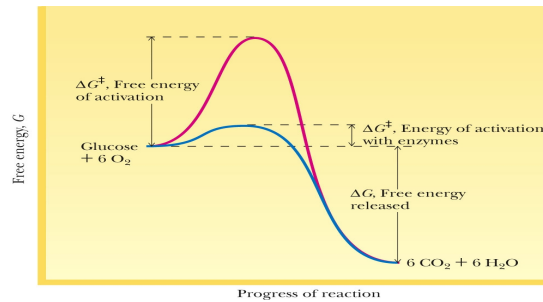


Catalytic Mechanisms

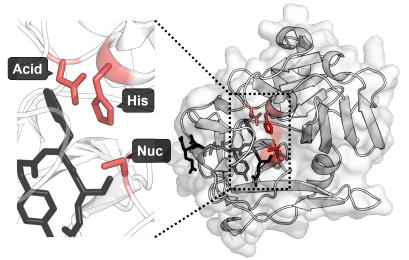
HOW do enzymes do their job?

- Reducing activation energy... sure, but HOW does an enzyme catalysis reduce the energy barrier ΔG^\ddagger ?
- Remember:

The rate of a chemical reaction of substrate S to product P is governed by the activation energy (E_{act}), which is the difference in free energy between the reagent(s) and the transition state for the reaction.

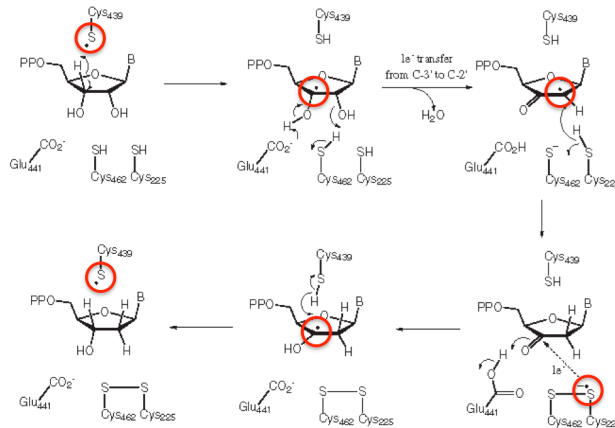


It's the amino acids!



Stabilization of reactive intermediates

Enzyme-catalyzed reactions generate **reactive intermediates**, such as carbanions, carbocations, and radicals, that normally would require **strong reaction conditions to generate in chemical reactions** and would be **very unstable in aqueous solution**.



It's about transition state!

Enzymes stabilize the transition state and catalyze the reaction by providing appropriate functional groups of the protein or cofactor spatially and temporally appropriate manner.

- There are five general mechanisms by which enzymes contribute to catalysis:

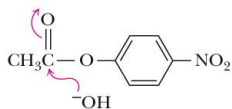
Acid/Base, covalent and metal ion catalysis. Proximity and orientation (strain) effects and preferential binding to transition state complex

Acid / Base catalysis

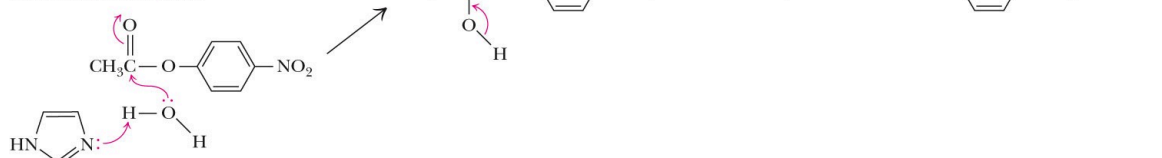
Chemical groups are made more reactive by adding or removing a proton from substrate to reduce (stabilizing) transition state free energy.

- Result of Acid/Base catalysis is making a reactive group more reactive by increasing its intrinsic electrophilic or nucleophilic character
- This can increase the rate 10-100 fold
- Microenvironment shifts in pKa allow for several amino acids to be involved: Asp, Glu, His, Cys, Tyr, and Lys often involved

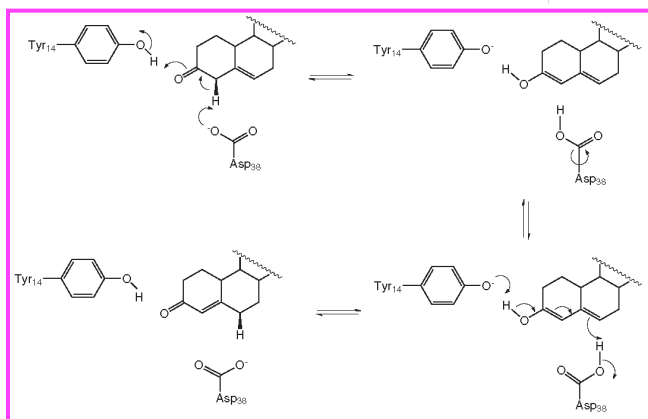
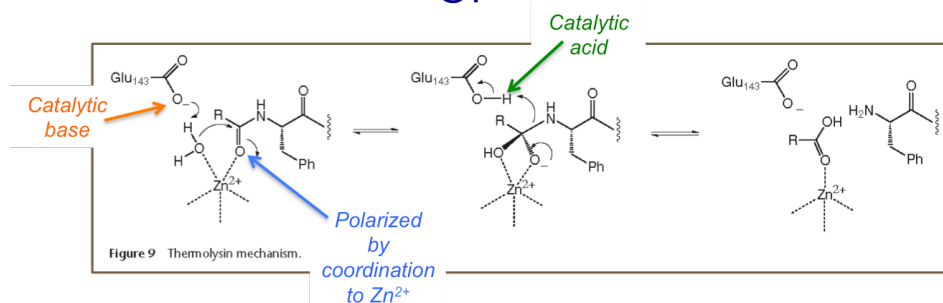
Specific base mechanism



General base mechanism



Metals can help too @ pH 7

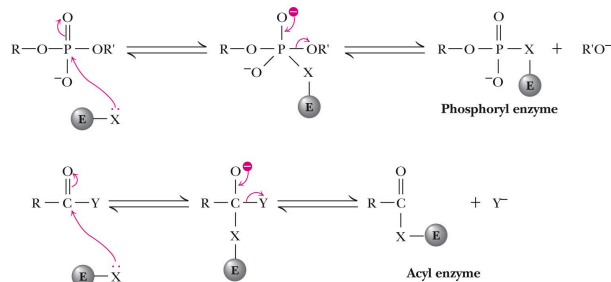


Acids deprotonated, act in general base catalysis
Bases protonated, act in general acid catalysis

Often happens in pairs
A concerted reaction

Covalent Catalysis

A covalent bond between substrate and enzyme is generated to lower transition state
Takes place with **enzyme side chain** nucleophiles with **substrates** that have electrophilic centers



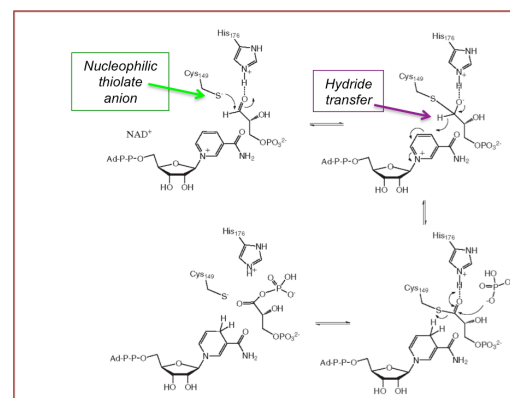
Important nucleophiles of enzyme and electrophiles of substrates (see table in book)

Covalent Catalysis

In some enzymes a nucleophilic side chain group forms an unstable covalent bond with the substrate. The enzyme-substrate complex then forms product. The pathway can require that the intermediate is more susceptible to nucleophilic attack by water than the original substrate.

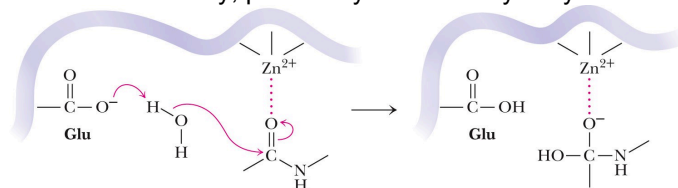
Three stages of covalent catalysis:

- Formation of a bond between substrate and enzyme
 - Removal of electrons to make a reactive center
 - Elimination of the bond that was formed in step one
- Bond formation between deprotonated Cys nucleophile (STRONG nucleophilic thiolate anion) and electrophilic substrate
 - Lewis base transferring H^+ & e^- Intermediate
 - Elimination of (phosphate anion attack) bond forms new product



Metal Ion Catalysis

metalloenzymes require metal ions for maintain the native structure/state of the enzyme
metal activated enzymes bind metals more weakly, potentially in the catalytic cycle



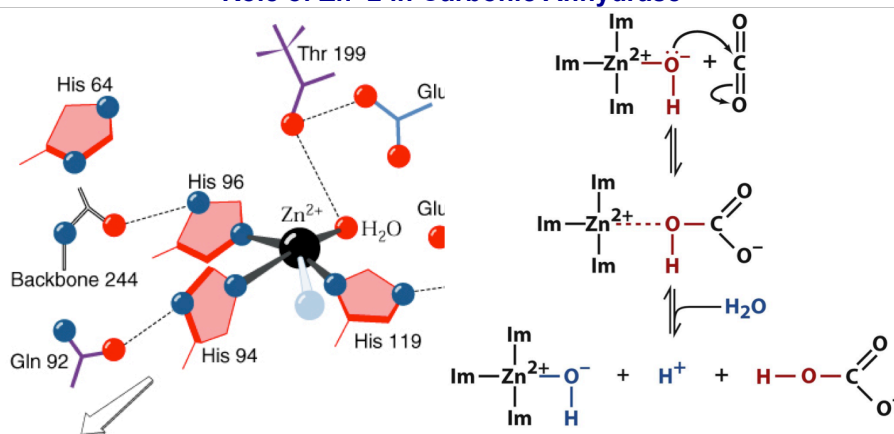
Initial step of thermolysin reaction

Metal Ion Catalysis

Transition metals as well as divalent cations (Mg^{+2} and Ca^{+2}) are useful for this type of catalysis. Metal ions act as a lewis acid and accept electrons. Therefore they are effective electrophiles. Another important reason for involving metals is the positive charge at any physiological pH.

- Called Metalloenzymes
- Involved in redox reactions
- Metals such as zinc activate water - to "acidify" or polarize the water so the OH group can act as a nucleophile.
- Electrostatically stabilize or shield negative charges (ATP and Mg^{+2})
- Carbonic anhydrase is an example of this type of reaction

Role of Zn²⁺ in Carbonic Anhydrase



After Sheridan, R.P. and Allen, L.C., J. Am. Chem. Soc. 103, 1545 (1981).
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Figure 11-13b
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Proximity and Orientation Effects

Efficiency of enzymes as catalysis occur due to where and how functional groups of enzyme and cofactors are physically enforced on the substrate

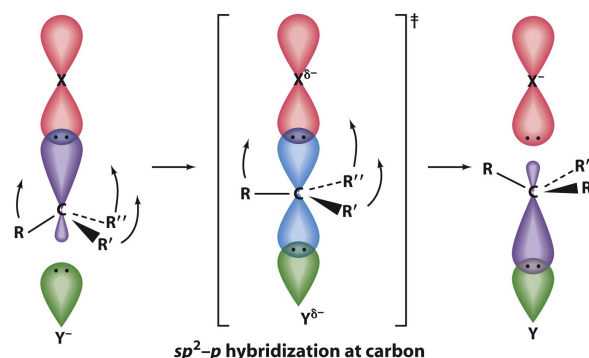
- Substrate – active site components must be precisely oriented (think $\text{S}_{\text{N}}2$ attacking and leaving orientation)
- Strain is often introduced to substrate as a result, bringing the complex to a transition state.

$\text{S}_{\text{N}}2$ and Orientation

$\text{S}_{\text{N}}2$ reactions must have the attacking nucleophile ($\text{Y}=\text{enzyme}$) approach the electrophile's SP^3 C opposite of the leaving group (X).

- Orientation permits this where the transition state becomes SP^2 -p hybridized.

Limiting orientation has an entropic penalty overcome by the enthalpy of bond formation

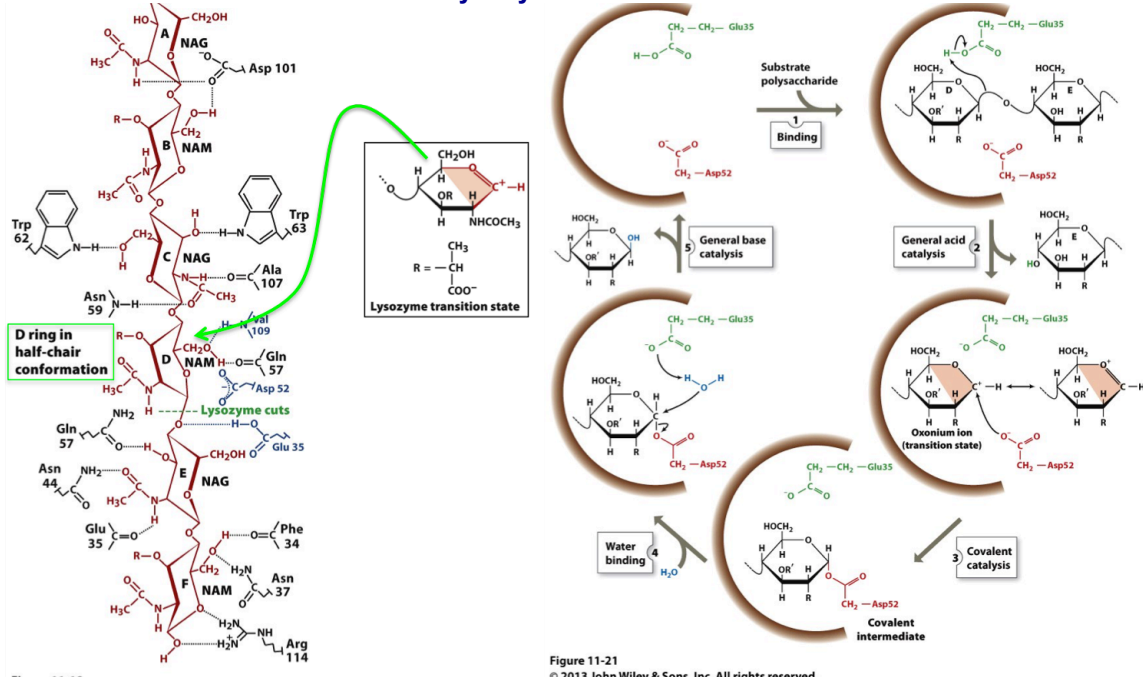


Proximity and Orientation Effects

Electrostatic Effects Recall that the strength of electrostatic interactions is related to the capacity of surrounding solvent molecules to reduce the attractive forces between chemical groups.

- Because water is largely excluded from the active site of most enzymes, the local dielectric constant is low. The charge distribution in the relatively anhydrous active site may influence the chemical reactivity of the substrate.
- In addition, weak electrostatic interactions such as those between permanent and induced dipoles in both the reactive site and the substrate are believed to contribute to catalysis. A more efficient binding of substrate lowers the free energy of the transition state, which accelerates the reaction.
- Thus enzymes act by stabilizing the distribution of electrical charge in the transition states.
- Many inhibitors of enzymes mimic the transition state. The enzyme binds the inhibitor at a stable energy state - these inhibitors bind with a much higher affinity than the normal substrate.

Lysozyme and Strain



Enzymatic Catalysis III

RNaseA Ribonuclease A (see book for reaction mechanism)

- An example of a general acid and base catalysis
- Digestive enzyme found in pancreas - involved in digestion of RNA. (ribonucleic acid) Both a general acid and general base catalyst
- RNA not DNA
- Cleaves between the 5' P of one sugar residue and the 2' O of the other ribose
- Pyrimidines are only real recognition site
- pH dependence of reaction indicates acid/bases with pKa around 5.4 and 6.4
- Iodoacetate - modifies His with acetate. This reaction kills RNaseA activity
- Two His residues act in concert in a two step reaction

This is a hydrolytic reaction yet the reaction begins with without water

- The reaction occurs by the following mechanism
 - His 12 (deprotonated) accepts the H of 2'OH, Followed by a nucleophilic attack by 2' O on P
 - Simultaneously - His 119 (protonated) donates H⁺ to other side of phosphate bond.
 - Lysine stabilizes (-) of phosphate
 - When His 12 and 119 are done cyclic O-P-O is formed
 - After cleavage and departure of RNA strand (leaving group) water adds into site for reversal of first step
 - Role of His 119 and His 12 are reversed when water is added onto 2'O and P
 - His 12 is general acid and His 119 acts as a general base
 - Last step regenerates original state for His to allow continued reactions

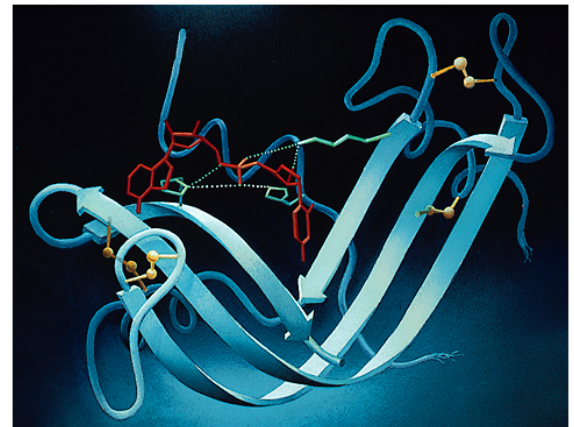
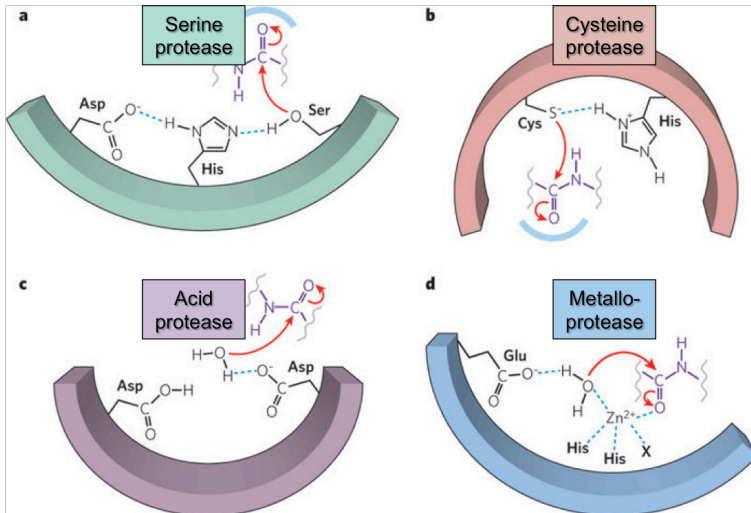


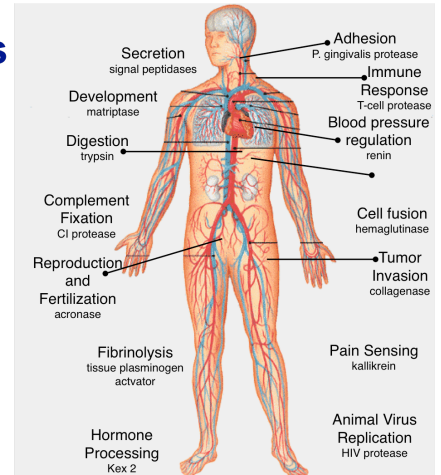
Figure copyrighted © by Irving Geis.

Covalent Cats - Proteases

4 classes of proteases: Serine, Thiol (Cys), Acid (Aspartyl), & Metal (Zn)



Roles

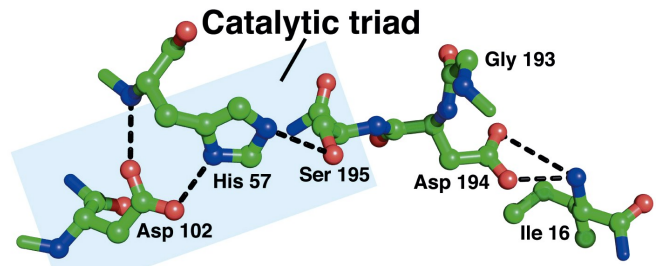


Function	Protease ex.
Nutrition	trypsin, subtilisin, α -lytic protease
Invasion	matrix metalloproteases
Evasion	IgA protease
Adhesion	ADAM (a disintegrin and metalloprotease)
Processing	signal peptidase, viral proteases, proteasome
Signaling	caspases, granzymes

Serine Proteases

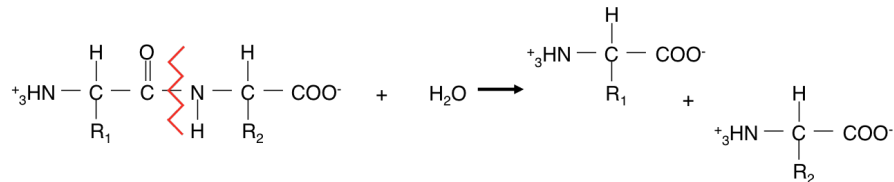
Ser, His and Asp in active site

- Zymogen proteases
- Binding site pocket creates substrate specificity
- Use several catalytic mechanisms



What are the proteases?

Hydrolyze peptide backbone of proteins



ΔG for the rxn is -2kcal/mol But...

Uncatalyzed rxn at neutral pH, 37°C: 1×10^{-10} /sec

Catalyzed rxn (chymotrypsin) at neutral pH, 37°C: 100/s

Conditions for chemically catalyzed reaction:

24hrs. @ 6M HCl, 110°C

Substrate Specificity

Binding pocket is responsible for affinity

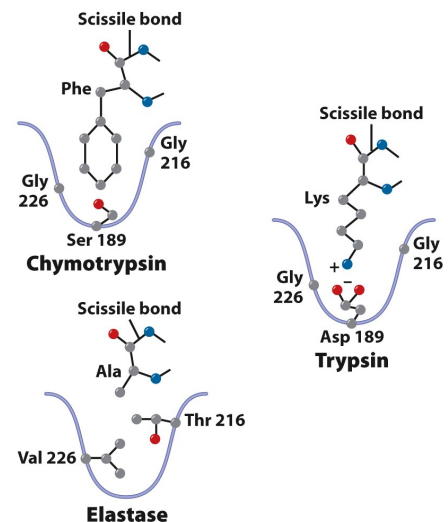
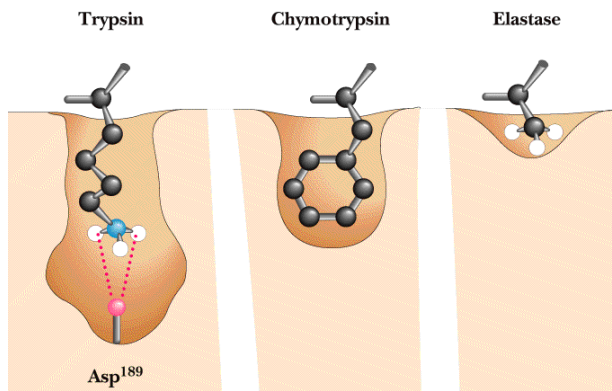
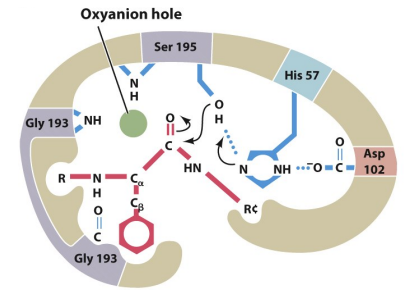


Figure 11-27
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Ser Proteases Multiple Mechanism

- Serine not generally an active amino acid for acid/base catalysis
- Catalytic triad of serine, histidine and aspartate responsible for the reactivity of serine in this active site
- Covalent catalysis, Acid/base Catalysis, transition state binding and Proximity mechanisms are used
- Two phase reaction when an ester is used
 - burst phase - E + S initial reactions
 - steady state phase - EP \rightarrow E + P (deacylation)
 - The first step is the covalent catalysis - where the substrate actually is bound to the enzyme itself



SEE BOOK FOR MECHANISM FIGURE

Importance of the Residues in the Active Site

Site-directed mutagenesis of Asp102 residue to Asn showed an enzyme with activity 1/10,000th of that of the native protein. Until this mutagenesis study, the role of Asp102 was only thought to be important because of its location in the active site as seen by x-ray crystal structures...

Transition State Binding

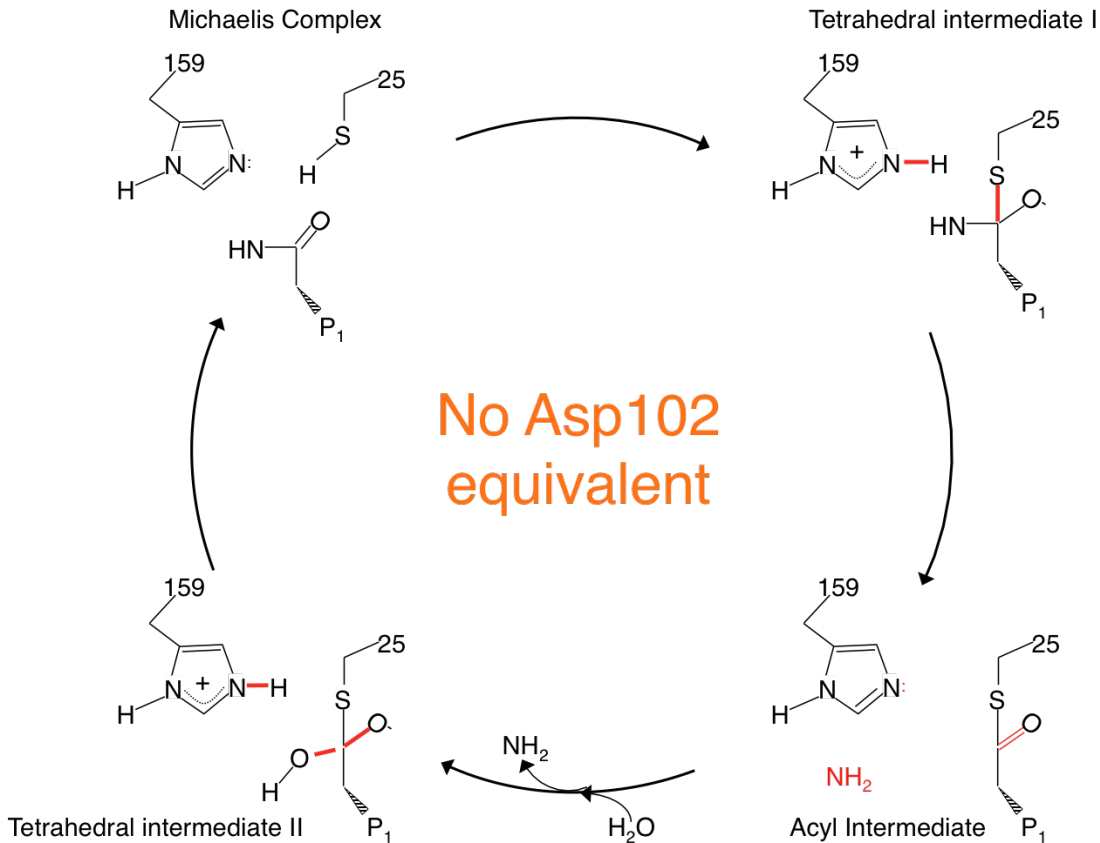
- After formation of oxyanion hole the peptide can move deeper into the active site
- H bonds formed in the oxyanion hole can only take place in this transition state form
- The distortion of the tetrahedral intermediate increases other H bonding between enzyme and NH of substrate peptide bond

Low-Barrier H Bonds

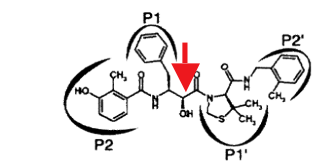
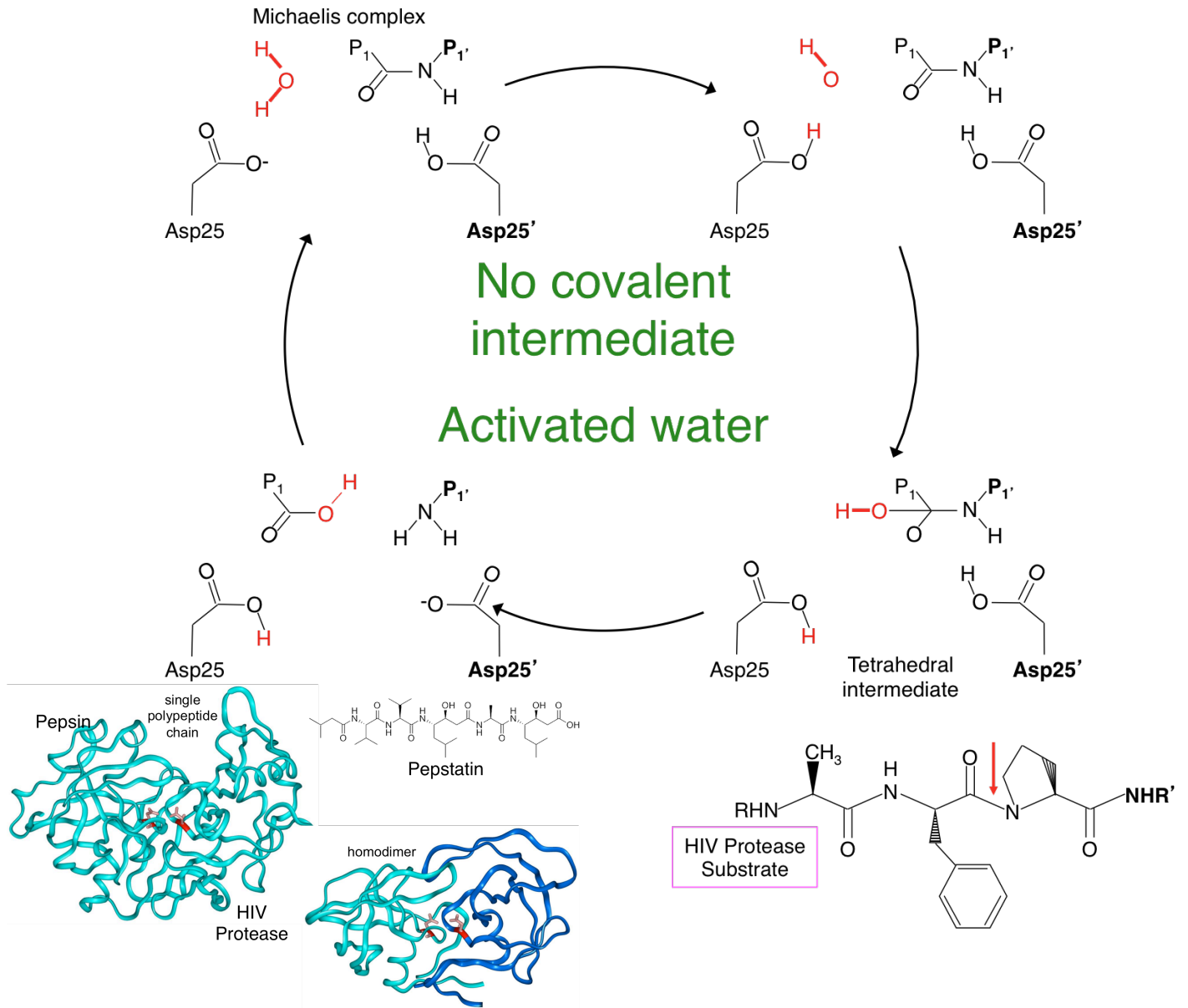
H bonds between heteroatoms occurs with very small distances between atoms

- These are short and very strong bonds
- Nearly equal pKa of His and Asp and distance of H bonds suggest a conversion of a weak H bond to a strong bond in transition state to promote proton transfer.

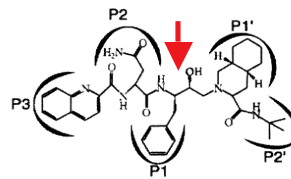
Cysteine Protease



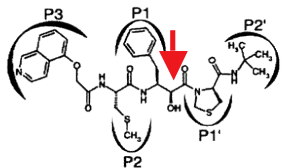
Aspartic Acid Protease



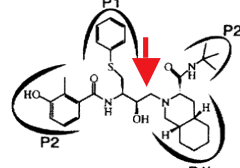
JE-2147
PDB: (p2₁2₁2₁)
Surface Area 781.1
Enthalpy: -7600
Entropy: -6556
Delta C_p: -370



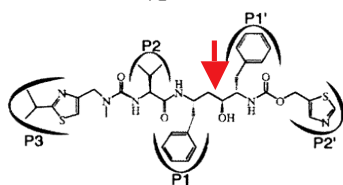
Saquinavir
PDB: 1HXB (p6₁)
Surface Area: 801.5
Enthalpy: +2200
Entropy: -14000
Delta C_p: -410



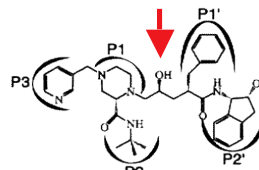
KNI-272
PDB: 1HPX (p2₁2₁2₁)
Surface Area 752.1
Enthalpy: -6300
Entropy: -8344
Delta C_p: -430



Nelfinavir
PDB: 1OHR (p2₁2₁2₁)
Surface Area 720.0
Enthalpy: +2800
Entropy: -14200
Delta C_p: -410

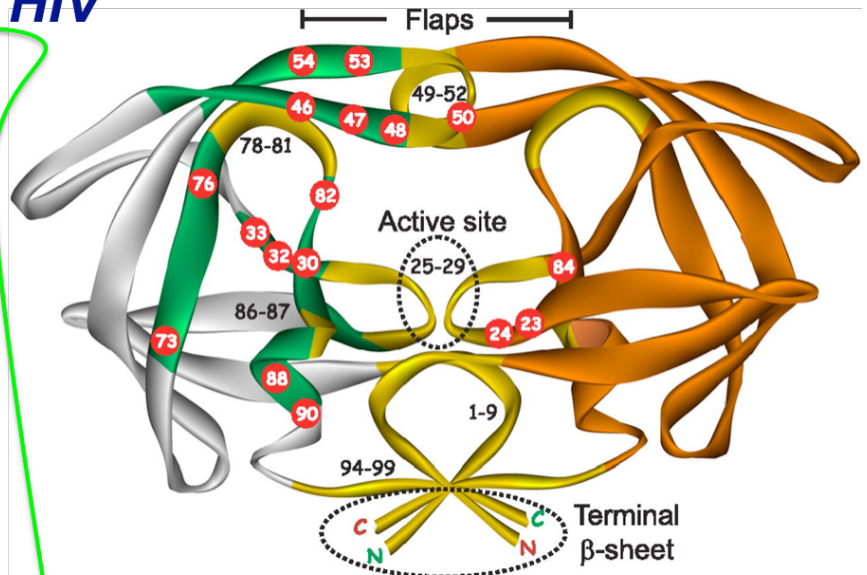
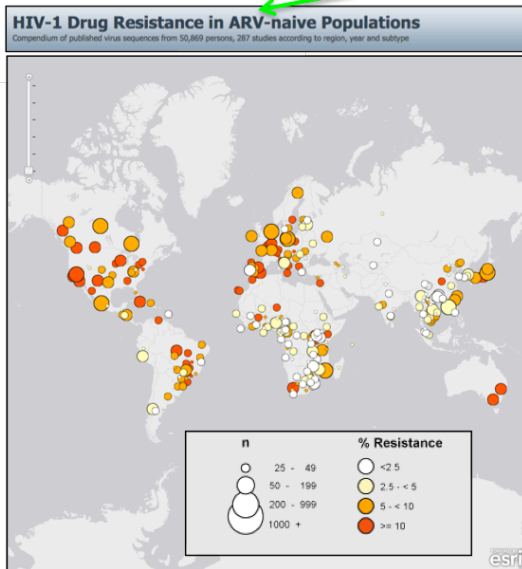


Ritonavir
PDB: 1HXW (p2₁2₁2₁)
Surface Area: 834.9
Enthalpy: -2300
Entropy: -11200
Delta C_p: -580



Indinavir
PDB: 1HSG (p2₁2₁2₁)
Surface Area: 827.0
Enthalpy: +3900
Entropy: -15700
Delta C_p: -440

Multi-drug resistant HIV

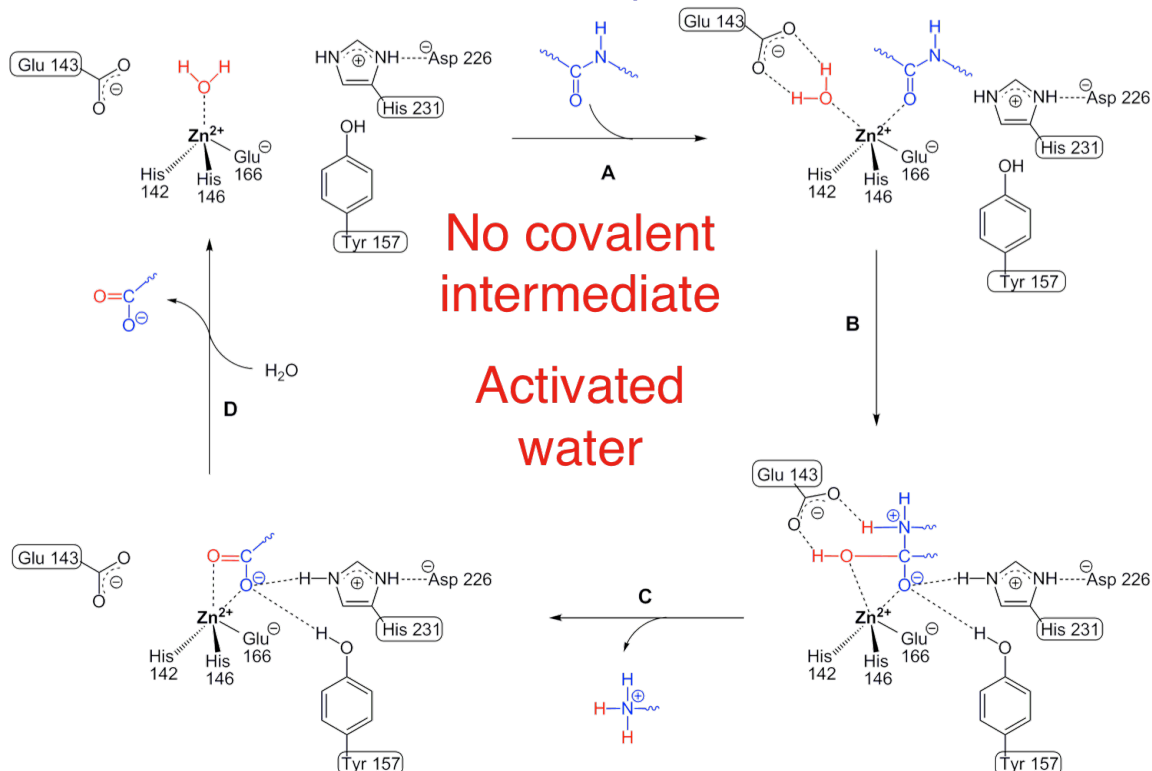


Did you know...

- ① 90% of HIV-positive people worldwide don't know that they have HIV.
- ② AntiRetroViral drugs increase the lifespan of an HIV-positive person by ~8 y.
- ③ HIV reproduces 10 bil to 1 tril times per day in the human body.
- ④ On average, a person can go up to 10 y or more without experiencing any symptoms of AIDS.
- ⑤ The strain of the virus responsible for the majority of HIV cases world wide originated from SE Cameroon chimpanzees.

http://youtu.be/UvEIXNo_aPw

Metalloprotease Mechanism



Serine

Ser-His Asp Catalytic Triad

covalent intermediate

Cysteine

Cys-His

covalent intermediate

Acid

Asp-Asp

Activated water

no covalent intermediate

Metallo

Zn^{2+} or equivalent-Glu

Activated Water

no covalent intermediate