PHRM 836 September 1, 2015

Protein structure-function relationship: Catalysis – example of serine proteases

Devlin, section 9.3

- Physiological processes requiring serine proteases
- Control of enzymatic activity
- Structural conservation of catalytic site

Assumed knowledge includes:

- The chemical change catalyzed
- Basic classification (exo/endoprotease; type)
- Catalytic triad of proteases
- Catalytic mechanism of proteases

Serine Proteases Function

- Serine protease family: critical role in many physiological processes:
  - digestion: trypsin, chymotrypsin, elastase
  - blood clotting/degradation: thrombin, plasmin, tissue plasminogen activator [myocardial infarction]
  - immune responses:complement proteases
  - hormone activation:nerve growth factor
  - cell migration: urokinase [cancer metastasis]

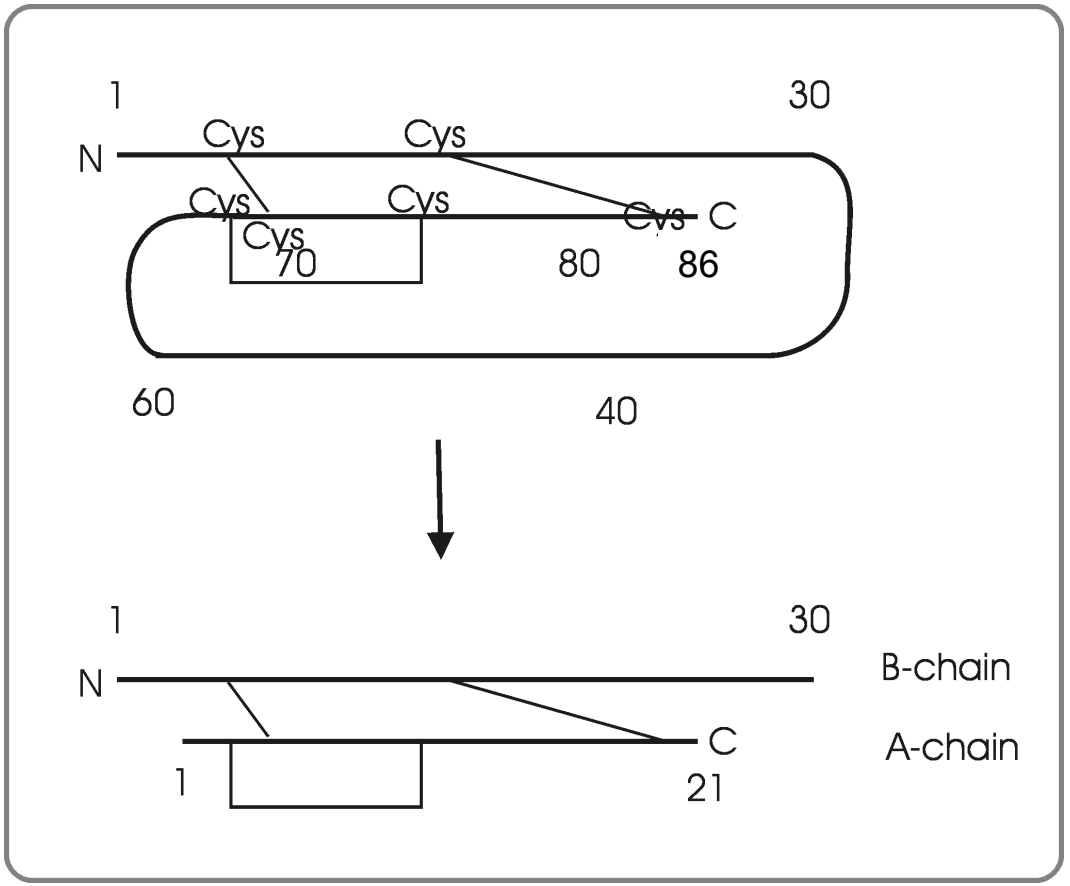
Serine Proteases Function

- to degrade proteins, including itself (autolysis)
- to activate proteins by specific peptide cleavage (limited proteolysis)
  - Processing of precursor forms of polypeptide chains. Some proteins are synthesized as inactive precursors, called zymogen or "pro" form:
    - Trypsin and trypsinogen
    - Thrombin and prothrombin
  - Protease cleaves 1 or more specific bonds

- This specific cleavage generates active form.
- Irreversible

Example of precursor molecule: proinsulin cleaved to the hormone insulin, the active form

cleavage by a protease produces mature insulin and peptide C

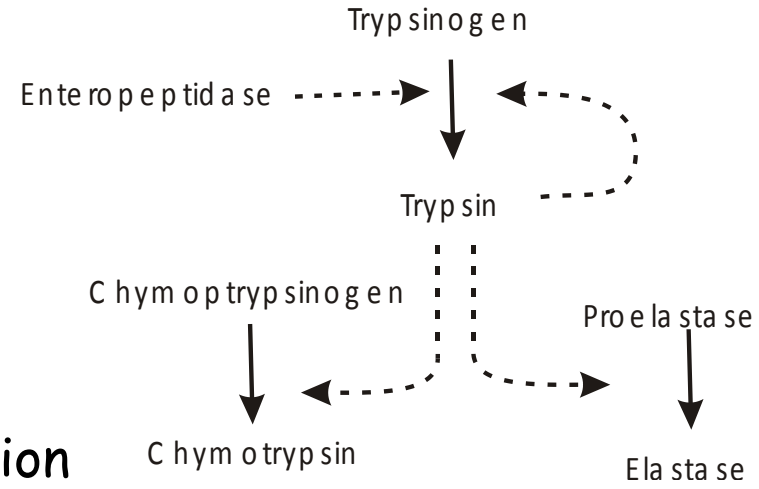


Cleavages:

1. 30-31
2. 65-66

proteolytic enzymes: role of zymogens

- Example: proteolytic enzymes of the digestive tract



  - Zymogen form stored in pancreas. After secretion to small intestine, they're activated by selective proteolysis: [enteropeptidase is secreted under hormonal control]
  - Activated serine proteases self cleave, cleave other serine proteases, and degrade ingested protein
- 2nd example: blood clotting cascade. Multiple activation steps amplify and allow rapid coagulation to occur.

Clinical Connections, an example

- Proteases are also targets in treatments. [Clinical correlation 9.4]:
  - Degradation of blood clots = Plasmin (a serine protease) degrades fibrin (x-linked fibrin makes up the clot).
  - Tissue plasminogen activator, t-PA, "activates" plasmin. Recombinant t-PA administered shortly after a myocardial infarction enhances recovery.

Specific Recognition for Proteases

- a given protease (e.g. trypsin, elastase, etc) exhibits a preference for a peptide bond adjacent to a particular type of amino acid
  - ➔ specific amino acids near the cleavage site are recognized. (Note: specificity describes relative reactivity, not absolute requirement)

Trypsin: basic amino acids (K or R)
Chymotrypsin: hydrophobic amino acids (W, F, Y, and L)
Elastase: small hydrophobic residues (A)

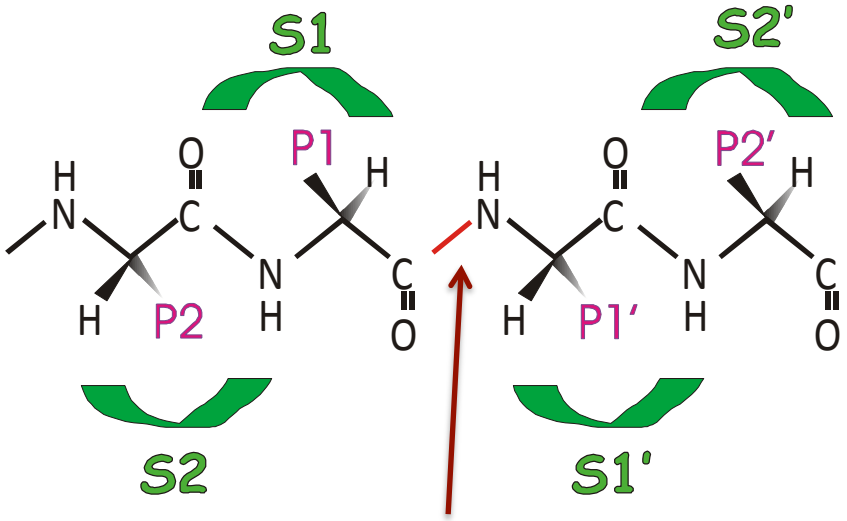
Specific Recognition

S5-S4-S3-S2-S1-S1'-S2'-S3' (protease)

P5-P4-P3-P2-P1-P1'-P2'-P3' (substrate protein)

↑

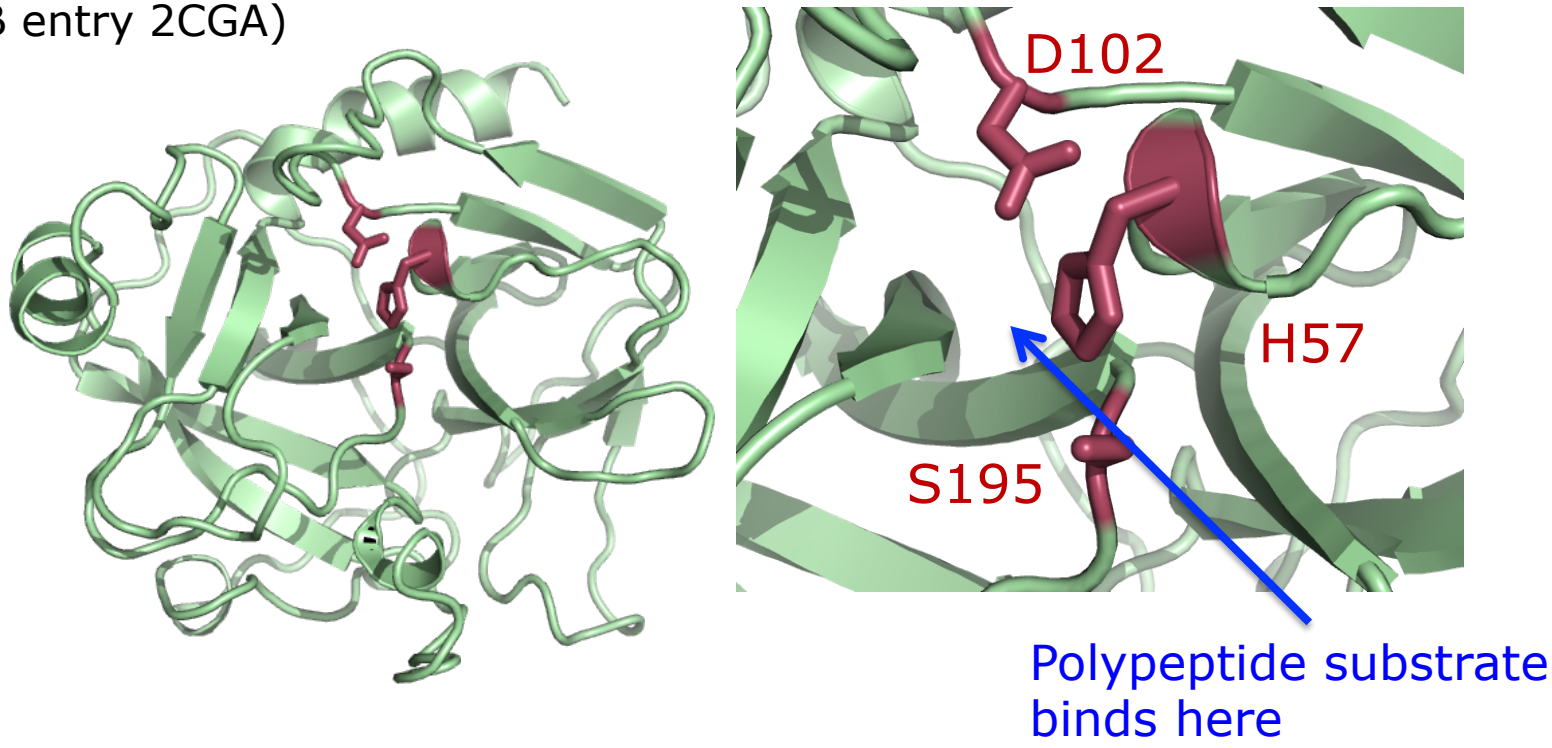
- Recognition is based on size, aromaticity, charge of the substrate residues P1-P1' according to a pocket in the serine protease



Cleavage site

Catalytic Triad of Serine Proteases

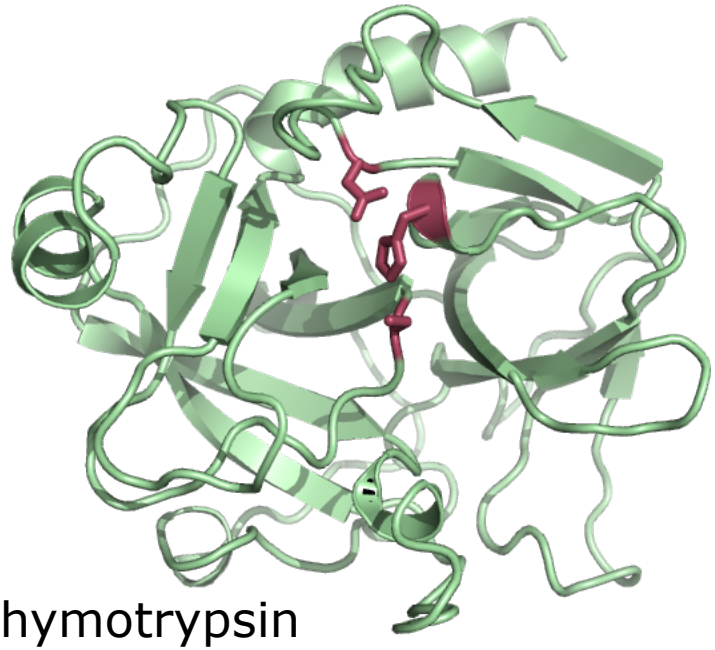
Chymotrypsin
(PDB entry 2CGA)



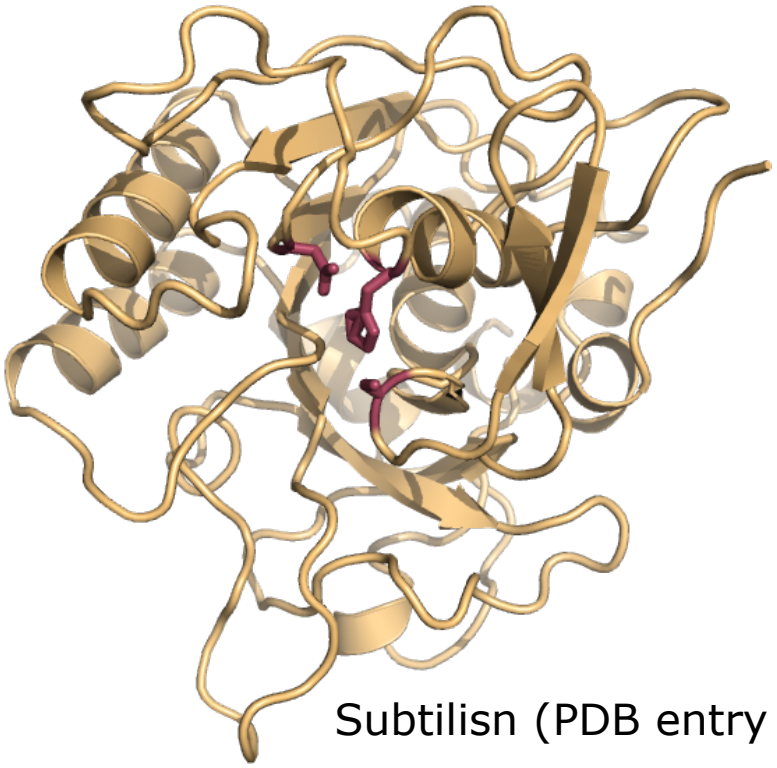
- S195,D102 and H57 residues of chrymotrypsin are primary residues involved in catalysis.
  - These 3 residues are in from different domains
- Other Serine proteases have same catalytic triad in the same spatial orientation

Structural Basis of Enzymatic Activity

- Some serine proteases have common sequence, structure and catalytic residues.
- Other serine proteases have same catalytic triad but with very different sequences/structures (e.g. chymotrypsin vs subtilisn)

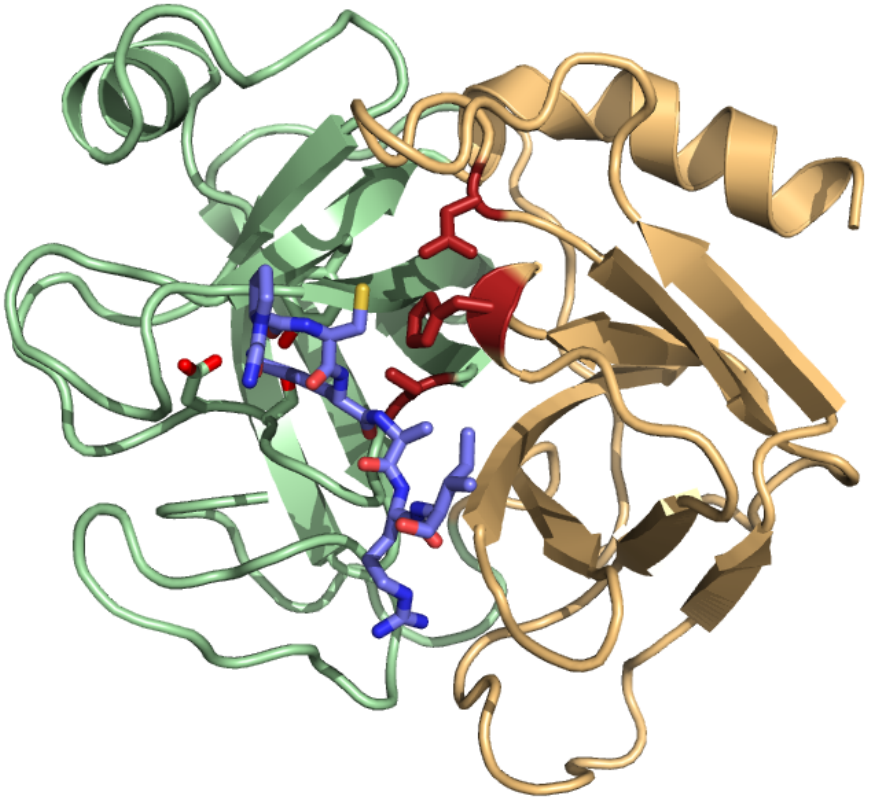


Chymotrypsin
(PDB entry 2CGA)

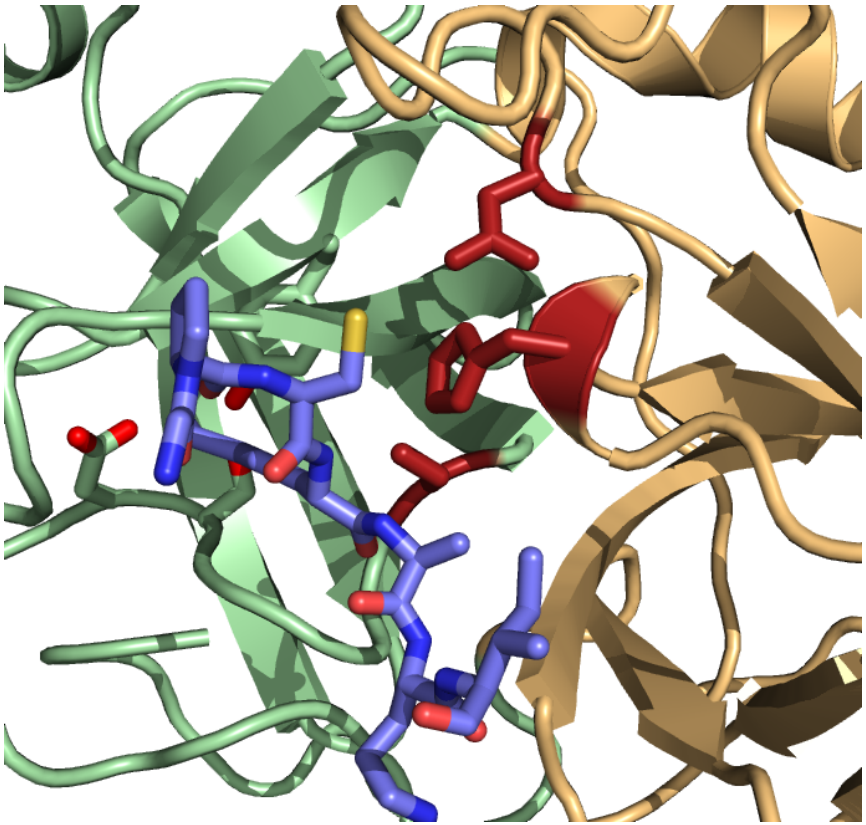


Subtilisn (PDB entry 1SBT)

Catalytic Triad of Serine Proteases



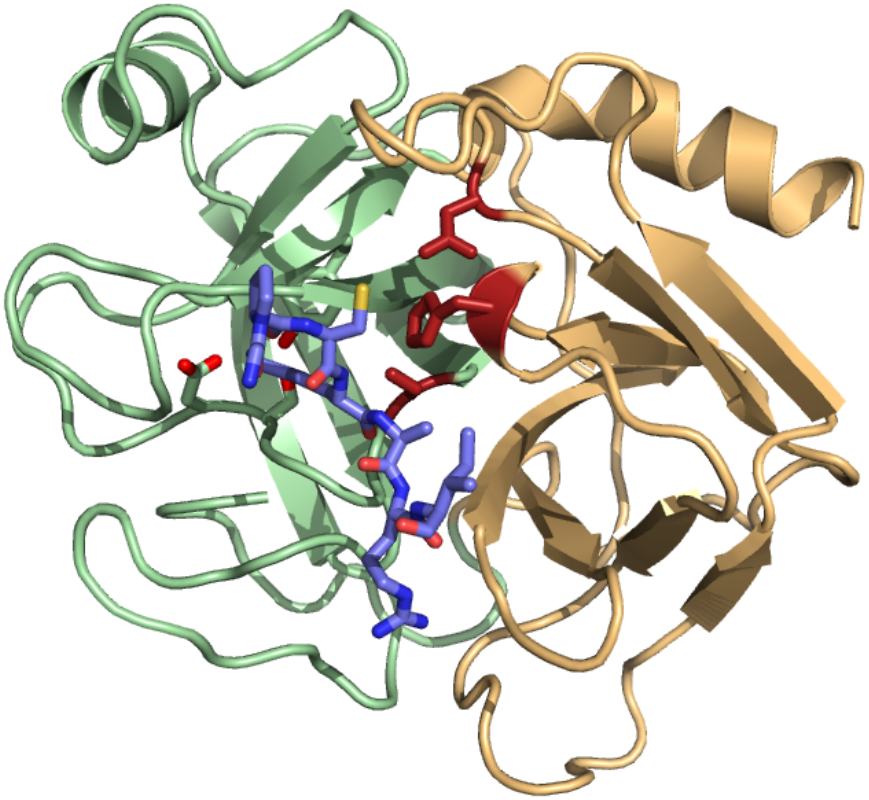
P3P2P1P1′P2′P3′



Substrate-analogue complex: reaction site

- Catalytic residues oriented to break the peptide bond

Catalytic Triad of Serine Proteases



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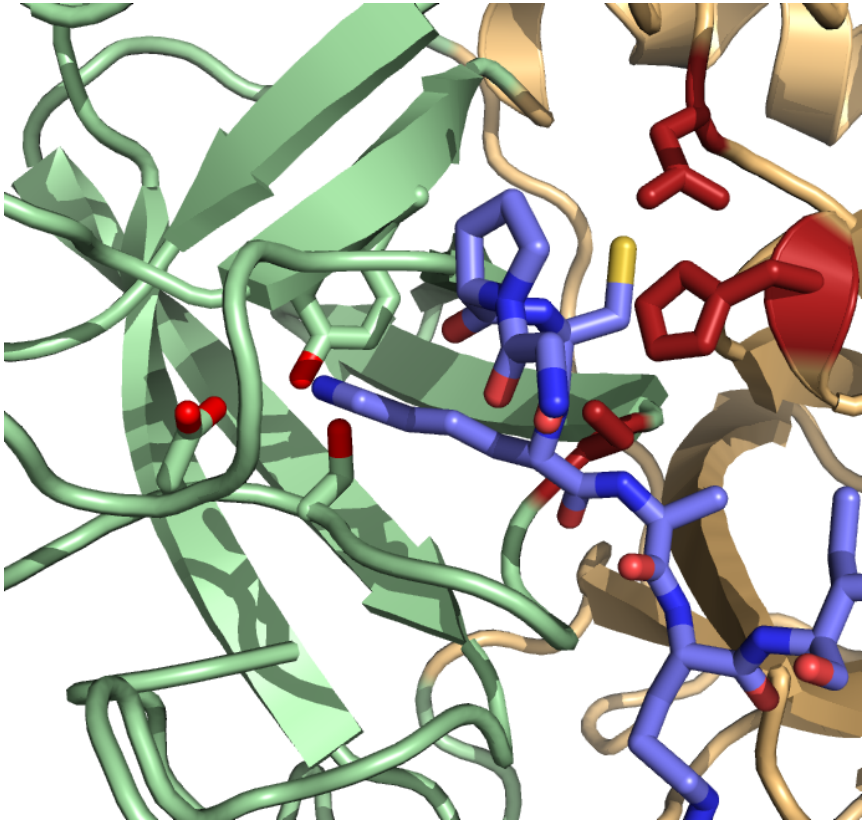
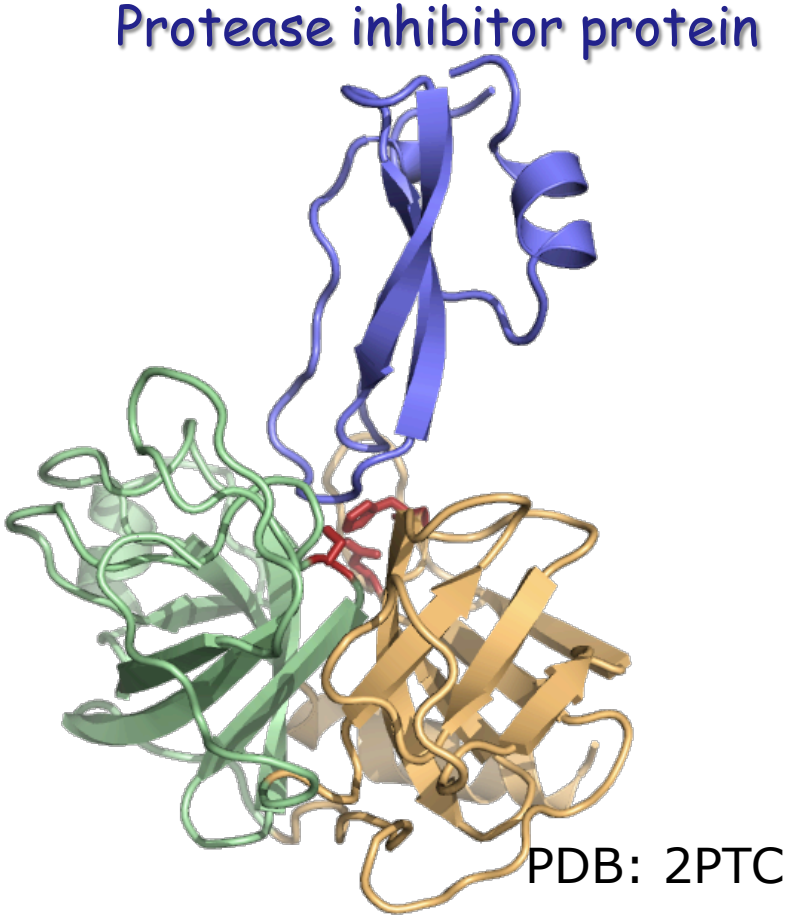


Substrate-analogue complex: S1-P1 interactions

- Specific recognition of P1
- P1 residue (lys) interacts with negatively polar side chains in S1 site

Inactivation of Serine Proteases

- must limit activity to certain sites in the body and turn it off once activated.
- Specific protein inhibitors exist to inhibit a given protease. Proteins (serpins) bind tightly to protease and block protease binding site.



PDB: 2PTC

When serpins fail:
Protease is uncontrolled.
Emphysema: alpha 1-antitrypsin is compromised and the protease elastase, destroys lung connective tissue.

Summary of Protease Structure/ Function

1. Serine proteases function in numerous biological processes.

2. Proteolysis generates the active forms of precursor molecules, such as prohormones to hormones, or proenzymes/zymogens to enzymes.

3. Proteases are selective for their substrates. Selectivity is based on spatial and chemical complementarity between residues on the substrate protein and the protease where it contacts these residues.

4. The catalytic triad is highly conserved in a spatial sense.

5. Activity of proteases must be tightly regulated: zymogens to activate; protease inhibitors to inactivate.