**Lecture 16: Serine Proteases**

**Goals:**
- Explain the role of the key catalytic residues in serine proteases.
- Describe enthalpy based transition state stabilization in serine proteases – oxyanion hole.
- Understand the structural basis of substrate specificity.
- Understand the kinetic basis of substrate specificity – a general concept.

**Proteases:** Enzymes that cleave peptide bonds.
- Serine proteases (e.g. trypsin)
- Thiol proteases (e.g. papain)
- Aspartyl proteases (e.g. HIV protease)
- Metalloproteases (Zn+2 containing).

**Serine proteases:** These enzymes play an important role in many processes, e.g. digestion of dietary protein, blood clotting cascade, and in several pathways of differentiation and development. Proteases active in digestion include:

- **Trypsin**
- **Chymotrypsin**
- **Elastase**

**Reactions Catalyzed:**
Serine proteases can hydrolyze either esters or peptide bonds:

**Ester Hydrolysis:** The bright yellow color of the p-nitrophenolate ion provides a convenient way to monitor the rate of product formation.

**Peptide Hydrolysis:**

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**Catalytic Residues:**
Key residues are Ser 195, His 57, and Asp 102. These three residues are called the catalytic triad. Serine is the nucleophile, for the first part of the reaction.

**Nucleophile:** group that is electron rich and can form bonds with electron deficient groups. In the case of the peptide bond (or ester) the electronegativity of the oxygen makes the carbonyl carbon electron deficient.

**Catalytic Cycle:** $E + S \rightarrow (ES) \rightarrow (EX_1^t) \rightarrow P_1 + (EX_2^t) \rightarrow (EX_3^t) \rightarrow P_2$

I. Substrate binds, forming (ES) complex.
   a) activation of Ser by proton transfer to His (stabilized by Asp)
   b) attack of Ser on electropositive C=O
   c) Formation of tetrahedral transition state (stabilized by amides of Ser195 and Gly 193 – enthalpic)
II. Nucleophilic attack of the side chain oxygen of Ser 195 on the carbonyl carbon of the scissile bond (bond to be cleaved) forming a tetrahedral intermediate.
   d) decay of transition state, breaking C-N bond (forward direction)
III: 1st product leaves. Acyl-intermediate remains - the substrate is covalently attached to the active site Serine.
   e) protonation of new amine by His
IV: Activation of water by His-Asp pair.
V: Nucleophilic attack of water on the acyl-enzyme intermediate with formation of the tetrahedral intermediate.
VI: Decomposition of acyl intermediate and release of the second product.
**Substrate Specificity:** Why are certain substrates preferred (hydrolyzed at a faster rate)?

Serine proteases utilize all of the intermolecular forces that we have discussed to bind their substrates. In addition to general recognition of the peptide by H-bonds, a particular serine protease is specific for certain amino acids. The molecular nature of this specificity can be inferred from the structure of the active site:

- **Trypsin** cleaves after Lys and Arg residues: Asp189 in the **specificity pocket** of the active site interacts with the positive charge on Arg and Lys.

- **Chymotrypsin** cleaves after aromatic (large hydrophobic) residues: The specificity pocket is hydrophobic due, in part, to Met192.

**Kinetics & Specificity — “kinetic editing”**

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E + S \xrightleftharpoons[k_{on}]{k_{off}} (ES) \xrightarrow{f} (EP) \xleftarrow{k_{off}} \text{“good substrates” have low } k_{off}\]

**Serine Proteases — Review**

Provide names and/or roles in the catalytic mechanism for each of the labeled items:

- **A:** pepthile bond
- **B:** nucleophilic
- **C:** Act. nucleophilic
- **D:** stabilize hist
- **E:** oxygen hole
- **F:** specificity pocket