Mechanism of Enzyme action

Dr Bela Goyal

Coenzymes

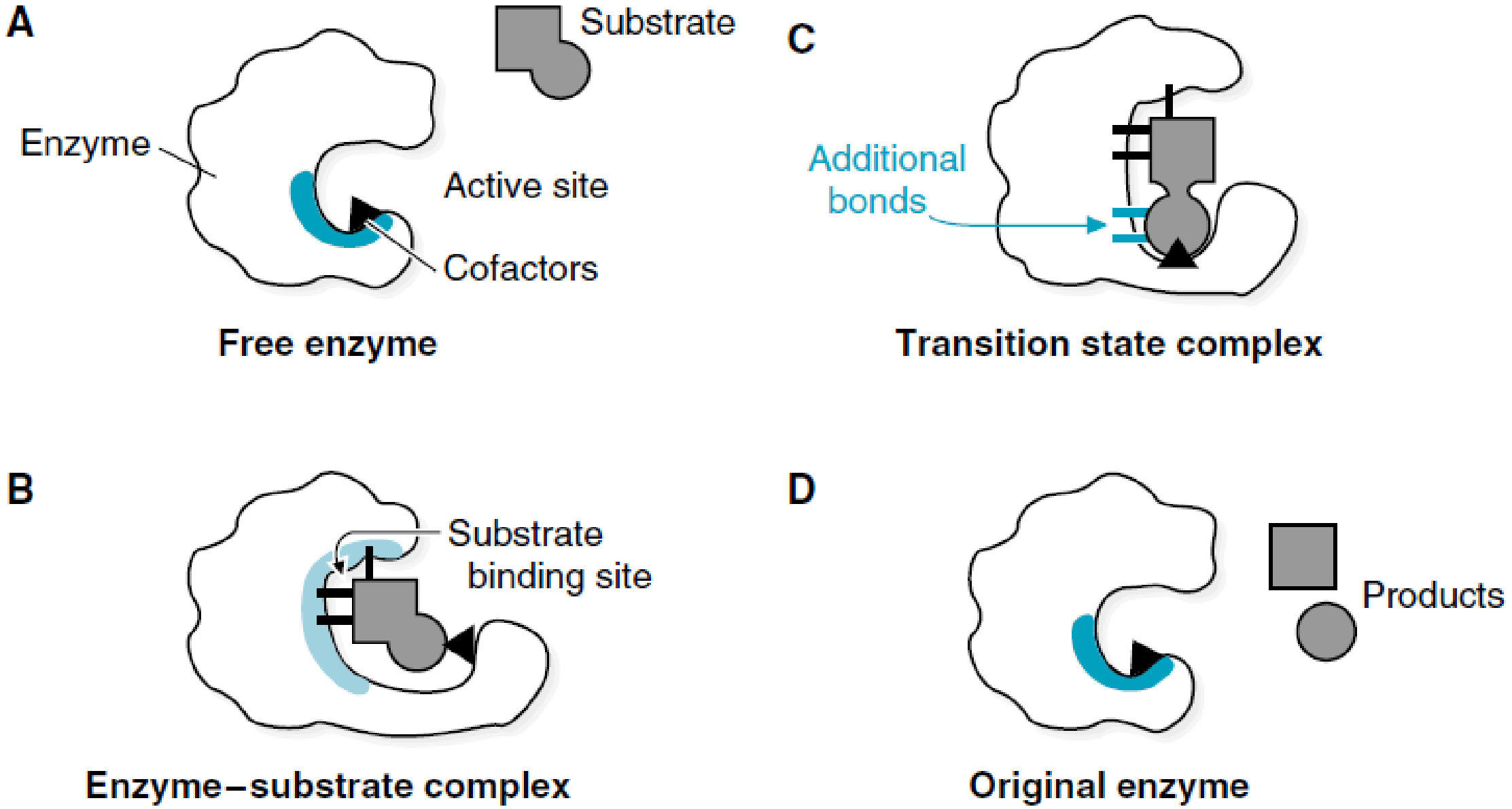
Table 6.1: Some common coenzymes and their functions

Vitamin	*Coenzyme*	*Function as coenzyme*
Thiamine (Vit B_1)	TPP (Thiamine pyrophosphate)	Oxidative decarboxylation and transketolase reaction
Riboflavin (Vit B_2)	FAD and FMN (Flavin Adenine Dinucleotide and Flavin Mononucleotide)	Oxidation and reduction reactions
Niacin	NAD^+ (Nicotinamide Adenine Dinucleotide), $NADP^+$ (Nicotinamide Adenine Dinucleotide Phosphate)	Oxidation and reduction reactions
Pyridoxine (Vit B_6)	PLP (Pyridoxal phosphate)	Transamination, deamination decarboxylation reactions of amino acids
Biotin	Biocytin	Carboxylation reactions
Folic acid	THF (Tetrahydrofolate)	Carrier of one carbon group
Pantothenic acid	Coenzyme A	Acyl carrier
Cynocobalamine	Methylcobalamine and Deoxyadenosylcobalamine	Transfer of CH_3 group and isomerizations

Inorganic co factors

Table 6.2: Enzymes requiring or containing inorganic elements as cofactors (activators)

Enzyme	*Cofactor (activator)*
Ferroxidase (ceruloplasmin), Ascorbic acid oxidase	Copper
Carbonic anhydrase, DNA-polymerase, Porphobilinogen synthase, Carboxypeptidase	Zinc
Cytochrome oxidase, Catalase	Iron
Glucose-6-Phosphatase, Hexokinase	Magnesium
Glutathione peroxidase	Selenium
Arginase, Pyruvate carboxylase	Manganese
Xanthine oxidase	Molybdenum


A
Substrate
Enzyme
Active site
Cofactors
Free enzyme
C
Additional
bonds
Transition state complex
B
Substrate
binding site
Enzyme–substrate complex
D
Products
Original enzyme

MCQ

- A patient was born with a congenital mutation in an enzyme, severely affecting its ability to bind an activation-transfer coenzyme.

As a consequence,

(A) the enzyme would be unable to bind the substrate of the reaction.

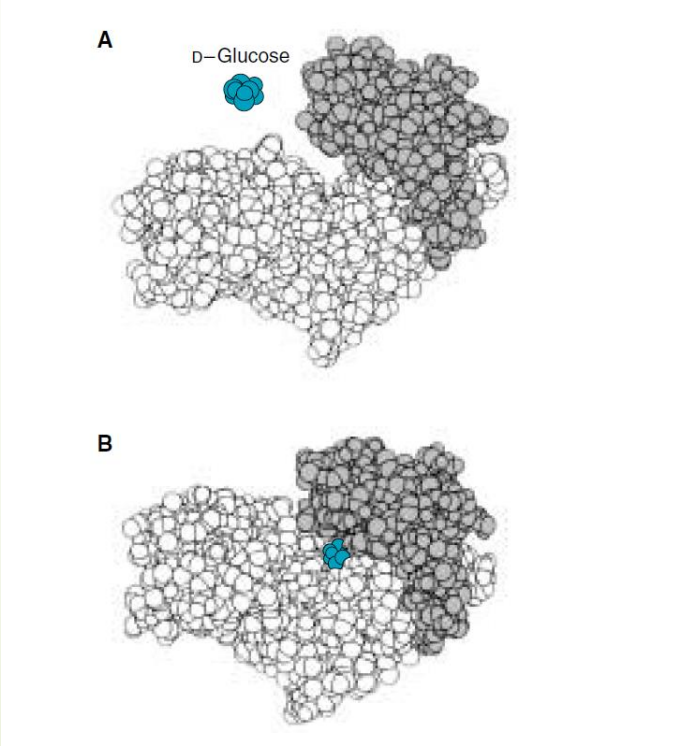
(B) the enzyme would be unable to form the transition state complex.

(C) the enzyme would normally use a different activation-transfer coenzyme.

(D) the enzyme would normally substitute the functional group of an active site amino acid residue for the coenzyme.

- 1. **LOCK-AND-KEY MODEL FOR SUBSTRATE BINDING: Emil Fisher**

- **2. "INDUCED FIT" MODEL FOR SUBSTRATE BINDING: Daniel E koshland**



The Transition State Complex

- Condition in which bonds in the substrate are maximally strained
- The highest energy level corresponds to the most unstable substrate configuration
- transition state analogs
- ?abzymes

Stabilization of transition state is the mechanism of enzyme action.

Active site is more complementary to transition state than substrate.

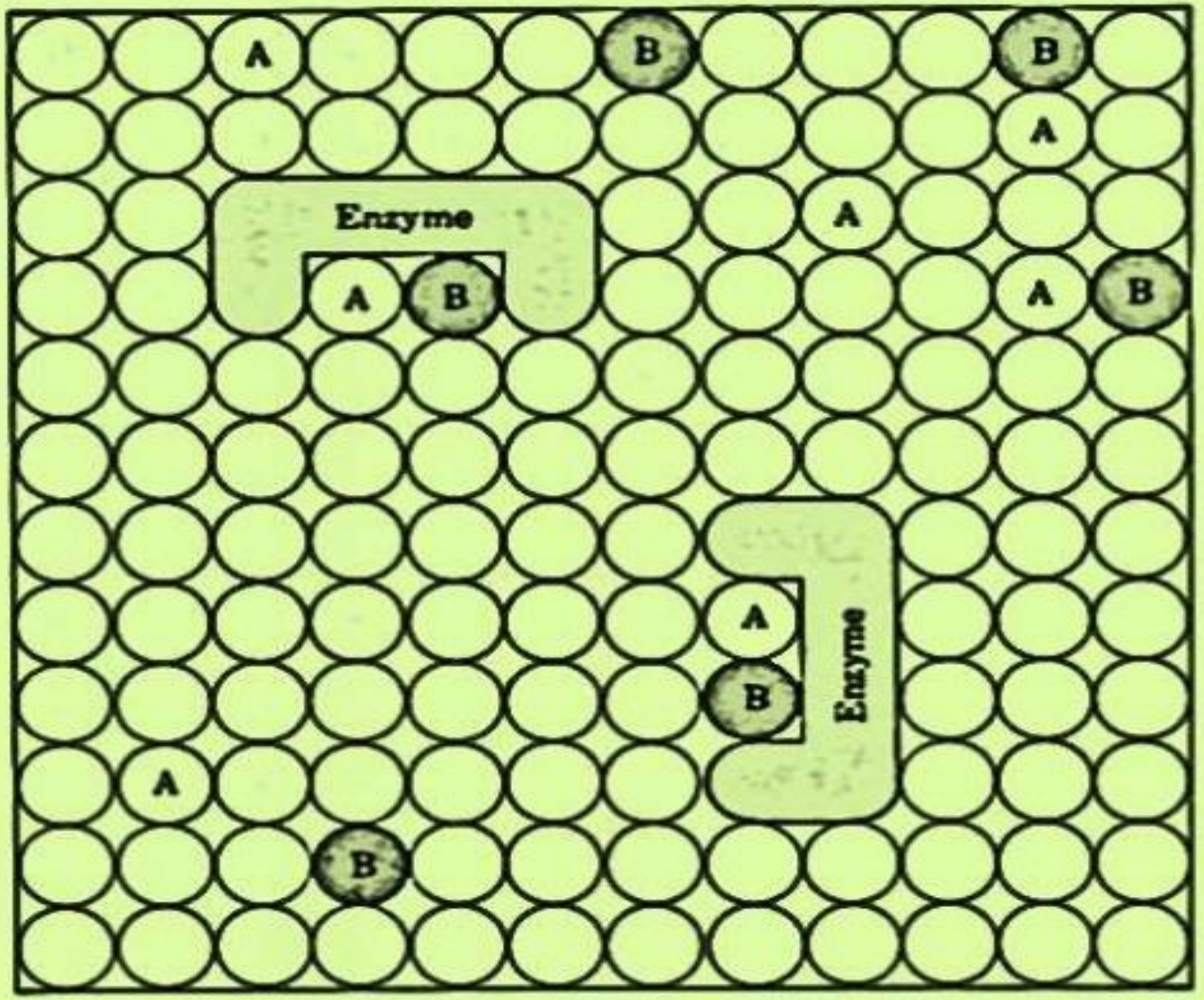
So, Transition state analogues are better competitive inhibitors than substrate analogues

How does enzymes increase the rate ?

- Proximity
- Straining
- Orientation Change
- Change of environment
- Transition state stabilization

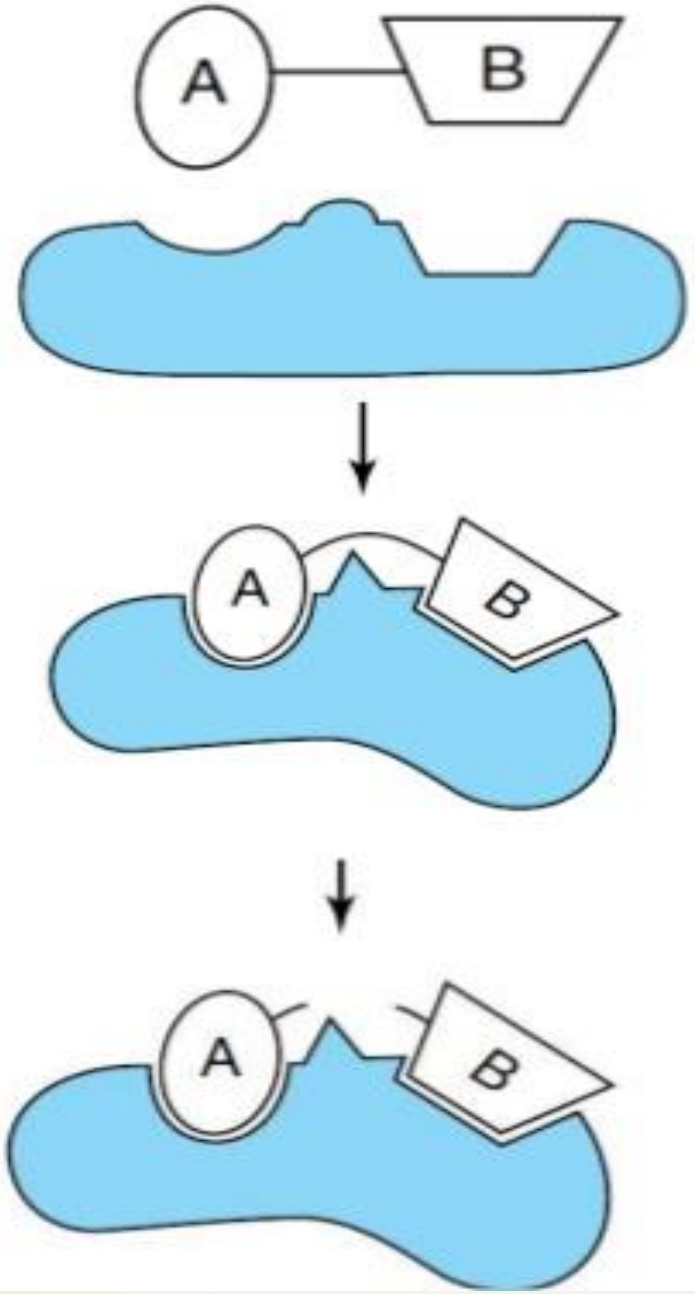
Proximity Effect

- higher their concentration, the more frequently they will encounter one another
- Concept of effective molarity



Proximity Effect

Straining



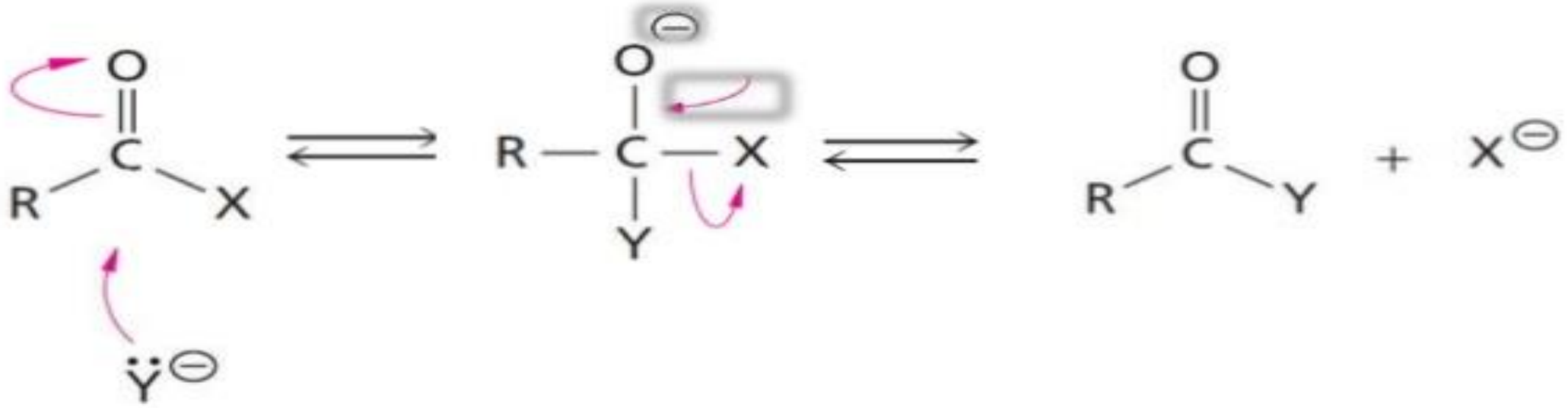
What are all the modes of catalysis ?

- Acid Base Catalysis
- Covalent Catalysis
- Metal Ion Catalysis

Chemical Reactions

- Nucleophilic Substitutions
- Cleavage Reactions
- Oxidation–Reduction Reactions

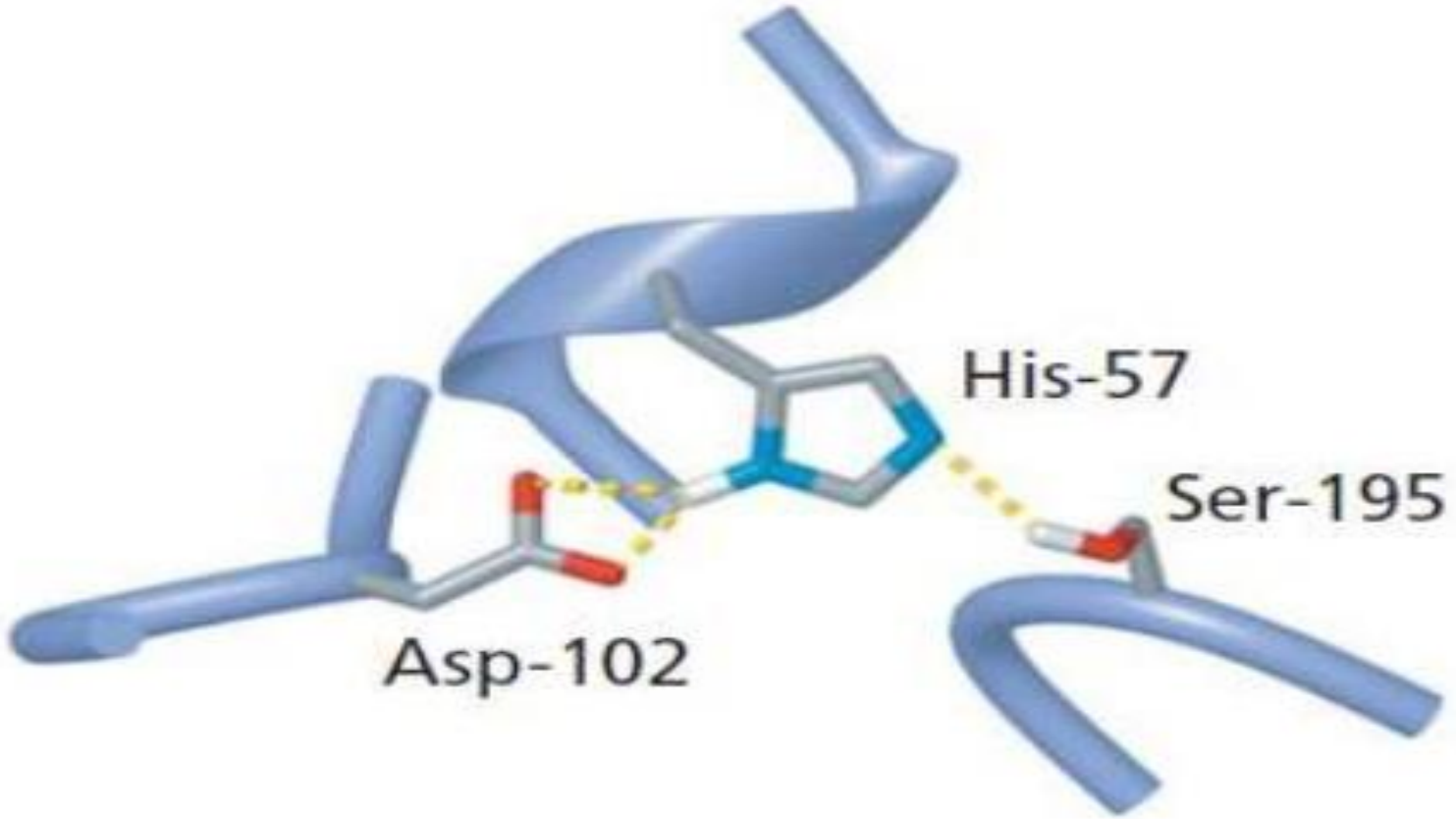
Nucleophilic Substitutions



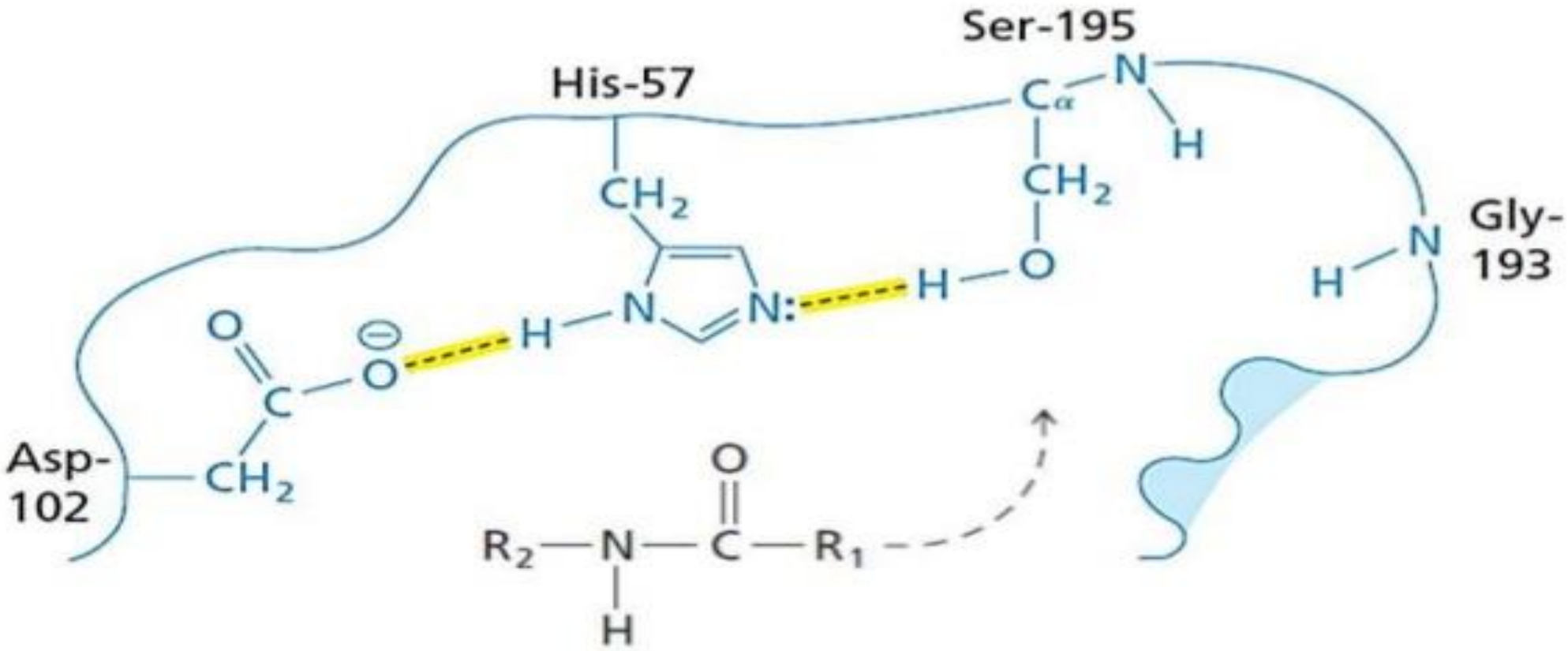
Covalent catalysis

Serine proteases

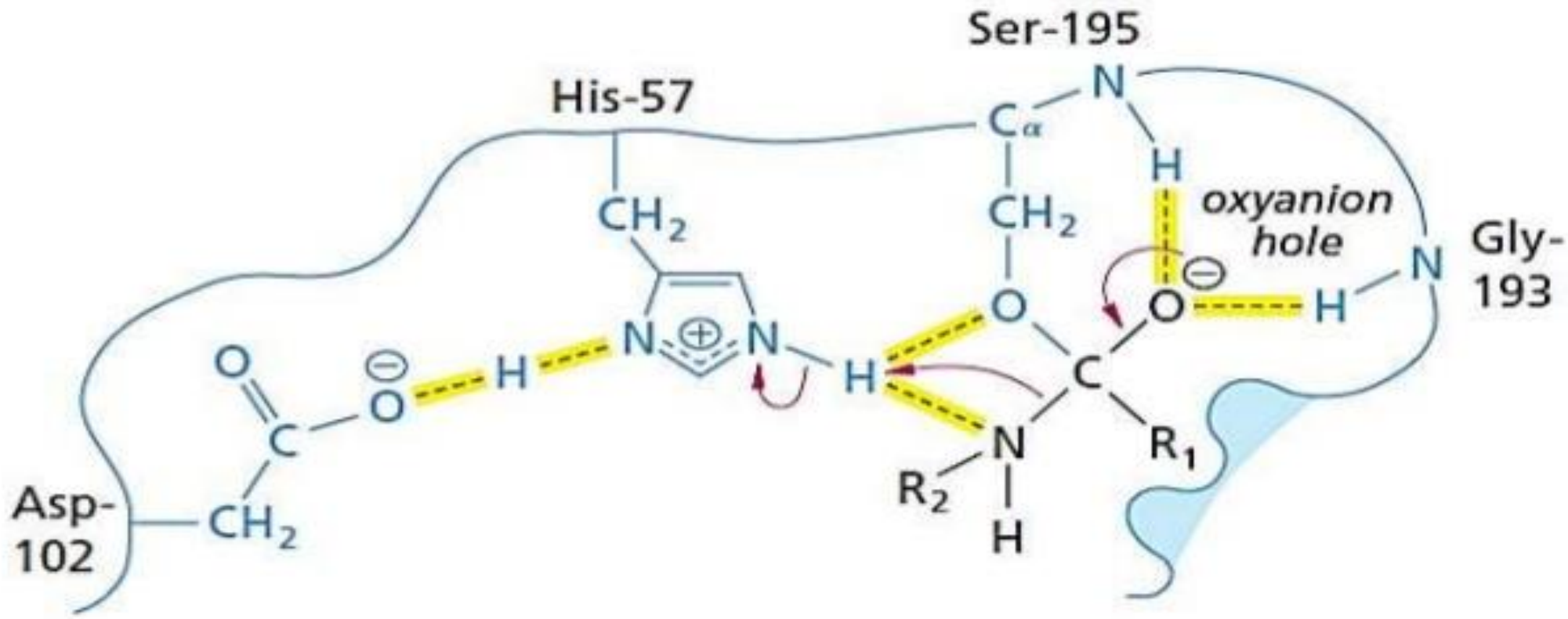
Catalytic Triad



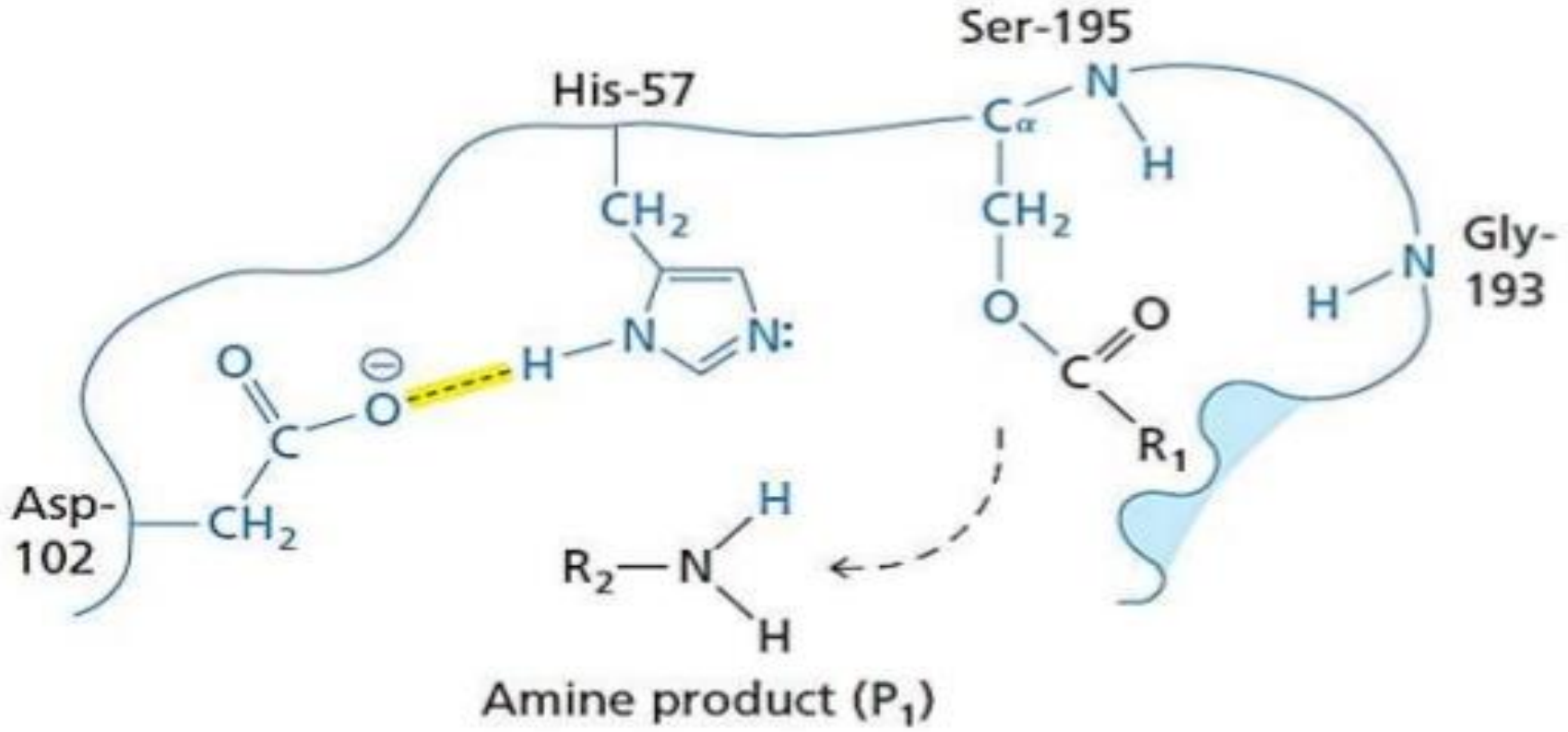
Ser-195 becomes a nucleophile



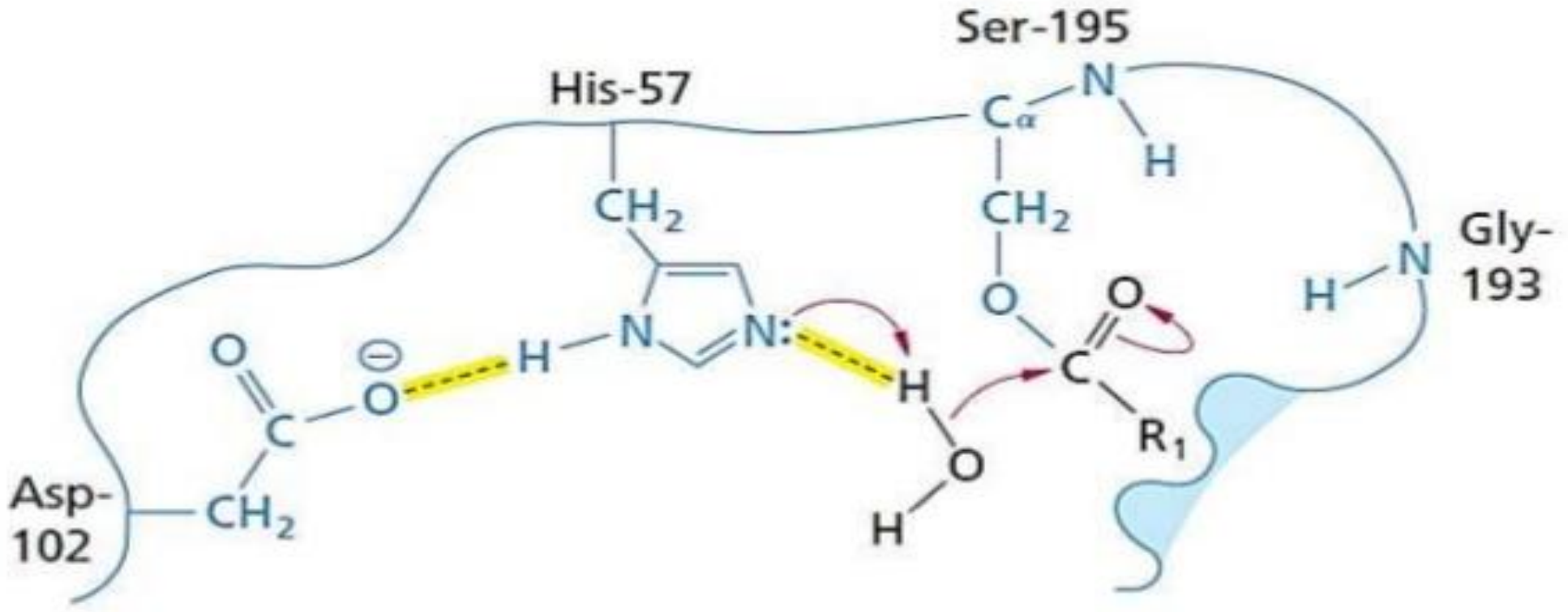
Ser-195 attacks the carbonyl group



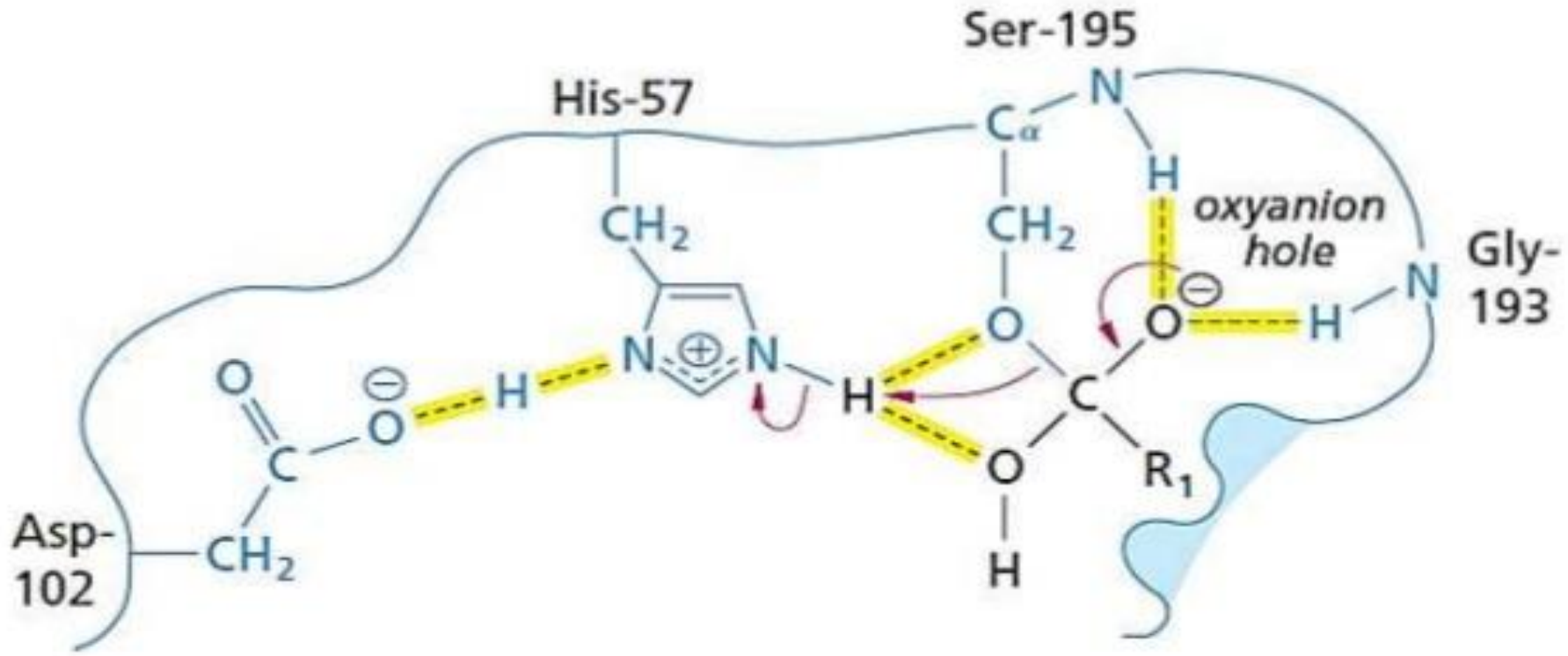
Acyl enzyme intermediate



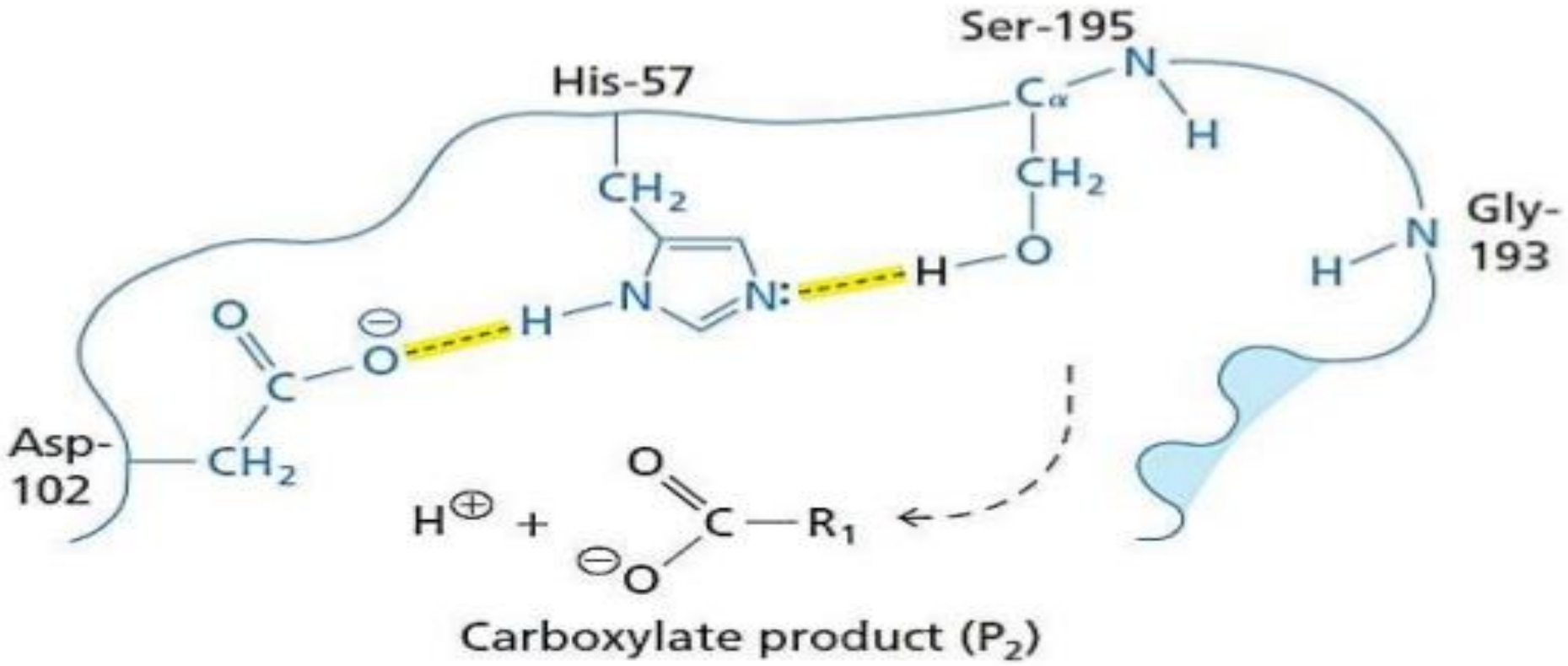
Binding of Water



Tetrahedral intermediate

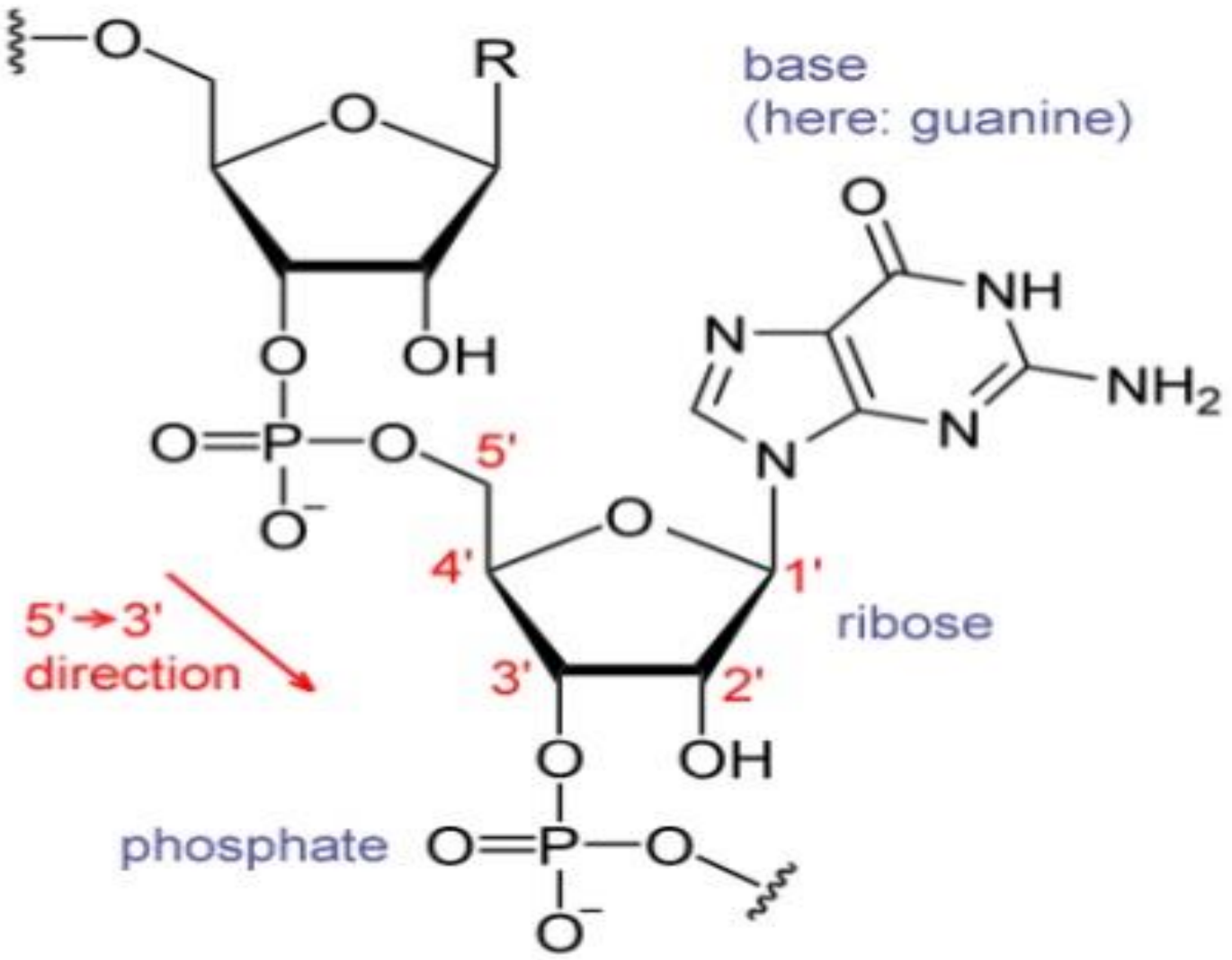


Regeneration of enzyme

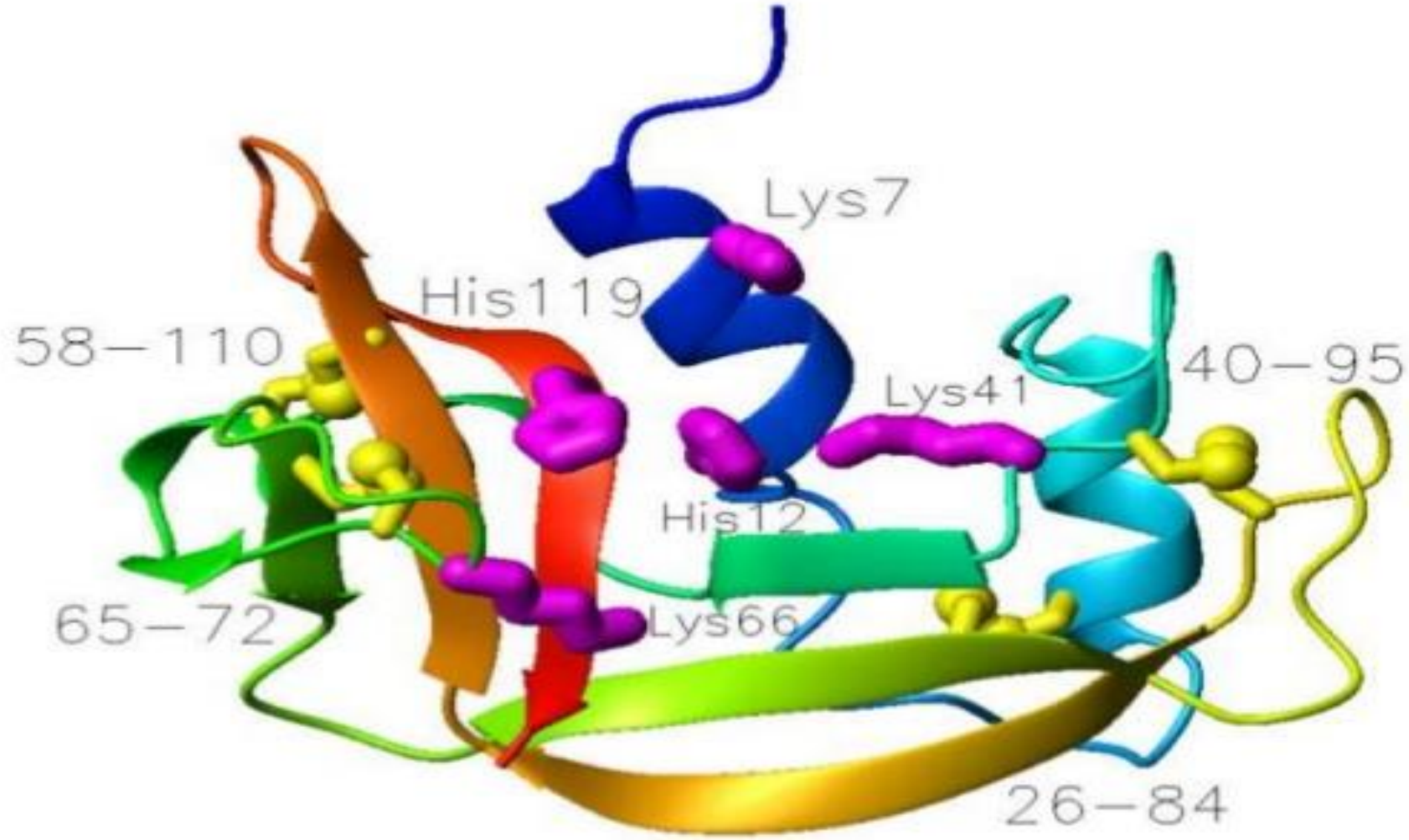


Acid-Base catalysis

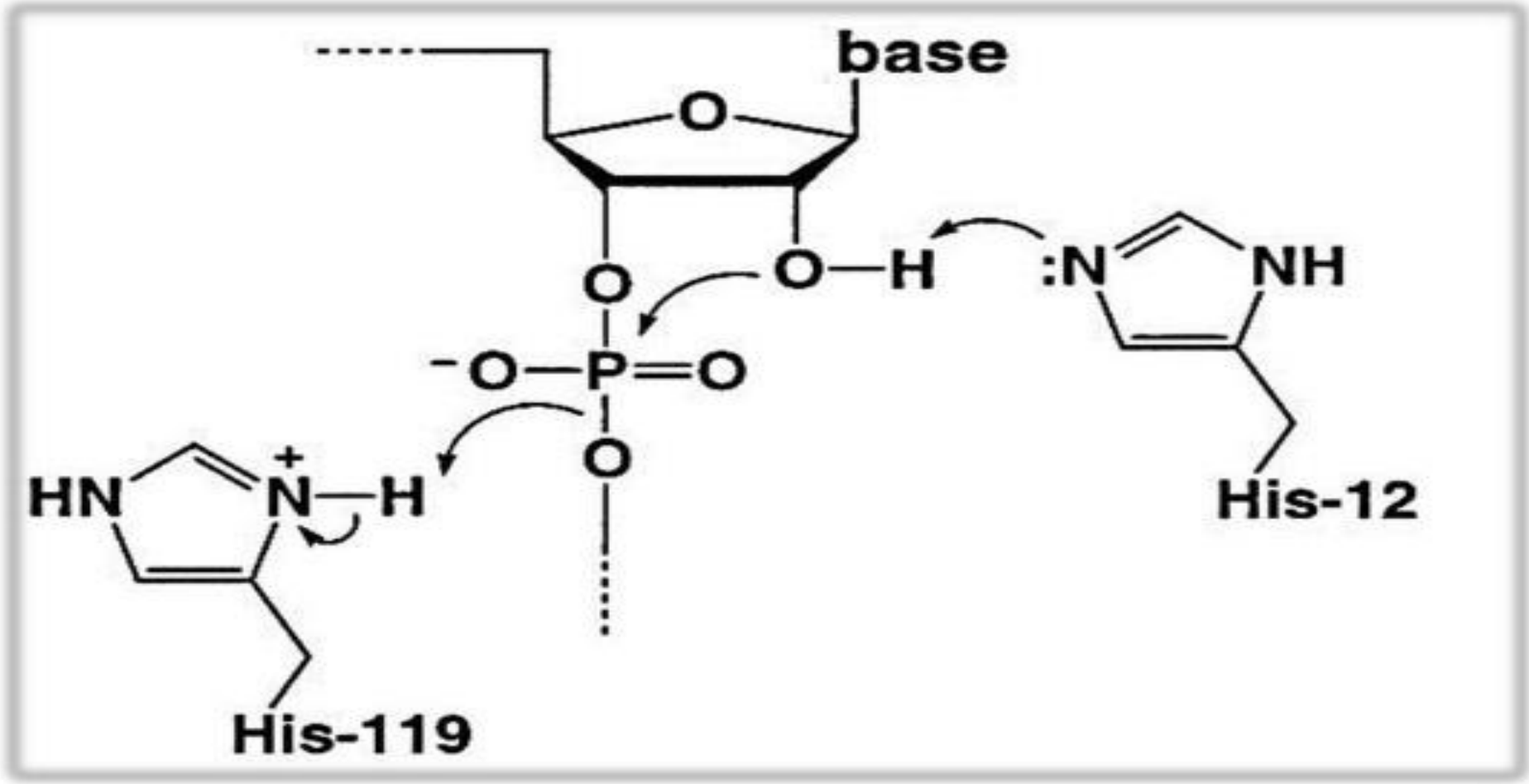
Structure of RNA

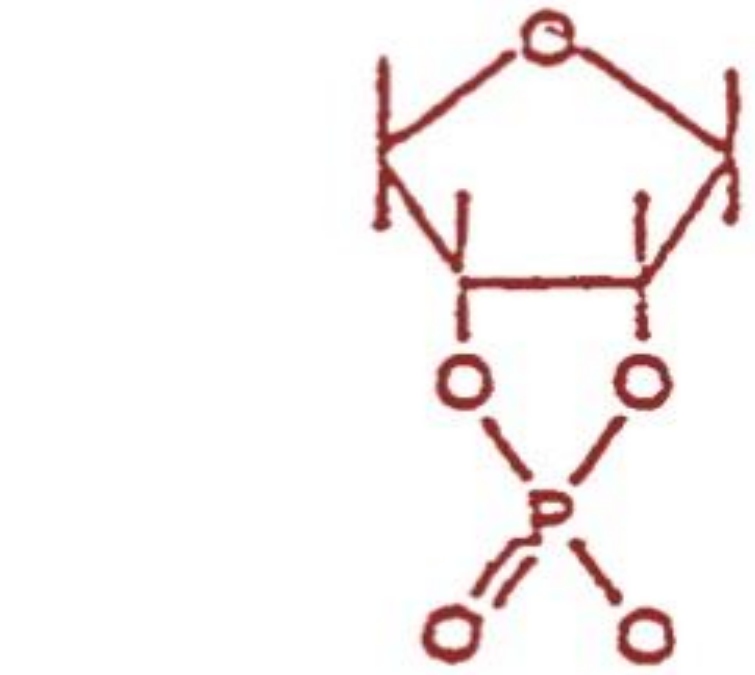


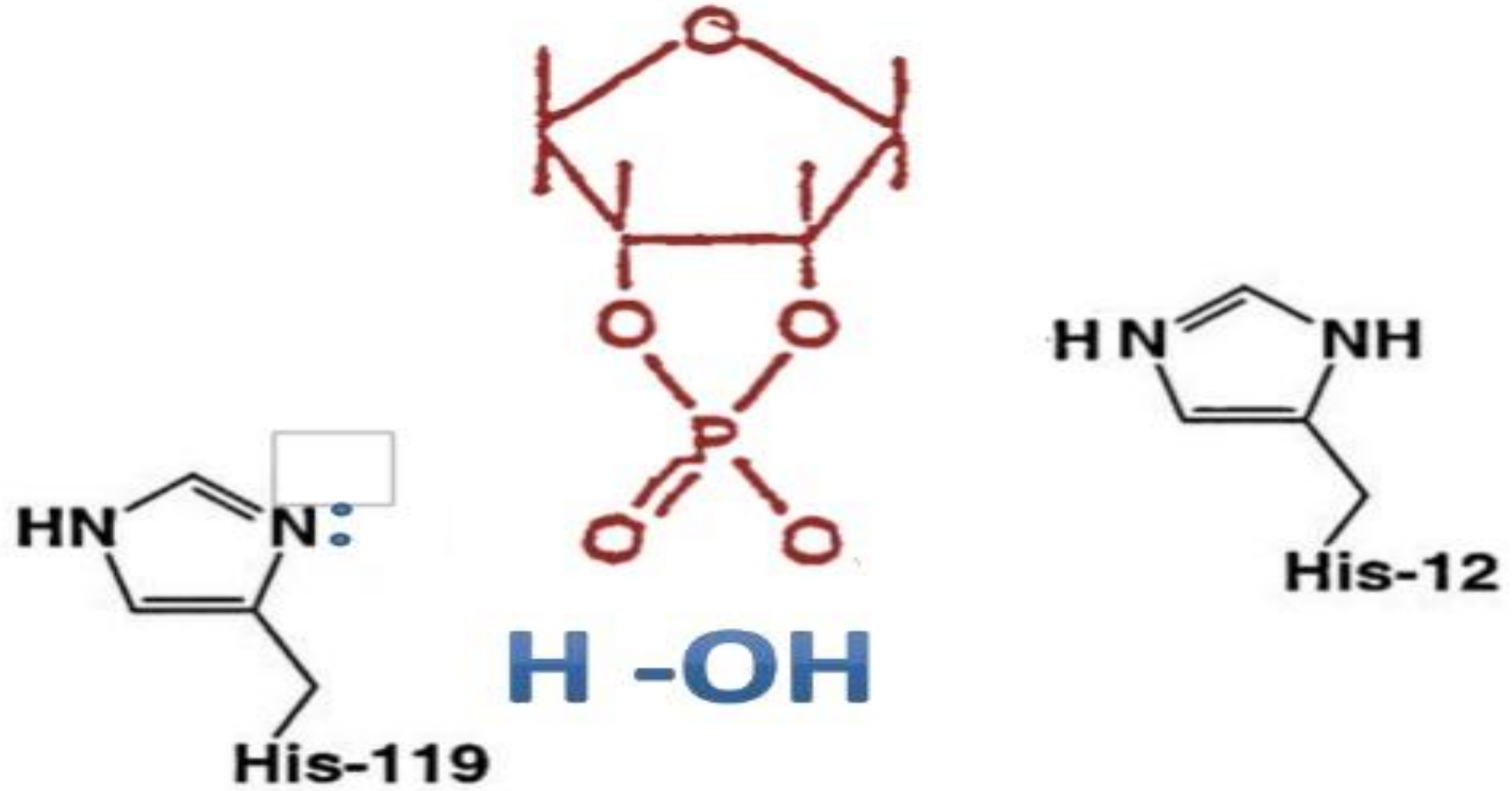
RNAse A



RNAse A



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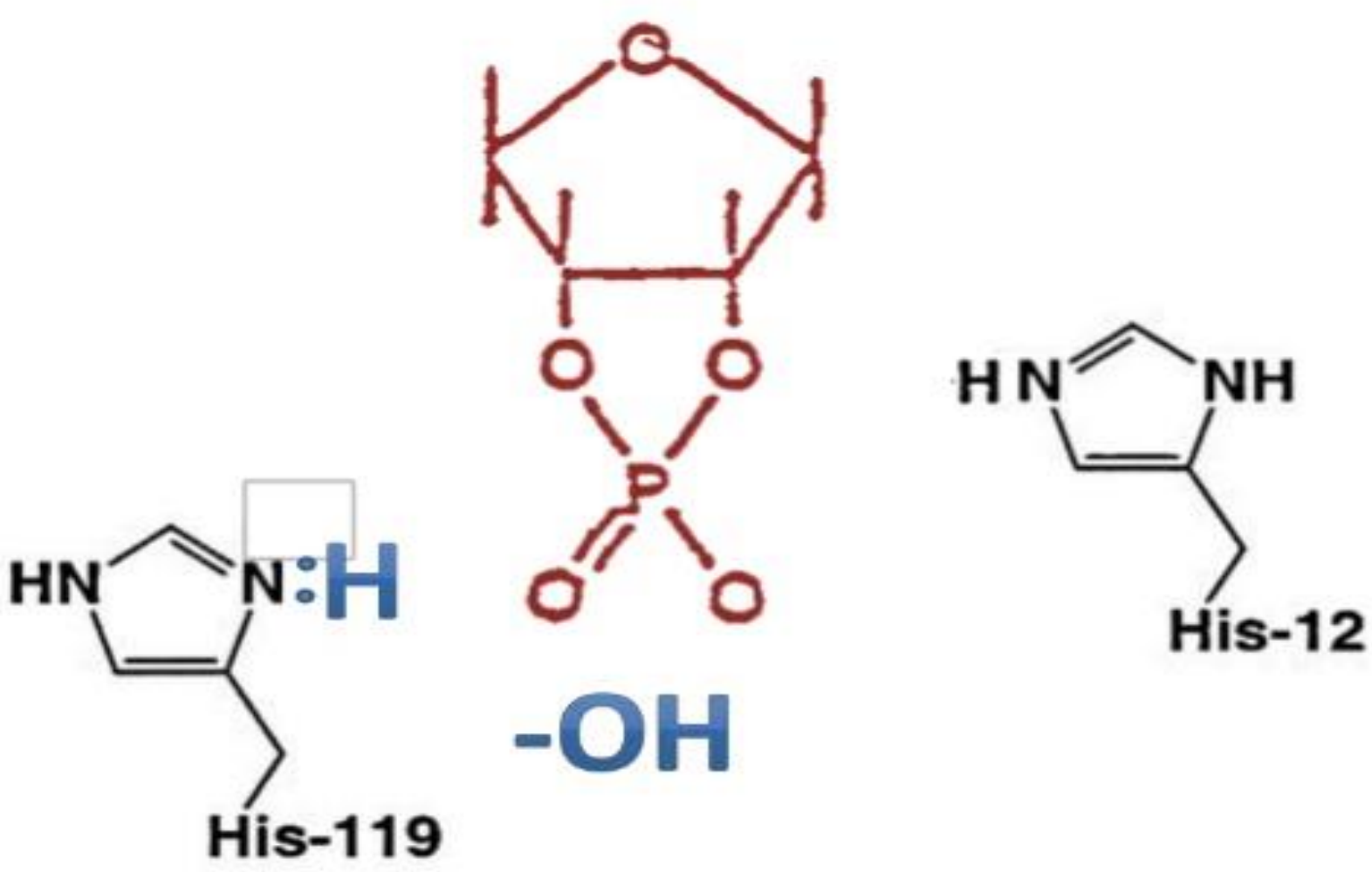
N
His-119
H -OH
HN
NH
His-12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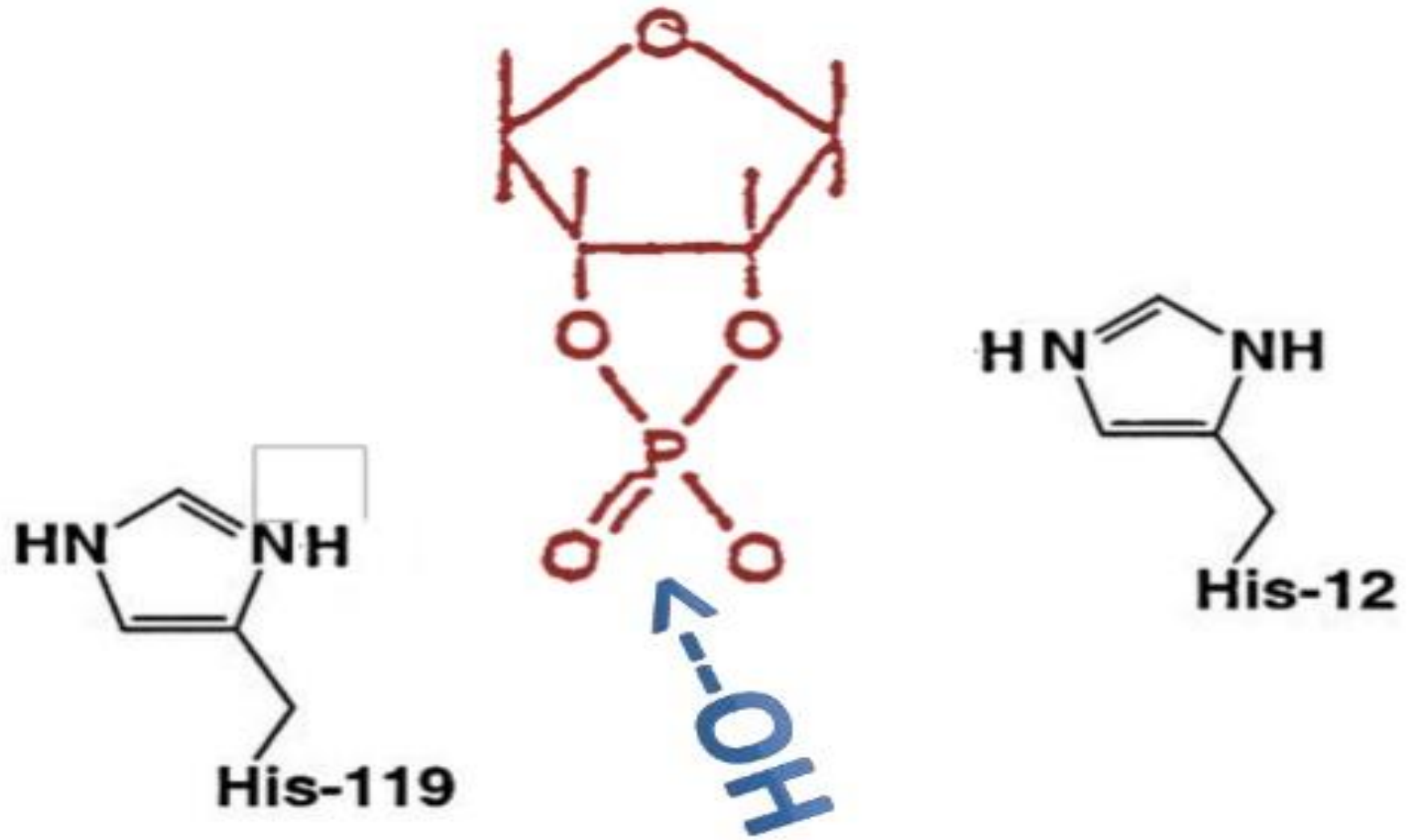
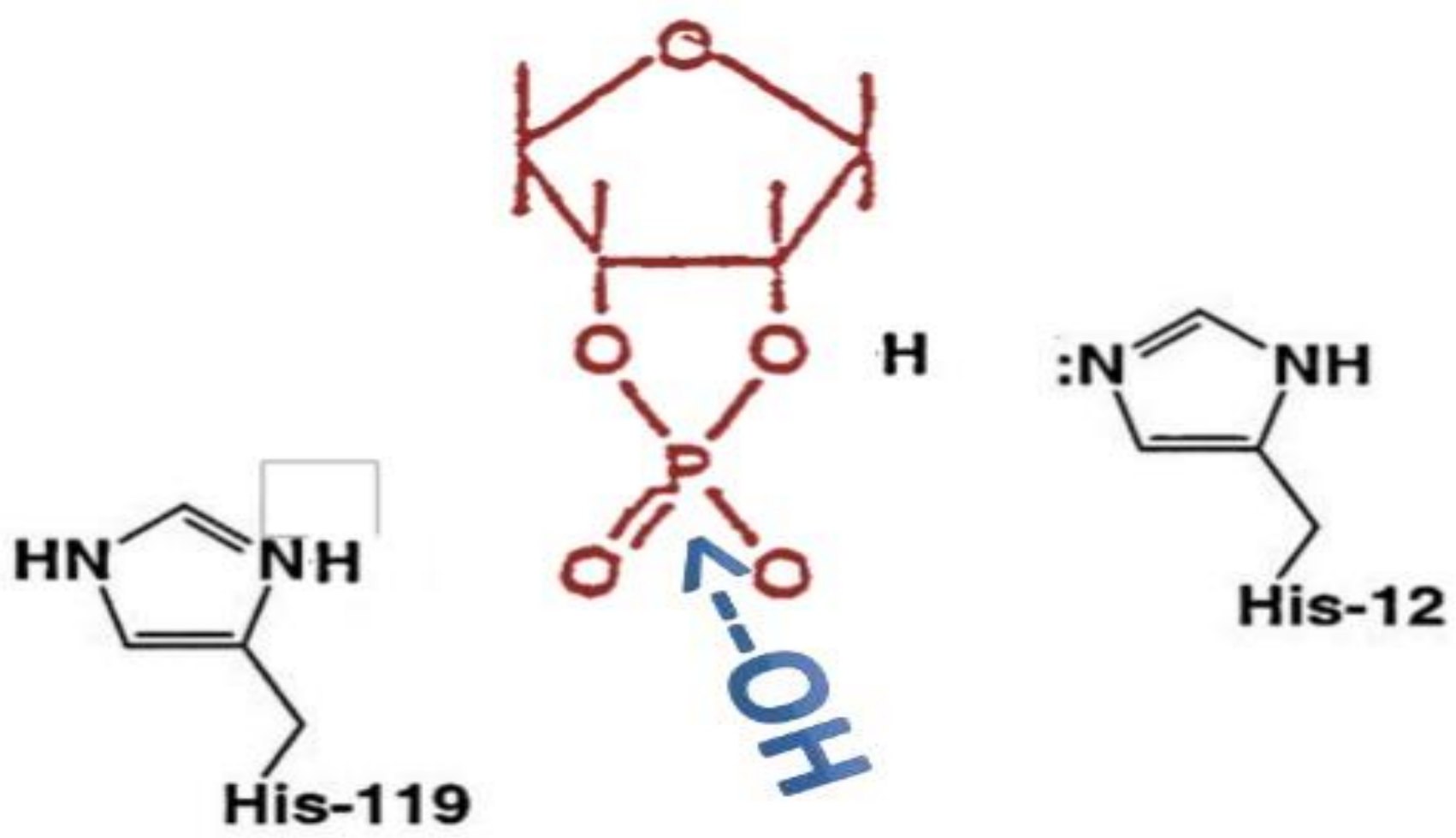
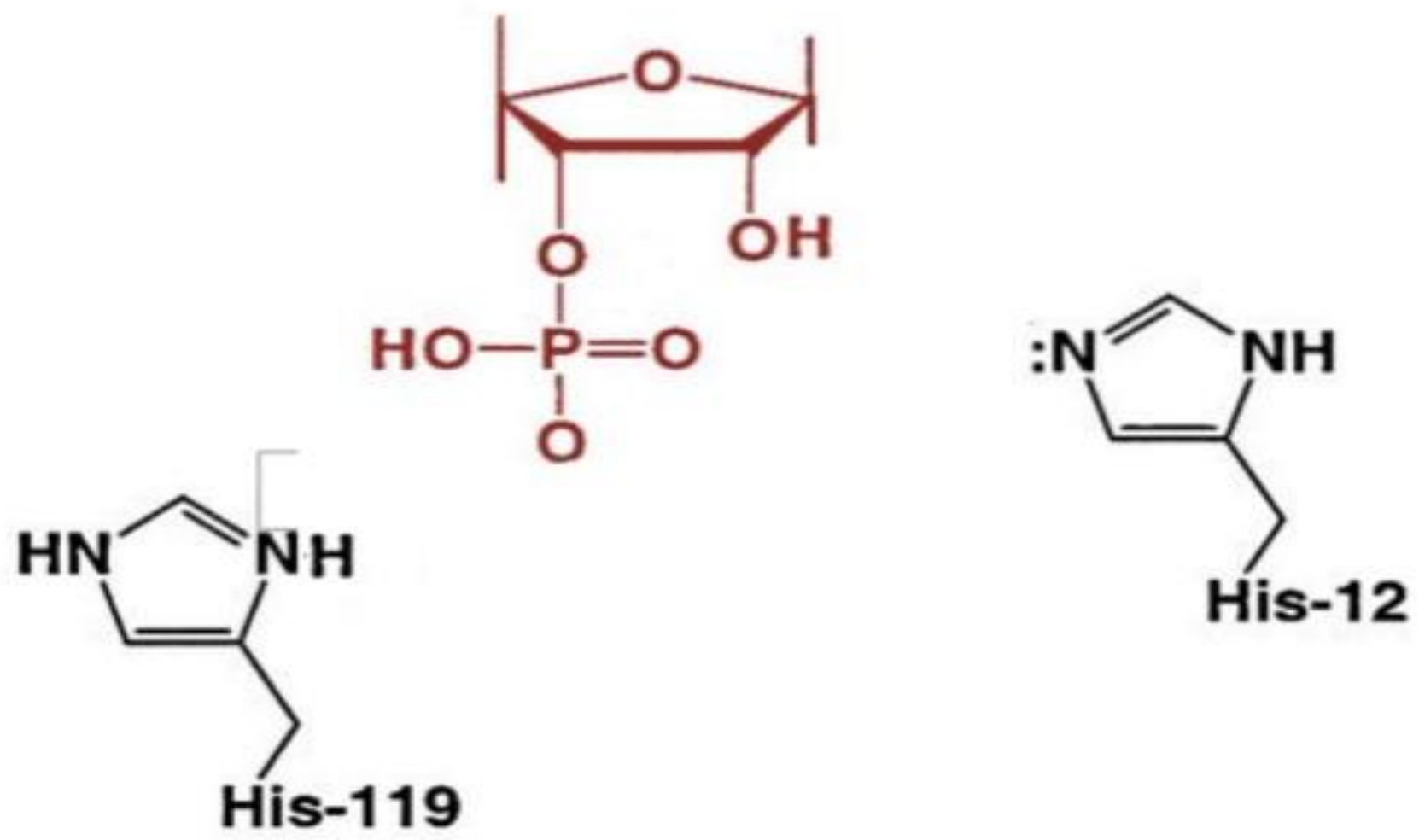
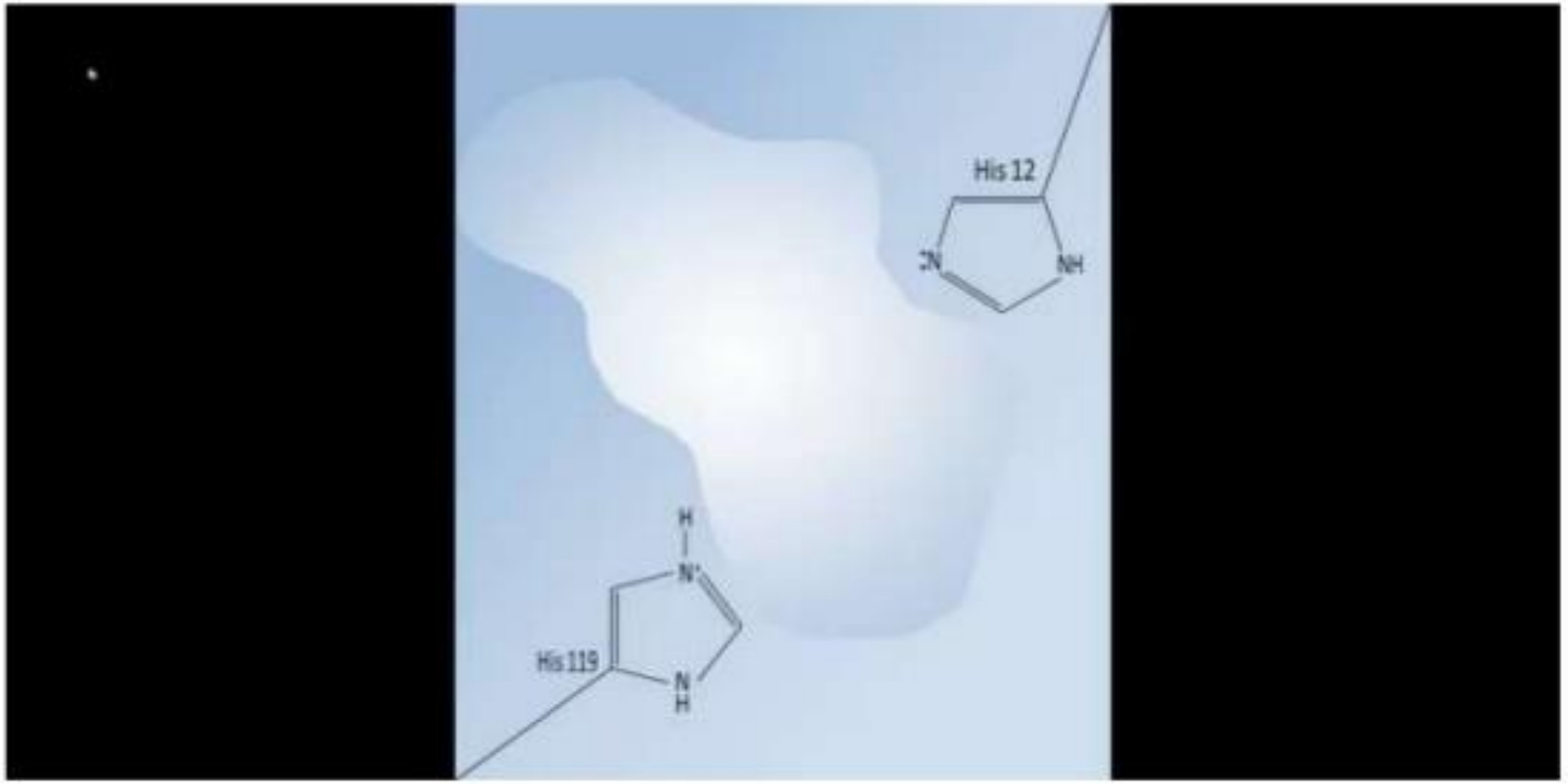
N:H
His-119
-OH
HN
NH
His-12


HN
NH
His-119
HO-
HN
NH
His-12


HN
NH
His-119
H
2'-OH
:N
NH
His-12

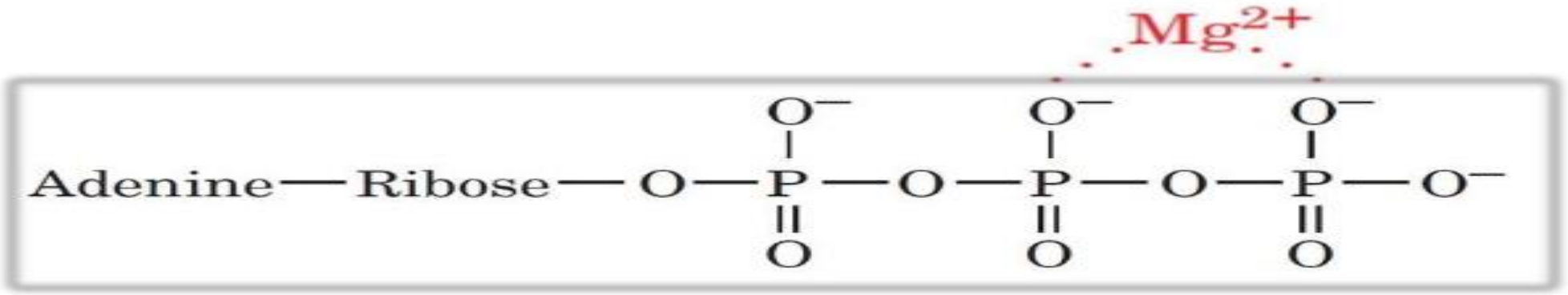

O
OH
O
HO—P=O
O
HN
NH
His-119
:N
NH
His-12


His 12
:N
NH
H
N
His 119
N
H

Why RNAse can’t act on DNA ?

Metal Ion catalysis

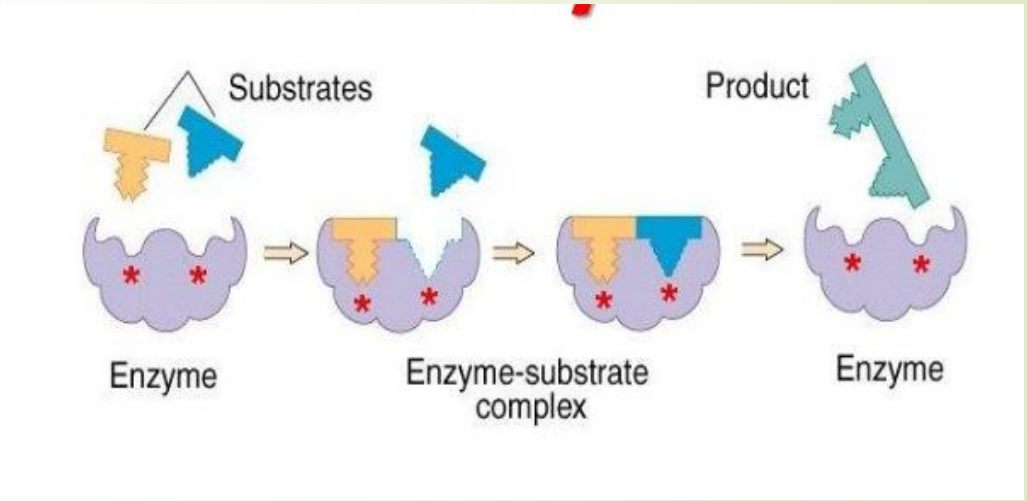
Charge Shielding by metals



- assist in binding of the substrate,
- stabilize developing anions in the reaction.
- can also accept and donate electrons in oxidation-reductio reactions.

Mechanism of enzyme action

- Catalysis by Proximity
- Acid-Base Catalysis
- Catalysis by Strain
- Covalent Catalysis
- Metal ion catalysis



MECHANISM-BASED INHIBITORS

Covalent Inhibitors

- **DFP** exerts its toxic effect by forming a covalent intermediate in the active site of acetylcholinesterase
- **Aspirin** (acetylsalicylic acid): covalent acetylation of an active site serine in the enzyme cycloxygenase

Transition State Analogs

- extremely potent and specific inhibitors
- PENICILLIN: suicide inhibitor
- ALLOPURINOL

Home work Question ?

What are Iso Enzymes ?

Why are they required ?

What are iso-forms of Enzymes

Post translational modification of proteins ?