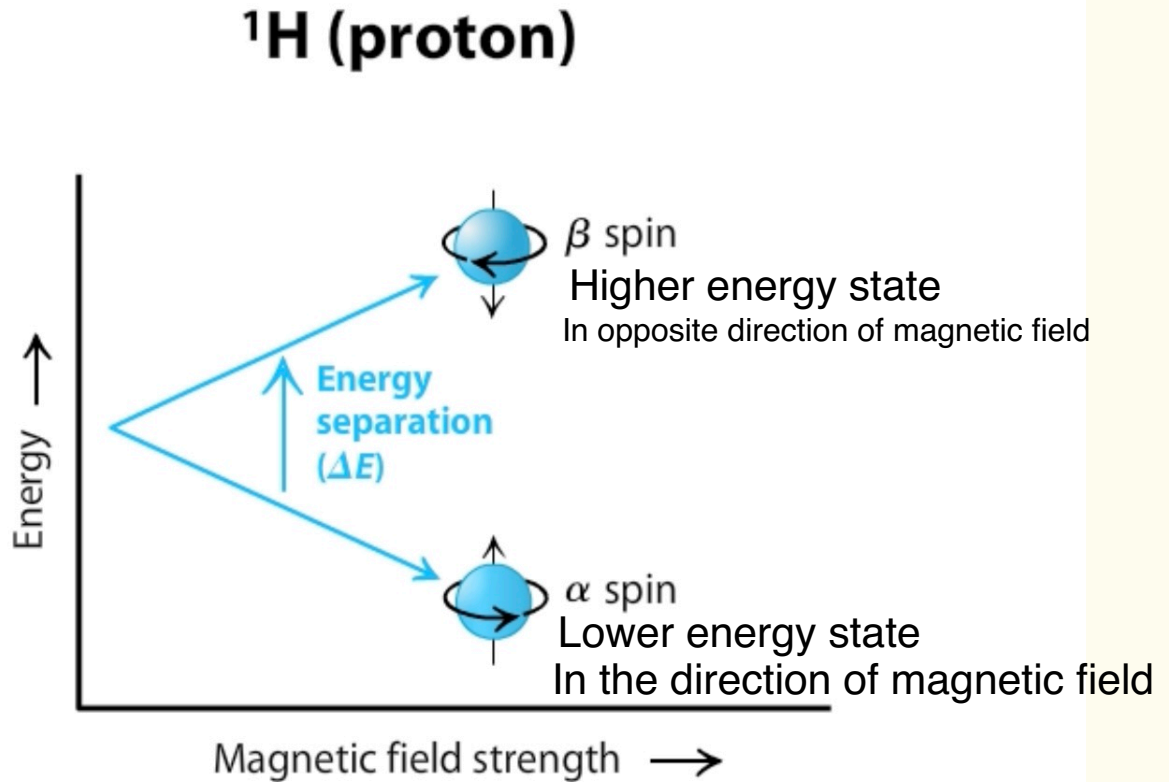


Nuclear magnetic resonance (NMR) spectroscopy

-Used to determine the structure of macromolecules



Type of radiation is radio frequency radiation

- * Energy separation is directly proportional to magnetic field strength
- * When electromagnetic radiation at specific frequency applied at alpha spin, alpha state can rise to excited beta state

≡ The energy that change the nuclear spin called magnetic resonance \longrightarrow Transition between spin states gives NMR line


- * Nuclear overhauser enhancement spectroscopy (NOESY)
- ≡ NOESY is useful for determining which signals arise from protons that are close to each other in space even if they are not bonded
- ≡ 2D NMR spectroscopy
- ≡ Protein that are far apart in the Linear structure may become close together in the folded structure
- ≡ Used to determine the structure of potential confirmation of protein

4 The Three-Dimensional Structure of Proteins

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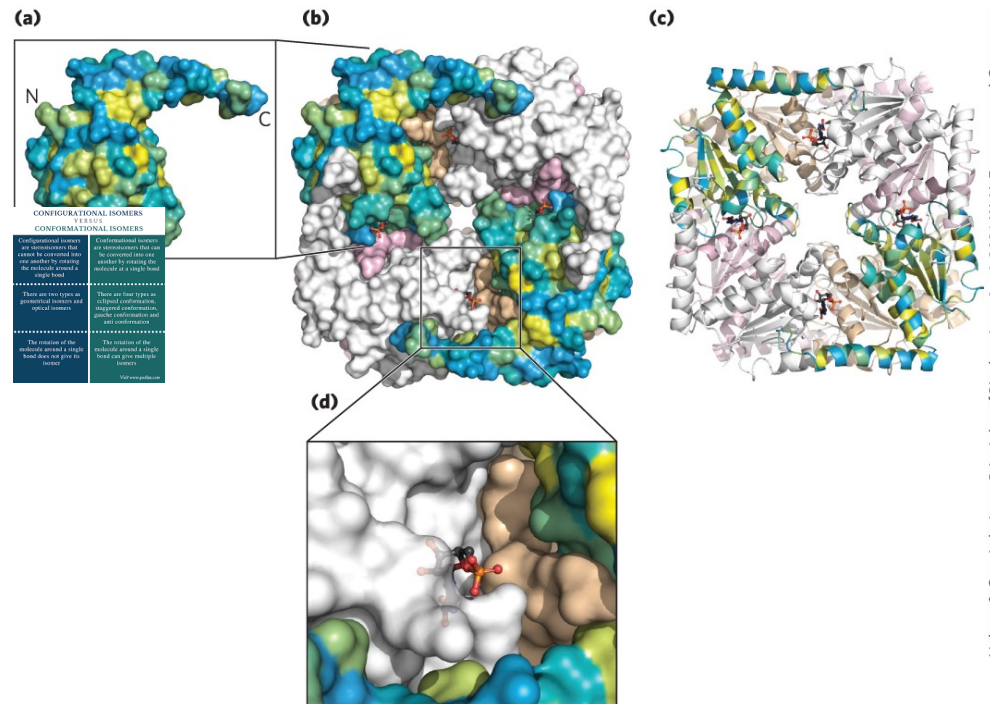


General Aspects of Protein Structure

1. ^{↗ Abbreviation of amino acid} aa sequence determines 3D structure
 2. Protein function depends on its structure
 3. An isolated protein usually exists in one or few stable structures (Native structure)
 4. Noncovalent interactions are the most important forces stabilizing protein structure
 5. There are common structural patterns in protein architecture بنية
- 

The Relationship between Protein Structure and Function

- in principle, proteins can assume an uncountable number of special arrangements, or **conformations**
- chemical or structural functions relate to unique three-dimensional structures



4.1 Overview of Protein Structure

CONFIGURATIONAL ISOMERS VERSUS CONFORMATIONAL ISOMERS

Configurational isomers are stereoisomers that cannot be converted into one another by rotating the molecule around a single bond

Conformational isomers are stereoisomers that can be converted into one another by rotating the molecule at a single bond

There are two types as geometrical isomers and optical isomers

There are four types as eclipsed conformation, staggered conformation, gauche conformation and anti conformation

The rotation of the molecule around a single bond does not give its isomer

The rotation of the molecule around a single bond can give multiple isomers

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Principle 1

Protein structures are stabilized by noncovalent interactions and forces. Formation of a thermodynamically favorable structure depends on the influences of the hydrophobic effect, hydrogen bonds, ionic interactions, and van der Waals forces. Natural protein structures are constrained by peptide bonds, whose configurations can be described by the dihedral angles φ and ψ .

Protein Conformations

- limited number of conformations predominate under biological conditions
- conformations = thermodynamically the most stable, that is, lowest free energy (G)
- **native** = proteins in any functional, folded conformations


A Protein's Conformation Is Stabilized Largely by Weak Interactions

- **stability** = tendency of a protein to maintain a native conformation
 - unfolded proteins have high conformational entropy
 - chemical interactions stabilize native conformations:
 - strong disulfide (covalent) bonds are uncommon
 - weak (noncovalent) interactions and forces are numerous
 - hydrogen bonds
 - hydrophobic effect
 - ionic interactions
-  Weak interactions are responsible for native structure
-  Protein conformation with the lowest free energy (most stable) is the one with MAXIMUM number of weak interactions

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

Packing of Hydrophobic Amino Acids Away from Water Favors Protein Folding

- **hydrophobic effect** = predominating weak interaction
- **solvation layer** = highly structured shell of H₂O around a hydrophobic molecule
 - decreases when nonpolar groups cluster together
 - decrease causes a favorable increase in net entropy
-  When two non-polar groups associate with each other, water molecules are liberated from the solvation shell, increasing the entropy.
- hydrophobic R chains form a hydrophobic protein core
 - When H₂O surrounds a hydrophobic molecule, a solvation layer of H₂O molecules is created
 - increased order of H₂O → ↓ entropy
 - BUT when non polar groups cluster together → ↓ solvation layer → ↑ entropy → ↓ ΔG → >more stable

Polar Groups Contribute Hydrogen Bonds and Ion Pairs to Protein Folding

- repeating secondary structures (α helices and β sheets) optimize hydrogen bonding
- interaction of oppositely charged groups = ion pair = salt bridge
 - strength increases in an environment of lower dielectric constant, ϵ
 - polar aqueous solvent: $\epsilon \sim 80$
 - nonpolar protein interior: $\epsilon \sim 4$



Clicker Question 1

Which item is the predominant factor in protein stability?

- A. disulfide bonds
- B. the hydrophobic effect
- C. salt bridges
- D. nonpeptide covalent bonds

Clicker Question 1, Response

Which item is the predominant factor in protein stability?

B. the hydrophobic effect

The hydrophobic effect, derived from the increase in entropy of the surrounding water when nonpolar molecules or groups are clustered together, makes the major contribution to stabilizing the globular form of most soluble proteins.

Individual van der Waals Interactions Are Weak but Combine to Promote Folding

- van der Waals interactions = dipole-dipole interactions over short distances
- individual interactions contribute little to overall protein stability
- high number of interactions can be **أساسي، مهم** **substantial**



P1

Clicker Question 2

Which statement regarding protein structure is true?

- A. Entropy is generally not a consideration when determining stability.
- B. Van der Waals interactions are insignificant.
- C. Hydrophilic side chains are never found in the interior.
- D. Hydrophobic side chains are usually in the interior of the native structure.

Clicker Question 2, Response

Which statement regarding protein structure is true?

D. Hydrophobic side chains are usually in the interior of the native structure.

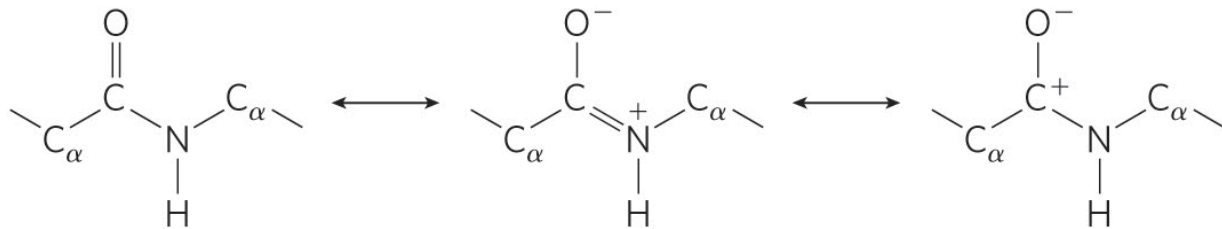
When nonpolar groups cluster together, the extent of the solvation layer decreases, because each group no longer presents its entire surface to the solution. The result is a favorable increase in entropy. Hydrophobic amino acid side chains therefore tend to cluster in a protein's interior, away from water.

Primary Structure: 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 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 have a **large dipole moment** in the favored **trans** configuration

The Peptide Bond Is Rigid and Planar

- 3 covalent bonds separate the α carbons of adjacent amino acid residues: $C_{\alpha}-C-N-C_{\alpha}$
- resonance between the carbonyl oxygen and the amide nitrogen
- partial negative charge and partial positive charge sets up a small electric dipole



(a)

Dihedral Angles Define Peptide Conformations

– ϕ (phi): angle around the α carbon—amide nitrogen bond

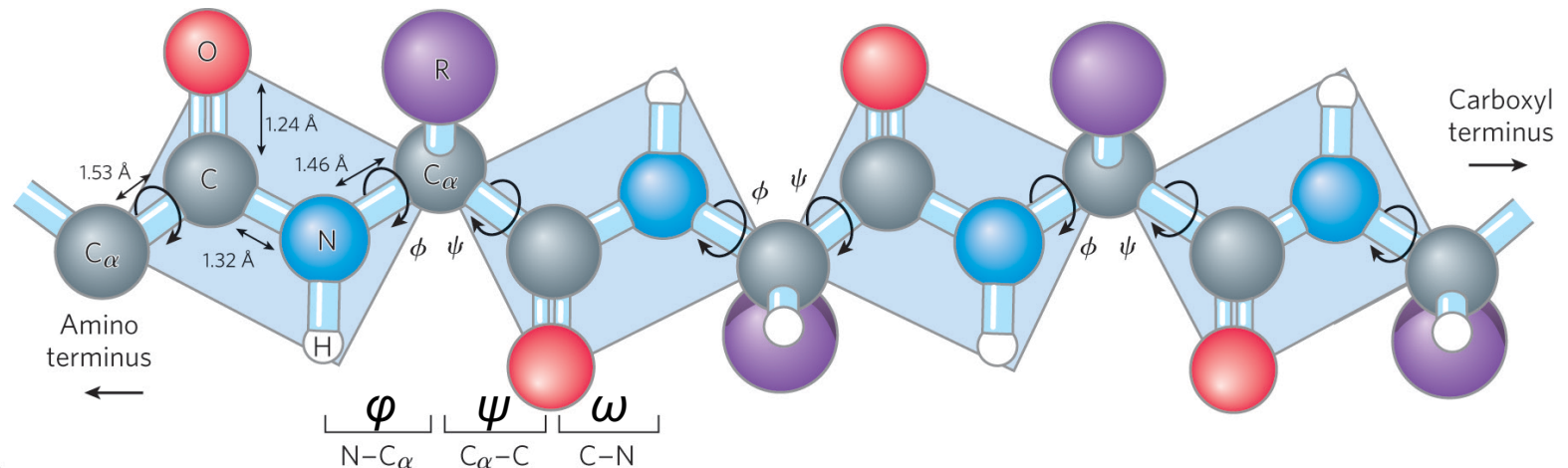
– ψ (psi): angle around the α carbon—carbonyl carbon bond

- 3 dihedral angles:

- ϕ (phi) = between -180 and $+180$ degrees

- ψ (psi) = between -180 and $+180$ degrees

- ω (omega) = ± 180 degrees for *trans* peptide bonds (0 for the rare *cis* peptide bonds)



(b)

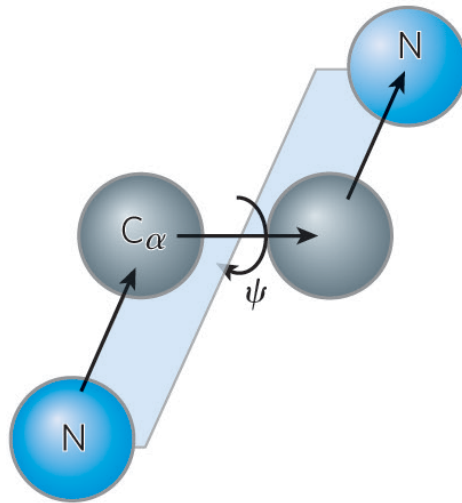
Key Convention

The important dihedral angles in a peptide are defined by the three bond vectors connecting four consecutive main-chain (peptide backbone) atoms (Fig. 4-2c): φ involves the C—N—C α —C bonds (with the rotation occurring about the N—C α bond), and ψ involves the N—C α —C—N bonds. Both φ and ψ are defined as $\pm 180^\circ$ when the polypeptide is fully extended and all peptide groups are in the same plane (Fig. 4-2d). As one looks down the central bond vector in the direction of the vector arrow (as depicted in Fig. 4-2c for ψ), the dihedral angles increase as the distal (fourth) atom is rotated clockwise (Fig. 4-2d). From the $\pm 180^\circ$ position, the dihedral angle increases from -180° to 0° , at which point the first and fourth atoms are eclipsed. The rotation can be continued from 0° to $+180^\circ$ (same position as -180°) to bring the structure back to the starting point. The third dihedral angle, ω , is not often considered. It involves the C α —C—N—C α bonds. The central bond in this case is the peptide bond, where rotation is constrained. The peptide bond is almost always (99.6% of the time) in the trans configuration, constraining ω to a value of $\pm 180^\circ$. For a rare cis peptide bond,

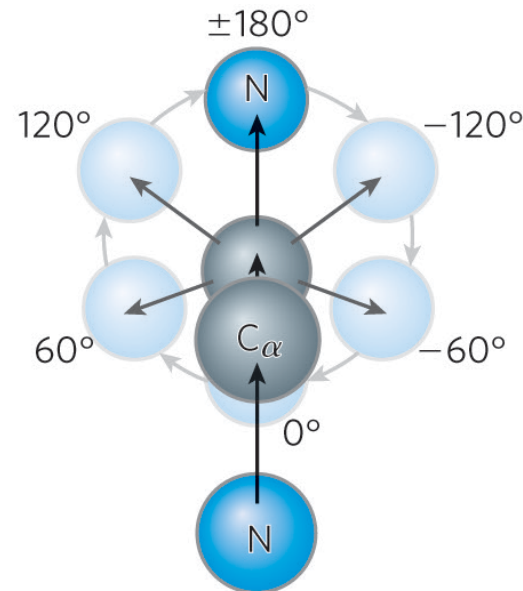
Prohibited Conformations

محظور، ممنوع

- many φ (phi) and ψ (psi) values are prohibited by steric interference
 - φ and ψ cannot both = 0 degrees
 - In a fully-extended polypeptide, both φ and ψ are 180 degrees



(c)



(d)



P1

Clicker Question 3

Why is there very little allowable rotation around the peptide bond?

- A. Peptide bonds are amide bonds.
- B. The R group prevents rotation about the peptide bond, except in glycine.
- C. The C—N bond is shorter.
- D. Peptide bonds are only formed in the cis configuration.
- E. The partial double-bond character makes the peptide bond planar.

Clicker Question 3, Response

Why is there very little allowable rotation around the peptide bond?

E. The partial double-bond character makes the peptide bond planar.

Because of their partial double-bond character, peptide C—N bonds cannot rotate freely. This keeps the entire six-atom peptide group in a rigid planar configuration.

4.2 Protein Secondary Structure



Principle 2

Protein segments can adopt regular secondary structures such as the α helix and the β conformation. These structures are defined by particular values of φ and ψ and their formation is impacted by the amino acid composition of the segment. All of the φ and ψ values for a given protein structure can be visualized using a Ramachandran plot.

Protein Secondary Structure

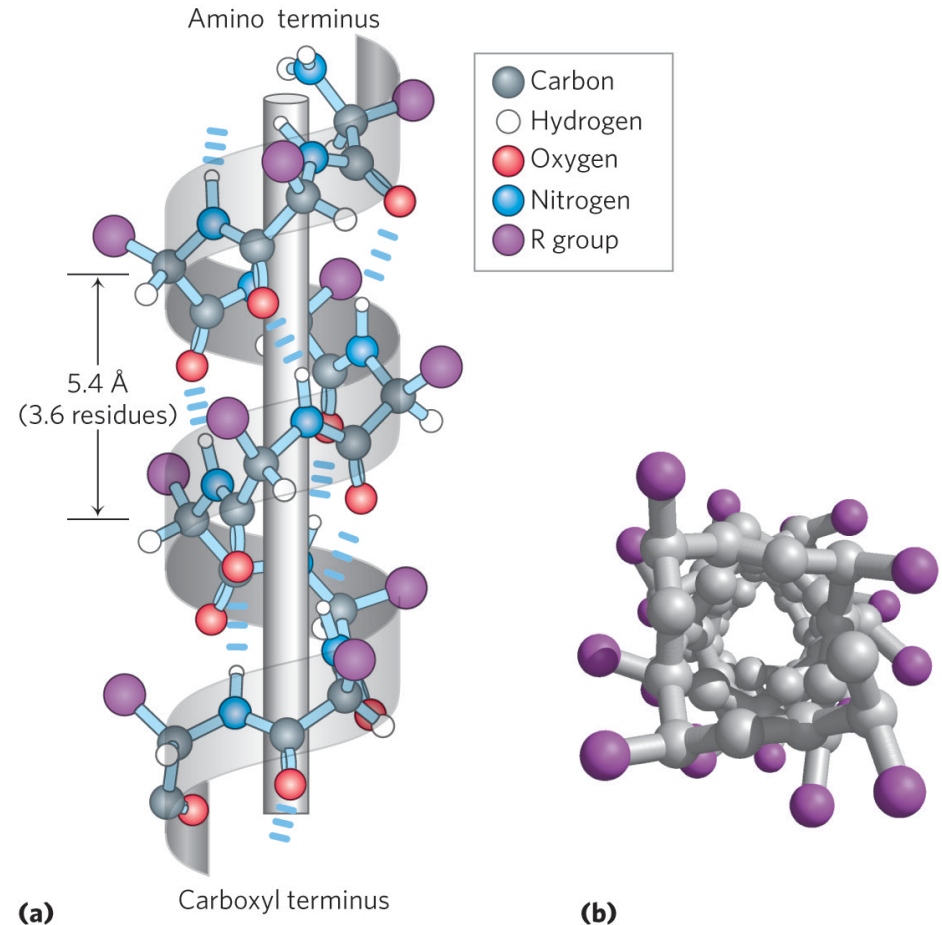
مكاني، فراغي

- **secondary structure** = describes the local spatial arrangement of the main-chain atoms (backbone) in a segment of a polypeptide chain
 - *regular* secondary structure: φ and ψ remain the same throughout the segment
 - common types: α helix, β conformation, β turn, random coils

The α Helix Is a Common Protein Secondary Structure

1/4 of all a residues in polypeptides
are found in alpha helix

- **α helix** = simplest arrangement, maximum number of hydrogen bonds
 - backbone wound around an imaginary longitudinal axis
 - R groups protrude out from the backbone
 - each helical turn = 3.6 residues, $\sim 5.4 \text{ \AA}$



(a)

(b)

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P2

Clicker Question 4

Given that an α helix has 3.6 amino acids per turn and a rise of 5.4 Å per turn, what is the length per amino acid of the α helix?

- A. 3.6 Å
- B. 5.4 Å
- C. 1.5 Å
- D. 19.4 Å

Clicker Question 4, Response

Given that an α helix has 3.6 amino acids per turn and a rise of 5.4 Å per turn, what is the length per amino acid of the α helix?

C. 1.5 Å

length per amino acid = (5.4 Å per turn)/(3.6 amino acids per turn)

$$= 1.5 \text{ Å}$$

Distribution of 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

Dihedral Angles Define Protein Conformations

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

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

* Alpha helix has a sharp angle and is more folded, but beta conformation has obtuse (منفرجة) angle and is more extended

Handedness of the α Helix

- right-handed:
 - R groups protruding away from the helical backbone
 - most common
- extended left-handed: theoretically less stable, not observed in proteins

Left-handed helix



Right-handed helix

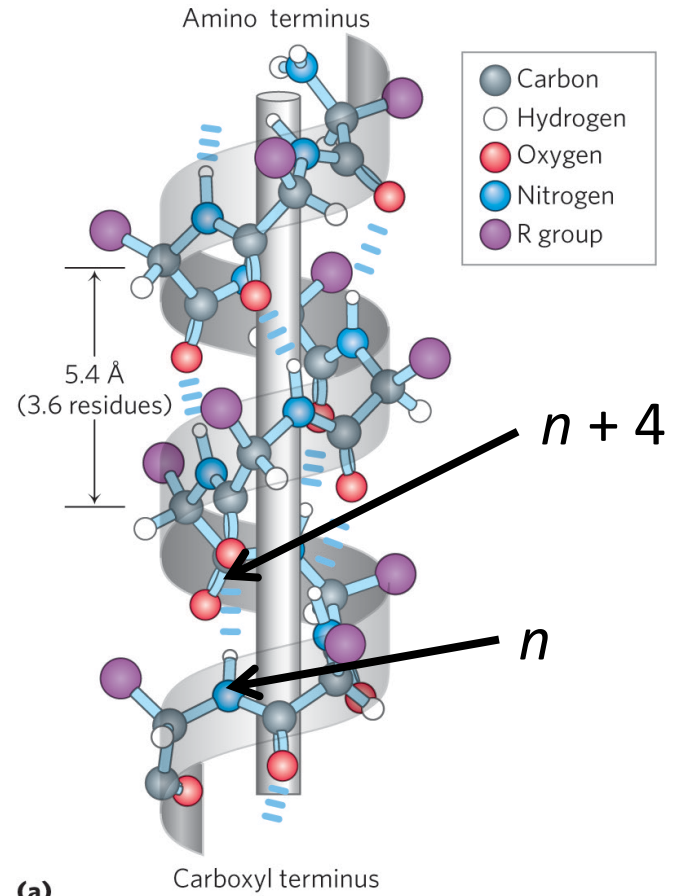


Intrahelical Hydrogen Bonds

- between hydrogen atom attached to the electronegative nitrogen atom of residue n and the electronegative carbonyl oxygen atom of residue $n + 4$ (or $n+3$)

يمنح

- confers significant stability



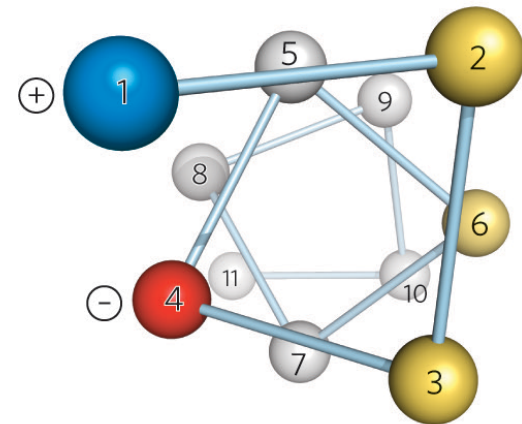
(a)

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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
 - Fits 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?**

Helical wheel



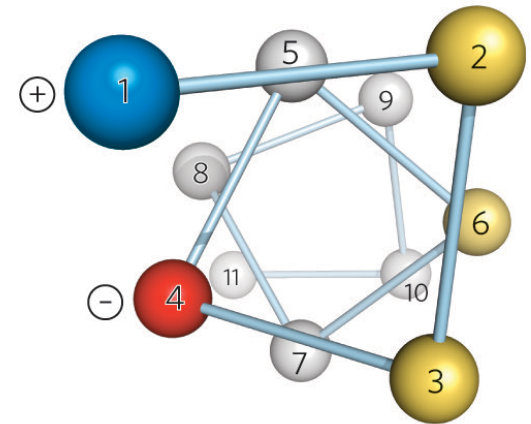
(d)

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Amino Acid Sequence Affects Stability of the α Helix

- amino acid residues have an intrinsic propensity to form an α helix
- interactions between R chains spaced 3–4 residues apart can stabilize or destabilize α helix
 - charge, size, and shape of R chains can destabilize
 - formation of ion pairs and hydrophobic effect can stabilize

Helical wheel



(d)

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Sequence affects helix stability

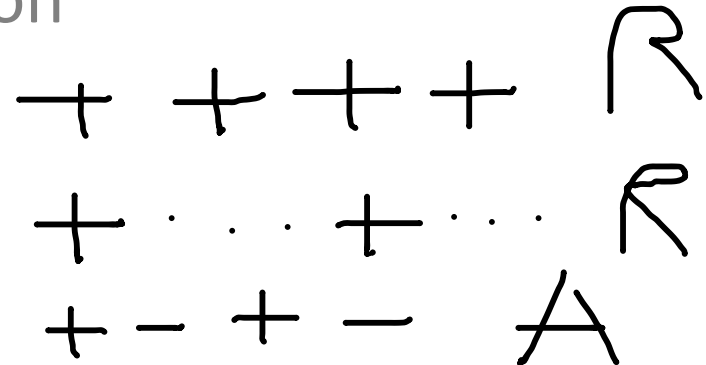
- Not all polypeptide sequences adopt α -helical structures
- Small hydrophobic residues such as **Ala** and **Leu** are strong helix formers Because when proline amine group lose on H to form the structure (N bond to R group and the other H lose through formation of residue
- **Pro** acts as a helix 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

- 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 \rightarrow increase stability
- Two hydrophobic side chains 3 or 4 aa apart \rightarrow hydrophobic interactions

Constraints to helix stability

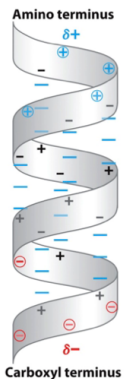
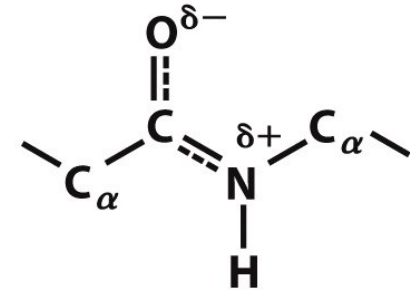
4. The presence of Pro or Gly

- **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

First four amino acids in chain → donor → positive charge

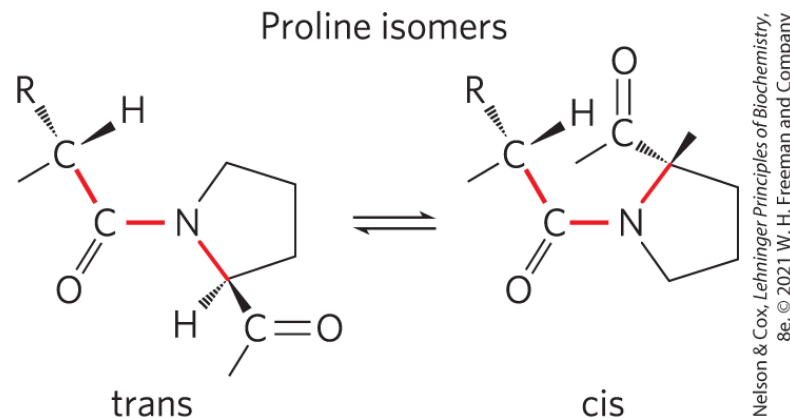
Final four amino acids in chain → acceptor → negative charge

Rest amino acids in chain → donor and acceptor at same time

Therefore, normally -vely charge aa are found near N-termini and vice versa (a +ve aa near N-terminus is destabilizing).

Proline and Glycine Occur Infrequently in an α Helix

- proline = introduces destabilizing kink in helix
 - nitrogen atom is part of rigid ring
 - rotation about N—C $_{\alpha}$ bond not possible



- glycine = high conformational flexibility, take up coiled structures

P2

Clicker Question 5

Which amino acid would destabilize an amphipathic α helix if placed in the middle of one?

- A. glycine
- B. proline
- C. glutamate
- D. valine
- E. All of these amino acids would potentially destabilize an amphipathic α helix.

Clicker Question 5, Response

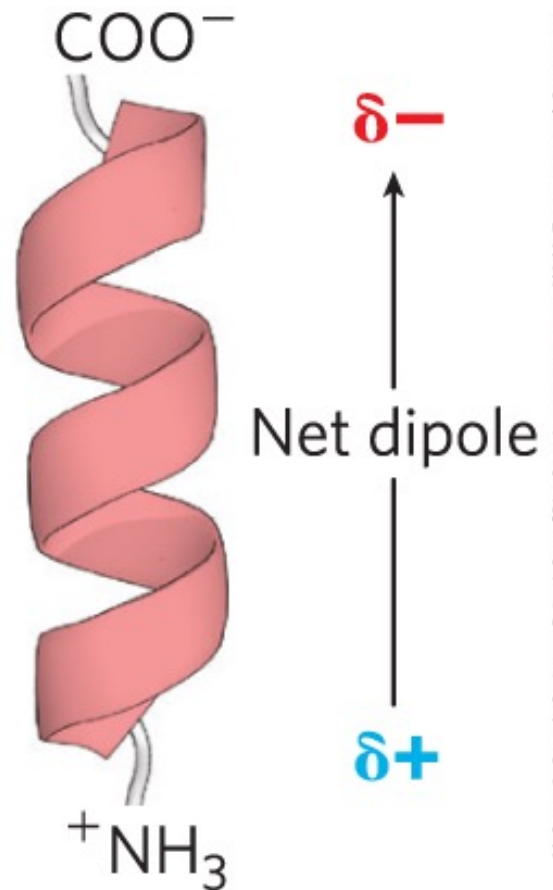
Which amino acid would destabilize an amphipathic α helix if placed in the middle of one?

E. All of these amino acids would potentially destabilize an amphipathic α helix.

Glycine has high conformational flexibility and tends to take up coiled structures. Proline introduces a destabilizing kink and the nitrogen atom of proline has no substituent hydrogen to participate in hydrogen bonds. The charge of glutamate and the branching of valine could destabilize the helix.

Amino Acid Residues Near the End of the α Helix Segment Affect Stability

- small electric dipoles in each peptide bond align through hydrogen bonds
- negatively charged amino acids often found near the NH_3^+ terminus
- positively charged amino acids often found near the COO^- terminus





P2

Clicker Question 6

The α helix is:

- A. affected by the identities of the residues near each end.
- B. stabilized by amino acid R-group interactions.
- C. a left-handed helix with 3.6 amino acids per turn.
- D. stabilized primarily by the hydrophobic effect.

Clicker Question 6, Response

The α helix is:

A. affected by the identities of the residues near each end.

There is a net dipole along the helical axis of an α helix. The partial positive and negative charges of the helix dipole reside on the peptide NH_3^+ and COO^- groups. For this reason, negatively charged amino acids are often found near the NH_3^+ terminus and positively charged amino acids are often found near the COO^- terminus.

The β Conformation Organizes Polypeptide Chains into Sheets

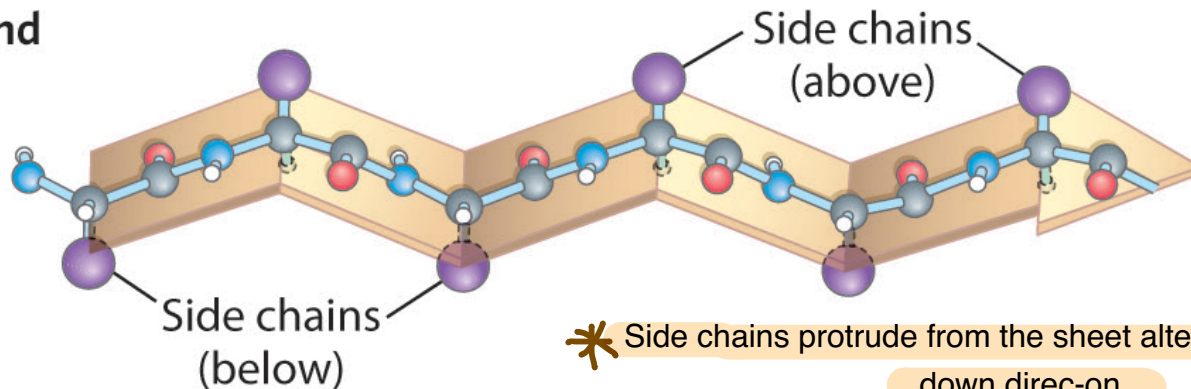
* Hydrogen bond in alpha helix occur at same helix(nitrogen that bond to hydrogen and the oxygen are found at same helix

* Hydrogen bond in beta sheet found between two sheets, one has a nitrogen that bond to hydrogen and other sheet has oxygen atom

- **β conformation** = backbone extends into a zigzag
 - β strand = single protein segment
 - **β sheet** = several strands in β conformation side by side

(a) β Strand

Side view



* Side chains protrude from the sheet alternating in up and down direction

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* **Important note:** Segments of beta sheet are usually near each other to form hydrogen bond (sometimes can be very far or even between different polypeptides)

Adjacent Polypeptide Chains in a β Sheet Can Be Antiparallel or Parallel

* Hydrogen bonds between strands are linear (stronger).

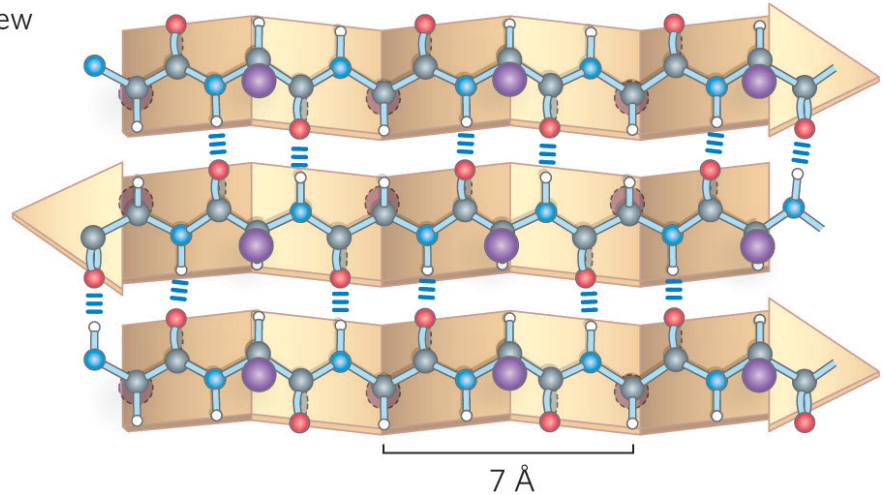
- antiparallel = opposite orientation
 - occur more frequently

* Hydrogen bonds between strands are bent (weaker).

- parallel = same orientation
- H bonds form between backbone atoms of adjacent segments

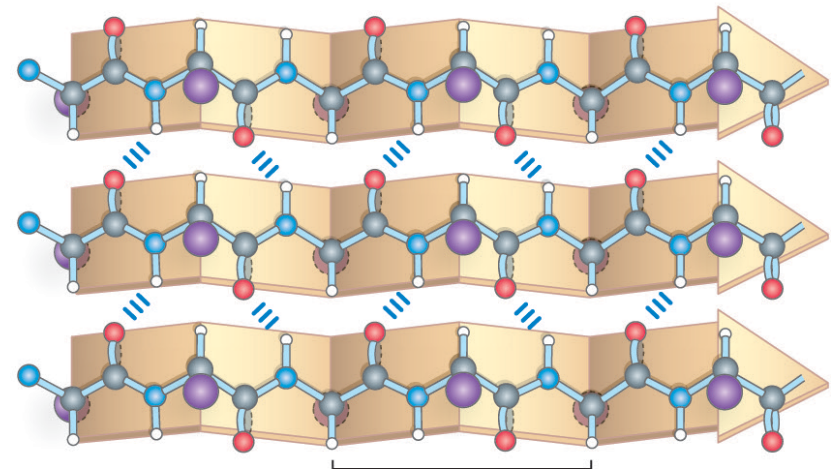
(b) Antiparallel β sheet

Top view



(c) Parallel β sheet

Top view





P2

Clicker Question 7

The β sheet:

- A. can be either parallel or perpendicular.
- B. has strands in which the R groups of the amino acids are all on the same side of the sheet.
- C. is composed of amino acid residues all very near each other in the primary structure.
- D. is primarily stabilized by hydrogen bonds.

Clicker Question 7, Response

The β sheet:

D. is primarily stabilized by hydrogen bonds.

Hydrogen bonds form between backbone atoms of adjacent segments of polypeptide chains within the sheet.

β Turns Are Common in Proteins

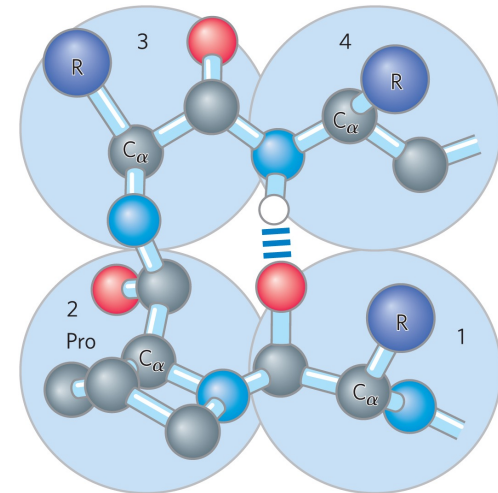
- β turns = connect ends of two adjacent segments of an antiparallel β sheet
 - 180° turn
 - involves 4 residues
 - hydrogen bond forms between first and fourth residue
 - Gly (residue 2) and Pro (residue 3) often occur in β turns

* Type I beta turn → contain proline at position 2 →

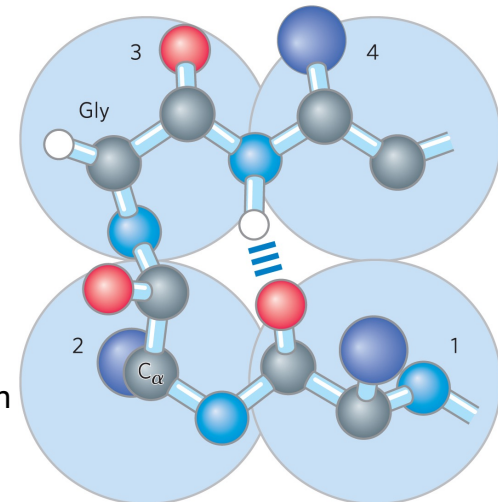
Rigidity of R group of proline is important to maintain best shape of type I beta turn

* Type II beta turn → contain glycine at position 3 →

Glycine important for the flexibility of type II beta turn



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**

Convert from cis to trans and vice versa

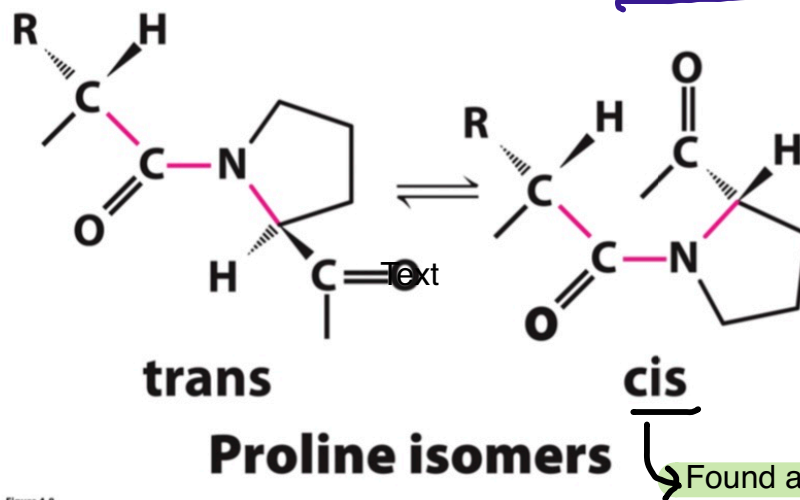
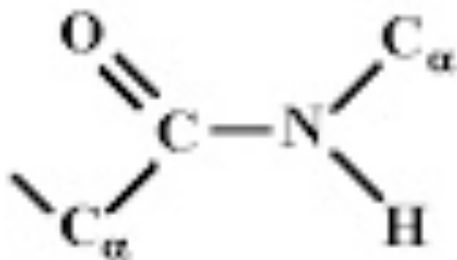
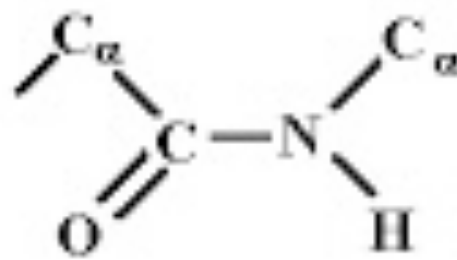


Figure 4-8
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Trans peptide bond

Carbonyl carbon up and hydrogen down



Cis peptide bond

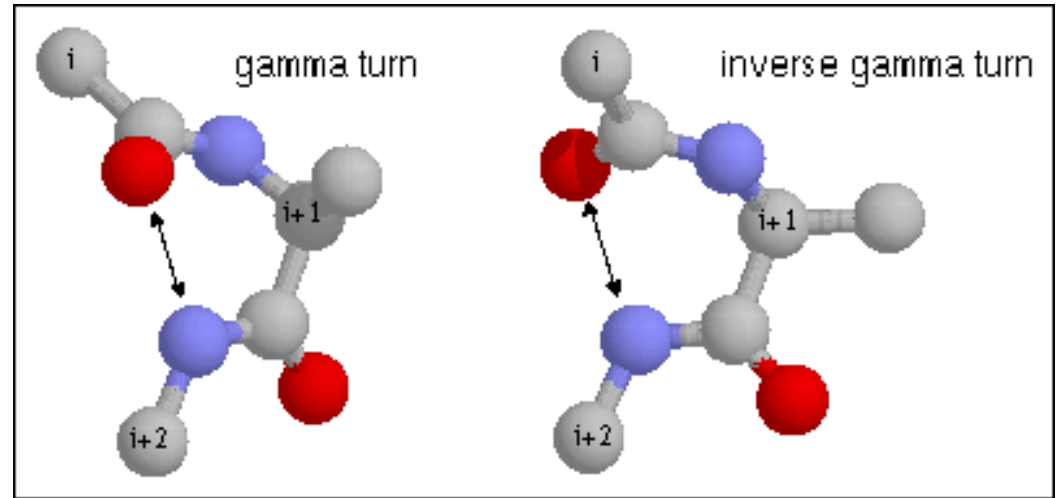
Carbonyl carbon down and hydrogen up

γ Turns Are Less Common

- Less common

- 3 aa turn

- H-bonding between 1st and 3rd aa



P2

Clicker Question 8

Which is the more stable β sheet: parallel or antiparallel?

- A. antiparallel due to more linear hydrogen bonds
- B. parallel due to the alignment of all terminal amino groups on one side and all carboxyl groups on the other side
- C. parallel due to a high concentration of proline residues, promoting more linear hydrogen bonds
- D. antiparallel due to a more elongated structure than parallel sheets
- E. antiparallel because β turns can join the strands, an arrangement that is not possible with parallel sheets

Clicker Question 8, Response

Which is the more stable β sheet: parallel or antiparallel?

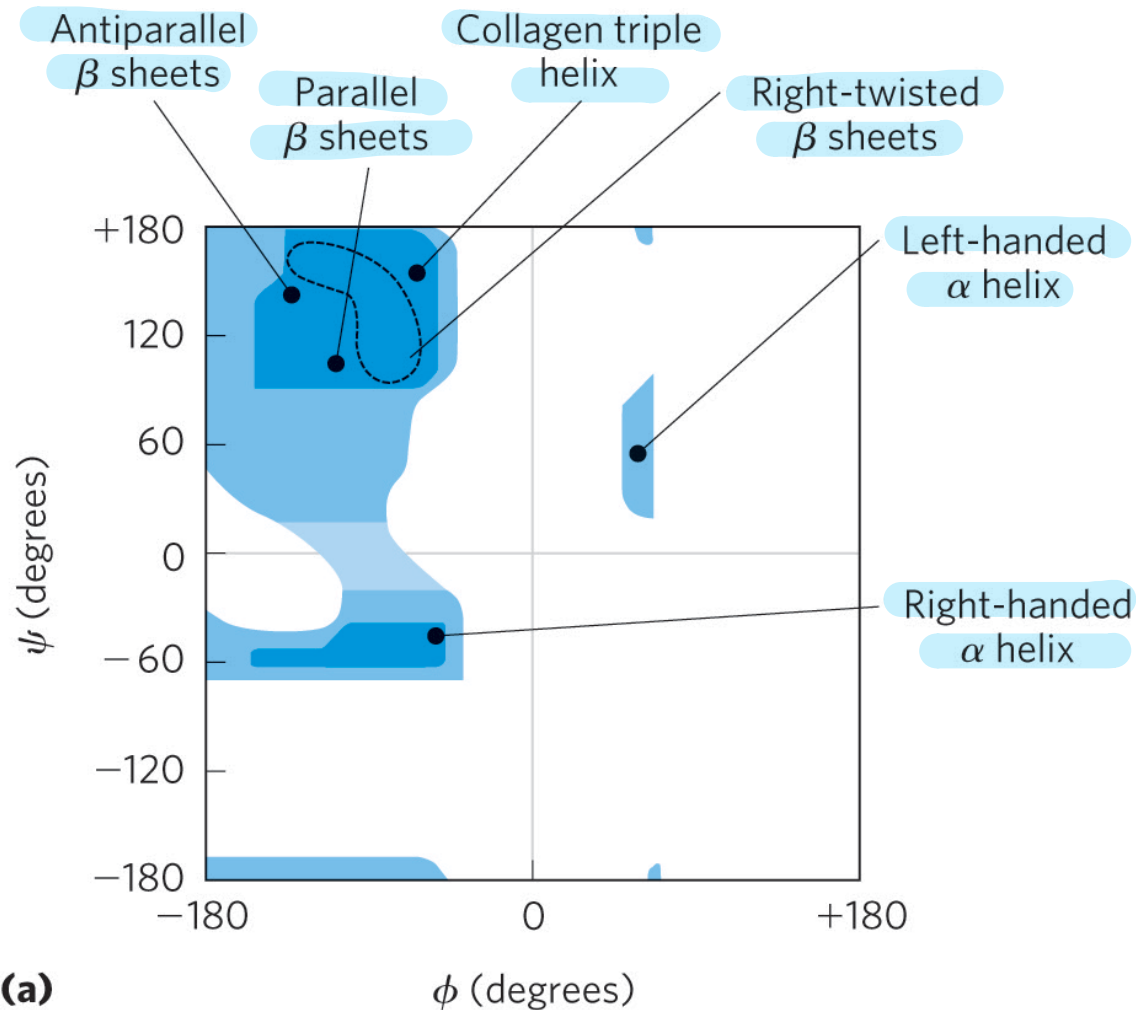
A. antiparallel due to more linear hydrogen bonds

The interstrand hydrogen bonds are essentially in-line in the antiparallel β sheet, whereas they are distorted or not in-line for the parallel variant.

Common Secondary Structures Have Characteristic Dihedral Angles

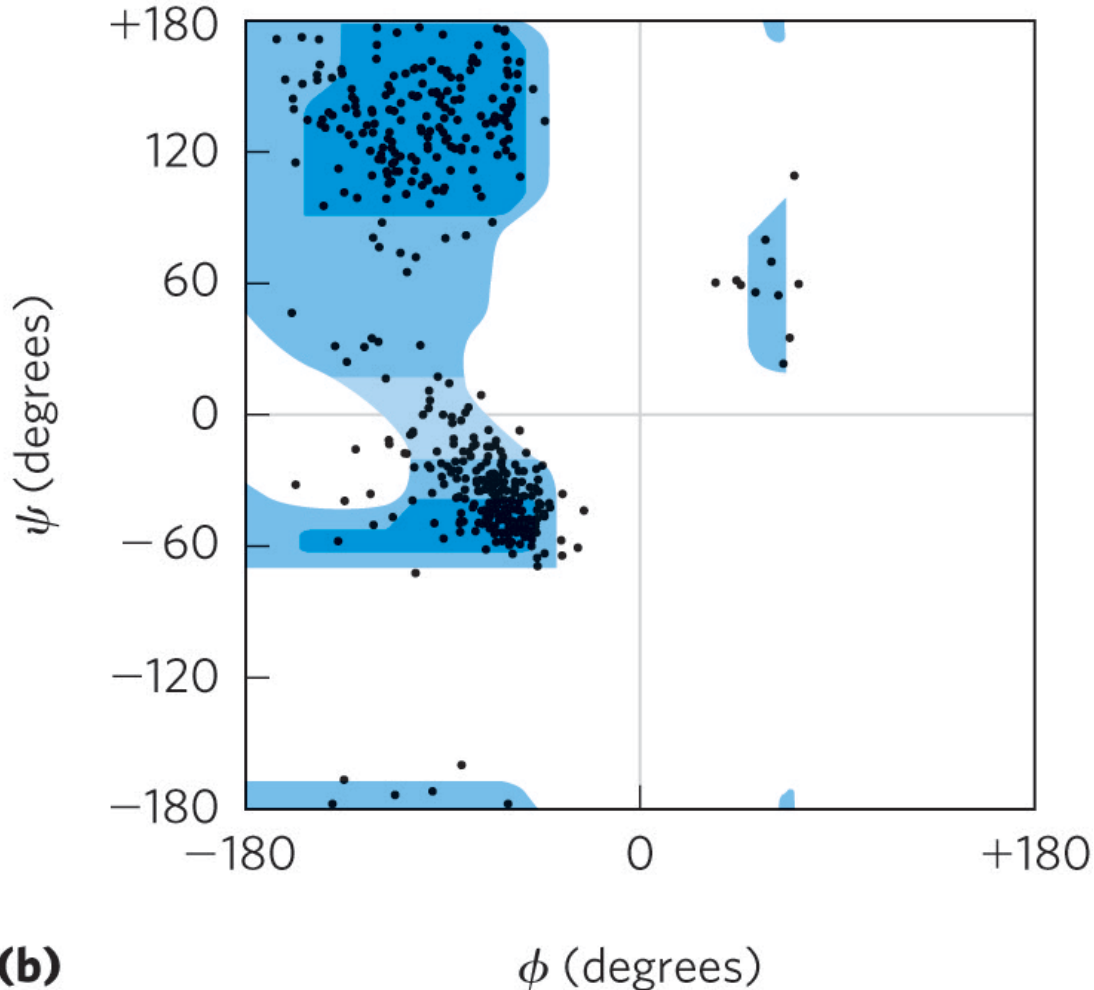
- dihedral angles φ (phi) and ψ (psi) associated with each residue completely described secondary structure
- **Ramachandran plots:**
 - visualize all φ and ψ angles
 - test quality of three-dimensional protein structures

Secondary Structure Conformations are Defined by ϕ and ψ Values



(a)

ϕ and ψ Values from Known Proteins Fall into Expected Regions



- glycine frequently falls outside the expected ranges

#Sample

(b)

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P2

Clicker Question 9

Ramachandran plots measure which dihedral angles?

- A. ϕ (phi) and ψ (psi) angles
- B. ψ (psi) and ω (omega) angles
- C. ϕ (phi) and ω (omega) angles
- D. ϕ (phi), ψ (psi), and ω (omega) angles

Clicker Question 9, Response

Ramachandran plots measure which dihedral angles?

A. ϕ (phi) and ψ (psi) angles

Ramachandran plots are useful tools for visualizing all of the ϕ and ψ angles observed in a particular protein structure and are often used to test the quality of three-dimensional protein structures.

Common Secondary Structures Can Be Assessed by Circular Dichroism

→ A technique used to determine the secondary structure fold of purified proteins

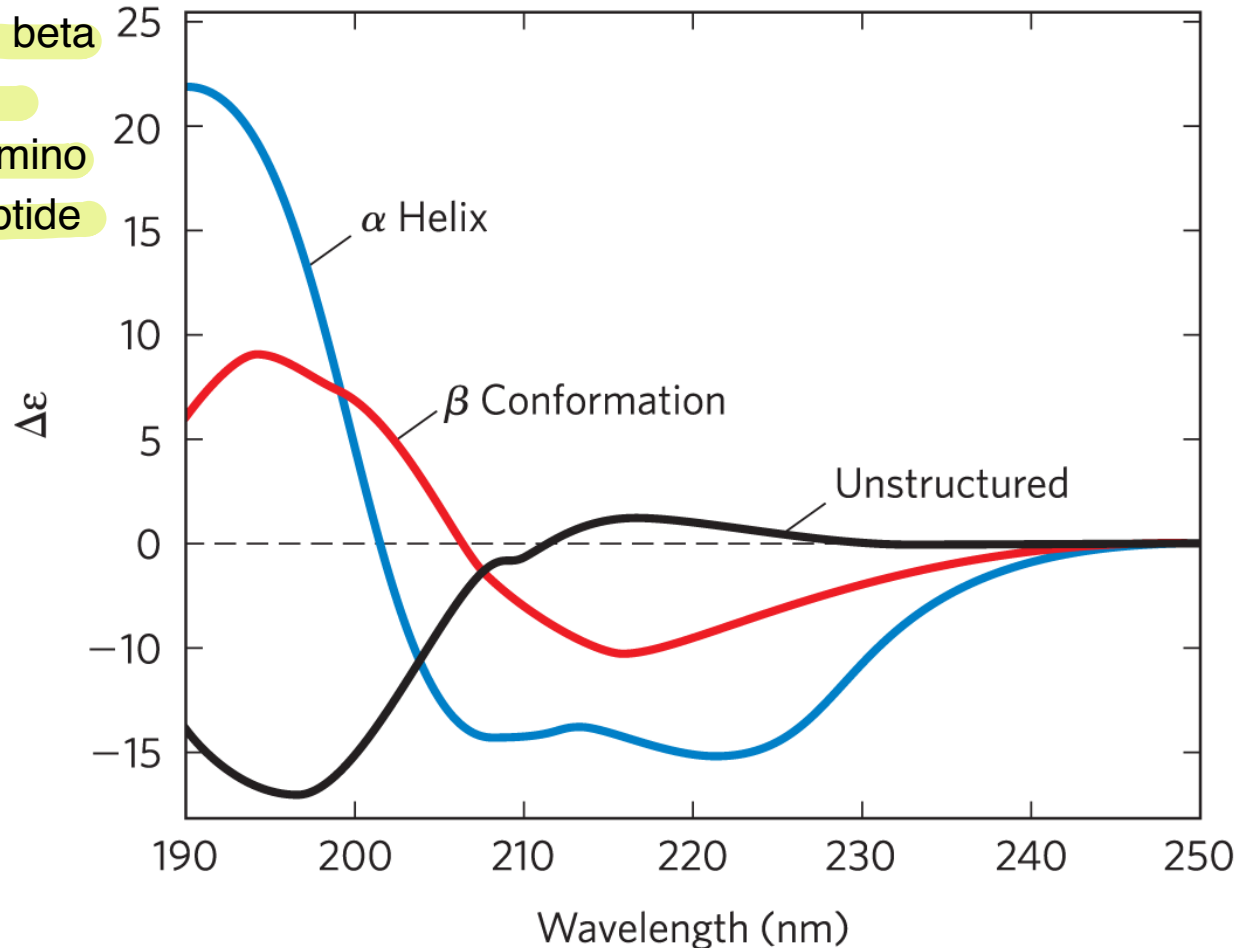
- circular dichroism (CD) spectroscopy = measures differences in the molar absorption of left-handed vs. right-handed circularly polarized light:

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R$$

- chromophore = peptide bond

Different Secondary Structures Have Different Circular Dichroism Spectra

* Use to determine the ratio between alpha helix and beta sheet and unstructured amino acid in polypeptide



P2

Clicker Question 10

Circular dichroism can NOT:

- A. provide a rough estimate for the fraction of the protein made up of the two common secondary structures.
- B. monitor the denaturation of a protein.
- C. monitor conformational changes in a protein.
- D. obtain a high-resolution three-dimensional structure.

Clicker Question 10, Response

Circular dichroism can NOT:

D. obtain a high-resolution three-dimensional structure.

Circular dichroism spectroscopy is a method for assessing common secondary structure and monitoring folding in proteins based on absorption of circularly polarized UV light. However, circular dichroism can not be used to obtain a high-resolution three-dimensional structure.

4.3 Protein Tertiary and Quaternary Structures



Principle 3

Tertiary structure describes the well-defined, three-dimensional fold adopted by a protein. Protein structures are often built by combinatorial use of common protein folds or motifs. Quaternary structure describes the interactions between components of a multisubunit assembly.

Protein Tertiary and Quaternary Structure

- **tertiary structure** = overall three-dimensional arrangement of all the atoms in a protein
 - weak interactions and covalent bonds hold interacting segments in position Make it more stable
- **quaternary structure** = arrangement of 2+ separate polypeptide chains in three-dimensional complexes

 Video:

1) What is a Protein? (from PDB-101)

Classifying Proteins

- four major types of protein groups based on polypeptide chains:
 - **fibrous proteins** = arranged in long strands or sheets
 - **globular proteins** = folded into a spherical or globular shape
 - **membrane proteins** = embedded in hydrophobic lipid membranes
 - **intrinsically disordered proteins** = lacking stable tertiary structures

- Fibrous proteins

- polypeptide chain arranged in long strands or sheets

- consist largely of 1 type of 2ry structure

- needed for structure and shape of cells

- water insoluble (hydrophobic)

- Globular proteins

- polypeptide chain folded into spherical shape

- many types of 2ry structures

- enzymes and regulators

- largely water soluble (hydrophilic)

P3

Clicker Question 11

Which statement regarding the tertiary structure of proteins is false?

- A. Amino acid residues far apart in primary structure may interact with each other in the tertiary structure.
- B. The tertiary structure of a protein is destabilized by the hydrophobic effect.
- C. Many proteins can be classified as either globular or fibrous (but not both).
- D. Quaternary structure is the description of how the tertiary structures in a multisubunit protein are arranged with respect to each other.

Clicker Question 11, Response

Which statement regarding the tertiary structure of proteins is false?

B. The tertiary structure of a protein is destabilized by the hydrophobic effect.

The tertiary structure of a protein is largely stabilized by the hydrophobic effect. In typical globular proteins, most of the hydrophobic R groups are in the interior of the molecule, hidden from exposure to water.

Fibrous Proteins Are Adapted for a Structural Function

- give strength and/or flexibility to structures
- simple repeating element of secondary structure
- H₂O insoluble due to high concentrations of hydrophobic residues

Table 4-2 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, Horns الريش القرون
β Conformation	Soft, flexible filaments, Without stretch	Silk fibron
<u>Collagen triple helix</u>	High tensile strength, without stretch	Collagen of tendons, bone matrix

Connected by flexible linker(Lys-HyLys)

Non-covalently held β -sheets
van der Waals interaction

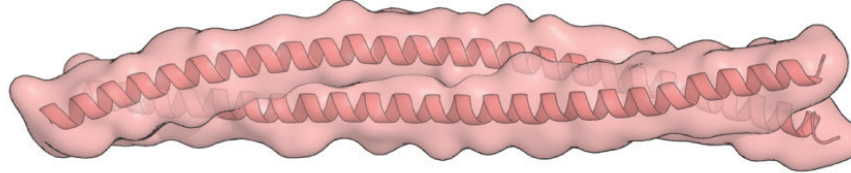
The Structure of α -Keratin in Hair

Part of a family called intermediate filaments (IF) that are important for structure of and support the cell

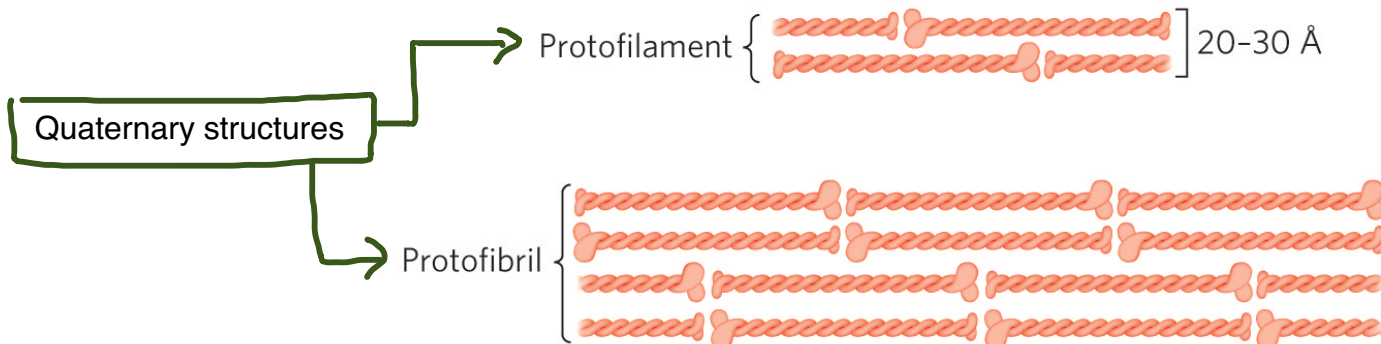
- α -keratin helix is a right-handed α helix
- two strands of α -keratin, oriented in parallel, wrap about each other to form a supertwisted coiled coil
 - supertwisted helical path is left-handed

Meaning superhelical structure

Two-chain coiled coil



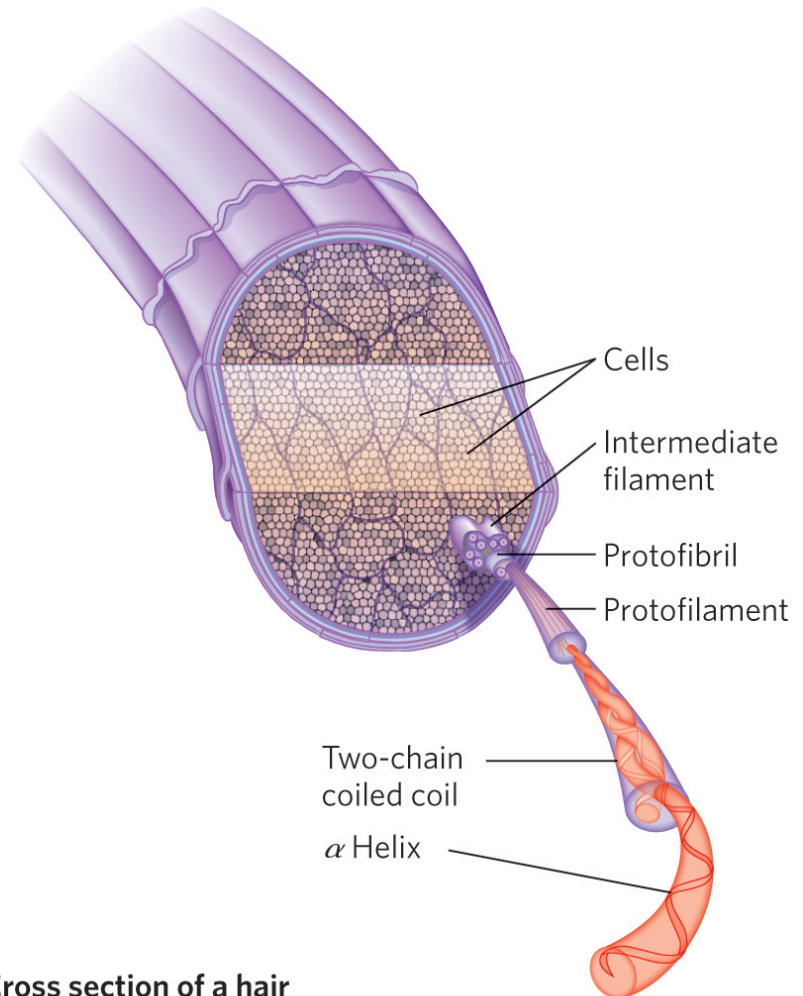
* Tertiary structure of alpha keratin in coiled coil



(a)

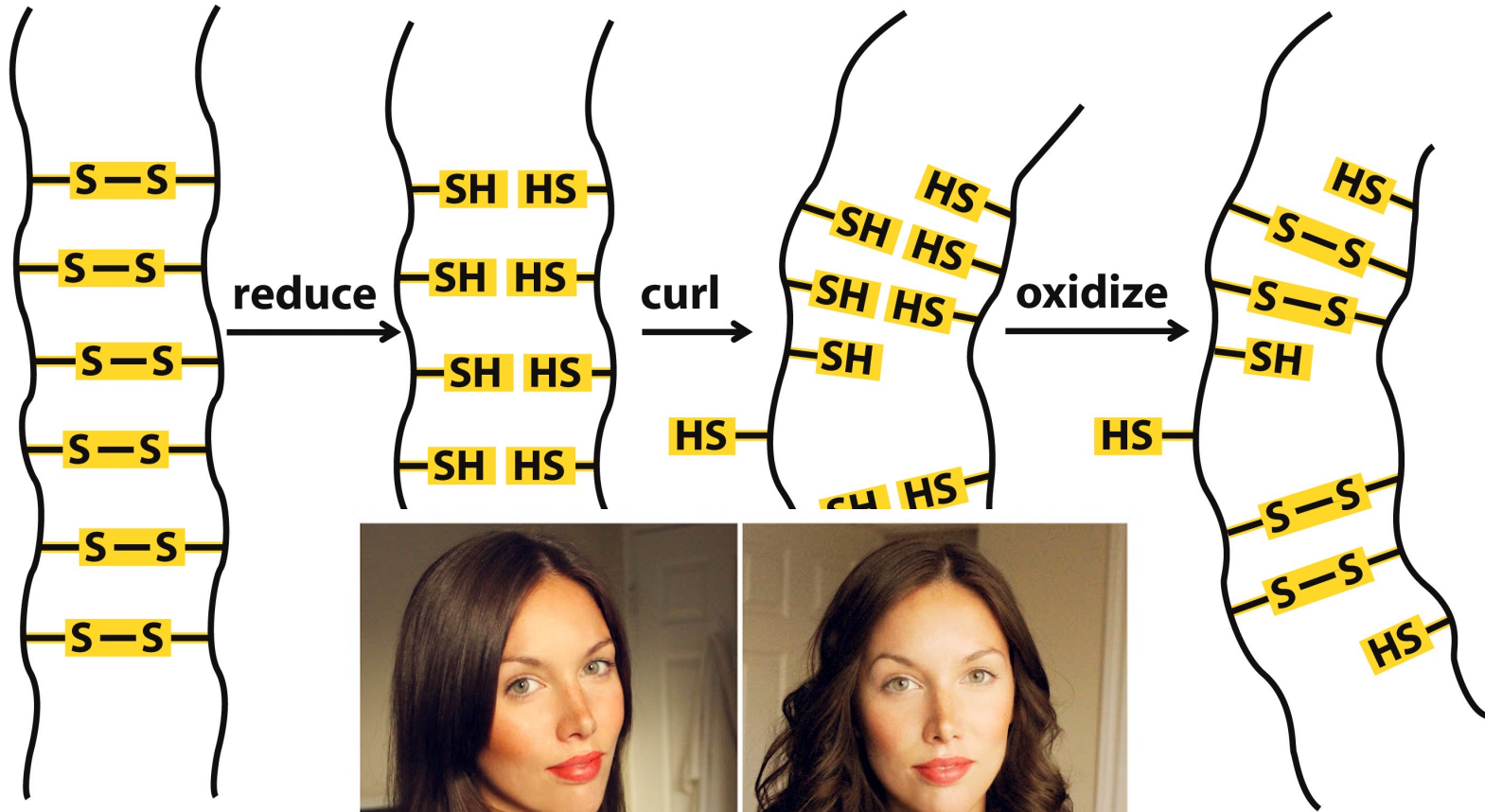
Hair Contains Many α -Keratin Filaments

- rich in hydrophobic residues: Ala, Val, Leu, Ile, Met, Phe
- cross-links stabilized by disulfide bonds



(b) Cross section of a hair

Chemistry of Permanent Waving



Box 4-2
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 YouTube Video:

1) Perming hair and tertiary structure of keratin

P3

Clicker Question 12

α -Keratin proteins constitute almost the entire dry weight of horns and hooves in mammals. The strength of α -keratin proteins comes from:

- A. cross-linking of α helices by disulfide bonds.
- B. a close packing of β sheets.
- C. a triple helix.
- D. high concentrations of charged amino acids.

Clicker Question 12, Response

α -Keratin proteins constitute almost the entire dry weight of horns and hooves in mammals. The strength of α -keratin proteins comes from:

A. cross-linking of α helices by disulfide bonds.

The α -keratin helix is a right-handed α helix, enhanced by covalent (disulfide) cross-links.

The Structure of Collagen

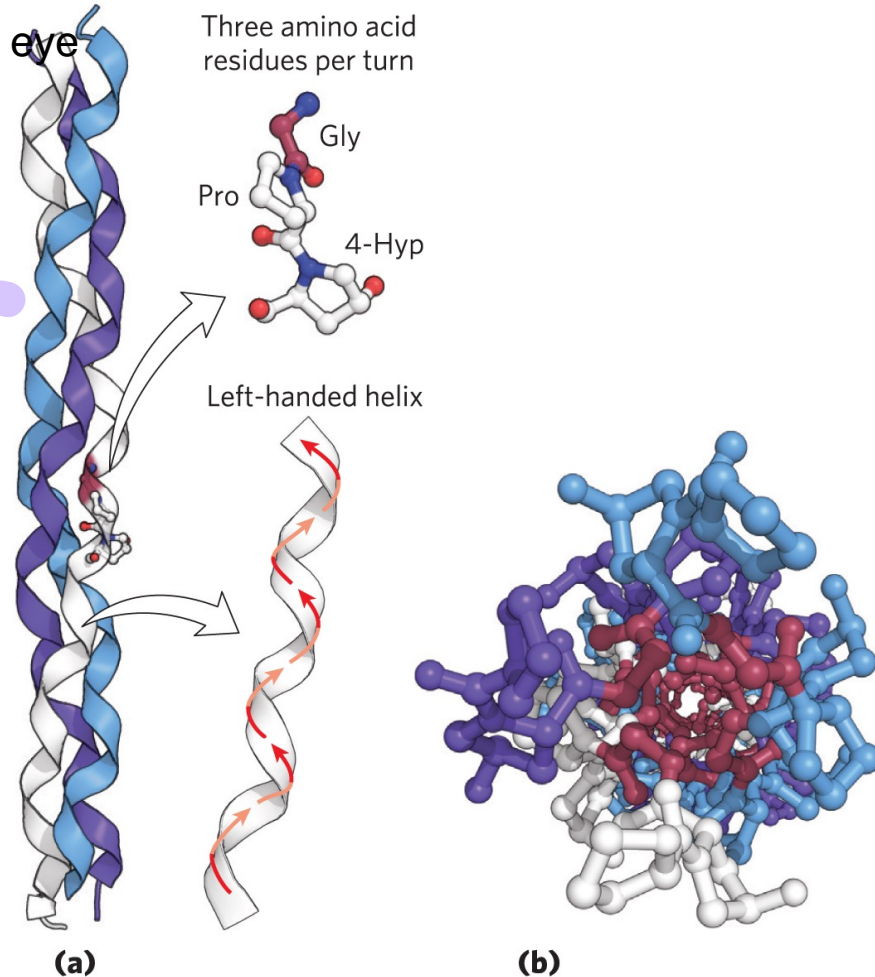
• **collagen** = found in connective tissue tendons, cartilage, bones, ^{قرنية} cornea of the eye

– secondary structure = **left-handed, repeating tripeptide unit Gly–X–Y, where X is often Pro and Y is often 4-Hyp**

– 3 aa per turn

– tertiary and quaternary structure = right-handed twisting of 3 separate polypeptides

– Higher tensile strength than a steel wire of equal cross section **Very strong**



The Structure of Collagen

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

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

often Pro

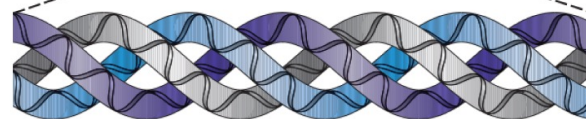
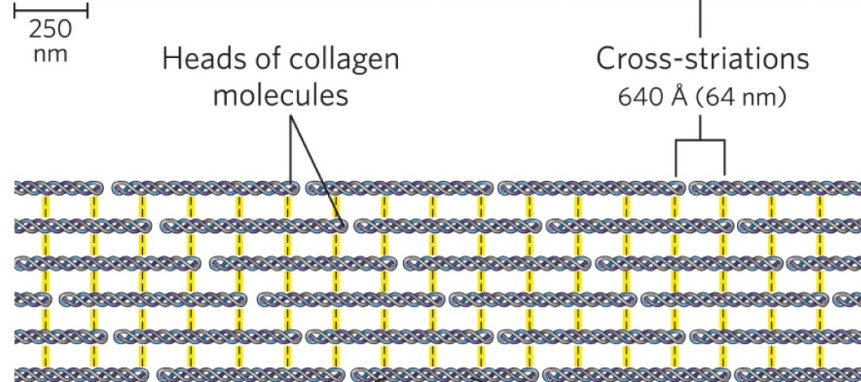
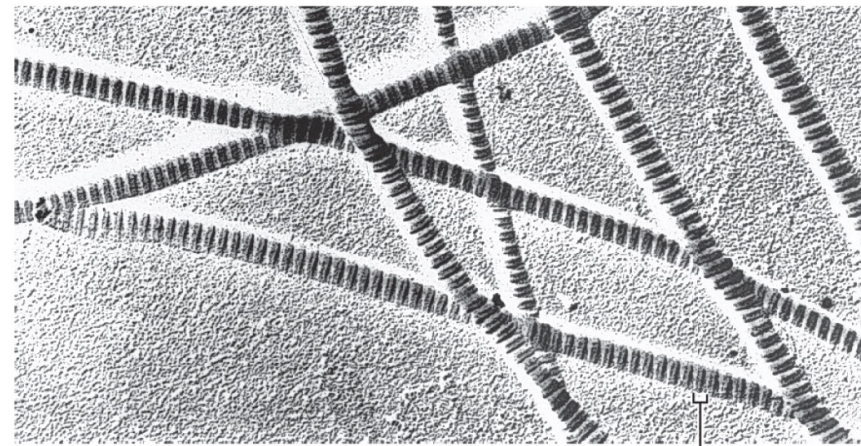
often 4-Hyp

- Many triple-helices assemble into a collagen fibril

- **Collagenase** is an enzyme that breaks down collagen

Found in bacteria that cause gangrene (غرغرينا)

The Structure of Collagen Fibrils

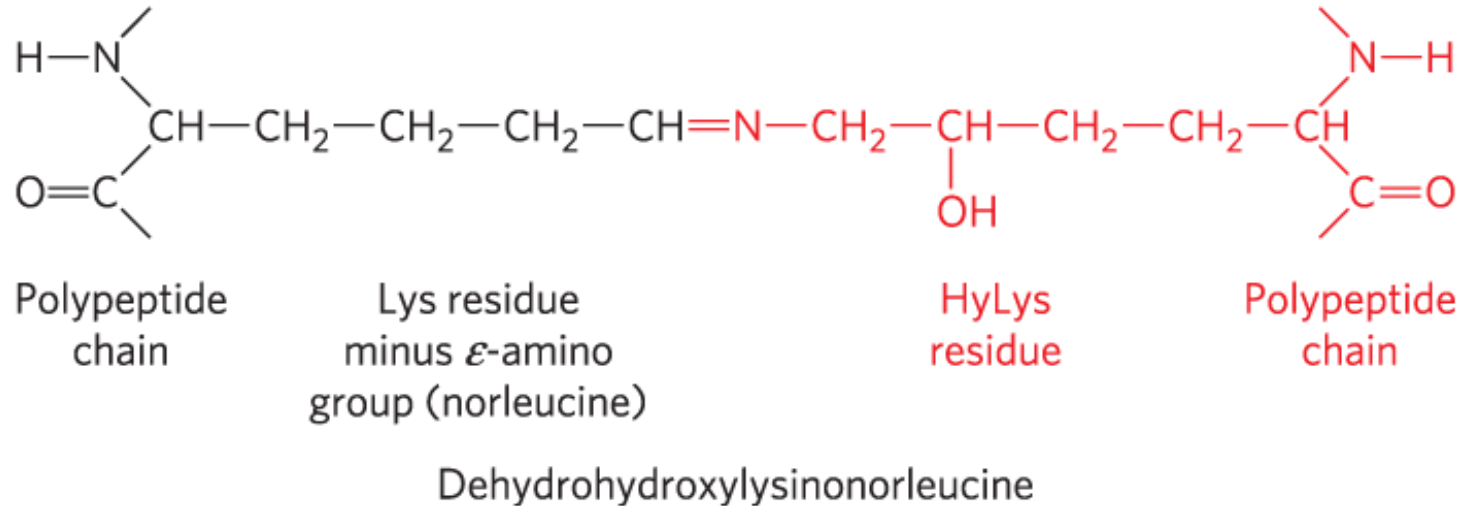


Section of collagen molecule

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J. Gross/Biozentrum, University of Basel/Science Source.

Covalent Cross-Links in Collagen Fibrils

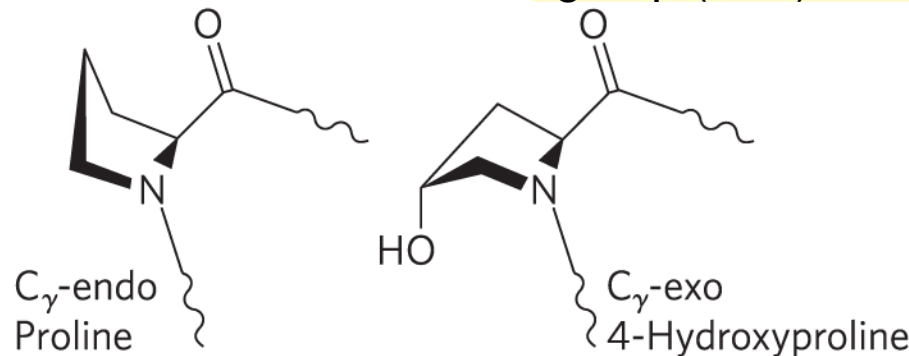
- cross-linked by covalent bonds involving Lys, HyLys (5-hydroxylysine), or His
 - links create uncommon amino acid residues



Scurvy, Vitamin C, and Collagen Formation

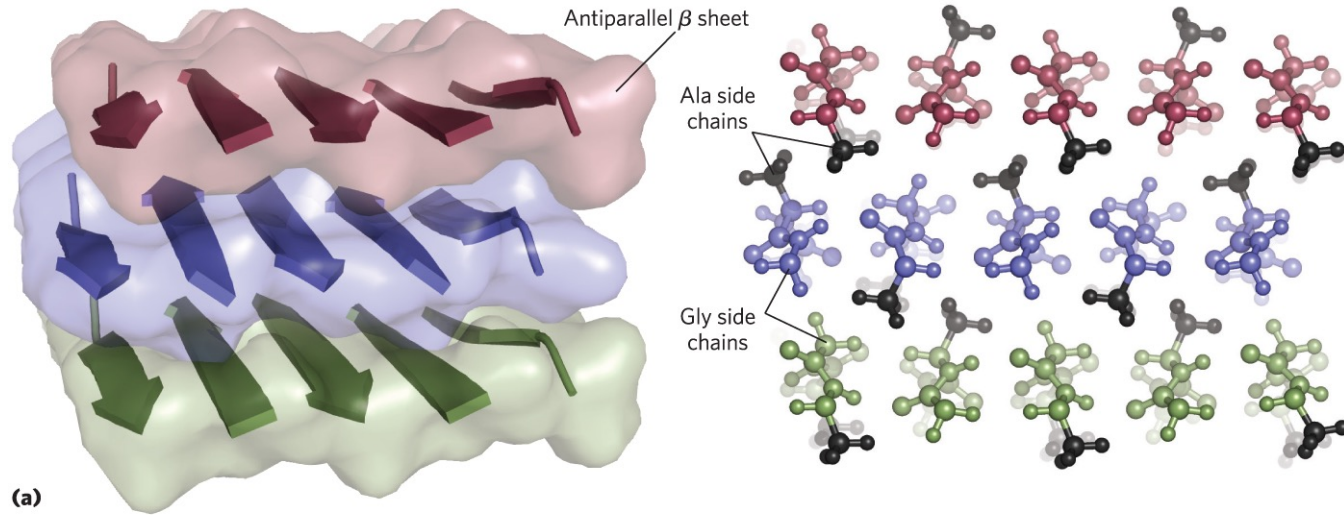
الاسقربوط

- scurvy is caused by a lack of vitamin C
 - characterized by general degeneration of connective tissue
- vitamin C is required for the hydroxylation of proline and lysine in collagen
 - ↳ a chemical process that introduces a hydroxyl group (-OH) into an organic compound



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The Structure of Fibroin



70 μm

- main protein in silk
- predominantly β conformation (Antiparallel beta sheet)
- rich in Ala and Gly
- stabilized by hydrogen bonding and van der Waals interactions (\rightarrow flexible)

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) Not stretch
 - rubber-like stretchy parts

P3

Clicker Question 13

Collagen:

- A. has fibrils consisting of cross-linked triple helices of polypeptide chains.**
- B. dose not have quaternary structure.**
- C. consists of α -helical subunits.**
- D. has a primary structure of repeating –Glu-X-Y-.**

Clicker Question 13, Response

Collagen:

A. has fibrils consisting of cross-linked triple helices of polypeptide chains.

Collagen is composed of three separate polypeptides twisted about each other. Collagen fibrils are made up of collagen molecules aligned in a staggered fashion and cross-linked for strength.

Structural Diversity Reflects Functional Diversity in Globular Proteins

* Have more than one secondary structure

- globular proteins:
 - fold back on each other
 - more compact than fibrous proteins
 - enzymes, transport proteins, motor proteins, regulatory proteins, immunoglobulins

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

α Helix
 $900 \times 11 \text{ \AA}$

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

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The Protein Data Bank

- The **Protein Data Bank (PDB)**: www.rcsb.org
 - archive of experimentally determined three-dimensional structures
 - structures assigned an identifier called the PDB ID
 - PDB data files describe:
 - the spatial coordinates of each atom
 - information on how the structure was determined
 - information on its accuracy
 - structure visualization software can convert atomic coordinates to an image of the molecule

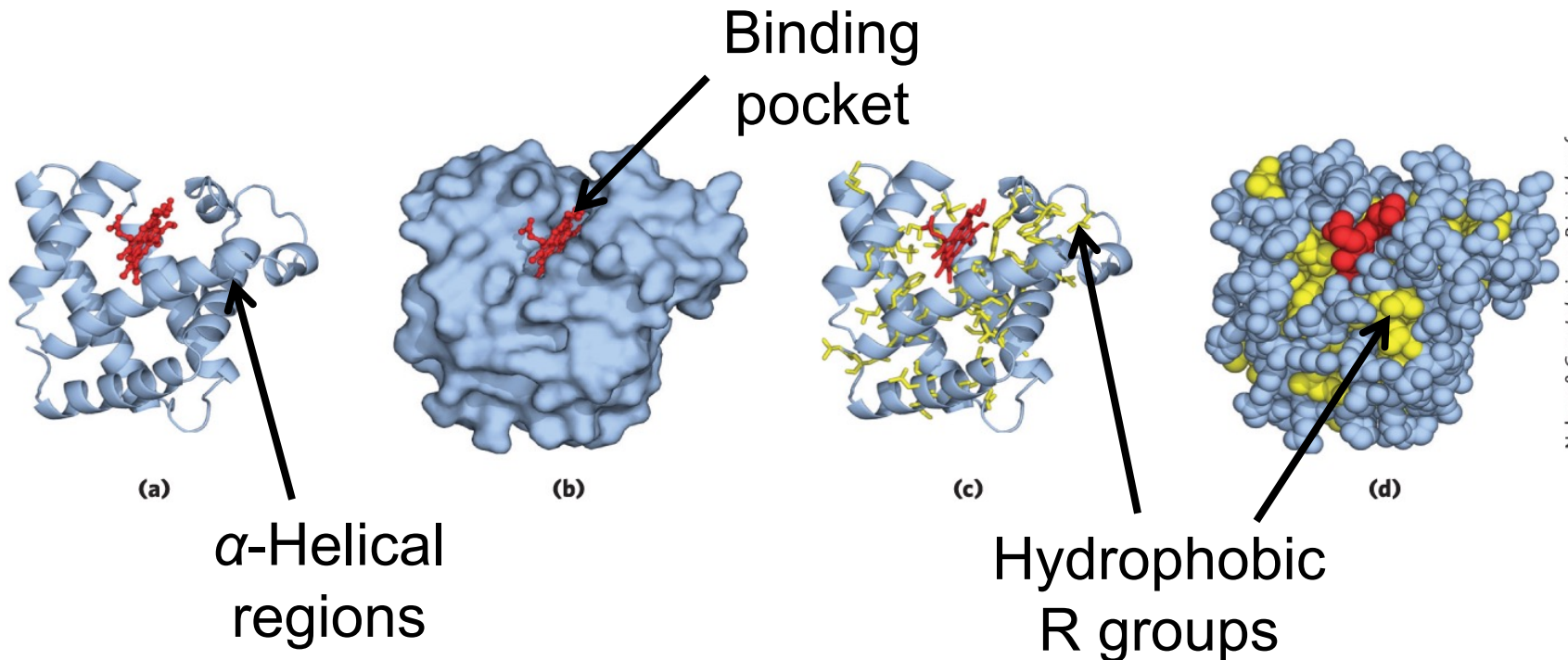


AlphaFold

- The **AlphaFold Data Base**: <https://alphafold.com>
 - AlphaFold DB provides open access to 992,316 protein structure predictions for the human proteome and other key proteins of interest, to accelerate scientific research
 - AlphaFold is an AI system developed by DeepMind that predicts a protein's 3D structure from its amino acid sequence. It regularly achieves accuracy competitive with experiment

Myoglobin Provided Early Clues about the Complexity of Globular Protein Structure

- several structural representations of myoglobin's tertiary structure:



Myoglobin

- Small (Mr 16700)
- O₂-binding protein of muscle cells
- Storage of O₂ 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

Globular Proteins Have a Variety of Tertiary Structures

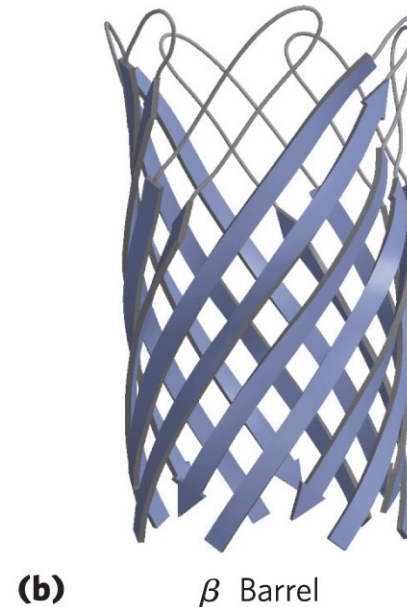
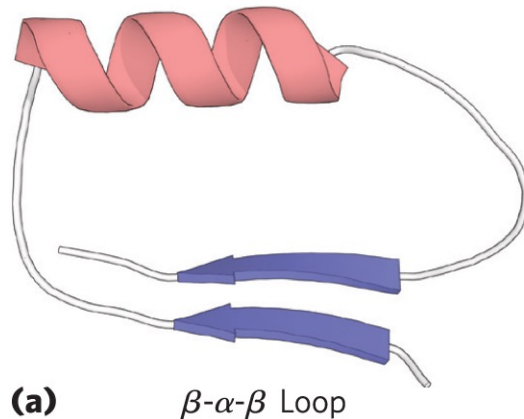
Table 4-3 Approximate Proportion of α Helix and β Conformation in Some Single-Chain Proteins

Protein (total residues)	Residues (%): α Helix	Residues (%): β Conformation
Chymotrypsin (247)	14	45
Ribonuclease (124)	26	35
Carboxypeptidase (307)	38	17
Cytochrome c (104)	39	0
Lysozyme (129)	40	12
Myoglobin (153)	78	0

- each globular protein has a distinct structure, adapted for its biological function

Folding Patterns of Proteins

- **motif = fold = super-secondary structure =** recognizable folding pattern involving 2+ elements of secondary structures and the connection(s)
 - can be simple, such as in a **β - α - β loop**
 - can be **elaborate**, such as in a **β barrel**



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

P3

Clicker Question 14

A *motif* in the glycolysis enzyme hexokinase could NOT contain:

- A. a β turn.
- B. a β sheet.
- C. an α helix.
- D. disulfide bonds
- E. All of these could be found in a motif.

Clicker Question 14, Response

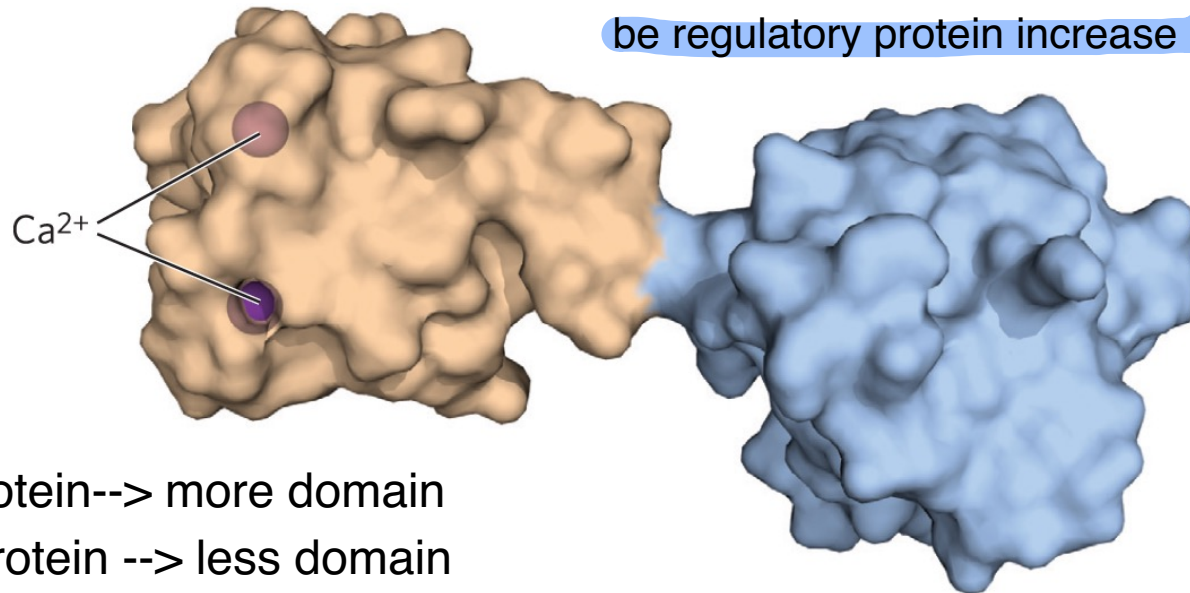
A *motif* in the glycolysis enzyme hexokinase could NOT contain:

E. All of these could be found in a motif.

A motif or fold is a recognizable folding pattern involving two or more elements of secondary structure and the connection(s) between them. A motif could include a β turn, a β sheet, an α helix, and disulfide bonds.

Protein Domains

- **domain** = part of a polypeptide chain that is independently stable or could undergo movements as a single entity
 - domains may appear as distinct or be difficult to **discern** (تحديد، تمييز)
 - small proteins usually have only one domain
 - * As the size of protein increase, the possibility of this protein to be regulatory protein increase



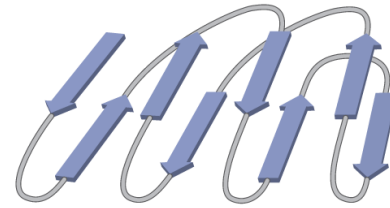
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- * Bigger protein --> more domain
- * Smaller protein --> less domain

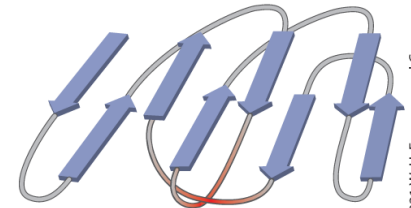
Not important

Protein-Folding Rules

- ^{دفن} burial of hydrophobic R groups requires 2+ layers of secondary structure
- α helices and β sheets are found in different layers
- adjacent amino acid segments are usually ^{مكدسة} stacked adjacent
- the β conformation is most stable with right-handed connections



(a) Typical connections in an all- β motif



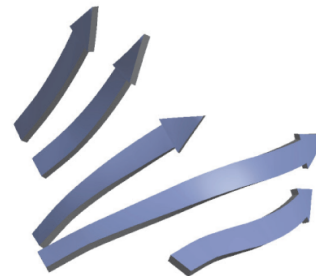
Crossover connection (not observed)



(b) Right-handed connection between β strands



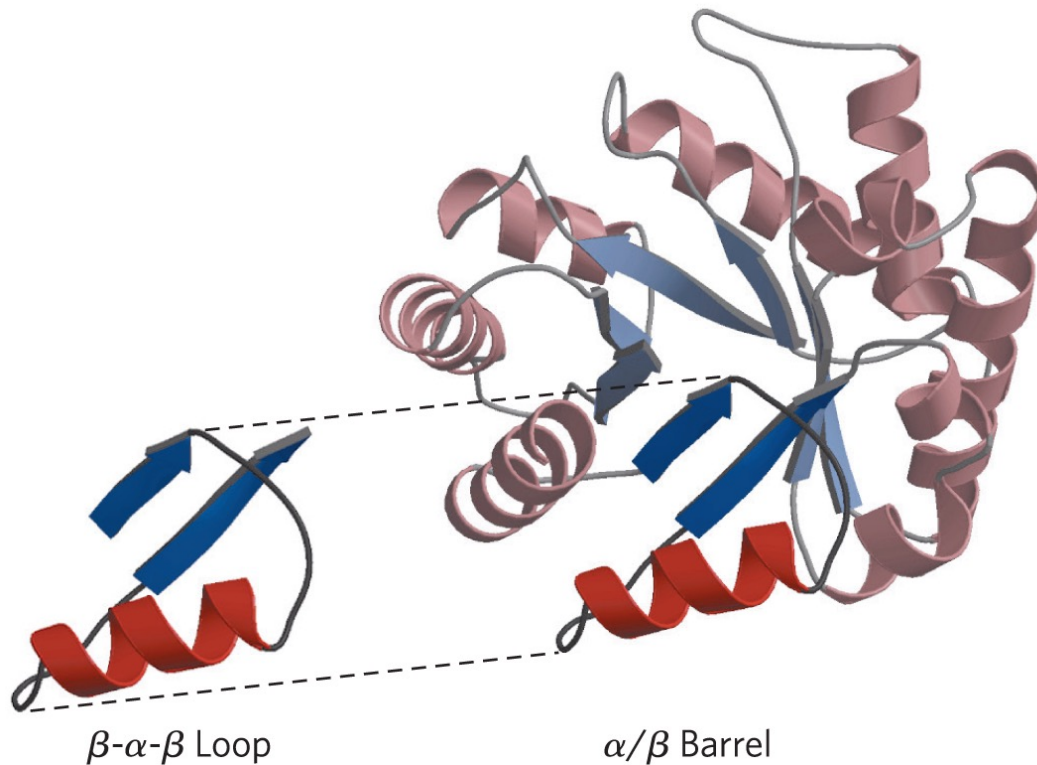
Left-handed connection between β strands (very rare)



(c) Twisted β sheet

Complex Motifs Are Built from Simple Motifs

- α/β barrel = series of β - α - β loops arranged such that the β strands form a barrel



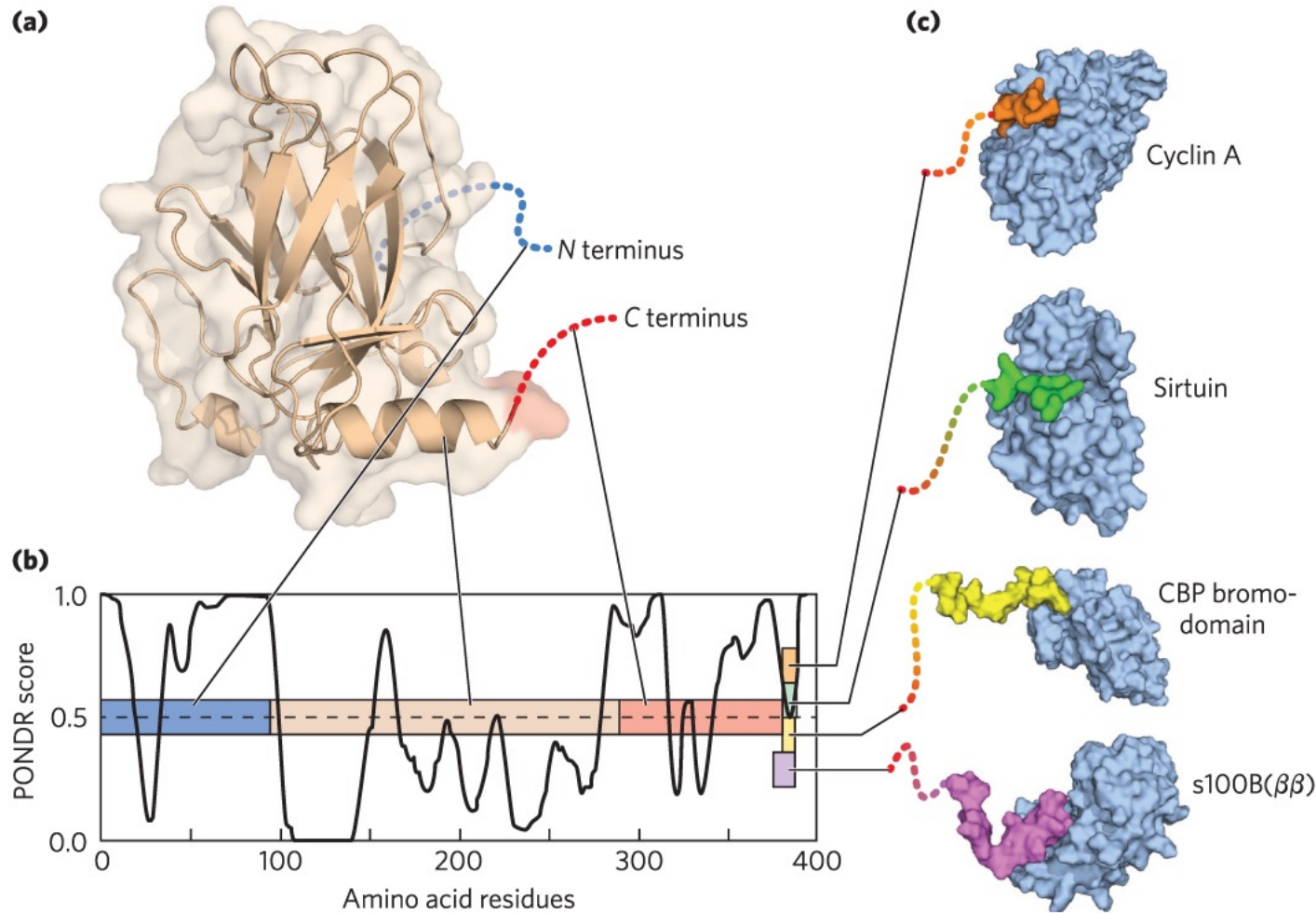
Some Proteins or Protein Segments Are Intrinsically Disordered

- intrinsically disordered proteins:
 - lack definable structure
 - often lack a hydrophobic core
 - high densities of charged residues (Lys, Arg, Glu) and Pro
 - facilitates a protein to interact with multiple binding partners

* P207 is an intrinsically disordered protein, it has the ability to bond around other proteins like protein kinases, it inhibit protein kinases so protein kinases stop work. Protein kinases play a very important role in cell division.

* When cancer occur, the concentration of p207 will be very low.

Intrinsically Disordered Segments Can Assume Different Structures





P3

Clicker Question 15

Which feature is NOT associated with globular proteins?

- A. domains
- B. motifs
- C. classified as either all α or all β
- D. intrinsically disordered regions of structure

Clicker Question 15, Response

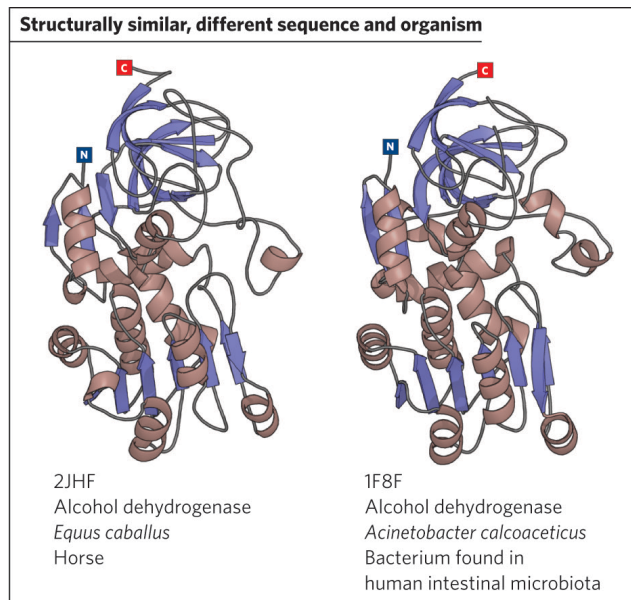
Which feature is NOT associated with globular proteins?

C. classified as either all α or all β

Globular proteins are folded into a spherical or globular shape. Globular proteins often contain several types of secondary structure and have a variety of tertiary structures. These proteins are not classified as either all α or all β .

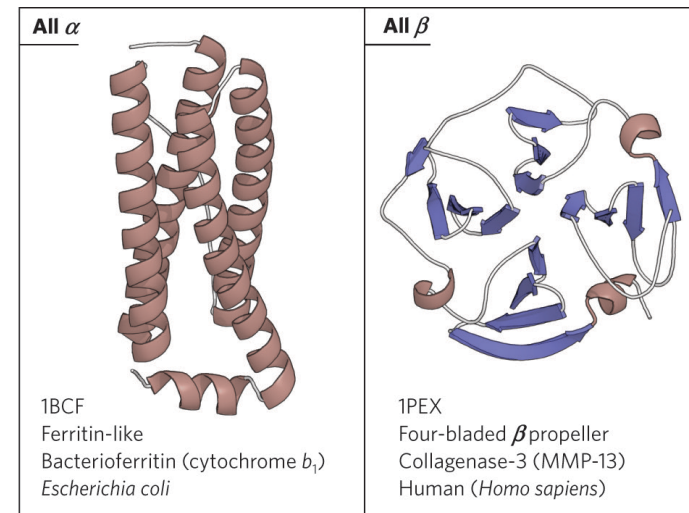
Protein Motifs Are the Basis for Protein Structural Classification

- Protein Data Bank (PDB) = 150,000+ structures archived
- Structural Classification of Proteins database (SCOP2) = searches protein information in the PDB



(a)

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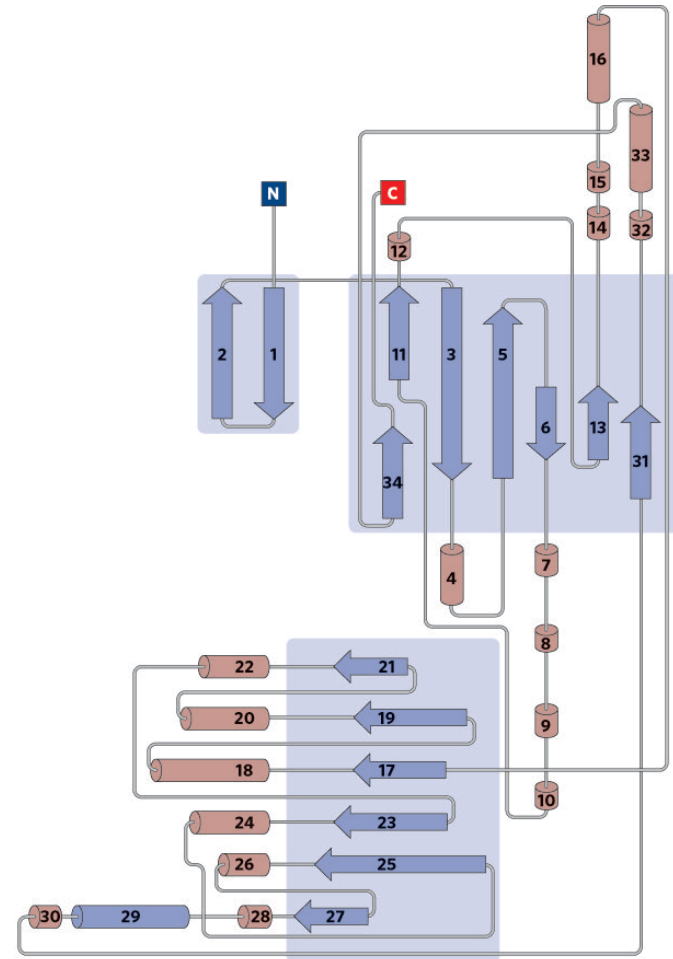


(c)

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Topology Diagrams

- **topology diagram** = represent elements of secondary structure and the relationships among segments of secondary structure in a protein



(b)

Protein Families and Superfamilies

- proteins with significant similarity in primary structure and/or tertiary structure and function are in the same **protein family**
 - ~4,000 different protein families in the PDB
 - strong evolutionary relationship within a family
- **superfamilies** = 2+ families that have little sequence similarity, but the same major structural motif and have functional similarities
 - evolutionary relationship is probable

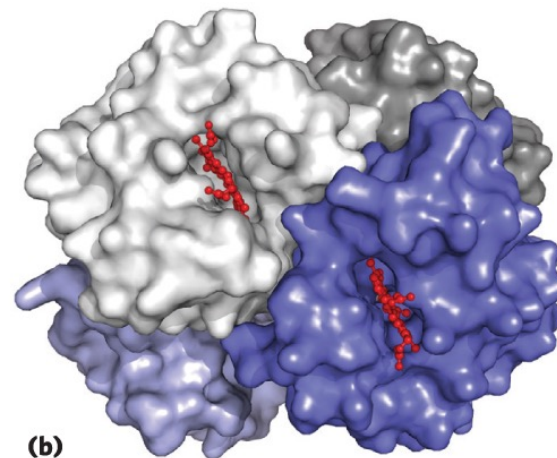
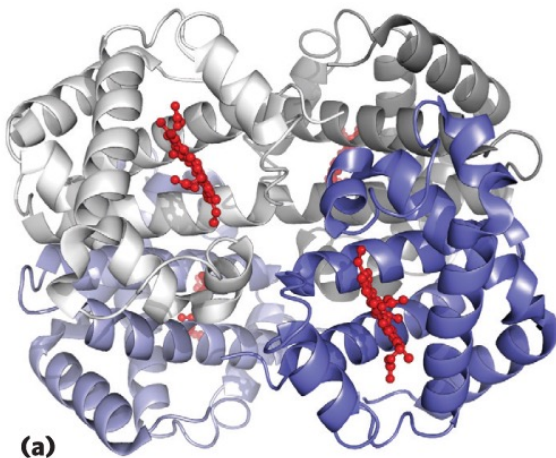
محتمل

Protein Quaternary Structures Range from Simple Dimers to Large Complexes

* Hemoglobin (Hb) is a tetramer (composed of Four subunits), two of them is alpha and the other two is beta, and each of this Subunits bond to hem Group

* Hemoglobin is an alpha beta dimer

- quaternary structure = assembly of multiple peptide subunits
- **oligomer = multimer = multisubunit protein**
 - repeating structural unit = **protomer**



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Hb is a tetramer, also a dimer of $\alpha\beta$ protomers



P3

Clicker Question 16

Quaternary structure:

- A. describes how oligomers are assembled into protomers.
- B. refers to proteins with identical subunits.
- C. refers to proteins with four subunits.
- D. refers to an arrangement of tertiary protein subunits in a three-dimensional complex.

Clicker Question 16, Response

Quaternary structure:

D. refers to an arrangement of tertiary protein subunits in a three-dimensional complex.

Some proteins contain two or more separate polypeptide chains, or subunits, which may be identical or different. The arrangement of these protein subunits in three-dimensional complexes constitutes a quaternary structure.

4.4 Protein Denaturation and Folding

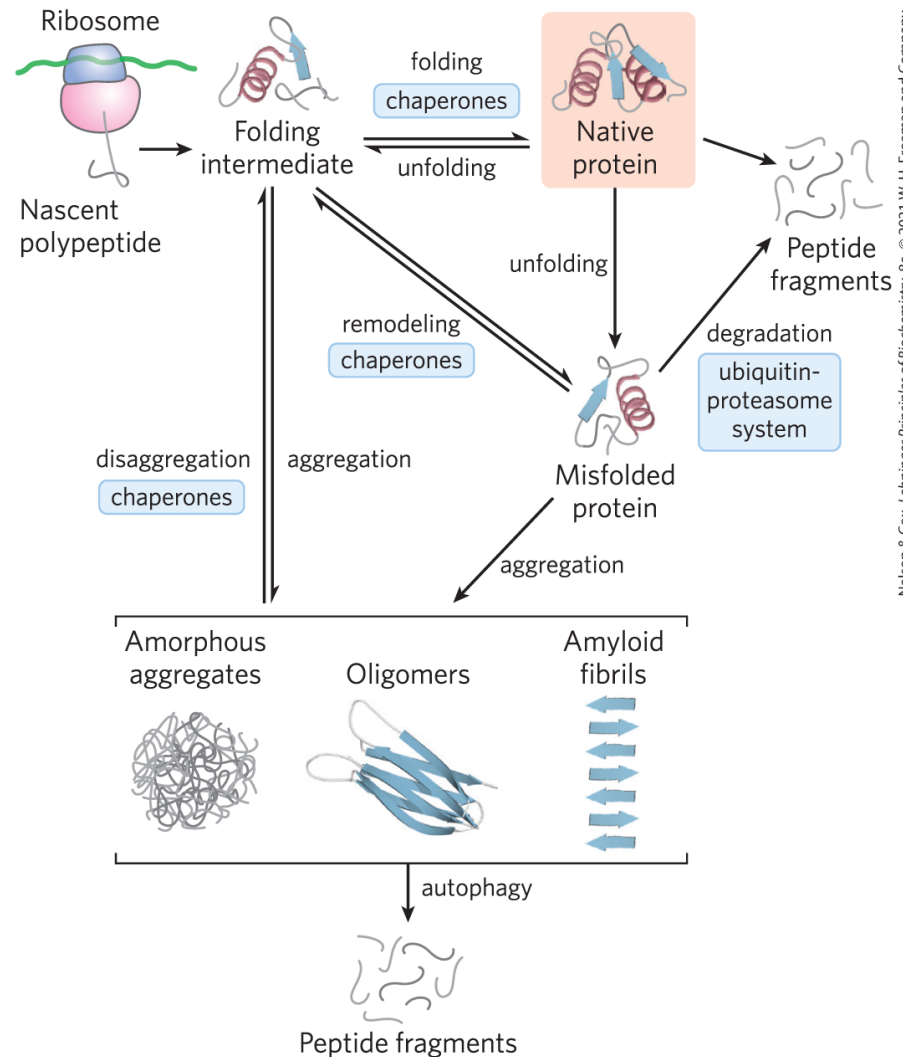


Principle 4

Tertiary structure is determined by amino acid sequence. Even though protein folding is complex, some denatured proteins can spontaneously refold into their active conformation based only on the chemical properties of their constituent amino acids. Cellular proteostasis involves numerous pathways that regulate the folding, unfolding, and degradation of proteins. Many human diseases arise from protein misfolding and defects in proteostasis.

Pathways Involved in Proteostasis

- **proteostasis** = **مستمر**
continual maintenance of the active set of cellular proteins required under a given set of conditions





Clicker Question 17

Which characteristic of protein is NOT associated with proteostasis?

- A. structure
- B. synthesis
- C. refolding
- D. degradation

Clicker Question 17, Response

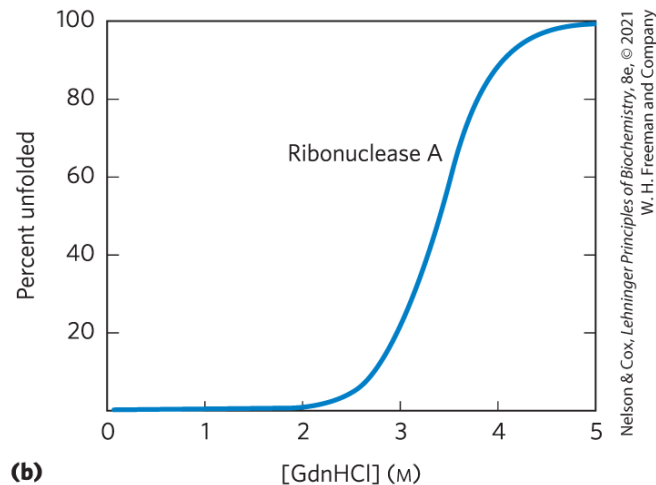
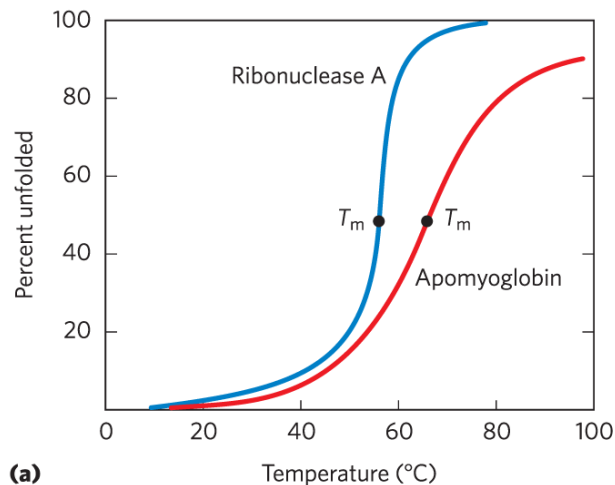
Which characteristic of protein is NOT associated with proteostasis?

A. structure

Proteostasis is the continual maintenance of the active set of cellular proteins required under a given set of conditions. This requires the coordinated function of pathways for protein synthesis and folding, the refolding of partially unfolded proteins, and the sequestration and degradation of proteins that are irreversibly unfolded or are no longer needed.

Loss of Protein Structure Results in Loss of Function

- **denaturation** = loss of three-dimensional structure sufficient to cause loss of function
 - can occur by heat, pH extremes, miscible organic solvents, certain solutes, detergents
 - often leads to protein precipitation



- 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

Cleansing agent

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

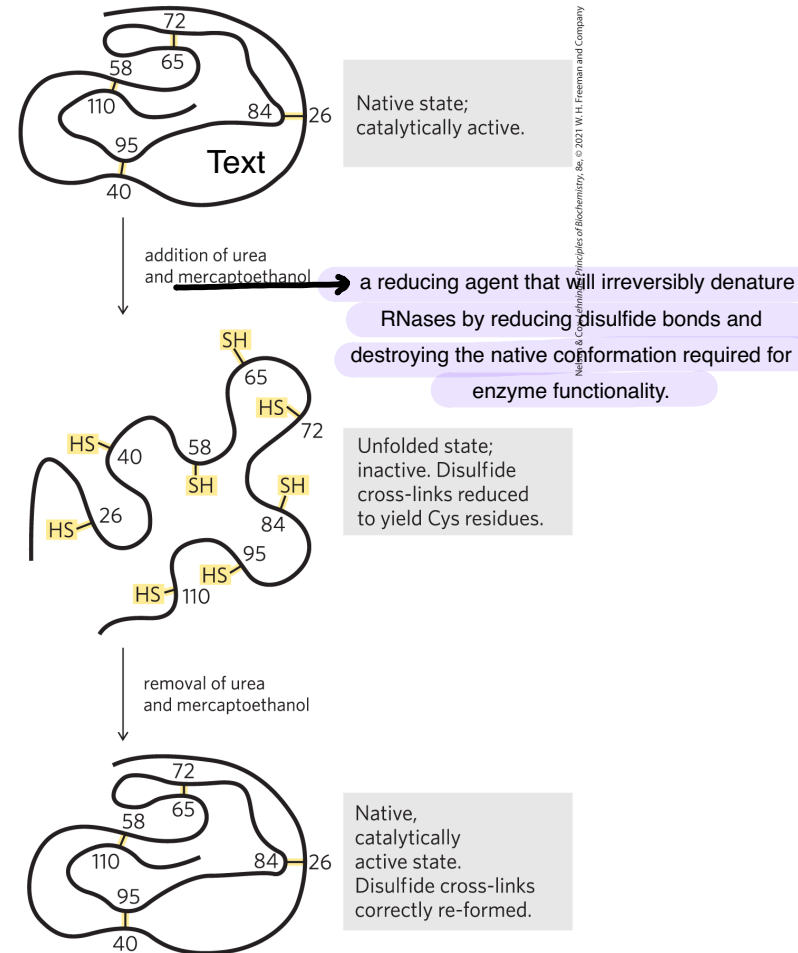
4) Chaotropic agents (urea and guanidine HCl): disruption of the arrangement of water molecules that solvate the proteins

* Melting temperature: the temperature at which half of proteins are folded and the other half of proteins are unfolding

* Proteins impossible melt

Amino Acid Sequence Determines Tertiary Structure

- **renaturation** = process by which certain denatured globular proteins regain their native structure and biological activity
- Anfinsen experiment showed the amino acid sequence contains all the information required to fold the chain
- *Quite “simple” experiment, but so important it earned Chris Anfinsen the 1972 Chemistry Nobel Prize*





P4

Clicker Question 18

Denaturing followed by renaturing of a protein:

- A. always results in the protein regaining its function.
- B. demonstrates that primary structure dictates tertiary structure.
- C. requires heat.
- D. requires breaking and reforming disulfide bonds.

Clicker Question 18, Response

Denaturing followed by renaturing of a protein:

B. demonstrates that primary structure dictates tertiary structure.

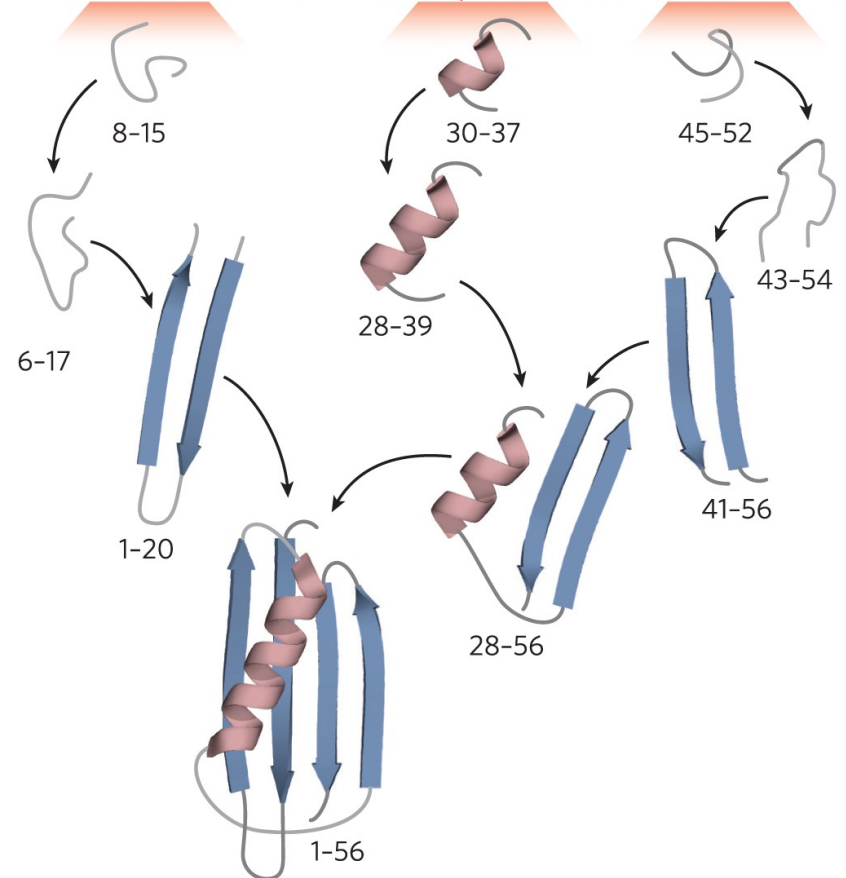
The Anfinsen experiment provided the first evidence that the amino acid sequence of a polypeptide chain contains all the information required to fold the chain into its native, three-dimensional structure.

Polypeptides Fold Rapidly by a Stepwise Process

- local secondary structures fold first
 - ionic interactions play an important role
- longer range interactions follow
 - hydrophobic effect plays a significant role
- process continues until the entire polypeptide folds

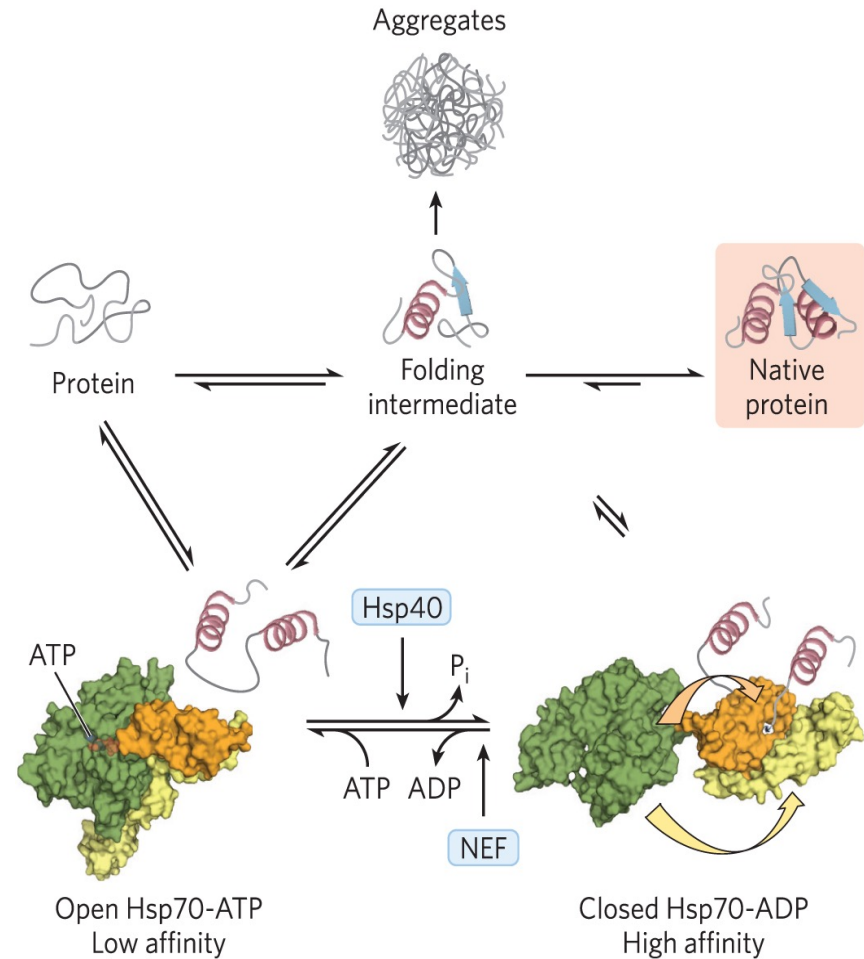
Amino acid sequence of a 56 residue peptide

MTYKLLILNGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFVTVE



Some Proteins Undergo Assisted Folding

- **chaperone** proteins = facilitate correct folding pathways or ideal microenvironments
 - **Hsp70** = bind to hydrophobic regions
 - **chaperonins** = required for the folding of proteins that do not fold spontaneously



P4

Clicker Question 19

What important function do molecular chaperones perform?

- A. assemble protein subunits into a quaternary structure
- B. fold proteins into a native conformation
- C. transport proteins to either the plasma membrane or release them from cells to the extracellular space
- D. add cofactors, coenzymes, or prosthetic groups to proteins as they are synthesized
- E. move proteins from endoplasmic reticulum to the Golgi apparatus

Clicker Question 19, Response

What important function do molecular chaperones perform?

B. fold proteins into a native conformation

Chaperone proteins interact with partially folded or improperly folded polypeptides, facilitating correct folding pathways or providing microenvironments in which folding can occur.

Some Folding Pathways Require Isomerization Reactions

- **protein disulfide isomerase (PDI)** = catalyzes interchange, or shuffling, of disulfide bonds
تبادل خلط
- **peptide prolyl cis-trans isomerase (PPI)** = catalyzes the interconversion of the cis and trans isomers of peptide bonds formed by Pro residues
التحول بين حالتين من حالة الى اخرى

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Clicker Question 20

Which statement about protein folding is false?

- A. It requires protein disulfide and peptide prolyl cis-trans isomerases.
- B. It is a stepwise process.
- C. It is sometimes assisted by proteins known as chaperones.
- D. It is driven by changes in free energy.

Clicker Question 20, Response

Which statement about protein folding is false?

A. It requires protein disulfide and peptide prolyl cis-trans isomerases.

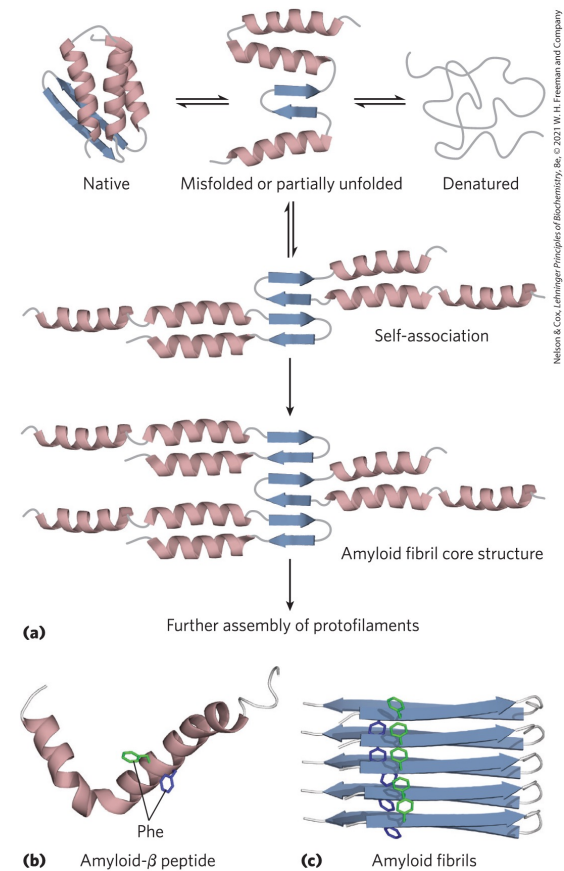
Only some folding pathways require protein disulfide and peptide prolyl cis-trans isomerases to catalyze isomerization reactions.

Defects in Protein Folding Are the Molecular Basis for Many Human Genetic Disorders

- **amyloid** fiber = protein secreted in a misfolded state and converted to an insoluble extracellular fiber
- **amyloidose** diseases: type 2 diabetes, Alzheimer disease, Huntington disease, and Parkinson disease

Formation of Disease-Causing Amyloid Fibrils

- native = high degree of β -sheet structure
- misfolded β amyloid promotes aggregation, forming an amyloid fibril



Neurodegenerative Conditions

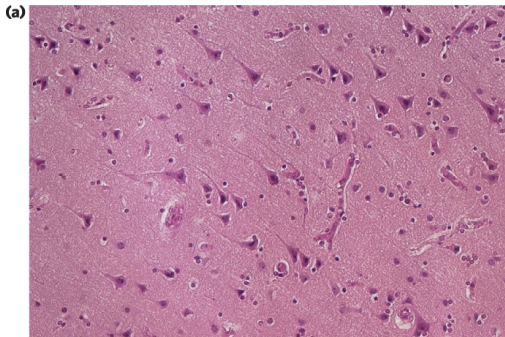
- Alzheimer disease = associated with extracellular amyloid deposition by neurons, involving the amyloid- β peptide
- Parkinson disease = misfolded form α -synuclein aggregates into spherical filamentous masses called Lewy bodies
- Huntington disease = involves the intracellular aggregation of huntingtin, a protein with long polyglutamine repeat

Cystic Fibrosis

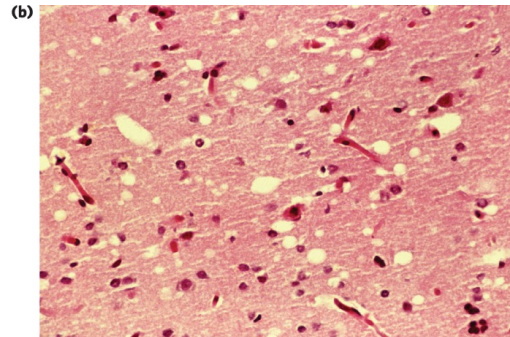
- cystic fibrosis = caused by defects in the membrane-bound protein *cystic fibrosis transmembrane conductance regulator* (CFTR)
 - deletion of a Phe residue causes improper protein folding

Death by Misfolding: The Prion Diseases

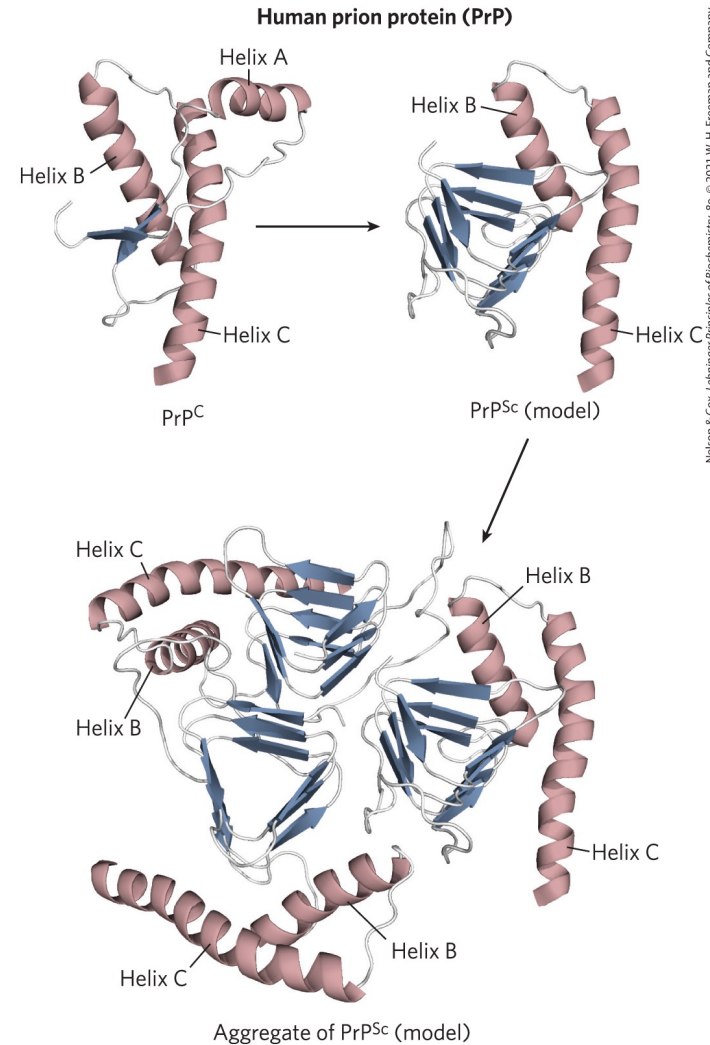
- **prion** protein (PrP) = misfolded brain protein



Pyramidal cells in the human cerebral cortex



Comparable section from a patient with Creutzfeldt-Jakob “mad cow” disease



P4

Clicker Question 21

Which disease is NOT associated with protein misfolding?

- A. cystic fibrosis
- B. amyloidosis
- C. Parkinson disease
- D. type 1 diabetes mellitus

Clicker Question 21, Response

Which disease is NOT associated with protein misfolding?

D. type 1 diabetes mellitus

Many conditions, including amyloidosis, Parkinson disease, and cystic fibrosis are associated with protein misfolding. Unlike type 2 diabetes, type 1 diabetes mellitus is not associated with protein misfolding.

Disulfide linkage occur between
disulfide residues