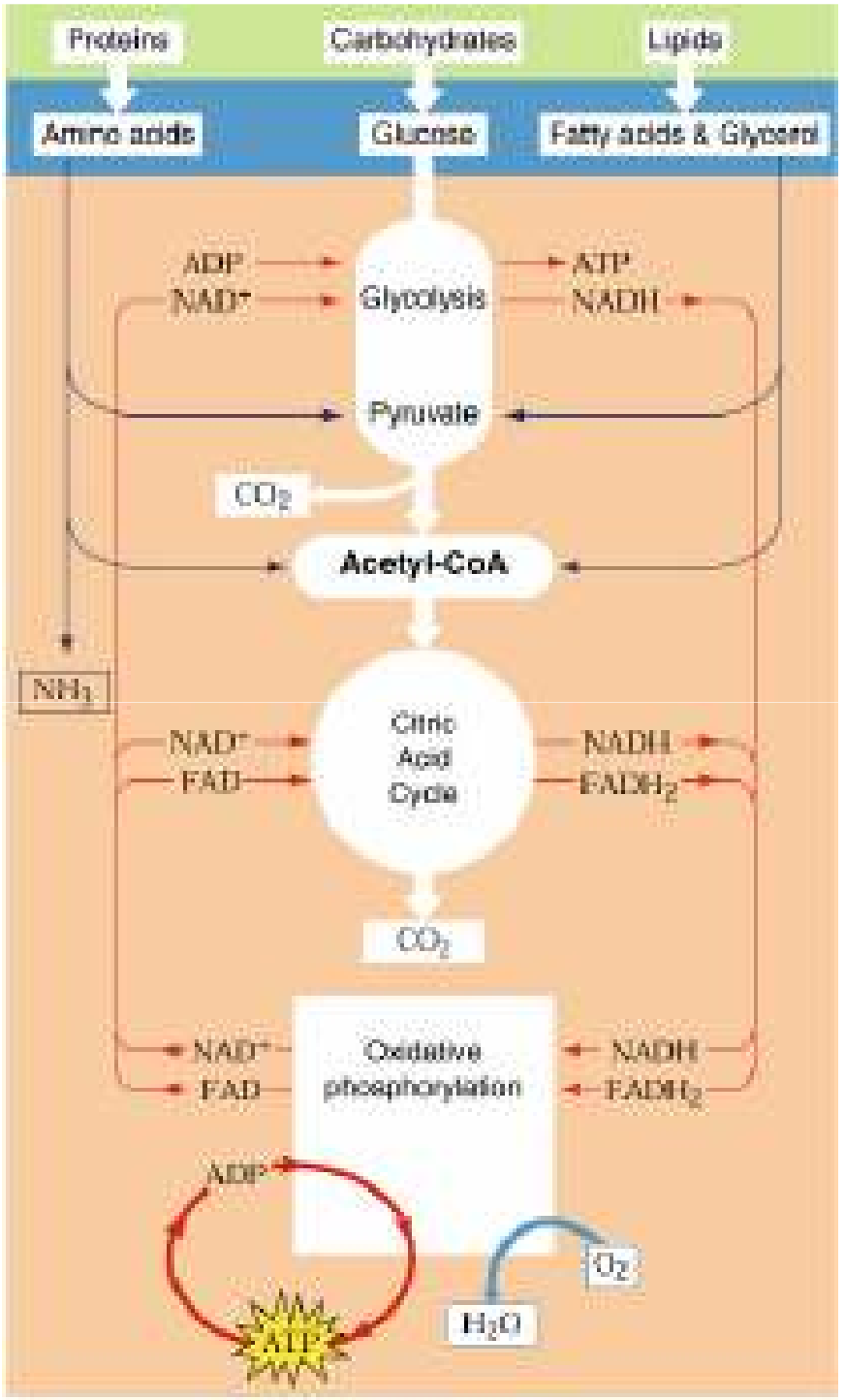
ELECTRON TRANSFER CHAIN

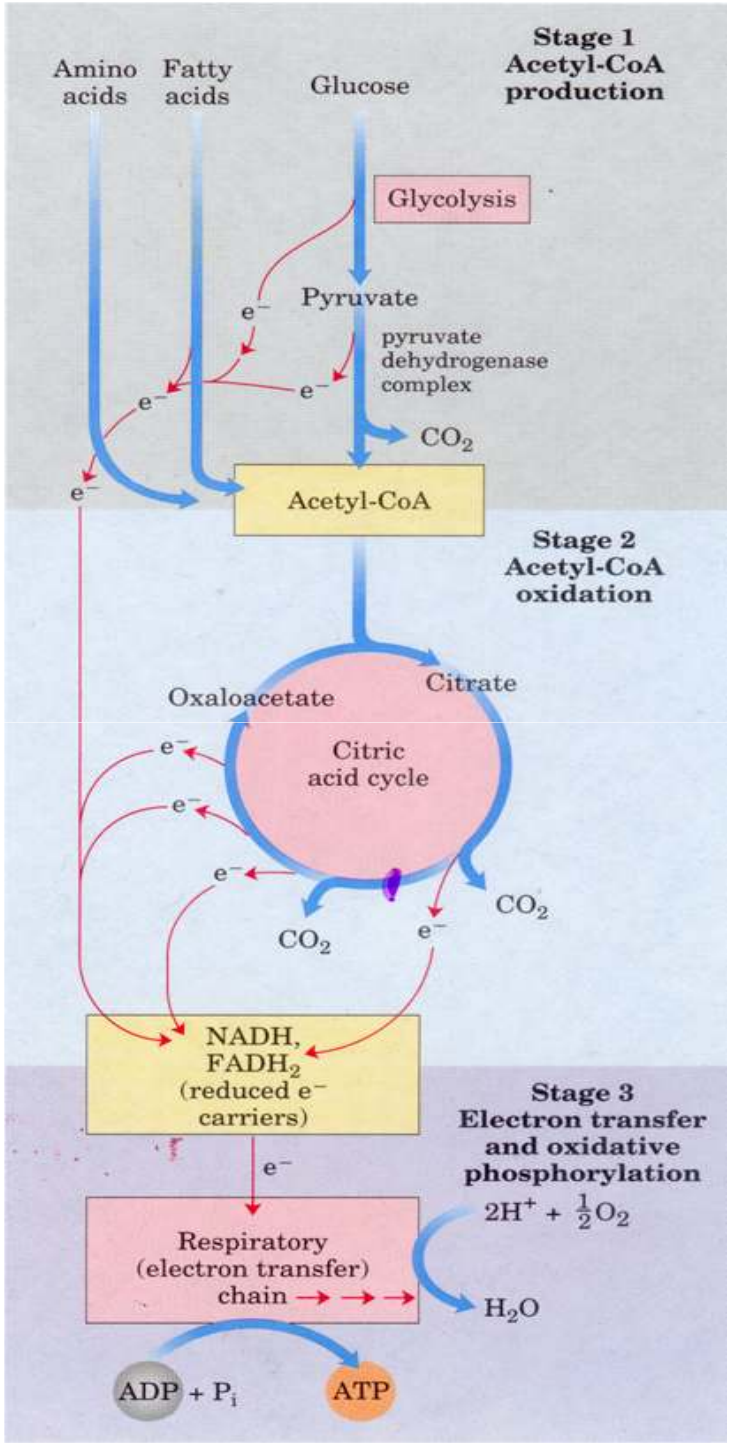
&

OXIDATIVE PHOSPHORYLATION

Željka Vukelić

OVERVIEW OF CATABOLISM





3 Stages of Catabolism

(substrate oxidation)

- Under aerobic conditions in the cell

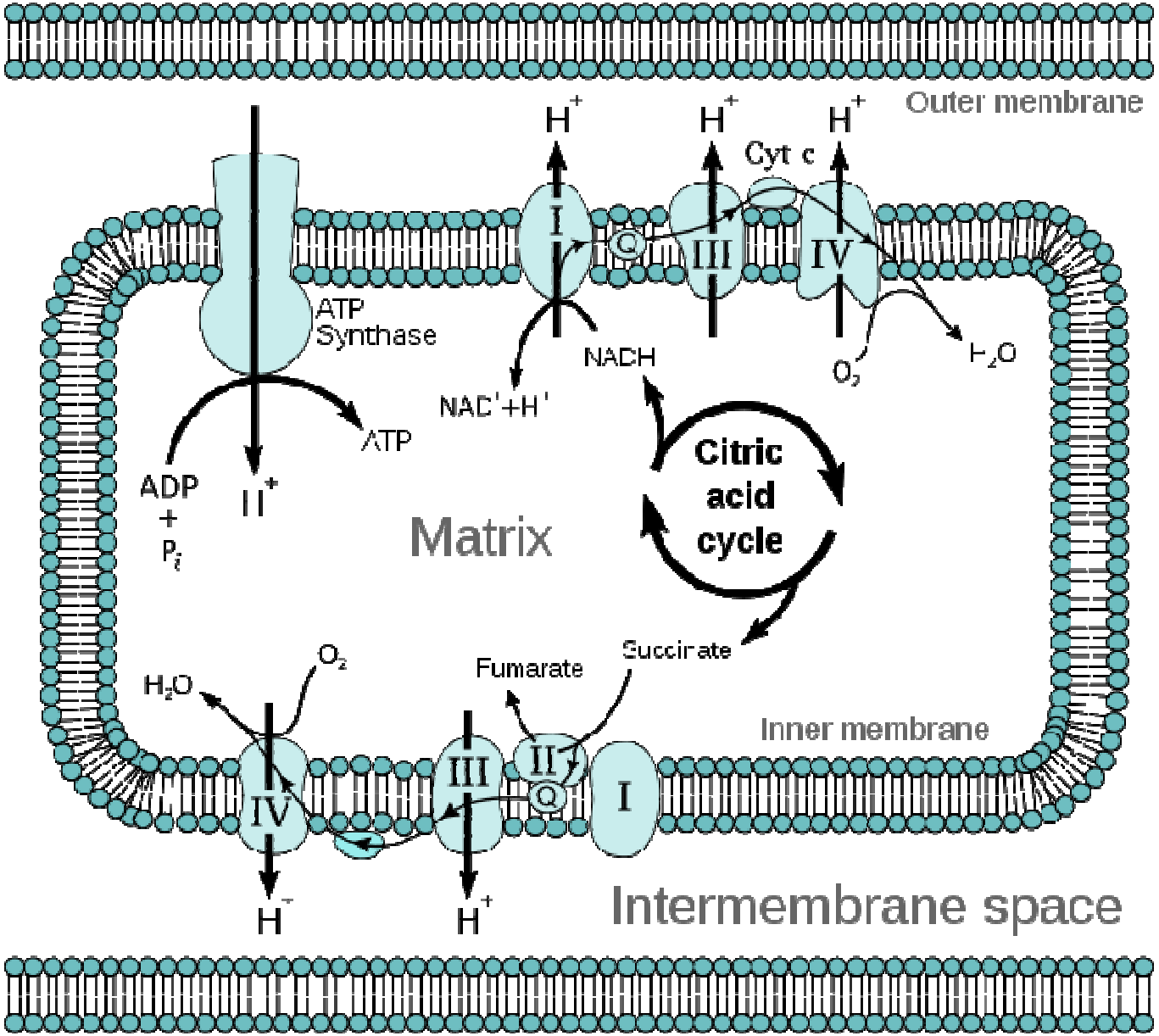
table 19–5

ATP Yield from Complete Oxidation of Glucose

Process	Direct product	Final ATP
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into mitochondria.

The electron transport chain and oxidative phosphorylation occur in the **MITOCHONDRION** in **eukaryotes**. The **NADH and $FADH_2$** (generated during oxidation of fuel molecules) **are oxidized, providing energy to power ATP synthesis.**



An **electron transport chain** couples a redox reaction between an **electron donor** (**NADH, $FADH_2$**) and an **electron acceptor** (**O_2**) to the **active transfer** of **H^+ ions** across the inner mitochondr. membrane, through a set of mediating redox reactions. Due to created **proton (H^+) gradient (electrochemical gradient)**, H^+ move back across the **ATP-synthase** (in the membrane). The **energy** from the proton gradient (**the proton-motive force**) is used to produce **ATP**.

ETC is used for **extracting and conserving energy** from **oxidation** of **fuel molecules**.

Standard Reduction Potentials of Some Biologically Important Half-Reactions, at 25 °C and pH 7

Half-reaction	E'° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome f (Fe^{3+}) + e^- ⟶ cytochrome f (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) + e^- ⟶ $Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 (Fe^{3+}) + e^- ⟶ cytochrome a_3(Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome a (Fe^{3+}) + e^- ⟶ cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + e^- ⟶ cytochrome c (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + e^- ⟶ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b (Fe^{3+}) + e^- ⟶ cytochrome b (Fe^{2+})	0.077
Ubiquinone + $2H^+ + 2e^-$ ⟶ ubiquinol + H_2	0.045
$Fumarate^{2-} + 2H^+ + 2e^- \longrightarrow succinate^{2-}$	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+ + 2e^-$ ⟶ butyryl-CoA	−0.015
$Oxaloacetate^{2-} + 2H^+ + 2e^- \longrightarrow malate^{2-}$	−0.166
$Pyruvate^- + 2H^+ + 2e^- \longrightarrow lactate^-$	−0.185
Acetaldehyde + $2H^+ + 2e^-$ ⟶ ethanol	−0.197
$FAD + 2H^+ + 2e^- \longrightarrow FADH_2$	−0.219*
Glutathione + $2H^+ + 2e^-$ ⟶ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid + $2H^+ + 2e^-$ ⟶ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
Acetoacetate + $2H^+ + 2e^-$ ⟶ β-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^-$ ⟶ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + e^- ⟶ ferredoxin (Fe^{2+})	−0.432

Standard state for the reaction in biological system is defined at pH=7 (Not at pH=0), i.e. at $a(H^+) = 10^{-7}$

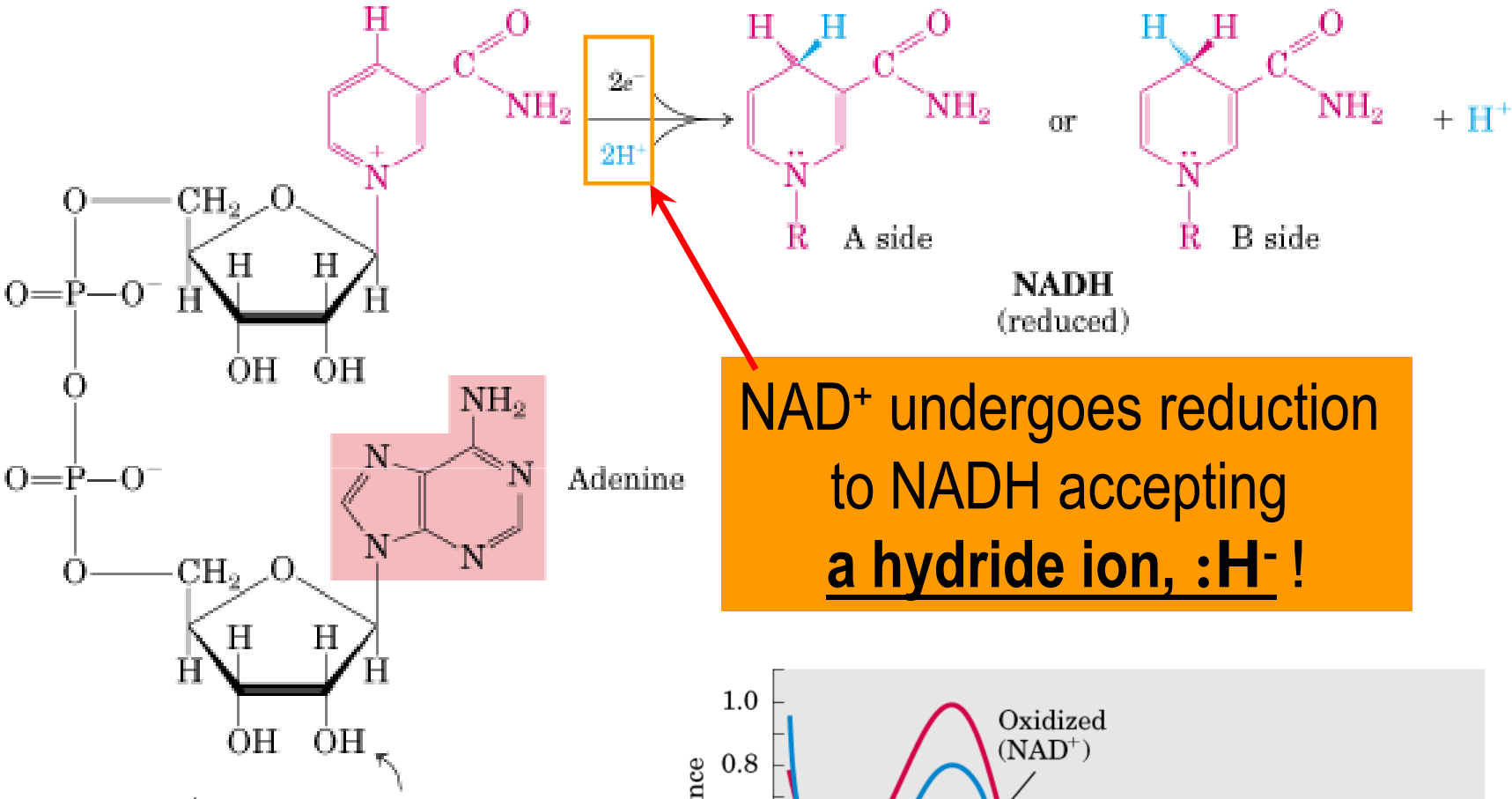
Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

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+})	0.55
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816

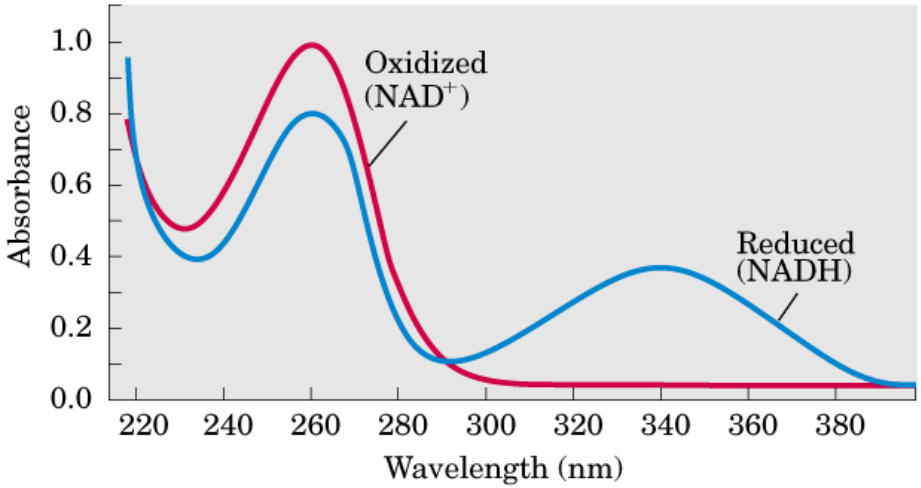
e^-

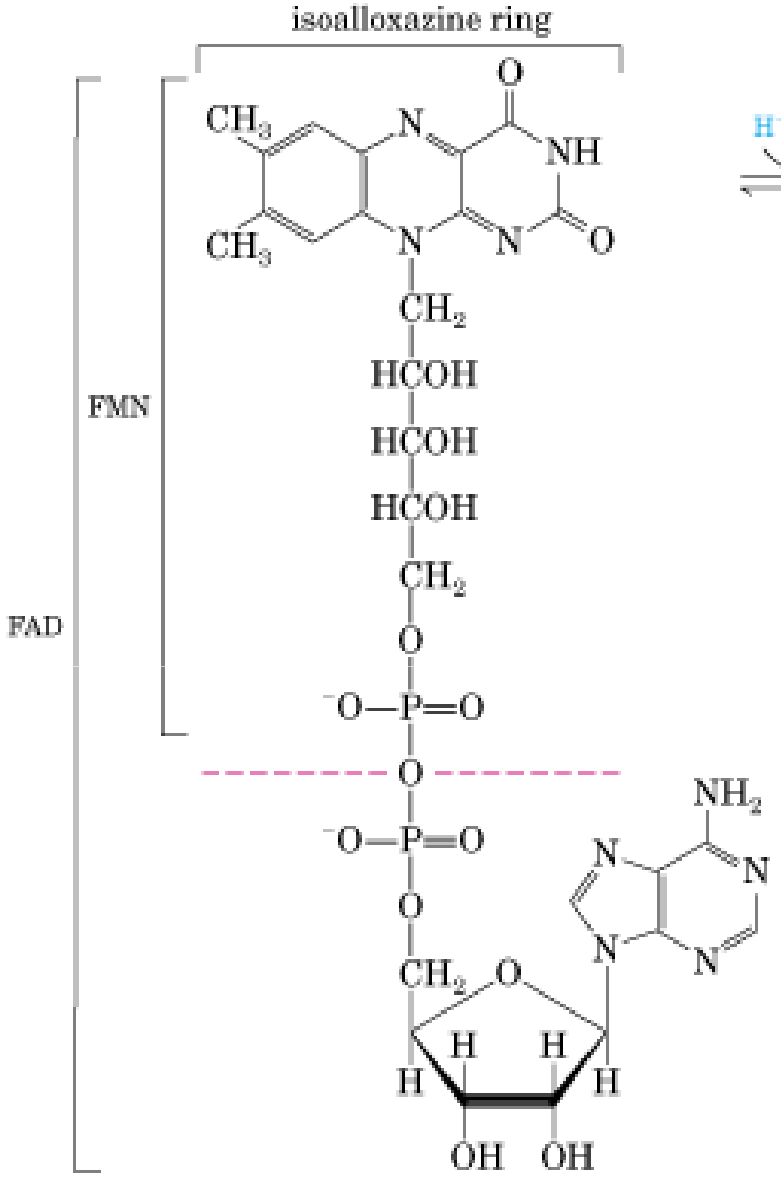
Key coenzymes as redox-systems...

$$NAD^+ + 2\,H^+ + 2\,e^- \rightarrow NADH + H^+, \qquad E_p'^{\ominus} = -0{,}32\ V$$

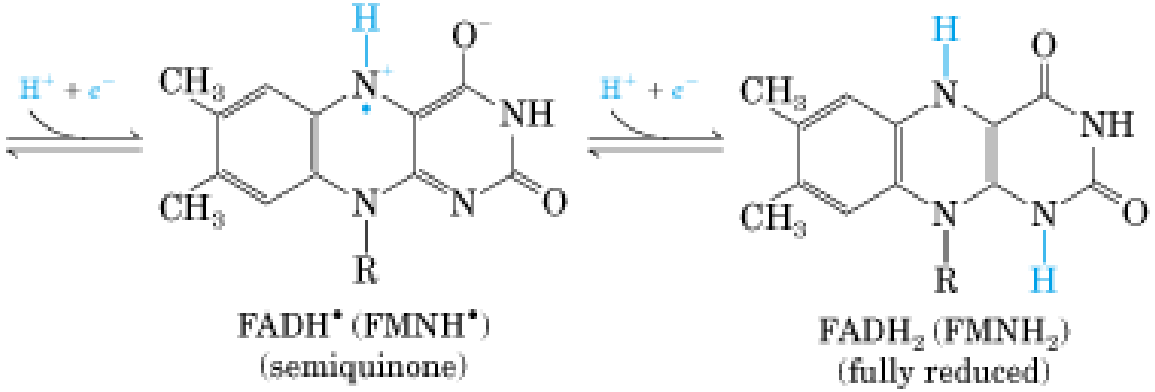


NAD⁺ undergoes reduction to NADH accepting **a hydride ion, :H⁻** !





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



$FADH^{\bullet}$ ($FMNH^{\bullet}$) (semiquinone)

$FADH_2$ ($FMNH_2$) (fully reduced)

$FAD/FADH_2$ REDOX-SYSTEM

The strongest oxidizing agent generally available in biological systems is

molecular oxygen!

$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O;$ $\quad E'^{\Theta} = +0{,}8147\ V$

(or $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O;$)

$NAD^+ + 2\ H^+ + 2\ e^- \rightarrow NADH + H^+,$ $\quad E_p'^{\Theta} = -\ 0{,}32\ V$

e^- donor

The **NET reaction of the respiratory chain:**

$$NADH + H^+ + \frac{1}{2}O_2 \rightarrow NAD^+ + H_2O$$

or $$2NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O$$

Calculate the standard free energy change for the reaction of electron transfer chain (respiratory chain)!

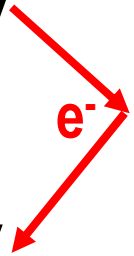
$$NADH + H^+ + \tfrac{1}{2}O_2 \rightarrow NAD^+ + H_2O$$

The half-reactions are:

$$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+ \qquad E'^{\ominus} = -0.320\ V$$

$$NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^-; \qquad -(E^{0'} = -0{,}320\ V)$$

$$\tfrac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O; \qquad E'^{\ominus} = +0{,}816\ V$$

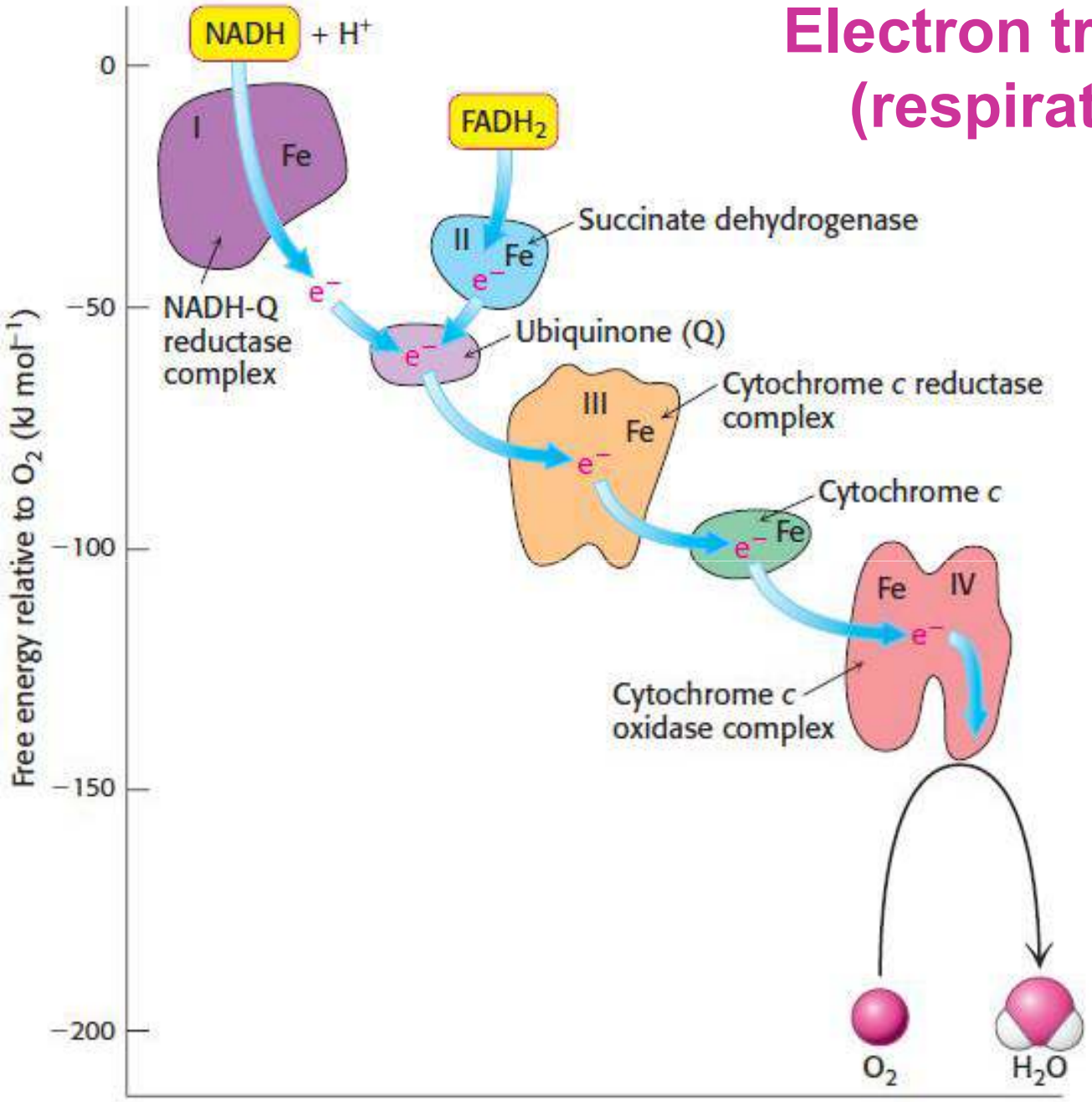


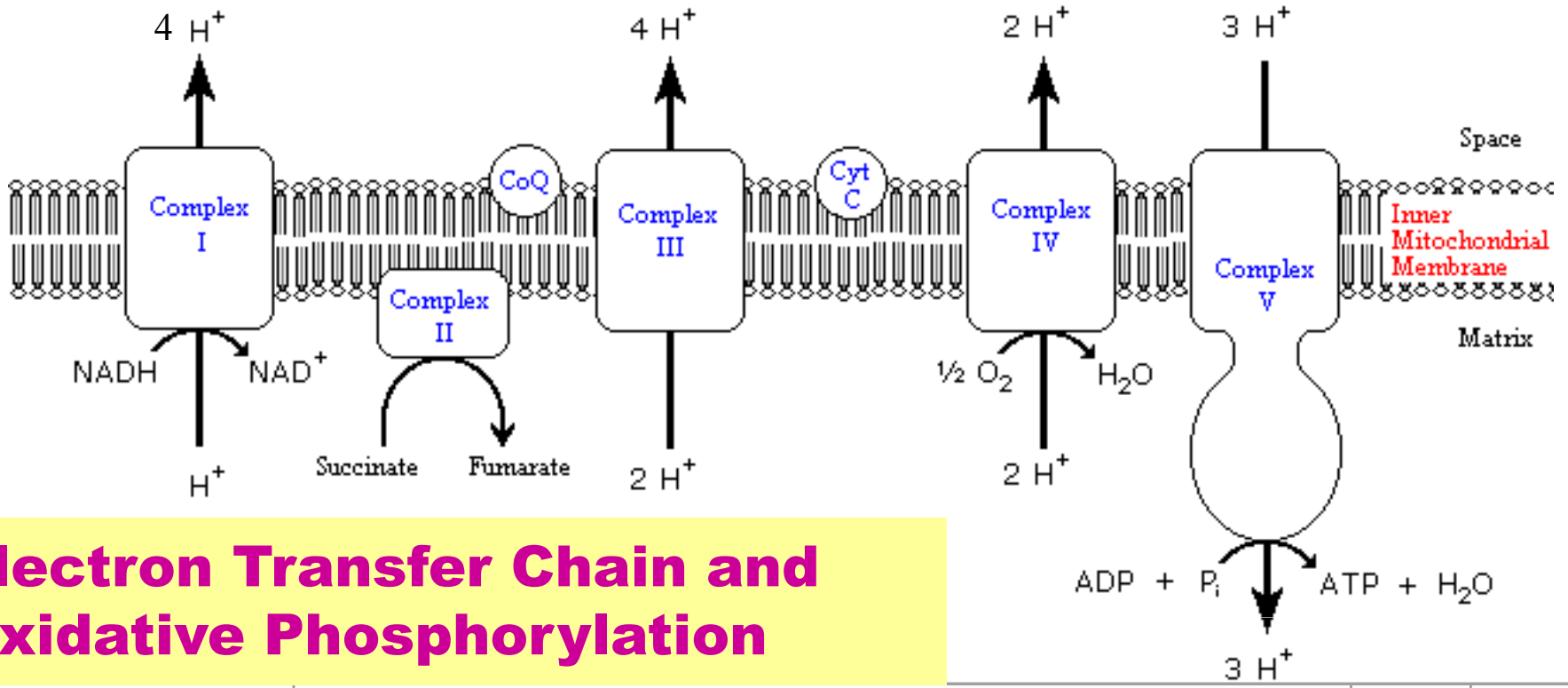
The potential difference (EMF) is:

$$\Delta E'^{\ominus} = 1.136\ V \quad \text{(or EMF)}$$

$$\Delta_r G'^{\ominus} = -\,z\,F\,\Delta E'^{\ominus} = -220\ kJ/mol$$

Electron transfer chain (respiratory chain)





Electron Transfer Chain and Oxidative Phosphorylation

Complex I	NADH dehydrogenase (or) NADH:ubiquinone oxidoreductase	kD 850	
Complex II	Succinate dehydrogenase (or) Succinate:ubiquinone oxidoreductase	140	
Complex III	Ubiquinone:cytochrome c oxidoreductase	250	
Complex IV	Cytochrome c oxidase	160	
Complex V	ATP synthase	380	

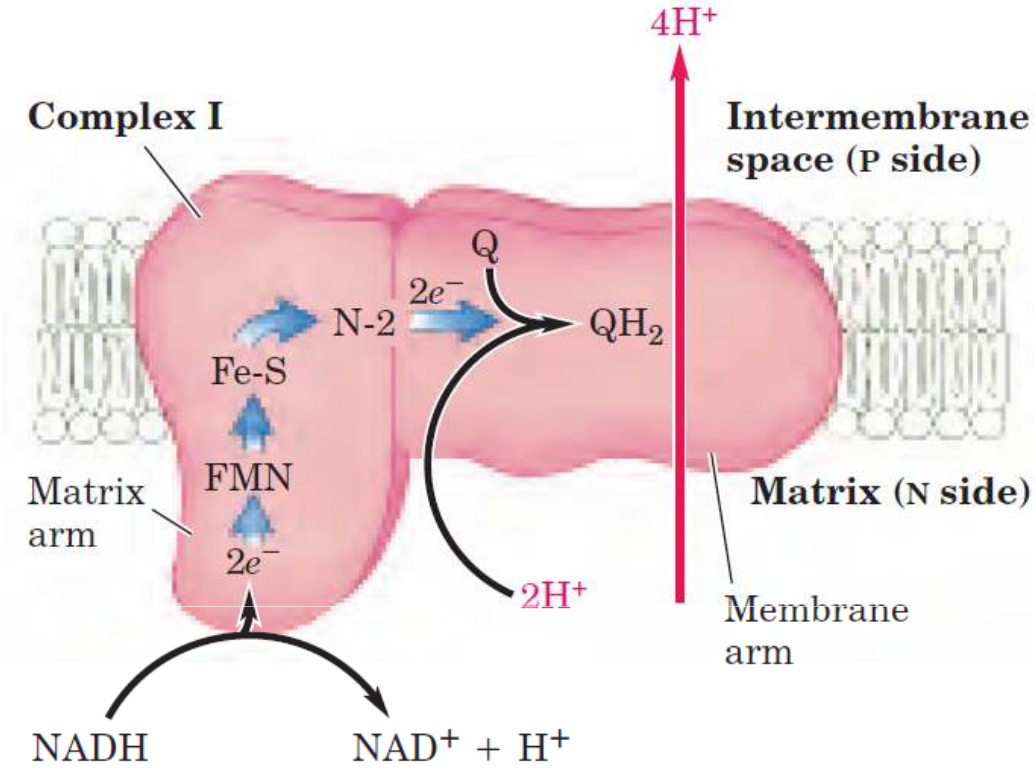
TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

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.

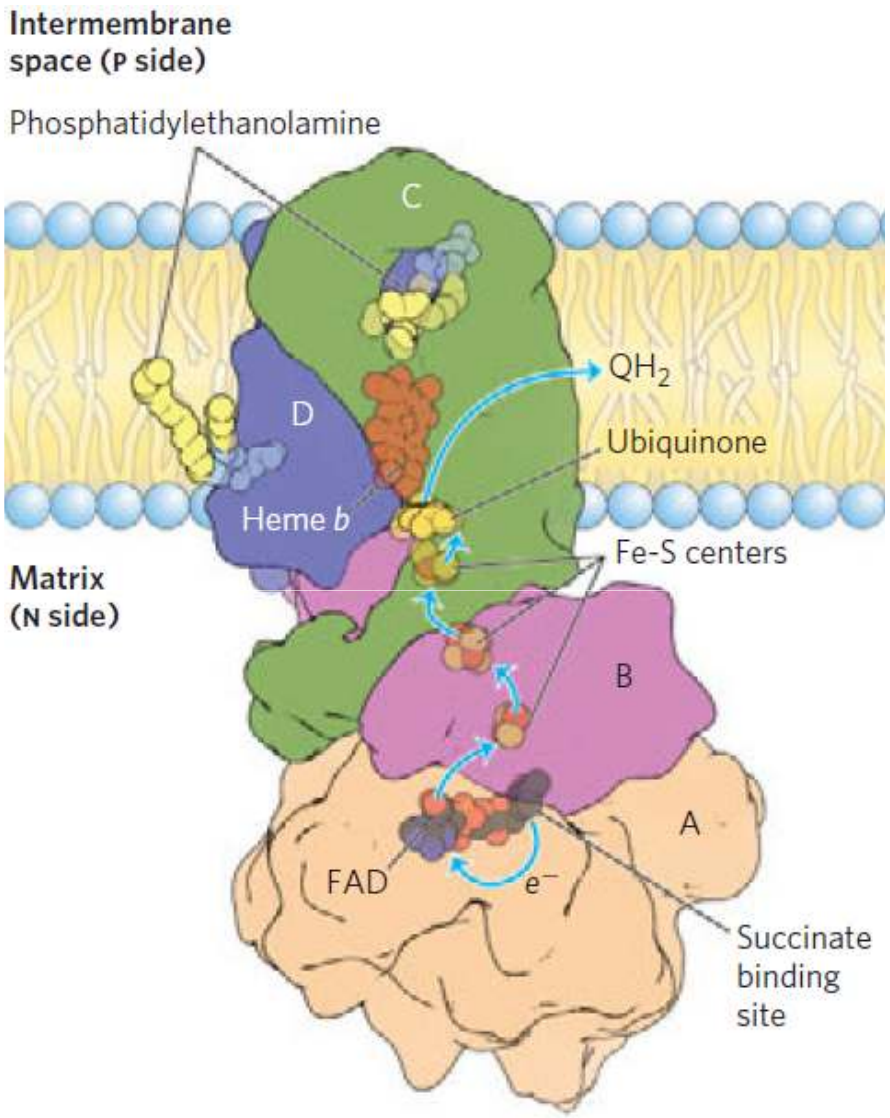
NADH:ubiquinone oxidoreductase (Complex I)



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

Complex I catalyzes the transfer of a hydride ion from NADH to FMN, from which two electrons pass through a series of Fe-S centers to the iron-sulfur protein N-2 in the matrix arm of the complex. **Electron transfer** from **N-2 to ubiquinone,** on the membrane arm **forms QH2, which diffuses into the lipid bilayer. This electron transfer also drives the expulsion from the matrix of four protons per pair of electrons.** The detailed mechanism that couples electron and proton transfer in Complex I is not yet known, but probably involves a Q cycle similar to that in Complex III in which QH_2 participates twice per electron pair. Proton flux produces an electrochemical potential across the inner mitoch. membrane (N side negative, P side positive), which conserves some of the energy released by the electron-transfer reactions. This electrochemical potential drives ATP synthesis.

Structure of Complex II (succinate dehydrogenase)



This complex (shown here is the porcine heart enzyme) has two transmembrane subunits, C and D; the cytoplasmic extensions contain subunits A and B. Just behind the **FAD** in subunit A is the binding site for succinate. Subunit B has three Fe-S centers, ubiquinone is bound to subunit B, and heme b is sandwiched between subunits C and D. Two phosphatidylethanolamine molecules are so tightly bound to subunit D that they show up in the crystal structure. **Electrons move** (blue arrows) **from succinate to FAD, then through the three Fe-S centers to ubiquinone.** The heme b is not on the main path of electron transfer but protects against the formation of reactive oxygen species (ROS) by electrons that go astray. FIGURE 19–10;

$$FADH_2 + Q \rightarrow FAD + QH_2$$

Net equation

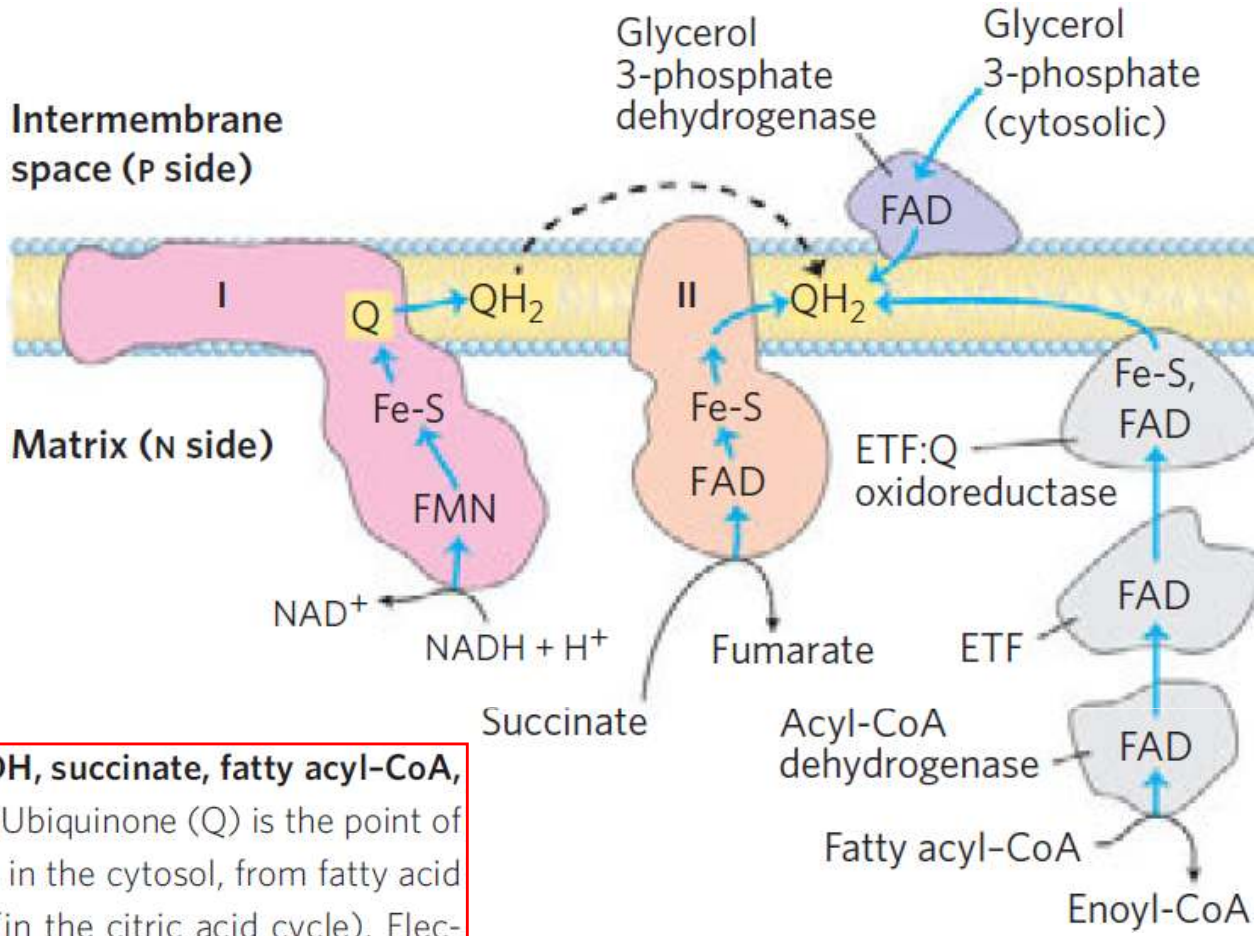


FIGURE 19–8 Path of electrons from NADH, succinate, fatty acyl-CoA, and glycerol 3-phosphate to ubiquinone. Ubiquinone (Q) is the point of entry for electrons derived from reactions in the cytosol, from fatty acid oxidation, and from succinate oxidation (in the citric acid cycle). Electrons from NADH pass through a flavoprotein with the cofactor FMN to a series of Fe-S centers (in Complex I) and then to Q. Electrons from succinate pass through a flavoprotein with the cofactor FAD and several Fe-S centers (in Complex II) on the way to Q. Glycerol 3-phosphate donates electrons to a flavoprotein (glycerol 3-phosphate dehydrogenase) on the outer face of the inner mitochondrial membrane, from which they pass to Q. Acyl-CoA dehydrogenase (the first enzyme of β oxidation) transfers electrons to electron-transferring flavoprotein (ETF), from which they pass to Q via ETF : ubiquinone oxidoreductase.

Complex III

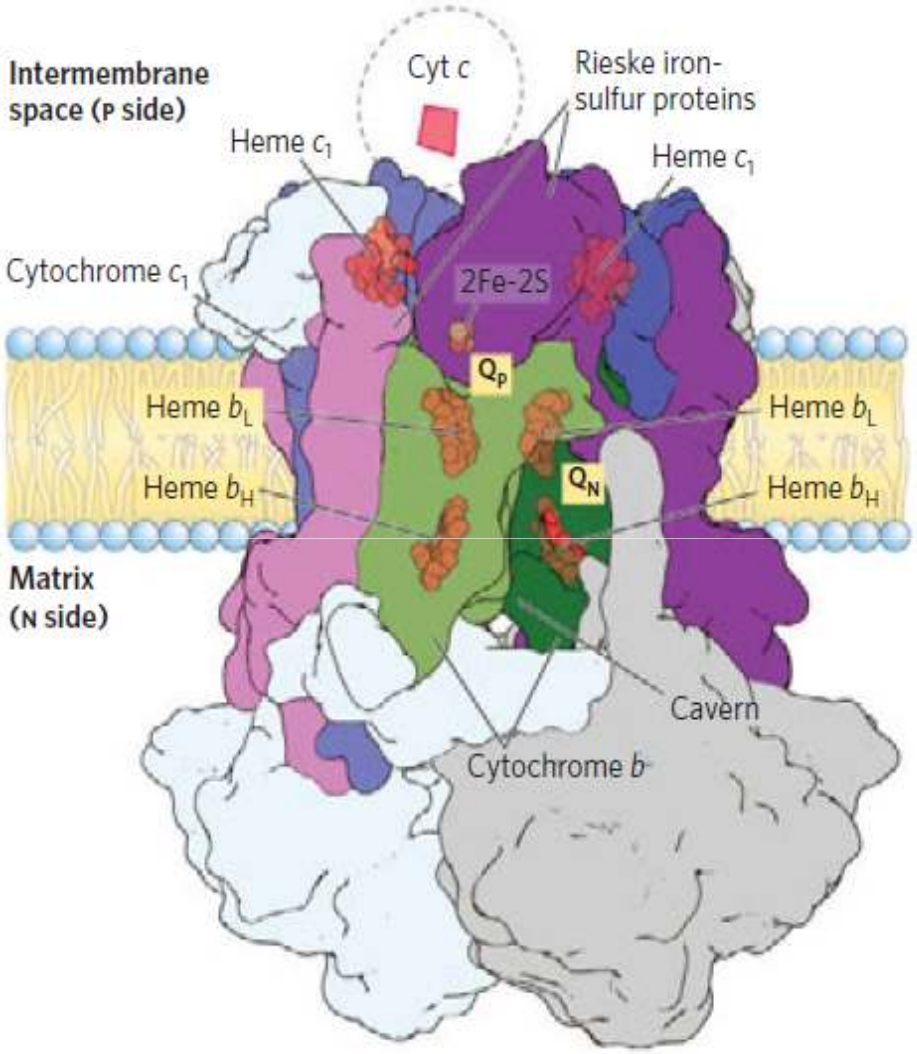
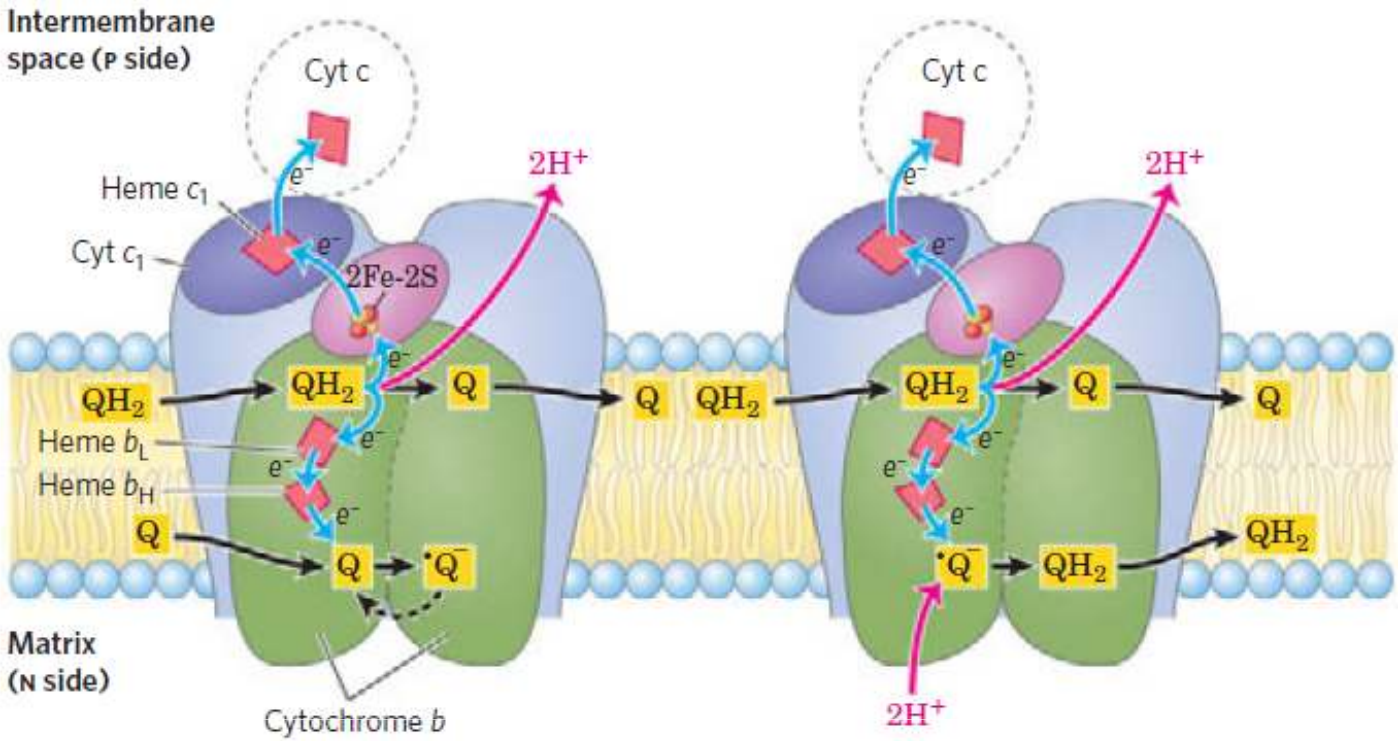


FIGURE 19–11 Cytochrome bc_1 complex (Complex III). (PDB ID 1BGY) The complex is a dimer of identical monomers, each with 11 different subunits. The functional core of each monomer is three subunits: cytochrome b (green) with its two hemes (b_H and b_L), the Rieske iron-sulfur protein (purple) with its 2Fe-2S centers, and cytochrome c_1 (blue) with its heme. This cartoon view of the complex shows how cytochrome c_1 and the Rieske iron-sulfur protein project from the P surface and can interact with cytochrome c (not part of the functional complex) in the intermembrane space. The complex has two distinct binding sites for ubiquinone, Q_N and Q_P, which correspond to the sites of inhibition by two drugs that block oxidative phosphorylation. Antimycin A, which blocks electron flow from heme b_H to Q, binds at Q_N, close to heme b_H on the N (matrix) side of the membrane. Myxothiazol, which prevents electron flow from QH_2 to the Rieske iron-sulfur protein, binds at Q_P, near the 2Fe-2S center and heme b_L on the P side. The dimeric structure is essential to the function of Complex III. The interface between monomers forms two caverns, each containing a Q_P site from one monomer and a Q_N site from the other. The ubiquinone intermediates move within these sheltered caverns.

Complex III crystallizes in two distinct conformations (not shown). In one, the Rieske Fe-S center is close to its electron acceptor, the heme of cytochrome c_1, but relatively distant from cytochrome b and the QH_2-binding site at which the Rieske Fe-S center receives electrons. In the other, the Fe-S center has moved away from cytochrome c_1 and toward cytochrome b. The Rieske protein is thought to oscillate between these two conformations as it is first reduced, then oxidized.

Complex III



$$QH_2 + Q + \text{cyt } c_1\,(\text{oxidized}) \longrightarrow Q + {}^{\bullet}Q^- + 2H_P^+ + \text{cyt } c_1\,(\text{reduced})$$

$$QH_2 + {}^{\bullet}Q^- + 2H_N^+ + \text{cyt } c_1\,(\text{oxidized}) \longrightarrow Q + 2H_P^+ + QH_2 + \text{cyt } c_1\,(\text{reduced})$$

$$\text{Net equation: } QH_2 + 2\ \text{cyt } c_1\,(\text{oxidized}) + 2H_N^+ \longrightarrow Q + 2\ \text{cyt } c_1\,(\text{reduced}) + 4H_P^+$$

FIGURE 19–12 The Q cycle, shown in two stages. The path of electrons through Complex III is shown by blue arrows. The movement of various forms of ubiquinone is shown with black arrows. In the first stage (left), Q on the N side is reduced to the semiquinone radical, which moves back into position to accept another electron. In the second stage (right), the semiquinone radical is converted to QH_2. Meanwhile, on the P side of the membrane, two molecules of QH_2 are oxidized to Q, releasing two protons per Q molecule (four protons in all) into the intermembrane space. Each QH_2 donates one electron (via the Rieske Fe-S center) to cytochrome c_1, and one electron (via cytochrome *b*) to a molecule of Q near the N side, reducing it in two steps to QH_2. This reduction also consumes two protons per Q, which are taken up from the matrix (N side). Reduced cyt c_1 passes electrons one at a time to cyt *c*, which dissociates and carries electrons to Complex IV.

Complex IV

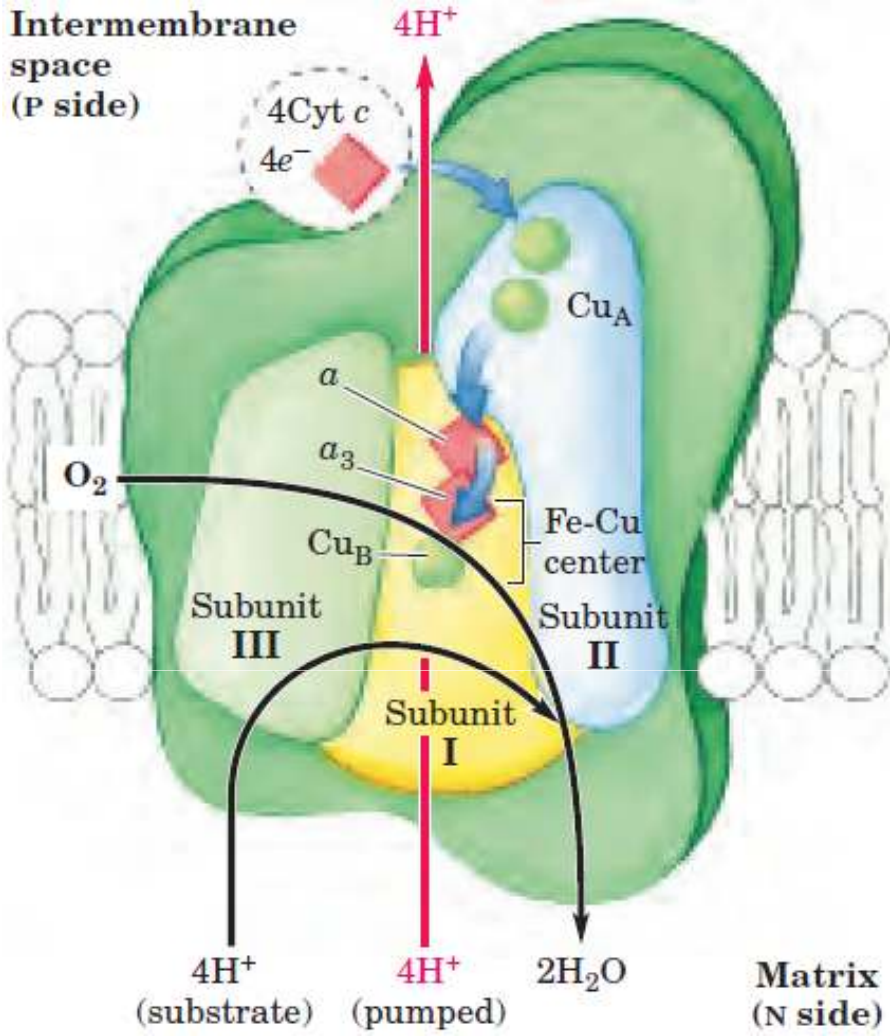
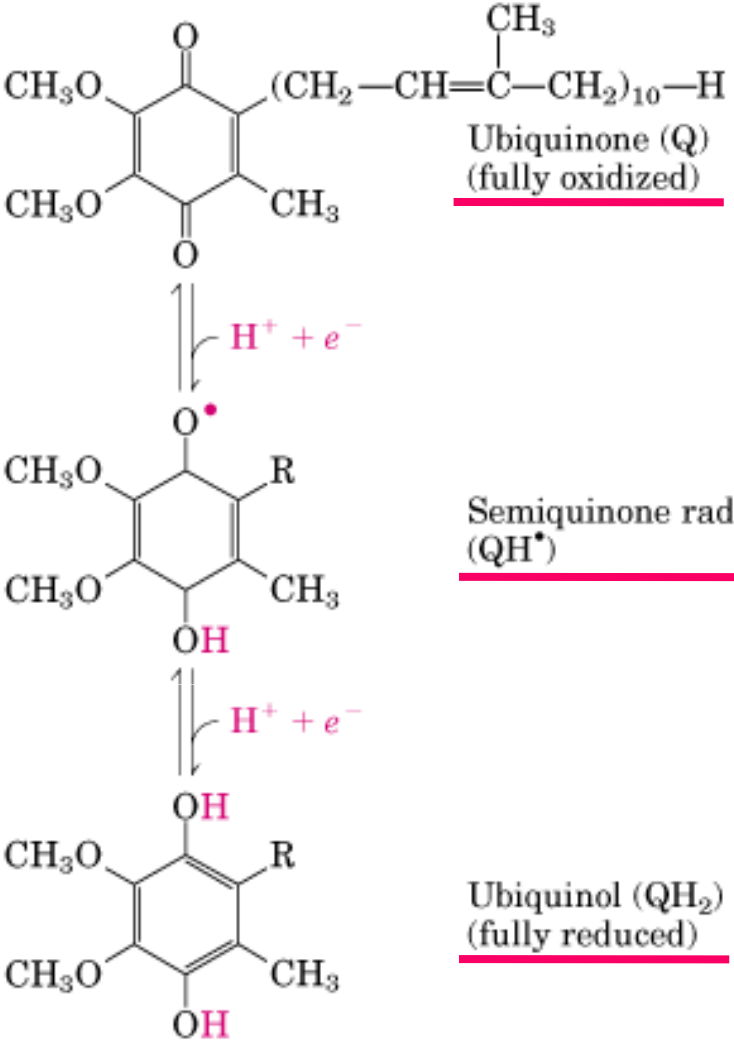


FIGURE 19–14 Path of electrons through Complex IV. The three proteins critical to electron flow are subunits I, II, and III. The larger green structure includes the other ten proteins in the complex. Electron transfer through Complex IV begins when two molecules of reduced cytochrome *c* (top) each donate an electron to the binuclear center Cu_A. From here electrons pass through heme *a* to the Fe-Cu center (cytochrome a_3 and Cu_B). Oxygen now binds to heme a_3 and is reduced to its peroxy derivative (O_2^{2-}) by two electrons from the Fe-Cu center. Delivery of two more electrons from cytochrome *c* (making four electrons in all) converts the O_2^{2-} to two molecules of water, with consumption of four "substrate" protons from the matrix. At the same time, four more protons are pumped from the matrix by an as yet unknown mechanism.

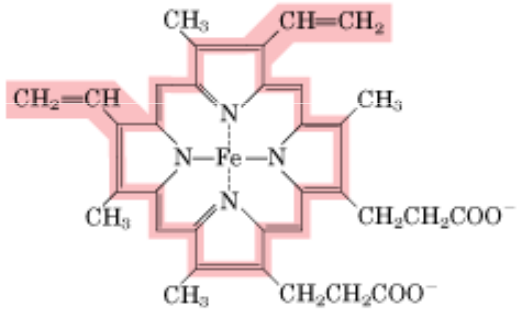
Net equation:

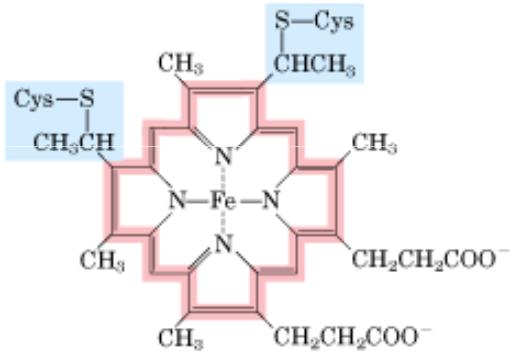
$$4\ \text{Cyt}\ c\ (\text{reduced}) + 8H^+_N + O_2 \longrightarrow 4\ \text{cyt}\ c\ (\text{oxidized}) + 4H^+_P + 2H_2O$$

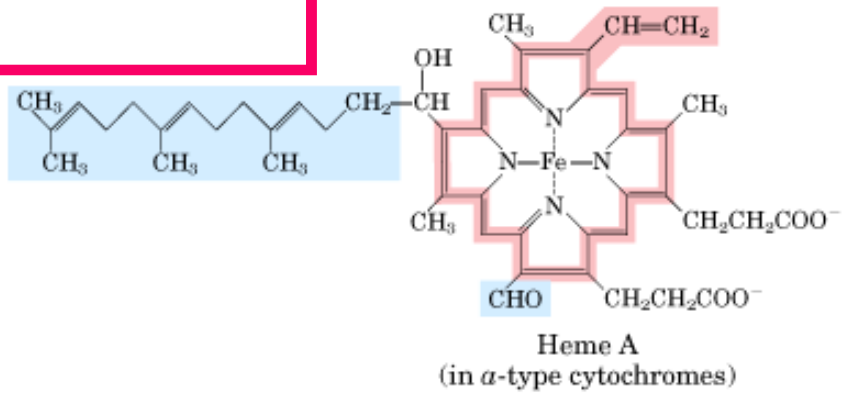
Ubiquinone (coenzyme Q)



Cytocrome c contains heme c

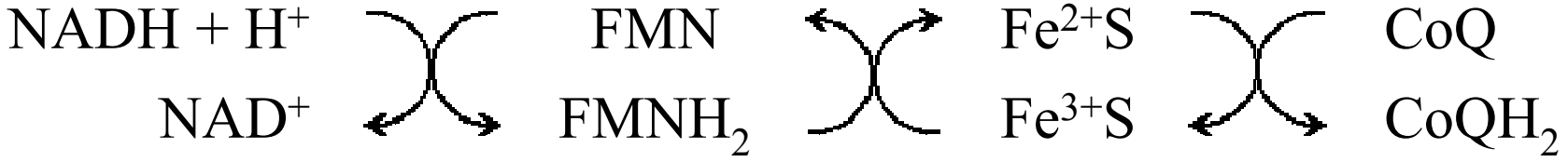




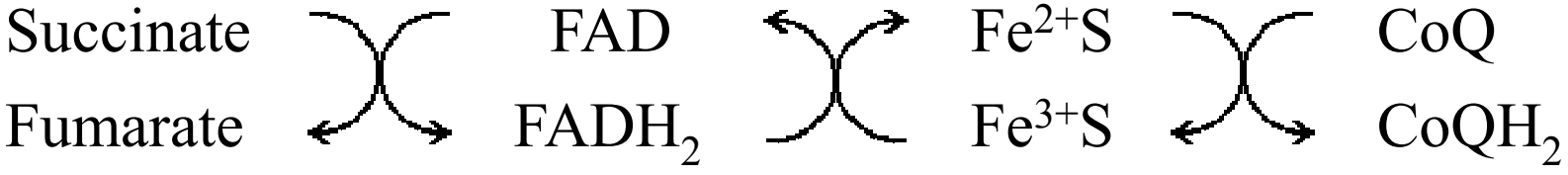


Prosthetic groups of cytochromes

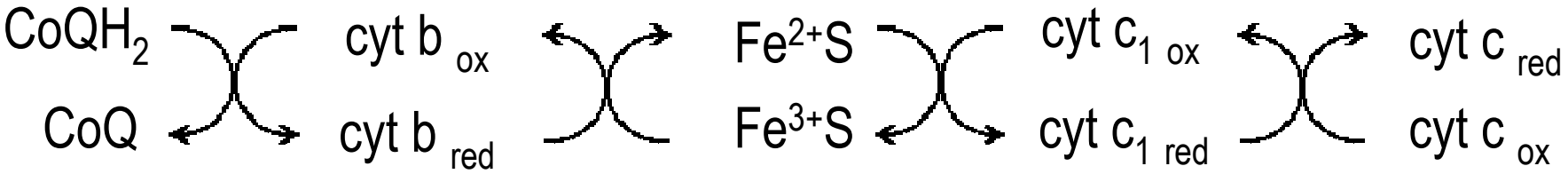
Complex I



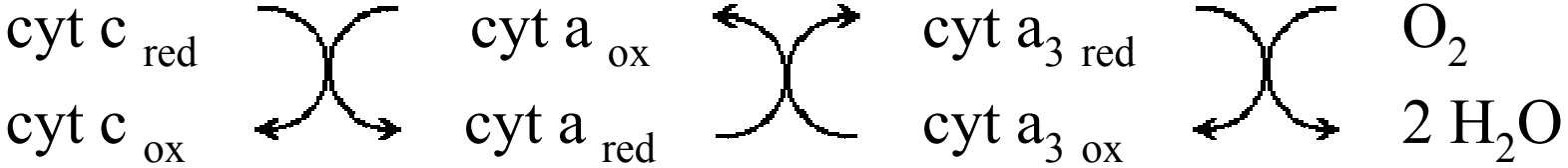
Complex II



Complex III



Complex IV



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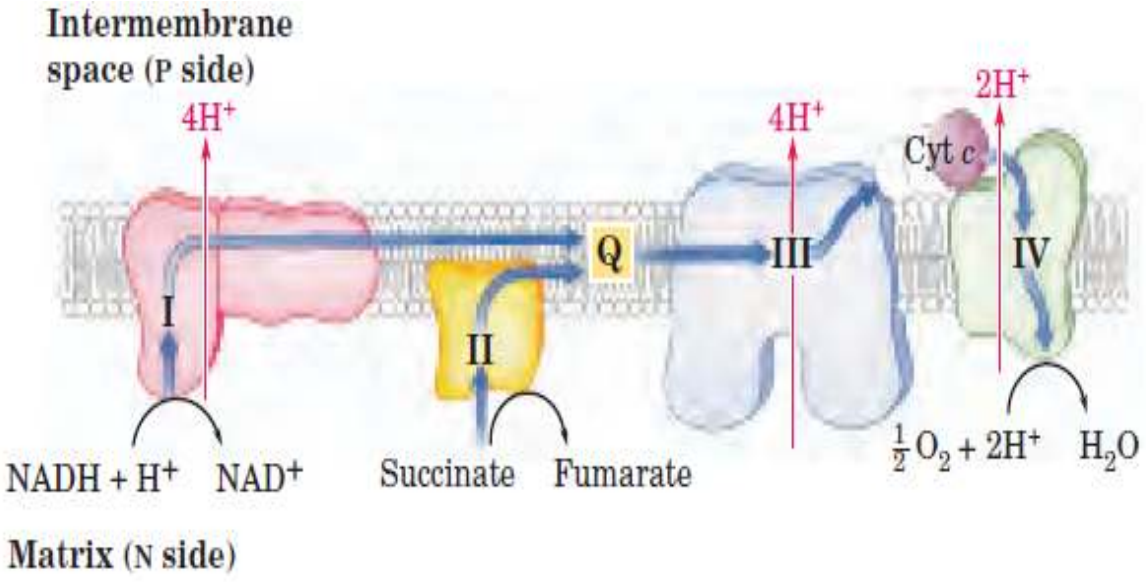


FIGURE 19–15 Summary of the flow of electrons and protons through the four complexes of the respiratory chain. Electrons reach Q through Complexes I and II. QH_2 serves as a mobile carrier of electrons and protons. It passes electrons to Complex III, which passes them to another mobile connecting link, cytochrome *c*. Complex IV then transfers electrons from reduced cytochrome *c* to O_2. Electron flow through Complexes I, III, and IV is accompanied by proton flow from the matrix to the intermembrane space. Recall that electrons from β oxidation of fatty acids can also enter the respiratory chain through Q (see Fig. 19–8).

Much of this energy is used to pump protons out of the matrix. For each pair of electrons transferred to O_2, four protons are pumped out by Complex I, four by Complex III, and two by Complex IV (Fig. 19–15). The *vectorial* equation for the process is therefore

$$NADH + 11H_N^+ + \tfrac{1}{2}O_2 \longrightarrow NAD^+ + 10H_P^+ + H_2O \qquad (19\text{–}7)$$

The electrochemical energy inherent in this difference in proton concentration and separation of charge represents a temporary conservation of much of the energy of electron transfer. The energy stored in such a gradient, termed the **proton-motive force,** has two components: (1) the *chemical potential energy* due to the difference in concentration of a chemical species (H^+) in the two regions separated by the membrane, and (2) the *electrical potential energy* that results from the separation of charge when a proton moves across the membrane without a counterion (Fig. 19–16).

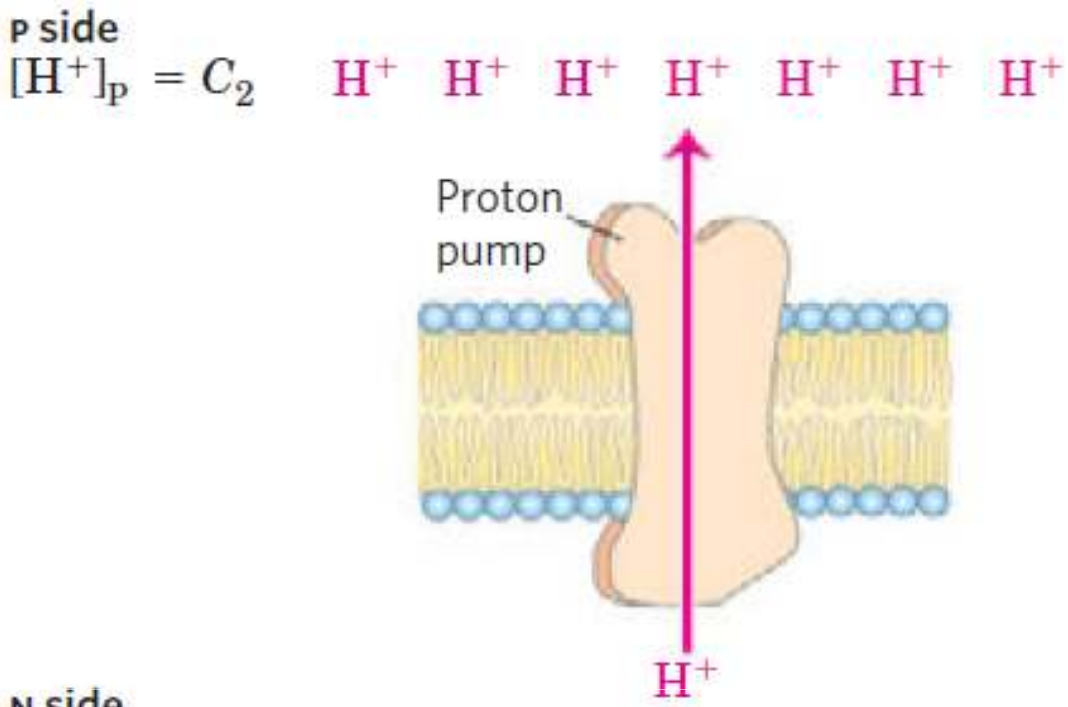
!!! !!!

As we showed in Chapter 11, the free-energy change for the creation of an electrochemical gradient by an ion pump is

!!!

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

where C_2 and C_1 are the concentrations of an ion in two regions, and $C_2 > C_1$; Z is the absolute value of its electrical charge (1 for a proton), and $\Delta\psi$ is the transmembrane difference in electrical potential, measured in volts.



$$\Delta G = RT \ln (C_2/C_1) + Z\mathcal{F}\Delta\psi$$
$$= 2.3RT\ \Delta\text{pH} + \mathcal{F}\Delta\psi$$

FIGURE 19–17 Proton-motive force. The inner mitochondrial membrane separates two compartments of different $[H^+]$, resulting in differences in chemical concentration (ΔpH) and charge distribution ($\Delta\psi$) across the membrane. The net effect is the proton-motive force (ΔG), which can be calculated as shown here. This is explained more fully in the text.

ATP SYNTHESIS – THE CHEMIOSMOTIC MODEL

How is a concentration gradient of protons transformed into ATP?

We have seen that electron transfer releases, and the proton-motive force conserves, more than enough free energy (about 200 kJ) per "mole" of electron pairs to drive the formation of a mole of ATP, which requires about 50 kJ (p. 519).

Mitochondrial oxidative phosphorylation therefore poses no thermodynamic problem.

But what is the chemical mechanism that couples proton flux with phosphorylation?

The **chemiosmotic model**, proposed by Peter Mitchell, is the paradigm for this mechanism.

According to the model, **the electrochemical energy** inherent in the difference in proton concentration and the separation of charge across the inner mitochondrial membrane — **the proton-motive force** — **drives the synthesis of ATP as protons flow** passively **back into the matrix** through **a proton pore in ATP synthase**. **(Fig. 19-19)**

To emphasize this crucial **role of the proton-motive force**, the equation for ATP synthesis is sometimes written

$$\mathrm{ADP} + \mathrm{P_i} + n\mathrm{H_P^+} \longrightarrow \mathrm{ATP} + \mathrm{H_2O} + n\mathrm{H_N^+}$$

Mitchell used "chemiosmotic" to describe enzymatic reactions that involve, simultaneously, a chemical reaction and a transport process, and the overall process is sometimes referred to as **"chemiosmotic coupling"**. **Coupling** refers to the obligate connection between mitochondrial ATP synthesis and electron flow through the respiratory chain; neither of the two processes can proceed without the other.

Chemiosmotic model

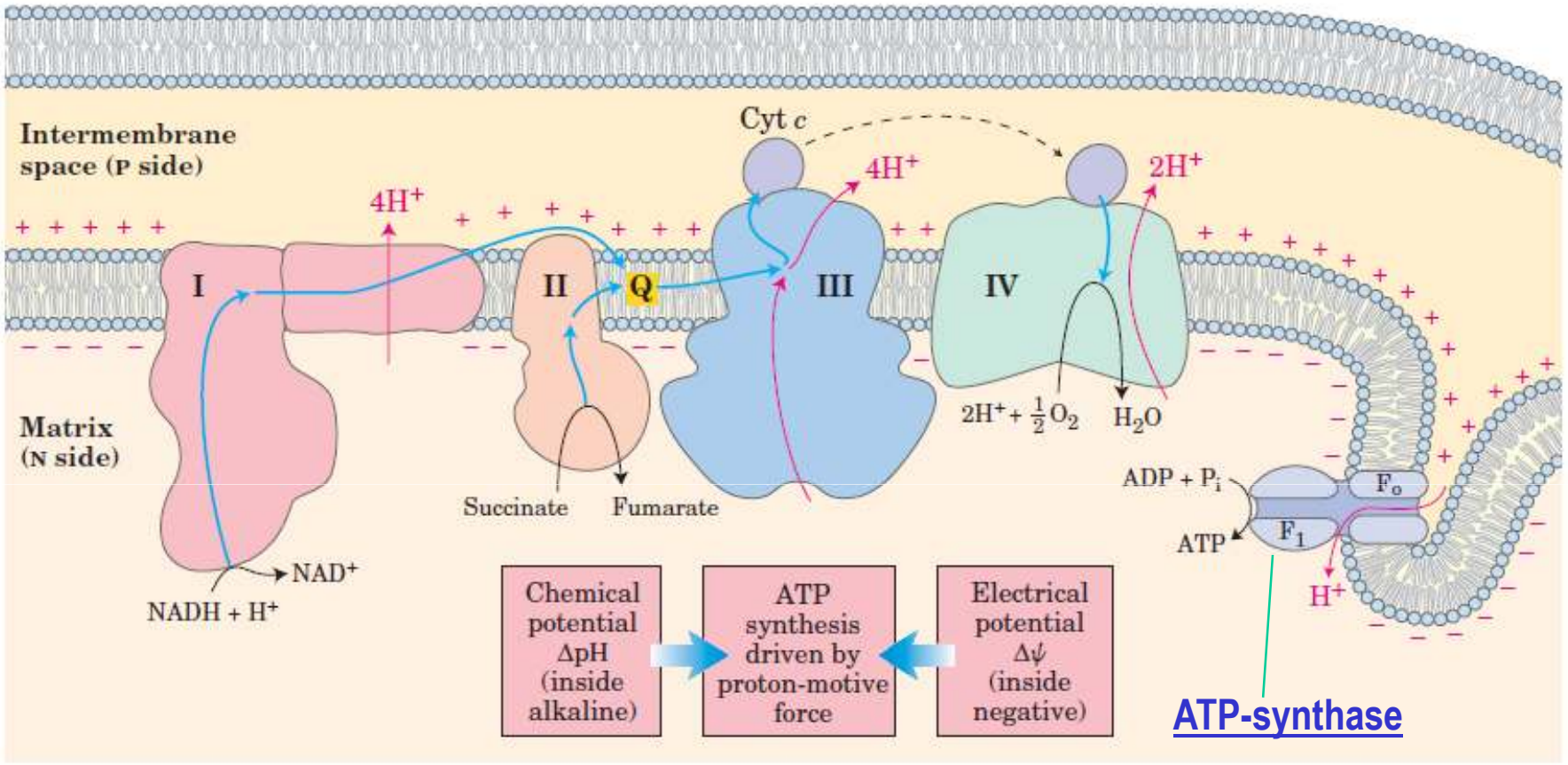
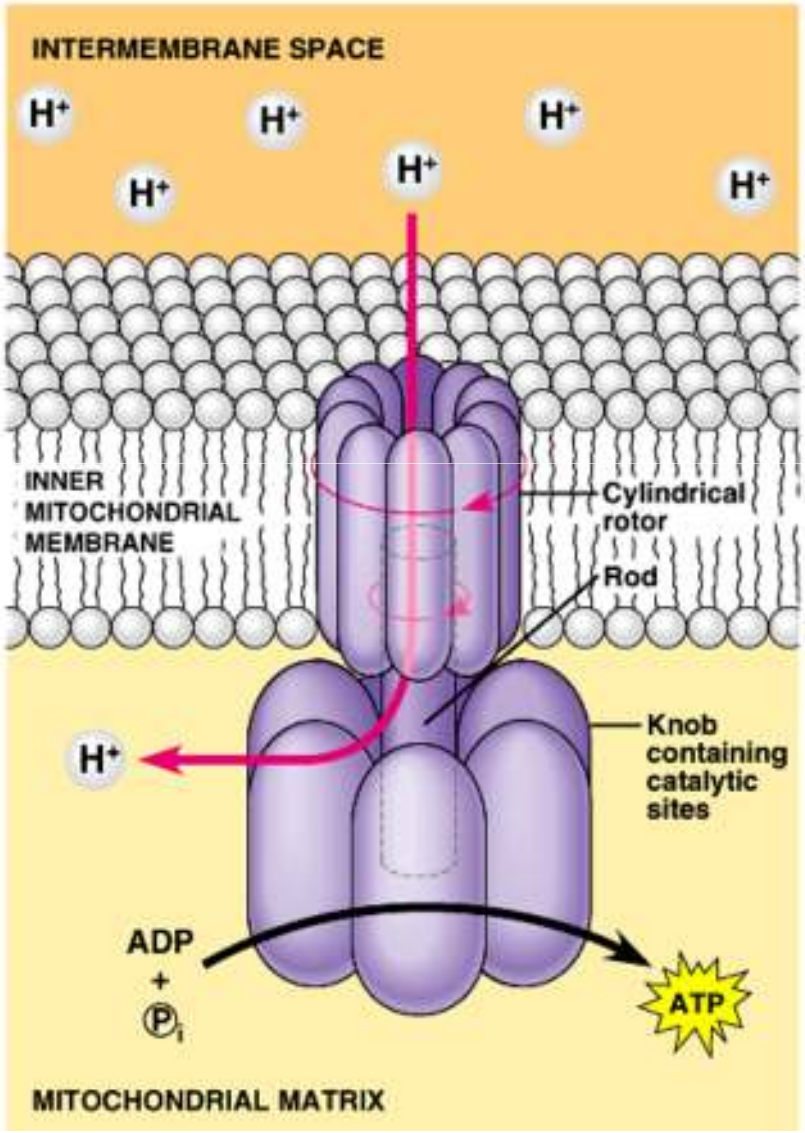
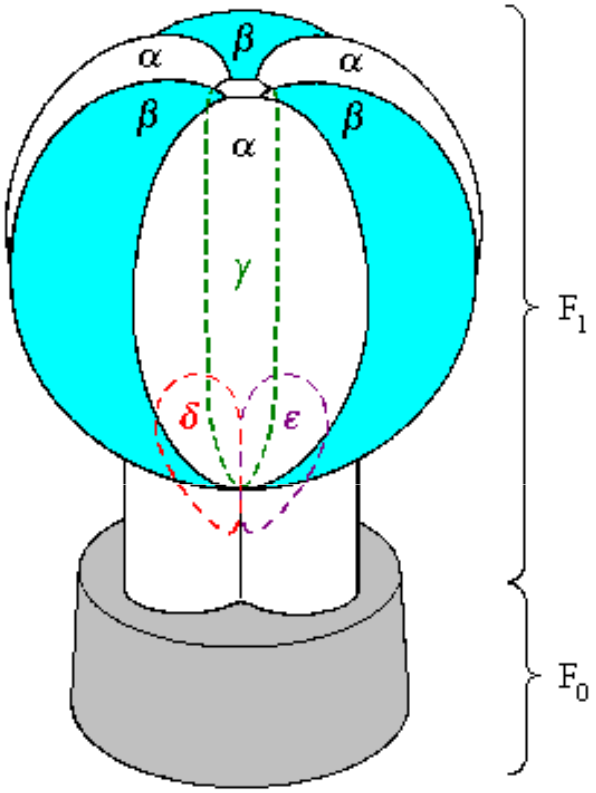


FIGURE 19–19 Chemiosmotic model. In this simple representation of the chemiosmotic theory applied to mitochondria, electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner membrane. Electron flow is accompanied by proton transfer across the membrane, producing both a chemical gradient (ΔpH) and an electrical gradient ($\Delta\psi$) (combined, the proton-motive force). The inner mitochondrial membrane is impermeable to protons; protons can reenter the matrix only through proton-specific channels (F_o). The proton-motive force that drives protons back into the matrix provides the energy for ATP synthesis, catalyzed by the F_1 complex associated with F_o.

Chemistry of ATP-synthase (Complex V)





Complexes **I, III, and IV** all **"pump" protons (H^+)** into the intermembrane space between the inner and outer mitoch. membrane, **establishing a proton gradient (electrical and concentration gradient) across the inner mitoch. membrane. As the protons pass through ATP-synthase, the proton-motive force (osmotic energy) of the gradient is converted into chemical energy, in the form of ATP. The use of this transmembrane proton gradient, created by the exergonic redox reactions occurring between Complex I and Complex IV, to drive the endergonic reaction of ATP synthesis is known as *chemiosmotic coupling.***

Chemiosmotic coupling is achieved through the unique structure of ATP-synthase. The enzyme complex is composed of two distinct protein component: F_1 and F_0. F_0 has a proton pore through which protons leak (passively flow into the mitoch. matrix). The F_1 consists of a knob-like structure, which is attached to stalk proteins, linked to the F_0 base. The F_1 has multiple subunits, 3 alpha, 3 beta, 1 gamma, 1 delta, and 1 epsilon. The site of ATP synthesis is the beta subunit.

The P/O ratio

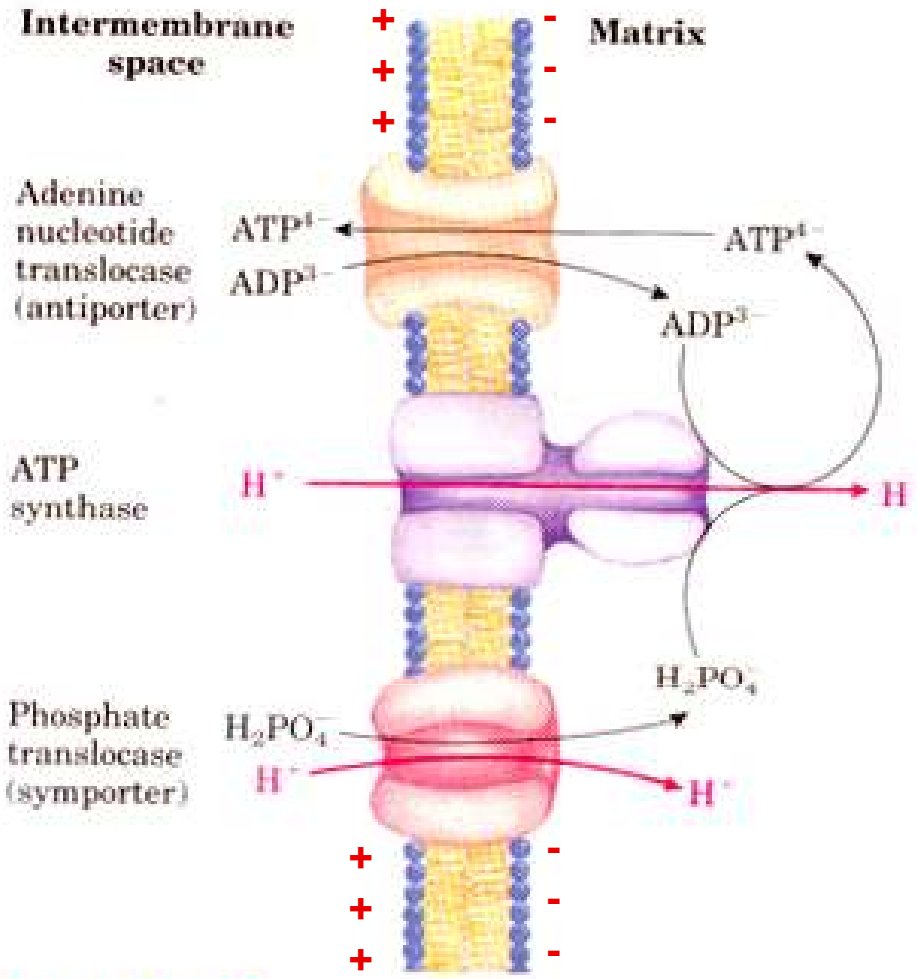
The **ratio of ATP synthesized per ½O_2 reduced to H_2O (the P/O ratio or P/2e^- ratio)** is about 2.5 when 2 electrons enter the respiratory chain (ETC) at Complex I, and 1.5 when 2 electrons enter at ubiquinone (Q).

If 10 protons are pumped out per NADH and 4 must flow in to produce 1 ATP, the proton-based P/O ratio is 2.5 for NADH as the electron pair (**2e^-**) donor and 1.5 (6/4) for succinate (i.e. $FADH_2$) as the electron pair donor.

Therefore:

2e^- from 1 NADH in ETC provide Gibbs energy sufficient for synthesis of 2.5 ATP by ATP-synthase;

2e^- from 1 $FADH_2$ in ETC provide Gibbs energy sufficient for synthesis of 1.5 ATP by ATP-synthase;



The proton-motive force also drives (energizes) active transport of ion species essential to oxidative phosphorylation

Adenine nucleotide and phosphate translocases

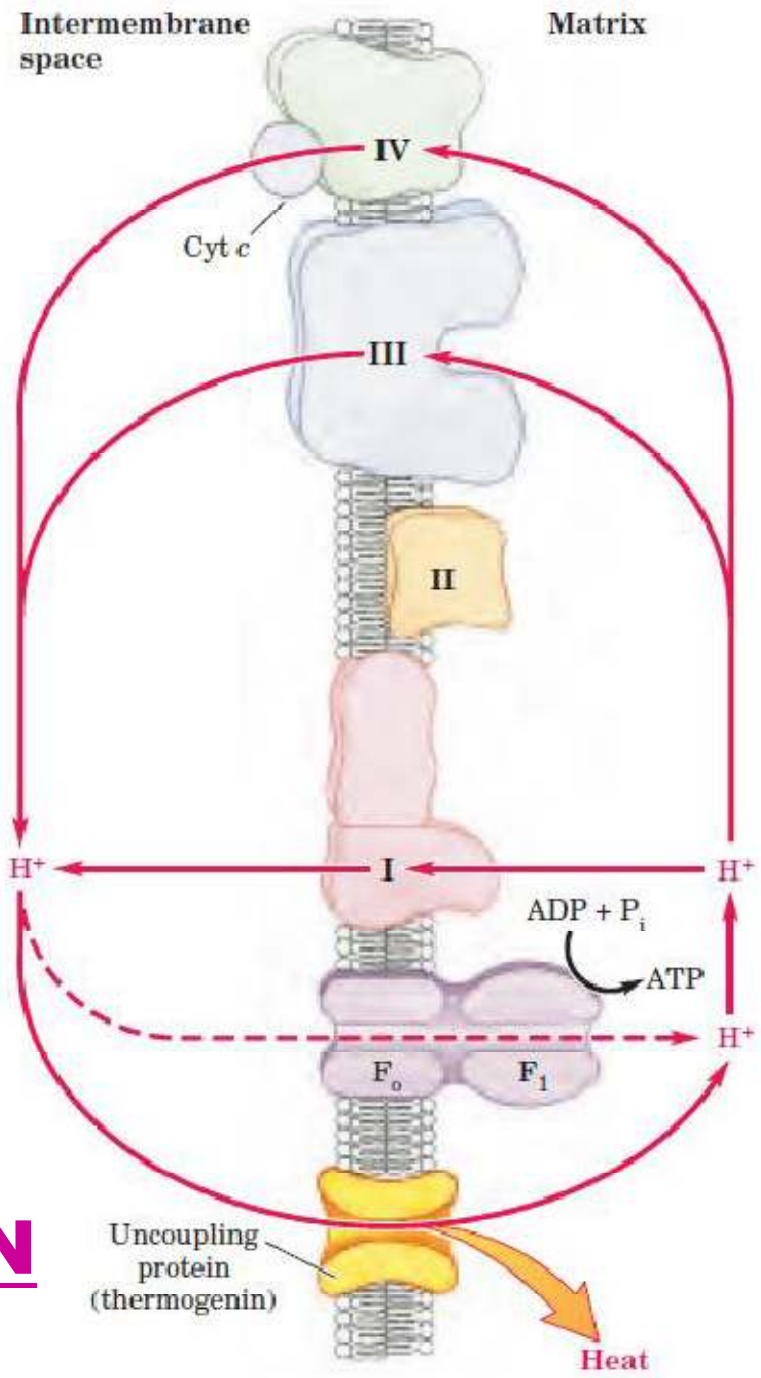
Transport systems of the inner mitochondrial membrane carry **ADP and P_i into the matrix** and newly synthesized **ATP into the cytosol**.

The adenine nucleotide **translocase is an antiporter**; the same protein moves ADP into the matrix and ATP out. The effect of replacing ATP with ADP is **the net efflux of one negative charge**, which is favored by the charge difference across the inner membrane (**outside positive**).

At pH 7, P_i is present as both HPO_4^{2-} and $H_2PO_4^-$; **the phosphate translocase is specific for $H_2PO_4^-$**. There is **no net flow of charge** during **symport of $H_2PO_4^-$ and H^+**, but the relatively low proton concentration in the matrix favors the inward movement of H^+. Thus **the proton-motive force** is responsible both for providing the energy for ATP synthesis and for transporting substrates (ADP and Pi) in and product (ATP) out of the mitochondrial matrix. All three of these transport systems can be isolated as a single membrane-bound complex (ATP synthasome). FIGURE 19–30

UNCOUPLERS:

Substances that uncouple oxidation and phosphorylation process, causing dissipation of a proton gradient, and therefore inhibit the ATP synthesis!



THERMOGENIN

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	Inhibit F_o and CF_o
	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.

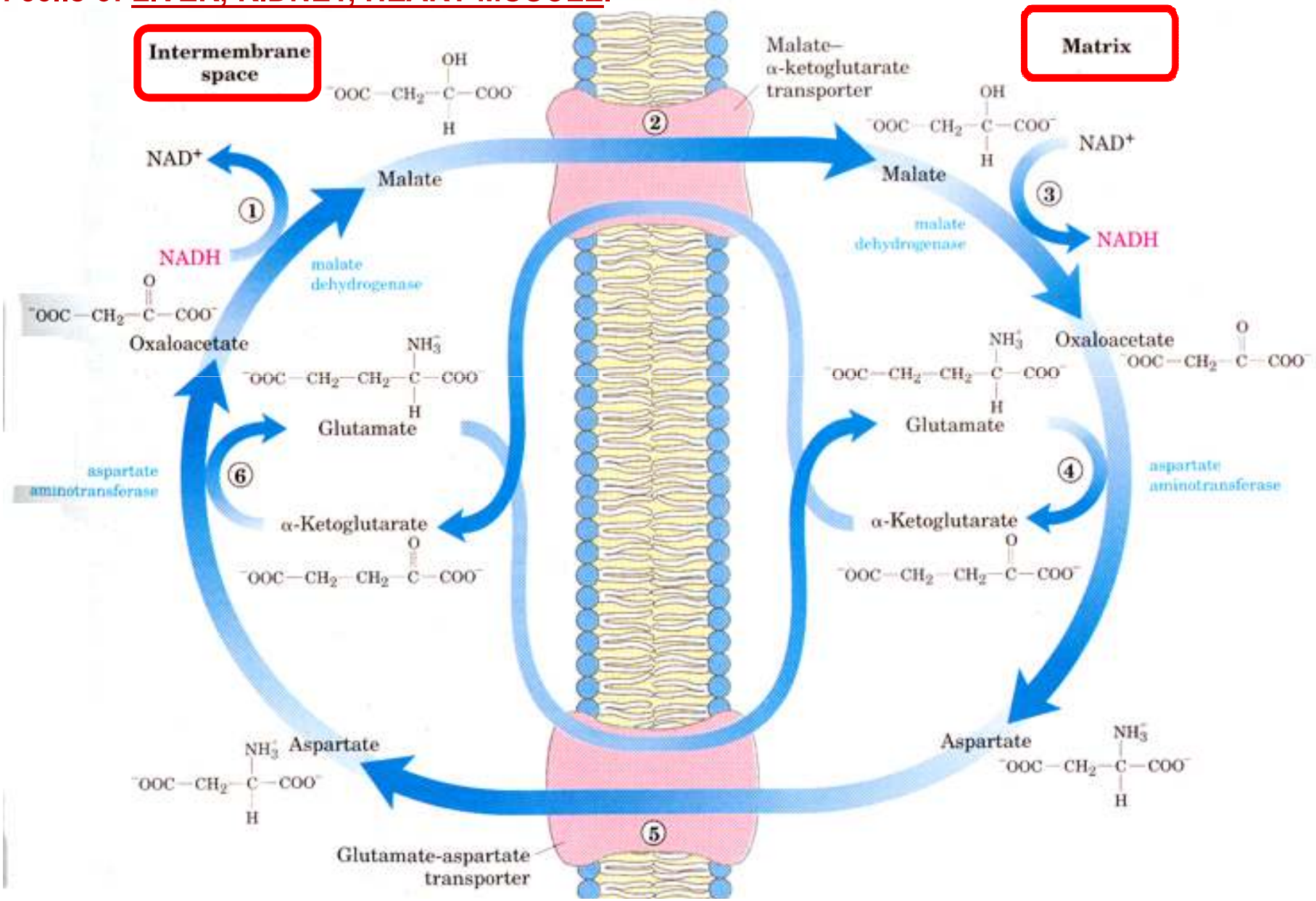
DNP: 2,4-dinitrophenol

Shuttle Systems

- Required for mitochondrial oxidation of cytosolic NADH
- **Our cells produce cytosolic NADH by glycolysis (occurres in cytosol)**
- They also have to be reoxidized to NAD^+
- **Inner mitoch. membrane is NOT permeable to cytosolic NADH**
- NADH dehydrogenase of the inner mitoch. membrane can accept electrons only from NADH in the matrix
- We therefore need shuttle systems

Malate-aspartate shuttle

For transport of e^- (reducing equivalents) from cytosolic NADH to NAD^+ in mitochondial matrix in cells of LIVER, KIDNEY, HEART MUSCLE.



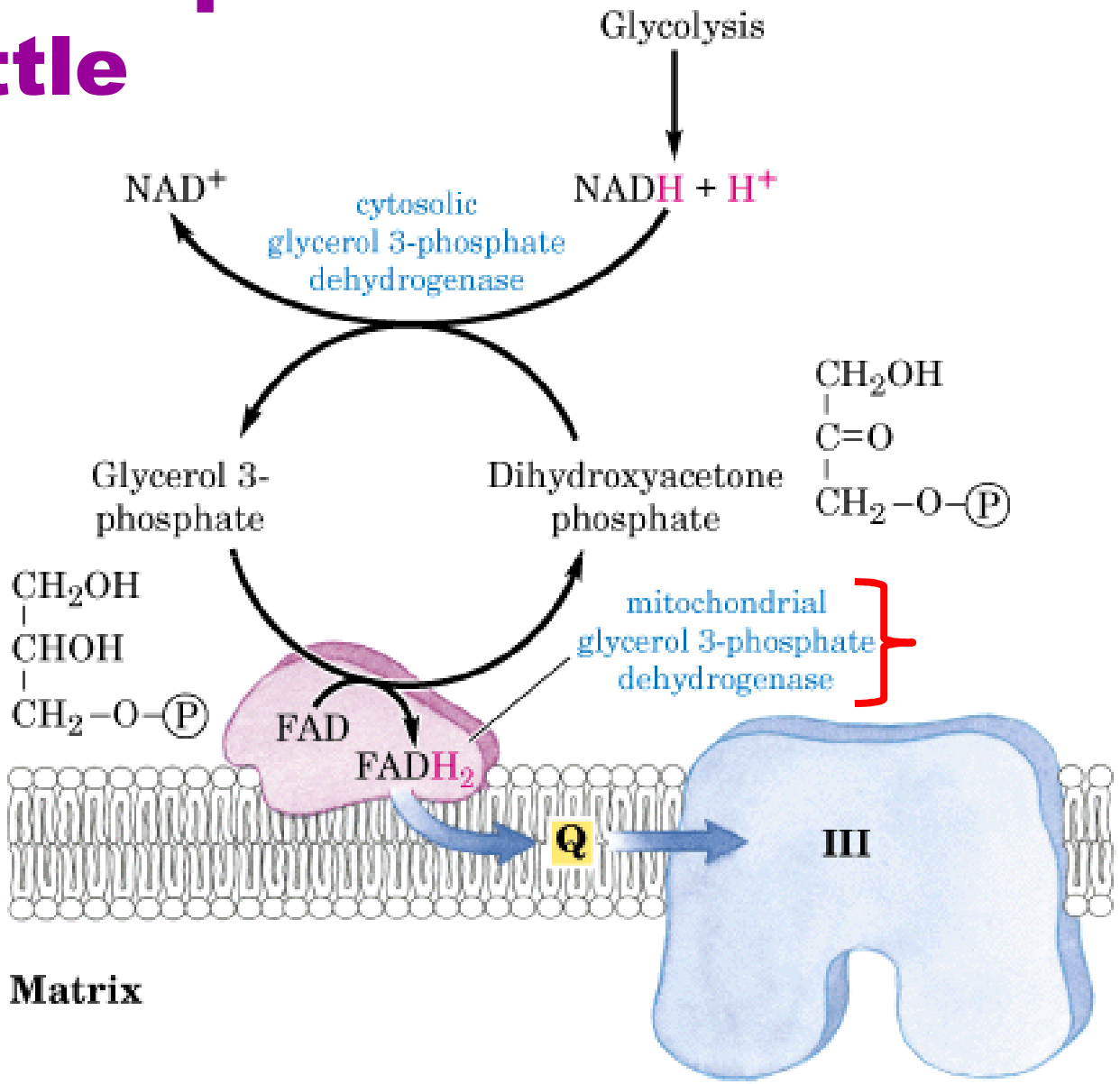
Malate-aspartate Shuttle

- Heart, liver, kidney
- **Transport of e^- (reducing equivalents) from cytosolic NADH to NAD^+ in mitochondial matrix** (cytoplasmic NADH is indirectly brought to mitochondria by this shuttle)
- This shuttle works only if [NADH]/ [NAD^+] increases in the cytosol (higher than in mitochondria)
- No energy consumed
- No ATP lost

Glycerol 3-phosphate shuttle

Electrons are transferred from cytosolic NADH to FAD forming $FADH_2$, and via ubiquinone to **Complex III**, providing (only) enough Gibbs free energy to produce **1.5 ATP per pair of electrons**.

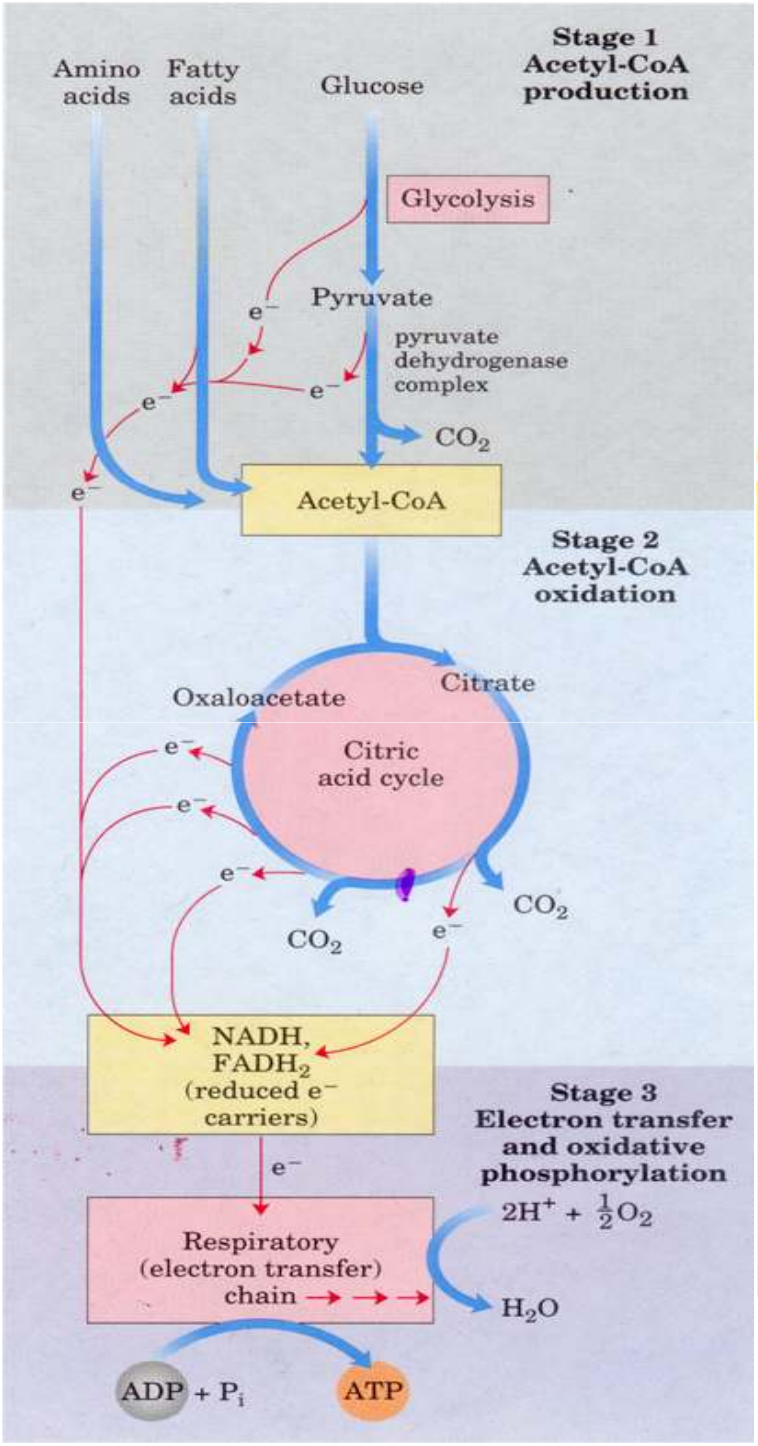
It is active **in muscle** and **brain**.



Net reaction:

$NADH + H^+ + E\text{-}FAD \rightarrow NAD^+ + E\text{-}FADH_2$

(cytosolic) (mitoch.) → (cyto.) (mitoch.)



3 catabolic stages (substrate oxidation)

- in aerobic conditions in cell;

table 19–5

ATP Yield from Complete Oxidation of Glucose

Process	Direct product	Final ATP
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into mitochondria.

!!!

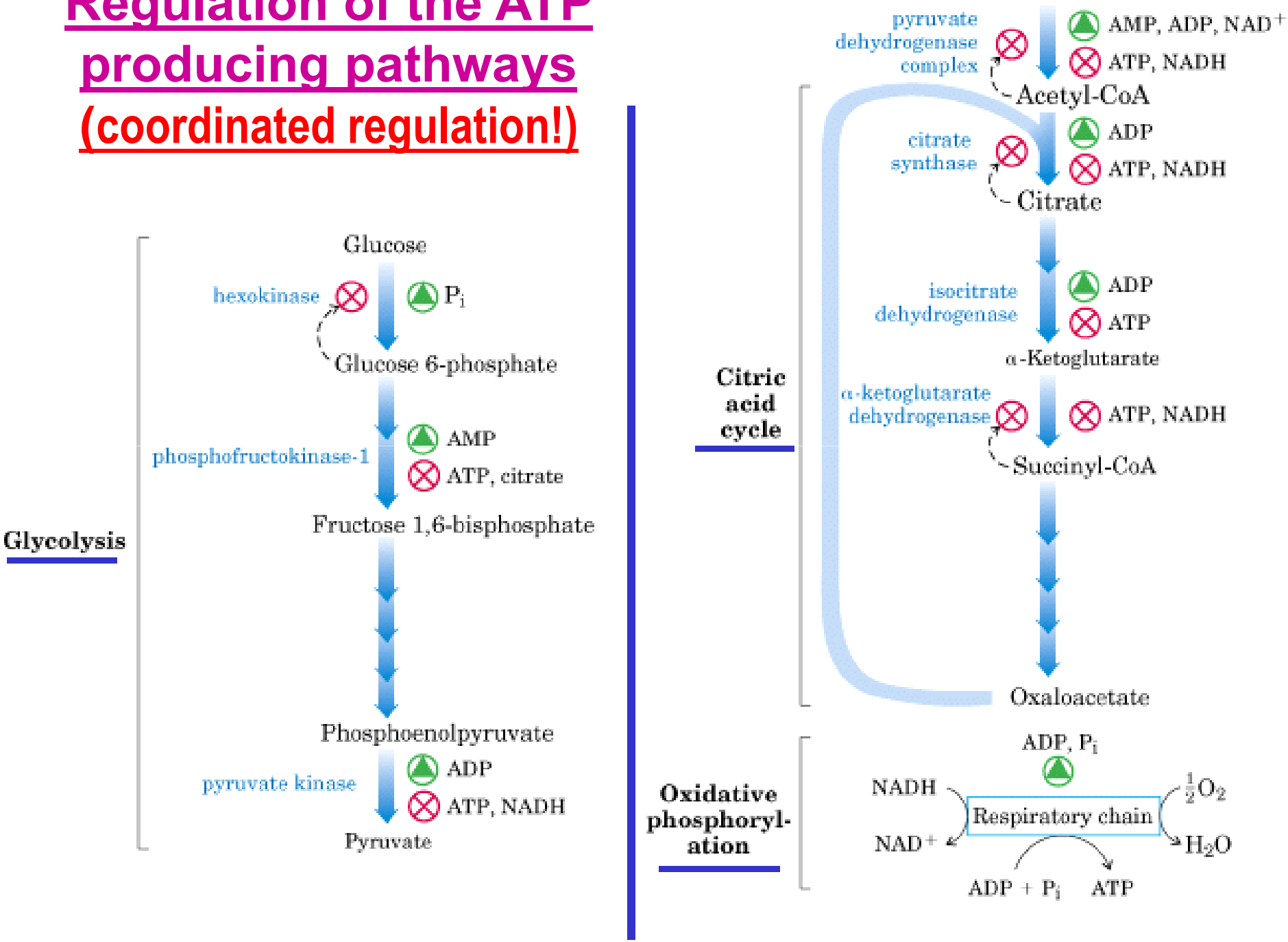
TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	*Number of ATP or reduced coenzyme directly formed*	*Number of ATP ultimately formed**
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH !	3 or 5† !
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32 !!!

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

† This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–27 and 19–28.

Regulation of the ATP producing pathways (coordinated regulation!)



HOMEWORK: Questions to be answered

1. (a) Write the net redox reaction of electron transfer chain! Represent the partial reactions (oxidation and reduction) of the initial electron donor and the final electron acceptor.
(b) Calculate the standard Gibbs free energy (at pH=7) of the electron transfer if the standard reduction potentials of two redox pairs are: $E'°$= -0.320 V and $E'°$= 0.816 V.

2. Briefly explain the chemical mechanism that couples the ETC proton flux with ATP synthesis, known as chemiosmotic model, proposed by Peter Mitchell!

3. Name and represent structural formula of the final product(s) synthesized by mitochondrial oxidative phosphorylation!

Required background knowledge from the Chemistry course!

Standard Reduction Potentials of Some Biologically Important Half-Reactions, at 25 °C and pH 7

Half-reaction	E'° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome f (Fe^{3+}) + e^- ⟶ cytochrome f (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) + e^- ⟶ $Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 (Fe^{3+}) + e^- ⟶ cytochrome a_3(Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome a (Fe^{3+}) + e^- ⟶ cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + e^- ⟶ cytochrome c (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + e^- ⟶ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b (Fe^{3+}) + e^- ⟶ cytochrome b (Fe^{2+})	0.077
Ubiquinone + $2H^+ + 2e^-$ ⟶ ubiquinol + H_2	0.045
$Fumarate^{2-} + 2H^+ + 2e^- \longrightarrow succinate^{2-}$	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+ + 2e^-$ ⟶ butyryl-CoA	−0.015
$Oxaloacetate^{2-} + 2H^+ + 2e^- \longrightarrow malate^{2-}$	−0.166
$Pyruvate^- + 2H^+ + 2e^- \longrightarrow lactate^-$	−0.185
Acetaldehyde + $2H^+ + 2e^-$ ⟶ ethanol	−0.197
$FAD + 2H^+ + 2e^- \longrightarrow FADH_2$	−0.219*
Glutathione + $2H^+ + 2e^-$ ⟶ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid + $2H^+ + 2e^-$ ⟶ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
Acetoacetate + $2H^+ + 2e^-$ ⟶ β-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^-$ ⟶ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + e^- ⟶ ferredoxin (Fe^{2+})	−0.432

Standard state for the reaction in biological system is defined at pH=7 (Not at pH=0), i.e. at $a(H^+) = 10^{-7}$

For all types of reactions:

At the STANDARD STATE:

$$\Delta_r G^{\Theta} = -RT \ln K^{\Theta}$$

$$\Delta_r G'^{\Theta} = -RT \ln K'$$

$Q \not\equiv K$

At the NONSTANDARD STATE:

$$\Delta_r G = \Delta_r G^{\Theta} + RT \ln \frac{\prod a_{\text{product}}}{\prod a_{\text{reactant}}} = \Delta_r G^{\Theta} + RT \ln Q$$

$$\Delta_r G = \Delta_r G'^{\Theta} + RT \ln \frac{\prod a_{\text{product}}}{\prod a_{\text{reactant}}} = \Delta_r G'^{\Theta} + RT \ln Q$$

For the redox-reactions at standard state:

$\Delta_r G^{\ominus} = - z \cdot F \cdot \Delta E^{\ominus}$ (at pH=0, t = 25 °C)

$\Delta_r G'^{\ominus} = - z \cdot F \cdot \Delta E'^{\ominus}$ (at pH=7, t = 25 °C)

$$\Delta E^{\ominus} = E^{\ominus}_{\text{more positive}} - E^{\ominus}_{\text{more negative}}$$

or $\Delta E^{\ominus} = E^{\ominus}_{\text{cathode}} - E^{\ominus}_{\text{anode}}$

For the redox-reactions at nonstandard state:

$\Delta_r G = - z \cdot F \cdot \Delta E$ *i.e.* $\Delta_r G' = - z \cdot F \cdot \Delta E'$

$$\Delta E = E_{\text{more positive}} - E_{\text{more negative}}$$

or $\Delta E = E_{\text{cathode}} - E_{\text{anode}}$

From the expression for $\Delta_r G$ of (redox-) reaction at <u>NONSTANDARD state</u>, the Nernst equation could be derived:

$$\Delta_r G = \Delta_r G^{\Theta} + RT \ln Q$$

$$-z \cdot F \cdot \Delta E = -z \cdot F \cdot \Delta E^{\Theta} + R \cdot T \cdot \ln Q \qquad /\div (-z \cdot F)$$

$$\Delta E = \Delta E^{\Theta} - \frac{R \cdot T}{z \cdot F} \ln Q$$

$$\Delta E = \Delta E^{\Theta} - \frac{RT}{zF} \ln \frac{a_{\mathrm{ox1}}{}^{\nu 2} \cdot a_{\mathrm{red2}}{}^{\nu 1}}{a_{\mathrm{red1}}{}^{\nu 2} \cdot a_{\mathrm{ox2}}{}^{\nu 1}}$$

For the reaction: ν_2 **red1 +** ν_1 **ox2** $\rightarrow$ ν_2 **ox1 +** ν_1 **red2**

- For *E* of a redox syst. at ***NONSTANDARD*** STATE:

NERNST EQUATION

For a redox-system: $ox_1 + \nu e^- \rightarrow red_1$

$$E = E^{\Theta} - \frac{RT}{zF} \ln \frac{(a_{red})^{\nu(r)}}{(a_{ox})^{\nu(o)}} = E^{\Theta} - \frac{RT}{zF} \ln Q$$

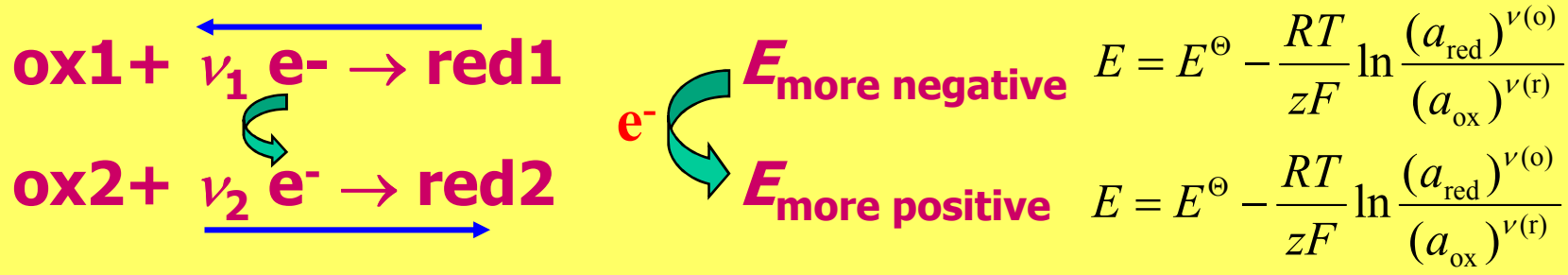
or

$$E = E^{\Theta} + \frac{RT}{zF} \ln \frac{(a_{ox})^{\nu(o)}}{(a_{red})^{\nu(r)}}$$

- E - actual value of reduction (electrode) potential (at nonstandard state);
- E^{Θ} - standard reduction (electrode) potential;
- R - universal gas constant, 8.314 $JK^{-1}mol^{-1}$;
- T - thermodynamic temperature;
- z - number of electrons exchanged in a single redox-reaction;
- F - Faraday constant, 96 485 $Cmol^{-1}$;
- a_{ox} - activity of the oxidized form of redox-system in solution;
- a_{red} - activity of the reduced form of redox-system in solution;
- $\nu(o)$ - stoichiometric coefficient of the oxidized form;
- $\nu(r)$ - stoichiometric coefficient of the reduced form.

NERNST EQUATION

For a redox-reaction (net reaction):



ν_2 red1 + ν_1 ox2 → ν_2 ox1 + ν_1 red2

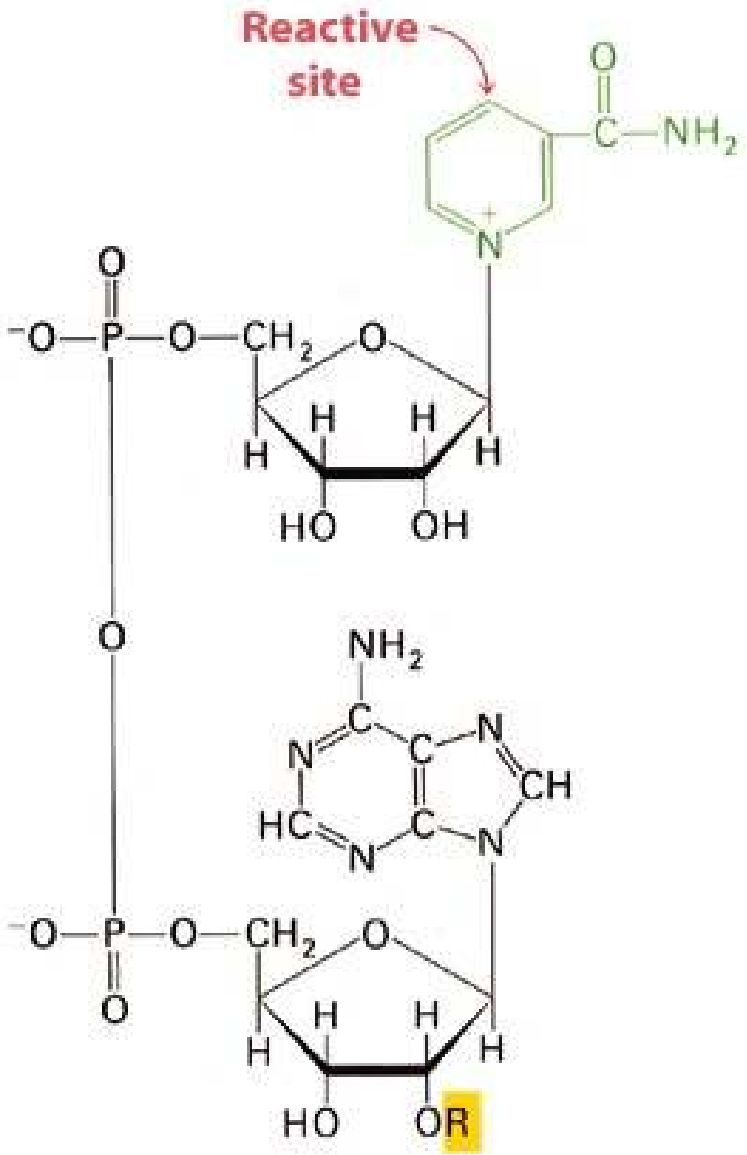
POTENTIAL DIFFERENCE (at actual nonstandard state):

$\Delta E = E_{rr}$ = EMF = $E_{\text{more positive}} - E_{\text{more negative}}$

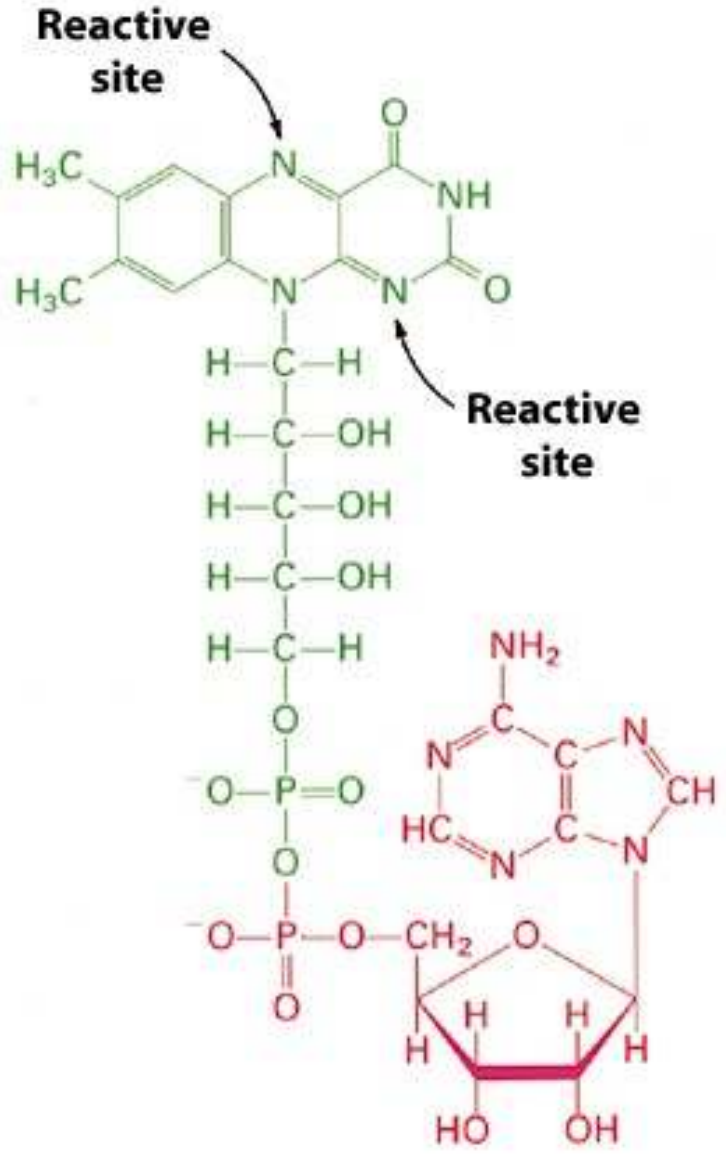
$$E_{rr} = E_{rr}^{\Theta} - \frac{RT}{zF} \ln \frac{a_{\text{ox1}}^{\nu 2} \cdot a_{\text{red2}}^{\nu 1}}{a_{\text{red1}}^{\nu 2} \cdot a_{\text{ox2}}^{\nu 1}}$$

or $$E_{rr} = E_{rr}^{\Theta} - \frac{RT}{zF} \ln \frac{\prod a_{\text{product}}}{\prod a_{\text{reactant}}} = E_{rr}^{\Theta} - \frac{RT}{zF} \ln Q$$

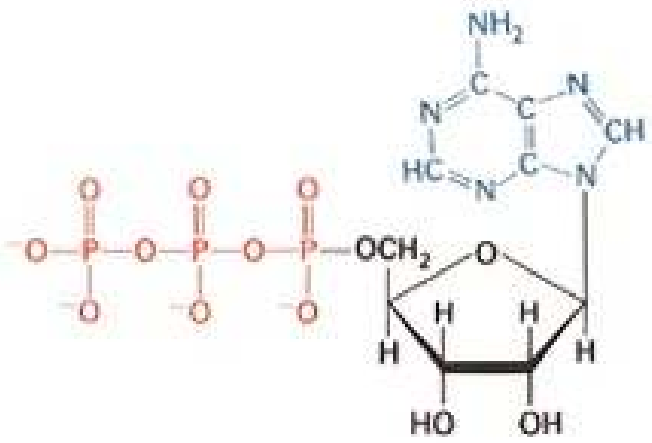
NAD^+


Reactive site
O
C—NH2
N+
O
⁻O—P—O—CH2
O
H
H
H
H
HO
OH
O
NH2
C
N
C
N
CH
HC
N
C
N
O
⁻O—P—O—CH2
O
H
H
H
H
HO
OR

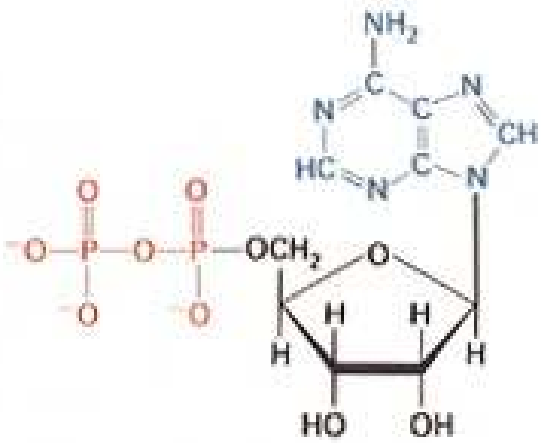
FAD


Reactive site
H3C
N
O
NH
H3C
N
N
O
H—C—H
H—C—OH
H—C—OH
H—C—OH
H—C—H
Reactive site
O
⁻O—P=O
O
⁻O—P—O—CH2
O
O
NH2
C
N
C
N
CH
HC
N
C
N
H
H
H
H
HO
OH

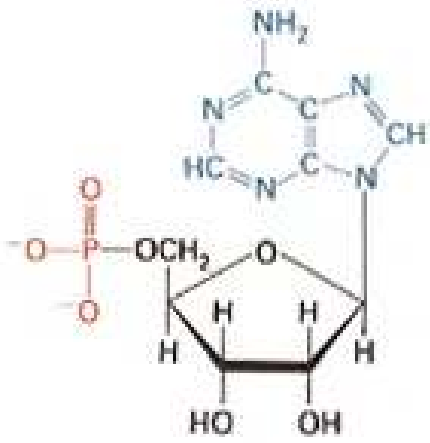
ATP



Adenosine triphosphate
(ATP)



Adenosine diphosphate
(ADP)



Adenosine monophosphate
(AMP)

ATP — A “High Energy” Compound