Enzymes I

General features, cofactors

Literature for Biochemistry I

- Lecture files on *is.muni.cz*.
- Tomandl J., Táborská E.: *Biochemistry I – Seminars*. MU, 2012
- Harvey R.A., Ferrier D.R.: *Biochemistry*. 5th ed., Lippincott Williams & Wilkins, 2011.
- Koolman J., Röhm K.H.: *Color atlas of biochemistry*, Thieme, 2013.

General features of enzymes

CAUTION: peptidyltransferase is **ribozyme**

$$(AK)_n\text{-}tRNA_1 + AK\text{-}tRNA_2 \xrightarrow{rRNA} tRNA_1 + (AK)_{n+1}\text{-}tRNA_2$$

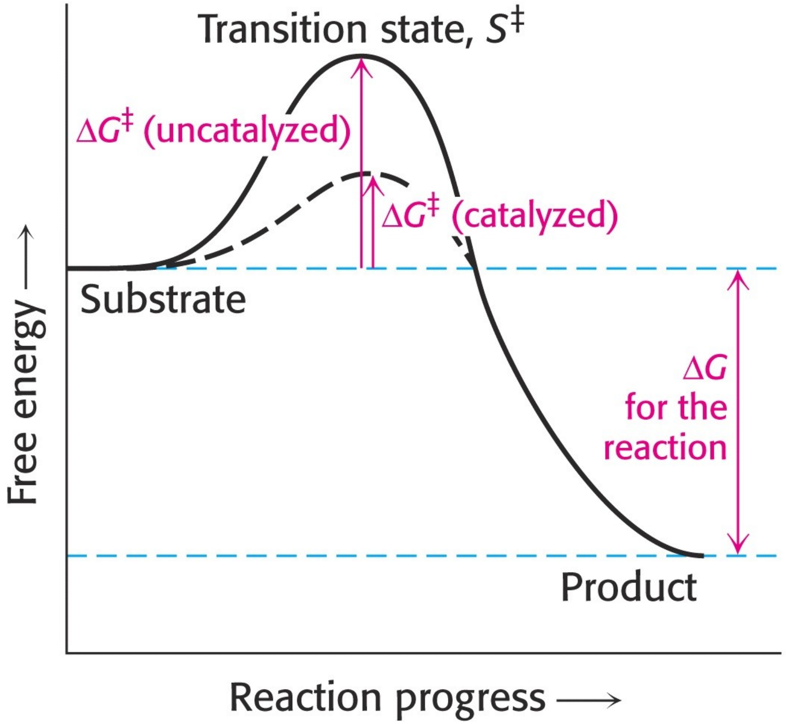
- biocatalysts
- different types of proteins / also RNA (ribozyme)

  with covalently attached prosthetic group and/or metal cation,

  oligomeric / multienzyme complexes / associated with membranes etc.
- different distribution in cell and in the body, make isoforms (isoenzymes)
- specific (towards substrate and reaction), highly effective
- work under mild conditions
- *in vivo* - can be regulated in two ways (activity of enzyme, quantity of enzyme)
- *in vitro* - sensitive to many factors

Enzymes are highly efficient catalysts

- decrease activation energy ⇒ **increase the reaction rate**
- much more efficient than other (inorganic) catalysts
- remain unchanged after reaction
- do not alter equilibrium constant K
- *in vitro* sensitive to many factors



Enzymes work under mild conditions

- narrow temperature range around **37 °C**
- over 50 °C become denaturated = inactivated
- narrow pH range ⇒ **pH optimum**
- most intracellular enzymes have pH optima around 7
- digestion enzymes function in rather stronger acidic / alkaline environment (pepsin 1-2, trypsin ~ 8)

Dual specifity of enzymes towards:

Reaction

catalyze just

one type of reaction

Substrate

work with **one substrate**

(or group of similar substrates)

often stereospecific

Enzymes are stereospecific catalysts

there are two types of stereospecific conversions:

1. **non-chiral substrate → chiral product$_{\text{(one enantiomer)}}$**

   pyruvate → L-lactate

   fumarate → L-malate

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2. **chiral substrate$_{\text{(one enantiomer)}}$ → product**

   L-alanine → pyruvate (D-alanine does not react)

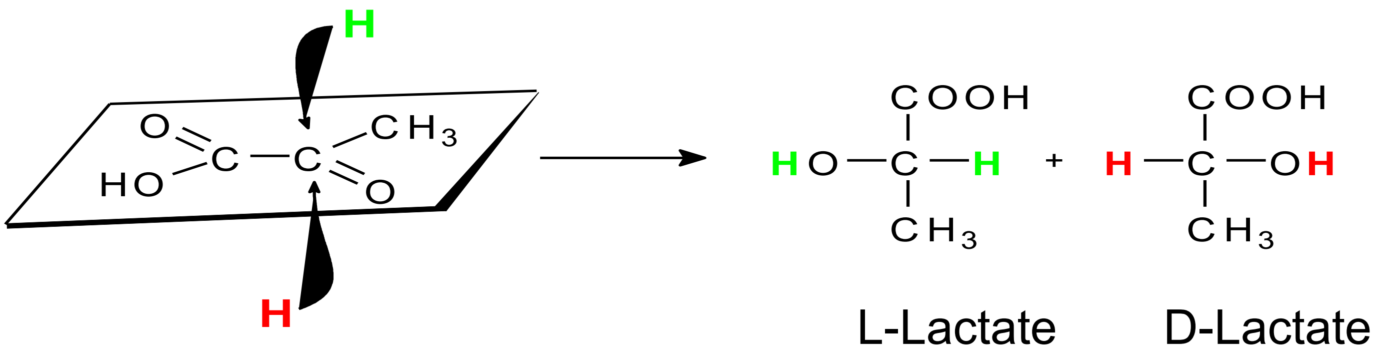
   D-glucose → → pyruvate (L-glucose does not react)

   chiral signal molecule → complex with receptor → biological response

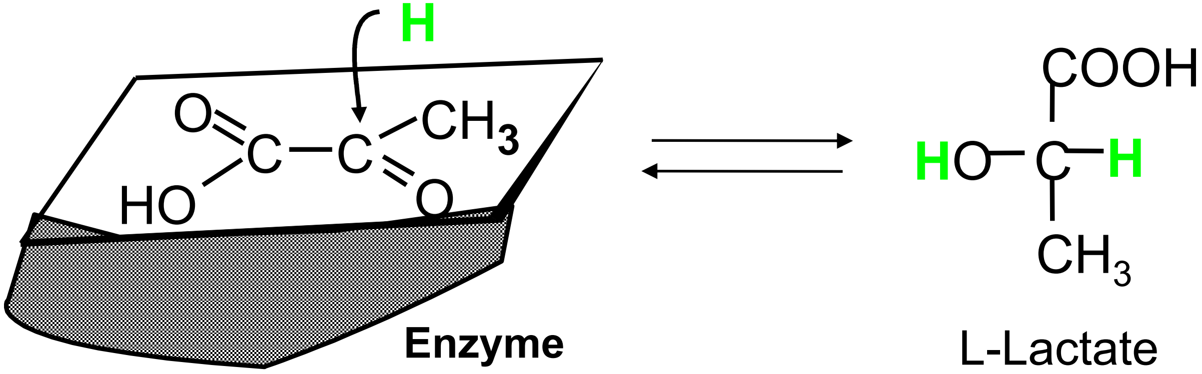
   chiral drug$_{\text{(ant)agonist}}$ → complex with receptor → pharmacological response

Hydrogenation of pyruvate

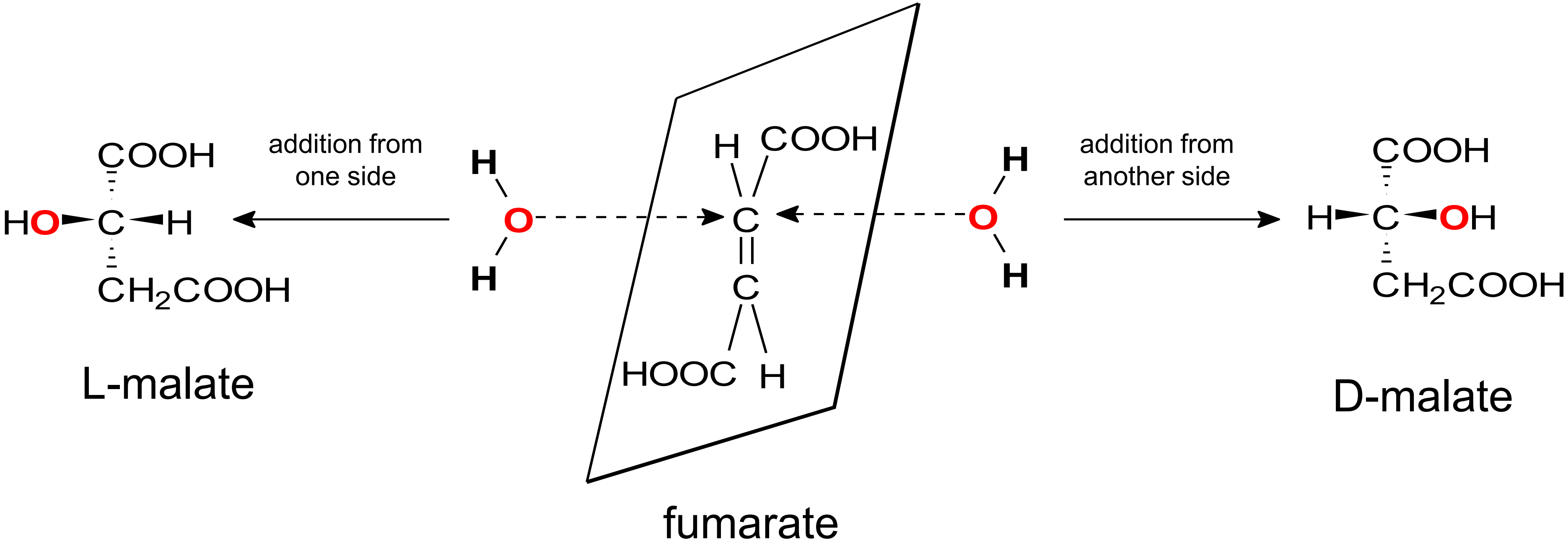
when pyruvate is hydrogenated without enzyme (*in vitro*),
reaction product is the **racemic mixture** of D-lactate and L-lactate:



in reaction catalyzed by lactate dehydrogenase (*in vivo*),
pyruvate is reduced stereospecifically to **L-lactate** only:

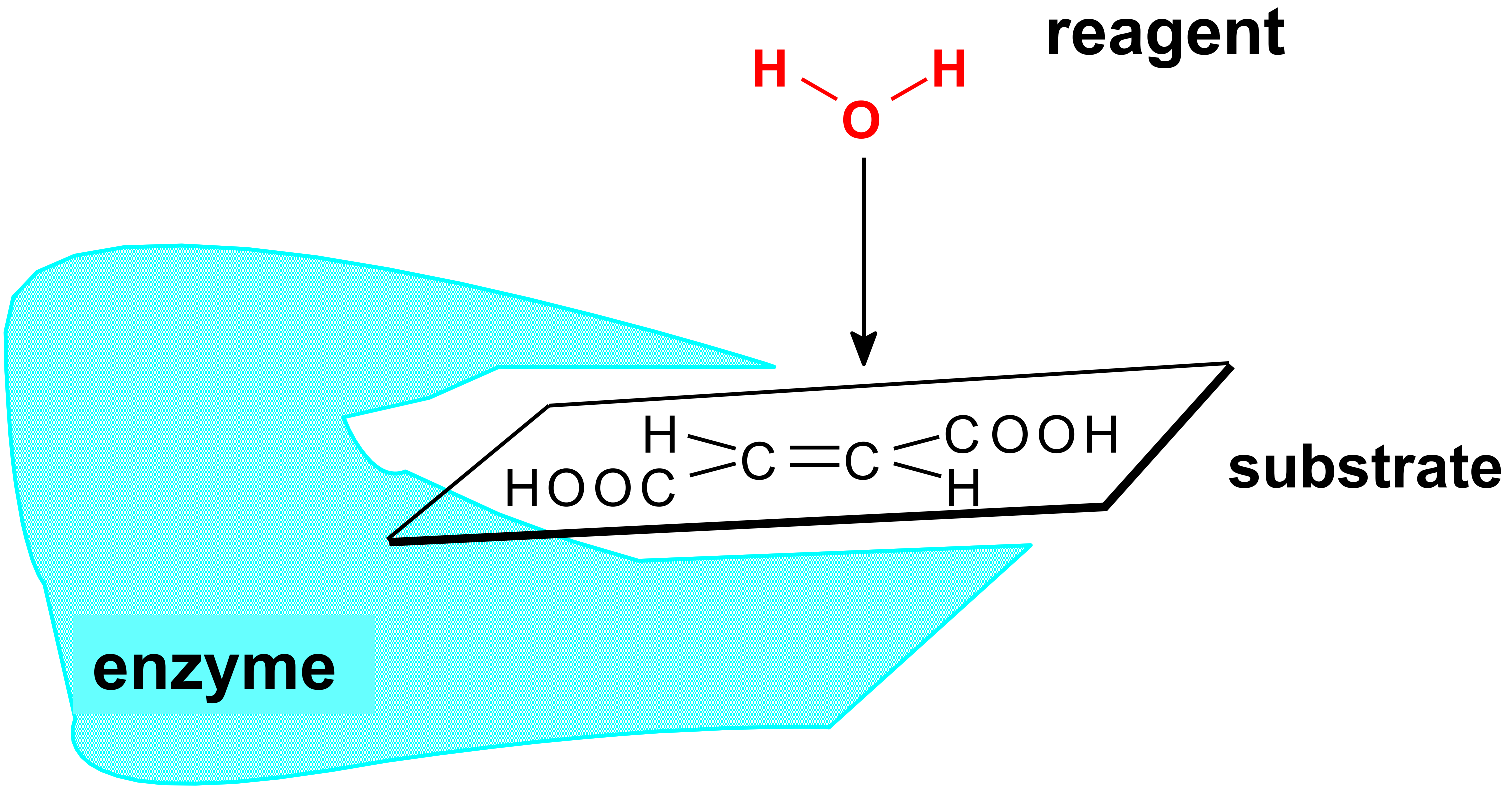


Non-enzymatic hydration of fumarate



in vitro reaction proceeds to racemic D,L-malate

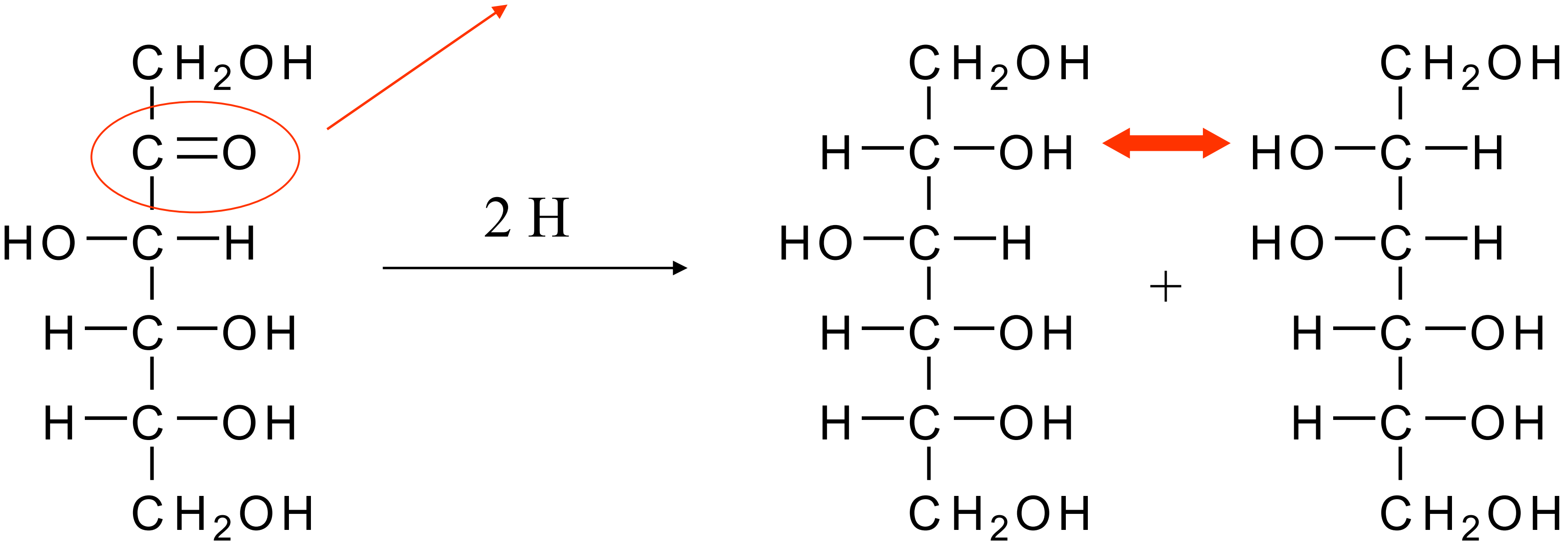
Enzymatic hydration of fumarate (citrate cycle)



in vivo just one enantiomer (L-malate) is produced

Hydrogenation of D-fructose *in vitro* gives two epimers

reaction site is planar



D-fructose D-glucitol D-mannitol

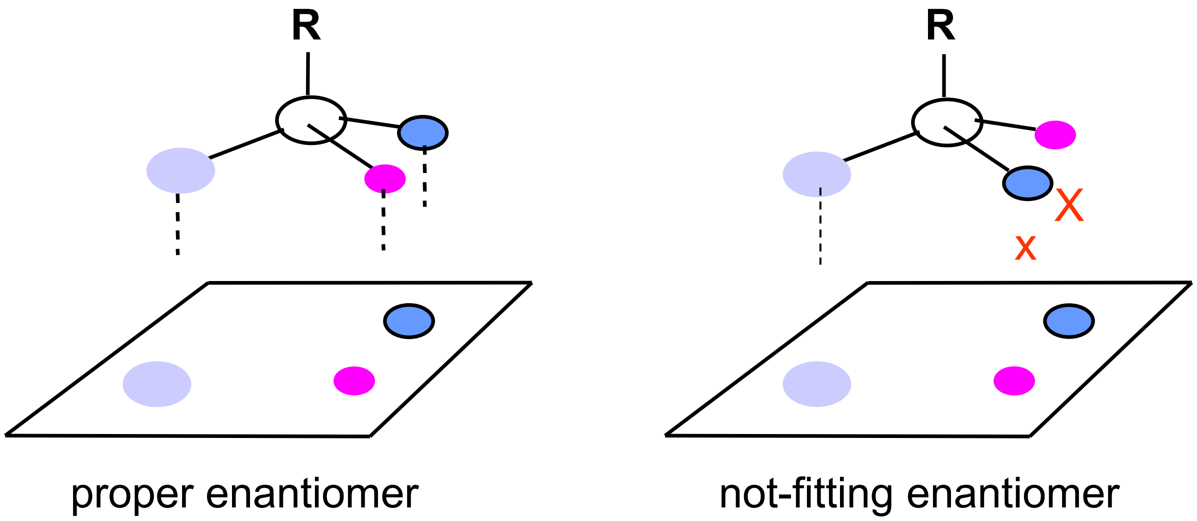
in vivo: enzymatic reaction gives just one product (D-glucitol)

Enzymes or receptors recognize only one enantiomer

If the reactant of enzymatic reaction is a chiral compound,
only one of two enantiomers is recognized as the specific substrate.

Chiral substrates/signal molecules are bound to the stereospecific enzymes/receptors at three sites:

see also
MCH II, p. 13



Enzyme nomenclature: the ending *-ase*

Systematic names identify the enzymes fully with the EC code number,

contain information about substrate and type of reaction,

not very convenient for everyday use.

Recommended (accepted) names are shorter than systematic names,

include also some historical names (pepsin, amylase)

EC (abbr. Enzyme Commission) of International Union of Biochemistry (IUB)
major class number
. subclass number
. sub-subclass number
. enzyme serial number

http://www.chem.qmul.ac.uk/iubmb/enzyme/

Examples of enzyme names

Recommended name: alcohol dehydrogenase

Systematic name: EC 1.1.1.1 ethanol:NAD$^+$-oxidoreductase

Reaction: ethanol + NAD$^+$ → acetaldehyde + NADH + H$^+$

Recommended name: alanine aminotransferase (ALT)

Systematic name: EC 2.6.1.2 L-alanine:2-oxoglutarate-aminotransferase

Reaction: L-alanine + 2-oxoglutarate → pyruvate + L-glutamate

Classification of enzymes: six classes according to reaction type

(each class comprises other subclasses)

Enzyme class	**General scheme of reaction**
1. Oxidoreductases	$A_{red} + B_{ox} \leftrightarrows A_{ox} + B_{red}$
2. Transferases	$A\text{-}B + C \rightarrow A + C\text{-}B$
3. Hydrolases	$A\text{-}B + H_2O \rightarrow A\text{-}H + B\text{-}OH$
4. Lyases	$A\text{-}B \leftrightarrows A + B$ (reverse reaction: synthases)
5. Isomerases	$A\text{-}B\text{-}C \leftrightarrows A\text{-}C\text{-}B$
6. Ligases (synthetases)	$A + B + ATP \rightarrow A\text{-}B + ADP + P_i$

1 Oxidoreductases

catalyze the oxidation or reduction of substrate

subclasses:

- **dehydrogenases** catalyze the transfers of two H atoms
- **oxygenases** catalyze the incorporation of one/two O atoms into the substrate (monooxygenases, dioxygenases)
- **oxidases** catalyze transfers of electrons between substrates (e.g. cytochrome *c* oxidase, ferroxidase)
- **peroxidases** catalyze the decomposition of peroxides

Example: lactate + NAD^+ $\leftrightarrows$ pyruvate + NADH + H^+

Recommended name: lactate dehydrogenase

Systematic name: (*S*)-lactate:NAD^+ oxidoreductase

2 Transferases

catalyze the transfer of a group from one to another substrate

subclasses:

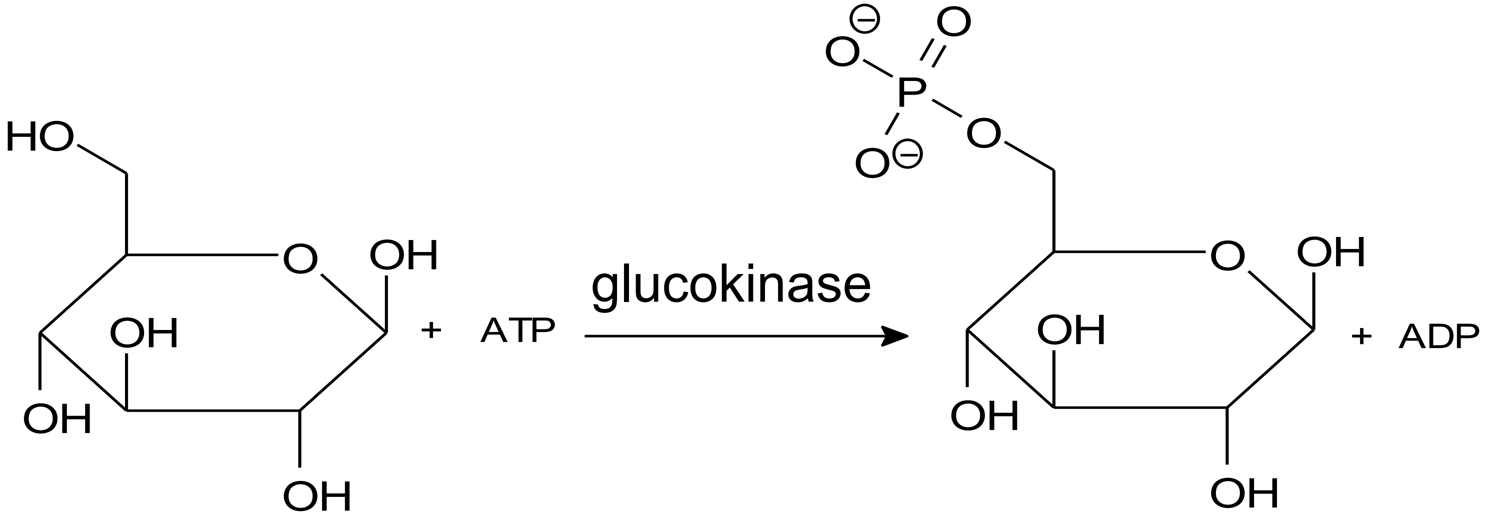
- **aminotransferases, methyltransferases, glucosyltransferases**
- **kinases** phosphorylate substrate by the transfer of phosphoryl group PO_3^{2-} from ATP (e.g. hexokinases, protein kinases)

Example: glucose + ATP → glucose 6-P + ADP

Recommended name: glucokinase

Systematic name: ATP:D-glucose phosphotransferase

Example: Phosphorylation of glucose



glucose

glucose 6-phosphate

3 Hydrolases

catalyze the hydrolytic splitting of esters, glycosides, amides, peptides etc.

subclasses:

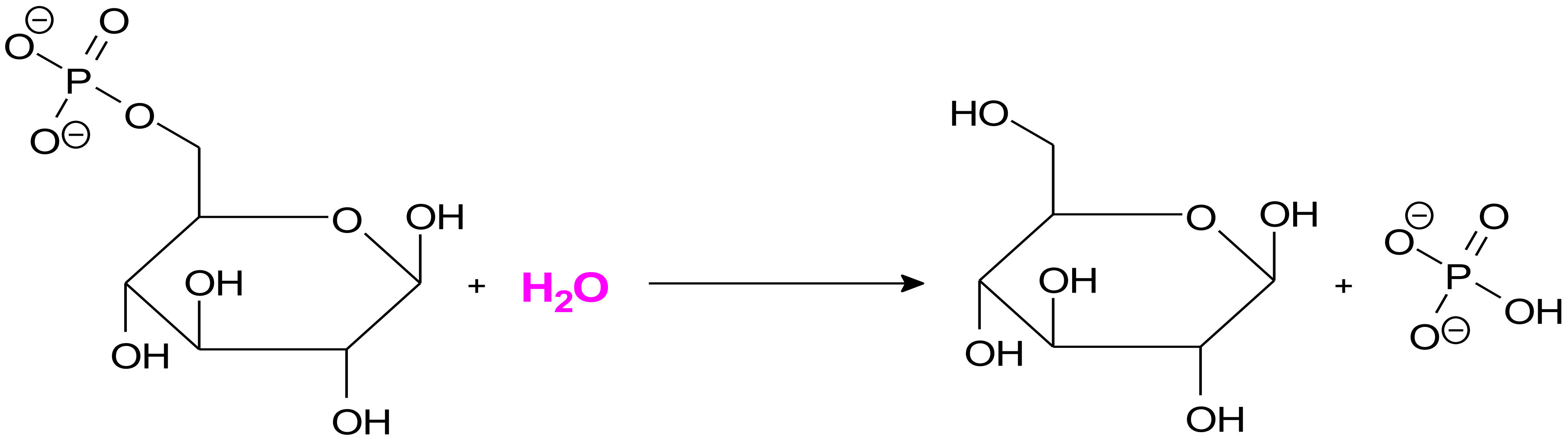
- **esterases** (lipases, phospholipases, ribonucleases, **phosphatases**)
- **glycosidases** (e.g. sucrase, maltase, lactase, amylase)
- **proteinases, peptidases** (pepsin, trypsin, cathepsins, caspases/apoptosis, dipeptidases, carboxypeptidases, aminopeptidases)
- **amidases** (glutaminase, asparaginase)
- **ATPases** (split anhydride bonds of ATP)

Example: glucose 6-P + $H_2O \rightarrow$ glucose + P_i

Recommended name: glucose 6-phosphatase

Systematic name: glucose 6-phosphate phosphohydrolase

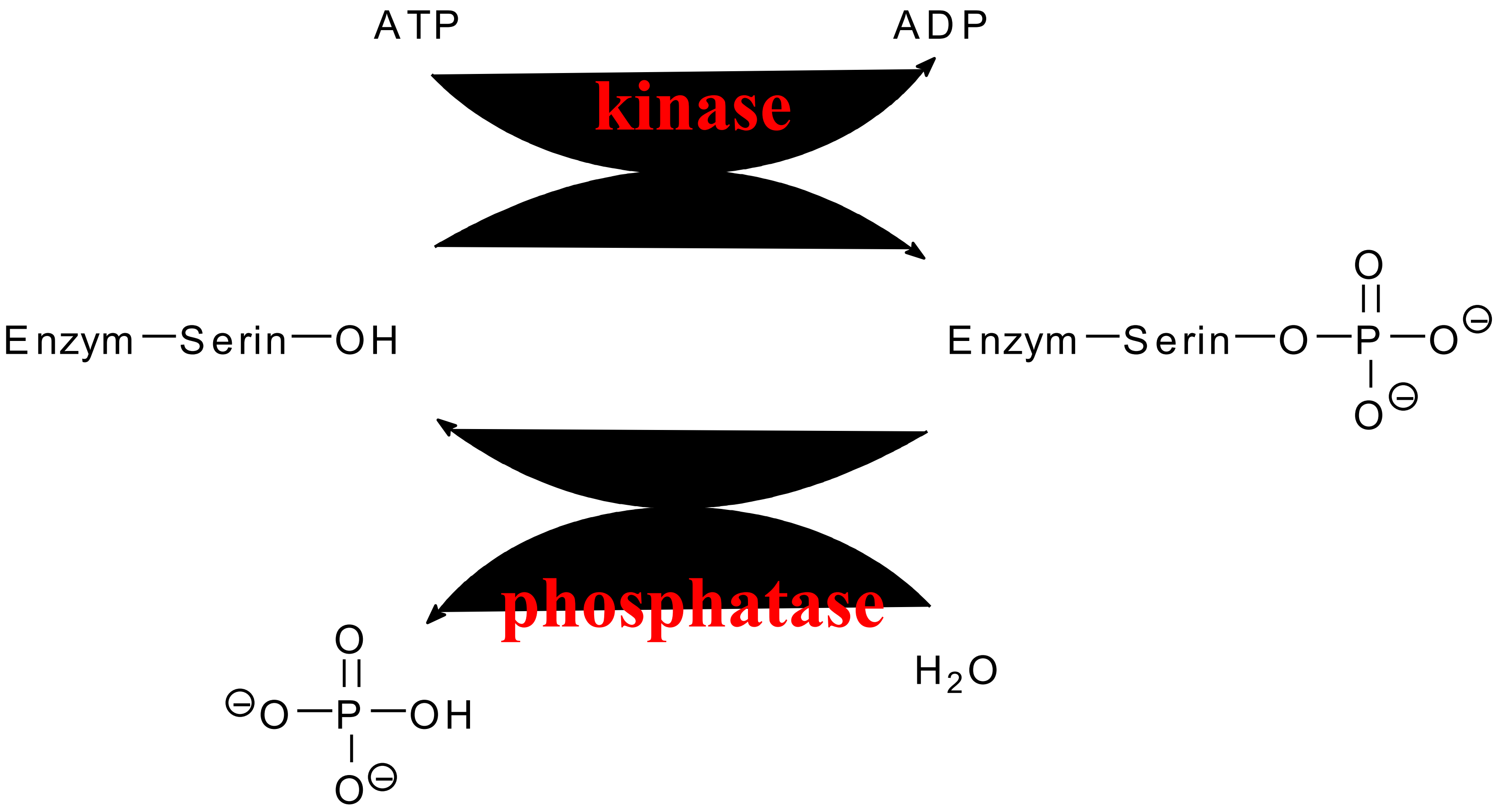
Example: glucose 6-phosphatase



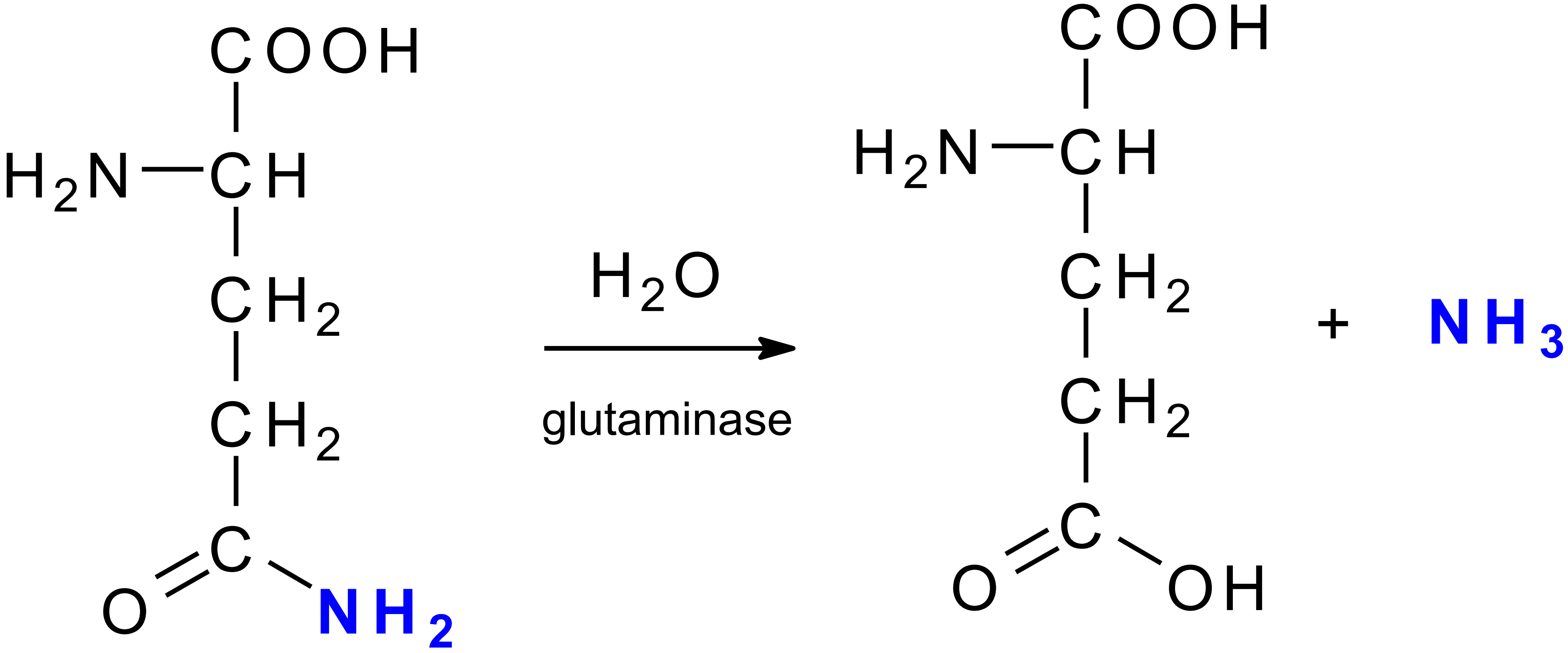
glucose 6-P

glucose

Compare two antagonistic enzymes



Glutaminase is amidase which catalyzes the <u>deamidation</u> of glutamine



$$HOOC\text{-}CH(NH_2)\text{-}CH_2\text{-}CH_2\text{-}C(=O)\text{-}NH_2 \xrightarrow[\text{glutaminase}]{H_2O} HOOC\text{-}CH(NH_2)\text{-}CH_2\text{-}CH_2\text{-}C(=O)\text{-}OH + NH_3$$

glutamine

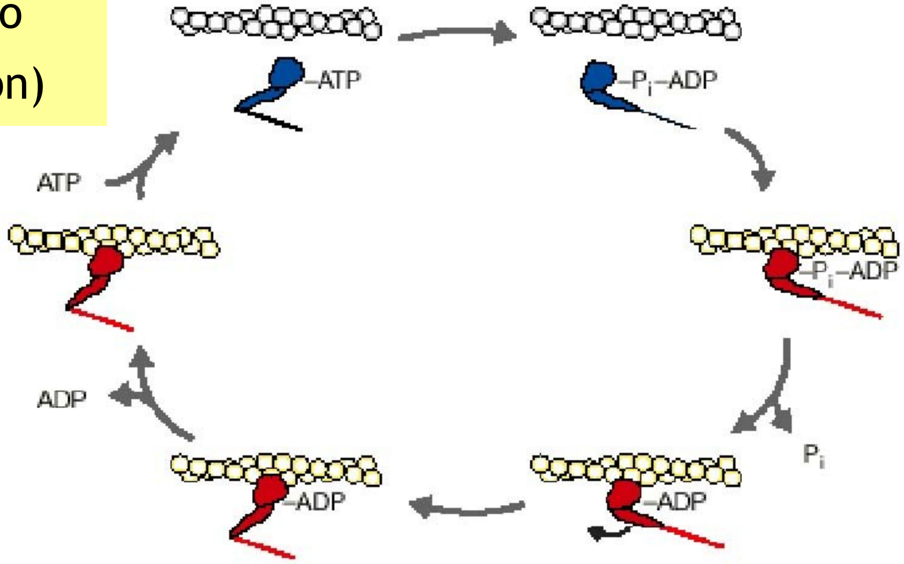
gluta glutamate

ATPase catalyzes the exergonic hydrolysis of phosphoanhydride bond in ATP

$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{P_i} + \textbf{energy}$$

Example: muscle contraction

myosine head exhibits ATPase activity, chemical energy of ATP is transformed into mechanical work (actin-myosin contraction)



Examples of lysosomal hydrolases

Hydrolase	Bond hydrolyzed
Glucosidase	glycoside
Galactosidase	glycoside
Hyaluronidase	glycoside
Arylsulfatase	sulfoester
Lysozyme	glycoside
Cathepsin	peptide
Collagenase	peptide
Elastase	peptide
Ribonuclease	phosphodiester
Lipase	ester
Phosphatase	phosphoester
Ceramidase	amide

Distinguish: lysozyme lysosome

Lysozyme is enzyme

- compound word, **lyso** (Greek *lysis*) + **zyme** (from *enzyme*)
- hydrolase, glycosidase, cleaves β-1,4-glycoside bond in bacterial heteropolysaccharides, antiseptic defense
- occurs in saliva, tears, and other body fluids

Lysosome is intracellular digestion organelle

- Greek compound word from *lysis* (to lyse) and *soma* (body)
- typical for animal cells
- acidic pH, contains many acidic hydrolases

4 Lyases

catalyze **non-hydrolytic splitting** or **forming** bonds C–C, C–O, C–N, C–S through removing or adding, respectively, a small molecule (H_2O, CO_2, NH_3)

Some frequent recommended names:

- **ammonia lyases** (e.g. histidine ammonia lyase: histidine → urocanate + NH_3)
- **decarboxylases** (amino acid → amine + CO_2)
- **aldolases** (catalyze aldol cleavage and formation)
- **(de)hydratases** (e.g. carbonate dehydratase: $CO_2 + H_2O \leftrightarrows H_2CO_3$)

Example: fumarate + H_2O ⇆ L-malate

Recommended name: fumarate hydratase

Systematic name: (*S*)-malate hydro-lyase (fumarate-forming)

5 Isomerases

catalyze intramolecular rearrangements of atoms

examples:

- **epimerases**
- **racemases**
- **mutases**

Example: UDP-glucose → UDP-galactose

Recommended name: UDP-glucose 4-epimerase

Systematic name: UDP-glucose 4-epimerase

6 Ligases

catalyze the formation of high-energy bonds C–C, C–O, C–N

in the reactions coupled with **hydrolysis of ATP**

Frequent recommended names:

carboxylases

synthetases

(e.g. glutamine synthetase: glutamate + ATP + NH_3 → glutamine + ADP + P_i)

Example: pyruvate + CO_2 + ATP + H_2O → oxaloacetate + ADP + P_i

Recommended name: pyruvate carboxylase

Systematic name: pyruvate:carbon-dioxide ligase (ADP-forming)

Three enzymes dealing with phosphate

!

Enzyme (Class)	**Reaction scheme / Reaction type**
Kinase (Transferase)	substrate-OH + ATP → substrate-O-P + ADP phosphorylation = transfer of phosphoryl PO_3^{2-} from ATP to substrate
Phosphatase (Hydrolase)	substrate-O-P + H_2O → substrate-OH + P_i the hydrolysis of phosphoester bond
Phosphorylase (Transferase)	$(\text{glycogen})_n + P_i \rightarrow (\text{glycogen})_{n-1}$ + glucose 1-P inosine + P_i → hypoxanthine + ribose 1-P phosphorolysis = the splitting of glycoside bond by phosphate = transfer of glucosyl to inorganic phosphate

Distinguish:
Three types of lysis (decomposition of substrate)

!

Hydrolysis	the decomposition of substrate **by water**, frequent in intestine: sucrose + $H_2O \rightarrow$ glucose + fructose $(\text{starch})_n + H_2O \rightarrow$ maltose + $(\text{starch})_{n-2}$
Phosphorolysis (see previous page)	the cleavage of *O*/*N*-glycoside bond **by phosphate**: $(\text{glycogen})_n + P_i \rightarrow (\text{glycogen})_{n-1}$ + glucose 1-P
Thiolysis	the cleavage of C-C bond **by sulfur atom** of coenzyme A in β-oxidation of FA or ketone bodies catabolism $RCH_2COCH_2CO\text{-}SCoA + CoA\text{-}SH \rightarrow RCH_2CO\text{-}SCoA + CH_3CO\text{-}SCoA$ $CH_3COCH_2CO\text{-}SCoA + CoA\text{-}SH \rightarrow 2\ CH_3CO\text{-}SCoA$

Cofactors of enzymes

- low-molecular non-protein compounds
- many of them are derived from B-complex vitamins
- many of them are nucleotides
- **transfer 2 H or e^- (cooperate with oxidoreductases)**
- **transfer groups (cooperate with transferases)**
- tightly (covalently) attached – prosthetic groups
- loosely attached – coenzymes (cosubstrates)

Three different components in enzyme reaction

$$\text{substrate} + \text{cofactor} \xrightleftharpoons{\text{enzyme}} \text{product} + \text{cofactor}_{\text{altered}}$$

$$\left.\begin{array}{l}\text{1. substrate(s)}\\ \text{2. cofactor}\end{array}\right\} \text{react to each other}$$

3. enzyme catalyzes the whole process

Notes:

- one or two substrates may be involved (dehydrogenation × transamination)
- substrate can be low / high molecular (hexokinase × protein kinase)
- some reactions proceed without cofactor (hydrolysis, isomeration)
- reaction can be reversible or irreversible (dehydrogenation × decarboxylation)

Cofactors of oxidoreductases

Oxidized form	**Reduced form**	**The function of cofactor**
NAD^+	$NADH+H^+$	NAD^+ acceptor of 2H
$NADP^+$	$NADPH+H^+$	$NADPH+H^+$ donor of 2H
FAD	$FADH_2$	FAD acceptor of 2H
Dihydrobiopterin (BH_2)	tetrahydrobiopterin (BH_4)	BH_4 donor of 2H
$\text{Molybdopterin}_{\text{oxid}}$	$\text{molybdopterin}_{\text{red}}$	electron transfer
Lipoate (-S-S-)	dihydrolipoate (2 -SH)	antioxidant / transfer of acyl
Ubiquinone (Q)	ubiquinol (QH_2)	transfer of 2 electrons + 2 H^+
Heme-Fe^{3+}	heme-Fe^{2+}	transfer of 1 electron
Non-heme-S-Fe^{3+}	non-heme-S-Fe^{2+}	transfer of 1 electron
$\text{Glutathione}_{\text{oxid}}$ (G-S-S-G)	$\text{glutathione}_{\text{red}}$ (GSH)	2 GSH donor of 2H

NAD^+ is the cofactor of dehydrogenases, derivative of nicotinamide (vitamin)

- NAD^+ is oxidant – takes off **2 H** from substrate
- one H adds as **hydride ion (H^-)**

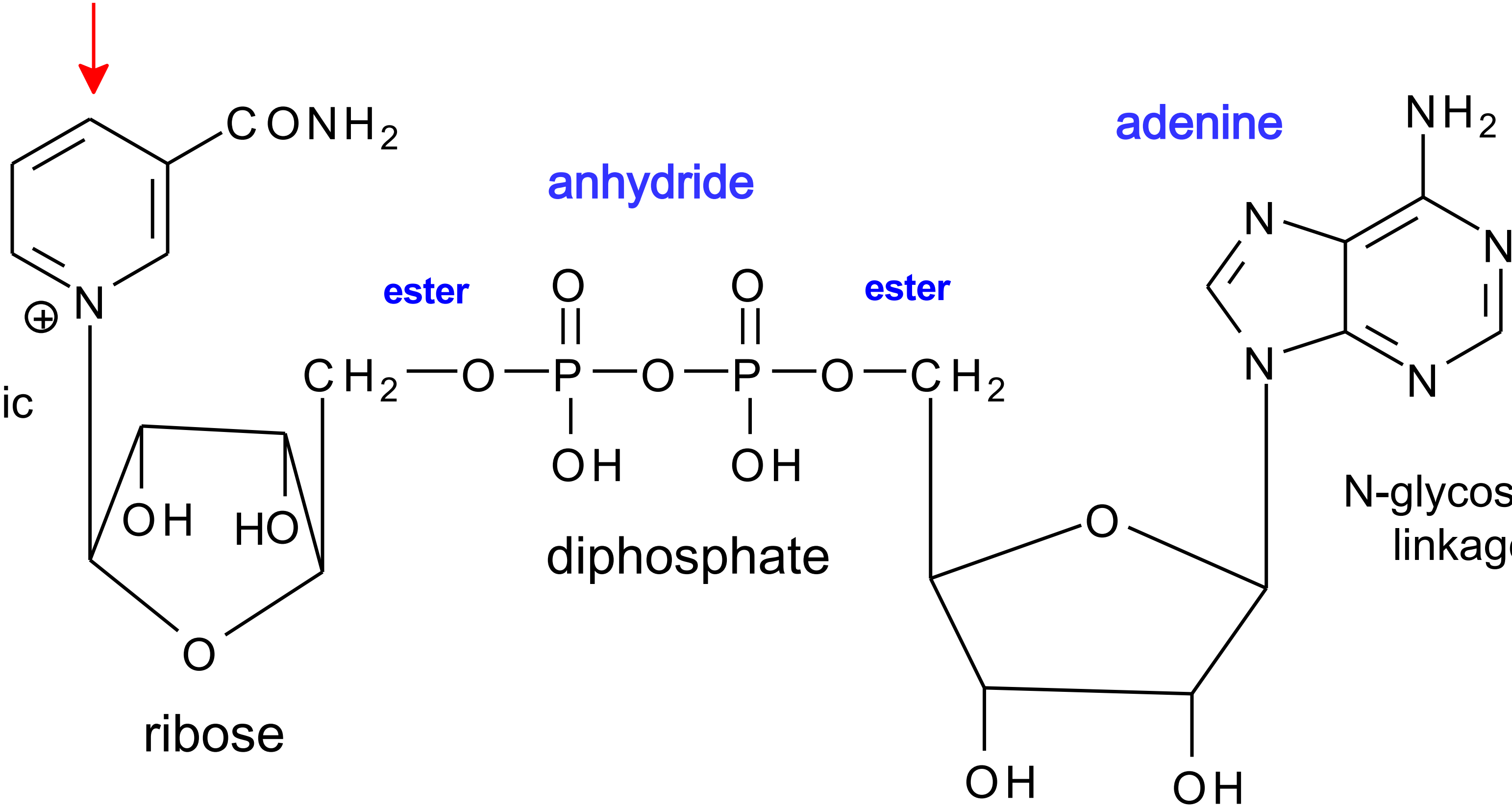
$2\ H = H^- + H^+$

  into *para*-position of pyridinium cation of NAD^+
- $NAD^+ + H^- = NADH$ = equivalent of two electrons
- the second H is released as **proton (H^+)** and

  binds to enzyme molecule

NAD$^+$ (nicotinamide adenine dinucleotide)

addition of hydride anion



pyridinium

ester

anhydride

ester

adenine

N-glycosidic linkage

diphosphate

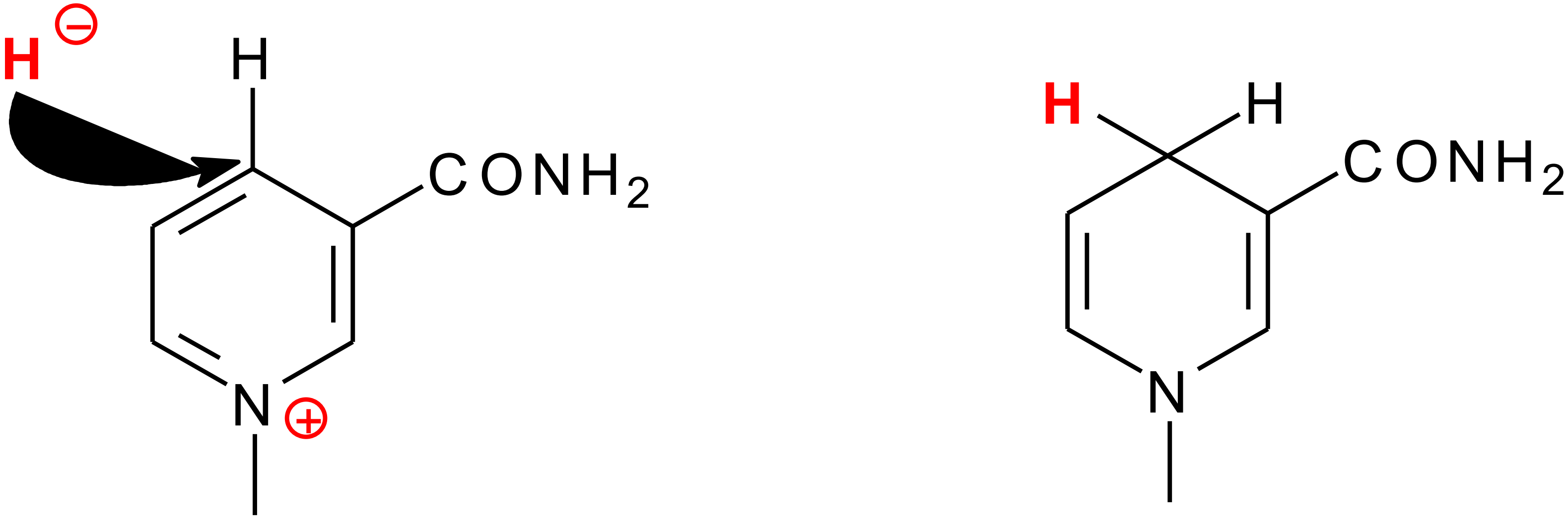
N-glycosidic linkage

ribose

ribose

Redox pair of cofactor

!



oxidized form NAD^+

aromatic ring

tetravalent nitrogen

positive charge on nitrogen

reduced form NADH

aromaticity **totally** disturbed

trivalent nitrogen

electroneutral species

high-energy compound

Dehydrogenation by NAD^+

- typical substrate groups:
- primary alcohol $-CH_2-OH$
- secondary alcohol $>CH-OH$
- secondary amine $>CH-NH_2$
- **double bond** ($C=O$, $C=N$) is produced

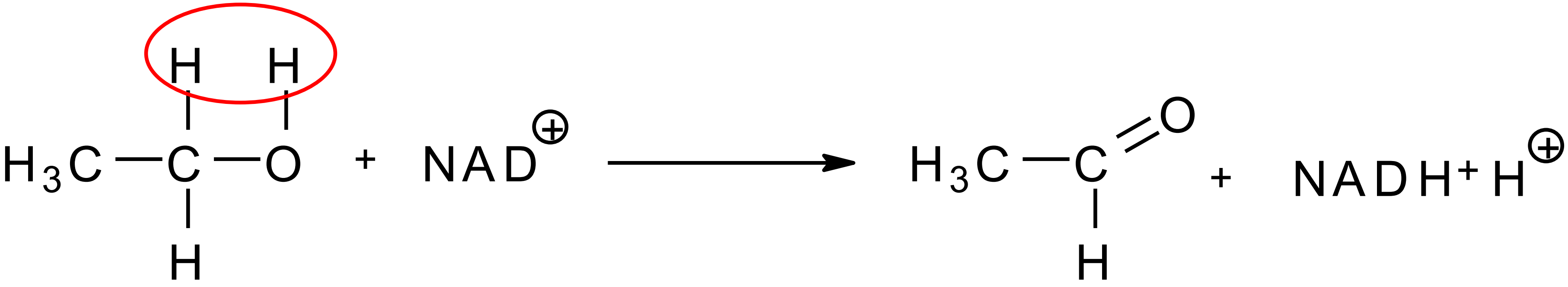
NAD$^+$ dehydrogenations form a double bond

Substrate	Product
primary alcohol	aldehyde
secondary alcohol	ketone
aldehyde hydrate	carboxylic acid
hemiacetal	ester
cyclic hemiacetal	lactone
hydroxy acid	oxo acid
amino acid	imino acid

compare
Med. Chem. II
Appendix 3

Dehydrogenation of ethanol

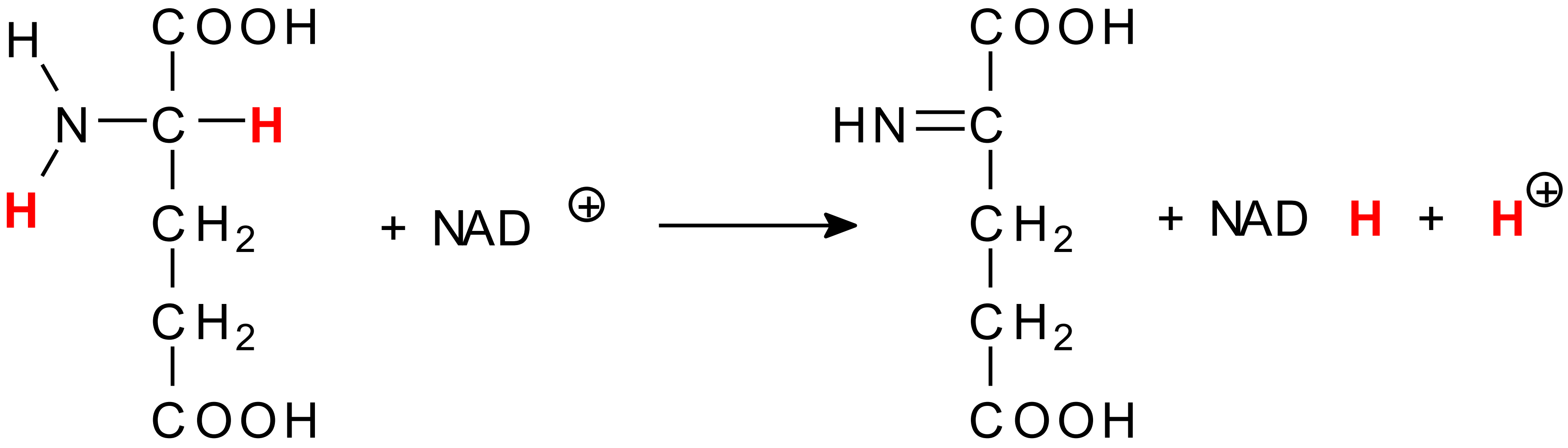
(alcohol dehydrogenase)



$$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$$

Dehydrogenation of glutamate

(glutamate dehydrogenase)



glutamate

2-imino glutarate

NAD$^+$-dependent enzymes are called pyridine dehydrogenases

- **Citrate cycle**
  isocitrate dehydrogenase
  2-oxoglutarate dehydrogenase
  malate dehydrogenase

- **Glycolysis**
  glyceraldehyde 3-P dehydrogenase
  lactate dehydrogenase

- **Oxidation of ethanol**
  alcohol dehydrogenase
  acetaldehyde dehydrogenase

Reduced cofactor NADPH+H$^+$ is hydrogenation agent

- donor of 2 H in hydrogenations
- cofactor of **reducing syntheses** (FA, cholesterol)
- regeneration of glutathione (GSH) in erythrocytes
- cofactor of **hydroxylation reactions:**

cholesterol → → bile acids

calciol → → calcitriol

xenobiotic → hydroxylated xenobiotic

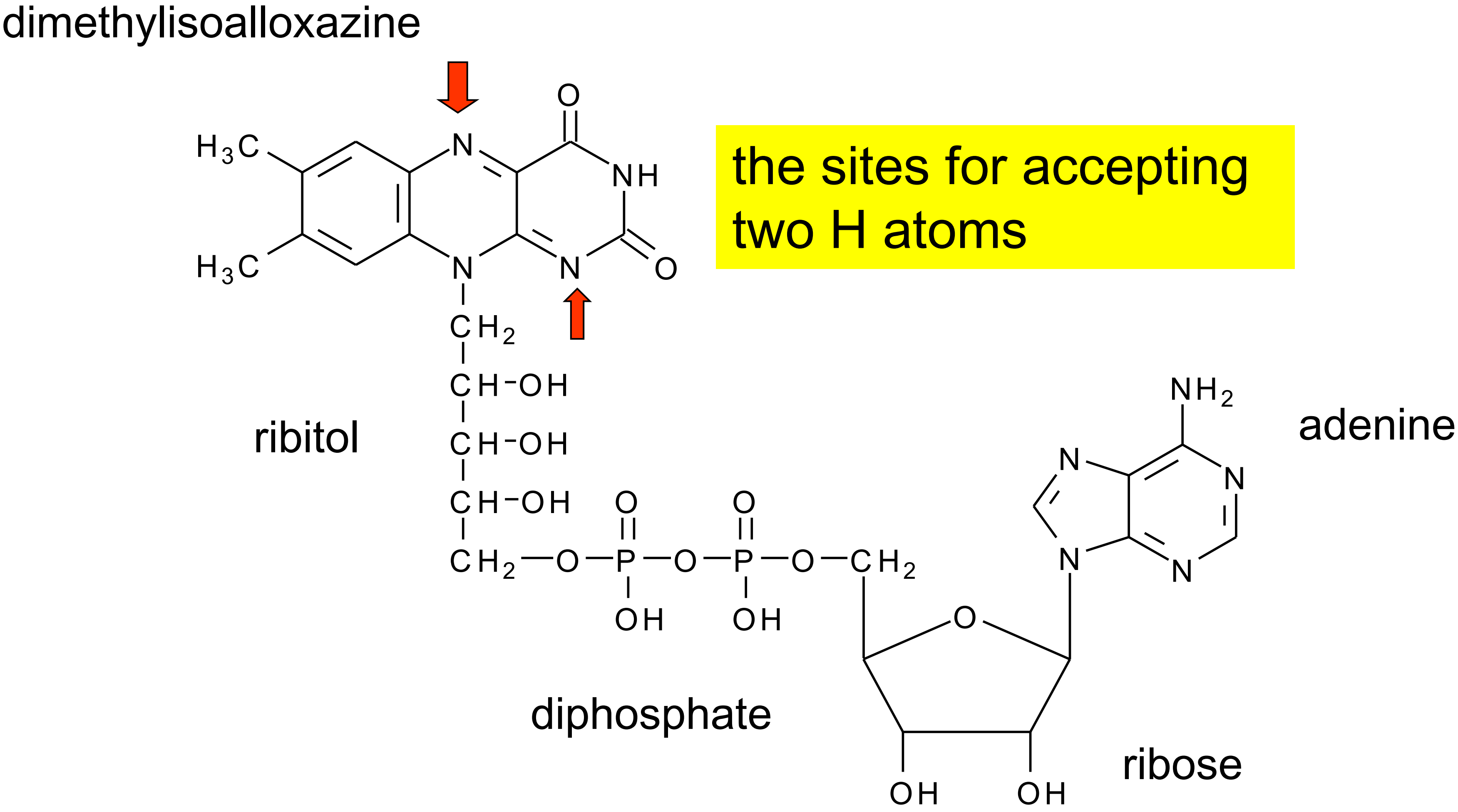
- general scheme of hydroxylation:

$$R\text{-}H + O_2 + NADPH{+}H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$$

FAD is cofactor of flavin dehydrogenases, derivative of riboflavin (vitamin B_2)

- flavin adenine dinucleotide
- dehydrogenation of $-CH_2-CH_2-$ group
- **two** H atoms are attached to **two** nitrogens of riboflavin (N-1 and N-10)
- $FAD + 2H \rightarrow FADH_2$

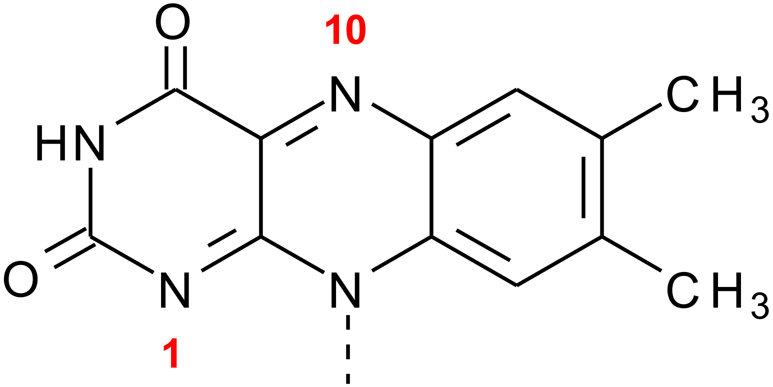
FAD (flavin adenine dinucleotide)

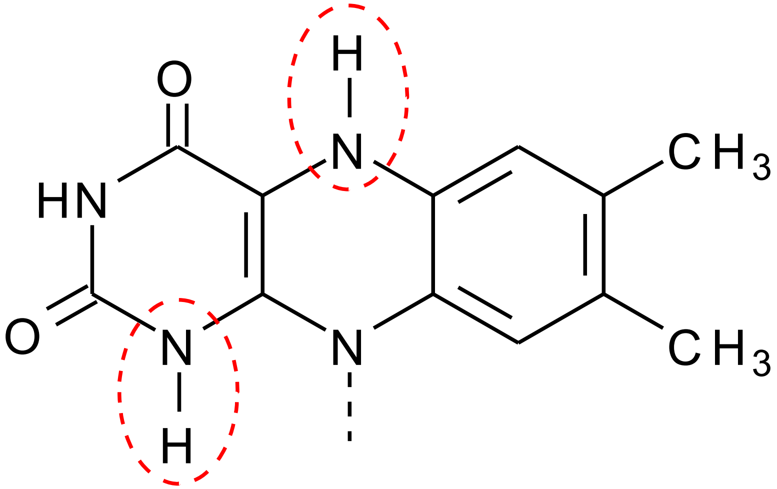


the sites for accepting two H atoms

Redox pair of cofactor

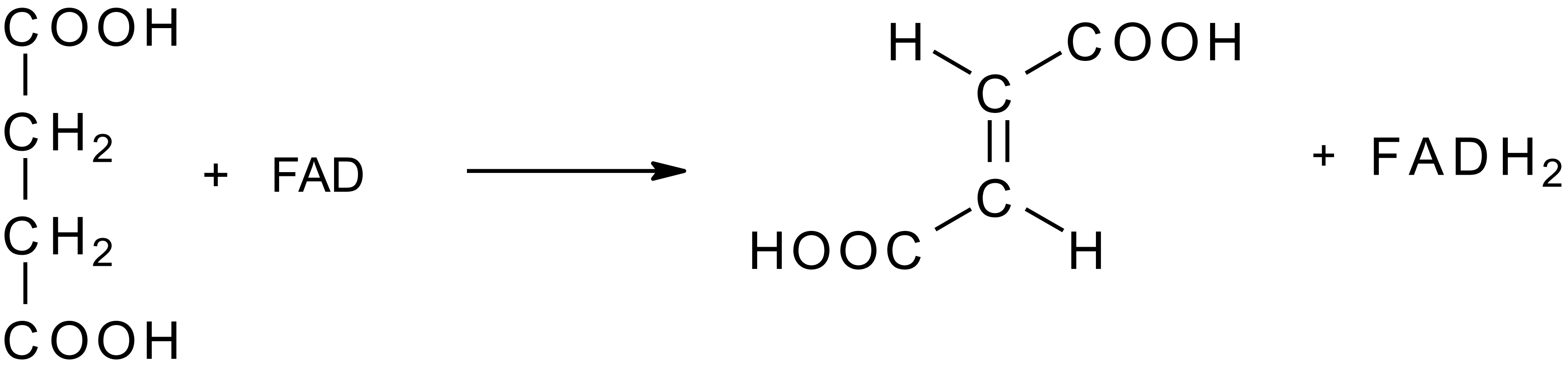
!





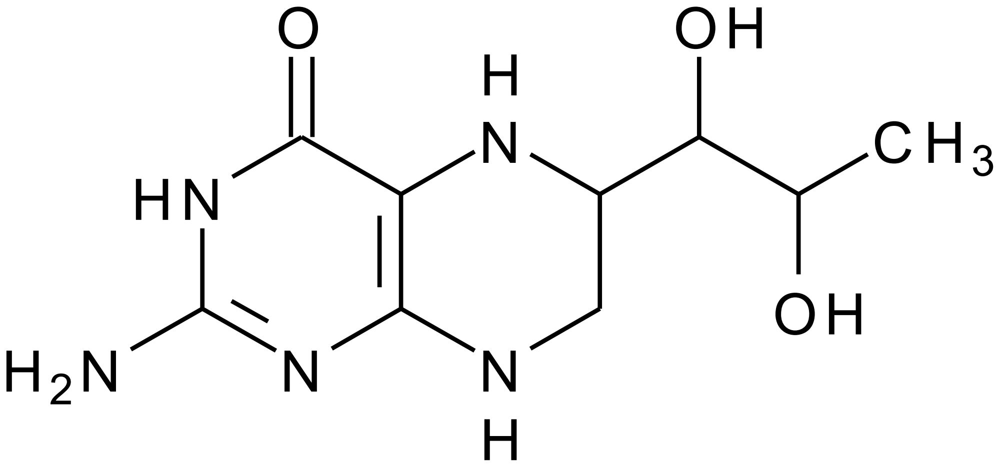
oxidized form FAD	reduced form $FADH_2$
aromatic system	aromaticity **partially** disturbed
electroneutral species	electroneutral species
	high-energy compound

Dehydrogenation of succinate to fumarate (flavin dehydrogenase)



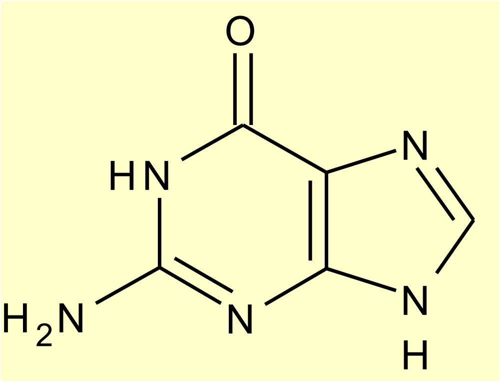
$$HOOC-CH_2-CH_2-COOH + FAD \longrightarrow HOOC-CH=CH-COOH + FADH_2$$

Tetrahydrobiopterin (BH_4) is a cofactor of hydroxylations



1,2-dihydroxypropyl

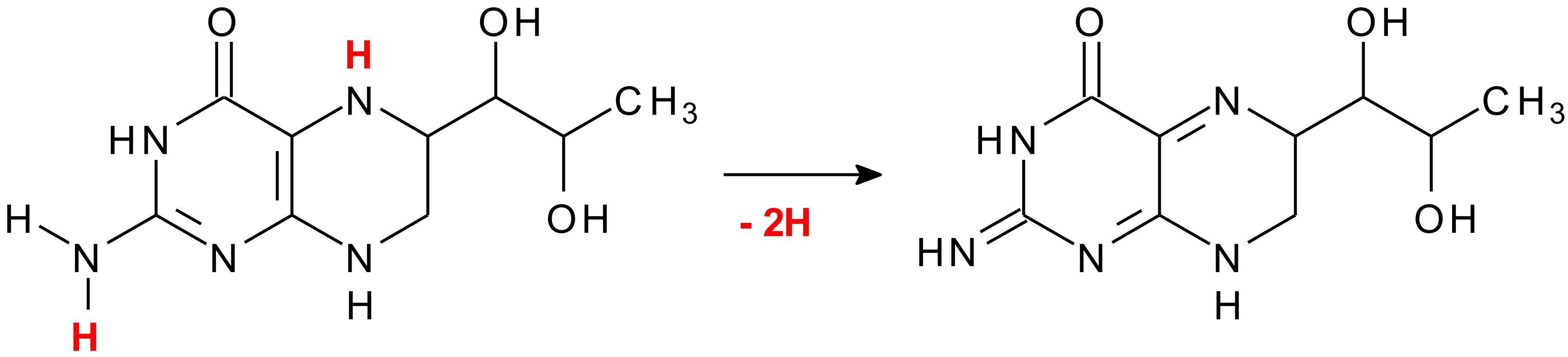
tetrahydropteridin



guanine

- made in the body from GTP
- **donor of 2H**
- oxidized to dihydrobiopterin (BH_2)

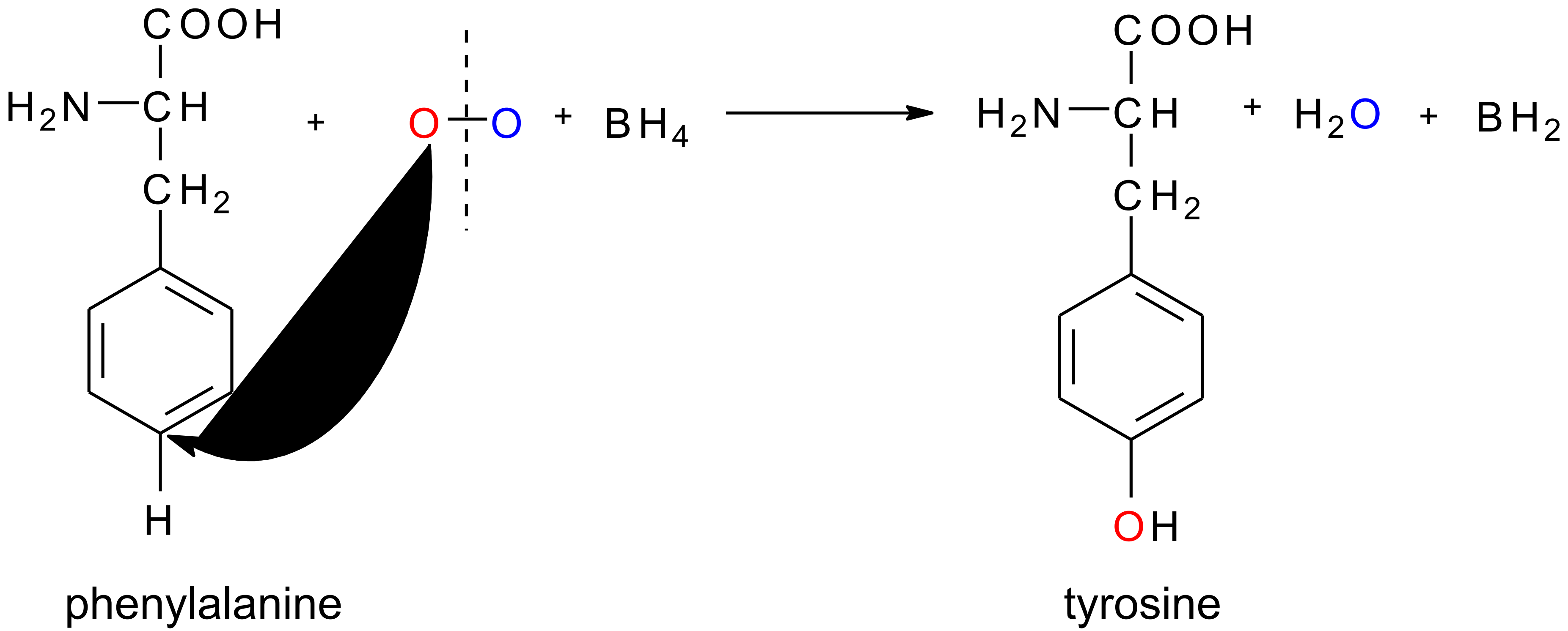
Redox pair of cofactor



tetrahydrobiopterin
(BH_4)

dihydrobiopterin
(BH_2)

Hydroxylation of phenylalanine

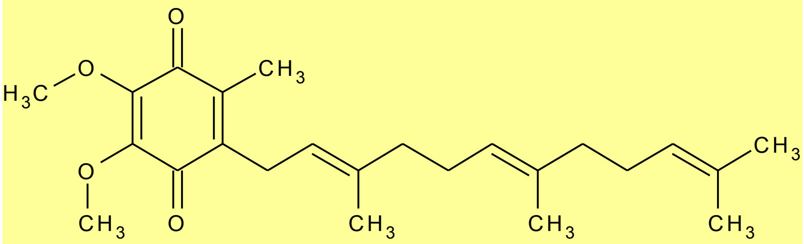


phenylalanine

tyrosine

Coenzyme Q (ubiquinone)

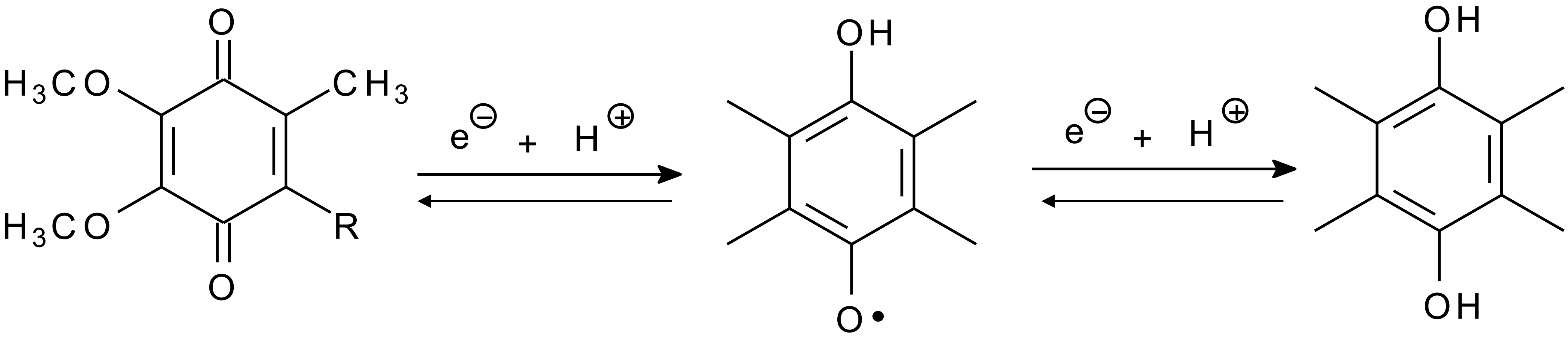
- derivative of 1,4-benzoquinone
- cyclic diketone, not aromatic



- component of respiratory chain
- gradually accepts electron and proton (2x)
- reduced to semiubiquinone and ubiquinol

Reversible reduction of ubiquinone

$Q \leftrightarrows {}^{\bullet}QH \leftrightarrows QH_2$



ubiquinone
(non-aromatic cycl. diketone)

semiubiquinone
(aromatic ring + radical)

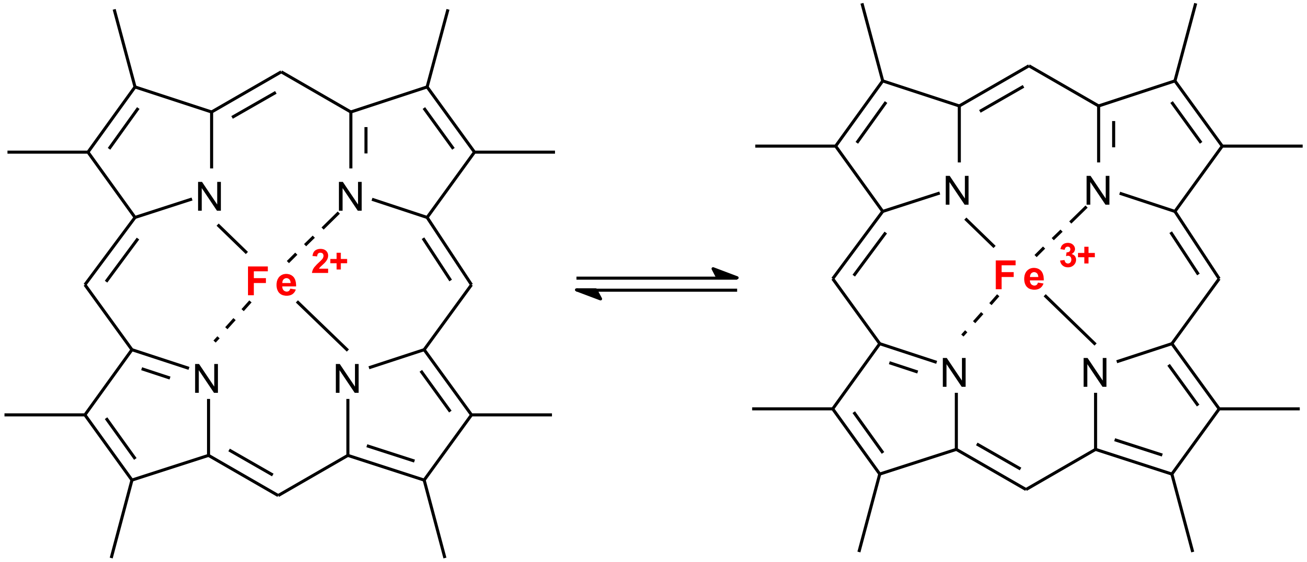
ubiquinol
(diphenol)

electron (e^-) and proton (H^+) have different origin:
electron comes from reduced cofactors, H^+ from matrix of mitochondria

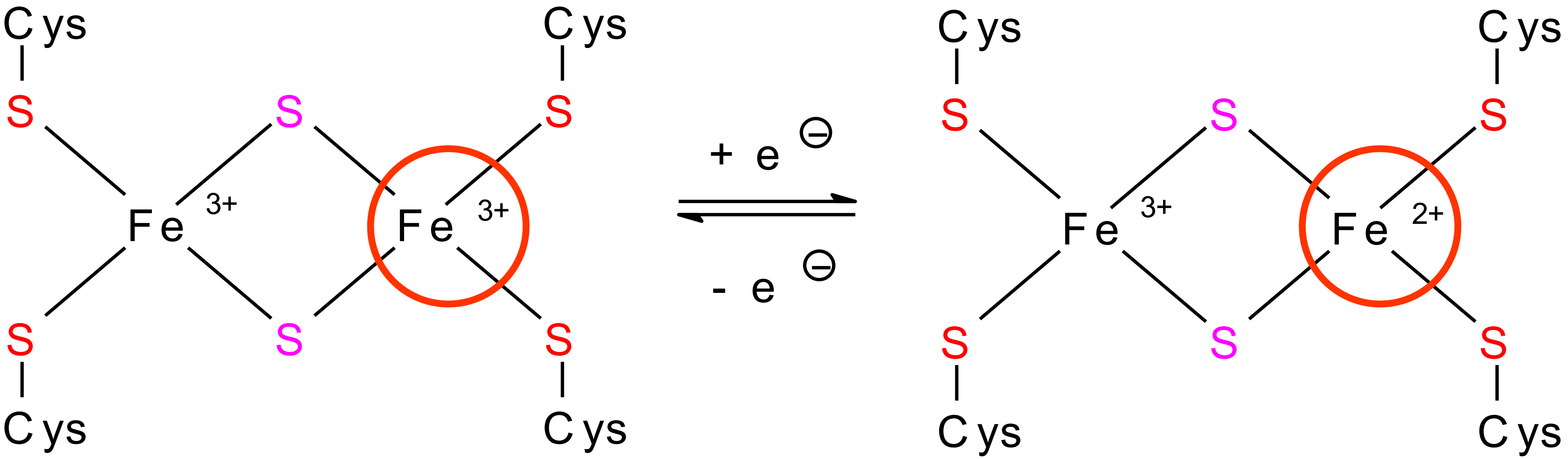
R = long polyisoprenoid chain ⇒ lipophilic character

Heme of various cytochromes

- transfers just 1 electron
- cytochromes are hemoproteins
- components of respiratory chain or other heme enzymes (cyt P-450)
- reversible redox reaction: $Fe^{2+} \leftrightarrows Fe^{3+}$



Non-heme iron (Fe_2S_2 cluster) transfers electron in R.CH.



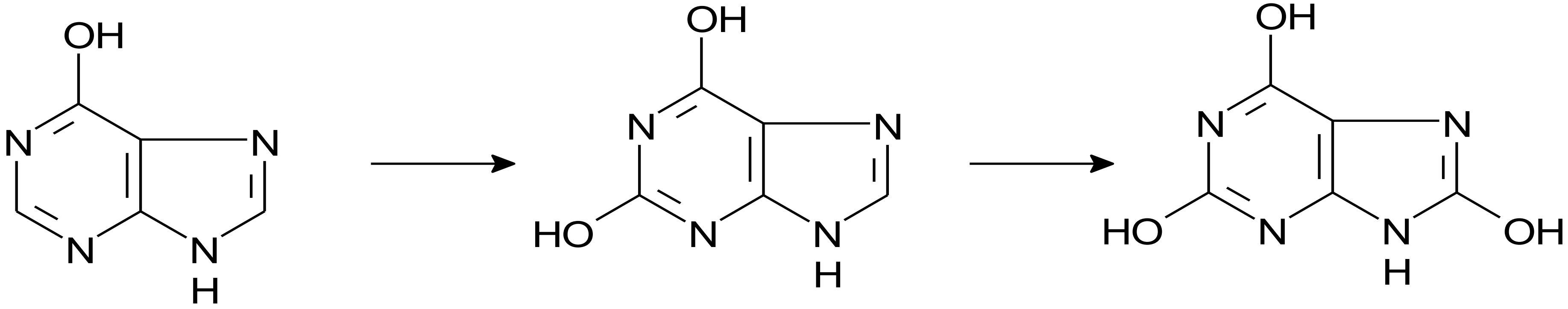
oxidized state

reduced state

just one iron cation changes oxidation number

Molybdopterin (formula in Seminars)

Xanthine oxidase catalyzes the oxygenation of purine bases (catabolism)

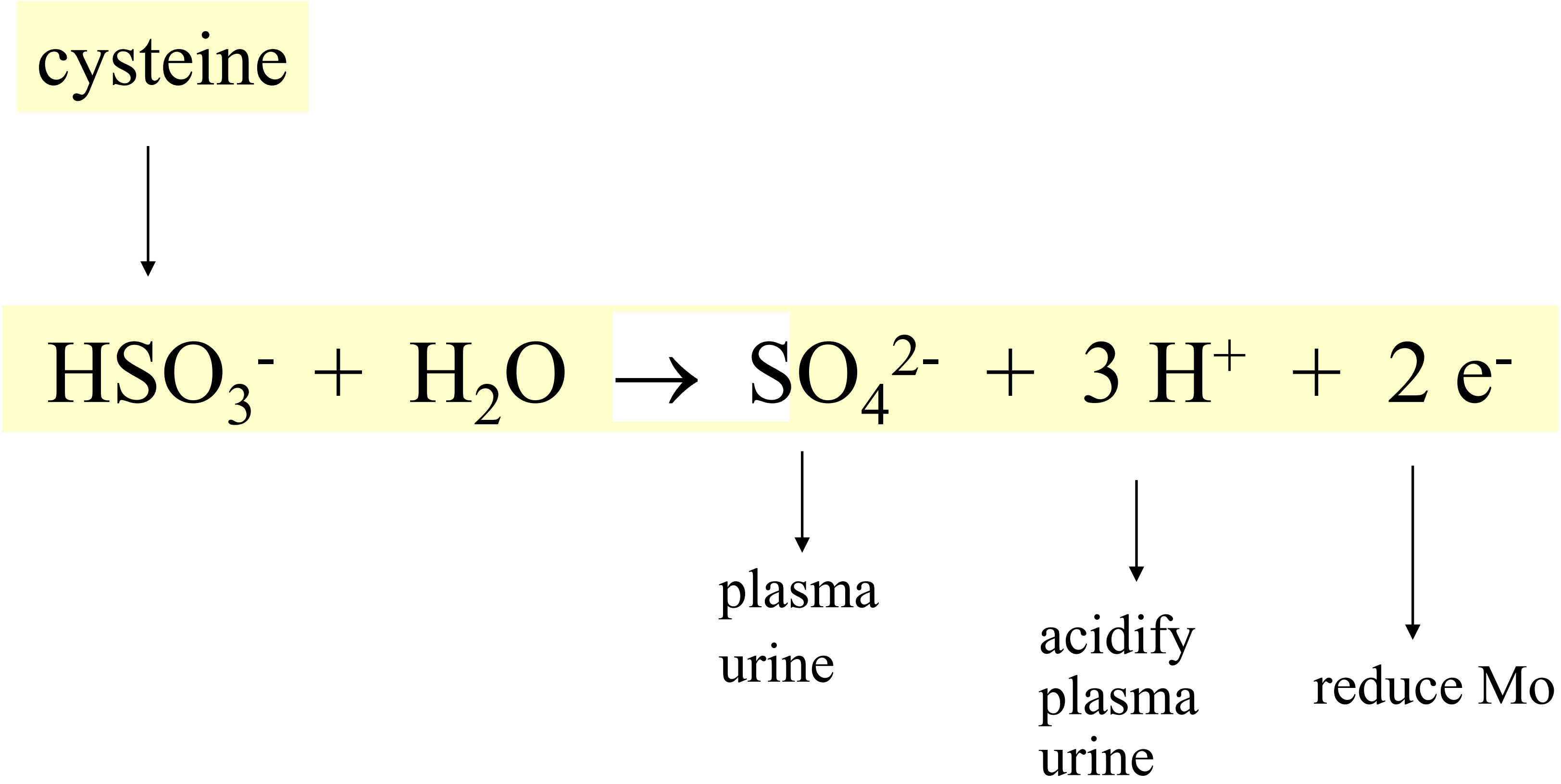


hypoxanthine ⟶ xanthine ⟶ uric acid

side product: H_2O_2

Molybdopterin

Sulfite oxidase:
sulfate is catabolite from cysteine



cysteine

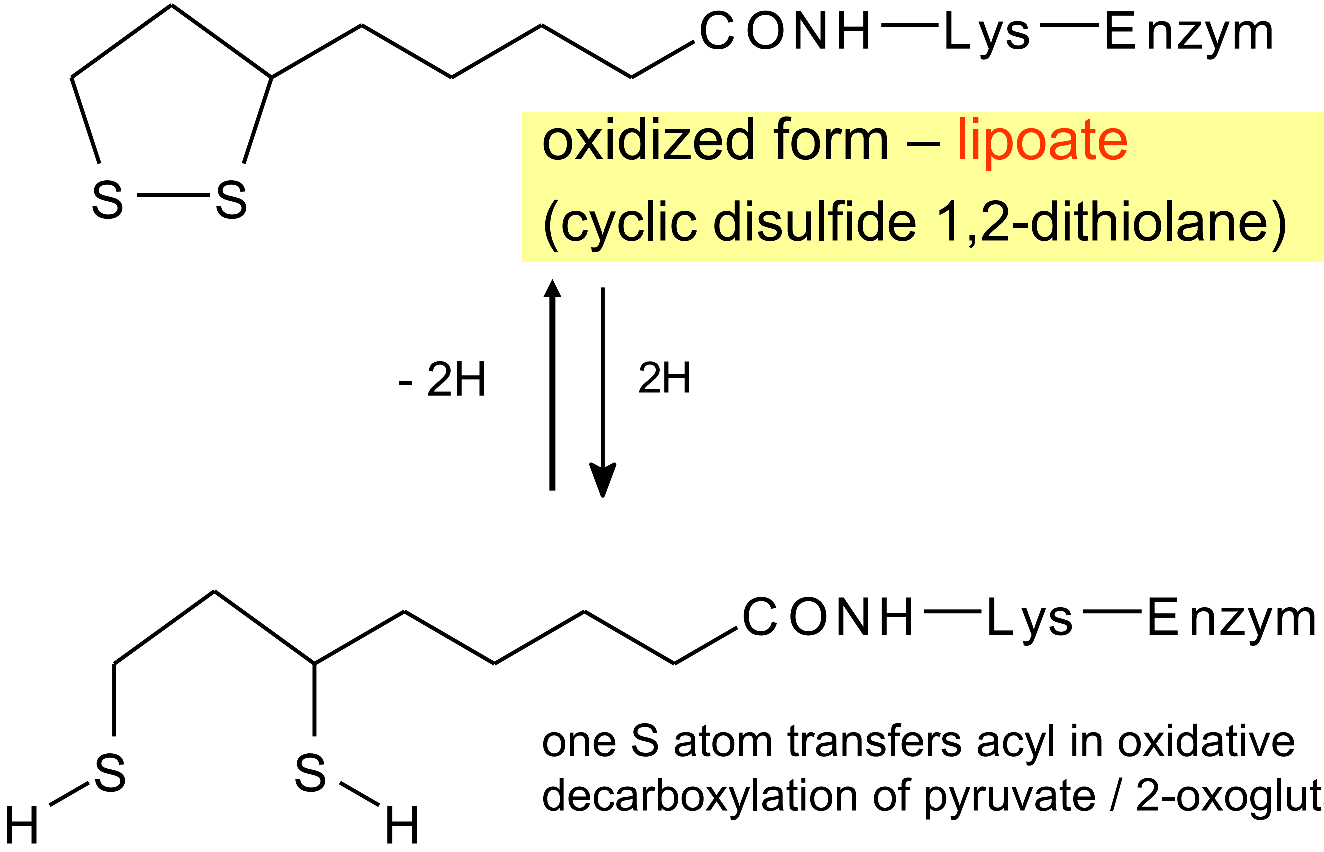
$$HSO_3^- + H_2O \rightarrow SO_4^{2-} + 3\,H^+ + 2\,e^-$$

plasma
urine

acidify
plasma
urine

reduce Mo

Redox pair lipoate/dihydrolipoate is antioxidant system. It is also involved in the acyl transfer (see later)



oxidized form – lipoate
(cyclic disulfide 1,2-dithiolane)

one S atom transfers acyl in oxidative decarboxylation of pyruvate / 2-oxoglutarate

reduced form - dihydrolipoate

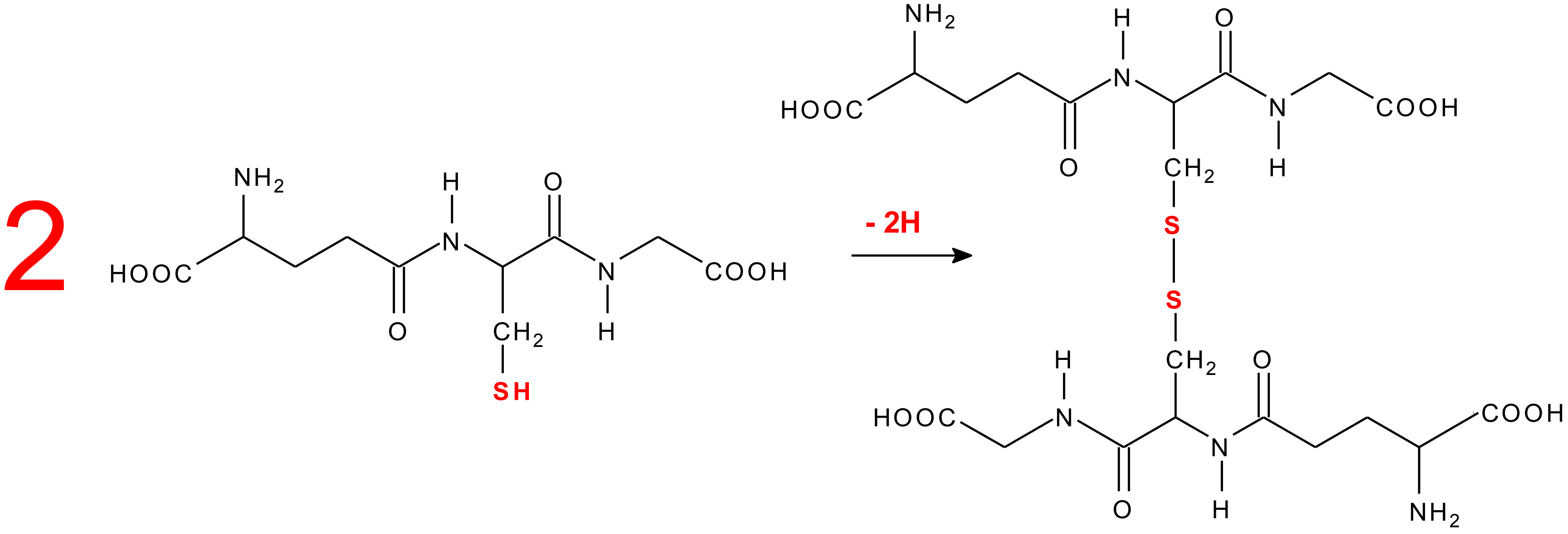
Glutathione (GSH)

- tripeptide
- γ-glutamyl-cysteinyl-glycine
- cofactor of glutathione peroxidase (contains selenocysteine)
- reduces H_2O_2 to water
- $2\ \text{G-SH} + \text{H-O-O-H} \rightarrow \text{G-S-S-G} + 2\ H_2O$

Remember:

The -SH compounds have generally reducing properties.

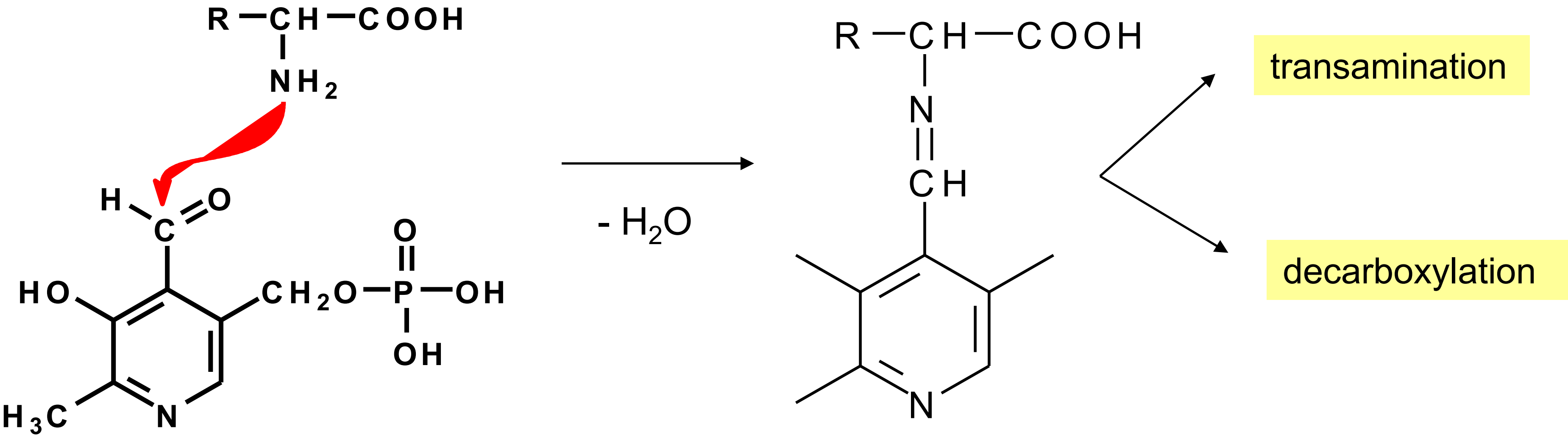
Dehydrogenation of <u>two</u> GSH molecules



Vitamins and cofactors of transferases

Vitamin	**Cofactor**	**Transferred group**
Pyridoxin	pyridoxal phosphate	$-NH_2$ (transamination)
(Made in body)	ATP	$-PO_3^{2-}$ (phosphoryl)
(Made in body)	PAPS	$-SO_3^{2-}$
Biotin	carboxybiotin	CO_2
Pantothenic acid	CoA-SH	acyl
(Made in body)	dihydrolipoate	acyl
(Methionine)	SAM	$-CH_3$
Folate	tetrahydrofolate	C_1 groups
Cyanocobalamin	methylcobalamin	$-CH_3$
Thiamin	thiamin diphosphate	residue of oxo acid

Pyridoxal phosphate is the cofactor of transamination and decarboxylation of AA



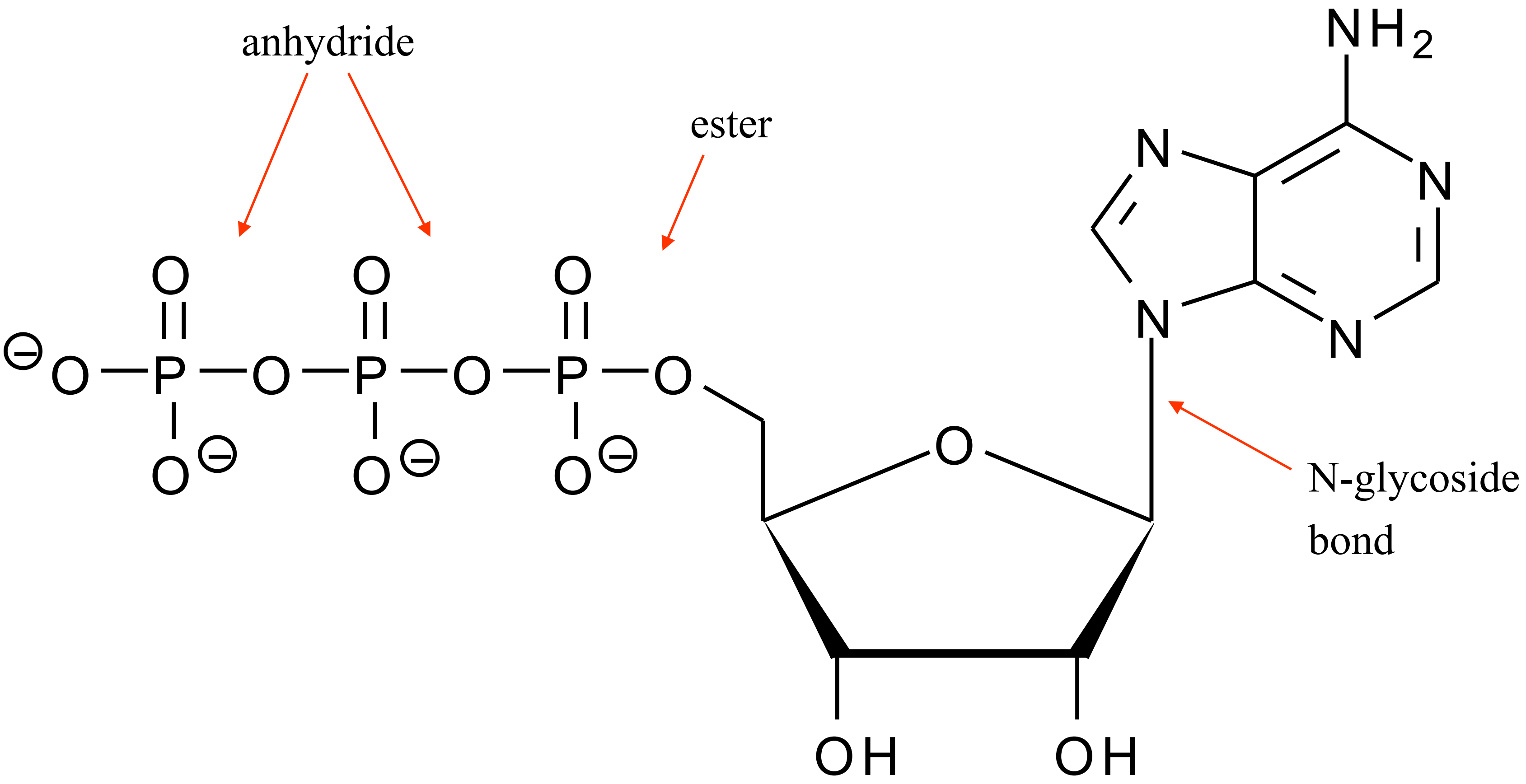
- H_2O

transamination

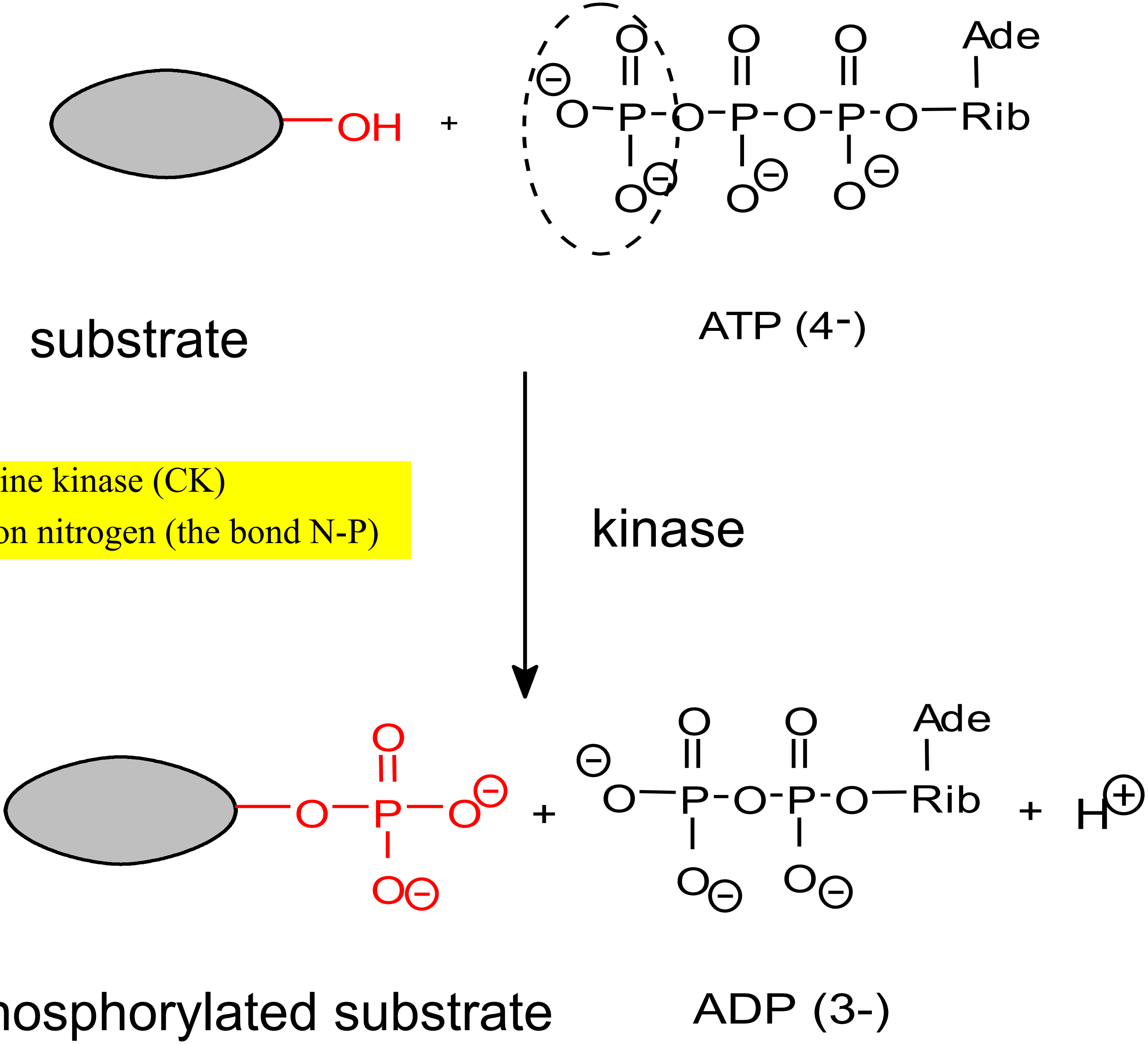
decarboxylation

aldimine
(Schiff base)

ATP is the cofactor of kinases (phosphorylation agent)



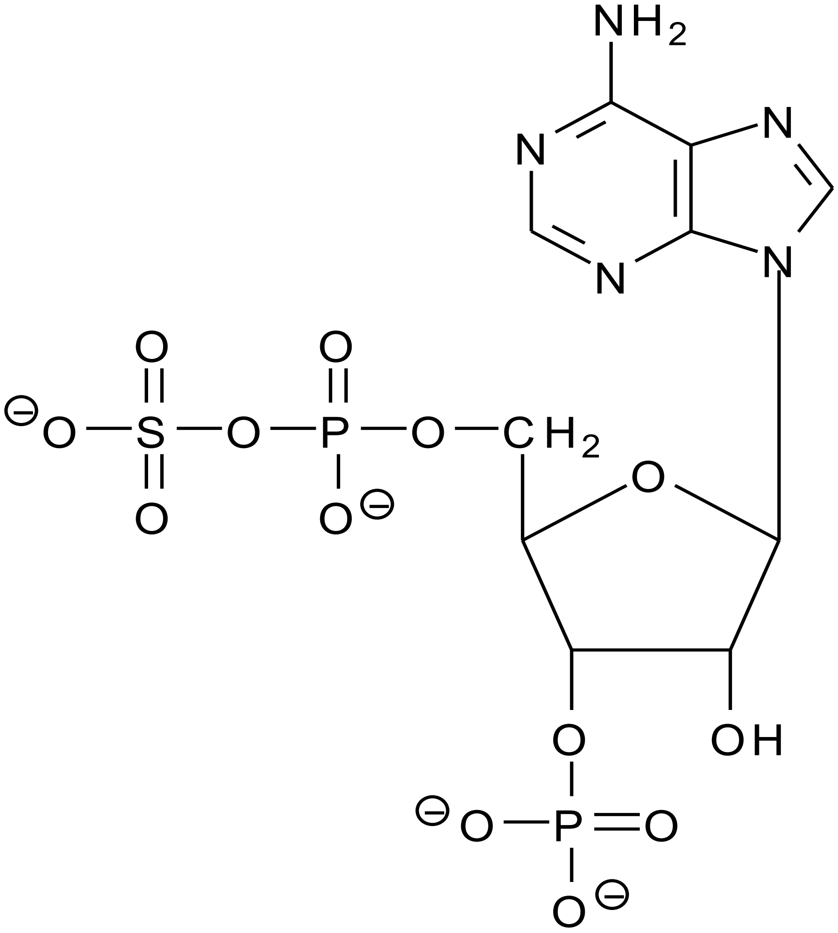
Phosphorylation of substrate



CAUTION: creatine kinase (CK)
phosphorylation on nitrogen (the bond N-P)

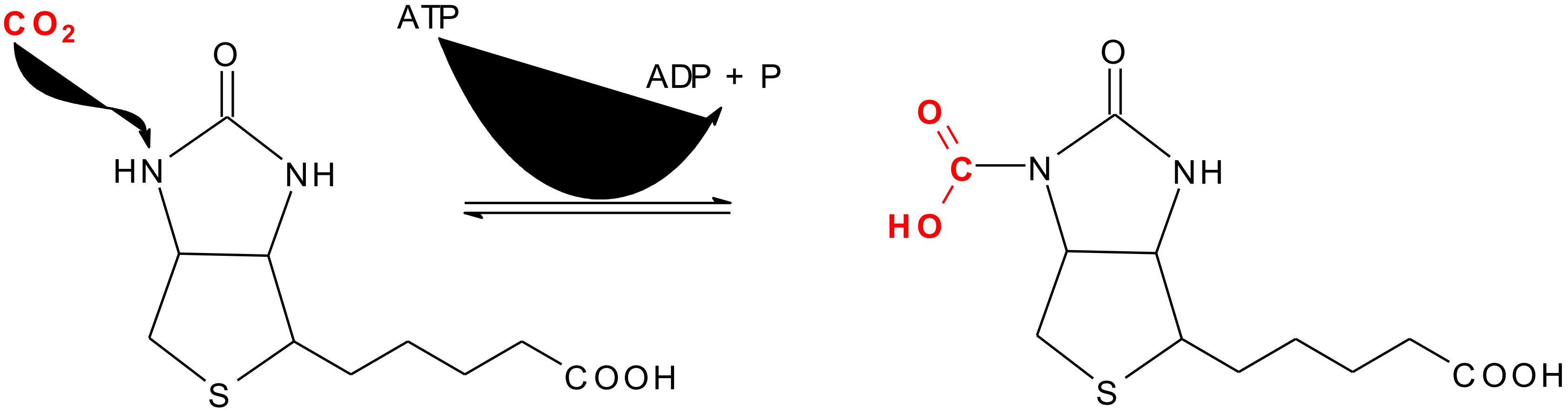
PAPS is sulfation agent

- 3’-phosfoadenosine-5’-phosphosulfate
- mixed anhydride of H_2SO_4 and H_3PO_4
- esterification of hydroxyl groups by sulfuric acid = sulfation
- sulfated sphingoglycolipids
- sulfated glycosaminoglycans (heparin, chondroitin sulfate, keratan sulfate)



Carboxybiotin

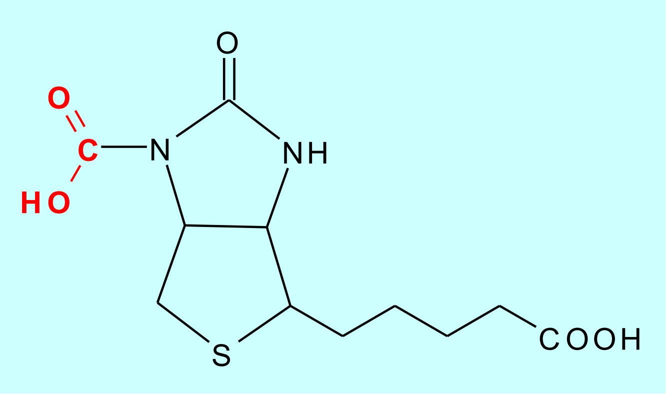
- cofactor of carboxylation reactions
- carboxylation of biotin needs ATP

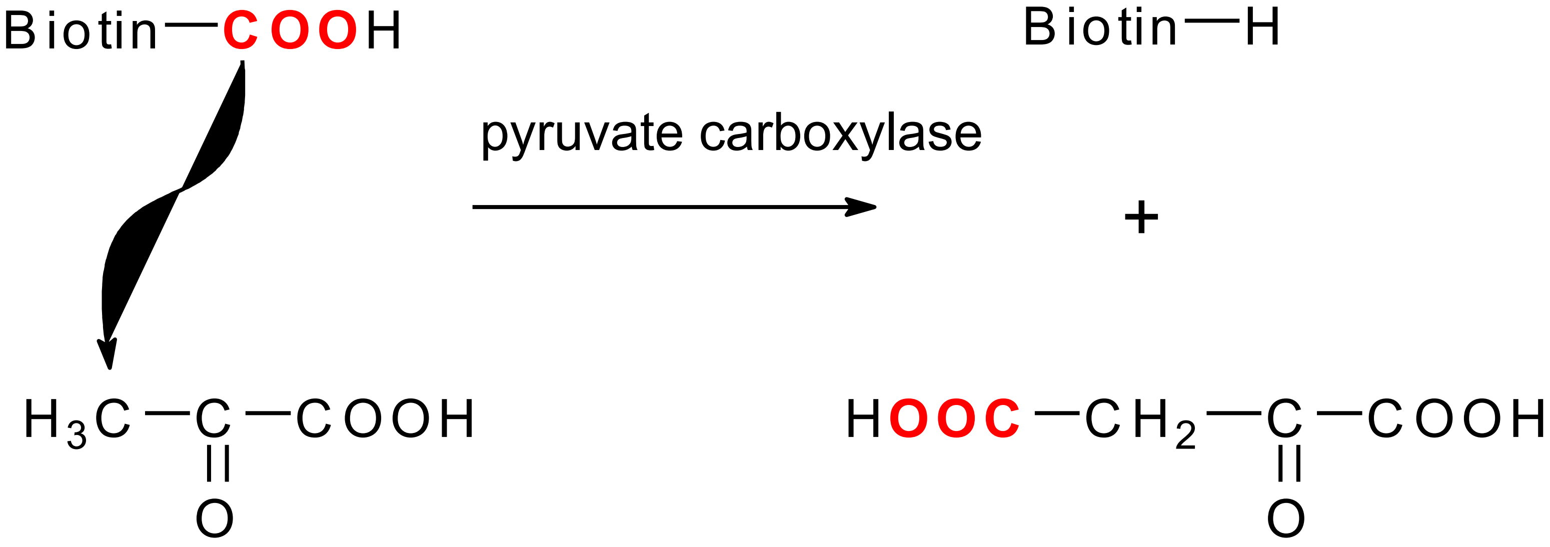


biotin

carboxybiotin

Carboxybiotin is the cofactor of carboxylation reactions





$$\text{Biotin}-\text{COOH} + \text{H}_3\text{C}-\underset{\underset{\text{O}}{\|}}{\text{C}}-\text{COOH} \xrightarrow{\text{pyruvate carboxylase}} \text{Biotin}-\text{H} + \text{HOOC}-\text{CH}_2-\underset{\underset{\text{O}}{\|}}{\text{C}}-\text{COOH}$$

pyruvate

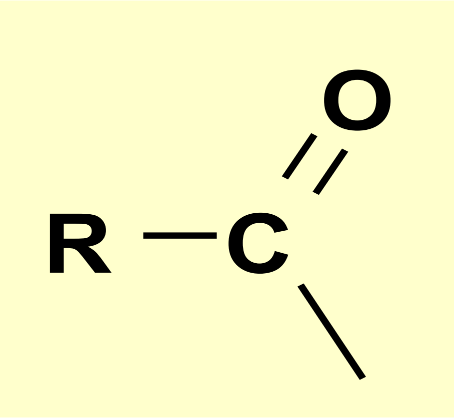
oxaloacetate

Distinguish: Decarboxylation *vs.* Carboxylation

!

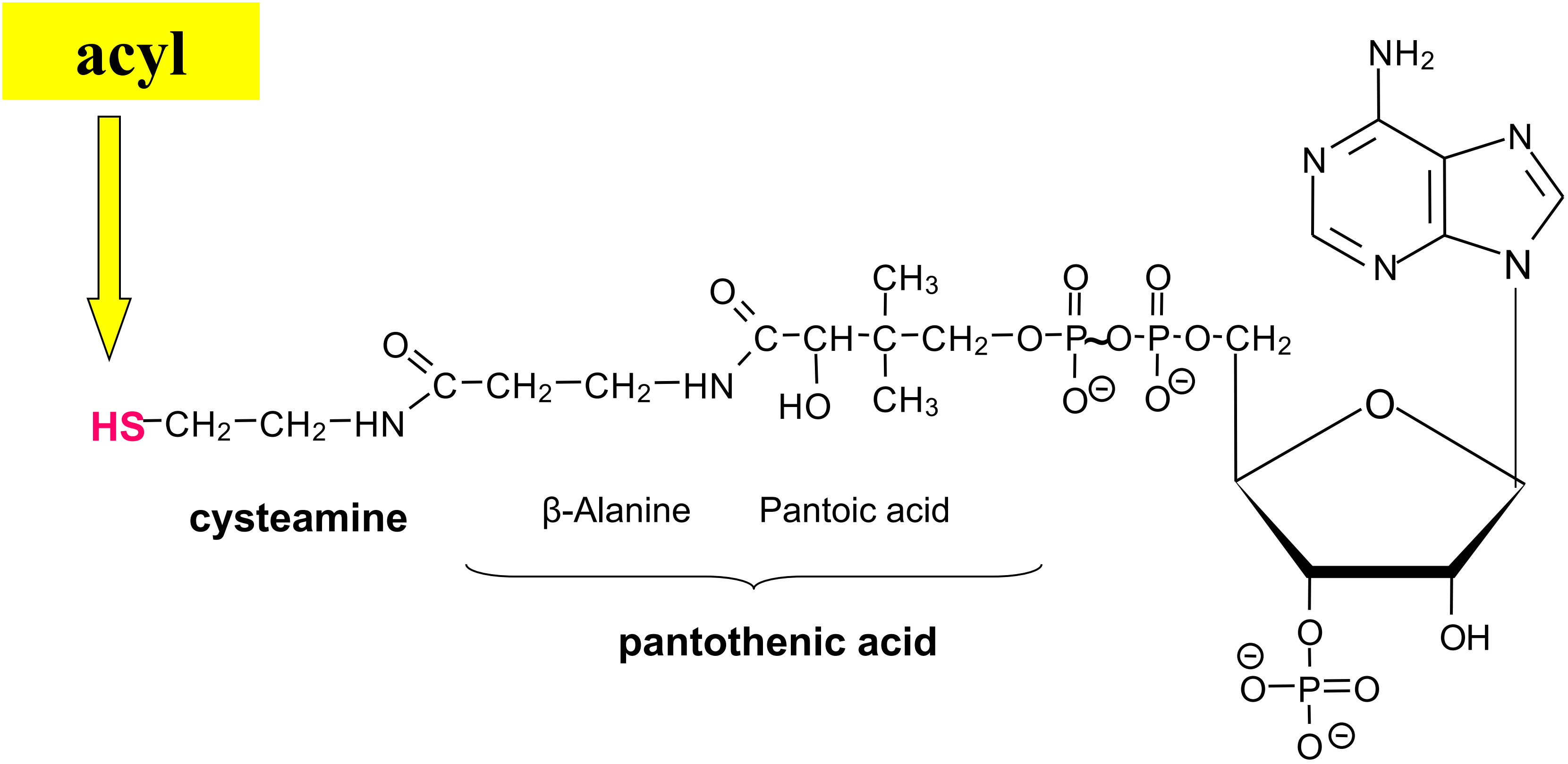
Cofactor	**Decarboxylation (does not require energy)**
Thiamin-diP	pyruvate → acetyl-CoA + CO_2 2-oxoglutarate → succinyl-CoA + CO_2
Pyridoxal-P	amino acid → amine + CO_2
None	acetoacetate → acetone + CO_2 (non-enzymatic, spontaneous)
Cofactor	**Carboxylation (requires energy)**
Biotin	pyruvate + CO_2 + ATP → oxaloacetate acetyl-CoA + CO_2 + ATP → malonyl-CoA propionyl-CoA + CO_2 + ATP → methylmalonyl-CoA → succinyl-CoA carboxylations (ATP) in the catabolism of Val, Leu, Ile
Phylloquinone (vitamin K)	protein-glutamate + O_2 + vit K_{red} + CO_2 → protein-γ-carboxyglutamate posttranslational carboxylation of glutamate → hemostasis
None	Hb-NH_2 + CO_2 → Hb-NH-COOH (unstable Hb-carbamate, spontaneous)

Coenzyme A (CoA-SH)



- transfers acyl
- attached to sulfur atom
- **thioester bond**
- acyl-CoA is activated acyl
- e.g. acetyl-CoA

Coenzyme A

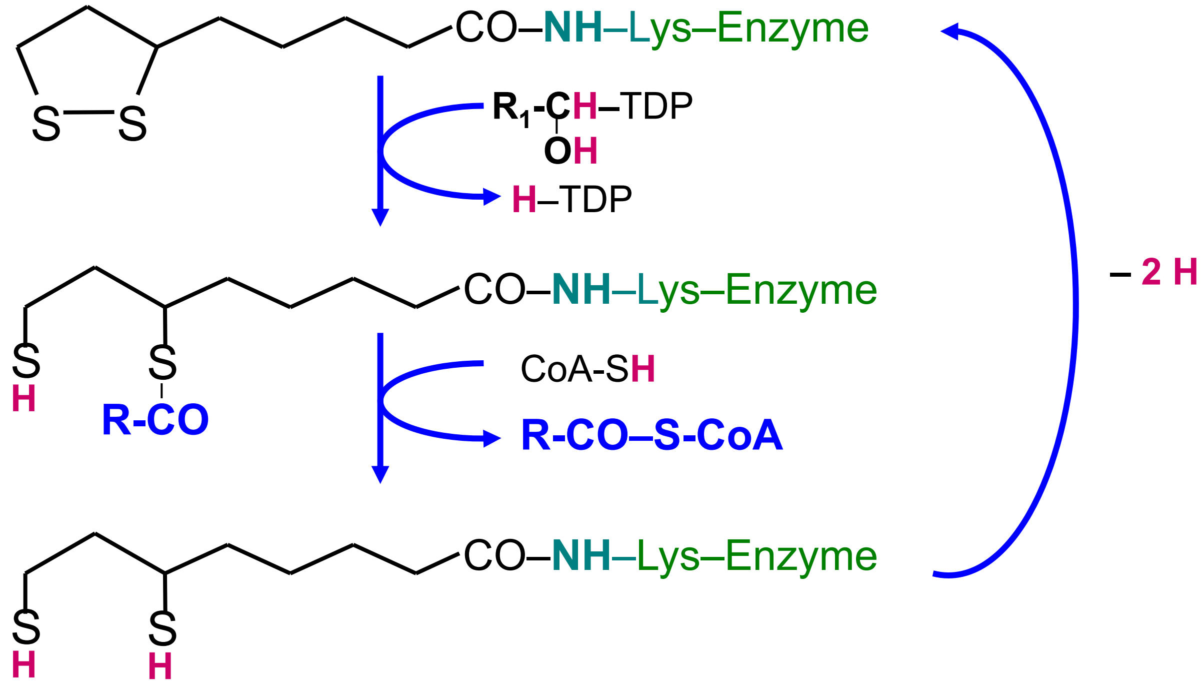


3´-PhosphoADP

Lipoate (lipoamide)

part of the 2-oxo acid dehydrogenase complex (see the following lectures)

it is oxidant of a group carried by thiamine diphosphate (TDP),
binds the resulting acyl as thioester and transfers the acyl to coenzyme A:



Lipoamide
(**oxidized** form)

***S*^{6}-Acyldihydrolipoamide**
(reduced form)

Dihydrolipoamide
(**reduced** form)

S-Adenosylmethionine (SAM)

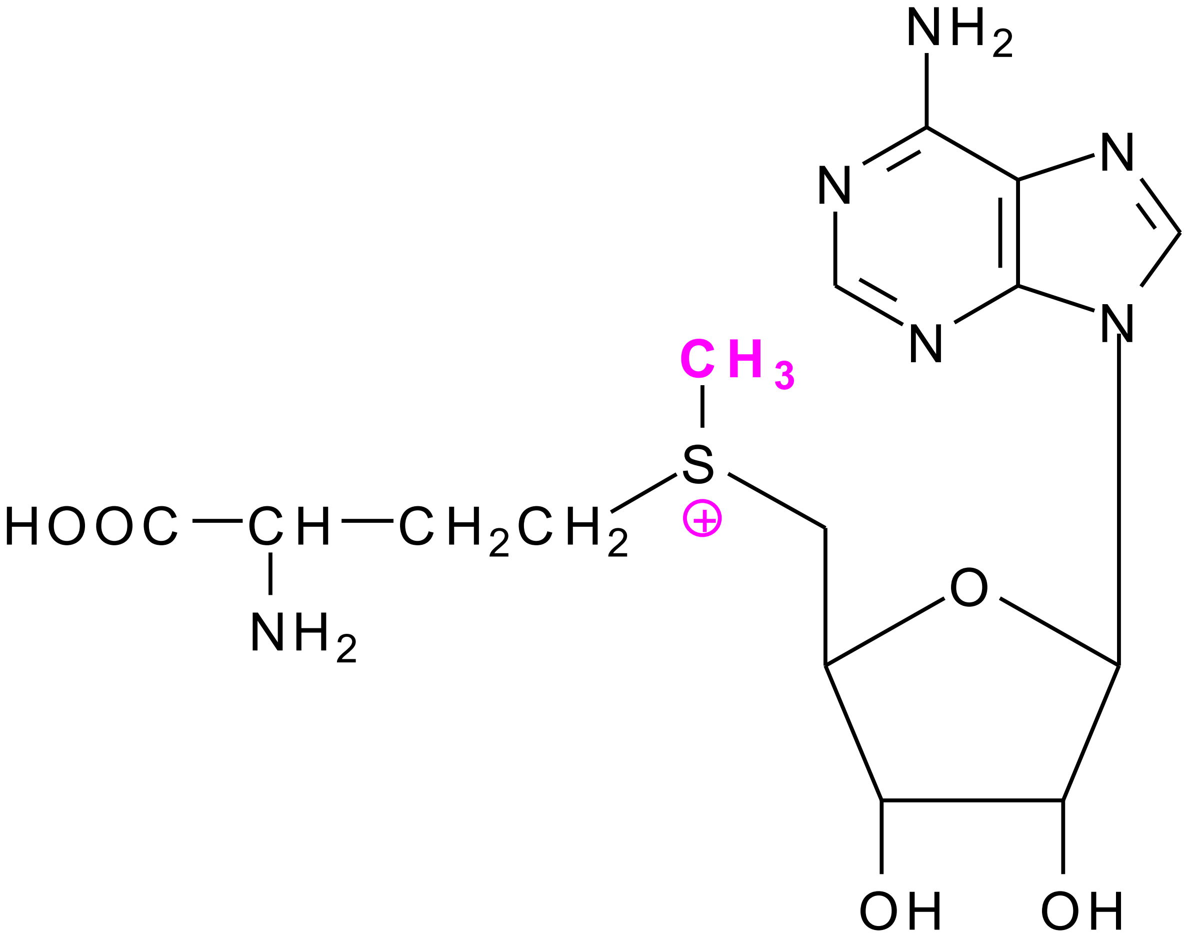
- „active methyl“, trivalent sulfur ⇒ **sulfonium cation**
- cofactor of methylation reactions:

  ethanolamine → choline (3 methylation)

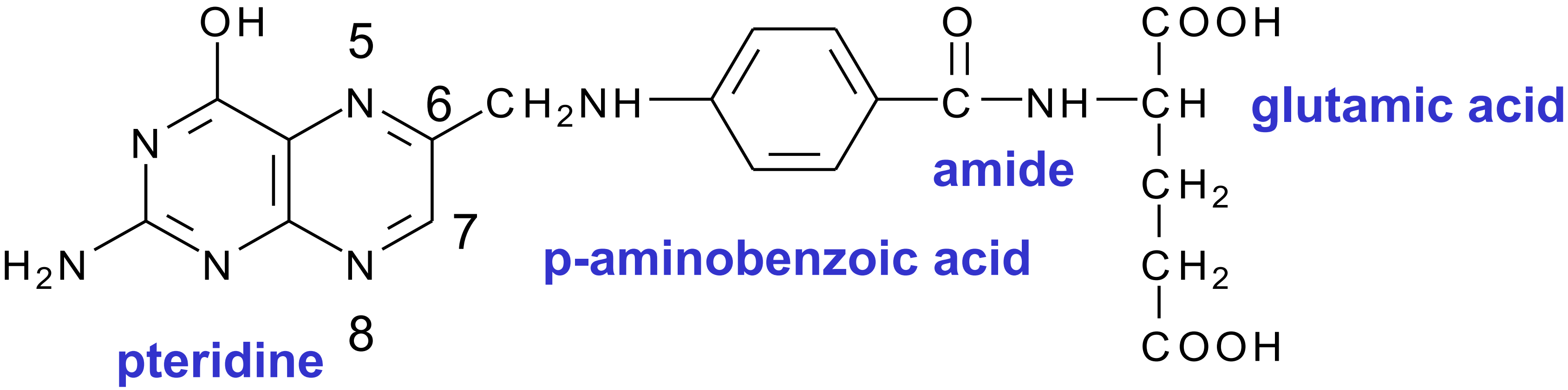
  guanidine acetate → creatine

  noradrenaline → adrenaline ..... and many others
- side product is **homocysteine**
- remethylation of homocysteine needs methyl-FH_4 + B_{12} cofactor (see Seminars)

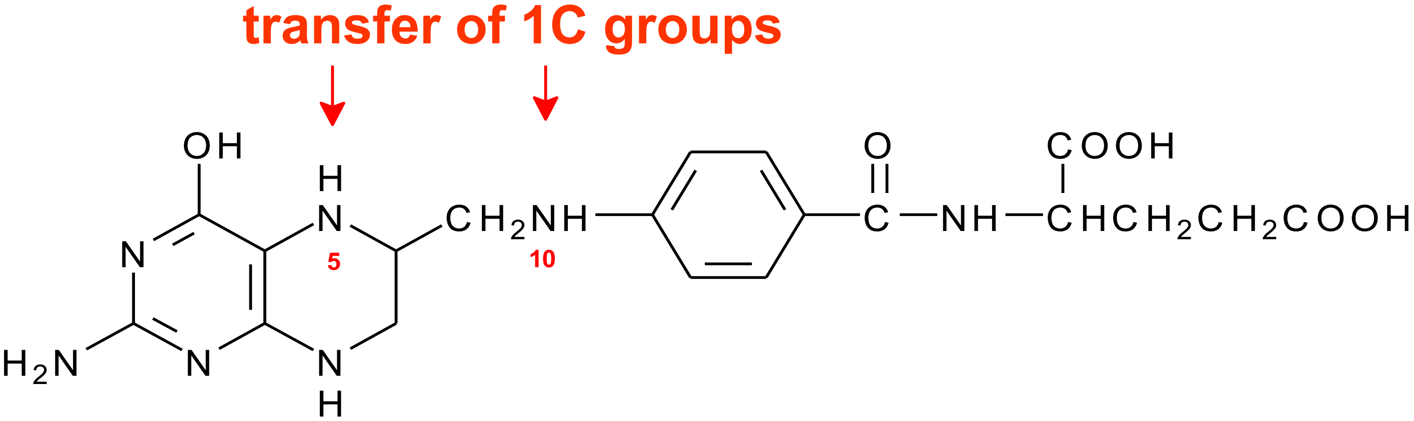
S-Adenosylmethionine (SAM)



Folic acid is vitamin.
In the body, it is hydrogenated to 5,6,7,8-tetrahydrofolate.



Tetrahydrofolate (FH_4) is cofactor for the transfer of C_1 groups



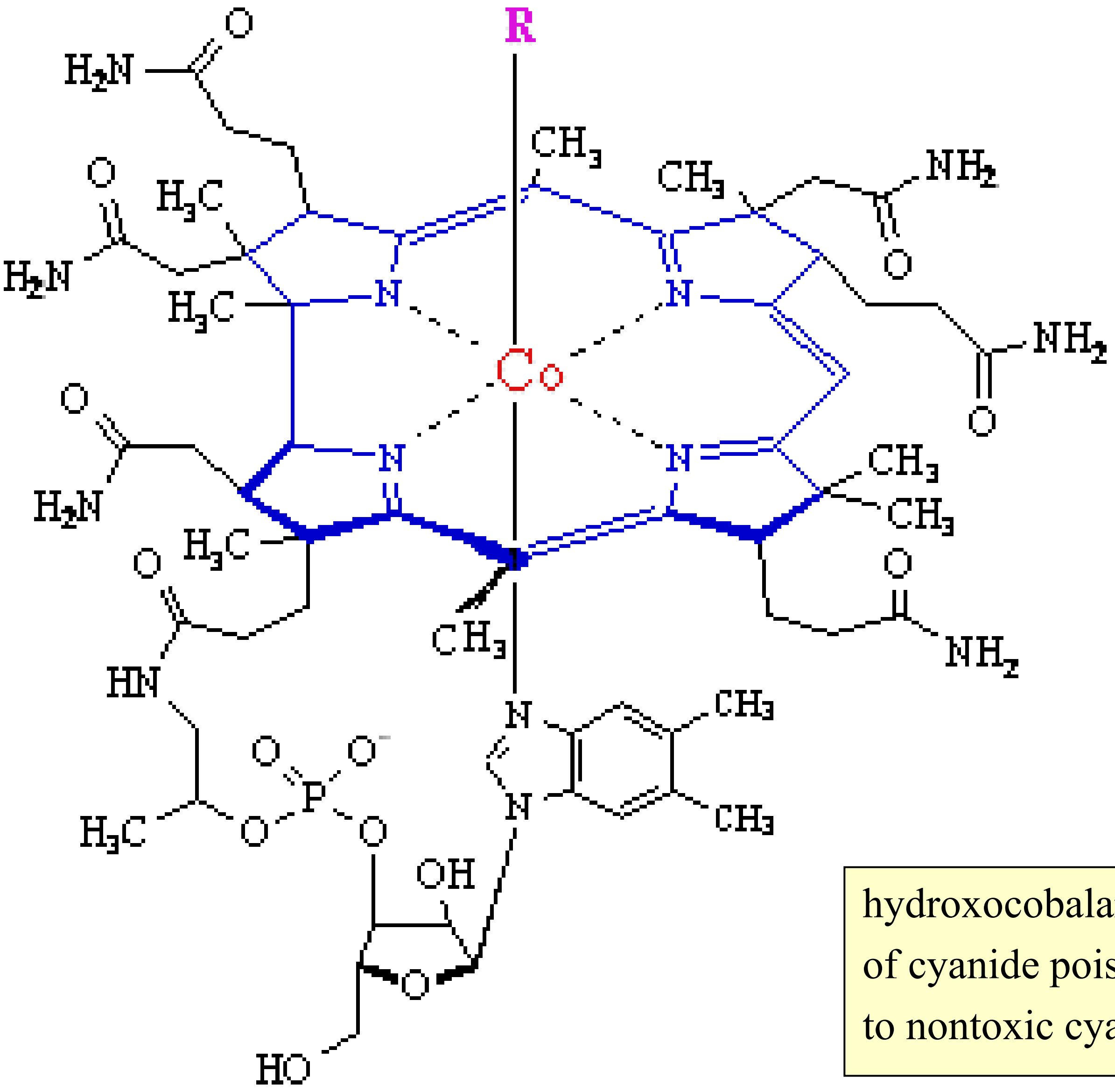
C_1 Groups transferred by FH_4

compare scheme

Seminars, p. 26

Oxidation number of C	**Formula**	**Name**	**Metabolic Origin / Comment**
-III	$-CH_3$	methyl	reduction of methylene-FH_4 (from serine, glycine) methyl-FH_4 cooperates with B_{12} cofactor in methylation
-II	$-CH_2-$	methylene	catabolism of serine, glycine used in synthesis of dTMP → DNA
-I	$-CH=$	methenyl	deamination of formimino-FH_4 (from histidine) used in synthesis of purine bases
+I	$-CH=O$	formyl	catabolism of tryptophan → formiate → formyl used in synthesis of purine bases
+I	$-CH=NH$	formimino	catabolism of histidine

B_{12} vitamin is cyano or hydroxocobalamin



R = CN or OH

corrin cycle

hydroxocobalamin is used in the treatment of cyanide poisoning, it binds cyanide ions to nontoxic cyanocobalamin

B_{12} cofactor is methyl or deoxyadenosylcobalamin, it is needed for two reactions in the body

1. homocysteine $\xrightarrow{FH_4 / B_{12}}$ methionine

   methylation of homocysteine (regeneration of methionine)

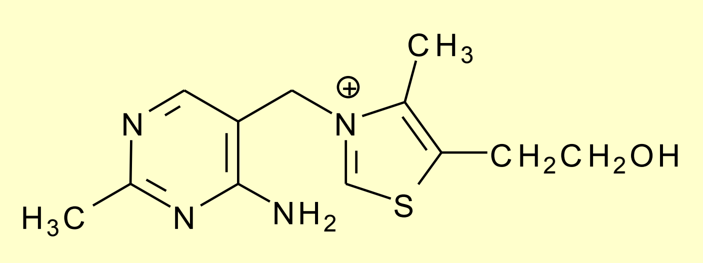
2. homocysteine → → propionyl-CoA $\xrightarrow{B_{12}} \rightarrow$ succinyl-CoA

Compare: Four different cofactors of methylations

Cofactor	**Origin of methyl**	**Examples of methylation reactions**
SAM	methionine	ethanolamine → choline guanidine acetate → creatine noradrenaline → adrenaline methylation of DNA (regulation of gene expression) methylation of bases in tRNA / mRNA (guanine-N^7 = cap) inactivation of catecholamines (COMT): • dopamine → methoxytyramine • noradrenaline → normetanephrine • adrenaline → metanephrine methylation of xenobiotics (II. phase - conjugation)
methyl-FH$_4$	methylene-FH$_4$	homocysteine → methionine
methyl-B$_{12}$	methyl-FH$_4$	
methylene-FH$_4$	serine, glycine	dUMP → dTMP dUMP + methylene-H$_4$F → dTMP + H$_2$F (thymidylate synthase)

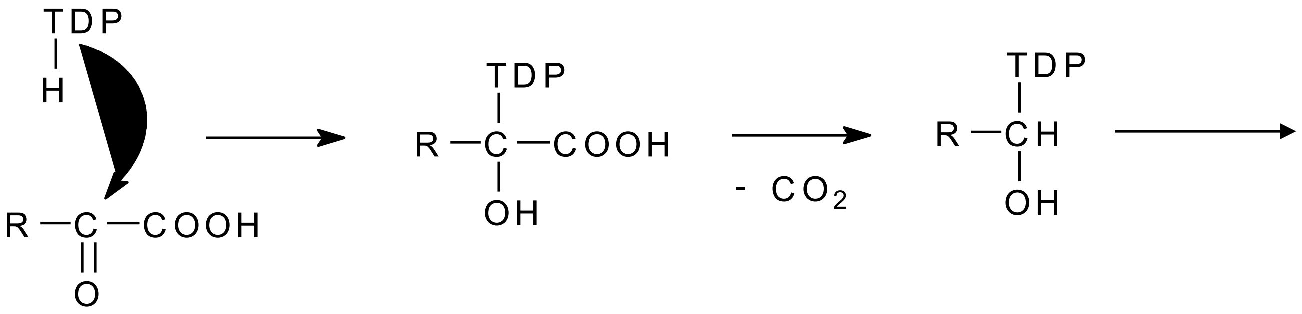
SAM = *S*-adenosylmethionine, FH$_4$ = tetrahydrofolate, COMT = catechol *O*-methyltransferase

Thiamin is vitamin B_1
Thiamin diphosphate (TDP) is cofactor



Oxidative decarboxylation of some 2-oxo acids

- pyruvate → acetyl-CoA
- 2-oxoglutarate → succinyl-CoA (citrate cycle)
- 2-oxo acids in the catabolism of branched amino acids (Val, Leu, Ile)



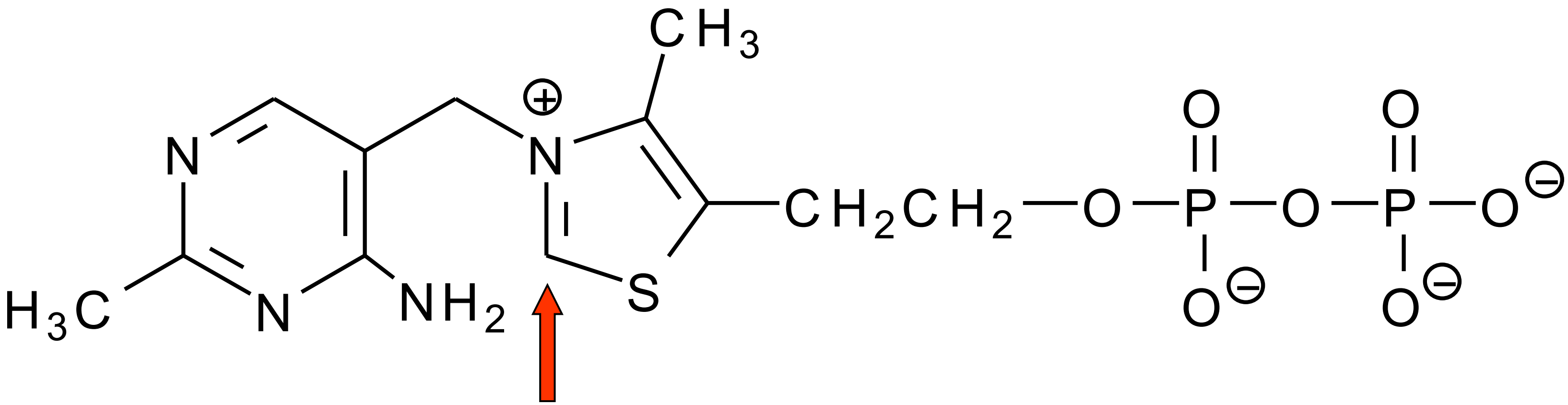
transfer to

dihydrolipoate and CoA

Transketolase reactions in pentose cycle

- ribose-5-P + xylulose-5-P ⇆ glyceraldehyde-3-P + sedoheptulose-7-P
- xylulose-5-P + erythrose-4-P ⇆ fructose-6-P + glyceraldehyde-3-P

Thiamin diphosphate (TDP) is cofactor in the oxidative decarboxylation of pyruvate



attachment of pyruvate and its decarboxylation

glucose → pyruvate $\xrightarrow{\text{TDP}}$ acetyl-CoA

↓

CAC

In human body, a number of non-enzymatic reactions proceeds

- decarboxylation of acetoacetate → acetone
- catabolism of creatine → creatinine (dehydration + cyclization)
- glycation / carbamylation / nitrosylation / nitration of proteins
- the reactions of reactive oxygen species (e.g. lipoperoxidation)
- spontaneous oxidation of hemoproteins (hemoglobin → methemoglobin)
- spontaneous oxidation of urobilinogens to urobilins (large intestine)
- condensation of amines with carbonyl compounds to heterocyclic derivatives
  dopamine + pyruvate → salsolinol (neurotoxin ?)
  tryptamine + pyruvate → harmane
  dopamine + dihydroxyphenylacetaldehyde → tetrahydropapaveroline
- binding ligands to proteins:
  bilirubin + albumin → bilirubin-albumin complex
  CO + hemoglobin → carbonylhemoglobin
- the interactions of macromolecules:
  antigen + antibody → immuno complex