

Lehninger

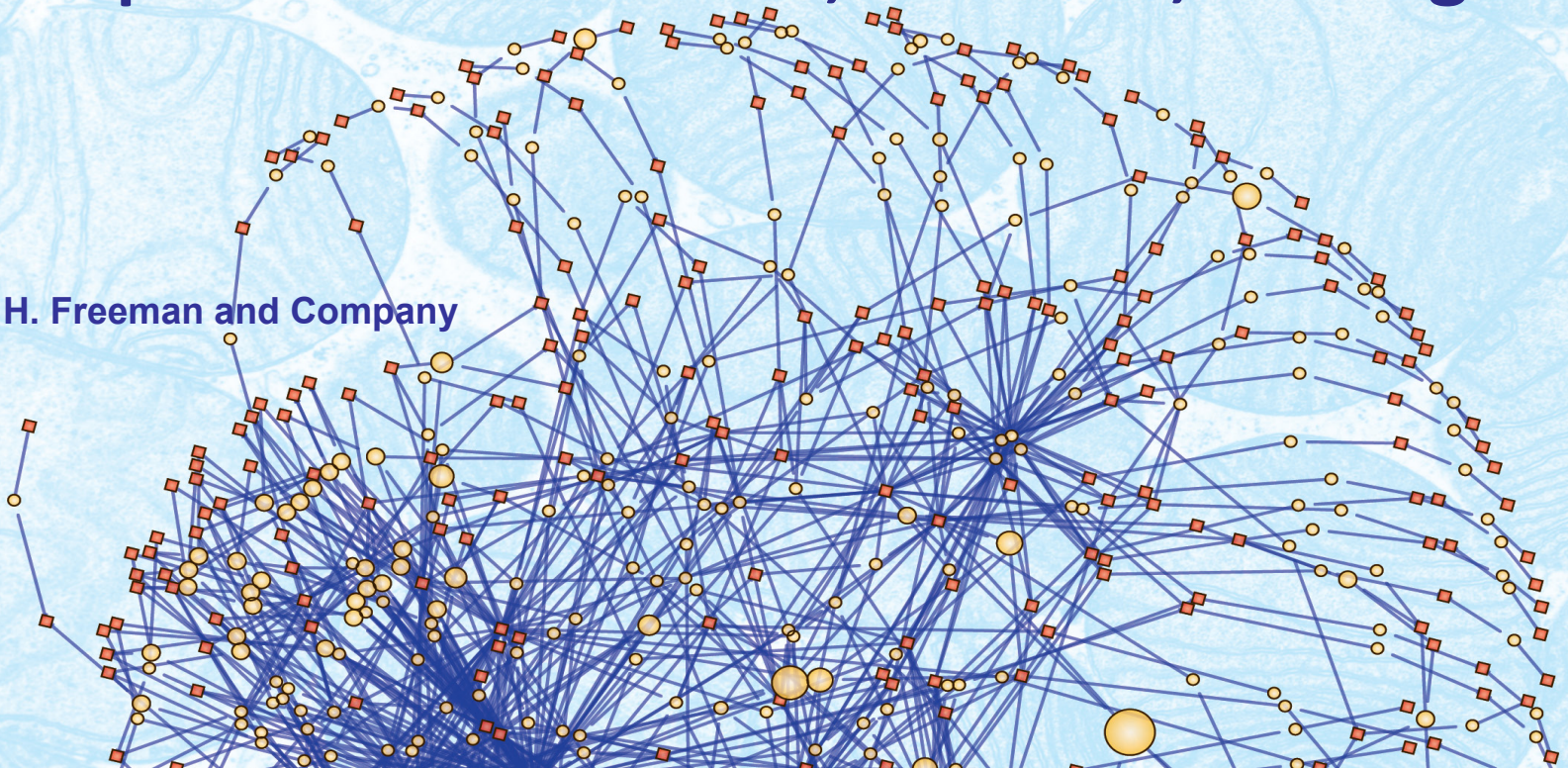
SIXTH EDITION

Principles of Biochemistry

David L. Nelson | Michael M. Cox

4 | Proteins: Structure, Function, Folding

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Structure of Proteins - General Aspects

1. aa sequence determines 3D structure
2. Protein function depends on its structure
3. An isolated protein usually exists in one or few stable structures
4. Noncovalent interactions are the most important forces stabilizing protein structure
5. There are common structural patterns in protein architecture

Structure of Proteins

- Unlike most organic polymers, protein molecules adopt a specific **three-dimensional conformation**.
- ✓ ***Conformation:** spatial arrangement of atoms in a protein (any structure that can exist without breaking covalent bonds)*
- This structure is able to fulfill a specific **biological function**
- This structure is called the **native fold**
- ✓ ***Native proteins:** proteins in any of their functional, folded conformations*
- 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

**Structure of the enzyme
chymotrypsin, a globular protein.**
A molecule of glycine (blue) is
shown for size comparison

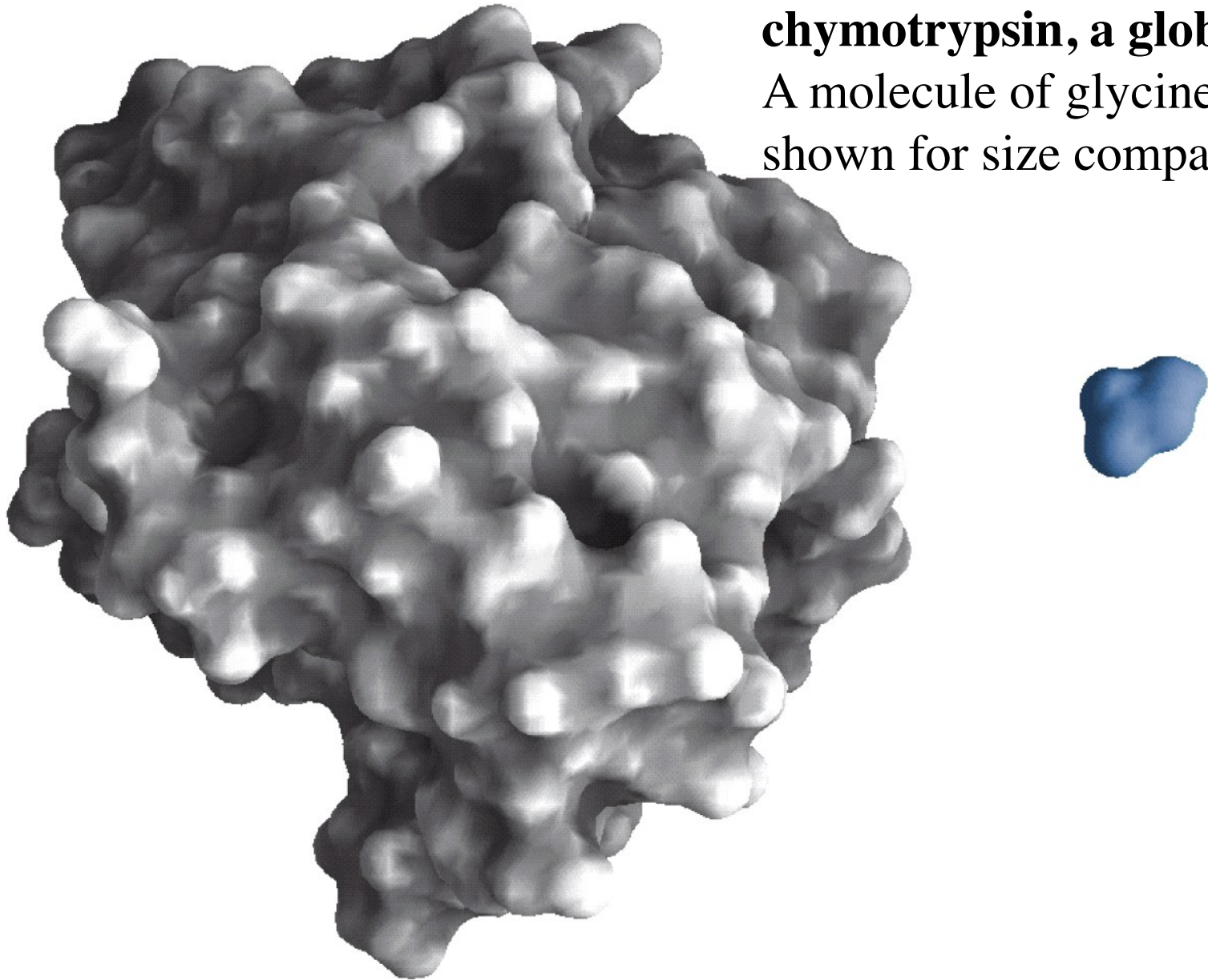


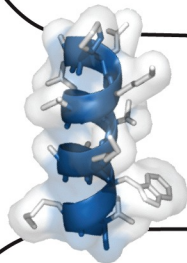
Figure 4-1
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4 Levels of Protein Structure

Primary structure

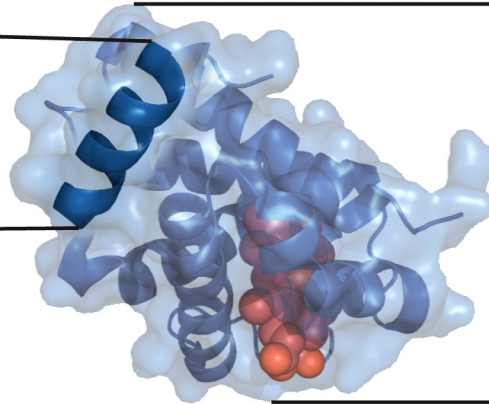
Pro
Ala
Asp
Lys
Thr
Asn
Val
Lys
Ala
Ala
Trp
Gly
Lys
Val

Secondary structure



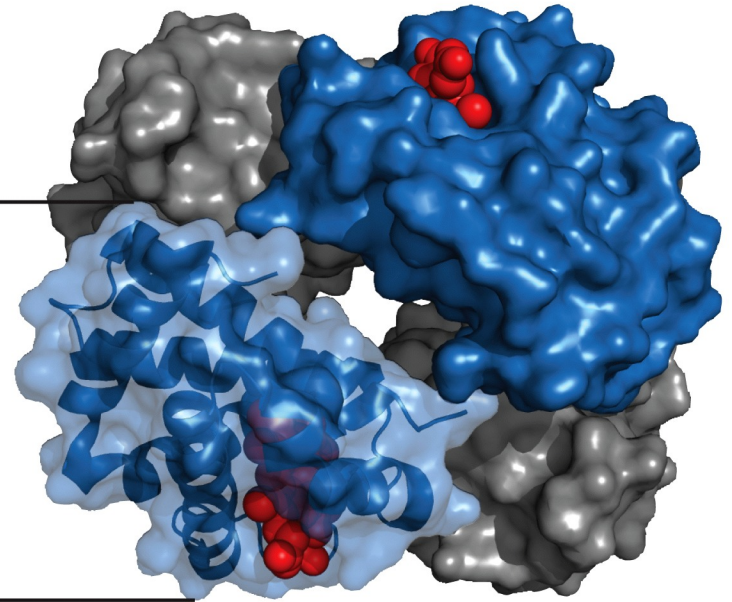
α Helix

Tertiary structure



Polypeptide chain

Quaternary structure



Assembled subunits

Amino acid residues

Figure 3-23
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Weak Interactions Stabilize Conformation

- **Stability:** tendency to keep native conformation Release of water molecules from the structured solvation layer around the molecule as protein folds increases the net entropy
 - To break 1 covalent bond → 200 – 460 kJ/mol
 - To break a weak interaction (H-bond; hydrophobic interaction, ionic bond, etc.) → 4 – 30 kJ/mol
- **Weak interactions are responsible for native structure**
 - Breaking individual covalent bonds that contribute to native structure of proteins (S-S bonds) is more difficult than breaking 1 weak interaction
 - BUT because there are so many weak interactions → they are responsible for keeping native structure
- **Protein conformation with the lowest free energy (most stable) is the one with MAXIMUM number of weak interactions**

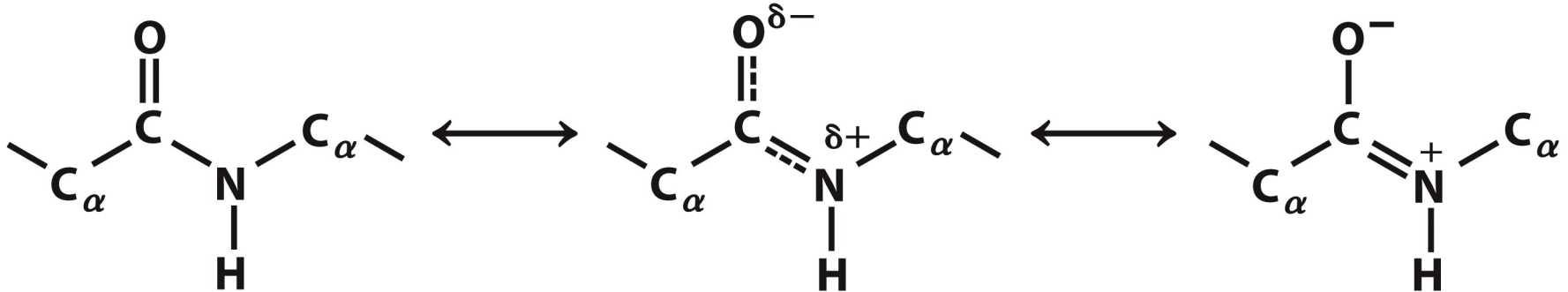
Weak Interactions Stabilize Conformation

- **When H_2O surrounds a hydrophobic molecule, a solvation layer of H_2O molecules is created**
 - increased order of H_2O \rightarrow \downarrow entropy
 - BUT when non polar groups cluster together \rightarrow \downarrow solvation layer
 \rightarrow \uparrow entropy \rightarrow \downarrow ΔG \rightarrow more stable
- **Two rules:**
 - 1) Hydrophobic residues are buried inside proteins
 - 2) The number of H-bonds within a protein is maximized

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 (electron sharing) causes the peptide bonds
 - to be less reactive compared to esters, for example
 - C-N bond in a peptide bond is shorter than C-N bond in a simple amine
 - 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.

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

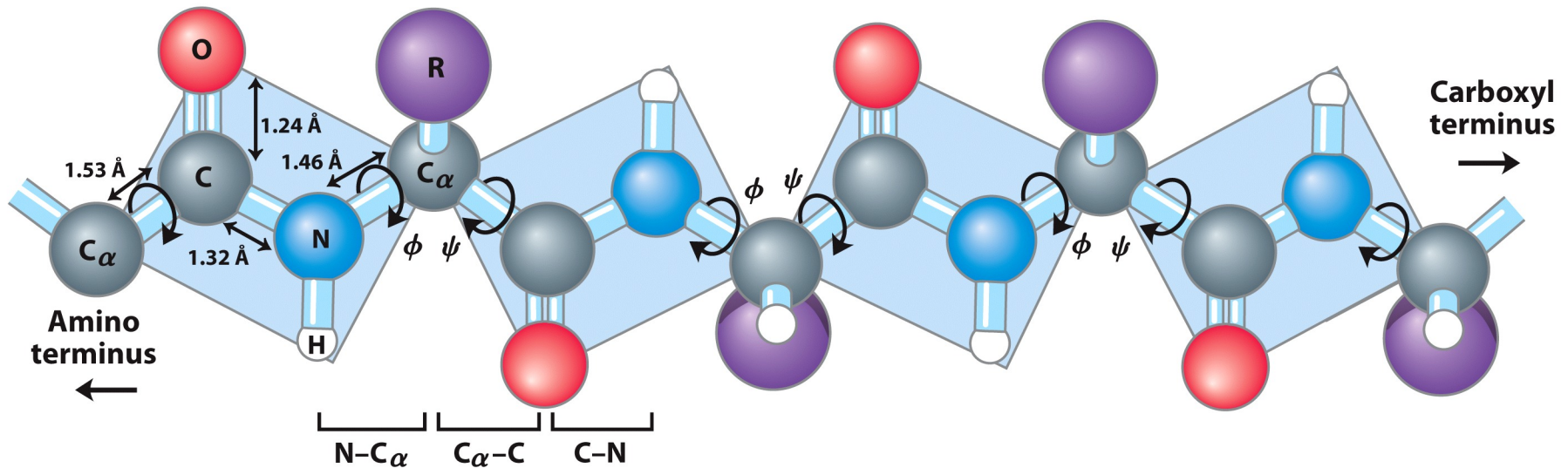


Figure 4-2b

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- The 6 atoms of the peptide group are in 1 plane
- O atom of carbonyl and H atom of amide N are *trans*
- Due to partial double bond, peptide bond cannot rotate freely
→ limiting the range of conformation of a polypeptide

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

Secondary Structures

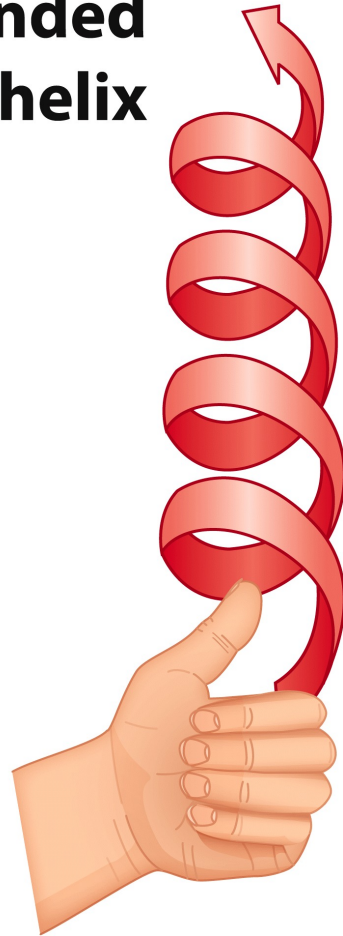
- 2^o structure refers to a local spatial arrangement of the polypeptide backbone (depends on common regular folding patterns of polypeptide backbone)
- Two regular arrangements are common:
- The **α helix**
 - stabilized by hydrogen bonds between nearby residues
- The **β sheet**
 - stabilized by hydrogen bonds between adjacent segments that may not be nearby
- Irregular arrangement of the polypeptide chain is called the **random coil**

The α Helix

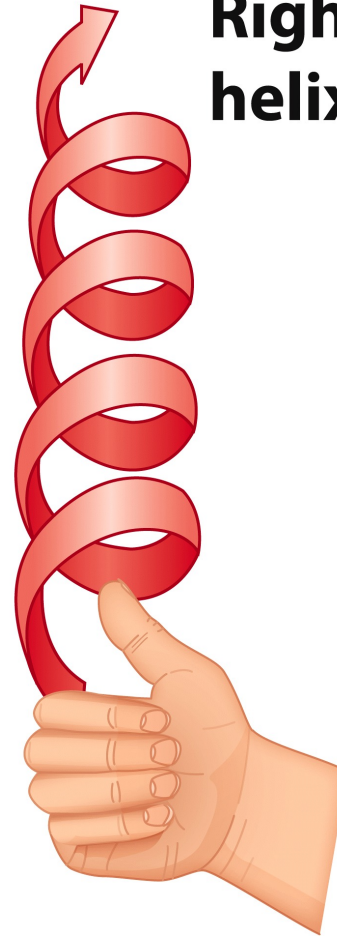
- The simplest arrangement a polypeptide can assume (with its rigid peptide bonds and other freely-rotating single bonds) is a helical structure
 - In general, $\sim \frac{1}{4}$ of all aa residues in polypeptides are found in α helices
 - E.g. in α -keratins, α helix is a predominant structure
- Helical 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?

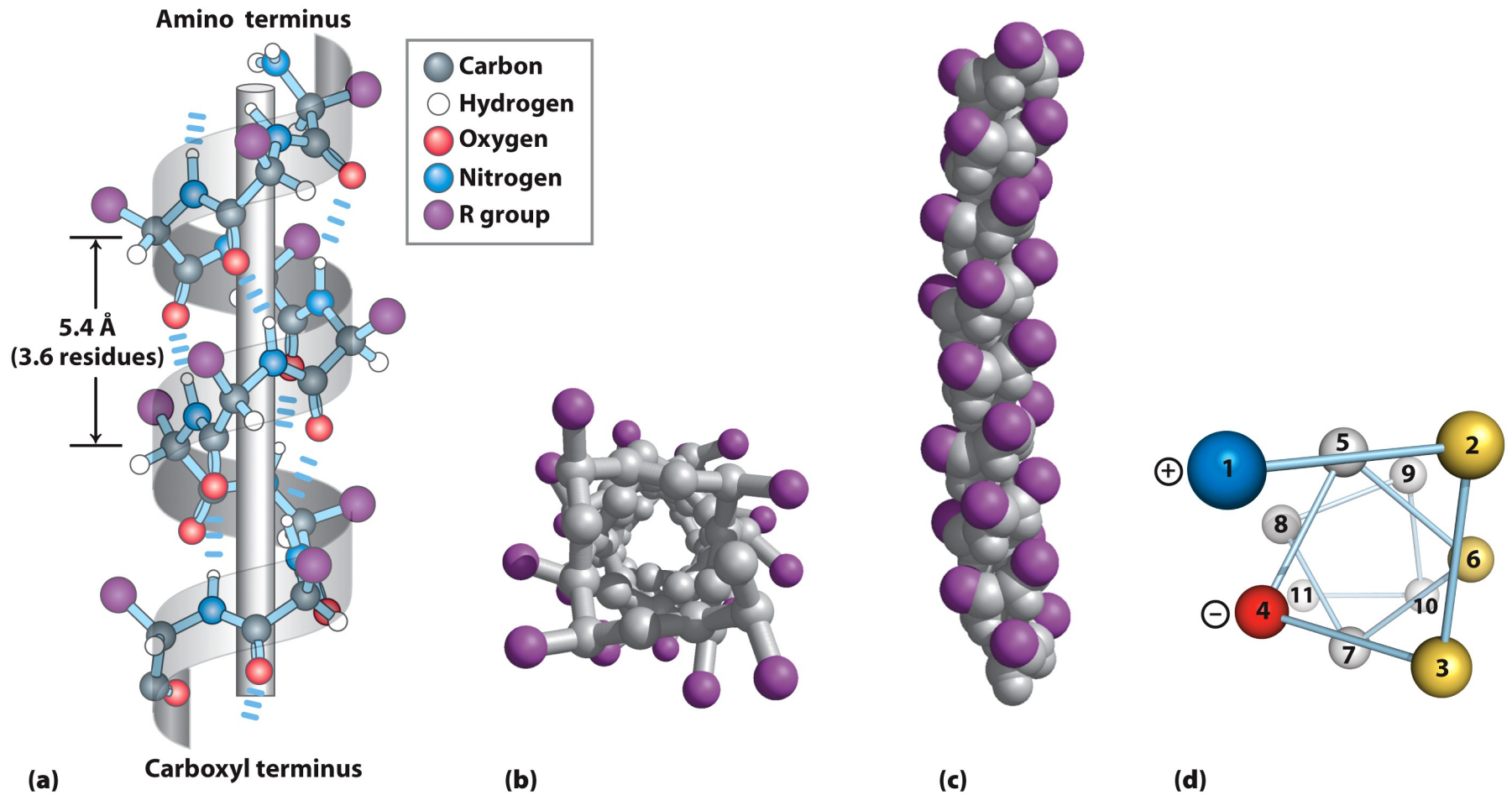


Figure 4-4
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Sequence affects helix stability

- 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

Constraints to helix stability

1. Electrostatic repulsion (or attraction) between successive charged aa
2. Bulkiness of adjacent R groups
3. Interactions between R groups 3 or 4 residues apart
4. Pro or Gly
5. Interactions of aa with dipoles of a helix

Constraints to helix stability

1. Electrostatic repulsion (or attraction) between successive charged aa
 - Interactions between aa side chains can stabilize or destabilize a helix:
 - E.g. long block of Glu (E) residues → no helix at pH 7 because of -ve charge repulsion
 - Same for +vely charged aa

Constraints to helix stability

2. Bulkiness of adjacent R groups

- Near each other, these aa and other bulky aa will not fit well due to steric hindrance
- Small hydrophobic residues such as **Ala** and **Leu** are strong helix formers

Constraints to helix stability

3. Interactions between R groups 3 or 4 residues apart

- Due to the twist of α helix \rightarrow critical interaction between aa side chain and side chain of aa 3 or 4 residues apart in both directions
- Often, +vely charged aa are 3 or 4 aa away from -ve charge \rightarrow forming ion pairs
- Two hydrophobic side chains 3 or 4 aa apart \rightarrow hydrophobic interactions

Constraints to helix stability

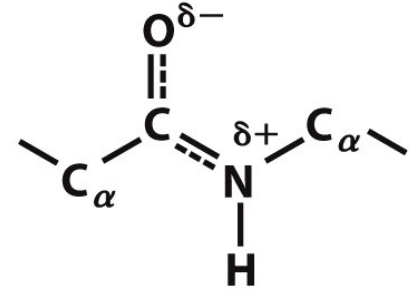
4. The presence of Pro or Gly

- **Pro:** rarely found in α helix
 - 1) rotation about N-C α is not possible because N is part of a rigid ring
 - 2) N has no H to participate in H-bonds
- **Gly:** infrequently found in α helix
- it has more conformational flexibility than other aa
- makes a different coiled structures than α helix

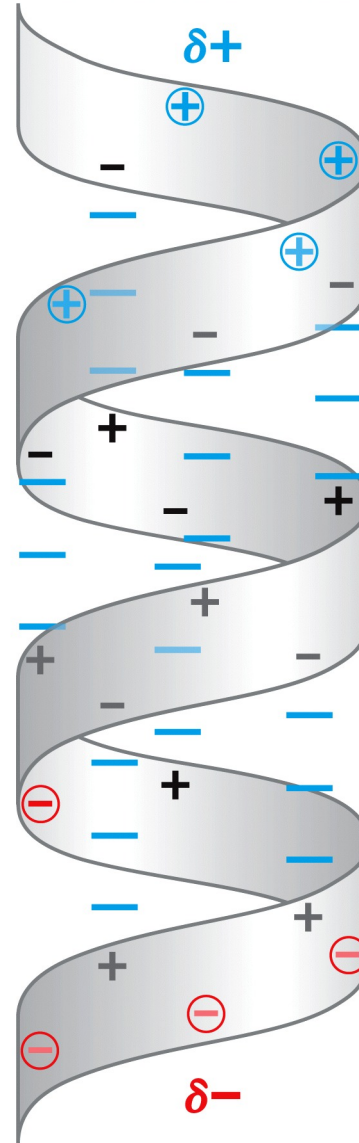
Constraints to helix stability

5. Dipoles

- A small electric dipole exists in each peptide bond
 - Carbonyl O negative
 - Amide H positive
- Dipoles are connected by H-bonds → large net macroscopic dipole moment extending along helix (increases with helix length)
- The 4 aa at the ends of the helix do not participate fully in H-bonds → on N-terminus partial +ve charge and on C-terminus partial -ve charge
- Therefore, normally -vely charge aa are found near N-termini and vice versa (a +ve aa near N-terminus is destabilizing).



Amino terminus



Carboxyl terminus

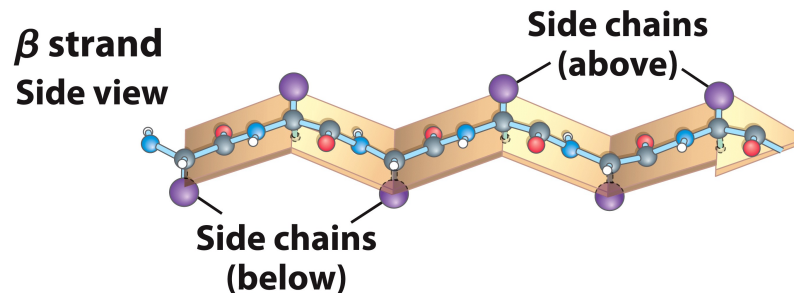
Figure 4-5

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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



β Sheets

- Backbone is extended into a zigzag
- Zigzag polypeptide chains are arranged side-by-side forming the β sheet
- H-bonds form between adjacent segments
- Segments are usually near each other (sometimes can be very far or even between different polypeptides)
- The R groups of adjacent aa come out opposite direction
- **Size limits:** nearby R groups must be small
- E.g. β -keratins (silk fibroin and fibroin of spider webs) have high content of Gly and Ala (smallest R groups)
- Adjacent chains can be parallel or anti-parallel

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

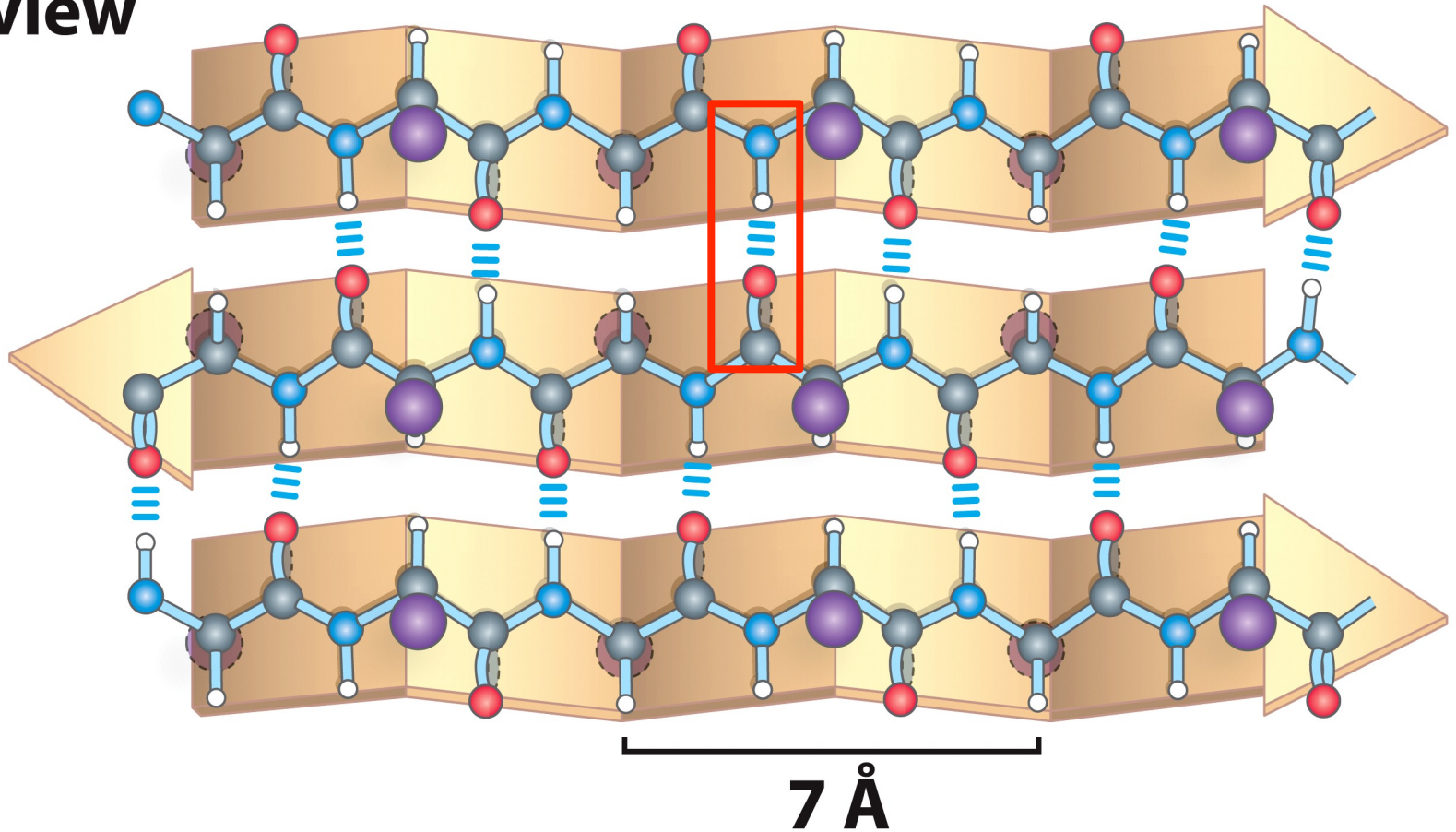


Figure 4-6b

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Parallel β sheet

Top view

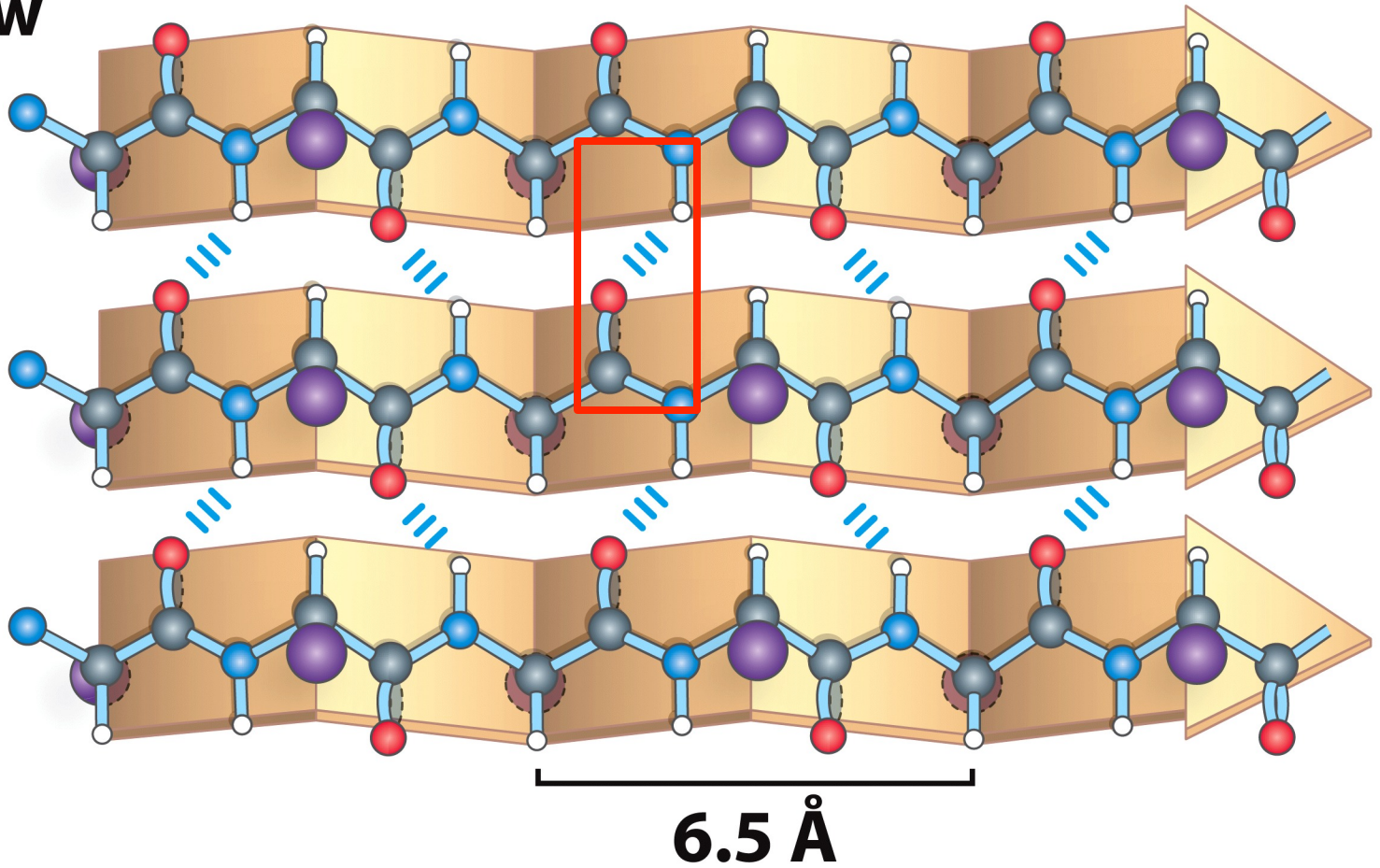
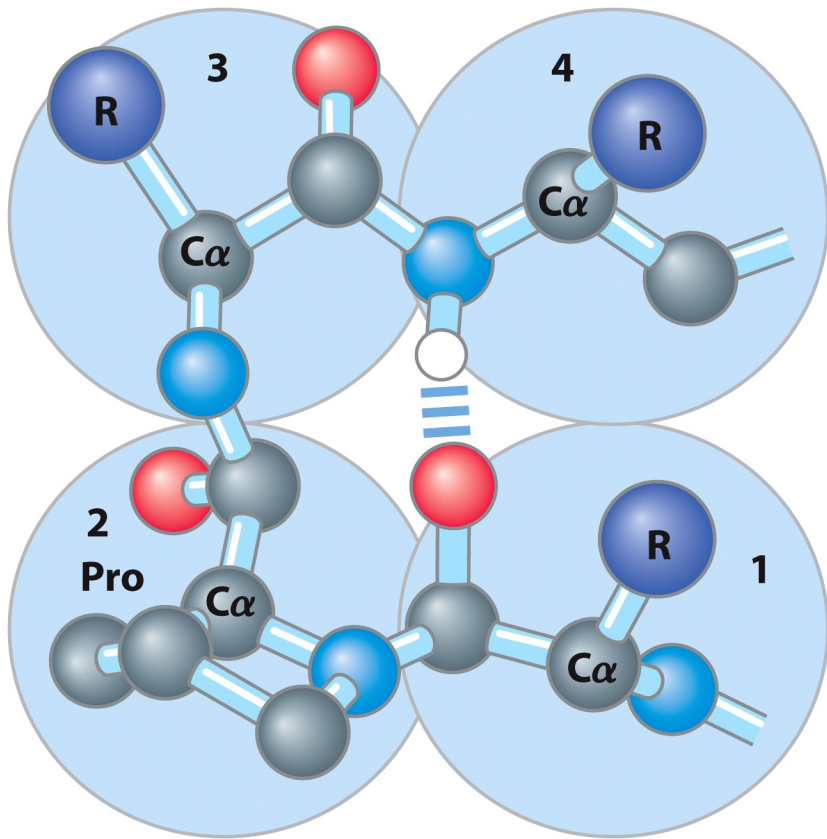


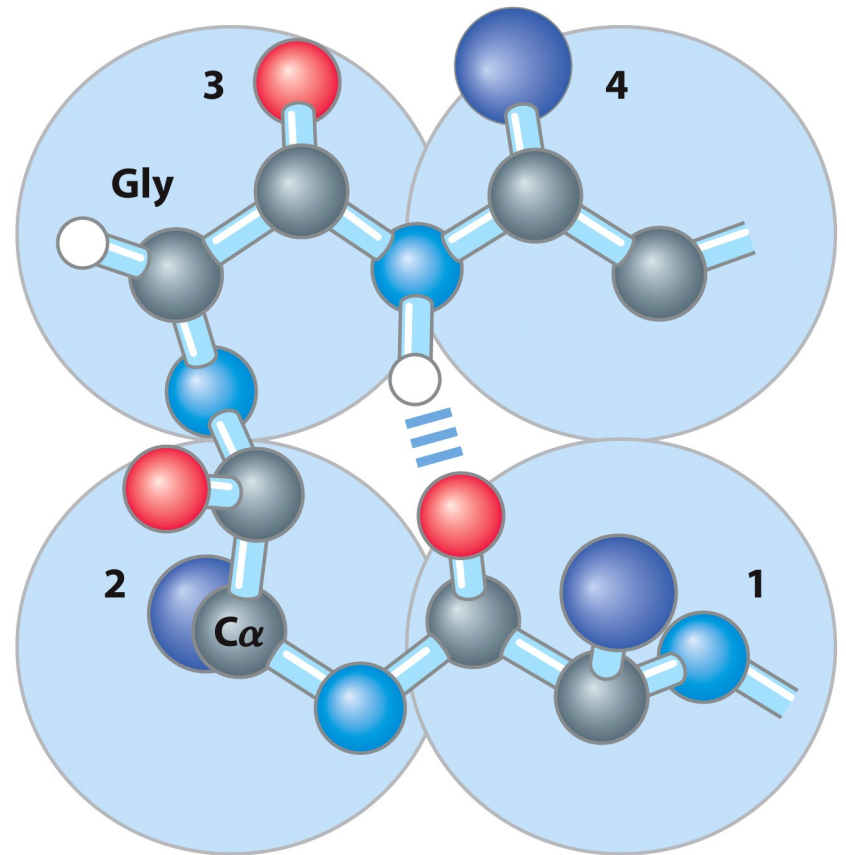
Figure 4-6c
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β Turns

- Globular proteins have compact folded structures
- $\sim 1/3$ aa are turns or loops (polypeptide reverses direction)
- β turns occur frequently whenever strands in β sheets change the direction
- Connecting elements linking successive runs of α helix or β sheet
- 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 (aa1 and aa4)
- **Proline** in position 2 or **glycine** in position 3 are common in β turns



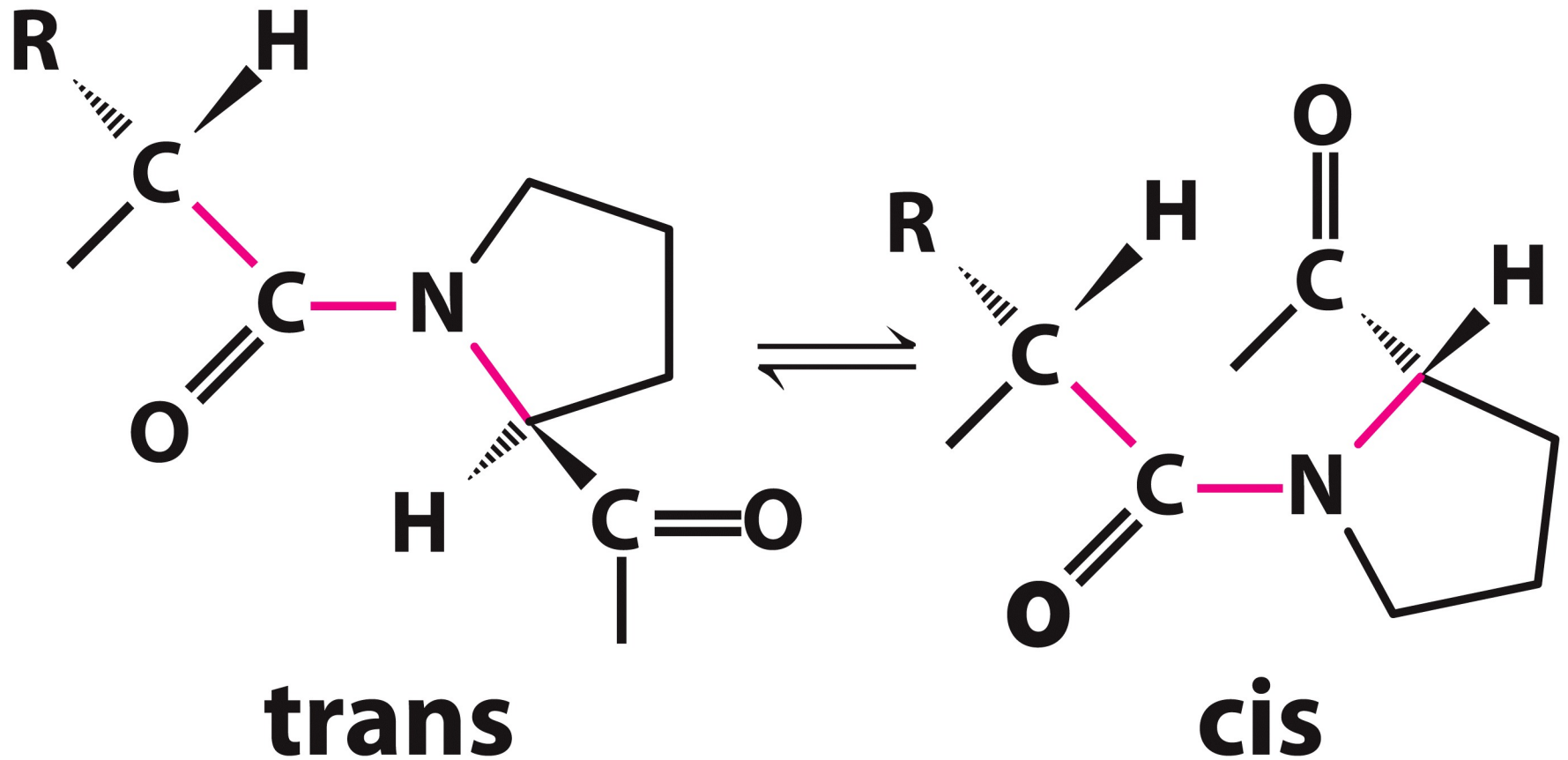
Type I β turn



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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γ turns

- Less common
- 3 aa turn
- H-bonding between 1st and 3rd aa

Circular Dichroism (CD) Analysis

- A technique used to determine the secondary structure fold of purified proteins
- CD measures the molar absorption difference $\Delta\varepsilon$ of left- and right-circularly polarized light: $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$
- Chromophores (mainly aromatic aa) in the chiral environment produce characteristic signals
- CD signals from peptide bonds depend on the chain conformation
- Characteristic spectra for different secondary structures
- Combined spectra give the percentage of α to β in a protein

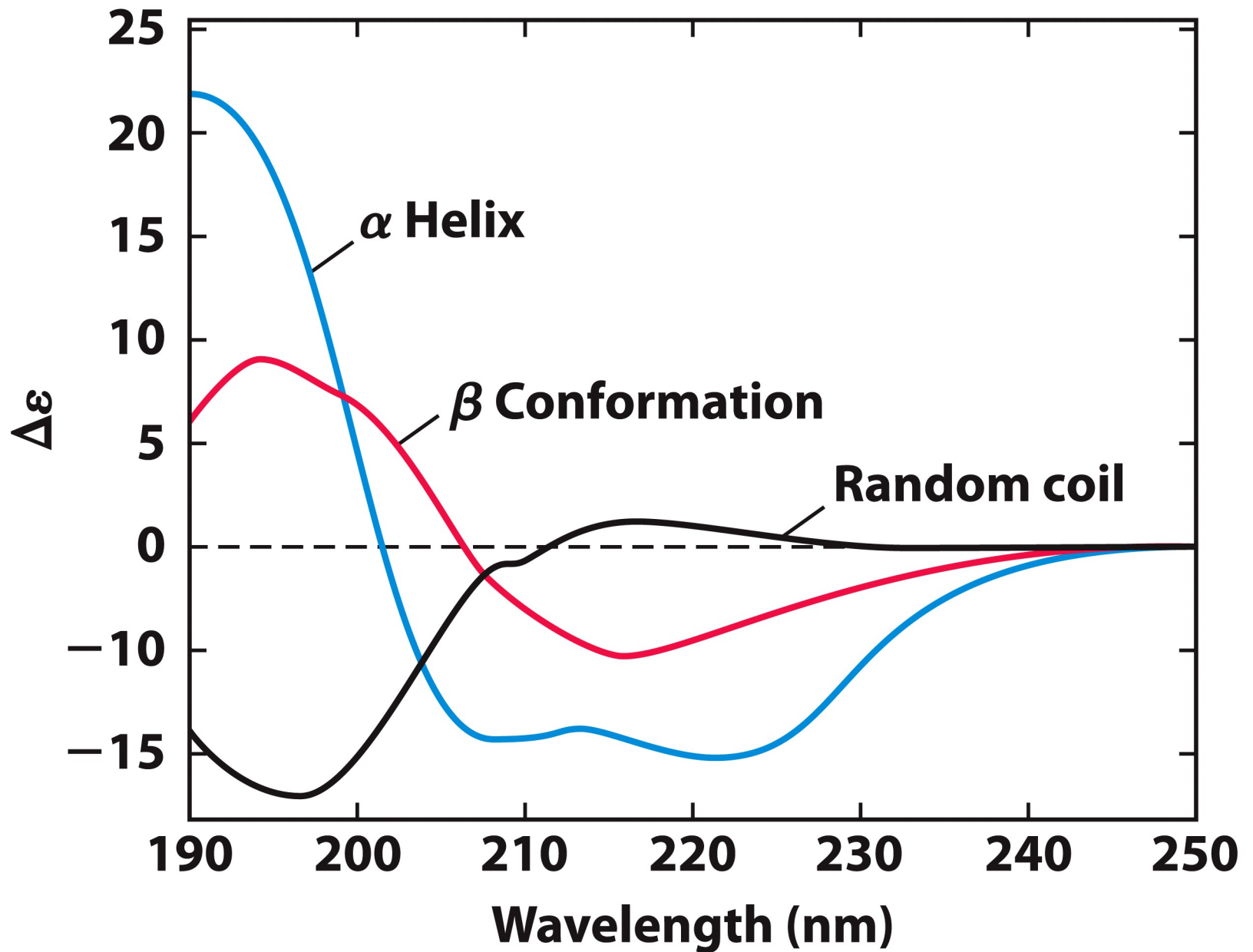


Figure 4-10

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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**

Protein Tertiary Structure

- **Fibrous** proteins – polypeptide chain arranged in long strands or sheets
 - consist largely of 1 type of 2^o structure
 - needed for structure and shape of cells
 - water insoluble (hydrophobic)
- **Globular** proteins – polypeptide chain folded into spherical shape
 - many types of 2^o structures
 - enzymes and regulators
 - largely water soluble (hydrophilic)

Fibrous Proteins: From Structure to Function

Function	Structure	Example
Tough, rigid, hard (nails, horns)	Cross-linked α -helixes Rigid linker (S—S)	α -keratin
Tensile strength, non-stretching (tendons, cartilage)	Cross-linked triple-helixes Flexible linker (Lys-HyLys)	Collagen
Soft, flexible non-stretchy (egg sac, nest, web)	Non-covalently held β -sheets van der Waals interaction	Silk fibroin

Fibrous Proteins

- Have properties that give them strength and flexibility
- All fibrous proteins are water insoluble (high concentration of hydrophobic aa inside and on protein surface)
- **α -keratin:** ~ all dry weight of hair, wool, nail, horns, hooves, etc.
- Part of a family called intermediate filaments (IF)
- **Coiled coil**, parallel (N-termini at same side)
- Supertwists are **left-handed**
- At the surface of a helix where supertwists exist there are many hydrophobic aa

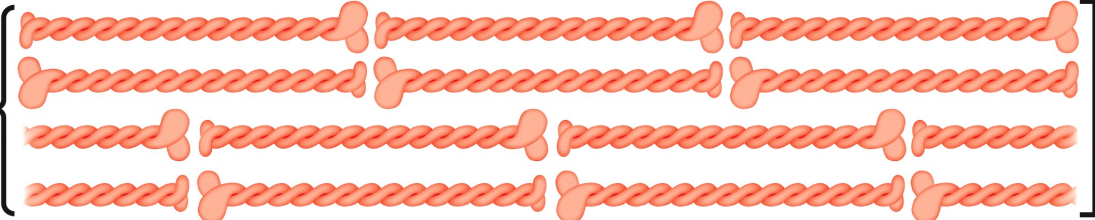
Structure of α -Keratin in Hair

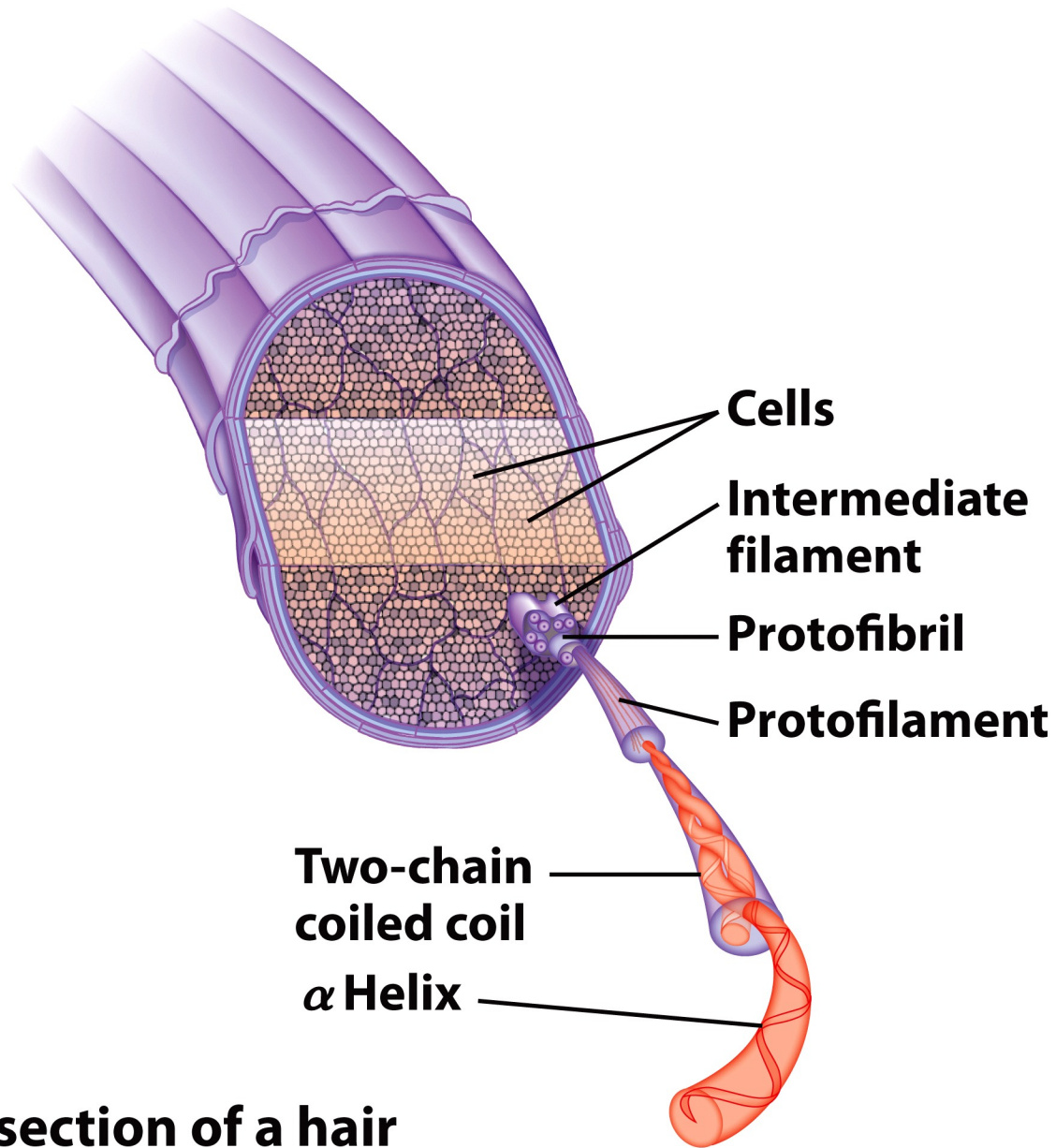
- **Simple 3^o structure:** dominated by α -helical 2^o structure with its helical axis twisted in a left-handed helix
- **Complex 4^o structure:** many coiled coils assembled into large supramolecular complexes

Keratin α helix — 

Two-chain coiled coil — 

Protofilament {  } 20–30 Å

Protofibril {  }



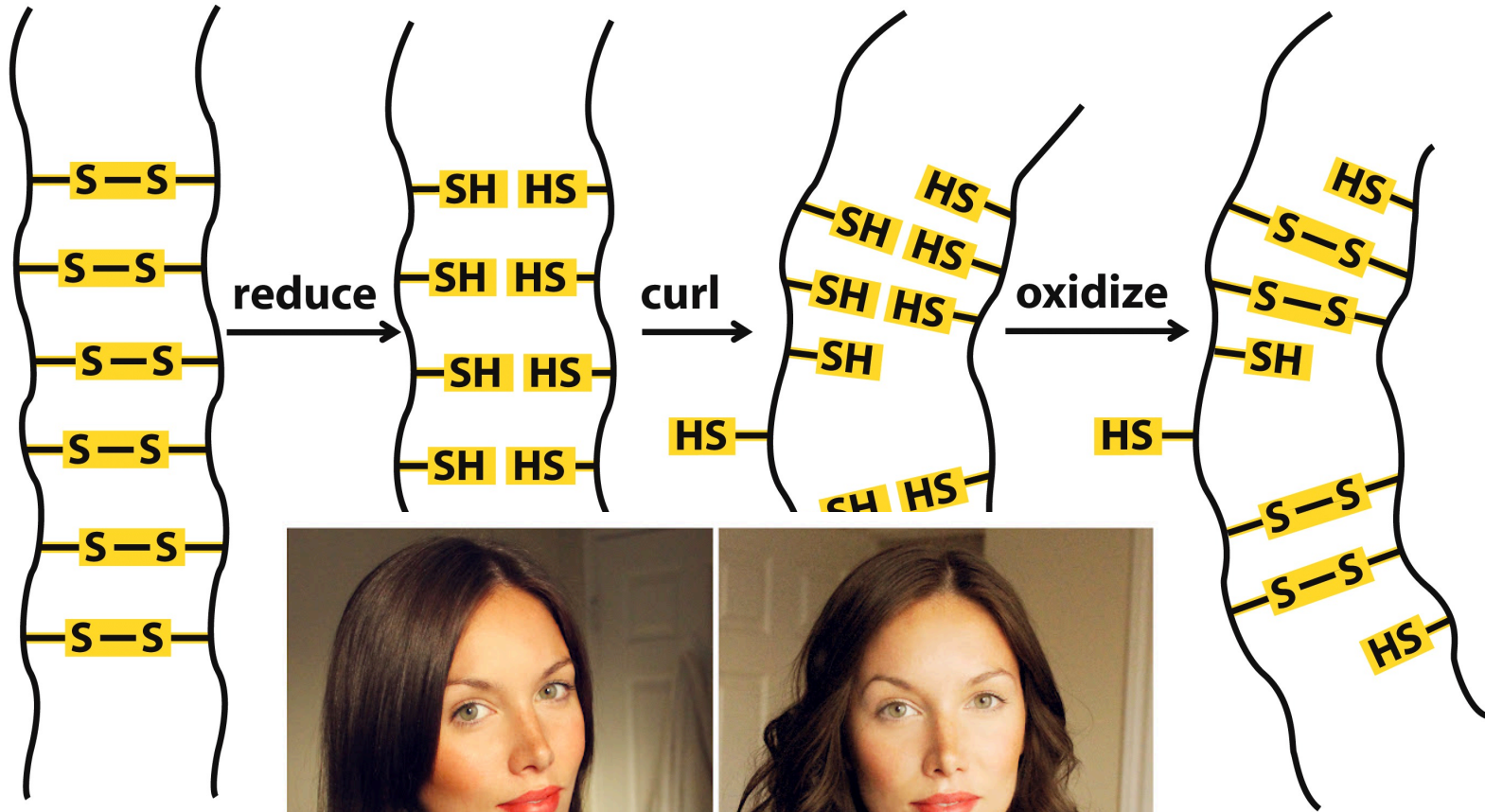
Cross section of a hair

Figure 4-11b

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Chemistry of Permanent Waving



Box 4-2

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Straight



to curly.

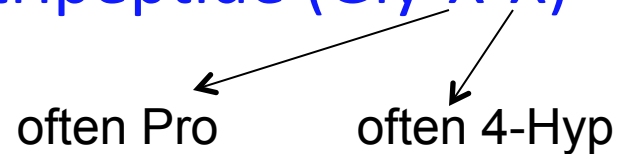
Structure of Collagen

- 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**
- Collagen helix is different from α helix:
 - 1) it is left handed
 - 2) it has 3 aa per turn
- 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

Structure of Collagen

- Typical collagen has ~35% Gly, 11% Ala and 21% Pro and 4-Hyp

– aa sequence is generally a tripeptide (Gly-X-X)



- Many triple-helices assemble into a collagen fibril
- Collagenase is an enzyme that breaks down collagen

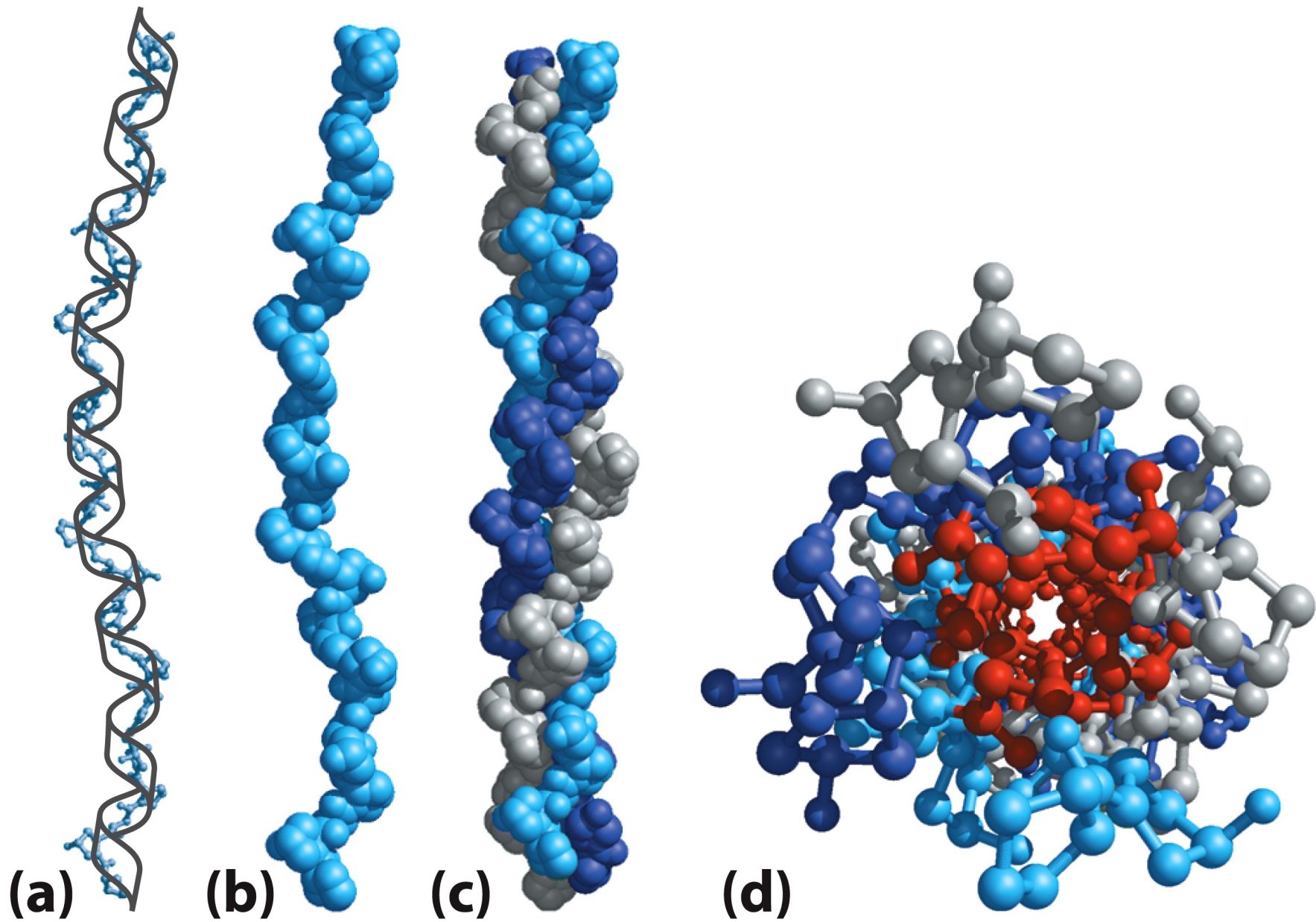


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Collagen Fibrils

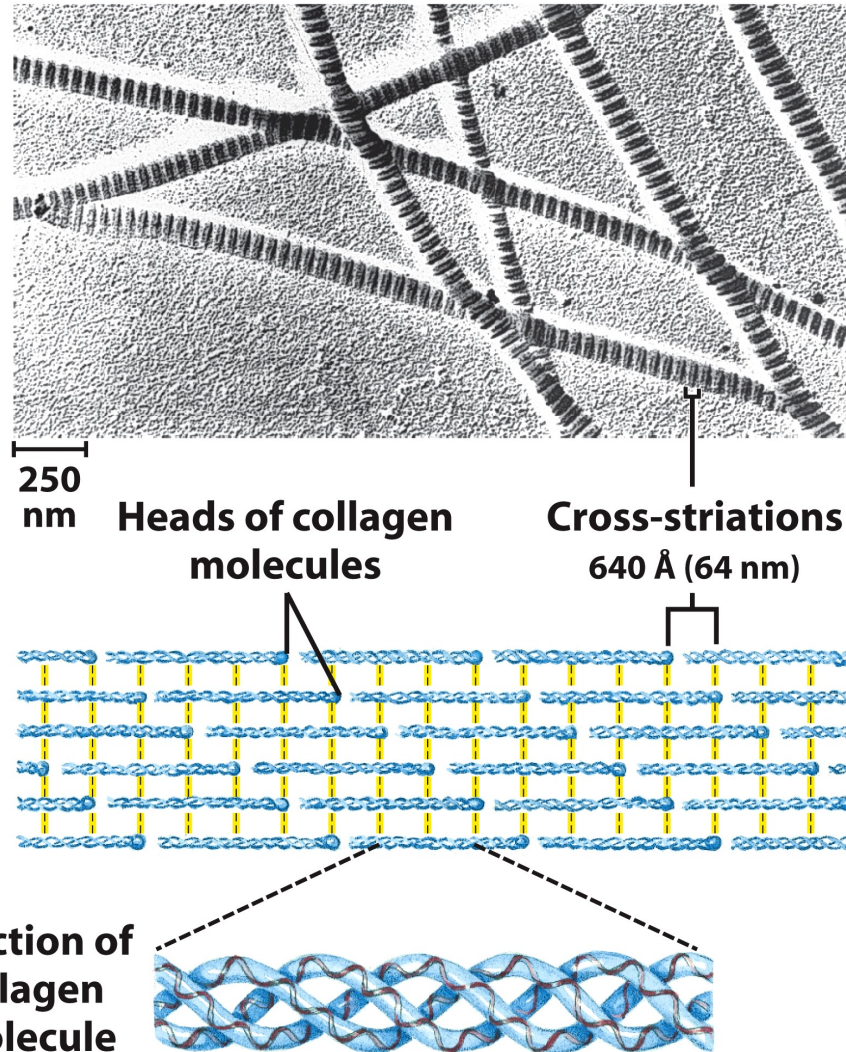


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Silk Fibroin

- **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
- Silk does not stretch (β conformation is highly extended)
- Flexible because it is held together by weak interactions
- Structure is stabilized by
 - hydrogen bonding within sheets
 - London dispersion interactions between sheets

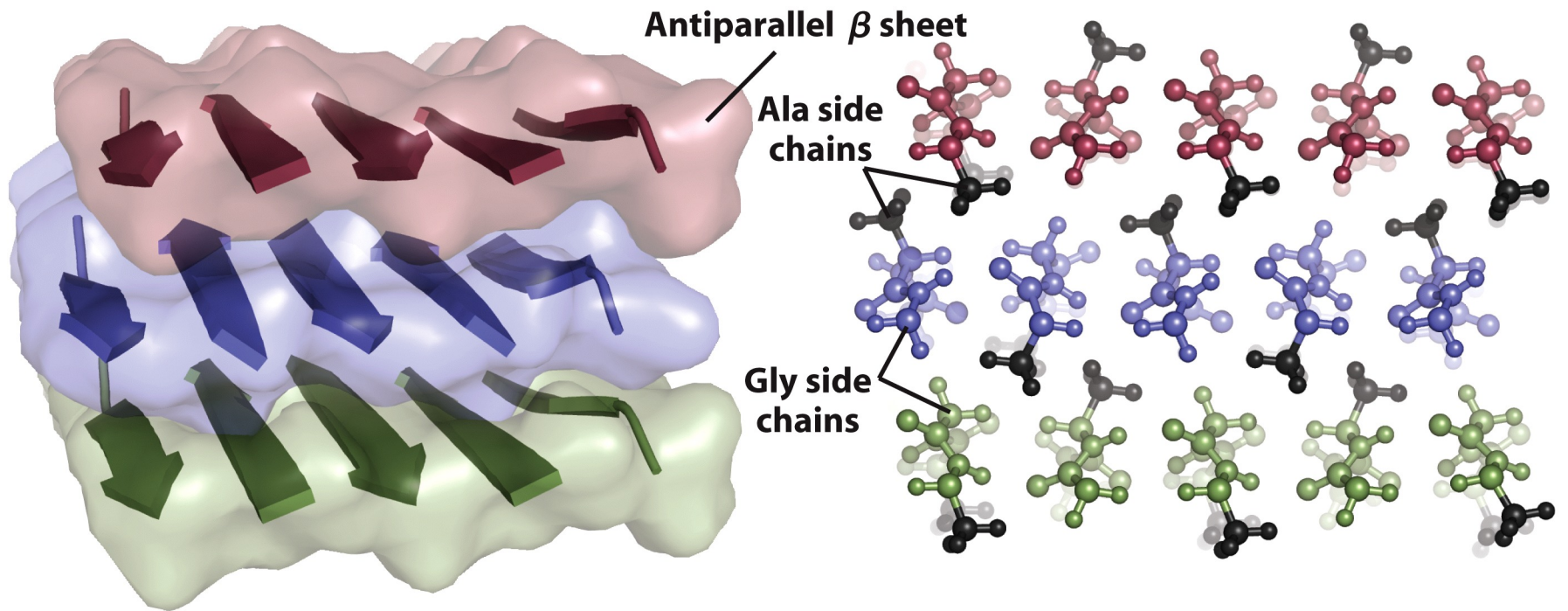


Figure 4-14a
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Globular Proteins

- Different segments of polypeptide chain (or multiple polypeptide chains) fold back on each other → compact form
- Provides structural diversity to carry out a wide range of biological functions

Human serum albumin

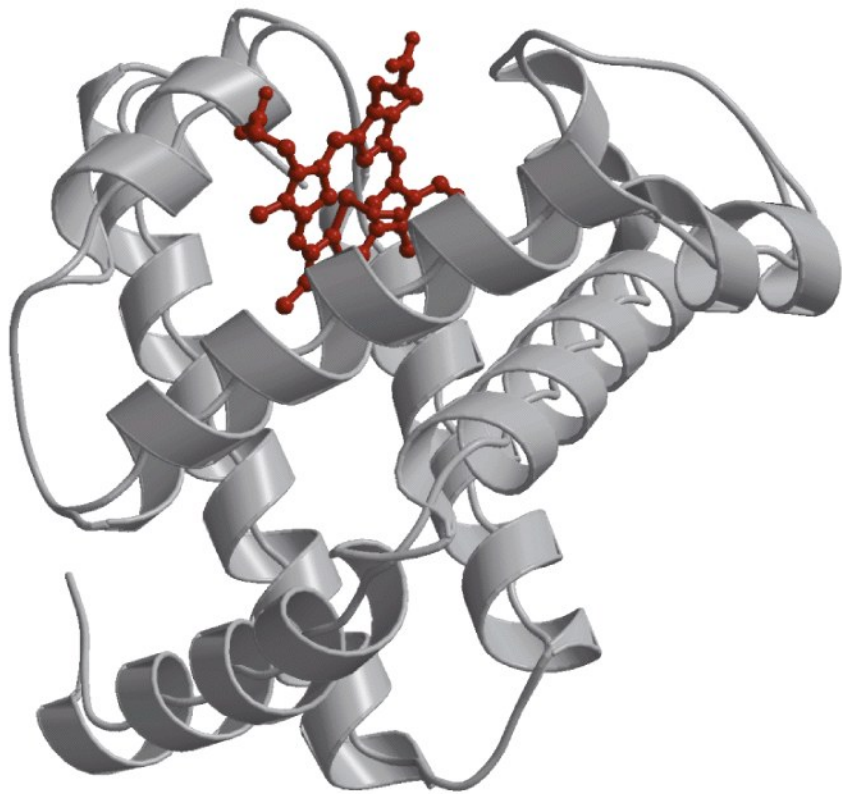
β Conformation
 $2,000 \times 5 \text{ \AA}$

α Helix
 $900 \times 11 \text{ \AA}$

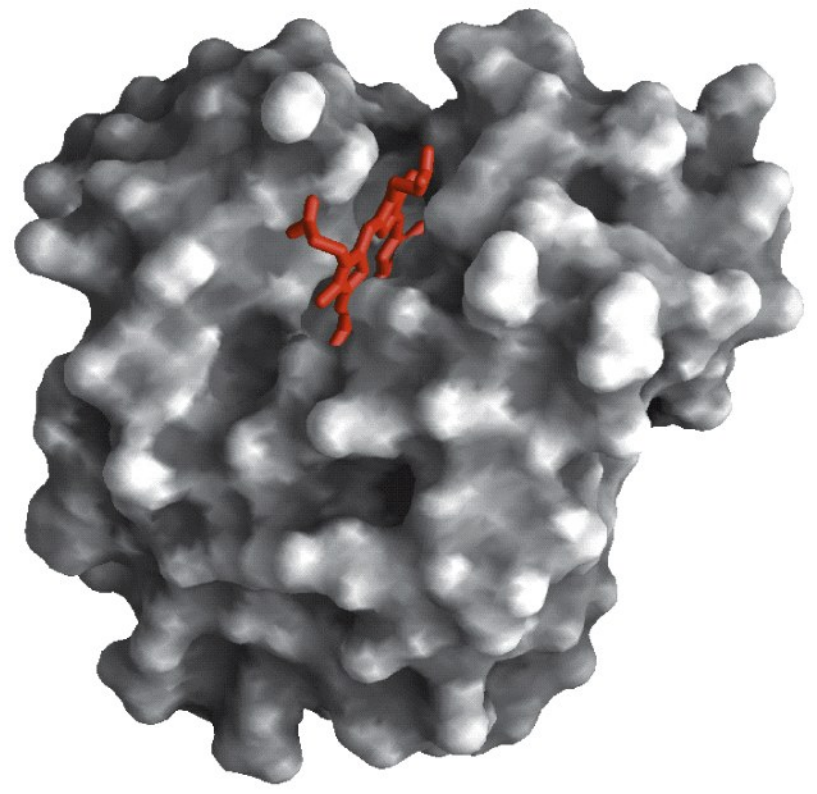
Native globular form
 $100 \times 60 \text{ \AA}$

Myoglobin

- Small (M_r 16700)
- O_2 -binding protein of muscle cells
- Storage of O_2 and rapid supply to contracting muscles
- Has a heme group
- Abundant in muscles of diving mammals
- 8 straight α helices interrupted by bends (some of which are β turns)
- Longest helix – 23 aa; shortest helix – 7 aa
- 70% of myoglobin is α helix
- Most hydrophobic R groups are inside the protein



(a)

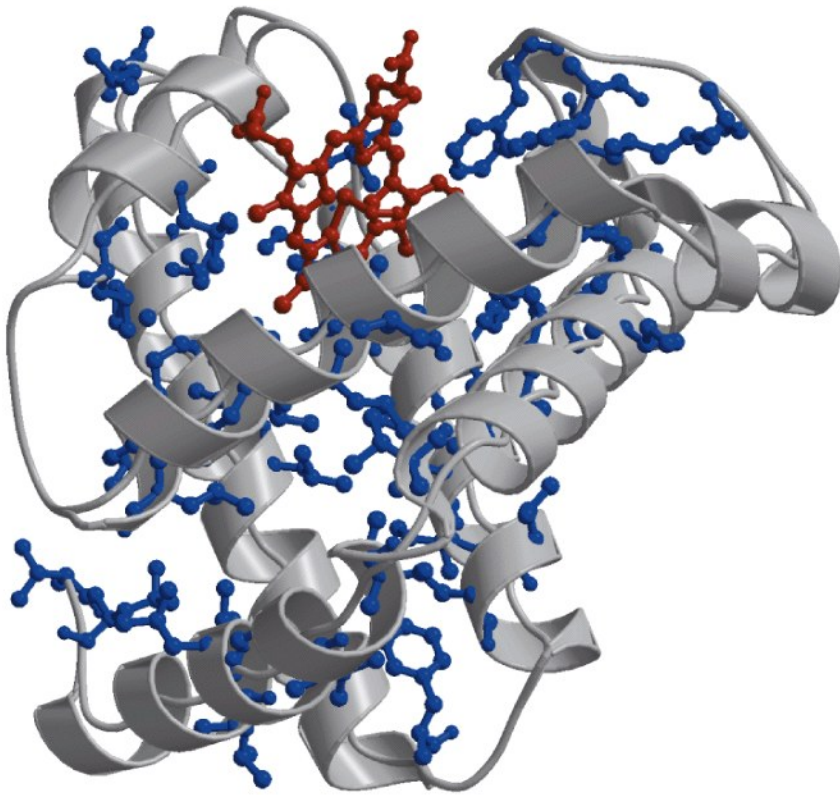


(b)

Figure 4-15ab

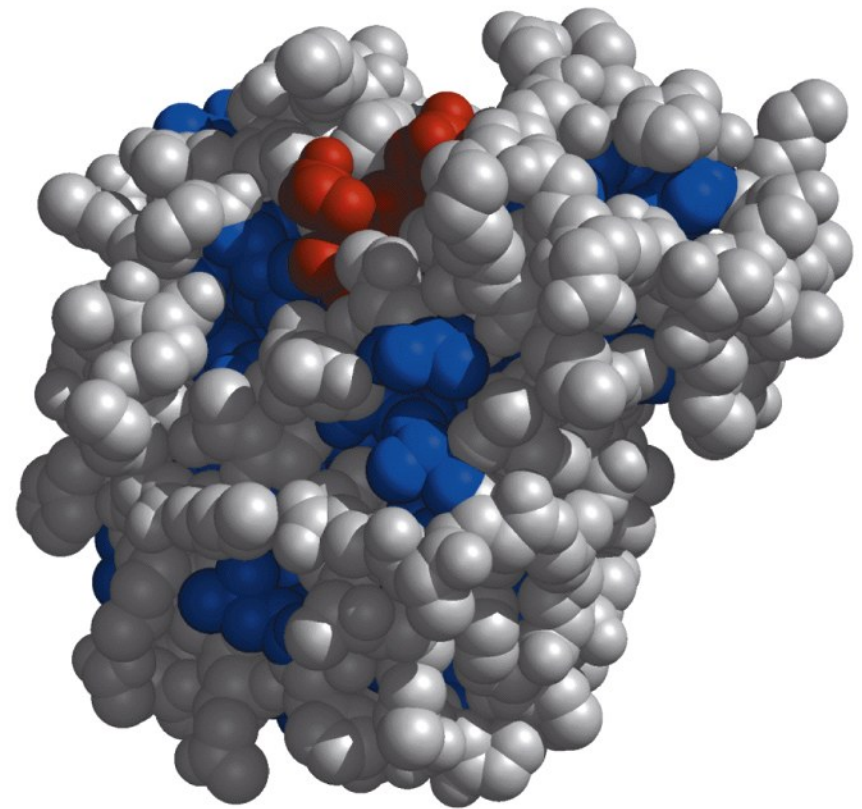
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(c)

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(d)

The hydrophobic residues are shown in blue; most are buried in the interior of the protein and thus not visible

Motifs (folds or supersecondary structures)

- 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

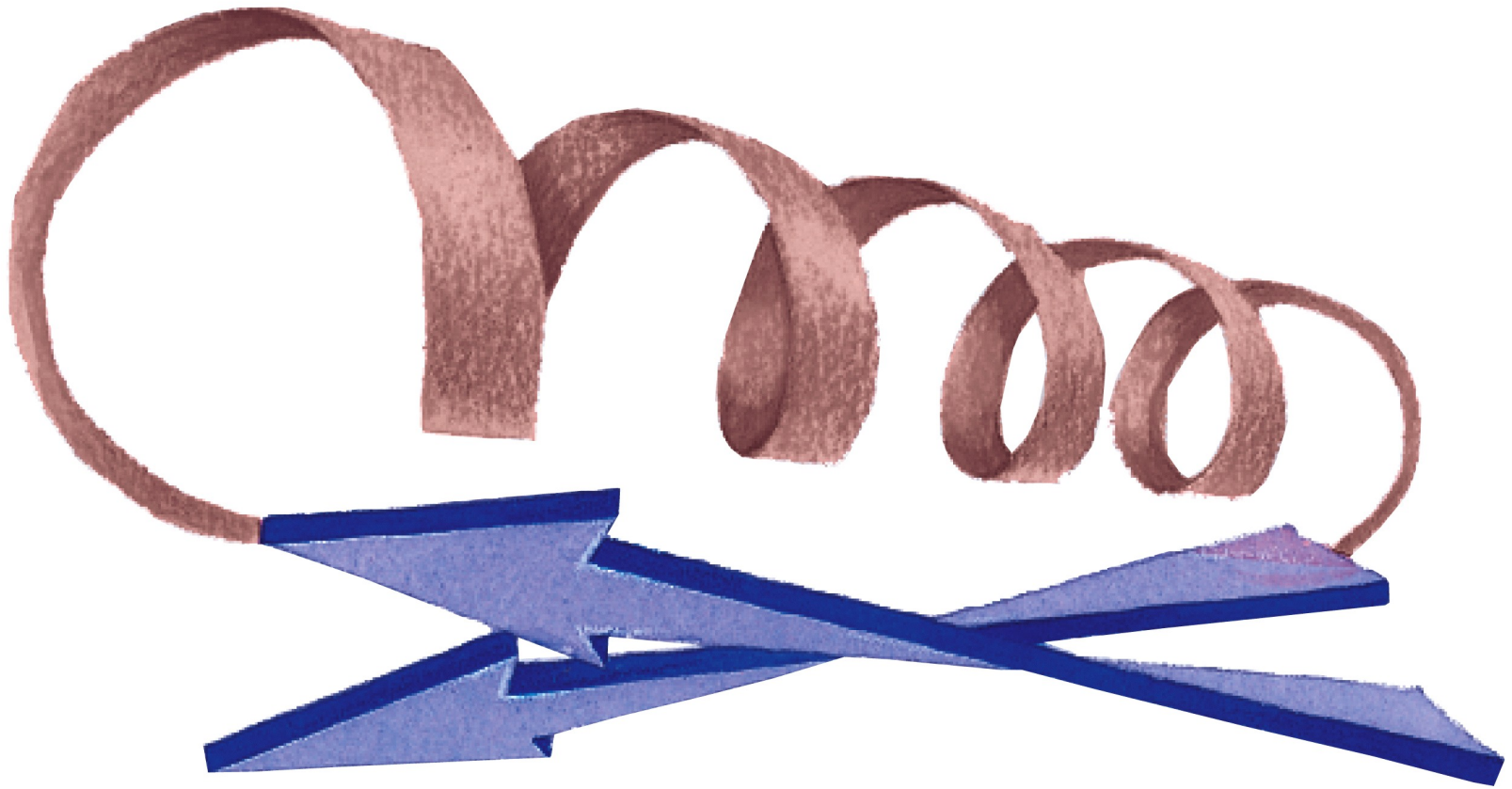


β Barrel

Figure 4-17b

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β - α - β Loop

Figure 4-18a

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- **Complex motifs:**
- A series of β - α - β loops can form a barrel, called α/β barrel
- All connections are right handed

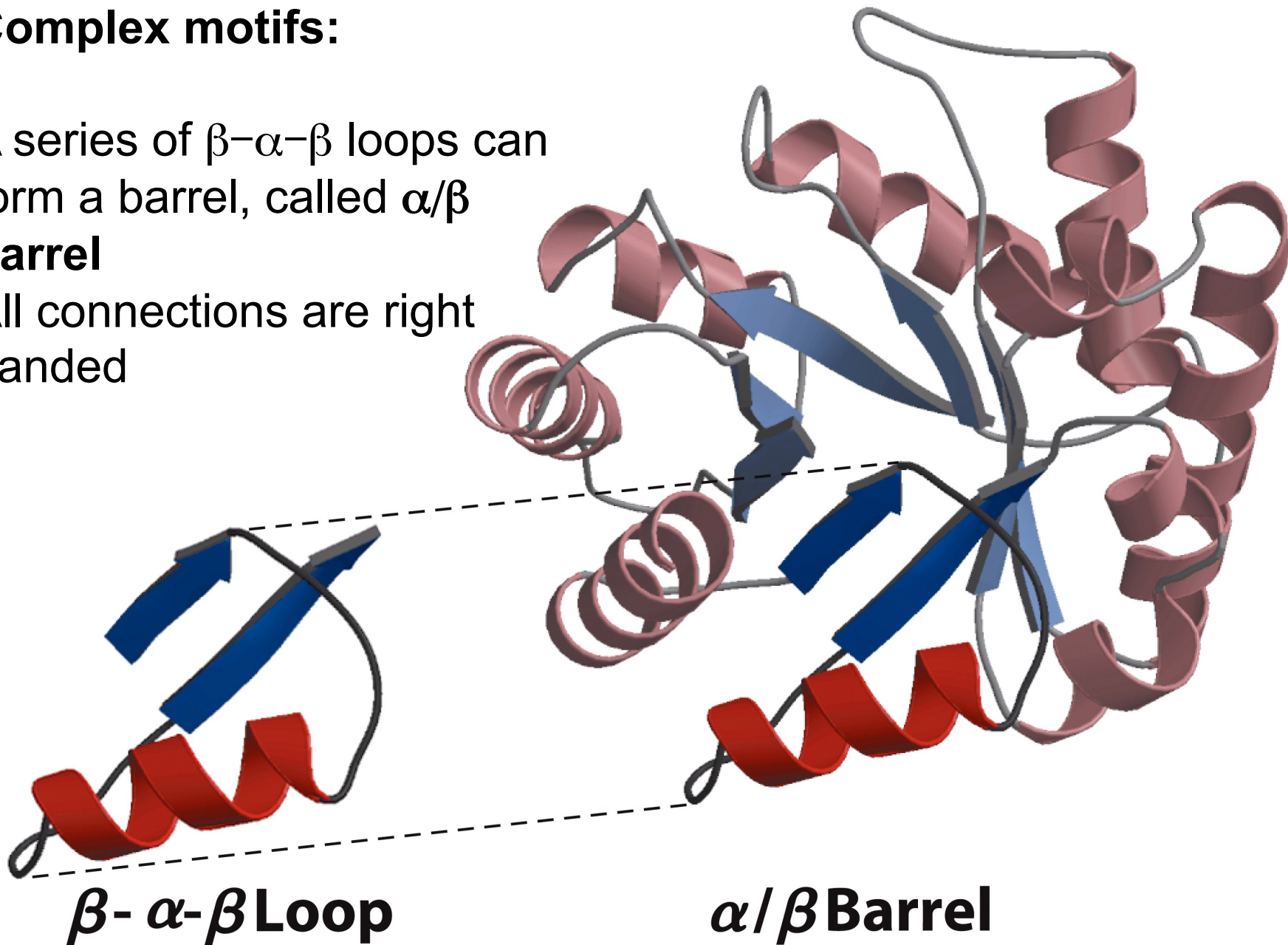


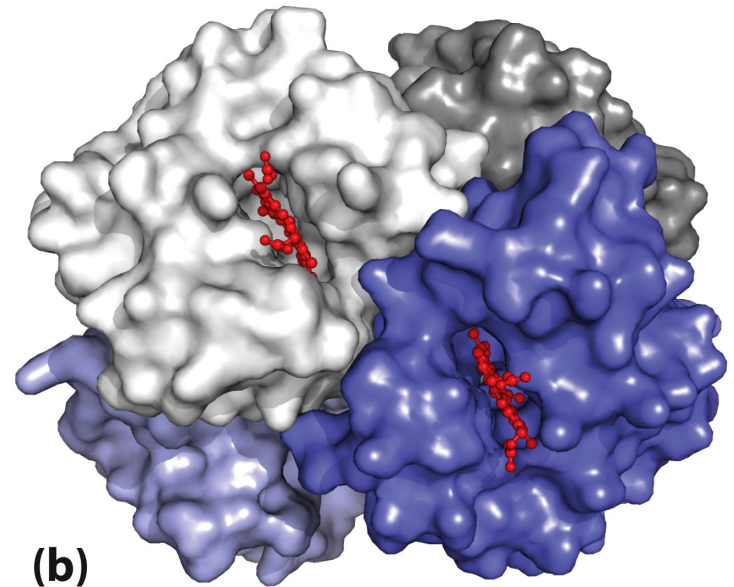
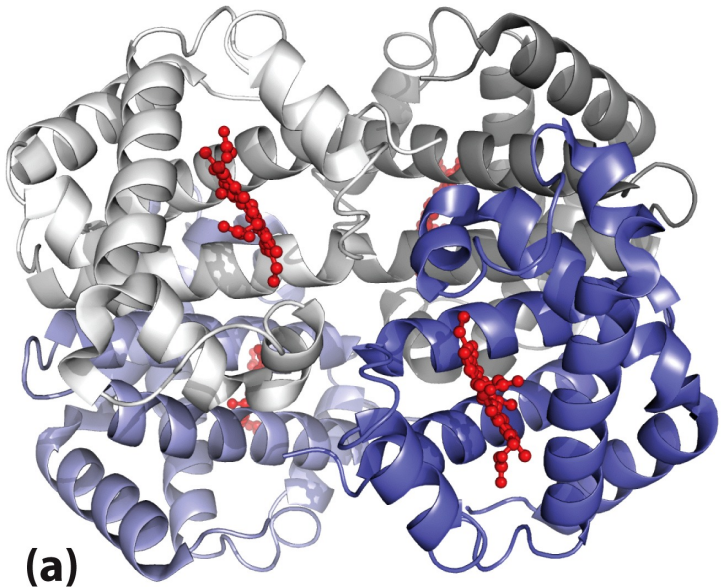
Figure 4-21

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Protein Quaternary Structure

- **Quaternary structure** is formed by the assembly of individual polypeptides into a larger functional cluster
- It describes the arrangement of protein subunits in 3D complexes
- **Only** in multisubunit proteins



Tetramer or dimer of $\alpha\beta$ protomers

Proteostasis

Maintenance of cellular protein activity is accomplished by the coordination of many different pathways.

3 processes contribute to proteostasis:

- 1) protein **synthesis** on ribosomes
- 2) protein **folding** (involving complexes called chaperones)
- 3) the **sequestration** and **degradation** of proteins that are irreversibly unfolded

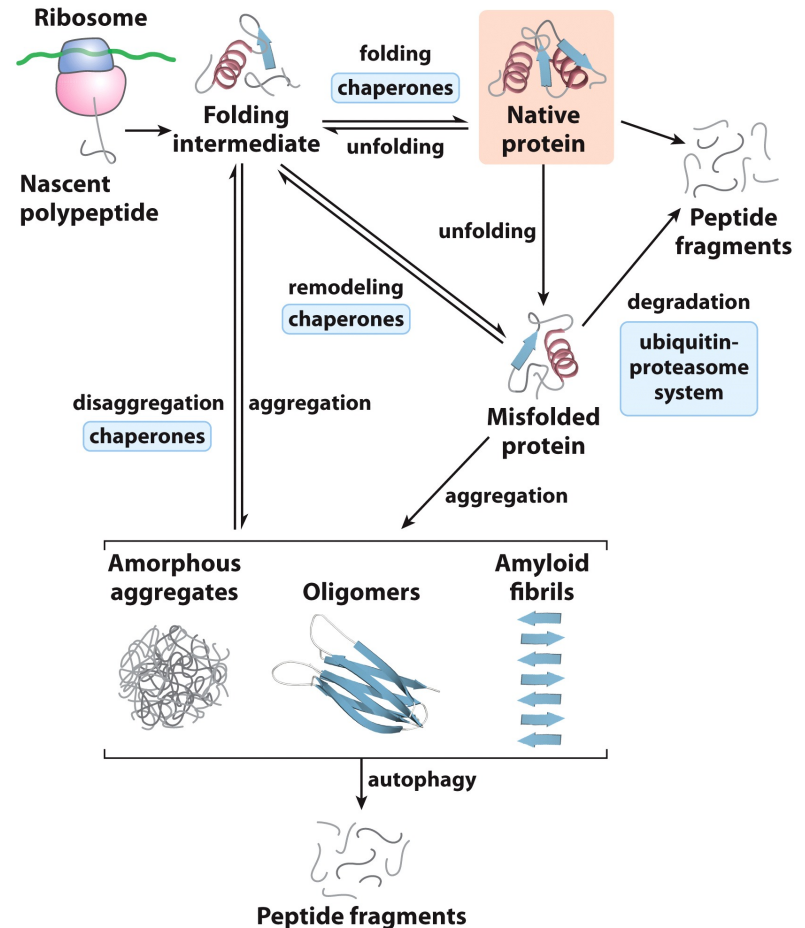


Figure 4-25
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Protein Stability and Folding

- A protein's function depends on its 3D-structure
- **Denaturation:** the loss of native structure sufficient to cause loss of function
- Does not mean that protein is always unfolded
- Under most conditions, denatured proteins exist in **partially** folded states

• **Loss of structure → Loss of function**

- Causes of denaturation:

- 1) Heat:** affecting weak interactions (mainly H-bonds)

- gradual increase in temp, conformation remains intact until an abrupt change occurs at a narrow temp range

- due to cooperativity (loss of structure in one part of the protein destabilizes other parts)

- 2) Extremes of pH:** change net charge on protein → electrostatic repulsion and disruption of some H-bonds

- 3) Organic solvents, detergents:** no covalent bonds in the protein are broken. Disruption of hydrophobic interactions

- 4) Chaotropic agents (urea and guanidine HCl):** disruption of the arrangement of water molecules that solvate the proteins

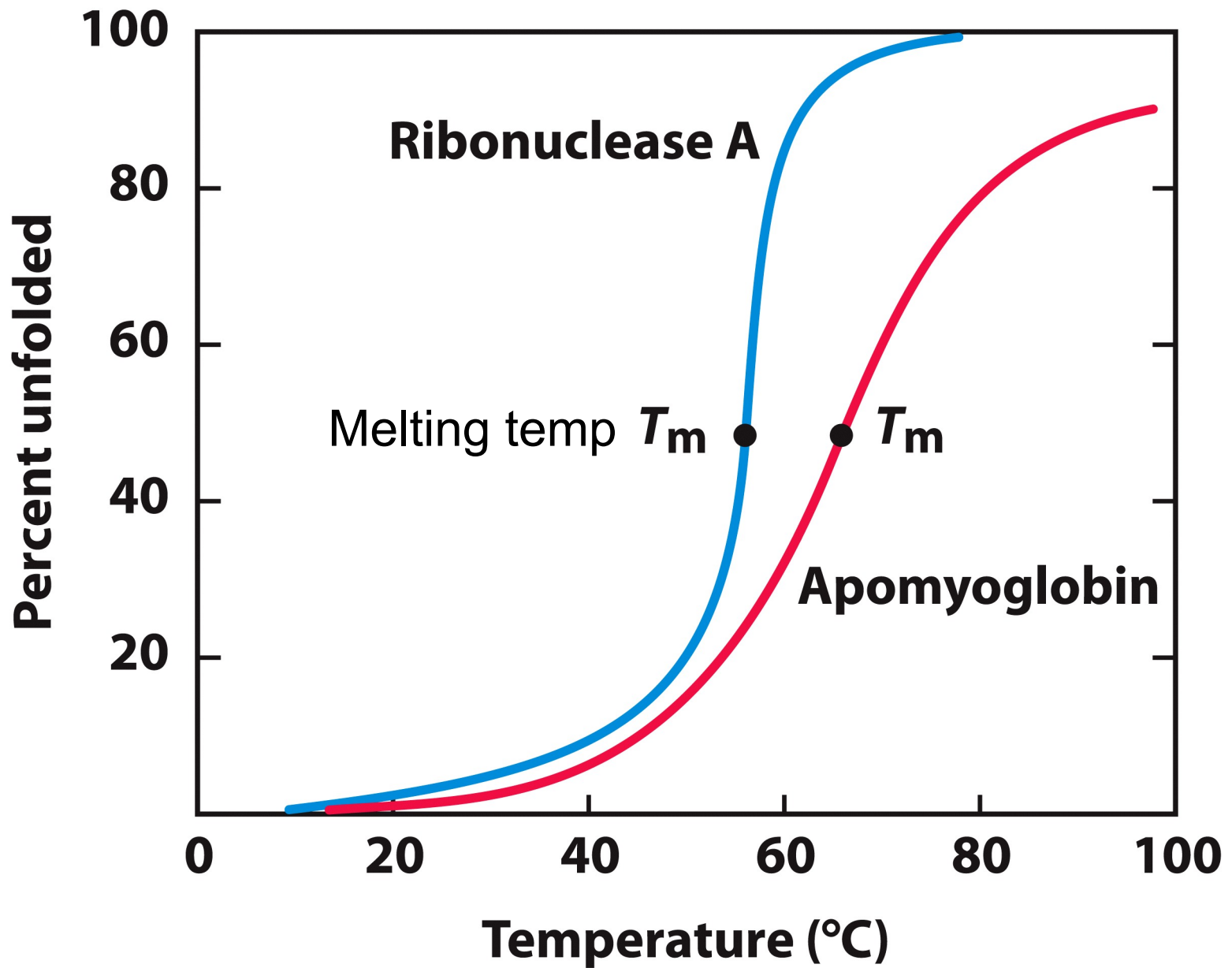


Figure 4-26a

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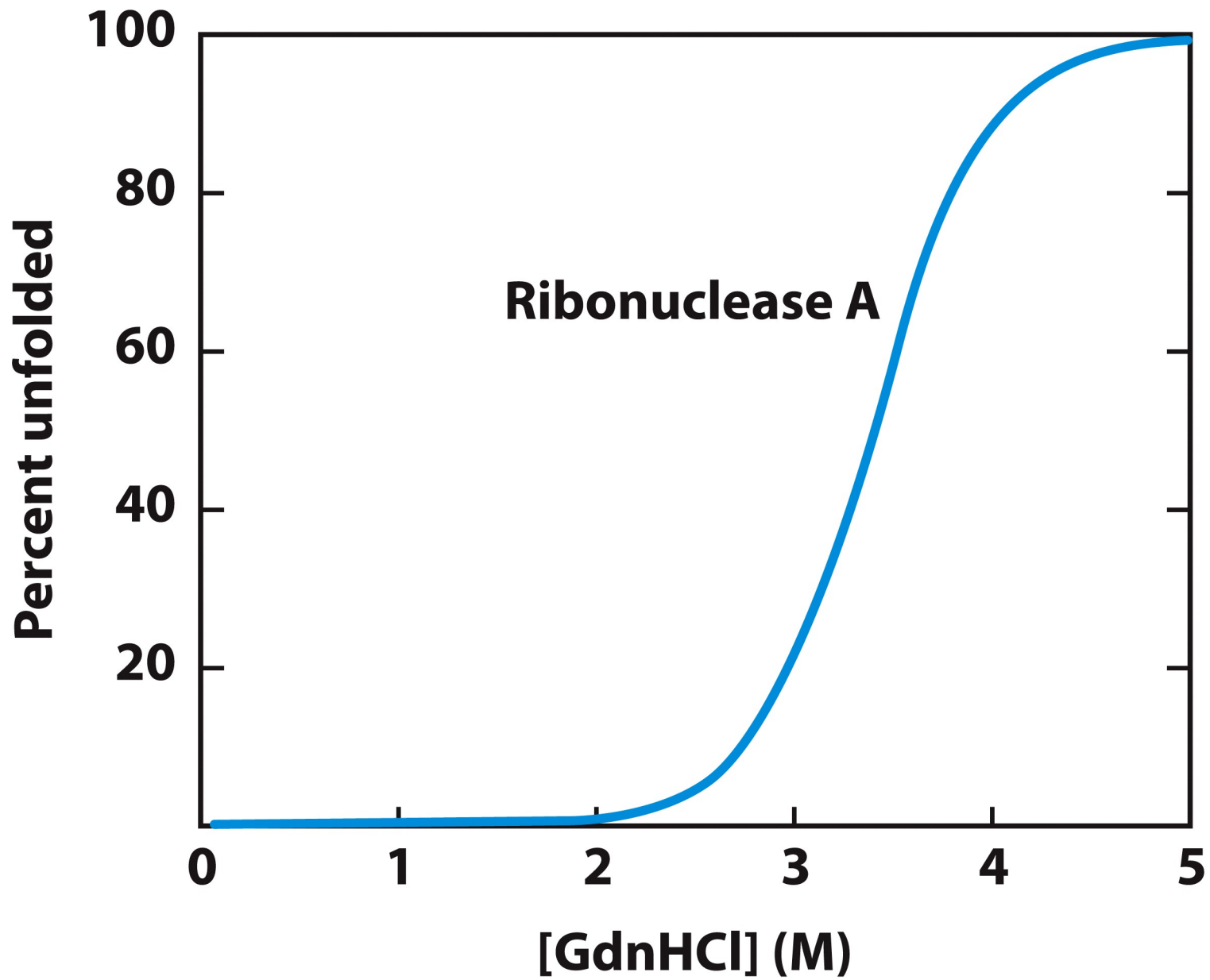


Figure 4-26b

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Ribonuclease Refolding Experiment

- **Renaturation:** some denatured proteins can get back to their native conformation and biological activity if returned to conditions where native conformation is stable
- E.g. **Ribonuclease A** is a small protein that contains 8 cysteines linked via 4 disulfide bonds. It can be *fully denatured* by urea and a reducing agent (to break its 4 disulfide bonds)
- When urea and 2-mercaptoethanol are removed, the protein *spontaneously* refolds, and the correct disulfide bonds are reformed
- *The sequence alone determines the native conformation*

Quite “simple” experiment, but so important it earned Chris Anfinsen the 1972 Chemistry Nobel Prize

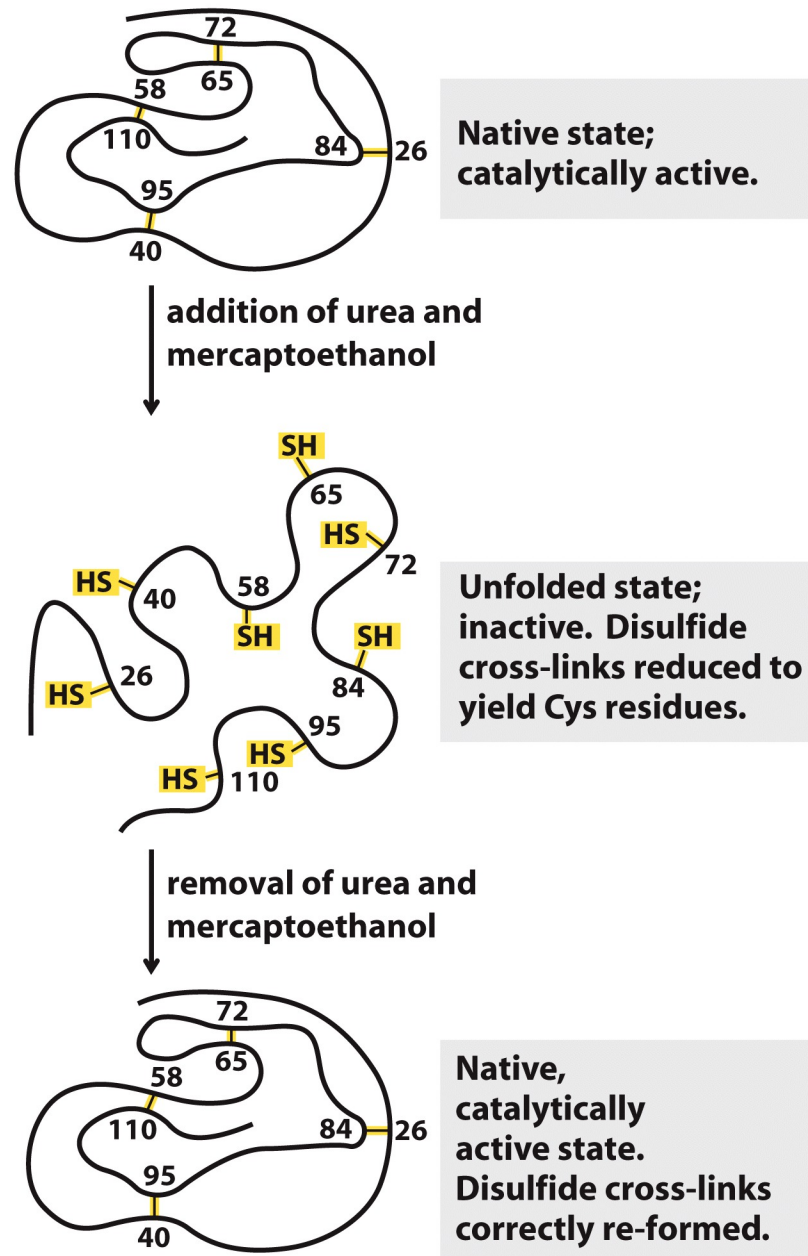


Figure 4-27

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Protein misfolding is the basis of numerous human diseases

- Defects in protein folding cause many diseases:
 - ❑ **Cystic fibrosis** (CF التليف الكيسي) is caused by a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR), a Cl⁻ channel. A mutation in this protein causes misfolding
 - ❑ **Mad cow disease** caused by a misfolded protein (prion)
 - ❑ Amyloid fibrils accumulate in **Alzheimer's disease** and many other **neurodegenerative diseases**. They arise from misfolded normal proteins and polypeptides

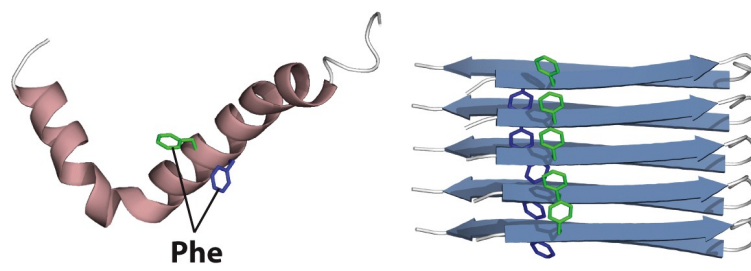
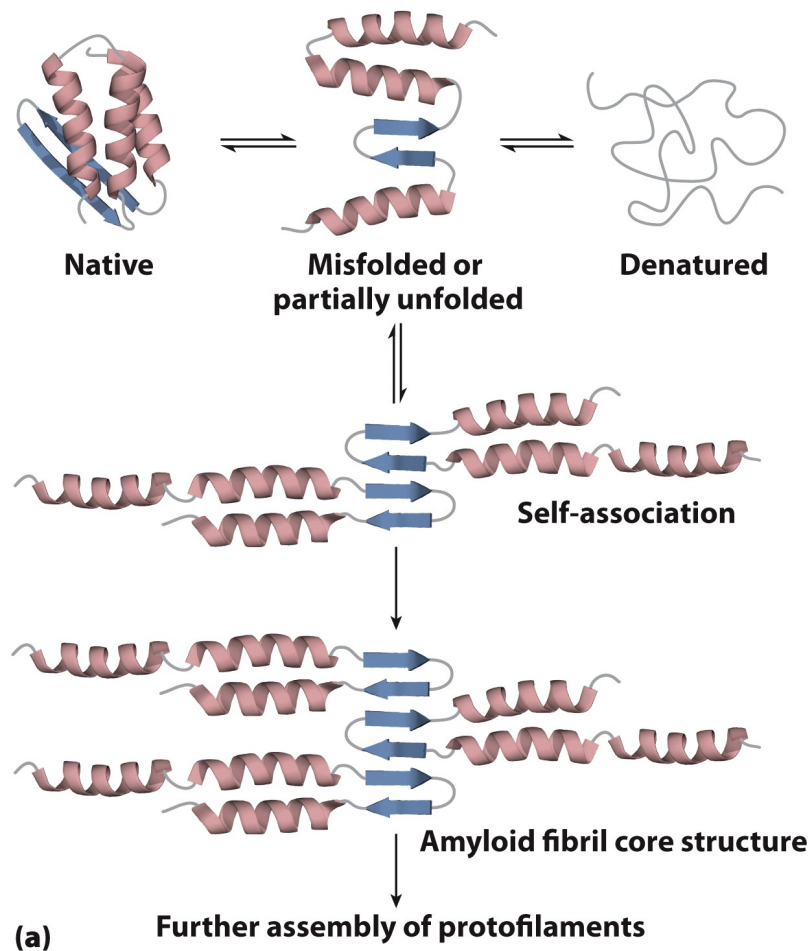


Figure 4-32

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Assisted Folding of Proteins

- Not all proteins fold spontaneously
- **Molecular chaperones** are proteins that help proteins fold by interacting with partially folded polypeptides and providing microenvironments for proper folding
- Two classes:
 - 1. Hsp70** – heat shock proteins of MW ~70 kDa
 - bind to unfolded polypeptides rich in hydrophobic aa
 - need another Hsp40
 - In bacteria, homologs are DnaK and DnaJ
 - prevent the aggregation of unfolded proteins
 - 2. Chaperonins** – (GroEL/GroES in bacteria)
 - barrel and lid, provide good microenvironment for proteins to fold properly

Chaperones prevent misfolding

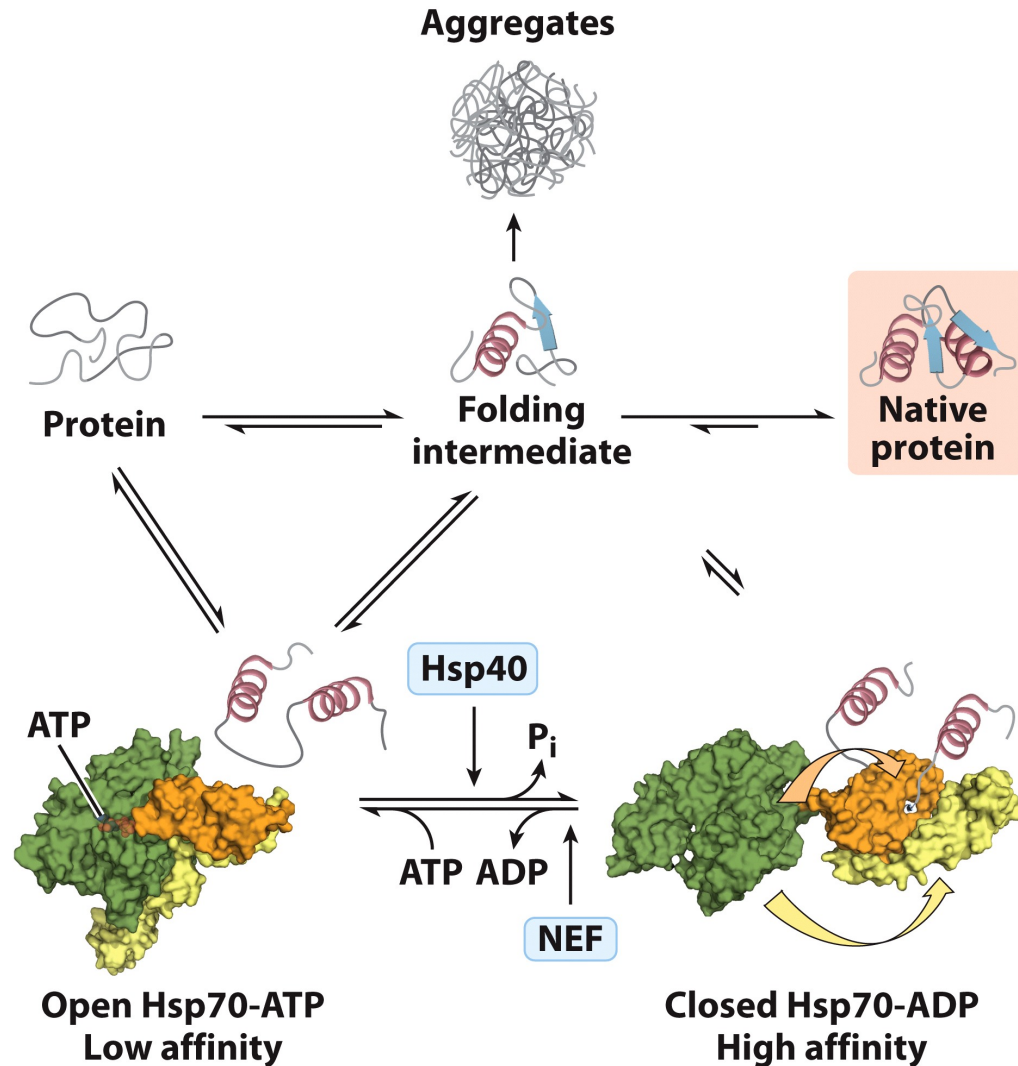


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Chaperonins facilitate folding

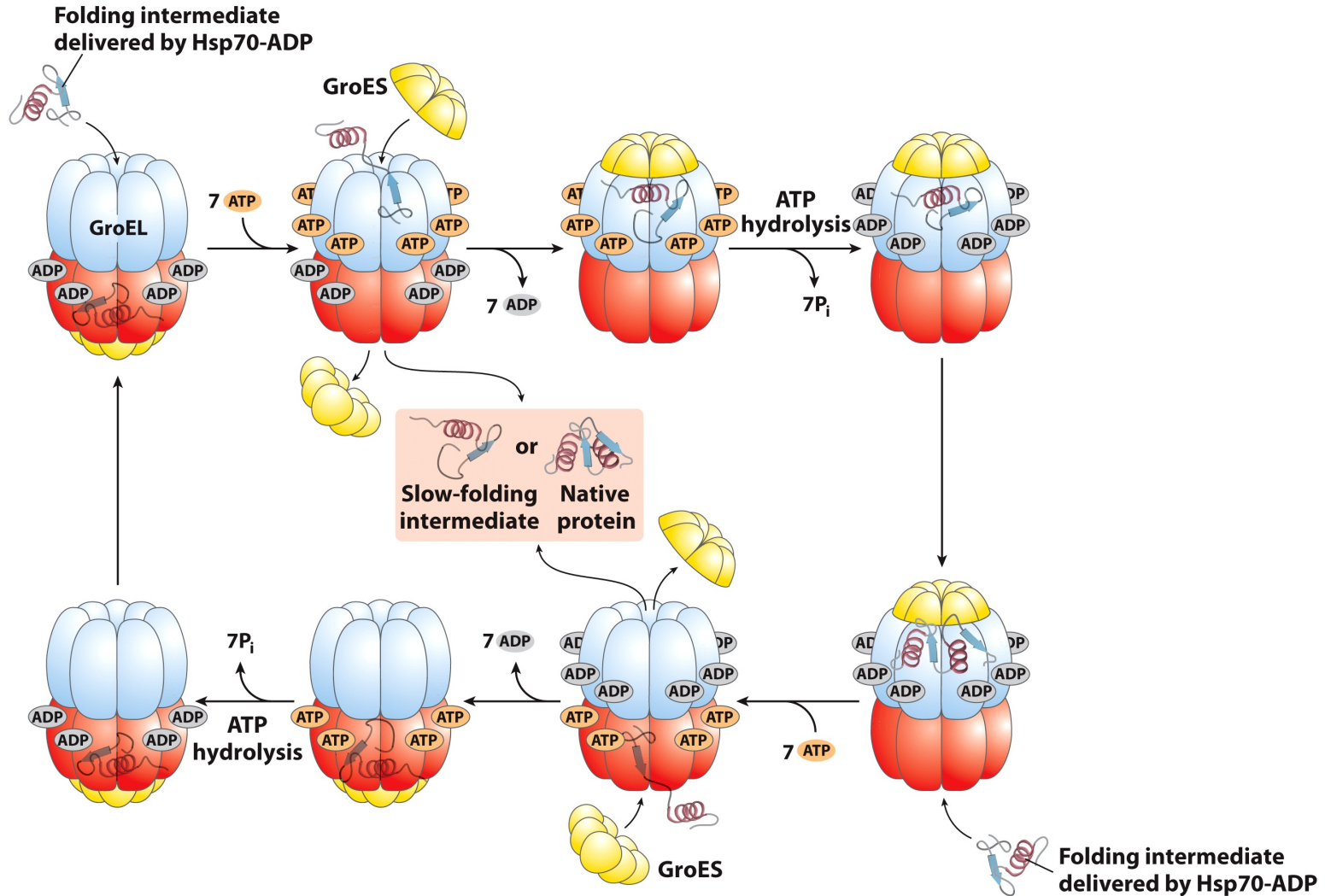


Figure 4-31a

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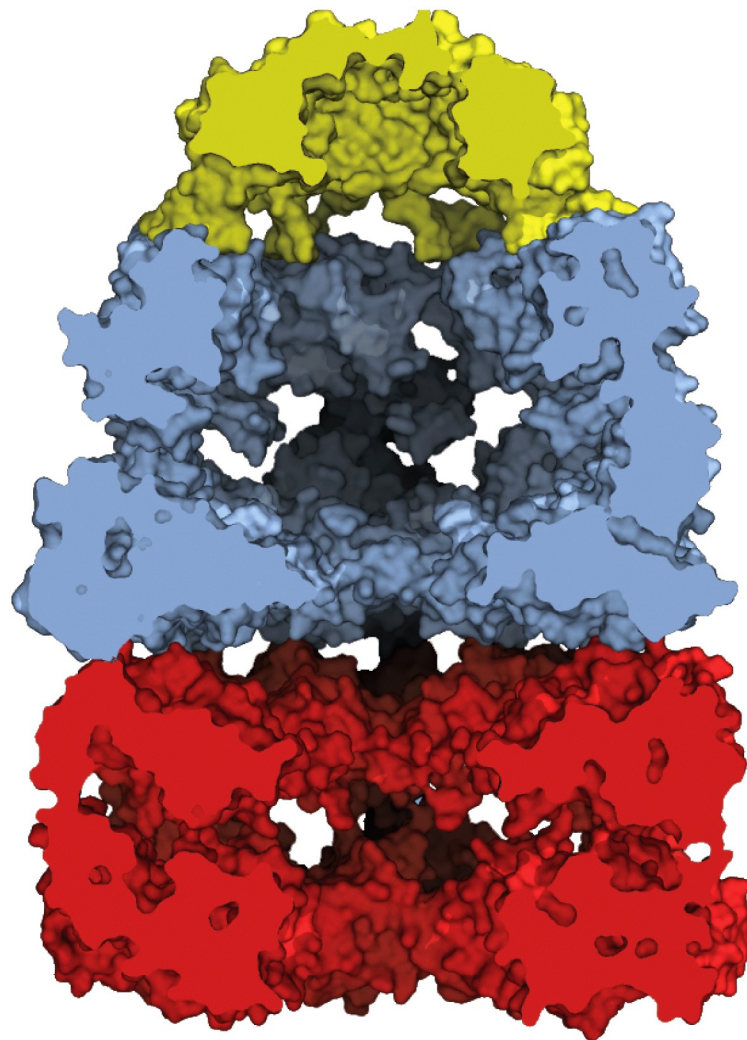
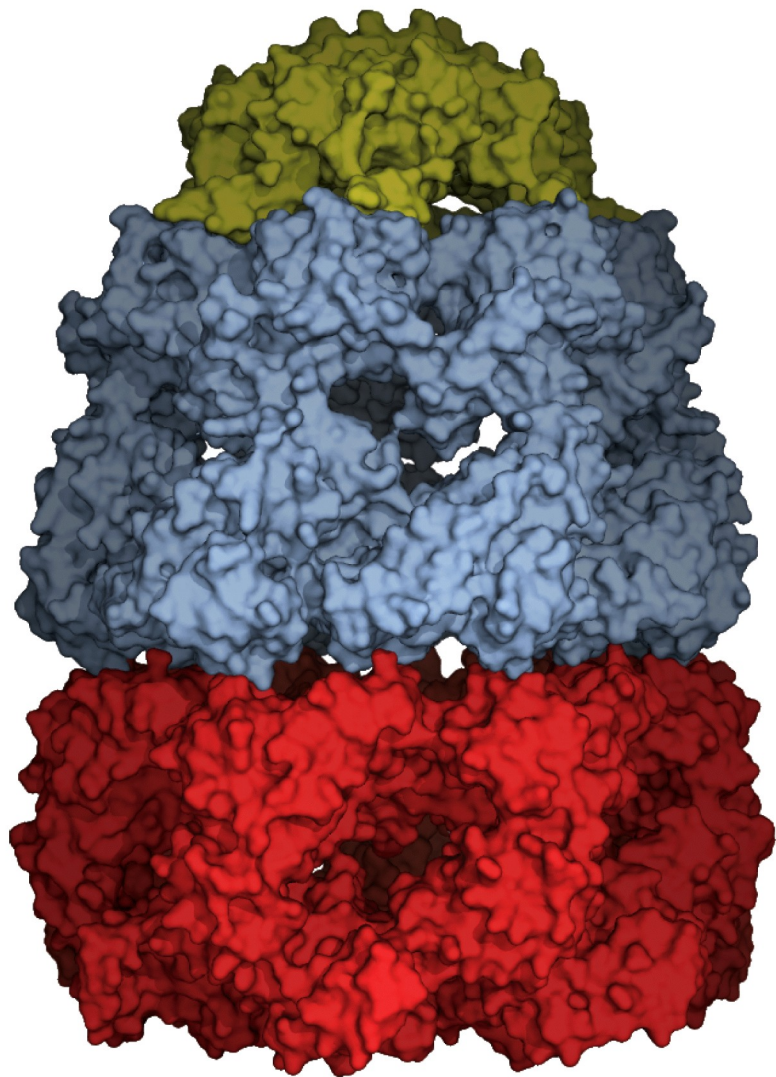


Figure 4-31b

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