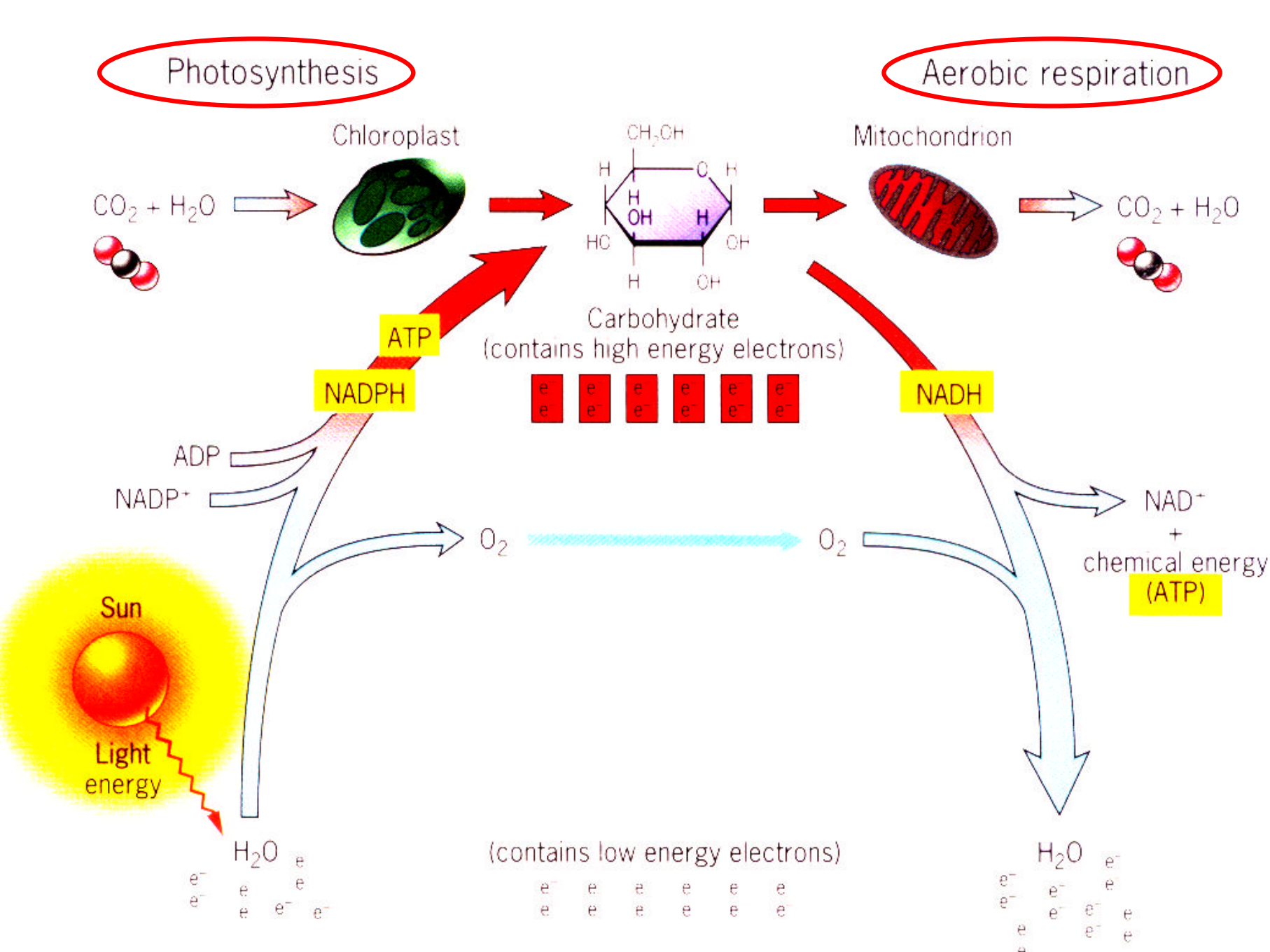
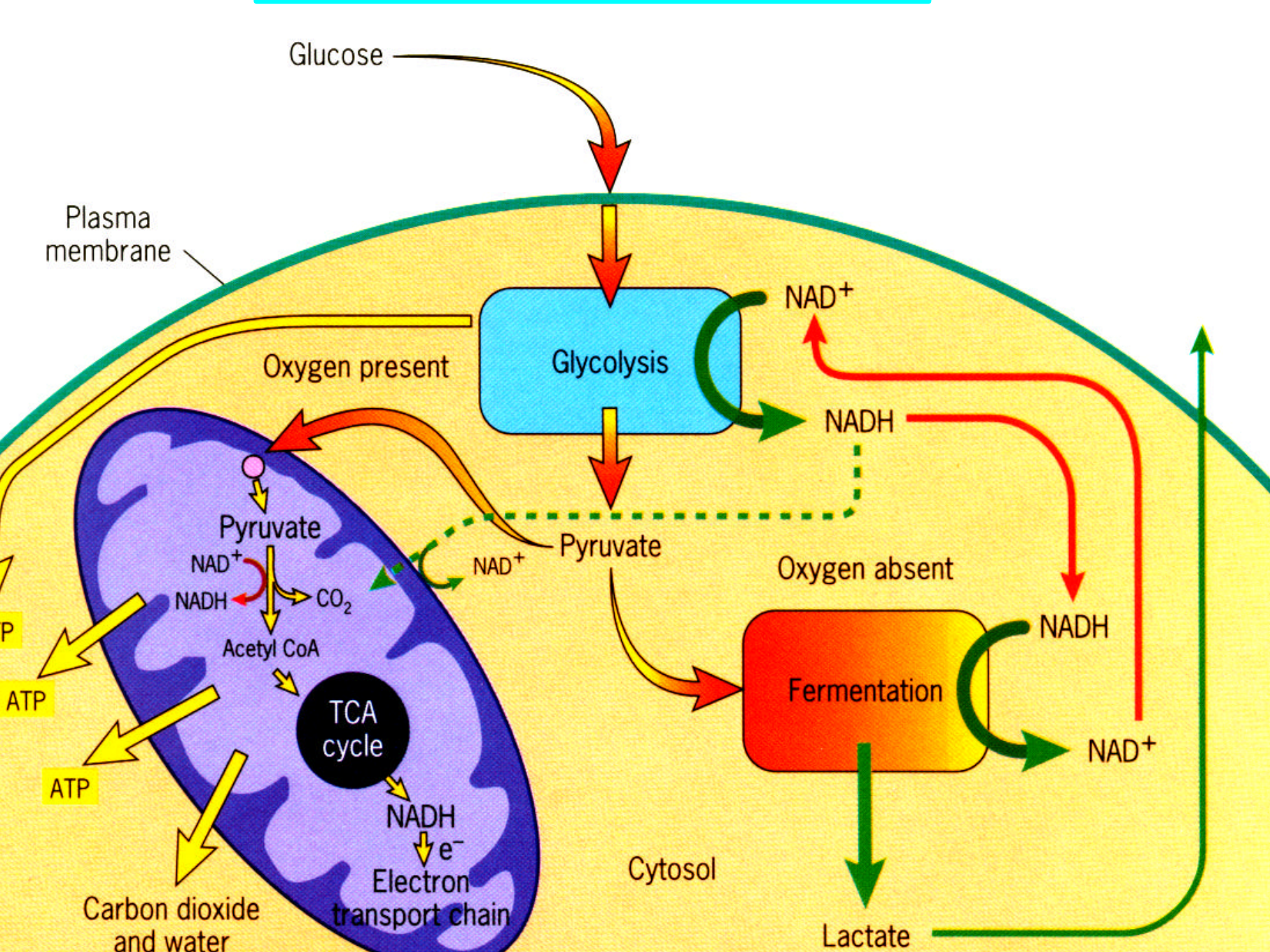
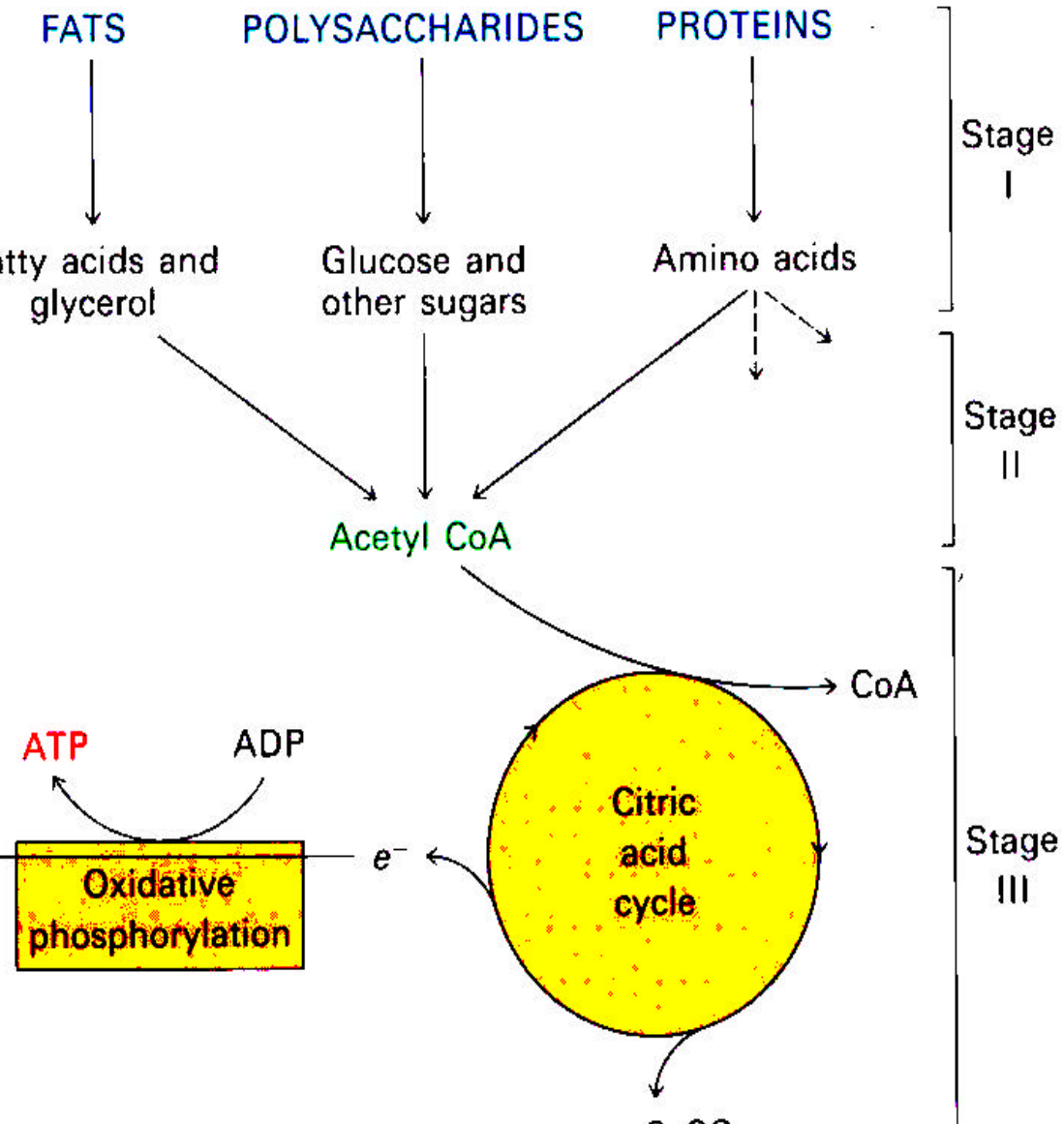
Chapter 3 Bioenergetics

Discussing about:

- What are the energy sources ?
- How is the energy conserved ?
- How does the energy converted to different forms ?
- How do chemical energy source metabolized and to what ?
- What are the components involved ?
- Where does the energy conversion take place ?
- What is the efficiency on each step of energy conversion


Photosynthesis
Aerobic respiration
Chloroplast
Mitochondrion
$CO_2 + H_2O$
$CO_2 + H_2O$
Carbohydrate
(contains high energy electrons)
ATP
NADPH
NADH
ADP
$NADP^+$
NAD^+
+
chemical energy
(ATP)
O_2
O_2
Sun
Light
energy
H_2O
(contains low energy electrons)
H_2O


Glucose
Plasma membrane
Oxygen present
Glycolysis
NAD+
NADH
Pyruvate
NAD+
NADH
CO2
Acetyl CoA
TCA cycle
ATP
ATP
NADH
e−
Electron transport chain
Carbon dioxide and water
Pyruvate
NAD+
Oxygen absent
Fermentation
NADH
NAD+
Cytosol
Lactate



Cytosol

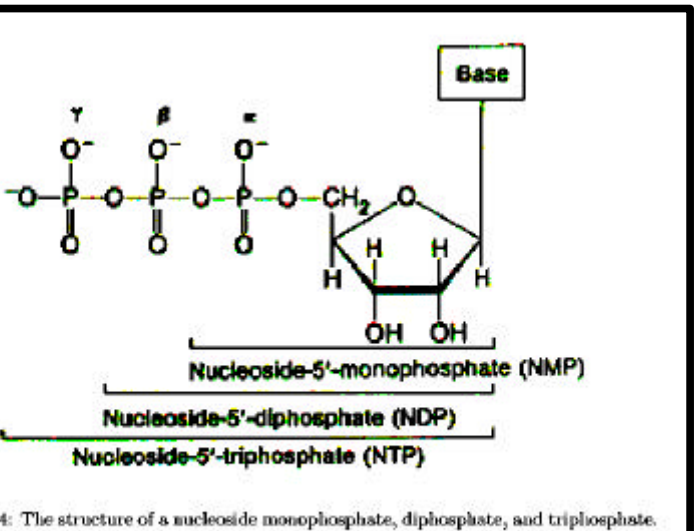
Glycolysis
Fermentation

In Mitochondria

1. Krebs Cycle - NADH production
2. Oxidative phophorylation
3. Electron transport
4. ATP synthesis

Figure 17-15
Stages in the extraction of e

ligh Energy Compounds



4: The structure of a nucleoside monophosphate, diphosphate, and triphosphate.

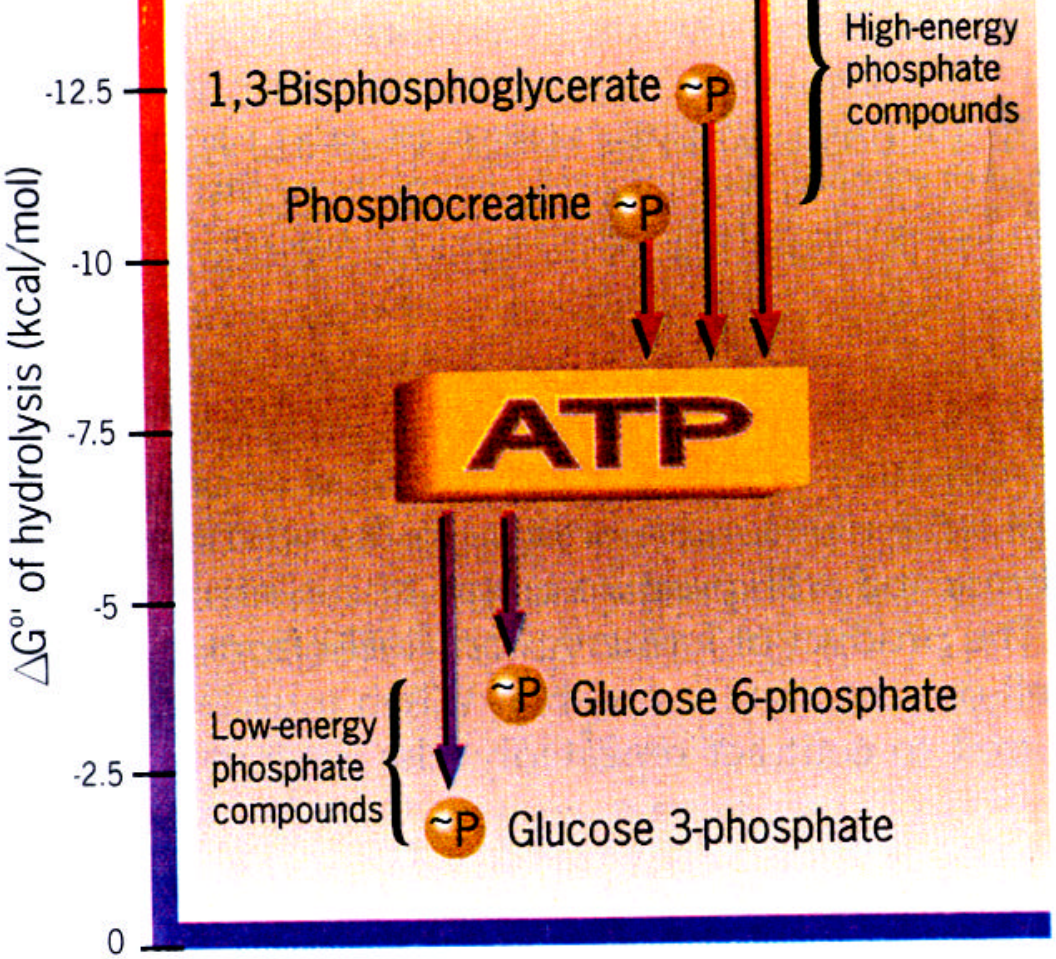
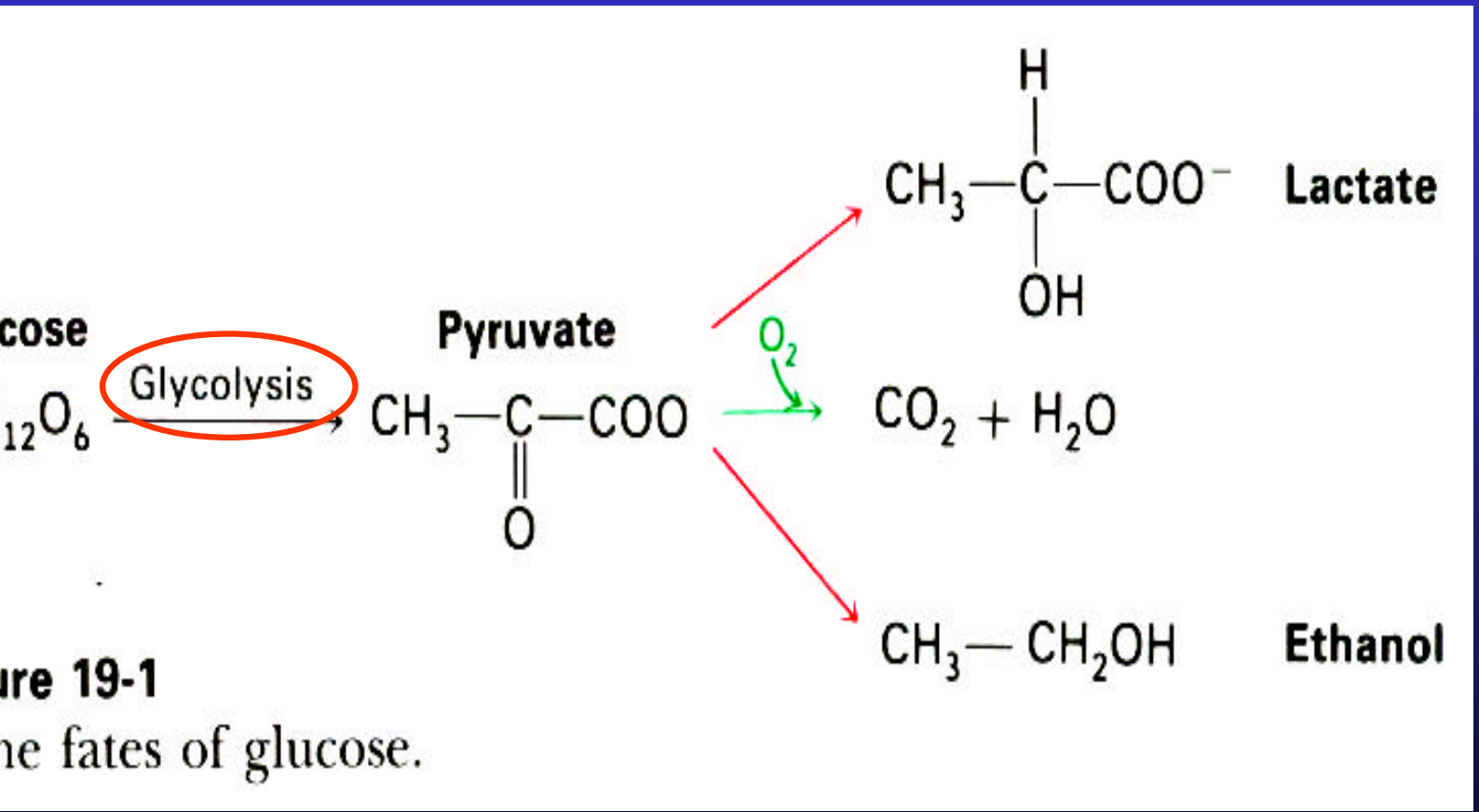


Figure 3.26 Ranking compounds by phosphate transfe potential. Those phosphate compounds higher on the scale (ones with a higher $\Delta G°'$ of hydrolysis) have a lower affinity for their phosphate group than those compounds that are lower on the scale. As a result, compounds higher on the scale readily transfer their phosphate group to form compounds that are lower on the scale. Thus, phosphate

Glycolysis

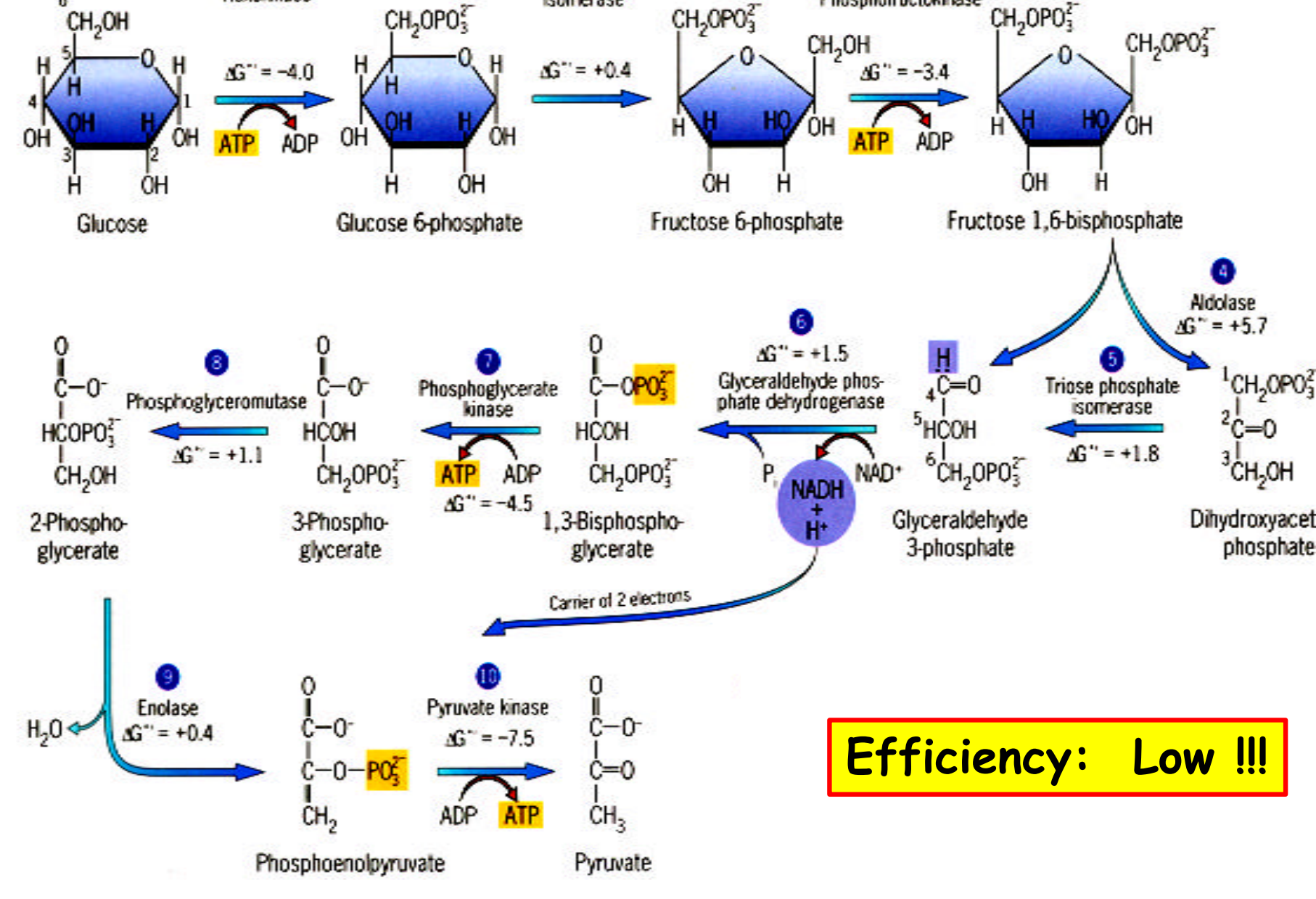


ure 19-1
ne fates of glucose.

Ferment

Aerobic oxidatio

Ferment



Efficiency: Low !!!

Glucose + 2 ADP + 2 NAD → 2 Pyruvate + 2 ATP + 2 NADH + 2 H^+ + 2 H_2O

Reaction	Enzyme	Type*	$\Delta G^{\circ\prime}$
Glucose + ATP $\longrightarrow$ glucose 6-phosphate + ADP + H^+	Hexokinase	a	−4.0
Glucose 6-phosphate $\rightleftharpoons$ fructose 6-phosphate	Phosphoglucose isomerase	c	+0.4
Fructose 6-phosphate + ATP $\longrightarrow$ fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	a	−3.4
Fructose 1,6-bisphosphate $\rightleftharpoons$ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	Aldolase	e	+5.7
Dihydroxyacetone phosphate $\rightleftharpoons$ glyceraldehyde 3-phosphate	Triose phosphate isomerase	c	+1.8
Glyceraldehyde 3-phosphate + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	f	+1.5
1,3-Bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	a	−4.5
3-Phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate	Phosphoglyceratmutase	b	+1.1
2-Phosphoglycerate $\rightleftharpoons$ phosphoenolpyruvate + H_2O	Enolase	d	+0.4
Phosphoenolpyruvate + ADP + H^+ $\longrightarrow$ pyruvate + ATP	Pyruvate kinase	a	−7.5

on type: (a) phosphoryl transfer; (b) phosphoryl shift; (c) isomerization; ydration; (e) aldol cleavage; (f) phosphorylation coupled to oxidation.

: $\Delta G^{\circ\prime}$ and ΔG are expressed in kcal/mol. ΔG, the actual free-energy change, has alculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiologic ons. Glycolysis can proceed only if the ΔG values of all reactions are negative. The

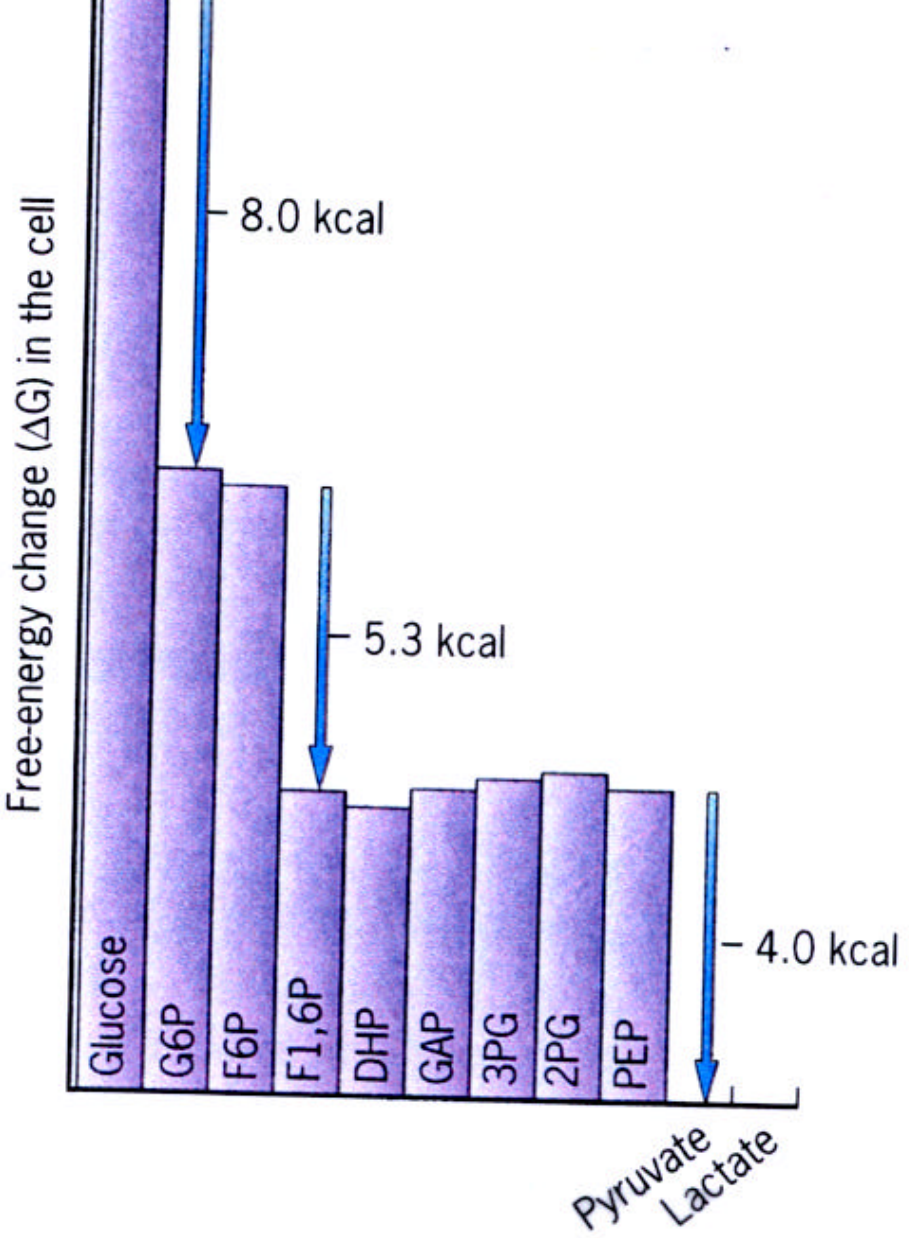


Figure 3.23 Free-energy profile of glycolysis in the hu-

$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2$ $\Delta G^{o'} = -686$ kcal/mol

$ADP + P_i \rightarrow ATP + H_2O$ $\Delta G^{o'} = +7.3$ kcal/mo

Efficiency = 2 x 7.3 / 686 = 0.021 = 2%

NADH

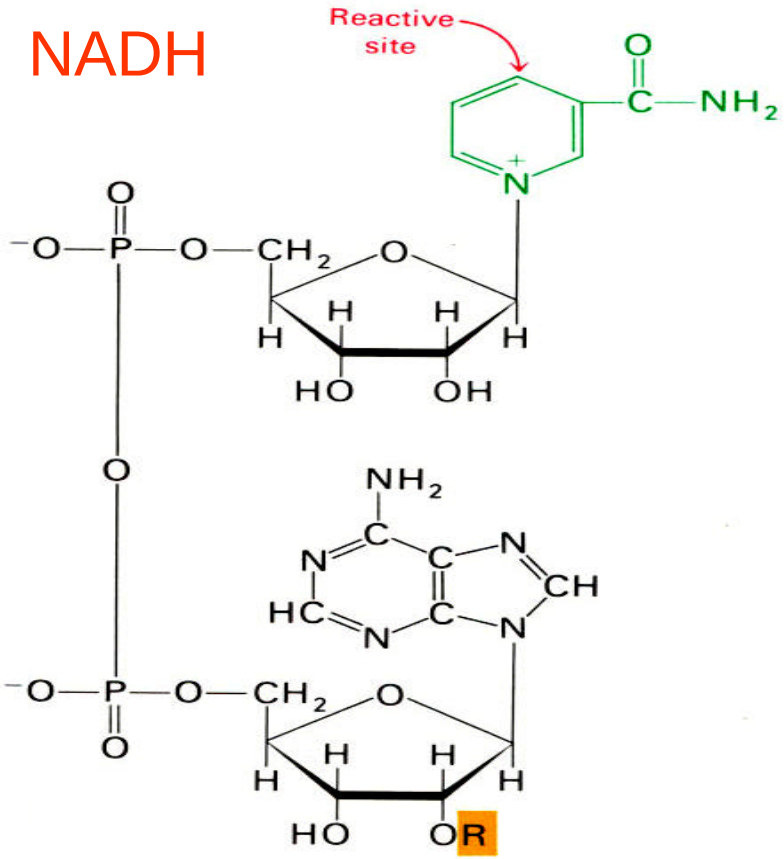


Figure 17-7
Structure of the oxidized form of nicotinamide adenine dinucleotide (NAD^+) and of nicotinamide adenine

$FADH_2$

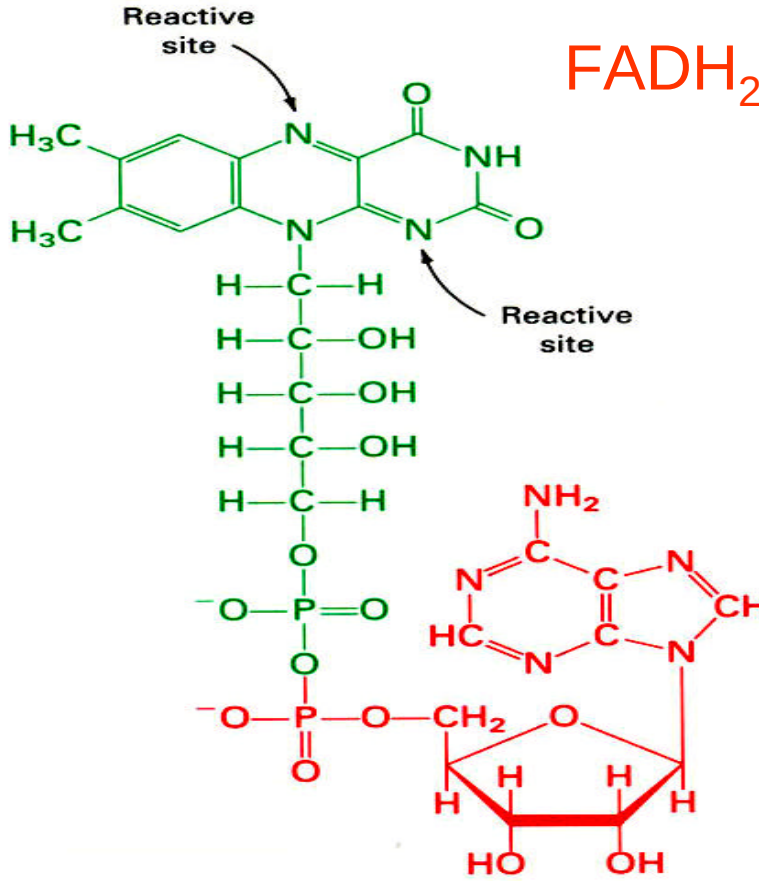


Figure 17-8
Structure of the oxidized form of fla-
vin adenine dinucleotide (FAD). Thi
electron carrier consists of a flavin

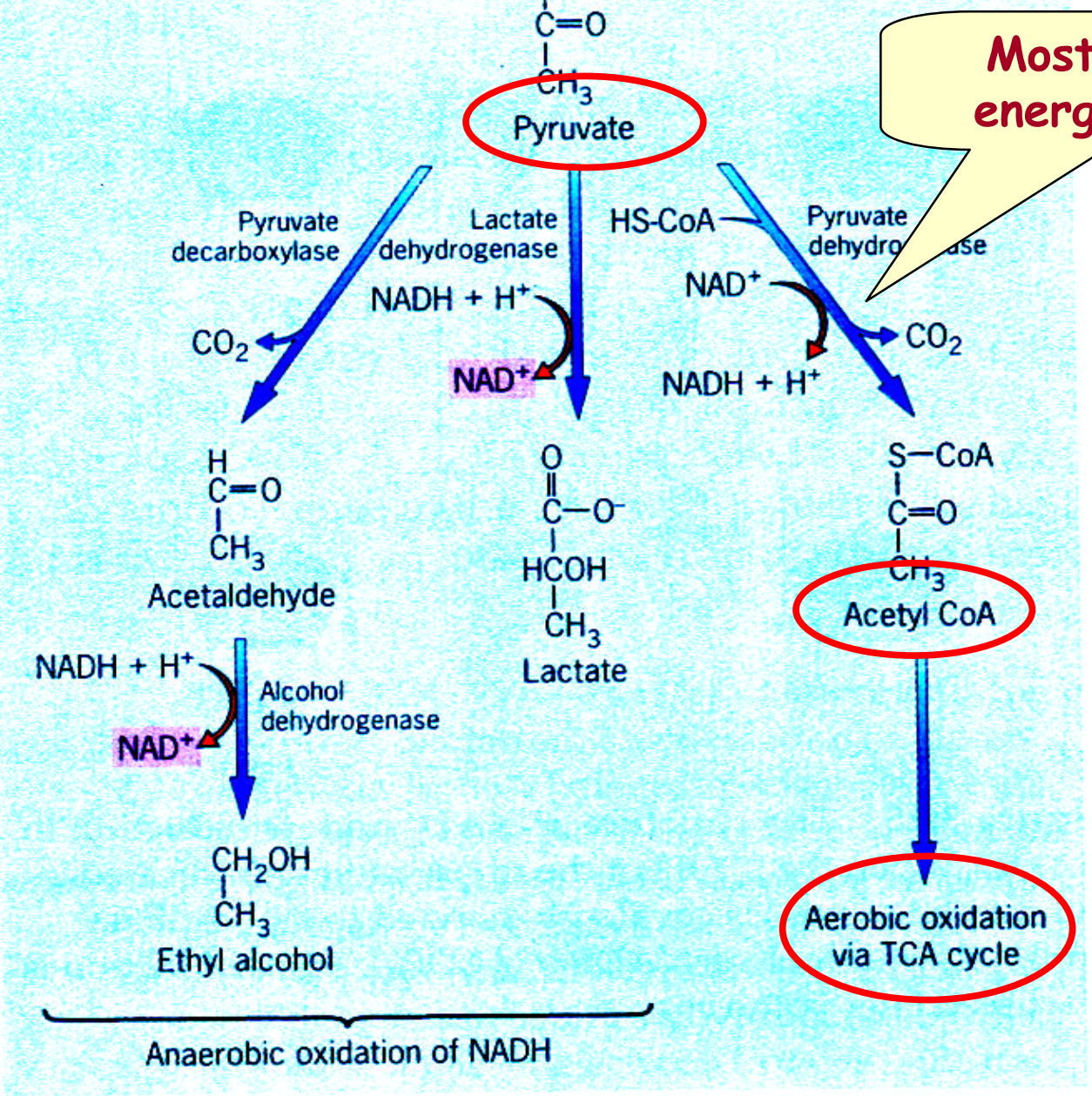


Figure 3.27 **Fermentation.** Most cells carry out aerobic

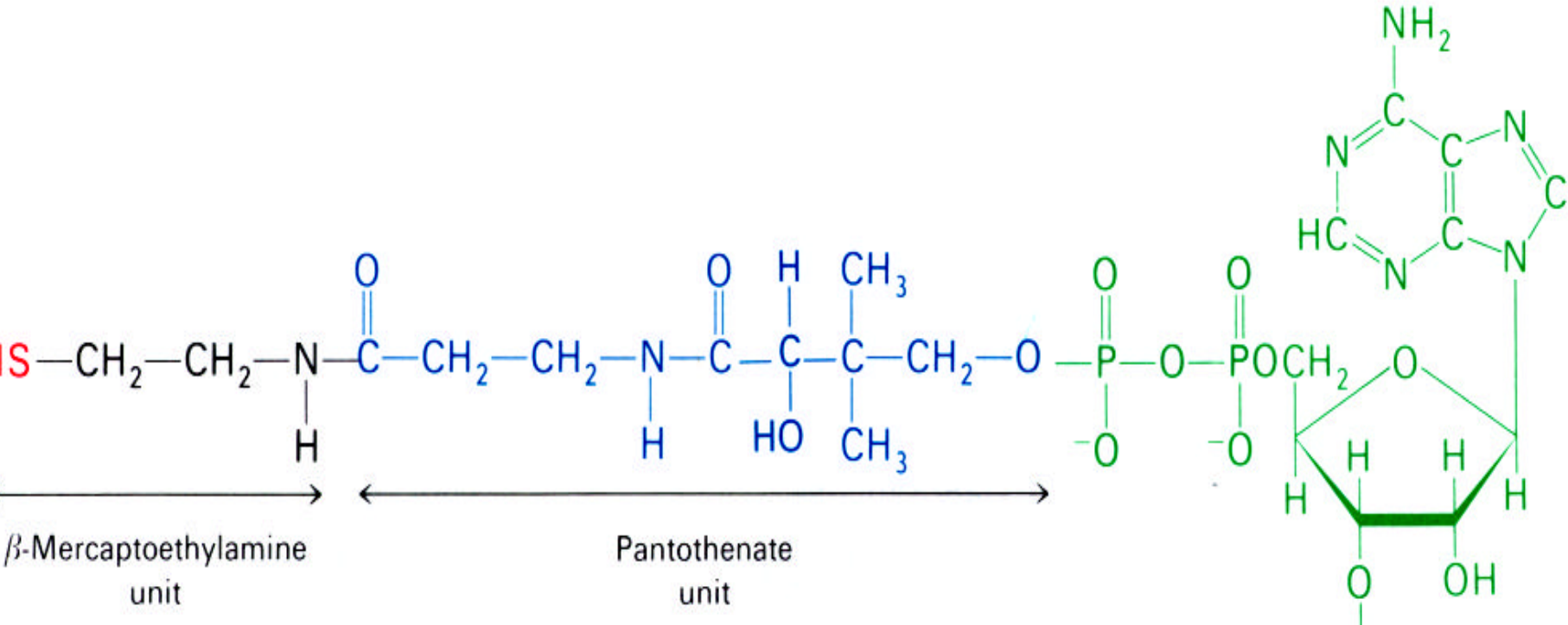


Figure 17-10
Structure of coenzyme A (CoA).

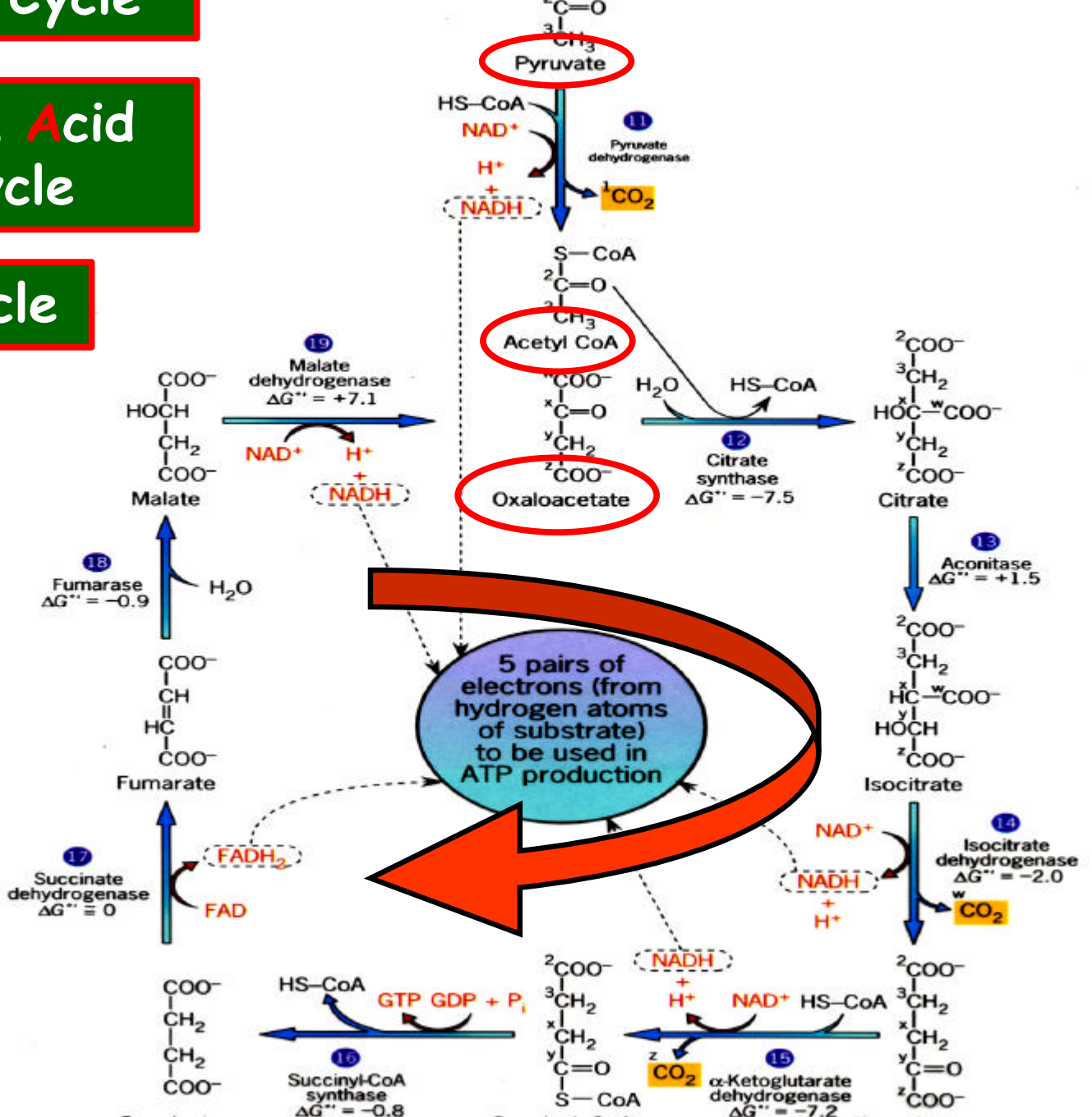
Acetyl group: CH_3 – CO -

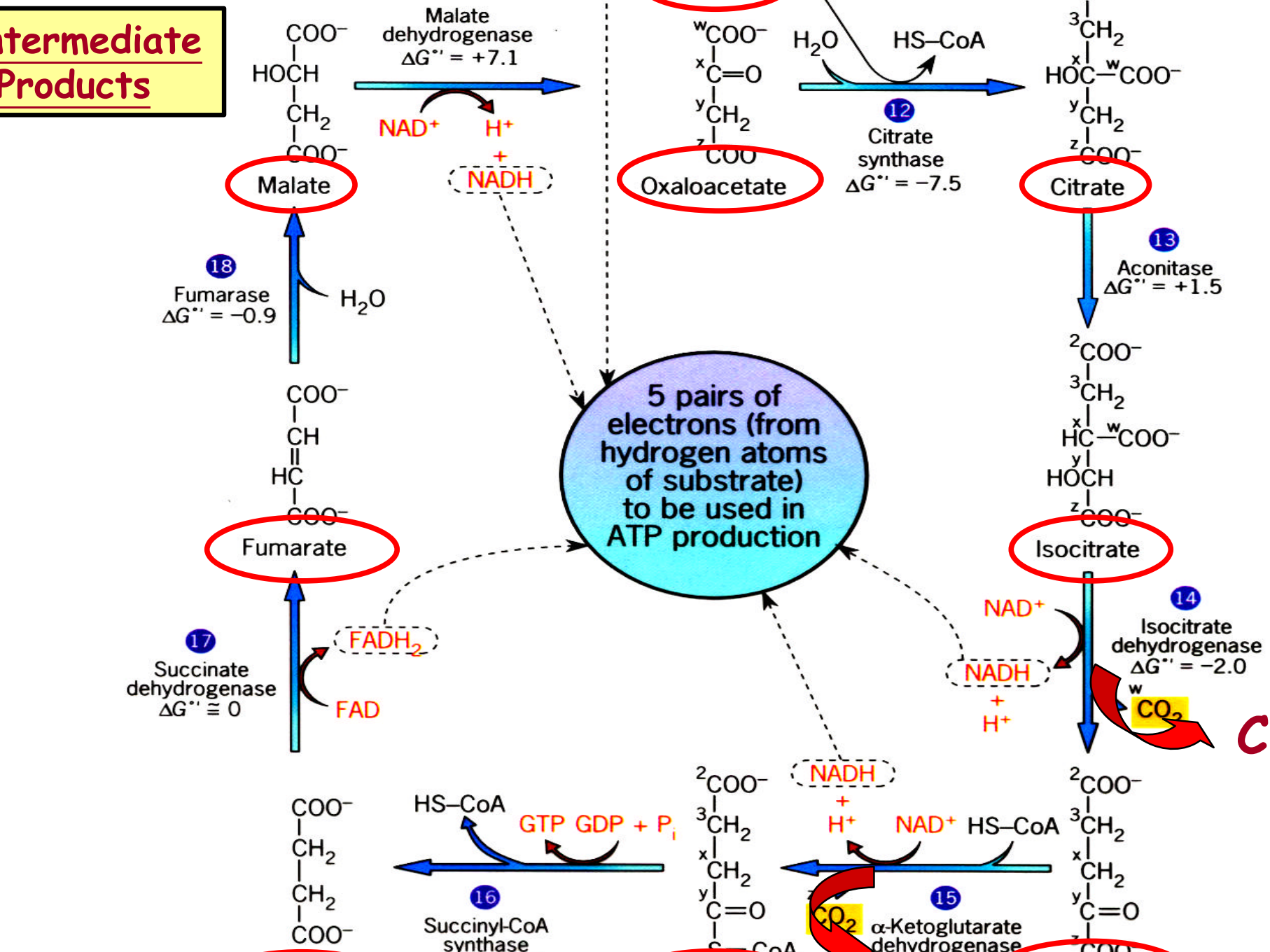
Acetyl CoA: CH_3 - CO – S – CoA (Universal acyl group carrier)

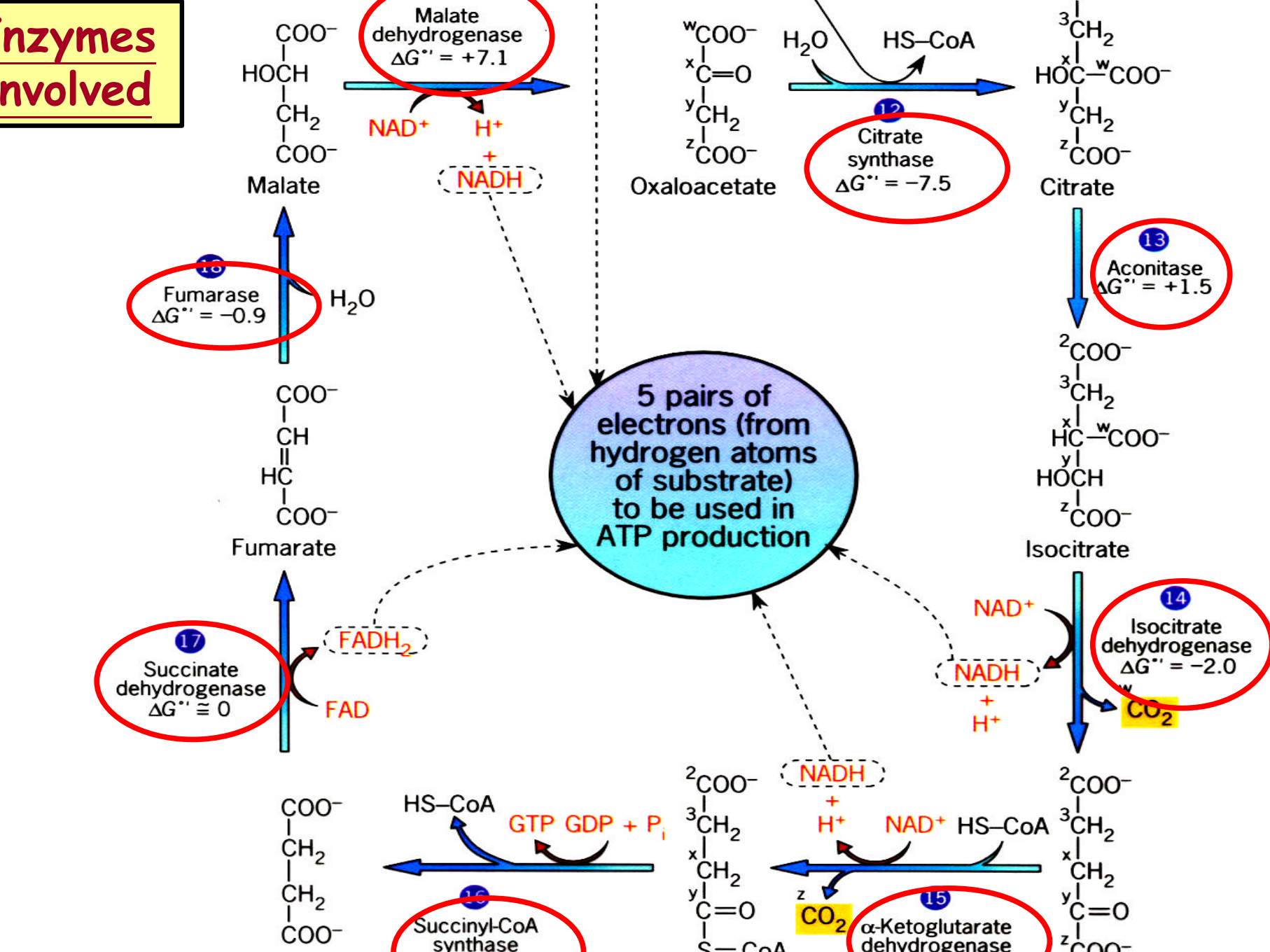
Citric Acid cycle

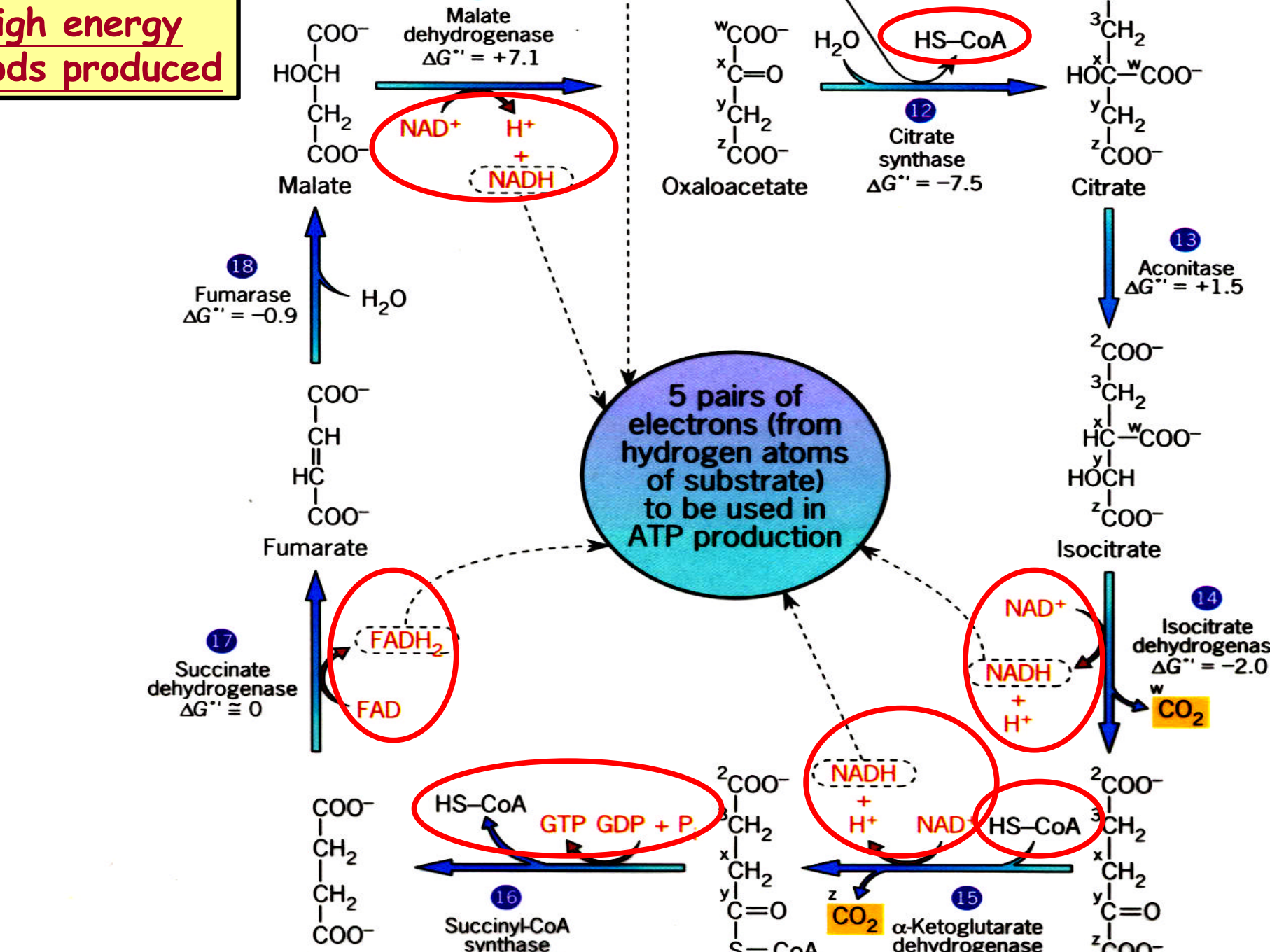
Tri-Carbon Acid (TCA) cycle

Krebs cycle




termediate
Products
Malate dehydrogenase
ΔG°' = +7.1
NAD+
H+
+
NADH
Malate
Oxaloacetate
H2O
HS–CoA
12
Citrate synthase
ΔG°' = −7.5
Citrate
13
Aconitase
ΔG°' = +1.5
Isocitrate
18
Fumarase
ΔG°' = −0.9
H2O
Fumarate
5 pairs of electrons (from hydrogen atoms of substrate) to be used in ATP production
17
Succinate dehydrogenase
ΔG°' ≅ 0
FADH2
FAD
NAD+
NADH
+
H+
14
Isocitrate dehydrogenase
ΔG°' = −2.0
CO2
C
HS–CoA
GTP GDP + Pi
16
Succinyl-CoA synthase
NADH
+
H+
NAD+
HS–CoA
15
CO2
α-Ketoglutarate dehydrogenase


nzymes
nvolved
Malate dehydrogenase
ΔG°' = +7.1
NAD+
H+
+
NADH
Malate
Oxaloacetate
H2O
HS–CoA
12
Citrate synthase
ΔG°' = −7.5
Citrate
13
Aconitase
ΔG°' = +1.5
Isocitrate
18
Fumarase
ΔG°' = −0.9
H2O
Fumarate
5 pairs of electrons (from hydrogen atoms of substrate) to be used in ATP production
17
Succinate dehydrogenase
ΔG°' ≅ 0
FADH2
FAD
NAD+
NADH
+
H+
14
Isocitrate dehydrogenase
ΔG°' = −2.0
CO2
NADH
+
H+
NAD+
HS–CoA
CO2
15
α-Ketoglutarate dehydrogenase
HS–CoA
GTP
GDP + Pi
Succinyl-CoA synthase


igh energy
ods produced
Malate dehydrogenase
ΔG°' = +7.1
NAD+
H+
+
NADH
Malate
Oxaloacetate
H2O
HS–CoA
12
Citrate synthase
ΔG°' = –7.5
Citrate
13
Aconitase
ΔG°' = +1.5
Isocitrate
18
Fumarase
ΔG°' = –0.9
H2O
Fumarate
5 pairs of electrons (from hydrogen atoms of substrate) to be used in ATP production
17
Succinate dehydrogenase
ΔG°' ≅ 0
FADH2
FAD
NAD+
NADH
+
H+
14
Isocitrate dehydrogenase
ΔG°' = –2.0
CO2
HS–CoA
GTP GDP + Pi
16
Succinyl-CoA synthase
NADH
+
H+
NAD+
HS–CoA
CO2
15
α-Ketoglutarate dehydrogenase

Net reaction of the TCA cycle

$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + GDP + P_i + 2H_2O \rightarrow$$

$$2CO_2 + 3NADH + FADH_2 + GTP + 2H^+ + CoA$$

- It remove two carbon (CH3-CO-) every cycle to generate two CO_2.
- The intermediate compounds are not affected.

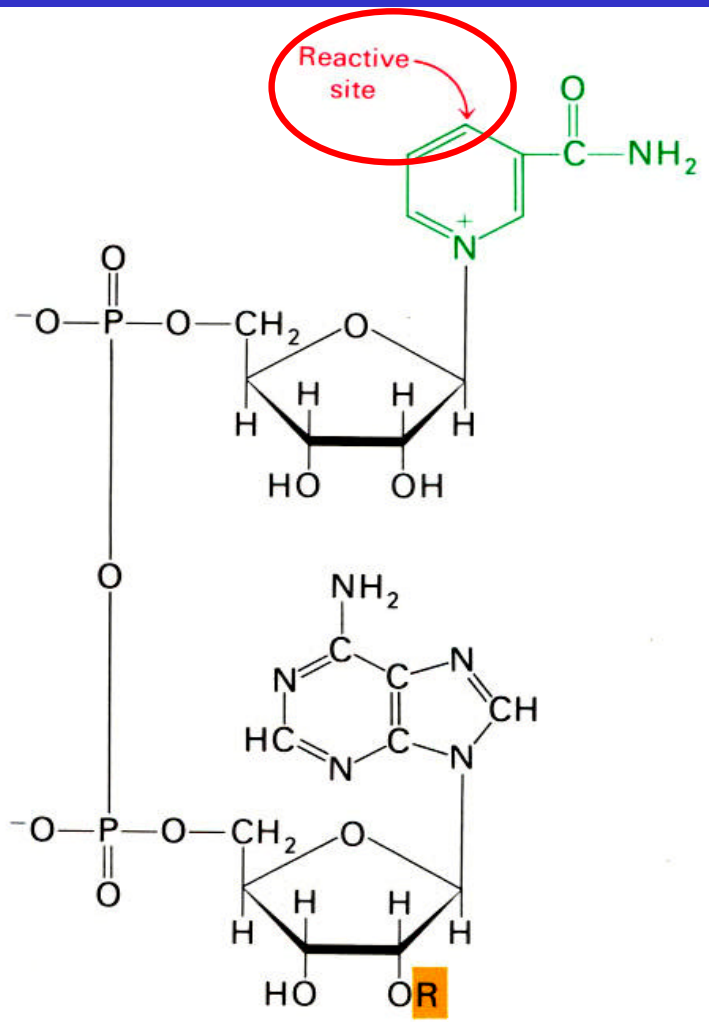


Figure 17-7
Structure of the oxidized form of nicotinamide adenine dinucleotide (NAD^+) and of nicotinamide adenine dinucleotide phosphate ($NADP^+$). In NAD^+, R = H; in $NADP^+$, R = PO_3^{2-}.

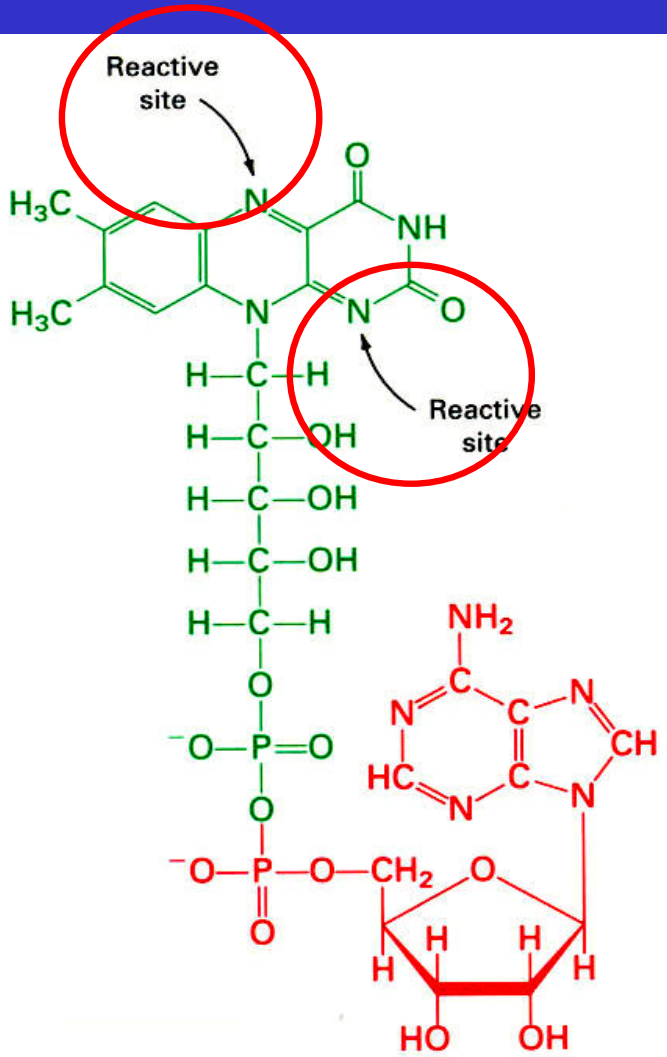
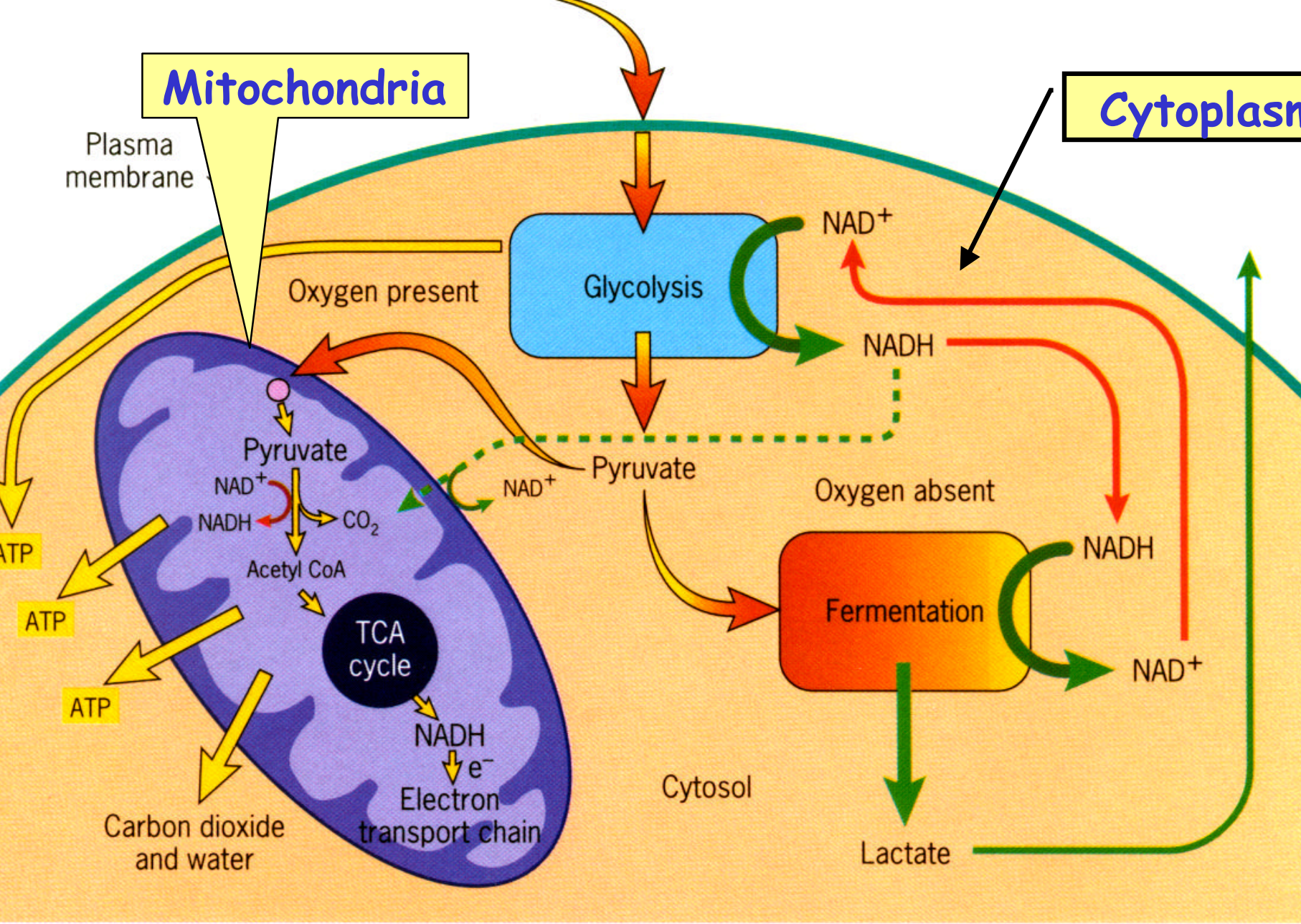


Figure 17-8
Structure of the oxidized form of flavin adenine dinucleotide (FAD). This electron carrier consists of a flavin mononucleotide (FMN) unit (shown in green) and an AMP unit (shown in


Mitochondria
Cytoplasm
Plasma membrane
Oxygen present
Glycolysis
NAD+
NADH
Pyruvate
NAD+
NADH
CO2
Acetyl CoA
TCA cycle
NADH
e−
Electron transport chain
ATP
ATP
ATP
Carbon dioxide and water
Pyruvate
NAD+
Oxygen absent
Fermentation
NADH
NAD+
Cytosol
Lactate

Utilization of the Coenzymes to generate ATP

1. High energy electrons are passed from $FADH_2$ or NADH to the first of a series of electron carriers, the Electron transport chain, with the concomitant generation of proton gradient across the inner mitochondrial membrane.

2. The controlled movement of protons back across the membrane through the ATP-synthesizing enzyme provides the energy required to phosphorylate ADP to ATP – Proton motive force, Mitchell's chemiosmotic theory.

$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + GDP + P_i + 2H_2O \rightarrow 2CO_2 + 3NADH + FADH_2 + GTP + 2H^+ + CoA$$

cetyl-CoA enters Krebs Cycle to generate NADH and $FADH_2$ which are used to ump H^+ outside mitochondria to create pH gradient which drives ATP synthesis nd exports to outside mitochondria.

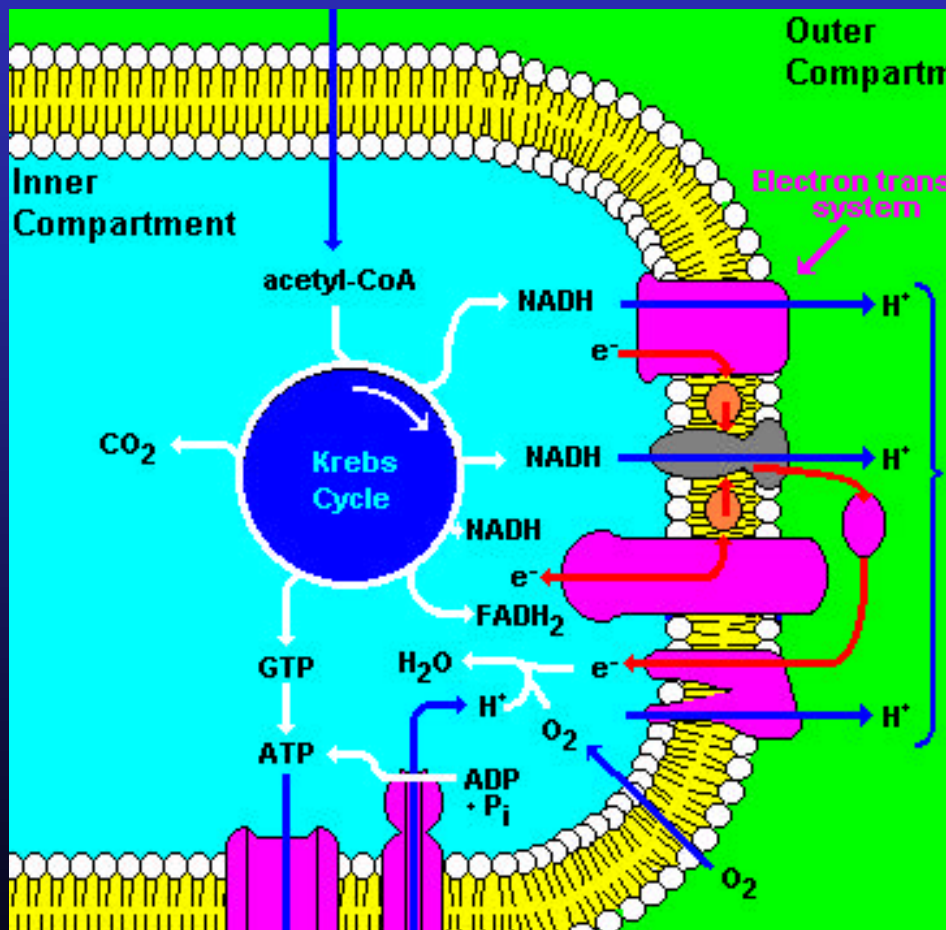
Michell's Chemiosmotic Theory

Proton chemical gradient is used to drive ATP synthesis.

→ **Proton Motive Force**

→ **Oxidative phosphorylation**

Reference: Karp Chap 5

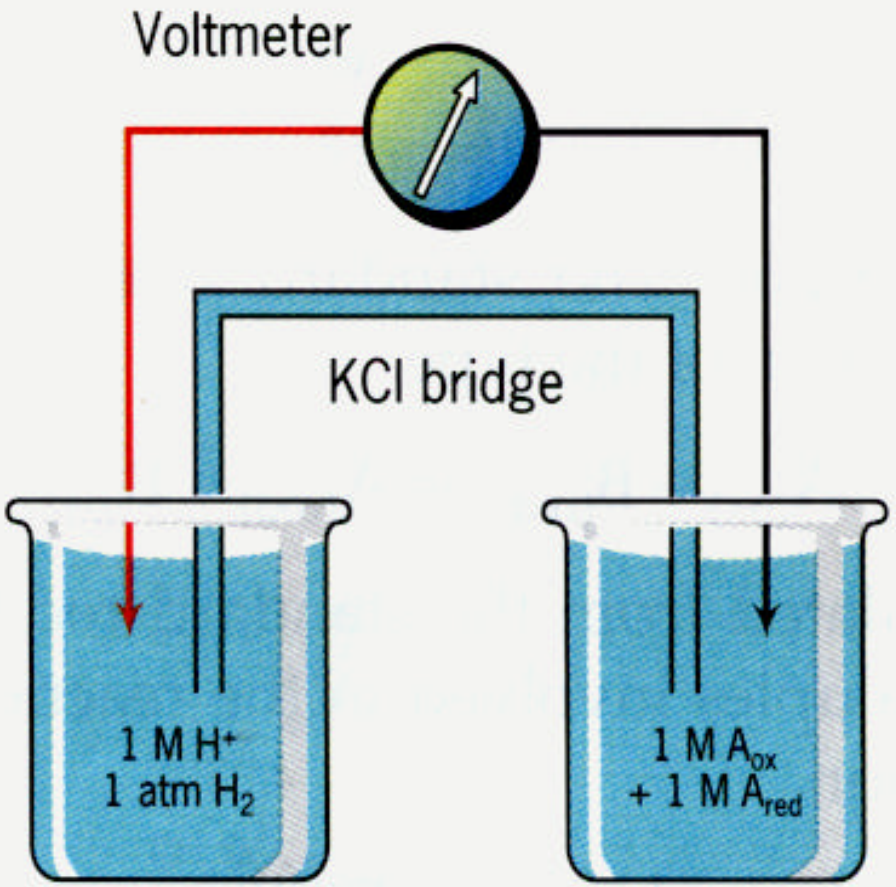


to drive ATP synthesis

Oxidative Phosphorylation: The formation of ATP driven by energy released from electrons removed during substance oxidation.($2x10^{2}$

Oxidation-Reduction potential (Redox Potential):

Half-Cell



$2H^+ + 2e^- \rightarrow H_2$

Reductant
igh energy)

Oxidant
ow energy)

Standard Redox Potentials of Selected Half-Reactions

(At pH 7.0)

Electrode equation	E'_0 (V
Acetate + $2H^+ + 2e^-$ ⇌ acetaldehyde	–0.58
$2H^+ + 2e^-$ ⇌ H_2	–0.42
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^-$ ⇌ isocitrate	–0.38
Acetoacetate + $2H^+ + 2e^-$ ⇌ β-hydroxybutyrate	–0.34
$NAD^+ + 2H^+ + 2e^-$ ⇌ NADH + H^+	–0.32
$NADP^+ + 2H^+ + 2e^-$ NADPH + H^+	–0.32
Acetaldehyde + $2H^+ + 2e^-$ ⇌ ethanol	–0.19
Pyruvate + $2H^+ + 2e^-$ ⇌ lactate	–0.18
Oxaloacetate + $2H^+ + 2e^-$ ⇌ malate	–0.16
FAD + $2H^+ + 2e^-$ ⇌ $FADH_2$ (in flavoproteins)	+0.03
Fumarate + $2H^+ + 2e^-$ ⇌ succinate	+0.03
2 cytochrome $b_{K(ox)} + 2e^-$ ⇌ 2 cytochrome $b_{K(red)}$	+0.03
Ubiquinone + $2H^+ + 2e^-$ ⇌ ubiquinol	+0.10
2 cytochrome $c_{ox} + 2e^-$ ⇌ 2 cytochrome $c_{(red)}$	+0.25
2 cytochrome $a_{3(ox)} + 2e^-$ ⇌ 2 cytochrome $a_{3(red)}$	+0.38
$\frac{1}{2}O_2 + 2H^+ + 2e^-$ ⇌ H_2O	+0.81

Redox Potential

ductant

xidant

Standard redox potential of some reactions:

Oxidant	⟷	Reductant	n	E'_0(V)
Succinate + CO_2	→	α-ketoglutarate	2	-0.67
$2H^+$	→	H_2	2	-0.42
NAD^+	⟷	NADH + H^+	2	-0.32
Cytochrome b (+3)	⟷	Cytochrome b (+2)	1	0.07
Ubiquinone(Oxidized)	⟷	Ubiquinone (Reduced)	2	0.10
Cytochrome c (+3)	⟷	Cytochrome c (+2)	1	0.22
Fe (+3)	⟷	Fe (+2)	1	0.77
½ O_2 + 2 H^+	→	H_2O	2	0.82

Thermodynamics

$$\Delta G = \Delta H - T\Delta S \quad \text{(kcal/mol)}$$

ΔH : Enthalpy change. Internal energy, binding energy, interactions or thermal energy;

ΔS : Entropy change. Randomness or degree of freedom and is related to molecular rearrangement (cal/°K/mol, kcal/°K/mol)

ΔG : Gibbs free energy. Determine the nature of the biological processes

1. Separation of energy into internal energy and randomness.

2. Biological process proceed in the direction of lowest energ and highest randomness

Redox Potential

Calculate the free energy of oxidation of the following reaction:

$$\tfrac{1}{2}\,O_2 + NADH + 2\,H^+ \longleftrightarrow H_2O + NAD+$$

Sol: $NAD^+ + H^+ + 2e^-$ $\longleftrightarrow$ $NADH$ $\quad E'_o = -0.32$ V -------- (a)

$\tfrac{1}{2}\,O_2 + 2\,H^+ + 2e^-$ $\longleftrightarrow$ H_2O $\quad E'_o = 0.82$ V -------- (b)

(b) - (a):

$\tfrac{1}{2}\,O_2 + NADH + 2\,H^+ \longleftrightarrow H_2O + NAD^+ \quad \Delta E'_o = 1.14$ V

Energy change:

$$\Delta G_{elec} = - n \cdot z \cdot F \cdot \Delta E$$

E: Electric potential (Volts); n: moles of ions (electro

F: (Faraday's constant) = 23 kcal.mol^{-1}.V^{-1} ; z: valence of the ion.

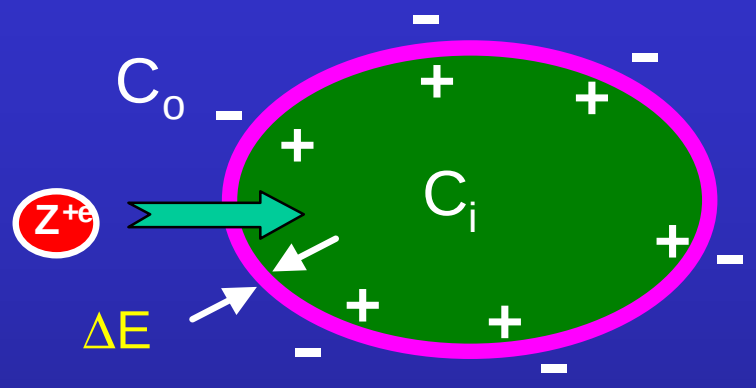
ΔE = 1.14 Volts; n = 2 moles; F = 23 kcal.mol^{-1}.V^{-1} ; z = 1:

$$\Delta G^{o'} = - nF\,\Delta E'_o = - 2 \times 23 \times 1.14 = - 52.5 \text{ kcal}$$

Transport of charge across a polarized membrane

Electrical work:

$$\Delta G_{elec} = - n \cdot z \cdot F \cdot \Delta E$$

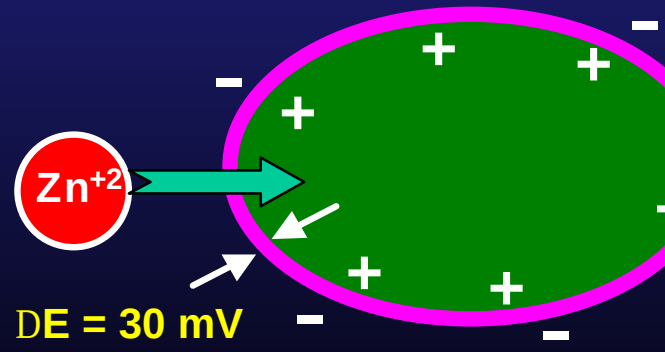


ΔE: Membrane potential (Volts);
n: moles of ions;
F: (Faraday's constant) = 23 kcal.mol^{-1}.V^{-1} ;
z: valence of the ion.

xample: Translocating 1mmol of Zn^{+2} across membrane

E = - 30 mV; n = 1 mM = 0.001 mole; z = +2;

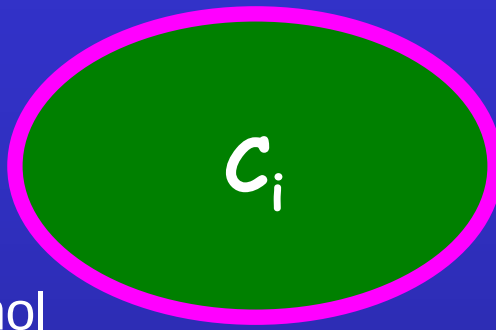
$$\Delta G_{elec} = - z \cdot n \cdot F \cdot \Delta E$$
$$= - 2 \cdot (0.001) \cdot 23 \cdot (-0.03)$$
$$= 1.38 \text{ cal}$$



➢ Need energy against potential gradient

Chemical potential: $\mu_i = (\partial G/\partial n_i)_{T,P,n}$

$$G_{chem} = \sum n_i \mu_i = \sum n_i \mu_i^{o} + \sum n_i RT \cdot \ln C_i$$



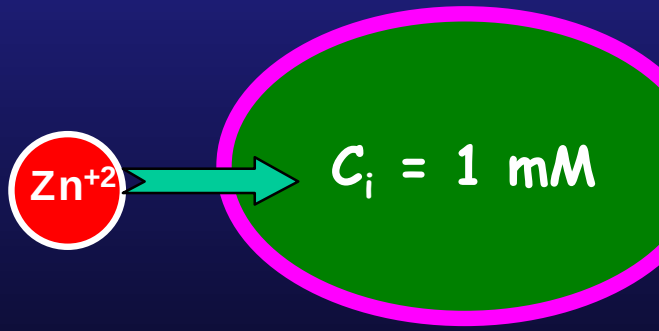
n_i: moles of compound i; R: gas constant = 2 cal/^{o}K/mol
T: absolute temperature; C_i = concentration (molar)
μ_i^{o} is the chem potential of the ideal solute at unit conc.

Example: Translocating 1mmol of Zn^{+2} across a neutral membrane

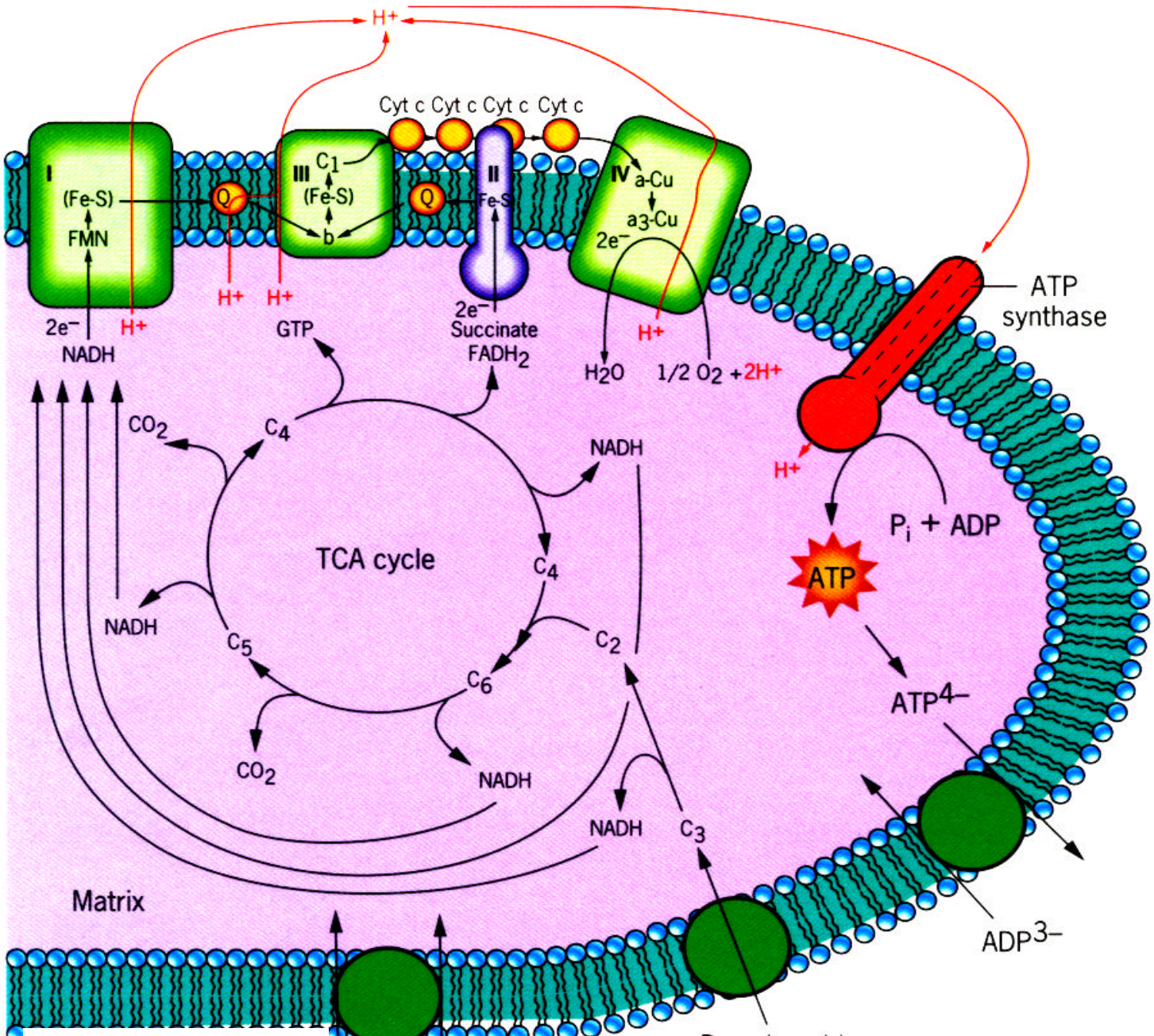
E = - 0mV; n = 1 mM = 0.001 mole; z = +2;
C_i = 0.001 M; C_o = 0.0001 M; T = 27 ^{o}C

C_o = 0.1 mM

$$\Delta G_{elec} = G_i - G_o = RT \cdot \ln(C_i/C_o)$$
$$= 2 \times (27 + 273) \times \ln (0.001/0.0001)$$
$$= 1382 \text{ cal} = 1.382 \text{ kcal}$$



- Need energy, against concentration gradient.
- Only apply to ideal solution.
- If both potential and concentration gradient exit


H+
Cyt c Cyt c Cyt c Cyt c
I
(Fe-S)
FMN
Q
III
C1
(Fe-S)
b
Q
II
Fe-S
IV
a-Cu
a3-Cu
2e−
H+ H+
2e−
NADH
H+
2e−
Succinate
FADH2
GTP
H2O
1/2 O2 +2H+
H+
ATP
synthase
CO2
C4
NADH
TCA cycle
C4
Pi + ADP
ATP
NADH
C5
C2
C6
ATP4−
CO2
NADH
NADH
C3
Matrix
ADP3−

1. **Flavoproteins:** Proteins contain either flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN).

2. **Cytochromes:** Proteins contain heme group

$$Fe^{+2} \longleftrightarrow Fe^{+3}$$

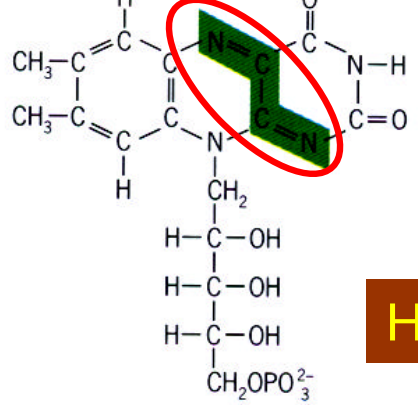
3. **Ubiquinone (UQ or coenzyme Q):** A lipid soluble molecule contining a long hydrophibic chain composed of five-cqrbon isoprenoid unit.

Quinone $\longleftrightarrow$ Ubisemiquinone $\longleftrightarrow$ Ubiquinone

4. **Iron-sulfer proteins:** Proteins contain irons which are linked to inorganic sulfur atoms as part irons sulfur center [2Fe-2S] or [4Fe-4S]-linked to cysteine.

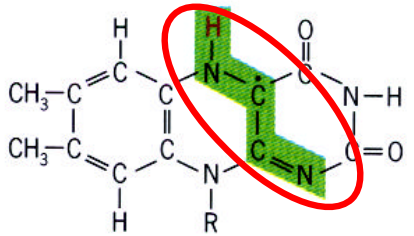
$$\Delta E^{o} = -700 \text{ mV} - +300 \text{ mV}$$

FMN



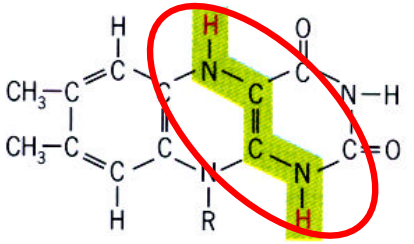
Oxidized form of FMN
(quinone state)

$-H^+ - e^-$ ⇌ $+H^+ + e^-$



Intermediate free radical
(semiquinone state)

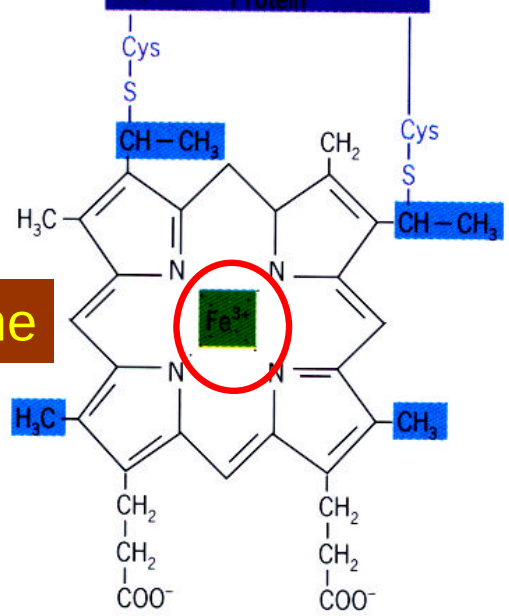
$-H^+ - e^-$ ⇌ $+H^+ + e^-$



Reduced form of FMN
(hydroquinone state)

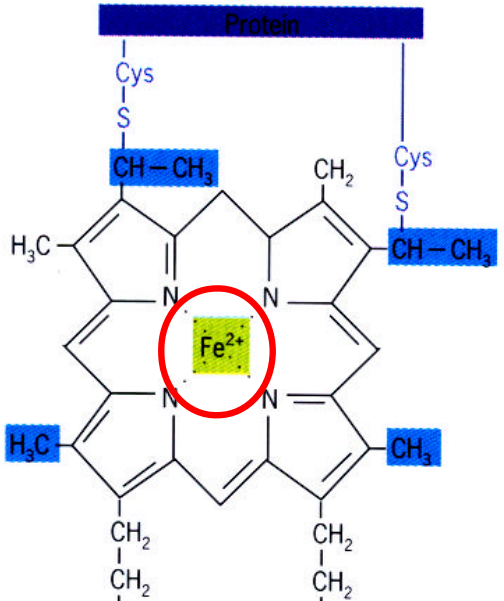
(a)

Heme

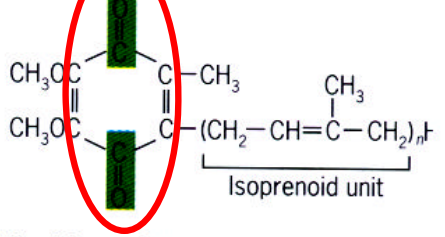


Oxidized form of Heme

$-1e^-$ ⇌ $+1e^-$

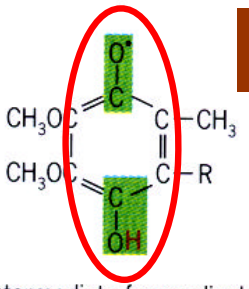


Ubiquinone



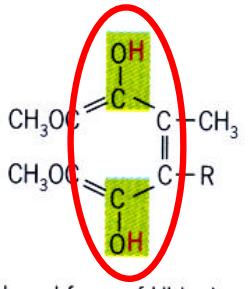
Oxidized form of Ubiquinone
(quinone state)

$-H^+ - e^-$ ⇌ $+H^+ + e^-$



Intermediate free radical
(ubisemiquinone)

$-H^+ - e^-$ ⇌ $+H^+ + e^-$



Reduced form of Ubiquinone
(ubiquinol)

(c)

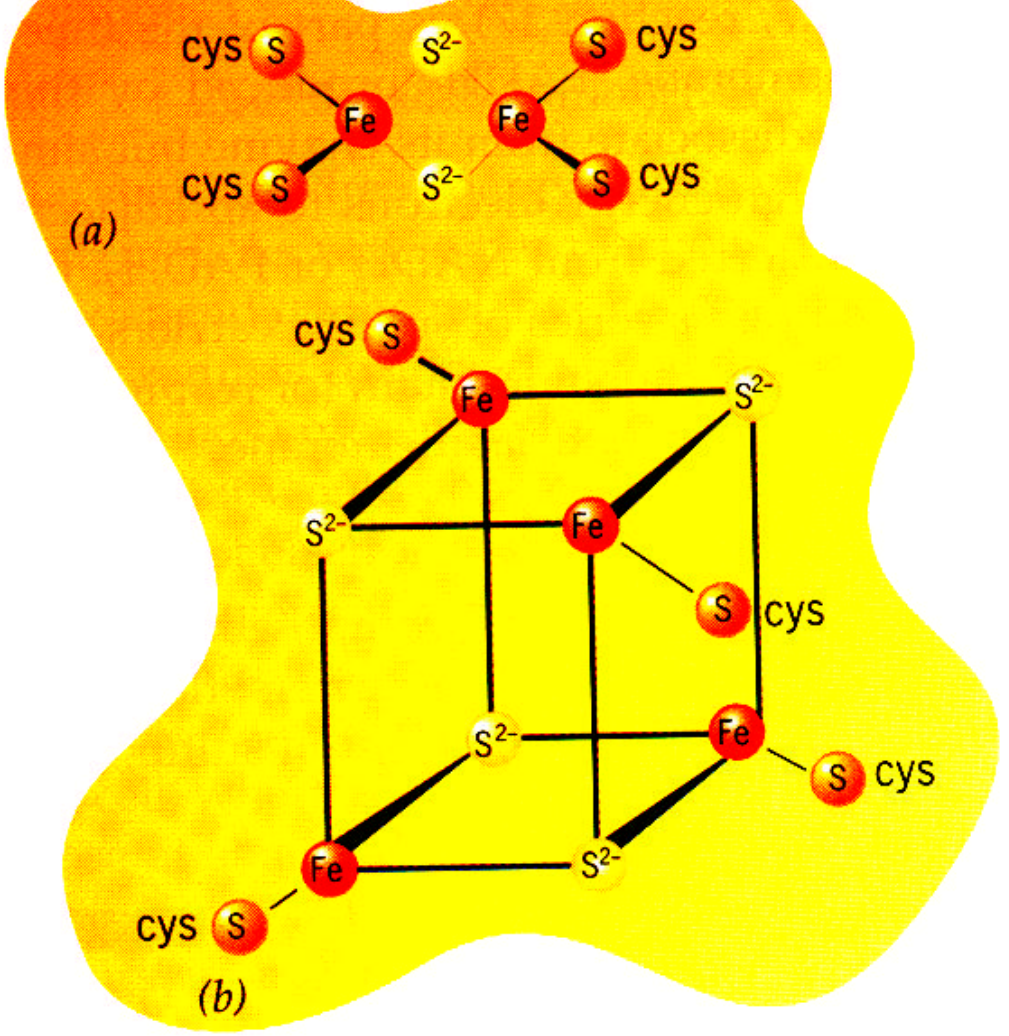


Figure 5.12 Iron-sulfur centers. Structure of a [2Fe-2S] *(a)* and a [4Fe-4S] *(b)* iron-sulfur center. Sulfur atoms are shown in yellow. Both types of iron-sulfur centers are joined to the protein by linkage to a sulfur atom of a cysteine residue. Both types of iron-sulfur centers accept only a

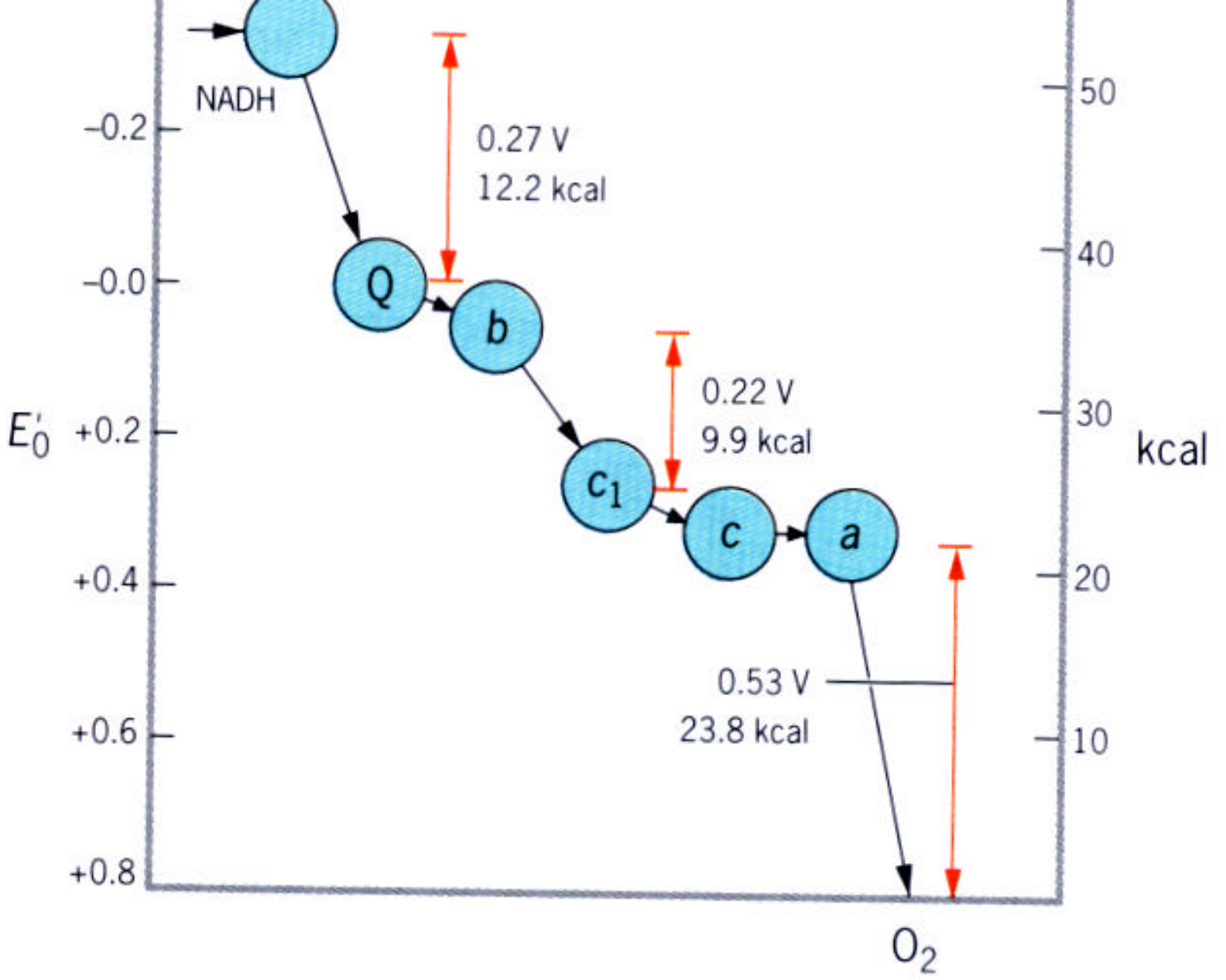


Figure 5.13 The arrangement of several carriers in the

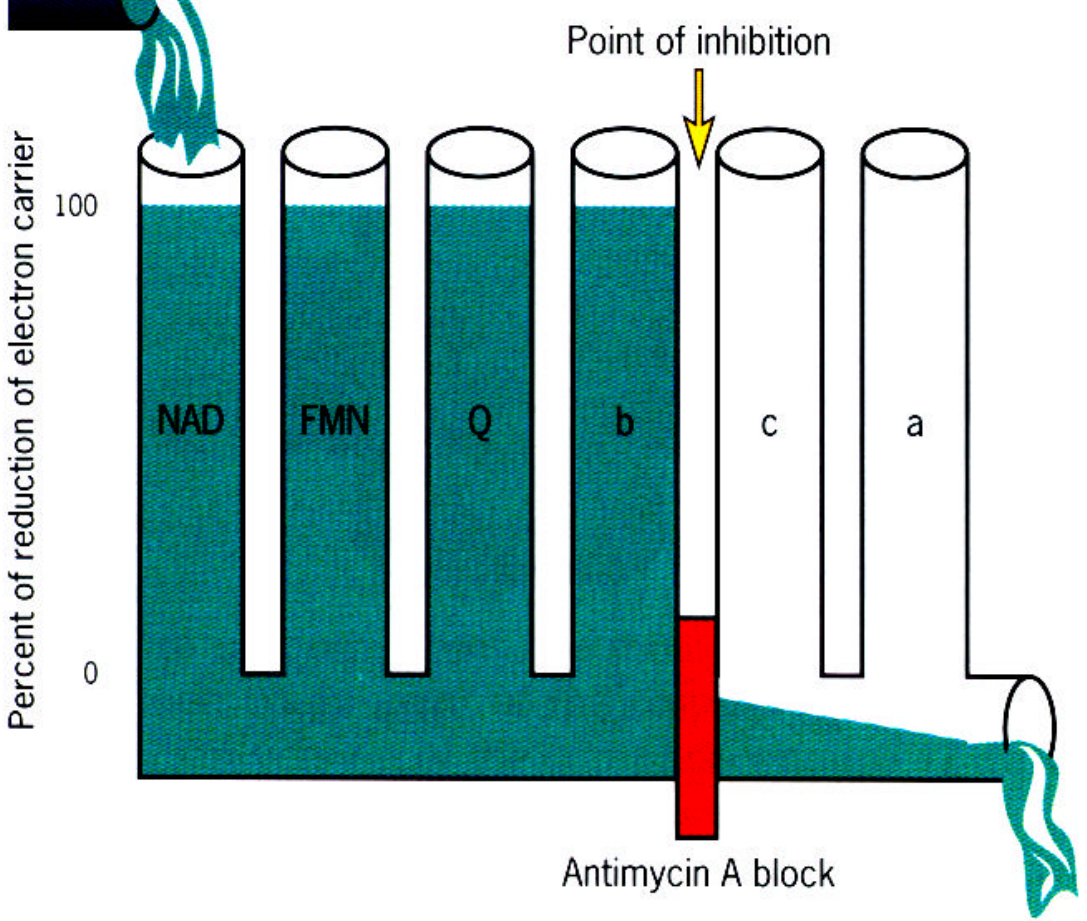
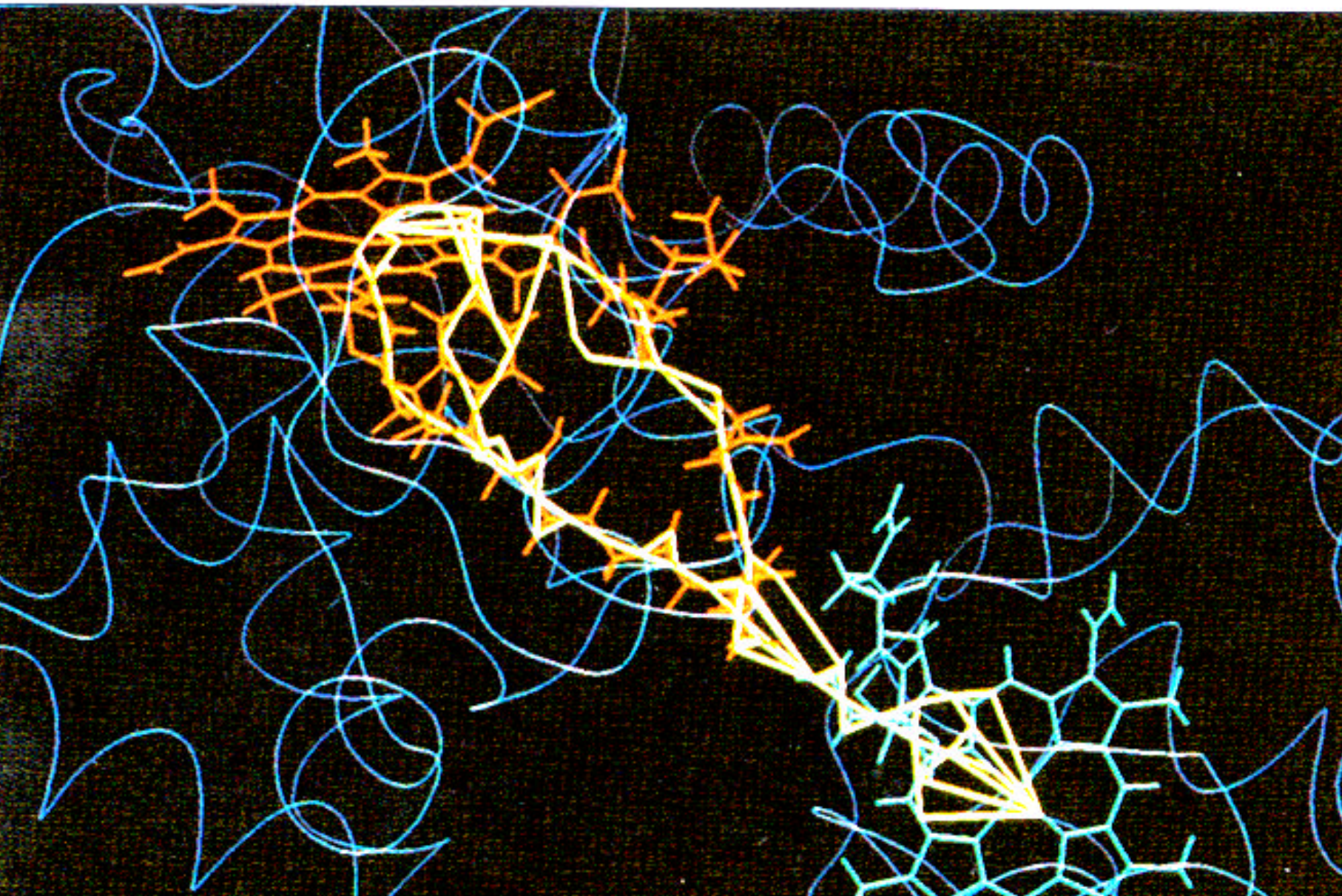
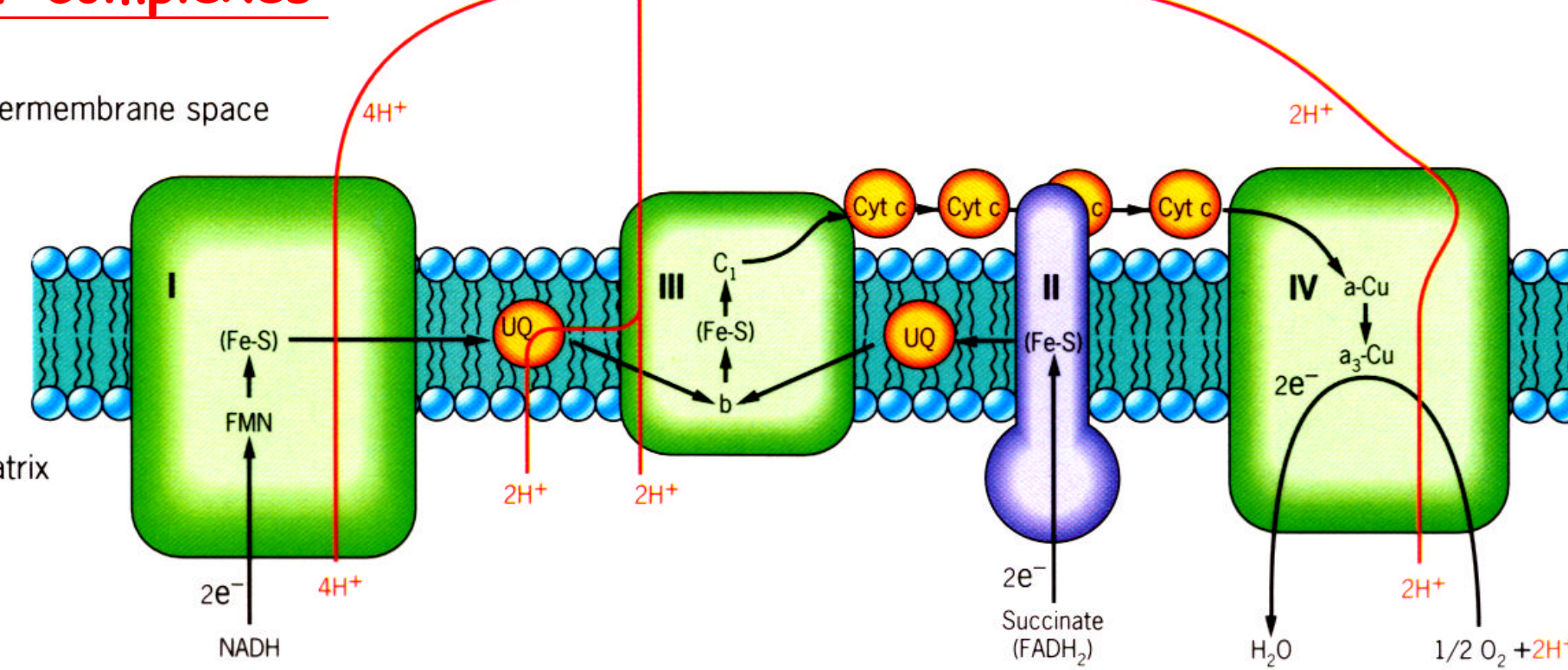


Figure 5.14 Experimental use of inhibitors to determine the sequence of carriers in the electron-transport chain. In this hydraulic analogy, treatment of mitochondria with the inhibitor antimycin A leaves those carriers on the upstream (NADH) side of the point of inhibition in the fully reduced state and those carriers on the downstream (O_2) side of inhibition in the fully oxidized state. Comparison of the effects of several inhibitors revealed the order of the carriers within

onds and covalent bonds for considerable distances (10 – 20



complexes



10 nm

UBUNITS	Complex I NADH Dehydrogenase Mammalian	Complex III Cytochrome bc_1	Complex II Succinate dehydrogenase	Complex IV Cytochrome *c* Oxidase
DNA	7	1	0	3
NA	35	10	4	10
TAL	42	11	4	13

$4cytc^{2+} + 4H^{+} + O_2 \rightarrow 4cyt\ c^{+3} + 2H_2O$

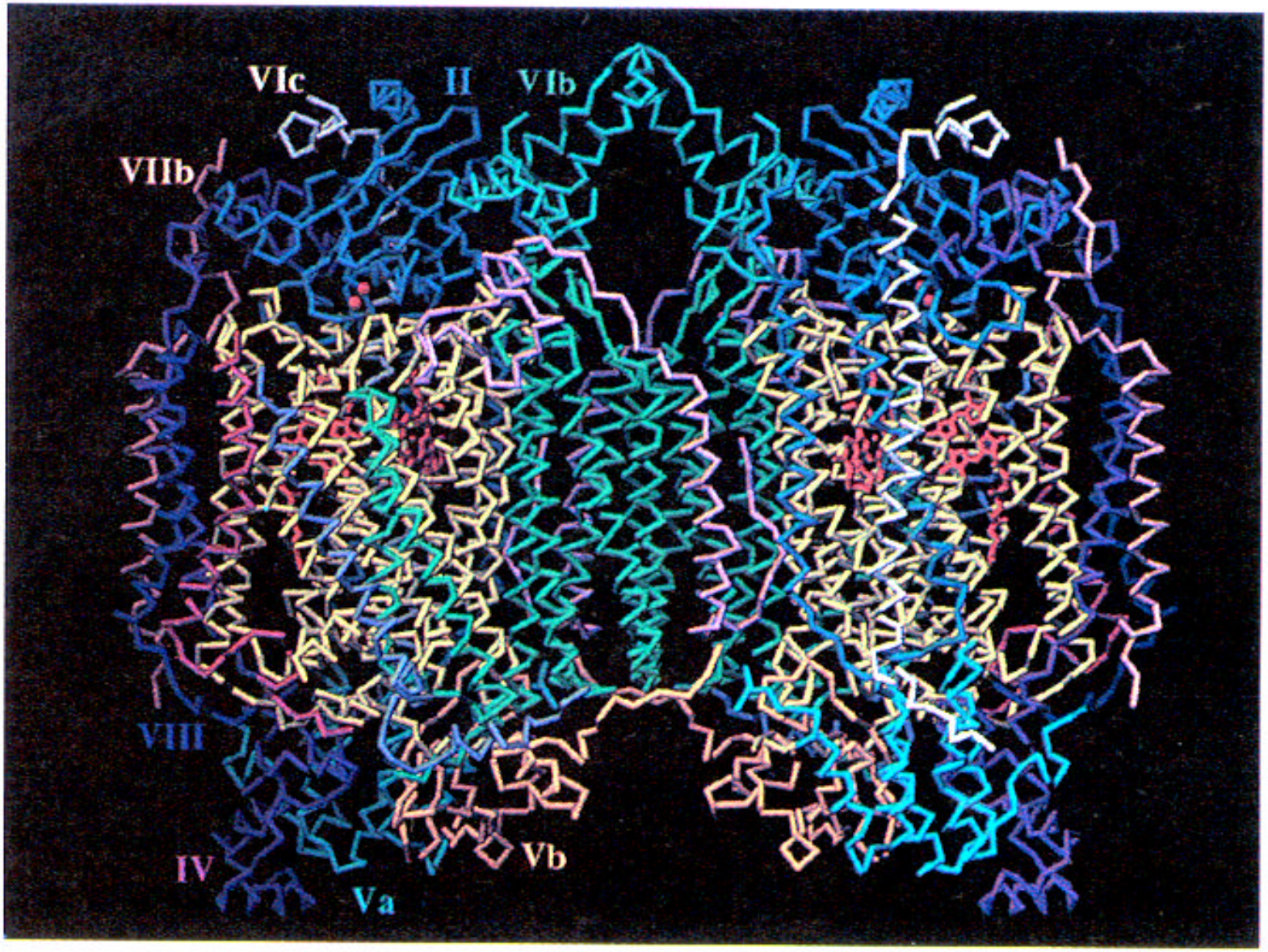


Figure 5.17 Three-dimensional structure of beef heart

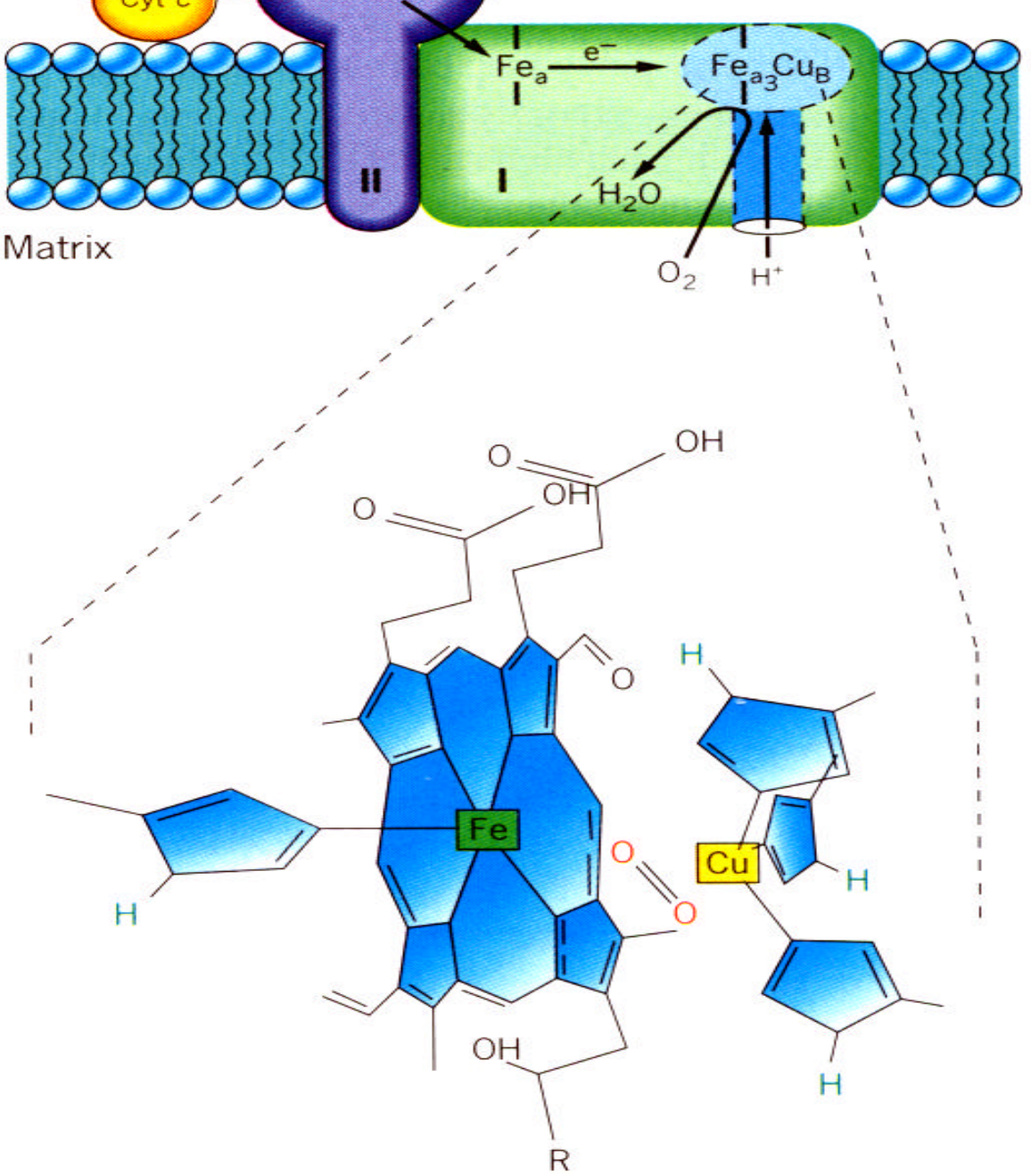


Figure 5.18 The mechanism of action of cytochrome ox-

Coupling of proton gradient to ATP synthesis

Chemical Equilibrium

Chemical reaction: $a\,A + b\,B + \cdots \longleftrightarrow c\,C + d\,D + \cdots$

$$\Delta G = \Delta G^{o} + 2.3RT \log K \quad \text{---------- (1)}$$

G : Gibbs free energy under experimental condition (cal, kcal)

G^{o} : Gibbs free energy in equilibrium = - 2.3 RT log K_{eq} (Equilibrium consta

Standard state free energy (All conc = 1 M)

R: Gas constant = 1.99 cal K^{-1} Mol^{-1} = 8.31 J K^{-1} mol^{-1};

$K = [C]^{c} \cdot [D]^{d} \cdot \cdot / [A]^{a} \cdot [B]^{b} \cdot \cdot$ (Not necessarily in equilibrium)

1. Sign of ΔG, not ΔG^{o}, determines the direction of a reactio
   - $\Delta G < 0$ Spontaneous reaction (proceeds in forward direction).
   - $\Delta G > 0$ Reaction proceeds in reverse direction.
   - $\Delta G = 0$ System in equilibrium (No change in reactant concentratio

Reaction	Enzyme	Type*	$\Delta G^{\circ\prime}$
Glucose + ATP $\longrightarrow$ glucose 6-phosphate + ADP + H^+	Hexokinase	a	−4.0
Glucose 6-phosphate $\rightleftharpoons$ fructose 6-phosphate	Phosphoglucose isomerase	c	+0.4
Fructose 6-phosphate + ATP $\longrightarrow$ fructose 1,6-bisphosphate + ADP + H^+	Phosphofructokinase	a	−3.4
Fructose 1,6-bisphosphate $\rightleftharpoons$ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate	Aldolase	e	+5.7
Dihydroxyacetone phosphate $\rightleftharpoons$ glyceraldehyde 3-phosphate	Triose phosphate isomerase	c	+1.8
Glyceraldehyde 3-phosphate + P_i + NAD^+ $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+	Glyceraldehyde 3-phosphate dehydrogenase	f	+1.5
1,3-Bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	a	−4.5
3-Phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate	Phosphoglyceratmutase	b	+1.1
2-Phosphoglycerate $\rightleftharpoons$ phosphoenolpyruvate + H_2O	Enolase	d	+0.4
Phosphoenolpyruvate + ADP + H^+ $\longrightarrow$ pyruvate + ATP	Pyruvate kinase	a	−7.5

on type: (a) phosphoryl transfer; (b) phosphoryl shift; (c) isomerization; ydration; (e) aldol cleavage; (f) phosphorylation coupled to oxidation.

: $\Delta G^{\circ\prime}$ and ΔG are expressed in kcal/mol. ΔG, the actual free-energy change, has alculated from $\Delta G^{\circ\prime}$ and known concentrations of reactants under typical physiologic ons. Glycolysis can proceed only if the ΔG values of all reactions are negative. The

of biological interest.

Species	Products	$\Delta G^{\circ\prime}$ (kJ/mol)
Phosphoenolpyruvate	$Pyruvate^- + HPO_4^{2-}$	-61.5
Carbamoyl phosphate		-51.4
Glycerate-1,3-bisphosphate		-49.3
Acetyl phosphate	$Acetate^- + HPO_4^{2-} + H^+$	-47.2
Phosphocreatine	$Creatine^+ + HPO_4^{2-}$	-42.6
Phosphoarginine	$Arginine^+ + HPO_4^{2-}$	-38.0
ADP^{3-}	$AMP^{2-} + HPO_4^{2-} + H^+$	-36.0
ATP^{4-}	$AMP^{2-} + HP_2O_7^{3-} + H^+$	-35.1
ATP^{4-}	$ADP^{3-} + HPO_4^{2-} + H^+$	-34.3
$HP_2O_7^{3-}$ (pyrophosphate, PP_i)	$2\ HPO_4^{2-} + H^+$	-33.0
Glucose-1-phosphate		-20.9
Glucose-6-phosphate		-13.8
AMP^{2-}	$Adenosine + HPO_4^{2-}$	-9.20
Glycerol-3-phosphate		-9.20

Table 1.4, taken from Zubay[16] and Stryer[17], lists the standard free energy of hydrolysis for some phosphate compounds. The table orders compounds in descending magnitude

xample The enzyme aldolase catalyzeds the conversion of fructose-1,6-diphosphat FDP, to dihydroxyacetone phosphate, DHAP, and glyceraldehyde-3-phosphate, GAP. Under physiological conditions in erythrocytes (red blood cells), the concentrations of these species are [FDP] $= 35\mu M$, [DHAP] $= 130\,\mu M$, and [GAP] $= 15\ \mu M$. Will the conversion occur spontaneously under these conditions?

olution The reaction quotient for the reaction considered,

$$\mathrm{FDP} \rightarrow \mathrm{DHAP} + \mathrm{GAP}, \qquad \Delta G'^{o\prime} = 23.8\ \mathrm{kJ/mol},$$

is

$$\mathrm{K}' = \frac{[\mathrm{DHAP}][\mathrm{GAP}]}{[\mathrm{FDP}]} = \frac{(130 \times 10^{-6})(15 \times 10^{-6})}{35 \times 10^{-6}} = 5.8 \times 10^{-5},$$

so the free energy change is

$$\Delta G' = \Delta G'^{o\prime} + RT \ln\ \mathrm{K} = -0.47\ \mathrm{J/K\text{-}mol}.$$

Hence, under the given conditions, the reaction will proceed spontaneously. ¶

- ATP hydrolysis

TP ➔ ADP + P_i + H^+

TP ➔ AMP + Pp_i + H^+

DP ➔ AMP + P_i + H^+

= A; ATP, ADP, AMP

denosine-5′-triphosphate
denosine-5′-diphosphate
denosine-5′-monophosphate

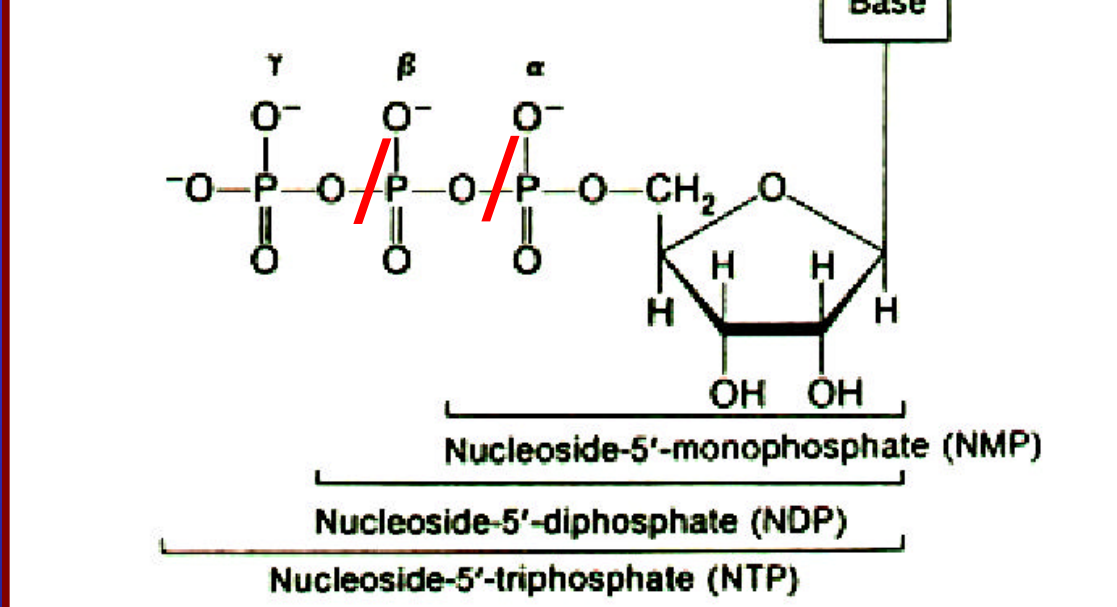


Figure 1.4: The structure of a nucleoside monophosphate, diphosphate, and triphospha
NMP, NDP, and NTP dissociate two, three, and four protons, respectively. The phospha
groups in NTPs are designated α, β, and γ according to their positions. [From G. Zub
ed., *Biochemistry*, 2nd ed., MacMillan, New York, New York, 1988, Fig.7-11.]

alculate ΔG of the hydrolysis of ATP inside the cell at 27°C.
ssume [ATP] = 100 mM, [P_i] = 10 mM and [ADP] = 1 mM inside the cell.

G $= \Delta G^o + 2.3\ RT \log K$; $K = [ADP]\bullet[P_i] / [ATP]$; $\Delta G^o = -34.3\ kJ\ mol^{-1}$

G $= -34.3 \times 10^3 + 2.3 \bullet 8.31 \bullet (273+27) \log(0.001 \bullet 0.01/0.1)$

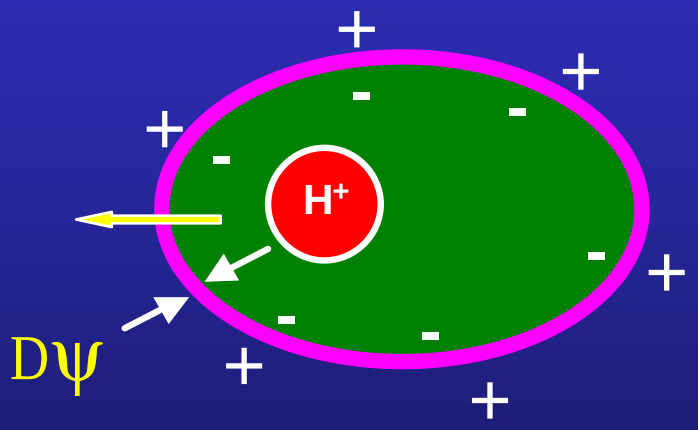
$= -57.2\ kJ\ mol^{-1} = -13.3\ kcal/mol$

Proton motive force (Electrochemical gradient):

Proton gradient generated by oxidative phosphorylation contains both chemical gradient and electric gradient.

Electromotive force (Δp):

$$\Delta p = \psi - 2.3\,(RT/F)\,\Delta pH$$
$$= \psi - 59\,\Delta pH \ (mV)$$



- ΔpH ~ 0.5 – 1 pH unit
- ΔpH contribute about 20% and $\Delta \psi$ contribute about 80% to Δp.

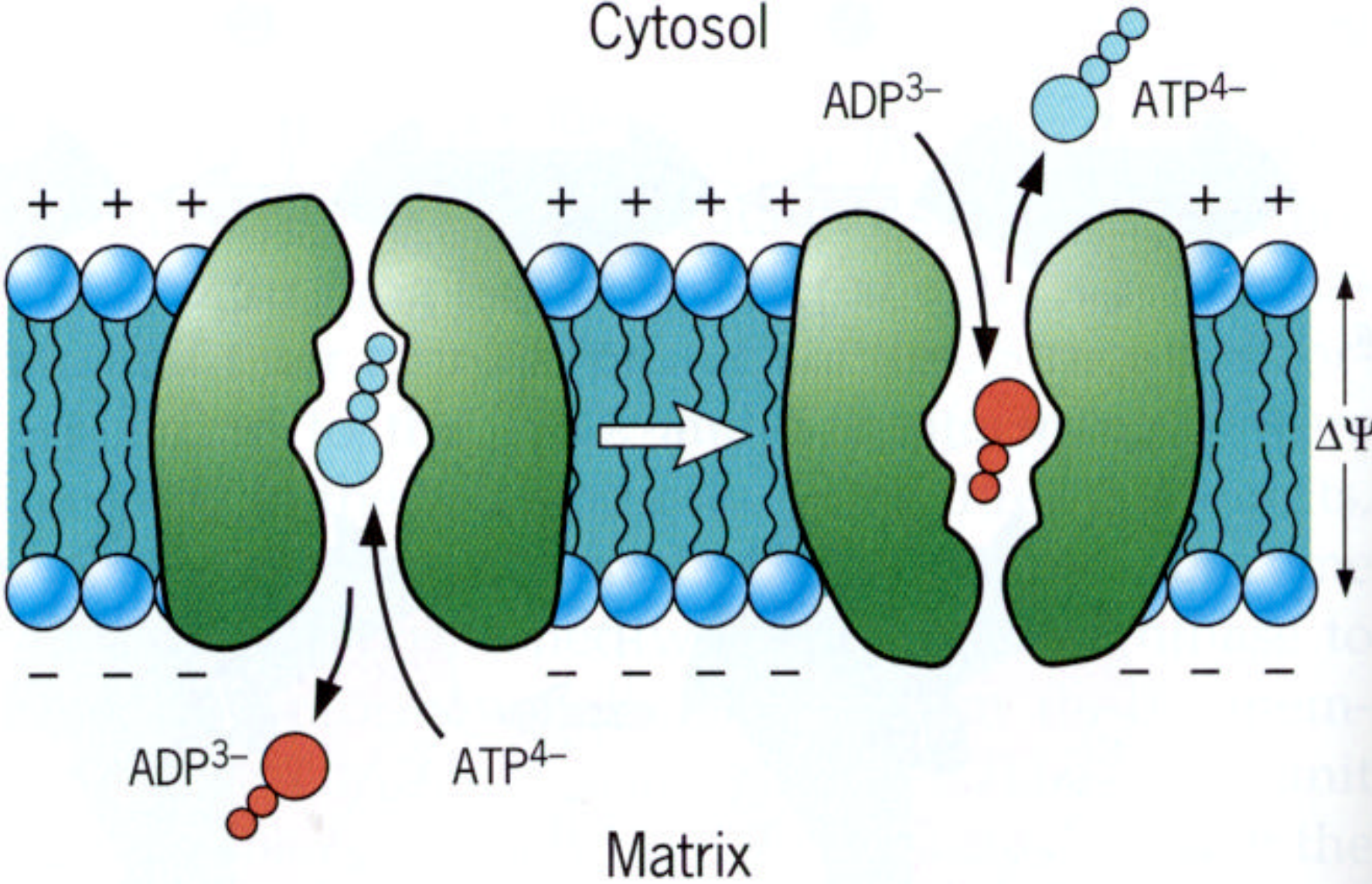
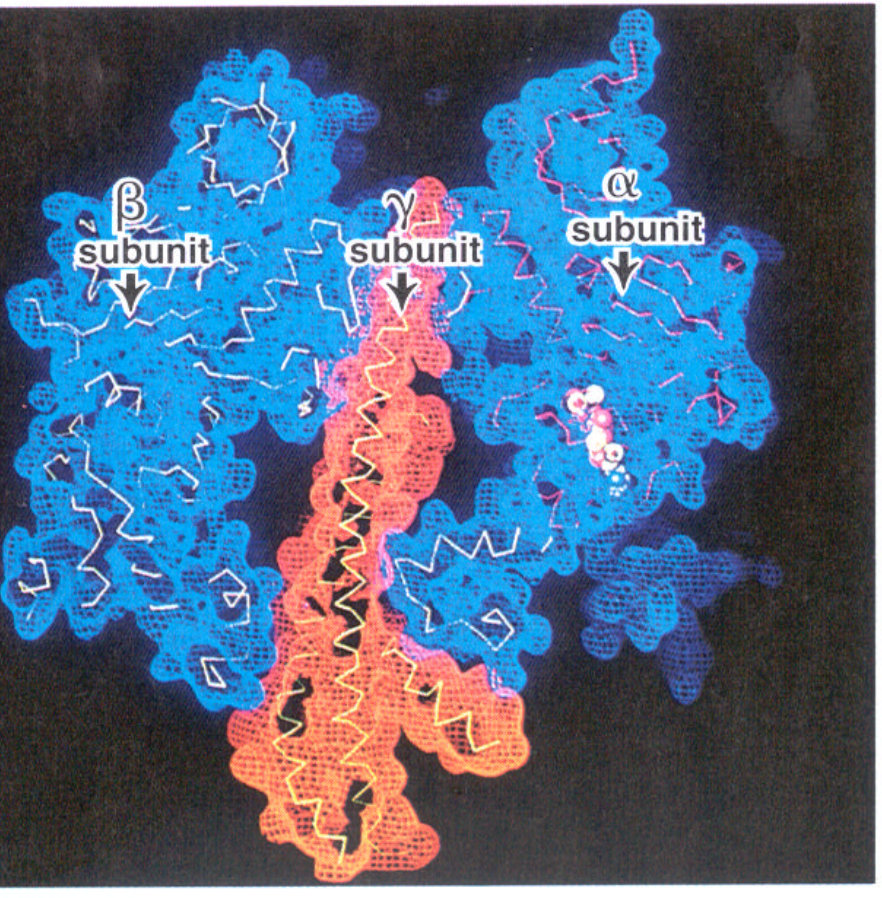
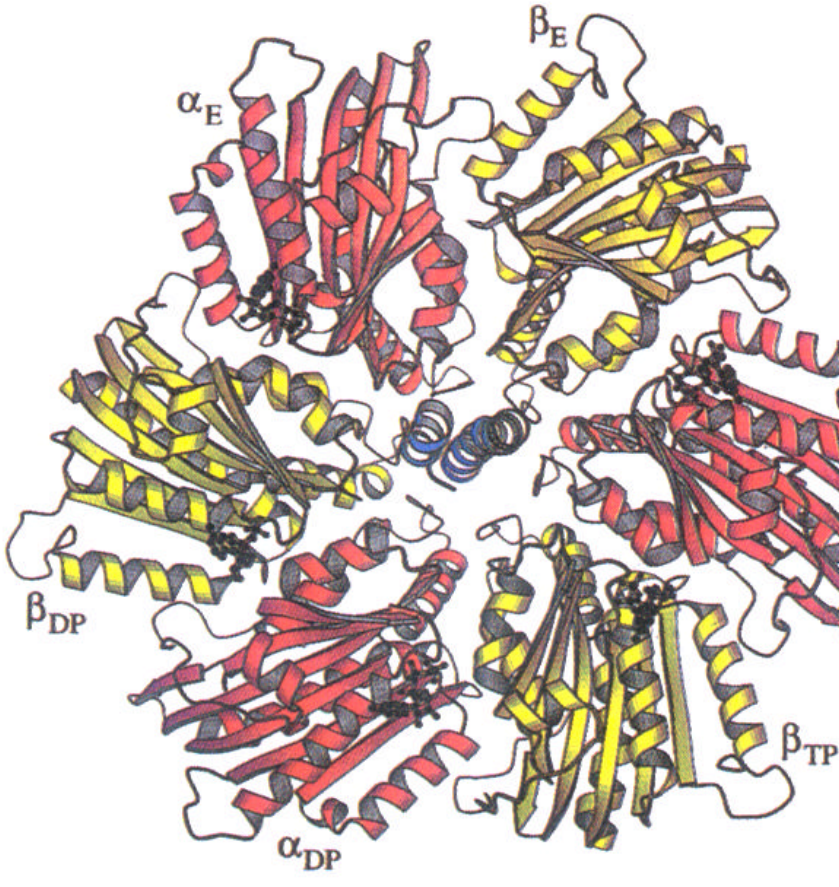


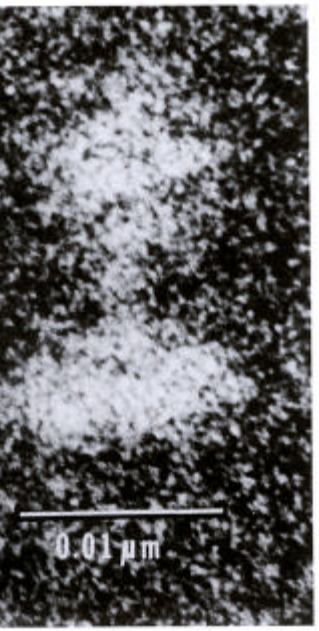
Figure 5.27 The use of the proton-motive force in moving ADP into the matrix and ATP into the cytosol.

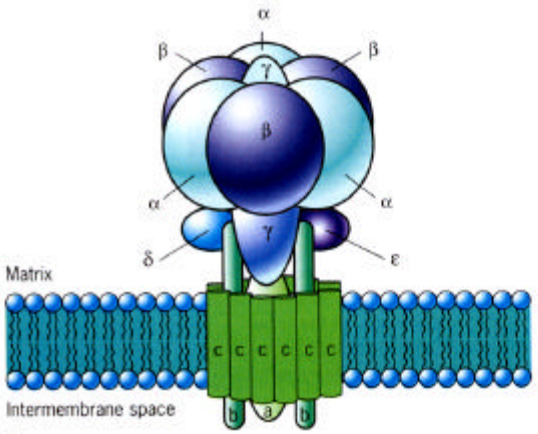




(b)

e 5.24 The structural basis of catalytic site confor-n. *(a)* A section through the F_1 head shows the spatial ization of three of its subunits. The α-helical γ subunit to project into the central cavity (black) of the F_1, and en the α and β subunits on each side. The conforma- f the catalytic site of the β subunit (shown on the left) ermined by its contact with the γ subunit. *(b)* A top of the F_1 head showing the arrangement of the six α and β subunits around the asymmetric γ subunit. Th subunit is in position to rotate relative to the surrou subunits. It is also evident that the γ subunit makes in a different way with each of the three β subunits, ing each of them to adopt a different conformation. *(Reprinted with permission from J. P. Abrahams, et al., c of John E. Walker,* Nature *370:624, 627, 1994. Copyrigh Macmillan Magazines Limited.)*





(b)

e 5.22 The structure of the ATP synthase. *(a)* Electron micrograph of the isolated rat liver ynthase. *(b)* Schematic diagram of the bacterial ATP synthase. The enzyme consists of two portions, called F_1 and F_0. The F_1 head consists of five different subunits in the ratio 3α $:1\gamma:1\epsilon$. The α and β subunits are organized in a circular array to form the spherical head of the le; the δ and ϵ subunits are thought to be localized in the stalk; and the γ subunit runs through re of the ATP synthase from the tip of F_1 down to F_0. The F_0 base, which is embedded in the rane, consists of three different subunits in the apparent ratio 1a:2b:12c. As discussed later, the nits are thought to form a movable ring within the membrane; the b subunits form part of the nd extend into the F_1 head where they may hold the α/β subunits in a fixed position; and the a it may contain the proton channel that allows protons to traverse the membrane. *(a: From J. W. G. L. Decker, and P. L. Pedersen,* J. Biol. Chem. *254:11173, 1979.)*

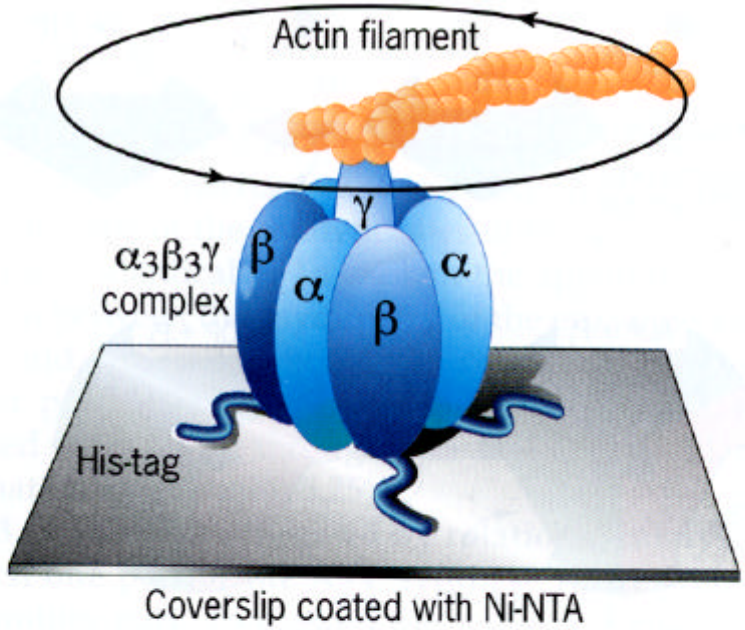
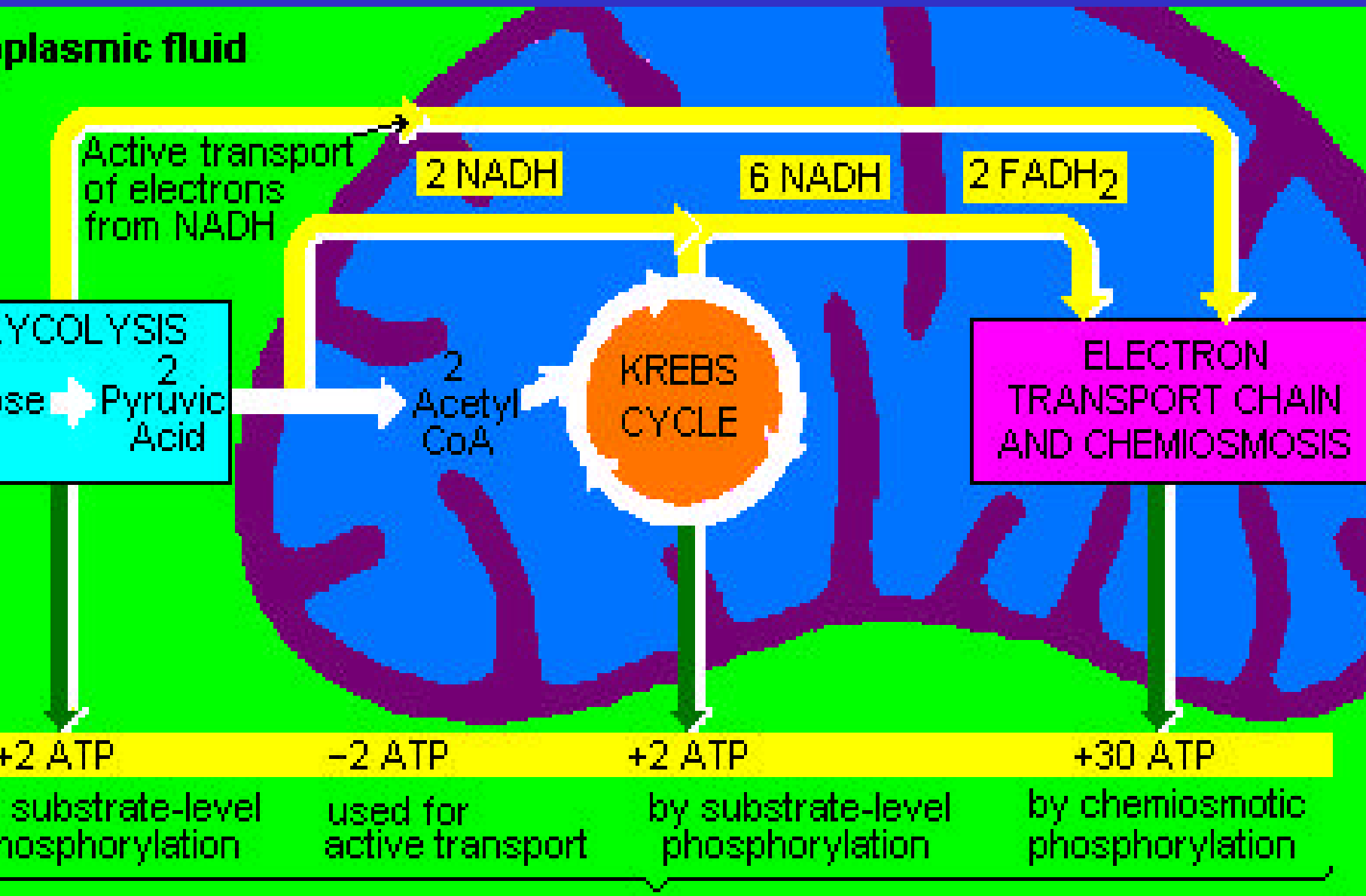


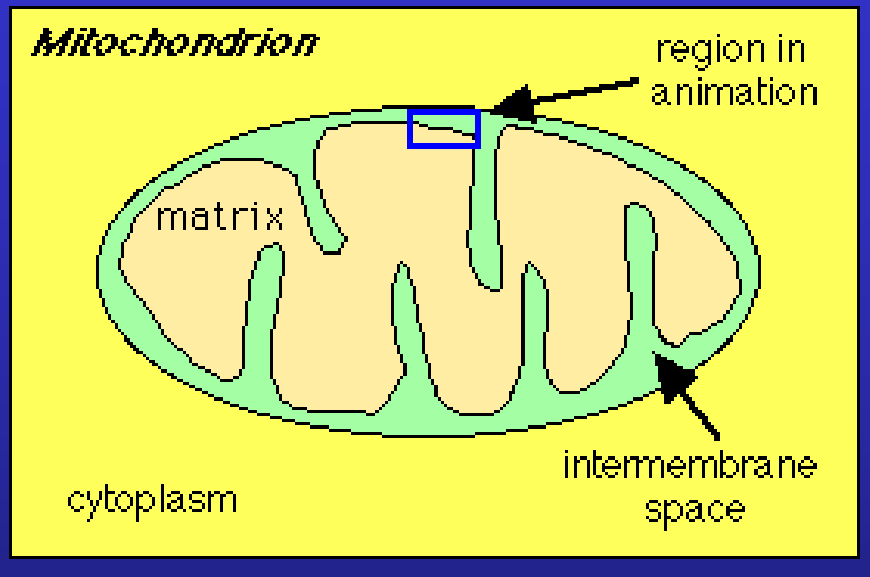
Figure 5.26 Direct observation of rotational catalysis. To carry out the experiment, a modified version of a portio of the ATP synthase consisting of $\alpha_3\beta_3\gamma$ was prepared. Eac β subunit was modified to contain 10 histidine residues at its N-terminus, a site located on the outer (matrix) face of the F_1 head. The side chains of histidine have a high affinit for a substance (Ni-NTA), which was used to coat the cove slip. The γ subunit was modified by replacing one of the serine residues near the end of the stalk with a cysteine residue, which provided a means to attach the fluorescentl labeled actin filament. In the presence of ATP, the actin fila ment was observed to rotate counterclockwise (when viewed from the membrane side) at a speed of less than 4 cycles per second. *(Reprinted with permission from H. Noji, et al., courtesy of Masasuke Yoshida,* Nature *386:300, 1997. Copyright 1997, Macmillan Magazines Limited.)*

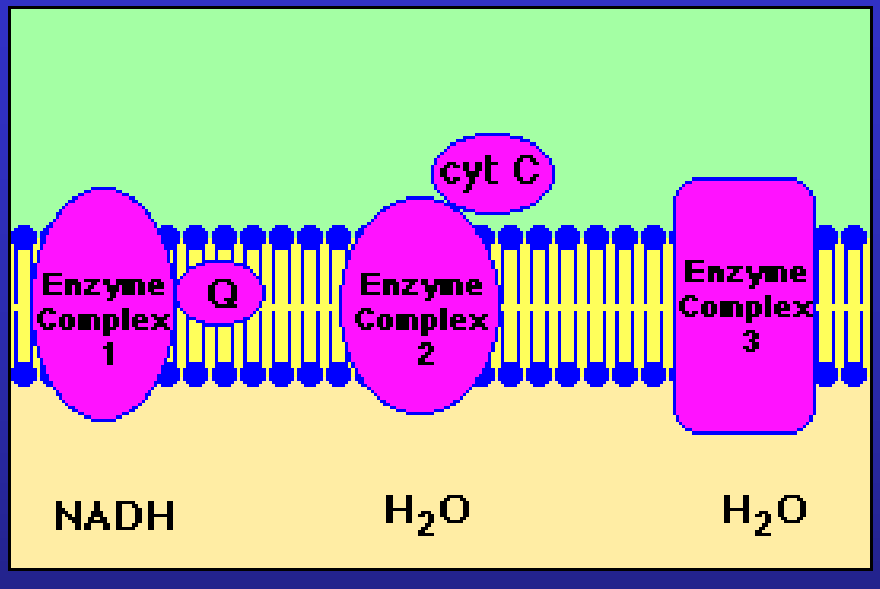
ATP yield from metabolism of a glucose molecule



About

Animation of Electron transport in Mitochondria



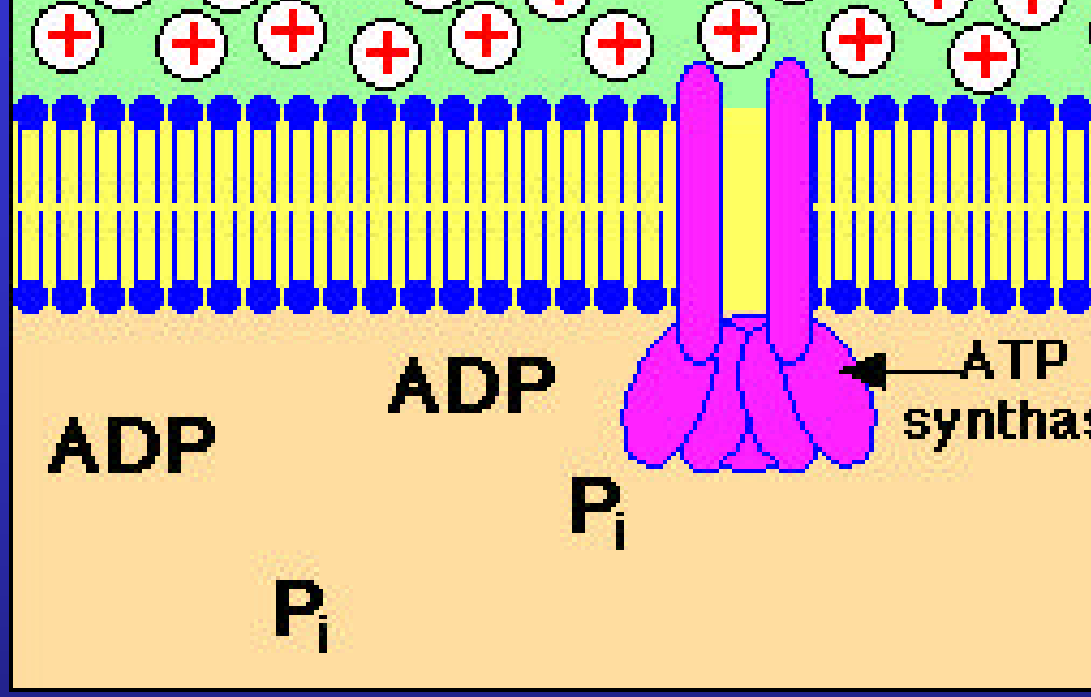


he schematic diagram above illustrates a mitochondrion. In the animation, wa
as NADH transfers H^+ ions and electrons into the electron transport system.

tep 1: Proton gradient is built up as a result of NADH (produced from oxidatio
eactions) feeding electrons into electron transport system.

tep 2: Protons (indicated by **+** charge) enter back into the mitochondrial matri
rough channels in ATP synthase enzyme complex. This entry is coupled to A

nt illustrates a mitochondrion. In
e animation, watch as H^+ ions
cumulate in the outer
tochondrial compartment
enever NADH is made from
dation reactions, generating a
oton gradient (upper image).
otons re-enter the cell through
e ATP synthase complex,
nerating ATP (lower image).



y points:

1. ATP synthase is a large protein complex with a proton channel that allows re-entry of protons.
2. Protons are translocated across the membrane, from the matrix to the intermembrane space, as a result of electron transport resulting from the formation of NADH by oxidation reactions. (See the animation of electron transport.) The continued buildup of these protons creates a proton gradie
3. ATP synthesis is driven by the resulting current of protons flowing through the membrane:

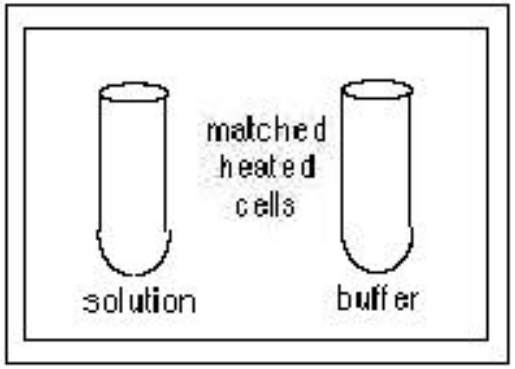
Determination of ΔH and ΔS by DSC

macromolecules:
nstant P)
= Dq.

equilibrium:
= 0 = DH - DS

DS = Dq/T
= C_p ΔT/T

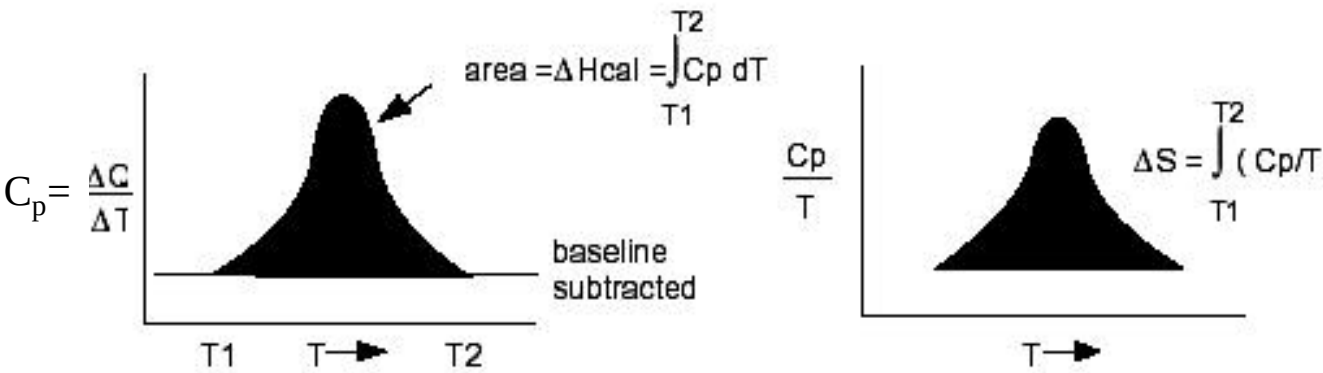
Biological samples are usually small and dilute, so conventional calorimetric measurem
have until recently required too much material and have lacked sensitivity. However, recent
technical advances have led to the development of *microcalorimeters*, which can detect the
small amounts of heat generated or consumed by the ligand binding and conformational ch
reactions undergone by proteins, nucleic acids, and membranes. Binding reactions are gene
studied by *isothermal titration calorimeters*, which will be described later. Here we conside
differential scanning calorimeters, in which processes such as protein unfolding and helix-
transitions can be studied as a function of temperature (3). A schematic diagram of a differe
scanning calorimeter is shown below:



Heat is supplied at the same rate to two matched cell
solution cell will generally absorb more heat than the
buffer cell, causing a slight difference in temperature
between the two cells. A feedback loop monitoring T
supply a small amount of heat ΔQ to the solution cell
to equalize the temperatures. The heat capacity ΔCp
ΔT.

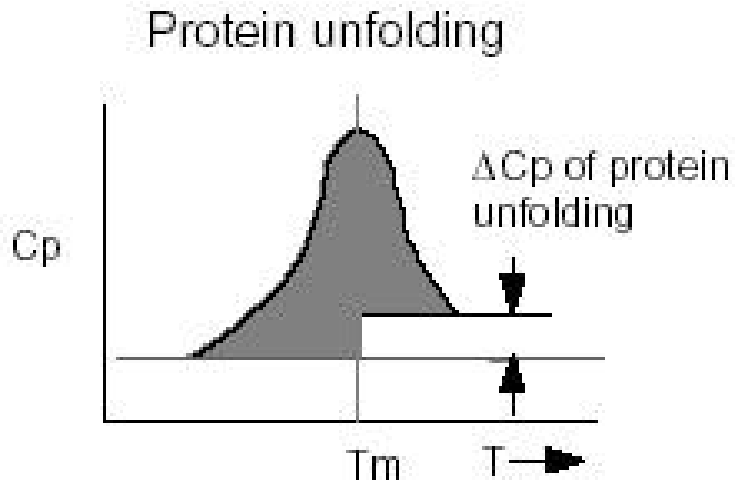
Determination of ΔH and ΔS from DSC

A schematic illustration of how DSC traces are integrated to obtain enthalpy and entrop



DSC of protein unfolding

As an example of how DSC can be used in a biologically relevant situation, consider the figur
elow which represents the typical thermal denaturation of a protein.



$$\Delta H^{\circ}_{cal} = \int_{T_1}^{T_2} \Delta C_p dT$$

$$\Delta S^{\circ} = \int_{T_1}^{T_2} \frac{\Delta C_p}{T} dT$$

$$T_M = \frac{\Delta H^{\circ}_{cal}}{\Delta S^{\circ}} \text{ (when } \Delta G^{\circ} = 0)$$

he shaded area represents the heat input to the system to unfold the protein; the difference
etween the baselines at low and high T represents the difference in heat capacities between
olded and unfolded forms.

xample:
rotein denaturation

ative (Folded) state
ow Δ H, low Δ S)

natured (unfolded) state
igh Δ H, high Δ S)

easure C_p and ΔH by
C
ferential scanning
rimeter)

licin E1 has 3 melting
ansitions, corresponding to
folding of 3 domains

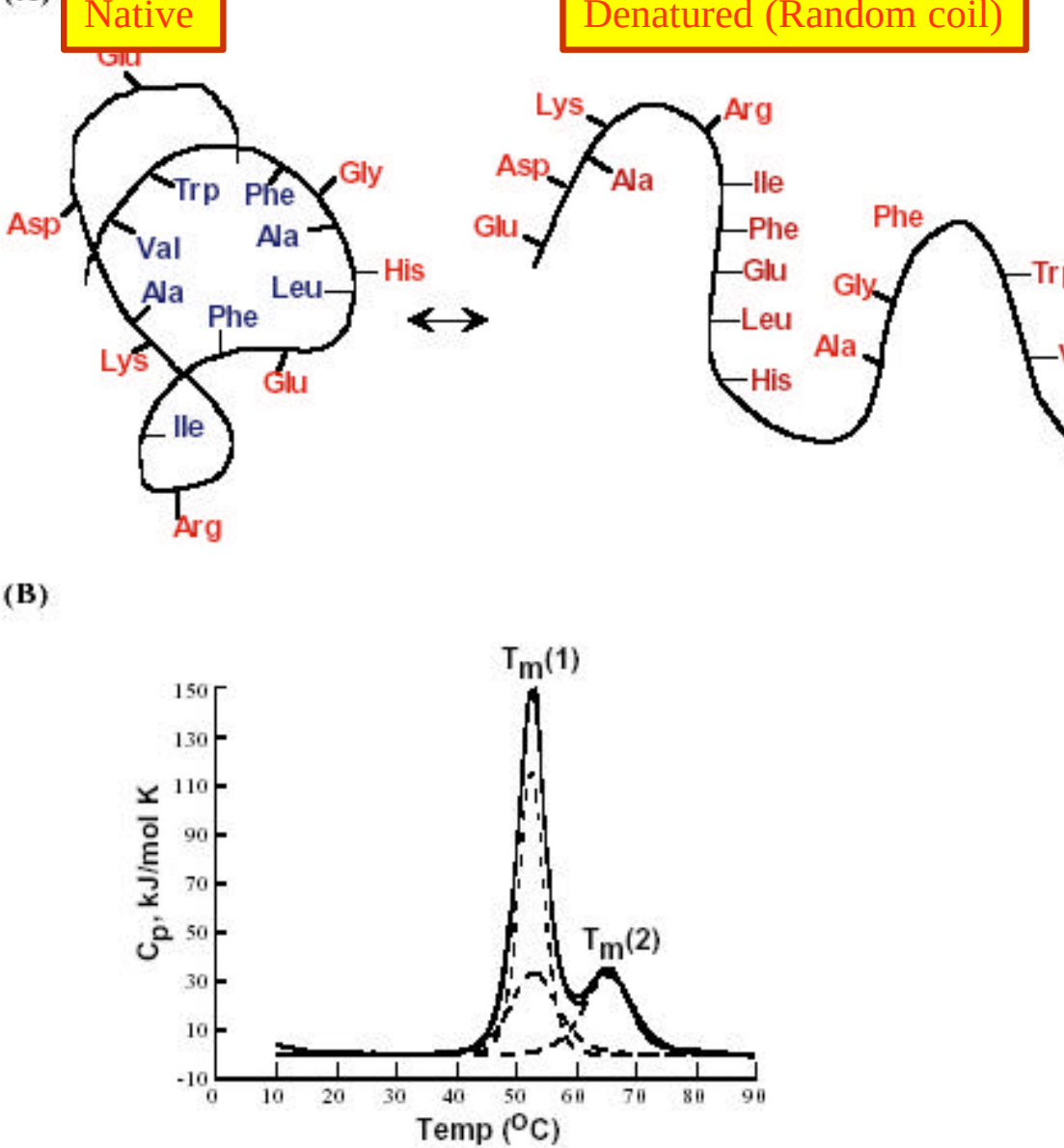
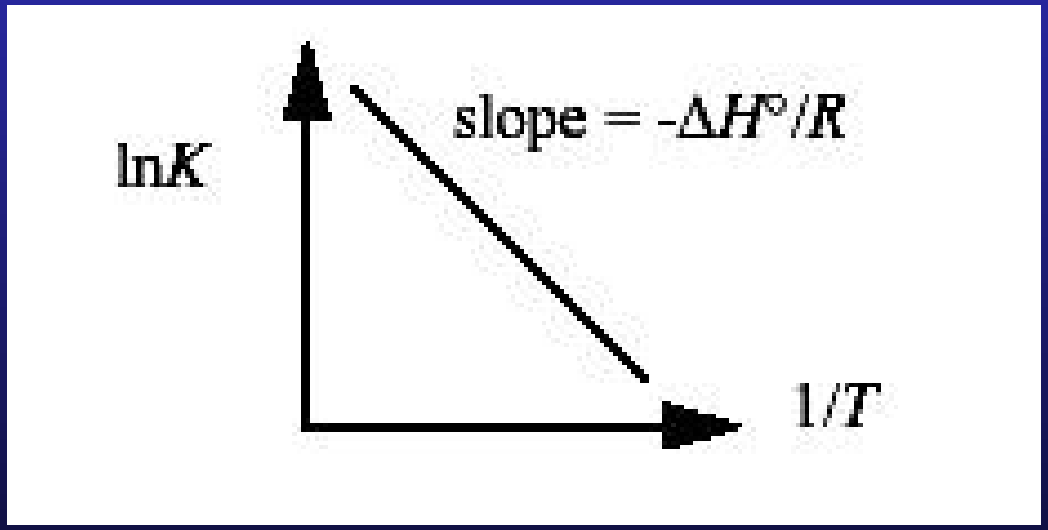


Fig.1-6. Protein denaturation. (A) Schematic diagram of the initial and final final states of
→ denatured transition; (B) Endothermic transitions associated with thermal denaturation
functional domains of colicin E1 modified from (Griko et al., 2000); deconvolution of th
endotherm into two melting transitions is shown. A representative value of ΔS° for
denaturation is ~100 cal/mol-°K.

$\Delta G^o = \Delta H^o - T\,\Delta S^o = RT\ln K$

➔ $\ln K = -\Delta H^o/RT + \Delta S^o/R = \Delta S^o/R - (\Delta H^o/R)(1/T)$

Measure K at different temperatures and determine ΔH (slope) and ΔS (intercept) from van't Hoff plot.



ΔH_{vH} and ΔH_{DSC} may not be the same if the process is cooperative or there are molecular interaction involved

Calculate the ΔG for ATP hydrolysis in a cell in which the [ATP]/[ADP] ratio had climbed to 100:1 while the P_i concentration remained at10 mM How does this compare to the ratio of [ATP]/[ADP] when the reaction i at equilibrium and P_i concentration remains at 10 mM ? What would be the value of ΔG when the reactants and prodcuts were all at standard state conditions of 1 M ?

Calculate the free energy released when $FADH_2$ is oxidized by molecular O_2 under standard conditions.

Of the following substances, ubiquinone, cytochrome c, NAD^+, NADH, O_2 H_2O, which is the strongest reducing agent ? Which is the strongest oxidizing agent ? Which has the greatest affinity for electrons ?

Suppose that you are able to manipulate th epotential of the inner membrane of an mitochondrion. You measure the pH of the mitochondrial matric and find it to be 8.0. You measure the bathing solution and find its pH to be 7.0. You clamp the inner membrane potential at +59 mV, i.e. you force the matrix to be 59 mV positive with respect to the bathing solution. Under these circumstances, can the mitochondrion use the proton gradient to drive the synthesis of ATP ?