Biochemistry I

2nd Semester
Academic Year 2008/2009

Biochemistry I - textbooks:

Lippincott´s Illustrated Reviews: **Biochemistry. 3rd** Edition 2004
Pamela C. **Champe**, R. A. Harvey, R. Denise
Lippincott Williams & Wilkins, Philadelphia, PA, ISBN 0-7817-2265-9

(Alternative for both Biochemistry I and Biochemistry II:)
Harper's Illustrated Biochemistry, 27th Edition 2006
R. K. Murray, D. K. Granner, P. A. Mayes, V. W. Rodwell et al.
Appleton & Lange – Prentice Hall, London, ISBN 0-071461973

Biochemistry I – Seminars, **1st** Edition 2008
J. **Tomandl**, E. **Táborská** et al.
Masaryk University, Brno, ISBN 978-80-210-4547-7

Alternatives:

J. Baynes, M. H. Dominiczak: **Medical Biochemistry,** 2nd Edition 2004, Mosby, London, ISBN 978-072343341-0

Colleen M. Smith, A. D. Marks, M. Lieberman: **Marks' Basic Medical Biochemistry** A Clinical Approach. – 2nd Edition 2004 Lippincott Williams & Wilkins, Philadelphia, PA, ISBN 0-7817-2145-8

D. K. Apps, B. B. Cohen, C. M. Steel: **Biochemistry** (A Concise Text for Medical Students). – 5th Ed., 1992, Baillière Tindall, London, ISBN 0-7020-1444-3

T. M. Devlin: **Textbook of Biochemistry with Clinical Correlations** 6th Ed., 2005, Wiley

P. Campbell, A. D. Smith, T. J. Peters: **Biochemistry Illustrated** (264 pp., 508 illustrations) 5th Ed., 2005, ISBN 0443100349

Enzymes – Part I

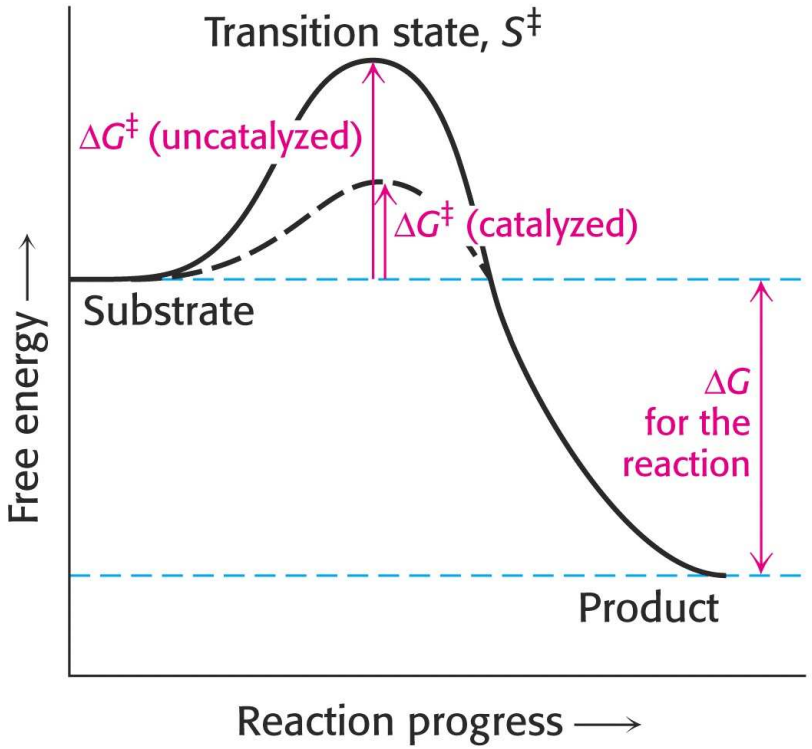
Biochemistry I
Lecture 1

2009 (J.S.)

Variety of biochemical reactions that comprise life are remarkable **biological catalysts** known as **enzymes**.

As any chemical catalysts at all, enzymes

- **remain unchanged** after the catalyzed reactions,
- **cannot alter reaction equilibria** (the values of *K*),
- **increase the rate** at which a reaction approaches equilibria by lowering the height of the kinetic barrier, i.e. **by lowering the free energy of activation $\Delta G^{\ddagger}$.**



Nearly all **enzymes are** **proteins**.
Some enzymes also have non-protein **prosthetic groups** (covalently bound coenzymes), some other enzymes associate with **metal ions** or organic **coenzymes** that are needed for catalytic activity.

The first enzyme prepared as pure substance in crystalline form was urease (Sumner 1926), the primary structure of it (complete amino acid sequence) was recognized in 1960.

Lysozyme was the first enzyme the tertiary structure of which had been estimated by X-ray diffraction in 1965.

Since the 80´s, some types of **ribonucleic acids** are known that catalyze splitting and certain polymerization of nucleic acids. These catalysts are named **ribozymes**.

About 3000 different molecular types of enzymes are present in an average cell. Many of them are localized **only in certain organelles** (compartments) within the cell. The **principle of compartmentalization** facilitates the control of different metabolic pathways.

The characteristic attributes of enzymes

Enzymes differ from ordinary chemical catalysts
in four important respects:

1 **High catalytic efficiency** resulting in high reaction rates.

2 **Enzymes function under mild reaction conditions**

3 **Specificity of the catalytic action**

4 **Capacity for regulation**

1 High catalytic activities

Reaction rates are at least **several orders of magnitude greater** than those of the corresponding reactions catalyzed by ordinary chemicals and 10^6–10^{17} times greater than those of non-catalyzed reaction.

On the other hand, enzymes like other proteins exhibit a **low degree of stability** and are subjects of biodegradation.

Rate enhancement by selected enzymes

Enzyme	Nonenzymatic half-life	Uncatalyzed rate (k_{un} s^{-1})	Catalyzed rate (k_{cat} s^{-1})	Rate enhancement (k_{cat}/k_{un})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wofenden. *Science* 267 (1995):90–93.

2 Enzymes function under mild reaction conditions:

- at atmospheric pressure,
- at low temperature
  (most of the enzymes are **denatured above 50 °C**),
- in limited range of pH values
  (denaturation of enzymes in extremely acidic as well as alkaline solutions),
- in dilute solutions (at very low enzyme concentrations).

3 Enzymes are highly specific catalysts

Specificity in the reaction that enzymes catalyze

An enzymes usually catalyzes a **single type of chemical reaction** or a set of closely related reactions:

Enzymes are classified on the basis of the types of reactions that they catalyze (oxidoreductases, transferases, hydrolases, etc.).

The type of catalyzed reaction depends very much of the coenzyme (if it is needed for the reaction).

Substrate specificity – enzymes are specific in the **choice of reactants**.

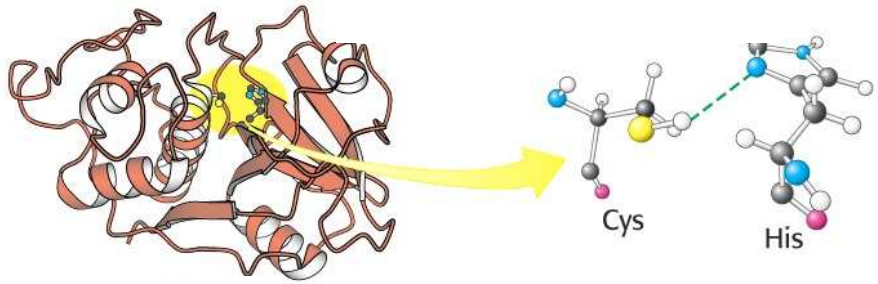
The first step in enzymatic catalysis is the binding of the proper reactant (called substrate) to the enzyme – the formation of an enzyme-substrate (ES) complex.

From many compounds that may react in a certain type of reaction, enzymes are usually able to bind a limited **group of similar compounds** of the same type; the existence of a **unique specific substrate** (so-called absolute substrate specificity) is rather exceptional.

Example:
Major classes of proteinases

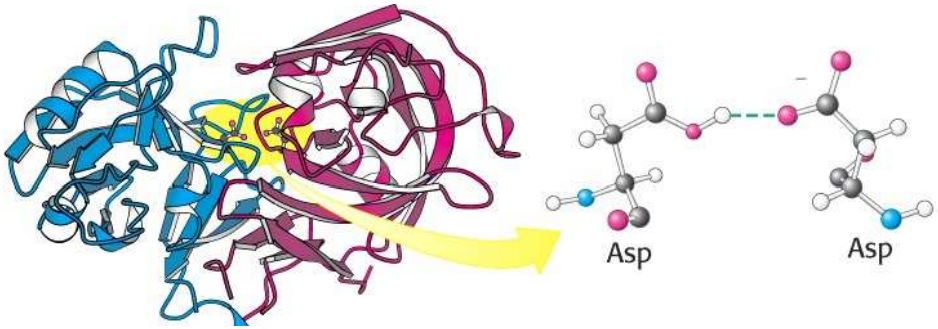
All of them are peptide-cleaving enzymes.
Due to the different arrangement of active sites, they exhibit **different substrate specificities** and **different catalytic mechanisms**.

CYSTEINE PROTEINASES



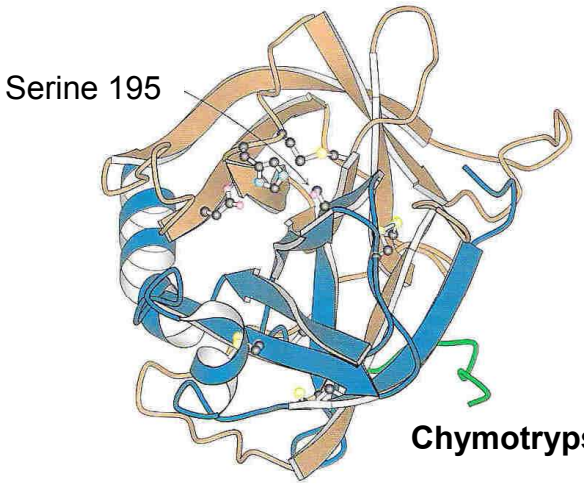
Cathepsins, papain

ASPARTYL PROTEINASES



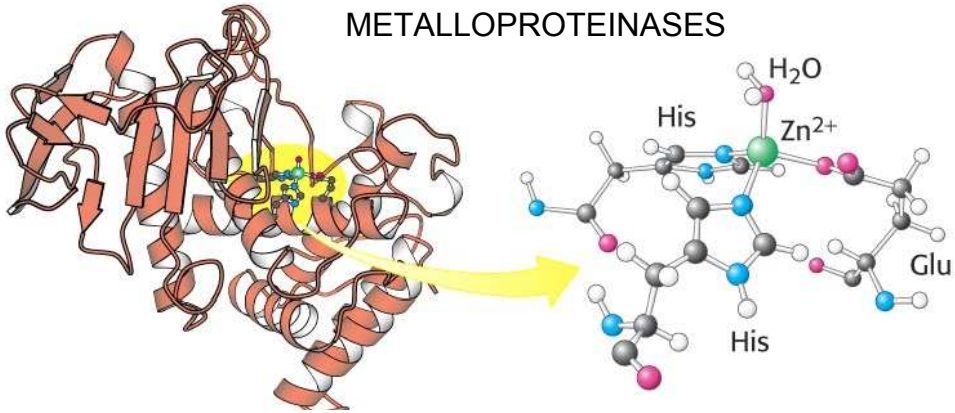
Pepsin, renin

SERINE PROTEASES



Chymotrypsin, trypsin

METALLOPROTEINASES



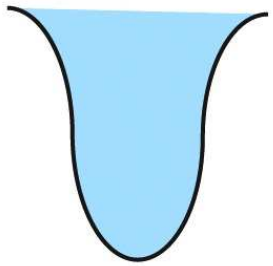
Carboxypeptidase A, thermolysin (bacterial)

The active sites of three homologous **serine proteinases**
- members of the chymotrypsin family:

Their overall structure is nearly the same, the amino acid sequences are approximately 40 % identical.

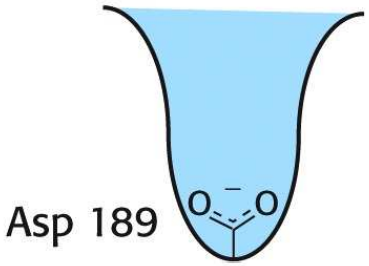
Certain residues other than serine in **"additional pockets"** play key roles in determining the substrate specificity of these enzymes.

The peptide bonds preferred to be cleaved
by **chymotrypsin** are those just past residues with large, hydrophobic, and uncharged side chains (e.g. Phe, Trp),
by **trypsin** those after residues with long, positively charged side chains (Arg, Lys),
by **elastase** those past residues with small hydrophobic side chains (Gly, Ala).



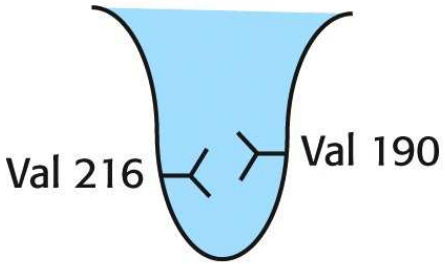
Chymotrypsin

A deep, relatively hydrophobic pocket



Trypsin

At the bottom of the deep pocket there is an acidic residue (Asp)



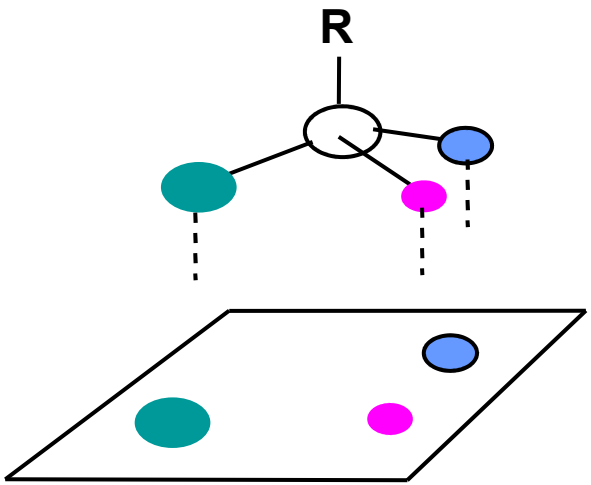
Elastase

Two residues of valine close off the mouth of the small hydrophobic pocket

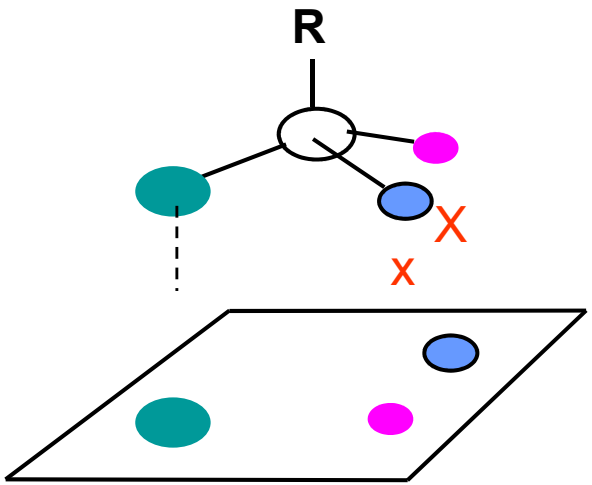
Enzymes are frequently **stereospecific catalysts**

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

Chiral substrates are bound to the stereospecific enzymes **at three sites**:



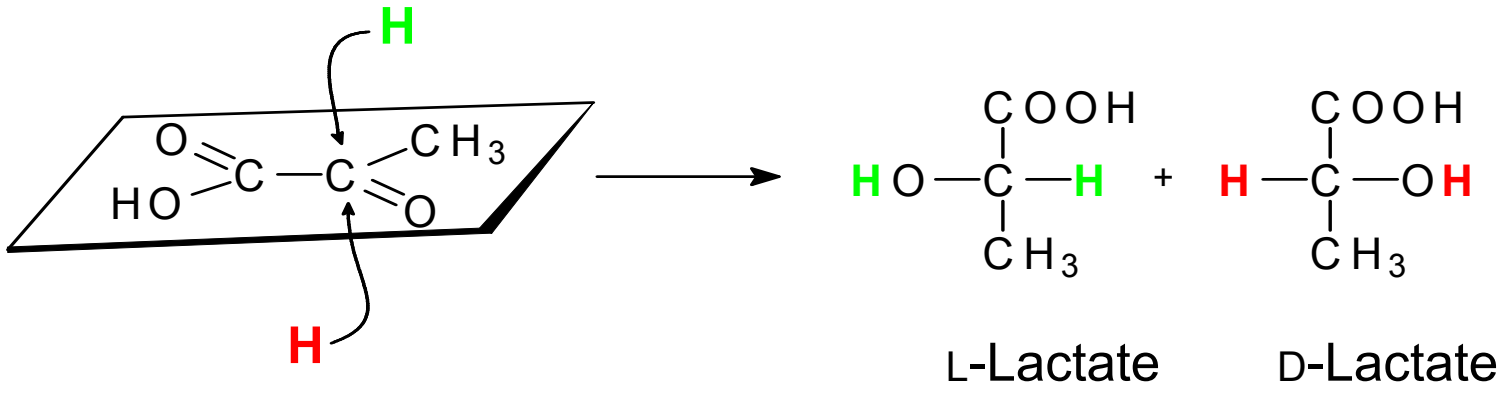
Proper substrate



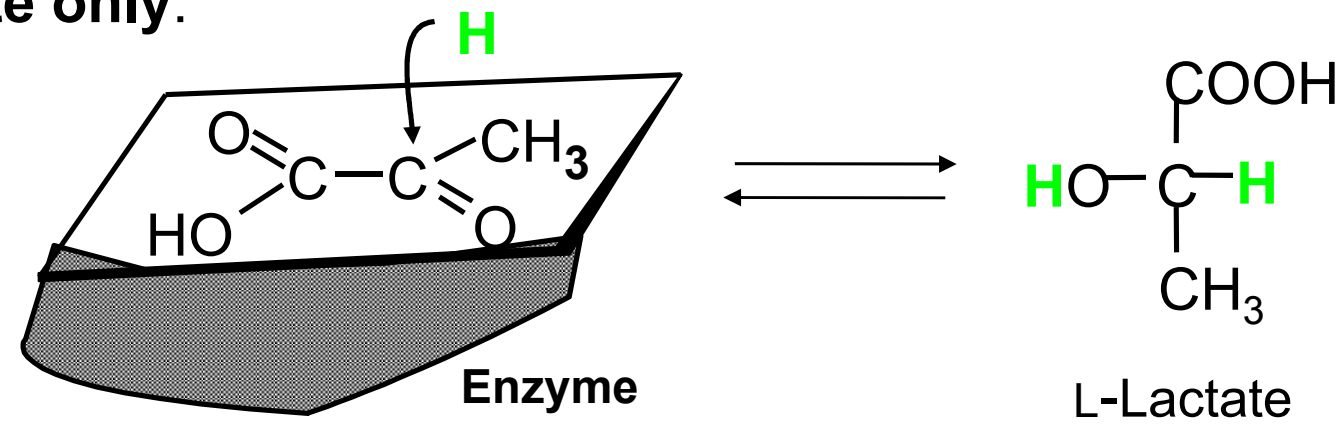
Not-fitting enantiomer

Example:

When pyruvate is hydrogenated without any catalyst (e.g. *in vitro*), the reaction product is the **racemic mixture of D-lactate and L-lactate**.



In the same reaction catalyzed by ***lactate dehydrogenase*** (in the presence of NADH), pyruvate is reduced stereospecifically to **L-lactate only**:



4 Enzymes are regulated catalysts

- **The catalytic activity** of enzymes can be inhibited or increased
  by the binding of **specific small molecules and ions**,
  by the binding of **regulatory proteins**,
  by **covalent modifications** of enzyme molecules
  (e.g. through phosphorylation controlled by extracellular signals).

- **The specificity** of few enzymes also can be changed
  (the specificity of galactosyl transferase during period of lactation)

- **The amount of the enzyme** in cells is controlled by
  induced expression of the particular genes ("adaptable" enzymes),
  control of the proteosynthesis of new enzyme molecules
  and/or by regulated proteolysis (breakdown) of enzymes.

Enzyme nomenclature

Some **historical** names are used for few enzymes till this time (without any respect to the function belonging to them, e.g. pepsin, trypsin, cathepsin, catalase).
The common ending ***–ase*** was settled for all enzymes later on.
The contemporary enzyme nomenclature is determined by the Enzyme Commission (EC) of the International Union of Biochemistry (IUB).
There are two types of names:

– **Systematic names** identifying the enzymes fully (with the enzyme **code number**, too) without ambiguity. Those names are not very convenient for everyday use. Only some examples will be introduced to the students.

– **Recommended names** (some of them are historical) are shorter than systematic names; recommended names will be used in this course.

Enzyme nomenclature was adapted according to the **IUB Enzyme classification**.

Six major classes of enzymes were constituted:

Class	Type of reaction	Example
1. Oxidoreductases	Oxidation-reduction	Lactate dehydrogenase
2. Transferases	Group transfer	Nucleoside monophosphate kinase (NMP kinase)
3. Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	Chymotrypsin
4. Lyases	Addition or removal of groups to form double bonds	Fumarase
5. Isomerases	Isomerization (intramolecular group transfer)	Triose phosphate isomerase
6. Ligases	Ligation of two substrates at the expense of ATP hydrolysis	Aminoacyl-tRNA synthetase

Major class 1 Oxidoreductases

catalyze oxidation-reduction of substrates.

Subclasses and frequent recommended names:

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

Example: ethanol + NAD+ $\rightleftharpoons$ acetaldehyde + NADH + H+

Recommended name of the enzyme *alcohol dehydrogenase*

Systematic name *ethanol*:*NAD*$^+$ *oxidoreductase*

(Classification number EC 1.1.1.1)

Major class 2 Transferases

catalyze transfers of an atomic group from one to another substrate.

Subclasses and frequent recommended names:

- **aminotransferases, methyltransferases, glucosyltransferases,**
- **phosphomutases** catalysing transfers of the groups PO_3^{2-} within certain molecules,
- **kinases** phosphorylating substrates by the transfer of the phosphoryl group PO_3^{2-} from ATP (e.g. hexokinases, proteinkinases, pyruvate kinase).

Example: glucose + ATP ⟶ glucose 6-phosphate + ADP

Recommended name of the enzyme *glucokinase*

Systematic name *ATP:D-glucose phosphotransferase*

(Classification number EC 2.7.1.1)

Major class 3 Hydrolases

catalyze hydrolytic splitting of esters, glycosides, ethers, amides and peptides, anhydrides of acids, etc.

Some subclasses and frequent recommended names:

- **esterases** (e.g. lipases, phospholipases, ribonucleases, phosphatases),
- **glycosidases** (e.g. saccharase, maltase, amylases),
- **proteinases** and **peptidases** (pepsin, trypsin, cathepsins, dipeptidases, carboxypeptidases and aminopeptidases),
- **amidases** (glutaminase, asparaginase),
- **ATPases** splitting anhydride bonds of ATP.

Example: glucose 6-phosphate + H_2O $\longrightarrow$ glucose + HPO_4^{2-}

Recommended name of the enzyme *glucose 6-phosphatase*

Systematic name *glucose 6-phosphate phosphohydrolase*

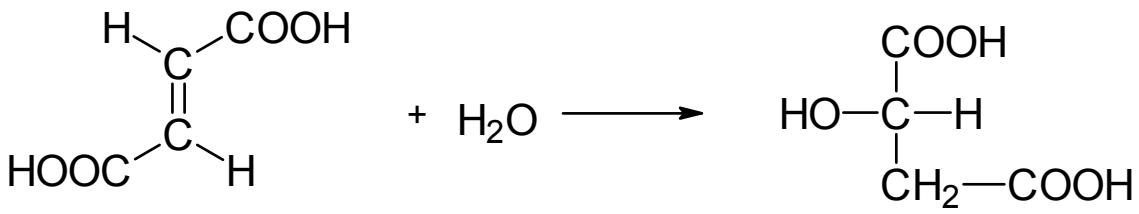
Major class 4 Lyases

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

Some frequent recommended names:

- **ammonia lyases** (e.g. histidine ammonia lyase – histidase),
- **decarboxylases** (carboxy lyases),
- **aldolases** (catalyzing aldol cleavage and formation),
- (**de**)**hydratases** (carbonate dehydratase, enolase),
- **synthases** (citrate synthase).

Example: fumarate + H_2O ⟶ L-malate



Recommended name of the enzyme *fumarate hydratase*

Major class 5 Isomerases

catalyze intramolecular rearrangements of atoms.

Frequent names:

- **epimerases**,
- **racemases**,
- (some types of) **mutases**.

Example: UDP-glucose ⟶ UDP-galactose

Recommended name *UDP-glucose 4-epimerase*

Major class 6 Ligases

catalyze formation of high-energy bonds C–C, C–O, C–N in the reaction coupled with hydrolysis of another high-energy compound (usually molecule ATP).

Frequent recommended names

- **carboxylases**
- **synthetases** (e.g. glutamine synthetase; don't change for synthases in class 4!)

Example: Pyruvate + CO_2 + ATP + H_2O $\longrightarrow$ oxaloacetate + ADP + Pi
Recommended name *pyruvate carboxylase*

Classification numbers of enzymes

EC (abbr. Enzyme Commission)
major class number
. subclass number
. sub-subclass number
. the enzyme's arbitrarily assigned serial number in its subsubclass

Example:

Recommended name: *Alanine aminotransferase* (ALT)

Code number: **EC 2.6.1.2**

- Major class	2	Transferases
- Subclass	6	- transferring nitrogenous groups
- Sub-subclass	1	- transferring amino groups
- Enzyme's number	2	*Alanine aminotransferase*

Systematic name: L-*Alanine*:*2-oxoglutarate aminotransferase*

The structure of enzymes

Not speaking of ribozymes, enzymes are **proteins** mostly of globular type. Some of them exhibit simple or complicated quaternary structures (oligomeric enzymes and multienzyme complexes).

The catalytic activity of many enzymes depends on the presence of small molecules – **cofactors**.

Such an enzyme without its cofactor is referred to as an **apoenzyme**, the complete active enzyme is **holoenzyme**.

Cofactors can be bound to an enzyme covalently as a **prosthetic group**, or non-covalently like a "co-substrate" named **coenzyme**.

Biochemists do not usually discriminate between the two types of cofactors and use for both the term **coenzymes**.

Enzymes the structure of which comprises ions or atoms of a metal that function as cofactors are classified as **metalloenzymes**.

The active sites of enzymes

The active site of an enzyme is a **relatively small part** of the structure that binds the substrates (and the cofactor, if any).

It is a **three-dimensional cleft** formed by groups that come from different parts of the amino acid sequence.

In the active site there are

- groups that provide the binding of substrates by multiple weak attractions,
- groups that take direct part in the catalytic mechanism (e.g. with an electric charge or donating protons),
- auxilliary groups that reinforce and finish the proper shape of the active site (remind the flexibility of protein tertiary structures).

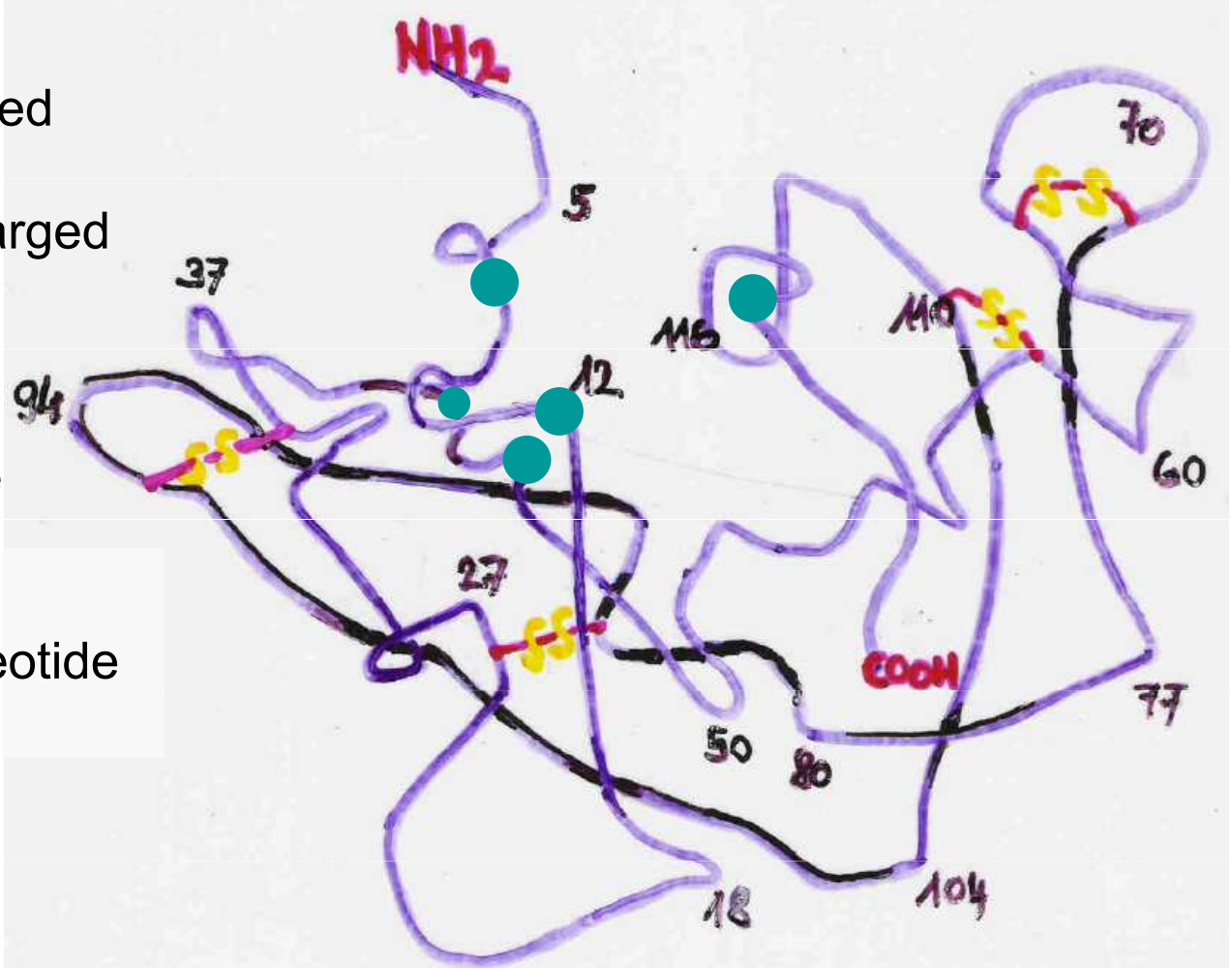
The precisely defined arrangement of atoms in an active site that is **finished after the substrate is bound** is the cause of high substrate specificity of enzymes.

This process of dynamic recognition of the proper substrate(s) is called **induced fit** (D. E. Koshland, Jr., 1958).

Ribonuclease (from bovine pancreas) – a diagram of the main chain.
The number represent the positions of α-carbons.

The green dots
are the positively charged
residues of Lys and His
that bind negatively charged
chain of the RNA.

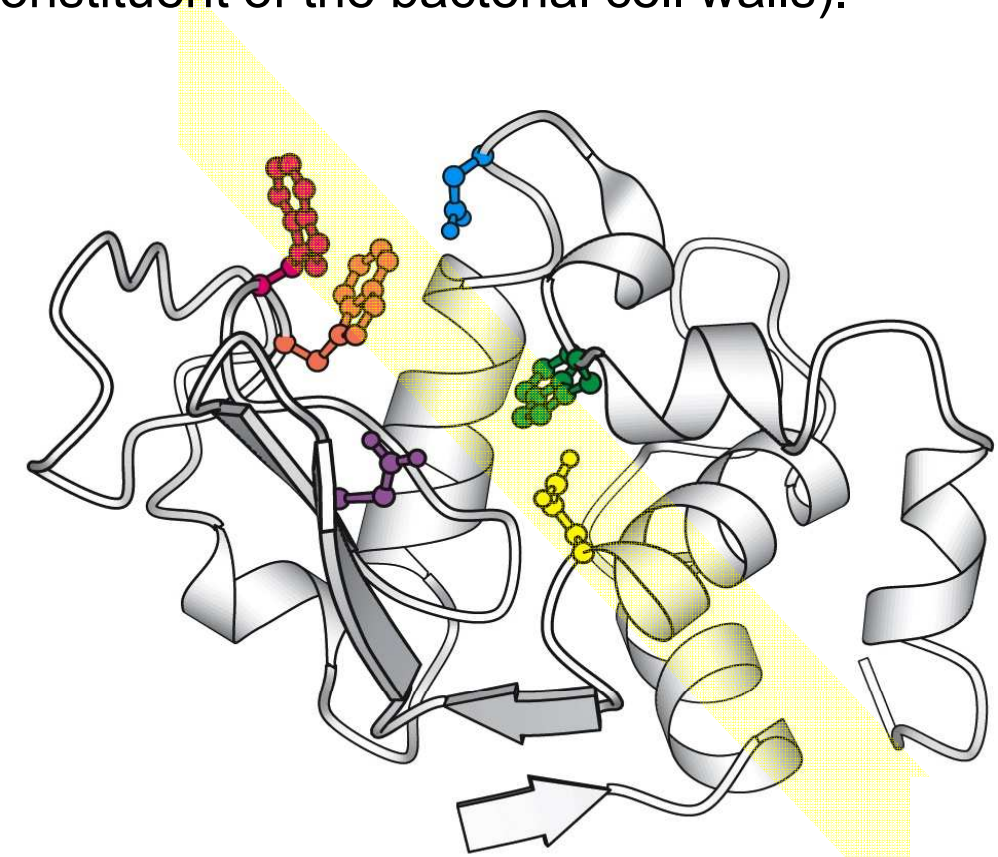
The shape of the active
site can be seen as
<u>a deep cut</u>. It enables
binding of the polynucleotide
chain.



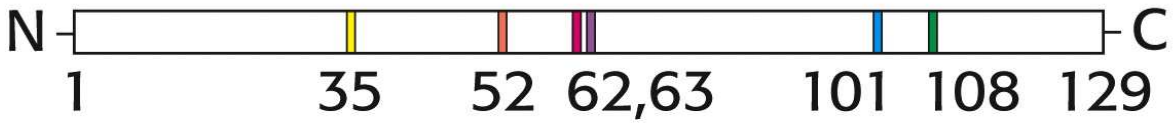
Lysozyme – The enzyme catalyzes hydrolytic decomposition of the long chains of the polysaccharide muramic acid (a constituent of the bacterial cell walls).

The active site again in the shape of **an incision** into the tertiary structure.

Ribbon diagram with several components of the active site.



A schematic representation of the primary structure – the active site is composed of residues that come from different parts of the chain.



Enzyme cofactors

Cofactors	Enzyme
Coenzymes	
Thiamine pyrophosphate	Pyruvate dehydrogenase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Ions or atoms of metals	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase
K^{+}	Propionyl CoA carboxylase

Numerous coenzymes are derivatives of vitamins:

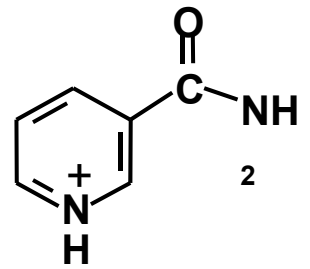
Vitamin	Coenzyme	Typical reaction type
Thiamine (B_1)	Thiamine pyrophosphate	Aldehyde transfer
Riboflavin (B_2)	Flavin adenine dinucleotide (FAD)	Oxidation–reduction
Pyridoxine (B_6)	Pyridoxal phosphate	Group transfer to or from amino acids
Nicotinic acid (niacin)	Nicotinamide adenine dinucleotide (NAD^+)	Oxidation–reduction
Pantothenic acid	Coenzyme A	Acyl–group transfer
Biotin	Biotin–lysine complexes (biocytin)	ATP-dependent carboxylation and carboxyl-group transfer
Folic acid	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis
B_{12}	5′-Deoxyadenosyl cobalamin	Transfer of methyl groups; intramolecular rearrangements

Cofactors of oxidoreductases (Major class 1)

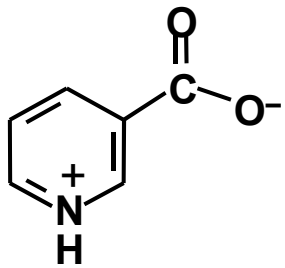
exist in the oxidized and the reduced forms (a redox half-cell).

NAD$^+$ - nicotinamide adenine dinucleotide

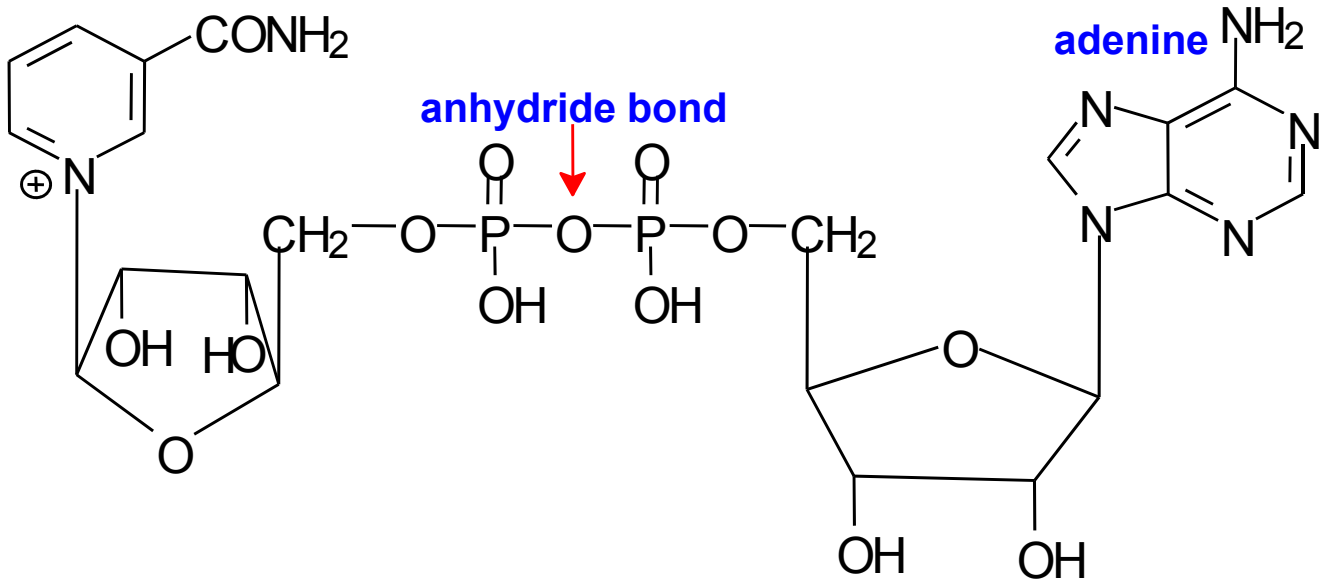
The constituent of NAD$^+$ (as well as of NADP$^+$) is nicotinamide:



Nicotinamide

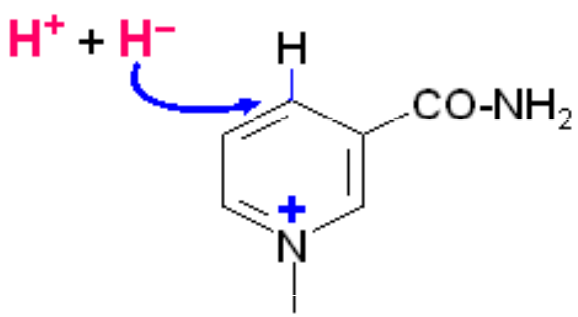


Nicotinic acid
(niacin, PPF)

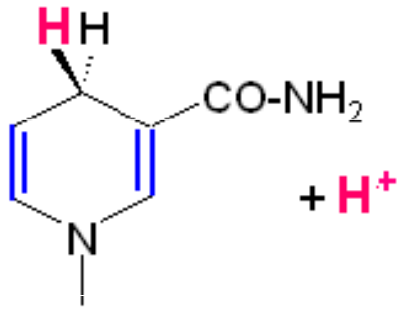


NAD^+ is the coenzyme of dehydrogenases

It acts as an **oxidant** that takes off **two atoms of hydrogen** from the substrate.
One atom plus one electron (**hydride anion H^-**) is added to the *para*-position of the pyridinium ring, the remaining H^+ binds to the enzyme.

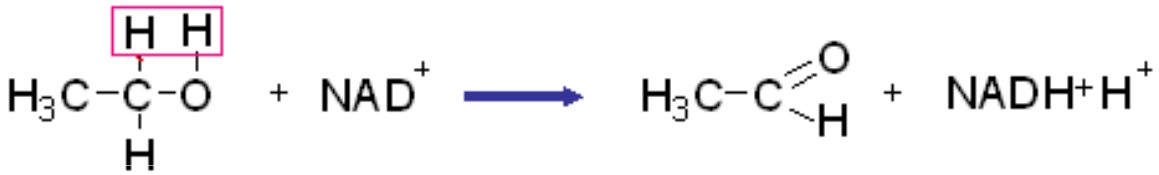


Oxidized form NAD^+
(aromatic ring, positive charge)



Reduced form $NADH + H^+$
(quinoid ring, no charge)

Example: Dehydrogenation of ethanol by ***alcohol dehydrogenase***:



NADPH + H^+

- nicotinamide adenine dinucleotide phosphate

acts as an **reductant** that supplies **two atoms of hydrogen**

– to the substrates **in reductive syntheses** of fatty acids or cholesterol,

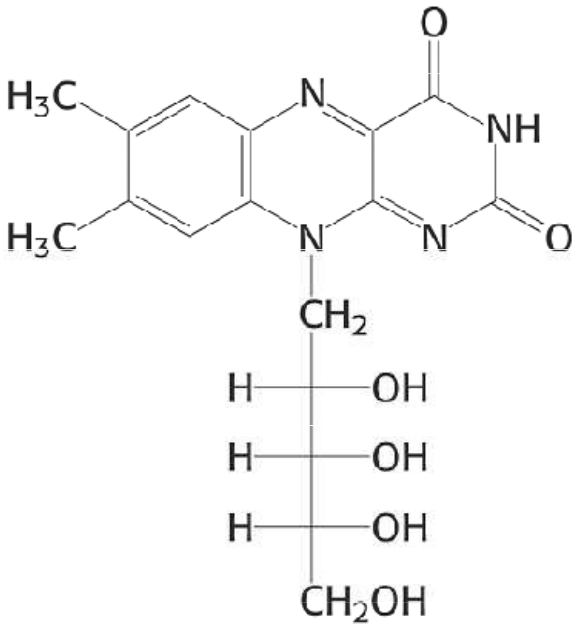
– to the **hydroxylating enzymatic systems** (e.g. synthesis of bile acids and other steroids, biotransformation of drugs) .

Schematic representation of the hydroxylations
of various biomolecules catalyzed by the hydroxylating monooxygenases:

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

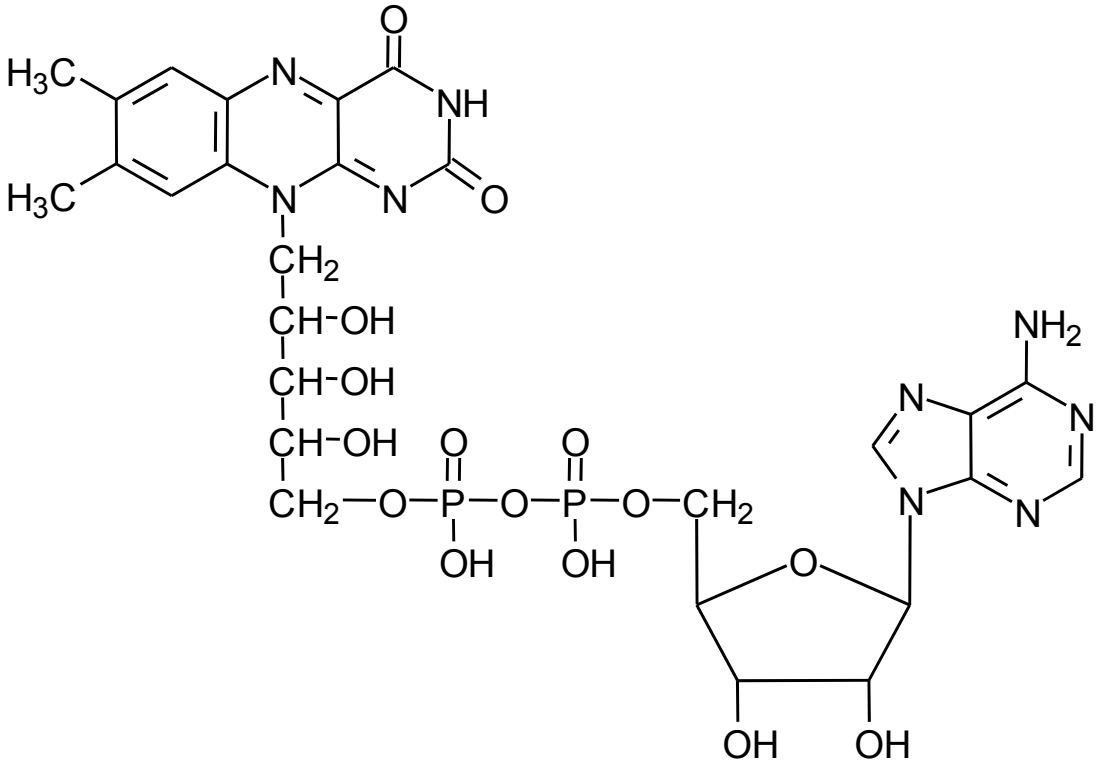
FAD - flavin adenine dinucleotide and
FMN - flavin mononucleotide

act as **oxidants** in certain types catalyzed by dehydrogenases.



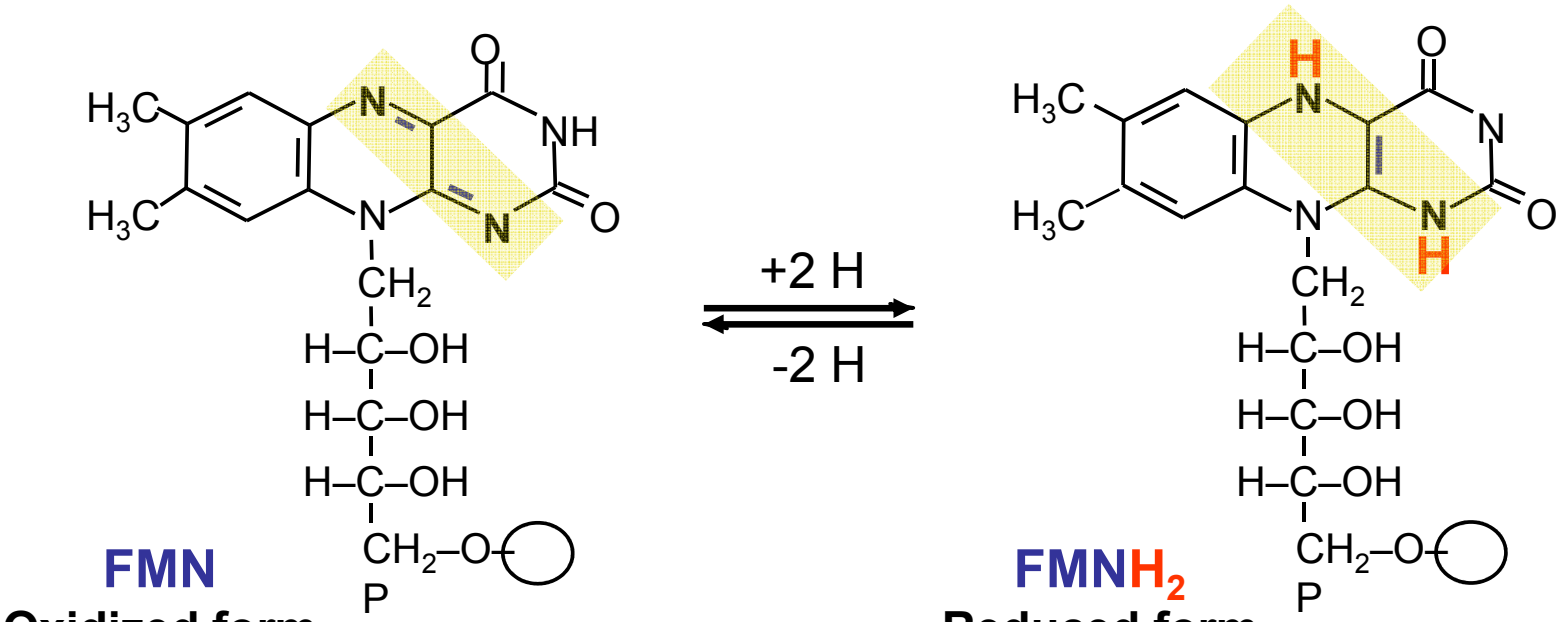
Riboflavin (vitamin B_2)

a part of coenzymes FAD and FMN



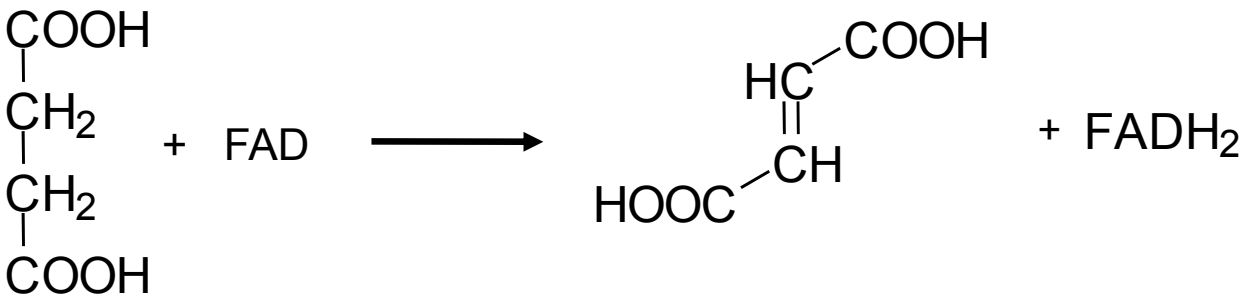
Coenzyme FAD

Oxidized form



Oxidized forms of coenzymes FAD and FMN také off two atoms of hydrogen (e.g. from the group $–CH_2\text{-}CH_2\text{-}$).

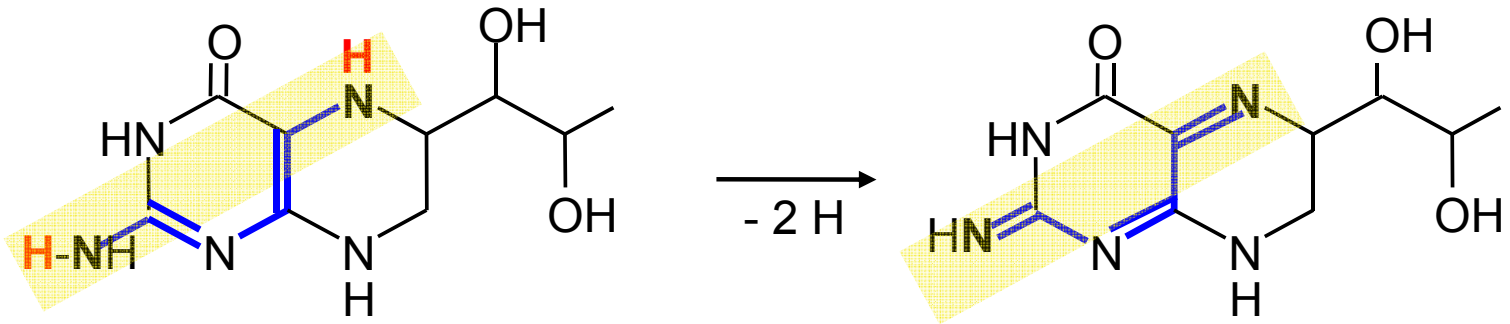
Example: Dehydrogenation of succinate to fumarate catalysed by ***succinate dehydrogenase***:



Tetrahydrobiopterin (BH_4)

acts as the coenzyme – a **reductant** – in certain hydroxylations catalyzed by monooxygenases.

It supplies **two atoms of hydrogen** and is oxidized to the quinoid form of dihydropterin:



Tetrahydrobiopterin
reduced form
(BH_4)

Dihydrobiopterin
oxidized (quinoid) form
(qBH_2)

Molybdopterin

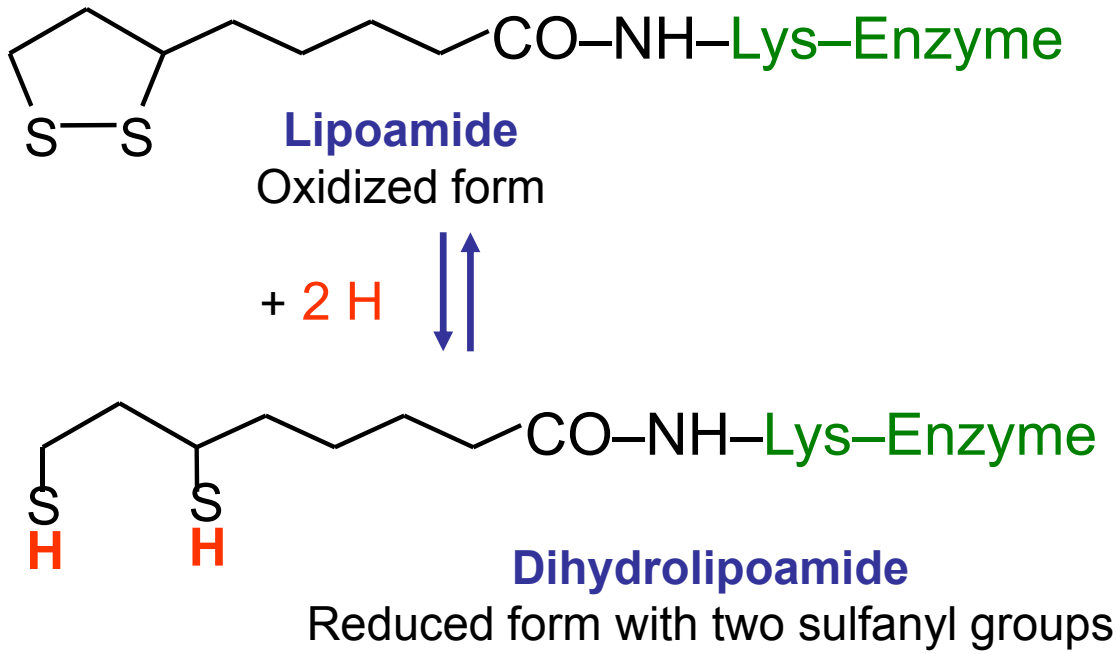
is the derivative of biopterin with the attached heterocycle that comprises a heteroatom of molybdenum.

It is an important coenzyme of few oxygenases (e.g. xanthine oxidase and sulfite oxidase).

Lipoic acid - 1,2-dithiolane-3-pentanoic acid (**thioctic acid**)

is a cyclic disulfide attached through an amide bond (**lipoamide**) to the transacylase subunit of the 2-oxoacid dehydrogenase complex.

This coenzyme acts as an **oxidant:** It accepts **two hydrogen atoms** from the activated form of an aldehyde, binds the resulting acyl as a thioester **and transfers the acyl** onto coenzyme A.

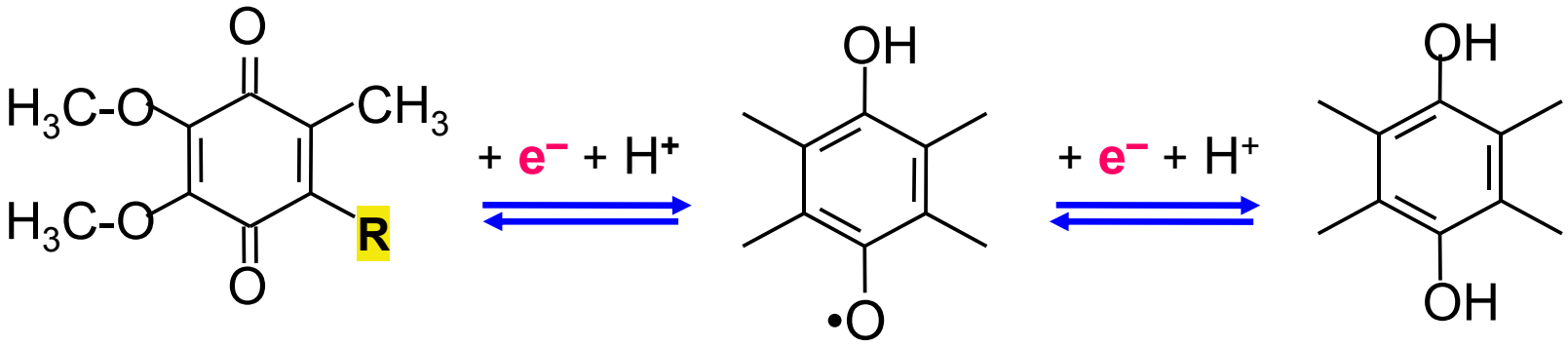


Ubiquinone (UQ, coenzyme Q)

is a **substituted 1,4-benzoquinone** that acts as the **electron acceptor** for flavoprotein dehydrogenases in the respiratory chain.

It has a side chain containing a variable number of (in animals usually 10) **isoprenoid units**. Because of its high hydrophobicity, it is entirely dissolved in the inner mitochondrial membrane.

Ubiquinone accepts stepwise **two electrons** (from the flavoprotein) and two protons from the mitochondrial matrix) and is so fully reduced to ubiquinol:

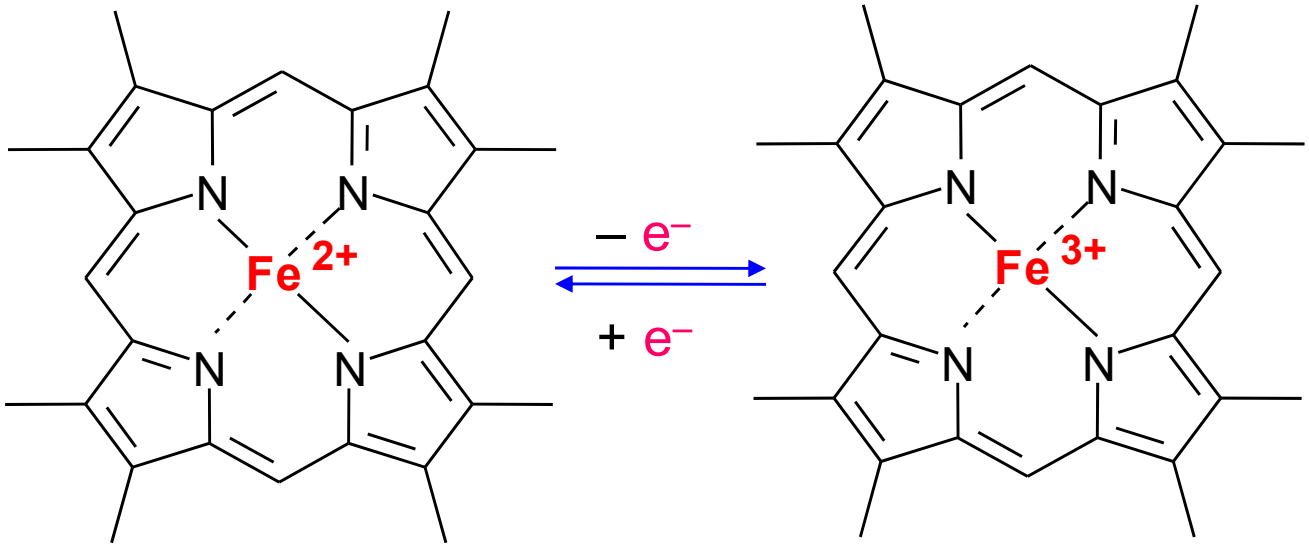


Ubiquin**one** Semiubiquinone Ubiquin**ol**

R = $-(CH_2\text{-}CH{=}C\text{-}CH_2)_{10}\text{-}H$ with CH_3 on the C=C

Cytochromes

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



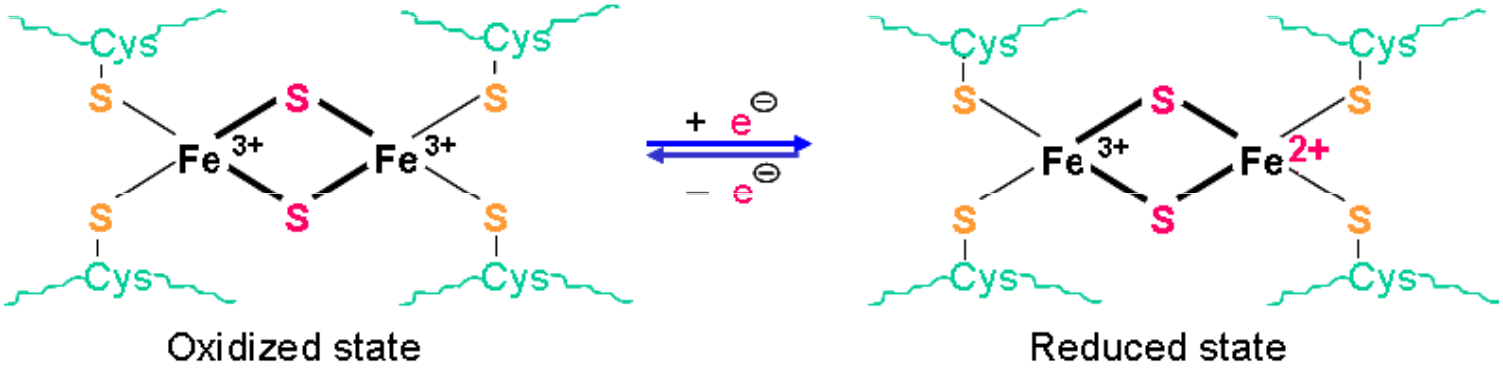
Mammalian cytochromes are of three types, called **a**, **b**, and **c**.

All these types of cytochromes occur in the mitochondrial respiratory (electron transport) chain. Cytochromes type **b** (including cytochromes class P450) occur also in membranes of endoplasmic reticulum and elsewhere.

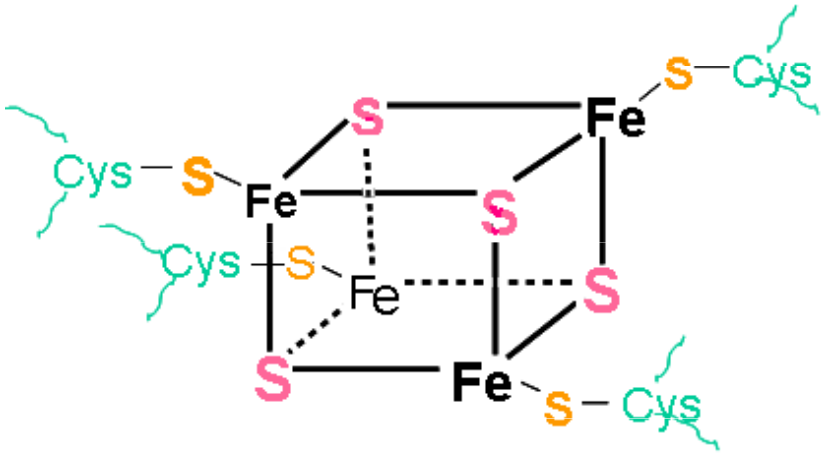
Iron-sulphur proteins (**FeS-proteins**, non-haem iron proteins)

are involved in electron transfer in the mitochondrial terminal respiratory chain or, e.g. in mitochondrial steroid hydroxylations. Despite the different number of iron atoms present, each cluster accepts or donates only one electron.

Fe_2S_2 cluster



Fe_4S_4 cluster



Cofactors of other enzymes

In addition to prosthetic groups or coenzymes of oxidoreductases (carriers of hydrogen atoms and electrons, resp.), there exist many enzyme cofactors and other molecules able to carry different groups in activated form. Surprisingly, most **interchanges of activated groups** in metabolism are accomplished by a rather small set of carriers

Carrier molecule in activated form	Group carried	Vitamin precursor
Coenzyme A	Acyl	Pantothenate
Lipoamide	Acyl	–
Thiamine pyrophosphate	Aldehyde	Thiamine (vitamin B_1)
Biotin	CO_2	Biotin
Tetrahydrofolate	One-carbon units	Folate
ATP	Phosphoryl	–
S-Adenosylmethionine	Methyl	–
Uridine diphosphate glucose	Glucose	–
Cytidine diphosphate diacylglycerol	Phosphatidate	–
Nucleoside triphosphates	Nucleotides	–

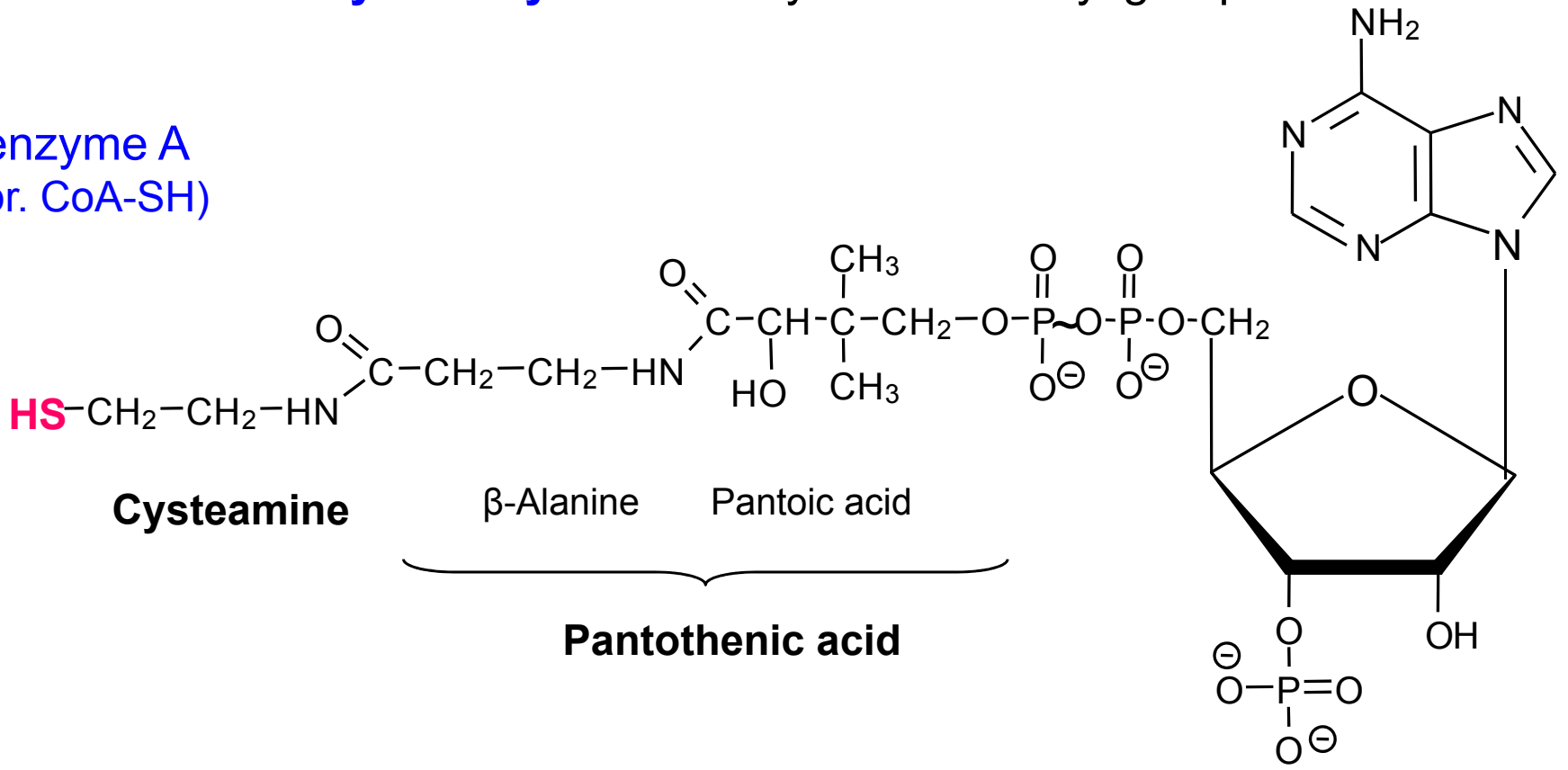
Coenzyme A

Acyl groups are important constituents in both catabolism and anabolism.

Coenzyme A serves as **carrier of acyl groups**. The terminal **sulfanyl group** (–SH) in CoA is the reactive site.

After the reaction with ATP and coenzyme A, the acyl group are linked to CoA by **thioester bonds**. **Acylcoenzymes A** carry activated acyl groups.

Coenzyme A
(abbr. CoA-SH)



Cysteamine β-Alanine Pantoic acid

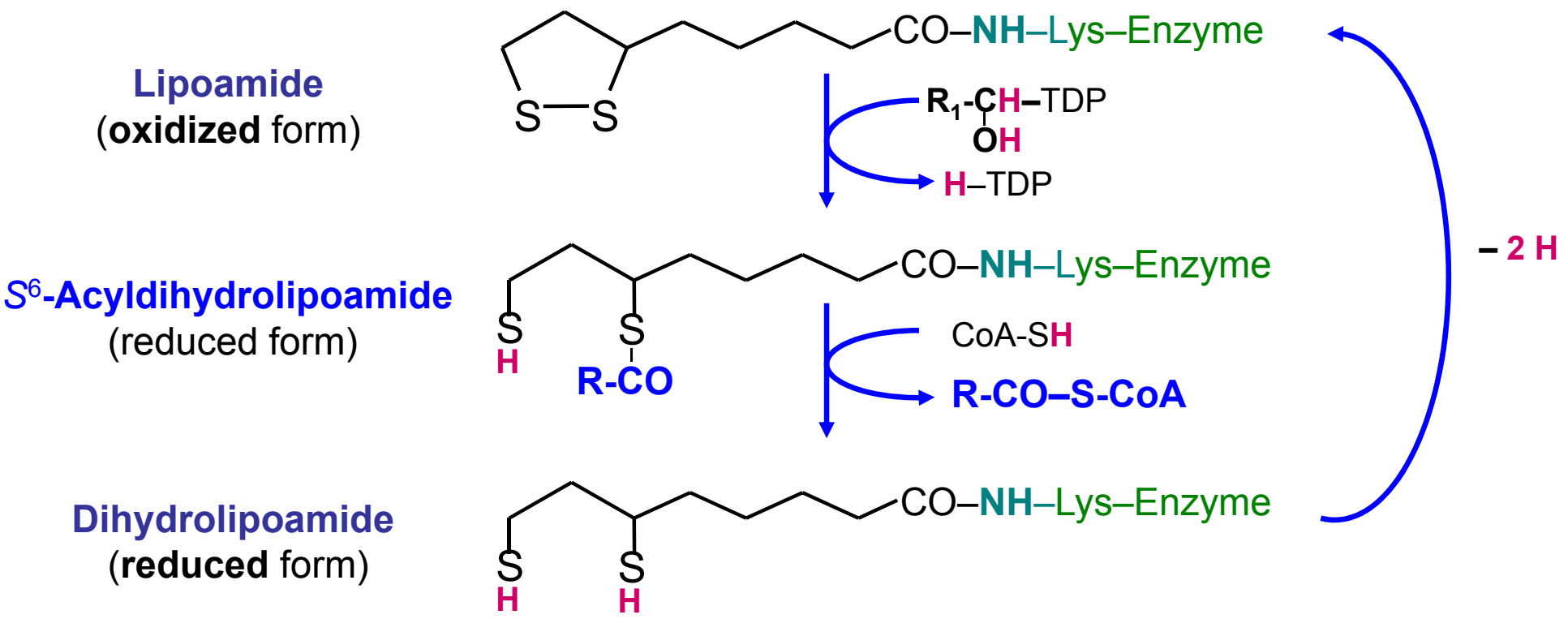
Pantothenic acid

3´-PhosphoADP

Lipoic acid (1,2-dithiolane-3-pentanoic acid, **thioctic acid**)

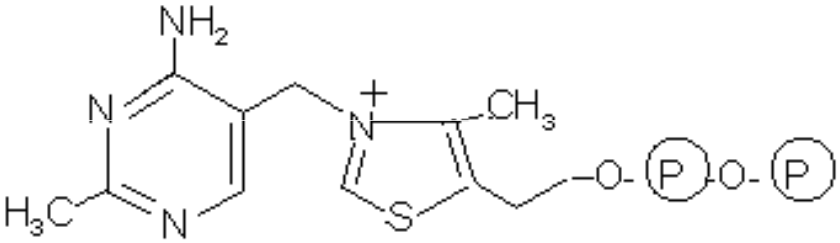
was mentioned earlier among prosthetic groups of oxidoreductases, as a cyclic disulfide attached to the transacylase subunit of the **2-oxoacid dehydrogenase complex** (as lipoamide).

It acts both as an **oxidant** of an activated aldehyde (carried by thiamine diphosphate), binds the resulting acyl as a thioester and **transfers the acyl onto coenzyme A**:

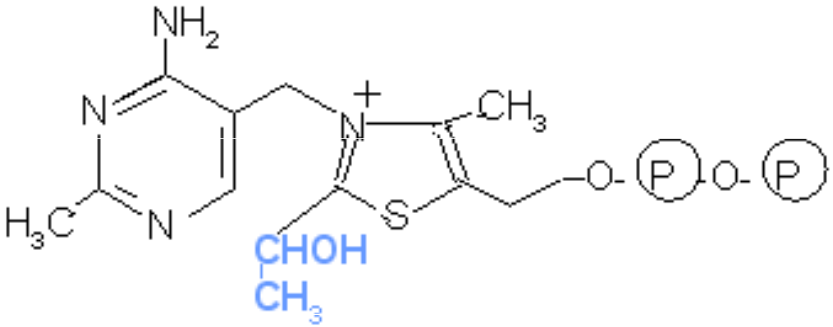


Thiamin diphosphate (TDP or TPP)

Thiamine diphosphate (TDP) is one of the five cofactors required for the full function of **2-oxoacid dehydrogenase complex.** In the first of the catalysed reaction, it acts at decarboxylation of the 2-oxoacid (pyruvate, 2-oxoglutarate, branched chain 2-oxoacid) as a **carrier of the resulting activated aldehyde** to the oxidized form of lipoamide.



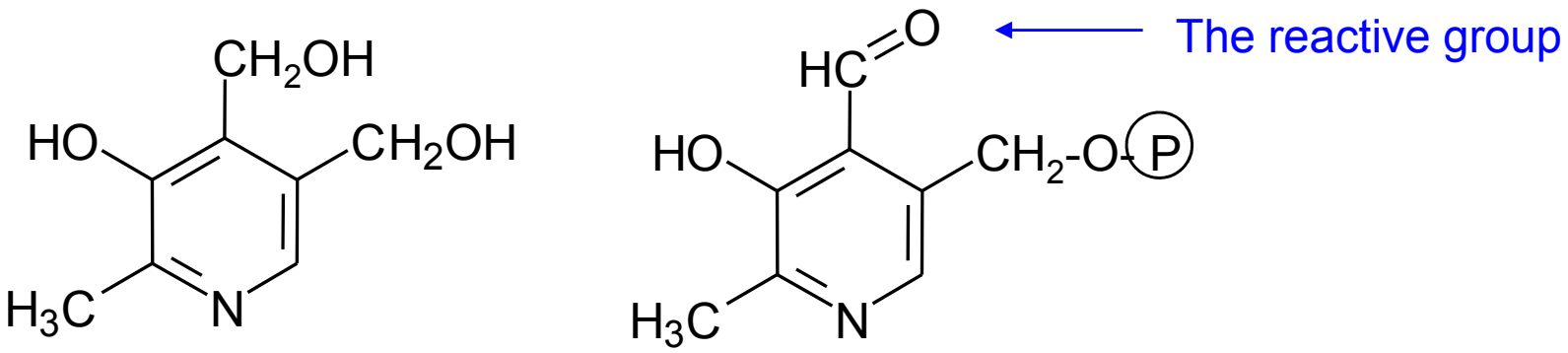
Thiamine diphosphate



Thiamine diphosphate to which activated acetaldehyde (α-hydroxyethyl group, product of decarboxylation of pyruvate) is attached at C-2 of thiazole ring.

Thiamine diphosphate serves similarly as the prosthetic group of **transketolase** (transfer of activated glycolaldehyde in the pentose phosphate pathway.

Pyridoxal 5-phosphate (PLP)



Pyridoxine (vitamin B_6) **Pyridoxal phosphate**

Pyridoxal phosphate is the prosthetic group in many enzymes taking part in α-amino acid metabolism, catalysing the following types of reactions:

- **transamination**,
- **decarboxylation of amino acids**,
- cleavage of the bond between α- and β-carbons of serine and threonine or their direct deamination, etc.

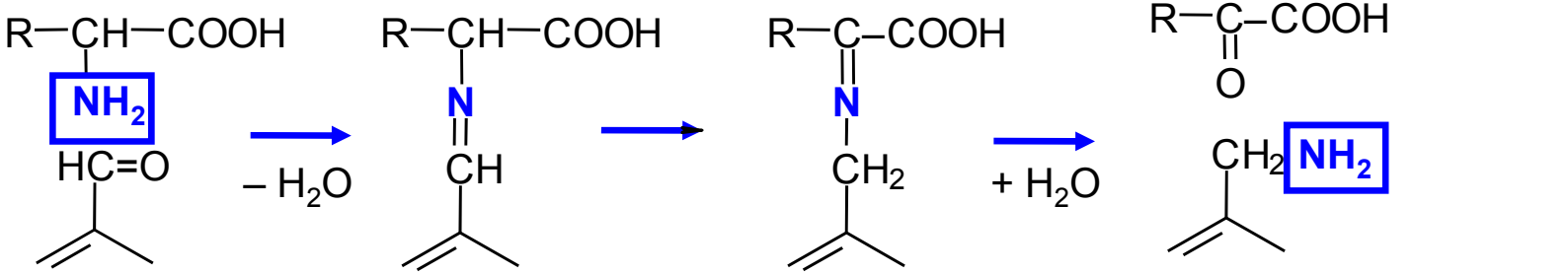
The aldehyde group binds to α-amino group of an amino acid forming an **aldimine (Schiff's base) intermediate** so that any of the bonds from the α-carbon of the amino acid may be unstabilized.

Details of **aminotransferase reaction** are shown on the next slide.

Transamination is the example of a "ping-pong" mechanism, in which the first product leaves before the second substrate binds, with the prosthetic group of the enzyme temporarily in an altered form.

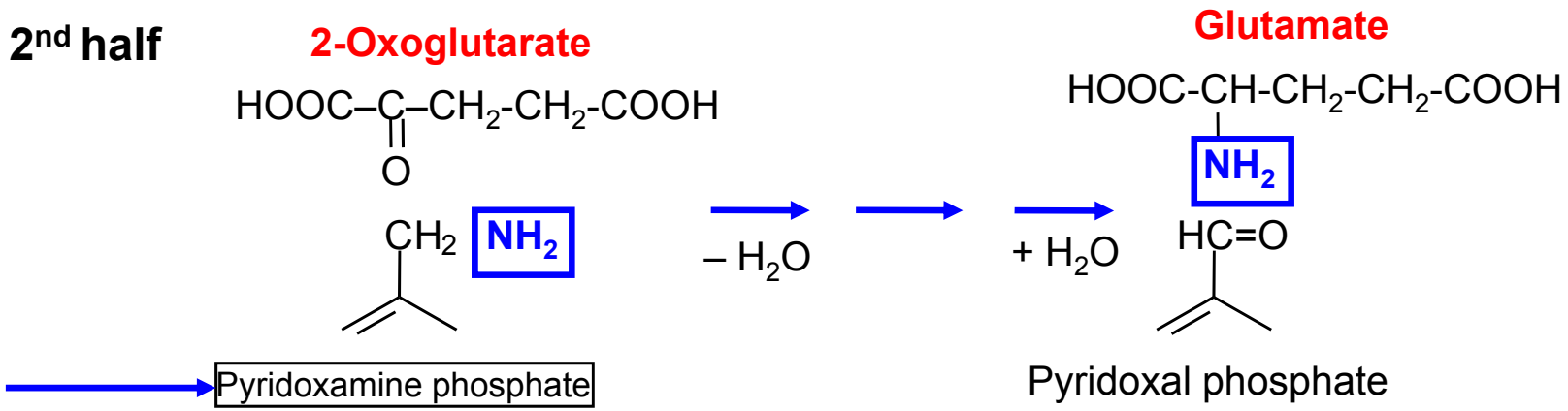
Transamination

1st half **Amino acid** **Oxoacid**

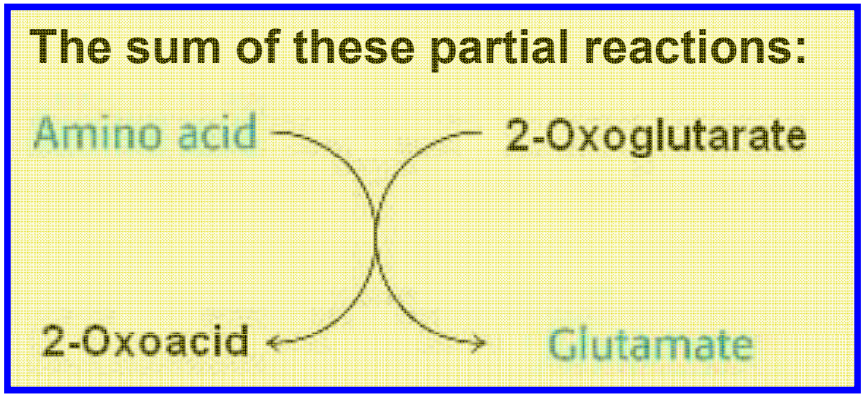


Pyridoxal phosphate — Aldimine of PLP (Schiff's bases) — Aldimine of AA — Pyridoxamine phosphate →

2nd half **2-Oxoglutarate** **Glutamate**

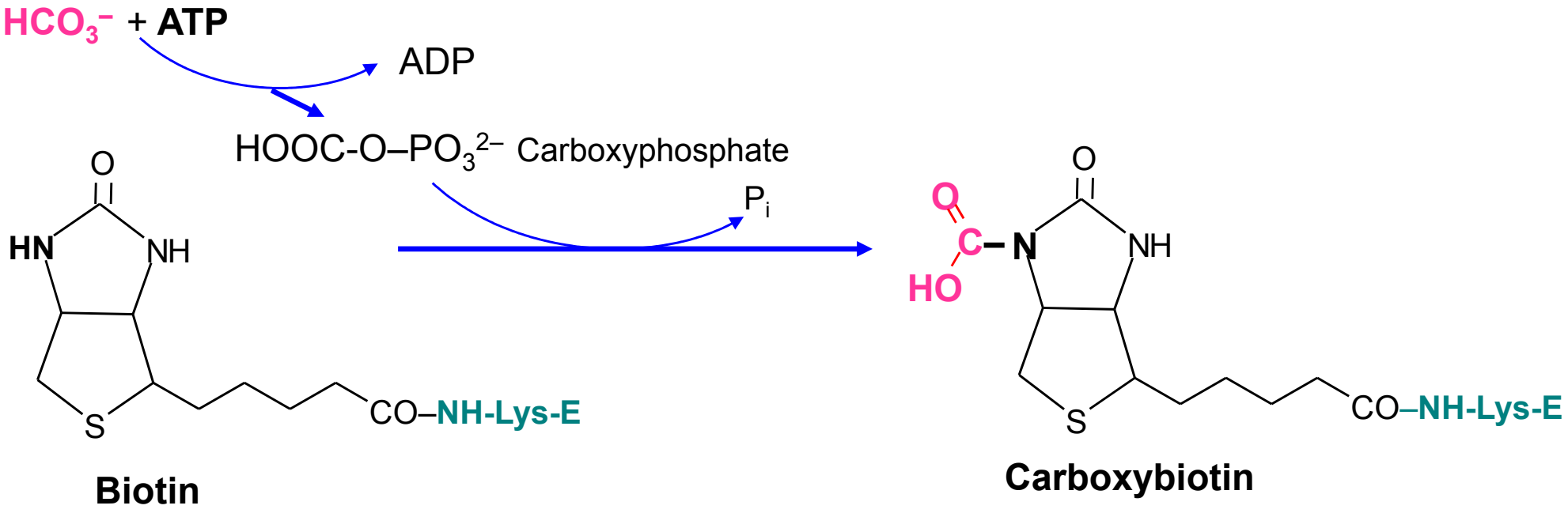


→ Pyridoxamine phosphate — Pyridoxal phosphate



Biotin

Biotin is the prosthetic group of many **carboxylases**. It is attached to the apoenzymes by an amide link between its carboxyl and the ε-amino group of a lysine residue.
After carboxylation, carboxybiotin serves as **carrier of activated carboxyl group**.



Carboxybiotin is able to transfer CO_2 to acceptors without the input of additional free energy. **Example** – mitochondrial ***pyruvate carboxylase***:

CO_2-biotin-enzyme + **pyruvate** → biotin-enzyme + **oxaloacetate**

Important recommendation:

Pay attention to the distinguishing of **decarboxylations** and **carboxylations**.

Decarboxylations of acids may be

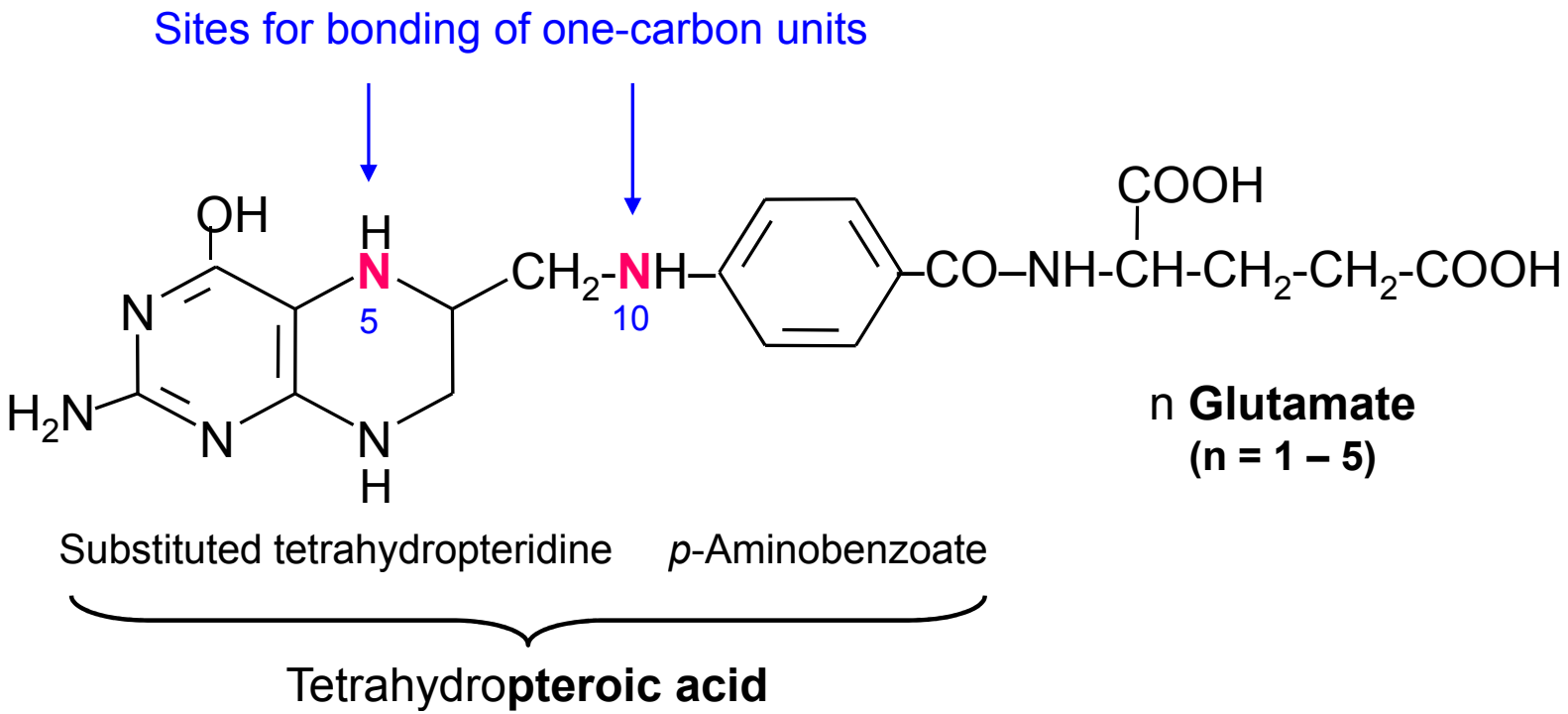
- **spontaneous** (not catalysed by enzymes),
- catalysed by **decarboxylases**, e.g.
  decarboxylation of amino acids (coenzyme **pyridoxal phosphate**),
  oxidative decarboxylation of pyruvate and other α-ketoacids (coenzyme **thiamin diphosphate**).

Carboxylations require the presence of **carboxybiotin** as donor of CO_2, e.g.
carboxylation of pyruvate to oxaloacetate (gluconeogenesis),
carboxylation of acetyl-CoA to malonyl-CoA (synthesis of fatty acids),
carboxylation of propionyl-CoA to methylmalonyl-CoA,
carboxylations in the branched-chain amino acid breakdown.

Tetrahydrofolate

(H_4folate, FH_4, tetrahydropteroylglutamate)

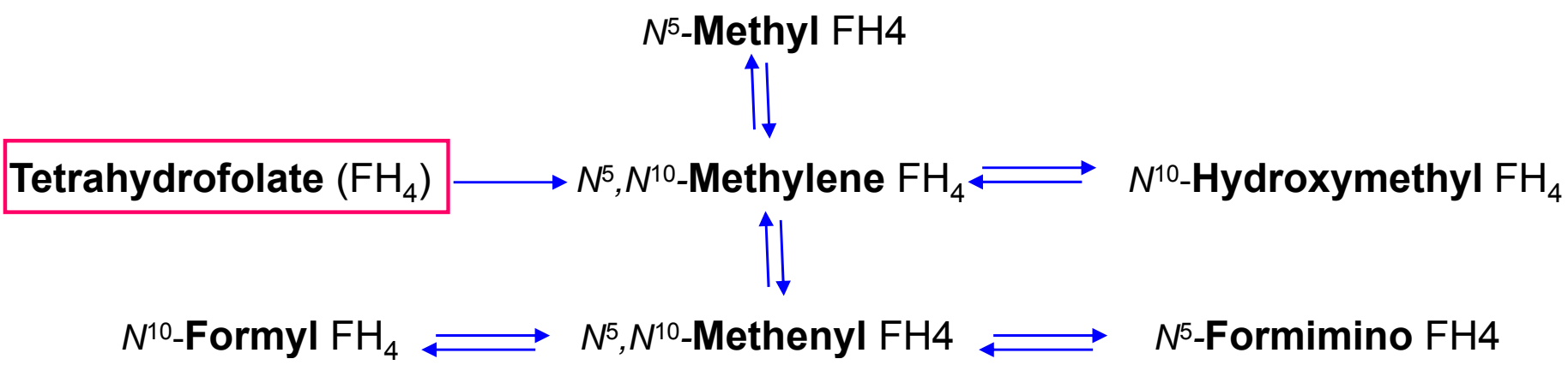
carries activated one-carbon units. Mammals can synthesize the pteridine ring, but they are unable to conjugate it to the other two units. They obtain FH_4 from their diets or from microorganisms in their intestinal tracts,



The transferred one-carbon units can exist in **three oxidation states.** The fully oxidized unit is CO_2, but it is carried by biotin rather than by FH_4.

One-carbon groups transferred by H_4folate

Oxidation state	Group	
Most reduced (= methanol)	$-CH_3$	Methyl
Intermediate (= formaldehyde)	$-CH_2-$	Methylene
Most oxidized (= formic acid)	–CHO	Formyl
	–CHNH	Formimino
	–CH═	Methenyl



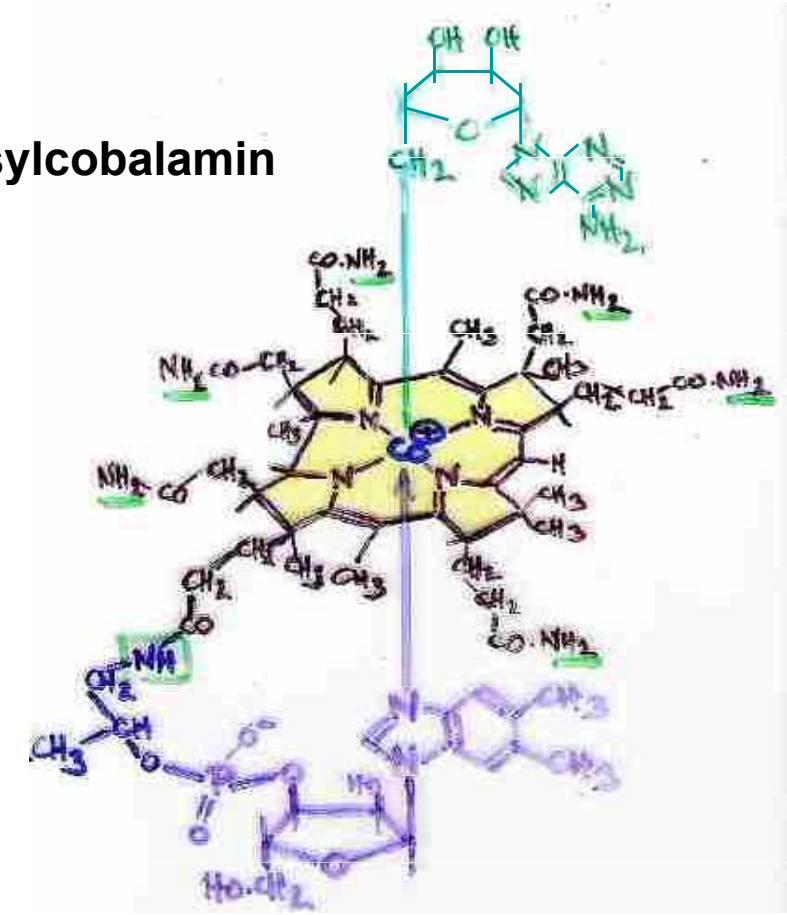
Coenzyme B_{12}

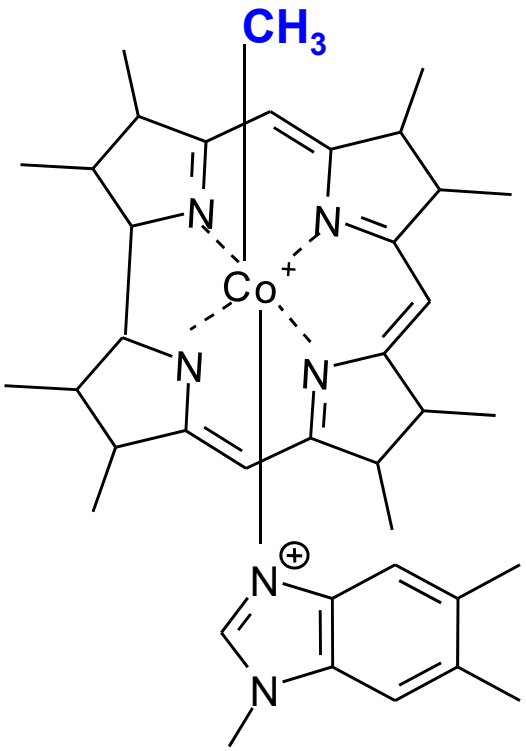
(cobamide cofactors 5´-deoxyadenosylcobalamin, methylcobalamin)

In mammals, only two reactions are known to require coenzyme B_{12}:

- intramolecular **rearrangement of methylmalonyl Co A into succinyl Co A**,
- formation of methionine by **remethylation of homocysteine**.

5´-Deoxyadenosylcobalamin





Methylcobalamin
(part of the molecule)