Respiratory chain
-Electron transport chain

The electron transport chain consists of a **spatially** separated series of redox reactions in which electrons are transferred from a donor molecule to an acceptor molecule. The underlying force driving these reactions is the Gibbs free energy of the reactants and products. The Gibbs free energy is the energy available ("free") to do work. Any reaction that decreases the overall Gibbs free energy of a system is thermodynamically spontaneous.
The function of the electron transport chain is to produce a transmembrane proton electrochemical gradient as a result of the redox reactions.[1] If protons flow back through the membrane, they enable mechanical work, such as rotating bacterial flagella. ATP synthase, an enzyme highly conserved among all domains of life, converts this mechanical work into chemical energy by producing ATP,[2] which powers most cellular reactions. A small amount of ATP is available from substrate-level phosphorylation, for example, in glycolysis. In most organisms the majority of ATP is generated in electron transport chains, while only some obtain ATP by fermentation

Transformation of energy in human body

Chemical E of nutrient = **work** + **heat**

E of nutrient = **BM + physical activity + reserves** + **heat**

Every work needs- ATP

chemical: synthesis of proteins.. ...

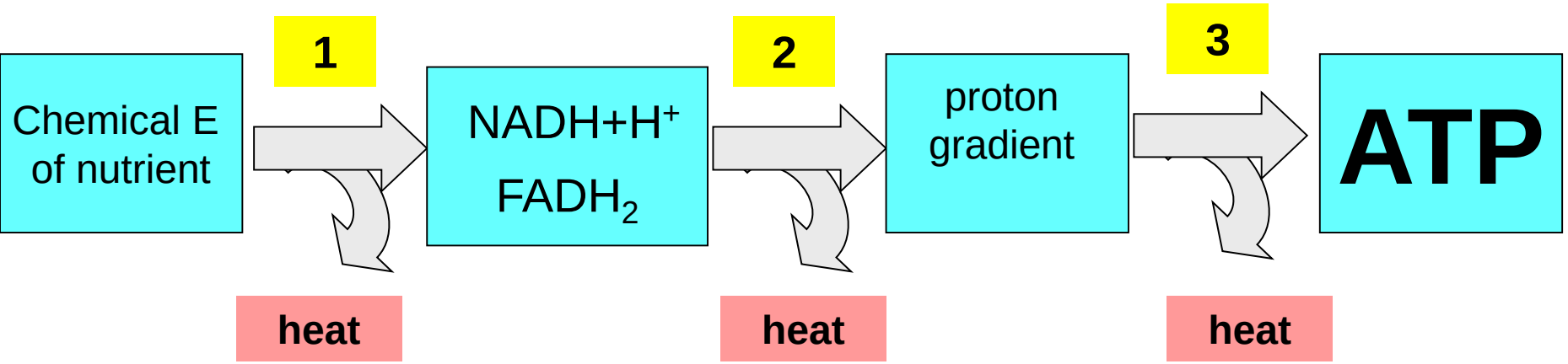
osmotical: transport of ions ...

mechanical: muscle contraction ...

BM = basal methabolism

reserves = adipose tissue, glycogene

Transformation of E – production of heat



1 ..... metabolically dehydrogenation

2 ..... RCH = oxidation of reduced cofactors and reduction of O_2 to H_2O

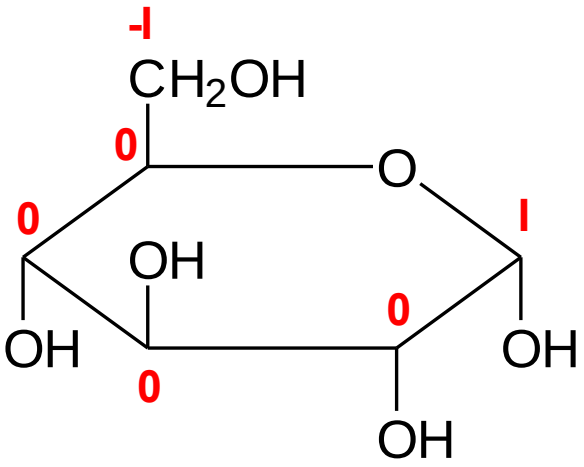
3 ..... Aerobic phosphorylation

..... High energetic system

Nutrients and E

nutrient	E (kJ/g)	Thermogenesis	Source of E/day
Lipids	38	4 %	≤ 30 % SAFA 5 %, MUFA 20 %, PUFA 5 %
CH + sugars	17	6 %	55 - 60 %
Proteins	17	30 %	10 - 15 %

Nutrients



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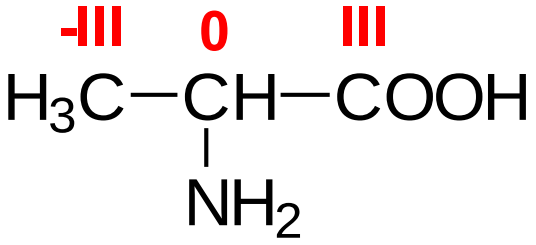

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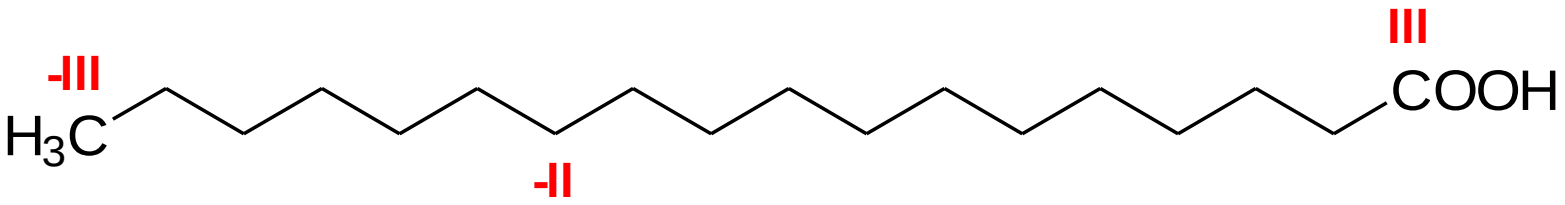
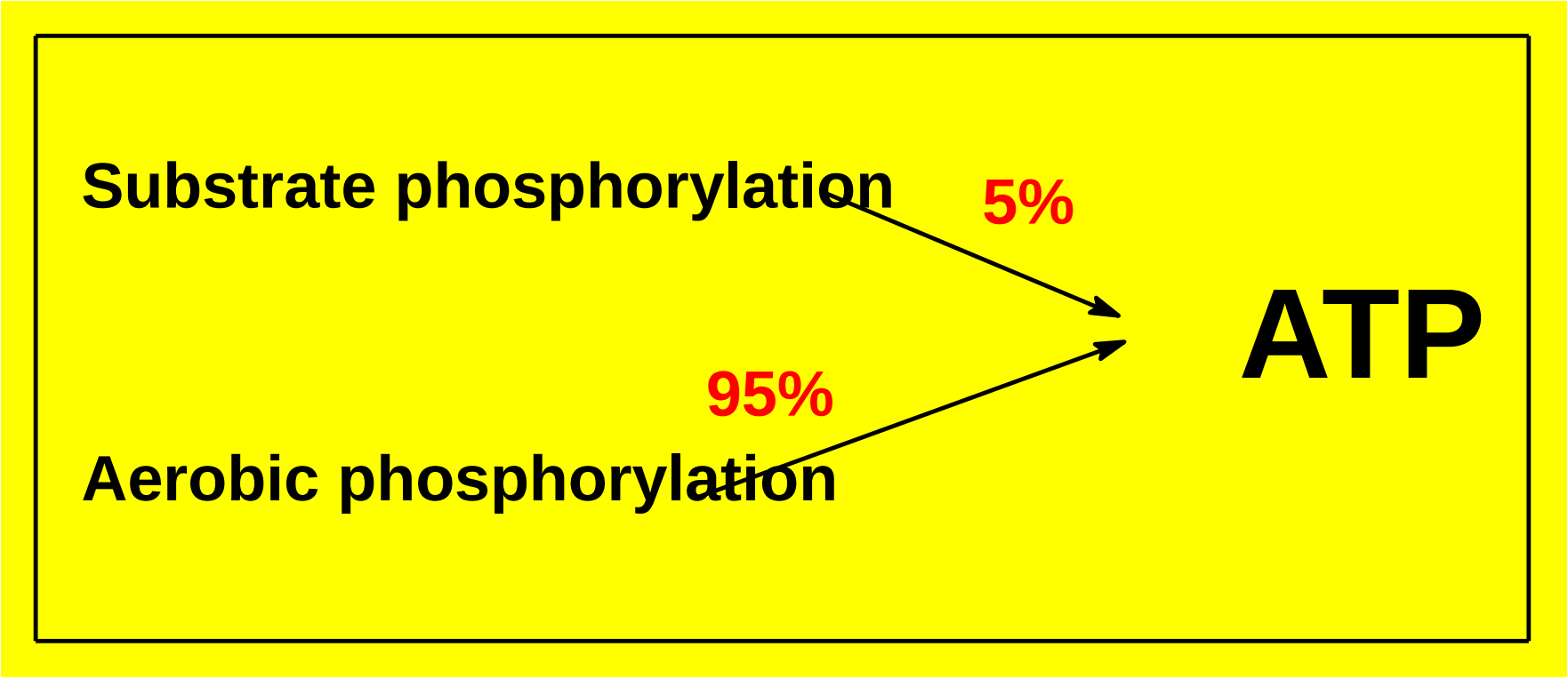


Event. ox.n. C = -1,8 ⇒best C

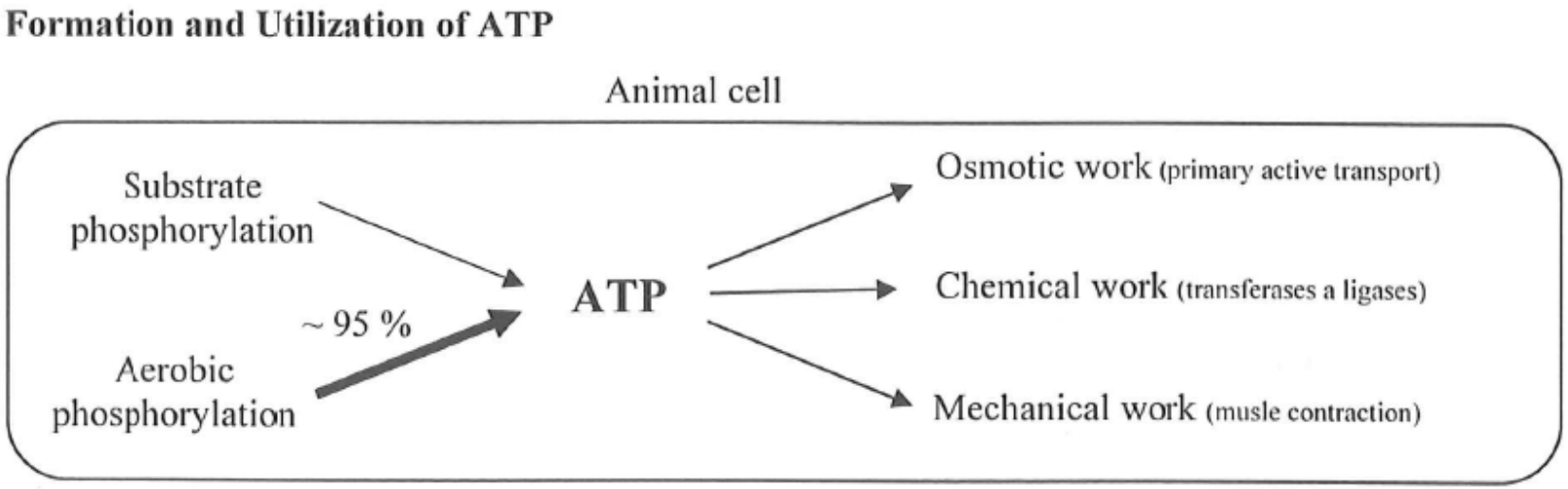
ATP

- **Adenosine-triphosphate** (**ATP**) is a nucleotide triphosphate used in cells as a coenzyme. It is often called the "molecular unit of currency" of intracellular energy transfer.[1] ATP transports chemical energy within cells for metabolism. It is one of the end products of photophosphorylation, cellular respiration, and fermentation and used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division.[2] One molecule of ATP contains three phosphate groups, and it is produced by a wide variety of enzymes, including ATP synthase, from adenosine diphosphate (ADP) or adenosine monophosphate (AMP) and various phosphate group donors. Substrate level phosphorylation, oxidative phosphorylation in cellular respiration, and photophosphorylation in photosynthesis are three major mechanisms of ATP biosynthesis.

Formation of ATP in body



Formation and utilization of ATP



Two ways of ATP formation

Aerobic phosphorylation (95 %)

ADP + P_i + energy H^+-gradient $\rightarrow$ ATP

Substrate phosphorylation (5 %)

macroergic ~P + ADP $\rightarrow$ ATP + second product

!! Different: common **phosphorylation**

X-OH + ATP $\rightarrow$ X-O-P + ADP

Two ways of ATP formation

Substrate phosphorylation

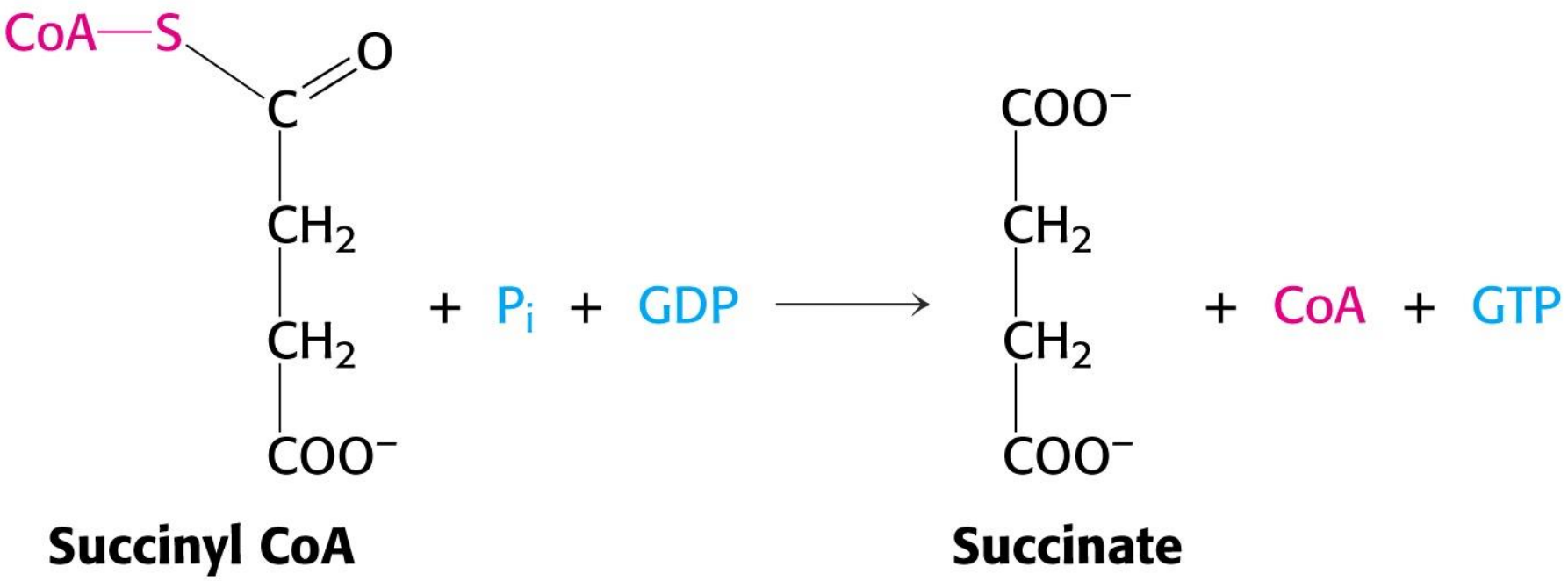
- ATP is produced after conversion of macroergic intermediates in metabolism of nutrients
- **succinyl-CoA (CC)**
- **1,3-bisphosphoglycerate** (glycolysis)
- **phosphoenolpyruvate** (glycolysis)

Aerobic phosphorylation

- Connected to RCh
- For ATP synthesis is used proton motive force

CC Succinate formation: step5

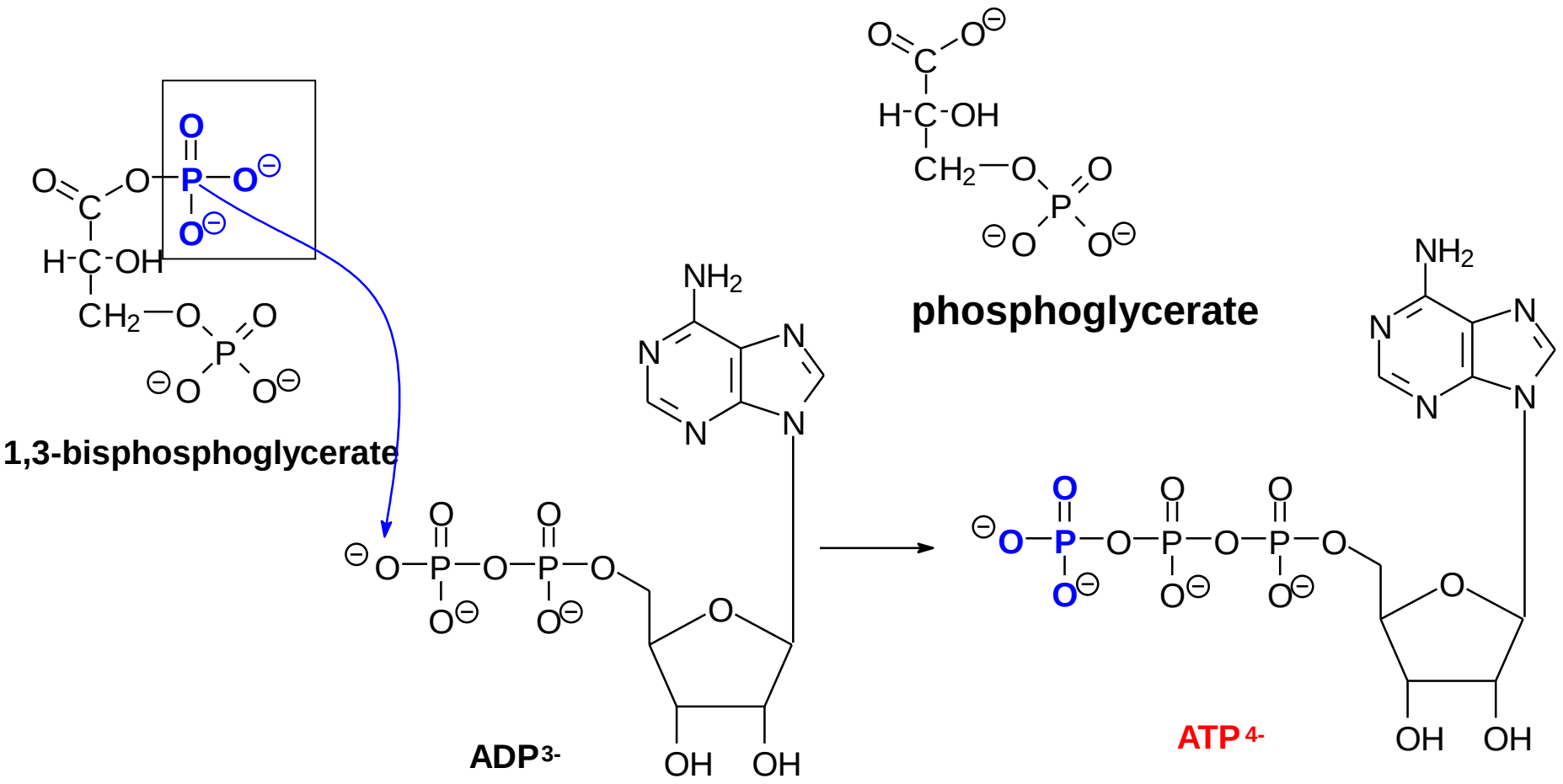
Enzyme: succinyl CoA synthetase



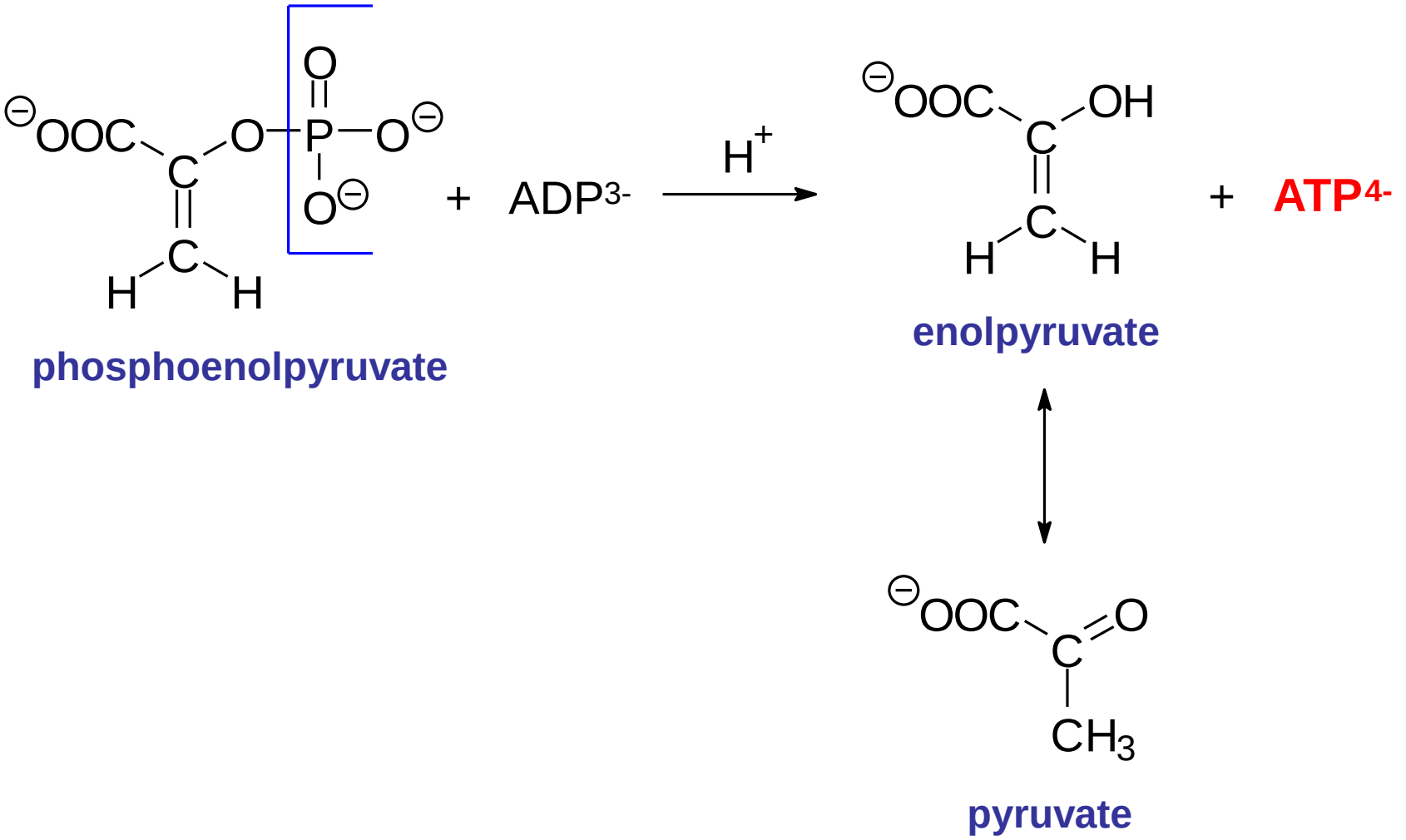
GTP produced

GTP + ADP ⇒ GDP + ATP (NPTase)

Phosphorylation of ADP by 1,3-bisphosphoglycerate



Phosphorylation of ADP by phosphoenolpyruvate



phosphoenolpyruvate

Mitochondrion

Double membrane

Outer membrane is permeable

Inner membrane has invaginations called cristae

The electron transport chain is located in the inner membrane

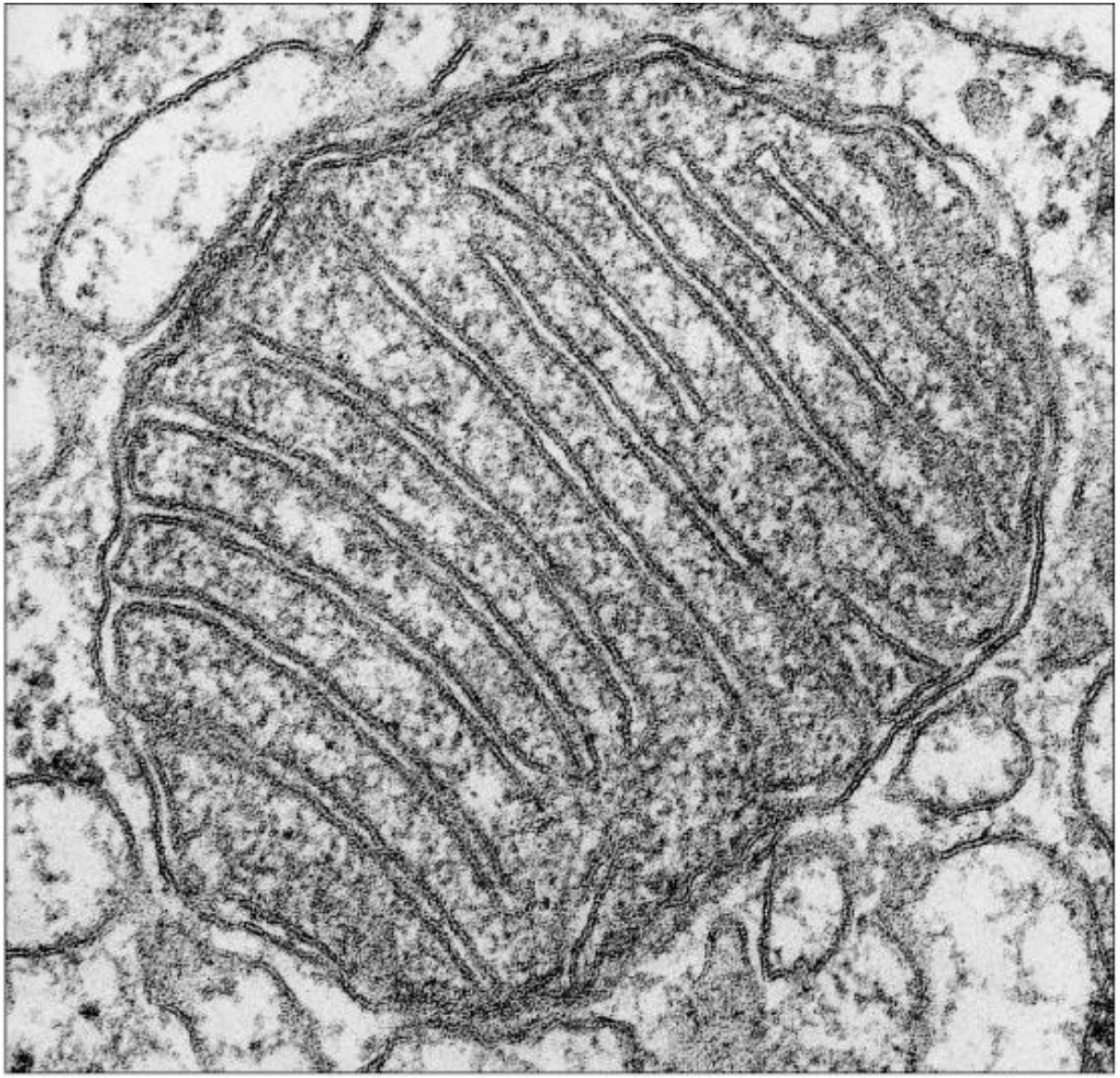
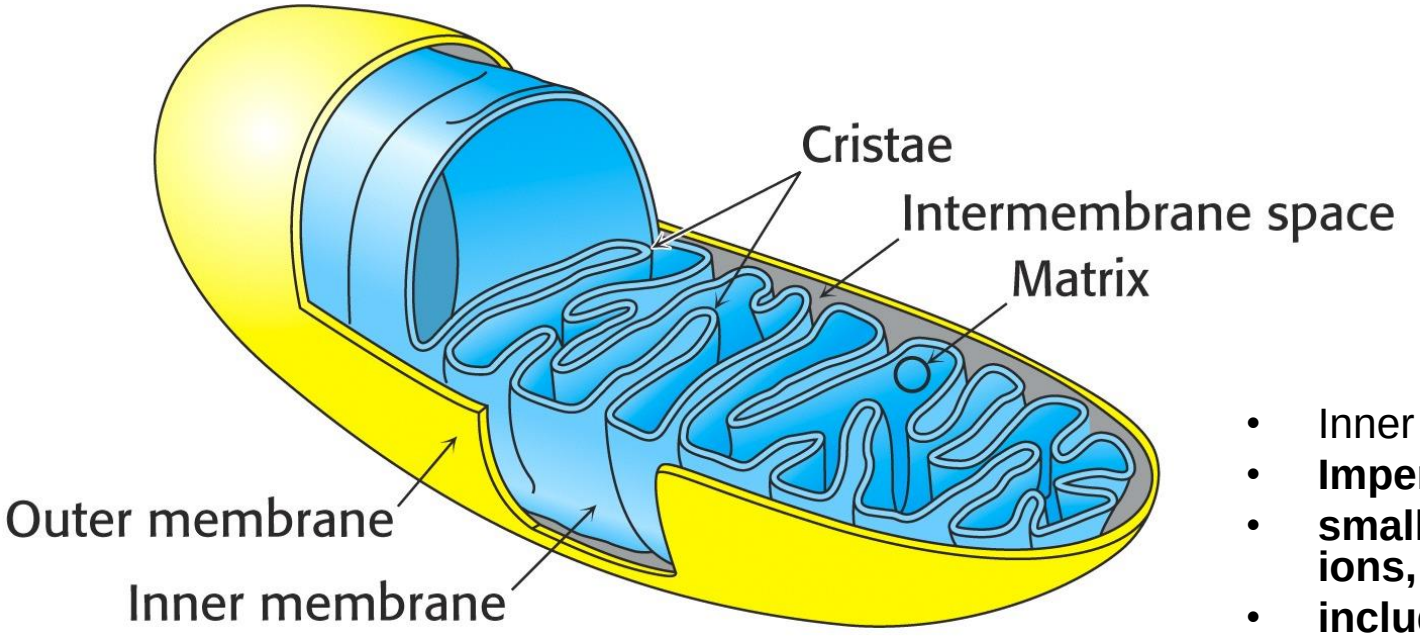
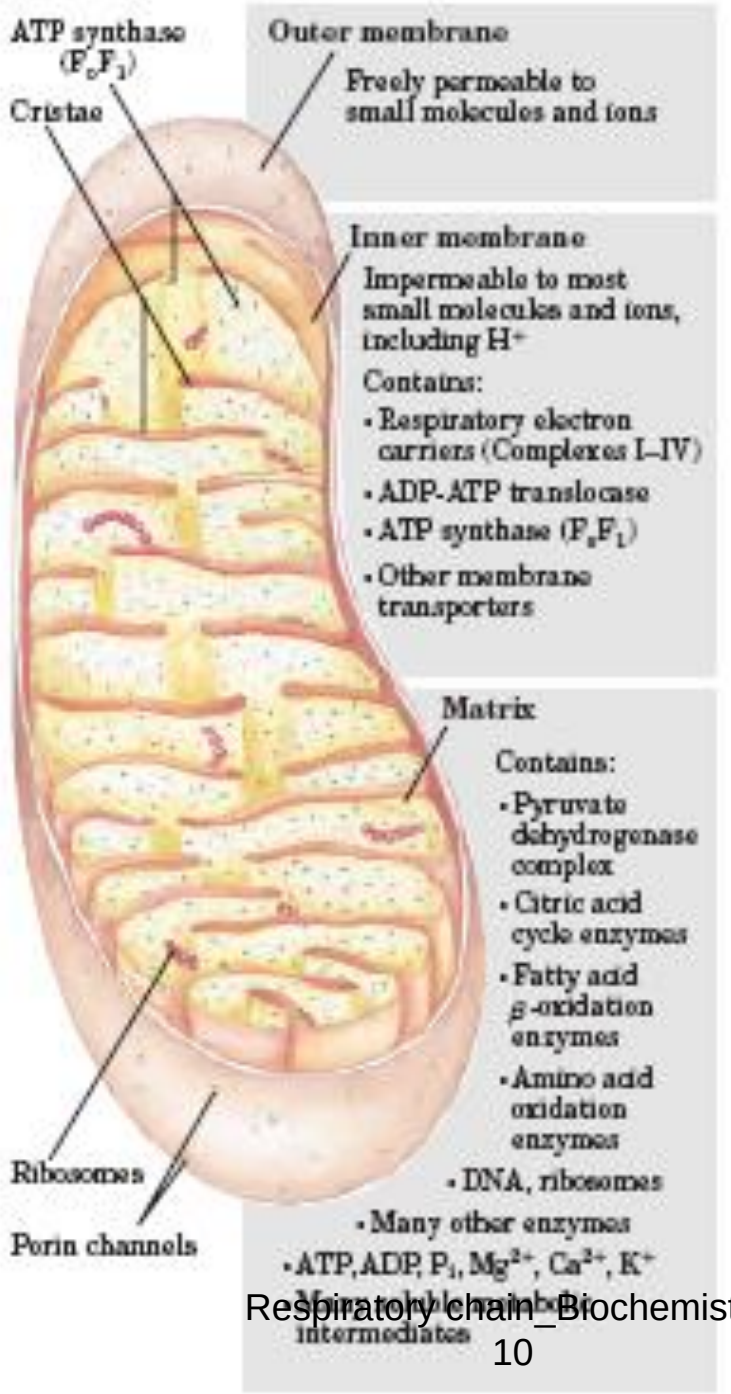


Figure 14–8 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

Diagram of a mitochondrion

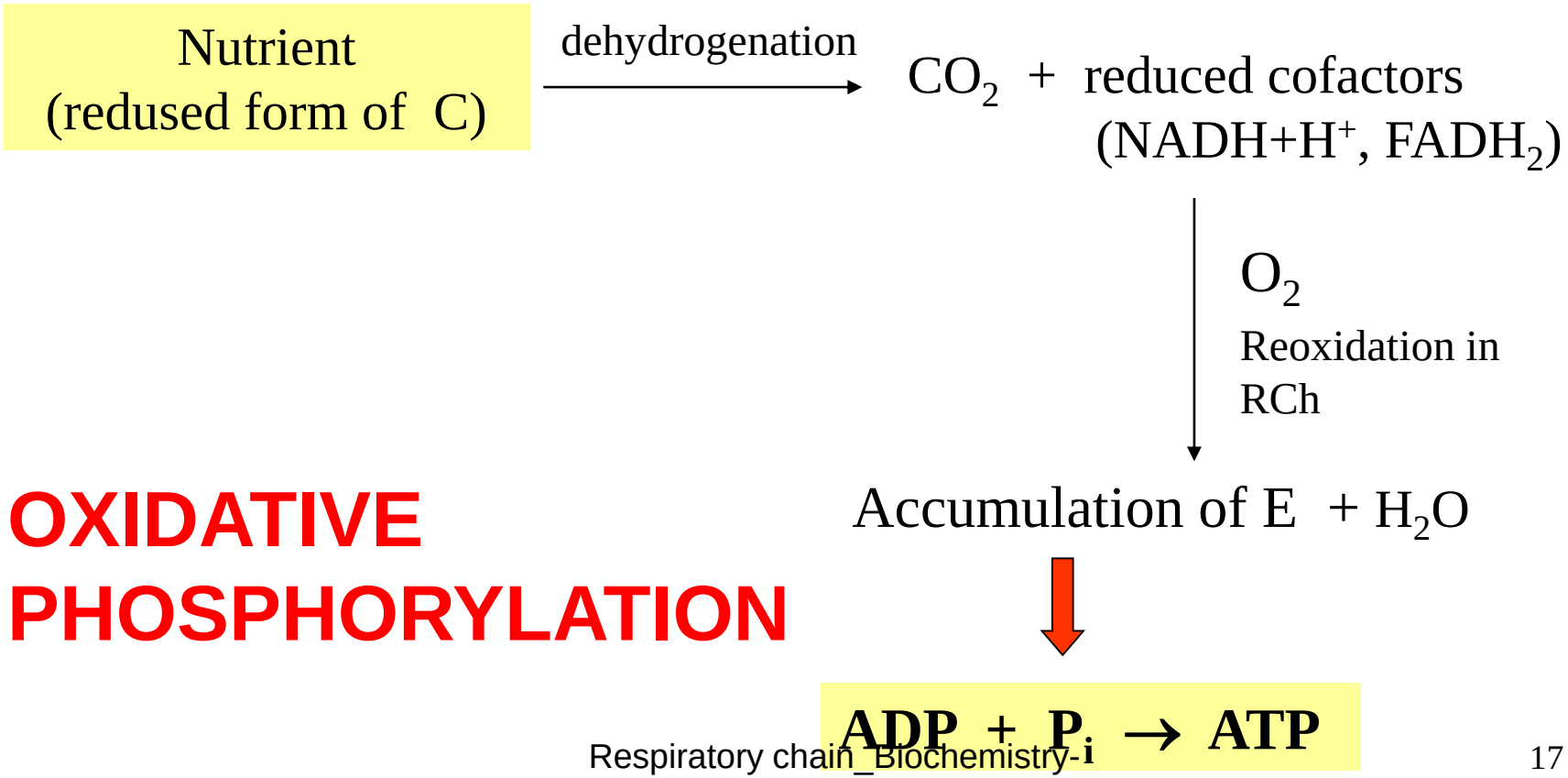


- Inner membrane
- **Impermeable to most**
- **small molecules and ions,**
- **including H+**
- Contains:
- **• Respiratory electron**
- **carriers (Complexes I–IV)**
- **• ADP-ATP translocase**
- **• ATP synthase (FoF1)**
- **• Other membrane**
- **Transporters**
- **80% of proteins**
- **Phospholipids (kardiolipin)**



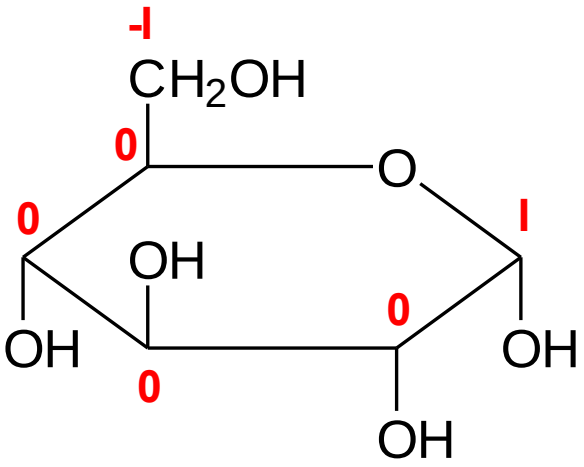
Outer membrane
Freely permeable to small molecules and ions

Aerobic phosphorylation



OXIDATIVE PHOSPHORYLATION

Nutrients



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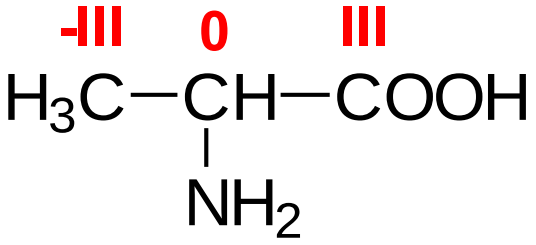

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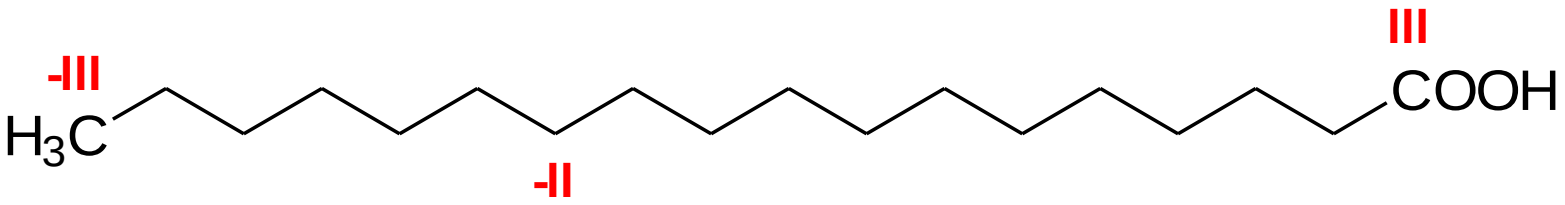
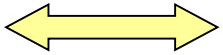


Event. ox.n. C = -1,8 ⇒best C

Formation of NADH in MM

- **TCA**
- isocitrate
  2-oxoglutarate
  malate
- **β-oxidation of FA**
  β-hydroxyacyl-CoA



- **Oxidation decarboxylation**
  pyruvate
  2-oxoglutarate
- 2-oxo acids from Val, Leu, Ile
- **Dehydrogenation of Ketone bodies**
- β-hydroxybutyrate
- **Dehydrogenation deaminatione**
  glutamate

Formation of NADH in cytoplasm

- **Glycolysis**

  (dehydrogenation of glyceraldehyde-3-P)
- **Gluconeogenesis**

  (dehydrogenation of lactate to pyruvate)
- **Dehydrogenation of ethanol**

  (to acetaldehyde)

Formation of $FADH_2$ in MM

- **β-Oxidation of FA**

  (dehydrogenation of alcyl-CoA)

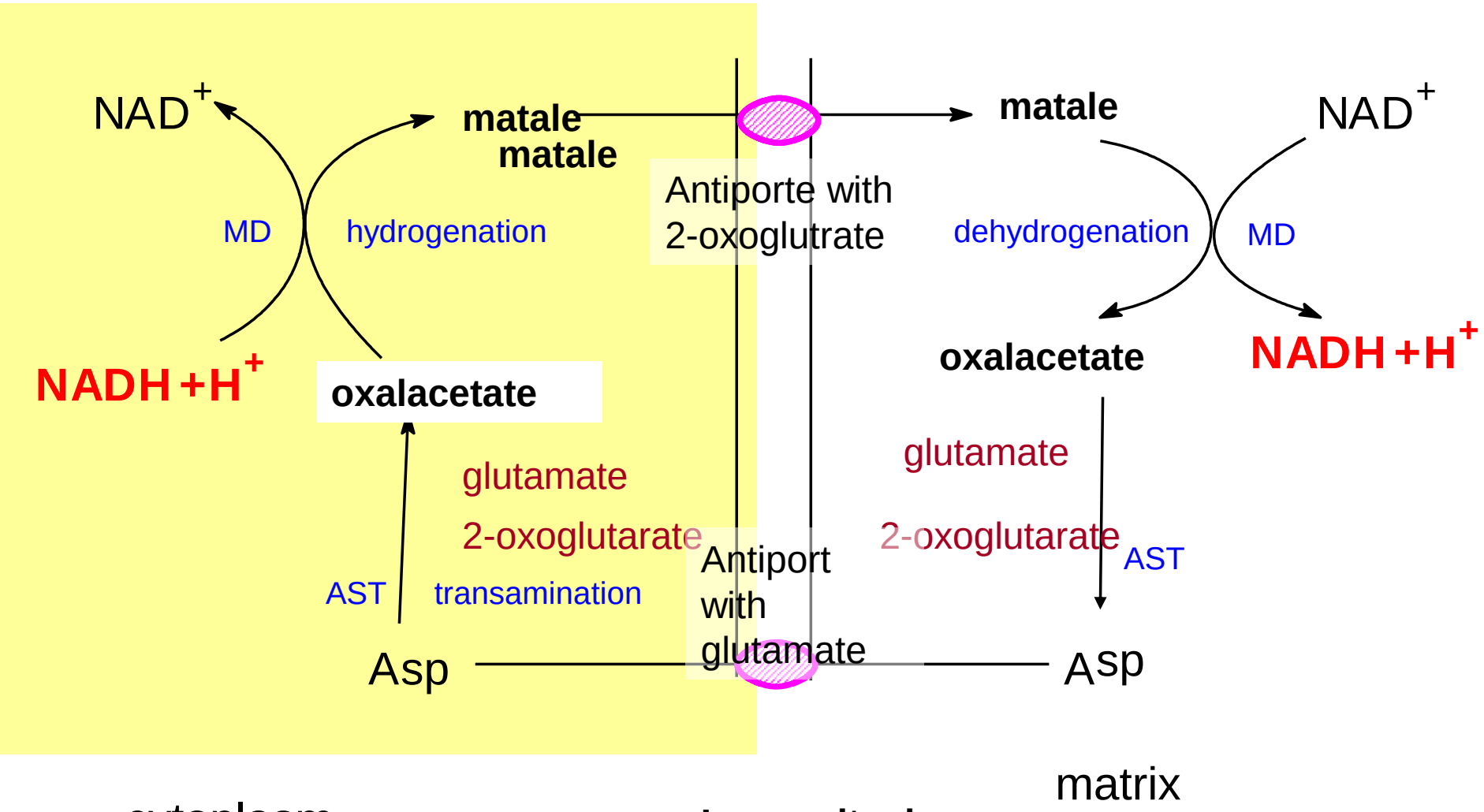
- **TCA**

  (dehydrogenation of succinate)

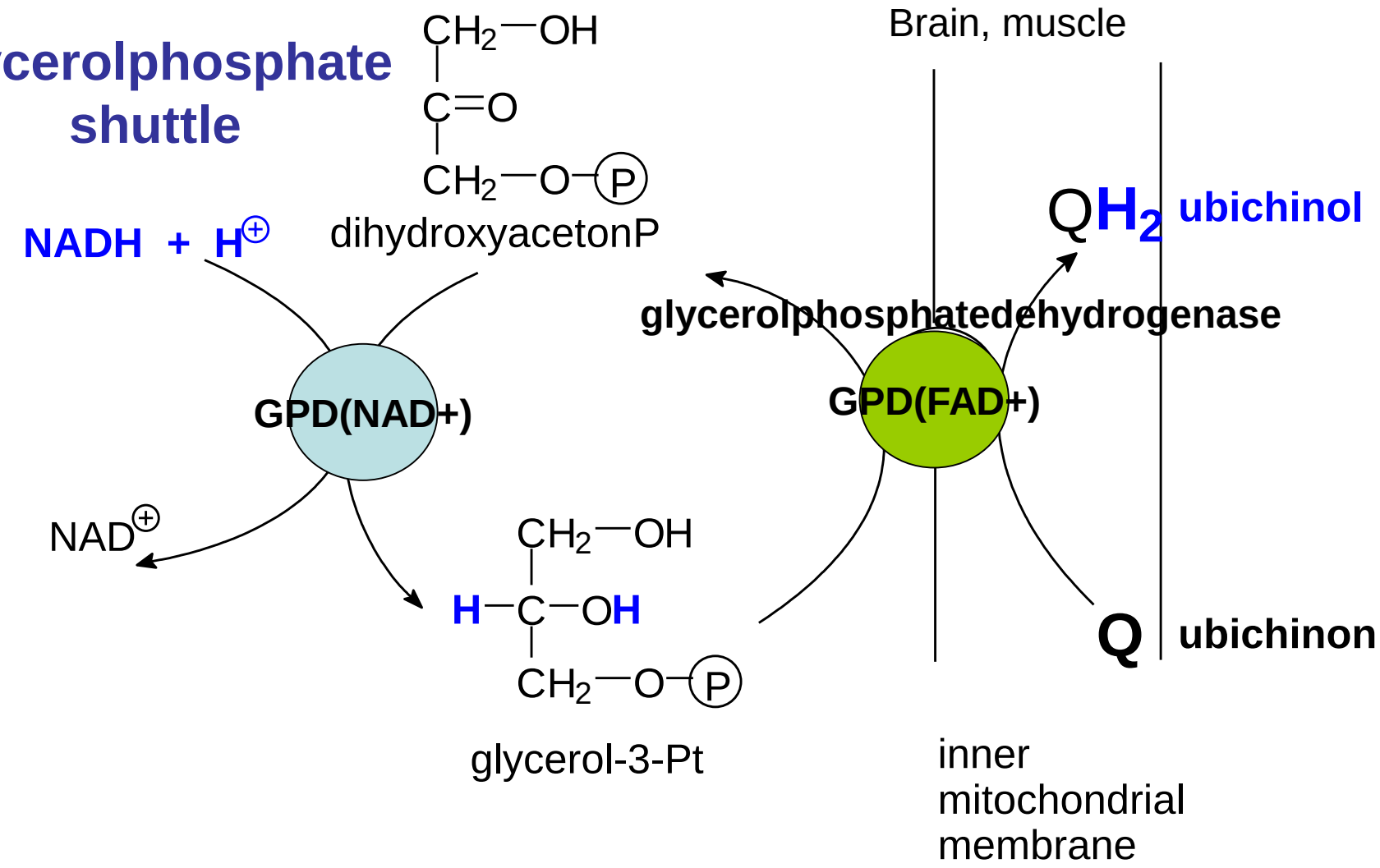
Transport of NADH from cytoplasm to matrix

- NADH **from cytoplasm to matrix**
- Inpermeable inner MM
- Change of H+ protones
- 2 shuttle **mechanism**
- aspartate/malate (heart, liver,kidney)
- glycerolphosphate (brain, muscle, kidney)

Aspartate/malate shuttle



Glycerolphosphate shuttle



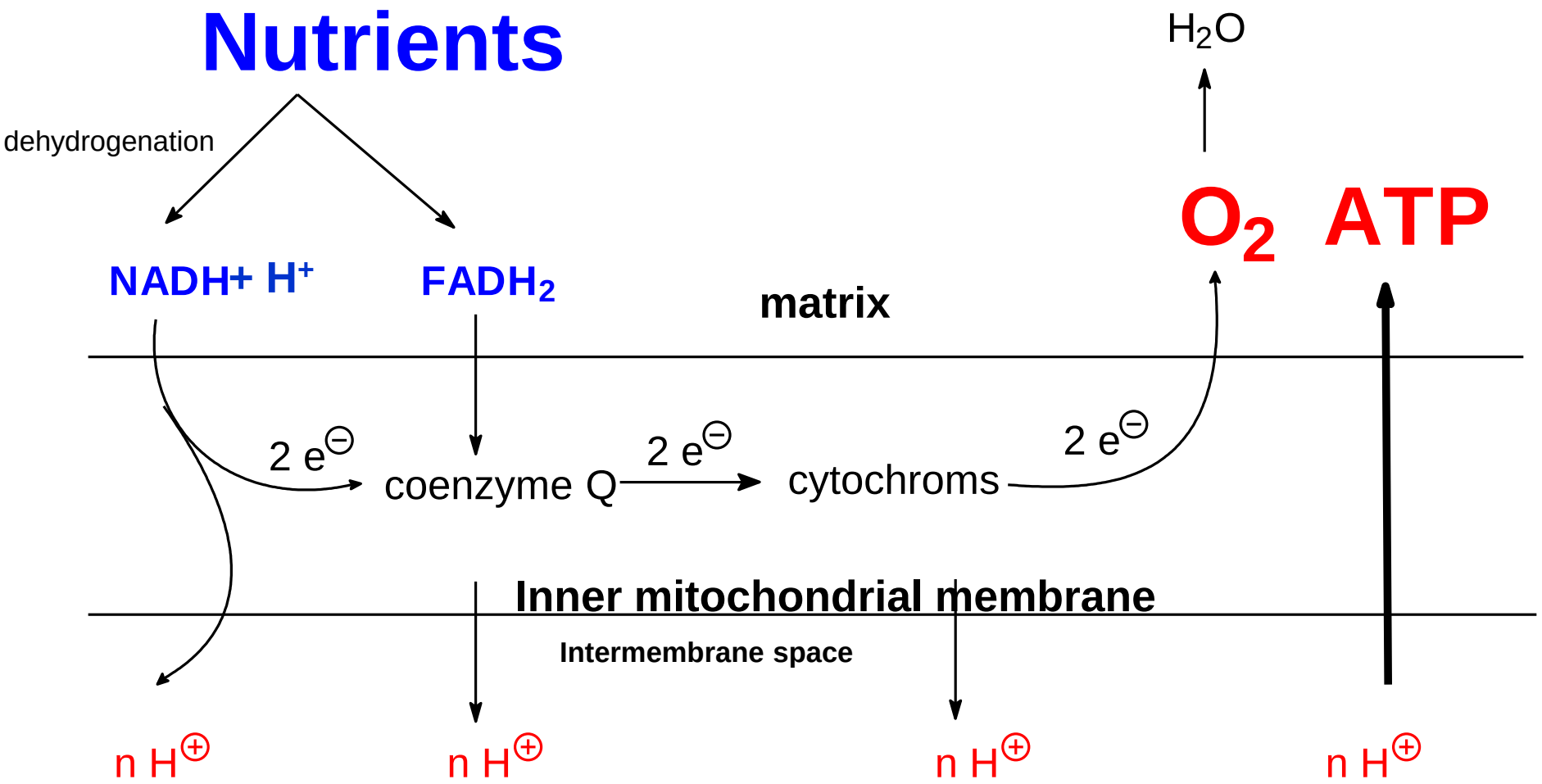
Respiratory chain

- An **electron transport chain (ETC)** couples electron transfer between an electron donor (such as NADH) and an electron acceptor (such as O2) with the transfer of H+ ions (protons) across a membrane. The resulting electrochemical proton gradient is used to generate chemical energy in the form of adenosine triphosphate (*ATP*). Electron transport chains are the cellular mechanisms used for extracting energy from sunlight in photosynthesis and also from redox reactions, such as the oxidation of sugars (respiration).

The Components of the Electron Transport Chain

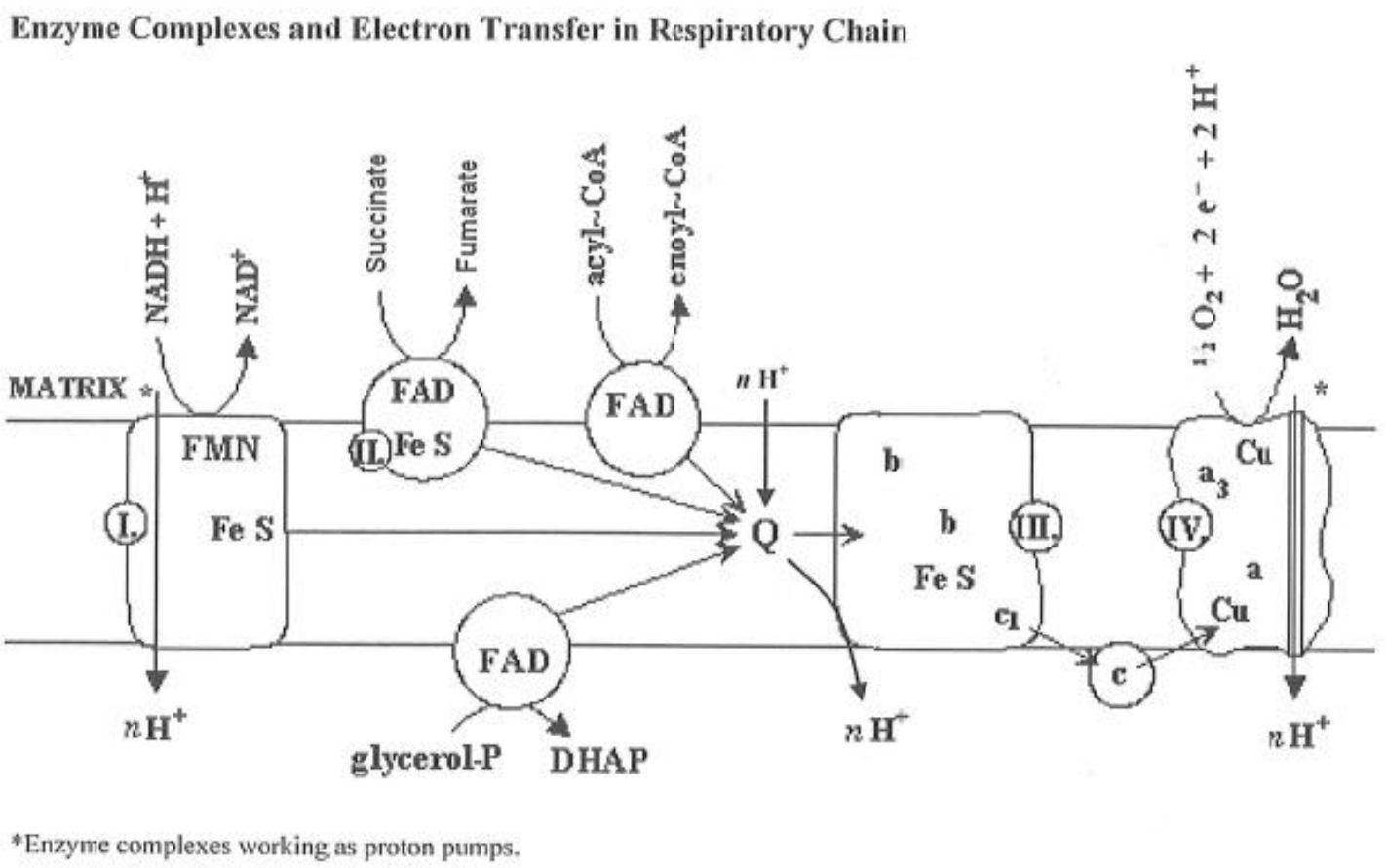
- The electron transport chain of the mitochondria is the means by which electrons are removed from the reduced carrier NADH and transferred to oxygen to yield H2O

Respiratory chain



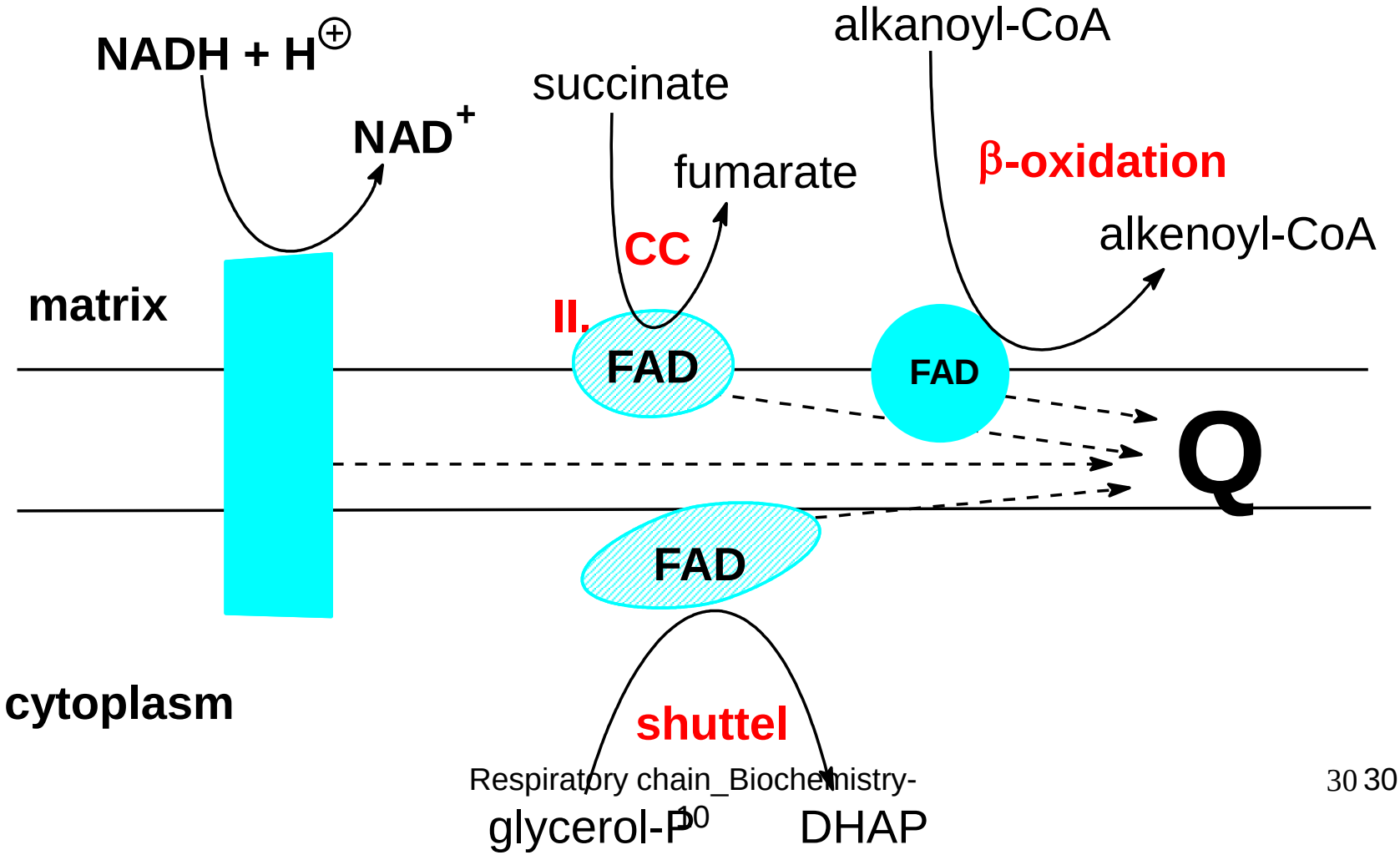
Inner mitochondrial membrane

- Large surface
- High concetration of proteins (enzymes, shuttles)
- Permeable for small no charge moleculs
- Non permeable for ions, organic substrates
- The **mitochondrial inner membrane** forms internal compartments known as cristae, which allow greater space for the proteins such as cytochromes to function properly and efficiently. The electron transport chain is located on the inner membrane of the mitochondria. Within the inner mitochondrial membrane are also transport proteins that transport in a highly controlled manner metabolites across this membrane.



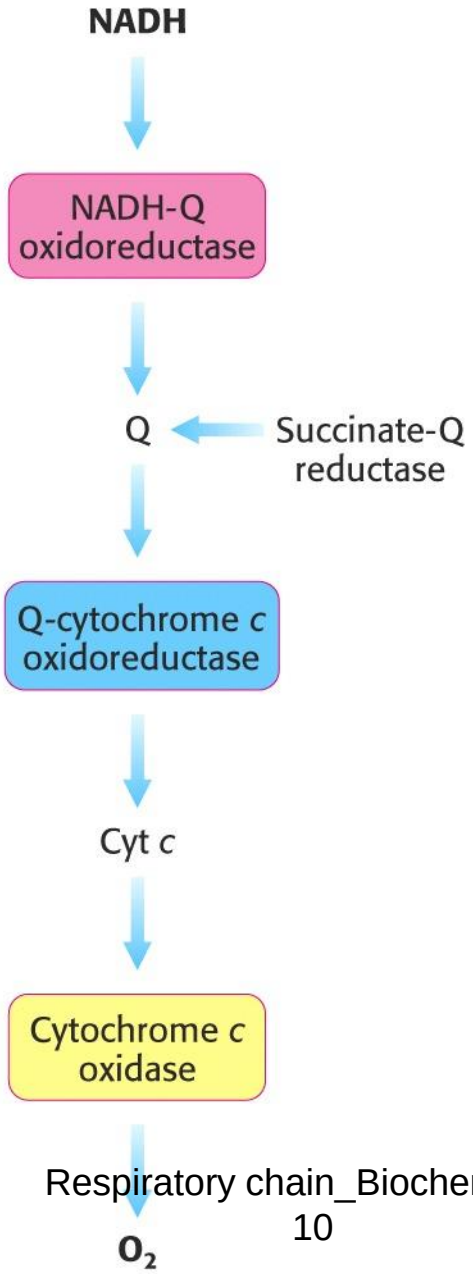
•**Complex I (NADH coenzyme Q reductase; labeled I)** accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme Q (ubiquinone; labeled UQ), which also receives electrons from **complex II (succinate dehydrogenase; labeled II).** UQ passes electrons to **complex III (cytochrome bc1 complex; labeled III),** which passes them to cytochrome *c* (cyt *c*). Cyt *c* passes electrons to **Complex IV (cytochrome *c* oxidase; labeled IV),** which uses the electrons and hydrogen ions to reduce molecular oxygen to water.

Reduced coffactors



- Energy obtained through the transfer of electrons (black arrows) down the ETC is used to pump protons (red arrows) from the mitochondrial matrix into the intermembrane space, creating an electrochemical proton gradient across the mitochondrial inner membrane (IMM) called ΔΨ. This electrochemical proton gradient allows ATP synthase (ATP-ase) to use the flow of H+ through the enzyme back into the matrix to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate. Complex I (NADH coenzyme Q reductase; labeled I) accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme Q (ubiquinone; labeled UQ), which also receives electrons from complex II (succinate dehydrogenase; labeled II). UQ passes electrons to complex III (cytochrome bc1 complex; labeled III), which passes them to cytochrome *c* (cyt *c*). Cyt *c* passes electrons to Complex IV (cytochrome *c* oxidase; labeled IV), which uses the electrons and hydrogen ions to reduce molecular oxygen to water.
- Four membrane-bound complexes have been identified in mitochondria. Each is an extremely complex transmembrane structure that is embedded in the inner membrane. Three of them are proton pumps. The structures are electrically connected by lipid-soluble electron carriers and water-soluble electron carriers. The overall electron transport chain:

Sequence of electron carriers in the respiratory chain



Coenzyme Q electron shuttle

Complex I proton pump

Complex II, does not pump protons

Complex III proton pump

Cytochrome c electron shuttle

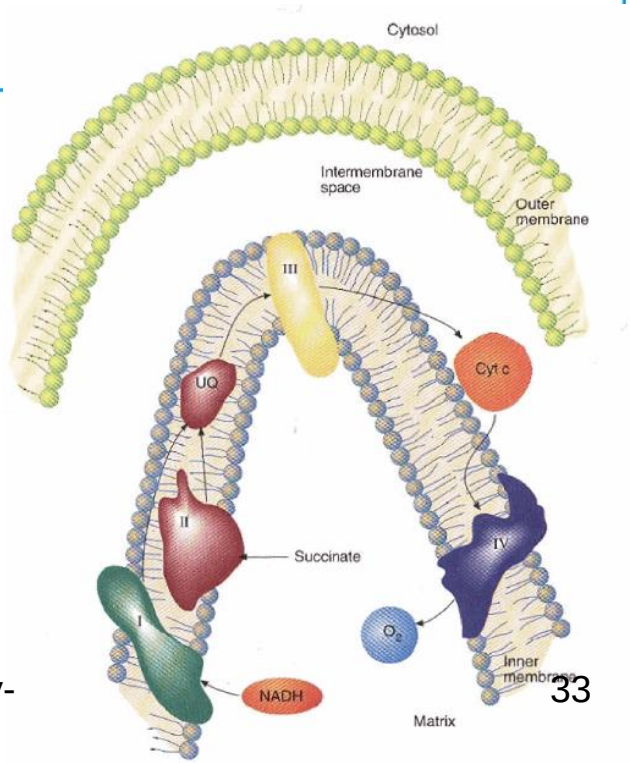
Complex IV proton pump

TABLE 18.2 Components of the mitochondrial electron-transport chain

Enzyme complex	Mass (kd)	Subunits	Prosthetic group	Oxidant or reductant: Matrix side	Oxidant or reductant: Membrane core	Oxidant or reductant: Cytosolic side
NADH-Q oxidoreductase	880	≥ 34	FMN Fe-S	NADH	Q	
Succinate-Q reductase	140	4	FAD Fe-S	Succinate	Q	
Q-cytochrome *c* oxidoreductase	250	10	Heme b_H Heme b_L Heme c_1 Fe-S		Q	Cytochrome *c*
Cytochrome *c* oxidase	160	10	Heme *a* Heme a_3 Cu_A and Cu_B			Cytochrome *c*

Sources: J. W. DePierre and L. Ernster, *Annu. Rev. Biochem.* 46(1977):215; Y. Hatefi, *Annu Rev. Biochem.* 54(1985);1015; and J. E. Walker, *Q. Rev. Biophys.* 25(1992):253.

Summary of electron-transport chain



Enzyme complexes of RCh

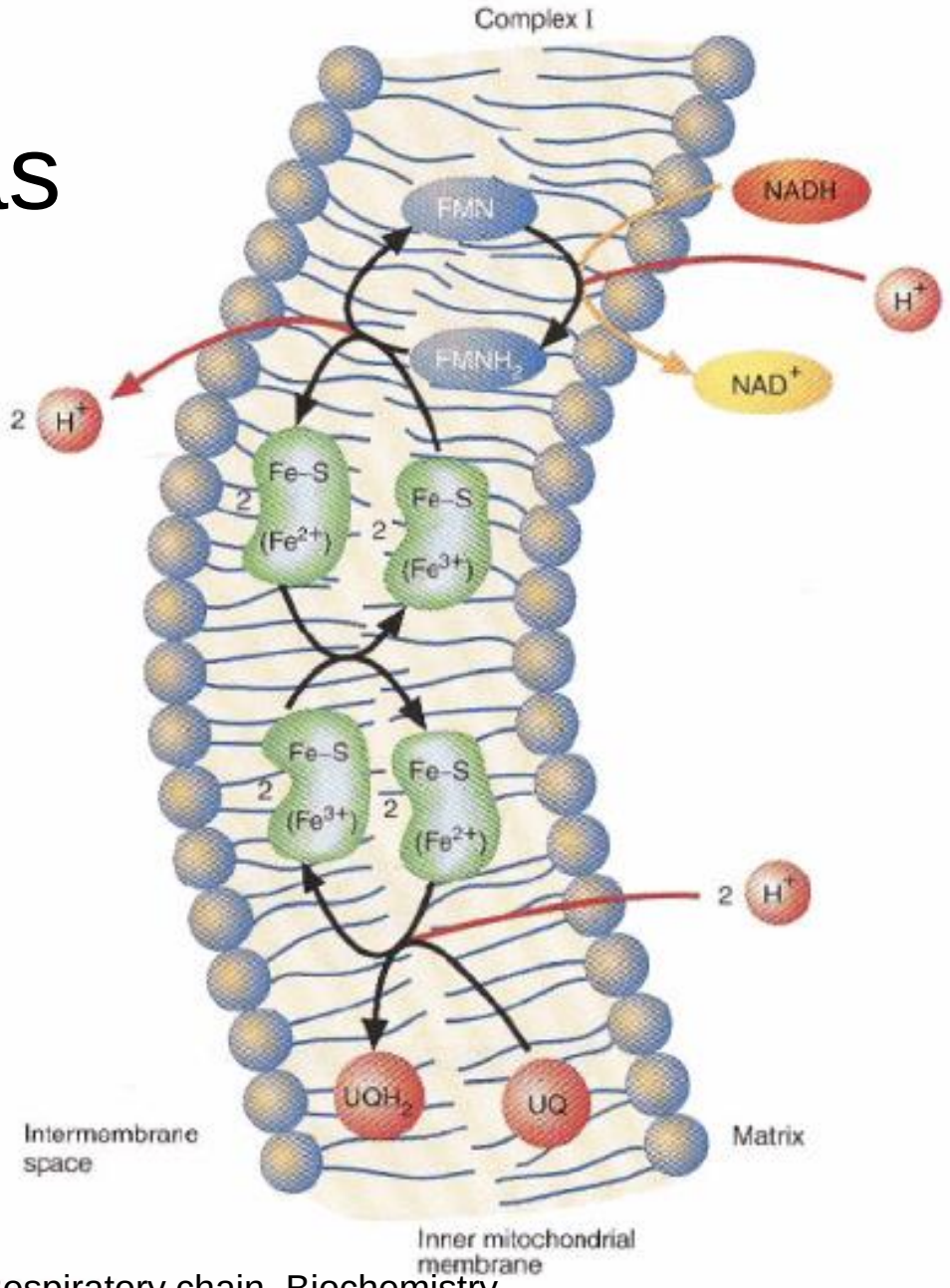
complexes	**name**	**cofactors**	**Transfer e^-**
I.	NADH-dehydrogenase	FMN, Fe-S	NADH $\rightarrow$ Q
II.	succinatedehydrogenase	FAD, Fe-S, cyt *b*	$FADH_2 \rightarrow$ Q
III.	cytochrom-*c*-reductase	Fe-S, cyt *b*, c_1	Q $\rightarrow$ cyt *c*
IV.	cytochrom-*c*-oxidase	cyt *a*, a_3, Cu	cyt *c* $\rightarrow O_2$

Redox pairs in RCh

Oxid / Red form	$E°'$ (V)
$NAD^+ / NADH+H^+$	-0,32
$FAD / FADH_2$	0,00
Ubichinon (Q) / Ubichinol (QH_2)	0,10
Cytochrom c_1 (Fe^{3+} / Fe^{2+})	0,22
Cytochrom c (Fe^{3+} / Fe^{2+})	0,24
Cytochrom a_3 (Fe^{3+} / Fe^{2+})	0,39
$O_2 / 2H_2O$	0,82

- **Complex I**
- In *Complex I* (NADH dehydrogenase, also called NADH:ubiquinone oxidoreductase; EC 1.6.5.3) two electrons are removed from NADH and transferred to a lipid-soluble carrier, *ubiquinone* (Q). The reduced product, *ubiquinol* (QH2) freely diffuses within the membrane, and Complex I translocates four protons (H+) across the membrane, thus producing a proton gradient. Complex I is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of the main sites of production of superoxide.[3]
- The pathway of electrons occurs as follows:
- NADH is oxidized to NAD+, by reducing Flavin mononucleotide to FMNH2 in one two-electron step. FMNH2 is then oxidized in two one-electron steps, through a semiquinone intermediate. Each electron thus transfers from the FMNH2 to an Fe-S cluster, from the Fe-S cluster to ubiquinone (Q). Transfer of the first electron results in the free-radical (semiquinone) form of Q, and transfer of the second electron reduces the semiquinone form to the ubiquinol form, QH2. During this process, four protons are translocated from the mitochondrial matrix to the intermembrane space. [3]

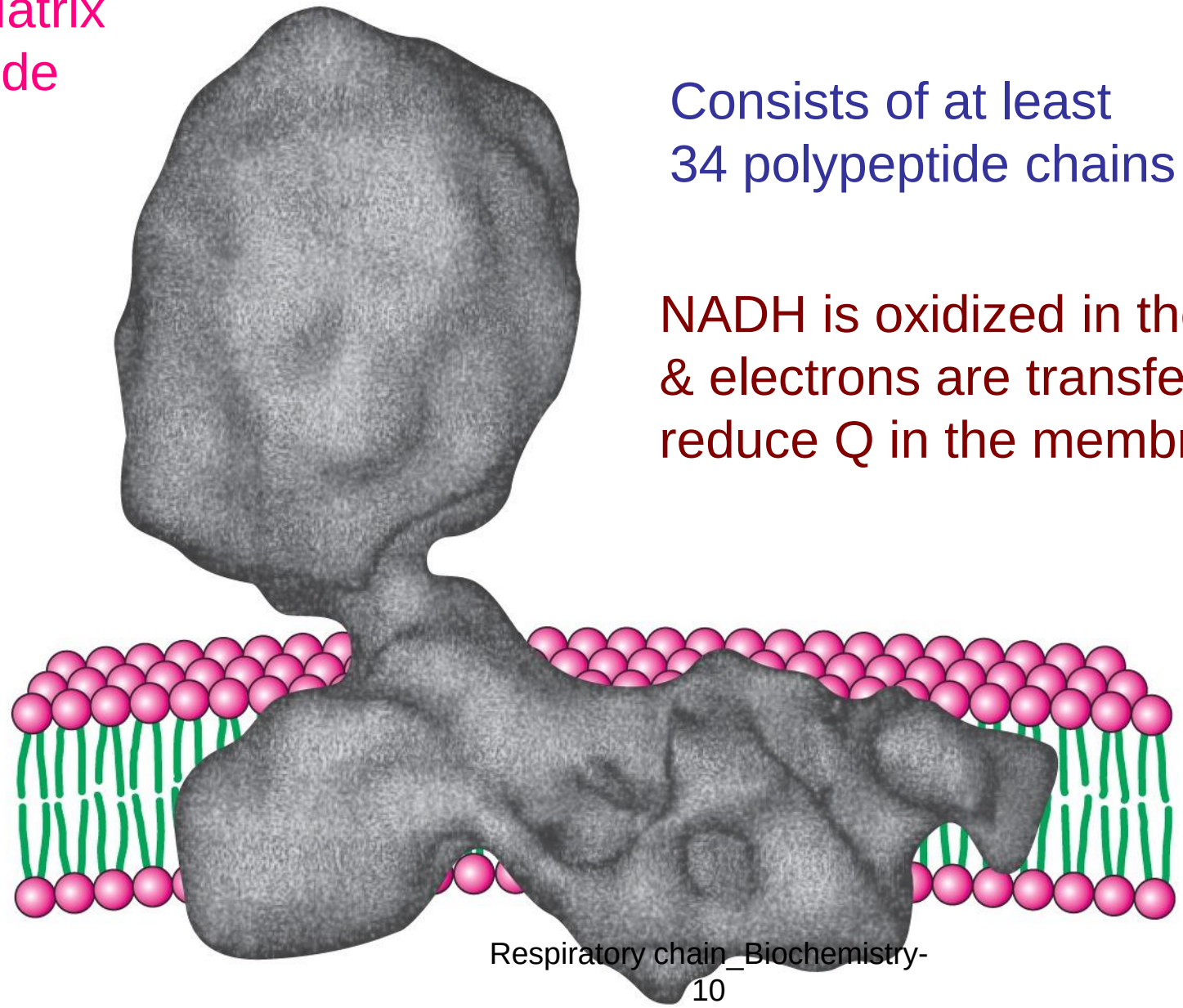
NADH-Q oxidoreductase



Structure of NADH-Q oxidoreductase

EM at moderate resolution

Matrix side

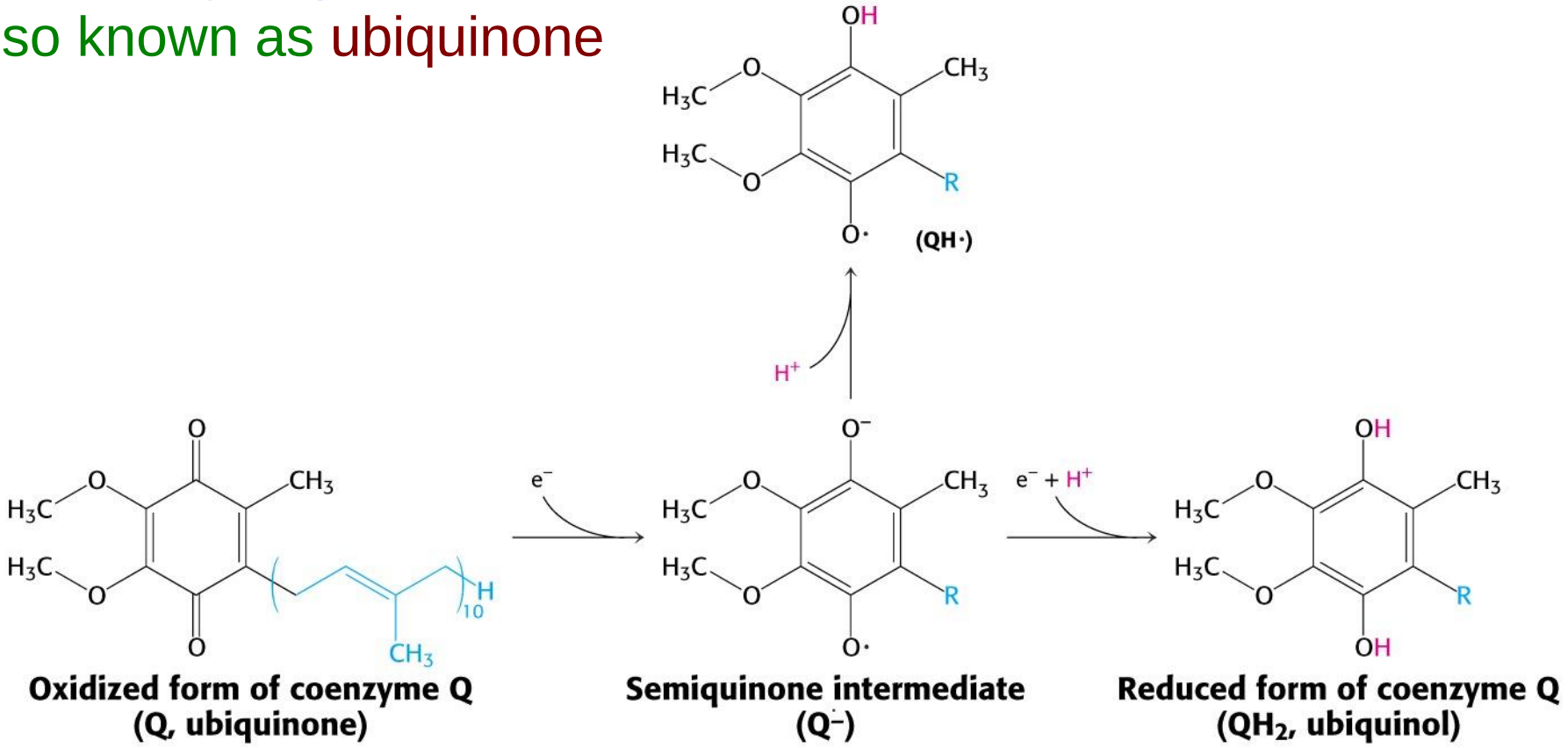


Consists of at least
34 polypeptide chains

NADH is oxidized in the arm, & electrons are transferred to reduce Q in the membrane

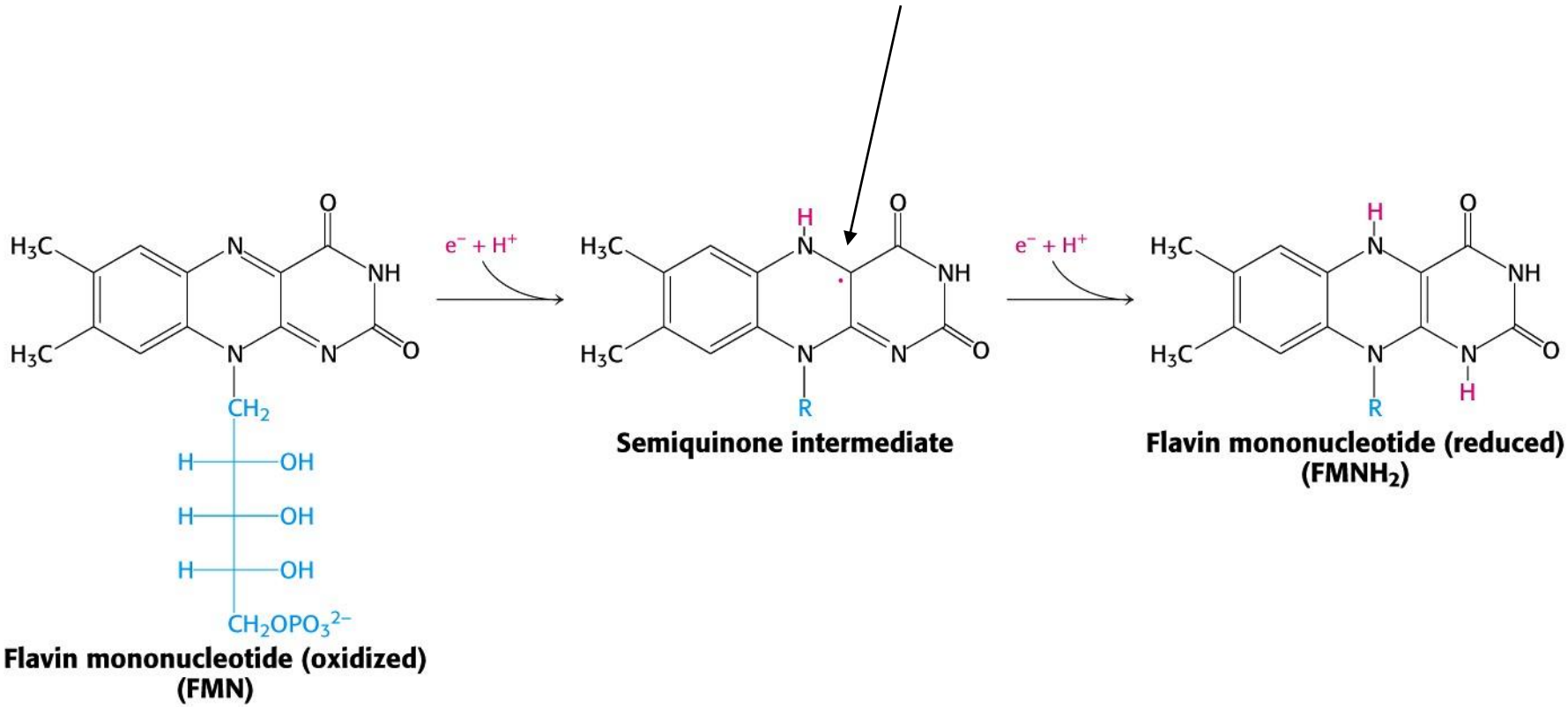
Oxidation states of quinones

Coenzyme Q (Q) is a quinone derivative
with a long isoprenoid tail,
also known as ubiquinone



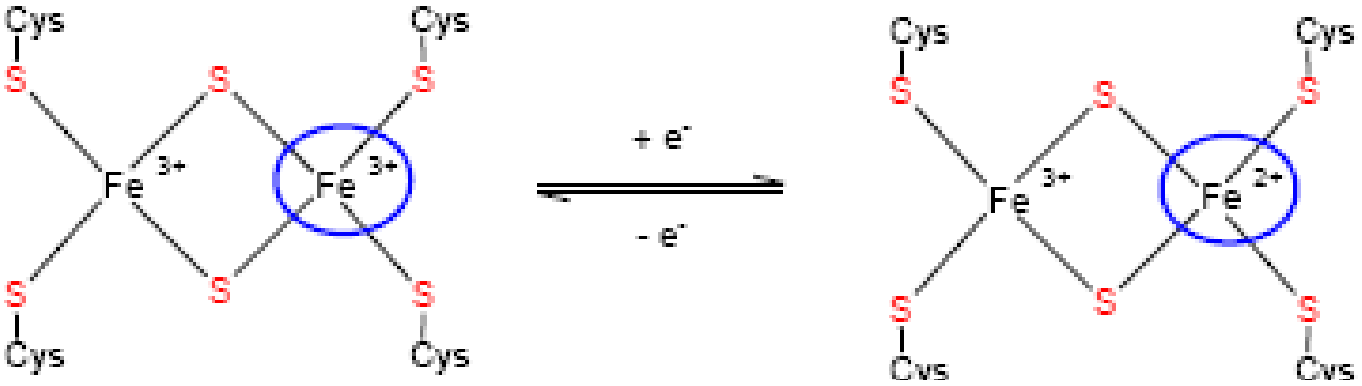
Reduction proceeds through
a semiquinone anion intermiediate ($Q^{\cdot -}$)

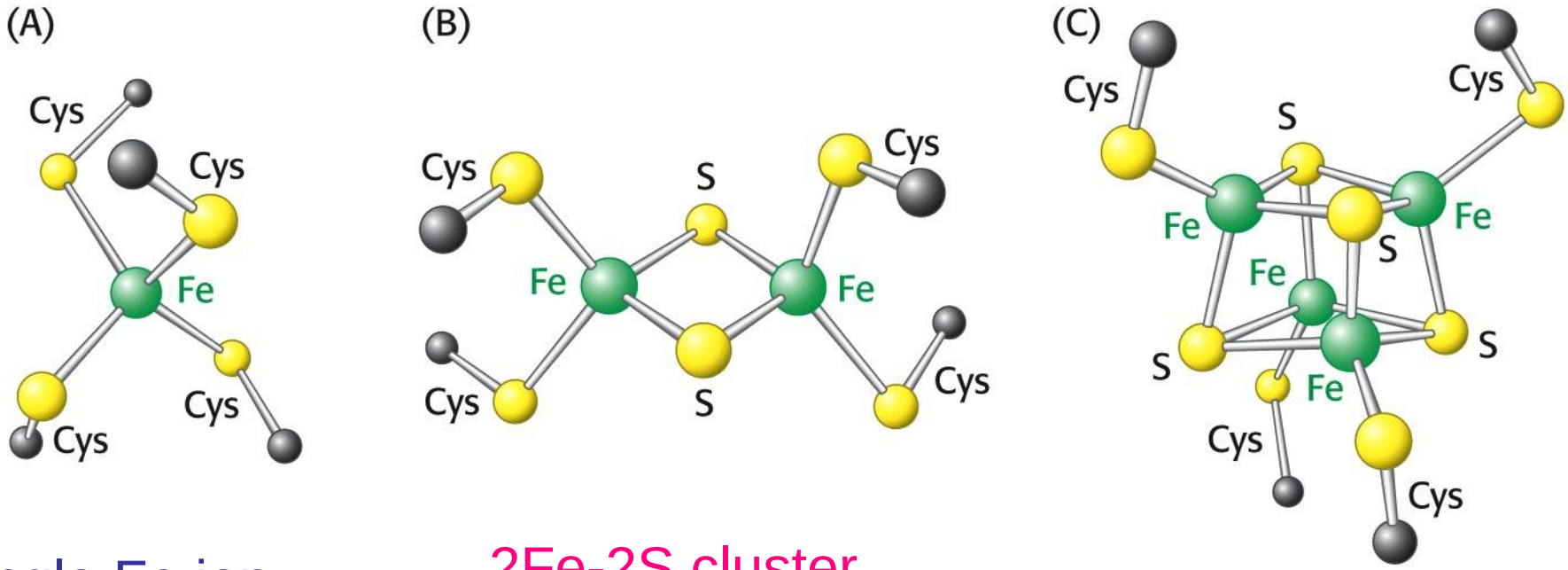
Oxidation states of flavins



Iron-sulfur clusters

Undergo oxidation-reduction reacti





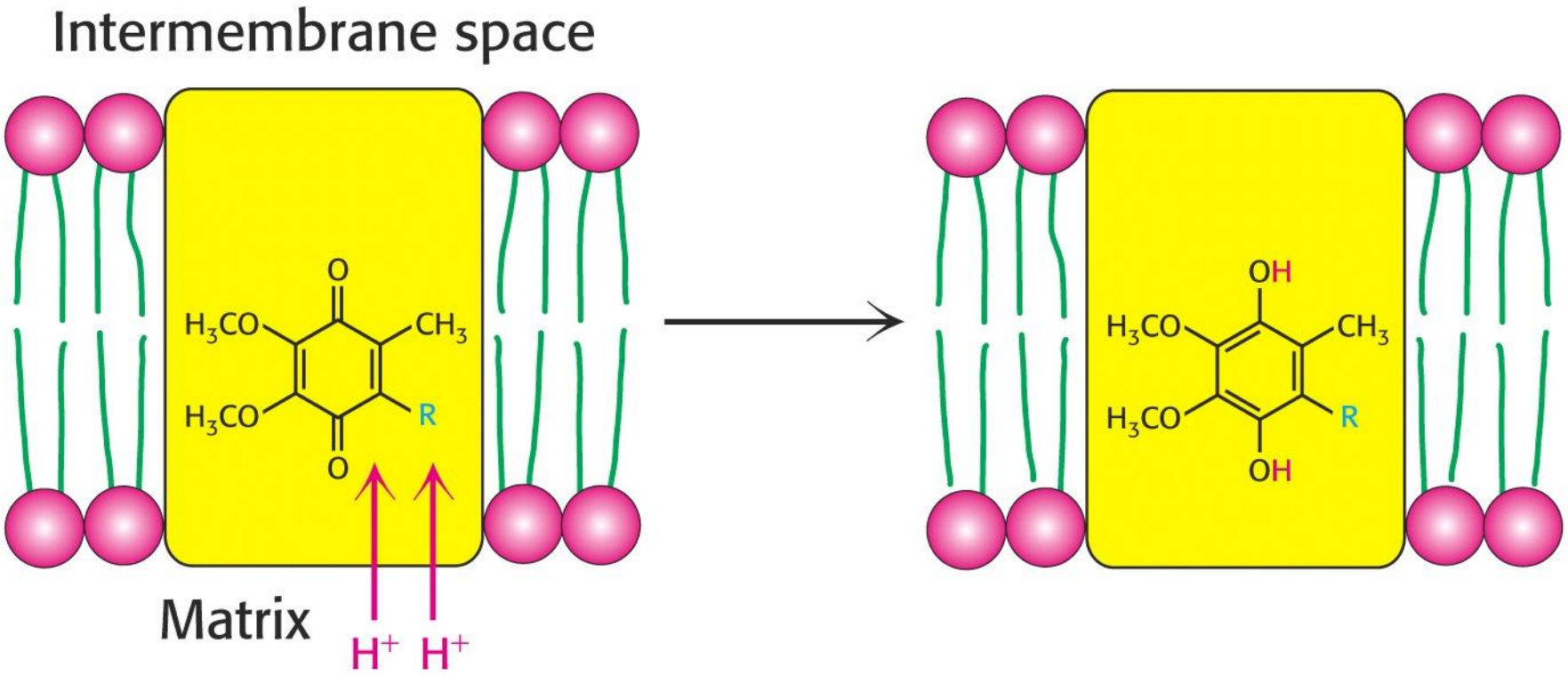
Single Fe ion
+ 4 Cys cluster

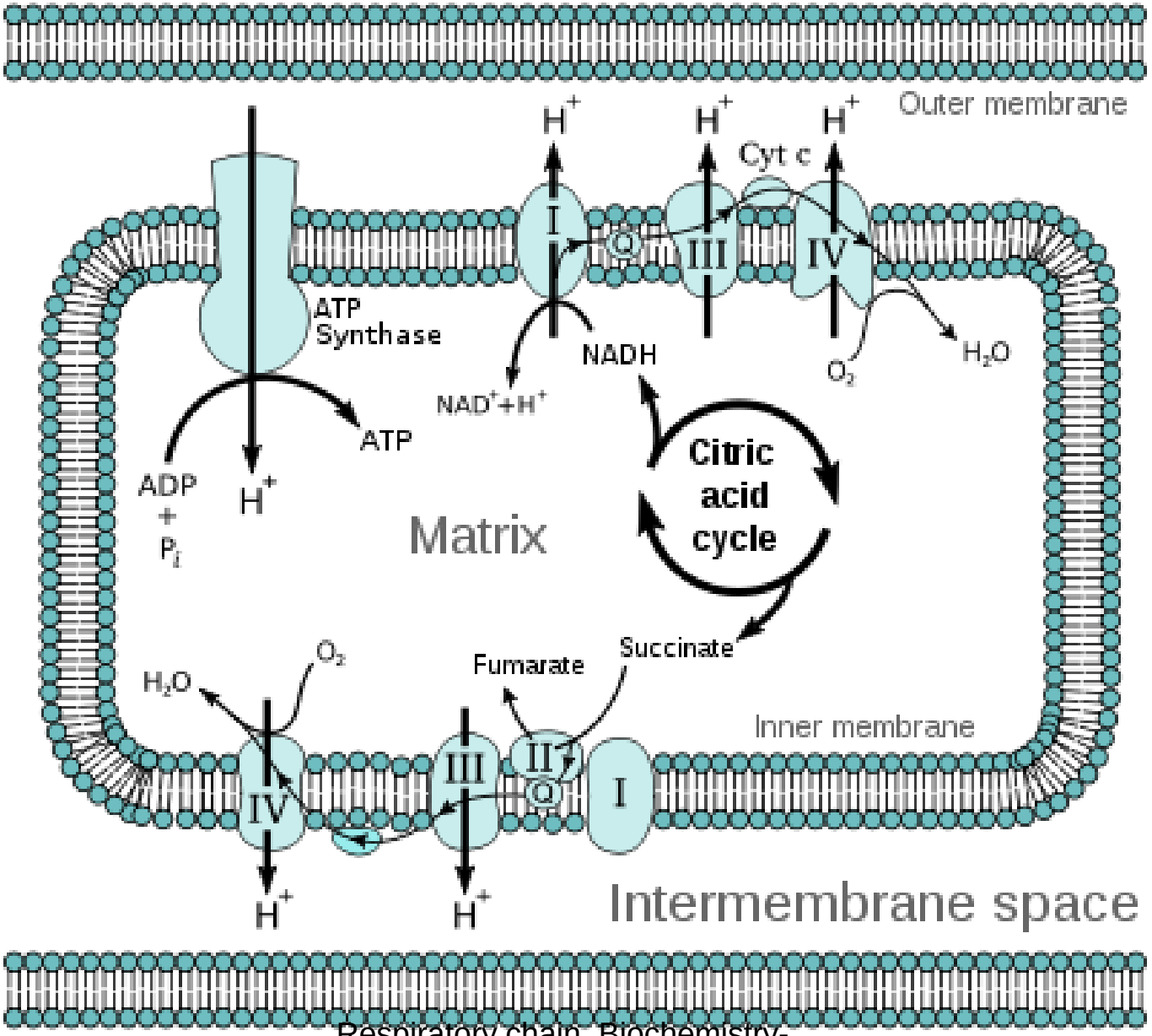
2Fe-2S cluster,
bridge of sulfide ions

4Fe-4S cluster

Coupled electron-proton transfer reactions

Reduction of Q can result in the uptake of 2 protons




Outer membrane
H+
H+
H+
Cyt c
I
Q
III
IV
ATP
Synthase
NADH
NAD+ + H+
H2O
O2
ATP
ADP
+
Pi
H+
Matrix
Citric
acid
cycle
Succinate
Fumarate
O2
H2O
Inner membrane
IV
III
II
Q
I
H+
H+
Intermembrane space

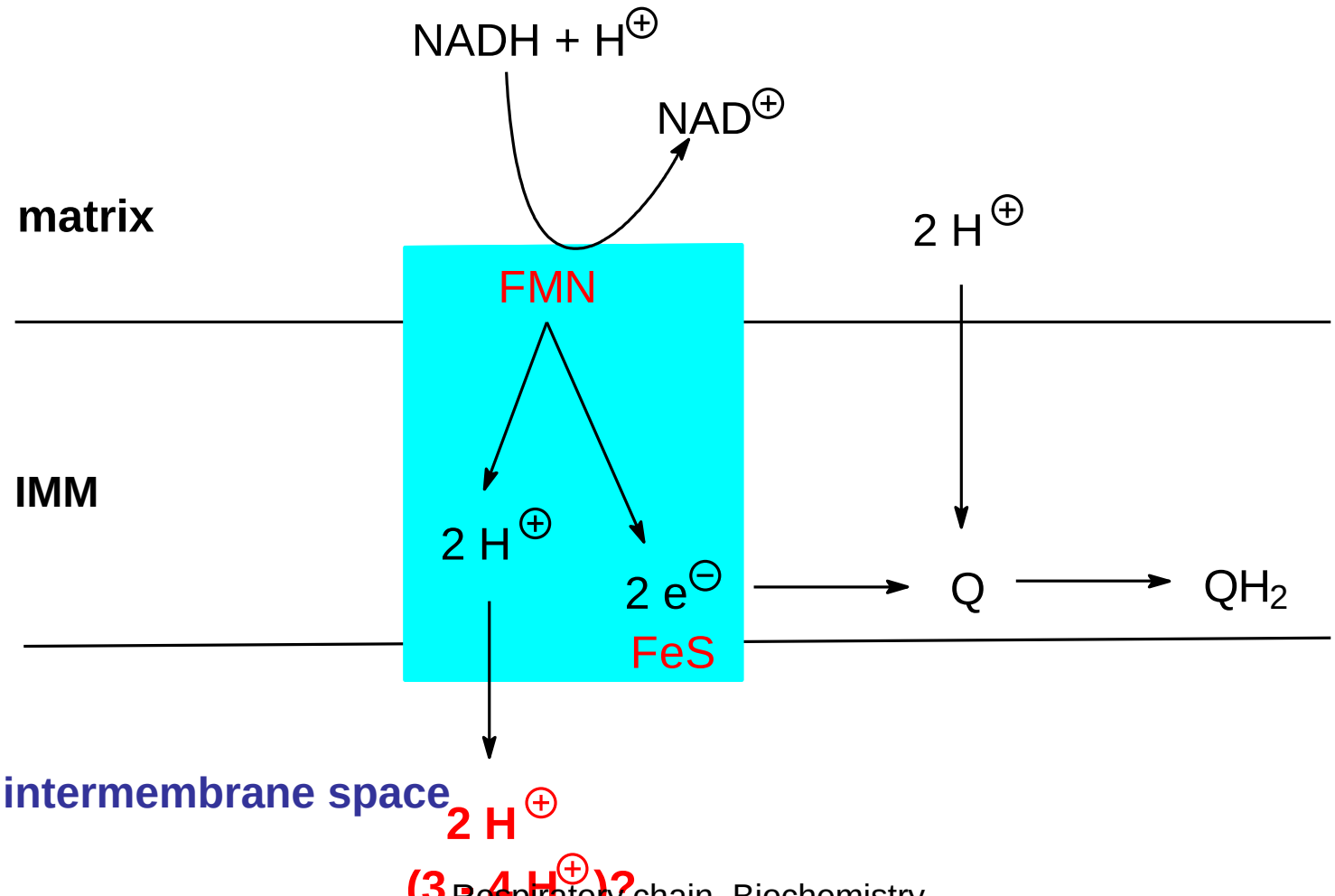
NADH-dehydrogenase

$$NADH+H^+ + FMN \rightarrow NAD^+ + FMNH_2$$

Fe-S

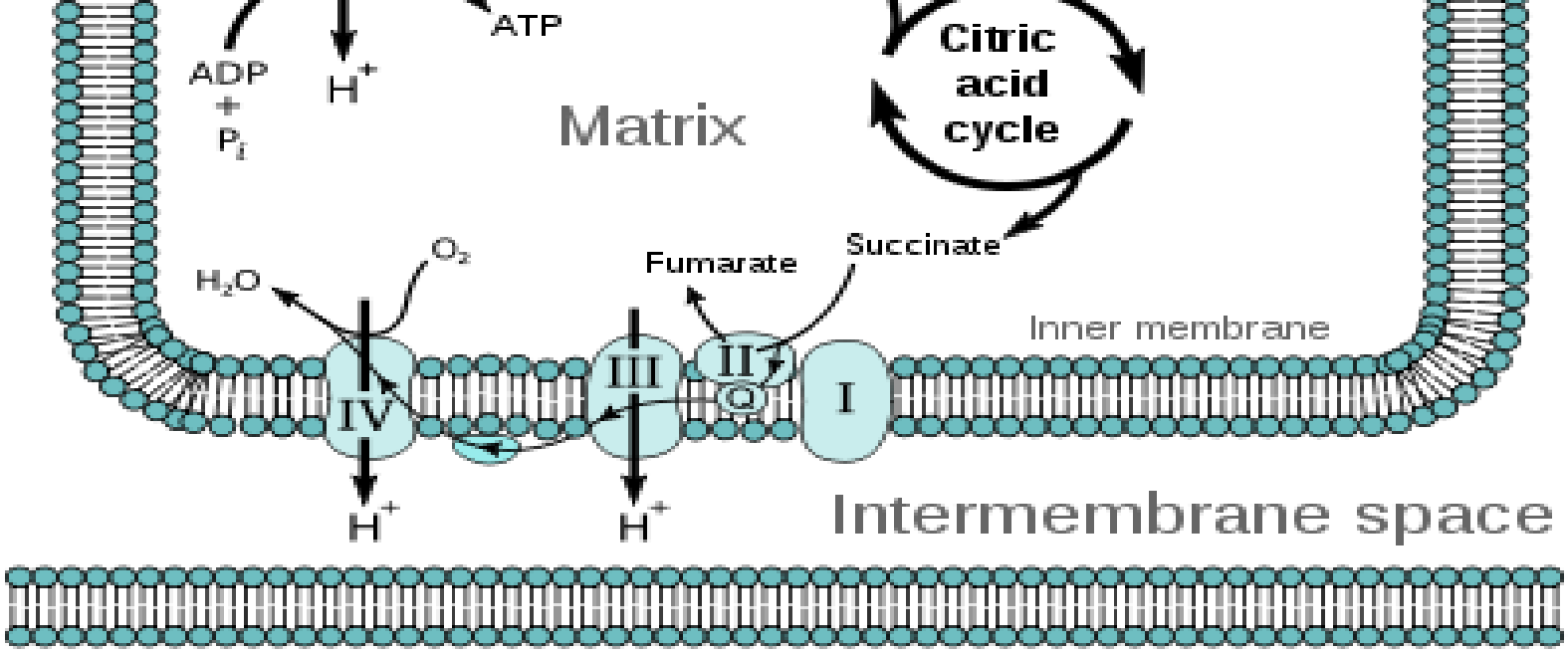
$$FMN + 2\,H^+ + 2\,e^-$$

complex I - first „proton pump“ to intermembrane space

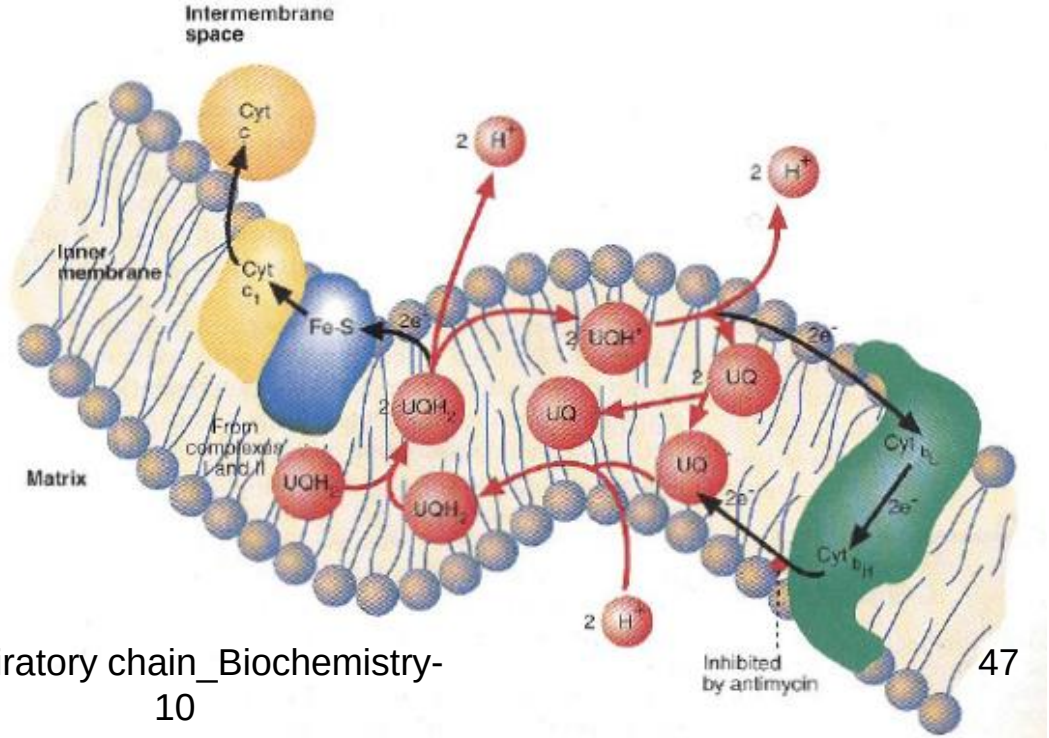


Complex II

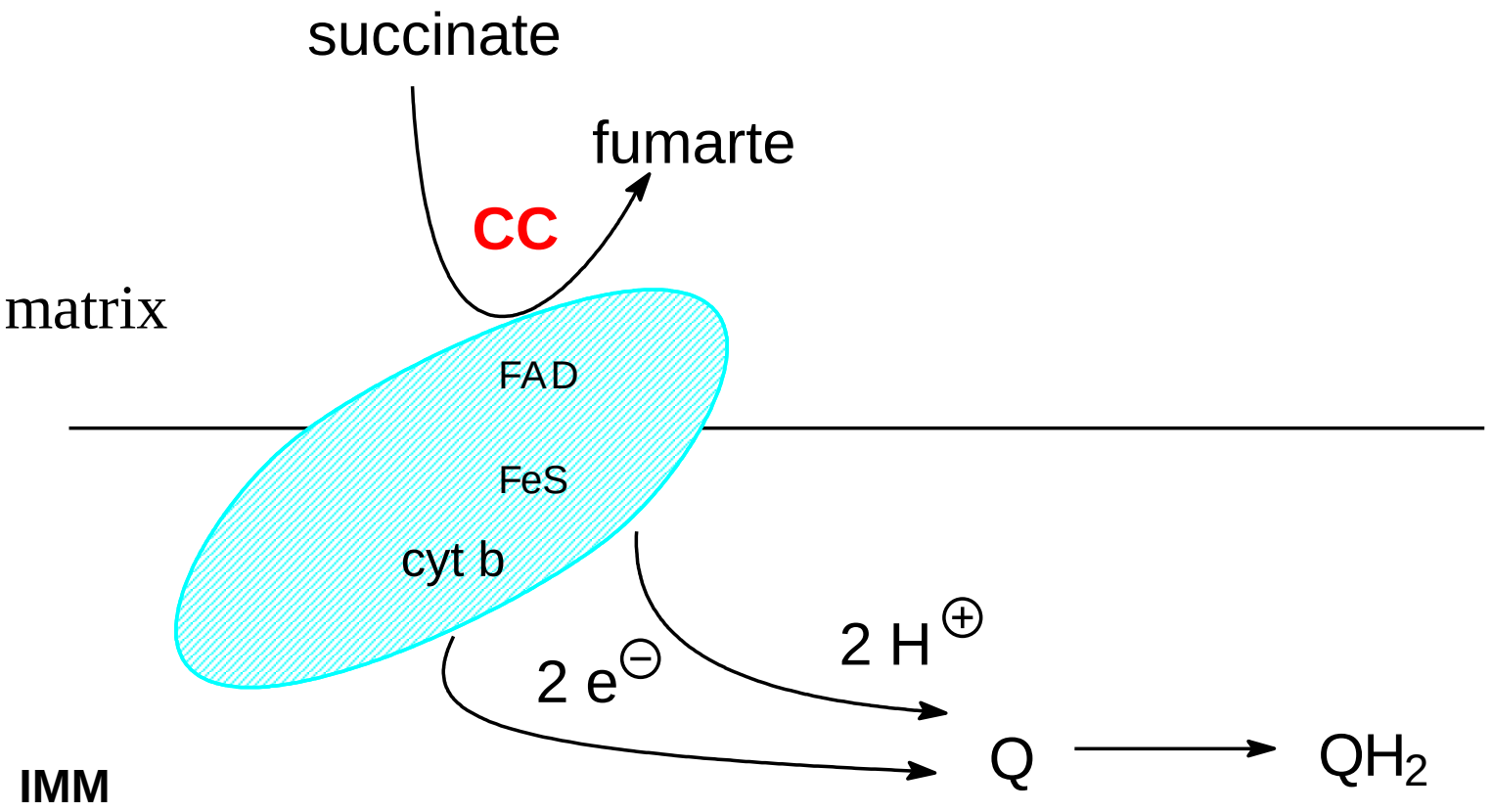
- In *Complex II* (succinate dehydrogenase; EC 1.3.5.1) additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via FAD) to Q. Complex II consists of four protein subunits: SDHA, SDHB, SDHC, and SDHD. Other electron donors (e.g., fatty acids an glycerol 3-phosphate) also direct electrons into Q (via FAD).



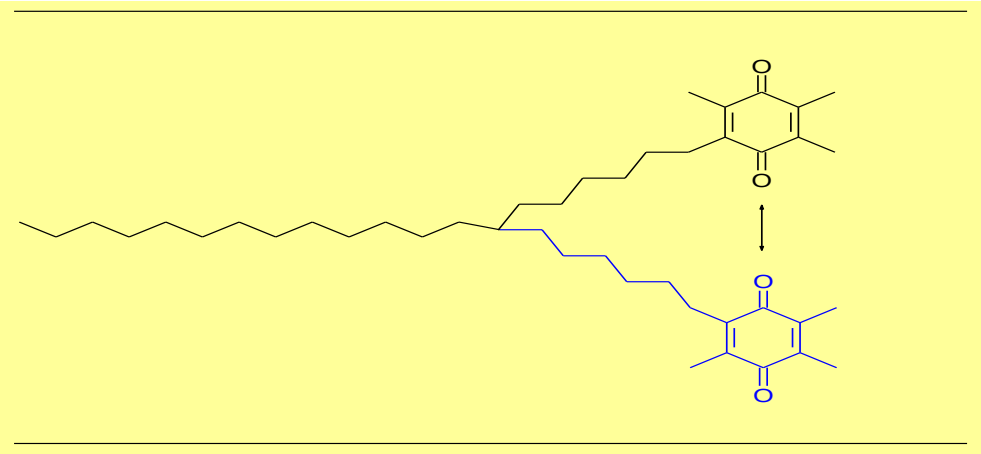
Succinate-Q reductase



Complex II - $FADH_2$



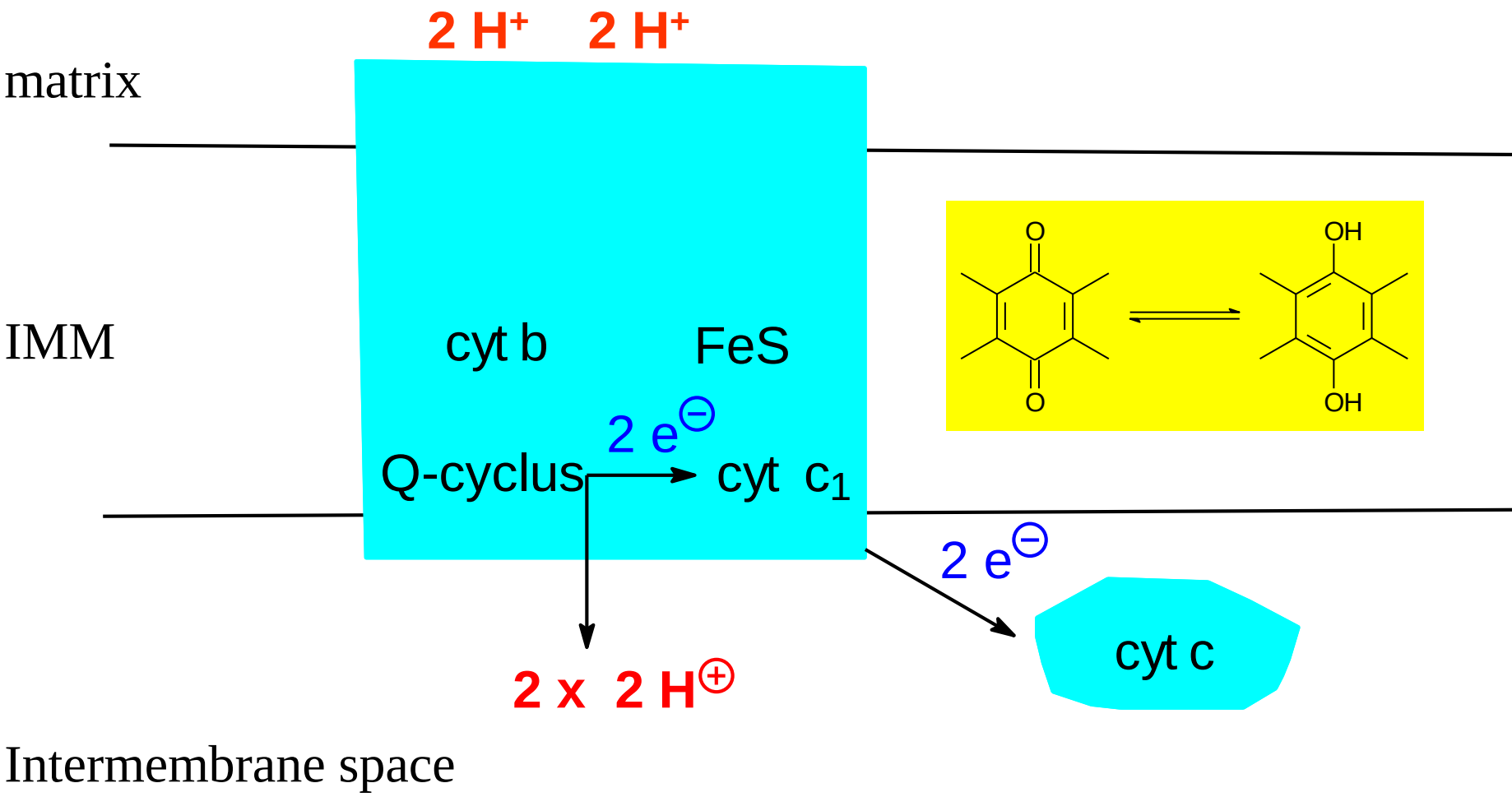
Ubichinon –mobile cofactor



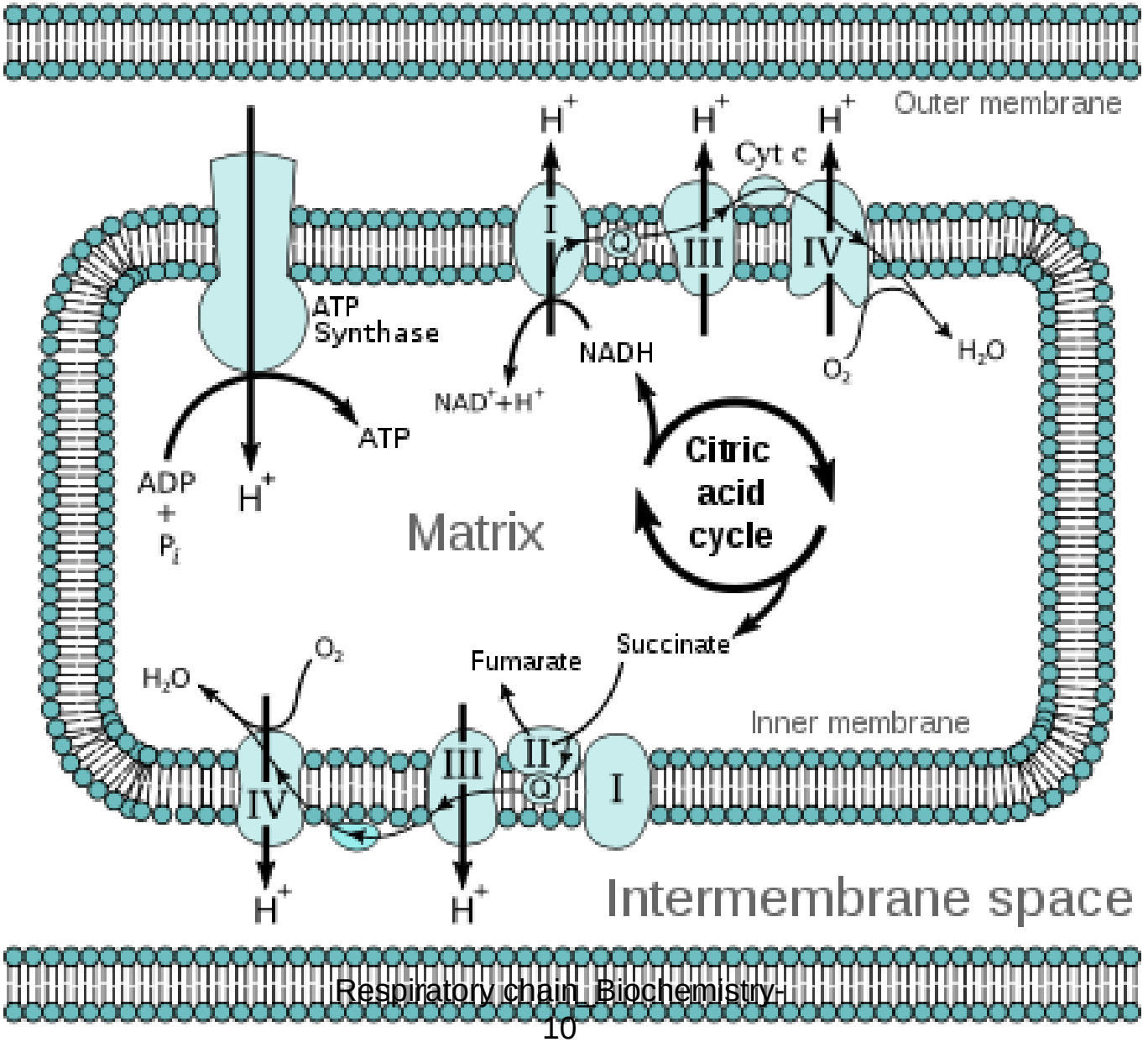
Complex III

- In *Complex III* (cytochrome *bc1* complex; EC 1.10.2.2), the Q-cycle contributes to the proton gradient by an asymmetric absorption/release of protons. Two electrons are removed from QH2 at the QO site and sequentially transferred to two molecules of cytochrome *c*, a water-soluble electron carrier located within the intermembrane space. The two other electrons sequentially pass across the protein to the Qi site where the quinone part of ubiquinone is reduced to quinol. A proton gradient is formed by two quinol (4H+4e-) oxidations at the Qo site to form one quinol (2H+2e-) at the Qi site. (in total six protons are translocated: two protons reduce quinone to quinol and four protons are released from two ubiquinol molecules).
- When electron transfer is reduced (by a high membrane potential or respiratory inhibitors such as antimycin A), Complex III may leak electrons to molecular oxygen, resulting in superoxide formation.

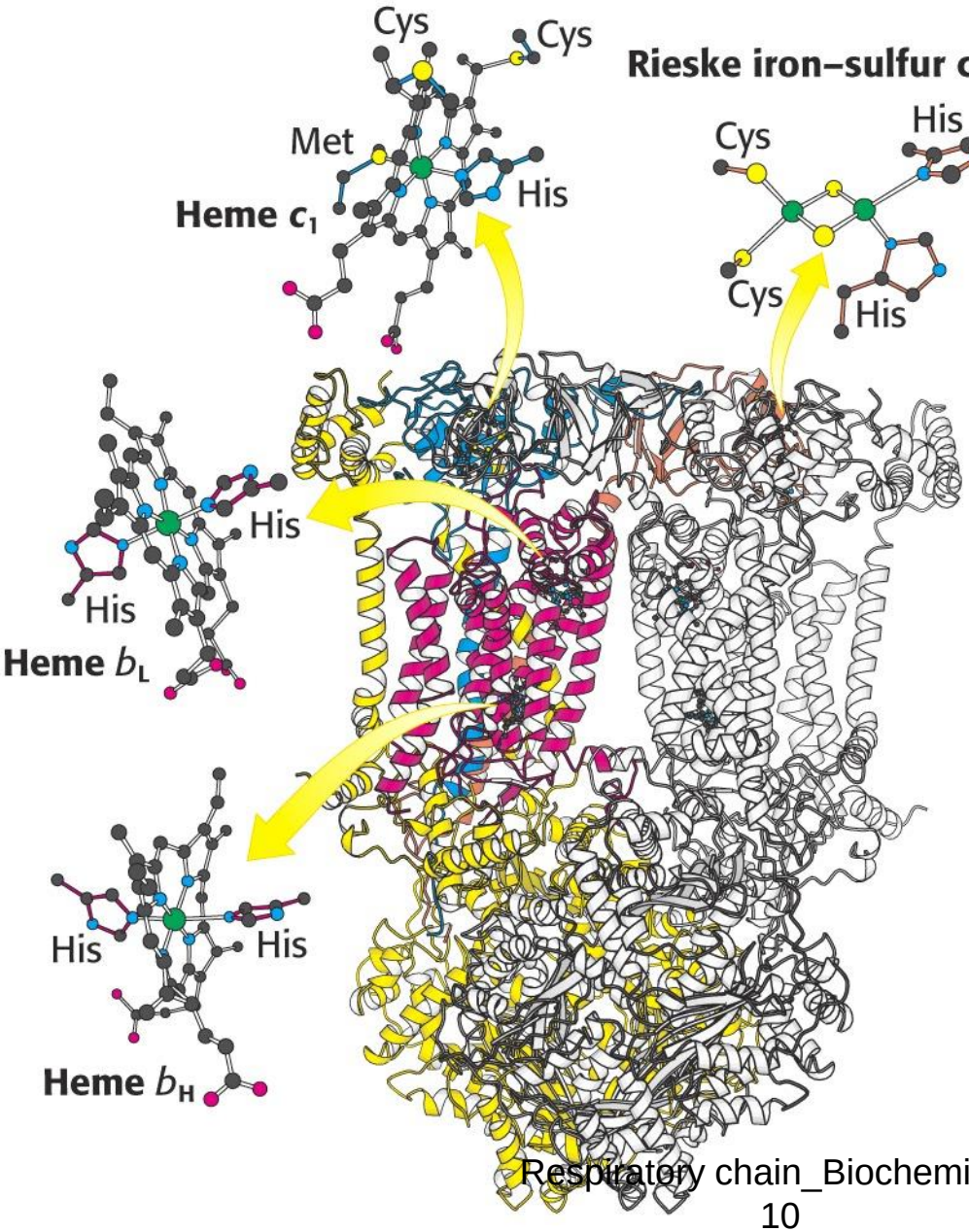
Complex III a Q-cyclus transfer 2 × 2H⁺



III- Q- Cytochrome c oxidoreductase



Q-cytochrome c oxidoreductase

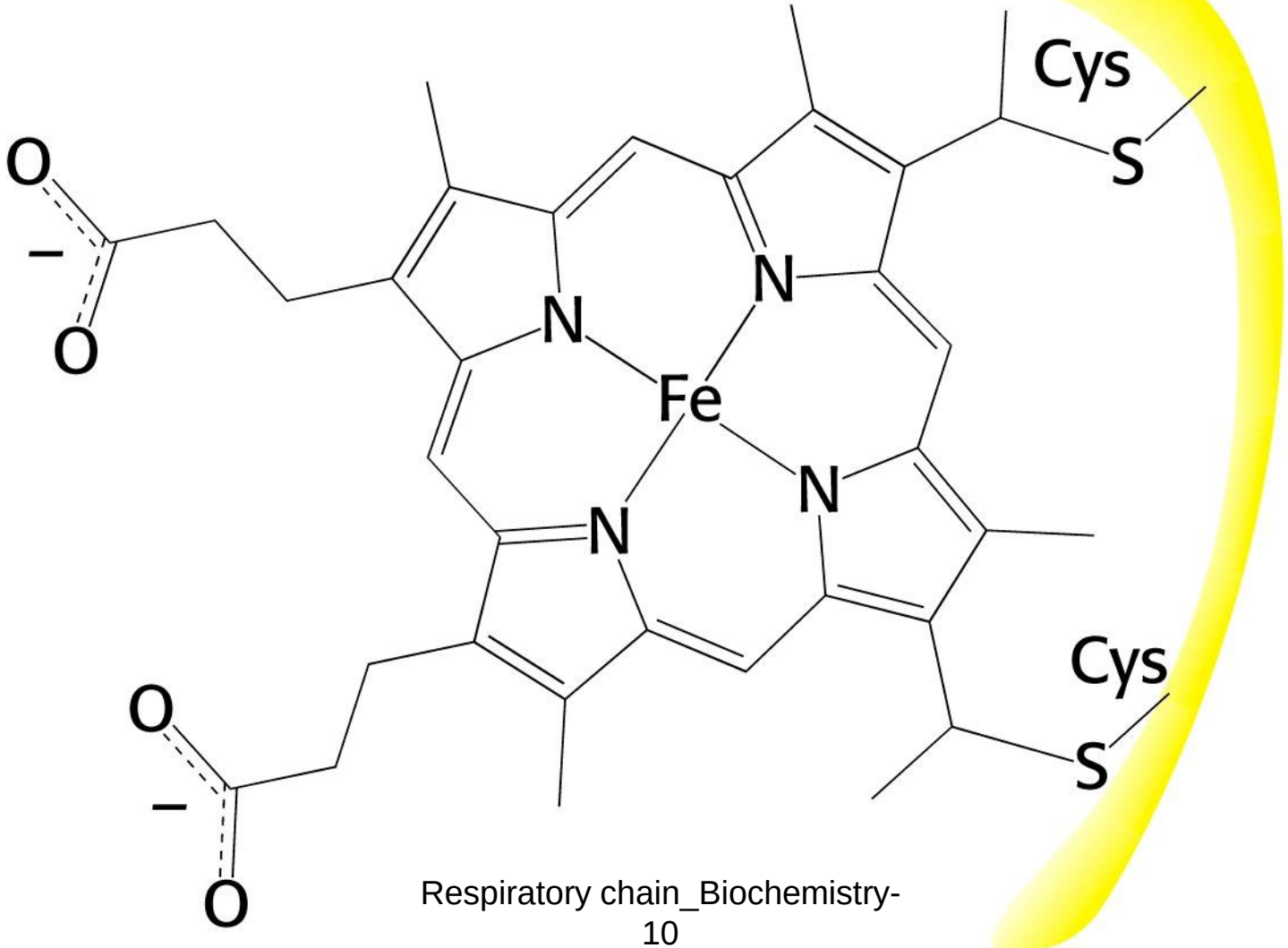


Homodimer with 11 distinct polypeptides

Major prosthetic guoups:
3 hemes, & a 2Fe-2S cluster,
they mediate electron-transfer between quinones in the membrane & cytochrome c in the intermembrane space

Attachment of heme group in c-type cytochromes

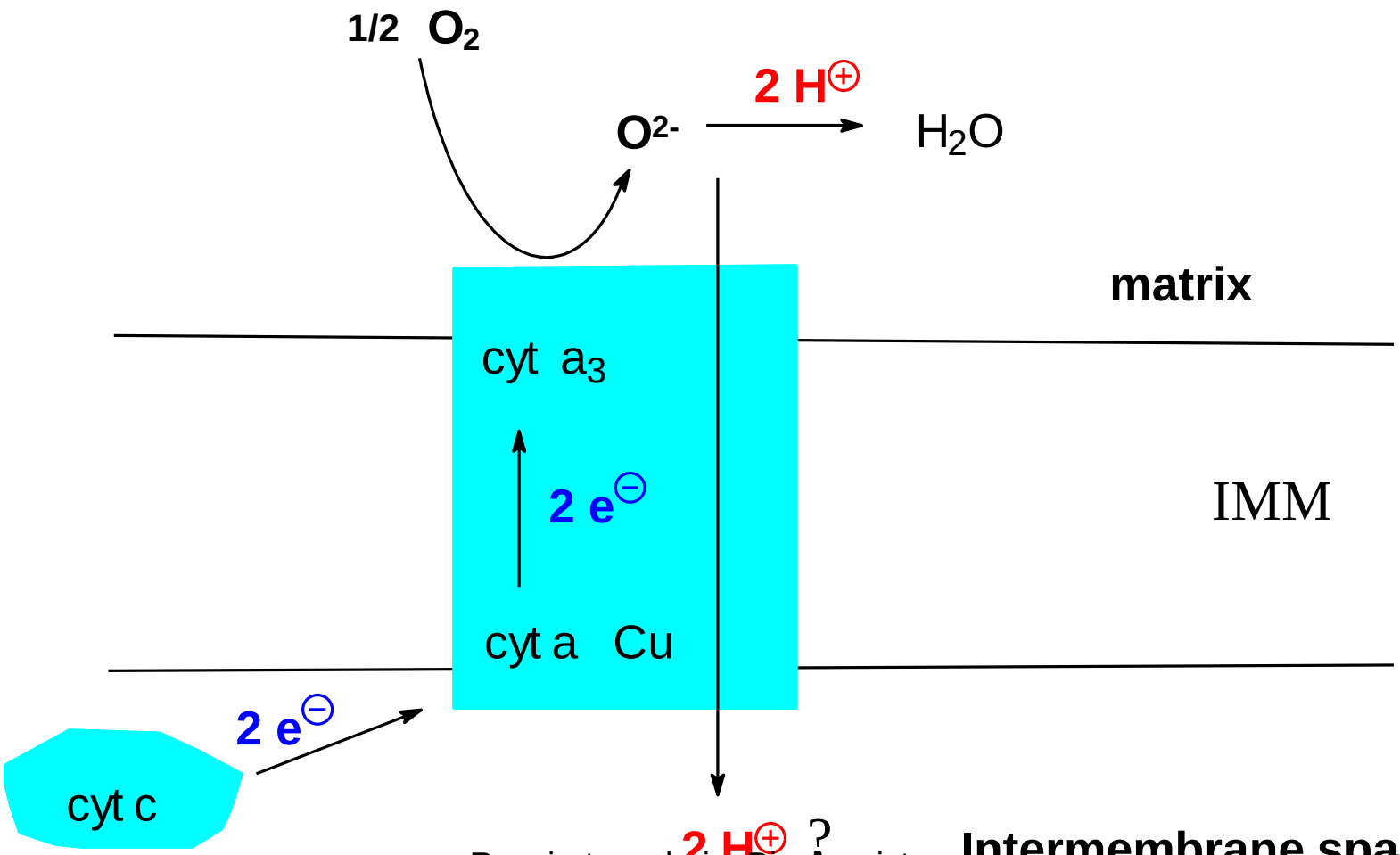
A cytochrome is an electron-transferring protein that contains a heme prosthetic group



Complex IV

- In *Complex IV* (cytochrome *c* oxidase; EC 1.9.3.1), sometimes called cytochrome A3, four electrons are removed from four molecules of cytochrome *c* and transferred to molecular oxygen (O2), producing two molecules of water. At the same time, four protons are removed from the mitochondrial matrix (although only two are translocated across the membrane), contributing to the proton gradient. The activity of cytochrome c oxidase is inhibited by cyanide.

Complex IV - second H^+ pump



Proton motive force :2 parts

Electric part
= differences of membrane potentials

$$\Delta\Psi = \Psi_{out} - \Psi_{in}$$

Concentration part = differences in pH

$$\Delta pH = pH_{out} - pH_{in}$$

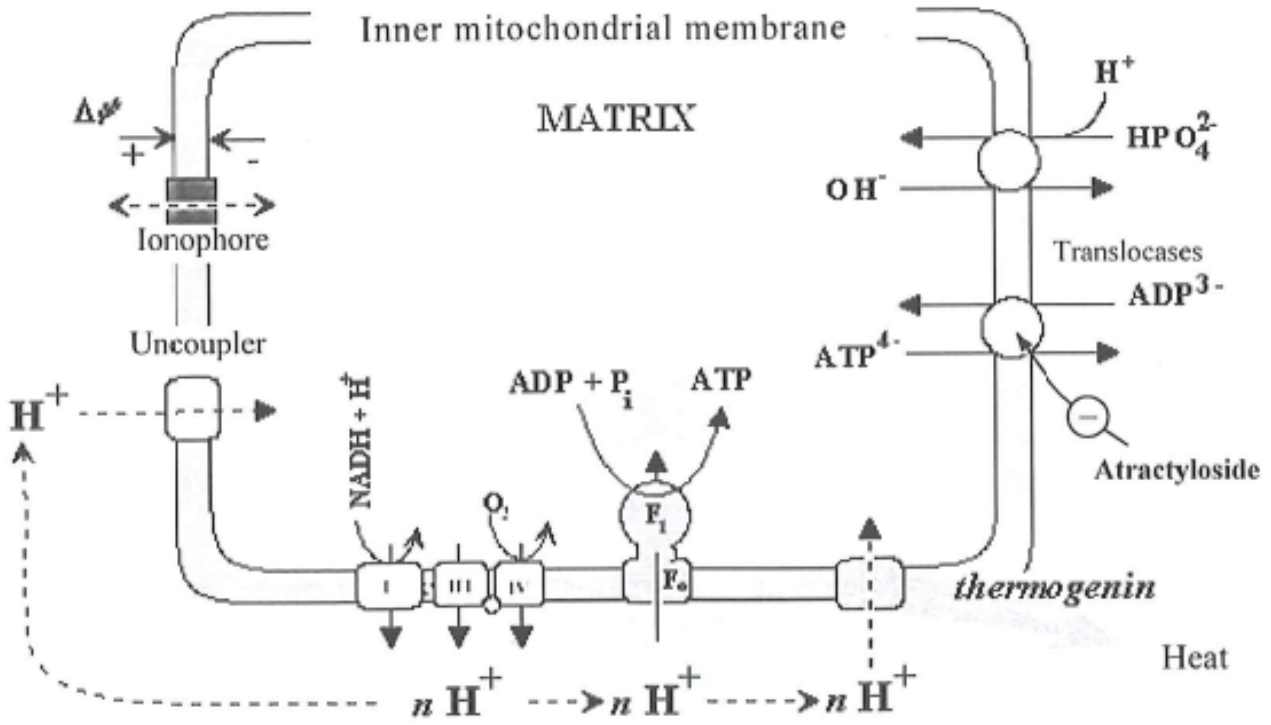
Using of proton motive force

- Synthesis ofATP
- Heat
- Active transports of metabolits -IMM

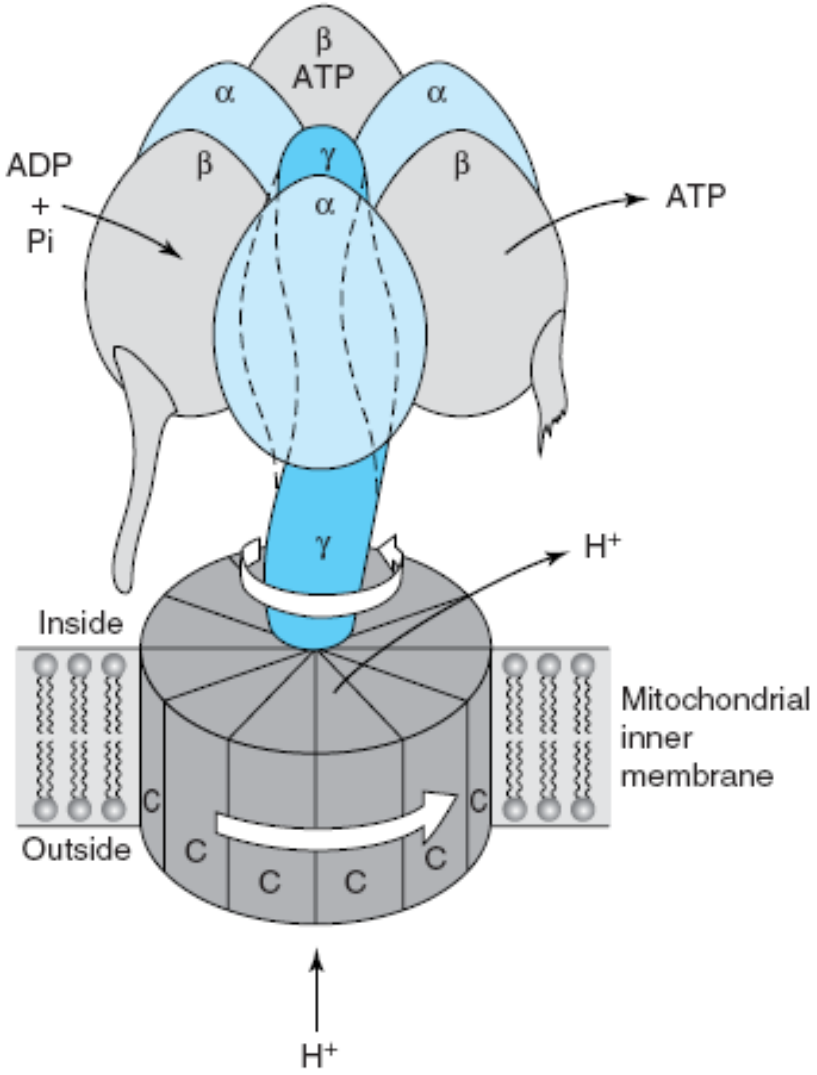
Proton motive force

Examples of proton motive force utilization:

a) ATP synthesis: ATP-synthase
b) Heat: thermogenin (brown adipose tissue)
c) Active transport through mitochondrial membrane



Mechanism of ATP production by ATP synthase.

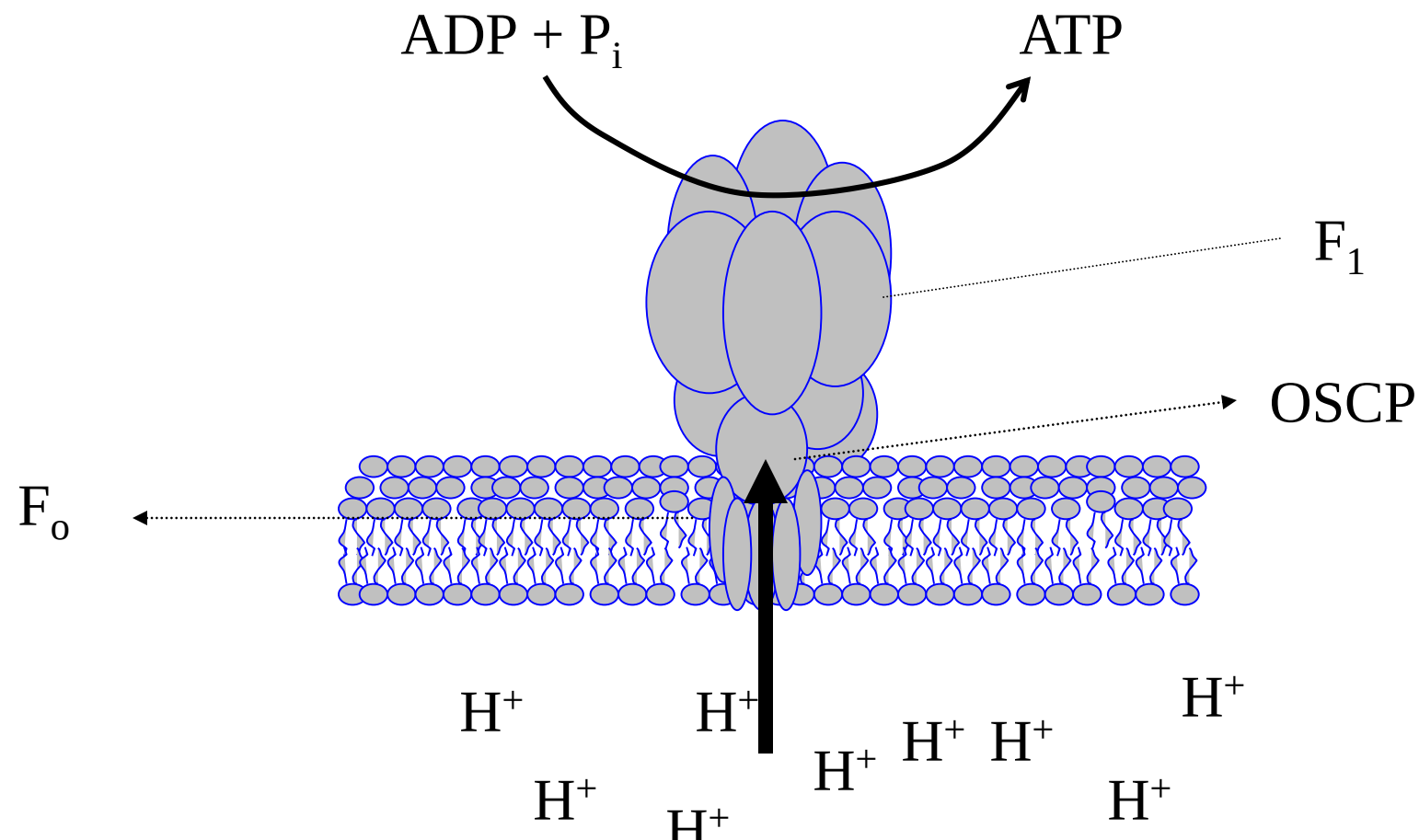


The enzyme complex consists of an F0 subcomplex which is a disk of "C" protein subunits. Attached is a γ-subunit in the form of a "bent axle."
Protons passing through the disk of "C" units cause it and the attached γ-subunit to rotate. The γ-subunit fits inside the F1 subcomplex of three α- and three β-subunits, which are fixed to the membrane and do not rotate.

ADP and Pi are taken up sequentially by the β-subunits to form ATP, which is expelled as the rotating γ-subunit squeezes each β-subunit in turn.

Thus, three ATP molecules are generated per revolution. For clarity, not all the subunits that have been identified are shown—eg, the "axle" also contains an ε-subunit.

Synthesis of ATP



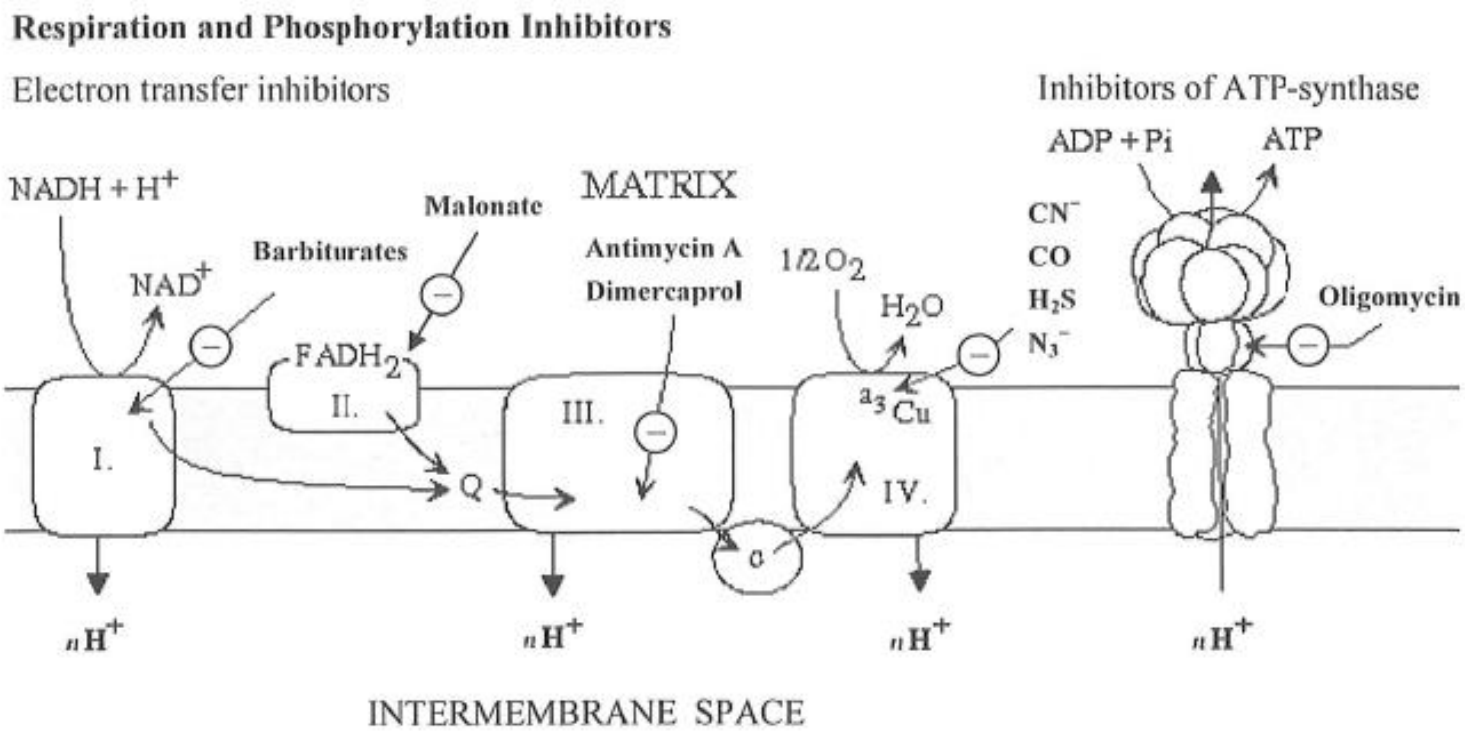
Synthesis of ATP

- ATP-syntaase -3 parts
- F_o channel for H^+
- F_1 in matrix, synthesis of ATP
- OSCP (oligomycin sensitivity conferring protein)

Connection of RCh and OPh

- **RCh and OPh** – non permeable IMM for H^+
- **Only way for H+ to matrix- F_o ATP-synthase**

Uncouplers and Inhibitors



Specific inhibitors were used to distinguish the electron transport system from the phosphorylation system and helped to define the sequence of redox carriers along the respiratory chain. If the chain is blocked then all the intermediates on the substrate side of the block become more reduced, while all those on the oxygen side become more oxidized. It is easy to see what has happened because the oxidized and reduced carriers often differ in their spectral properties. If a variety of different inhibitors are available then many of the respiratory carriers can be placed in the correct order.

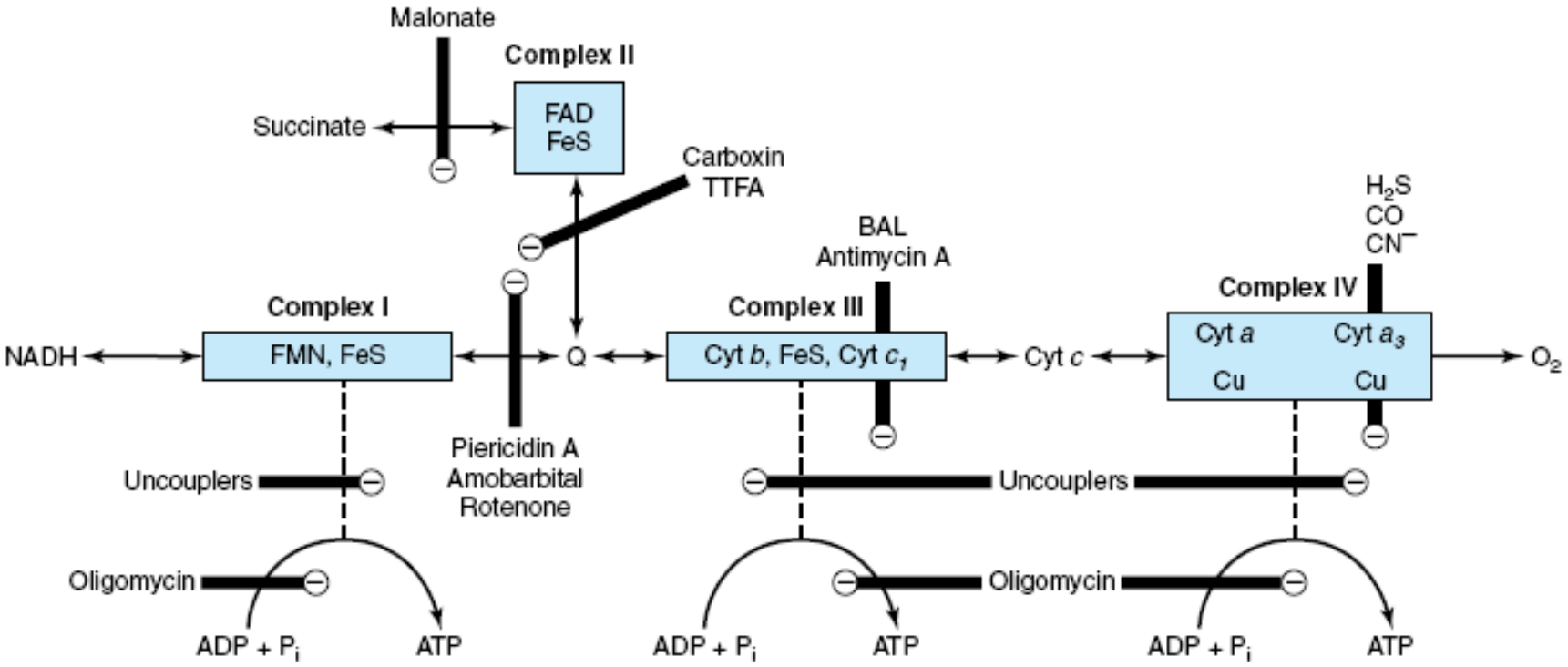


Figure 12–7. Proposed sites of inhibition (⊖) of the respiratory chain by specific drugs, chemicals, and antibiotics. The sites that appear to support phosphorylation are indicated. BAL, dimercaprol. TTFA, an Fe-chelating agent. Complex I, NADH:ubiquinone oxidoreductase; complex II, succinate:ubiquinone oxidoreductase; complex III, ubiquinol:ferricytochrome c oxidoreductase; complex IV, ferrocytochrome c:oxygen oxidoreductase. Other abbreviations as in Figure 12–4.

There are six distinct types of poison which may affect mitochondrial function:

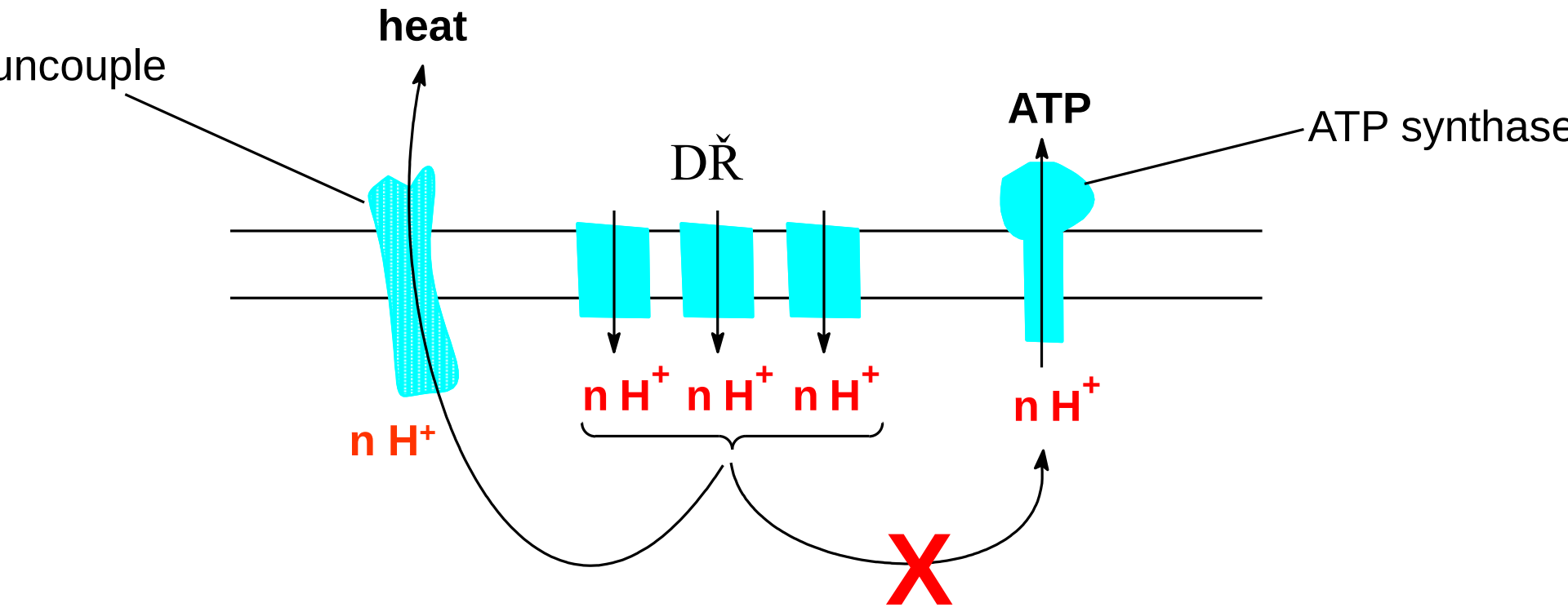
- 1) **Respiratory chain inhibitors** (e.g. cyanide, antimycin, rotenone & TTFA) block respiration in the presence of either ADP or uncouplers.
- 2) **Phosphorylation inhibitors** (e.g. oligomycin) abolish the burst of oxygen consumption after adding ADP, but have no effect on uncoupler-stimulated respiration.
- 3) **Uncoupling agents** (e.g. dinitrophenol, CCCP, FCCP) abolish the obligatory linkage between the respiratory chain and the phosphorylation system which is observed with intact mitochondria.
- 4) **Transport inhibitors** (e.g. atractyloside, bongkrekic acid, NEM) either prevent the export of ATP, or the import of raw materials across the the mitochondrial inner membrane.
- 5) **Ionophores** (e.g. valinomycin, nigericin) make the inner membrane permeable to compounds which are ordinarily unable to cross.
- 6) **Krebs cycle inhibitors** (e.g. arsenite, aminooxyacetate) which block one or more of the TCA cycle enzymes, or an ancillary reation.
- Some of the best-known compounds are listed below:

Uncouples

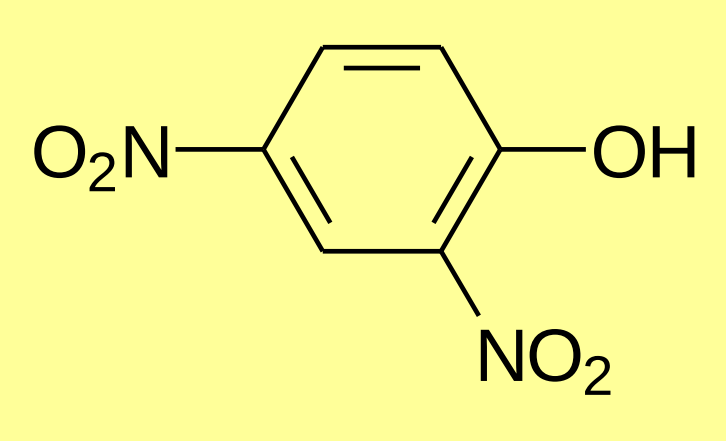
- abolish the obligatory linkage between the respiratory chain and the phosphorylation system
- abolish proton gradient without gain of ATP
- Creation of heat
- RCh is running
- OPh is closed

- **Uncoupling agents** (e.g. dinitrophenol, CCCP, FCCP) abolish the obligatory linkage between the respiratory chain and the phosphorylation system which is observed with intact mitochondria.

Uncouples



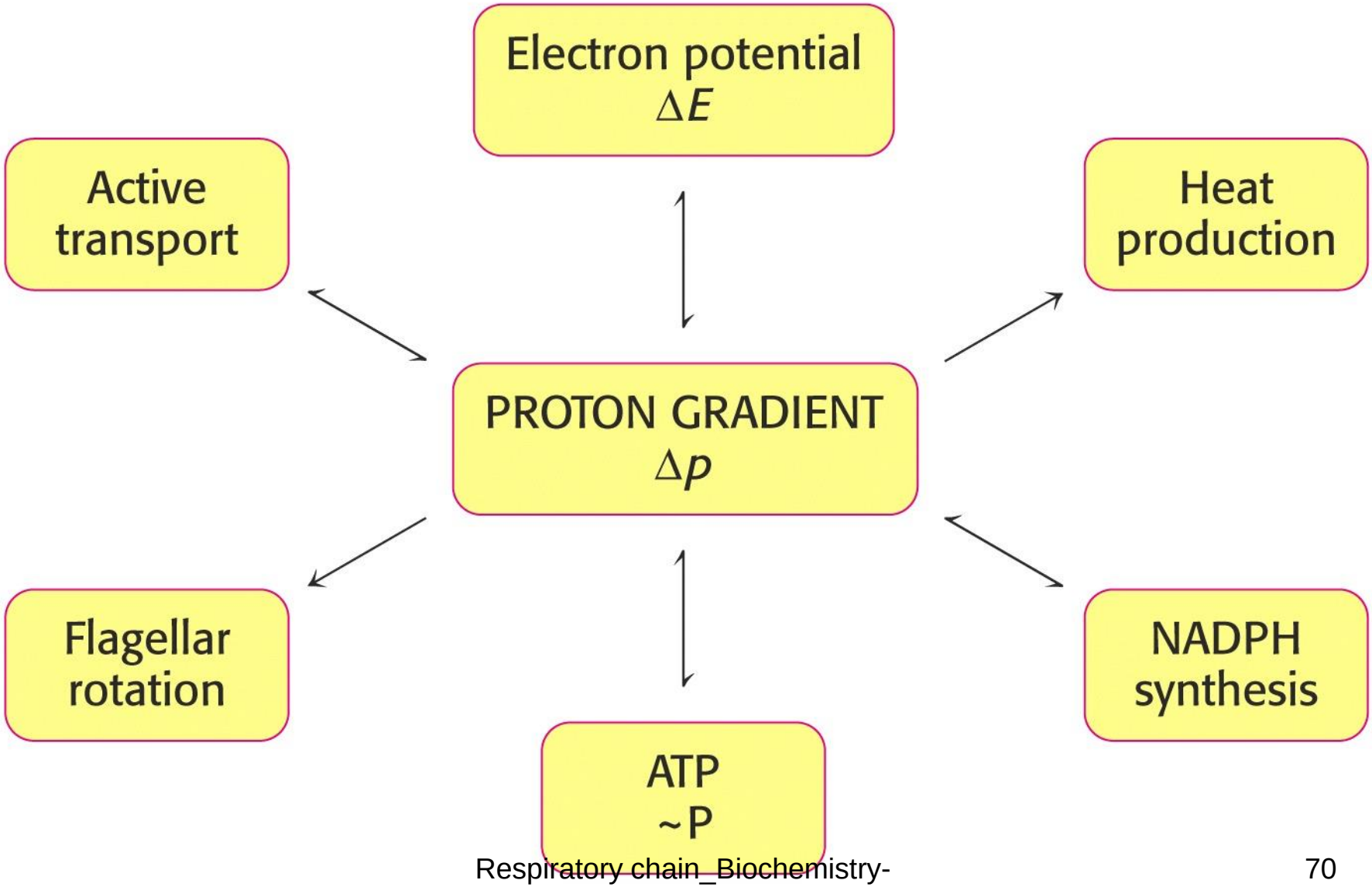
2,4-Dinitrophenol



Thermogenin-Biological Uncoupling

- Protein with channel for H^+, adipose tissue,
- Brown adipose tissue, new born child,
- Uncoupling the ETS from oxidative phosphorylation speeds metabolism, and generates heat. Some mammals lacking fur use this function in brown adipose tissue as a way of generating heat.
- One such process is called nonshivering thermogenesis that occurs in cells in the neck and upper back. The mitochondria of brown adipose tissue cells contain a protein called thermogenin (or uncoupling protein - UCP). Thermogenin acts as a channel to permeabilize these cells' inner mitochondrial membrane to protons. Normally, ADP, ATP, GDP, and GTP are present in high enough concentrations to block the flow of protons through it. However, thermogenin in the mitochondria of these cells is activated to uncoupling by the presence of free fatty acids. Free fatty acids can be generated in these cells by the hormone norepinephrine, which through second messengers (including cAMP) activates hormone-sensitive triacylglycerol lipase to cleave fats to release fatty acids. Thus, brown adipose tissue cells respond to norepinephrine by uncoupling the ETS from oxidative phosphorylation, speeding metabolism and generating heat, at the expense of metabolic energy.

Proton gradient is interconvertible form of free energy

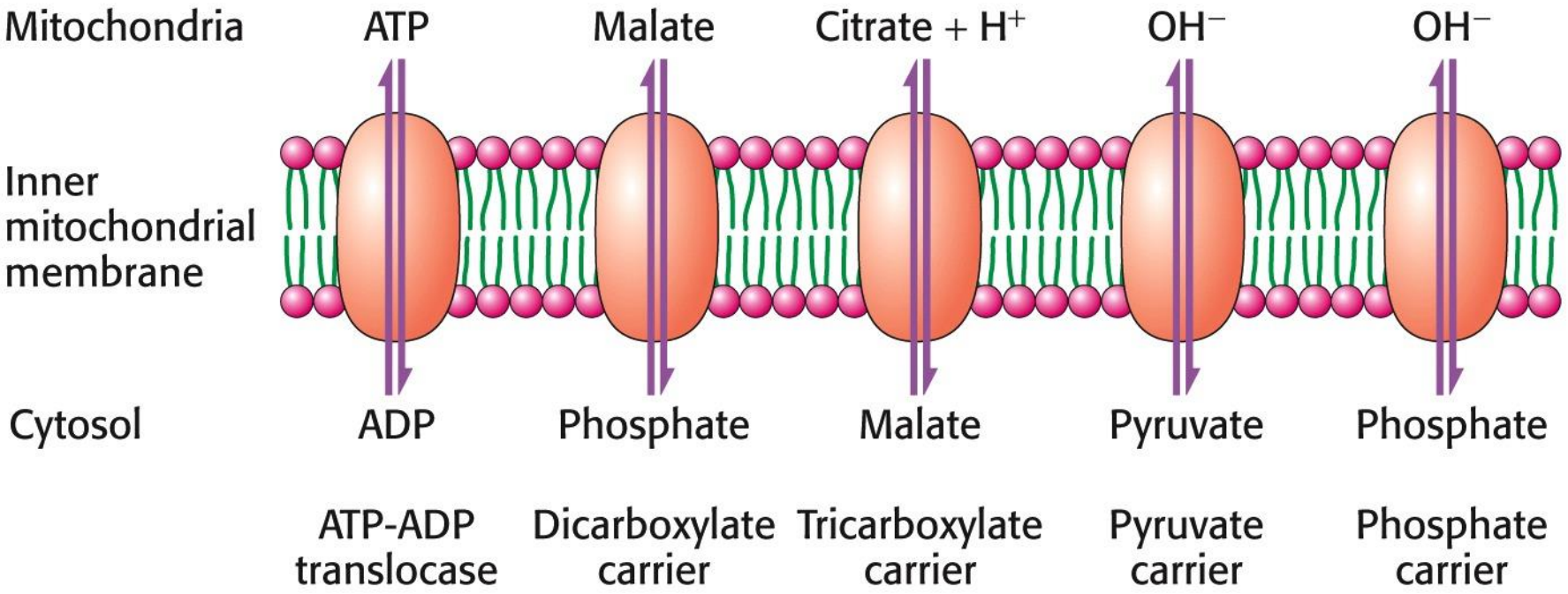


Transport of metabolits over IMM

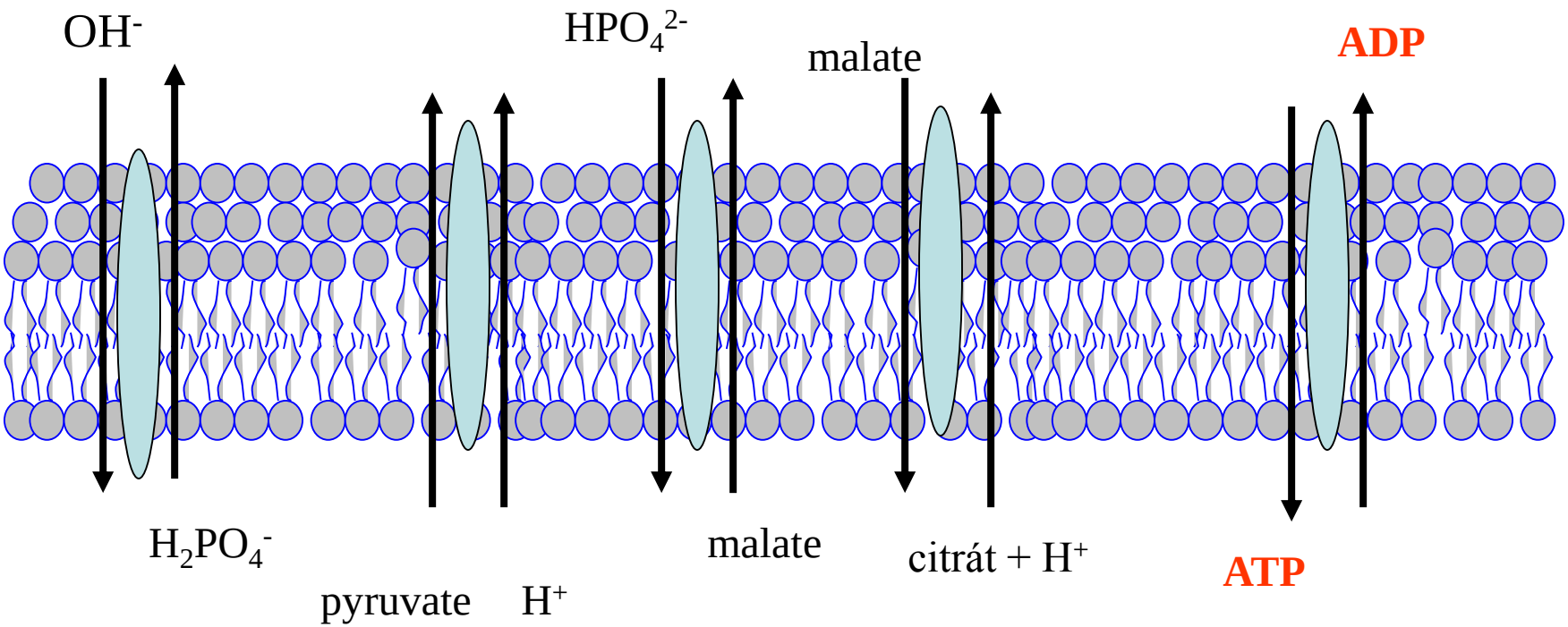
- Non permeable – shuttle syntems
- Source of E- proton motive force of RCh
- Secondary active transport

- O_2, H_2O, NH_3 - free
- FA - carnitin
- pyruvare - symport with H^+
- CC, AA acids - specific transporters
- hydrogenphosphate – exchange for OH^-
- malate- exchange for 2-oxoglutarate (shuttle)
- aspartate – exchange for glutamate (shuttle)
- **ATP – exchange for ADP**

Mitochondrial transporters

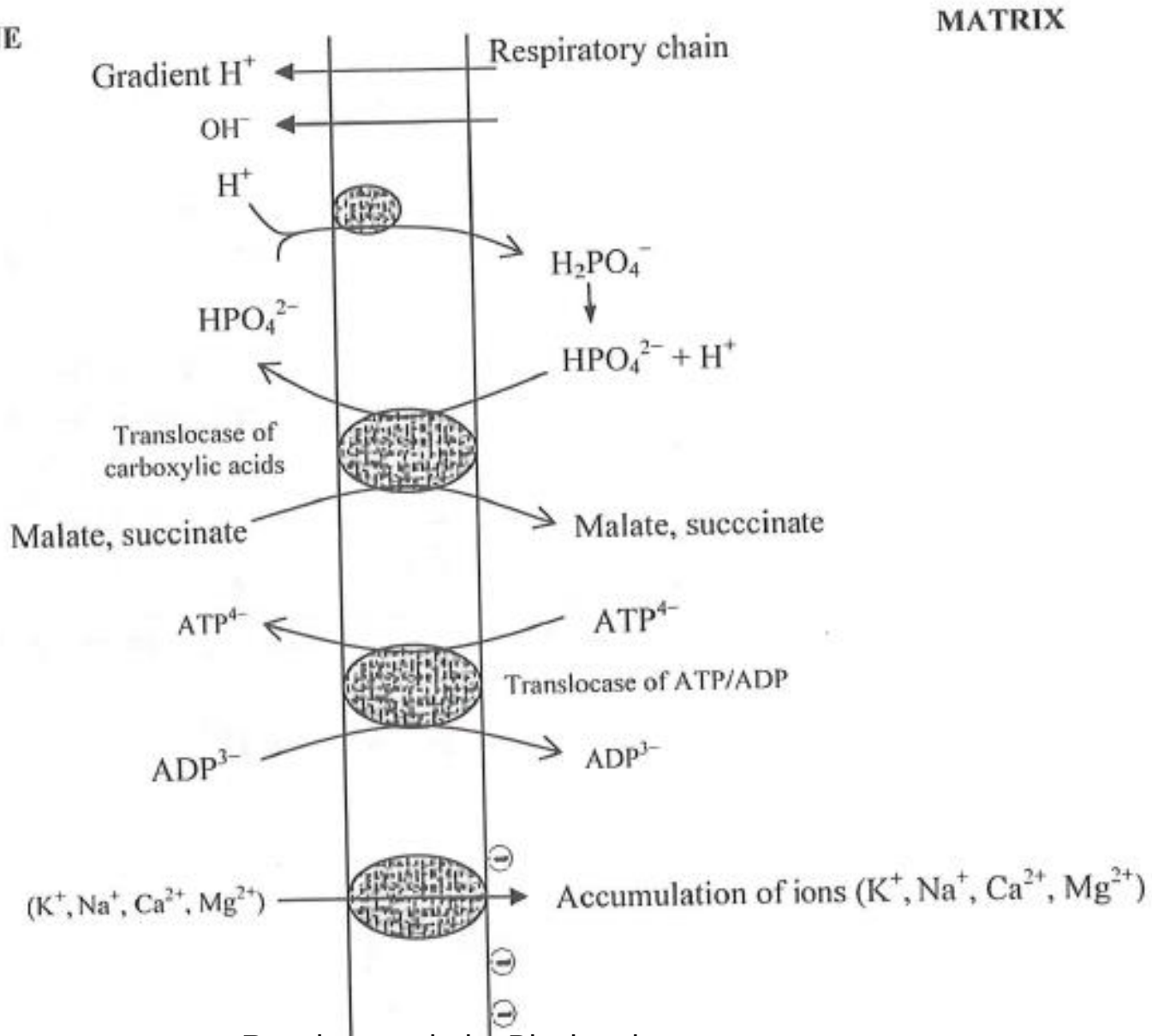


matrix

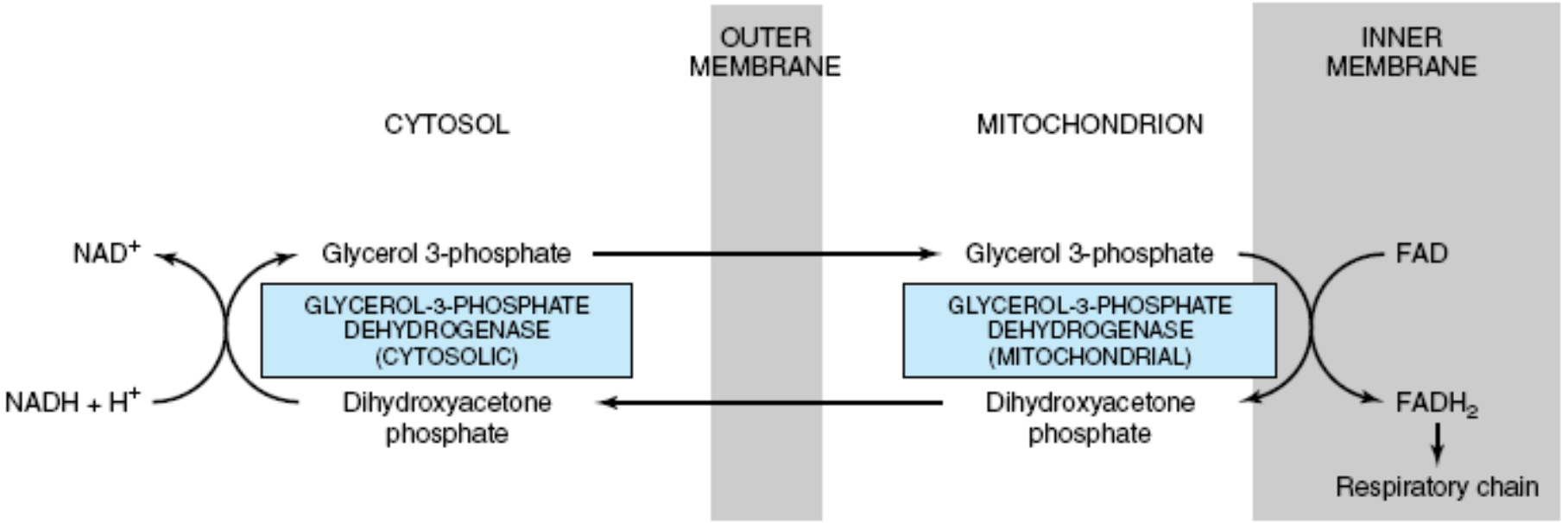


cytosol

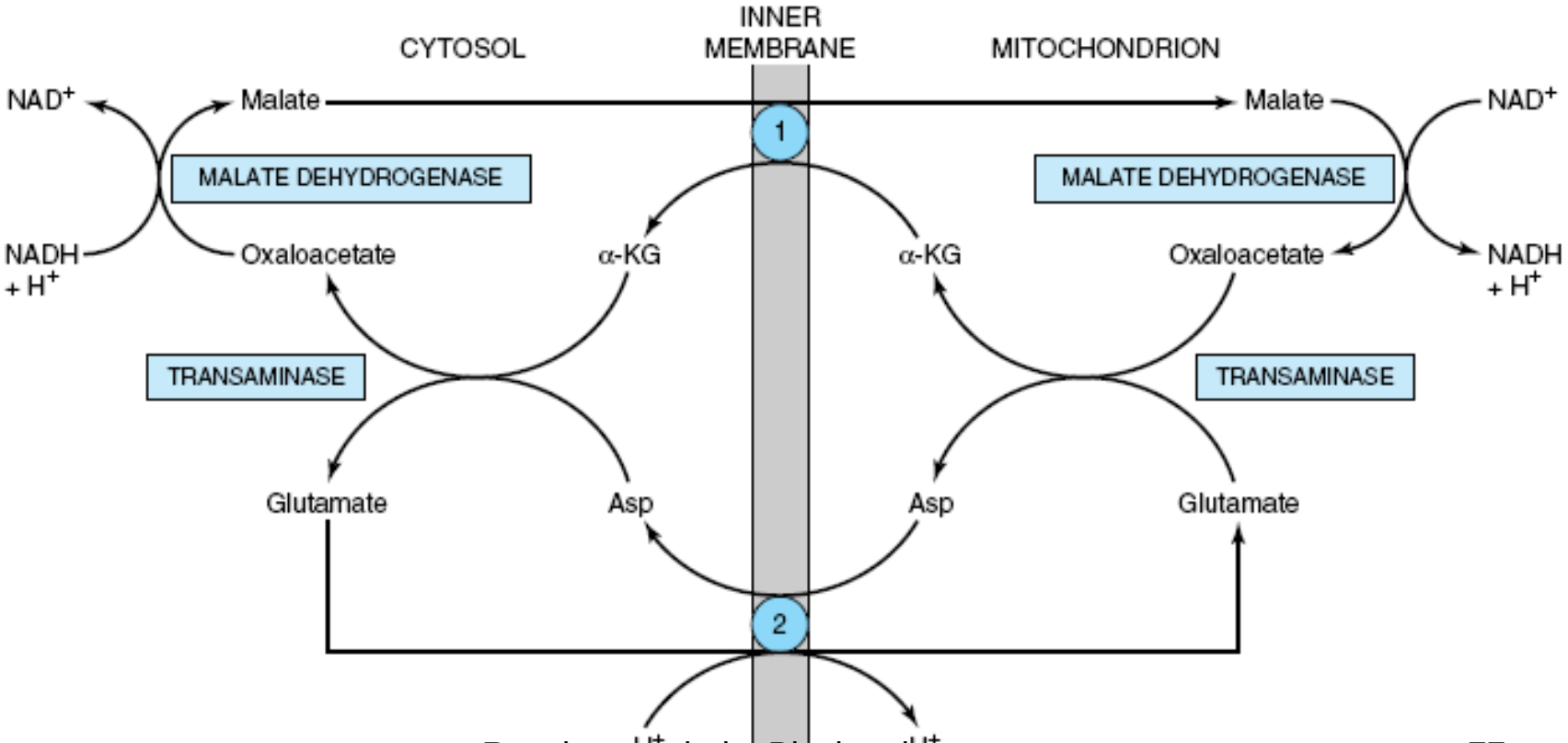
Examples of Active Transport through Inner Mitochondrial Membrane



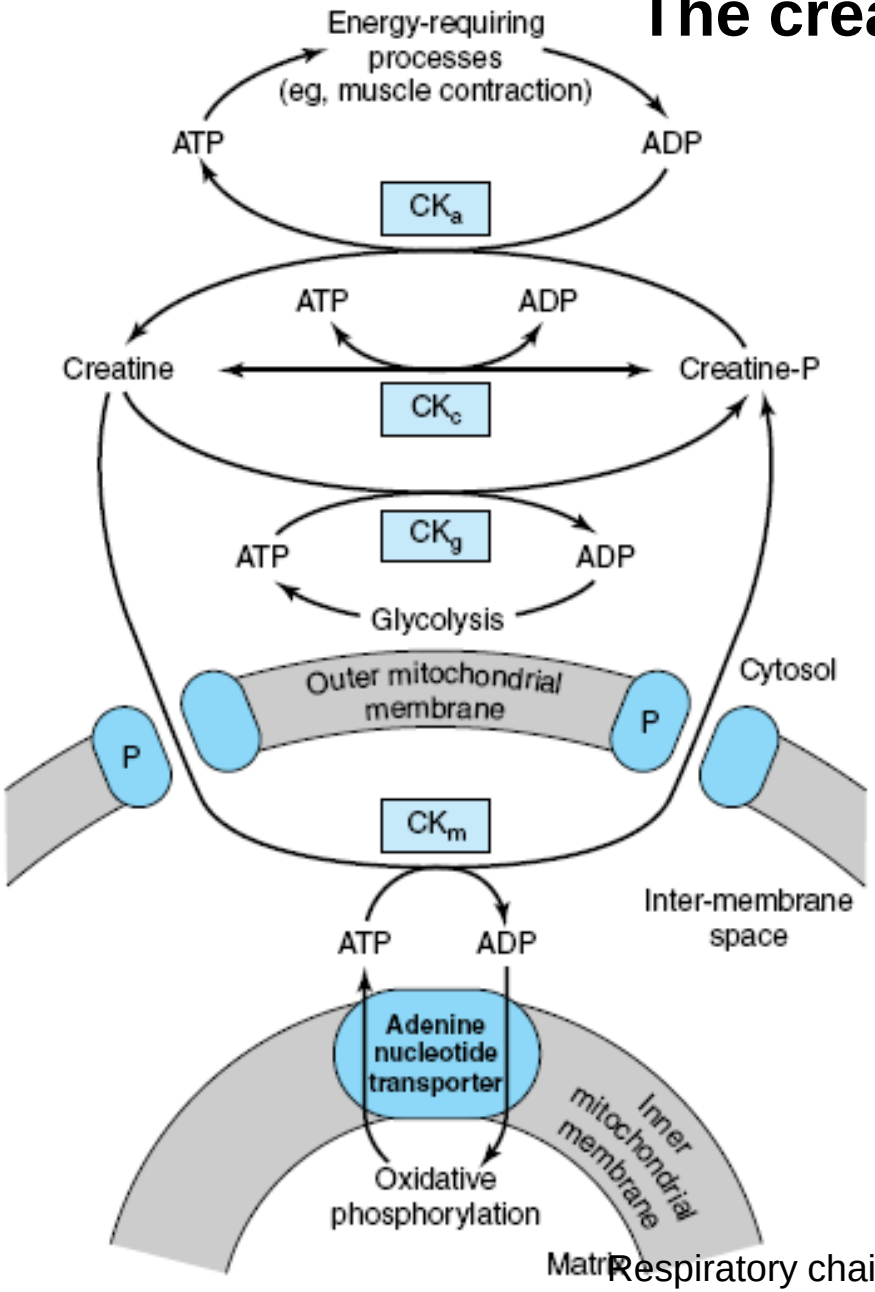
Glycerophosphate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion.



Malate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion. 1 Ketoglutarate transporter; 2 , glutamate/aspartate transporter (note the proton symport with glutamate).



The creatine phosphate shuttle



The creatine phosphate shuttle of heart and skeletal muscle. The shuttle allows rapid transport of high-energy phosphate from the mitochondrial matrix into the cytosol.

CKa, creatine kinase concerned with large requirements for ATP, eg, muscular contraction; CKc, creatine kinase for maintaining equilibrium between creatine and creatine phosphate and ATP/ADP;

CKg, creatine kinase coupling glycolysis to creatine phosphate synthesis; CKm, mitochondrial creatine kinase mediating creatine phosphate production from ATP formed in oxidative phosphorylation; P, pore protein in outer mitochondrial membrane.

Inhibitors

There are several well-known drugs and toxins that inhibit oxidative phosphorylation. Although any one of these toxins inhibits only one enzyme in the electron transport chain, inhibition of any step in this process will halt the rest of the process. For example, if oligomycin inhibits ATP synthase, protons cannot pass back into the mitochondrion.[84] As a result, the proton pumps are unable to operate, as the gradient becomes too strong for them to overcome. NADH is then no longer oxidized and the citric acid cycle ceases to operate because the concentration of NAD+ falls below the concentration that these enzymes can use.

RCh

- rotenon, barbital (I)
- malonate (II)
- antimycin A (III)
- dimerkaprol (III)
- CO, **CN^-,** SH^-, N_3^- (IV)

ATP-synthase

- oligomycin

ATP/ADP-translocase

Bongcrecid acid

- atractylosid

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- 5) **Ionophores** (e.g. valinomycin, nigericin) make the inner membrane permeable to compounds which are ordinarily unable to cross.
- 6) **Krebs cycle inhibitors** (e.g. arsenite, aminooxyacetate) which block one or more of the TCA cycle enzymes, or an ancillary reation.
- Some of the best-known compounds are listed below:

Cyanide poisoning

- Many cyanides are highly toxic. The cyanide anion is an inhibitor of the enzyme cytochrome c oxidase (also known as aa3) in the fourth complex of the electron transport chain (found in the membrane of the mitochondria of eukaryotic cells). It attaches to the iron within this protein. The binding of cyanide to this cytochrome prevents transport of electrons from cytochrome c oxidase to oxygen. As a result, the electron transport chain is disrupted, meaning that the cell can no longer aerobically produce ATP for energy.[18] Tissues that depend highly on aerobic respiration, such as the central nervous system and the heart, are particularly affected. This is an example of histotoxic hypoxia.[19]
- The most hazardous compound is hydrogen cyanide, which is a gas at ambient temperatures and pressure and can therefore be inhaled. For this reason, an air respirator supplied by an external oxygen source must be worn when working with hydrogen cyanide. Hydrogen cyanide is produced when a solution containing a labile cyanide is made acidic, because HCN is a weak acid. Alkaline solutions are safer to use because they do not evolve hydrogen cyanide gas. Hydrogen cyanide may be produced in the combustion of polyurethanes; for this reason, polyurethanes are not recommended for use in domestic and aircraft furniture. Oral ingestion of a small quantity of solid cyanide or a cyanide solution as little as 200 mg, or to airborne cyanide of 270 ppm is sufficient to cause death within minutes.[19]
- Organic nitriles do not readily release cyanide ions, and so have low toxicities. By contrast, compounds such as trimethylsilyl cyanide (CH3)3SiCN readily release HCN or the cyanide ion upon contact with water.[*citation needed*]

- **Antidote**
- Hydroxocobalamin reacts with cyanide to form cyanocobalamin, which can be safely eliminated by the kidneys. This method has the advantage of avoiding the formation of methemoglobin (see below). This antidote kit is sold under the brand name Cyanokit and was approved by the FDA in 2006.[20]
- An older cyanide antidote kit included administration of three substances: amyl nitrite pearls (administered by inhalation), sodium nitrite, and sodium thiosulfate (administered by infusion). The goal of the antidote was to generate a large pool of ferric iron (Fe3+) to compete with cyanide cytochrome a3 (so that cyanide will bind to the antidote rather that the enzyme). The nitrites oxidize hemoglobin to methemoglobin, which competes with cytochrome oxidase for the cyanide ion. Cyanmethemoglobin is formed and the cytochrome oxidase enzyme is restored. The major mechanism to remove the cyanide from the body is by enzymatic conversion to thiocyanate by the mitochondrial enzyme rhodanese. Thiocyanate is a relatively non-toxic molecule and is excreted by the kidneys. To accelerate this detoxification, sodium thiosulfate is administered to provide a sulfur donor for rhodanese, needed in order to produce thiocyanate.

ROS, reactive oxygen species

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include oxygen ions and peroxides. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis.[1] However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically.[1] This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation.

Reactive Oxygen Species (ROS)

Radicals:

$O_2^{\cdot -}$	Superoxide
$OH^{\cdot}$	Hydroxyl
$RO_2^{\cdot}$	Peroxyl
$RO^{\cdot}$	Alkoxyl
$HO_2^{\cdot}$	Hydroperoxyl

Non-Radicals:

H_2O_2	Hydrogen peroxide
$HOCl^-$	Hypochlorous acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite

Reactive Nitrogen Species (RNS)

Radicals:

$NO^{\cdot}$	Nitric Oxide
$NO_2^{\cdot}$	Nitrogen dioxide

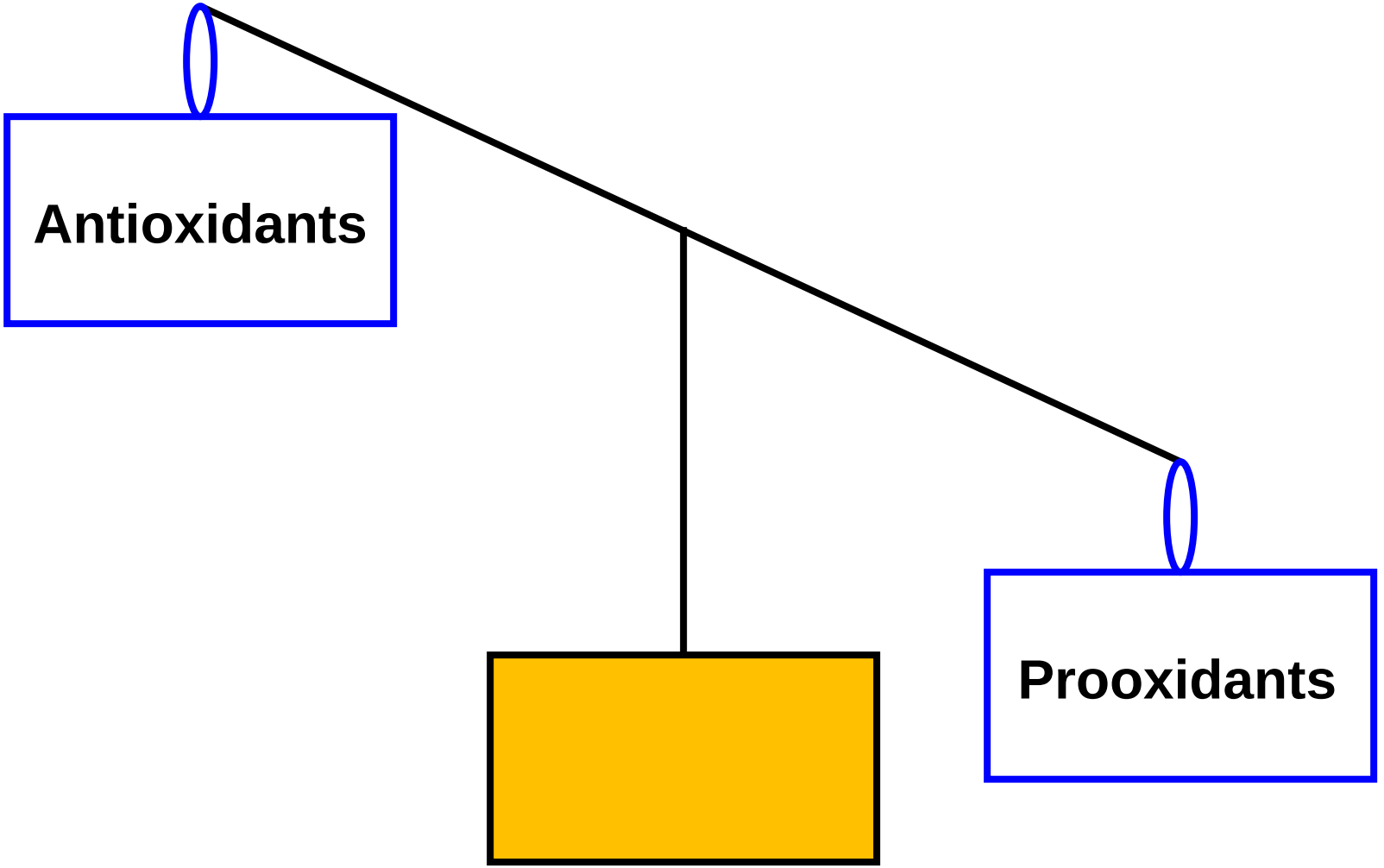
Non-Radicals:

$ONOO^-$	Peroxynitrite
ROONO	Alkyl peroxynitrites
N_2O_3	Dinitrogen trioxide
N_2O_4	Dinitrogen tetroxide
HNO_2	Nitrous acid
NO_2^+	Nitronium anion
NO^-	Nitroxyl anion
NO^+	Nitrosyl cation
NO_2Cl	Nitryl chloride

"Longevity" of reactive species

Reactive Species	Half-life
Hydrogen peroxide Organic hydroperoxides Hypohalous acids	~ minutes
Peroxyl radicals Nitric oxide	~ seconds
Peroxynitrite	~ milliseconds
Superoxide anion Singlet oxygen Alcoxyl radicals	~ microsecond
Hydroxyl radical	~ nanosecond

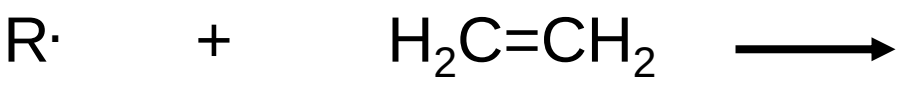
Oxidative Stress



"An imbalance favoring prooxidants and/or disfavoring antioxidants, potentially leading to damage" -H. Sies

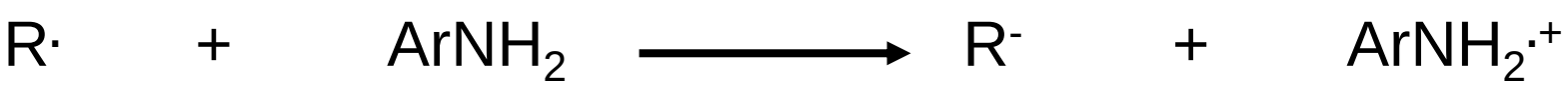
Radical-mediated reactions

Addition

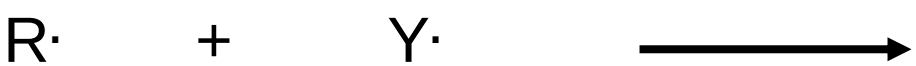


Hydrogen abstraction

Electron abstraction



Termination



Disproportionation

Hydroxyl radical (·OH)

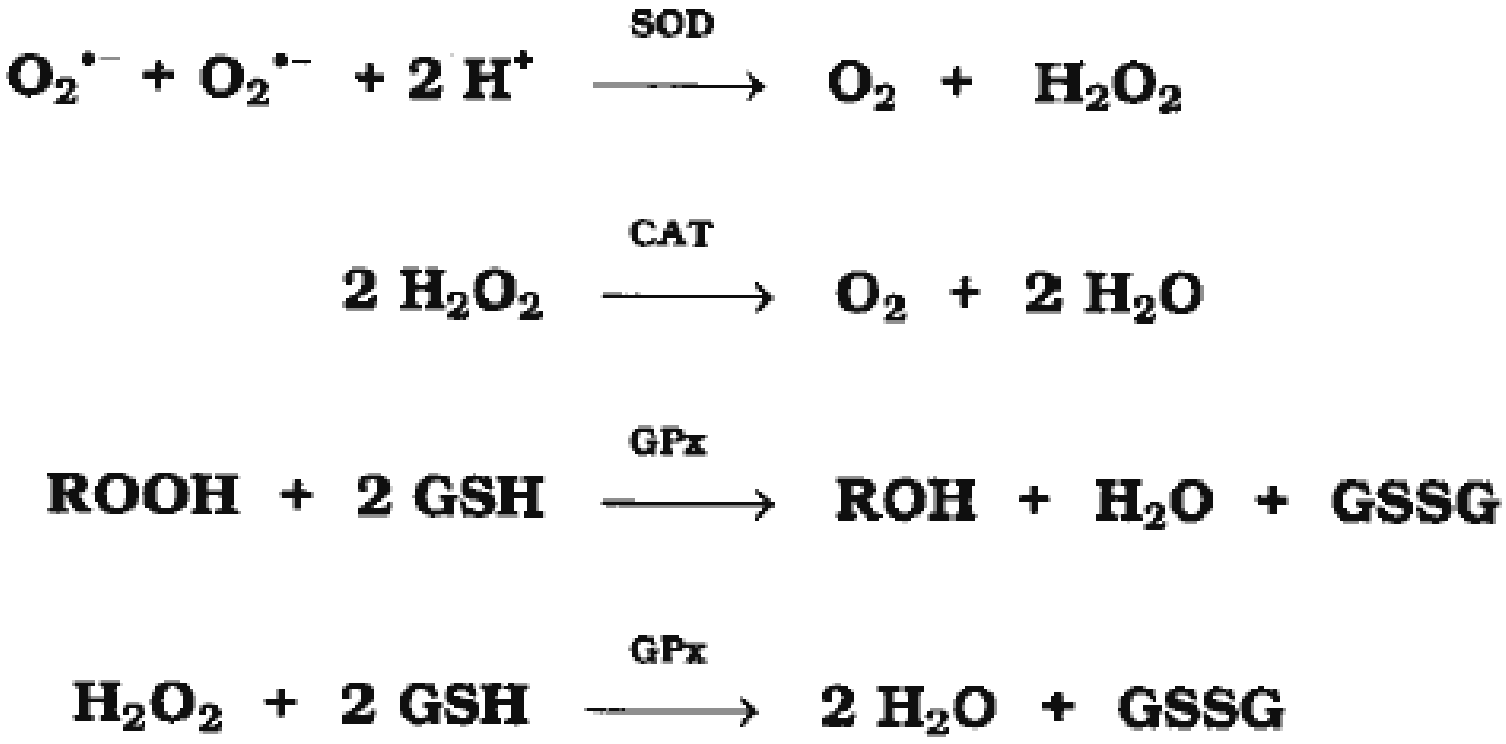
$O_2^{\cdot -} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$ (ferrous)

Fenton: $H_2O_2 + Fe^{2+} \rightarrow OH^- + {\cdot}OH + Fe^{3+}$ (ferric)

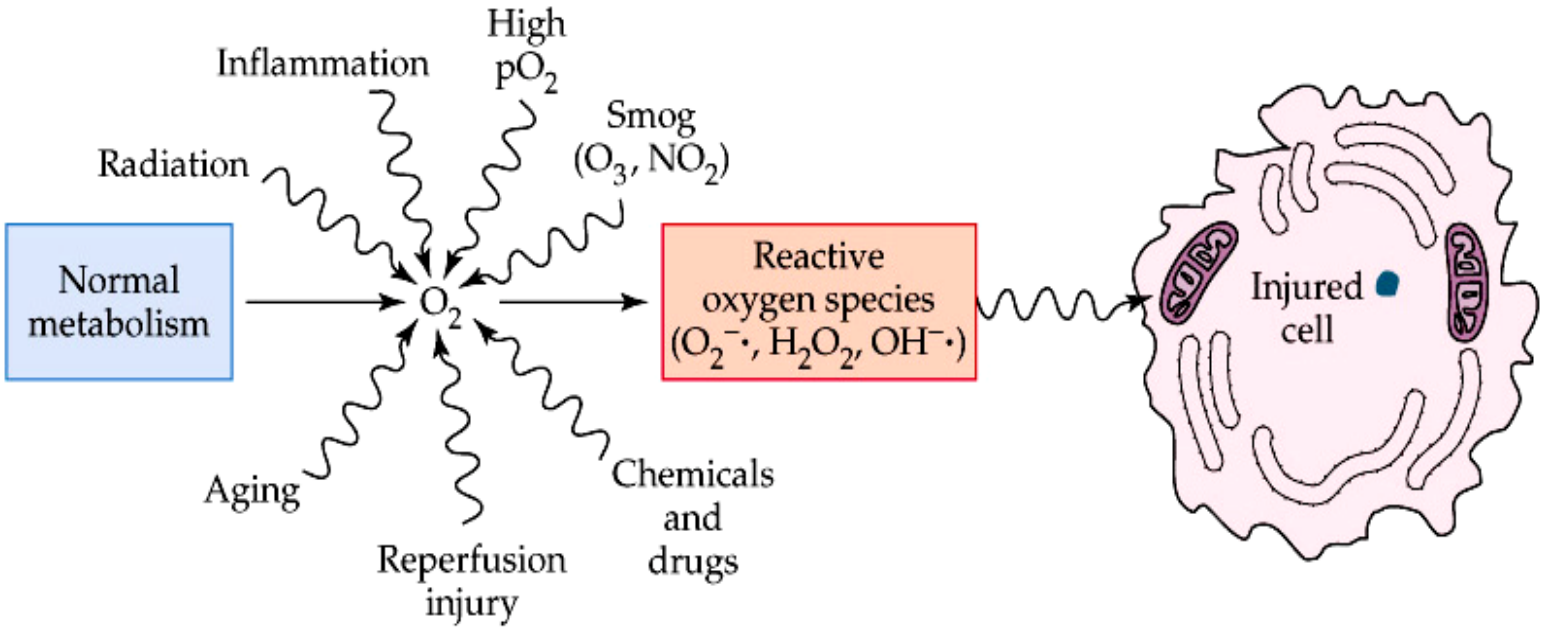
Haber-Weiss: $O_2^{\cdot -} + H_2O_2 \rightarrow OH^- + O_2 + {\cdot}OH$

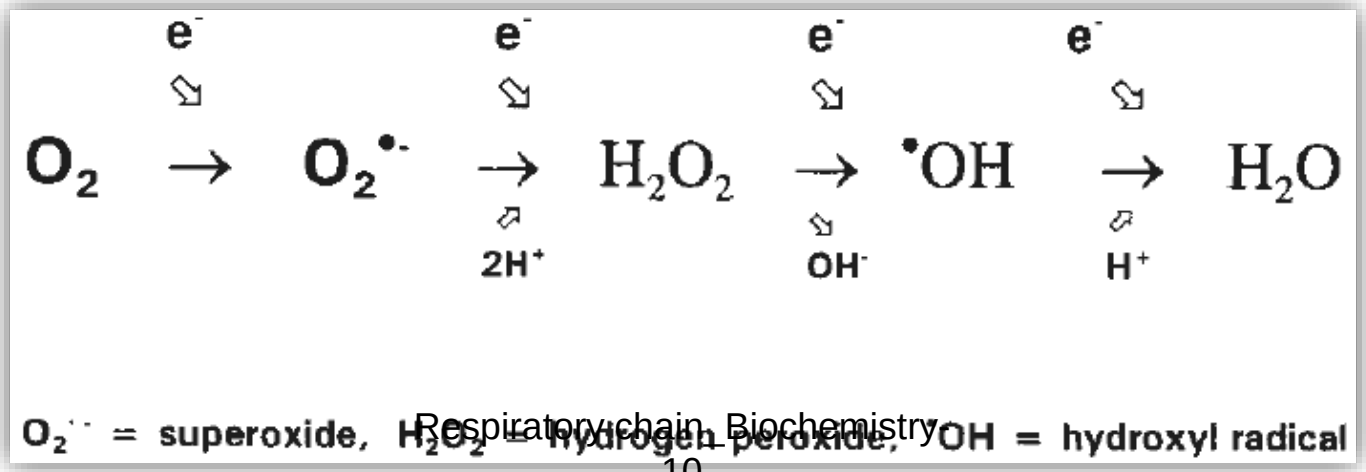
- **Transition metal catalyzed**
- **Other reductants can make Fe^{2+}** (e.g., GSH, ascorbate, hydroquinones)
- **Fe2+ is an <u>extremely</u> reactive oxidant**

Important Enzyme-Catalyzed Reactions



Biological Pathways for Oxygen Reduction

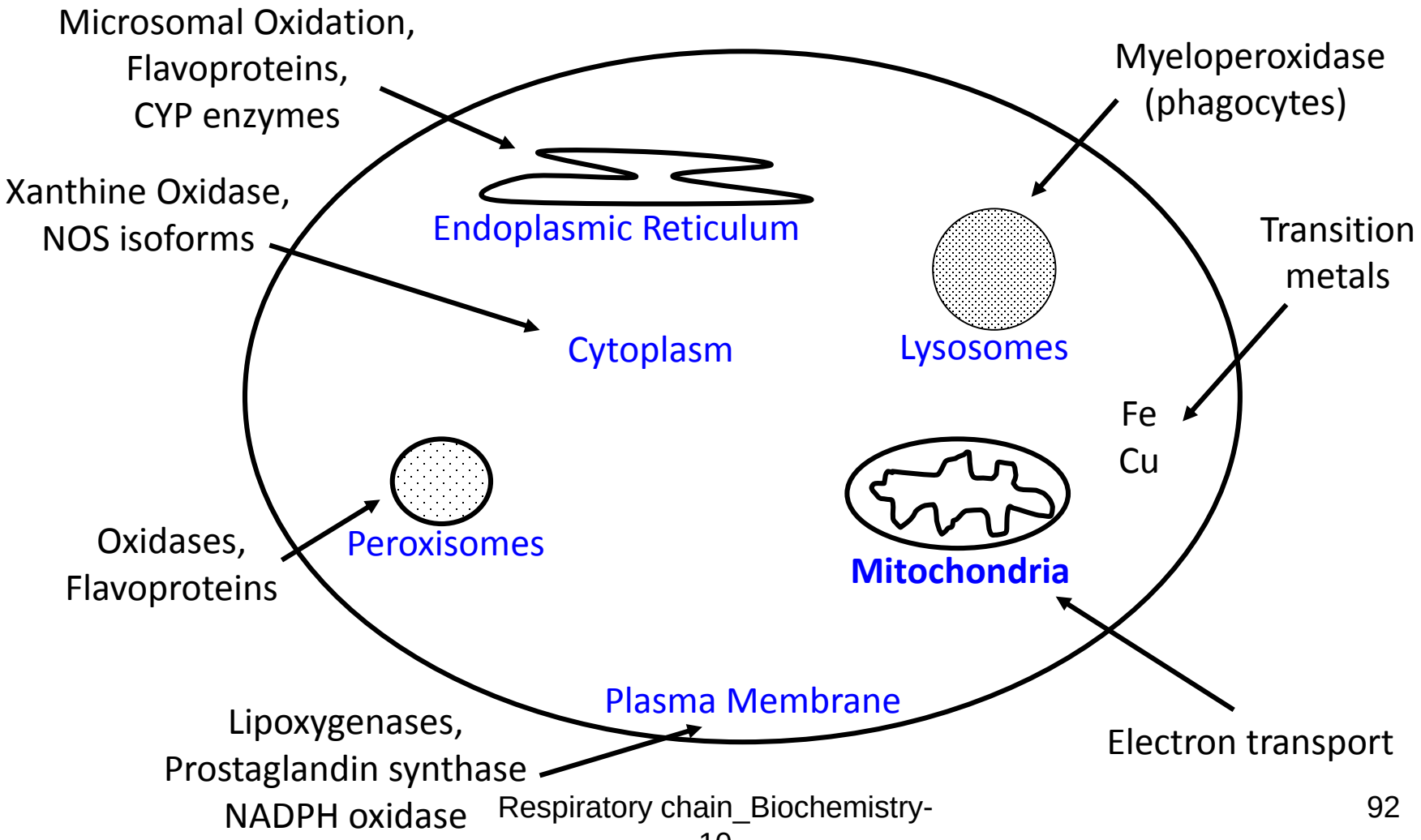




From: McMurry and Castellion "Fundamentals of general, organic and biological chemistry"

II. Sources of ROS

Endogenous sources of ROS and RNS



Antioxidant systems of the organism

1. **Enzymes** (endogenous), superoxide dismutase , catalase , glutathione peroxidase
2. **Second high molecular weight** antioxidants (endogenous), transferrin, ferritin , ceruloplasmin al., Bind free metal ions
3. **Third low molecular weight antioxidants** (exogenous , endogenous),

-reducing substances with the phenolic -OH (tocopherol , flavonoids, urate)

- reducing substances with enolic OH (ascorbate)

- reducing substance having -SH group (glutathione, dihydrolipoát)

- substances with an extensive system of conjugated double bonds (carotenoids, retinol, bilirubin)