Covalent Mechanisms of Protein Instability

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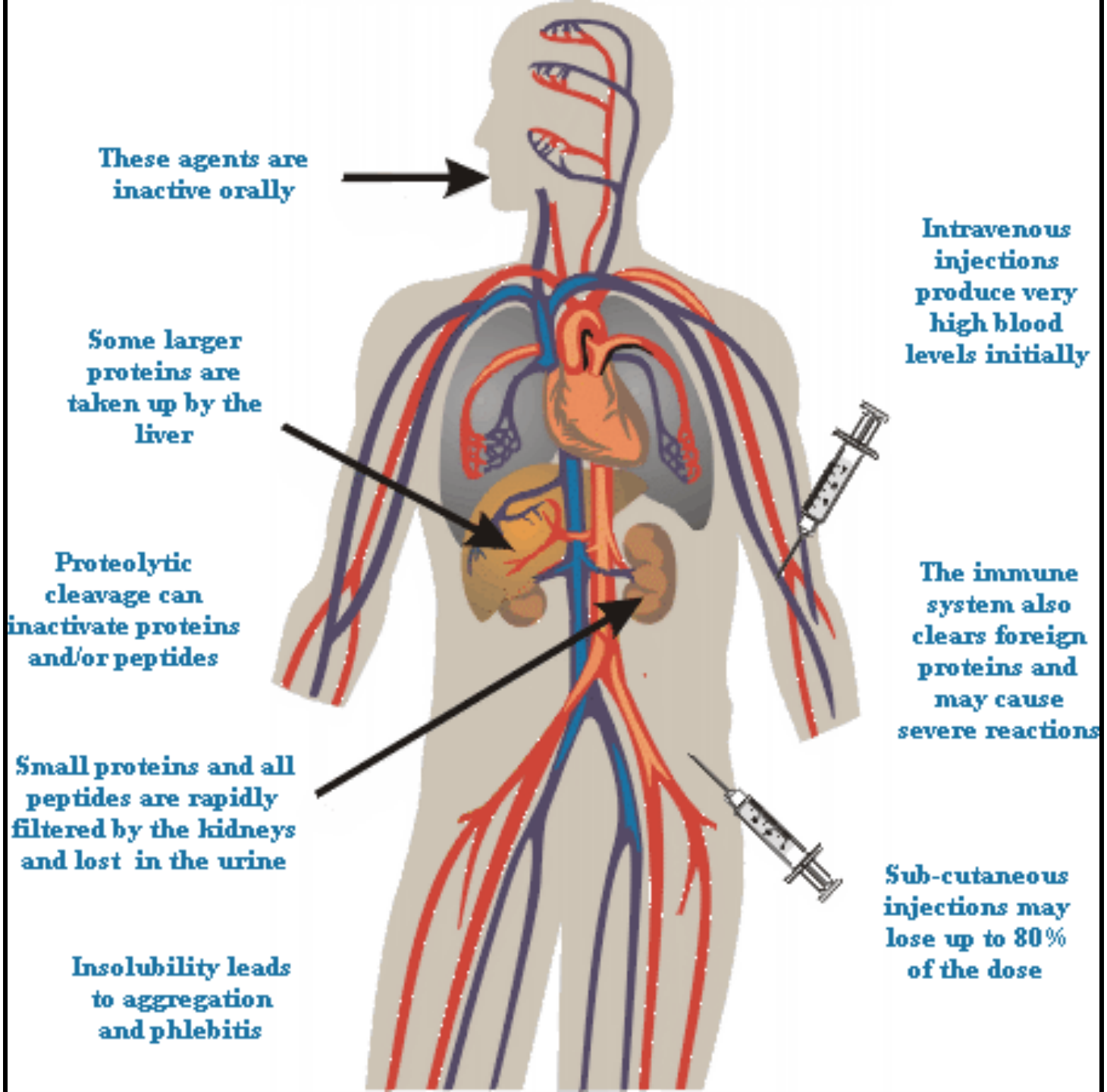
Objectives of this lecture

By the end of this lecture you will be able to:

1. Describe the challenges in pharmaceutical proteins production
2. Distinguish between the different mechanisms of protein instability
3. Predict the mechanism of degradation from peptide primary structure

Protein administration

Oral		Parenteral		
Amylase & Mucosa	HCl & Proteases	Plasma Proteases	Albumin & Lipoproteins	Immune System
Deglyosylation, Adsorption & Denaturation	Denaturation & Degradation	Proteolysis	Adsorption & Clearance	Response


These agents are inactive orally
Some larger proteins are taken up by the liver
Proteolytic cleavage can inactivate proteins and/or peptides
Small proteins and all peptides are rapidly filtered by the kidneys and lost in the urine
Insolubility leads to aggregation and phlebitis
Intravenous injections produce very high blood levels initially
The immune system also clears foreign proteins and may cause severe reactions
Sub-cutaneous injections may lose up to 80% of the dose

Challenges

1. Conventional pharmaceutical formulations would destroy most proteins and eliminate their activities:
   - Pharmaceutical manufacturing includes harsh processing steps that would be harmful for proteins e.g. heating, high shear force, granulation, compression, etc.

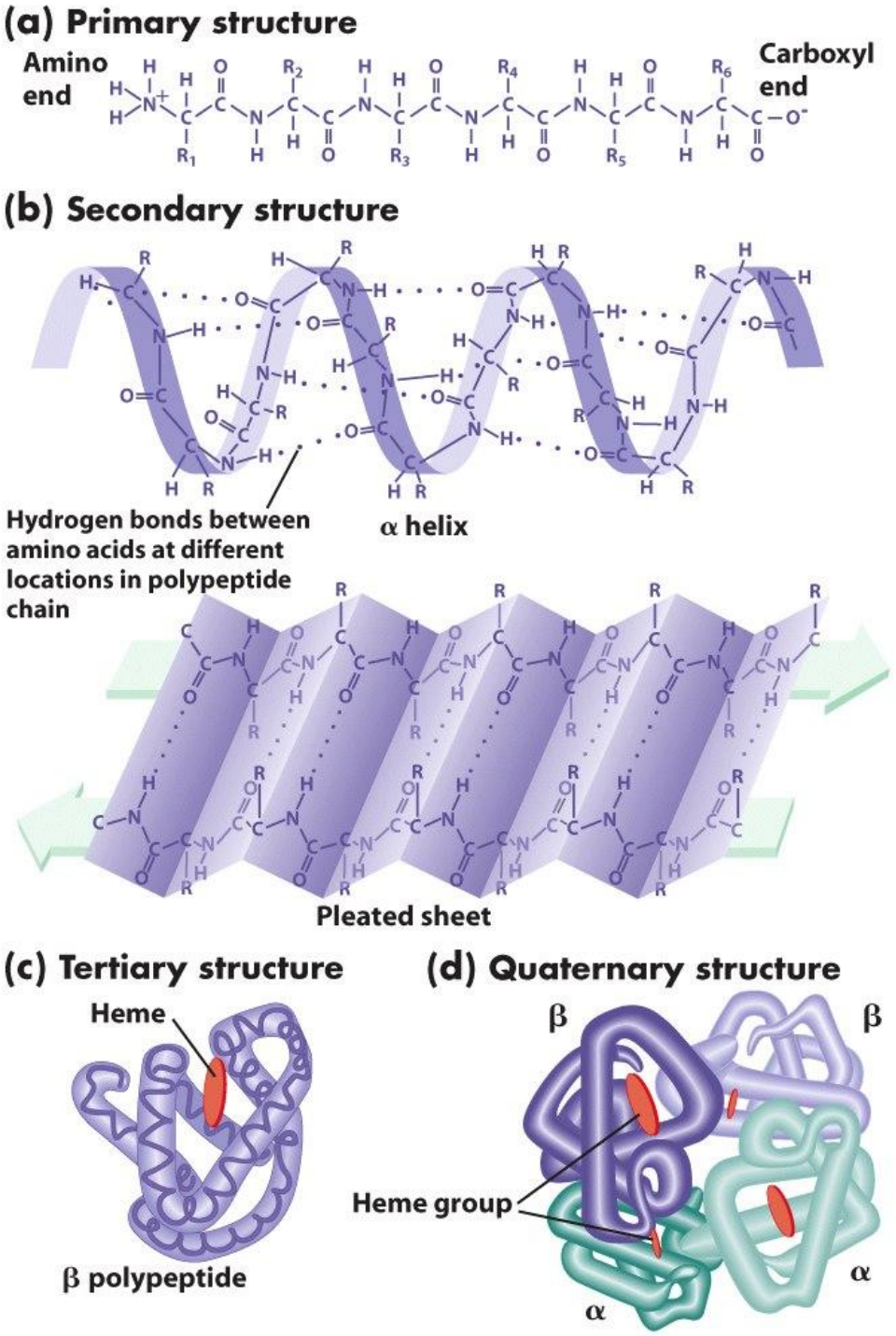
2. Short shelf life of protein preparations:
   - If kept in correct conditions, must not be used after 4 weeks post opining

Protein Structure

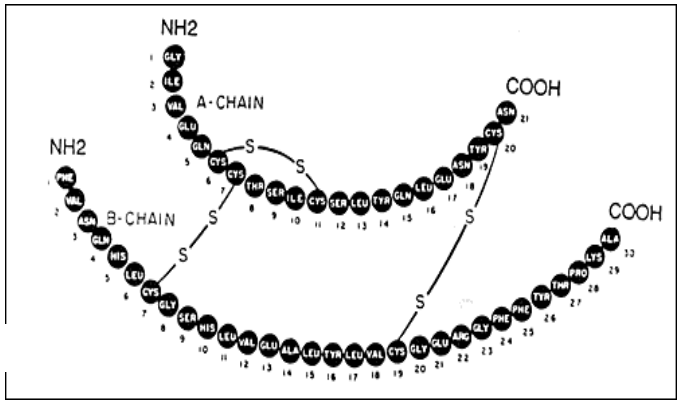
- The tertiary structure of the protein is essential for its function

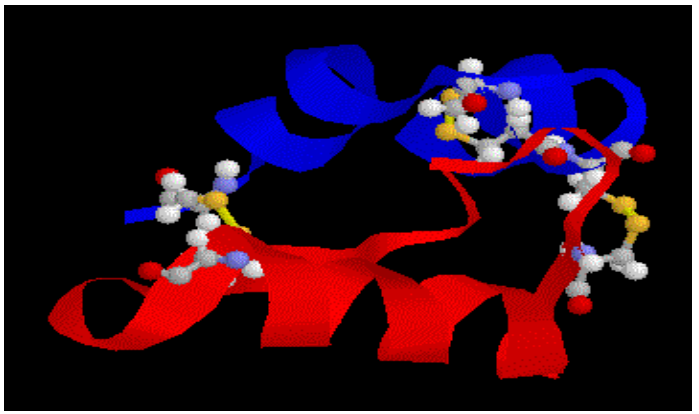
- Intramolecular:
  - Disulfide bonds
  - Ionic bonds

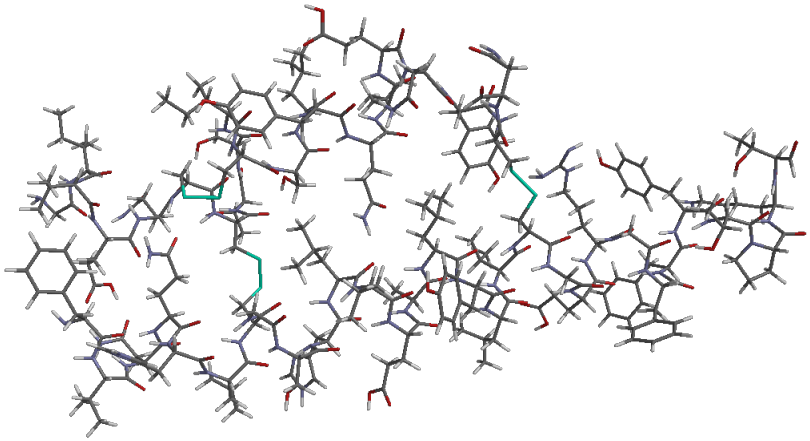
- Intermolecular:
  - Hydrogen bonds
  - Hydrophobic effect



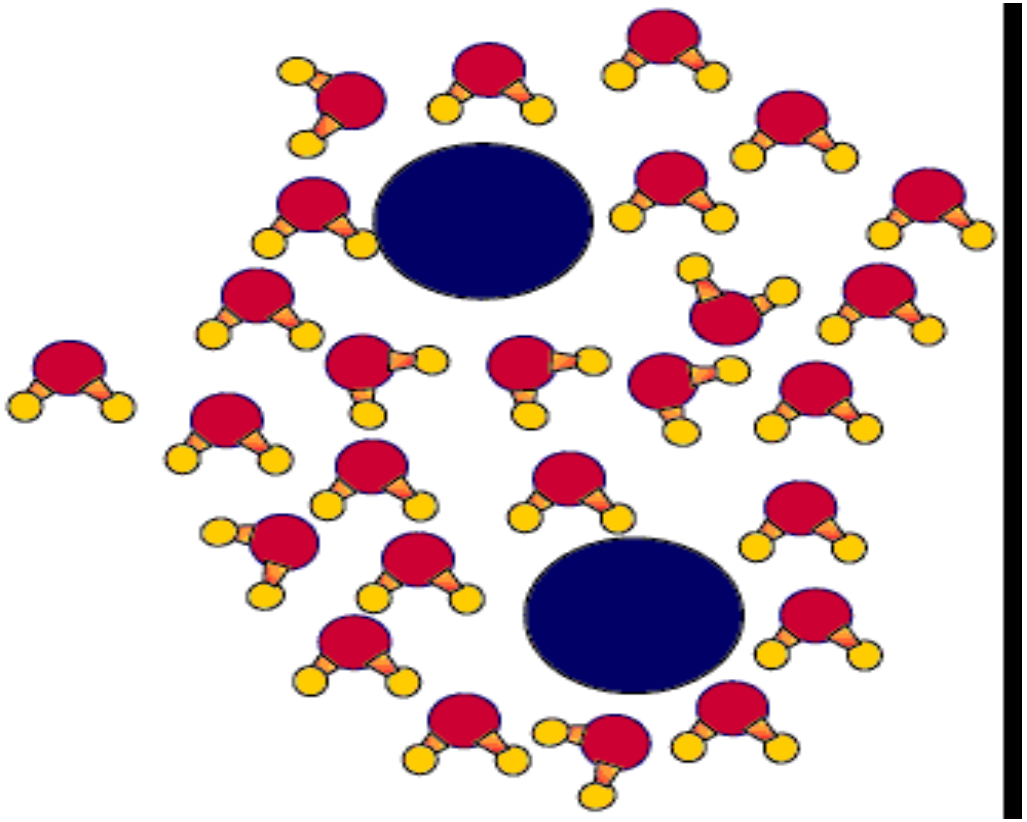
Insulin shows it all …







Hydrophobic effect



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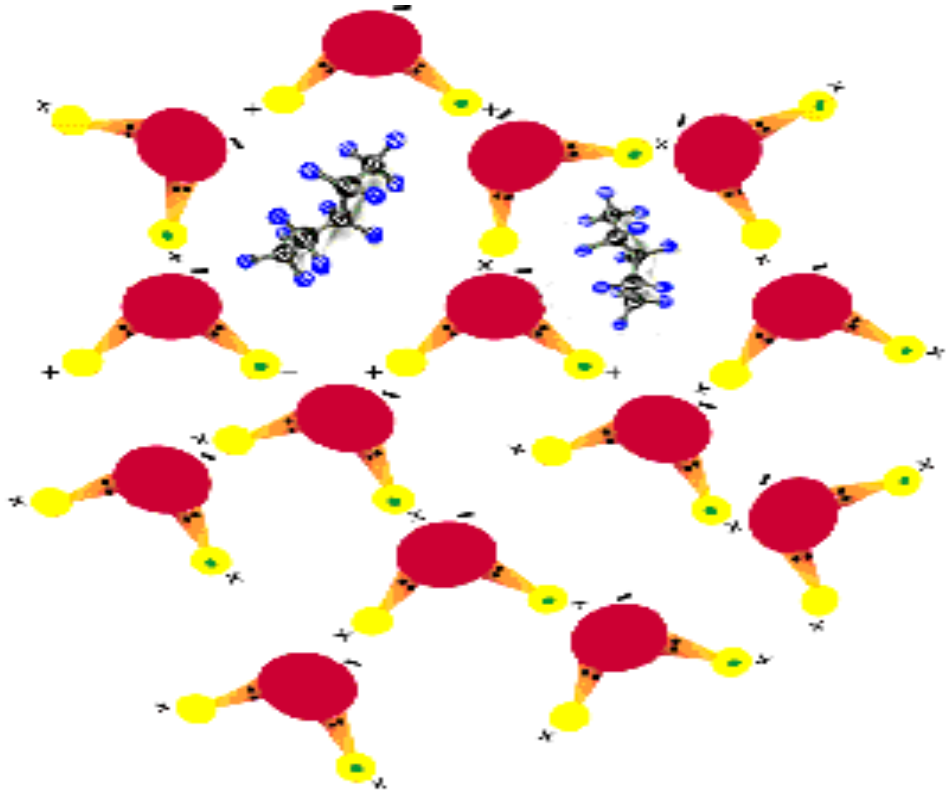
Hydrophobic effect



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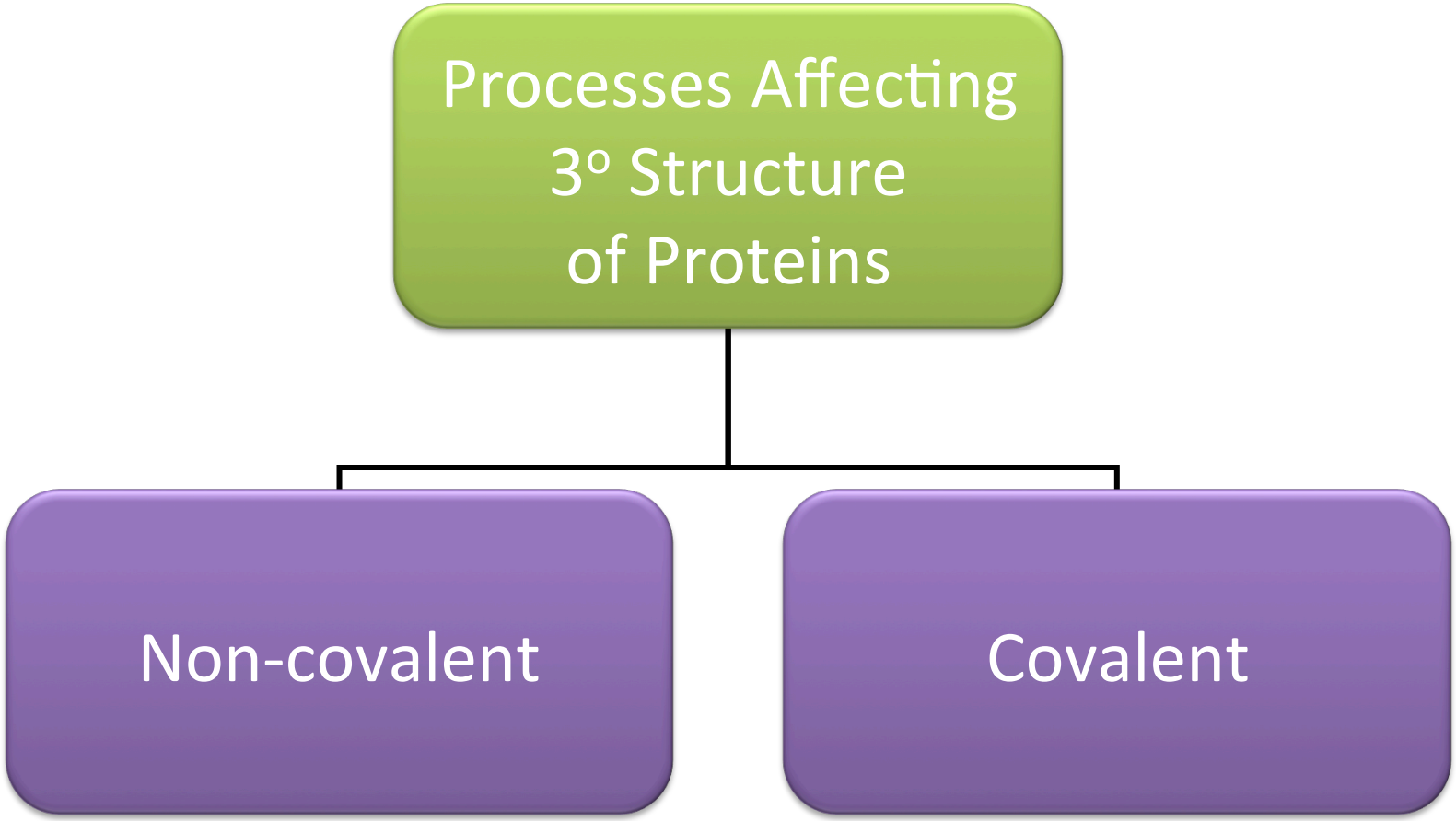
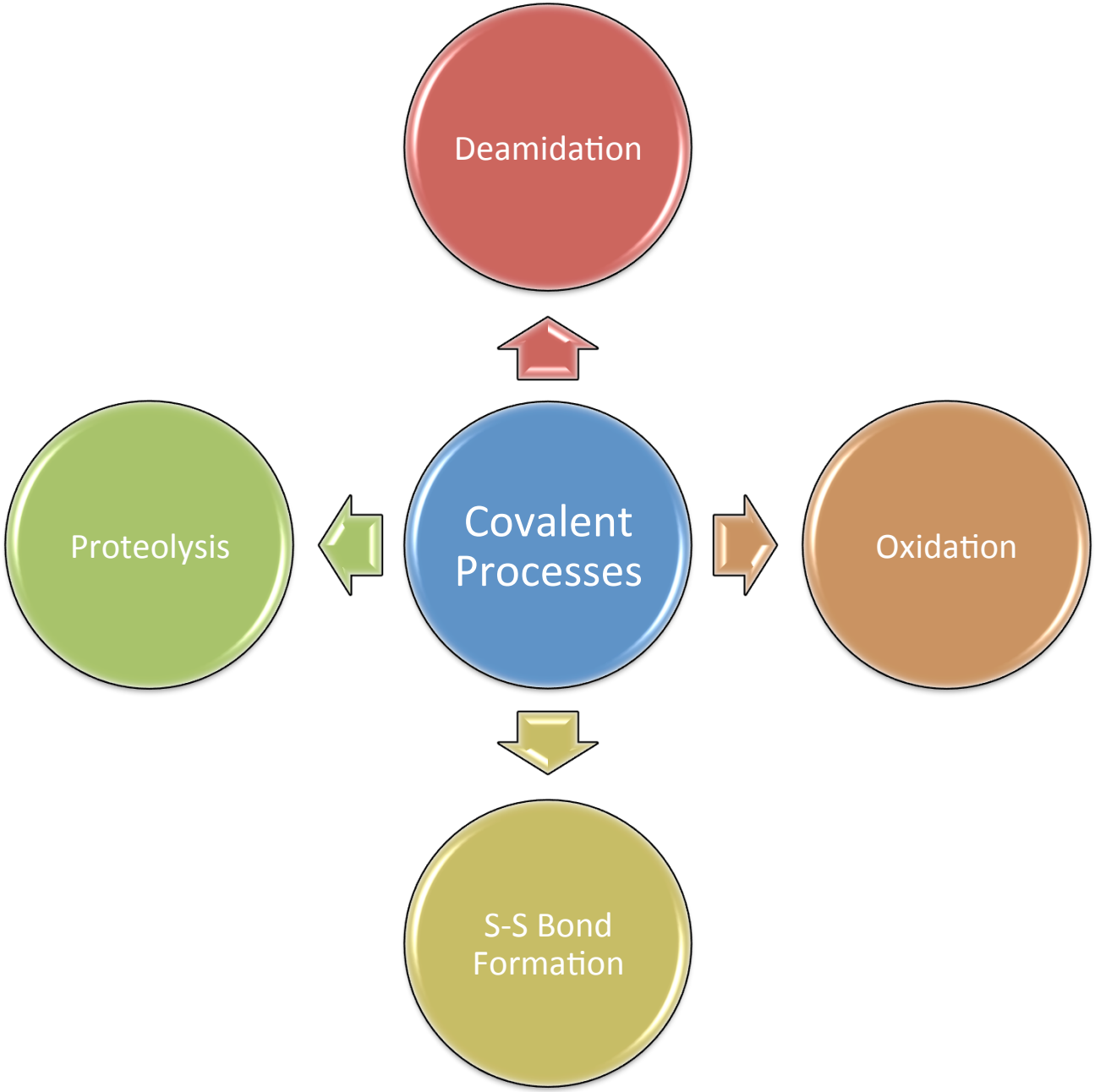
Protein Stability

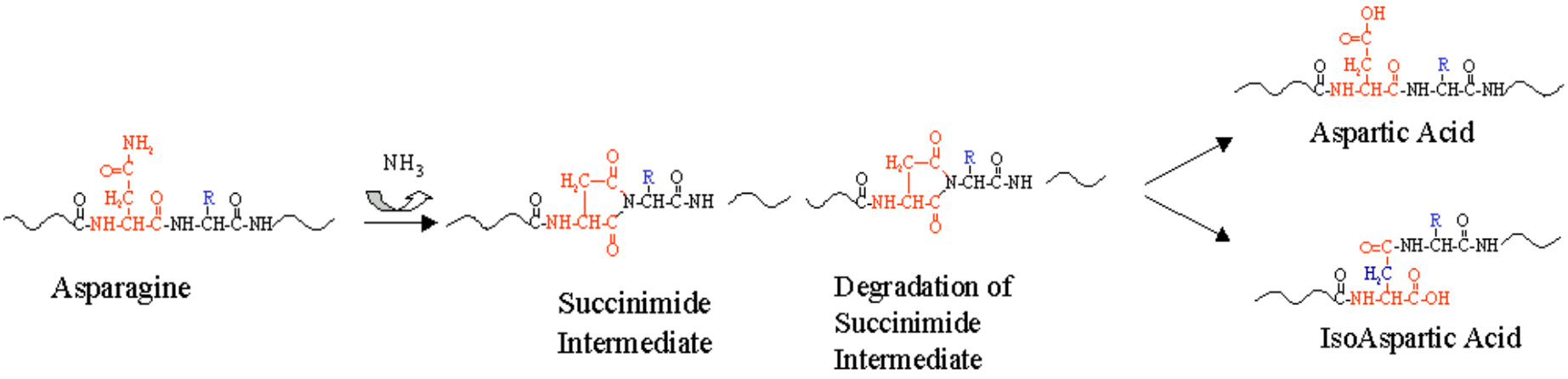



Deamidation
Proteolysis
Covalent Processes
Oxidation
S-S Bond Formation

Deamidation

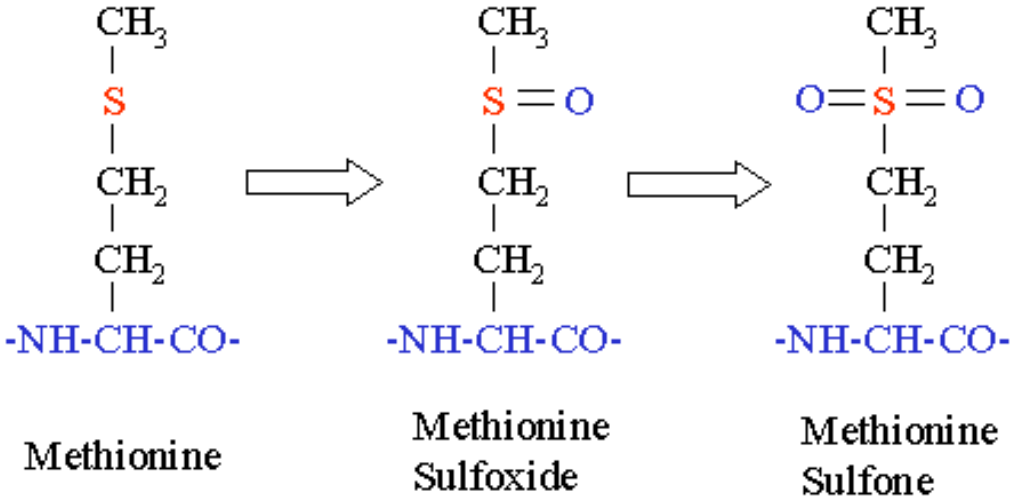
- Loss of the side-chain amide
- Occurs in Asn or Gln
- The sequence **Asn-Gly** is most common site for deamidation

- Spontaneous reaction
- Rapid
- Most likely occurs after denaturation
- β-Asp derivative is unnatural



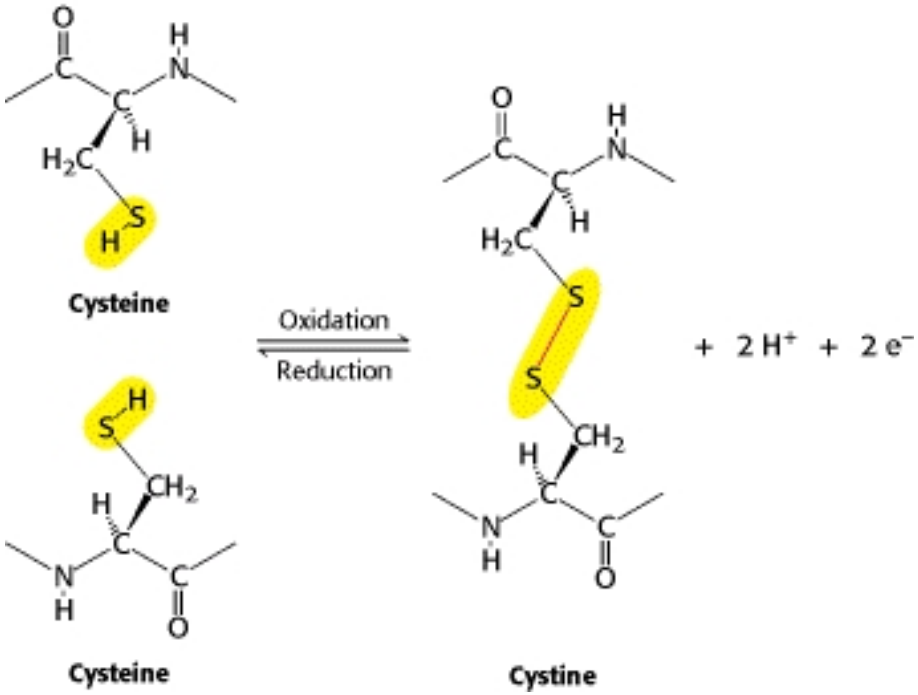
Oxidation

- Aromatic a.a:
  - **Histidine**
  - **Tryptophan**
  - **Tyrosine**
- Sulfur-containing a.a:
  - **Methionine**
  - **Cysteine**
- Methionine is the most easily oxidized
  - Sulfoxide (mild)
  - Sulfone (strong)
- Leads to loss of activity



Disulfide bond formation

- Two cysteine residues
- Redox reaction
  - Enhanced by O_2
  - Fe^{++}
  - Cu^{+}
- Disulfide exchange occurs spontaneously
  - Could lead to:
    - Denaturation
    - Aggregation
    - Precipitation
- Mechanism depends on the pH



Proteoelysis

- Peptide bond breakdown
- Most serious change
- Catalytic mechanism:
  - Enzymatic *in vivo*
- Autocatalytic:
  - In the bottle *in vitro*
  - On N or C terminal **Asp** residue
  - **Asp-Pro** are most susceptible to autohydrolysis

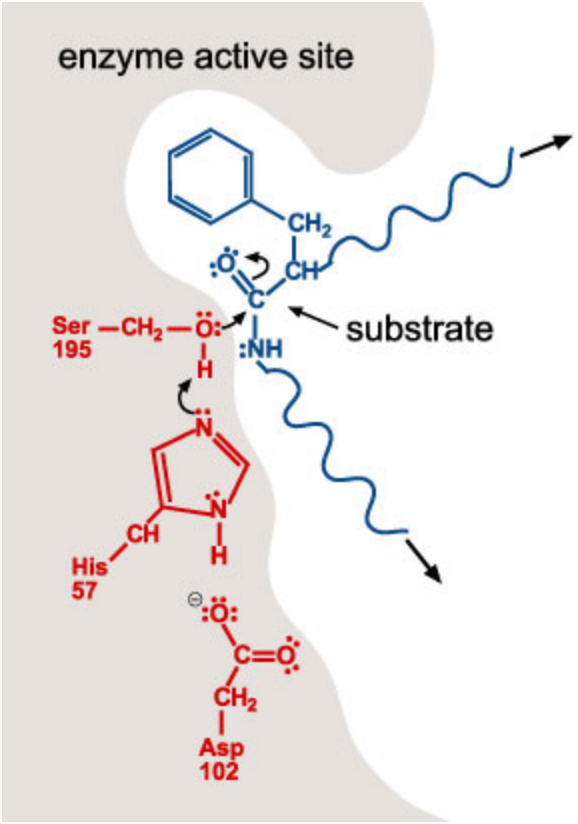
Proteases

- The most serious limiting factor *in vivo*
- In different compartments:
  - GIT: Proteases attack orally administered proteins
  - Plasma: Proteases hydrolyze proteins given parenterally
  - Cytoplasm: Intracellular proteases e.g. Proteasome

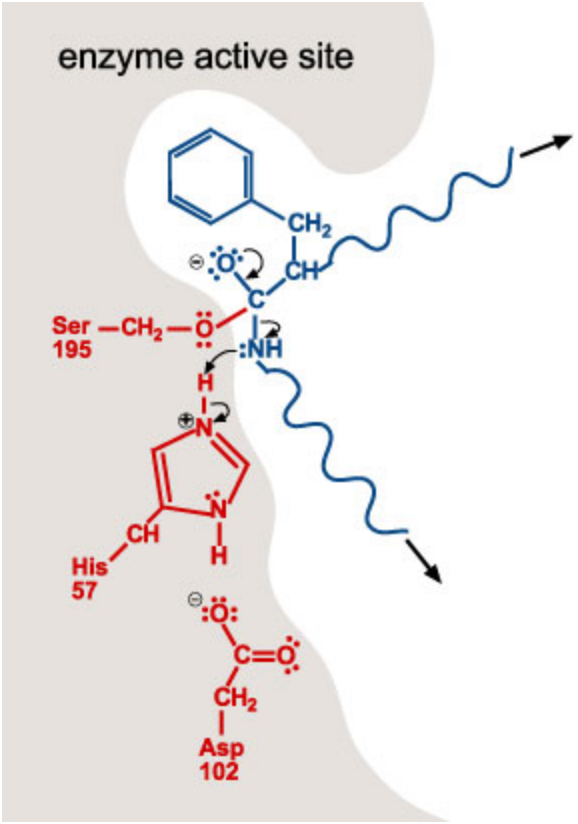
Proteases

- Endo- or exo-proteases
- Classes:
  1. Serine proteases
  2. Threonine proteases
  3. Cysteine proteases
  4. Aspartic acid proteases
  5. Metalloproteases
  6. Glutamic acid proteases
- They act specifically:
  1. Chymotrypsin: aromatic a.a. (**Trp, Tyr, Phe**)
  2. Trypsin: **Lys** residues
  3. Factor Xa: **Ile**-(**Glu** or **Asp**)-**Gly-Arg**
  4. Carboxypeptidase: C-terminal

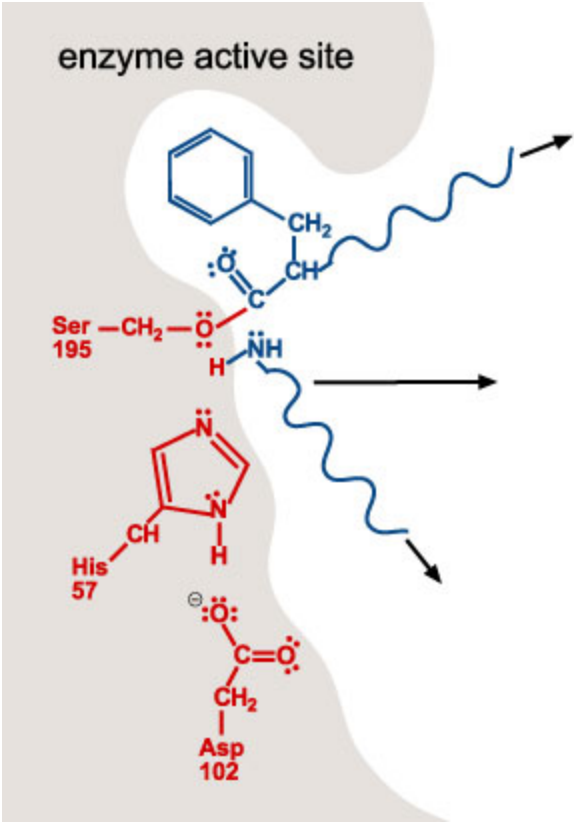
Chymotrypsin 1



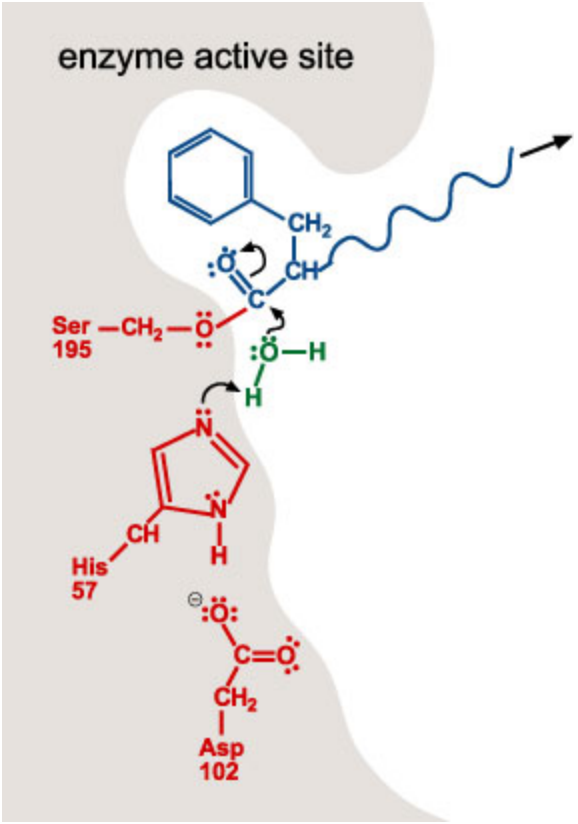
Chymotrypsin 2



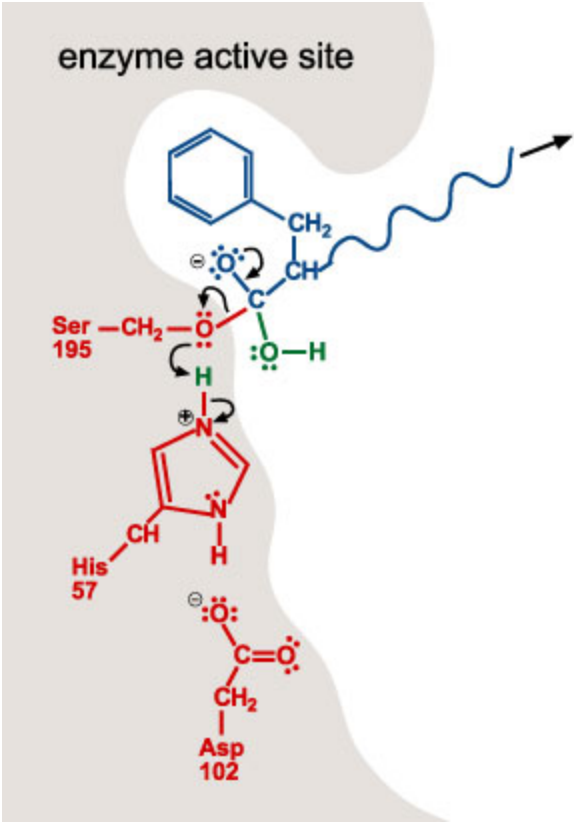
Chymotrypsin 3



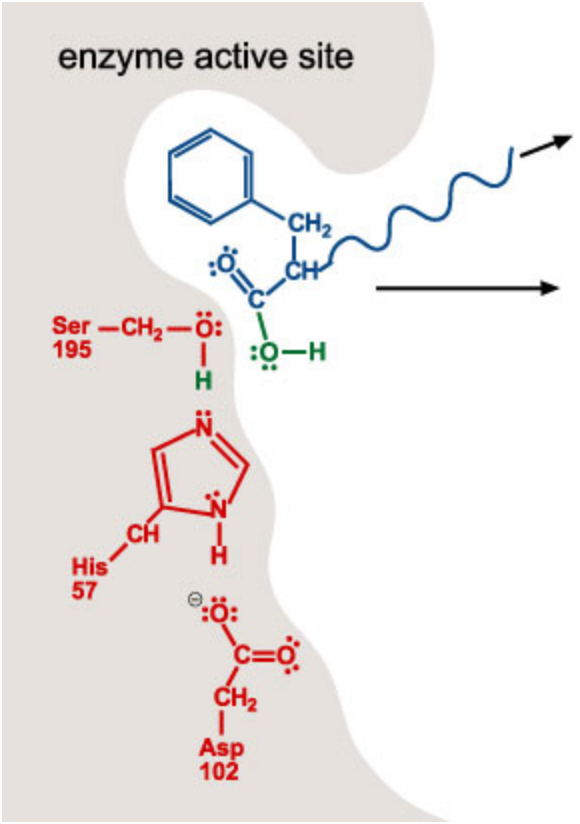
Chymotrypsin 4



Chymotrypsin 5



Chymotrypsin 6



Asp 102, His 57, and Ser 195 are known as the Catalytic Triad

Now you are able to:

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