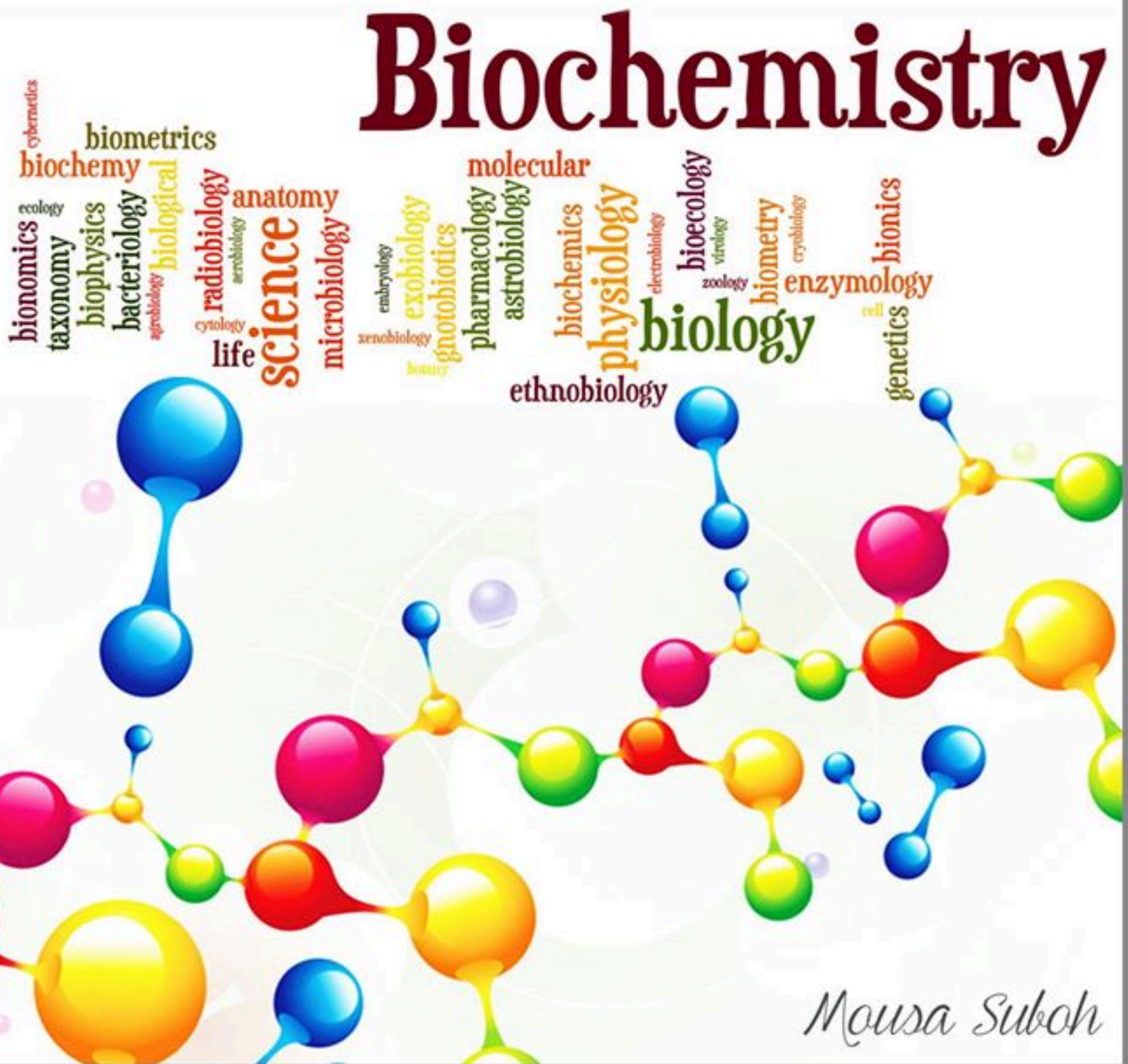
Slide : Enzymes Part 2

Dr. Name : Dr. Nafez Abu Tarboosh

Sections : 1,2,3

■ Slide □ Sheet

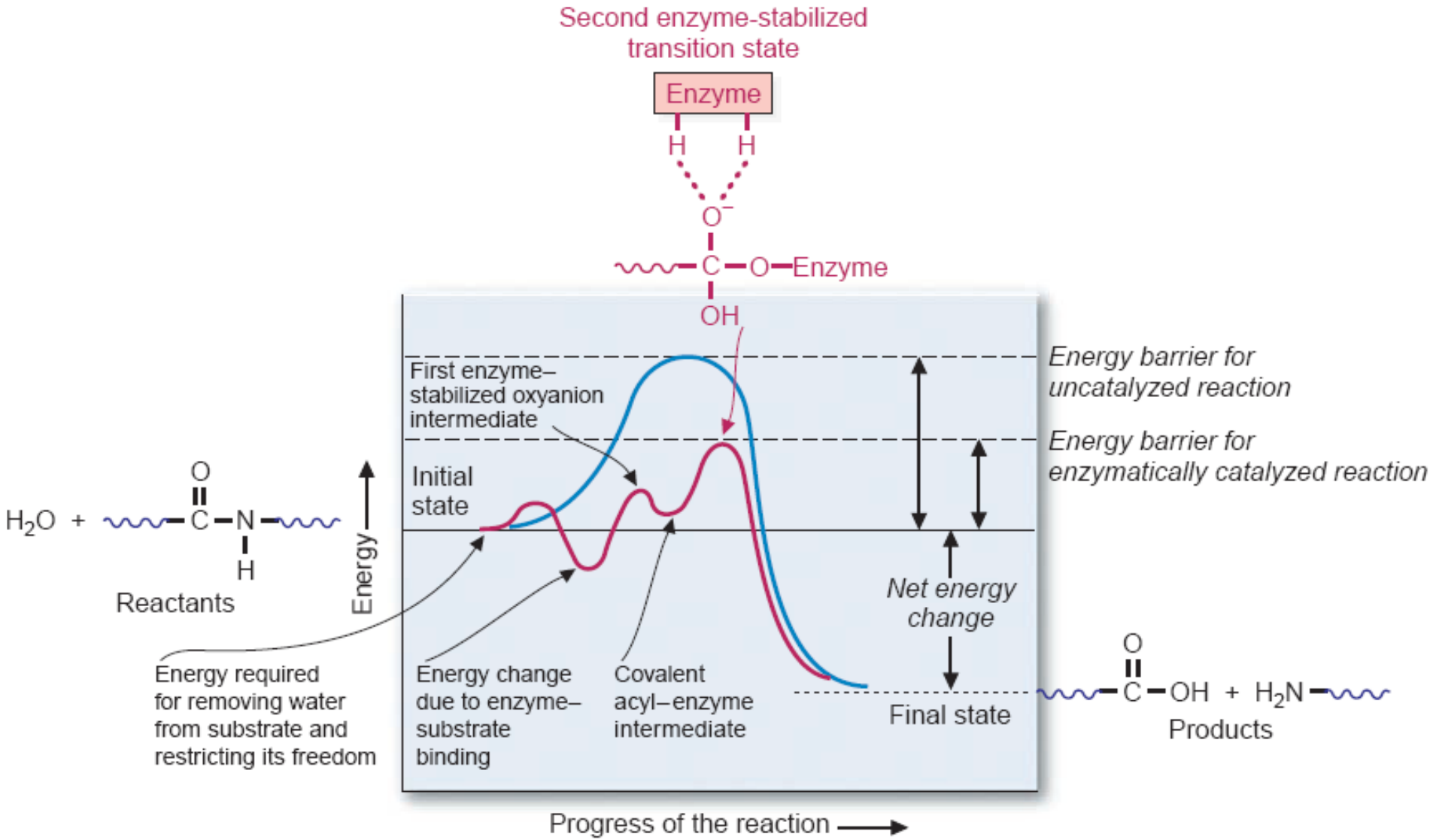
Medical Committee
The University of Jordan



Nafith Abu Tarboush
DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

ENZYMES

Energy Diagram in the Presence of Chymotrypsin



CATALYSIS

FUNCTIONAL GROUPS IN CATALYSIS

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

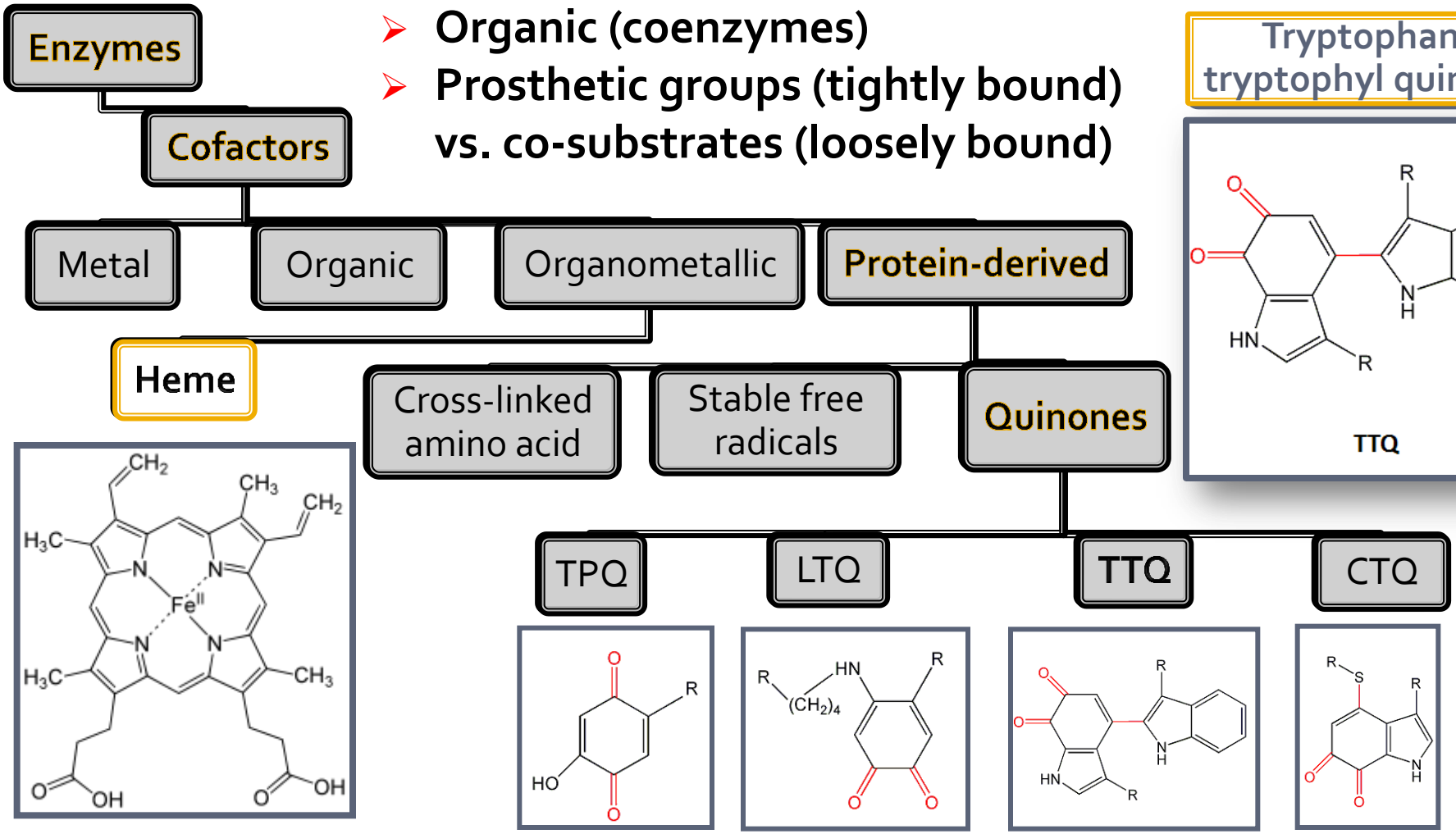
A. **Functional Groups on Amino Acid Side Chains:**
   - **Almost all polar amino acids (nucleophilic catalysis)**
   - **Ser, Cys, Lys, & His can participate in covalent catalysis**
   - **Histidine: pKa, physiological pH & acid–base catalysis**

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**

Enzymes-cofactors

Enzyme cofactors

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

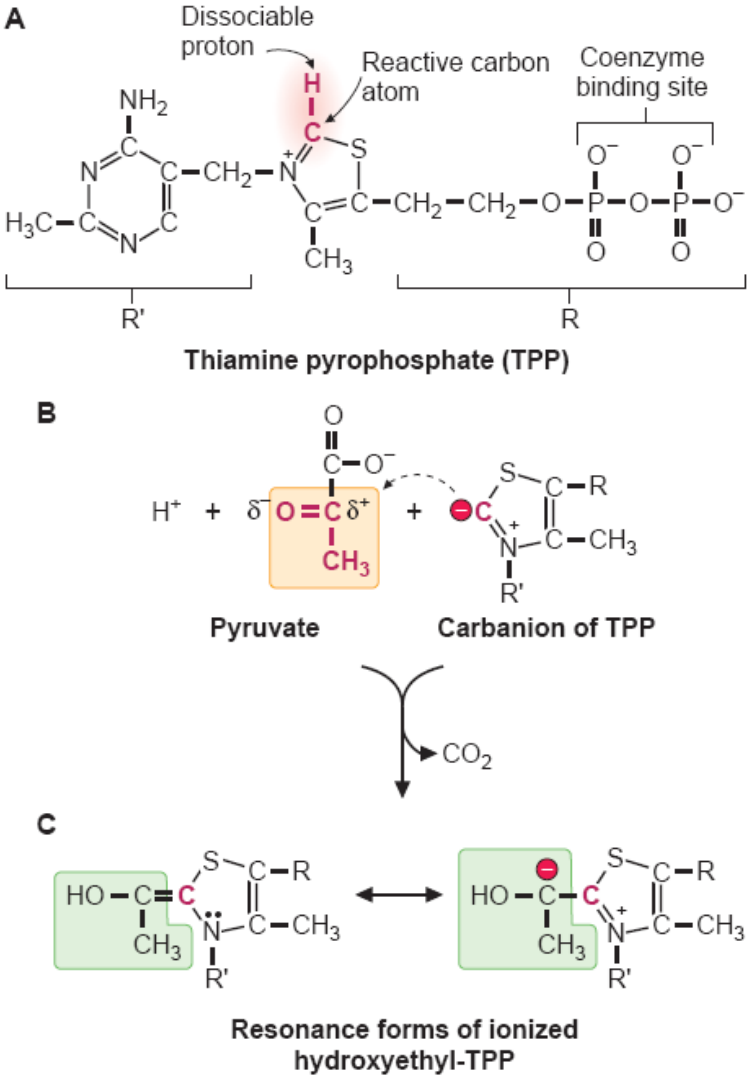


ACTIVATION-TRANSFER COENZYMES

- **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**

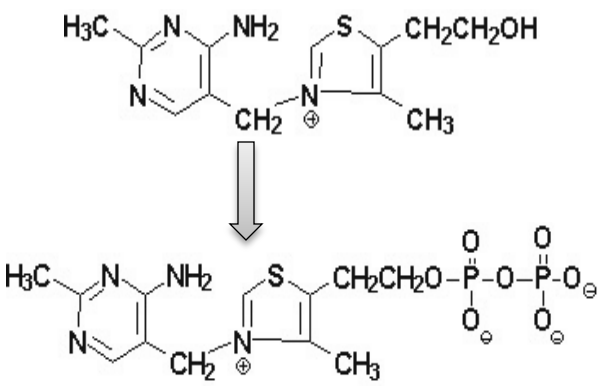
ACTIVATION-TRANSFER COENZYMES
1 - TPP

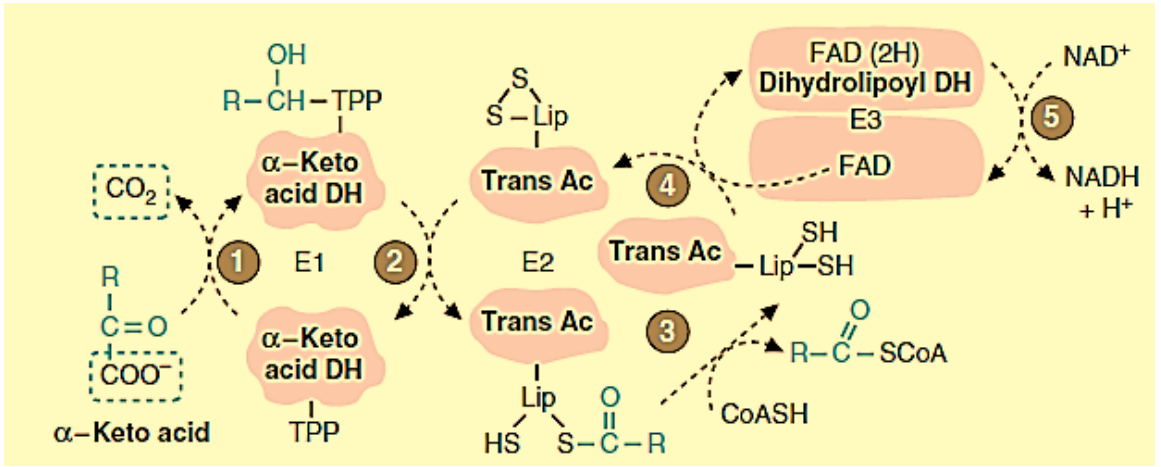
- Thiamine pyrophosphate
- Source: thiamine (B1)
- Decarboxylation reactions
- Pyrophosphate:
  - Provides negatively charged oxygen atoms
  - Chelate Mg^{2+} (tight binding)
- Functional group (reactive carbon atom)
- Reactive thiamine carbon forms a covalent bond with a substrate keto group while cleaving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

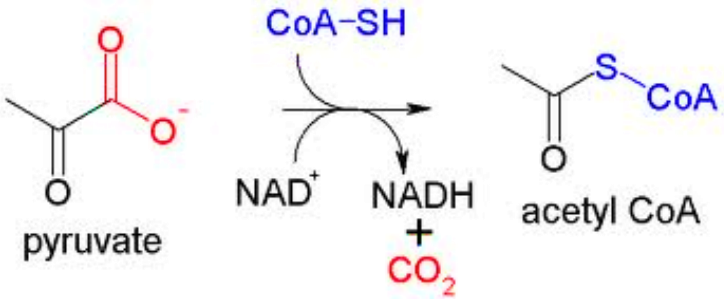


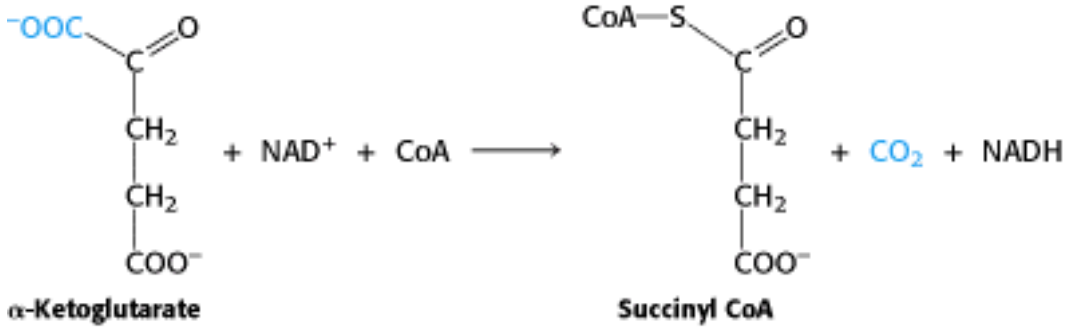
Thiamin (vitamin B1)

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



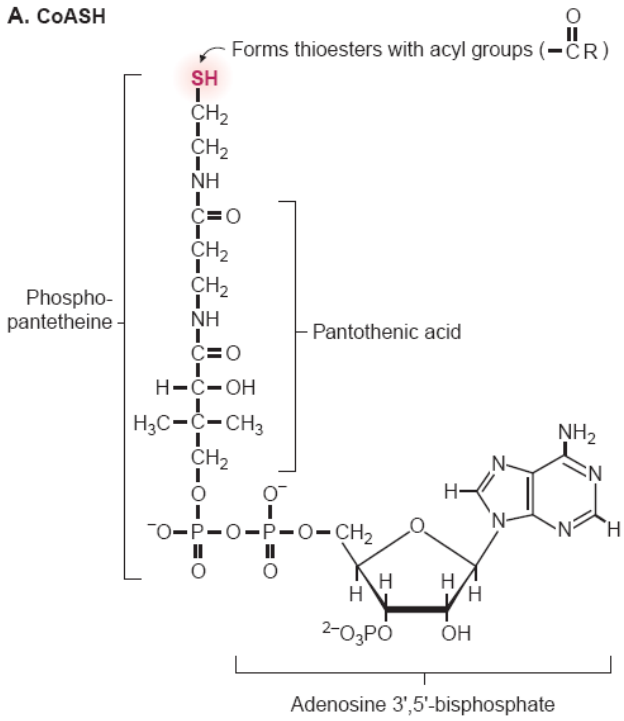






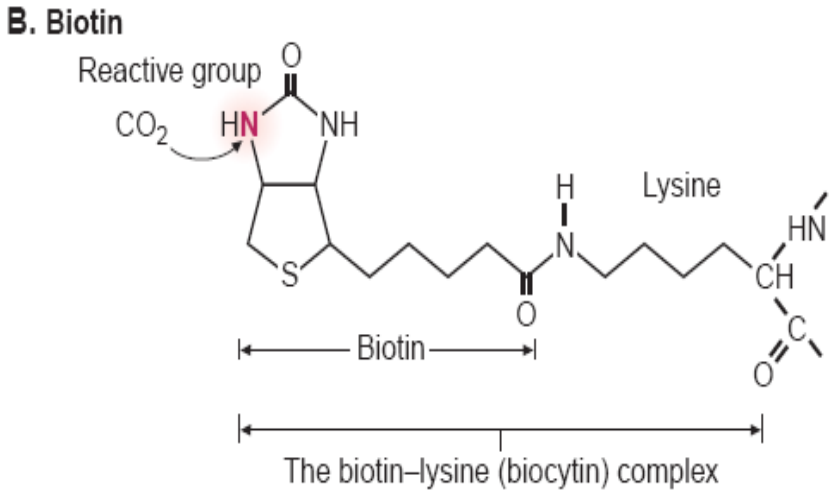
ACTIVATION-TRANSFER COENZYMES
2 - Coenzyme A (CoA)

- 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



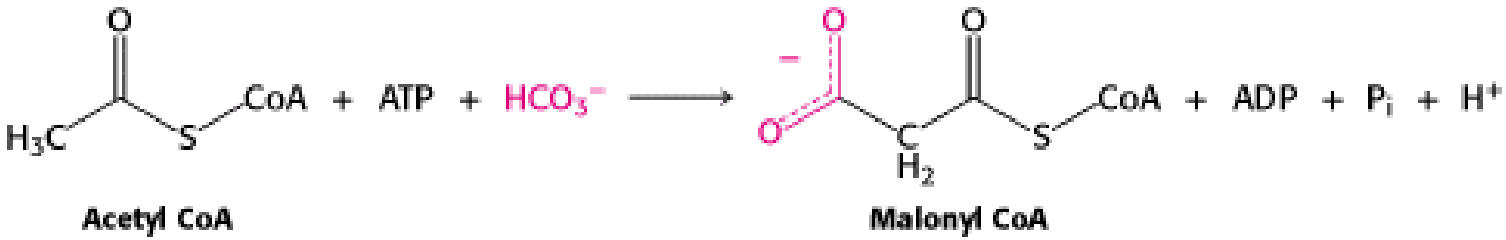
ACTIVATION-TRANSFER COENZYMES

3 – Biotin (B7)



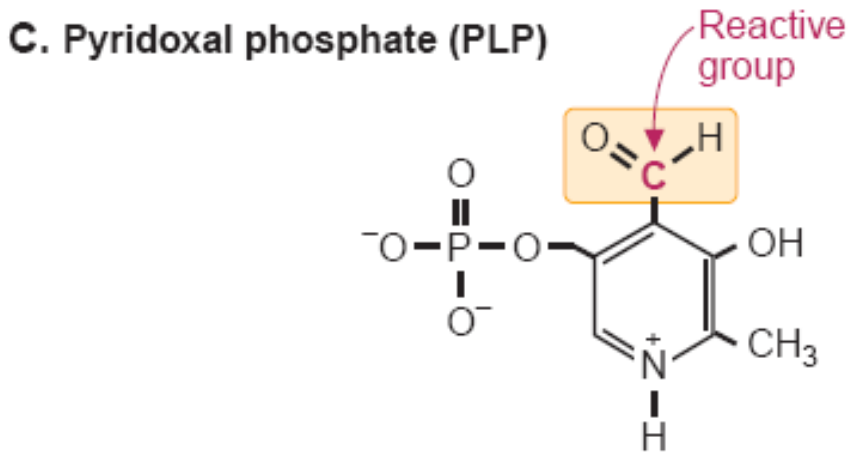
- Biotin is required for carboxylation reactions (covalently bound to Lys)
- Source: food & intestinal bacteria
- Deficiencies are generally seen
  - Long antibiotic therapies
  - Excessive consumption of raw eggs (egg white protein, avidin, high affinity for biotin)
- Pyruvate carboxylase
- Acetyl CoA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

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

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

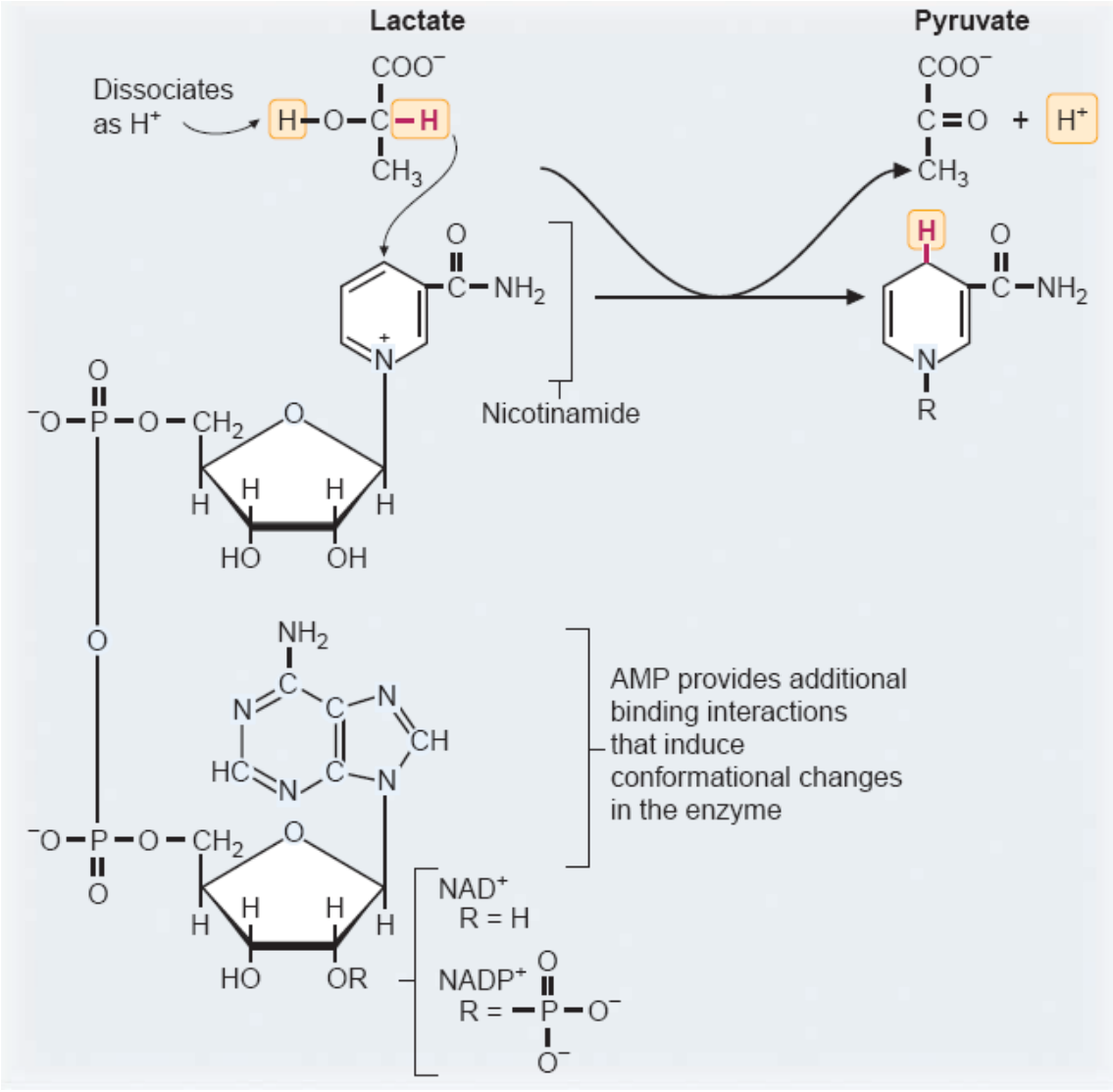
$$\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

OXIDATION–REDUCTION COENZYMES

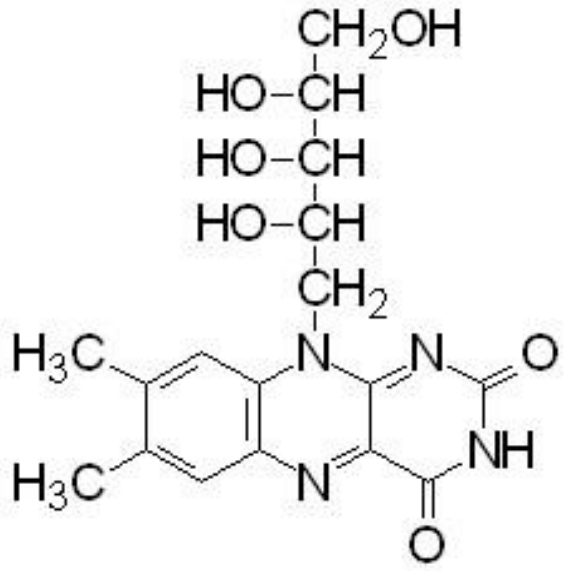
- **A large number of coenzymes**
- **Do not form covalent bonds with the substrate**
- **Most common: NAD^+ (niacin, B3) & FAD (riboflavin, B2)**
- **Others: work with metals to transfer single electrons to O2 (Vitamins E & C)**
- **Again: Dependence on the enzyme for additional specificity of substrate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

OXIDATION–REDUCTION COENZYMES
1 – NAD^+

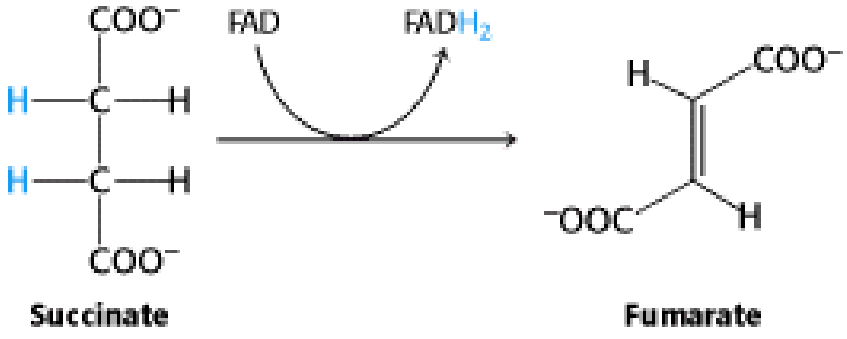
- Functional group (C opposite to N)
- Accepts a hydride ion
- The H^+ from substrate dissociates, & a keto group (CO) is formed
- (ADP) portion of the molecule binds tightly
- The role of enzymes' Histidine

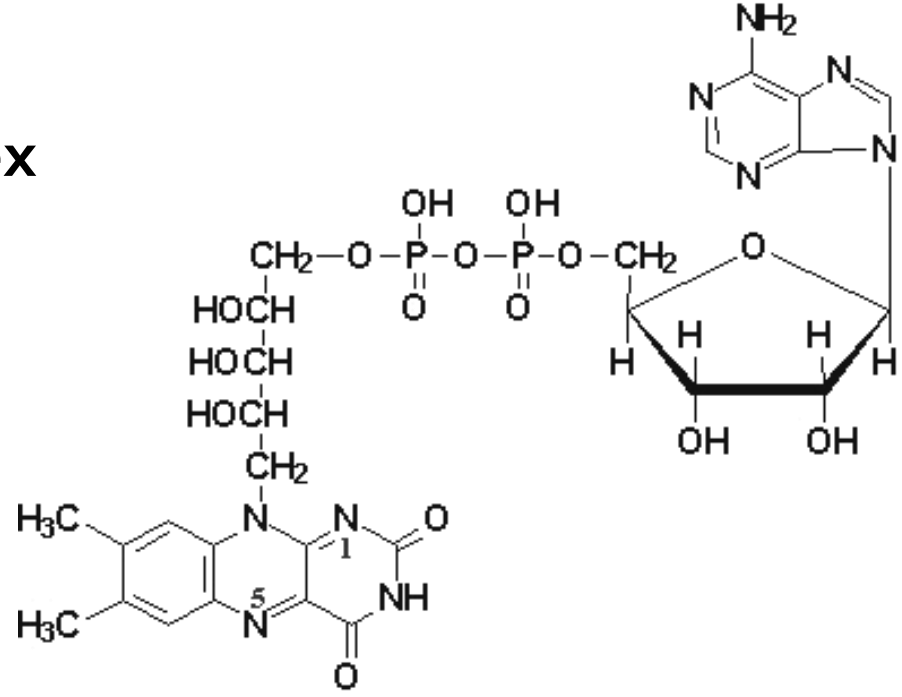


OXIDATION–REDUCTION COENZYMES
2 – FAD & FMN



- 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)	Hydrogen-Atom (electron) transfer
	Flavin adenine dinucleotide (FAD)	Hydrogen-Atom (electron) transfer
Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD)	Hydrogen-Atom (electron) transfer
	Nicotinamide adenine dinucleotide phosphate (NADP)	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^{+})

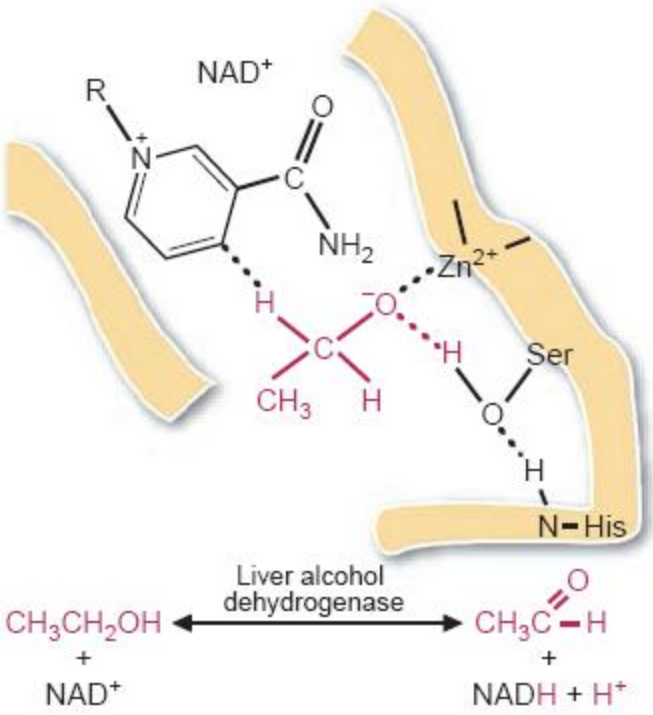
- Phosphofructokinase & TPP; (Mg^{2+}) is required to coordinate the phosphate groups on the ATP for a successful reaction (chelation)

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

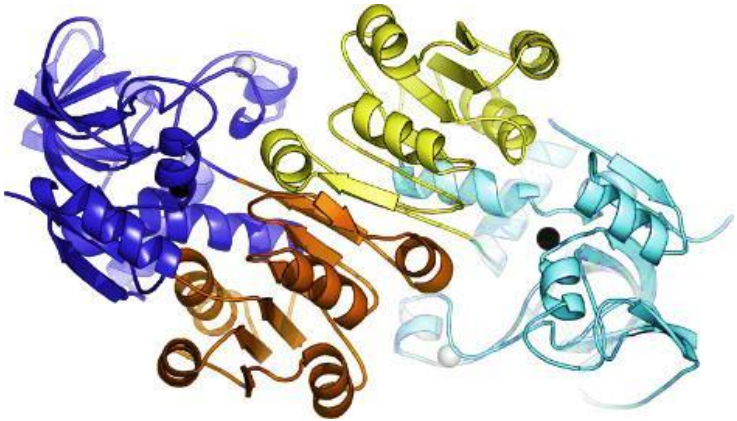
Catalytic Metals

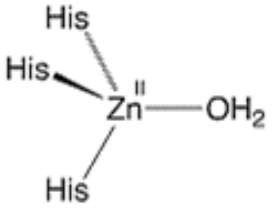
- Alcohol dehydrogenase (ADH)
- Activated serine (pulls a proton off –OH)
- Oxyanion is stabilized by zinc
- Transfer of a hydride ion to NAD^+
- Zinc in ADH as His in lactate dehydrogenase



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