Lehninger

SIXTH EDITION

Principles of Biochemistry

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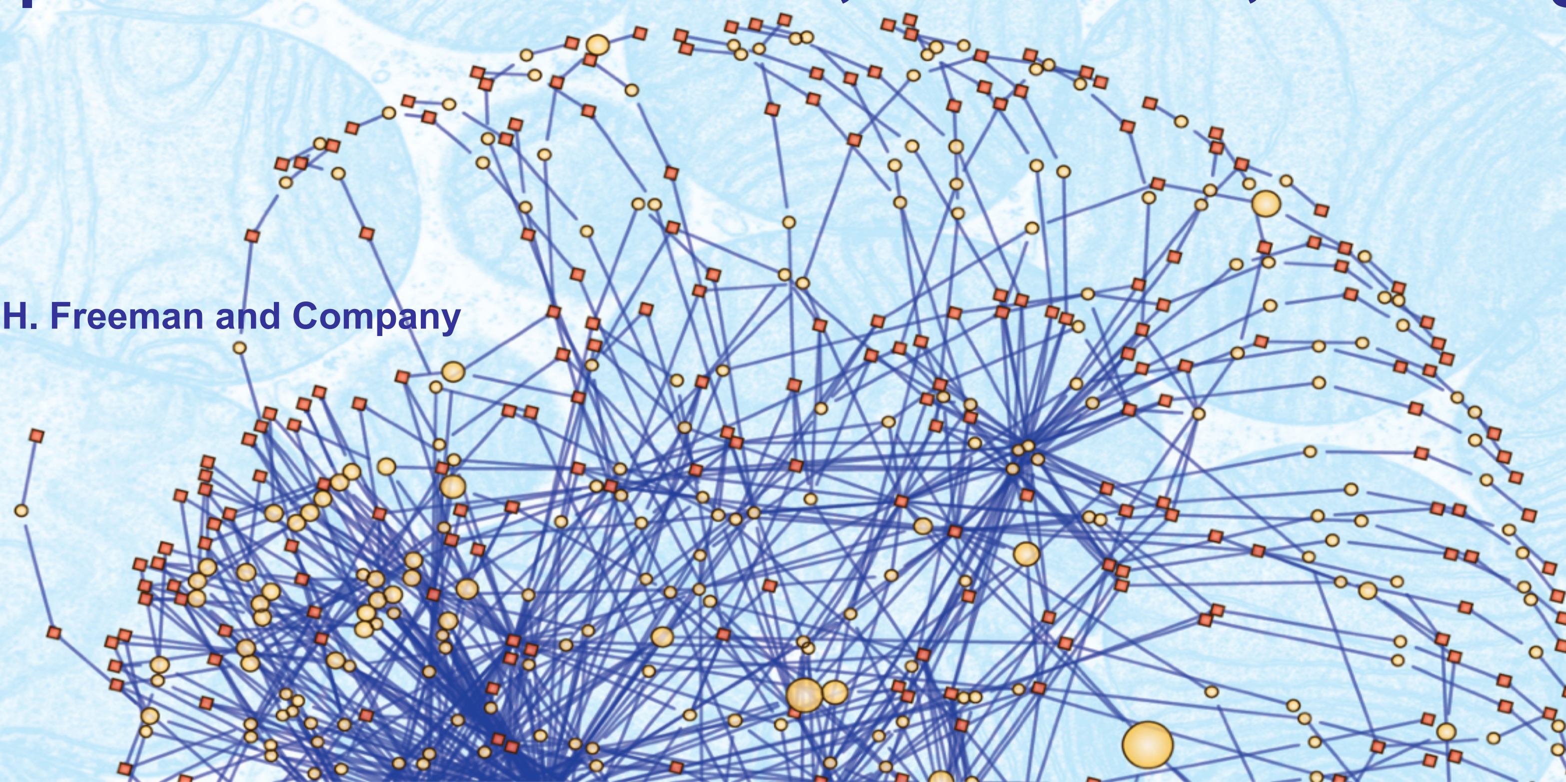
4 | Proteins: Structure, Function, Folding



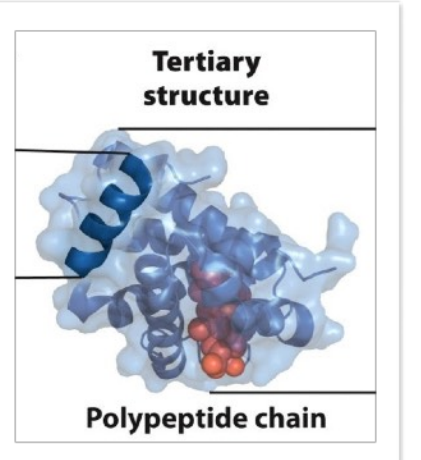
CHAPTER 4
Proteins: Structure, Function, Folding

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

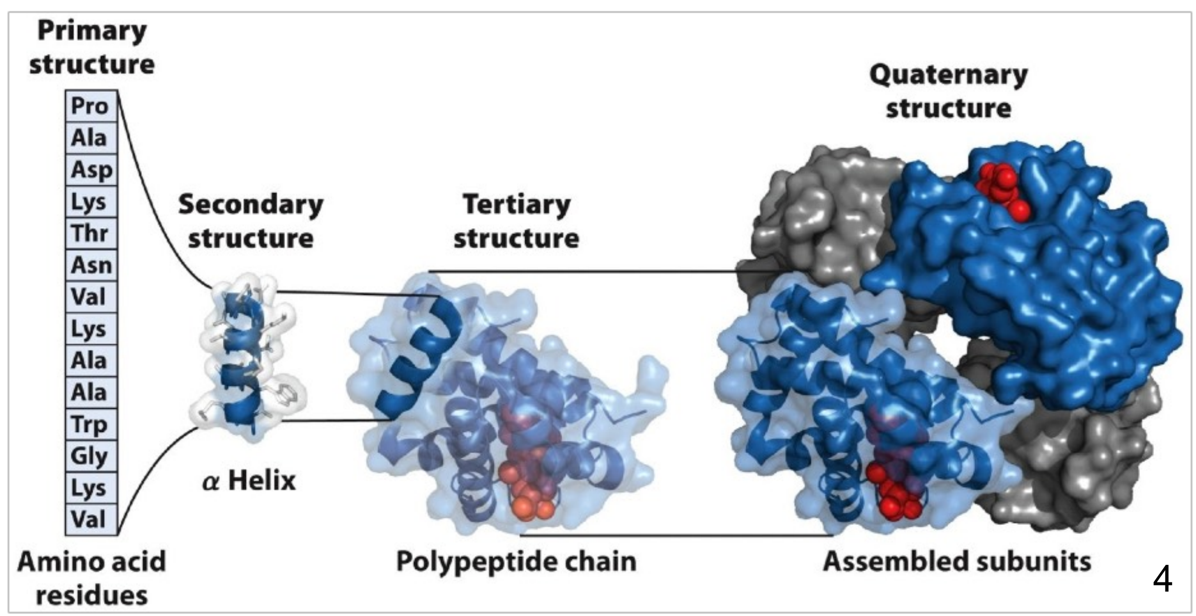
- Protein adopts a specific 3D conformation.
  - In principle, an uncountable number of conformations.
  - In reality, one or a few unique structures.
  - Structure fulfills a specific biological function.



- This structure is called the native fold.
  - A large number of favorable interactions within the protein.
  - A cost in conformational entropy of folding into native fold.

Six Themes About Protein Structure

1. 3D structure determined by amino acid sequence.
2. Function depends on structure.
3. One or a few stable structures.
4. Most important force is noncovalent bond.
5. Structural patterns can be organized.
6. Not static.



Three-Dimensional Structure of Proteins

4.1 Overview of Protein Structure

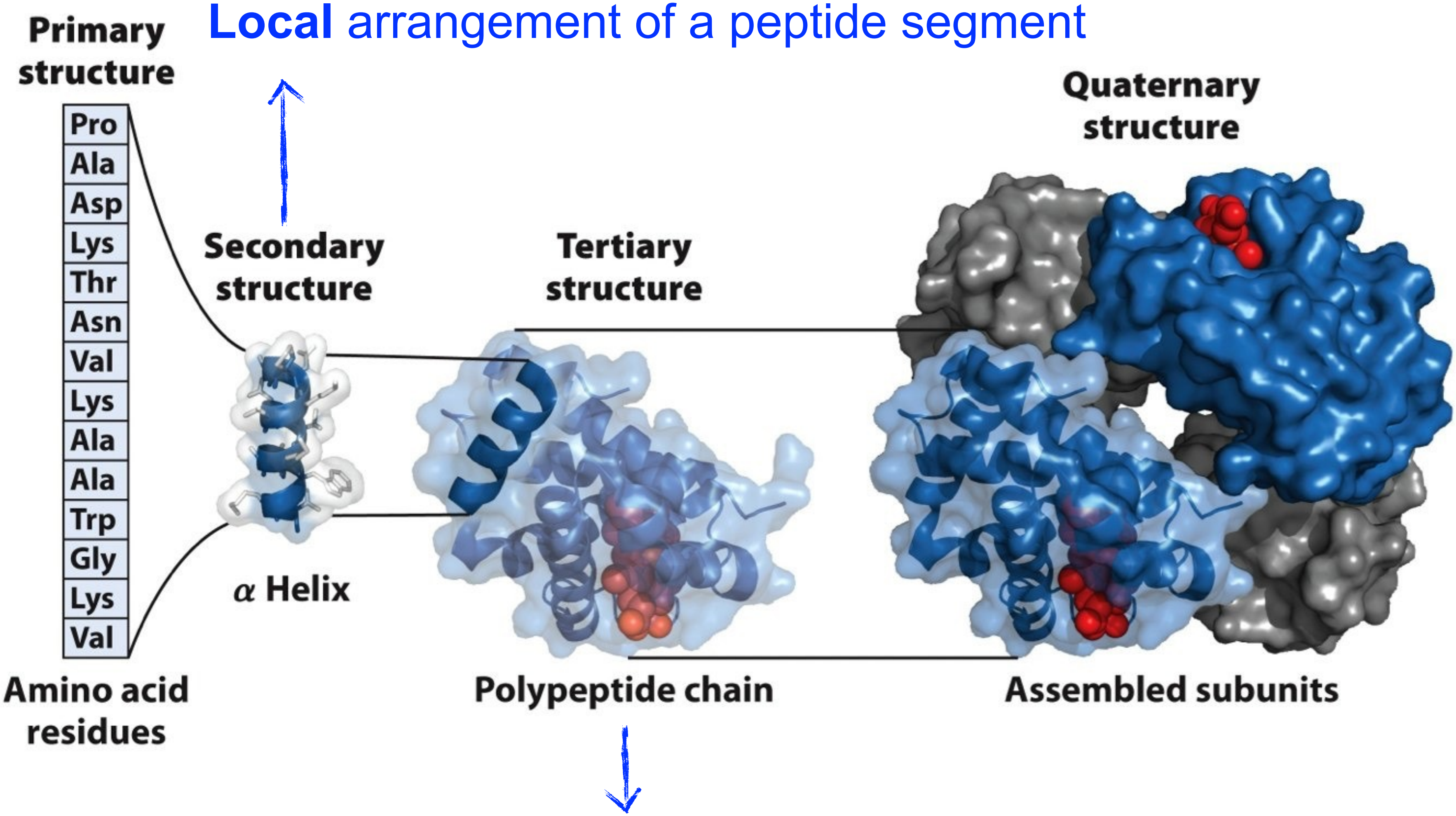
4.2 Protein Secondary Structure

4.3 Protein Tertiary and Quaternary Structure

4.4 Protein Denaturation and Folding

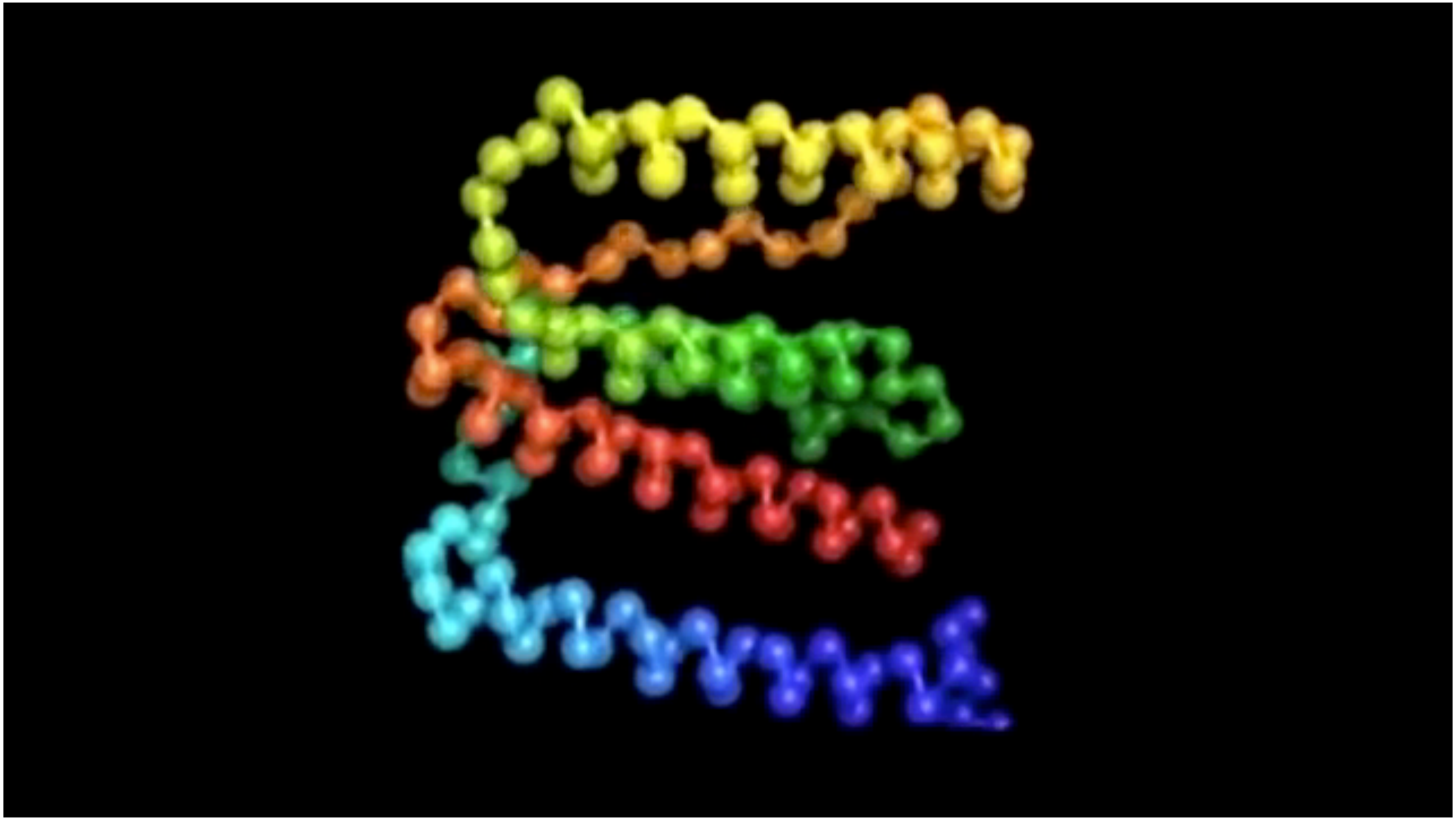
4 Levels of Protein Structure

Local arrangement of a peptide segment



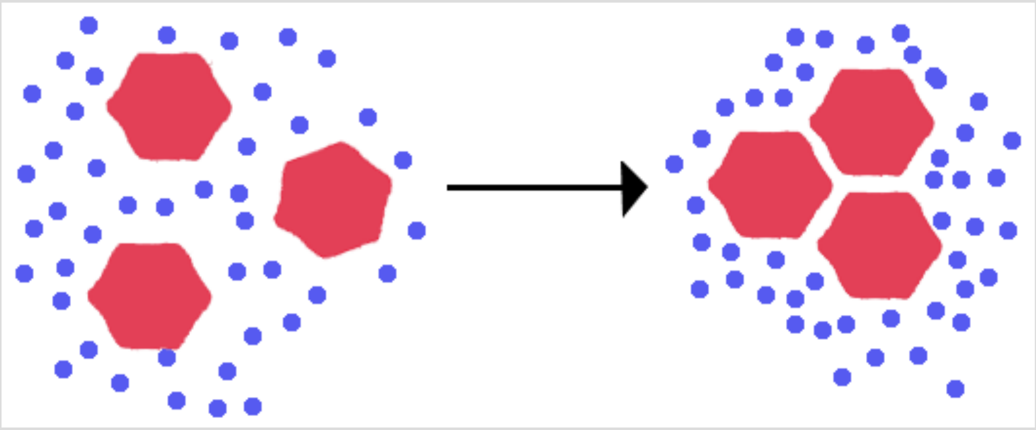
Overall arrangement of the whole polypeptide

From Primary to Tertiary Structure



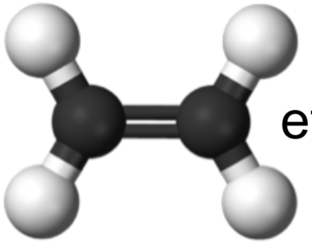
Structure Stabilized by **Weak** Interactions

- Covalent bond (disulfide)
- Noncovalent bond
  - **hydrophobic interaction**
  - hydrogen bond
  - ionic interaction

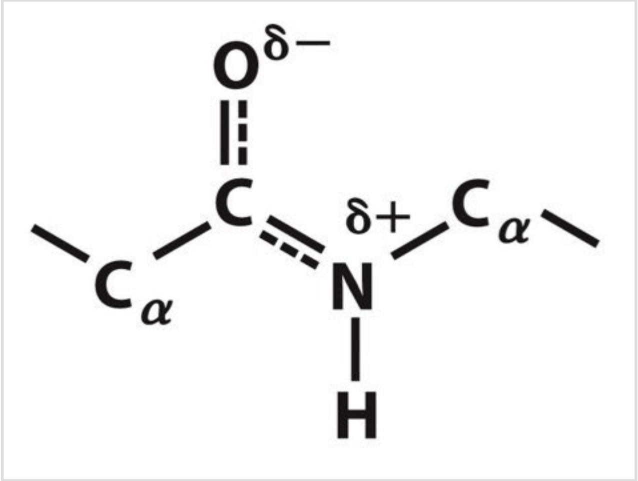


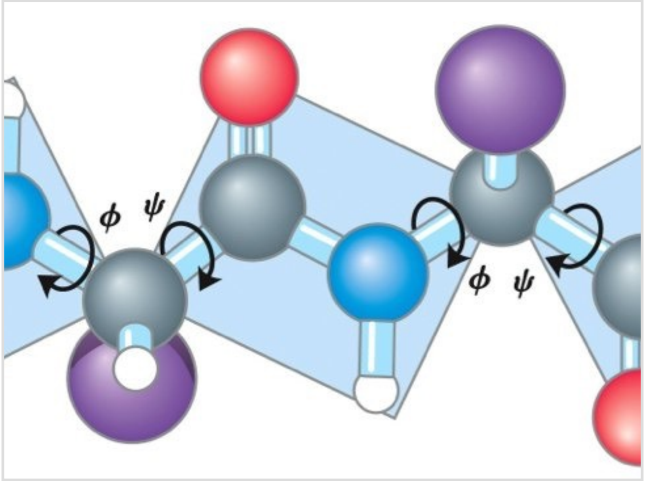
Structure of Peptide Bond

- Structure partially dictated by peptide bond.
- A resonance hybrid of two canonical structures.
- The resonance causes the peptide bonds:
  - to be less reactive.
  - to be quite **rigid** and nearly **planar**.

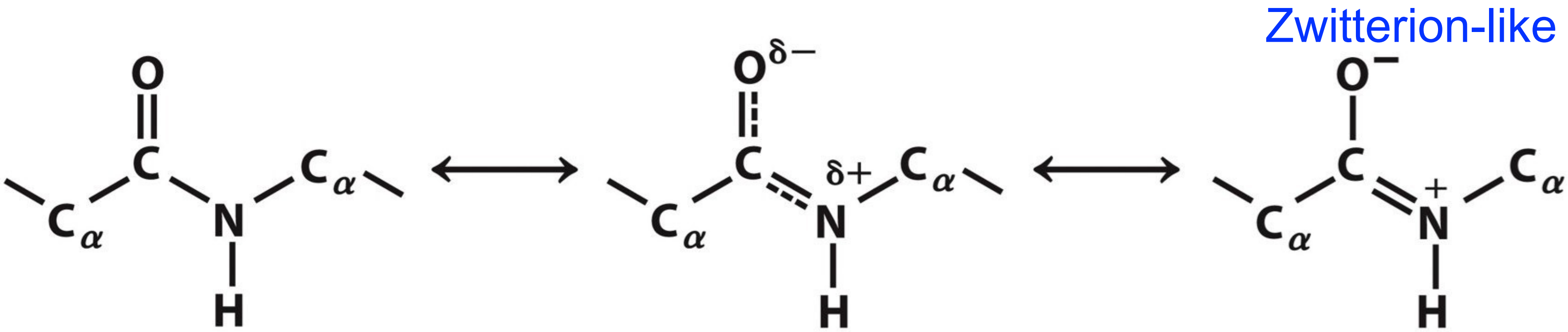


ethylene

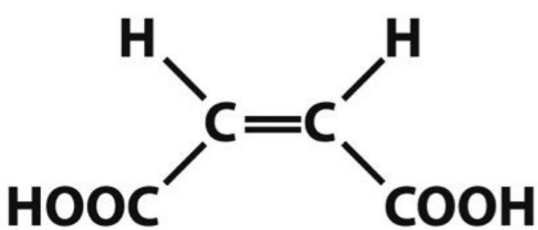




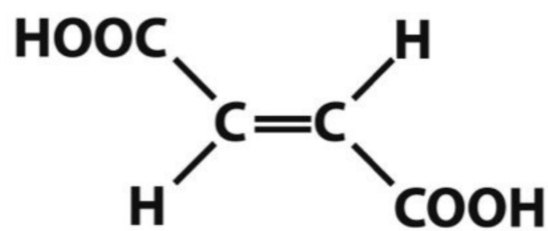
Resonance in Peptide Bond



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



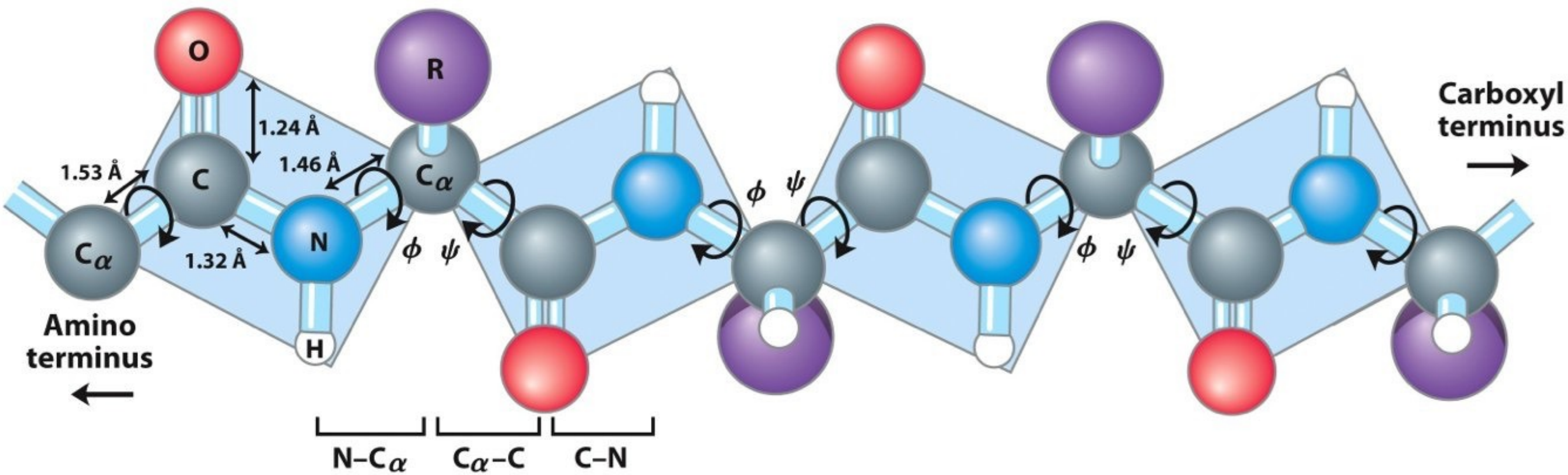
Maleic acid (cis)



Fumaric acid (trans)

Polypeptide Made up of A Series of Planes Linked at α Carbons

Two C-N bond lengths are different?



on the SAME plane

α-carbon

carbonyl group (C and O)

amino group (N and H)

α-carbon

Summary 4.1 Overview of Protein Structure

- A typical protein has one or more stable three-dimensional structures or conformations.
- Protein structure is stabilized largely by multiple weak interactions, with hydrophobic interactions being the major contributors.
- The peptide bond has a partial double-bond character that keeps it in a rigid planar configuration.

Example Question

Any given protein is characterized by a unique amino acid sequence (primary structure) and three-dimensional (tertiary) structure. How are these related?

Example Question

All of the following are considered "weak" interactions in proteins *except:*

A) hydrogen bonds.

B) hydrophobic interactions.

C) ionic bonds.

D) peptide bonds.

E) all above interactions are weak interactions.

Example Question

Which statement about the peptide bond is correct?

A) peptide bonds have many different conformations that are equally probable.

B) peptide bonds are essentially planar, with no rotation about the C-N axis.

C) peptide bonds in proteins are uncommon.

D) peptide bond structure is extraordinarily complex and poorly understood.

E) primary structure of all proteins is similar, although the secondary and tertiary structure may differ greatly.

Three-Dimensional Structure of Proteins

4.1 Overview of Protein Structure

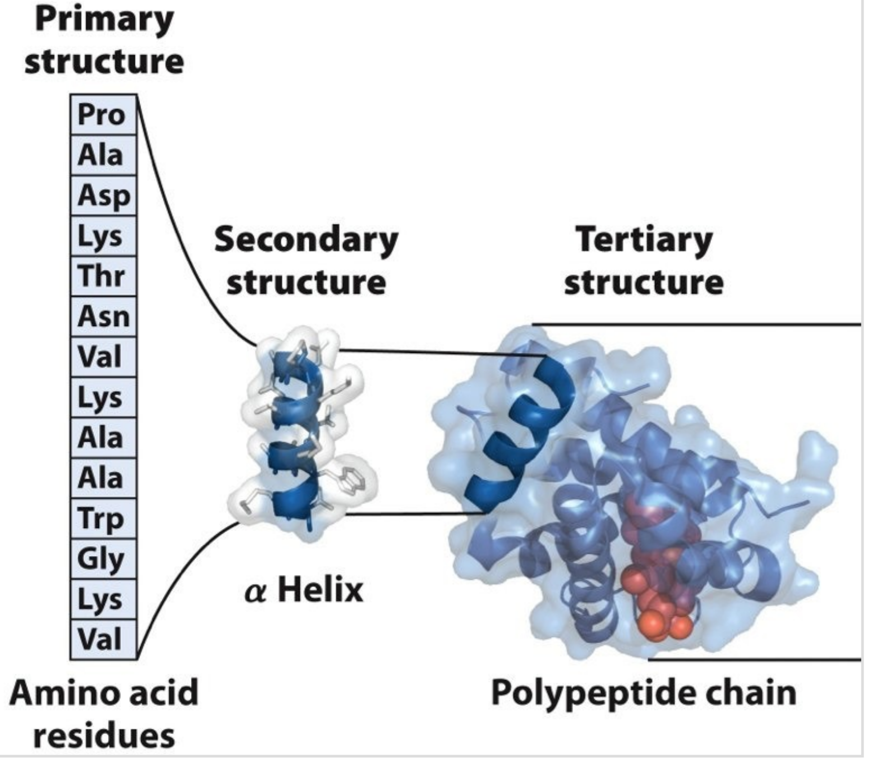
4.2 Protein Secondary Structure

4.3 Protein Tertiary and Quaternary Structure

4.4 Protein Denaturation and Folding

Secondary Structures

- Any chosen segment of a polypeptide chain.
- Local spatial arrangement of main-chain atoms.
- NOT positioning of side chains.
- NOT relationship to other segments.



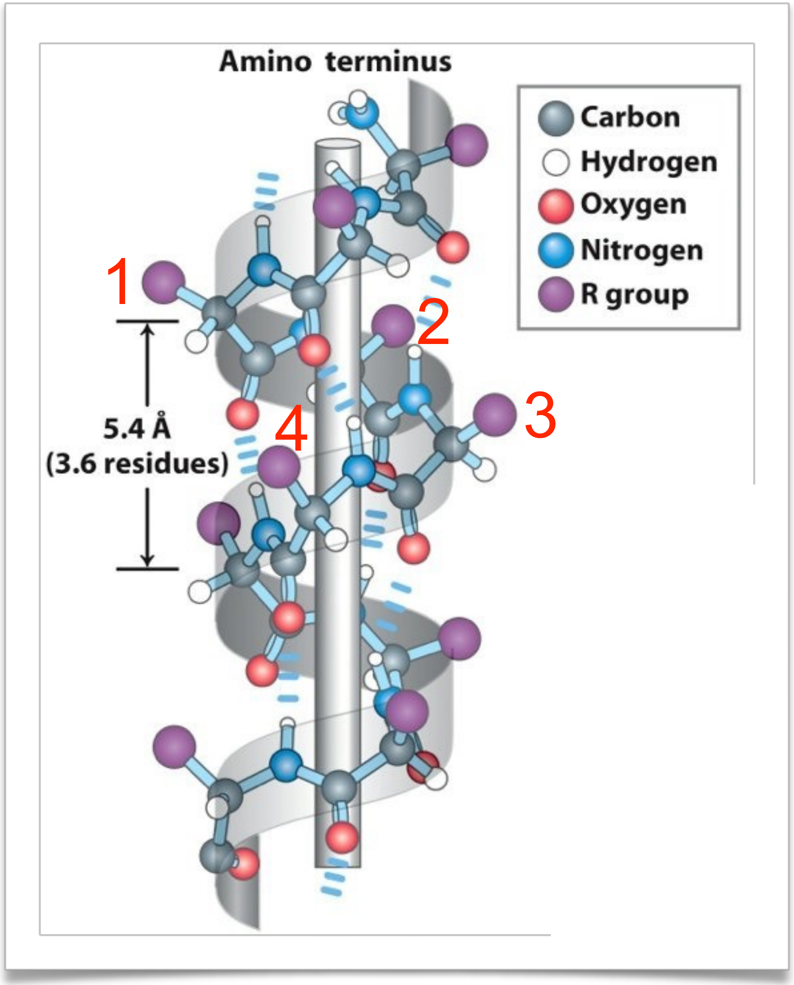
Secondary Structures

Three common secondary structures:

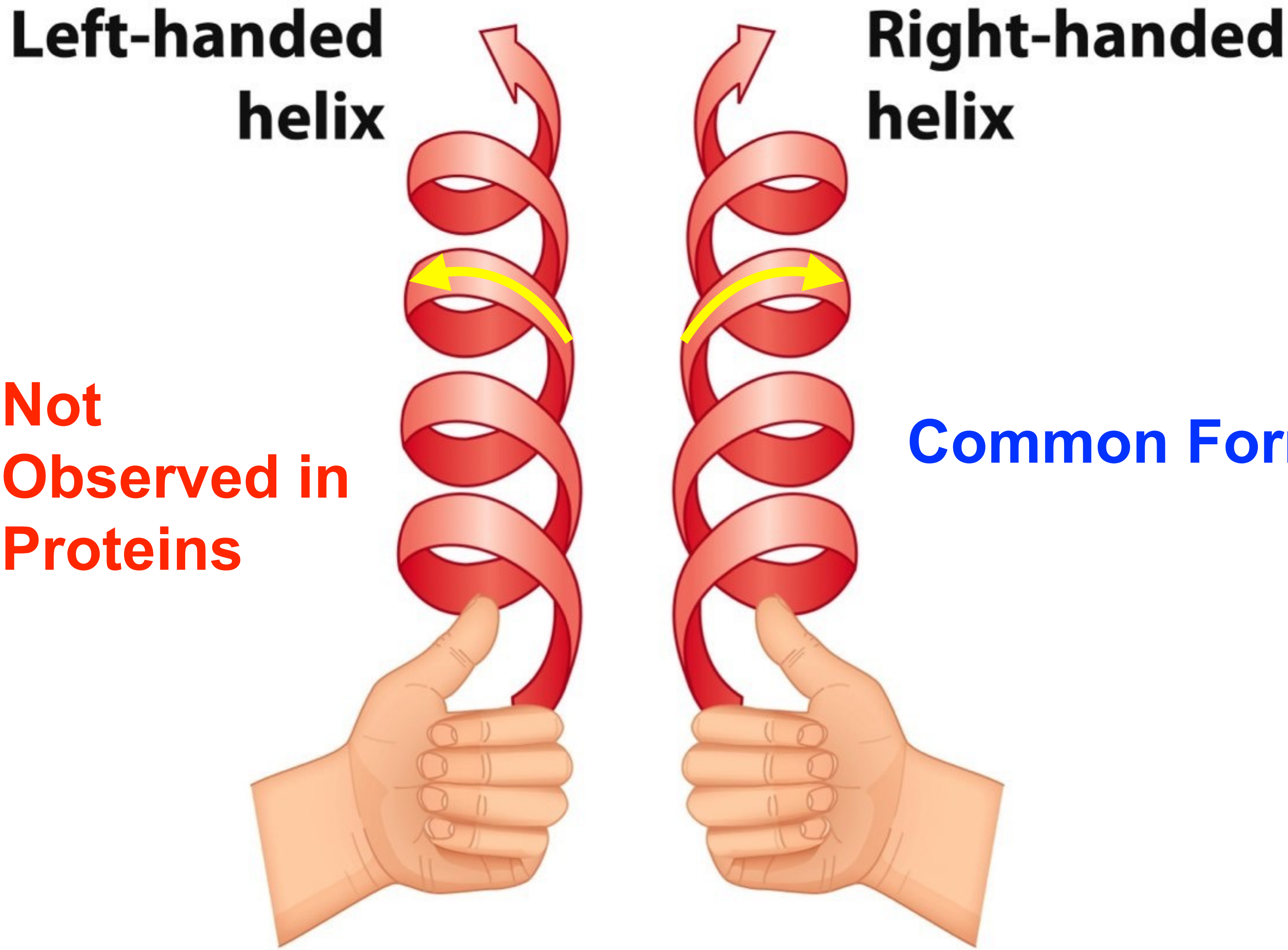
- α helix
  - stabilized by hydrogen bonds between **nearby** residues.
- β sheet
  - stabilized by hydrogen bonds between adjacent segments that **may not be nearby** in primary sequence.
- β turn
  - used to connect two adjacent β sheets.

α Helix

- Side chains point outward and roughly perpendicular with the axis.
- 3.6 residues per turn.
  - 5.4 Å along the axis
  - 1 Å (angstrom) = 10^{-10} m = 0.1 nm.
- Hydrogen bonds between the backbone amides of 1st and 4th peptide bonds.



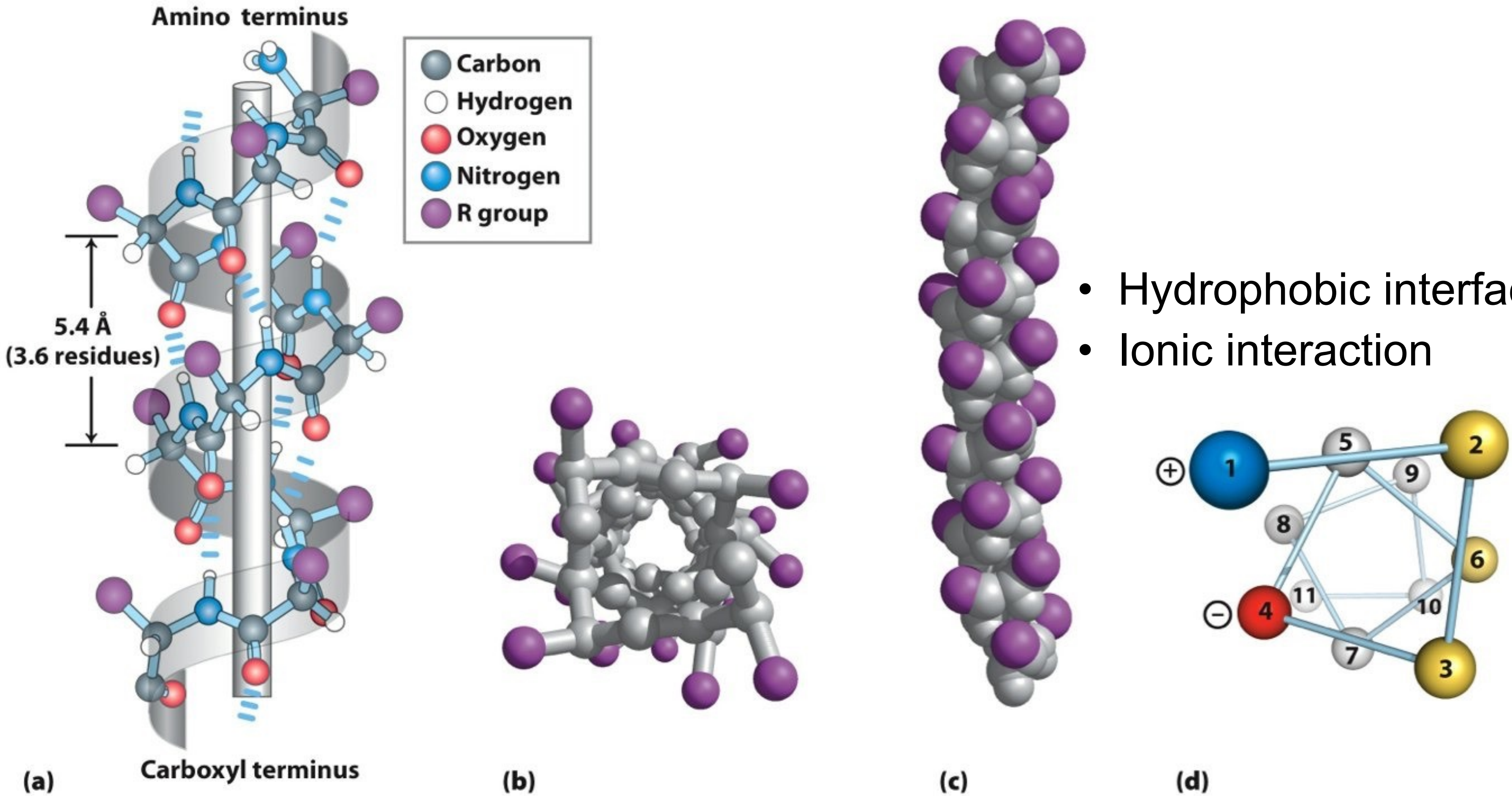
What is a Right-handed Helix?



Not Observed in Proteins

Common Form

α Helix



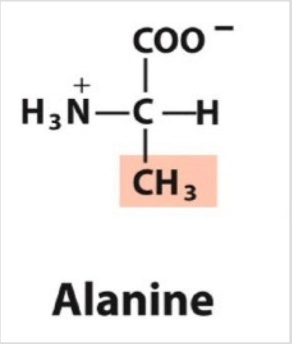
- Hydrophobic interface
- Ionic interaction

Side view Top view Space-filling Interactions

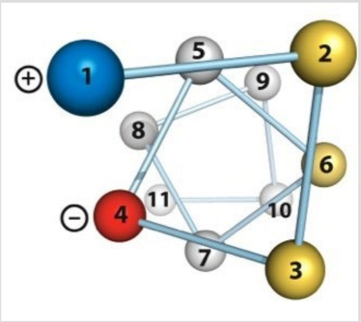
False impression that helix interior is hollow

Sequence Affects Helix Stability

- Not all polypeptide sequences adopt α-helical structures.
- Ala is the strongest helix former.
  - Small hydrophobic residues.



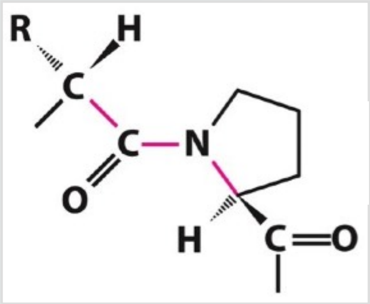
- Pro acts as a helix breaker.
  - Rotation around the N-C_α bond is impossible.



- Gly acts as a helix breaker.
  - the tiny R-group supports other conformations.
- Attractive or repulsive interactions between side chains 3-4 amino acids apart will affect formation.

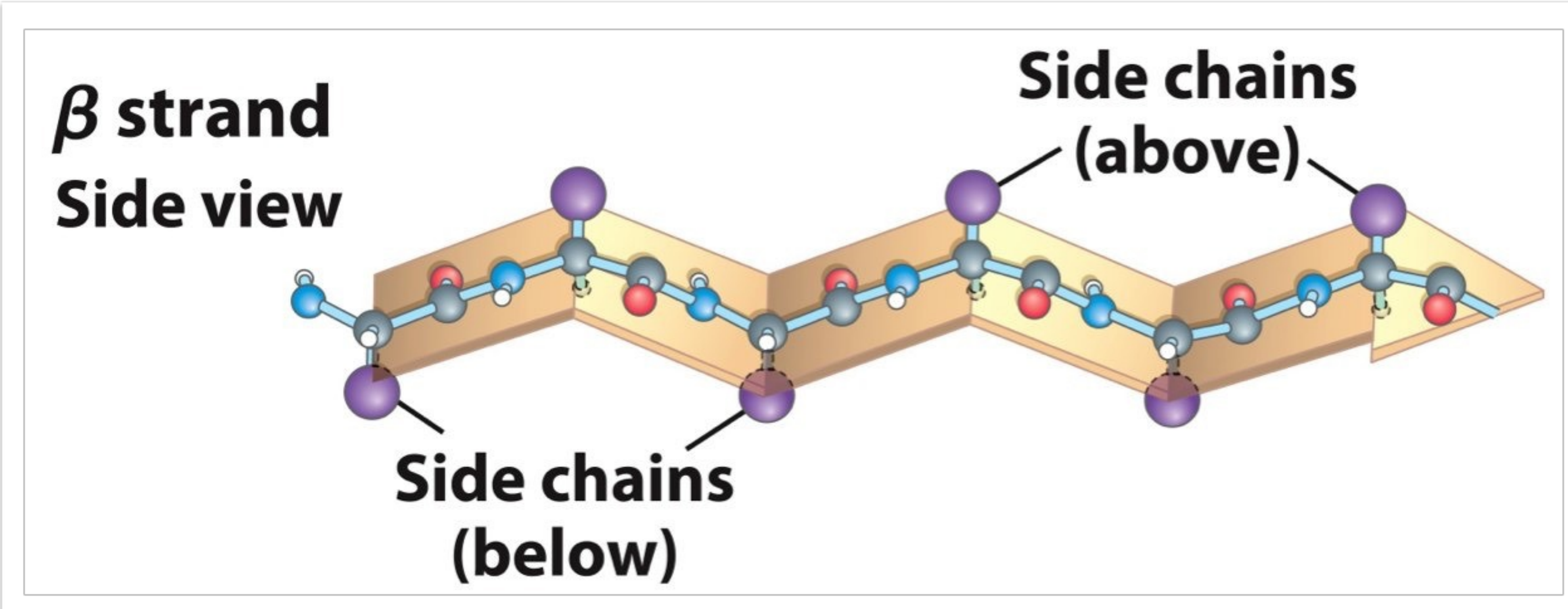
TABLE 4–2 **Propensity of Amino Acid Residues to Take Up an α-Helical Conformation**

Amino acid	ΔΔG° (kJ/mol)*	Amino acid	ΔΔG° (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



β Sheet

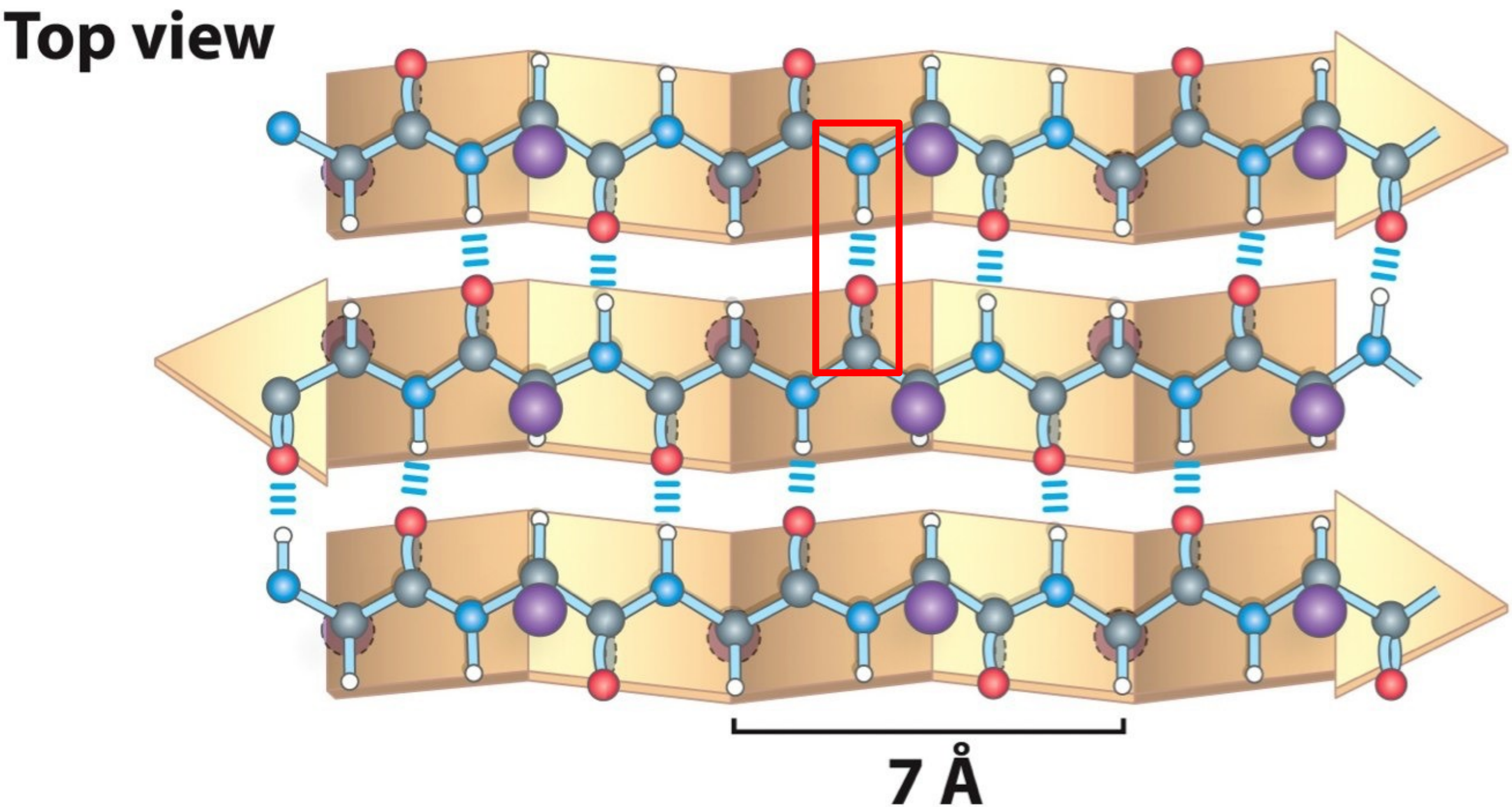
- A pleated sheet-like structure.
- Held together by hydrogen bonds between the backbone amides in different segments.
- Side chains protrude from the sheet alternating in up and down direction.



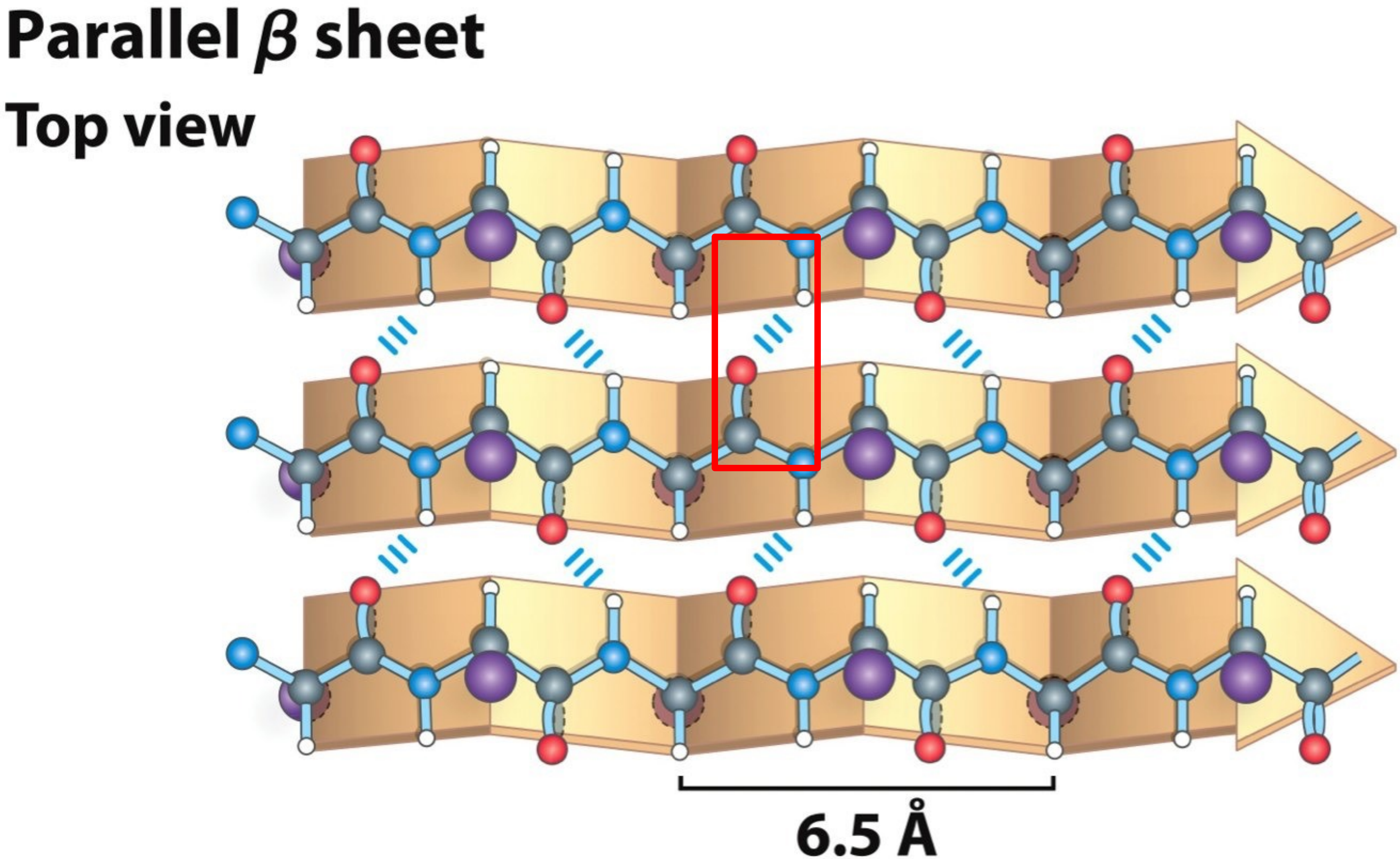
Parallel and Antiparallel β Sheets

- 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



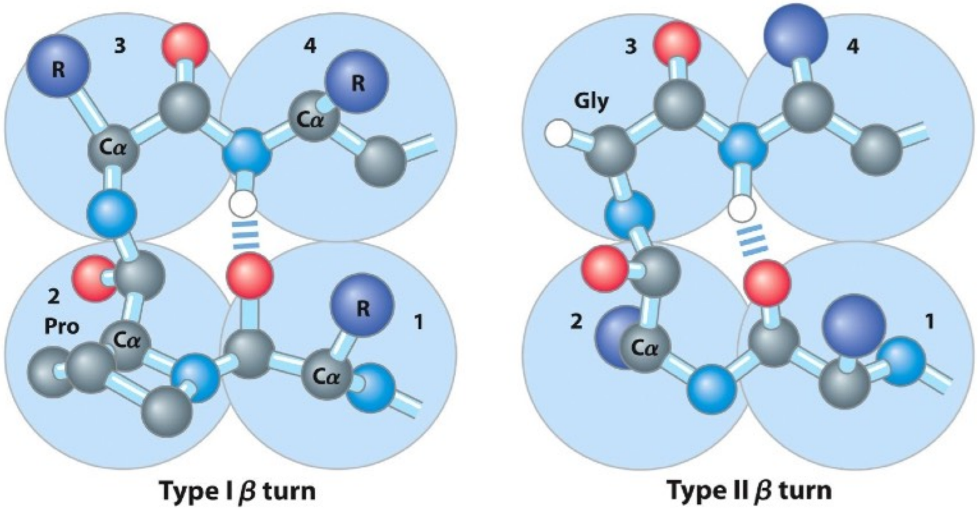
hydrogen bonds: **In-line.**



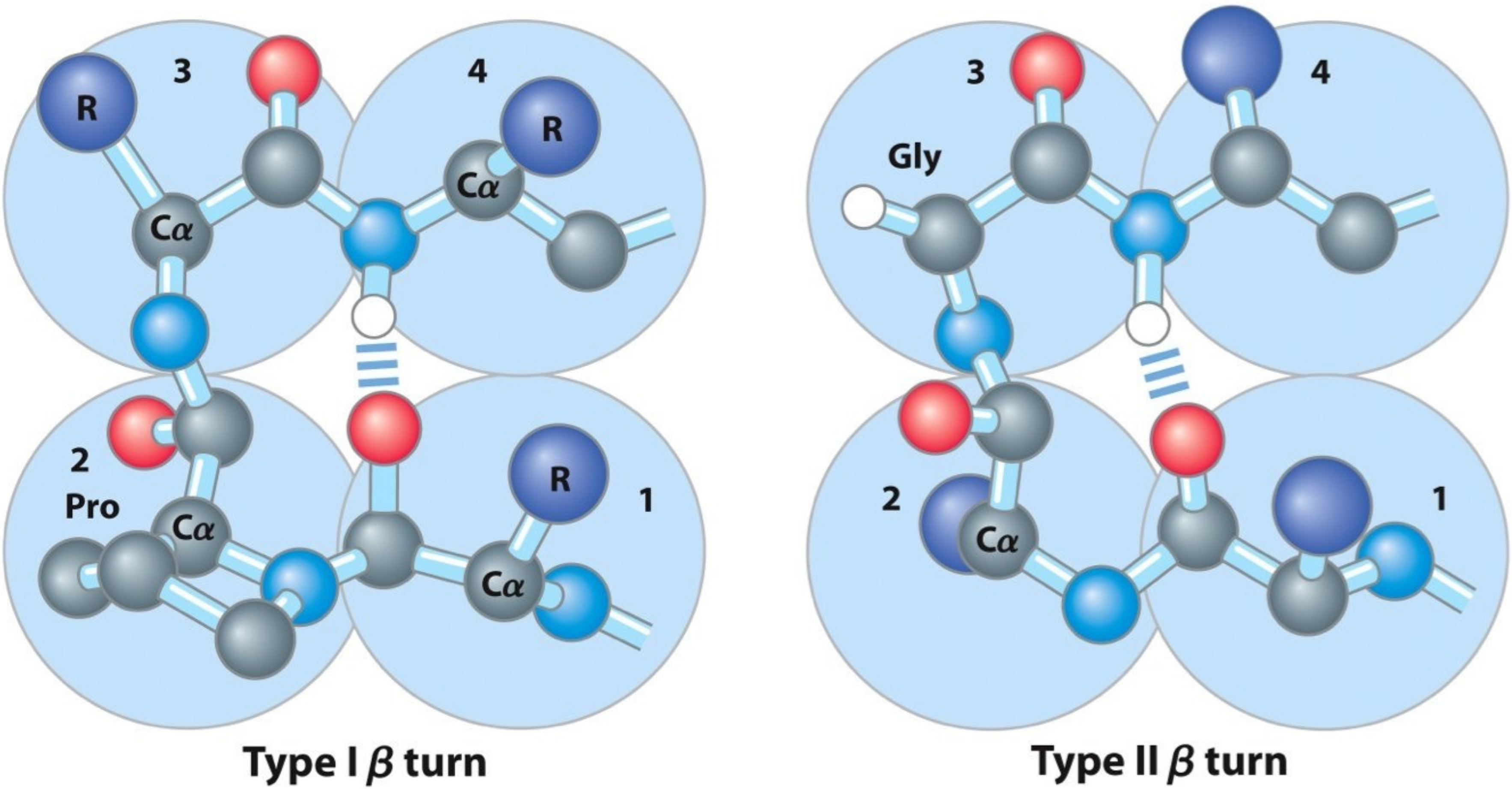
hydrogen bonds: **Not in-line, or distorted.**

β Turn

- 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.
- Proline in position 2 or glycine in position 3 are common in β turns

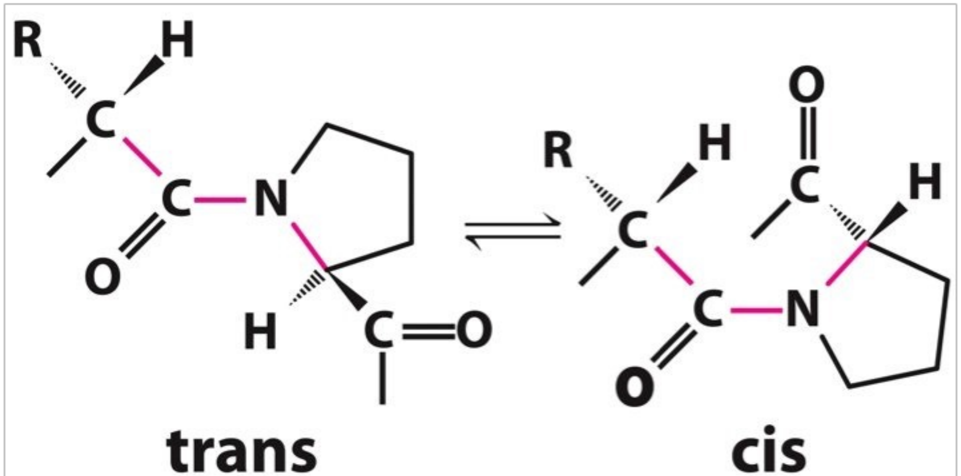


β Turn



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

Summary 4.2 Secondary Structure

- Secondary structure is the **local** spatial arrangement of main-chain atoms in a selected segment of polypeptide chain.
- The α helix (3.6 residues, 5.4 Å, right-handed, Ala).
- The β sheet (parallel vs antiparallel).
- The β turn (180°, four residues, Pro).

Example Question

Secondary structure describes the relationship and interaction of amino acid residues that are:

A) always side by side.

B) generally near each other in sequence.

C) restricted to about 7 of the 20 standard amino acids.

D) often on different polypeptide strands.

E) usually near the polypeptide chain's amino terminus or carboxyl terminus.

Example Question

Roughly how many amino acids are there in one turn of an α−helix?

A) 1

B) 2.8

C) 3.6

D) 4.2

E) 10

Example Question

What is the length of a polypeptide with 80 amino acid residues in a single contiguous α helix?

Example Question

A D-amino acid would interrupt an α−helix made of L-amino acids. Another naturally occurring hindrance to the formation of an α−helix is the presence of:

A) a negatively charged Arg residue.

B) a nonpolar residue near the carboxyl terminus.

C) a positively charged Lys residue.

D) a Pro residue.

E) two Ala residues side by side.

Example Question

A sequence of amino acids in a certain protein is found to be -Ser-Gly-Pro-Gly-. The sequence is most probably part of a(n):

A) antiparallel β sheet.

B) parallel β sheet.

C) α helix.

D) β sheet.

E) β turn.

Three-Dimensional Structure of Proteins

4.1 Overview of Protein Structure

4.2 Protein Secondary Structure

4.3 Protein Tertiary and Quaternary Structure

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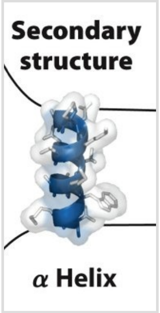
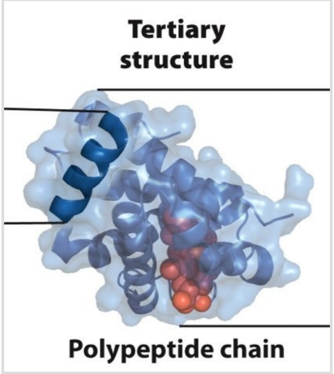
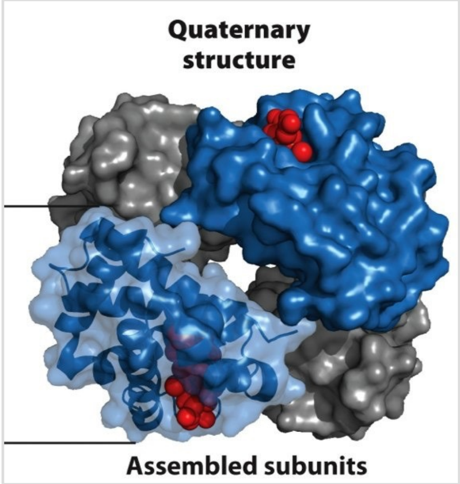
Protein Tertiary and Quaternary Structure

- 3° structure refers to the overall spatial arrangement of atoms in a protein
  - Secondary structure: adjacent residues
  - Tertiary structure: longer-range interactions
- 4° structure refers to the arrangement of subunits in a multisubunit protein in three-dimensional complex.
- Stabilized by numerous weak interactions between amino acid side chains.
  - Largely hydrophobic and polar interactions
  - Can be stabilized by disulfide bonds



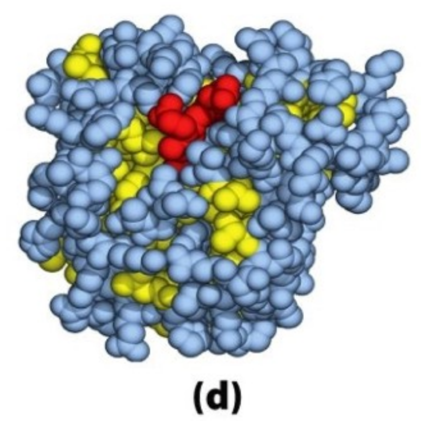




Fibrous Proteins and Globular Proteins

- Fibrous Proteins
  - Long strands or sheets
  - A single type of secondary structure
  - Provide support, shape, and external protection
- Globular Proteins
  - Spherical or globular shape
  - Several types of secondary structure
  - Enzymes and regulatory protein

(c)

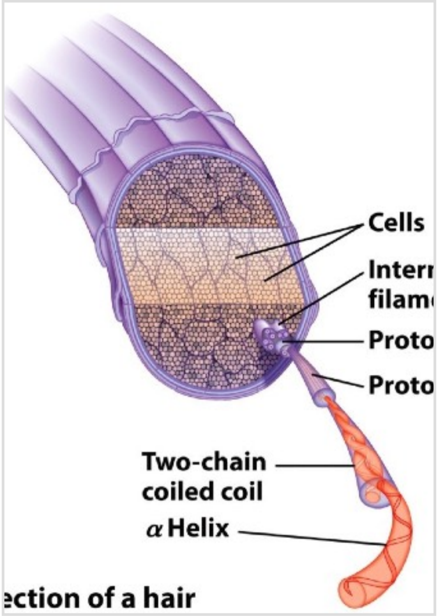


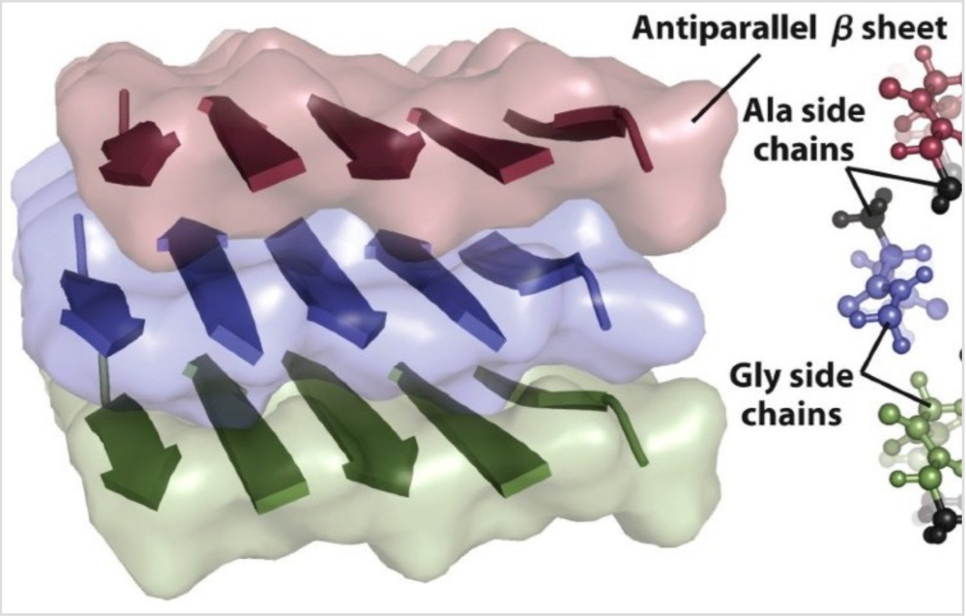
(d)

Fibrous Proteins: From Structure to Function

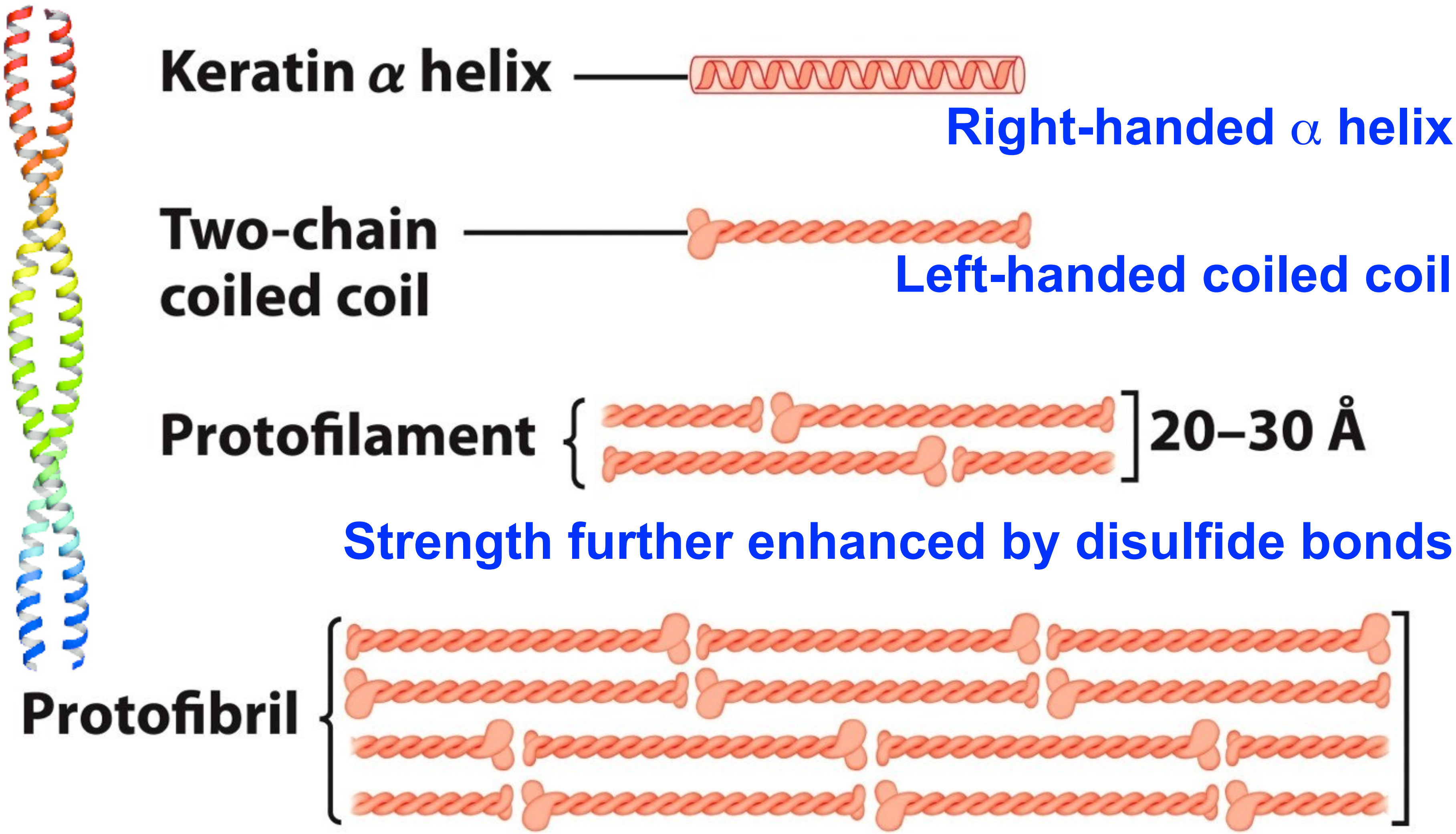
TABLE 4–3 Secondary Structures and Properties of Some Fibrous Proteins

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

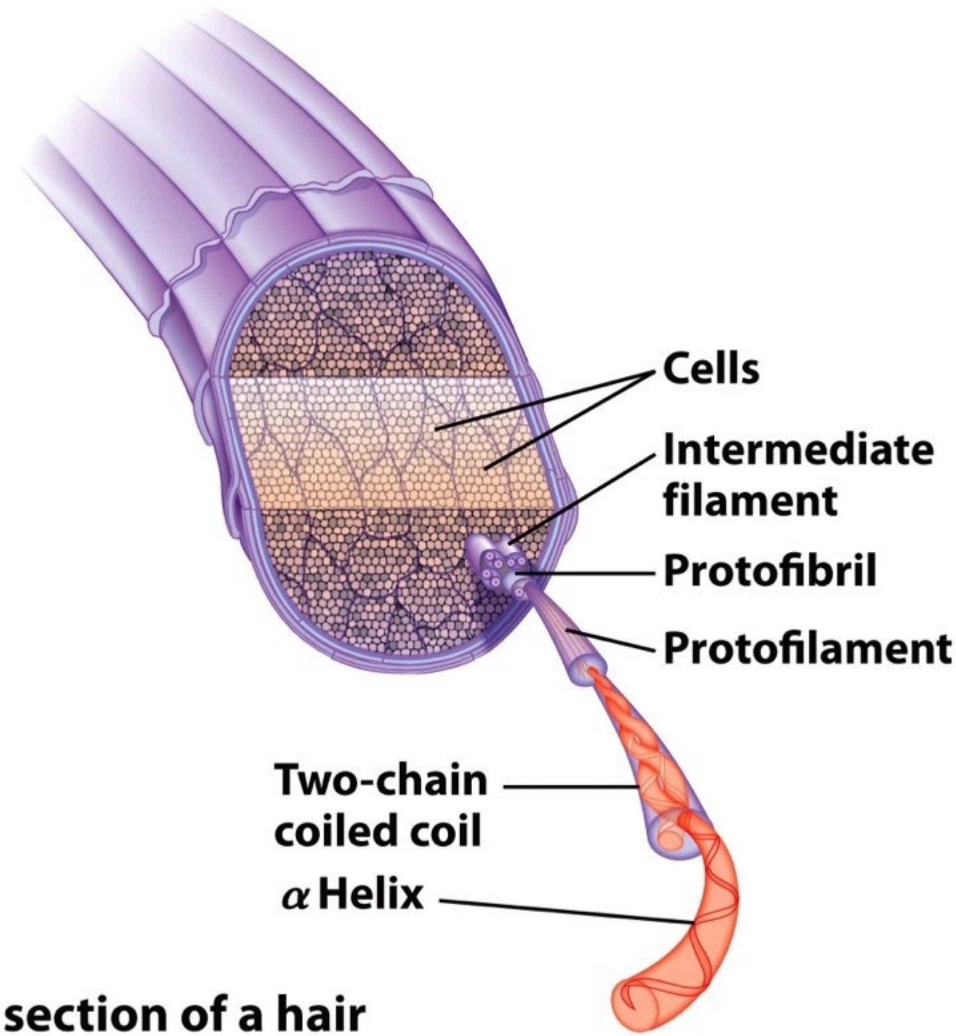




Structure of α-Keratin in Hair



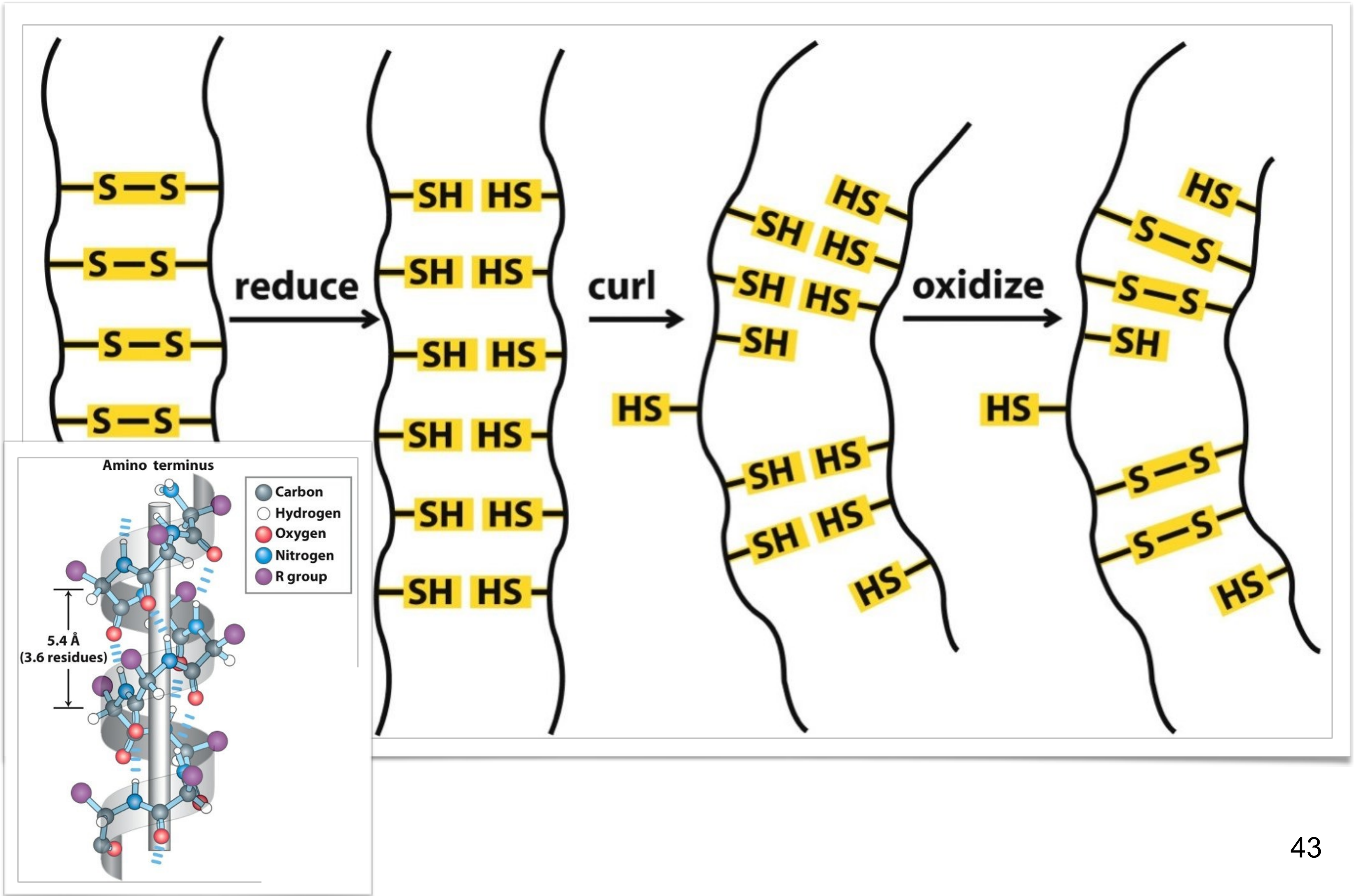
Structure of Hair



Cross section of a hair

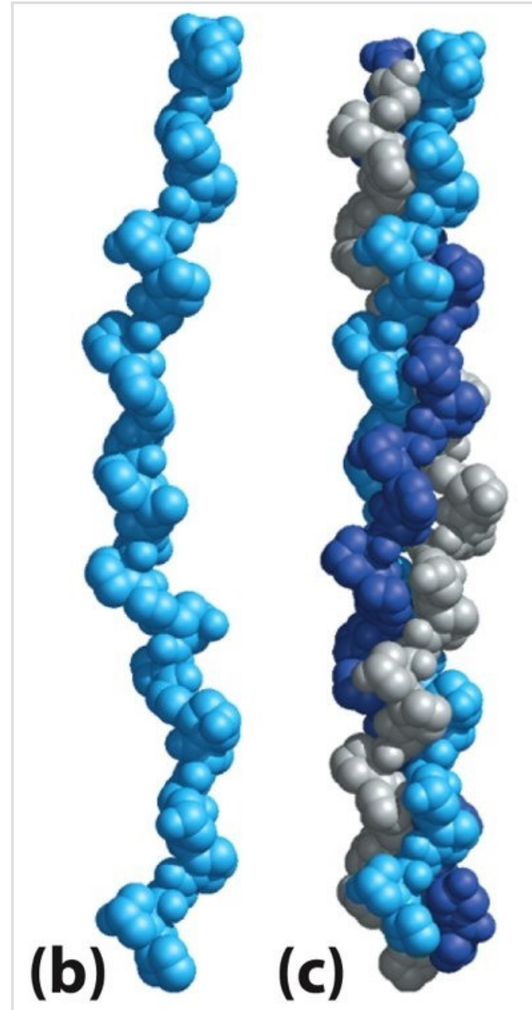
- Right-handed α helix
- Left-handed coiled coil
- Cross-linked by disulfide bonds

Chemistry of Permanent Waving



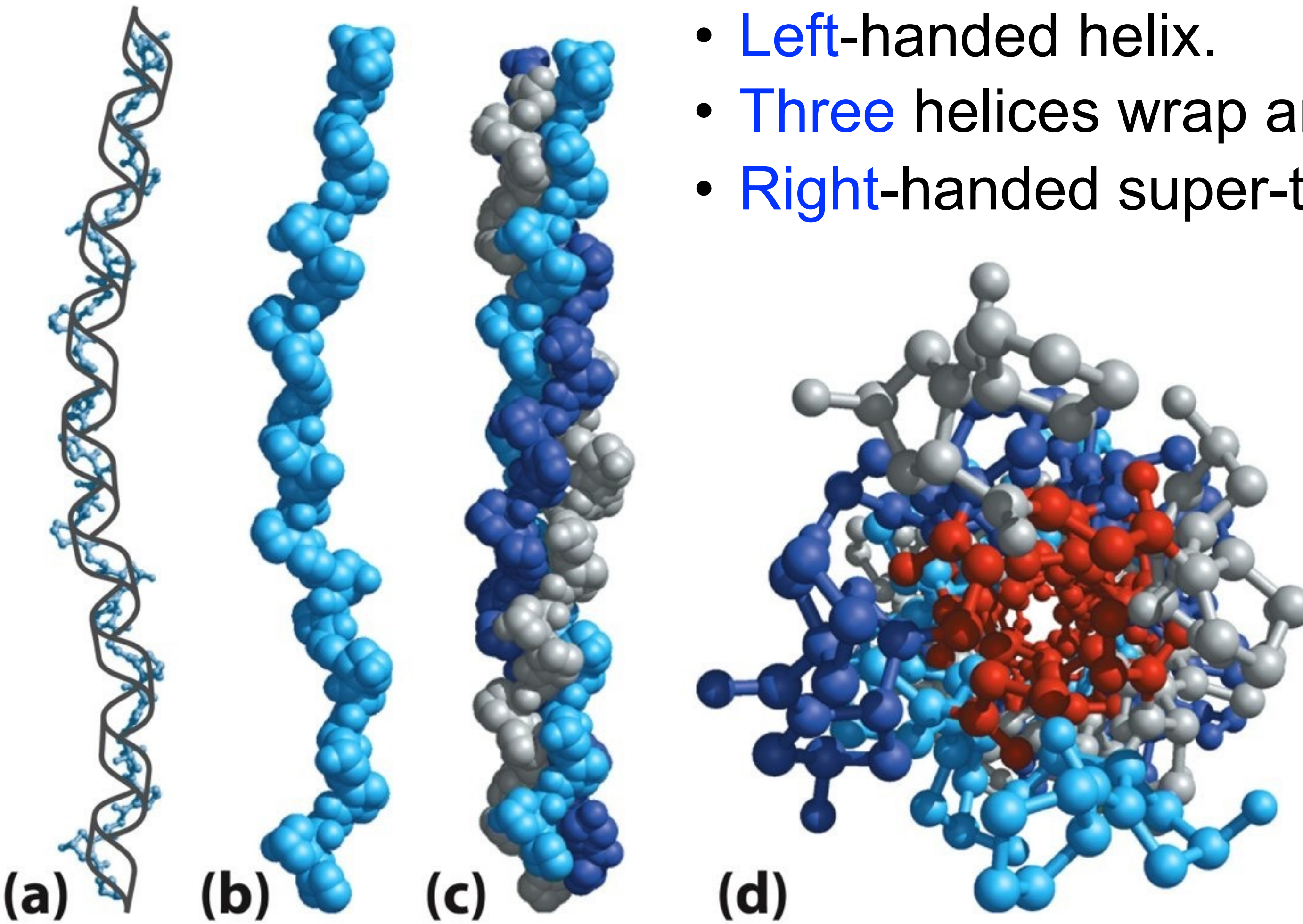
Structure of Collagen

- Each collagen chain is a long Gly-rich and Pro-rich **left**-handed helix.
- Three collagen chains intertwine into a **right**-handed superhelical triple helix.
- Many triple-helices assemble into a collagen fibril.



Structure of Collagen

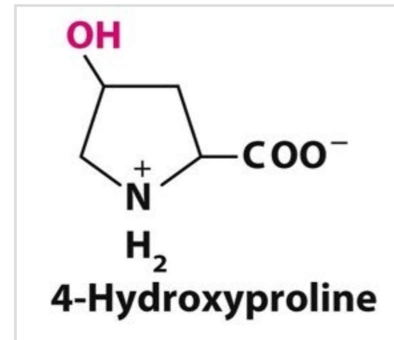
- NOT α-helix.
- Left-handed helix.
- Three helices wrap around.
- Right-handed super-twisting.



Glycine shown in red

Proline & 4-Hydroxyproline in Collagen

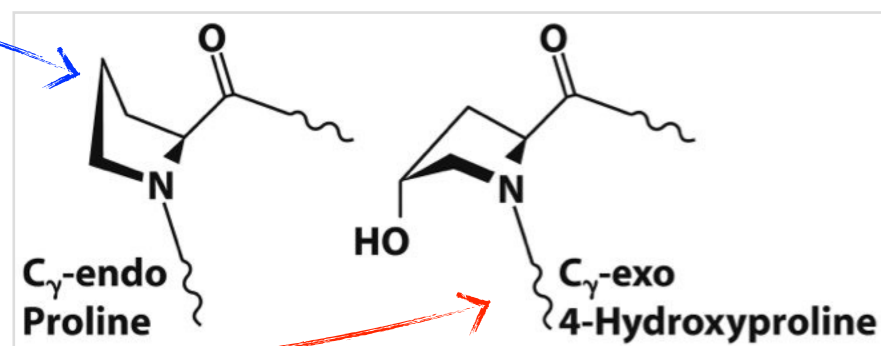
- Repeating unit in collagen: Gly-X-Y (X is often Pro, Y is often 4-Hyp).
  - Three amino acid residues per turn.
  - Only Gly can be accommodated at very tight junctions.
  - Pro and 4-Hyp permit sharp twisting of collagen helix.



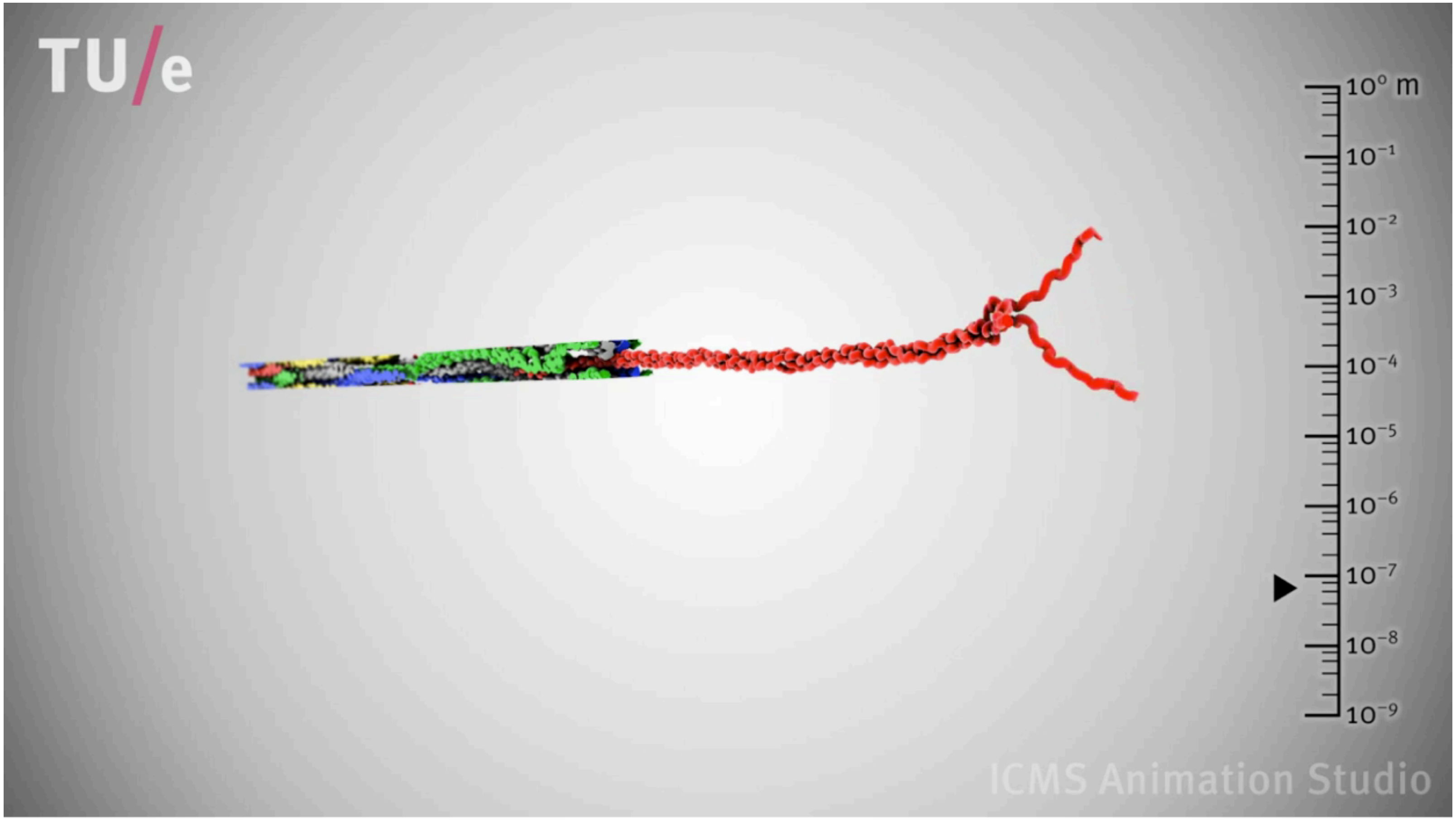
- 4-Hyp forces proline ring into a favorable pucker.
- 4-Hyp offers more hydrogen bonds between three strands of collagen.
- Post-translational processing is catalyzed by prolyl hydroxylase and requires ascorbate (vitamin C).

C_γ-endo required in X position

C_γ-exo required in Y position



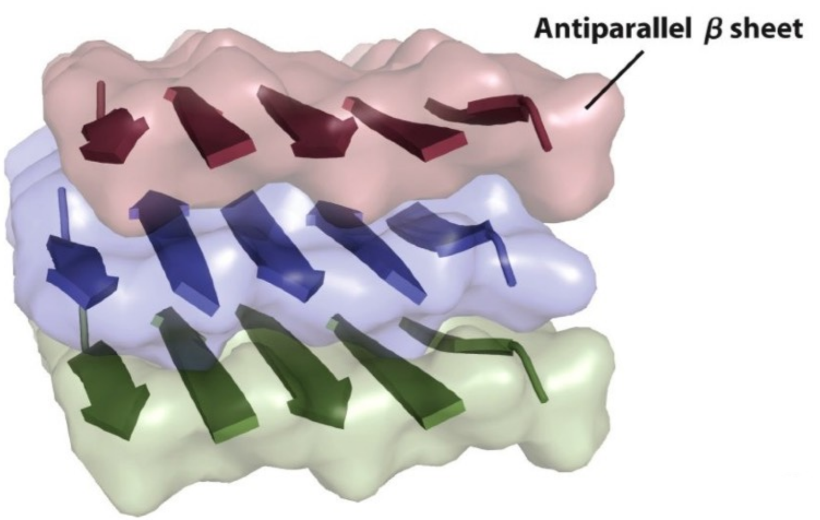
Collagen



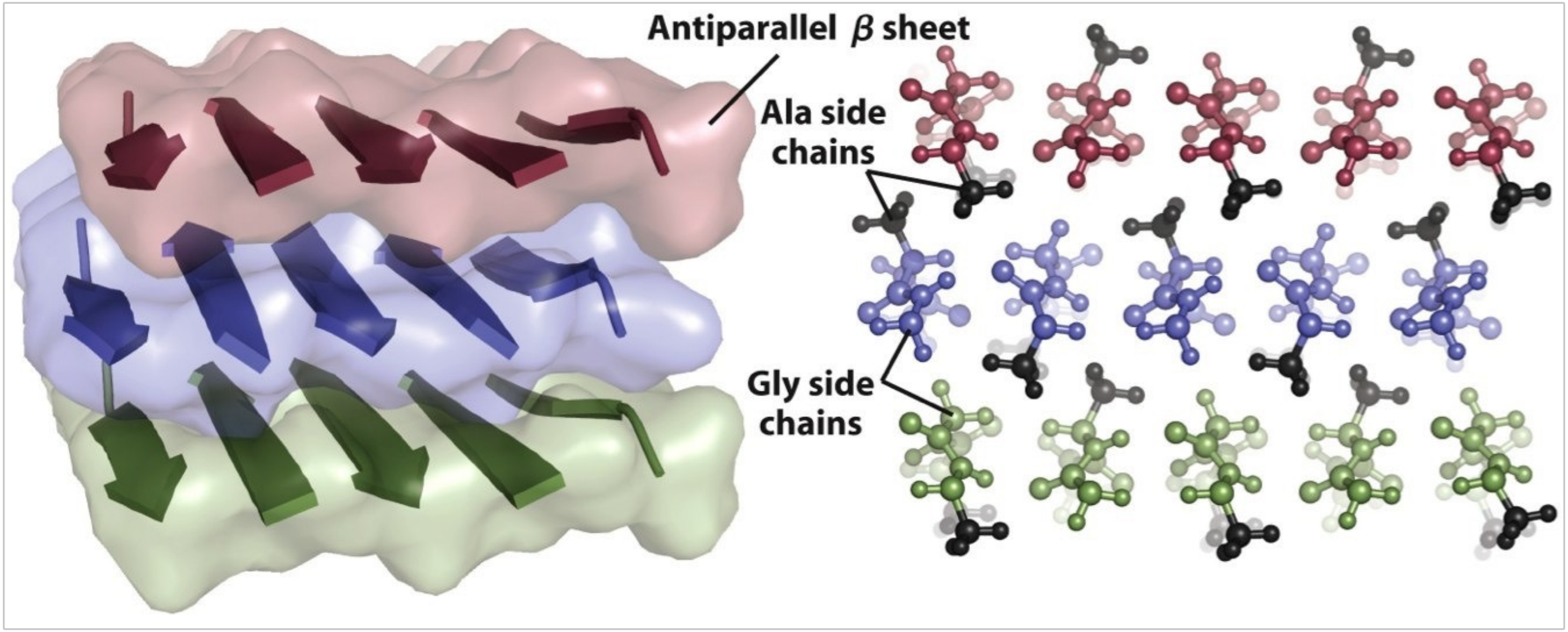
blood vessel 血管 These filaments are NOT individual protein strands. Each collagen monomer consists of three left-handed helical protein strands, wound around each other to form a right-handed triple helix.

Silk Fibroin

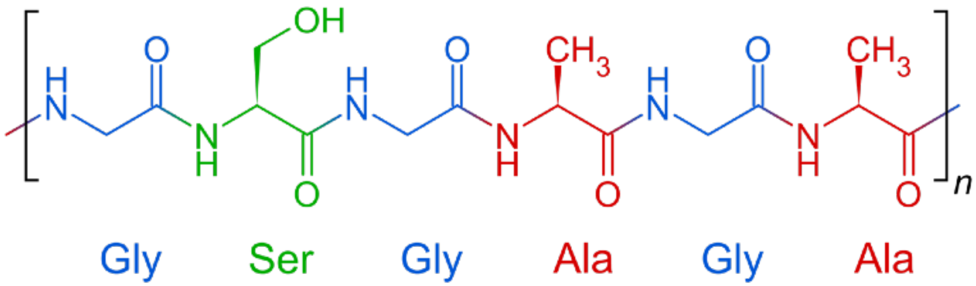
- Fibroin is the main protein in silk from insects and spiders.
- Antiparallel β sheet structure.
- Small side chains (Ala and Gly) allow the close packing of sheets.
- Structure is stabilized by
  - Hydrogen bonding.
  - NOT covalent bonds.



Structure of Silk



recurrent amino acid sequence Gly-Ser-Gly-Ala-Gly-Ala



Fibrous Proteins Review

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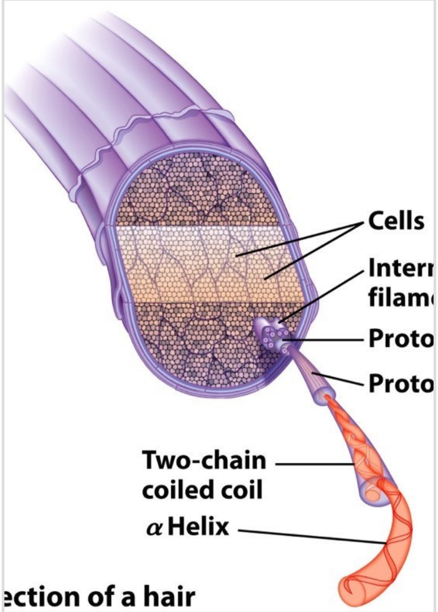
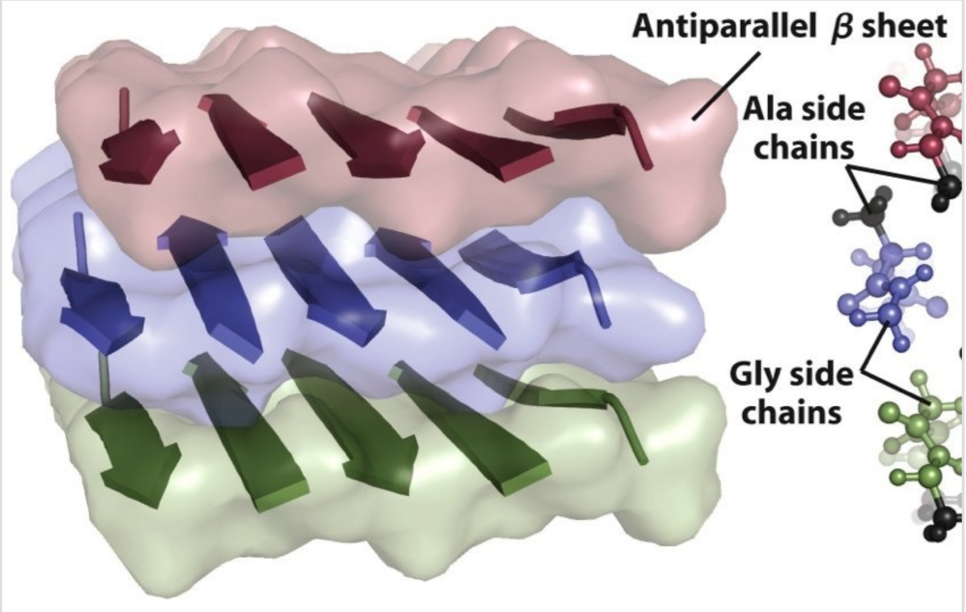
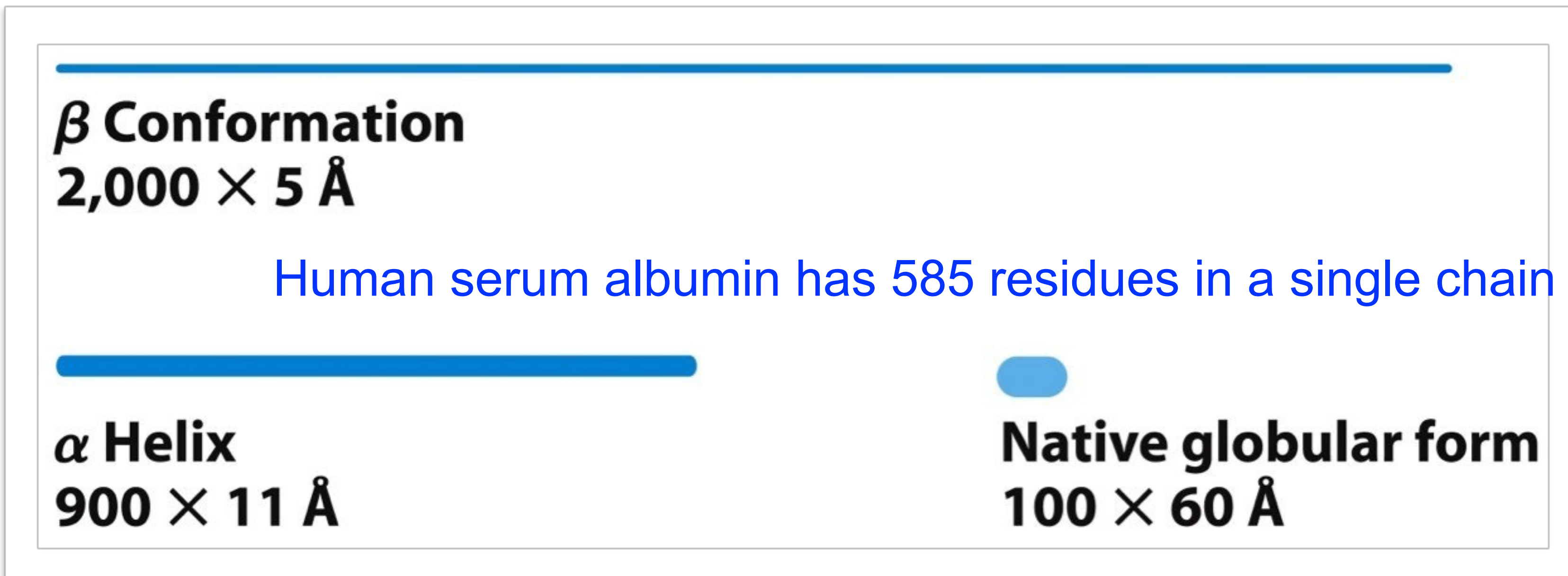




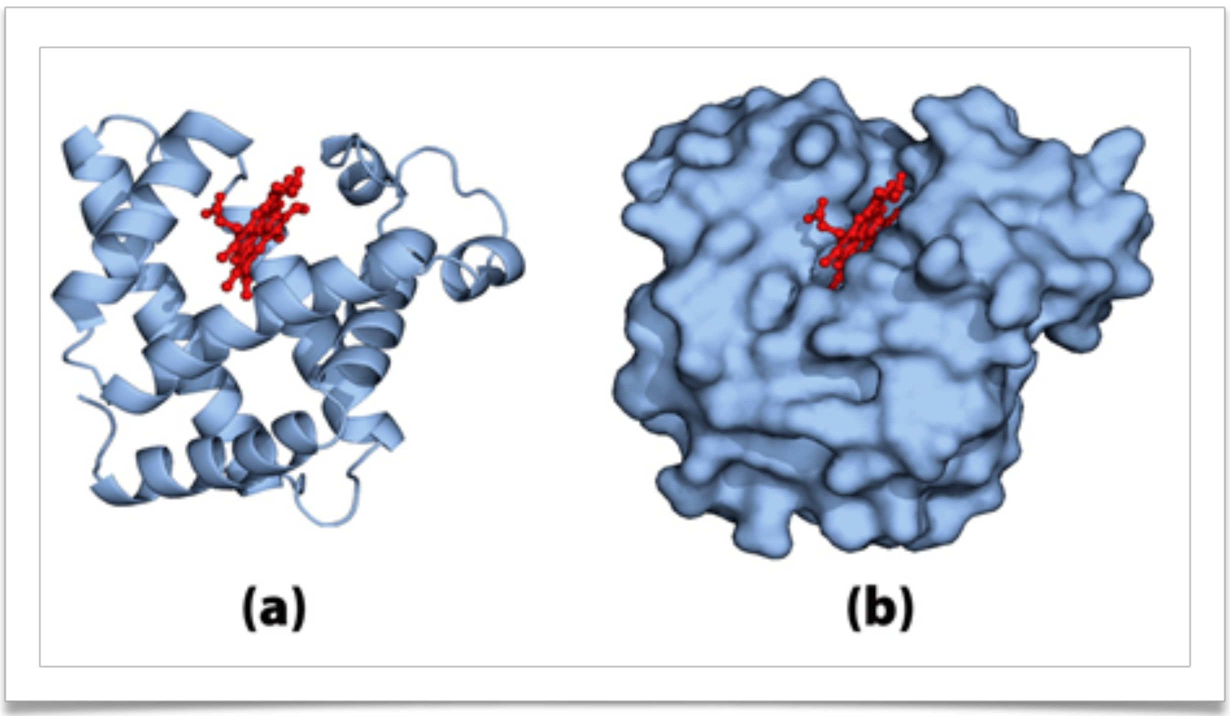
Structural Diversity -> Functional Diversity

- Globular proteins more compact than fibrous proteins.
- A wide array of biological functions.
  - Enzymes, regulatory proteins, transport proteins, etc.



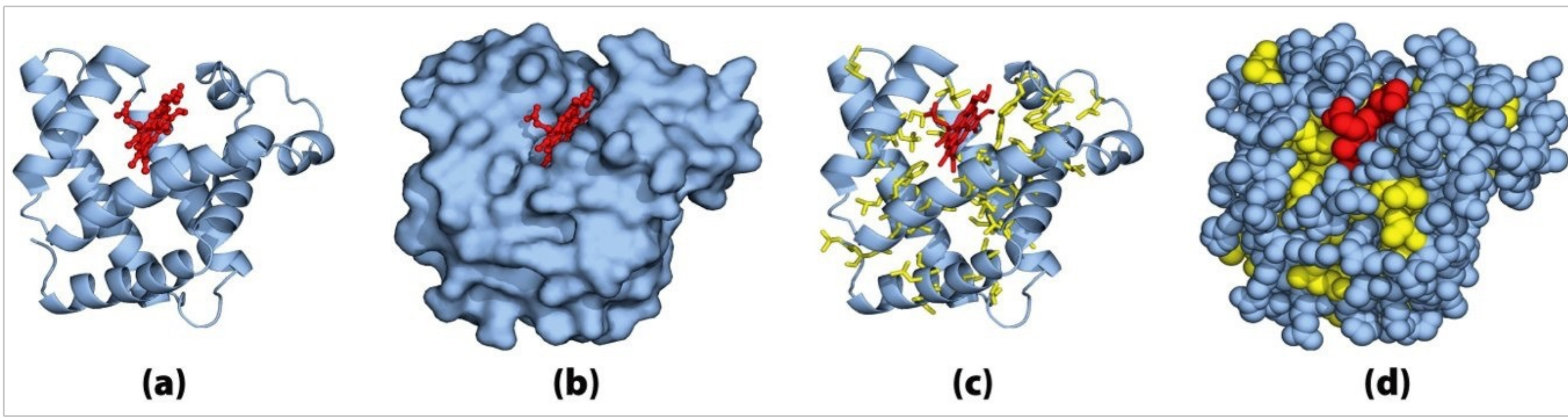
Myoglobin

- A single peptide chain with 153 residues.
- Oxygen binding.
- Contains a single heme group (iron protoporphyrin).



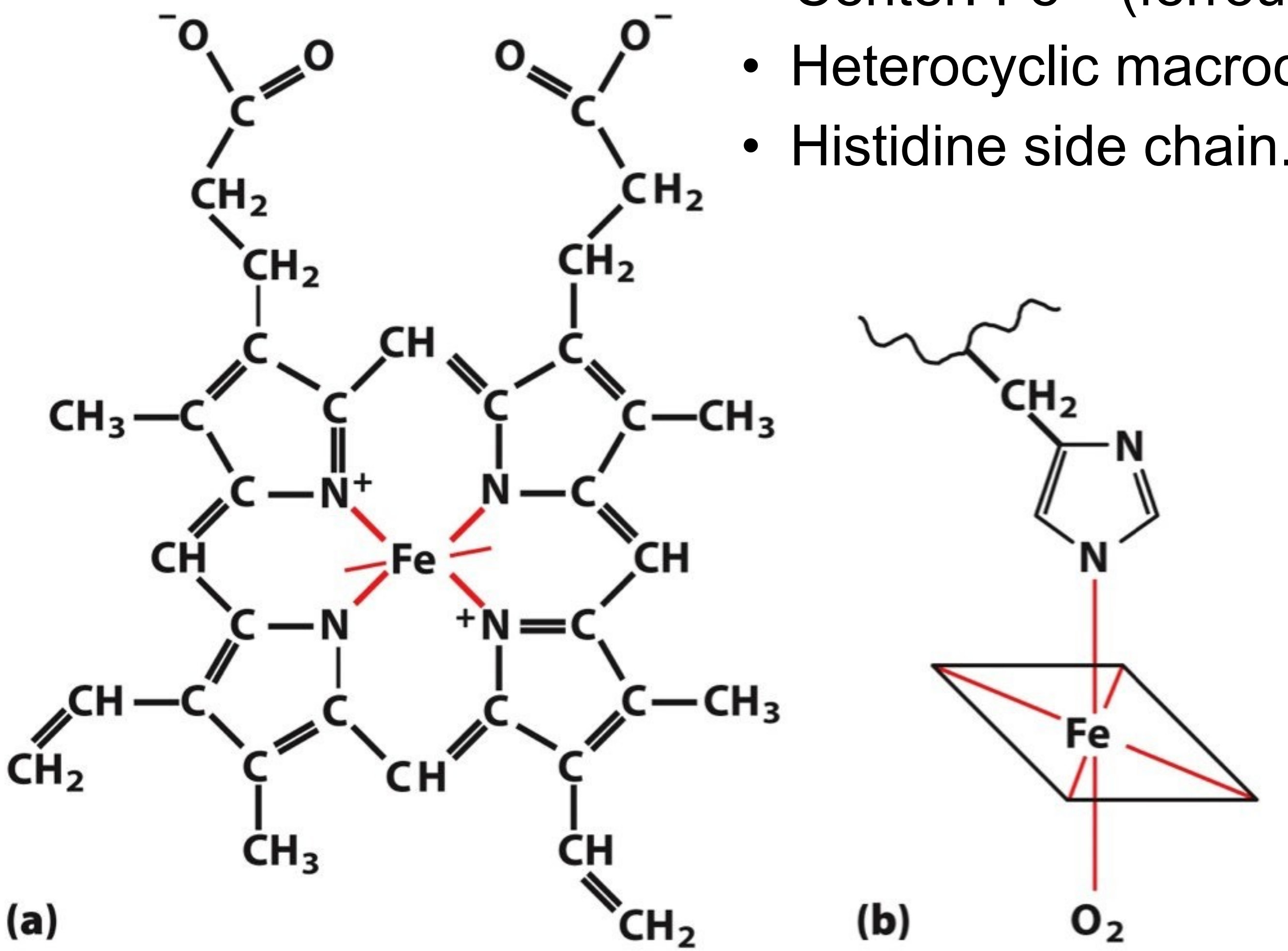
Structure of Myoglobin

(a) Ribbon representation
(b) Surface representation
(c) Ribbon representation with several side chains shown as sticks
(d) Space-filling model



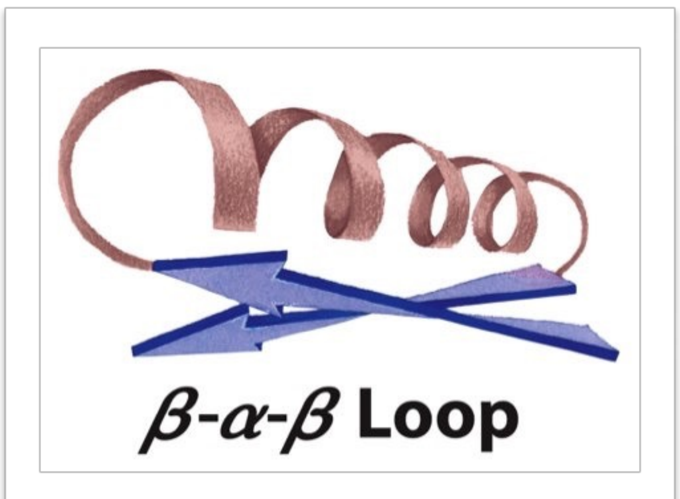
Structure of Heme

- Center: Fe^{2+} (ferrous) ion.
- Heterocyclic macrocycle porphyrin.
- Histidine side chain.

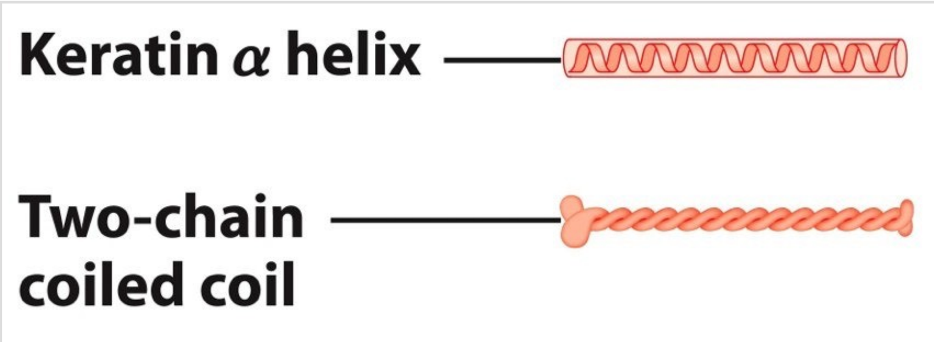


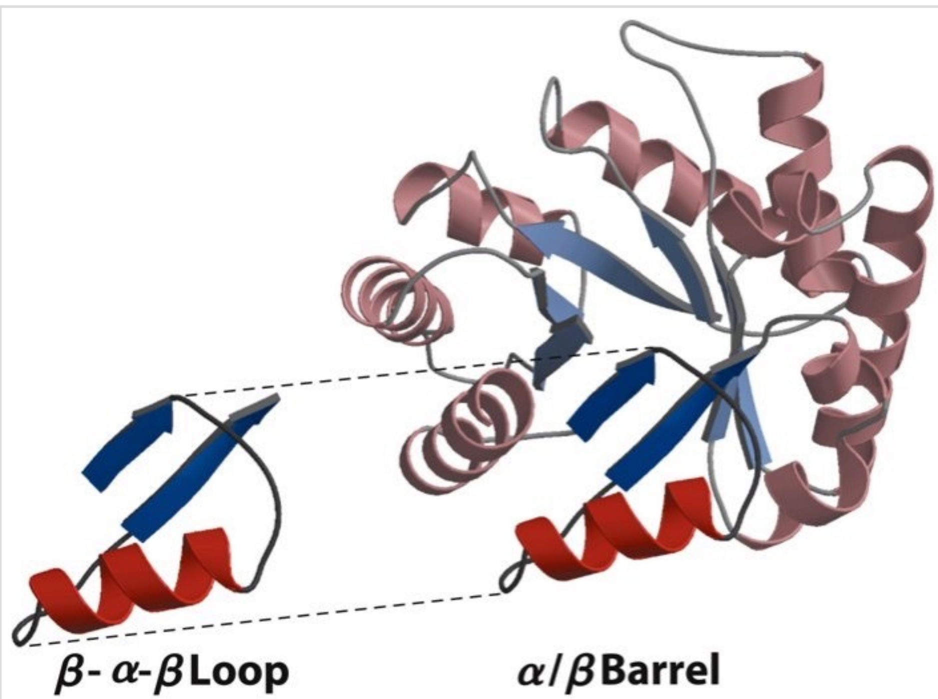
Motif or Fold

- A recognizable folding pattern
- Involves two or more elements of secondary structure
- Can be very simple or complex



Examples of Motifs



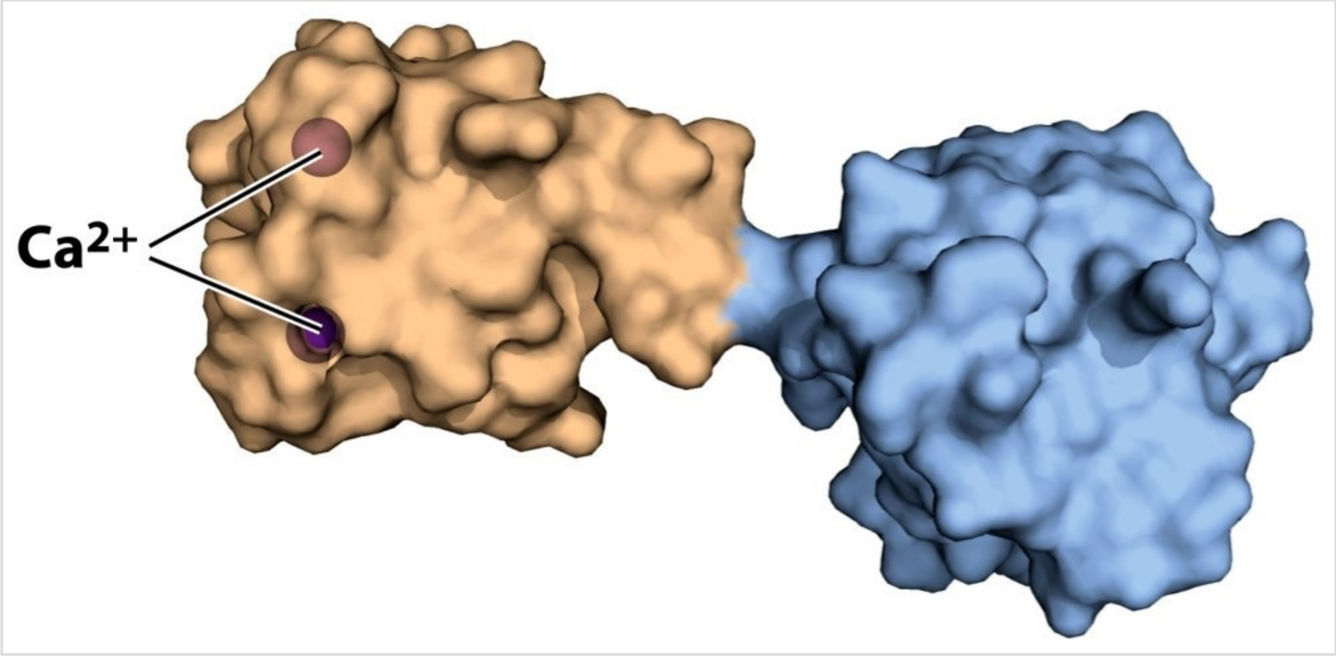


Globin Fold

- Reoccurring in many proteins.
- A single large motif may be the entire protein.
  - NOT a hierarchical structural element.
  - A folding pattern.

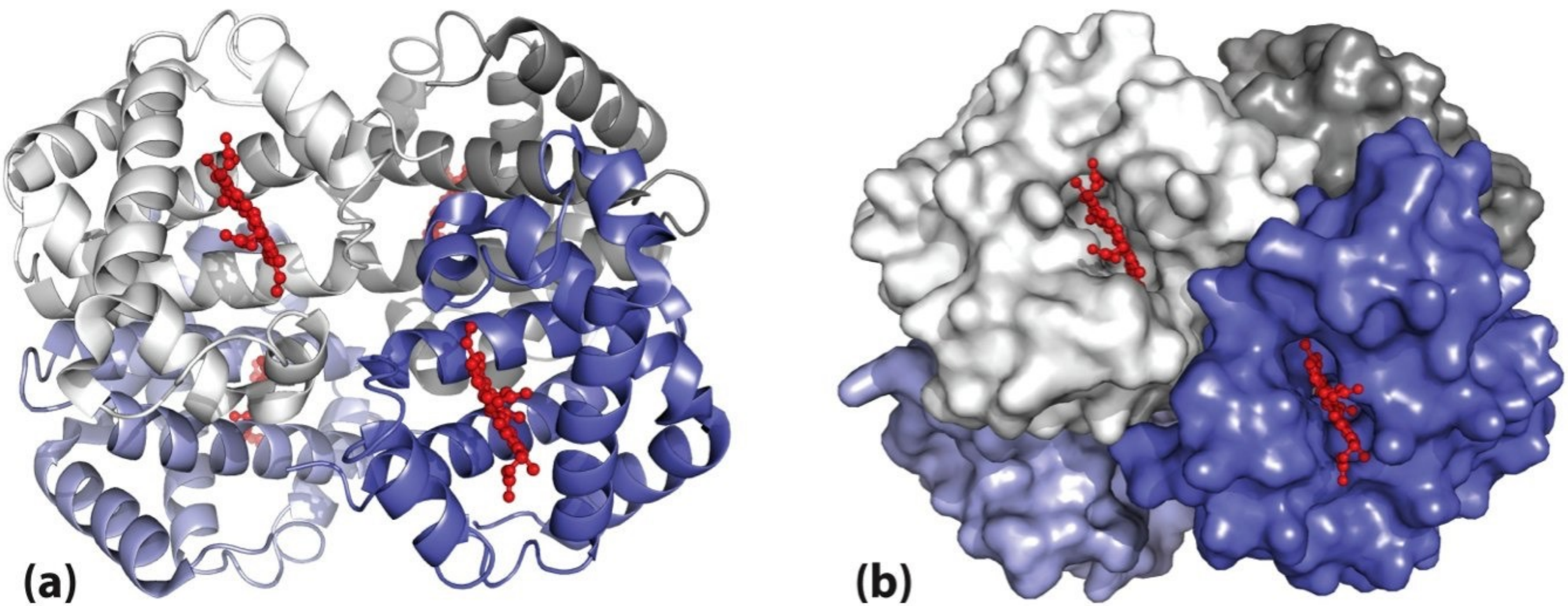
Domain

- A part of a polypeptide chain.
- Independently stable or move as a single entity.
- Maintain structure and function when separated.
- For small proteins, the domain is the protein.



Quaternary Structure

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



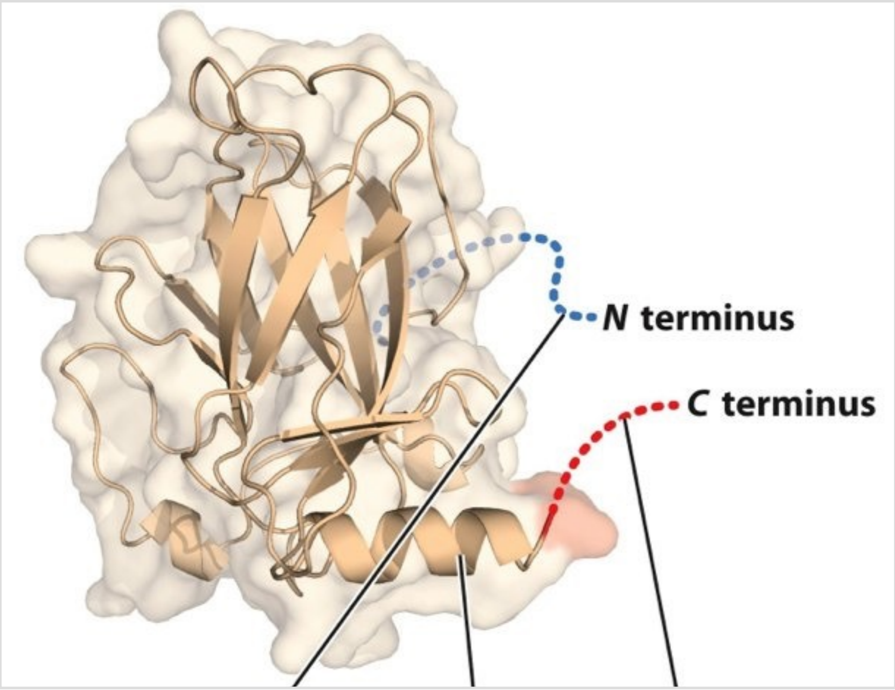
Ribbon representation

Surface model

Hemoglobin

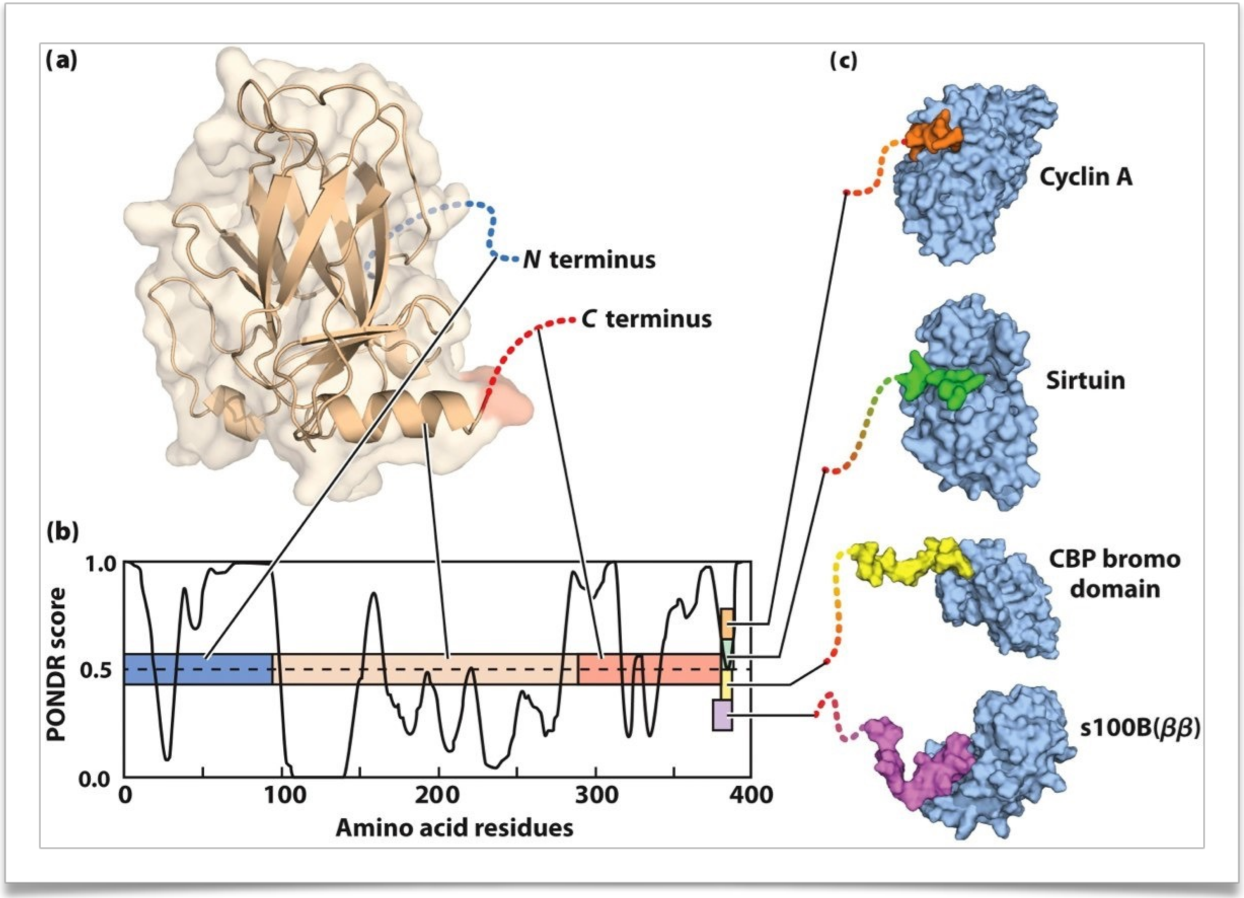
Intrinsically Disordered Proteins

- Contain protein segments lack definable structure.
- Composed of amino acids whose higher concentration forces less-defined structure.
  - Lys, Arg, Glu, and Pro
- Disordered regions can interact with many different partner proteins

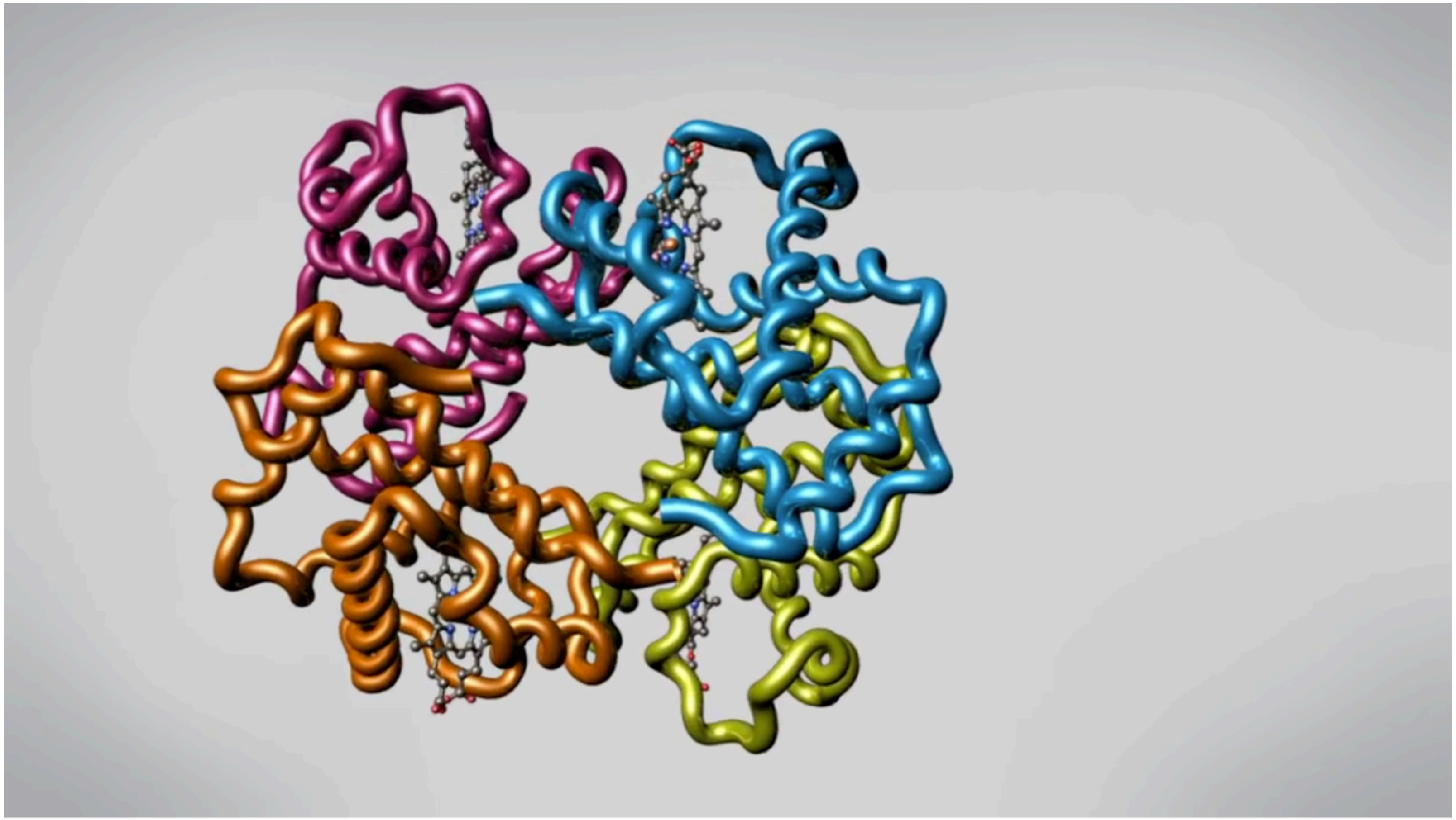


Intrinsically Disordered p53 Protein

- Inhibit by wrapping around target proteins.
- Multiple targets.
- Bind different targets in different ways.



What is A Protein?

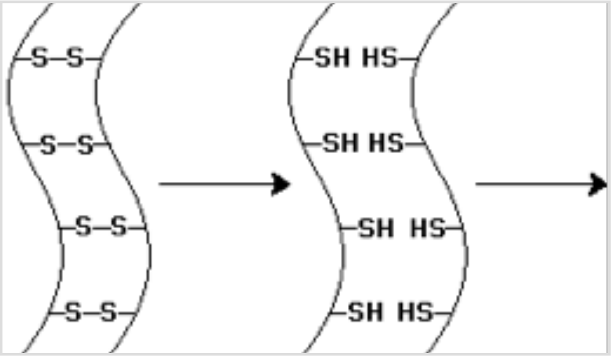


Summary 4.3 Tertiary & Quaternary Structure

- Tertiary structure is the complete three-dimensional structure of a polypeptide chain. Quaternary structure describes interactions between subunits of multisubunit proteins.
- Fibrous proteins mainly serve structural roles. Globular proteins have more complicated tertiary structures.
- Some proteins are intrinsically disordered. Some function as versatile inhibitors.

Example Question

The α-keratin chains indicated by the diagram below have undergone one chemical step. To alter the shape of the α-keratin chains—as in hair waving—what subsequent steps are required?



A) Chemical oxidation and then shape remodeling

B) Chemical reduction and then chemical oxidation

C) Chemical reduction and then shape remodeling

D) Shape remodeling and then chemical oxidation

E) Shape remodeling and then chemical reduction

Example Question

Which of the following statements concerning protein domains is *true*?

A) They are a form of secondary structure.

B) They are examples of structural motifs.

C) They consist of separate polypeptide chains (subunits).

D) They have been found only in prokaryotic proteins.

E) They may retain their correct shape even when separated from the rest of the protein.

Example Question

Which statement about intrinsically disordered proteins is *true*?

A) They contain small hydrophobic cores.

B) They represent misfolded conformations of cellular proteins.

C) They have no stable three-dimensional structure and therefore have no cellular function.

D) They can interact with multiple protein-binding partners and are central to protein interaction networks.

Three-Dimensional Structure of Proteins

4.1 Overview of Protein Structure

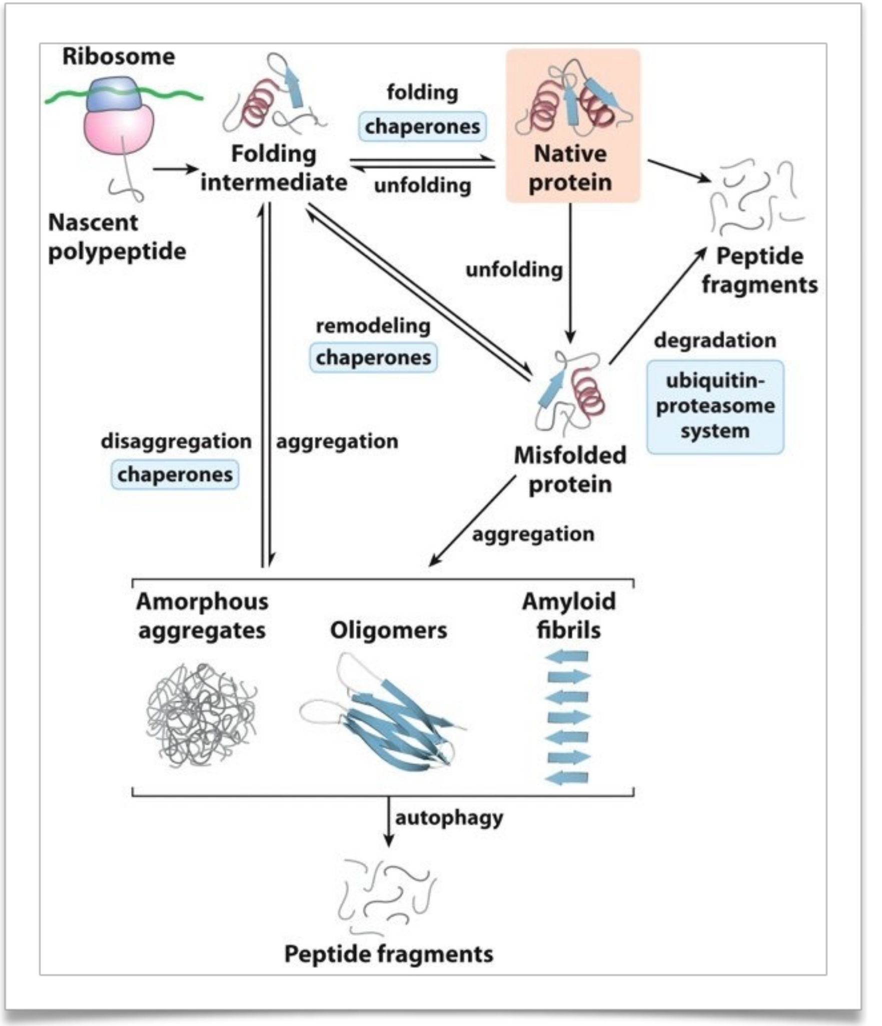
4.2 Protein Secondary Structure

4.3 Protein Tertiary and Quaternary Structure

4.4 Protein Denaturation and Folding

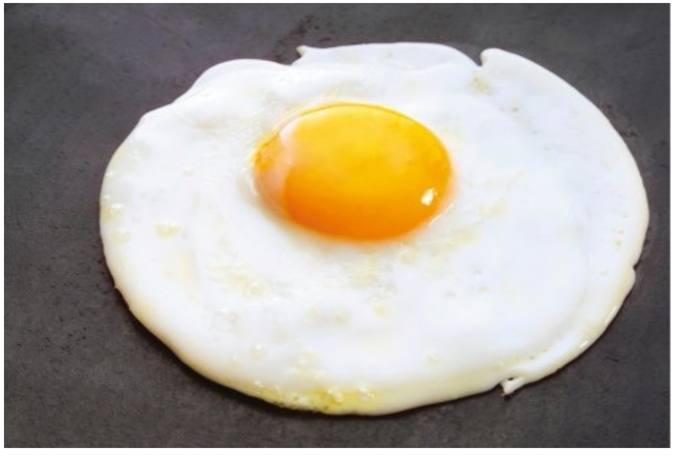
Proteostasis

- Continual maintenance of an active set of cellular proteins under a given set of conditions.
- Three processes:
  - Synthesis
  - Folding
  - Degradation

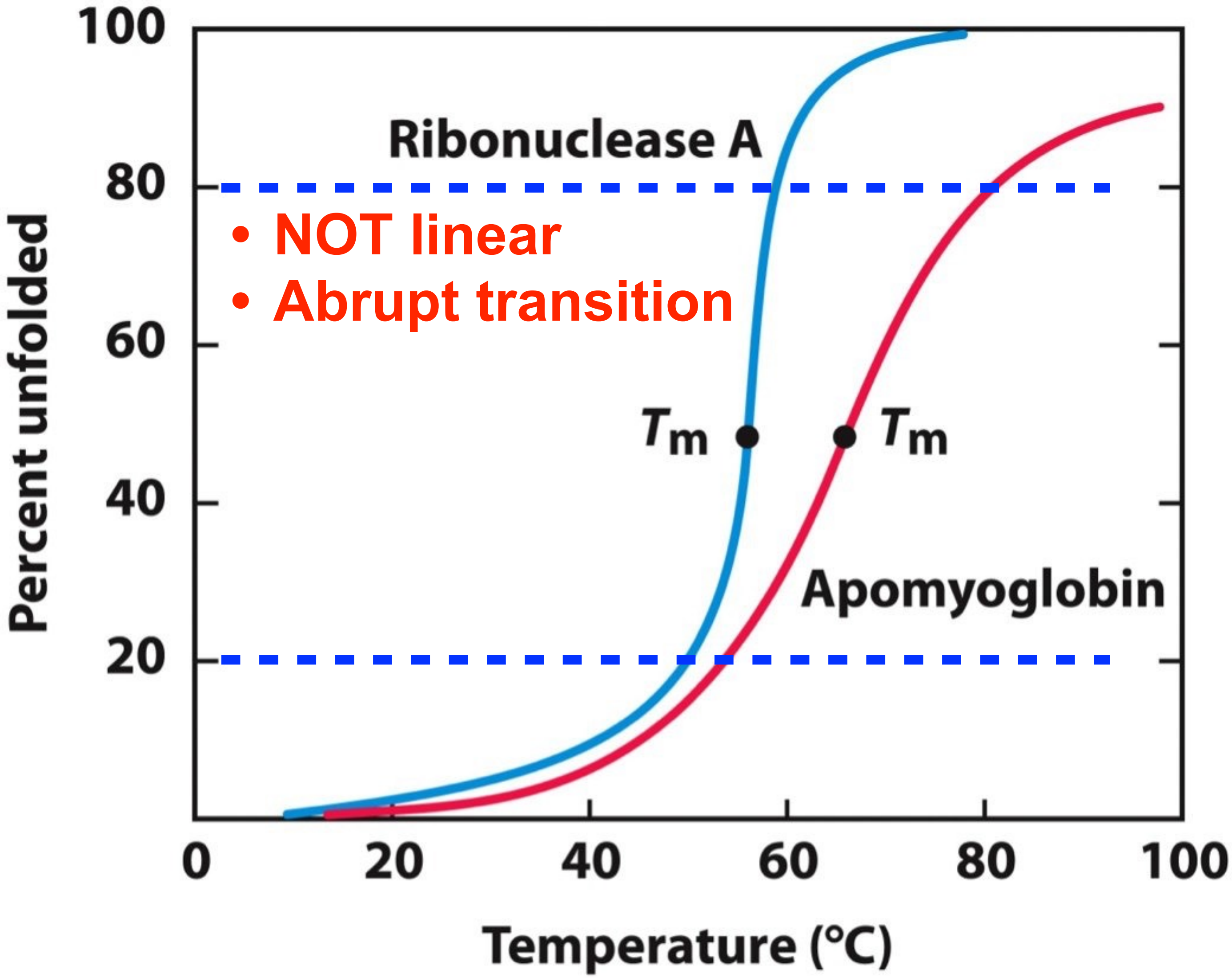


Loss of Structure -> Loss of Function

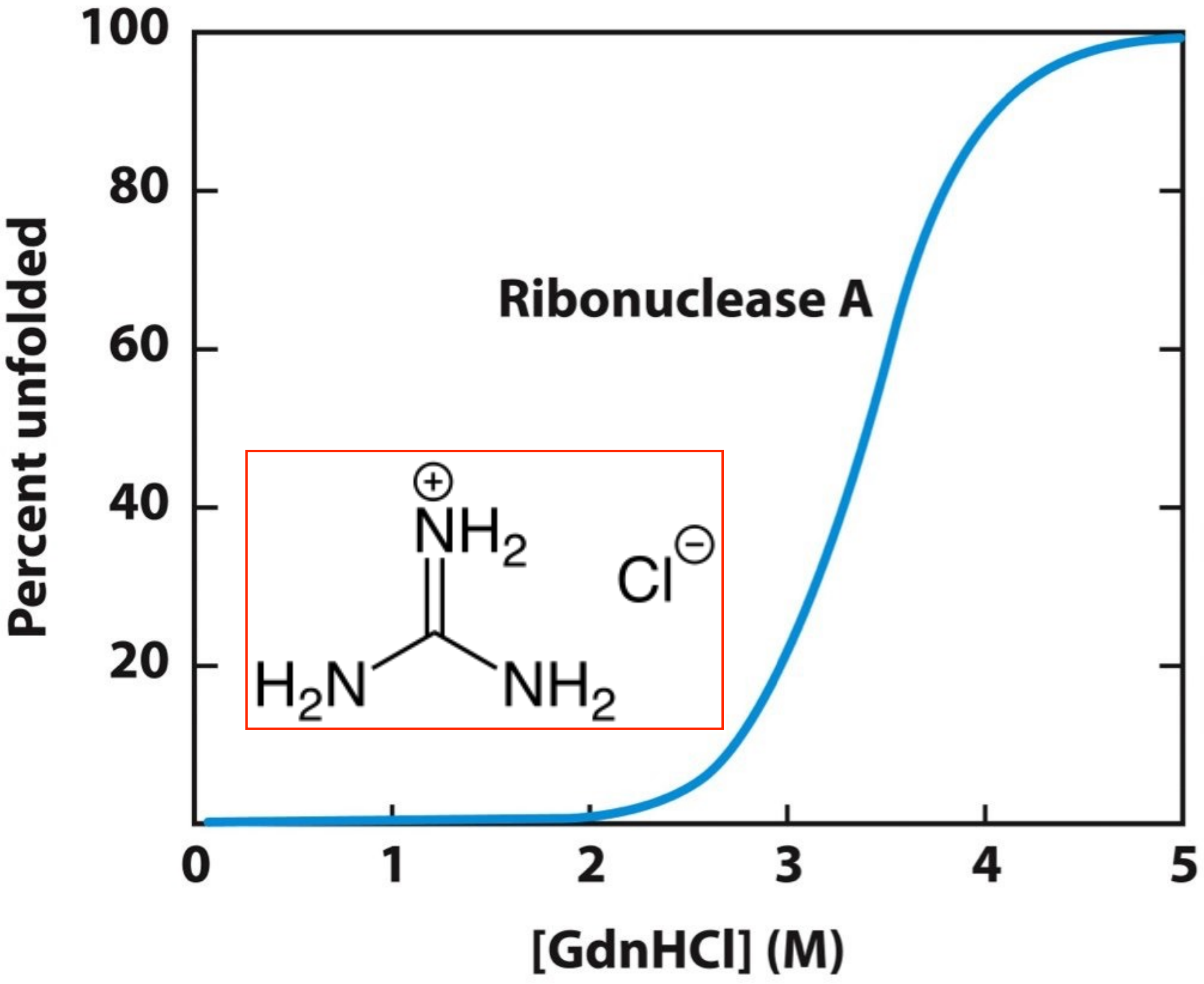
- A protein's function depends on its 3D-structure.
- Loss of structural integrity with accompanying loss of activity is called denaturation.
- Proteins can be denatured by:
  - Heat
  - pH extremes
  - Organic solvents
  - Urea, detergent and guanidinium hydrochloride



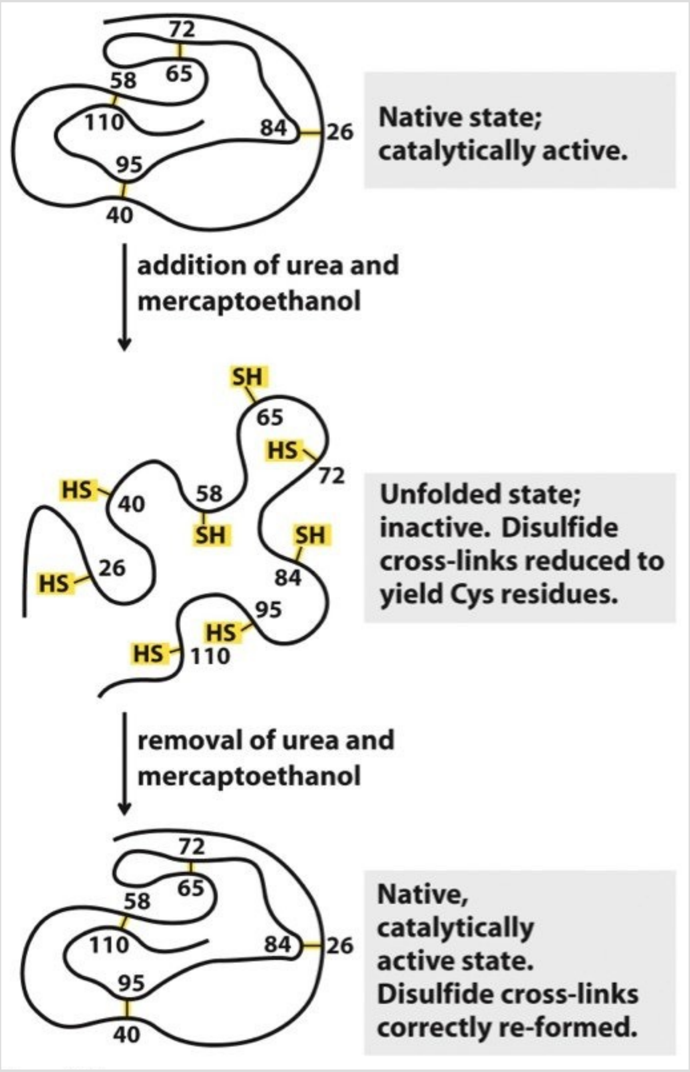
Protein Denaturation by Heat



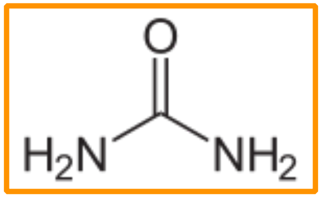
Denaturation by Guanidine Hydrochloride

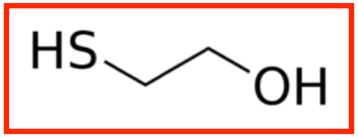


Ribonuclease Refolding Experiment

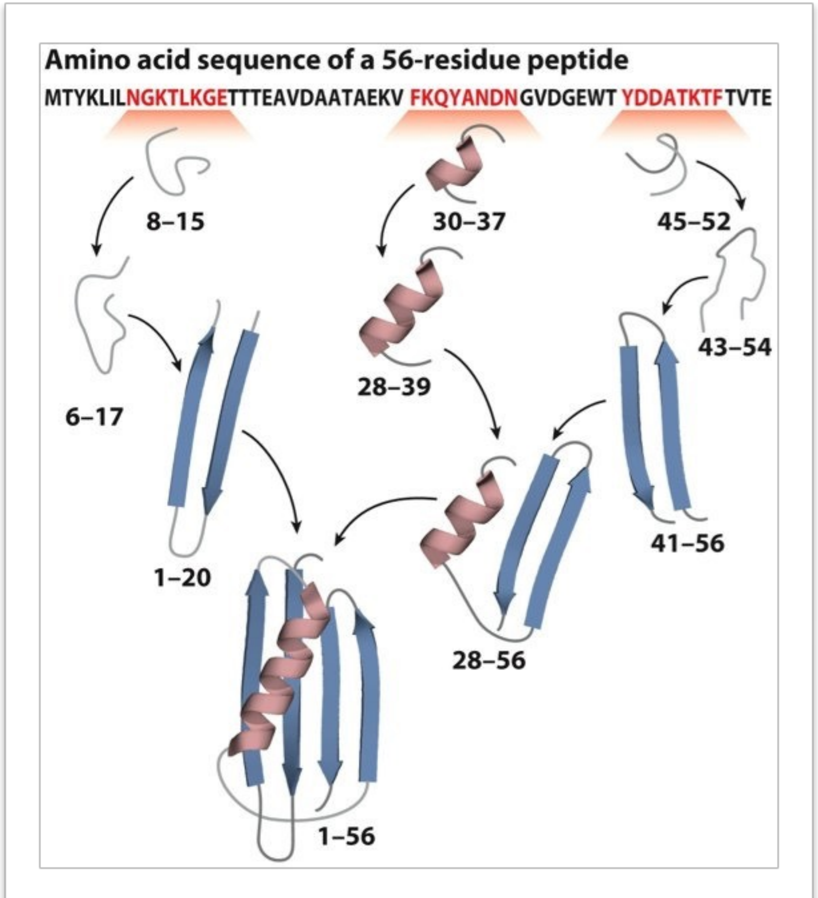


- A small protein
- 8 cysteines
- 4 disulfide bonds
- Urea and BME fully denatures
- When urea and BME are removed, the protein spontaneously refolds, and the correct disulfide bonds are reformed
- **Sequence alone determines the native conformation.**





Proteins Fold by a Stepwise Process



- Local structures first.

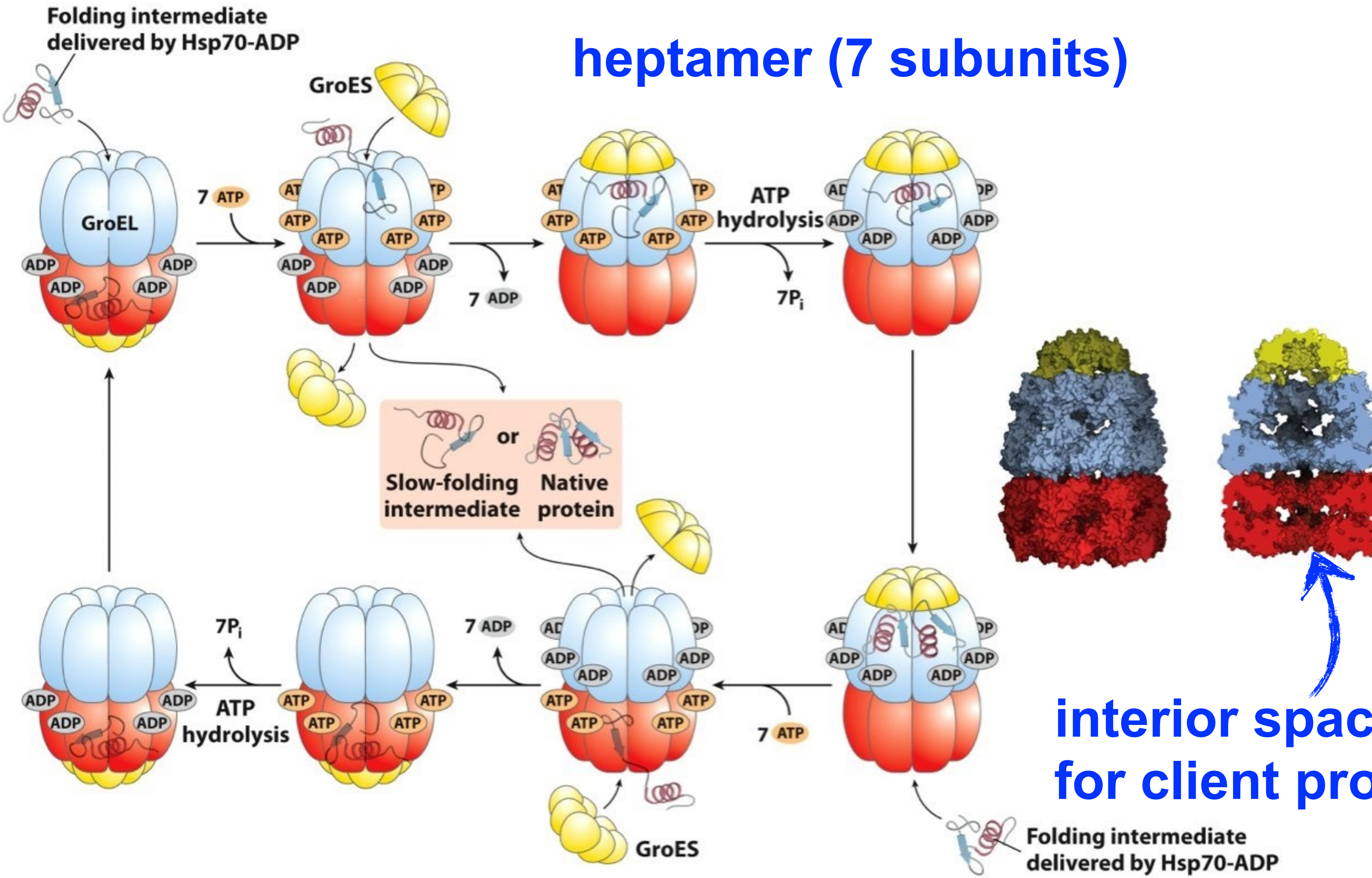
  2° structures assembled.

- Then long-range interactions.
  - 2° structures further interact to form 3° structure.

- Hydrophobic interactions play a significant role throughout the process.

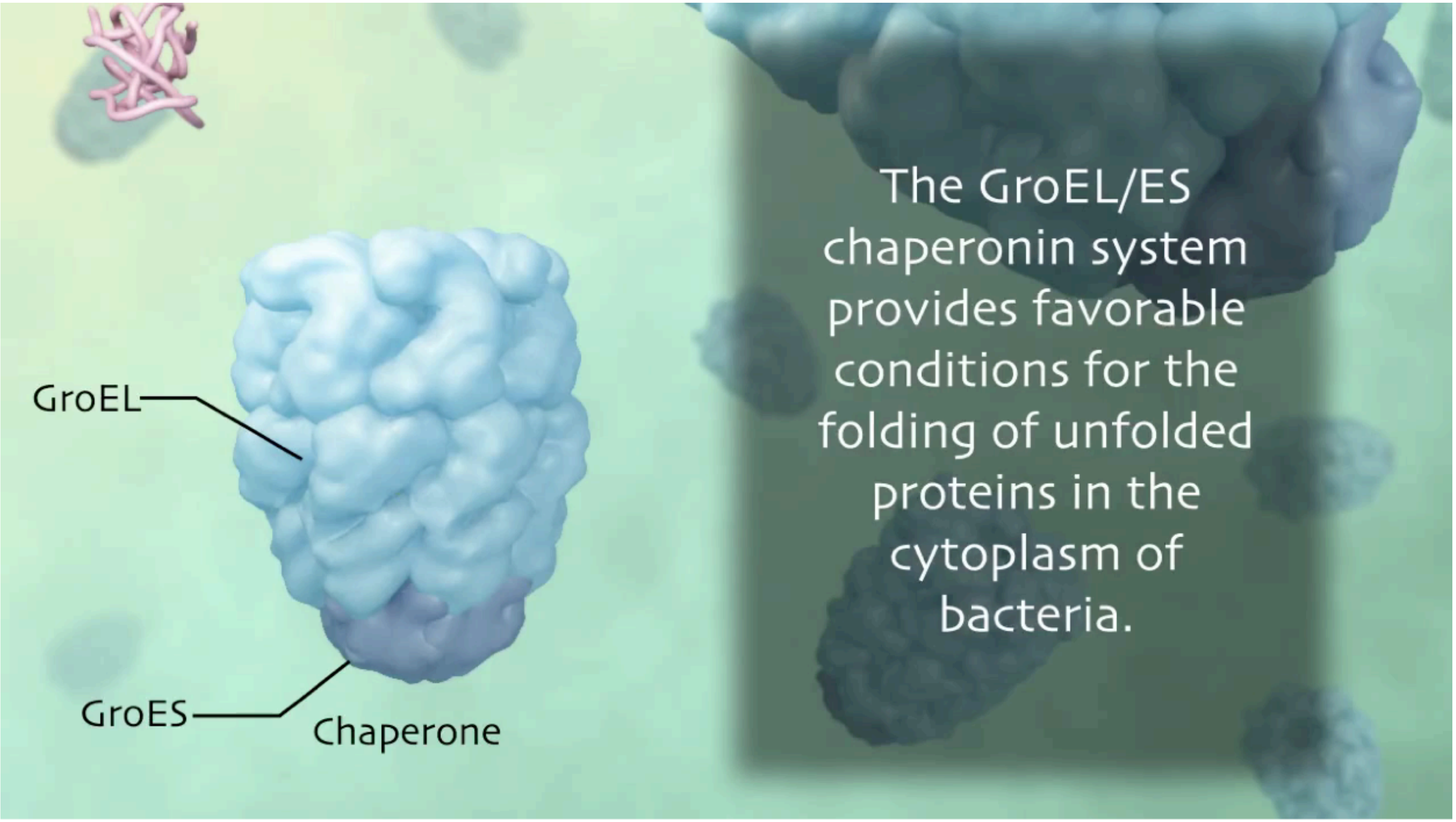
Assisted Folding by Chaperones

heptamer (7 subunits)



interior space
for client protein

Assisted Folding by Chaperones



Summary 4.4 Protein Folding & Denaturation

- Maintenance of a collection of active cellular proteins is called proteostasis. It involves three processes: synthesis, folding, and degradation.
- Structure and function can be destroyed by denaturation. Some denatured proteins can renature spontaneously. 3° structure is determined by 1° sequence.
- Protein folding is hierarchical and often assisted by chaperones.

Example Question

Experiments on denaturation and renaturation after the reduction and re-oxidation of the —S—S— bonds in the enzyme ribonuclease (RNase) have shown that:

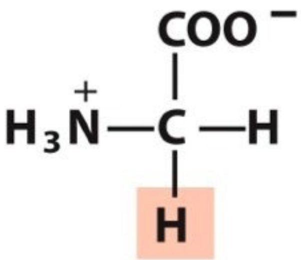
A) folding of denatured RNase into the native, active conformation, requires input of energy in the form of heat.

B) native ribonuclease does not have a unique secondary and tertiary structure.

C) the completely unfolded enzyme, with all —S—S— bonds broken, is still enzymatically active.

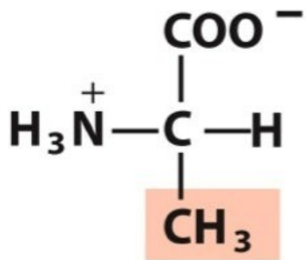
D) the enzyme, dissolved in water, is thermodynamically stable relative to the mixture of amino acids whose residues are contained in RNase.

E) the primary sequence of RNase is sufficient to determine its specific secondary and tertiary structure.



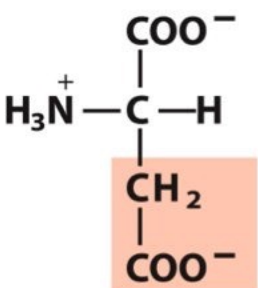
Glycine

Gly, G



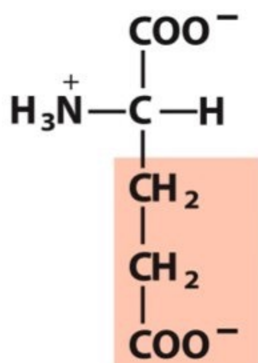
Alanine

Ala, A



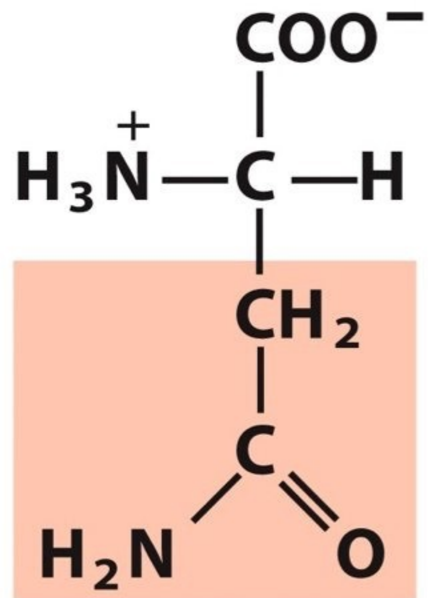
Aspartate

Asp, D



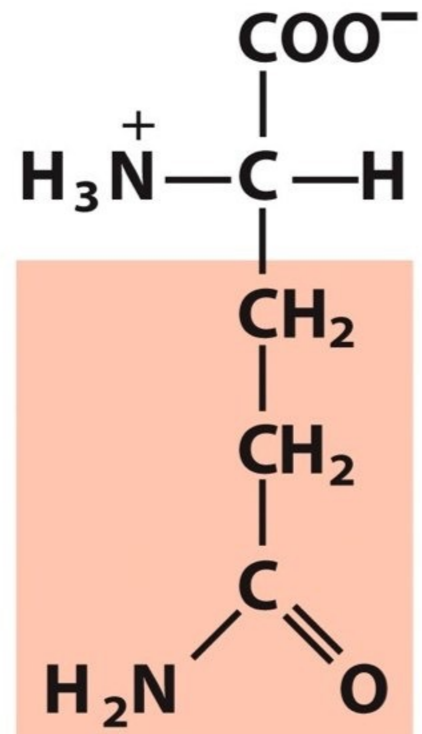
Glutamate

Glu, E



Asparagine

Asn, N



Glutamine

Gln, Q

Amino acid for 3rd week

Chapter 4: Summary

In this chapter, we learned about:

- Two most important secondary structures.
  - α helices
  - β sheets
- Three common fibrous proteins and one globular protein.
  - α keratin
  - collagen
  - silk fibroin
  - myoglobin
- Protein denaturation & folding.