Respiratory chain

~

Reactive oxygen species

Transformation of energy in human body

chemical energy of nutrients = **work** + **heat**

energy of nutrients = **BM + phys. activity + reserves** + **heat**

BM = basal metabolism

reserve = adipose tissue, glycogen

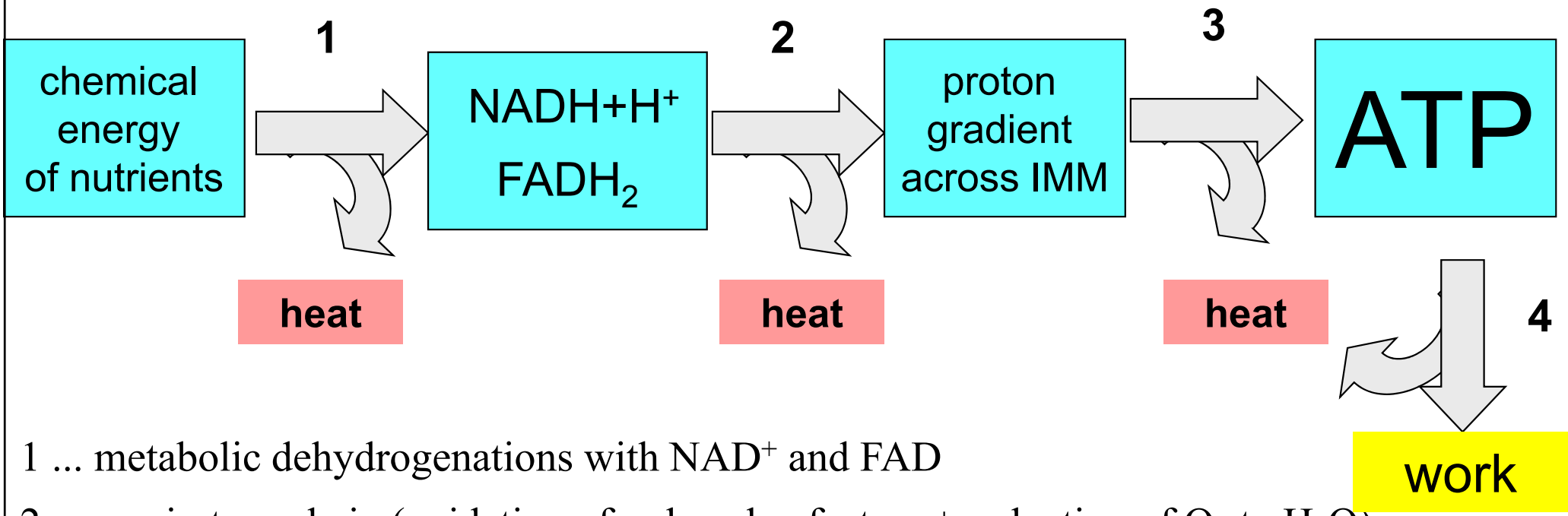
any work requires ATP

chemical: synthesis of proteins, urea ...

osmotic: transport of ions against gradient ...

mechanical: muscle contraction ...

Energy transformations in the human body are accompanied with continuous production of heat



1 ... metabolic dehydrogenations with NAD^+ and FAD

2 ... respiratory chain (oxidation of reduced cofactors + reduction of O_2 to H_2O)

3 ... oxidative phosphorylation, IMM inner mitochondrial membrane

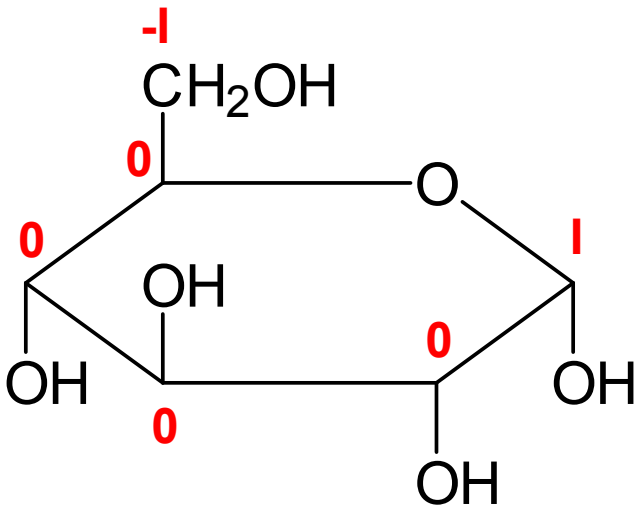
4 ... transformation of chemical energy of ATP into work + some heat

... high energy systems

Energetic data about nutrients

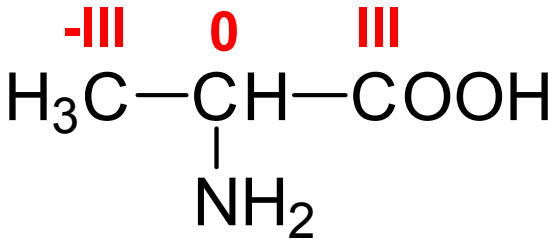
Nutrient	Energy (kJ/g)	Thermogenesis	Energy supply/day
Lipids	38	4 %	30 % SAFA 5 %, MUFA 20 %, PUFA 5 %
Saccharides	17	6 %	60 %
Proteins	17	30 %	10 %

Oxidation numbers of carbon and the content of hydrogen in nutrients



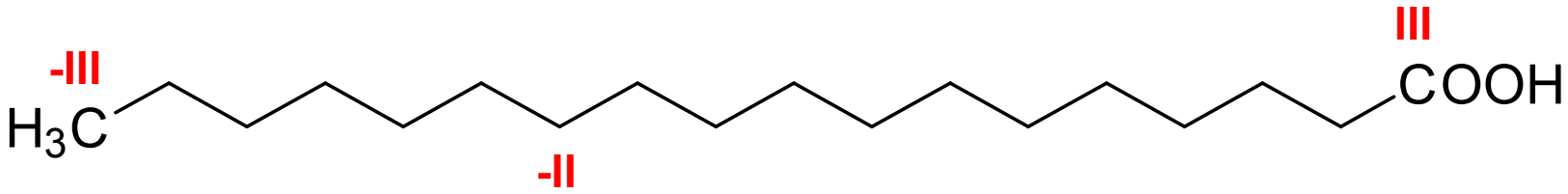
glucose: 6.7 % H

average ox. num. of C = 0.0



alanine: 7.9 % H

average ox. num. of C = 0.0



stearic acid: 12.8 % H

average ox. num. of C = -1.8 $\Rightarrow$ C is the most reduced

Two ways of ATP formation in body

1. Oxidative phosphorylation in the presence of O_2 (~ 95 % ATP)

ADP + P_i + energy of H^+gradient → ATP

2. Substrate-level phosphorylation (~ 5 % ATP)

ADP + macroergic phosphate-P → ATP + second product

higher energy content than ATP

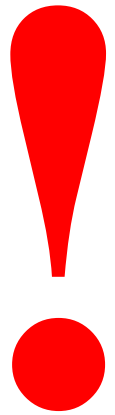
Compare: Phosphorylation

substrate-OH + ATP → substrate-O-P + ADP

(e.g. phosphorylation of glucose, proteins, etc., catalyze kinases)

Distinguish

Process	ATP is
Oxidative phosphorylation	produced
Substrate-level phosphorylation	produced
Phosphorylation of a substrate	consumed

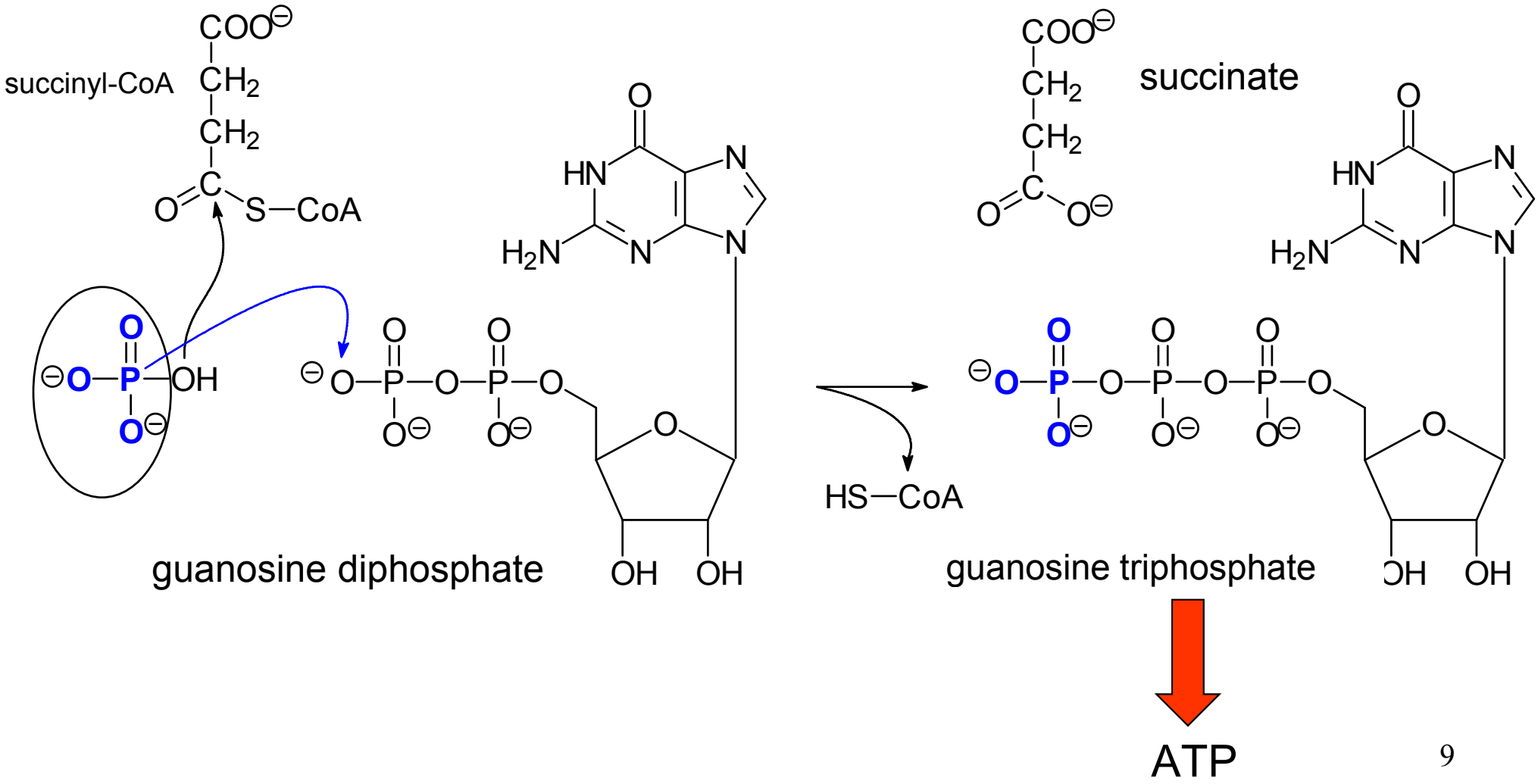


Substrate level phosphorylation

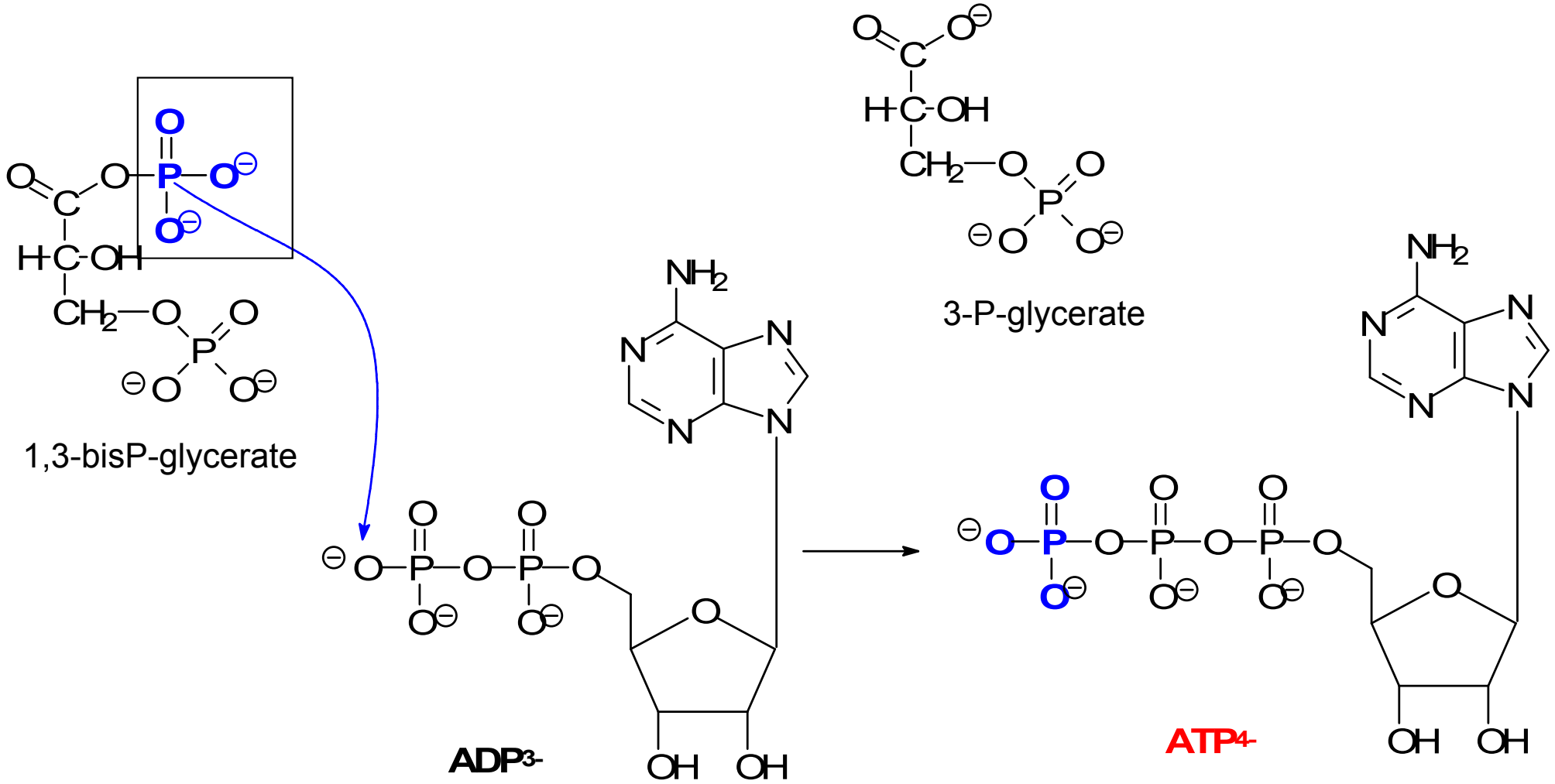
- phosphorylation of ADP (GDP) is performed by the high-energy intermediates
- succinyl-CoA (CAC)
- 1,3-bisphosphoglycerate (glycolysis)
- phosphoenolpyruvate (glycolysis)

Phosphorylation of GDP in citrate cycle

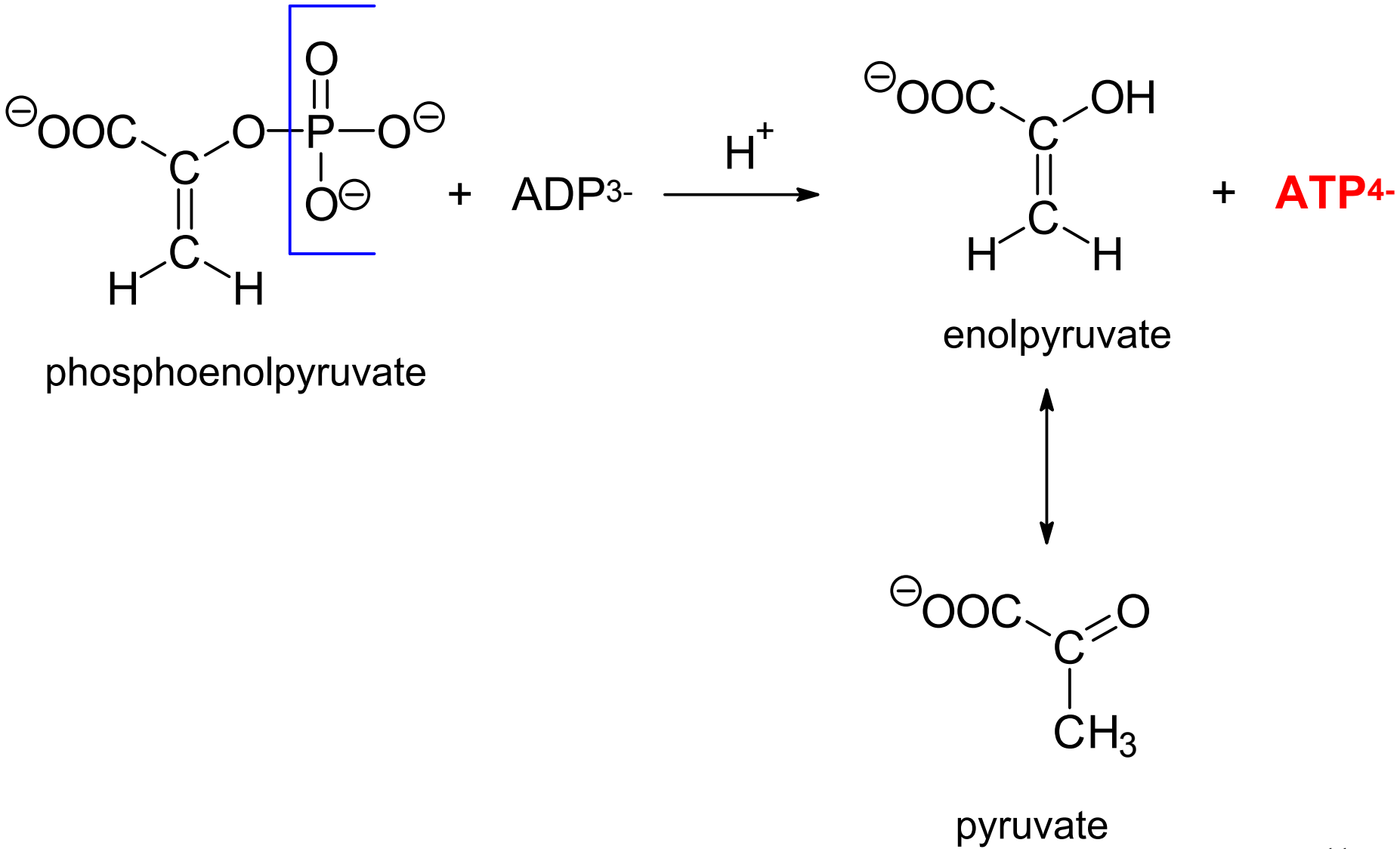
succinyl phosphate is made from succinyl-CoA + P_i



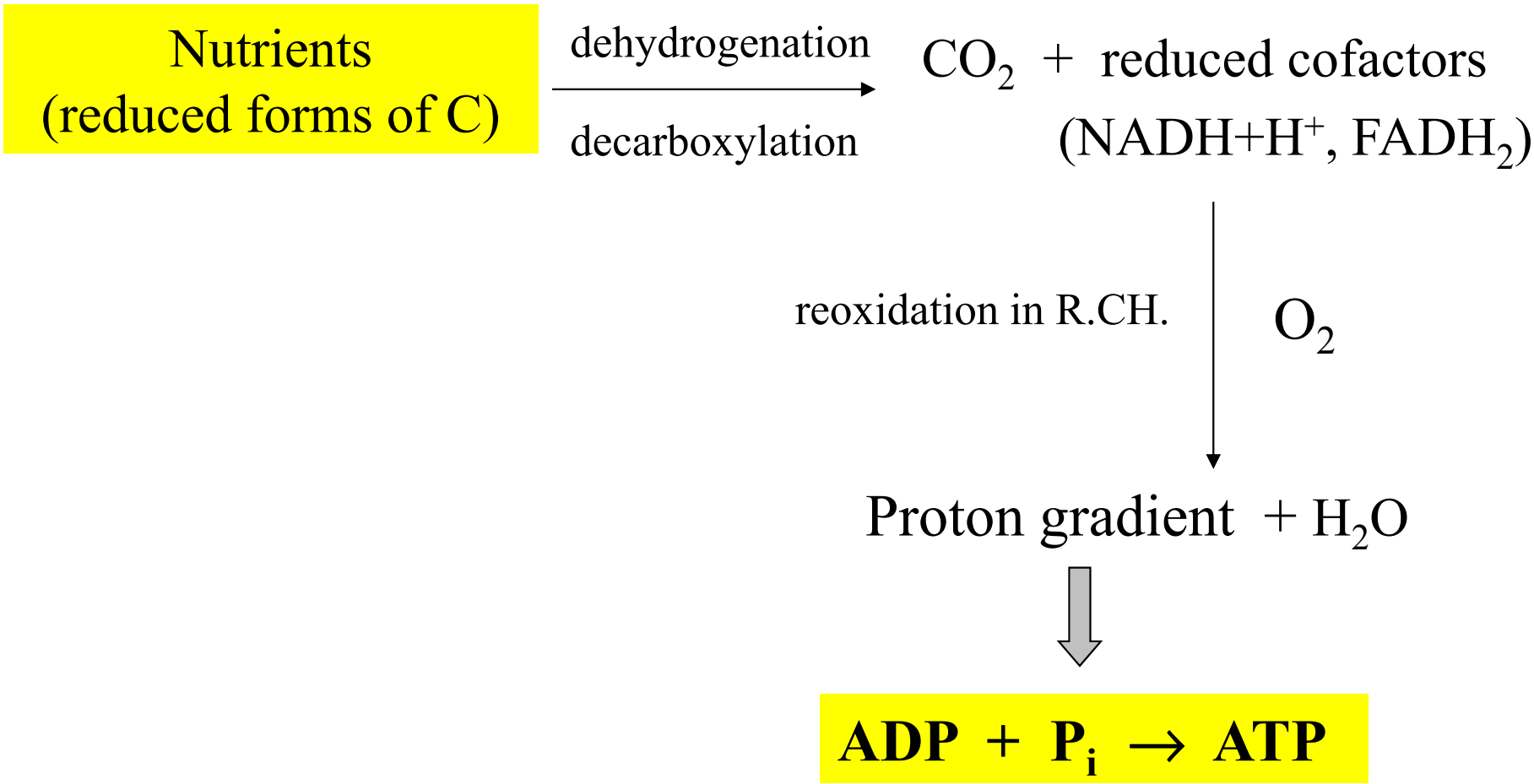
Phosphorylation of ADP by 1,3-bisphosphoglycerate



Phosphorylation of ADP by phosphoenolpyruvate



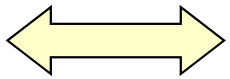
Aerobic phosphorylation follows the reoxidation of reduced cofactors in R.CH.



NADH formation in mitochondrial matrix

(substrates of the important reactions)

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



- **Oxidative decarboxylation**
  pyruvate
  2-oxoglutarate
  2-oxo acids from Val, Leu, Ile
- **Dehydrogenation of KB**
  β-hydroxybutyrate
- **Oxidative deamination**
  glutamate

NADH formation in cytoplasm

- **Glycolysis**

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

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

  (to acetaldehyde)

$FADH_2$ formation in matrix of mitochondria

- **β-Oxidation of FA**

  (dehydrogenation of saturated acyl-CoA)

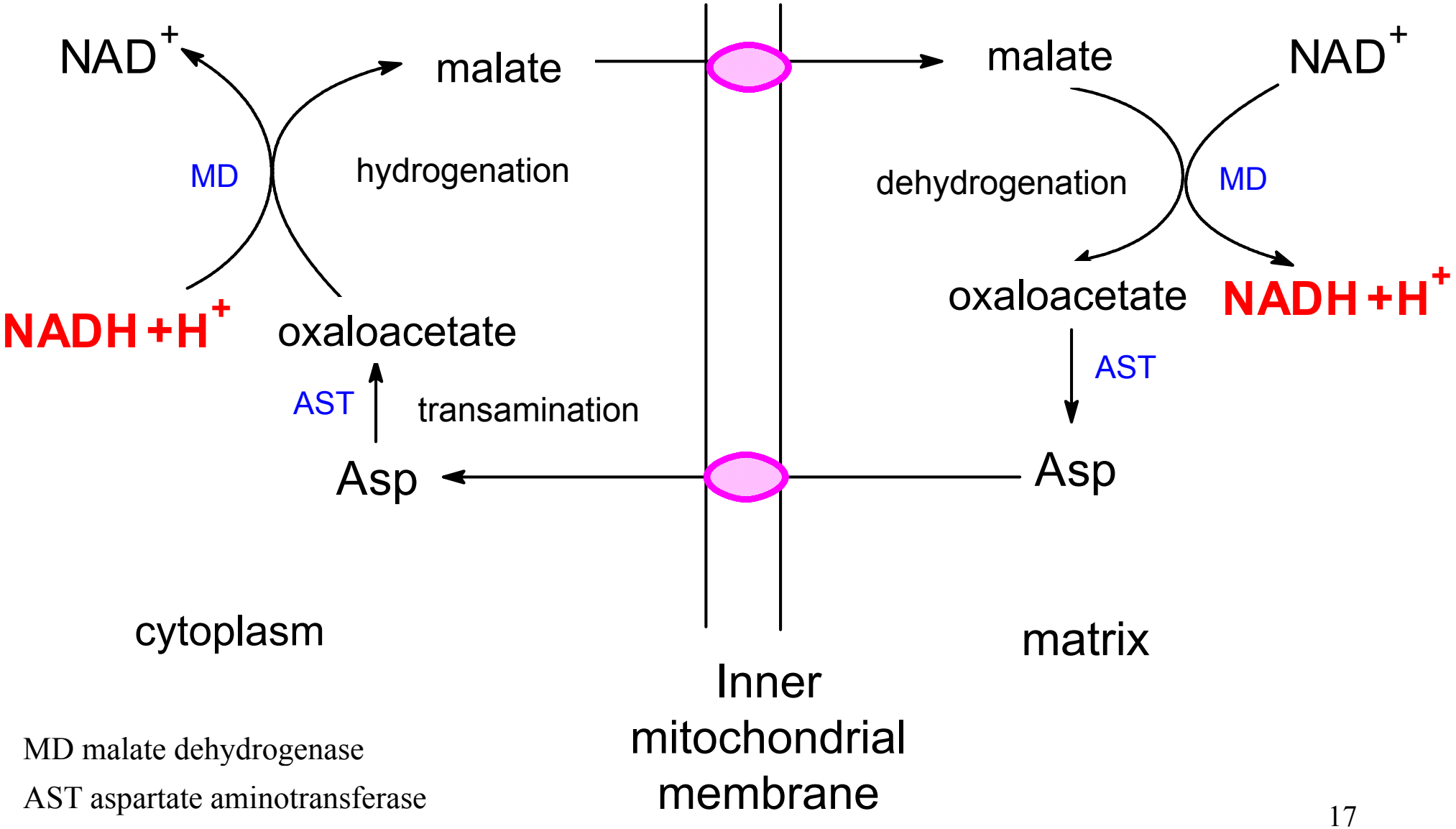
- **Citrate cycle**

  (dehydrogenation of succinate)

Transport of NADH from cytoplasm to matrix

- NADH produced in cytoplasm is transported into matrix to be reoxidized in R.CH.
- inner mitochondrial membrane is impermeable for NADH
- two shuttle systems:
- aspartate/malate shuttle (universal)
- glycerol phosphate shuttle (brain, kidney)

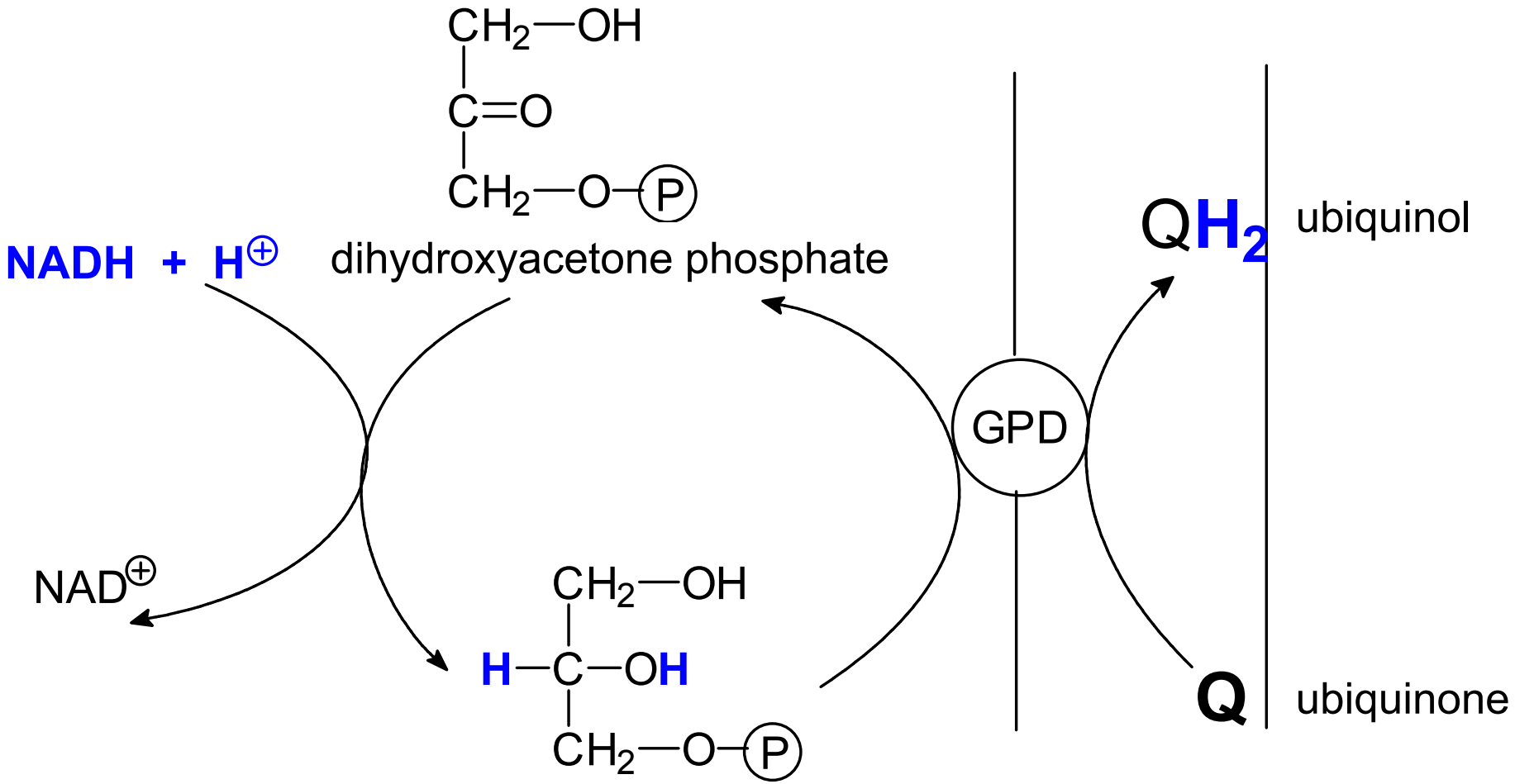
Aspartate/malate shuttle



MD malate dehydrogenase

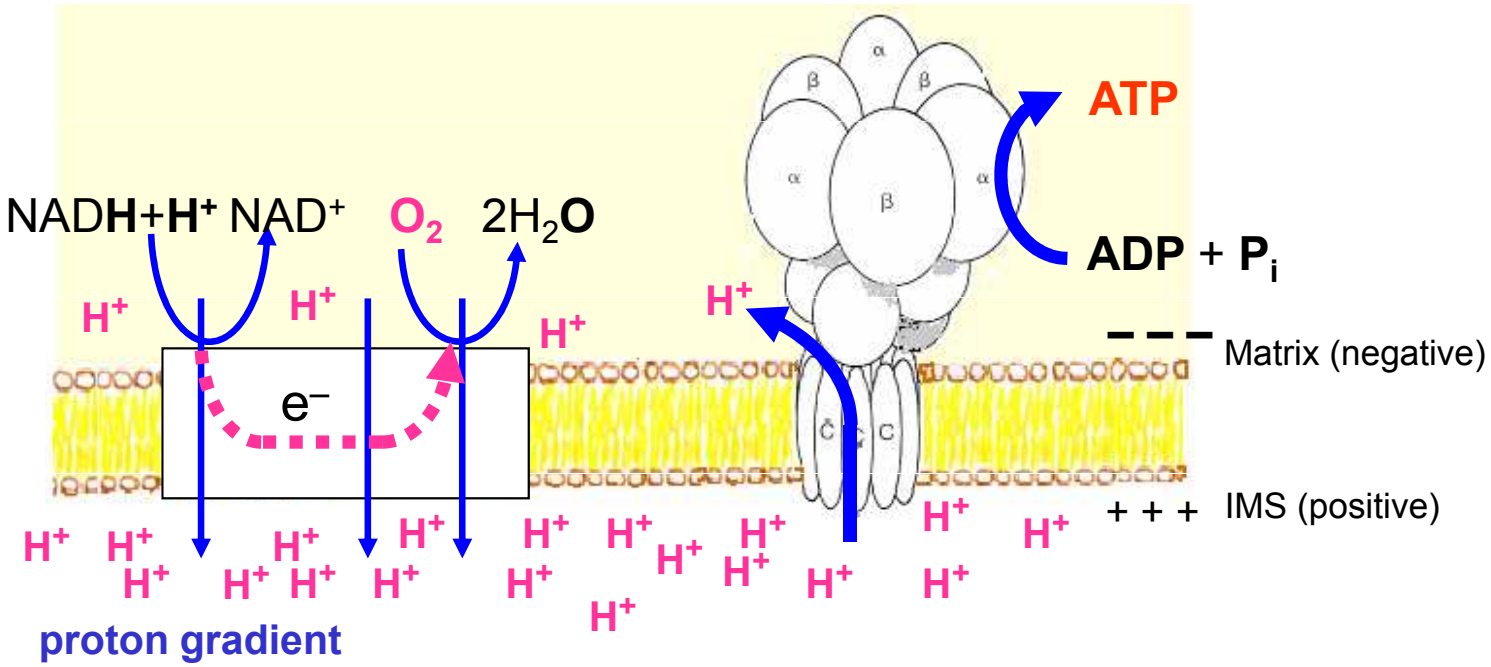
AST aspartate aminotransferase

Glycerol phosphate shuttle



GPD = glycerol 3-P dehydrogenase

Respiratory chain is the system of redox reactions in IMM which starts by the NADH oxidation and ends with the reduction of O_2 to water.



The free energy of oxidation of $NADH/FADH_2$ is utilized for pumping protons to the outside of the inner mitochondrial membrane.
The proton gradient across the inner mitochondrial membrane represents **the energy for ATP synthesis**.

Four types of cofactors in R.CH.

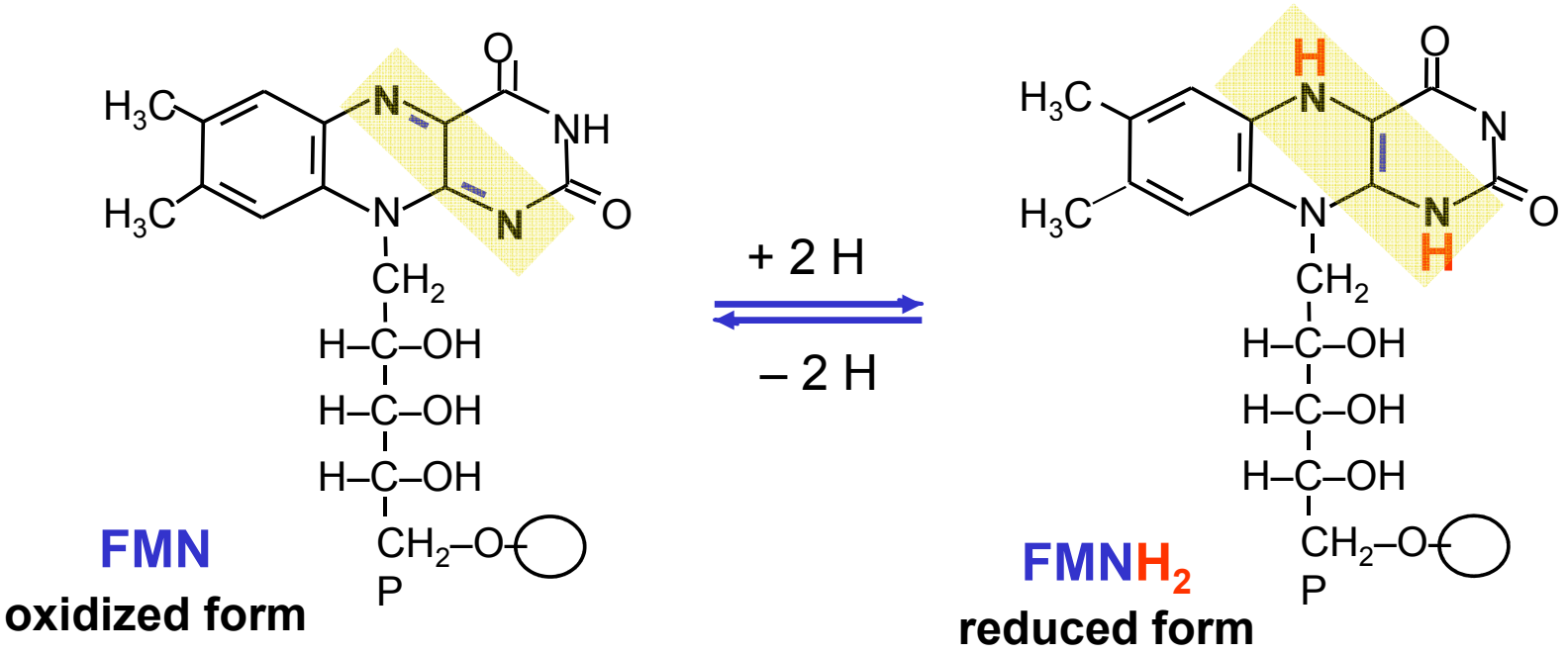
- flavine cofactors (FMN, FAD)
- non-heme iron with sulfur (Fe-S)
- ubiquinone (Q)
- heme (in cytochromes)

Distinguish:

heme (cyclic tetrapyrrol) × cytochrome (hemoprotein)

Flavoproteins

contain flavin prosthetic group as **flavin mononucleotide** (FMN, complex I) or **flavin adenine dinucleotide** (FAD, complex II):

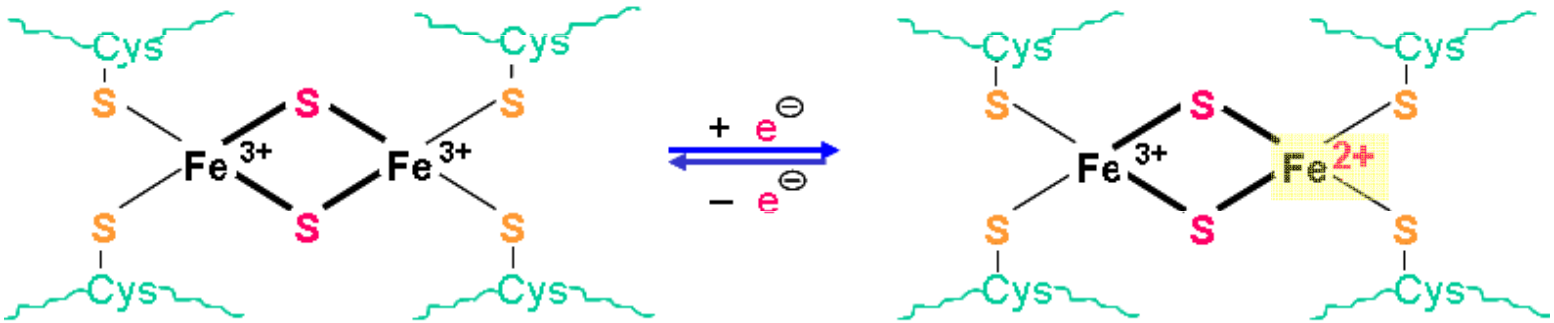


Coenzyme FMN (as well as FAD) transfers **two atoms of hydrogen**.

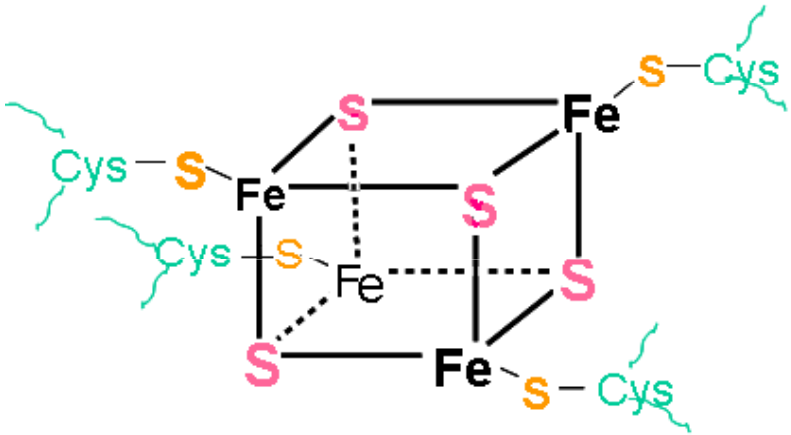
Iron-sulfur proteins (FeS-proteins, non-heme iron proteins)

Despite the different number of iron atoms present,
each cluster accepts or donates **only one electron**.

Fe_2S_2 cluster

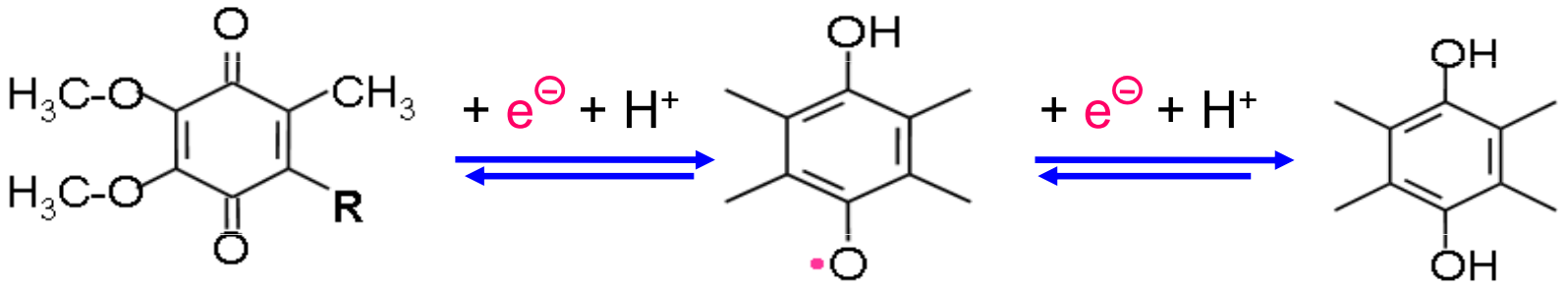


Fe_4S_4 cluster



Ubiquinone (coenzyme Q)

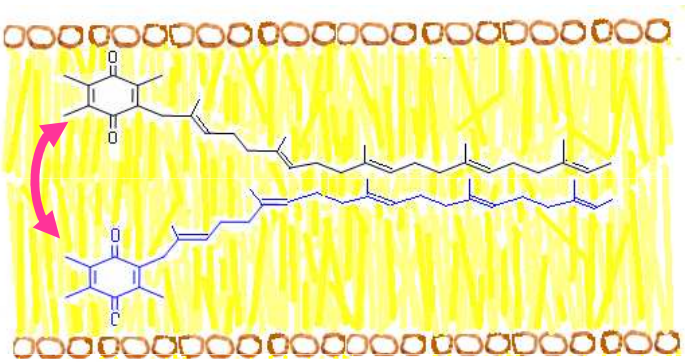
It accepts stepwise **two electrons** (one from the complex I or II and the second from the cytochrome *b*) **and two protons** (from the mitochondrial matrix), so that it is fully reduced to ubiquinol:



ubiquin**one**, **Q** — semiquinone, •QH — ubiquin**ol**, QH_2

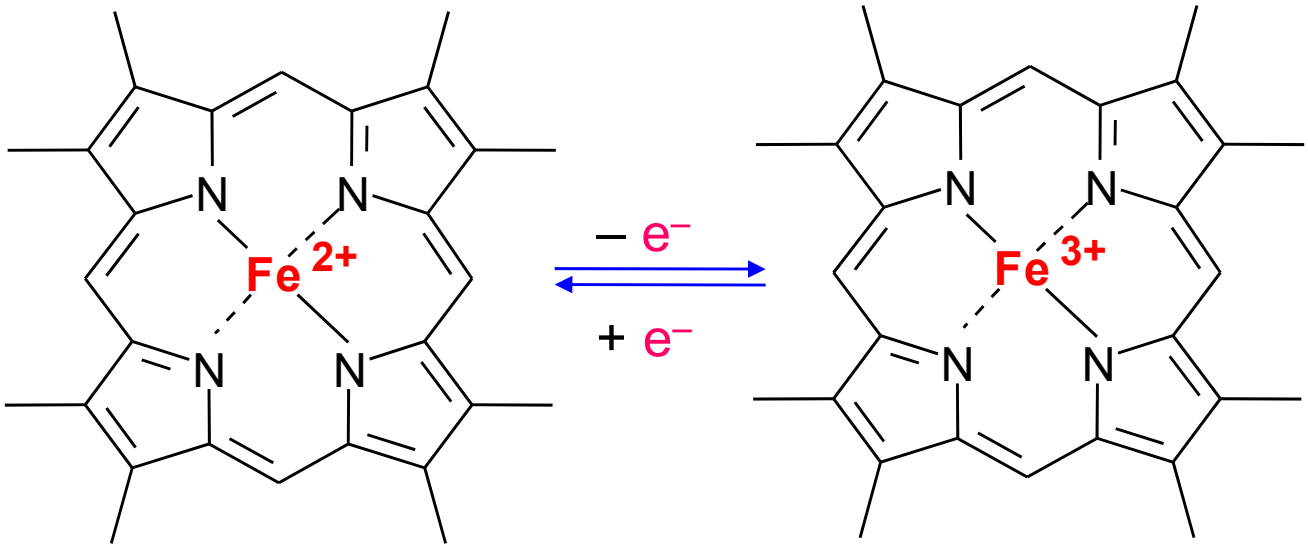
R = $-(CH_2-CH=C(CH_3)-CH_2)_{10}-H$

The very lipophilic polyisoprenoid chain is anchored within phospholipid bilayer. The ring of ubiquinone or ubiquinol (not semiquinone) moves from the membrane matrix side to the cytosolic side and translocates electrons and protons.



Cytochromes

are **heme proteins**, which are **one-electron carriers** due to reversible oxidation of the iron atom:



Mammalian cytochromes are of three types – ***a***, ***b***, ***c***. They differ in the substituents attached to the porphin ring. All these types of cytochromes occur in the mitochondrial respiratory chain.

Cytochromes type ***b*** (including cytochromes class P-450) occur also in membranes of endoplasmic reticulum and the outer mitochondrial membrane.

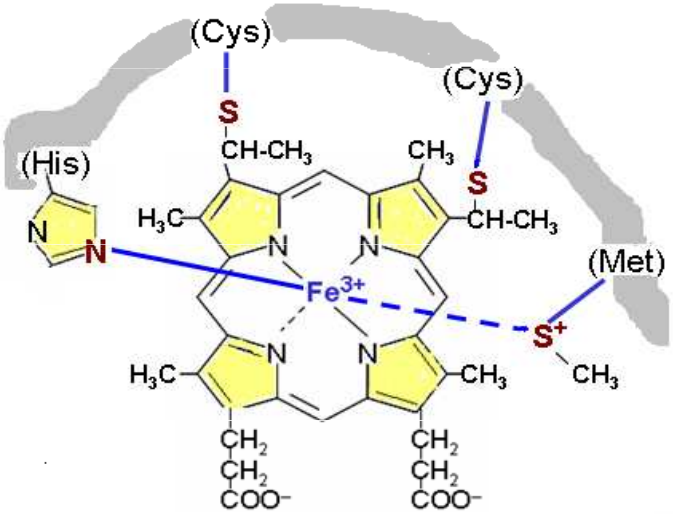
Some differences in cytochrome structures

Cytochrome *c*

central Fe ion is attached by coordination
to N-atom of His_{18} and to S-atom of Met_{80};
two vinyl groups bind covalently S-atoms of Cys_{14}+Cys_{17}.

The heme is dived deeply in the protein terciary
structure so that it is unable to bind O_2, CO.
Cyt *c* is water-soluble, peripheral protein that moves
on the outer side of the inner mitochondrial membrane.

Heme of cytochrome *c*

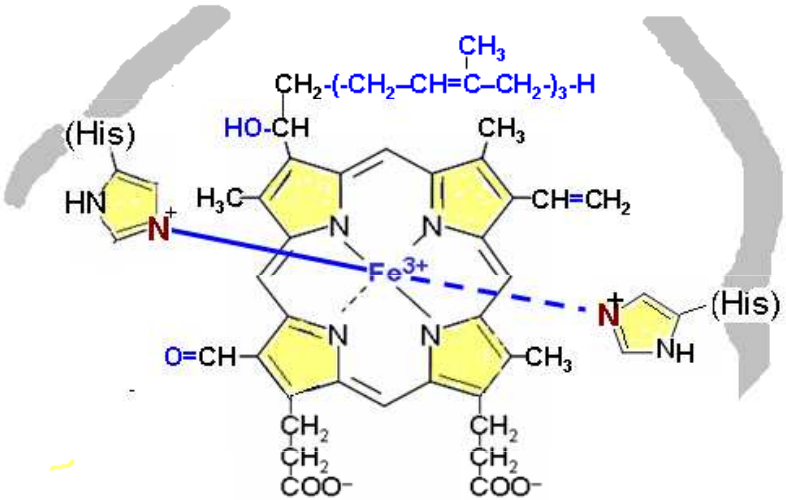


Cytochrome *aa*$_3$

central Fe ion is attached by coordination to two
His residues; one of substituents is a hydrophobic
isoprenoid chain, another one is oxidized to formyl.
The heme *a* is the accepts an electrons from
the copper centre A (two atoms Cu_A).

Its function is inhibited by carbon monoxide,
CN^-, HS^-, and N_3^- anions.

Heme *a* of cytochrome *aa*$_3$

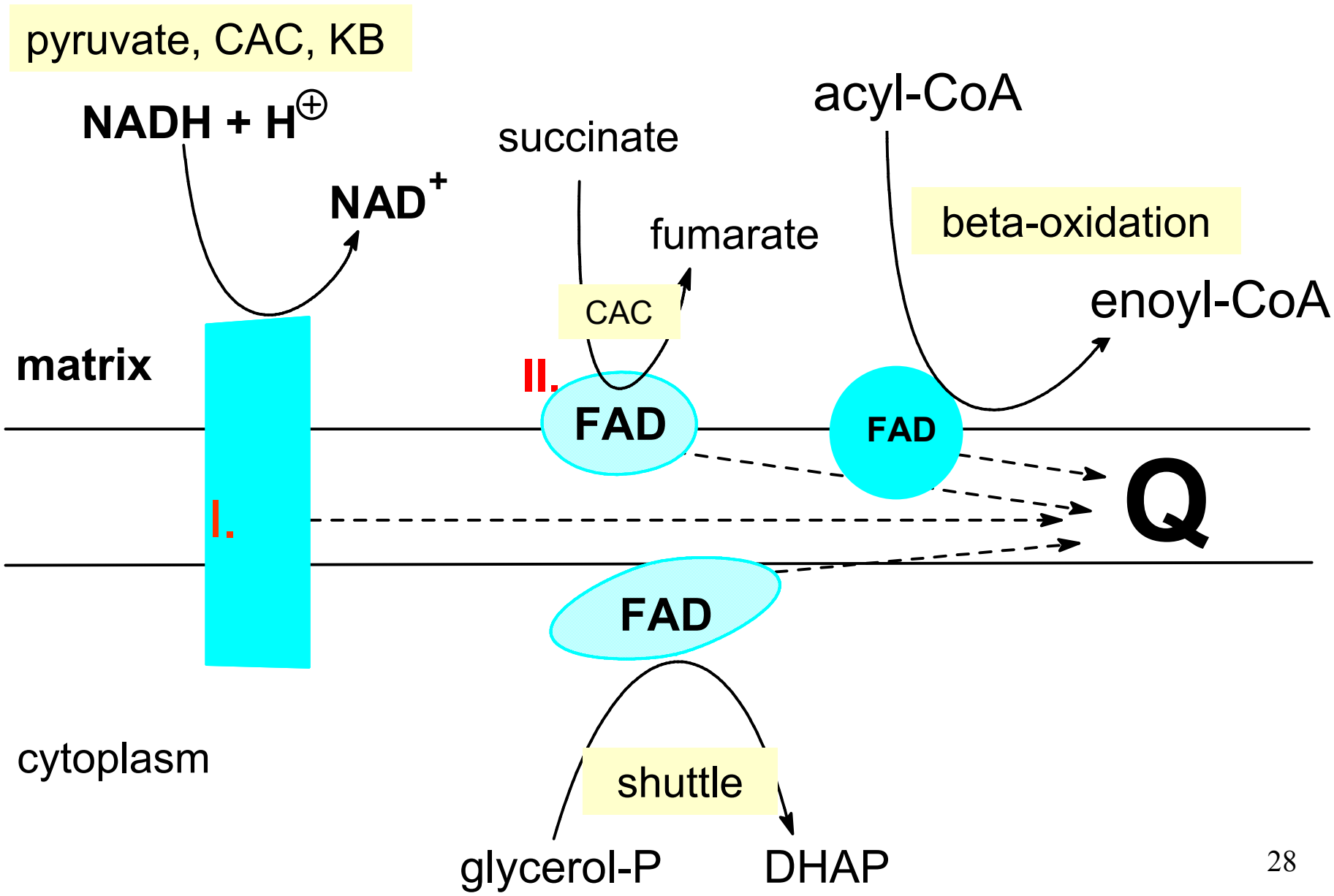


Redox pairs in the respiratory chain

Oxidized / Reduced form	$E°'$ (V)	
$NAD^+ / NADH+H^+$	-0,32	←
$FAD / FADH_2$	0,00	
Ubiquinone (Q) / Ubiquinol (QH_2)	0,10	
Cytochrome c_1 (Fe^{3+} / Fe^{2+})	0,22	
Cytochrome c (Fe^{3+} / Fe^{2+})	0,24	
Cytochrome a_3 (Fe^{3+} / Fe^{2+})	0,39	
$O_2 / 2\ H_2O$	0,82	←

- redox pairs are listed according to increasing $E^{\circ\prime}$
- they are standard values (1 mol/l), real cell values are different
- the strongest reducing agent in R.CH. is NADH
- the strongest oxidizing agent in R.CH. is O_2
- the value of potential depends on protein molecule (compare cytochromes)

Entry points for reducing equivalents in R.Ch.



Enzyme complexes in respiratory chain

No.	Name	Cofactors	Oxidation	Reduction
I.	NADH-Q oxidoreductase*	FMN, Fe-S	$NADH \rightarrow NAD^+$	$Q \rightarrow QH_2$
II.	succinate-Q reductase	FAD,Fe-S,cyt *b*	$FADH_2 \rightarrow FAD$	$Q \rightarrow QH_2$
III.	Q-cytochrome-*c*-reductase	Fe-S, cyt *b*, c_1	$QH_2 \rightarrow Q$	cyt $c_{ox} \rightarrow$ cyt c_{red}
IV.	cytochrome-*c*-oxidase	cyt *a*, a_3, Cu	cyt $c_{red} \rightarrow$ cyt c_{ox}	$O_2 \rightarrow 2\ H_2O$

* also called NADH dehydrogenase

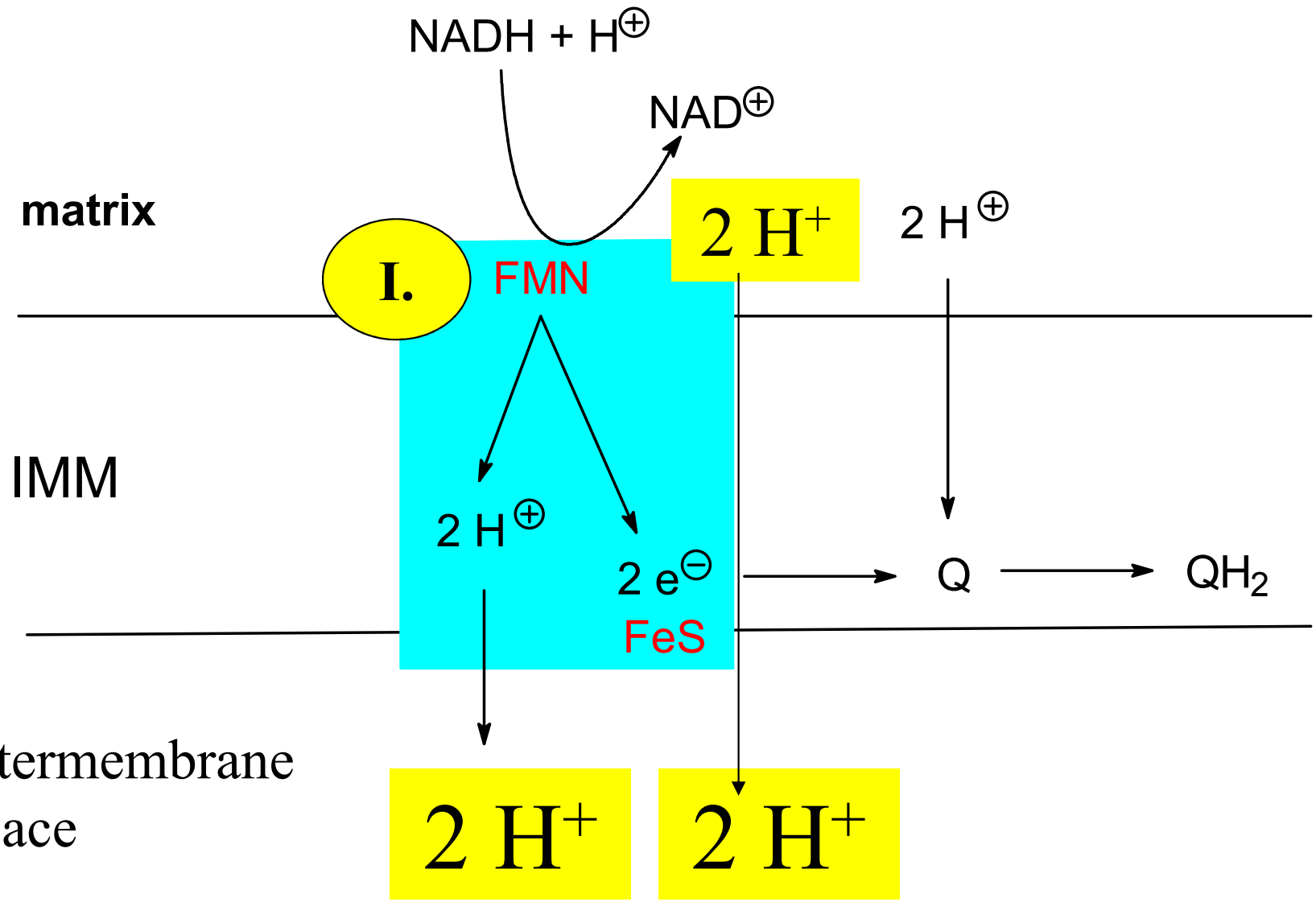
Complex I oxidizes NADH and reduces ubiquinone (Q)

FMN, Fe-S

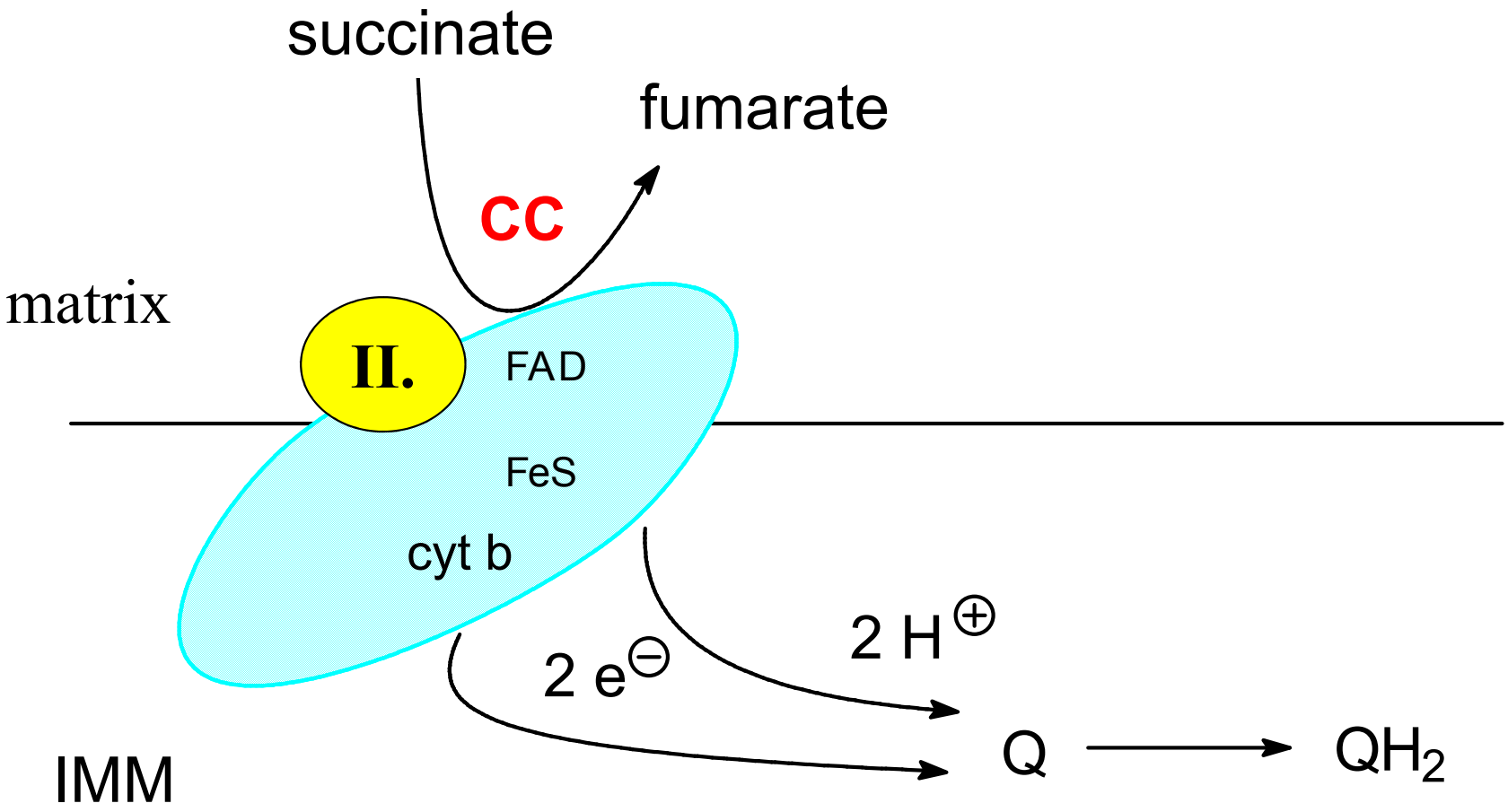
$$NADH+H^+ + Q + \mathbf{4\ H^+_{matrix}} \rightarrow NAD^+ + QH_2 + \mathbf{4\ H^+_{ims}}$$

The four H^+ are translocated from matrix to intermembrane space (ims)

Complex I oxidizes NADH and translocates 4 H^+ into intermembrane space

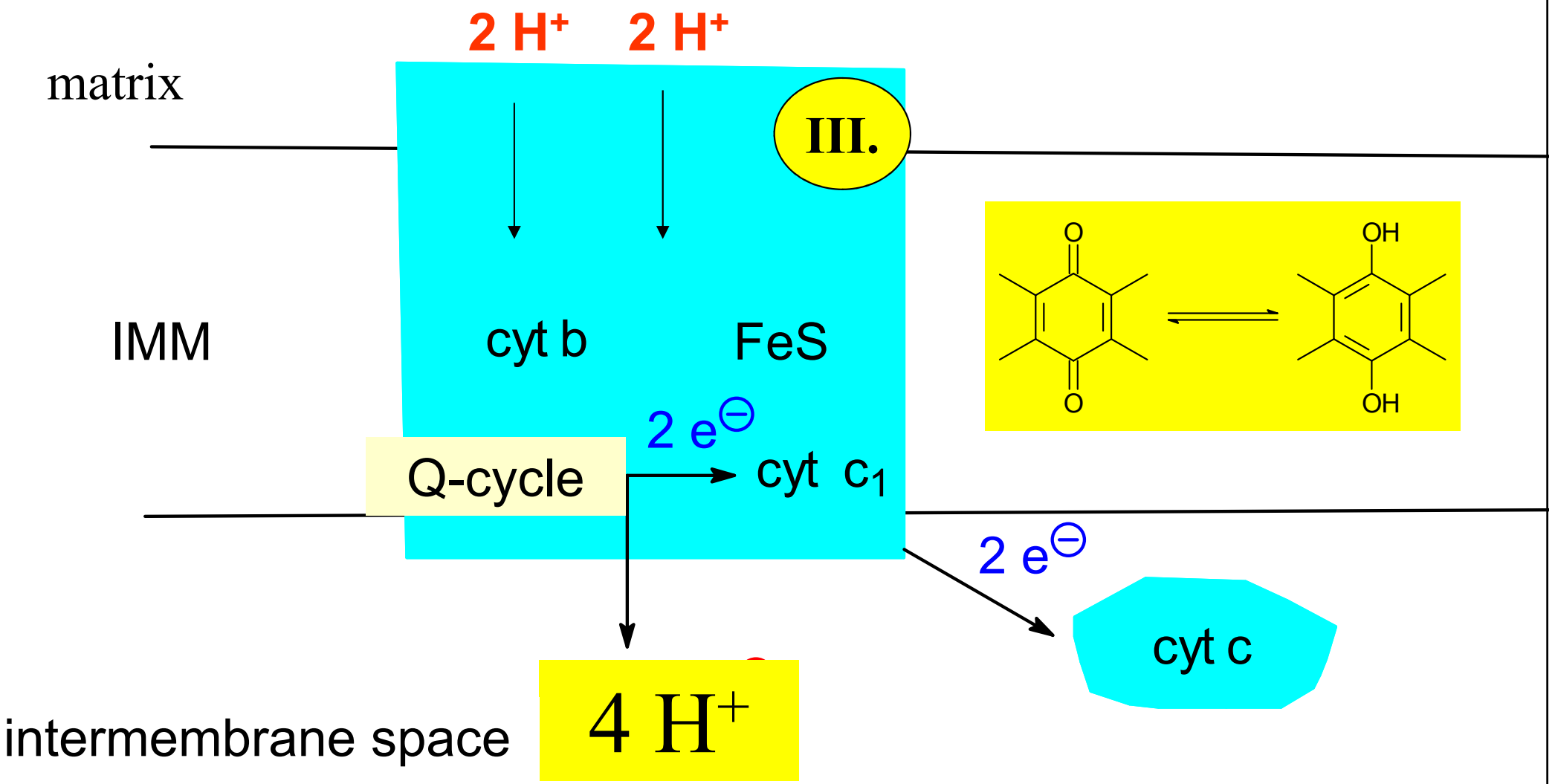


Complex II (independent entry) oxidizes $FADH_2$ from citrate cycle and reduces ubiquinone

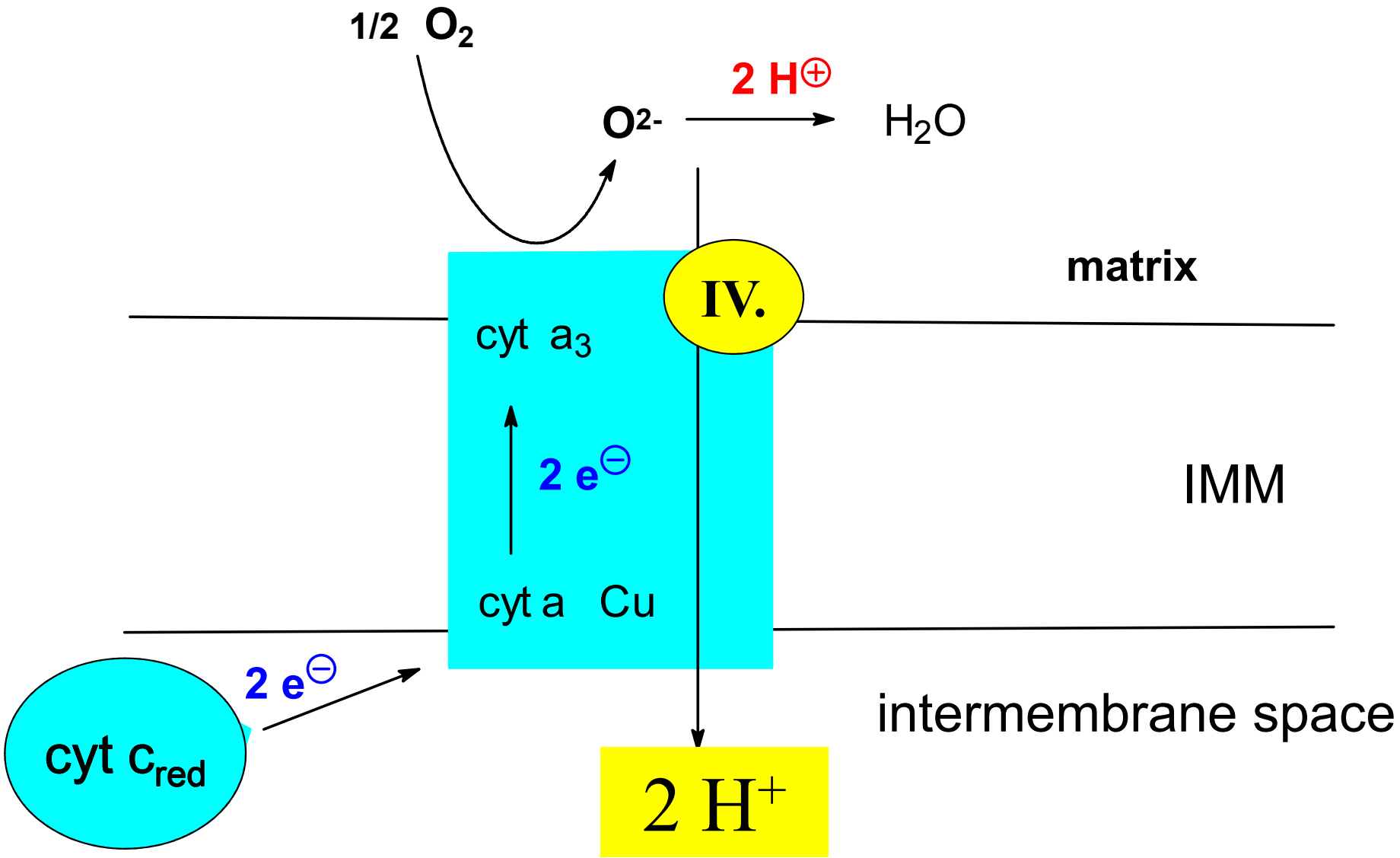


Complex II, in contrast to complex I, does not transport H^+ across IMM. Consequently, less proton gradient (and less ATP) is formed from the oxidation of $FADH_2$ than from NADH.

Complex III oxidizes QH_2, reduces cytochrome *c*, and translocates 4 H^+ across IMM



Complex IV oxidizes cyt c_{red} and two electrons reduce monooxygen ($½O_2$)



Complex IV:
real process is four-electrone reduction of dioxygen

partial reaction (redox pair):

$$O_2 + 4\ e^- + 4\ H^+ \rightarrow 2\ H_2O$$

complete reaction:

$$4\ \text{cyt-}Fe^{2+} + O_2 + 8\ H^+_{matrix} \rightarrow 4\ \text{cyt-}Fe^{3+} + 2\ H_2O + 4\ H^+_{ims}$$

metabolic water

For every 2 electrons, 2 H^+ are pumped into intermembrane space

Three times translocated protons create electrochemical H^+ gradient across IMM

It consists of two components:

1) difference in **pH**, $\Delta G = RT \ln ([H^+]_{out}/[H^+]_{in}) = 2.3\ RT(pH_{out} - pH_{in})$

2) difference in **electric potential** ($\Delta \Psi$, negative inside), depends not only on protons, but also on concentrations of other ions, $\Delta G = -n F \Delta \Psi$

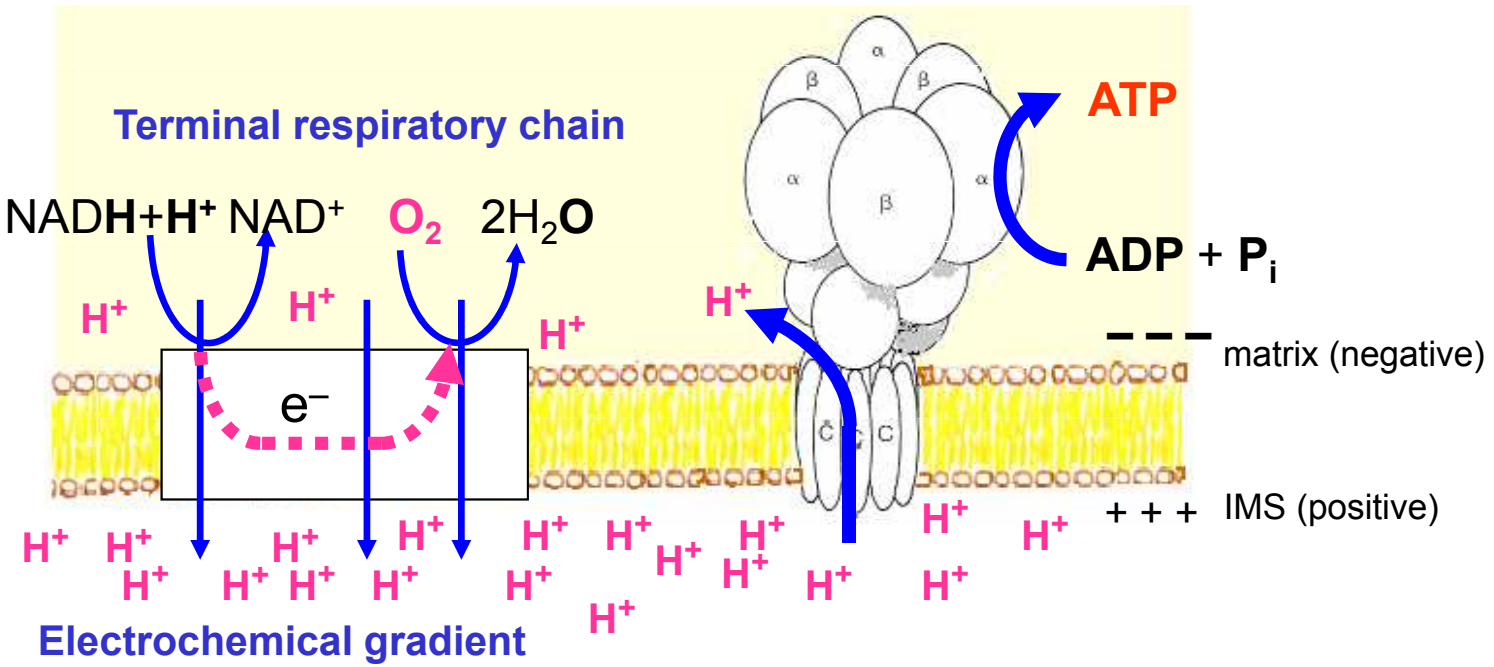
The **proton motive force Δp** is the quantity expressed in the term of potential (milivolts per mole of H^+ transferred): $\Delta p = -\Delta G / nF = \Delta \Psi + 60\ \Delta pH$.

Utilization of proton motive force

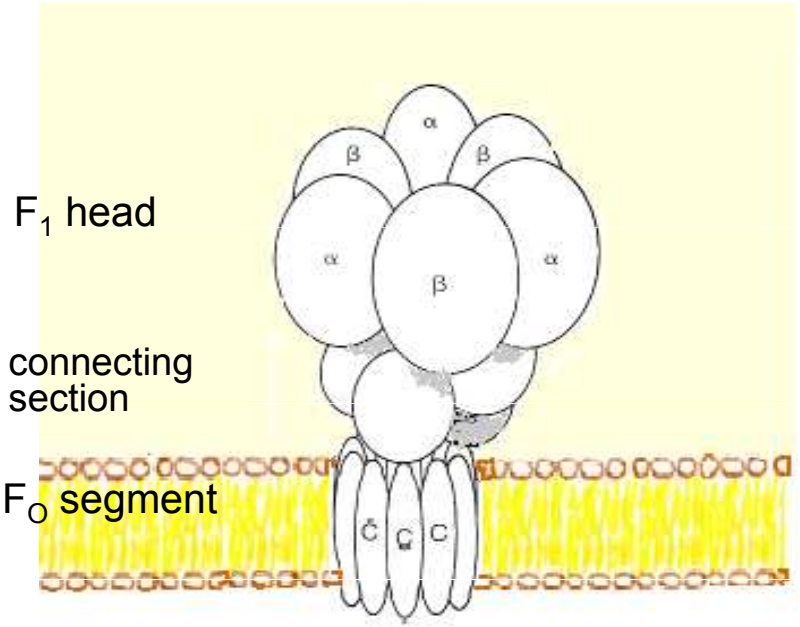
- synthesis of ATP = aerobic phosphorylation
- heat production - especially in brown adipose tissue
- active transport of ions and metabolites across IMM

Aerobic phosphorylation of ADP by ATP synthase

The endergonic phosphorylation of ADP is driven by the flux of protons back into the matrix along the electrochemical gradient through ATP synthase.



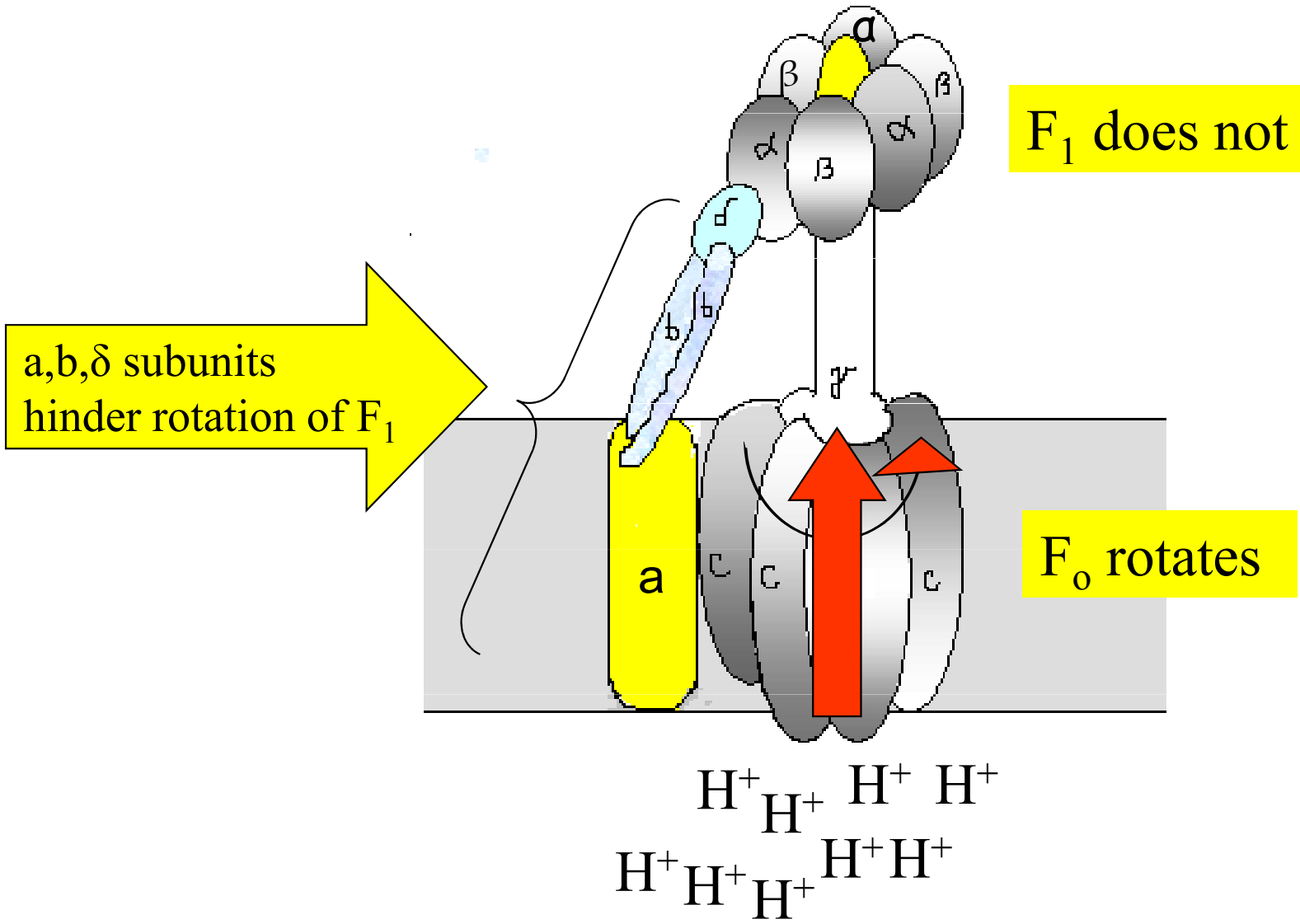
ATP synthase consists of three parts



1) F_1 complex projects into the matrix, 5 subunit types (α_3, β_3, γ, δ, ε) catalyze the ATP synthesis
2) connecting section
3) F_o inner membrane component, several *c*-units form a rotating proton channel

ATP-synthase is a molecular rotating motor: 3 ATP/turn

$$ADP + P_i \rightarrow ATP$$



Stoichiometry of ATP synthesis is not exactly recognized

- transfer of 2 e^- from NADH to ½ O_2 .... **3 ATP**
- transfer of 2 e^- from $FADH_2$ to ½ O_2 .... **2 ATP**

new research data indicate somewhat lower values (see Harper)

- transfer of 2 e^- from NADH to ½ O_2 .... 2.5 ATP
- transfer of 2 e^- from $FADH_2$ to ½ O_2 .... 1.5 ATP

Control of the oxidative phosphorylation

Production of ATP is strictly coordinated so that ATP is never produced more rapidly than necessary.

Synthesis of ATP depends on:

– supply of substrates (mainly $NADH+H^+$)

– supply of dioxygen

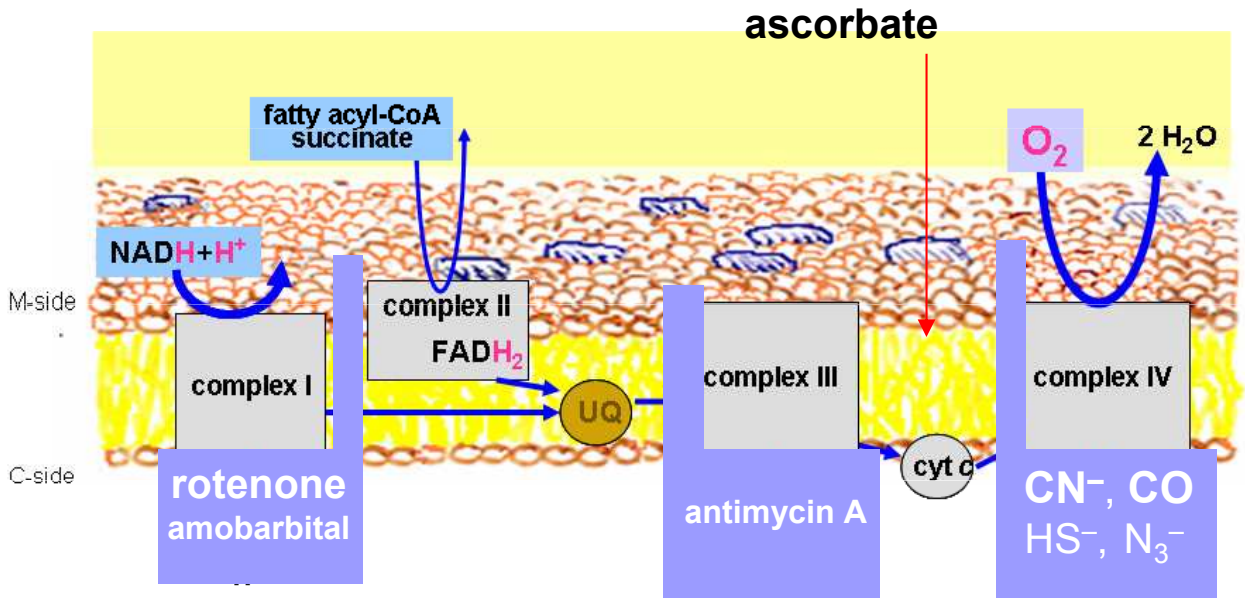
– the energy output of the cell; hydrolysis of ATP increases the concentration of ADP in the matrix, which activates ATP production. This mechanism is called respiratory control.

Inhibitors of the terminal respiratory chain

Complex I is blocked by an insecticide rotenone. A limited synthesis of ATP exists due to electrons donated to ubiquinone through complex II.

Complex III is inhibited by antimycin A – complexes I and II become reduced, complexes III and IV remain oxidized. Ascorbate restores respiration, because it reduces cyt *c*.

Complex IV is blocked by carbon monoxide, cyanide ion, HS^- (sulfane intoxication), azide ion N_3^-. Respiration is disabled.



Cyanide poisoning

occurs after ingestion of alkali cyanides or inhalation of hydrogen cyanide.
Bitter almonds or apricot kernels contain amygdalin, which can release HCN.

Cyanide ion, besides inhibition of cytochrome *c* oxidase, binds with high affinity onto methemoglobin (Fe^{3+}).

The lethal dose LD_{50} of alkali cyanide is about 250 mg. Symptoms - dizziness, gasping for breath, cramps, and unconsciousness follow rapidly.

Antidotes may be effective, when applied without any delay:
Hydroxycobalamin (a semisynthetic compound) exhibits high affinity to CN^- ions, binds them in the form of harmless cyanocobalamin (B_{12}).
Sodium nitrite $NaNO_2$ or **amyl nitrite** oxidize hemoglobin (Fe^{II}) to methemoglobin (Fe^{III}), which is not able to transport oxygen, but binds CN^- and may so prevent inhibition of cytochrome *c* oxidase.
Sodium thiosulfate $Na_2S_2O_3$, administered intravenously, can convert cyanide to the relatively harmless thiocyanate ion: $CN^- + S_2O_3^{2-} \rightarrow SCN^- + SO_3^{2-}$.

Carbon monoxide poisoning

CO binds primarily to hemoglobin (Fe^{II}) and inhibits oxygen transport, but it also blocks the respiratory chain by inhibiting cytochrome oxidase (complex IV).

Oxygenotherapy improves blood oxygen transport, administered **methylene blue** serves as acceptor of electrons from complex III so that limited ATP synthesis can continue.

Uncoupling of the respiratory chain and phosphorylation

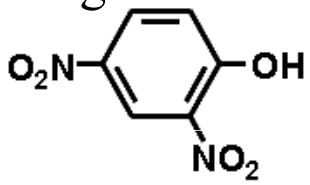
is the **wasteful oxidation of substrates without concomitant ATP synthesis:** protons are pumped across the membrane, but they re-enter the matrix using some other way than that represented by ATP synthase.

The free energy derived from oxidation of substrates appears as heat..

There are **four types** of artificial or natural uncouplers:

1 **"True" uncouplers** – compounds that transfer protons through the membrane.

A typical uncoupler is **2,4-dinitrophenol** (DNP):



DNP is very toxic, the lethal dose is about 1 g.

More than 80 years ago, the long-term application of small doses (2.5 mg/kg) was recommended as a "reliable" drug in patients seeking to lose weight. Its use has been banned, because hyperthermia and toxic side effect (with fatal results) were excessive.

2 **Ionophors** that do not disturb the chemical potential of protons, but diminish the electric potential $\Delta\Psi$ by enabling free re-entry of K^+ (e.g. valinomycin) or both K^+ and Na^+ (e.g. gramicidin A).

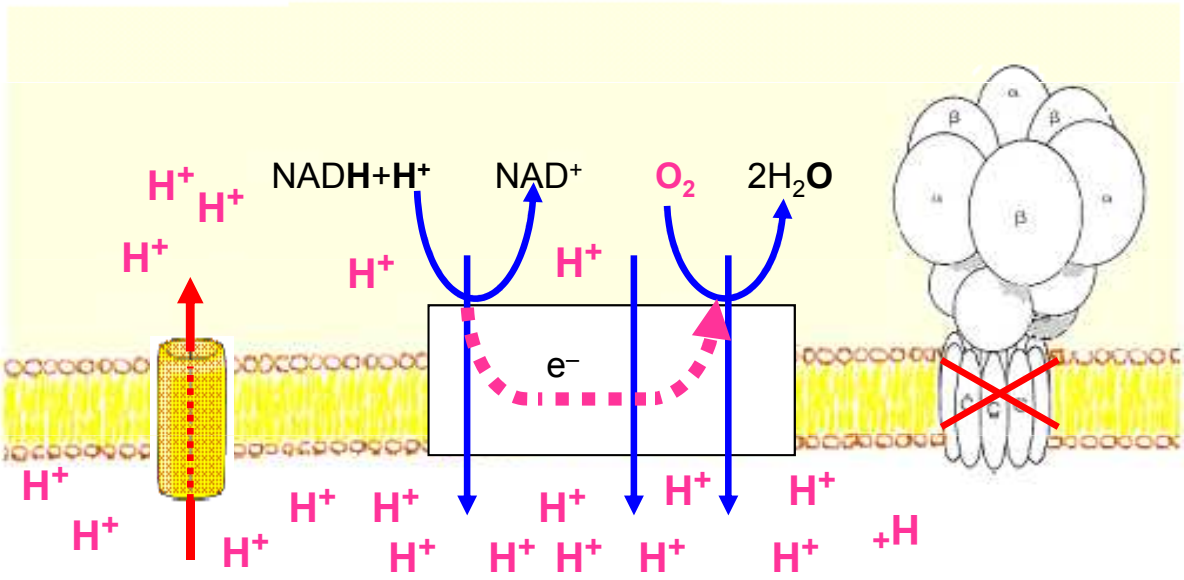
3 **Inhibitors of ATP synthase** – oligomycin.

4 **Inhibitors of ATP/ADP translocase** like unusual plant and mould toxins bongkrekic acid (irreversibly binds ADP onto the translocase) and atractylate (inhibits binding of ATP to the translocase). ATP synthase then lacks its substrate.

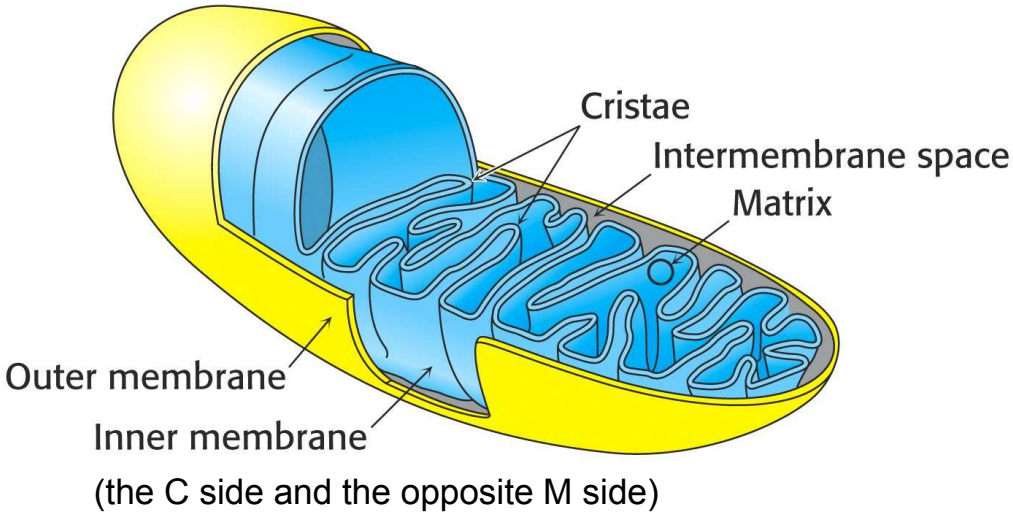
Thermogenin is a natural uncoupler

is a inner mitochondrial membrane protein that transports protons back into the matrix, bypassing so ATP synthase.

It occurs in **brown adipose tissue** of newborn children and hibernating animals, which spend the winter in a dormant state.



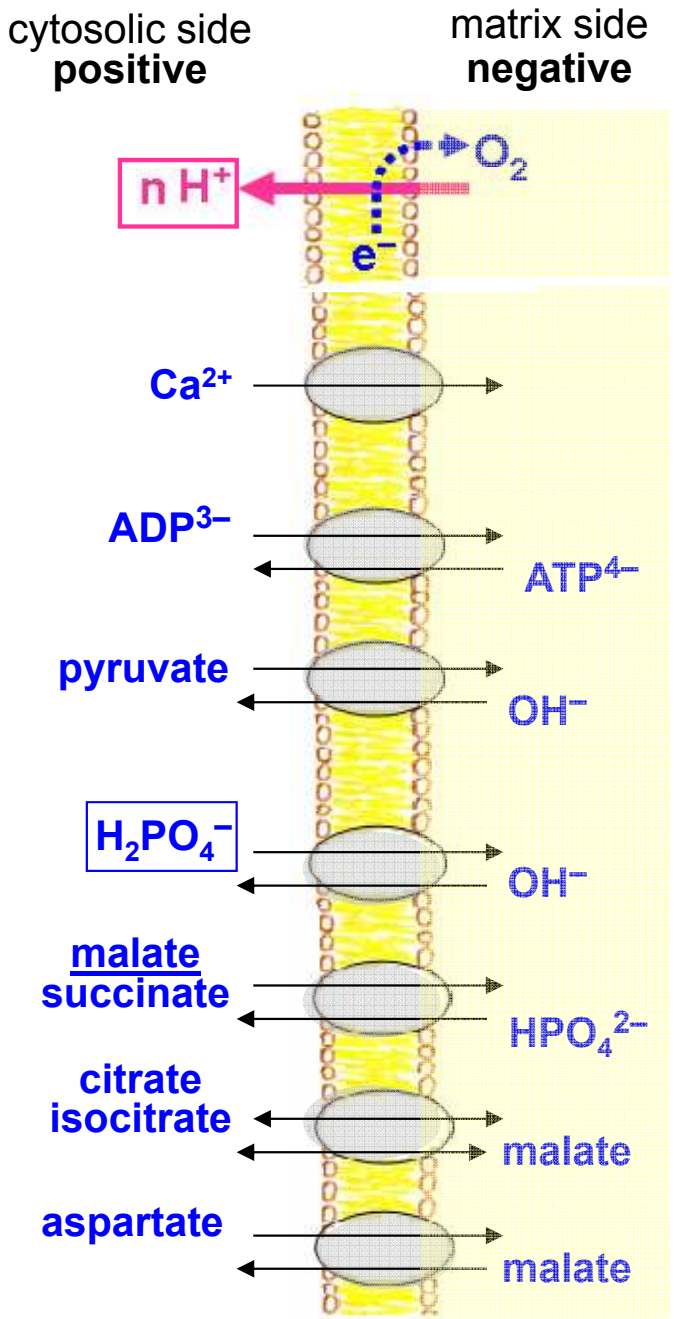
Mitochondrial metabolite transport



The outer membrane is quite permeable for small molecules and ions – it contains many copies of **mitochondrial porin** (voltage-dependent anion channel, VDAC).

The inner membrane is intrinsically impermeable to nearly all ions and polar molecules, but there are many **specific transporters** which shuttles **metabolites** (e.g. pyruvate, malate, citrate, ATP) and **protons** (terminal respiratory chain and ATP synthase) across the membrane.

Transport through the inner mitochondrial membrane



Free diffusion of O_2, CO_2, H_2O, NH_3

Primary active H^+ transport forms the proton motive force (the primary gradient)

Secondary active transports driven by a H^+ gradient and dissipating it:

ATP/ADP translocase

pyruvate transporter

phosphate permease
– forms a (secondary) phosphate gradient

dicarboxylate carrier

tricarboxylate carrier

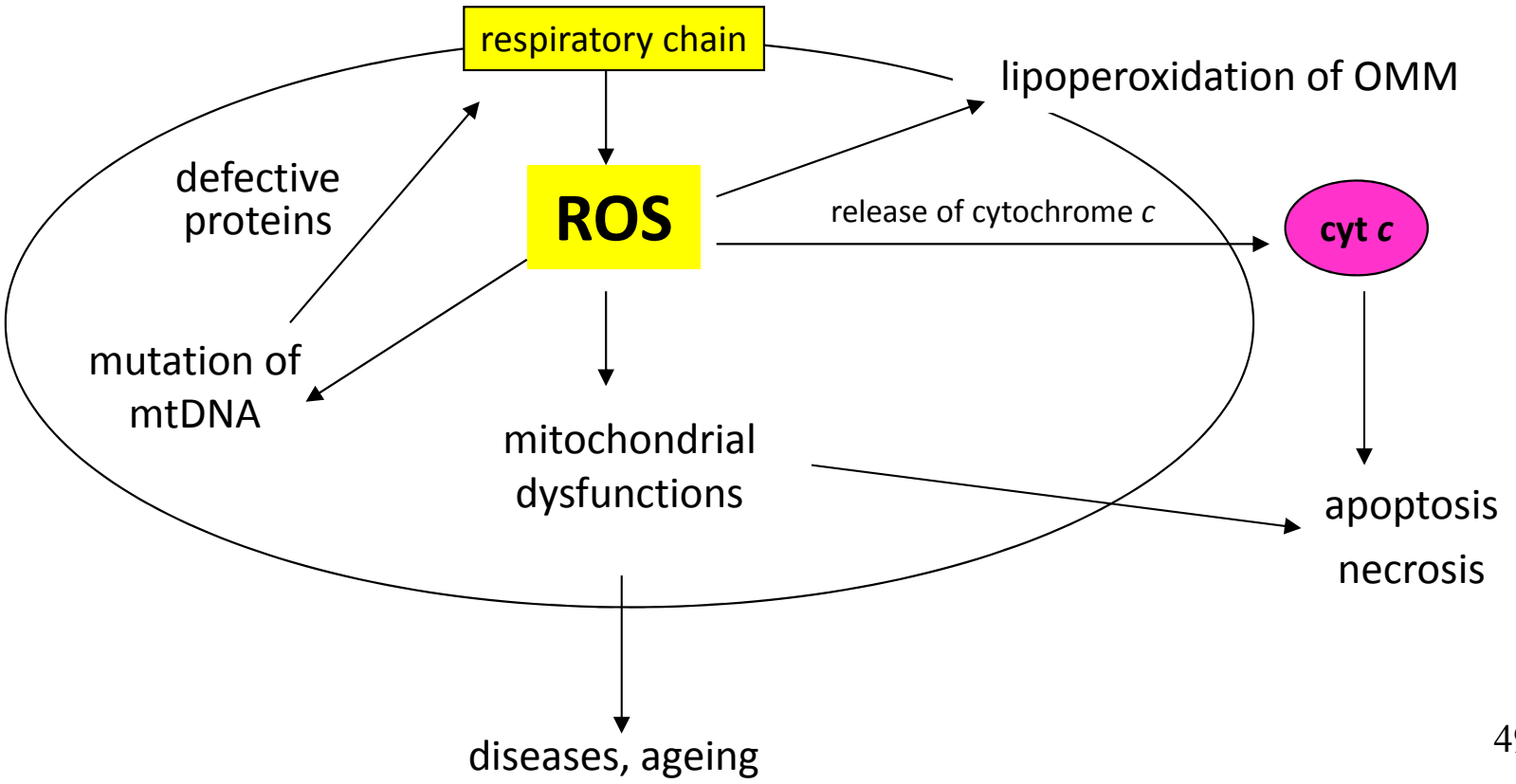
the malate shuttle for NADH + H^+

Mitochondria and apoptosis

- apoptosis is a controlled process of cell death with minimal effect on surrounding tissue
- apoptosis is important for physiological tissue turnover
- apoptosis is regulated by a number of cell signals
- regulatory protein family Bcl-2 (B-cell lymphoma 2)
- some proteins are anti-apoptotic (Bcl-xl), other pro-apoptotic (Bax, Bak)
- Bax and Bak proteins oligomerize and make a pore in outer mitochondrial membrane
- cytochrome c is released into cytosol, binds with inactive caspases and other pro-apoptotic factors - creates **apoptosome** - and triggers the executive phase of apoptosis (caspase cascade)

Mitochondria and oxidative stress

- about 98 % of O_2 is consumed in respiratory chain for the complete reduction to water (cytochrome-*c*-oxidase)
- however, other partly reduced oxygen species are also produced
- they are called reactive oxygen species (ROS)
- mainly in compl. I, III, especially, if electron trasport is slowned down or reversed
- mitochondria contains a number of antioxidants (GSH, QH_2, superoxide dismutase)



Reactive oxygen species in human body

Radicals	Neutral / Anion / Cation
Superoxide $\cdot O_2^-$	Hydrogen peroxide H-O-O-H
Hydroxyl radical ·OH	Hydroperoxide* R-O-O-H
Peroxyl radical* ROO·	Hypochlorous acid HClO
Alkoxyl radical RO·	Singlet oxygen $^1O_2$
Hydroperoxyl radical HOO·	Peroxynitrite $ONOO^-$
Nitric oxide NO·	Nitronium NO_2^+

* Typically phospholipid-PUFA derivatives during lipoperoxidation:

PUFA-OO·, PUFA-OOH

Superoxide anion-radical $\bullet O_2^-$

- One-electrone reduction of dioxygen

$$O_2 + e^- \rightarrow \bullet O_2^-$$

[one redox pair]

Superoxide formation

- **Respiratory burst** (in neutrophils)

  $2\ O_2 + \ NADPH \longrightarrow 2\ \bullet O_2^- + \ NADP^+ + H^+$

- **Spontaneous oxidation of heme proteins**

  $heme\text{-}Fe^{2+} + \ O_2 \longrightarrow heme\text{-}Fe^{3+} + \bullet O_2^-$

[complete redox reactions, combinations of two redox pairs]

Radical •OH is the most reactive species; it is formed from superoxide and hydrogen peroxide

$$\bullet O_2^- + H_2O_2 \rightarrow O_2 + OH^- + \bullet OH$$

Catalyzed by reduced metal ions (Fe^{2+}, Cu^{+})

(Fenton reaction)

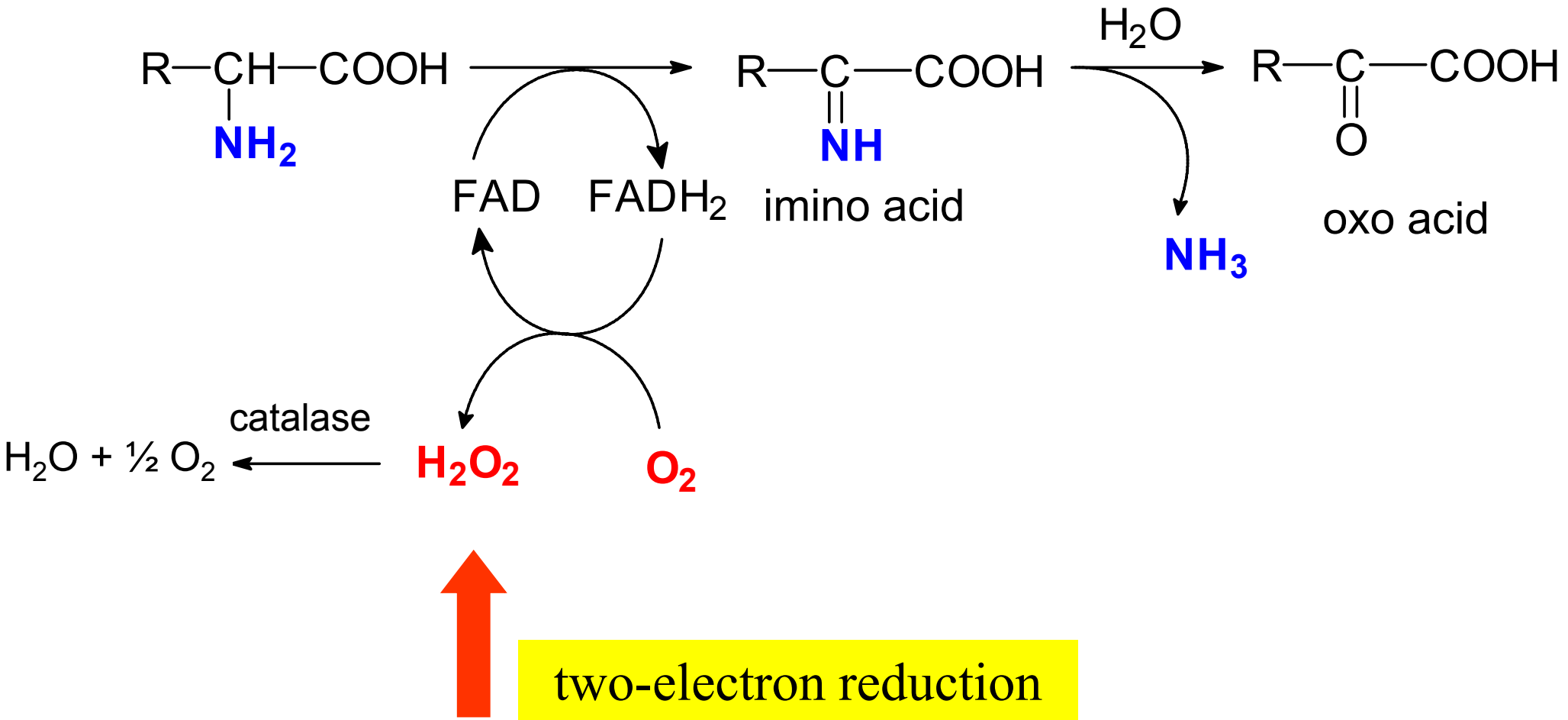
Singlet oxygen $^1O_2$

- excited form of triplet dioxygen
- formed after absorption of light by some compounds (porphyrins)

$$^3O_2 \rightarrow {}^1O_2$$

for electron configuration see Medical Chemistry I, p. 18

Hydrogen peroxide H_2O_2 is a side product in the deamination of certain amino acids



Xanthin oxidase reaction produces hydrogen peroxide

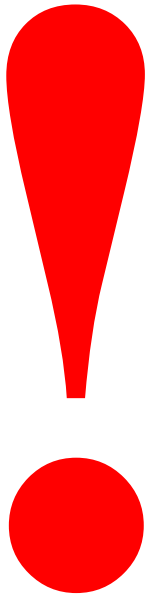
hypoxanthin + O_2 + H_2O $\rightarrow$ xanthin + **H_2O_2**

xanthin + O_2 + H_2O $\rightarrow$ uric acid + **H_2O_2**

most tissues, mainly liver

Compare: reduction of dioxygen

Type of reduction	Redox pair
Four-electron	$O_2 + 4\ e^- + 4\ H^+ \rightarrow 2\ H_2O$
One-electron	$O_2 + e^- \rightarrow \cdot O_2^-$
Two-electron	$O_2 + 2\ e^- + 2\ H^+ \rightarrow H_2O_2$



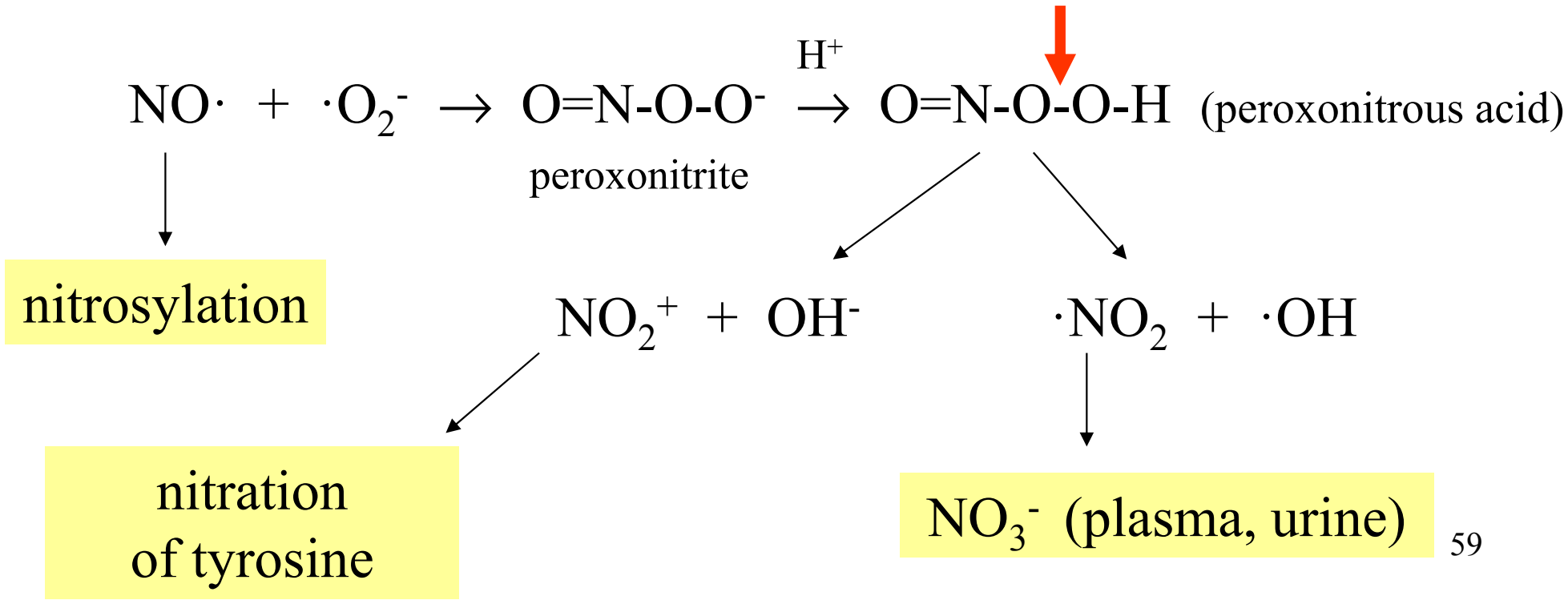
Hypochlorous acid HClO

- in some neutrophils
- myeloperoxidase reaction
- HClO has strong oxidative and bactericidal effects

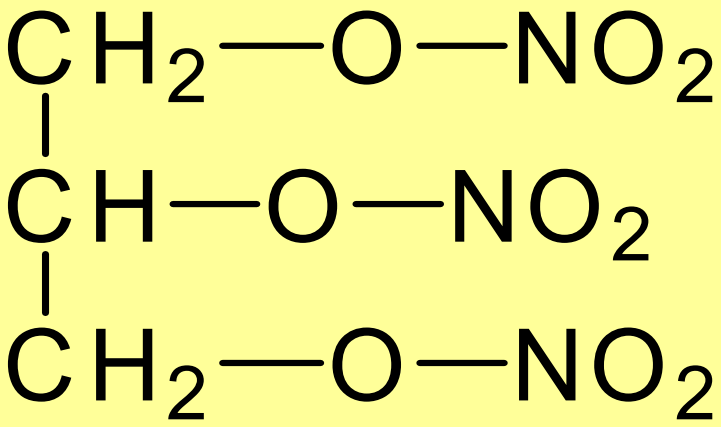
$$H_2O_2 + Cl^- + H^+ \rightarrow HClO + H_2O$$

Nitric oxide NO· is released from arginine

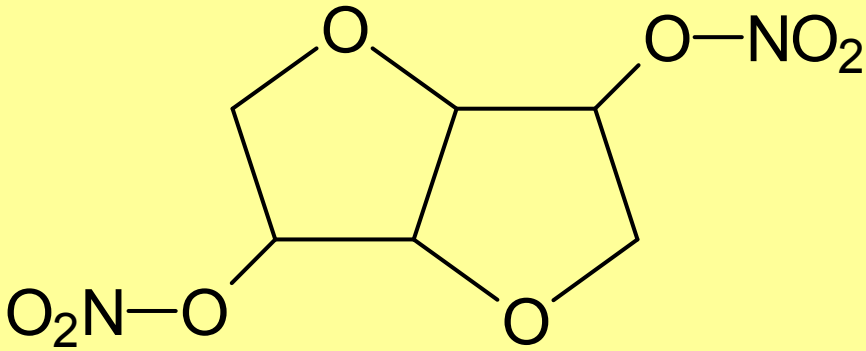
- exogenous sources: drugs - vasodilators
- NO· activates guanylate cyclase ⇒ cGMP ⇒ relaxation of smooth muscles
- NO· is a radical and affords other reactive metabolites:



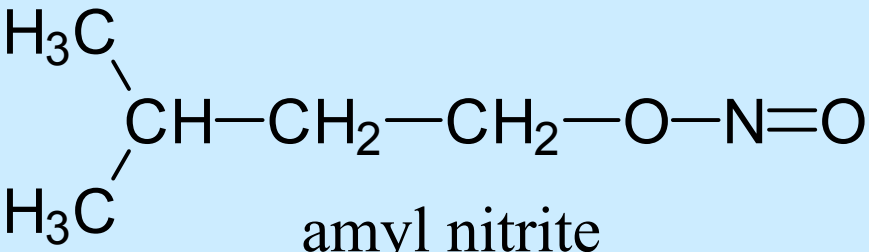
Compounds releasing NO



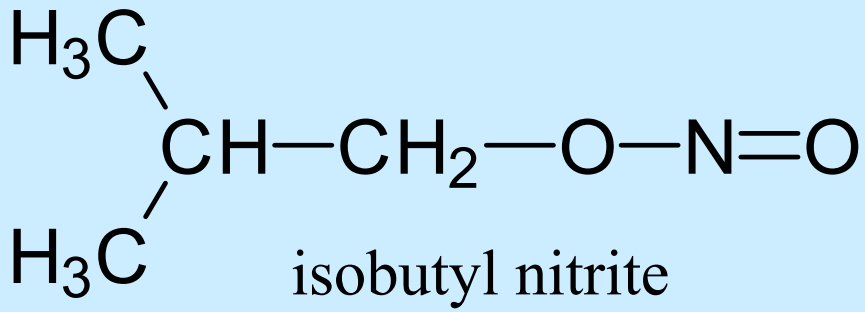
glycerol trinitrate
(glyceroli trinitras)



isosorbid dinitrate
(isosorbidi dinitras)



amyl nitrite



isobutyl nitrite

$Na_2[Fe(CN)_5NO]$

sodium nitroprusside

(natrii nitroprussias)

sodium pentacyanonitrosylferrate(III)

Good effects of ROS

- **intermediates** of oxidase and oxygenase reactions (cyt P-450), during reactions the radicals are trapped in enzyme molecule so that they are not harmful
- **bactericidal effect –** fagocytes, respiratory burst (NADPH-oxidase)
- **signal molecules –** clearly proved in NO·, perhaps other radical species can have similar action

Bad effects of ROS

Substrate	Damage	Consequences
PUFA	formation of aldehydes (MDA) and peroxides	changes in membrane permeability, damage of membrane enzymes
Proteins	aggregation, cross-linkage fragmentation oxidation of –SH, phenyl	changes in ion transport influx of Ca^{2+} into cytosol altered enzyme activity
DNA	deoxyribose decomposition modification of bases chain breaks	mutations translations errors inhibition of proteosynthesis

Antioxidant systems in the body

Enzymes

- superoxide dismutase, catalase, glutathione peroxidase

Low molecular antioxidants = reducing compounds with

- phenolic -OH (tocopherol, flavonoids, urates)
- enolic -OH (ascorbate)
- -SH (glutathione GSH, dihydrolipoate)
- or compounds with extended system of conjugated double bonds (carotenoids)

Elimination of superoxide

- Superoxide dismutase
- Catalyzes the dismutation of superoxide

  $2\ \bullet O_2^- + 2\ H^+ \longrightarrow O_2 + H_2O_2$

- Oxidation numbers of oxygen

  $-\frac{1}{2} \longrightarrow 0 \qquad -I$

- two forms:

  SOD1 (Cu, Zn, cytosol), SOD2 (Mn, mitochondria)

Dismutation is a special type of redox reaction
in which an element is simultaneously reduced
and oxidized so as to form two different products.

Elimination of H_2O_2

- **catalase** - in erythrocytes and other cells

  $H_2O_2 \rightarrow \frac{1}{2} O_2 + H_2O$

- **glutathione peroxidase**

3% H_2O_2 aplied to a wound releases bubbles

- contains selenocystein, reduces H_2O_2 and hydroperoxides of phospholipids (ROOH)

$$2\ G\text{-}SH + H\text{-}O\text{-}O\text{-}H \rightarrow G\text{-}S\text{-}S\text{-}G + 2\ H_2O$$

$$2\ G\text{-}SH + R\text{-}O\text{-}O\text{-}H \rightarrow G\text{-}S\text{-}S\text{-}G + R\text{-}OH + H_2O$$

Lipophilic antioxidants

Antioxidant	Sources
Tocopherol	Plant oils, nuts, seeds, germs
Carotenoids	Fruits, vegetables (most effective is lycopene)
Ubiquinol	Formed in the body from tyrosine

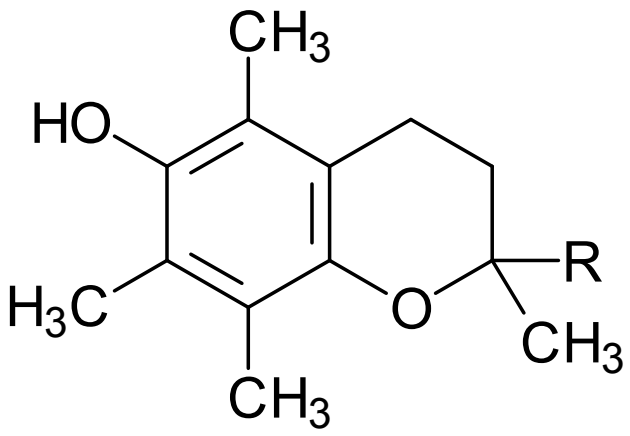
Hydrophilic antioxidants

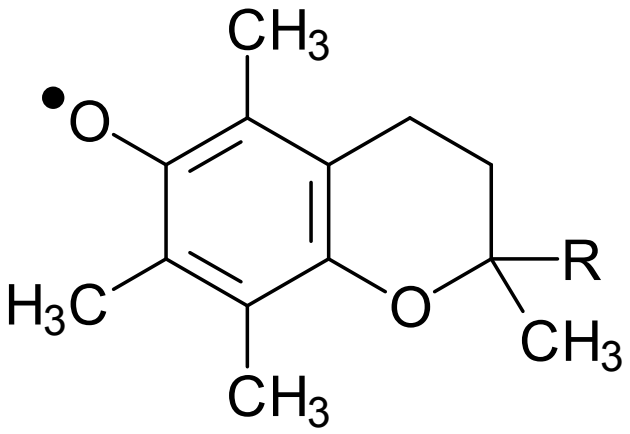
Antioxidant	Sources
L-ascorbate	Fruits, vegetables, potatoes
Flavonoids	Fruits, vegetables, tea, wine
Dihydrolipoate	Made in the body from cysteine
Uric acid	Catabolite of purine bases
Glutathione	Made in the body from cysteine

Tocopherol (Toc-OH)

- Lipophilic antioxidant of cell membranes and lipoproteins
- Reduces peroxyl radicals of phospholipids to hydroperoxides which are further reduced by GSH, tocopherol is oxidized to stable radical Toc-O·

  PUFA-O-O· + Toc-OH → PUFA-O-O-H + Toc-O·
- Toc-O· is partially reduced to Toc-OH by ascorbate or GSH

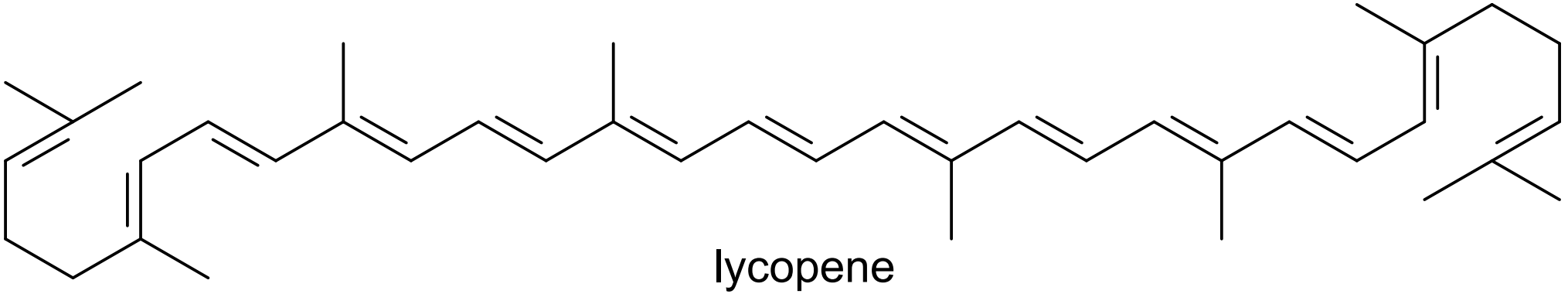




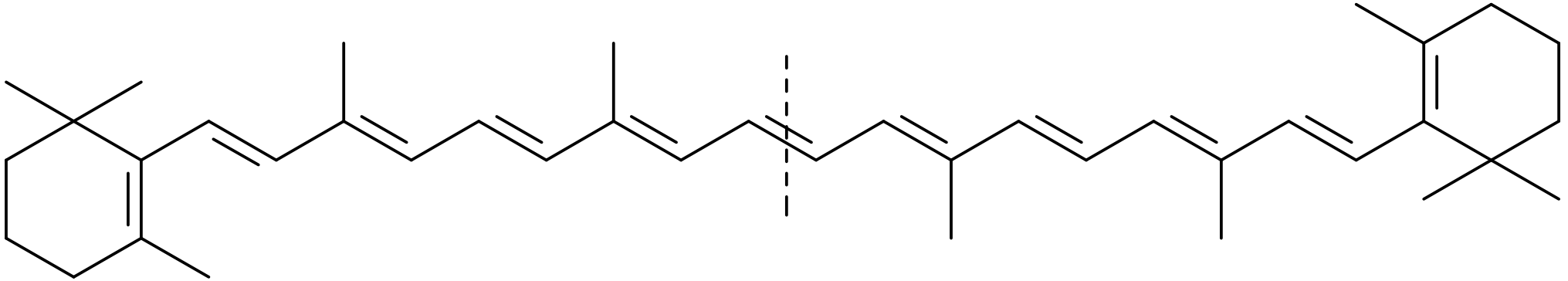
Carotenoids

- polyisoprenoid hydrocarbons (tetraterpens)
- eliminate peroxyl radicals
- they can quench singlet oxygen
- food sources: green leafy vegetables, yellow, orange, red vegetables and fruits
- very potent antioxidant is **lycopene** (tomatoes, more available from cooked tomatoes, ketchup etc.)

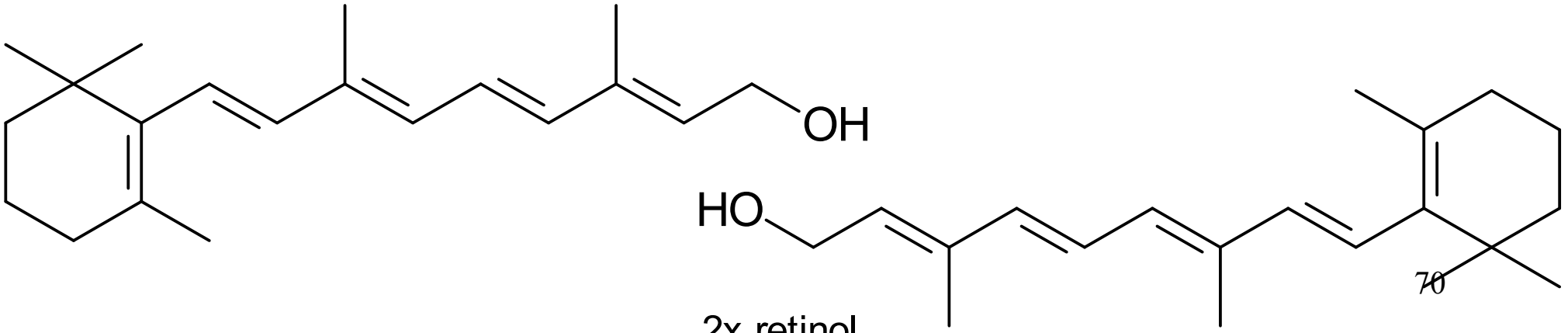
Lycopene does not have the β-ionone ring



lycopene



beta-carotene



2x retinol

Lycopene in food (mg/100 g)

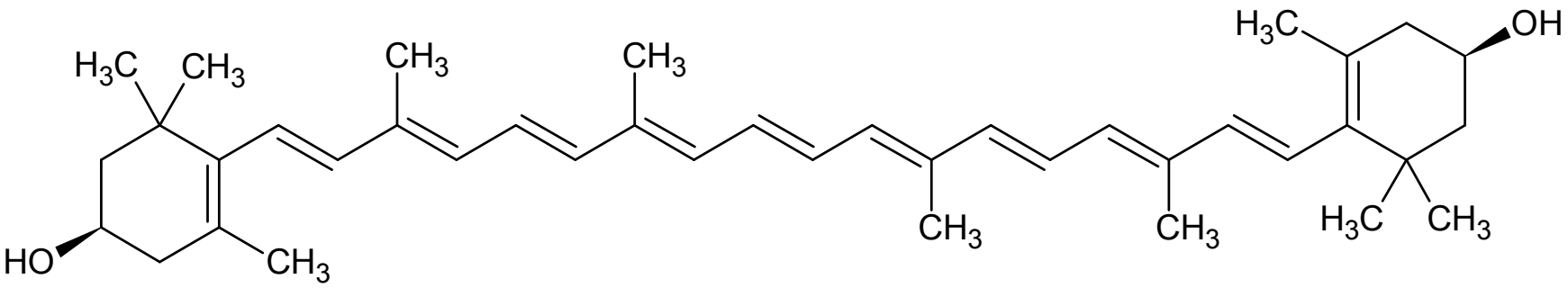
Tomato purée	10-150
Ketchup	10-14
Tomato juice/sauce	5-12
Watermelon	2-7
Papaya	2-5
Tomatoes fresh	1-4
Apricots canned	~ 0.06
Apricots fresh	~ 0.01

In order to effectively absorb lycopene, tomatoes should be

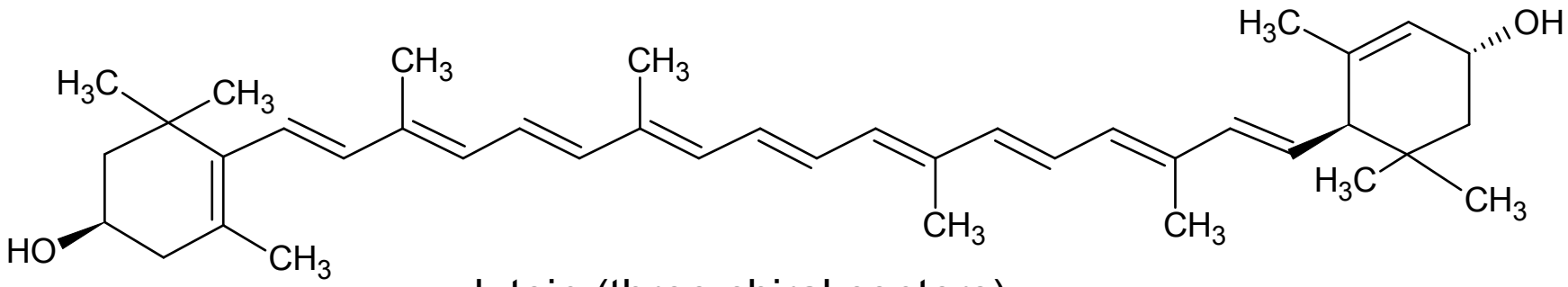
- chopped and mashed
- stewed slowly
- combined with oil

Zeaxanthin and lutein

- belong to xanthophylls – oxygen derivatives of carotenoids
- they differ in the position of double bond and in the number of C*
- occur mainly in green leafy vegetables (spinach, cabbage, kale)
- contained in *macula lutea*, prevents it against degeneration
- many pharmaceutical preparations available



zeaxanthin (two chiral centers)



lutein (three chiral centers)

Ubiquinol (QH_2)

- occurs in all membranes
- Endogenous synthesis by intestinal microflora from tyrosine and farnesyl diphosphate (biosyntheis of cholesterol)
- Exogenous sources: liver, meat and other foods
- Reduced form QH_2 regenerates tocopherol
- Toc-O$\cdot$ + QH_2 $\rightarrow$ Toc-OH + $\cdot$QH

L-Ascorbate (vitamin C)

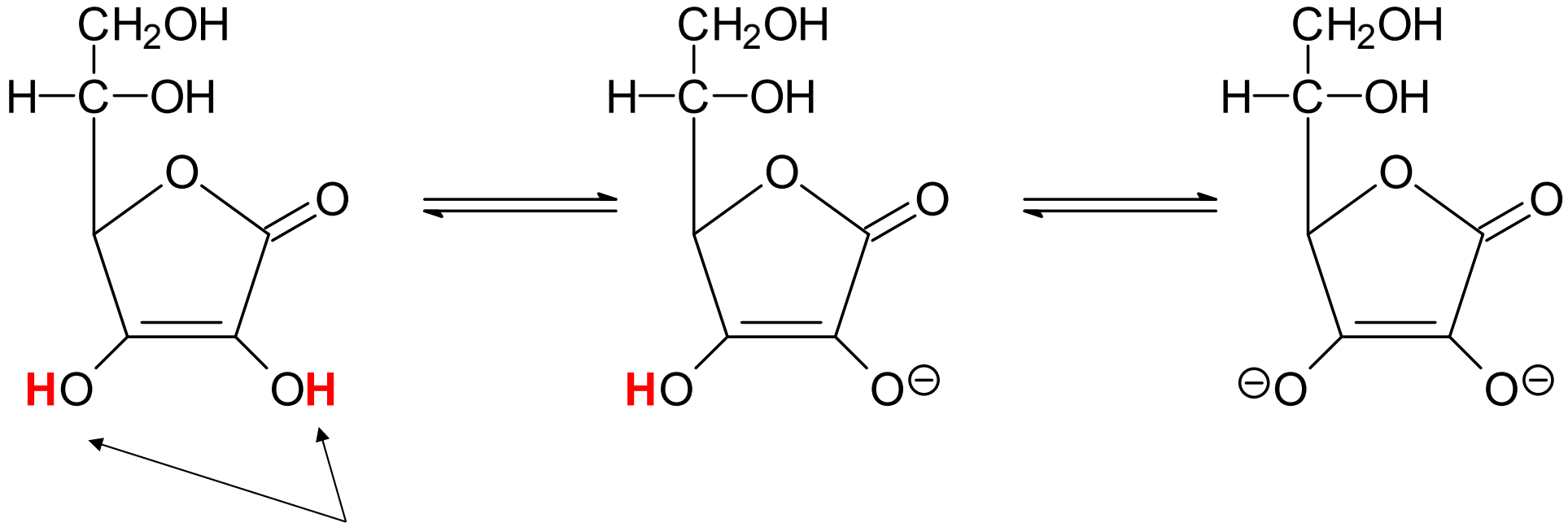
- cofactor of proline hydroxylation (maturation of collagen)
- cofactor of dopamine hydroxylation (to noradrenaline)
- potent reducing agent ($Fe^{3+} \rightarrow Fe^{2+}$, $Cu^{2+} \rightarrow Cu^{+}$)
- supports intestinal absorption of iron
- Reduces many radicals: $\cdot OH$, $\cdot O_2^-$, $HO_2\cdot$, $ROO\cdot$ ....
- Regenerates tocopherol
- It is catabolized to oxalate!! (high doses are not recomended)
- excess of ascorbate has pro-oxidative effects:

  Fe^{2+} and Cu^{+} catalyze the formation of hydroxyl radical

  ascorbate + O_2 $\rightarrow$ $\cdot O_2^-$ + $\cdot$monodehydroascorbate

L-Ascorbic acid is a weak diprotic acid

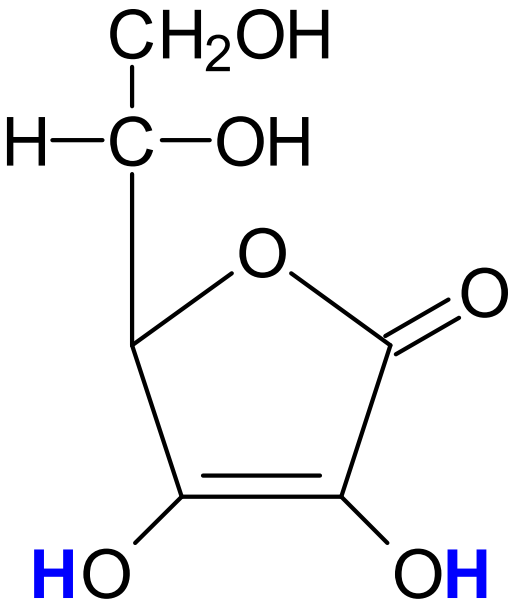
$pK_{A1} = 4.2$ $pK_{A2} = 11.6$



two enol hydroxyls

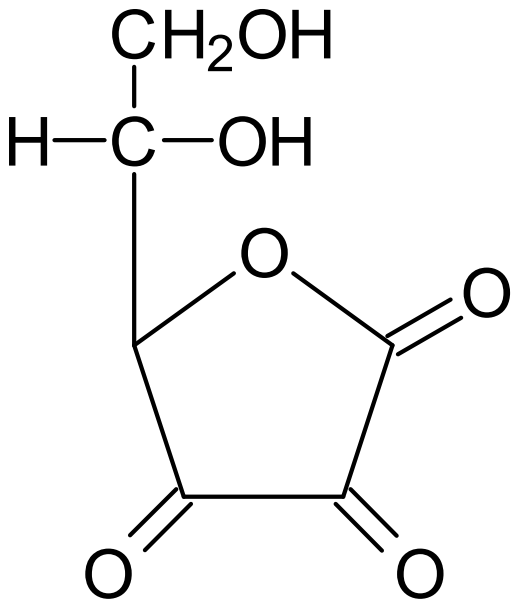
Two conjugate pairs:
Ascorbic acid / hydrogen ascorbate
Hydrogen ascorbate / ascorbate

L-Ascorbic acid has reducing properties (antioxidant)



ascorbic acid

(reduced form)



dehydroascorbic acid

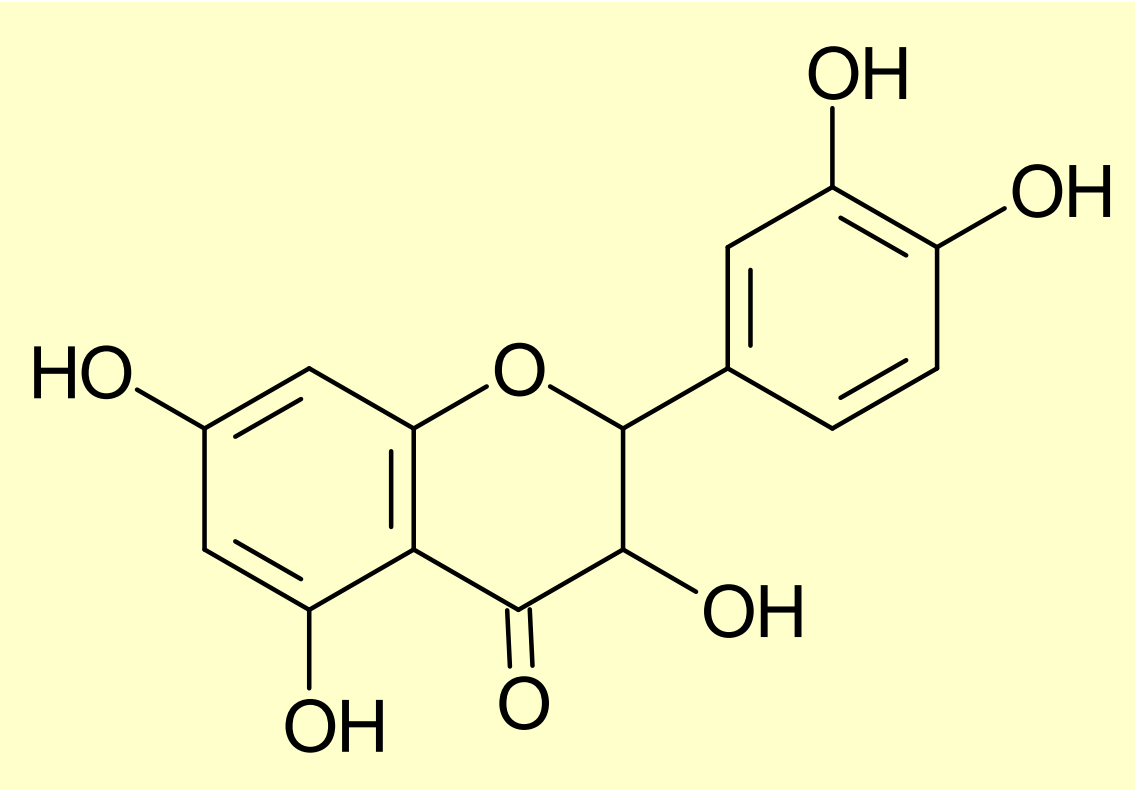
(oxidized form)

Flavonoids and other polyphenols

- commonly spread in plant food
- total intake about 1 g (higher than in vitamins)
- derivatives of chromane (benzopyrane), many phenolic hydroxyls
- a main example: **quercitin** (see also Med. Chem. II, p. 76)
- they reduce free radicals, themselves are converted to unreactive phenoxyl radicals
- they chelate free metal ions (Fe^{2+}, Cu^{+}) blocking them to catalyze Fenton reaction and lipoperoxidation

Main sources of flavonoids

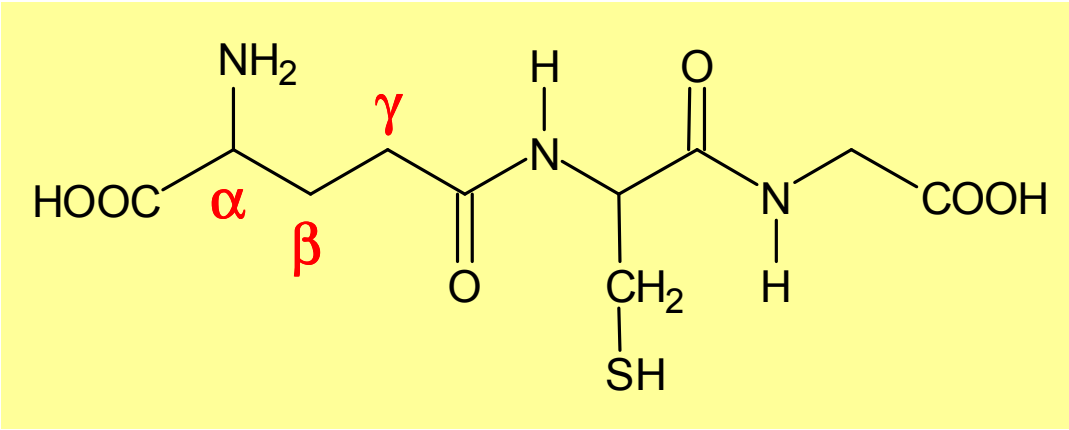
- vegetable (onion)
- fruits (apples, grapes)
- green tea
- cocoa, quality chocolate
- olive oil (Extra Virgin)
- red wine



quercitin

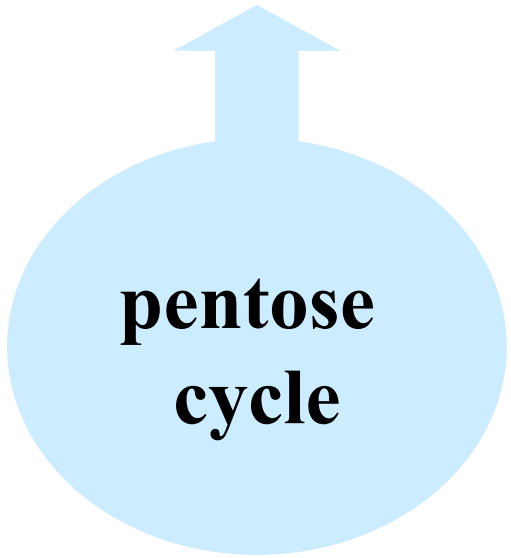
Glutathione (GSH)

- tripeptide
- γ-glutamylcysteinylglycine
- made in all cells
- reducing agent (-SH)
- reduces H_2O_2 and ROOH (glutathione peroxidase)
- reduces many ROS
- regenerates -SH groups of proteins and coenzyme A
- regenerates tocopherol and ascorbate



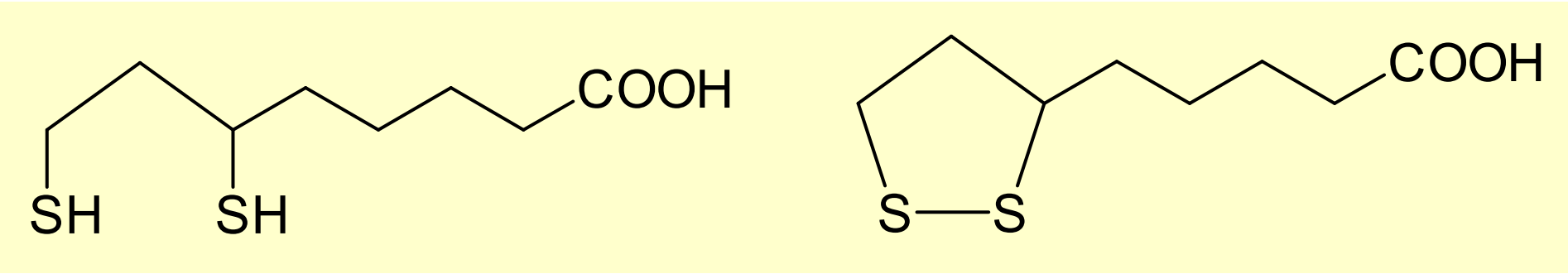
Regeneration of reduced form of GSH

- continuous regeneration of GSH proceeds in many cells
- glutathione reductase, esp. in erythrocytes
- $GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$



Dihydrolipoate

- cofactor of oxidative decarboxylation of pyruvate, 2-OG
- reduces many radicals (mechanism not well understood)



dihydrolipoate
(reduced form)

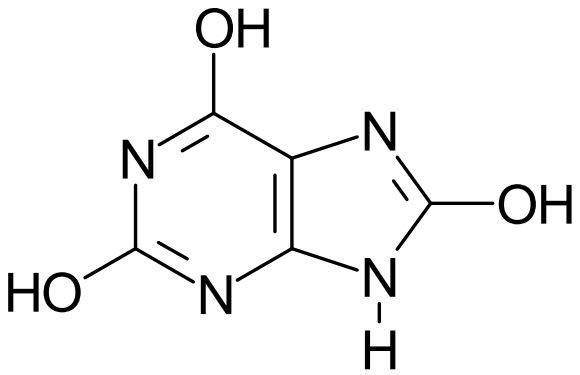
lipoate
(oxidized form)

Uric acid

- final catabolite of purine bases
- in kidney, tubular cells, 90 % of urates are resorbed
- **the most abundant antioxidant of blood plasma**
- reducing properties, reduces various radicals
- has ability to chelate iron and copper ions

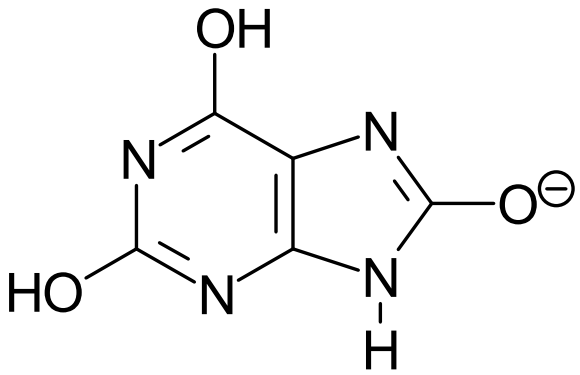
Uric acid (lactim) is a weak diprotic acid

$pK_{A1} = 5.4$ $pK_{A2} = 10.3$

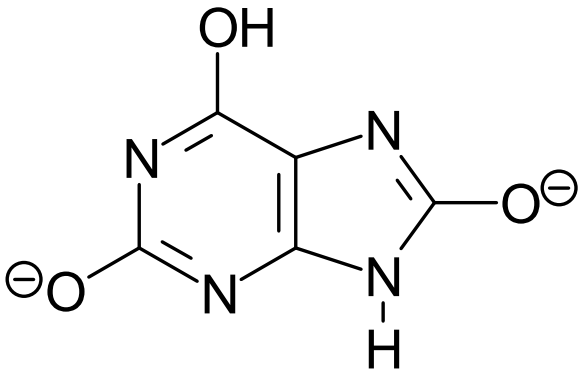


uric acid

2,6,8-trihydroxypurine

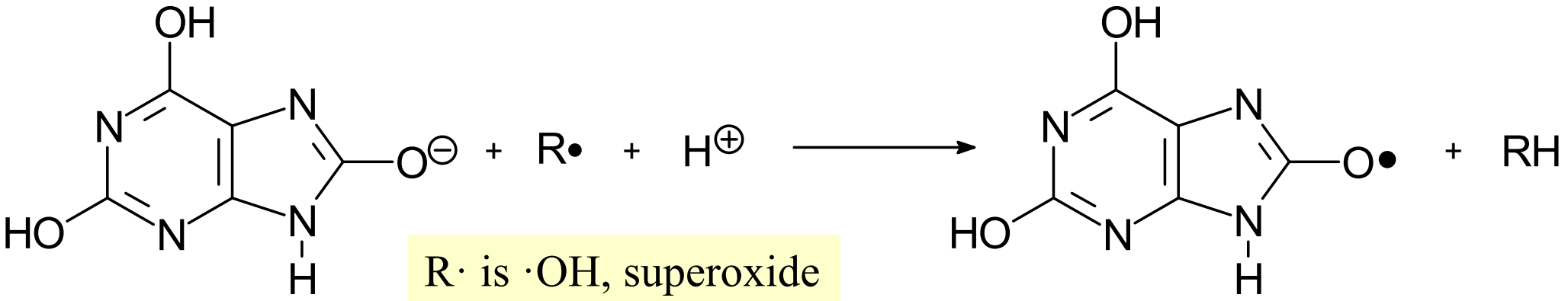


hydrogen urate



urate

Uric acid is the most abundant plasma antioxidant



R· is ·OH, superoxide

hydrogen urate
(reduced form)

stable radical
(oxidized form)

Compare plasma concentrations

Ascorbic acid: 10 - 100 µmol/l

Uric acid: 200 - 420 µmol/l