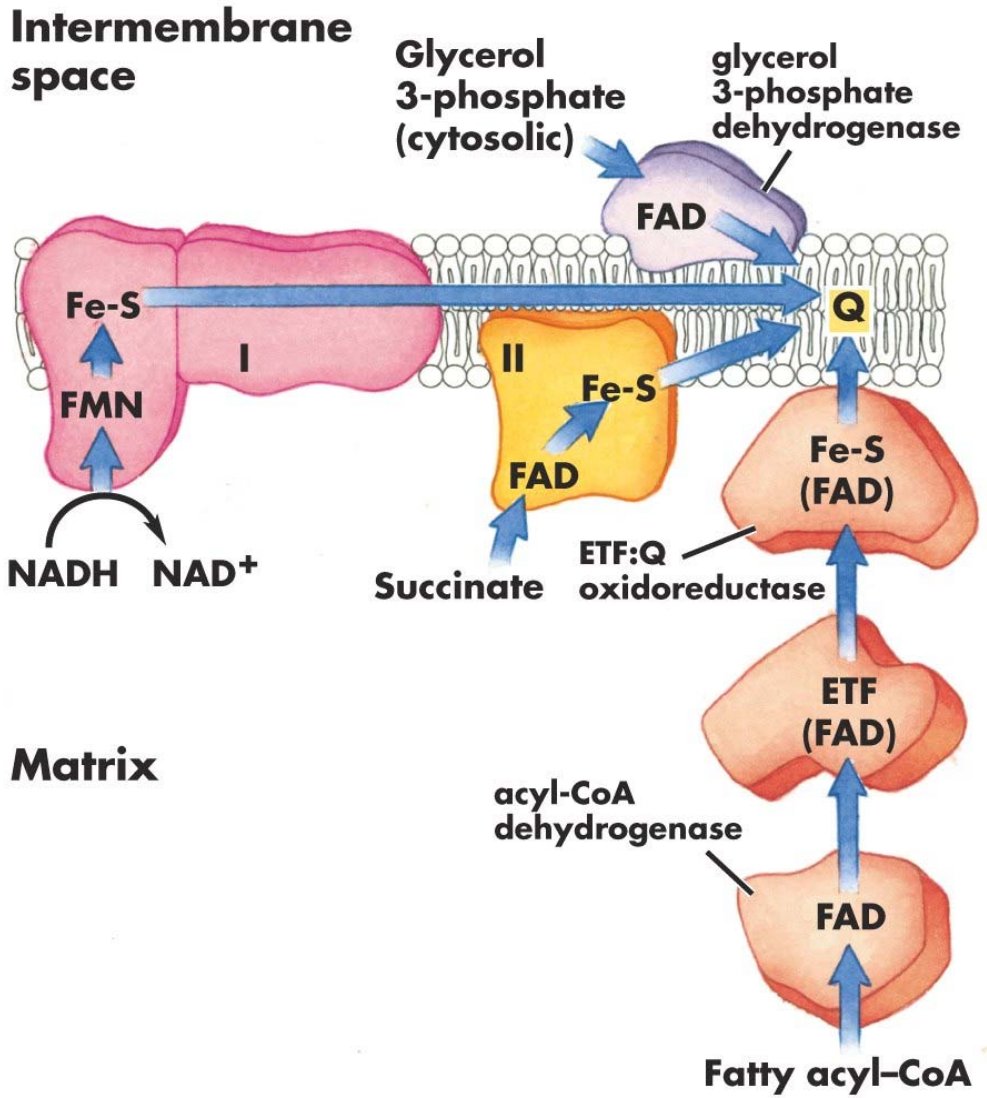
*Numbers of subunits in the bacterial equivalents in parentheses.
†Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

Separation of functional complexes of the respiratory chain

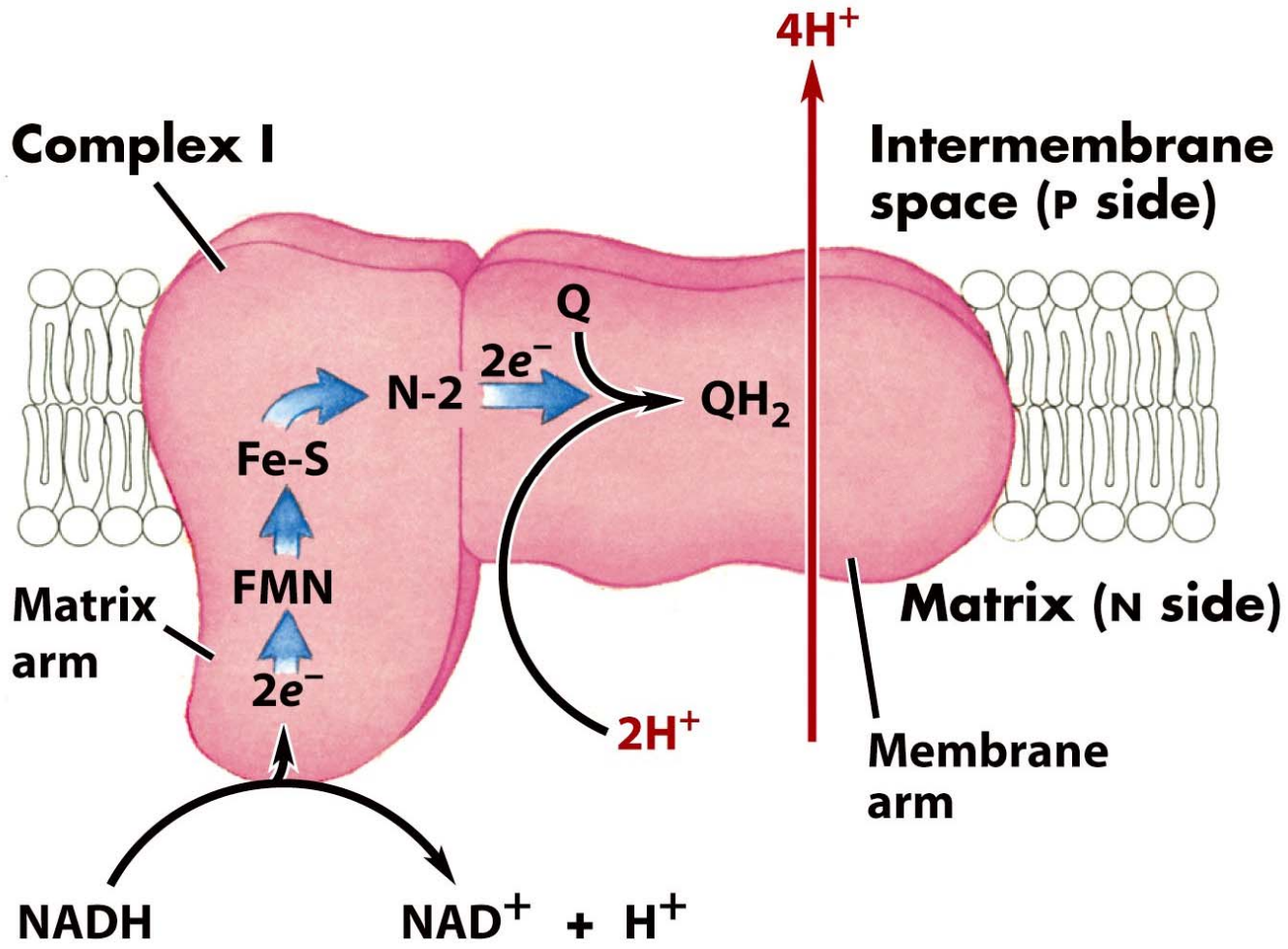


Path of electrons:

(from NADH, succinate, fatty acyl-CoA and G3P to Q)



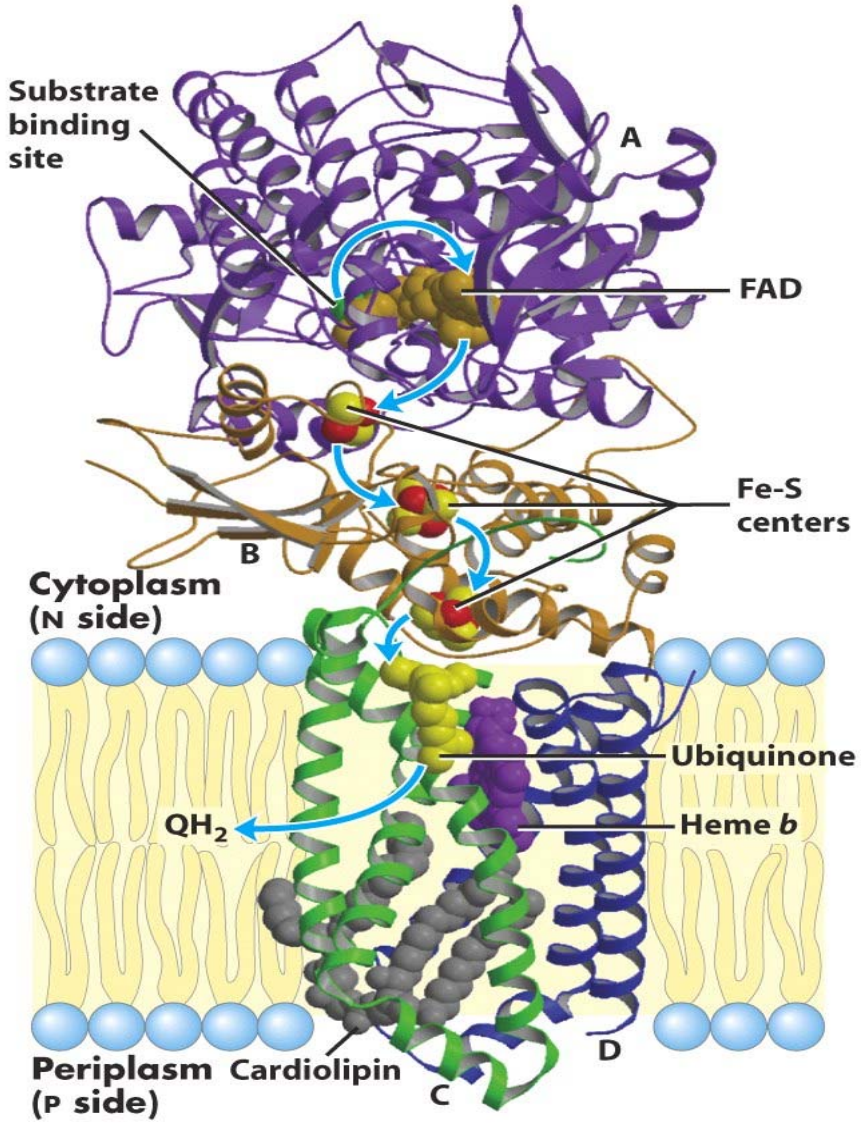
NADH:ubiquinone oxidoreductase (Complex I)

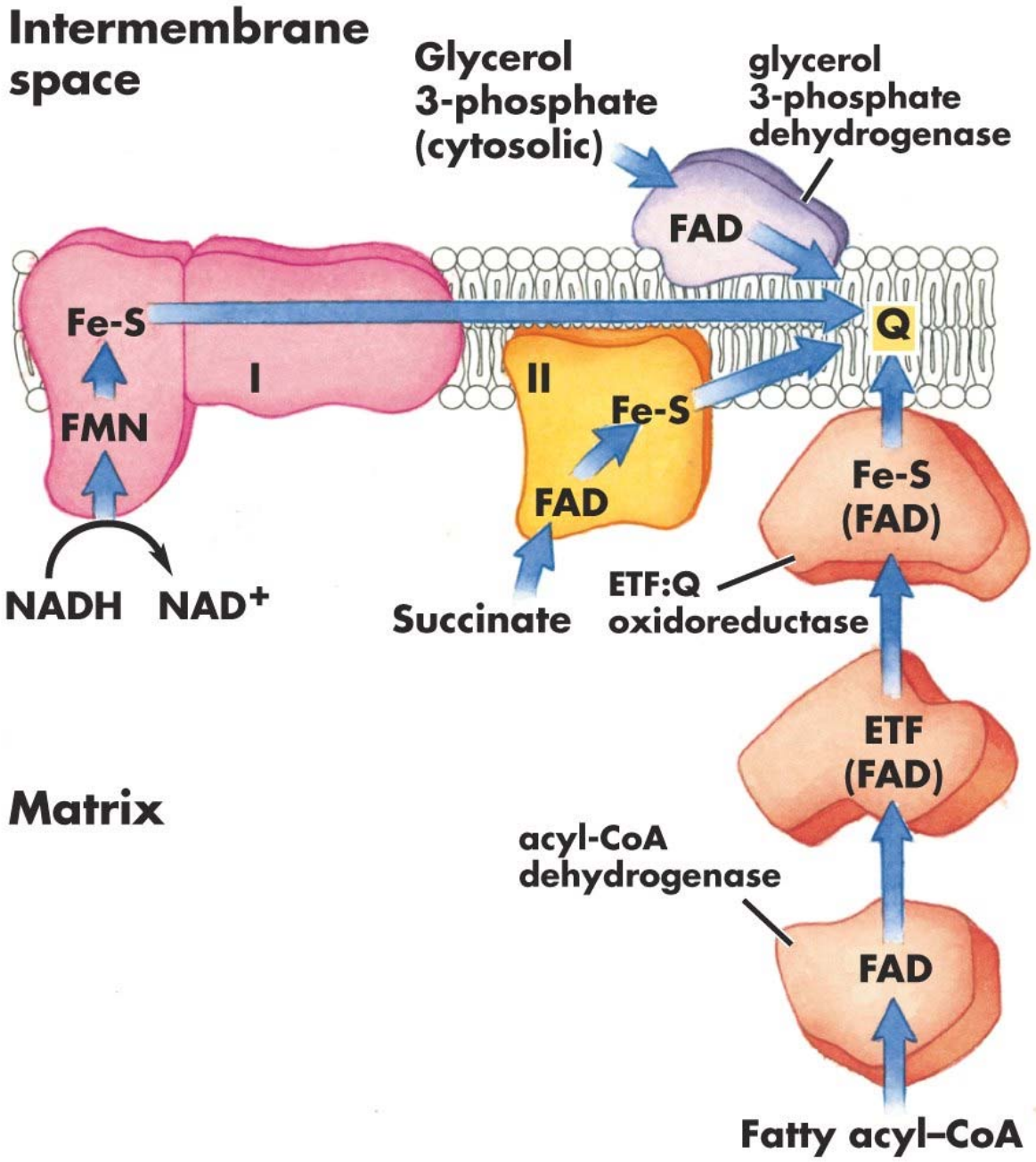


$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$

$NADH + 5H^+_N + Q \longrightarrow NAD^+ + QH_2 + 4H^+_P$

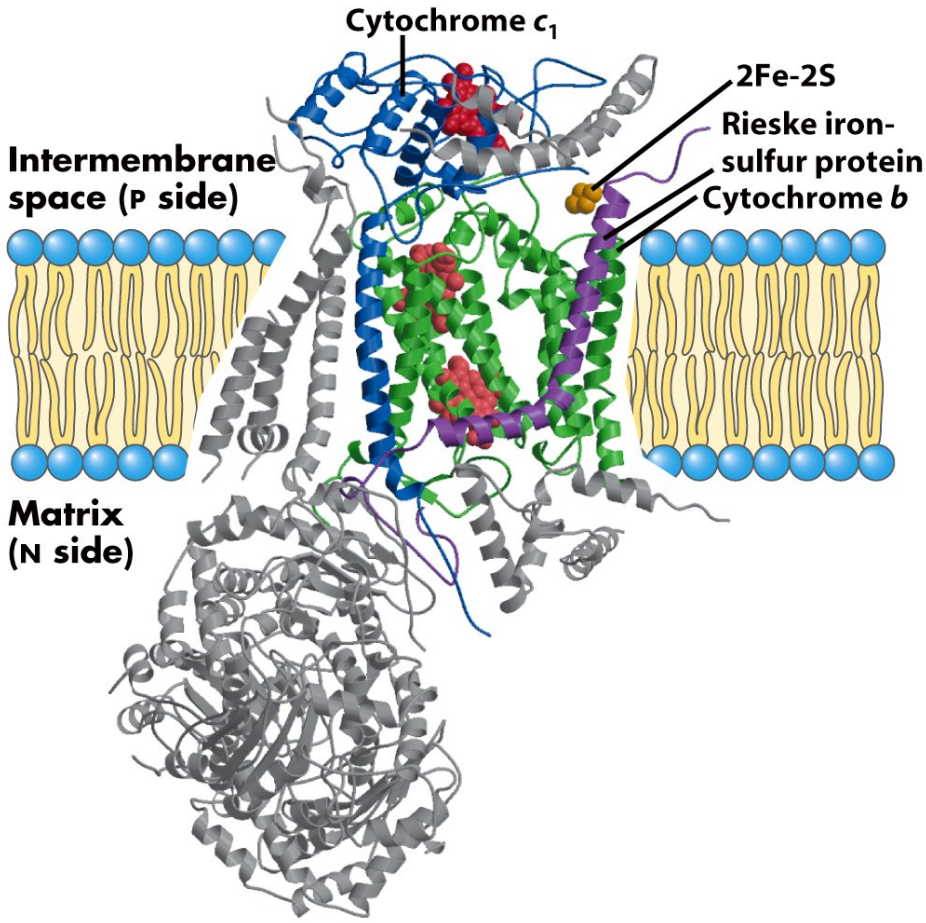
Structure of succinate dehydrogenase (Complex II)

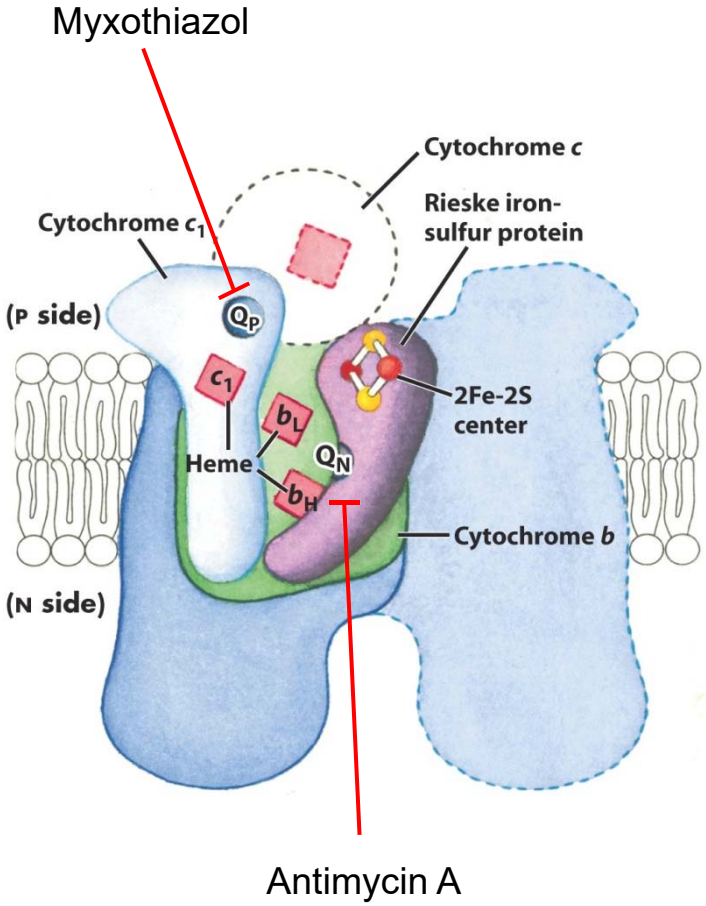



Intermembrane space
Glycerol 3-phosphate (cytosolic)
glycerol 3-phosphate dehydrogenase
FAD
Fe-S
I
FMN
NADH
NAD+
II
Fe-S
FAD
Succinate
Q
Fe-S (FAD)
ETF:Q oxidoreductase
ETF (FAD)
Matrix
acyl-CoA dehydrogenase
FAD
Fatty acyl–CoA

Cytochrome bc_1 complex (Complex III)

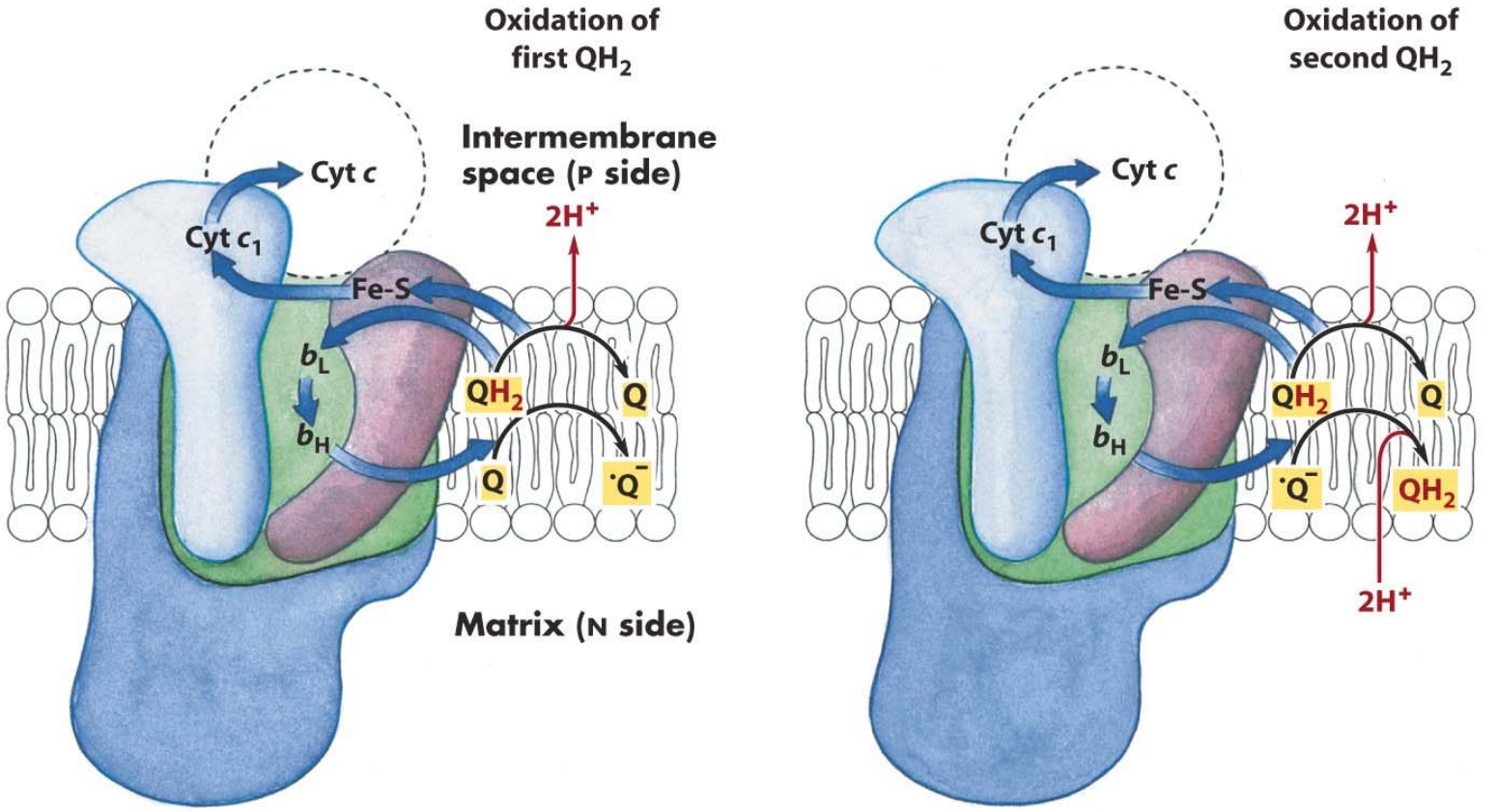
Ubiquinone:cytochrome *c* oxidoreductase





(dimeric functional unit)

The Q cycle



QH_2 + cyt c_1 (oxidized) ⟶ $\cdot Q^-$ + $2H_P^+$ + cyt c_1 (reduced)

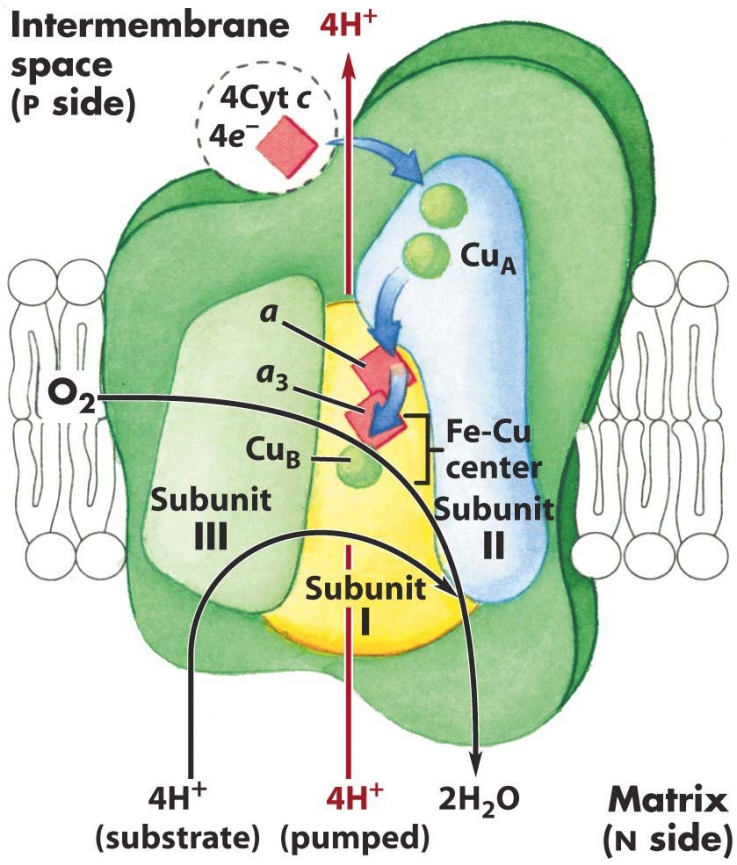
QH_2 + $\cdot Q^-$ + $2H_N^+$ + cyt c_1 (oxidized) ⟶ QH_2 + $2H_P^+$ + Q + cyt c_1 (reduced)

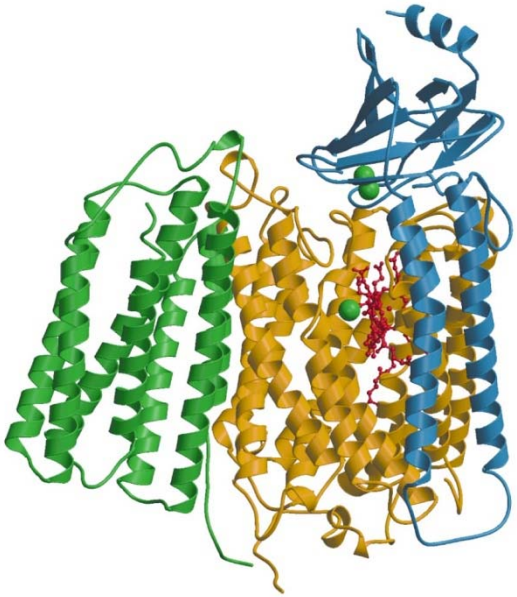
Net equation:

QH_2 + 2 cyt c_1 (oxidized) + $2H_N^+$ ⟶ Q + 2 cyt c_1 (reduced) + $4H_P^+$

Cytochrome oxidase (Complex IV)

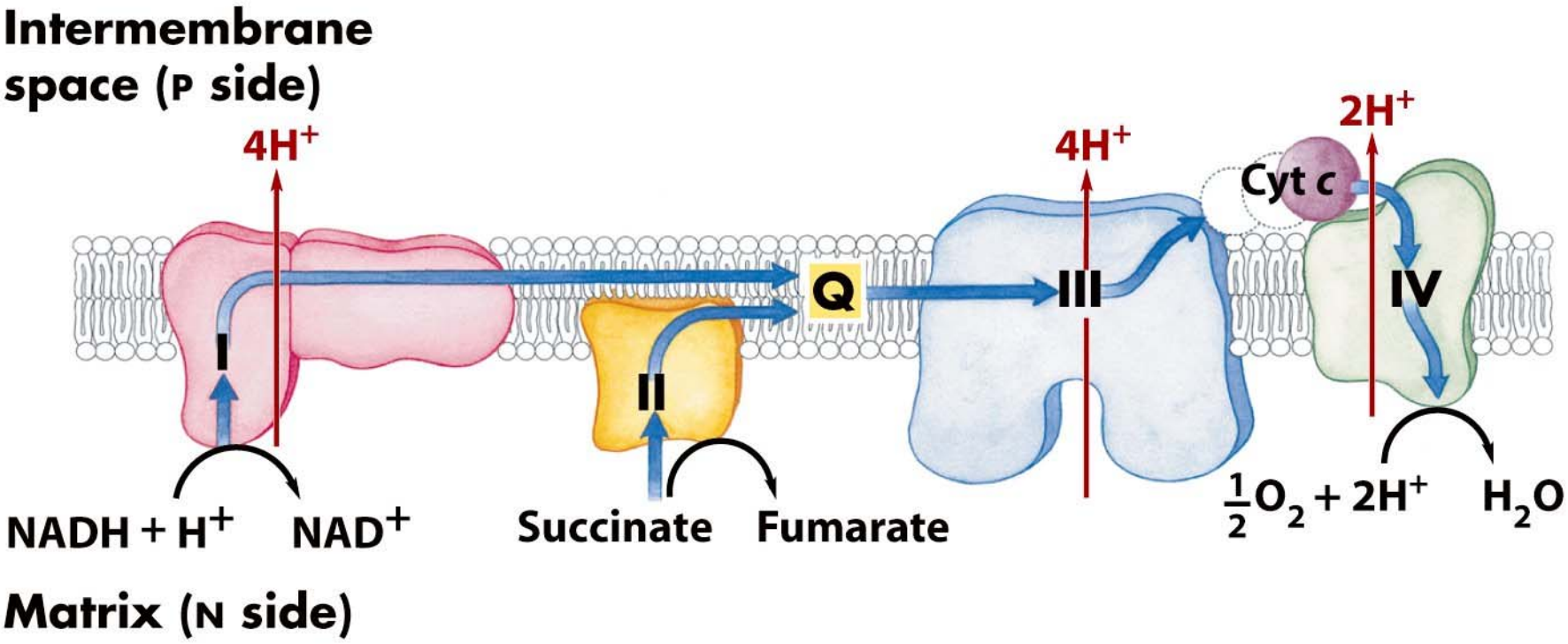
Cytochrome *c* (e^-) → H_2O (with O_2)





$$4 \text{ cyt } c_{(R)} + 8H^+_N + O_2 \longrightarrow 4 \text{ cyt } c_{(O)} + 4H^+_P + 2H_2O$$

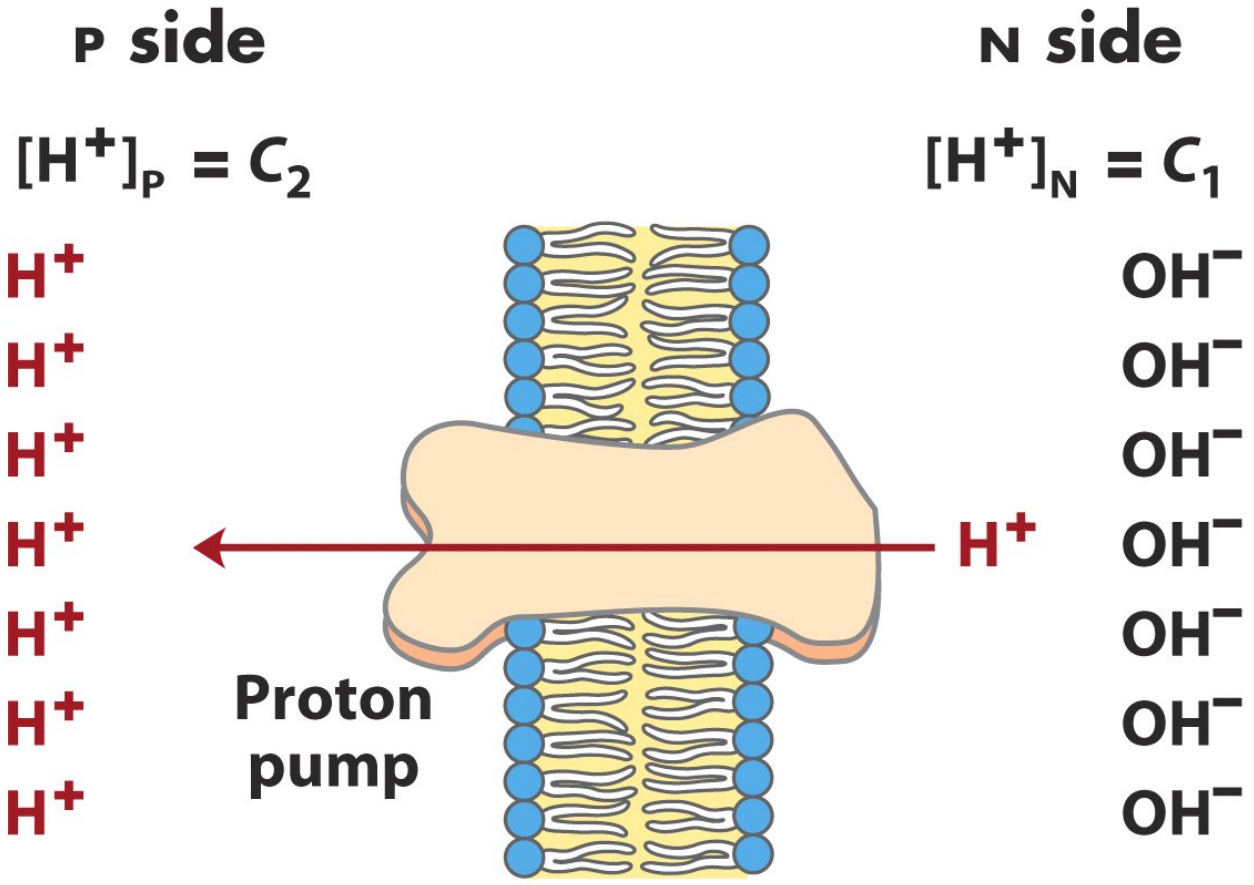
Summary of the flow of electrons and protons



$$NADH + 11H^+_N + \tfrac{1}{2}O_2 \longrightarrow NAD^+ + 10H^+_P + H_2O$$

Proton-motive force: ΔpH (~ 0.75 units)

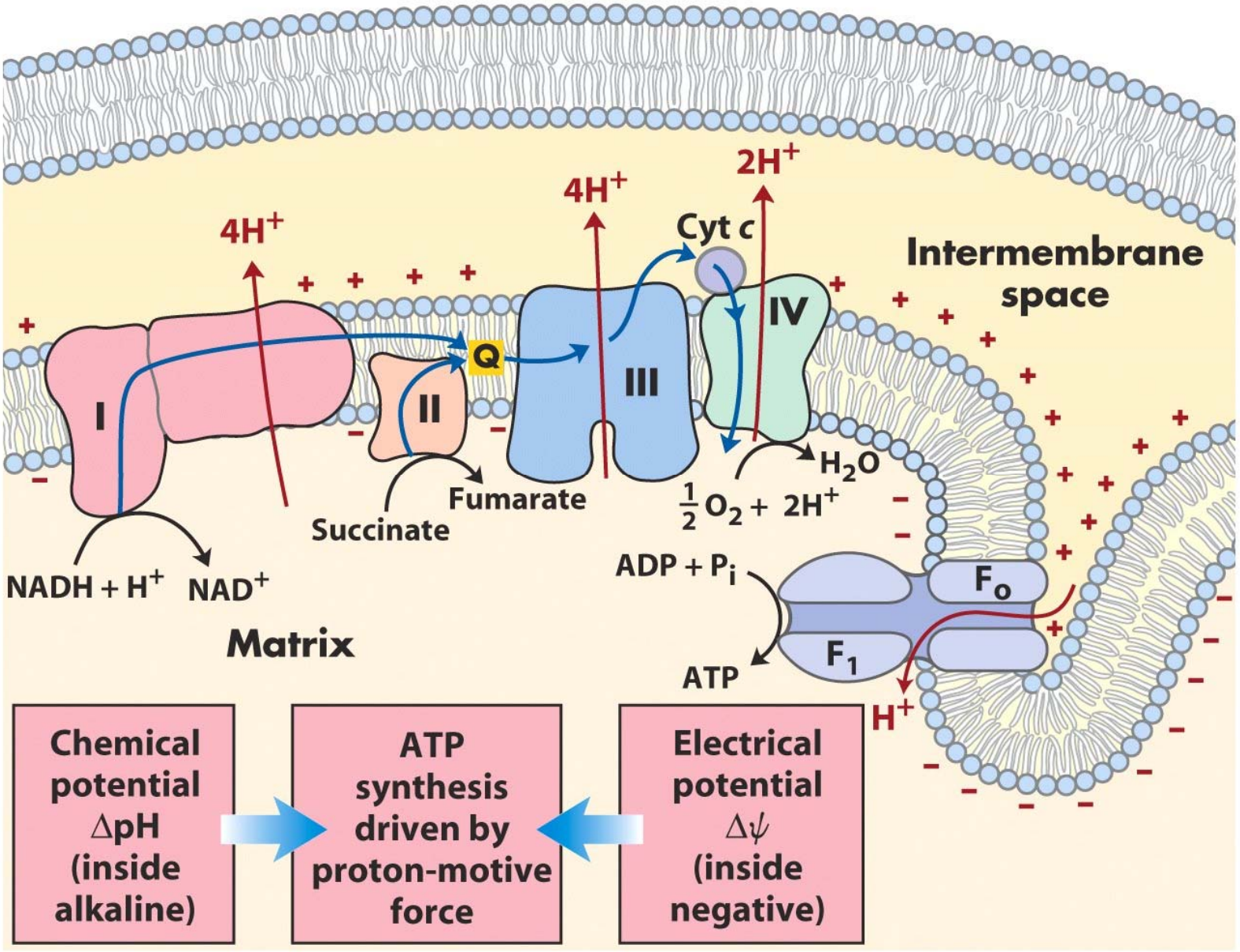
$\Delta\psi$ (0.15 V ~ 0.2 V) → 200 ~ 220 kJ



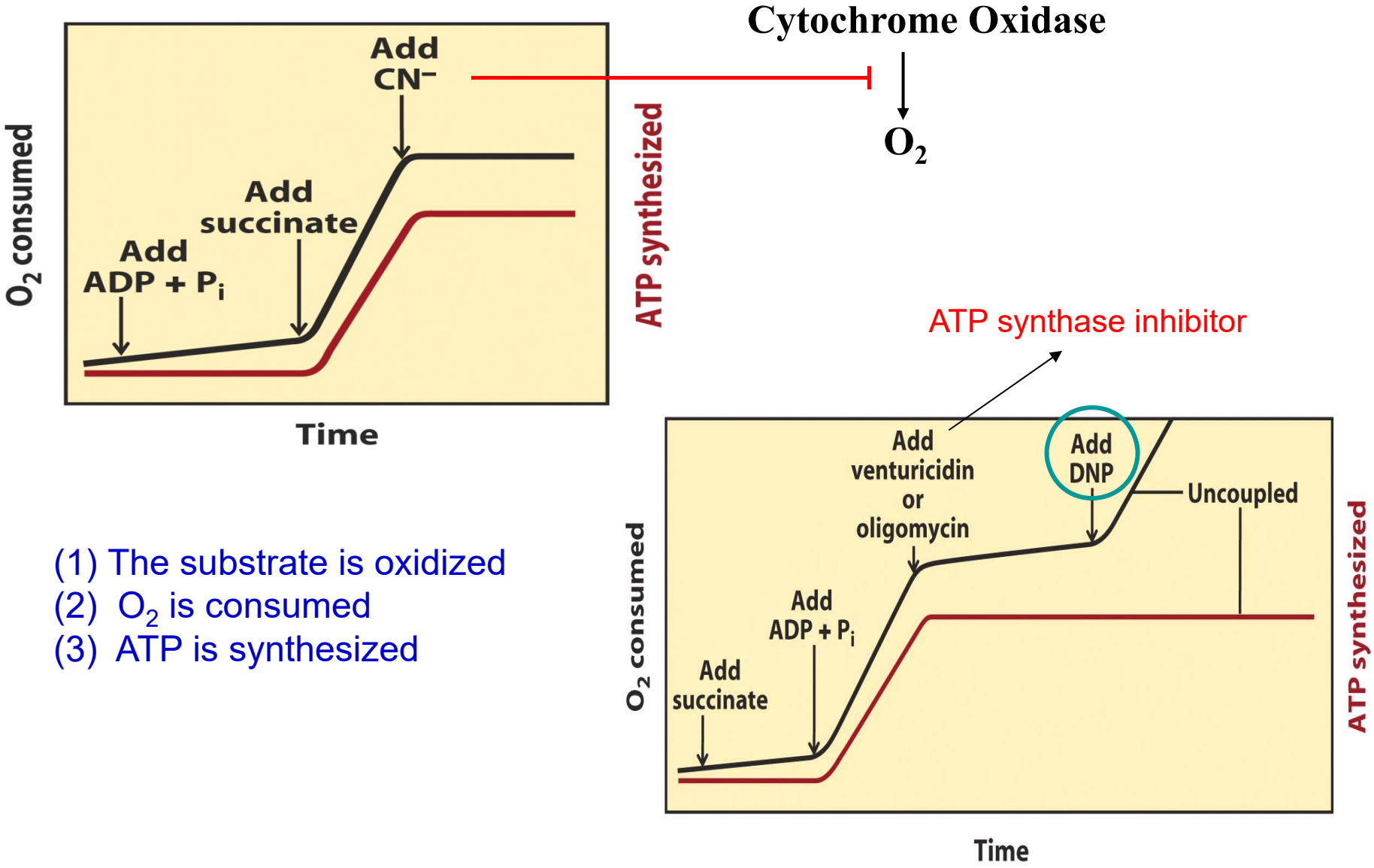
$$\Delta G = RT \ln (C_2/C_1) + Z\mathcal{F}\,\Delta\psi$$

$$= 2.3RT\,\Delta pH + \mathcal{F}\,\Delta\psi$$

Chemiosmotic model

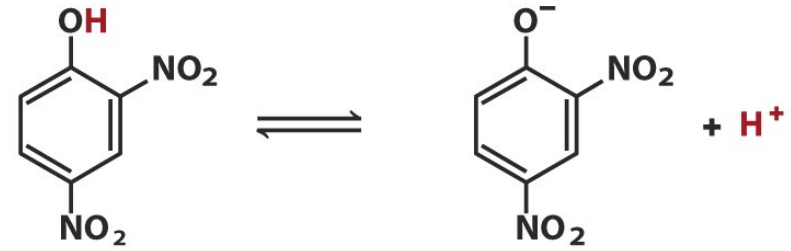


Coupling of electron transfer and ATP synthesis in mitochondria

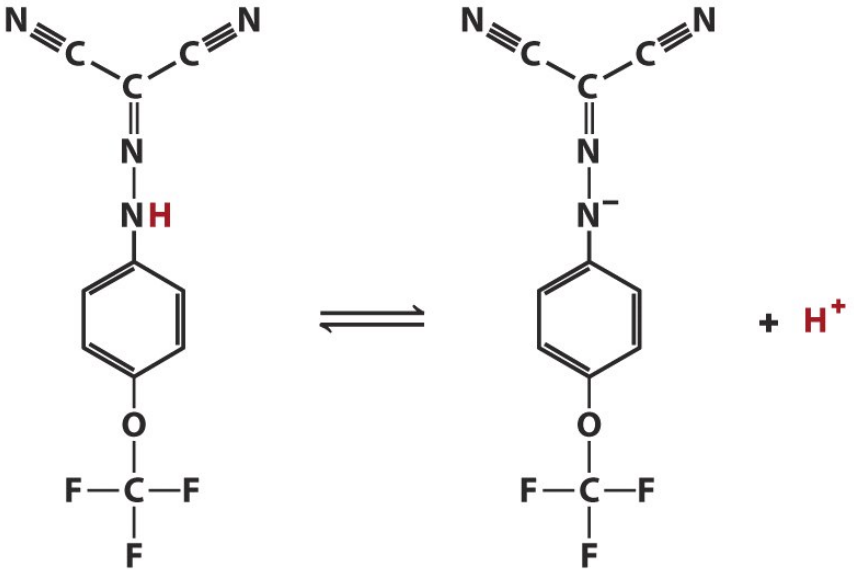


(1) The substrate is oxidized
(2) O_2 is consumed
(3) ATP is synthesized

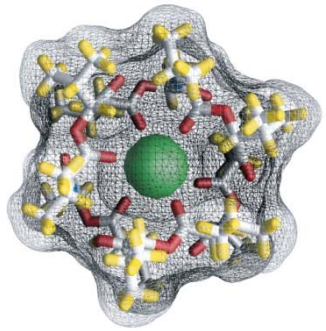
Two chemical uncouplers of oxidative phosphorylation



2,4-Dinitrophenol
(DNP)

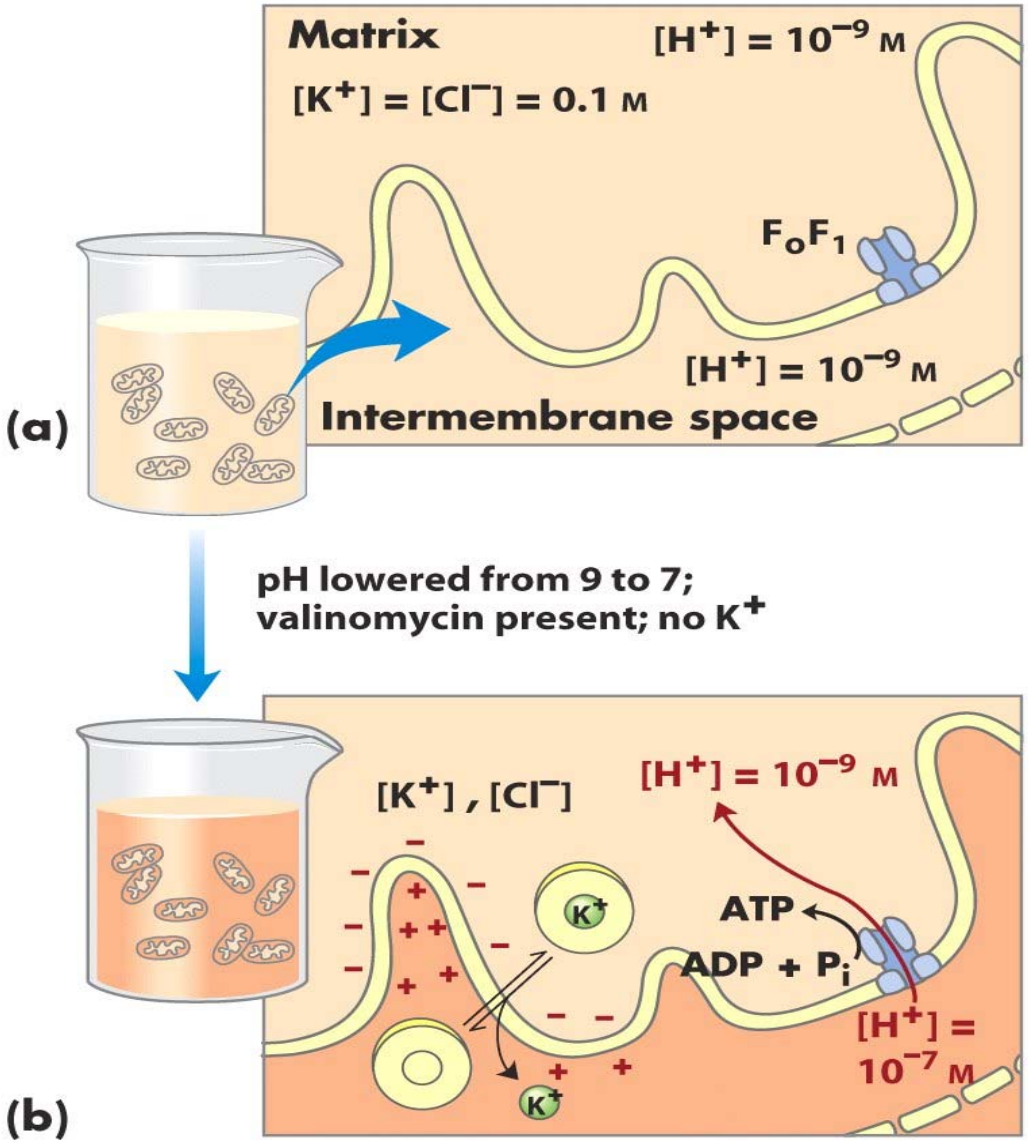


Carbonylcyanide-*p*-
trifluoromethoxyphenylhydrazone
(FCCP)

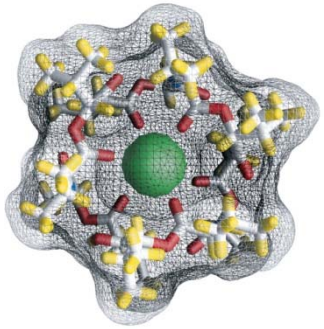


Valinomycin (ionophore)

Evidence for the role of a proton gradient in ATP synthesis

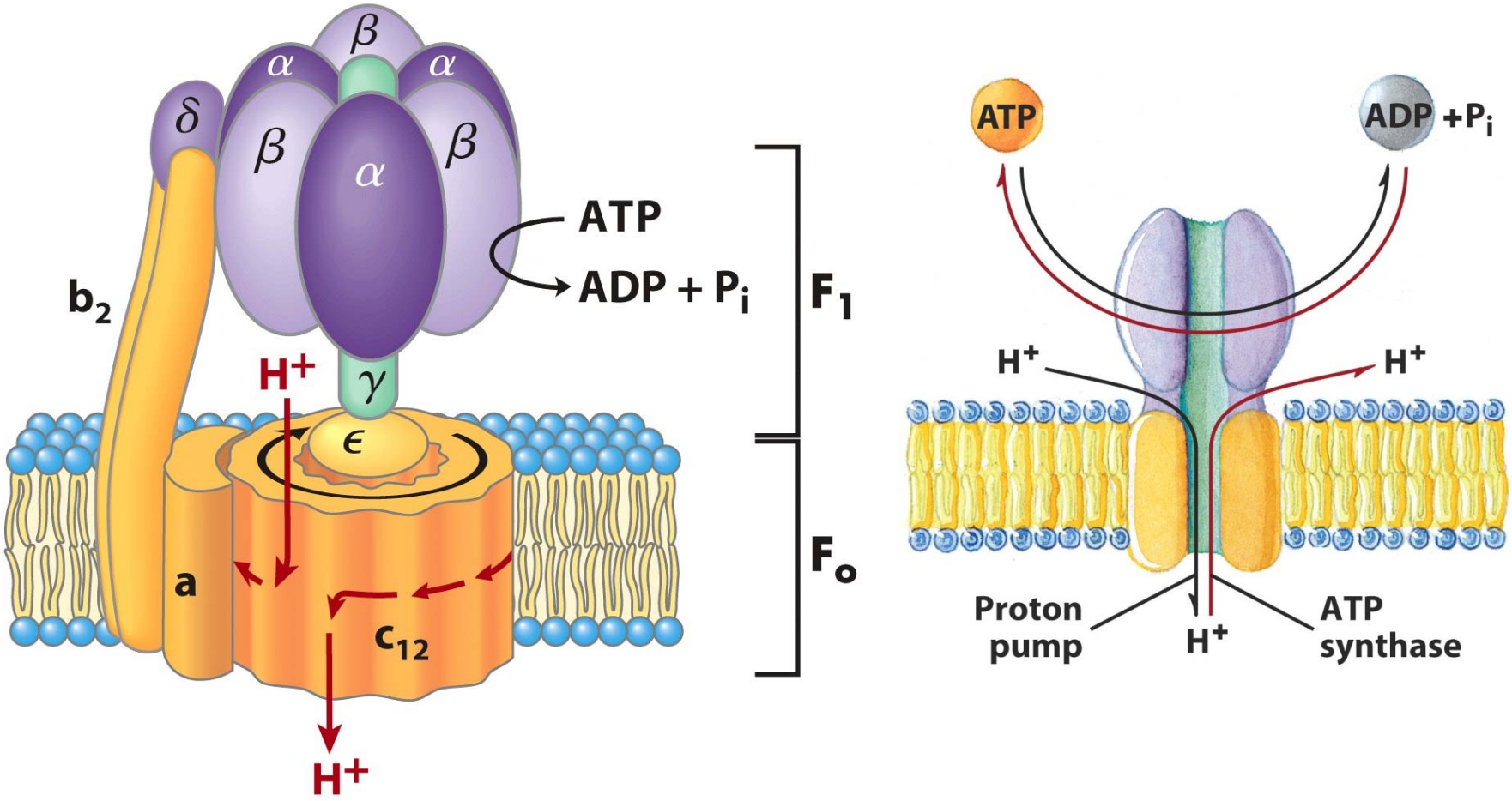


In the absence of an oxidizable substrate

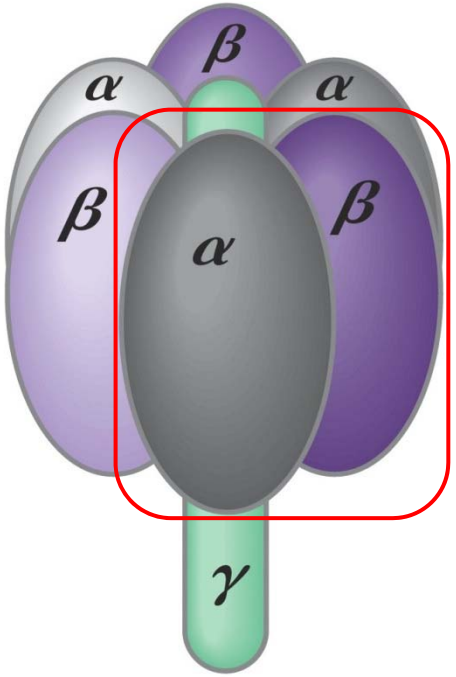


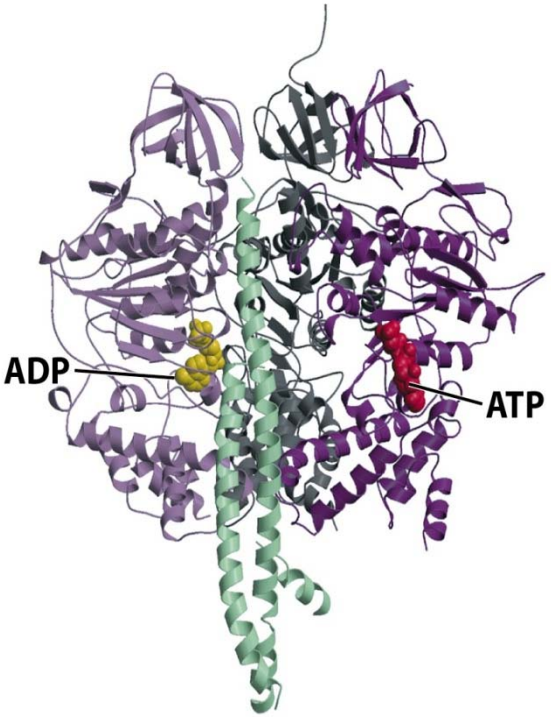
The proton-motive force alone suffices to drive ATP synthesis

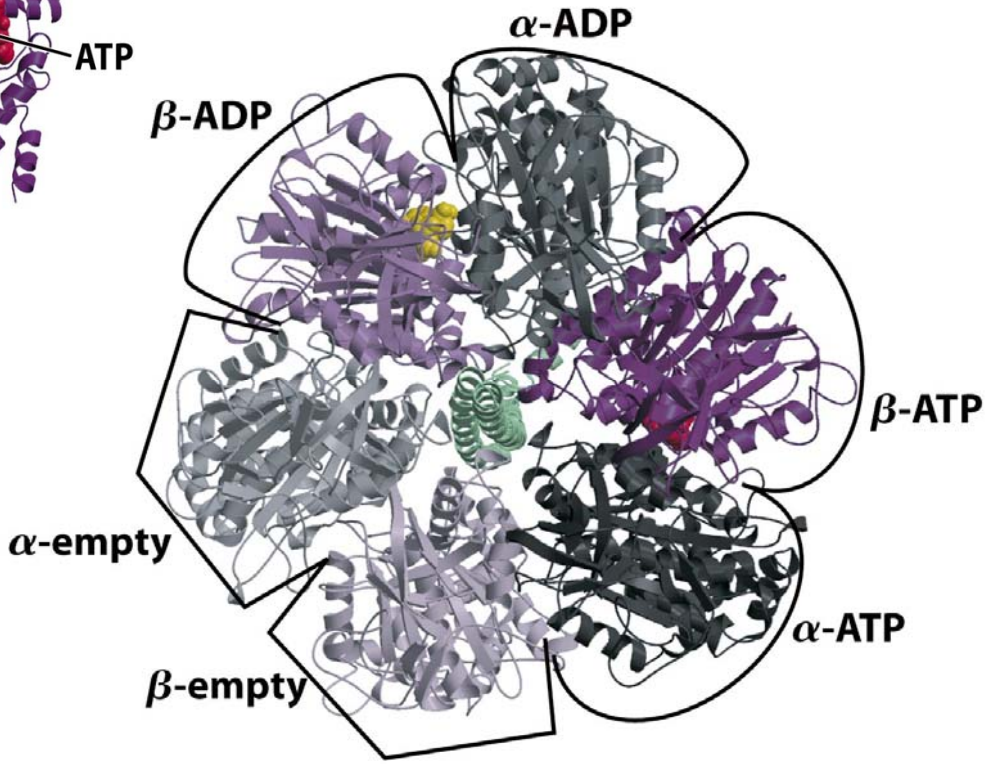
Structure of the F_oF_1 ATPase/ATP synthase (Complex V)



Mitochondrial ATP synthase complex

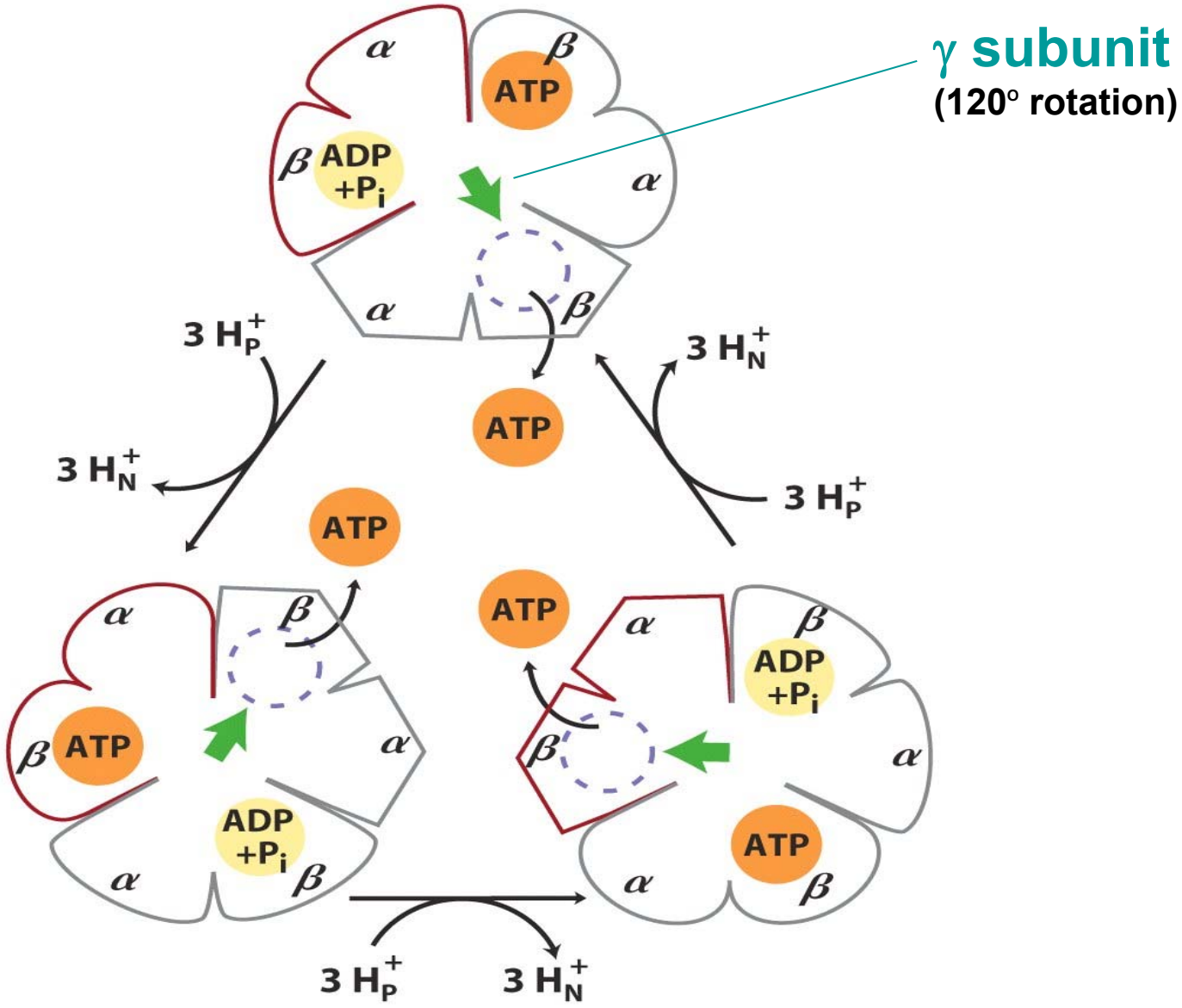




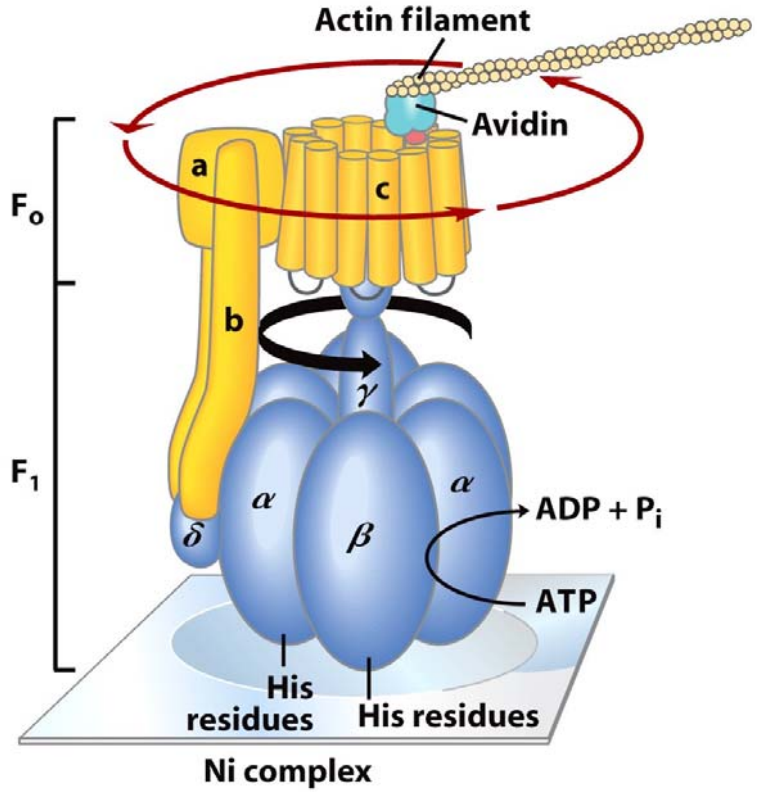


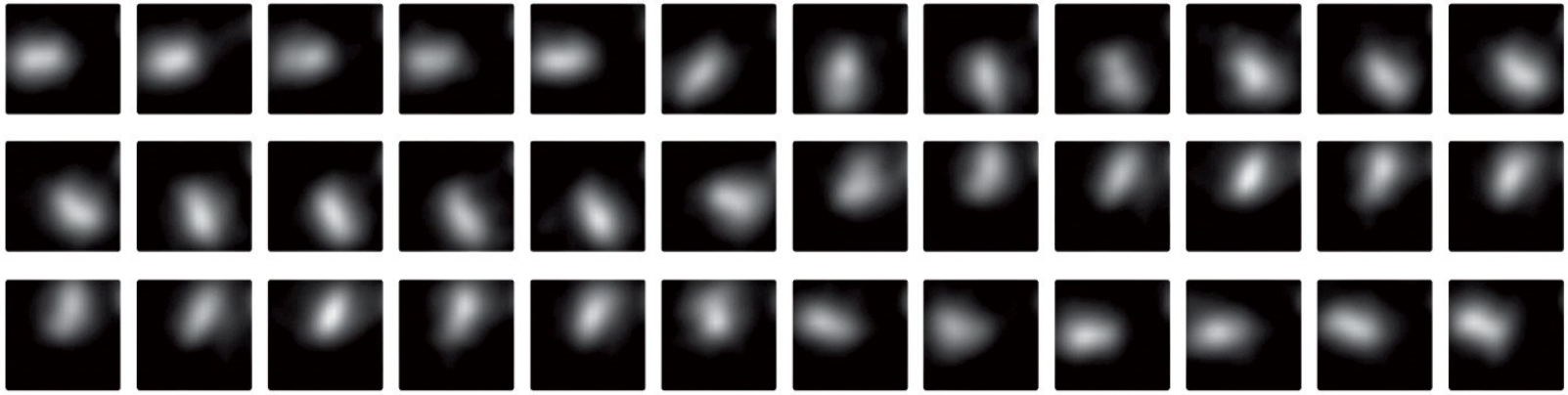
$\alpha_3\beta_3\gamma\delta\varepsilon$

Binding-change model for ATP synthase

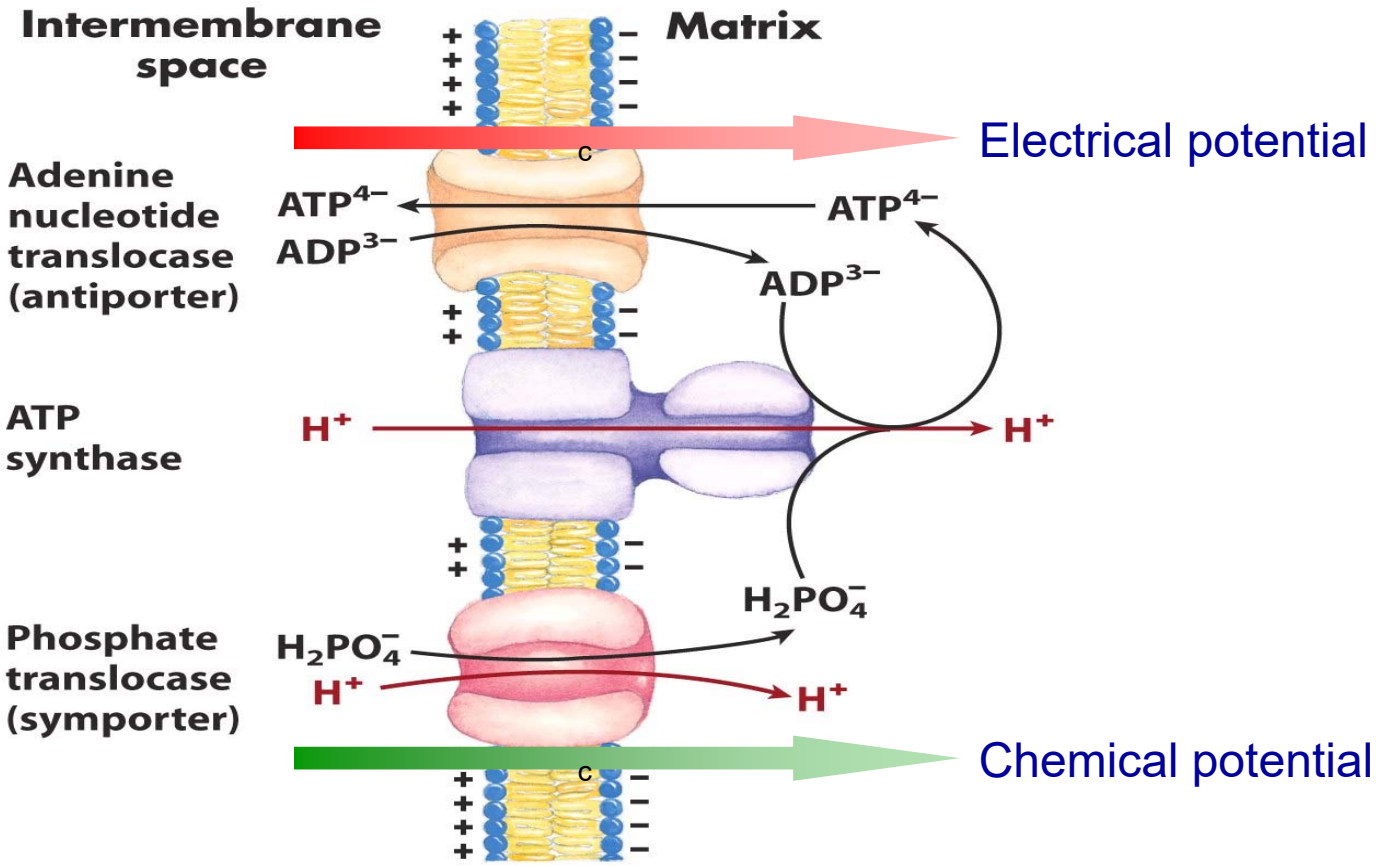


Rotation of F_o and γ experimentally demonstrated





Adenine nucleotide and phosphate translocases

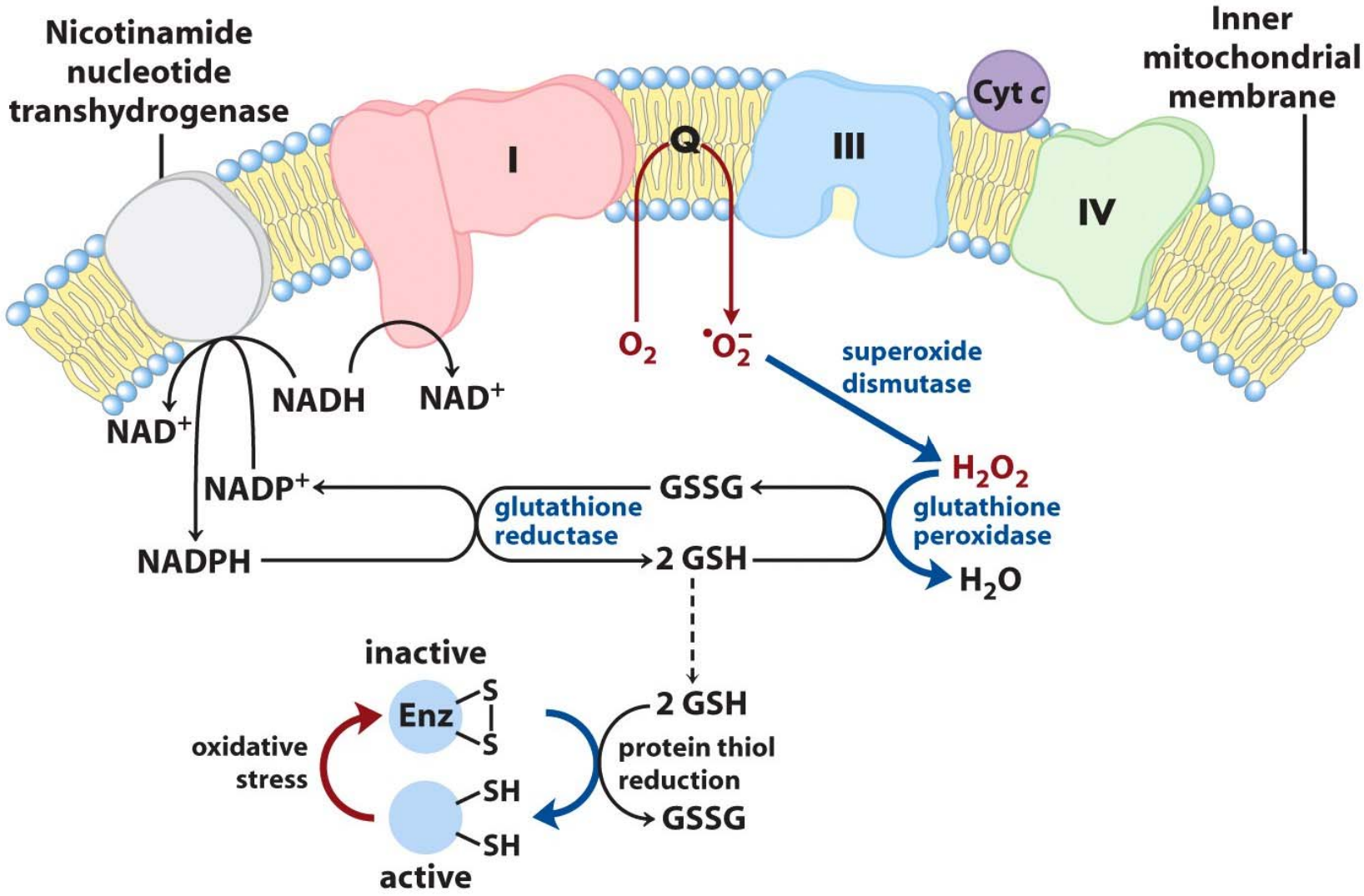


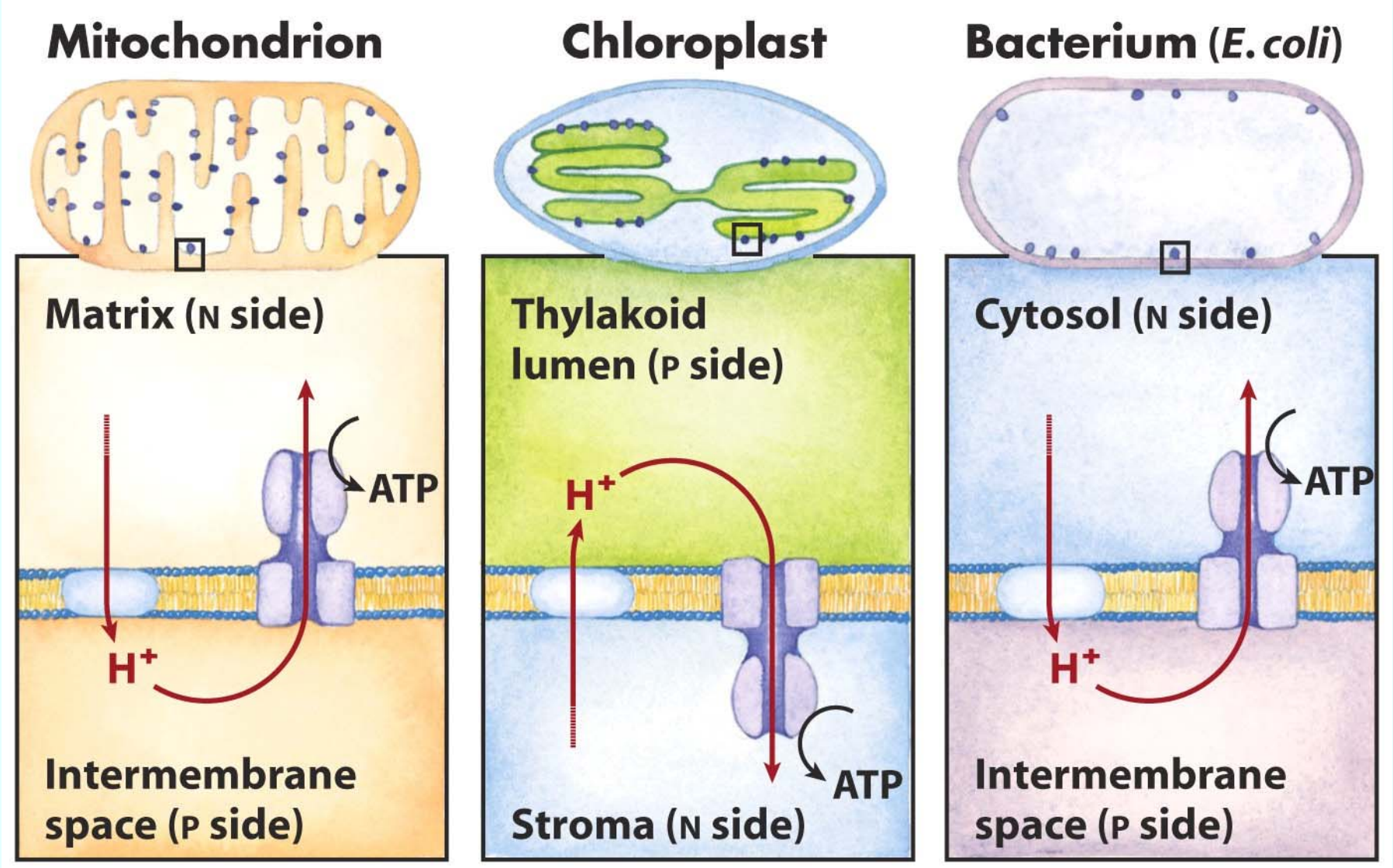
Pronton-motive force is responsible for:

(1) Providing the E for ATP synthesis

(2) Transporting substrates (ADP + Pi) in,
product (ATP) out of the mitochondria matrix.

Mitochondrial production and disposal of superoxide




Mitochondrion
Matrix (N side)
ATP
H+
Intermembrane space (P side)
Chloroplast
Thylakoid lumen (P side)
H+
ATP
Stroma (N side)
Bacterium (E. coli)
Cytosol (N side)
ATP
H+
Intermembrane space (P side)