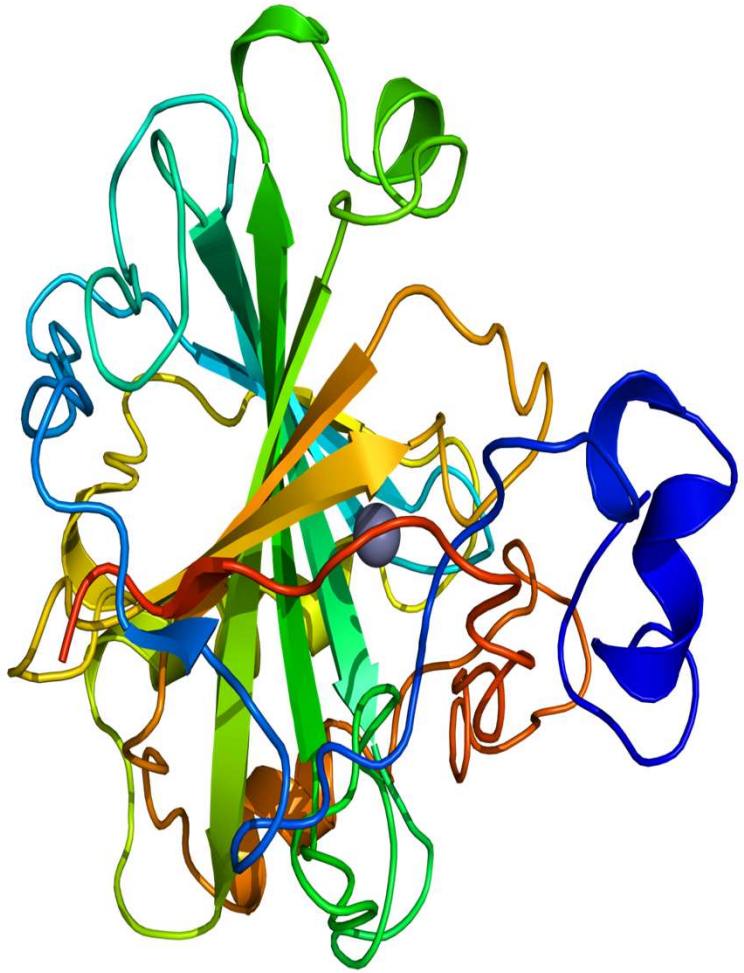
Enzymes

Suma Somasekharan

What are Enzymes?

- Enzymes are biological catalysts – they accelerate the rate of a reaction
- They are themselves not altered or consumed in the process.
- Functionally they are the largest group of Proteins
- The exception is Ribozymes which are RNA based enzymes
- Catalyze innumerable reactions- e.g. breakdown of food to generate, energy, DNA synthesis



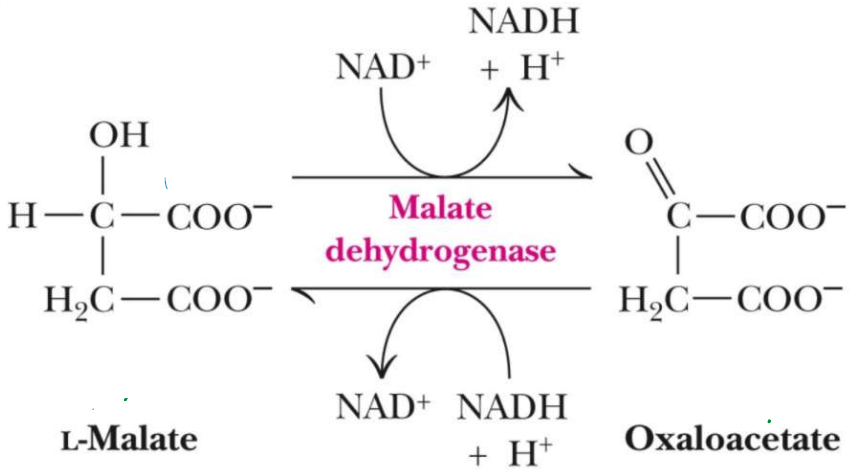
Structure of Carbonic anhydrase

Björn Sjöblom et al. PNAS 2009;106:10609-10613

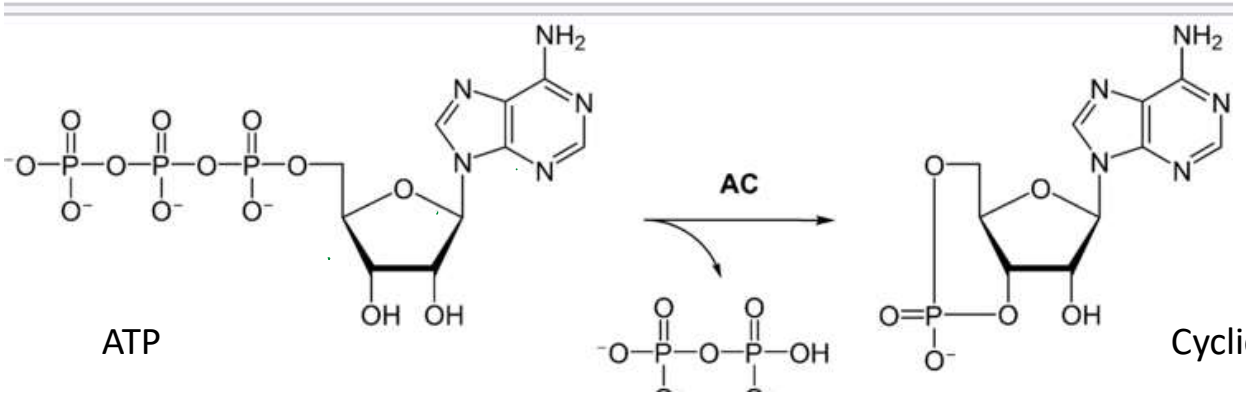
Enzyme classes-Types of reactions catalyzed by Enzymes

Enzymes	Types of reactions	Examples
Oxidoreductases	Oxidation - Reduction	Pyruvate dehydrogenase, Carbonic anhydrase
Transferases	Group transfers	Aminotransferase, Kinases
Hydrolases	Hydrolysis	Chymotrypsin, Carboxypeptidase
Lyases	Group elimination or elimination of double bond	Adenyl cyclase, Decarboxylases
Isomerases	Isomerization	Isomerases
Ligases	Bond formation coupled with ATP	DNA ligase

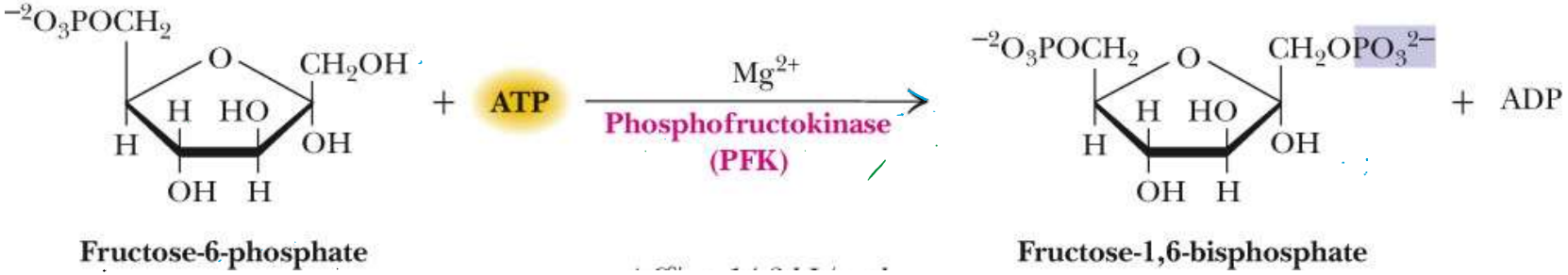
Oxidoreductases



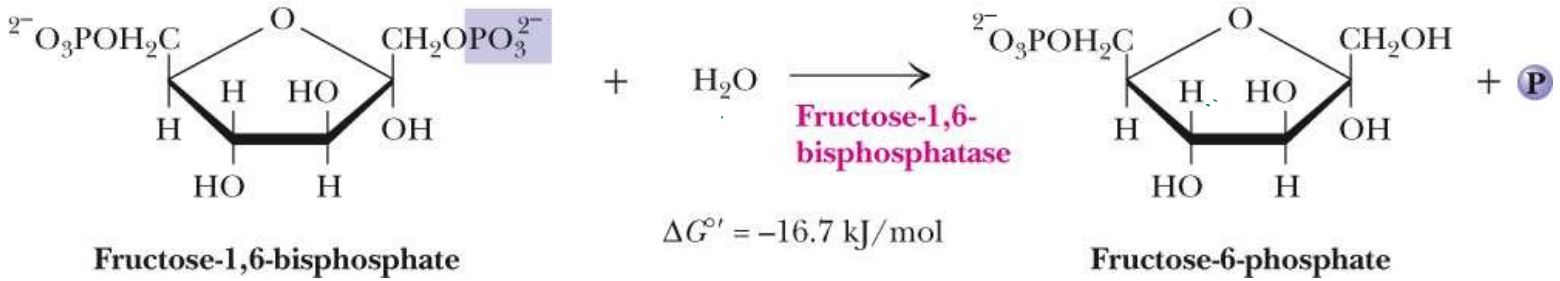
Lyases



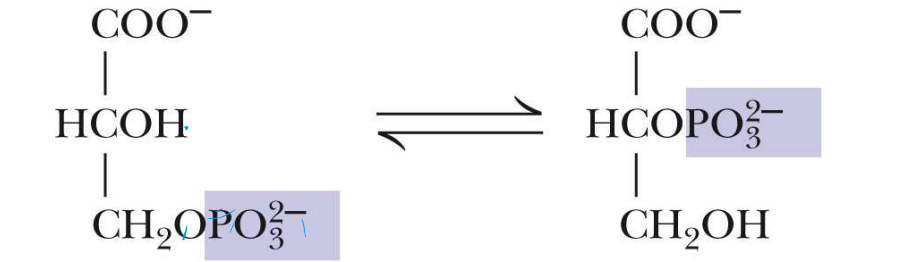
Transferases



Hydrolases



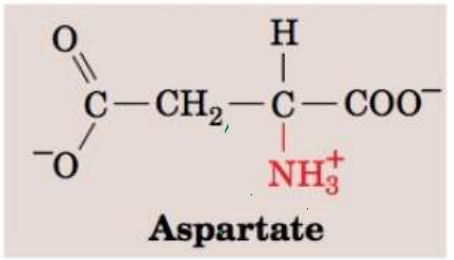
Isomerases



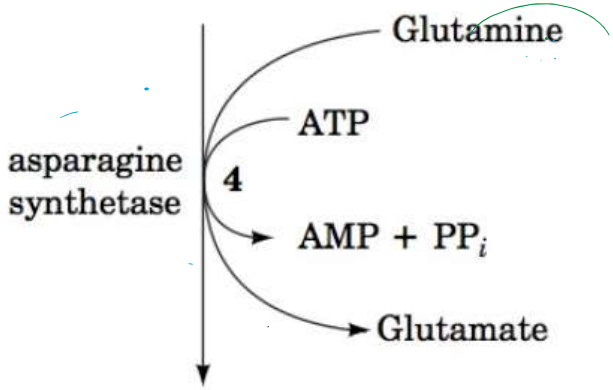
3-Phosphoglycerate (3-PG) ⇌ **2-Phosphoglycerate (2-PG)**

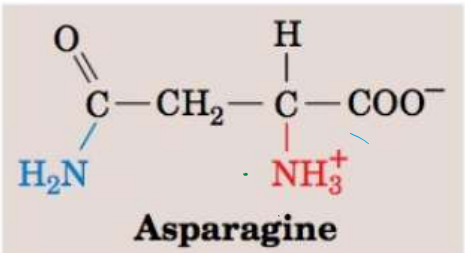
Phosphoglycerate mutase

Ligases



Aspartate





Asparagine

Catalytic Efficiency

Enzymes bind one more substrates and catalyze their rapid conversion to products.

The rate of an enzyme catalyzed reaction is many orders of magnitude greater than an uncatalyzed reaction

Turnover rate: The number of molecules of substrate converted to product per enzyme molecule per second

$$\mathbf{CO_2 + H_2O \leftrightarrow H_2CO_3 \rightarrow HCO3- + H^+}$$

The enzyme Carbonic anhydrase.

It catalyzes this reaction at a rate million times faster than the uncatalyzed reaction

$$\mathbf{Starch \rightarrow Glucose + Maltose}$$

The enzyme in saliva- Alpha amylase

Catalyzed reaction is million times faster than the uncatalyzed

Properties of Enzymes

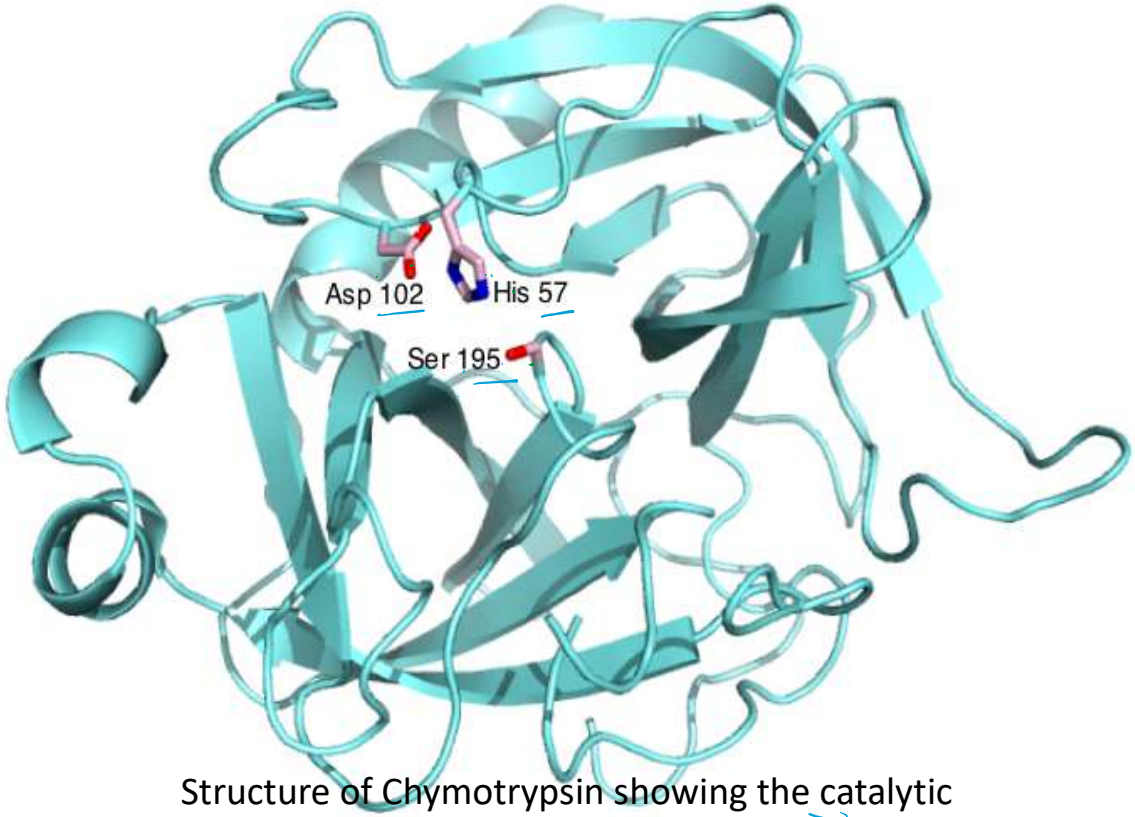
- Enzymes show great specificity for one or more substrates and kinds of reactions they catalyze.
- Enzymes catalyze reactions under fairly mild conditions of temperature and pH
- Most enzymes have optimal activity at 37 degrees
- Most enzymes have optimal activity in the pH range of 5 to 9
- Enzyme activity can be regulated-

➢ by limiting expression of the enzyme

➢ by another molecule other than substrate which binds the enzyme often at a site other than the active site-Allosteric regulation

Structure of Enzymes and their Active site

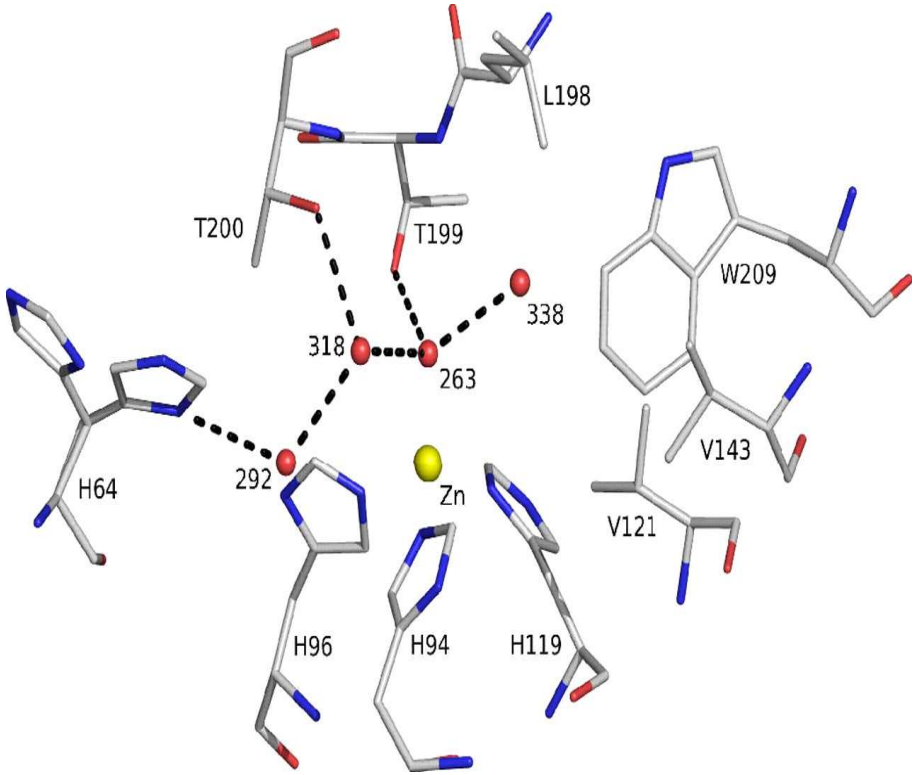
- The three dimensional structure of enzymes –large globular protein with a small Active site
- Active site- small pocket in the protein which binds substrate
- The active site brings together key residues that are far apart in the primary structure
- The residues are essential in the catalytic process-e.g. the catalytic triad in Chymotrypsin



Structure of Chymotrypsin showing the catalytic triad in the active site

Active site

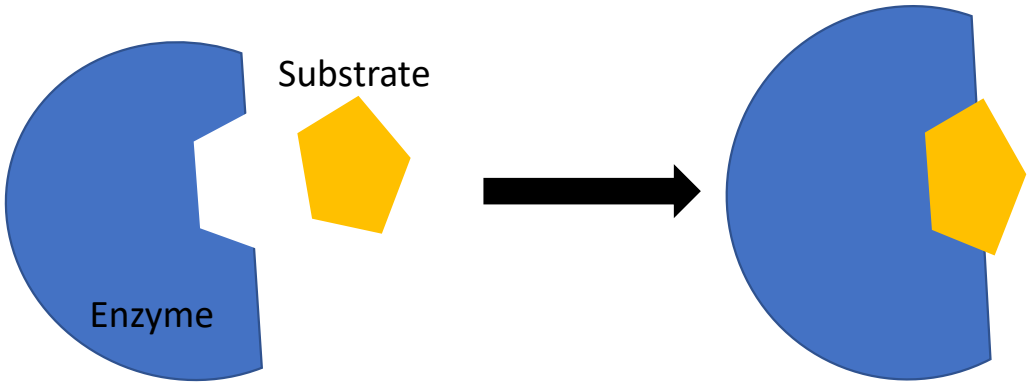
- The active site excludes water except for catalytic water
- Can have metal ions (cofactors) that are bound to the enzyme and participate in the catalytic process
- Substrate binding and stabilization of the transition state involve non-covalent bonding between enzyme and substrate-hydrogen bonding, Vander Waals forces and the hydrophobic effect



The active site of Human Carbonic anhydrase II. The zinc ion is tetrahedrally coordinated by 3 histidine's (His-94, His-96, and His-119) and catalytic water (Wat-263).

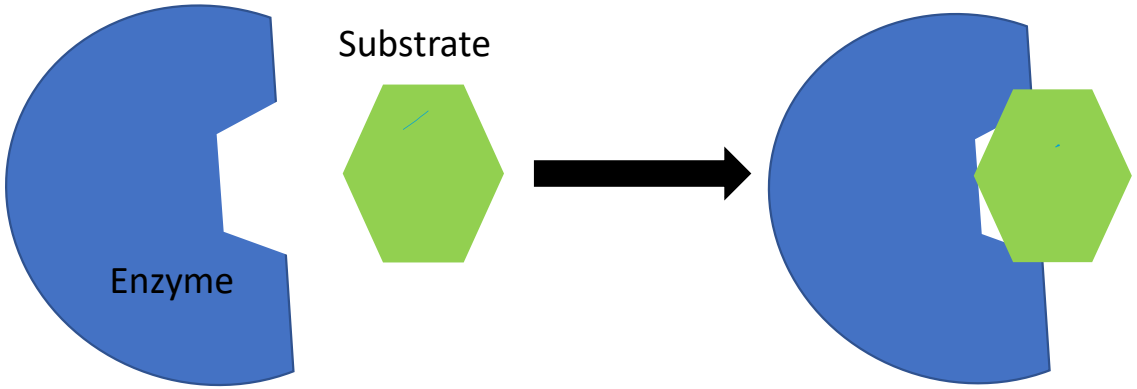
Björn Sjöblom et al. PNAS 2009;106:10609-10613

Substrate Binding



Lock and Key model

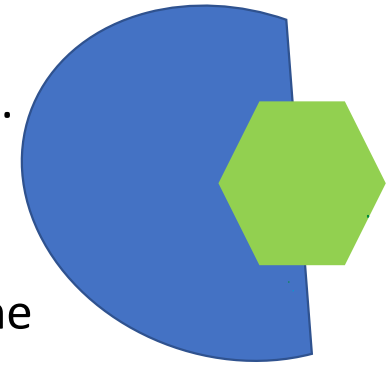
Substrate is a perfect fit for the Enzyme's active site



Induced fit model

Substrate is not a perfect fit for the Enzyme's active site.

The Enzyme's active site changes to fit the substrate



Substrate specificity

- Enzymes catalyze specific type of reactions and products are defined-no side reactions

➢ Specific substrate or a group of substrates

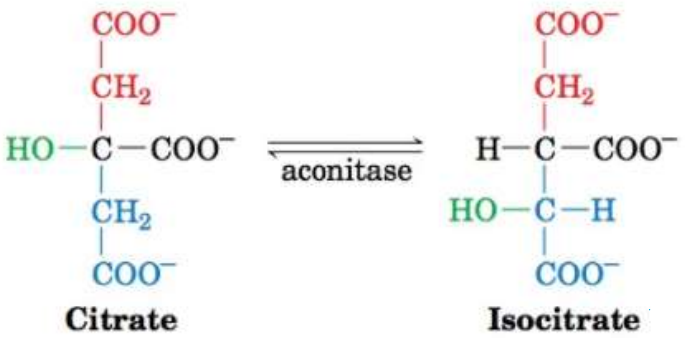
➢ Stereospecificity- Specific for one kind of chiral molecule

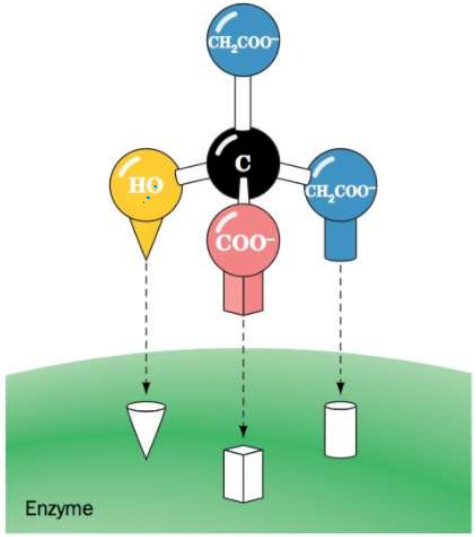
  ➢ Enzymes involved in glucose metabolism are specific for D-glucose

➢ Trypsin will only hydrolyze L-amino acids

➢ Geometric specificity- specific for type of chemical groups in their substrates

  Yeast alcohol dehydrogenase shows a strong specificity for ethanol

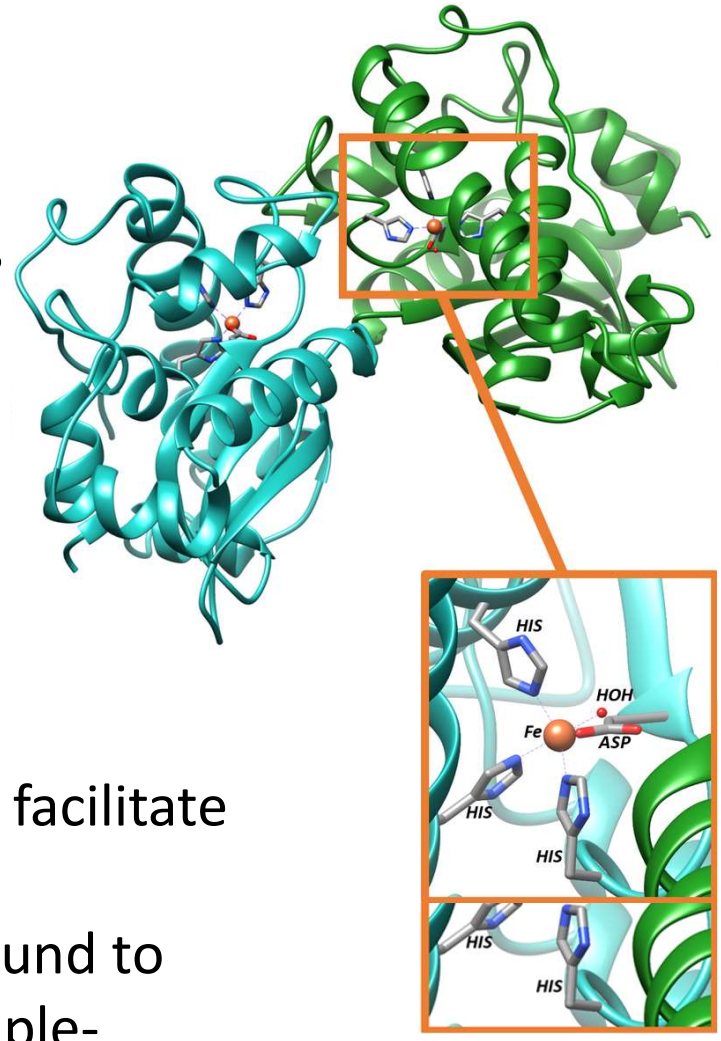




Coenzyme and Cofactors

In addition to the amino acid sidechains, enzymes use Cofactors-inorganic and organic molecules to aid in catalysis.
Cofactors can be metal ions as in the case of Carbonic anhydrase (CA) and Superoxide dismutase(SOD) and are called Metalloenzymes

Coenzymes are organic cofactors.
Often they transiently interact in the active site to facilitate catalysis (co-substrates)
Some enzymes have an organic cofactor that is bound to the enzyme and is called a prosthetic group. Example- Horseradish peroxidase(Hrp) has Heme

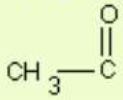


Fe-SOD

Coenzymes can carry groups or electrons that are transferred to the substrate.

See a list of some common coenzymes

The coenzyme is regenerated after the reaction takes place, sometimes by another reaction.

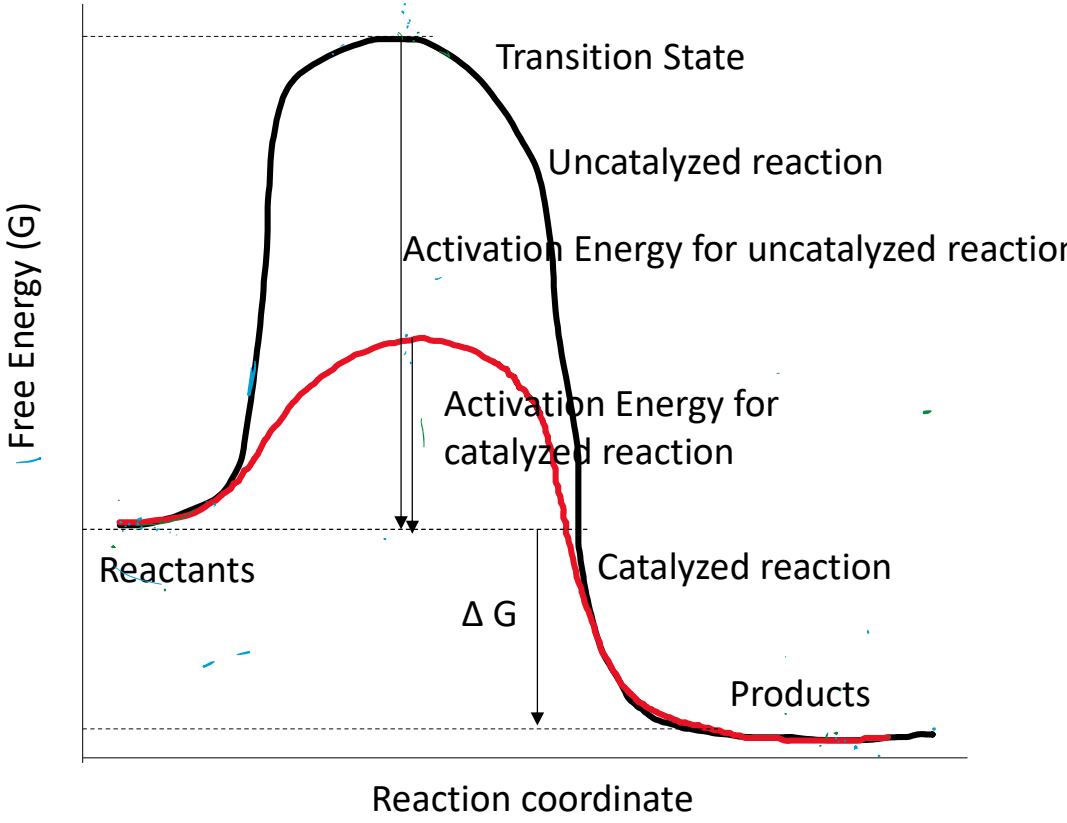
coenzyme	abbreviation	entity transfered
nicotine adenine dinucelotide	NAD - partly composed of niacin	electron (hydrogen atom)
nicotine adenine dinucelotide phosphate	NADP -Partly composed of niacin	electron (hydrogen atom)
flavine adenine dinucelotide	FAD - Partly composed of riboflavin (vit. B2)	electron (hydrogen atom)
coenzyme A	CoA	Acyl groups 
coenzymeQ	CoQ	electrons (hydrogen atom)
thiamine pyrophosphate	thiamine (vit. B1)	aldehydes
pyridoxal phosphate	pyridoxine (vit B6)	amino groups
biotin	biotin	carbon dioxide
carbamide coenzymes	vit. B12	alkyl groups

Mechanism of Enzyme Catalysis

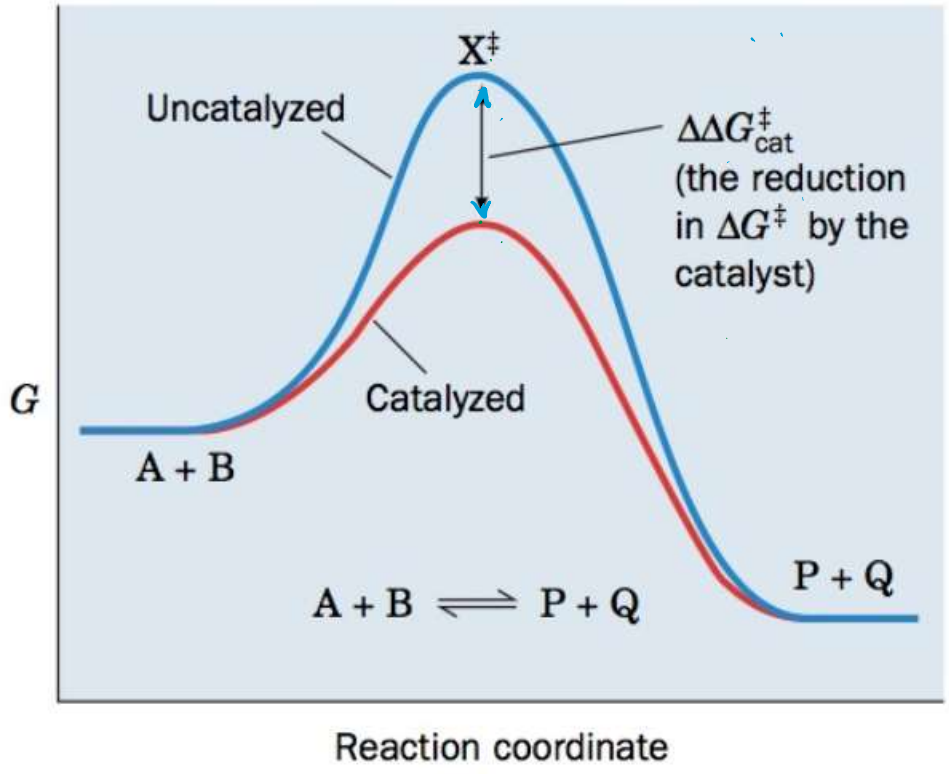
- Stabilization of the transition state
- Proximity and Orientation Effects
- Acid Base Catalysis
- Covalent Catalysis
- Metal ion Catalysis

Thermodynamics of Enzyme action

- A+B ⇌ P+Q
- A+B → $X^{\#}$ → P+Q
- Where the $X^{\#}$ is called the Transition State
- ΔG is negative –reaction is favorable
- The difference in energy between the transition state and the reactants is called the Activation Energy – $G^{\#}$



- The uncatalyzed reaction occurs at a fairly low rate because of the high activation energy
- The enzyme increases the rate of this reaction by lowering the activation energy- $G^{\#}$
- The enzyme lowers the activation energy by stabilizing the transition state
- The $\Delta\ \Delta G^{\#}_{cat}$ is a measure of the enzymes efficiency
- The rate enhancement of a reaction by an enzyme is calculated by
- Rate enhancement = $e^{\Delta\ \Delta G\#\ cat\ /RT}$



SAMPLE CALCULATION 11-1

By how much must an enzyme reduce the activation energy of a reaction at 37°C for the reaction to occur 50 times faster than in the absence of the enzyme?

Rate enhancement $= 50 = e^{\Delta\Delta G^{\ddagger}_{cat}/RT}$. Rearranging the equation gives

$$\ln 50 = \Delta\Delta G^{\ddagger}_{cat}/RT$$

$$\Delta\Delta G^{\ddagger}_{cat} = RT \ln 50$$

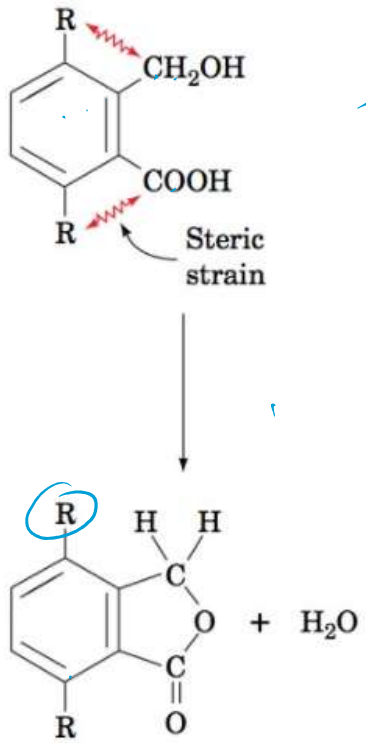
$$= (8.3145\ \text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1})\ (37 + 273)\text{K} \ln 50$$

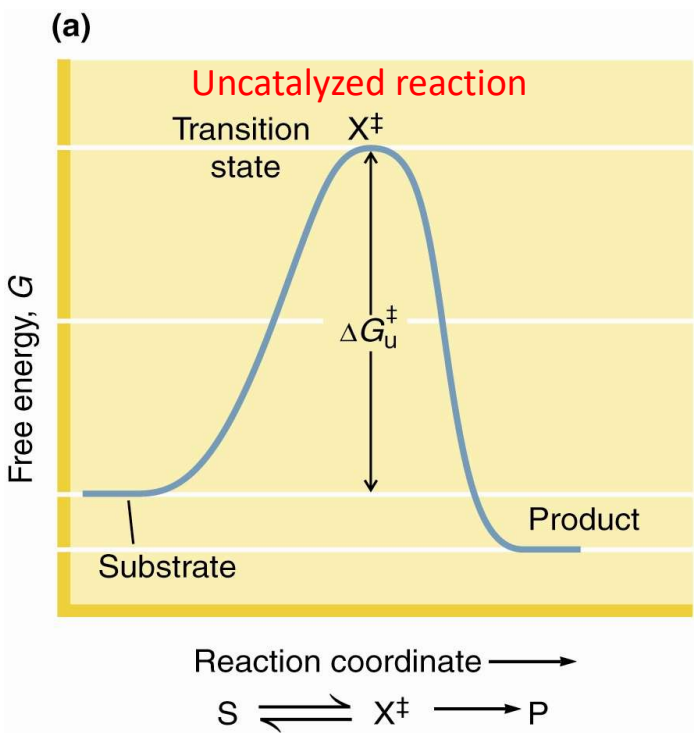
$$= 10{,}000\ \text{J} \cdot \text{mol}^{-1} = 10\ \text{kJ} \cdot \text{mol}^{-1}$$

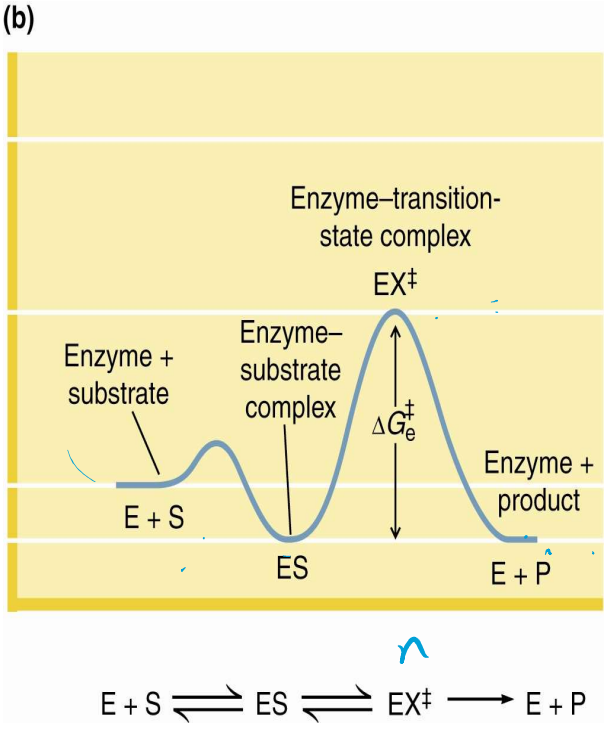
WPLS See Sample Calculation Videos.

Transition state

- The rate of the reaction shown here is 315 times faster if the R group is a CH_3 molecule than if it's a H molecule.
- The transition state closely resembles the strained reactant than the unstrained reactant.
- The transition state is a state where both bond breaking and bond formation are happening.
- The bonds that are forming and those that are breaking are weak bonds.
- The transition state for most enzymes is a fleeting state occurring in the femtosecond time scale

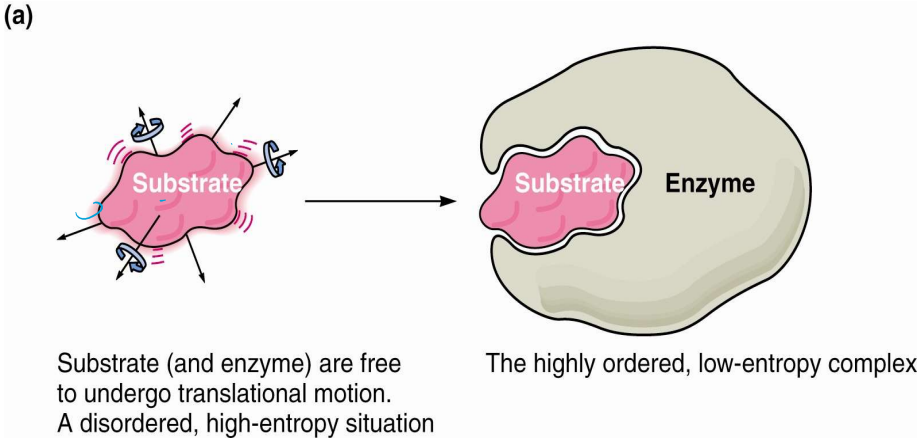


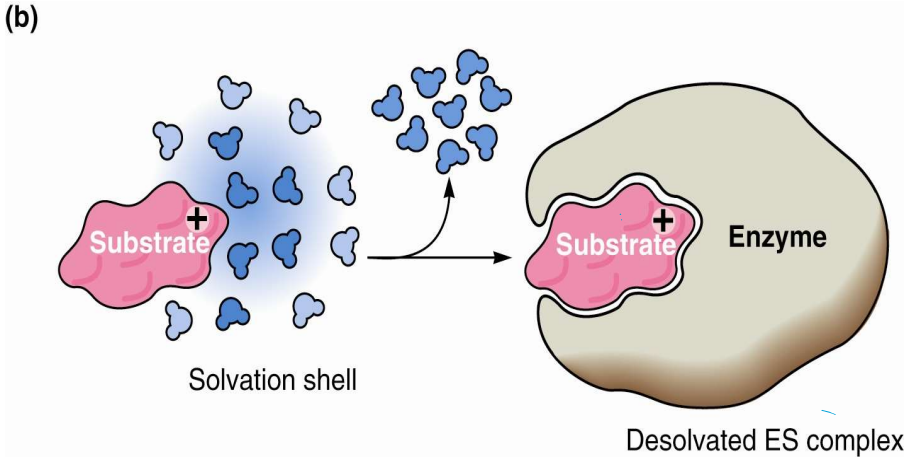




The enzyme has to **bind the transition state (T) more tightly** than the substrate or reactants to enhance the rate of the reaction.

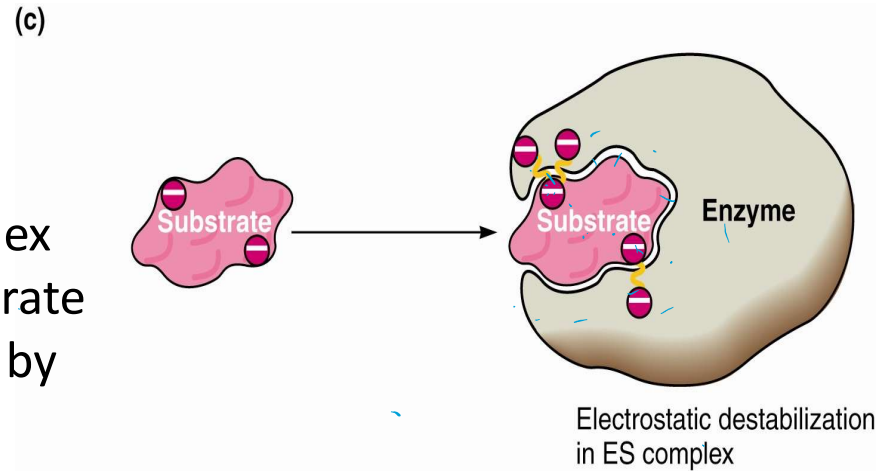
Destabilization of the ES complex





The favorable binding energy of substrate and Enzyme is offset by destabilizing effects such as

1) Loss of entropy upon binding
2) Destabilization of the Enzyme substrate complex by desolvation of charged groups on the substrate
3) Electrostatic destabilization of the ES complex by charged groups in the active site



Note: These factors actually are favorable in the case of the transition state

Proximity and Orientation effects

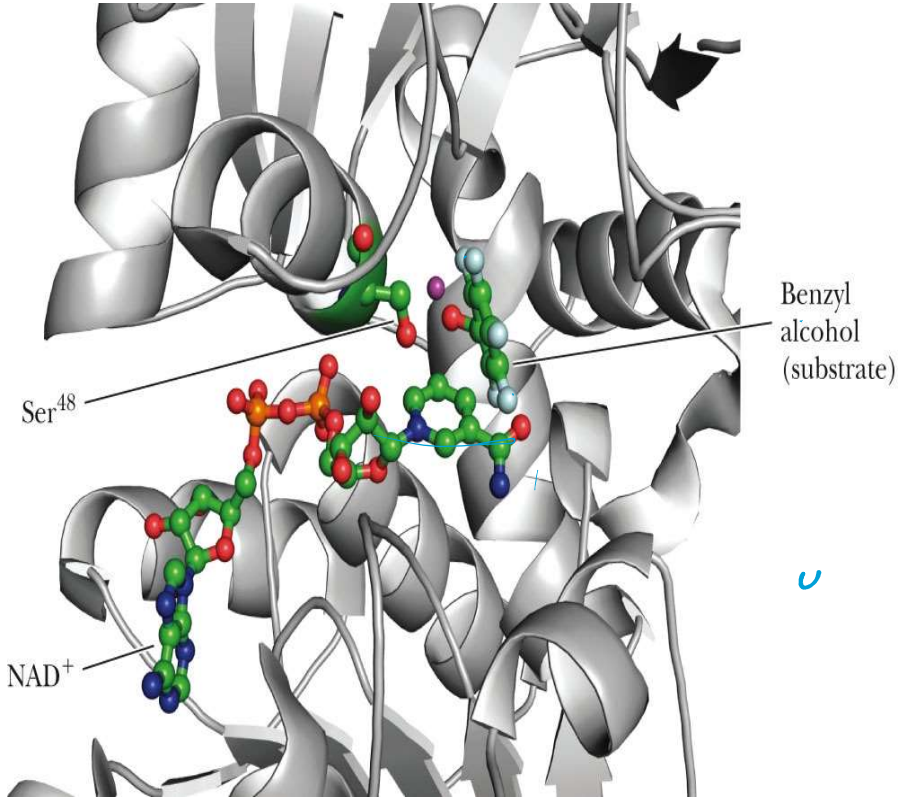
- Enzyme bring substrates in close proximity with one another
- Enzymes orient the molecules in such a way that it tremendously increases the likelihood of a successful reaction occurring
- Charged groups in the enzymes active site can stabilize the transition state and this phenomenon is called electrostatic catalysis which is enhanced by the expulsion of water from the active site
- Enzyme active sites freeze the motions within the transition state to significantly enhance the rate of the reaction.

Near attack conformation

- Enzyme active sites are referred to as "Preorganized" meaning the residues in the active site are placed in such as way that the reacting atoms are properly oriented and in vanderwaals contact and are in an angle that will resemble the bonds formed in the transition state.
- This is called the Near attack conformation.

- Thomas Bruice has proposed that near-attack conformations are precursors to transition states
- In the absence of an enzyme, potential reactant molecules adopt a NAC only about 0.0001% of the time
- On the other hand, NACs have been shown to form in enzyme active sites from 1% to 70% of the time

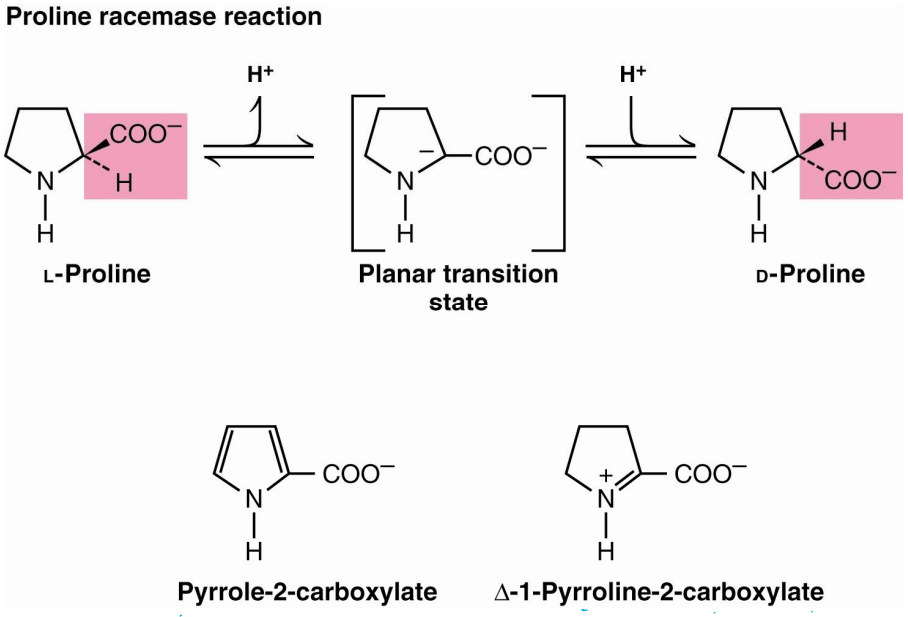
The active site of liver alcohol dehydrogenase – a near-attack conformation.



- The Benzyl alcohol is oriented in such a way that
- It is oriented in such a way that the Serine can abstract a proton from the Benzyl alcohol, and the NAD can accept the hydrogen from a pro-R position on the alcohol.
- There is a Zn^{2+} ion in the active site which stabilizes the negative charges formed on the substrate during the reaction
- https://pdb101.rcsb.org/motm/13

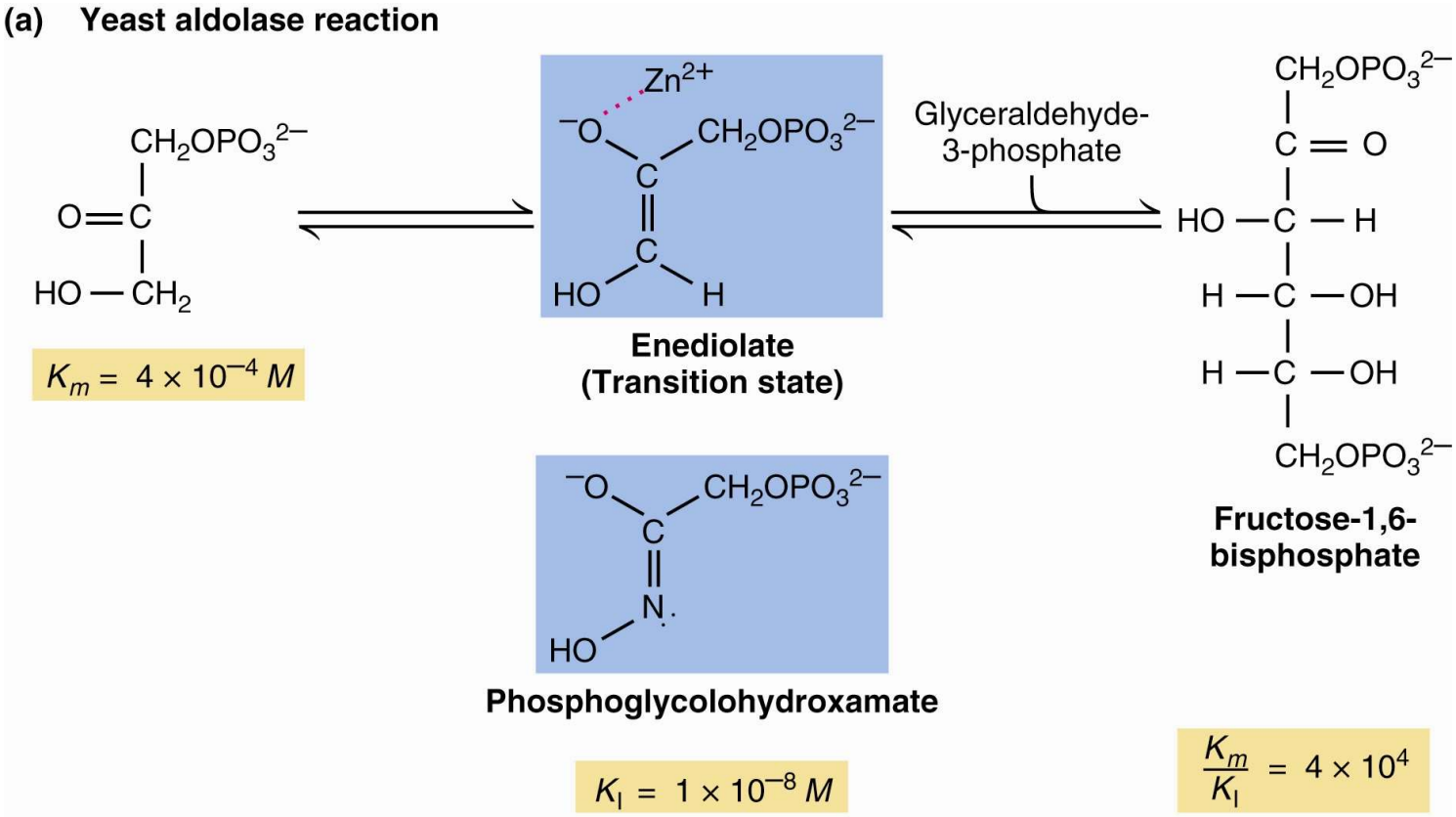
Transition State Analogs

- The binding constant of the enzyme for the transition state may be 10^{-20} to 10^{-26} *M*
- Transition state analogs (TSAs) are stable molecules that are chemically and structurally similar to the transition state



Transition state analogs

How Tightly Do Transition-State Analogs Bind to the Active Site?

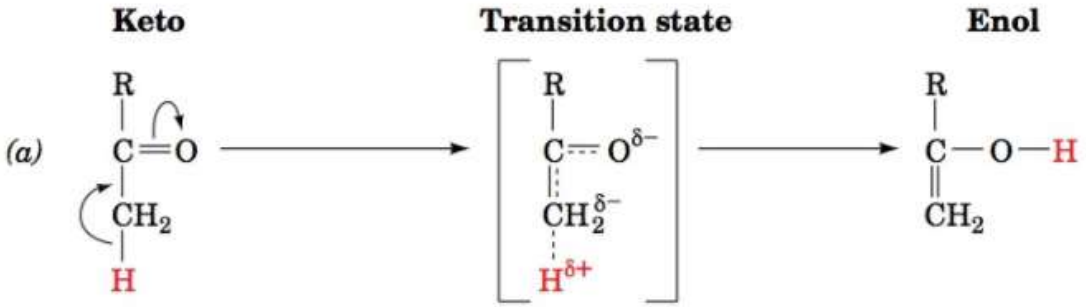


Phosphoglycolohydroxamate is an analog of the enediolate transition state of the yeast aldolase reaction.

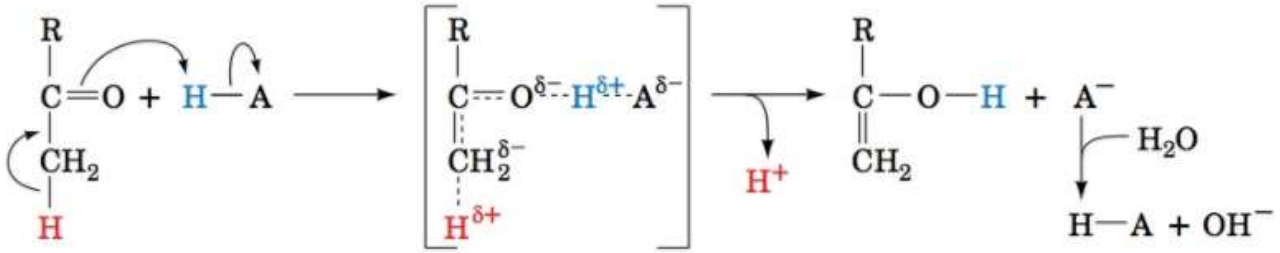
Transition State Analogs as therapeutics

- Transition-state analogs often make ideal enzyme inhibitors
- Statins such as Lipitor are powerful cholesterol-lowering drugs, because they are transition-state analog inhibitors of HMG-CoA reductase, a key enzyme in the biosynthetic pathway for cholesterol.
- Protease inhibitors are AIDS drugs. Invirase (saquinavir) by Roche and similar "protease inhibitor" drugs are transition-state analogs for the HIV-1 protease.
- Tamiflu is a neuraminidase inhibitor and antiviral agent based on the transition state of the neuraminidase reaction

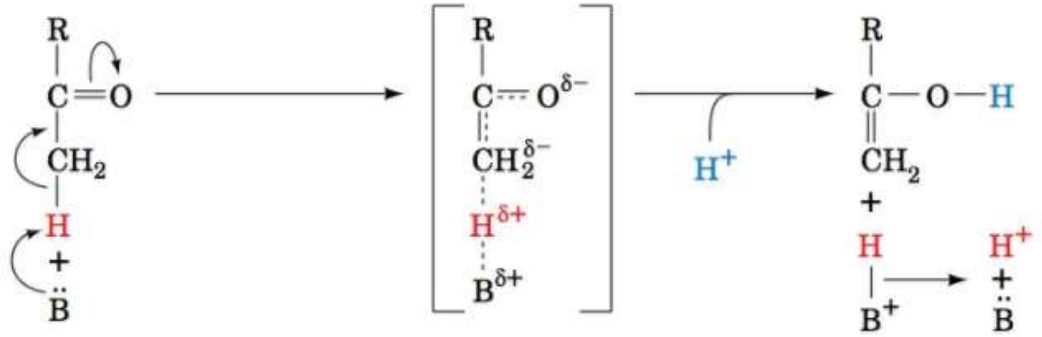
Acid Base Catalysis



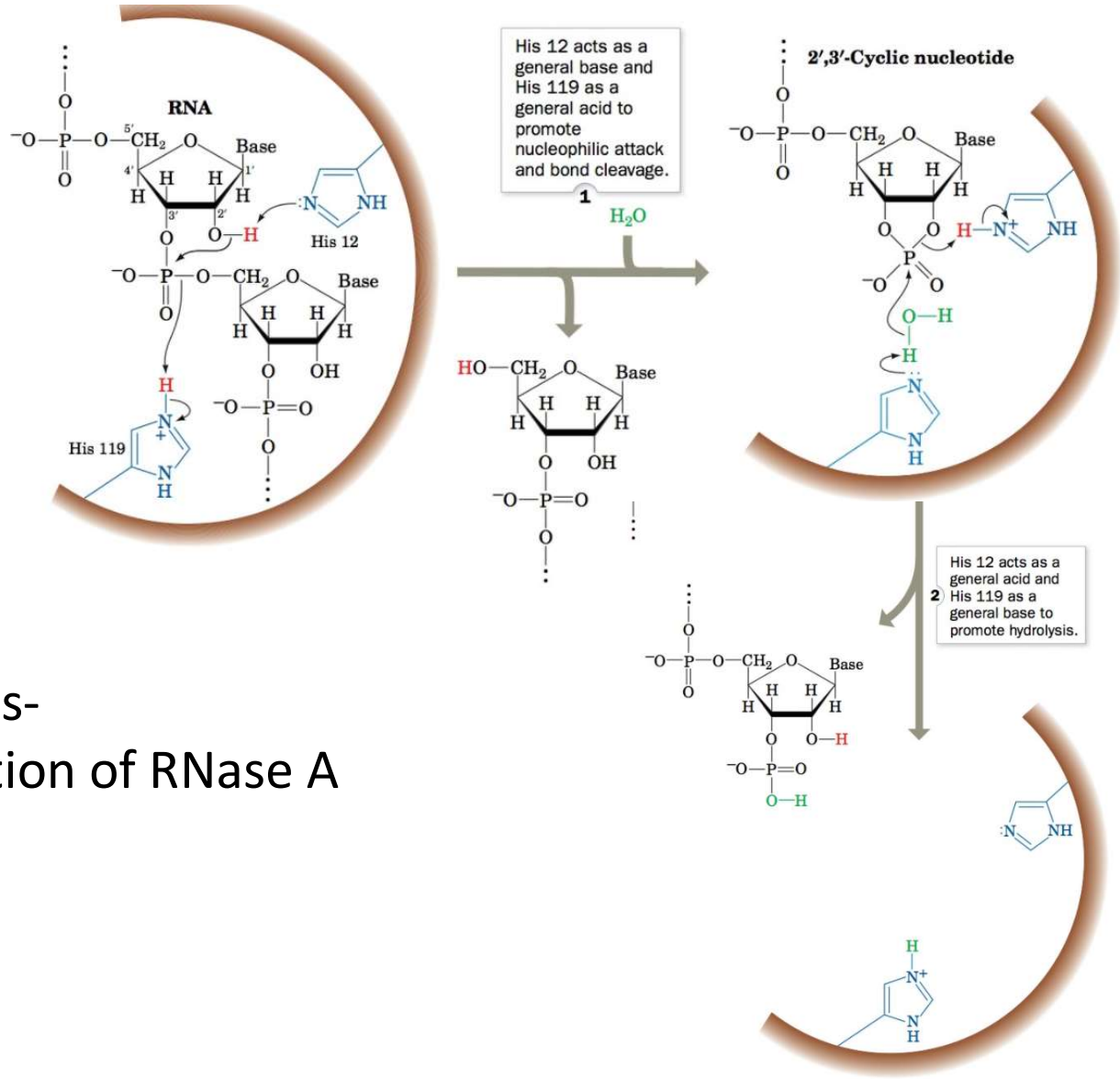
General Acid Catalyzed reaction



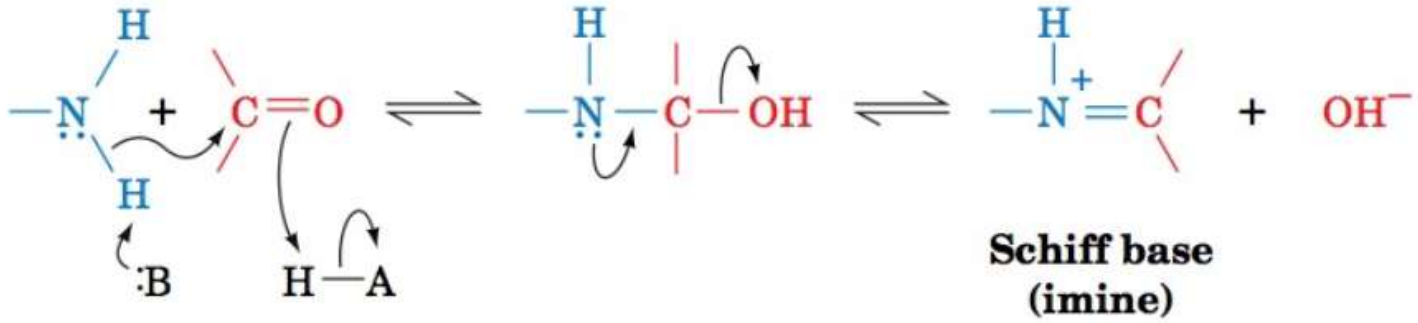
General Base Catalyzed reaction



Acid base Catalysis-
Mechanism of action of RNase A

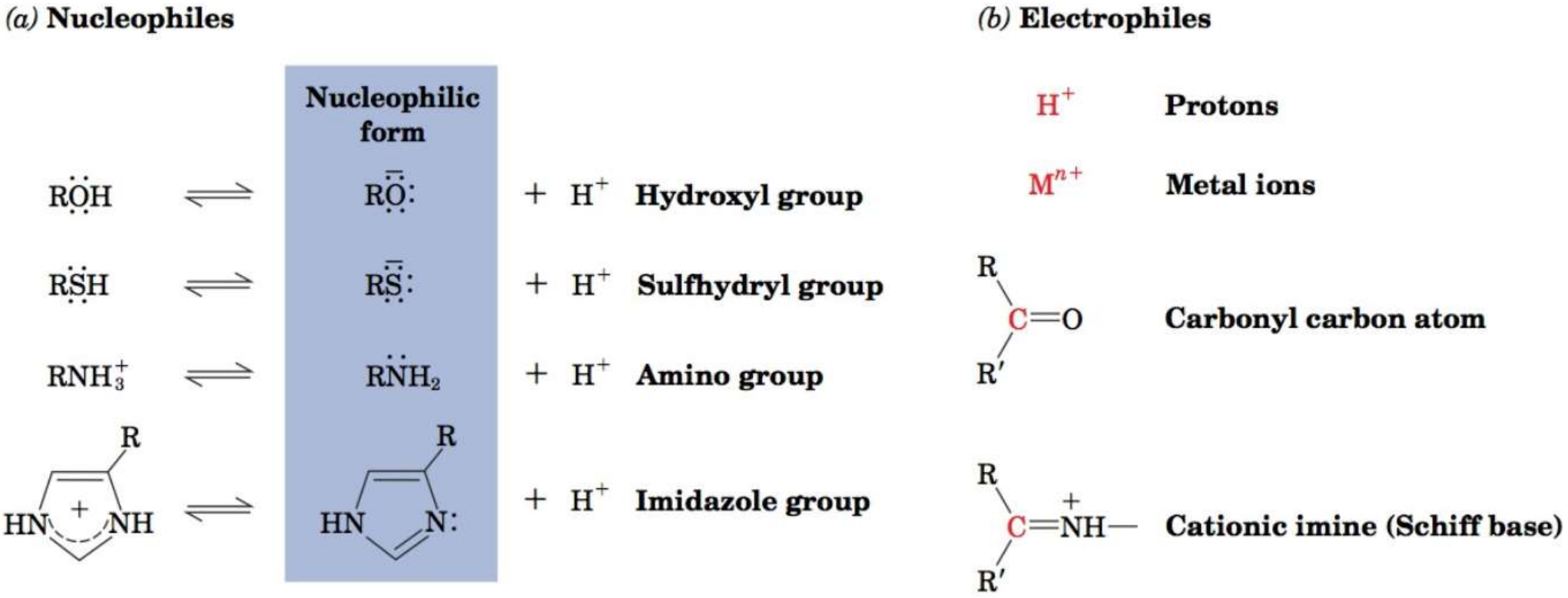


Covalent Catalysis

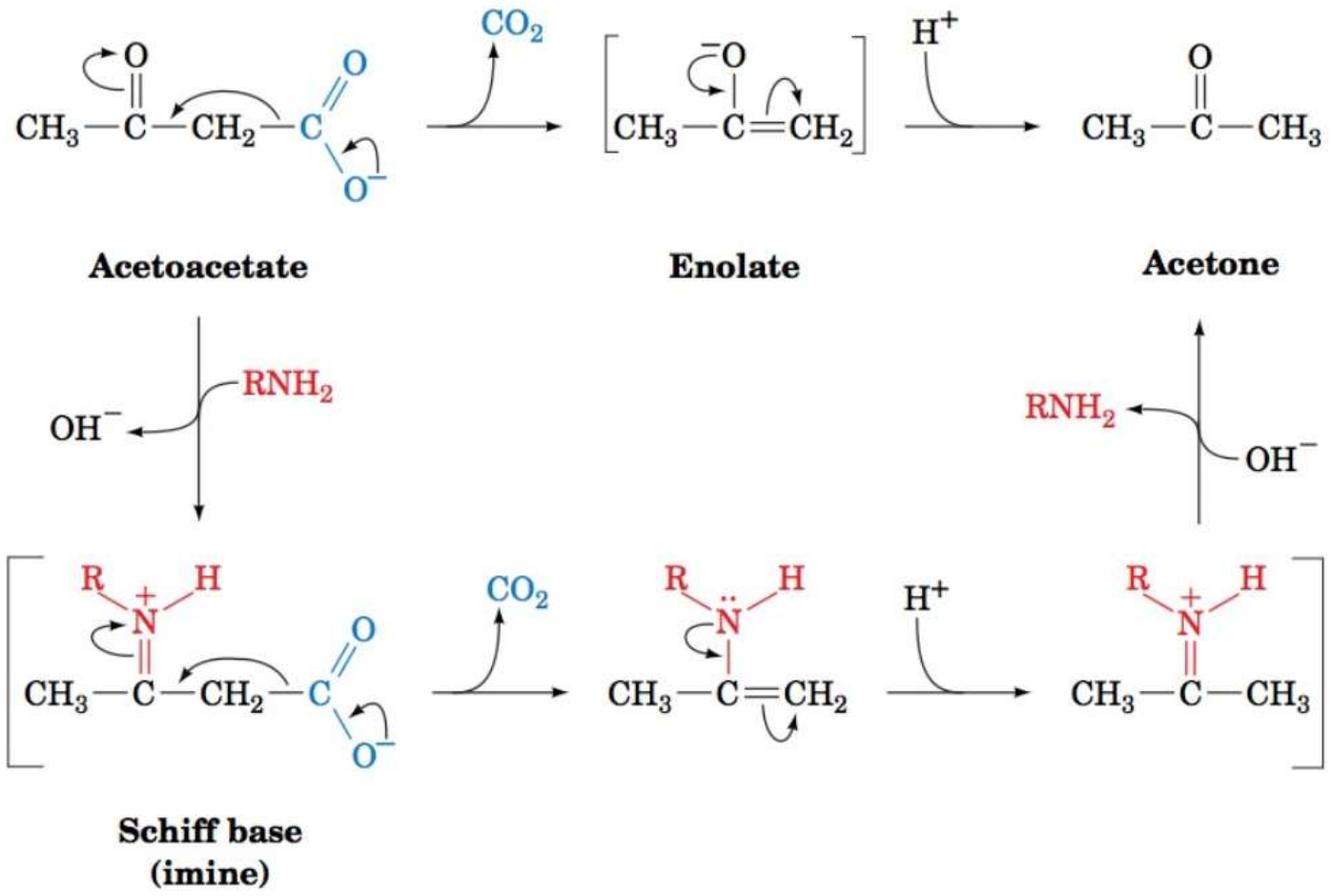


- Some enzymes derive much of their rate acceleration from formation of covalent bonds between enzyme and substrate
- The side chains of amino acids in proteins offer a variety of **nucleophilic** centers for catalysis
- These groups readily attack electrophilic centers of substrates, forming covalent enzyme-substrate complexes
- The covalent intermediate can be attacked in a second step by water or by a second substrate, forming the desired product

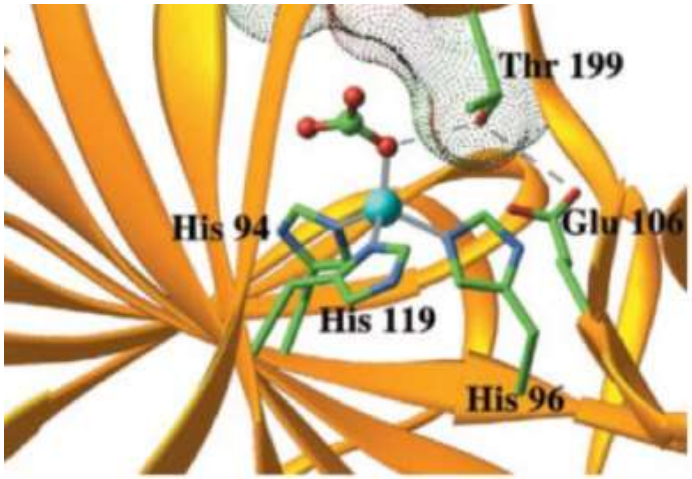
Nucleophiles and Electrophiles



Covalent Catalysis of Acetoactate to Acetone

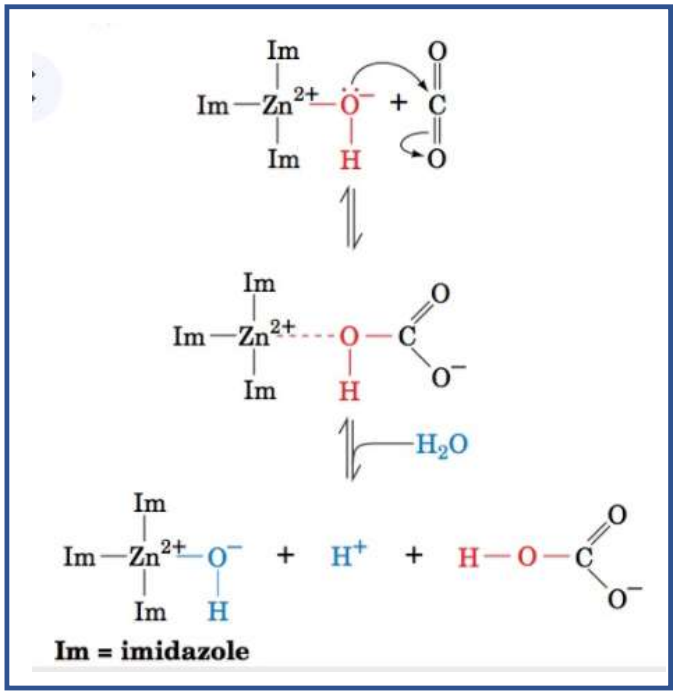


Metal ion catalysis



Carbonic anhydrase with Zn^{2+} in its active site

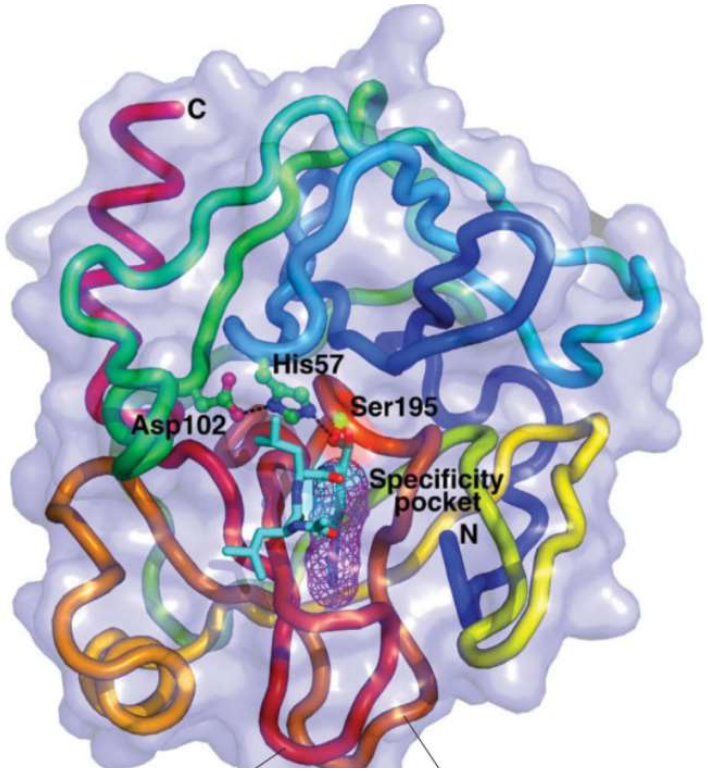
$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

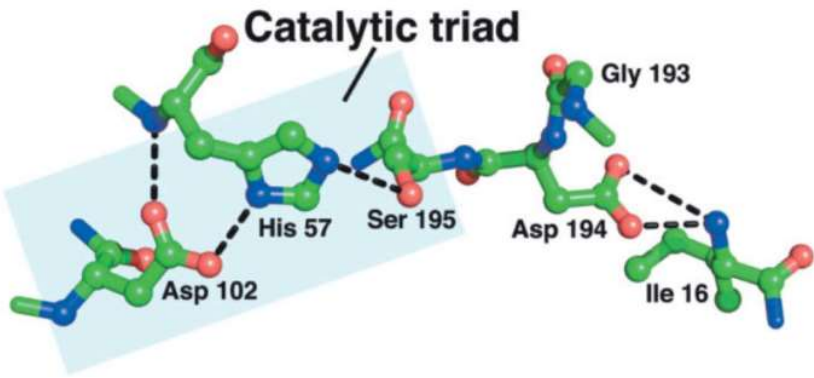


- The Zn^{2+} ion polarizes a water molecule (not shown) so it ionizes to form OH^-, which nucleophilically attacks the substrate CO_2 to yield HCO^-_3
- Metal ions will help orient the substrate in the active site
- Metal ions can participate in oxidation reduction reactions by changes in the ionization state of the metal
- Metal ions electrostatically shield the negative charges and hence stabilize transition state

Serine Proteases

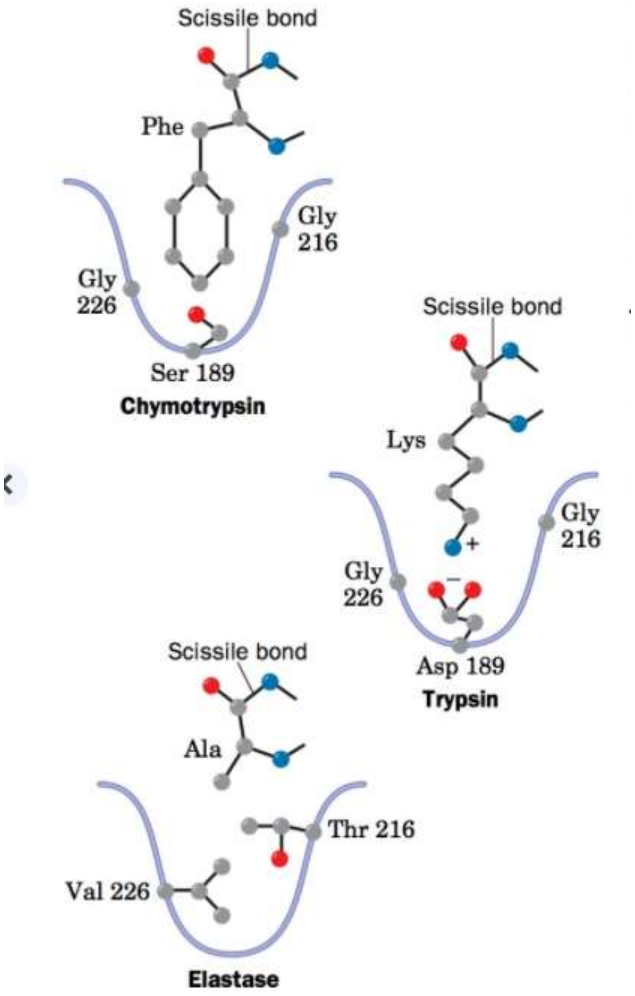
- Serine Proteases are a group of proteolytic enzymes which include digestive enzymes and the enzymes of the coagulation cascade
- E.g: Trypsin, Chymotrypsin and Elastase
- Shown here is the Active site of Bovine trypsin with 3 key residues which are referred to as the catalytic triad.
- The catalytic triad is found in all Serine proteases with serine always being one of the residues.
- Significant sequence identity and structural similarity

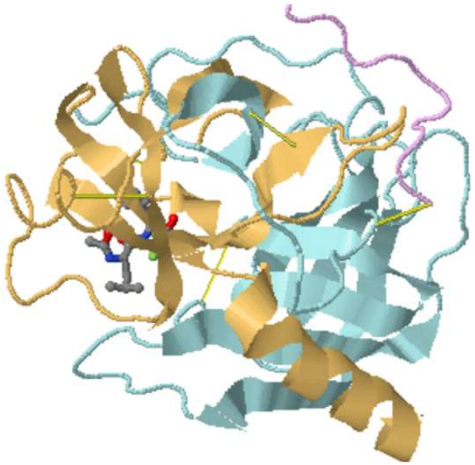


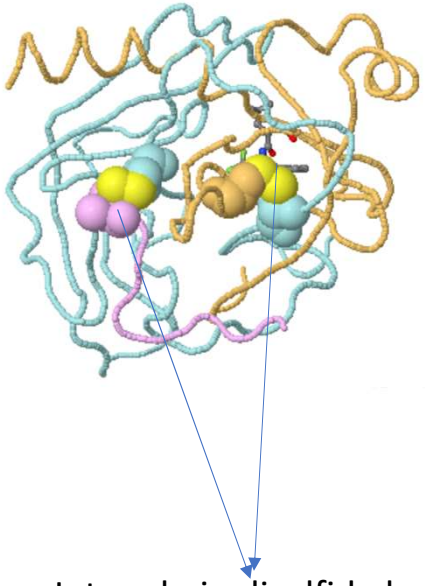


Substrate specificity

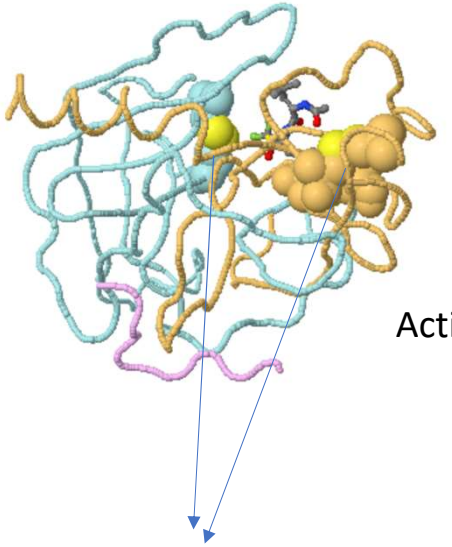
- The specificity pocket shown in the fig on right defines the choice of substrate for each of these enzymes
- However mutating these residues of the specificity pocket alone is not sufficient to interchange substrate specificity



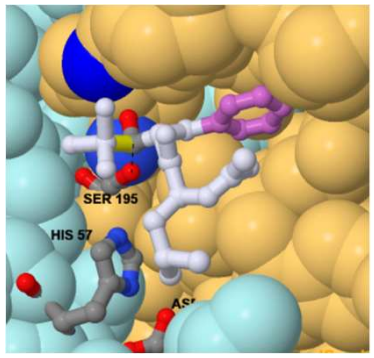




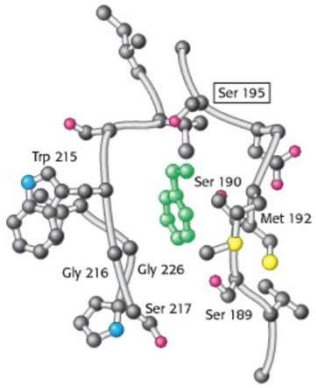
Inter-chain disulfide bonds



Intra-chain disulfide bonds

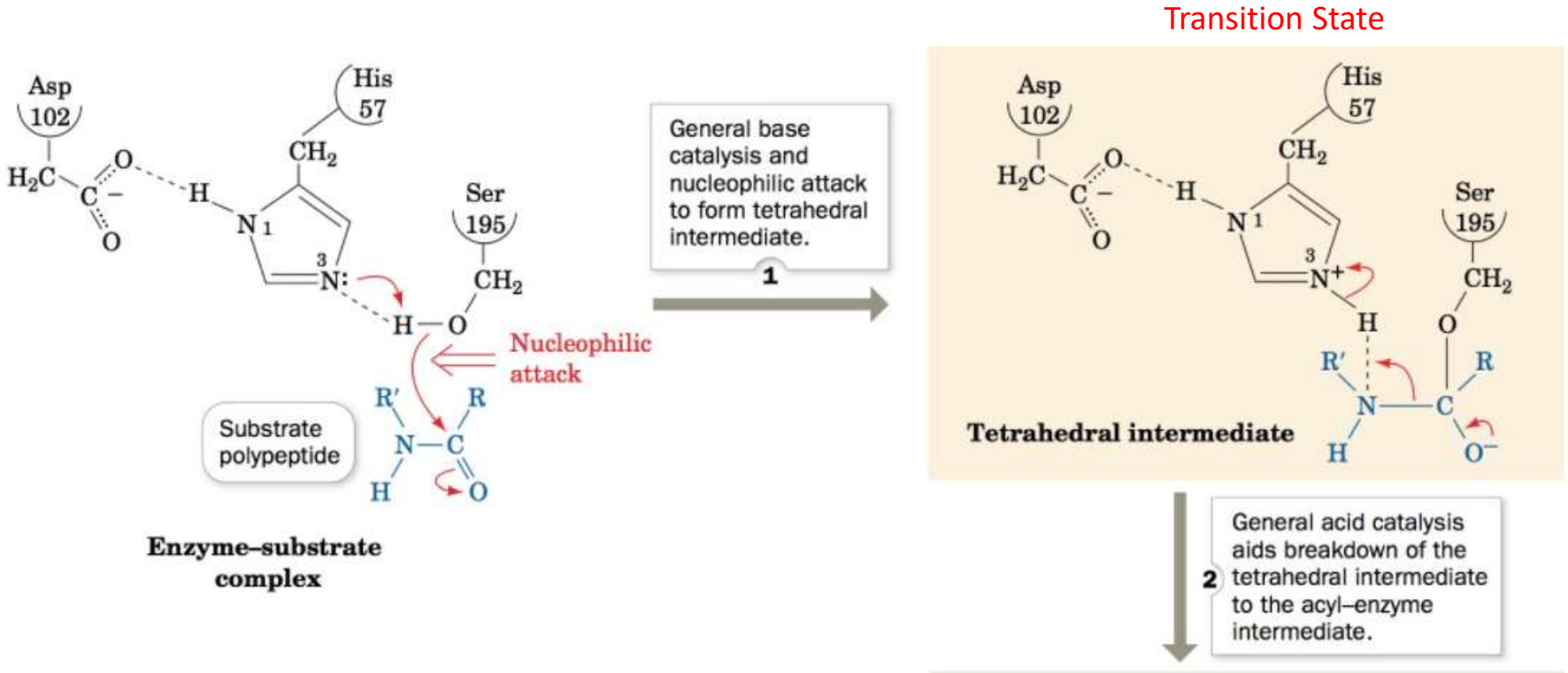


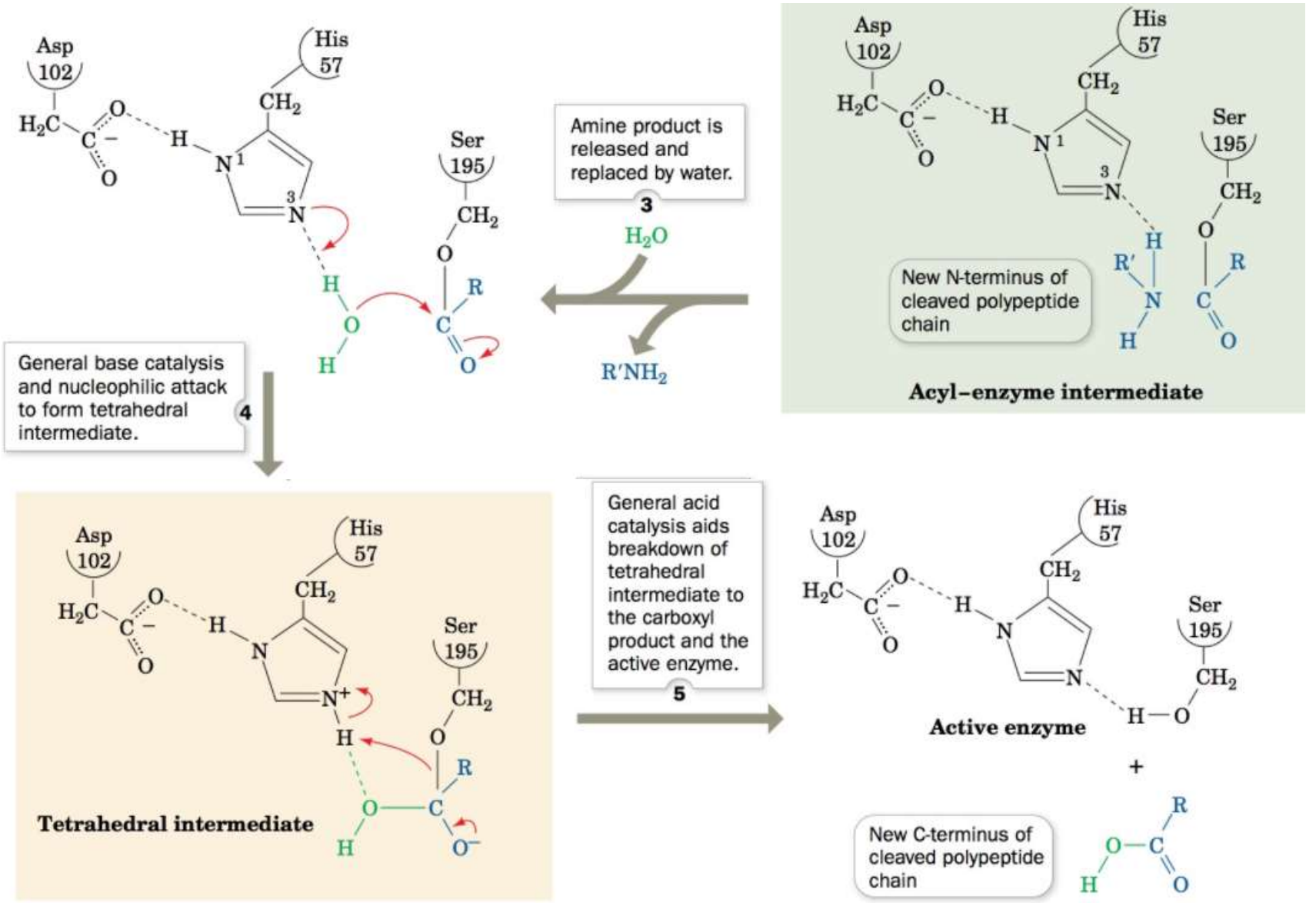
Active site and substrate binding pocket



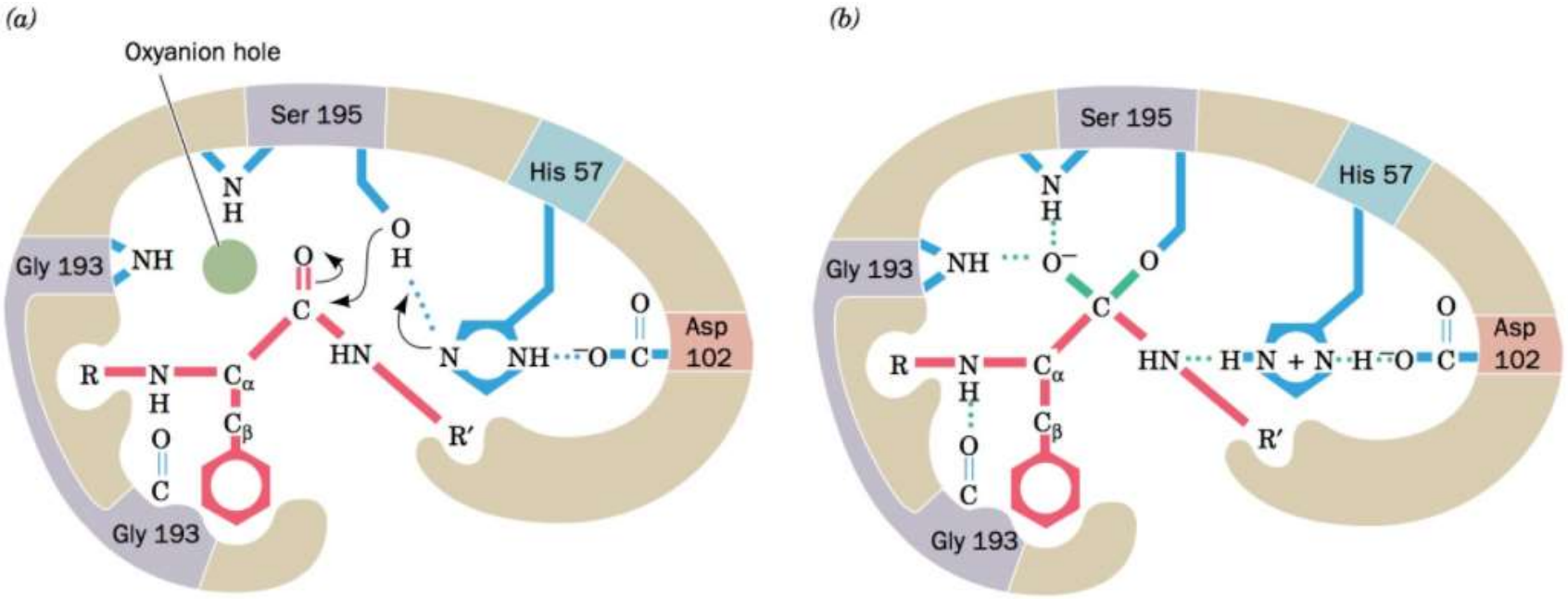
Chymotrypsin is made up of three polypeptide chains indicated in blue, beige and pink. These three polypeptide chains are linked by 2 interchain disulfide bonds. There are also three intrachain disulfide bonds. Chymotrypsin structure has 2 beta barrel domains and one alpha helix has its primary secondary structural units.

Mechanism of Serine Proteases (e.g Chymotrypsin)




Asp 102
His 57
Ser 195
Amine product is released and replaced by water.
3
H_2O
$R'NH_2$
New N-terminus of cleaved polypeptide chain
Acyl–enzyme intermediate
General base catalysis and nucleophilic attack to form tetrahedral intermediate.
4
Tetrahedral intermediate
General acid catalysis aids breakdown of tetrahedral intermediate to the carboxyl product and the active enzyme.
5
Active enzyme
+
New C-terminus of cleaved polypeptide chain

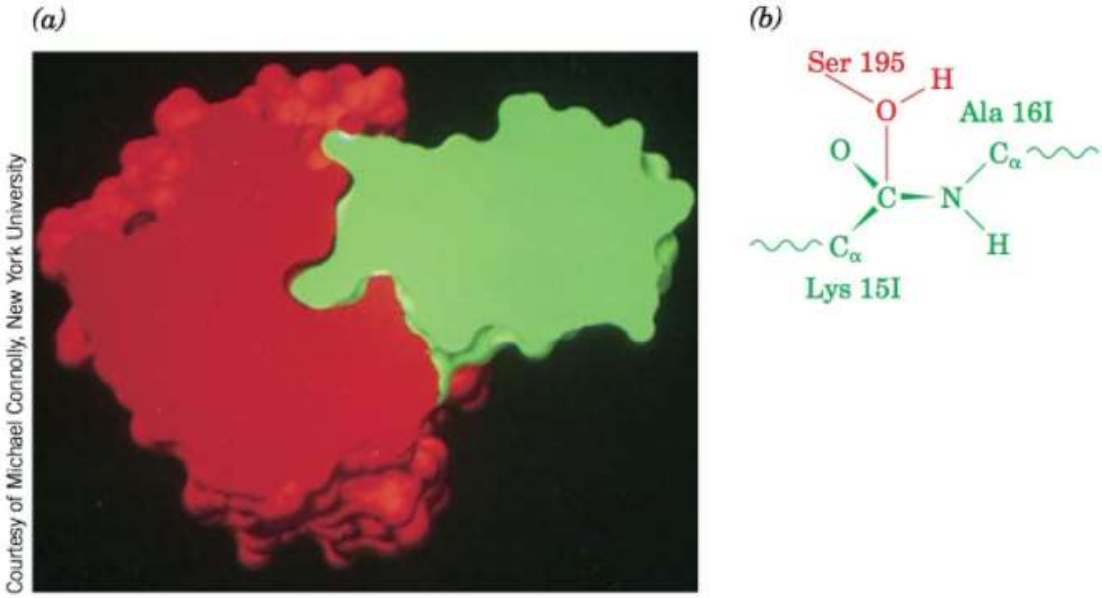
Preferential binding of the transition state



The conformational rearrangement that occurs with the formation of the tetrahedral intermediate causes the now anionic carbonyl oxygen of the scissile peptide to move deeper into the active site so as to occupy a previously unoccupied position called the oxyanion hole. The tetrahedral distortion, moreover, permits the formation of an otherwise unsatisfied hydrogen bond between the enzyme and the backbone NH group of the substrate residue preceding the scissile peptide bon

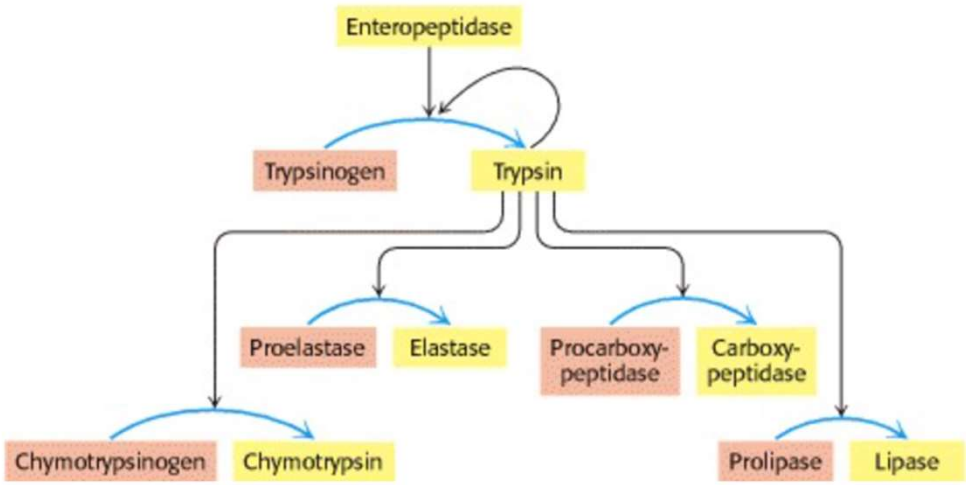
Proteolytic Inhibitors are Transition state analogs

- Bovine trypsin inhibitor is an proteolytic enzyme inhibitor that naturally exists
- The inhibitor bound to the enzyme (fig) resembles the transition state.
- The Ser 195 forms a bond with the scissile peptide and the distortion in this case is such that the cleaved group is constrained the active site and cannot leave and water is unable to enter the active site



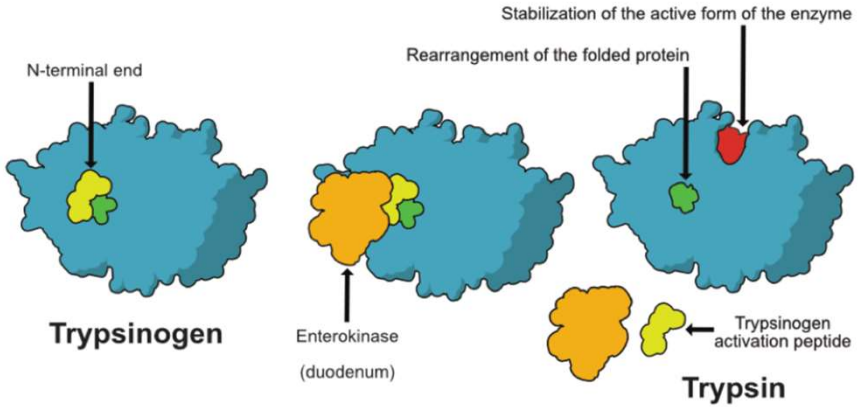
Zymogens

- Serine Proteases exist in an inactive precursor state called proenzymes or Zymogens
- This is to prevent the enzymes from digesting tissues
- Eg is Trypsinogen is the precursor of Trypsin
- It is activated by the enzyme Enteropeptidase which is under hormonal control
- Trypsin then autocatalytically cleaves more Tryspinogen
- Trypsin also activates Chymotrypsinogen, proelastase and other proteolytic enzymes

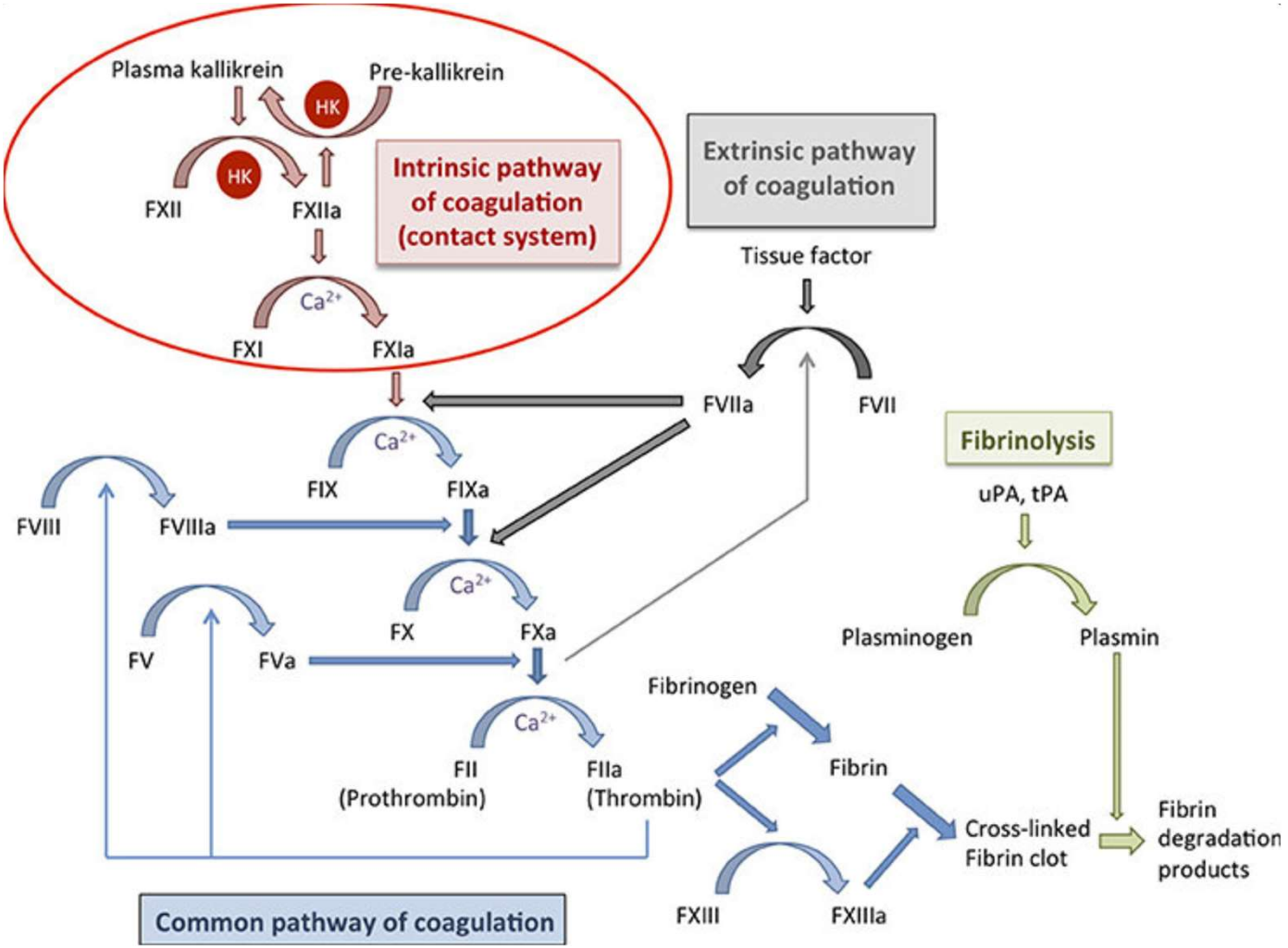


Zymogen activation

- Zymogens have distorted active sites and upon losing the small peptide that distorts the active site, the enzyme changes conformation and the active site is restored
- The enzymes of the coagulation cascade are Zymogens and are activated in a cascade fashion



Coagulation Cascade



Activation of Chymotrypsinogen

Chymotrypsinogen is a single polypeptide consisting of 245 aminoacids. It is converted into a fully active form called π Chymotrypsin by cleaving between Arg 15 and Ile 16.

π Chymotrypsin further cleaves out two dipeptides Ser 14-Arg 15 and Thr 147-Asn 148.

The newly formed amino-terminal group of Ile 16 turns inward and forms an ionic bond with Asp 194 in the interior of the chymotrypsin molecule.

The formation of the ionic bond triggers a series of conformational changes that results in the creation of the substrate specificity pocket. The changes also completes the formation of the oxyanion hole.

This form of Chymotrypsin is called α-Chymotrypsin.