

Catalysis

➤ Catalysis is a process that increases the rate at which a reaction approaches equilibrium

- Amino acid side chains that can donate or accept **protons** can participate in chemical reactions as **acid or base catalysts**.
- Nucleophilic groups can catalyze reactions through the transient formation of **covalent bonds** with the substrate.
- In **metal ion catalysis**, the unique electronic properties of the metal ion facilitate the reaction.
- Enzymes** accelerate reactions by bringing reacting groups together and orienting them for reaction.
- Transition state stabilization can significantly lower the **activation energy** for a reaction.

The types of catalytic mechanisms that enzymes employ have been classified as:

- 1) Acid- base catalysis.
- 2) Covalent catalysis.
- 3) Metal ion catalysis.
- 4) Electrosatic catalysis.
- 5) Proximity and orientation effects.
- 6) Preferential binding of the transition state complex.

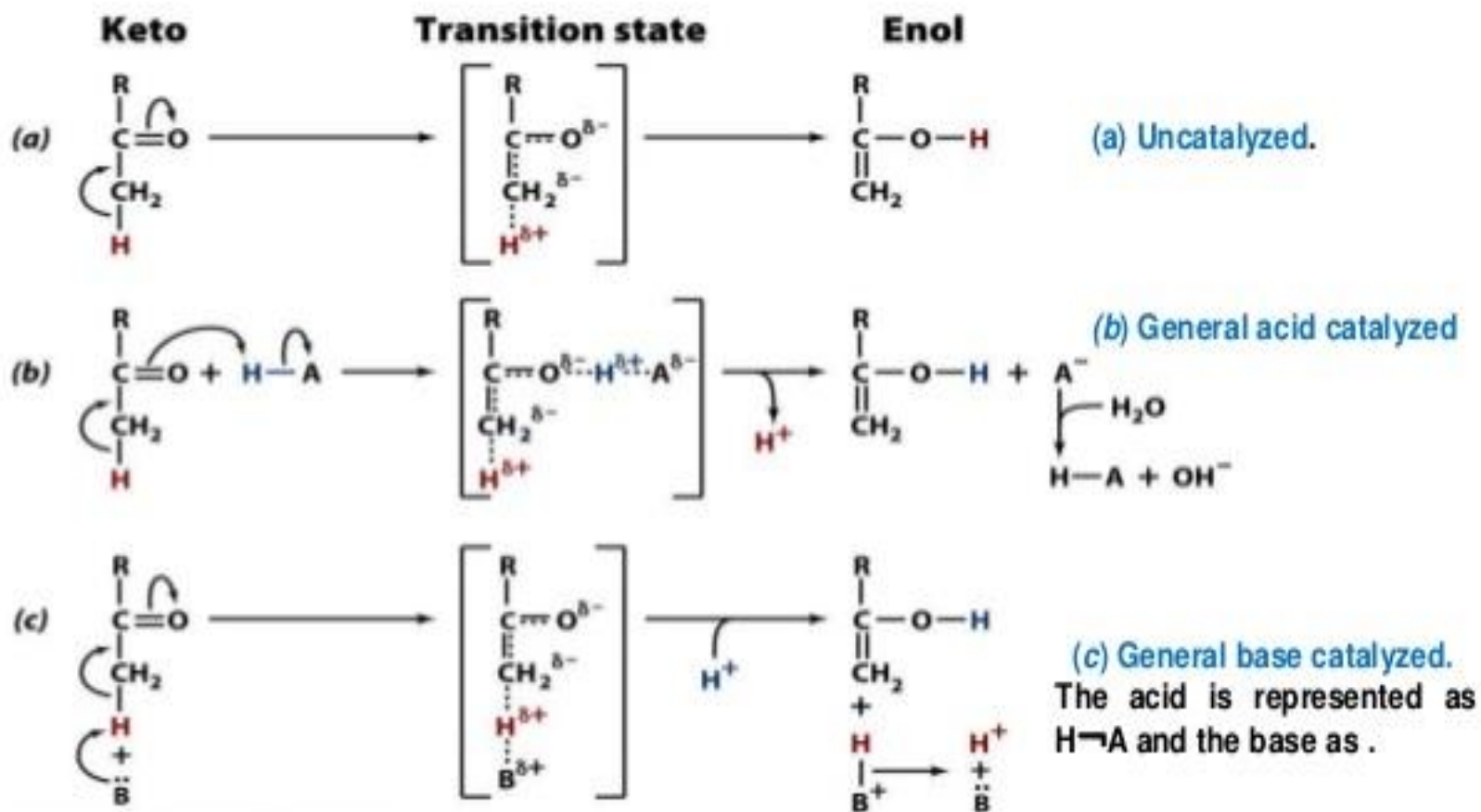
Acid- base catalysis

- **General acid catalysis:** Proton transfer from an acid lowers the free energy of a reaction's transition state

Example: an uncatalyzed keto-enol tautomerization reaction occurs quite slowly as a result of the high free energy of its carbanion-like transition state.

- A reaction may also be stimulated by **general base catalysis** if its rate is increased by proton abstraction by a base.
- Some reactions may be simultaneously subject to both processes; these are **concerted acid-base catalyzed reactions**.

Mechanisms of keto-enol tautomerization



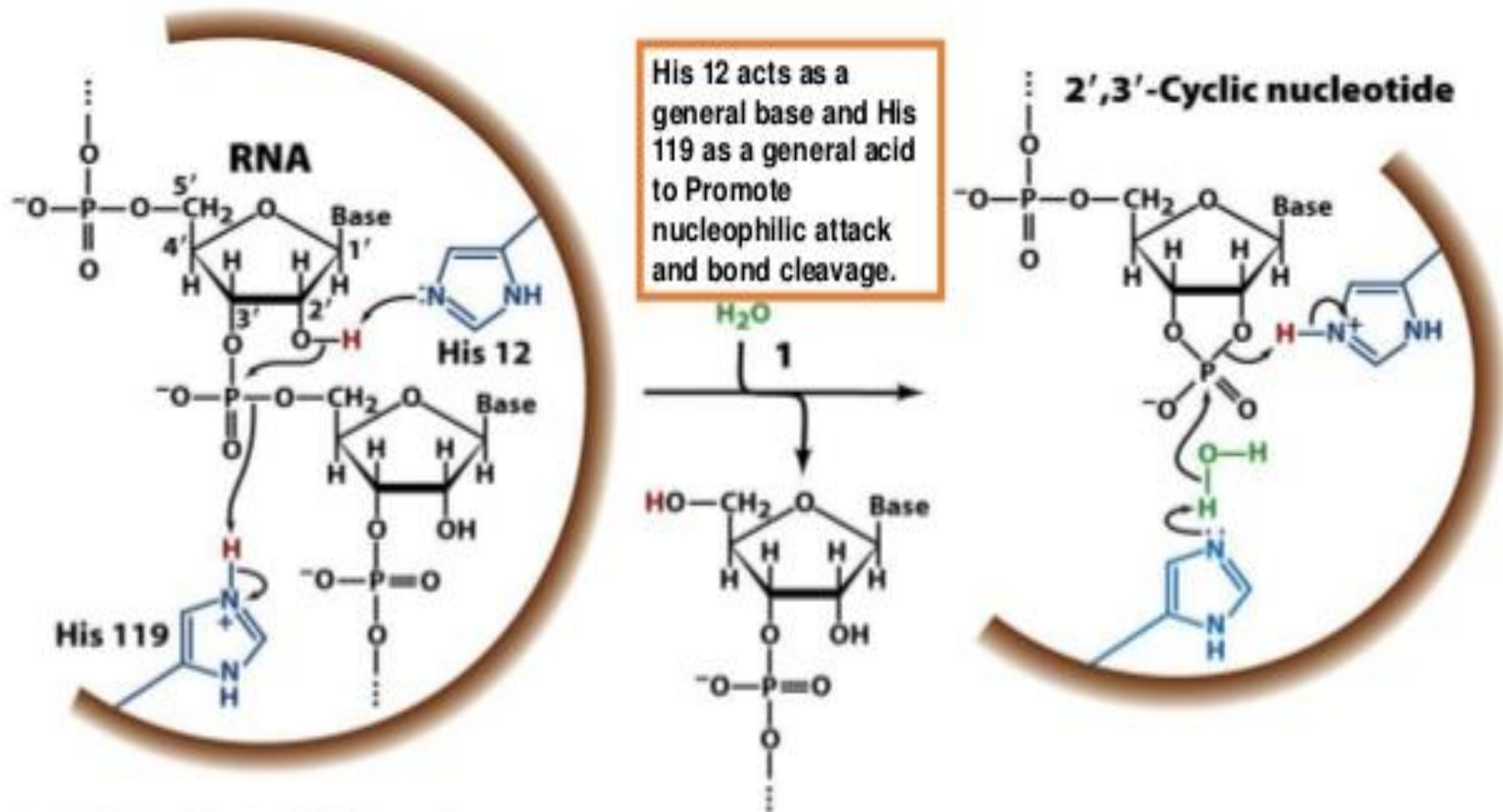
Concerted Acid-Base Catalysis

- Many types of biochemical reactions are susceptible to acid and/or base catalysis.
- These include the hydrolysis of peptides and esters, the reactions of phosphate groups, tautomerizations, and additions to carbonyl groups.
- The side chains of the amino acid residues Asp, Glu, His, Cys, Tyr, and Lys have pK 's in or near the physiological pH range

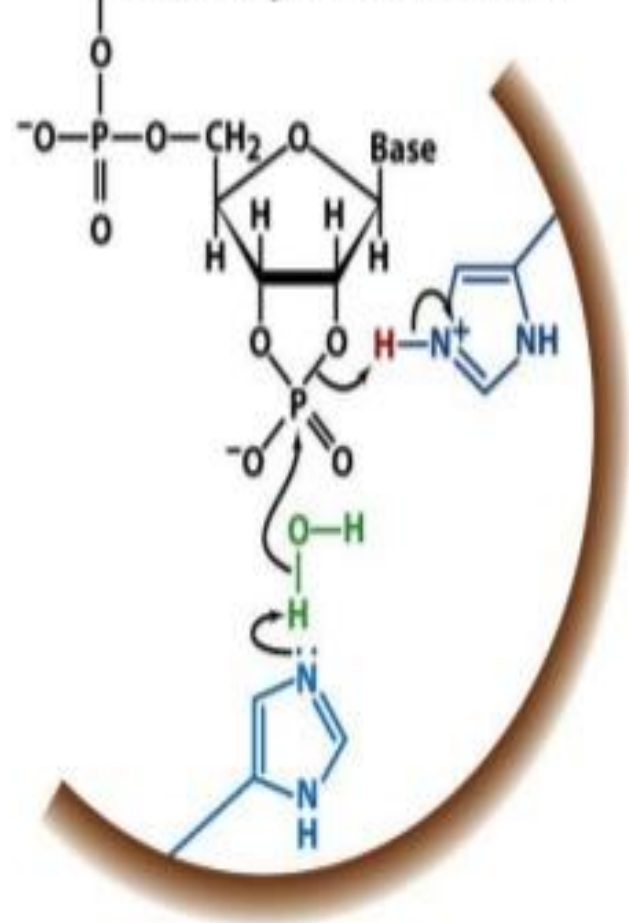
RNase A is an acid-base catalyst

- Bovine pancreatic RNase A: Digestive enzyme secreted by pancreas into the small intestine
- The isolation of 2',3' cyclic nucleotides from RNase A digests of RNA indicates that the enzyme intermediates.
- pH-dependence indicates 2 important His, 12, 119 that act in a concerted manner as general acid and base catalysts to catalyze a **two-step reaction**

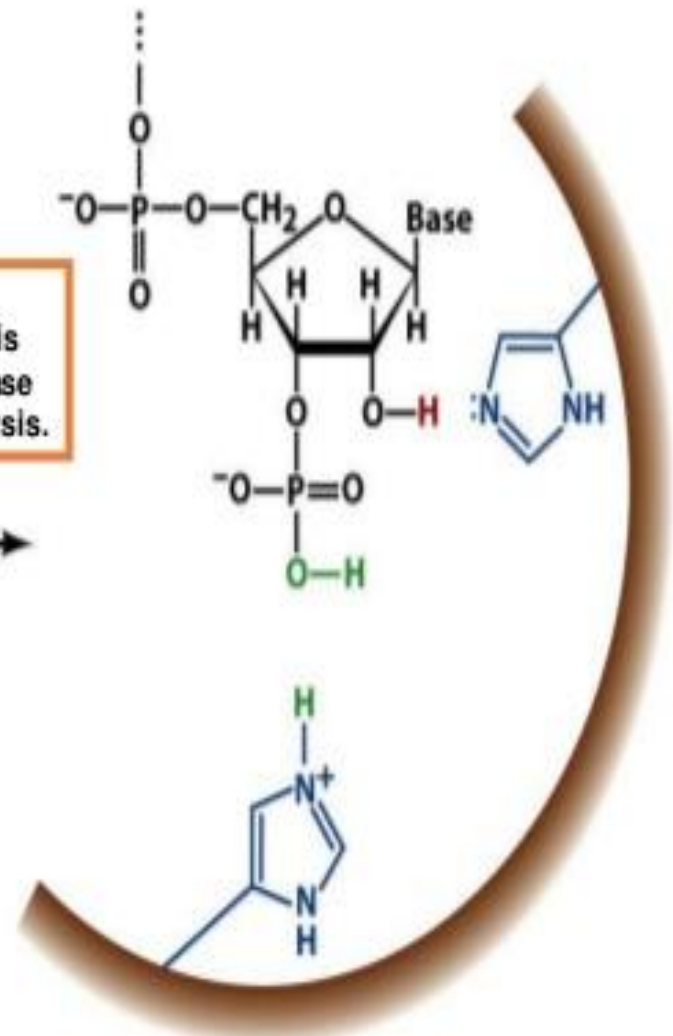
The RNase A mechanism



2',3'-Cyclic nucleotide



His 12 acts as a general acid and His 119 as a general base to promote hydrolysis.



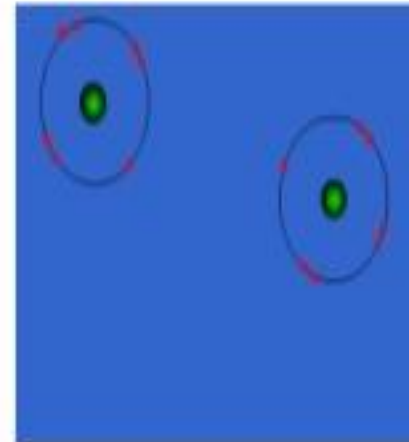
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Covalent Catalysis Usually Requires a Nucleophile

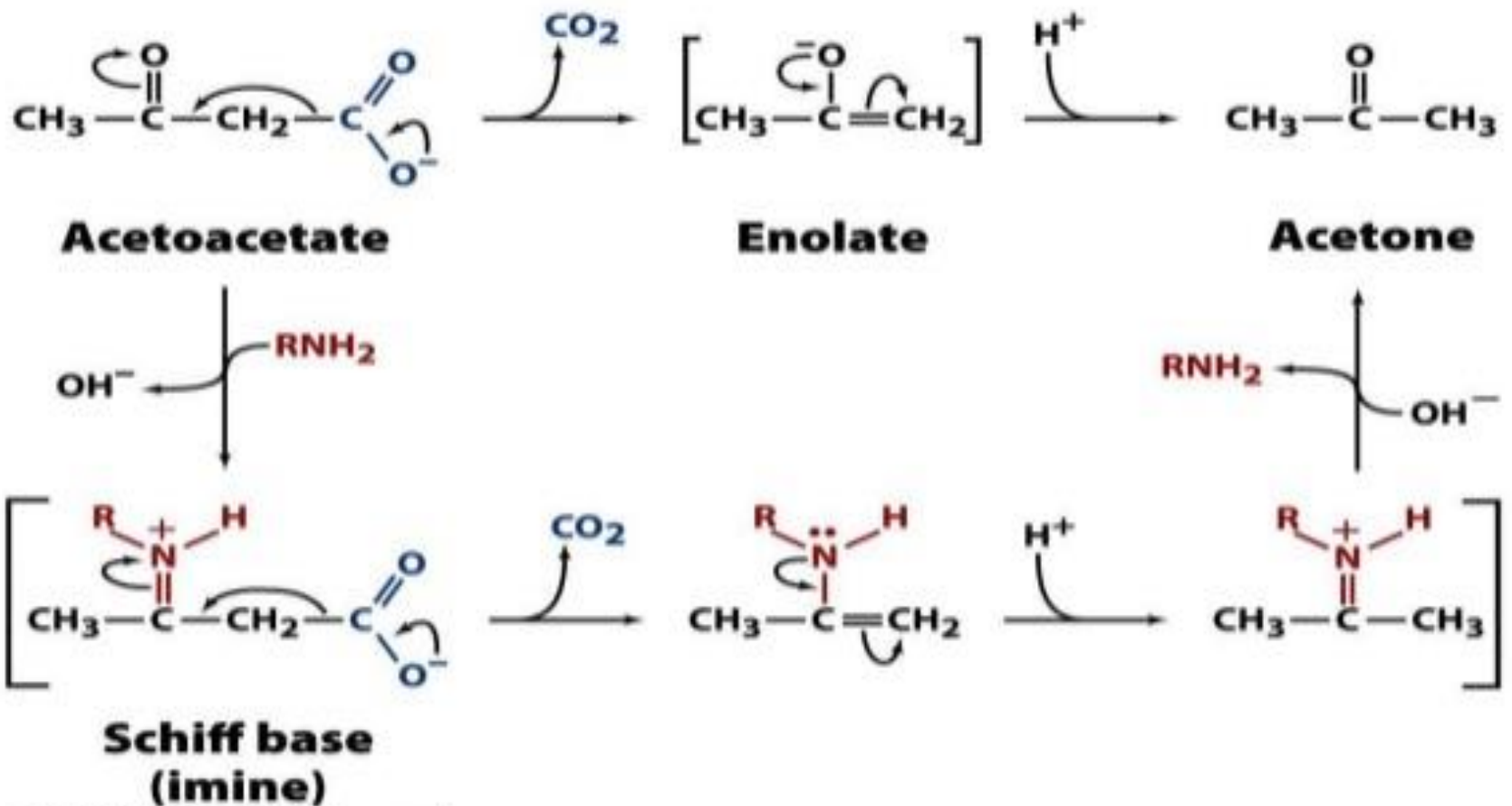
Covalent Catalysis accelerates reaction rates through the transient formation of a catalyst-substrate covalent bond

Usually, nucleophilic group on enzyme attacks an electrophilic group on the substrate = nucleophilic catalysis

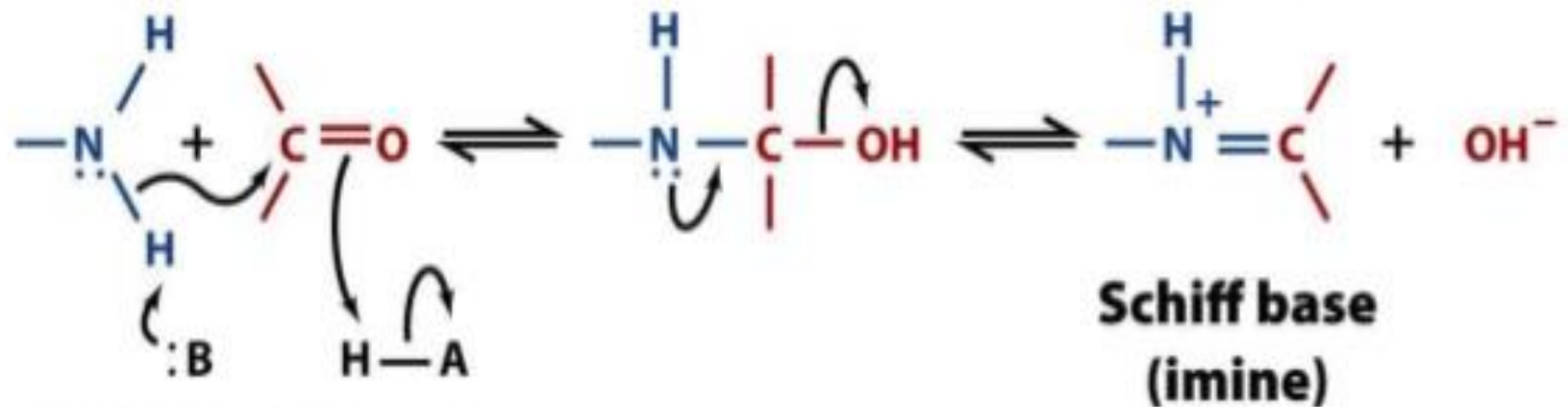
Example: decarboxylation of acetoacetate



Decarboxylation of acetoacetate



Three stages of Covalent Catalysis



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1. Nucleophilic attack of enzyme on substrate
2. Withdrawal of electrons from the reaction center by the now electrophilic catalyst.
3. Elimination of catalysts by reversion of step 1.

Important aspect of covalent catalysis

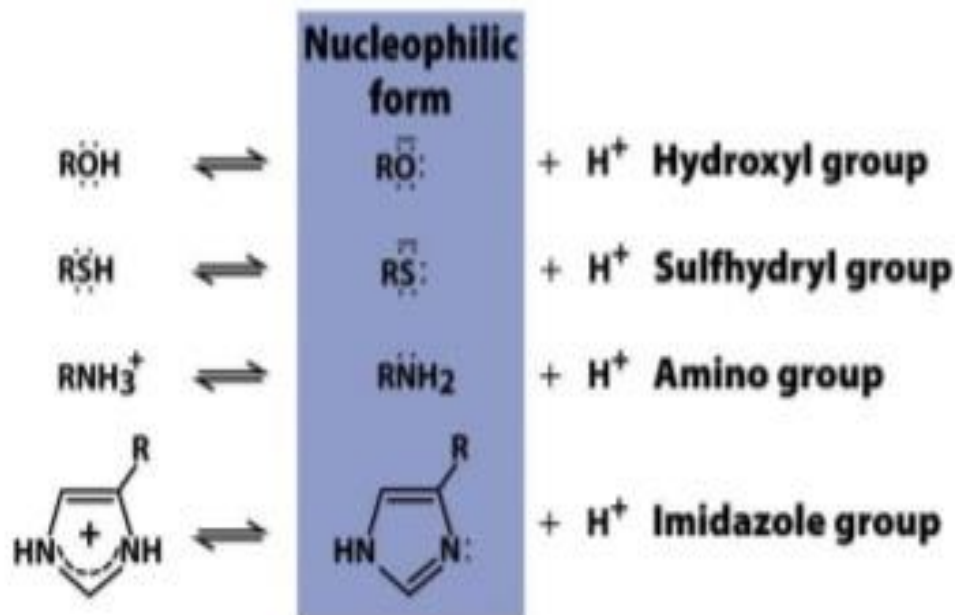
The more stable the covalent bond formed, the less easily it can be decomposed in the final step of a reaction

Good covalent catalysis must be:

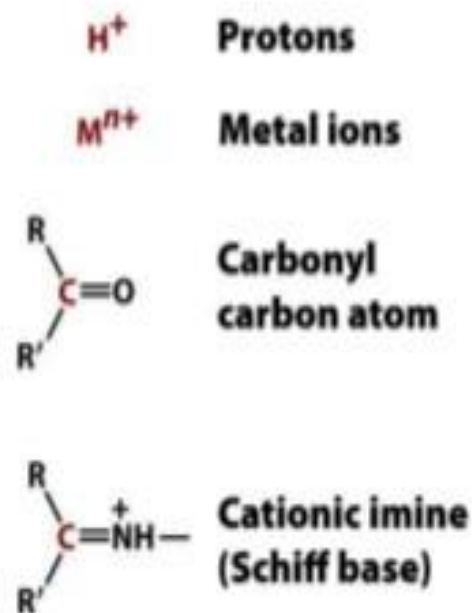
- (i) highly nucleophile
- (ii) the ability to form a good leaving group. Groups with high polarizability (highly mobile electrons), such as :
 - Functional groups in proteins include (the unprotonated amino group of Lys, the imidazole group of His, the thiol group of Cys, the carboxyl group of Asp, and the hydroxyl group of Ser).
 - In addition, several coenzymes, notably (thiamine pyrophosphate and pyridoxal phosphate).

Nucleophilicity of a substance is related to its **basicity**:

(a) Nucleophiles



(b) Electrophiles



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Metal ion catalysis base catalysis

1/3 of all known enzymes require the presence of metal ions for catalytic activity.

There are two classes of metal ion – requiring enzymes that are distinguished by the strengths of their ion-protein interactions:

- **Metalloenzymes** contain tightly bound metal ion (Fe^{2+} , Fe^{3+} , Cu^{2+} , Zn^{+2} , Mn^{2+} , Co^{3+}),
- **Metal- activated enzymes**: loosely bind metal ions from solution, usually the alkali and alkaline earth metal ions (Na^+ , K^+ , Mg^{2+} or Ca^{2+}).

Metal Ion Cofactors Act as Catalysts

Metal ions participate in the catalytic process in three major ways:

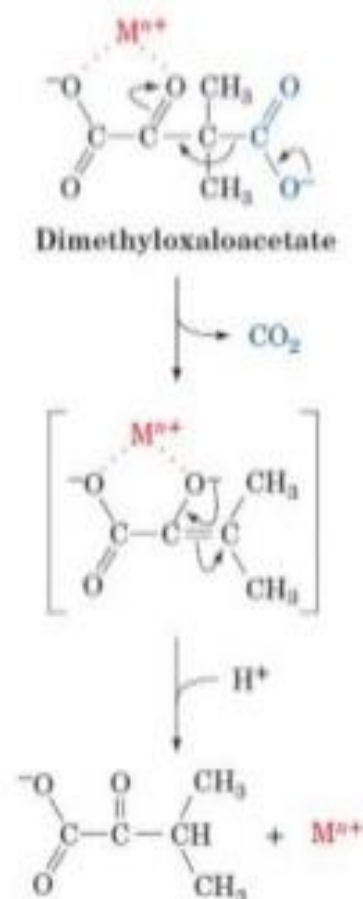
- 1. By binding to substrate to orient them properly for reaction**
- 2. By mediating oxidation-reduction reactions through reversible changes in the metal ions oxidation state**
- 3. By electrostatically stabilizing or shielding negative charges**

Often: Metal ion acts similar to a proton, or polarizes water to generate OH

❖ Metal Ion Promote Catalysis through charge stabilization

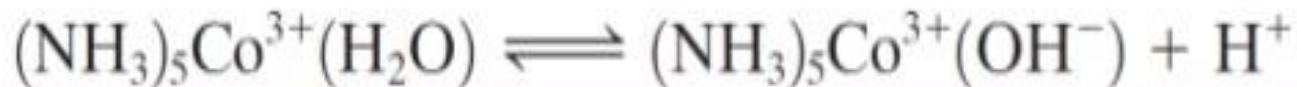
- In many metal ion–catalyzed reactions, the metal ion acts in much the same way as a proton to neutralize negative charge.
- Yet metal ions are often **much more effective catalysts than protons** because metal ions can be present in high concentrations at neutral pH and may have charges greater than +1.

An example non-enzymatic catalysis by a metal ion is the decarboxylation of **dimethyloxaloacetate** catalyzed by metal ions such as Cu^{+2} and Ni^{+2}



❖ Metal Ions Promote Nucleophilic Catalysis via Water Ionization

- When a metal ion binds with water molecules, the charge of the metal ion causes that water molecule to be more acidic than free H₂O and therefore a source of OH⁻ ions even below neutral pH's.
- **For example**, the water molecule of (NH₃)₅Co³⁺(H₂O) ionizes according to the reaction:



with a pK of 6.6, which is ~9 pH units below the pK of free H₂O. The resulting metal ion-bound hydroxyl group is a potent nucleophile.

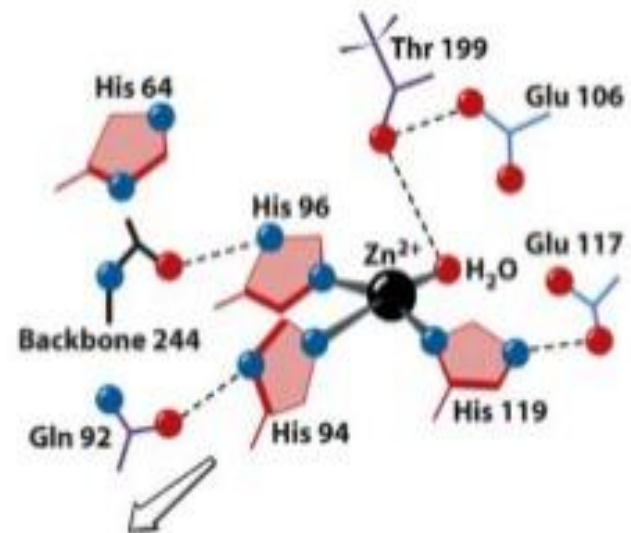
An example of mechanism of metal ion catalysis

is catalytic mechanism of the following reaction by

carbonic anhydrase:

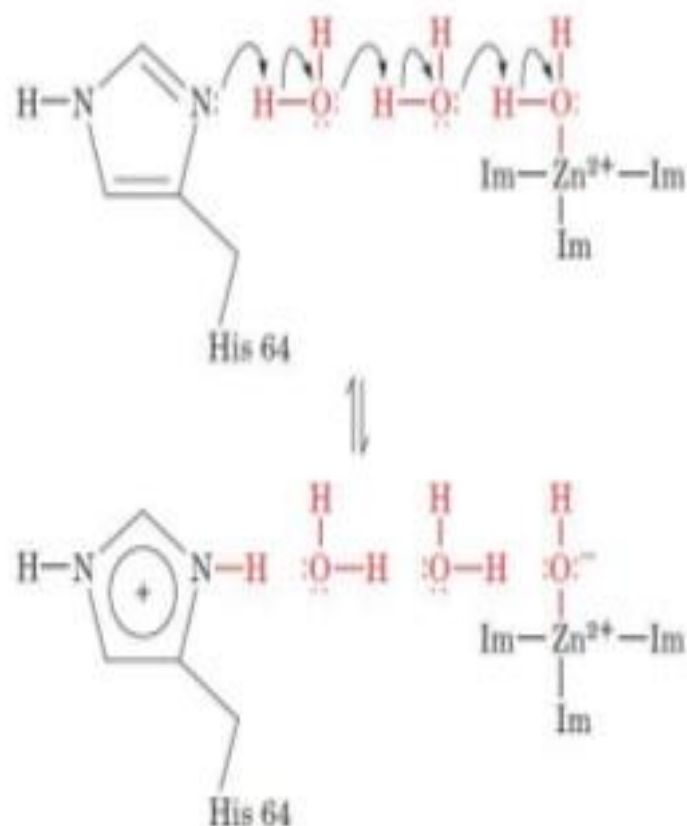


In carbonic anhydrase a Zn^{2+} ion is present at the active site cleft. The central metal atom is tetrahedrally coordinated by three imidazole (Im) ring of three His side chains and an O atom of either an HCO_3^- ion or a water molecule.



The role of Zn^{2+} in carbonic anhydrase

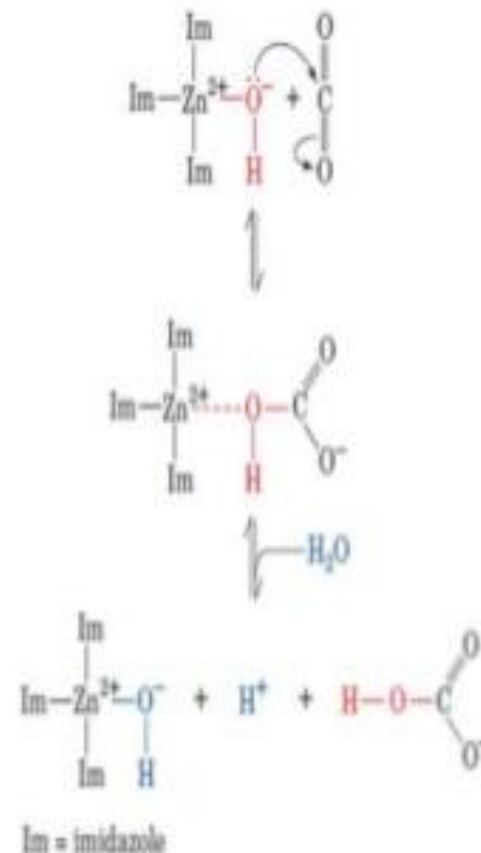
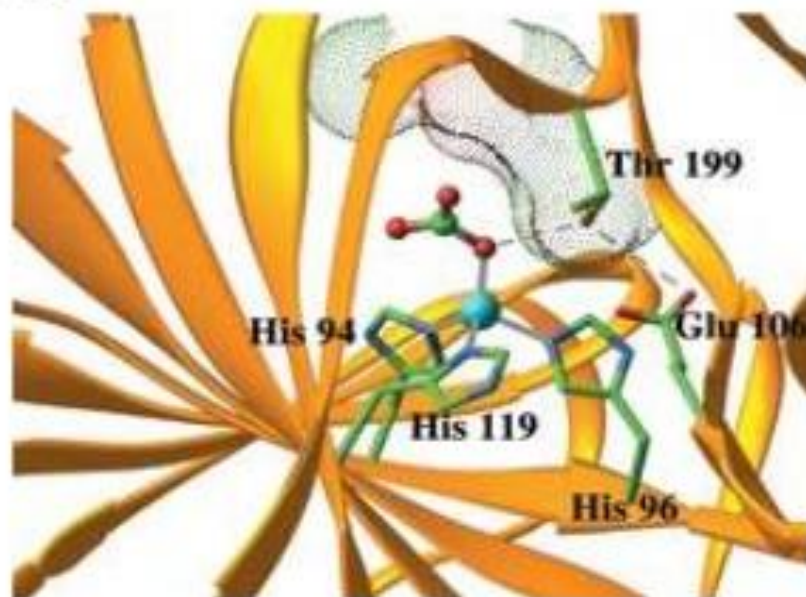
1- We begin with a water molecule bound to the protein in the Zn^{2+} ion's fourth liganding position. This Zn^{2+} -polarized H_2O ionizes in a process facilitated through general base catalysis by His 64 in its "in" conformation.



The role of Zn²⁺ in carbonic anhydrase

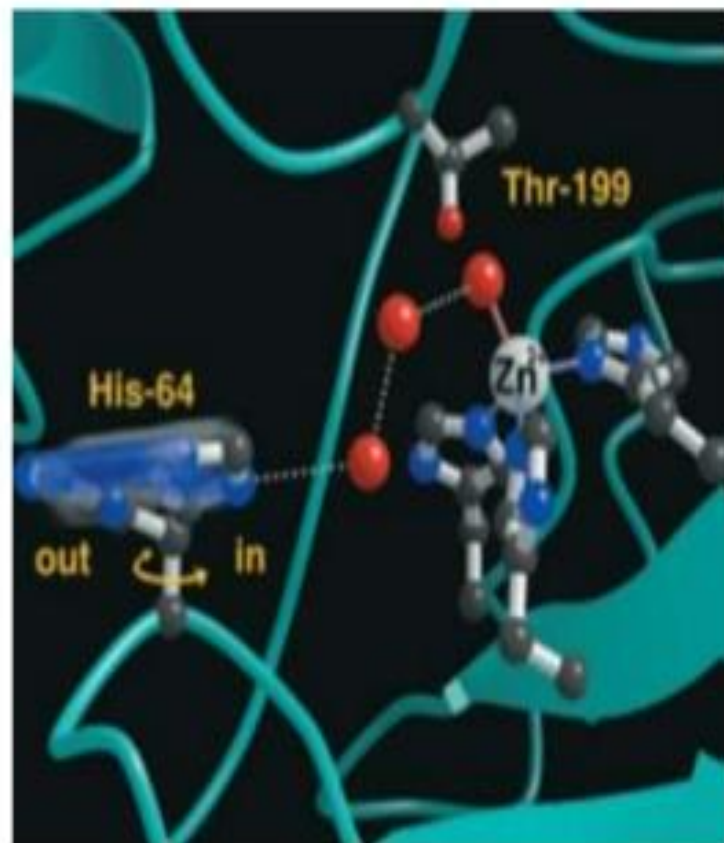
2. The resulting Zn²⁺-bound OH⁻ ion nucleophilically attacks the nearby enzymatically bound CO₂, thereby converting it to HCO₃⁻

In doing so, the Zn²⁺-bound OH group donates a hydrogen bond to Thr 199, which in turn donates a hydrogen bond to Glu 106.



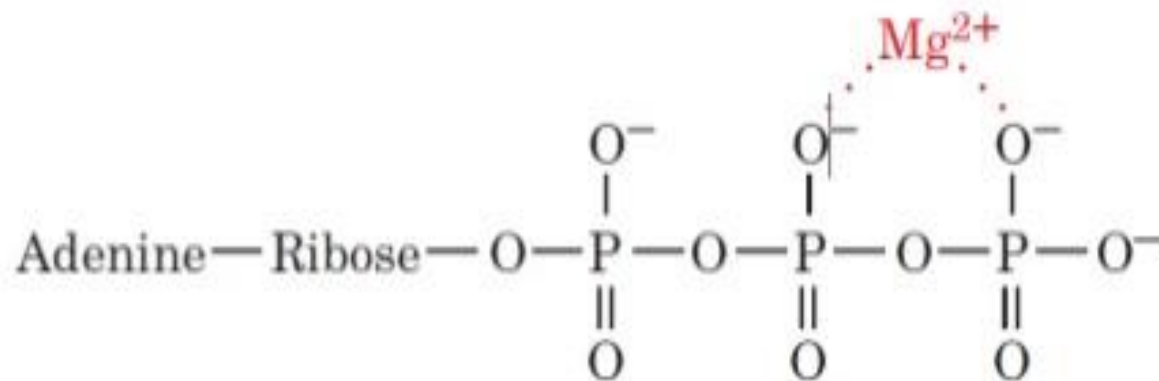
The role of Zn^{2+} in carbonic anhydrase

3. The catalytic site is regenerated by the exchange of the Zn^{2+} -bound HCO_3^- reaction product for H_2O together with the deprotonation of His 64. In the latter process, His 64 swings to its "out" conformation, which may facilitate proton transfer to the bulk solvent.



❖ Metal Ions Promote Reactions through Charge Shielding

- **For example**, the actual substrates of kinases (phosphoryl-transfer enzymes utilizing ATP) are Mg^{+2} -ATP complexes such as:



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Electrostatic catalysis

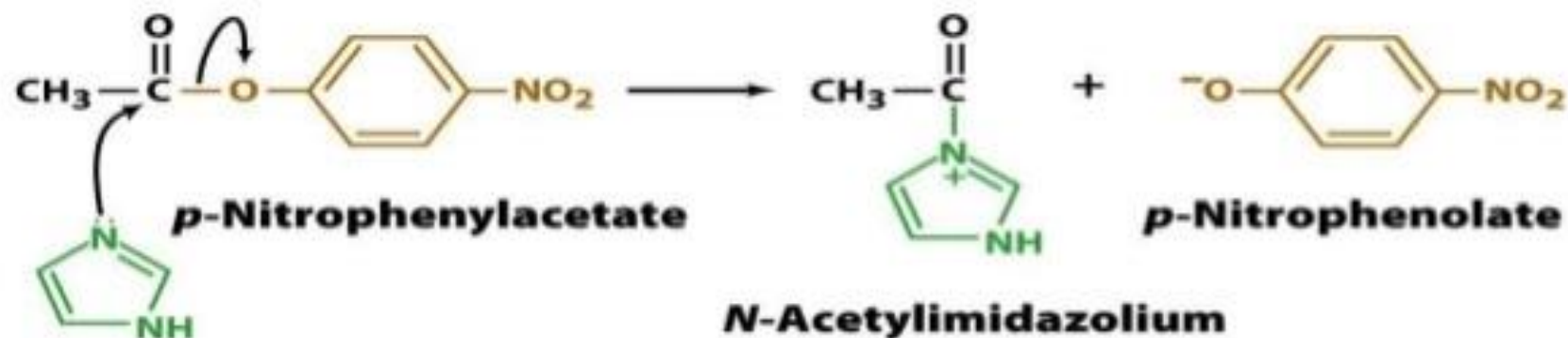
- concept not always "listed" separately because it's involved in many other aspects of catalytic mechanisms
- **Some examples:**
 - providing lower dielectric constant of environment in active site (hydrophobic environment)
 - altering pK values of specific functional groups
 - stabilizing a particular conformation of critical groups in active site by electrostatic interactions
 - stabilizing (binding) a charged intermediate or transition state by providing an oppositely charged enzyme group nearby.

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Catalysis can occur through proximity and orientation effects

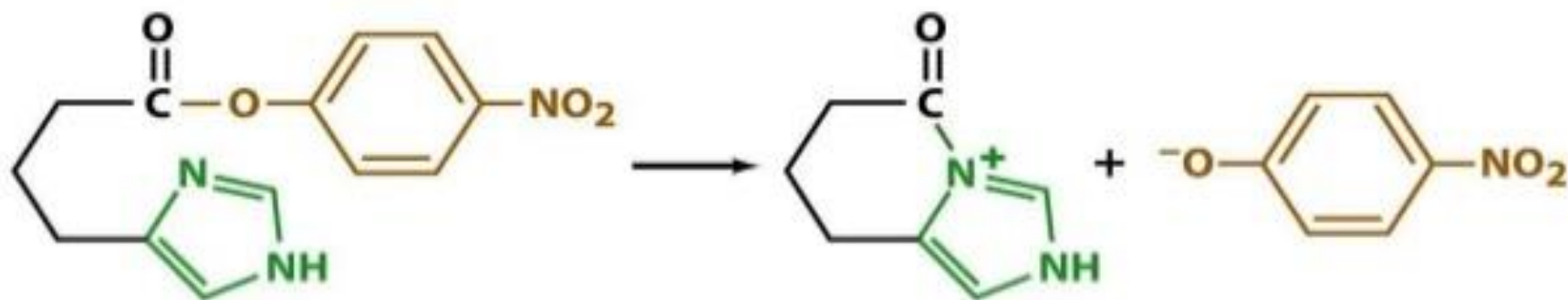
- Enzymes are much more efficient catalysts than organic model compounds
- Due to **proximity** and **orientation** effects
- Reactants come together with proper spatial relationship
- Example: the bimolecular reaction of imidazole with ***p*-nitrophenylacetate**.
intramolecular reaction is 24 times faster

Inter- versus intramolecular reaction



Imidazole

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Catalysis can occur through proximity and orientation effects

Enzymes are usually much bigger than their substrates

By oriented binding and immobilization of the substrate, enzymes facilitate catalysis by four ways:

1. bring substrates close to catalytic residues
2. Binding of substrate in proper orientation (up to 10^2 -fold)
3. Stabilization of transition state by electrostatic interactions
4. freezing out of translational and rotational mobility of the substrate (up to 10^7 -fold)

Proximity Alone Contributes Relatively Little to Catalysis

- 1. Reactant species, that is, functional groups, are about the size of water molecules.**
- 2. Each reactant species in solution has 12 nearestneighbor molecules, as do packed spheres of identical size.**
- 3. Chemical reactions occur only between reactants that are in contact.**
- 4. The reactant concentration in solution is low enough so that the probability of any reactant species being in simultaneous contact with more than one other reactant molecule is negligible.**