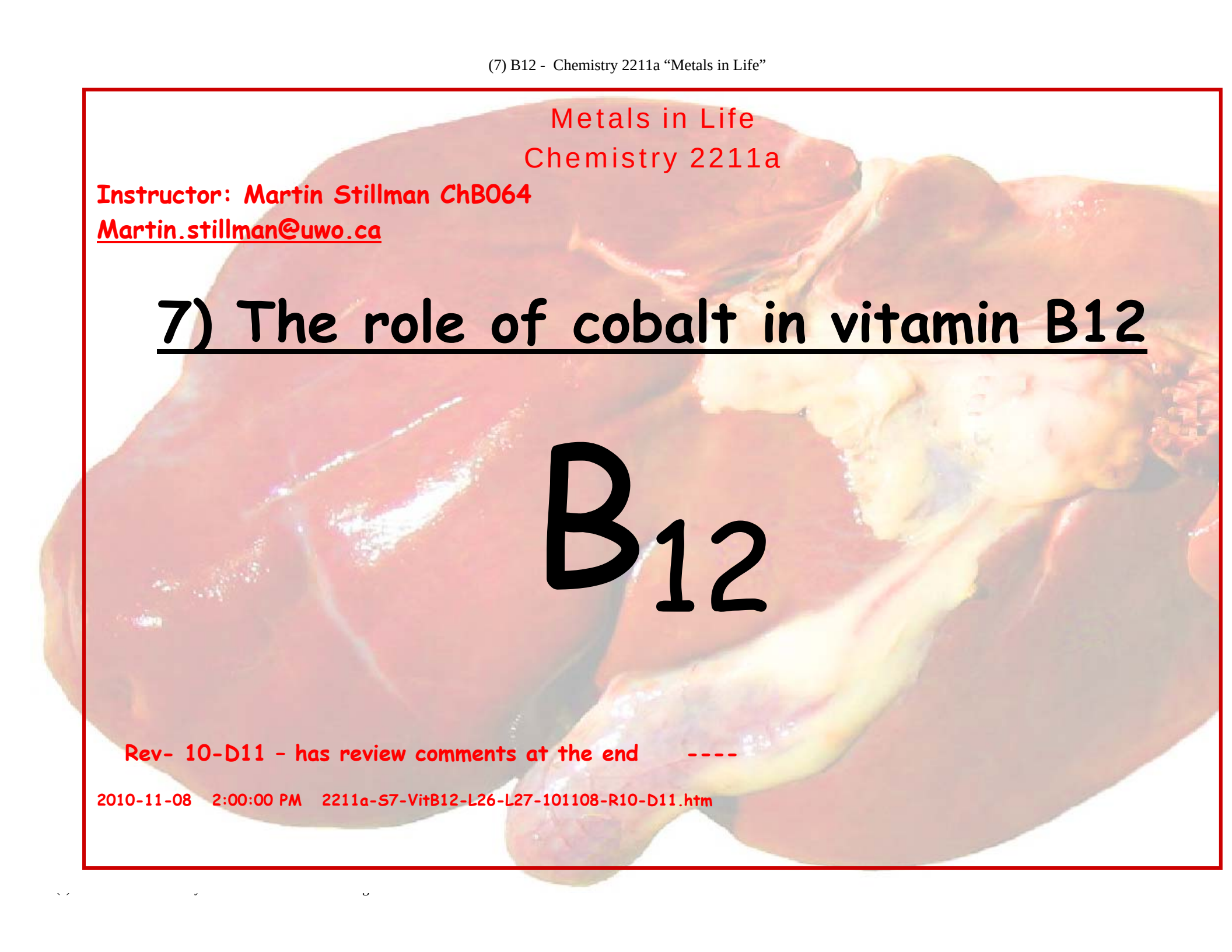
Metals in Life

Chemistry 2211a

Instructor: Martin Stillman ChB064

Martin.stillman@uwo.ca

7) The role of cobalt in vitamin B12



Rev- 10-D11 – has review comments at the end ----

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To start then

Vitamin B12 is the only, metal-containing vitamin –
it is also a water-soluble vitamin that is stored in the liver and must come from the diet.

Vitamin B12 is a collective term for a group of cobalt-containing compounds known as corrinoids which when assembled with 5^{th} and 6^{th} position ligands are known as cobalamins.

The principal cobalamins are:

cyanocobalamin, hydroxocobalamin

and the two coenzyme forms:

methylcobalamin and 5-deoxyadenosylcobalamin (adenosylcobalamin).

Vitamins are organic compounds required in the diet in, usually, very small amounts.

They are conveniently classed as fat soluble (A, D, E and K) or water soluble (C and the B complex).

We need to consider

1. Vitamins in general
2. Chemical nature of Vit B12
3. Sources
4. Function in brief
5. Consequences of deficiency
6. Function of Vit B12 in detail

Vitamins in general The 13 Essential Vitamins

Vitamins – are organic compounds essential to health that must be supplied in small amounts in the diet

Provide no energy and are unchanged by the reaction they catalyse

Body does not synthesize vitamins – must be part of the diet

Vitamins are essential molecules for enzyme activity – deficiency leads to death

Vitamins a Definition

A group of substances essential to normal metabolism (see below), growth and development, and regulation of cell function; vitamins work together with enzymes, co-factors, and other substances.

Each vitamin has specific functions. If a certain vitamin is deficient, a deficiency disease results

Enzymes – are proteins that serve as catalysts in biological synthesis and degradation reactions

Called a Coenzyme because: it is a chemical that is required for an enzyme to function –

remembering: Apoenzyme + Cofactor[1] → Holoenzyme

(protein only = inactive) (active)

Light-mass and small Cofactor/CoEnzyme[2] (determines the type of reaction that the HOLOenzyme carries out) + Heavy-large ApoEnzyme (determines the biochemistry)

→ Heavier-larger (?-see next page) HOLOenzyme (fully functional)

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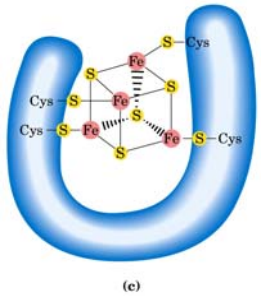
[1] Cofactor: An atom, organic molecule, or molecular group that is necessary for the catalytic activity (*see* catalysis) of many enzymes. A cofactor may be tightly bound to the protein portion of an enzyme and thus be an integral part of its functional structure, or it may be only loosely associated and free to diffuse away from the enzyme. Cofactors of the integral kind include metal atoms — such as iron, copper, or magnesium — or moderately sized organic molecules called prosthetic groups; many of the latter contain a metal atom, often in a coordination complex (*see* transition element). Removal of the cofactor from the enzyme's structure causes loss of its catalytic activity. From: Britannica Concise Encyclopedia.

[2] Loosely associated cofactors are sometimes called coenzymes; examples include most members of the vitamin B complex. Rather than directly contributing to the catalytic ability of an enzyme, coenzymes participate with the enzyme in the catalytic reaction. From: Britannica Concise Encyclopedia.

Larger (?) – not necessarily – the Coenzyme might fit – probably fits – into a pocket in the apo-enzyme – so may actually reduce the size/volume of the enzyme

Metabolism

1. Physical and chemical processes within the body related to body functions.
2. Processes of energy generation and use.
3. Processes that include digestion of food, absorption, elimination, respiration (the process or organs of breathing), blood circulation, and temperature regulation.



(e)

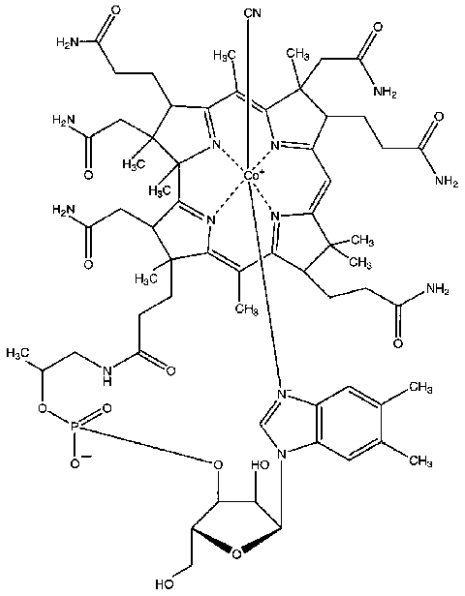
Coenzymes (cofactors) act as group-transfer reagents

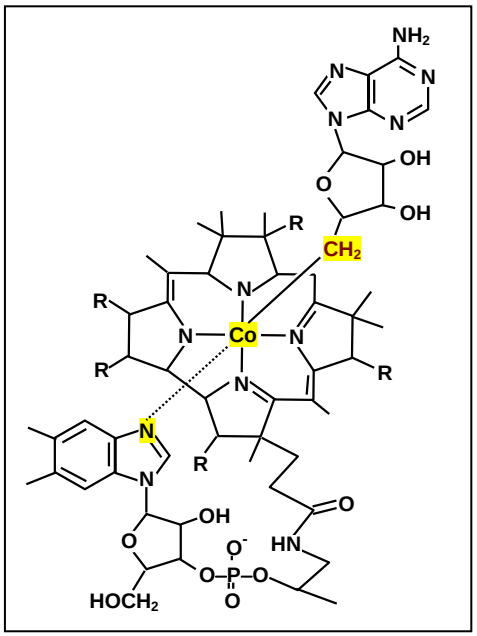
- Hydrogen, electrons, or other groups can be transferred
- Two types of coenzymes:
  - **metabolite coenzymes**
  - **vitamin-derived coenzymes**

Functional aspects of cofactors

Activator ions - transient	Permanent metals – part of the enzyme	Transient cofactors – coenzymes – molecular species Cosubstrates – altered during the reaction but are regenerated at the end	Permanent cofactors – part of the enzyme – coenzymes – molecular species Prosthetic groups – remain unchanged
Na^+ ; Ca^{++} K^+; Mg^{++} Mn^{++}	**Fe-S centres – see the Fe_4S_4 from aconitase [3] Zinc; copper; cobalt Add your enzyme here:…….**	**ATP**	**adenosylcobalamin AND methyl-cobalamin**

[3] Aconitase – has 4 Fe's, 2Fe's were seen in the photosystem of chlorophyll.





Vitamin-derived coenzymes

Vitamins are conveniently classed as fat soluble (A, D, E and K) or water soluble (C and the B complexes).

Water-soluble B-complex vitamins - thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pyridoxine (vitamin B6), folic acid, vitamin B12, and biotin, pantothenic acid and vitamin C – odd ones

Chemical nature of Vitamin B12

Vitamin B12 is also called cobalamin based on cobalt-containing compounds known as corrinoids.

Structure: Co-bound to a corrin ring; 5th position is bound a **dimethylbenzimidazole** –connected via a ribose sugar unit to one of the reduced pyrroles of the corrin ring. The 6th position of the cobalt can have one of 4 different groups attached. The cobalt cycles between Co^{3+} , Co^{2+} and Co^{1+} in its catalytic activity.

cobalamins are **cyanocobalamin**, **hydroxocobalamin** and the two coenzyme forms **methylcobalamin** (note CH_3^-) and **5-deoxyadenosylcobalamin (adenosylcobalamin)**.

Vitamin B12 is a dark red multiple ring complex structure – see right

Different derivatives $N\text{-}Co^{3+}\text{-}R$:

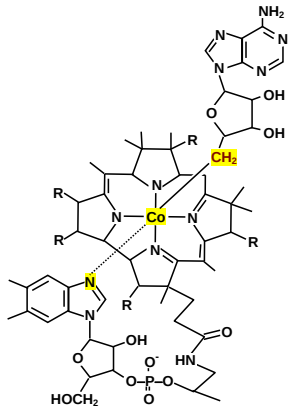
methylcobalamin (R=CH_3) (= coenzyme B12 form), cyanocobalamin (R=CN) – the vitamin , hydroxycobalamin (R=OH) , aquacobalamin (R=H_2O); and, 5'-deoxyadenosylcobalamin (adenosylcobalamin) R= alkyl attached- 5'-deoxyadenosine (= coenzyme B12 →)

As we can see from the structure on the right,

cobalamins comprise a nucleotide (base, ribose and phosphate) attached to a corrin ring.

The corrin ring is made up of four pyrrole groups and an atom of cobalt in its centre.

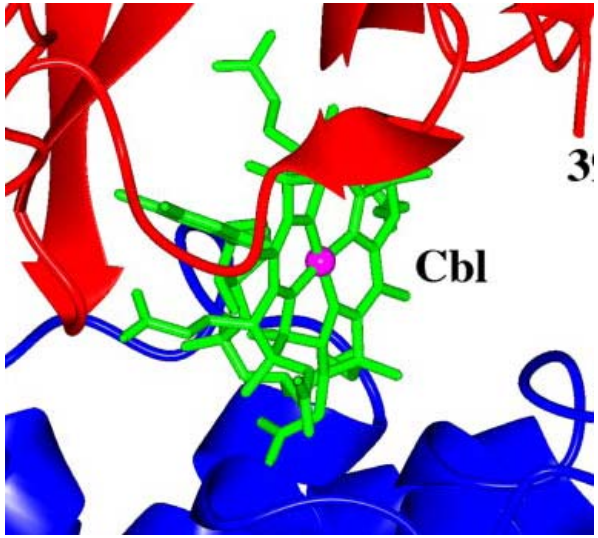
The cobalt atom attaches to one of: a methyl group, a deoxyadenosyl group, an hydroxyl group, water or a cyano group, to yield the five cobalamin forms mentioned above.

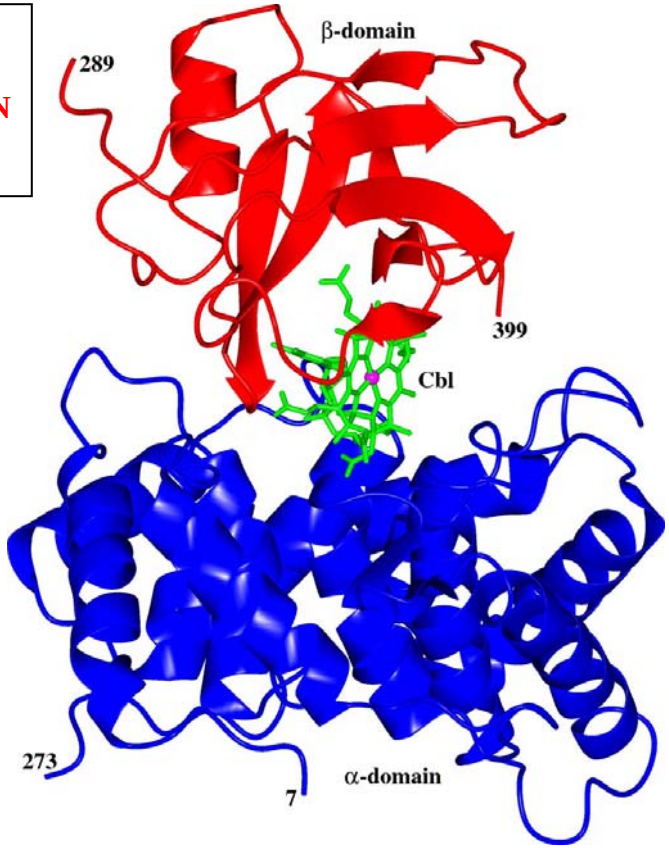


Sources and requirements

Vitamin B12 (cobalamin) only synthesised by bacteria and micro-organisms. Animals ingest it from their natural bacterial flora or eat other animals who have stores of the vitamin.

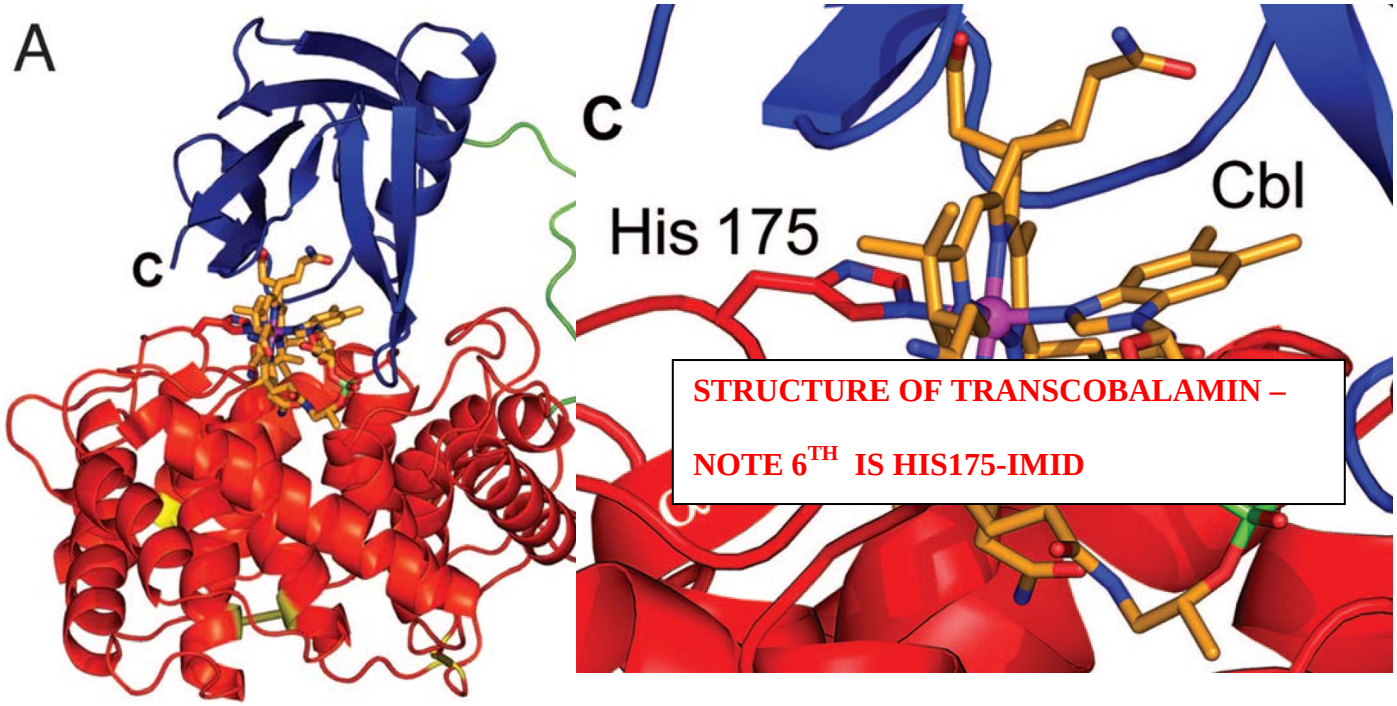
STRUCTURE OF THE INTRINSIC FACTOR –
NOTE: THERE IS NO 6TH POSITION LIGAND





Cyanocobalamin is the principal form of the vitamin used for fortification of foods and in supplements.
Liver, brain, kidneys richest sources, then eggs; and lower in general meats, milk, tuna, cottage cheese, yogurt. Not in plants which makes it a problem for vegans who are at risk from deficiency.
Recommend Daily Allowance (USA): 2-3 µg/day•

Hydrochloric acid in the stomach releases B12 from proteins in foods during digestion.
Vitamin B12 is then absorbed from the ileum as a complex with **intrinsic factor** which is produced by the gastric mucosa – for a large figure see last few pages.5



STRUCTURE OF TRANSCOBALAMIN –

NOTE 6TH IS HIS175-IMID

Transport: Vitamin B12 binds to a glycoprotein, the intrinsic factor, in the stomach – deficiency of the intrinsic factor leads to B12 deficiency and pernicious anaemia. The vitamin B12-intrinsic factor complex recognises surface receptors of mucosal cells in the ileum and is absorbed.

The Cbl molecule is bound at the interface between the α and β domains of IF.[5]

It is transported around the body bound to **transcobalamin**, a specific Vit B12 binding protein[6].
The Cbl molecule is bound to TC between the α and β domains in a manner very similar to that in IF, with its cobalt ion coordinated by the four nitrogen atoms of the corrin ring and by the N3B atom of the **dimethylbenzimidazole** ring as the fifth ligand on the α side of the corrin ring.

The sixth coordination site of the cobalt is occupied by the N ε2 atom of a histidine side chain, at position 175 in TC, which is located on a loop between helices α 7 and α 8

B12 enters the blood stream and is taken up by the liver, bone marrow and blood cells prior to intracellular release. The vitamin is then converted into its coenzyme forms, as above.

3-5 mg is stored mainly in the liver in amounts sufficient to last up to 5 years.

The requirement of vitamin B12 is (with vitamin D) the smallest of all the vitamins, only 2-3 micrograms per day.

4. Function in brief

Vitamin B_{12} is important for the normal functioning of the brain and nervous system and for the formation of blood.

It is involved in the metabolism of every cell of the body, especially affecting DNA synthesis and regulation but also fatty acid synthesis and energy production.

Its effects are still not completely known.

Vitamin B12 (cobalamin) derived cofactors are used for two important reactions in humans:

1. Methylmalonyl CoA mutase requires 5-deoxyadenosylcobalamin (known as adenosylcobalamin – mol structure above) - Mutase carbon chain rearrangement – shifts CH_3's about. – involved in fat metabolism.
2. Methionine synthase (cofactor methylcobalamin) catalyses the conversion of homocysteine to methionine and tetrahydrofolate is also formed – see below) – this is a Methyl Transferase (MTA) – important in production of red blood cells. The second reaction taking Methionine back to Homocysteine (see last slide) is catalysed by Methionine Synthase Reductase (cofactor adenosylcobalamin) and forms S-adenosylmethionine (SAM) – SAMs control myelin sheath covering of nerves – this deficiency leads to neurological problems – depression – because of neural problems.

Therefore, Vit B12 controls:

1. production of blood platelets and red and white blood cells
2. normal nerve cell activity – formation of myelin basic protein,
3. DNA and RNA replication, and
4. production of the mood-affecting substance SAM (S-adenosyl-L-methionine).
5. One electron reduction and oxidation

Co(III) is d^6 – the Co(III) can be reduced in 1e steps to Co(I) – d^8

So starting with (N)4-Co(III) – CH_2R homolysis results in $\cdot CH_2R$ a radical and leaves a low spin d^7 the alkyl radical is very reactive

6. Vitamin B12 works closely together with vitamin B9 (folate) to regulate the formation of red blood cells and to assist in the function of iron.
7. Vitamin B12 is important for the activity of enzymes within cells that control fat, amino acid and carbohydrate metabolism.
8. Both vitamin B12 and the vitamin folate are essential for the production of DNA and RNA.
9. Cyanocobalamin promotes normal growth and development; treats pernicious anaemia.
10. Vitamin B12 helps in the maintenance of the central nervous system:
11. B12 plays a vital role in the metabolism of fatty acids essential for the maintenance of myelin. Nerves are surrounded by an insulating fatty sheath comprised of a complex protein called myelin.

Vitamin B12 participates with folic acid in DNA synthesis so its deficiency leads to a similar anaemia.

L-B	R-M	K-S	Problems to do
		Ch 3 – p 39	If blank – see later

5. Summary of the Effects of Vit B12 Deficiency (11 points)

1. Deficiency results in fatigue, anaemia, impaired nervous system functioning, and can increase the risk of infection. Anaemia due to a true folic secondary folic acid deficiency caused by primary B12 deficiency is pernicious anaemia
2. Folic acid deficiency is common in hospital patients – lack of absorption of B12 containing foods
3. Deficiency most often afflicts elderly whose diets are not sufficiently rich in B12 foods.
4. Impaired methylmalonyl CoA mutase causes accumulation of unusual odd number carbon fatty acids. These accumulate in nerve cell membranes causing irreversible neurological disorders.
5. Vitamin B12 also has a separate biochemical role, unrelated to folate, in the synthesis of fatty acids in the myelin sheath that surrounds nerve cells. So, vitamin B12 deficiency has a severe effect on the nervous system
6. Degeneration of spinal cord neurons
7. Prolonged B12 deficiency can lead to nerve degeneration and irreversible neurological damage.
8. Vitamin B12 deficiency leads to irregular destruction of the myelin sheaths, which eventually causes paralysis and death.
9. Vitamin B9 (folate) and vitamin B12 are critical to the health of the nervous system and to a process that clears homocysteine from the blood. Elevated plasma homocysteine concentrations are considered to be a risk factor for vascular disease and birth defects

such as neural tube defects..[4]

10. Vitamins B12, B6, and B9 (folate) work closely together to control blood levels of the amino acid homocysteine.
11. The most important use of vitamin B12 is to treat the symptoms of pernicious anaemia.
12. Vitamin B12 levels decrease with age and various measures of cognitive impairment are associated with reduced B12 status.

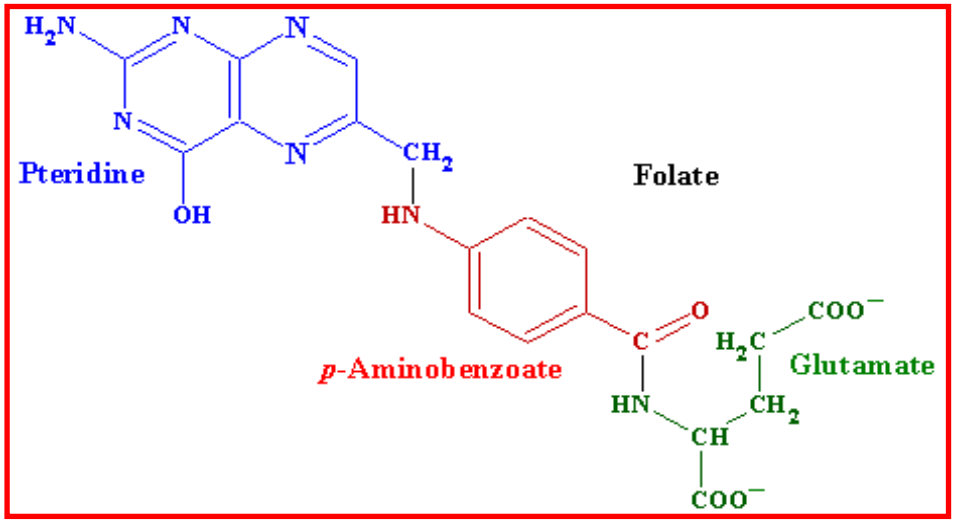
What is vitamin B12 deficiency anaemia?

This is a blood problem that occurs when there are not enough red blood cells to carry the required amount of oxygen via hemoglobin. Vitamin B12 is key to red blood cell synthesis.

What causes vitamin B12 deficiency anaemia? Vitamin B12 deficiency anaemia usually develops when the body cannot absorb B12 efficiently from food.

This occurs with pernicious anaemia. In pernicious anaemia, the body destroys the cells in the stomach needed to help absorb vitamin B12. (In case you ask - Pernicious anaemia is the result of an autoimmune process in which parietal cell autoantibodies against the gastric H^+/K^+-adenosine triphosphatase (the gastric proton pump) cause loss of gastric parietal cells. The loss of parietal cells results in diminished production of the intrinsic factor. The intrinsic factor is necessary for B_{12} absorption. Deficiency of intrinsic factor results in B_{12} deficiency.)

[4] The American Heart Association, and the National Institutes of Health's (NIH) National Heart, Lung, and Blood Institute (NHLBI), have both recognized that elevated homocysteine levels are a potential risk factor for cardiovascular disease.



Pernicious anaemia due to a primary deficiency of vitamin B12 gives rise to a secondary deficiency of folic acid because all the folate ends up trapped as N5-methyl-tetrahydrofolate

Or following stomach surgery the small intestine (the ileum) has been removed… and more..

Or in the case of severe digestive problems, such as sprue (also called celiac disease), Crohn's disease, bacteria growth in the small intestine, or a parasite.

Or when drugs are taken to treat heartburn and ulcers for a long time. For example, omeprazole (Prilosec) and lansoprazole (Prevacid) – these are generally proton pump inhibitors.

Or people who eat a strict vegetarian (vegan) diet, and older adults who don't eat a variety of foods, and people with chronic alcoholism. Breast fed infants of vegan mothers are particularly at risk for B12 deficiency.

FOLIC ACID

The importance of folic acid during early pregnancy is the closure of the neural tube occurs around the 28th day of pregnancy.

Incidence of neural tube defects (spina bifida and anencephaly) is reduced by 400μg folic acid supplement/day before conception and during the first month of pregnancy.

Function: Folic acid is a precursor for tetrahydrofolate that is used as a carrier of one carbon units at different levels of oxidation.

Effect of Deficiency: Megaloblastic anaemia, GI

Adequate levels of folate are particularly critical during the development of the nervous system. A shortage of folate in this period can cause neural-tube defects, including spina bifida. Anaemia caused by folate deficiency is very similar to that caused by B12 deficiency (pernicious anaemia).

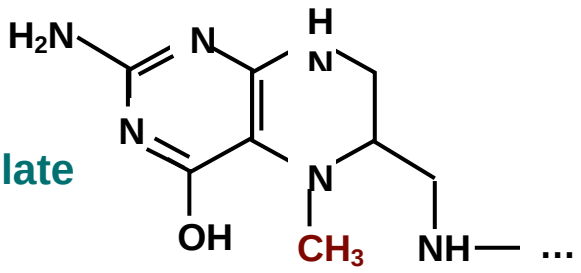
Sources of folic acid: Intestinal bacteria, liver, yeast and green vegetables

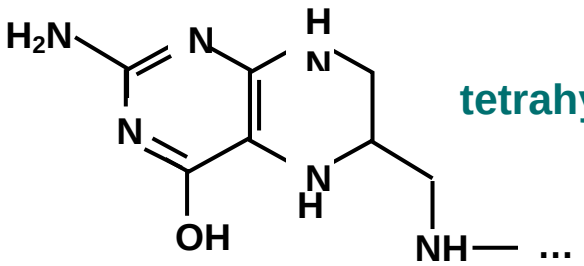
Folate deficiency is probably the most common vitamin deficiency. Since addition of folate to flour in the U.S. began in 1996, neural-tube birth defects have decreased by ~20%.

RDA: 200 μg/day.

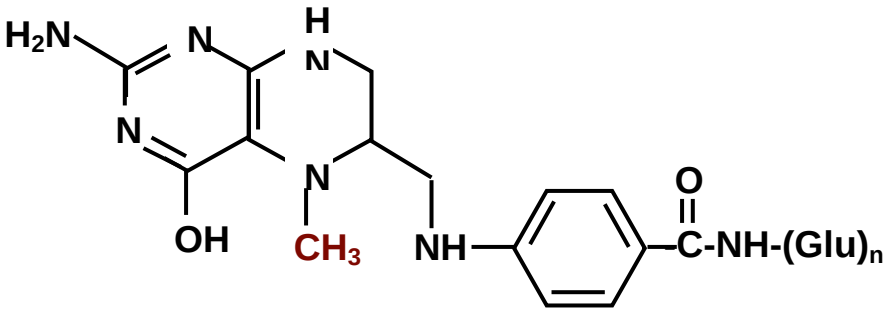
For the next slides:

N^5-methyl - tetrahydrofolate





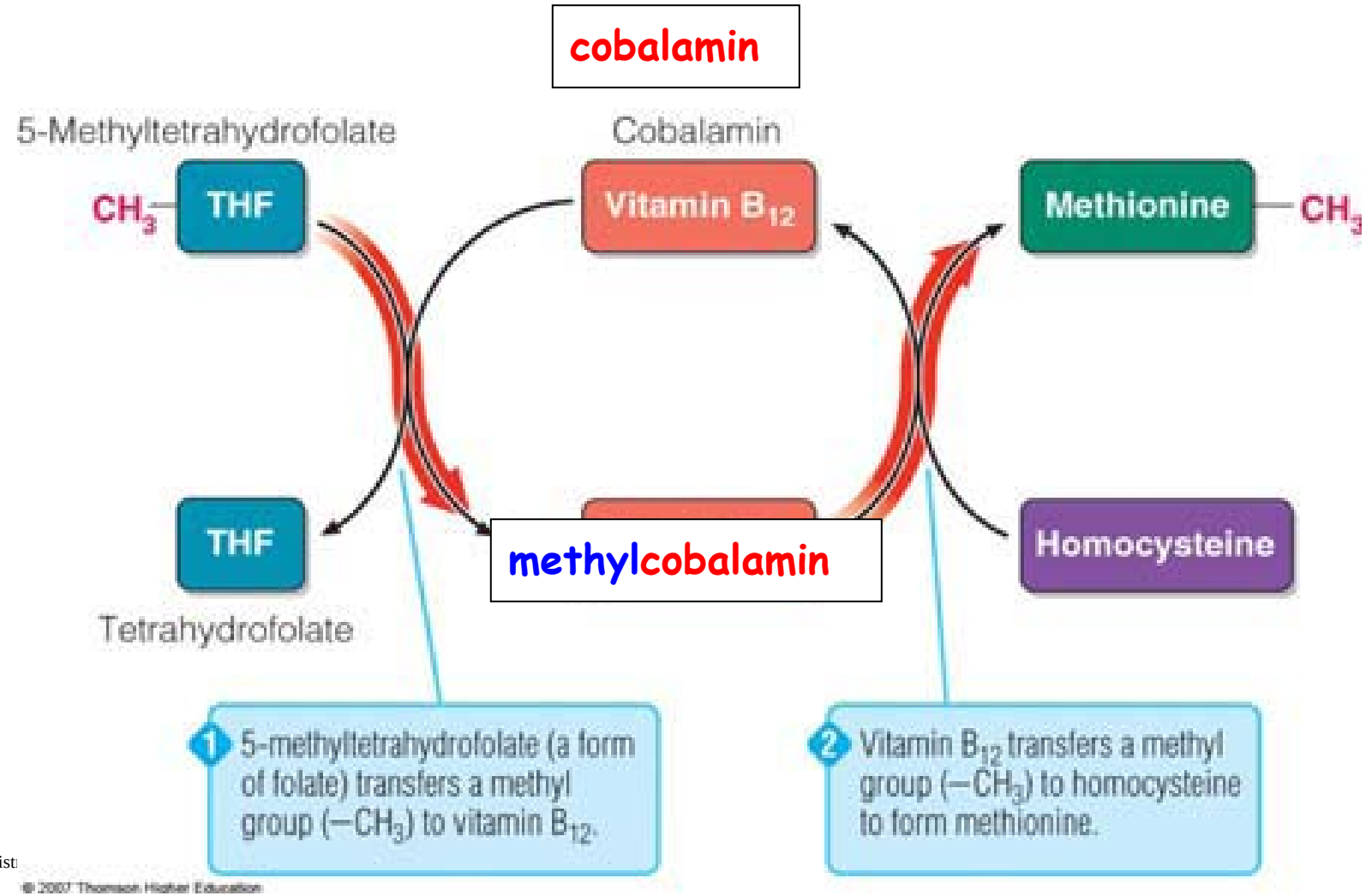
tetrahydrofolate



6. A function of coenzyme B12 - in detail

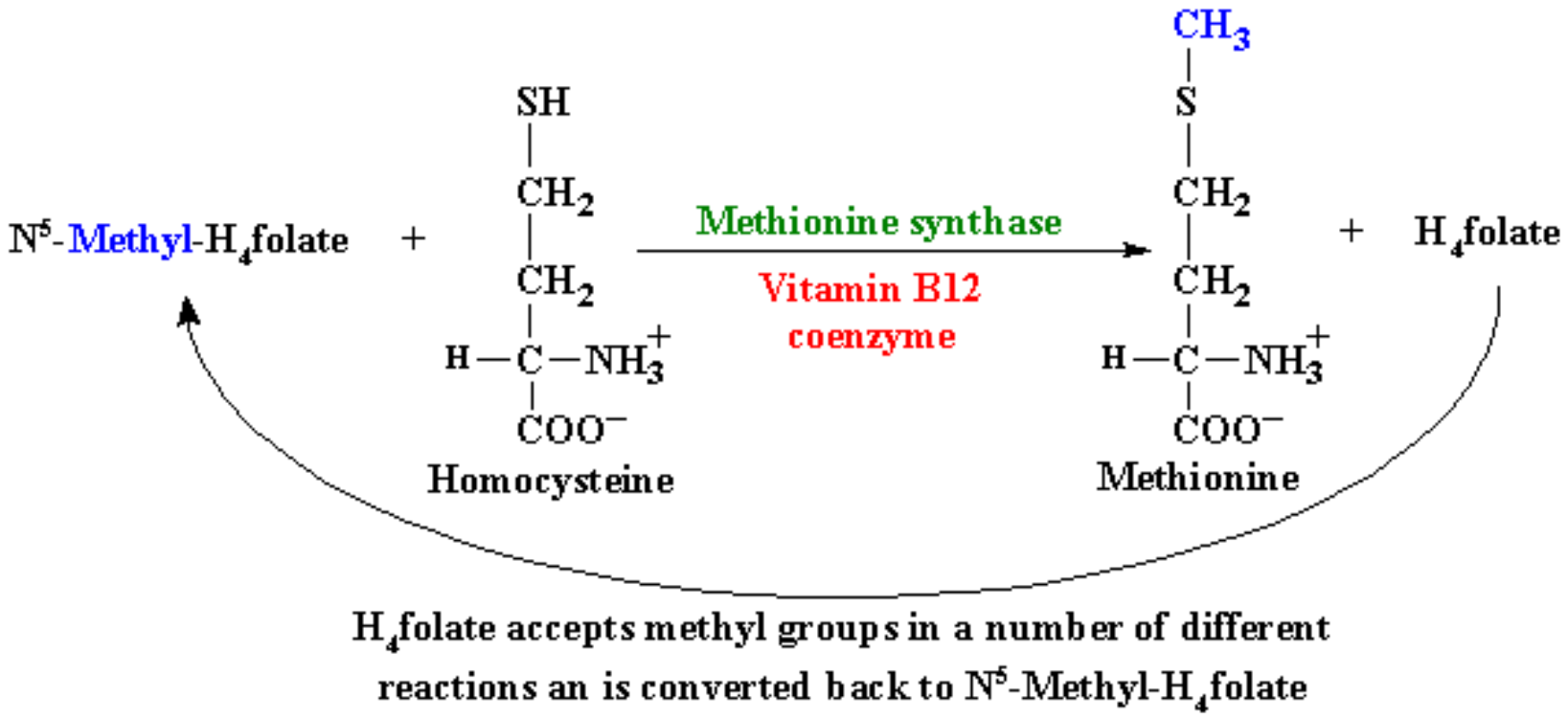
In summary here – molecular details next page and at the end.

Only about 15 reactions in humans absolutely require cobalamin coenzymes - but without it – death. Methylcobalamin is the cofactor or coenzyme in Methionine Synthase (MS) – formation of methionine.



Adenosylcobalamin (B12) is a coenzyme in the reaction involved in methionine metabolism using methionine synthase reductase (MSR) – that is further reactions after methionine has been formed.

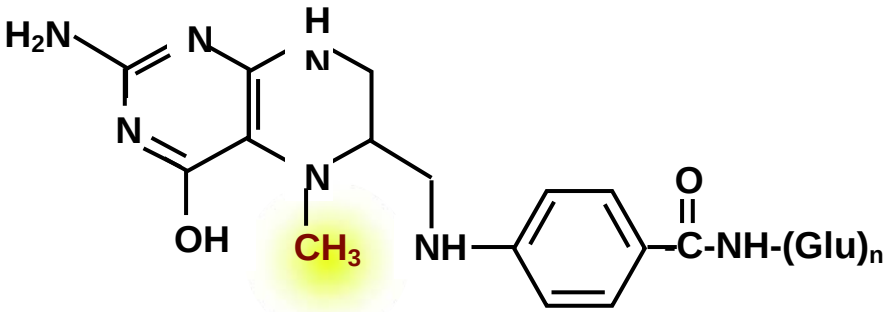
In the MS reaction below – CH_3-cobalamin is the cofactor.



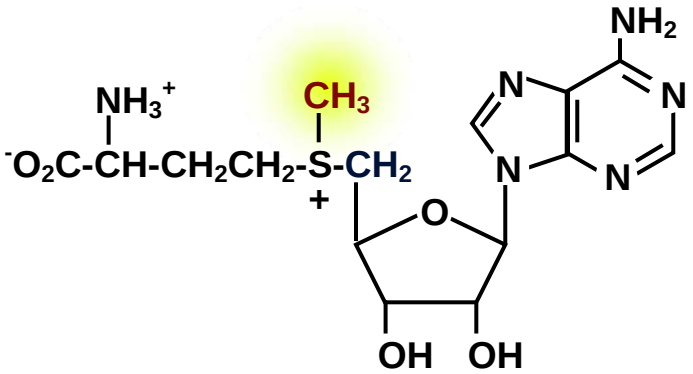
H_4folate is converted to N_5-methyl-H_4folate in a number of different reactions as it accepts methyl groups. The methyl group can only be removed and the H_4folate regenerated by the above reaction. (See folic acid).

We have seen that there are three main carriers of reduced one-carbon units- THF, SAM and cobalamin

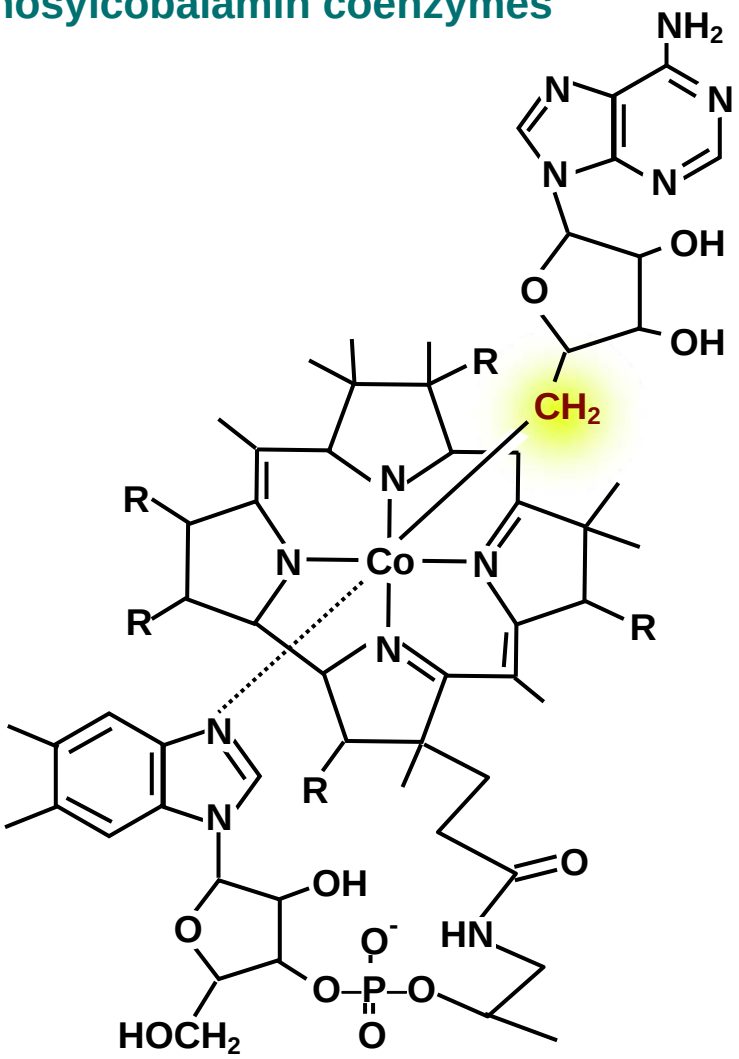
derivatives of tetrahydrofolic acid (= THF) or here N_5-methyl-H_4-folate



S-adenosylmethionine (= SAM)



adenosylcobalamin coenzymes



More detail on the MS and MSR cycles mentioned above:
Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS) and methionine synthase reductase (MSR or sometimes MTRR) in the folate cycle. The three enzymes of folate metabolism play an essential role in both DNA synthesis and methylation processes.

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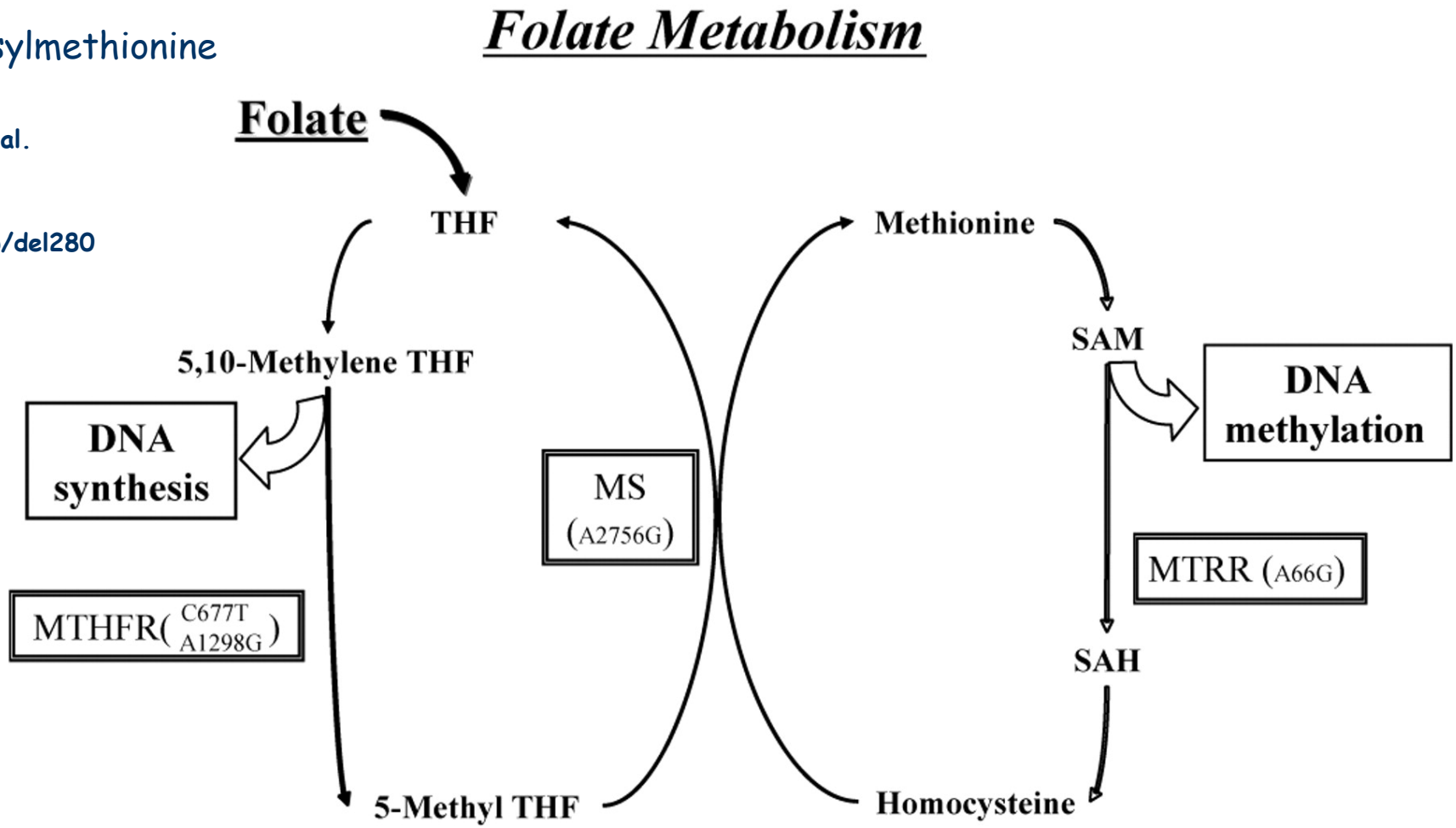
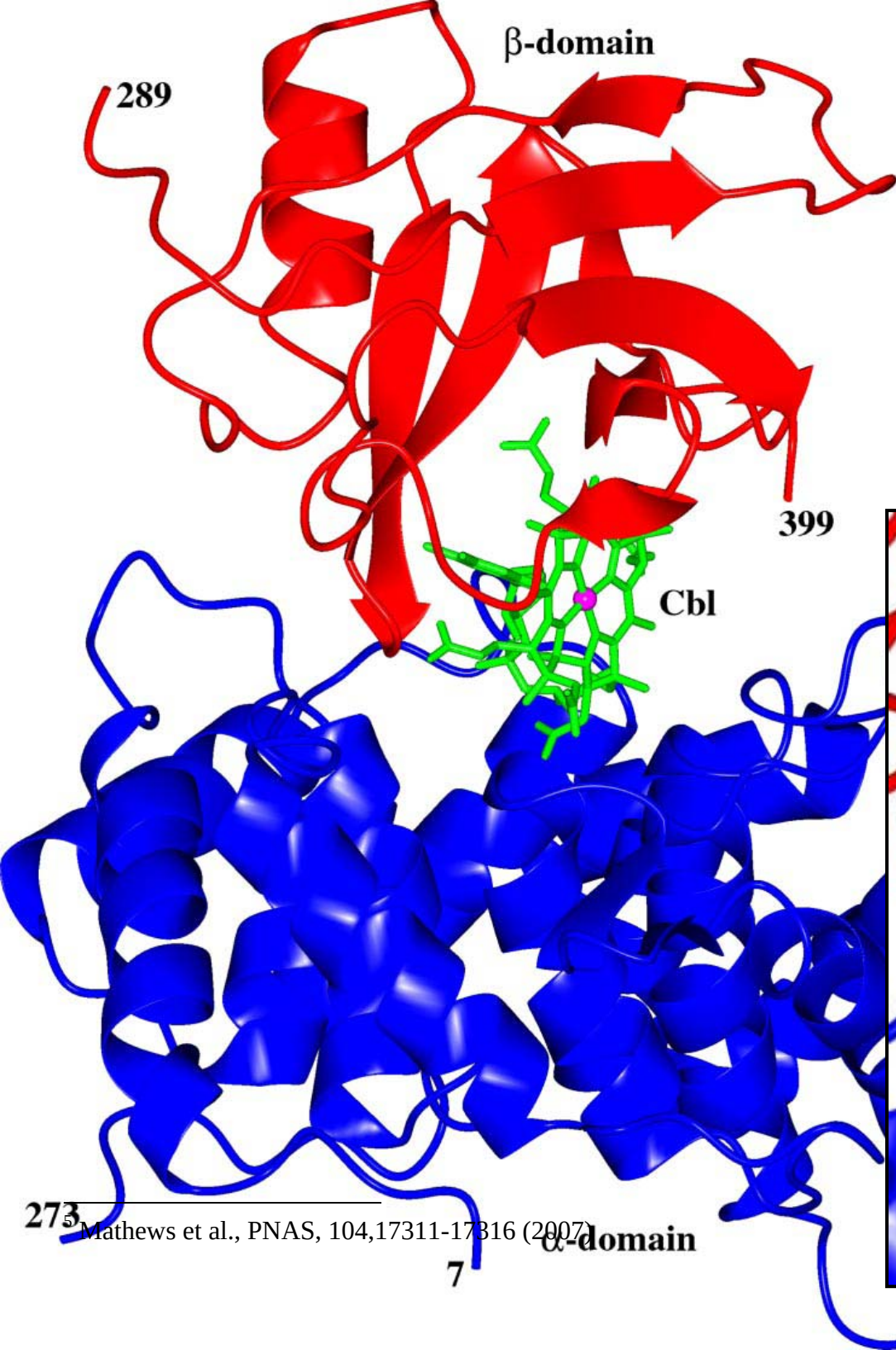
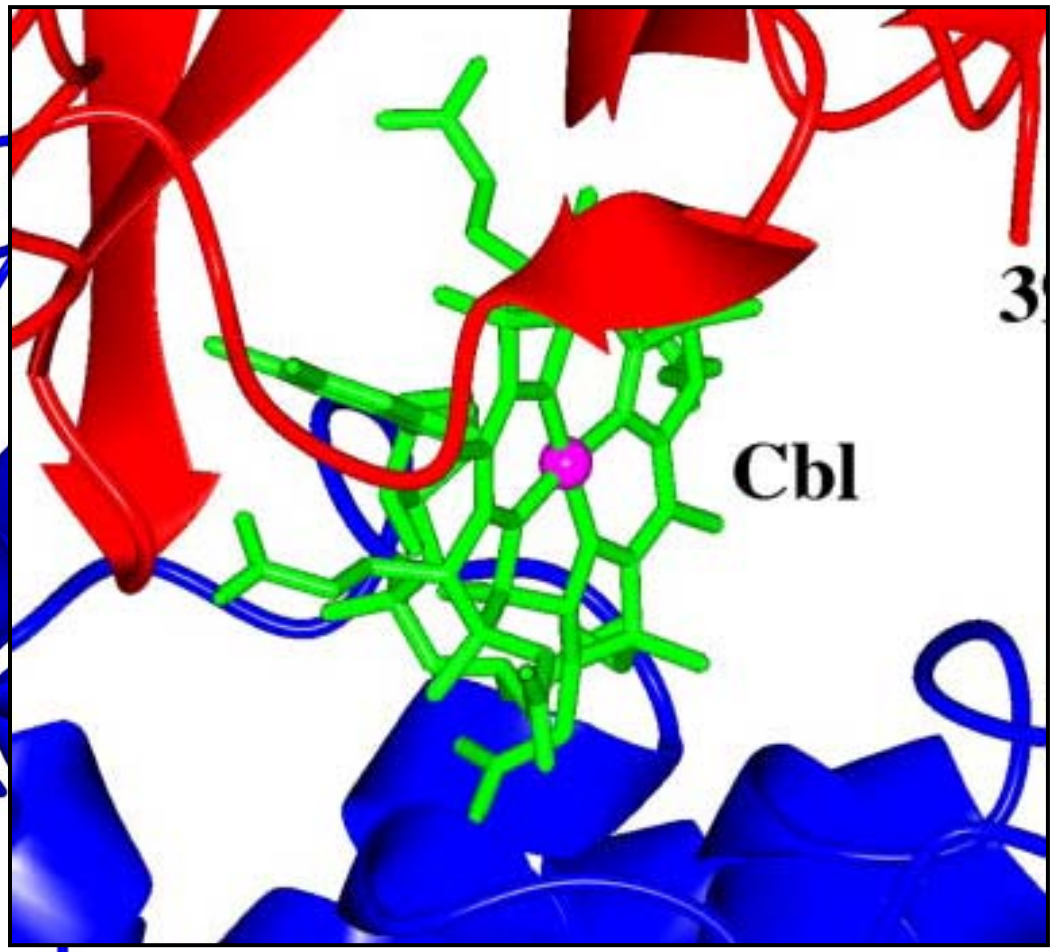
SAM: *S*-adenosylmethionine

From: **Lee, H.-C. et al. Hum. Reprod. 2006 21:3162-3170; doi:10.1093 /humrep/del280**

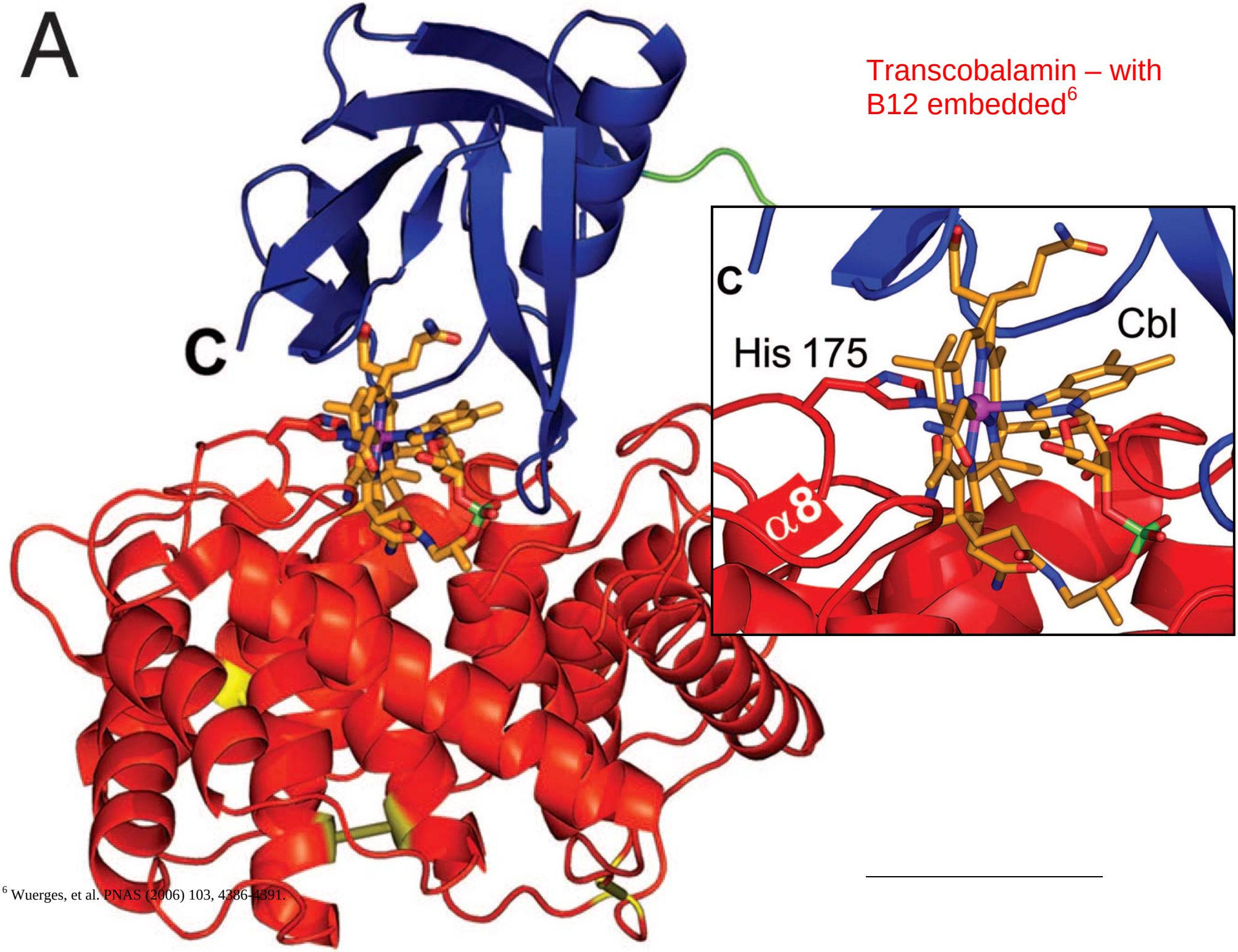




Intrinsic factor with bound B12[5]
notice no 6th position binding of the cobalt, contrast transcobalamin below



[5] Mathews et al., PNAS, 104,17311-17316 (2007)

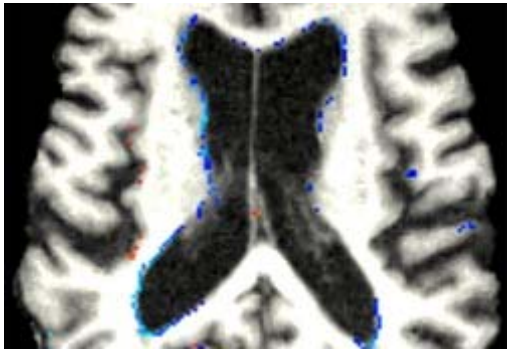


Transcobalamin – with B12 embedded[6]

[6] Wuerges, et al. PNAS (2006) 103, 4386-4391.

Vitamin B12 may protect older brains from shrinking: study

Last Updated: Monday, September 8, 2008 | 6:55 PM CBC News



The blue colour shows where the brain tissue has shrunk by more than 1 mm. (Courtesy David Smith)

Elderly people who have higher levels of vitamin B12 in their blood may gain some protection against brain shrinkage, a new study suggests.

The brain normally shrinks about 0.5 per cent per year in normal elderly brains, compared with 1 per cent in those with mild cognitive impairment and 2 per cent in the same time for those with Alzheimer's, said the study's lead author, pharmacology Prof. David Smith of the University of Oxford in Britain.

In Tuesday's issue of the journal Neurology, Smith and his colleagues report that people in the upper third of vitamin B12 levels were six times less likely to experience brain shrinkage compared with those who had the lowest levels.

All 107 participants in the study were between the ages of 61 and 87 and were not deficient in vitamin B12 based on usual criteria. The subjects had scans to measure the volume of their brains, as well as memory tests and physical exams including blood tests to assess their B12 levels once a year for up to five years.

"We can only advise that it makes good sense to eat plenty of the foods that are a good source of B12, such as fish, milk (low-fat is fine) and meat and, in North America, fortified breakfast cereals," Smith said in an e-mail interview.

The team is carrying out a trial of B vitamins in elderly people with memory imp

'It is the first time that something in the blood which is related to our diet has been shown to be related to brain shrinkage.' *— Prof. David Smith*

airment to see if the vitamins can slow the rate of brain shrinkage.

The results are expected in 2009.

While the latest results suggest that modifying B12 might protect the brain and possibly prevent cognitive decline, the researchers cautioned the findings to date do not prove a benefit.

"Many factors that affect brain health are thought to be out of our control, but this study suggests that simply adjusting our diets …may be something we can easily adjust to prevent brain shrinkage and so perhaps save our memory," agreed study author Anna Vogiatzoglou, also at Oxford.

Smith and Vogiatzoglou used a more accurate way of testing for vitamin B12 compared with previous studies that showed mixed results.

"The result is novel as it is the first time that something in the blood which is related to our diet has been shown to be related to brain shrinkage," Smith said.

Vitamin B12 helps in the formation of red blood cells and is important to help maintain the central nervous system.

A full-blown deficiency of vitamin B12 is rare in developed countries, but elderly people may not absorb it well and vegetarians may not get enough from their diet.

Vitamin B12 deficiency is a serious problem in less developed countries such as India. Deficiency can lead to anemia and neurological damage.

Summary of information about B12 from these notes

1. Vitamin B12 is the only, metal-containing vitamin –
2. The principal cobalamins are:

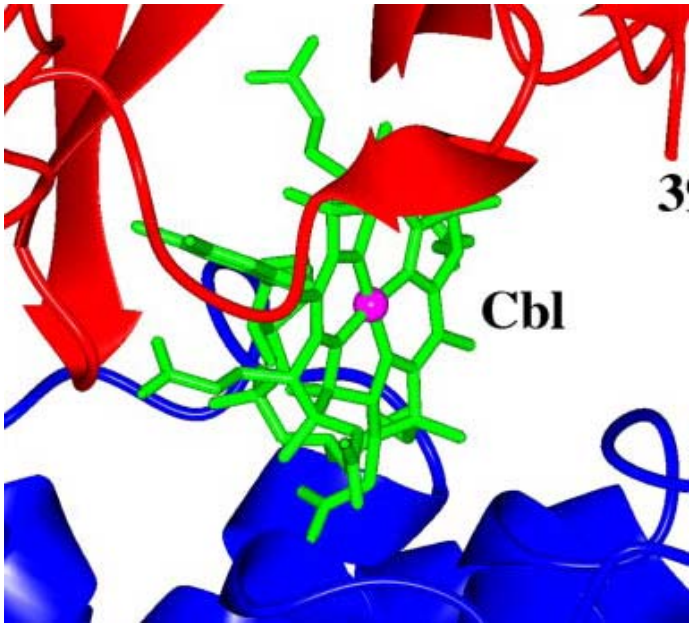
cyanocobalamin, hydroxocobalamin and the two coenzyme forms methylcobalamin and 5-deoxyadenosylcobalamin (adenosylcobalamin).

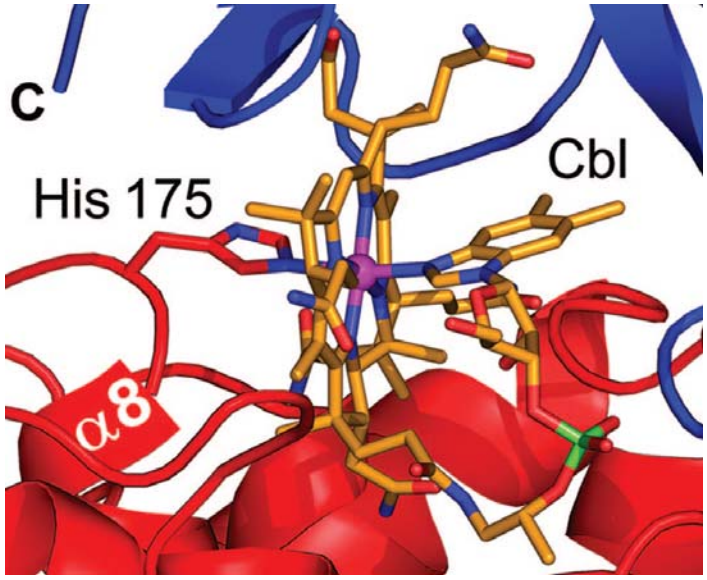
3. Structure: Co-bound to a corrin ring; 5th position is bound a dimethylbenzimidazole –connected via a ribose sugar unit to one of the reduced pyrroles of the corrin ring. The cobalt cycles between Co^{3+}, Co^{2+} and Co^{1+} in its catalytic activity.
4. Called a Coenzyme because: it is a chemical that is required for an enzyme to function –

remembering: Apoenzyme+ Cofactor[7] $\rightarrow$ Holoenzyme

5. Transport: Vitamin B12 binds to a glycoprotein, the intrinsic factor, in the stomach. The vitamin B12-intrinsic factor complex recognises surface receptors of mucosal cells in the ileum[8] and is absorbed. The Cbl molecule is bound at the interface between the α and β domains of IF as shown.5

It is transported around the body bound to transcobalamin (TC) – see RHS .- bottom – all 6 positions occupied now…





[8] The **ileum** is the final section of the small intestine. It is about 2-4 m long in humans, follows the duodenum and jejunum and is separated from the cecum by the ileocecal valve (ICV). The pH in the ileum is usually between 7 and 8 (neutral or slightly alkaline). Its function is mainly to absorb vitamin B12 and bile salts and whatever products of digestion were not absorbed by the jejunum. Ref: Wikipedia.

1.

6. The Cbl molecule is bound to TC between the α and β domains in a manner very similar to that in IF, with its cobalt ion coordinated by the four nitrogen atoms of the corrin ring and by the N3B atom of the dimethylbenzimidazole ring as the fifth ligand on the α side of the corrin ring. The sixth coordination site of the cobalt is occupied by the N ε2 atom of a histidine side chain, at position 175 in TC.

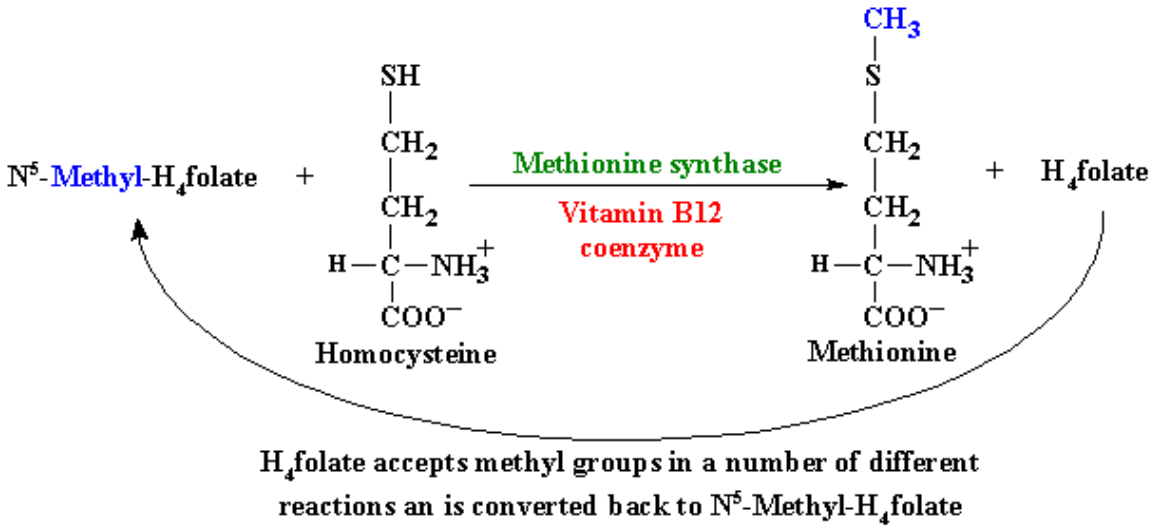
7. Vitamin B12 (cobalamin) derived cofactors are used for two important reactions in humans:

Methylmalonyl CoA mutase requires 5-deoxyadenosylcobalamin (known as adenosylcobalamin) - Mutase= carbon chain rearrangement – shifts CH_3's about. – involved in fat metabolism.

Methionine synthase (cofactor methylcobalamin) catalyses the conversion of homocysteine to methionine and tetrahydrofolate is also formed) – this is a Methyl Transferase (MTA) – important in production of red blood cells. The second reaction taking Methionine back to Homocysteine (see last slide) is catalysed by Methionine Synthase Reductase (cofactor adenosylcobalamin) and forms S-adenosylmethionine (SAM) – SAMs control myelin sheath covering of nerves – this deficiency leads to neurological problems – depression – because of neural problems.

Adenosylcobalamin (B12) is a coenzyme in the reaction involved in methionine metabolism using methionine synthase reductase (MSR). In the MS reaction – CH_3-cobalamin is the cofactor.

8. Vitamin B12 works closely with vitamin B9 (folate) to regulate the formation of red blood cells and to assist in the function of iron, and in the production of DNA and RNA, and in the maintenance of the central nervous system and a vital role in the metabolism of fatty acids essential for the maintenance of myelin.



Expectations from this unit	
1	Coenzyme B12 is based on the Co(III)-corrin ring with a 5'-deoxyadenosyl group bound to the Co(III).
2	Many reactions involve moving a CH_3 group around – called mutase reactions. Reactions involve adding a CH_3 group (methyl transferase - MTA) include the formation of methionine from homocysteine.
3	Other MTA reactions involve addition of CH_3^- to Hg^{2+} in anaerobic bacteria. B12 deficiency reduces folic acid so setting off folic-acid deficiency symptoms.
4	Key features – the vitamin taken as a pill has a CN^- as the 6^{th} position. This is replaced by the 5'-deoxyadenosyl group in the coenzyme (one of the active form of the molecules (in mutases)). Coenzymes bind to an apo-enzyme to form a holoenzyme that is biologically active. The Co changes between Co(I), Co(II), and Co(III) in its reactions.

Study questions from the lectures to date and from the books (S-L; R-M; K-S)	
Lectures	
L-B	**See p 336-343.**
R-M	
K-S	**Ch 3 – p 39-54**