

# **Citric acid cycle**

## **Synthesis of heme. Hemoproteins**

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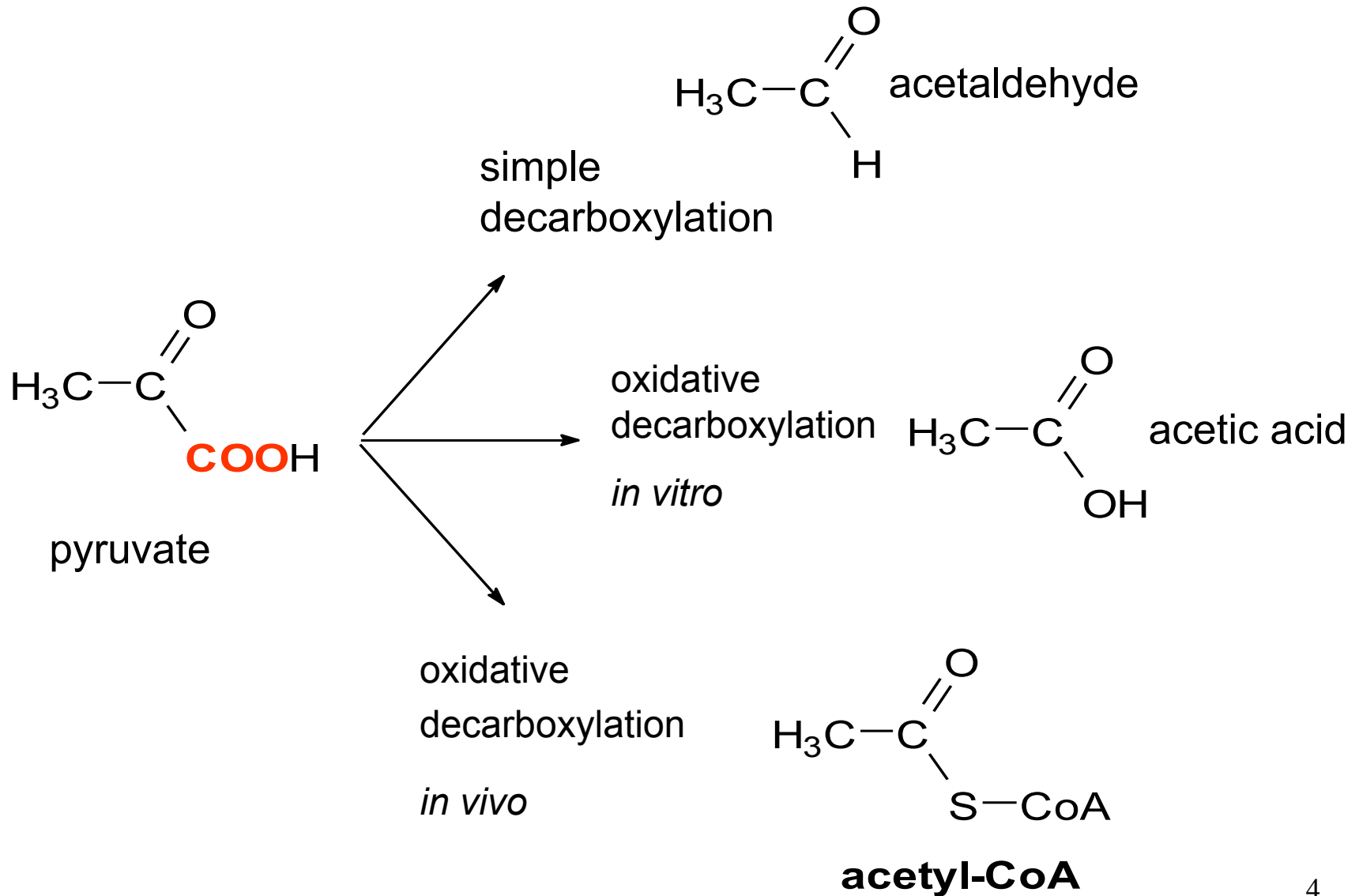
# Three phases of nutrient catabolism

Phase	The conversions of nutrients	ATP yield
I.	Hydrolytic cleavage of nutrients during digestion: Starch → maltose → glucose Proteins → peptides → amino acids Lipids → fatty acids	none
II.	Intracellular catabolism of nutrients: Glc, FA, AA → → → pyruvate → acetyl-CoA Production of ATP in glycolysis (2 ATP/Glc) Production of reduced cofactors (NADH+H <sup>+</sup> , FADH <sub>2</sub> )	small
III.	Citrate cycle: acetyl-CoA → 2 CO <sub>2</sub> + red. cofactors + ATP Respiratory chain: oxidation of reduced cofactors Aerobic phosphorylation: synthesis of ATP from ADP + P <sub>i</sub>	the biggest

# Sources of acetyl-CoA

- oxidative decarboxylation of pyruvate (from glucose and 6 AA)
- $\beta$ -oxidation of fatty acids
- catabolism of some amino acids (Thr, Trp, Lys, Leu, Ile)
- ketone bodies utilization in extrahepatal tissues:  
acetoacetate  $\rightarrow$  acetoacetyl-CoA  $\rightarrow$  2 acetyl-CoA
- catabolism of ethanol  $\rightarrow$  acetaldehyde  $\rightarrow$  acetate  $\rightarrow$  acetyl-CoA

# Compare different ways of pyruvate decarboxylation



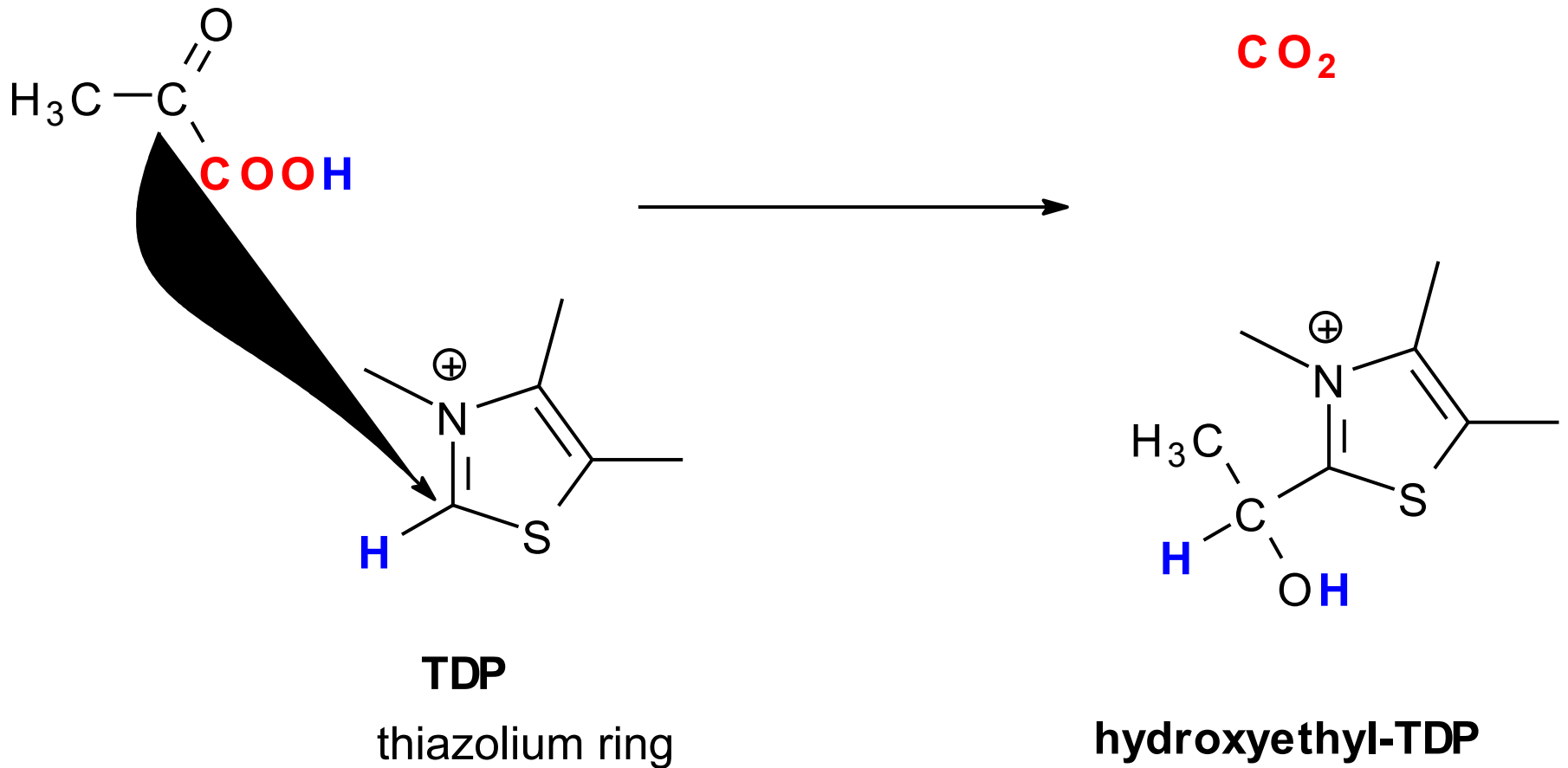
**Oxidative decarboxylation of pyruvate is catalyzed by pyruvate dehydrogenase complex: three enzymes and five cofactors**

1. thiamin diphosphate (TDP)
2. lipoate
3. coenzyme A
4. FAD
5. NAD<sup>+</sup>

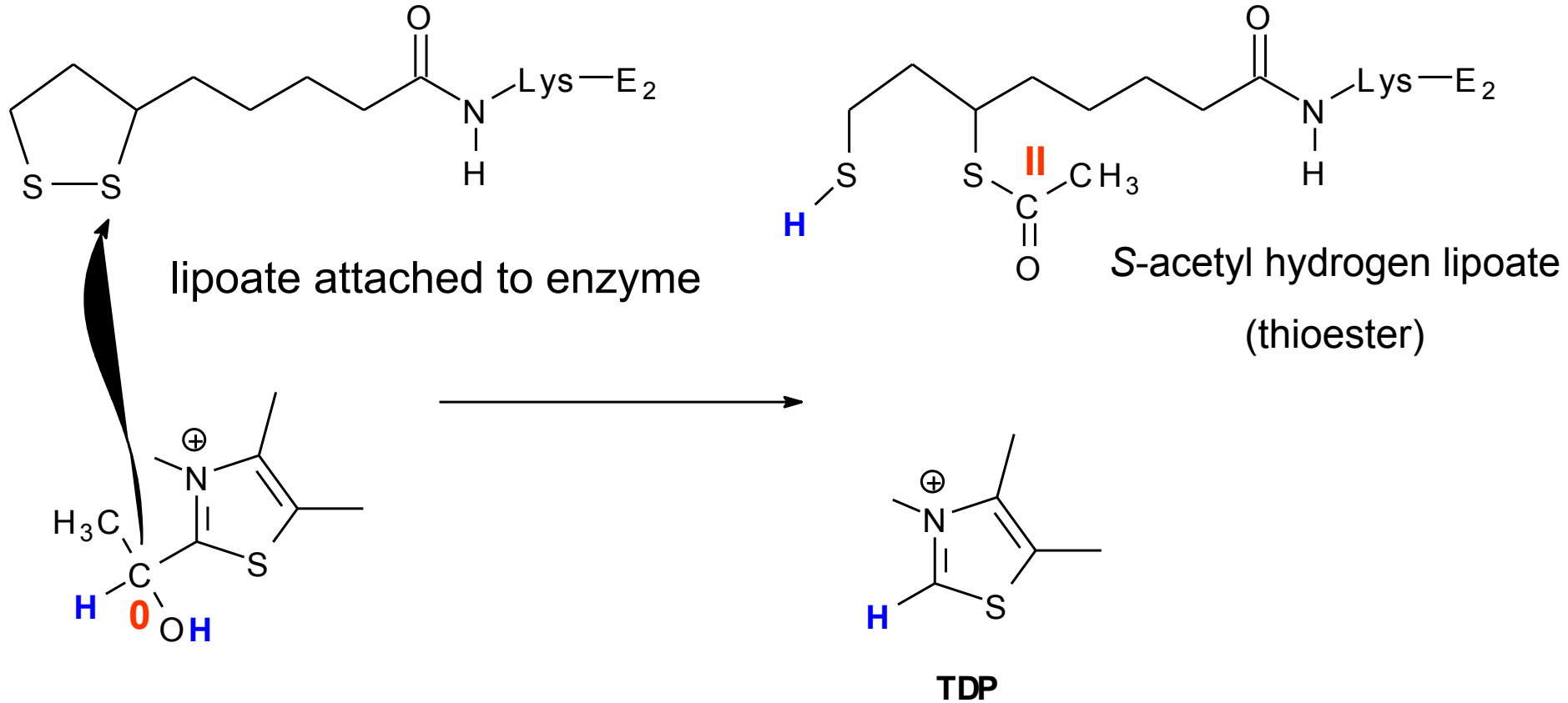


mitochondria

# (1) Decarboxylation of pyruvate

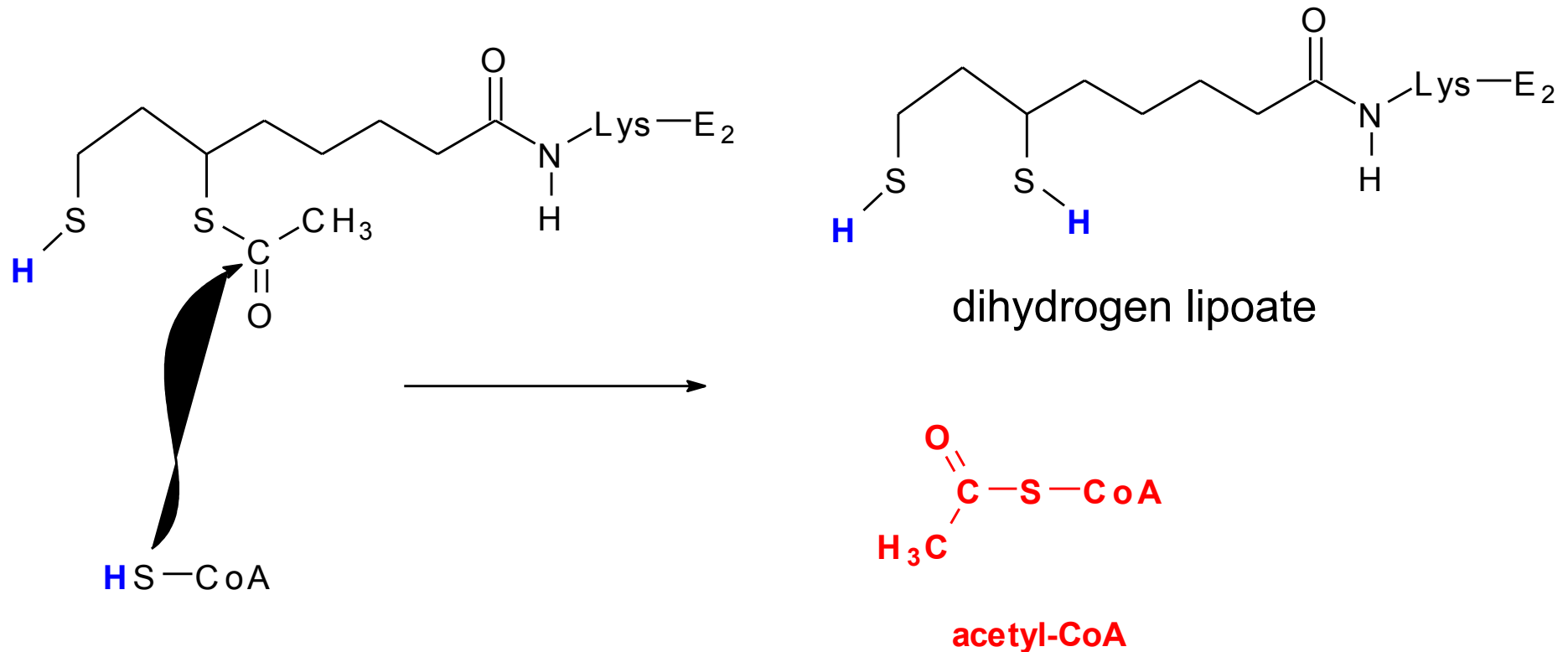


## (2) Transfer of acetyl to lipoate is redox reaction



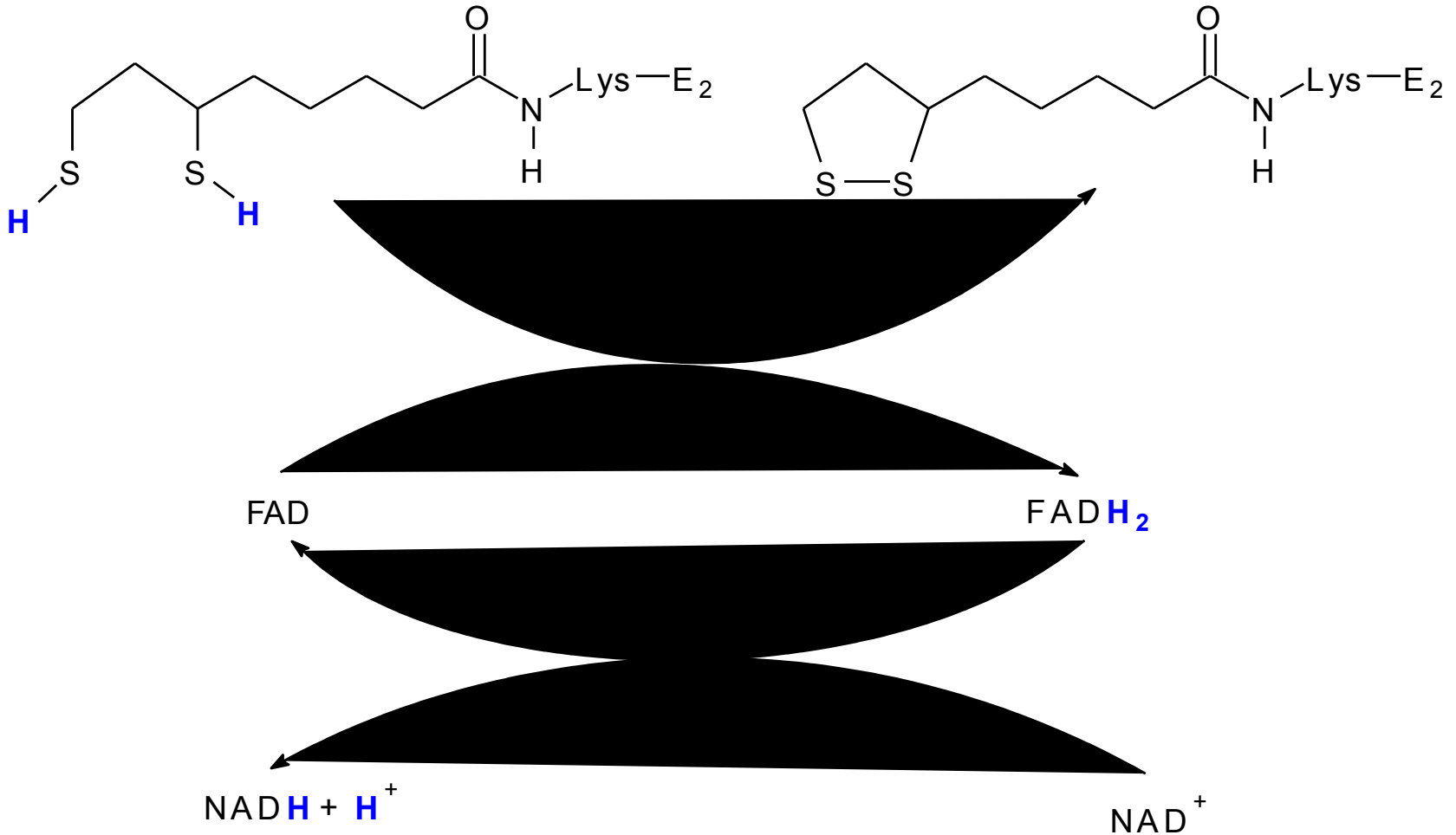
- hydroxyethyl group is dehydrogenated to thioester during transfer
- one H atom reduces sulfur atom of lipoate to –SH group
- the second H atom goes back to TDP

### (3) Transfer of acetyl to coenzyme A

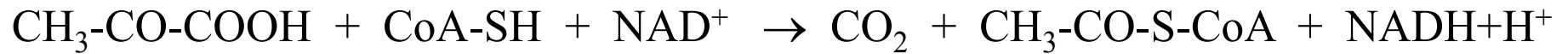




# (4) Transfer of 2H to NAD<sup>+</sup> via FAD



# Balance reaction

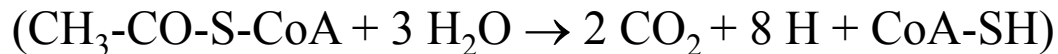


Pyruvate dehydrogenase is allosterically inhibited  
by end products: acetyl-CoA + NADH

# Citric acid cycle (CAC)

Krebs cycle, tricarboxylic acid cycle (TCA)

- final common pathway for oxidation of all major nutrients
- located in mitochondria, active in all cells that possess mitochondria
- **acetyl-CoA** from metabolism of nutrients is **oxidized to two molecules of CO<sub>2</sub>**



- CAC products:

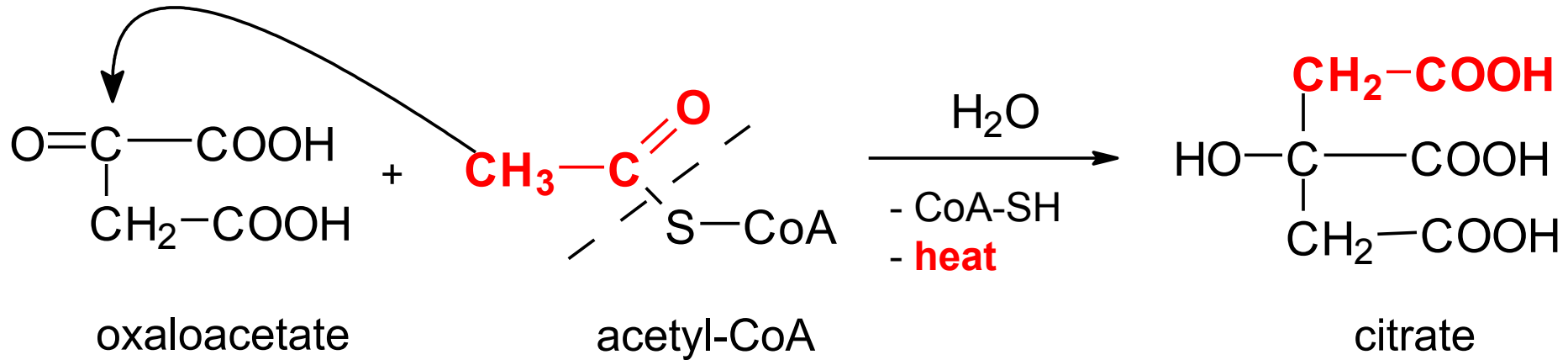
CO<sub>2</sub> → expired by lungs

four oxidative steps → reduced cofactors → respiratory chain

GTP → ATP

- most reactions are reversible, only **three** reactions are irreversible

# (1) Oxaloacetate + Acetyl-CoA



Reaction type: condensation

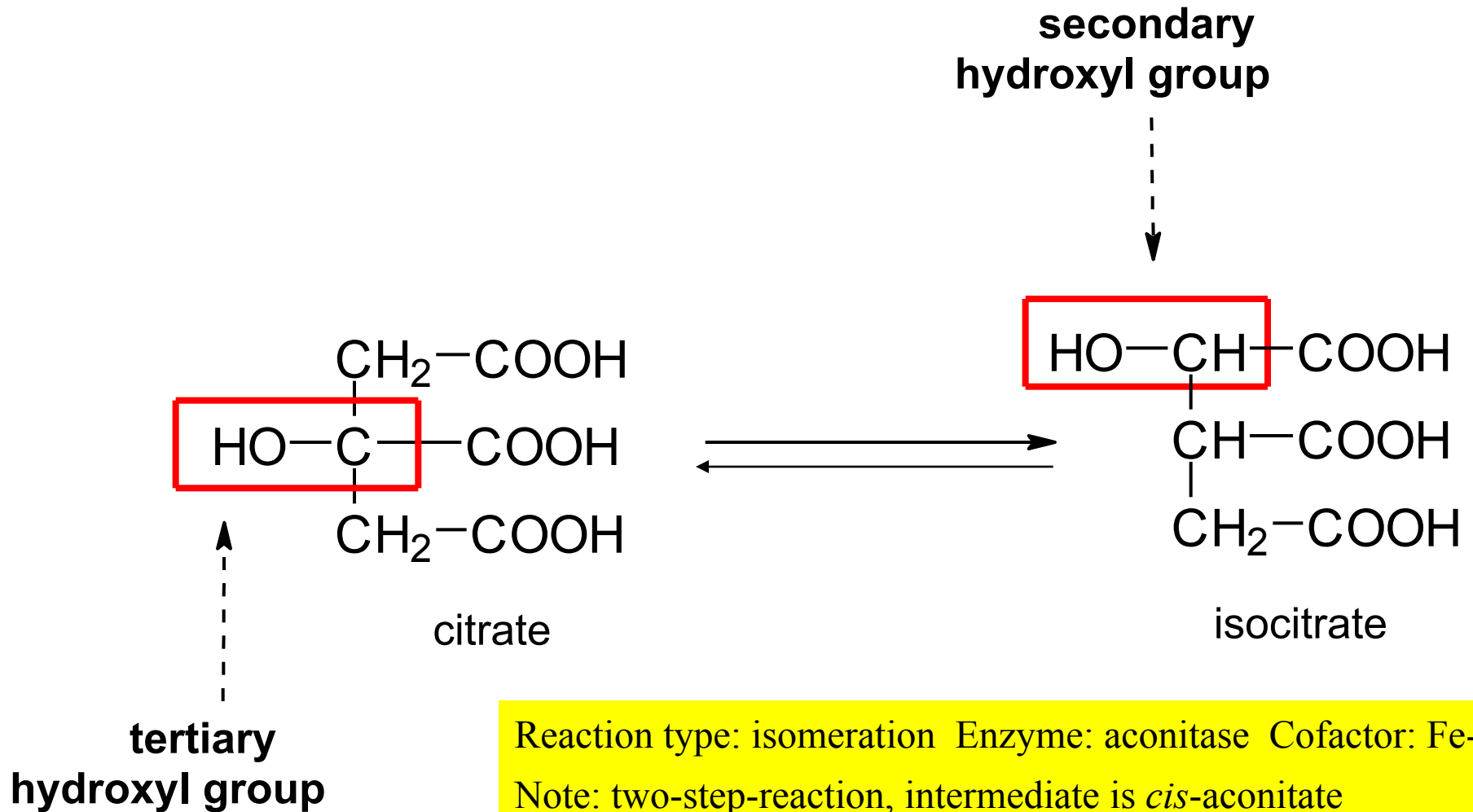
Enzyme: citrate synthase

Cofactor: coenzyme A

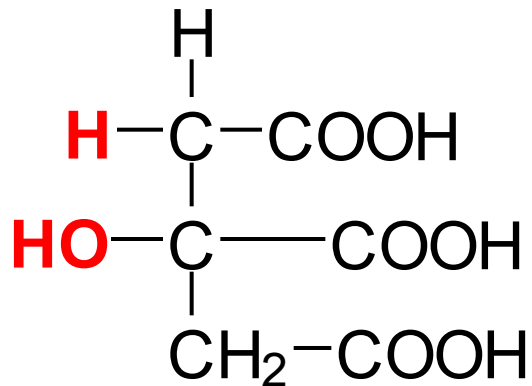
Note: **exothermic + irreversible**

low-energy compound  $\Rightarrow$   
for backward reaction  
in cytosol ATP needed

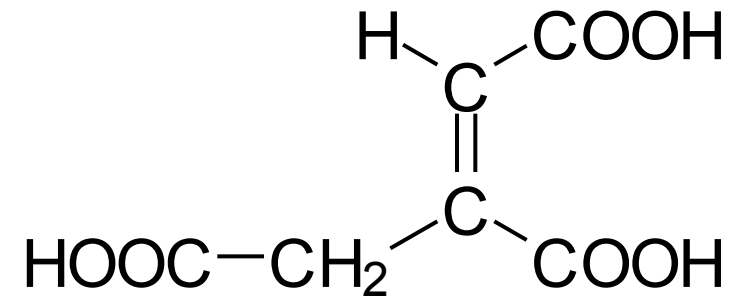
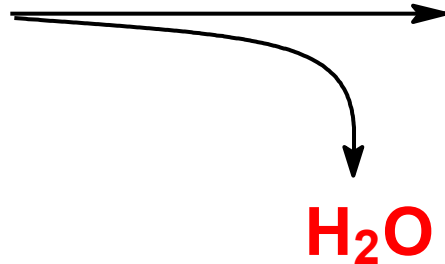
## (2) Citrate → Isocitrate



## (2a) Dehydration of citrate

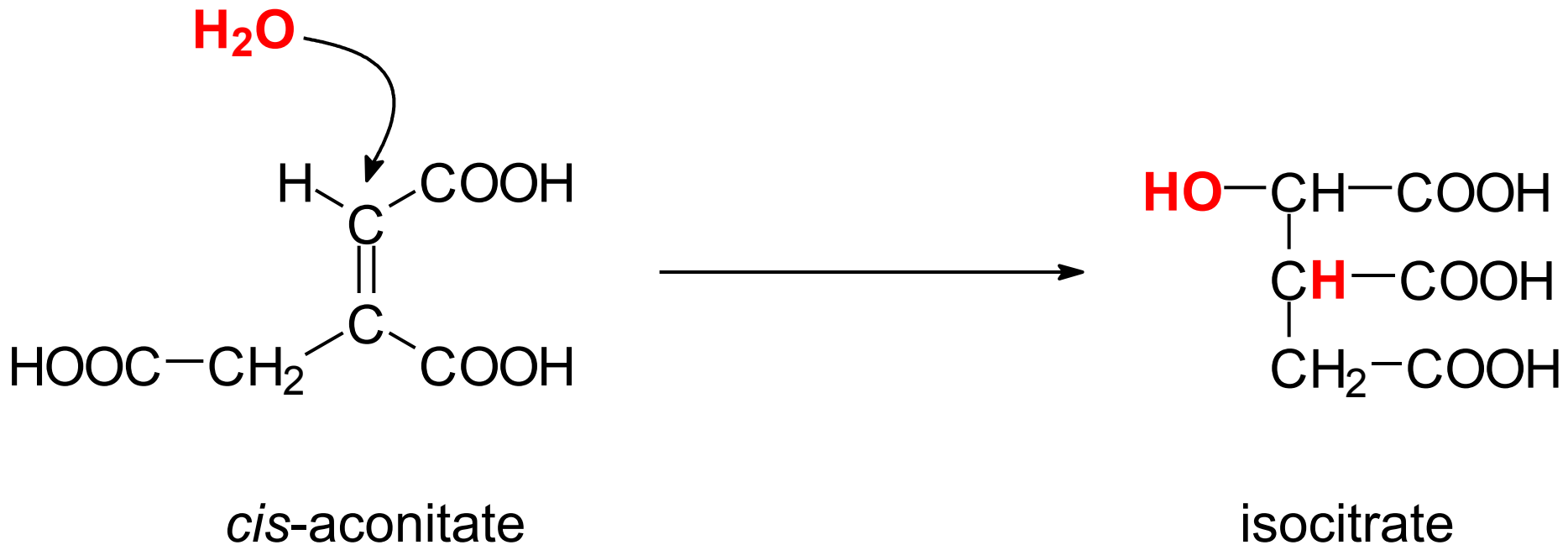


citrate



*cis*-aconitate

## (2b) Hydration of cis-aconitate



stereospecific reaction

# Aconitase is inhibited by fluoroacetate



reacts with oxaloacetate  
to give fluorocitrate

CAC is stopped

LD<sub>50</sub> for human is 1 mg/kg

rat poison

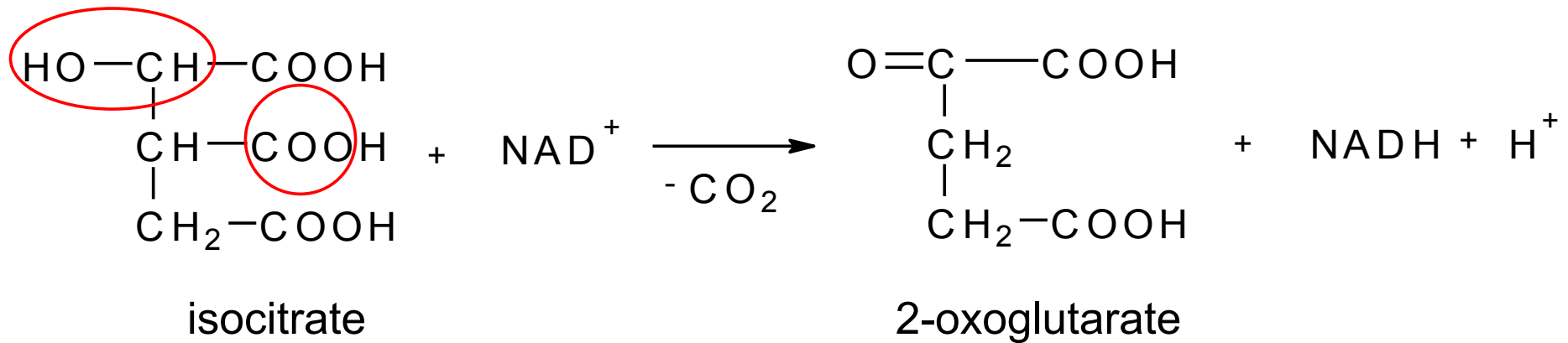
*Dichapetalum cymosum*

(see also Med. Chem. II, p. 65)





### (3) Isocitrate → 2-oxoglutarate

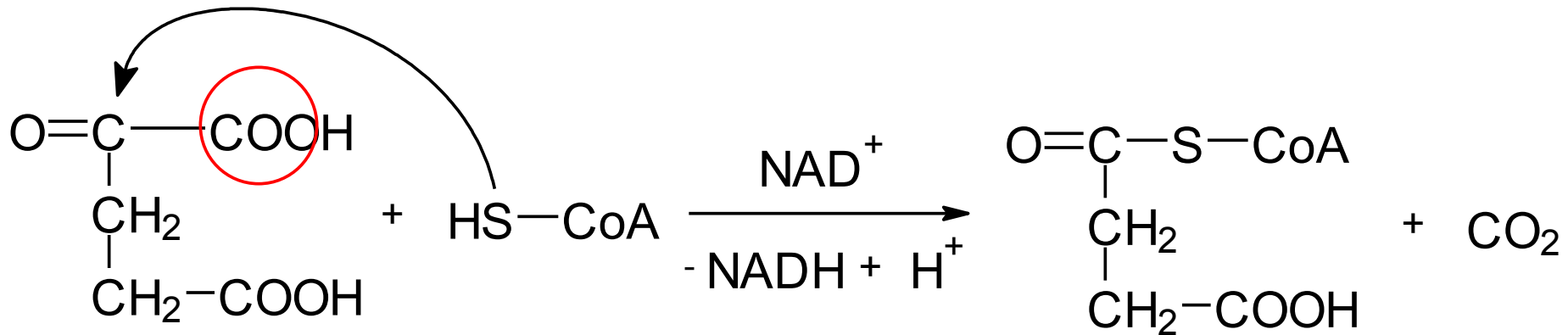


Reaction type: dehydrogenation + decarboxylation

Enzyme: isocitrate dehydrogenase

Cofactor: NAD<sup>+</sup> Note: **irreversible**

## (4) 2-Oxoglutarate → succinyl-CoA



2-oxoglutarate

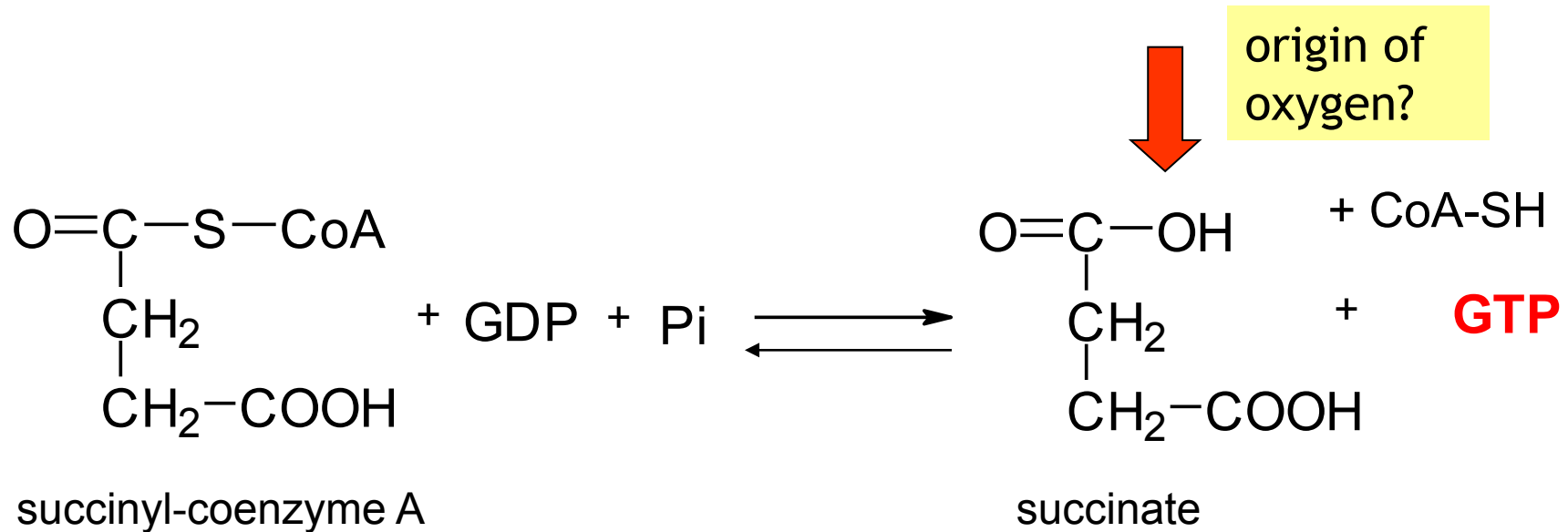
**succinyl-coenzyme A  
thioester  
macroergic intermediate**

Reaction type: oxidative decarboxylation    Enzyme: 2-oxoglutarate dehydrogenase complex

Cofactors: TDP, lipoate, CoA-SH, FAD, NAD<sup>+</sup>

Note: **irreversible**, similar to pyruvate dehydrogenase reaction (five coenzymes)

# (5) Succinyl-CoA + GDP + P<sub>i</sub>



Reaction type: substrate phosphorylation

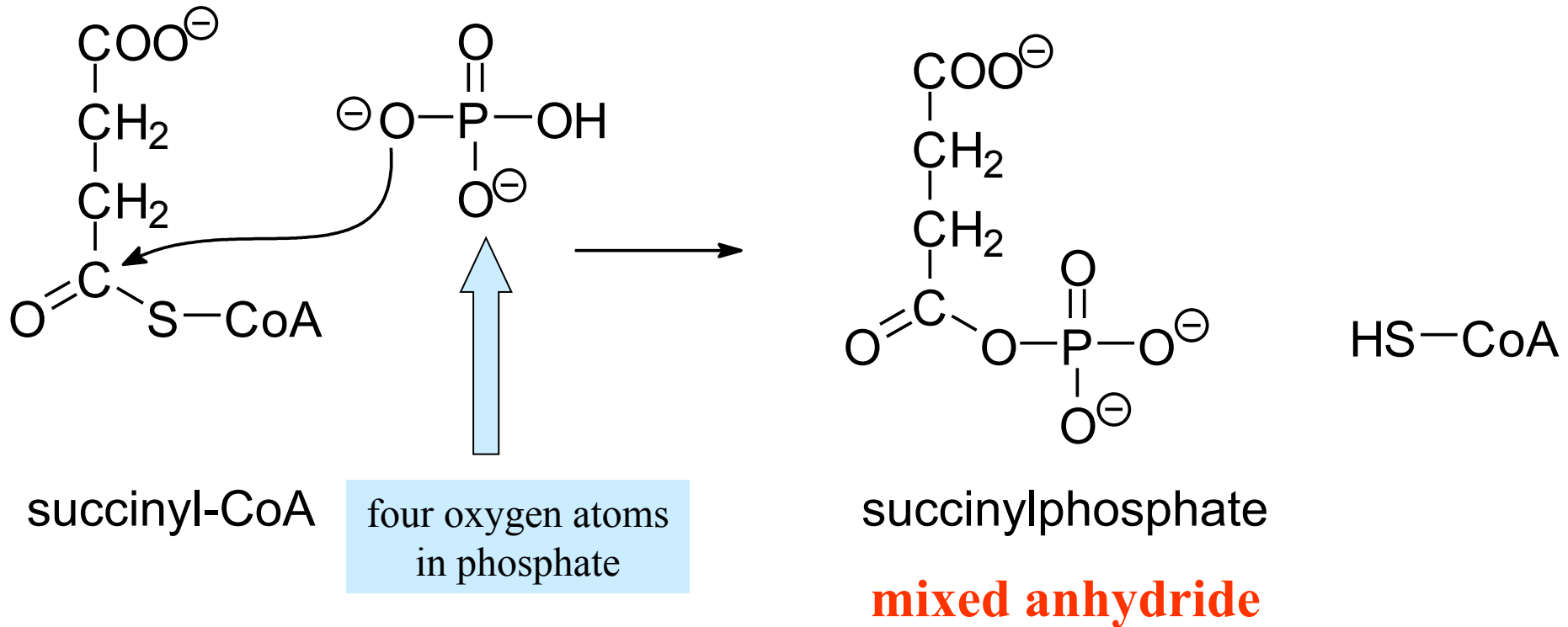
Enzyme: succinyl-CoA synthetase (succinate thiokinase)

Cofactor: coenzyme A

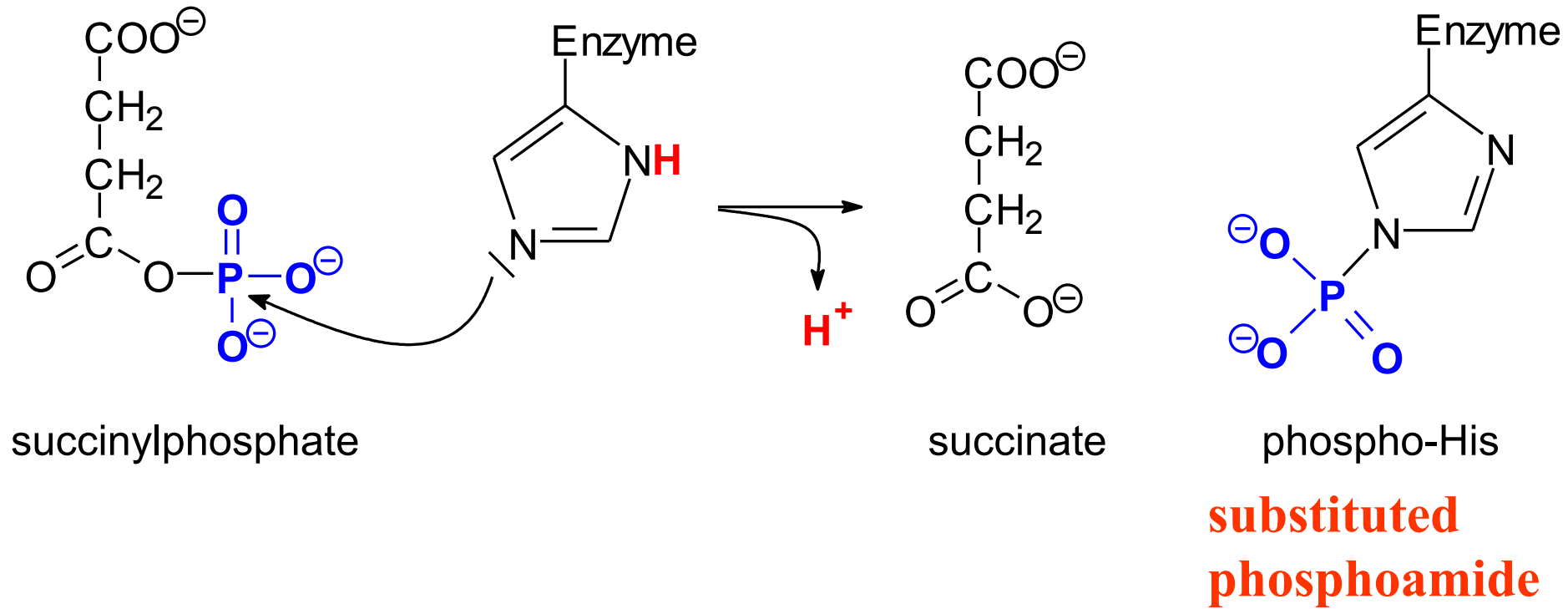
## **GTP is formed in three-step reaction**

Chemical energy of macroergic succinyl-CoA is gradually transformed into two macroergic intermediates and finally to macroergic GTP  
(Passing a hot potato)

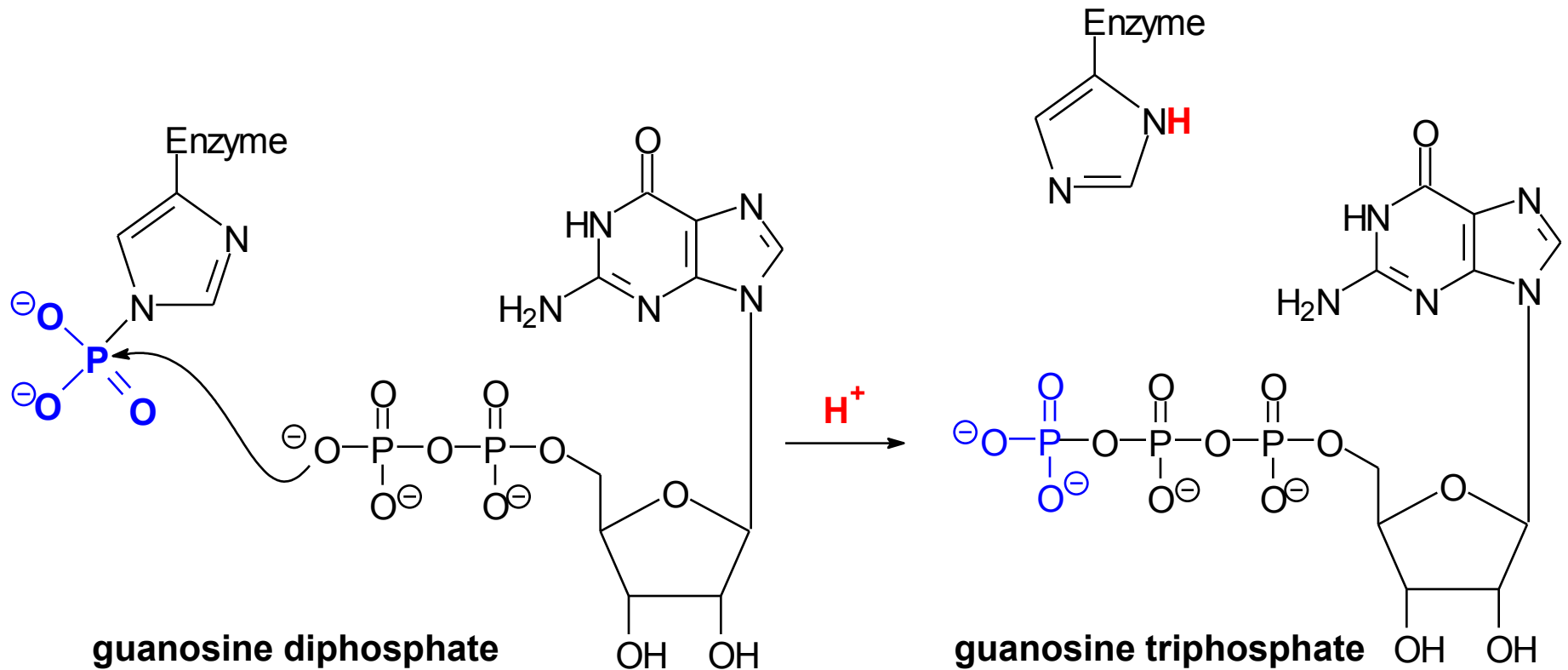
# (5a) Addition of phosphate to succinyl-CoA



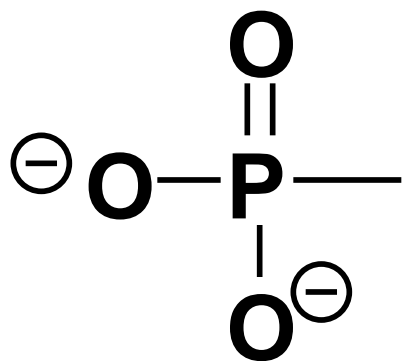
## (5b) Phosphorylation of His in the active site of enzyme



# (5c) Phosphorylation of GDP



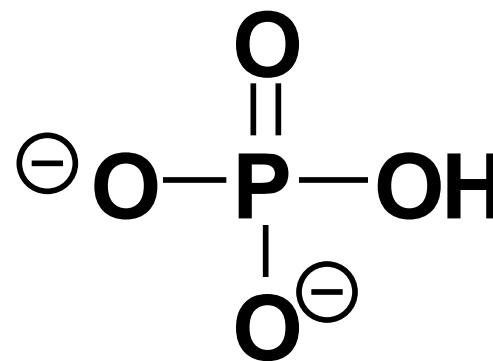
# Distinguish



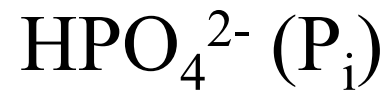
**phosphoryl**



virtual group



**phosphate**

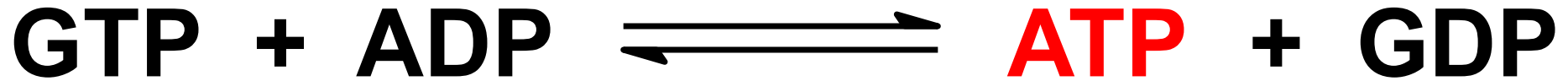


phosphate inorganic  
real compound

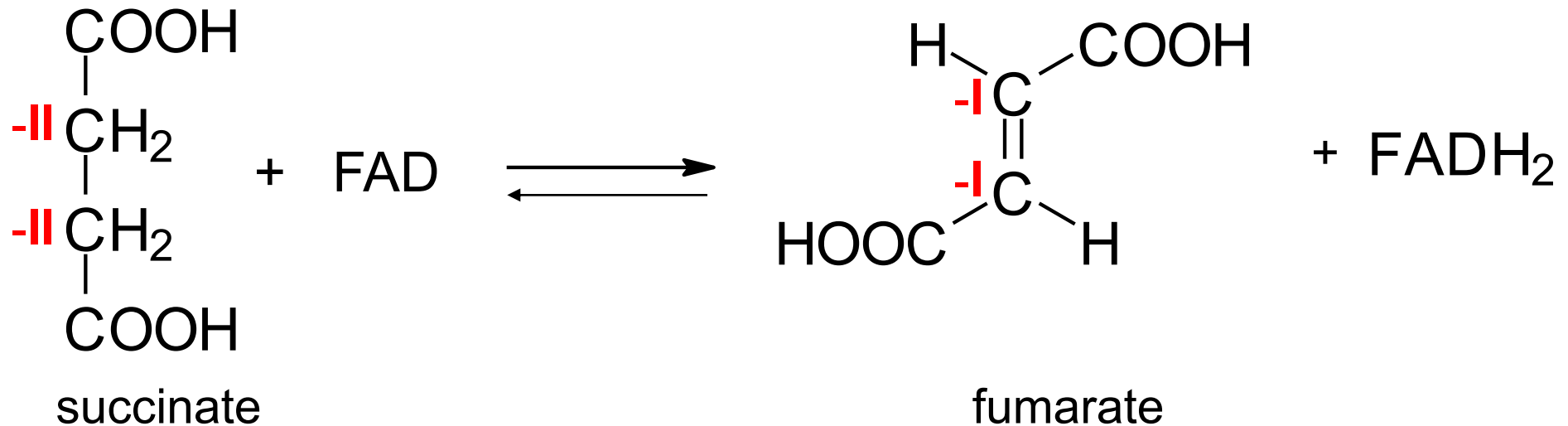


# GTP is quickly converted to ATP

nucleoside-diphosphate kinase



## (6) Succinate → fumarate

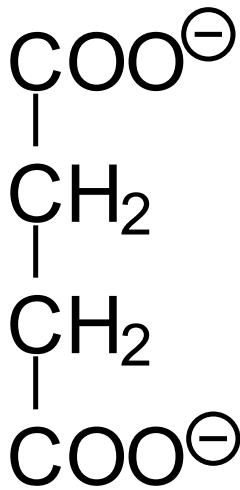


Reaction type: dehydrogenation (-CH<sub>2</sub>-CH<sub>2</sub>- bond)

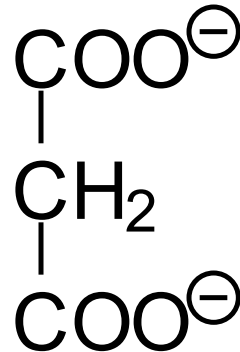
Enzyme: succinate dehydrogenase

Cofactor: FAD

# Malonate is competitive inhibitor of succinate dehydrogenase



succinate

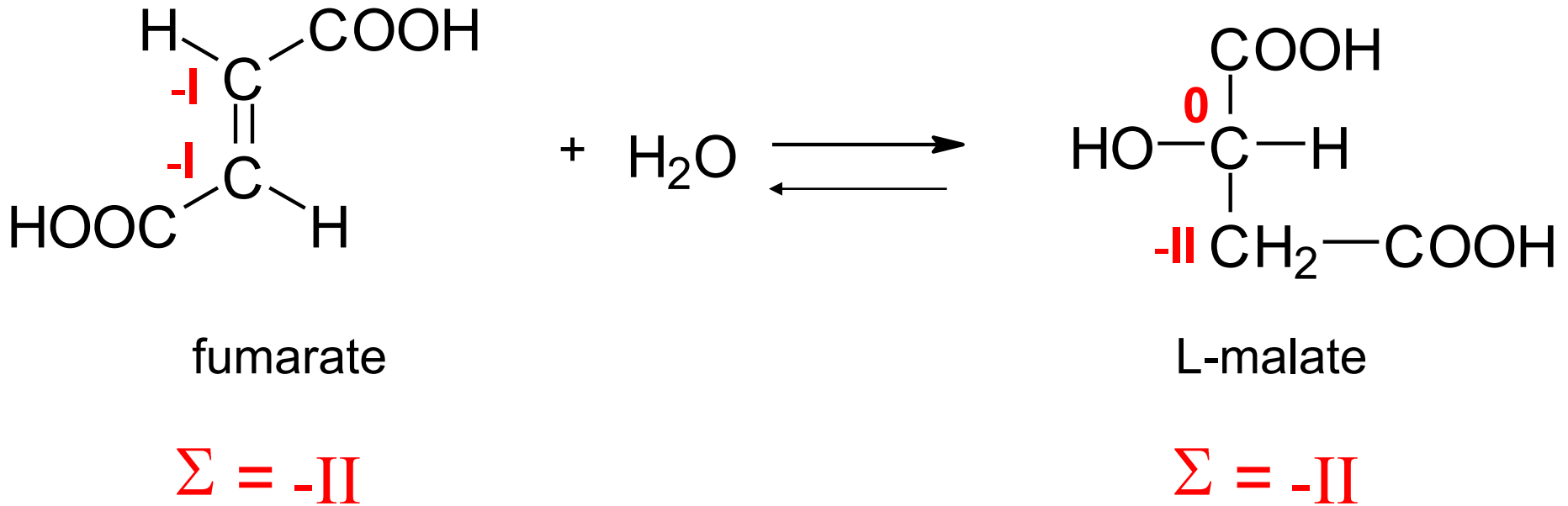


malonate



Do not confuse:  
malonate malate

# (7) Fumarate $\rightarrow$ L-malate



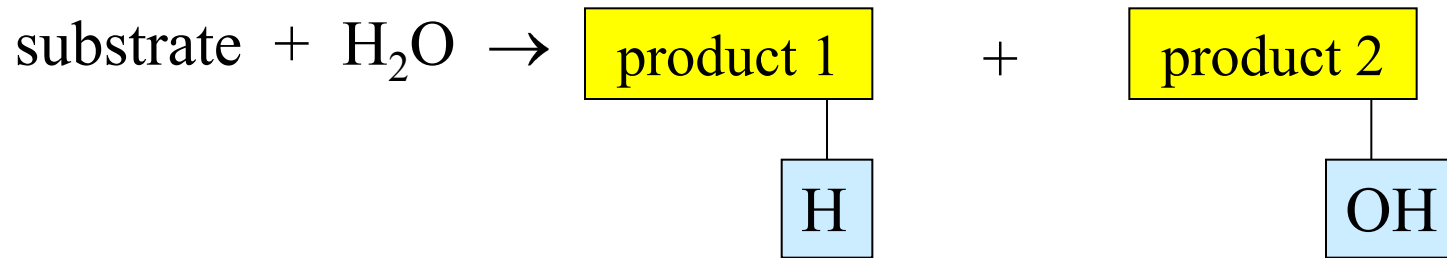
Reaction type: hydration    Enzyme: fumarase    Cofactor: none

Notes: 1) addition of water on double bond is **stereospecific**

2) hydration is not redox reaction

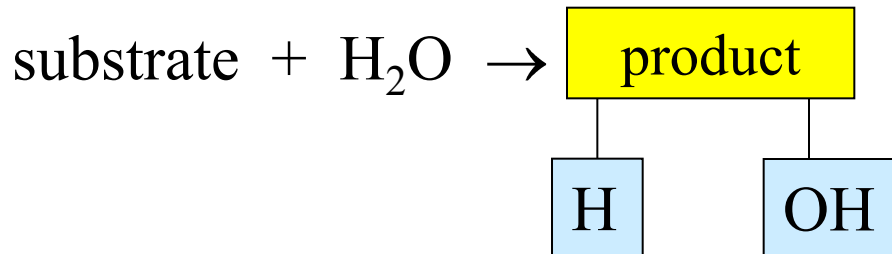
# Distinguish: hydrolysis      hydration

**Hydrolysis = decomposition of substrate by the action of water**  
(typical in esters, amides, peptides, glycosides, anhydrides)

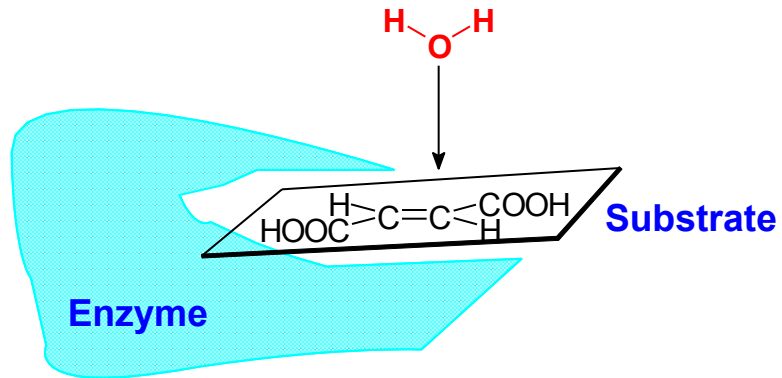


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**Hydration = addition of water** (to unsaturated substrates)

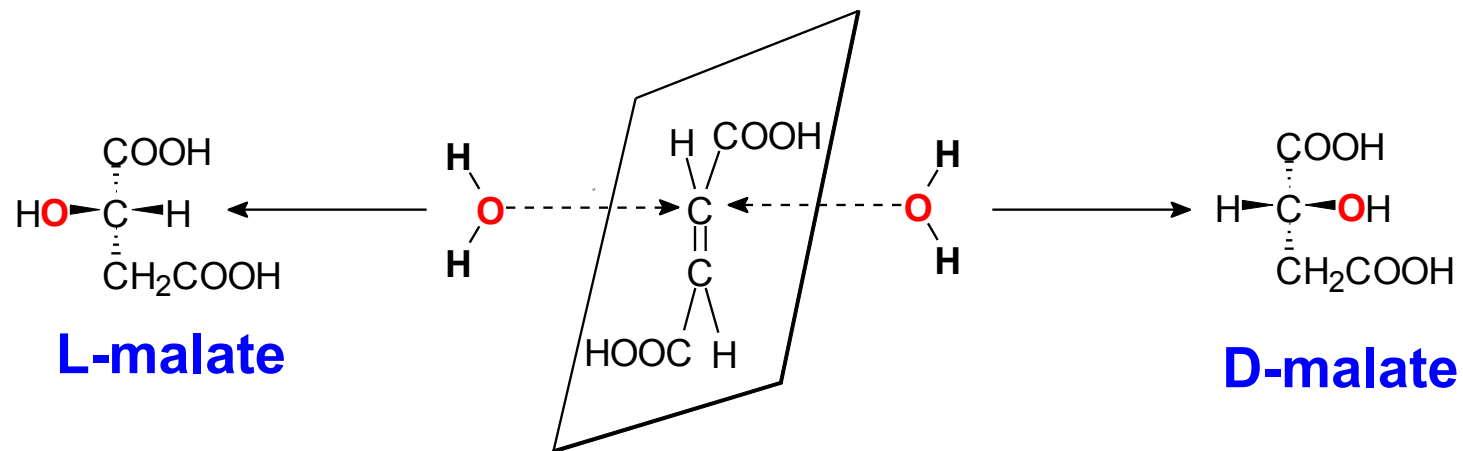


# Compare: Hydration of fumarate *in vivo* and *in vitro*



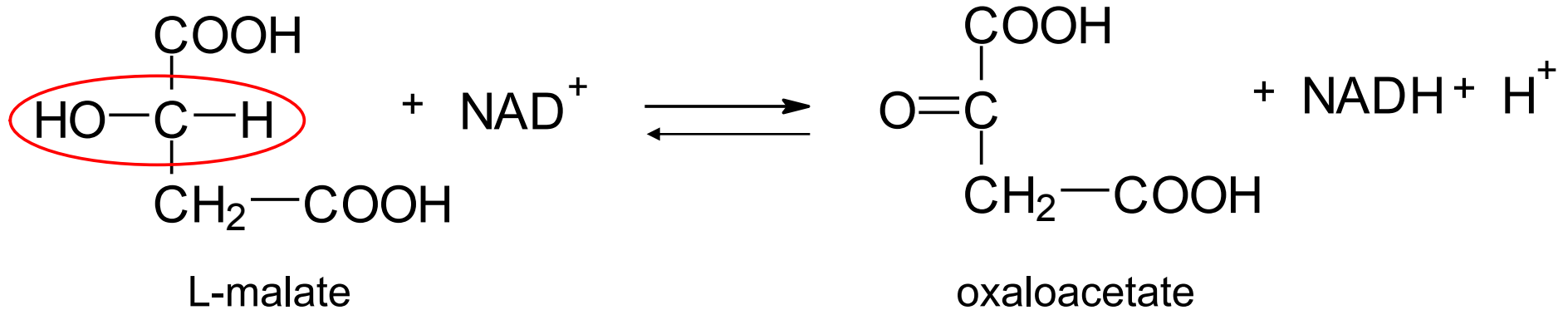
*in vivo*: (enzymatic reaction):

only one enantiomer is formed (L-malate)



*in vitro*: formation of racemate

## (8) L-malate → oxalacetate

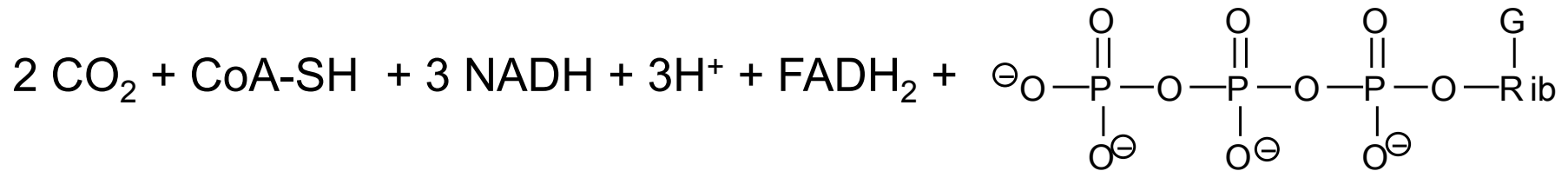
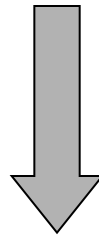
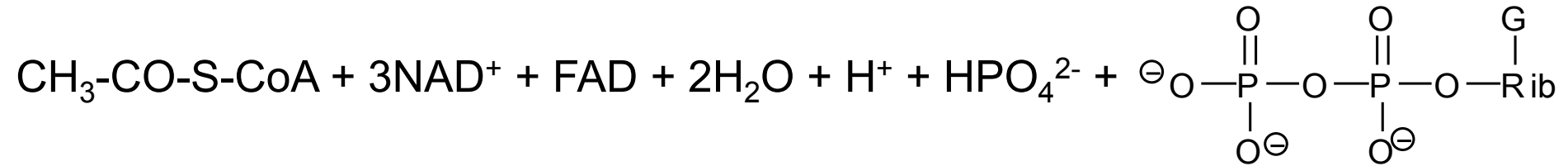


Reaction type: dehydrogenation

Enzyme: malate dehydrogenase

Cofactor:  $\text{NAD}^+$

# The net equation of citrate cycle



- two C atoms are completely oxidized to 2 CO<sub>2</sub>
- 8 H atoms are released in the form of reduced cofactors  
(3 NADH+H<sup>+</sup>, 1 FADH<sub>2</sub>)



# The energetic yield

## Products of CAC

## Equivalent of ATP (Resp. chain)

1 × GTP

1

3 × NADH + H<sup>+</sup>

9

1 × FADH<sub>2</sub>

2

**Total: 12 ATP\***

\* new calculations: 10 ATP

# Factors affecting CAC

- Energy charge of the cell:
- ATP/ADP ratio and NADH/NAD<sup>+</sup> ratio
- Allosteric inhibition
- Inhibition by products
- Supply of oxygen - CAC can proceed only at aerobic conditions  
(reduced cofactors must be reoxidized in respiratory chain)

# Key enzymes for regulation of citrate cycle: irreversible reactions

Enzyme	ATP <sup>a</sup>	NADH <sup>a</sup>	Other effect
Pyruvate dehydrogenase	⊖	⊖	⊖ acetyl-CoA <sup>b</sup>
Citrate synthase	⊖		⊖ citrate <sup>b</sup>
Isocitrate dehydrogenase	⊖	⊖	⊕ ADP <sup>c</sup>
2-OG dehydrogenase		⊖	⊖ succinyl-CoA <sup>b</sup>

<sup>a</sup> allosteric inhibitor – signal of high energy status of cell

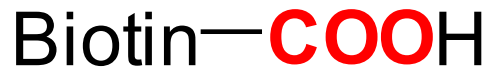
<sup>b</sup> feed-back inhibitor (inhibition by a product)

<sup>c</sup> allosteric activator

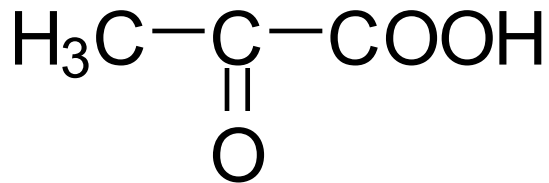
# Anaplerotic reactions of CAC

- Reactions that supply the intermediates of citrate cycle
- **Carboxylation of pyruvate → oxalacetate**
- (Reductive carboxylation of pyruvate → malate)
- Transamination of aspartate → oxaloacetate
- Catabolism of Phe + Tyr → fumarate
- Aspartate in the synthesis of urea/purines → fumarate
- Catabolism of Val, Ile, Met → succinyl-CoA
- Transamination of glutamate → 2-oxoglutarate

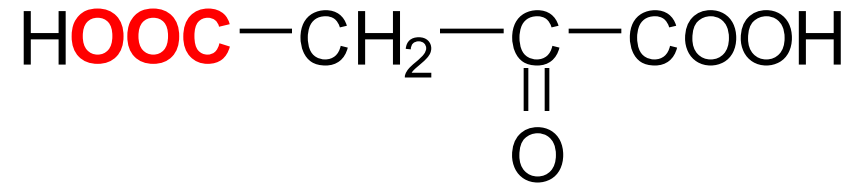
# Carboxylation of pyruvate (biotin)



pyruvate carboxylase

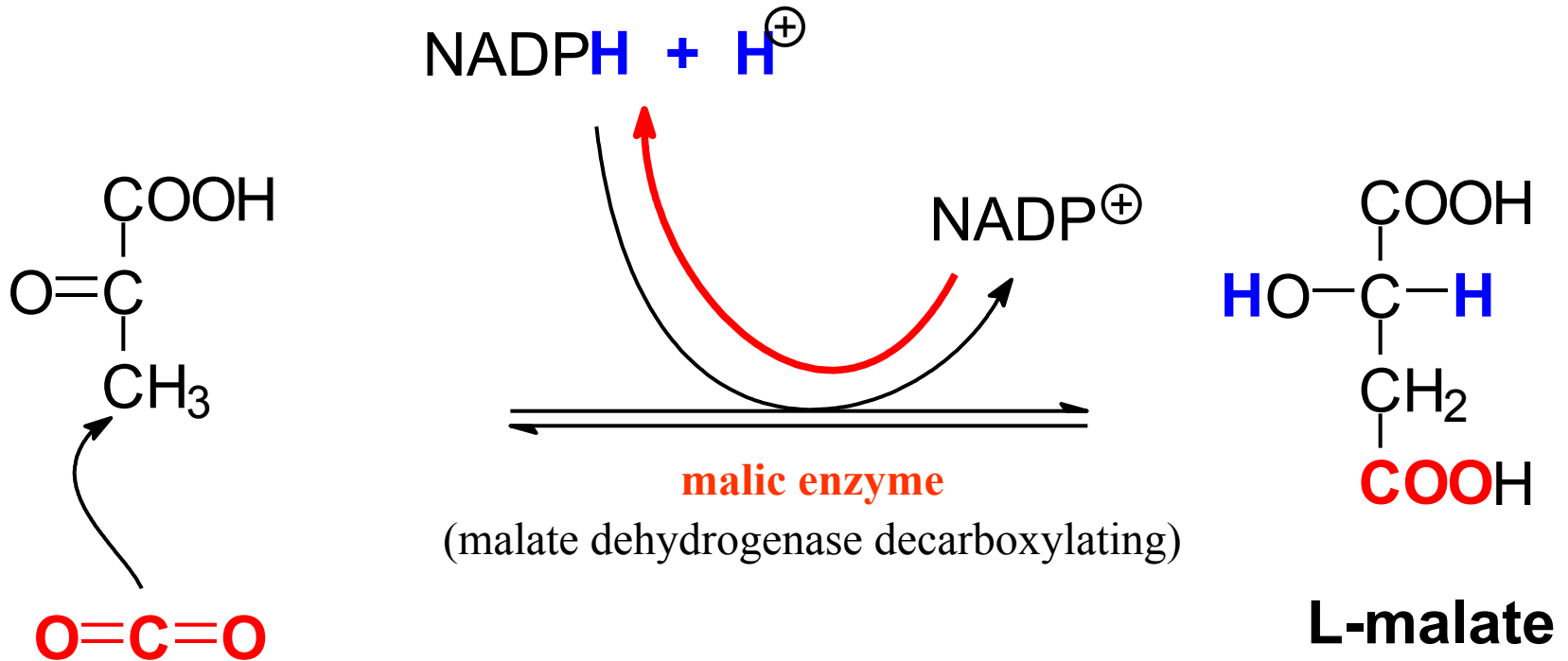


pyruvate



oxaloacetate

# Reductive carboxylation of pyruvate



Reaction is more important for production of NADPH for reductive synthesis (FA, cholesterol)

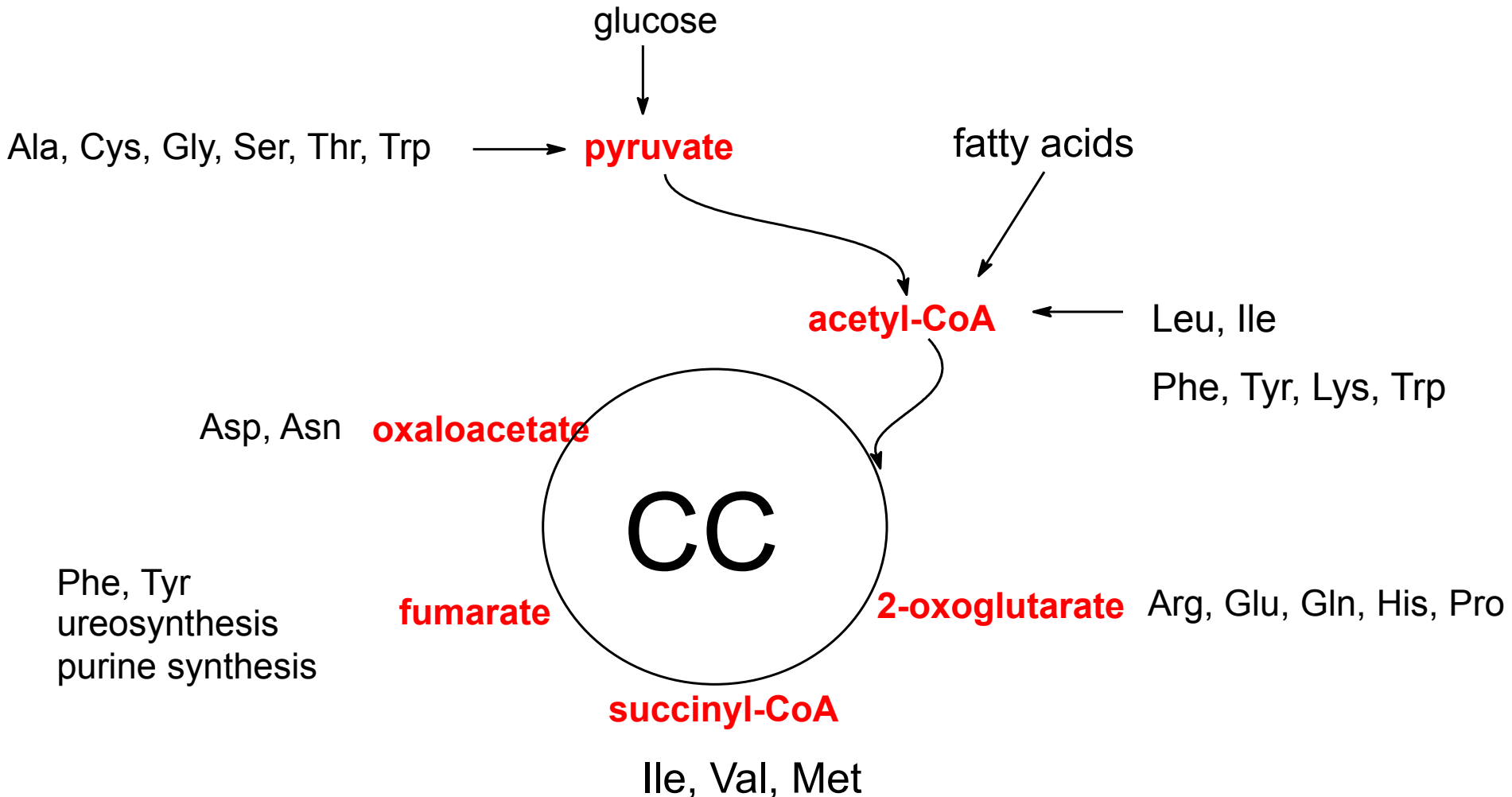
# Amphibolic character of CAC

Final **catabolic** pathway:  
oxidation of acetyl-CoA to  $2 \text{CO}_2$

Also other compounds,  
which are metabolized to CAC  
intermediates, can serve as substrates  
of the cycle

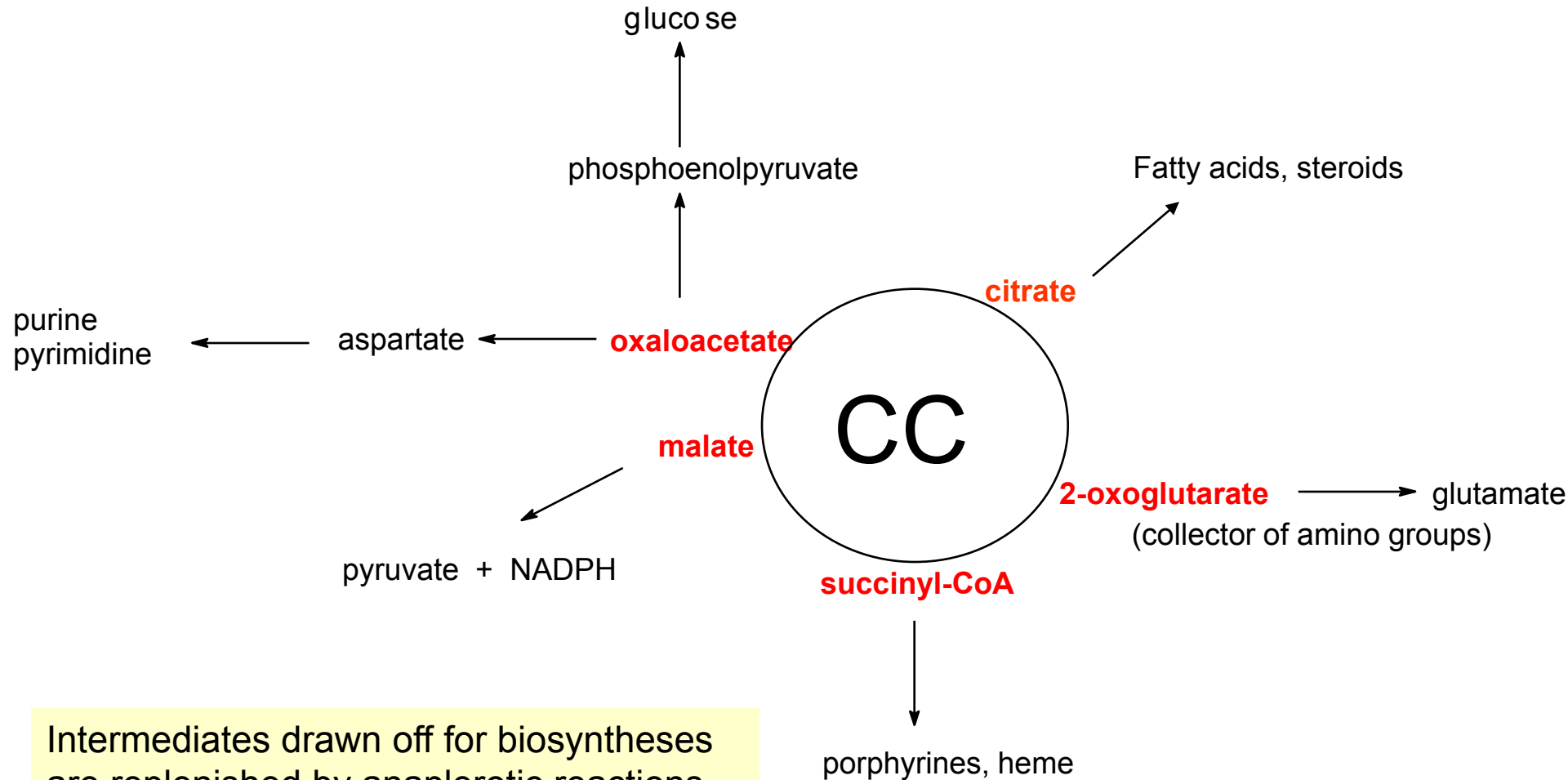
CAC provides important  
metabolic intermediates for  
**anabolic** processes:  
gluconeogenesis, transamination

# Catabolic processes - entries into the cycle

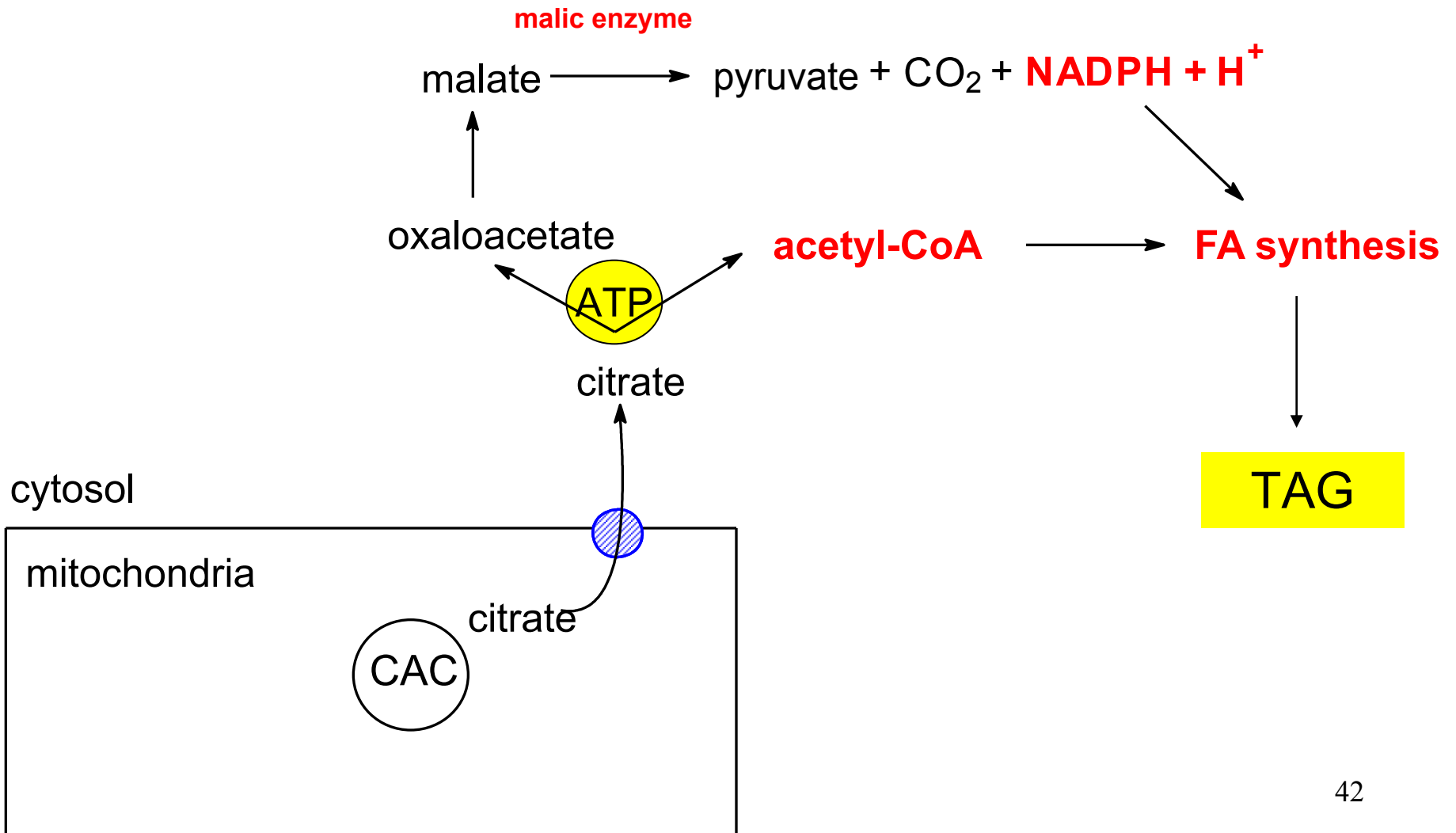




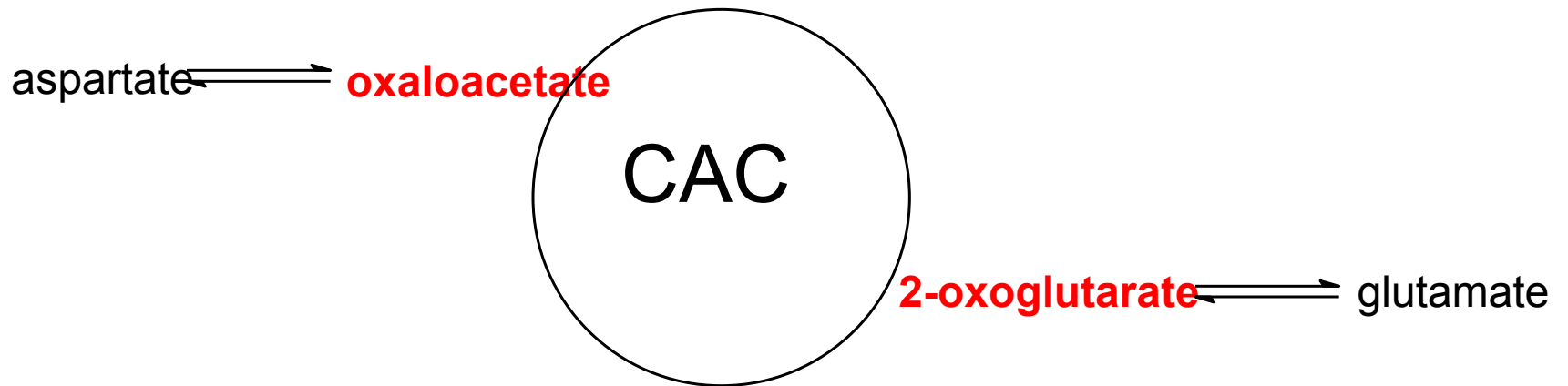
# Anabolic processes – intermediates for syntheses



# CAC and the synthesis of lipids



# CAC and transamination

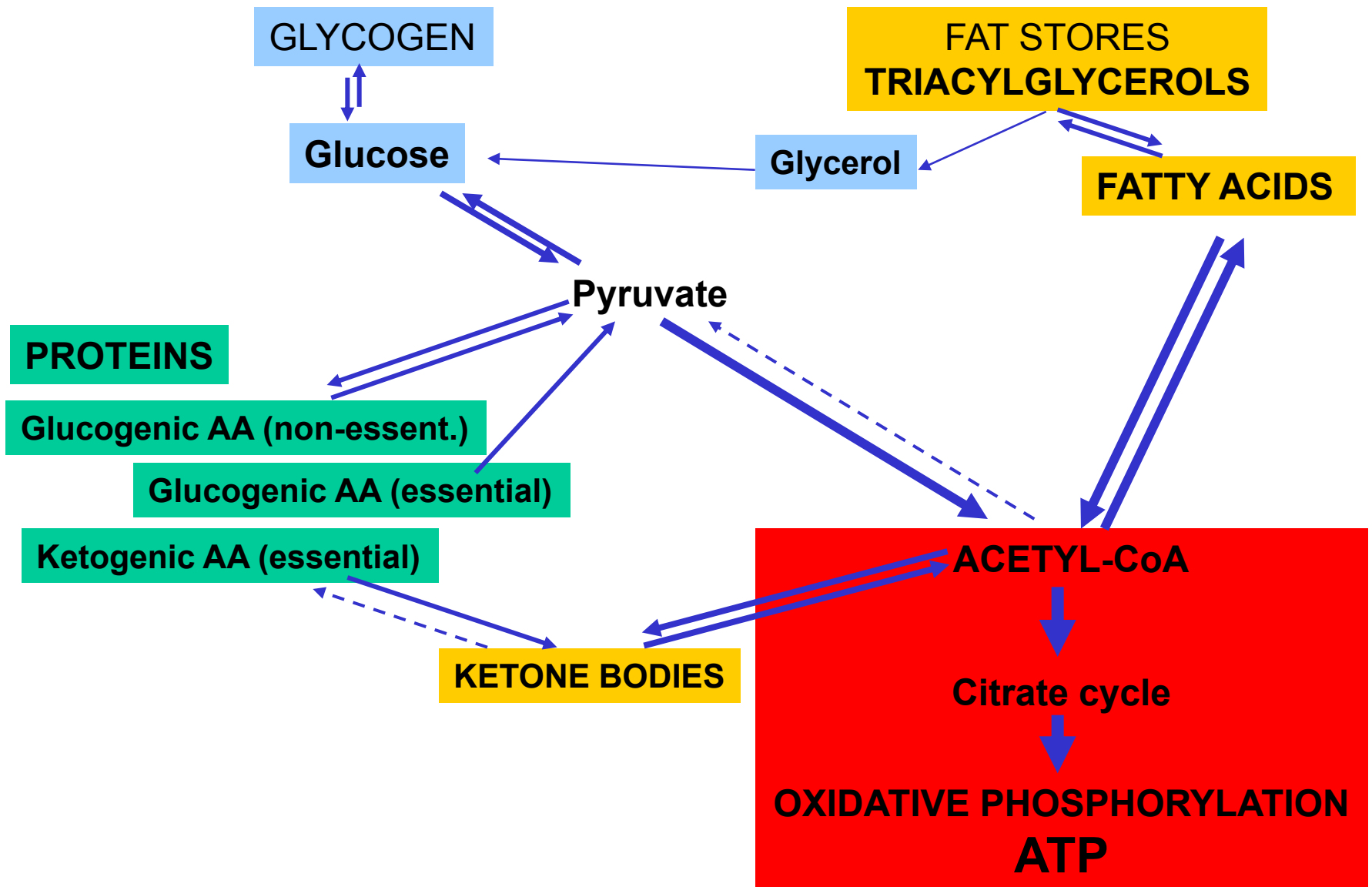


# CAC and vitamins

**Complete**

<b>Vitamin</b>	<b>Reaction in citrate cycle</b>
Riboflavin	
Niacin	
Thiamin	
Pantothenic acid	

# Relationships in major metabolic pathways



# Interconversions between nutrients

Interconversion	Commentary
Sugars → lipids	very easy and quickly
Lipids <del>→</del> glucose	not possible, pyruvate dehydrogenase reaction is irreversible
Amino acids → glucose	most AA are glucogenic
Glucose intermediates → AA	pyruvate and CAC intermediate provide carbon skeleton for some amino acids
Amino acids → lipids	in excess of proteins
Lipids <del>→</del> amino acids	pyruvate dehydrogenase reaction is irreversible ketogenic AA and most mixed AA are essential

**Saccharides** are the most **universal nutrients** –  
the overdose is transformed in the fat stores,  
carbon skeleton of non-essential amino acids can originate from saccharides.

**Triacylglycerols** exhibit the highest **energetic yield** –  
but **fatty acids cannot convert into saccharides** or the skeleton of amino acids.

**Amino acids** represent the unique **source of nitrogen** for proteosynthesis  
that serves as fuel rather when the organism is lacking in other nutrients -  
glucogenic amino acids can convert into glucose,  
a overdose of diet protein may be transformed in fat stores.

The metabolism of nutrients is sophisticatedly controlled with different mechanisms  
in the **well-fed state** (absorptive phase),

**short fasting** (post-absorptive phase), and in **prolonged starvation**.

It also depends on **energy expenditure** (predominantly muscular work) –  
either of maximal intensity (anaerobic, of short duration only)  
or aerobic work of much lower intensity (long duration).

## The tissues differ in their enzyme equipment and metabolic pathways

Pathway	Liver	CNS	Kidneys	Muscles	Adipocyte	Ery
CAC	+	+	+	+	+	-
FA $\beta$ -oxidation	++	-	+	++	-	-
FA synthesis	+++				+++	-
Ketogenesis	+	-	-	-	-	-
KB oxidation*	-	+	+	+++	+	-
Glycolysis	+	+++	+	+++	+	+++
Gluconeogenesis	+++	-	+	-	-	-

\* KB = ketone bodies



# Cellular compartmentation of major metabolic pathways

<b>Nucleus</b>	DNA replication, RNA synthesis (= DNA transcription)
<b>Mitochondria</b>	oxidative decarboxylation of pyruvate, CAC, RCh, FA $\beta$ -oxidation, synthesis of KB / urea / heme / Gln, AST reaction
<b>Rough ER</b>	proteosynthesis on ribosomes (translation of mRNA)
<b>Smooth ER</b>	synthesis of TAG / chol., FA desaturation, hydroxylations of xenobiotics
<b>Lysosomes</b>	non-specific hydrolysis of various substrates
<b>Cell membrane</b>	transport of molecules/ions/information = transporters/channels/receptors
<b>Golgi apparat.</b>	glycosylation of proteins, sorting and export of proteins
<b>Peroxisomes</b>	formation and decomposition of H <sub>2</sub> O <sub>2</sub> and peroxides
<b>Cytosol</b>	glycolysis, gluconeogenesis, glycogen metabolism, pentose cycle, transamination, synthesis of FA / urea / urate / heme; ethanol $\rightarrow$ acetaldehyde

# Metabolic effects of insulin

<b>Liver</b>	↑ Glucose phosphorylation
	↑ Glycolysis
	↓ Gluconeogenesis
	↑ Synthesis of glycogen
	↓ Glycogenolysis
	↑ Synthesis of fatty acids
	↑ Pentose phosphate cycle

<b>Adipose tissue</b>	↑ Glucose uptake (GLUT 4)
	↑ Glycolysis
	↑ Pentose phosphate pathway
	↑ Ox. decarboxylation of pyruvate
	↑ Hydrolysis of TG in lipoproteins
	↑ Synthesis of TG
	↓ Lipolysis

<b>Muscle</b>	↑ Glucose uptake (GLUT 4)
	↑ Glycolysis
	↑ Synthesis of glycogen
	↓ Glycogenolysis
	↑ Synthesis of proteins

# Metabolic effects of glucagon [not on muscles]

<b>Liver</b>	↓ Glycolysis
	↑ Gluconeogenesis
	↓ Synthesis of glycogen
	↑ Glycogenolysis
	↓ Synthesis of fatty acids
	↑ Oxidation of fatty acids
<b>Adipocytes</b>	↑ Lipolysis (HSL, hormone sensitive lipase)

**Biosynthesis of heme**

~

**Hemoproteins**

# Heme

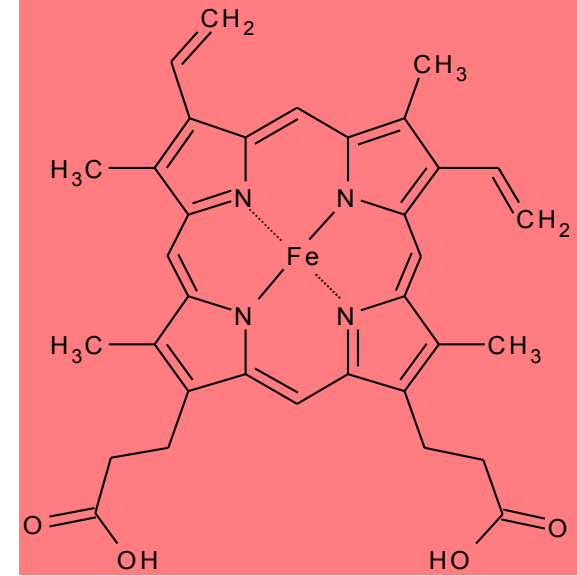
Prosthetic group of many proteins  
(hemoglobin, myoglobin, cytochromes)

Synthesis in the body:

70-80 % in erythroid cells in bone marrow - hemoglobin

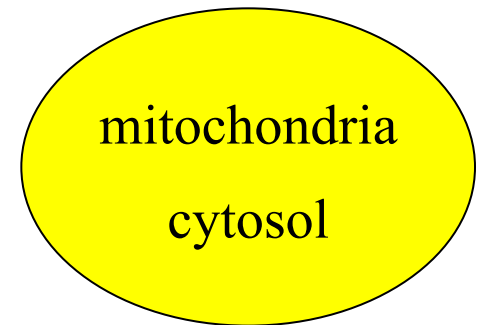
15 % liver – cytochromes P450 and other hemoproteins

Heme consists of porphyrin ring coordinated with iron cation



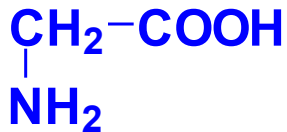
# Biosynthesis of heme

- initial compound for synthesis is succinyl-CoA (intermediate of CAC)
- source of nitrogen is glycine
- reactions are located in mitochondria and cytosol
- regulation: ALA-synthase

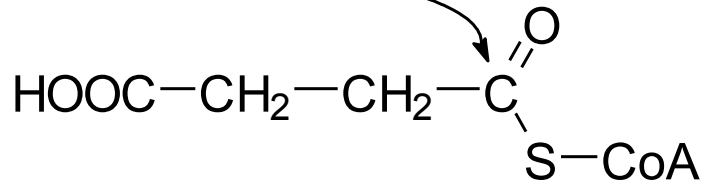


# Synthesis of $\delta$ -aminolevulinate (ALA)

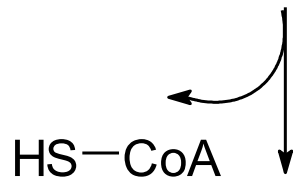
mitochondria



glycin

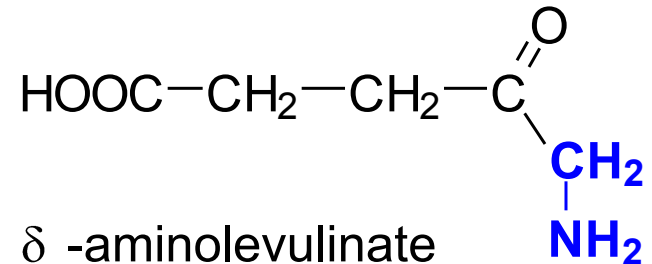
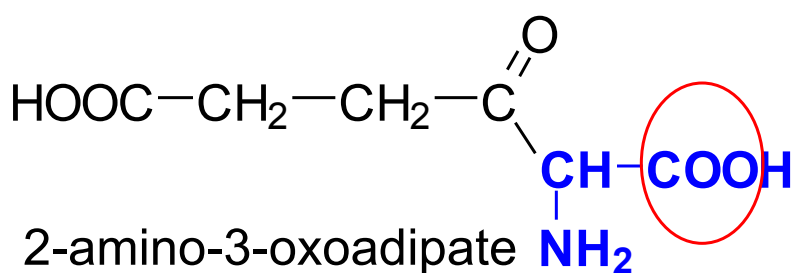


succinyl-CoA



ALA-synthase

pyridoxalphosphate  
is cofactor



(5-amino-4-oxobutanoic acid)

# ALA-synthase is the rate-controlling enzyme of porphyrine biosynthesis

Half-life about 1 hour

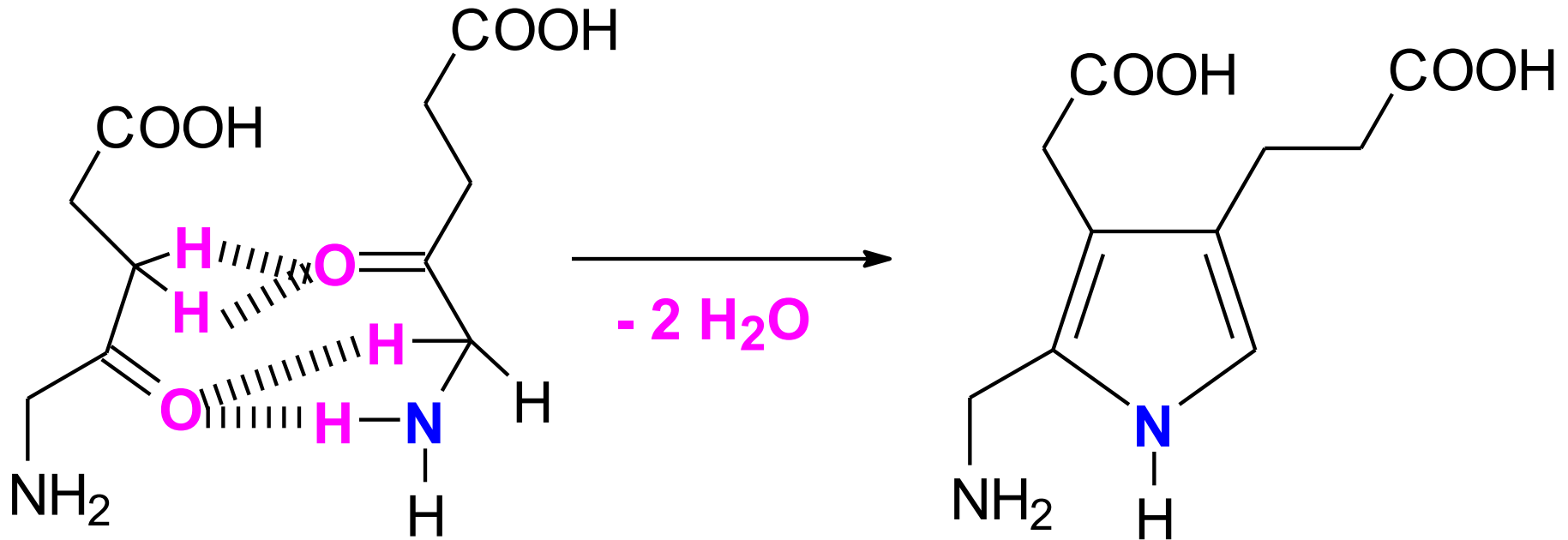
ALA-synthase

- is inhibited by heme (allosteric inhibition)
- synthesis of enzyme is repressed by heme
- is induced by some drugs (barbiturates, phenytoin, griseofulvin)
- cytochrome P-450 is needed for biotransformation of drugs/xenobiotics



# Condensation to substituted pyrrole

cytosol

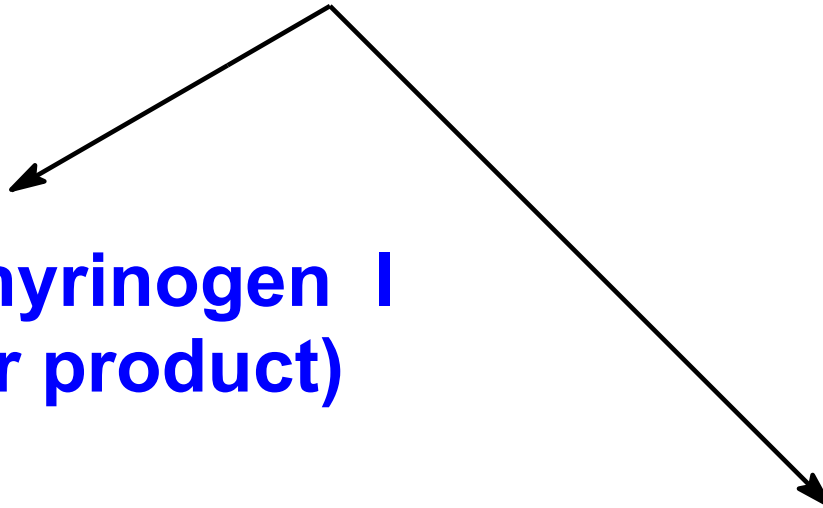


$\delta$ -aminolevulinate

porphobilinogen

# Condensation of porphobilinogen

## Porphobilinogen



**uroporphyrinogen I**  
**(minor product)**

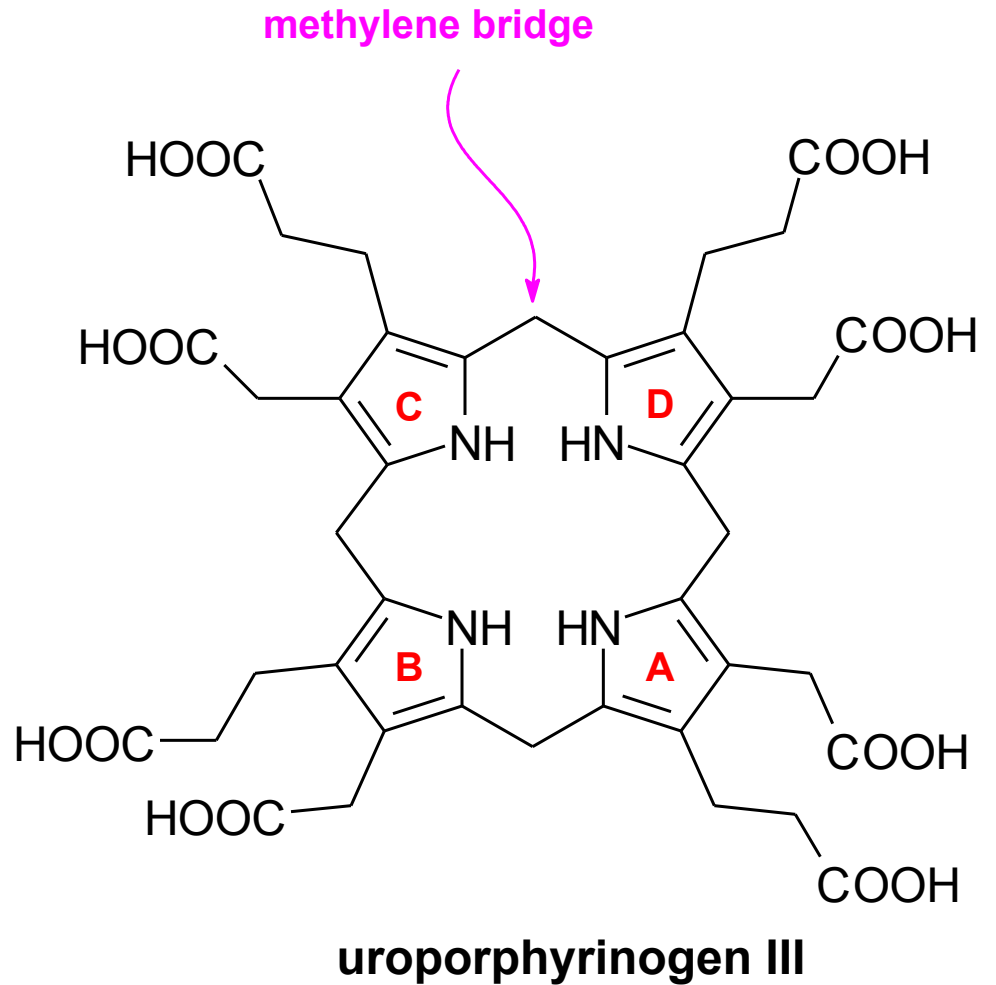
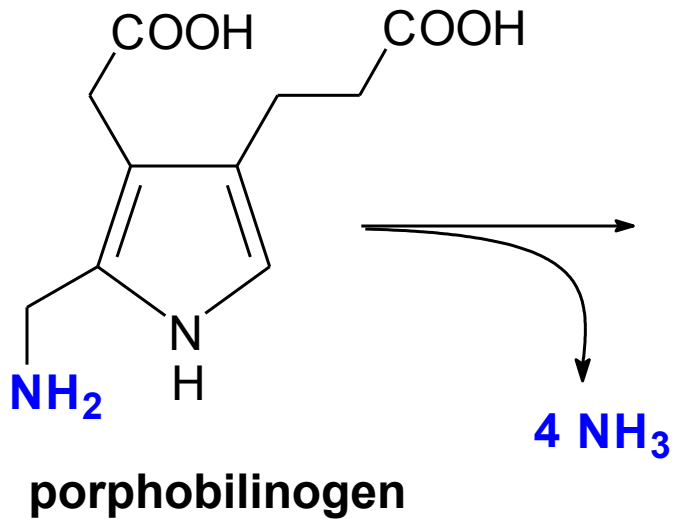
**uroporphyrinogen III**  
**(main product)**

Under physiological circumstances, due to the presence of a protein modifier called co-synthase, uroporphyrinogen III with an **asymmetrical** arrangement of side chains of the ring D is formed. Only traces of symmetrical uroporphyrinogen I are produced

# Condensation of porphobilinogen

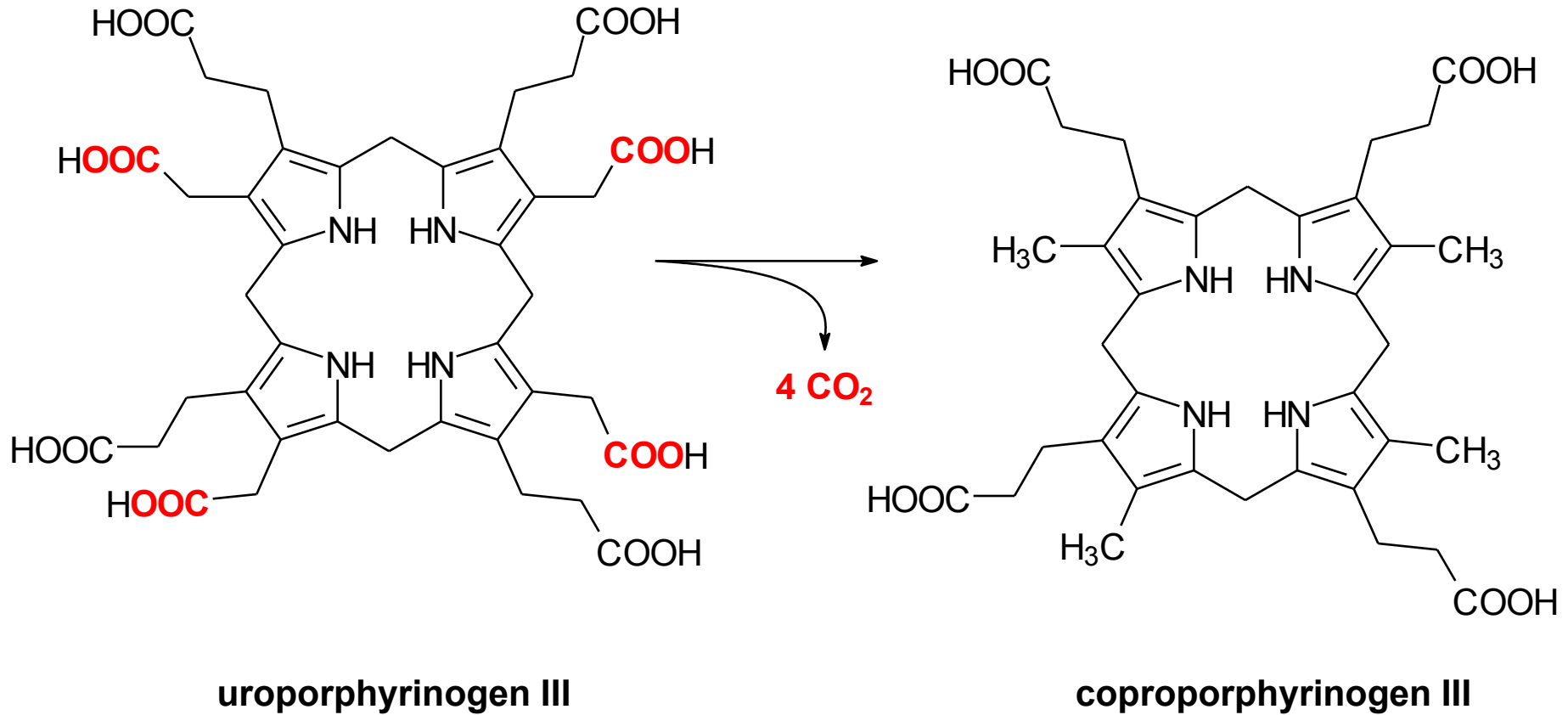
cytosol

4



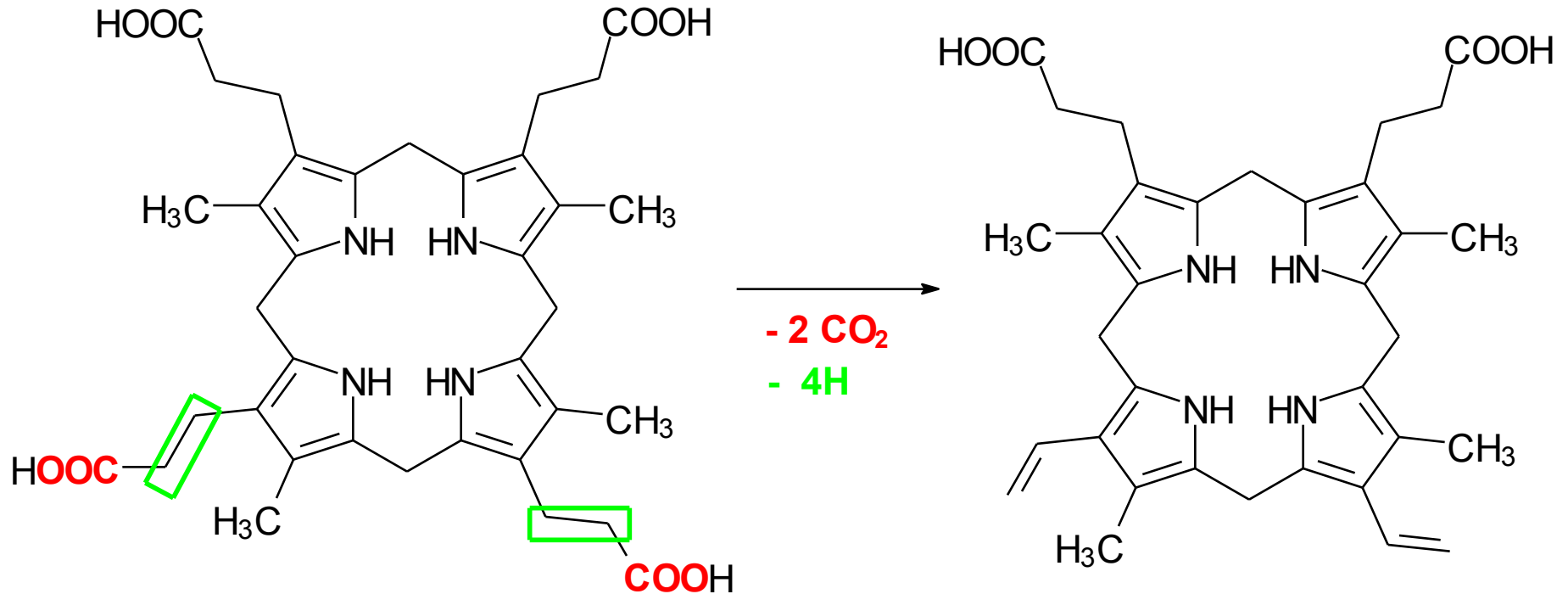
# Decarboxylation of four acetates – formation of methyl groups

cytosol



# Formation of vinyl groups from two propionates

mitochondria

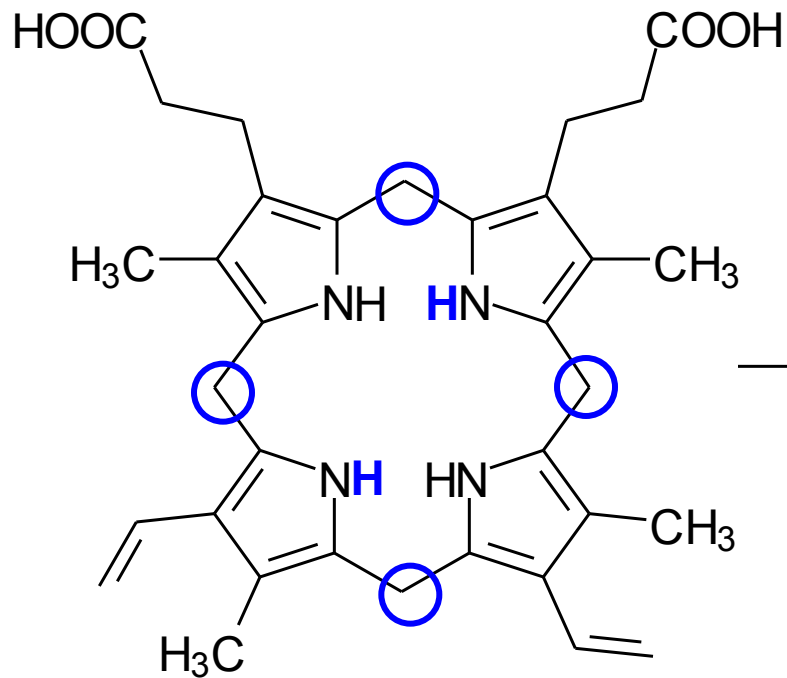


coproporphyrinogen III

protoporphyrinogen IX

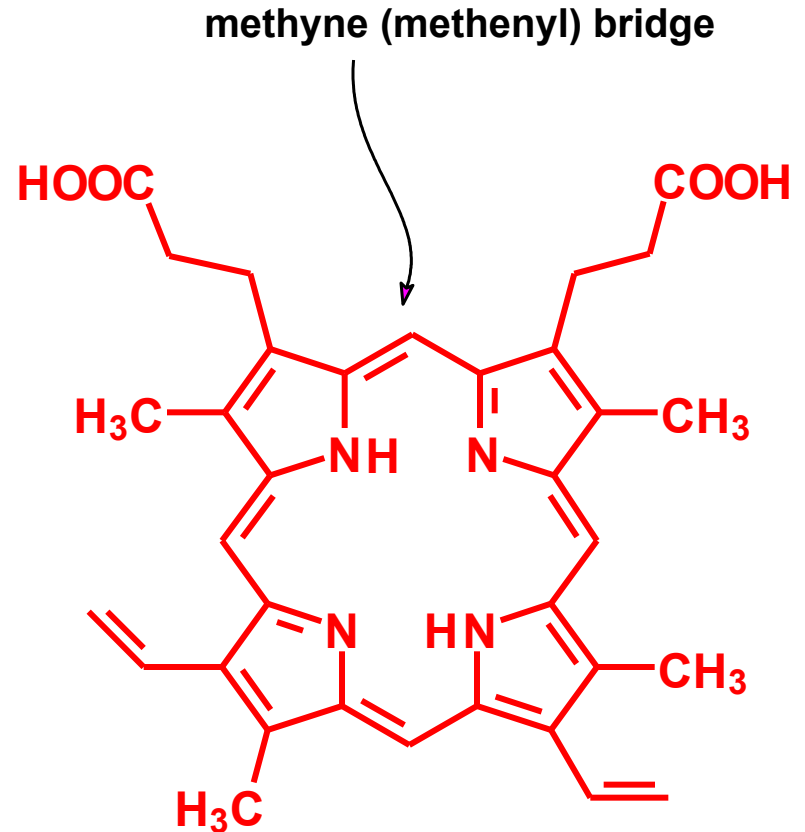
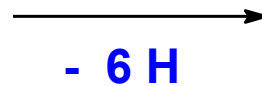
# Formation of conjugated system

mitochondria



protoporphyrinogen IX

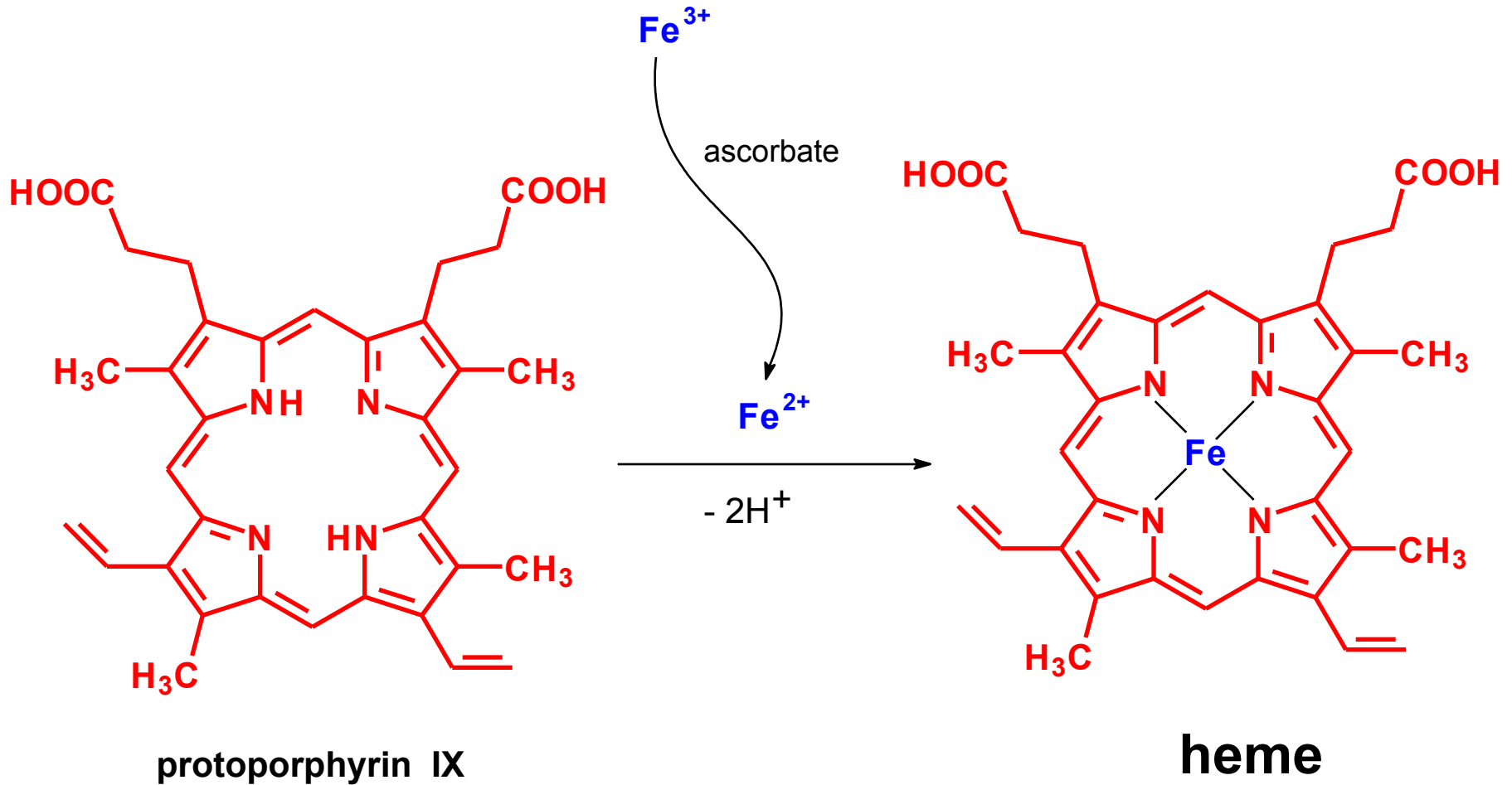
colourless



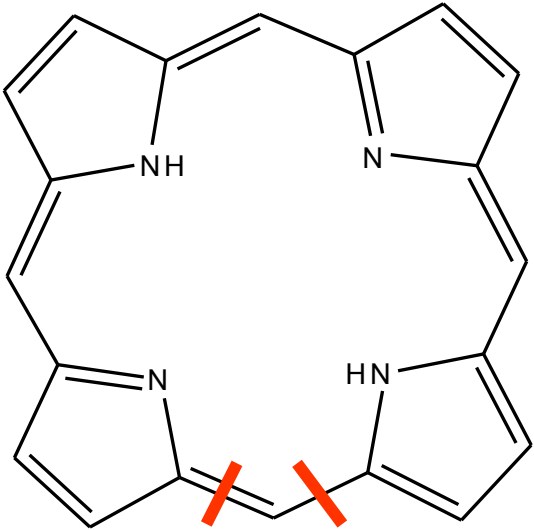
protoporphyrin IX

red

# Heme is coloured chelate with $\text{Fe}^{2+}$



# CO and bilirubin are formed by the degradation of heme



$3 \text{ O}_2$   
 $3 \text{ NADPH} + \text{H}^+$       ↓      oxidative cleavage (heme oxygenase)

CO + biliverdin

↙  
carbonyl-hemoglobin

↓  
bilirubin

more details  
in the 4<sup>th</sup> semester



# Porphyrias are caused by partial deficiency of one of the heme synthesizing enzymes

## Primary (genetic)

- Defective enzyme of heme biosynthesis
- Overproduction and accumulation of intermediates (ALA, PBG)
- Porphyrinogens in skin - photosensitivity

## Secondary

- Inactivation of enzymes as a consequence of disease or poisoning
- Similar symptoms

# Hemoproteins

Protein	Redox state of Fe	Function
Hemoglobin	$\text{Fe}^{2+}$	Transport of $\text{O}_2$ in blood
Myoglobin	$\text{Fe}^{2+}$	Deposit of $\text{O}_2$ in muscles
Catalase	$\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+}$	Decomposition of $\text{H}_2\text{O}_2$
Peroxidase	$\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+}$	Decomposition of peroxides
Cytochroms	$\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+}$	Components of resp. chain
Cytochrom P-450	$\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+}$	Hydroxylation
Desaturases of FA	$\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+}$	Desaturation of FA

# Oxidation number of Fe in various hemes

## Does not change

- $\text{Fe}^{2+}$
- prosthetic group for  $\text{O}_2$  transport
- hemoglobin, myoglobin
- heme is hidden in hydrophobic pocket of globin
- oxidation of  $\text{Fe}^{2+}$  means the loss of function

## Does change

- $\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+}$
- cofactor of oxidoreductases
- cytochromes, heme enzymes
- heme is relatively exposed
- reversible redox change is the primary function = the transfer of one electron

# Hemoglobin and myoglobin bind O<sub>2</sub>

## Hemoglobin

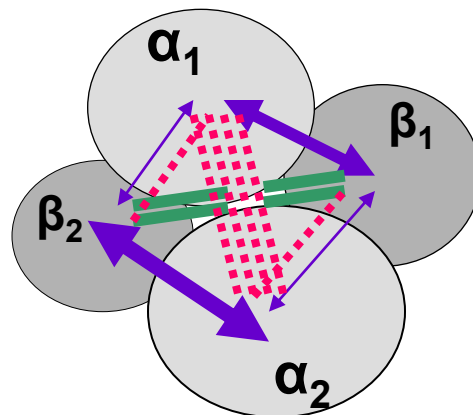
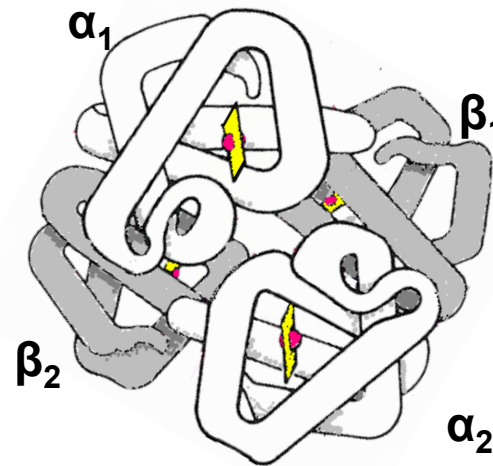
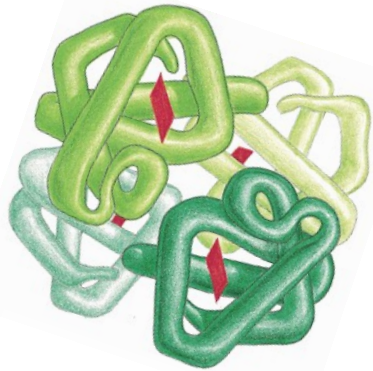
- transports O<sub>2</sub> from lungs to tissues
- from tissues to lungs, it transports some H<sup>+</sup> and CO<sub>2</sub> (carbamino-Hb)
- tetramer ⇒ sigmoidal saturation curve
- two conformations: T-deoxyHb(2,3-BPG), R-oxyHb
- binding O<sub>2</sub> in lungs → release of H<sup>+</sup>
- binding H<sup>+</sup> in tissues → release of O<sub>2</sub>
- buffer system in erythrocytes (His)

Bohr  
effect !

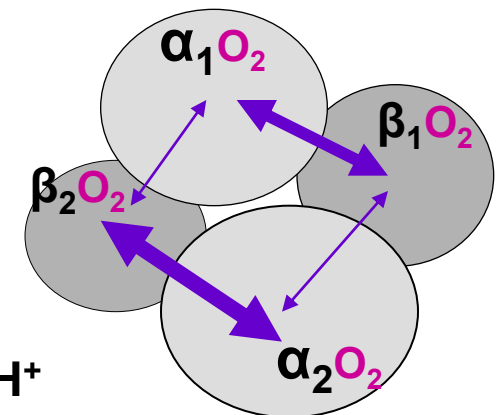
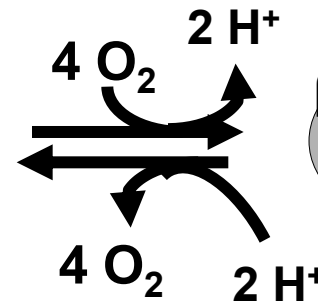
## Myoglobin

- muscle hemoprotein – deposition (reserve) of oxygen
- monomer ⇒ saturation curve hyperbolic = stronger binding O<sub>2</sub>

# Quaternary structure of hemoglobin



deoxygenated hemoglobin  
(2,3-bisphosphoglycerate)  
T-conformation



oxyhemoglobin  
R-conformation

# Derivatives of hemoglobin

## Carbonylhemoglobin

- CO has great affinity to  $\text{Fe}^{2+}$  in heme
- physiological level: 1 - 15 % from total Hb (environment, smokers etc.)

## Glycated hemoglobin

- non-enzymatic reaction with free glucose,  $-\text{NH}_2$  group of Hb (N-terminus, Lys) and aldehyde group of glucose
- physiological level: 2.8 – 4.0 % (from total Hb)

## Methemoglobin (hemoglobin)

- oxidation of heme iron,  $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ , physiol. level: 0.5 - 1.5 %
- oxidation agents: nitrites, alkyl nitrites, aromatic amines, nitro compounds
- Hb mutation: hemoglobin M (HbM), the replacement of F8<sup>His</sup>→<sup>Tyr</sup>
- deficit of methemoglobin reductase

# Language note: Methemoglobin

- **it has nothing to do with methyl group !!!**
- abbreviated from *metahemoglobin*
- the prefix *meta-* (from Greek) indicates change, transformation, alteration
- other examples with the prefix *meta*:  
metabolic (= catabolic + anabolic)  
metamorphosis, metazoan ...

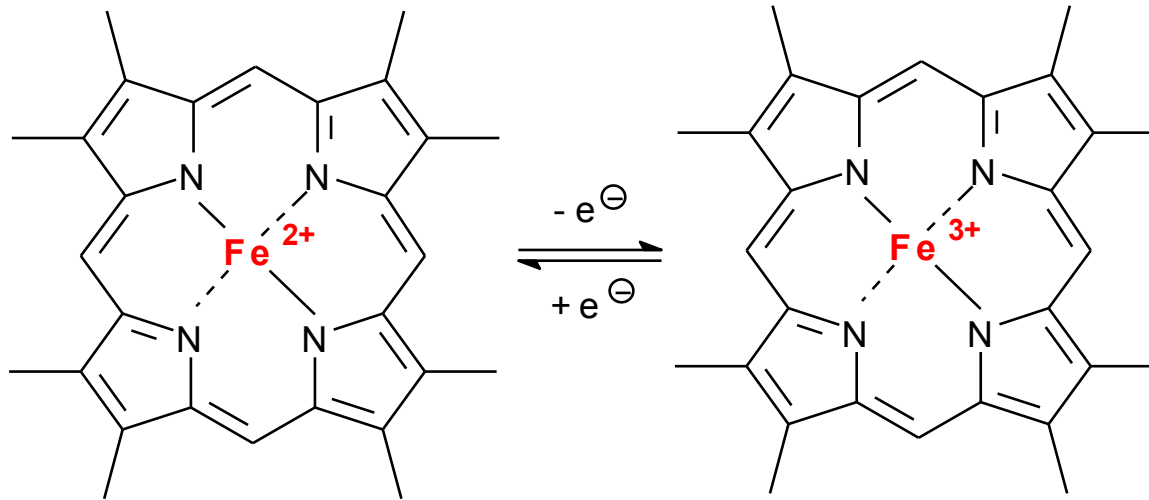
# Linguistic note:

## How to express two redox states of iron

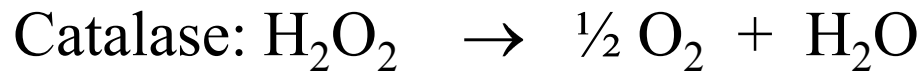
	Fe <sup>2+</sup>	Fe <sup>3+</sup>
Infix	<b>-o</b>	<b>-i</b>
Biochemical names	hem <u>o</u> globin, ferro <u>o</u> portin, ferro <u>o</u> xidase	hem <u>i</u> globin = methemoglobin, ferr <u>i</u> tin, transferr <u>i</u> n, lactoferr <u>i</u> n, gastroferr <u>i</u> n, ferr <u>i</u> c reductase
Chemical names		
Latin	ferro <u>o</u> si chloridum	ferr <u>i</u> chloridum
Old English	ferr <u>o</u> s chloride	ferr <u>i</u> c chloride
New English	iron(II) chloride	iron(III) chloride



# Heme as cofactor of oxidoreductases transfers one electron



## Examples of heme enzymes



# Cytochrome P450 (CYP)

- superfamily of **heme** enzymes (many isoforms)
- catalyze mainly **hydroxylation** of various substrates
- exhibits wide substrate specificity (advantage for the body)
- can be induced and inhibited by many compounds
- occurs in most tissues (except of muscles and erythrocytes)
- the highest activity in the liver (ER)

Abbreviation: P = pigment, 450 = wave length (nm) of a absorption peak after binding CO

# Hydroxylation by CYP 450 occurs in endogenous and exogenous substrates

- Endoplasmic reticulum:

squalene, cholesterol, bile acids, calciol,

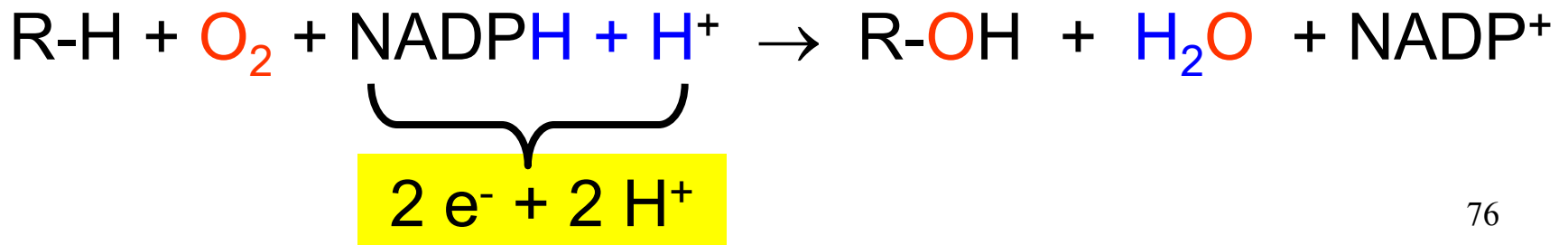
FA desaturation, prostaglandins, xenobiotics

- Mitochondria:

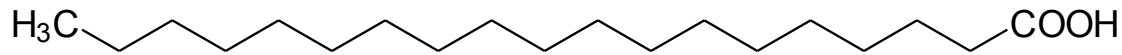
steroidal hormones

# Mechanism of hydroxylation

- the formation of hydroxyl group
- monooxygenase: one O atom from O<sub>2</sub> molecule is incorporated into substrate between C and H (R-H → R-OH )
- the second O atom and 2H from NADPH+H<sup>+</sup> produce water



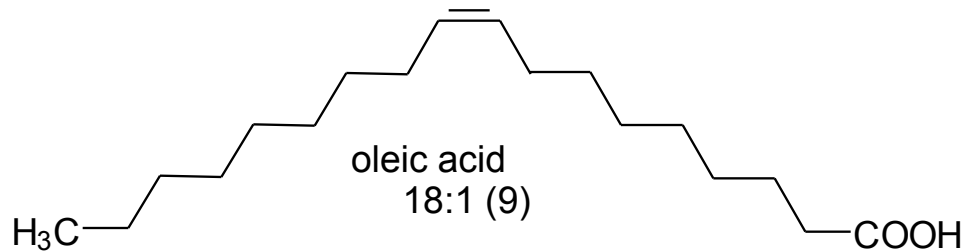
# Desaturation of fatty acids



stearic acid  
18:0

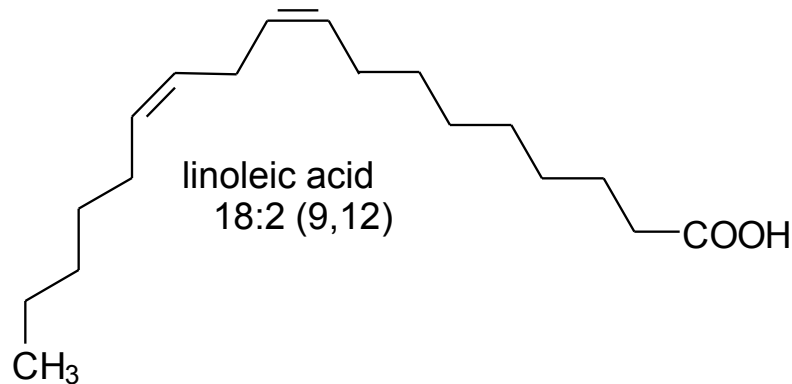
9-10 desaturation (humans)

$\Delta^9$  desaturase



oleic acid  
18:1 (9)

12-13 desaturation (plants)



linoleic acid  
18:2 (9,12)

# Desaturation of FA requires cytochrome b<sub>5</sub>

