CATALYSIS

FUNCTIONAL GROUPS IN CATALYSIS

- **Not all enzymes rely on their active site for catalysis (chymotrypsin vs. conjugated enzymes)**
- **Cojugated: coenzymes, metal ions, & metallocoenzymes**

A. Functional Groups on Amino Acid Side Chains:

- **Almost all polar amino acids (nucleophilic catalysis)**
- **Ser, Cys, Lys, & His can participate in covalent catalysis**
- **Histidine: pKa, physiological pH & acid–base catalysis**

B. Coenzymes in Catalysis

- **Usually (but not always) synthesized from vitamins**
- **Each coenzyme is specific for a type of reaction**
- **They are either:**
  - *** Activation-transfer coenzymes**
  - ***Oxidation–reduction coenzymes**

Enzyme cofactors

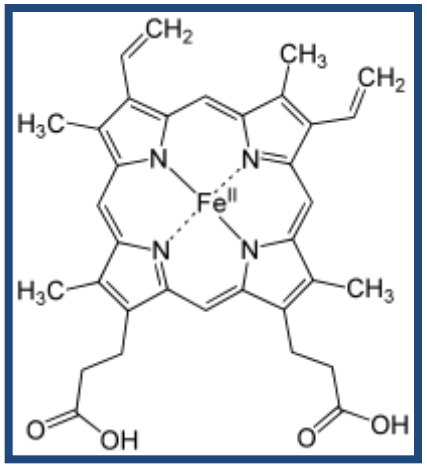
- **Apoenzyme vs. holoenzyme**
- **Organic (coenzymes)**
- **Prosthetic groups (tightly bound) vs. co-substrates (loosely bound)**

Enzymes

Cofactors

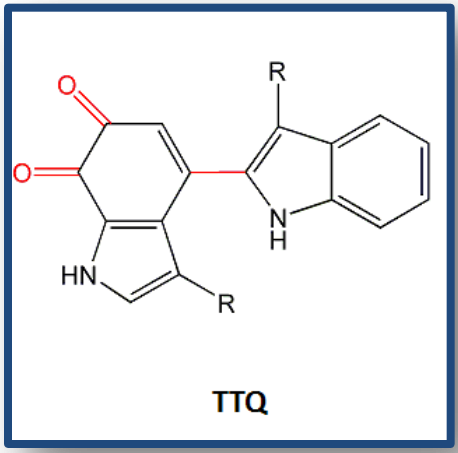
Metal | Organic | Organometallic | **Protein-derived**

Heme

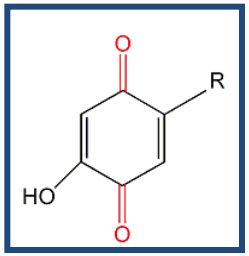


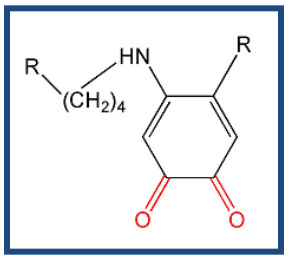
Cross-linked amino acid | Stable free radicals | **Quinones**

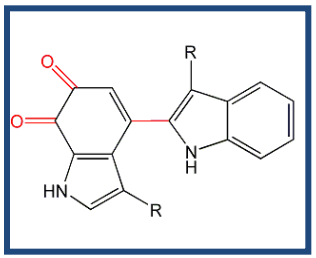
Tryptophan-tryptophyl quinone

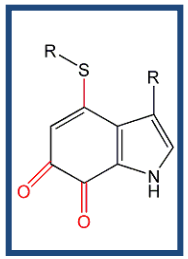


TPQ | LTQ | **TTQ** | CTQ









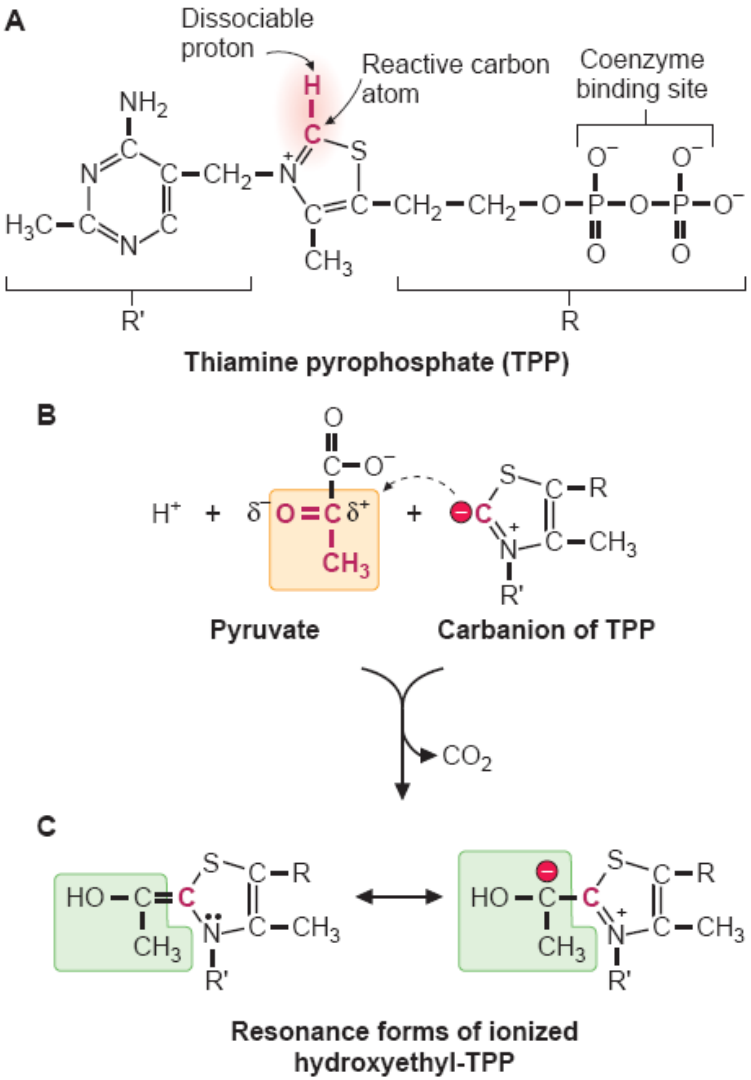
ACTIVATION-TRANSFER COENZYMES

- **Usually participate directly in catalysis by forming a covalent bond**

- **Characteristics:**
  - **Two groups in the coenzyme:**
    - **Forms a covalent bond (functional group)**
    - **Binds tightly to the enzyme (binding group)**
  - **Dependence on the enzyme for additional specificity of substrate & additional catalytic power**

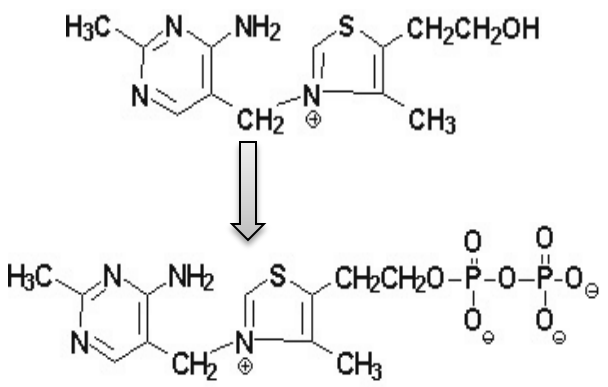
ACTIVATION-TRANSFER COENZYMES
1 - TPP

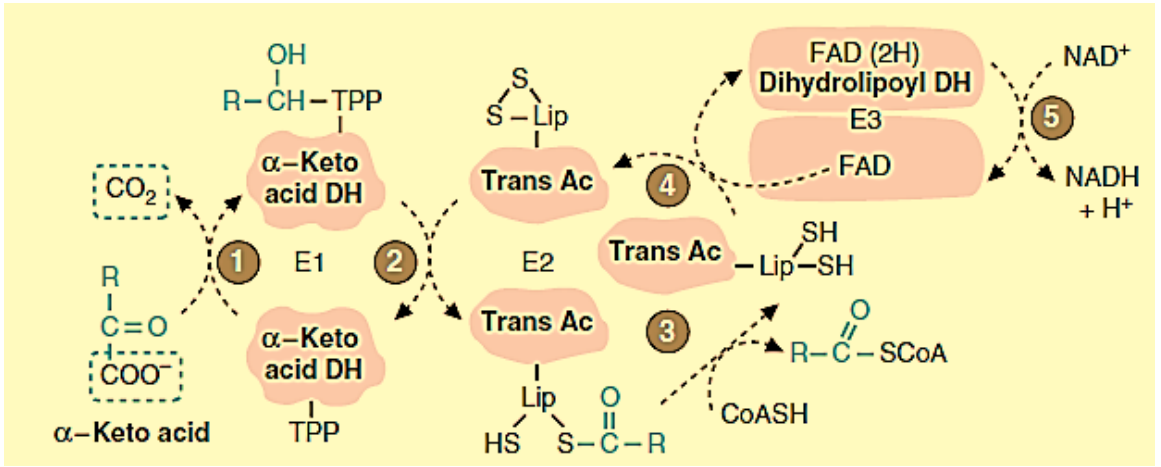
- **Thiamine pyrophosphate**
- **Source: thiamine (B1)**
- **Decarboxylation reactions**
- **Pyrophosphate:**
  - **Provides negatively charged oxygen atoms**
  - **Chelate Mg^{2+} (tight binding)**
  - **Functional group (reactive carbon atom)**
- **Reactive thiamine carbon forms a covalent bond with a substrate keto group while cleaving the adjacent carbon–carbon bond**

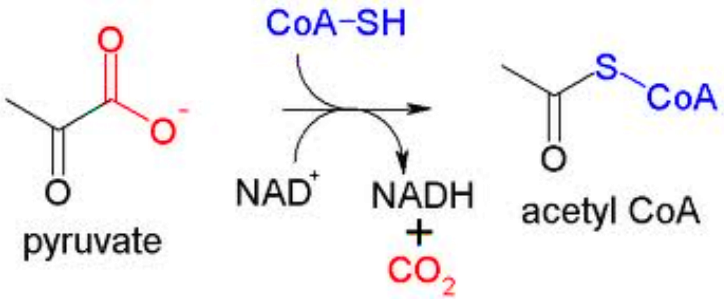


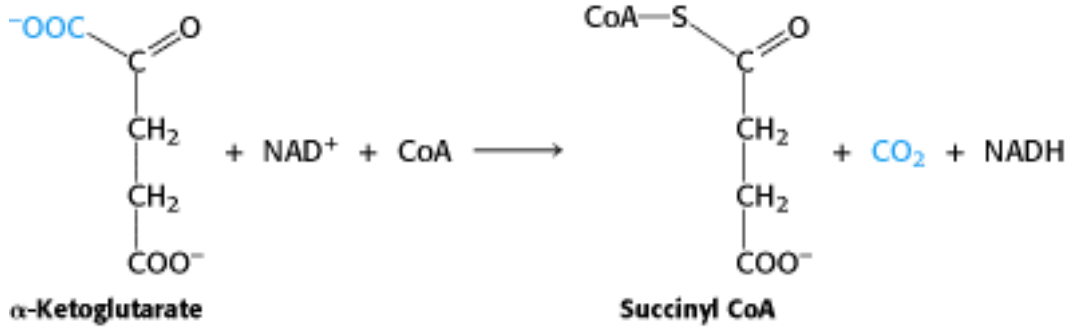
Thiamin (vitamin B1)

- **Thiamin (vitamin B1) is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver**
- **Required by pyruvate dehydrogenase & α-ketoglutarate dehydrogenase**





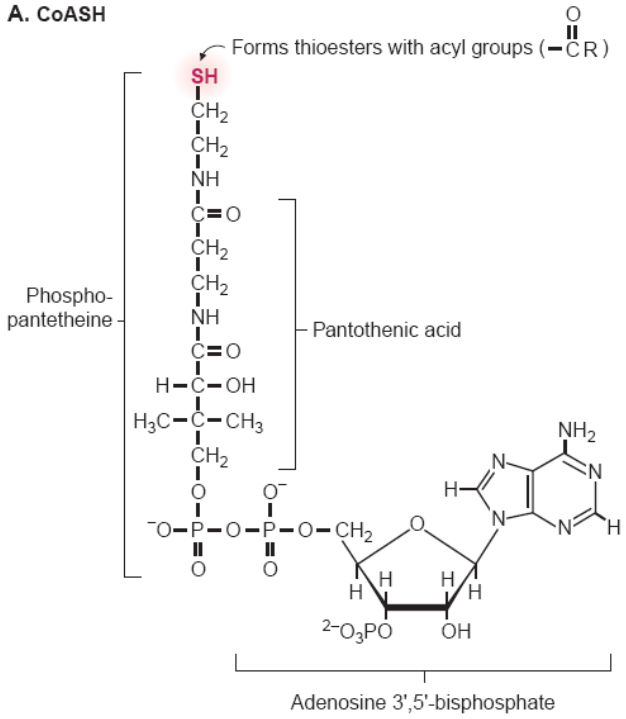




ACTIVATION-TRANSFER COENZYMES
2 - Coenzyme A (CoA)

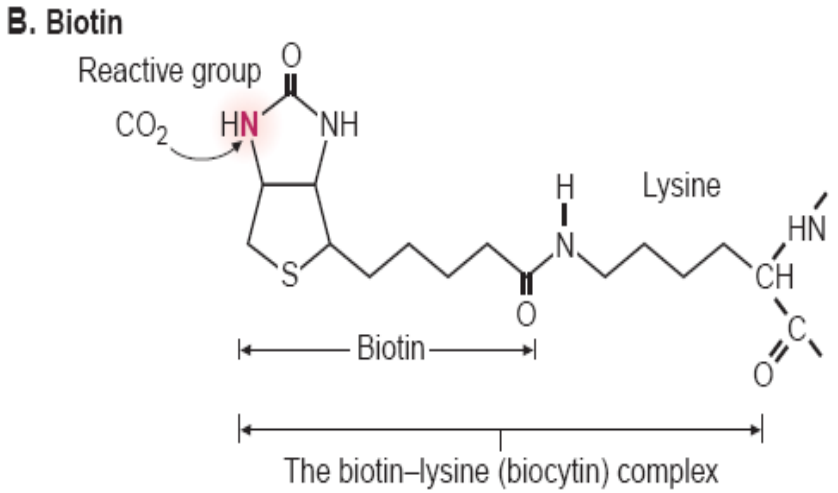
- **Source: pantothenate (B5)**

- **Binding group: adenosine 3',5'-bisphosphate (tight & reversible)**
- **Functional group: sulfhydryl group (nucleophile)**
  - **Attacks carbonyl groups & forms acyl thioesters (the "A")**
  - **How it is different from usual? (regeneration & acyl-CoA derivative)**
  - **Like some others (NAD^+), why do they call them coenzymes?**
    - **Common to so many reactions**
    - **The original form is regenerated by subsequent reactions**
    - **Synthesized from vitamins**
    - **The amount in the cell is nearly constant**



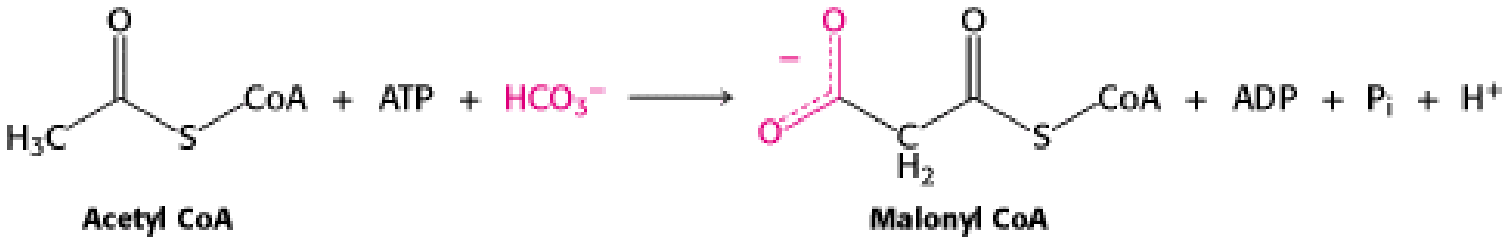
ACTIVATION-TRANSFER COENZYMES

3 – Biotin (B7)



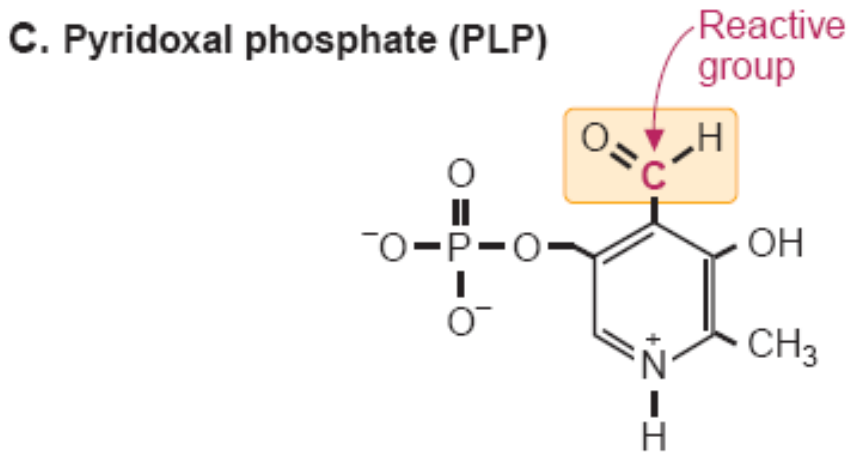
- **Biotin is required for carboxylation reactions (covalently bound to Lys)**
- **Source: food & intestinal bacteria**
- **Deficiencies are generally seen**
  - **Long antibiotic therapies**
  - **Excessive consumption of raw eggs (egg white protein, avidin, high affinity for biotin)**
- **Pyruvate carboxylase**
- **Acetyl CoA carboxylase (fatty acid synthesis)**

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \rightleftharpoons \text{oxaloacetate} + \text{ADP} + P_i + 2\,H^+$$



ACTIVATION-TRANSFER COENZYMES
4 - PLP

- **Synthesis: Pyridoxine (B6)**
- **Functions in the metabolism of amino acids (transaminases)**
- **Reversible reactions**



- **Mechanism:**
  - **Reactive aldehyde forms a covalent bond with the amino groups**
  - **Ring nitrogen withdraws electrons from bound amino acid (cleavage of bond)**

$$\text{Amino acid}_1 + \alpha\text{-ketoacid}_2 \rightleftharpoons \text{amino acid}_2 + \alpha\text{-ketoacid}_1$$

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

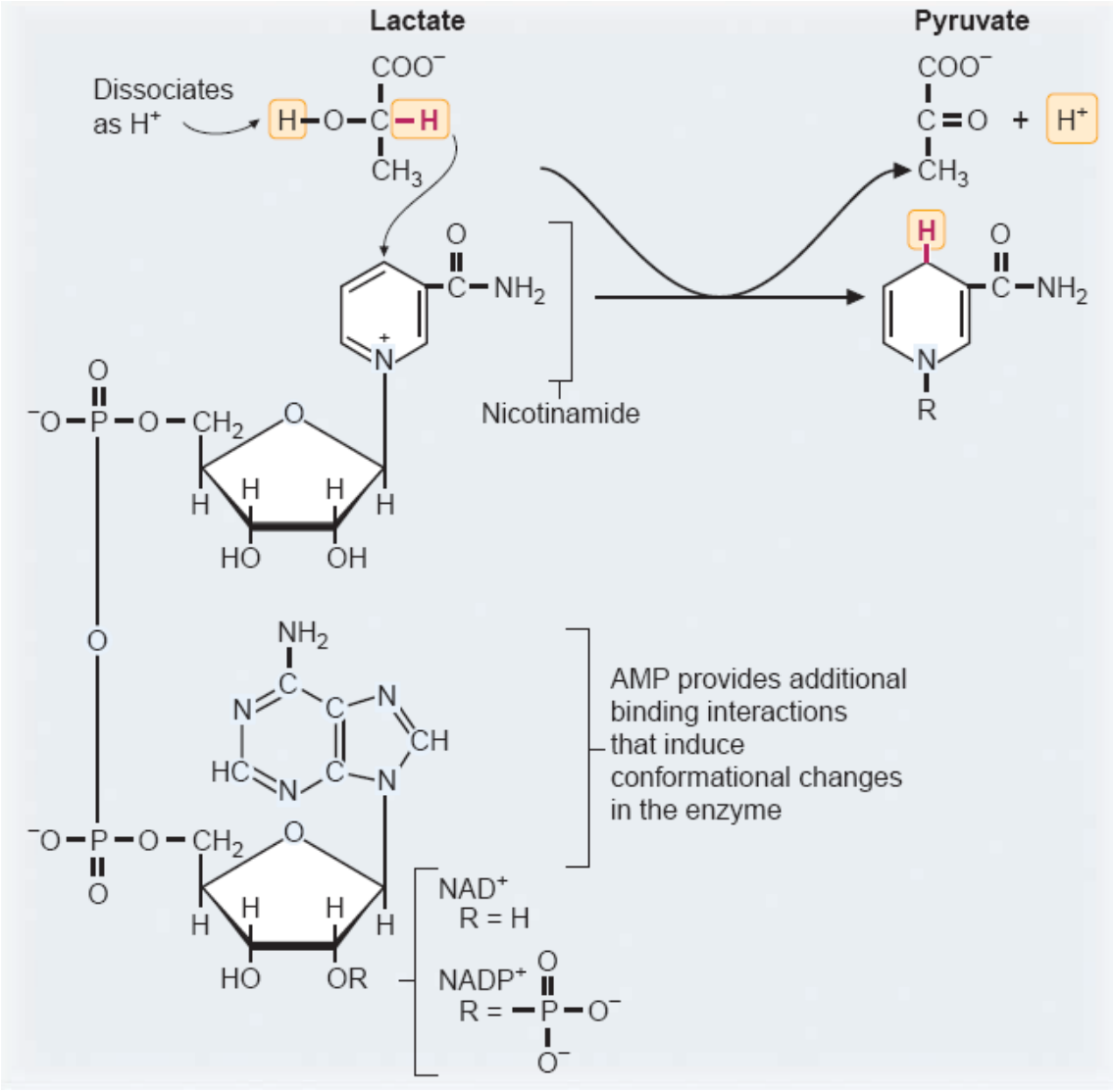
$$\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

OXIDATION–REDUCTION COENZYMES

- **A large number of coenzymes**
- **Do not form covalent bonds with the substrate**
- **Most common: NAD^+ (niacin, B3) & FAD (riboflavin, B2)**
- **Others: work with metals to transfer single electrons to O2 (Vitamins E & C)**
  - **Again: Dependence on the enzyme for additional specificity of substrate & additional catalytic power**

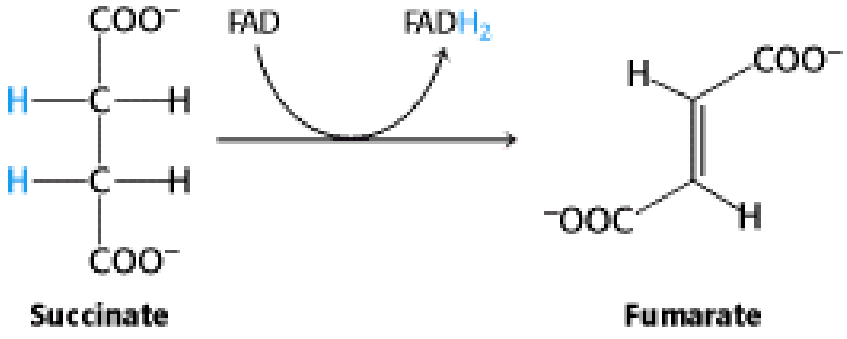
OXIDATION–REDUCTION COENZYMES
1 – NAD^+

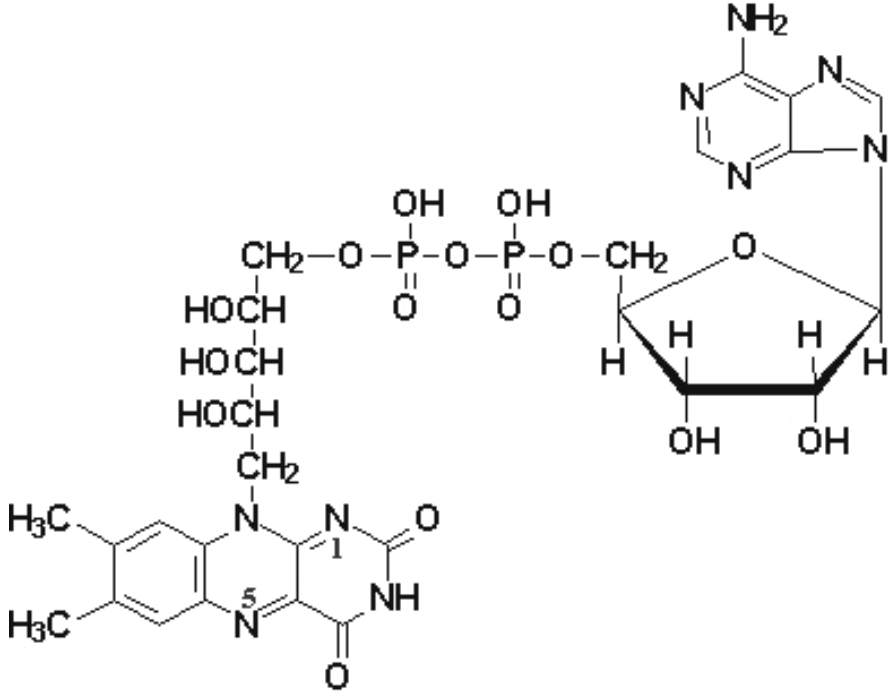
- **Functional group (C opposite to N)**
- **Accepts a hydride ion**
- **The H^+ from substrate dissociates, & a keto group (CO) is formed**
- **(ADP) portion of the molecule binds tightly**
- **The role of enzymes' Histidine**



OXIDATION–REDUCTION COENZYMES
2 – FAD & FMN

- **Source: Riboflavin (B2)**
- **$FMNH_2$ and $FADH_2$**
- **Flavoproteins**
- **FAD and FMN are prosthetic groups (tightly bound)**
- **Succinate dehydrogenase**
- **Pyruvate dehydrogenase complex**





Water-Soluble Vitamins

Name	Coenzyme or Active Form	Primary biochemical function
Thiamin	Thiamine pyrophosphate (TPP)	Aldehyde-group transfer
Riboflavin	Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer
Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer
Pantothenic Acid	Coenzyme A (CoA)	Acyl-group transfer
Pyridoxine	Pyridoxal Phosphate	Amino-group transfer
Biotin	Biocytin	Carboxyl transfer
Folate	Tetrahydrofolate	One-Carbon group transfer
Vitamin B_{12}	Coenzyme B_{12}	1,2 shift hydrogen atoms
Lipoic Acid	Lipoyllysine	Hydrogen-Atom and Acyl-group transfer
Ascorbic Acid	Ascorbic acid, dehydroascorbic acid	Cofactor in hydroxylation

Catalytic Metals

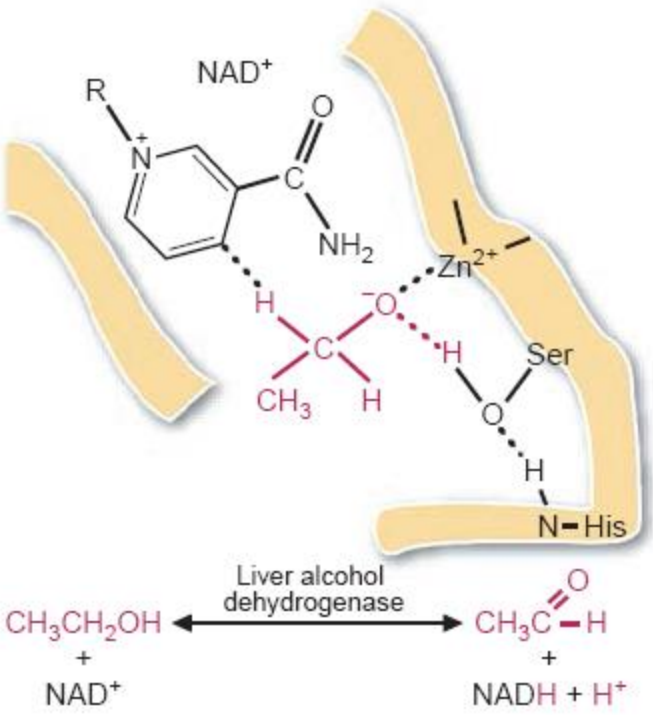
- **Metals can be tightly bound (metalloenzymes) or loosely bound (metal-activated enzymes)**
- **Acting as electrophiles**
- **Metal-activated enzymes; the metal either required or enhances activity (Mg^{2+}, Mn^{2+}, Ca^{2+}, & K^{+})**
- **Phosphofructokinase & TPP; (Mg^{2+}) is required to coordinate the phosphate groups on the ATP for a successful reaction (chelation)**

Metal	Enzyme
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	Hexokinase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase

Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP

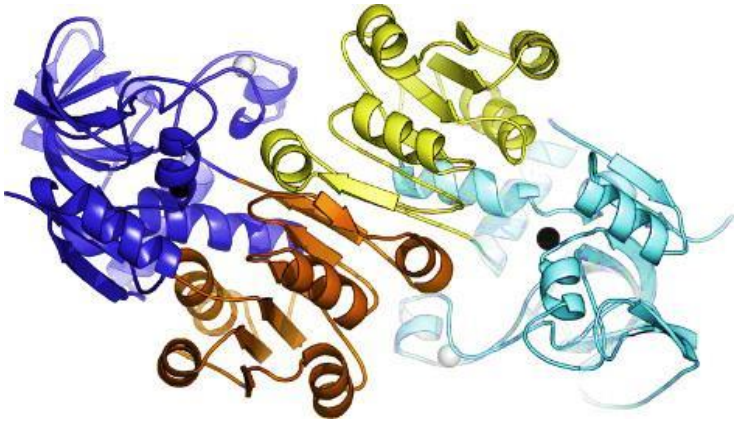
Catalytic Metals

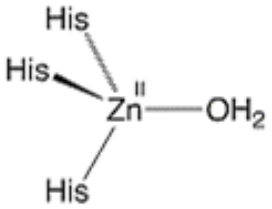
- **Alcohol dehydrogenase (ADH)**
- **Activated serine (pulls a proton off –OH)**
- **Oxyanion is stabilized by zinc**
- **Transfer of a hydride ion to NAD^+**
- **Zinc in ADH as His in lactate dehydrogenase**



Metalloenzymes

- **Metal ions are usually incorporated during synthesis & removal of the metal causes denaturation**
- **These metal ions may contribute either to the structure or the catalytic mechanism**
- **Liver alcohol dehydrogenase (dimer); 2 Zn^{+2} in each monomer; one for structural maintenance (joins the two subunits), the other is catalytic**
- **Carbonic anhydrase; A zinc atom is essentially always bound to four or more groups**





$[NNN]Zn^{II}–OH_2$

Carbonic Anhydrase