Chapter 5

Overview

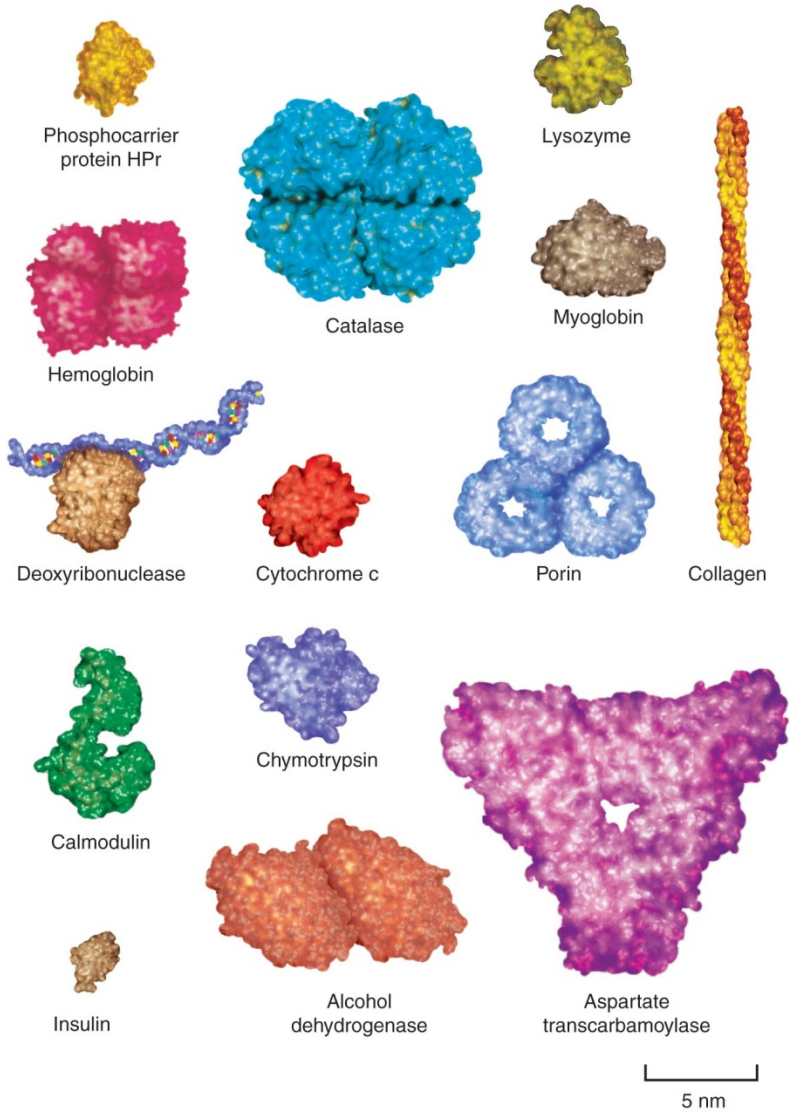
Proteins – molecular tools of life

- Functions
  - Structural – cell shape, connective tissue (cartilage, bond)
  - Catalysis – enzymes
  - Metabolic regulation – regulation of cellular metabolism
  - Transport – move substances back & forth across cell membrane
  - Defense – antibodies in immune response
- 3 General types based on 3-D structure & functional role
  - Fibrous – structural
  - Membrane – several roles associated with cells
  - Globular - transportation

Section 5.1: Amino Acids

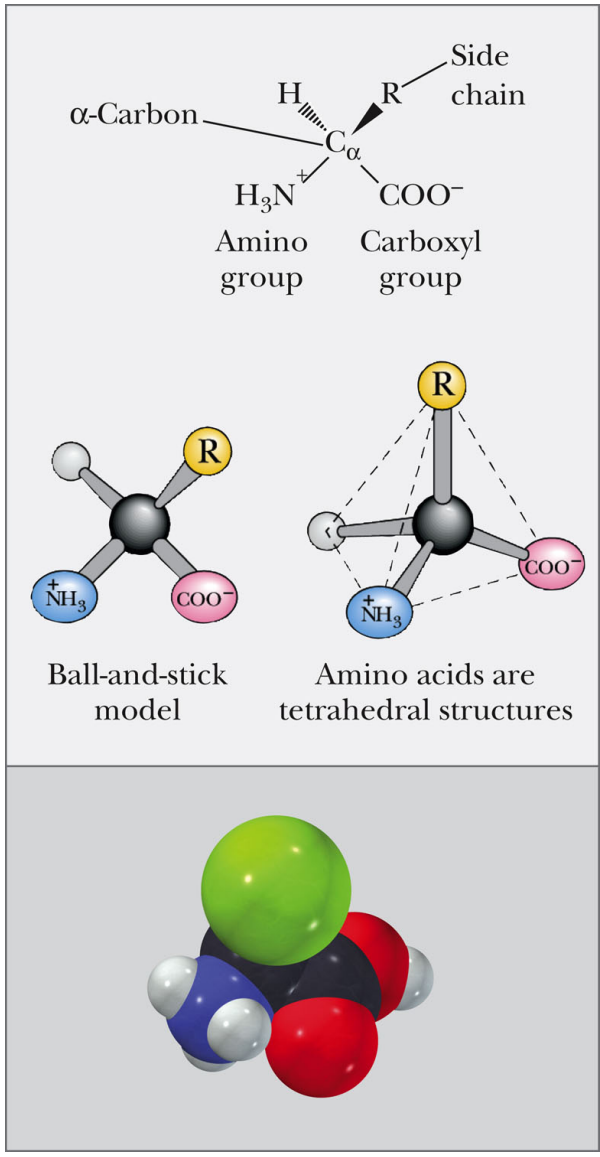
- Produced from only 20 amino acids
  - 100 amino acids can produce 20^{100} potential sequences
- Subset structure & function is result of selection pressure
  - Structure features facilitating folding
  - Presence of binding site
  - Balance of structural flexibility and rigidity
  - Appropriate surface structure
  - Vulnerability to degradation
- Distinguished based on number and sequence of amino acids
- **Polypeptides** – MW thousands to millions Daltons
- **Peptides** – lower MW, <50 amino acids
- **Proteins** - >50 amino acids; 1 or more polypeptide chains

Figure 5.1 Protein Diversity



Section 5.1: Amino Acids

- **Amino acid:** amino group and a carboxyl group bonded to alpha carbon
  - Amino group attached to the carbon adjacent to the carboxyl group
  - Side chain, R, bound to α-carbon
  - R identifies amino acid
- **Amphoteric –** behave as acid or base
  - pH 7, carboxyl group-conjugate base form ($-COO^-$) amino group-conjugate acid form ($-NH_3^+$)
  - **Zwitterions** – have both positive & negative charges
- **Nonstandard amino acids**
  - Chemically modified after incorporation
  - Occur in living organisms but are not in proteins



Section 5.1: Amino Acids

- All protein-derived amino acids have at least one stereocenter (α-carbon)
  - Superimposable mirror images – achiral,
    - ✓ glycine lacks center of asymmetry, H as R group
  - Chiral - stereoisomers are not superimposable
  - 4 groups bonded to α-carbon
- Side-chain carbons designated with Greek symbols, starting at α-carbon (β–beta, γ–gamma, δ–delta, ε–epsilon… etc)

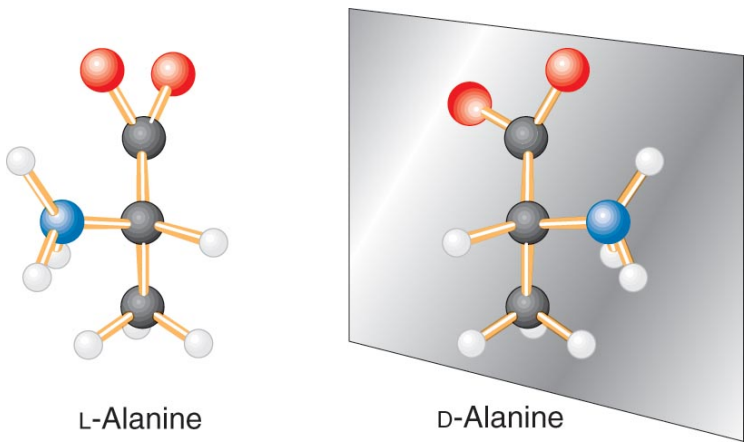


Figure 5.7 Two Enantiomers

- **Enantiomers** - molecules are mirror images of one another
- **Optical isomers – not** superimposeable and rotate plane- polarized light in opposite directions
  - L alanine, amino group on L
  - R alanine amino group on R
  - Mostly L-amino acids found in proteins

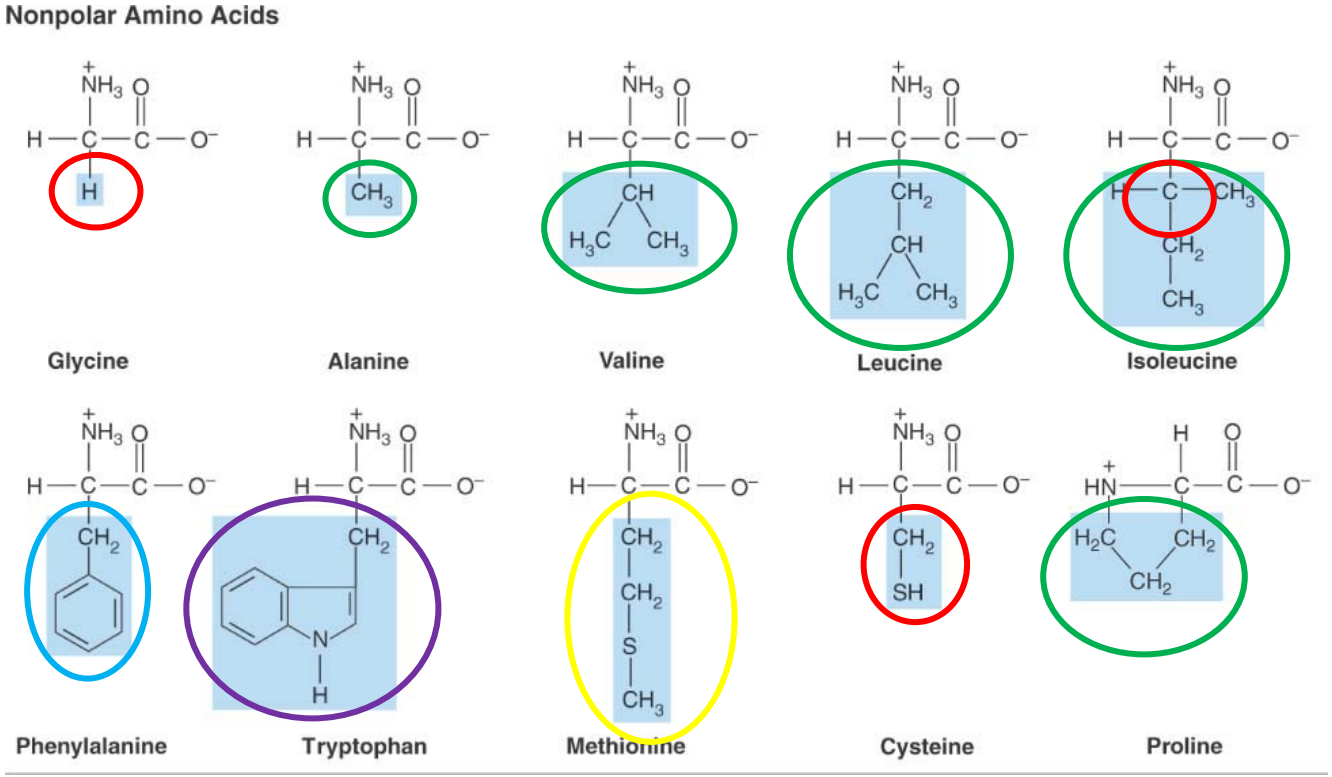
TABLE 5.1 Names and Abbreviations of the Standard Amino Acids

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

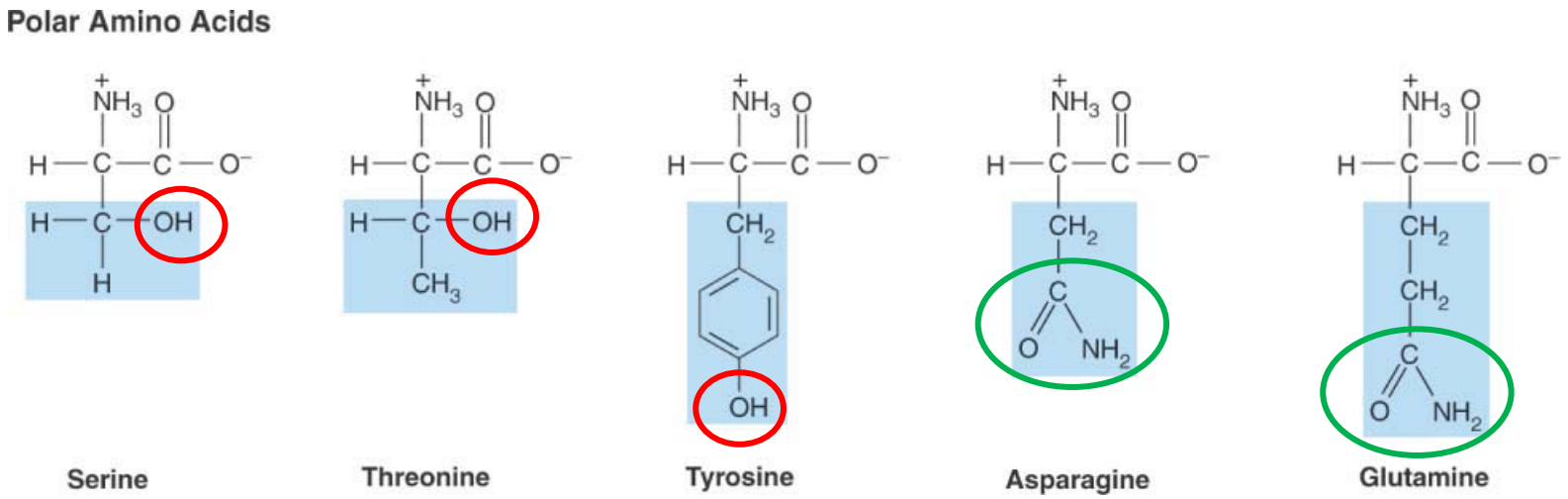
Section 5.1: Amino Acids

Group A: Nonpolar side chains- Ala, Val, Leu, Ile, Pro. Phe, Trp, Met.

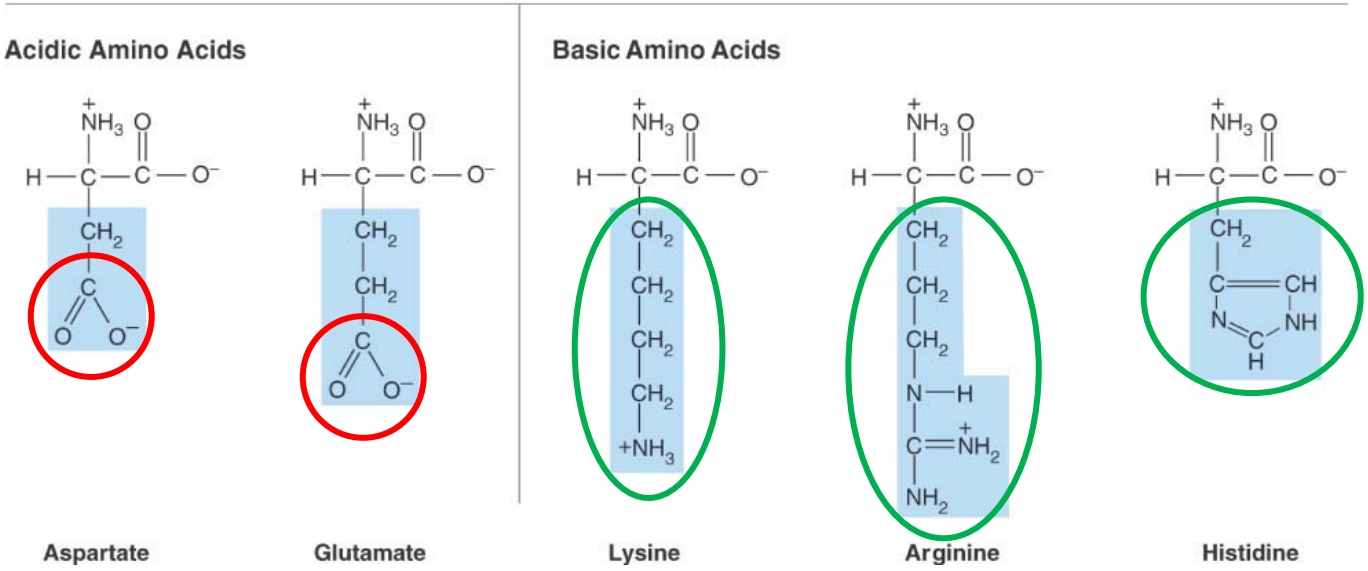
- Ala, Val, Leu, Ile, Pro- contain aliphatic hydrocarbon group. Pro has cyclic structure.
- Phe- hydrocarbon aromatic ring.
- Trp- Indole ring side chain, aromatic.
- Met- Sulfur atom in side chain
- Cys- side chain contains thiol group (-SH)



- **Group B:** Neutral Polar side chains; easily interact with water through hydrogen bonding
  - Ser, Thr- side chain is polar hydroxyl group
  - Tyr- hydroxyl group bonded to aromatic hydrocarbon group
  - Gln, Asn- contain amide bonds in side chain



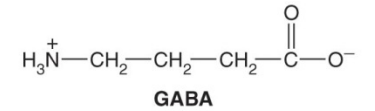
- **Group C:** Acidic Side Chains: Glu, Asp
  - Both have a carboxyl group in side chain
  - Can lose a proton, forming a carboxylate ion
  - Negatively charged at neutral pH
- **Group D:** Basic side chains: His, Lys, Arg
  - Side chains are positively charged at pH 7
  - Arg-side chain is a guanidino group
  - His-side chain is an imidazole group
  - Lys-side chain NH_3 group is attached to an aliphatic hydrocarbon chain

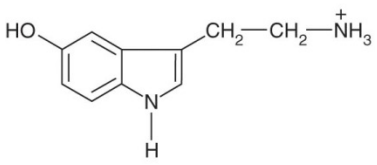


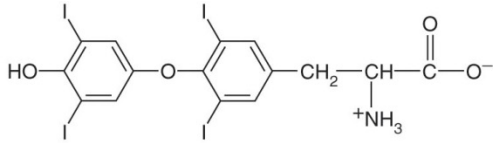
Biologically Active Amino Acids

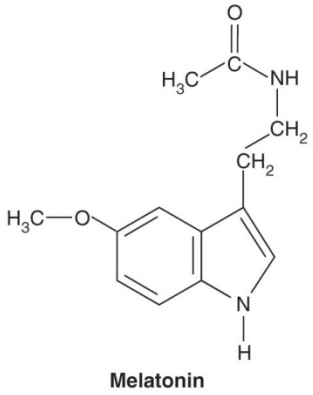
1. Some amino acids or derivatives can act as chemical messengers

- **Neurotransmitters** – glycine, glutamate, γ-amino butyric acid, GABA, serotonin, melatonin









- **Hormones** – thyroxine
  - ✓ Signal molecule produced in 1 cell, regulates function of other cells

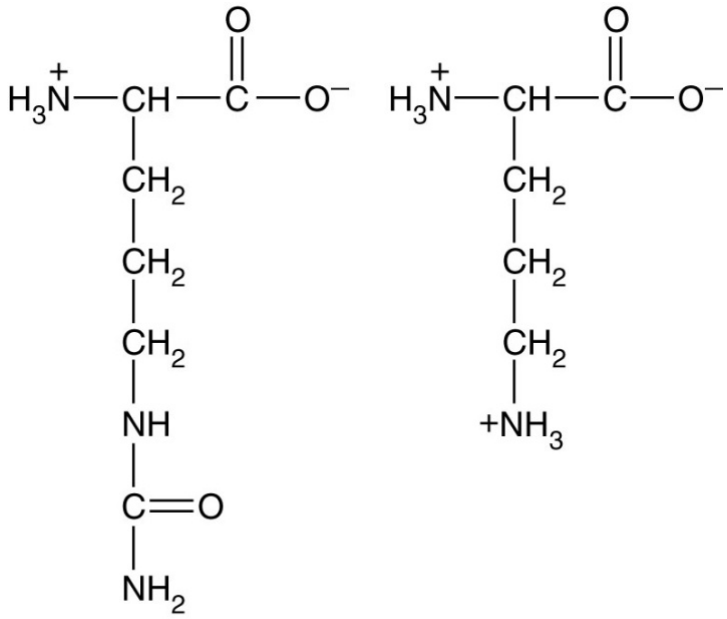
Figure 5.4 Some Derivatives of Amino Acids

2. Act as precursors for other molecules

- Nitrogenous base components of nucleotides & nucleic acids
- Heme, chlorophyll

3. Metabolic intermediates

- Arginine, ornithine, and citrulline in urea cycle



Modified Amino Acids in Proteins

- Derivatives of amino acids formed after protein synthesis
  - Serine, threonine, and tyrosine can be phosphorylated
  - γ-Carboxyglutamate (prothtrombin)
  - Collagen (4-hydroxyproline & 5-hydroxylysine)
    - ✓ Structural protein, most abundant protein in connective tissue

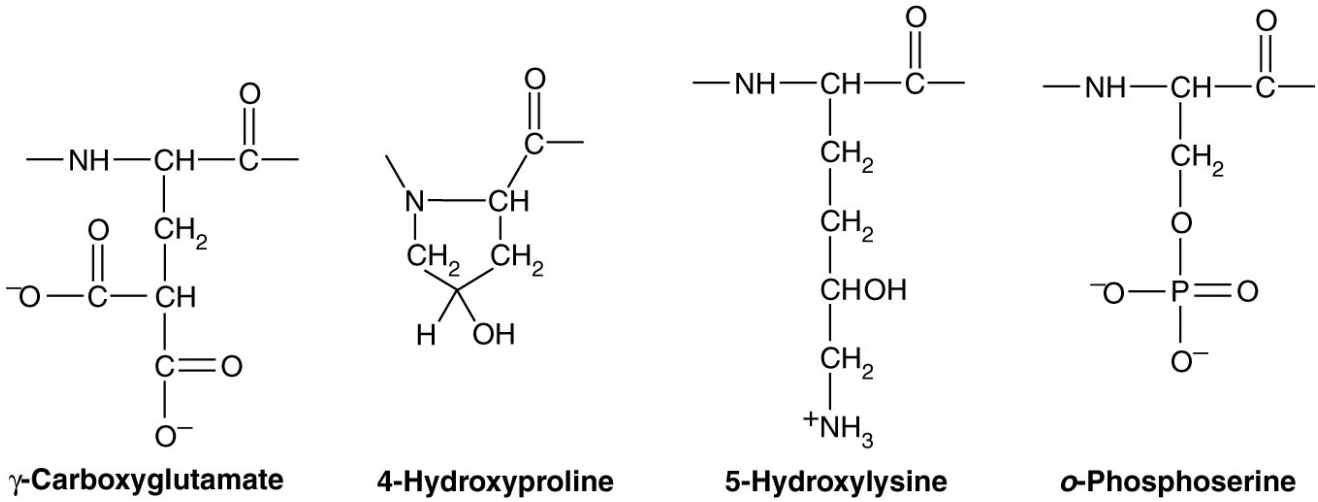
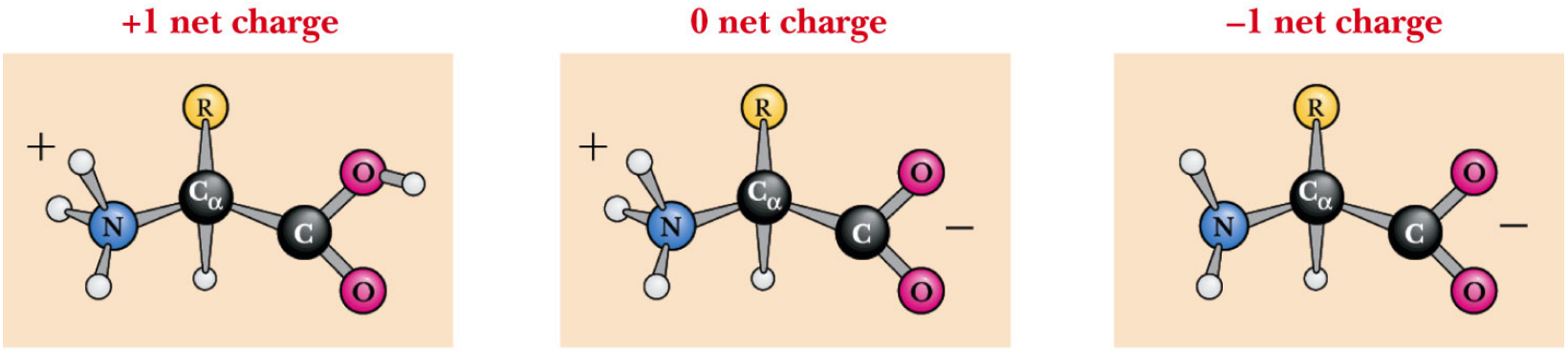


Figure 5.6 Modified Amino Acid Residues Found in Polypeptides

Titration of Amino Acids

- Amino acids without charged groups on side chain exist in neutral solution as **zwitterions** with no net charge



A The ionic forms of the amino acids, shown without consideration of any ionizations on the side chain. The cationic form is the low-pH form, and the titration of the cationic species with base yields the zwitterions and finally the anionic form.

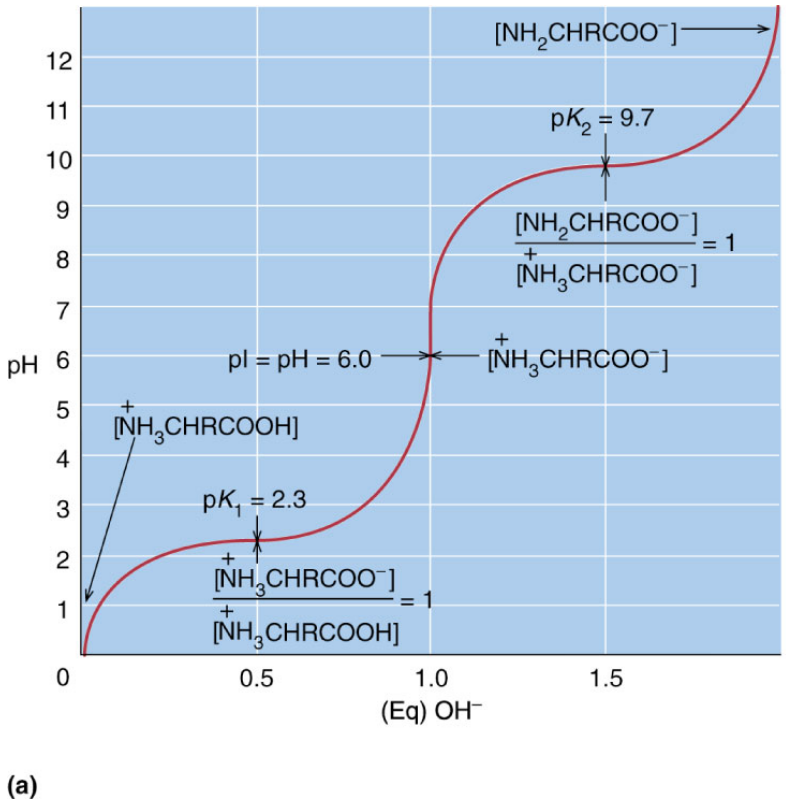


Figure 5.9 Titration of Two Amino Acids: Alanine

- Simple amino acid - two ionizable groups
  - Loses two protons in a stepwise fashion upon titration with NaOH
  - Isoelectric point is reached with deprotonation of the carboxyl group

$$pI = \frac{pK_1 + pK_2}{2}$$

TABLE 5.2 pK_a Values for the Ionizing Groups of the Amino Acids

Amino Acid	pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R
Glycine	2.34	9.6	
Alanine	2.34	9.69	
Valine	2.32	9.62	
Leucine	2.36	9.6	
Isoleucine	2.36	9.6	
Serine	2.21	9.15	
Threonine	2.63	10.43	
Methionine	2.28	9.21	
Phenylalanine	1.83	9.13	
Tryptophan	2.83	9.39	
Asparagine	2.02	8.8	
Glutamine	2.17	9.13	
Proline	1.99	10.6	
Cysteine	1.71	10.78	8.33
Histidine	1.82	9.17	6.0
Aspartic acid	2.09	9.82	3.86
Glutamic acid	2.19	9.67	4.25
Tyrosine	2.2	9.11	10.07
Lysine	2.18	8.95	10.79
Arginine	2.17	9.04	12.48

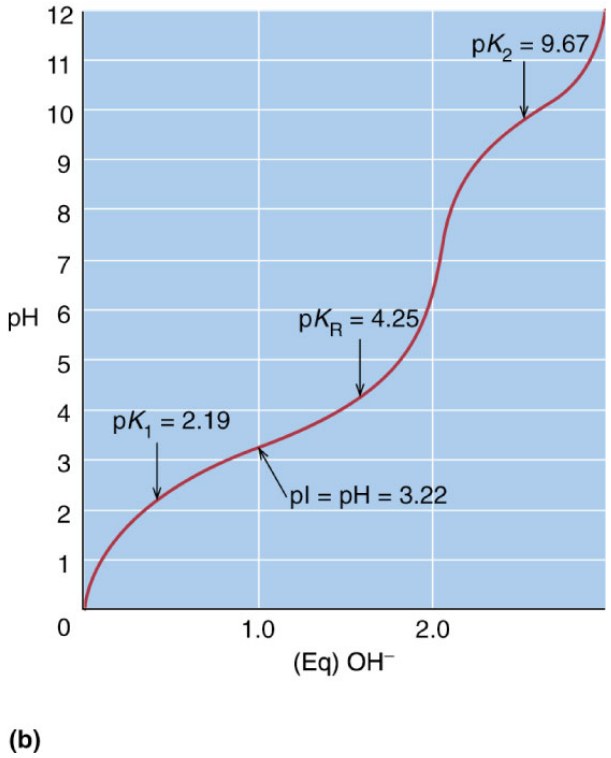


Figure 5.9 Titration of Two Amino Acids: Glutamic Acid

- Glutamic acid has a carboxyl side chain group
  - +1 charge at low pH
    - Isoelectric point between lose of α-carboxyl proton & R grp carboxyl proton
    - More base is added, it loses protons to a final net charge of -2

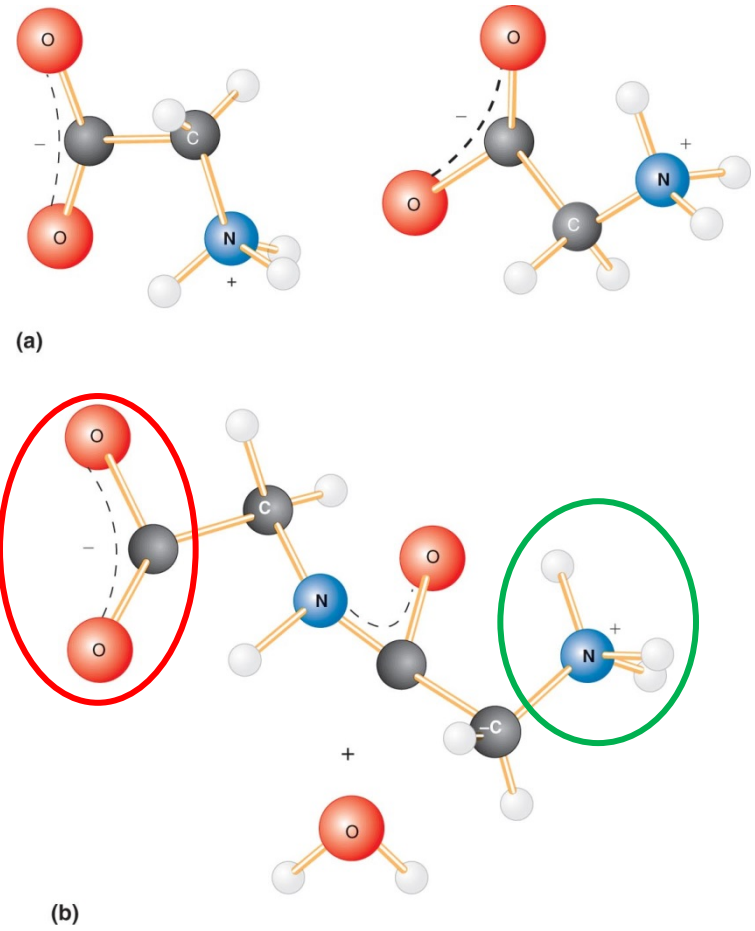
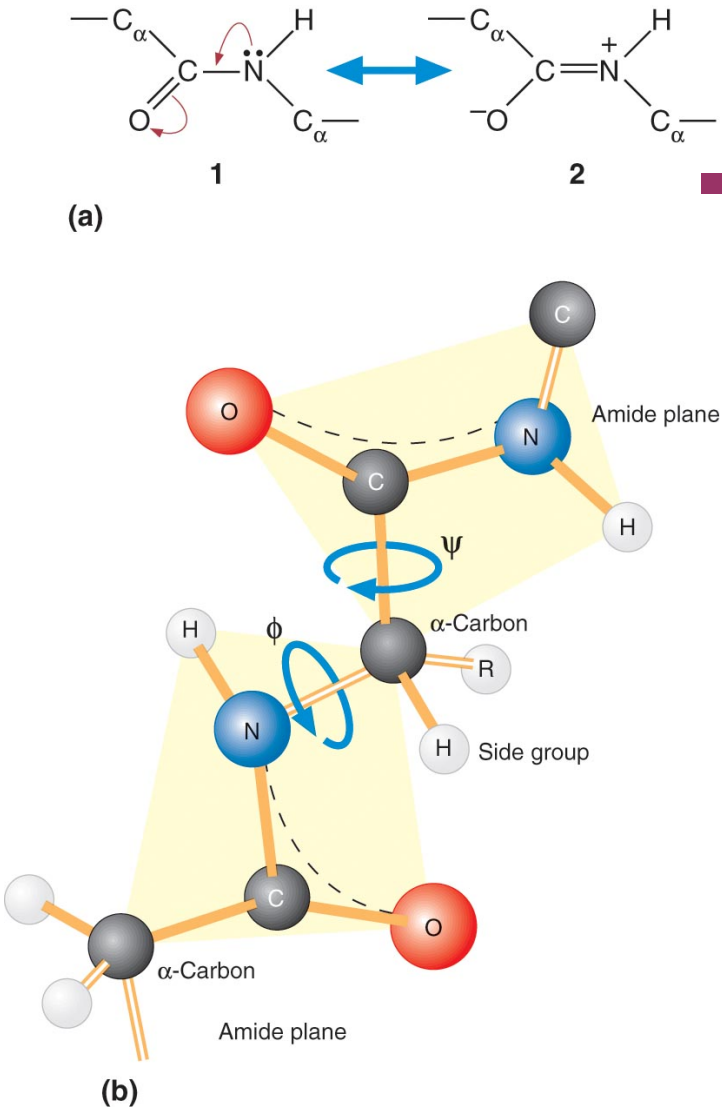


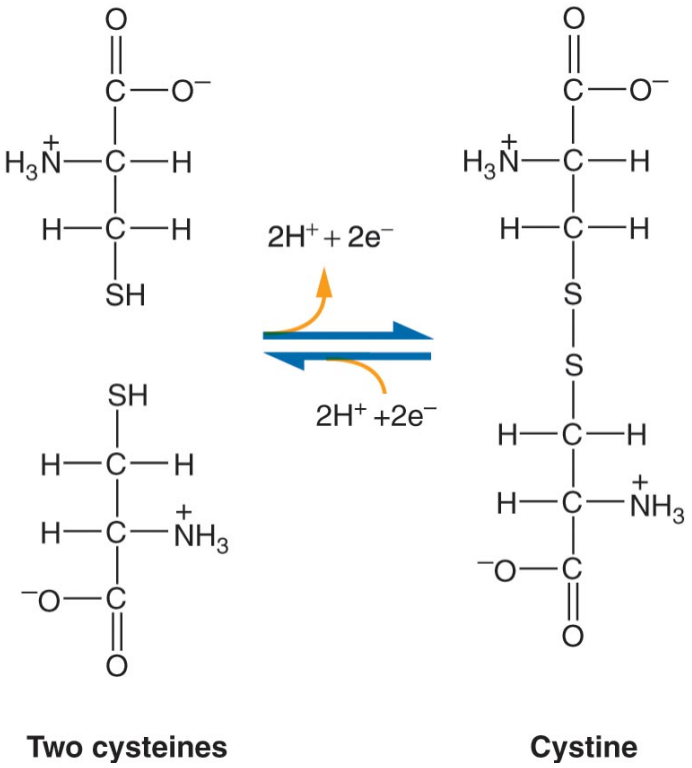
Figure 5.10 Formation of a Dipeptide

- **Peptide Bond Formation:** polypeptides are linear polymers of amino acids linked by **peptide bonds**
  - Amide linkages formed by nucleophilic acyl substitution
    - N-terminal amino acid has the free amino group
    - C-terminal has a free carboxyl group
    - Dehydration reaction
  - Resulting **amino acid residues** are named by number of amino acids
  - Amino acid sequence leads directly to the protein's **native conformation**



- Peptide bond as rigid and flat
  - C-N bonds between two amino acids are shorter than other C-N bonds
  - Partial double-bond characteristics (resonance hybrids)
  - Due to rigidity, one-third of the bonds in a polypeptide backbone cannot rotate freely
  - Limits the number of conformational possibilities

Figure 5.11 The Peptide Bond



- **Cysteine oxidation** leads to a reversible disulfide bond
- **Disulfide bridge** forms when two cysteine residues form this bond
  - ✓ Cystine – nonstandard amino acid
  - ✓ Helps stabilize polypeptides and proteins

Figure 5.12 Oxidation of Two Cysteine Molecules to Form Cystine

- **Peptides** have biologically important functions
  - **Glutathione** is a tripeptide found in most all organisms
    - Involved in protein and DNA synthesis, toxic substance metabolism, and amino acid transport
  - **Vasopressin** is an antidiuretic hormone
    - Regulates water balance, appetite, and body temperature
  - **Oxytocin** is a signal peptide
    - Aids in uterine contraction
    - Stimulates ejection of milk by mammary glands

- Proteins diverse set of functions:
  - **Catalysis** (enzymes)
  - **Structure** (cell and organismal)
  - **Movement** (amoeboid movement)
  - **Defense** (antibodies)
  - **Regulation** (insulin is a peptide hormone)
  - **Transport** (membrane transporters)
  - **Storage** (ovalbumin in bird eggs)
  - **Stress Response** (heat shock proteins)

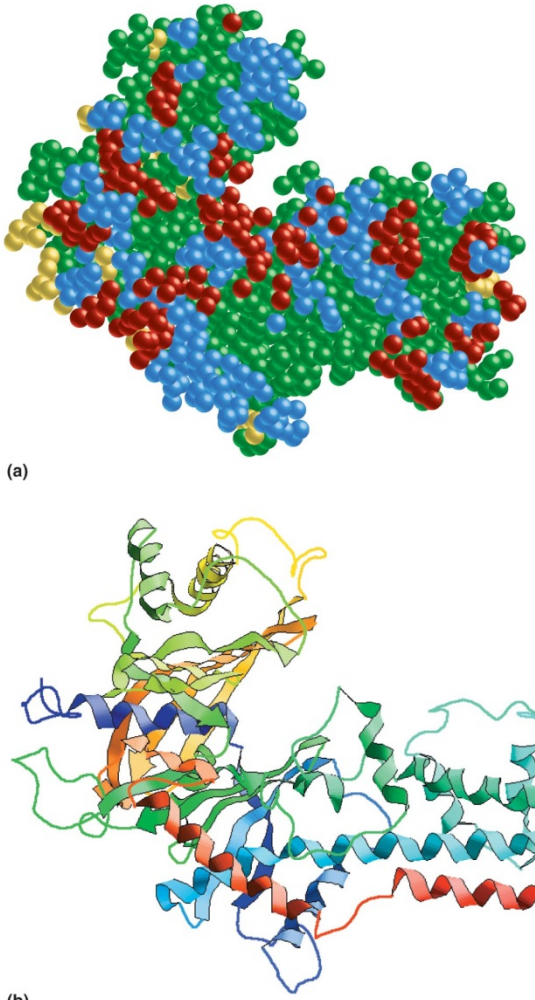


Figure 5.14 The Enzyme Adenylate Kinase

Two main classes: Fibrous, Globular

Composition classification

Simple – contain only amino acids
Conjugated – simple protein with **prosthetic grp**

Levels of structure

1°structure: order of amino acids in a polypeptide chain read from the N-terminal end to the C-terminal end (L to R)

2°structure: arrangement in space of the backbone atoms secondary structures: α-helix and β-pleated sheet

3° structure: 3-D arrangement of all atoms including those in the side chains and prosthetic groups

Prosthetic groups – atoms other than amino acids

4° structure: interaction of several polypeptide chains in a multi-subunit protein

- **Primary Structure** is the specific amino acid sequence of a protein
  - **Homologous** proteins share a similar sequence and arose from the same ancestor gene
  - Comparing amino acid sequences of a protein between species, those that are identical are **invariant** and presumed to be essential for function
- **Primary Structure, Evolution, and Molecular Diseases**
  - Due to evolutionary processes, the amino acid sequence of a protein can change due to alterations in DNA sequences called **mutations**
    - Many mutations lead to no change in protein function
      - Some sequence positions are less stringent (**variable**) because they perform nonspecific functions
    - Some changes are said to be conservative, because it is a change to a chemically similar amino acid

- Mutations can be deleterious, leading to **molecular diseases**
  - Sickle cell anemia is caused by a substitution of valine for a glutamic acid in β-globin subunit of hemoglobin
    - Valine is hydrophobic, unlike the charged glutamic acid
  - Substitution for hydrophobic valine HbS: molecules aggregate to form sickle-shaped cells
  - Cells have low oxygen- binding capacity and are susceptible to hemolysis

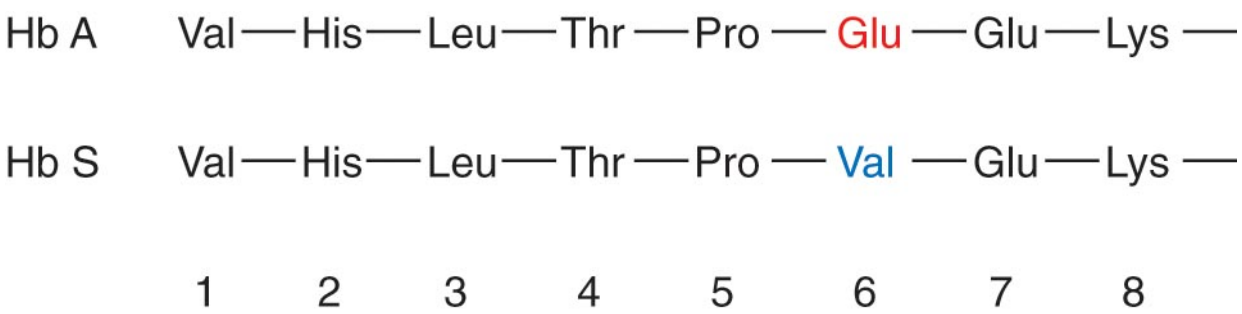


Figure 5.15 Segments of β-chain in HbA and HbS

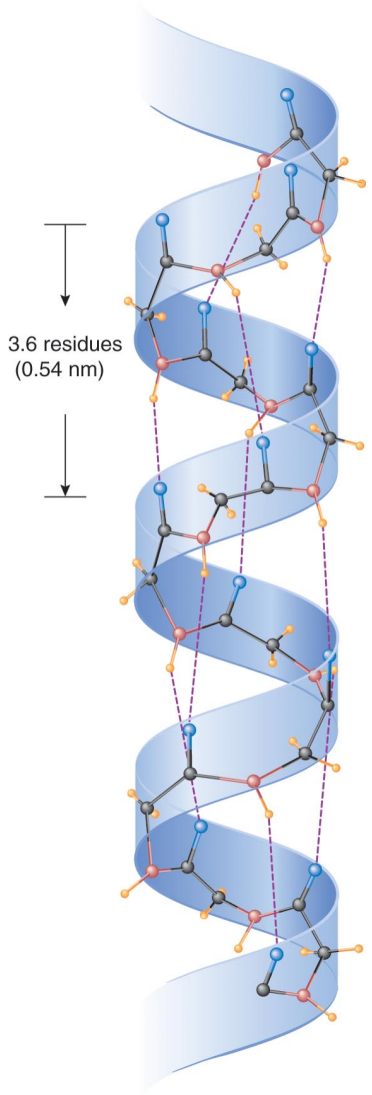


Figure 5.17 The α-Helix

- **Secondary Structure:** -variety of repeating structures
  - Most common include the α-helix and β-pleated sheet
  - Stabilized by hydrogen bonding between the carbonyl and the N-H groups of the polypeptide's backbone

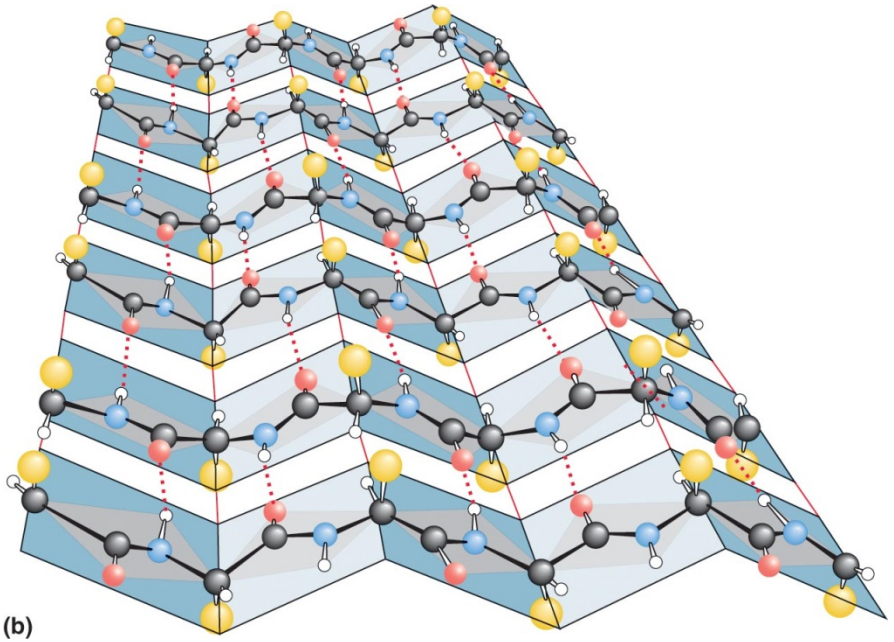
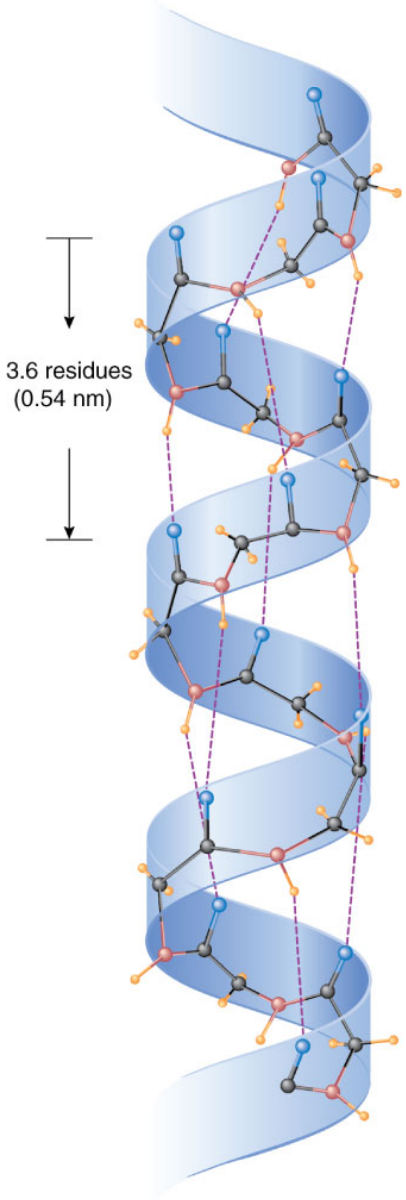


Figure 5.18 β-Pleated Sheet



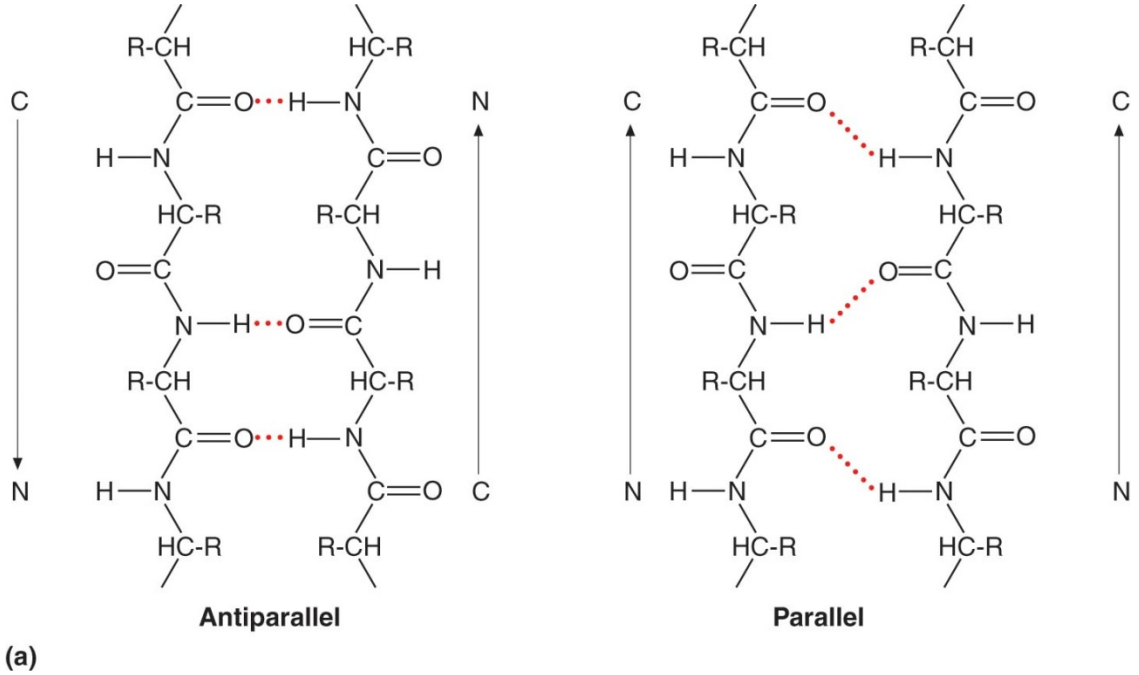
α-helix- rigid, rodlike structure

- Coil of the helix is clockwise or right-handed
- 3.6 amino acids per turn; repeat distance is 5.4Å
- Peptide bond is s-trans and planar
- C=O of peptide bond is H-bonded to the N-H of 4th amino acid away
- C=O----H-N hydrogen bonds are parallel to helical axis
- All R groups point outward from helix
- **Factors that disrupt**
  - Proline creates a bend
    - Restricted rotation due to its cyclic structure
    - α-amino group has no N-H for hydrogen bonding
  - Strong electrostatic repulsion
    - Lys and Arg or Glu and Asp
  - Steric repulsion
    - Val, Ile, Thr

Section 5.3: Proteins

- β-pleated sheets form when polypeptide chains lie adjacent to one another
  - Parallel – N terminal to C terminal
  - Anti-parallel – 1 chain N to C, other C to N
- R groups alternate
  - Above polypeptide chain; next below polypeptide chain
- C=O and N-H groups of each peptide bond are perpendicular to axis of the sheet
- C=O---H-N hydrogen bonds are between adjacent sheets and perpendicular to the direction of the sheet
  - Intrachain bonding – chain double back on itself
  - Interchain bonding – H bonds between 2 different chains

Figure 5.18 β-Pleated Sheet



- Parallel sheets are much less stable than antiparallel sheets

- **Supersecondary structures**: the combination of α- and β-sections
  - **βαβ unit**: two parallel strands of β-sheet connected by a stretch of a-helix
  - **β-meander**: an antiparallel sheet formed by a series of tight reverse turns connecting stretches of a polypeptide chain
  - **αα unit**: two antiparallel α-helices
  - **β-barrel**: created when β-sheets are extensive enough to fold back on themselves
  - **Greek key**: repetitive supersecondary structure formed when an antiparallel sheet doubles back on itself

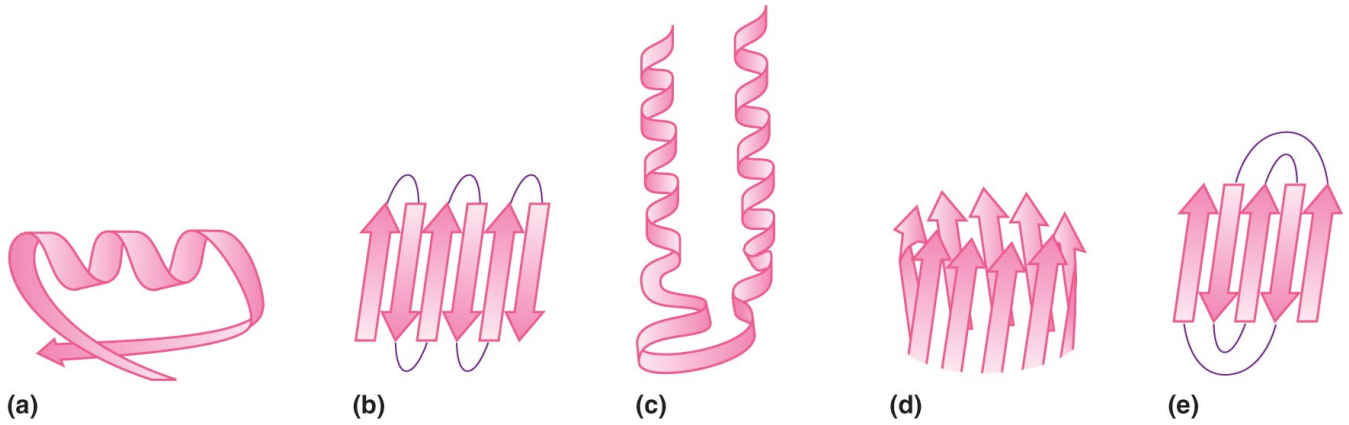


Figure 5.19 Selected Supersecondary Structures

- **Superfamilies** are more distantly related proteins (e.g., hemoglobin and myoglobin to neuroglobin)
- Proteins are also classified by shape
  - **Globular** - proteins which are folded to a more or less spherical shape
  - **Fibrous** - contain polypeptide chains organized approximately parallel along a single axis.
- Proteins can be classified by composition:
  - **Simple** (contain only amino acids)
  - Conjugated proteins have a protein and nonprotein component (**prosthetic group**) (i.e., **lipoprotein**, **glycoprotein, or hemoprotein**)
    - **Apoprotein – without prosthetic group**
    - **Holoprotein – with prosthetic group**

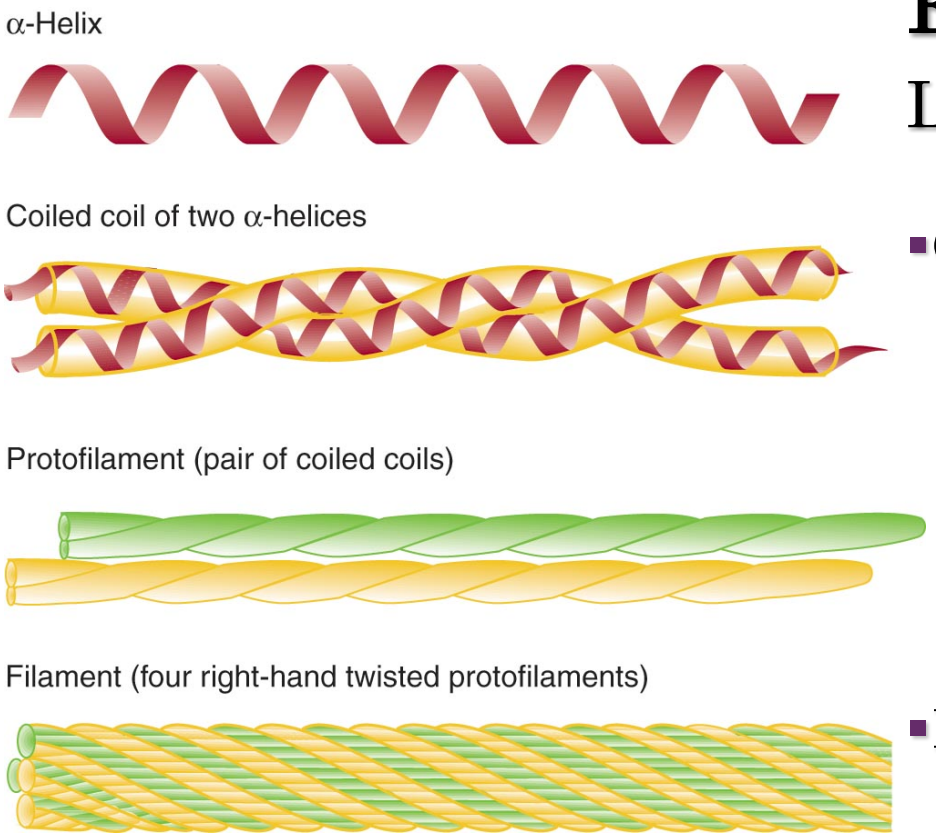


Figure 5.32 α-Keratin

Fibrous Proteins

Large amounts of α-helix & β-pleated sheets

- Contain polypeptide chains organized approximately parallel along a single axis.
  - Consist of long fibers or large sheets
  - Tend to be mechanically strong
  - Insoluble in water and dilute salt solutions
  - Play important structural roles in nature
- Examples are
  - keratin of hair and wool
  - collagen of connective tissue of animals including cartilage, bones, teeth, skin, and blood vessels

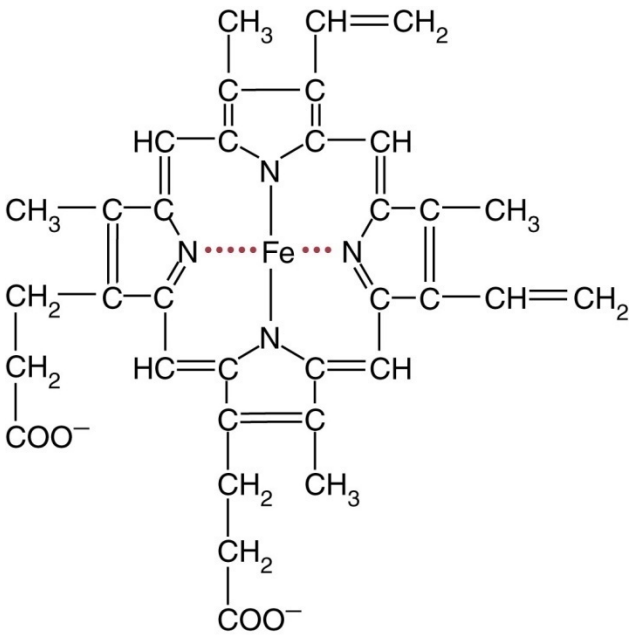


Figure 5.35 Heme

Globular Proteins

- Folded to a more or less spherical shape
  - Tend to be soluble in water and salt solutions
  - Most polar side chains are on the outside
    - ✓ interact with the aqueous environment by hydrogen bonding
    - ✓ ion-dipole interactions
  - Nonpolar side chains are buried inside
  - Nearly all have substantial sections of a-helix and b-sheet

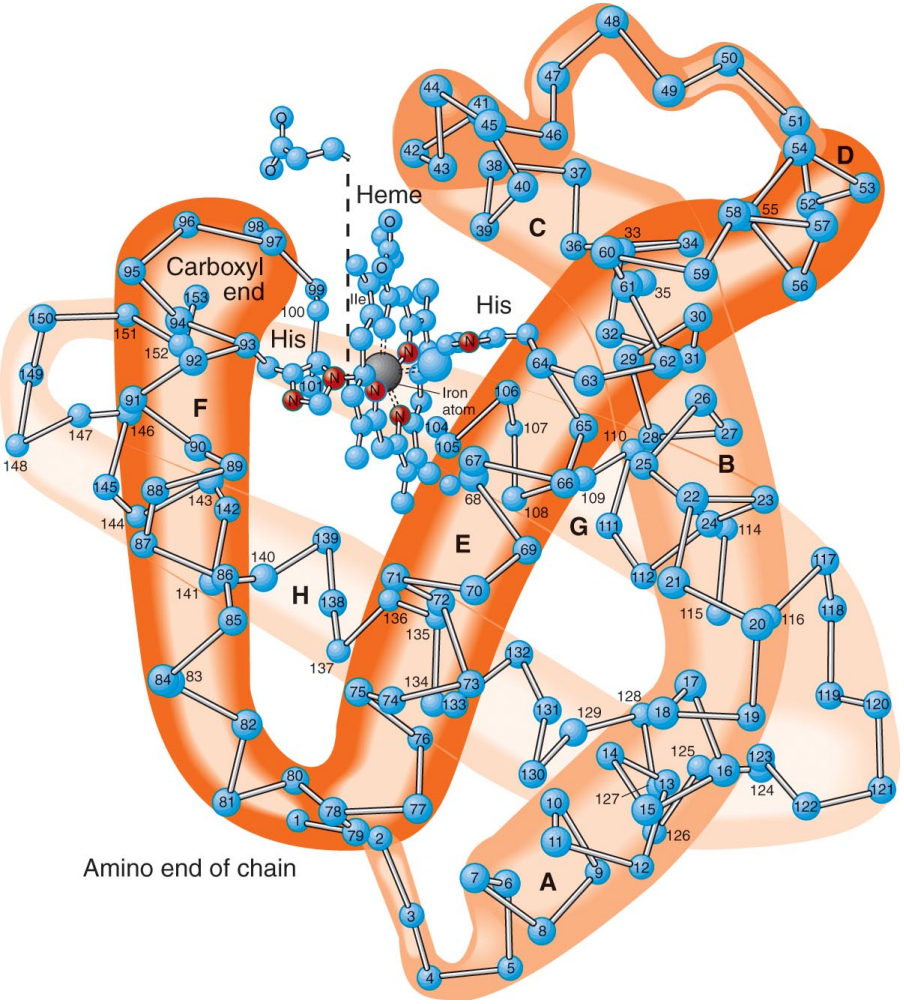


Figure 5.36 Myoglobin

- **Myoglobin:** found in high concentrations in cardiac and skeletal muscle
  - Protein component of myoglobin, **globin**, is a single protein with eight α-helices
  - Encloses a heme [Fe^{2+}] that has a high affinity for O_2

- **Tertiary Structure:** 3-dimensional arrangement of atoms in the molecule
  - ✓ Side chains and prosthetic groups
  - ✓ Arrangement of helical and pleated-sheet sections
- Fibrous protein – much of 3° specified by 2° structure
- Globular protein – 3° structure provides information
  - ✓ How helical and pleated-sheet sections fold back on each other
  - ✓ Positions of side-chain atoms & prosthetic groups
- Interactions between side chains also plays a role.
  - ✓ Folding brings widely separated residues into proximity to help stabilize

Noncovalent interactions

- Hydrophobic interactions between non-polar side chains, e.g., Val and Ile
- Electrostatic interactions:
  - Attraction between side chains of opposite charge, e.g., Lys and Glu
  - Repulsion between side chains of like charge, e.g., Lys and Arg, Glu and Asp
- Hydrogen bonding between polar side chains, e.g., Ser and Thr
- Hydration shell stabilizes structure

Covalent interactions

Disulfide (-S-S-) bonds between side chains of cysteines

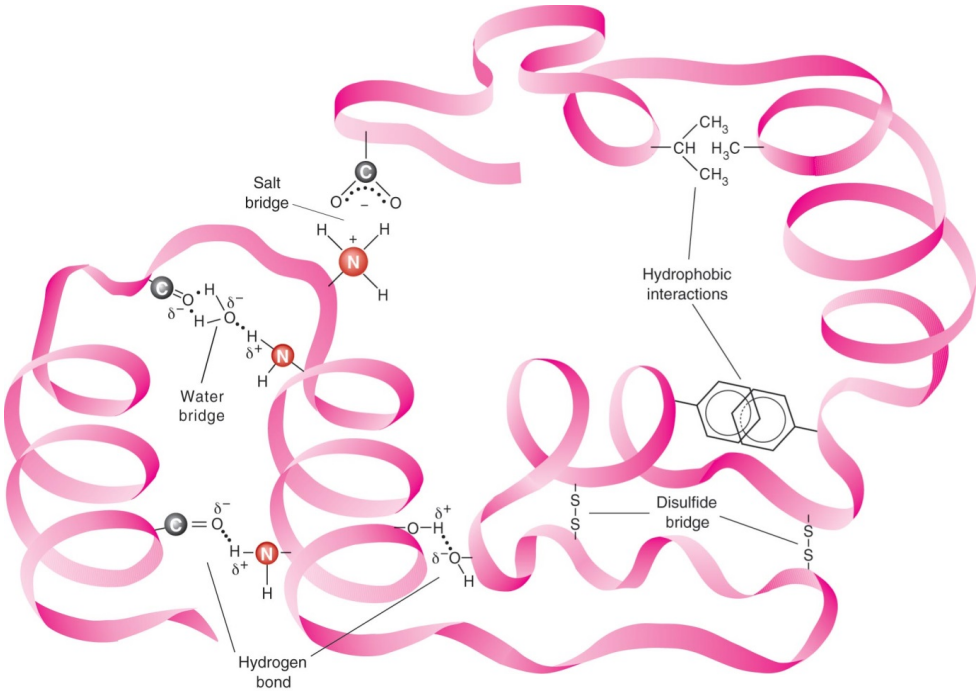
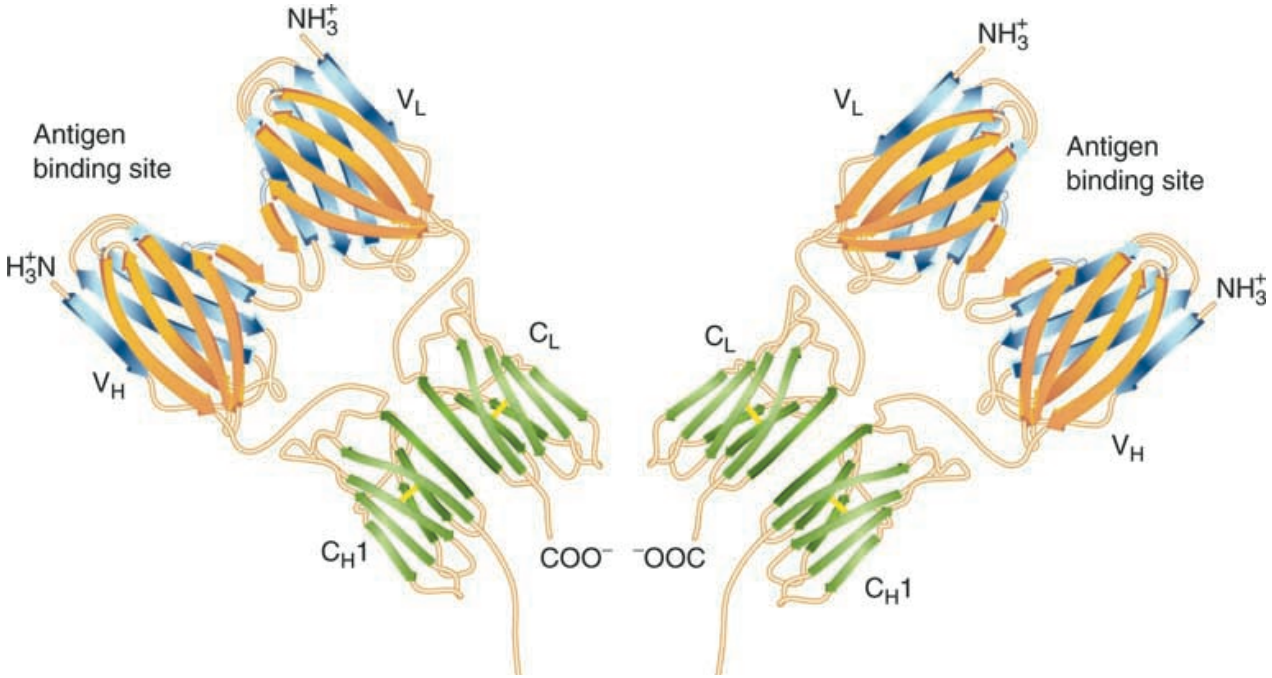


Figure 5.22 Interactions That Maintain Tertiary Structure

Figure 5.24 Structure of Immunoglobulin G



- **Quaternary structure:** final arrangement for proteins having multiple-subunits
  - **Oligomers:** multi-subunit proteins where some or all subunits are identical
    - Composed of **protomers** – may contain 1 or more subunits

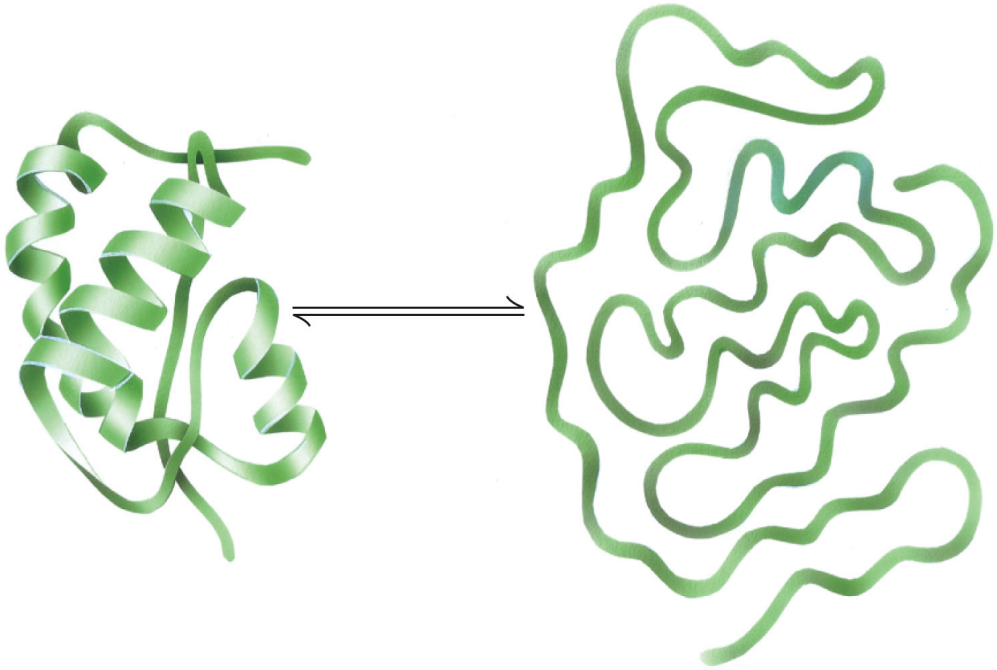
Interactions between subunits

- **Allostery**: control of protein function by ligand binding
  - **Allosteric transitions**: can change conformation and function
    - **Allosteric effectors or modulators**
      - Positive if increases affinity
      - Negative if decreases affinity
- Hemoglobin and oxygen affinity
  - ✓ 4 subunits, each with heme group
  - ✓ Oxygen binding promotes conformation change
    - ✓ Increasing affinity in other subunits

Section 5.3: Proteins

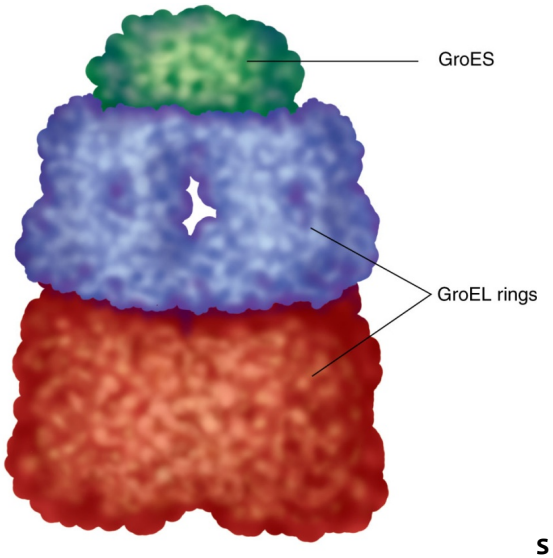
- **Denaturation:** the loss of the structural order (2°, 3°, 4°, or a combination of these) resulting in loss of biological activity

- Denaturation conditions
  1. Strong acid or base – alter pH, may precipitate
  2. Organic solvents – disrupt hydrophobic interactions
  3. Detergents – disrupt hydrophobic interactions
  4. Reducing agents – disrupts disulfide bridges, hydrogen bonds, hydrophobic interactions
  5. Salt concentration – protein aggregation, precipitation
  6. Heavy metal ions – changes structure and function
  7. Temperature – disrupts hydrogen bonds
  8. Mechanical stress – disrupts delicate balance of forces



- **Protein Folding Assistance**
  - Final 3-dimensional conformation comes directly from protein's primary
- **Molecular chaperones**
  - **Ribosome-Associated chaperones** – binds to emerging polypeptide preventing folding until entire polypeptide emerges
  - **Hsp70s –** bind and stabilize proteins during the early stages of folding; usually works with *co-chaperones*
  - **Hsp90s** – finalize folding of a limited set of partially unfolded molecules known as *client proteins*
  - **Chaperonins –** increase speed and efficiency of the folding process

(Note: Hsp – Heat shock protein)

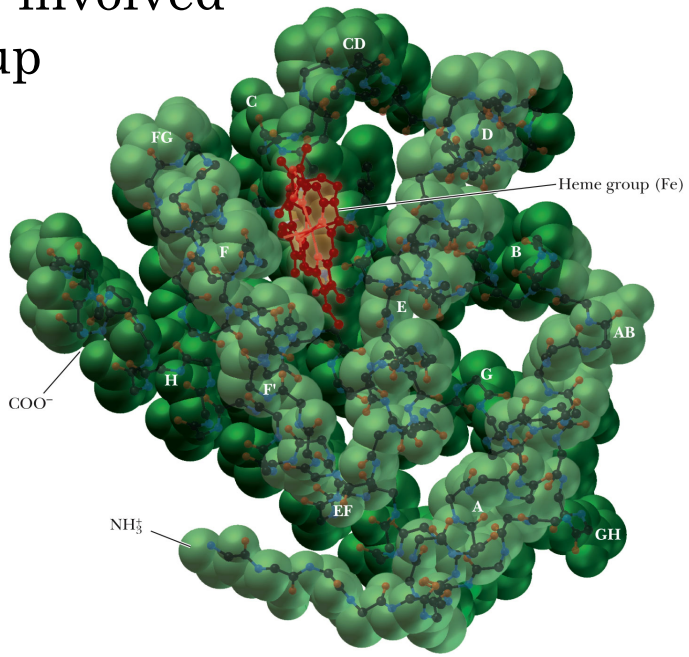


Model of the *E. Coli* Chaperonin

From McKee and McKee, *Biochemistry*, 5th E

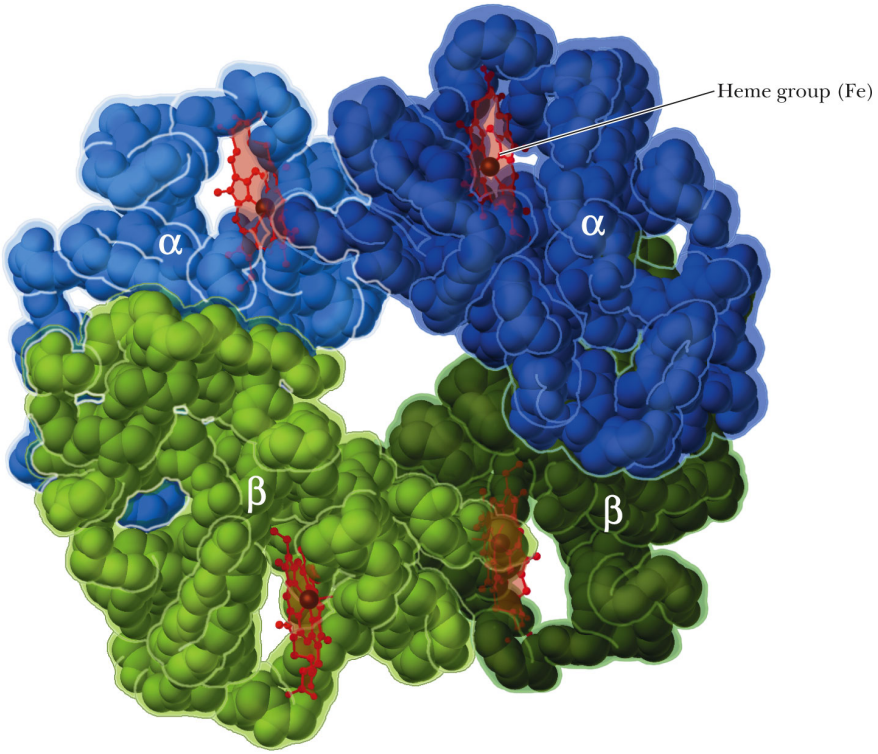
Myoglobin

- Single polypeptide chain of 153 amino acids
- 8 regions of a-helix
- Single heme group in a hydrophobic pocket
- Most polar side chains are on the surface; nonpolar side chains are folded to the interior
- Two His side chains are in the interior, involved with interaction with the heme group



Hemoglobin

- A tetramer of two α-chains (141 amino acids each) and two β-chains (153 amino acids each); α2β2
- Each chain has 1 heme group
  - Binds up to 4 molecules of O_2
- Function of hemoglobin is to transport oxygen
- **Positive cooperativity** - binding of O_2 increases affinity
  - ✓ Structure of oxygenated Hb is different from that of unoxygenated Hb



- Binding of ligands other than oxygen affects hemoglobin's oxygen-binding properties
  - pH decrease enhances oxygen release from hemoglobin (**Bohr effect**)
  - Waste product CO_2 also enhances oxygen release by increasing H^+ concentration
  - Binding of H^+ to several ionizable groups on hemoglobin shifts it to its T state
- 2,3-Bisphosphoglycerate (BPG) is also an important regulator of hemoglobin function
  - Red blood cells have a high concentration of BPG, which lowers hemoglobin's affinity for O_2
  - In the lungs, these processes reverse

- **Molecular Machines**
  - Purposeful movement is a hallmark of living things
  - This behavior takes a myriad of forms
  - **Biological machines** are responsible for these behaviors
  - Usually ATP or GTP driven
    - Motor proteins fall into the following categories:

1. **Classical motors** (myosins, dyneins, and kinesin)
2. **Timing devices** (EF-Tu in translation)

   Note: EF-Tu – elongation factor, thermo unstable
3. **Microprocessing switching devices** (G proteins)

   Note: transmit signal from outside to inside cell
4. **Assembly and disassembly factors** (cytoskeleton assembly and disassembly affects cell mechanics)