

# Coenzymes and prosthetic groups

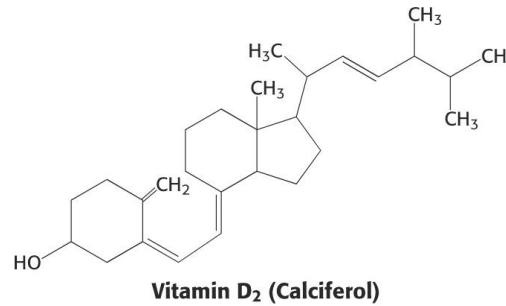
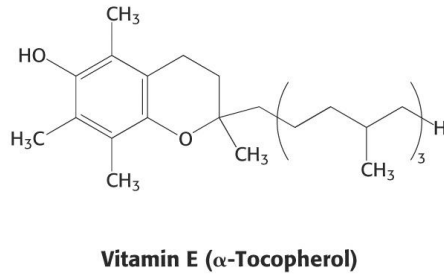
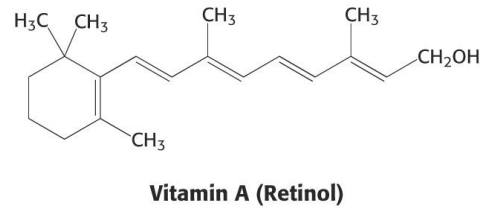
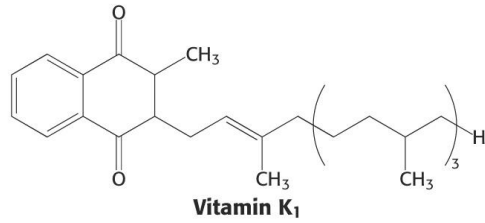
# Nomenclature

- Cofactor: nonprotein component of enzymes
- Cofactor - a co-catalyst required for enzyme activity
- Coenzyme - a dissociable cofactor, usually organic
- Prosthetic group - non-dissociable cofactor
- Vitamin - a required micro-nutrient (organism cannot synthesize adequate quantities for normal health - may vary during life-cycle).
  - water soluble - not stored, generally no problem with overdose
  - lipid soluble - stored, often toxic with overdose.
- Apoenzyme - enzyme lacking cofactor (inactive)
- Holoenzyme - enzyme with cofactors (active)

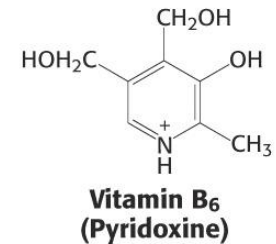
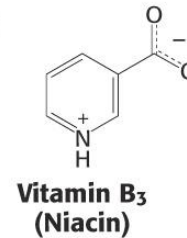
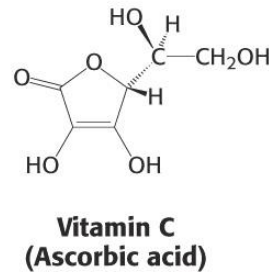
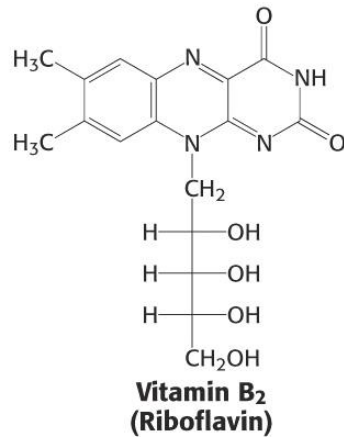
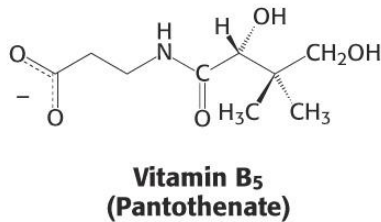
# Vitamins are precursors of cofactors

**TABLE 8.9** Water-Soluble Vitamins

Vitamin	Coenzyme	Typical reaction type	Consequences of deficiency
Thiamine (B <sub>1</sub> )	Thiamine pyrophosphate	Aldehyde transfer	Beriberi (weight loss, heart problems, neurological dysfunction)
Riboflavin (B <sub>2</sub> )	Flavin adenine dinucleotide (FAD)	Oxidation–reduction	Cheliosis and angular stomatitis (lesions of the mouth), dermatitis
Pyridoxine (B <sub>6</sub> )	Pyridoxal phosphate	Group transfer to or from amino acids	Depression, confusion, convulsions
Nicotinic acid (niacin)	Nicotinamide adenine dinucleotide (NAD <sup>+</sup> )	Oxidation–reduction	Pellagra (dermatitis, depression, diarrhea)
Pantothenic acid	Coenzyme A	Acyl–group transfer	Hypertension
Biotin	Biotin–lysine complexes (biocytin)	ATP-dependent carboxylation and carboxyl–group transfer	Rash about the eyebrows, muscle pain, fatigue (rare)
Folic acid	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis	Anemia, neural-tube defects in development
B <sub>12</sub>	5'-Deoxyadenosyl cobalamin	Transfer of methyl groups; intramolecular rearrangements	Anemia, pernicious anemia, methylmalonic acidosis
C (ascorbic acid)		Antioxidant	Scurvy (swollen and bleeding gums, subdermal hemorrhages)



# Why cofactors?



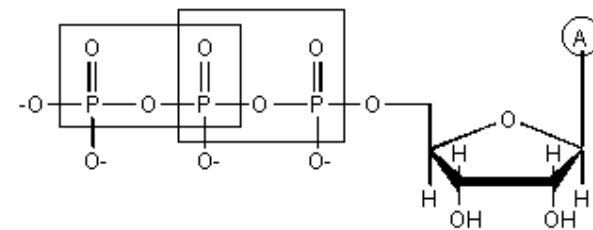
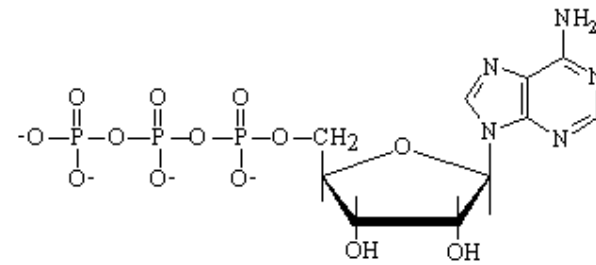
# Adenine Nucleotide Coenzymes

**All use the adenine nucleotide group  
solely for binding to the enzyme!**

- pyridine dinucleotides (NADH, NADPH)
- flavin mono- and dinucleotides (FMN, FADH)
- **coenzyme A**

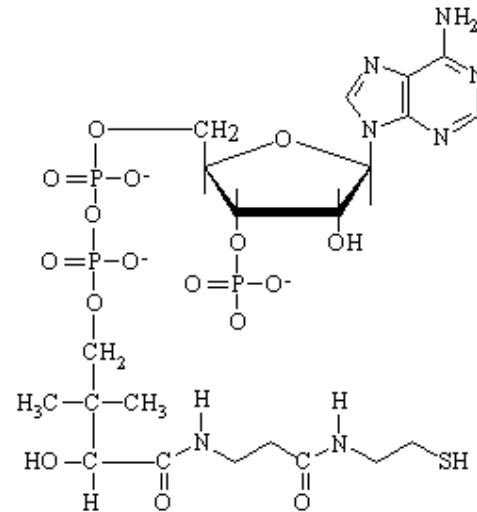
# Nucleotide triphosphates

- ATP hydrolysis
  - resonance stabilizes products
  - reactants cannot be resonance stabilized because of competition with adjacent bridging anhydrides
  - charge density greater on reactants than products

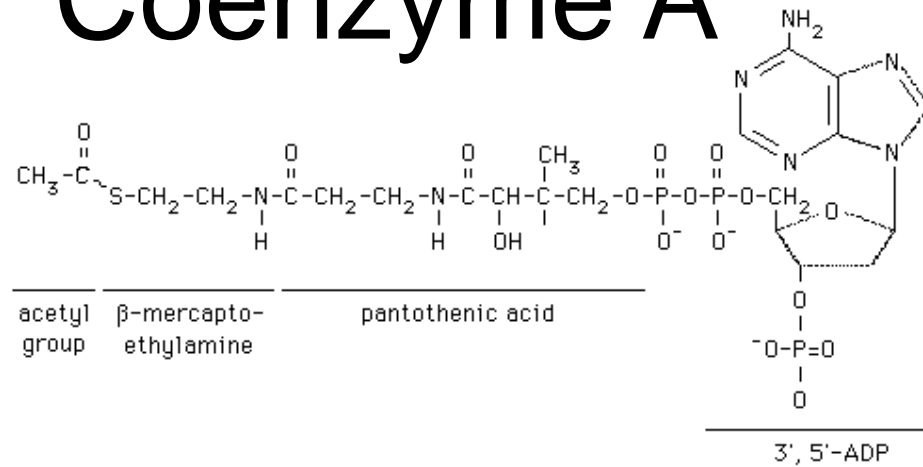


# Coenzyme A

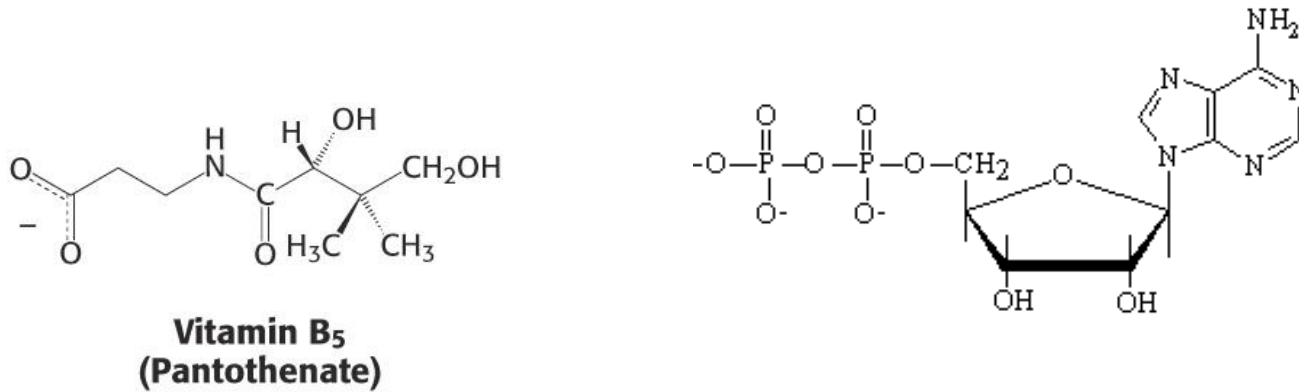
- Activation of acyl groups for transfer by nucleophilic attack
- activation of the alpha-hydrogen of the acyl group for abstraction as a proton
- Both these functions are mediated by the reactive -SH group on CoA, which forms thioesters



# Coenzyme A



Acetyl coenzyme A, showing its constituents

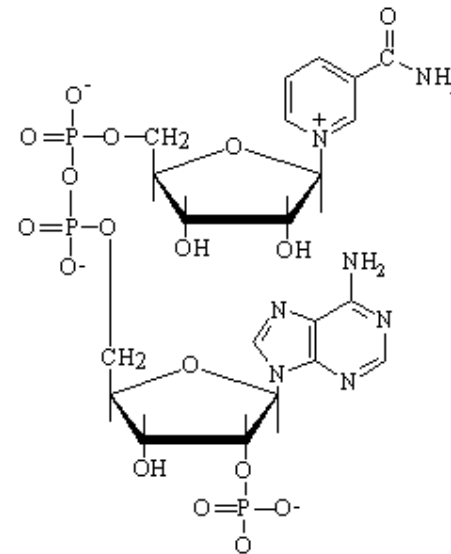
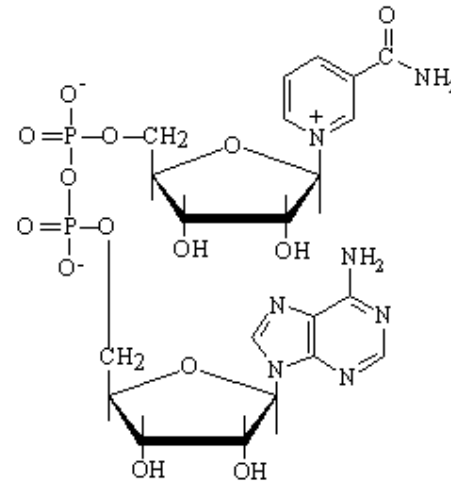


# Nicotinic Acid/Nicotinamide Coenzymes

- These coenzymes are two-electron carriers
- They transfer hydride anion ( $\text{H}^-$ ) to and from substrates
- Two important coenzymes in this class:
- Nicotinamide adenine dinucleotide ( $\text{NAD}^+$ )
- Nicotinamide adenine dinucleotide phosphate ( $\text{NADP}^+$ )

# NAD, NADP

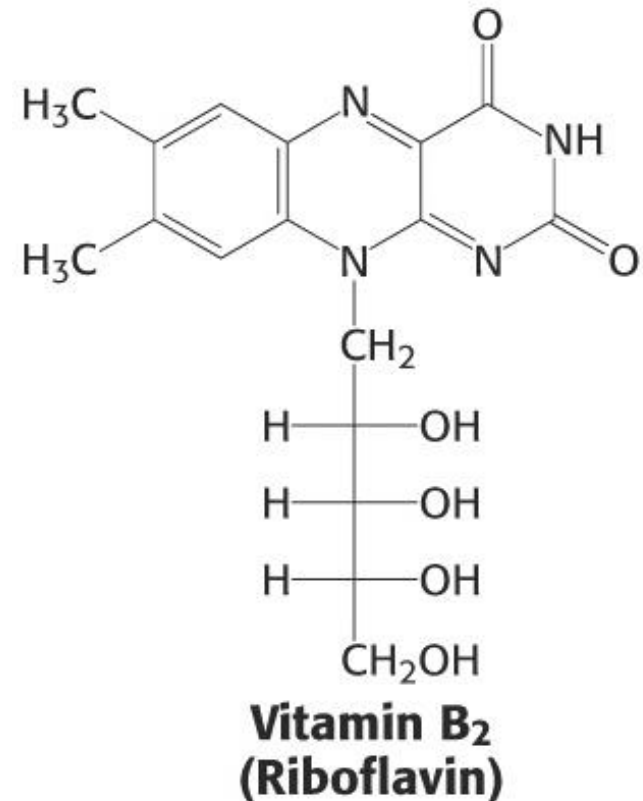
- The quaternary nitrogen of the nicotinamide ring acts as an electron sink to facilitate hydride transfer
- The site (on the nicotinamide ring) of hydride transfer is a prochiral center!
- Hydride transfer is always stereospecific!



# Riboflavin and the Flavins

## Vitamin B2

- All these substances contain ribitol and a flavin or isoalloxazine ring
- Active forms are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)
- FMN is not a true nucleotide
- FAD is not a dinucleotide
- But the names are traditional and they persist!



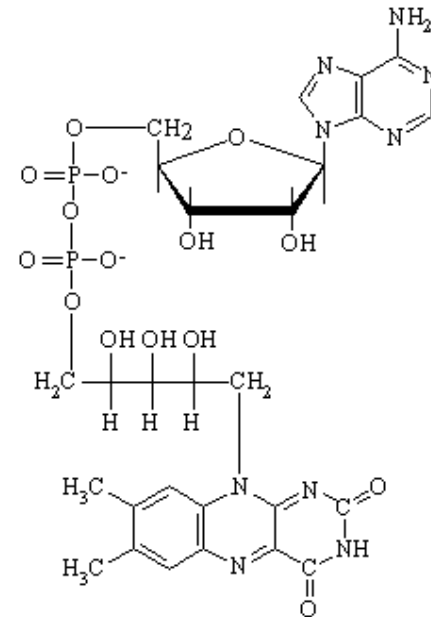
# Flavin Mechanisms

**Flavins are one- or two-electron transfer agents**

- Name "flavin" comes from Latin flavus for "yellow"
- The oxidized form is yellow, semiquinones are blue or red and the reduced form is colorless

# Flavin adenine dinucleotide

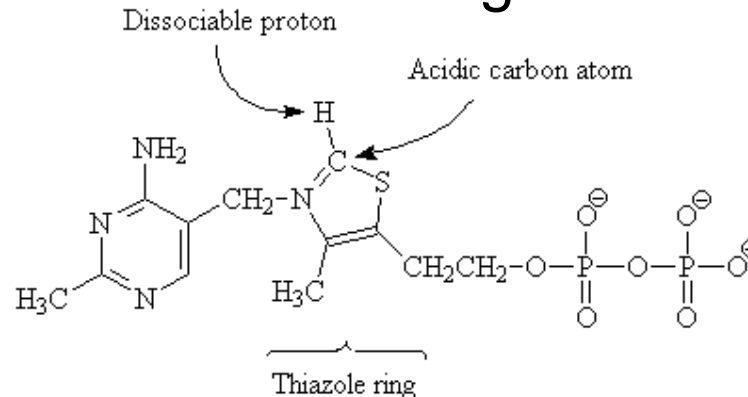
- FAD



# Thiamine pyrophosphate

## Vitamin B1

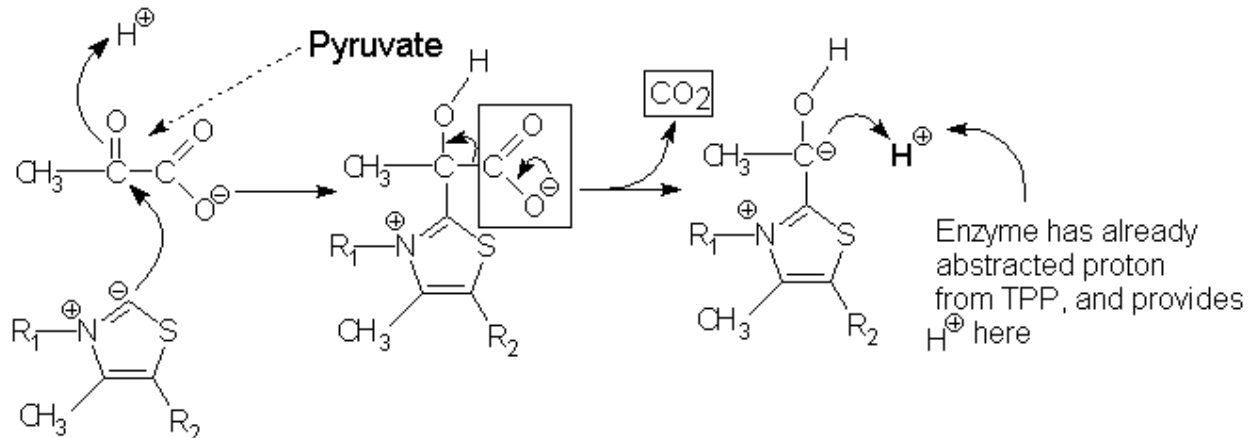
- Thiamine - a thiazole ring joined to a substituted pyrimidine by a methylene bridge
- Thiamine-PP is the active form
- TPP is involved in carbohydrate metabolism
- Catalyzes decarboxylations of  $\alpha$ -keto acids and the formation and cleavage of  $\alpha$ -hydroxyketones



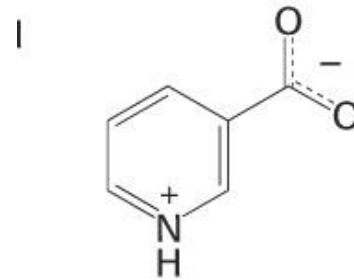
**THIAMINE PYROPHOSPHATE (TPP)**

# Thiamine pyrophosphate TPP

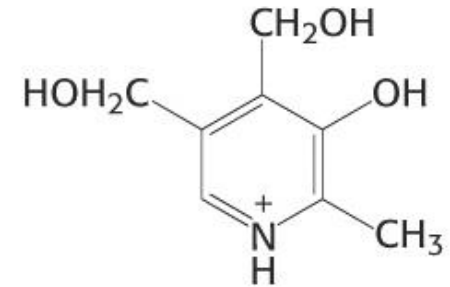
- Yeast pyruvate decarboxylase, acetolactate synthase, transketolase, phosphoketolase
- All these reactions depend on accumulation of negative charge on the carbonyl carbon at which cleavage occurs!
- Thiamine pyrophosphate facilitates these reactions by stabilizing this negative charge
- The key is the quaternary nitrogen of the thiazolium group
  - provides electrostatic stabilization of the carbanion formed by removal of the C-2 proton
  - acts as an electron sink via resonance interactions



- Vitamin B3
- Vitamin B6
  - Catalyzes reactions involving amino acids
  - Transaminations, decarboxylations, eliminations, racemizations and aldol reactions

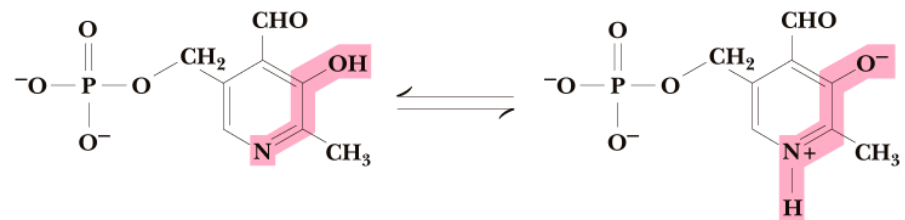


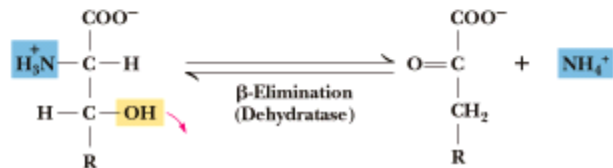
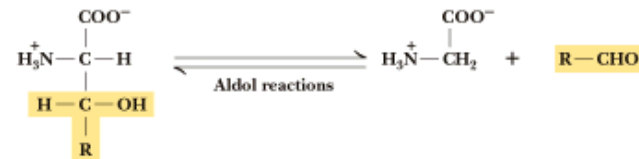
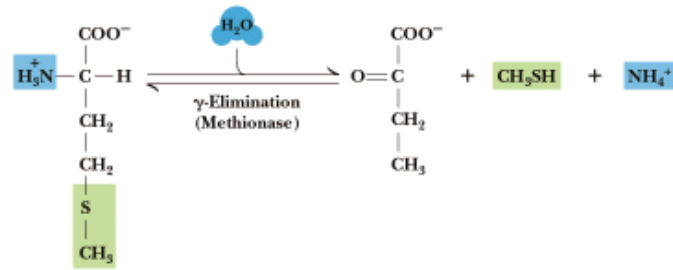
**Vitamin B<sub>3</sub>**  
**(Niacin)**

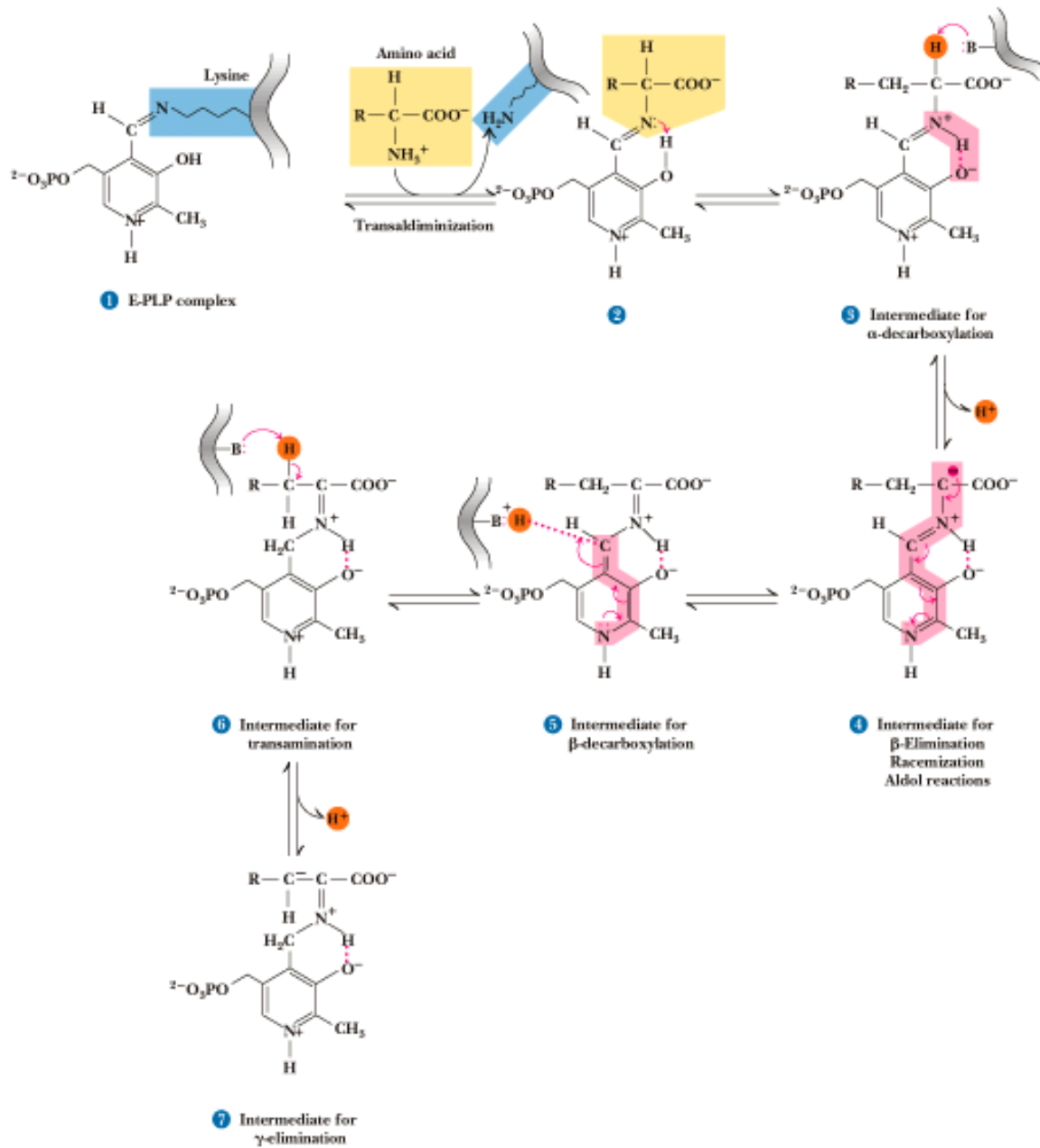


**Vitamin B<sub>6</sub>**  
**(Pyridoxine)**

- formation of stable Schiff base adducts
- a conjugated electron sink system that stabilizes reaction intermediates



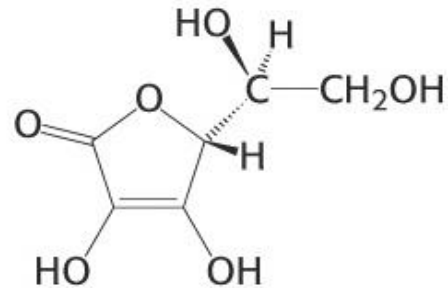




# Ascorbic Acid

- Vitamin C

- Most plants and animals make ascorbic acid - for them it is not a vitamin
- Only a few vertebrates - man, primates, guinea pigs, fruit-eating bats and some fish (rainbow trout, carp and Coho salmon) cannot make it!
- Vitamin C is a reasonably strong reducing agent
- It functions as an electron carrier



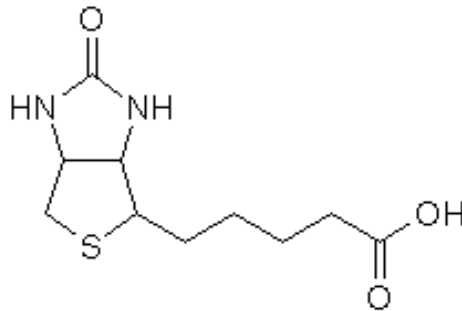
**Vitamin C**  
**(Ascorbic acid)**

- Hydroxylations of proline and lysine (collagen)
- Metabolism of Tyr in brain
- Fe mobilization from spleen
- May prevent the toxic effects of some metals
- Ameliorates allergic responses
- Can stimulate the immune system

# Biotin

## “chemistry on a tether”

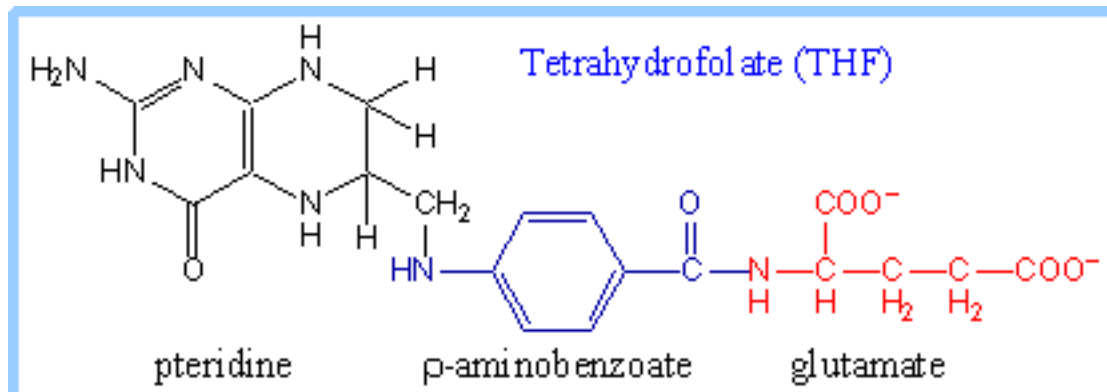
- Mobile carboxyl group carrier
- Bound covalently to a lysine
- The biotin-lysine conjugate is called biocytin
- The biotin ring system is thus tethered to the protein by a long, flexible chain
- Whenever you see a carboxylation that requires ATP and CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup>, think biotin!
- Activation by ATP involves formation of carbonyl phosphate (aka carboxyl phosphate)
- Carboxyl group is transferred to biotin to form N-carboxy-biotin
- The "tether" allows the carboxyl group to be shuttled from the carboxylase subunit to the transcarboxylase subunit of ACC-carboxylase

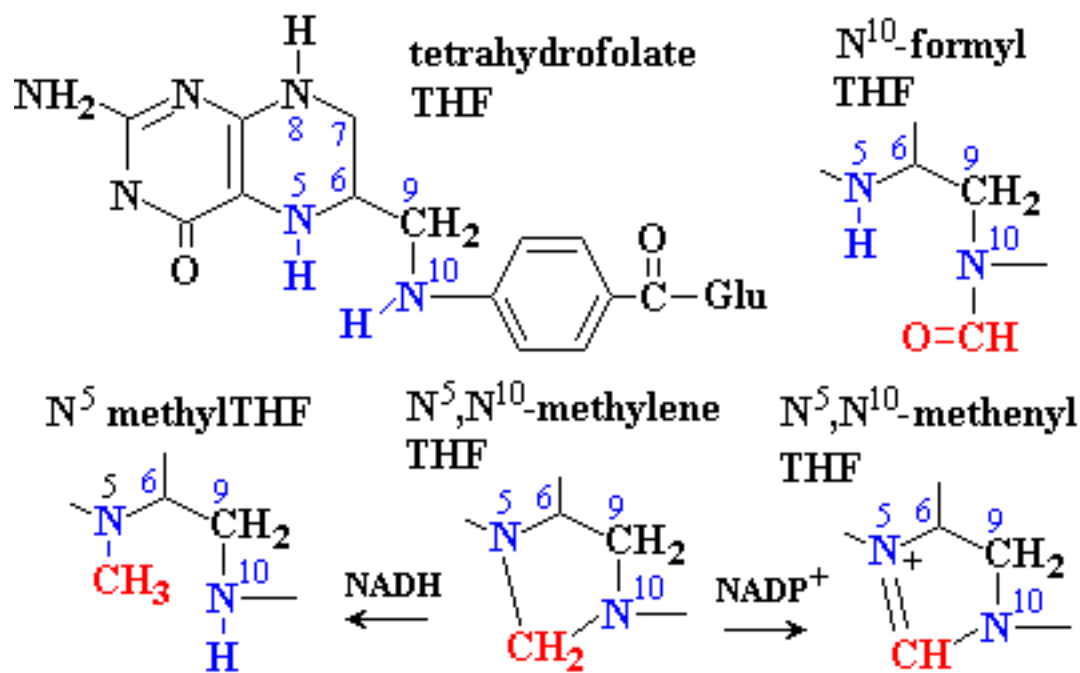


# Folic Acid

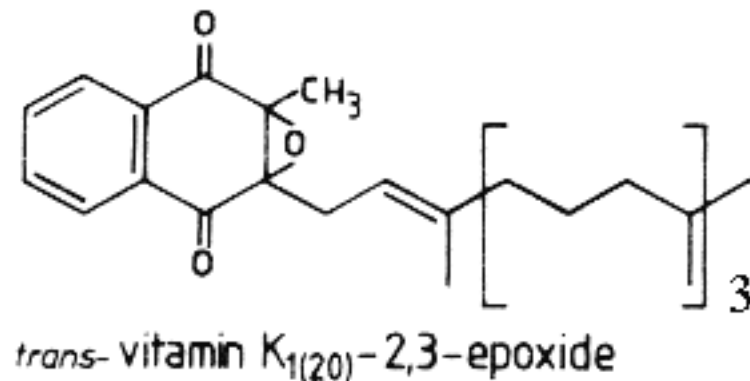
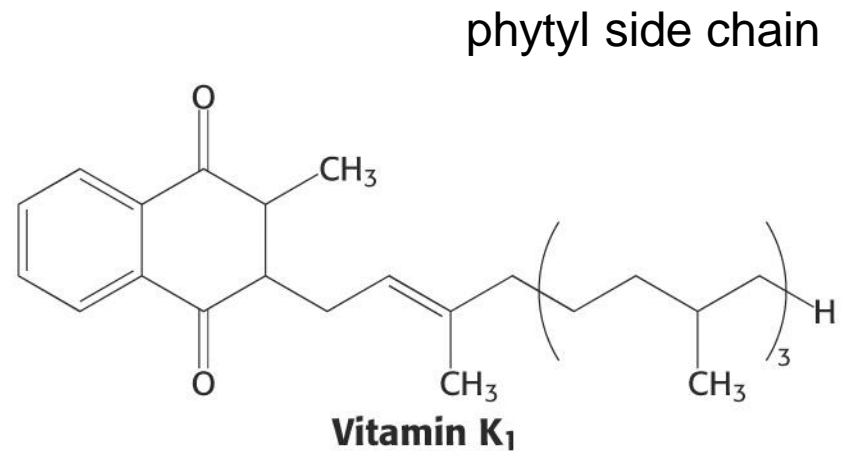
**Folates are donors of 1-C units for all oxidation levels of carbon except that of CO<sub>2</sub>**

- Active form is tetrahydrofolate (THF)
- THF is formed by two successive reductions of folate by dihydrofolate reductase

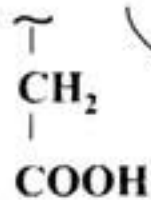




- Vitamin K
  - essential for blood clotting
- Carboxylation of 10 Glu on prothrombin ( $\gamma$  carboxy-Glu) is catalyzed by a vitamin K-dependent enzyme, liver microsomal glutamyl carboxylase
- Extra carboxyl enables calcium binding

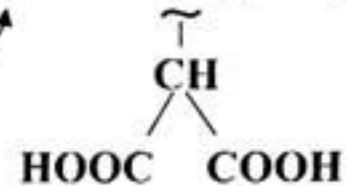


Prothrombin precursor (Glu)

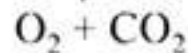


**PIVKA-II**

Native prothrombin (Gla)



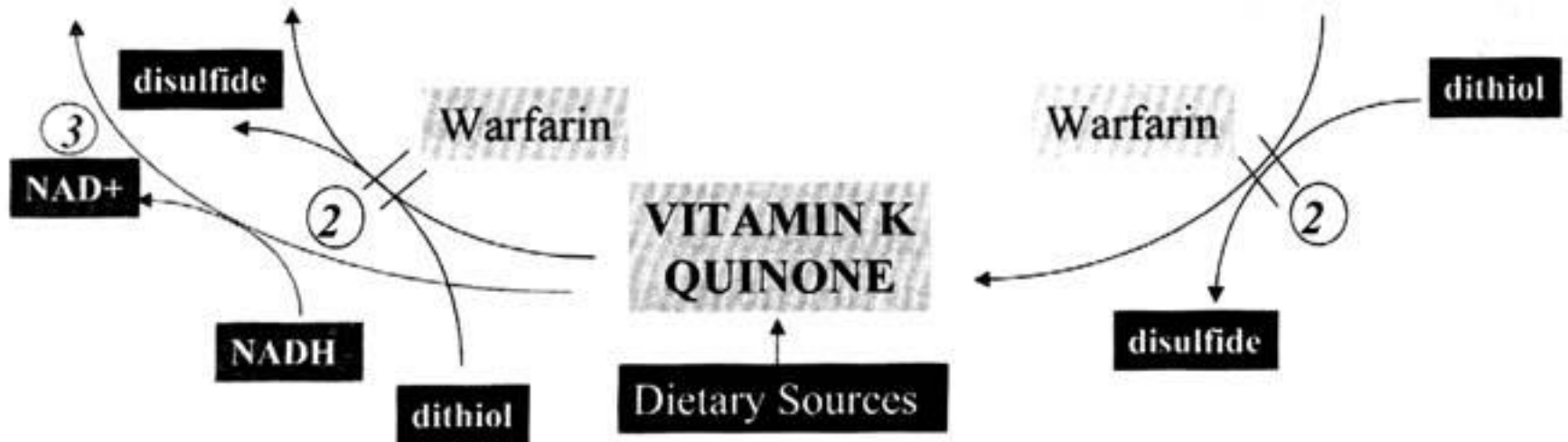
①



**VITAMIN K  
QUINOL**

- ① Vitamin K  $\gamma$ - glutamyl carboxylase
- ② Vitamin K epoxide reductase
- ③ Vitamin K reductase

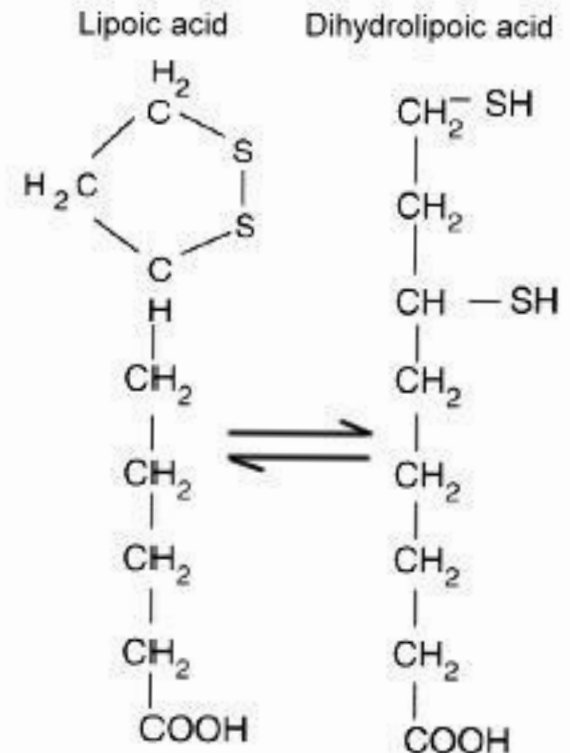
**VITAMIN K  
EPOXIDE**



# Lipoic Acid

## Another example of "chemistry on a tether"!

- Lipoic acid, like biotin, is a ring on a chain and is linked to a lysine on its protein
- Lipoic acid is an acyl group carrier
- Found in pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase
- Lipoic acid functions to couple acyl-group transfer and electron transfer during oxidation and decarboxylation of  $\alpha$ -keto acids



# Retinol

## Vitamin A

- Retinol-binding proteins (RBPs) help to mobilize and transport vitamin A and its derivatives
- Retinol is converted to retinal in the retina of the eye and is linked to opsin to form rhodopsin, a light-sensitive pigment protein in the rods and cones
- Vitamin A also affects growth and differentiation

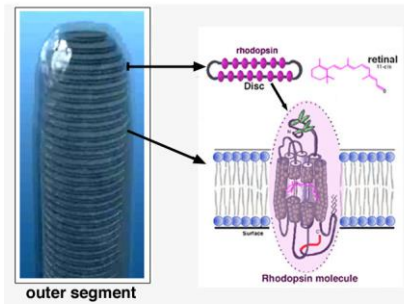
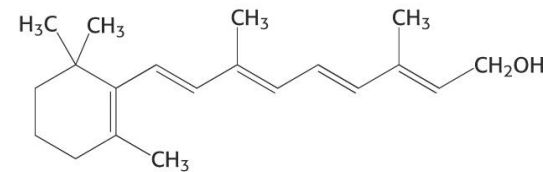
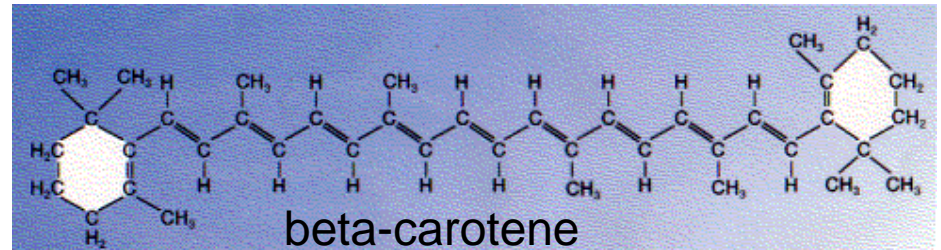
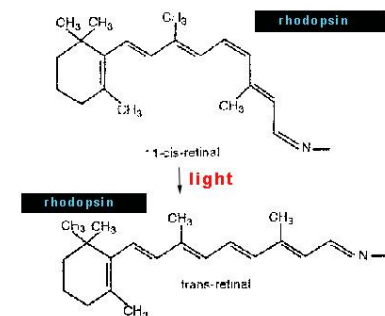
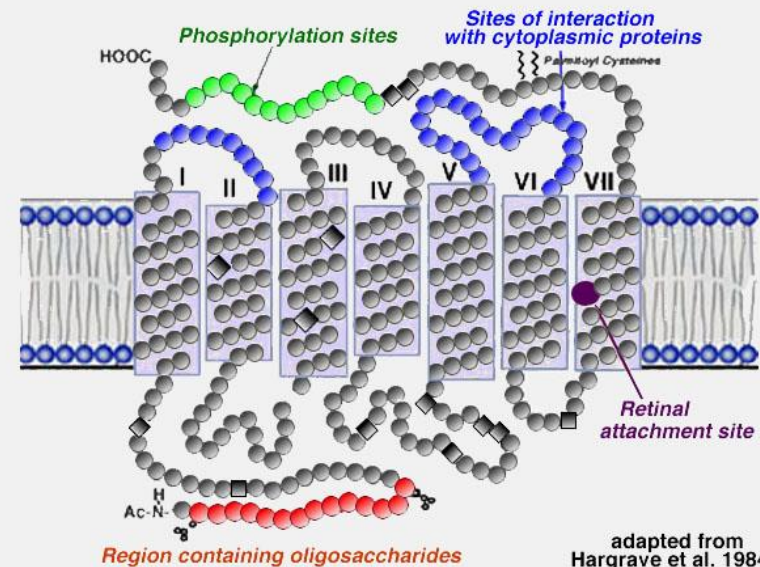
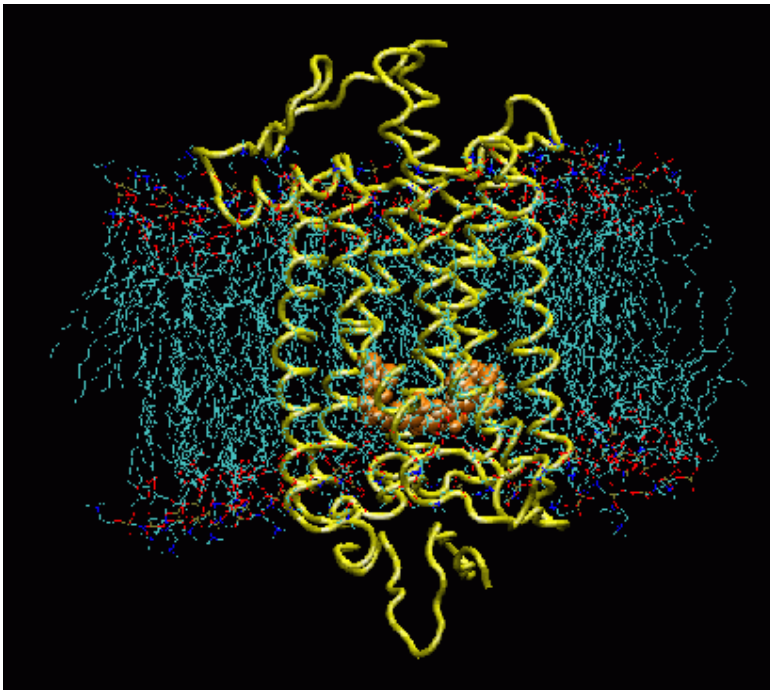
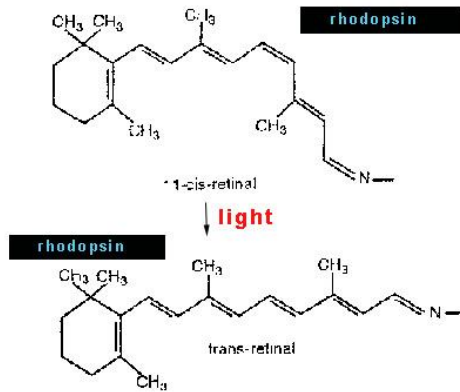


Fig 8. Schematic diagram of Rhodopsin in the outer segment discs.



# Retinal in rhodopsin

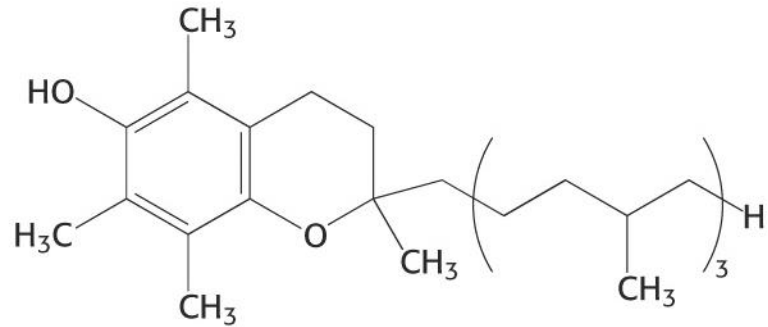


adapted from Hargrave et al. 1984 Plantanida, 1991

Fig. 9. Structural model of rhodopsin showing seven transmembrane components and the attachment site for retinal.

# Tocopherol

- Vitamin E
  - Potent antioxidant
  - Molecular details are almost entirely unknown
  - May prevent membrane oxidations

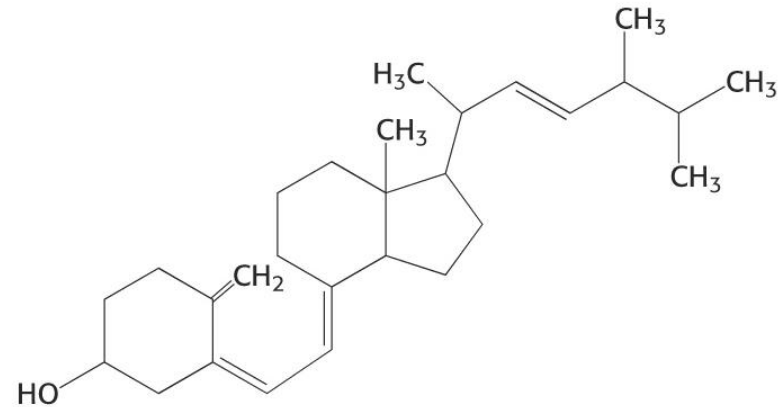


**Vitamin E ( $\alpha$ -Tocopherol)**

# Calciferol

- Vitamin D

- Cholecalciferol is made in the skin by the action of UV light on 7-dehydrocholesterol
- Major circulating form is 25-hydroxyvitamin D
- 1,25-dihydroxycholecalciferol (1,25-dihydroxyvitamin D<sub>3</sub>) is the most active form



Vitamin D<sub>2</sub> (Calciferol)

- regulates calcium homeostasis
- role in phosphorus homeostasis

# Metal cofactors

- Single metal sites
  - mostly structural sites  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$
  - exceptions  $\text{Cu}^{2+}$
- Metal clusters
  - Fe,S ( $\text{Fe}_4\text{S}_4$ ,  $\text{Fe}_2$ )
  - FeMoCo
  - $\text{Mn}_4$ ,  $\text{Mn}_2$ ,  $\text{Cu}_2$ , mixed metal clusters
- Organometallic cofactors
  - Porphyrins
  - Cobalamin

# Metal chelation by amino acids

Ligands are determined by electronic affinity and geometrical constraints

Small, “hard” metals prefer “hard” ligands

e.g.  $\text{Ca}^{2+}$  ---  $-\text{OOC}-\text{R}$  (Asp, Glu)

Large “soft” metals prefer “soft” ligands

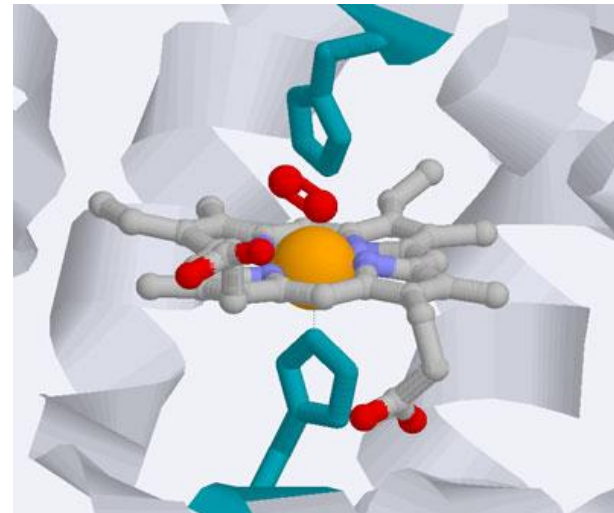
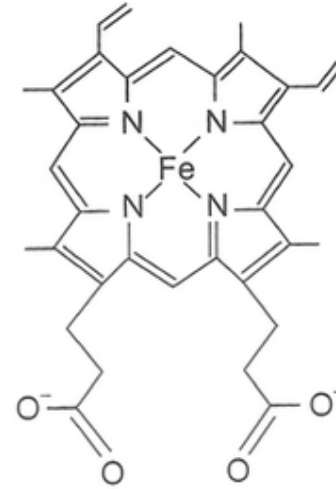
e.g.  $\text{Hg}^{2+}$  ---  $\text{S}-\text{R}$  (Cys)

Iron and copper in between

e.g.  $\text{Fe}^{2+}$  ---  $\text{N}<$  (His)

# Heme iron complexes

- porphyrin (pyrrole) ring
- iron prefers hexacoordination
- 5th coordinate position protein amino acid (usually His)
- 6th coordinate substrate binding or protein binding





# Cobalamin (B12)

- B12 is converted into two coenzymes in the body
  - 5'-deoxyadenosylcobalamin
  - methylcobalamin
- Catalyzes three reaction types
  - Intramolecular rearrangements
  - Reductions of ribonucleotides to deoxyribonucleotides
  - Methyl group transfers (assisted by tetrahydrofolate)
- B12 X-ray structure in 1961 by Dorothy Hodgkin - at the time it was the most complicated structure ever elucidated by X-ray diffraction and she won a Nobel prize
- Cobalamin is needed in the maturation of red blood cells and is used in carbohydrate metabolism and DNA synthesis
- Only found in animal products...not made by plants!

# Cobalamin

