MACROMOLECULE : PROTEIN

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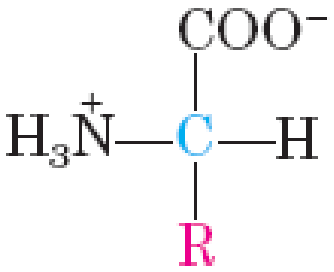
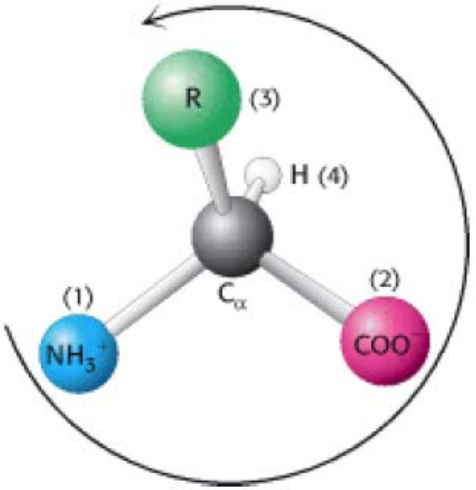
Functions of PROTEIN

- Catalysis → enzymes
- Immunity → antibodies
- Growth & development → DNA binding proteins
- Transport of metabolites → carrier proteins
- Relaying biological signals – hormones
- Biological structures → fibrous proteins
- etc

PROTEIN

- Proteins are polymers of amino acids, with each **amino acid residue joined to its neighbor by a specific type** of covalent bond.

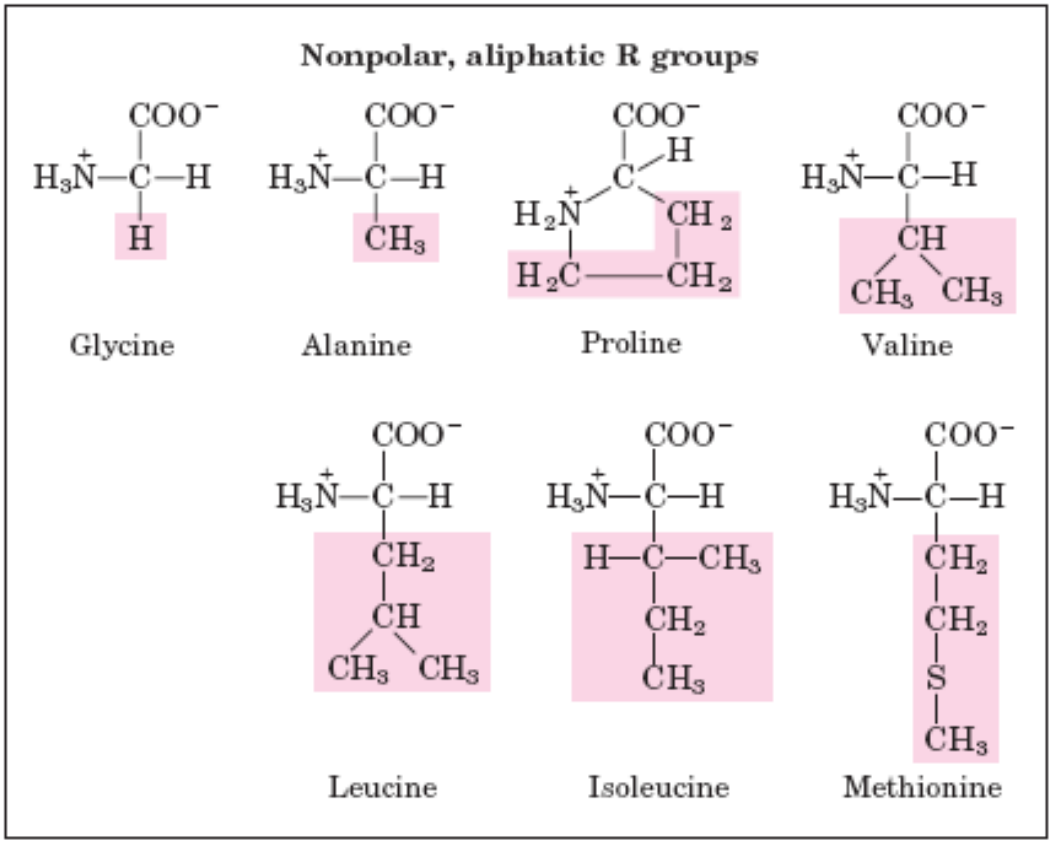


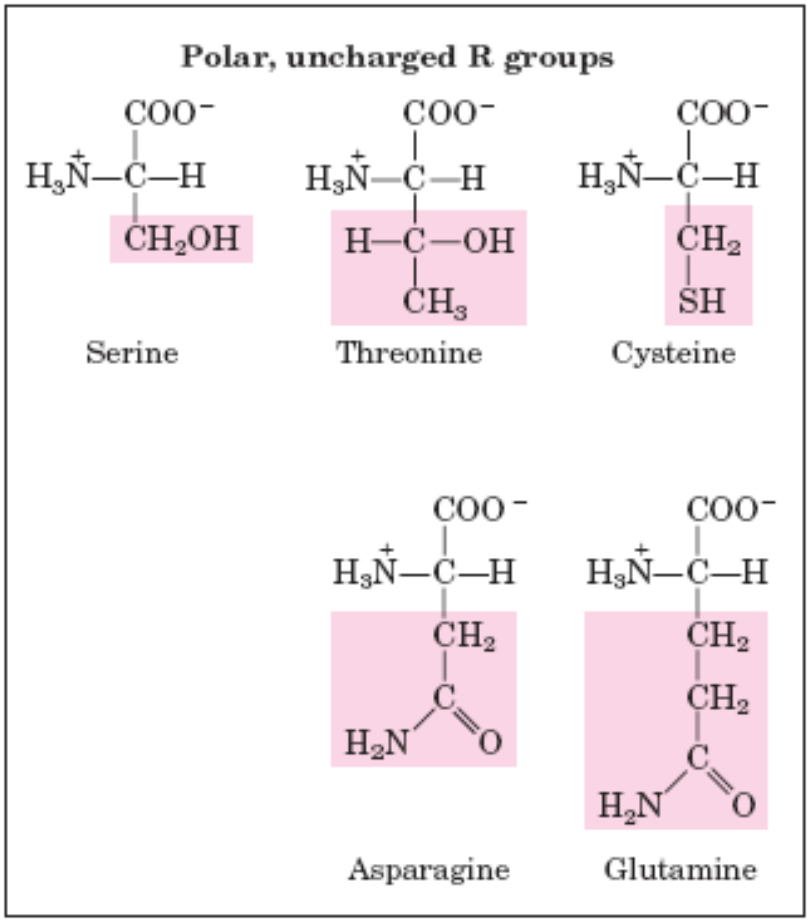


Only L-Amino Acids Are Found in Proteins

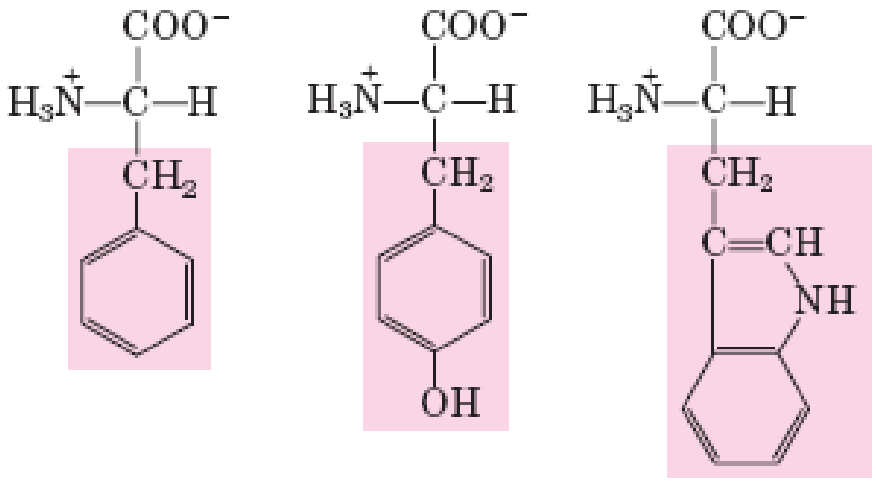
Cells are able to specifically synthesize the L isomers of amino acids because the active sites of enzymes are asymmetric, causing the reactions they catalyze to be stereospecific.

AMINO ACID


Nonpolar, aliphatic R groups
Glycine
Alanine
Proline
Valine
Leucine
Isoleucine
Methionine

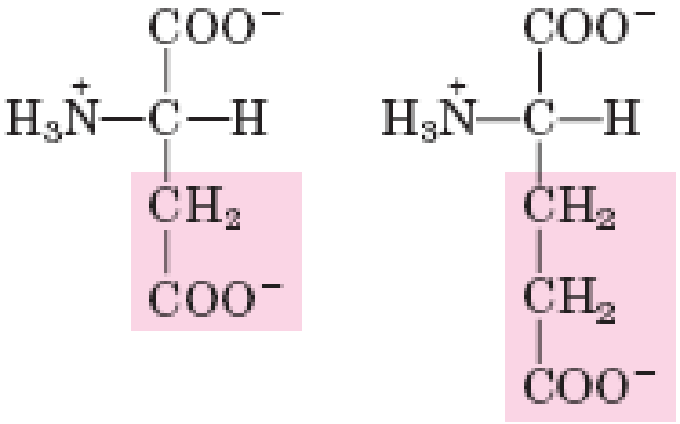

Polar, uncharged R groups
Serine
Threonine
Cysteine
Asparagine
Glutamine

Aromatic R groups



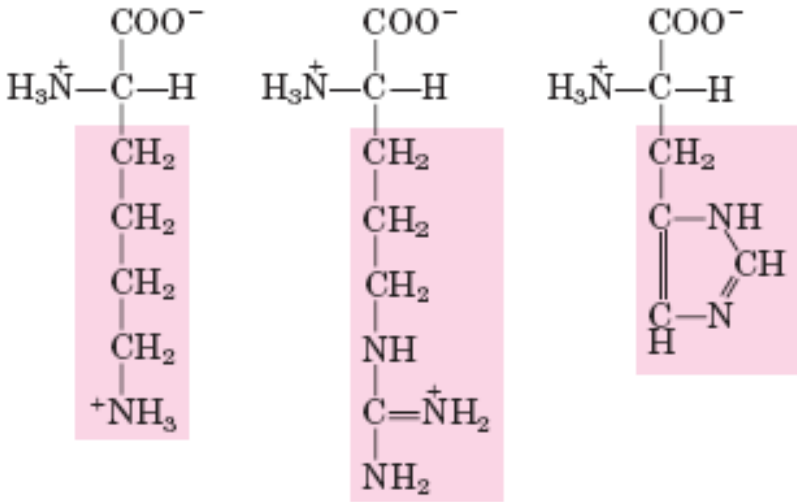
Phenylalanine Tyrosine Tryptophan

Negatively charged R groups



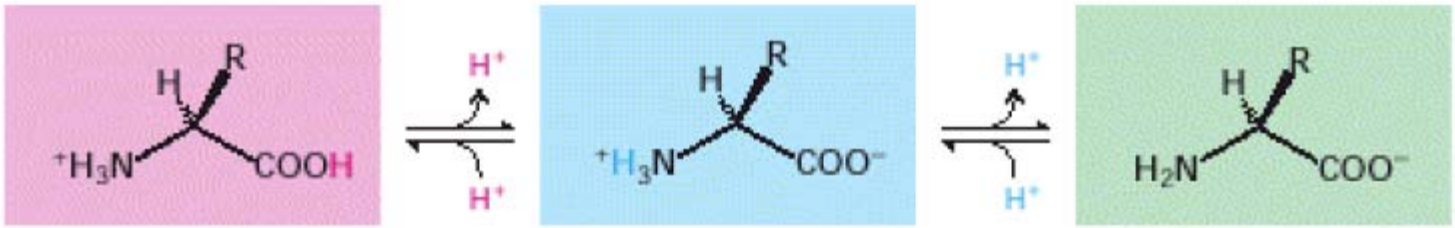
Aspartate Glutamate

Positively charged R groups



Lysine Arginine Histidine

When an amino acid is dissolved in water, it exists in solution as the dipolar ion, or **zwitterion**



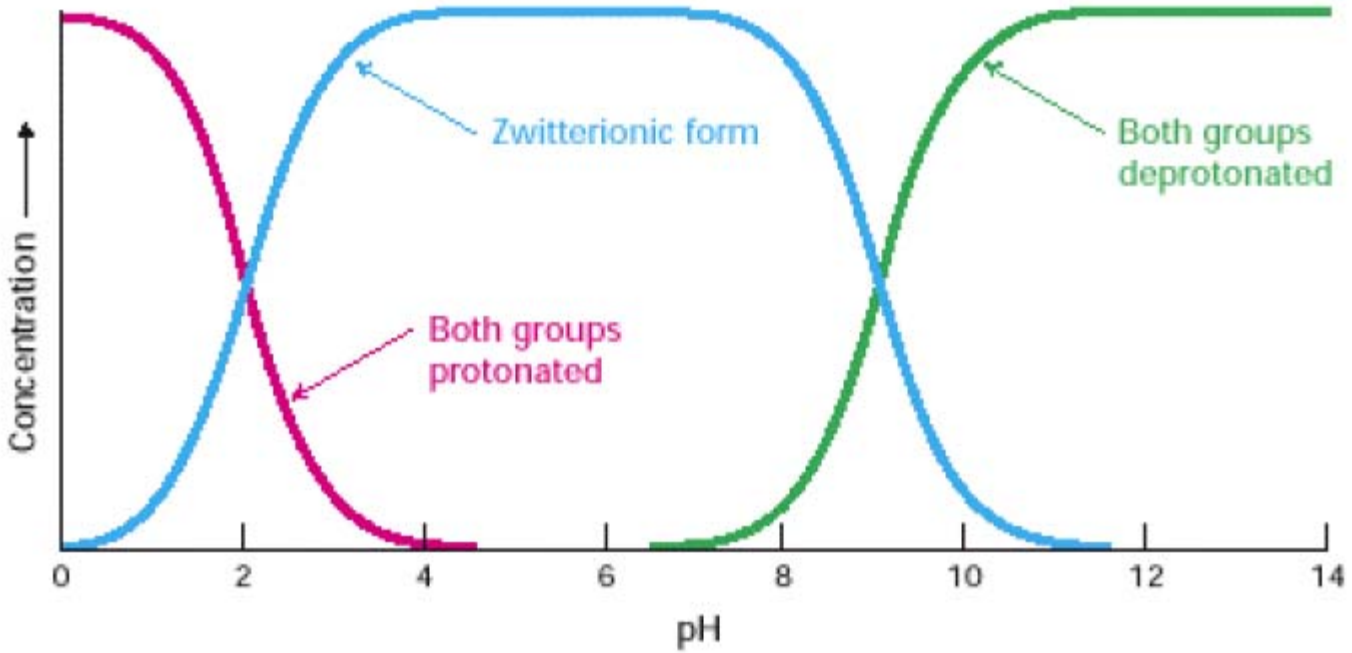
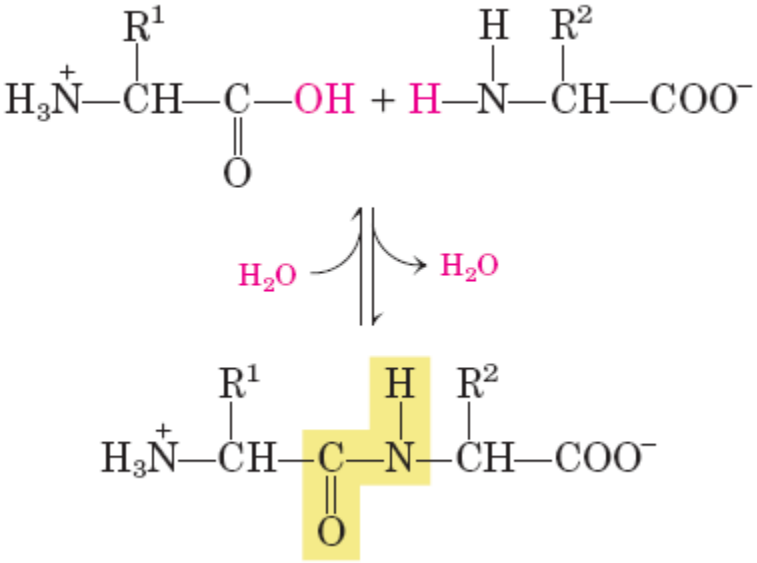


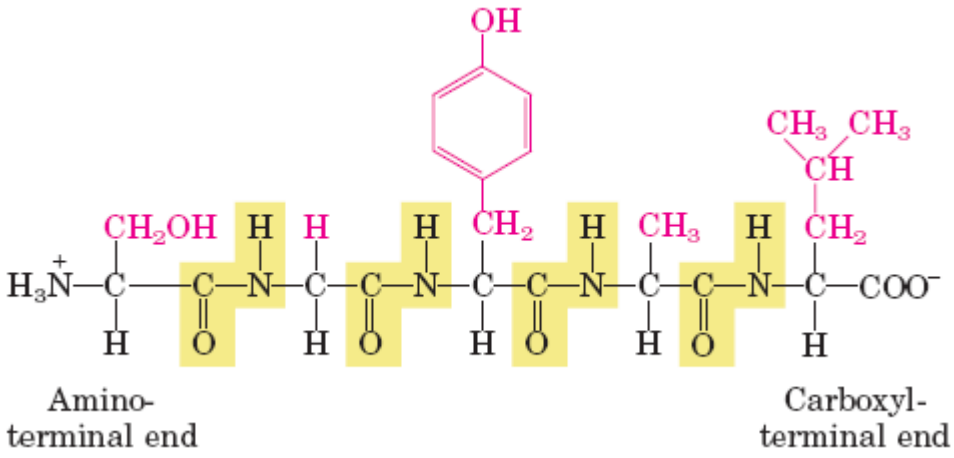
TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol	M_r	pK_a values			pI	Hydropathy index*	Occurrence in proteins (%)†
			pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)			
Nonpolar, aliphatic R groups								
Glycine	Gly G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups								
Serine	Ser S	105	2.21	9.15		5.68	−0.8	6.8
Threonine	Thr T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5	6.3

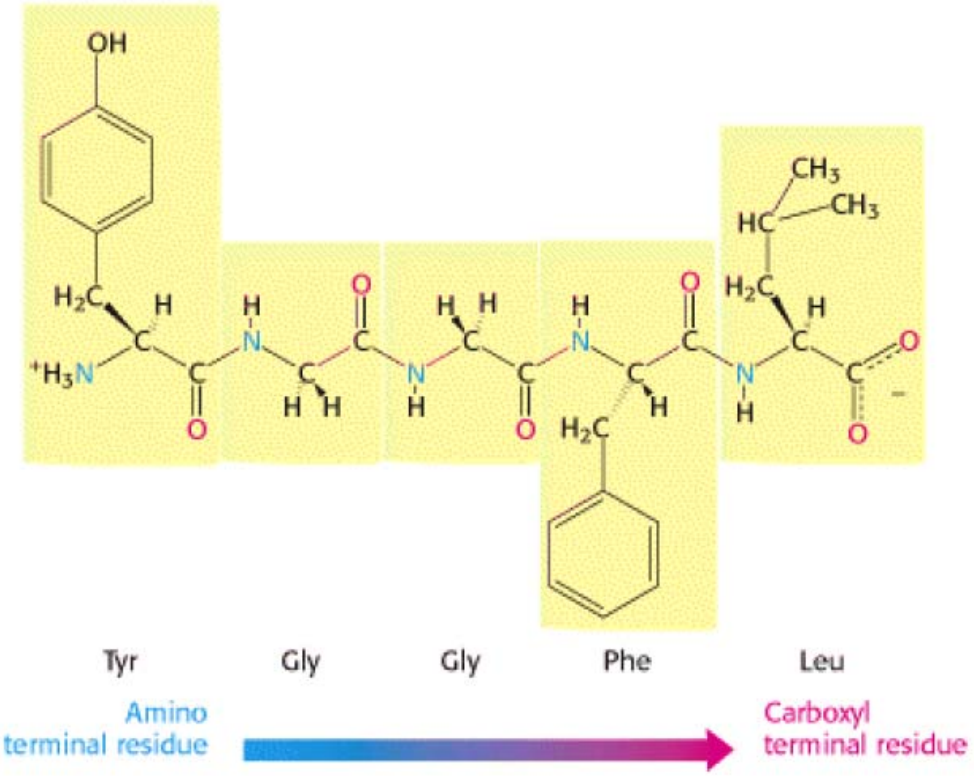
- Formation of a peptide bond by condensation



A series of amino acids joined by peptide bonds form a *polypeptide chain*



By convention, the amino end is taken to be the beginning of a polypeptide chain



Pentapeptide (YGGFL)→ Leu-enkephalin → an opioid peptide that modulates the perception of pain.

Peptides are named beginning with the aminoterminal residue

PROTEIN

- Most natural polypeptide chains → 50 and 2000 amino acid residues → *proteins.*
- the mass of a protein → units of daltons
- one *dalton = one* atomic mass unit →A unit of mass very nearly equal to that of a hydrogen atom

A protein with a molecular weight of 50,000 has a mass of 50,000 daltons

TABLE 3–2 Molecular Data on Some Proteins

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

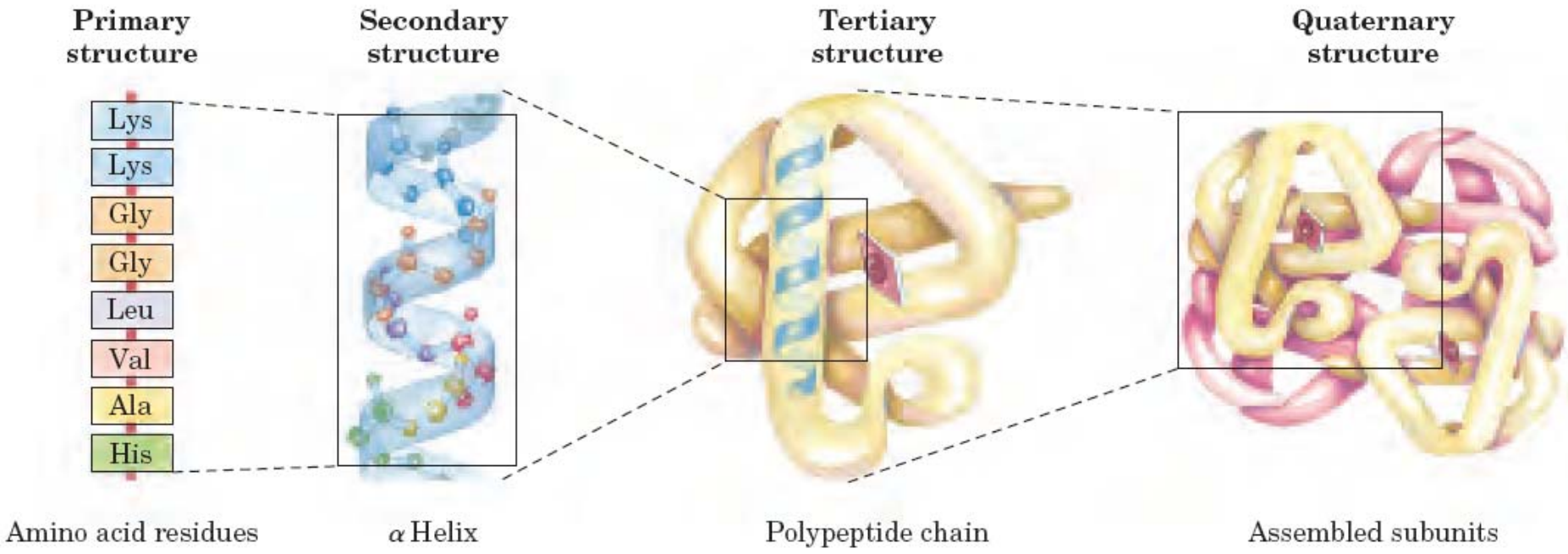
Conjugated protein

TABLE 3–4 Conjugated Proteins

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

The non–amino acid part of a conjugated protein → prosthetic group.

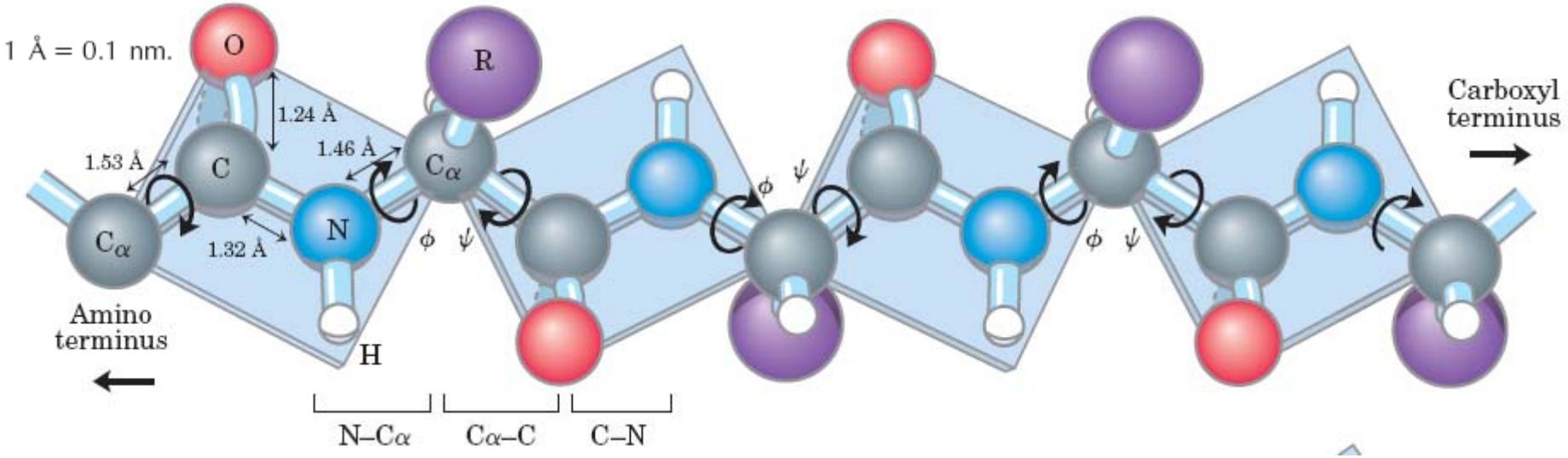
PROTEIN STRUCTURE



- **Primary structure** → all covalent bonds (mainly peptide bonds and disulfide bonds) linking amino acid residues in a polypeptide chain → the *sequence of amino* acid residues.
- **Secondary structure** → stable arrangements of amino acid residues giving rise to recurring structural patterns.
- **Tertiary structure** → the three-dimensional folding of a polypeptide.
- **Quaternary structure** → Arrangement in space of protein which has two or more polypeptide subunits.

PRIMARY STRUCTURE

Peptide Bonds Are Planar

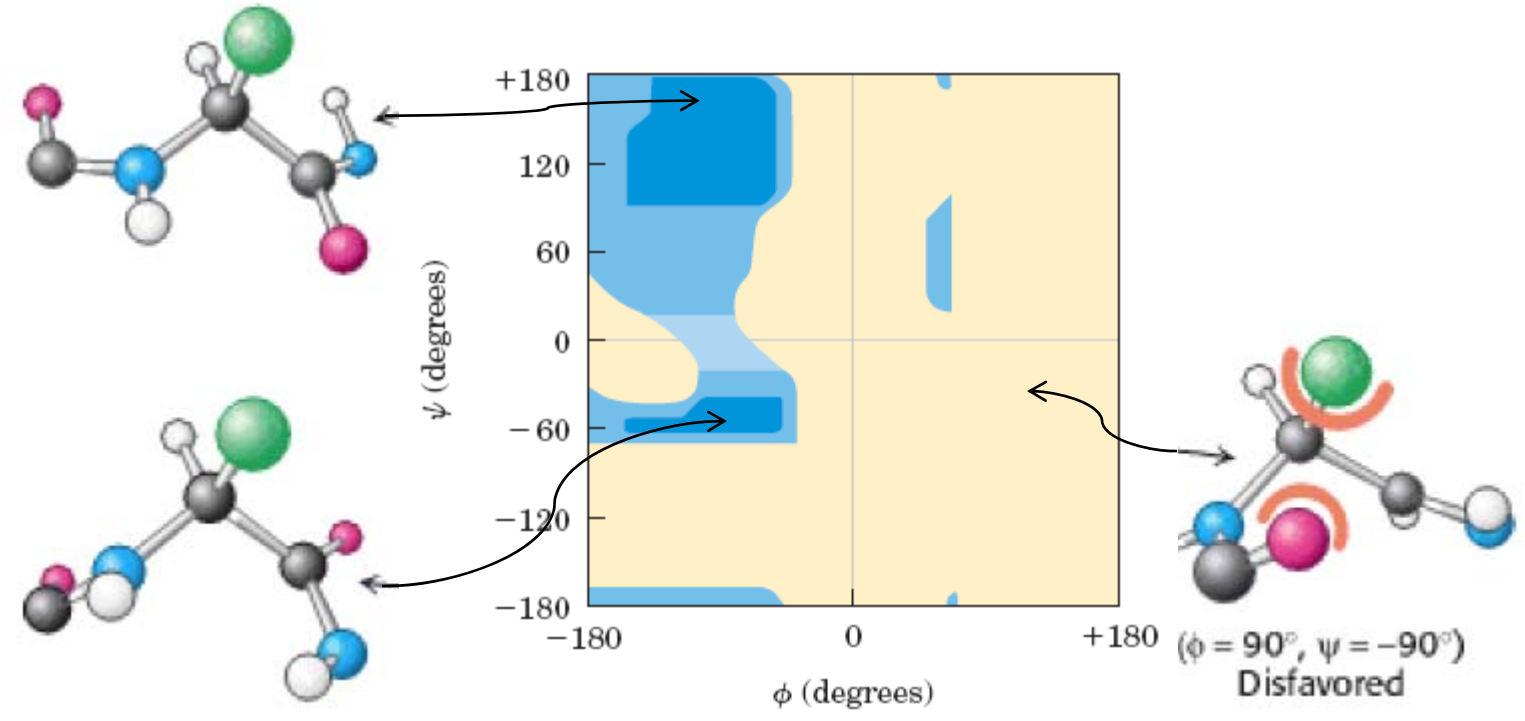


In a pair of linked amino acids, six atoms (C α , C, O, N, H, and C α) lie in a plane.

The N-Cα and Cα -C bonds → can rotate
The peptide C-N bond → not free to rotate.

The conformations of peptides are defined by the values of ϕ and ψ.

A RAMACHANDRAN DIAGRAM : Value of ϕ and ψ

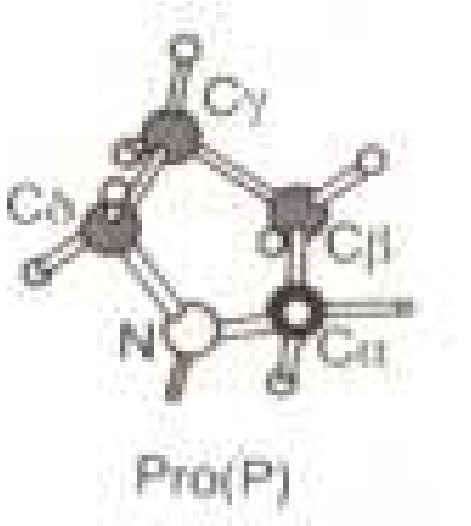


Area of :

- Dark blue → conformations that involve no steric overlap and thus are fully allowed.
- Medium blue → conformations allowed at the extreme limits for unfavorable atomic contacts.
- The lightest blue → conformations that are permissible if a little flexibility is allowed in the bond angles.

Glycine :

- **Hanya punya rantai samping 1 H**
- **Φ & Ψ tidak terbatas**
- **punya kebebasan konformasi besar**



Proline :

- **gugus NH terkunci dalam cincin pyrolidine**
- **Φ ~ 60º → konformasi sangat terbatas**
- ***. Proline punya kontribusi terkecil thd entropy – unfolded state.**

Misal: Penggantian Gly→Ala & Ala→Pro diharapkan meningkatkan stabilitas ± 1 kcal/mol. ; T4-lysozyme , *B.stearothermophilus* neutral protease

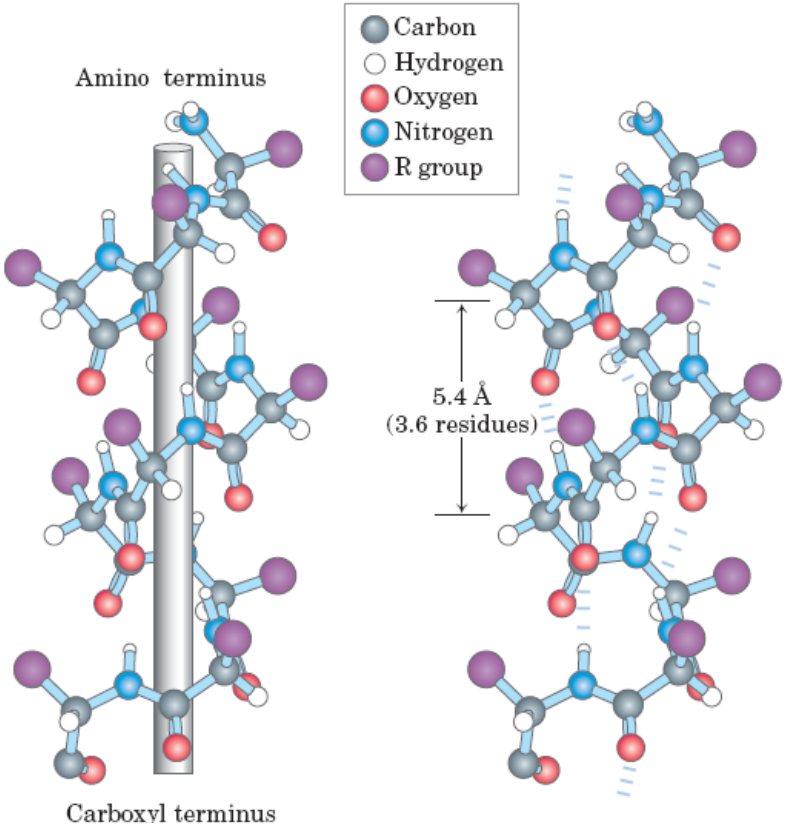
SECONDARY STRUCTURE

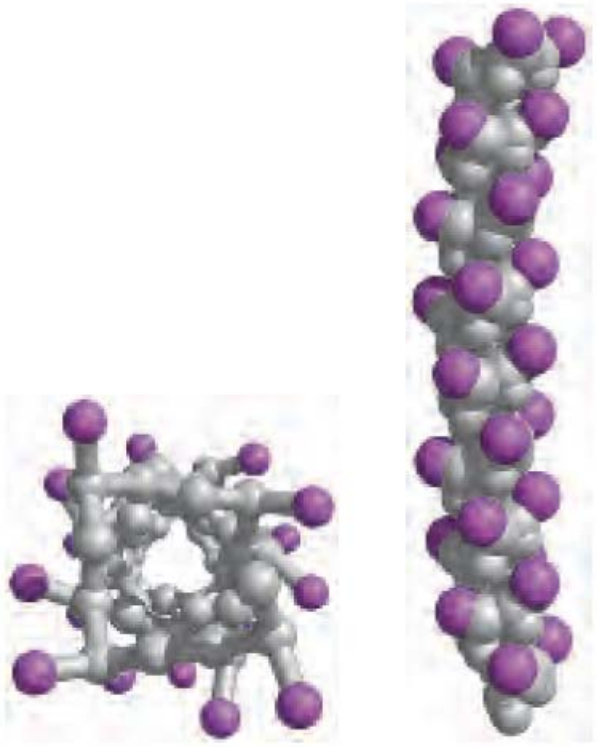
Linus Pauling, 1901–1994

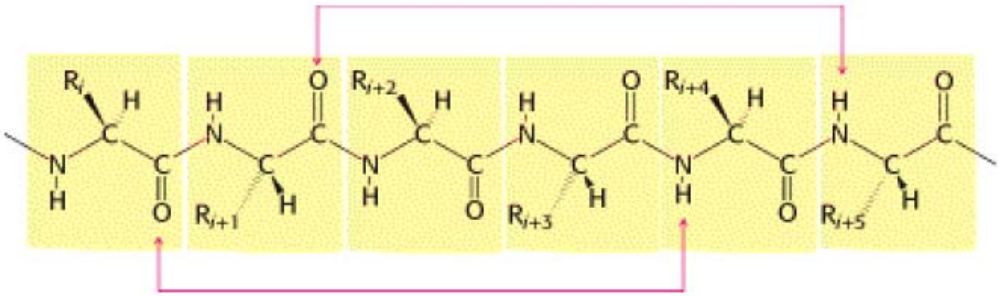
Robert Corey, 1897–1971

The α-HELIX structure

The Alpha Helix Is a Coiled Structure Stabilized by Intrachain Hydrogen Bonds



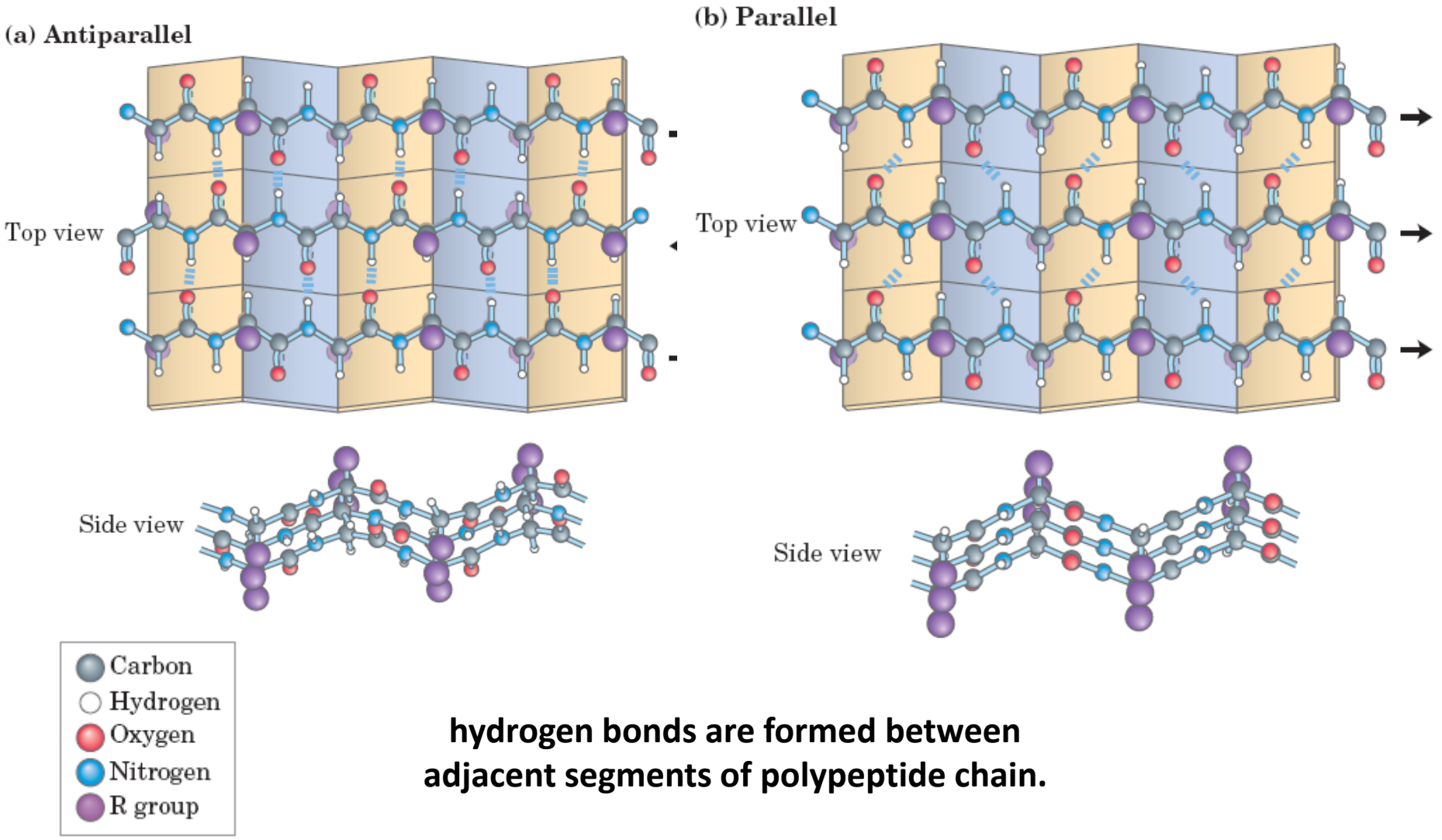




In the α helix, the CO group of residue n forms a hydrogen bond with the NH group of residue n+ 4.

The β-SHEETS Structure

interchain hydrogen bonding



hydrogen bonds are formed between adjacent segments of polypeptide chain.

TERTIARY STRUCTURE

- The overall three-dimensional arrangement of all atoms in a protein
- specific bend-producing residues → Pro, Thr, Ser, and Gly.

Tertiary contd..

- **Fibrous proteins, having polypeptide chains arranged in** long strands or sheets
  - consist largely of a single type of secondary structure
  - the structures that provide support, shape, and external protection to vertebrates
- **Globular proteins, having** polypeptide chains folded into a spherical or globular shape.
  - contain several types of secondary structure
  - most enzymes and regulatory proteins

- Proteins with significant primary sequence similarity, and/or with demonstrably similar structure and function, are said to be in the same **protein family.**
- Two or more families with little primary sequence similarity sometimes make use of the same major structural motif and have functional similarities; these families are grouped as **superfamilies**

Organization of proteins based on motifs

- All α
- All β
- α/β

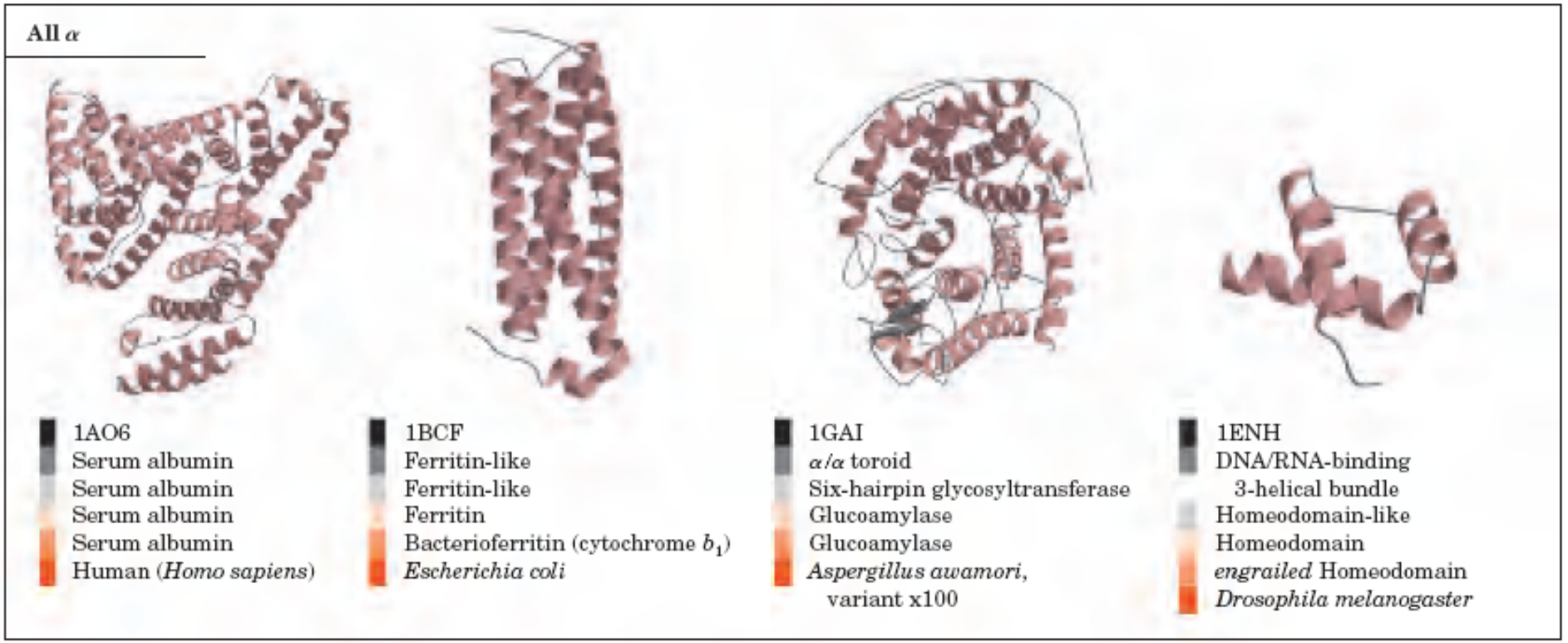
in which the α *and* β *segments are interspersed or* alternate

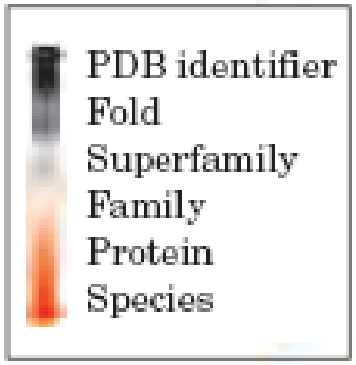
- α+ β

in which the α *and* β *regions are* somewhat segregated

domains exhibiting similar folding patterns are said to have the same motif even though their constituent *helices and sheets may differ* in length.

All α





- The top two levels of organization, **class and fold, are purely structural.**
- Below the fold level, categorization is based on evolutionary relationships.

All β

All β

1HOE
α-Amylase inhibitor tendamistat
α-Amylase inhibitor tendamistat
α-Amylase inhibitor tendamistat
α-Amylase inhibitor tendamistat
Streptomyces tendae

1LXA
Single-stranded left-handed β helix
Trimeric LpxA-like enzymes
UDP *N*-acetylglucosamine acyltransferase
UDP *N*-acetylglucosamine acyltransferase
Escherichia coli

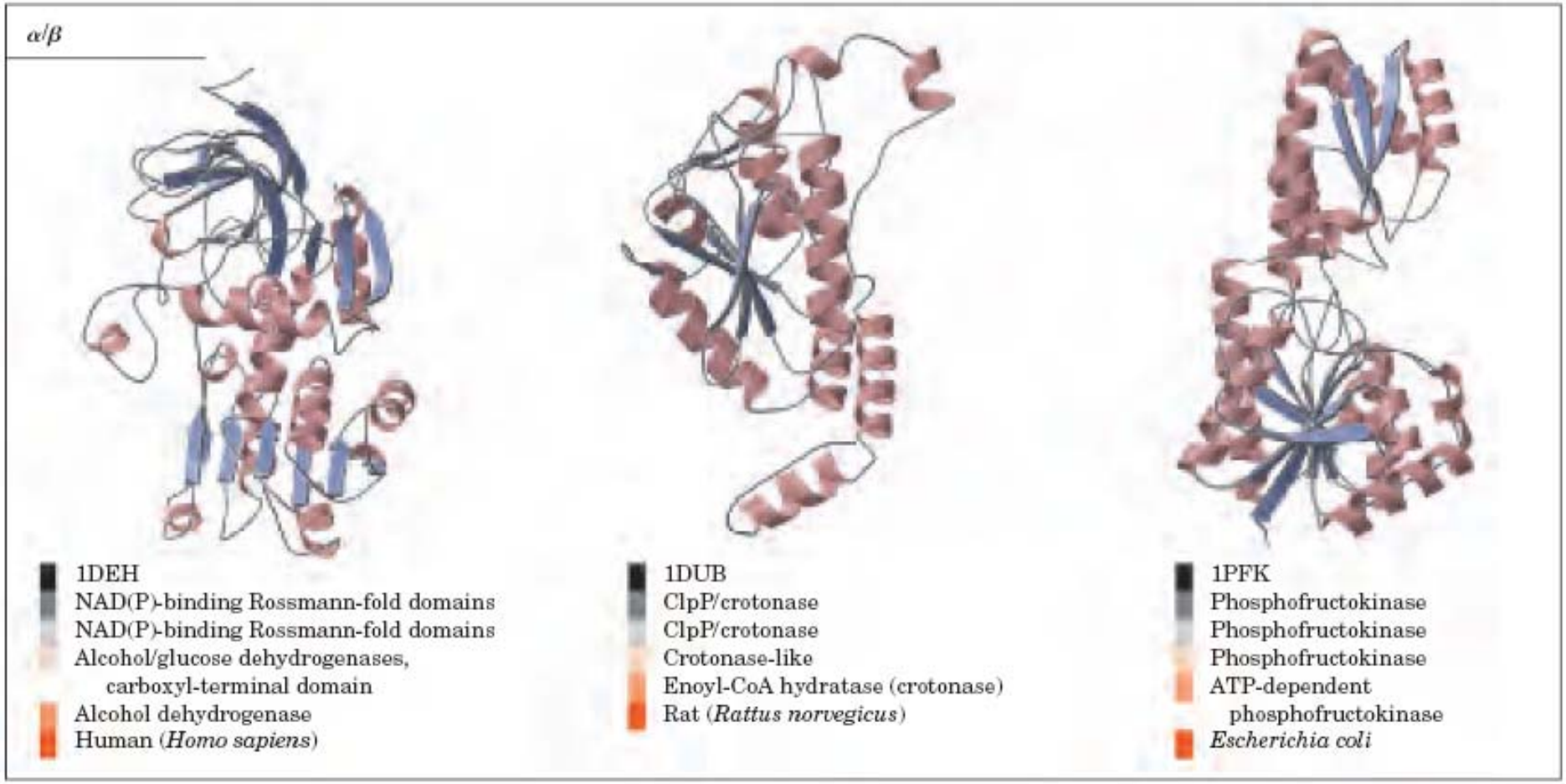
1PEX
Four-bladed β propeller
Hemopexin-like domain
Hemopexin-like domain
Collagenase-3 (MMP-13), carboxyl-terminal domain
Human (*Homo sapiens*)

1JPC
β-Prism II
α-D-Mannose-specific plant lectins
α-D-Mannose-specific plant lectins
Lectin (agglutinin)
Snowdrop (*Galanthus nivalis*)

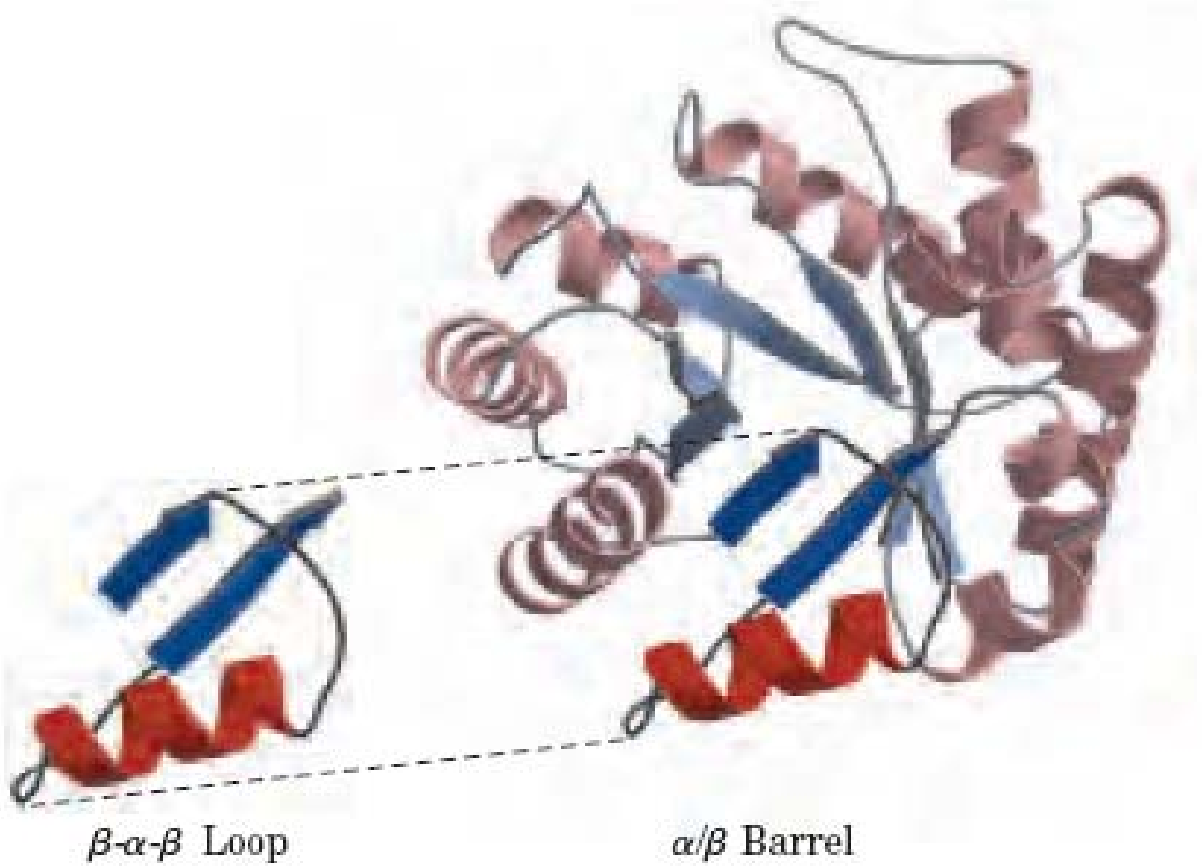
1CD8
Immunoglobulin-like β sandwich
Immunoglobulin
V set domains (antibody variable domain-like)
CD8
Human (*Homo sapiens*)

PDB identifier
Fold
Superfamily
Family
Protein
Species

α/β

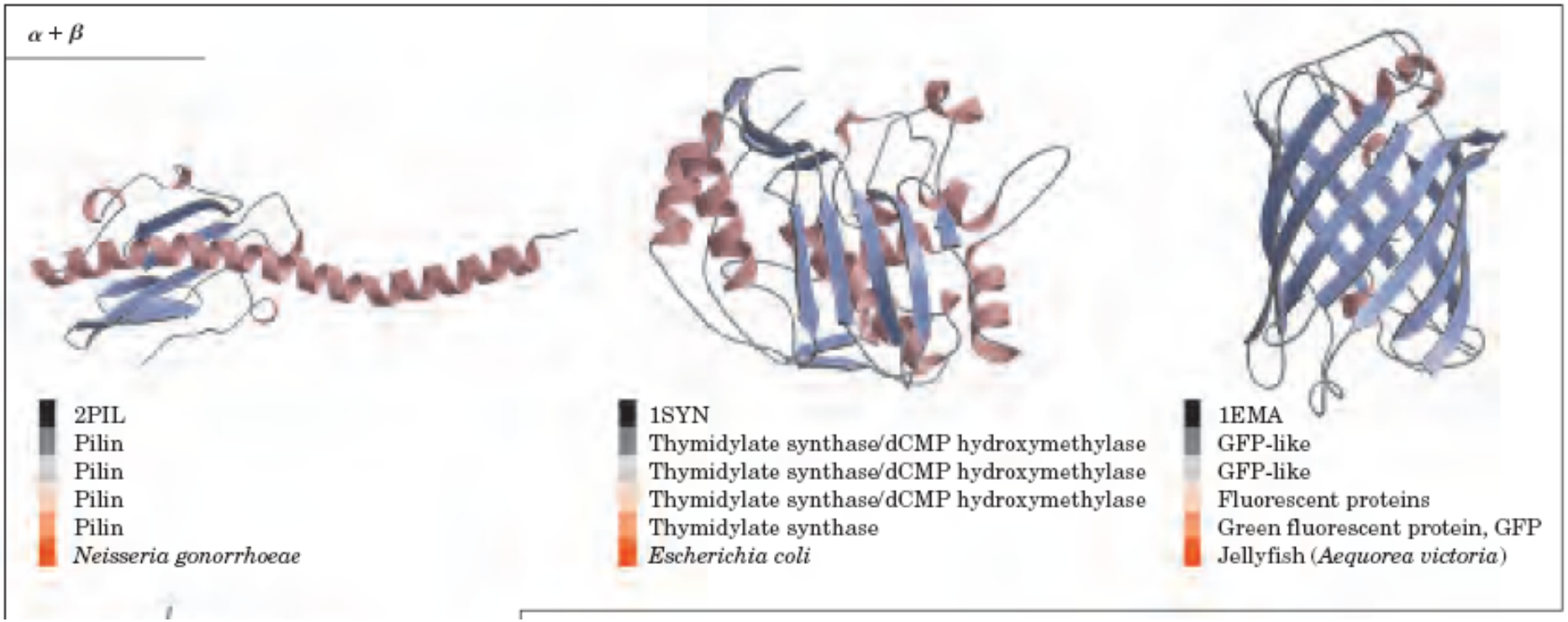


- The α / β barrel is a common motif constructed from repetitions of the simpler β-α-β *loop motif.*



a domain of the pyruvate kinase (a glycolytic enzyme) from rabbit

α+ β

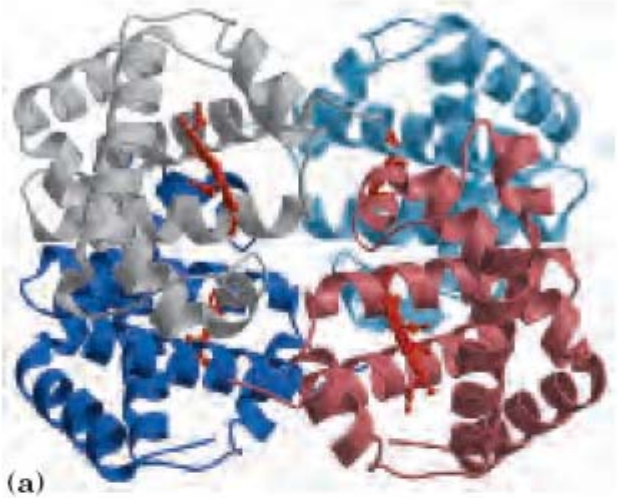


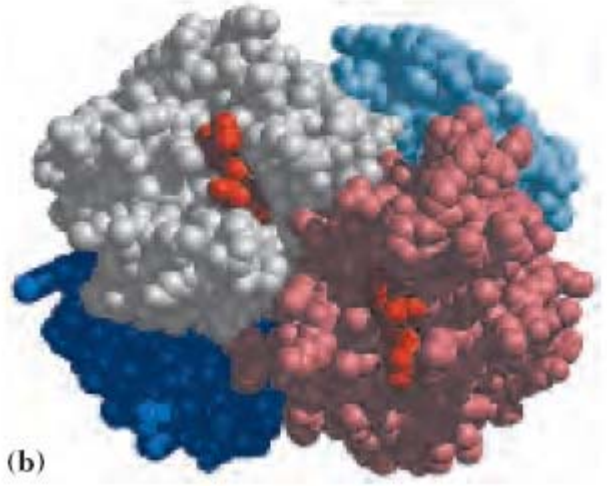
the α *and* β *regions are* somewhat segregated

QUATERNARY STRUCTURE

- Some proteins contain two or more separate polypeptide chains, or subunits, which may be identical or different.
- Quaternary structure results from interactions between the subunits of multisubunit (multimeric) proteins or large protein assemblies.

Quaternary structure of deoxyhemoglobin.



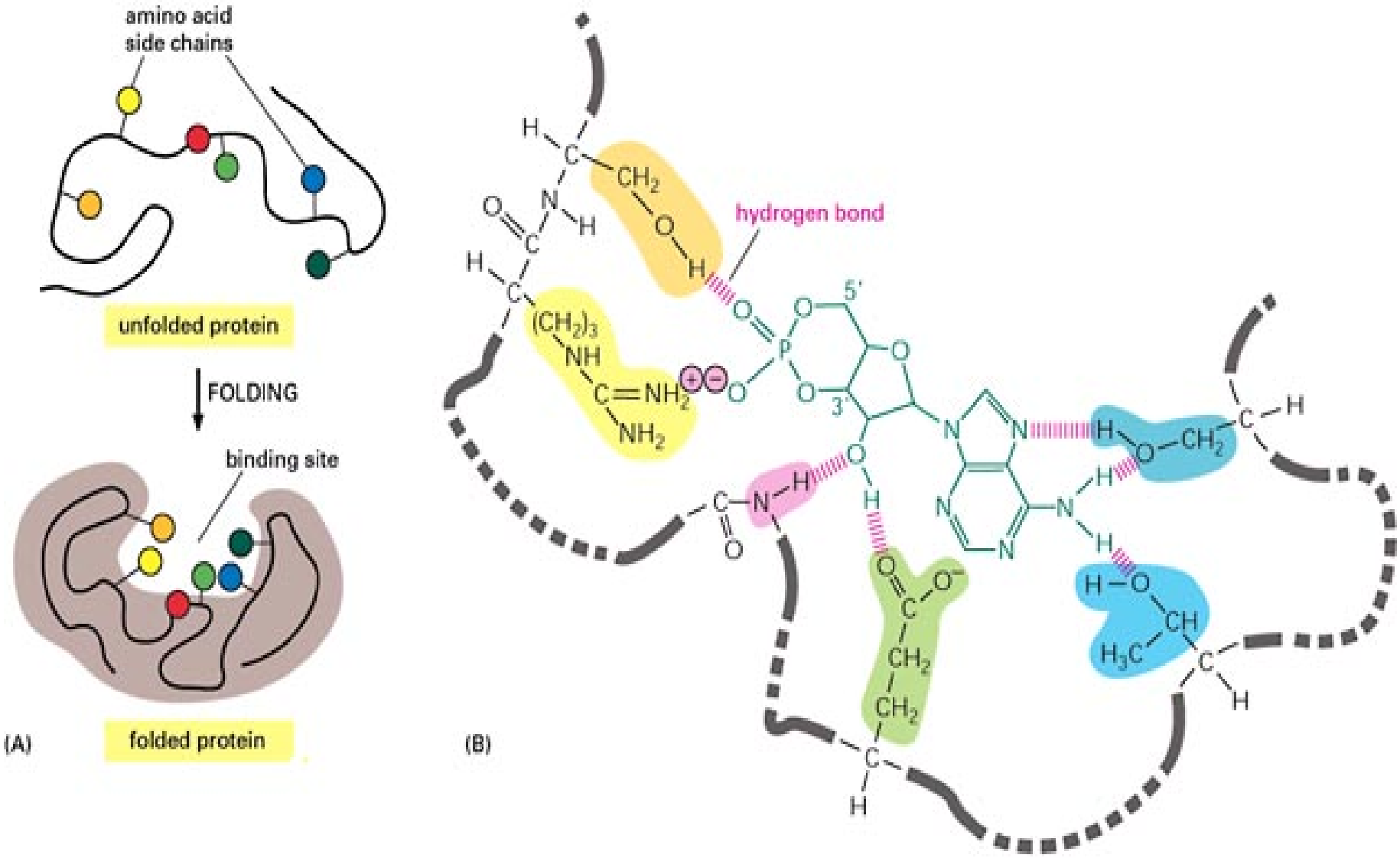


- The α *subunits are shown in gray and* light blue
- the β *subunits in pink and dark blue*

Protein Quaternary Structures Range from Simple Dimers to Large Complexes

- A multisubunit protein = a **multimer** → two to hundreds of subunits.
- A multimer with just a few subunits is often called an **oligomer.**
- If a multimer is composed of a number of **nonidentical subunits**, the overall structure of the protein can be **asymmetric** and quite complicated.
- most multimers have **identical subunits** or repeating groups of nonidentical subunits, usually in **symmetric** arrangements.
- Some multimeric proteins have a repeated unit consisting of a single subunit or a group of subunits referred to as a **protomer**.

PROTEIN FOLDING

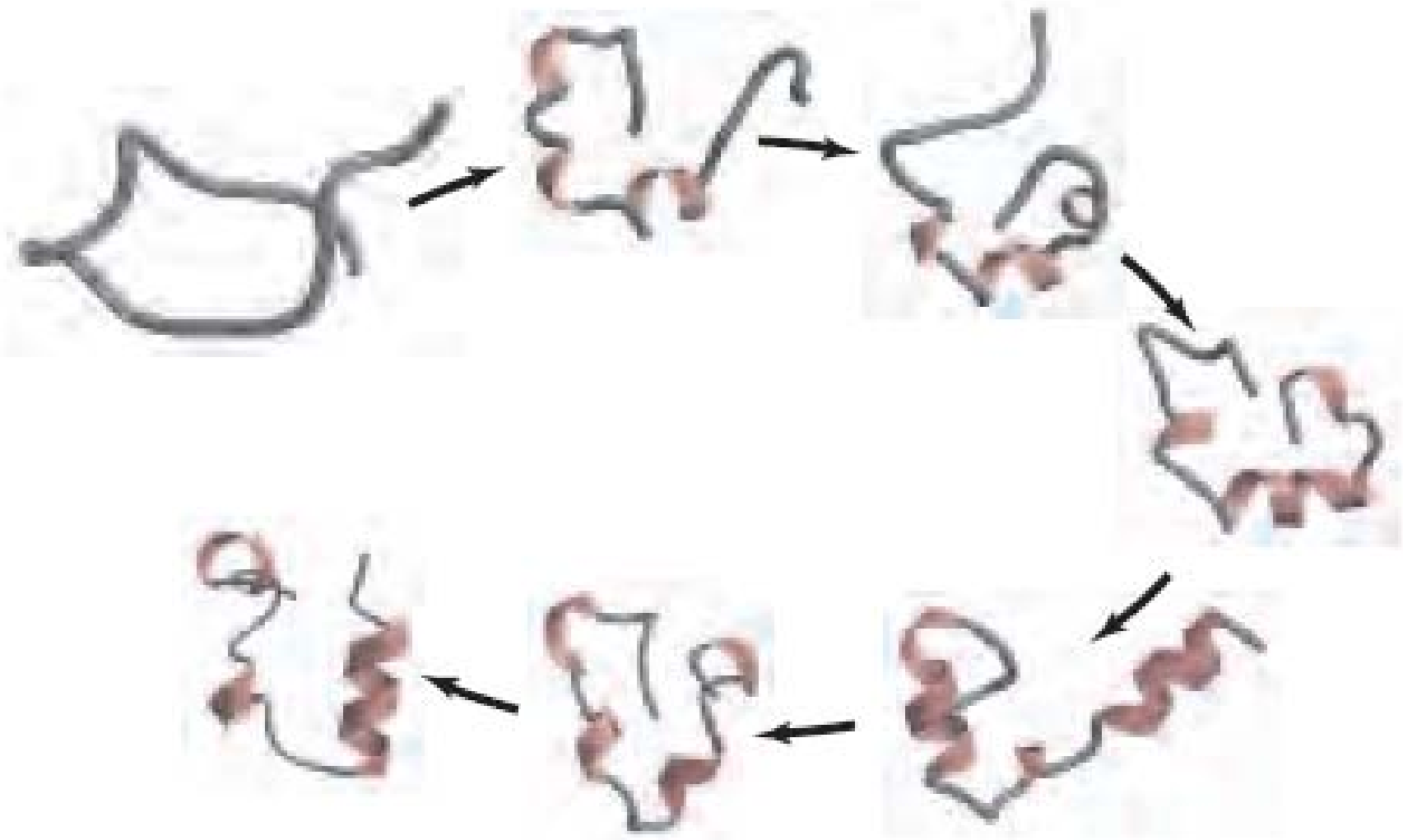


Proteins fold into compact structures.

- with nominal bond lengths and angles

Key to folding lies in…

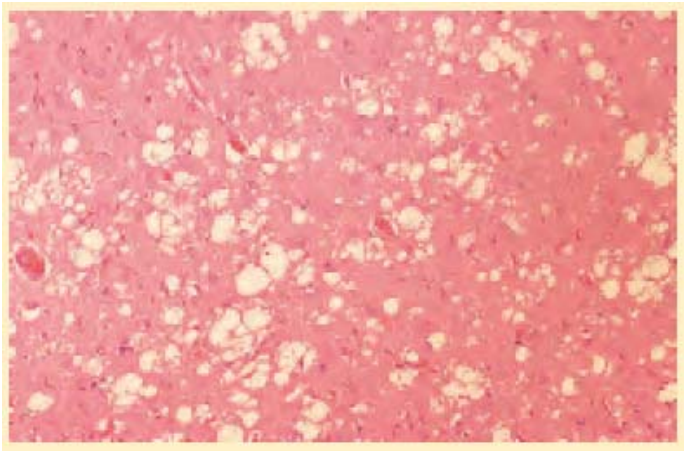
- rotations about main-chain bonds
- interactions between amide bond atoms along the chain
- interactions between sidechain groups



AreThere Limits to the Size of Proteins ?

- the genetic coding capacity of nucleic acids
- the accuracy of the protein biosynthetic process.

Death by Misfolding: The Prion Diseases



Stanley Prusiner

The infectious agent has been traced to a single protein (*Mr 28,000),* **Prion** (from *proteinaceous infectious only) protein (PrP).*

Prion protein is a normal constituent of brain tissue in all mammals. → molecular signaling function.

Illness occurs only when the normal cellular PrP, or PrPC, occurs in an altered conformation called PrPSc (Sc denotes scrapie)

The interaction of PrPSc with PrPC converts the latter to PrPSc, initiating a domino effect in which more and more of the brain protein converts to the disease-causing form.

A mutation in the gene encoding PrP produces a change in one amino acid residue → the conversion of PrPC to PrPSc

Protein Denaturation

- **Loss of Protein Structure Results in Loss of Function → denaturation**
- Most proteins can be denatured by heat, which affects the weak interactions in a protein (primarily hydrogen bonds)

The very heat-stable proteins of thermophilic bacteria have evolved to function at the temperature of hot springs (~100 C). Why??

PROTEIN SIDE CHAIN : STRUCTURE, STABILITY, BINDING CAPACITY

Residu hidrofobik tersembunyi di dlm, determinan penting dlm struktur & stabilitas prot.

Reduksi permukaan hidrofobik terekspos → menaikkan thermostabilitas

- **Substitusi residu hidrofilik dg hidrofobik → menaikkan thermostability: Asn24 → Leu24 *B.subtilis* neutral protease**
- **His133 →Tyr 133 *B.licheniformis* α-amylase**

What else?

Proteins can be denatured by :

- **extremes of pH**,
- by certain **miscible organic solvents** such as alcohol or acetone,
- by certain solutes such as **urea and guanidine hydrochloride,**
- by **detergents.**

no covalent bonds in the polypeptide chain are broken

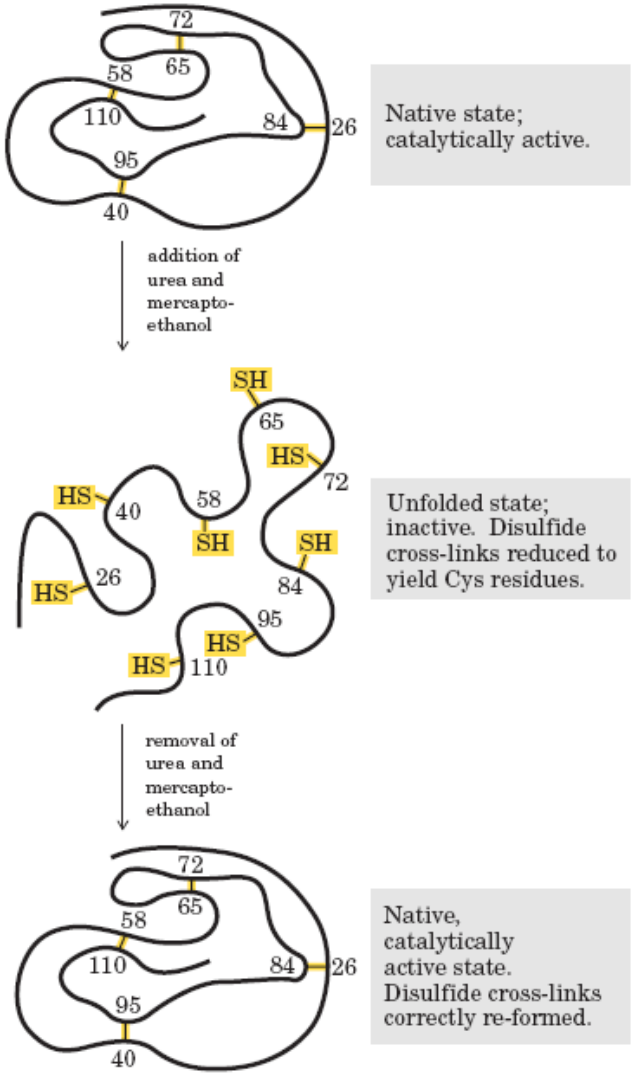
- extremes of pH alter the **net charge** on the protein, causing electrostatic repulsion and the disruption of some **hydrogen bonding**.
- Organic solvents, urea, and detergents act primarily by disrupting the **hydrophobic interactions** that make up the stable core of globular proteins;

Denaturation of some proteins = reversible or irreversible?

- Certain globular proteins denatured by heat, extremes of pH, or denaturing reagents will regain their native structure and their biological activity…

if returned to conditions in which the native conformation is stable → **renaturation.**

Renaturation of unfolded, denatured ribonuclease



Most Enzymes Are Proteins

Their catalytic activity depends on the integrity of their native protein conformation.

What will happen if an enzyme is denatured or dissociated into its subunits or is broken down into its component amino acids ?

TABLE 6–3 International Classification of Enzymes

No.	*Class*	*Type of reaction catalyzed*
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

Note: Most enzymes catalyze the transfer of electrons, atoms, or functional groups. They are therefore classified, given code numbers, and assigned names according to the type of transfer reaction, the group donor, and the group acceptor.

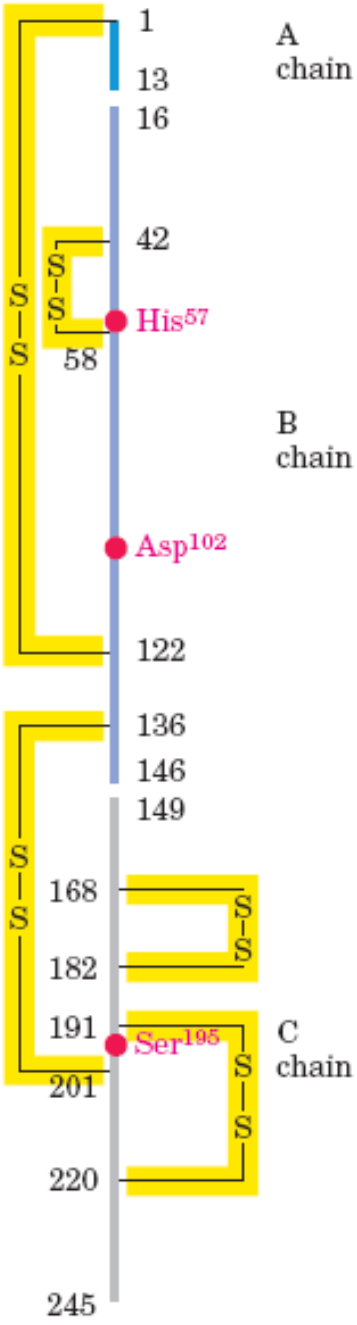
Enzyme	*Substrate*
Catalase	H_2O_2
Carbonic anhydrase	HCO_3^-
Acetylcholinesterase	Acetylcholine
β-Lactamase	Benzylpenicillin
Fumarase	Fumarate
RecA protein (an ATPase)	ATP

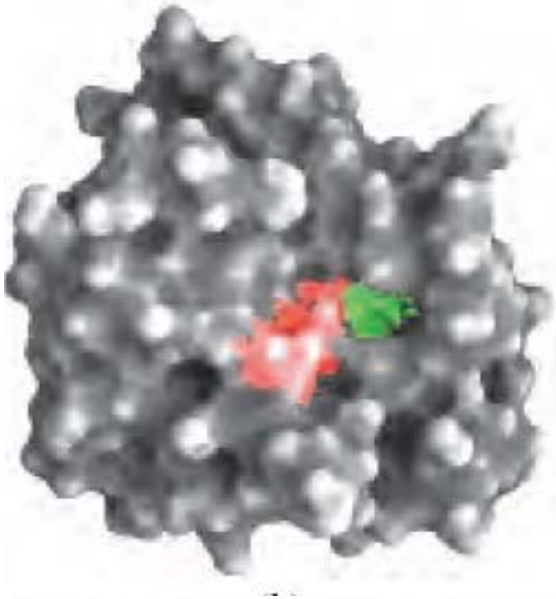
Bovine pancreatic chymotrypsin

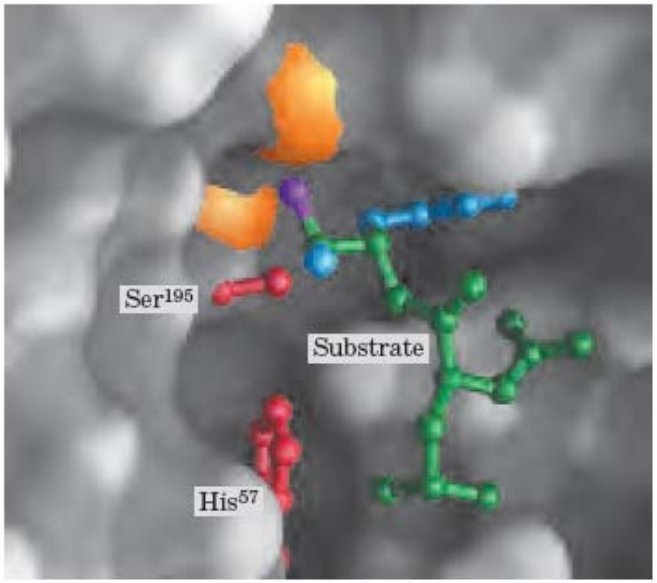
(*Mr 25,191)*

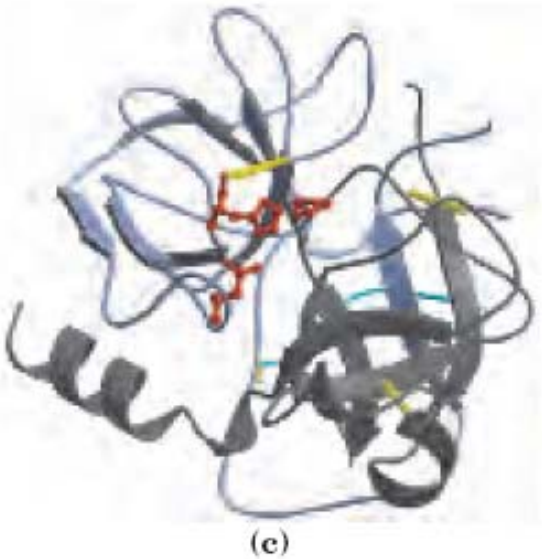
- *Is a protease,* an enzyme that catalyzes the hydrolytic cleavage of peptide bonds.
- specific for Trp, Phe, Tyr.

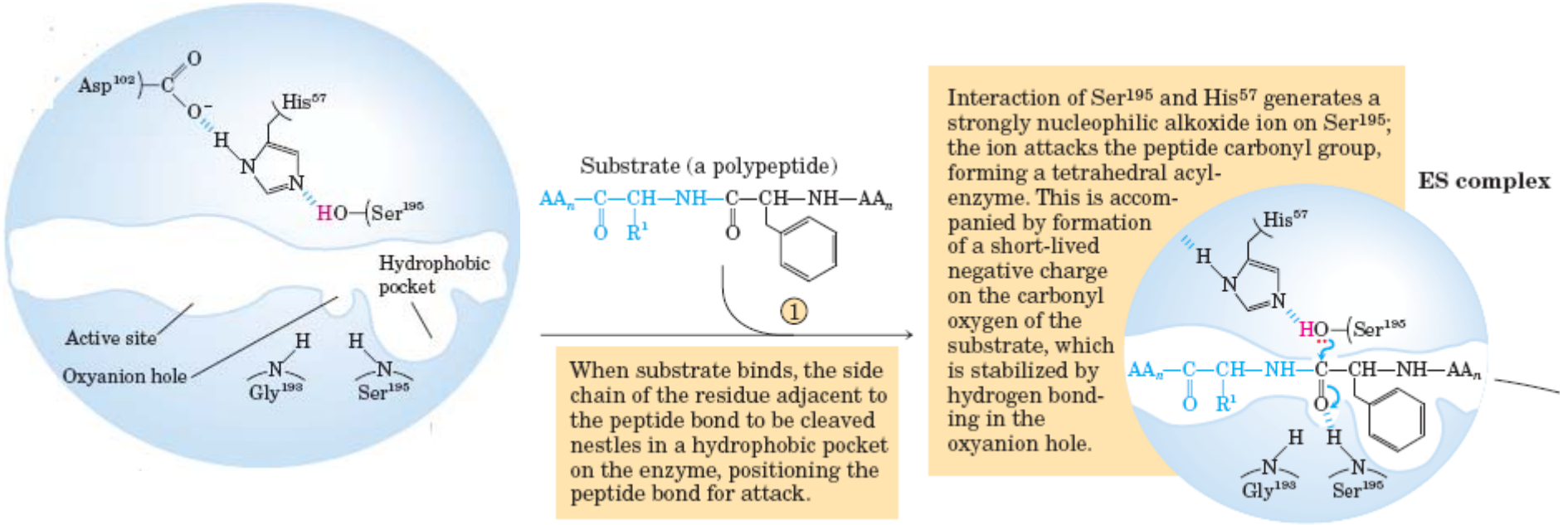
Chymotrypsin structure



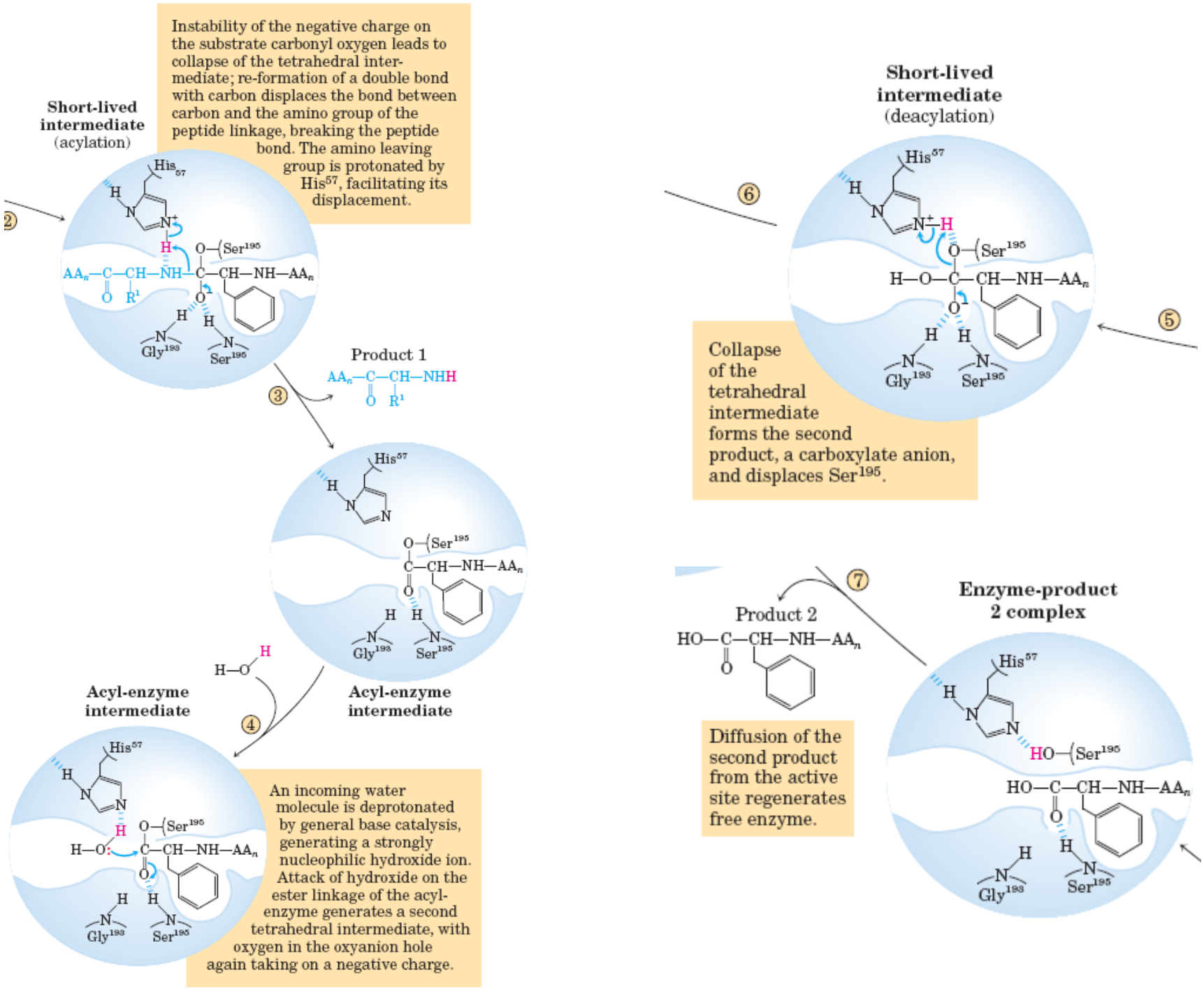





(c)



The Chymotrypsin Mechanism Involves Acylation and Deacylation of a Ser Residue


Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His57, facilitating its displacement.
Short-lived intermediate (acylation)
2
Product 1
3
Acyl-enzyme intermediate
Acyl-enzyme intermediate
4
An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.
Short-lived intermediate (deacylation)
6
5
Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser195.
7
Product 2
Diffusion of the second product from the active site regenerates free enzyme.
Enzyme-product 2 complex