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OXIDATIVE PHOSPHORYLATION

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BIOENERGETICS

Mechanisms of ATP synthesis phosphorylation (of ADP):

$$ADP + P_i + energy \rightarrow ATP$$

- <u>Substrate-level phosphorylation</u>

$AH_2 + B \rightarrow A + BH_2$ $\Delta G < 0$

$ADP + P_i \rightarrow ATP$ $\Delta G > 0$

Mechanism:

$AH_2 + B + P_i \rightarrow A\sim P + BH_2$

$A\sim P + ADP \rightarrow A + ATP$

- **Oxidative phosphorylation** (at the level of electron transfer chain).

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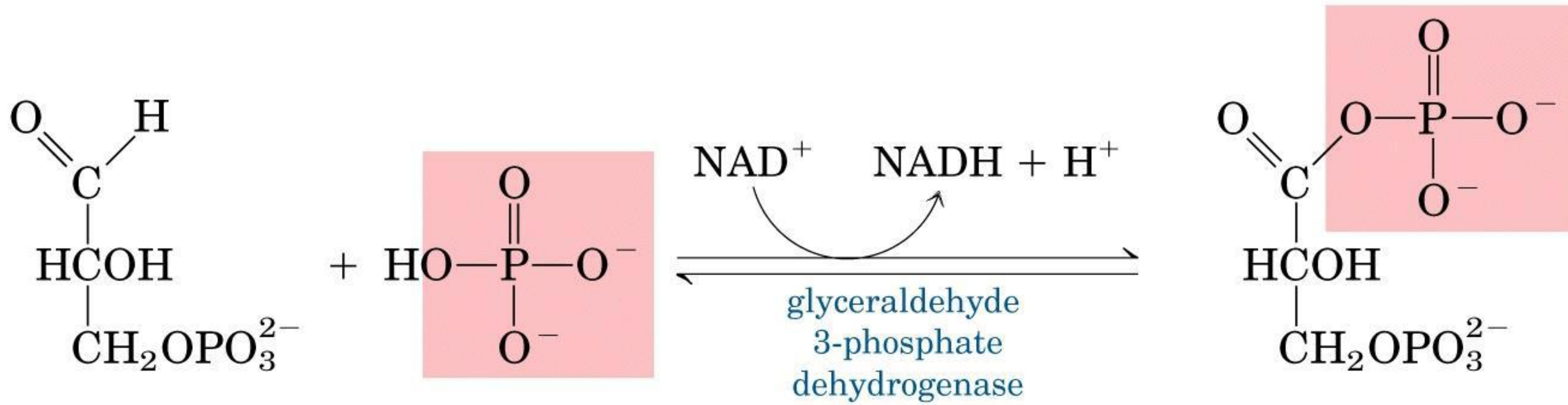
BIOENERGETICS



Glyceraldehyde 3-phosphate

GA3P

Inorganic phosphate

1,3-Bisphosphoglycerate

1,3BPG

$\Delta G'^{\circ} = 6.3$ kJ/mol

The oxidation of GA3P is very exergonic: the energy is used to incorporate P_i to form high-energy bond of 1,3BPG.

Two steps: oxidation of the aldehyde to a carboxylic group ($\Delta G°'$=-50 kJ/mol) + attach of P_i.

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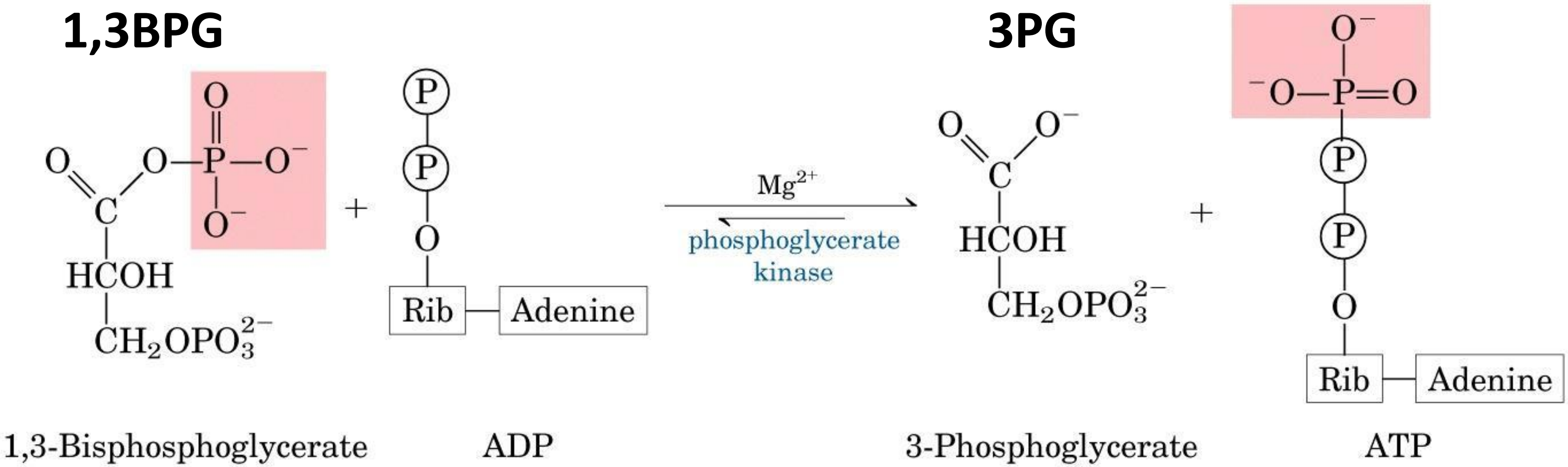
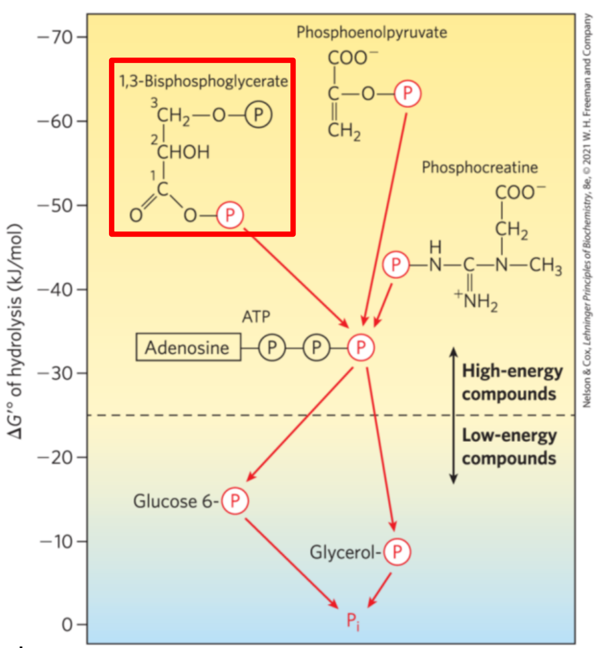
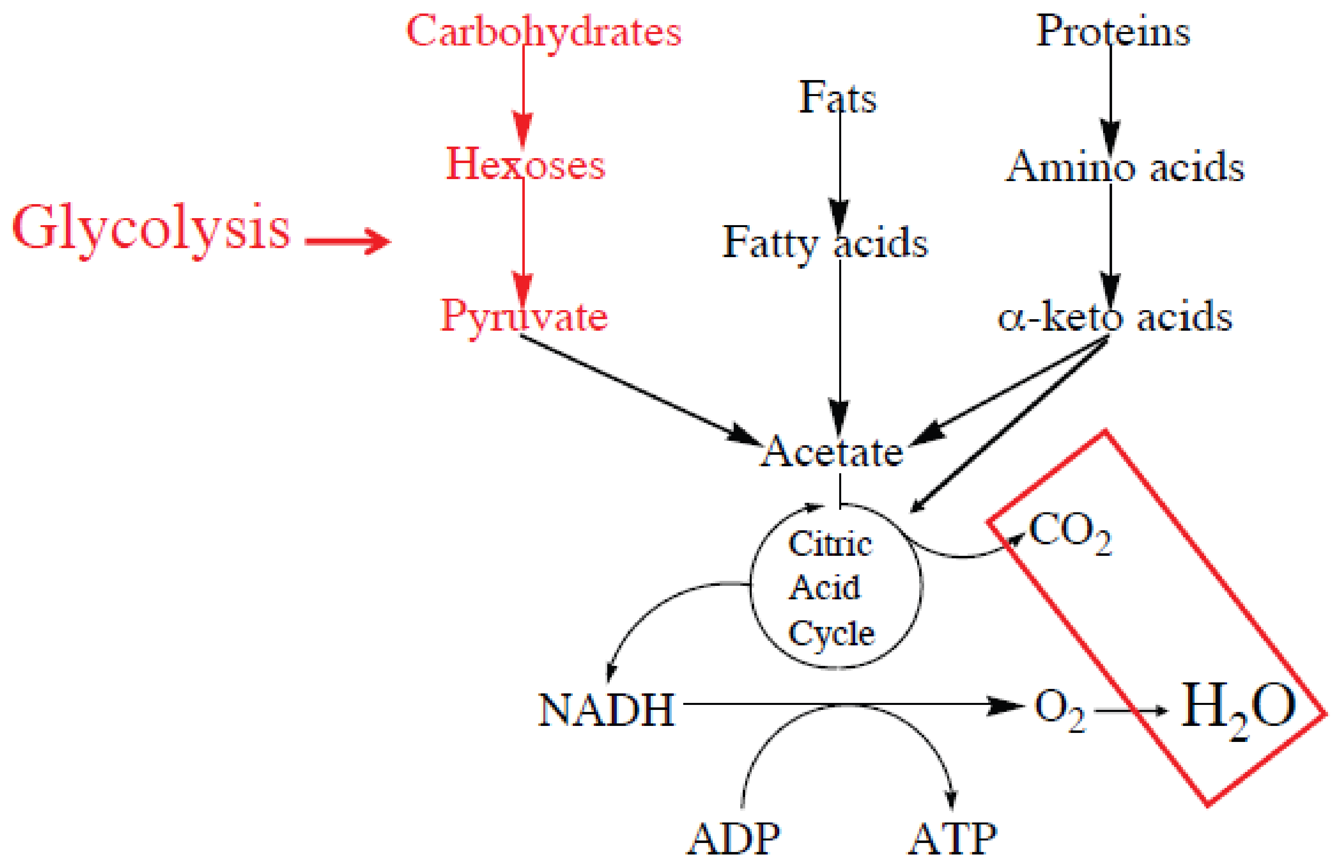




$$\Delta G'^\circ = -18.5 \text{ kJ/mol}$$

BIOENERGETICS

Catabolism (slow combustion) overview:

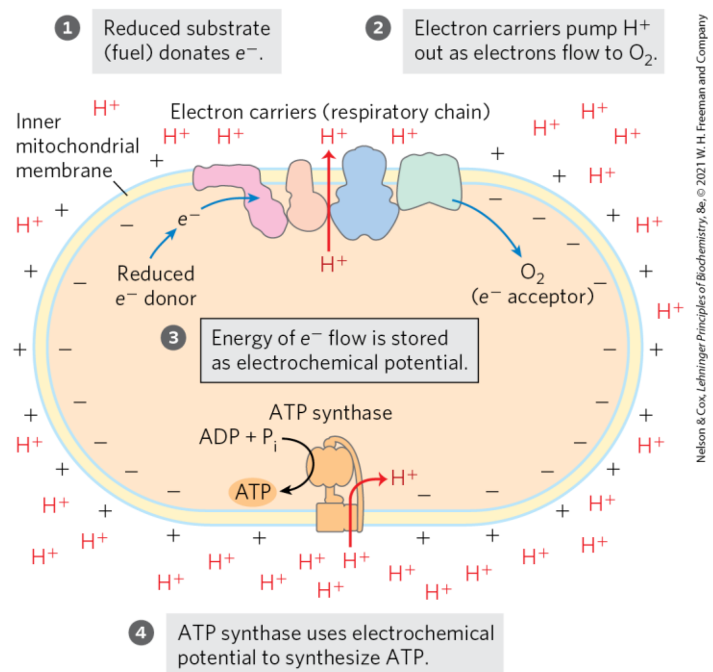


PRIMARY FUNCTION OF MITOCHONDRIA

Mitochondria are the power houses of the cell.

Mitochondria perform the transfer to oxygen of electrons derived from intermediates of oxidative metabolism of foodstuffs with production of water (*Respiratory Chain*).

They exploit the energy derived from electron transfer for the synthesis of ATP (*Oxidative Phosphorylation*).



ADDITIONAL FUNCTIONS OF MITOCHONDRIA

- Most catabolic pathways (TCA cycle, fatty acid β-oxidation, amino acid oxidation)
- Some biosynthetic pathways or reactions
- Mitochondrial DNA and protein synthesis
- Generation of Reactive Oxygen Species (ROS)
- Involved in signal transduction networks
- Involved in apoptosis (programmed cell death)

RESPIRATORY CHAIN

A sequence of redox coenzymes that carries electrons from substrates to oxygen.

Overall reaction:

$SH_2 + ½\ O_2 \rightarrow S + H_2O$

The respiratory chain:

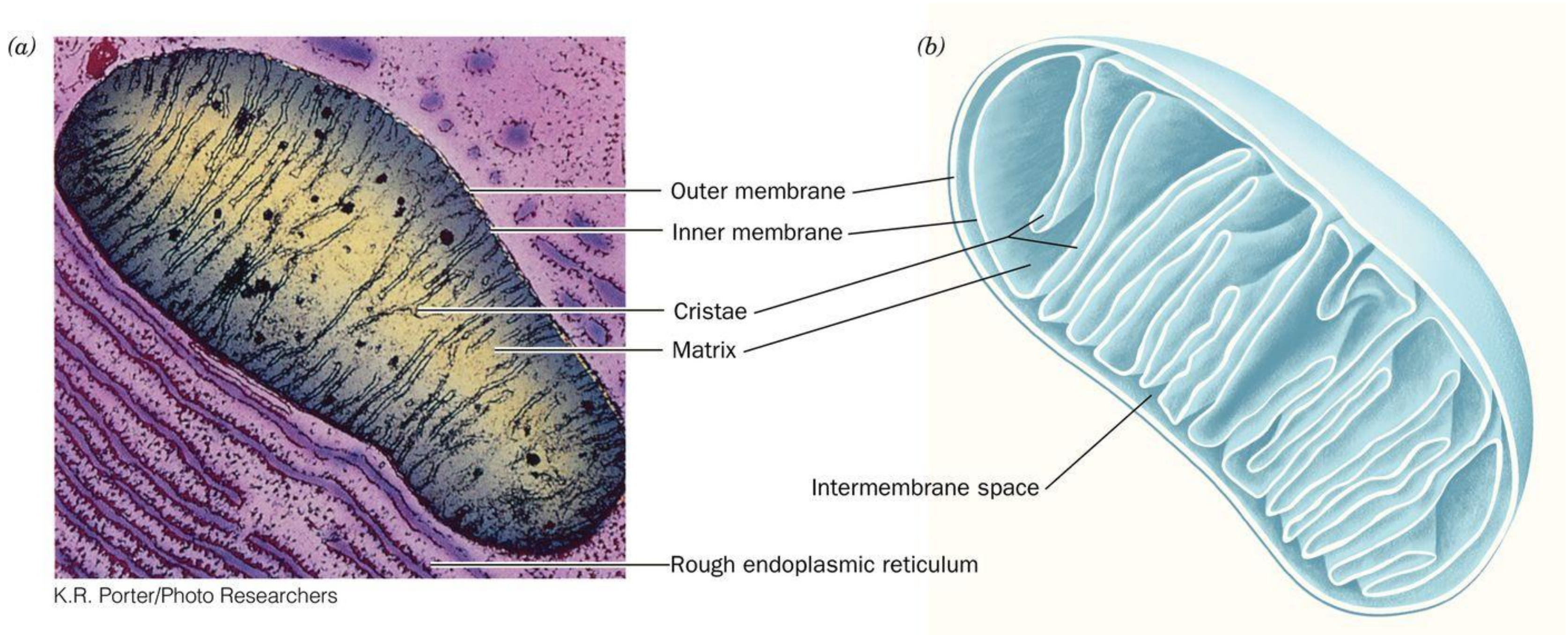
$SH_2 \rightarrow NAD \rightarrow FMN \rightarrow (CoQ) \rightarrow b \rightarrow c_1 \rightarrow c \rightarrow a \rightarrow a_3 \rightarrow O_2$

By subdividing the energy loss in several steps, the energy can be conserved in the form of the chemical bonds of ATP.

SUMMARY

- *Structure of Mitochondria*
- *The Respiratory Chain*
- *ATP Synthase*
- *Chemio-Osmotic Coupling*
- *Respiratory Control and Uncoupling*
- *Mitochondrial DNA*
- *Mitochondria and Reactive Oxygen Species (ROS)*

MITOCHONDRIAL STRUCTURE



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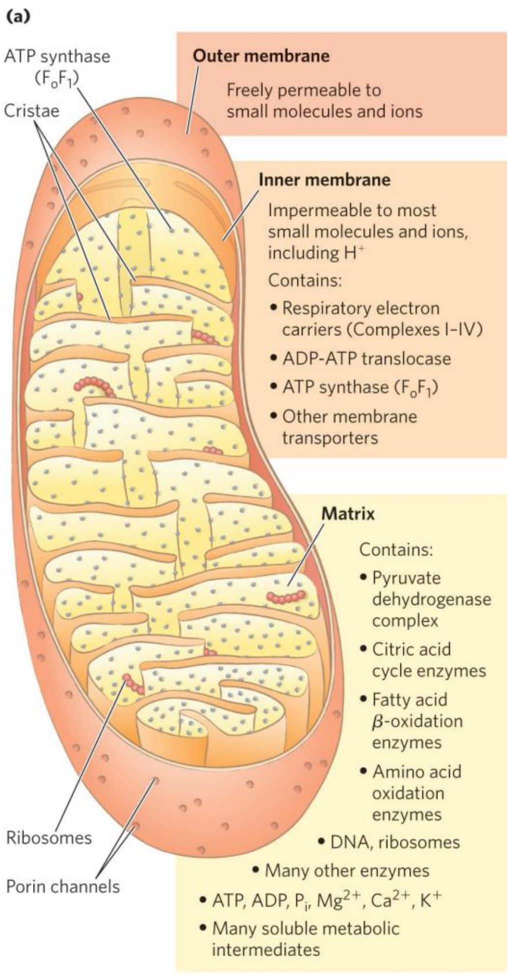
MITOCHONDRIAL STRUCTURE

Outer membrane: permeable to small molecules and ions **Inner membrane**: impermeable to small molecules and ions (specific transporters).

Mitochondrial matrix: enzymes of carbohydrate (downstream PDH), fatty acids and amino acid catabolism.



(b) Talley Lambert/Science Source. (c) Thomas Deerinck, NCMIR/Science Source. (d) Don W. Fawcett/Science Source. Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 202 W. H. Freeman and Company

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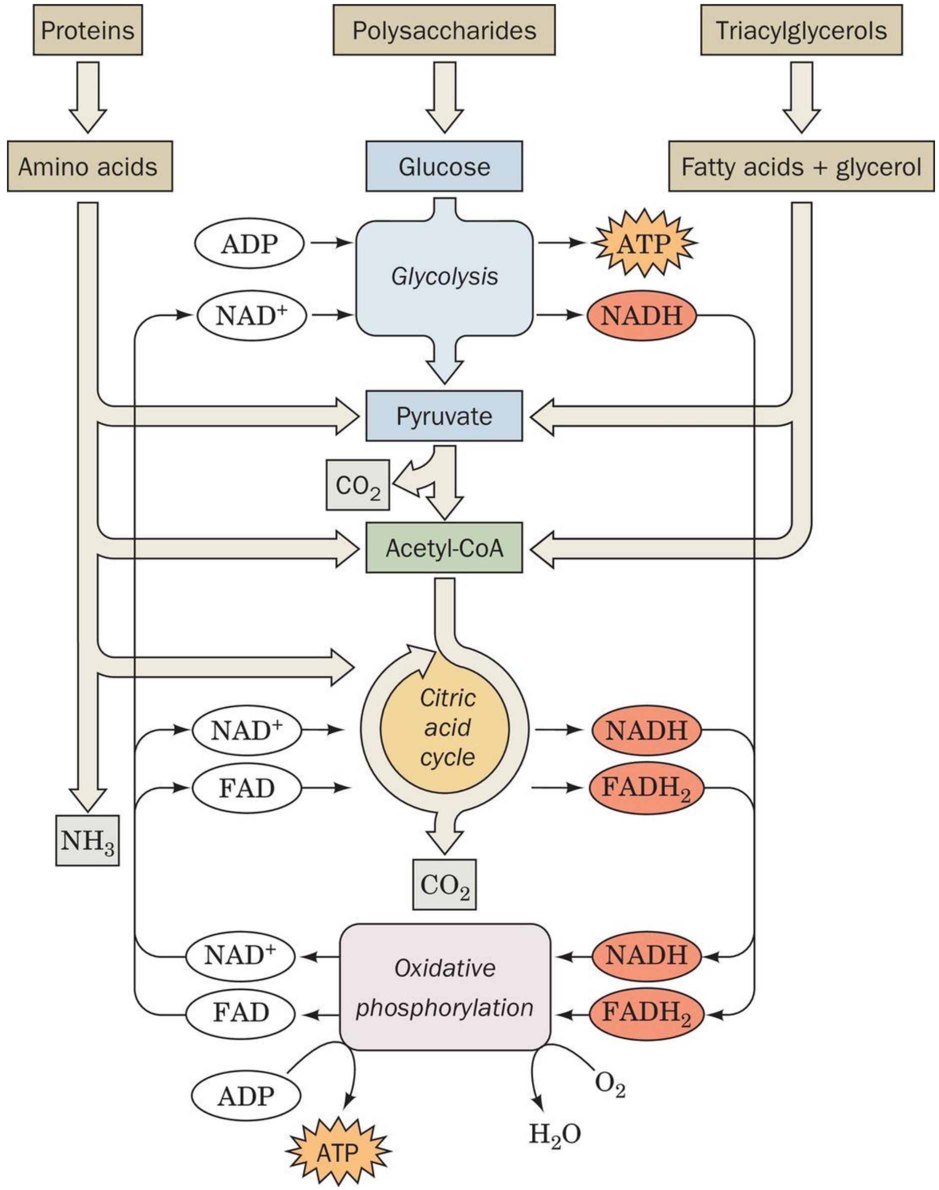
MITOCHONDRIAL ELECTRON TRANSPORT



Stage 1

Stage 2

Stage 3

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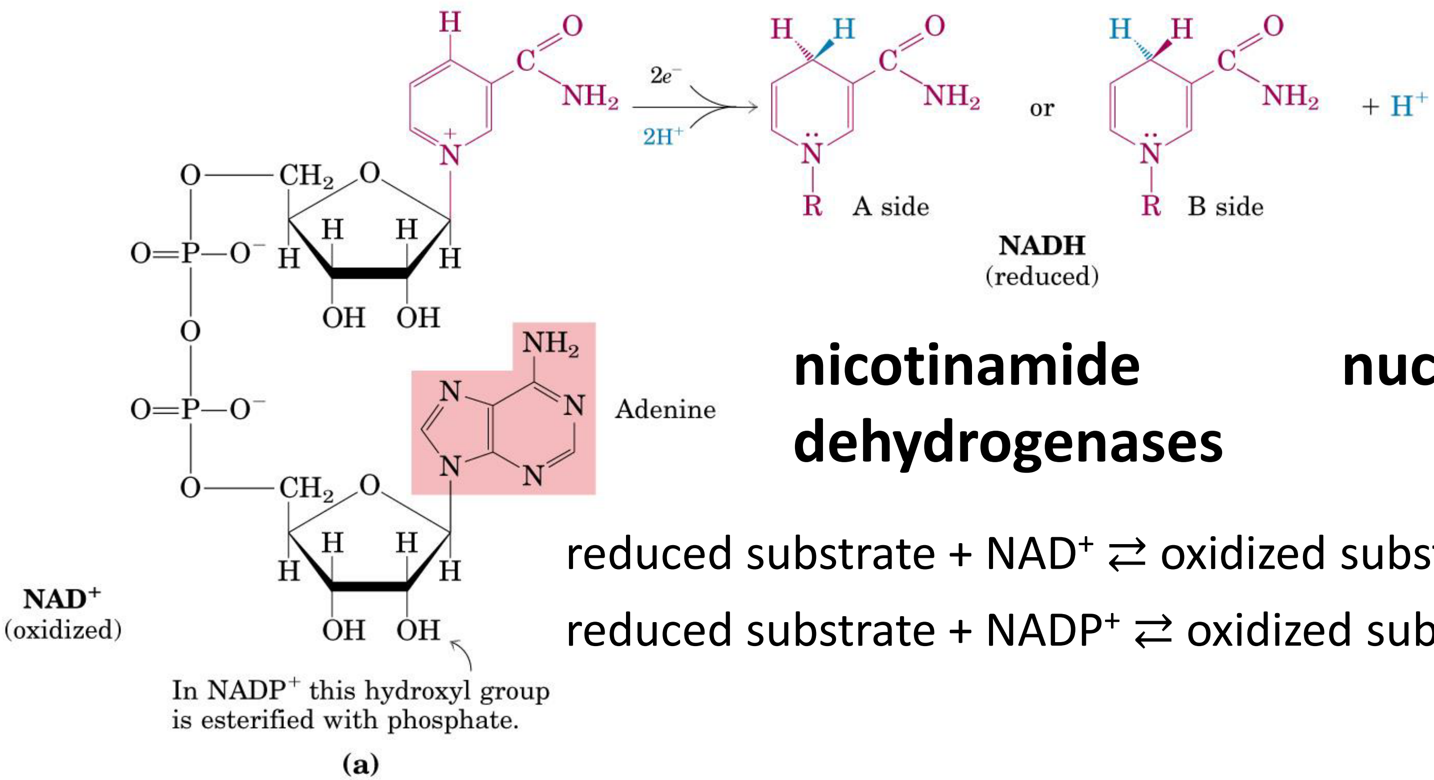
MITOCHONDRIAL RESPIRATORY CHAIN

Coenzymes of respiratory chain

5 types of electron-carrying molecules:

- NAD^+/NADH
- FMN/$FMNH_2$ FAD/$FADH_2$ (flavoproteins)
- CoQ/$CoQH_2$ (ubiquinone or coenzyme Q)
- FeS clusters proteins (Fe^{3+}/Fe^{2+})
- Cytochromes b, c_1, c, a, a_3 (Fe^{3+}/Fe^{2+}; Cu^{2+}/Cu^+)

MITOCHONDRIAL RESPIRATORY CHAIN



nicotinamide nucleotide-linked dehydrogenases

reduced substrate + $NAD^+ \rightleftarrows$ oxidized substrate + NADH + H^+

reduced substrate + $NADP^+ \rightleftarrows$ oxidized substrate + NADPH + H^+

two hydrogen atoms are removed from the substrates:

- one is transferred as a hydride ion ($:H^-$) to $NAD(P)^+$
- one is released as H^+ in the medium

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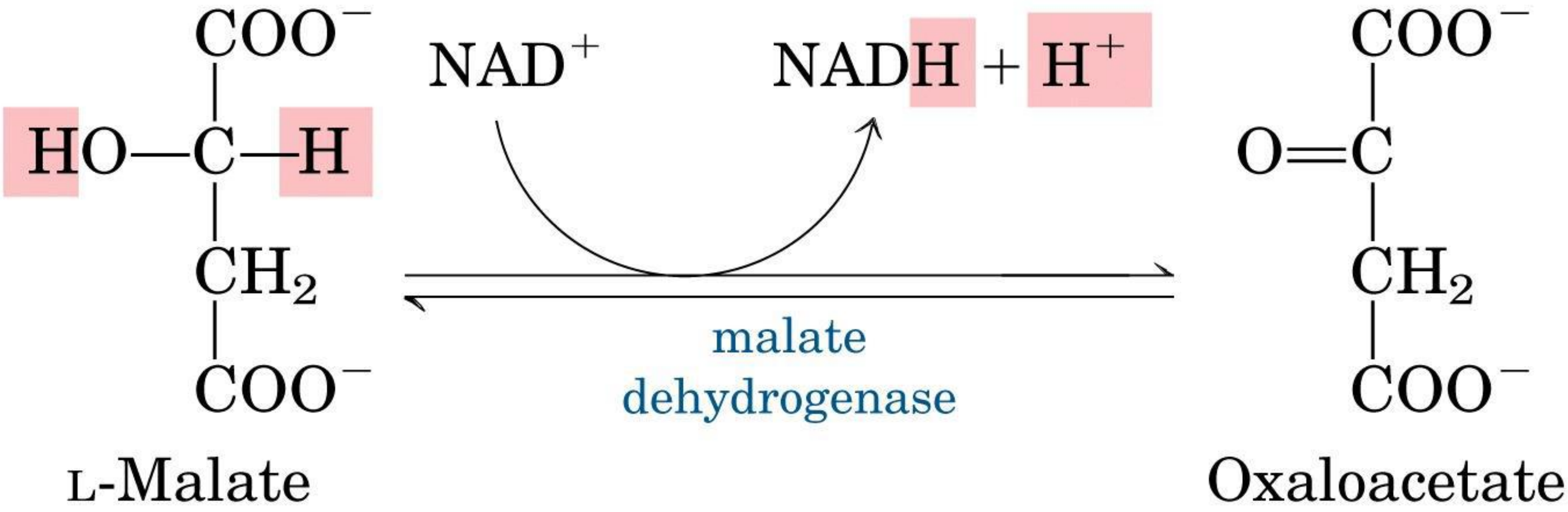
MITOCHONDRIAL RESPIRATORY CHAIN



$$\Delta G'^{\circ} = 29.7 \text{ kJ/mol}$$

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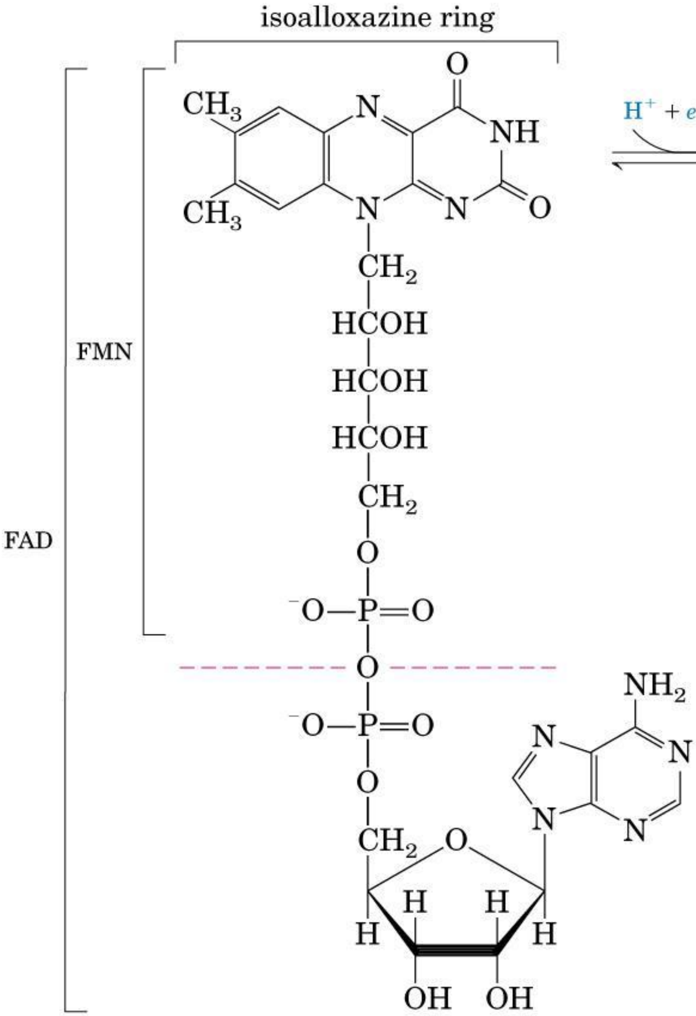
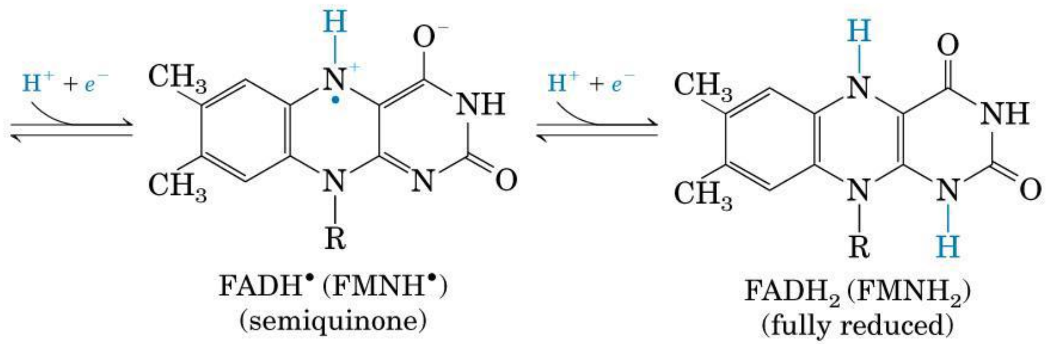
MITOCHONDRIAL RESPIRATORY CHAIN



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



Flavoproteins contain a flavin nucleotide (FMN or FAD)

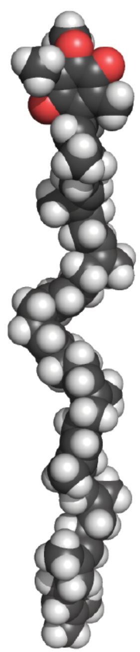
the oxidized flavin nucleotide can accept either:

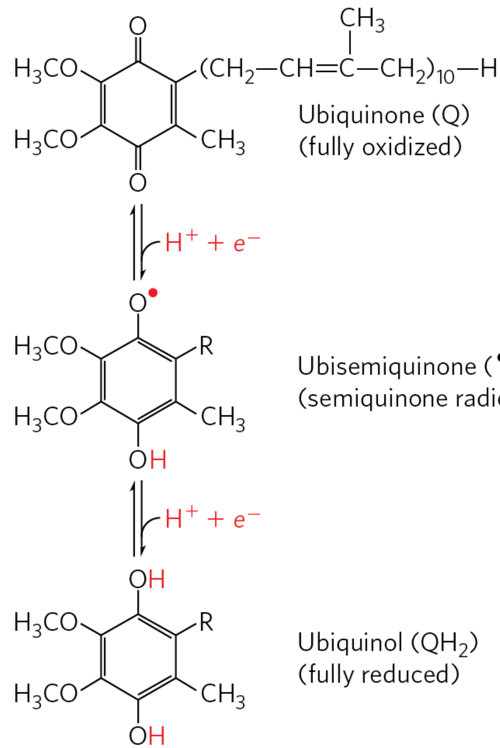
- one e^- (yielding the semiquinone form)
- two e^- (yielding $FADH_2$ or $FMNH_2$)

MITOCHONDRIAL RESPIRATORY CHAIN

Ubiquinone (coenzyme Q) is a lipid-soluble benzoquinone with a long isoprenoid side chain:

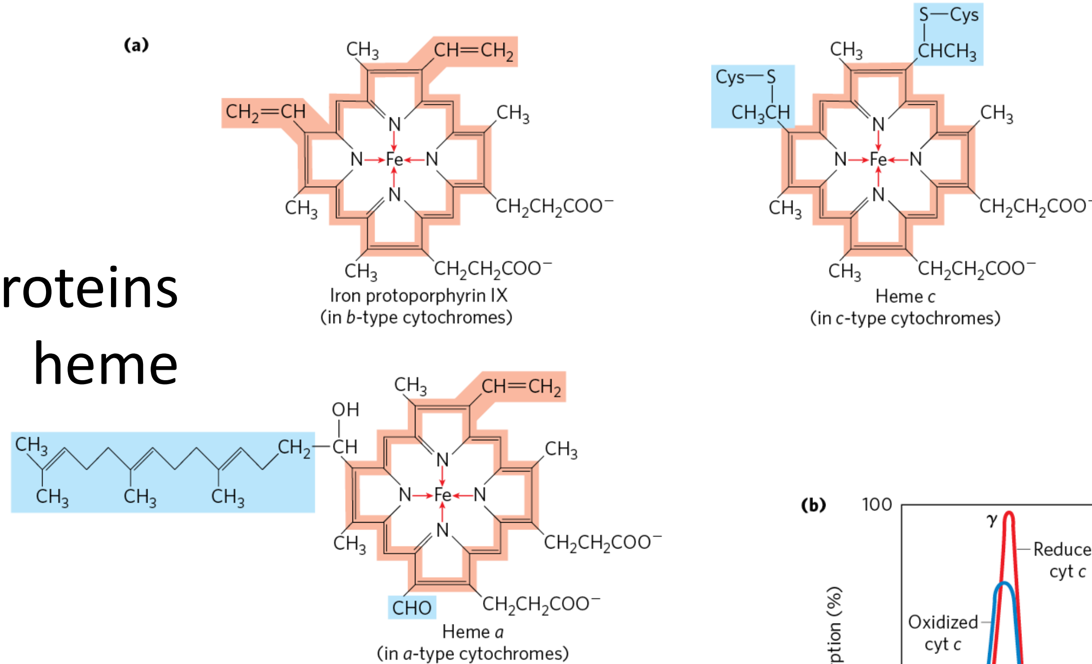
- can accept one or two e^-
- freely diffusible within the inner mitochondrial membrane
- plays a central role in coupling electron flow to proton movement



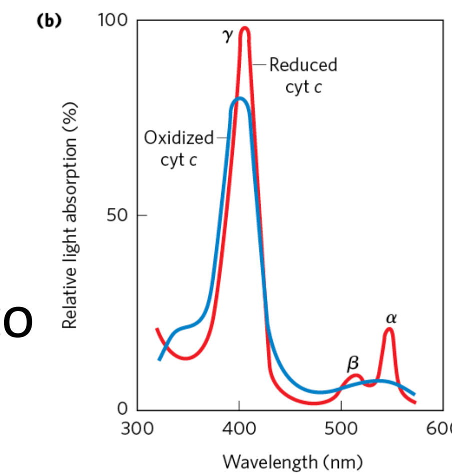


MITOCHONDRIAL RESPIRATORY CHAIN

Cytochromes are proteins with iron-containing heme prosthetic groups:



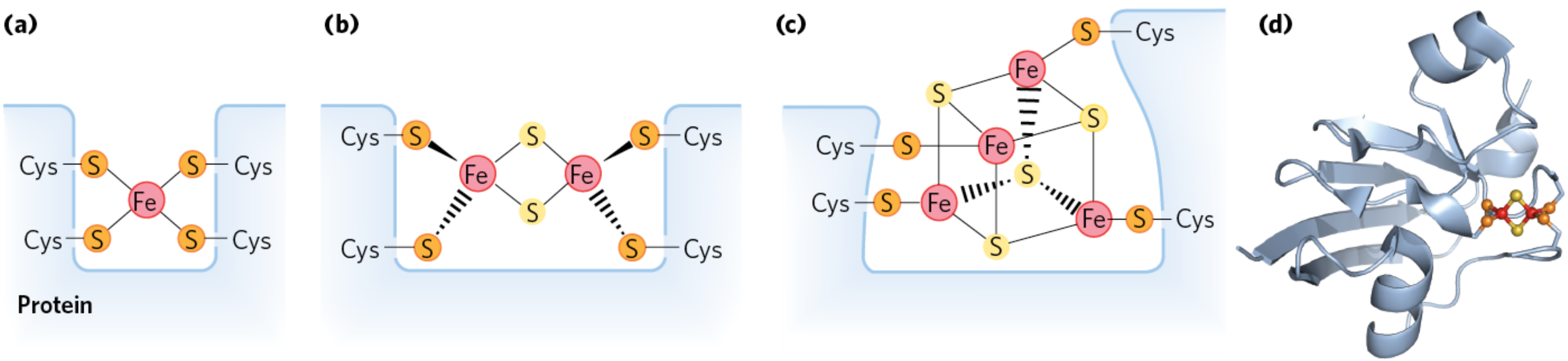
- one e^- carriers
- 3 classes in mitochondria: a, b, and c
- hemes of a and b are not covalently bound to associated proteins
- c is covalently attached through Cys residues



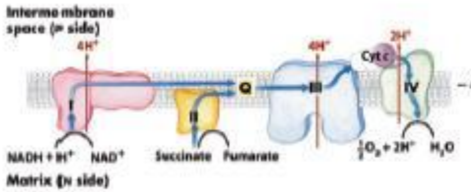
MITOCHONDRIAL RESPIRATORY CHAIN

Iron-sulfur proteins contain Fe in association with inorganic S atoms and/or with the S atoms of Cys residues in the protein:

- participate in one e^- transfers
- **Rieske iron-sulfur proteins** in which one Fe atom is coordinated to two His residues

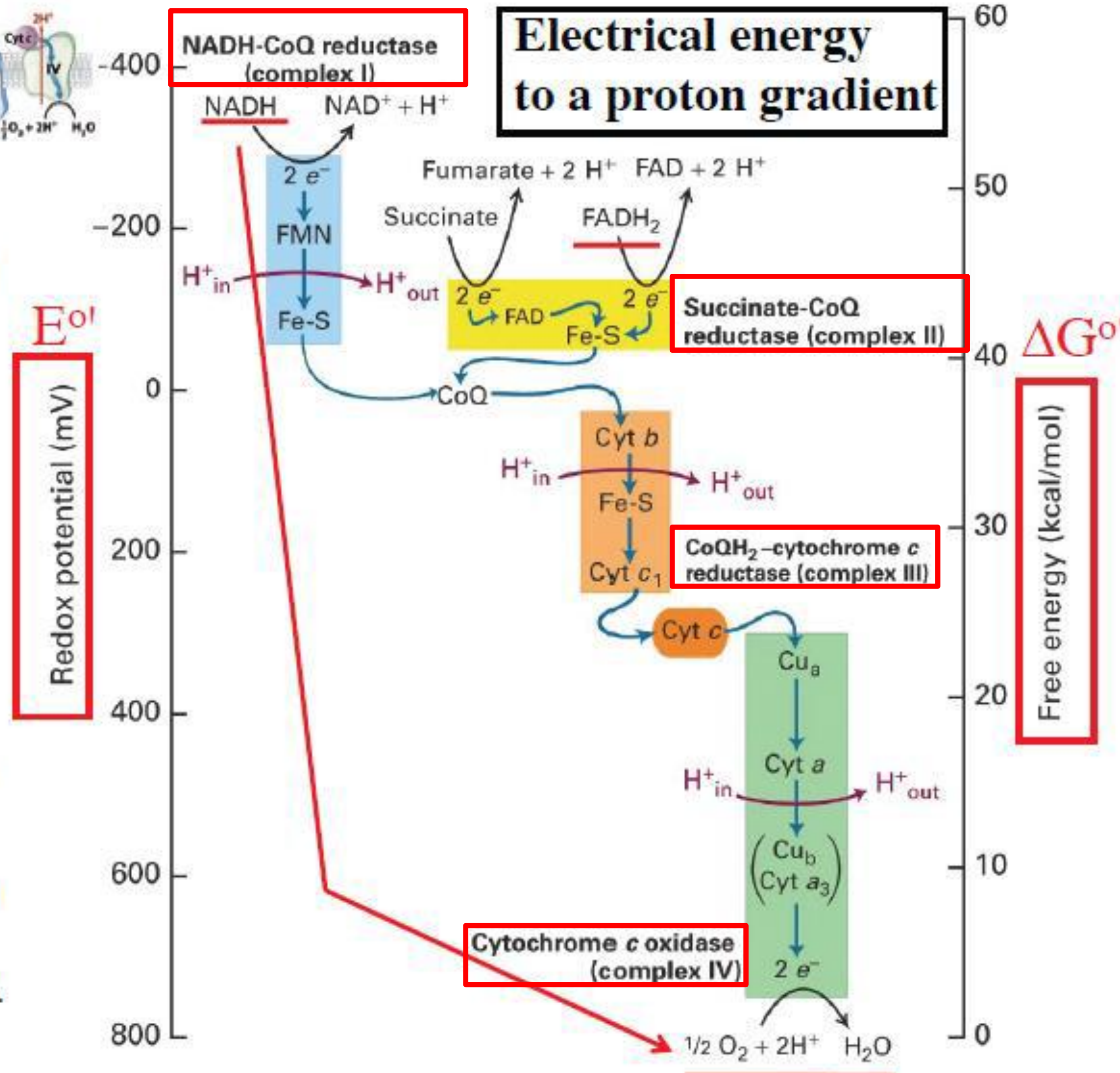


MITOCHONDRIAL RESPIRATORY CHAIN



High E

Low E



From *Molecular Cell Biology*, 5e by Harvey Lodish et al.

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THE RESPIRATORY CHAIN

First depicted as a series of prosthetic groups of increasing redox potential.

1° pathway:

$AH_2 \rightarrow NAD \rightarrow FMN \rightarrow \mathbf{CoQ} \rightarrow b \rightarrow c_1 \rightarrow c \rightarrow a \rightarrow a_3 \rightarrow O_2$

$AH_2 + \frac{1}{2}\, O_2 \rightarrow A + H_2O$

2° pathway

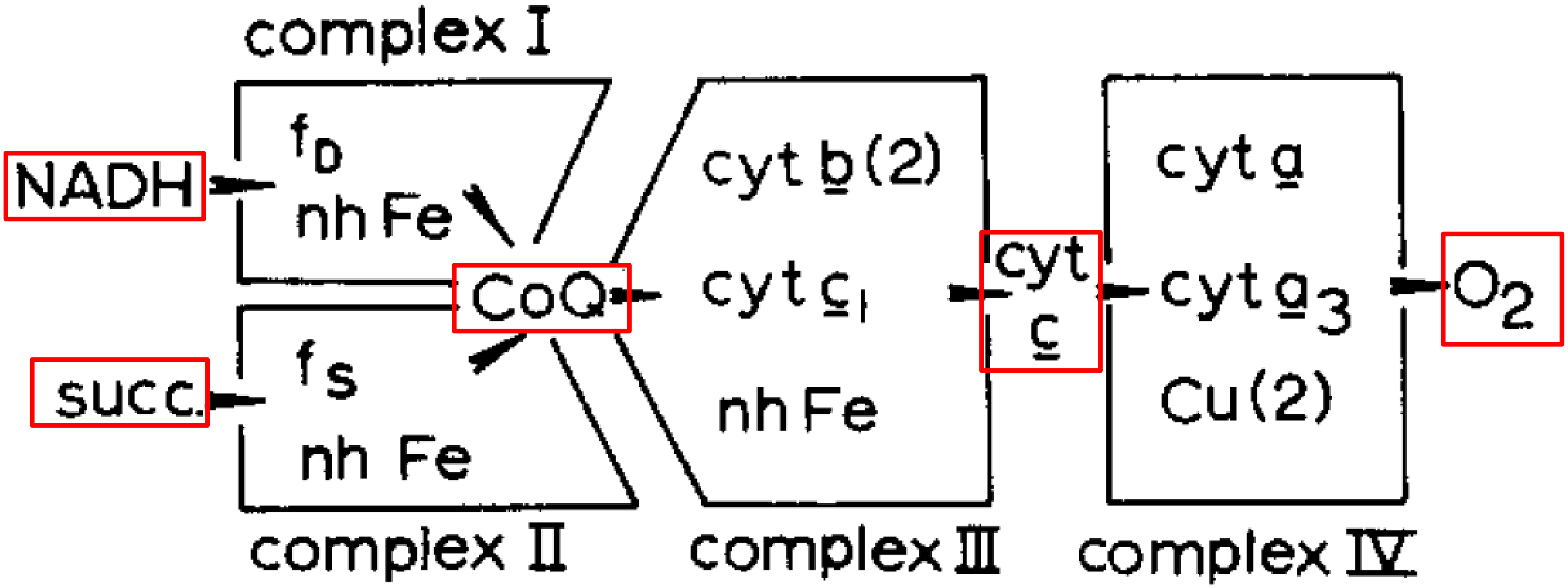
$Succinate \rightarrow FAD \rightarrow \mathbf{CoQ} \rightarrow b \rightarrow c_1 \rightarrow c \rightarrow a \rightarrow a_3 \rightarrow O_2$

Succinate + ½ O_2 → Fumarate + H_2O

Green demonstrated that these prosthetic groups are contained in individual enzymes called **respiratory complexes**.

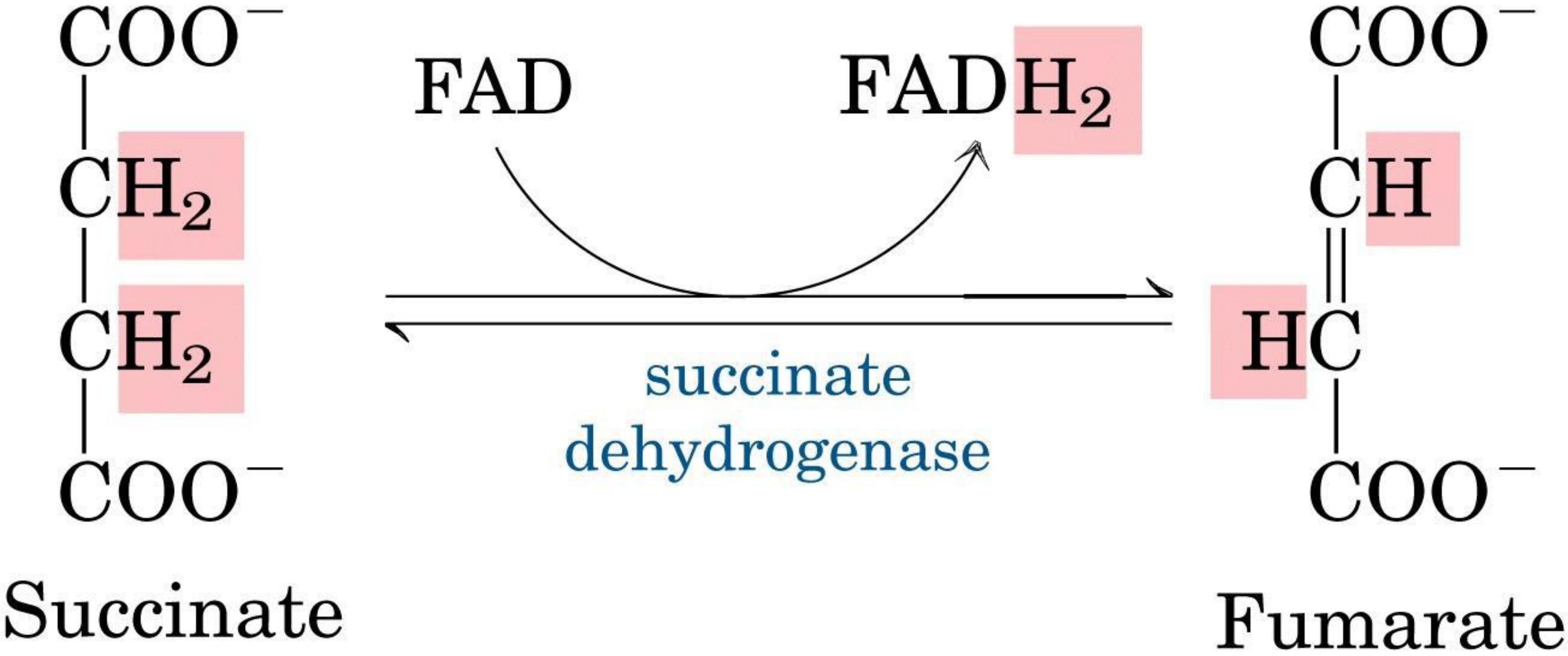
MITOCHONDRIAL RESPIRATORY CHAIN

Green and Tzagaloff 1966: the respiratory chain is made of 4 respiratory multiprotein complexes containing redox coenzymes and connected by mobile intermediates



MITOCHONDRIAL RESPIRATORY CHAIN

Natural acceptor is CoQ: succinate CoQ reductase is Complex II. The only membrane-bound enzyme of TCA cycle.



RESPIRATORY CHAIN ENZYMATIC COMPLEXES

In parenthesis number of subunits encoded by mitochondrial DNA

Enzyme Complex	MW (kDa)	Nr. subunits *	Prosthetic groups
I (NADH-CoQ)	1000	45-46 (7)	1 FMN, 8 Fe-S
II (succinate-CoQ)	140	5	1 FAD, 3 Fe-S (1 cyt.b)
III (ubiquinol-cyt. c)	250	11 (1)	2 heme b, 1 heme c_1, 1 Fe-S
IV (cyt. c- O_2)	160	13 (3)	1 heme a, 1 heme a_3, Cu_A, Cu_B

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RESPIRATORY CHAIN REACTIONS

- **Complex I** (NADH-CoQ oxidoreductase)

$NADH + H^{+} + CoQ + 4\ H^{+}_{in} \rightarrow NAD^{+} + CoQH_2 + 4\ H^{+}_{out}$

- **Complex III** ($CoQH_2$-cyt. c oxidoreductase)

$CoQH_2 + 2\ cyt.\ c\ Fe^{3+} + 4\ H^{+}_{in} \rightarrow CoQ + 2\ cyt.c\ Fe^{2+} + 4\ H^{+}_{out}$

- **Complex IV** (Cytochrome c oxidase)

$2\ cyt.\ c\ Fe^{2+} + \frac{1}{2}\ O_2 + 2\ H^{+}_{in} \rightarrow 2\ cyt.c\ Fe^{3+} + H_2O + 2\ H^{+}_{out}$

TOTAL

$NADH + H^{+} + \frac{1}{2}\ O_2 + 10\ H^{+}_{in} \rightarrow NAD^{+} + H_2O + 10\ H^{+}_{out}$

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RESPIRATORY CHAIN REACTIONS

TOTAL

$NADH + H^+ + ½ O2 + 10\ H^+_{in} \rightarrow NAD^+ + H_2O + 10\ H^+_{out}$

The standard reduction potential change is:

$\Delta E'° = E'°_{(electron\ acceptor)} - E'°_{(electron\ donor)}$

$E'°$ for O_2/H_2O = 0.816 V (electron acceptor)

$E'°$ for $NAD^+/NADH$ = −0.320 V (electron donor)

$\Delta E'°$ = 0.816 V − (−0.320 V) = 1.14 V

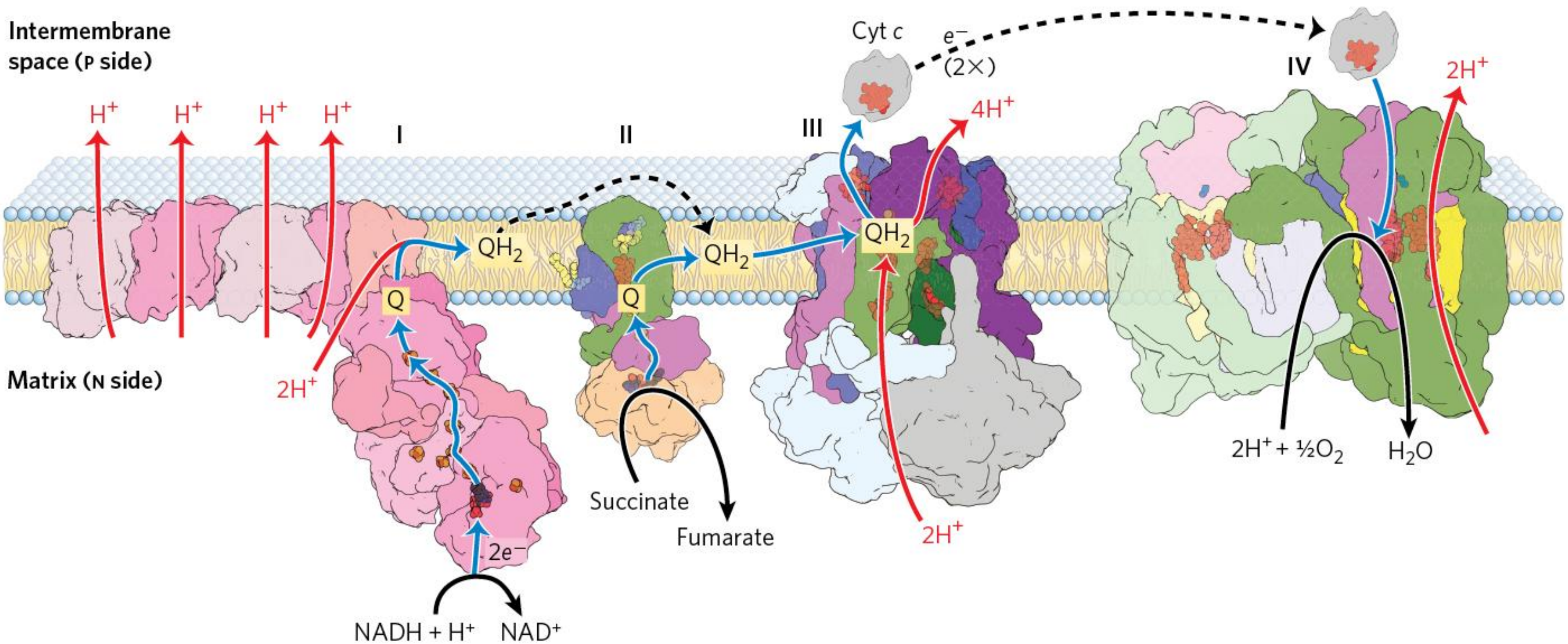
The standard free-energy change is:

$\Delta G'° = -nF\Delta E'°$

= −2 (96.5 kJ V^{-1} mol^{-1})(1.14 V) = −220 kJ/mol (of NADH)

RESPIRATORY CHAIN REACTIONS

Much of the free energy generated is recovered and stored in the form of an electrochemical proton gradient across the mitochondrial inner membrane.



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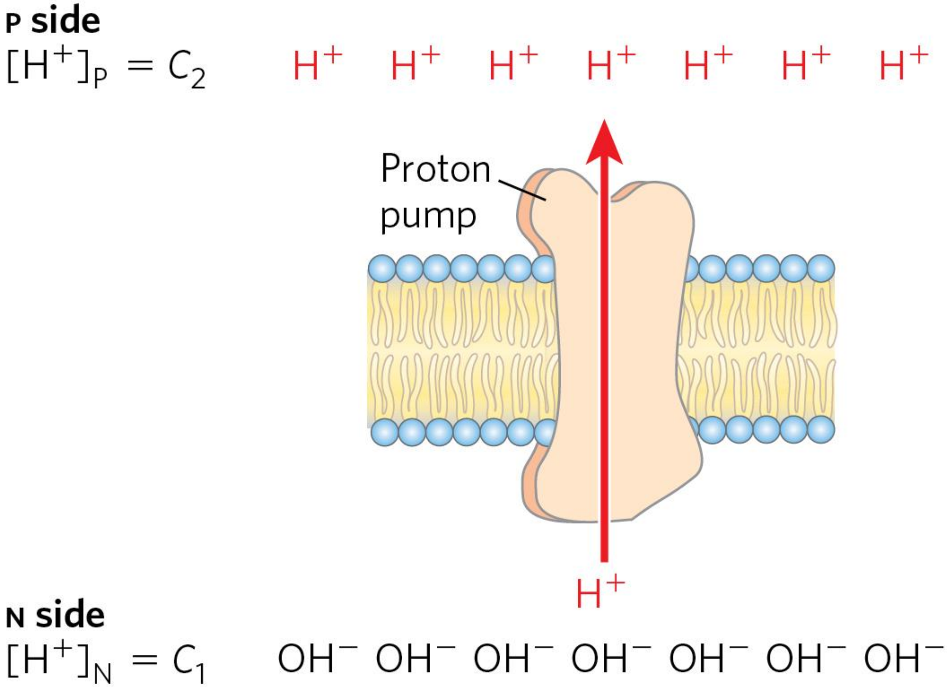
PROTON-MOTIVE FORCE

Complexes I, III and IV are proton (H^+) pumps toward the exterior exploiting the energy of redox reactions.

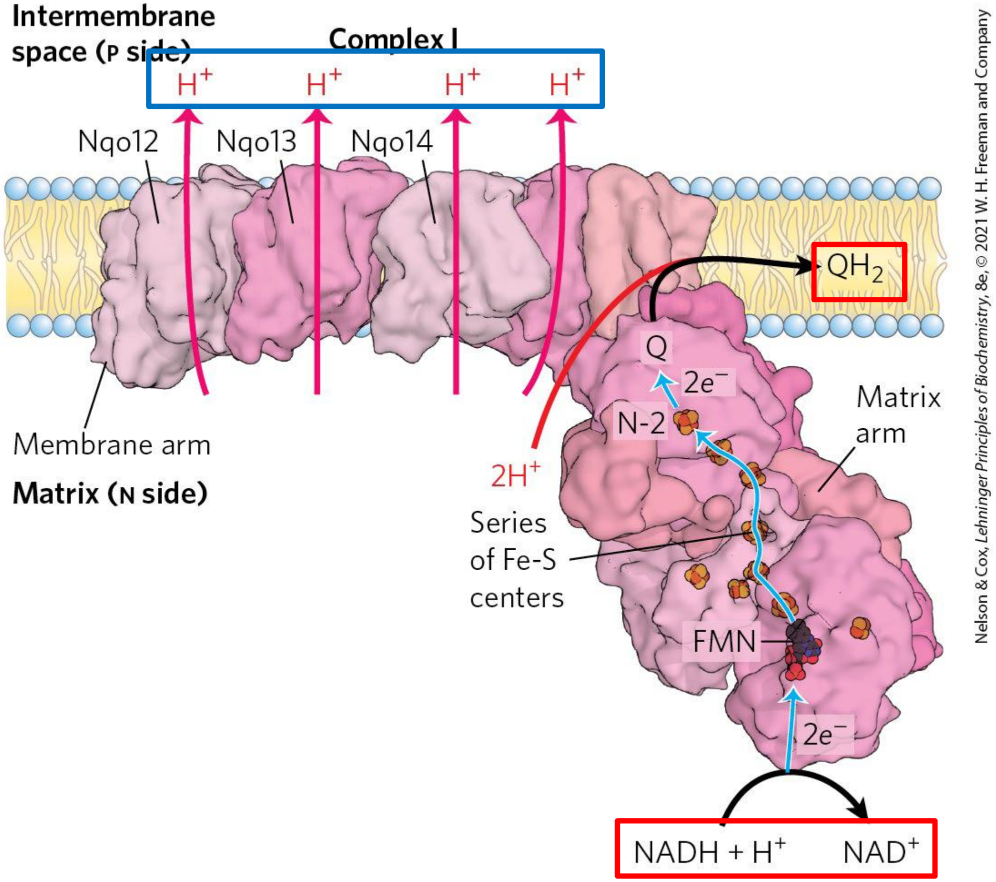


$$\Delta G = RT \ln (C_2/C_1) + ZF\Delta\psi$$
$$= 2.3RT\,\Delta\text{pH} + F\Delta\psi$$

$$\Delta G = RT \ln (C_2/C_1) + ZF\,\Delta\psi$$

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

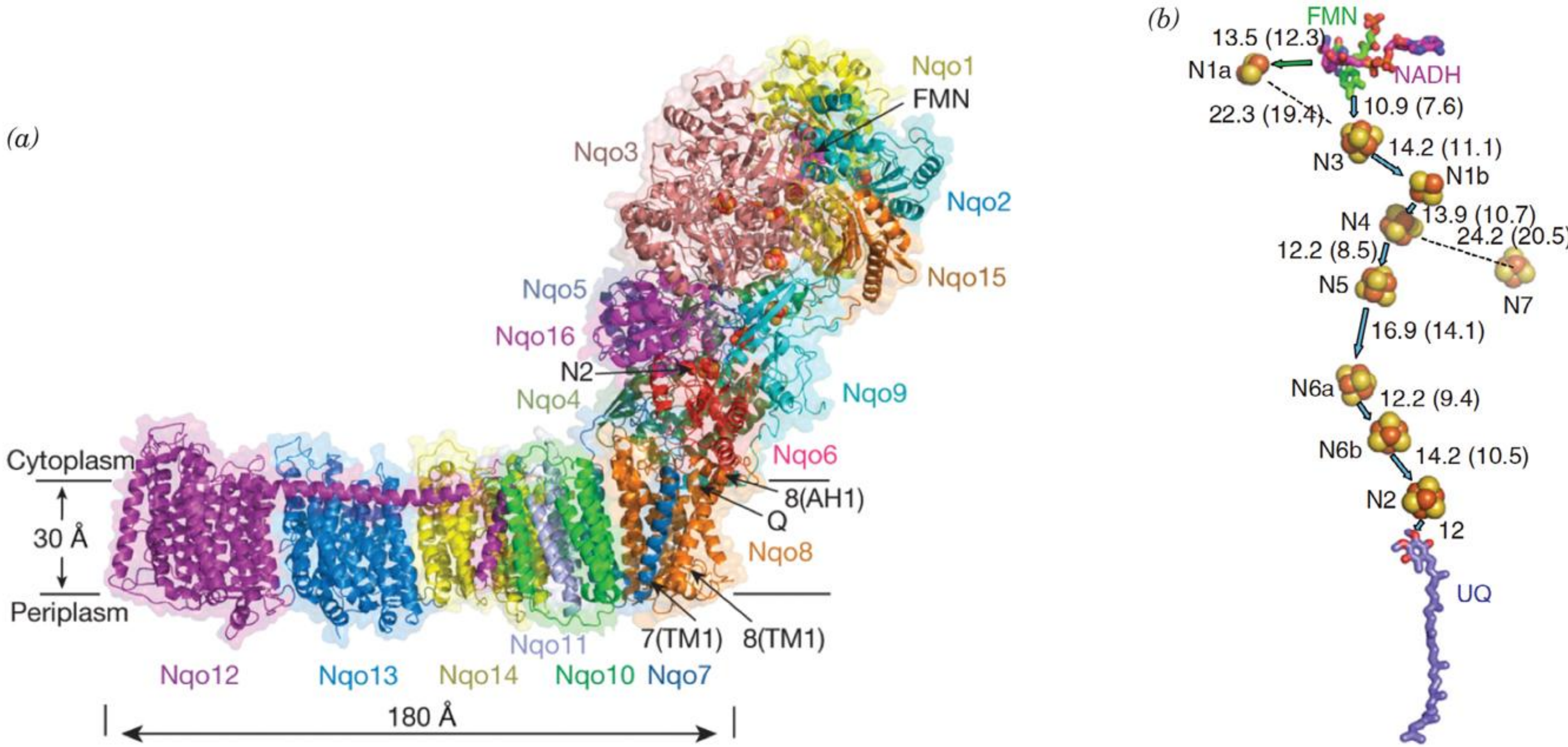
RESPIRATORY COMPLEXES



Schematic structure of Complex I (NADH:ubiquinone oxidoreductase)

RESPIRATORY COMPLEXES

Crystal structure of Complex I



Courtesy of Leonid Sazanov, Medical Research Council, Cambridge, U.K.

From Sazanoff, L.H., J. Bioenergetics and Biomembranes 46, 247 (2014) Fig. 1B.

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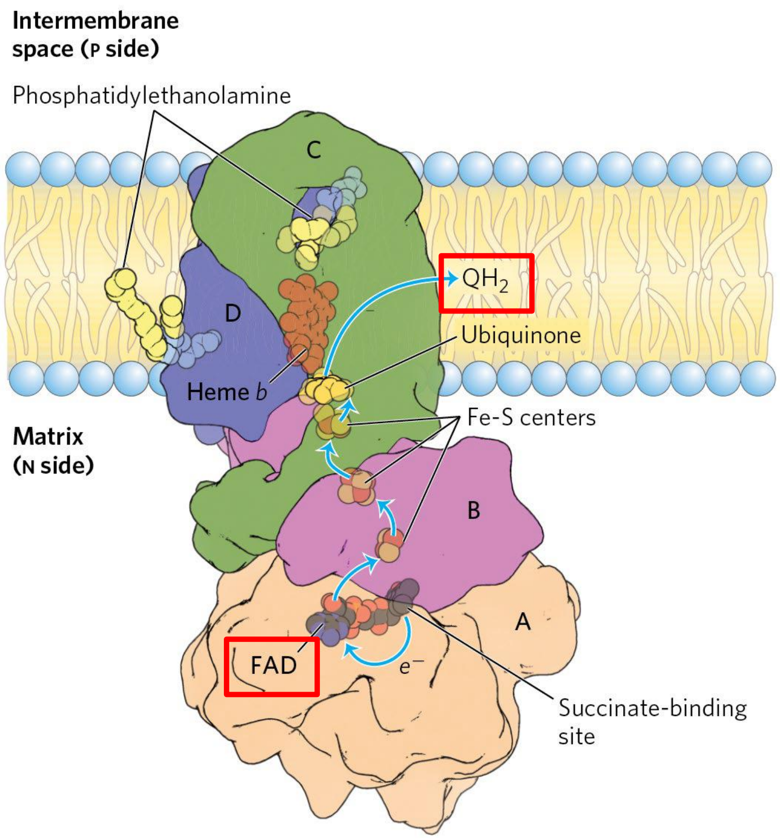
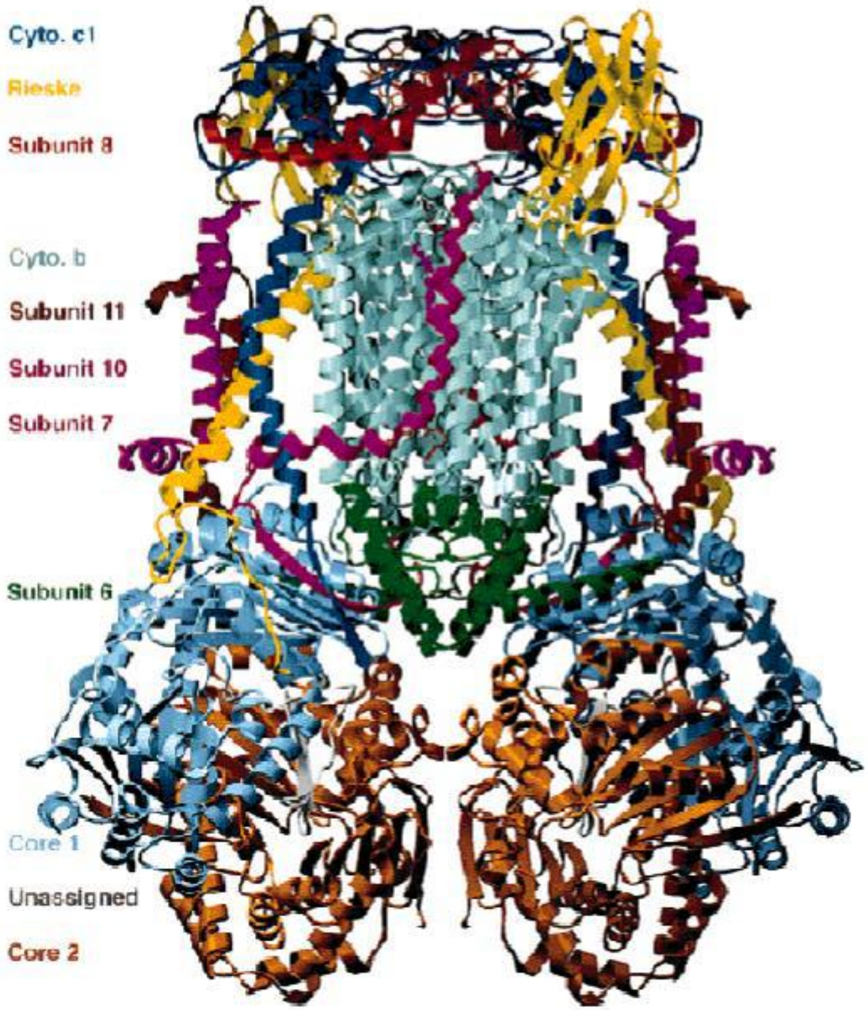
RESPIRATORY COMPLEXES

Schematic structure of Complex II (Succinate dehydrogenase)



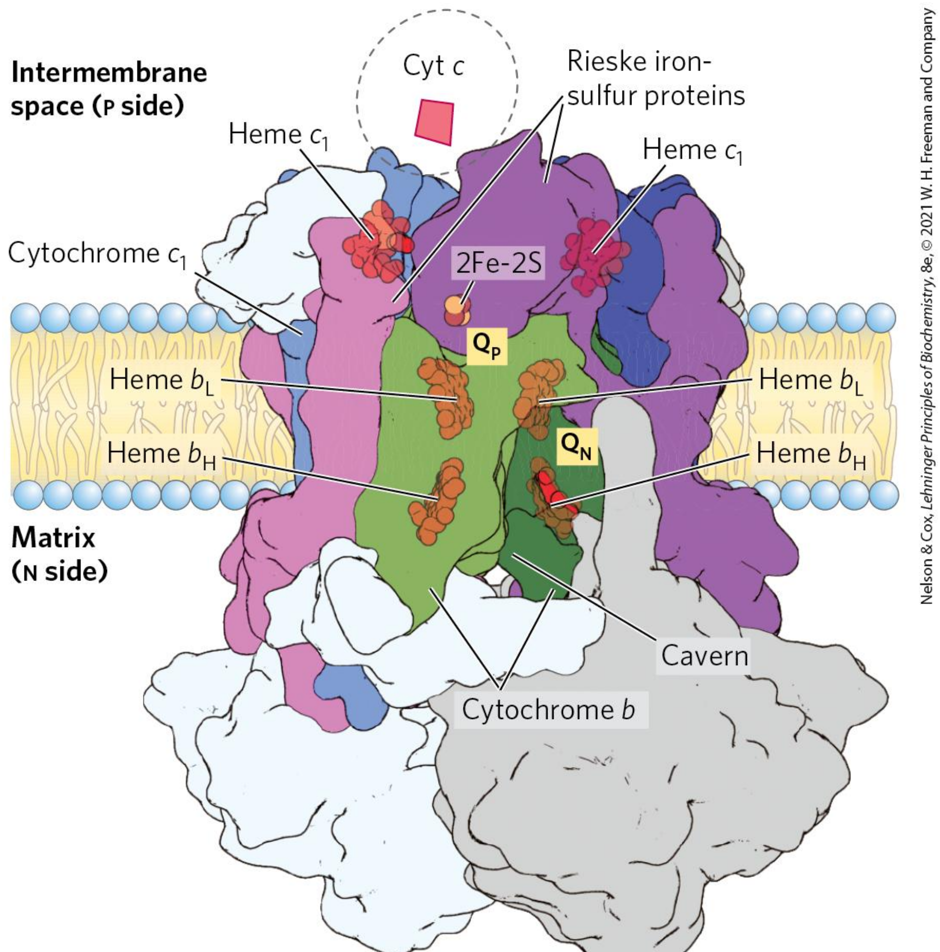
RESPIRATORY COMPLEXES

Crystal structure of Complex III



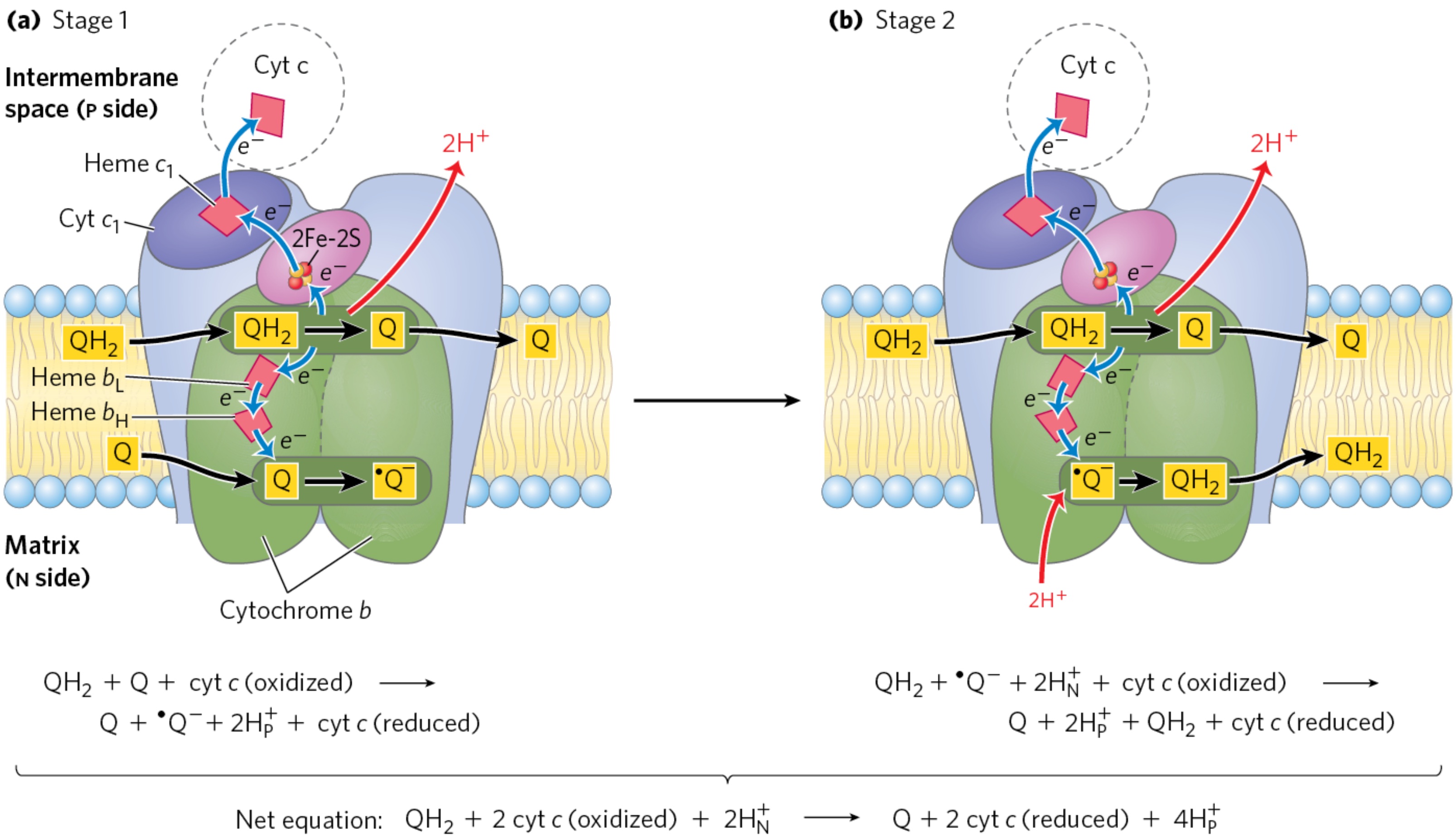
RESPIRATORY COMPLEXES

Schematic structure of Complex III (cytochrome bc_1 complex, ubiquinone: cytochrome c oxidoreductase)



RESPIRATORY COMPLEXES

Complex III: the Q cycle



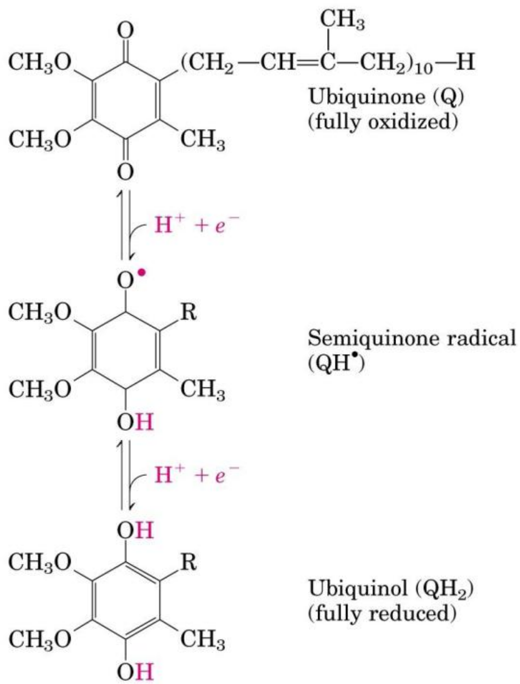
Q-CYCLE

Summary:

1) $QH_2 + c(ox) \rightarrow Q^{\bullet -} + c(red) + 2\ H^+_{out}$

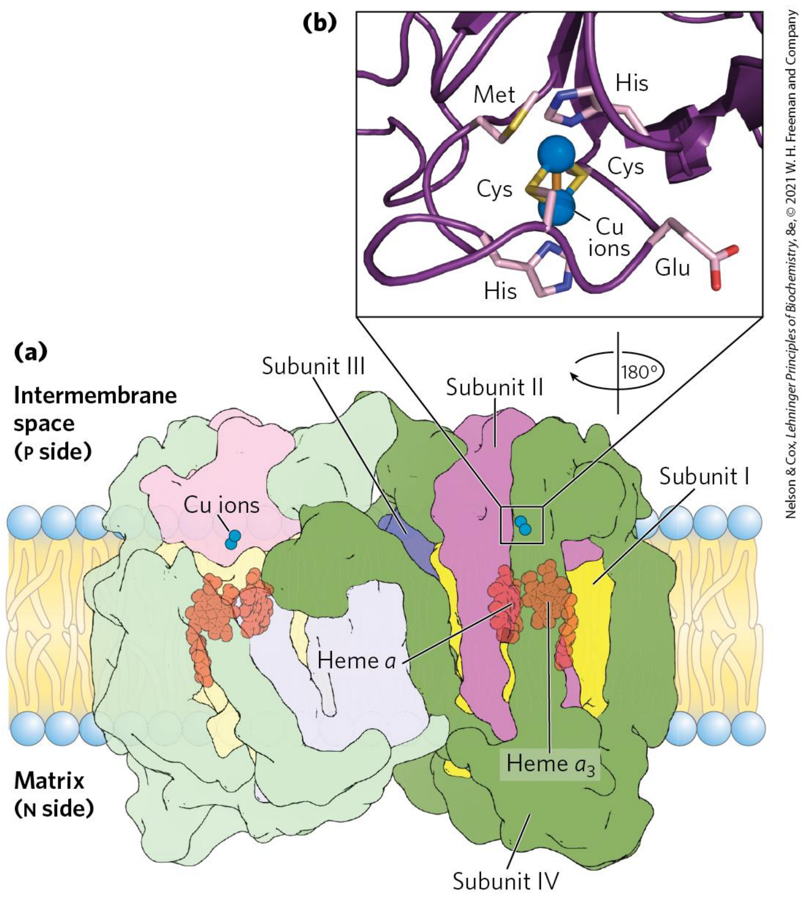
2) $QH_2 + c(ox) + Q^{\bullet -} + 2\ H^+_{in} \rightarrow Q + QH_2 + c(red) + 2\ H^+_{out}$

TOTAL: $QH_2 + 2\ c(ox) + 2\ H^+_{in} \rightarrow Q + 2\ c(red) + 4\ H^+_{out}$

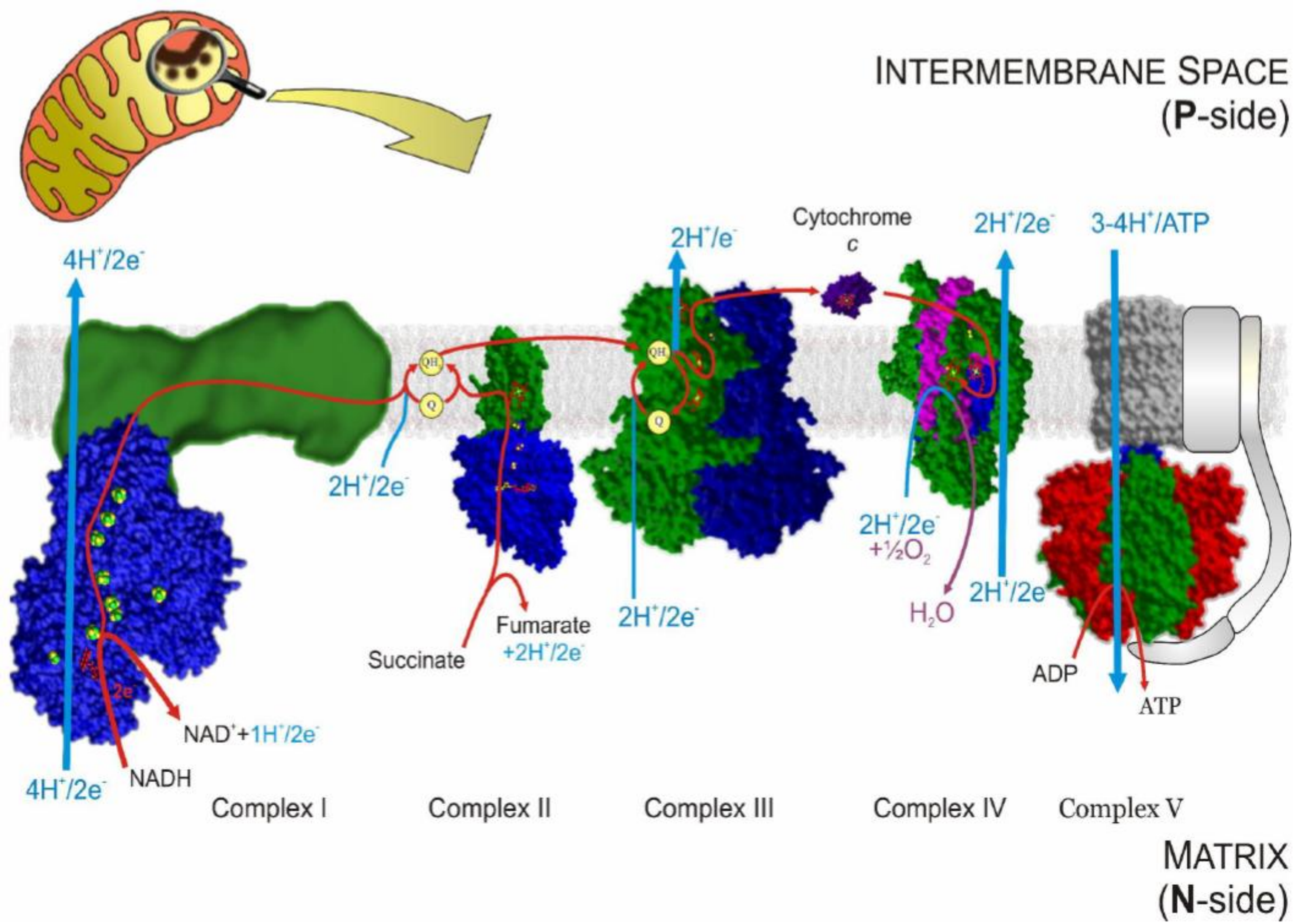


RESPIRATORY COMPLEXES

Schematic structure of Complex IV (cytochrome oxidase)



RESPIRATORY COMPLEXES



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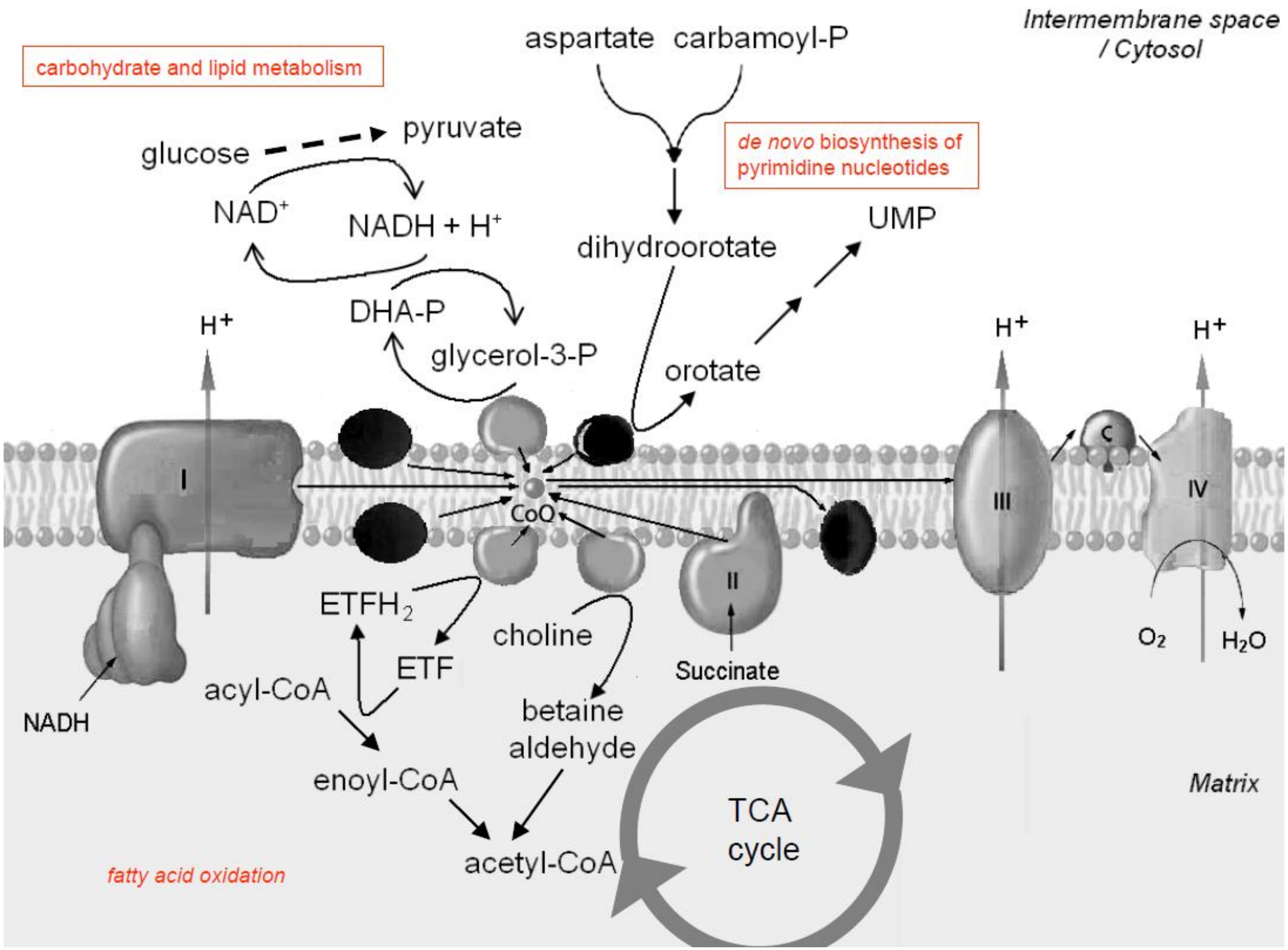
RESPIRATORY COMPLEXES



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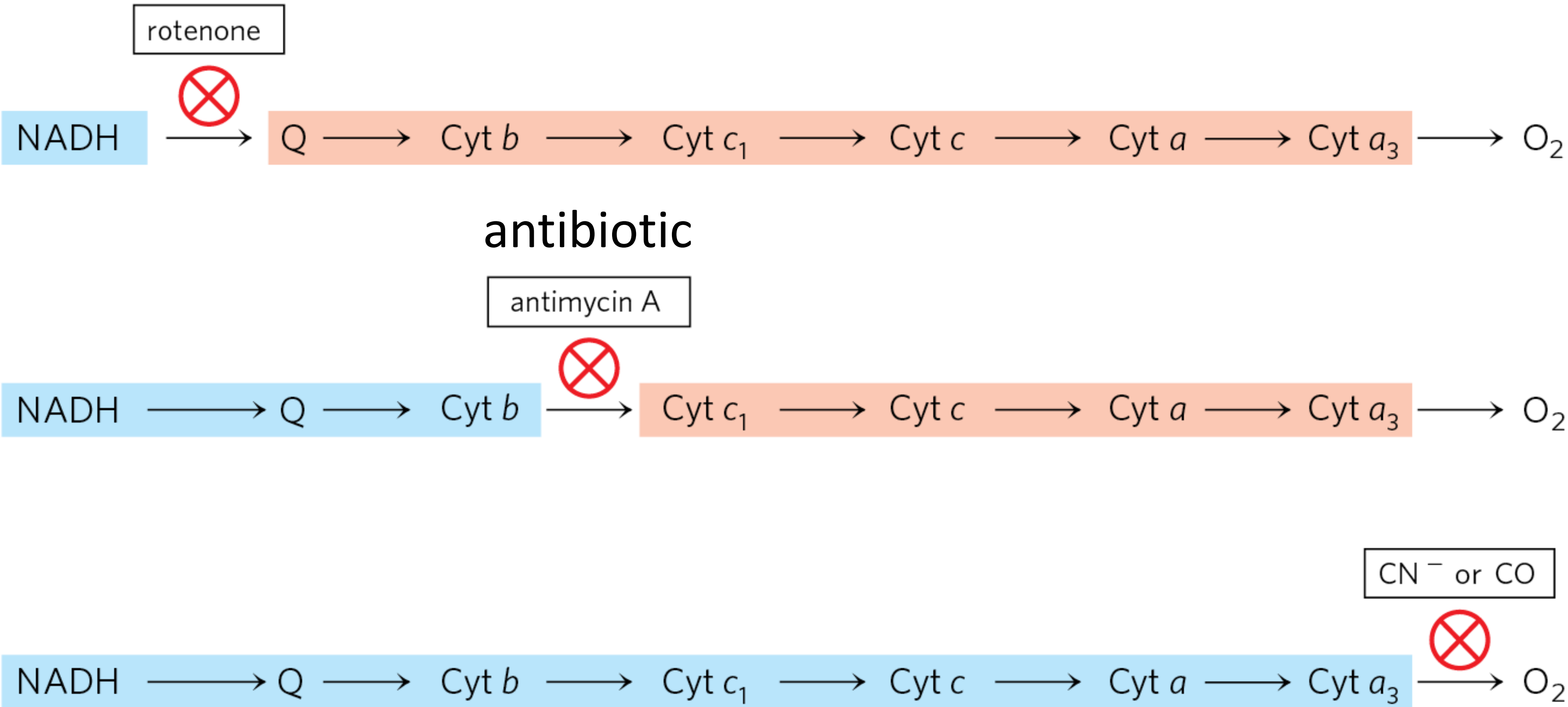
RESPIRATORY INHIBITORS

insecticide



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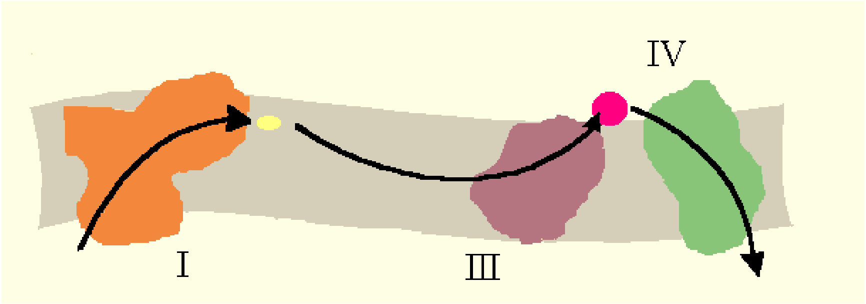
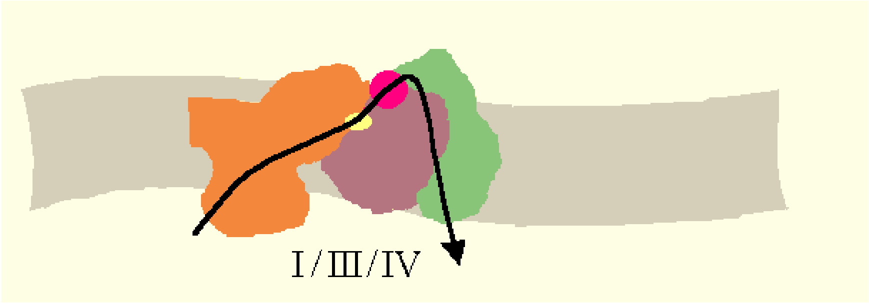
STRUCTURAL ORGANIZATION OF THE RESPIRATORY CHAIN

Two models:

1) **Fluid state**: random collisions between respiratory complexes and mobile components (CoQ and Cyt. c)

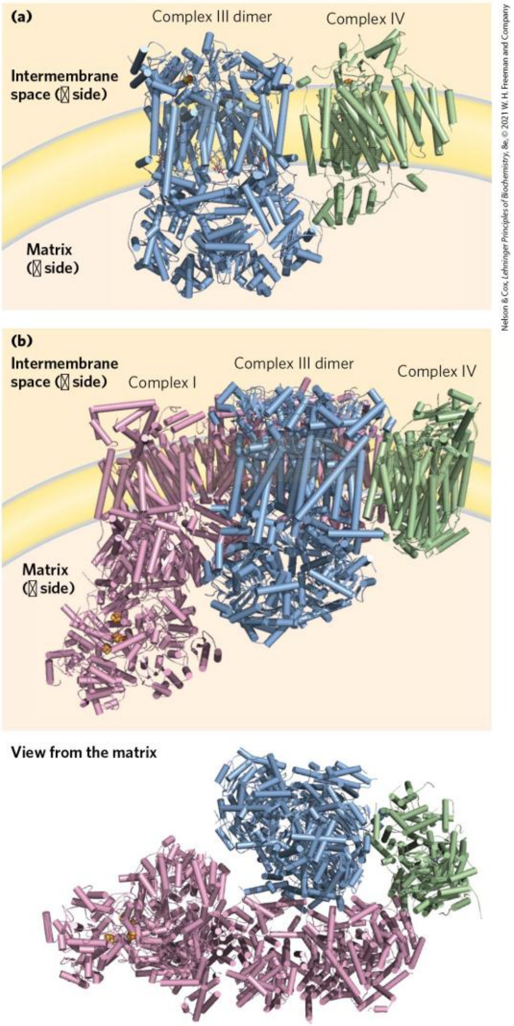


2) **Solid-state** organization: supercomplexes/respirasomes substrate channelling



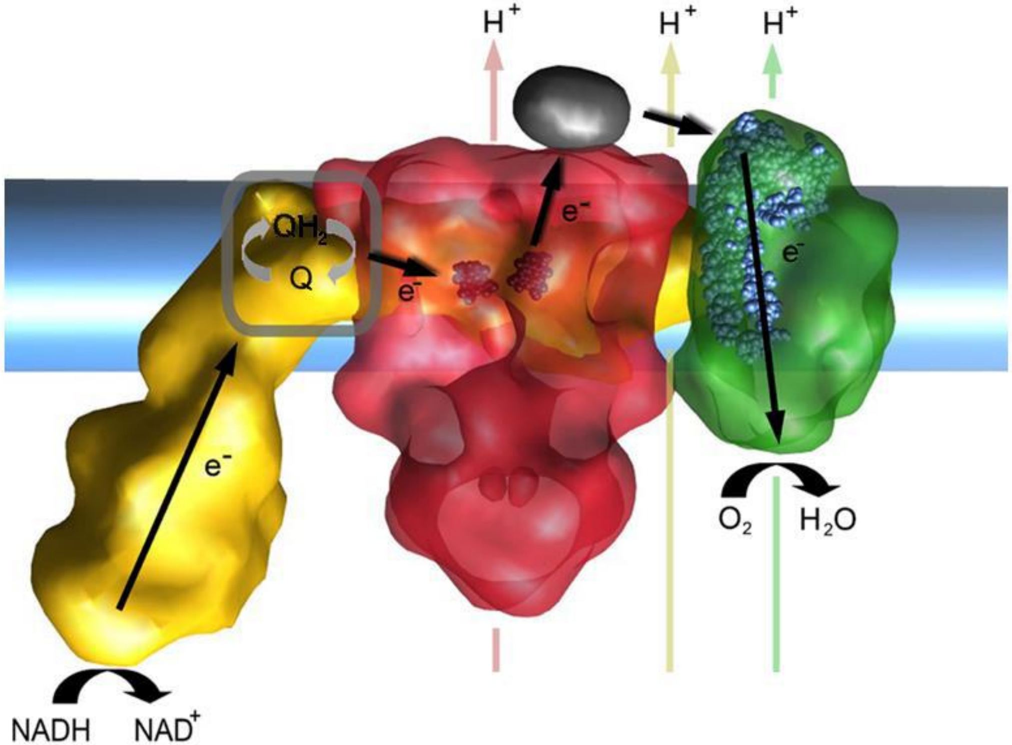
STRUCTURAL ORGANIZATION OF THE RESPIRATORY CHAIN

Respirasome is a supercomplex containing complexes I, III, and IV in which cytochrome c and ubiquinone readily diffuse between the supercomplexes.



STRUCTURAL ORGANIZATION OF THE RESPIRATORY CHAIN

The respiratory complexes tightly associate with each other in the inner membrane of the mitochondrion.



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