Catalytic devices or strategies

Catalytic Devices

Enzymes may use one or a combination of the following:

- acid-base catalysis: both give and take protons
- covalent catalysis: change reaction pathways
- metal ion catalysis: use redox cofactors, pK_a shifters

General Acid-Base Catalysis

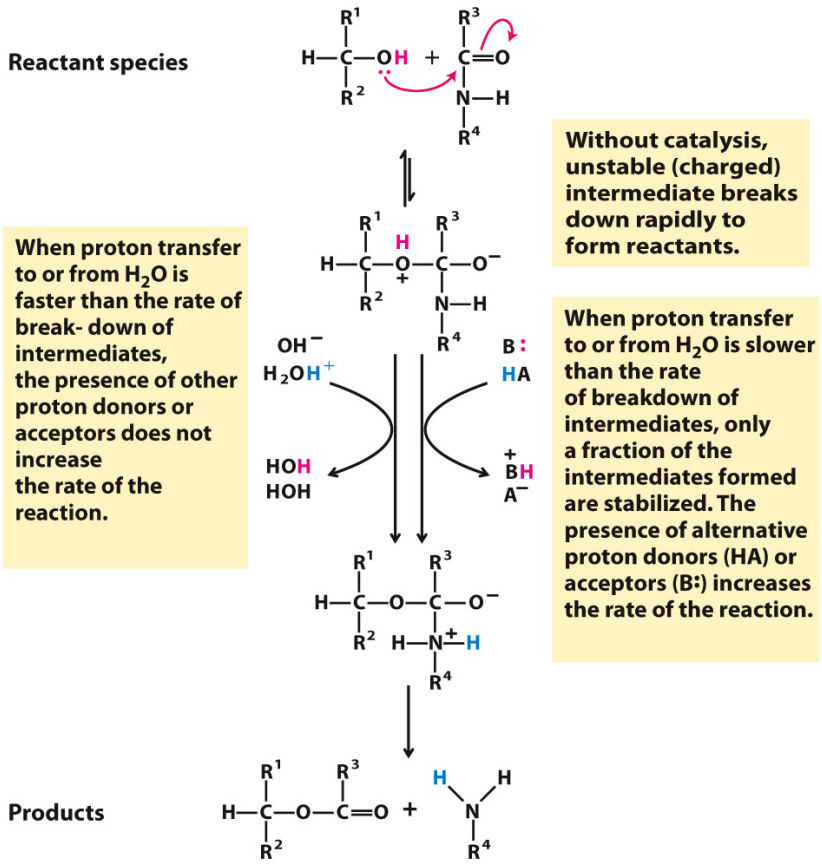


Figure 6-8
Lehninger Principles of Biochemistry, Seventh Edition

Amino Acids in General Acid-Base Catalysis

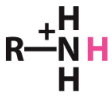
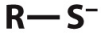
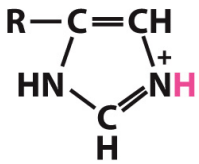
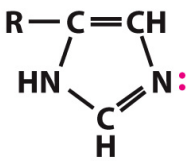
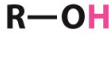
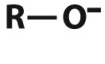
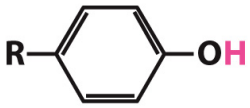
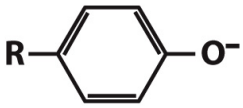
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp		
Lys, Arg		
Cys		
His		
Ser		
Tyr		

Figure 6-9
Lehninger Principles of Biochemistry, Seventh Edition

Covalent Catalysis

- A transient covalent bond forms between the enzyme and the substrate
- Changes the reaction pathway
  - uncatalyzed:

$$A — B \xrightarrow{H_2O} A + B$$

  - catalyzed (X = catalyst):

$$A — B + X: \rightarrow A — X + B \xrightarrow{H_2O} A + X: + B$$

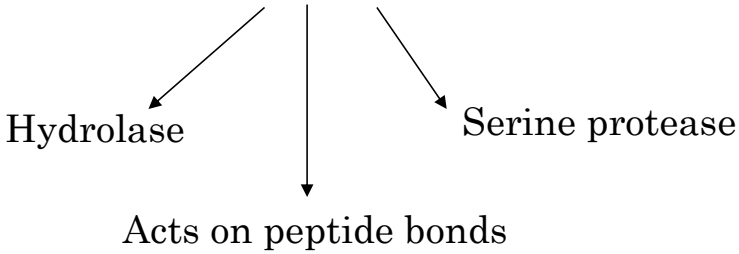
- Requires a nucleophile on the enzyme
  - can be a reactive serine, thiolate, amine, or carboxylate

Metal Ion Catalysis

- Involves one or more metal ions, bound to the enzyme
- Interacts with substrate to facilitate binding
  - stabilizes negative charges
- Accepts and or donates electrons (in redox reactions)

EXAMPLE OF HOW ENZYMES WORK

Example: Serine protease
Chymotrypsin (EC 3.4.21.1)
Trypsin (EC 3.4.21.4)



How Do Enzymes Work?

For the class we will study one type of enzyme (a serine protease) as an example of how all enzymes work.

how an enzyme

- Stabilizes a transition state
- Uses lock and key mechanism
- Uses acid-base catalysis: both gives and takes protons
- Uses covalent catalysis: changes the reaction pathway compared to the uncatalyzed reaction

Serine proteases do not use metal ions in catalytic mechanism.

Serine proteases do not use induced fit.

- During digestion, dietary proteins must be broken down into small peptides by proteases.
- Trypsin and Chymotrypsin are among a group of proteases that cut peptides at specific locations on the peptide backbone.
- Chymotrypsin cleaves the peptide bond adjacent to aromatic amino acids.
- Trypsin cleaves the peptide bond adjacent to basic amino acids.

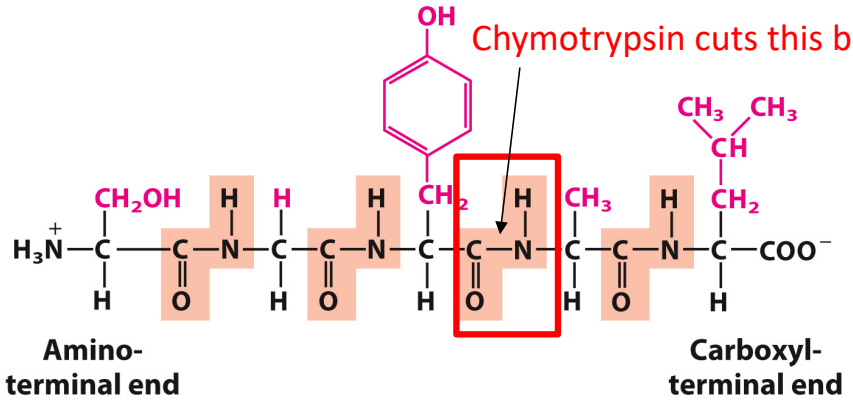
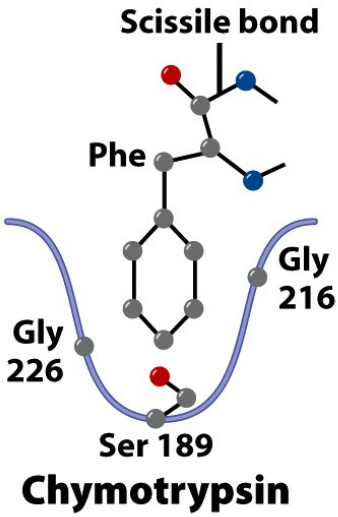
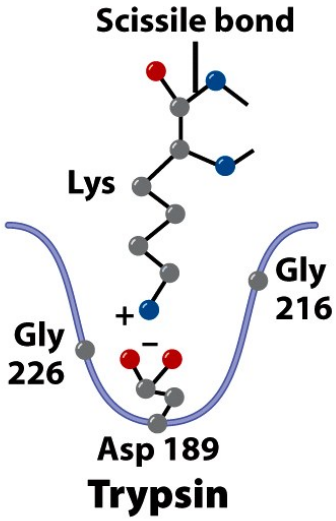


Figure 3-14
Lehninger Principles of Biochemistry, Seventh Edition

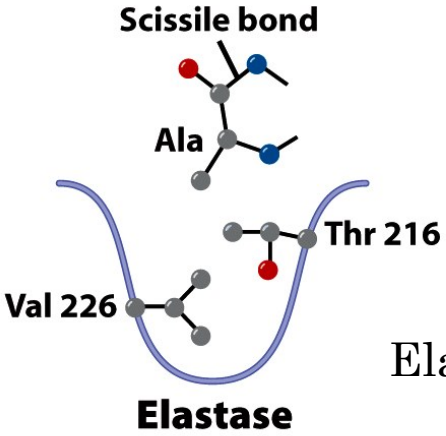
Nature of residues in binding pocket help define specificity.

ChT: cuts at Phe, Trp or Tyr





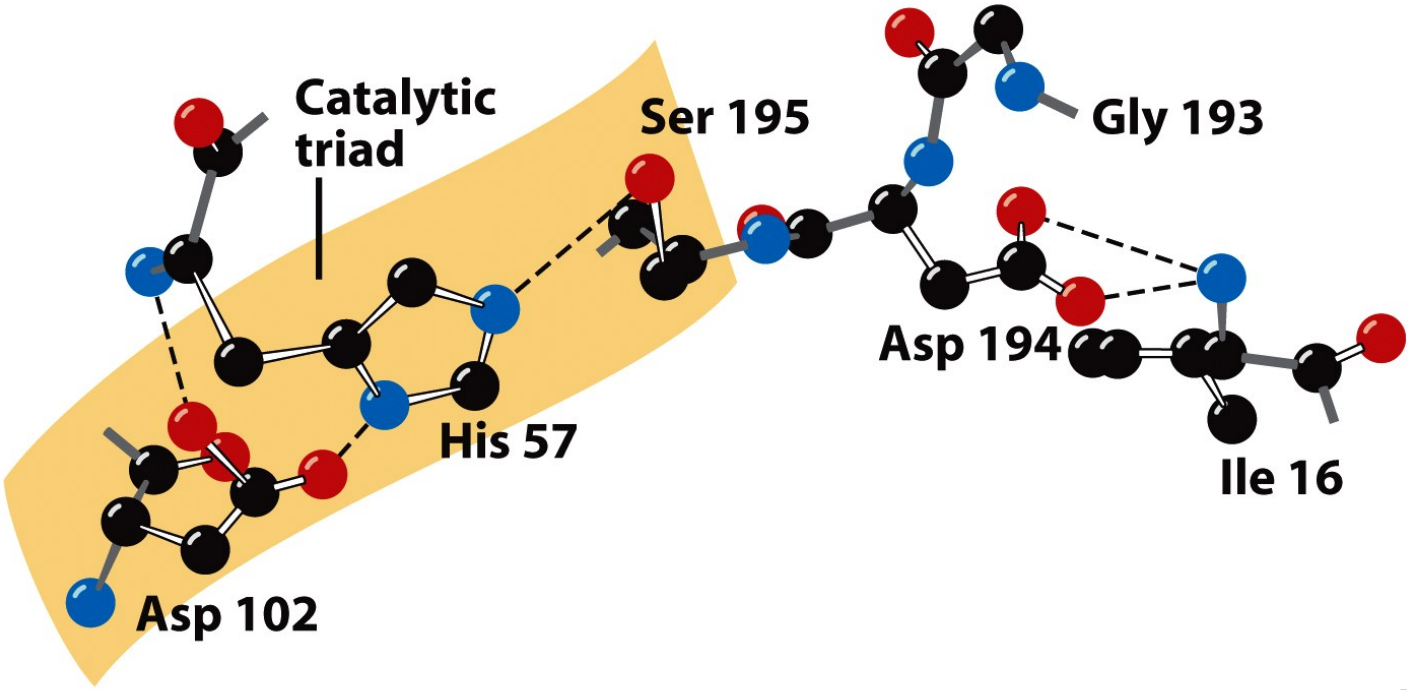
Trypsin: cuts at Lys or Arg



Elastase: cuts at Ala, Gly, Val.

Figure 11-27

Active site residues of chymotrypsin: The Catalytic triad



Trypsin and elastase, two other proteases, share 40% identity of their ~240 residues with ChT, including the catalytically important His and Ser.

Divergent evolution after duplication of an ancestral gene.

Convergent evolution: Same catalytic triad mechanism, no homology.

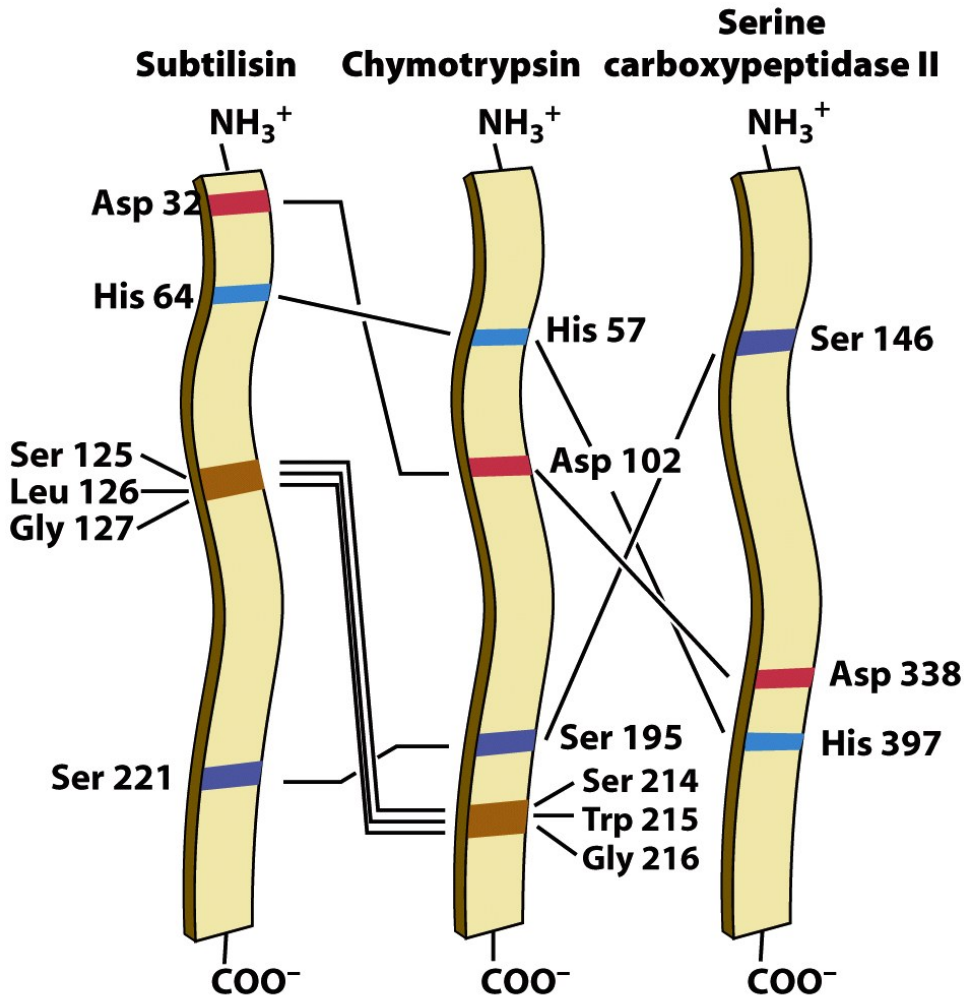
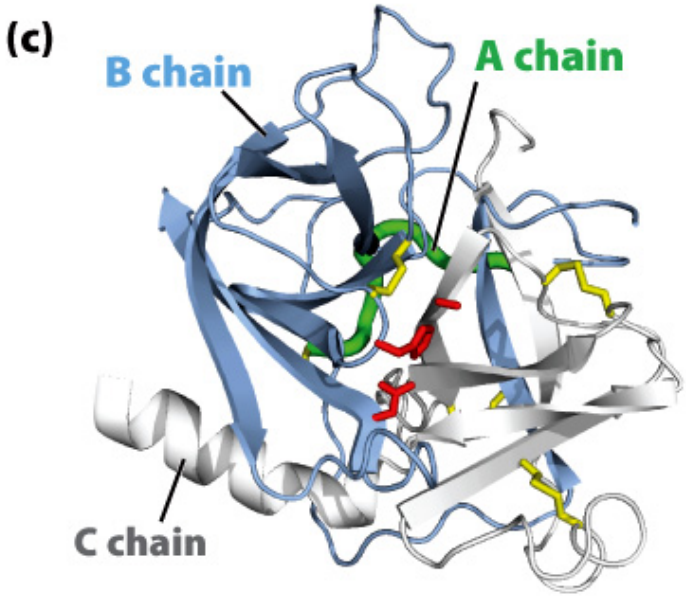
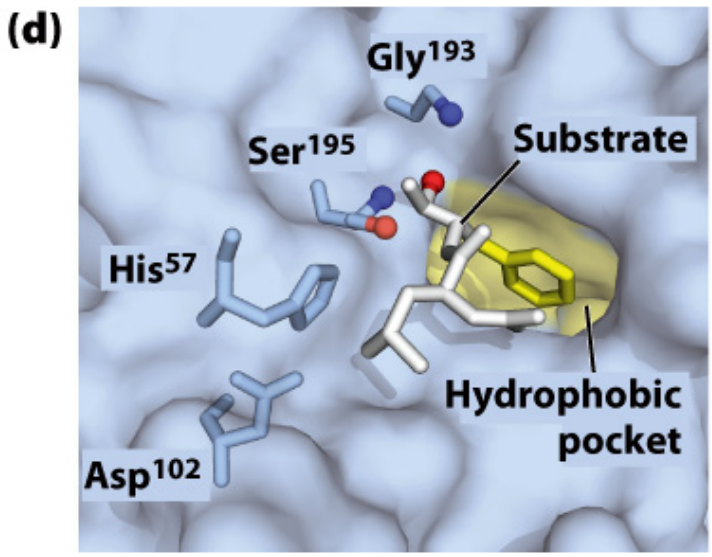
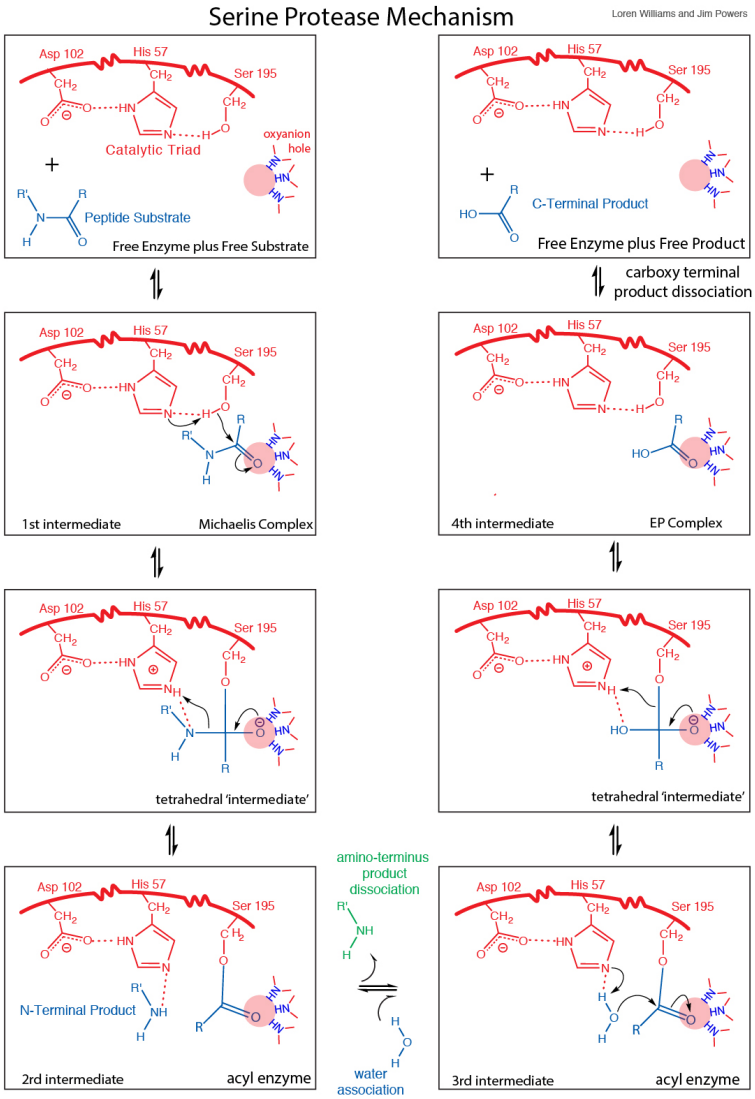


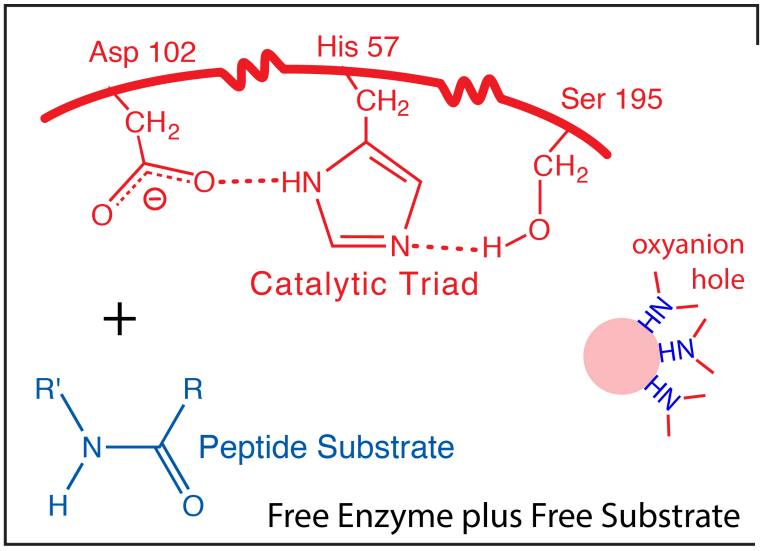
Figure 11-28

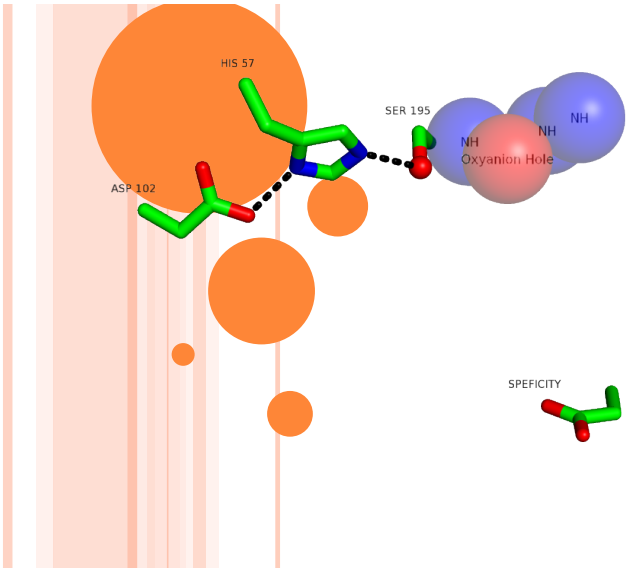
Trypsin/Chymotrypsin Use Several Enzymatic Devices

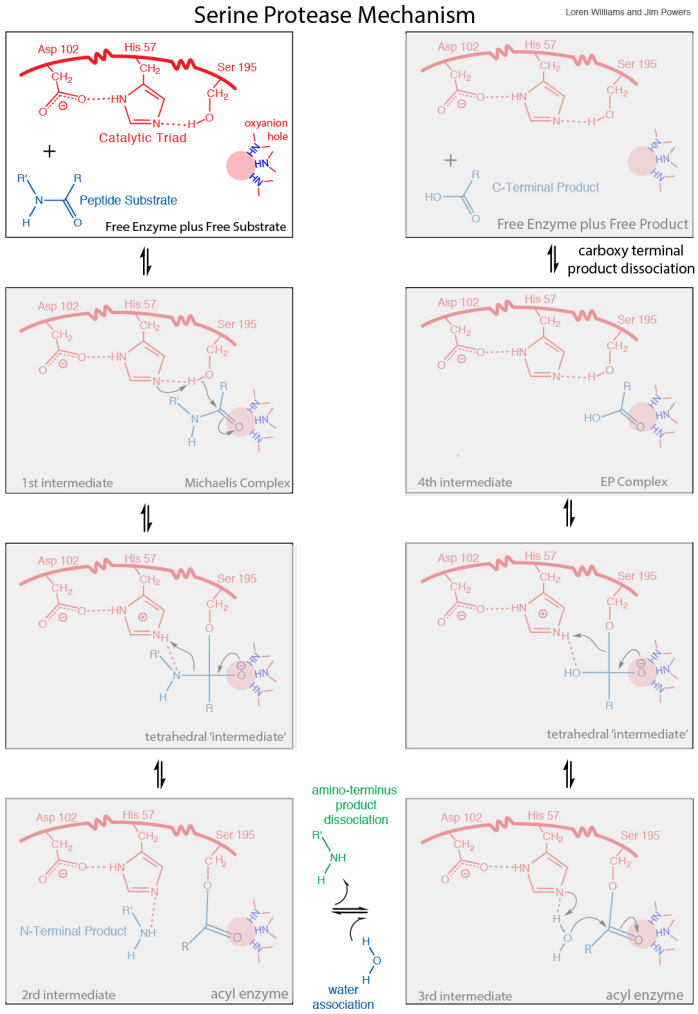





Serine Protease Mechanism
Loren Williams and Jim Powers
Asp 102
His 57
Ser 195
Catalytic Triad
oxyanion hole
Peptide Substrate
Free Enzyme plus Free Substrate
C-Terminal Product
Free Enzyme plus Free Product
carboxy terminal product dissociation
1st intermediate
Michaelis Complex
4th intermediate
EP Complex
tetrahedral 'intermediate'
tetrahedral 'intermediate'
N-Terminal Product
2rd intermediate
acyl enzyme
amino-terminus product dissociation
water association
3rd intermediate
acyl enzyme


Asp 102
His 57
Ser 195
Catalytic Triad
oxyanion
hole
Peptide Substrate
Free Enzyme plus Free Substrate


HIS 57
SER 195
ASP 102
NH
Oxyanion Hole
SPEFICITY


Serine Protease Mechanism
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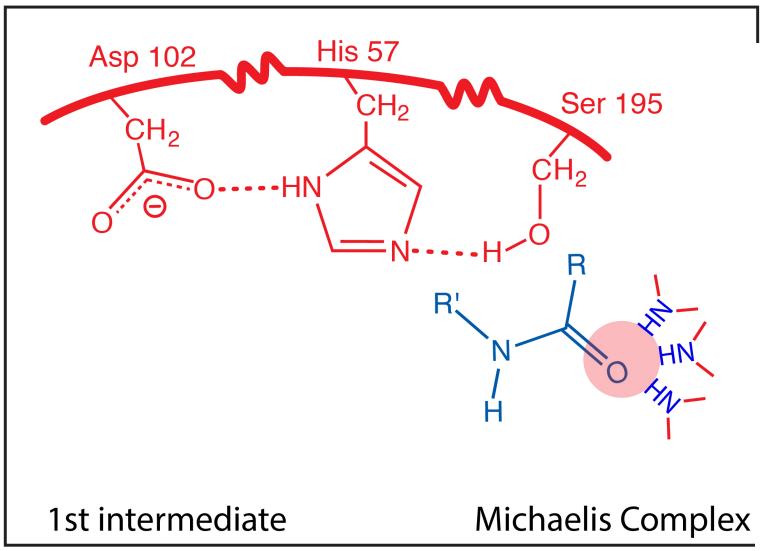
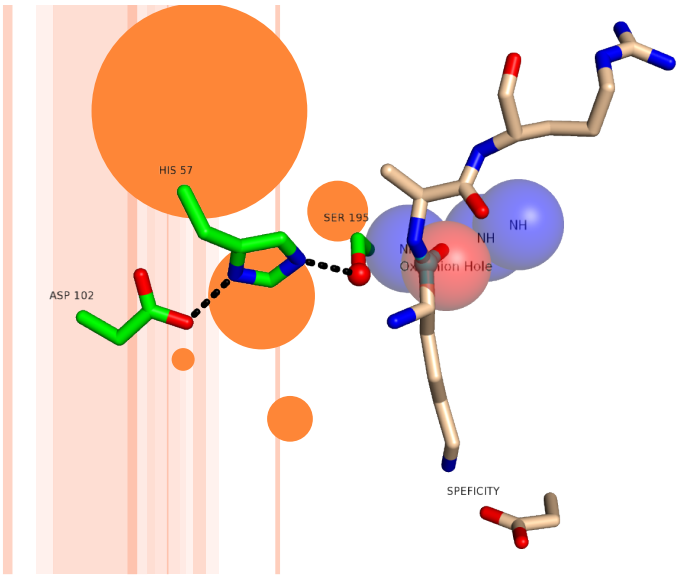
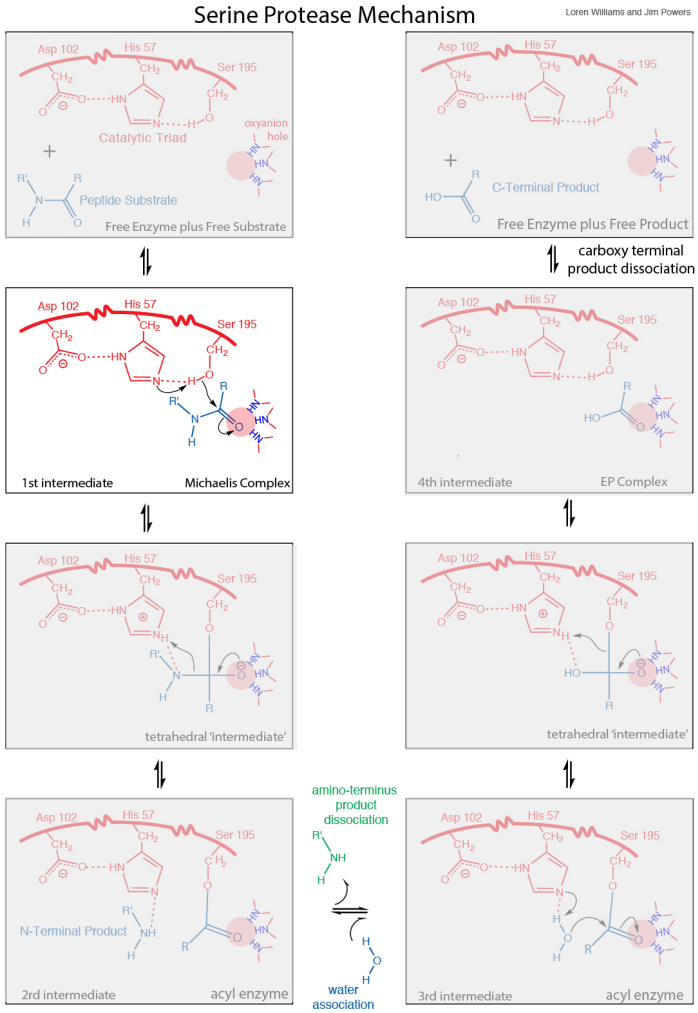
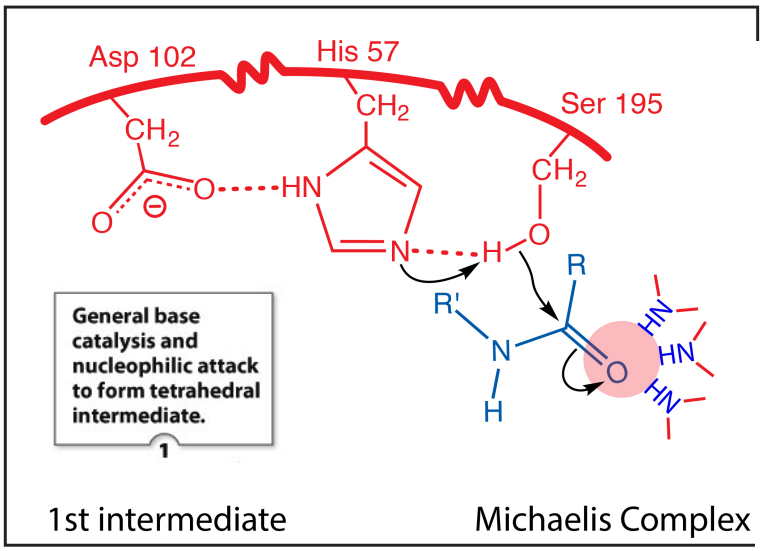
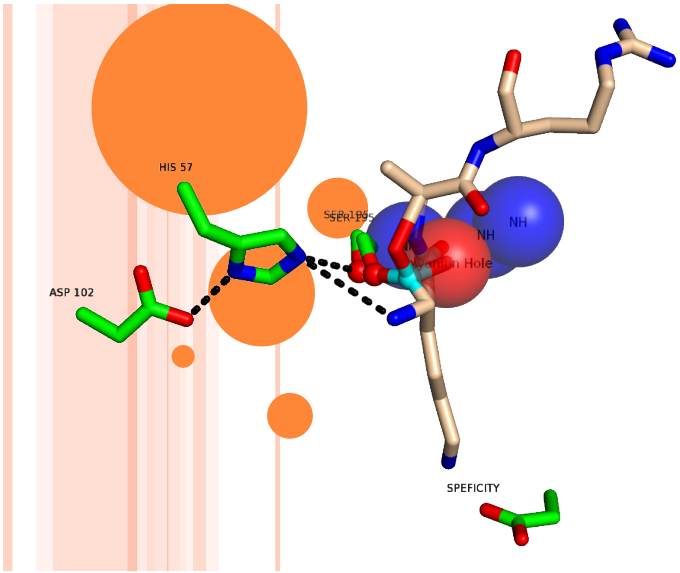
Ser 195
Catalytic Triad
oxyanion hole
Peptide Substrate
Free Enzyme plus Free Substrate
C-Terminal Product
Free Enzyme plus Free Product
carboxy terminal product dissociation
1st intermediate
Michaelis Complex
4th intermediate
EP Complex
tetrahedral 'intermediate'
tetrahedral 'intermediate'
amino-terminus product dissociation
water association
N-Terminal Product
2rd intermediate
acyl enzyme
3rd intermediate
acyl enzyme

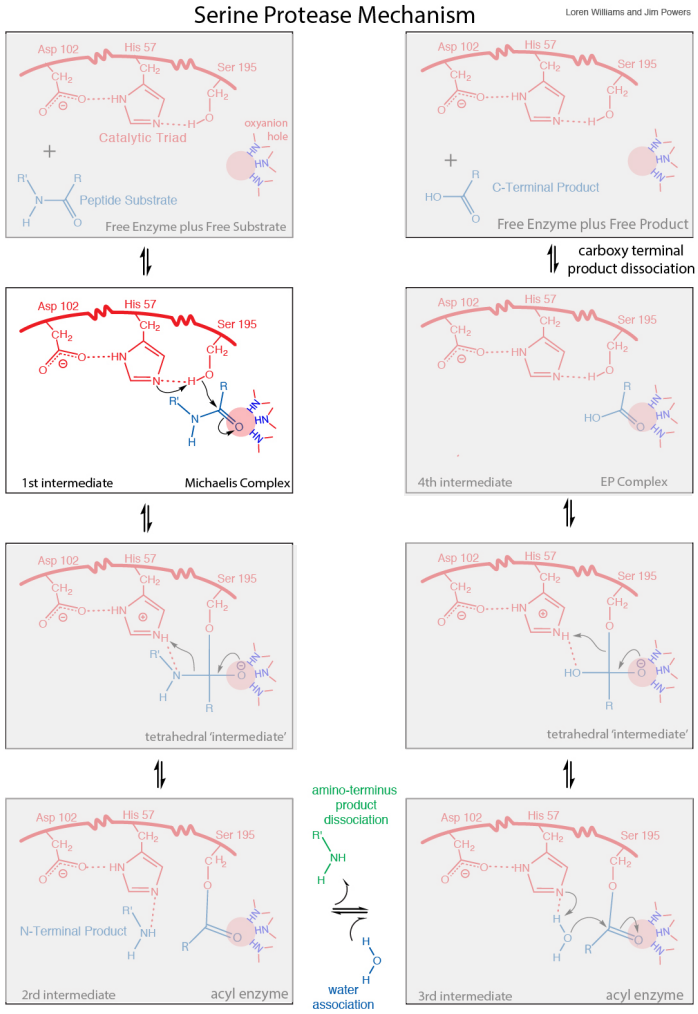

Asp 102
His 57
Ser 195
1st intermediate
Michaelis Complex

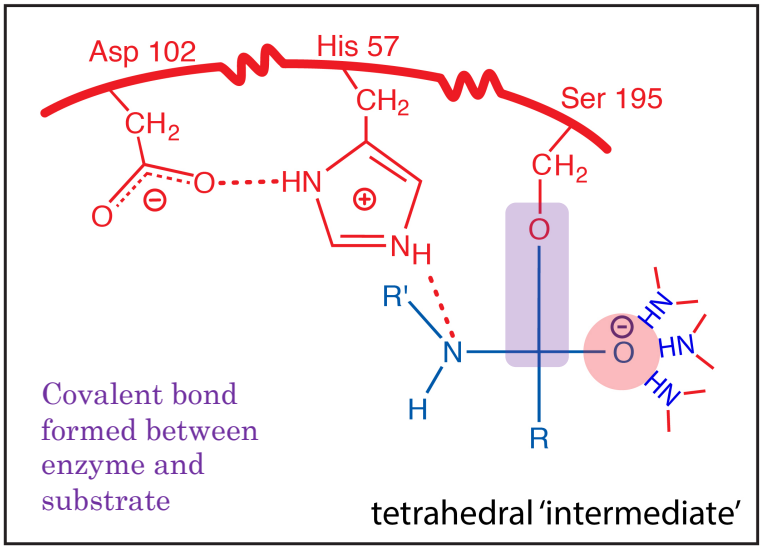

HIS 57
SER 195
ASP 102
SPEFICITY

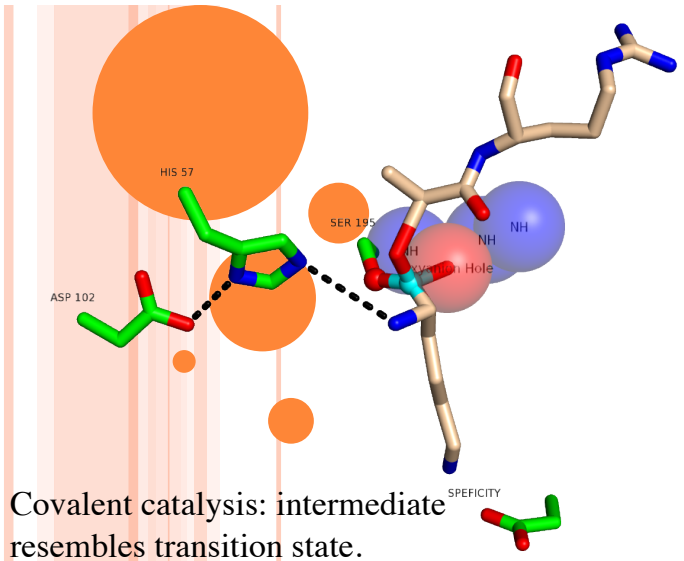

Serine Protease Mechanism
Loren Williams and Jim Powers
Catalytic Triad
oxyanion hole
Peptide Substrate
Free Enzyme plus Free Substrate
C-Terminal Product
Free Enzyme plus Free Product
carboxy terminal product dissociation
1st intermediate
Michaelis Complex
4th intermediate
EP Complex
tetrahedral 'intermediate'
tetrahedral 'intermediate'
amino-terminus product dissociation
N-Terminal Product
2rd intermediate
acyl enzyme
water association
3rd intermediate
acyl enzyme


Asp 102
His 57
Ser 195
General base catalysis and nucleophilic attack to form tetrahedral intermediate.
1
1st intermediate
Michaelis Complex

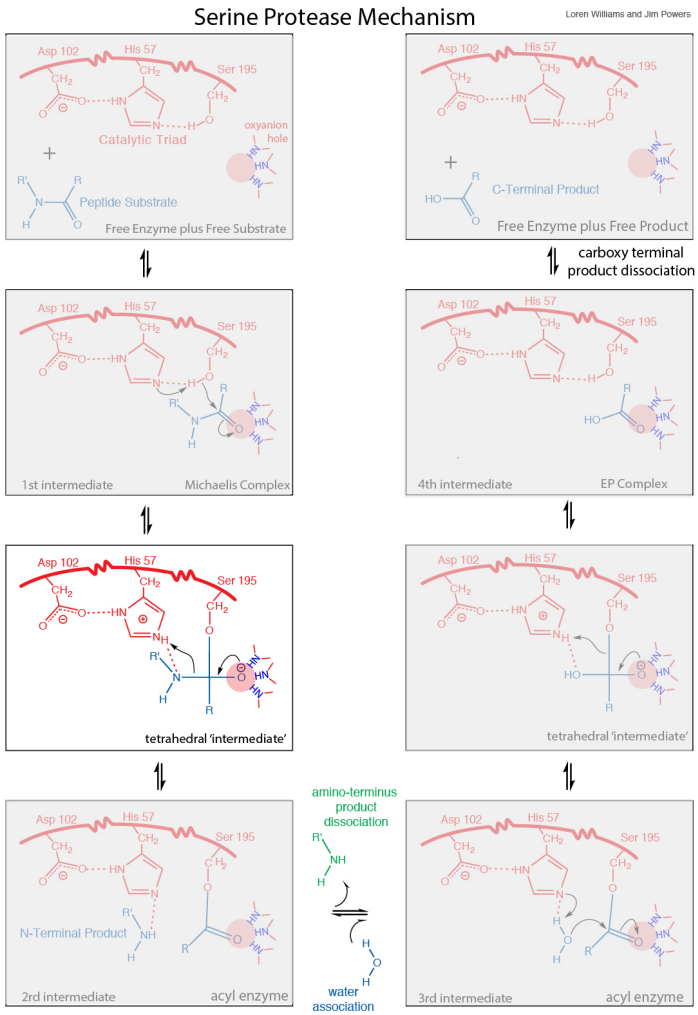

HIS 57
ASP 102
SPEFICITY

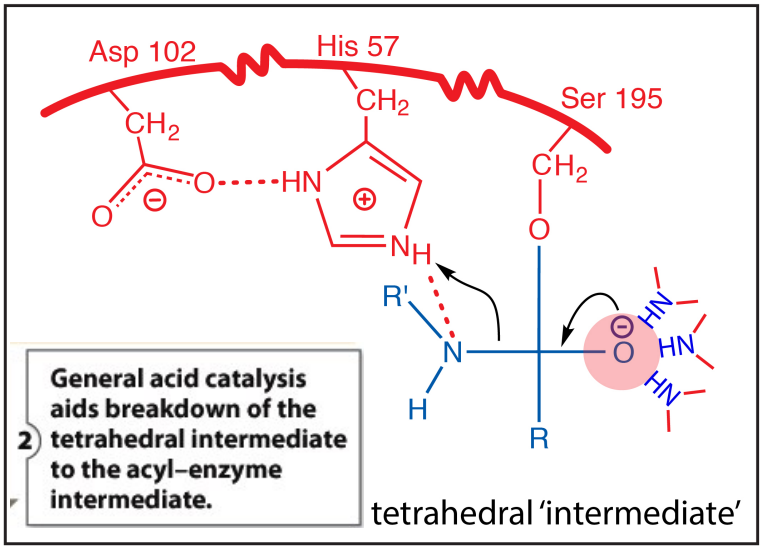

Serine Protease Mechanism
Loren Williams and Jim Powers
Catalytic Triad
oxyanion hole
Peptide Substrate
Free Enzyme plus Free Substrate
C-Terminal Product
Free Enzyme plus Free Product
carboxy terminal product dissociation
1st intermediate
Michaelis Complex
4th intermediate
EP Complex
tetrahedral 'intermediate'
tetrahedral 'intermediate'
amino-terminus product dissociation
N-Terminal Product
2rd intermediate
acyl enzyme
water association
3rd intermediate
acyl enzyme

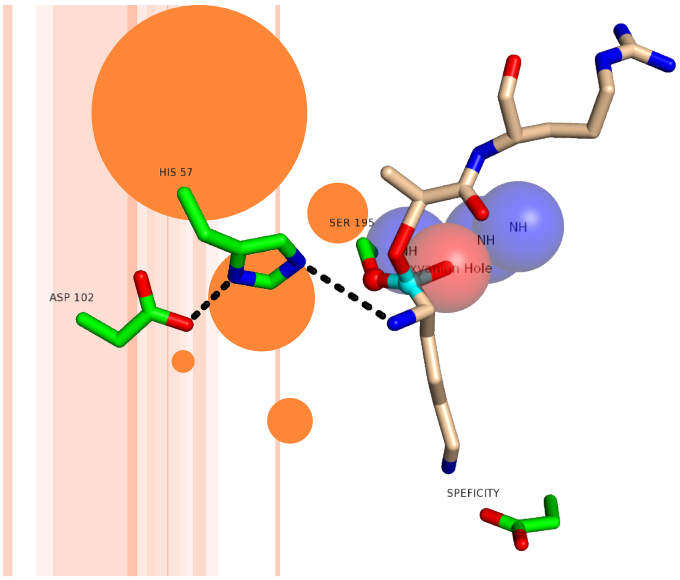


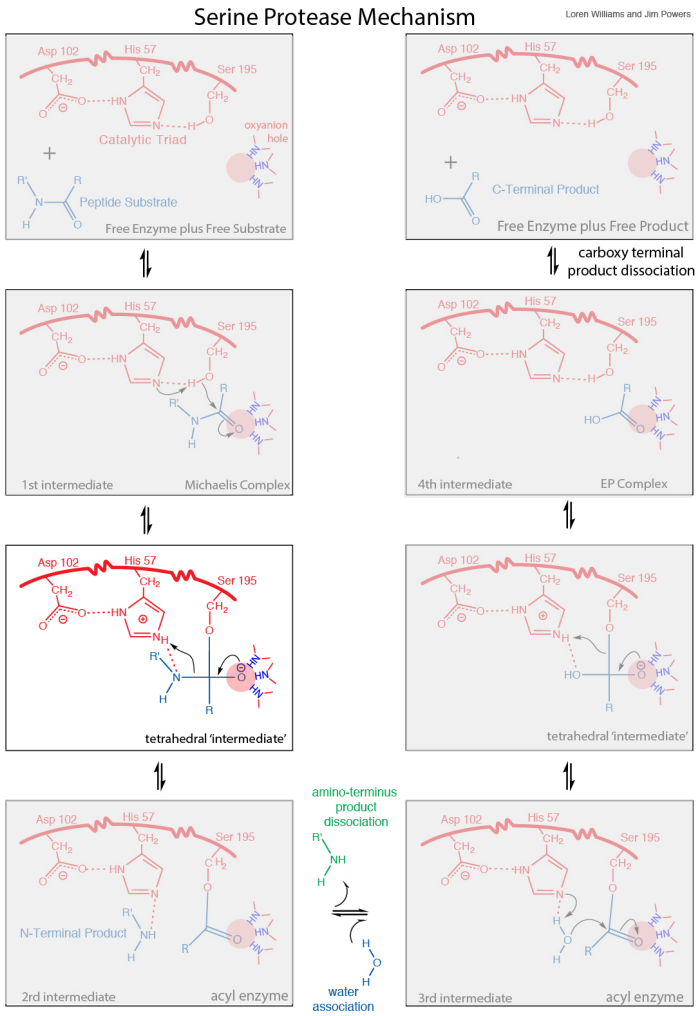


Covalent catalysis: intermediate resembles transition state.




Asp 102
His 57
Ser 195
CH2
O
HN
NH
R'
N
H
R
HN
HN
HN
2
General acid catalysis aids breakdown of the tetrahedral intermediate to the acyl–enzyme intermediate.
tetrahedral 'intermediate'


HIS 57
SER 195
ASP 102
NH
NH
SPEFICITY


Serine Protease Mechanism
Loren Williams and Jim Powers
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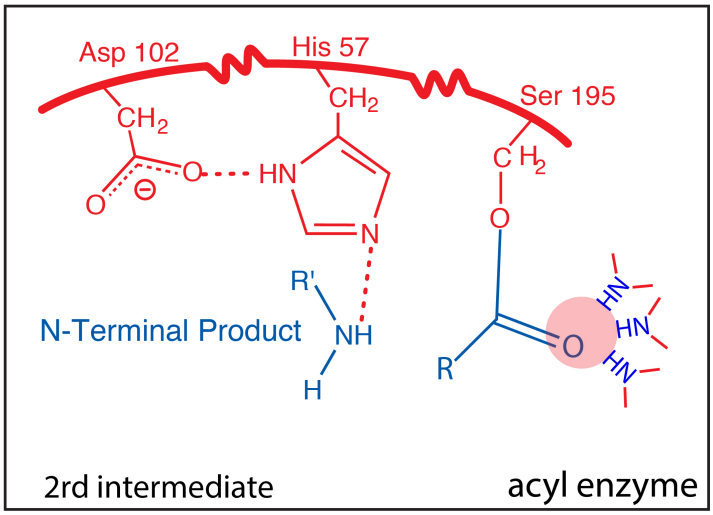
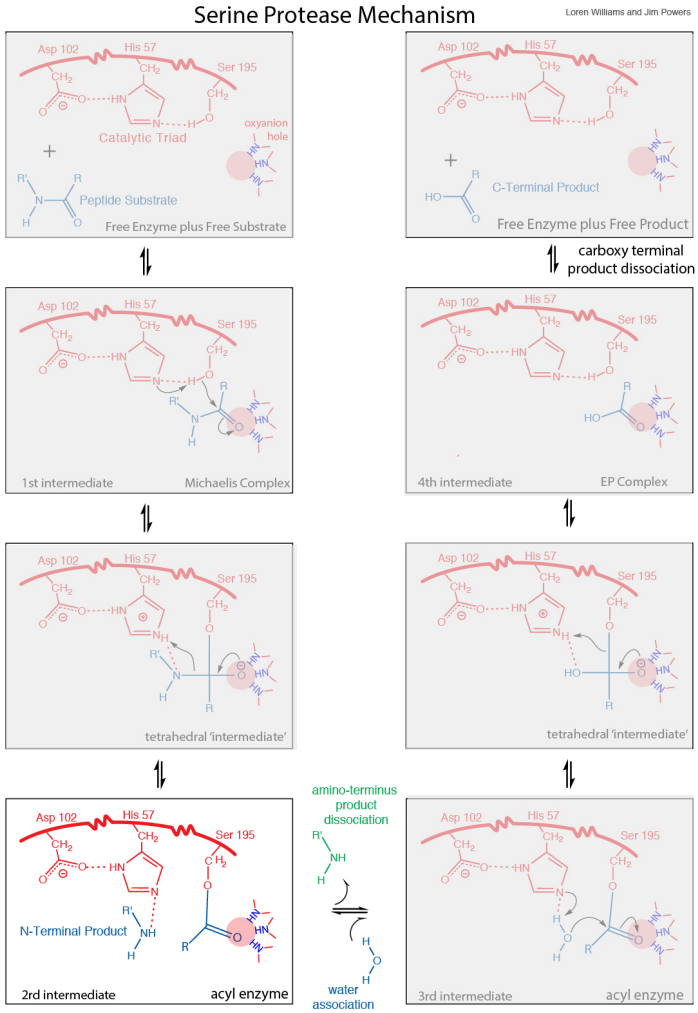
His 57
Ser 195
Catalytic Triad
oxyanion hole
Peptide Substrate
Free Enzyme plus Free Substrate
C-Terminal Product
Free Enzyme plus Free Product
carboxy terminal product dissociation
1st intermediate
Michaelis Complex
4th intermediate
EP Complex
tetrahedral 'intermediate'
tetrahedral 'intermediate'
amino-terminus product dissociation
water association
N-Terminal Product
2rd intermediate
acyl enzyme
3rd intermediate
acyl enzyme


Asp 102
His 57
Ser 195
CH2
HN
N
O
R'
NH
H
N-Terminal Product
R
HN
2rd intermediate
acyl enzyme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oxyanion hole
Peptide Substrate
Free Enzyme plus Free Substrate
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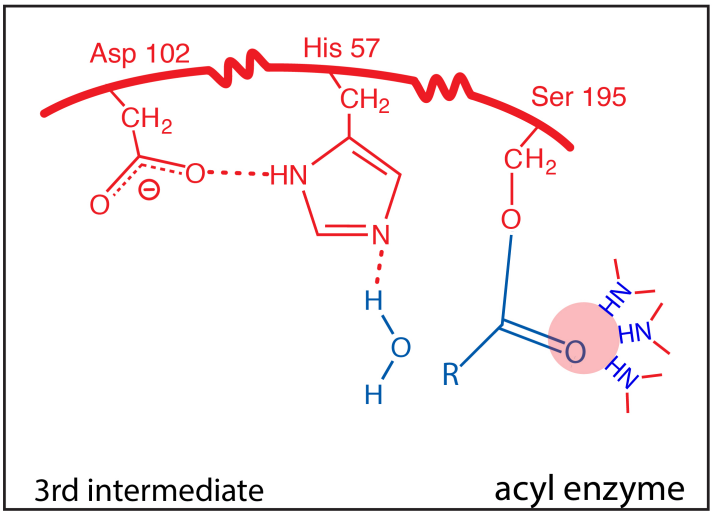
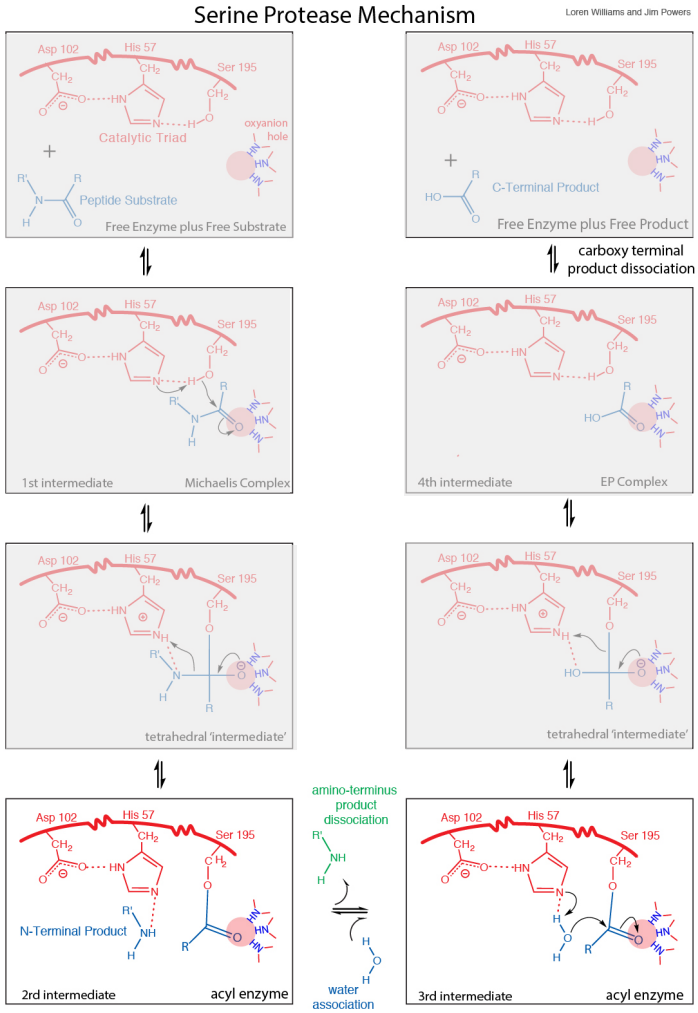
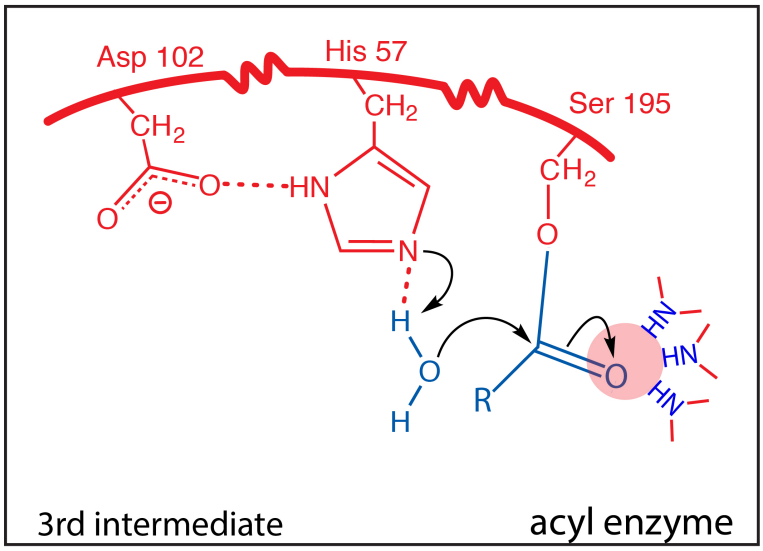
carboxy terminal product dissociation
1st intermediate
Michaelis Complex
4th intermediate
EP Complex
tetrahedral 'intermediate'
tetrahedral 'intermediate'
N-Terminal Product
2rd intermediate
acyl enzyme
amino-terminus product dissociation
water association
3rd intermediate
acyl enzyme



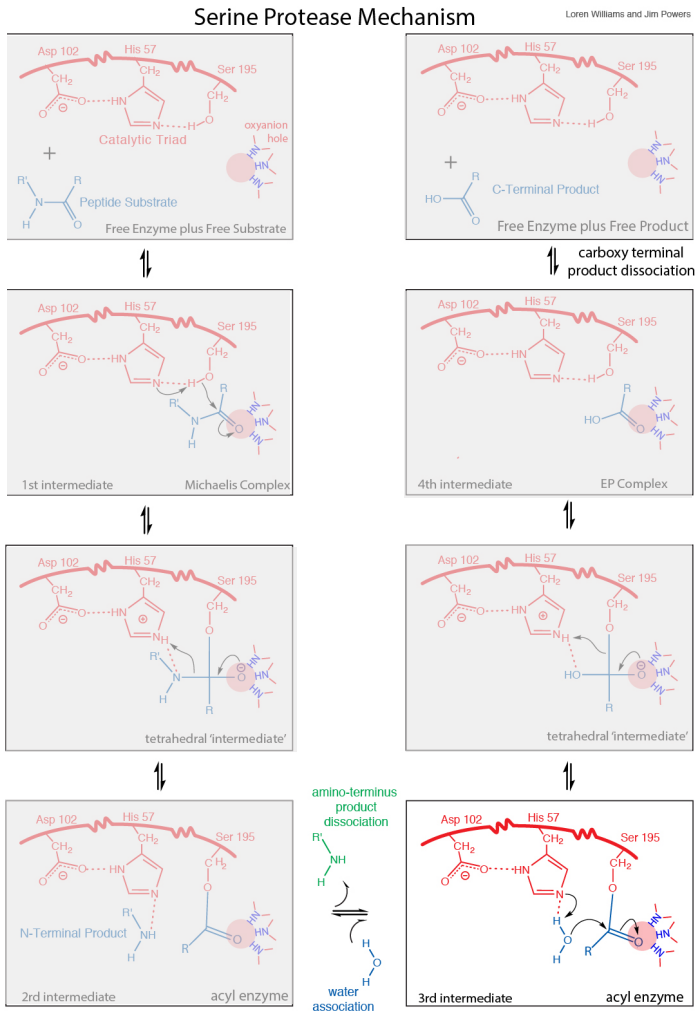
Amine product is released and replaced by water.

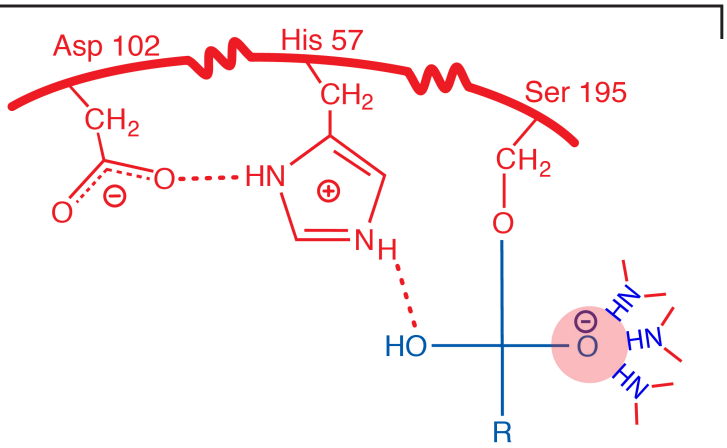
3

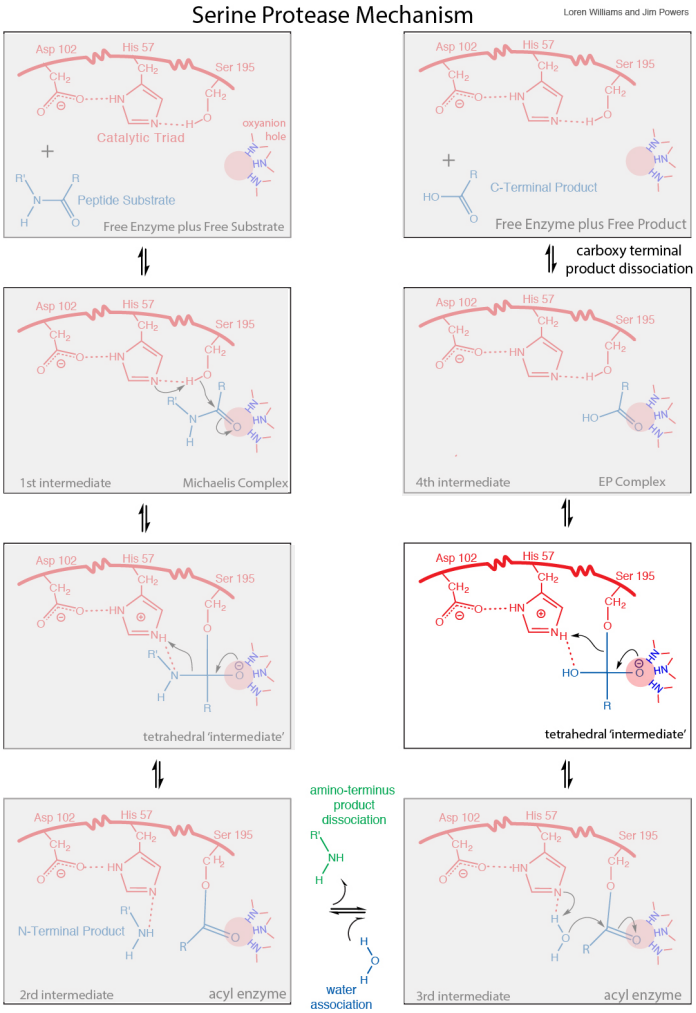




General base catalysis and nucleophilic attack to form tetrahedral intermediate. 4




Asp 102
His 57
Ser 195
tetrahedral 'intermediate'


Serine Protease Mechanism
Loren Williams and Jim Powers
Catalytic Triad
oxyanion hole
Peptide Substrate
Free Enzyme plus Free Substrate
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Free Enzyme plus Free Product
carboxy terminal product dissociation
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tetrahedral 'intermediate'
amino-terminus product dissociation
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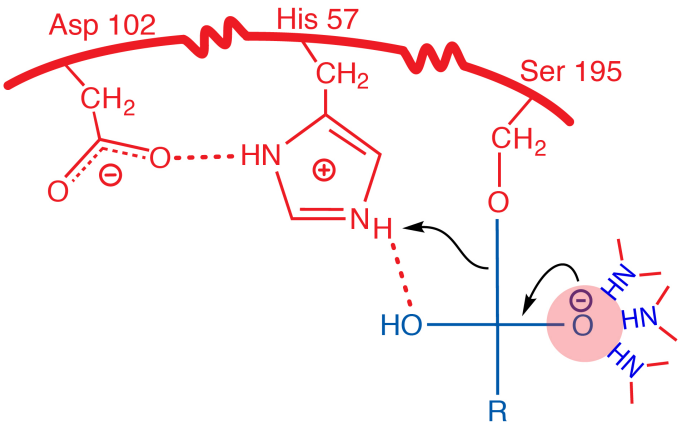
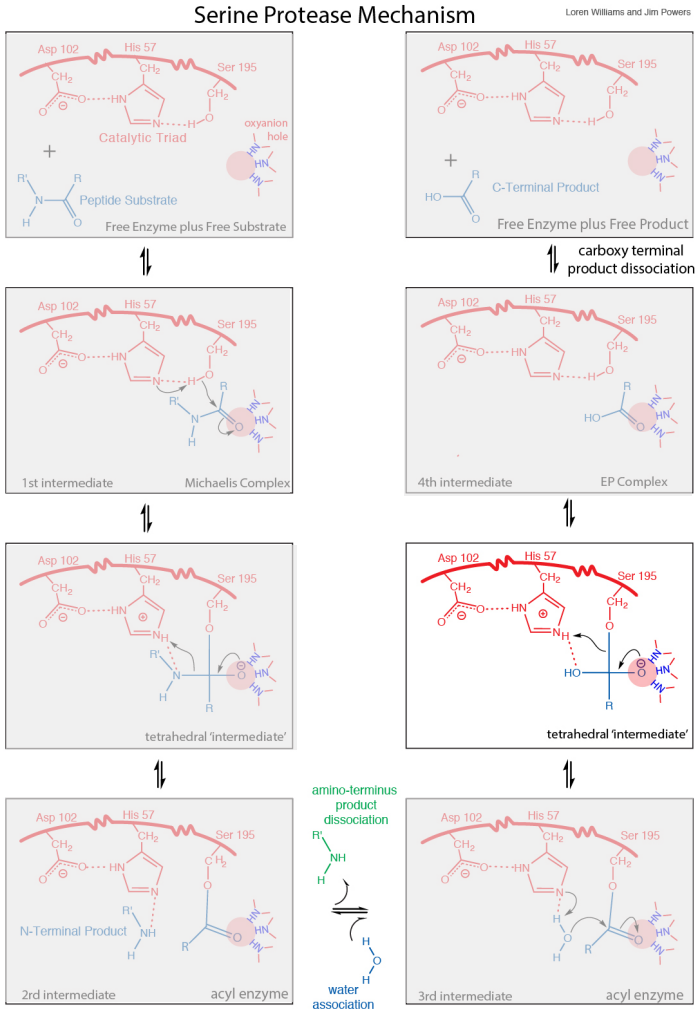
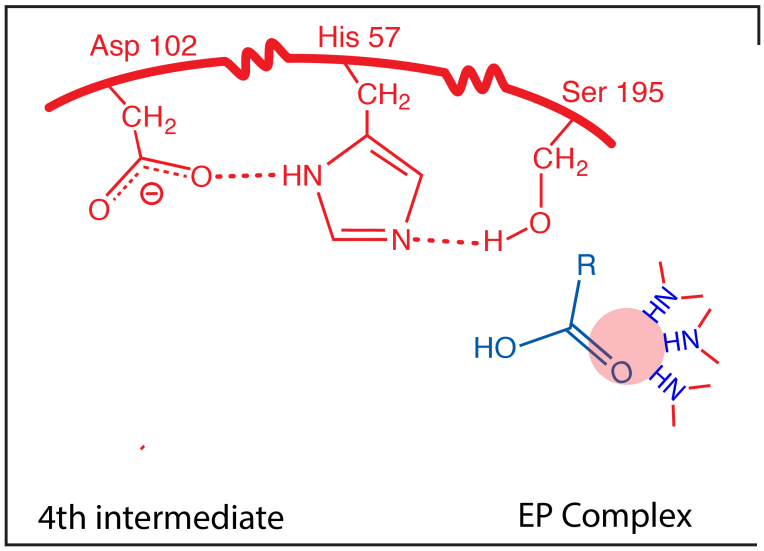
2rd intermediate
acyl enzyme
water association
3rd intermediate
acyl enzyme

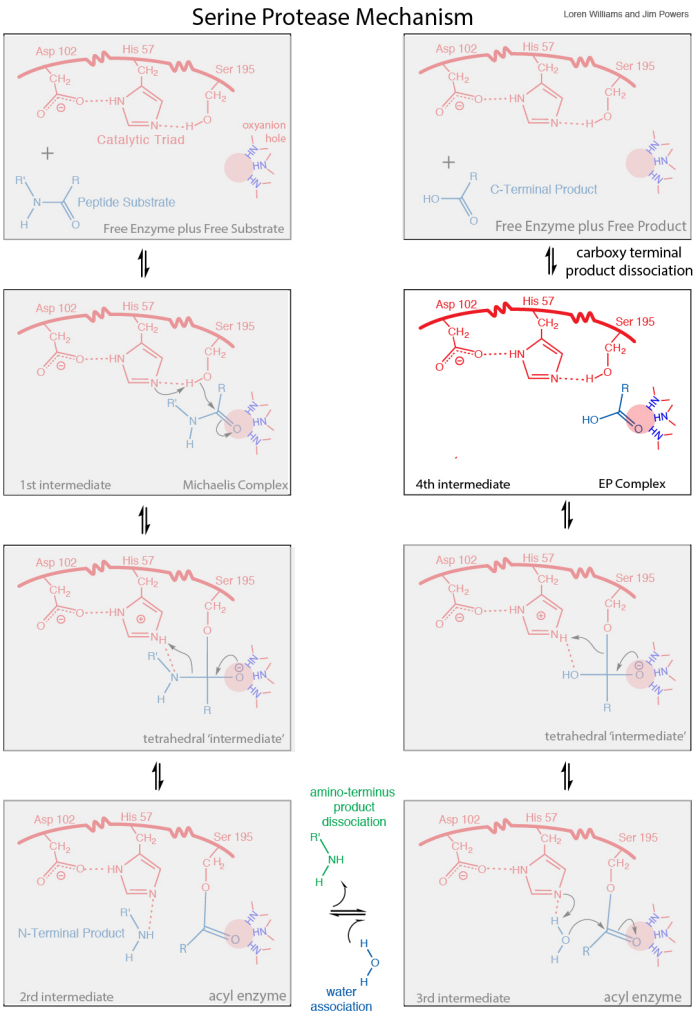


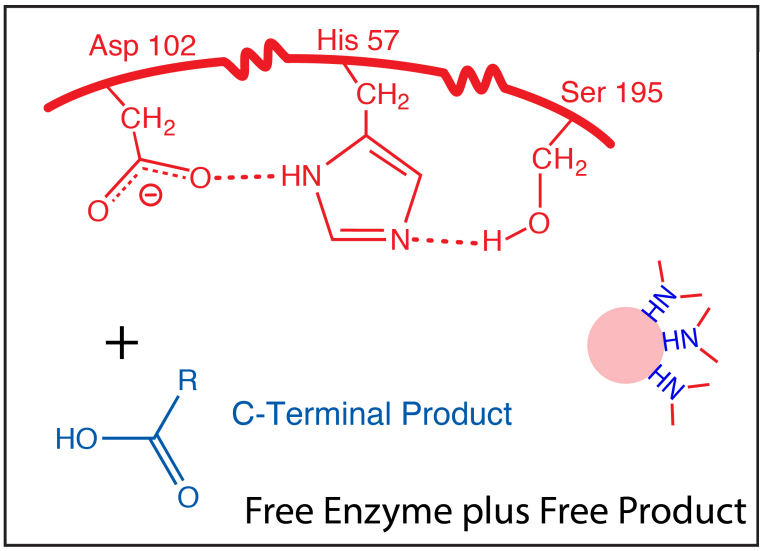
General acid catalysis aids breakdown of tetrahedral intermediate to the carboxyl product and the active enzyme.

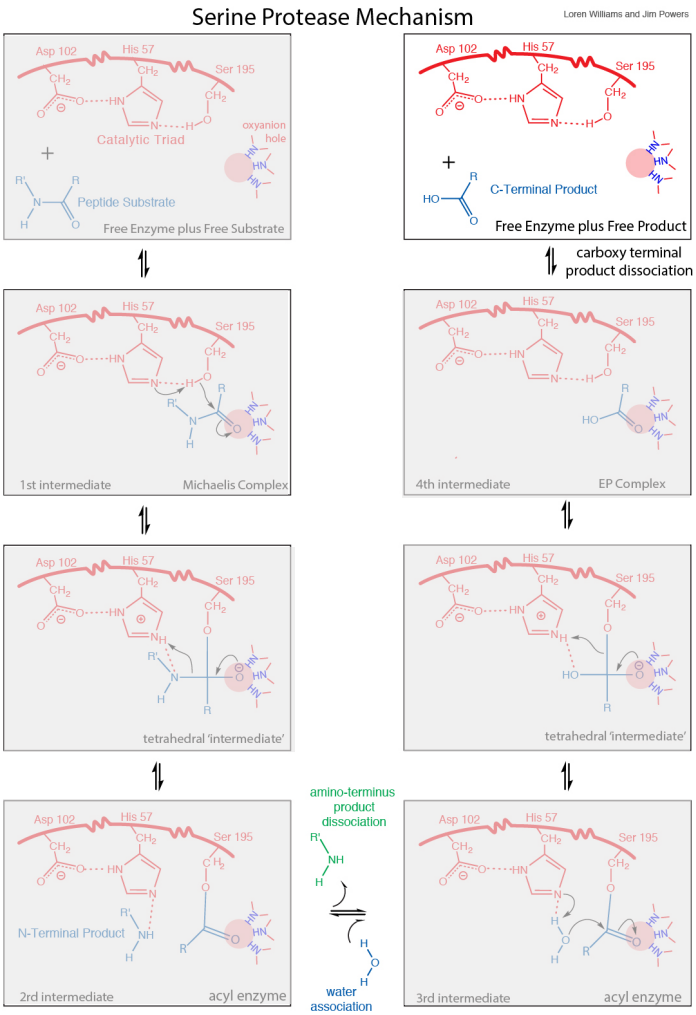
5




Asp 102
His 57
Ser 195
4th intermediate
EP Complex


Serine Protease Mechanism
Loren Williams and Jim Powers
Catalytic Triad
oxyanion hole
Peptide Substrate
Free Enzyme plus Free Substrate
C-Terminal Product
Free Enzyme plus Free Product
carboxy terminal product dissociation
1st intermediate
Michaelis Complex
4th intermediate
EP Complex
tetrahedral 'intermediate'
tetrahedral 'intermediate'
amino-terminus product dissociation
N-Terminal Product
2rd intermediate
acyl enzyme
water association
3rd intermediate
acyl enzyme


Asp 102
His 57
Ser 195
C-Terminal Product
Free Enzyme plus Free Product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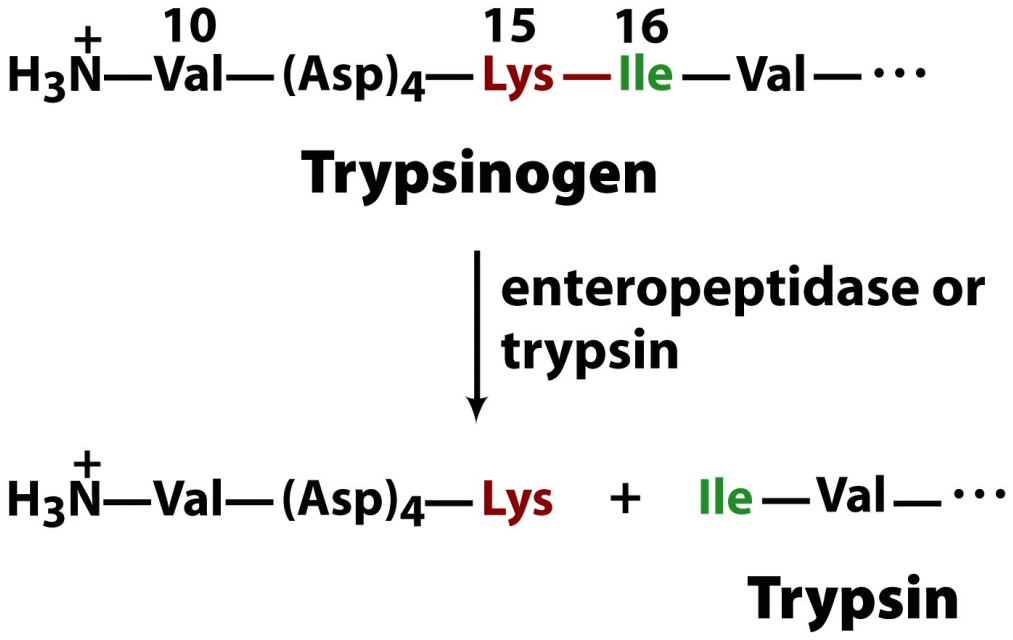
EP Complex
tetrahedral 'intermediate'
tetrahedral 'intermediate'
amino-terminus product dissociation
N-Terminal Product
2rd intermediate
acyl enzyme
water association
3rd intermediate
acyl enzyme

Zymogens: Inactive ezyme precursors
(if active at start, would digest tissue of origin.)

The activation of trypsinogen to trypsin (happens in duodenu



Autocatalytic!

Figure 11-33

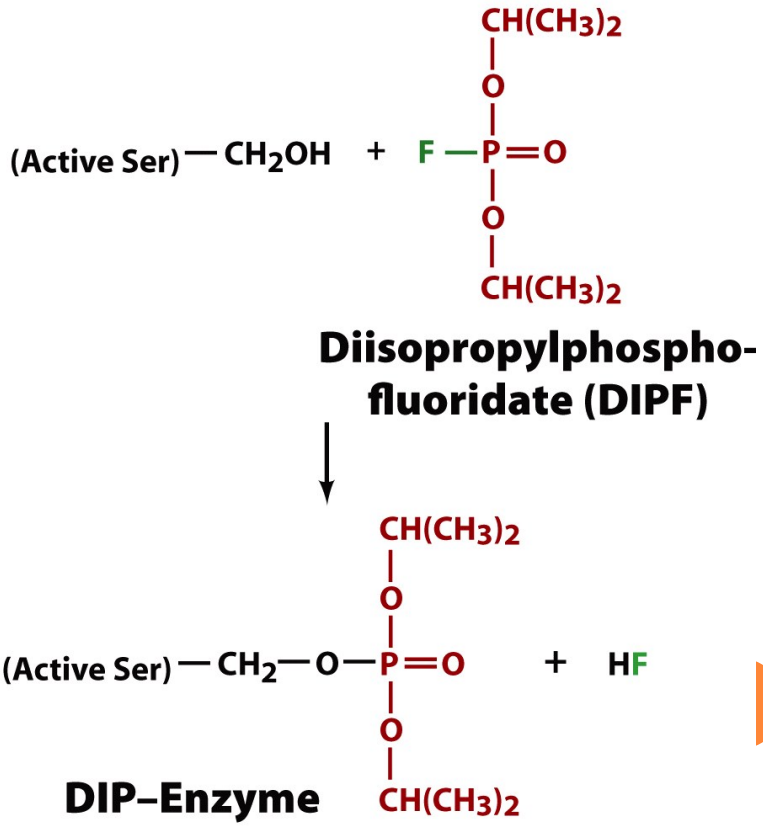
The mechanism of enzymatic reactions can be elucidated by using different tools, for example:

- X-ray crystallography
- Use of inhibitors (substrate analogs)
- Mutations
- Mass spectrometry

Active site residues of *chyrmotrypsin*, a serine protease, were identified by chemical modification.

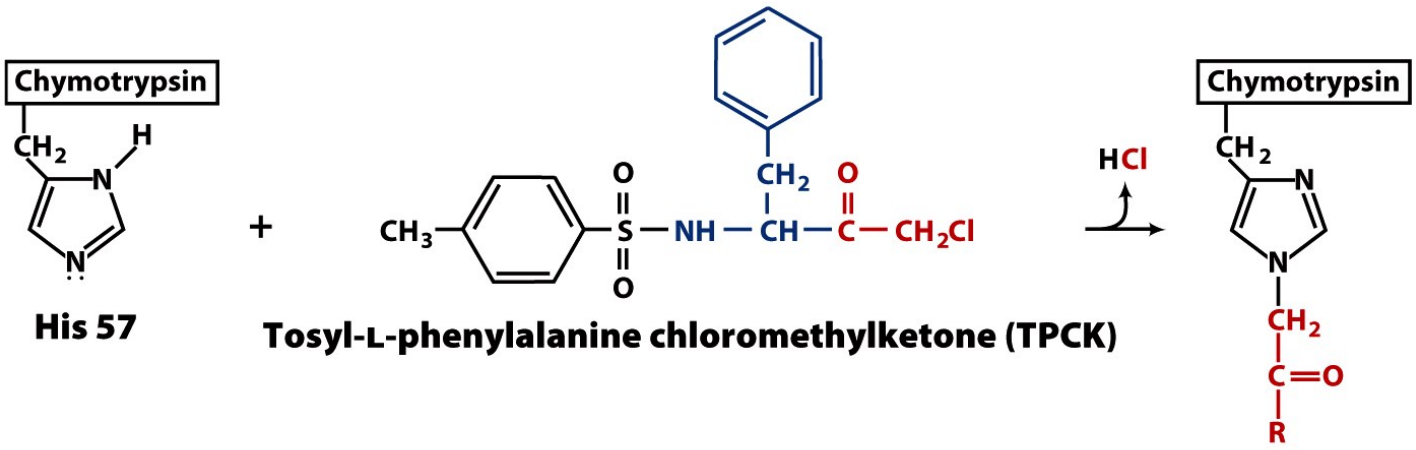
DIPF reacts on only with Ser 195 of chymotrypsin.

Identification of catalytic serine and tetrahedral intermediate.



Irreversible reaction.
Other Ser of ChT do not react

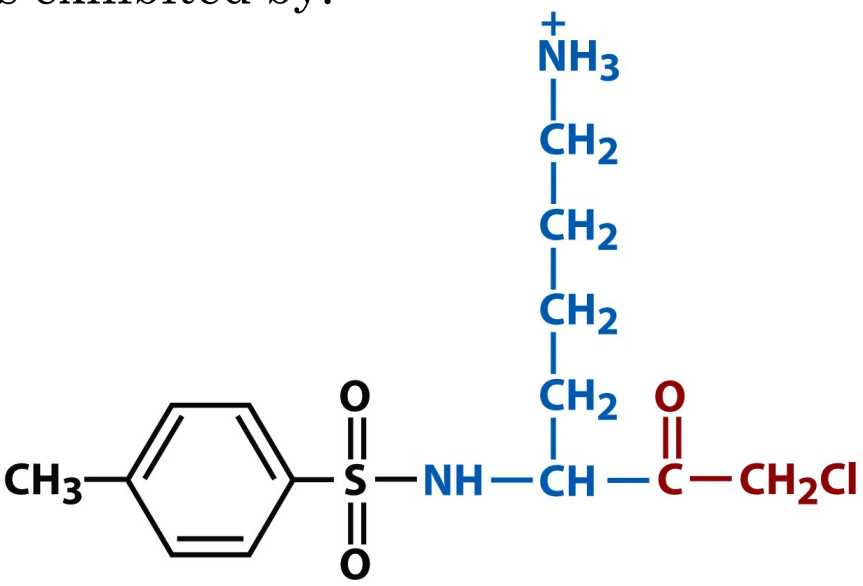
Identification of other active site residues with peptide analog



TPCK resembles a Phe residue and reacts with His 57 of Chymotrypsin. A "Trojan horse."

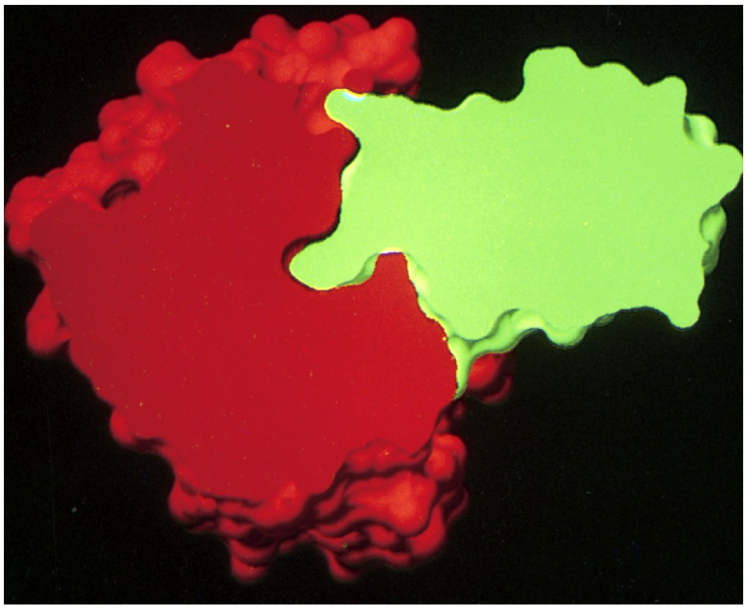
Figure 11-24

A similar activity against *trypsin*, another serine protease, is exhibited by:



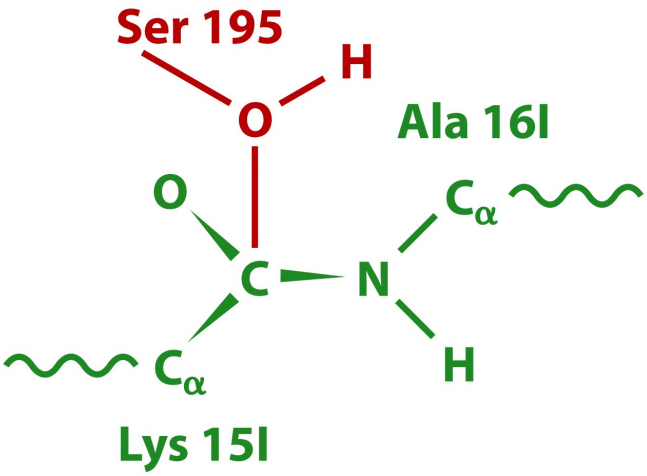
Tosyl-L-lysine chloromethylketone

Trypsin-BPTI → evidence of tetrahedral intermediate



Courtesy of Michael Connolly, New York University

Figure 11.31a



Trypsin(red)-bovine trypsin inhibitor (BPTI-green) complex.
Prevents premature activation of trypsin in pancreas.

Lys15 of BPTI is pyramidyally distorted towards Ser195.
Shape is similar to transition state of trypsin.

Elastase

Structure of porcine pancreatic elastase (the acyl-enzyme) and *direct observation of tetrahedral intermediate*. Structure stable at pH 5.0, so it was possible to obtain crystals.

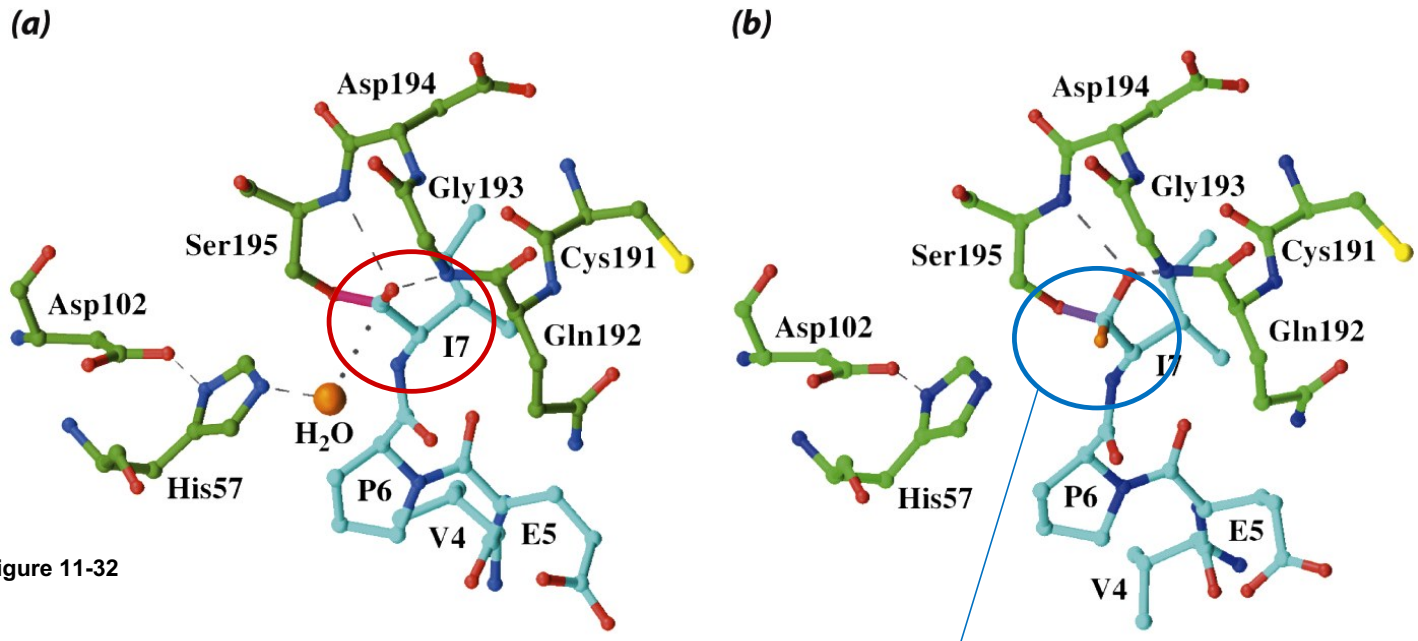
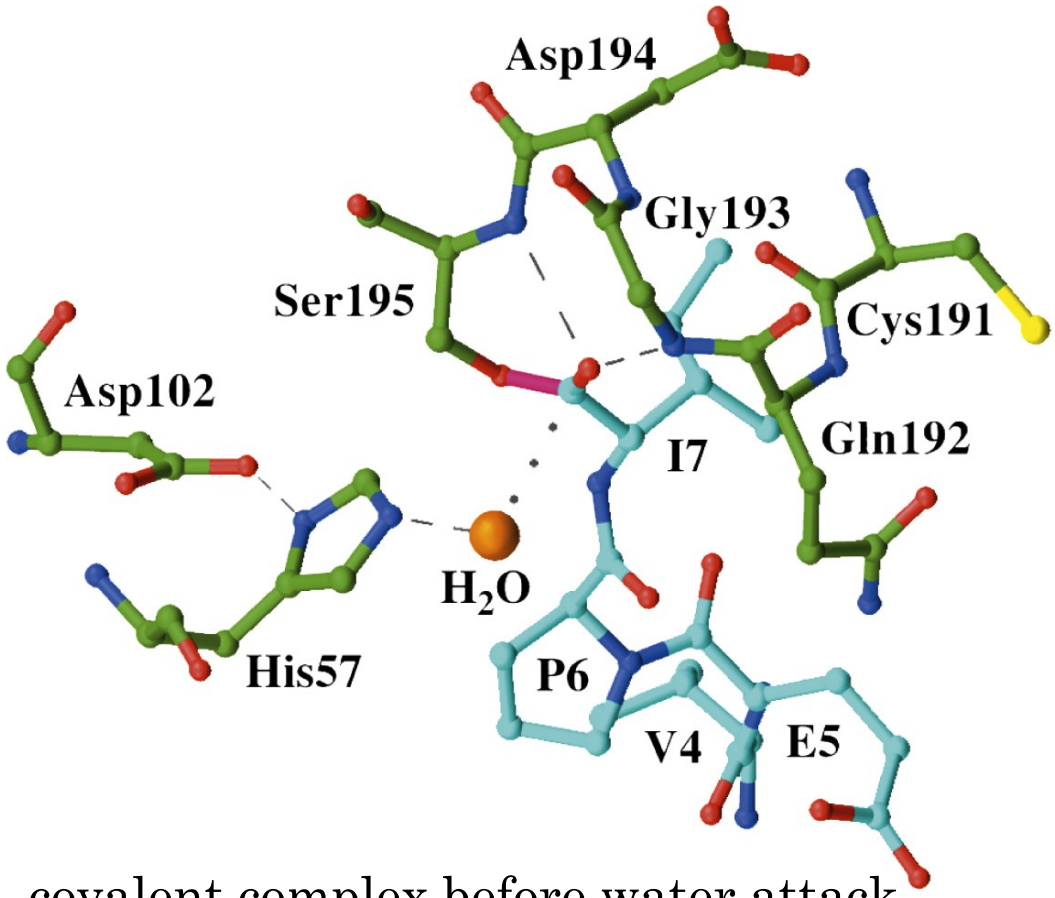


Figure 11-32

C-terminus of cleaved chain (acyl-enzyme intermediate)

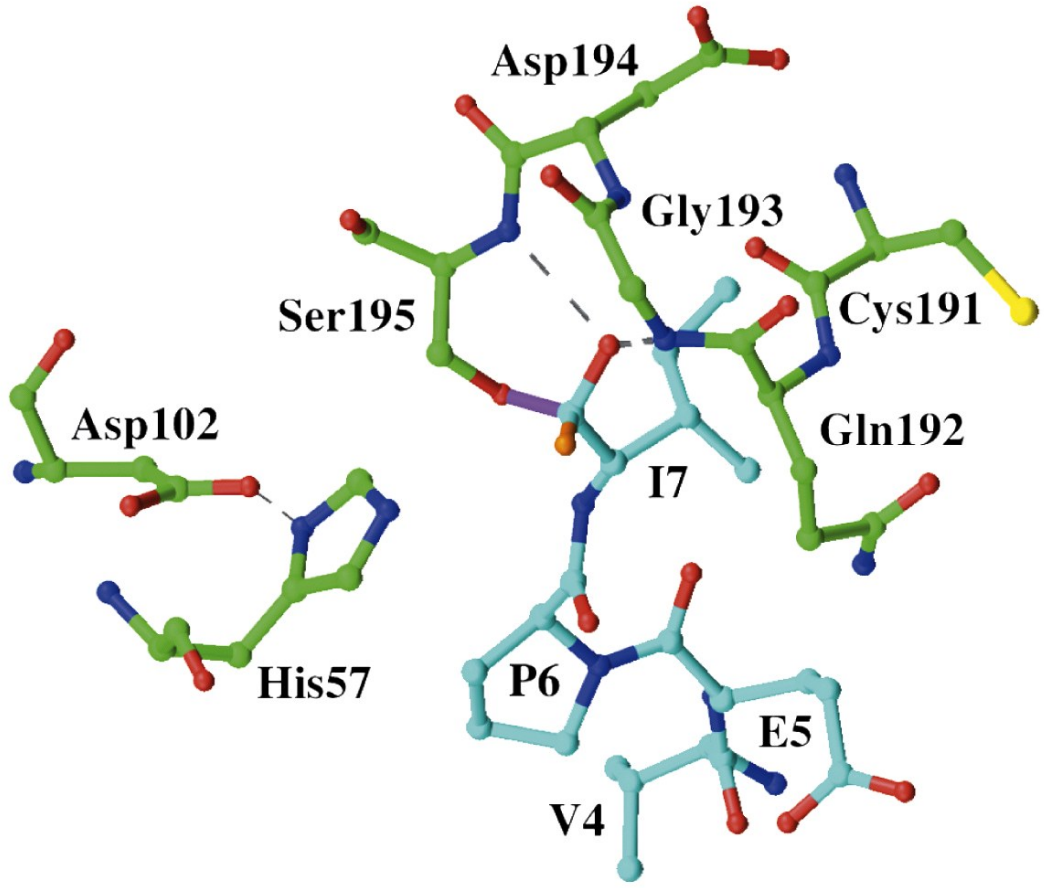
Tetrahedral intermediate

Step 4 from figure 11-29



At pH 5, covalent complex before water attack.
Protonation of His57 bars activity in base catalysis.

Figure 11-32a



Movement to pH 9 buffer activates His57, and water attacks.
Freezing halted further reaction (i.e. release of peptide fragment

Summary

- Enzymes are biopolymers that increase the rate of a chemical reaction (catalysis)
- They can bind to their substratehrough lock and key or induced fit
- Enzymes stabilize the transition state (Decrease $\Delta G^{\ddagger}$), but do not alter ΔG of the reaction
- Enzymes can use acid-base catalysis, covalent catalysis, metal ion catalysis