OXIDATIVE PHOSPHORYLATION AND ATP SYNTHESIS

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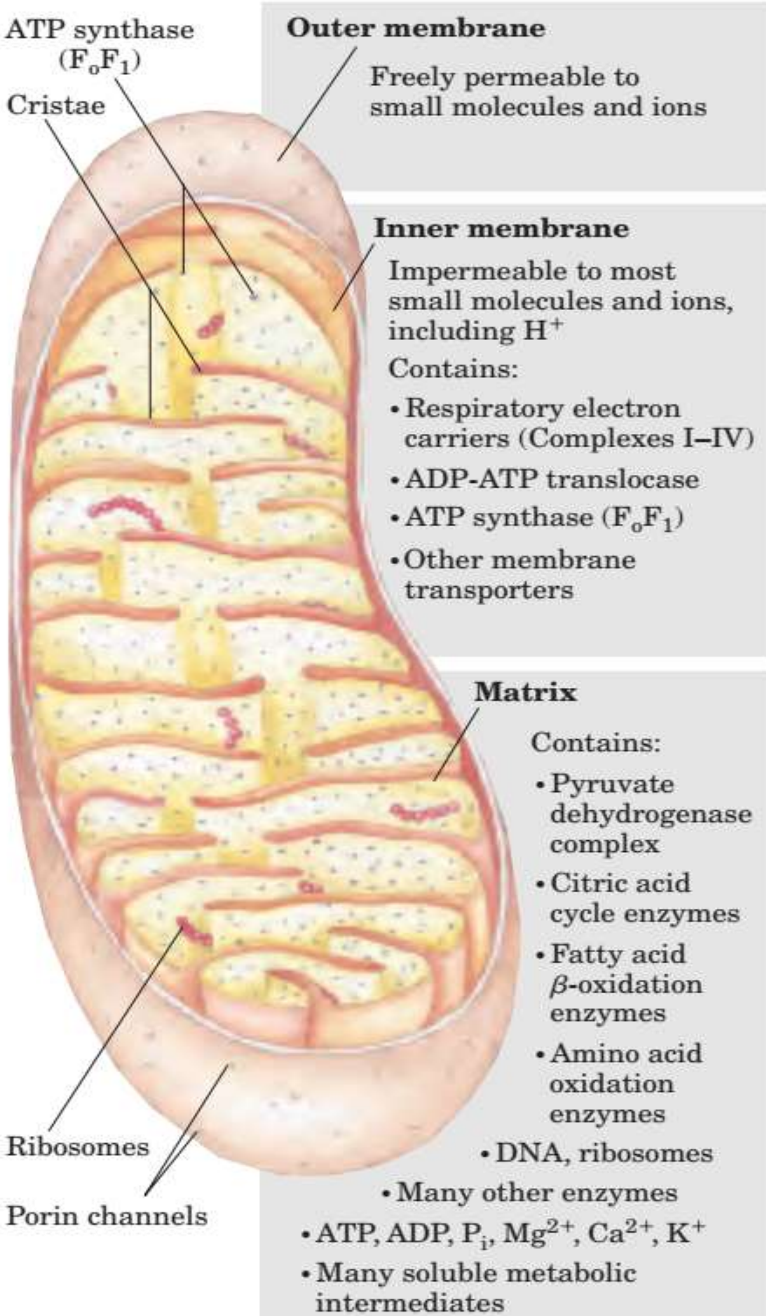
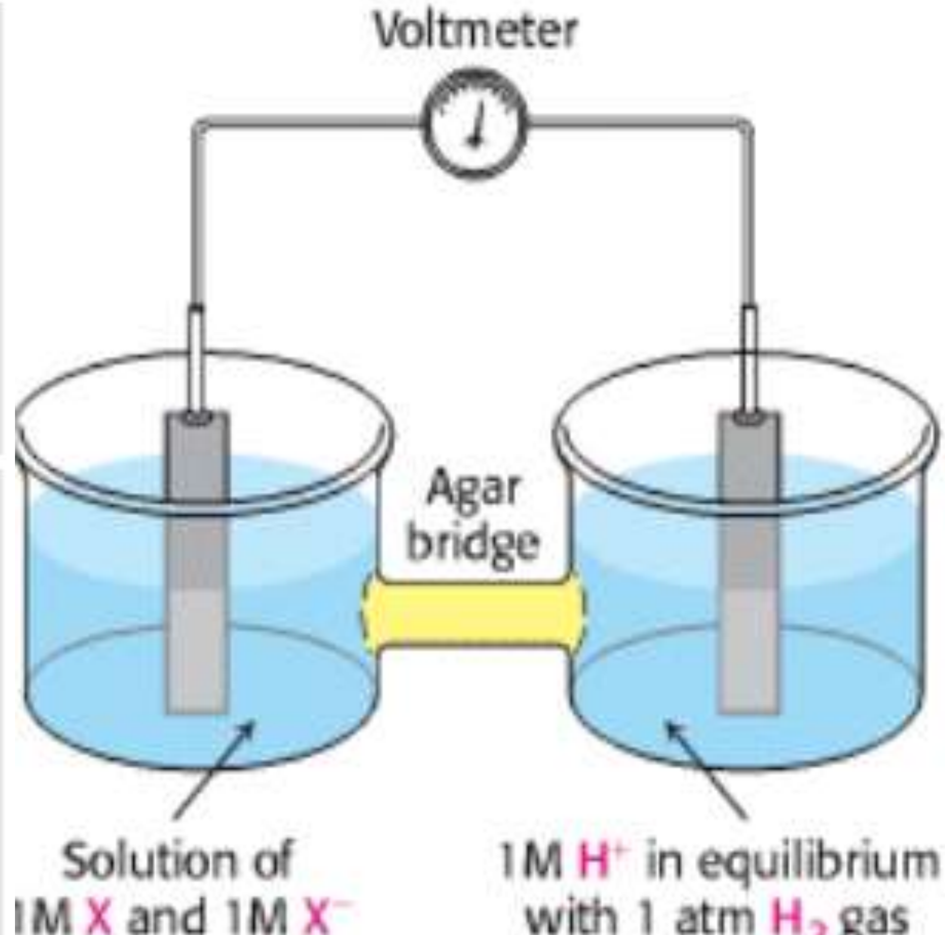
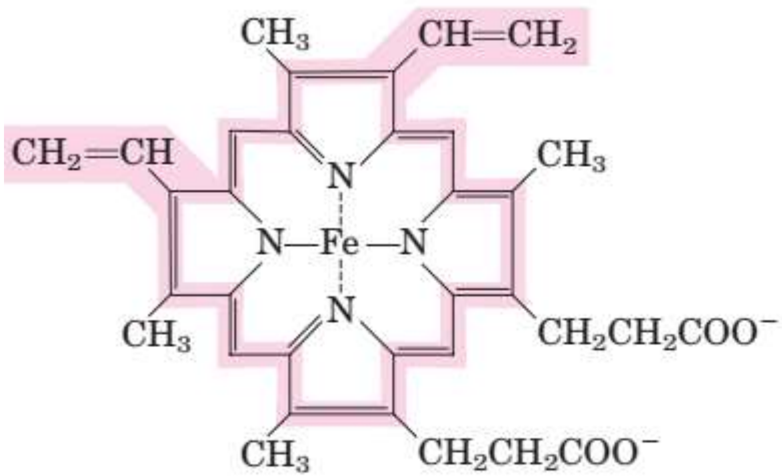


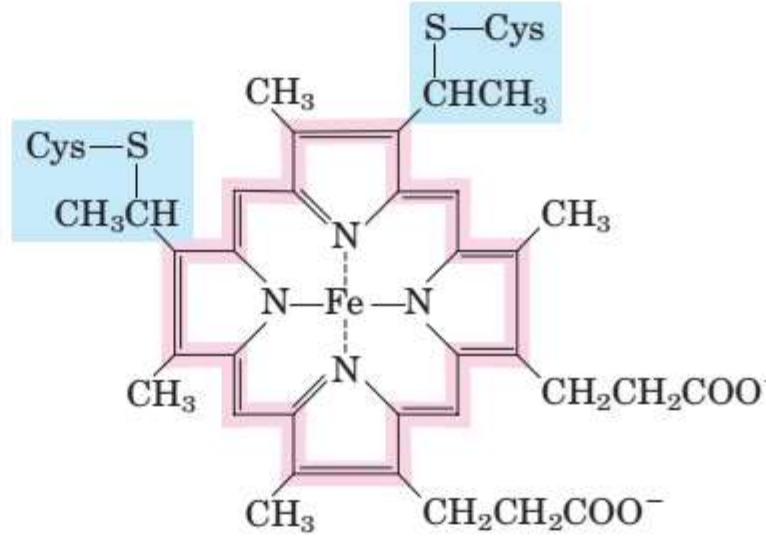
ATP synthase (F_oF_1)
Cristae
Ribosomes
Porin channels
Outer membrane
Freely permeable to small molecules and ions
Inner membrane
Impermeable to most small molecules and ions, including H^+
Contains:
• Respiratory electron carriers (Complexes I–IV)
• ADP-ATP translocase
• ATP synthase (F_oF_1)
• Other membrane transporters
Matrix
Contains:
• Pyruvate dehydrogenase complex
• Citric acid cycle enzymes
• Fatty acid β-oxidation enzymes
• Amino acid oxidation enzymes
• DNA, ribosomes
• Many other enzymes
• ATP, ADP, P_i, Mg^{2+}, Ca^{2+}, K^+
• Many soluble metabolic intermediates



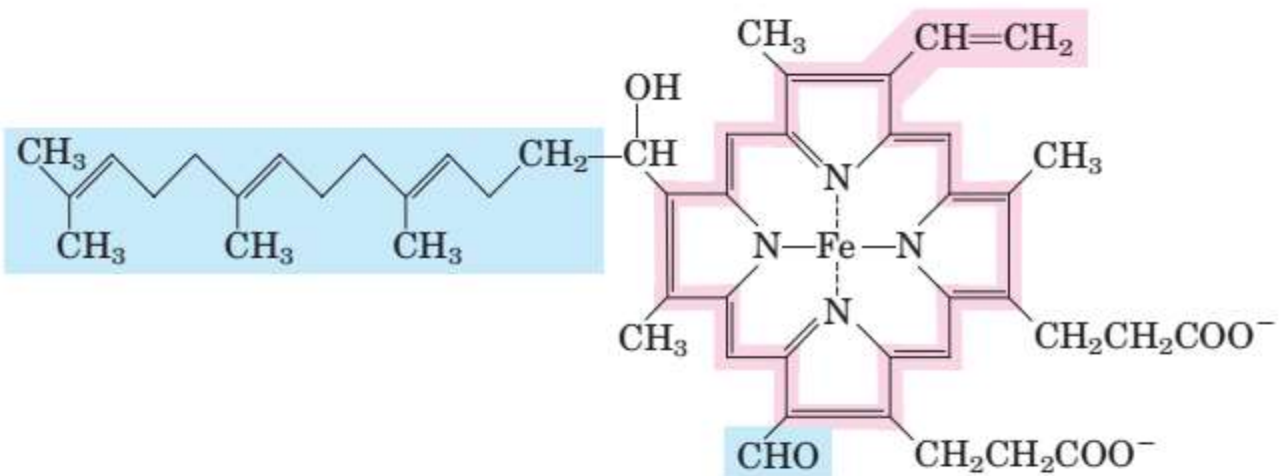
Voltmeter
Agar bridge
Solution of 1M X and 1M X^-
1M H^+ in equilibrium with 1 atm H_2 gas



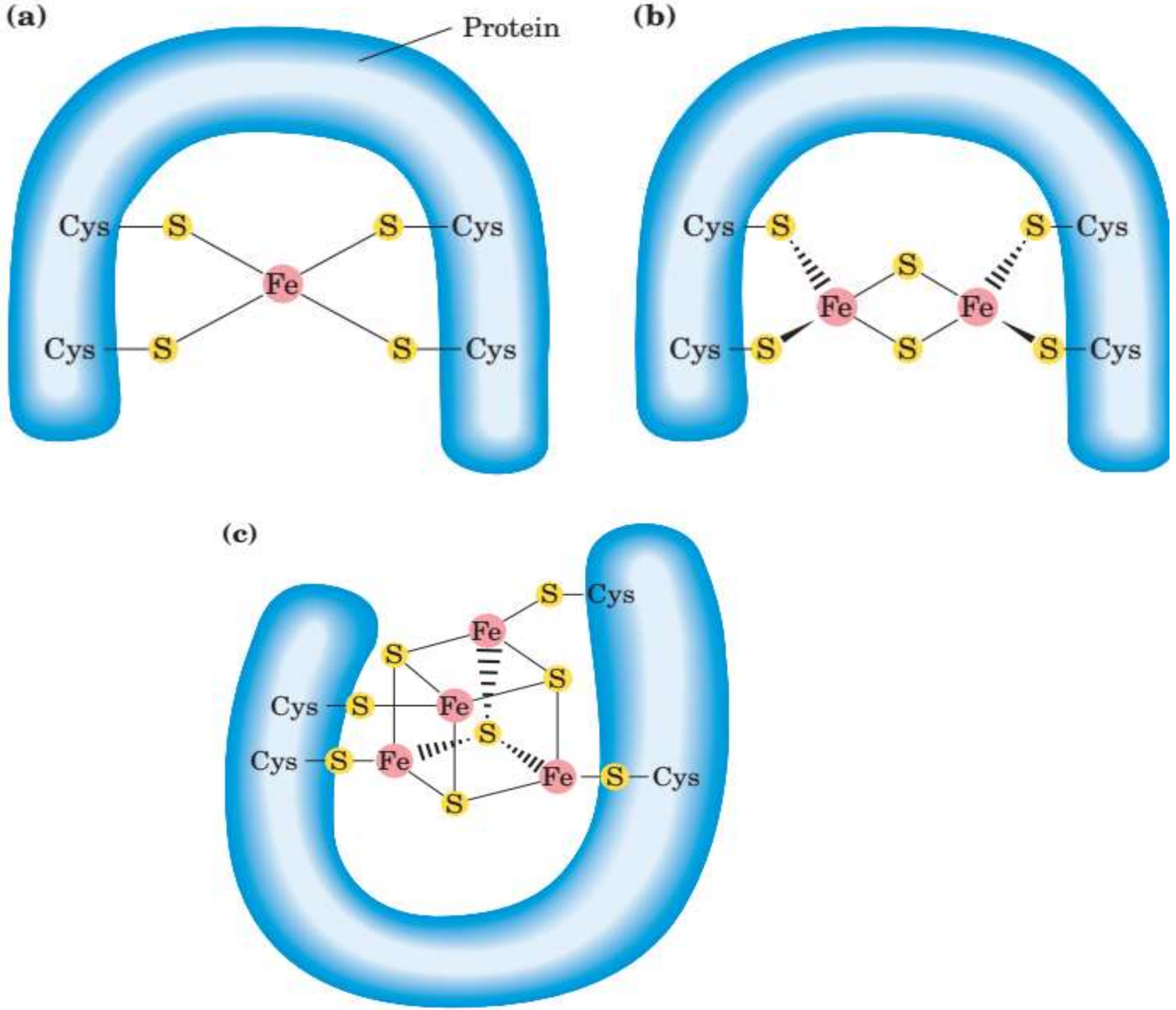
Iron protoporphyrin IX
(in *b*-type cytochromes)



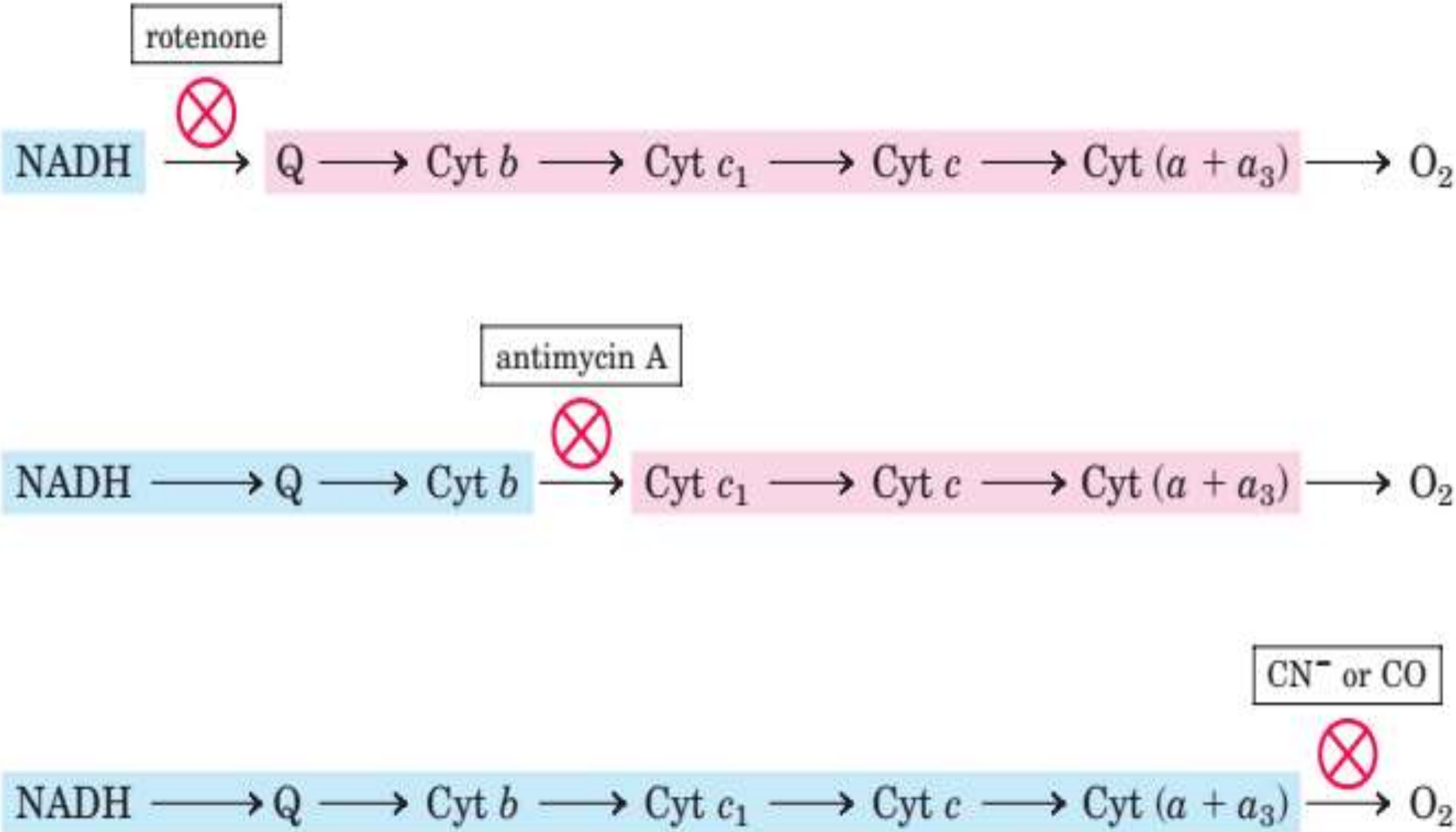
Heme C
(in *c*-type cytochromes)

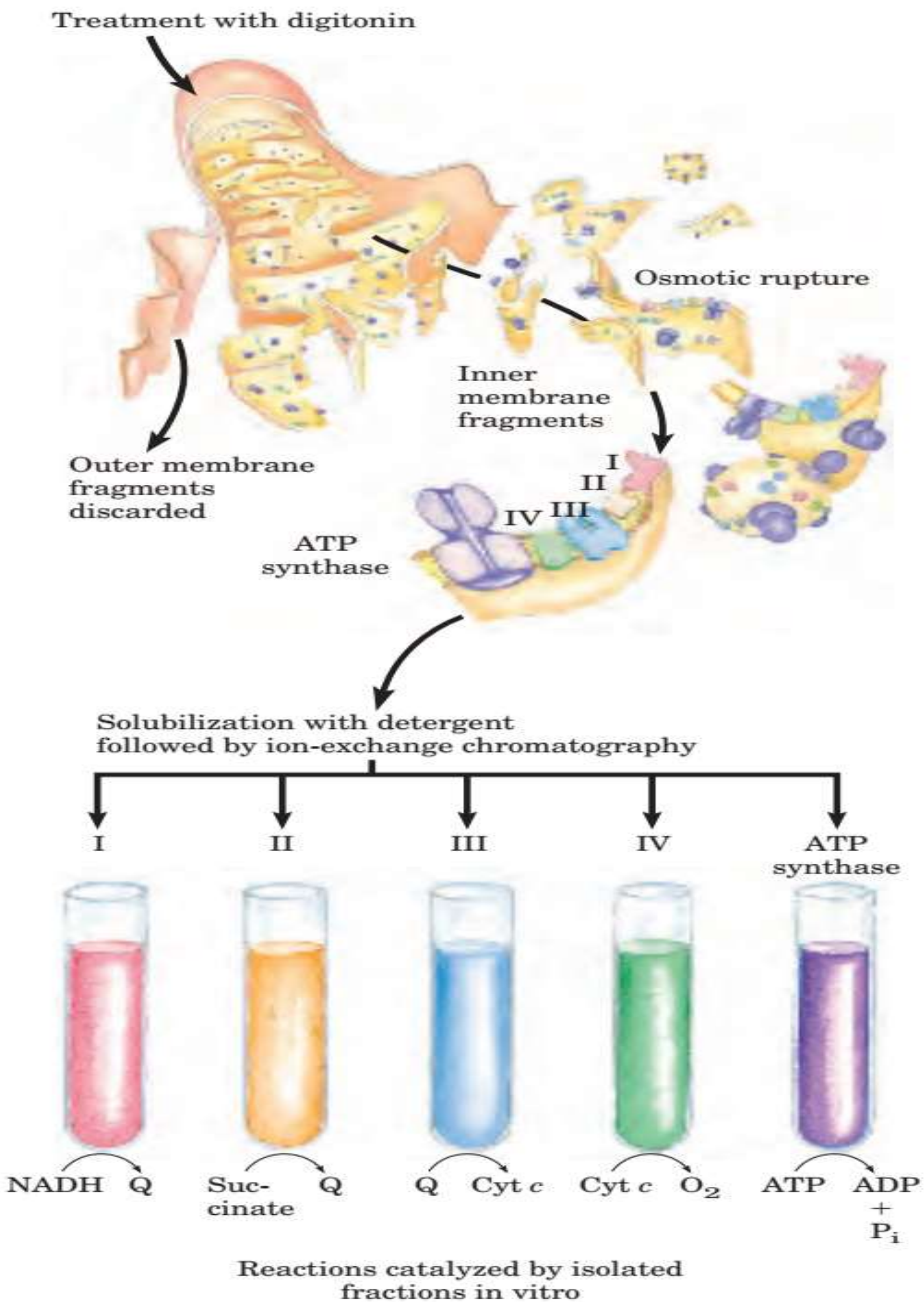


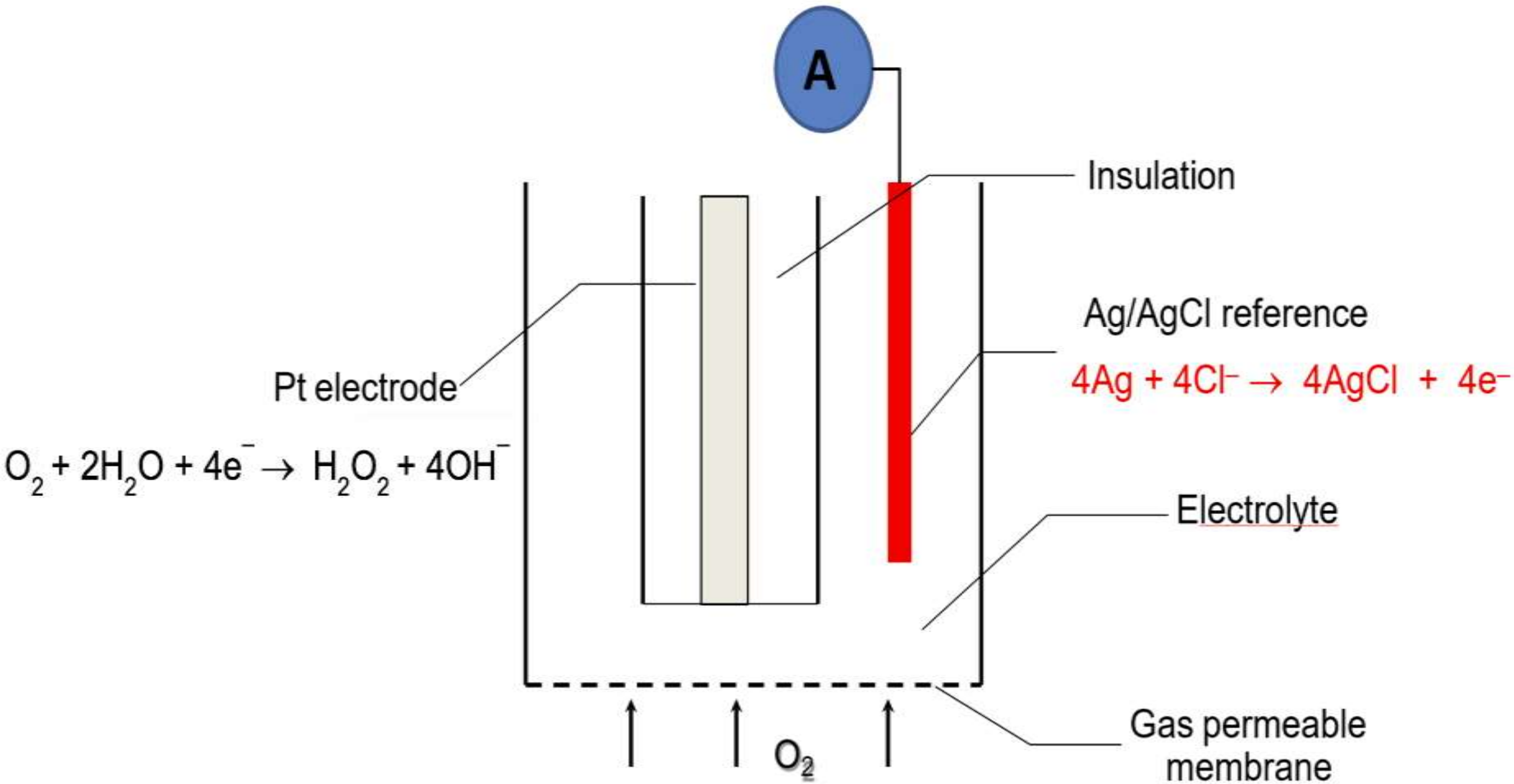
Heme A
(in *a*-type cytochromes)

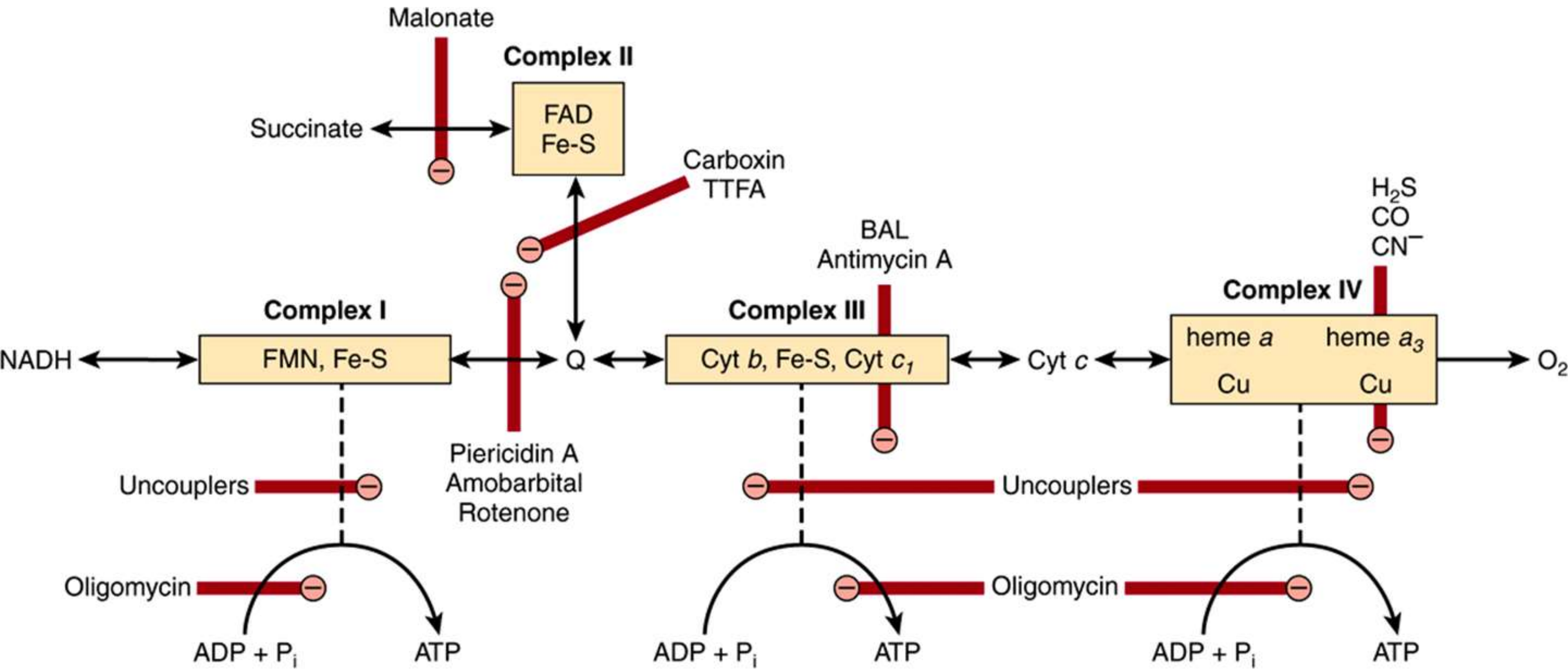


(a)
Protein
Cys
S
S
Cys
Fe
Cys
S
S
Cys
(b)
Cys
S
S
Cys
S
Fe
Fe
Cys
S
S
S
Cys
(c)
S
Cys
Fe
S
S
Cys
S
Fe
S
Cys
S
Fe
Fe
S
Cys
S

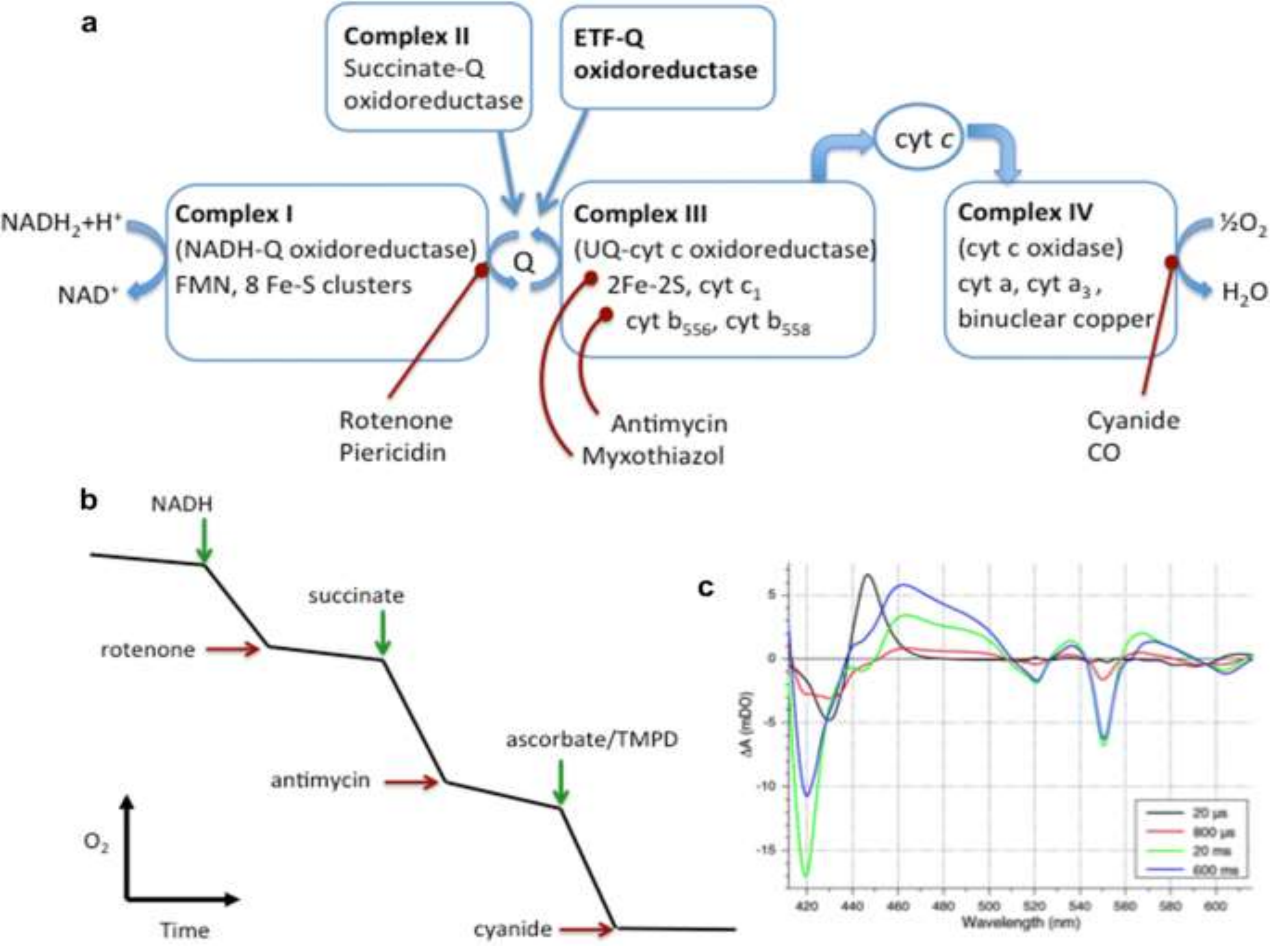

rotenone
NADH → Q → Cyt b → Cyt c1 → Cyt c → Cyt (a + a3) → O2
antimycin A
NADH → Q → Cyt b → Cyt c1 → Cyt c → Cyt (a + a3) → O2
CN⁻ or CO
NADH → Q → Cyt b → Cyt c1 → Cyt c → Cyt (a + a3) → O2

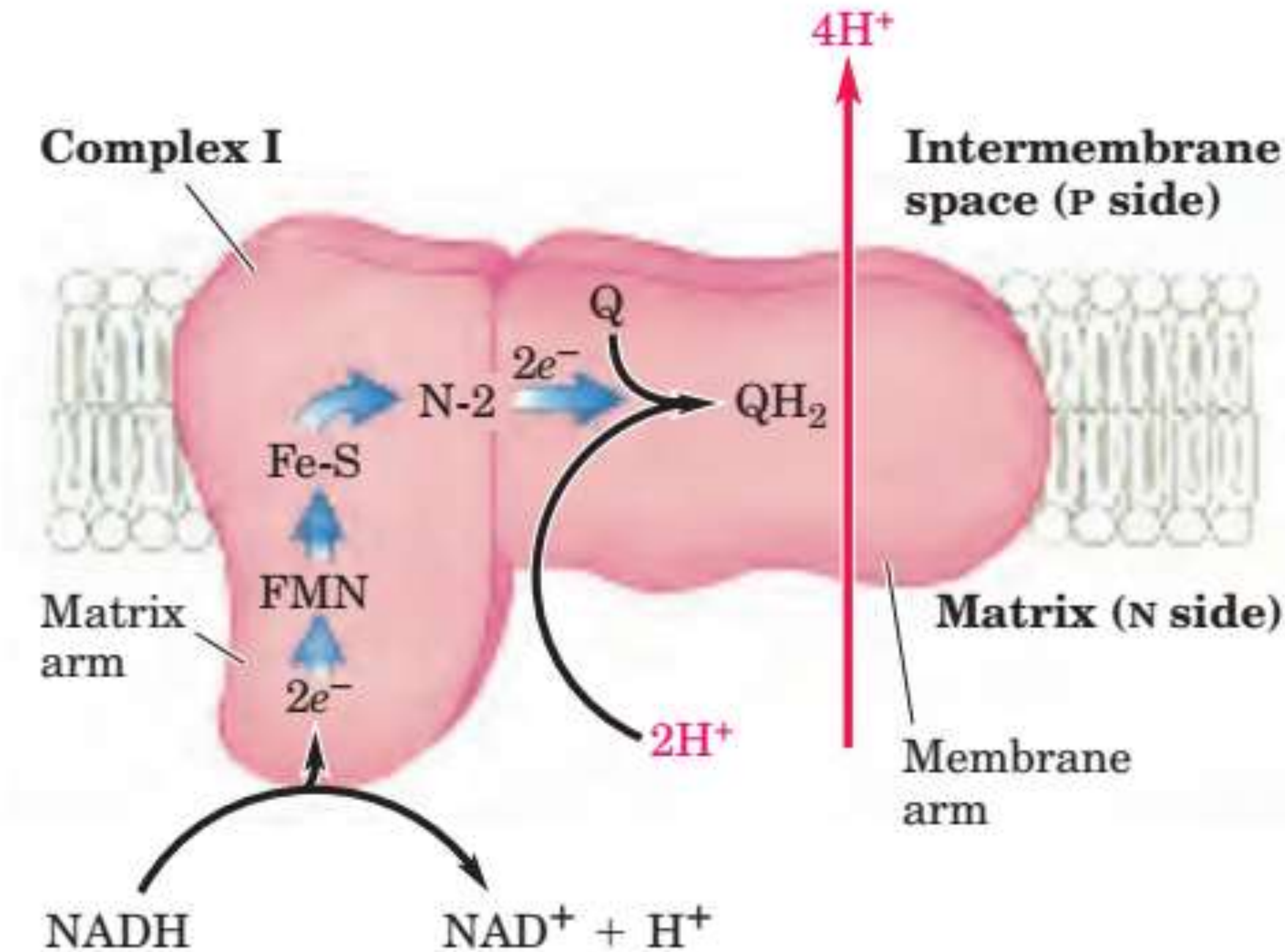

Treatment with digitonin
Osmotic rupture
Inner
membrane
fragments
Outer membrane
fragments
discarded
I
II
III
IV
ATP
synthase
Solubilization with detergent
followed by ion-exchange chromatography
I
II
III
IV
ATP
synthase
NADH Q
Suc-
cinate
Q
Q Cyt *c*
Cyt *c* O_2
ATP ADP
+
P_i
Reactions catalyzed by isolated
fractions in vitro

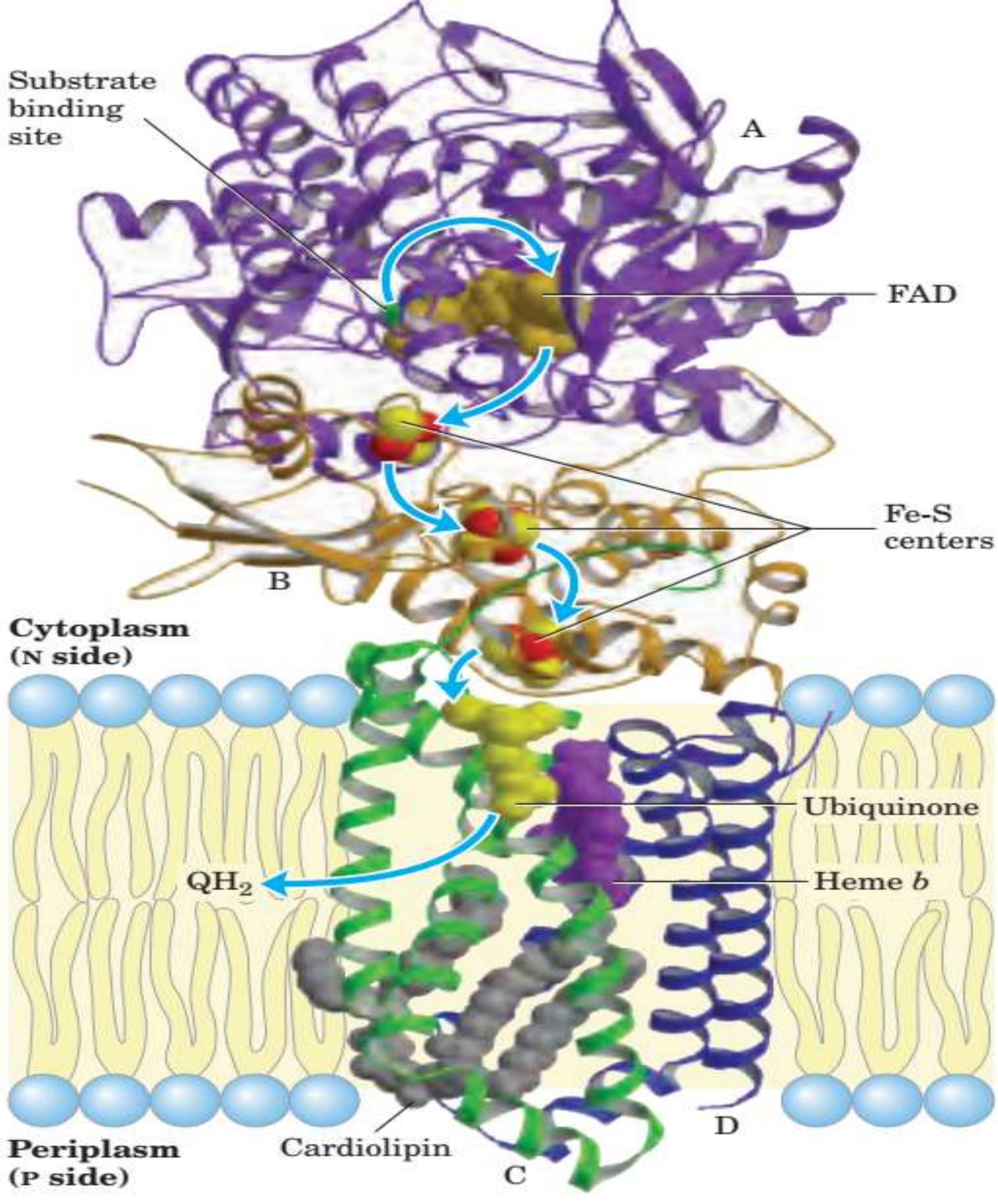

A
Insulation
Ag/AgCl reference
4Ag + 4Cl⁻ → 4AgCl + 4e⁻
Pt electrode
O2 + 2H2O + 4e⁻ → H2O2 + 4OH⁻
Electrolyte
O2
Gas permeable
membrane

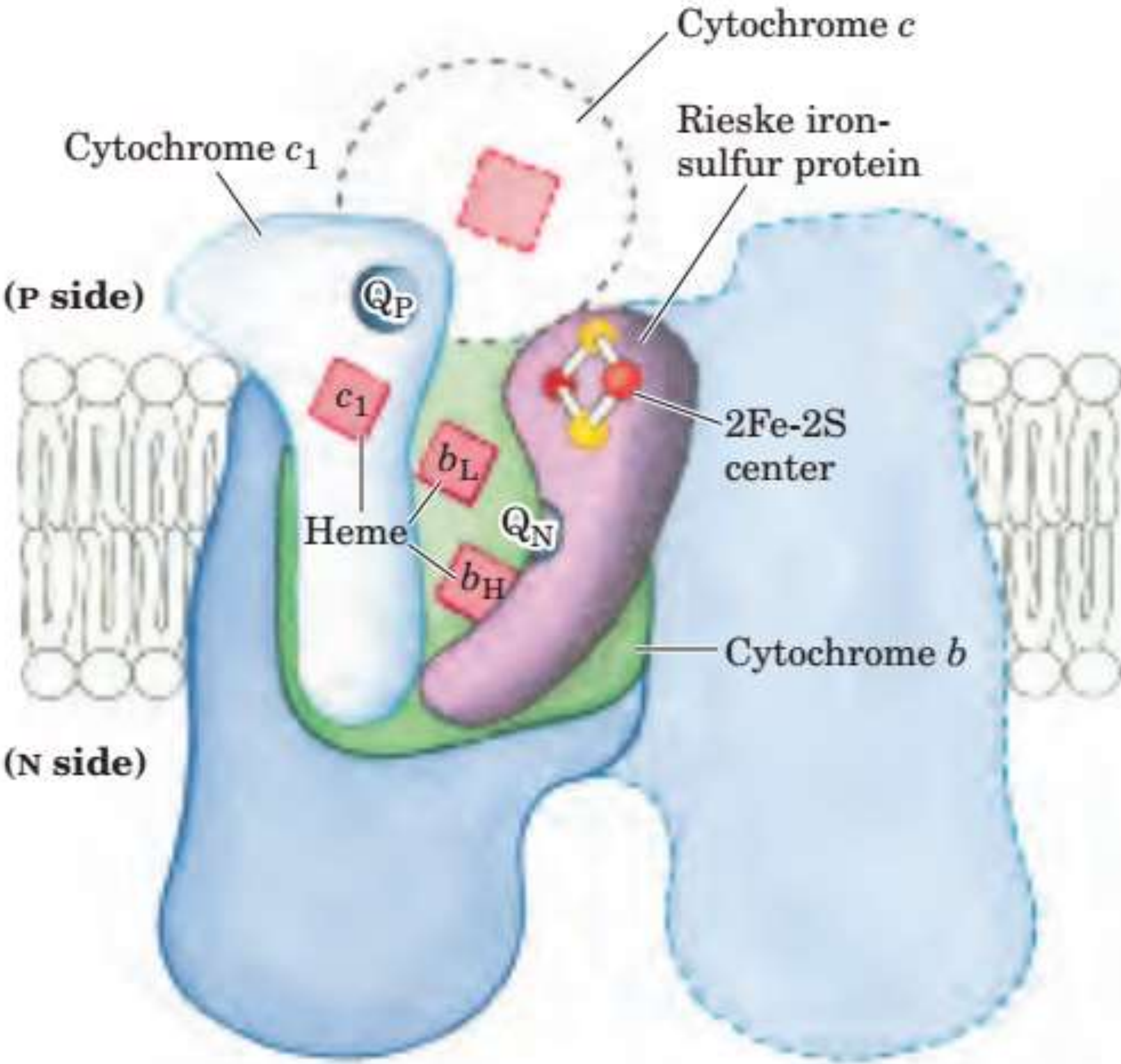


Malonate
Complex II
Succinate
FAD
Fe-S
Carboxin
TTFA
H_2S
CO
CN^-
BAL
Antimycin A
Complex I
Complex III
Complex IV
heme *a*
heme a_3
NADH
FMN, Fe-S
Q
Cyt *b*, Fe-S, Cyt c_1
Cyt *c*
Cu
Cu
O_2
Piericidin A
Amobarbital
Rotenone
Uncouplers
Uncouplers
Oligomycin
Oligomycin
ADP + P_i
ATP
ADP + P_i
ATP
ADP + P_i
ATP

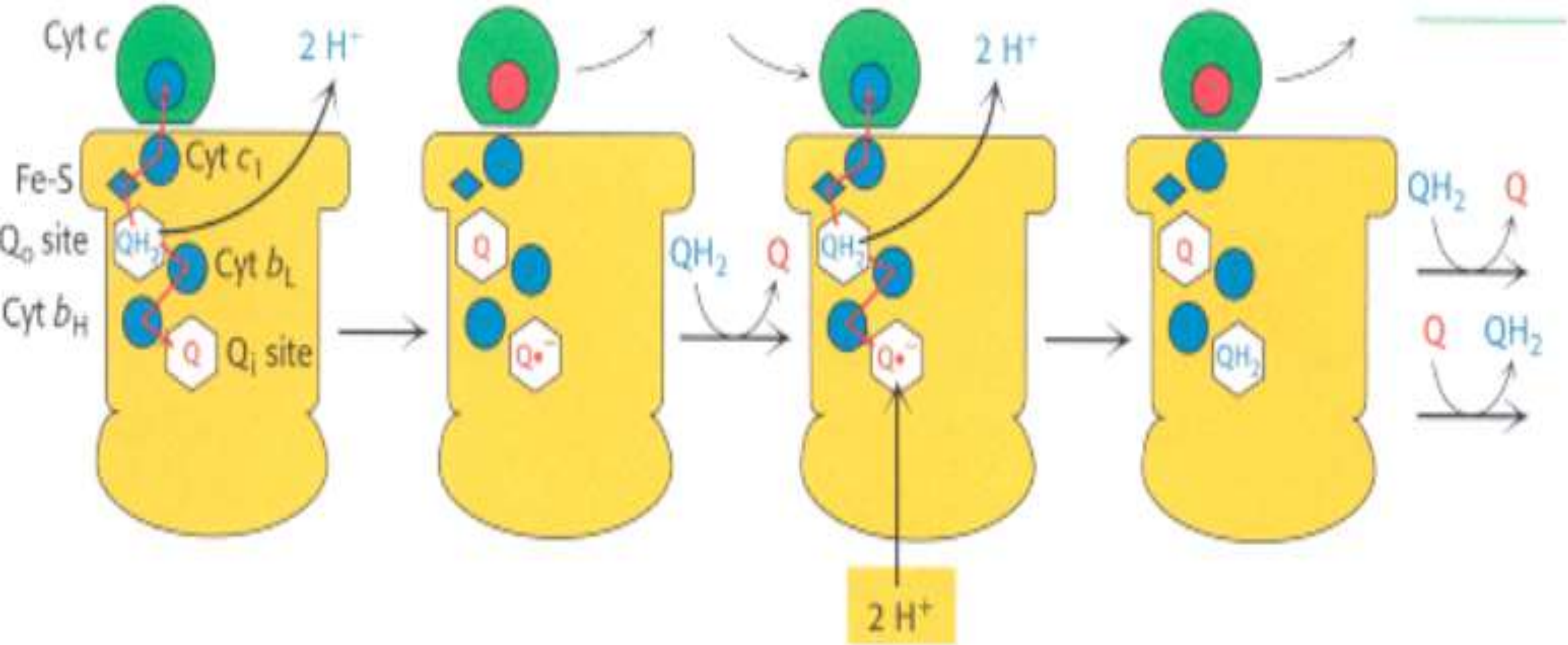

a
Complex II
Succinate-Q
oxidoreductase
ETF-Q
oxidoreductase
cyt c
NADH2+H+
NAD+
Complex I
(NADH-Q oxidoreductase)
FMN, 8 Fe-S clusters
Q
Complex III
(UQ-cyt c oxidoreductase)
2Fe-2S, cyt c1
cyt b556, cyt b558
Complex IV
(cyt c oxidase)
cyt a, cyt a3 ,
binuclear copper
½O2
H2O
Rotenone
Piericidin
Antimycin
Myxothiazol
Cyanide
CO
b
NADH
rotenone
succinate
antimycin
ascorbate/TMPD
cyanide
O2
Time
c
ΔA (mDO)
Wavelength (nm)
20 µs
800 µs
20 ms
600 ms


4H+
Complex I
Intermembrane space (P side)
Q
2e−
N-2
QH2
Fe-S
FMN
Matrix arm
Matrix (N side)
2e−
2H+
Membrane arm
NADH
NAD+ + H+

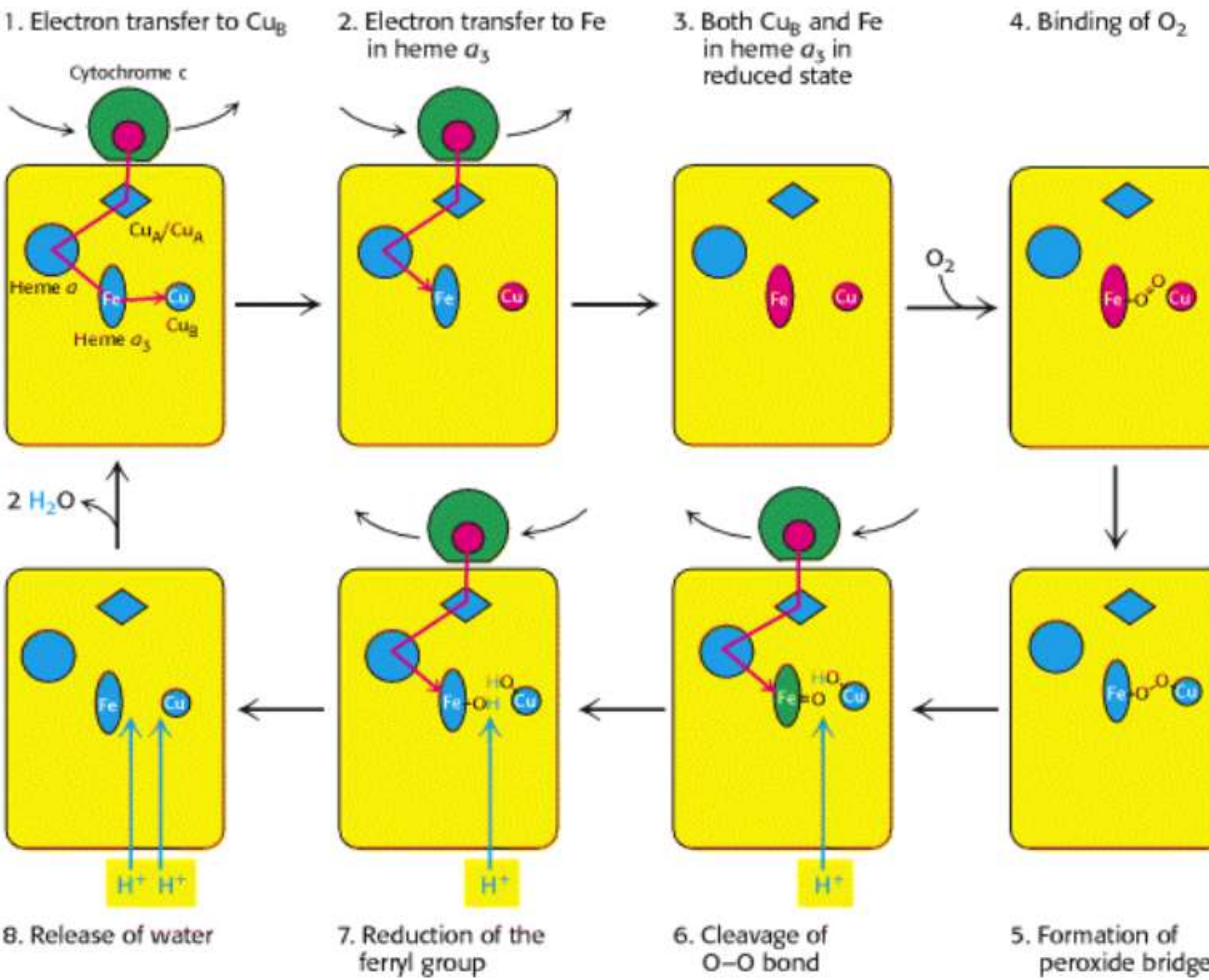

Substrate binding site
A
FAD
Fe-S centers
B
Cytoplasm (N side)
Ubiquinone
QH2
Heme b
D
Periplasm (P side)
Cardiolipin
C



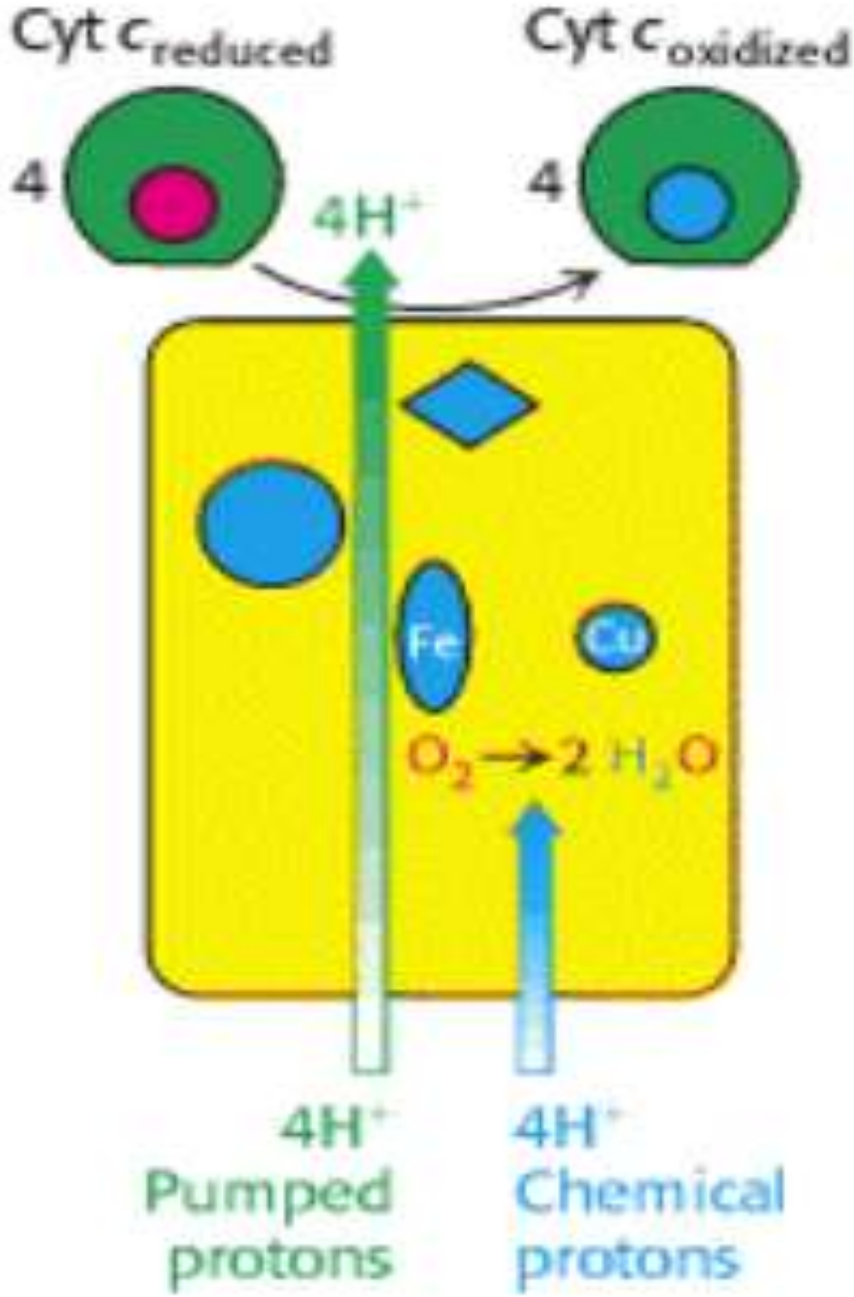
Cytochrome c
Rieske iron-
sulfur protein
Cytochrome c_1
(P side)
Q_P
c_1
b_L
2Fe-2S
center
Heme
Q_N
b_H
Cytochrome b
(N side)



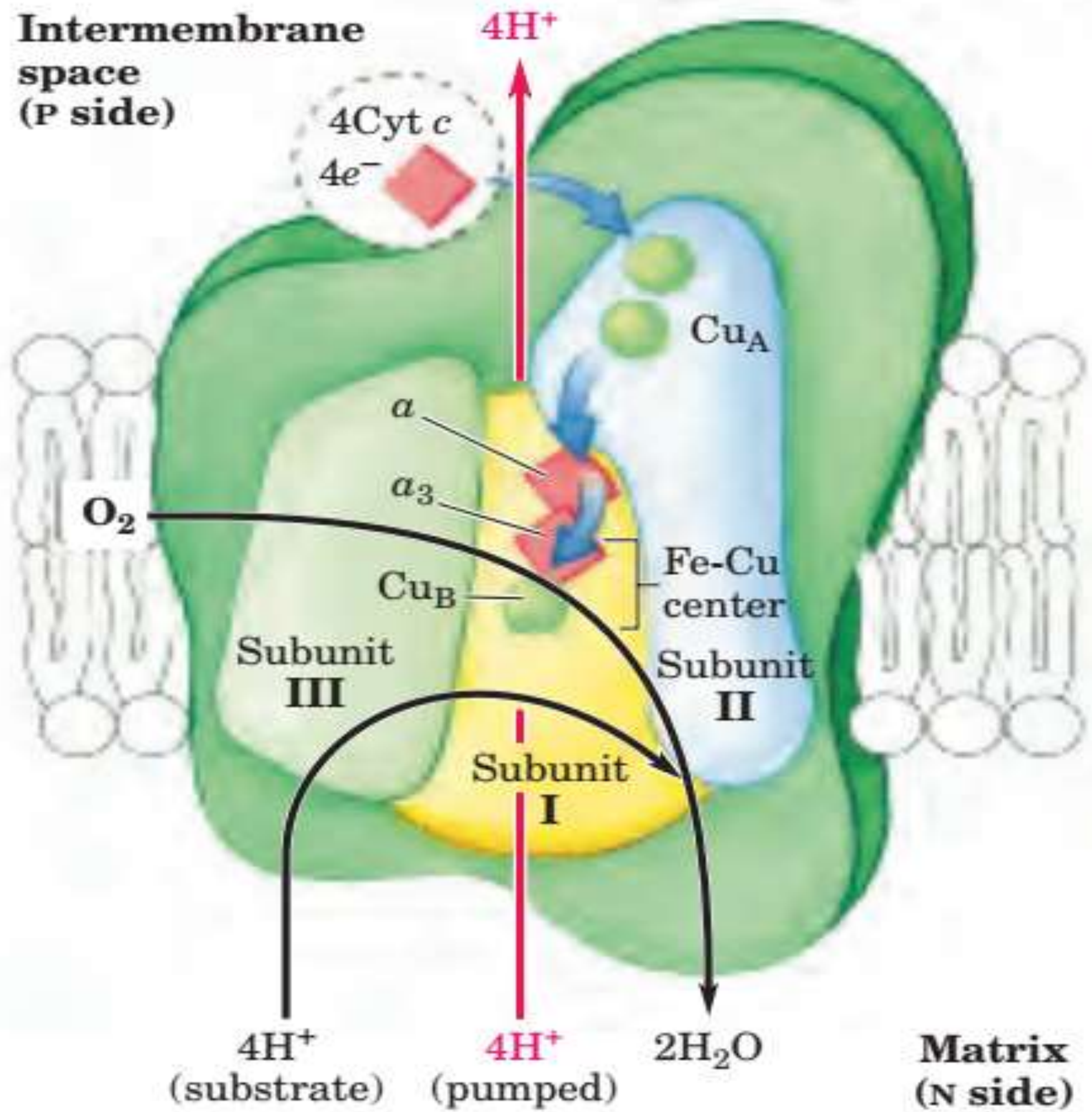
Cyt c
2 H+
Fe-S
Cyt c1
Qo site
QH2
Cyt bL
Cyt bH
Q
Qi site
Q•−
QH2
Q
2 H+
2 H+
QH2
Q
Q
QH2

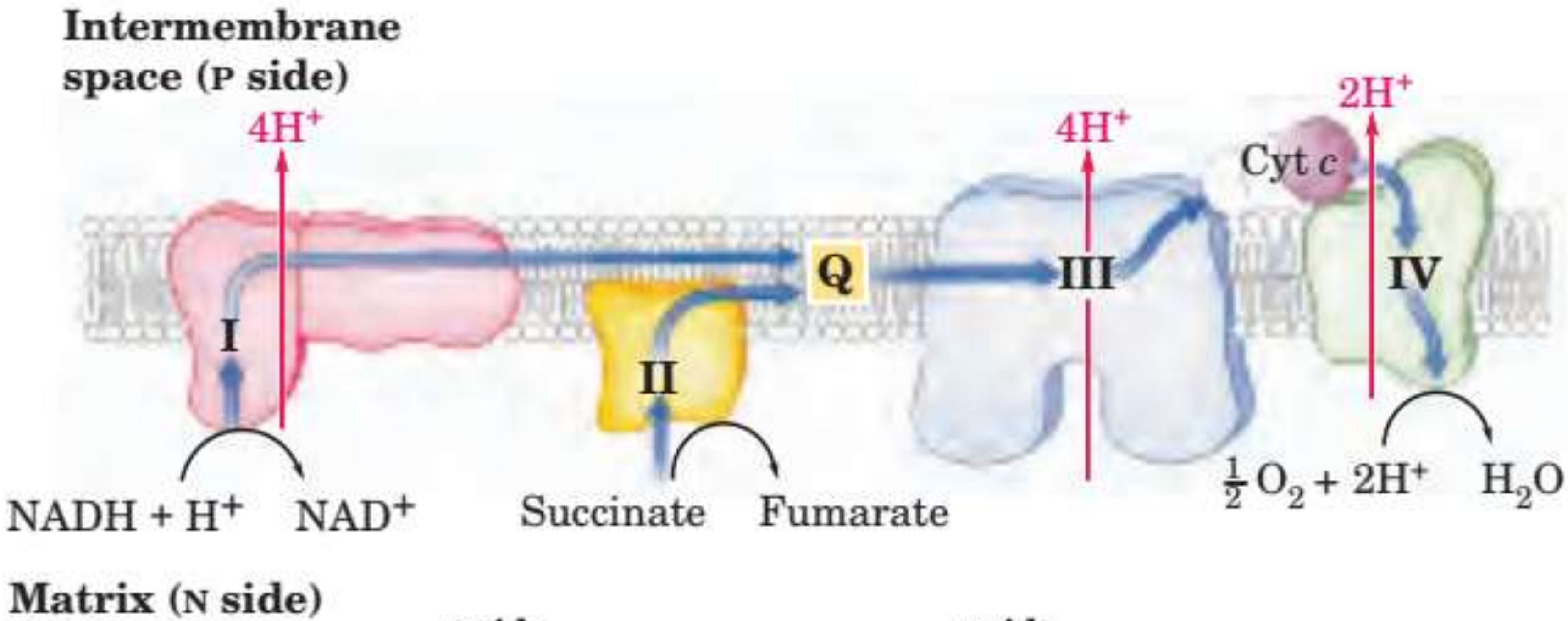


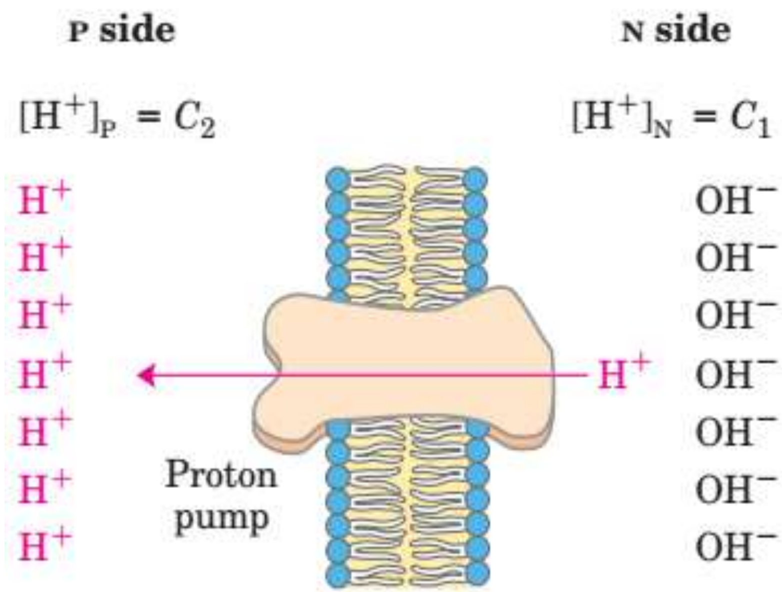
1. Electron transfer to Cu_B
2. Electron transfer to Fe in heme a_3
3. Both Cu_B and Fe in heme a_3 in reduced state
4. Binding of O_2
Cytochrome c
Cu_A/Cu_A
Heme a
Fe
Cu
Cu_B
Heme a_3
O_2
$2\ H_2O$
H⁺ H⁺
H⁺
H⁺
8. Release of water
7. Reduction of the ferryl group
6. Cleavage of O–O bond
5. Formation of peroxide bridge



Cyt c reduced
Cyt c oxidized
4
4
4H+
Fe
Cu
O2 → 2 H2O
4H+
Pumped
protons
4H+
Chemical
protons


Intermembrane space (P side)
4H⁺
4Cyt c
4e⁻
CuA
a
a3
O2
Fe-Cu center
CuB
Subunit III
Subunit II
Subunit I
4H⁺ (substrate)
4H⁺ (pumped)
2H2O
Matrix (N side)





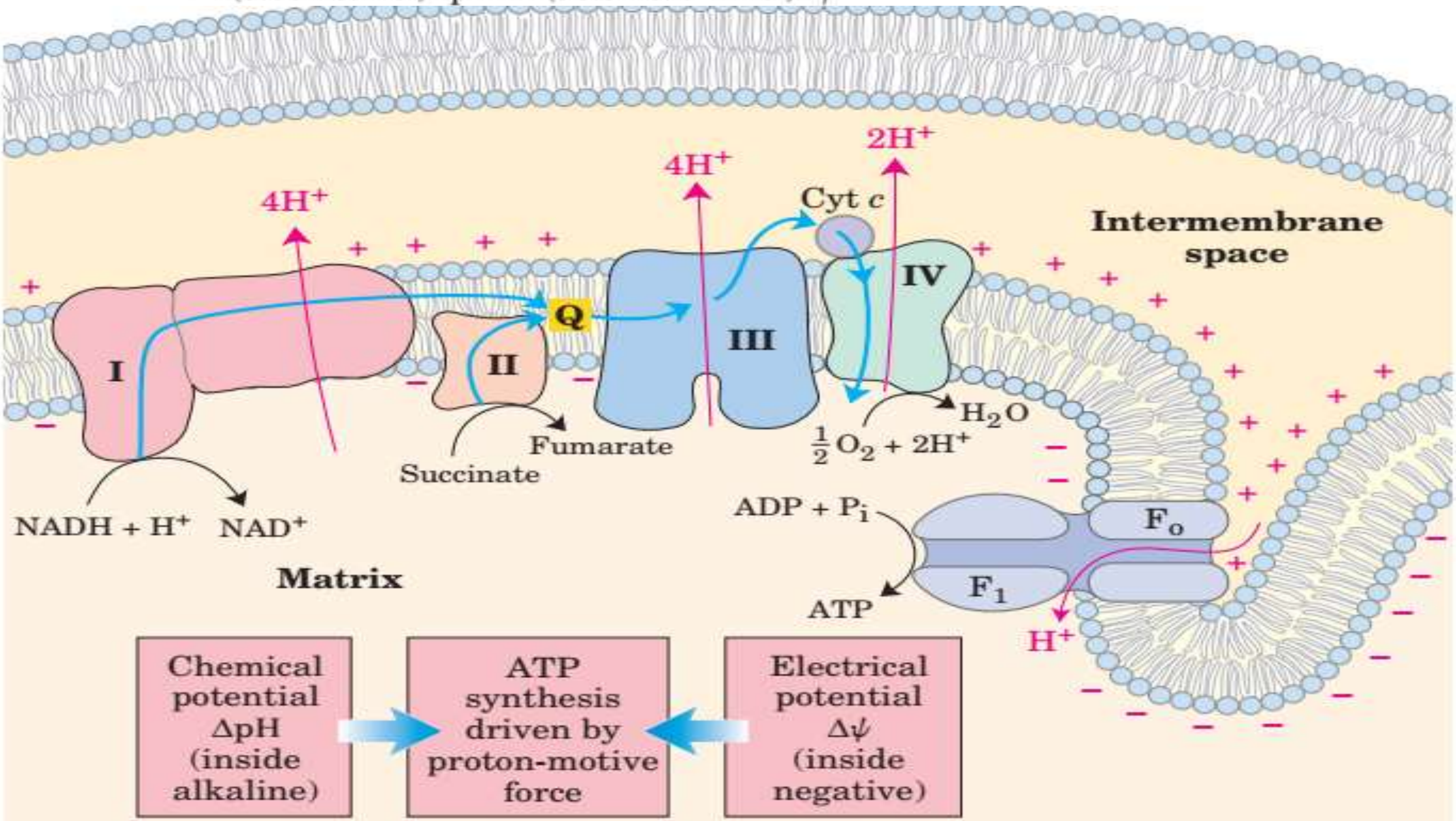
$$\Delta G = RT \ln (C_2/C_1) + Z\mathcal{F}\,\Delta\psi$$

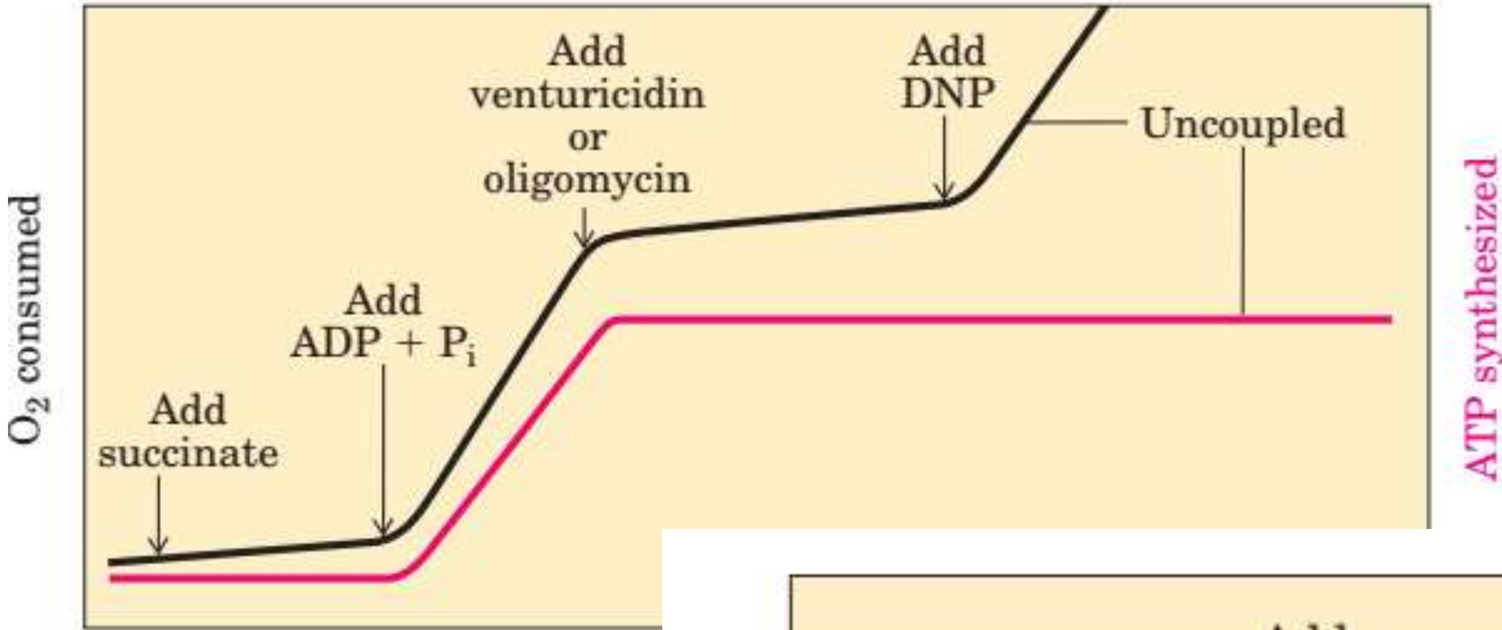
$$= 2.3RT\,\Delta\text{pH} + \mathcal{F}\,\Delta\psi$$

$$\ln\left(\frac{C_2}{C_1}\right) = 2.3(\log\,[\mathrm{H}^+]_\mathrm{P} - \log\,[\mathrm{H}^+]_\mathrm{N})$$
$$= 2.3(\mathrm{pH_N} - \mathrm{pH_P}) = 2.3\ \Delta\mathrm{pH}$$

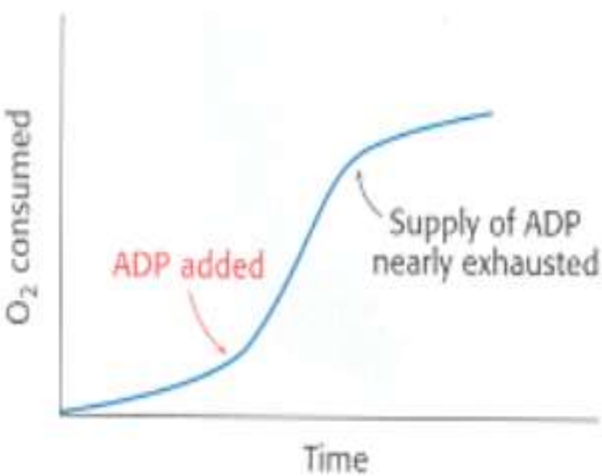
and Equation 19–8 reduces to

$$\Delta G = 2.3RT\,\Delta\mathrm{pH} + \mathcal{F}\,\Delta\psi$$
$$= (5.70\ \mathrm{kJ/mol})\Delta\mathrm{pH} + (96.5\ \mathrm{kJ/V\cdot mol})\Delta\psi$$



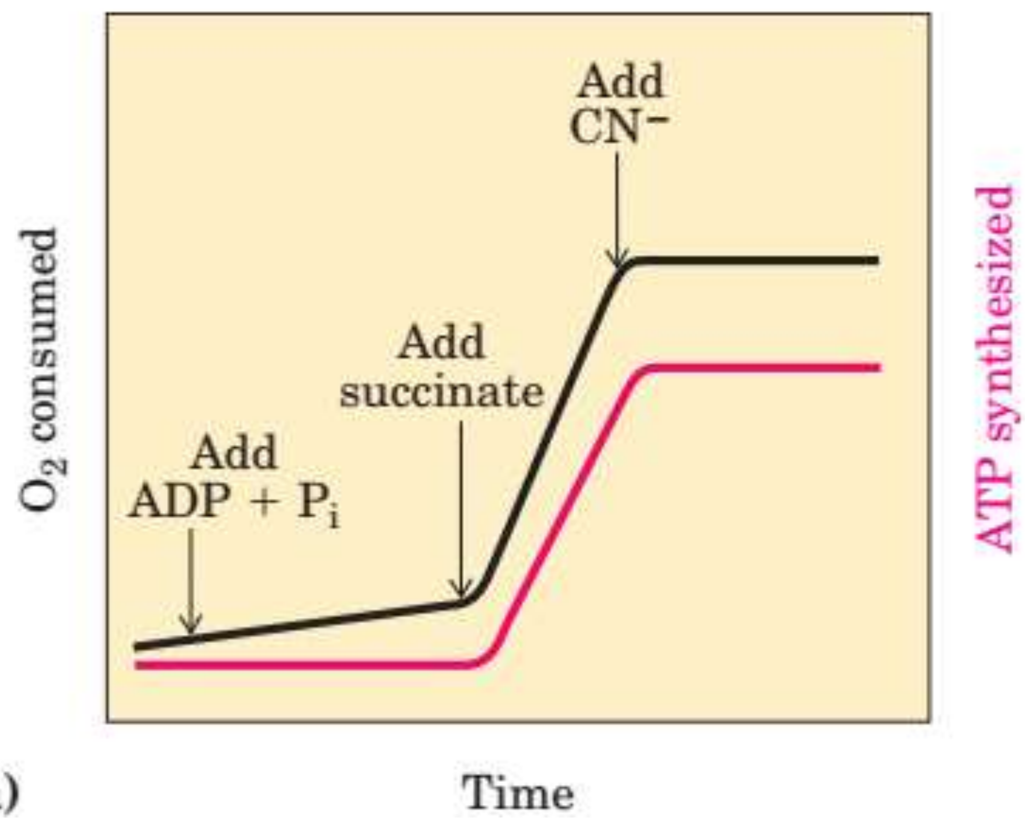


Add succinate
Add ADP + P_i
Add venturicidin or oligomycin
Add DNP
Uncoupled
O_2 consumed
ATP synthesized

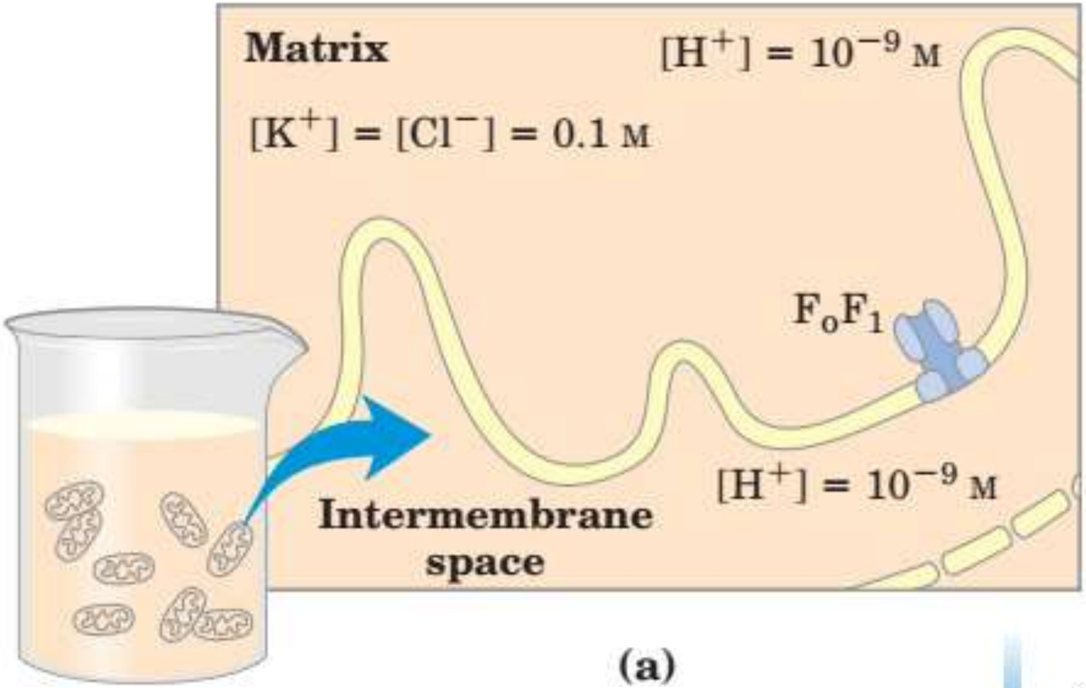


O_2 consumed
ADP added
Supply of ADP nearly exhausted
Time

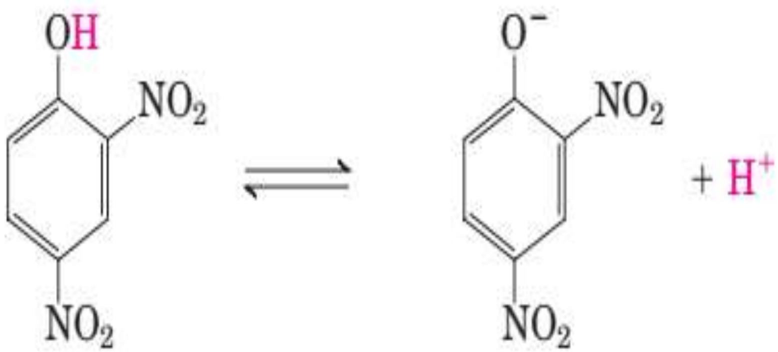
a)



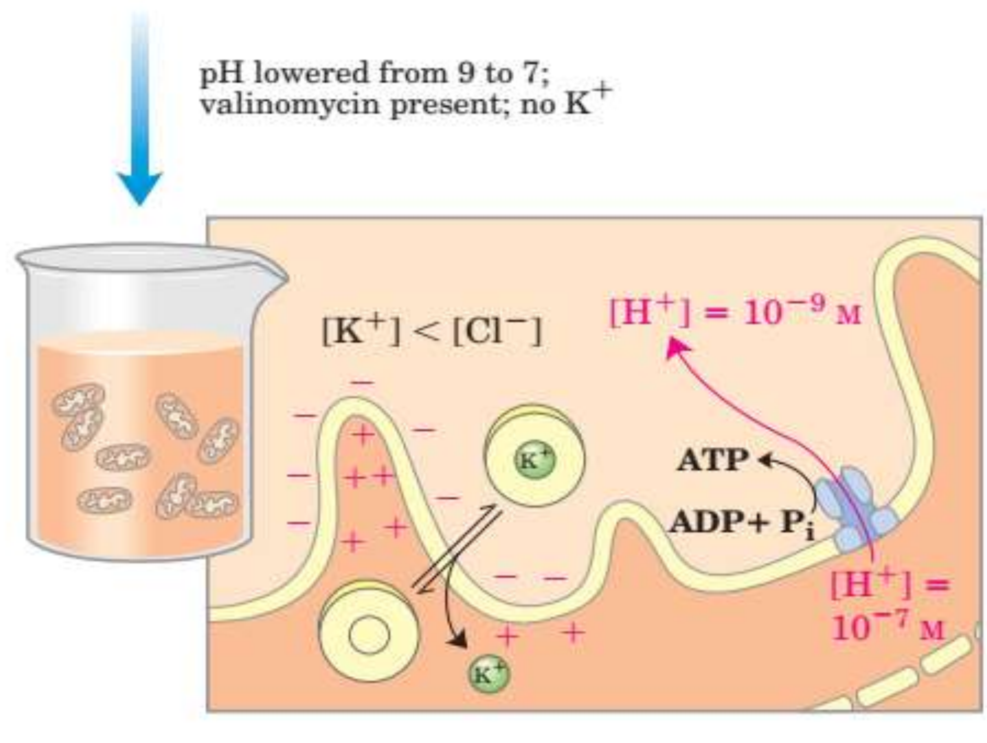
Add ADP + P_i
Add succinate
Add CN^-
O_2 consumed
ATP synthesized
Time



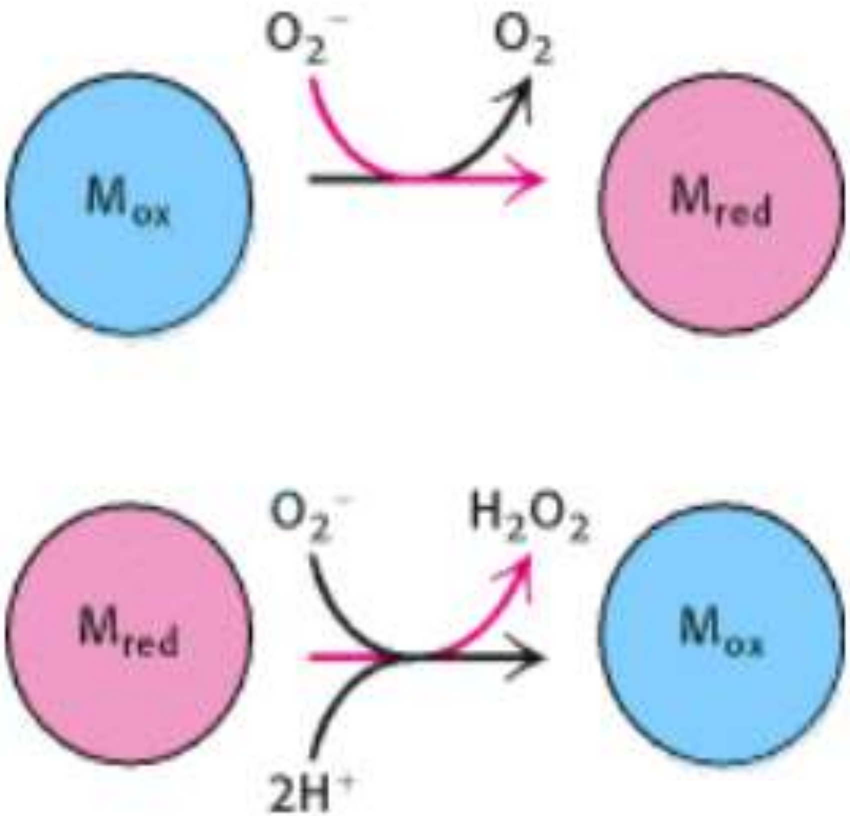
(a)



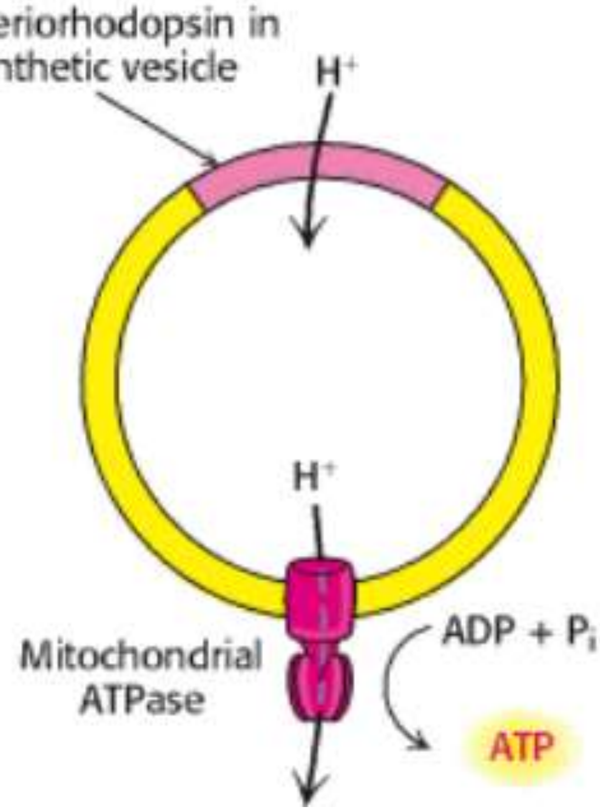
2,4-Dinitrophenol
(DNP)



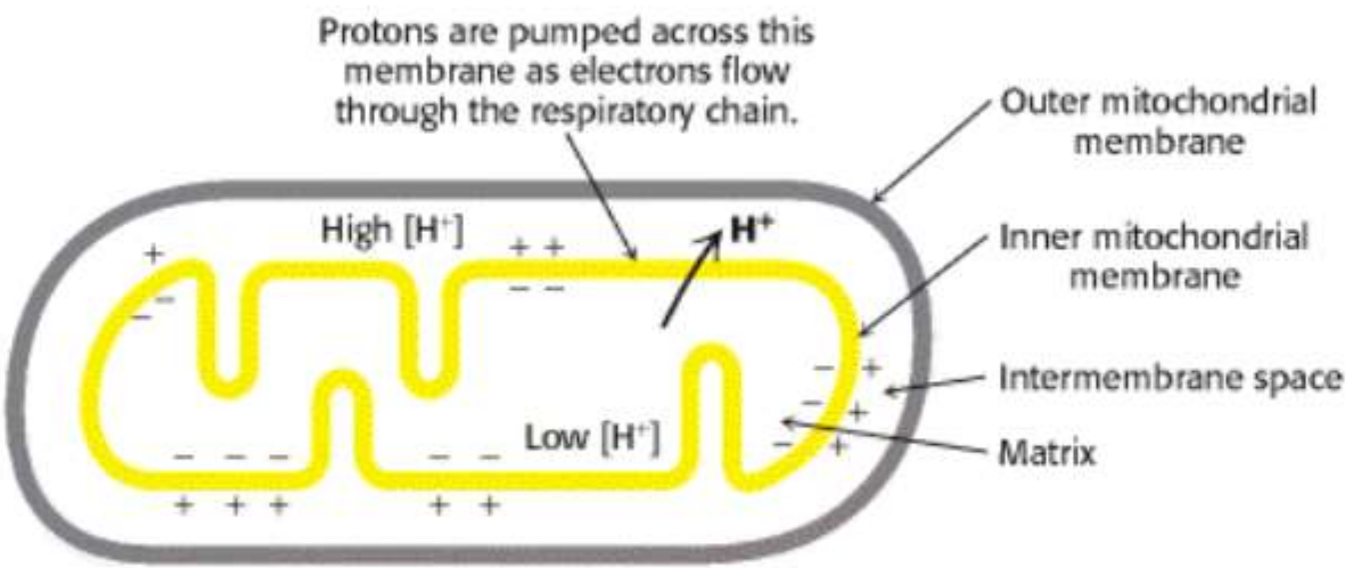
(b)



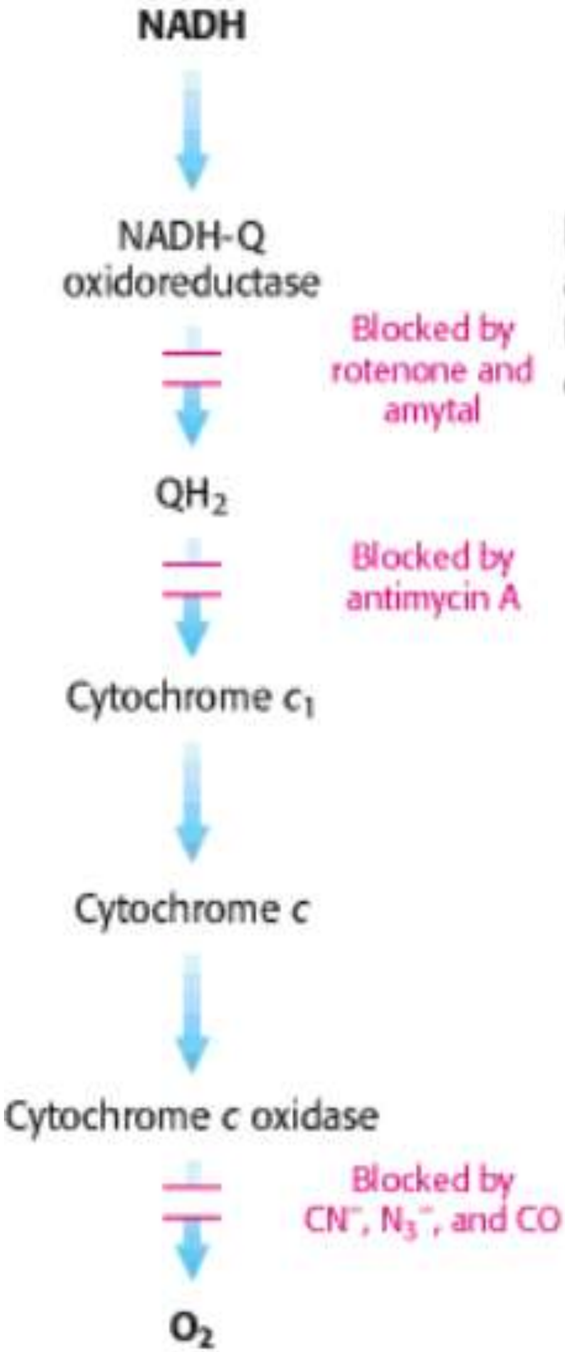
M_{ox}
O_2^-
O_2
M_{red}
M_{red}
O_2^-
H_2O_2
$2H^+$
M_{ox}

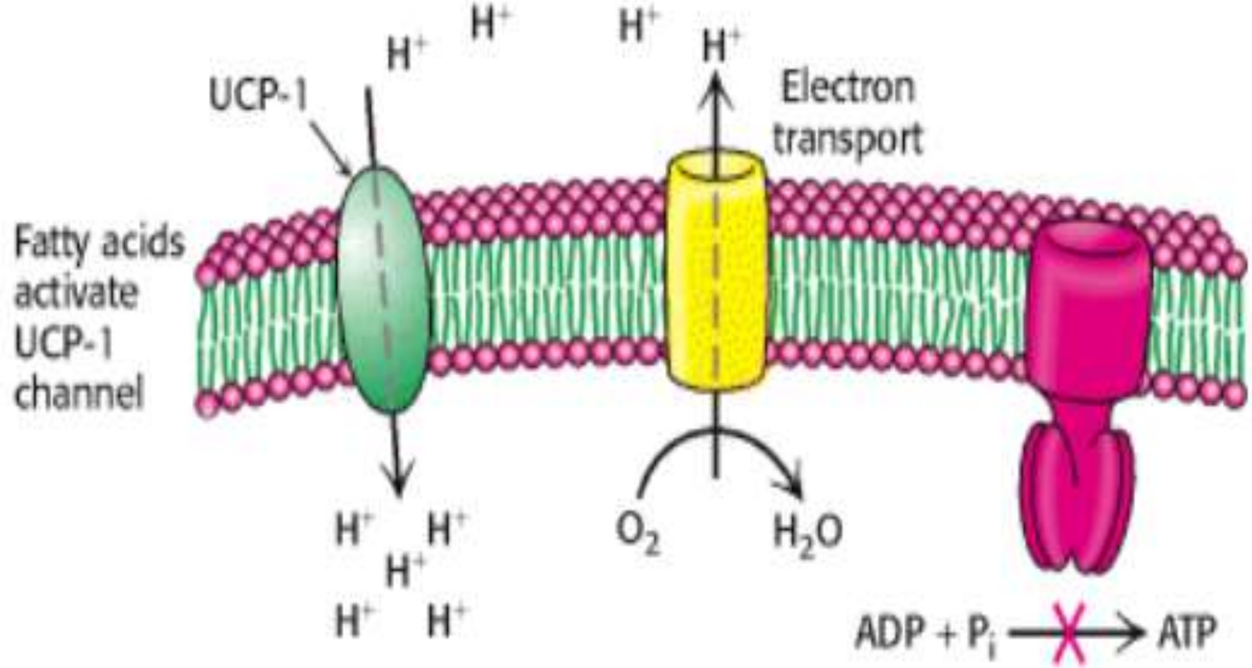


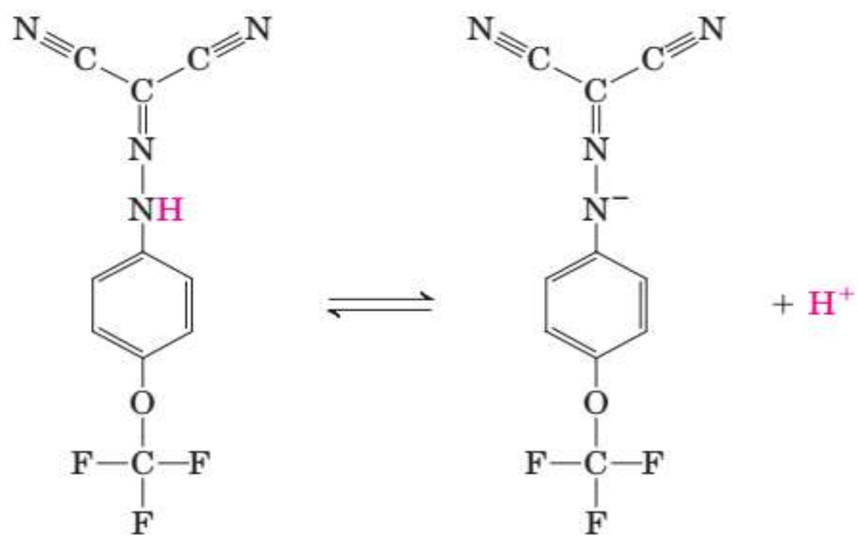
Bacteriorhodopsin in synthetic vesicle
H^+
H^+
Mitochondrial ATPase
ADP + P_i
ATP



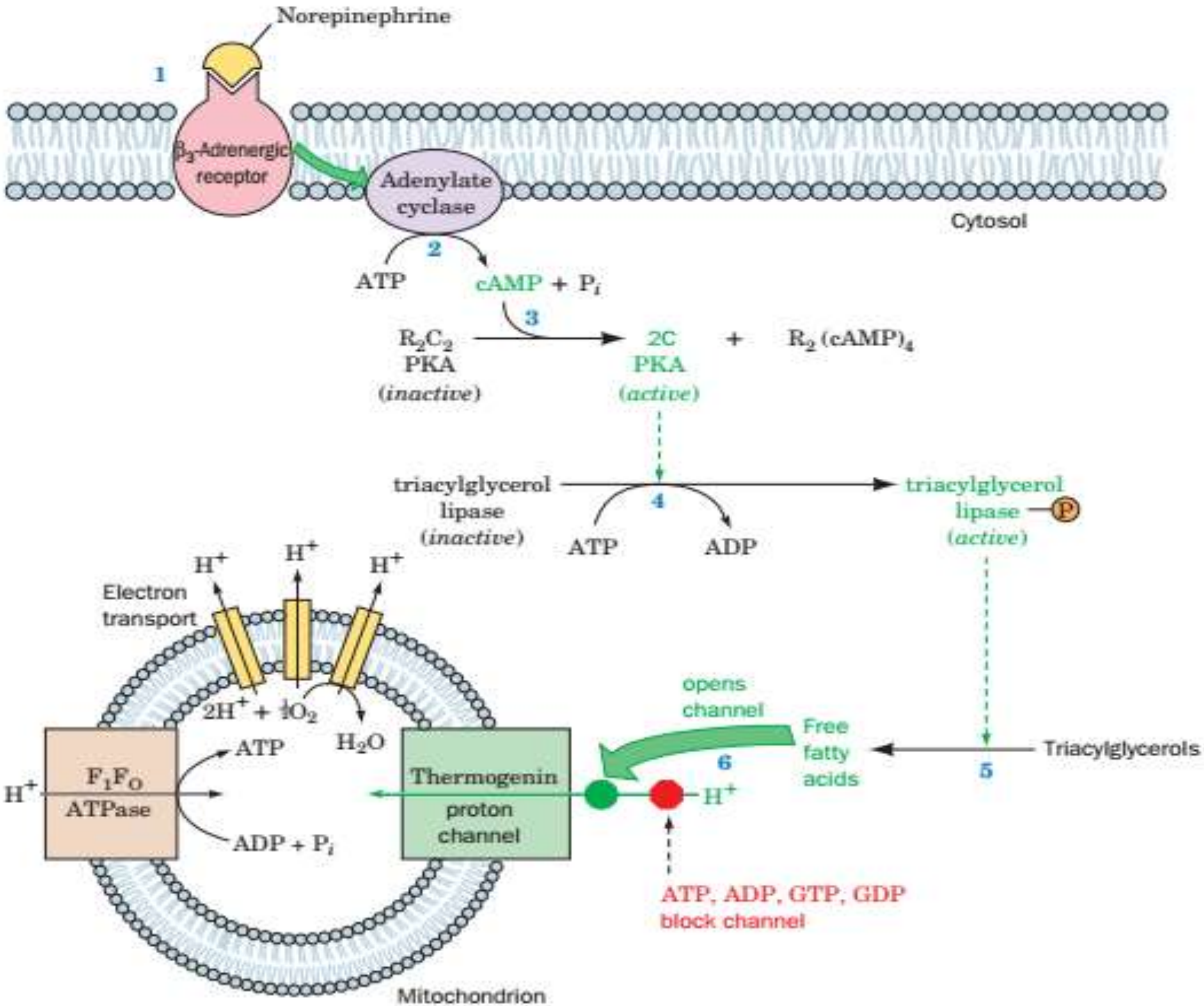
Protons are pumped across this membrane as electrons flow through the respiratory chain.
Outer mitochondrial membrane
High [H^+]
H^+
Inner mitochondrial membrane
Intermembrane space
Low [H^+]
Matrix

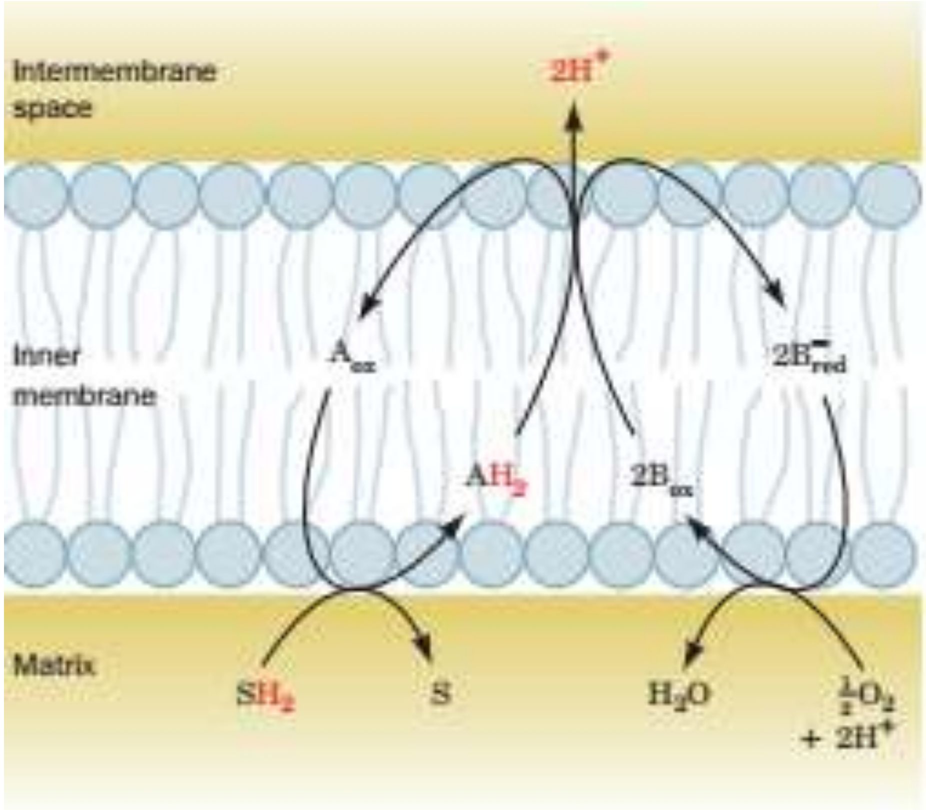






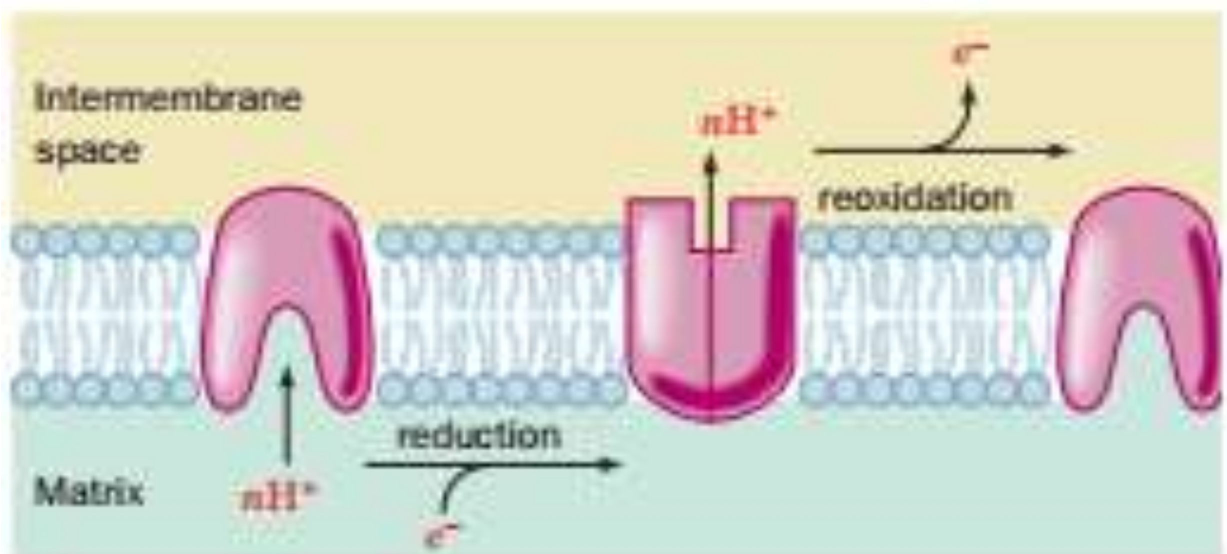
Carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP)

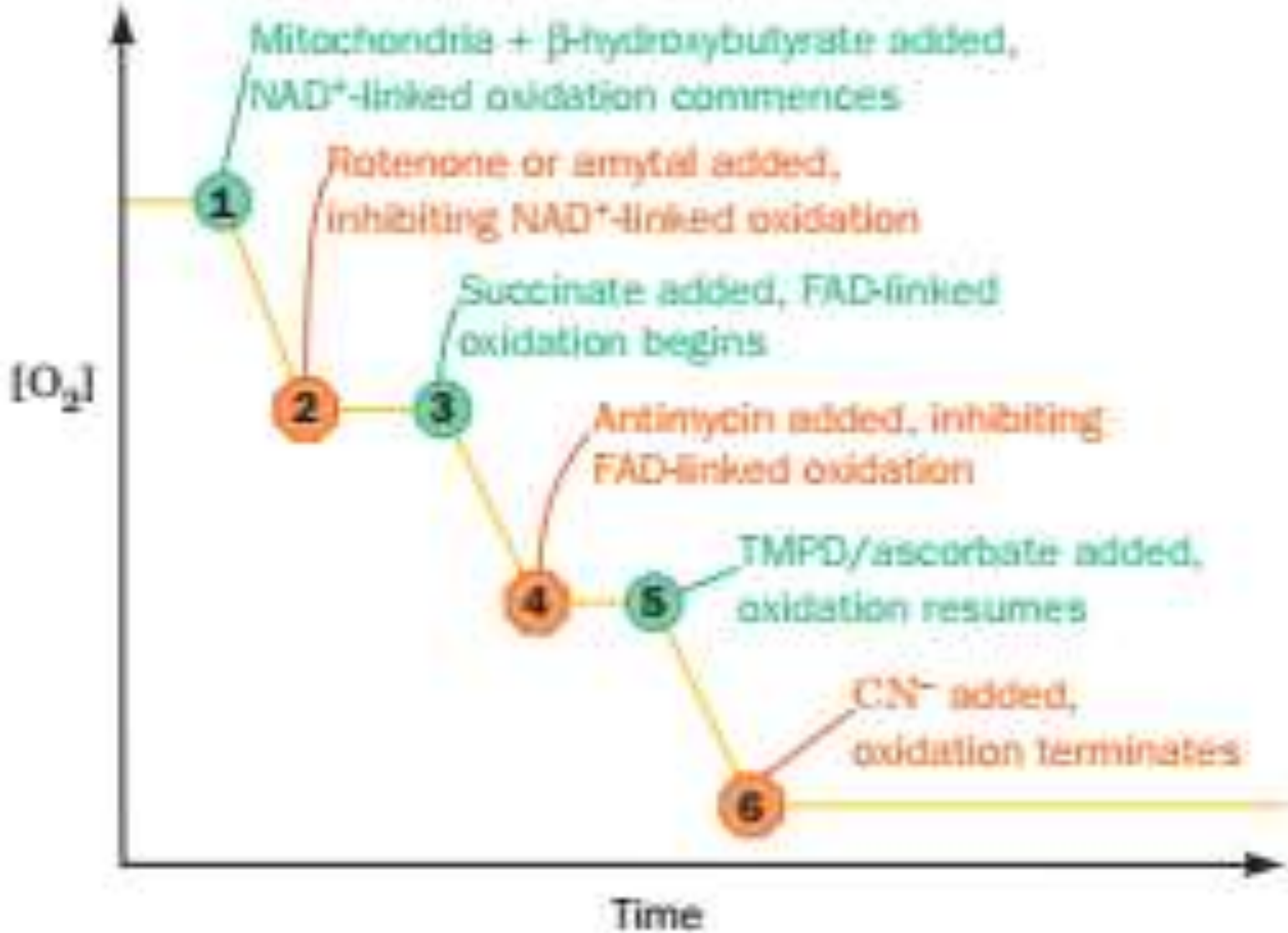

Norepinephrine
1
β3-Adrenergic receptor
Adenylate cyclase
Cytosol
2
ATP
cAMP + Pi
3
R2C2 PKA (inactive)
2C PKA (active)
R2 (cAMP)4
triacylglycerol lipase (inactive)
4
ATP
ADP
triacylglycerol lipase (active)
P
H+
H+
H+
Electron transport
2H+ + ½O2
H2O
ATP
ADP + Pi
F1FO ATPase
H+
Thermogenin proton channel
opens channel
Free fatty acids
6
H+
5
Triacylglycerols
ATP, ADP, GTP, GDP block channel
Mitochondrion



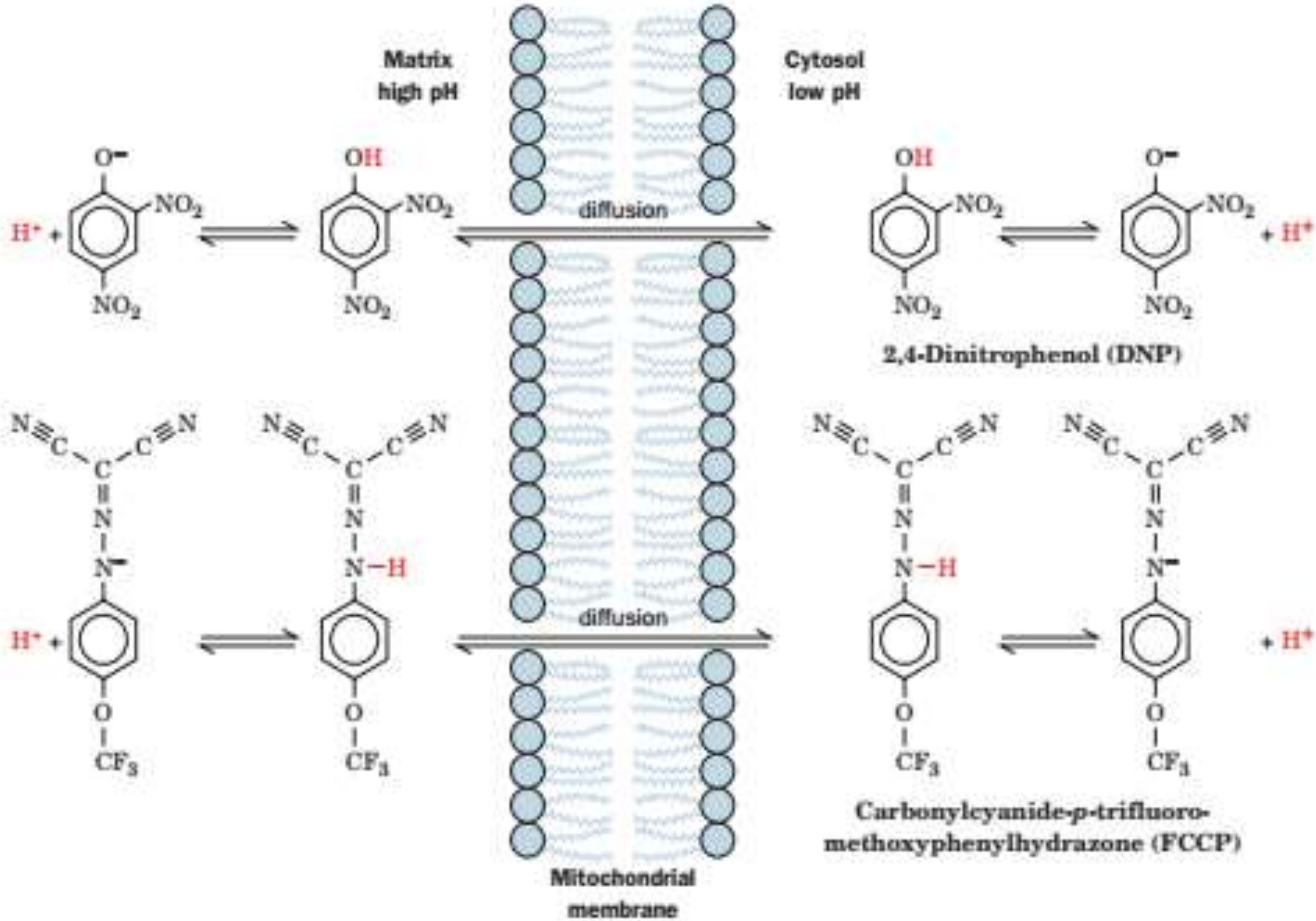
Redox Loop mechanism

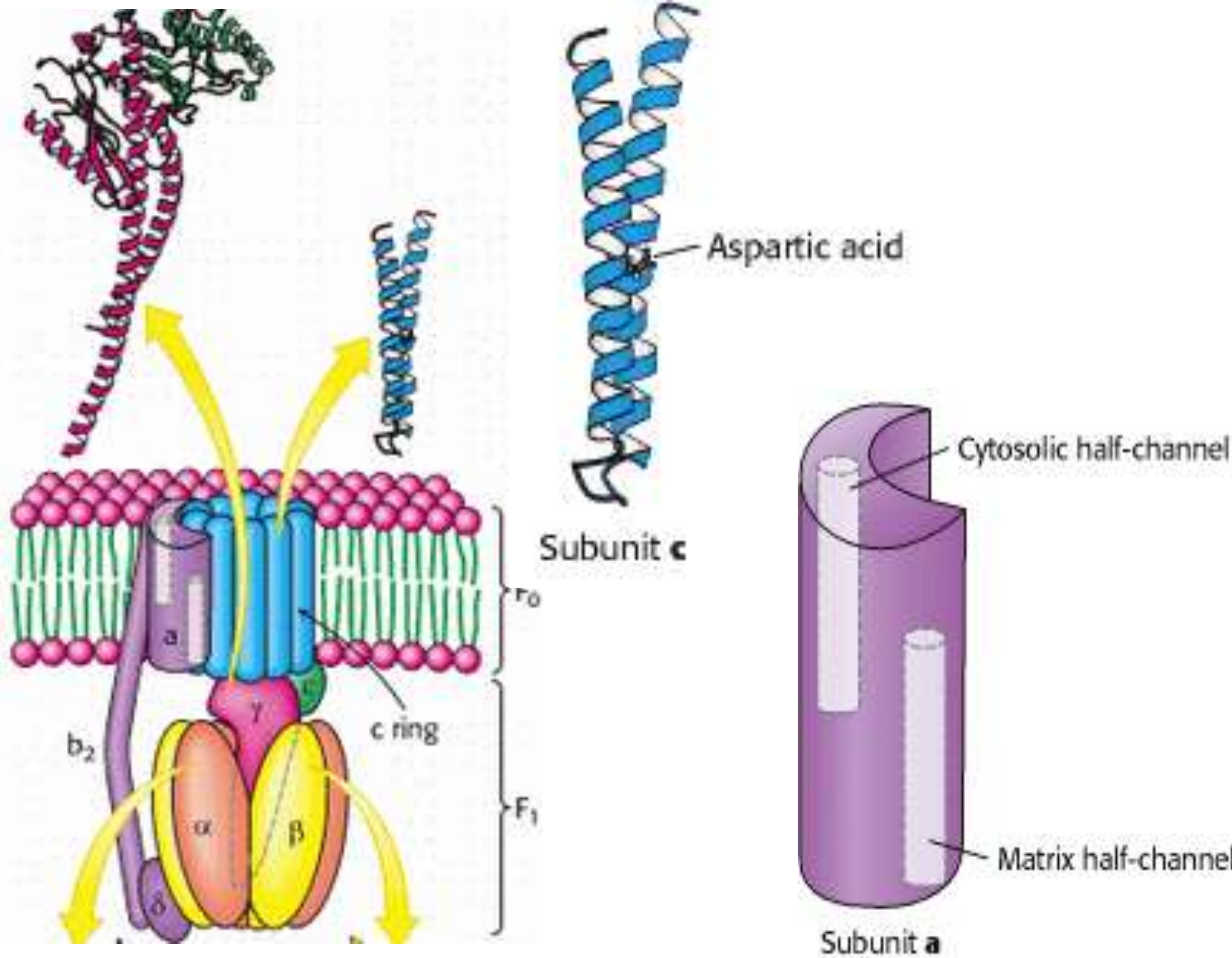
Proton pump mechanism



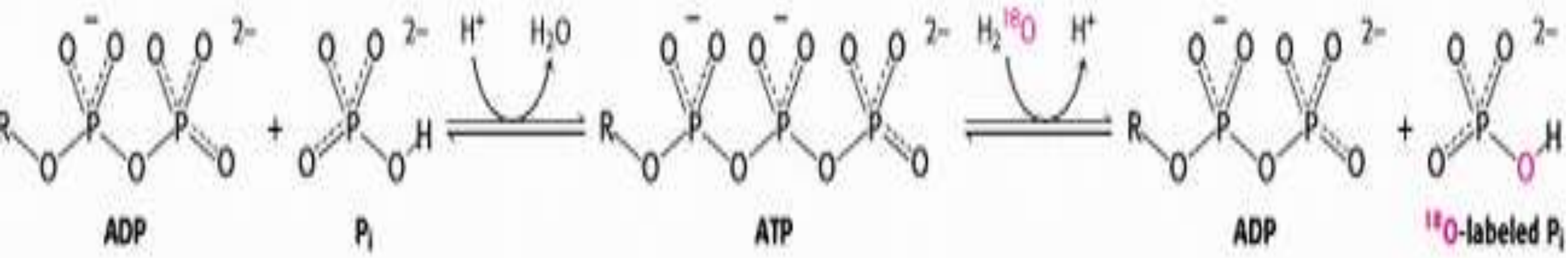


Mitochondria + β-hydroxybutyrate added,
NAD⁺-linked oxidation commences
Rotenone or amytal added,
inhibiting NAD⁺-linked oxidation
Succinate added, FAD-linked
oxidation begins
Antimycin added, inhibiting
FAD-linked oxidation
TMPD/ascorbate added,
oxidation resumes
CN⁻ added,
oxidation terminates
[O2]
Time
1
2
3
4
5
6

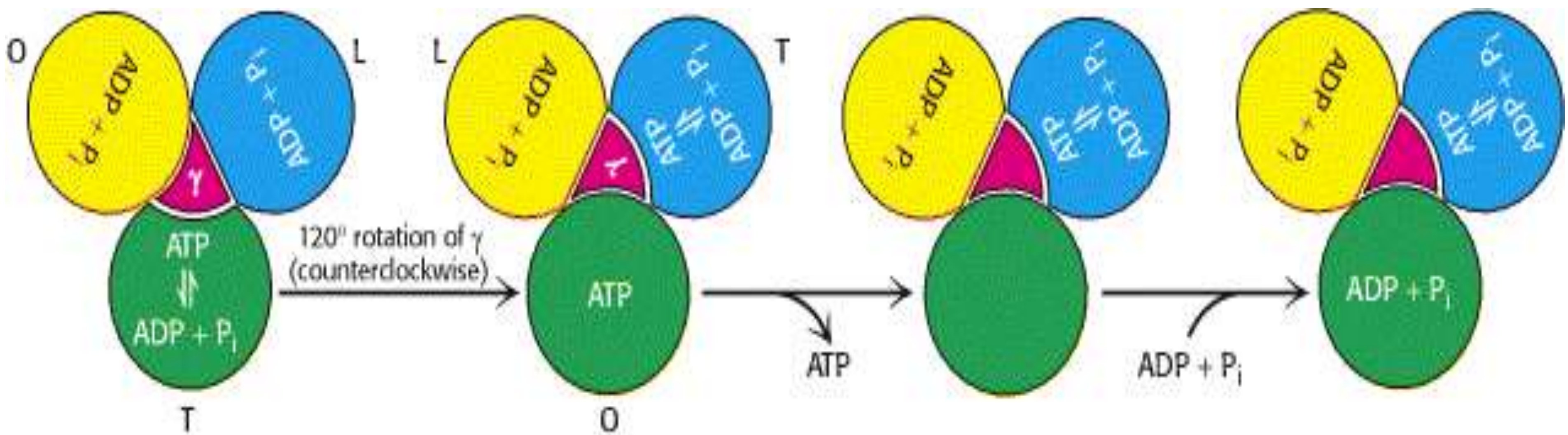

Matrix
high pH
Cytosol
low pH
diffusion
2,4-Dinitrophenol (DNP)
diffusion
Carbonylcyanide-p-trifluoro-
methoxyphenylhydrazone (FCCP)
Mitochondrial
membrane



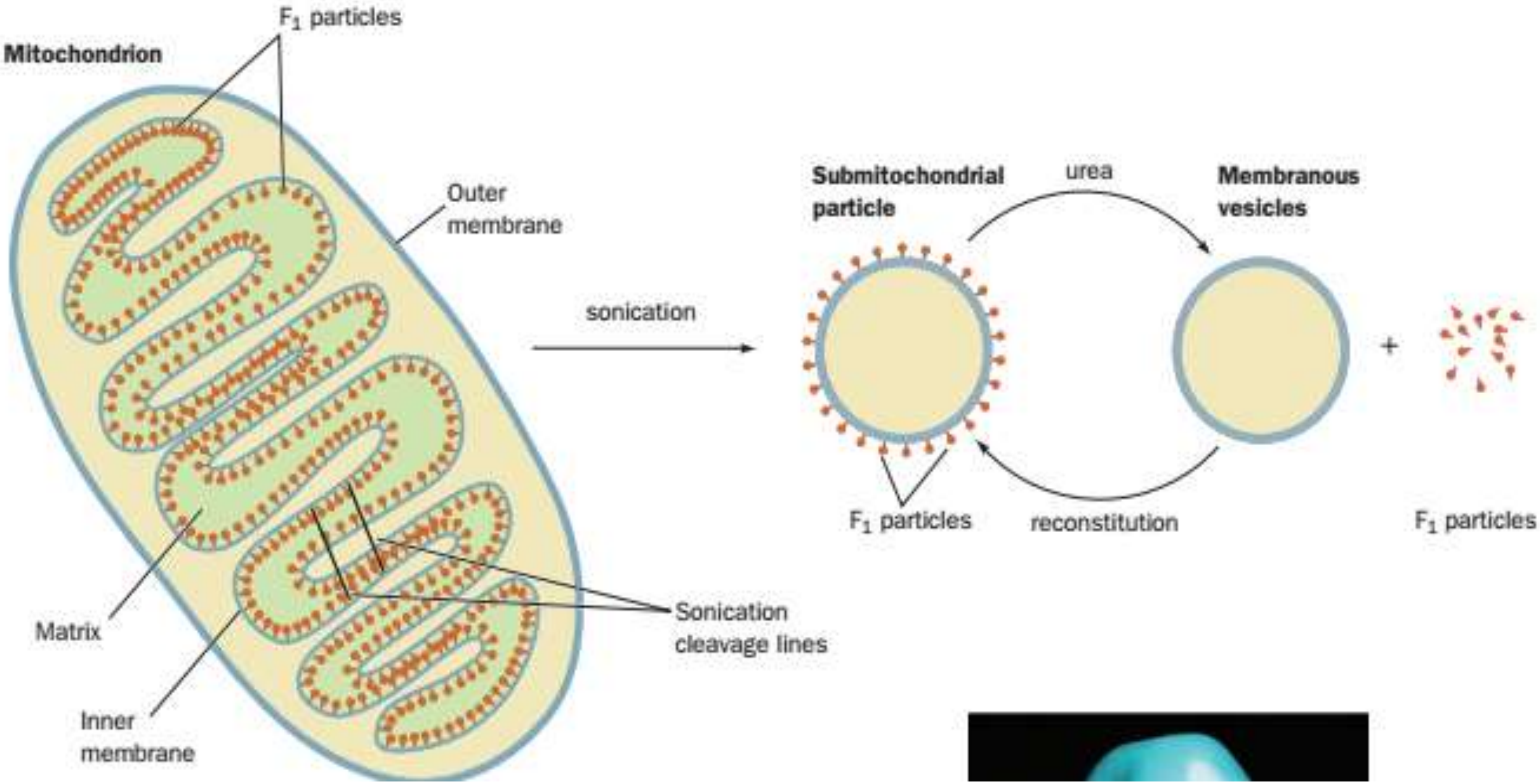
Aspartic acid
Subunit c
Cytosolic half-channel
Matrix half-channel
Subunit a
a
b_2
γ
α
β
δ
c ring
F_0
F_1

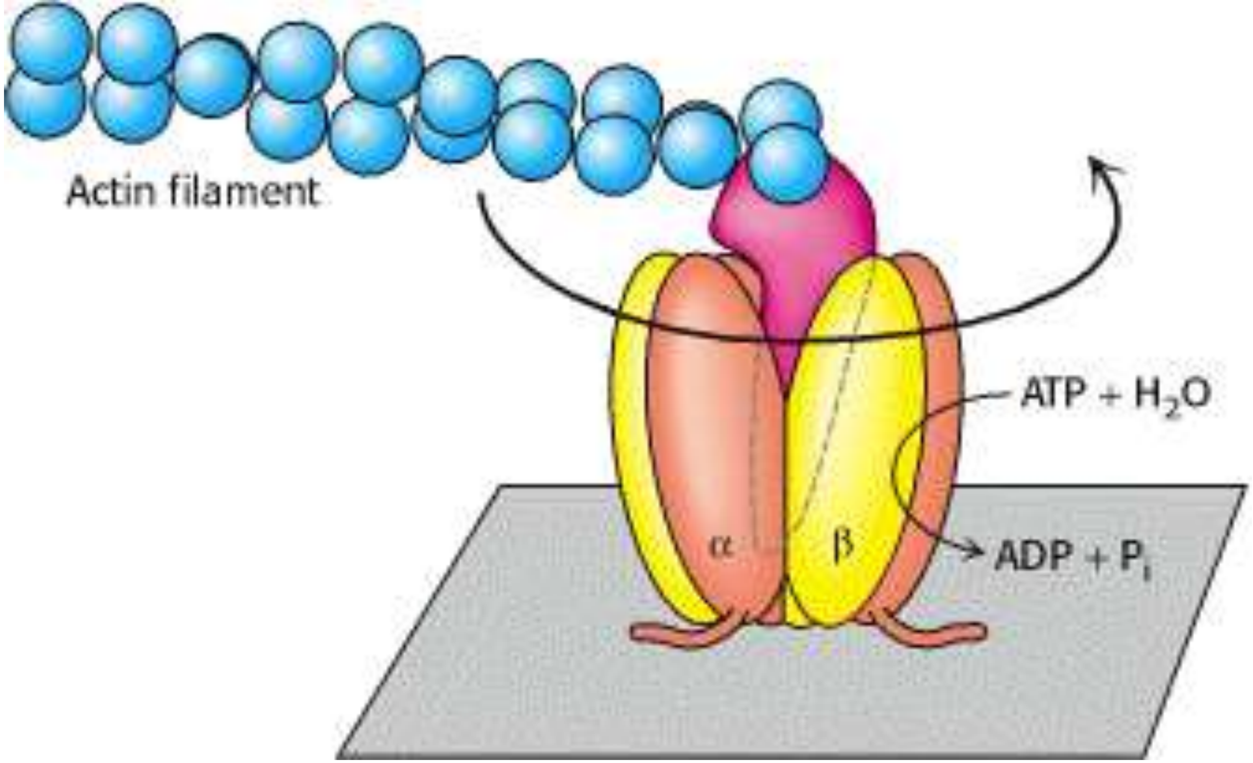


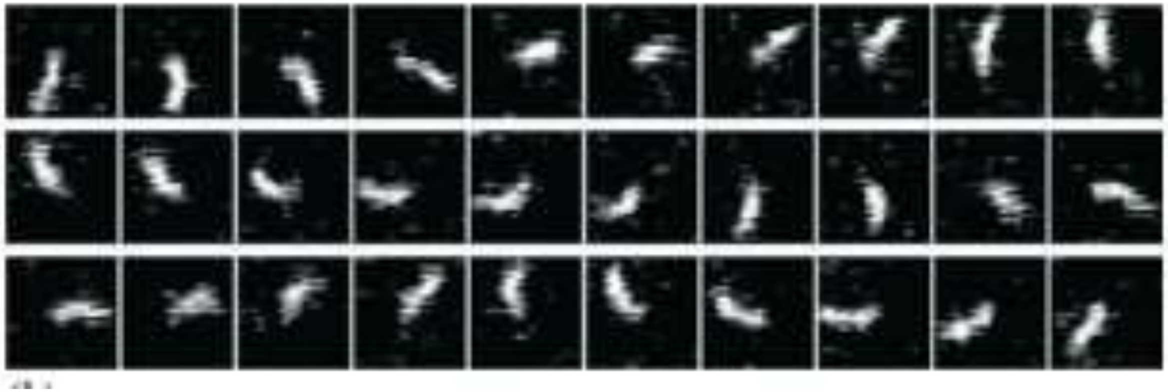
ADP
Pi
ATP
ADP
18O-labeled Pi

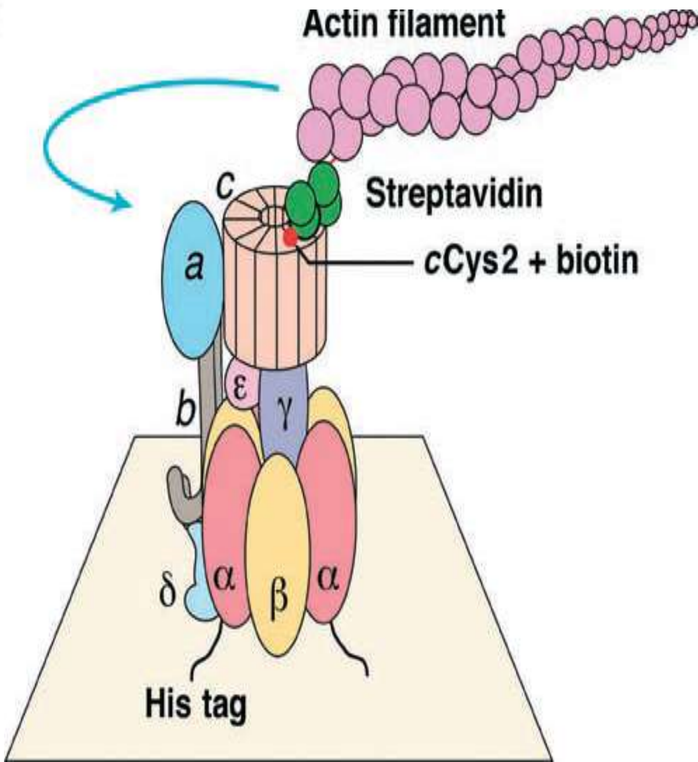


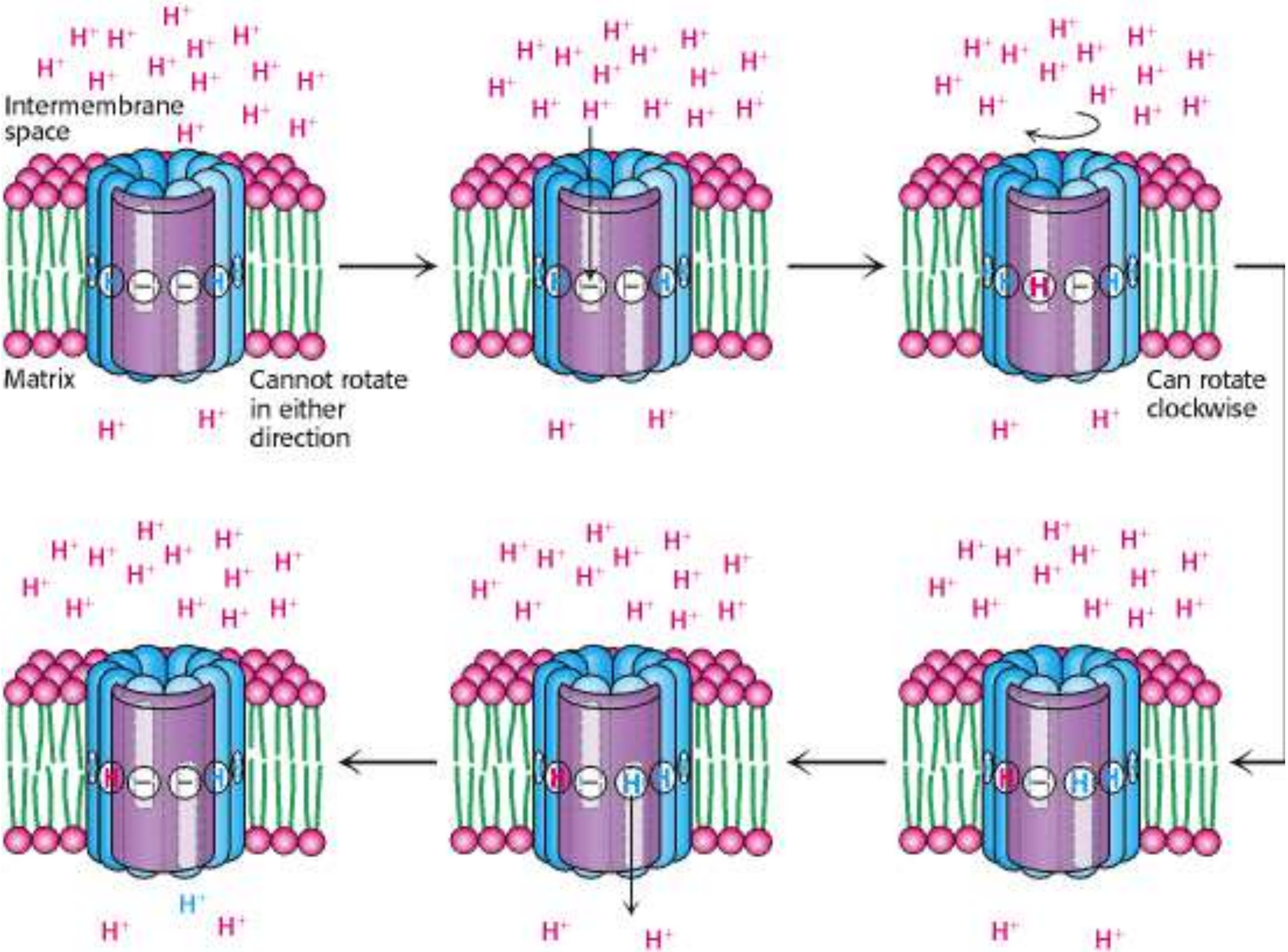
O
L
T
ATP
ADP + Pi
120° rotation of γ
(counterclockwise)
L
T
O
ATP
ADP + Pi
ATP
ADP + Pi

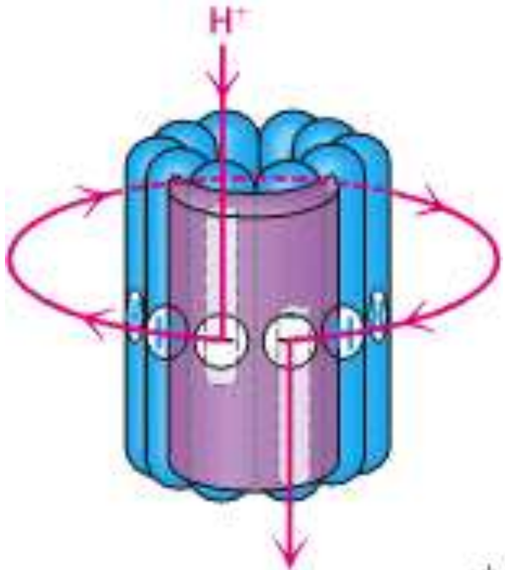

Mitochondrion
F1 particles
Outer membrane
Matrix
Inner membrane
Sonication cleavage lines
sonication
Submitochondrial particle
urea
Membranous vesicles
+
F1 particles
reconstitution
F1 particles

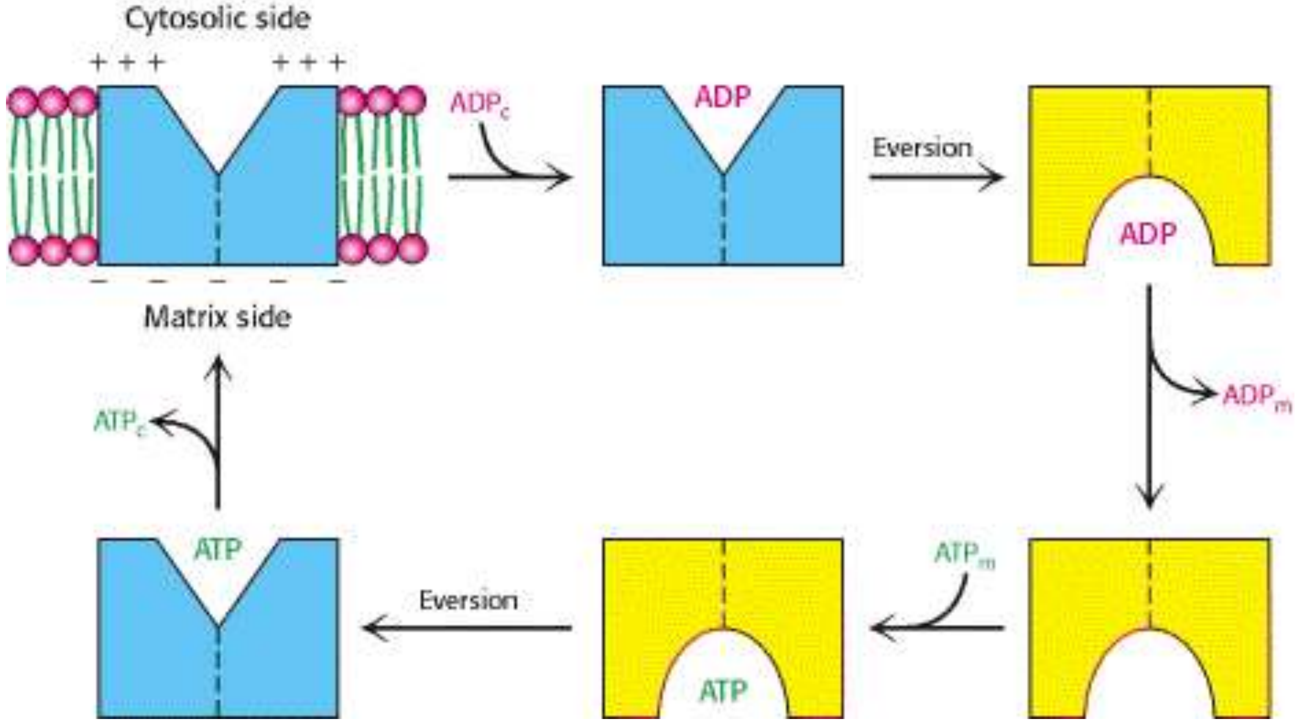

Actin filament
ATP + H_2O
ADP + P_i
α
β




Actin filament
Streptavidin
cCys2 + biotin
c
a
b
ε
γ
δ
α
β
α
His tag

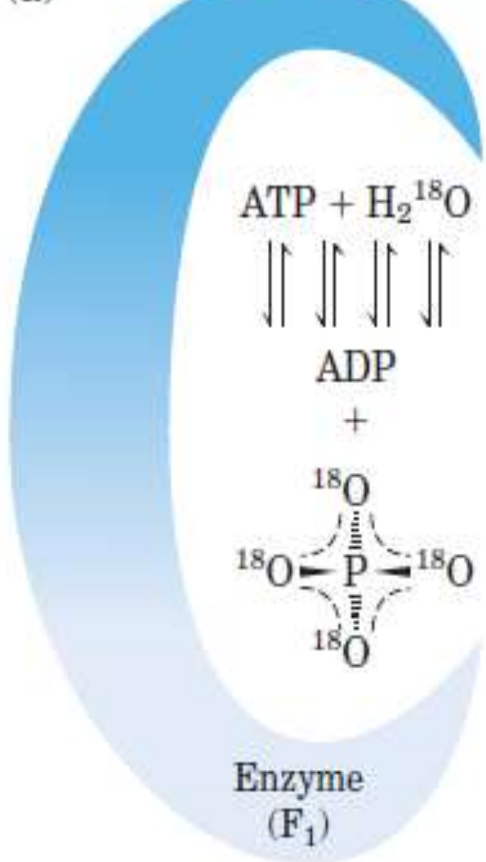

Intermembrane space
Matrix
Cannot rotate in either direction
Can rotate clockwise


H+


Cytosolic side
+ + +
+ + +
− − − − −
Matrix side
ADPc
ADP
Eversion
ADP
ADPm
ATPm
ATP
Eversion
ATP
ATPc

Efraim Racker,
1913–1991

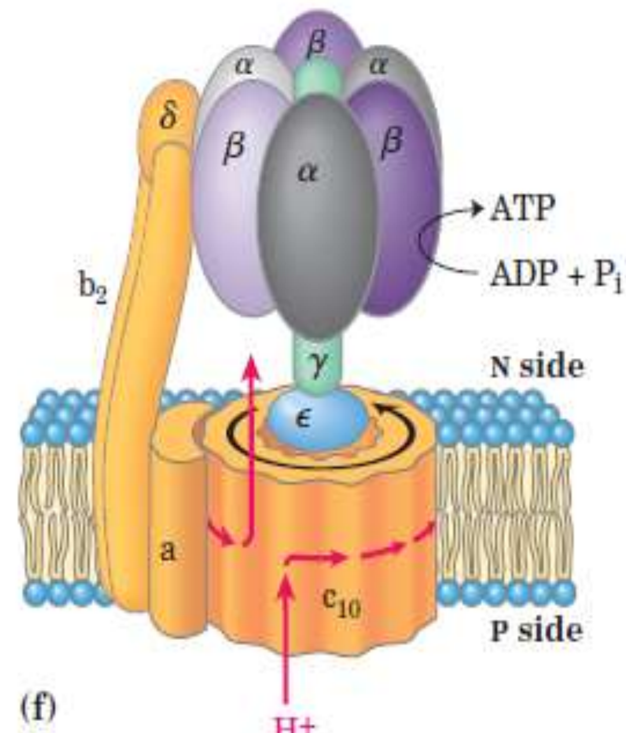
(a)



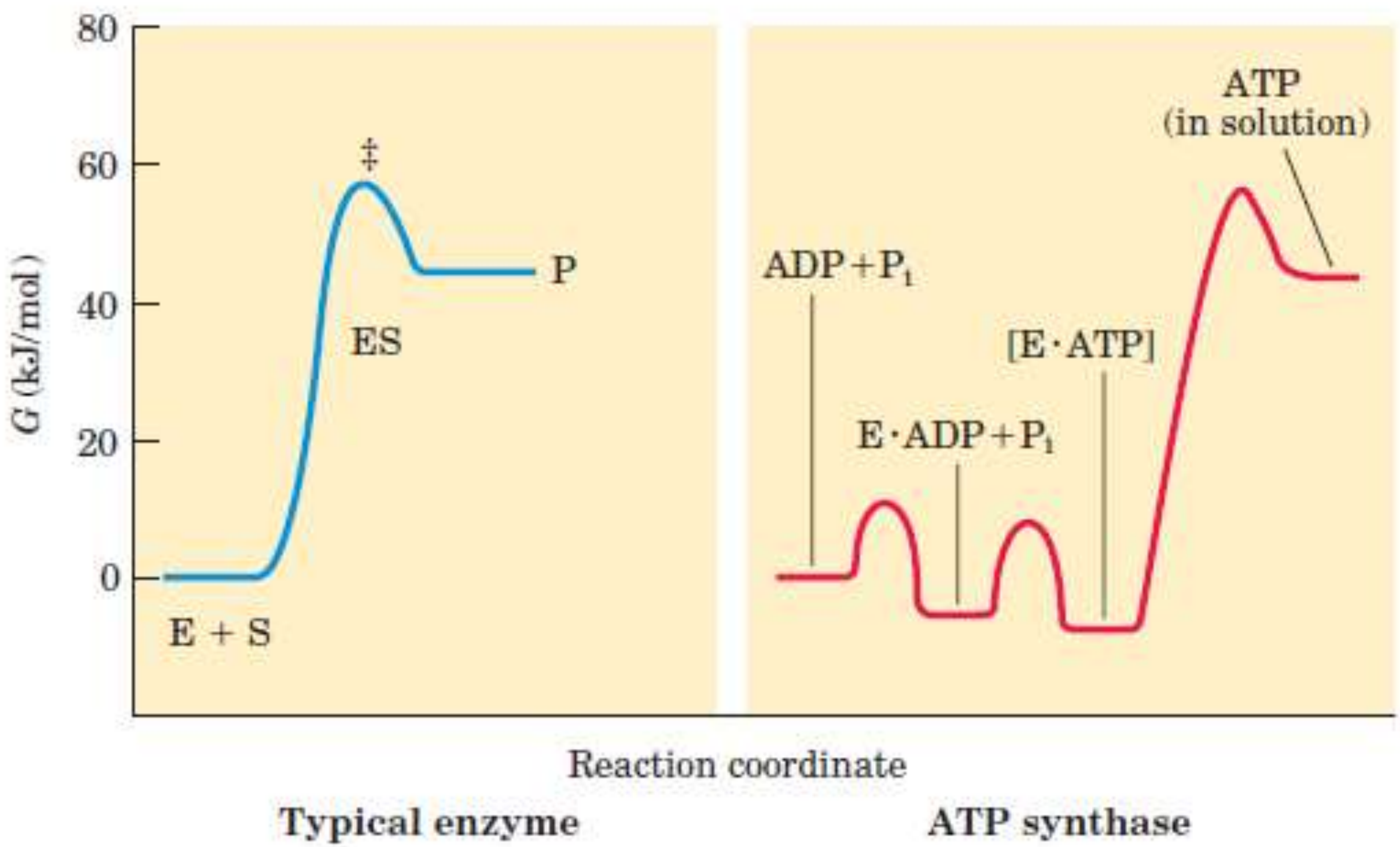
$$\text{Enz-ATP} \rightleftharpoons \text{Enz–(ADP + P}_i\text{)}$$

$$K'_{eq} = \frac{k_{-1}}{k_1} = \frac{24\ s^{-1}}{10\ s^{-1}} = 2.4$$

F_oF_1 binds ATP with very high affinity (*Kd* $\leq 10^{-12}$ M) and ADP with much lower affinity (*Kd* $\approx 10^{-5}$ M).



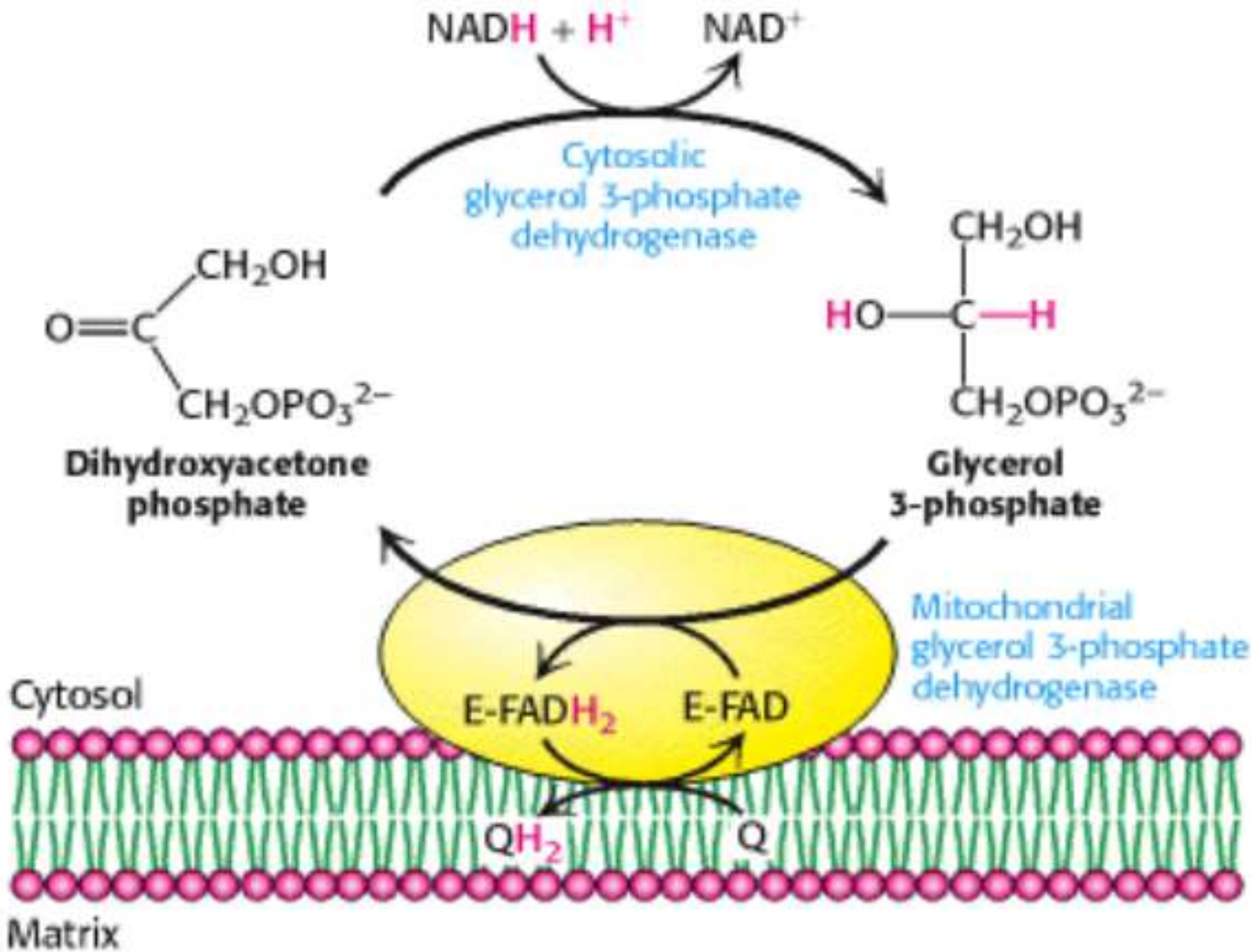
(f)

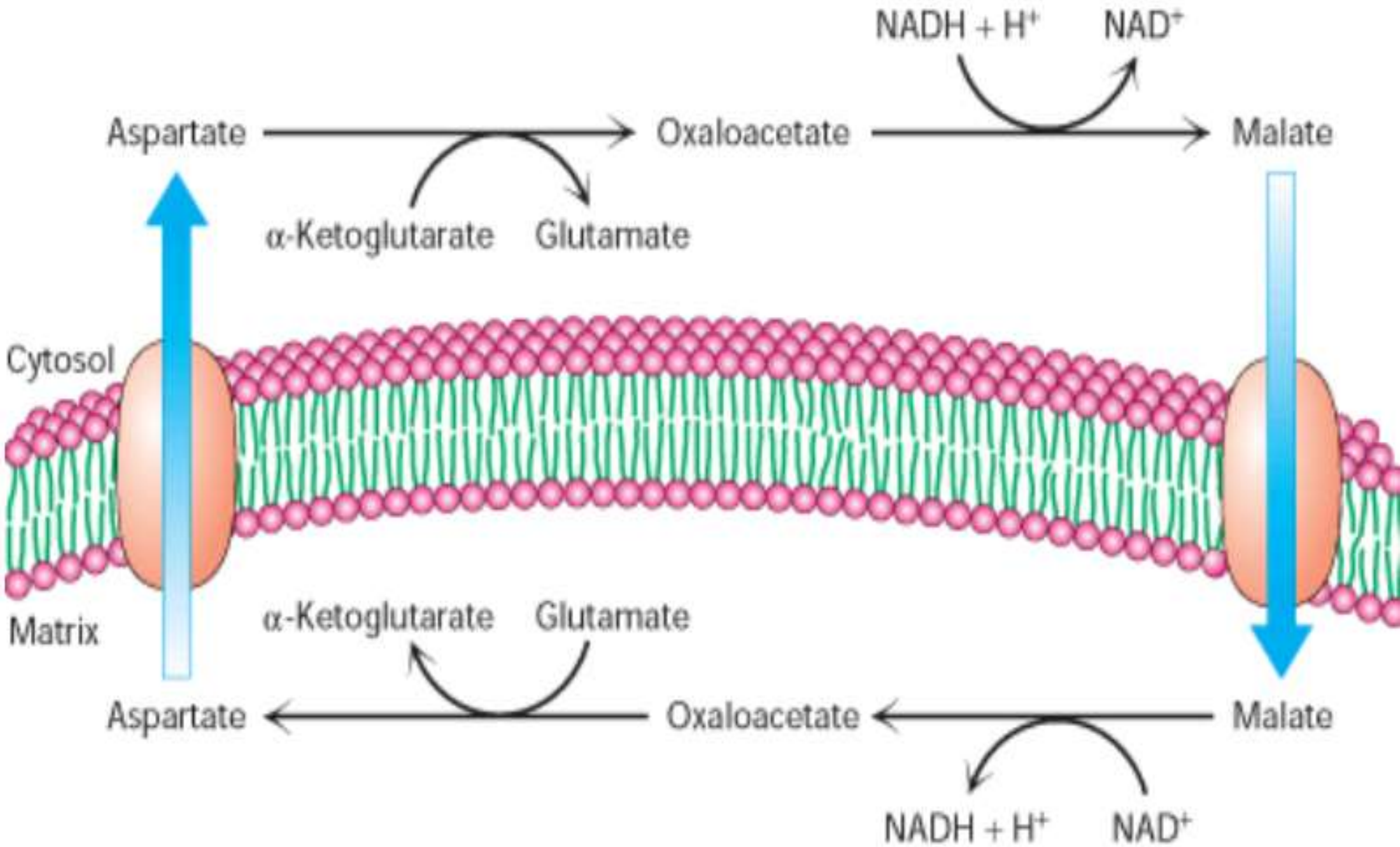


acceptor control ratio

mass-action ratio $[ATP]/([ADP][P_i])$

An Inhibitory Protein (IF1) Prevents ATP Hydrolysis during Ischemia


NADH + H+
NAD+
Cytosolic glycerol 3-phosphate dehydrogenase
CH2OH
O═C
CH2OPO3 2−
Dihydroxyacetone phosphate
CH2OH
HO—C—H
CH2OPO3 2−
Glycerol 3-phosphate
Mitochondrial glycerol 3-phosphate dehydrogenase
Cytosol
E-FADH2
E-FAD
QH2
Q
Matrix

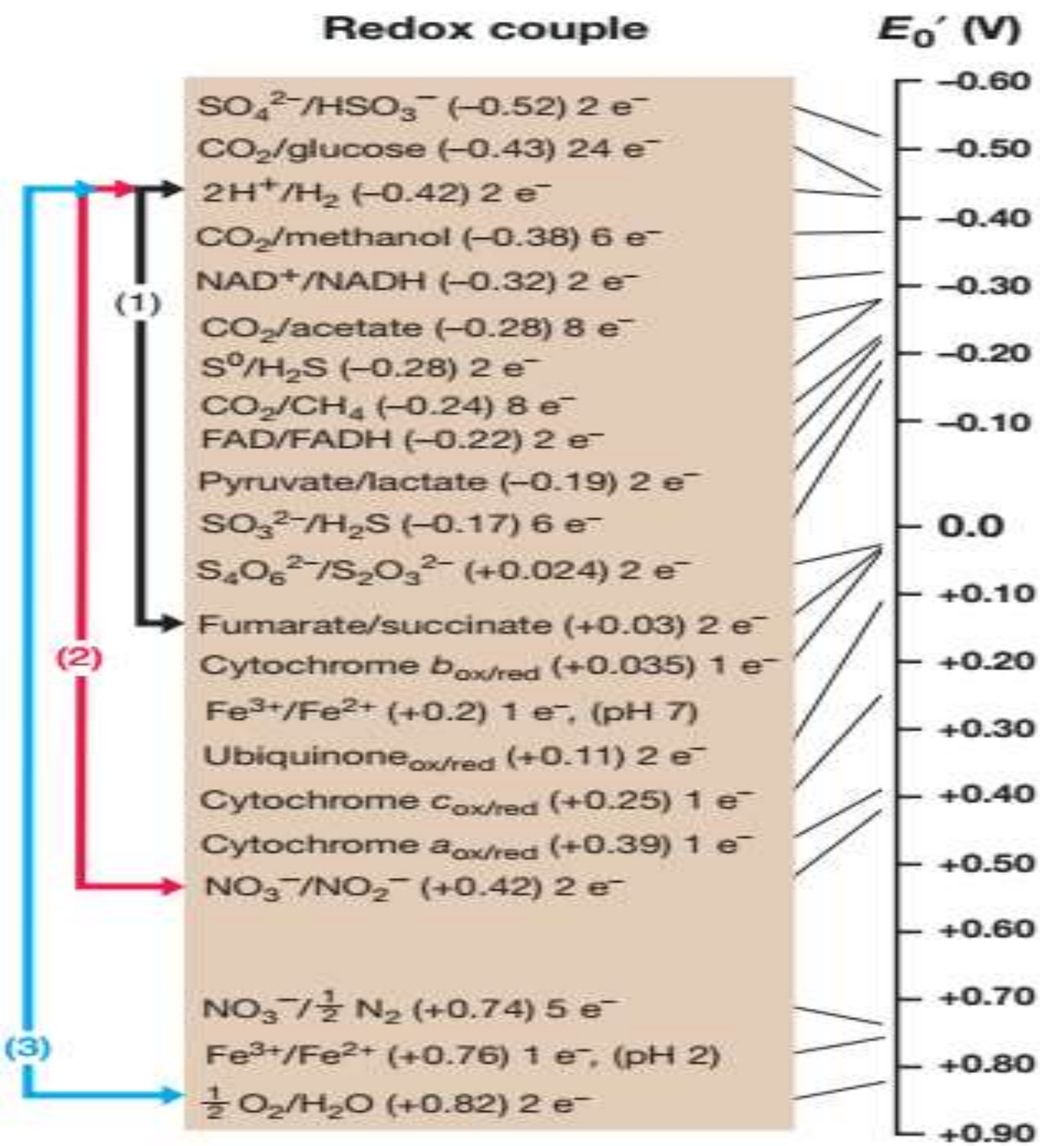

NADH + H+
NAD+
Aspartate
Oxaloacetate
Malate
α-Ketoglutarate
Glutamate
Cytosol
Matrix
α-Ketoglutarate
Glutamate
Aspartate
Oxaloacetate
Malate
NADH + H+
NAD+

Chemolithotrophy

- ✓ **Oxidation of Reduced inorganic molecule**
- ✓ **Mixotroph**
- ✓ **NAD(P)H synthesis and Calvin cycle**
- ✓ **Reverse electron flow**

Table 13.1 *Energy yields from the oxidation of various inorganic electron donors*[a]

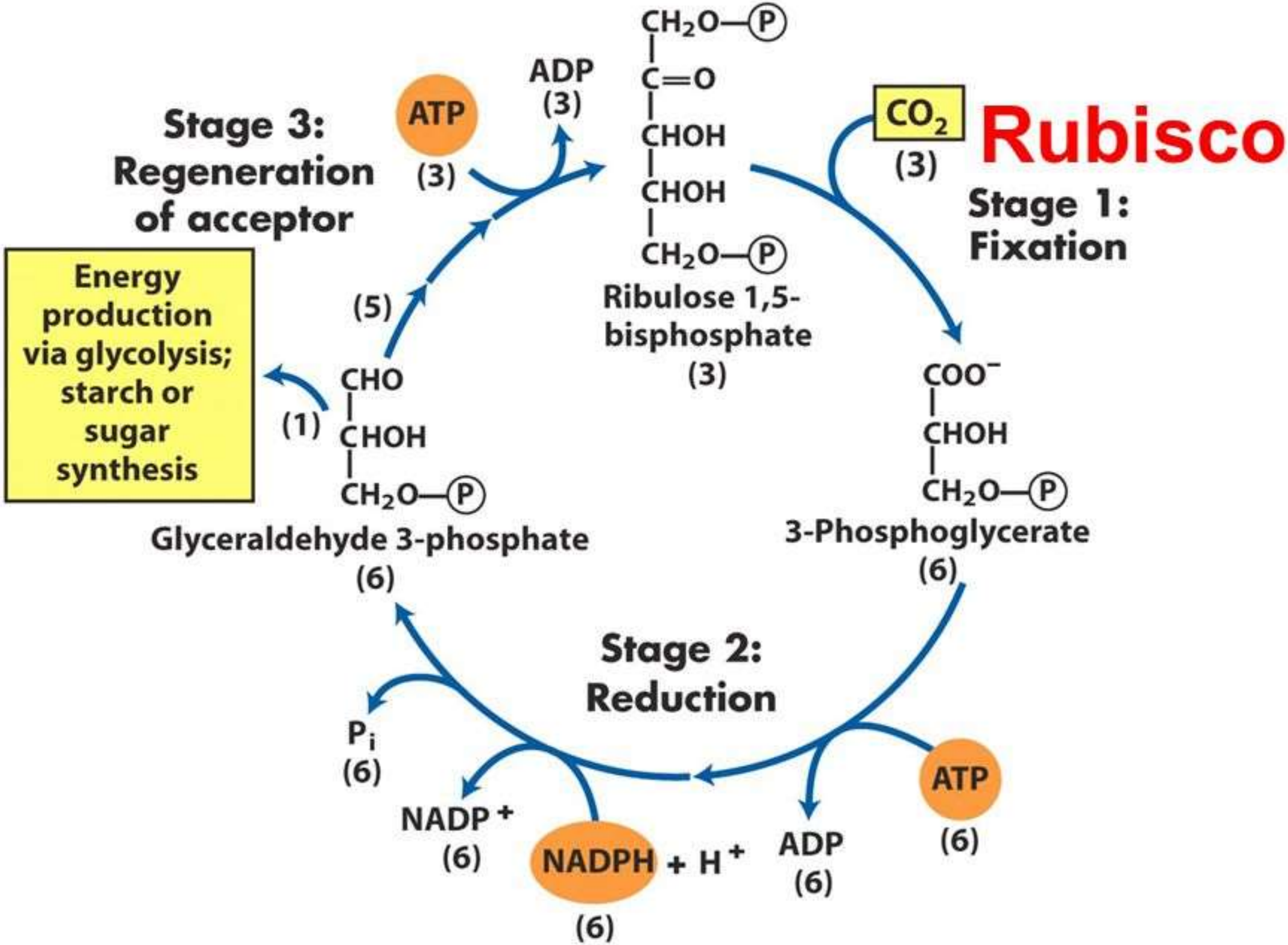
Electron donor	*Chemolithotrophic reaction*	*Group of chemolithotrophs*	*E_0' of couple (V)*	*$\Delta G^{0\prime}$ (kJ/reaction)*	*Number of electrons/reaction*	*$\Delta G^{0\prime}$ (kJ/2e$^-$)*
Phosphite[b]	$4\ HPO_3^{2-} + SO_4^{2-} + H^+ \rightarrow 4\ HPO_4^{2-} + HS^-$	Phosphite bacteria	−0.69	−91	2	−91
Hydrogen[b]	$H_2 + \frac{1}{2} O_2 \rightarrow H_2O$	Hydrogen bacteria	−0.42	−237.2	2	−237.2
Sulfide[b]	$HS^- + H^+ + \frac{1}{2} O_2 \rightarrow S^0 + H_2O$	Sulfur bacteria	−0.27	−209.4	2	−209.4
Sulfur[b]	$S^0 + 1\frac{1}{2} O_2 + H_2O \rightarrow SO_4^{2-} + 2\ H^+$	Sulfur bacteria	−0.20	−587.1	6	−195.7
Ammonium[c]	$NH_4^+ + 1\frac{1}{2} O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$	Nitrifying bacteria	+0.34	−274.7	6	−91.6
Nitrite[b]	$NO_2^- + \frac{1}{2} O_2 \rightarrow NO_3^-$	Nitrifying bacteria	+0.43	−74.1	2	−74.1
Ferrous iron[b]	$Fe^{2+} + H^+ + \frac{1}{4} O_2 \rightarrow Fe^{3+} + \frac{1}{2} H_2O$	Iron bacteria	+0.77	−32.9	1	−65.8

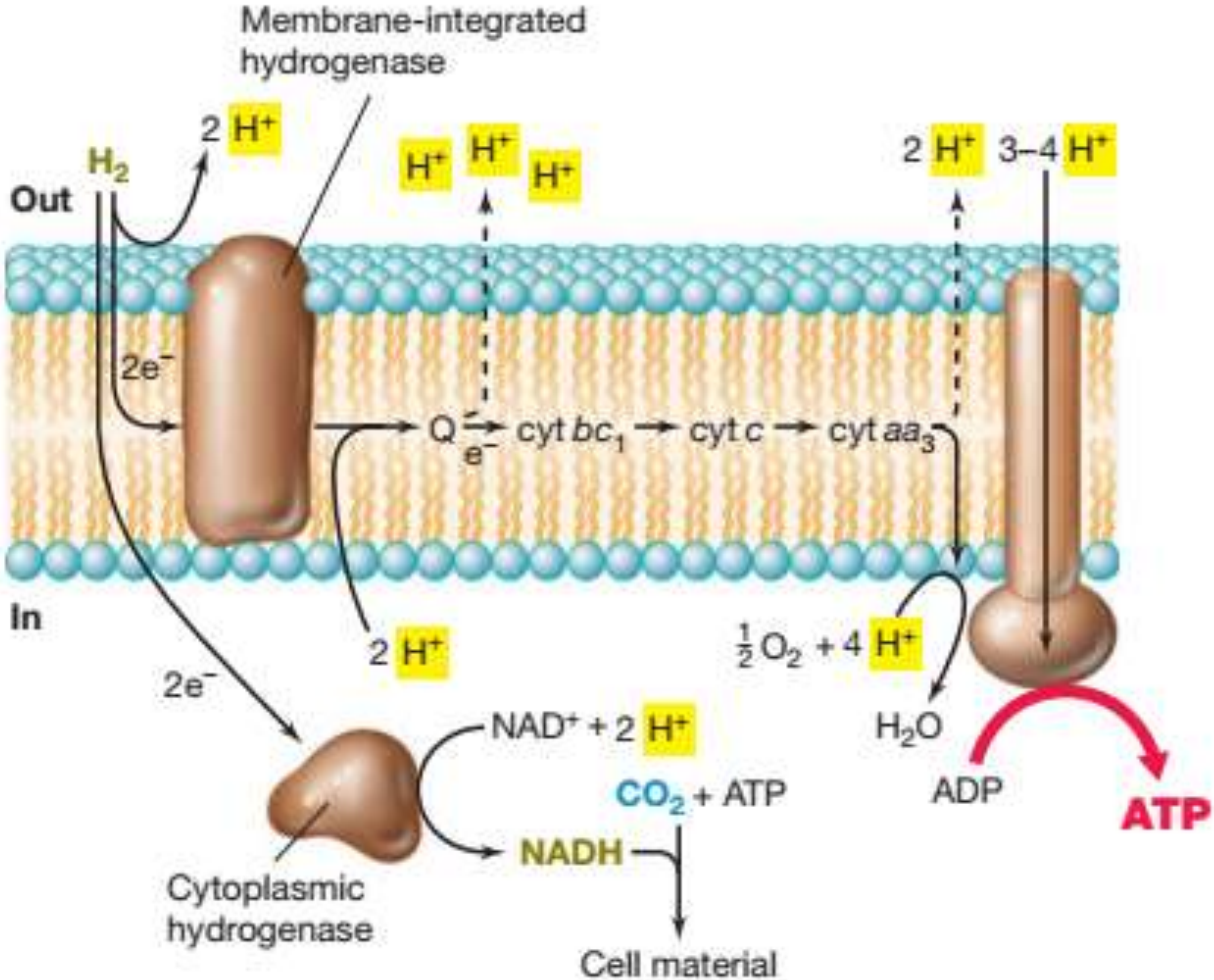


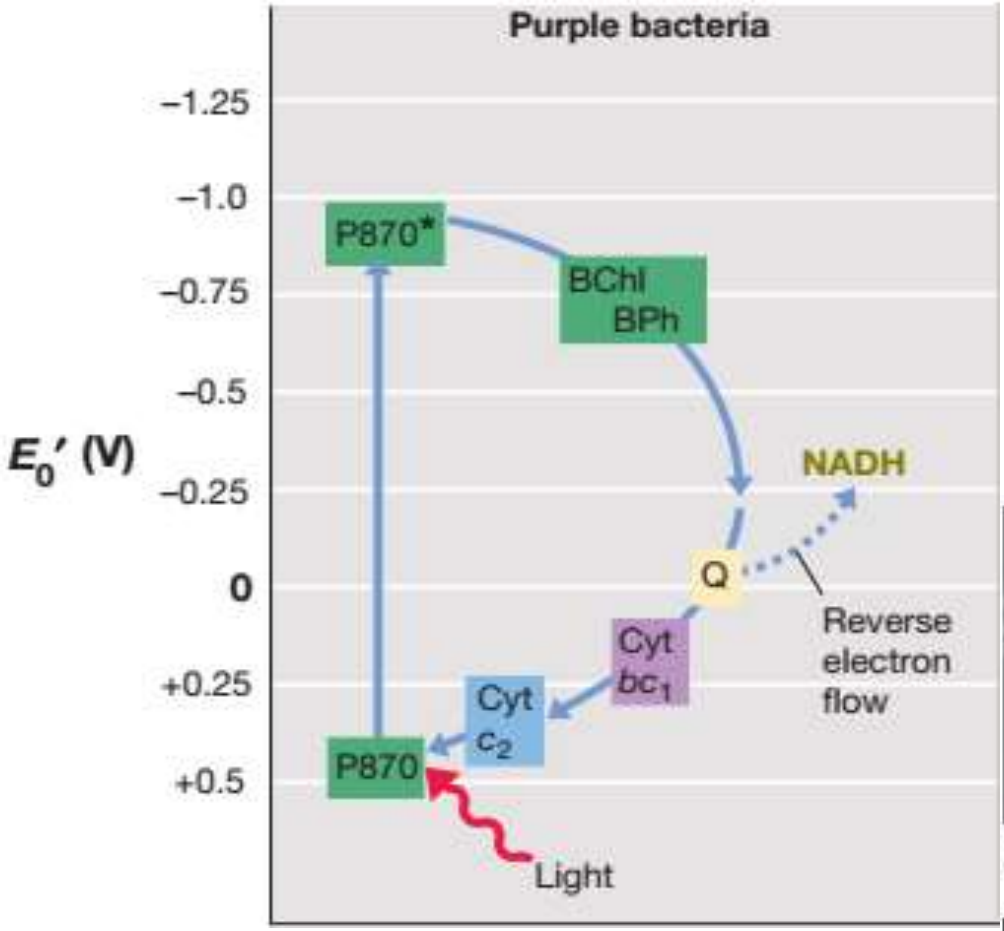
(1) H_2 + fumarate $\longrightarrow$ succinate $\quad \Delta G^{0\prime} = -86$ kJ

(2) $H_2 + NO_3^- \longrightarrow NO_2^- + H_2O \quad \Delta G^{0\prime} = -163$ kJ

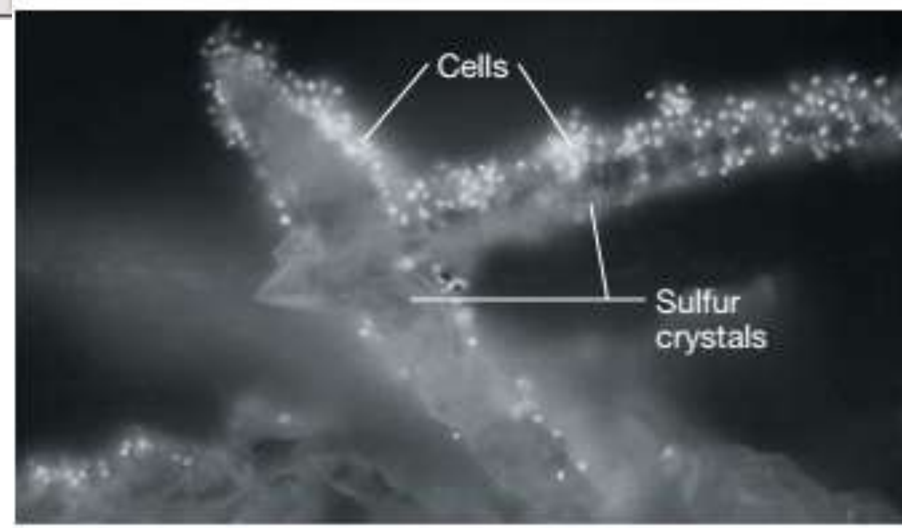
(3) $H_2 + \frac{1}{2} O_2 \longrightarrow H_2O \quad \Delta G^{0\prime} = -237$ kJ

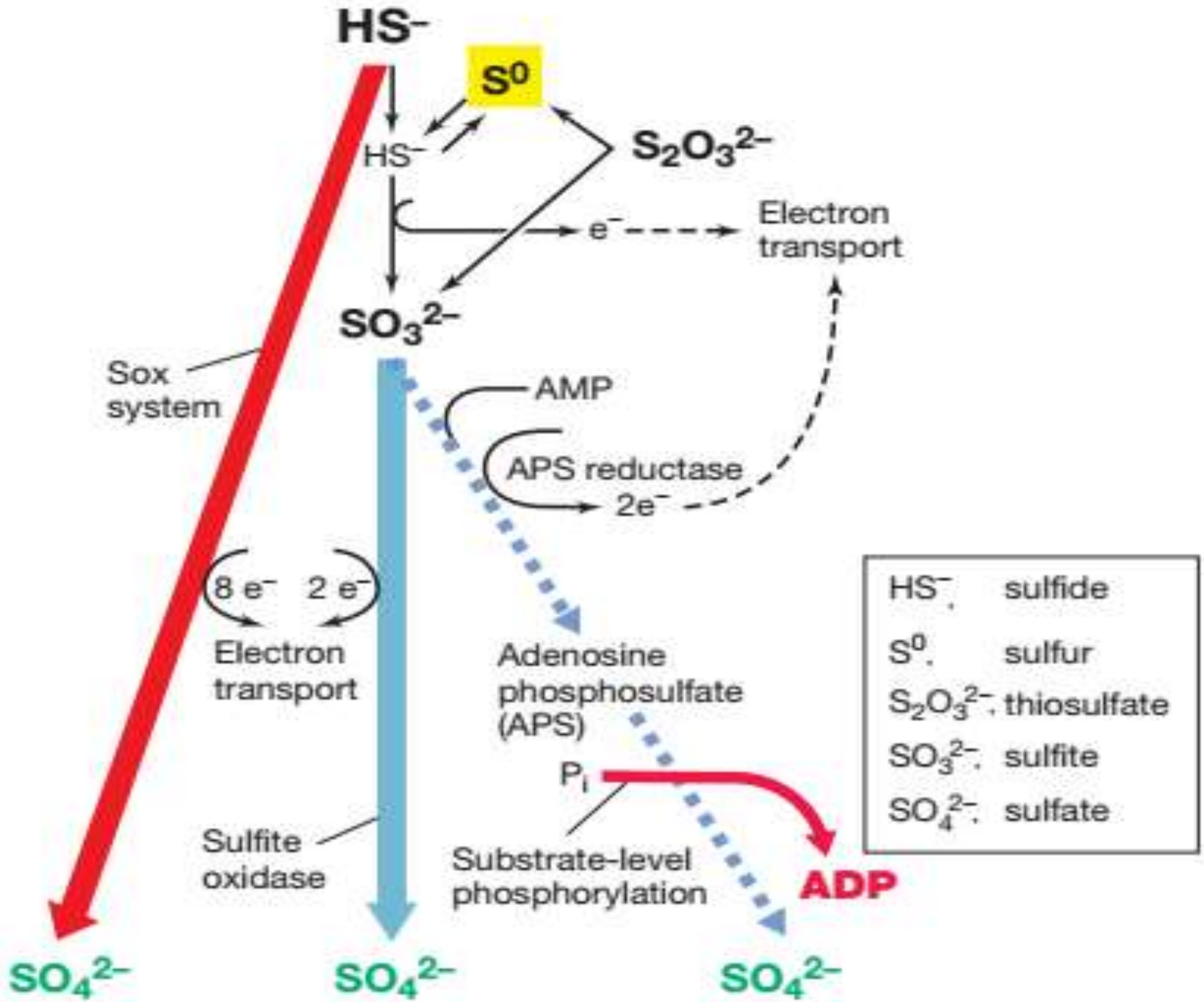

Rubisco
Stage 1:
Fixation
Stage 2:
Reduction
Stage 3:
Regeneration
of acceptor
CO_2
(3)
ATP
(3)
ADP
(3)
CH₂O—P
C=O
CHOH
CHOH
CH₂O—P
Ribulose 1,5-
bisphosphate
(3)
COO⁻
CHOH
CH₂O—P
3-Phosphoglycerate
(6)
ATP
(6)
ADP
(6)
NADPH + H⁺
(6)
NADP⁺
(6)
Pᵢ
(6)
CHO
CHOH
CH₂O—P
Glyceraldehyde 3-phosphate
(6)
(5)
(1)
Energy
production
via glycolysis;
starch or
sugar
synthesis


Membrane-integrated hydrogenase
Out
H_2
2 H^+
H^+ H^+ H^+
2 H^+ 3–4 H^+
2e⁻
Q
e⁻
cyt bc_1
cyt c
cyt aa_3
In
2 H^+
$\frac{1}{2}O_2 + 4\ H^+$
H_2O
2e⁻
NAD⁺ + 2 H^+
CO_2 + ATP
NADH
ADP
ATP
Cytoplasmic hydrogenase
Cell material

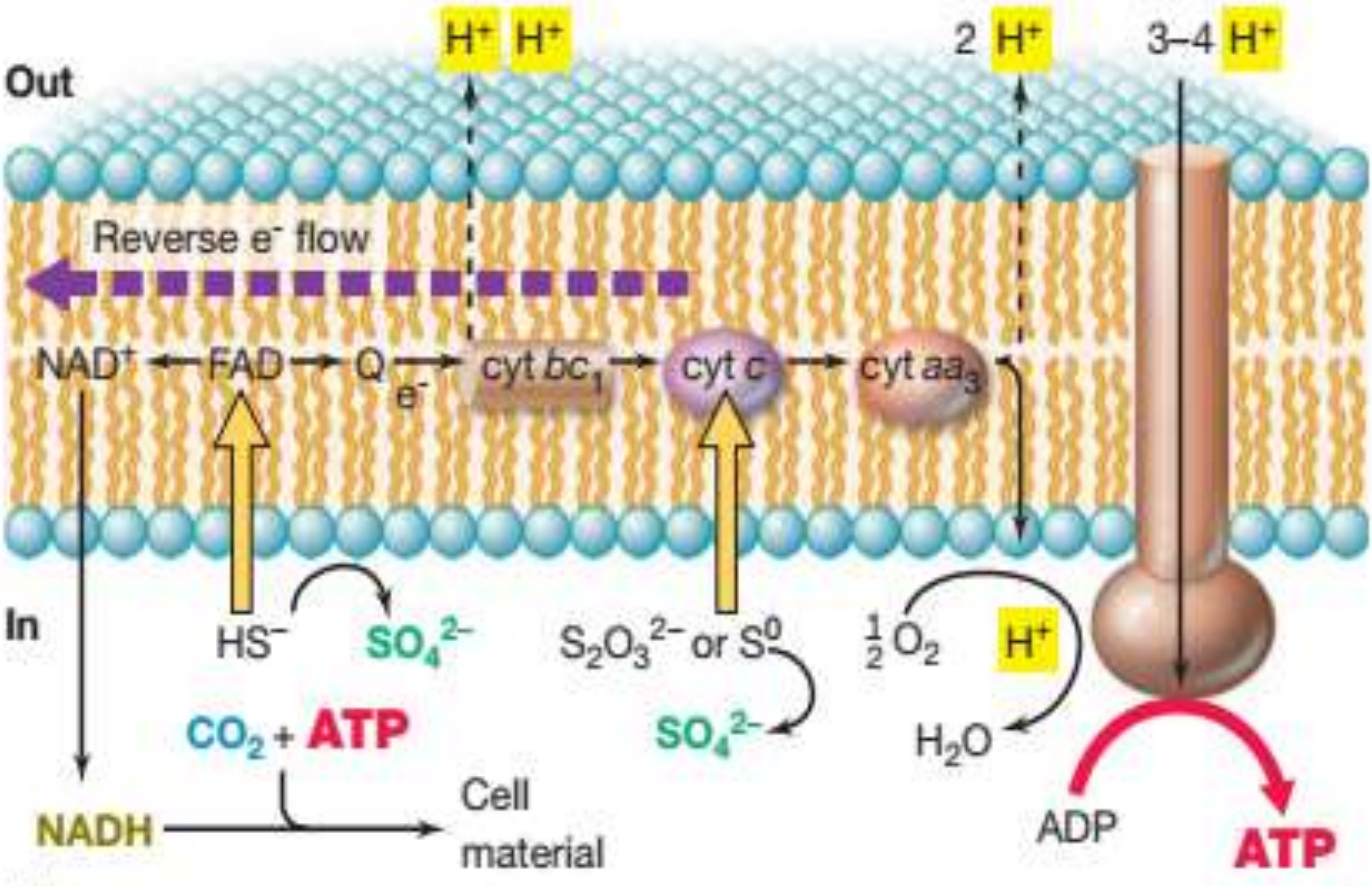

Purple bacteria
−1.25
−1.0
−0.75
−0.5
−0.25
0
+0.25
+0.5
E0′ (V)
P870*
BChl
BPh
NADH
Q
Reverse
electron
flow
Cyt
bc1
Cyt
c2
P870
Light

(a)


Cells
Sulfur
crystals

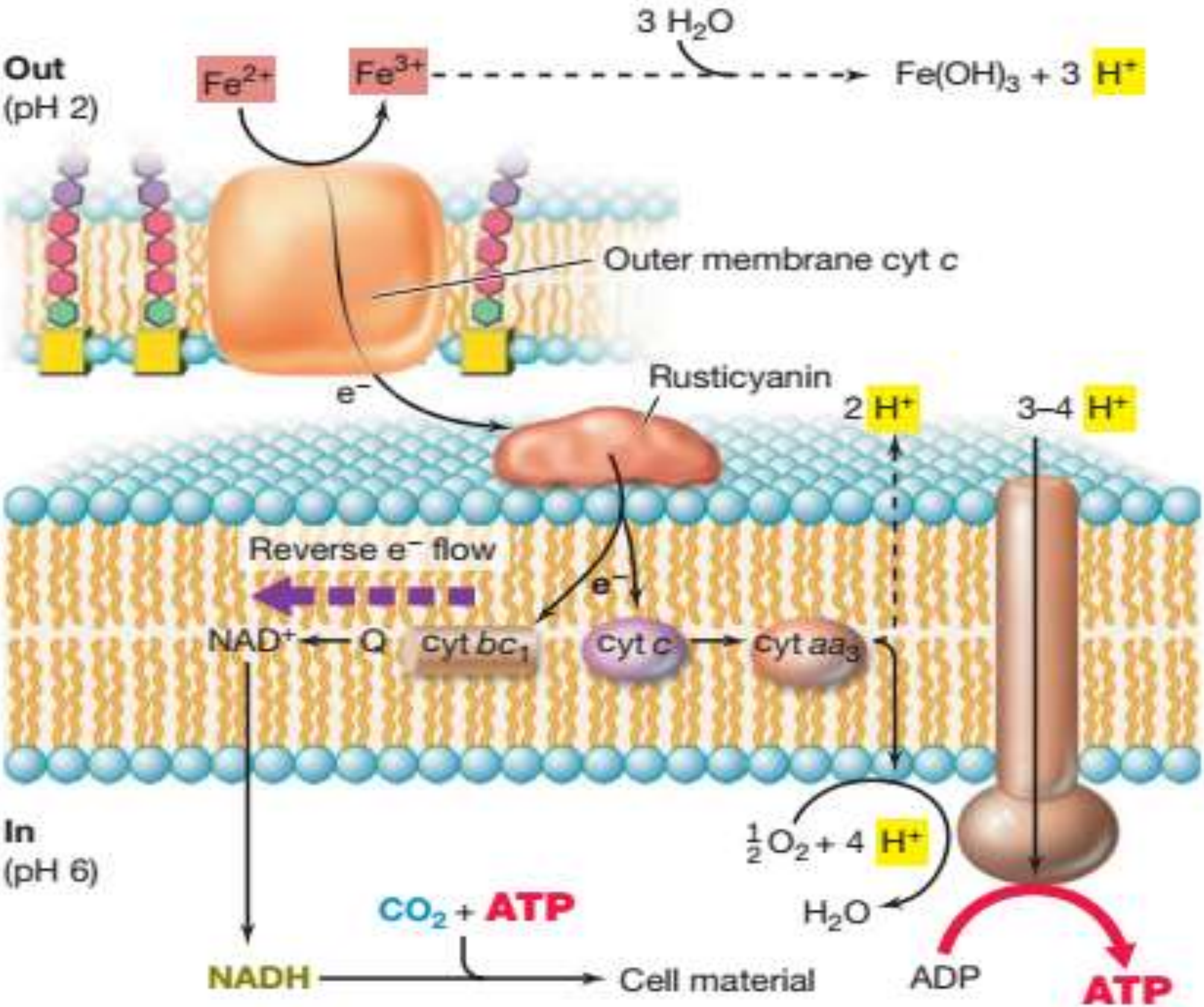


HS⁻
S⁰
HS⁻
S₂O₃²⁻
e⁻
Electron transport
SO₃²⁻
Sox system
AMP
APS reductase
2e⁻
8 e⁻
2 e⁻
Electron transport
Adenosine phosphosulfate (APS)
Pᵢ
Sulfite oxidase
Substrate-level phosphorylation
ADP
SO₄²⁻
SO₄²⁻
SO₄²⁻
HS⁻, sulfide
S⁰, sulfur
S₂O₃²⁻, thiosulfate
SO₃²⁻, sulfite
SO₄²⁻, sulfate

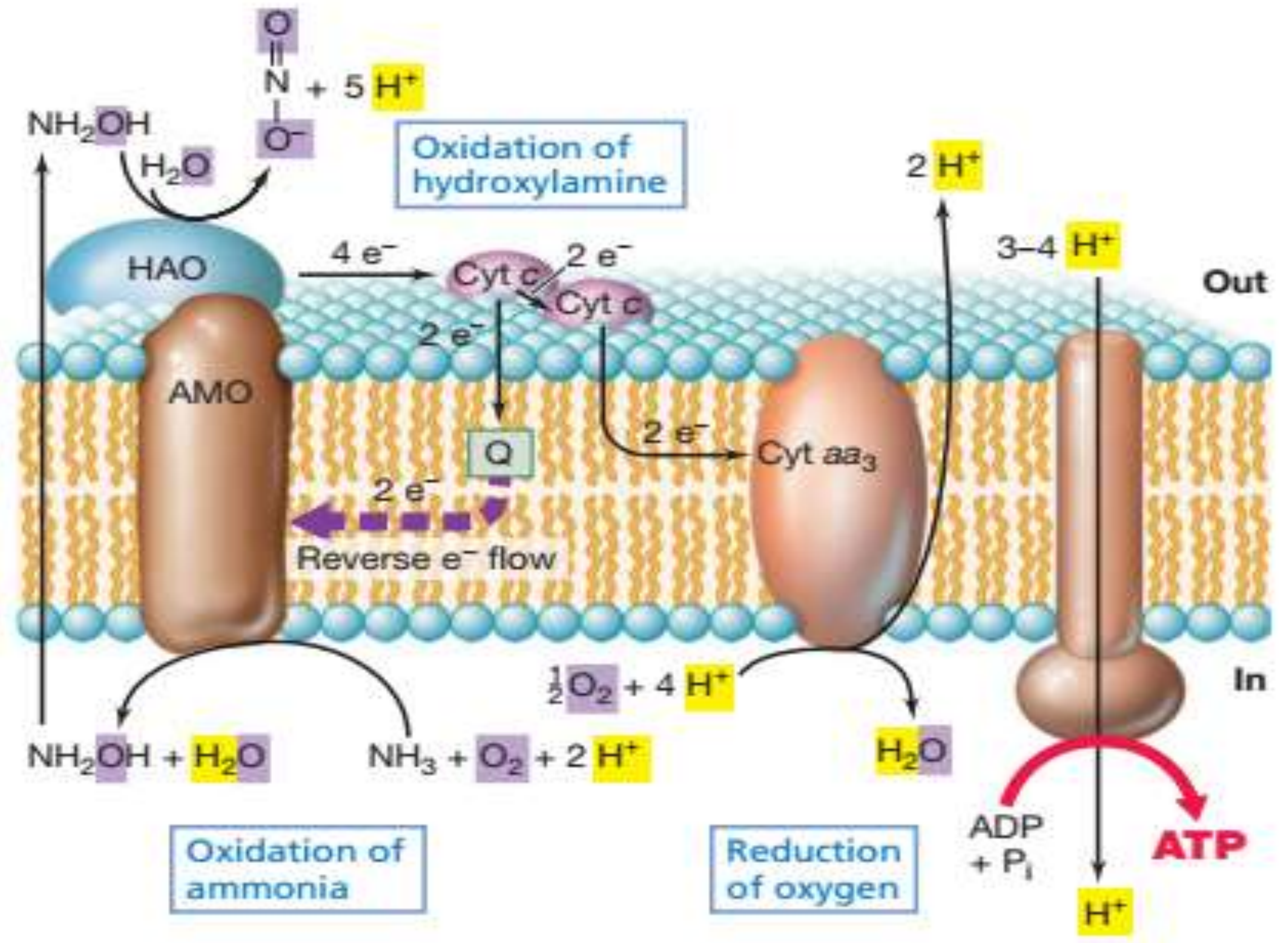


Out
H+ H+
2 H+
3–4 H+
Reverse e- flow
NAD+
FAD
Q
e-
cyt bc1
cyt c
cyt aa3
In
HS−
SO4 2−
S2O3 2− or S0
SO4 2−
1/2 O2
H+
H2O
CO2 + ATP
NADH
Cell material
ADP
ATP

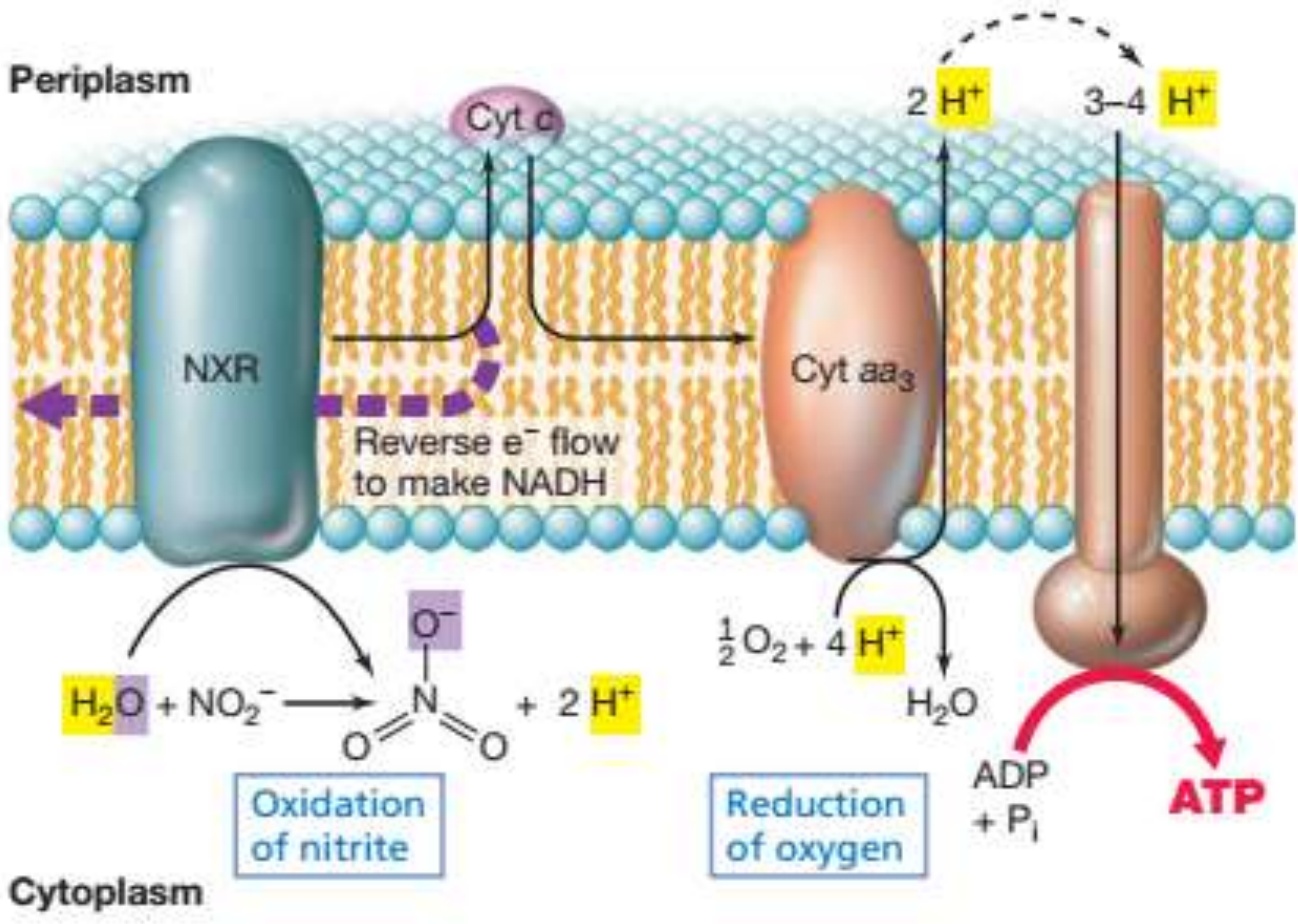
(b)



$3\ H_2O$
Out
(pH 2)
Fe^{2+}
Fe^{3+}
$Fe(OH)_3 + 3\ H^+$
Outer membrane cyt c
e^-
Rusticyanin
$2\ H^+$
3–4 H^+
Reverse e^- flow
e^-
NAD^+
Q
cyt bc_1
cyt c
cyt aa_3
In
(pH 6)
$\frac{1}{2}O_2 + 4\ H^+$
H_2O
CO_2 + ATP
NADH
Cell material
ADP
ATP



O
N + 5 H⁺
O⁻
NH₂OH
H₂O
Oxidation of hydroxylamine
2 H⁺
HAO
4 e⁻
Cyt c
2 e⁻
Cyt c
3–4 H⁺
Out
2 e⁻
AMO
2 e⁻
Q
Cyt aa₃
2 e⁻
Reverse e⁻ flow
½O₂ + 4 H⁺
In
NH₂OH + H₂O
NH₃ + O₂ + 2 H⁺
H₂O
ADP + Pᵢ
ATP
H⁺
Oxidation of ammonia
Reduction of oxygen


Periplasm
Cyt c
2 H+
3–4 H+
NXR
Reverse e⁻ flow to make NADH
Cyt aa3
½ O2 + 4 H+
H2O
H2O + NO2⁻ → O⁻ N O O + 2 H+
Oxidation of nitrite
Reduction of oxygen
ADP + Pi
ATP
Cytoplasm