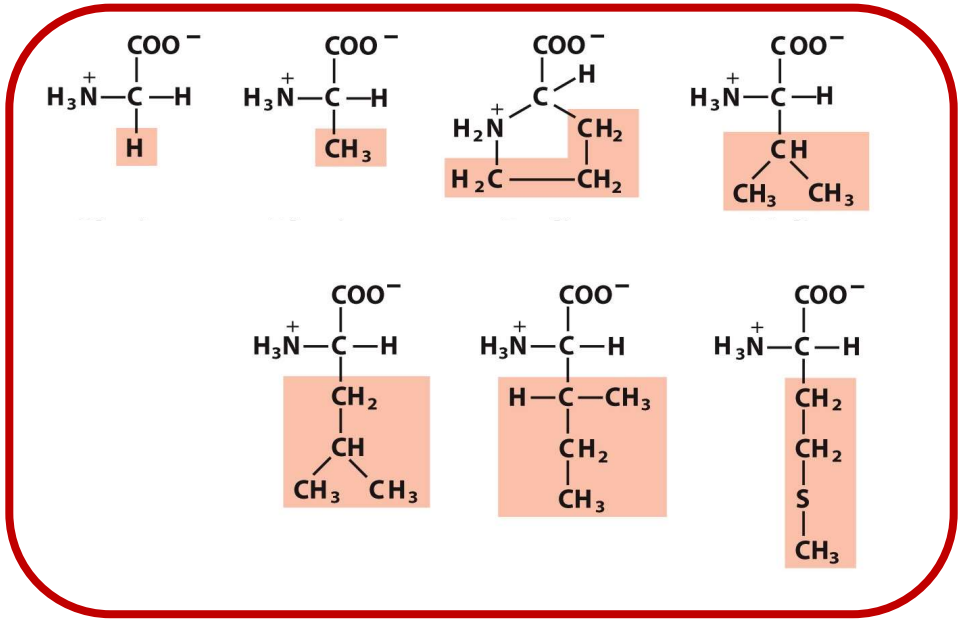

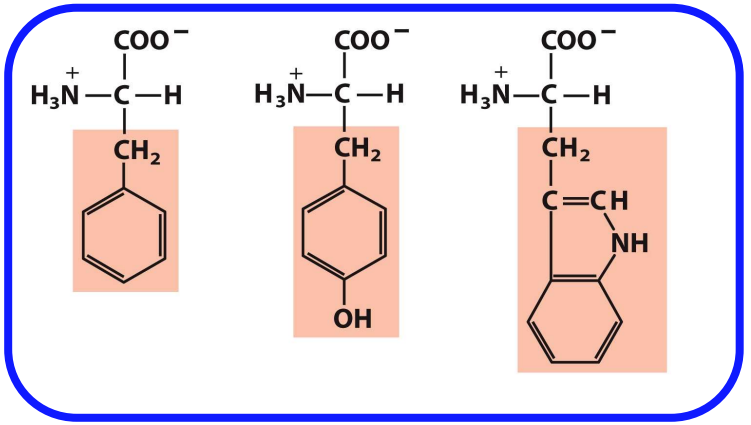

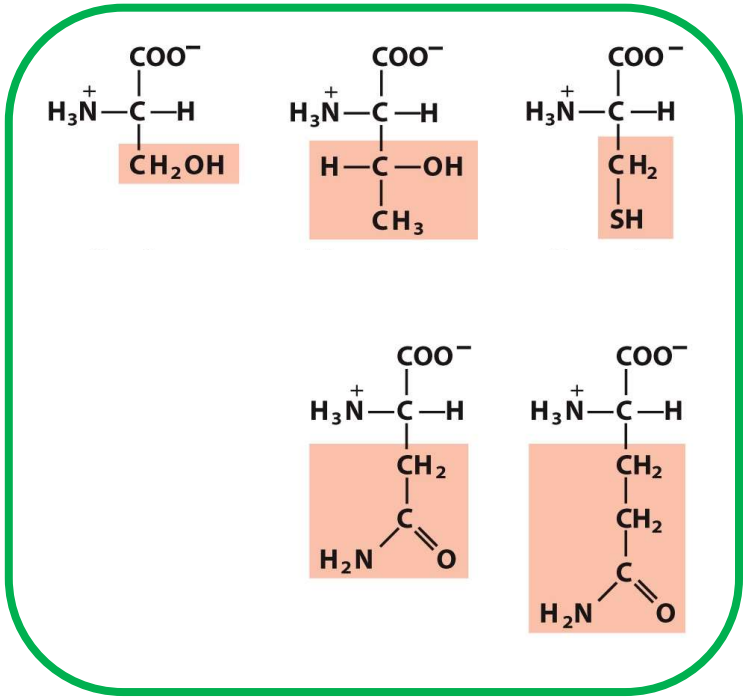

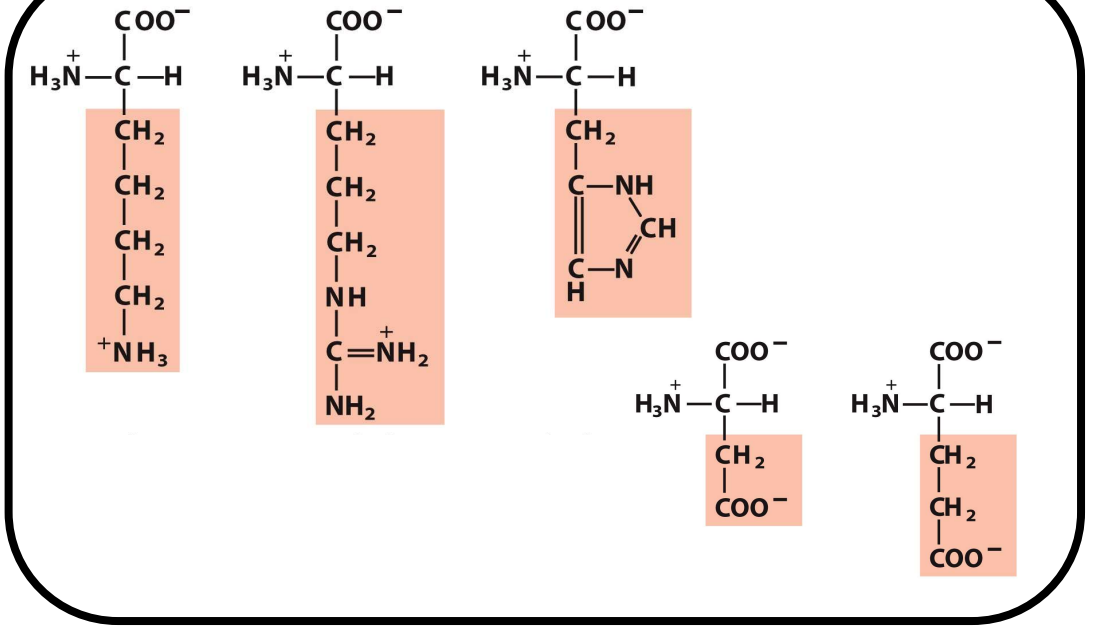

Ionization of Amino Acids

- Amino acids contain at least two ionizable protons, each with its own pK_a.
- The carboxylic acid has an acidic pK_a and will be protonated at an acidic (low) pH: $-COOH \leftrightarrow -COO^- + H^+$
- The amino group has a basic pK_a and will be protonated when basic pH (high) is achieved: $-NH_3^+ \leftrightarrow -NH_2 + H^+$
- At low pH, the amino acid exists in a positively charged form (cation).
- At high pH, the amino acid exists in a negatively charged form (anion).
- Between the pK_a for each group, the amino acid exists in a zwitterion form, in which a single molecule has both a positive and a negative charge.

Amino Acids Carry a Net Charge of Zero at a Specific pH (the pI)

- Zwitterions predominate at pH values between the pK_a values of the amino and carboxyl groups.
- For amino acids without ionizable side chains, the Isoelectric Point (equivalence point, pI) is:

$$pI = \frac{pK_1 + pK_2}{2}$$

- At this point, the net charge is zero.
  - AA is least soluble in water.
  - AA does not migrate in electric field.

Amino Acids Can Act as Buffers

Amino acids with uncharged side chains, such as glycine, have two pK_a values:

- The pK_a of the α-carboxyl group is 2.34.
- The pK_a of the α-amino group is 9.6.

As buffers prevent change in pH close to the pK_a, glycine can act as a buffer in two pH ranges.

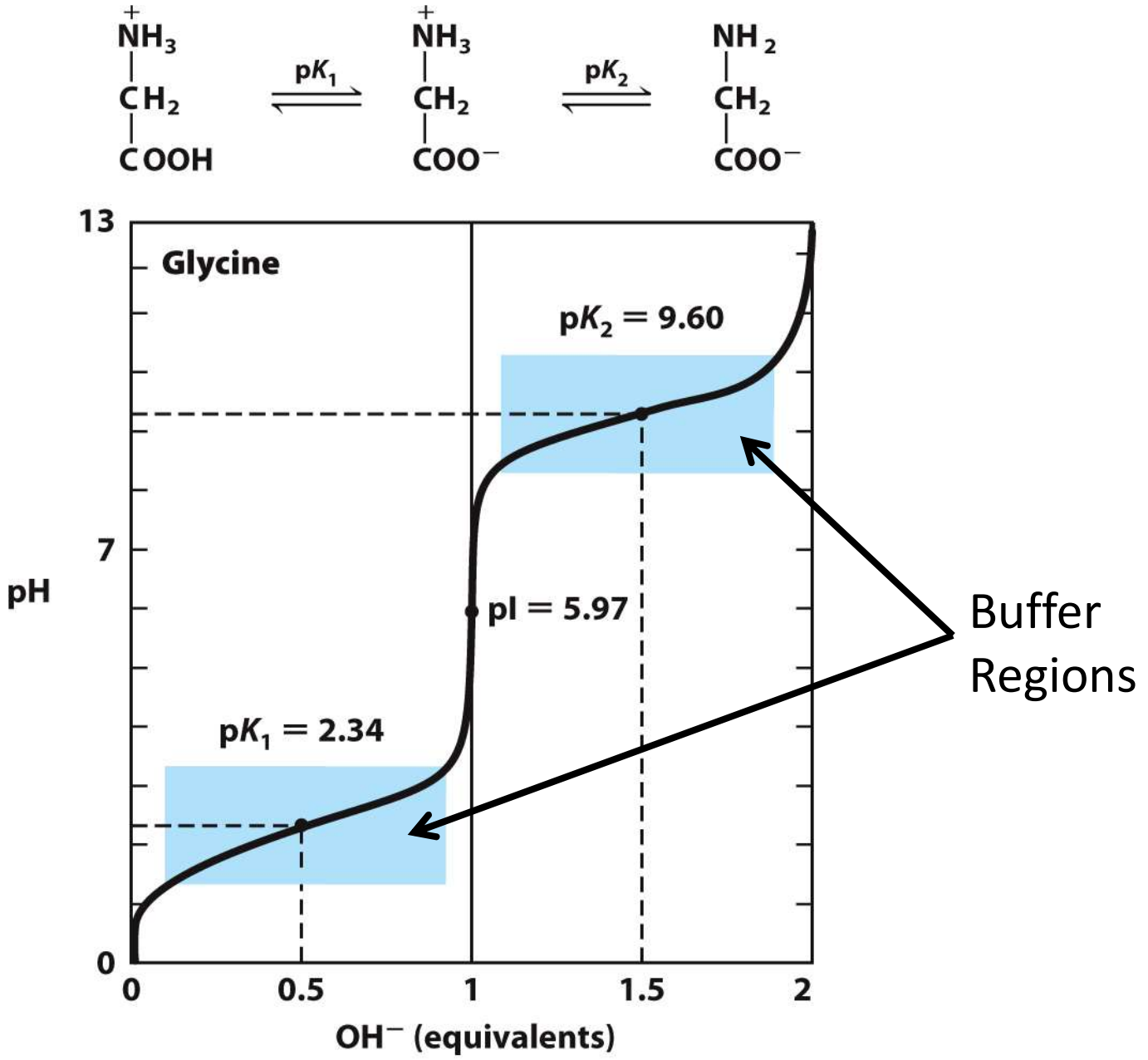

Figure 3-10
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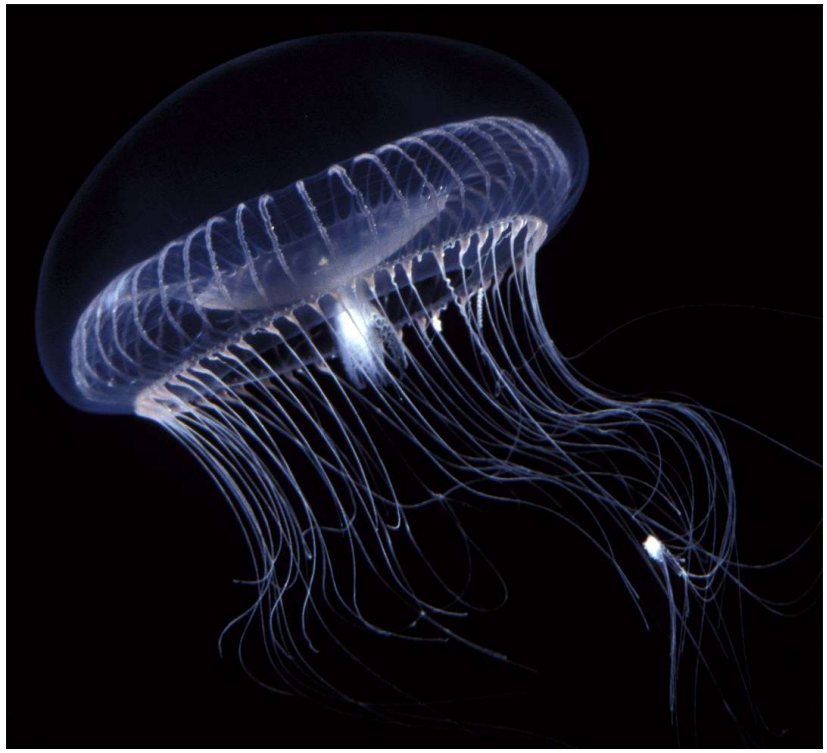
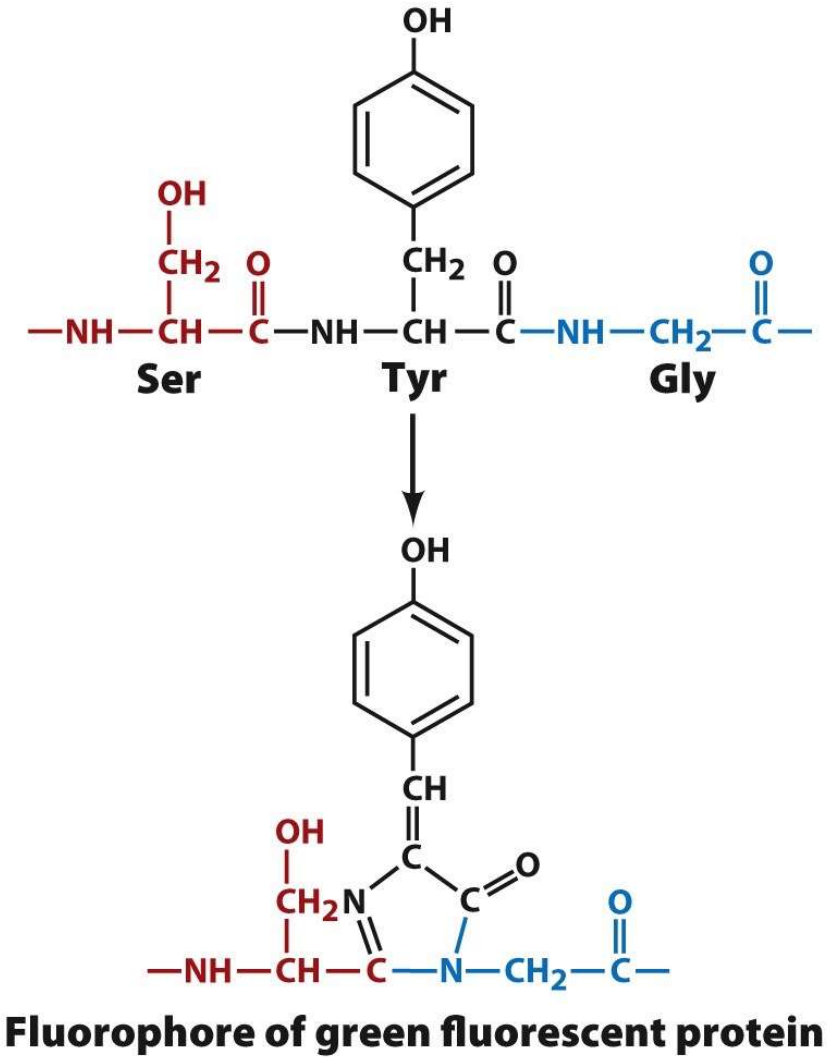
Green Fluorescent Protein



Box 4-3a
Dr. Kevin Raskoff

Box 4-3a
Dr kevin Raskoff



Fluorophore of green fluorescent protein

Biologically Active Amino Acid Derivatives

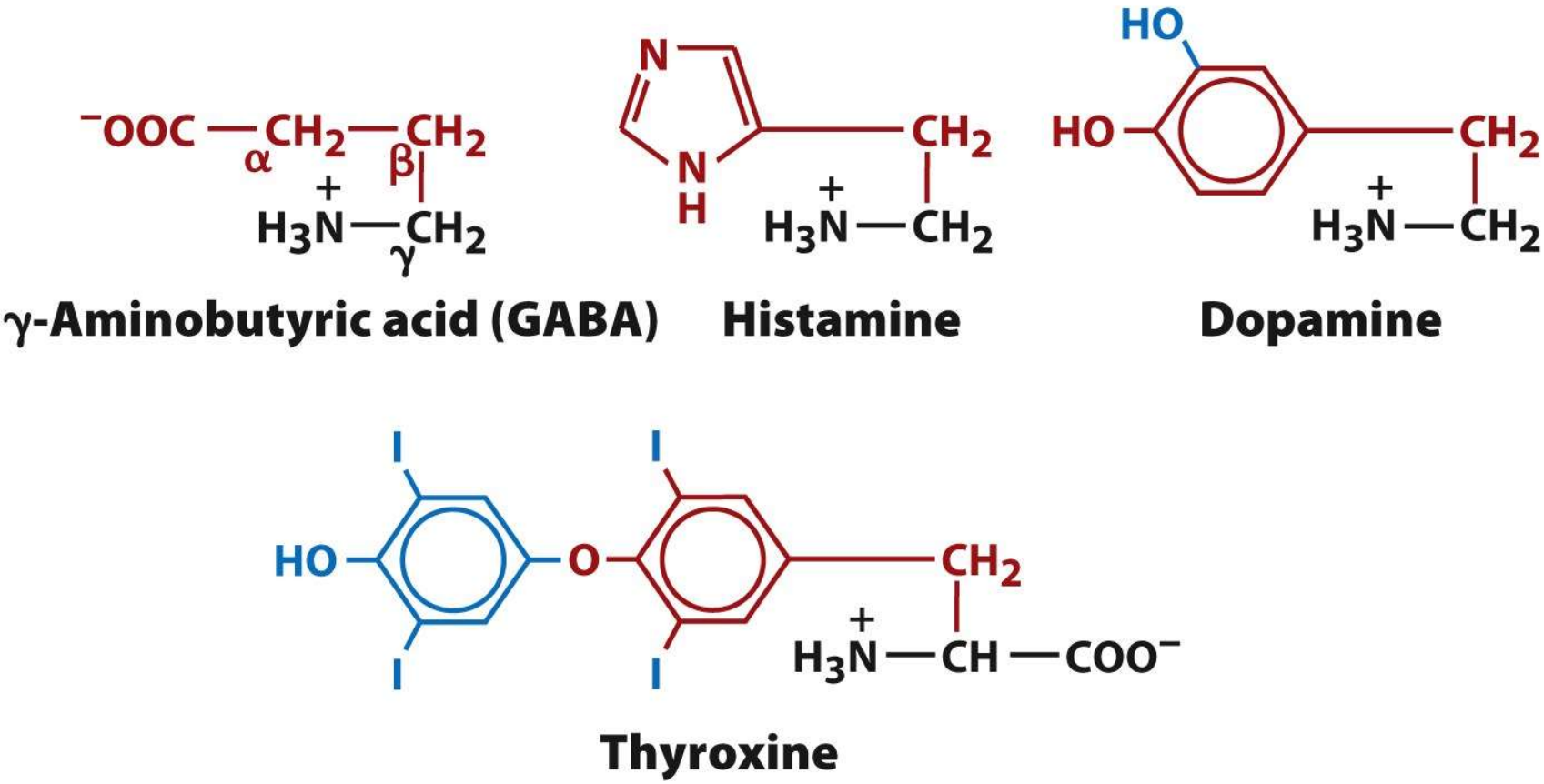


Figure 4-15

Common Questions About Peptides and Proteins

What is its sequence and composition?

What is its three-dimensional structure?

How does it achieve its biochemical role?

How is its function regulated?

How does it interact with other macromolecules?

How is it related to other proteins?

Where is it localized within the cell?

What are its physico-chemical properties?

A Mixture of Proteins Can Be Separated

- Separation relies on differences in physical and chemical properties:
  - charge
  - size
  - affinity for a ligand
  - solubility
  - hydrophobicity
  - thermal stability
- Chromatography is commonly used for preparative separation in which the protein is often able to remain fully folded.

Column Chromatography

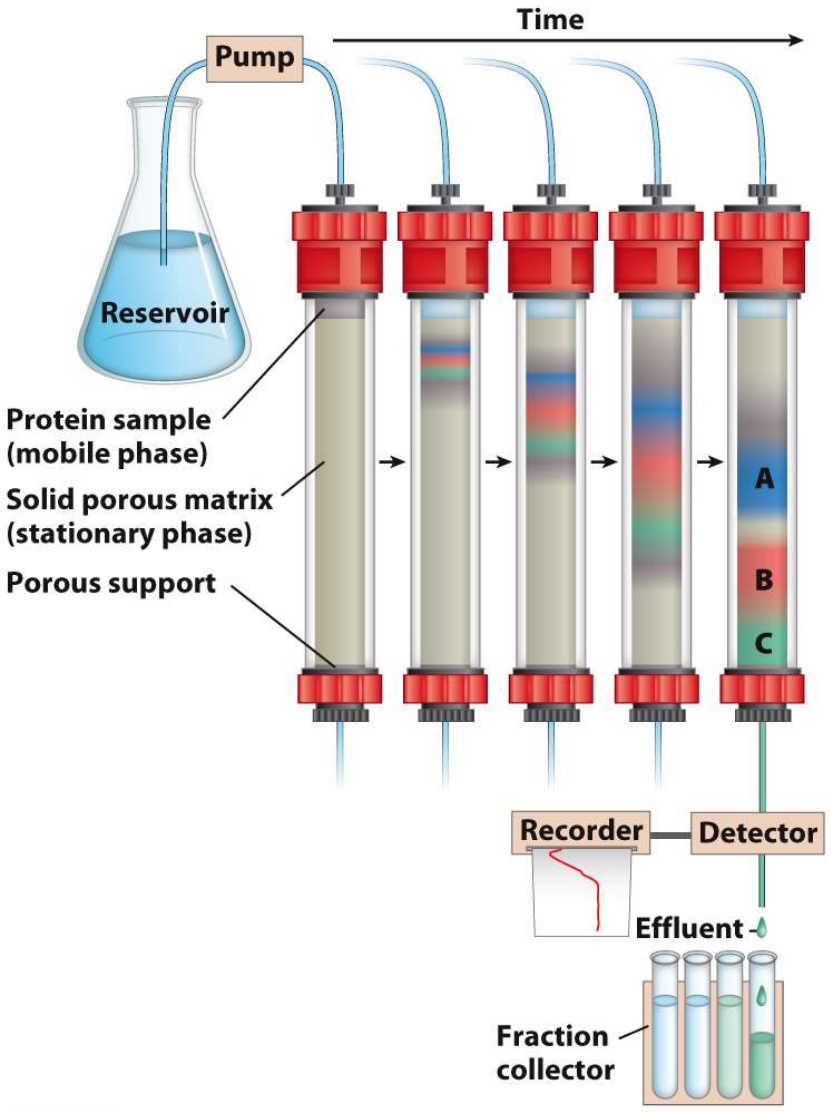


Figure 3-16
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- Column chromatography allows separation of a mixture of proteins over a solid phase (porous matrix) using a liquid phase to mobilize the proteins.
- Proteins with a lower affinity for the solid phase will wash off first; proteins with higher affinity will retain on the column longer and wash off later.

Separation by Charge: Ion Exchange

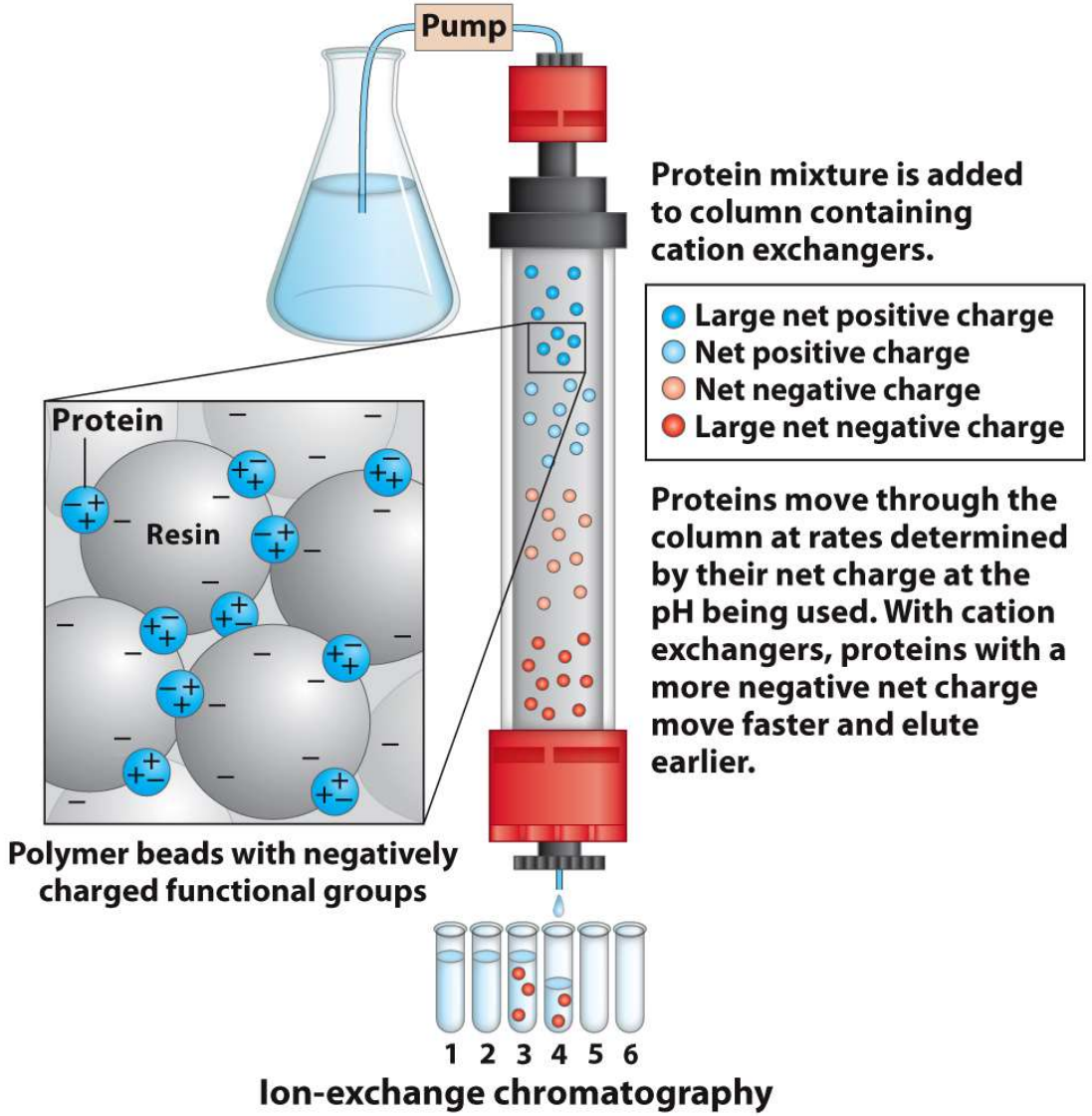

Figure 3-17a
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Separation by Size: Size Exclusion

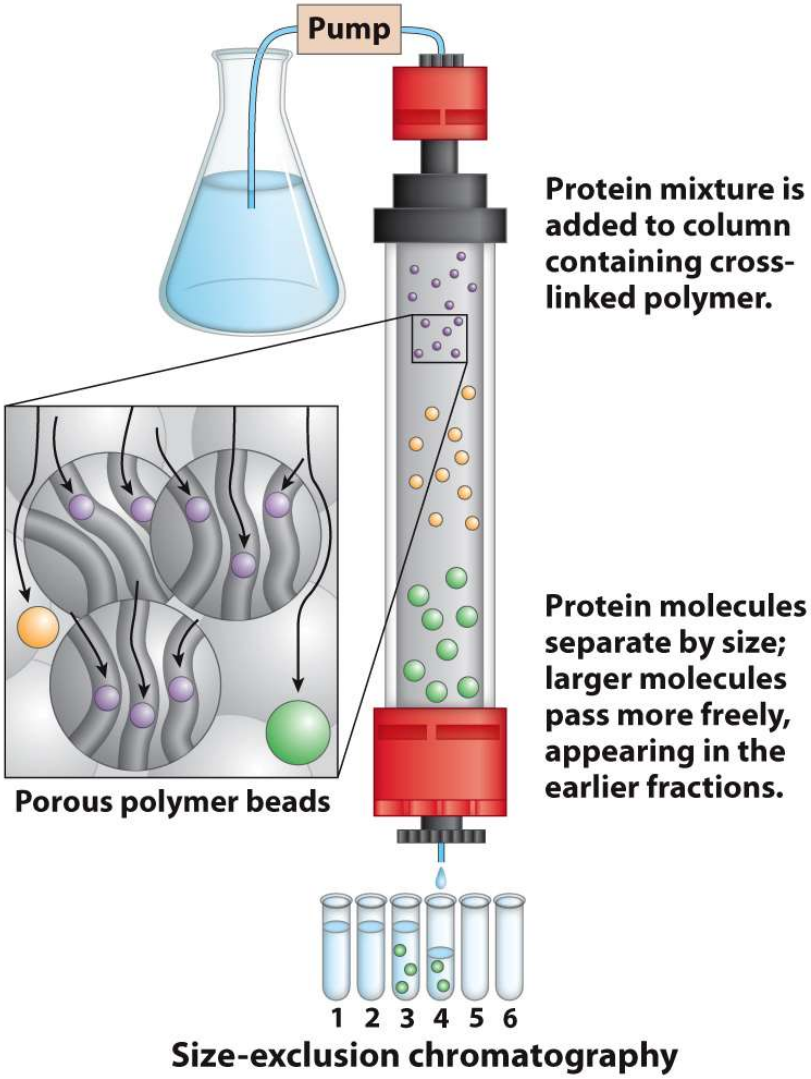

Figure 3-17b
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Separation by Binding: Affinity

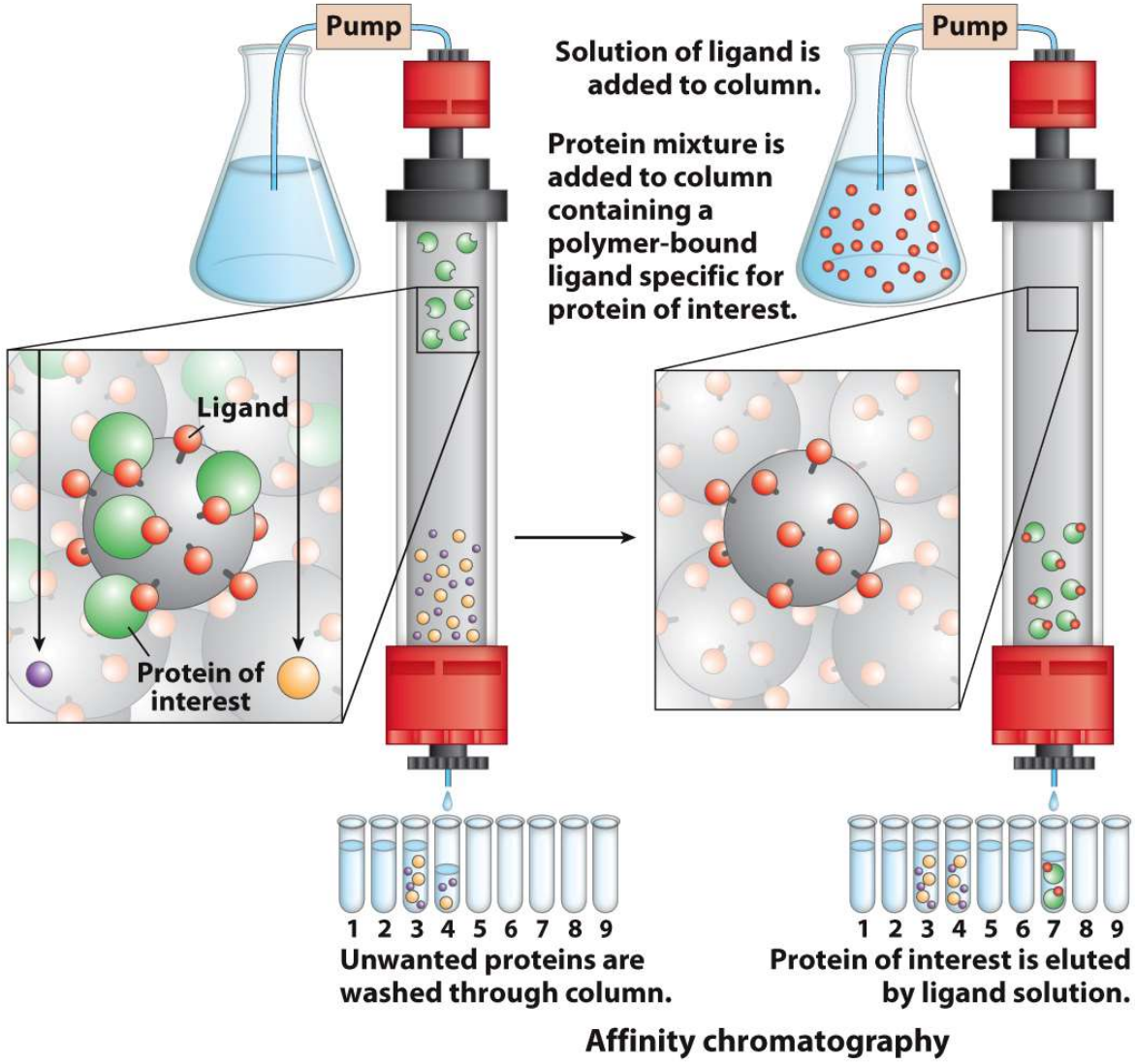

Figure 3-17c
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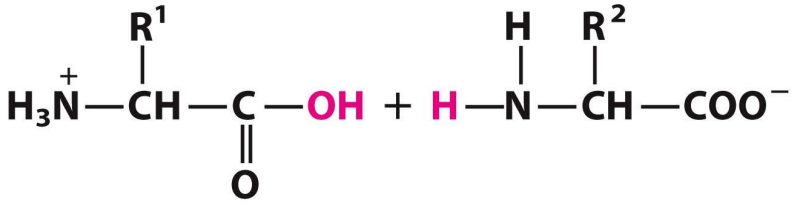
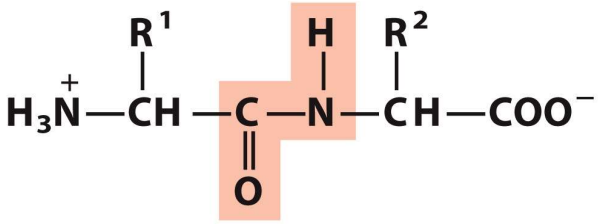
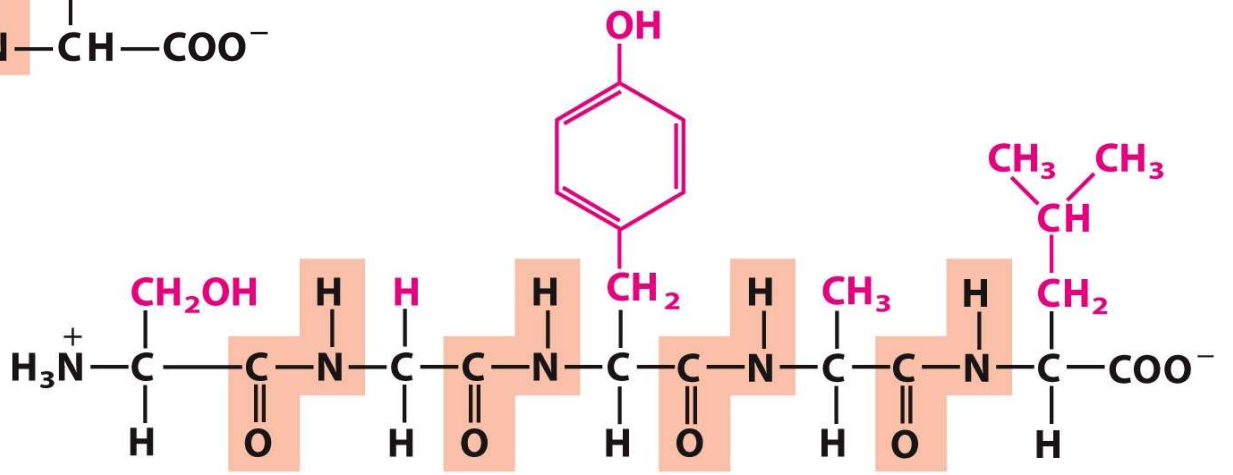
Amino Acids Polymerize to Form Peptides

Amino acids → peptides → proteins





- serylglycyltyrosylalanylleucine
- Ser-Gly-Tyr-Ala-Leu
- SGYAL



The Three-Dimensional Structure of Proteins

- Structure and properties of the peptide bond
- Structural hierarchy in proteins
- Structure and function of fibrous proteins
- Structure analysis of globular proteins
- Protein folding and denaturation

Structure of Proteins

- Unlike most organic polymers, protein molecules adopt a specific three-dimensional conformation.
- This structure is able to fulfill a specific biological function.
- This structure is called the native fold.
- The native fold has a large number of favorable interactions within the protein.
- There is an entropy cost to folding the protein into one specific native fold.

Favorable Interactions in Proteins

- **Hydrophobic effect**
  - The release of water molecules from the structured solvation layer around the molecule as protein folds increases the net entropy.
- **Hydrogen bonds**
  - Interaction of N−H and C=O of the peptide bond leads to local regular structures such as α helices and β sheets.
- **Van der Waals force**
  - Attraction between all atoms contributes significantly to the stability in the interior of the protein.
- **Electrostatic interactions**
  - long-range strong interactions between permanently charged groups
  - Salt bridges, especially those buried in the hydrophobic environment, strongly stabilize the protein.

yggfmsseks qtplvtlfkn aiiknahkkg q (31 aa)

Four Levels of Protein Structure

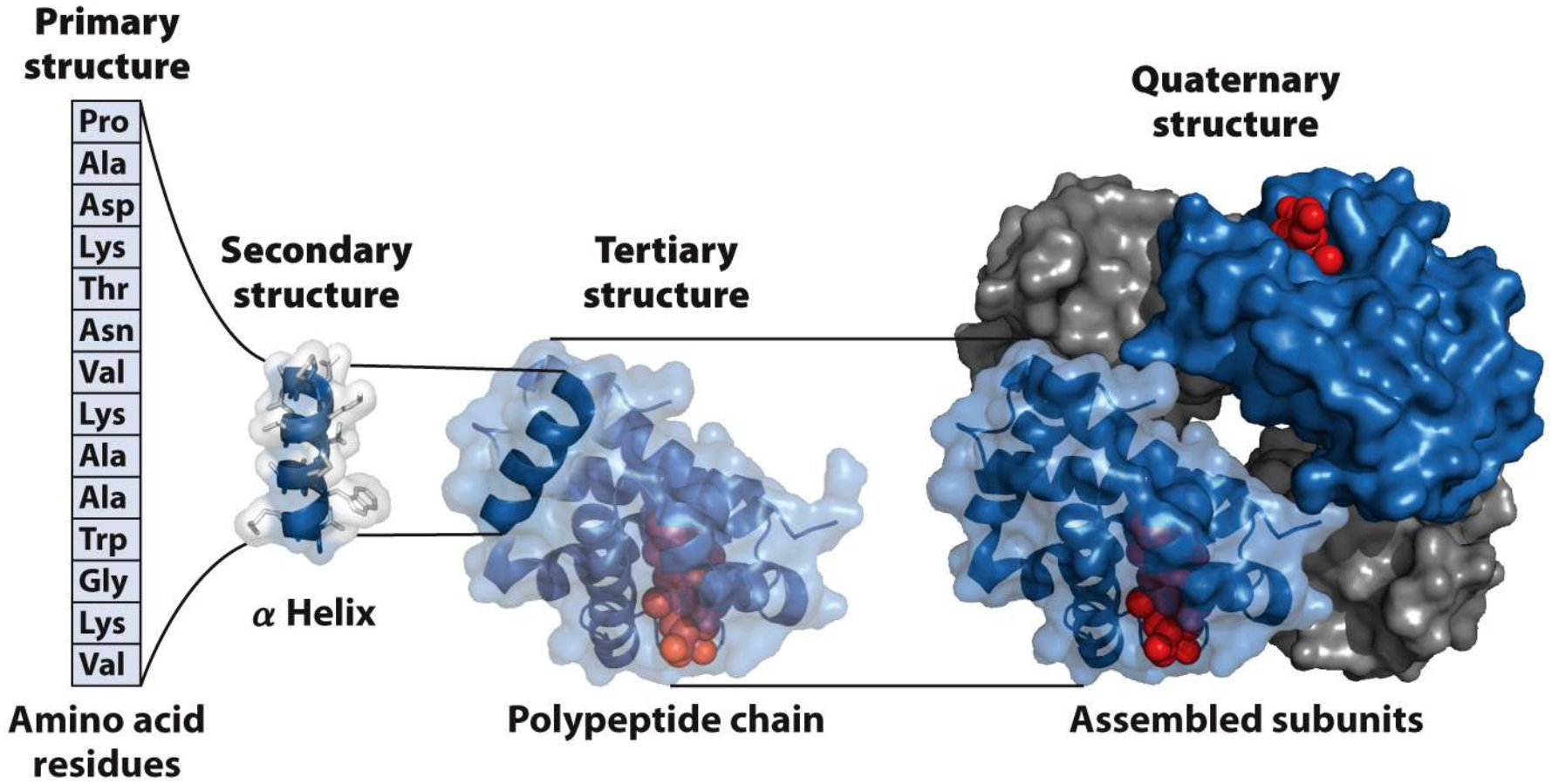


Figure 3-23
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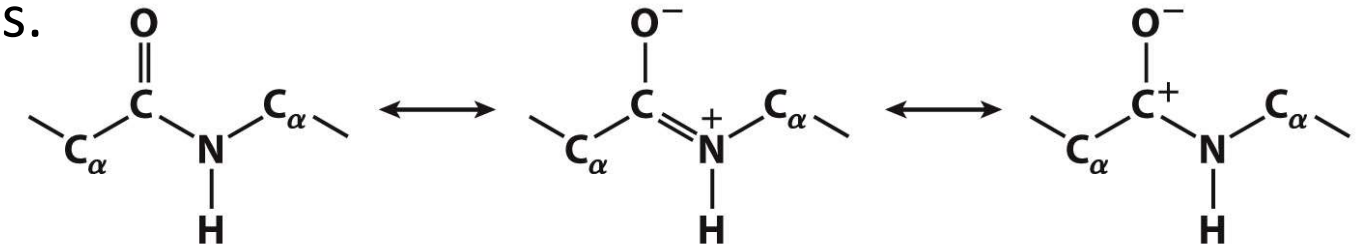
Primary Structure: The Peptide Bond

- The structure of the protein is partially dictated by the properties of the peptide bond.
- The peptide bond is a resonance hybrid of two canonical structures.



- The resonance causes the peptide bonds:
  - to be less reactive compared with esters, for example
  - to be quite **rigid** and nearly **planar**
  - to exhibit a large dipole moment in the favored trans configuration

The Rigid Peptide Plane and the Partially Free Rotations

- Rotation around the peptide bond is not permitted due to resonance structure.
- Rotation around bonds connected to the α carbon is permitted.
  - ϕ (phi): angle around the α carbon—amide nitrogen bond
  - ψ (psi): angle around the α carbon—carbonyl carbon bond
- In a fully extended polypeptide, both ψ and ϕ are 180° .

The organization around the peptide bond, paired with the identity of the R groups, determines the secondary structure of the protein.

The Polypeptide Is Made Up of a Series of Planes Linked at α Carbons

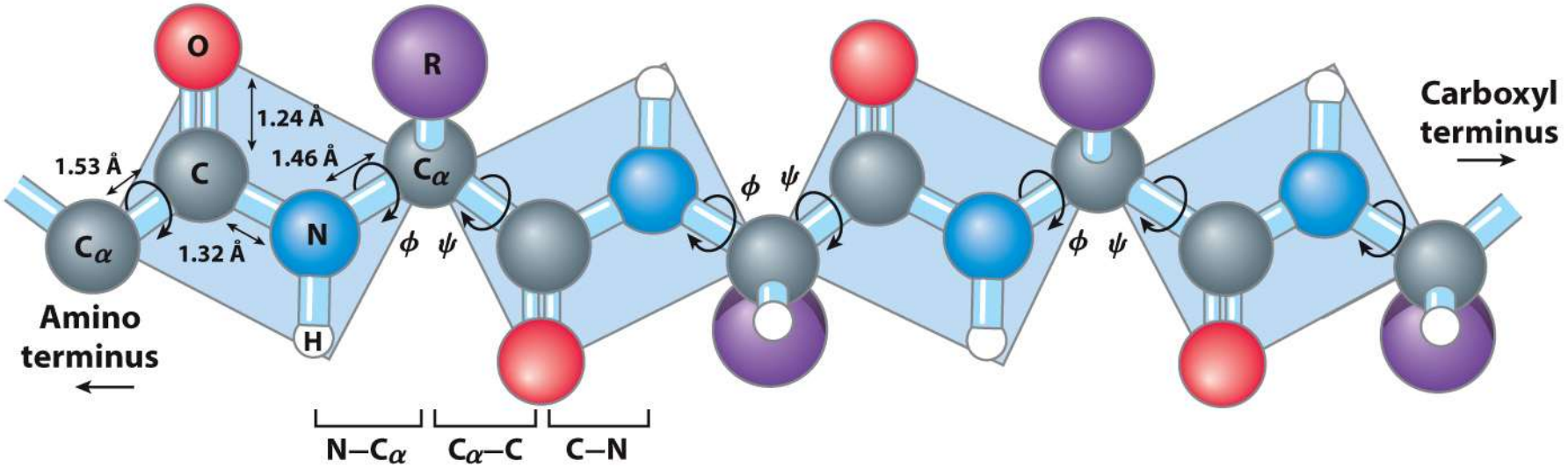


Figure 4-2b
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Distribution of ϕ and ψ Dihedral Angles

- Some ϕ and ψ combinations are very unfavorable because of steric crowding of backbone atoms with other atoms in the backbone or side chains.
- Some ϕ and ψ combinations are more favorable because of chance to form favorable H-bonding interactions along the backbone.
- **A Ramachandran plot shows the distribution of ϕ and ψ dihedral angles that are found in a protein:**
  - shows the common secondary structure elements
  - reveals regions with unusual backbone structure

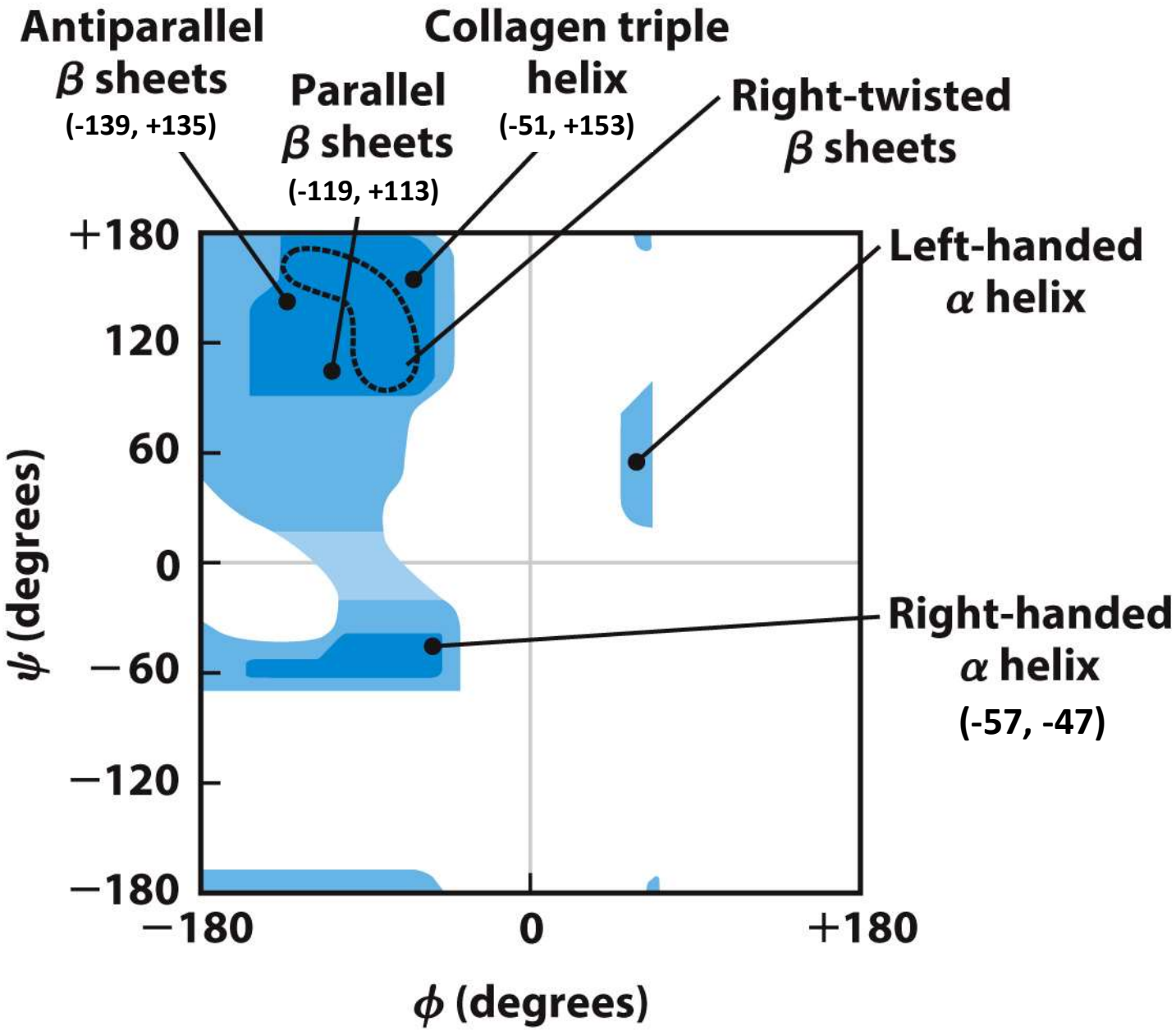

Figure 4-9a
Lehninger Principles of Biochemistry, Seventh Edition

Secondary Structures

- Secondary structure refers to a local spatial arrangement of the polypeptide backbone.
- Two regular arrangements are common:
  - the **α helix**
    - stabilized by hydrogen bonds between nearby residues
  - the **β sheet**
    - stabilized by hydrogen bonds between adjacent segments that may not be nearby
- Irregular arrangement of the polypeptide chain is called the random coil.

The α Helix

- Helical backbone is held together by hydrogen bonds between the backbone amides of an *n* and *n* + 4 amino acids.
- It is a right-handed helix with 3.6 residues (5.4 Å) per turn.
- Peptide bonds are aligned roughly parallel with the helical axis.
- Side chains point out and are roughly perpendicular with the helical axis.

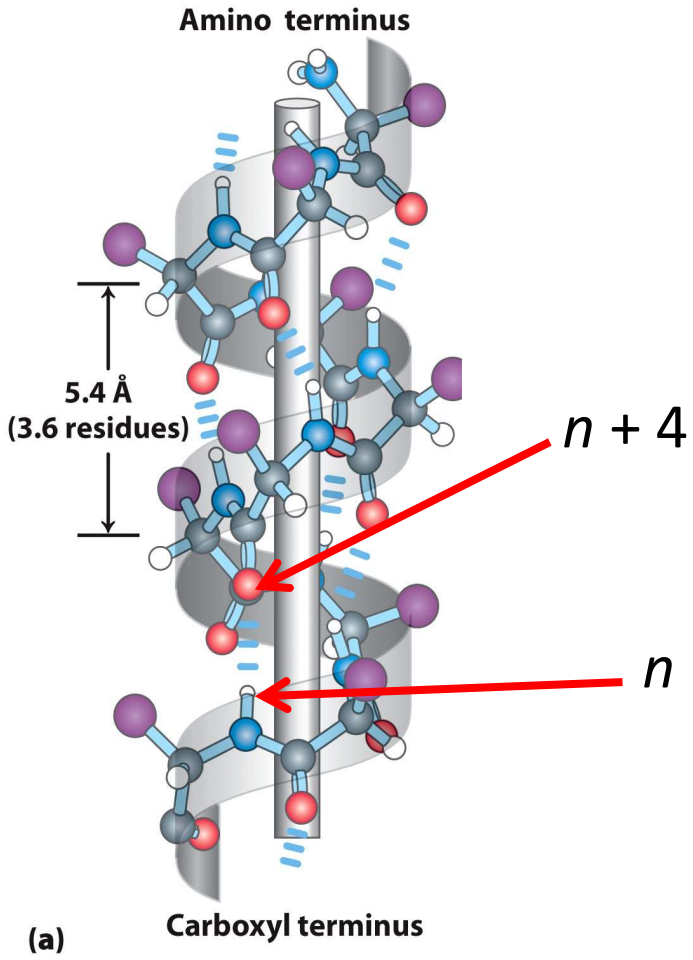

Figure 4-4
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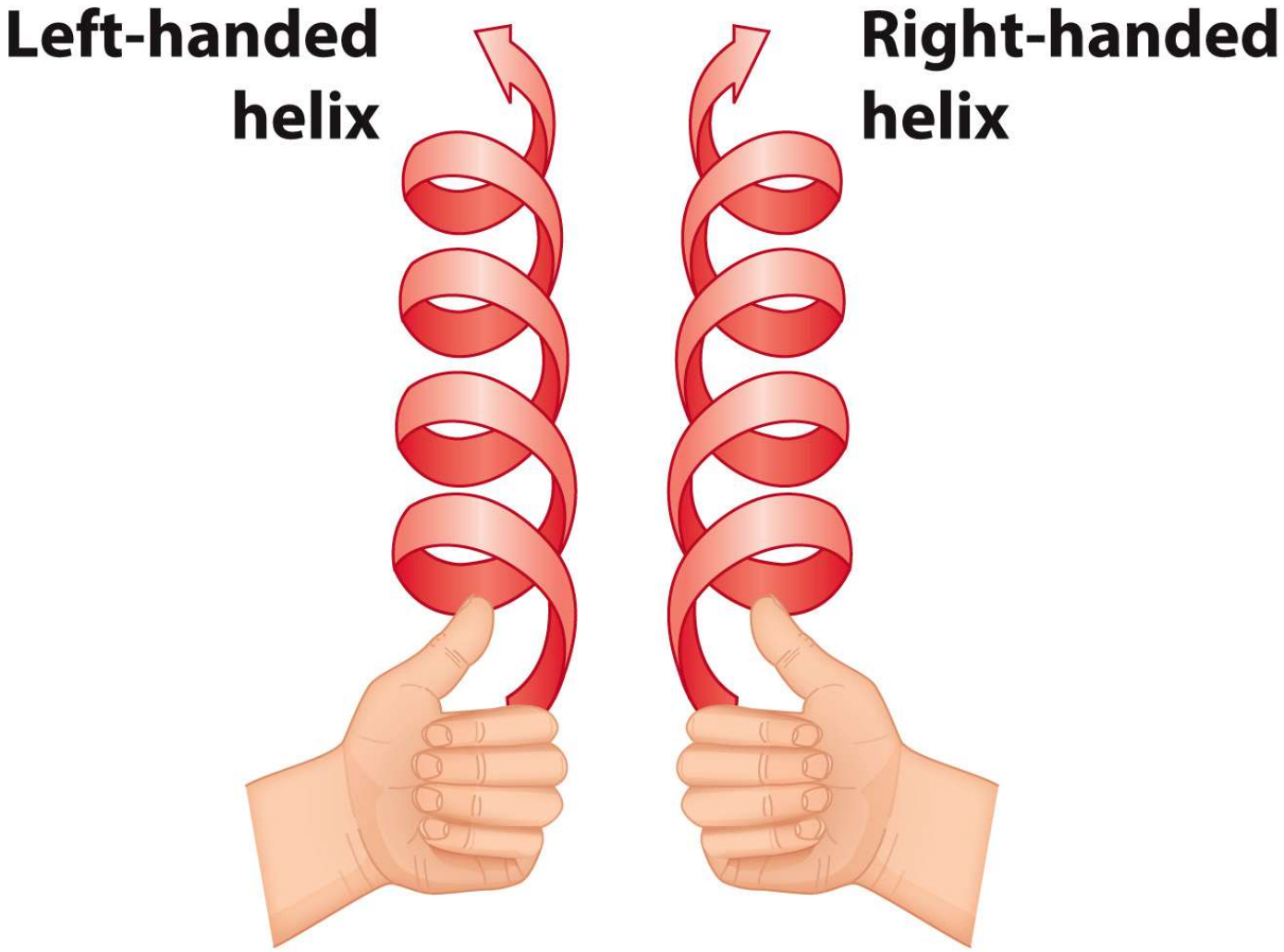
What Is a Right-Handed Helix?



Box 4-1
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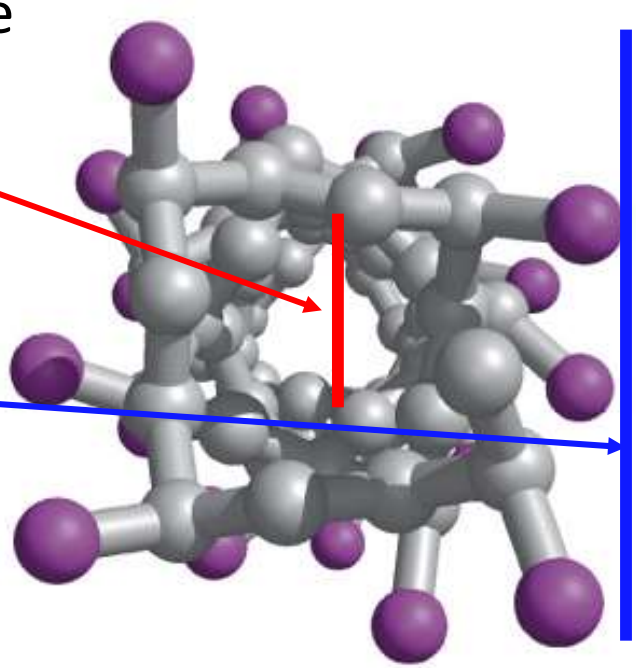
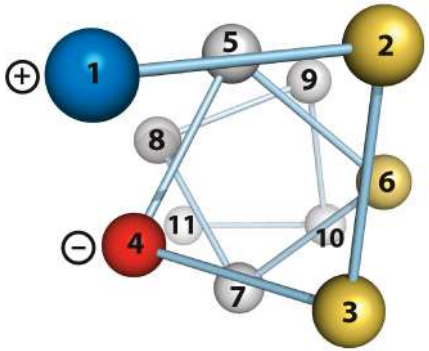
The α Helix: Top View

- The inner diameter of the helix (no side chains) is about 4–5 Å.
  - too small for anything to fit "inside"
- The outer diameter of the helix (with side chains) is 10–12 Å.
  - happens to fit well into the major groove of dsDNA
- Amino acids #1 and #8 align nicely on top of each other.





Sequence Affects Helix Stability

- Not all polypeptide sequences adopt α-helical structures.
- Small hydrophobic residues such as Ala and Leu are strong helix formers.
- Pro acts as a helix breaker because the rotation around the N-C_a (φ-angle) bond is impossible.
- Gly acts as a helix breaker because the tiny R group supports other conformations.
- Attractive or repulsive interactions between side chains 3 to 4 amino acids apart will affect formation.

The Helix Dipole

- Recall that the peptide bond has a strong dipole moment.
  - C–O (carbonyl) negative
  - N–H (amide) positive
- All peptide bonds in the α helix have a similar orientation.
- The α helix has a large macroscopic dipole moment that is enhanced by unpaired amides and carbonyls near the ends of the helix.
- Negatively charged residues often occur near the positive end of the helix dipole.

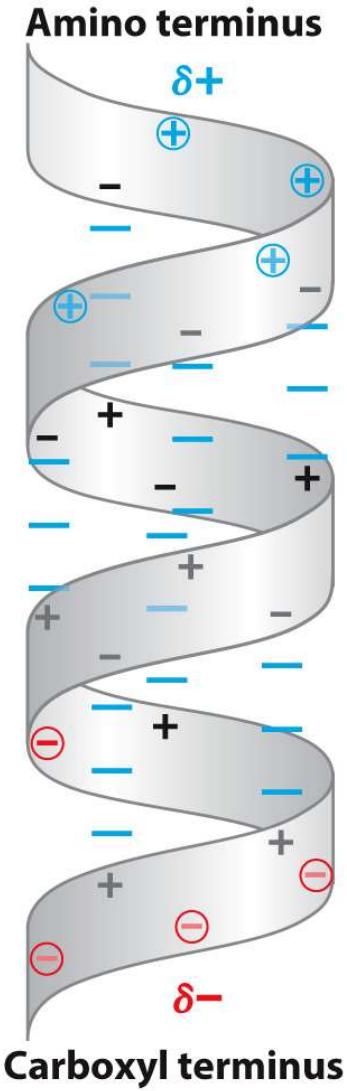


Figure 4-5
Lehninger Principles of Biochemistry, Seventh Edition

β Sheets

- The planarity of the peptide bond and tetrahedral geometry of the α carbon create a pleated sheet-like structure.
- Sheet-like arrangement of the backbone is held together by hydrogen bonds between the backbone amides in different strands.
- Side chains protrude from the sheet, alternating in an up-and-down direction.

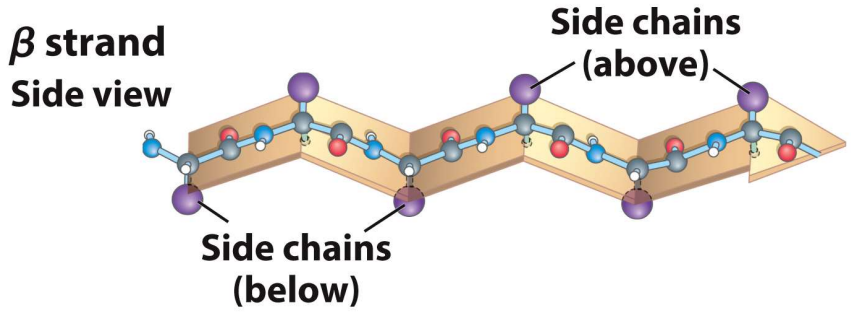


Figure 4-6a
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Parallel and Antiparallel β Sheets

- Multi β-strand interactions are called sheets.
- Sheets are held together by the hydrogen bonding of amide and carbonyl groups of the peptide bond from opposite strands.
- Two major orientations of β sheets are determined by the directionality of the strands within:
  - Parallel sheets have strands that are oriented in the same direction.
  - Antiparallel sheets have strands that are oriented in opposite directions.

In parallel β sheets, the H-bonded strands run in the same direction.

- Hydrogen bonds between strands are bent (weaker).

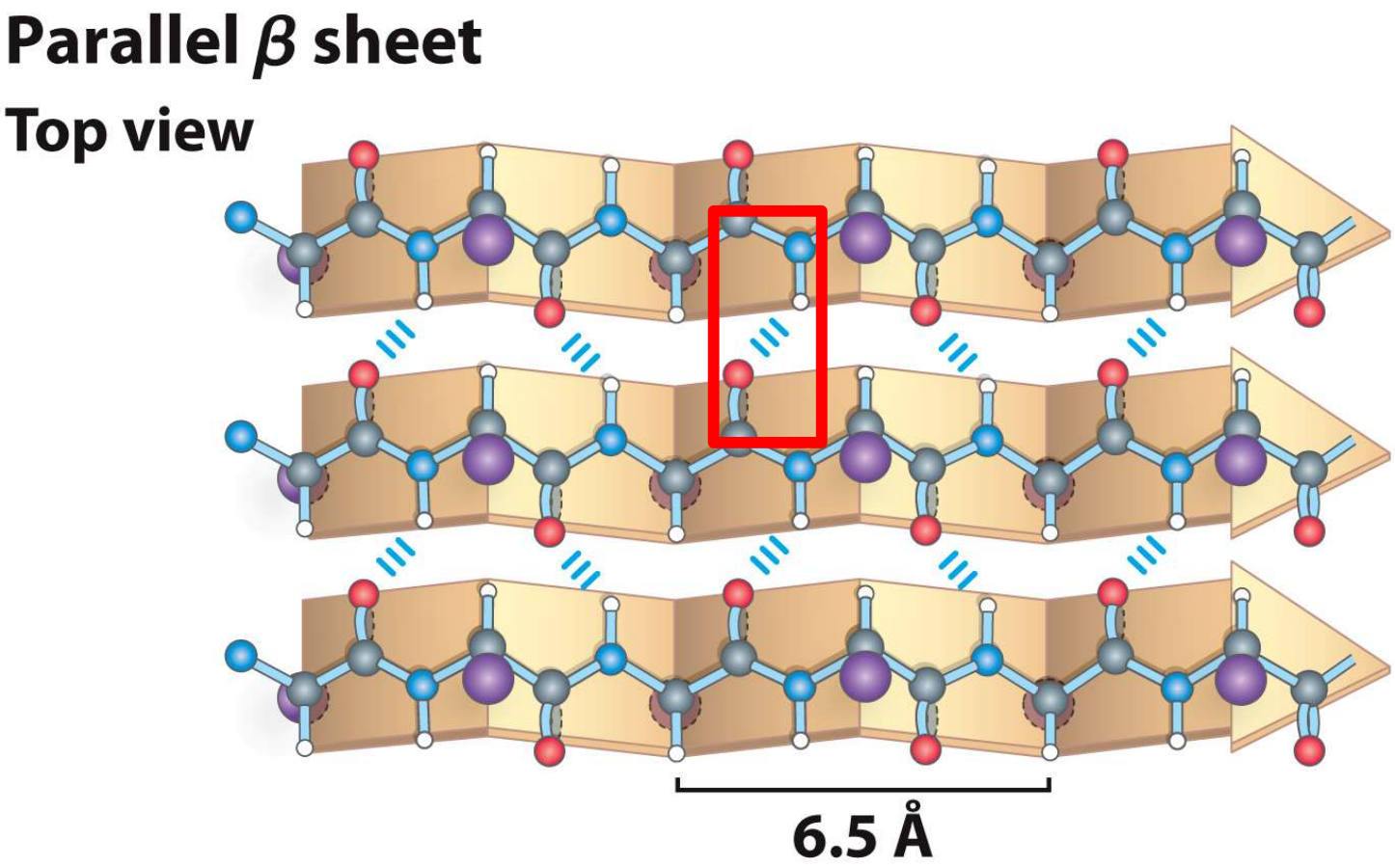


Figure 4-6c
Lehninger Principles of Biochemistry, Seventh Edition

In antiparallel β sheets, the H-bonded strands run in opposite directions.

- Hydrogen bonds between strands are linear (stronger).

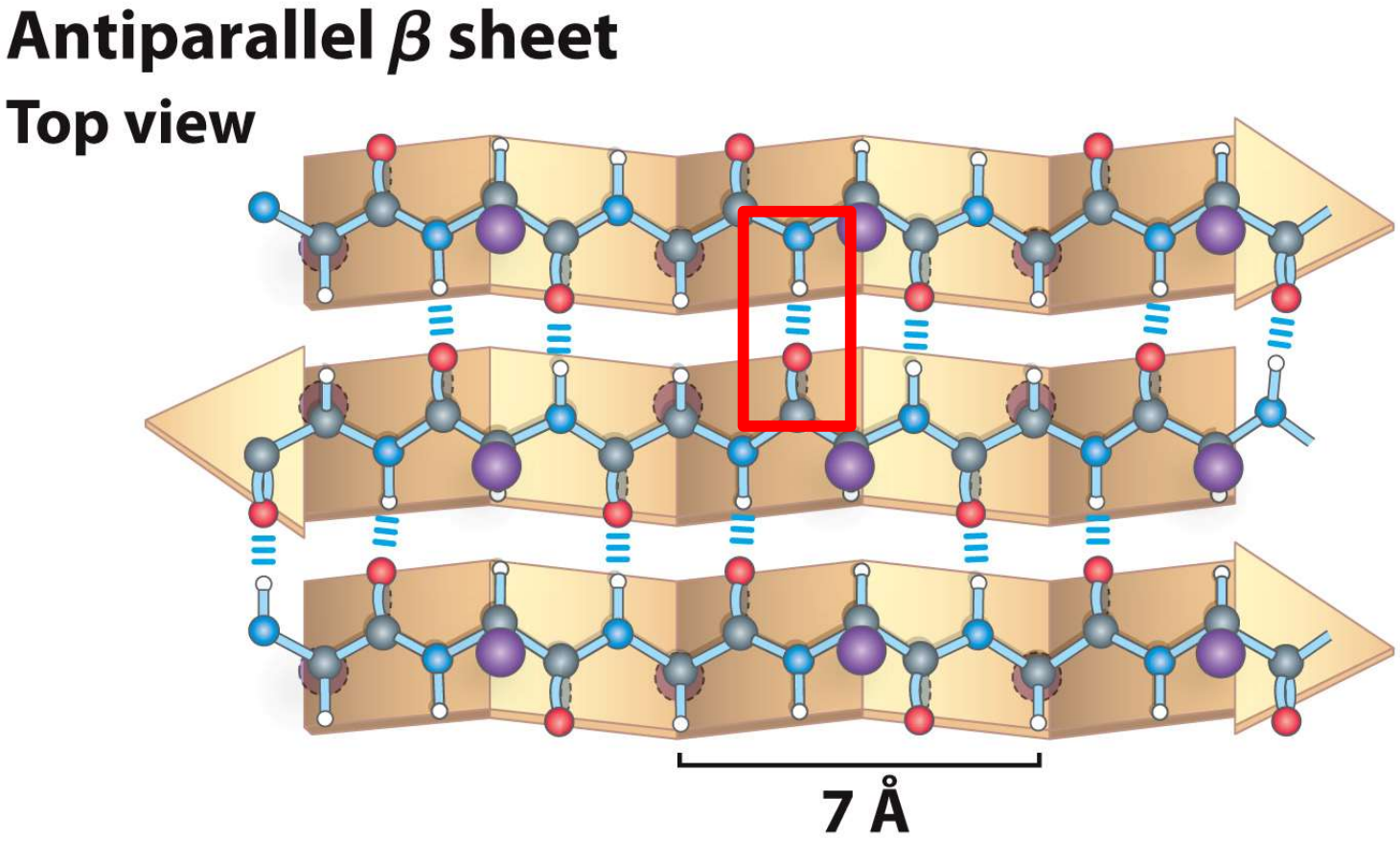
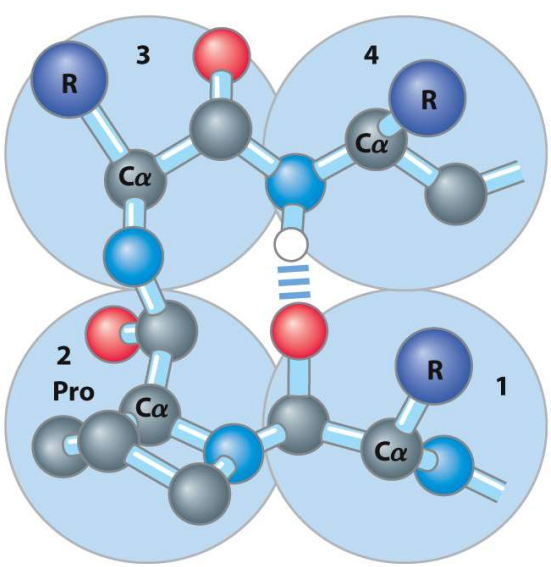


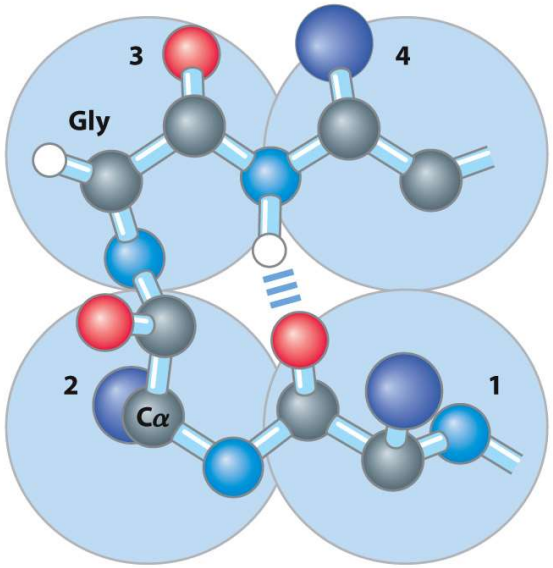
Figure 4-6b
Lehninger Principles of Biochemistry, Seventh Edition

β Turns

- β turns occur frequently whenever strands in β sheets change the direction.
- The 180° turn is accomplished over four amino acids.
- The turn is stabilized by a hydrogen bond from a carbonyl oxygen to amide proton three residues down the sequence.
- Proline in position 2 or glycine in position 3 are common in β turns.



Type I β turn



Type II β turn