جامعة الملك سعود
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1957

College of Science

Department of Biochemistry

Protein Biochemistry (BCH 303)

Chapter 3

Protein Structure

PROTEINS

- Proteins are the most abundant biological macromolecules, occurring in all cells and all parts of cells.
- Proteins also occur in great variety; thousands of different kinds, ranging in size from relatively small peptides to huge polymers with molecular weights in the millions, may be found in a single cell.
- Moreover, proteins exhibit enormous diversity of biological function and are the most important final products of the information pathways.

- Proteins are made up of large numbers of amino acids linked into chains by peptide bonds joining the carboxyl group of one amino acid to amino group of the next.
- The number of amino acids present varies from about a hundred to several thousands in different proteins.
- Some proteins are composed of only one polypeptide chain while others are composed of two or more polypeptide chains (multisubunit proteins) held together by non-covalent bonds

- The individual polypeptide chains in a multisubunit protein may be identical or different.

- If at least two are identical, the protein is said to be oligomeric and the identical units are referred to as protomers.

- example, two subunits (homodimer or heterodimer), four subunits (homotetramer, or heterotetramer).

- Each protein has a unique amino acid sequence and a well-defined three-dimensional structure known as the conformation.

Size and shape of proteins

The number , type and arrangement of amino acids differ in different proteins.

TABLE 3–2 Molecular Data on Some Proteins

	Molecular weight	*Number of residues*	*Number of polypeptide chains*
Cytochrome *c* (human)	13,000	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (chicken egg white)	13,930	129	1
Myoglobin (equine heart)	16,890	153	1
Chymotrypsin (bovine pancreas)	21,600	241	3
Chymotrypsinogen (bovine)	22,000	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	68,500	609	1
Hexokinase (yeast)	102,000	972	2
RNA polymerase (*E. coli*)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (*E. coli*)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

The general properties of proteins

1. High molecular weight substances.
2. It constitutes more than 50% of the dry weight of the cell.
3. It presents in different shapes; fibrous and globular.
4. The globular is soluble in water and diluted salt solution with different degrees
5. The chemical and physical properties depend on the amino acids forming the protein.
6. The biological properties and 3D conformation depend also on the constituting amino acids.
7. All proteins are *amphoteric* compounds.
8. They precipitate by heat, in alcohols and in their *isoelectric* point.

Classification of proteins

Proteins can be classified according to three different criteria:

A) proteins can be classified on the basis of the chemical composition.

B) proteins can be classified on the basis of shape.

C) proteins can be classified on the basis of their biological function.

Classification of proteins

a- According to their chemical composition:

Proteins can be classified on the basis of their chemical composition into two main classes:

1- Simple proteins

2- conjugated proteins

1- Simple proteins:

are those proteins which upon hydrolysis give only amino acids.

Example: ribonuclease A, chymotrypsin.

2- Conjugated proteins:

are proteins which yield upon hydrolysis amino acids and non-protein part is called **the prosthetic group**.

-Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups:

i) nucleoproteins

ii) Glycoproteins (contains carbohydrate part)

iii) Lipoproteins (contains lipid part)

iv) Hemoproteins (contains heme)

v) Metalloproteins (contains metal)

vi) Phosphoproteins (phosphorylated protein)

Examples of conjugated proteins

TABLE 3–4 Conjugated Proteins

Class	*Prosthetic group*	*Example*
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

Classification of proteins (Cont.)

b- According to their shape

Proteins can be classified on the basis of their chemical composition into two main classes:

1- Globular proteins

2- Fibrous proteins

1- Globular proteins:

- They are generally soluble in water.
- The polypeptide chains are tightly folded into a globular shape.

Example:

enzymes, hemoglobin, myoglobin

2- Fibrous proteins:

- They are insoluble in water
- Their polypeptide chains are arranged in long strands (elongated in the form of fibers).

Example:

Collagen, elastin, keratin

Classification of proteins (Cont.)

c- According to their biological function

Proteins can be classified on the basis of their biological function into:

1- Catalytic function (enzymes)
2- Transport function (hemoglobin, albumin, transferrin)
3- Nutrient and storage proteins [e.g., casein & ferritin]
4- contractile or mobile proteins [e.g., actin, myosin]
5- Structural function [Keratin, elastin, collagen]
6- Defense proteins [e.g., immunoglobulins, fibrinogen and thrombin]
7- Regulatory function, some hormones are proteins (Growth hormone [GH, somatotropin])
8- Some toxins are proteins
9- Defense (Antibodies and coagulating factors)

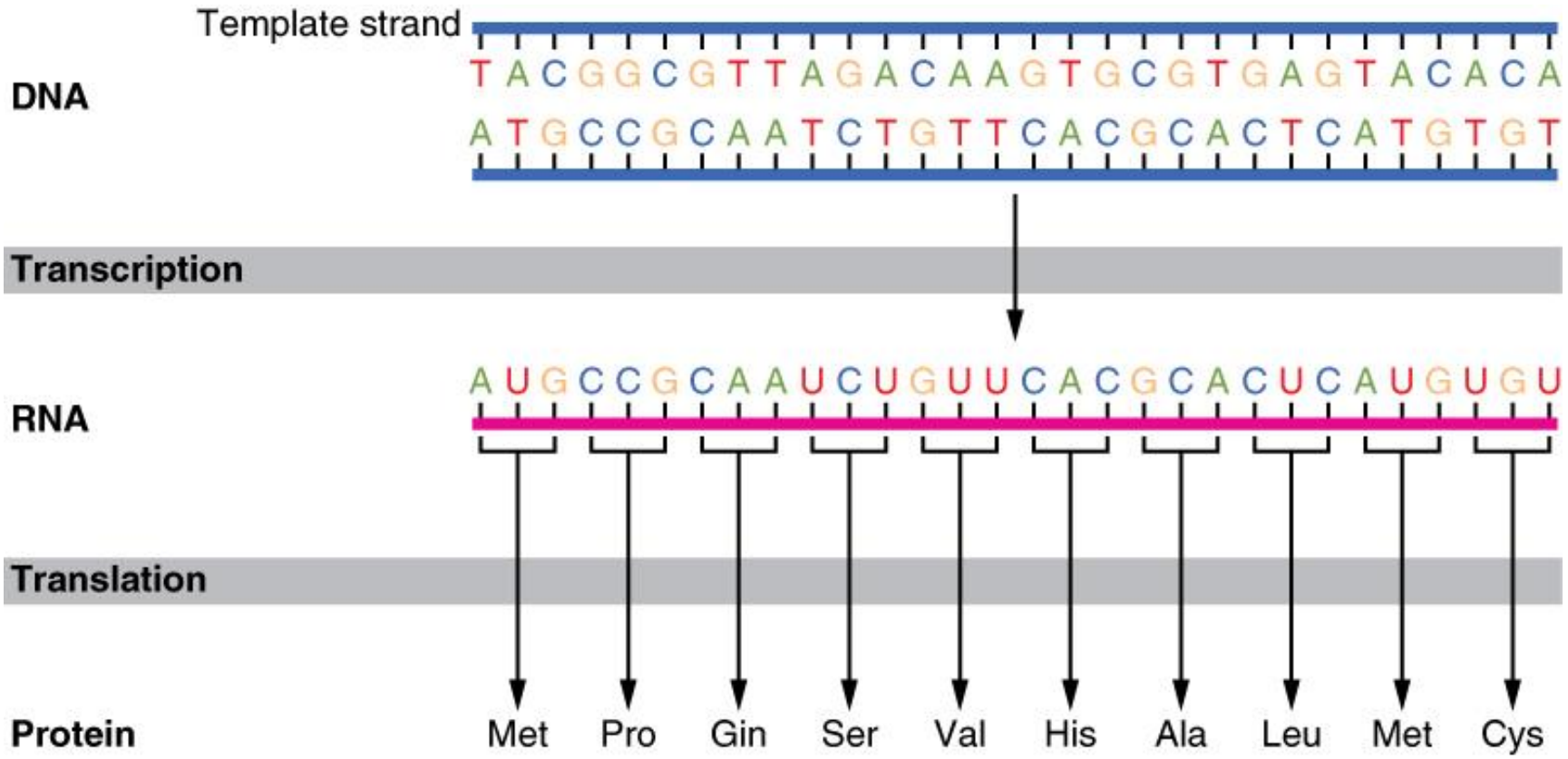
Ferritin
https://www.youtube.com/watch?v=FbDgDbl7LYE

Protein synthesis

The relation between DNA, RNA & proteins

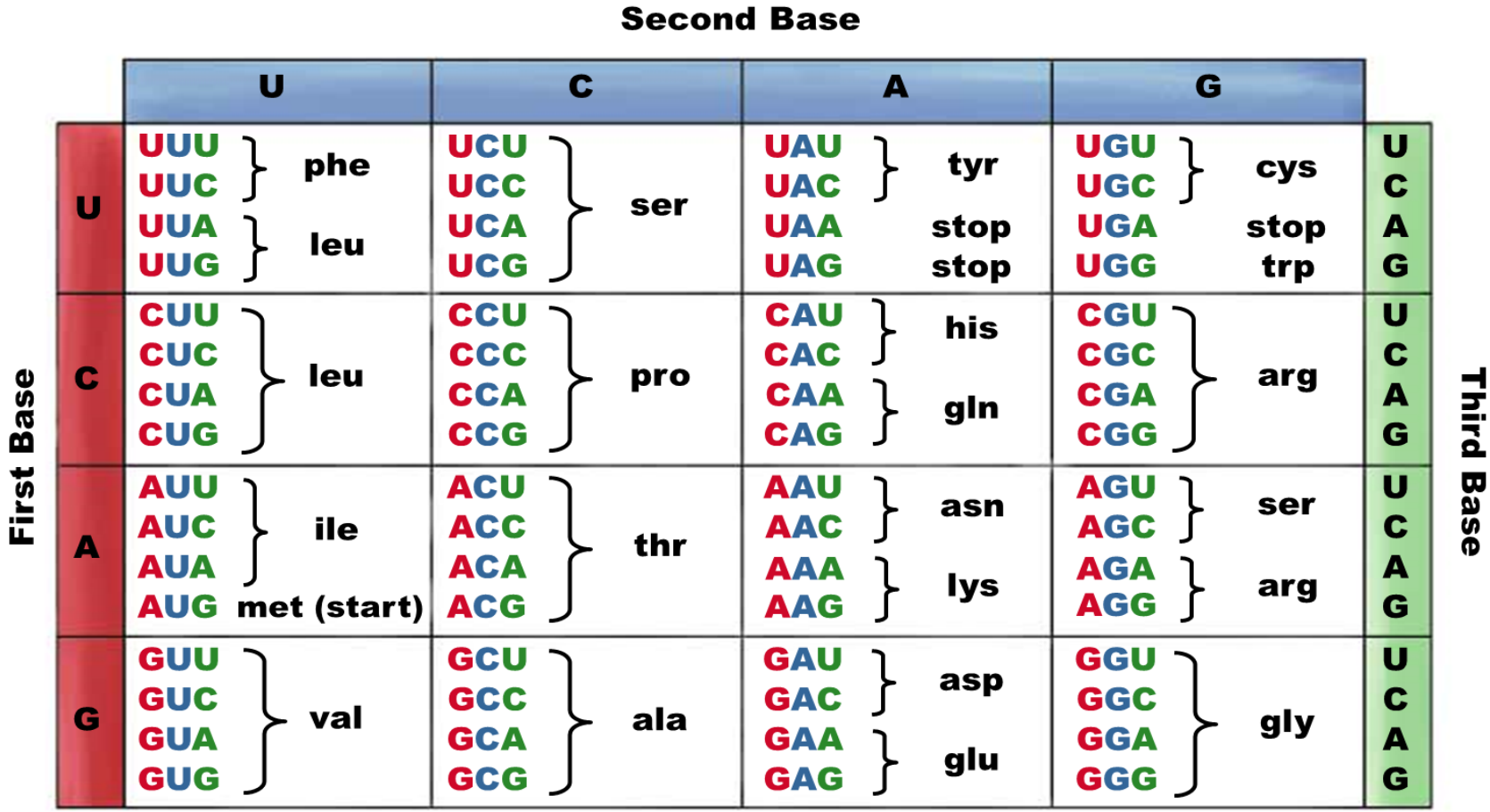
- The flow of information from DNA to RNA to protein is termed the *"central dogma".*
- The genetic information corresponding to certain protein is stored in the form of nucleotide sequence in DNA called gene.
- The gene is *transcribed* in the nucleus into the specific nucleotide sequence called mRNA molecule than contains the genetic code.
- mRNA is translated in the cytoplasm by the ribosome which bind the aminoacids charged in tRNA in a sequence determined by mRNA.

Central Dogma of Molecular Genetics

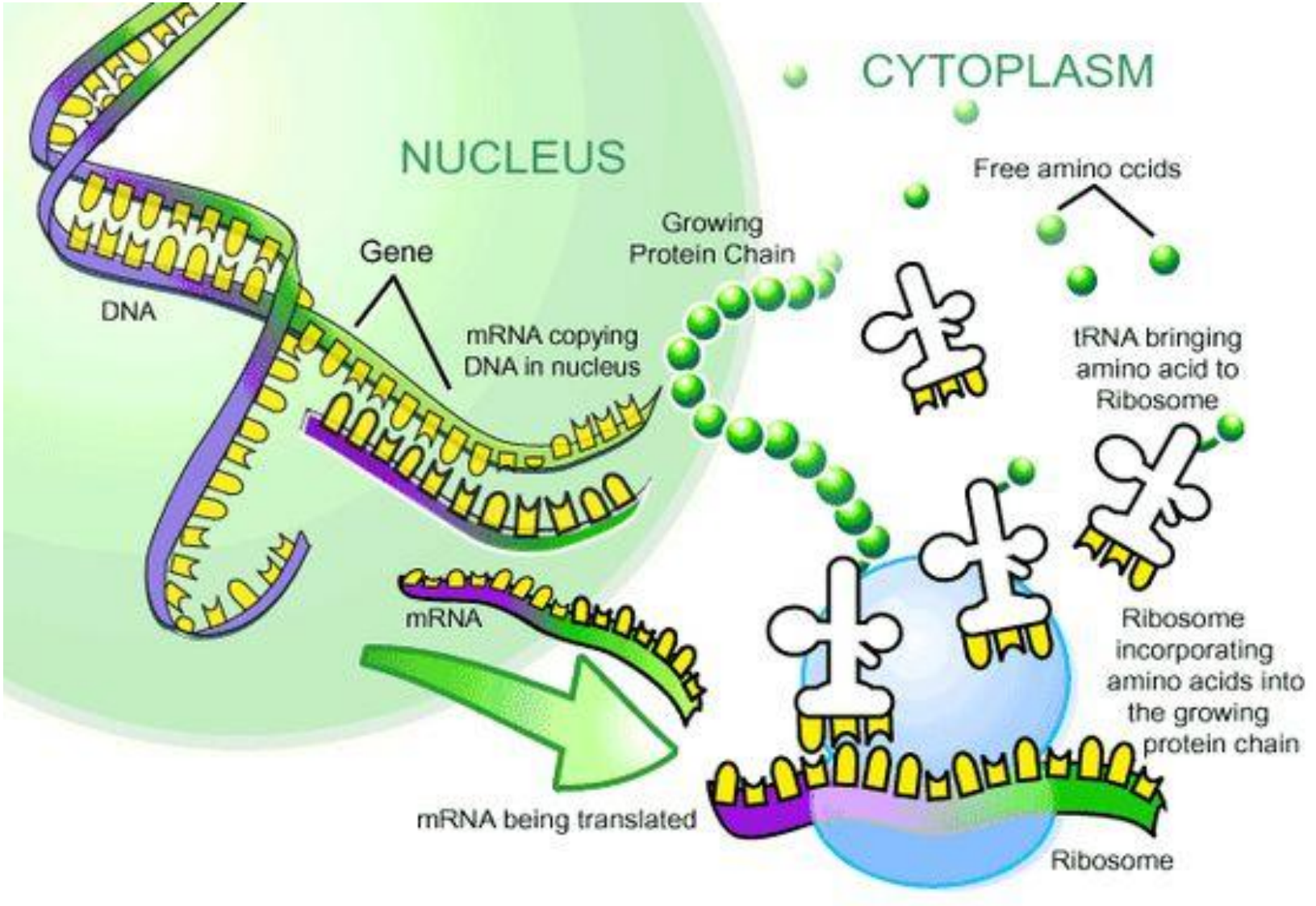


Genetic code

- ✓ Each amino acids has at least one code in the mRNA, some has 6 codons.



Transcription and Translation



HTTPS://WWW.YOUTUBE.COM/WATCH?V=GG7UCSKUORA

HTTPS://WWW.YOUTUBE.COM/WATCH?V=-ZB6R1MMTKC

- Post Translation Modification (hydroxylation, phosphorylation, methylation, disulfide bridge, etc)
- Protein folding
- Chaperones
- Misfolding

To be edited

Protein structure

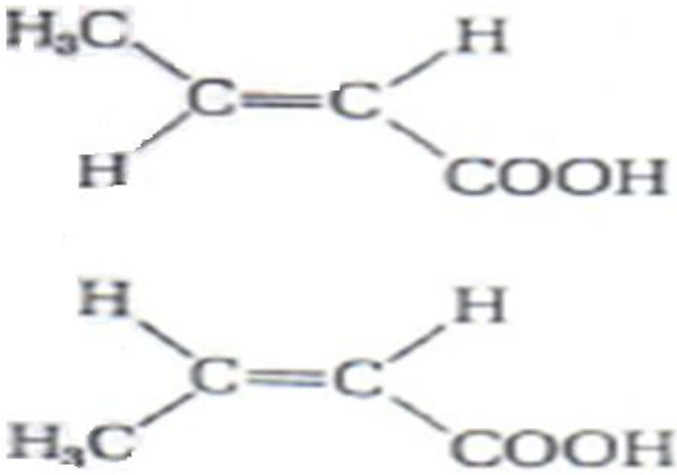
The amount and type of amino acids found in a protein and the sequence in which they are arranged in the polypeptide chains is a unique characteristic of each protein.

- The amino acid sequence of a number of proteins has been determined and it is established that the sequence of amino acids is the force that determines the protein conformation, which in turn is responsible for the biological function of protein.

Configuration and conformation:

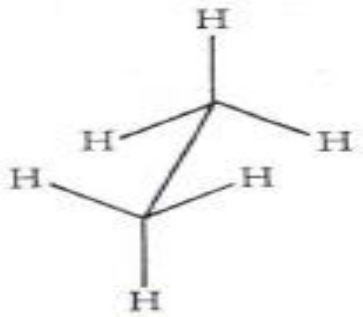
Configuration:

It is the arrangement in space of substituent groups in steroisomers; such structures can not be interconverted without breaking one or more covalent bond.

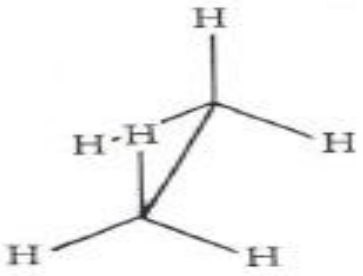


Conformation:

refers to the spatial arrangement of substituent groups that are free to assume many different positions, without breaking bonds, because of rotation about single bonds in the molecule.



Staggered



Eclipsed

- Of the many conformation, one or (more commonly) a few generally predominant under biological conditions.

[the need for multiple stable conformations reflects the changes that must take place in most proteins as they bind to other molecules or catalyze reaction].

- Proteins in any of their functional, folded conformations are called native proteins.

Protein structure (Cont.)

Protein structure can be considered at four levels:

1) Primary structure

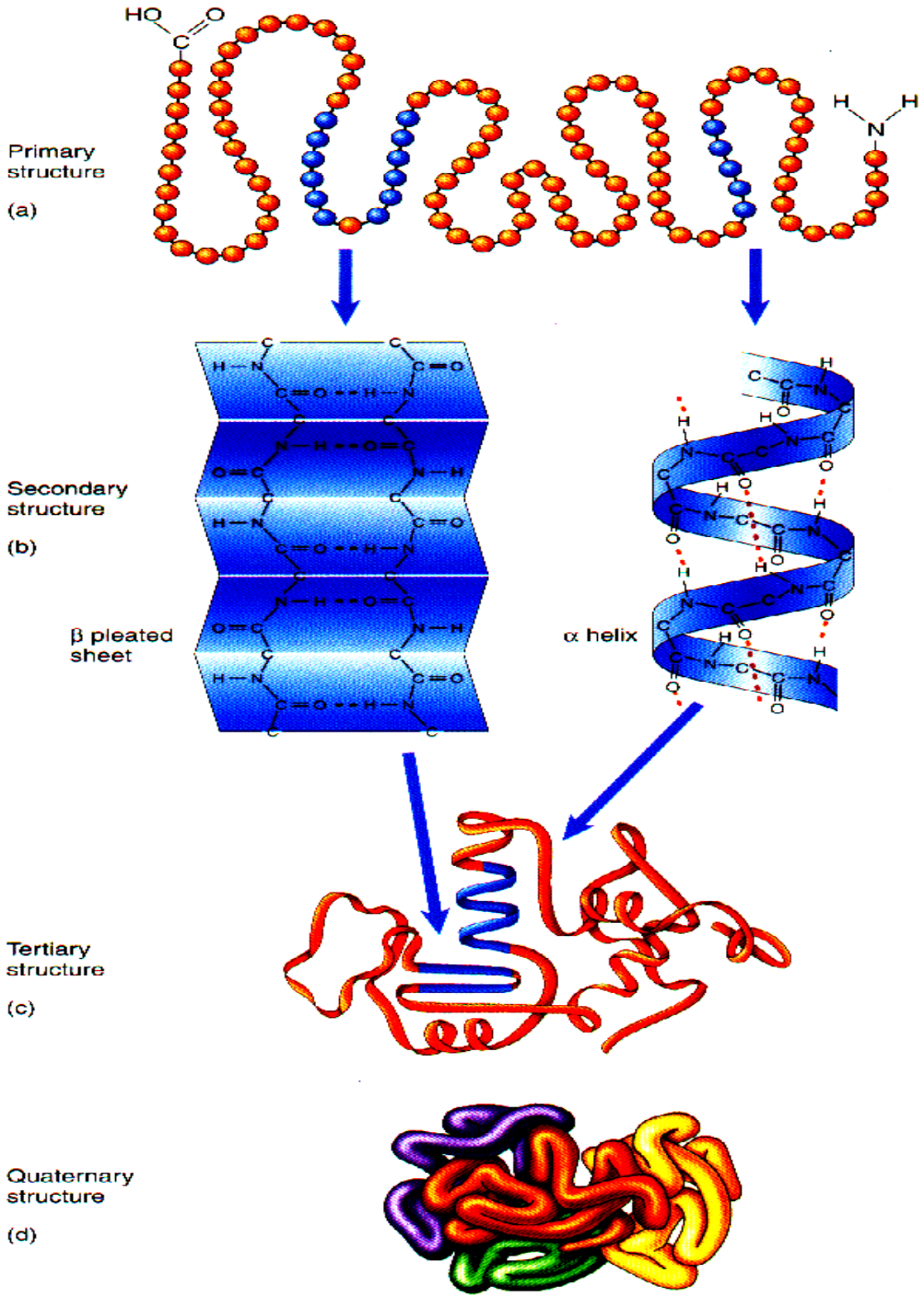
2) Secondary structure

3) Tertiary structure

4) Quaternary structure

Note:

- All proteins have their own specific primary structure, amino acids sequence, determined by their genes
- Different proteins have different extent of secondary structure. Some have none.
- All intracellular globular proteins have a tertiary structure.
- Proteins made of more than one subunit [polypeptide] have quaternary structure.



-Primary structure

- Secondary structure

- Tertiary structure

- Quaternary structure

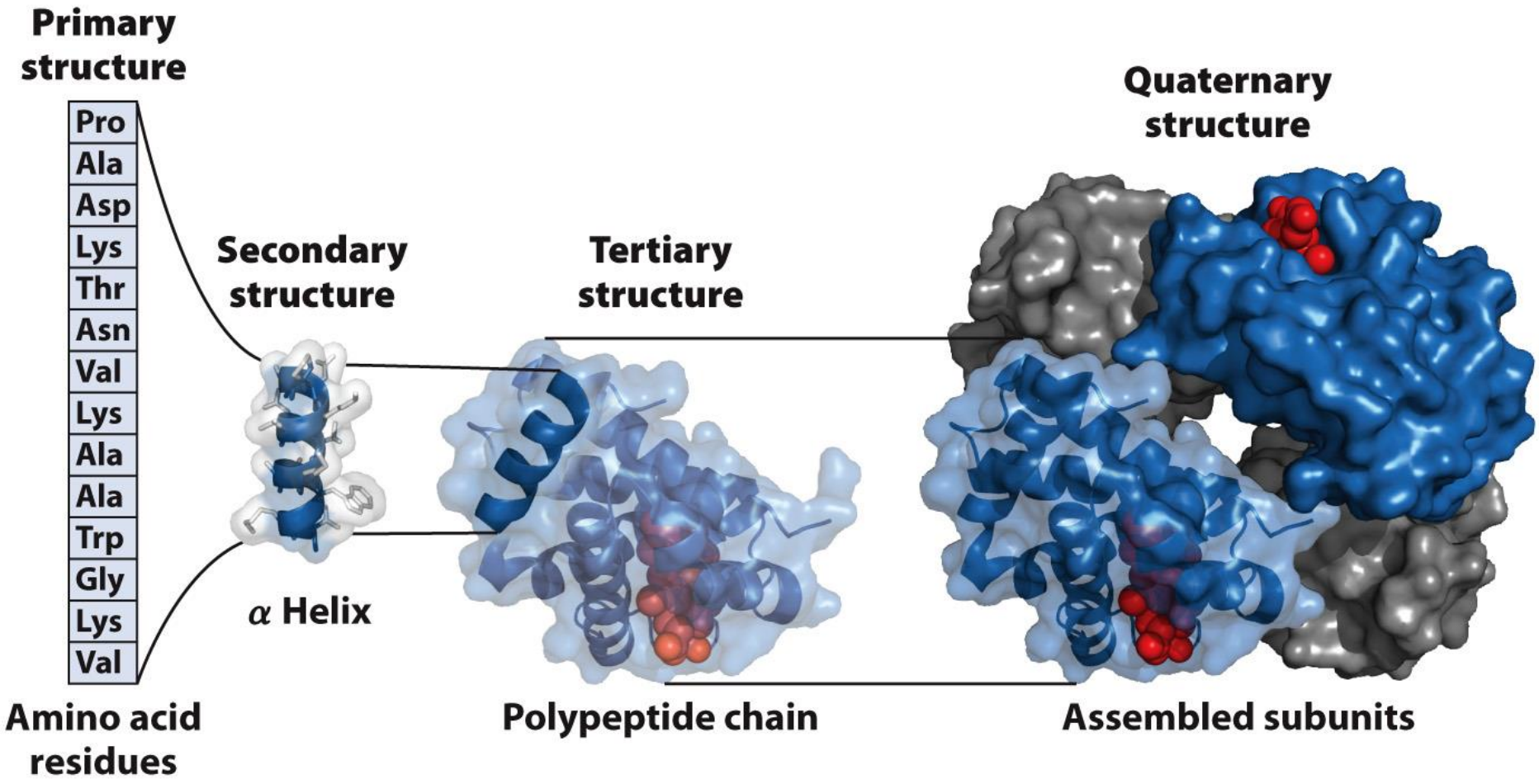


Figure 3-23

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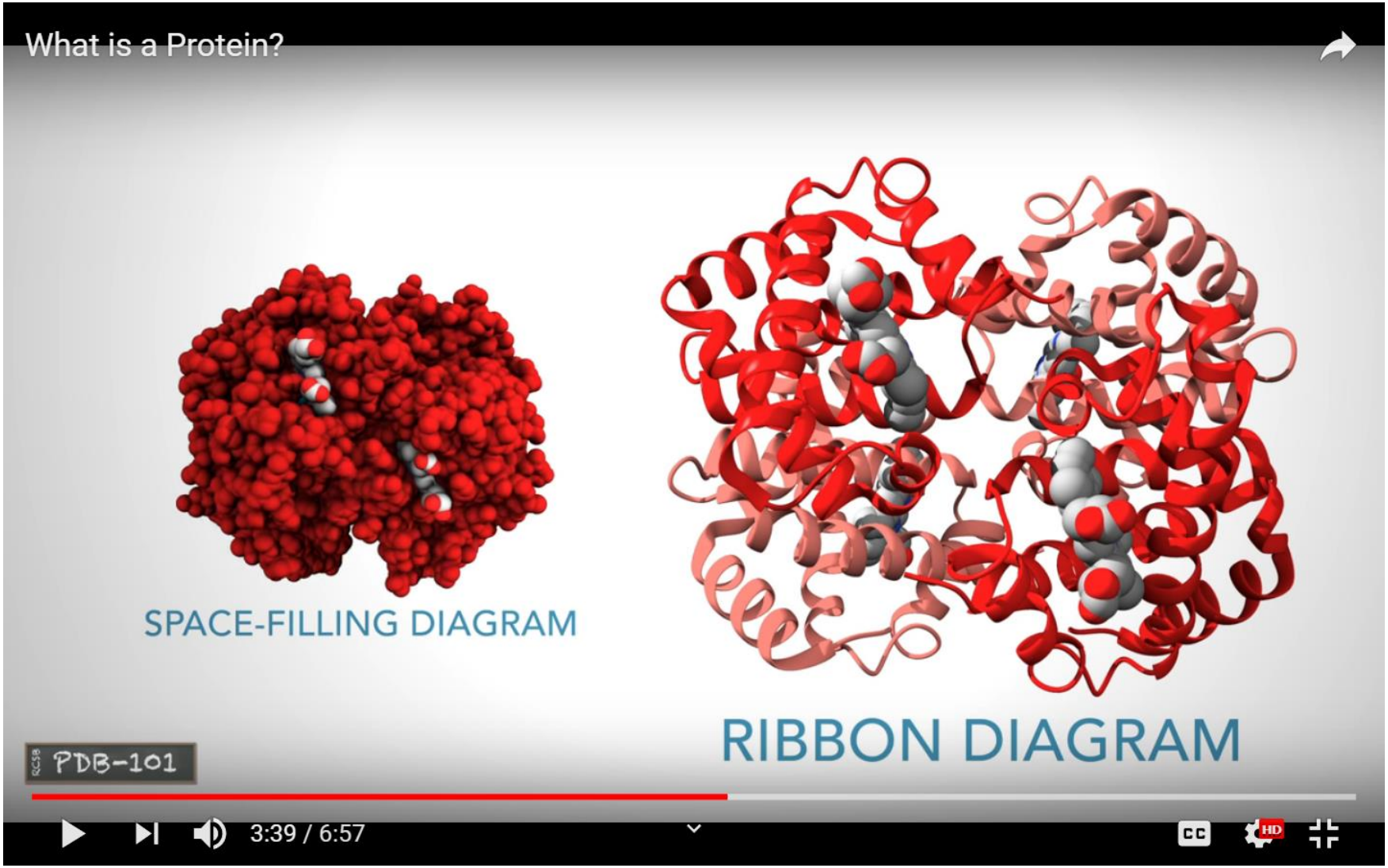
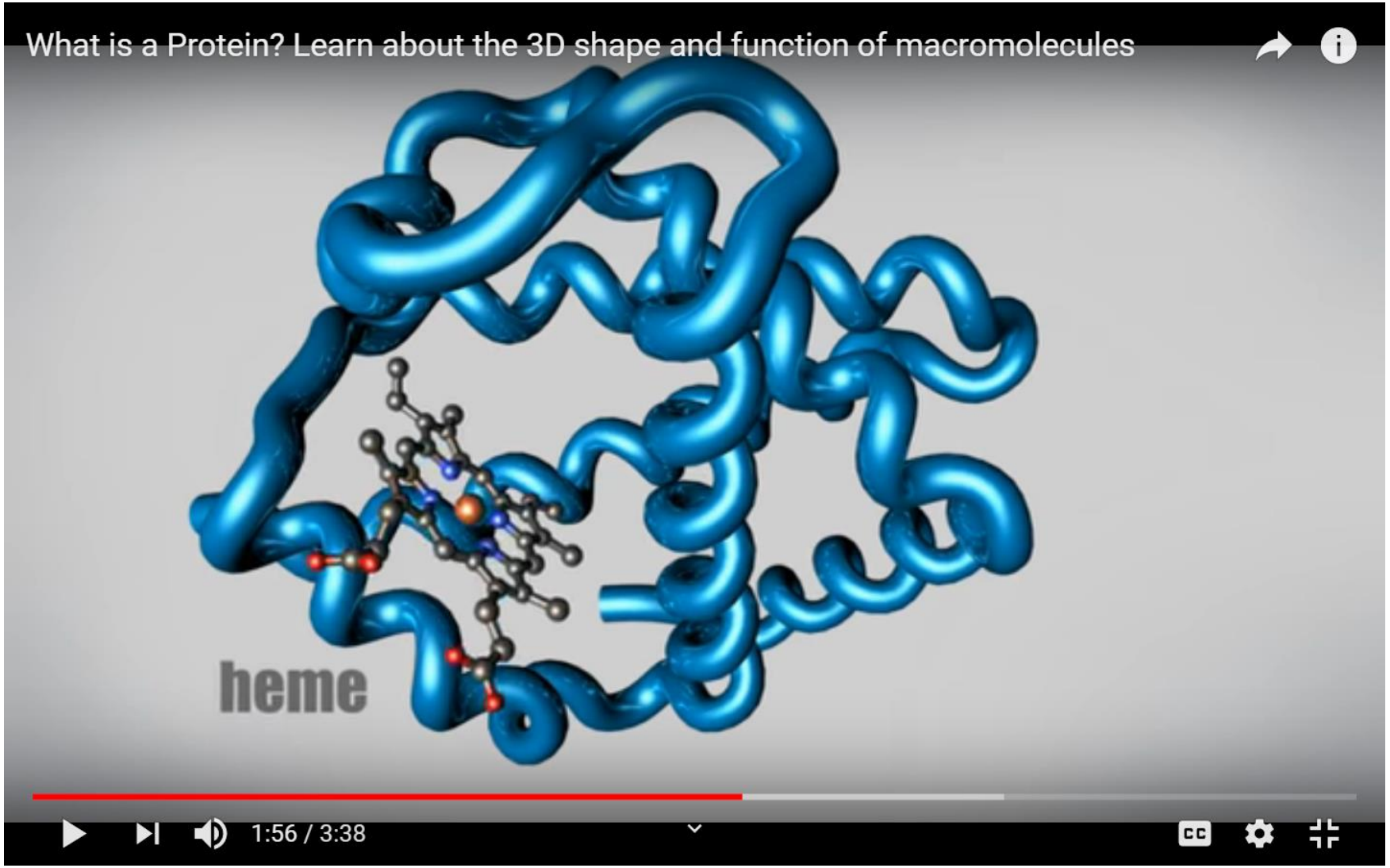


All about ptoteins (amazing video)
https://www.youtube.com/watch?v=wvTv8TqWC48



https://www.youtube.com/watch?v=qBRFIMcxZNM

Forces that stabilize the different protein structures

Level	Interactions that stabilize the structure
Primary	Covalent bond (amide/peptide bond)
Secondary	Hydrogen bonds **(C=O with NH)** to form α-helix and β-sheet
Tertiary	Ionic bonds, disulfide bonds, hydrophobic interactions, hydrogen bonding **(between R groups in the same chains)**
Quaternary	Ionic bonds, disulfide bonds, hydrophobic interactions, hydrogen bonding **(between R groups in different chains)**

1) Primary structure

It is the sequence of amino acids in a protein.

- Different proteins differ from each other in their primary structure but every protein has a free carboxyl end terminal and a free amino end terminal.

- The bonds responsible for the primary structure are:

the covalent bonds → The peptide bonds (C(=O) — N(H))

the covalent bonds → The disulfide bonds (S — S)

- The disulfide bonds (if present) between two cysteine residues of the same polypeptide chain or between different polypeptide chains.

  ⟹ The primary structure is therefore very stable due to these bonds.

- Peptide bonds are not broken by normal handling nor that conditions that denature proteins such as heating.
- Prolonged exposure to strong acid or base at elevated temperature is required to hydrolyze these bonds (peptide bond), non-enzymatically.
- Treatment with strong reducing agents disrupts the disulfide bridges and effects the biological properties of the proteins.

Characteristics of the peptide bond:

a) The peptide bond is rigid and planar:

- The peptide bond has a partial double bond character – that is, it is shorter than a single bond [i.e peptide bond is intermediate in bond length (0.132 nm) between a single bond (0.147 nm) and a double bond (0.123 nm)] and is, therefore, rigid and planar.

⇒ This prevents free rotation around the bond between the carbonyl carbon and the nitrogen of the peptide bond.

-The rigid peptide bonds limit the number of conformations by polypeptide chains.

[However, the bond between α-carbon and the α-amino or α-carboxyl groups can be freely rotated].

b) Trans configuration:

The peptide bond is generally a trans bond (instead of cis), because of steric interference of the R-groups when in the cis position.

c) Uncharged but polar:

(The - C = O and –NH groups of the peptide bond are polar and are involved in hydrogen bonds).

The rigid peptide plane and the partially free rotations

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

The polypeptide is made up of a series of planes linked at α carbons

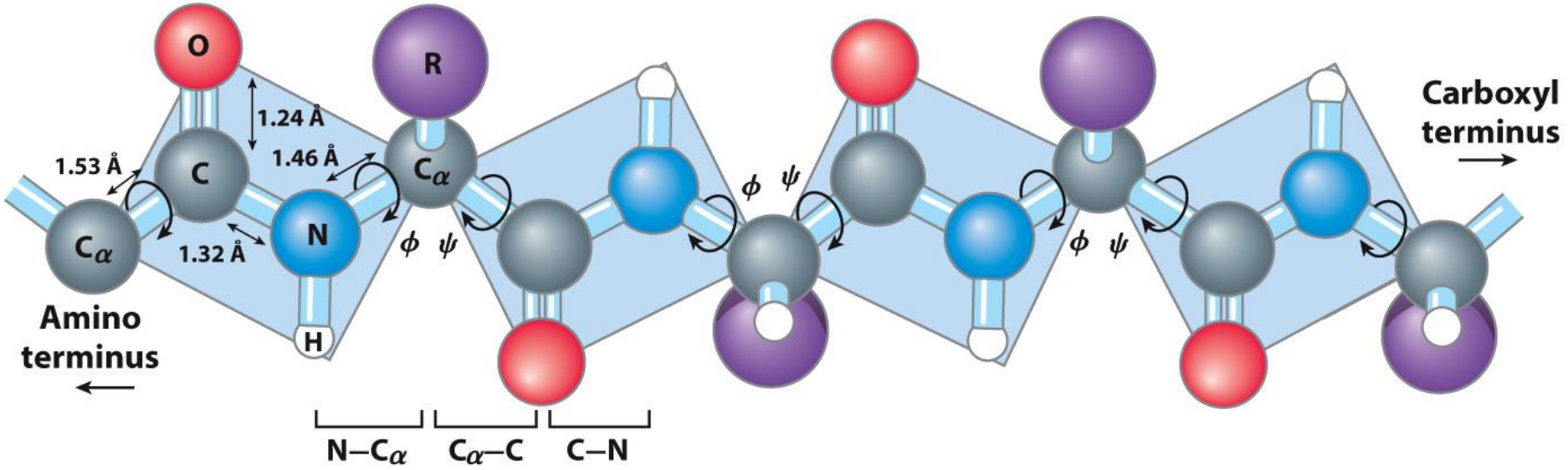


Figure 4-2b
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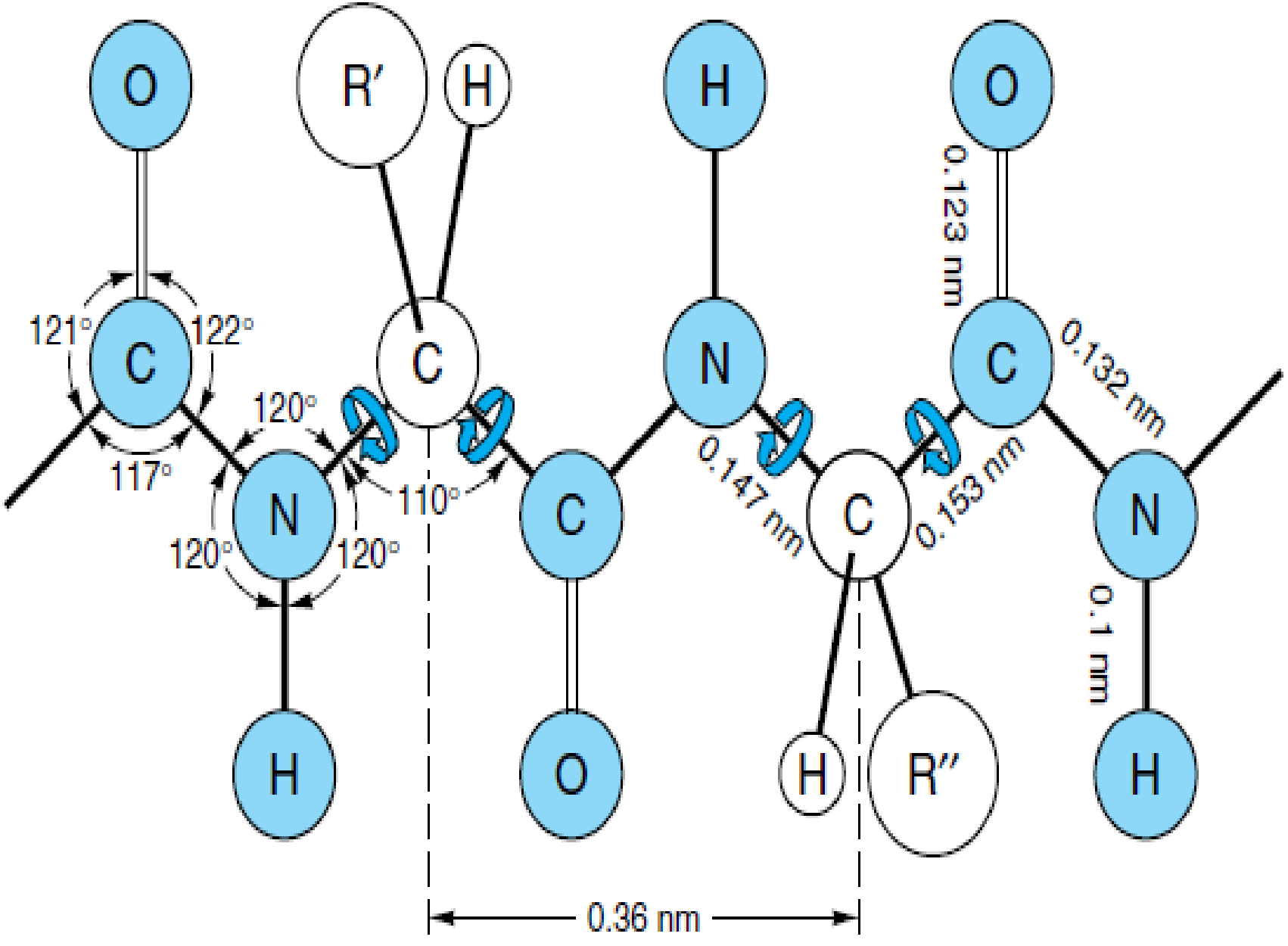
The planar peptide group. (b) Three bonds separate sequential α carbons in a polypeptide chain. The N—C_α and C_α—C bonds can rotate, described by dihedral angles designated φ and ψ, respectively. The peptide C—N bond is not free to rotate. Other single bonds in the backbone may also be rotationally hindered, depending on the size and charge of the R groups


O
R′
H
H
O
0.123 nm
121°
122°
C
C
N
C
0.132 nm
120°
117°
110°
0.147 nm
0.153 nm
N
C
C
120°
120°
N
0.1 nm
H
O
H
R″
H
0.36 nm

Distribution of *f* and *y* Dihedral Angles

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

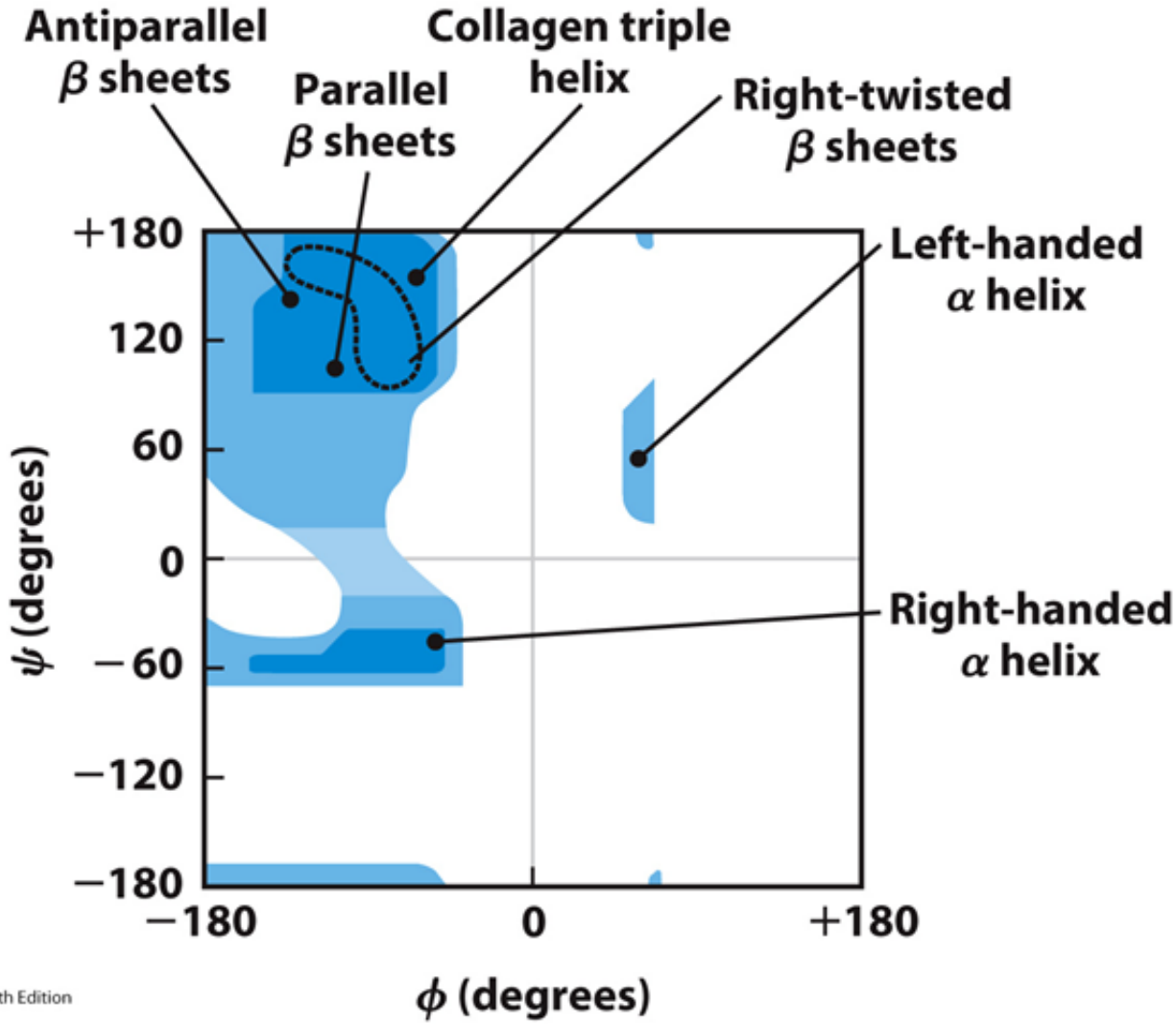


Figure 4-9a
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Ramachandran plots showing a variety of structures. (a) The values of φ and ψ for various allowed secondary structures are overlaid on the plot from Figure 4-3. Although left-handed α helices extending over several amino acid residues are theoretically possible, they have not been observed in proteins.

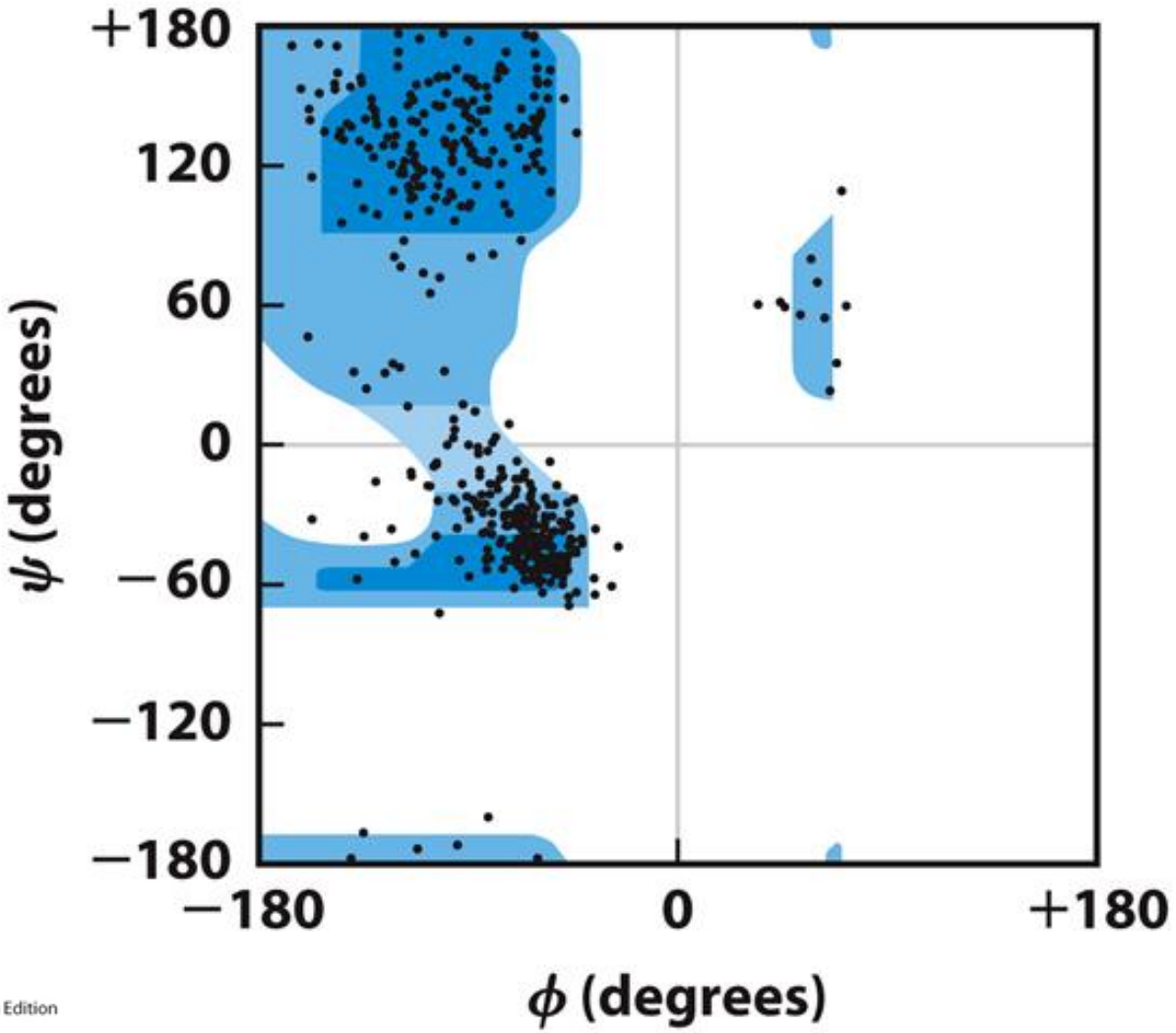

Figure 4-9b
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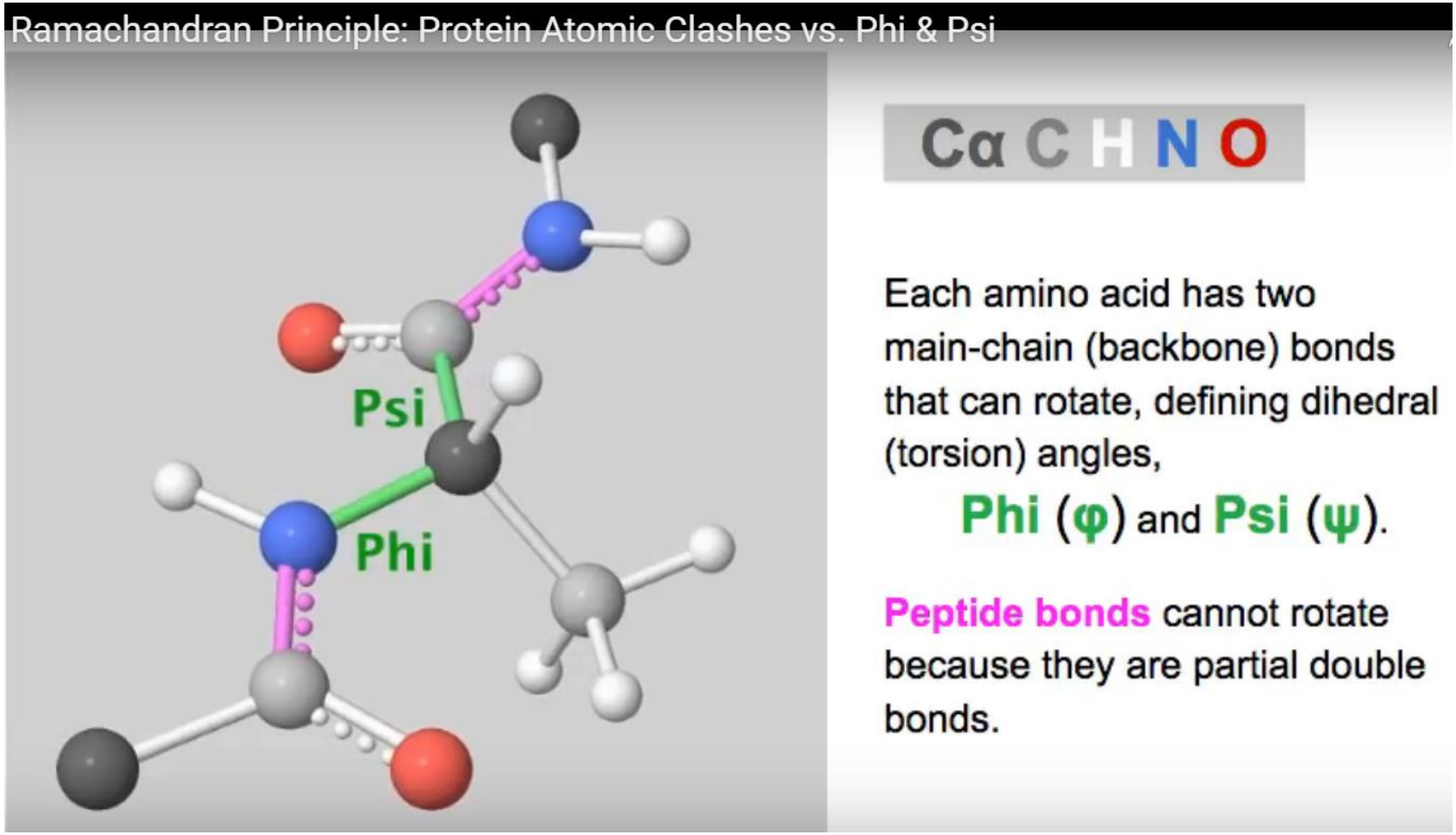
Ramachandran plots showing a variety of structures. (b) The values of φ and ψ for all the amino acid residues except Gly in the enzyme pyruvate kinase (isolated from rabbit) are overlaid on the plot of theoretically allowed conformations (Figure 4-3). The small, flexible Gly residues were excluded because they frequently fall outside the expected (blue) ranges.

https://www.youtube.com/watch?v=Q1ftYq13XKk



2) Secondary structure

It is the spatial arrangement of amino acid residues that are near one another in the linear sequence of the polypeptide chain.

The secondary structure are of three types:

a) The α-helix

b) β-pleated sheets

c) Collagen helix

The bonds responsible for stability of secondary structure:

The secondary structure is stabilized by a hydrogen bonds between peptide bond groups (i.e. between NH group of one amino acid residue and the carbonyl oxygen (C=O) of other amino acid).

a) α-helix:

- It is a spiral structure.

- The polypeptide backbone is tightly wound around the long axis of the molecule and the R-groups of amino acid residues protrude outward from the helical backbone.

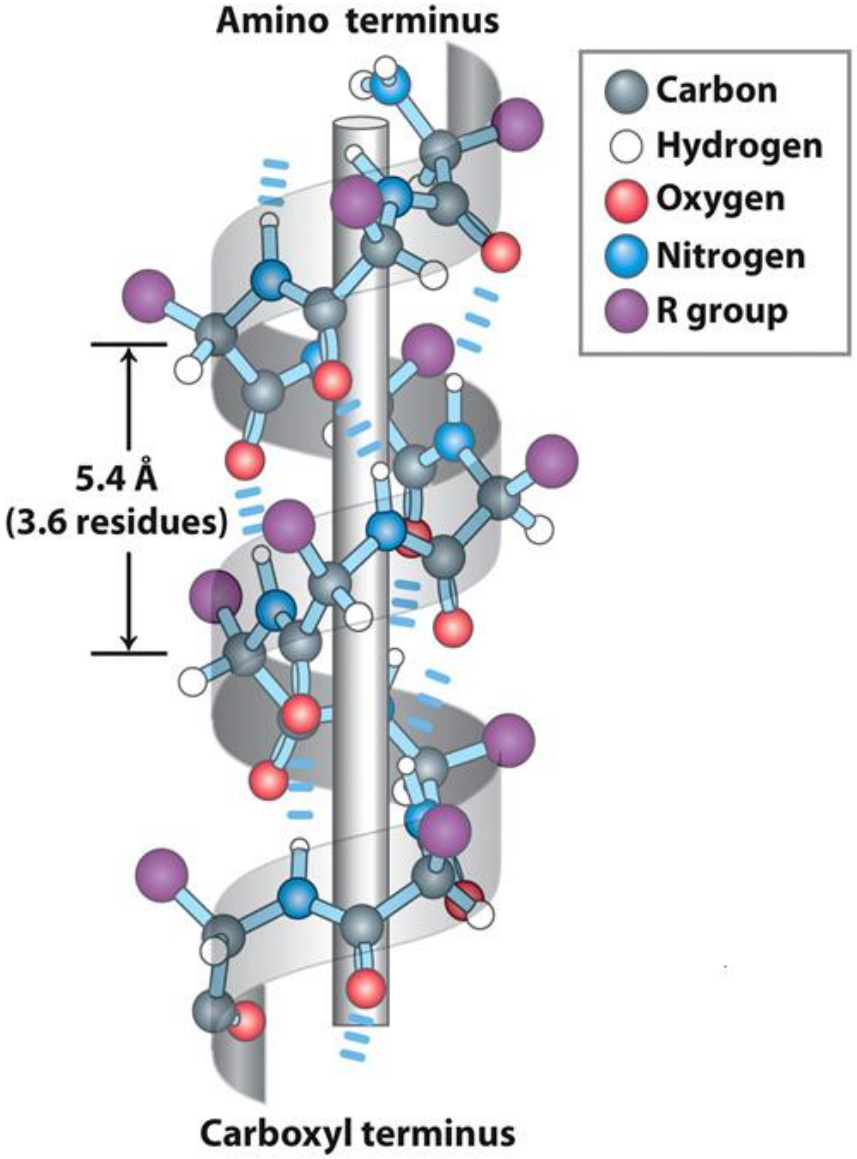
- The repeating unit is a single turn of the helix, which extends about 5.4°A along the long axis.

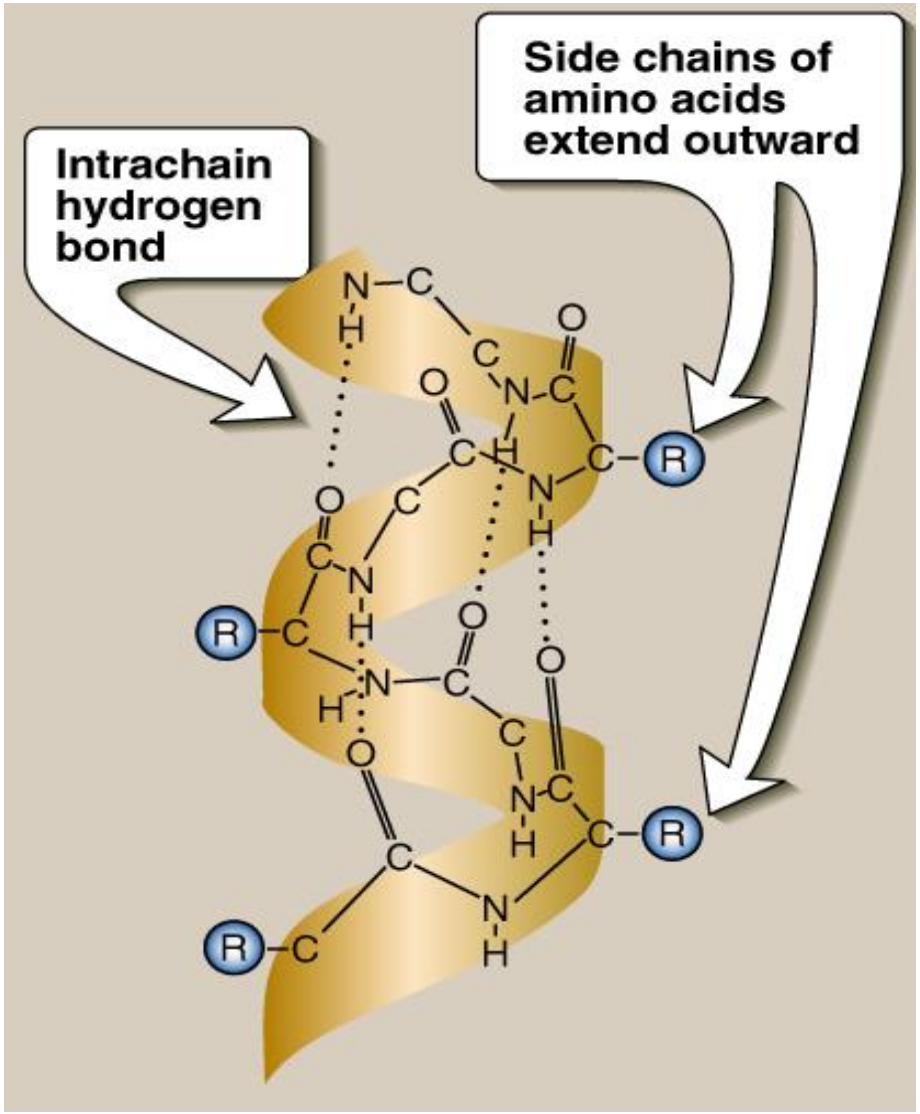
-Each helical turn includes 3.6 amino acid residues

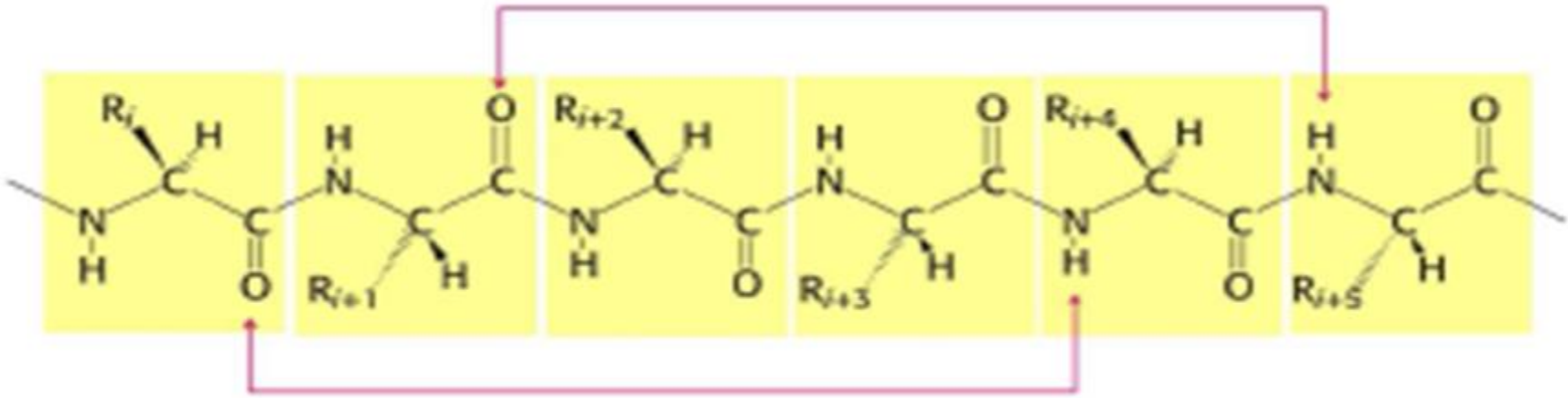
- In all proteins, the helical twist of the α-helix is **right-handed**.

The bonds responsible for stability of α-helix :

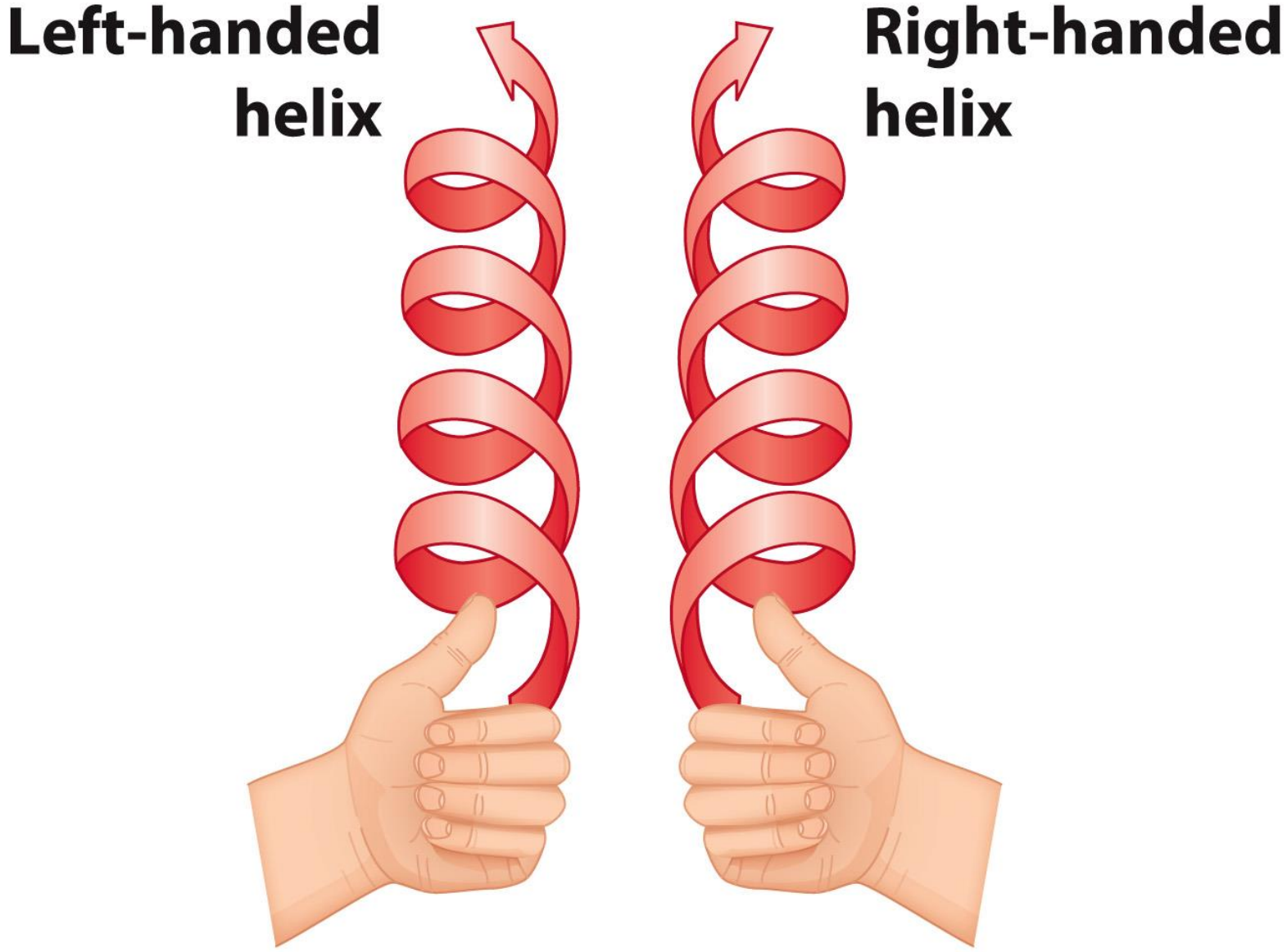
It is stabilized by a hydrogen bonds between peptide bond groups.


Amino terminus
Carbon
Hydrogen
Oxygen
Nitrogen
R group
5.4 Å
(3.6 residues)
Carboxyl terminus


Intrachain hydrogen bond
Side chains of amino acids extend outward
R


Ri
H
C
N
H
C
O
O
H
N
C
Ri+1
H
O
Ri+2
Ri+3
Ri+4
Ri+5

- An α-helix permits the formation of intrachain hydrogen bonds between successive coils of the helix, parallel to the long axis of the helix.
-The hydrogen bond formed between the hydrogen atom attached to the electronegative nitrogen atom of each peptide linkage and the electronegative carbonyl oxygen atom of the fourth amino acid on the amino terminal side of it in the helix.
- Every polypeptide bond of the chain participates in such hydrogen bonding.
- Each successive turn of the α-helix is held to the adjacent turns by three to four hydrogen bonds, conferring significant stability in the overall structure.



Box 4-1
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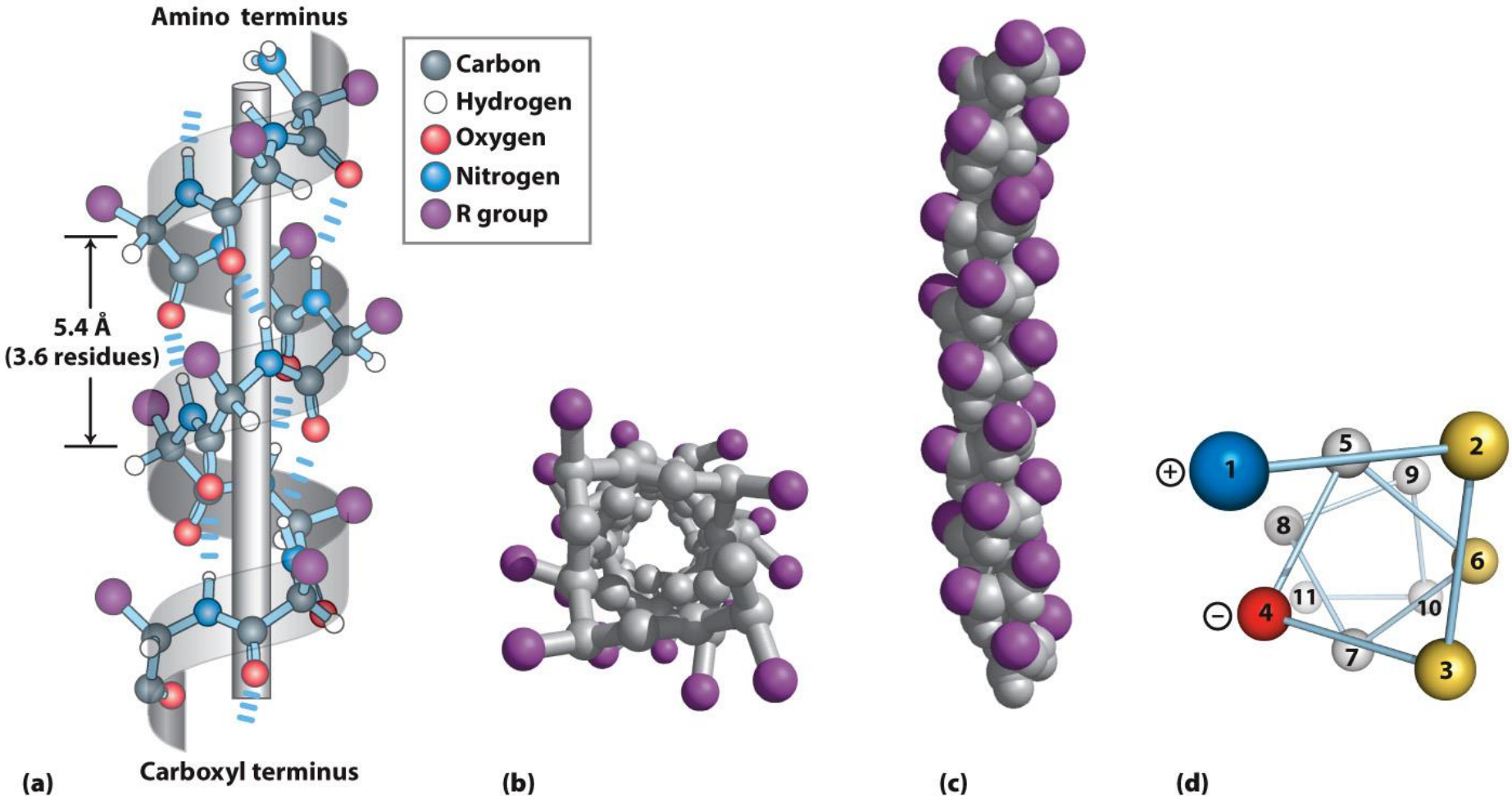

Figure 4-4
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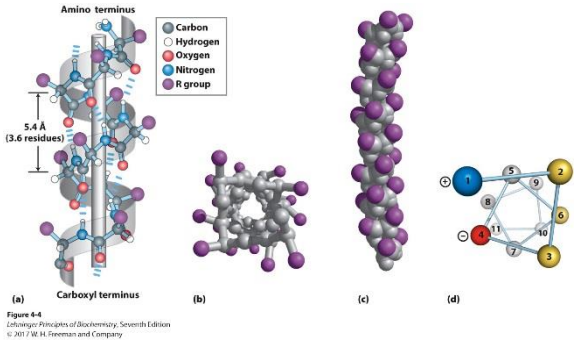
Models of the α helix, showing different aspects of its structure.



(a) Ball-and-stick model showing the intrachain hydrogen bonds. The repeat unit is a single turn of the helix, 3.6 residues.

(b) The α helix viewed from one end, looking down the longitudinal axis (derived from PDB ID 4TNC). Note the positions of the R groups, represented by purple spheres. This ball-and-stick model, which emphasizes the helical arrangement, gives the false impression that the helix is hollow, because the balls do not represent the van der Waals radii of the individual atoms.

(c) As this space-filling model shows, the atoms in the center of the α helix are in very close contact.

(d) Helical wheel projection of an α helix. This representation can be colored to identify surfaces with particular properties. The yellow residues, for example, could be hydrophobic and conform to an interface between the helix shown here and another part of the same or another polypeptide. The red and blue residues illustrate the potential for interaction of negatively and positively charged side-chains separated by two residues in the helix.

The α Helix: Top View

- The inner diameter of the helix (no side chains) is about 4–5 Å.
  - too small for anything to fit “inside”
- The outer diameter of the helix (with side chains) is 10–12 Å.
  - happens to fit well into the major groove of dsDNA
- Amino acids #1 and #8 align nicely on top of each other.
  - What kind of sequence gives an α helix with one hydrophobic face?

- Hydrogen bonds are individually weak but collectively serve to stabilize the helix.

Example:

- α-keratin:
  - it is a fibrous protein whose structure is entirely α-helix
  - it is rich in cysteine residues.
  - major component of tissues such as hair and skin.
- hemoglobin:
  - it is a globular protein.
  - 80% of hemoglobin structure is α-helix.

Factors that affect the stability of α-helix:

a) Electrostatic repulsion (or attraction) between amino acid residues with charged R-groups.

[e.g., if there is a large numbers of charged amino acids (for example, glutamate, aspartate, histidine, lysine, or arginine) they disrupt the helix by forming ionic bonds, or by electrostatically repelling each other].

b) The bulkiness of adjacent R-groups.

[e.g., tryptophan, or amino acids, such as valine or isoleucine, that branch at the β-carbon (the first carbon in the R-group, next to the α-carbon) can interfere with formation of the α-helix if they are present in large numbers and close to each others].

c) The interaction between amino acid side chains spaced three (or four) residues apart.

[e.g two aromatic amino acids are resulting in hydrophobic interaction].

d) The occurrence of proline residues:

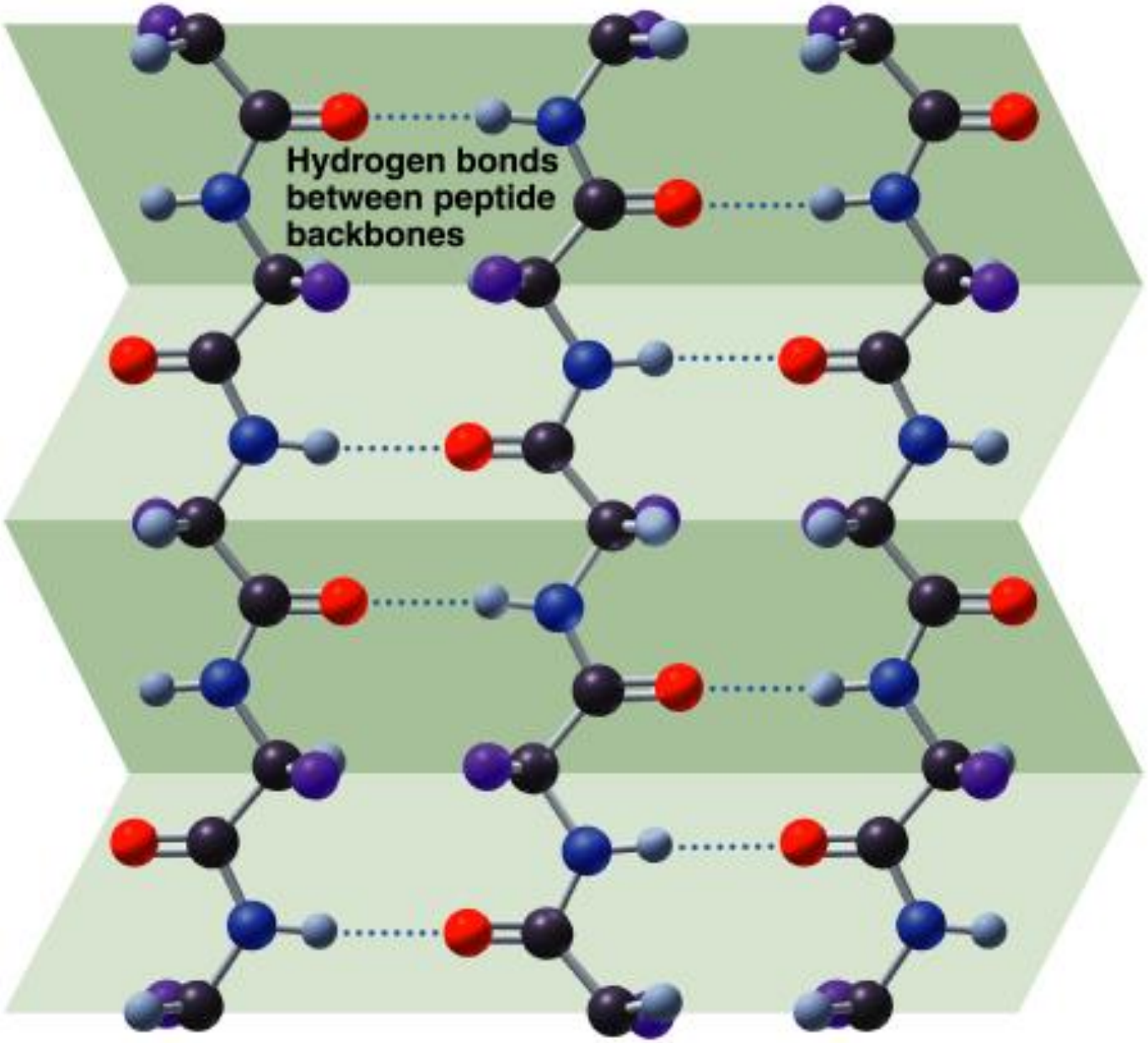
In proline the nitrogen atom is part of a rigid ring and the rotation around N-α C bond is not possible .

In addition , the nitrogen atom of a proline residue in peptide linkage has no substituent hydrogen to form hydrogen bond with other residues

⇒ So, it inserts a kink in the chain that disrupts the smooth, helical structure.

b) β-pleated sheet:

- In the β-conformation, the backbone of the polypeptide chain is extended into a zigzag rather than a helical structure.
- The zigzag polypeptide chains can be arranged side by side to form a structure resembling a series of pleats, such a structure is called a β-pleated sheet.
- The R-groups of adjacent amino acids protrude from the zigzag structure in opposite directions, creating the alternating pattern.
- Hydrogen bonds form between adjacent segments of polypeptide chain β- pleated sheet are composed of two or more β-strands.

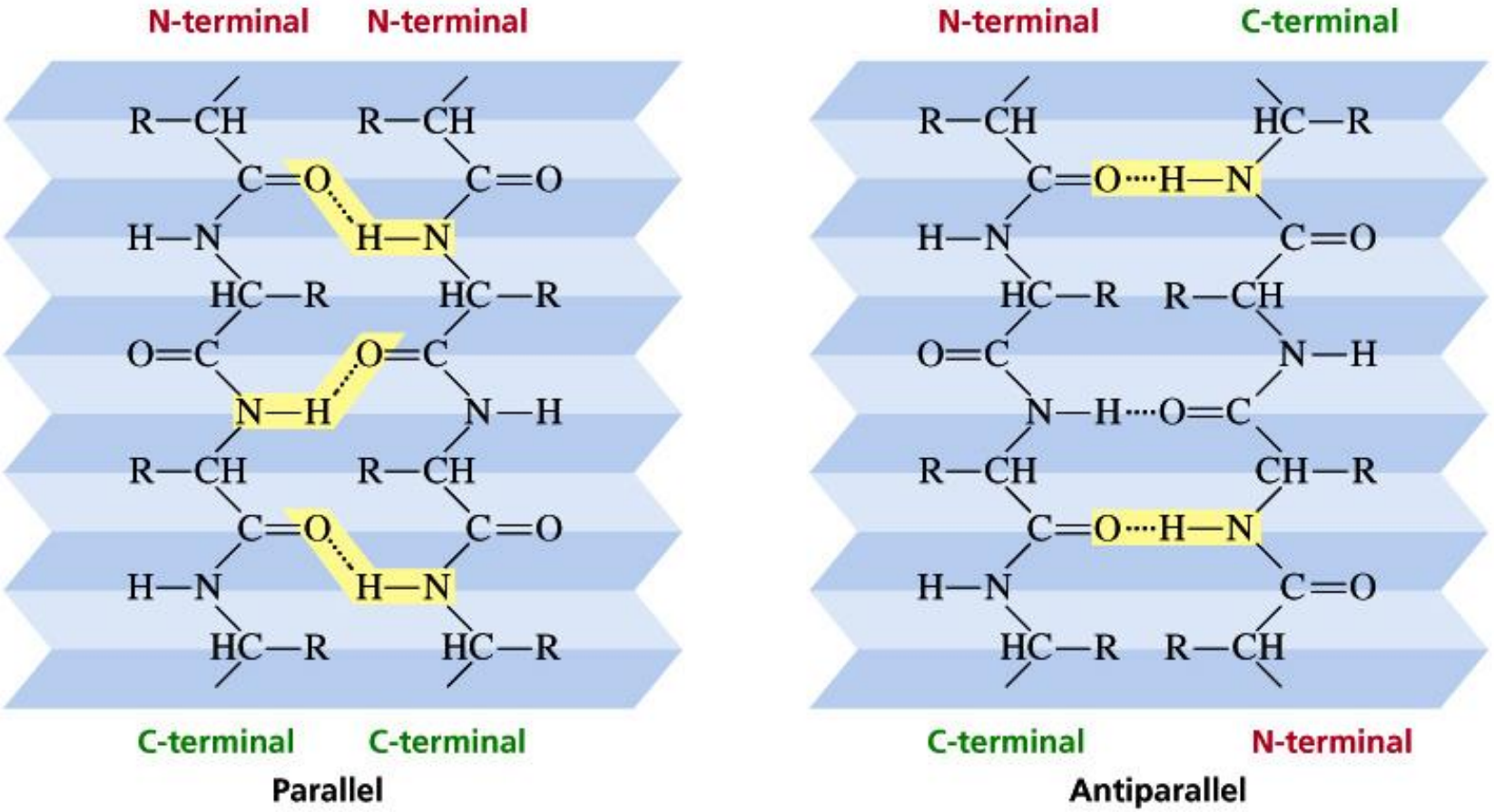

Hydrogen bonds
between peptide
backbones

- The adjacent polypeptide chains in a β sheet can be either

Parallel (having the same amino to carboxyl orientations) OR **antiparallel** (having the opposite amino to carboxyl orientations)

- When two or more β-sheets are layered close together within a protein, the R-groups of the amino acid residues on the touching surfaces must be relatively small.

H-bond : Pleated sheet structure



Notice the hydrogen bond angle in parallel and antiparallel b-sheet
In the antiparallel, the hydrogen bonds between strands are linear (stronger).

The bonds responsible for stability of β-pleated sheet :

It is stabilized by a hydrogen bonds between peptide bond groups.

Example:

β-keratins (such as silk fibroin):

- consist almost entirely stacks of antiparallel β-sheets.
- rich in amino acids having relatively small R-groups, particularly glycine and alanine.
- the β-keratin such as fibroin, have no S – S cross linkages.

β - turns

- β turns occur frequently whenever strands in β sheets change the direction.
- The 180° turn is accomplished over four amino acids.
- **There are two types of β turns:** type I and II
  - Type I occurs more than twice as frequently as type II.
  - Type II β turns usually have Gly as the third residue.
  - The turn is stabilized by a hydrogen bond from a carbonyl oxygen to amide proton three residues down the sequence.
- Proline in position 2 or glycine in position 3 are common in β turns.

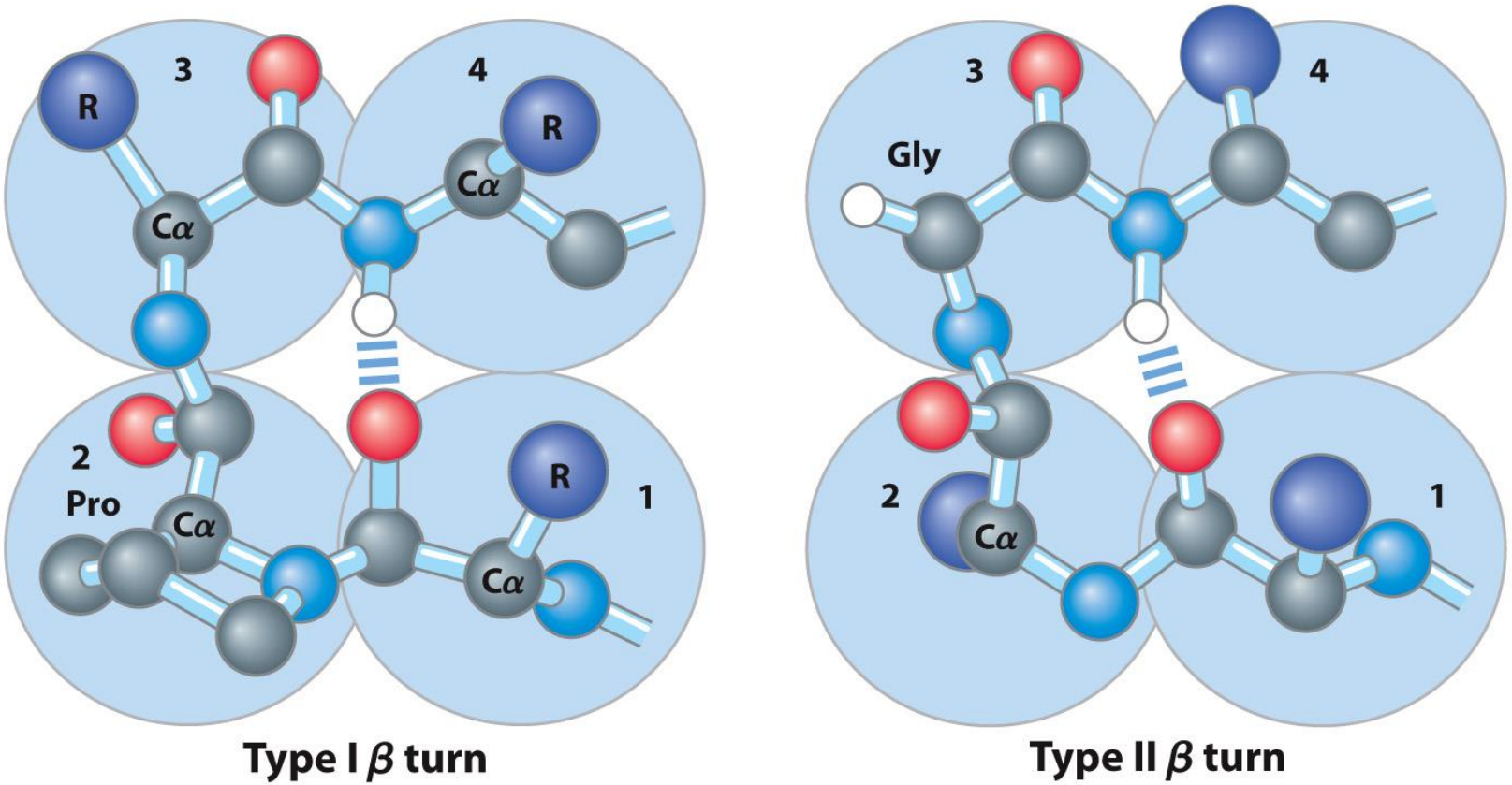


Figure 4-7
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- Note the hydrogen bond between the peptide groups of the first and fourth residues of the bends. (Individual amino acid residues are framed by large blue circles.)

TABLE 4-2 Approximate Amounts of α Helix and β Conformation in Some Single-Chain Proteins

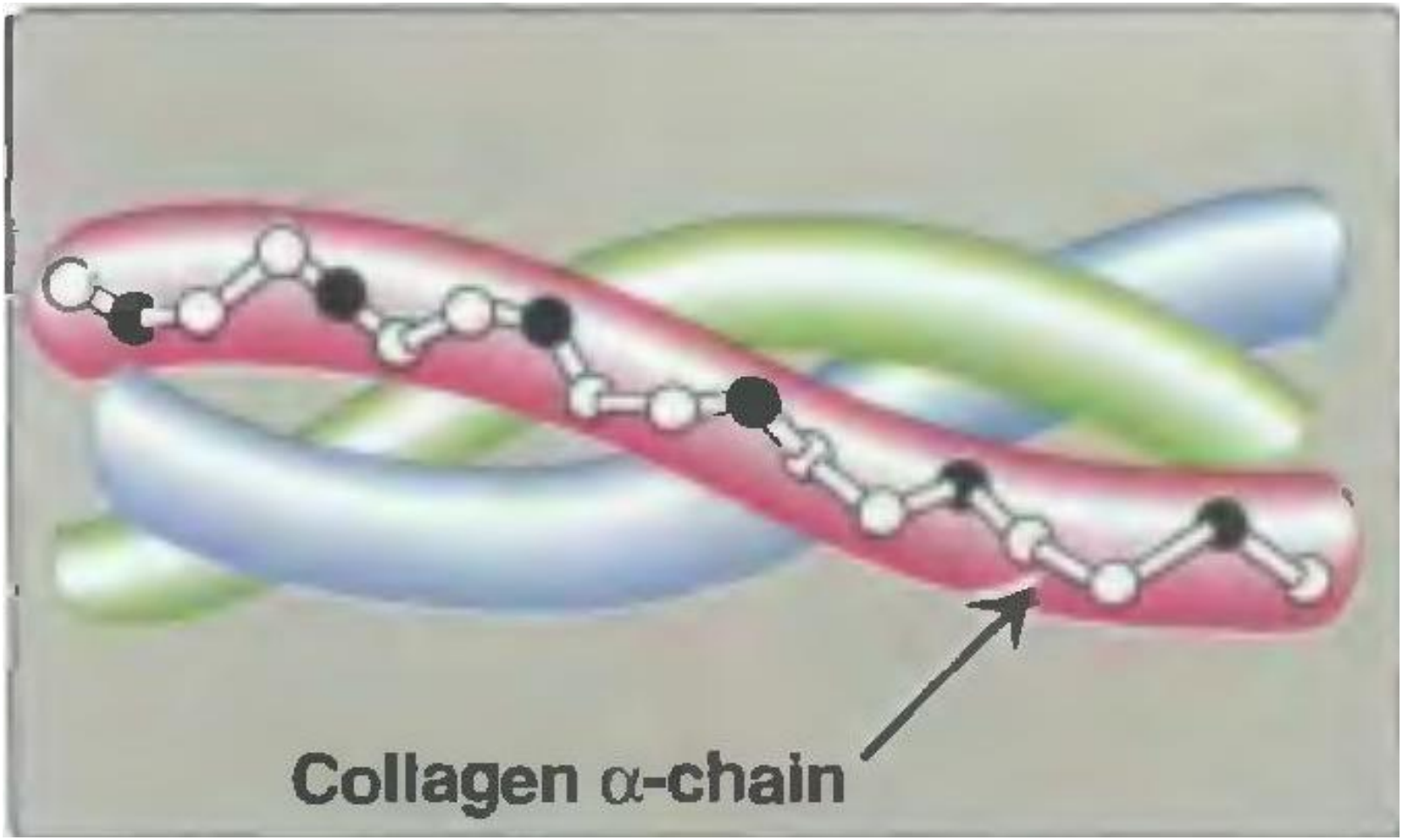
	*Residues (%)**	
Protein (total residues)	*α Helix*	*β Conformation*
Chymotrypsin (247)	14	45
Ribonuclease (124)	26	35
Carboxypeptidase (307)	38	17
Cytochrome *c* (104)	39	0
Lysozyme (129)	40	12
Myoglobin (153)	78	0

c) Collagen helix:

Collagen is found in connective tissue such as tendons, cartilage, the organic matrix of bone and the cornea of the eye.

- Collagen molecules (tropocollagen – basic unit) consist of three polypeptide, called α-chains (each α-chain is twisted into a **left handed helix** of 3 residue per turn) which wrap around each other in a triple helix (**right handed triple helix**) forming a rope-like structure.

- Collagen is built of recurring subunit structure, triple -stranded tropocollagen (triple helix) molecules, having distinctive heads.



Triple-stranded helix of collagen.

- These arranged head to tail in many parallel bundles, but the heads are staggered.
- Between the end of one triple helix and the beginning of the next is a gap that may provide a site for deposition of hydroxyapatite crystals in bone formation.
- The polypeptide chains of tropocollagen are covalently cross linked by dehydrolysinonorleucine residues formed by an enzymatic reaction between two lysine residues of adjacent tropocollagen subunits.

- The three polypeptide chains are held together by hydrogen bonds between chains.

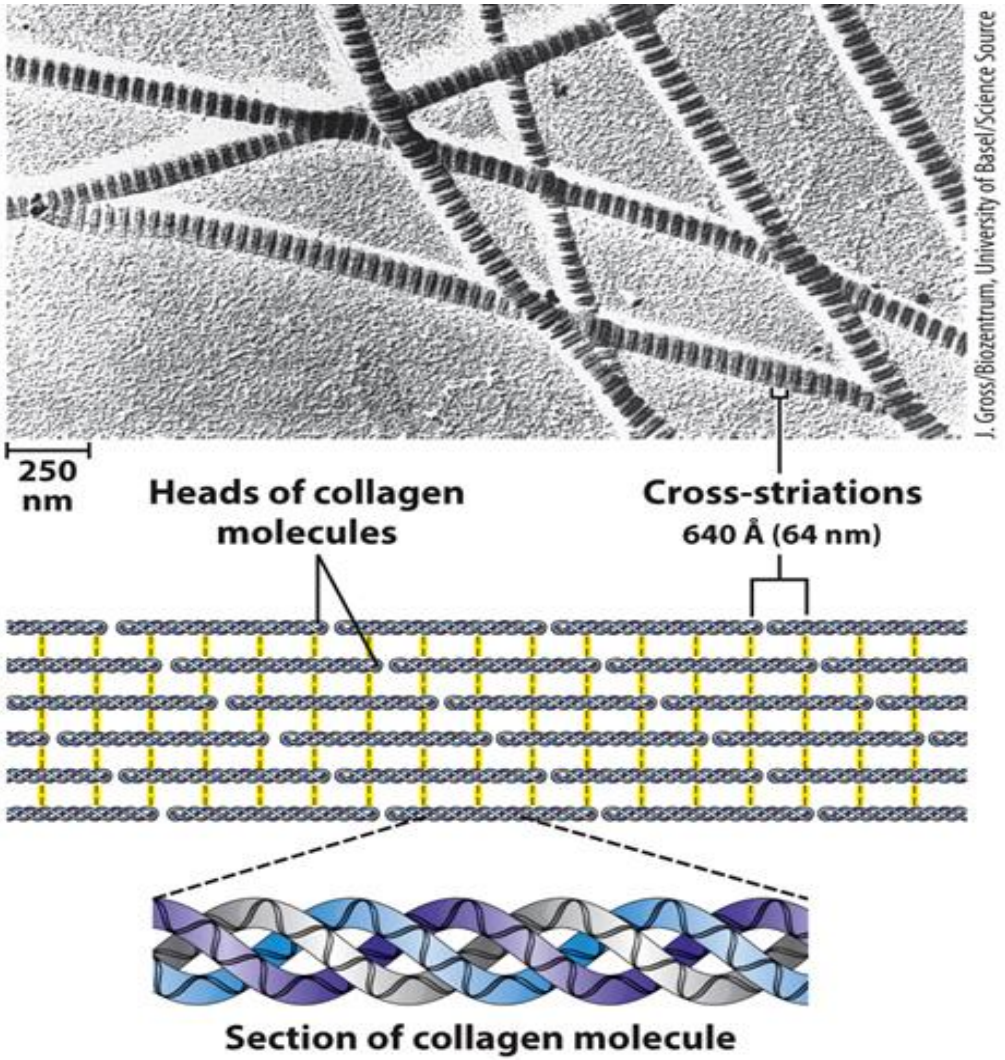


Figure 4-13
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FIGURE 4-13 Structure of collagen fibrils. Collagen (*Mr* 300,000) is a rod-shaped molecule, about 3,000 Å long and only 15 Å thick. Its three helically intertwined α chains may have different sequences; each chain has about 1,000 amino acid residues. Collagen fibrils are made up of collagen molecules aligned in a staggered fashion and cross-linked for strength. The specific alignment and degree of cross-linking vary with the tissue and produce characteristic cross-striations in an electron micrograph. In the example shown here, alignment of the head groups of every fourth molecule produces striations 640 Å (64 nm) apart.

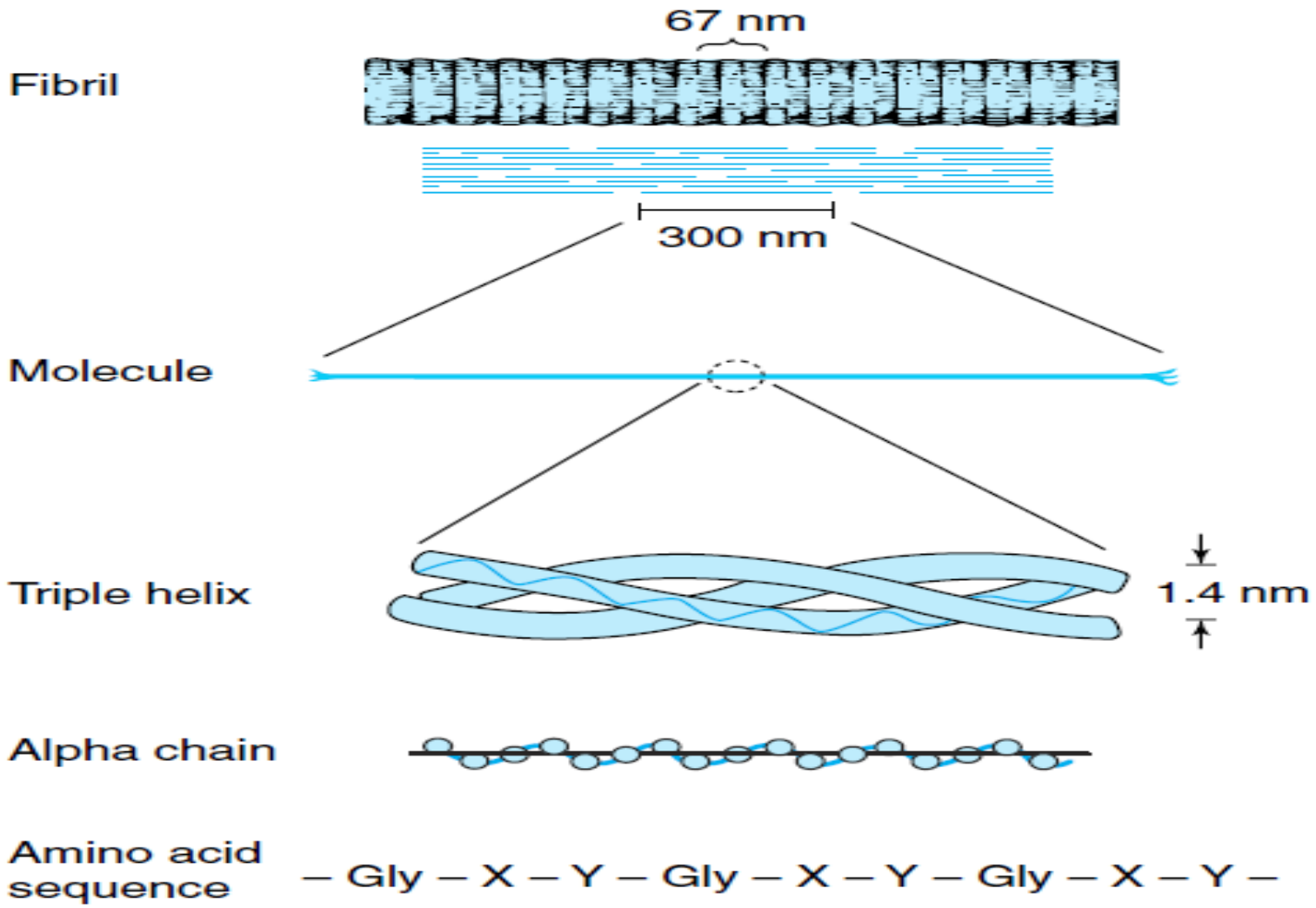
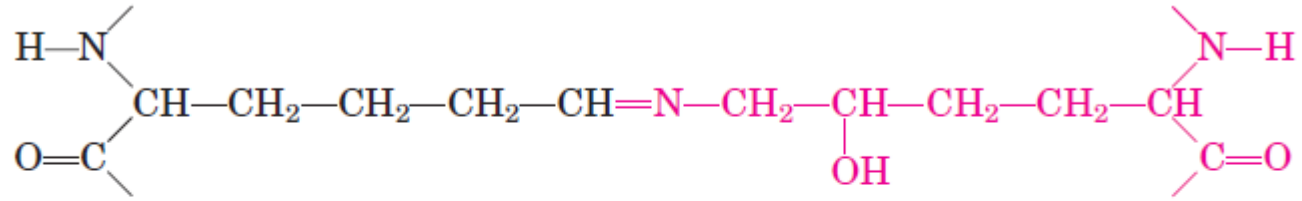


Figure 48–1. **Molecular features of collagen structure from primary sequence up to the fibril.** (Slightly modified and reproduced, with permission, from Eyre DR: Collagen: Molecular diversity in the body's protein scaffold. Science 1980;207:1315. Copyright © 1980 by the American Association for the Advancement of Science.)



Dehydrohydroxylysinonorleucine

- The triple helical molecule – unique to collagen – are then associated bilaterally and longitudinally into fibrils.

- The amino acid sequence in collagen is generally a repeating tripeptide unit (Gly – X – Y)

  where X is frequently proline

  Y is often hydroxyproline or hydroxylysine

  ⟹ Thus, most of the molecule can be regarded as a polytripeptide.

- Glycine constitute every third residue in the triple helical portion of each α-chain.

- Collagen contains hydroxyproline and hydroxylysine, which are not present in most other proteins.
- Although collagen of different species differ in amino acid sequence, most contain about 35% glycine and 11% alanine, resembling the β-keratin in this respect.
- Collagen is distinctive in containing about 12% proline and 9% hydroxyproline, are amino acids rarely found in proteins other than collagen.

3) Tertiary structure

Refer to the spatial arrangement of amino acid residues that are far apart in the linear sequence (i.e in the primary structure), so the polypeptide chain is folded into three dimension.

-Tertiary structure is three-dimensional conformation of a polymer in its native folded state.

- The unique three-dimensional structure of each polypeptide is determined by its amino acids sequence.
- Interaction of the amino acid side chains guide the folding of the polypeptide chain to form a compact structure.

- Hydrophobic side chains are burried in the interior whereas hydrophilic groups are generally found on the surface of the molecule.

Bonds that stabilize tertiary structure:

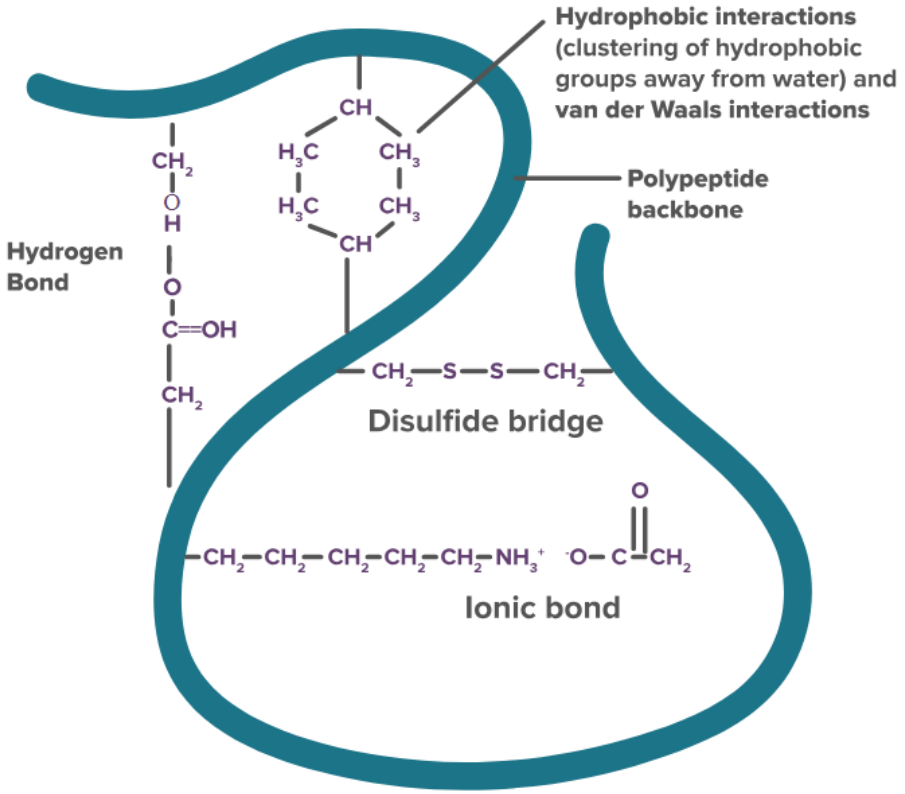
Non-covalent bonds between R-groups (i.e between groups in the side chains). [weak bonds]

a) Hydrophobic interaction

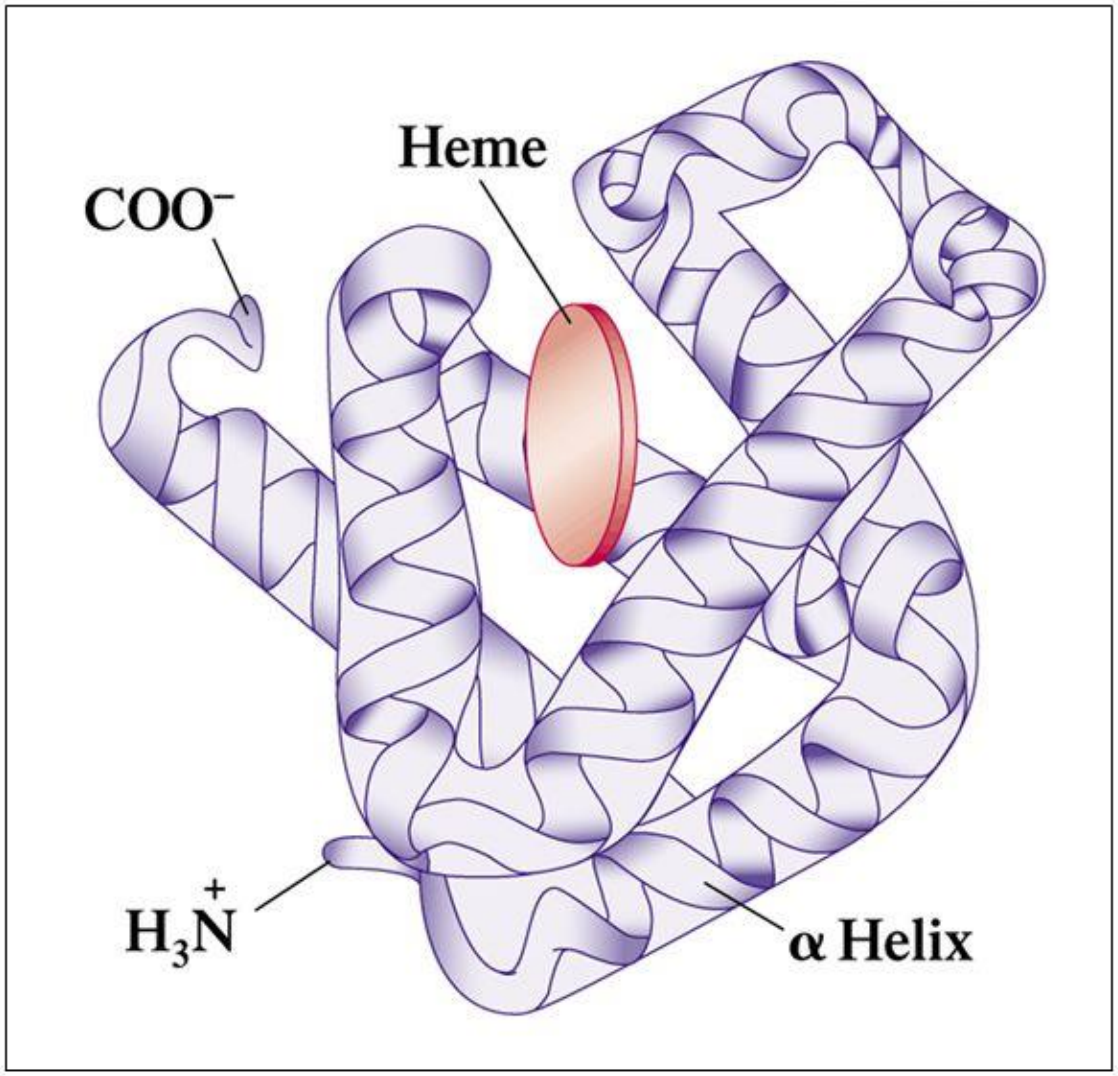
b) Hydrogen bonds

c) Ionic interaction

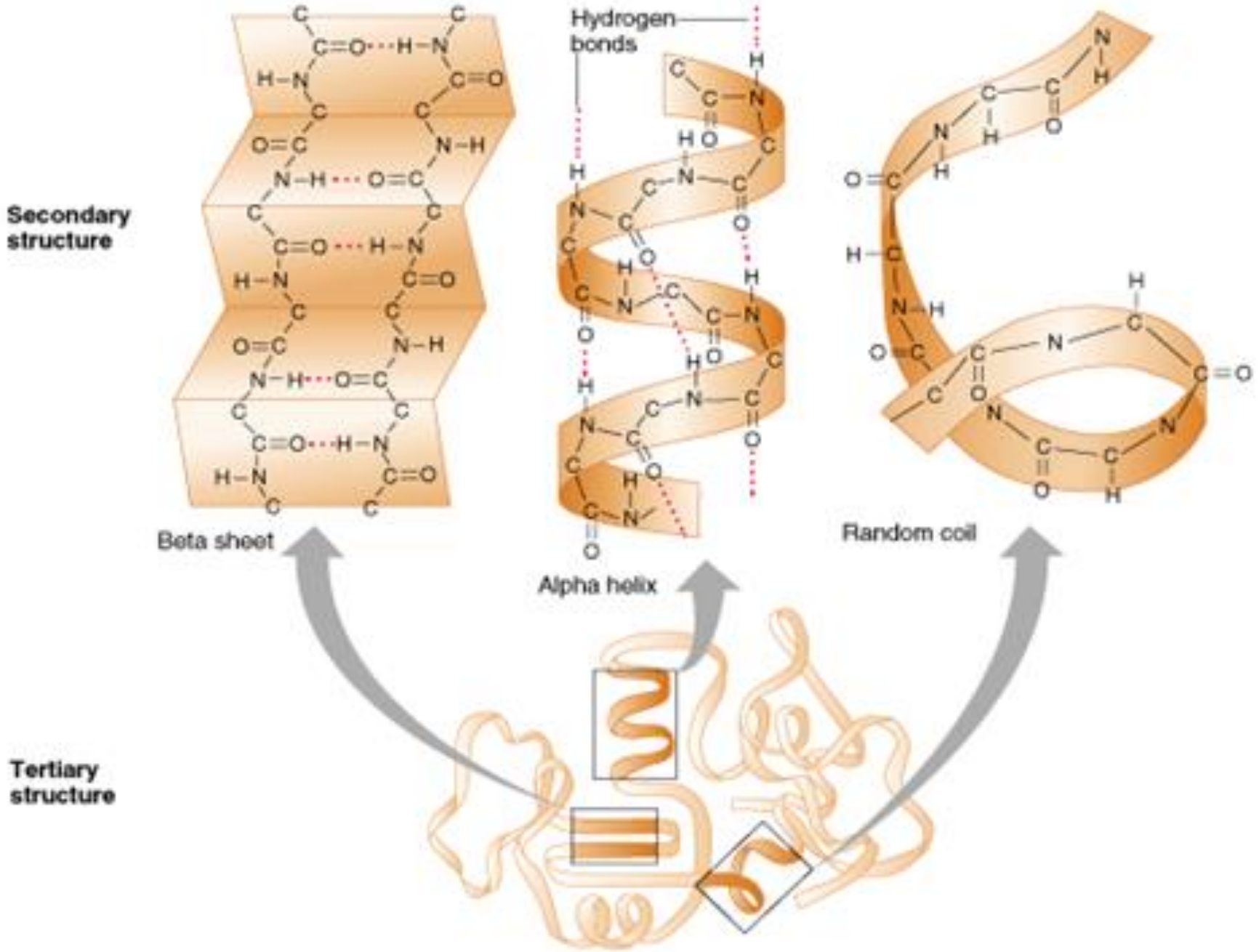
d) Disulphide bonds



Tertiary structure of the single-chain protein: **myoglobin**.



found mainly in muscle tissue where it serves as an intracellular storage site for oxygen


Secondary structure
Hydrogen bonds
Beta sheet
Alpha helix
Random coil
Tertiary structure

a) Hydrophobic interactions:

it is the association between non-polar groups (hydrophobic groups) in the side chains of amino acids.

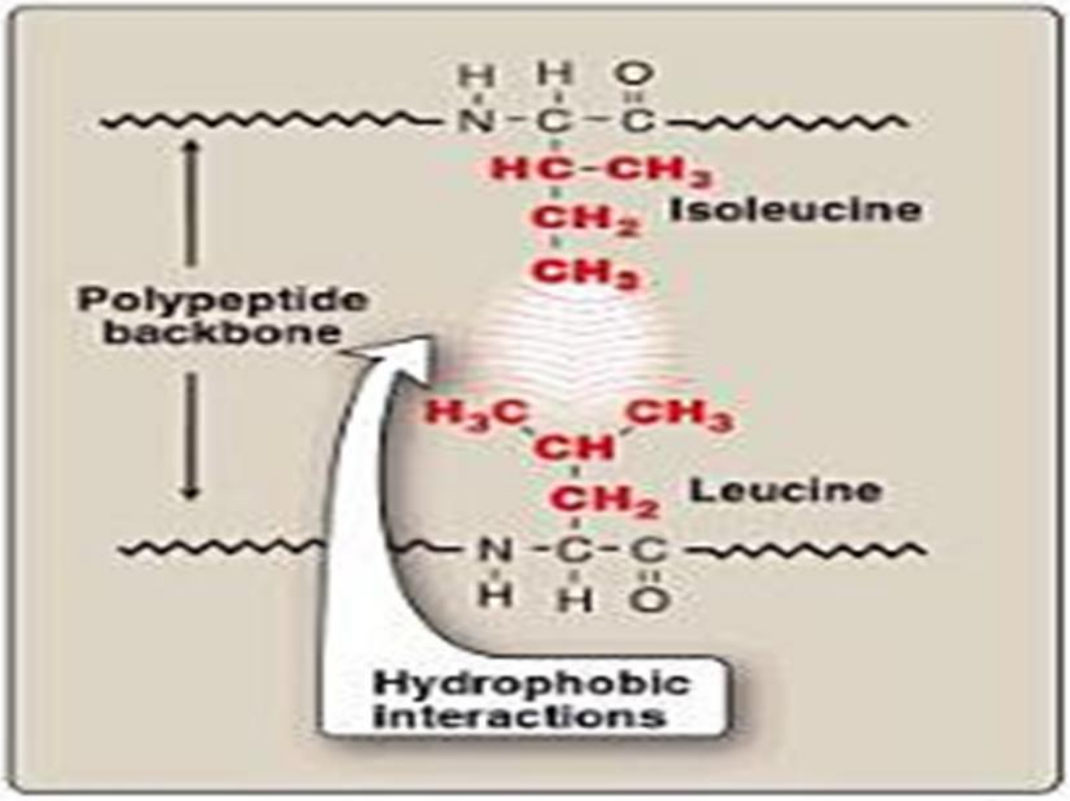
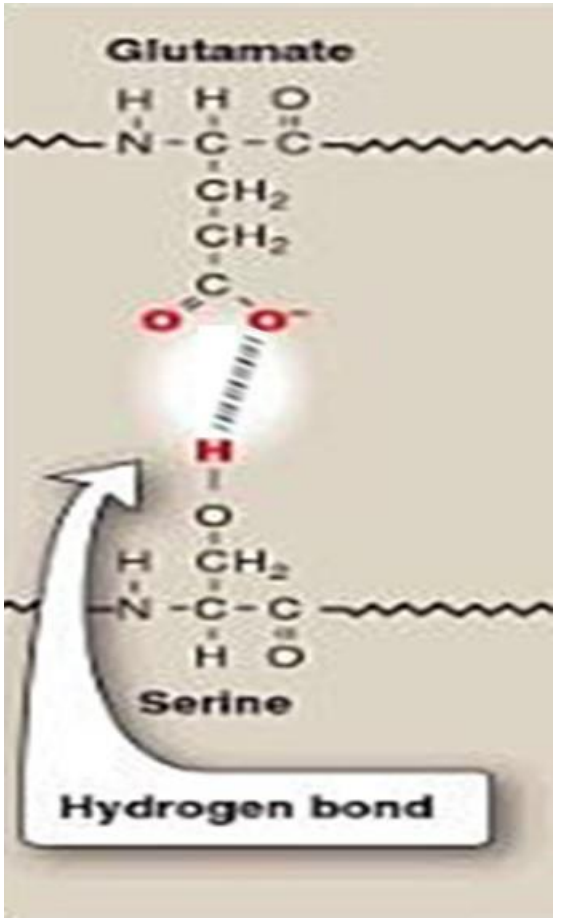


Figure 2.10
Hydrophobic interactions between amino acids with nonpolar side chains.

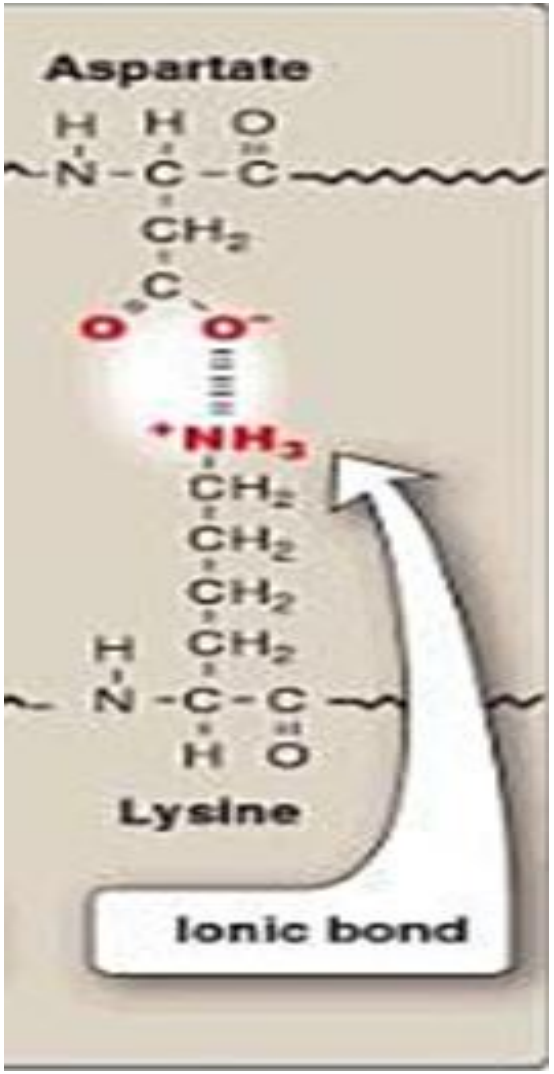
b) Hydrogen bonds:

A hydrogen bond is a type of attractive interaction between an electronegative atom (such as oxygen or nitrogen) and a hydrogen atom bonded covalently to another electronegative atom.



c) Ionic interaction (electrostatic interaction):

Interaction between opposite charged groups of the side chains of amino acids.



Example of 3ry structure
The structure of Myoglobin

-It is small globular protein (hemoprotein), present in heart and skeletal muscle.

- It functions both as a reservoir for oxygen and as an oxygen carrier that increases the rate of transport of oxygen within the muscle cell.

- Myoglobin consists of a single polypeptide chain of 153 amino acid residues of known sequence and a single heme group.

The structure of Myoglobin (Cont.)

- Myoglobin is a compact molecule with approximately 80% of its polypeptide chain folded into eight stretches of α-helix.

- The backbone of the myoglobin molecule is made up of 8 relatively straight segments of α-helix interrupted by bends.

- The longest α-helix has 23 amino acid residues and the shortest only 7.

- All the α-helices are right handed.

- More than 70% of the amino acids in the myoglobin molecule are in these α-helical regions.

- The eight α-helical regions, labeled A to H, are terminated either by:
  - - the presence of proline, whose five-membered ring can not be accommodated in α-helix, or
  - - β-bends and loops stabilize by hydrogen bonds and ionic bonds.

The structure of Myoglobin (Cont.)

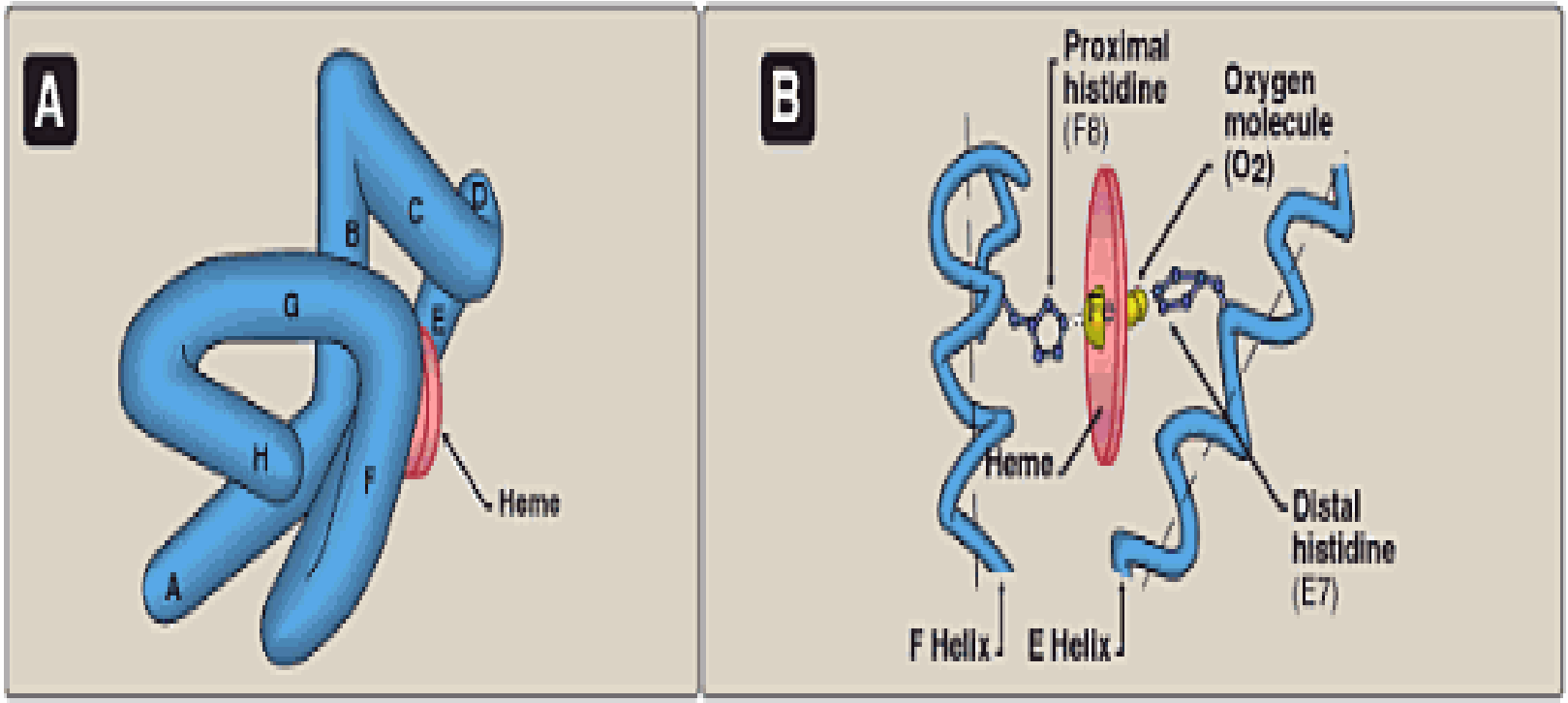


Figure 3.2
A. Model of myoglobin showing helices A to H. B. Schematic diagram of the oxygen-binding site of myoglobin.

The structure of Myoglobin (Cont.)

Location of polar and non-polar amino acid residues:

-The interior of the myoglobin molecule is composed almost entirely of non-polar amino acids.

- They are packed closely together, forming a structure stabilized by hydrophobic interactions among these clustered residues.

- In contrast, charged amino acids are located almost exclusively on the surface, where they can form hydrogen bonds with water.

The structure of Myoglobin (Cont.)

The Heme group:

Heme is prosthetic group that tightly bound to hemoprotein.

- It is ferroprotoporphyrin.

heme → protoporphyrin

→ iron (Fe^{+2})

protoprphyrin:

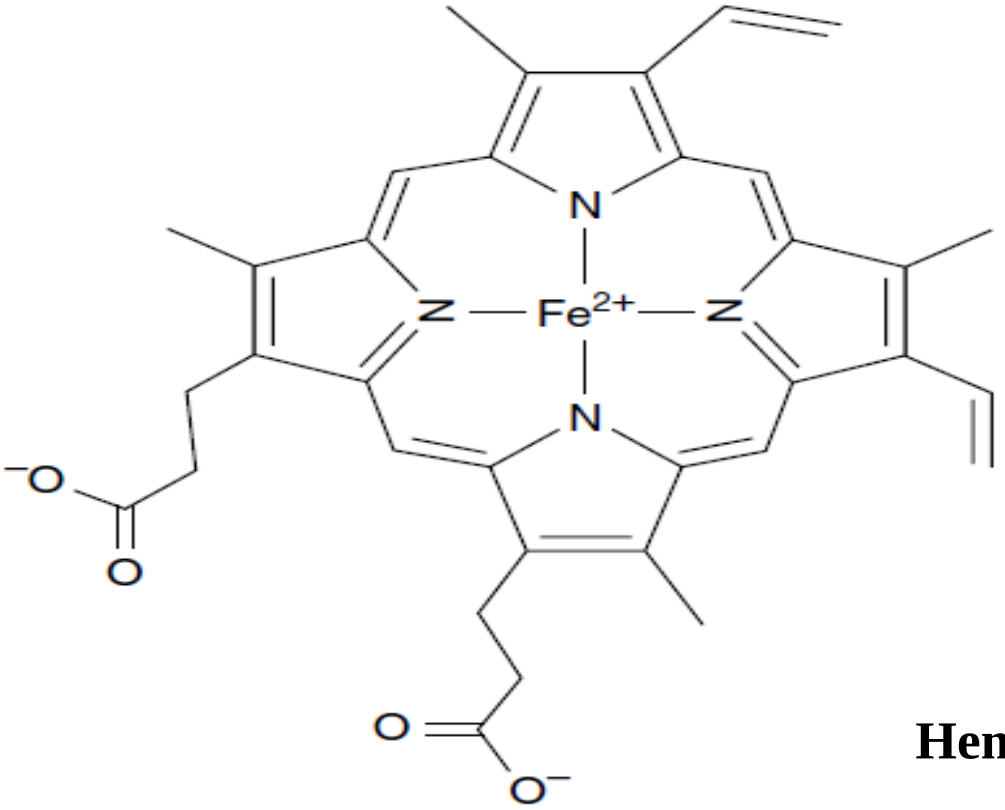
It is tetrapyrrol ring linked by four methene bridge (= CH- groups) in an alternating double bond ring system.

- It contains methyl, vinyl and propionic acid groups on the pyrrole rings.

The structure of Myoglobin (Cont.)

The Heme group in protoporphyrin :

- If the iron is Fe^{+2} ⇨ ferroprotoporphyrin or **heme**
- If the iron is Fe^{+3} ⇨ ferriprotoporphyrin or **hemin**



Heme structure

Binding of heme group to the protein:

- The heme group of myoglobin sits in a pocket in the molecule,
- The non-polar vinyl groups of the heme are buried in the hydrophobic non-polar amino acids located in the interior of the pocket, and
- the hydrophilic propionate project out of the pocket toward the surface.

Binding of iron:

-Iron bind to the 4 nitrogens in the center.

- Notable exception are two histidine residues:

- The first, termed the proximal histidine **(F8),** binds directly to the iron of heme.
- The second, or distal histidine **(E7)** does not directly interact with the heme group but helps stabilize the binding of oxygen to the ferrous iron.

-**Binding of Oxygen:**

-Oxygen binding site is between iron and His E7(distal His).

4) Quaternary structure

Myoglobin structure is stabilized by Non-covalent bonds.

- Proteins that are composed of more than one polypeptide chain (subunits) show a fourth level of protein structure which is the quaternary structure.

Quaternary structure is the three-dimensional structure of a multisubunit protein, particularly the manner in which the subunits fit together.

- Subunits may either function independently of each other or may work cooperatively, as in hemoglobin.

-The interaction between subunits are stabilized by the same forces that stabilize tertiary structure (non-covalent bonds).

Bonds that stabilize quaternary structure:

a) Hydrophobic interaction
b) Hydrogen bonds
c) Ionic interaction

} **Non-covalent bonds** between R-groups (i.e between groups in the side chains). [weak bonds]

Example of 4ry structure
The structure of Hemoglobin

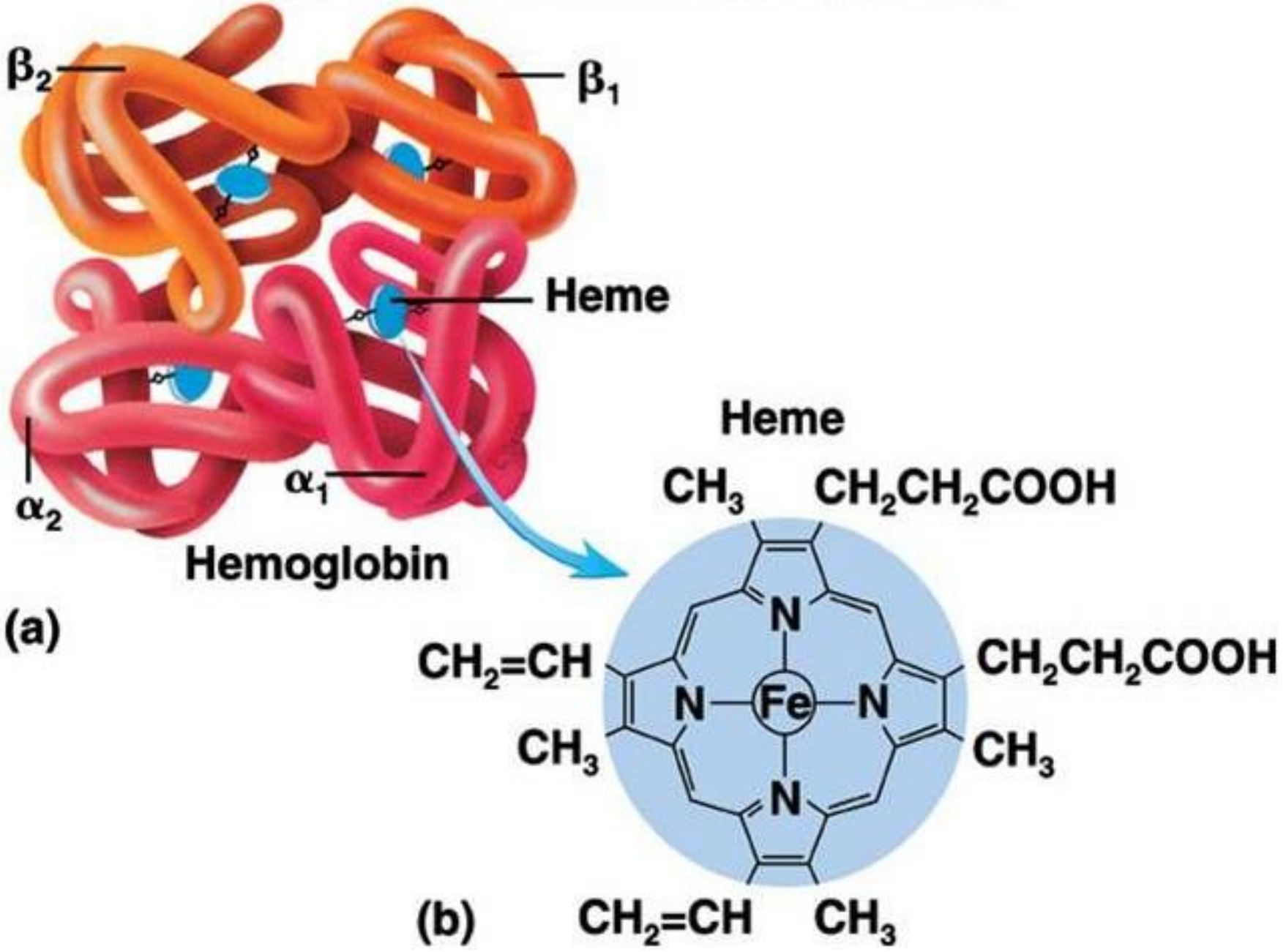
Hemoglobin is simple oligomeric protein.

-It is found in red blood cells (RBC), where its main function is to transport oxygen from the lungs to the capillaries of the tissues.

- Hemoglobin is hemoprotein, composed of four polypeptide chains and four heme groups.

- The protein portion (globin), consist of four polypeptide chains (two α-chains and two β-chains; $\alpha_2\beta_2$) held together by non-covalent interactions.

-The α and β chains contain several segments of α-helix separated by bends, with a tertiary structure very similar to that of the single polypeptide of myoglobin.


β2
β1
Heme
α2
α1
Hemoglobin
(a)
Heme
CH3
CH2CH2COOH
CH2=CH
CH2CH2COOH
N
N
Fe
N
CH3
CH3
N
(b)
CH2=CH
CH3

- Each subunit has stretches of α-helical structure and a heme-binding pocket similar to that described for myoglobin.
- One heme is bound to each polypeptide chain of hemoglobin.
- Each heme is partially burried in a pocket lined with hydrophobic amino acid side chains.
- It is bound to its polypeptide chain through a coordination bond of the iron atom to the R-group of His residue.

Quaternary structure of hemoglobin:

The hemoglobin tetramer composed of two identical dimers:

a) (αβ) dimer 1

b) (αβ) dimer 2

- The two polypeptide chains within each dimer are held tightly together by:
  - hydrophobic interaction between the monomer of each dimer (between α and β subunits in the dimers).
  - Ionic and hydrogen bonds also occur between the members of the dimer.

- The two dimers are held together primarily by polar bonds.
  - The weaker interactions between these mobile dimers result in the two dimers occupying different relative positions in deoxyhemoglobin compared to oxyhemoglobin namely T form and R form.

i) T form:

- The deoxy form of hemoglobin is called the "T" or taut (tense) form .
- In the T form, the two αβ dimers interact through a network or ionic bonds and hydrogen bonds that constrain the movement of the polypeptide chains.
- The T form is the **low oxygen-affinity** form of hemoglobin.

ii) R form :

- The binding of oxygen to hemoglobin causes rupture of some of the ionic bonds and hydrogen bonds between the αβ dimers.

⟹ this leads to a structure called the "R" or relaxed form, in which the polypeptide chains have more freedom of movement.

- The R form is the **high oxygen affinity** form of hemoglobin.

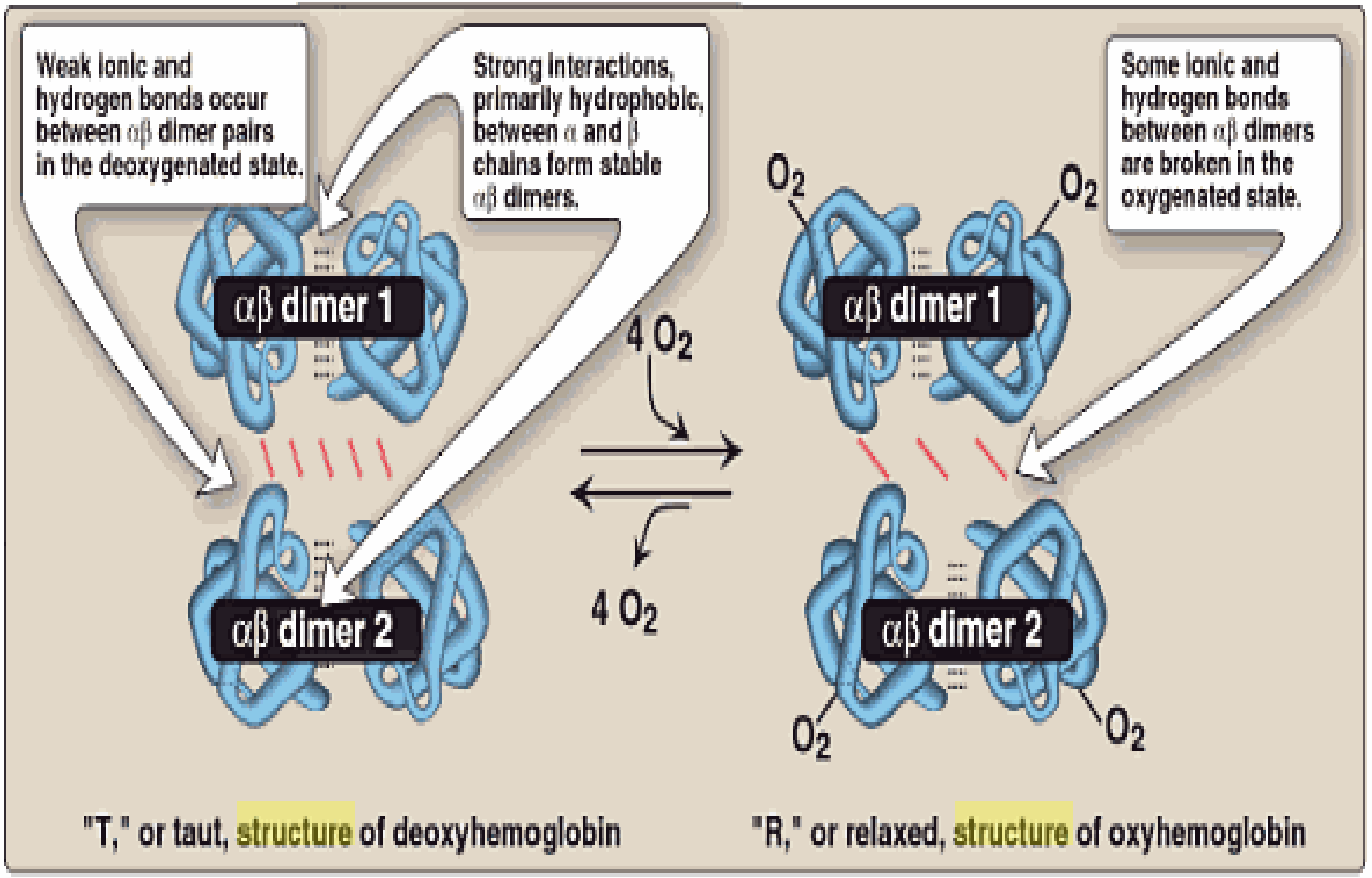


Figure 3.4
Schematic diagram showing structural changes resulting from oxygenation and deoxygenation of hemoglobin.

Binding of oxygen to hemoglobin:

- Myoglobin can bind only one molecule of oxygen because it contains only one heme group.
- In contrast, hemoglobin can bind one oxygen molecule (O_2) at each of its four heme groups.

- The degree of saturation (Y) of these oxygen-binding sites on all myoglobin or hemoglobin molecules can vary between zero (all sites are empty) and 100% (all sites are full).

Oxygen dissociation curve:

- A plot of Y is measured at different partial pressures of oxygen (pO_2) is called the oxygen dissociation curve.

The degree of saturation of myoglobin vs hemoglobin

- myoglobin has a higher oxygen affinity than does hemoglobin.

-The partial pressure of oxygen needed to achieve half- saturation of the binding sites (P_{50}) is approximately

- 1mm Hg for myoglobin
- 26 mm Hg for hemoglobin.

[note: The higher the oxygen affinity (that is, the more tightly oxygen binds), the lower the P_{50}].

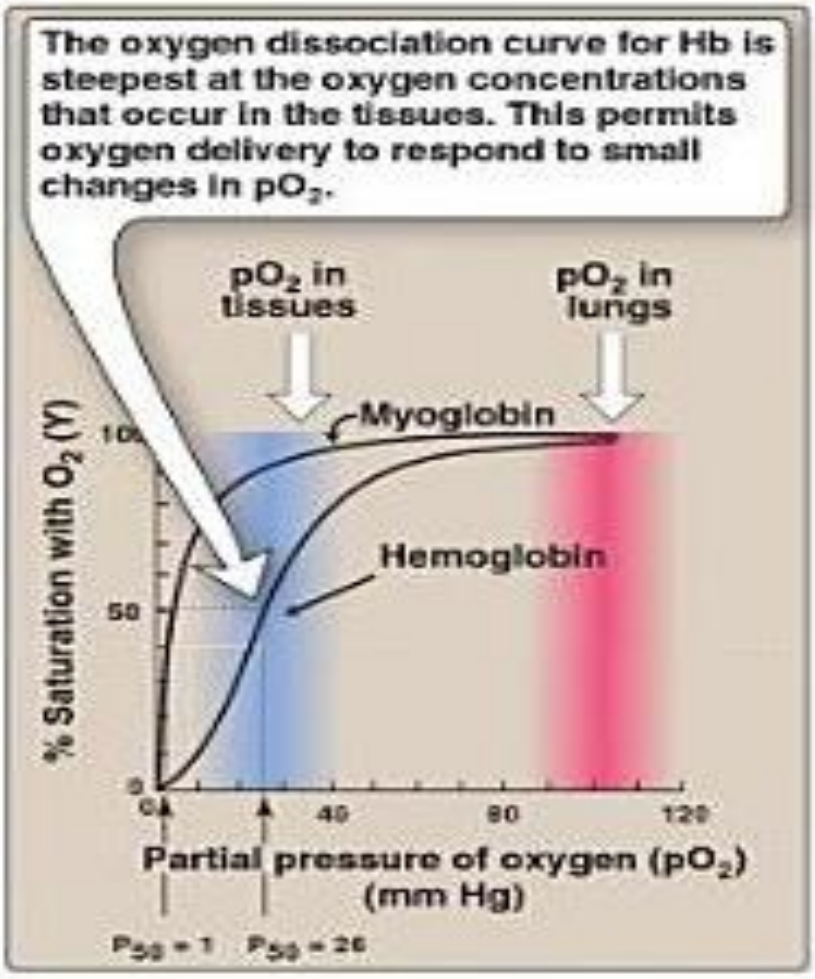


Figure 3.5
Oxygen dissociation curves for myoglobin and hemoglobin (Hb).

The degree of saturation of myoglobin vs hemoglobin (Cont.)

a) Myoglobin:

-The oxygen dissociation curve for myoglobin has a hyperbolic shape. This reflects the fact that myoglobin reversibly binds a single molecule of oxygen.

b) Hemoglobin:

-The oxygen dissociation curve for hemoglobin has sigmoidal shape, indicating that the subunits cooperate in binding oxygen.

- The cooperative binding of oxygen by the four subunits of hemoglobin means that:

  the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin molecule.

-This effect is referred to heme-heme interaction

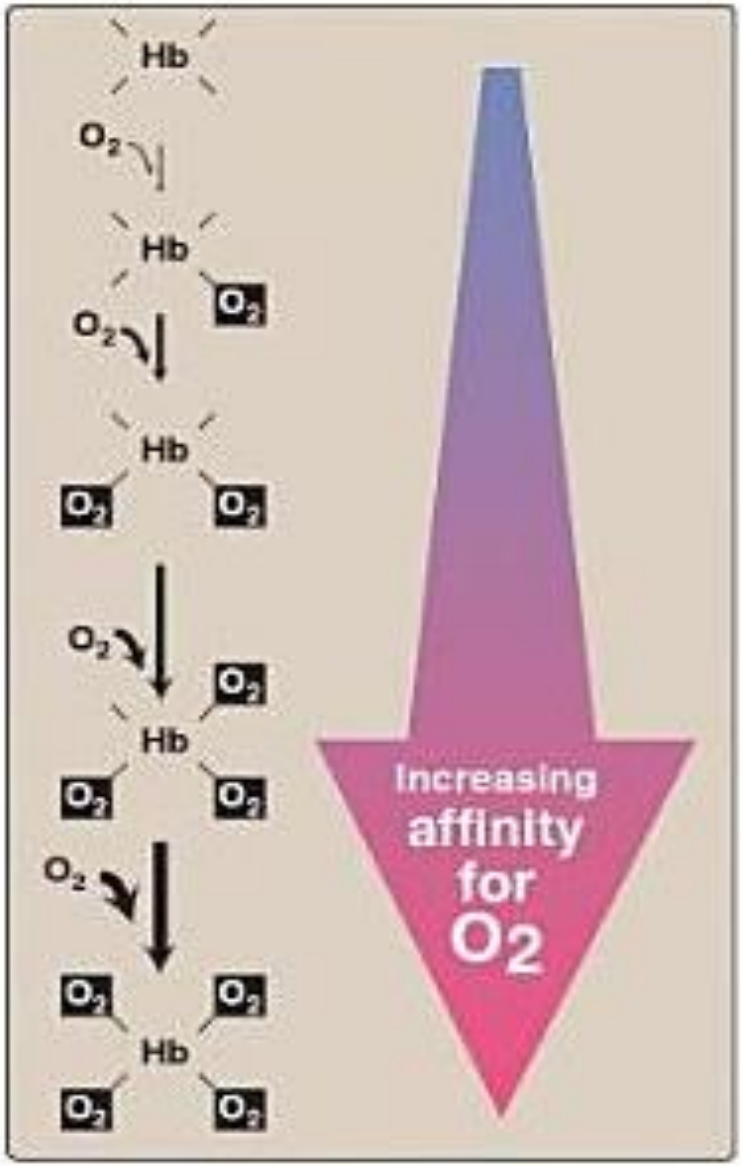


Figure 3.6
Hemoglobin (Hb) binds oxygen with increasing affinity.

MOTIFS (FOLDS)

- Specific arrangement of several secondary structure elements
  - all α helix
  - all β sheet
  - both
- Motifs can be found as recurring structures in numerous proteins.
- Globular proteins are composed of different motifs folded together.

Motifs (folds)

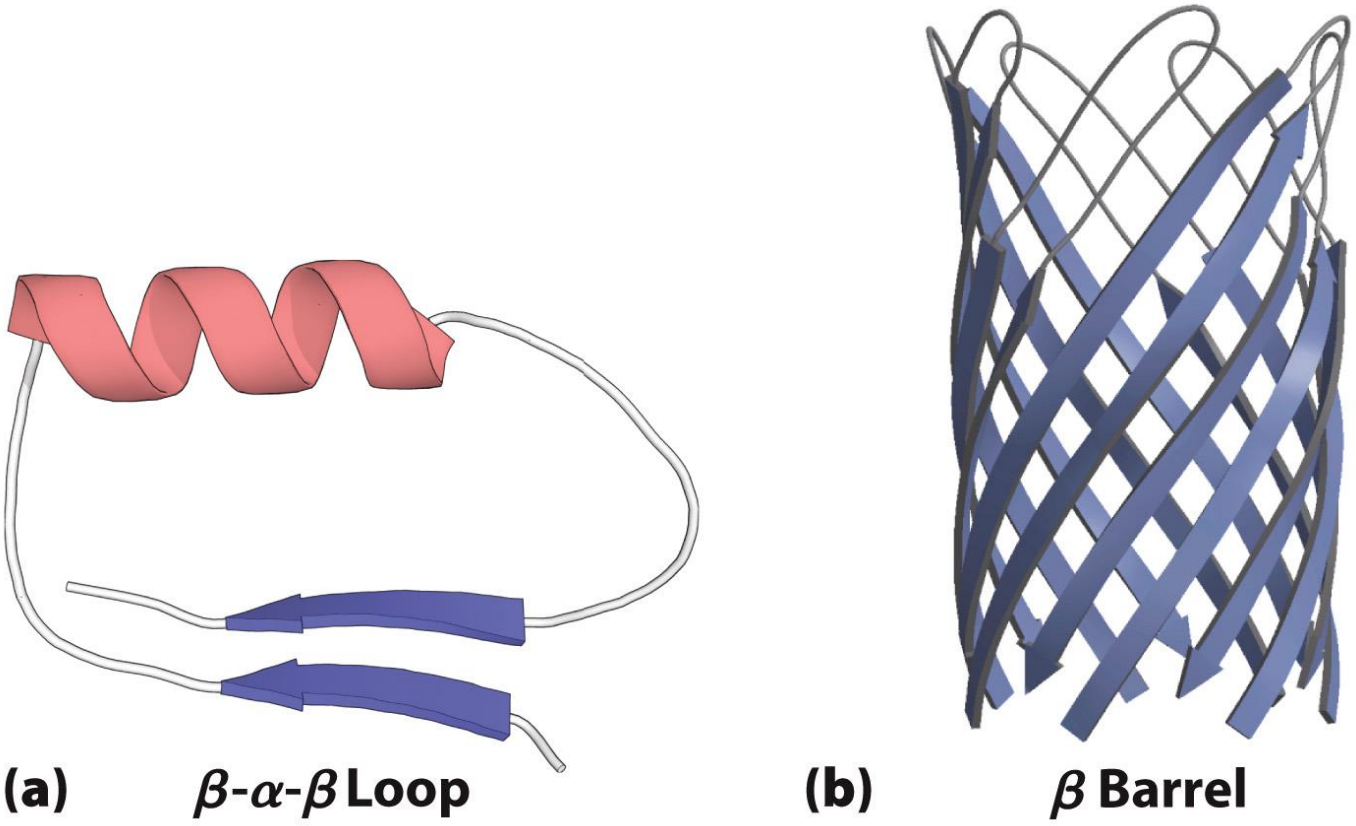


Figure 4-18
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(a) A simple motif, the β-α-β loop.
(b) A more elaborate motif, the β barrel. This β barrel is a single domain of α-hemolysin (a toxin that kills a cell by creating a hole in its membrane) from the bacterium *Staphylococcus aureus.*

Repeated Motifs Contribute to Final Fold

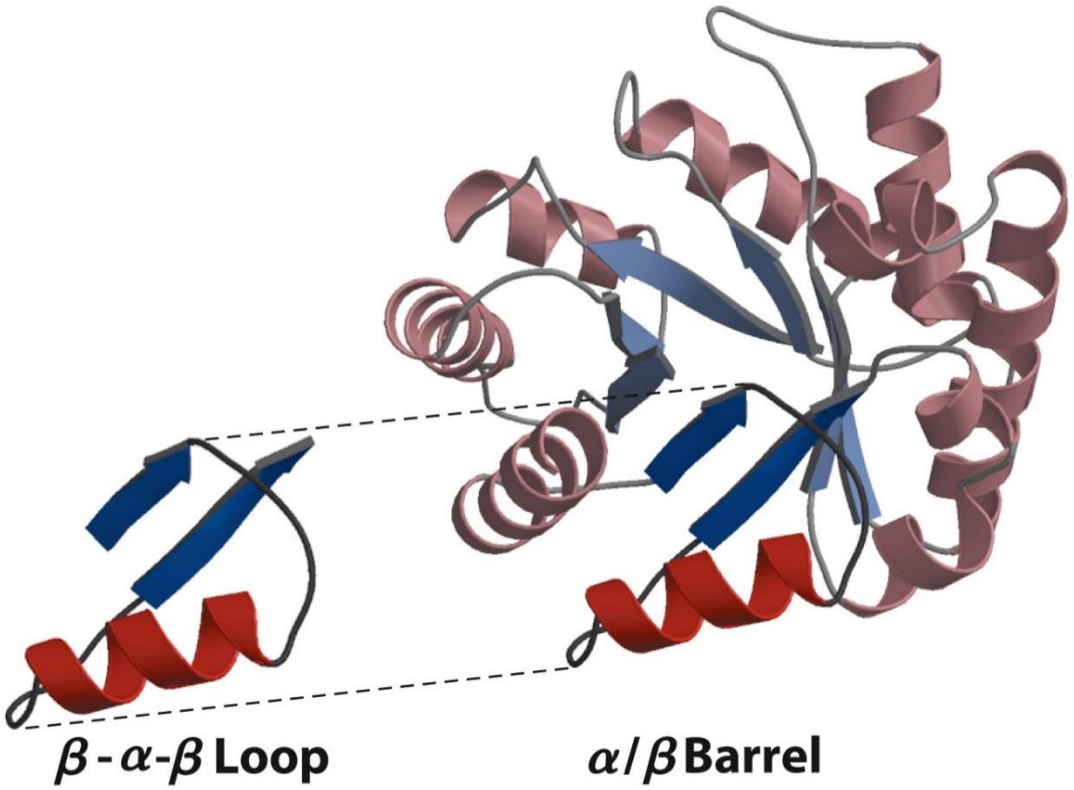

Figure 4-21
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Constructing large motifs from smaller ones.

The α/β barrel is a commonly occurring motif constructed from repetitions of the β-α-β loop motif.

This α/β barrel is a domain of pyruvate kinase (a glycolytic enzyme) from rabbit (derived from PDB ID 1PKN).

Intrinsically Disordered Proteins

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

Intrinsically Disordered Proteins (Cont.)

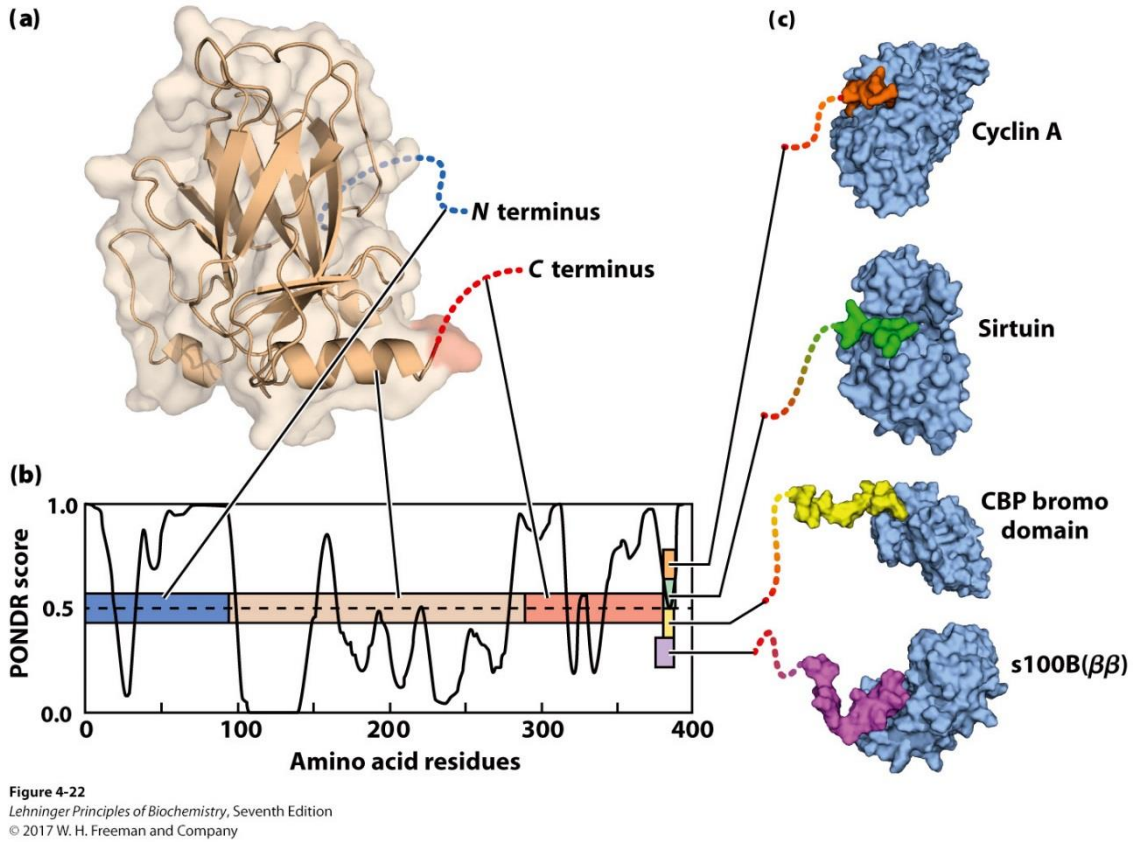

Figure 4-22
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FIGURE 4–22 Binding of the intrinsically disordered carboxyl terminus of p53 protein to its binding partners. (a) The p53 protein is made up of several different segments (PDB ID 1XQH). Only the central domain is well ordered. (b) The linear sequence of the p53 protein is depicted as a colored bar. The overlaid graph presents a plot of the PONDR (Predictor of Natural Disordered Regions) score versus the protein sequence.

Intrinsically Disordered Proteins (Cont.)

PONDR is one of the best available algorithms for predicting the likelihood that a given amino acid residue is in a region of intrinsic disorder, based on the surrounding amino acid sequence and amino acid composition.

A score of 1.0 indicates a probability of 100% that a protein will be disordered.

In the actual protein structure, the tan central domain is ordered.

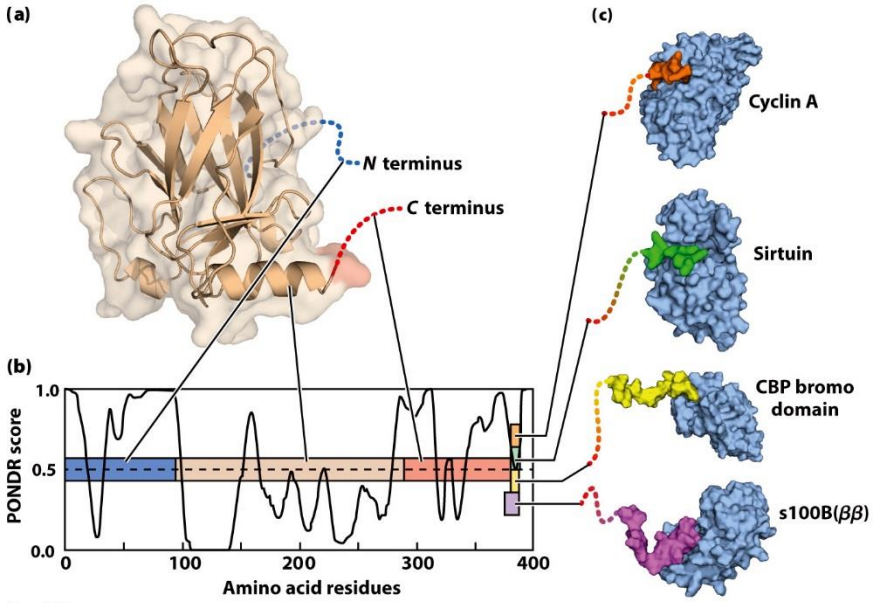

Figure 4-22
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The amino-terminal (blue) and carboxyl-terminal (red) regions are disordered. The very end of the carboxyl-terminal region has multiple binding partners and folds when it binds to each of them; however, the three-dimensional structure that is assumed when binding occurs is different for each of the interactions shown, and thus the color of this carboxyl-terminal segment (11 to 20 residues) is shown in a different color in each complex.

- Introduction to enzymes and metabolism

- Introduction to metabolism
- Overall Metabolic pathways for protein
- Urea cycle