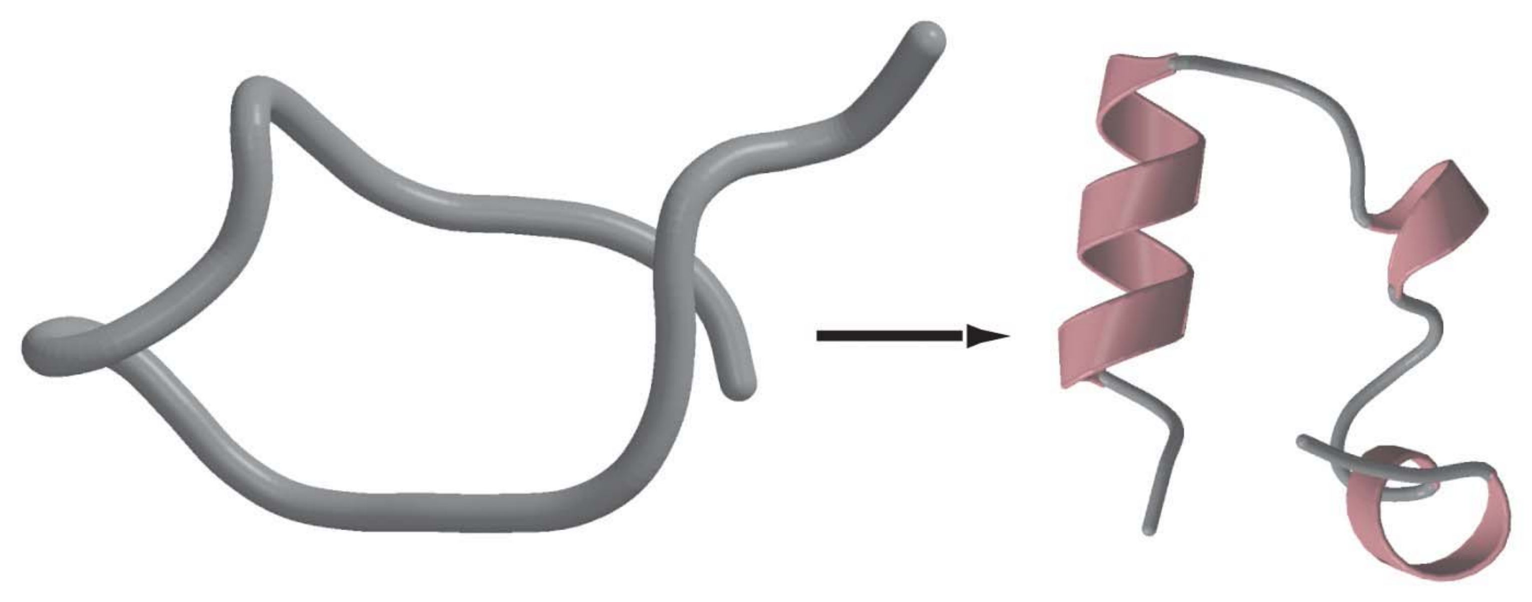
Chapter 4-1:

The Three-Dimensional Structure of Proteins



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http://neuron.pusan.ac.kr/

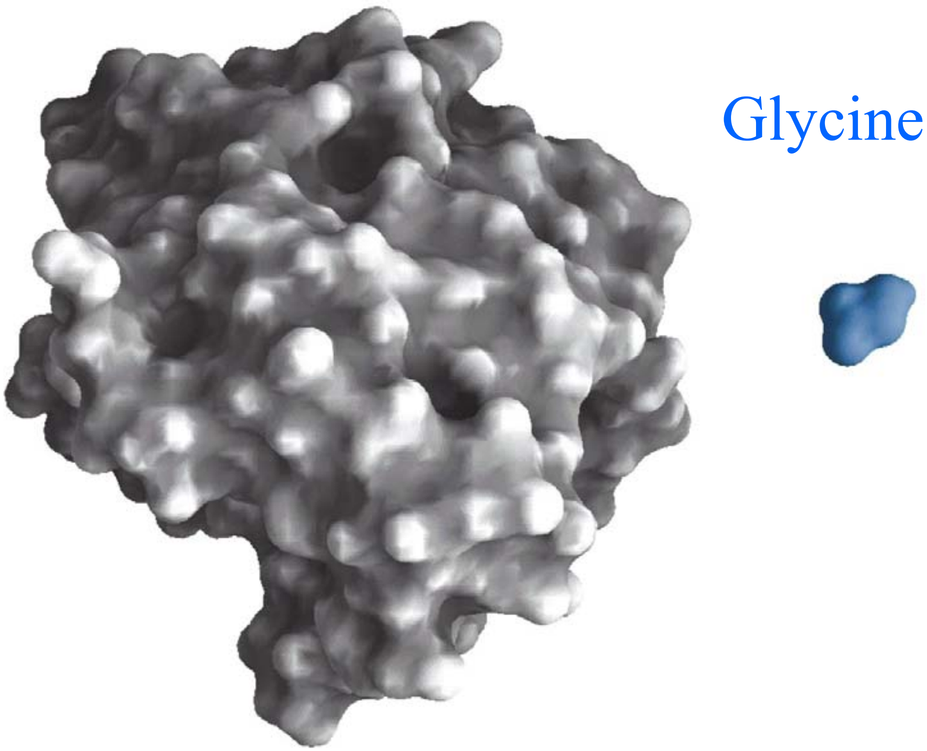
John Kendrew
article in Nature, 1958

- Perhaps, the more remarkable features of myoglobin are its complexity and its lack of symmetry. The arrangement seems to be almost totally lacking in the kind of regularities which one instinctively anticipates, and it is more complicated than has been predicted by any theory of protein structure.

The three dimensional structure of proteins

3D structure

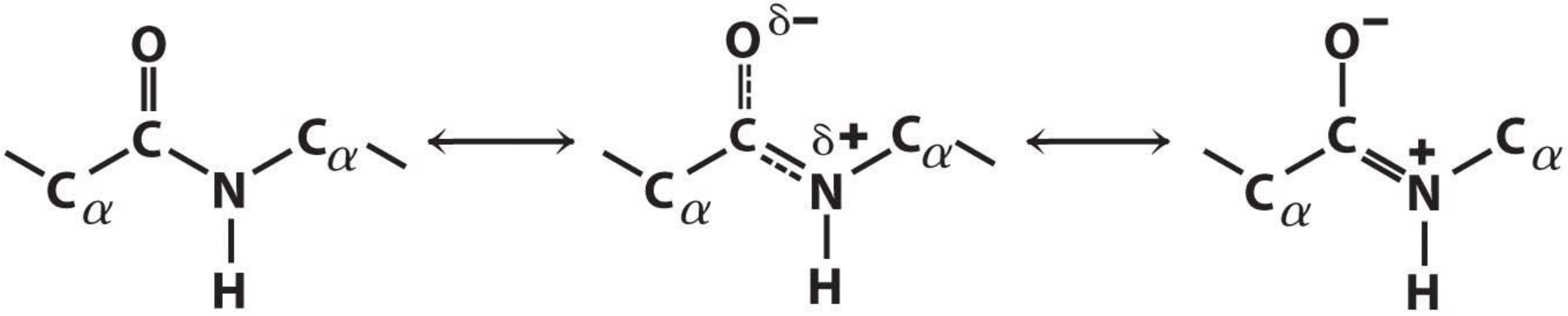
1. Determined by amino acids sequence
2. Function of a protein
3. Unique structure
4. Noncovalent linkage: stabilization
5. Complex but common pattern
   - Protein architecture



Protein Conformation

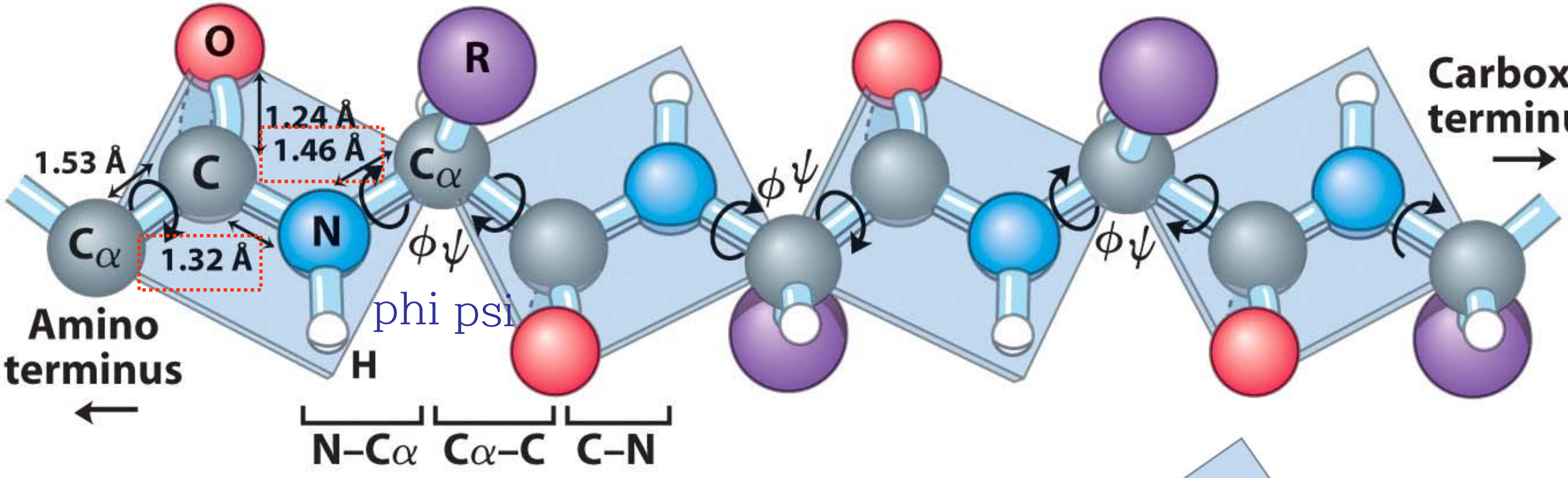
- Conformation: the spatial arrangement of atoms in a protein
- Gibbs free energy: thermodynamically the most stable
  - Having the lowest Gibbs free energy
- Native protein: proteins holding their functional, folded conformation
- Protein's conformation is stabilized **largely** by weak interactions
  - Hydrogen bond vs disulfide bond, weak and noncovalent interactions
  - So numerous weak interactions predominate to stabilize protein structure
  - Hydrophobic interactions are important in stabilizing a protein conformation
  - Solvation layer and entropy
- Structural pattern
  - Hydrophobic residues are buried in the protein interior
  - Numbers of H-bond within protein is maximized.

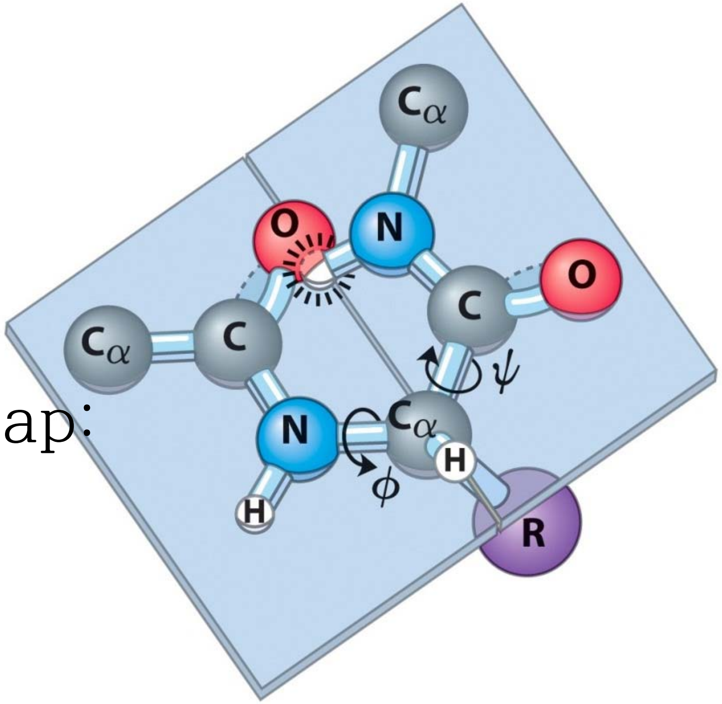
The peptide bond is rigid and planar



The carbonyl oxygen has a partial negative charge and the amide nitrogen a partial positive charge, setting up a small electric dipole. Virtually all peptide bonds in proteins occur in this trans configuration; an exception is noted in Figure 4–8b. proline

The planar peptide group





Prohibited by steric overlap:
Both ϕ and ψ are 0°

The atoms and planes defining ψ

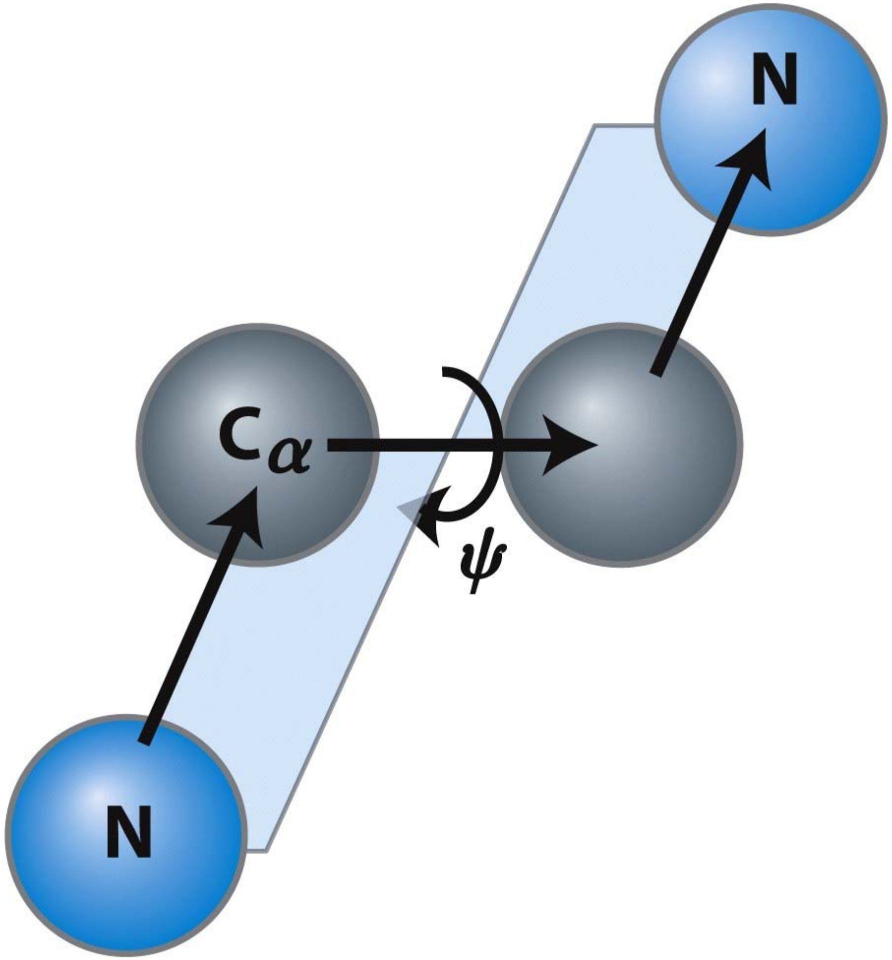


Figure 4-2c
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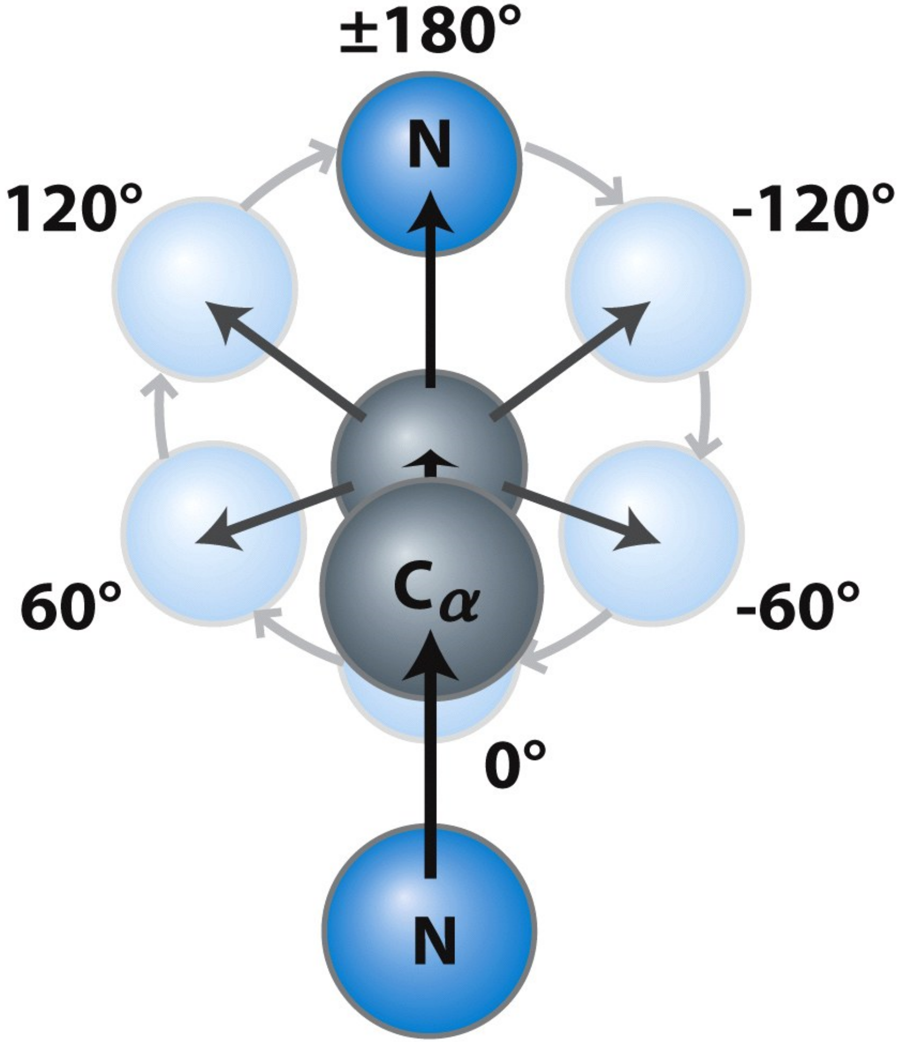

Figure 4-2d
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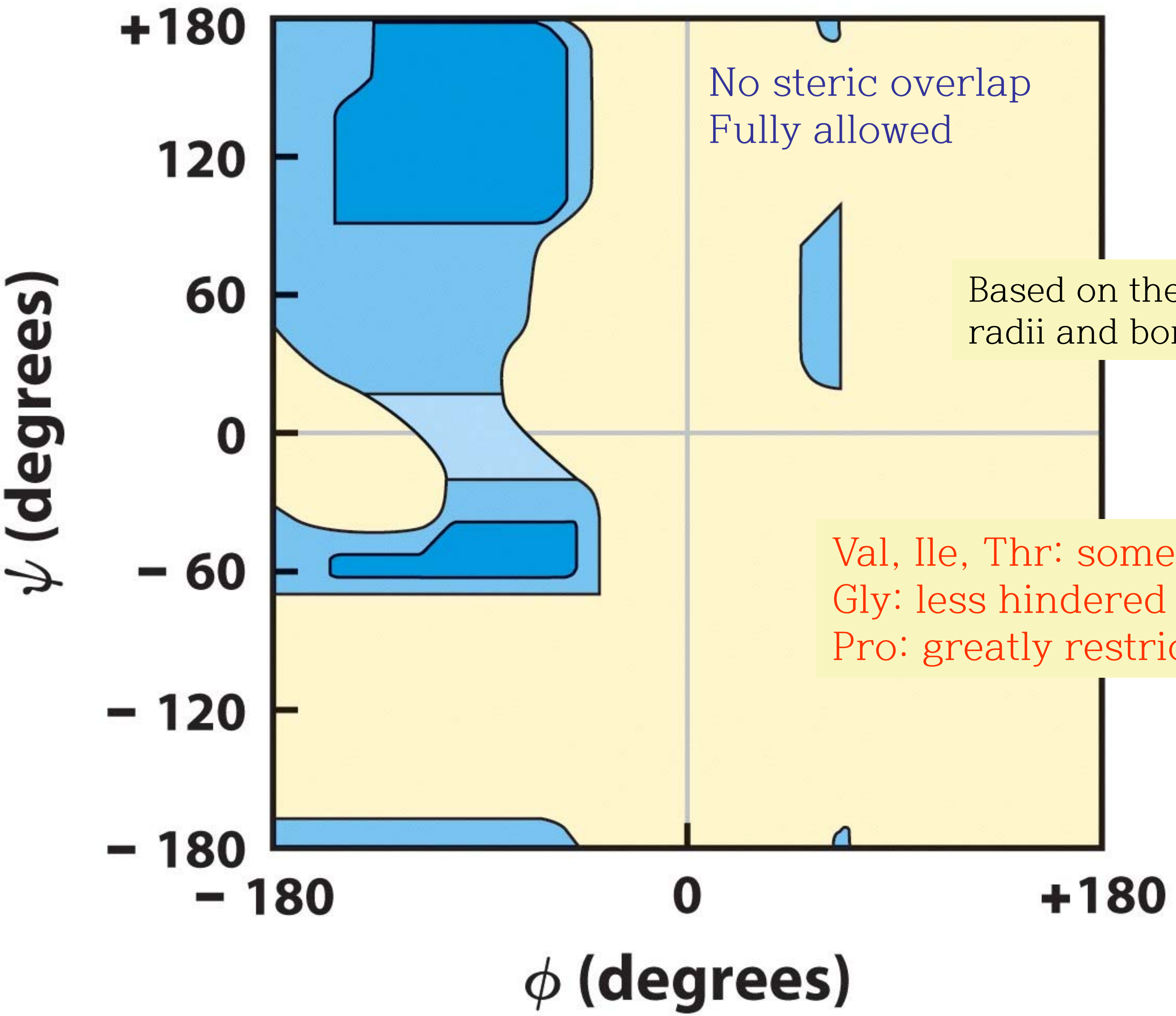
Ramachandran plot for L-Ala residue



Based on the van der Waals radii and bond angles

Val, Ile, Thr: somewhat smaller
Gly: less hindered
Pro: greatly restricted

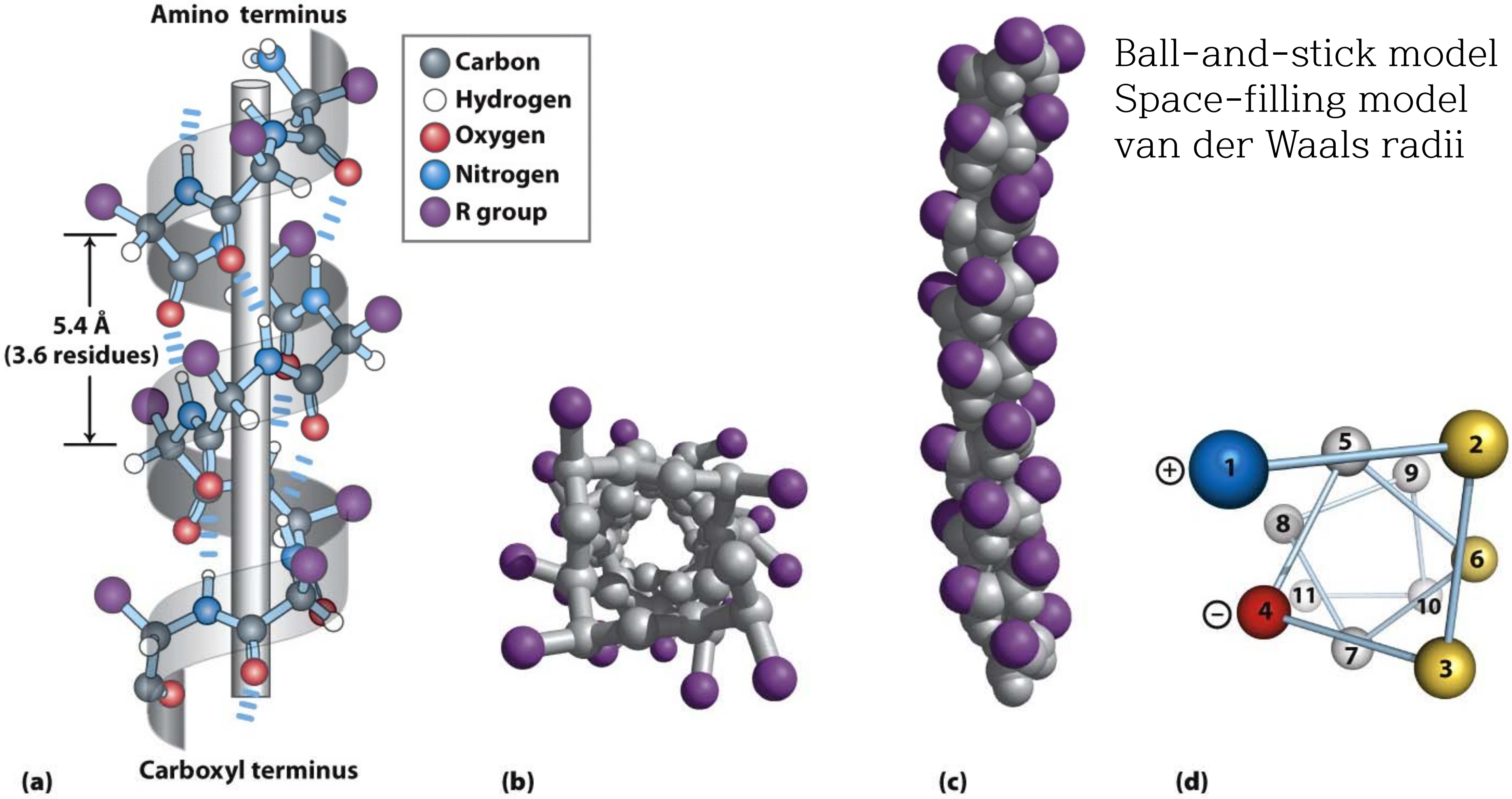
Protein secondary structure

- Local conformation of some part of a polypeptide
- α helix and β conformation
- Pauling and Corey predicted the structures in 1951
- 1st complete protein structure
    - 1959
    - Myoglobin
    - John Kendrew
    - Max Ferdinand Perutz
    - Nobel Prize for chemistry in 1962

α helix

- α helix: 5.4 Å, 3.6 residues/turn, helical structure
  - ψ = -45° to -50°, ϕ = -60°
  - Fibrous protein: predominant structure in α-keratin
  - ¼ of all amino acids are found in α helices
  - Hydrogen bond: between C=O and N-H groups of peptide bond
- Optimal use of internal H-bonds
- One stereoisomeric series: L- or D-amino acids
- Extended left-handed helices have not been observed in proteins.

Right-handed α helix



Ball-and-stick model
Space-filling model
van der Waals radii

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

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

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

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

Table 4-1
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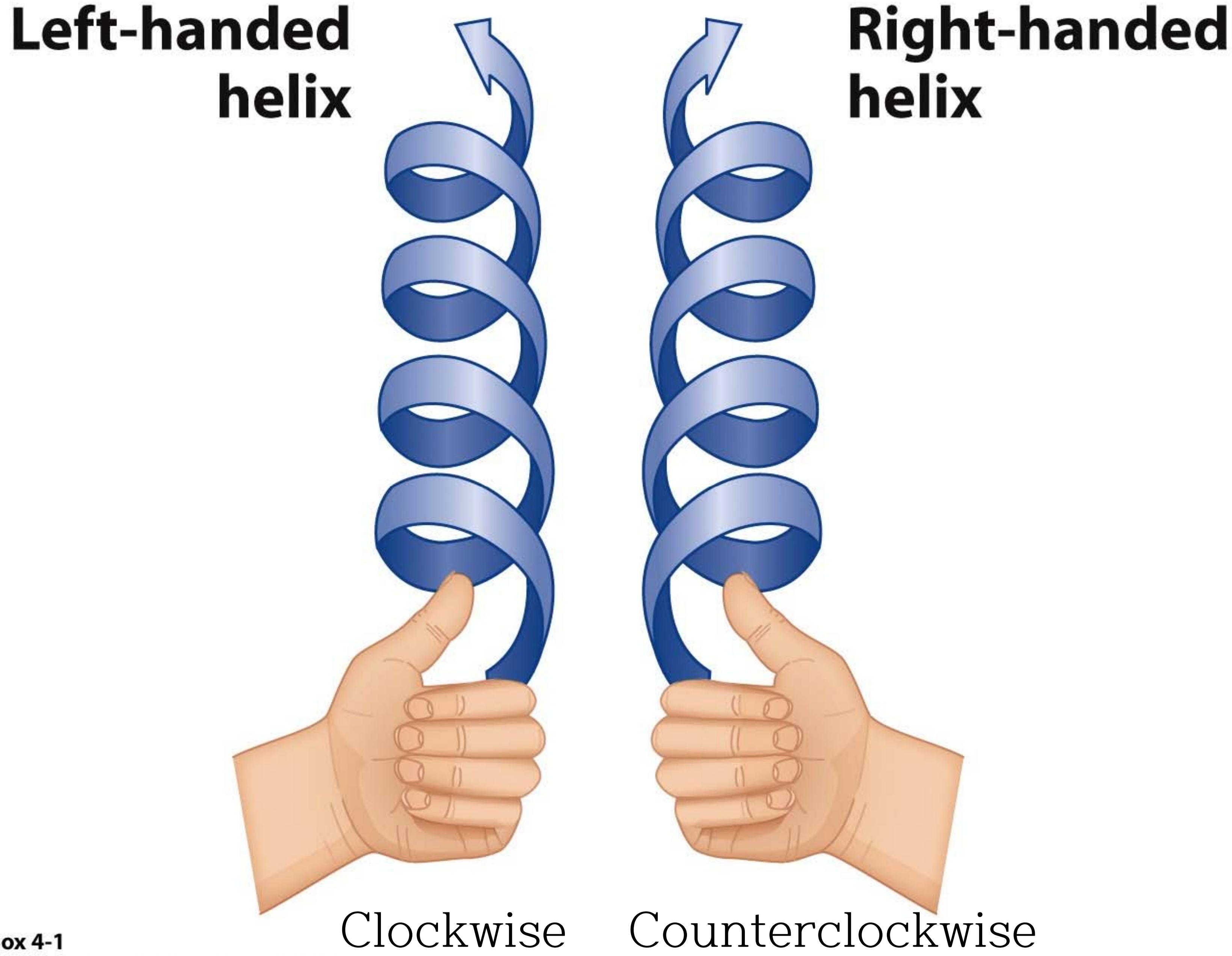

Box 4-1

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α helix stability

- Side chains can stabilize or destabilize the structure
  - Electrostatic repulsion (Glu / Lys, Arg)/attraction at pH 7.0
  - Bulk and shape: Asn, Ser, Thr, Cys
- Interactions between amino acid side chains spaced 3-4 residues apart
  - ion pair, hydrophobic interaction
- Constraint (Occurrence of Pro and Gly residues)
  - Pro: destabilizing kink, no H-bond (rarely found within α helix) Gly: more conformational flexibility (coiled structure)
- Interaction btw aa residues at the end of the helical segment and the electric dipole inherent to the helix
  - Use of internal H-bonds
  - Positively charged amino acids at the amino-terminal end
    - Stabilizing? destabilizing?

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

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

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

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

Table 4-2
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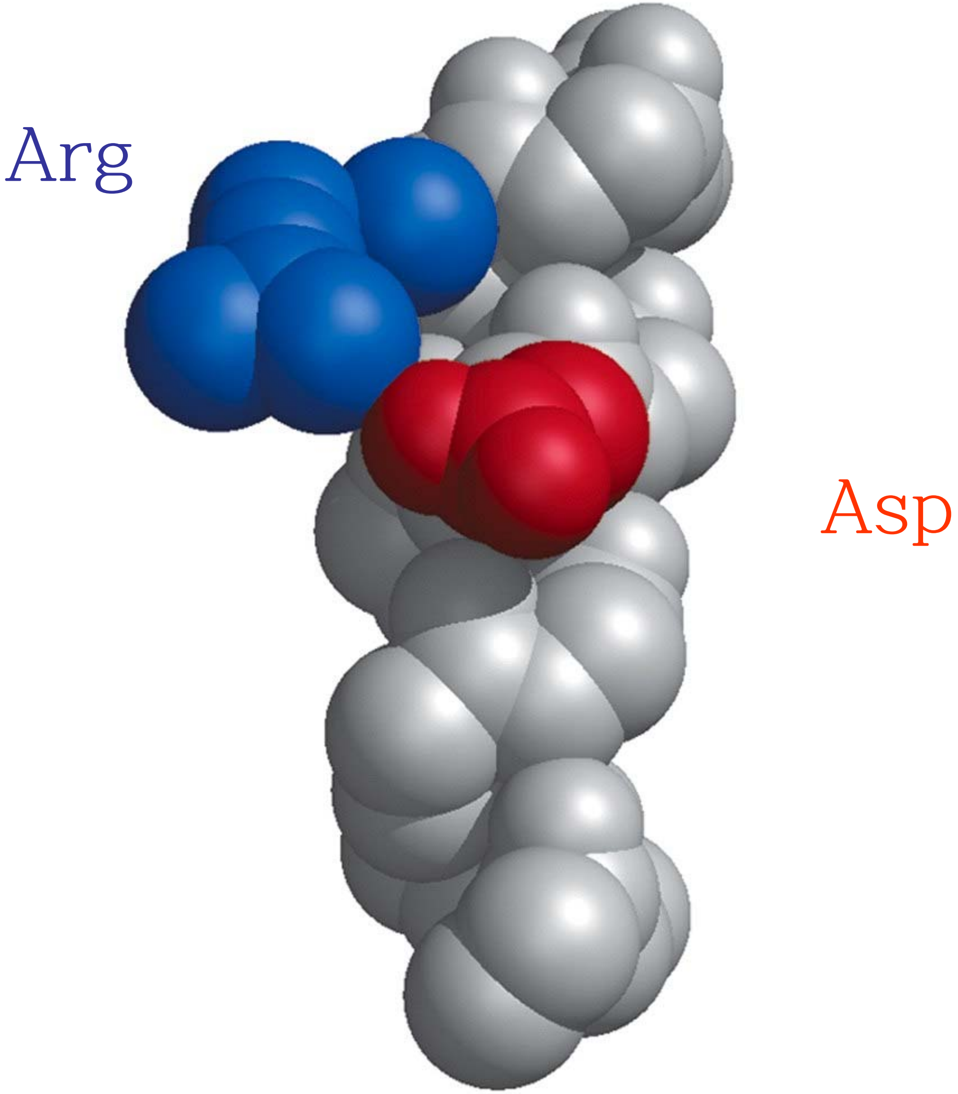
Interactions between R groups of amino acids three residue apart in an α helix



Helix dipole

Negatively charged amino acids

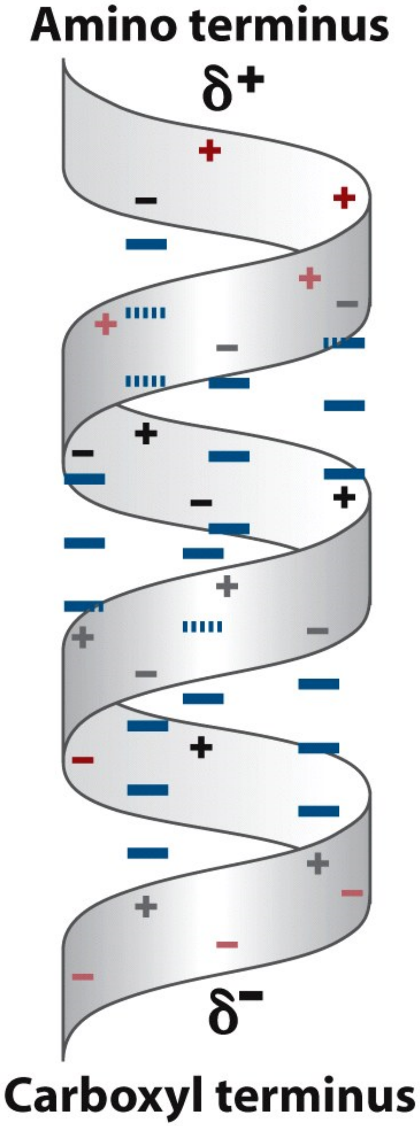


Figure 4-5
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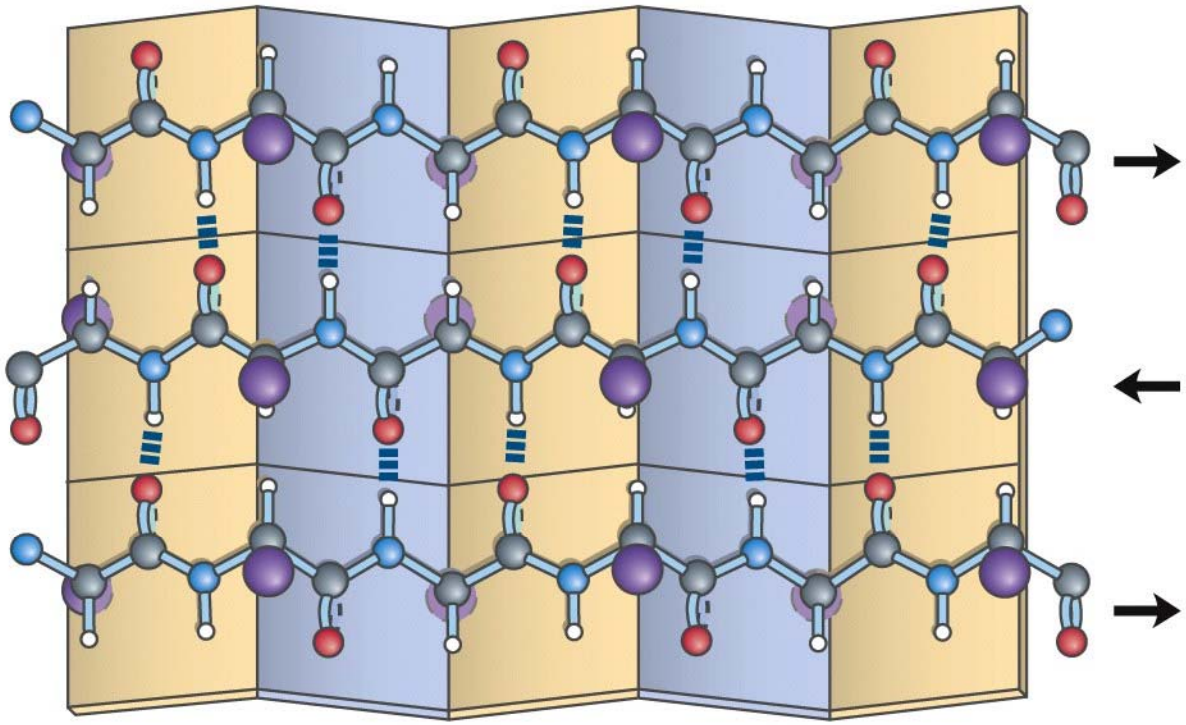
Positively charged amino acids

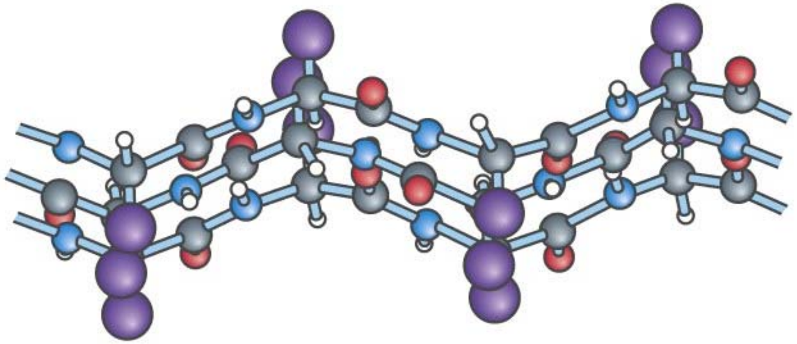
The β conformation organizes polypeptide chains into sheets.

- β conformation (fibroin, β-Keratin)
  - Second type of repetitive structure
  - More extended conformation
  - Zigzag
- β sheet: a series of pleats
- Hydrogen bond between adjacent segments of polypeptide chains
- Parallel (0.65 nm) and anti-parallel (0.7 nm) conformation
  - Similar but different in the repeat period and H-bonding pattern
- Some protein structures limit the kinds of amino acids
  - Gly and Ala (β-Keratin: silk fibroin, spider webs)

Antiparallel



Top view



Side view

Figure 4-6a

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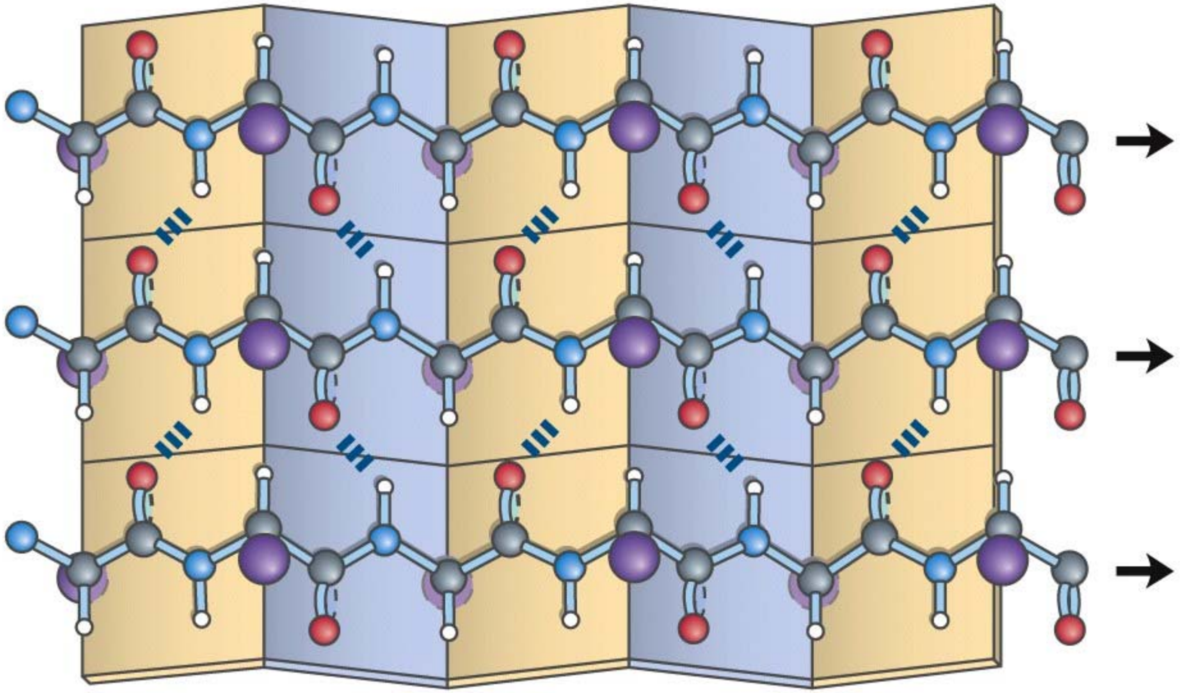
Parallel

Top view



Side view

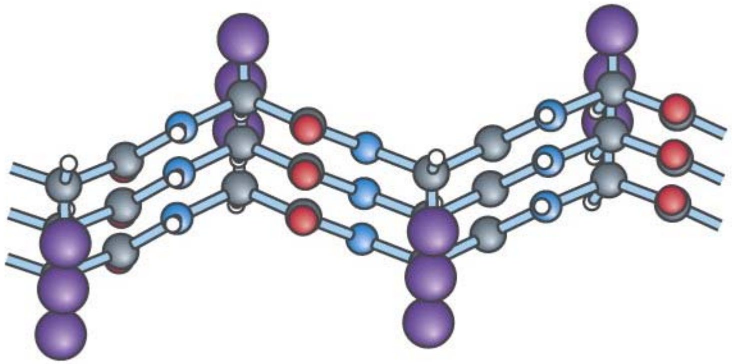


Figure 4-6b
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β turns (beta bend)

- In globular proteins
  - 1/3 are in turns or loops: reverses direction
- Link successive runs of α helix and β conformation
  - Reverse direction,180º turn: 4 amino acid residues
  - Hydrogen bond: 1st and 4th
  - Gly: small and flexible
  - Pro: imino nitrogen (cis configuration: amenable to a tight turn
- γ turn: less common, 3 residue turn
  - H-bond: 1st and 3rd amino acids

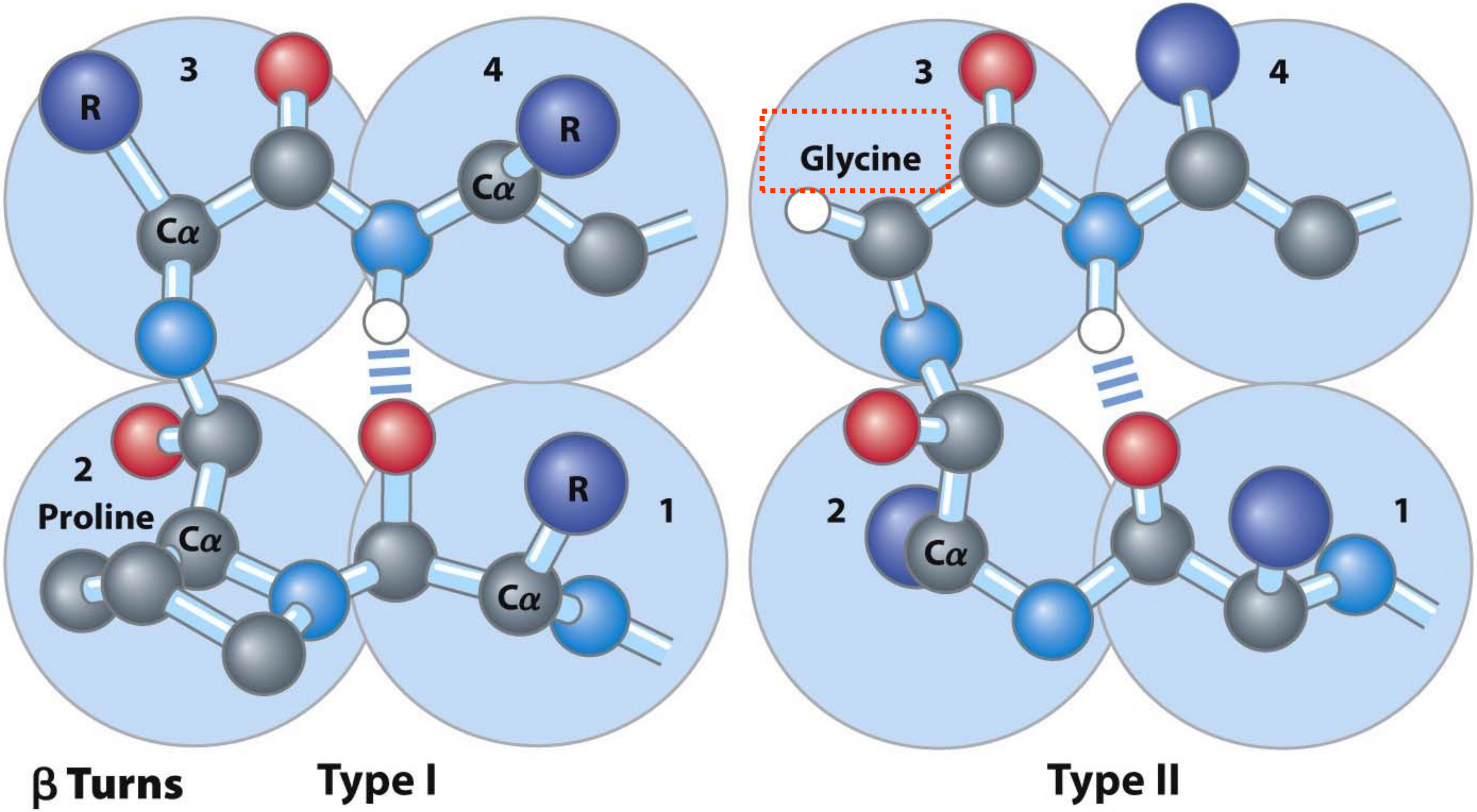

Figure 4-7a

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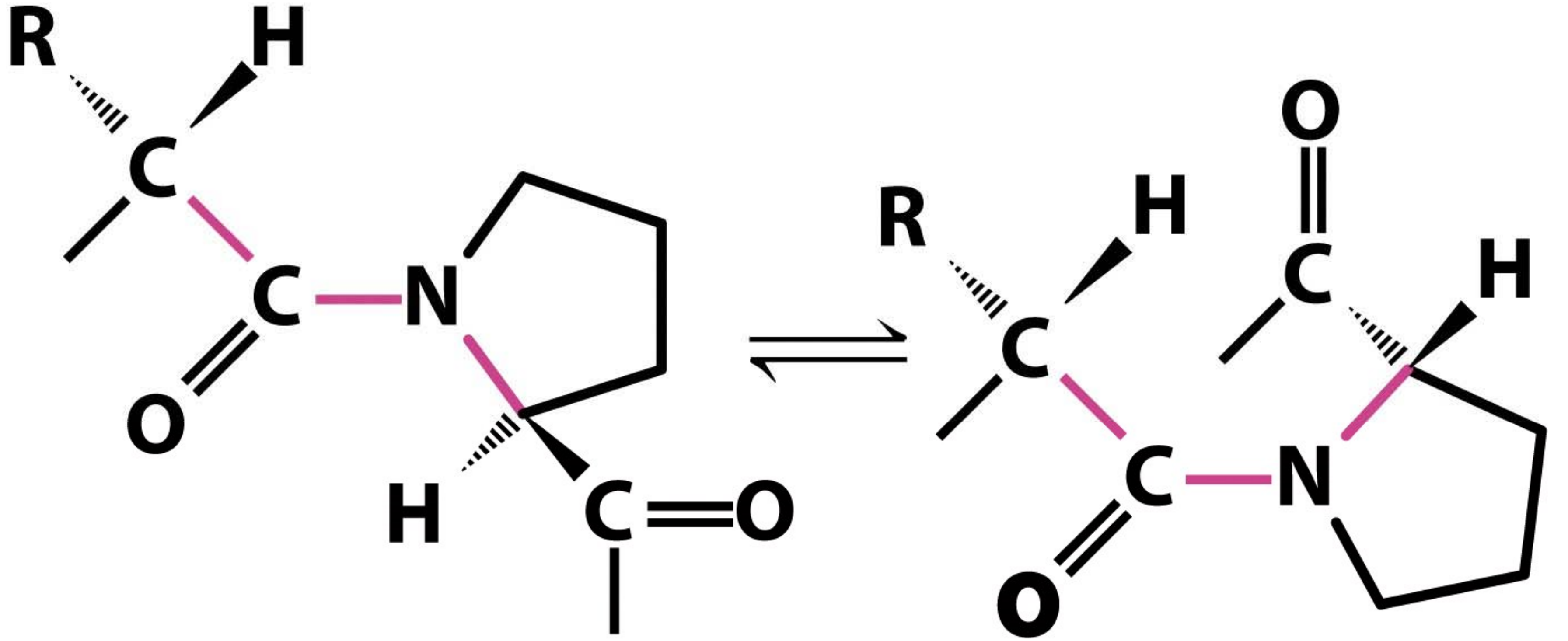
Proline isomer



trans **cis**

Figure 4-7b
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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.

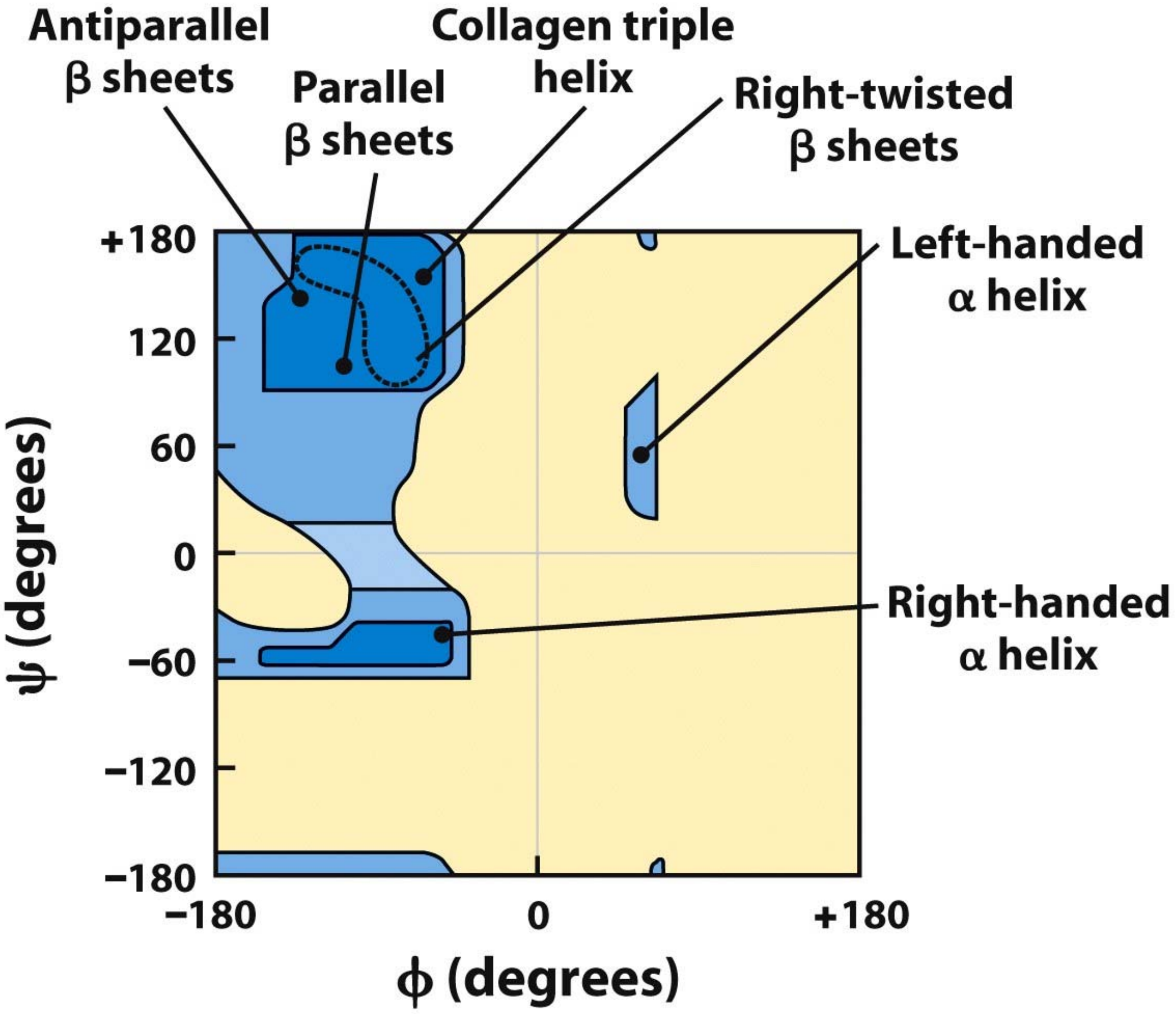

Figure 4-8a

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(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.

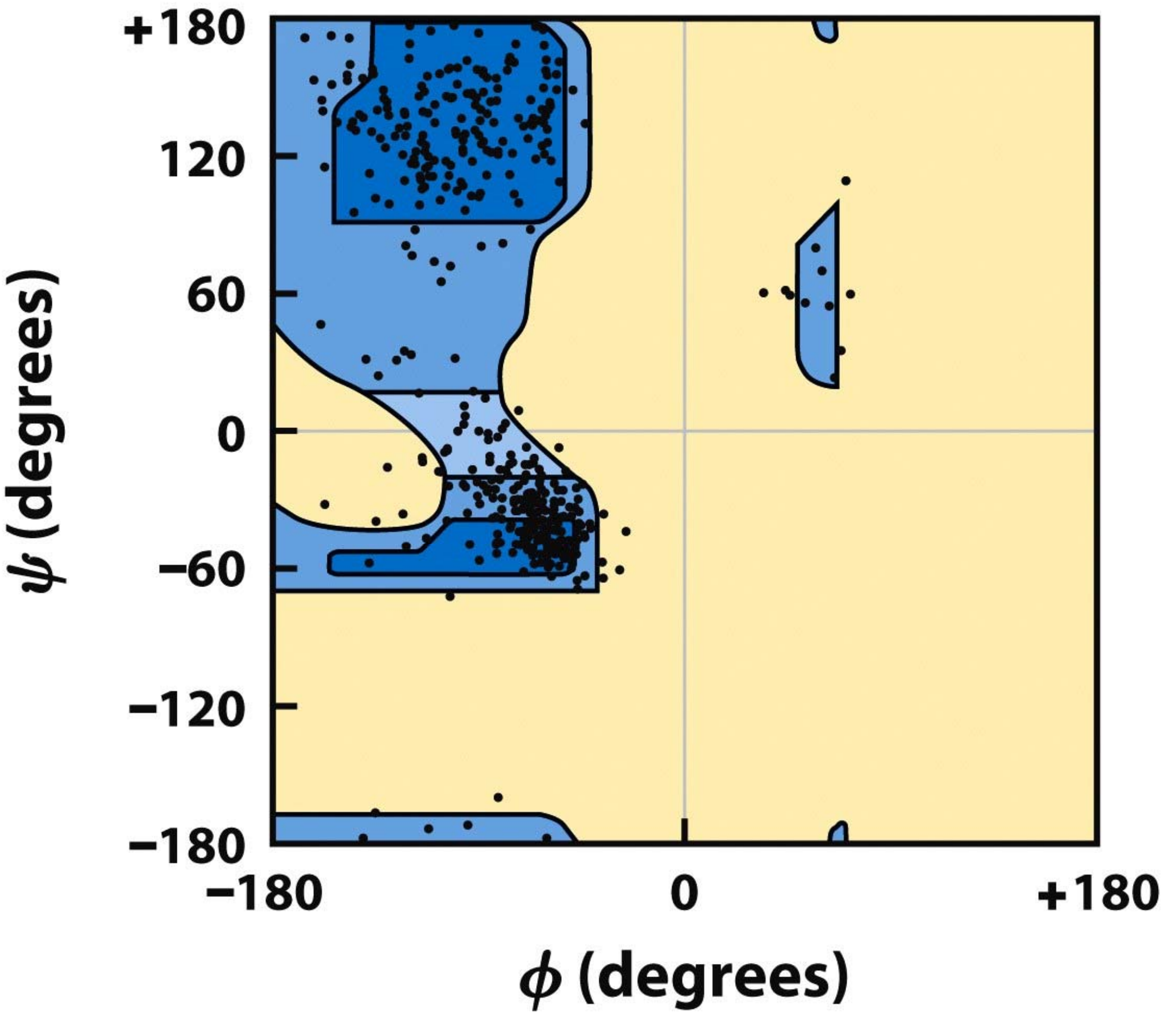

Figure 4-8b
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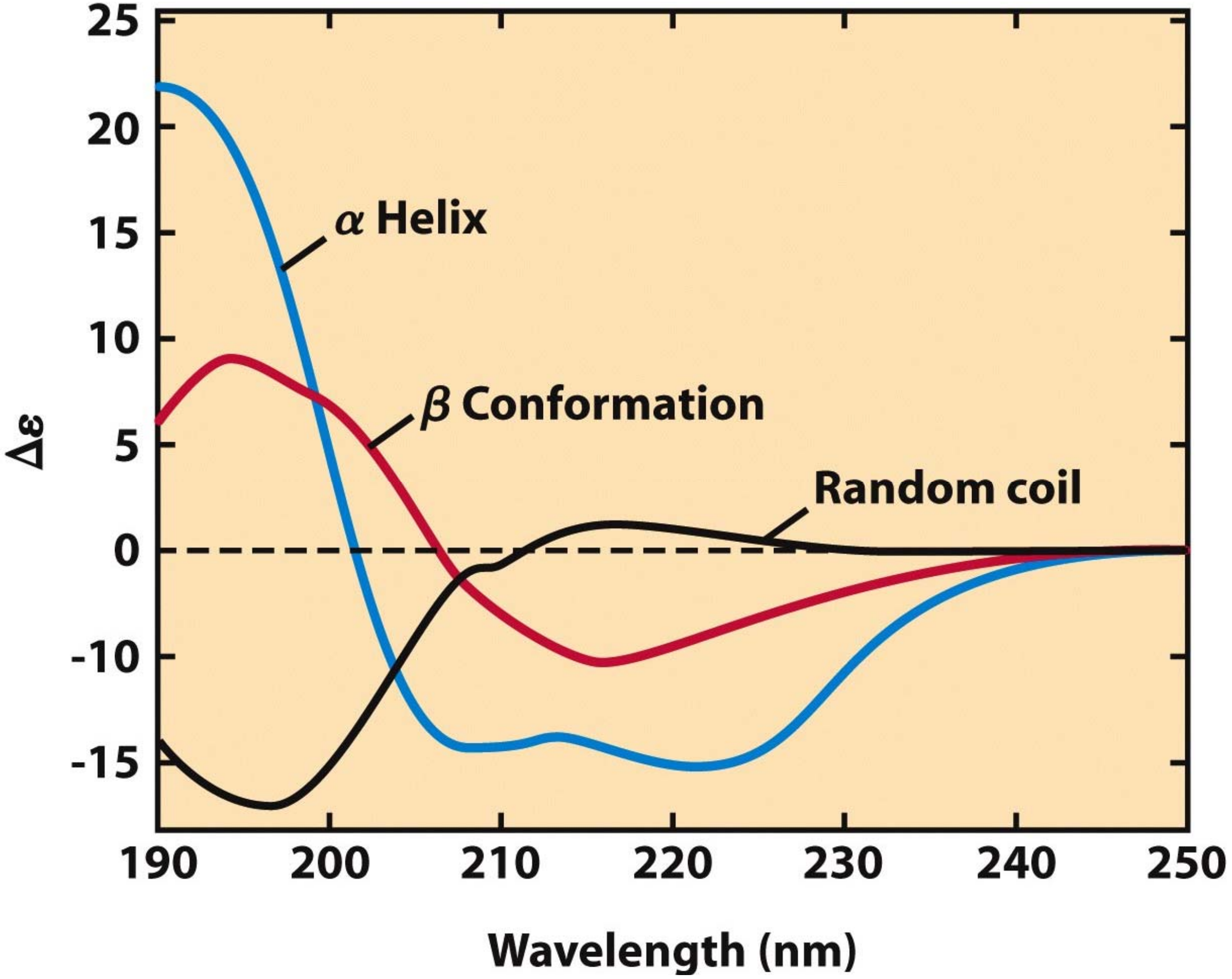
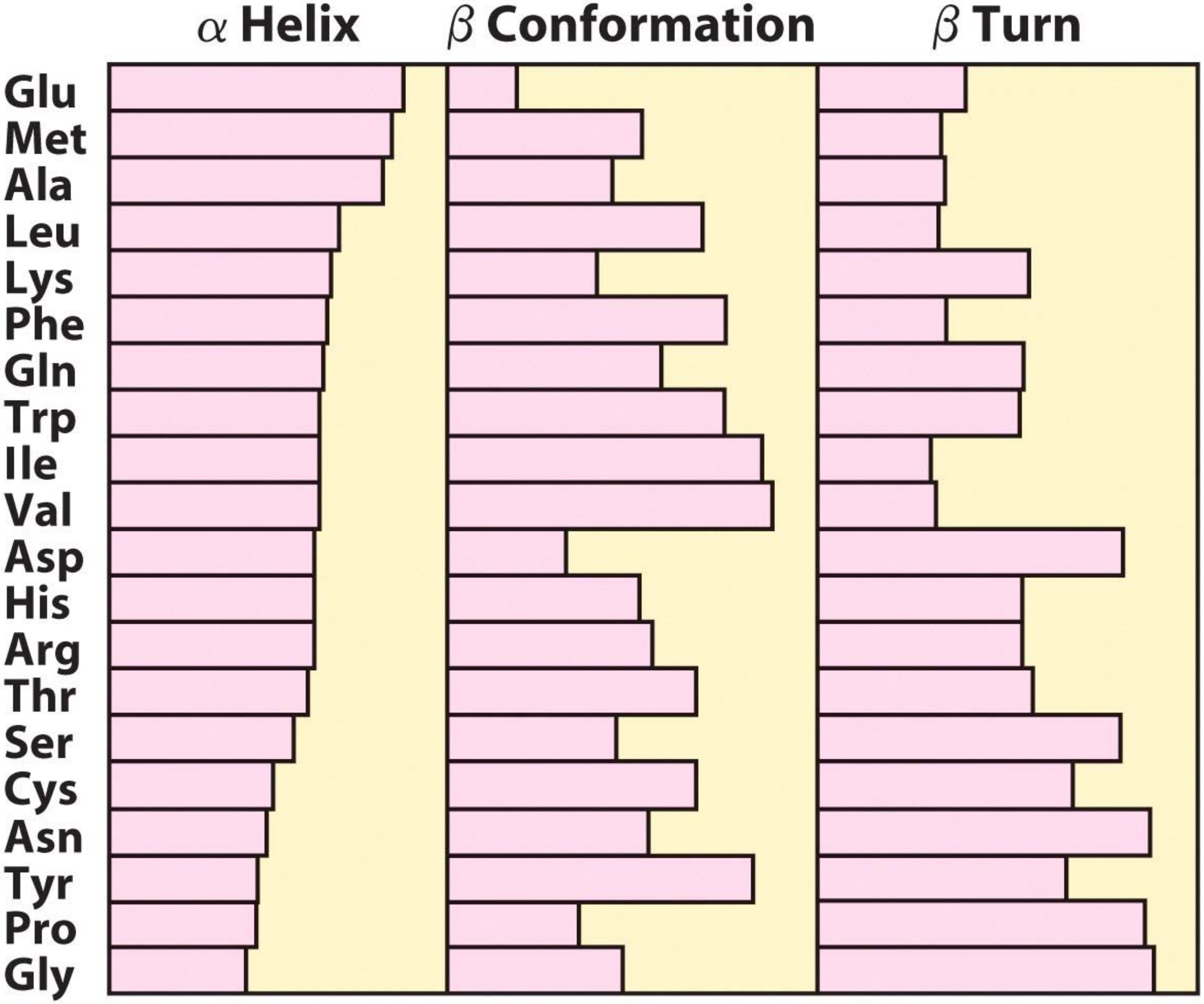
Circular dichroism spectroscopy. These spectra show polylysine entirely as α helix, as β conformation, or as a denatured, random coil. The y axis unit is a simplified version of the units most commonly used in CD experiments. Since the curves are different for α helix, β conformation, and random coil, the CD spectrum for a given protein can provide a rough estimate for the fraction of the protein made up of the two most common secondary structures. The CD spectrum of the native protein can serve as a benchmark for the folded state, useful for monitoring denaturation or conformational changes brought about by changes in solution conditions.



Relative probabilities



Protein tertiary and quanternary structures

- Secondary structure
  - Spatial arrangement of amino acid residues
- Tertiary structure
  - Overall 3-dimensional arrangement of all atoms in a protein
  - Longer-range aspects of Amino Acids sequence
- Quanternary structure
  - 2 or more separate polypeptide chains or subunits in 3-dimensional complexes

Protein classification

- Fibrous proteins
  - Polypeptide chains arranged in long strands or sheets
  - Single type of secondary structure
  - Support, shape, external protection
  - α-keratin,collagen, fibroin, elastin
  - Insoluble in water (Why?)
- Globular Proteins
  - Polypeptide chains folded into a spherical or globular shape
  - Several types of secondary structure
  - Different segments of a polypeptide chain (or multiple polypeptide chains)
  - Biological function
  - Enzymes, transport proteins, motor proteins, regulatory proteins, immunoglobulins…

Protein classification

- Fibrous proteins
  - 동식물의 해부학 생리학상 매우 중요한 기능
  - 몸 전체 단백질의 1/3 (몸 전체의 1/6)
  - 피부 (각질층), 머리카락, 깃털, 비늘, 뿔 등 몸의 외면을 보호
  - Tendon, cartilage, 뼈, 피부의 내층 등의 결합조직의 주요 성분
  - protein의 native conformation은 매우 안정하다.
- Globular proteins
  - 복잡 다양한 생물학적 기능
  - 작용이 Dynamic (Enzymes, transport proteins, motor proteins, regulatory proteins, immunoglobulins)

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

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

Table 4-3
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Fibrous proteins

- A. keratin
  - α-keratin: 척추동물의 외면을 보호
    - 머리카락, 양모, 깃털, 손톱, 비늘, 뿔, 거북이 껍질, 피부의 외층
    - Evolved for strength
    - Intermediate filament proteins
    - α-helix의 polypeptide: right-handed
    - Supertwisted coiled coil: rope, left-handed supertwist
    - Rich in Ala, Val, Leu, Ile, Met, Phe
    - Cysteines residue 다량 함유 (왜?): rhino horn (18%)
    - Supercoiled structure (머리카락)
    - 불용성 (왜?) compared to globular protein
    - 표피에서 유래되었음

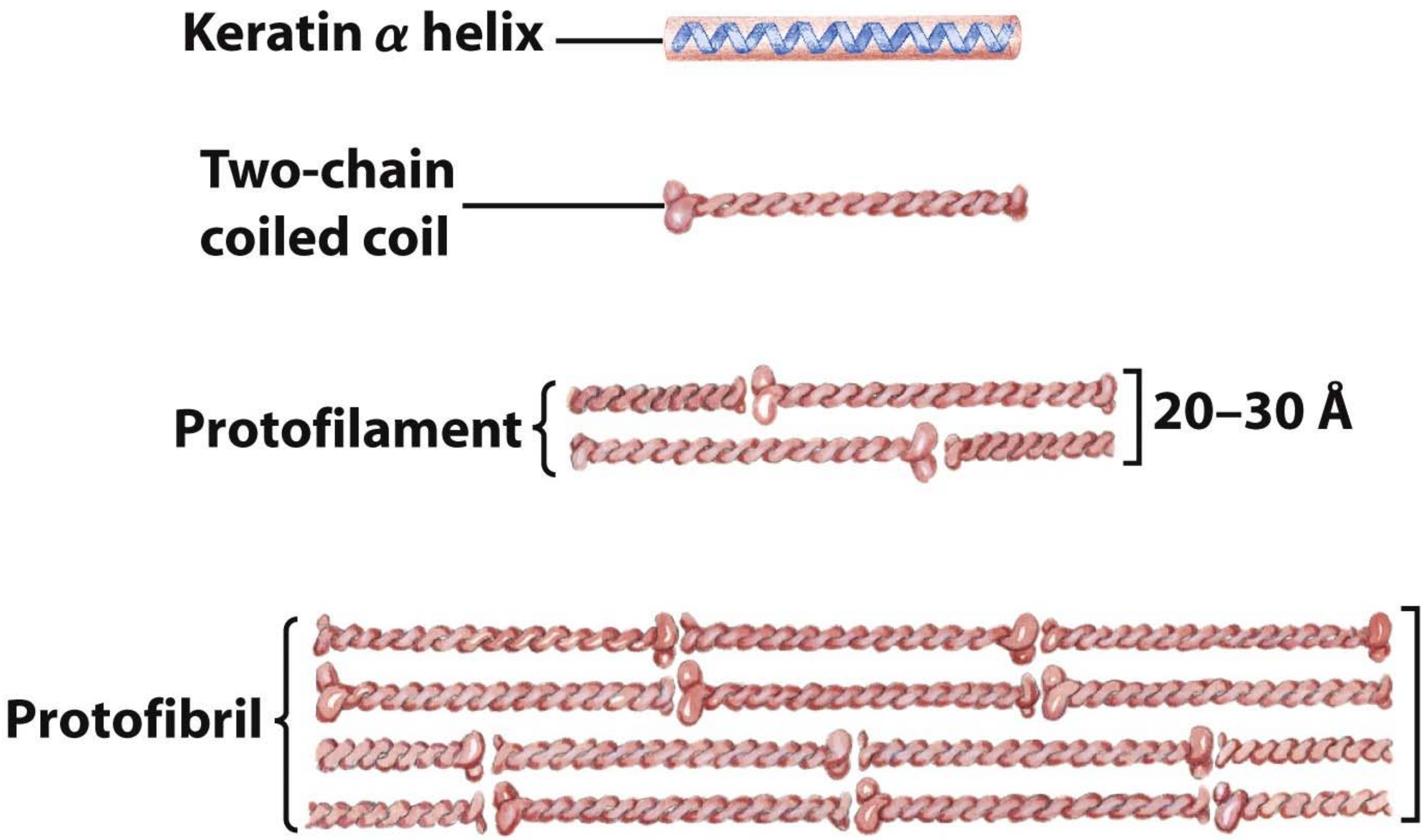

Figure 4-10a
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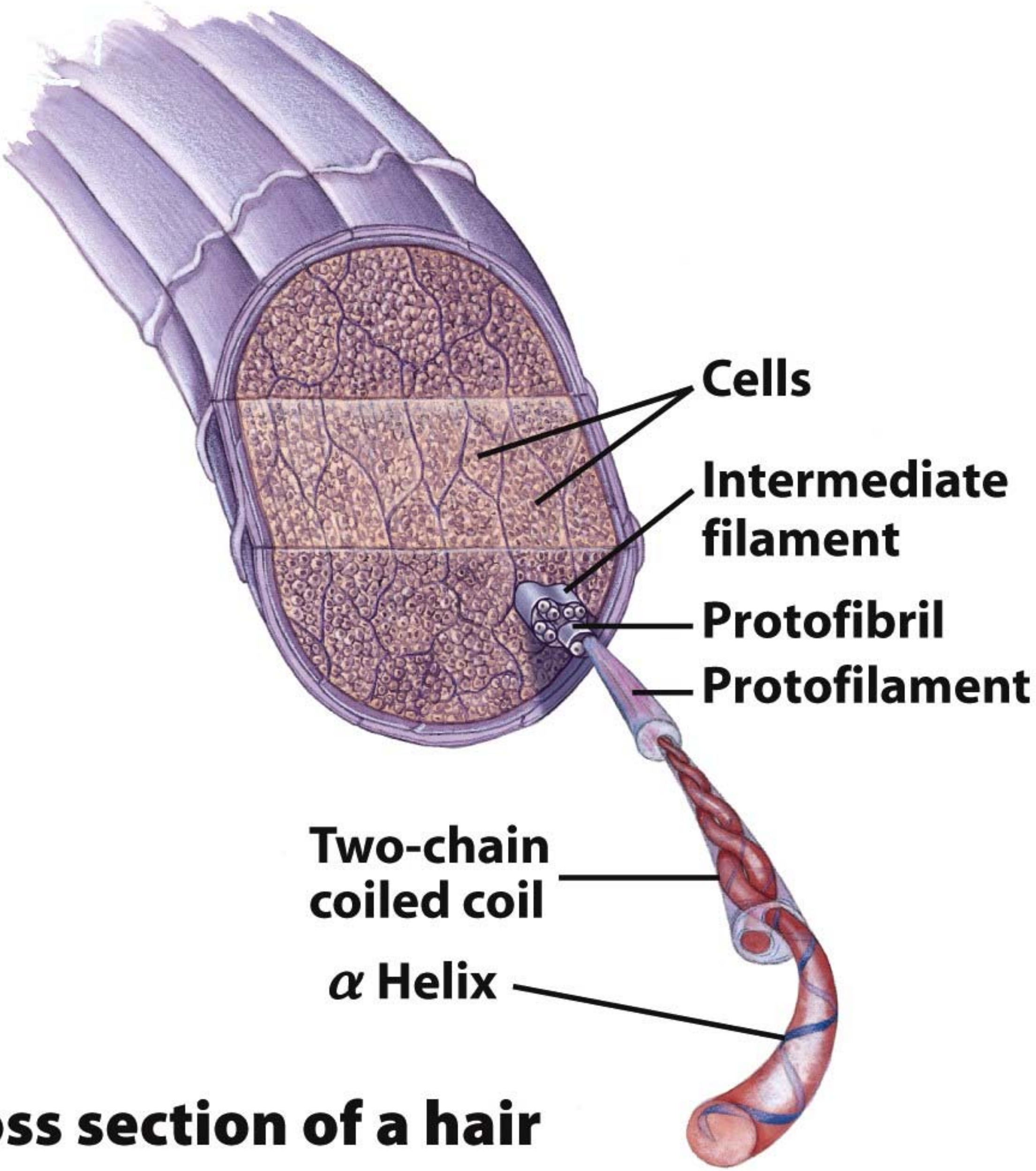

Cross section of a hair

Figure 4-10b
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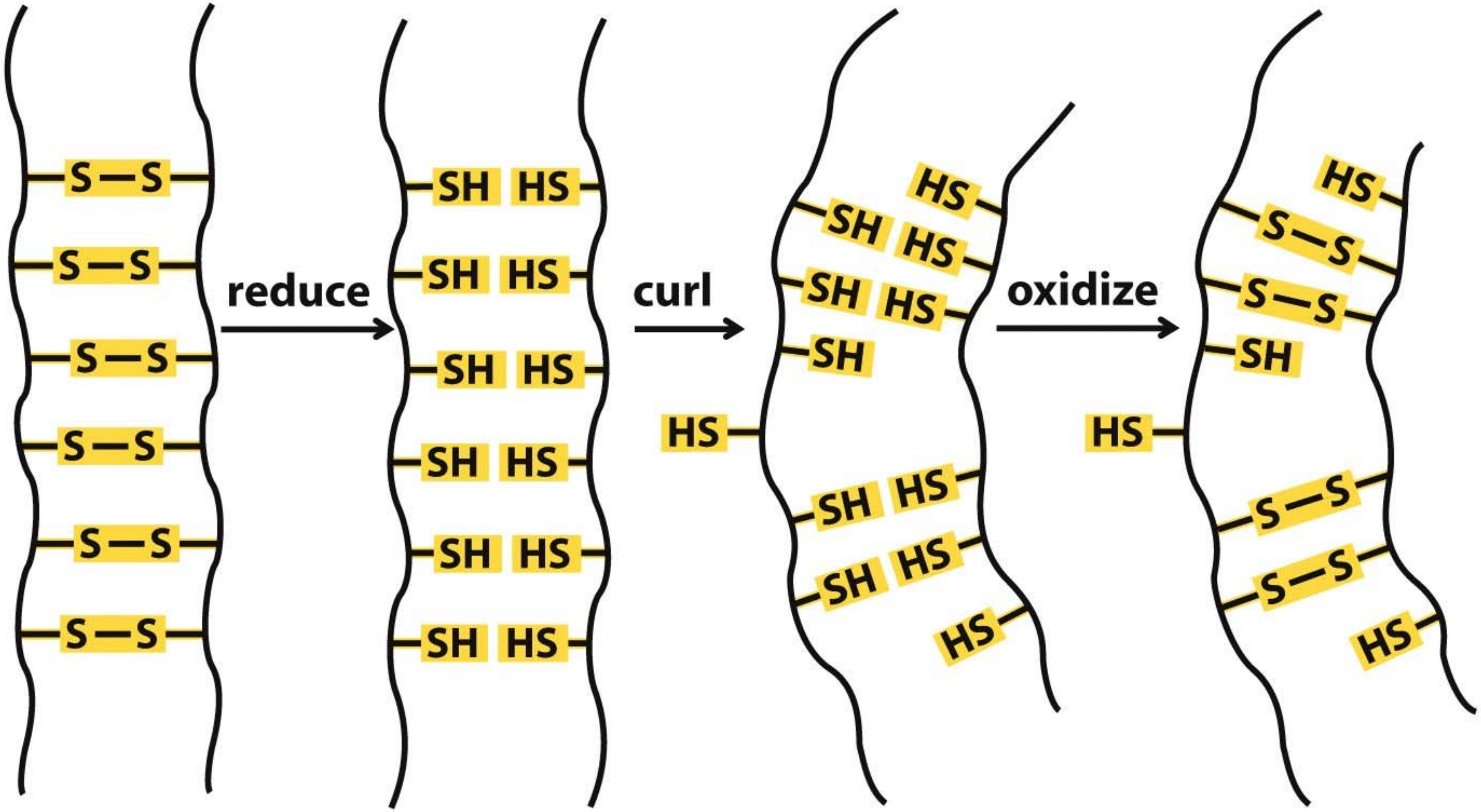
Stretchability and disulfide linkages: Basis of permanent waving



Box 4-2
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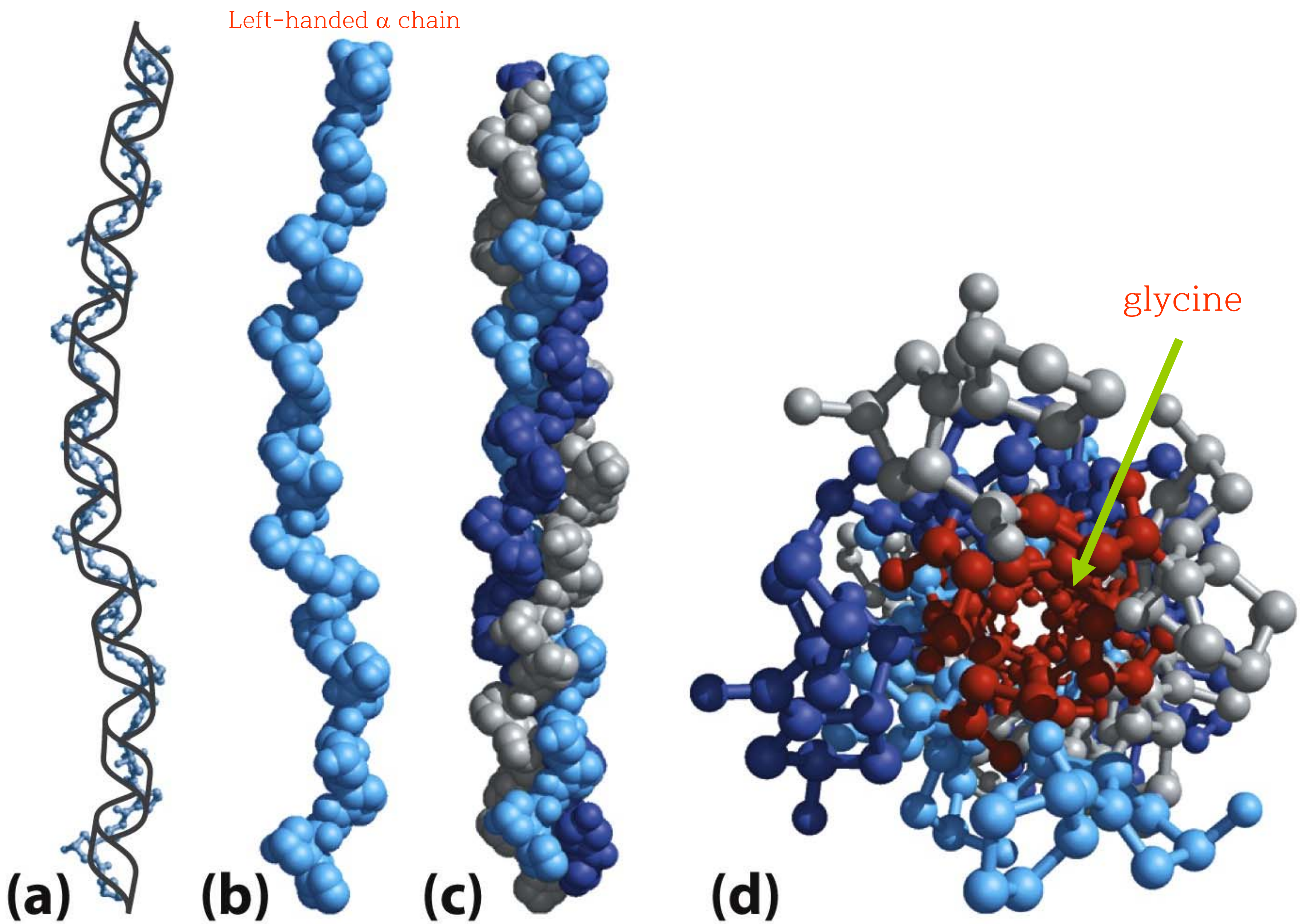
Fibrous proteins

B. Connective tissues (fibroblast: 결합조직, chondrocyte: 연골조직, osteoblast: 뼈조직)

- Connective tissue는 몸의 바깥구조와 지지를 위한 요소
- Tendon (힘줄), ligament (인대), cartilage (연골), bone의 organic matrix, 혈관의 지지체, 피부내의 구조층
- Collagen: 늘어나지 않는다 (tendon)
- Elastin: 늘어남 (ligament: 관절을 잇고 보정해줌)
- Proteoglycan: 다량의 다당류와 소량의 protein, ground substance (a type of glycoprotein: mucin 점액에 많은 단백질)

Collagen

1. Collagen:몸 구성 성분 중 가장 풍부함. Fibril로 구성 (간: 4%, 피부: 70%)
   - Tendon: 늘어지지 않는 인장강도가 크다. 평행된 다발구조
   - 가죽: 망상구조, 눈동자의 각막: +자로 배열된 collage섬유의 판, Connective tissue는 몸의 바깥구조와 지지를 의한 요소
   - 노화에 의한 cross-linkage 많음/어린동물조직은 가용화 가능
   - collagen의 성질
     - When boiled, it yields gelatin (가용성 polypeptide의 혼합물)
     - 요리에서의 응용: Lys-Lys결합, gelation (gelatin)
   - collagen의 구조
     - Left-handed and 3 amino acids/turn
     - Gly-X-Y (X is often Pro, Y is often 4-Hyp)
     - α helix와 다른 collagen helix: 3 separate polypeptides, α chain
     - Tropocollagen: triple-helical collagen molecules
     - H-bond, covalent cross-link, triple helix
     - 노화에 의한 covalent cross-link (Lys-Lys). 따라서 collagen fibril은 굳어지고 부러지기 쉽다. (뼈가 부러지기 쉽고 각막이상 등)



Left-handed α chain

glycine

Figure 4-11
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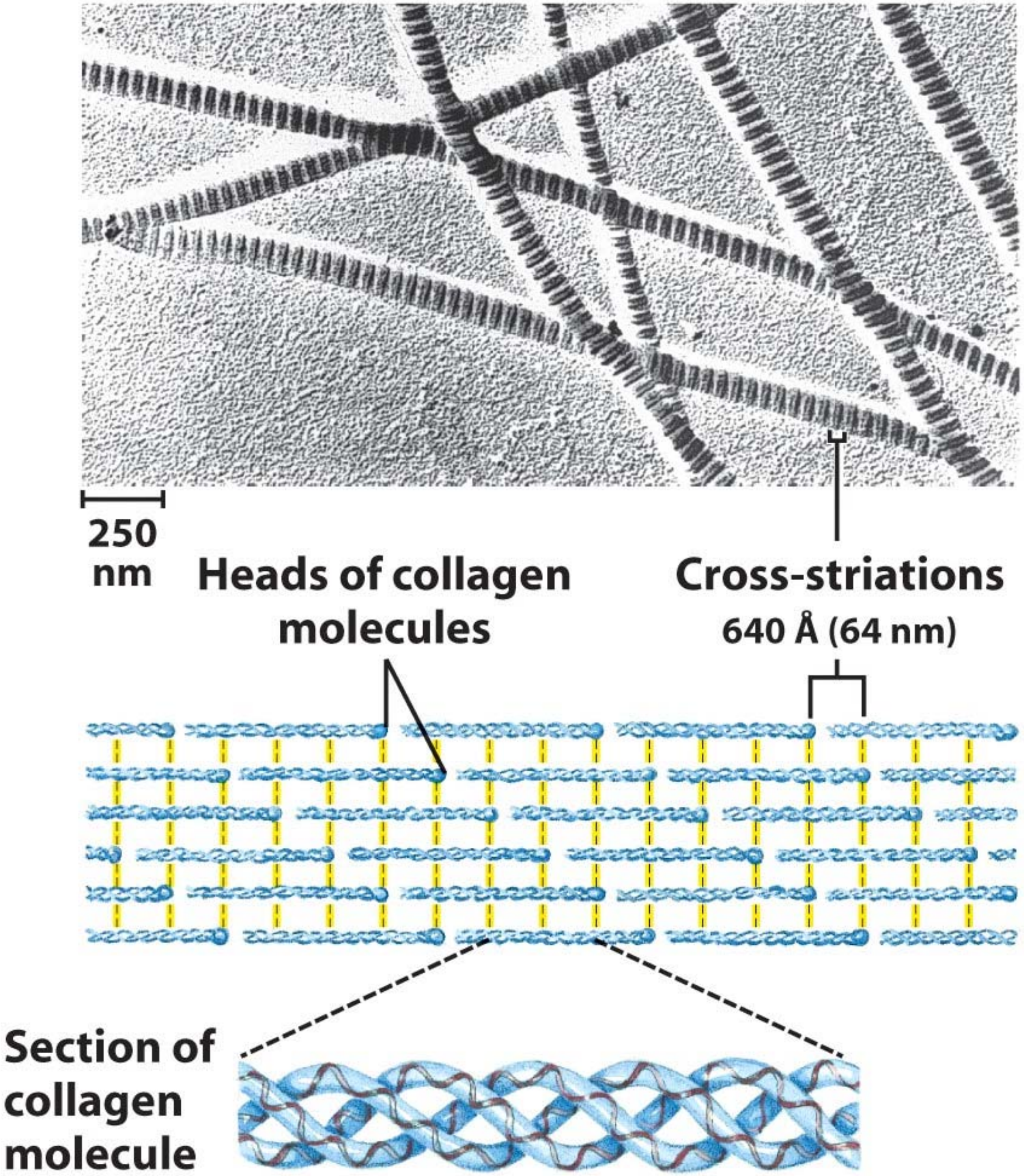
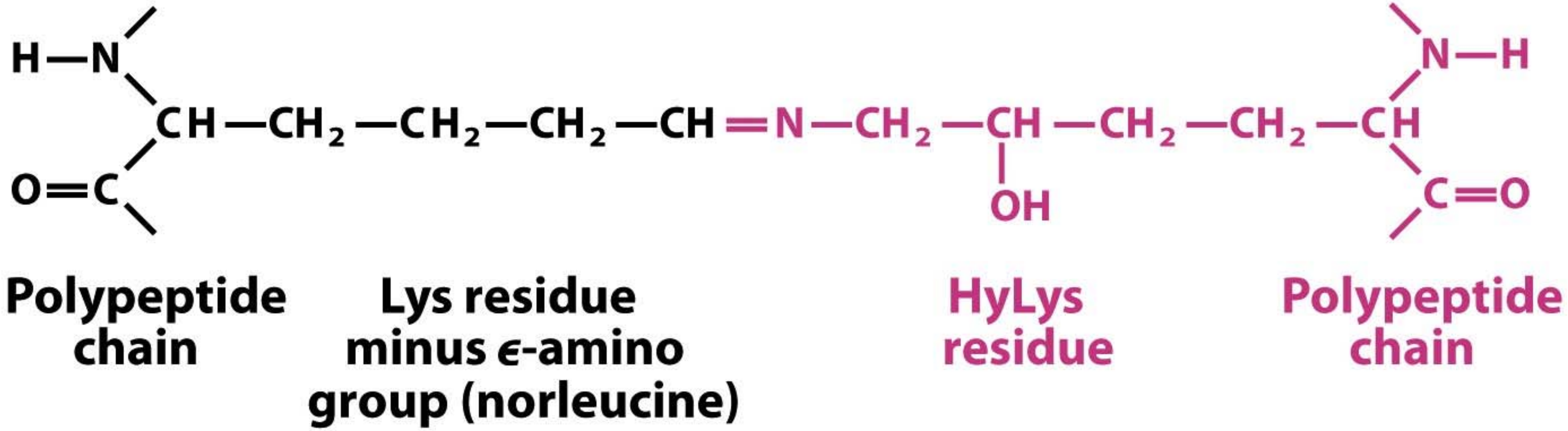
right-handed collagen

Structure of collagen fibrils

Mr, 300,000
3000 Å long, 15 Å thick



Rigid and brittle character of aging connective tissue



Dehydrohydroxylysinonorleucine

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Human genetic defects in collagen structure

- Osteogenesis imperfecta (골형성 부전증)
  - Abnormal bone formation in babies
- Ehlers-Danlos syndrome
  - Loose joint
- Result from the substitution of an amino acid with a larger R group (Cys, Ser) for Gly
  - Substantial deleterious effects on collagen structure

James Lind, Surgeon in the Royal Navy
British sailor : Limeys

James Lind, 1716–1794; naval surgeon, 1739–1748

Box 4-3
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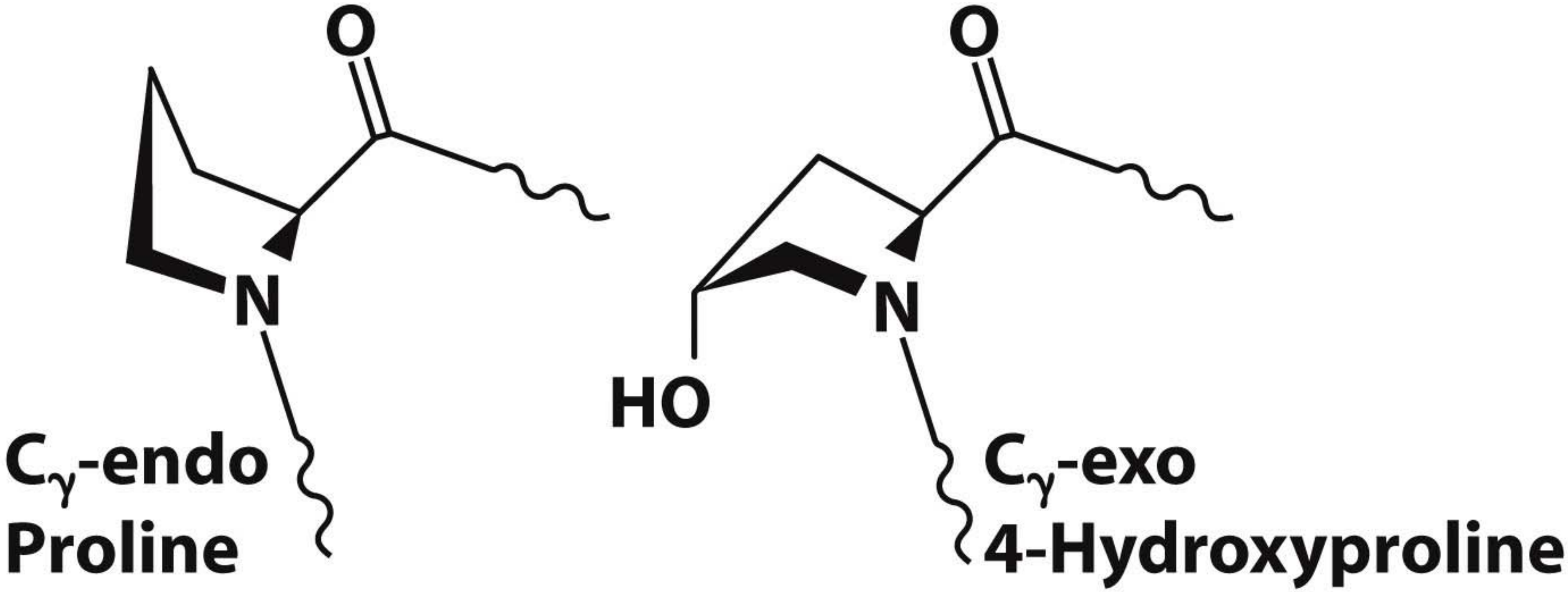

Box 4-3 figure 1

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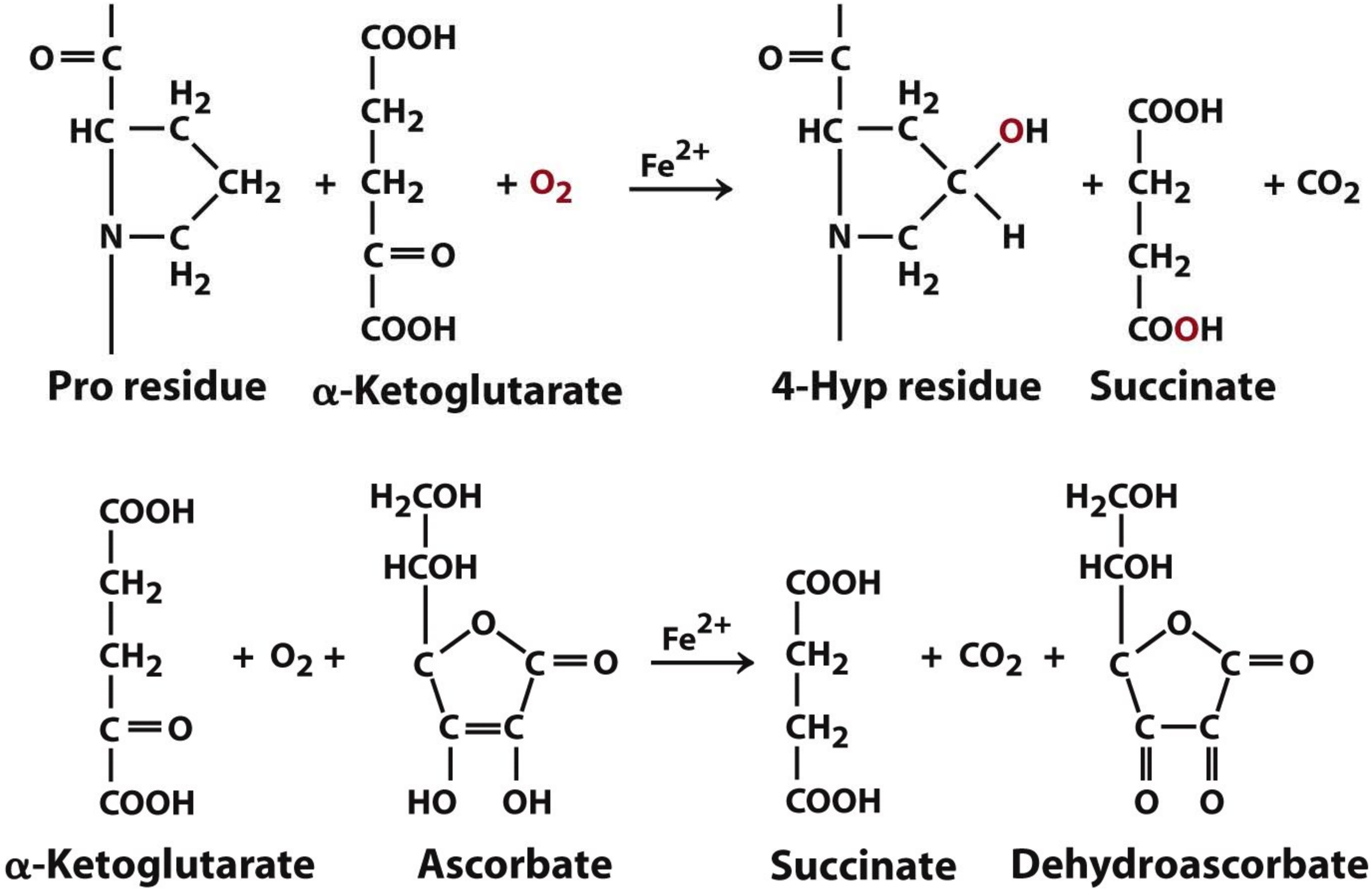

Box 4-3 figure 2

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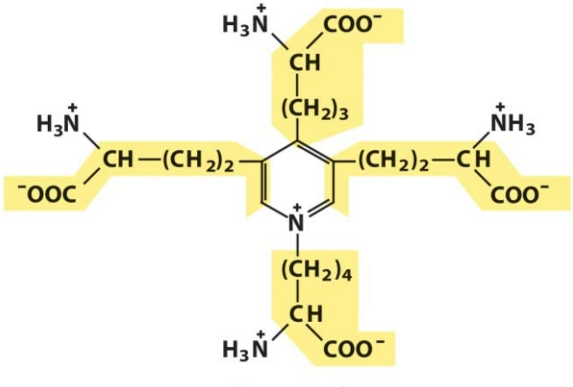
Fibrous proteins

2. Elastin: elastic tissue에 특이성 부여 (고무와 같은 탄성)
   - Ligament (인대)나 대동맥의 elastic tissue layer
   - Subunit: tropoelastin, Mw. 72000, 약 800개의 amino acids
   - 4개의 lys이 결합되어 있는 R기가 모여 효소에 의해서 desmosine, isodesmosine 형성. 따라서 어느 방향이든지 가역적으로 뻗을 수 있는 배치를 이루면서 tropoelastin 사슬과 결합

3. 골격근: Myosin, Actin

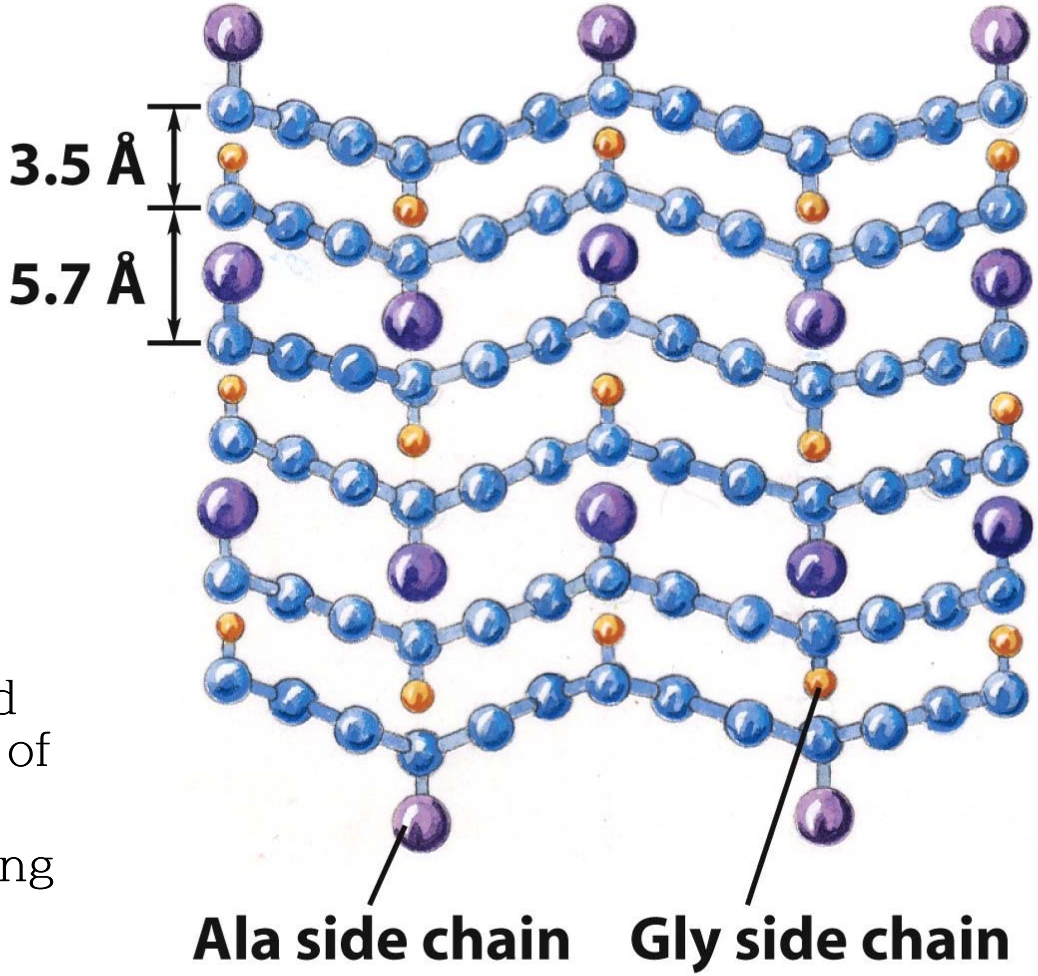


Desmosine

Structure of silk

Silk fibroin

- protein of silk
- β-keratin:
  - 명주실, 거미집의 단백질인 fibroin
  - β-conformation: β-keratin (0.7 nm 간격으로 반복)
  - Silk does not stretch
  - Rich in Gly, Ala
    - Close packing of sheet and interlocking arrangement of R groups
    - Extensive hydrogen bonding
    - Optimization of van der Waals interactions



Structure of silk. The fibers in silk cloth and in a spider web are made up of the protein fibroin. (a) Fibroin consists of layers of antiparallel β sheets rich in Ala and Gly residues. The small side chains interdigitate and allow close packing of the sheets, as shown in the ball and stick view.

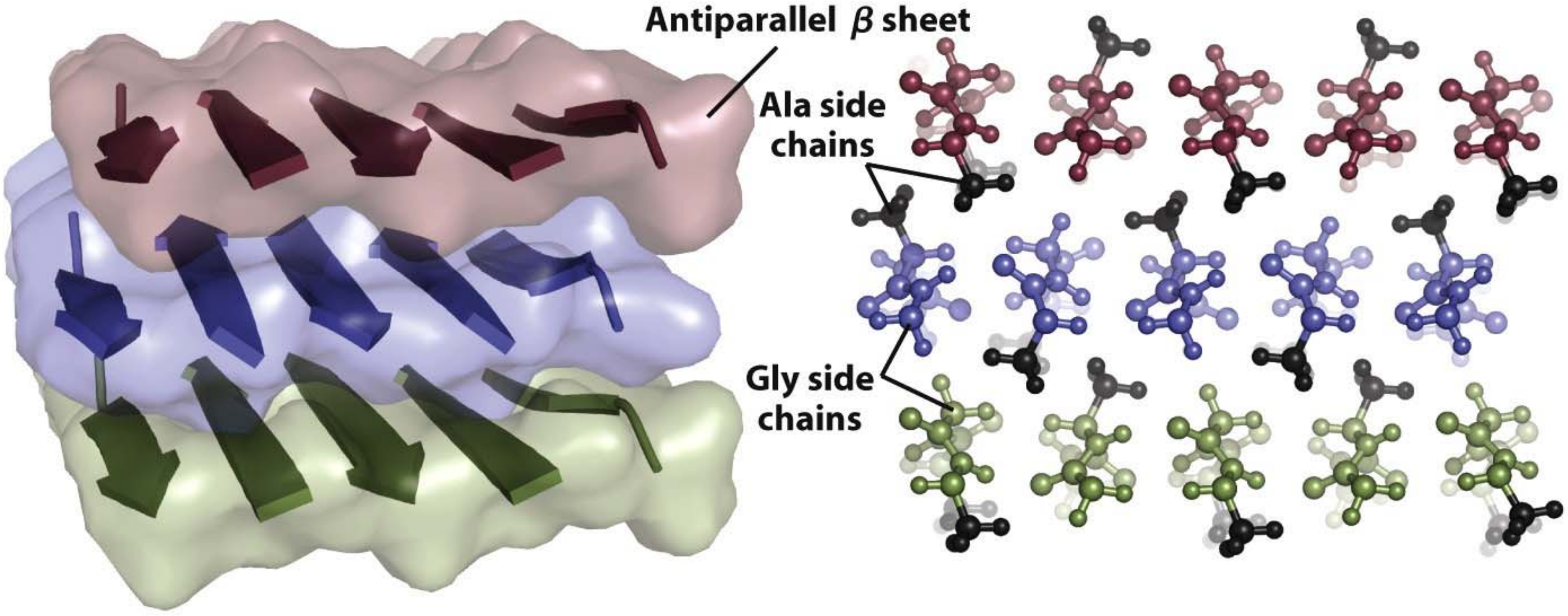


Figure 4-13a
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