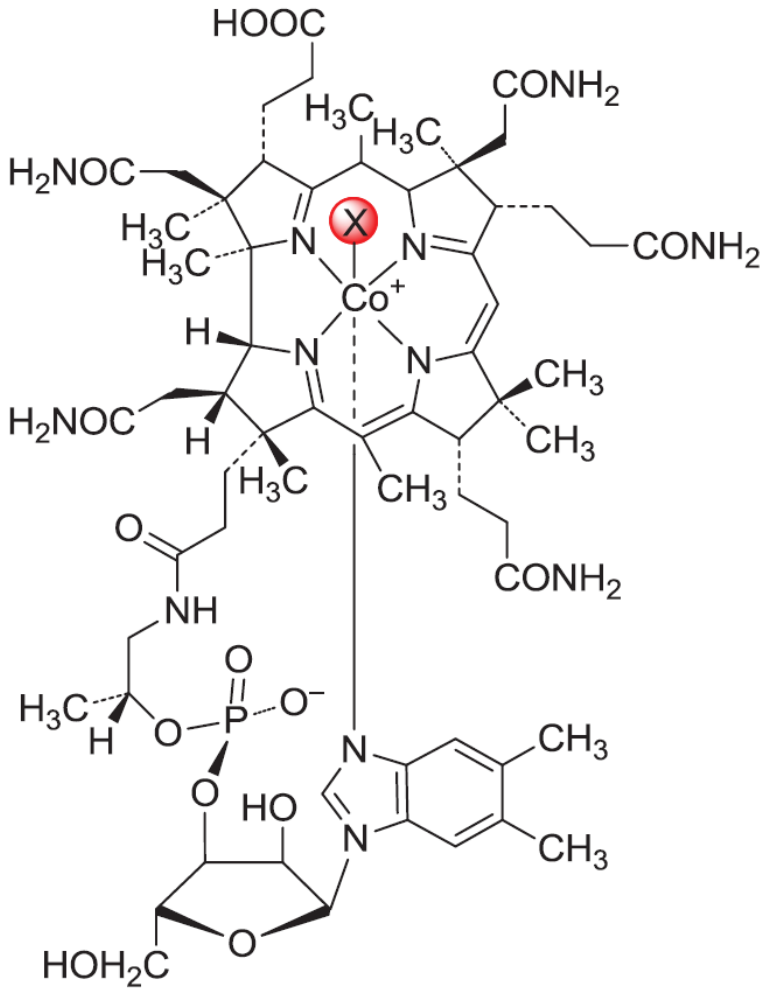
Cobalamins

Coenzyme B_{12} is a medium-sized molecule with a molecular mass of about 1350 Da.

It consists in a substituted **Co-containing corrinic ring.**

The sixth, axial metal coordination site in coenzyme B_{12} and methylcobalamin features a, primary alkyl group

"natural" **organometallic compound**



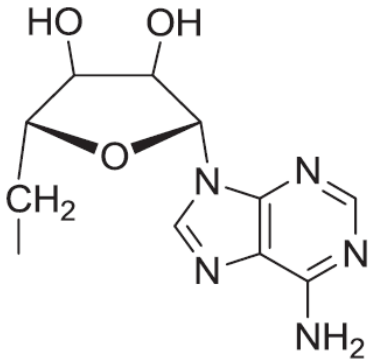
X = CH_3: methylcobalamin (MeCbl or MeB_{12})

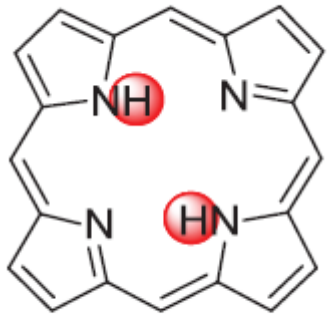
CN: cyanocobalamin (vitamin B_{12})

OH: hydroxycobalamin (vitamin B_{12a})

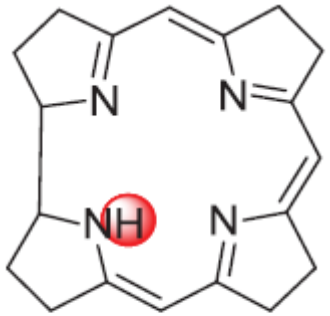
R: 5´-deoxyadenosyl-cobalamin (coenzyme B_{12} or AdoCbl)

R = 5´-deoxyadenosyl





porphyrin



corrin

The corrin ring is similar to the porphyrin ring found in the heme proteins but lacks one bridging carbon atom.

Furthermore, whereas the **porphyrin ring contains a full complement of double bonds**, the **corrin system is quite reduced**.

porphyrin has no chiral centers, whereas the corrin ring of **vitamin B_{12} has nine**

The Co−CH_2R configuration in alkylcobalamins is unusually stable towards hydrolysis in neutral aqueous solution.

On the other hand, this **cobalt−carbon bond shows a very special reactivity**, namely the **enzymatically controlled formation of reactive primary alkyl radicals**.

characteristic specificity and high reactivity only in combination with corresponding apoenzymes

The production of radicals is afforded by **homolytic cleavage of the Co-C bond**.

$$RCH_2\text{-}B_{12}Co(III) \rightarrow RCH_2^{\bullet} + B_{12}Co(II)$$

Since humans cannot produce vitamin B12 it must be obtained from the diet.

The average American ingests 5–15 μg of vitamin B_{12} per day; less than 1 μ g is necessary for normal function.

B_{12} is **not found in plant-based foods**!

Vitamin B_{12} deficiency can result from:

- insufficient dietary intake
- defects in metabolism of vitamin B_{12}
- pernicious anemia.

Cobalamin deficiency results in **megoblastic anemia**, whose symptoms are indistinguishable from a folic acid deficiency.
The symptoms include: anemia, decreased white blood cell count, hypercellular bone marrow, and abnormal maturation.

Cobalamin deficiency can also result in neuropsychiatric disturbances due to demyelinization of nerves and the spinal cord.

Crystallographic studies shows axial ligands:

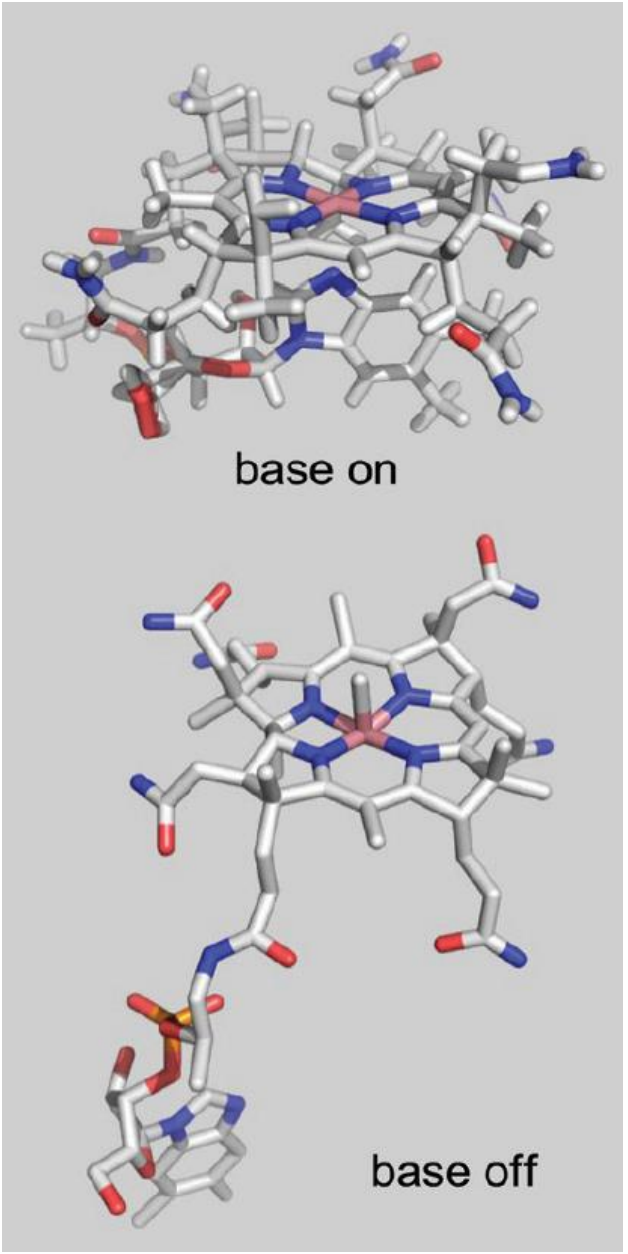
alkyl-bound 5-deoxyadenosine group
an N(1)-coordinated 5,6-dimethylbenzimidazole

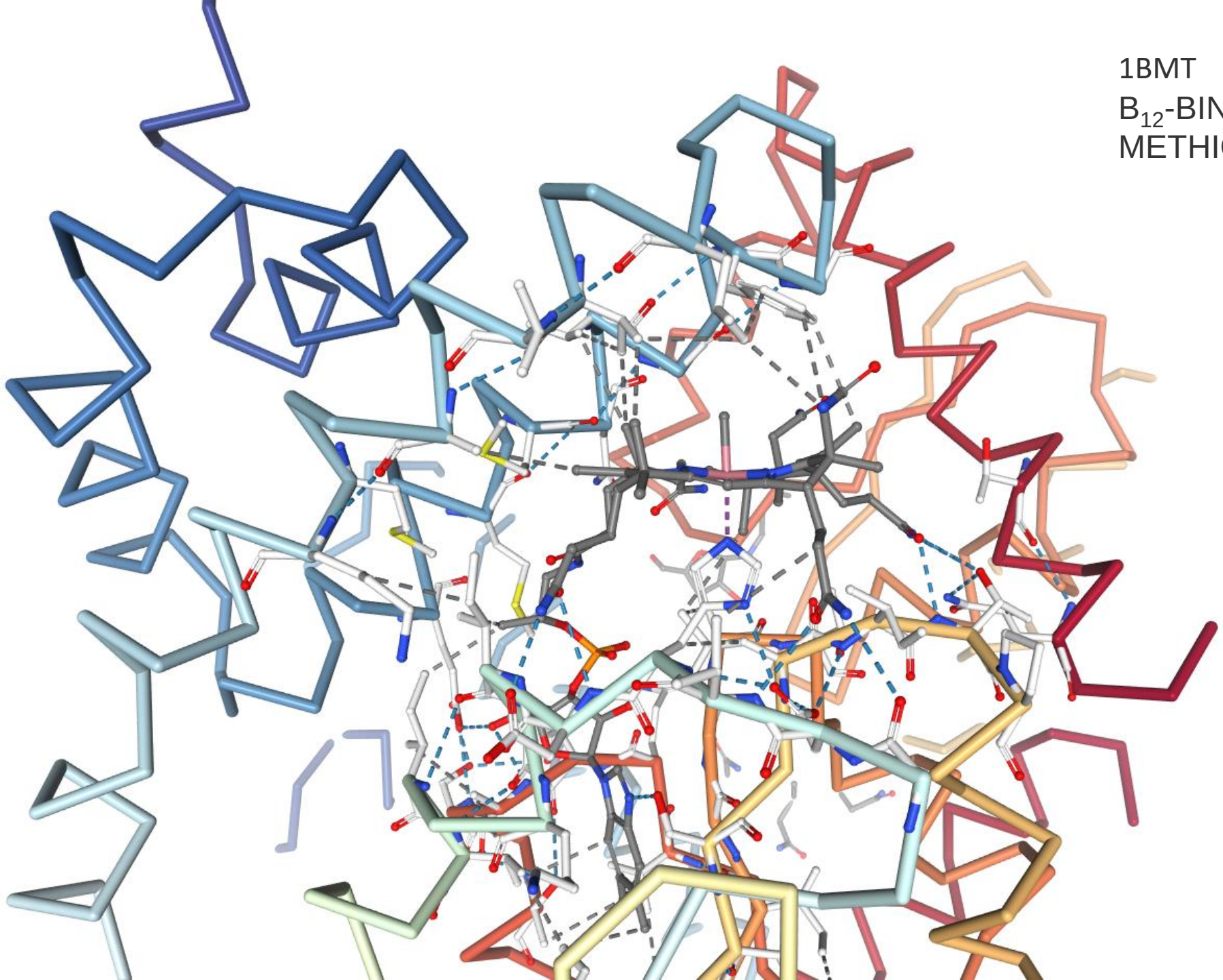
The latter is connected to the corrin macrocycle via a long pendant chain, so that this **corrin derivative** effectively functions as a **pentadentate chelate ligand**.

The benzimidazole ligand can switch between the **cobalt-coordinated "base-on"** form
and the **decoordinated** so-called **"base-off"** form

with possible **replacement** by a **donor** from the protein
- E.g. nitrogen imidazole in B_{12} binding domains of methionine synthase -

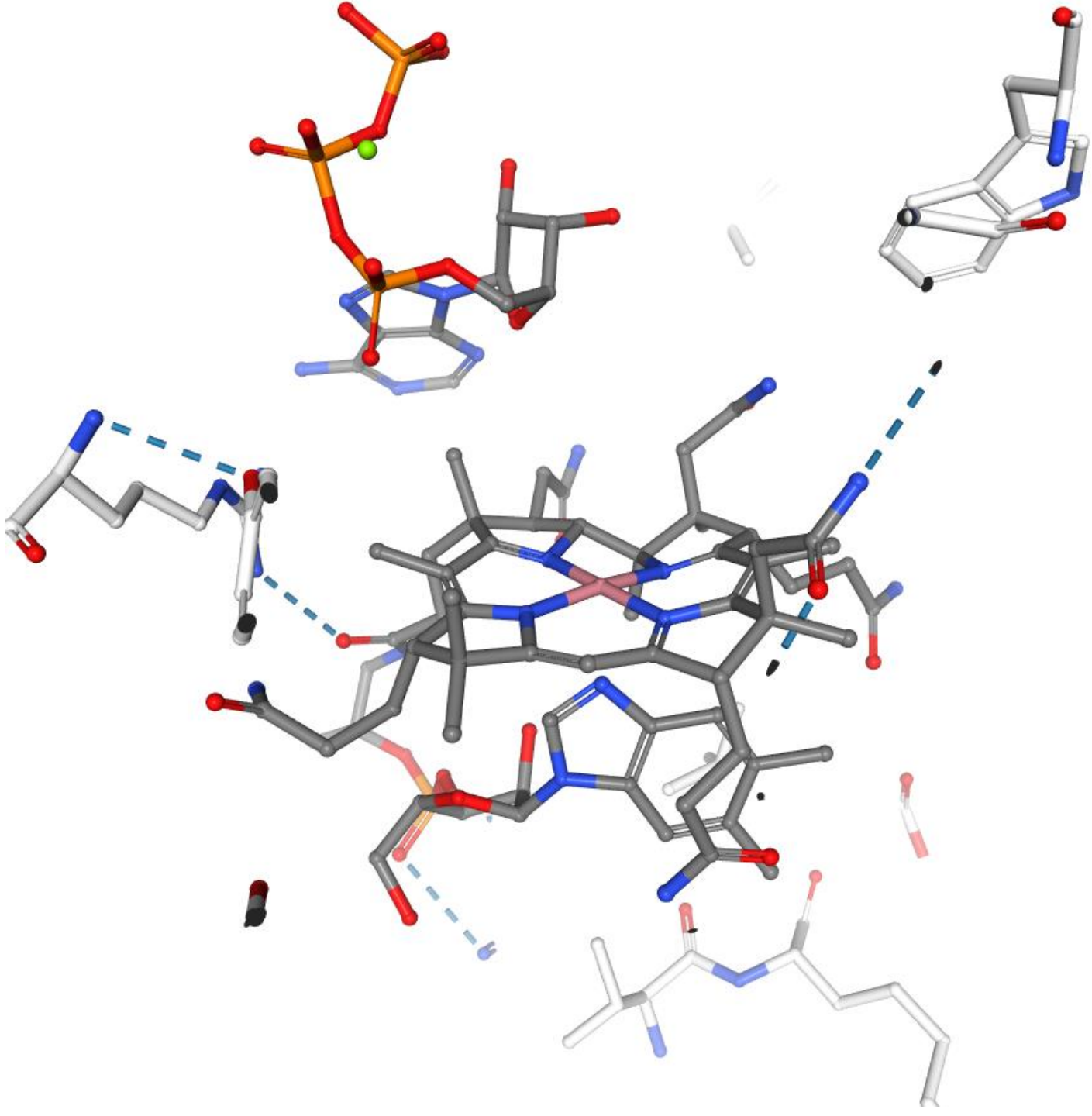
Coenzyme B_{12} can thus be considered a "**molecular switch**"





1BMT

B_{12}-BINDING DOMAINS OF METHIONINE SYNTHASE



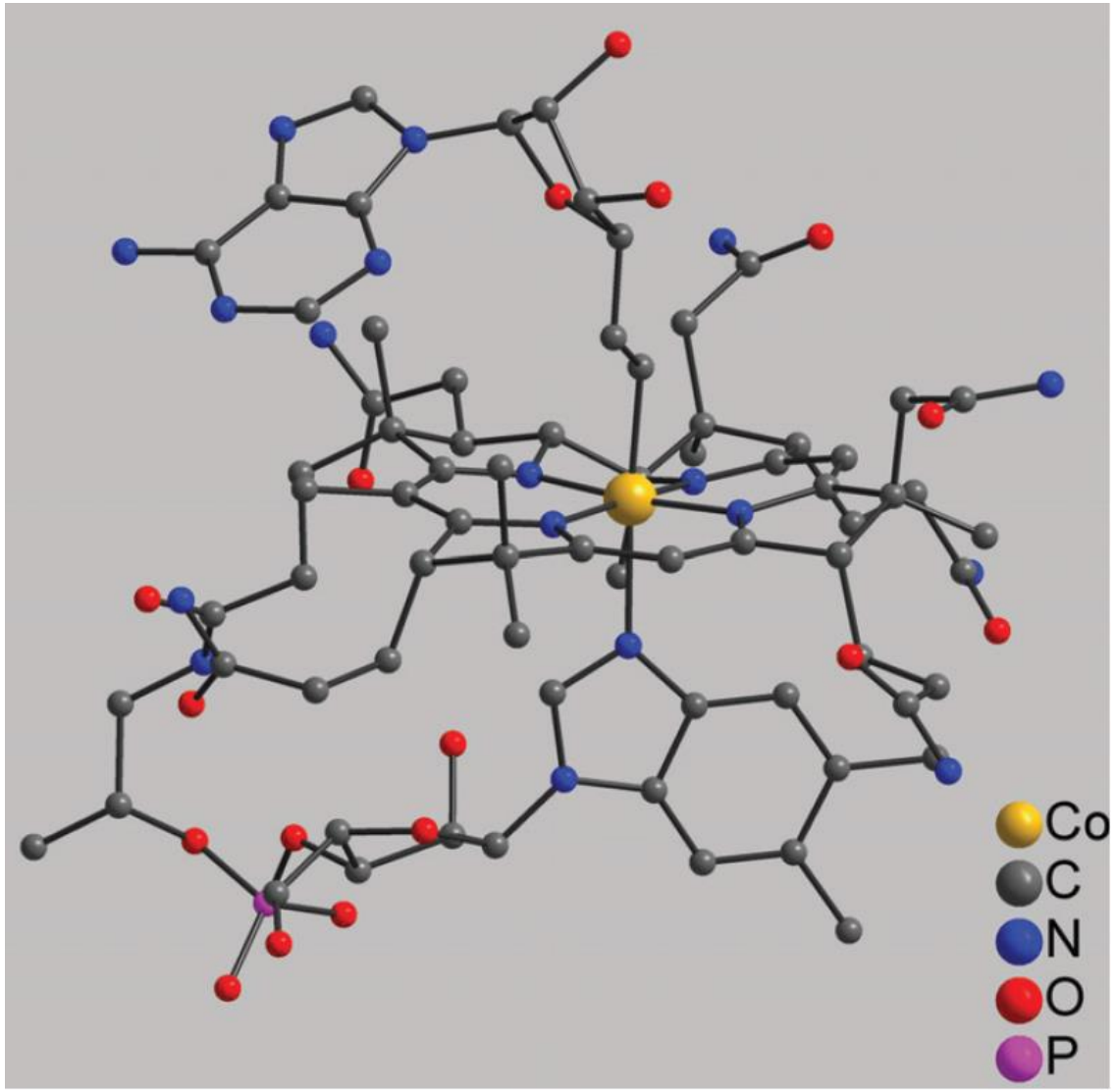
1G64
ADENOSYLTRANSFERASE FROM
SALMONELLA TYPHIMURIUM

The unsaturated macrocycle is not completely flat but adopts a slightly bent conformation

"**butterfly**" or "**saddle**"

Model studies have demonstrated the relevance of this structural feature for reactivity in terms of an **entatic state** structure.

The non planarity results from the fact that the relatively large metal ion is encapsulated by a 15-membered instead of a 16-membered macrocycle.



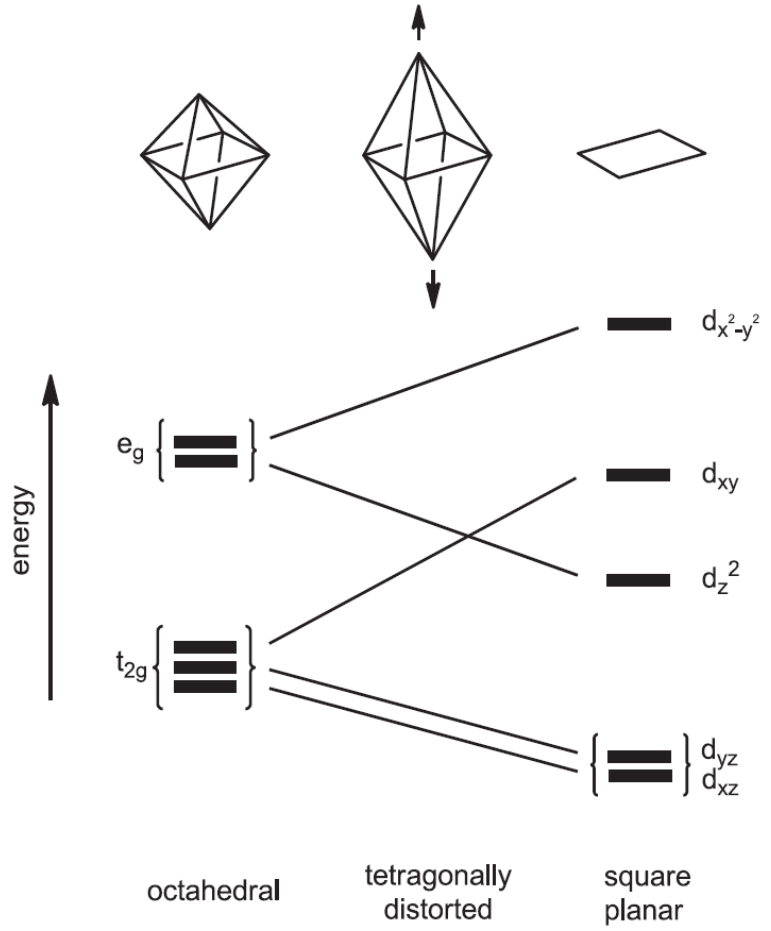
The existence of a **relatively inert bond between a transition metal and a primary alkyl ligand is quite remarkable**, especially since this true organometallic compound is stable under physiological conditions (aqueous solution at pH 7 and in the presence of oxygen).

The corrin macrocycle creates a strong ligand field, resulting in a **low-spin situation** with considerable **stabilization of the d_6 configuration** (Co^{III}) in an approximately octahedral environment. However, the six-coordinate arrangement shows distinctive tetragonal distortion, and a **splitting of the d orbitals** is to be expected.

Considering the ideally suited low-spin d_6 situation for Co(III) with accessible lower oxidation states Co(II) and Co(I),
WHY the equally d_6-configured neighbors of Co(III) are less suitable?

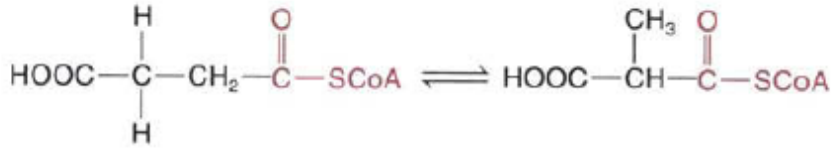
Fe(II) is too labile with physiological ligands and tends to cross over to the less inert high-spin state

Ni(IV) will have a too-positive redox potential for a biological environment.

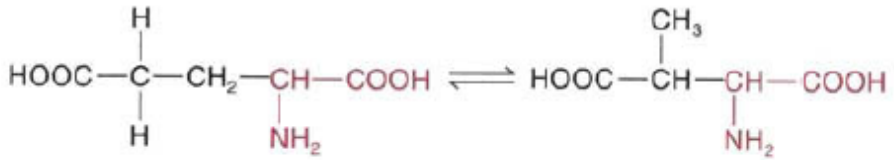


Reactions of Alkylcobalamins

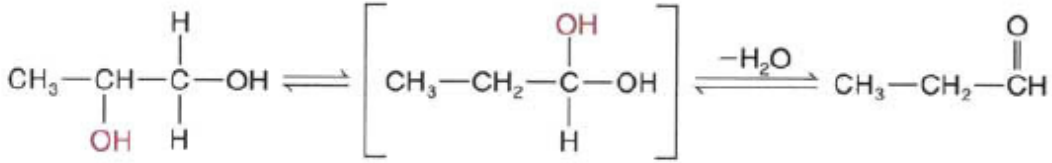
Whereas **several coenzyme-B_{12}-promoted reactions constitute true oxidation-reduction chemistry**, for example, the reduction of ribonucleoside to deoxyribonucleoside triphosphates, **many are simply rearrangements**



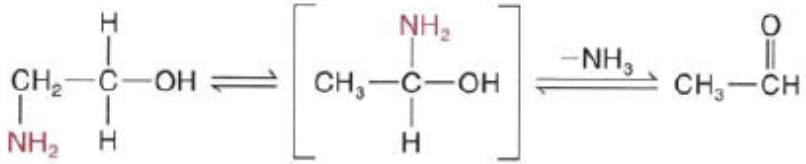
Methylmalonyl-CoA mutase



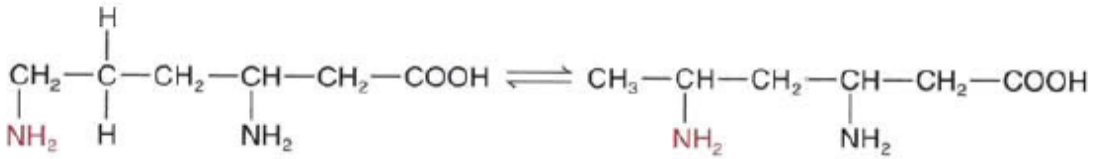
Glutamate mutase



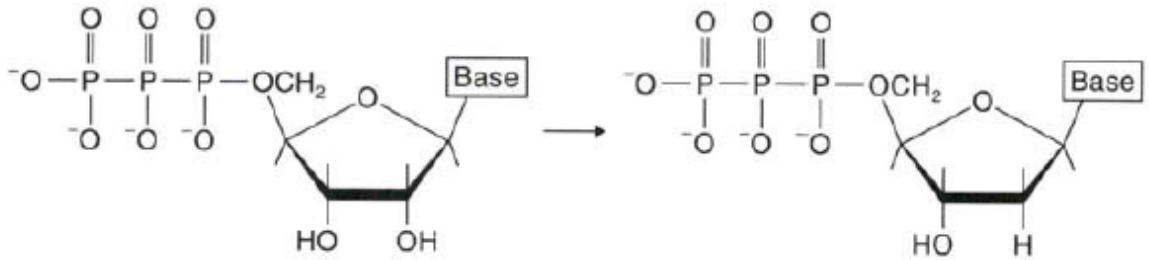
Diol dehydrase



Ethanolamine ammonia lyase



L-β-Lysine mutase



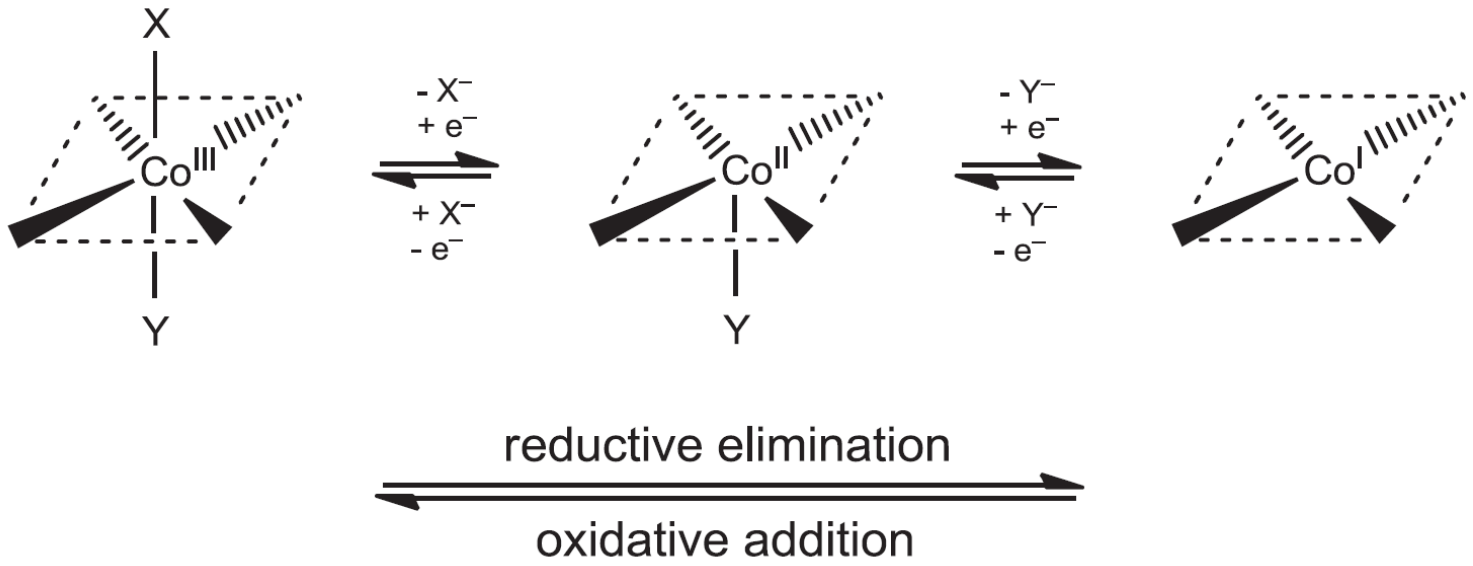
Ribonucleotide reductase (*lactobacillus leichmanni*)

One-electron Reduction and Oxidation

Starting configuration:
trivalent cobalt ion as a six-coordinate metal center with the corrin monoanion, an anionic axial group (e.g. alkyl) and the neutral dimethylbenzimidazole base as ligands; a negatively charged phosphate in the side chain completes the charge balance.

Starting from this configuration, two one-electron reduction steps are possible

The reduction of the metal from a Co^{III} (d_6) (3.3, left) to a Co^{I} (d_8) configuration is accompanied by a tendency towards decreased axial coordination, with the ultimate case being a square planar arrangement



One-electron reduction of Co(III)-methylcobalamin thus leads to a more than 50% decreased Co−C bond strength

rate enhancement for the homolysis

Why?

half-filled antibonding σ∗(Co−CH_3) orbital (dz^2 component)

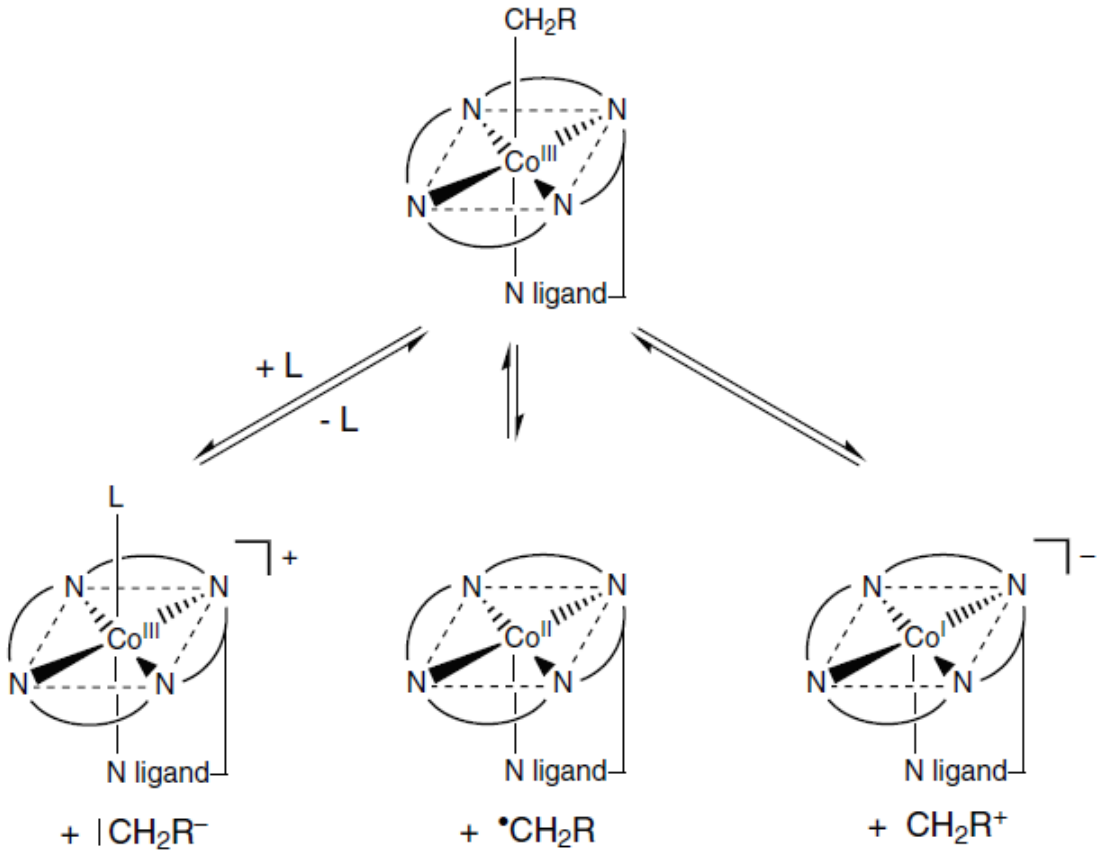
Excitation with light can also lead to a population of this σ∗(Co−CH_3) orbital and thus to a cleavage of the Co−C bond; however, this is probably not relevant for enzymatic reactions.

Non-occupation of the strongly antibonding dx^2-y^2 orbital in a d_8 system such as Co(I) favors the sterically less favorable **square planar configuration**

Co–C Bond Cleavage

The reactivity of the physiologically relevant alkyl cobalamins is characterized by the fact that the **reactive alkyl groups** are made available in a controlled fashion for **follow-up reactions**.

Three formal alternatives are conceivable for a cleavage of the Co–CH_2R bond, which may be induced by the interaction of the coenzymes with the apoenzyme and the substrate.



type of reaction:	heterolysis	homolysis	heterolysis
metal configuration in the product	d^6 low-spin, stable, inert	d^7 low-spin, 1 unpaired electron $(d_{z^2})^1$	d^8, "super-nucleophilic" $(d_{z^2})^2$
alkyl ligand, eliminated as:	"carbanion", nucleophilic	primary alkyl radical, very reactive	"carbocation", electrophilic
app. electrochemical potential equivalent[a]:	> 0 V	0 to -0.4 V	< -0.9 V

Heterolytic bond cleavage can lead either to **low-spin Co(III) and a carbanion equivalent CH_2R^-**, involving substitution (e.g. by water), or to **Co(I) and a carbocationic alkyl moiety, CH_2R^+**.

In the latter case, a d8-configurated metal center is formed, which behaves as a electron-rich "supernucleophile"; that is, its filled antibonding dz^2 orbital results in a high affinity towards σ electrophiles.

A typical d8 metal reactivity is thus the "oxidative addition" of organic compounds R−X. The carbanionic or carbocationic alkyl groups will not be produced as free ions but will be transferred in the presence of a reaction partner and a polar reaction medium in the transition state of the reaction.

The third alternative is the **homolytic bond cleavage**, which leads to paramagnetic, electron paramagnetic resonance (EPR) spectroscopically detectable **Co(II) with a low-spin d7 configuration** (one unpaired electron) and a **primary alkyl radical**.

Importance of the medium and axial coordination!

In the absence of a special base in the axial position:

the carbanionic mechanism is realized at potentials above 0V versus the normal hydrogen electrode (NHE).

The Co(I)/carbocation cleavage occurs only below approximately−0.9V and thus beyond physiological conditions.

Homolytic bond cleavage is a viable reaction in the physiologically interesting potential range between 0 and −0.4V

The low-spin Co(II) complex (d7) features an EPR signal for one unpaired electron.

Interaction (coupling) of the electron spin occurs with the nuclear spin of the metal center (^{59}Co: 100% natural isotopic abundance, nuclear spin I = 7/2) and the nuclear spin of one nitrogen atom (^{14}N: 99.6% natural abundance, I = 1).

The **unpaired electron is assumed to occupy the dz_2 orbital**, interacting mainly with the single, axially coordinated nitrogen atom of the benzimidazole ligand ("base-on"). If the $dx^2–y^2$ orbital were occupied by the unpaired electron, all four nitrogen centers of the macrocyclic corrin ligand would contribute with essentially similar nuclear spin/electron spin coupling.

The order of d orbitals corresponds to a relatively small distortion of octahedral symmetry and is in accordance with the observed **supernucleophilicity** in **axial direction** after double occupation of the dz^2 orbital.

Enzyme Functions of Cobalamins

Three different enzyme functions (enzyme classes) are connected with B_{12} cofactors

Co(B_{12})dependent proteins	Organisms
(a) adenosylcobalamin(AdoCbl)-dependent isomerases	
methylmalonyl-*CoA* mutase (MCM)	archaea, bacteria, eukaryotes
isobutyryl-*CoA* mutase (ICM)	archaea, bacteria, eukaryotes
ethylmalonyl-*CoA* mutase (ECM)	archaea, bacteria, eukaryotes
glutamate mutase (GM)	archaea, bacteria
methyleneglutarate mutase (MGM)	archaea, bacteria
D-lysine 5,6-aminomutase (5,6-LAM)	bacteria
diol dehydratase (DDH)	bacteria
glycerol dehydratase (GDH)	bacteria
ethanolamine ammonia lyase (EAL)	bacteria
(b) methylcobalamin(MeCbl)-dependent methyltransferases	
methionine synthase (MetH)	bacteria, eukaryotes
methyltransferases (Mta, Mtm, Mtb, Mtt, Mts, and Mtv)	archaea, bacteria
methyltetrahydromethanopterin *CoM* methyltransferase subunit A (MtrA)	archaea
(c) B_{12}-dependent reductive dehalogenase (CprA)	bacteria

First enzyme class contains **adenosylcobalamin-dependent isomerases**, which occur in all types of organisms. They rely on the **homolytical splitting** of the Co–C bond of AdoCbl to form **highly reactive radicals**.

The second enzyme class involves **methylcobalamin** and exhibits **alkylating activity**. The methionine synthase is essential for the biosynthesis of methionine in many organisms, including humans.

The third **class,B12-dependent reductive dehalogenases** (CprA) of **anaerobic microbes**, plays an important role in the detoxification of aromatic and aliphatic organo-chloro compounds

B_{12}-dependent reductive dehalogenases also contain Fe/S-clusters
- B_{12} cofactor plays the role of a redox catalyst-

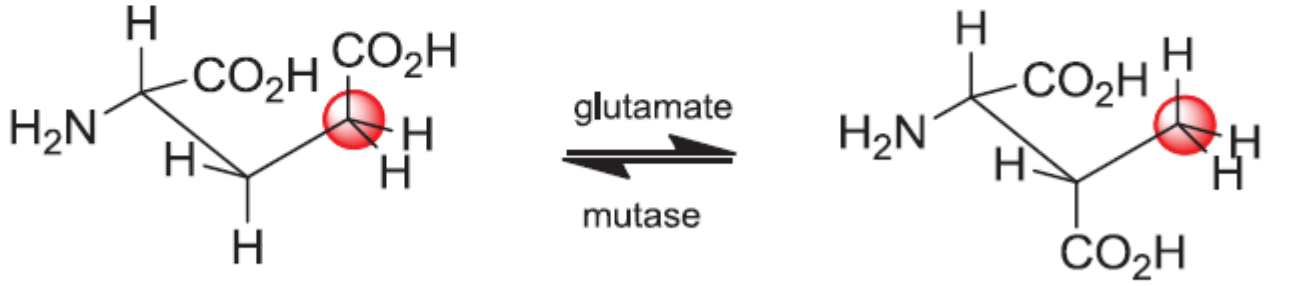
Adenosylcobalamin (AdoCbl)-dependent Isomerases

This class of B_{12}-dependent enzymes can be divided into three subclasses, distinguished by the nature of the migrating group and the kind of substituent on the carbon atom to which the group migrates.

The Class Ia enzymes are mutases, which catalyze **rearrangements of the carbon skeleton**.

in general:

example:



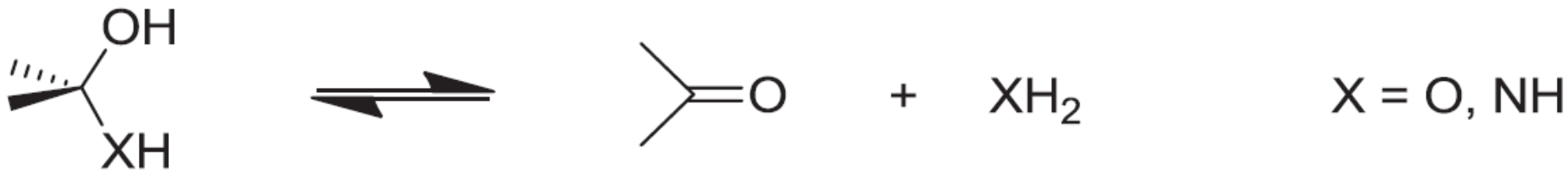
glutamic acid

β-methylaspartic acid

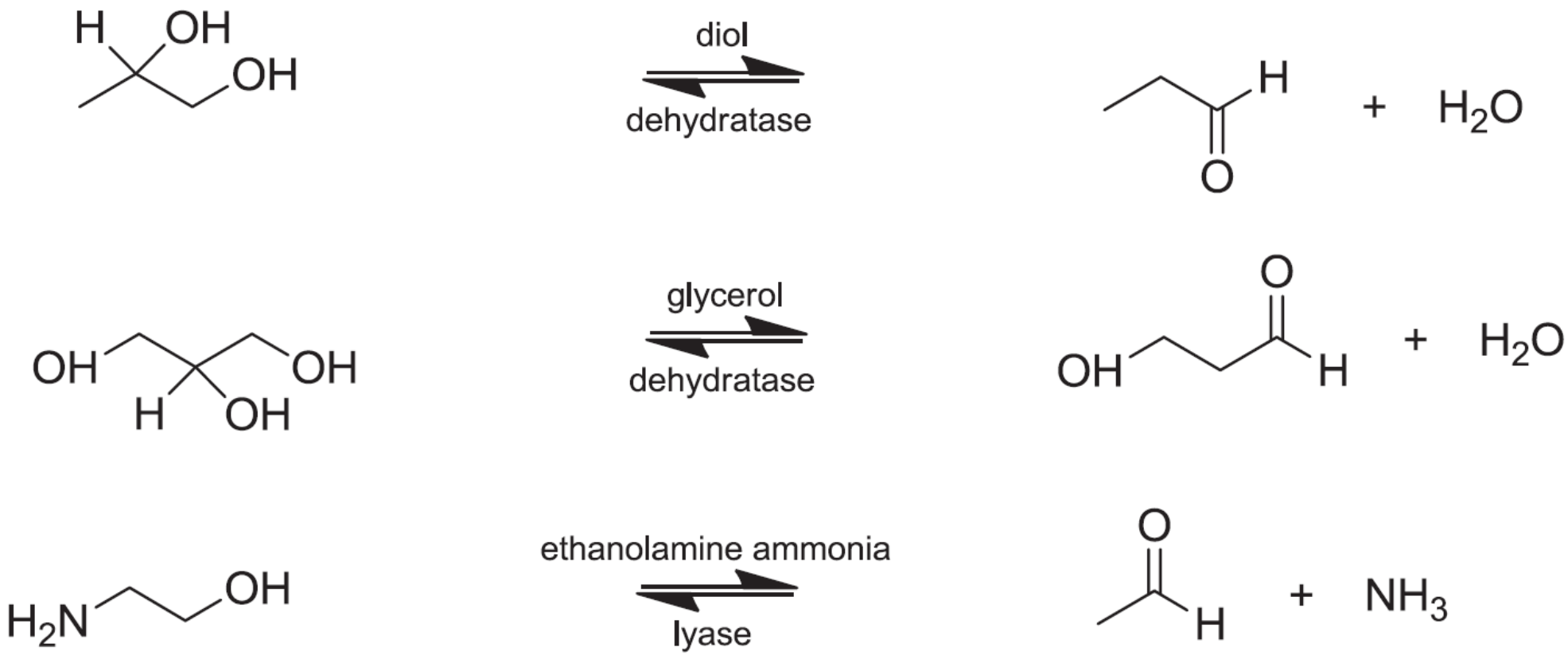
The **eliminases (Class Ib)** include **dehydratases or lyases** and are coenzyme B_{12}- dependent enzymes, since a 1,2-shift in 1,2-diols or 2-aminoalcohols leads to geminal (1,1)-isomers, which readily lose water or ammonia to form carbonyl compounds.

Also the **AdoCbl-dependent ribonucleotide reductases** can be added to these Class Ib enzymes, catalyzing the **reduction of ribonucleotides to their corresponding deoxy forms in certain bacteria**. The B12-dependent ribonucleotide reductase requires coenzymatic dithiol, which is oxidized to disulfide.

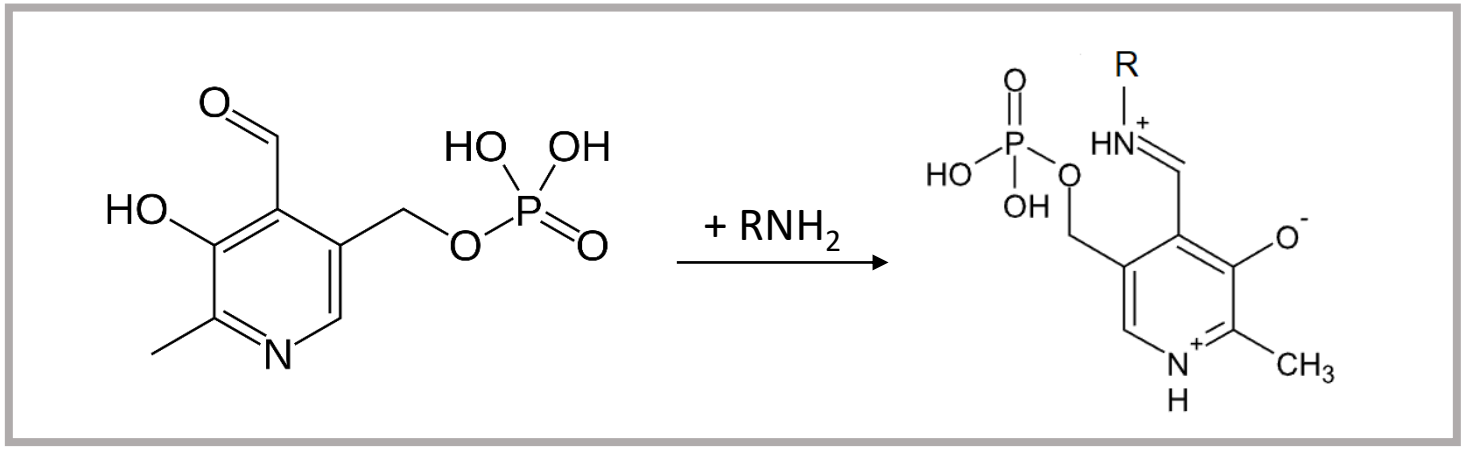
For this biochemically very important reaction (RNA→DNA), other organisms and bacteria such as *E. coli* use manganese- or iron-containing ribonucleotide reductases, which also require radicals for proper function.

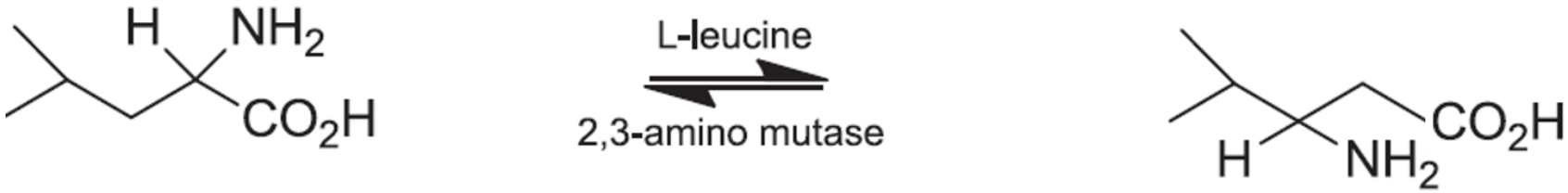


Examples

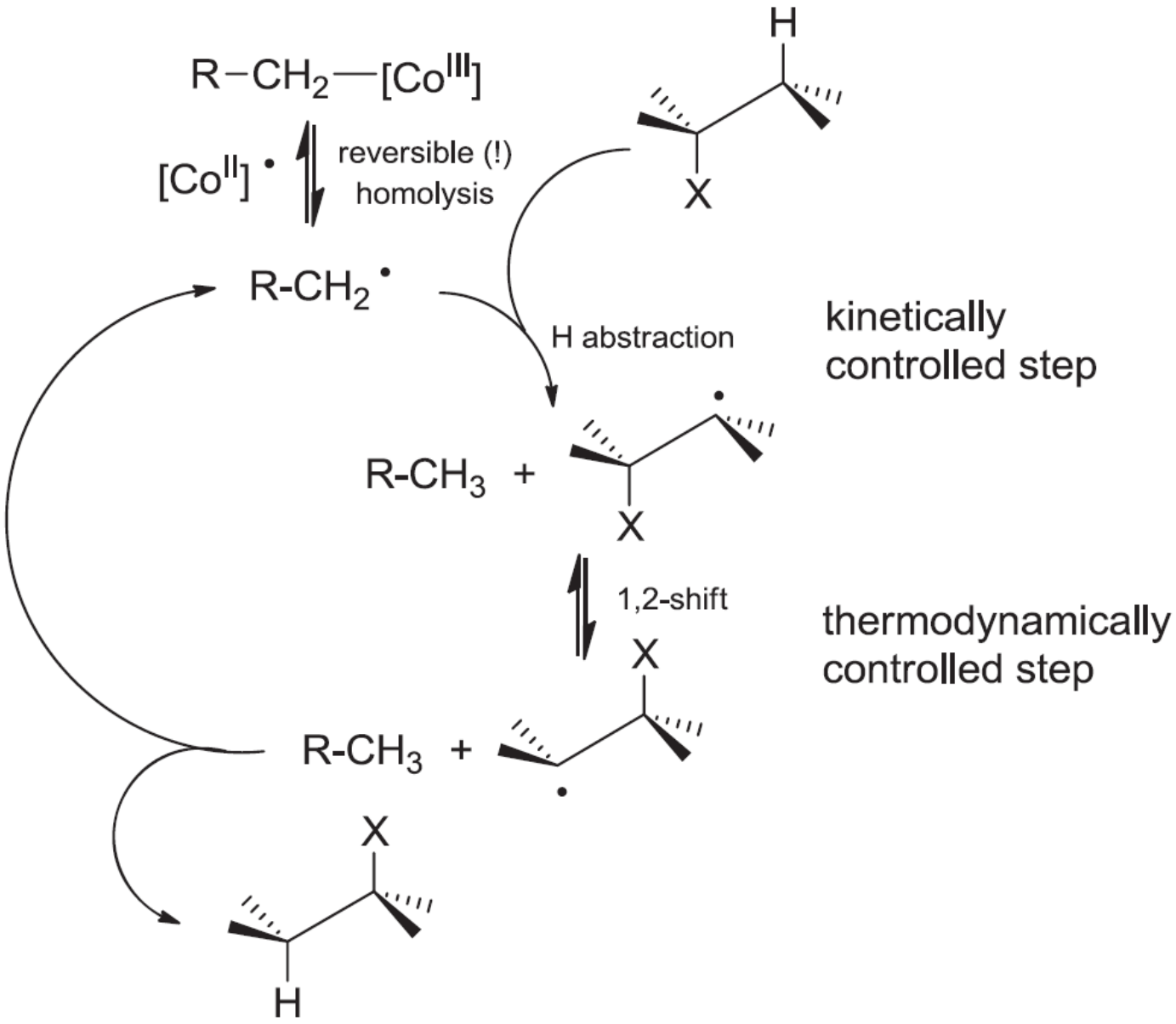

H
OH
OH
diol
dehydratase
H
O
+ H_2O
OH
OH
H
OH
glycerol
dehydratase
O
OH
H
+ H_2O
H_2N
OH
ethanolamine ammonia
lyase
O
H
+ NH_3

Class Ic enzymes are **aminomutases**, which catalyze amino group migrations and require pyridoxal phosphate in addition to AdoCbl.



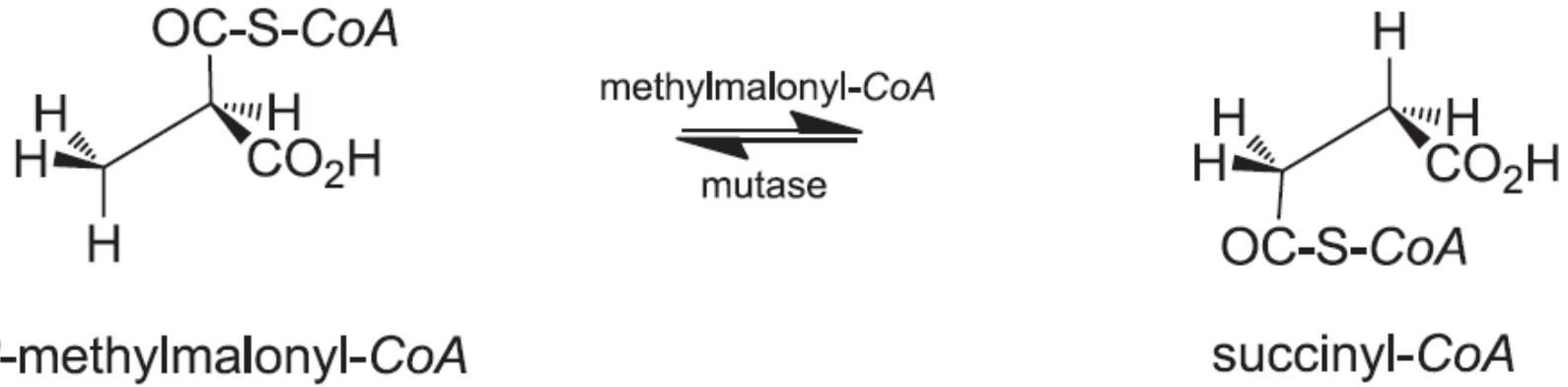


Numerous results from spectroscopic (EPR) and mechanistic (e.g. isotopic labeling) studies now point to the following, **radical-based reaction cycle**



Most reactions catalyzed by enzymes containing the AdoCbl cofactor are restricted to microorganisms that are also able to synthesize this coenzyme. For mammals, the **methylmalonyl-coenzyme A (*CoA*) mutase** is particularly important: it is required in the metabolism of amino acids in the liver and its absence due to genetic defects is lethal.

Important step in **propionate catabolism**, this reaction is required for the **degradation of odd-chain fatty acids**, the amino acids **valine, isoleucine, methionine, and threonine**, and **cholesterol**



R-methylmalonyl-*CoA*

succinyl-*CoA*

Substrate- and stereospecificity are not guaranteed in the absence of the apoenzyme

The initial step in the catalytic cycle is the enzyme-induced homolysis of the Co−C bond of the coenzyme to form a 5-deoxy-5-adenosyl radical and a Co(II) species [Co(II)]• (also called cob(II)alamin), a reaction which can be **accelerated by these enzymes 10^9- to 10^{14}-fold**.

The enzymatic activation lowers the dissociation energy from about 110 kJ/mole in the isolated coenzyme to less than 65 kJ/mol in the active enzyme, and the long-lasting debate over how this is achieved (electron transfer, "mechanochemical" triggering, adenosinebinding pockets) has **not yet been conclusively resolved**.

The primary alkyl radical can selectively attack an exposed H−C center in a kinetically (i.e. activation energy-controlled) step and abstract a hydrogen atom, which is the typical behavior of reactive alkyl radicals.

The second step is the actual rearrangement (1,2-shift), which may be determined by the equilibrium position, favoring for instance a secondary alkyl radical over a primary one.

The **re-abstraction** of a hydrogen atom from enzymebound 5-deoxyadenosine by such a **secondary substrate radical inside the "radical cage" system** will lead to a rearranged reaction product that, in the case of a geminal diol, rapidly loses water and forms the carbonyl compound.

Elimination of water, however, can also be imagined for the radical itself; 1,2-dihydroxyalkyl radicals tend to lose OH^- or H_2O (after protonation) under the formation of carbonylalkyl radicals.

Incidentally, the radical-induced transformation of glycols to aldehydes is not unknown in organic synthesis; such reactions can be triggered by hydroxyl radicals generated from Fenton's reagent.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{III}(OH^-) + OH^{\bullet}$$

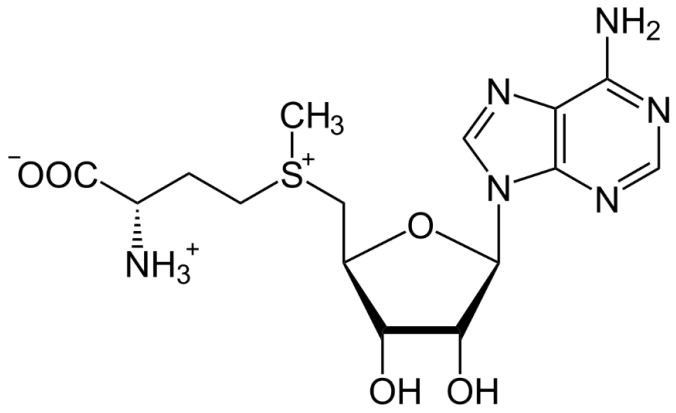
If not immediately used, the **5-deoxyadenosyl radical can recombine with the Co(II)** species, so alkylcobalamins can be described as reversibly-acting radical carriers.

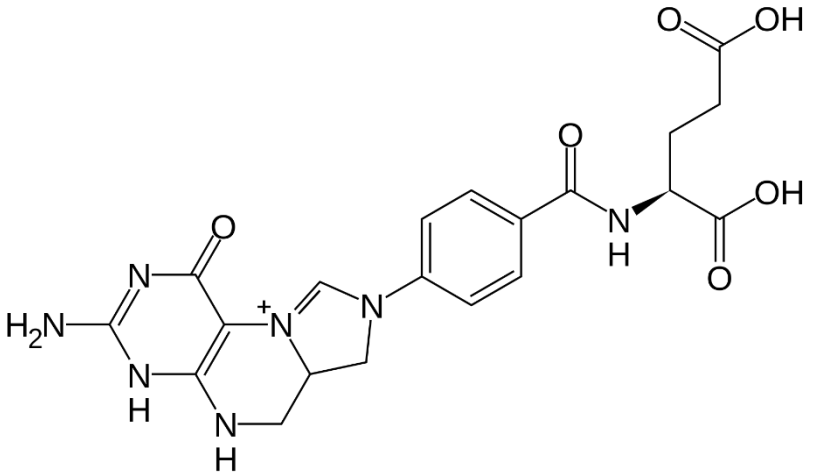
The protein (apoenzyme) in the B12-dependent enzymes has at least three functions.

- After substrate binding, it effects a drastic attenuation of the Co−C bond energy in an as yet unknown manner, causing an acceleration of this initial reaction step by a factor of 10^9 to 10^{14}.
- It protects the reactive primary alkyl radical from the multitude of other, undesired reactants (negative catalysis).
- The protein guarantees a high stereoselectivity of the isomerization by controlling the reaction space

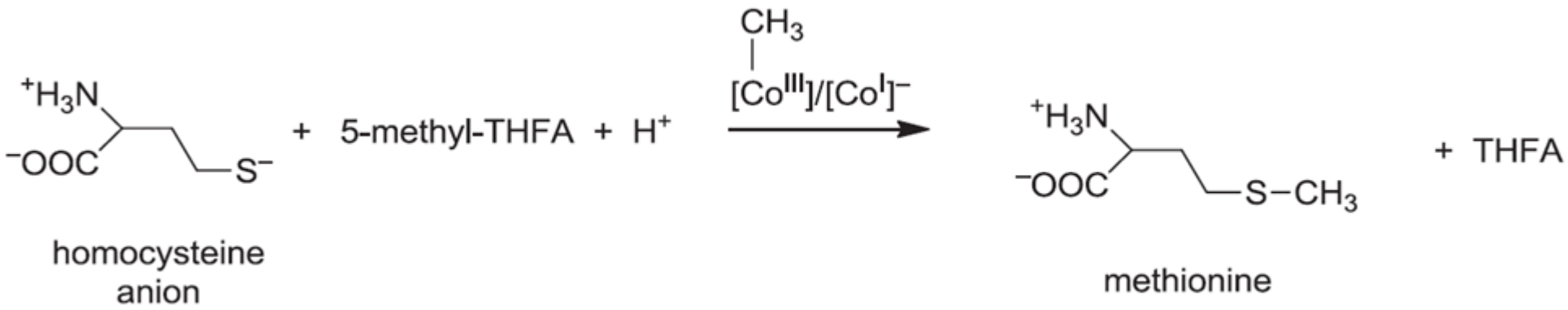
Alkylation Reactions of Methylcobalamin (MeCbl)-dependent Alkyl Transferases

Methyl groups with an electrophilic character, with a "positive partial charge", are **biochemically available through the sulfonium species** S-adenosyl methionine ("SAM"), (adenosyl)(CH_3)S^+($CH_2CH_2CH(NH_3^+)CO^-$), or through 5-methyltetrahydrofolic acid.





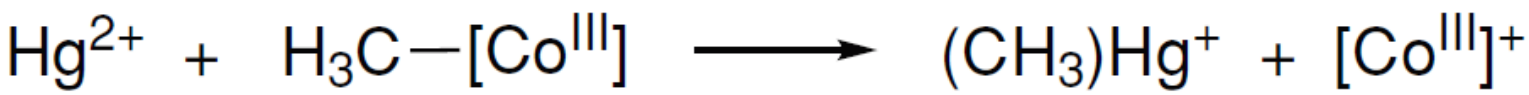
A biologically valuable **methylation requiring cobalamin-dependent enzymes** has been established for the **substrate homocysteine** ("homo": extended by one CH_2 chain link); the essential and often "limiting" amino acid **methionine is biosynthesized** by this reaction (methionine synthetase from *E. coli*)



The **methylation of electrophilic substrates** typically requires an **organometallic compound** that can react either in a **carbanionic fashion (S_N2 reaction)** or **as a radical**, involving a single-electron-transfer process.

The methylation of compounds of **less electropositive "soft" elements** such as selenium or mercury with oxidation potentials **(E_0 > 0V)** presumably occurs via a **carbanionic mechanism**, while **less noble elements** such as arsenic, tin and cadmium **(E_0 < 0 V)** are alkylated in their compounds via **radical pathways**.

In some instances, very toxic species like the methylmercury cation $(CH_3)Hg^+$ are formed by these reactions.



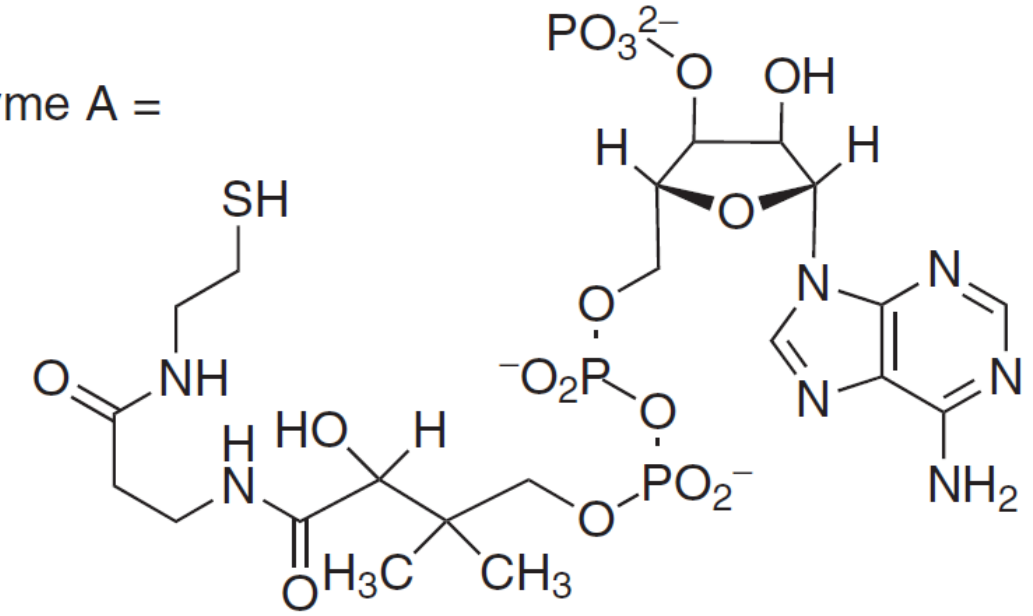
If sufficiently stable under physiological conditions, such **mixed hydrophilic/hydrophobic** organometallic cations are able to **penetrate the blood–brain barrier** and **deactivate sulfur-containing enzymes**

In microorganisms, especially in “acetogenic” or “methanogenic” bacteria, which produce acetic acid and methane, respectively, methyl-transferring “corrinoid” (cobaltcorrin-containing) enzymes are of great importance.

During bacterial CO_2 fixation, they participate in the catalytic formation of acetyl-*CoA* as “activated acetic acid”, a process that involves a nickel enzyme-requiring carbonylation (acetyl-*CoA* synthase (ACS)) and a methyl-group transfer from a 5-methyltetrahydropterin (Pter-N5)−CH_3 to *CoA* via a methylcobalt–corrin intermediate.

$$CO_2 + CO + 6\ \text{"H"} + \text{HS-}CoA \rightleftharpoons CH_3C(O)S\text{-}CoA + 2\ H_2O$$

HS-*CoA* = coenzyme A =



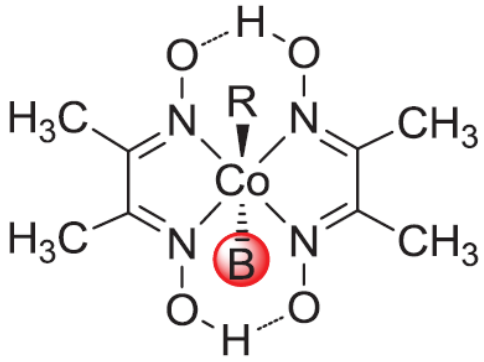
A possible cobalt–porphinoid factor has been described in the context of decarbonylation of long-chain aldehydes.

$$R(CH_2)_nCH_2CHO \rightarrow R(CH_2)_nCH_3 + CO$$

This transformation, which would not be untypical for cobalt- or nickel-containing catalysts, is **important in the biosynthesis of alkanes** (→ waterproofing of leaves or feathers); the **long-chain aldehyde precursors can be formed via peroxidasecatalyzed reactions.**

Model Systems and the Enzymatic Activation of the Co–C Bond

Model systems of the cobalamins are of considerable interest because of the organicsynthetic attraction of coenzyme B_{12}-catalyzed reactions; the actual cobalamins are very sensitive and exhibit only limited solubility.

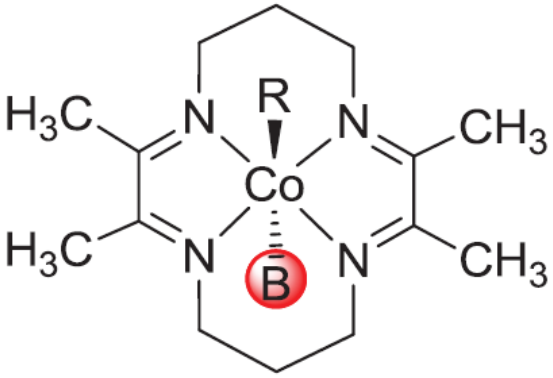


cobaloxime complex

It was realized quite early that **simple bis(diacetyldioxime)** complexes ("cobaloximes") represent surprisingly good models for B_{12} systems; these complexes contain a **"quasimacrocyclic" chelate ring structure** as a consequence of two strong hydrogen bonds

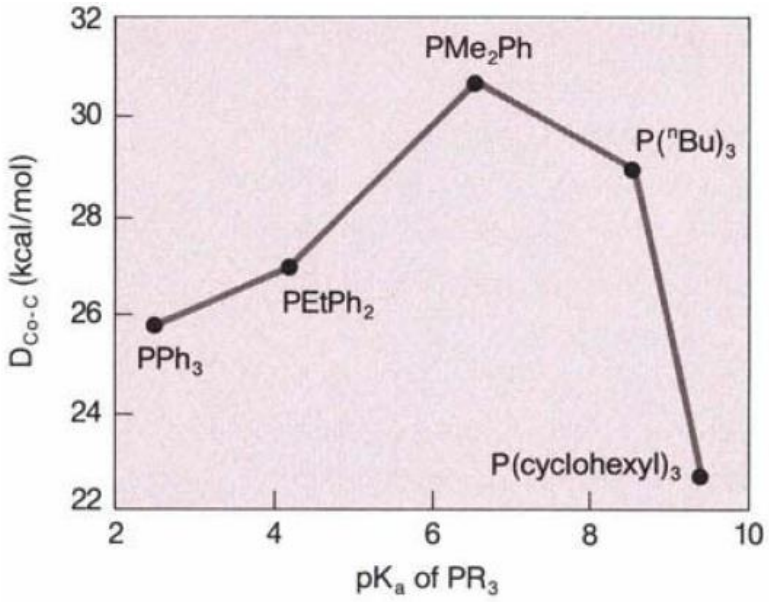
B: base

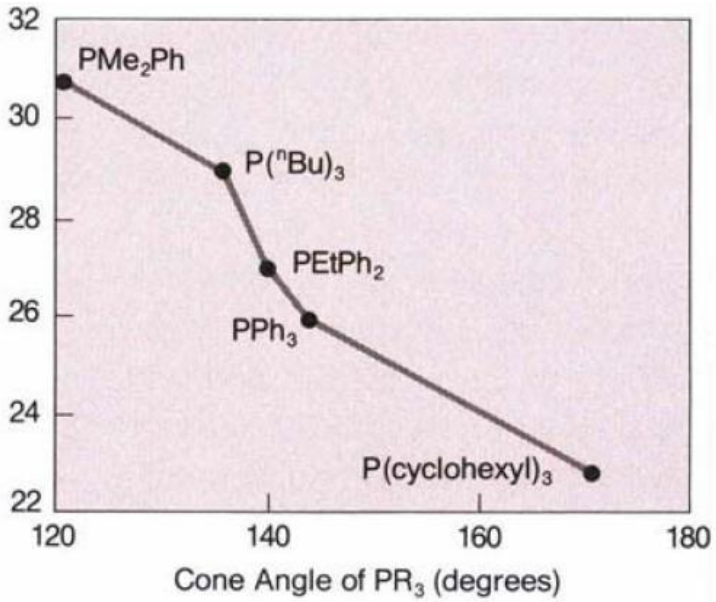
Even better suited with respect to redox properties are the **Costa complexes**, with covalently linked -diimine moieties



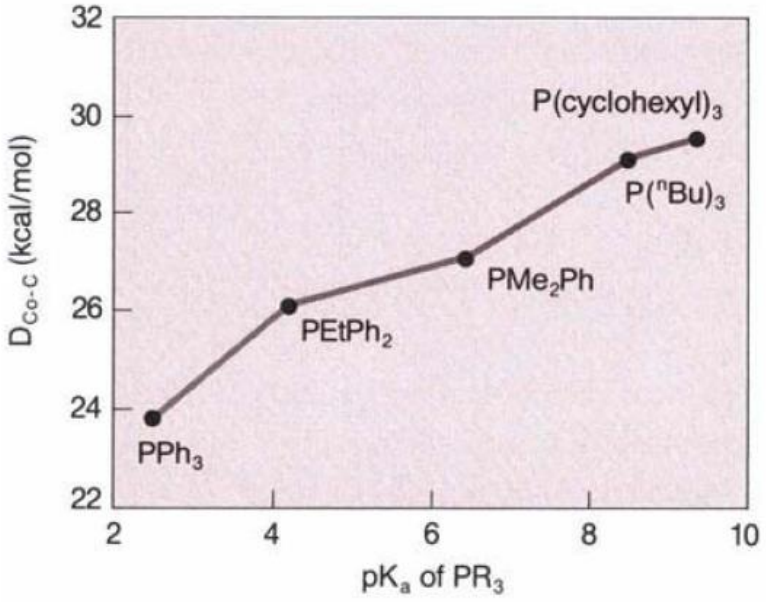
COSTA complex

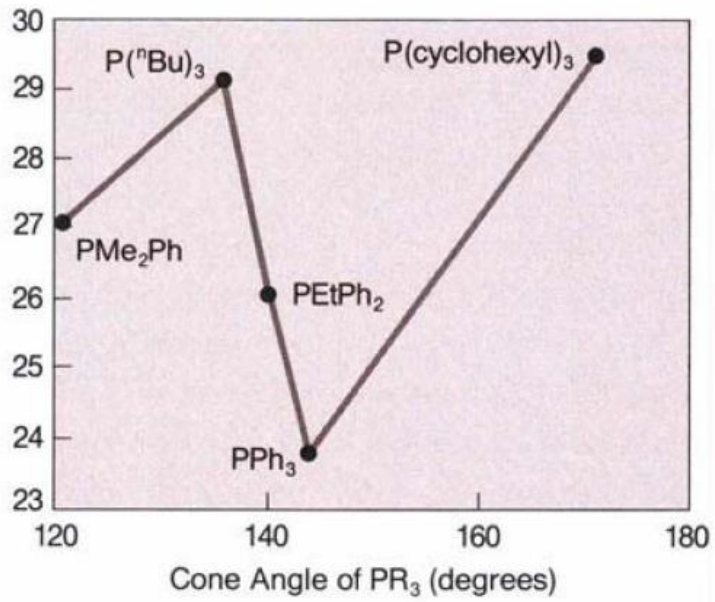
Dependence of Co-C bond dissociation energy on pK_a values and cone angles of various phosphine ligands. The top two panels are for **dimethylglyoxime cobalt benzyl complexes** and the bottom two are for the corresponding **cobalt octaethylporphyrin benzyl derivatives**.





The results suggest that the enzymatically relevant activation, the Co–C bond cleavage, **is influenced by electronic effects** from the PR_3 ligands in porphyrin complexes, while in cobaloximes the steric bulk of the axial PR_3 ligands is more essential.





Significance of the nonplanarity of cobalt–corrin complexes

Radical formation in the enzyme is sterically controlled

The term "**mechanochemical triggering**" was introduced to describe the assumption that base-on/base-off movements in conjunction with corrin deformations trigger the Co−C bond cleavage.

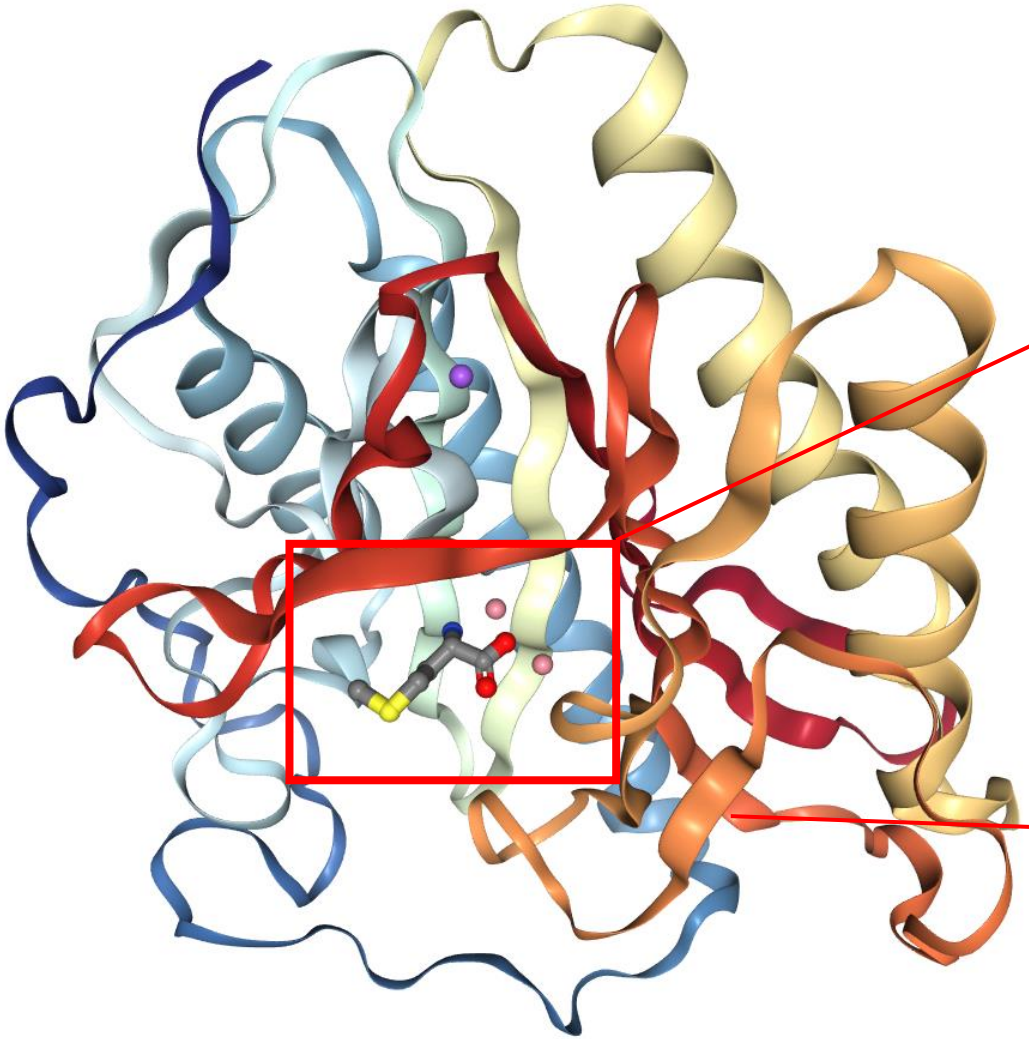
Additional activation can occur through binding of the nucleotide part of the 5-deoxyadenosyl group to the enzyme (adenosine-binding pockets)

The unique function of cobalt in B_{12} systems is its **tolerance of a bond from the redox-active transition metal to primary alkyl groups**, which can be set free through well-defined activation processes and then act as specific, reactive and reversibly transferable species.

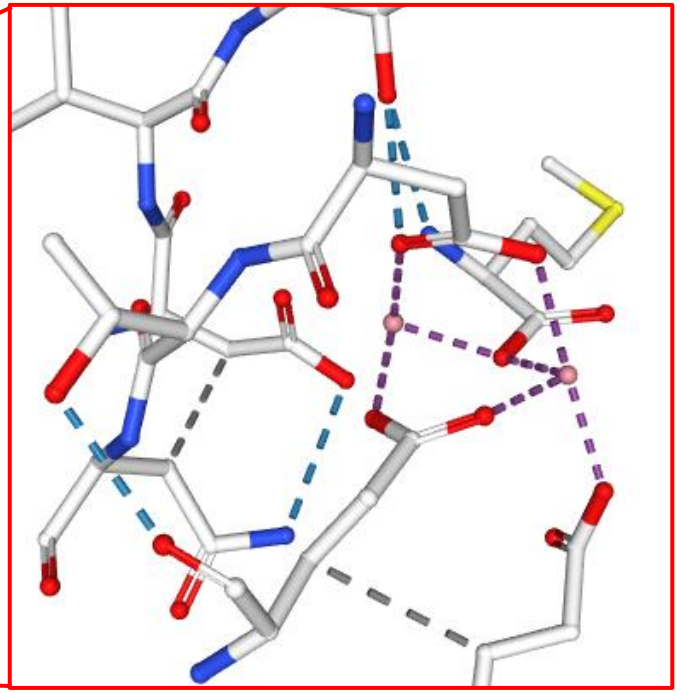
Most cobalt proteins contain a corrin ring, but recent studies have shown that there are a handful of noncorrin cobalt-containing enzymes, including methionine aminopeptidase, prolidase, and nitrile hydratase

Little is known about their action mechanism

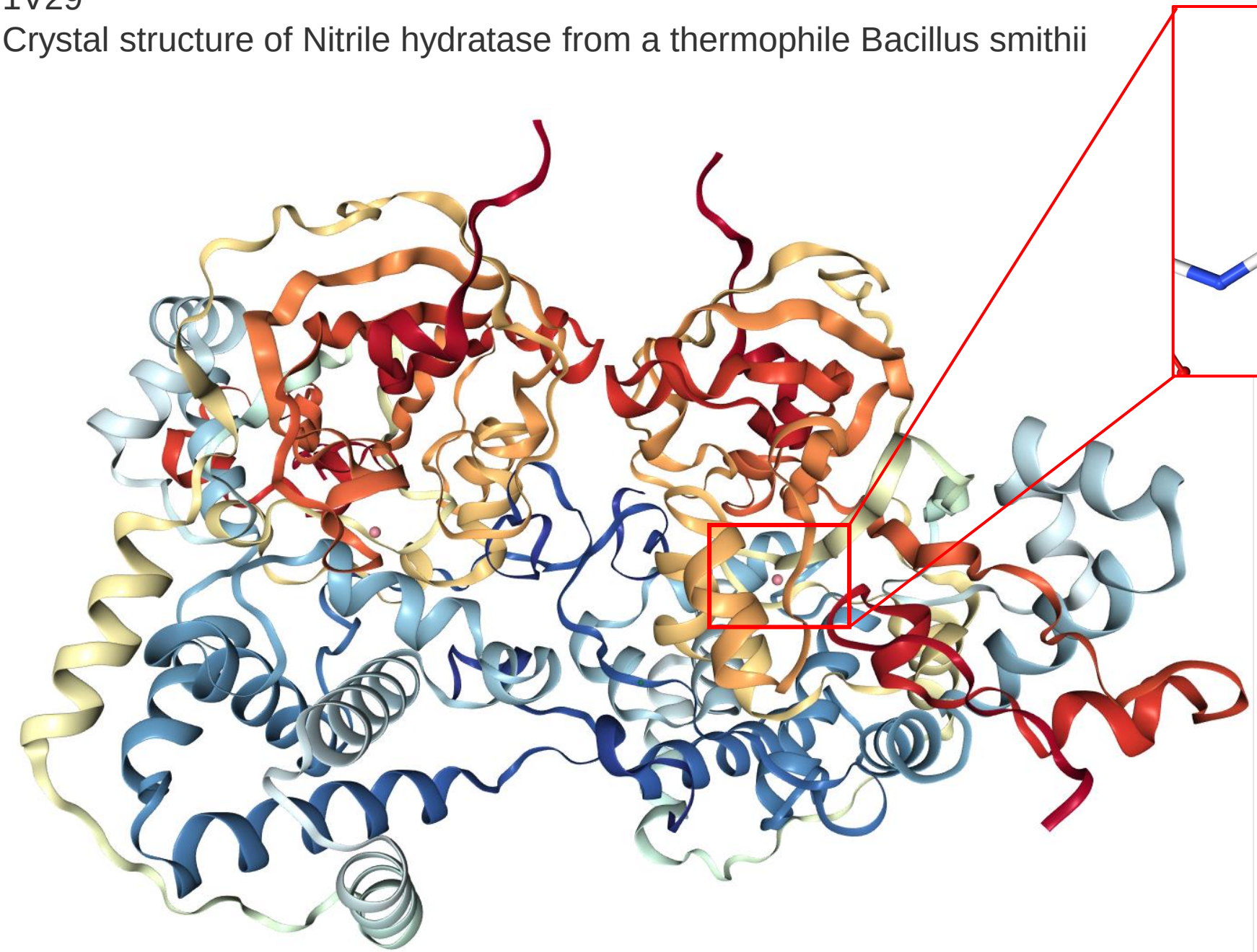
5YXF
Mycobacterium Tuberculosis Methionine aminopeptidase

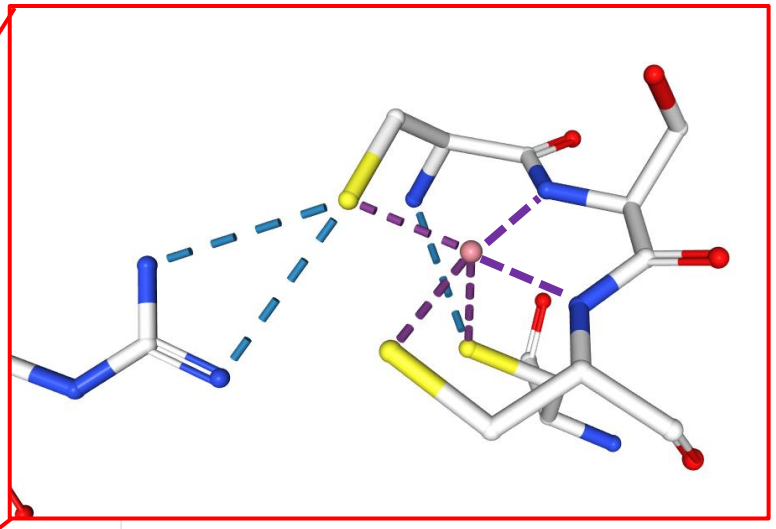


Release of N-terminal amino acids,
preferentially methionine,
from peptides and arylamides.



1V29
Crystal structure of Nitrile hydratase from a thermophile Bacillus smithii





Mononuclear iron or non-corrinoid cobalt enzymes that catalyse the hydration of diverse nitriles to their corresponding amides.

$R\text{-}C{\equiv}N + H_2O \rightarrow R\text{-}C(O)NH_2$