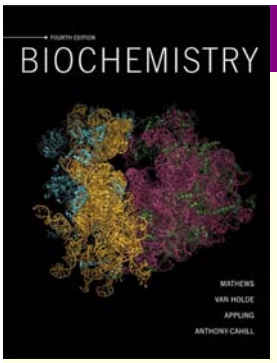


Chapter 6

The Three-Dimensional Structure of Proteins

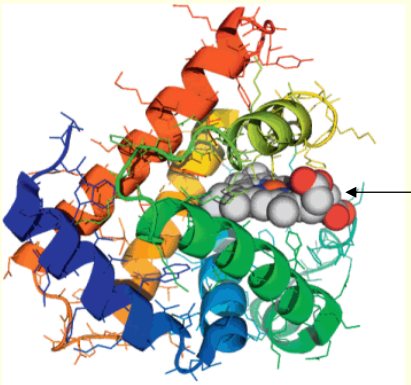
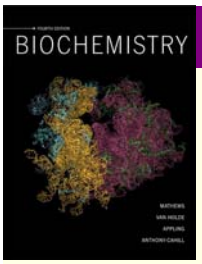


FIGURE 6.1
Three-dimensional folding of the protein myoglobin.

生化分生科 游佳融

2014/09



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Chapter 6 Outline:

- Secondary Structure: Regular Ways to Fold the Polypeptide Chain
- Fibrous Proteins: Structural Materials of Cells
- Globular Proteins: Teritary Structure and Functional Diversity
- Factors Determining Secondary and Tertiary Structure
- Dynamics of Globular Protein Structure
- Prediction of Secondary and Tertiary Protein Structure
- Quaternary Structure of Proteins

Protein molecules have ***four levels*** of structural organization

- **Primary structure** - the amino acid sequence
- **Secondary structure** - local folding into regularly repeating units
- **Tertiary structure -** overall folding of a monomeric protein or subunit
- **Quaternary structure -** subunit association 次單位體

摺疊: folding

The levels of protein structure

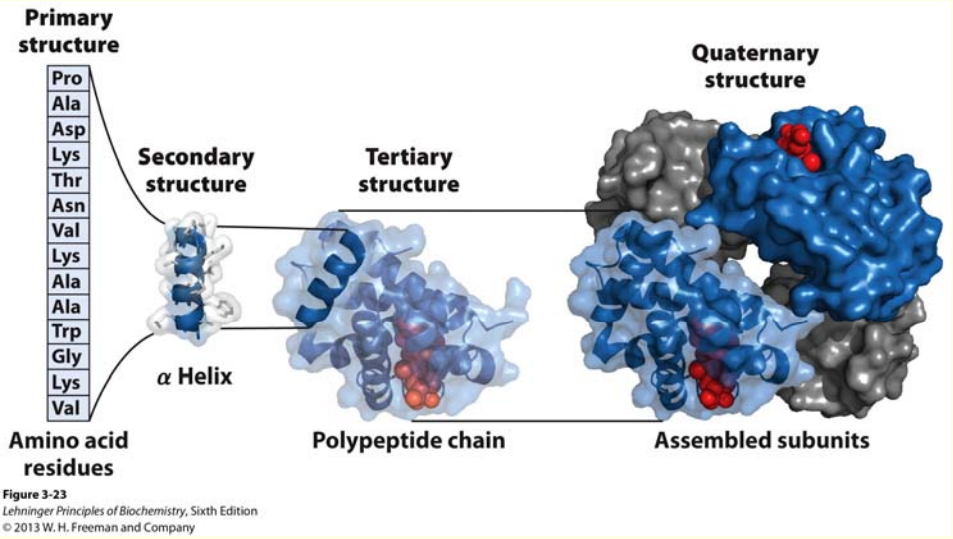

Figure 3-23
Lehninger Principles of Biochemistry, Sixth Edition
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1. AA. sequence

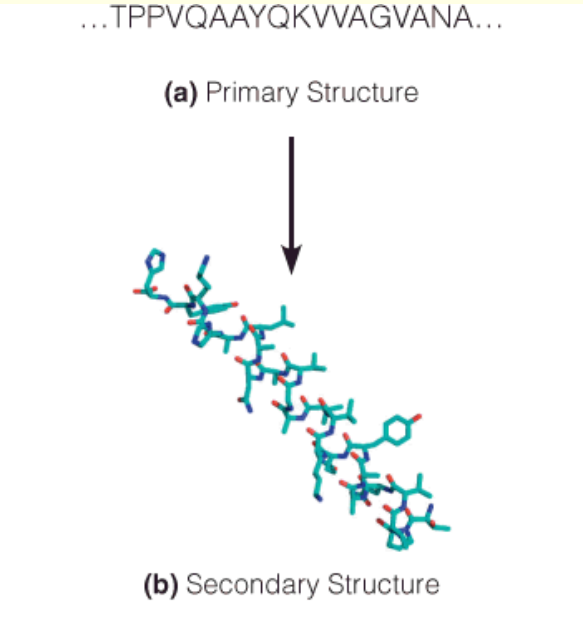
2. α-helix , the β-sheet , turns and loops

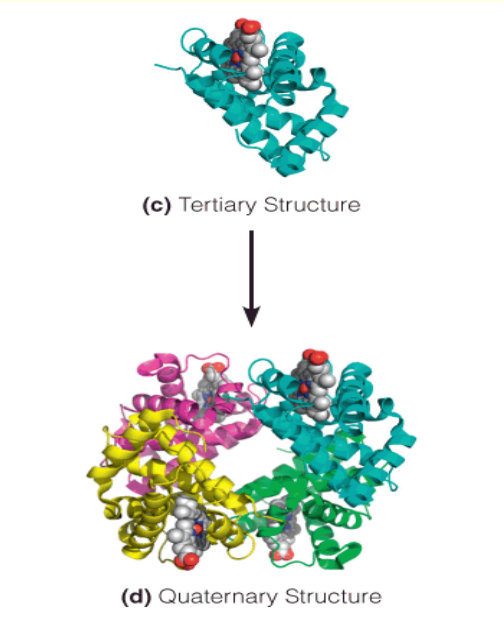
Motif & domain & fold

3. spatial arrangement of atoms

4. Multiple subunits

The levels of protein structure





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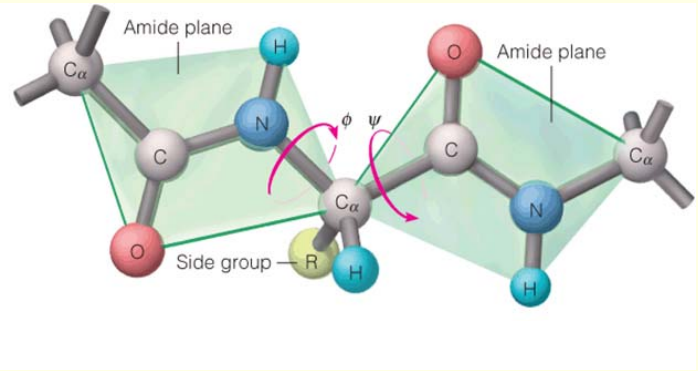
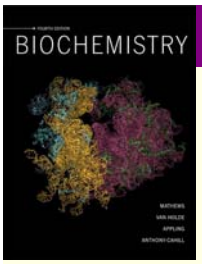
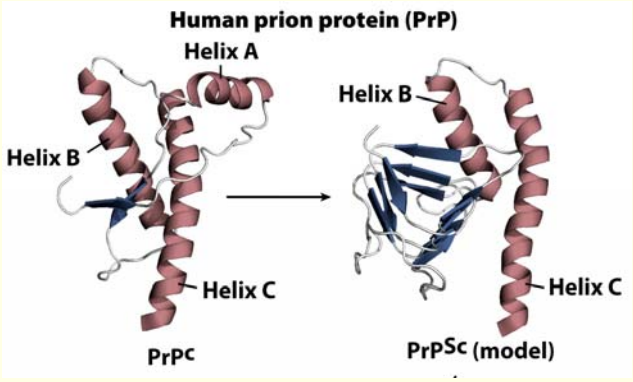
Regular Ways to Fold the Polypeptide Chain



- Rotation around the bonds in a polypeptide backbone.
- Two adjacent amide planes are shown in light green.
- Rotation is allowed only about the N_{amide}–C_α (ϕ, phi) and C_α–$C_{carbonyl}$ (ψ, psi) bonds.
- Positive rotation is clockwise as seen from the α-carbon.



Of the several possible secondary structures for polypeptides, the most frequently observed are:

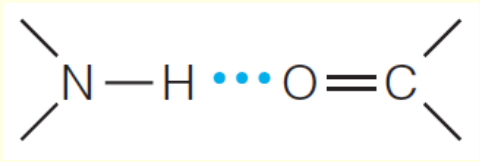


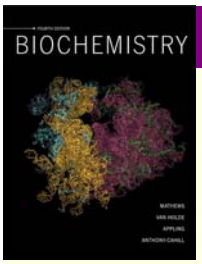
α helix 螺旋

β sheet 摺板

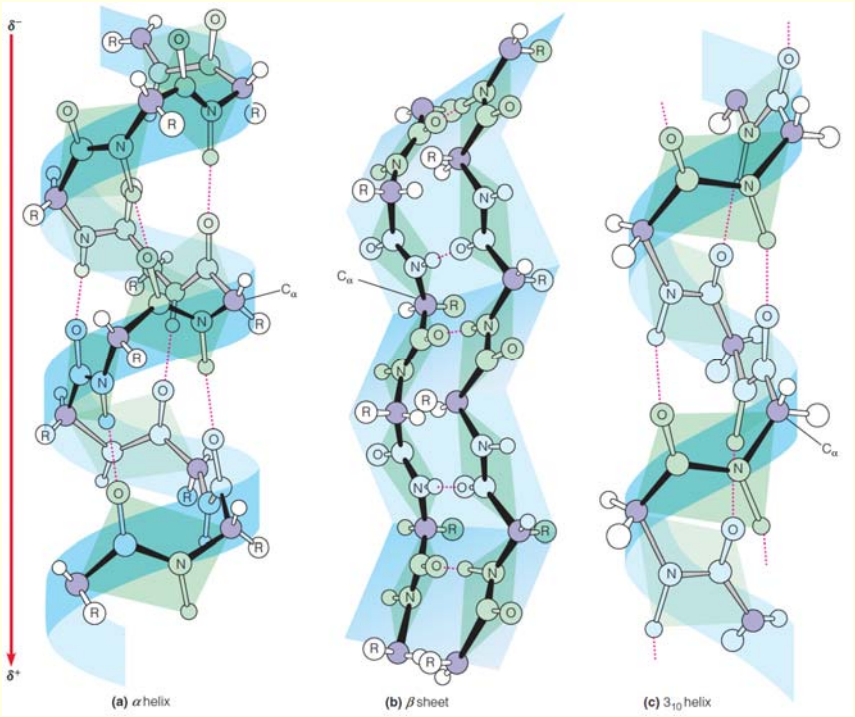
turn and loop 轉折及迴圈

Stabilize a regular folding by hydrogen bonding between amide protons and carbonyl oxygens.



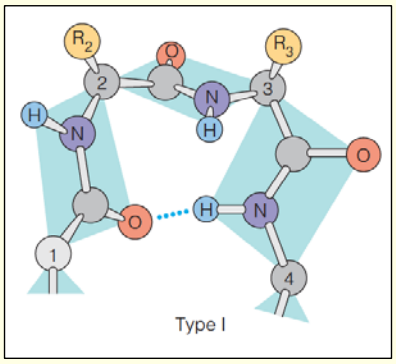


α helix **β sheet** **3_{10} helix**

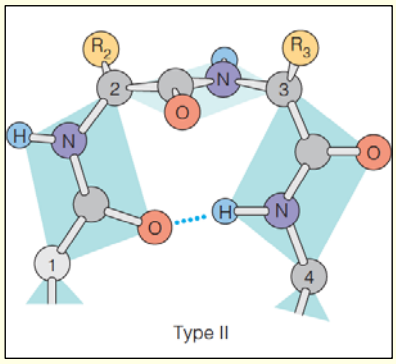


…..H-bound

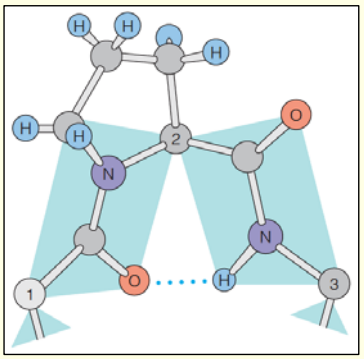
Turn 轉折



β-turns



β-turns
In the **type II turn**, residue 3 is usually *glycine*.



γ-turn
Residue 3 is usually *proline*.

.....H-bound

α-helix

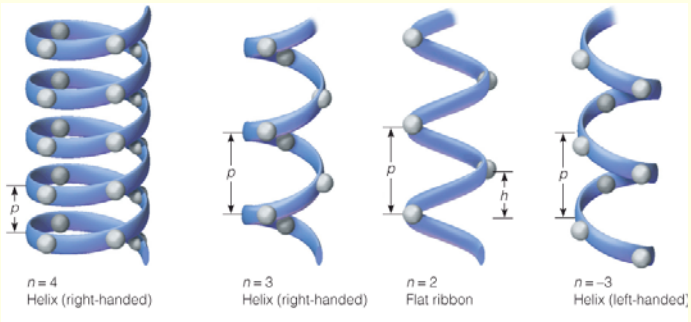
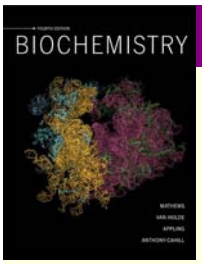


TABLE 6.1 Parameters of some polypeptide secondary structures

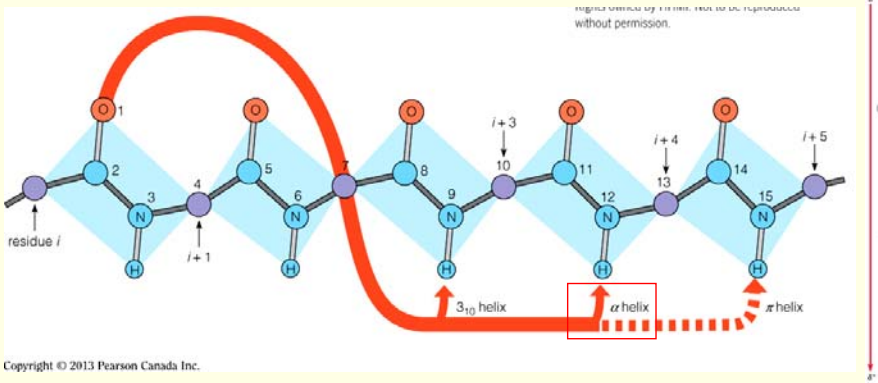
Structure Type	Residues/ Turn	Rise (h) per residue	Pitch (p)
β Strand (antiparallel)	2.0	0.34 nm	0.68 nm
β Strand (parallel)	2.0	0.32 nm	0.64 nm
α helix	3.6	0.15 nm	0.54 nm
3_{10} helix	3.0	0.20 nm	0.60 nm
Polypeptide II helix ("polyproline II helix")	3.0	0.47 nm	0.94 nm

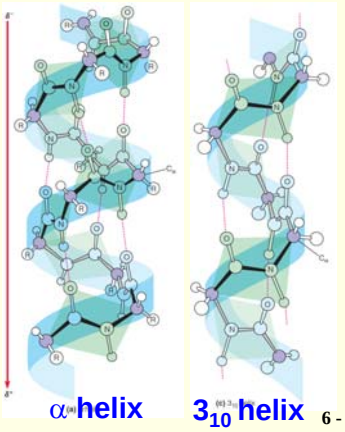


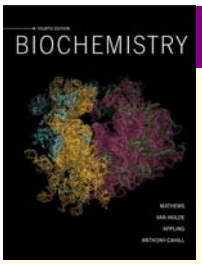
Parameters of an α-helix

•The carbonyl oxygen, on residue *i*, is **hydrogen-bonded to the amido proton** that is **four residues** removed in the direction of the *C*-terminus (i.e., on residue *i* + 4).

•A loop of **13 atoms** is formed.
•The helix could also be called a 3.6_{13} helix.
•The 3_{10} helix has exactly 3.0 residues per turn and a 10-member hydrogen-bonded loop.
•Hydrogen bonds tend to be linear, so the atoms in polypeptide helices should lie on a straight line.

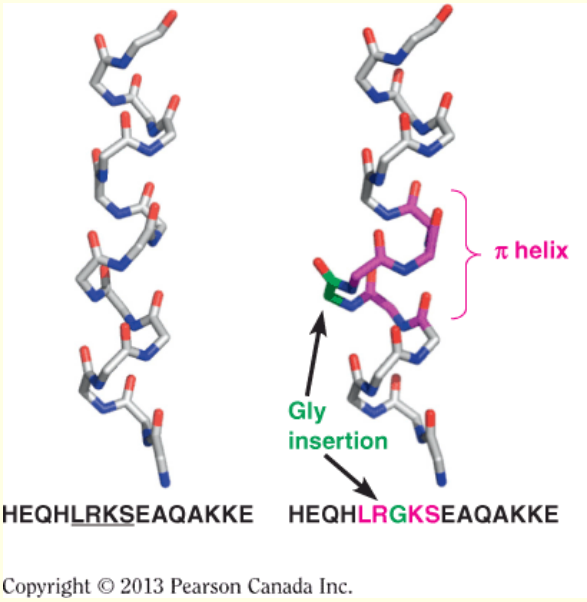






The π-helix conformation

Also known as an "α-bulge" or "α-aneurism" or "π-bulge."腫脹, 凸塊



• Left: a main-chain rendering of the *C*-terminal α-helix from *S. aureus.*

•Right: the analogous π-helix from a Gly insertion mutant is shown.

•Note that the inserted Gly carbonyl does not form an intrahelical H-bond.

The π helix:

- It is found in ~15% of protein sequences in the Protein Data Bank.
- It occurs only once in any given sequence.
- 85% appears to be the result of a mutation event that results in the insertion of an amino acid into an α–helix.
- This creates a bulge in the helical structure.

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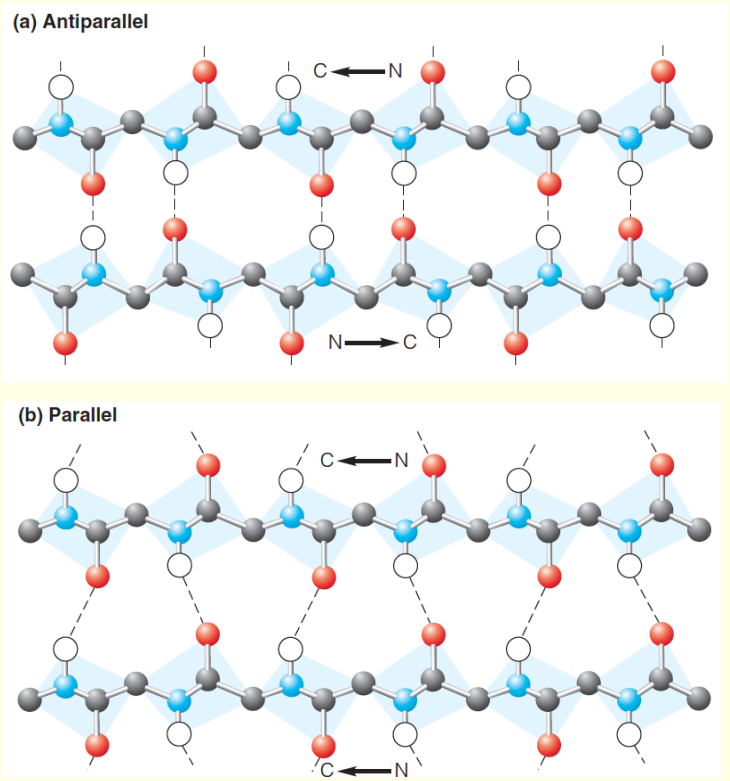
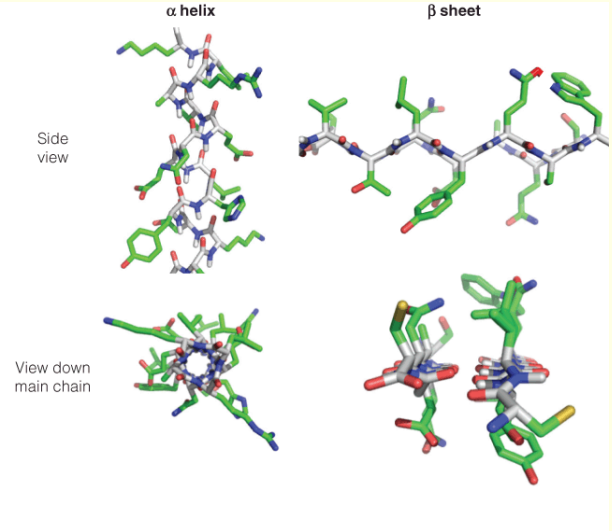
β-sheets



- α-helix will have side chains of similar polarity every 3-4 residues
- β-strand will have alternating polar and nonpolar side chains.



Secondary structures that display a predominantly ***hydrophobic face opposite a predominantly hydrophilic face*** are said to be "***amphiphilic*** " (or "***amphipathic***"). **雙極性**

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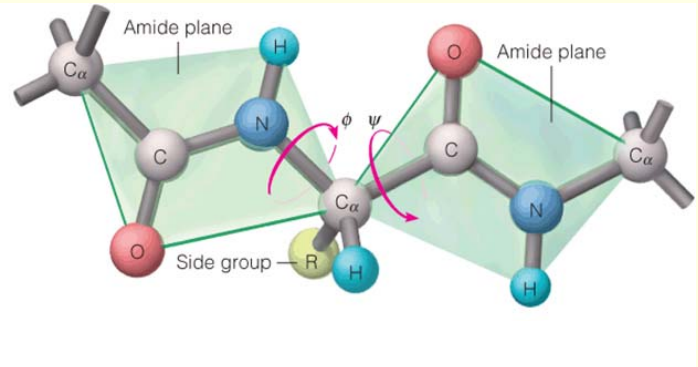
Dose secondary structure of protein can be predicted?

Yes!

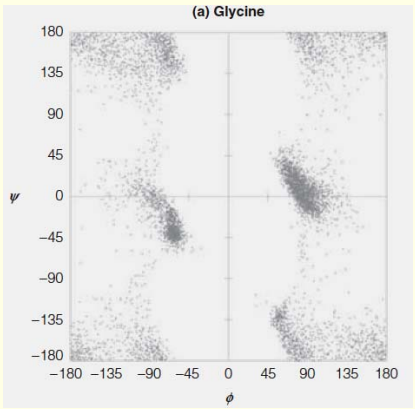
Why?

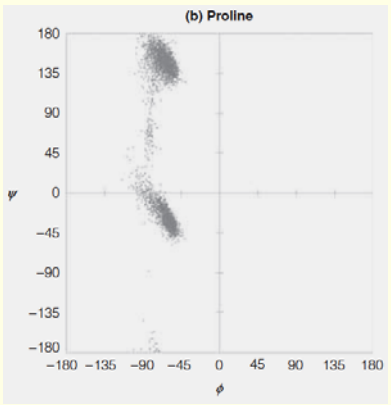
Regular Ways to Fold the Polypeptide Chain



- Rotation around the bonds in a polypeptide backbone.
- Two adjacent amide planes are shown in light green.
- Rotation is allowed only about the N_{amide}–C_α (ϕ, phi) and C_α–$C_{carbonyl}$ (ψ, psi) bonds.
- Positive rotation is clockwise as seen from the α-carbon.

Conformational preferences of the amino acids





•**Glycine has the greatest number** of allowed ϕ and ψ angle combinations, whereas **proline has the fewest.**

Prediction of Secondary Protein Structure

TABLE 6.8 Correspondence of amino acid residues to protein secondary structure

Relative probabilities of amino acid residue occurrence in different globular protein secondary structures[a]

Amino Acid	α Helix (P_α)	β Sheet (P_β)	Turn (P_t)	
Ala	1.29	0.90	0.78	Favor α helices
Cys	1.11	0.74	0.80	
Leu	1.30	1.02	0.59	
Met	1.47	0.97	0.39	
Glu	1.44	0.75	1.00	
Gln	1.27	0.80	0.97	
His	1.22	1.08	0.69	
Lys	1.23	0.77	0.96	
Val	0.91	1.49	0.47	Favor β sheets
Ile	0.97	1.45	0.51	
Phe	1.07	1.32	0.58	
Tyr	0.72	1.25	1.05	
Trp	0.99	1.14	0.75	
Thr	0.82	1.21	1.03	
Gly	0.56	0.92	1.64	Favor turns
Ser	0.82	0.95	1.33	
Asp	1.04	0.72	1.41	
Asn	0.90	0.76	1.23	
Pro	0.52	0.64	1.91	
Arg	0.96	0.99	0.88	

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Fibrous Proteins: Structural Materials of Cells

Fibrous proteins 纖維狀蛋白質:

•Have a **filamentous**, or **elongated**, form.

•Most of them play **structural roles** in animal cells and tissues.

•include the major proteins of skin and connective tissue and of animal fibers like hair and silk.

•The amino acid sequence of each of these proteins favors a particular kind of secondary structure

- Predominantly α-helical in structure.
- Built on a coiled-coil α–helical structure.

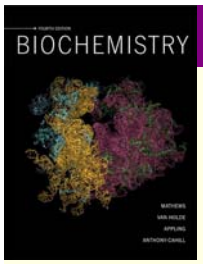

TABLE 6.3 Amino acid compositions of some fibrous proteins

Amino Acid	α-Keratin (wool)	Fibroin (silk)	Collagen (Bovine tendon)	Elastin (Pig aorta)	All proteins[f]
Gly	8.1	44.6	32.7	32.3	7.9
Ala	5.0	29.4	12.0	23.0	8.7
Ser	10.2	12.2	3.4	1.3	5.8
Glu + Gln	12.1	1.0	7.7	2.1	6.6 (3.7)
Cys	11.2	0	0	—[e]	1.3
Pro	7.5	0.3	22.1[a]	10.7[c]	4.7
Arg	7.2	0.5	5.0	0.6	5.0
Leu	6.9	0.5	2.1	5.1	8.9
Thr	6.5	0.9	1.6	1.6	5.6
Asp + Asn	6.0	1.3	4.5	0.9	5.9 (4.2)
Val	5.1	2.2	1.8	12.1	7.2
Tyr	4.2	5.2	0.4	1.7	3.5
Ile	2.8	0.7	0.9	1.9	5.5
Phe	2.5	0.5	1.2	3.2	4.0
Lys	2.3	0.3	3.7[b]	3.6[d]	5.5
Trp	1.2	0.2	0	—[e]	1.5
His	0.7	0.2	0.3	—[e]	2.4
Met	0.5	0	0.7	—[e]	2.0

Note: The three most abundant amino acids in each protein are indicated in red. Values given are in mole percent.

[a]About 39% of this is hydroxyproline.
[b]About 14% of this is hydroxylysine.
[c]About 13% of this is hydroxyproline.
[d]Most (about 80%) is involved in cross-links.

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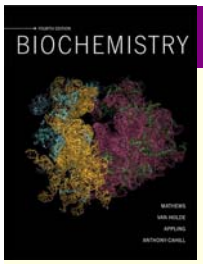
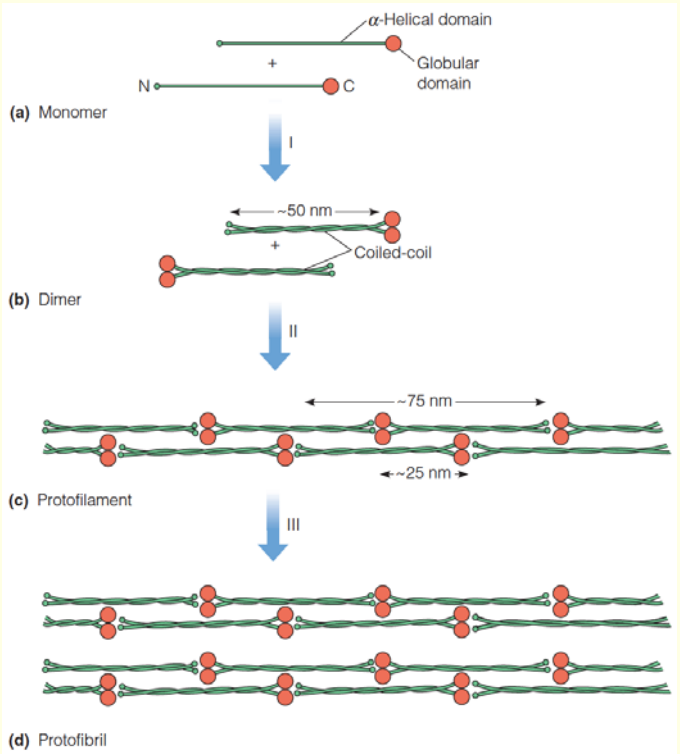


Proposed structure for keratin-type intermediate filaments



角質
中間絲

Fibroin 纖維蛋白

Almost half of its residues are glycine.

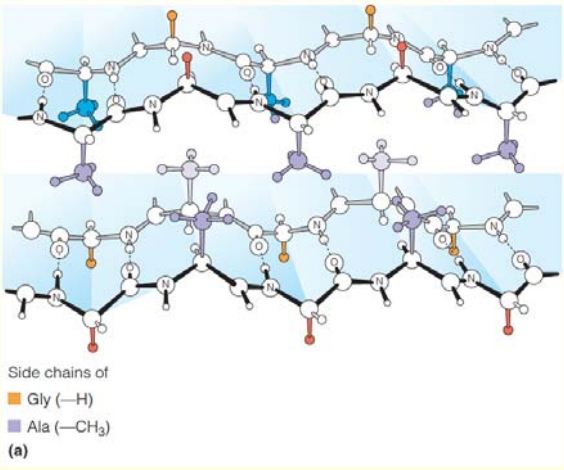
[Gly-Ala-Gly-Ala-Gly-Ser-Gly-Ala-Ala-Gly-(Ser-Gly-Ala-Gly-Ala-Gly)8]

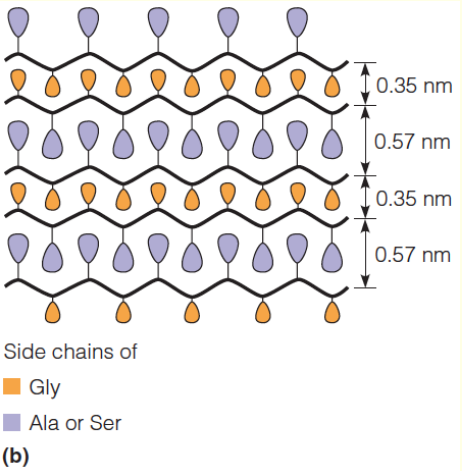
Silkworm fibroin contains long regions of antiparallel β-sheet, with the polypeptide chains running parallel to the fiber axis.

The β-sheet regions comprise almost exclusively multiple repetitions of the sequence:

In silkworm fibroin almost every other residue is Gly and that between them lie either Ala or Ser residues.

Fibroin 纖維蛋白





[Gly-Ala-Gly-Ala-Gly-Ser-Gly-Ala-Ala-Gly-(Ser-Gly-Ala-Gly-Ala-Gly)8]

The structure of silk fibroin.
(a) A three-dimensional view of the stacked sheets of fibroin. The region shown contains only alanine and glycine residues.

(b) Interdigitation of alanine or serine side chains and glycine side chains in fibroin. The plane of the section is perpendicular to the folded sheets.

Collagen structure 膠原蛋白的結構

•Collagen fibers are built from triple helices of polypeptides rich in glycine and proline.

•A **triple helix** of three polypeptide chains, ~1000 residues in length.

•**Left-handed** helices, with about **3.3 residues/turn**.

•The chains wrap around one another in a right-handed sense.

•Hydrogen bonds are ***between*** the chains.

•Every third residue can be only glycine

•Hydroxyproline and hydroxylysine are present.

•A repetitive motif in the sequence is of the form Gly–X–Y, where X is often proline and Y is proline or hydroxyproline.

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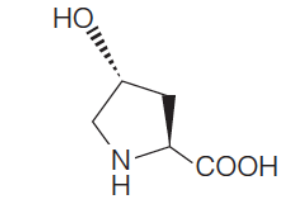
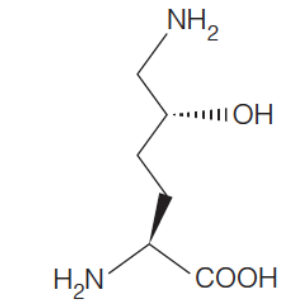


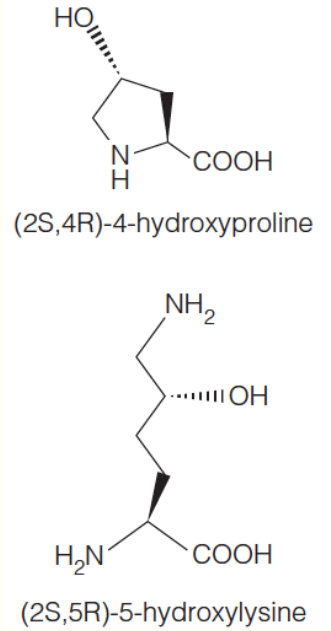
(2S,4R)-4-hydroxyproline

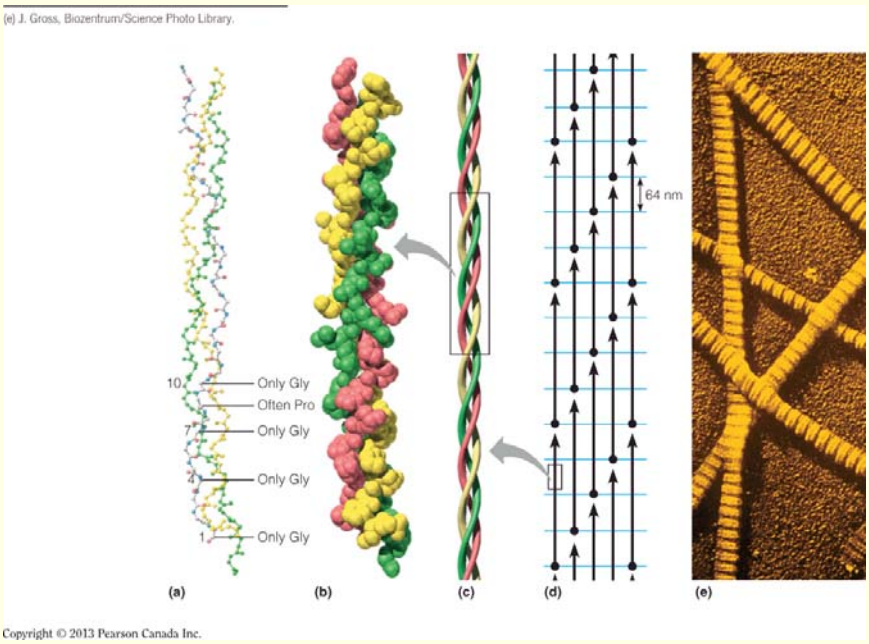


(2S,5R)-5-hydroxylysine

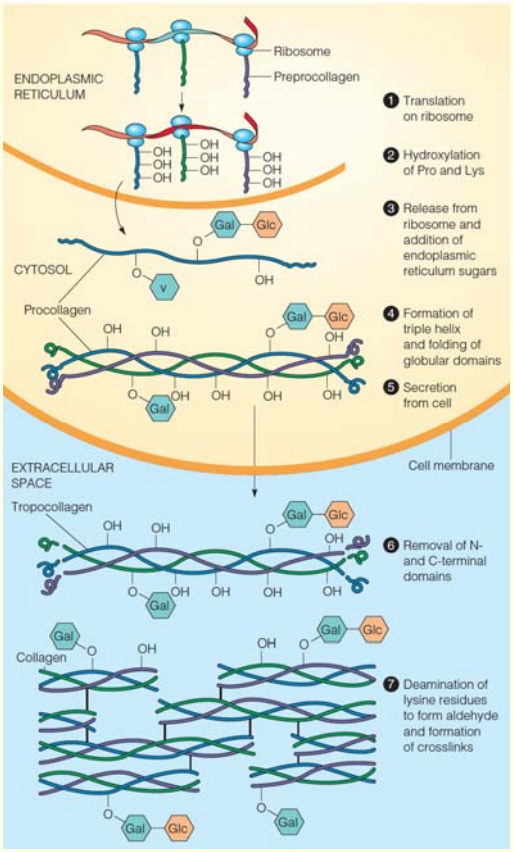
- Scurvy is a connective tissue disease from a deficiency in Vitamin C. 敗血病
- Collagen fibers are weakened.
- It is caused by failure to hydroxylate prolines and lysines in collagen.
- There is less H-bonding between the chains of tropocollagen.

Collagen structure 膠原蛋白的結構





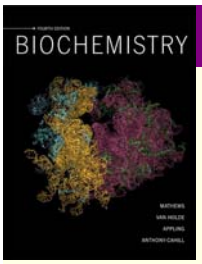
Triple helix



Biosynthesis and assembly of collagen:

•Steps 1-4 occur in the endoplasmic reticulum and cytosol of collagen-synthesizing cells.

•Steps 6 and 7 occur in the extracellular region.



Elastin 彈性蛋白

The protein **elastin** forms elastic fibers found in ligaments and blood vessels.

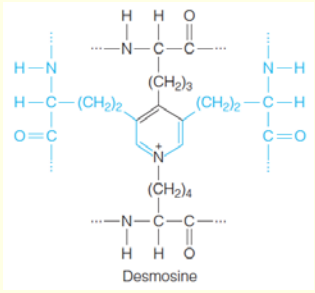
• Rich in glycine, alanine, and valine.

• Very flexible and easily extended.

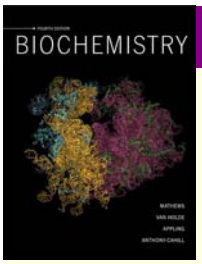
• Has little secondary structure at all in a conformation that approximates a random coil.

• Contains lysine side chains, which can cross-link.

• Four lysine side chains can be combined to yield a **desmosine** cross-link.



鎖鏈素



Globular proteins 球形蛋白質

•Carry out most of the chemical work of the cell

- Synthesis
- Transport
- Metabolism

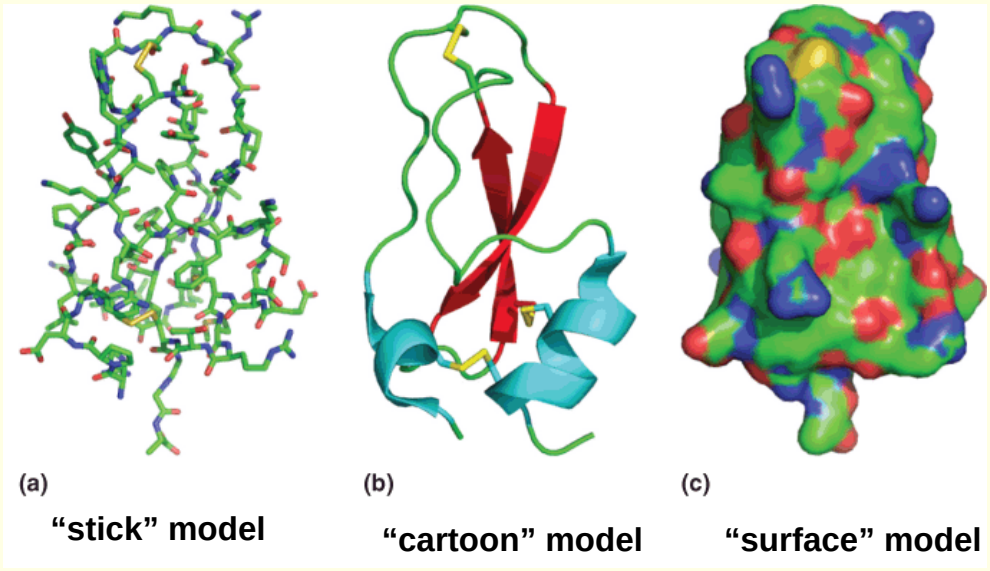
•Possess ***secondary structures***

•Folded into compact ***tertiary structures***

•Many carry ***prosthetic groups***

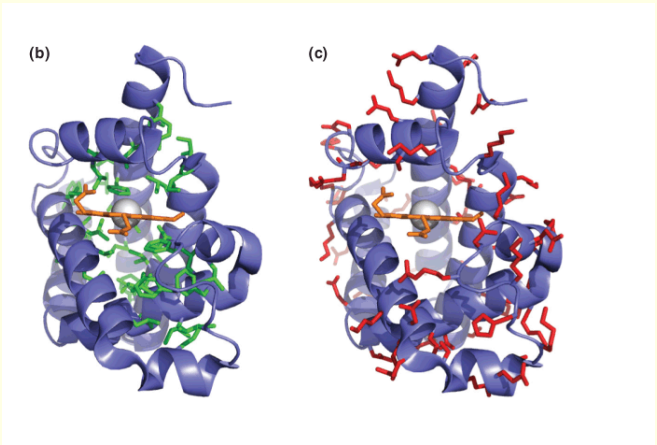
- small molecules that may be noncovalently or covalently bonded to the protein (*e.g.*, heme in myoglobin).

Teritary Structure of Globular Proteins



"stick" model "cartoon" model "surface" model

Globular proteins 球形蛋白質

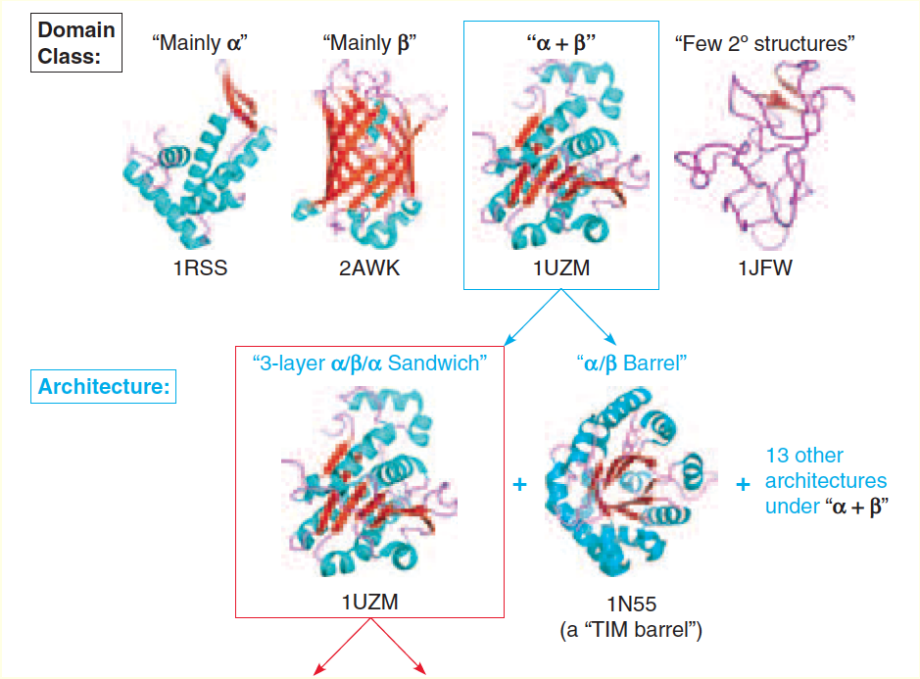


For a water soluble protein

- The hydrophobic amino acids (green) cluster about the hydrophobic heme cofactor (orange) and on the inside of the molecule.
- The hydrophilic residues (red) are on the solvent-exposed surface of the protein.

Domain 作用區

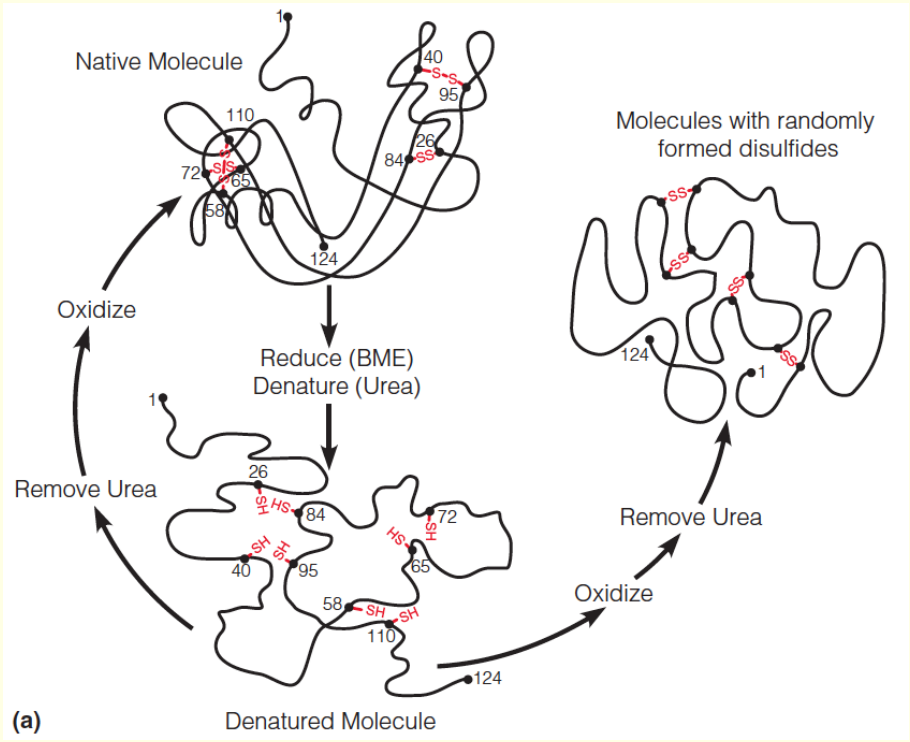
A compact, ***locally folded region*** of tertiary structure



Factors Determining Secondary and Tertiary Structure

- Most of the information for determining the **3-D structure** of a protein is carried in the **amino acid sequence** of that protein.
- Under harsh conditions, a protein loses its functional 3-D structure.
- This process is called ***denaturation.*** 變性
- Denaturing conditions include:
  - Increased temperature
  - pH becomes extremely acidic or alkaline
  - Organic solvents or urea

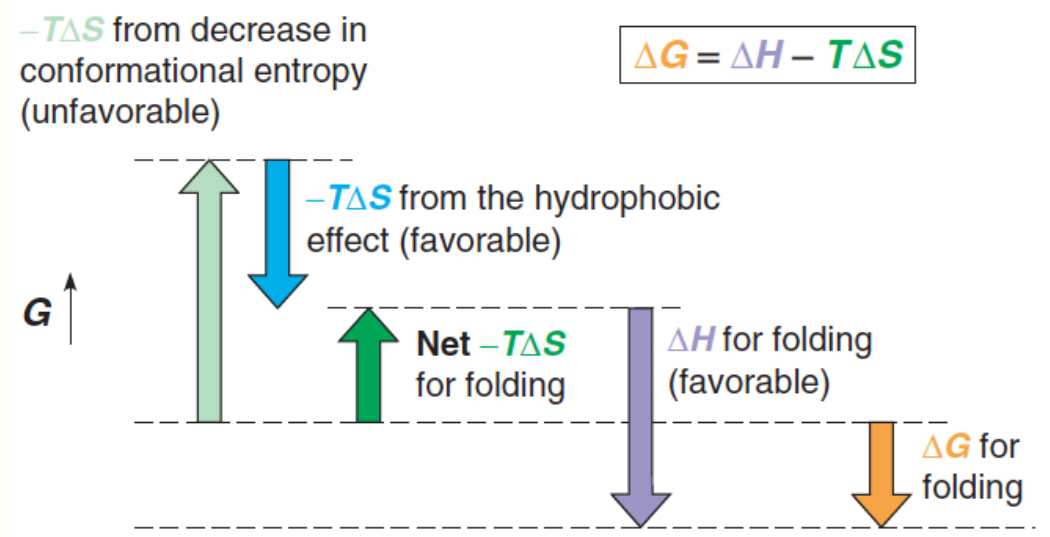
The denaturation and refolding of ribonuclease A



The Thermodynamics of Folding

- The folding of a globular protein is clearly a *thermodynamically favorable* process under physiological conditions.
- The overall free energy change for folding must be *negative*.
- This negative free energy change is achieved by a *balance of several thermodynamic factors:*
  - Conformational Entropy
  - Charge–Charge Interactions
  - Internal Hydrogen Bonds
  - van der Waals Interactions

Contributions to the free energy of folding of globular proteins



Protein Folding Is A Highly Cooperative Process

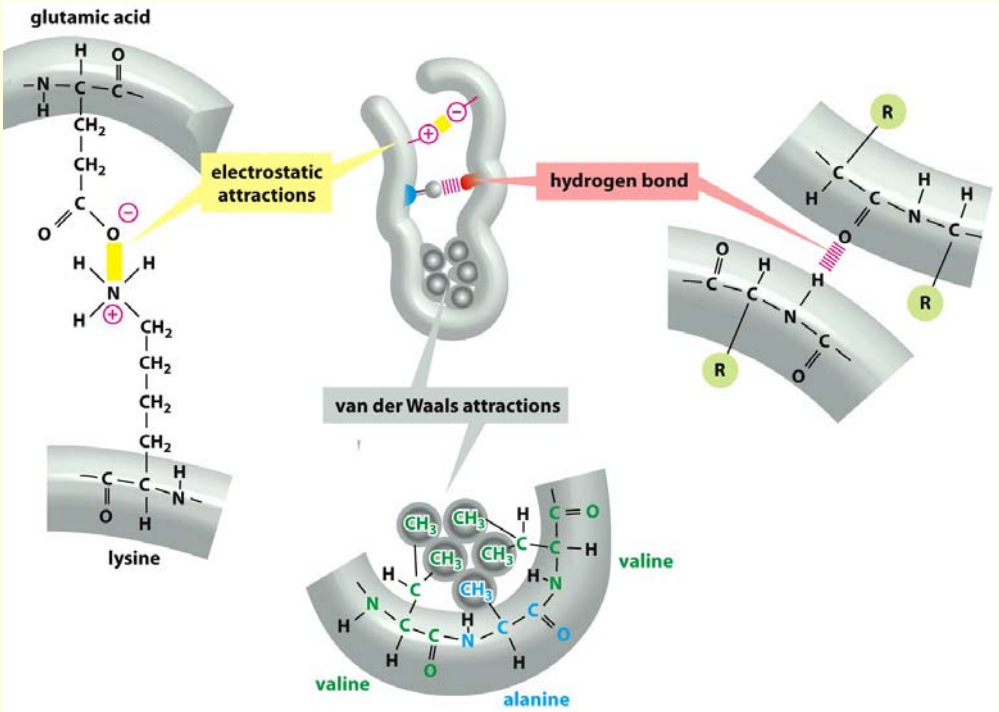
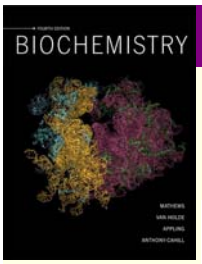
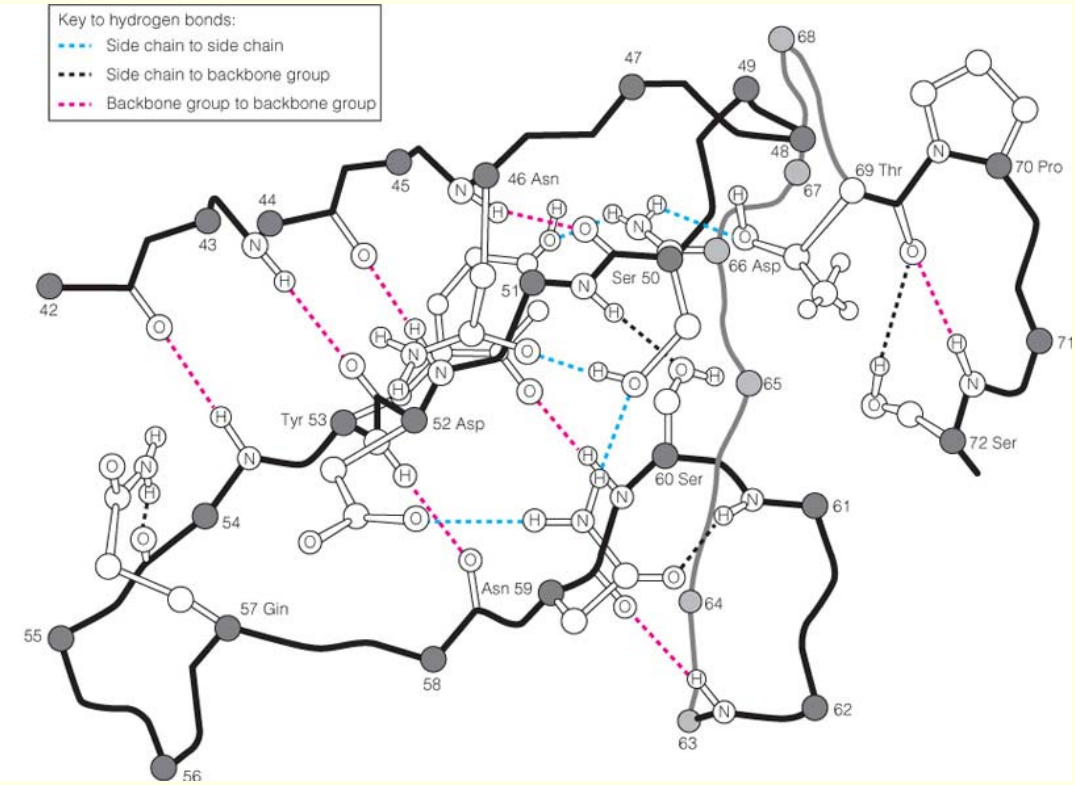
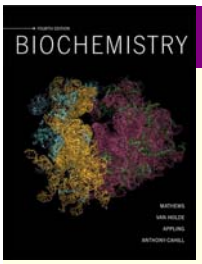


Figure 4-4 *Essential Cell Biology* (© Garland Science 2010)



Detail of Hydrogen Bonding in A Typical Protein





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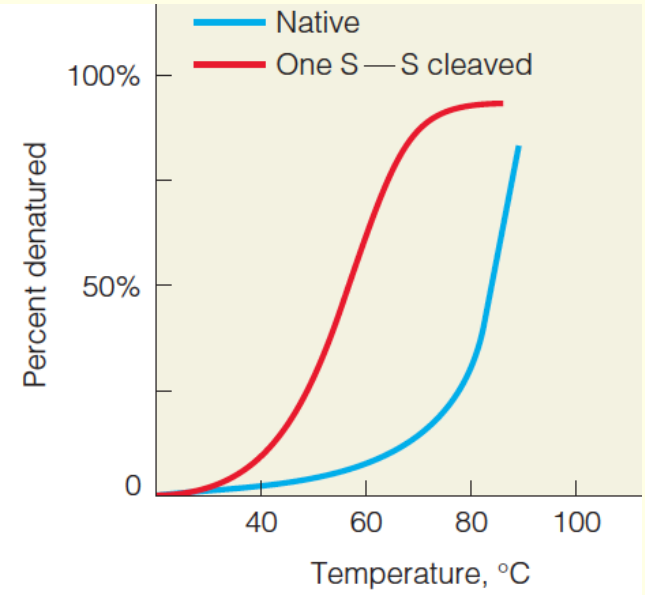
The hydrophobicity of amino acids *vs.* van der Waals Interactions

TABLE 6.5 Two examples of hydrophobicity scales

Amino Acid	Scale of Engelman, Steitz, and Goldman[a]	Scale of Kyte and Doolittle[b]
Phe	3.7	2.8
Met	3.4	1.9
Ile	3.1	4.5
Leu	2.8	3.8
Val	2.6	4.2
Cys	2.0	2.5
Trp	1.9	−0.9
Ala	1.6	1.8
Thr	1.2	−0.7
Gly	1.0	−0.4
Ser	0.6	−0.8
Pro	−0.2	−1.6
Tyr	−0.7	−1.3
His	−3.0	−3.2
Gln	−4.1	−3.5
Asn	−4.8	−3.5
Glu	−8.2	−3.5
Lys	−8.8	−3.9
Asp	−9.2	−3.5
Arg	−12.3	−4.5

The Role of Disulfide Bonds in Protein Denaturation



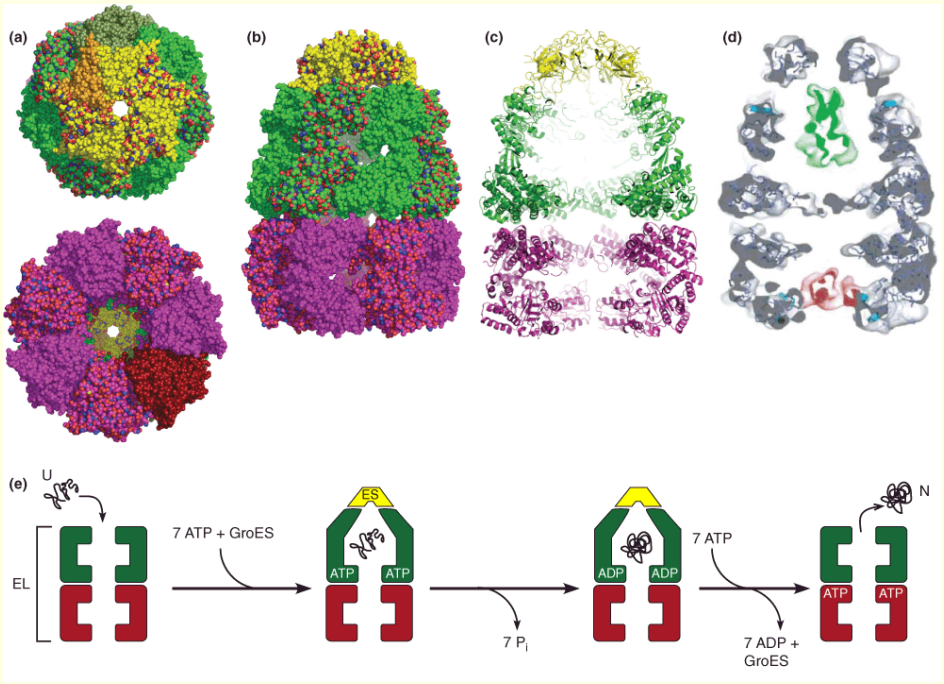
***Molecular* Chaperones** 分子伴侣

•Some proteins require the action of specialized proteins called ***molecular chaperones*** to achieve proper folding.

•Functions to keep the newly formed protein out of trouble.

- o improper folding
- o aggregation

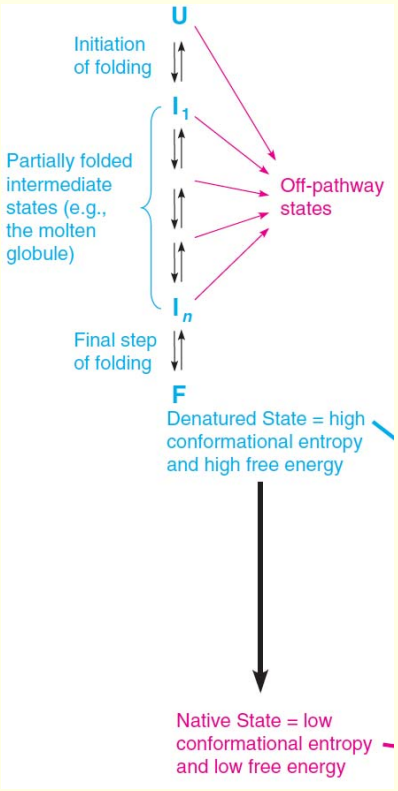
The GroEL-GroES chaperonin



Kinetics of Protein Folding 蛋白質摺疊的動力學

•The folding of globular proteins from their denatured conformations is a remarkably rapid process, often complete in less than a second.

•Protein folding is not a completely random search through a vast conformational space.



A simplified representation of the folding pathway for a protein:

- "U" is the unfolded or denatured state.
- "F" is the folded or native state.
- "I" "on-pathway" are intermediate states.
- Off-pathway states include aggregates and other non-native states that may be kinetic or thermodynamic "dead-ends"
- Thus, the paths to these states are generally shown as irreversible.
- In fact, not all pathways leading to such states are irreversible.
- Folding can be delayed by trapping of molecules in "off-pathway" states.

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In the "energy landscape" model, the trajectory of protein folding is "downhill"— it proceeds with a decrease in free energy.

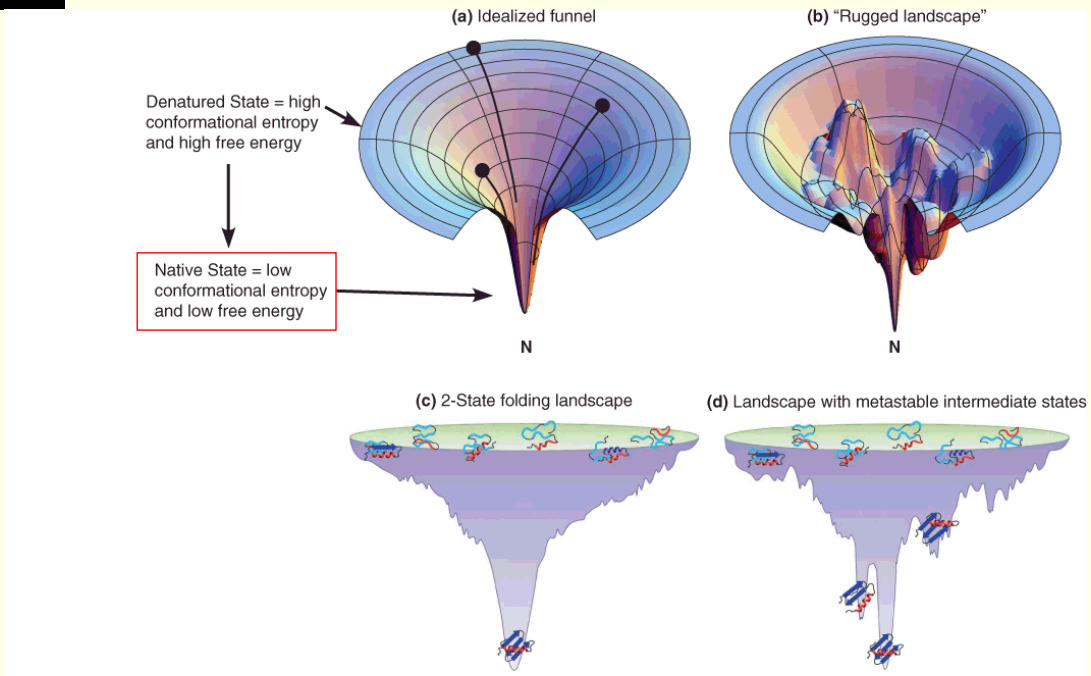
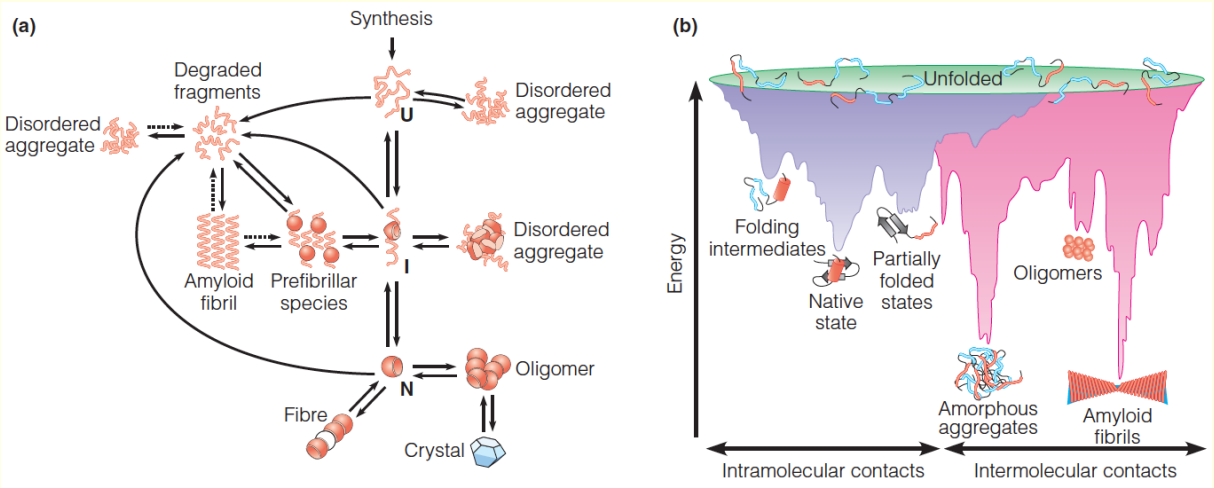


FIGURE 6.29

Protein folding and aggregation

•A pathway model showing various protein conformations and their interconversions.

•The same information shown in an energy landscape model.



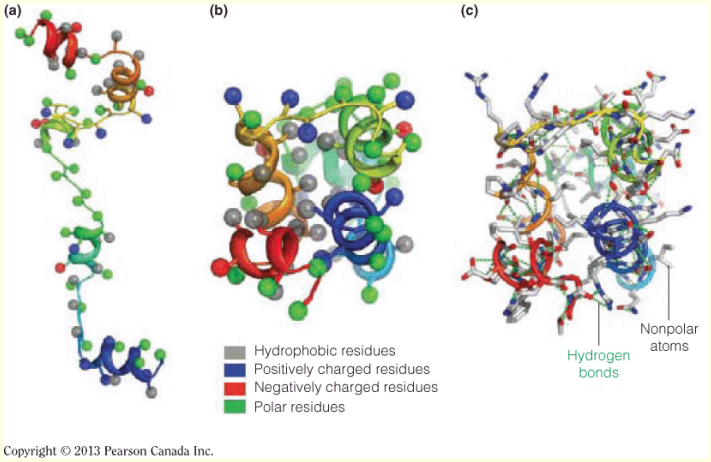
Protein misfolding 折疊錯誤

Protein misfolding is the basis for several diseases, including Alzheimer's disease and Parkinson's disease.

TABLE 6.6 Examples of amyloid-related human diseases

Disease	Associated Protein
Alzheimer's disease	Amyloid β or "Aβ" peptide
Parkinson's disease	α-Synuclein
Spongiform encephalopathies (e.g., Creutzfeldt-Jakob disease; kuru; etc.)	prion protein
Amyotrophic lateral sclerosis (Lou Gehrig's disease)	Superoxide dismutase I
Huntington's disease	Huntingtin with polyQ tracts
Cataract	γ-Crystallins
Type II diabetes	Islet amyloid polypeptide (IAPP)
Injection-localized amyloidosis	Insulin

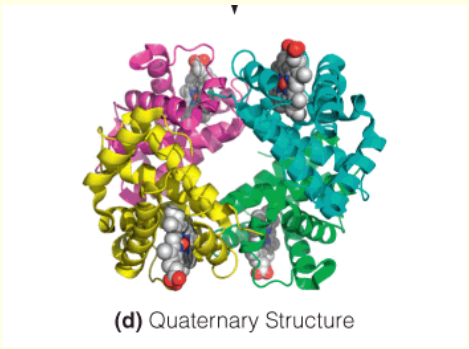
Prediction of Tertiary Protein Structure



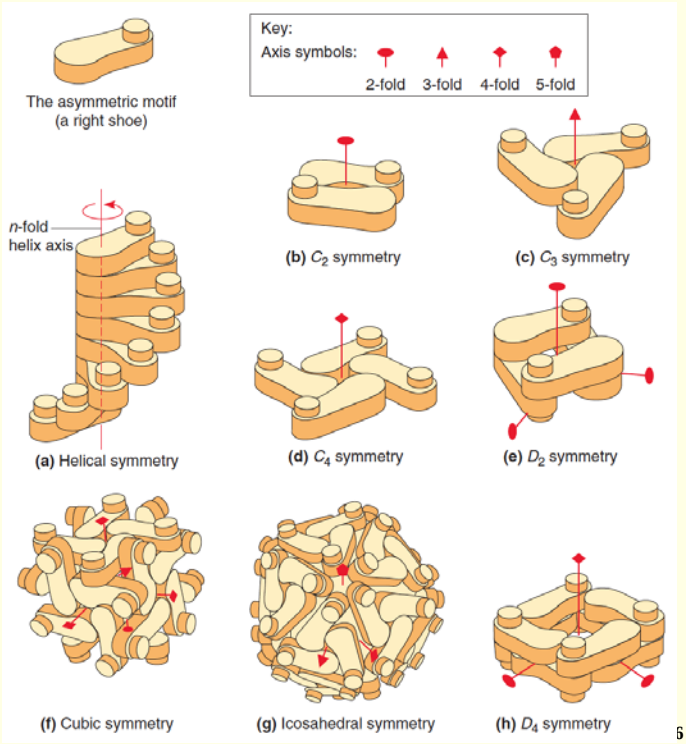
Schematic of *de novo* structure prediction using Rosetta:

- Assembly of fragments of local secondary structure.
- Final low-energy conformation produced by fragment packing.
- All-atom model produced after high-resolution refinement.

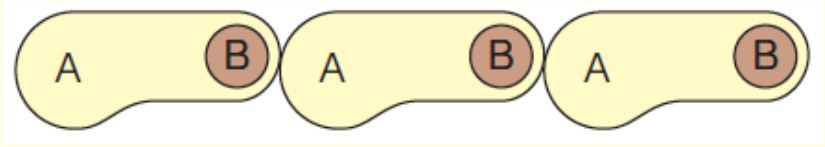
Quaternary Structure of Proteins



Tetramer



Multisubunit Proteins: Homotypic Protein–Protein Interactions



•The interactions between the folded polypeptide chains in multisubunit proteins are of the same kinds that stabilize tertiary structure

- o salt bridges
- o hydrogen bonding
- o van der Waals forces
- o the hydrophobic effect
- o disulfide bonding

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Protein Structure Methods (I)

X-Ray Crystallography X-光繞射

Steps needed:

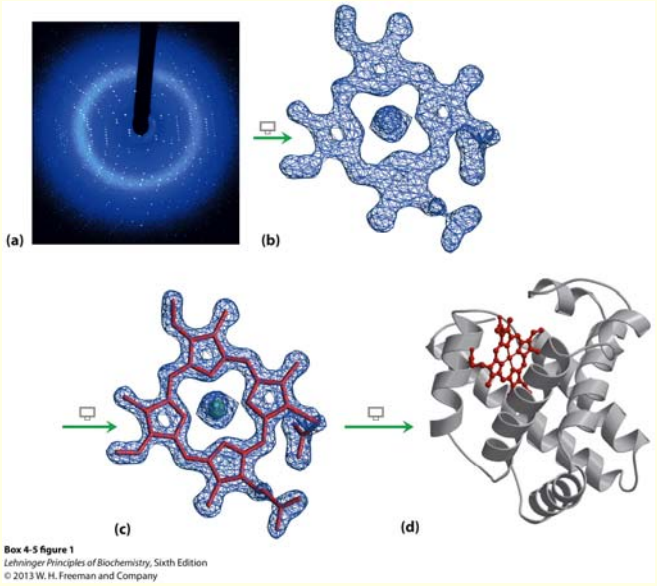
- Purify the protein
- Crystallize the protein
- Collect diffraction data
- Calculate electron density
- Fit residues into density

Pros:

- No size limits
- Well-established

Cons:

- Difficult for membrane proteins
- Cannot see hydrogens



Box 4-5 figure 1
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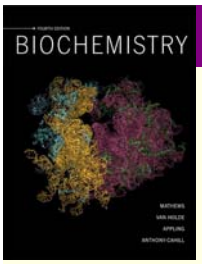


Protein Structure Methods (II)

Nuclear Magnetic Resonance (NMR) 核磁共振

Steps needed:

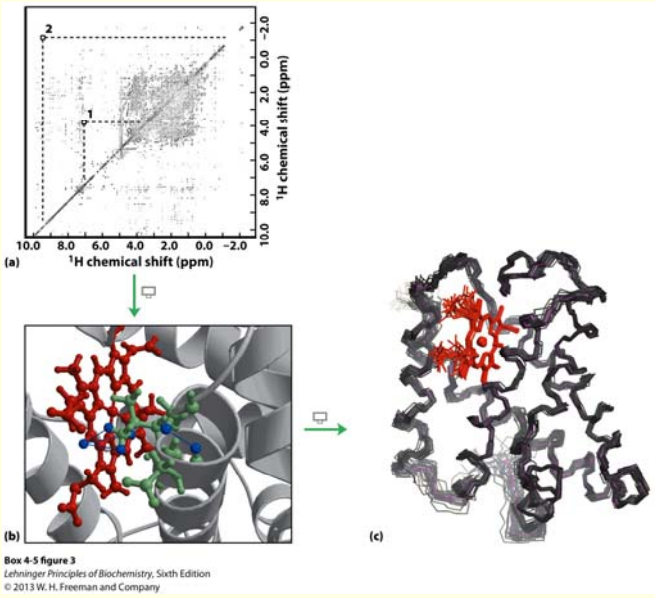
- Purify the protein
- Dissolve the protein
- Collect NMR data
- Assign NMR signals
- Calculate the structure

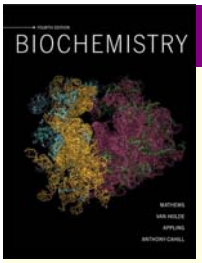
Pros:

- No need to crystallize the protein
- Can see many hydrogens

Cons:

- Difficult for insoluble proteins
- Works best with small proteins





Protein Structure Methods (III)

Circular dichroism (CD) 圓偏光二色光譜

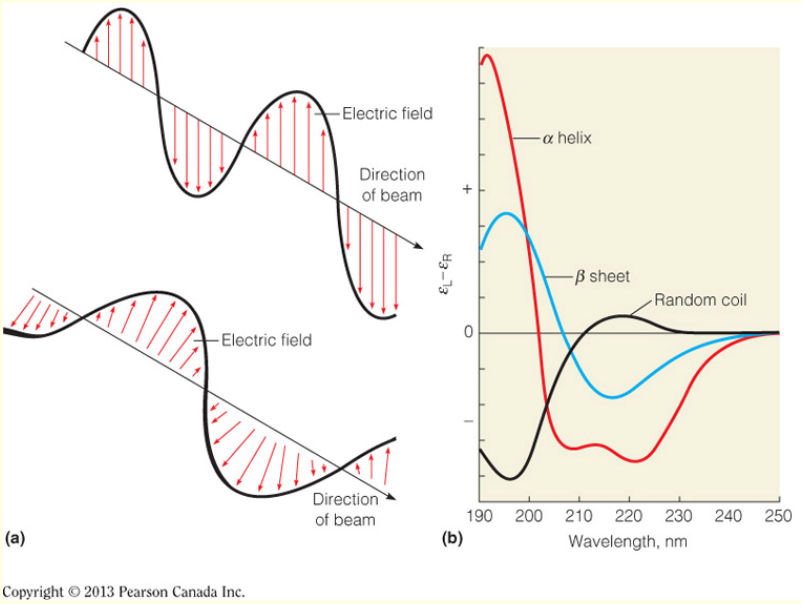


FIGURE 6A.9
Circular dichroism. (a) Polarization of light. Above: Plane polarized light, in which the amplitude of the electric field oscillates in a single plane. Below: In circularly polarized light, the oscillation of the electric field follows a helical path around the axis describing the direction of the beam. **(b)** Circular dichroism spectra for polypeptides in various conformations. Here the *y*-axis records differences in molar absorptivity (ε) between left- and right-circularly polarized light.

Thank you