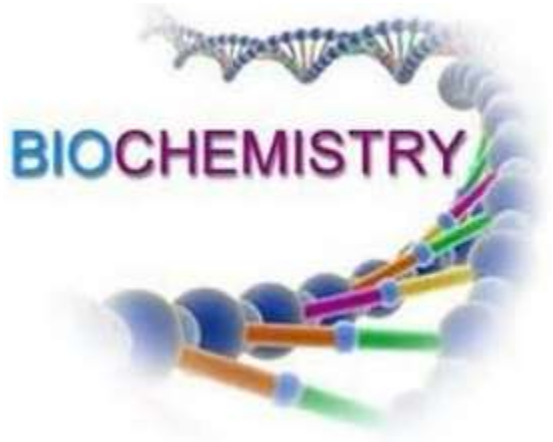
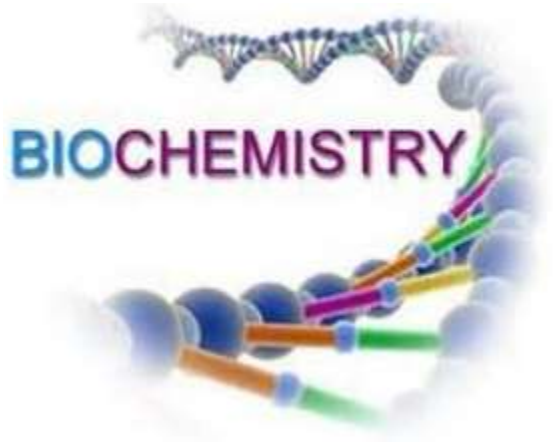
CHAPTER 3

Amino Acids, Peptides, and Proteins



CHAPTER 4

The Three-Dimensional Structure of Proteins

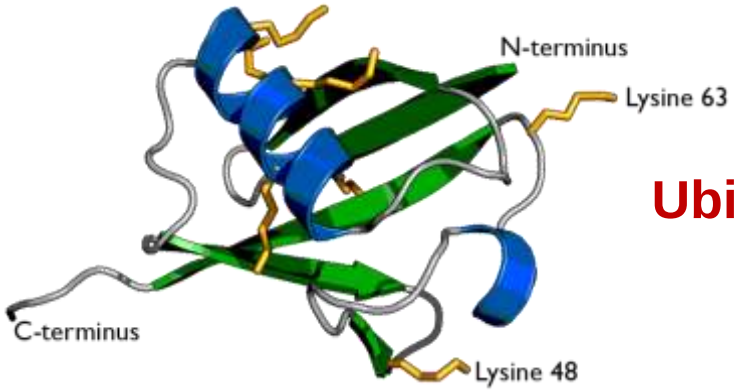


4.1 Overview of Protein Structure

■ Classification of Protein

Proteins are composed of one or more polypeptide chains

monomeric proteins（单体蛋白质）

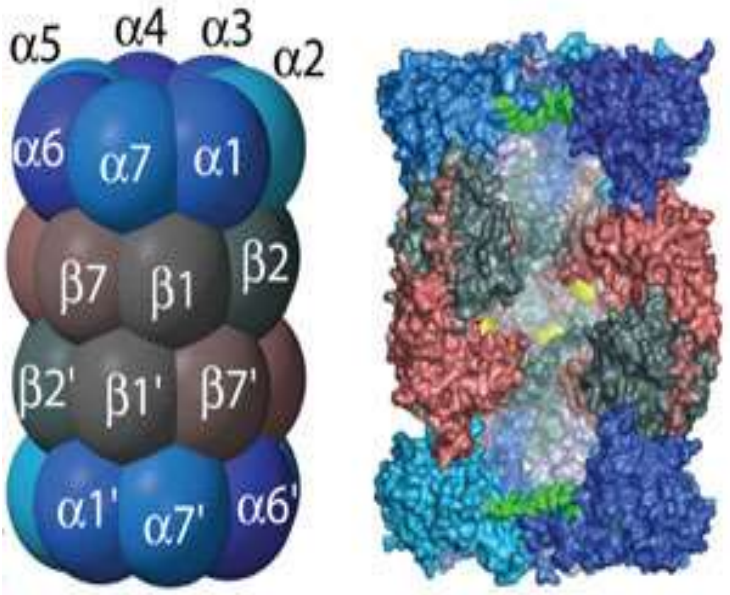


Ubiquitin

multimeric proteins (多亚基蛋白质)

homomultimeric proteins

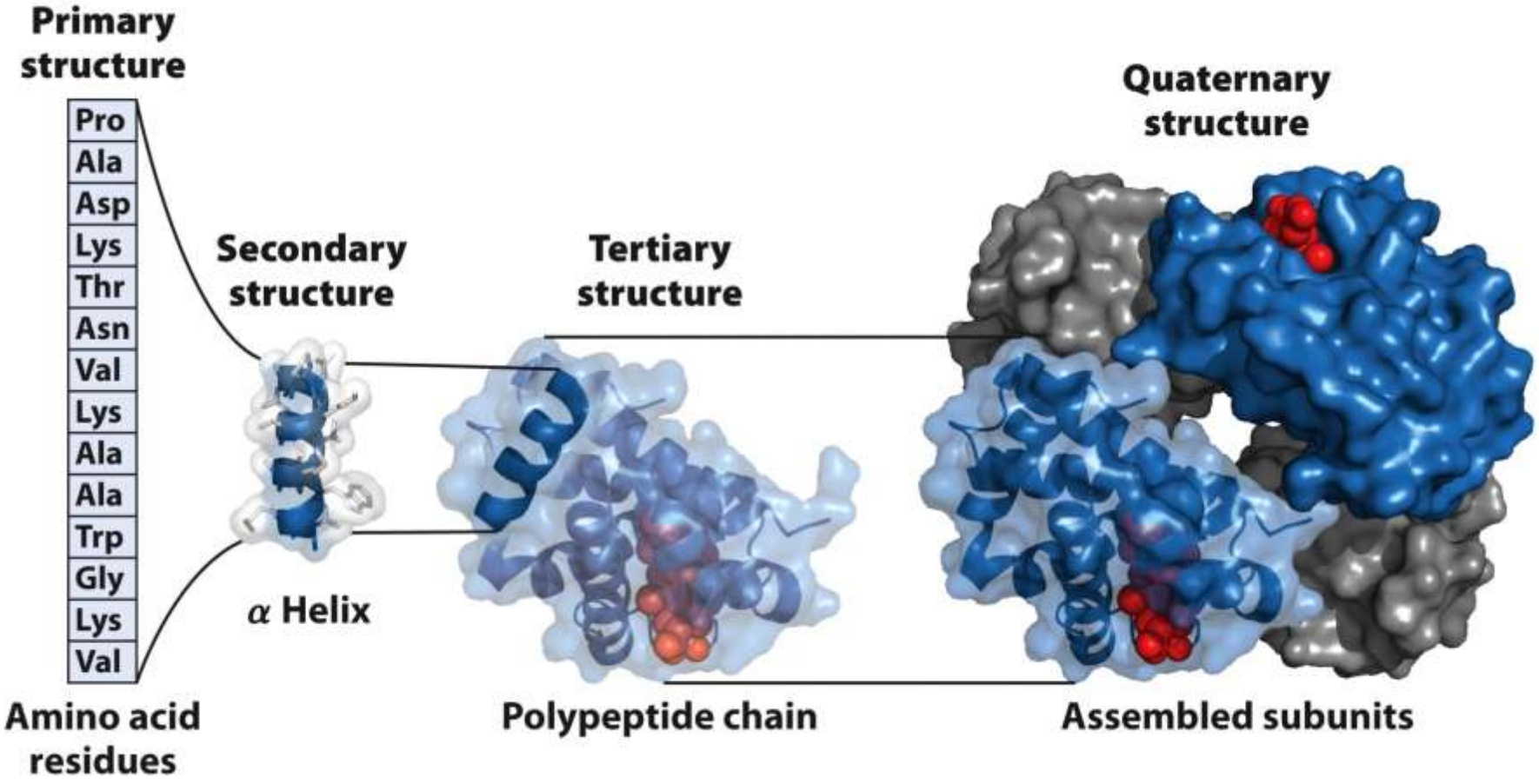
heteromultimeric proteins



20S proteasome

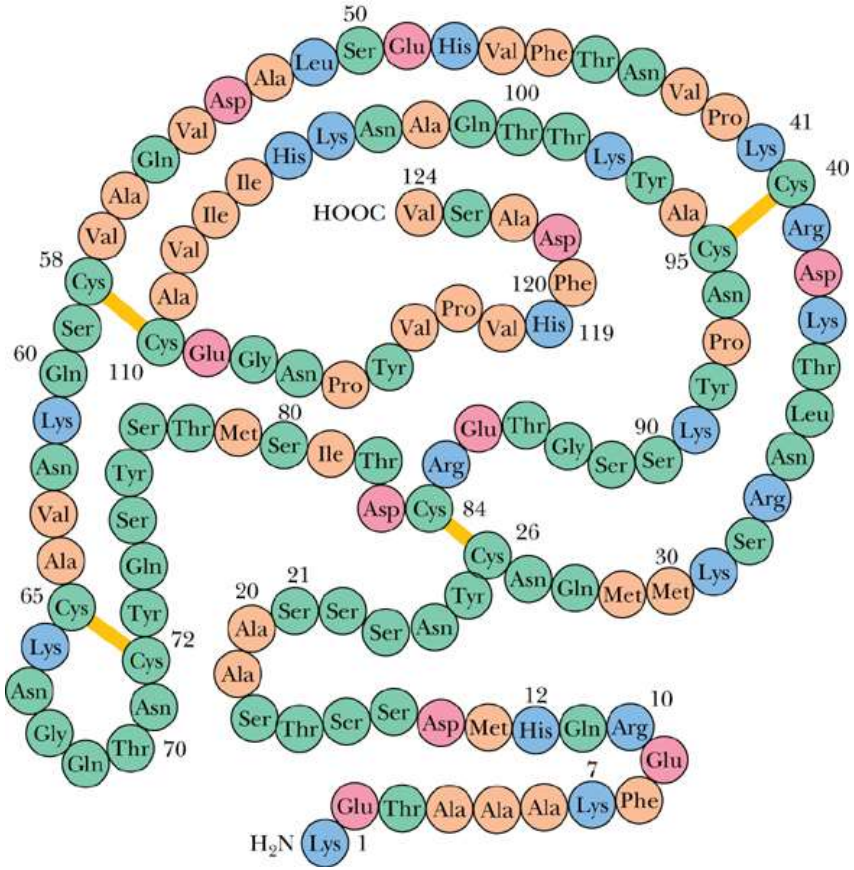
4.1 Overview of Protein Structure

■ Levels of structure in proteins



4.1 Overview of Protein Structure

- **Primary Structure: the *sequence* of amino acid residues**



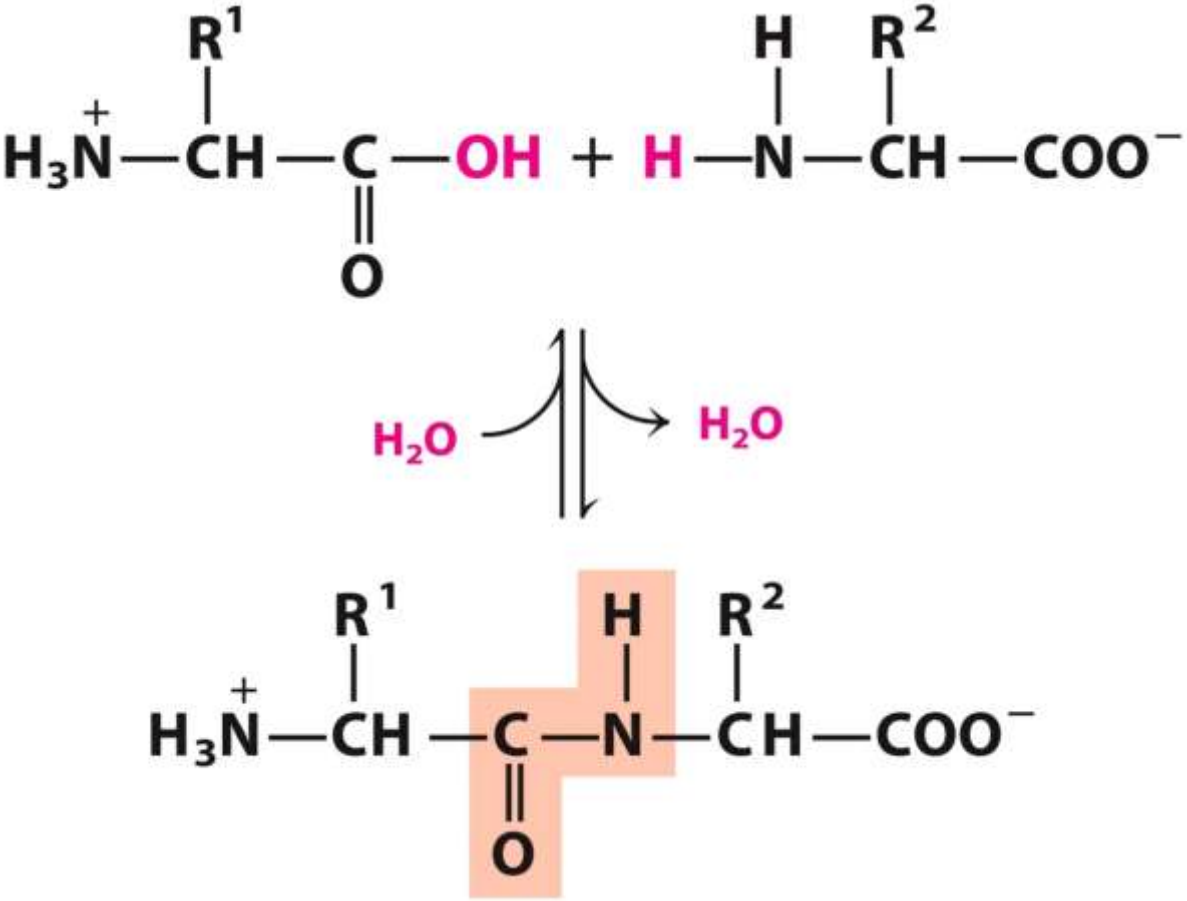
```
KETAAAKFER  QHMDSSTSAA
SSSNYCNQMM  KSRNLTKDRC
KPVNTFVHES  LADVQAVCSQ
KNVACKNGQT  NCYQSYSTMS
ITDCRETGSS  KYPNCAYKTT
QANKHIIVAC  EGNPYVPVHF
DASV
```

All covalent bonds (mainly peptide bonds and disulfide bonds) linking amino acid residues in a polypeptide chain is its **primary structure**.

Bovine pancreatic ribonuclease A

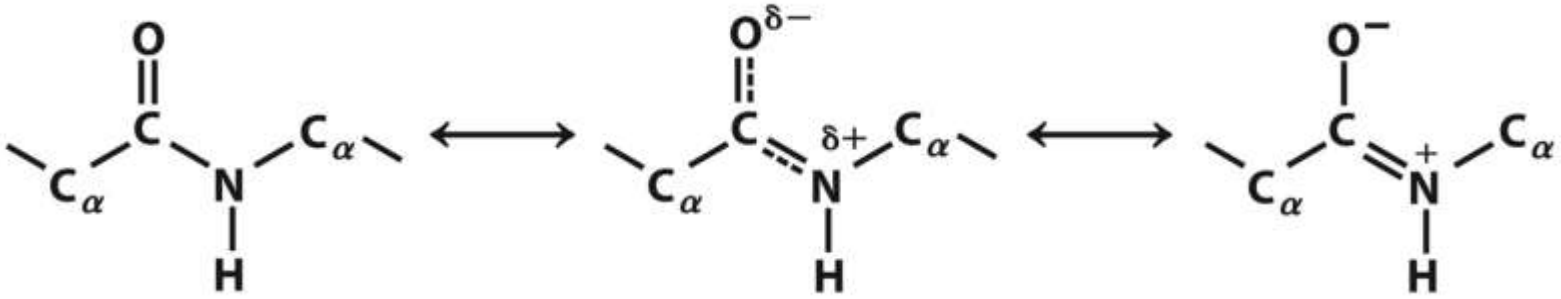
4.2 Protein Secondary Structure

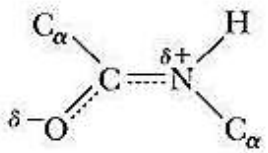
- **Formation of peptide bond**

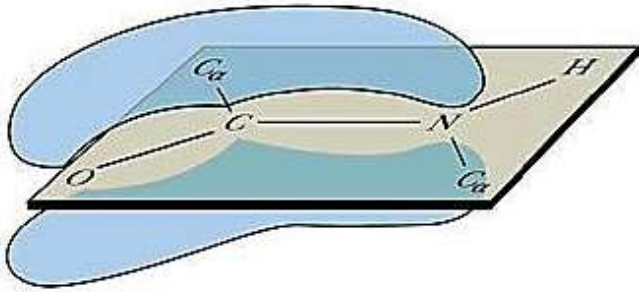


4.2 Protein Secondary Structure

- **Peptide bond has partial double-bond character**



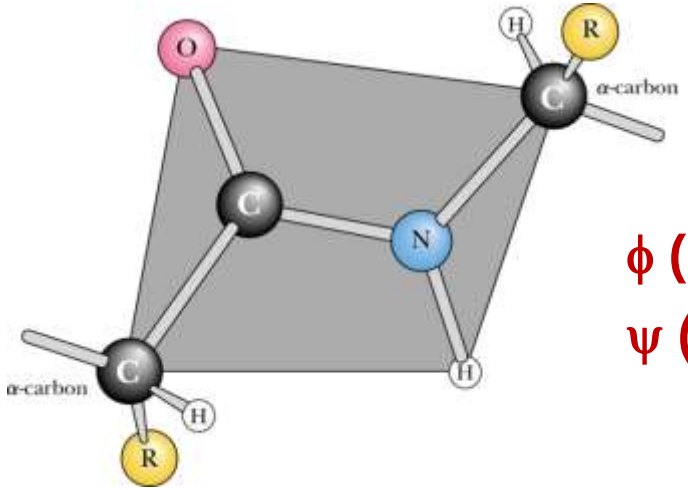




The true electron density is intermediate. The barrier to C—N bond rotation of about 88 kJ/mol is enough to keep the amide group planar.

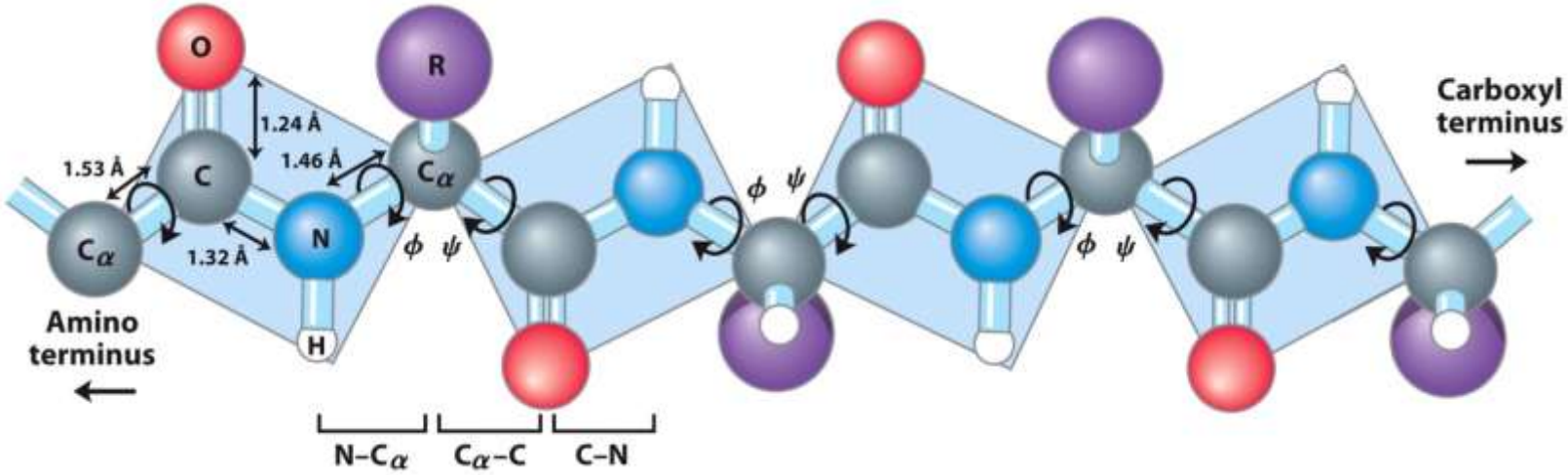
4.2 Protein Secondary Structure

- **The peptide bond is rigid and planar**



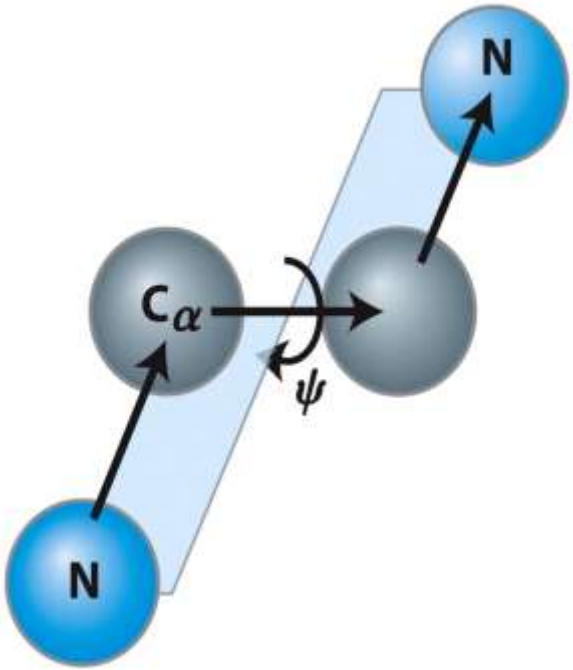
ϕ (phi): angle of rotation of N-C_α bond

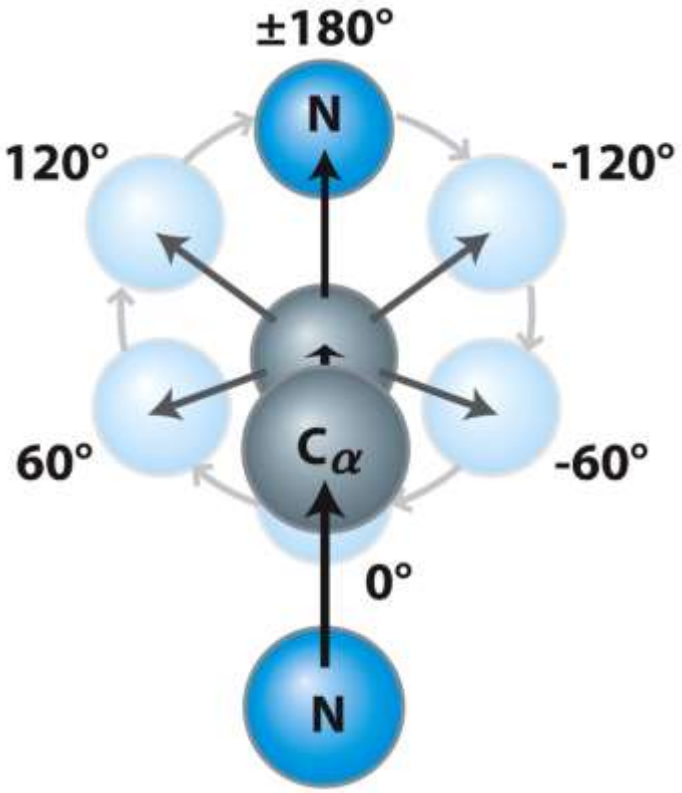
ψ (psi): angle of rotation of C_α-C_o bond



4.2 Protein Secondary Structure

- **Possible conformations of two peptide planes**

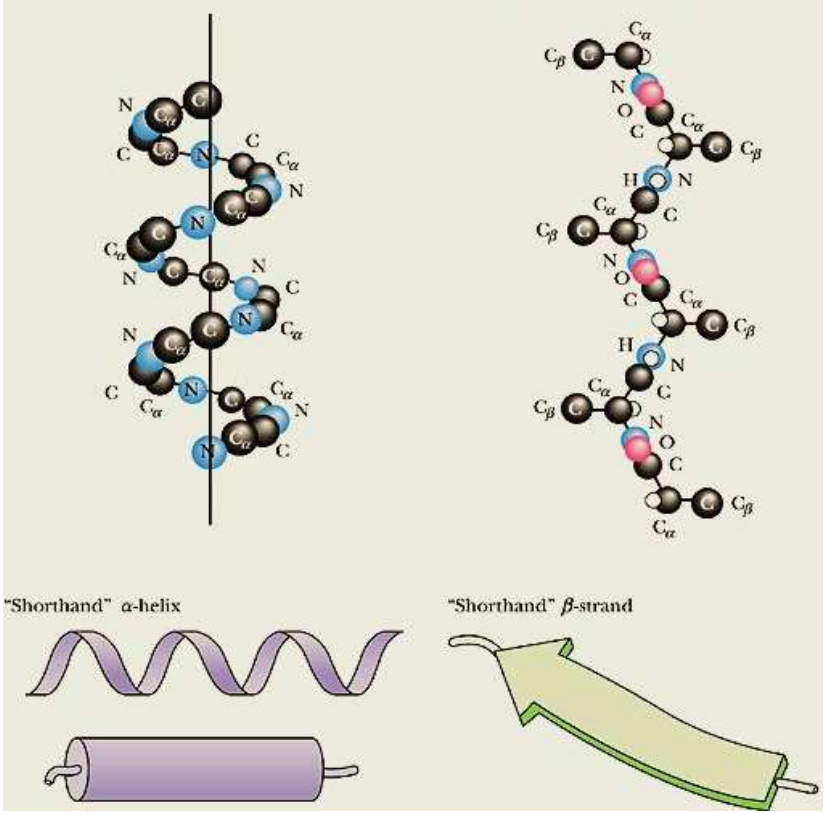




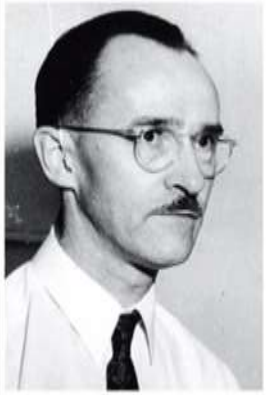
ϕ And ψ are 180° (or -180°) when the first and fourth atoms are farthest apart and the peptide is fully extended.

4.2 Protein Secondary Structure

- **Protein secondary structure: local conformation of some part of a polypeptide**




Linus Pauling, 1901–1994


Robert Corey, 1897–1971

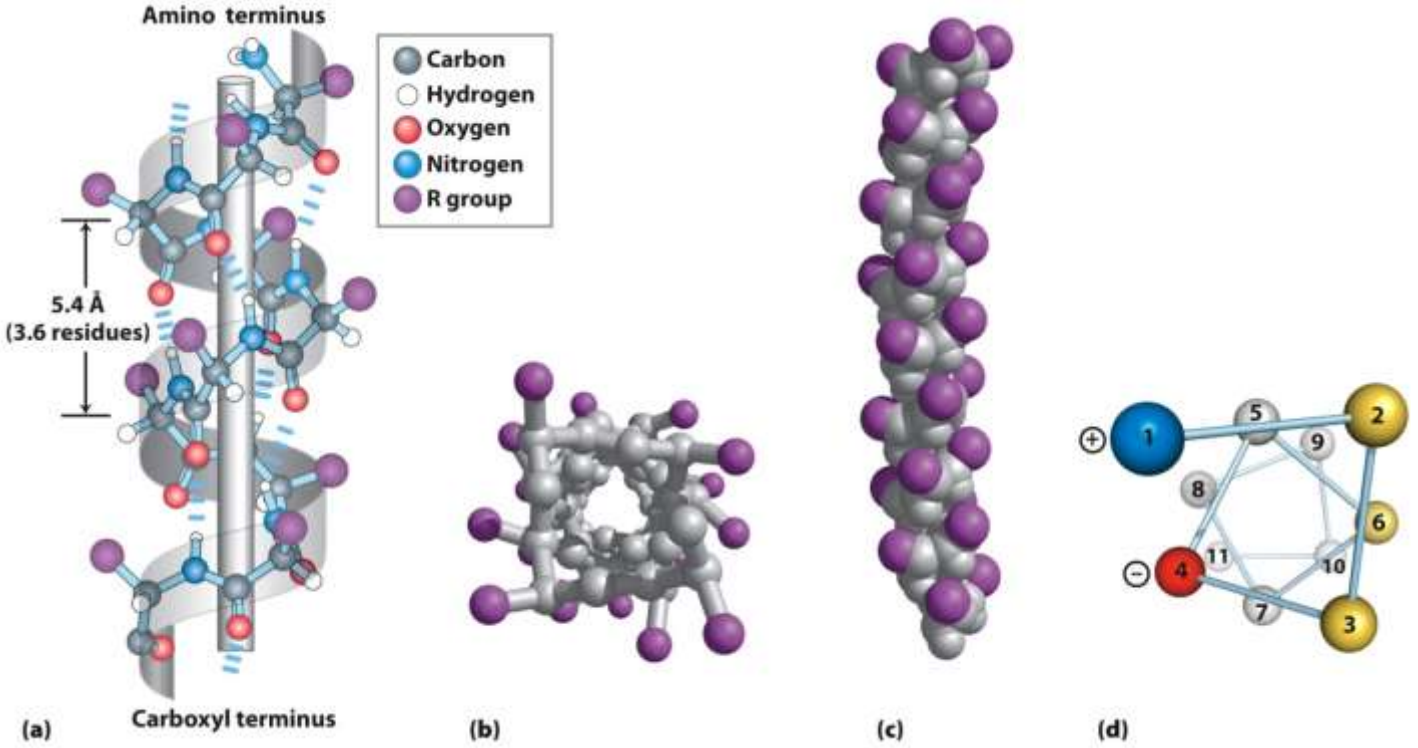
α-helix

β-sheet

Secondary structure refers to any chosen segment of a polypeptide chain and describes the local spatial arrangement of its main-chain atoms, without regard to the positioning of its side chains or its relationship to other segments.

4.2 Protein Secondary Structure

α-helix



1.The helix is formed when the values of ϕ are approximately -60° and the values of ψ are in the range of -45° to -50° .

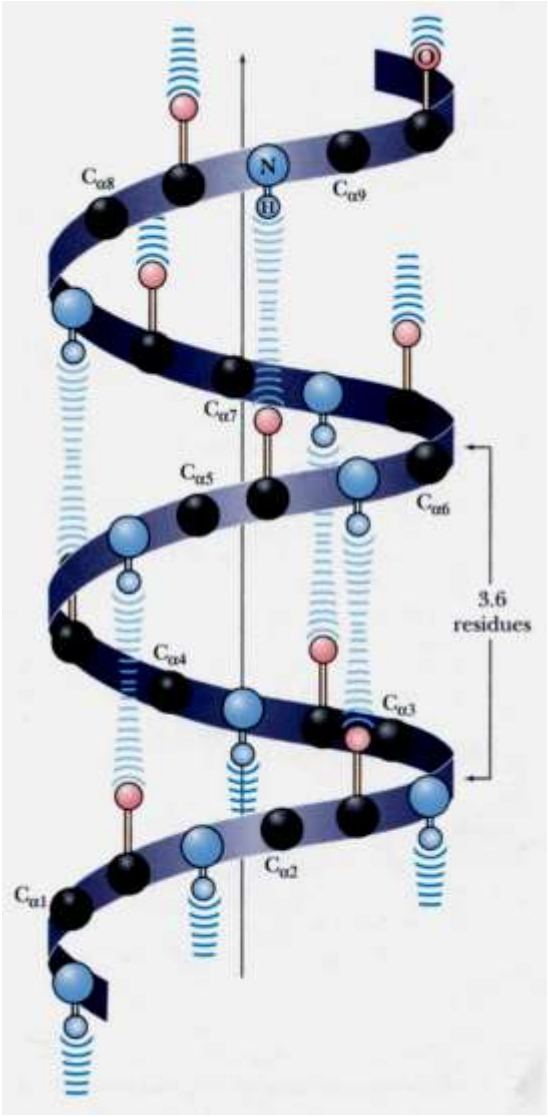
2. Each amino acid residue extends 1.5 Å along the helix axis, and there are 3.6 residues per turn , this amounts to 0.54 nm of travel along the helix axis per turn.

4.2 Protein Secondary Structure

3. One turn of the helix represents 3.6 amino acid residues, and a single turn of the α-helix involves 13 atoms from the O to the H of the H bond.

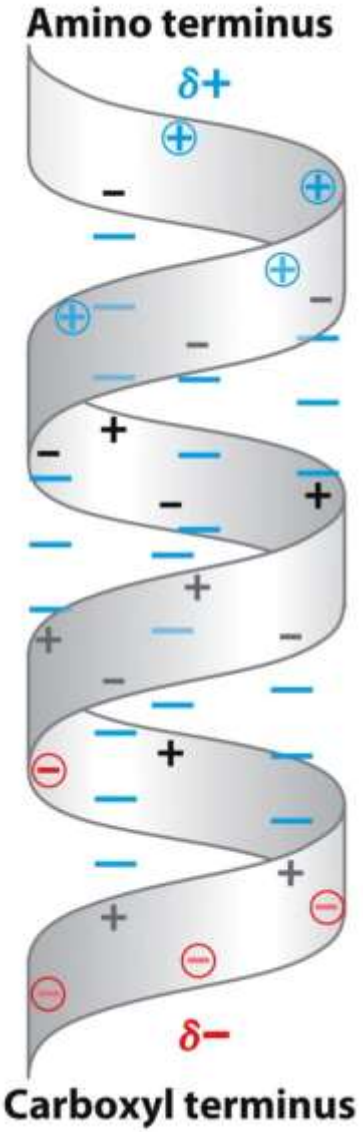
4. Each peptide C=O is hydrogen bonded to the peptide N-H group four residues farther up the chain, and all the hydrogen bonds point in the same direction along the helix axis.

5. The side chains extend outward from the core structure of the helix, the helix is about 0.6 nm in diameter without regarding the side chains.



4.2 Protein Secondary Structure

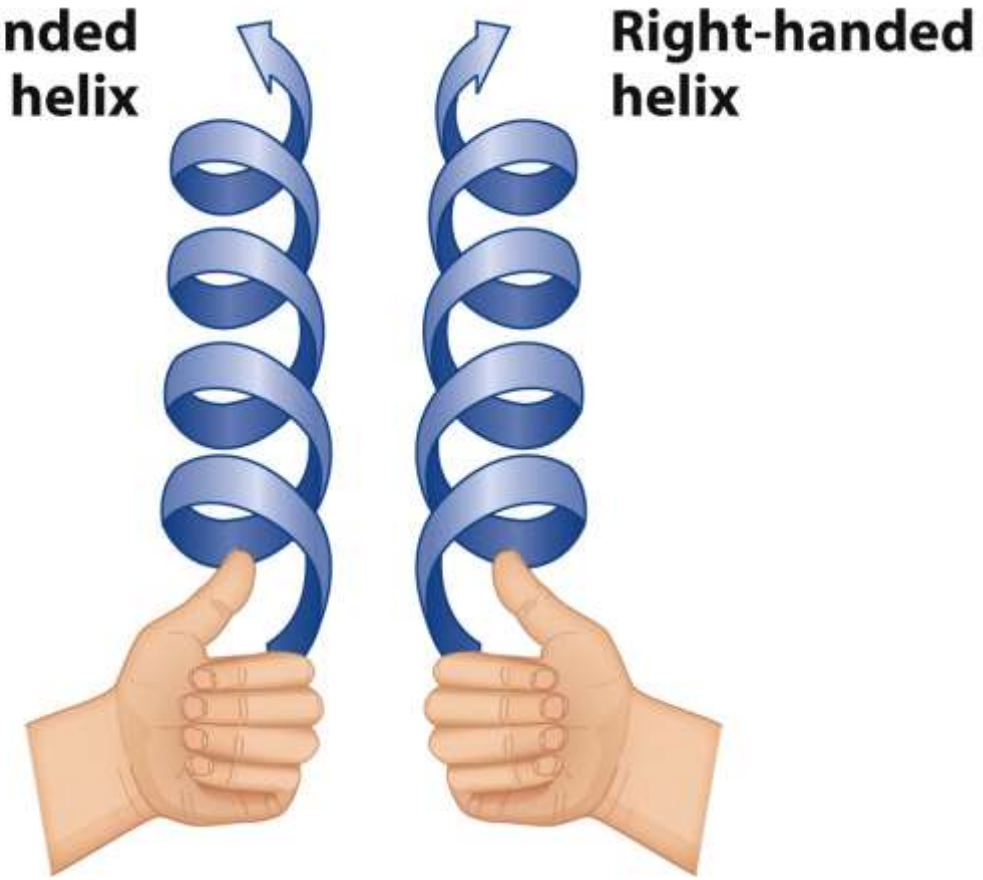
6. Because N-H and C=O groups are all aligned along the helix axis, the helix itself has a partial negative charge at the C-terminus and positive charge at the N-terminus.



4.2 Protein Secondary Structure

- **Right-handed or left-handed helical structure**

All a-helix in proteins are right handed. Extended left-handed helices have not been observed in proteins.



4.2 Protein Secondary Structure

- **Amino acid sequence affects α-helix stability**

Factors that affect the stability of a helix:

1. The electrostatic repulsion (or attraction) between successive amino acid residues with charged R groups,
2. The bulkiness of adjacent R groups,
3. The interactions between R groups spaced three (or four) residues apart,
4. The occurrence of Pro and Gly residues,
5. The interaction between amino acid residues at the ends of the helical segment and the electric dipole inherent to the helix.

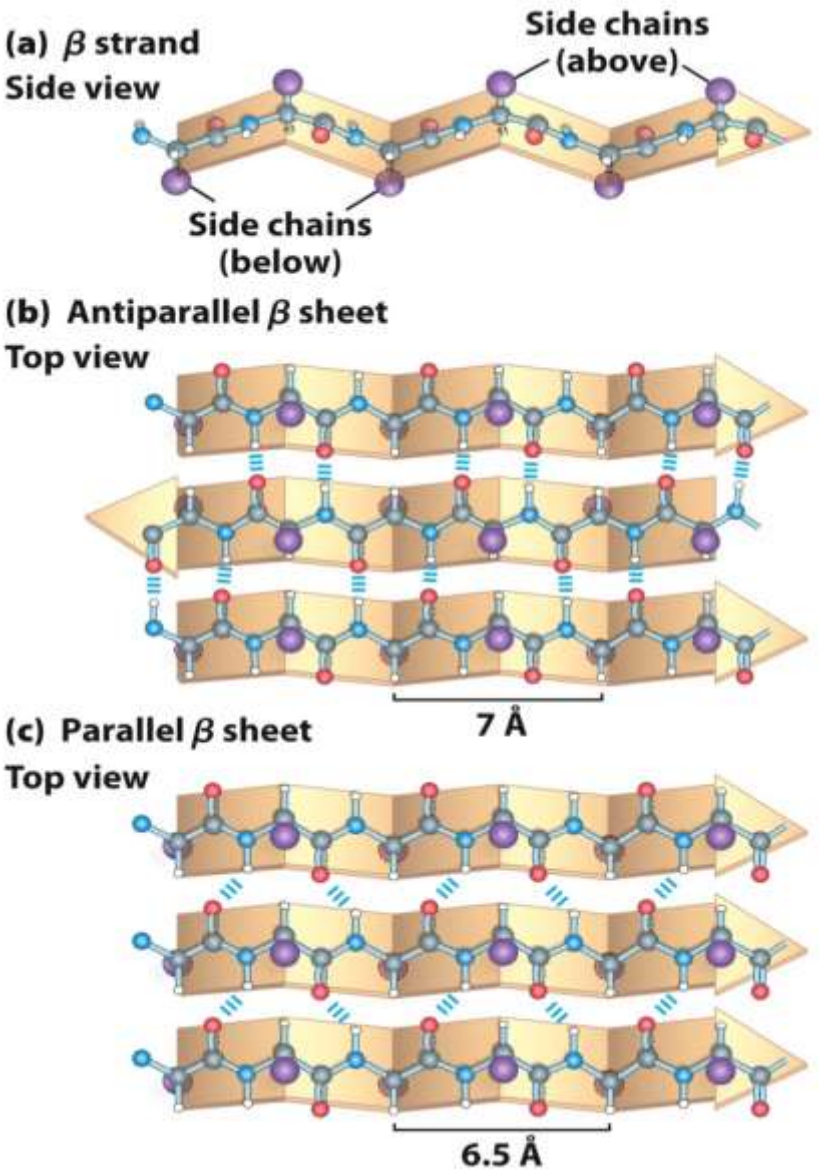
4.2 Protein Secondary Structure

β-sheet

The backbone of polypeptide chain extended into a zigzag structure is called **β conformation**.

The arrangement of several segments side by side, all of which are in the β conformation, is called a **β sheet**.

Hydrogen bonds are formed between adjacent segments of polypeptide chain within the sheet.



4.2 Protein Secondary Structure

- **The characteristics of β-pleated sheet**

1. Laying thin, extended strips of amide plans make a pleated sheet. The hydrogen bonds in this structure are essentially inter-strand rather than intra-strand.

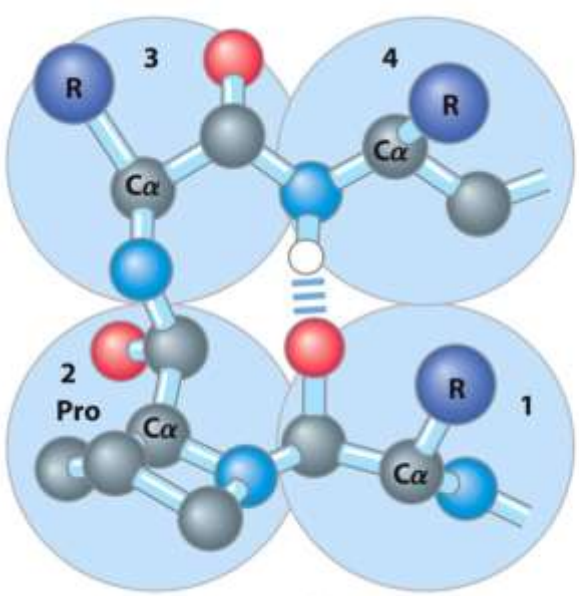
2. The R groups of adjacent amino acids protrude in opposite directions from the zigzag structure, creating an alternating pattern as seen in the side view.

3. The adjacent polypeptide chains in a β pleated sheet can be either **parallel** (having the same amino-to-carboxyl polypeptide orientation) or **antiparallel** (having the opposite amino-to-carboxyl orientation). The structures are similar, although the repeat period is shorter for the parallel conformation (0.65 nm, as opposed to 0.7694 nm for antiparallel).

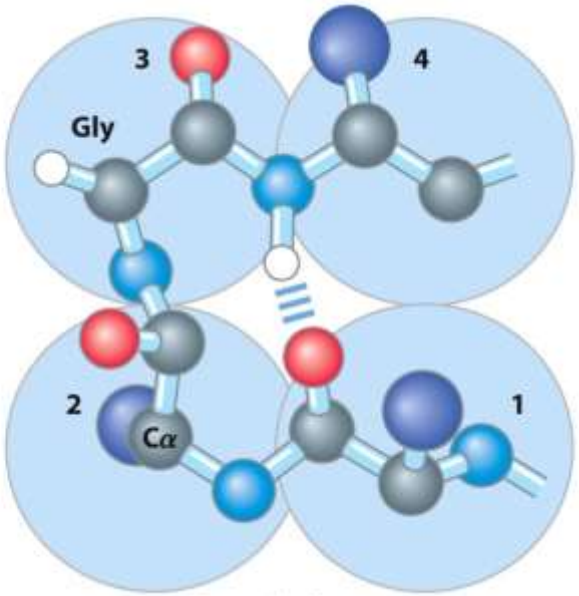
4.2 Protein Secondary Structure

β-turn

The **β-turn** structure is a 180° turn involving four amino acid residues, with the carbonyl oxygen of the first residue forming a hydrogen bond with the amino-group hydrogen of the fourth.

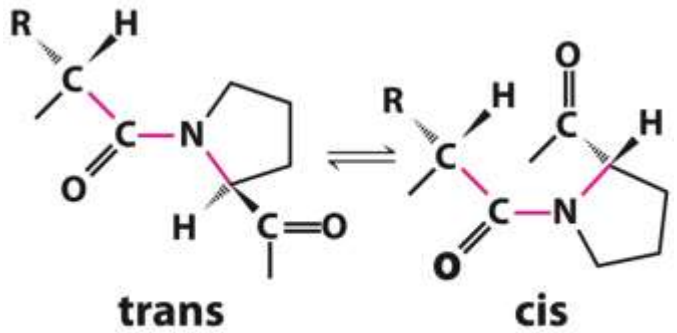


Type I β turn



Type II β turn

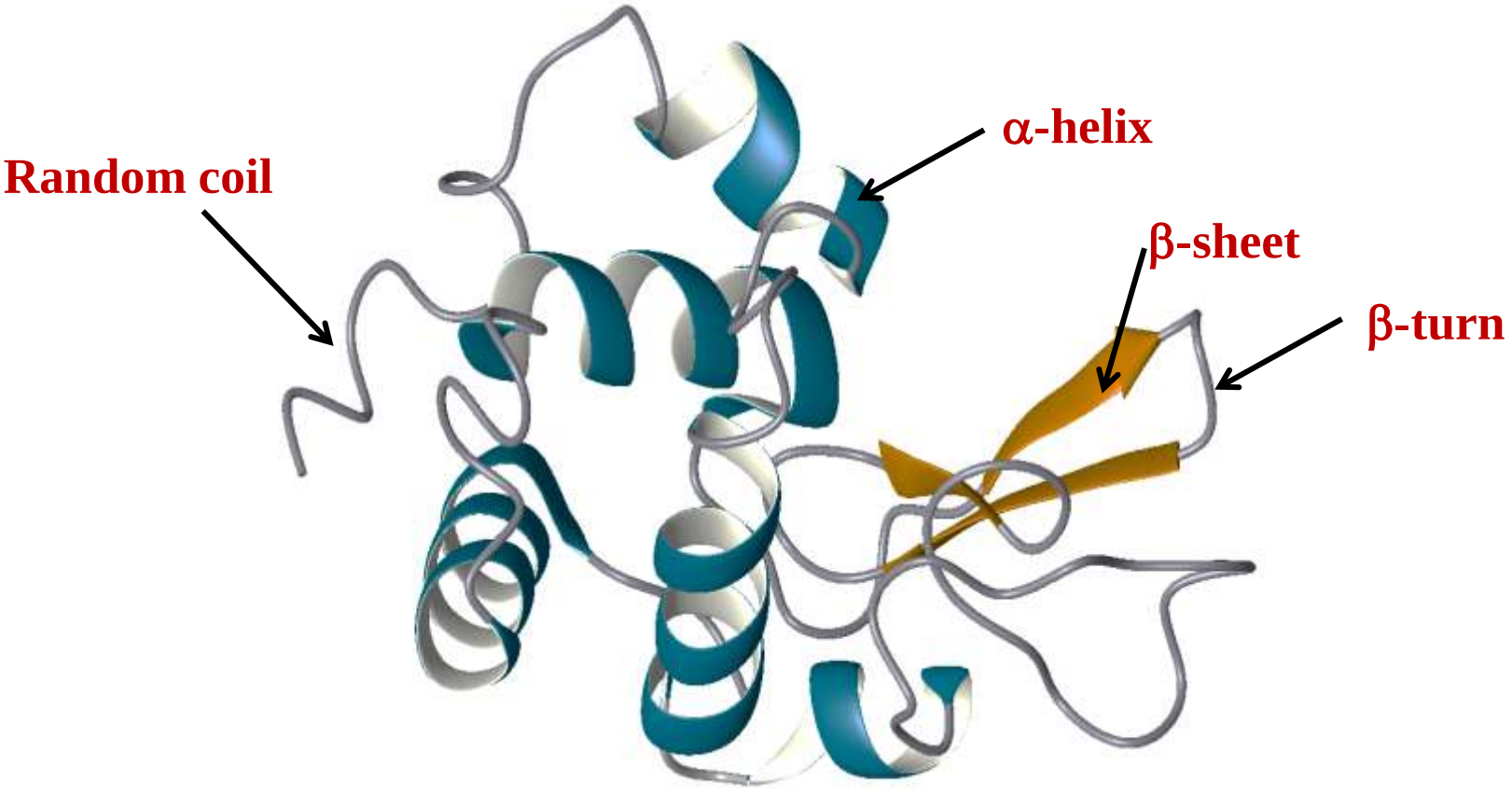
Gly and **Pro** residues often occur in turns, the former because it is small and flexible, the latter because peptide bonds involving the imino nitrogen of proline readily assume the ***cis*** configuration



Proline isomers

4.2 Protein Secondary Structure

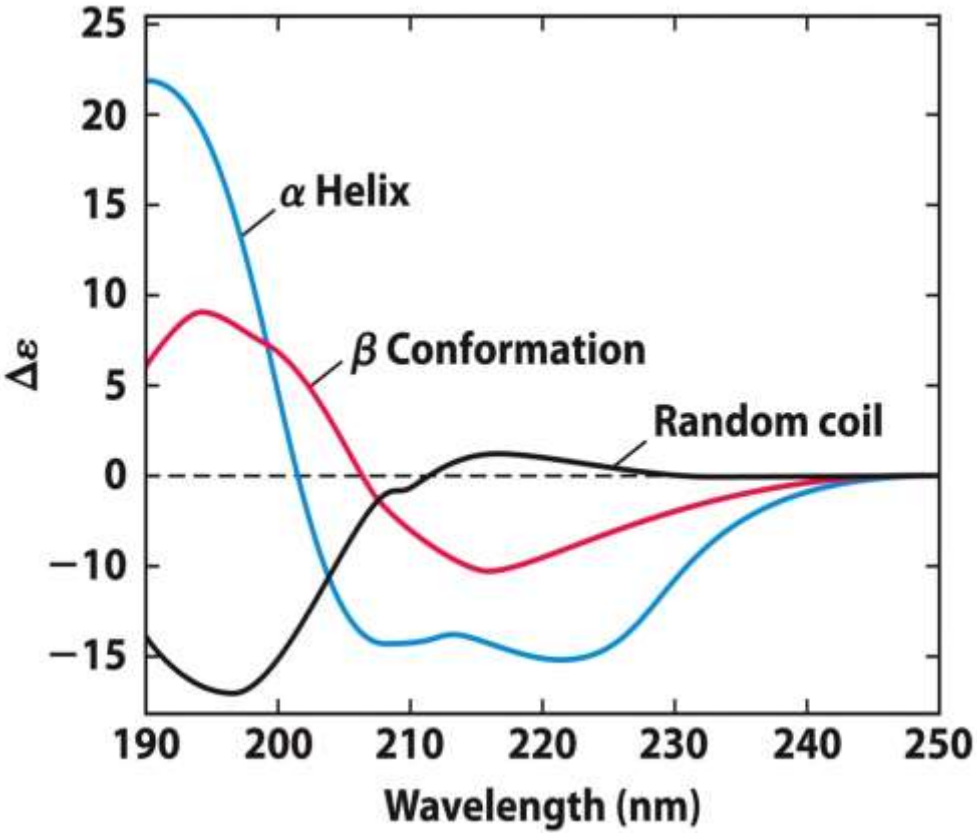
- **Random coil**



4.2 Protein Secondary Structure

- **Assessment of protein secondary**

Any form of structural asymmetry in a molecule gives rise to differences in absorption of left-handed versus right-handed circularly polarized light. Measurement of this difference is called **circular dichroism (CD) spectroscopy.**

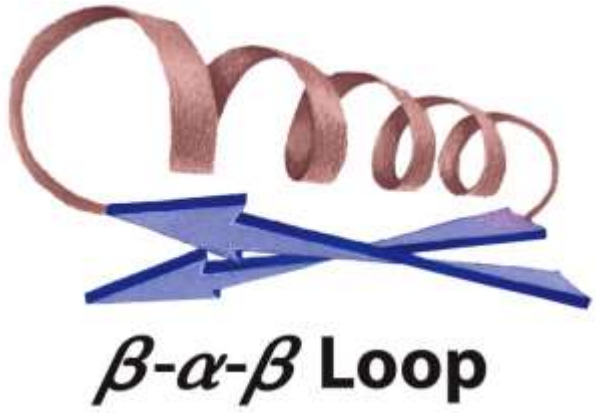


4.3 Protein Tertiary and Quaternary Structures

■ Tertiary structure

The overall three-dimensional arrangement of all atoms in a protein

- **Motif** (also called **fold** or **supersecondary structure**), is stable arrangements of two or more elements of secondary structure and the connection(s) between them.

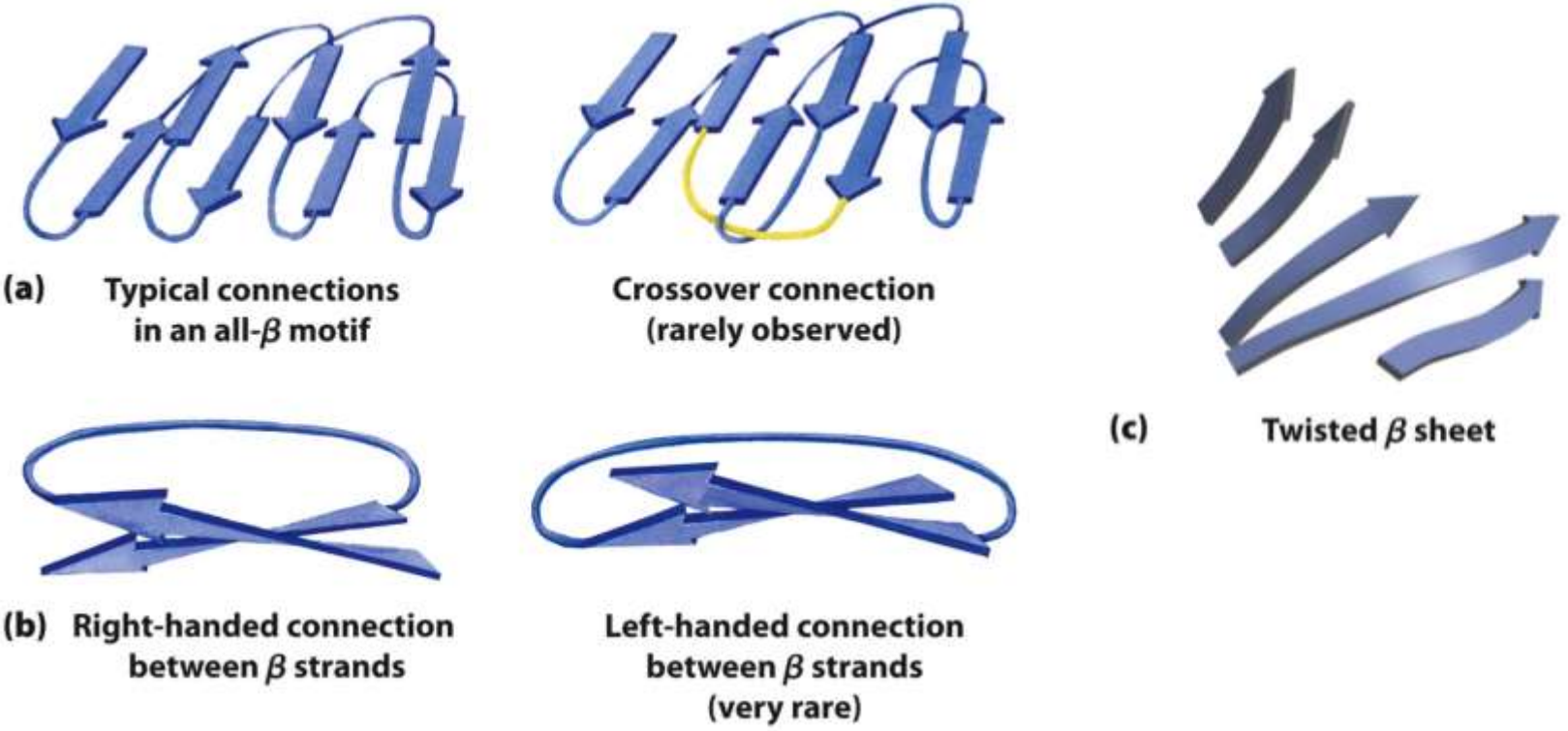


β-α-β Loop

β Barrel

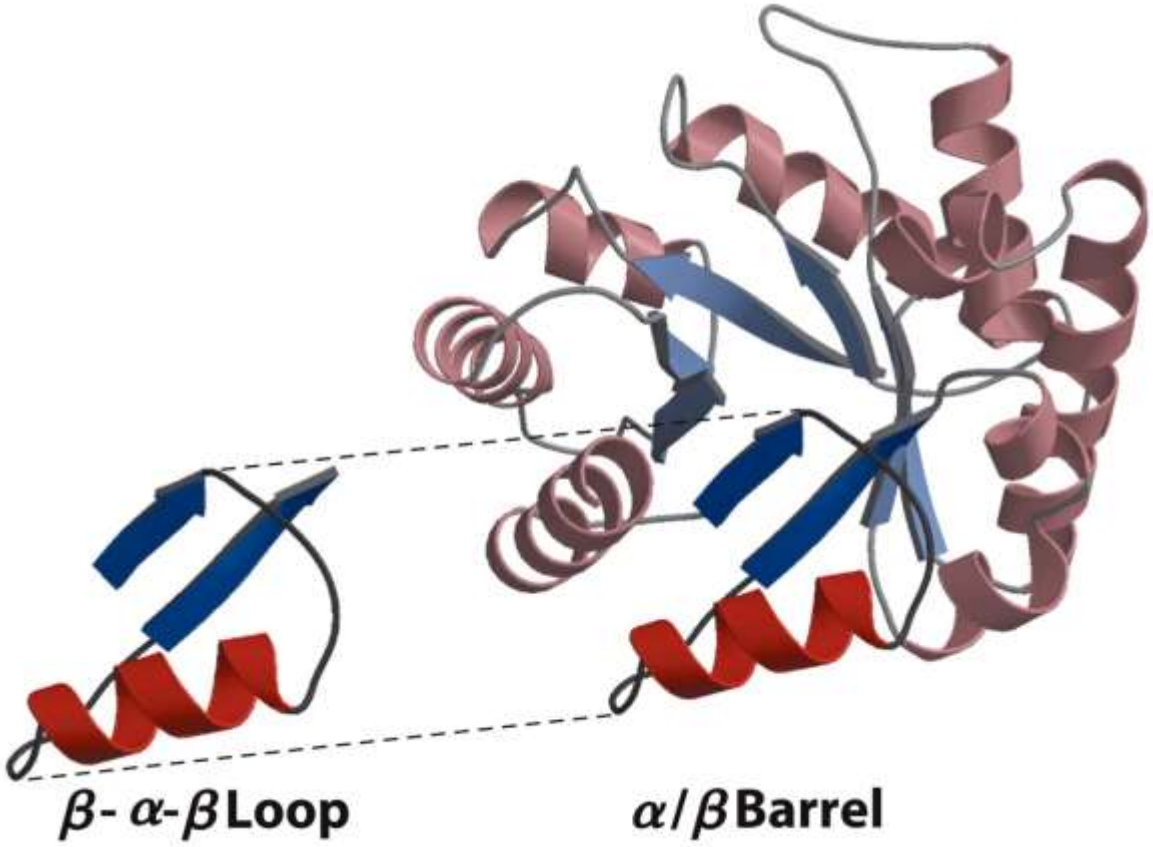
4.3 Protein Tertiary and Quaternary Structures

- **Some stable folding patterns in proteins**



4.3 Protein Tertiary and Quaternary Structures

- **Constructing large motifs from smaller ones**

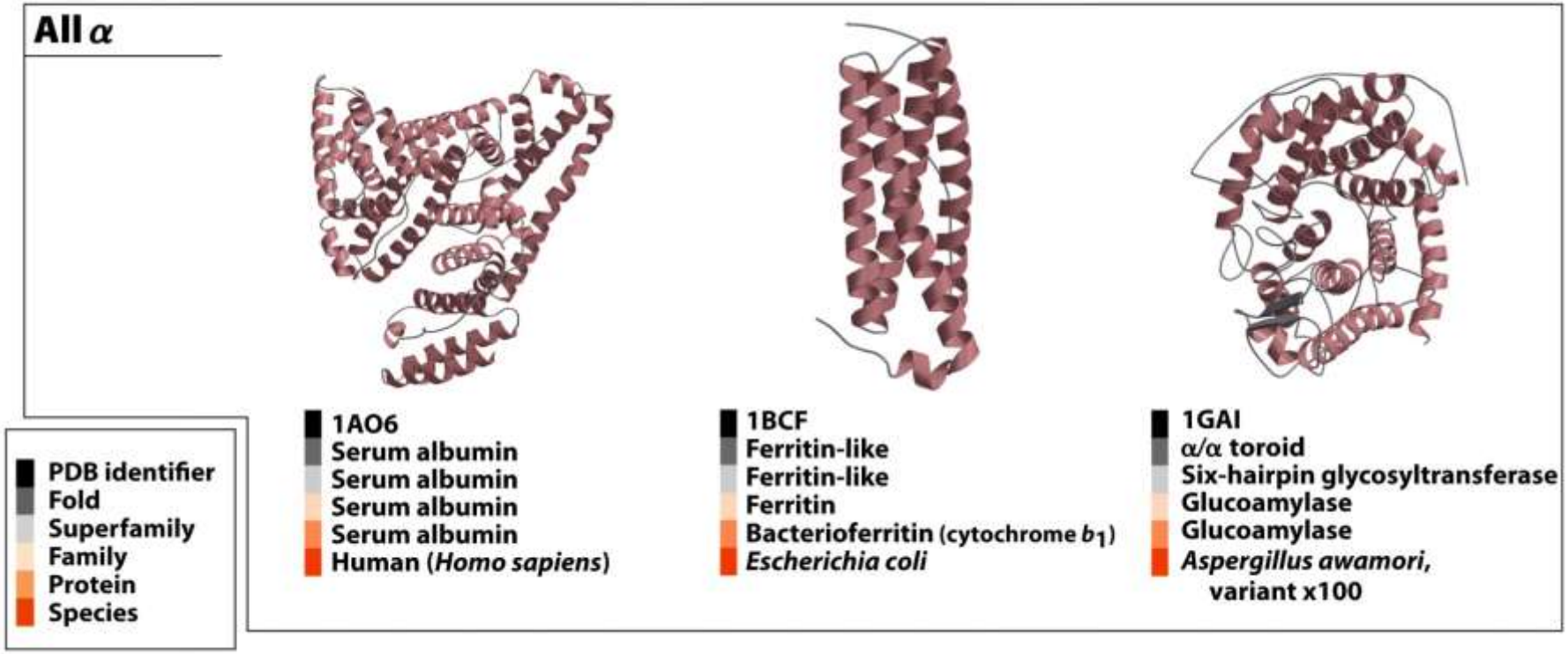


Complex motifs can be built up from simple ones.

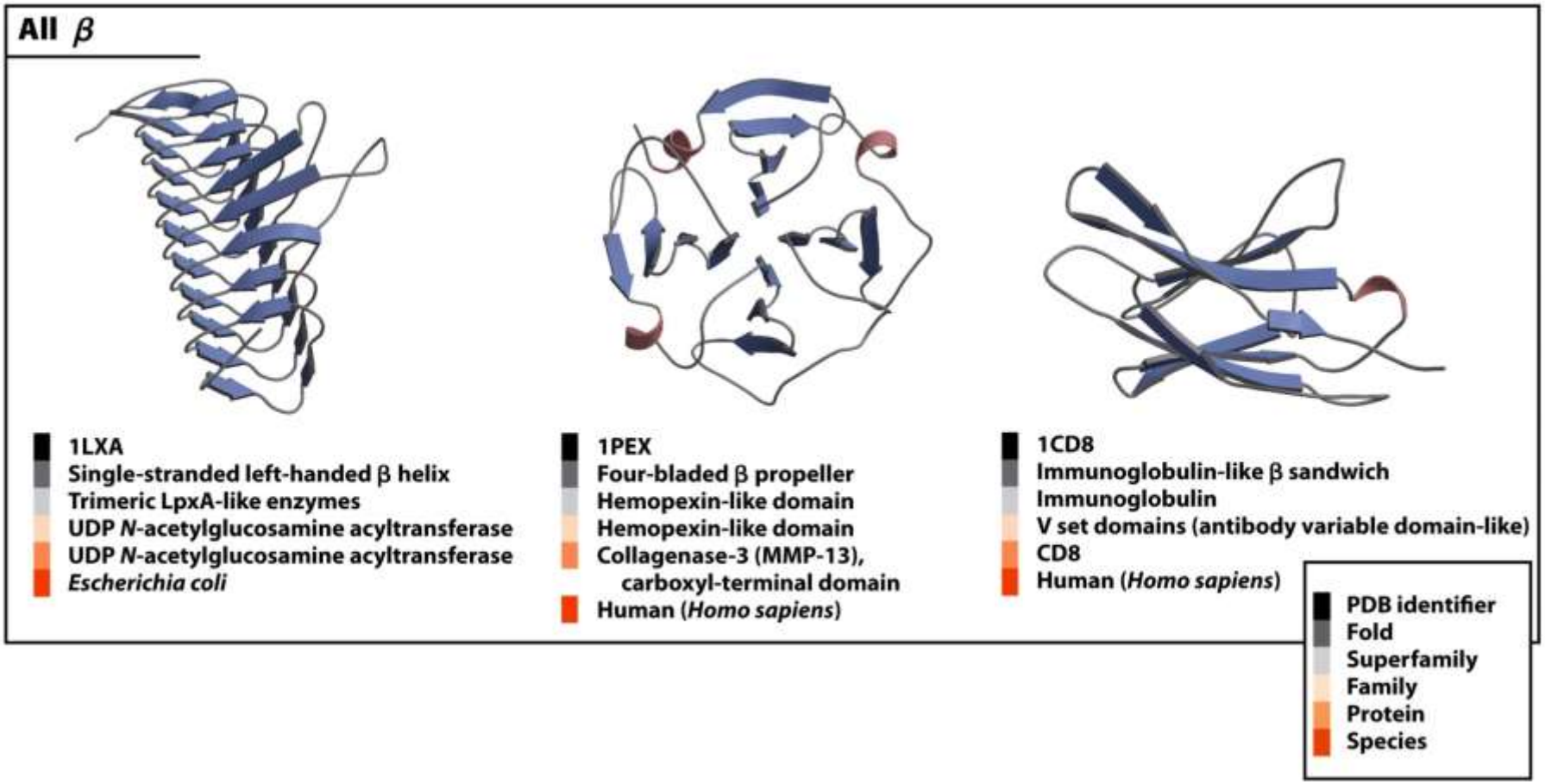
4.3 Protein Tertiary and Quaternary Structures

- **Protein motifs are the basis for protein structural classification**

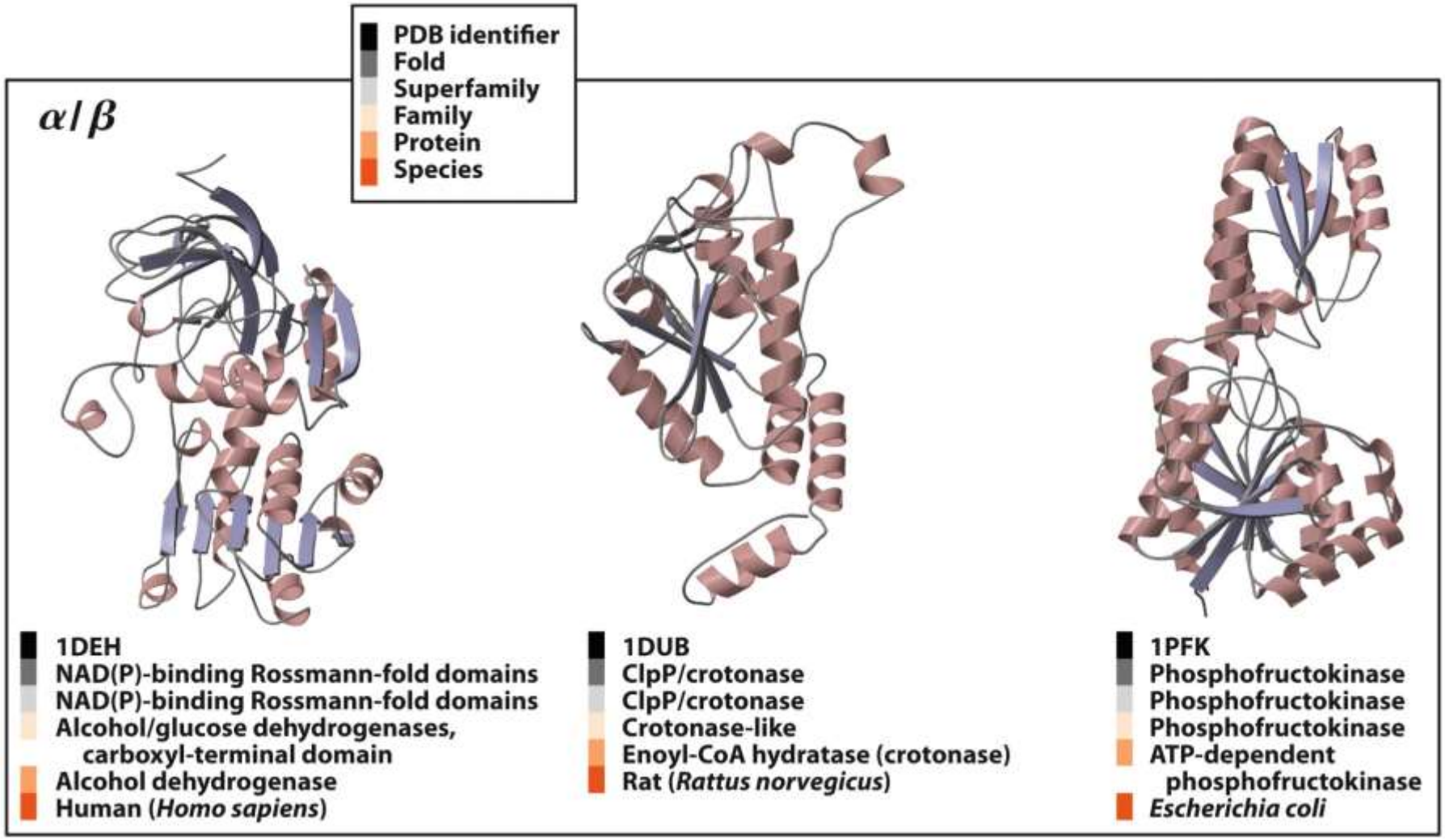
  Four classes of protein structure:
  all α, all β, α/β (with α and β segments interspersed or alternating), **and α+β** (with α and β regions somewhat segregated).



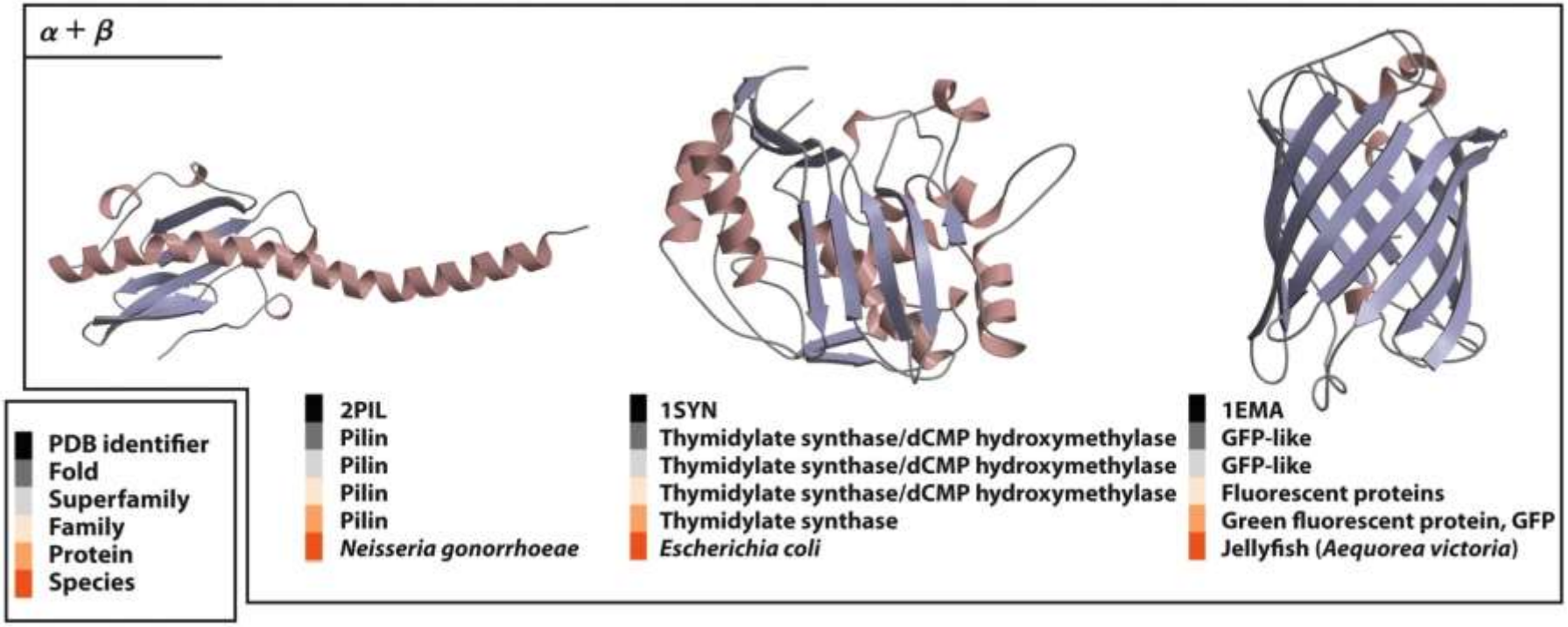
4.3 Protein Tertiary and Quaternary Structures



4.3 Protein Tertiary and Quaternary Structures



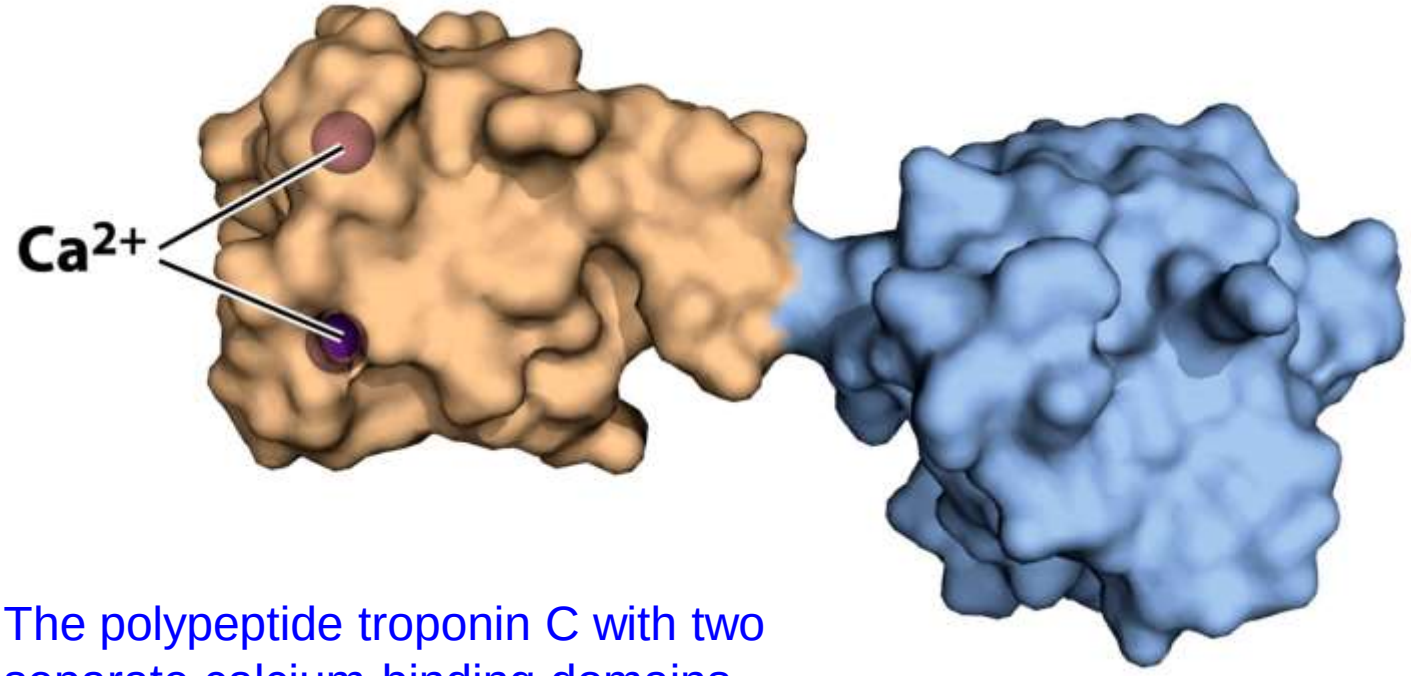
4.3 Protein Tertiary and Quaternary Structures



4.3 Protein Tertiary and Quaternary Structures

- **Domain**

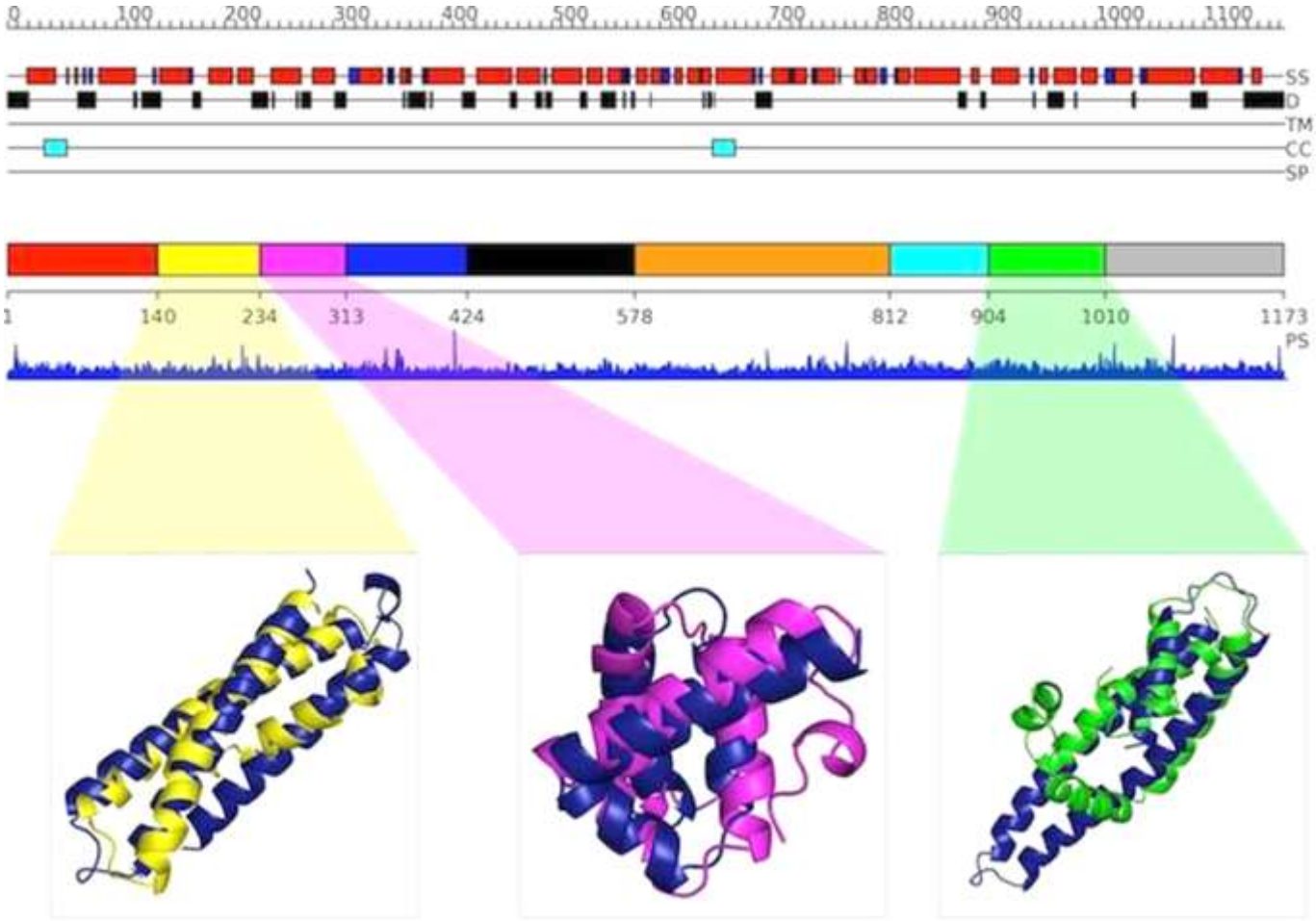
  Stable units that retain its correct three-dimensional structure even when it is separated from the remainder of the polypeptide chain.



. The polypeptide troponin C with two separate calcium-binding domains

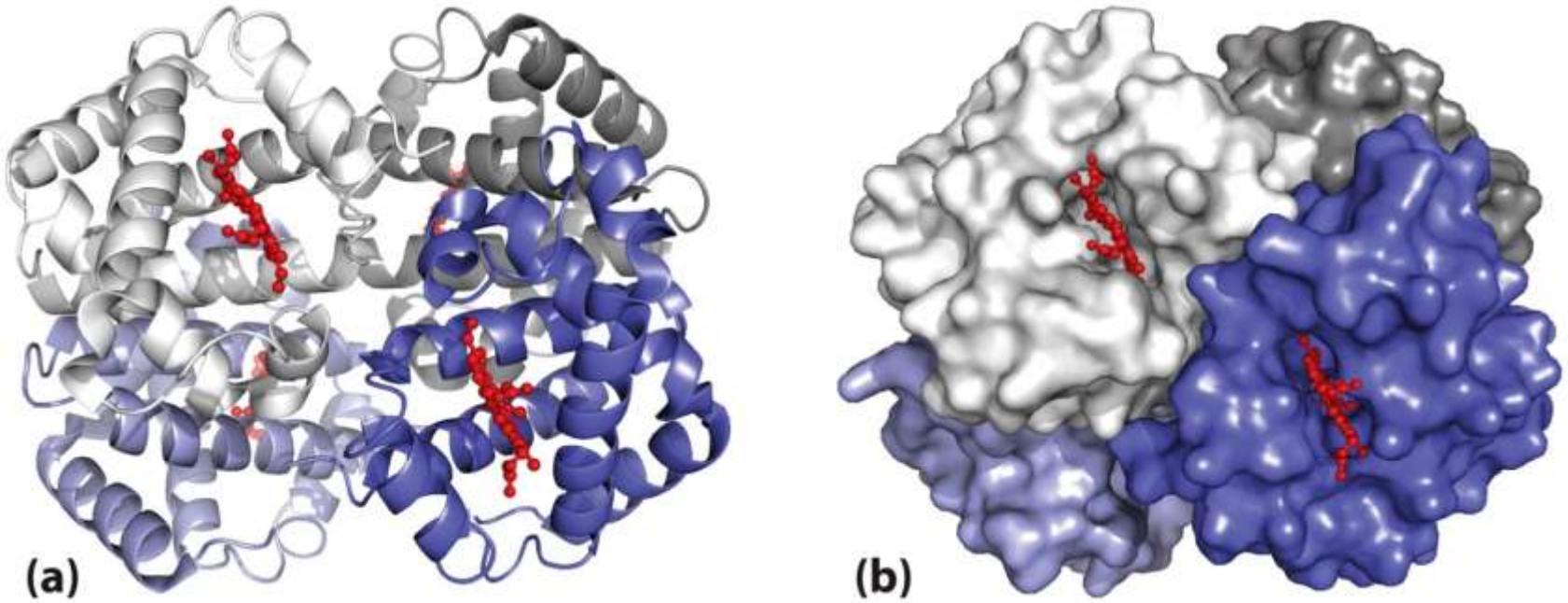
4.3 Protein Tertiary and Quaternary Structures

- **Sequence for domain prediction**



4.3 Protein Tertiary and Quaternary Structures

- **Protein quaternary structures**

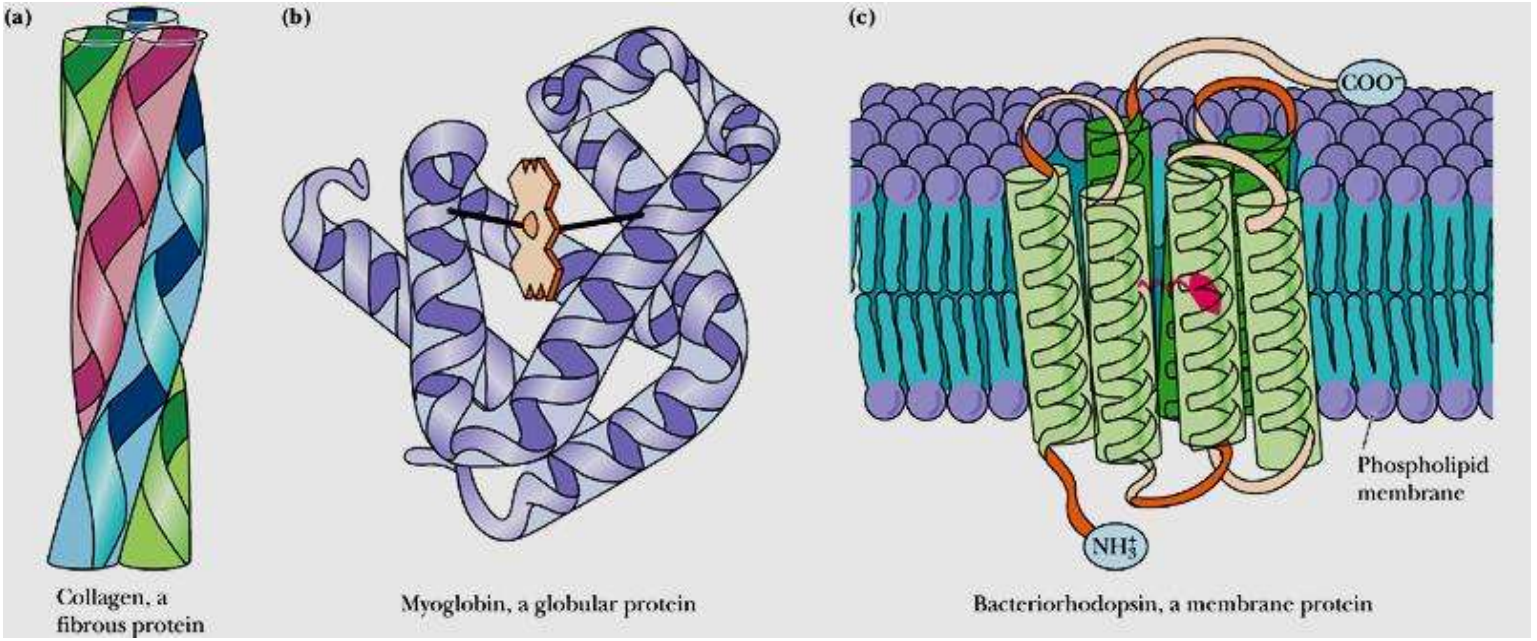


Hemoglobin
(2 α + 2 β)

The subunits of hemoglobin are arranged in symmetric pairs, each pair having one α and one β subunit. Hemoglobin can therefore be described either as a tetramer or as a dimer of αβ protomers.

4.3 Protein Tertiary and Quaternary Structures

■ Shape and solubility of proteins



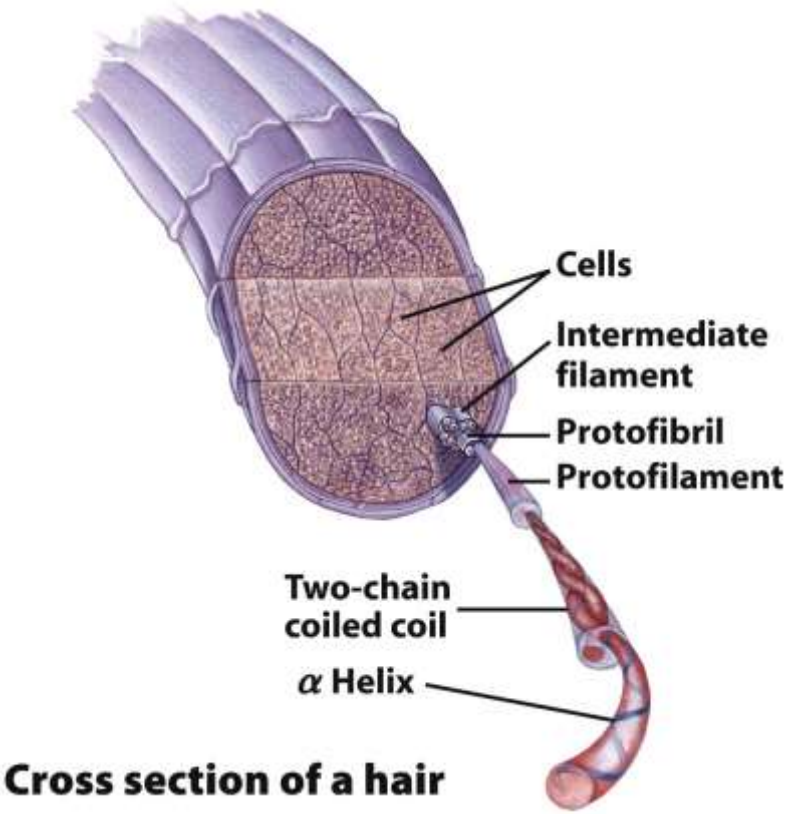
Fibrous proteins (纤维状蛋白质): arranged in long strands or sheets, usually consist largely of a single type of secondary structure.

Globular proteins (球蛋白质): folded into a spherical or globular shape, often contain several types of secondary structure.

Membrane proteins (膜蛋白): proteins that interact with biological membranes.

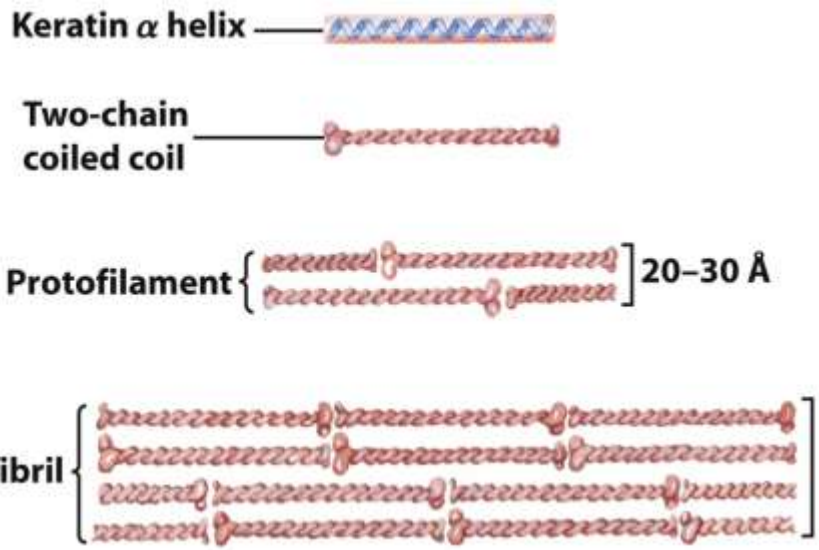
4.3 Protein Tertiary and Quaternary Structures

- **Fibrous proteins**



Cross section of a hair

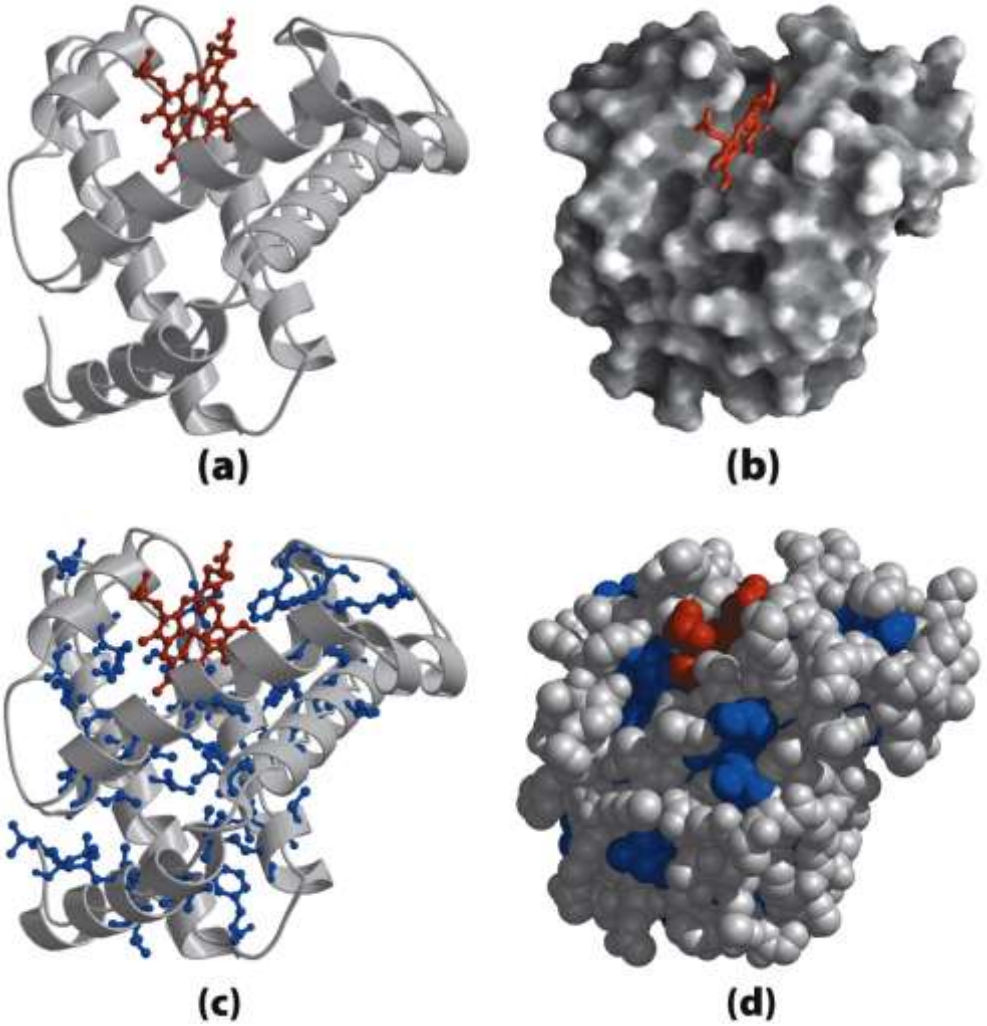
Keratin



4.3 Protein Tertiary and Quaternary Structures

- **Globular proteins**

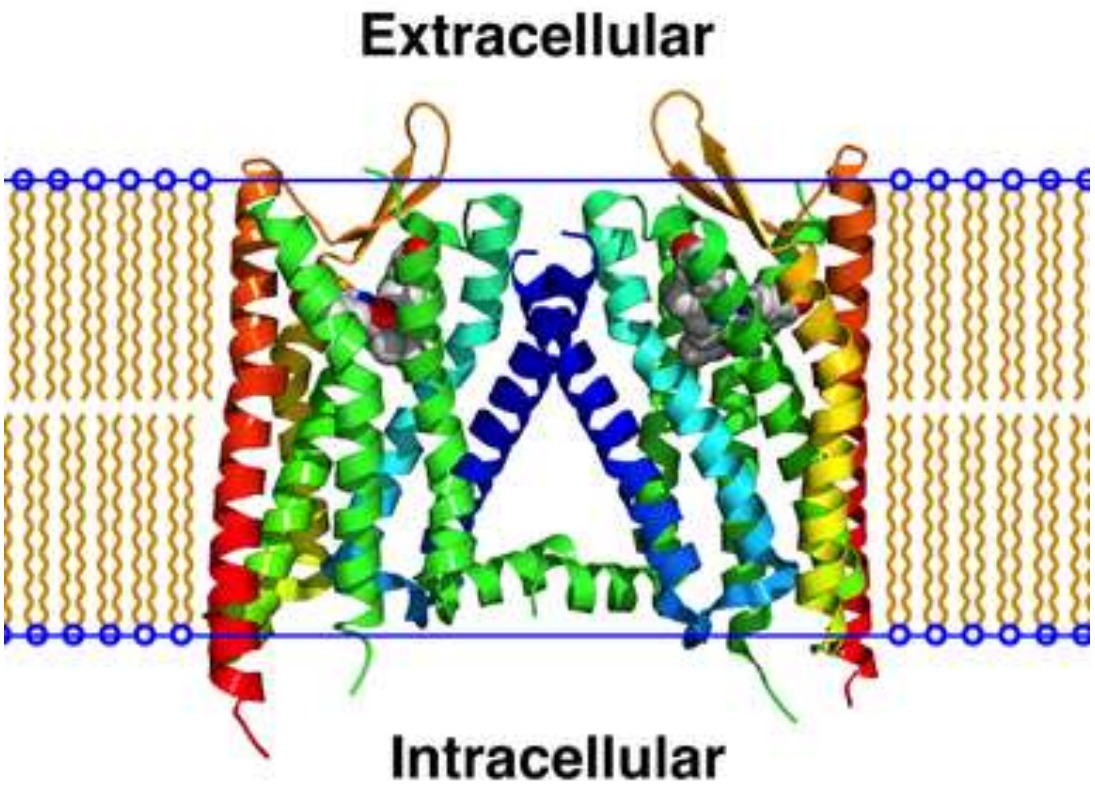
Myoglobin
肌球蛋白



4.3 Protein Tertiary and Quaternary Structures

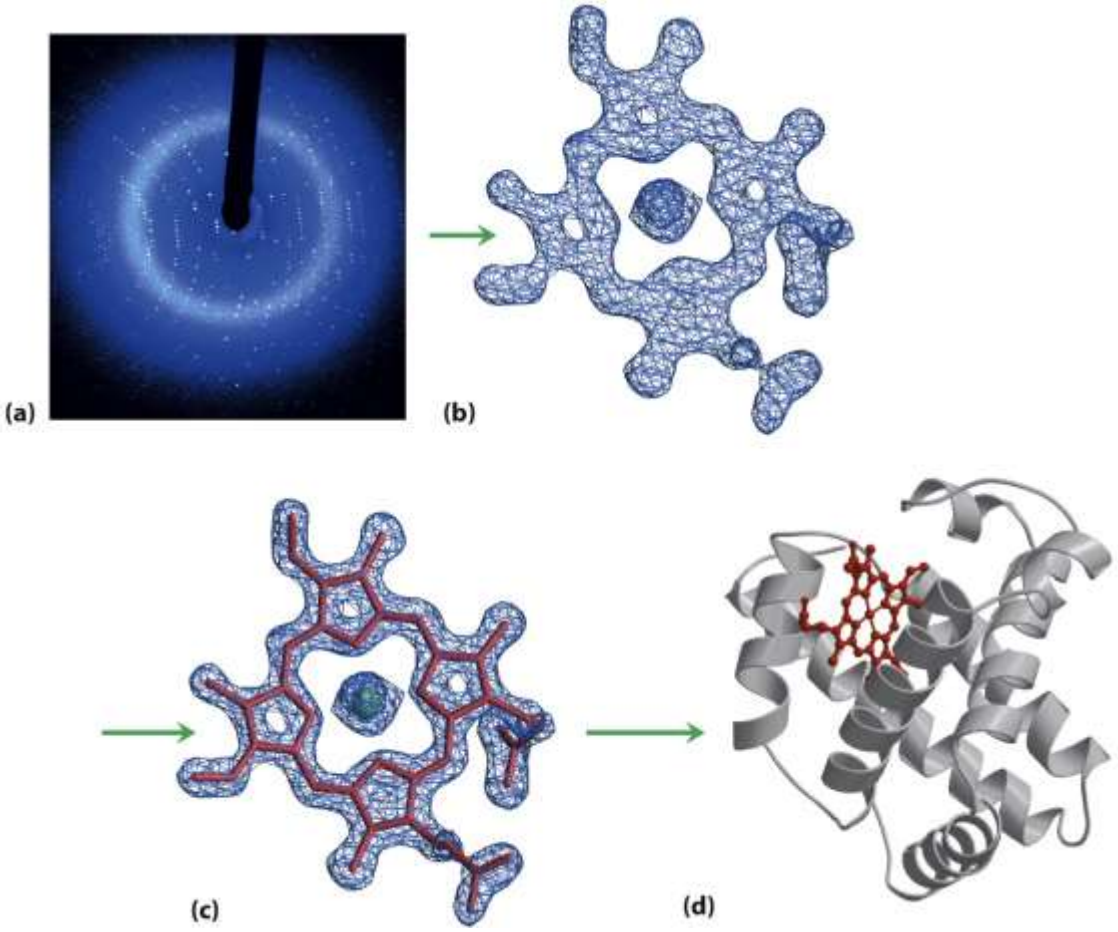
- **Membrane proteins**

GPCR



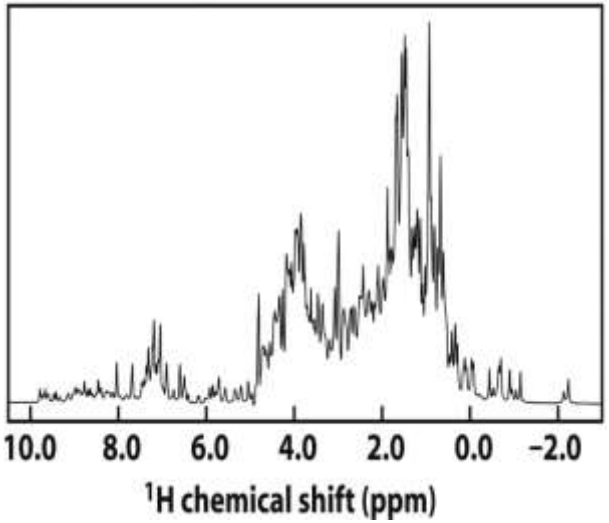
4.3 Protein Tertiary and Quaternary Structures

- **Determination of protein structure**
  - **X-ray crystallography**

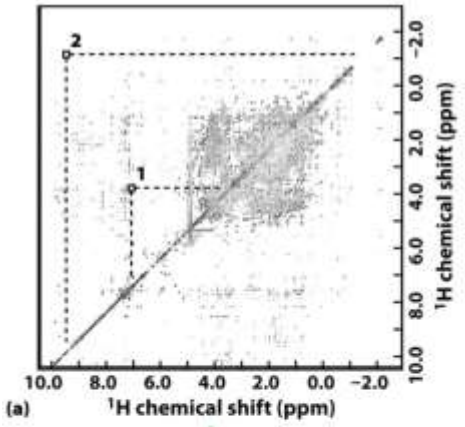


4.3 Protein Tertiary and Quaternary Structures

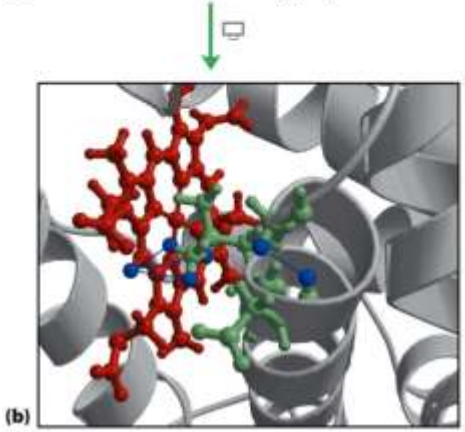
◆ Nuclear Magnetic Resonance (NMR)

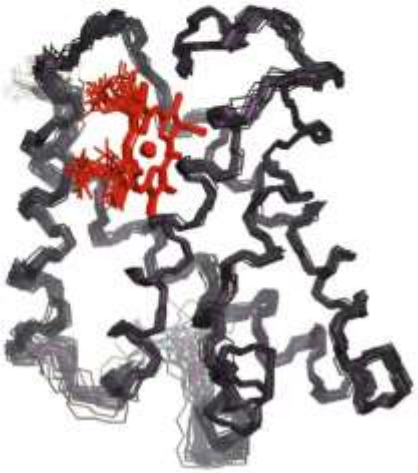


One-dimensional
NMR spectrum



Two-dimensional
NMR spectrum





4.3 Protein Tertiary and Quaternary Structures

■ Forces to stabilize the protein conformation

Conformation: the spatial arrangement of atoms of a protein

A protein's conformation is stabilized largely by weak interactions:

Hydrogen bonds

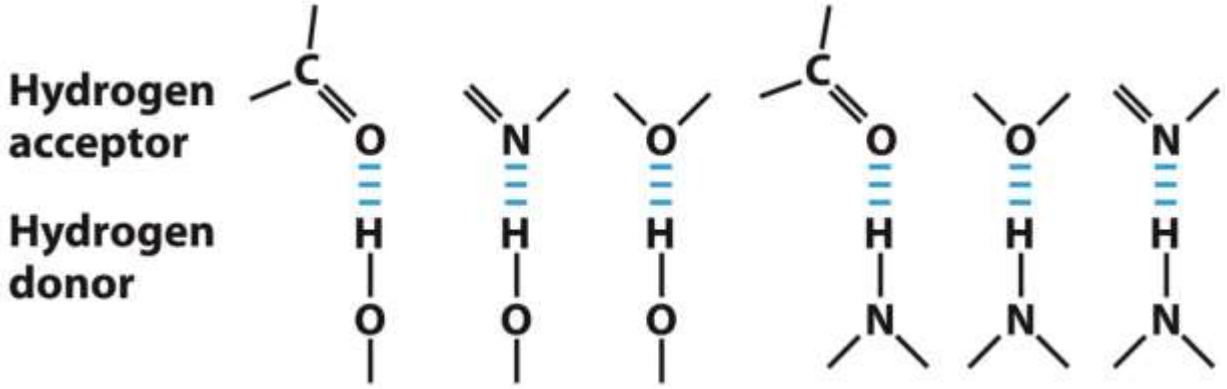
Hydrophobic interactions

Ionic bonds

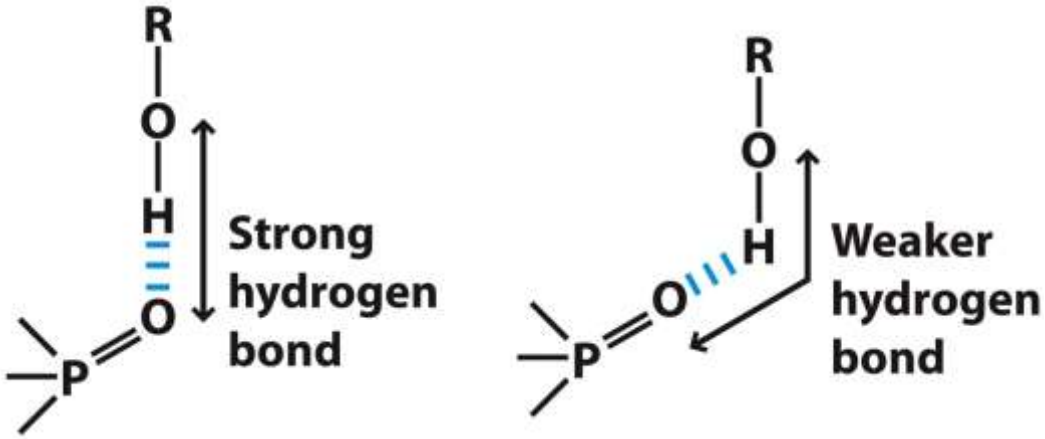
van der Waals interactions

4.3 Protein Tertiary and Quaternary Structures

- **Hydrogen bonds**



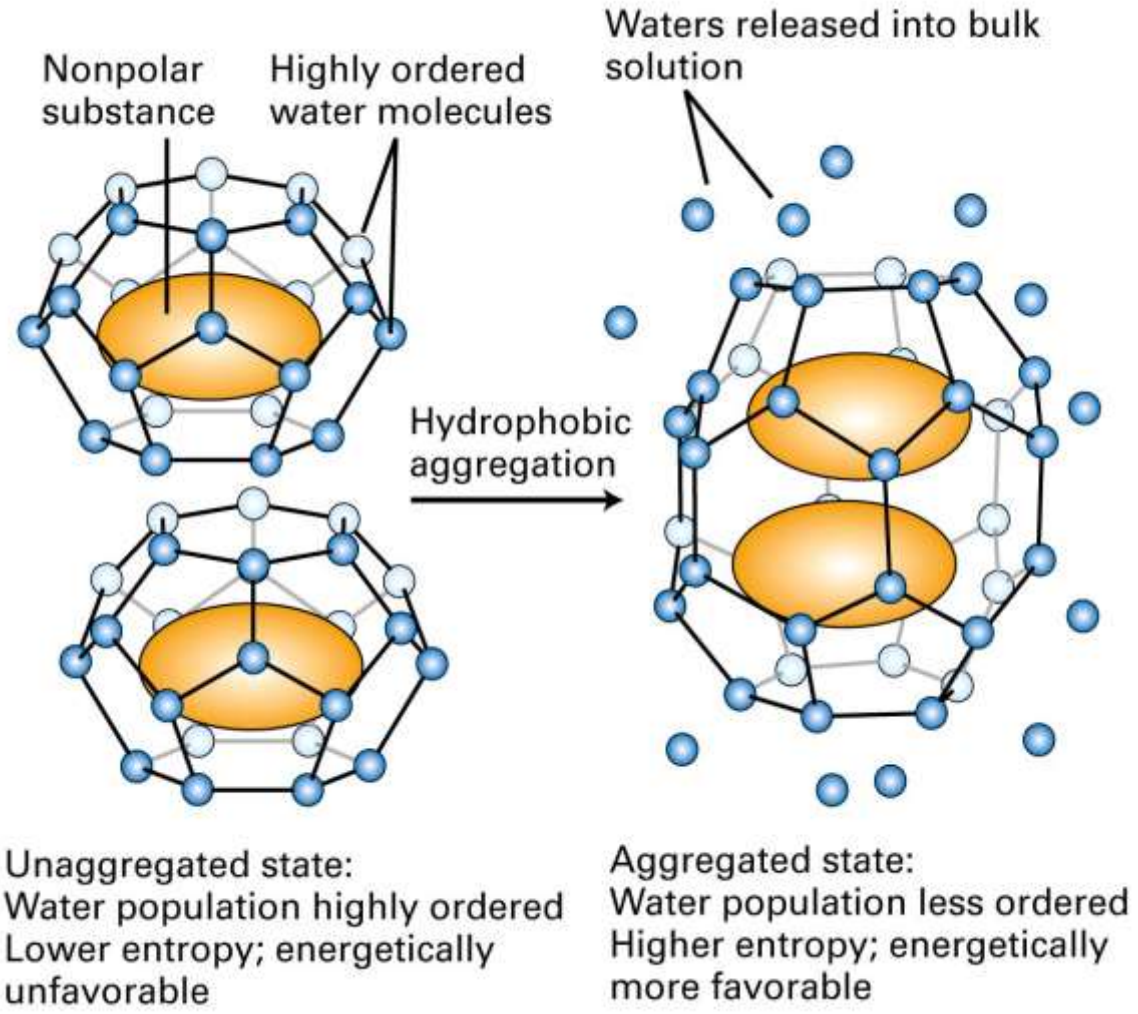
The interaction of a partially positively charged hydrogen atom in a molecule with unpaired electrons from another atom.



4.3 Protein Tertiary and Quaternary Structures

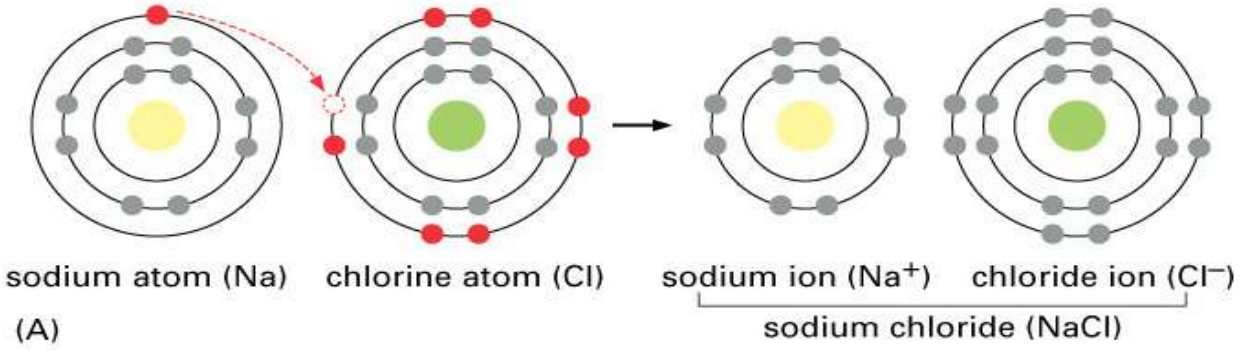
- **Hydrophobic interactions**

Water forces non-polar (uncharged) surfaces out of solution to minimize the number of ordered water molecules required to surround hydrophobic portions of the solute molecules.



4.3 Protein Tertiary and Quaternary Structures

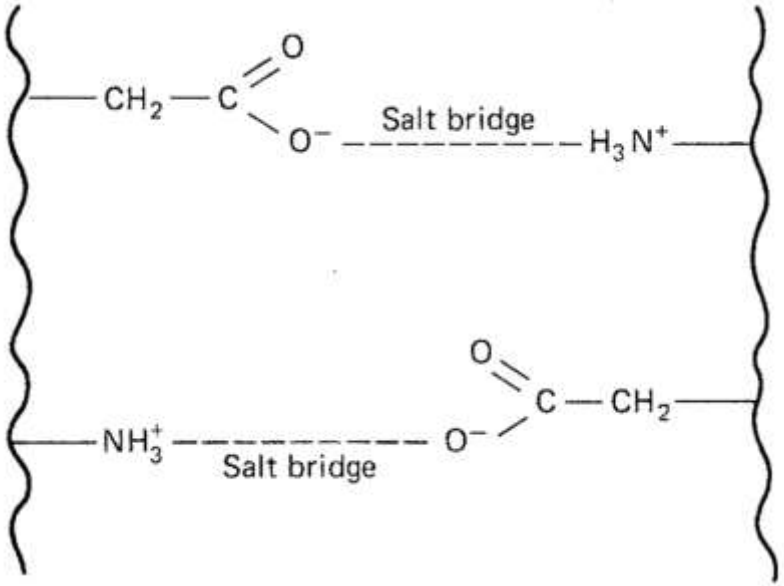
- **Ionic bonds**



Strong attractive forces between **+** and **-** charged atoms

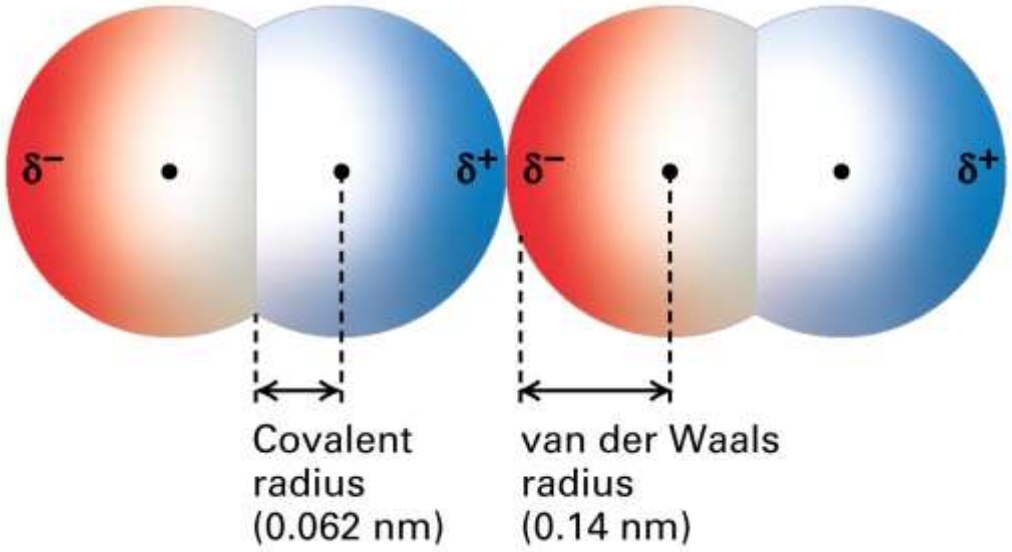
Electrons are donated/accepted by atoms rather than shared

Strong in the absence, weak in the presence of water



4.3 Protein Tertiary and Quaternary Structures

- **van der Waals interactions**

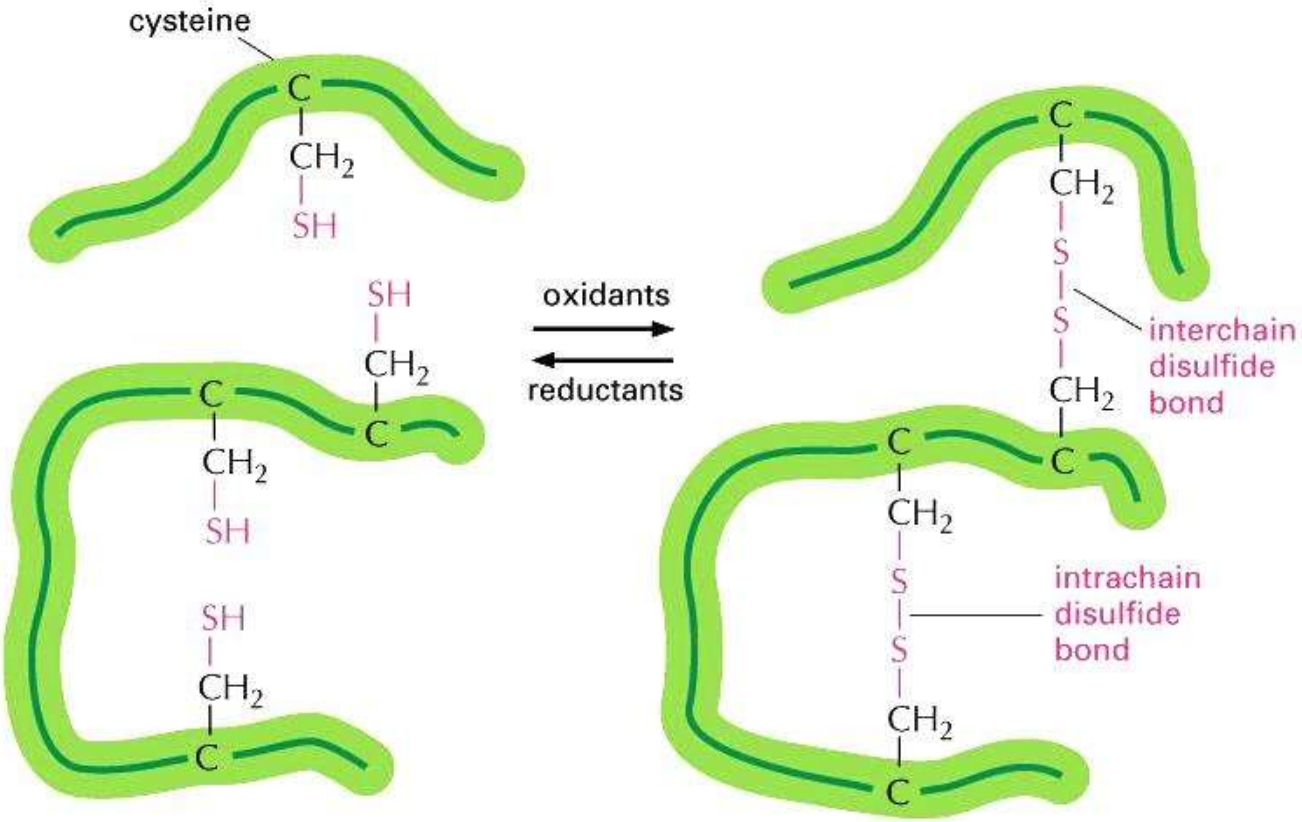


Weak force produced by fluctuations in electron clouds of atoms that are brought in close proximity.

Individually very weak, but may become important when two macromolecular surfaces are brought close together.

4.3 Protein Tertiary and Quaternary Structures

- **Disulfide bond**



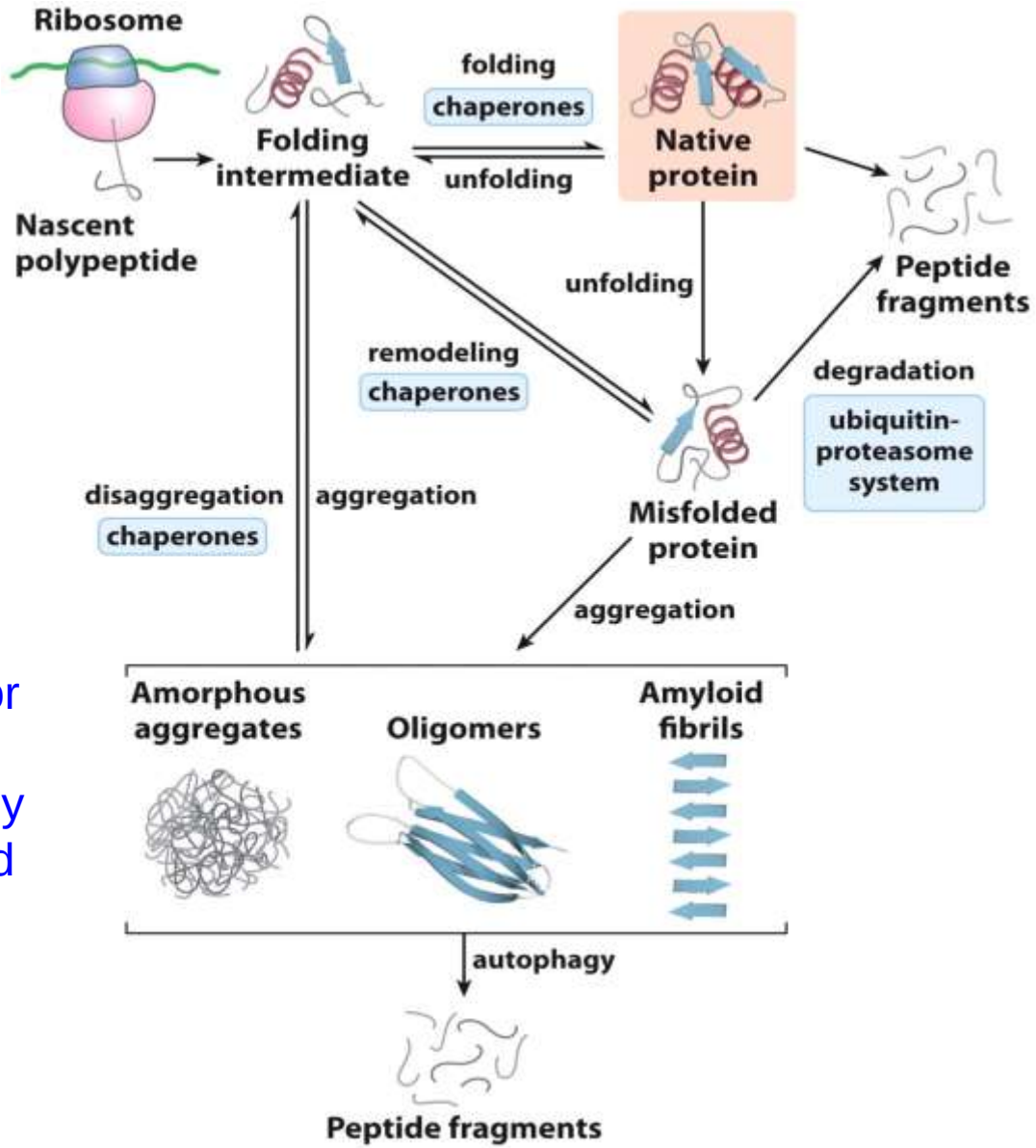
Disulfide bonds between Cys residues stabilize the structures of many proteins.

4.4 Protein Denaturation and Folding

■ Proteostasis

The continual maintenance of the active set of cellular proteins required under a given set of conditions is called **proteostasis.**

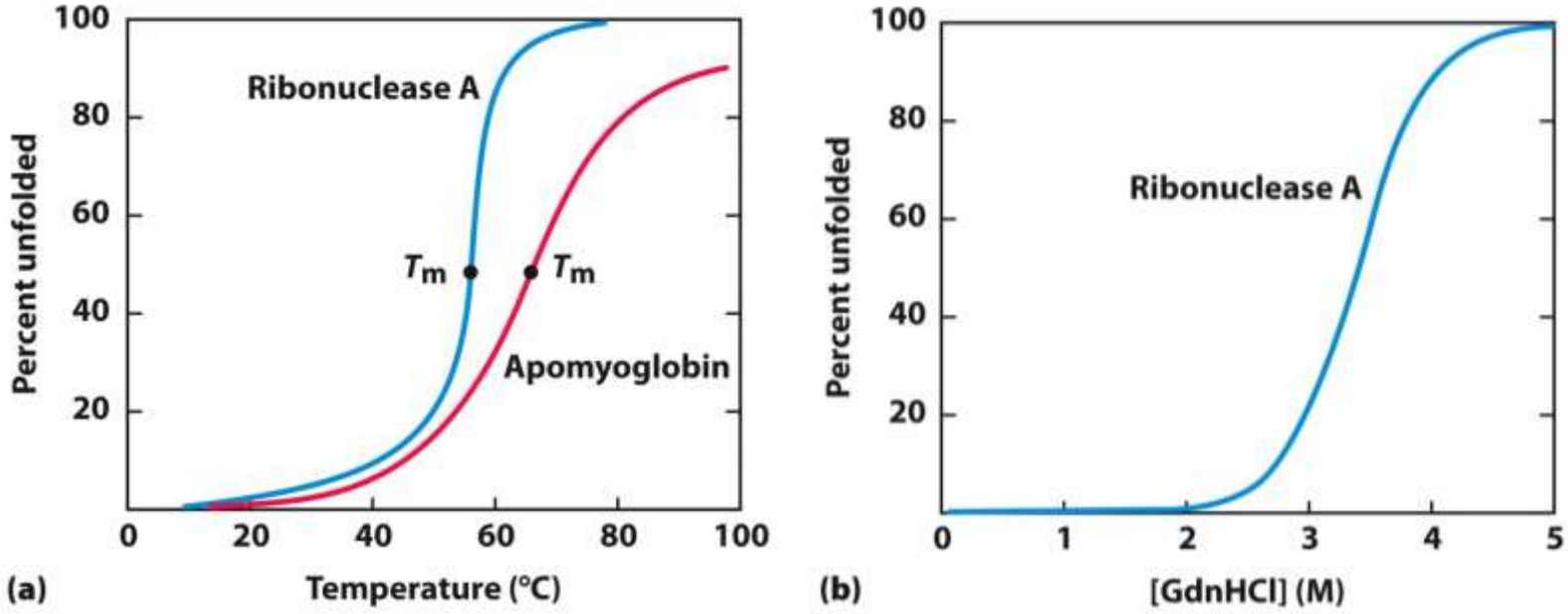
Cellular proteostasis requires the coordinated function of pathways for protein synthesis and folding, the refolding of proteins that are partially unfolded, and the sequestration and degradation of proteins that have been irreversibly unfolded.



4.4 Protein Denaturation and Folding

- **Loss of protein structure results in loss of function**

Protein function is dependent on its structure



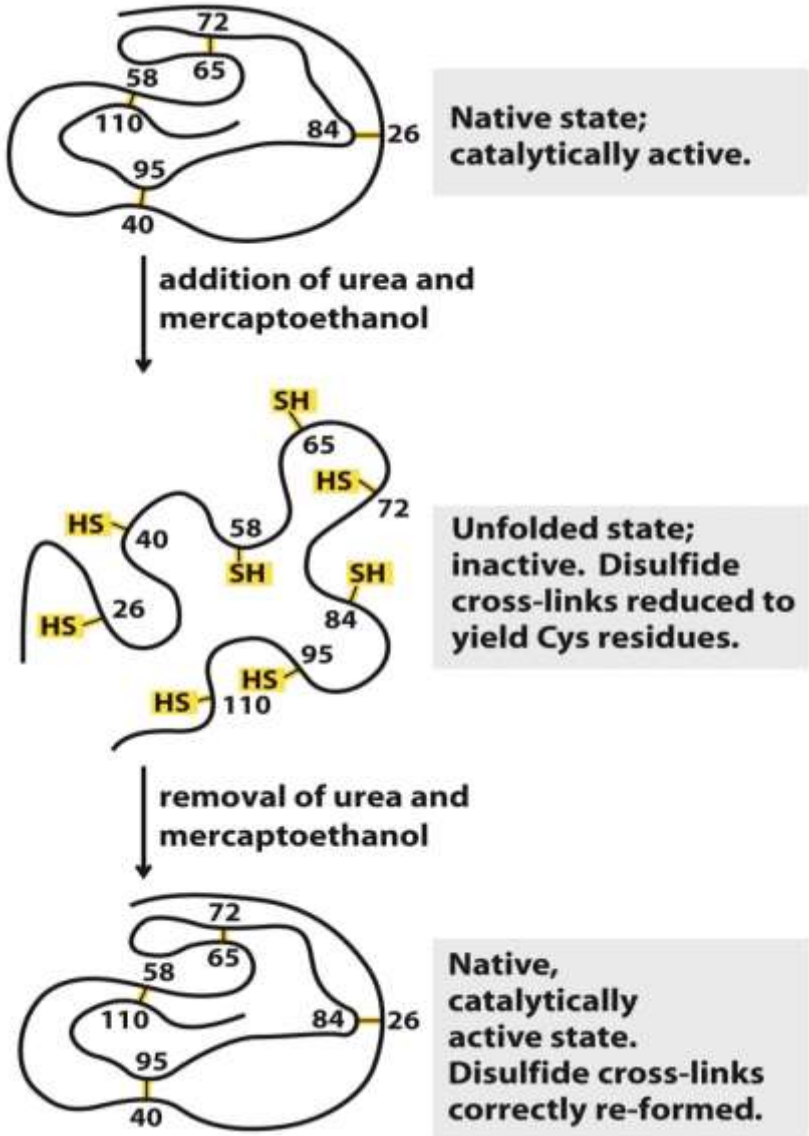
A loss of three-dimensional structure sufficient to cause loss of function is called **denaturation.**

4.4 Protein Denaturation and Folding

- **Amino acid sequence determines tertiary structure**

Denaturation of some proteins is reversible.

Proteins denatured by heat, extremes of pH, or denaturing reagents will regain their native structure and their biological activity if returned to normal conditions is called **renaturation**.

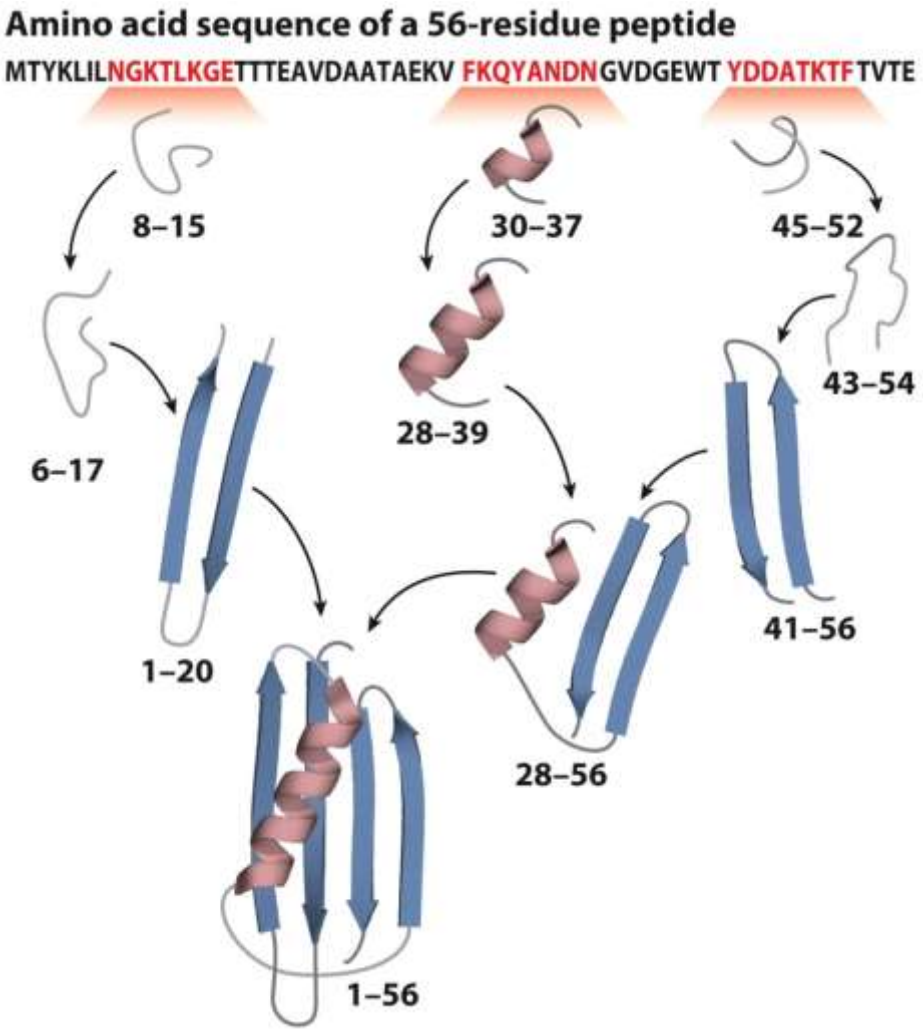


Renaturation of denatured ribonuclease

4.4 Protein Denaturation and Folding

- **Polypeptides fold rapidly by a stepwise process**

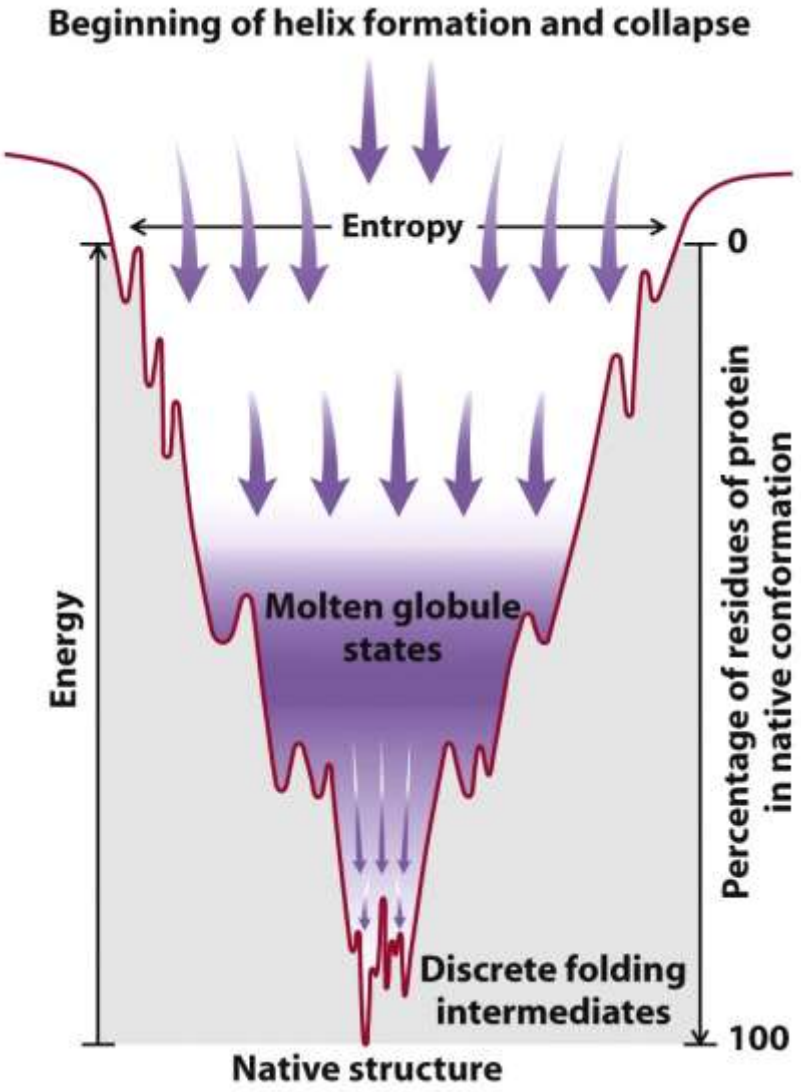
Small regions of secondary structure are assembled first and then gradually incorporated into larger structures.



4.4 Protein Denaturation and Folding

- **The thermodynamics of protein folding depicted as a free-energy funnel**

The unfolded states are characterized by a high degree of conformational entropy and relatively high free energy. As folding proceeds, the narrowing of the funnel reflects the decrease in the conformational space that must be searched as the protein approaches its native state.

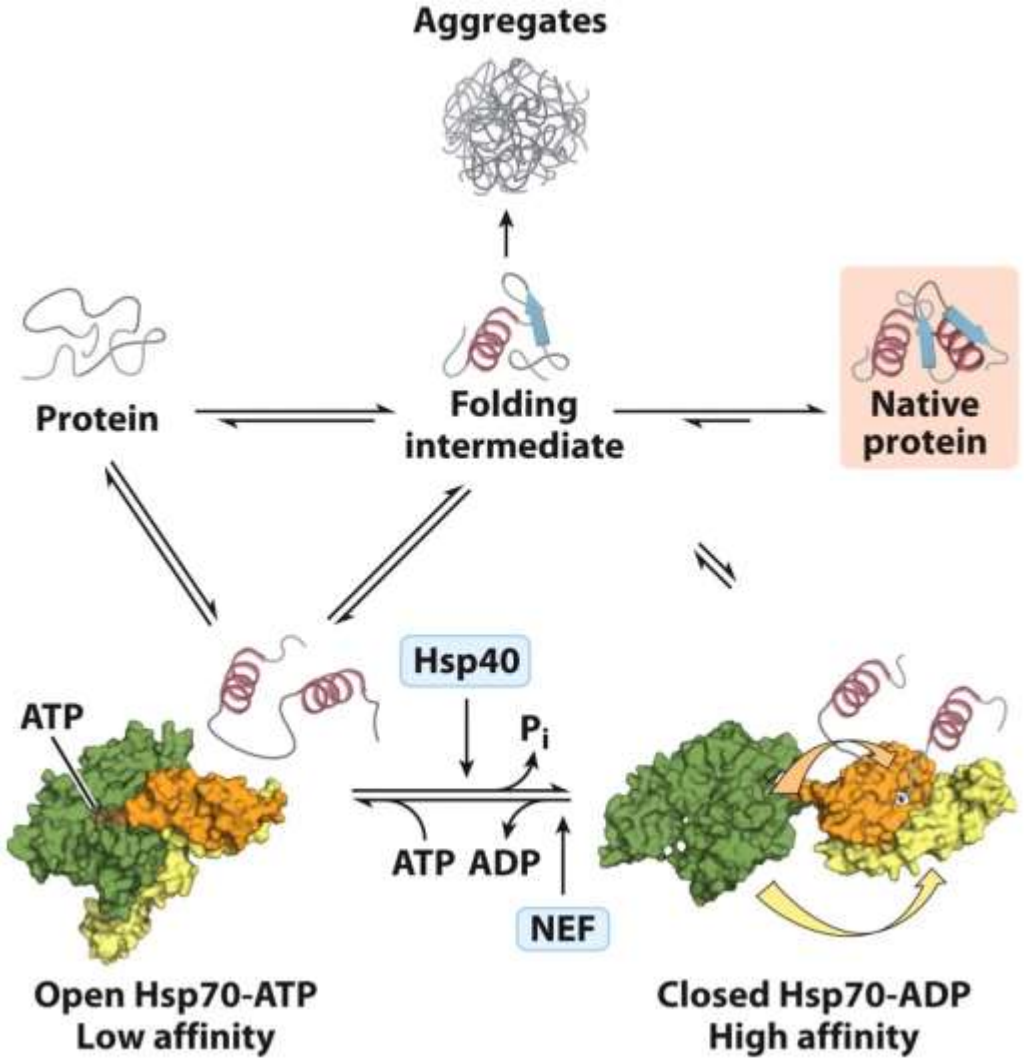


4.4 Protein Denaturation and Folding

- **Some proteins undergo assisted folding**

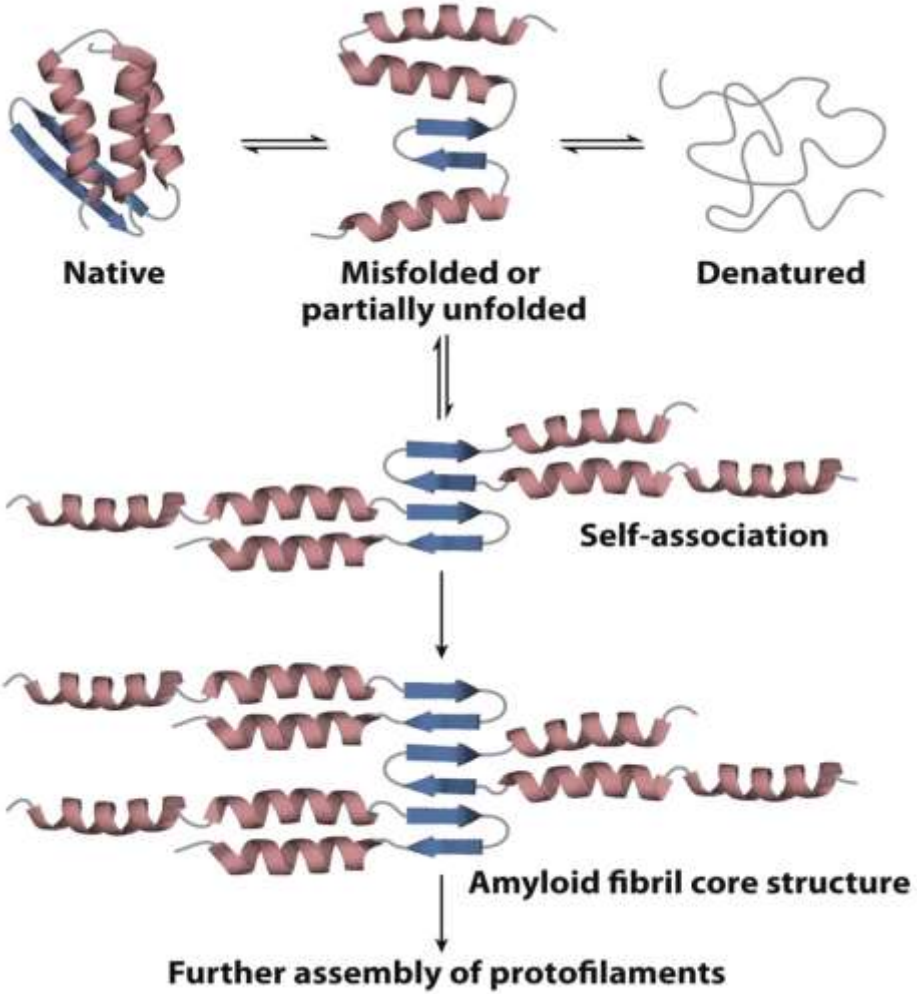
Chaperones are proteins that interact with partially folded or improperly folded polypeptides, facilitating correct folding pathways or providing microenvironments in which folding can occur.

Folding for many proteins requires **chaperones**.



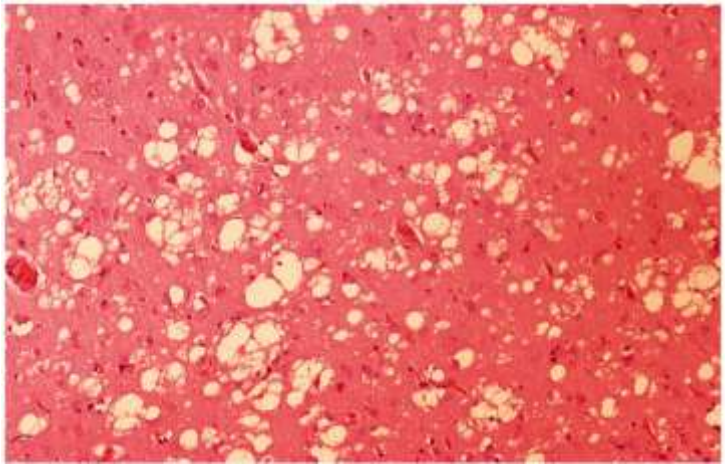
4.4 Protein Denaturation and Folding

- **Formation of disease-causing amyloid fibrils**

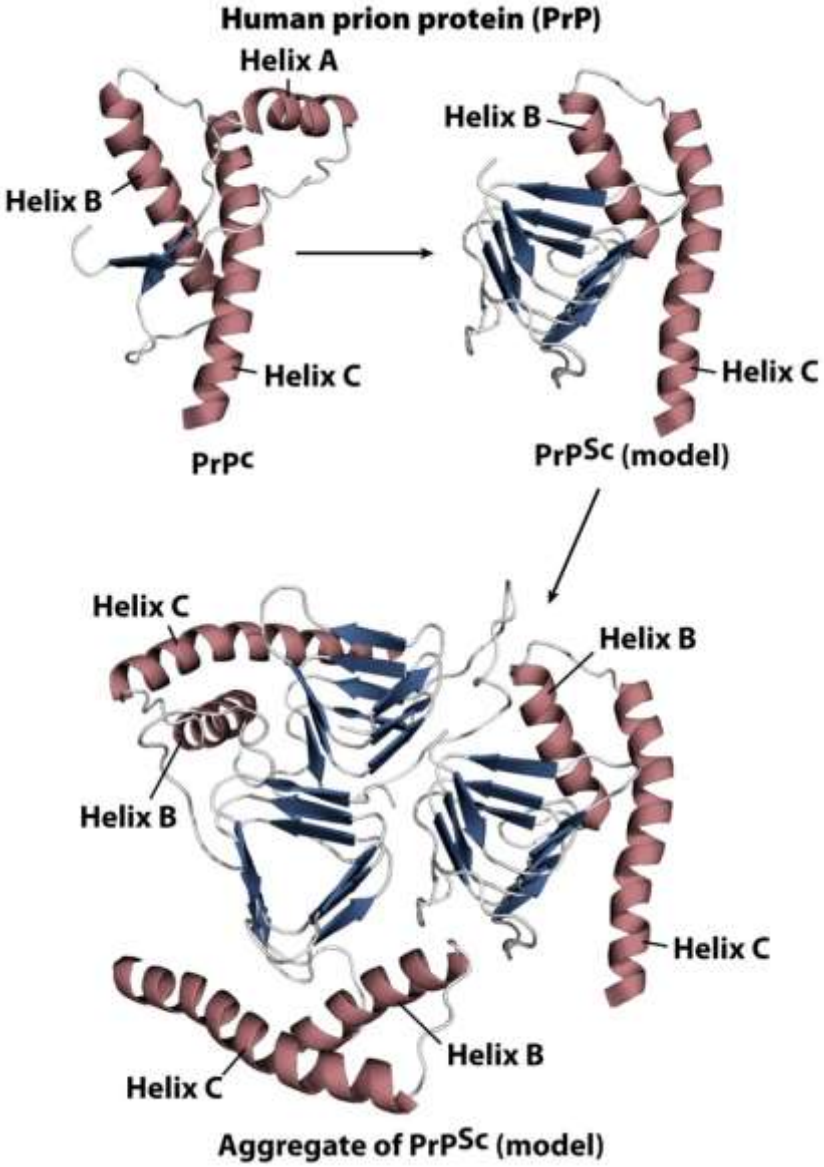


4.4 Protein Denaturation and Folding

- **Death by misfolding: the prion diseases**



Spongiform encephalopathies



Biological Functions of Proteins

- **Proteins are executors of various biological functions**

1. Biological catalytic activity
2. Biological regulation activity
3. Transport function
4. Motor function
5. Structural component
6. Scaffold function
7. Protect and attack
8. …….

Biological Functions of Proteins

1. Biological catalytic activity

Enzymes: proteins that catalyze chemical reactions

Enzymes are central to every biochemical process.

Biological Functions of Proteins

2. Biological regulation activity

Regulatory proteins

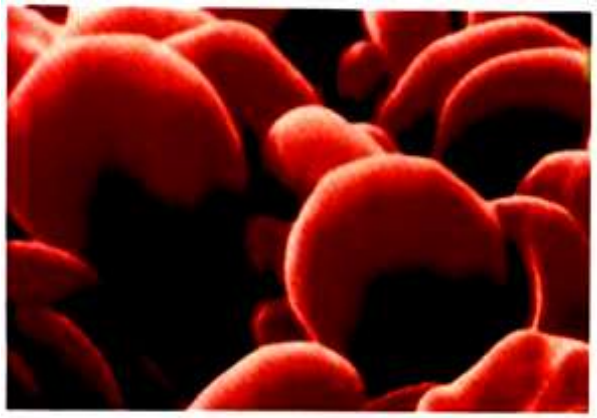
a. To regulate the ability of other proteins

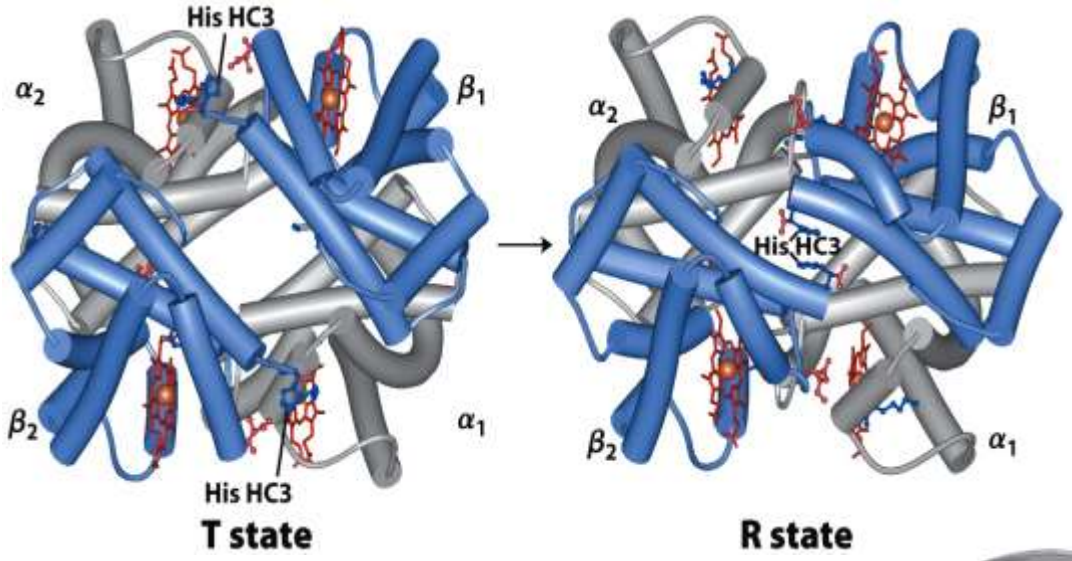
b. To regulate the gene expression

Biological Functions of Proteins

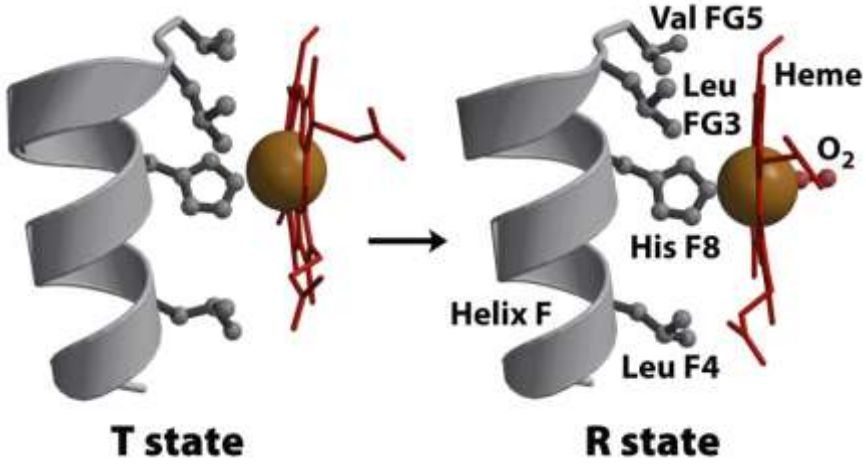
3. Transport function

1) Transport between different cells or tissues





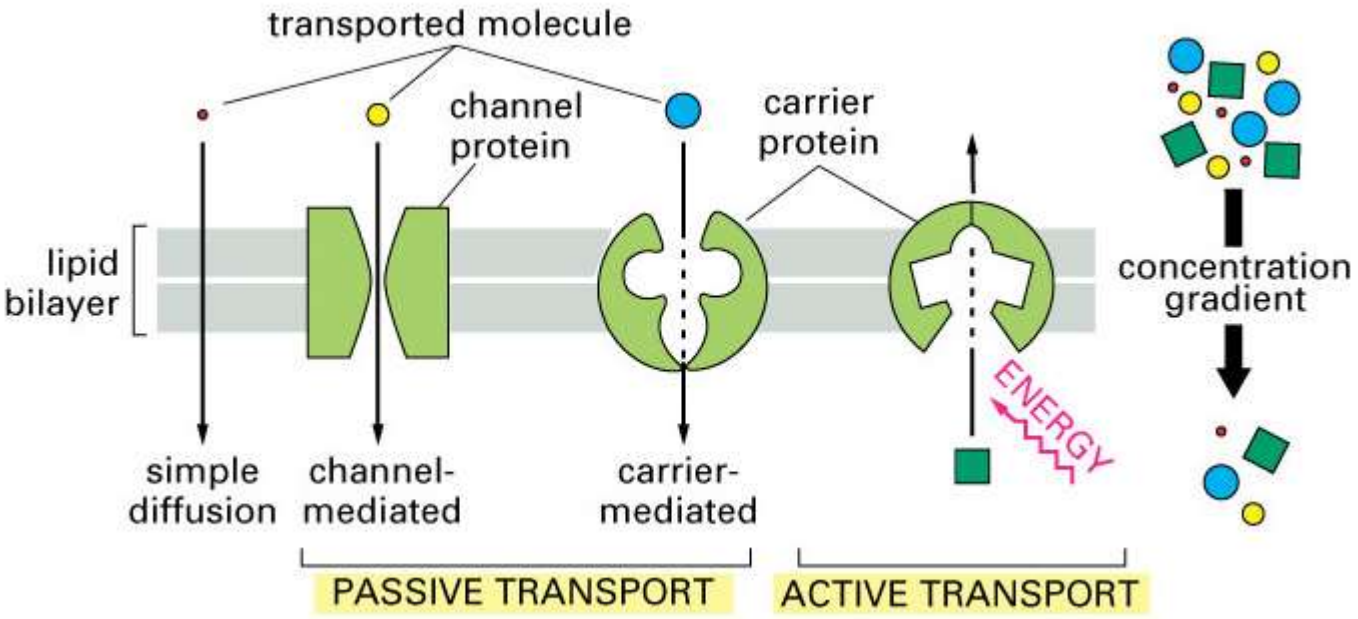
Hemoglobin



Biological Functions of Proteins

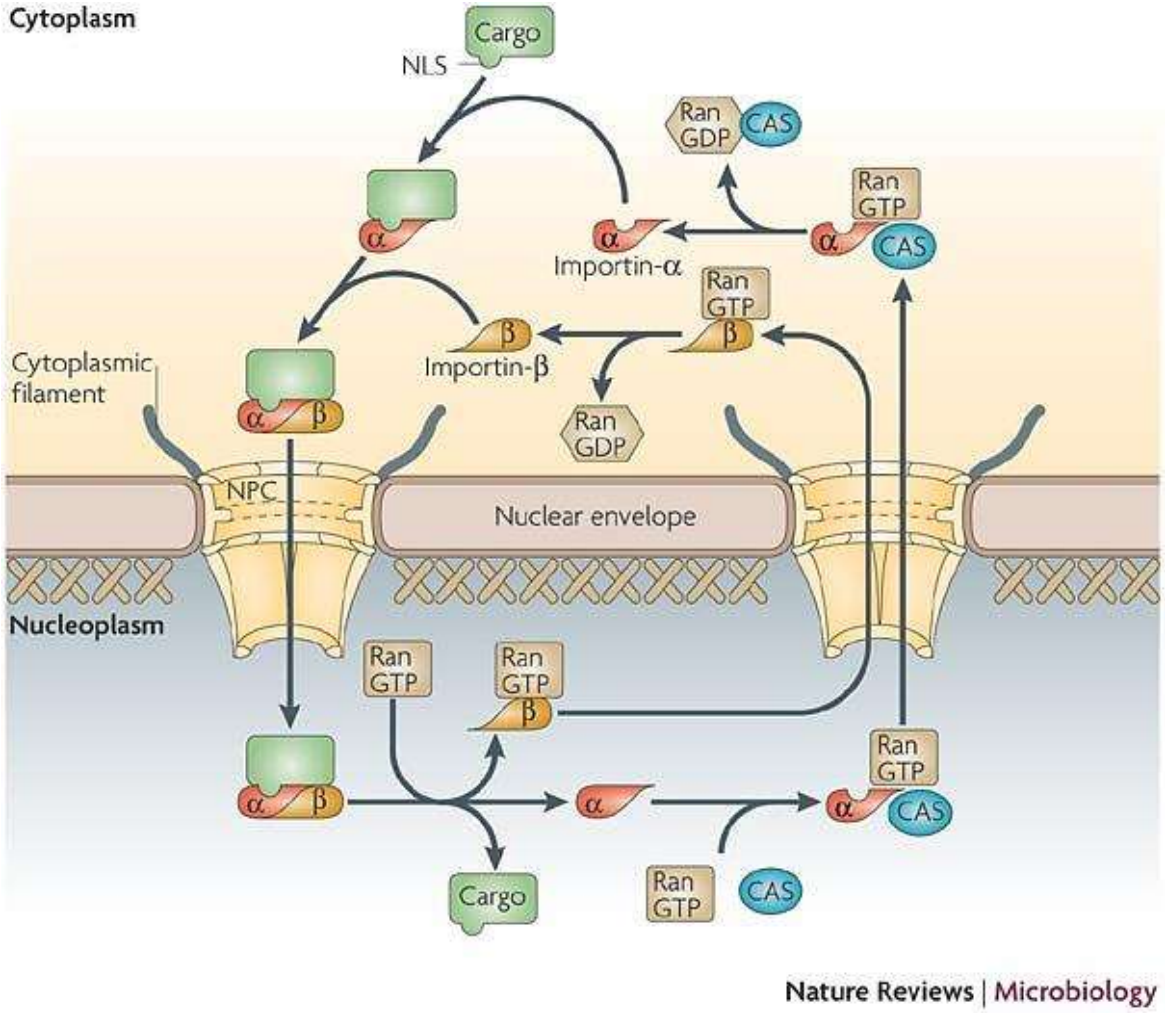
2) Transport into or out of cells

Membrane transport protein



Biological Functions of Proteins

3) Transport within a cell



Biological Functions of Proteins

4. Motor function

Contractile and motile proteins

Actin (肌动蛋白)

Myosin (肌球蛋白)

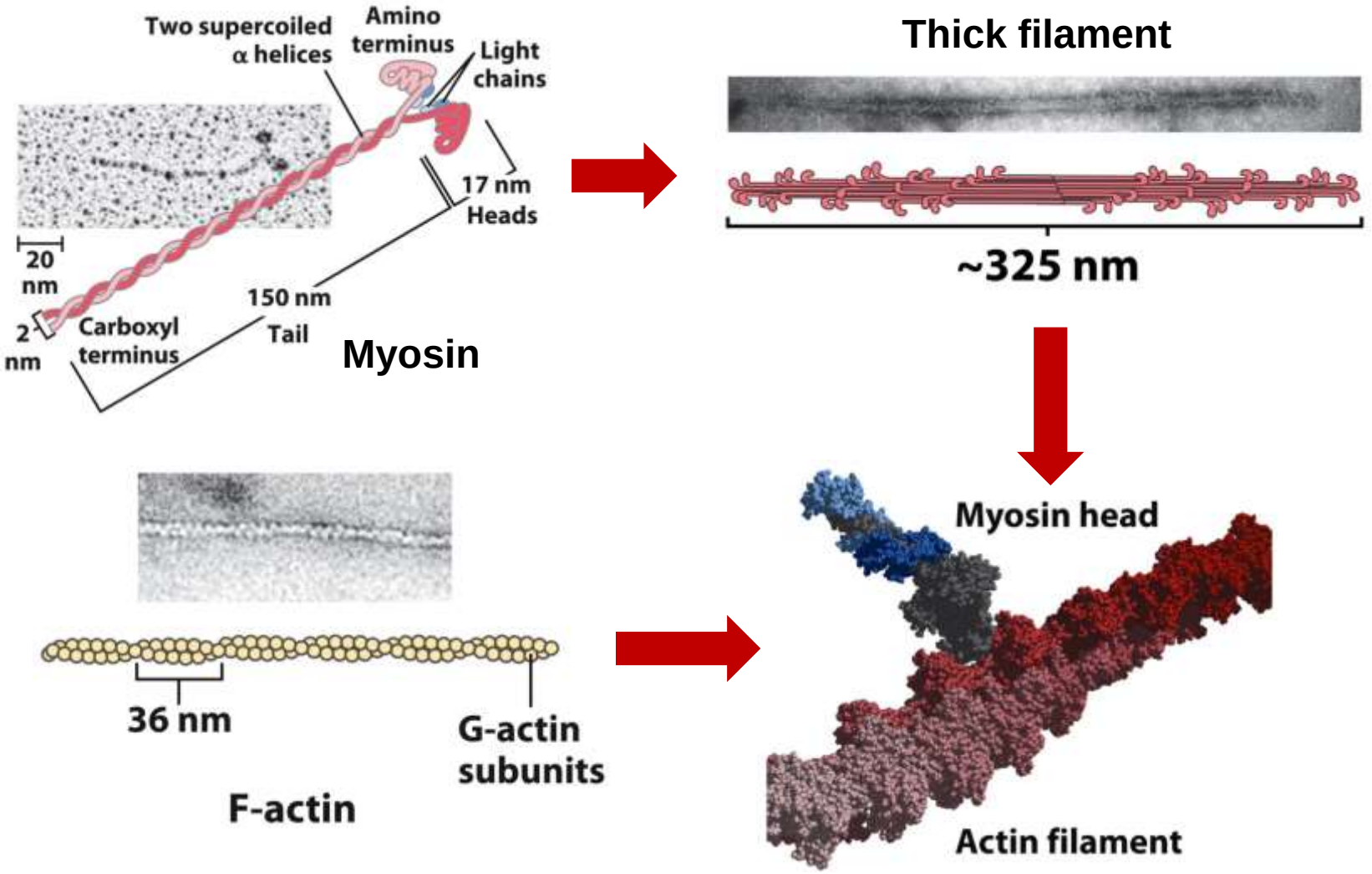
Tubulin (微管蛋白)

Motor protein

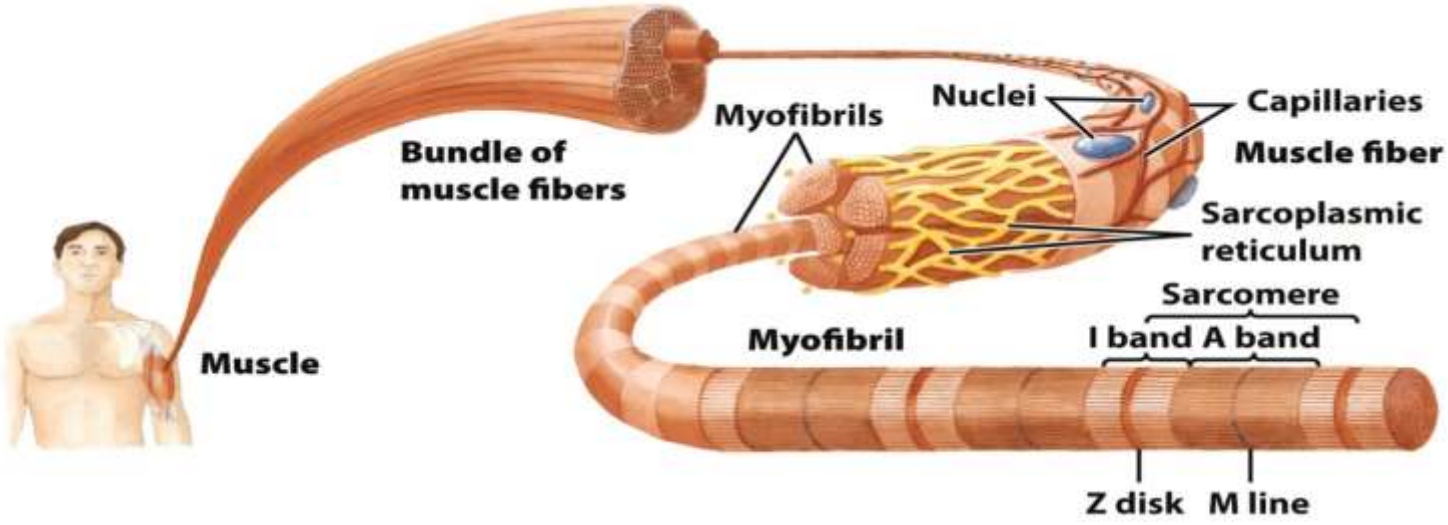
Kinesin (驱动蛋白)

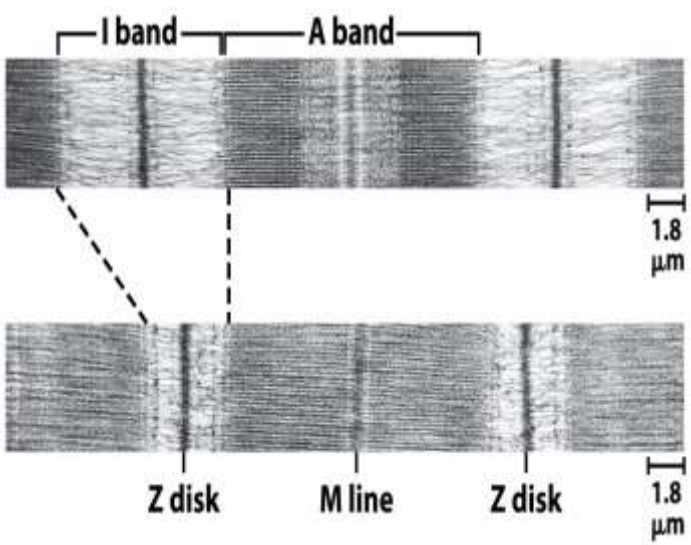
Biological Functions of Proteins

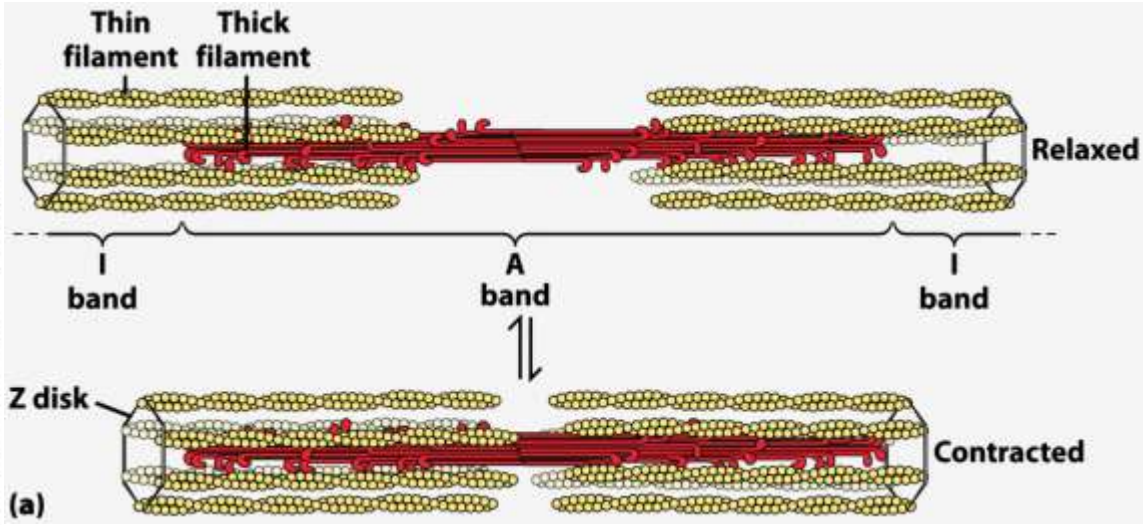
- How muscle works



Biological Functions of Proteins



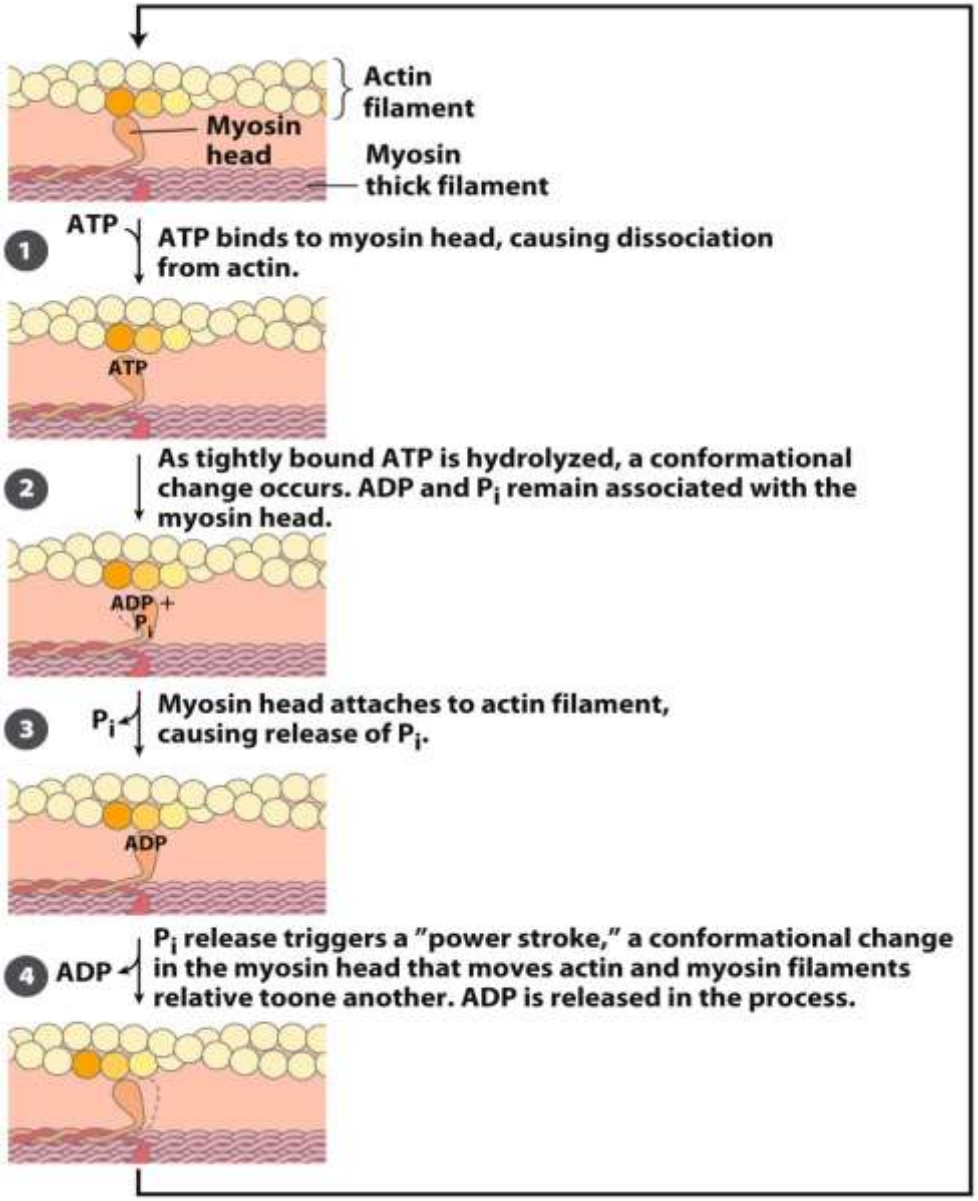




Biological Functions of Proteins

- **Molecular mechanism of muscle contraction**

Conformational changes in the myosin head that are coupled to stages in the ATP hydrolytic cycle cause myosin to successively dissociate from one actin subunit, then associate with another farther along the actin filament. In this way the myosin heads slide along the thin filaments, drawing the thick filament array into the thin filament array



Biological Functions of Proteins

5. Structural component

collagen (胶原蛋白)

elastin (弹性蛋白)

keratin (角蛋白)

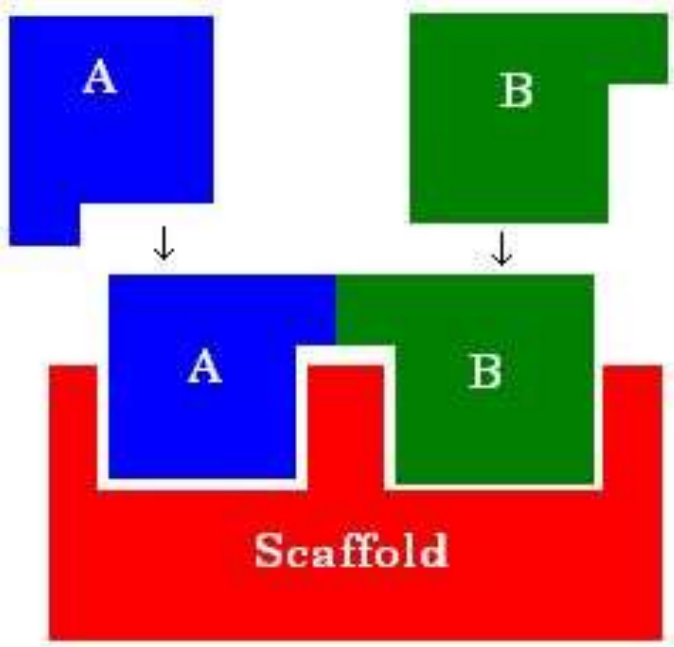
fibroin (蚕丝蛋白)

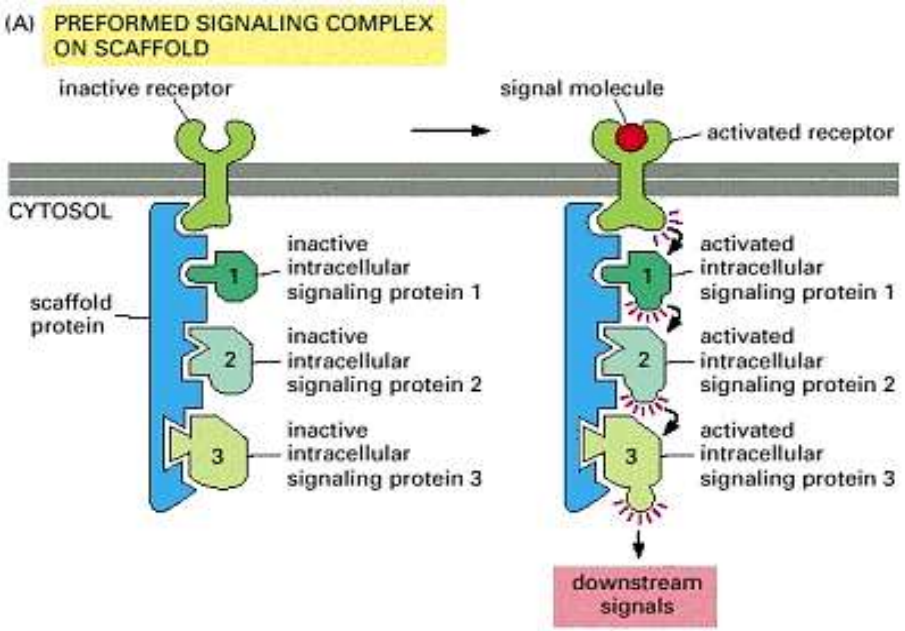
proteoglycan (蛋白聚糖)

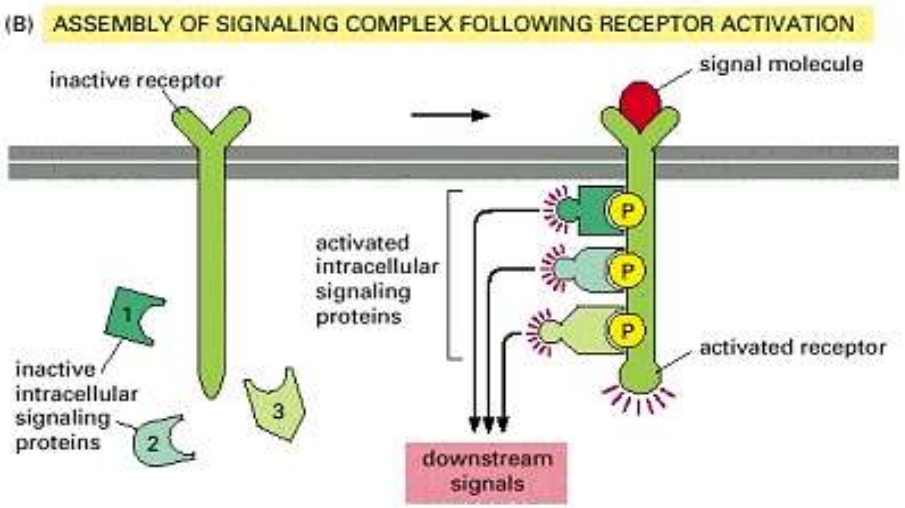
Biological Functions of Proteins

6. Scaffold function

Scaffold proteins (Adapter proteins) (支架蛋白或衔接蛋白)







Biological Functions of Proteins

7. Protective and attack

immunoglobulins (免疫球蛋白)

Thrombin (凝血酶)

fibrinogen (纤维蛋白原)

antifreeze protein (抗冻蛋白)

lytic and neurotoxic proteins

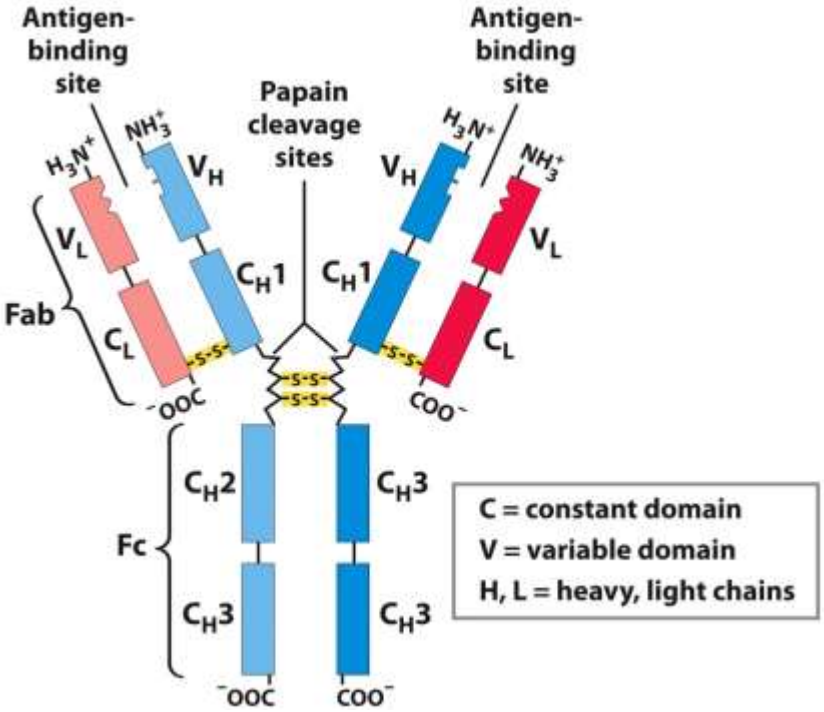
snake and bee venoms

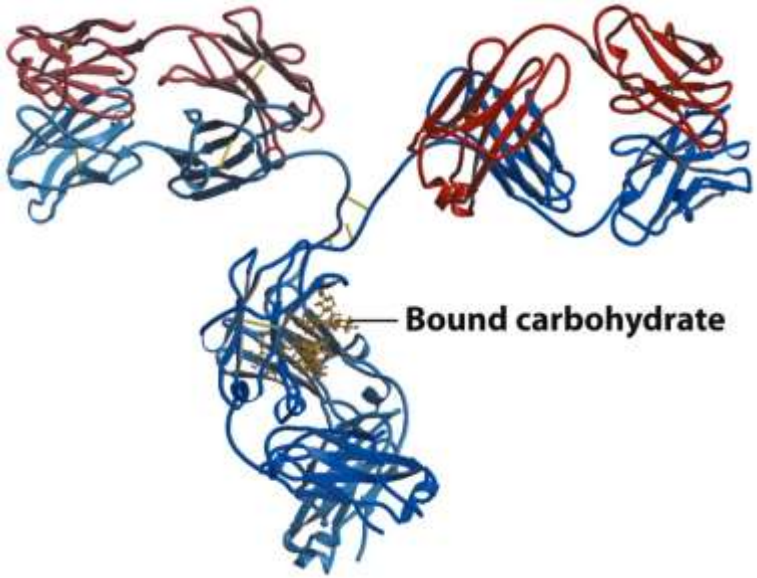
ricin (蓖麻毒素)

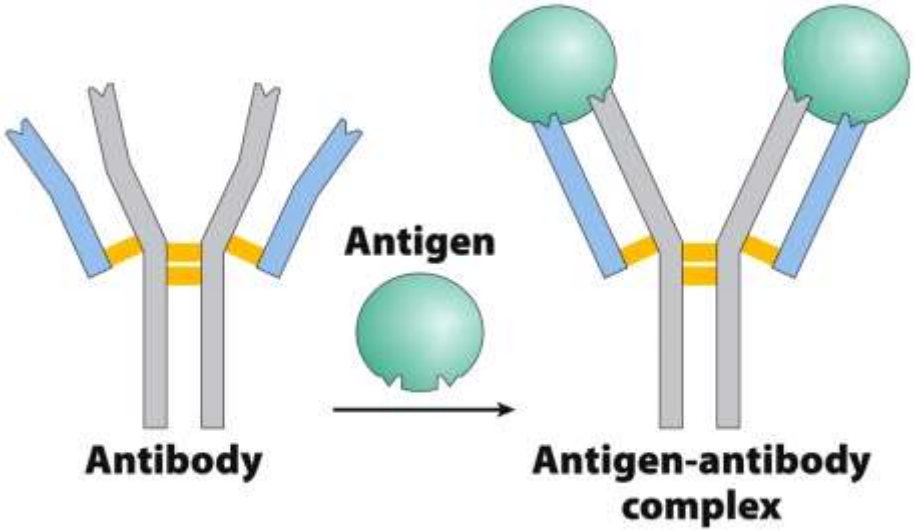
diphtheria (白喉毒素)

Biological Functions of Proteins

Immunoglobulin G.

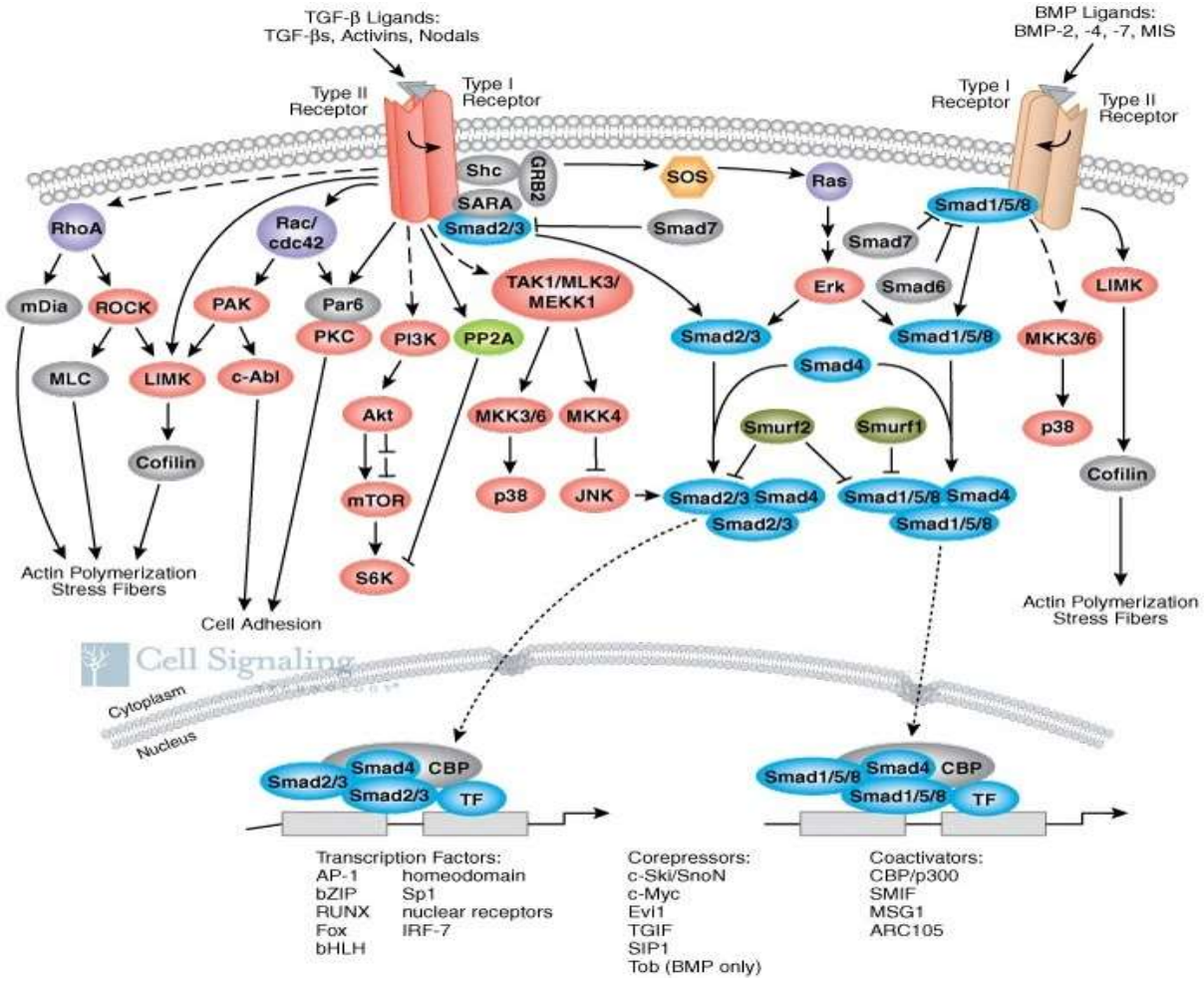




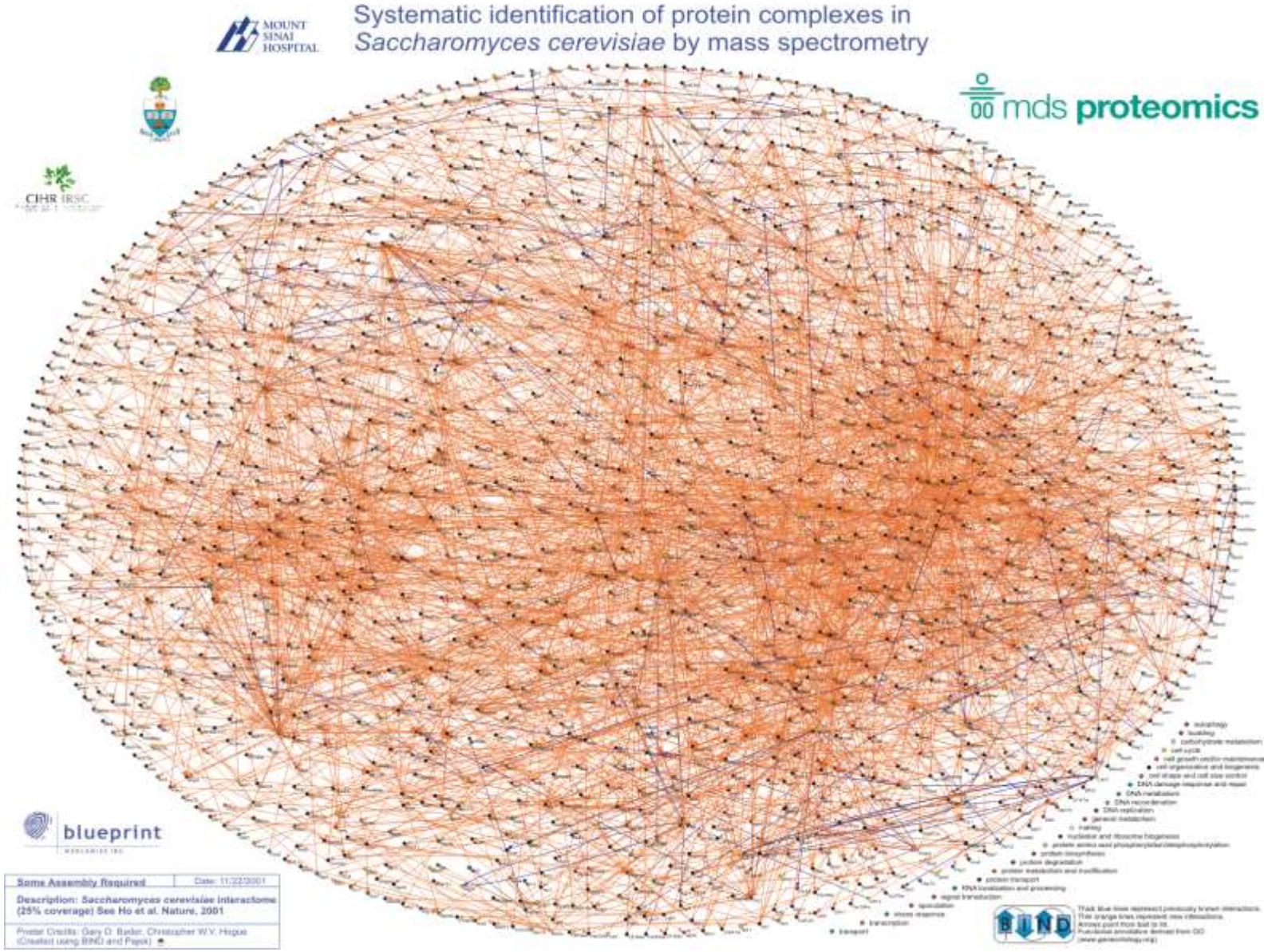


Biological Functions of Proteins

Proteins exert their functions through protein-protein interaction



Biological Functions of Proteins



3.3 Working with Proteins

■ Protein purification

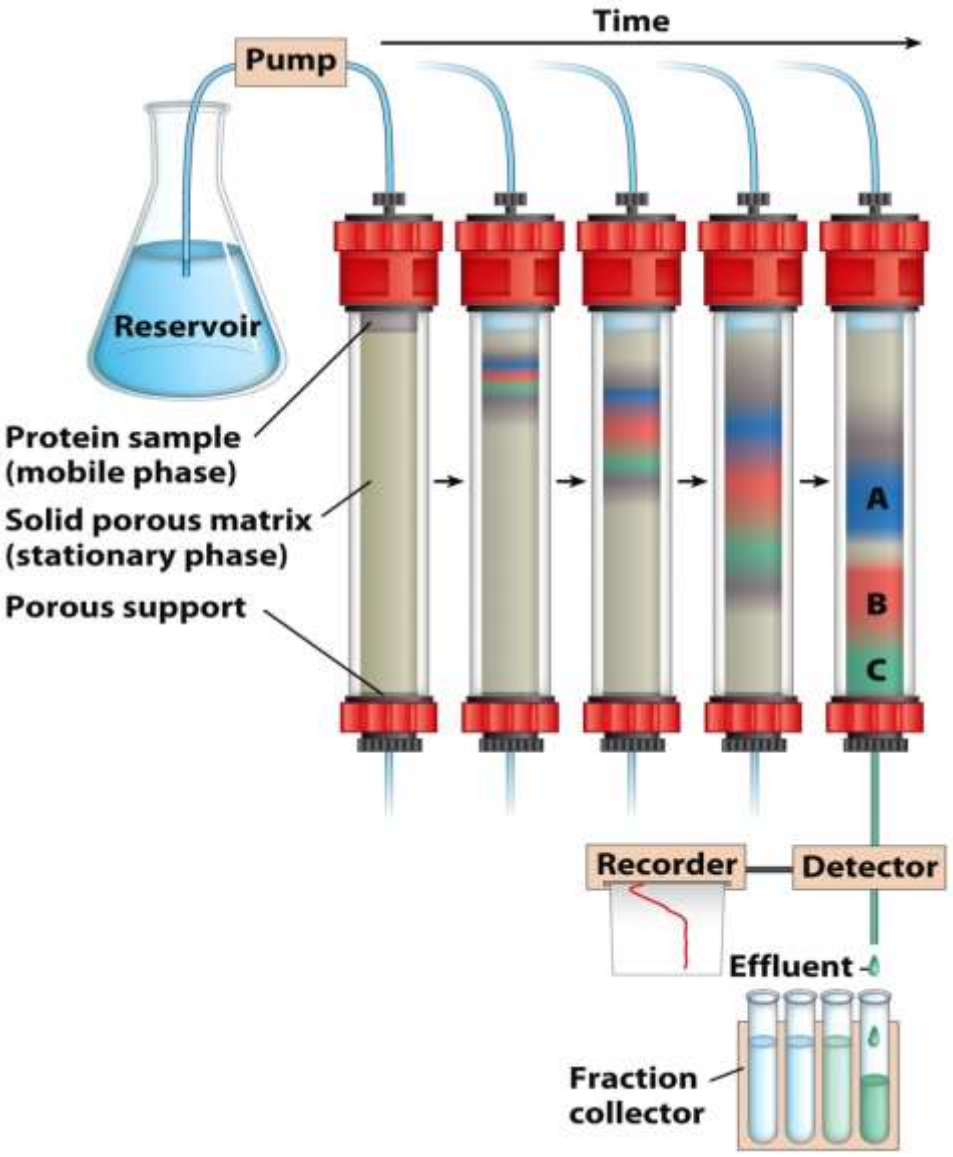
General procedure of protein purification

1. **Crude extract:** the first step in any protein purification procedure is to break open cells, releasing their proteins into a solution called a crude extract.

2. **Fractionation:** the extract is subjected to treatments that separate the proteins into different **fractions** based on a property such as size or charge. Early fractionation steps in a purification utilize differences in protein solubility, which is a complex function of pH, temperature, salt concentration, and other factors.

3.3 Working with Proteins

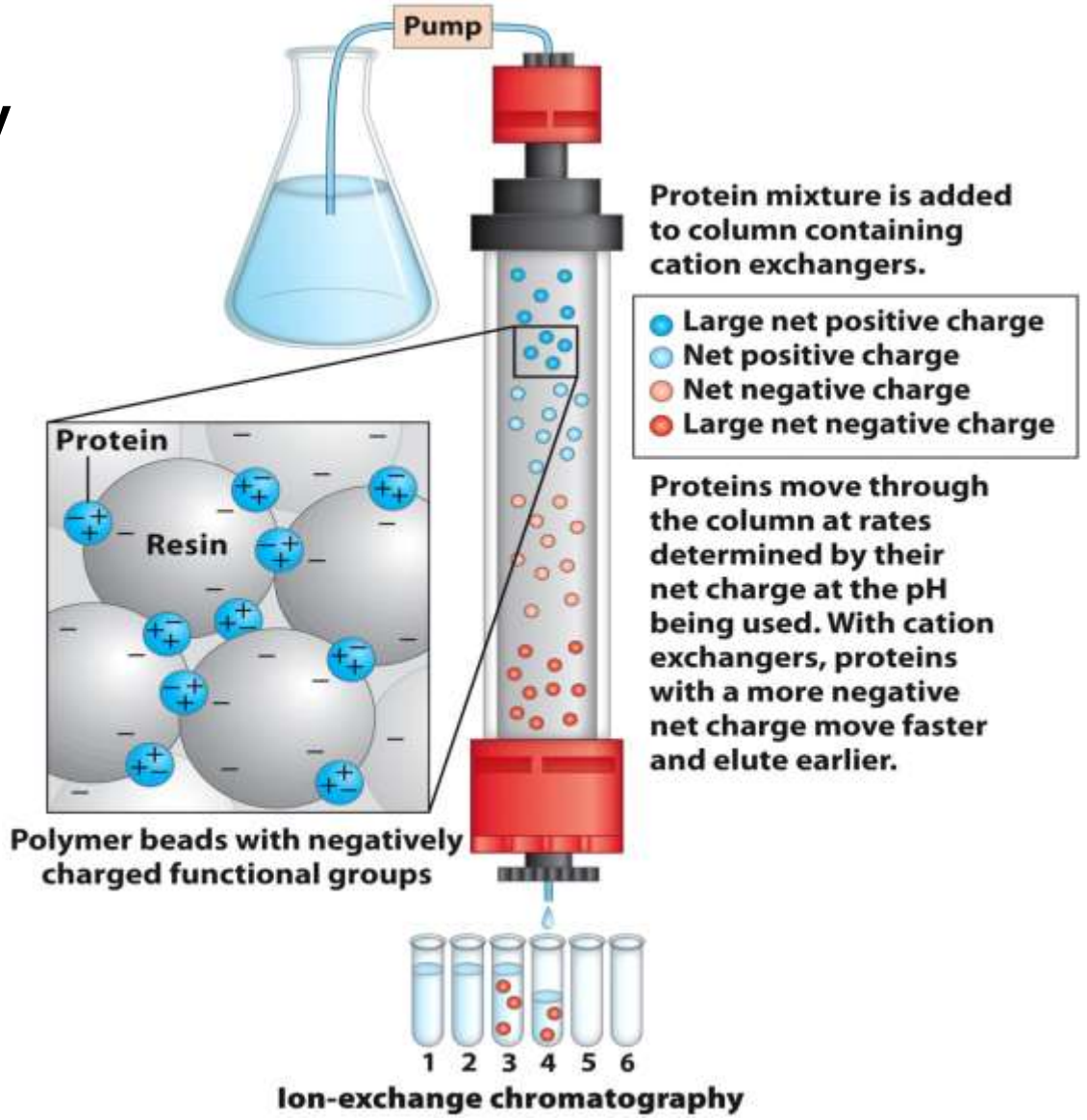
- **Column chromatography**



To fractionate proteins by the differences in size, charge, binding affinity, and other properties.

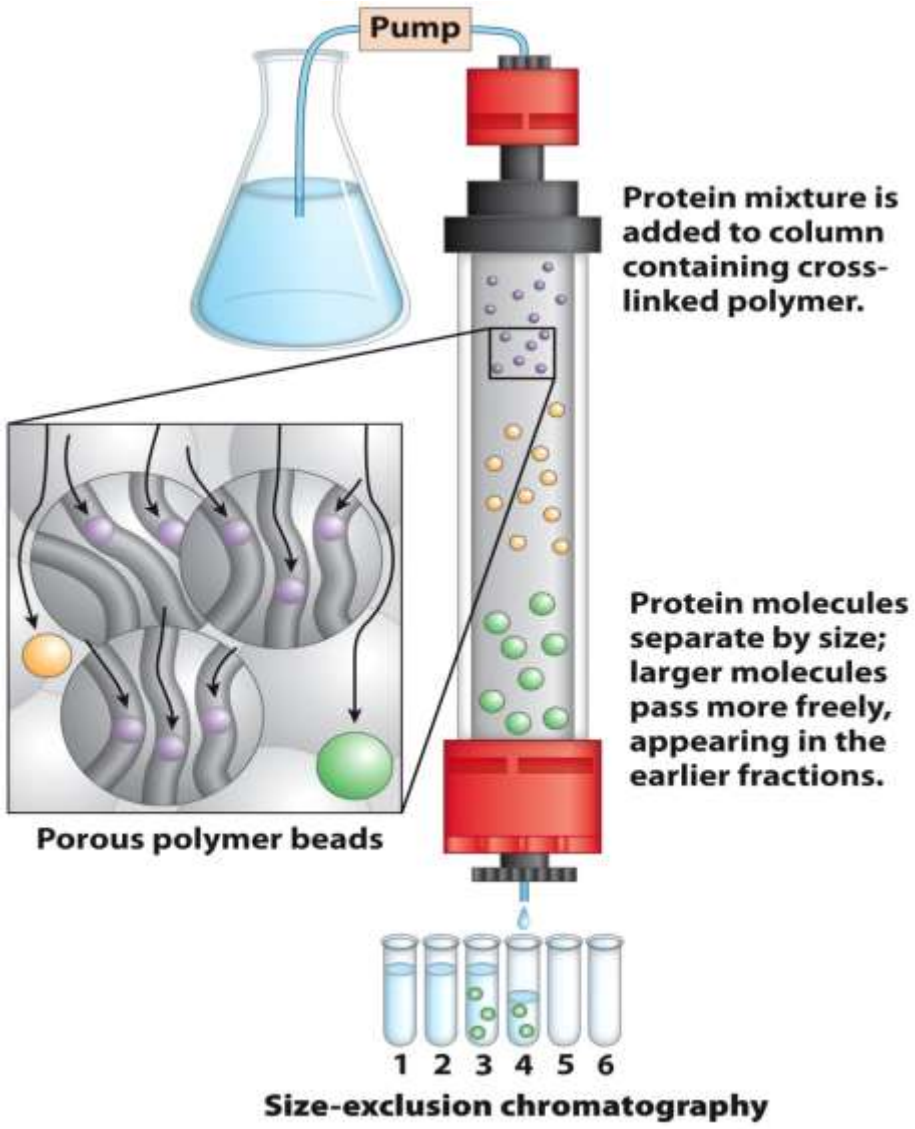
3.3 Working with Proteins

- **Ion-exchange chromatography**



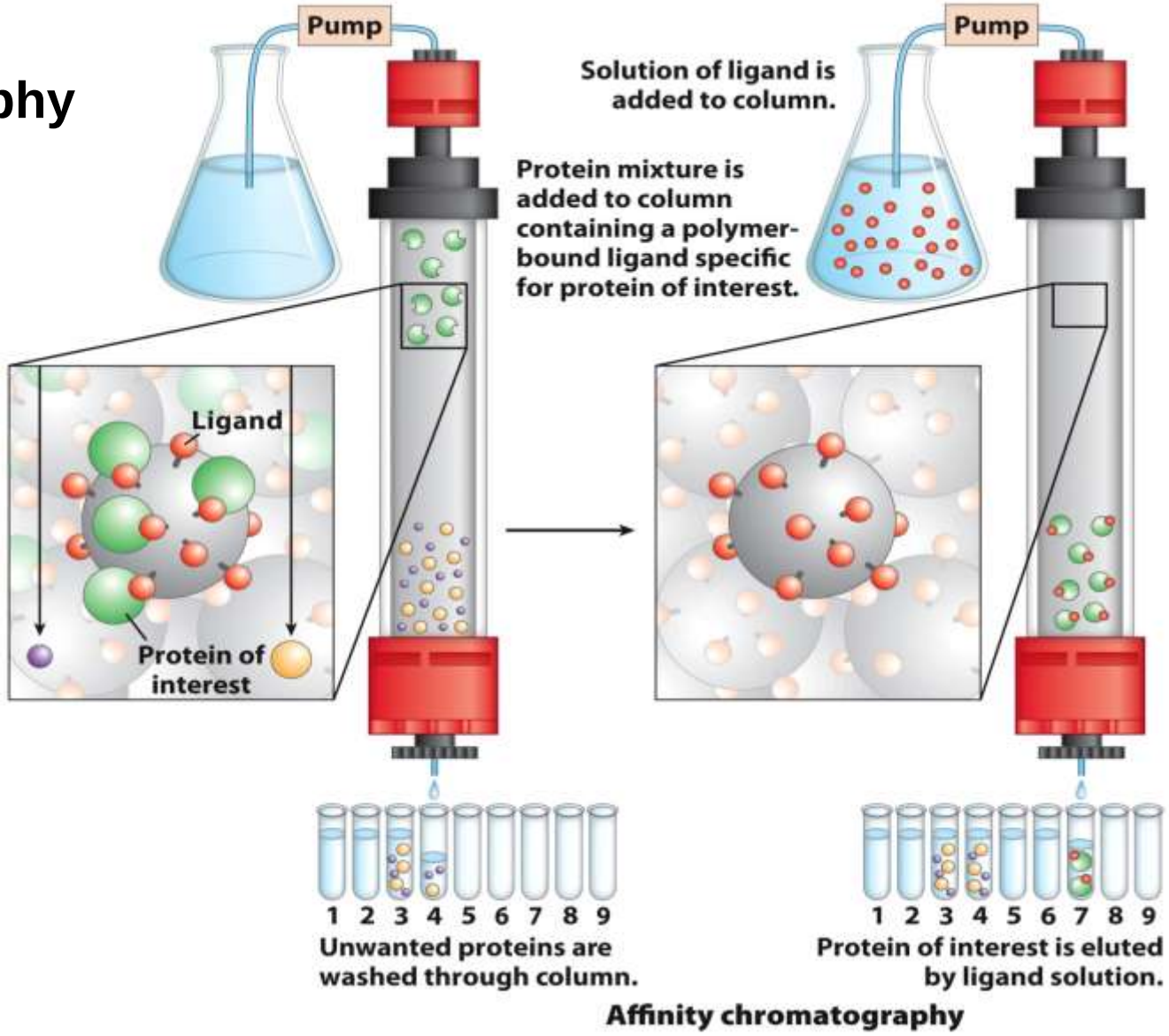
3.3 Working with Proteins

- **Size-exclusion chromatography**



3.3 Working with Proteins

- **Affinity chromatography**



3.3 Working with Proteins

TABLE 3–5 **A Purification Table for a Hypothetical Enzyme**

Procedure or step	Fraction volume (mL)	Total protein (mg)	Activity (units)	Specific activity (units/mg)
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	60,000	600
5. Affinity chromatography	6	3	45,000	15,000

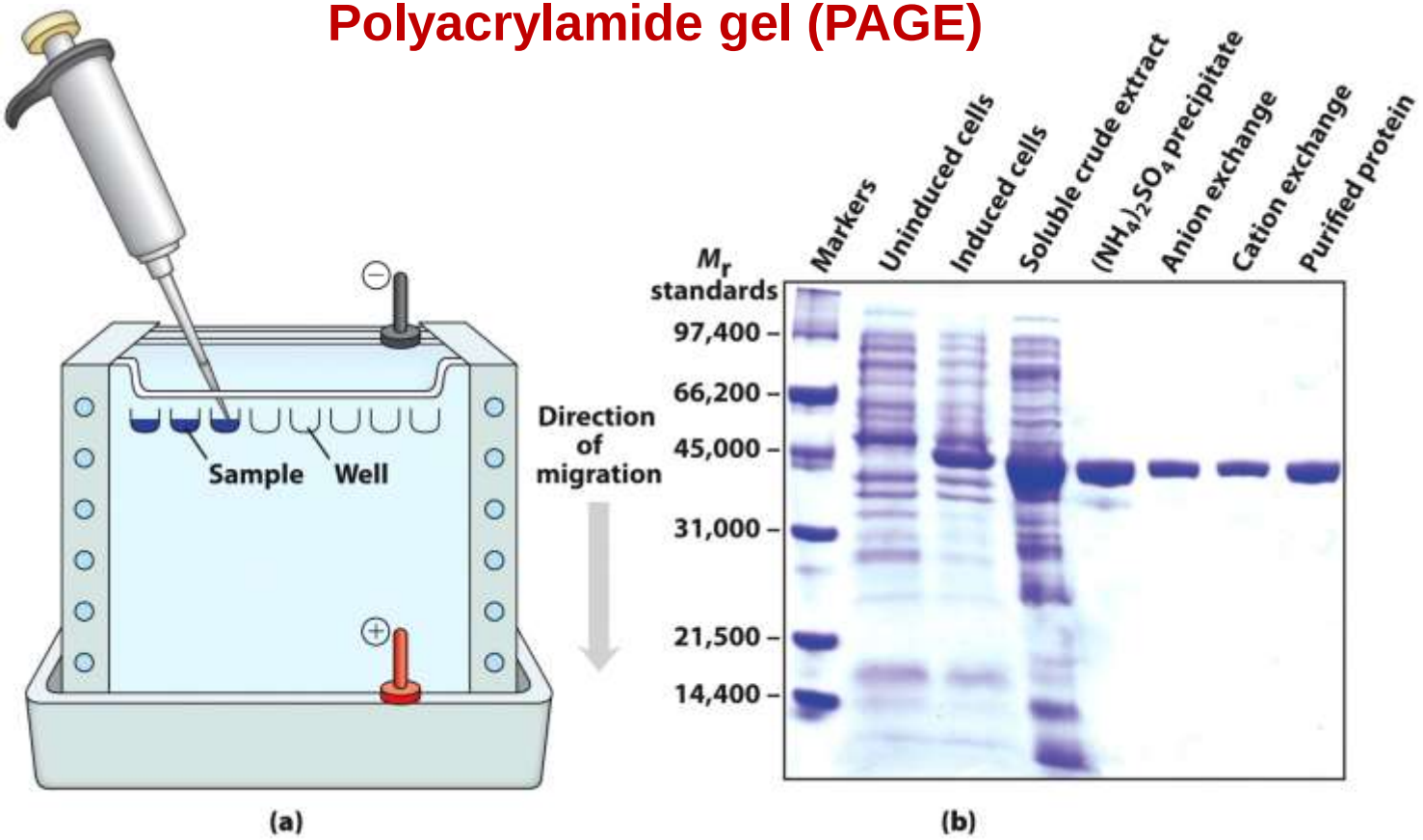
Note: All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 95.

3.3 Working with Proteins

- **Characterization of protein**

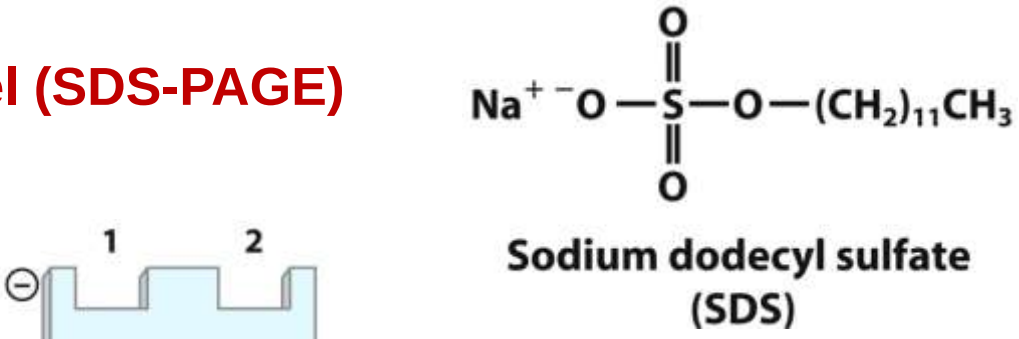
  Electrophoresis

Polyacrylamide gel (PAGE)

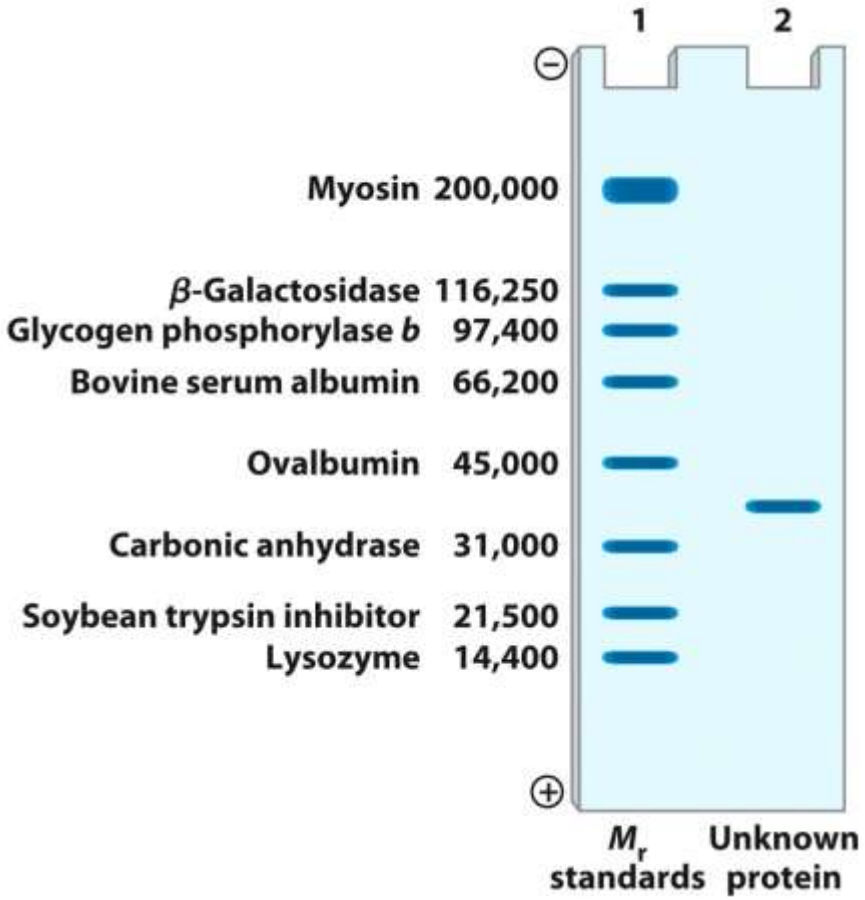


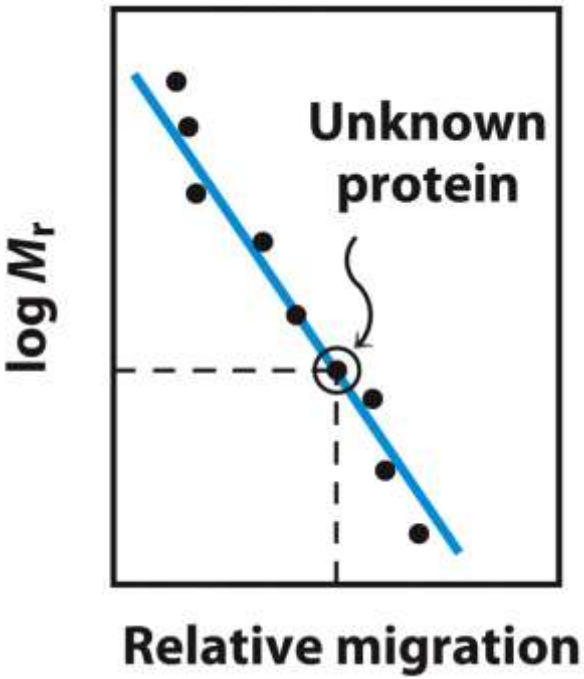
3.3 Working with Proteins

SDS-polyacrylamide gel (SDS-PAGE)



Sodium dodecyl sulfate (SDS)

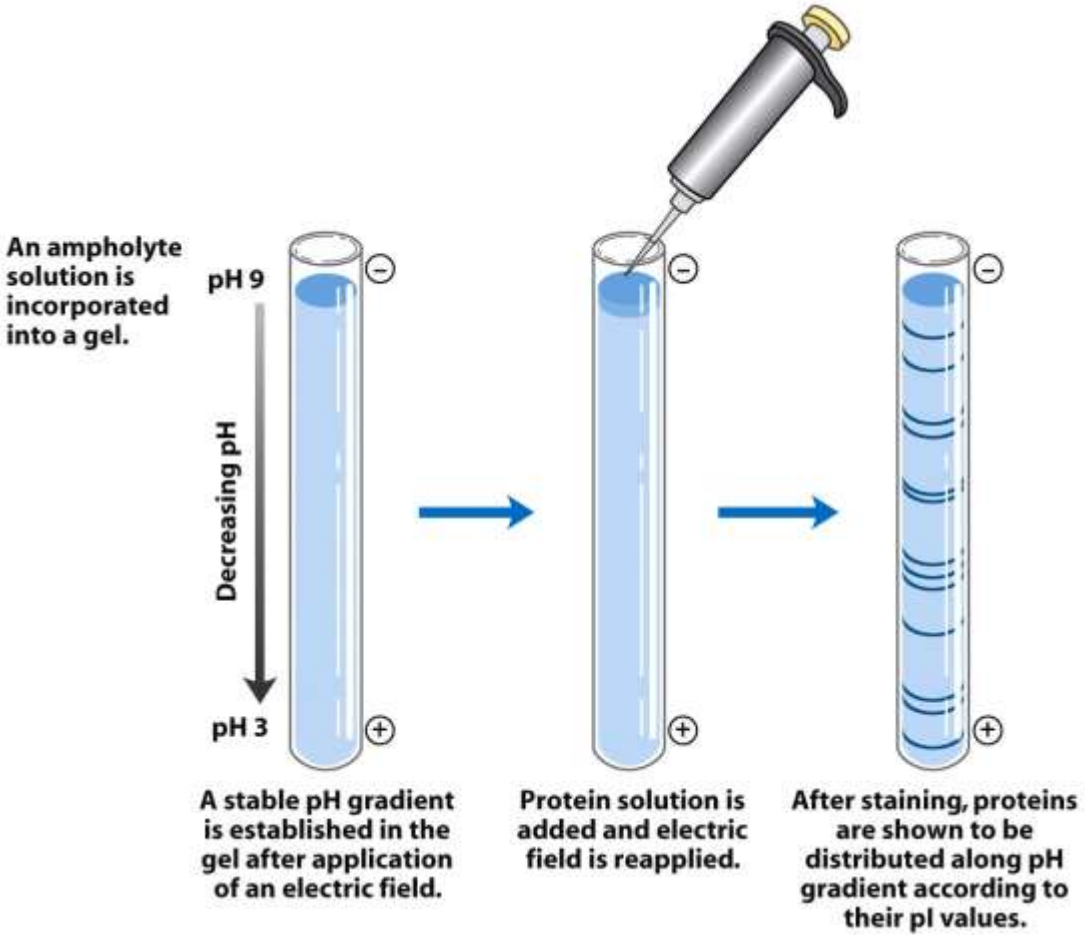




3.3 Working with Proteins

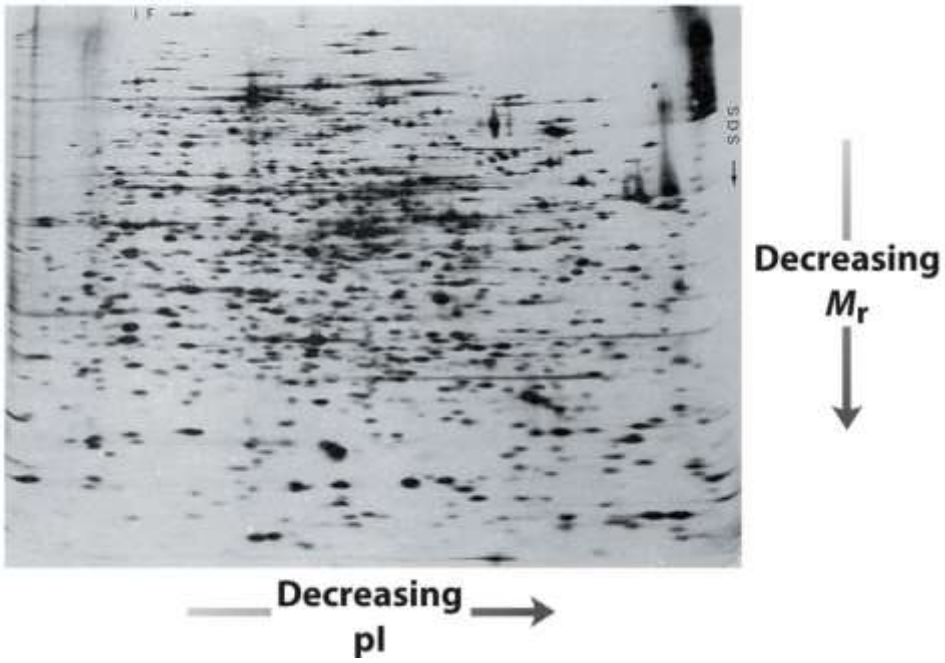
Isoelectric focusing

TABLE 3–6	The Isoelectric Points of Some Proteins
Protein	**pI**
Pepsin	<1.0
Egg albumin	4.6
Serum albumin	4.9
Urease	5.0
β-Lactoglobulin	5.2
Hemoglobin	6.8
Myoglobin	7.0
Chymotrypsinogen	9.5
Cytochrome *c*	10.7
Lysozyme	11.0

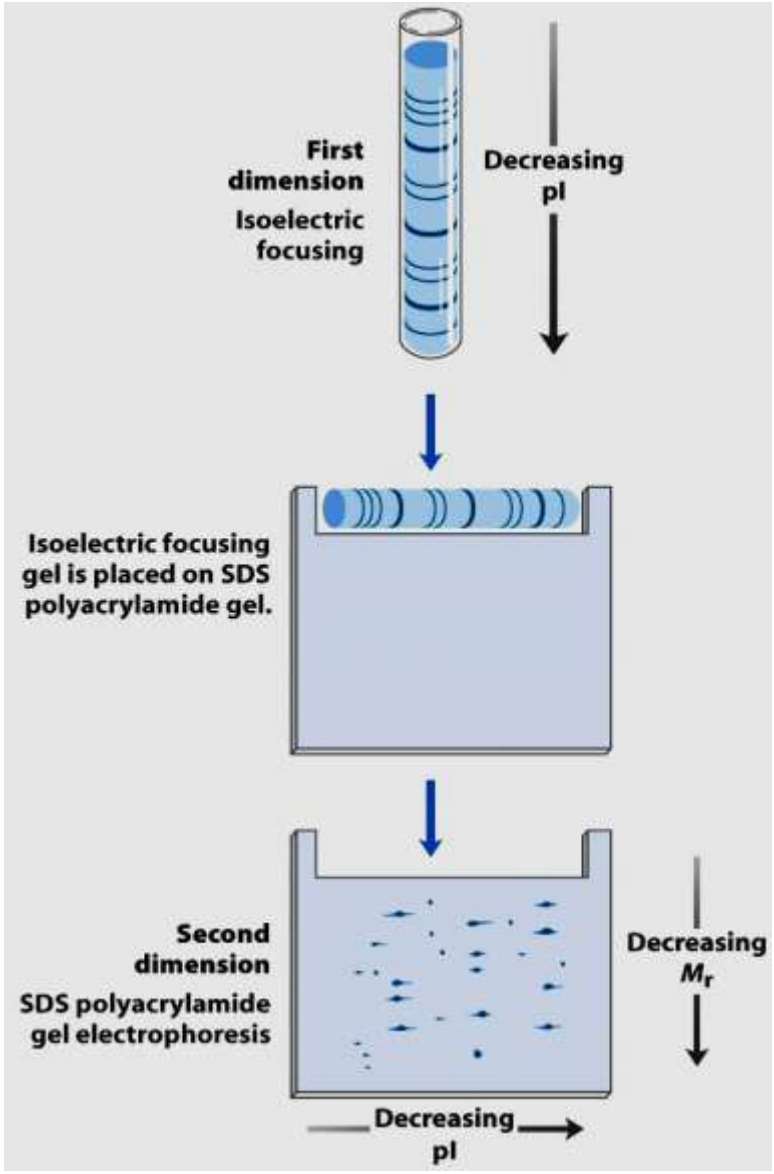


3.3 Working with Proteins

Two-dimensional electrophoresis



Proteins in *E. coli*



Take home messages …

- ✓ **Protein structures**

  The secondary, tertiary and quaternary structures, forces for protein structure, denaturation and folding

- ✓ **Protein functions**

  Variety of protein functions

- ✓ **Working with Proteins**

  Column chromatography, electrophoresis