

CHAPTER 4

Proteins: Structure, Function, Folding

Learning goals:

- 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 a **cost in conformational entropy** of folding the protein into one specific native fold

Favorable Interactions in Proteins

- **Hydrophobic effect**

- 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

- **London dispersion**

- Medium-range weak 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, esp. buried in the hydrophobic environment strongly stabilize the protein

4 Levels of Protein Structure

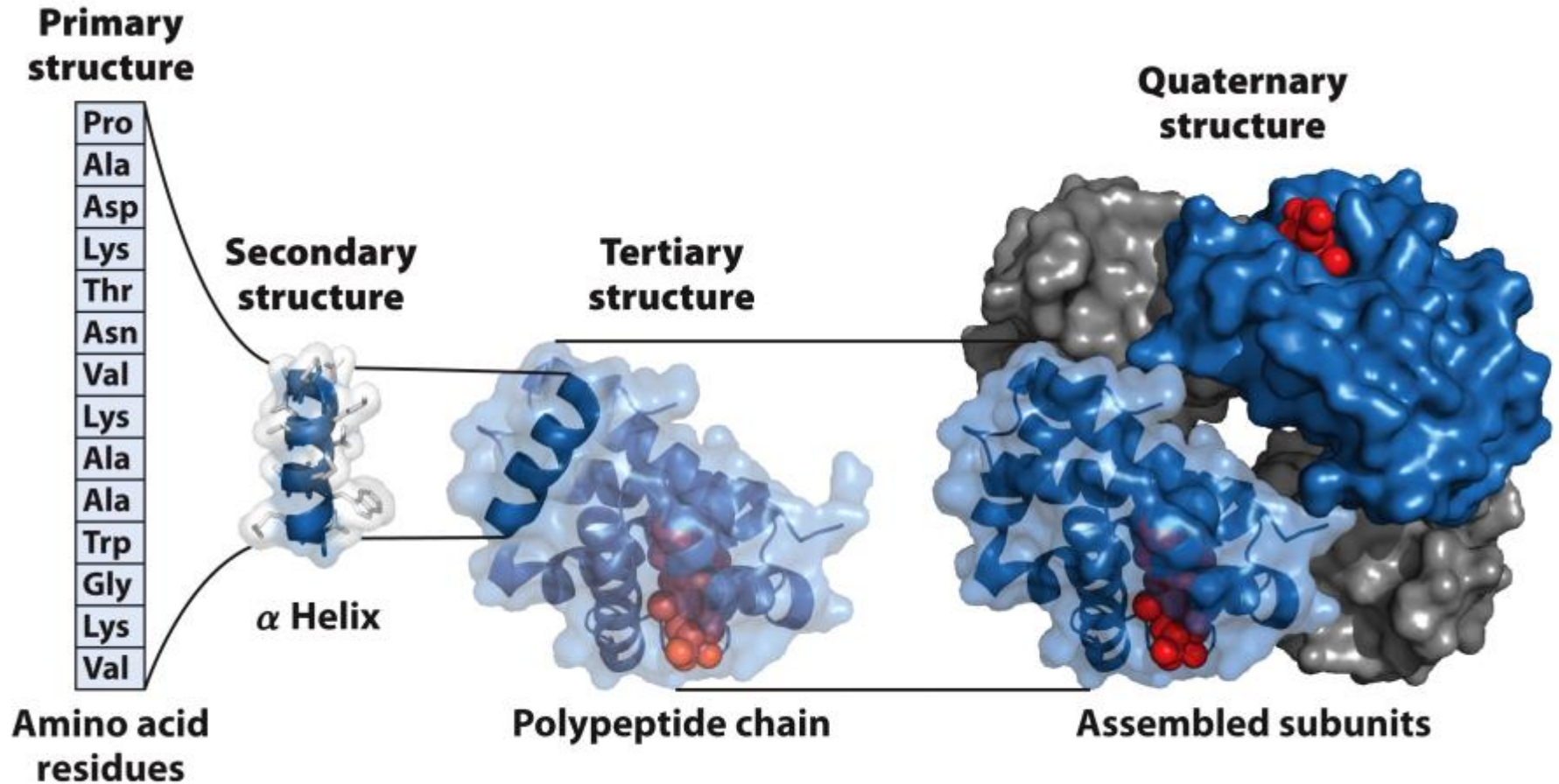
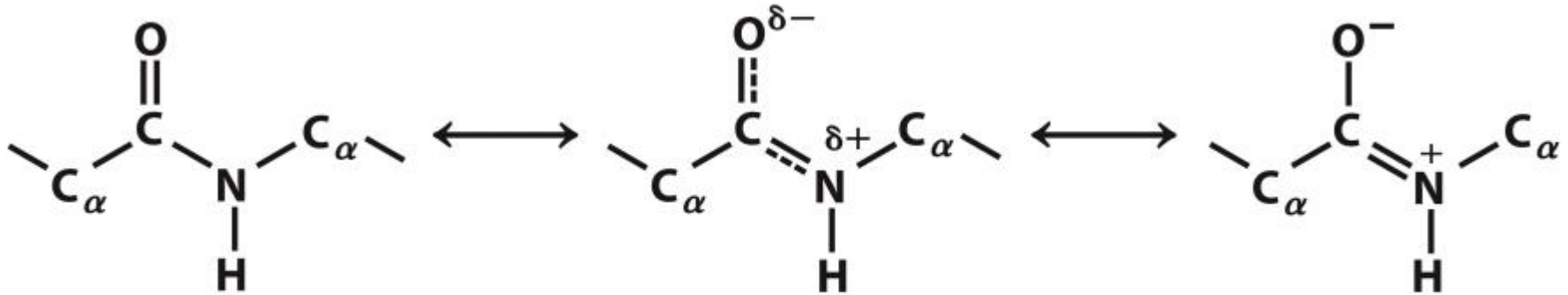


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Structure of the Peptide Bond

- 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 to esters, for example
 - to be quite **rigid** and nearly **planar**
 - to exhibit a large dipole moment in the favored trans configuration

Resonance in the Peptide Bond



The carbonyl oxygen has a partial negative charge and the amide nitrogen a partial positive charge, setting up a small electric dipole. Virtually all peptide bonds in proteins occur in this trans configuration; an exception is noted in Figure 4-7b.

Figure 4-2a
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The Rigid Peptide Plane and the Partially Free Rotations

- Rotation around the peptide bond is not permitted
- Rotation around bonds connected to the alpha 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 polypeptide is made up of a series of planes linked at α carbons

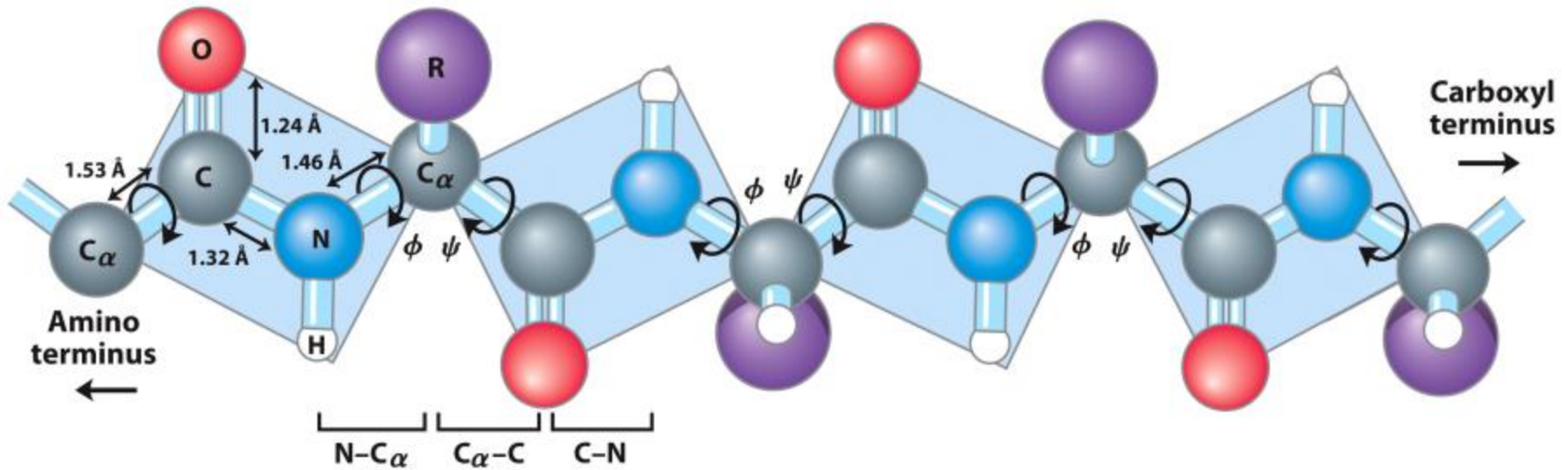


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Distribution of ϕ and ψ Dihedral Angles

- 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

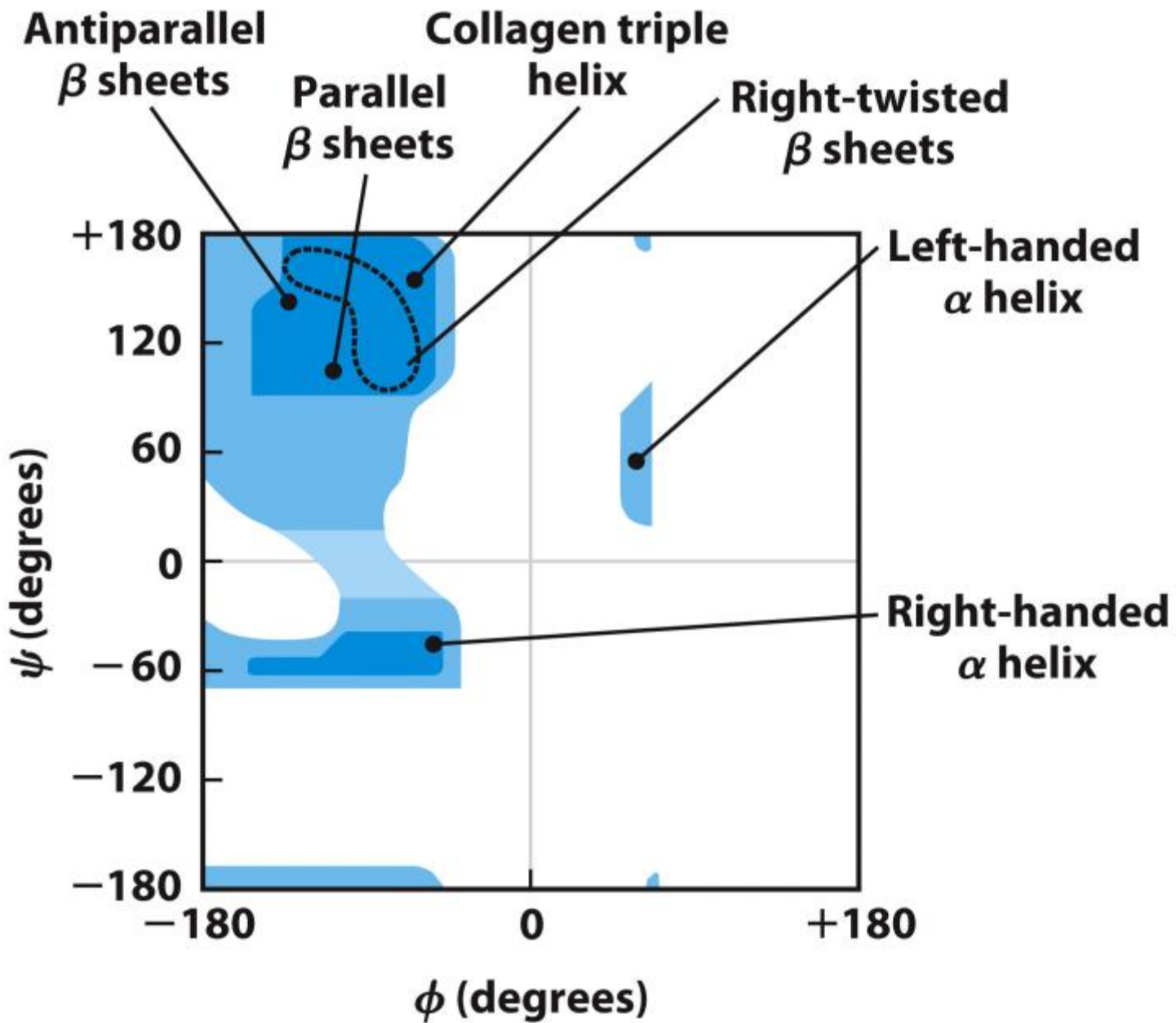


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Ramachandran Plot

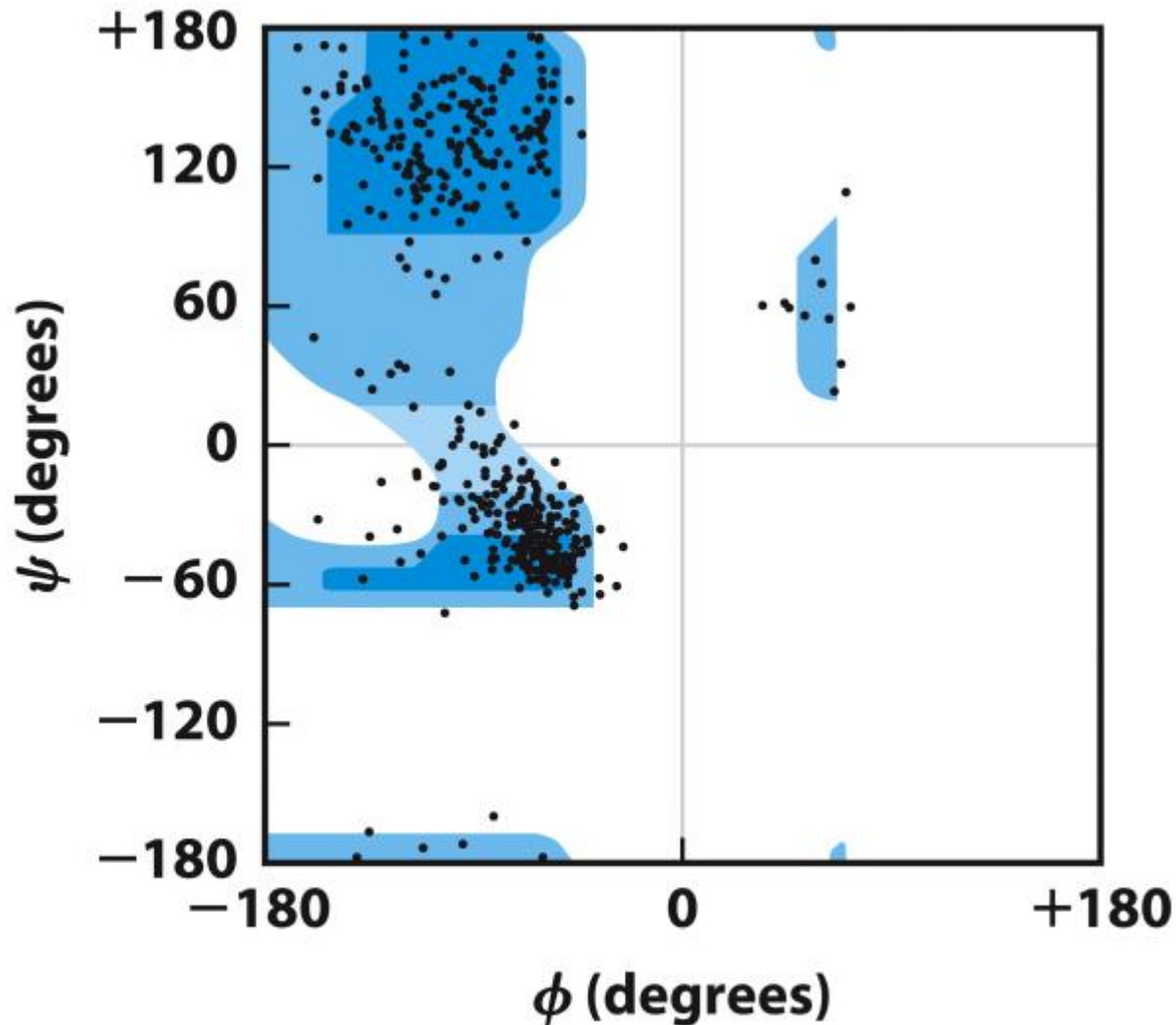


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Secondary Structures

- 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

TABLE 4-1**Idealized ϕ and ψ Angles for Common Secondary Structures in Proteins**

Structure	ϕ	ψ
α Helix	-57°	-47°
β Conformation		
Antiparallel	-139°	$+135^\circ$
Parallel	-119°	$+113^\circ$
Collagen triple helix	-51°	$+153^\circ$
β Turn type I		
i + 1*	-60°	-30°
i + 2*	-90°	0°
β Turn type II		
i + 1	-60°	$+120^\circ$
i + 2	$+80^\circ$	0°

Note: In real proteins, the dihedral angles often vary somewhat from these idealized values.

*The i+1 and i+2 angles are those for the second and third amino acid residues in the β turn, respectively.

Table 4-1

The α Helix

- Helical backbone is held together by hydrogen bonds between the backbone amides of an n and $n+4$ amino acids
- **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

What is a right-handed helix?

**Left-handed
helix**



**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
- Residues 1 and 8 align nicely on top of each other
 - What kind of sequence gives an α helix with one hydrophobic face?

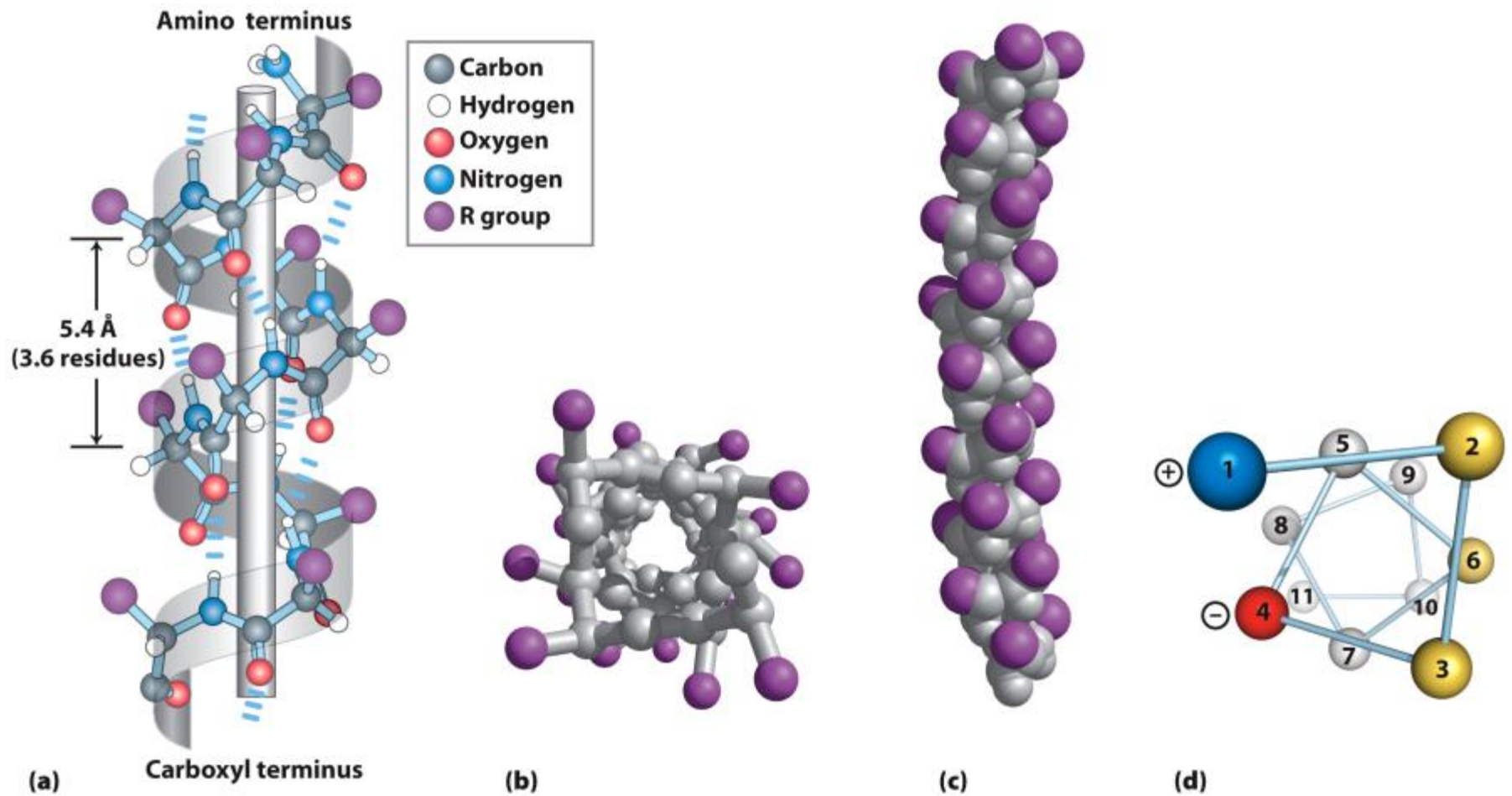


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Sequence affects helix stability

- 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 _{α} bond is impossible
- **Gly** acts as a helix breaker because the tiny R-group supports other conformations
- Attractive or repulsive interactions between side chains 3–4 amino acids apart will affect formation

TABLE 4-2 Propensity of Amino Acid Residues to Take Up an α -Helical Conformation

Amino acid	$\Delta\Delta G^\circ$ (kJ/mol)*	Amino acid	$\Delta\Delta G^\circ$ (kJ/mol)*
Ala	0	Leu	0.79
Arg	0.3	Lys	0.63
Asn	3	Met	0.88
Asp	2.5	Phe	2.0
Cys	3	Pro	>4
Gln	1.3	Ser	2.2
Glu	1.4	Thr	2.4
Gly	4.6	Tyr	2.0
His	2.6	Trp	2.0
Ile	1.4	Val	2.1

Sources: Data (except proline) from Bryson, J.W., Betz, S.F., Lu, H.S., Suich, D.J., Zhou, H.X., O'Neil, K.T., & DeGrado, W.F. (1995) Protein design: a hierarchic approach. *Science* 270, 935. Proline data from Myers, J.K., Pace, C.N., & Scholtz, J.M. (1997) Helix propensities are identical in proteins and peptides. *Biochemistry* 36, 10,926.

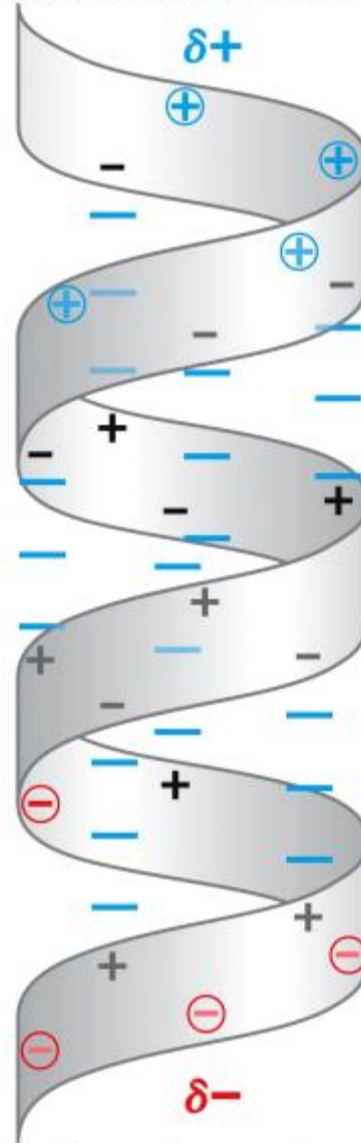
* $\Delta\Delta G^\circ$ is the difference in free-energy change, relative to that for alanine, required for the amino acid residue to take up the α -helical conformation. Larger numbers reflect greater difficulty taking up the α -helical structure. Data are a composite derived from multiple experiments and experimental systems.

Table 4-2

The Helix Dipole

- Recall that the peptide bond has a strong dipole moment
 - Carbonyl O negative
 - Amide H positive
- All peptide bonds in the α helix have a similar orientation
- The α helix has a large macroscopic dipole moment
- Negatively charged residues often occur near the positive end of the helix dipole

Amino terminus



Carboxyl terminus

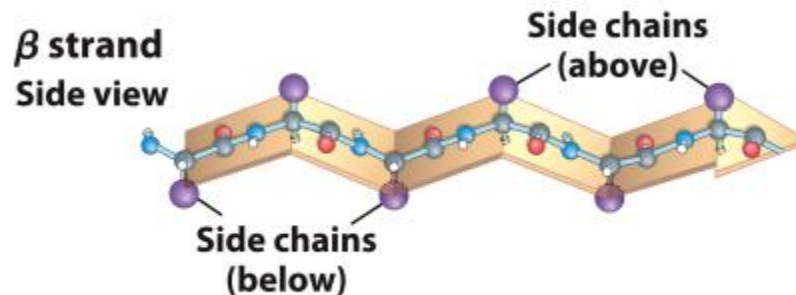
Figure 4-5

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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 backbone is held together by hydrogen bonds between the backbone amides in different strands
- Side chains protrude from the sheet alternating in up and down direction



Parallel and Antiparallel β Sheets

- Parallel or antiparallel orientation of two chains within a sheet are possible
- In parallel β sheets the H-bonded strands run in the same direction
 - Resulting in bent H-bonds (weaker)
- In antiparallel β sheets the H-bonded strands run in opposite directions
 - Resulting in linear H-bonds (stronger)

Antiparallel β sheet

Top view

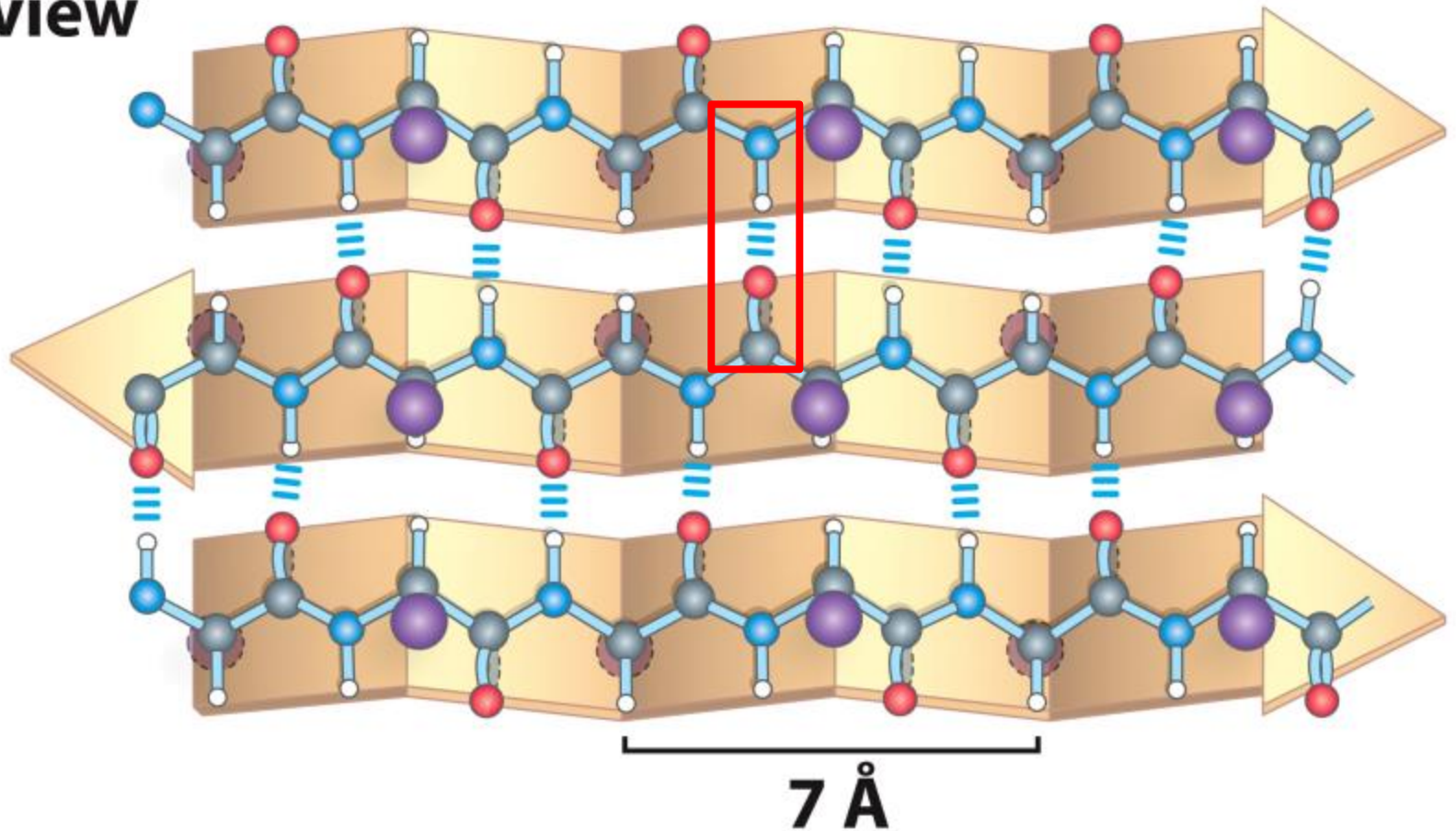


Figure 4-6b

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Parallel β sheet

Top view

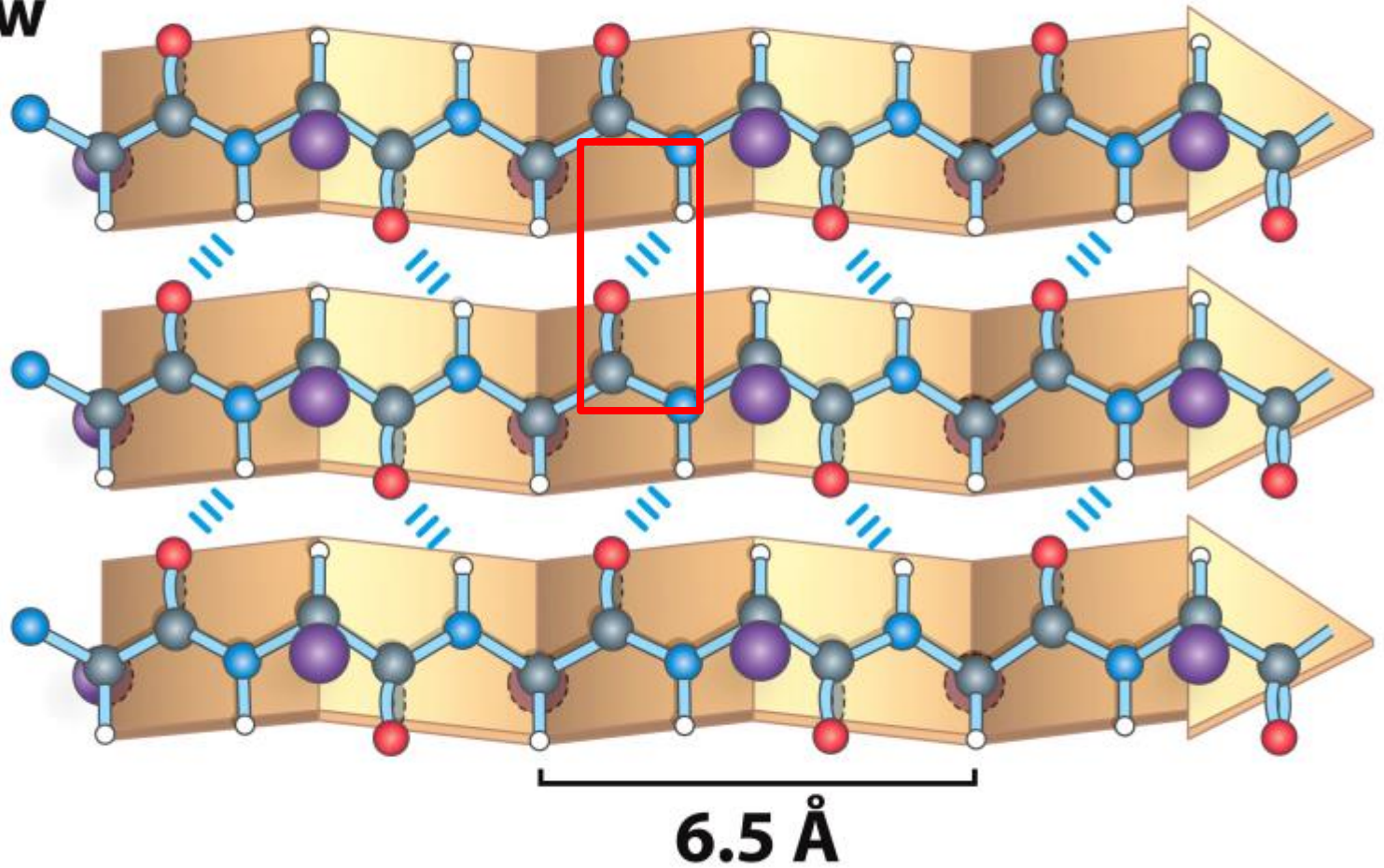
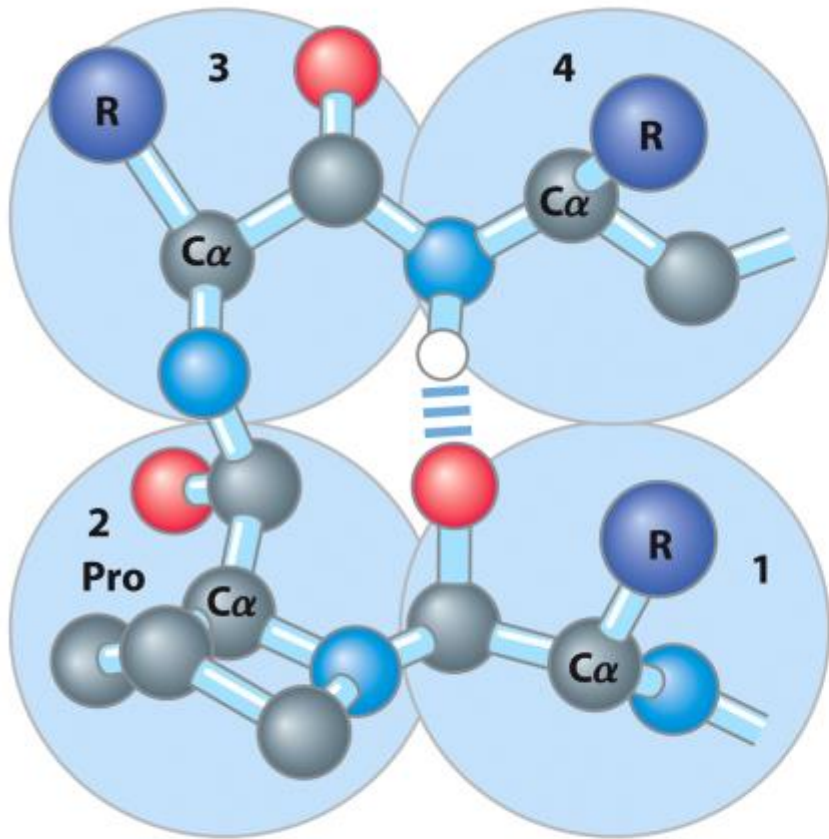


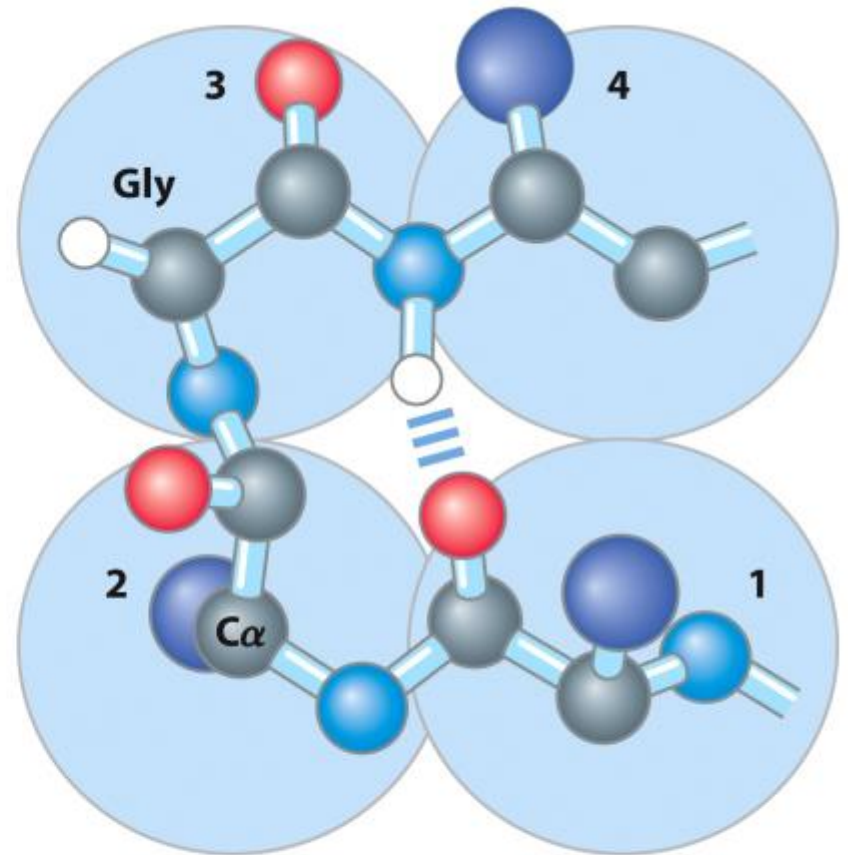
Figure 4-6c
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β Turns

- 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



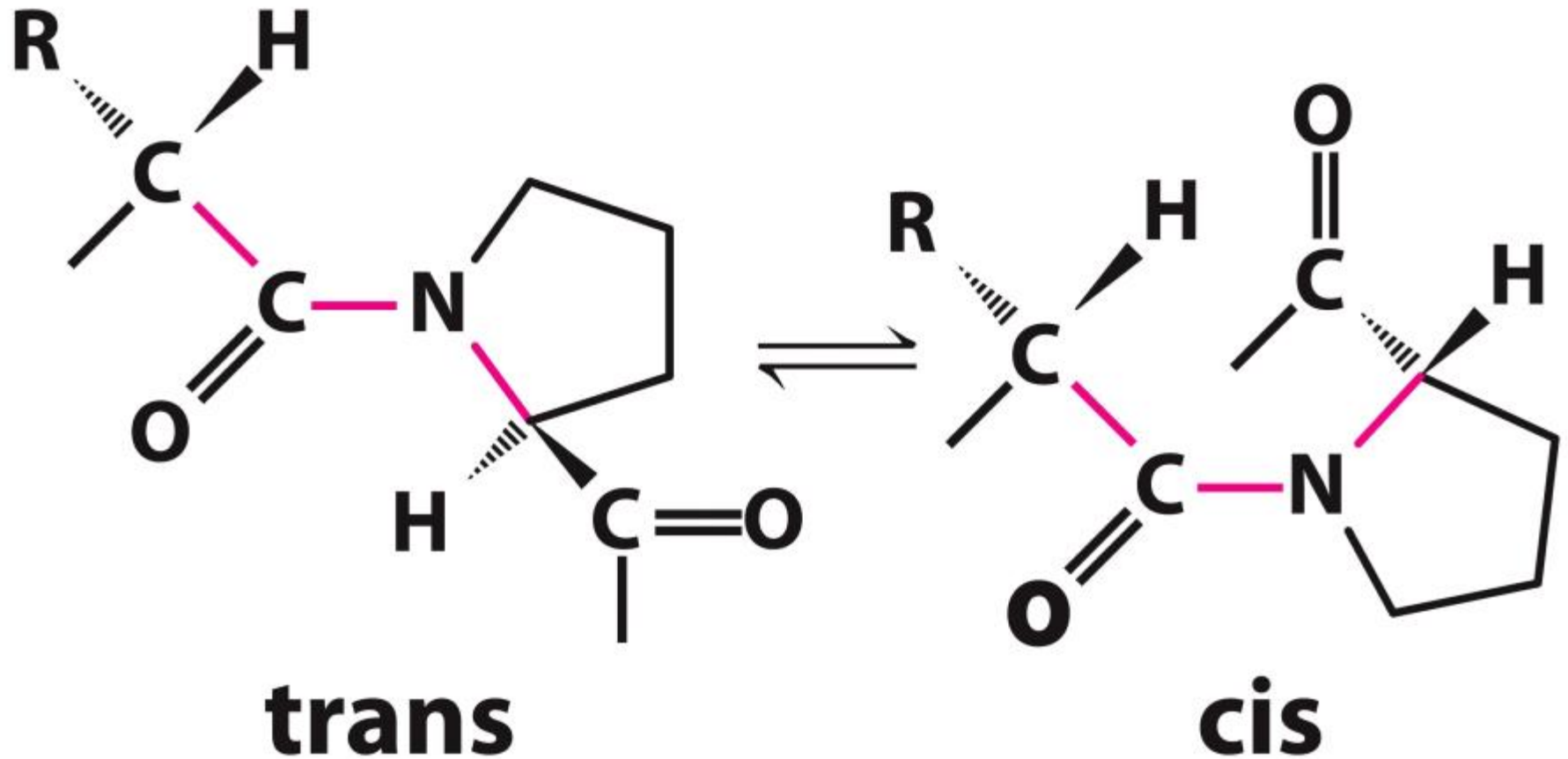
Type I β turn



Type II β turn

Proline Isomers

- Most peptide bonds **not involving proline** are in the trans configuration (>99.95%)
- For peptide bonds **involving proline**, about 6% are in the cis configuration. Most of this 6% involve β -turns
- Proline isomerization is catalyzed by proline isomerases



Proline isomers

Figure 4-8

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Circular Dichroism (CD) Analysis

- CD measures the molar absorption difference $\Delta\varepsilon$ of left- and right-circularly polarized light: $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$
- Chromophores in the chiral environment produce characteristic signals
- CD signals from peptide bonds depend on the chain conformation

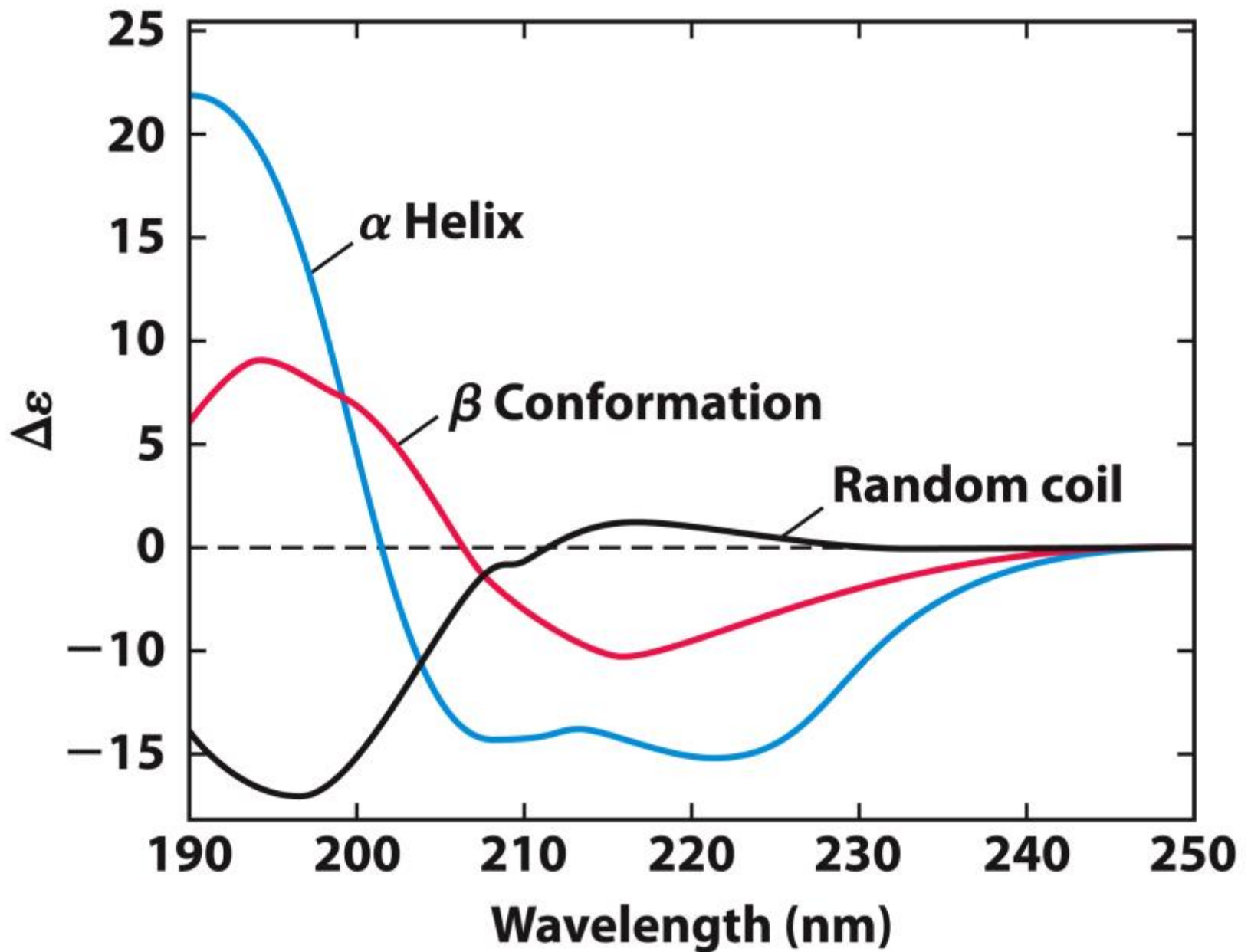


Figure 4-10

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Protein Tertiary Structure

- Tertiary structure refers to the overall spatial arrangement of atoms in a protein
- Stabilized by numerous weak interactions between amino acid side chains.
 - Largely hydrophobic and polar interactions
 - Can be stabilized by disulfide bonds
- Interacting amino acids are not necessarily next to each other in the primary sequence.
- Two major classes
 - Fibrous and globular (water or lipid soluble)

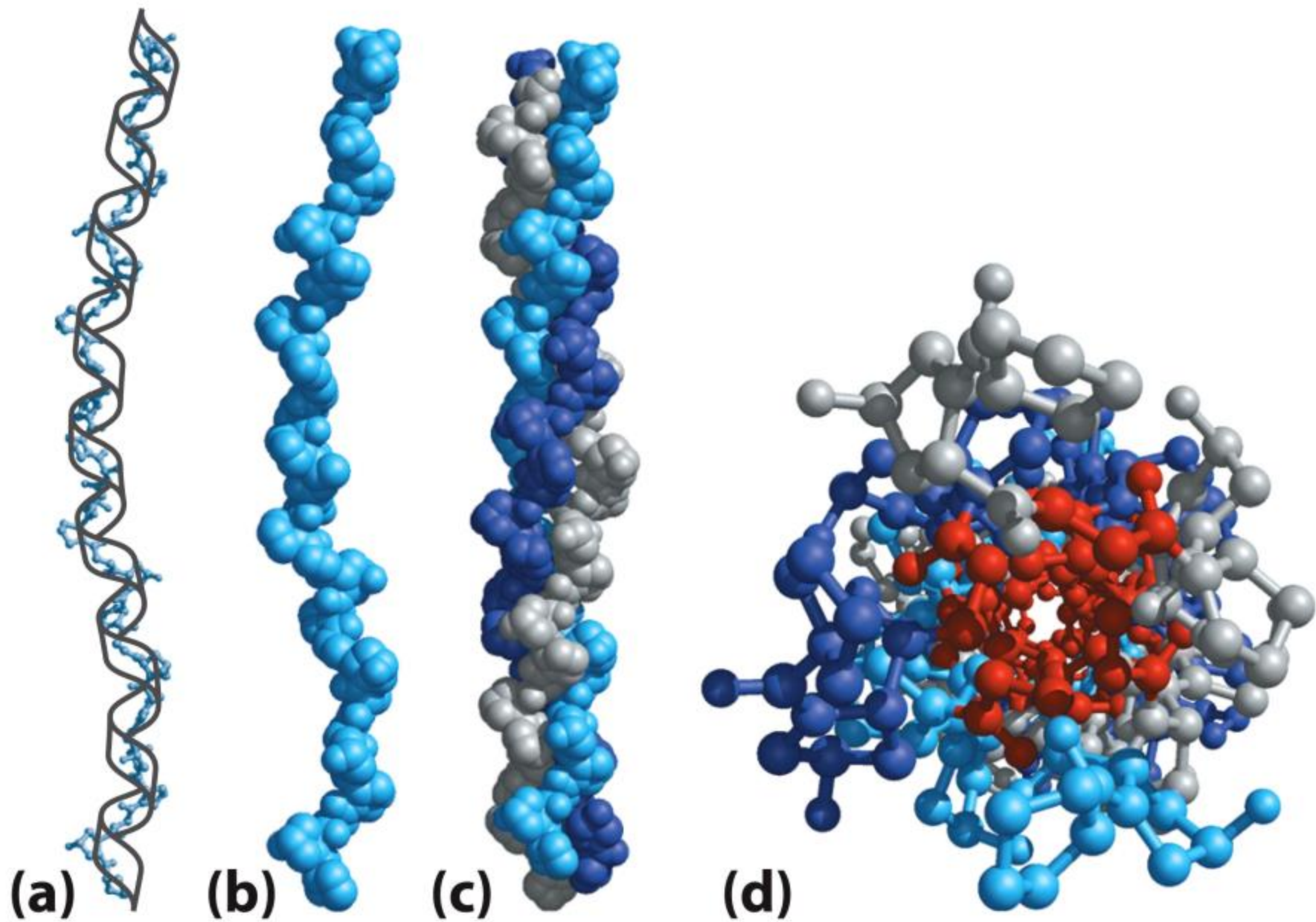


Figure 4-12

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Water-Soluble Globular Proteins

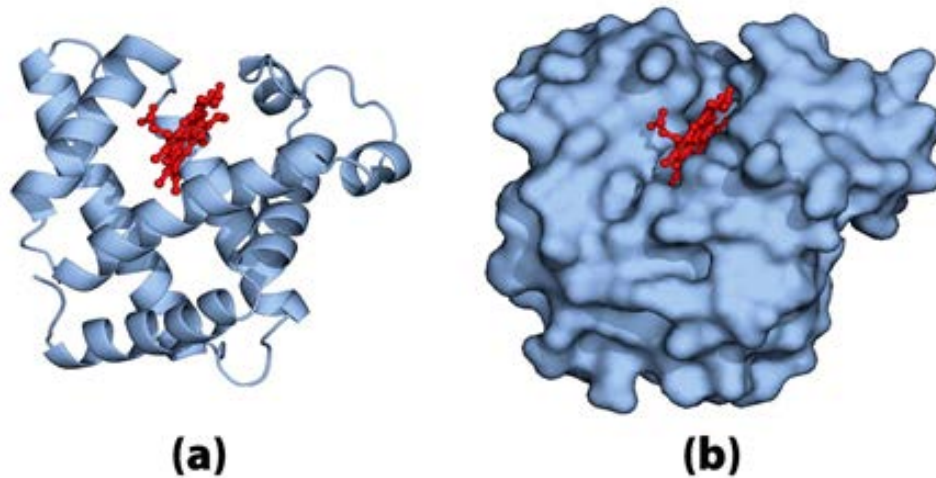


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Fibrous Proteins: From Structure to Function


TABLE 4-3 Secondary Structures and Properties of Some Fibrous Proteins

Structure	Characteristics	Examples of occurrence
α Helix, cross-linked by disulfide bonds	Tough, insoluble protective structures of varying hardness and flexibility	α -Keratin of hair, feathers, nails
β Conformation	Soft, flexible filaments	Silk fibroin
Collagen triple helix	High tensile strength, without stretch	Collagen of tendons, bone matrix

Table 4-3
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Structure of α -Keratin in Hair

Keratin α helix — 

Two-chain coiled coil — 

Protofilament {  } **20–30 Å**

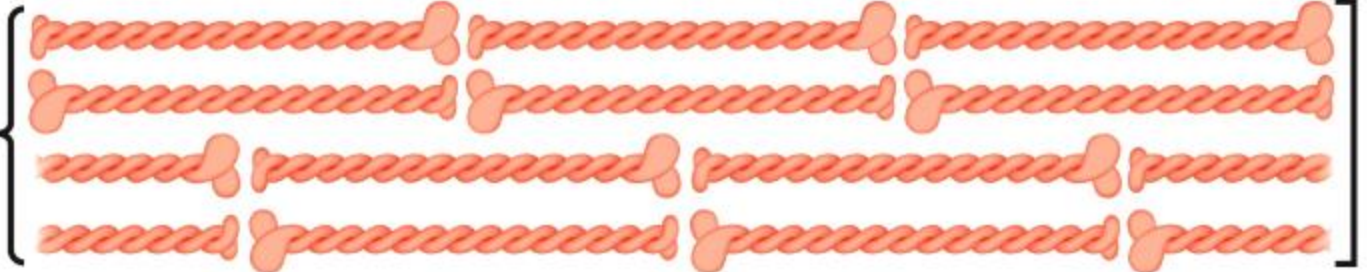
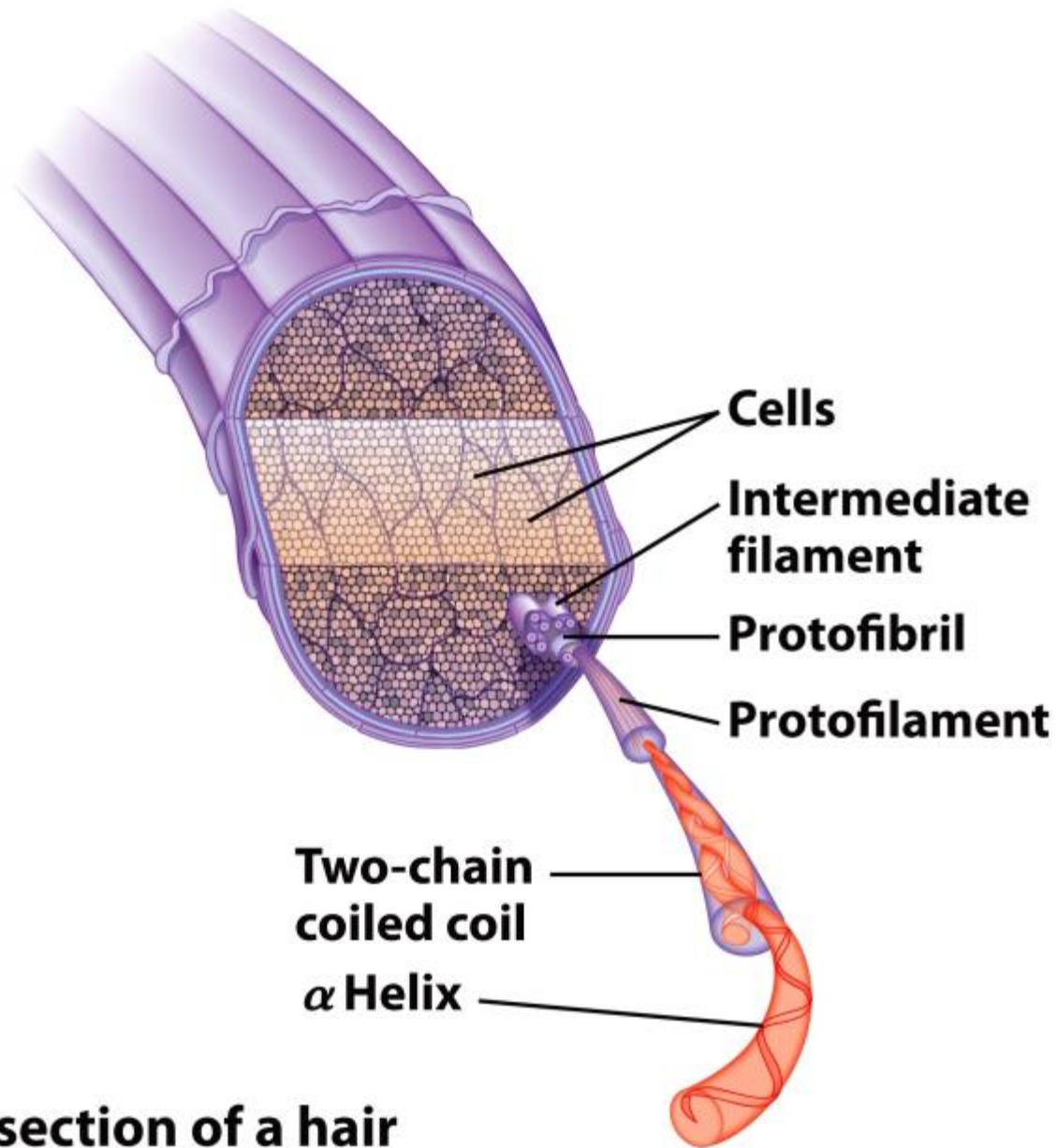
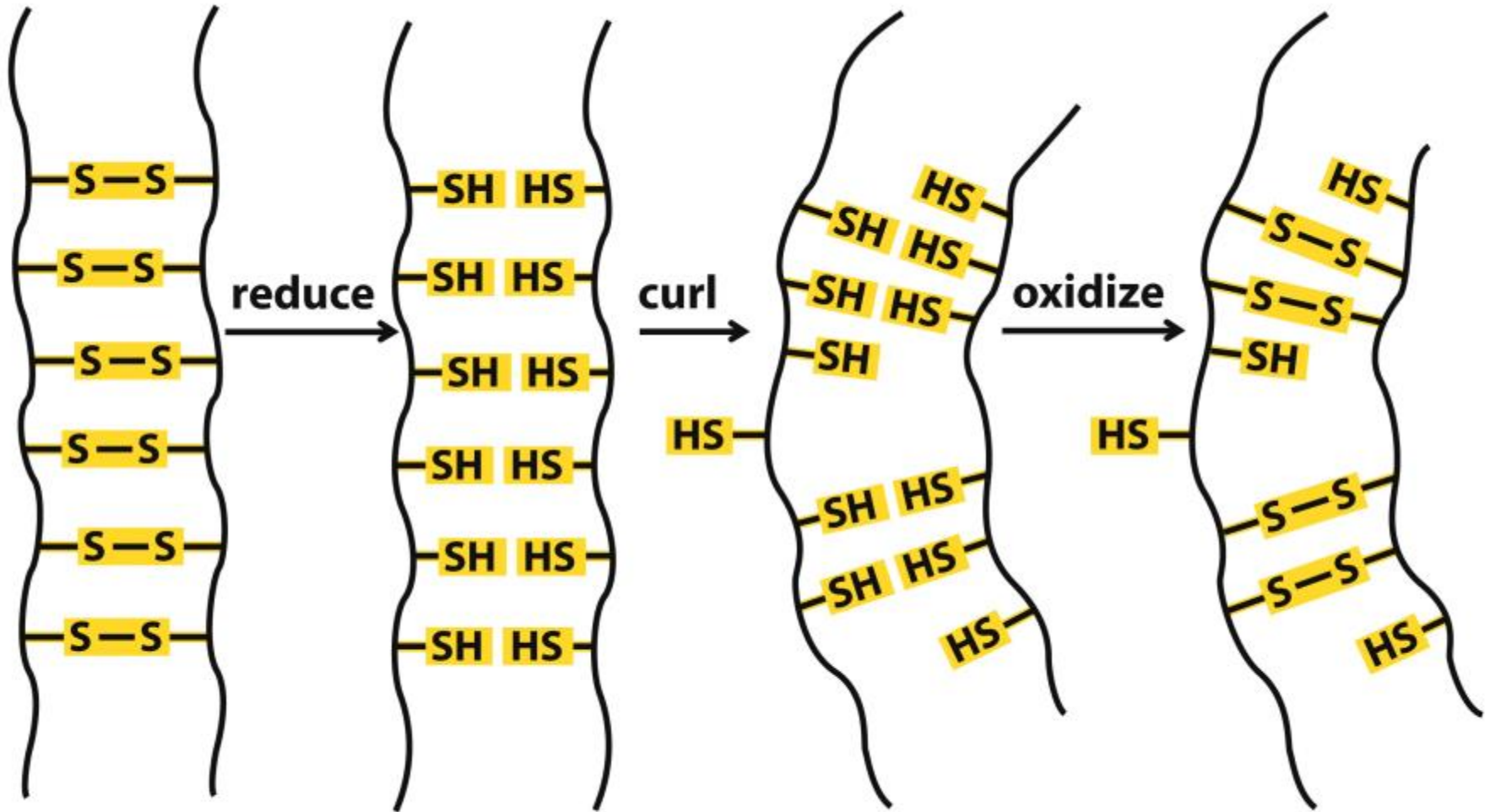
Protofibril {  }

Figure 4-11a



Cross section of a hair

Chemistry of Permanent Waving



Box 4-2

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Structure of Collagen

- Collagen is an important constituent of **connective tissue**: tendons, cartilage, bones, cornea of the eye
- Each collagen chain is a long Gly- and Pro-rich **left-handed helix**
- Three collagen chains intertwine into a **right-handed superhelical triple helix**
- The triple helix has higher tensile strength than a steel wire of equal cross section
- Many triple-helices assemble into a collagen fibril

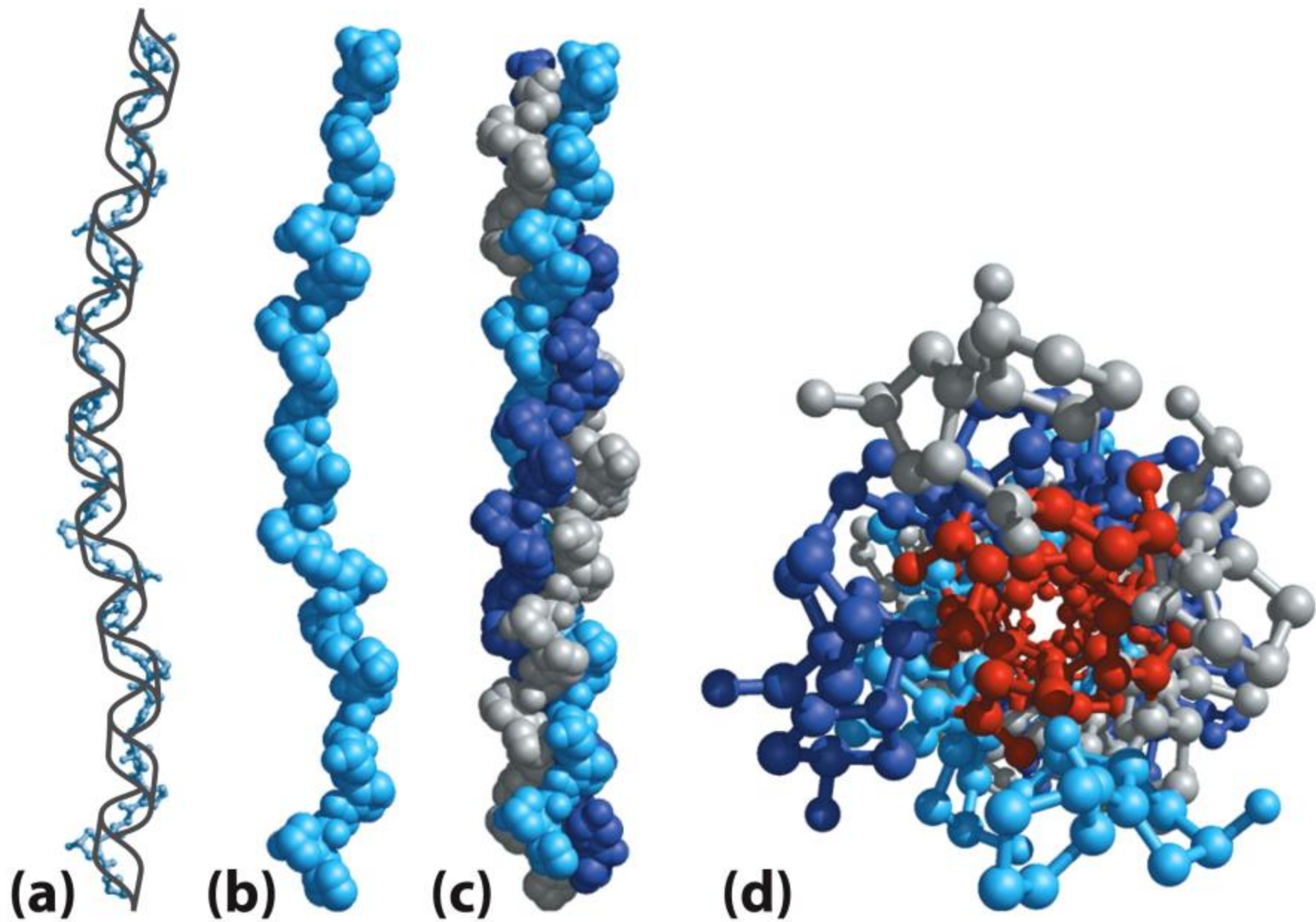


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Collagen Fibrils

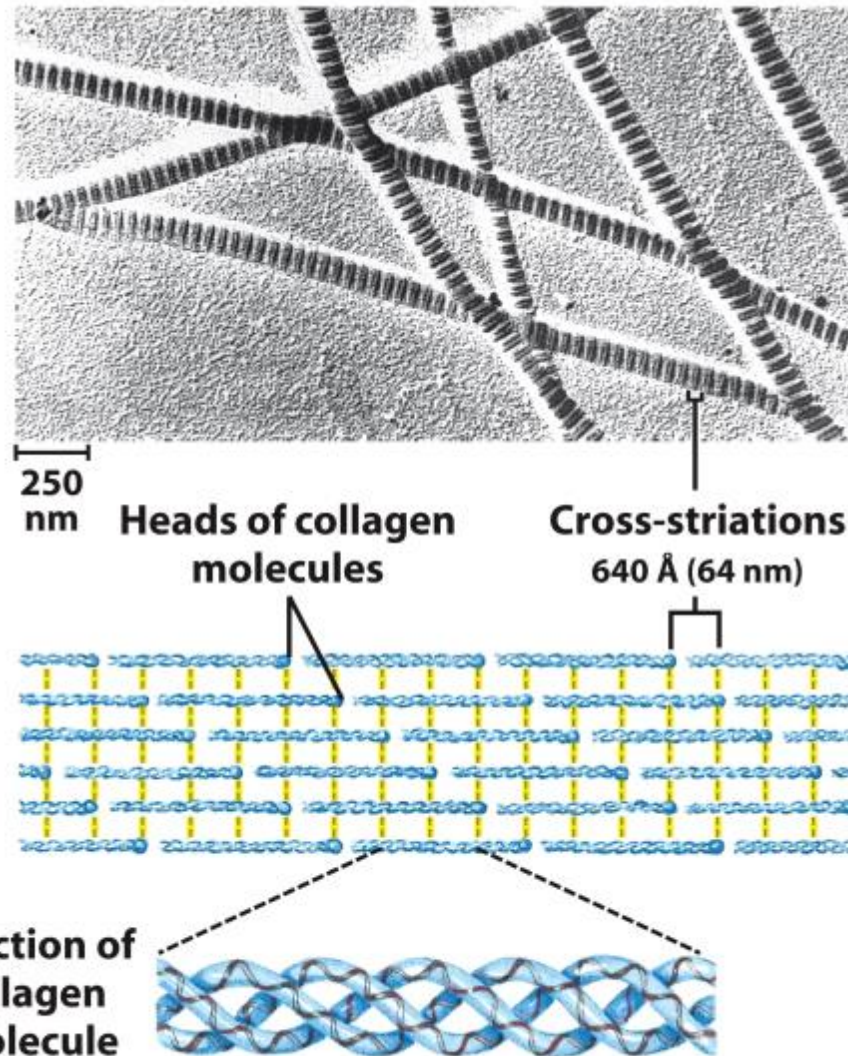
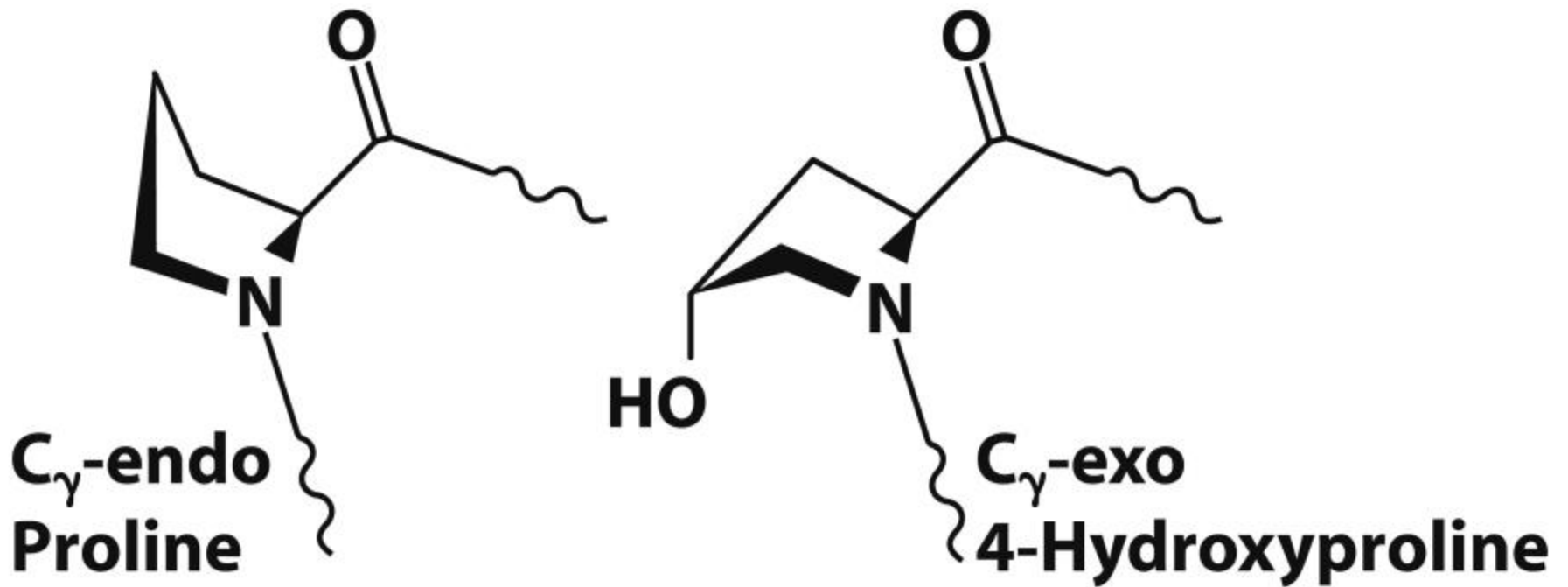


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4-Hydroxyproline in Collagen

- Forces the proline ring into a favorable pucker
- Offer more hydrogen bonds between the three strands of collagen
- The **post-translational processing** is catalyzed by prolyl hydroxylase and requires α -ketoglutarate, molecular oxygen, and **ascorbate (vitamin C)**

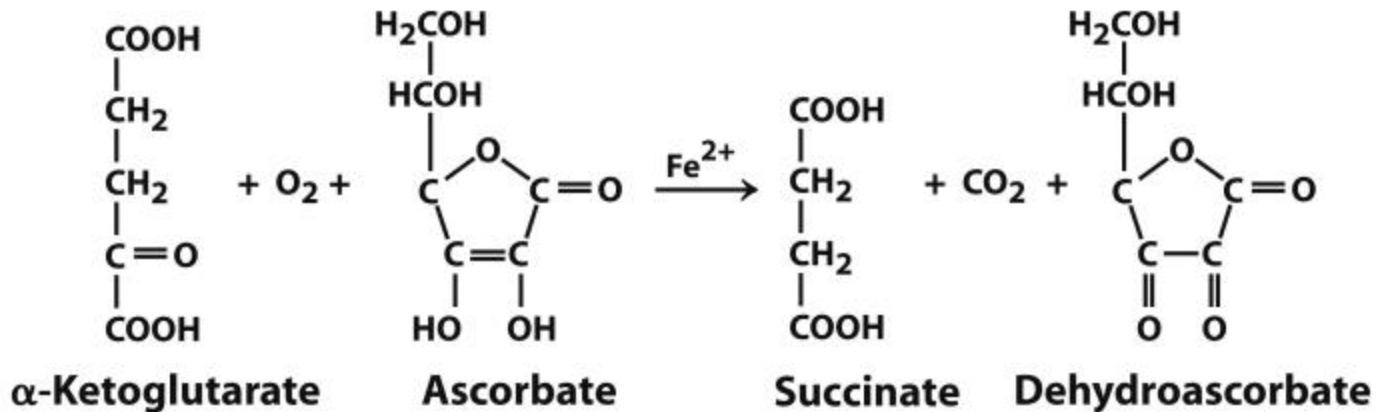
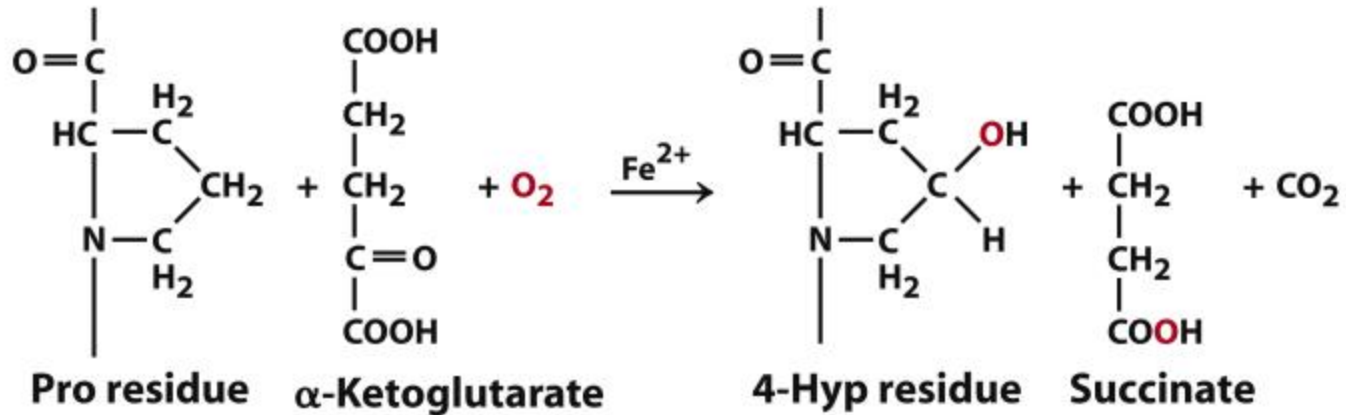


Box 4-3 figure 1

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Vitamin C in prolyl 4-hydroxylase restores Fe²⁺ state



Box 4-3 figure 2

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Silk Fibroin

- **Fibroin** is the main protein in silk from moths and spiders
- Antiparallel β sheet structure
- Small side chains (**Ala** and **Gly**) allow the close packing of sheets
- Structure is stabilized by
 - hydrogen bonding within sheets
 - London dispersion interactions between sheets

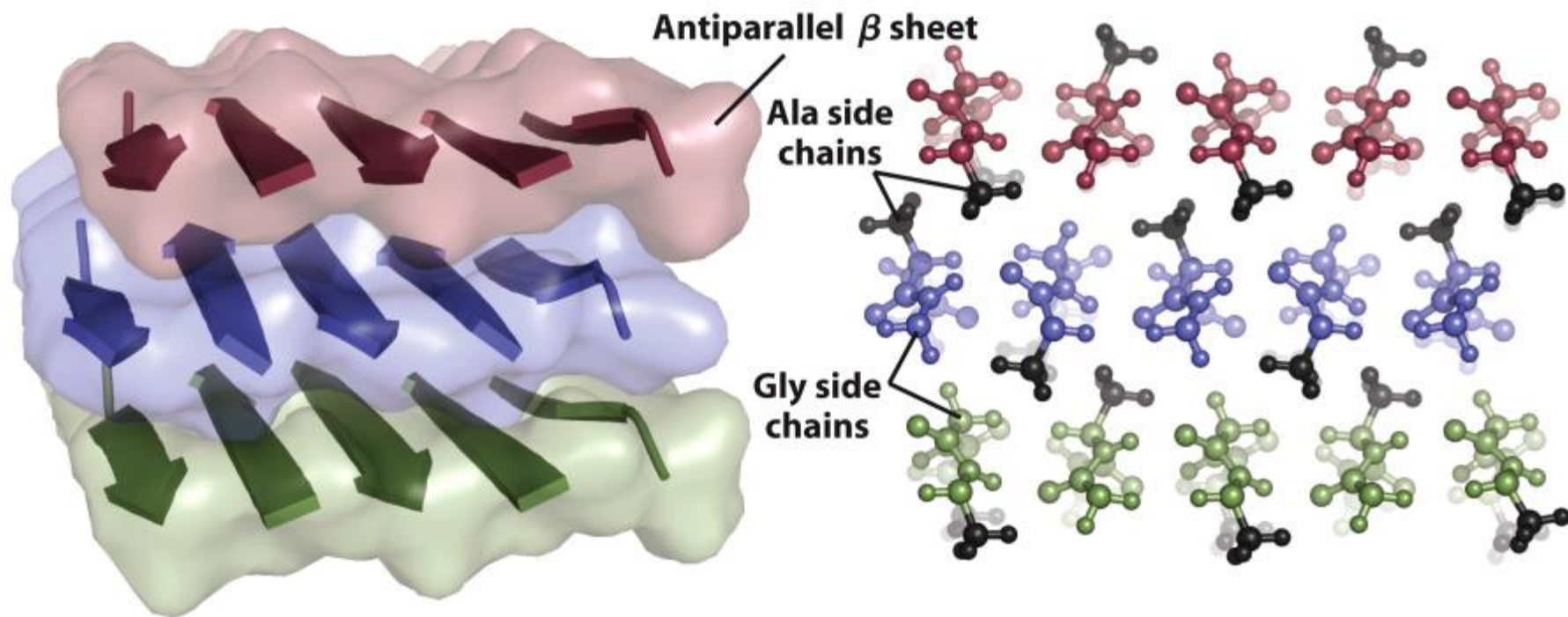


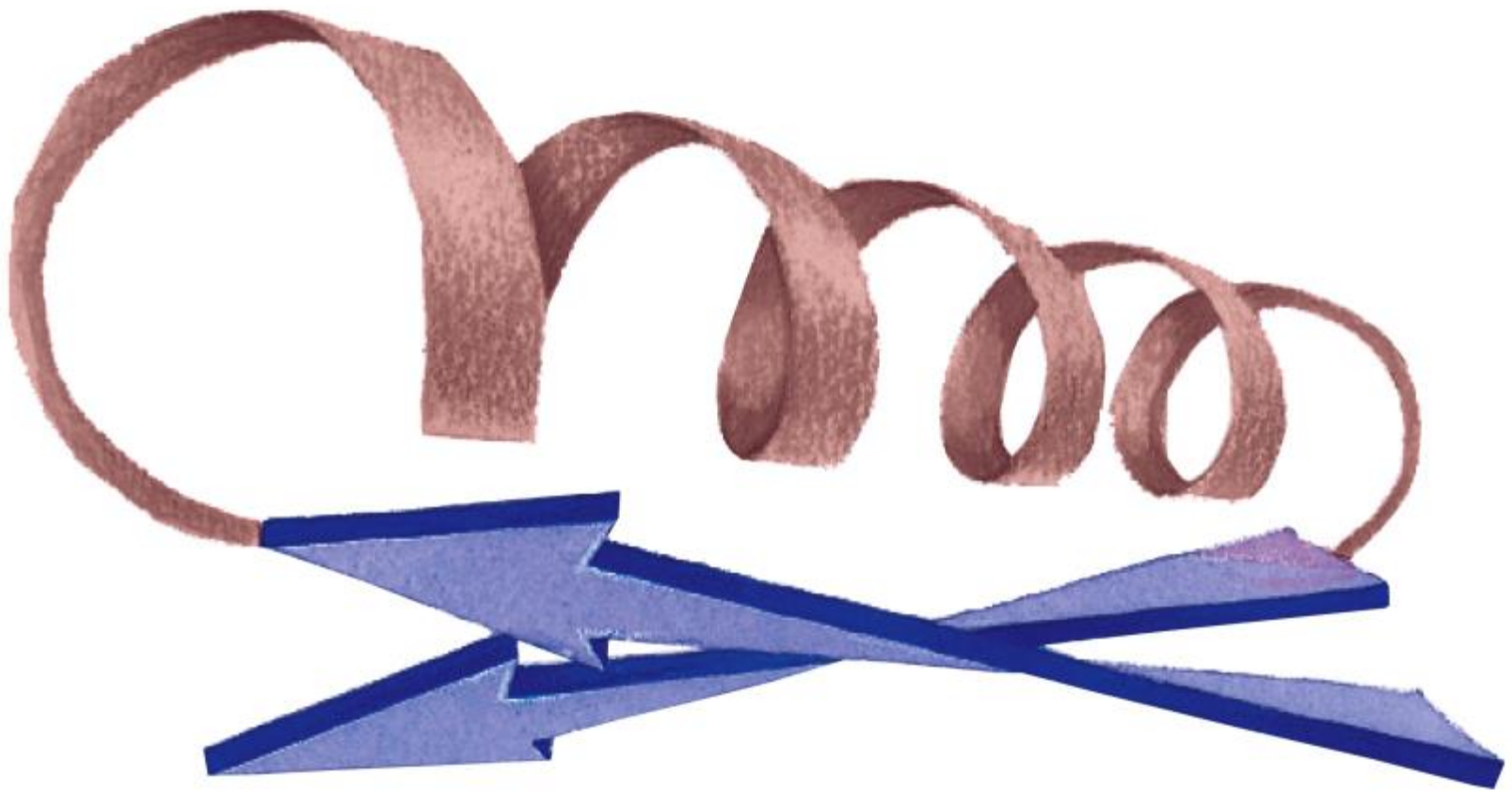
Figure 4-14a
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Spider Silk

- Used for webs, egg sacks, and wrapping the prey
- Extremely strong material
 - stronger than steel
 - can stretch a lot before breaking
- A composite material
 - crystalline parts (fibroin-rich)
 - rubber-like stretchy parts

Motifs (folds)

- Specific arrangement of several secondary structure elements
 - All alpha-helix
 - All beta-sheet
 - Both
- Motifs can be found as reoccurring structures in numerous proteins
- Proteins are made of different motifs folded together



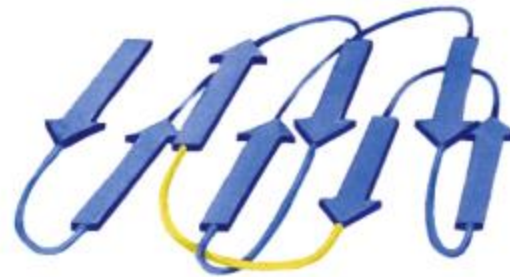
β - α - β Loop

Figure 4-18a

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(a) Typical connections in an all- β motif



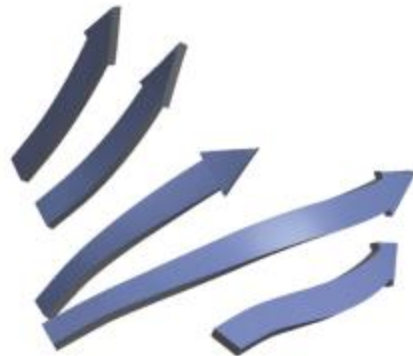
Crossover connection (rarely observed)



(b) Right-handed connection between β strands



Left-handed connection between β strands (very rare)



(c) Twisted β sheet

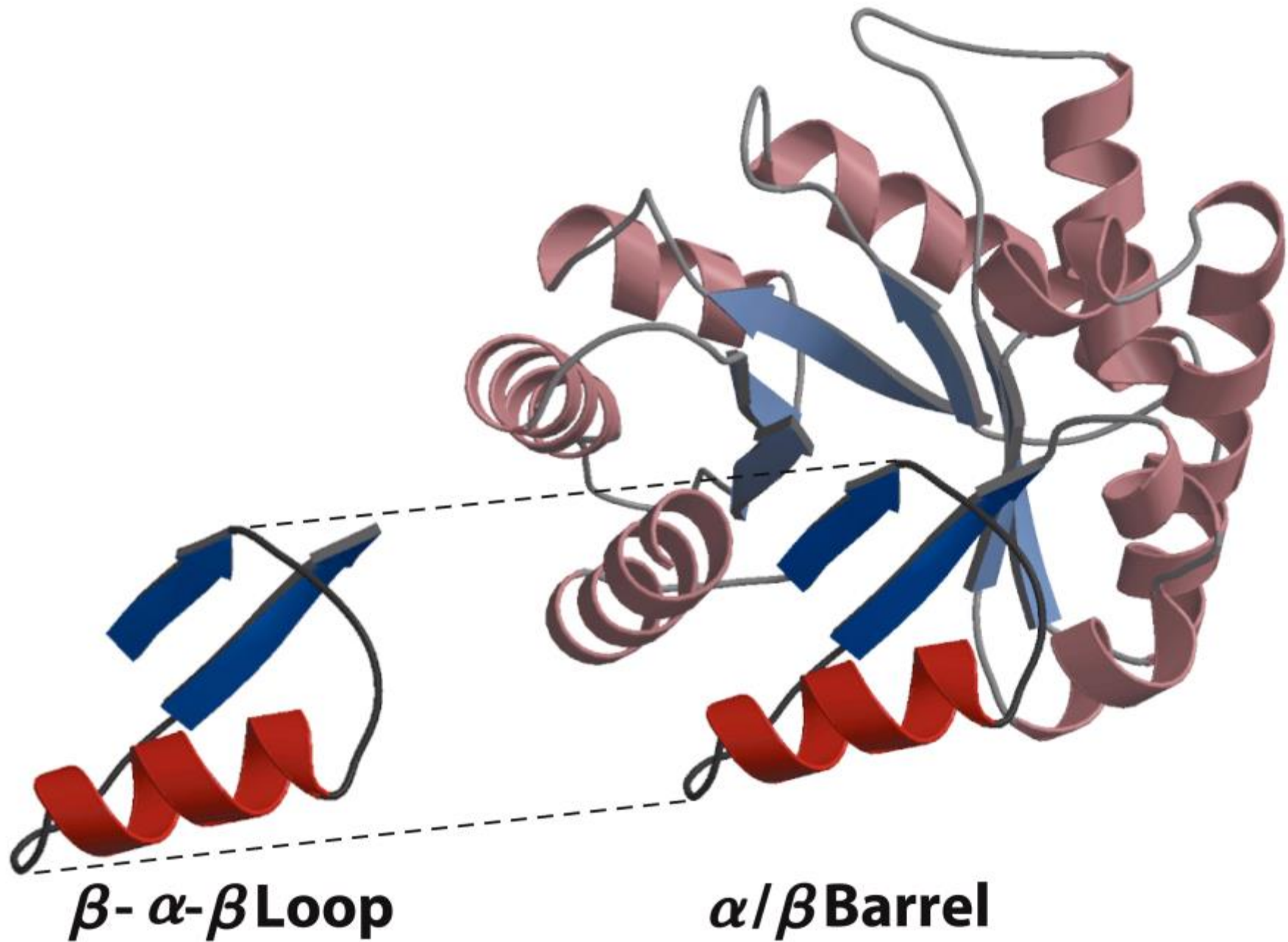


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Quaternary Structure

- **Quaternary structure** is formed by the assembly of individual polypeptides into a larger functional cluster

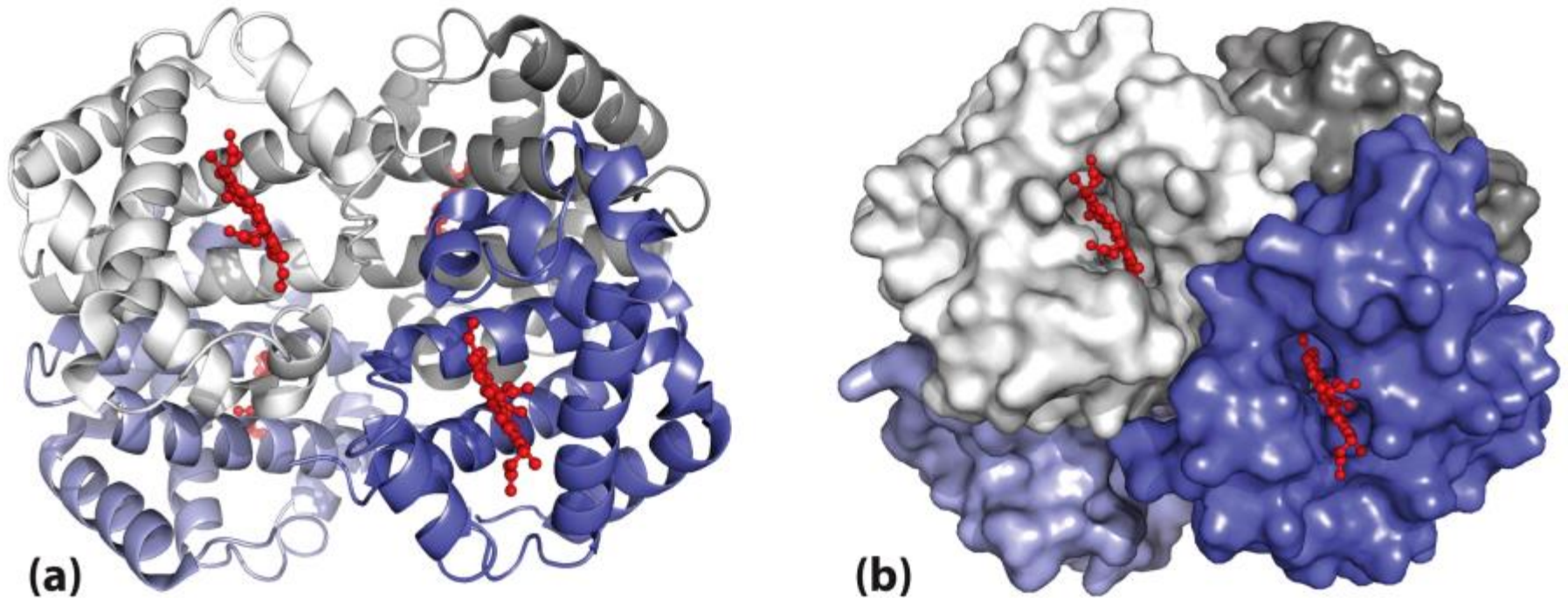


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Protein Structure Methods: X-Ray Crystallography

Steps needed

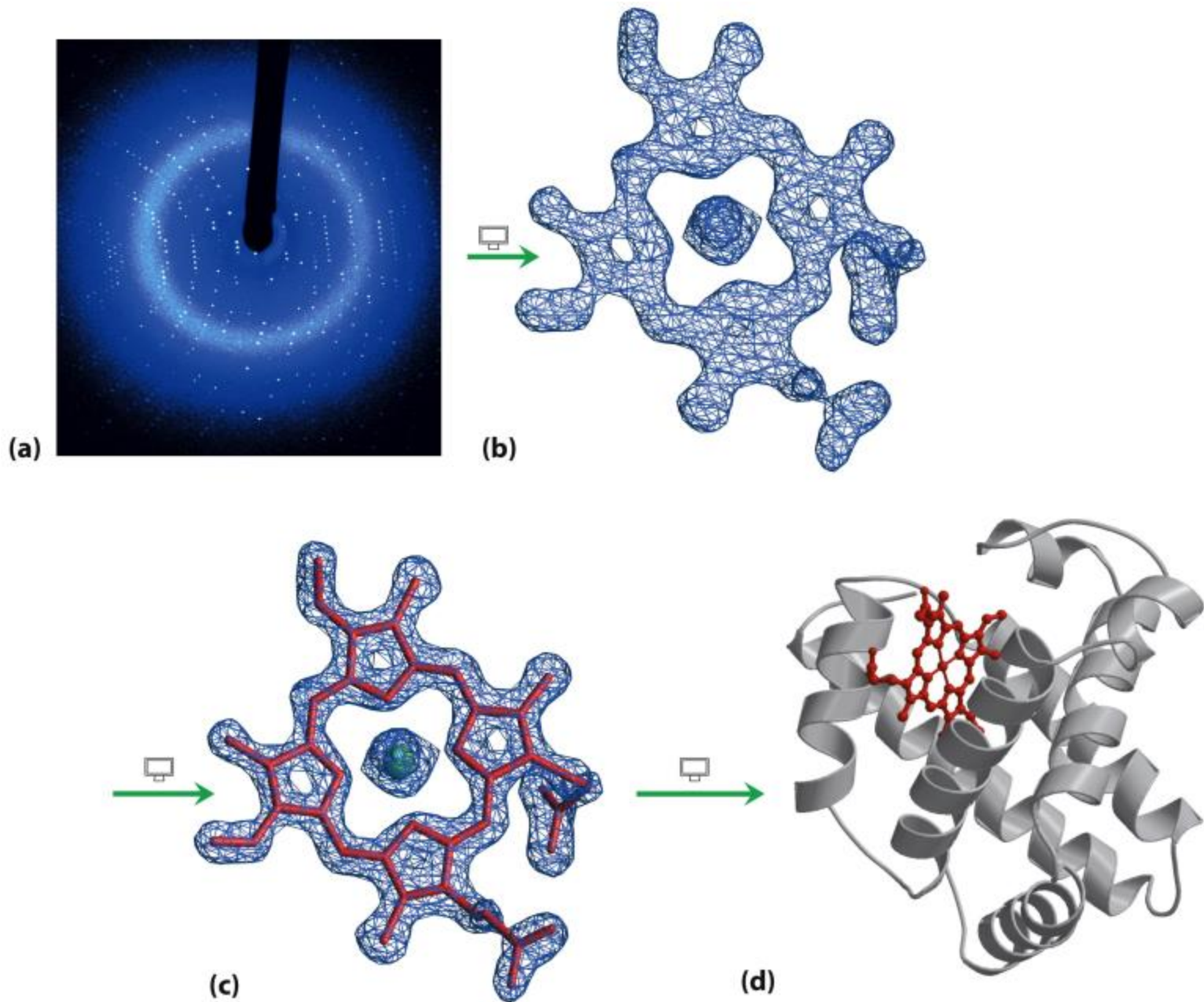
- Purify the protein
- **Crystallize the protein**
- Collect diffraction data
- Calculate electron density
- Fit residues into density

Pros

- No size limits
- Well-established

Cons

- Difficult for membrane proteins
- Cannot see hydrogens



Box 4-5 figure 1

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