BIOL 158: PROTEINS

by

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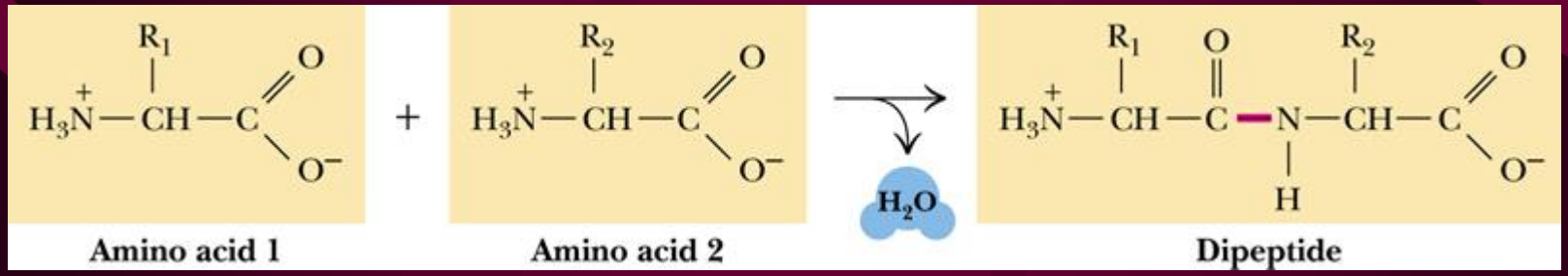
Structure and function of Proteins

- **The fundamental structural pattern in proteins.**
- **Architectural arrangements of protein structure.**
- **Isolation and purification of proteins from cells.**
- **Determination of the primary structure of a proteins.**
- **Functions of proteins.**

- Proteins are a diverse and abundant class of biomolecules, constituting more than 50% of the dry weight of cells.
- Composition: 20 kinds of amino acids linked in series; polymers composed of hundreds or even thousands of amino acids

The fundamental structural pattern in proteins

Covalent peptide bonds: amide linkage formed between a carboxyl group of an amino acid and an amino group of another amino acid.



The classification of peptide

- Peptide is the name assigned to short polymers of amino acids.
- **Amino acid residue:** basic amino acid units in peptide
- Classification of peptide:

  The number of amino acid residues

  - 2 ~ 12 : dipeptides, tripeptides, tetrapeptides, quint-, hex-, hepta-, octa-, nino-, deca-, hendeca-, dodeca-
  - 12~ 20: oligopeptides
  - > 20 : polypeptides

Proteins are composed of one or more polypeptide chains

The term **protein** broadly defines molecules composed of one or more polypeptide chains.

proteins
- Monomeric proteins (one polypeptide chain)
- Multimeric proteins (more than one chain)
  - homomultimeric proteins (only one kind of chain)
  - heteromultimeric proteins (more than one kind of chains)

Greek letters and subscripts are used to denote the polypeptide composition of multimeric proteins.

Eg. Haemoglobin: $\alpha_2\beta_2$

Insulin

αβ; 21, 30

Cytochrome *c*

α_1; 104

Ribonuclease

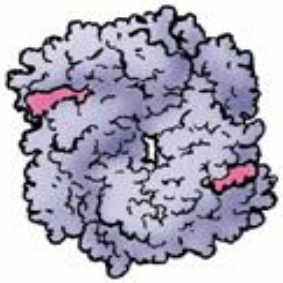
α_1; 124

Lysozyme

α_1; 129

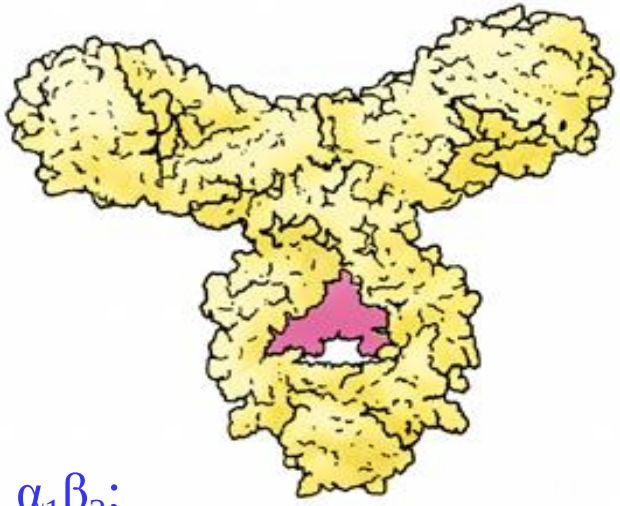
Myoglobin

α_1; 153



Hemoglobin

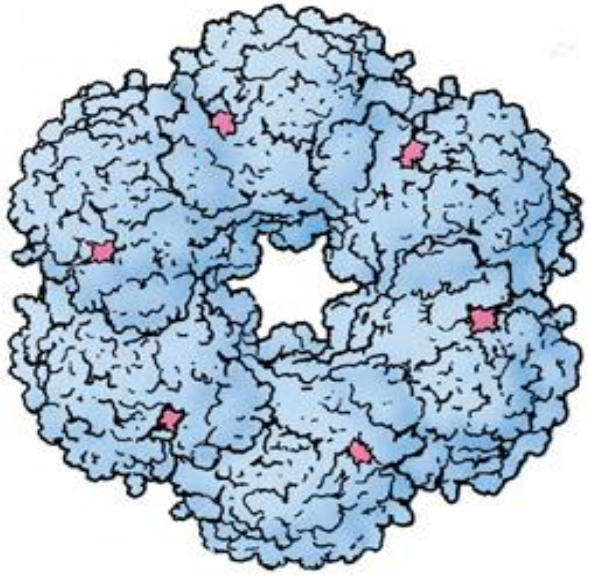
$\alpha_2\beta_2$;
141, 146



Immunoglobulin

$\alpha_1\beta_2$;
214, 446

α_{12};
468



Glutamine synthetase

- Polypeptide chains of proteins typically range in length: 100~2000 aa residues.
- The average molecule weight of polypeptide chains in eukaryotic cells is about 31,700, corresponding to about 270 amino acid residues.

The architectural arrangements of structure

Proteins fall into three basic classes according to shape and solubility.

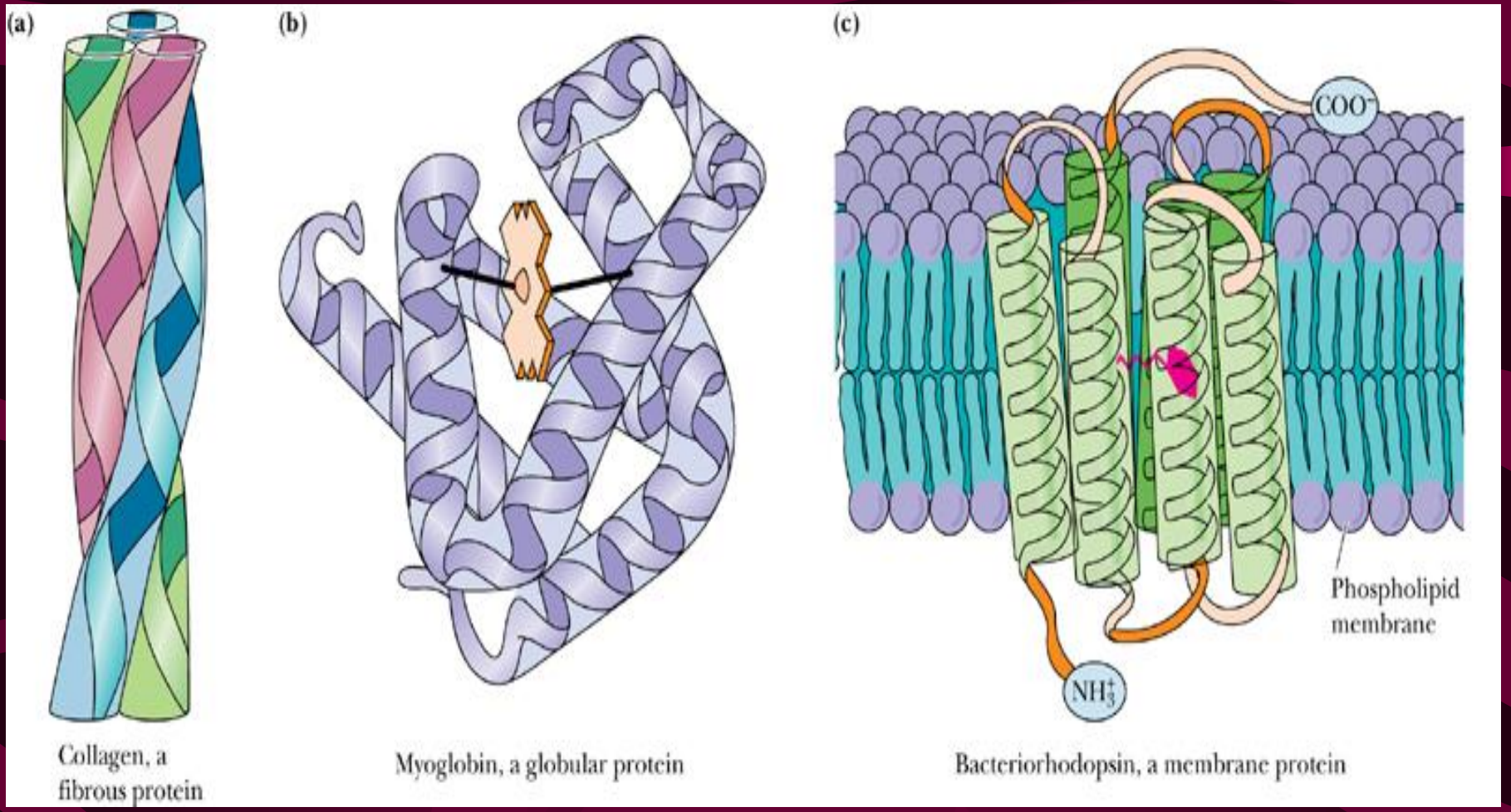
Fibrous proteins: tend to have relatively simple, regular linear structures, insoluble in water and several structural roles in cell.

Globular proteins: roughly spherical in shape,

Outside: hydrophilic side chain; interior: hydrophobic ~

Membrane proteins: multi-shape, insoluble in water,

Outside: hydrophobic side chain; interior: hydrophilic ~



Fibrous protein; Globular protein; Membrane protein

Proteins structure is described in terms of four levels of organization

Primary structure: amino acid sequence

Secondary structure: “backbone” shape of the polypeptide

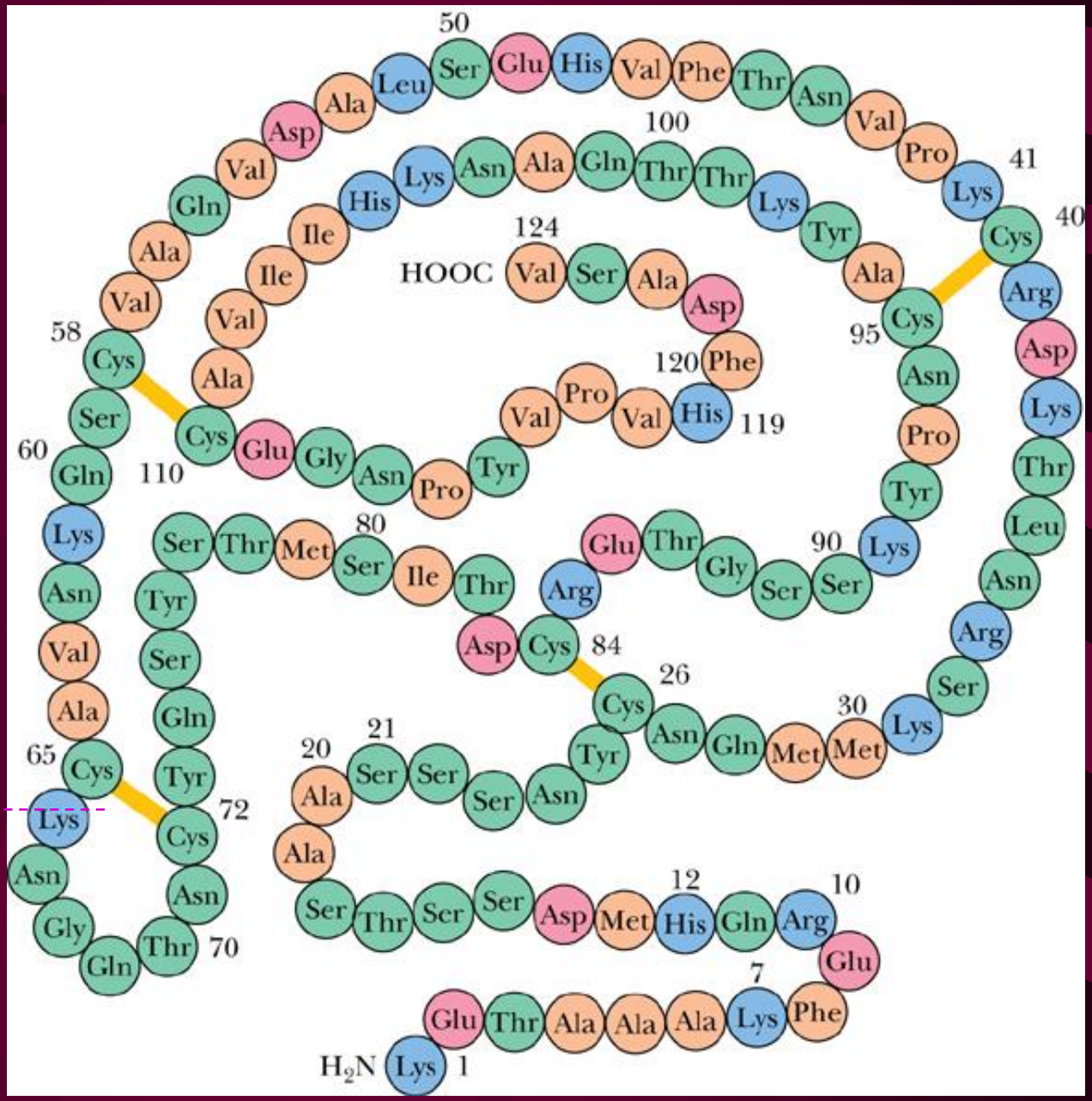
Tertiary structure: complete three-dimensional shape of a polypeptide

Quaternary structure: complete three-dimensional shape of all polypeptides

Only in multimeric proteins; chain: **subunit**

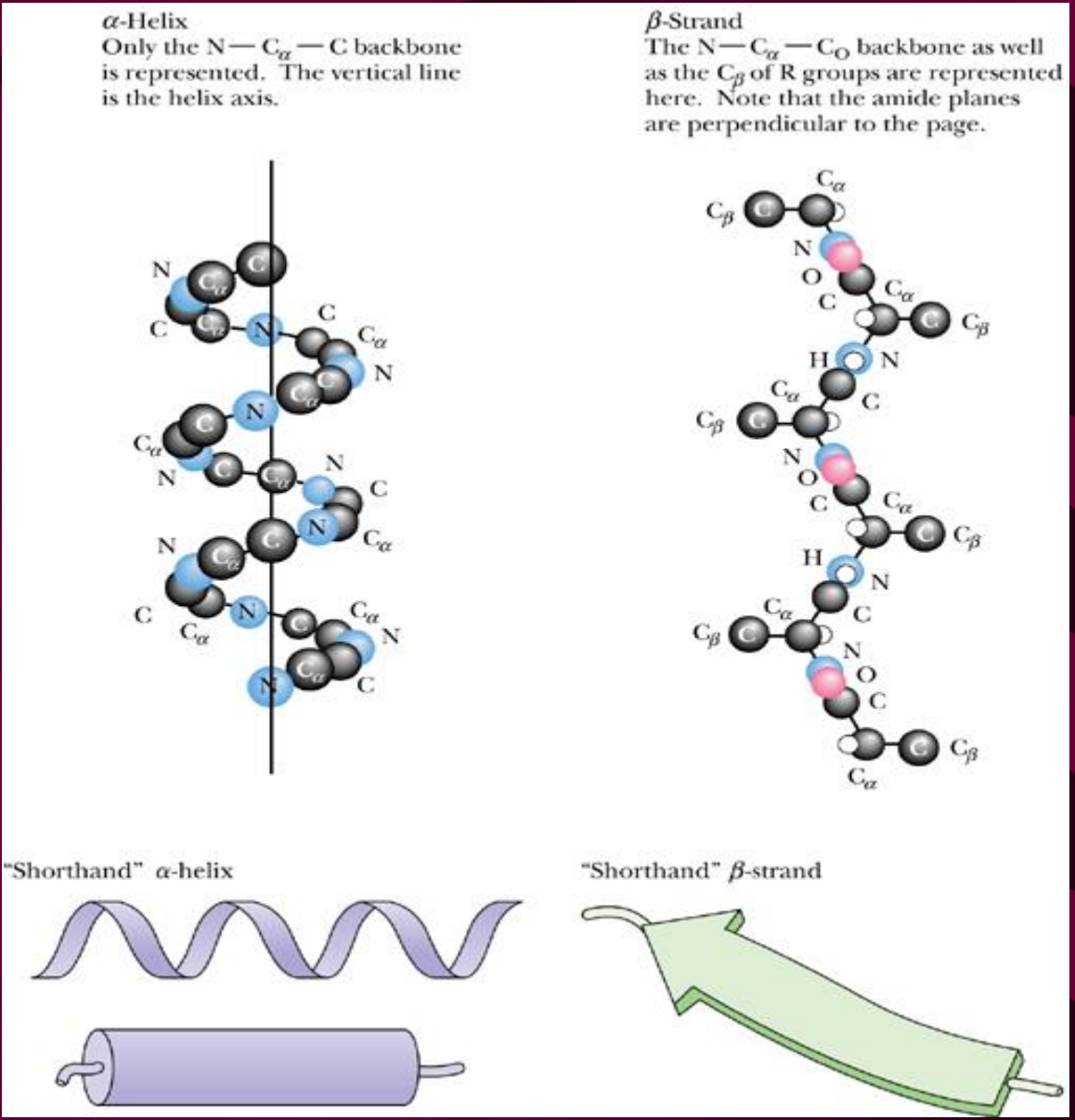
Primary structure:
Bovine pancreatic ribonuclease A (124 amino acid residues)

Disulfide bridge
—S—S—



Secondary structure

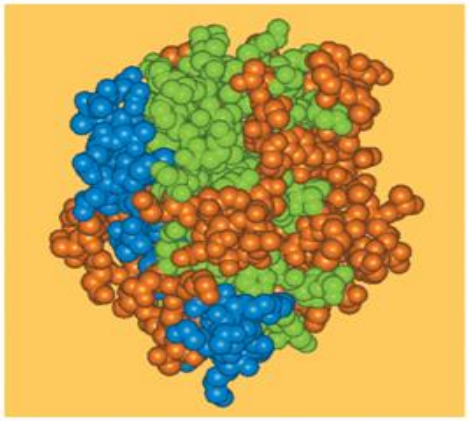
"backbone" shape of the polypeptide



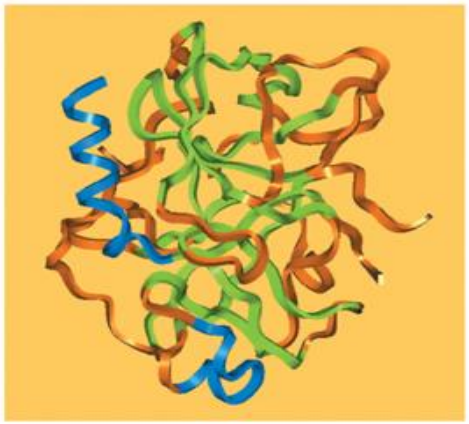
Tertiary structure

(a) Chymotrypsin primary structure

H_2N–CGVPAIQPVL$_{10}$SGL[SR]IVNGE$_{20}$EAVPGSWPWQ$_{30}$VSLQDKTGFH$_{40}$GGSLINEN$_{50}$WVVTAAHCGV$_{60}$TTSDVVVAGE$_{70}$FDQGSSSEKI$_{80}$QKLKIA
KVFK$_{90}$NSKYNSLTIN$_{100}$NDITLLKLST$_{110}$AASFSQTVSA$_{120}$VCLPSASDDF$_{130}$AAGTTCVTTG$_{140}$WGLTRY[TN]AN$_{150}$LPSDRLQQASL$_{160}$PLLSNTNCK
K$_{170}$YWGTKIKDAM$_{180}$ICAGASGVSS$_{190}$CMGDSGGPLV$_{200}$CKKNGAWTLV$_{210}$GIVSWGSSTC$_{220}$STSTPGVYAR$_{230}$VTALVNWVQQ$_{240}$TLAAN–COOH

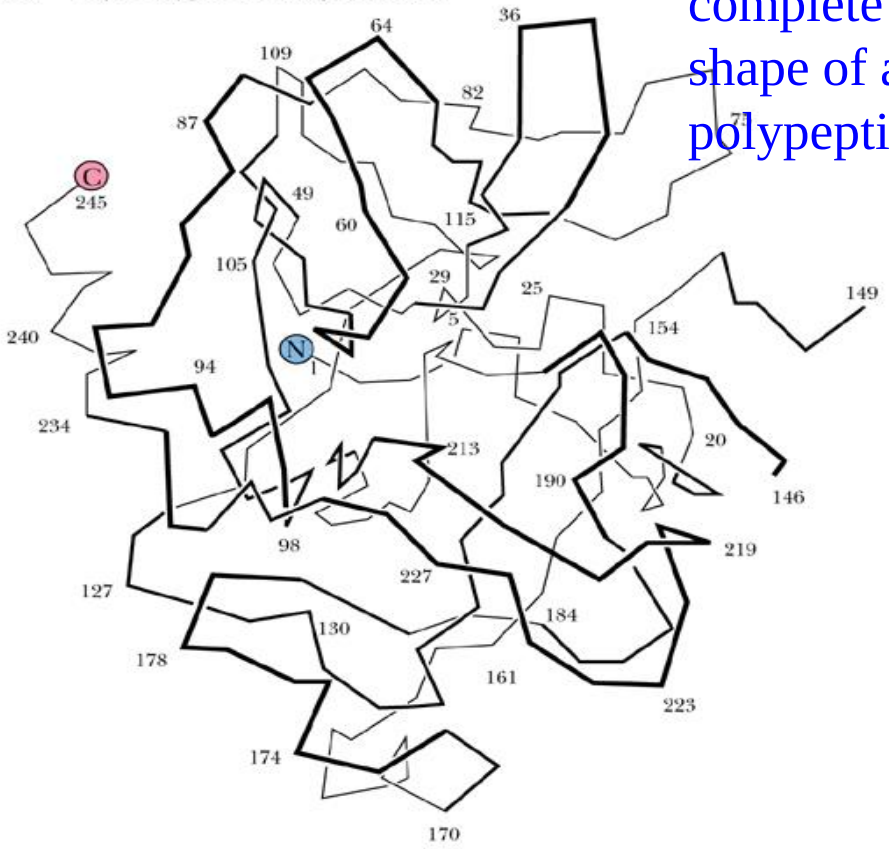


Chymotrypsin space-filling model



Chymotrypsin ribbon

(b) Chymotrypsin tertiary structure



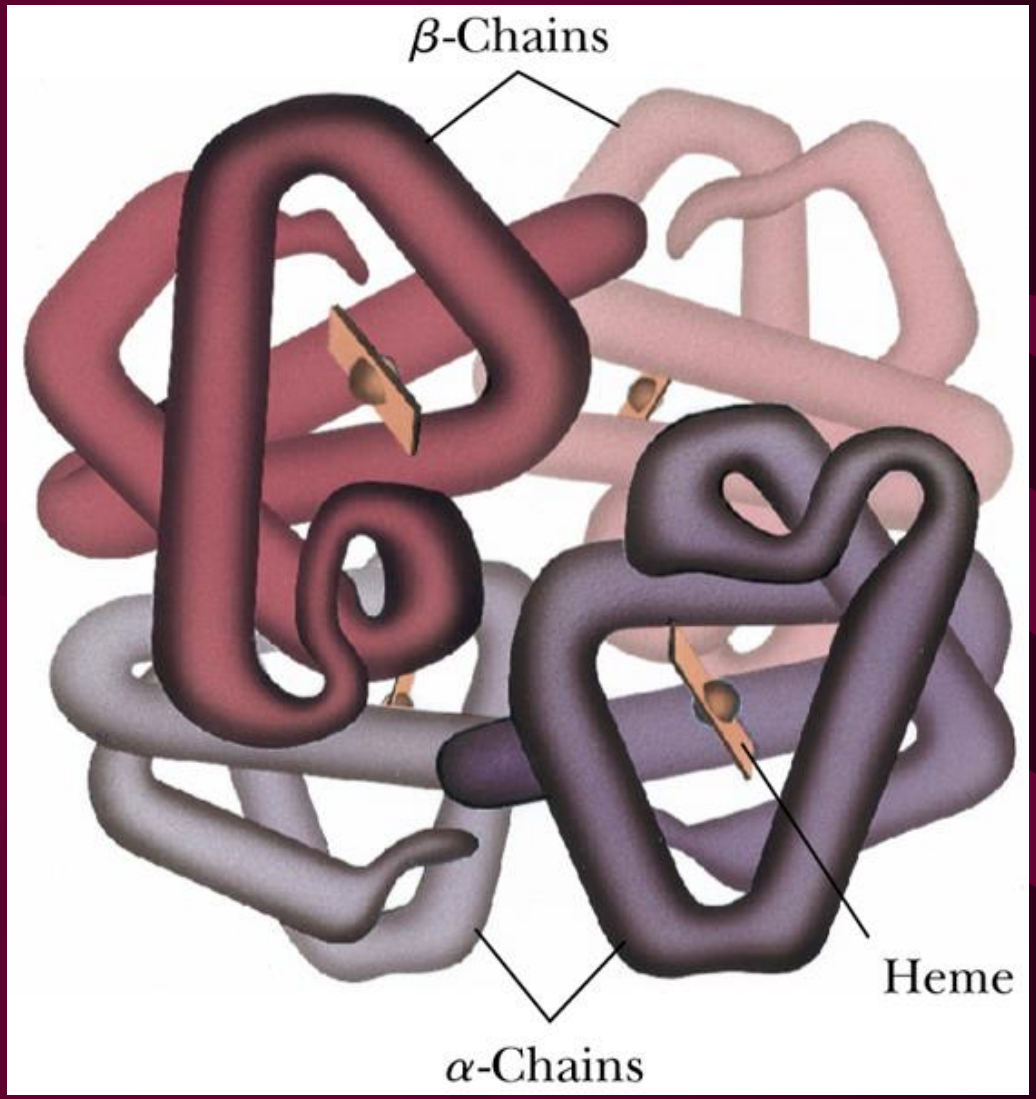
complete 3-D shape of a polypeptide

ribbon

Quaternary structure

Complete 3-D shape of all polypeptides

Multimeric protein

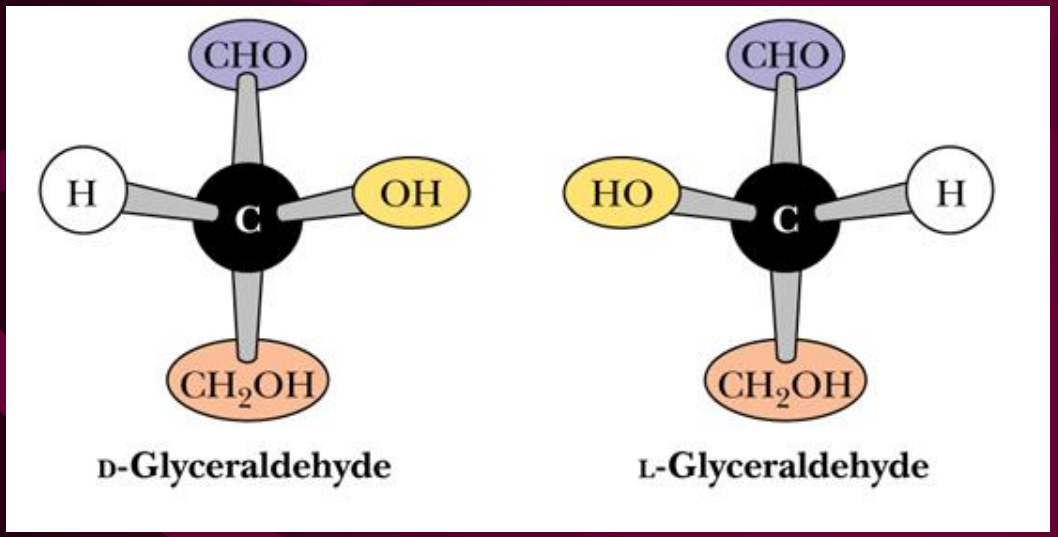


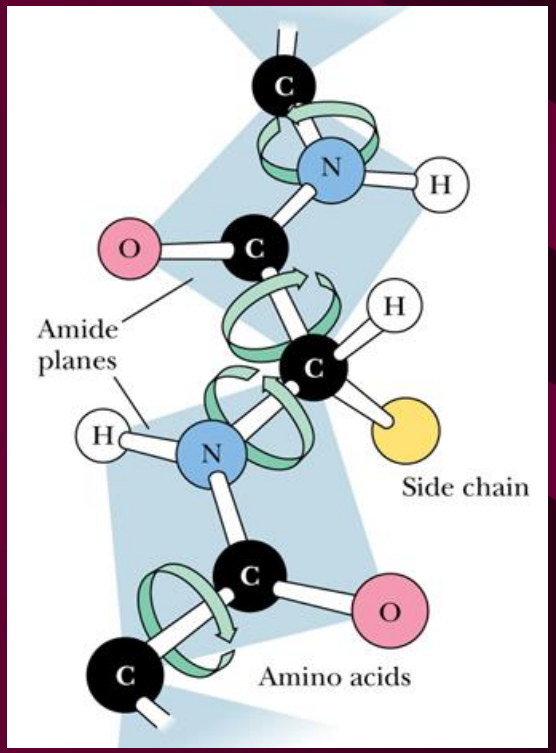
Protein's Conformation

A protein's **conformation** can be described as its overall three-dimensional structure. The term is not to be confused with **configuration**, which denotes the geometric possibilities for a particular set of atoms.

Configuration change: covalent bonds must be broken and rearranged

Conformation change: without breaking any covalent bonds.





How are proteins isolated and purified from cells?

Cells contain thousands of different proteins.

Purification of a chosen protein is very important for research.

Proteins can be separated and purified on the basis of their two prominent physical properties: **size** and **electrical charge**.

Protein size: ultrafiltration, ultracentrifugation

Protein electrical charge: electrophoresis, ion exchange chromatography, and solubility (precipitation under high level salt, partition chromatography)

Estimation of Protein concentration in solutions of biological origin

- Protein concentrations cannot be expressed on a molar basis (g/L, mg/ml)
- Detection of protein using the characters of amino acids (affected by composition of aa)

- UV absorption in 280nm (Phe, Tyr)
- Chemical reaction:
  Lowry Procedure and BCA (bicinchoninic acid) method
  (reductive ability of Tyr and His)
- Dye binding:
  Coomassie Brilliant Blue G-250

Protein Techniques (appendix, P.148-152)

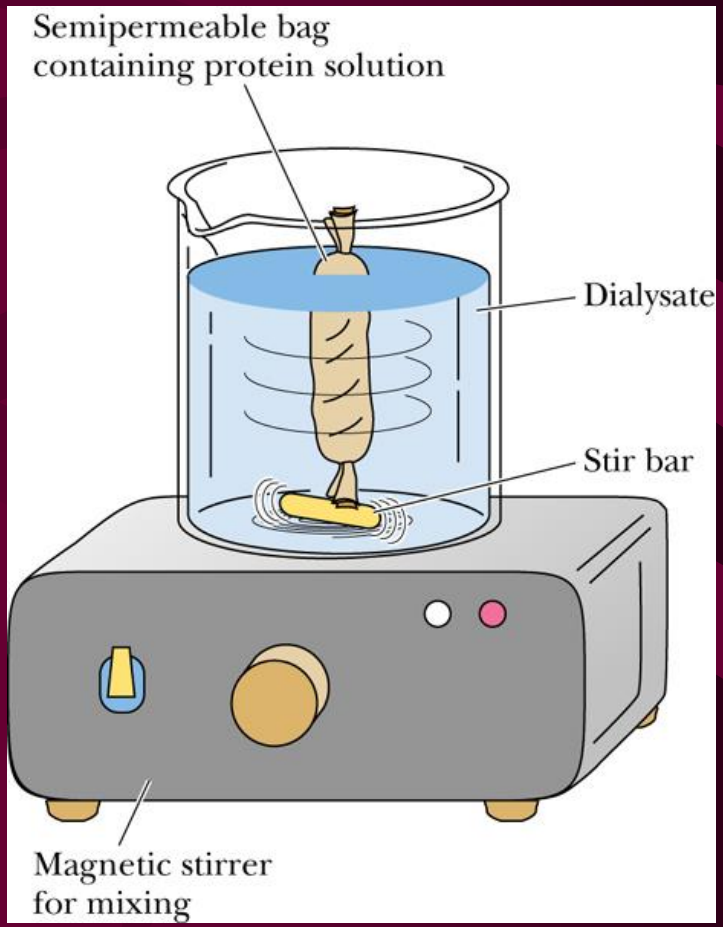
Isolation and purification of protein from a mixture

Dialysis and Ultrafiltration

Dialysis: Separating large and small molecular proteins with semipermeable membrane

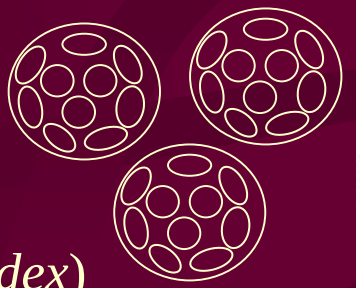
Ultrafiltration: improvement on the dialysis principle

Filters with different pore sizes



Size Exclusion Chromatography

Gel filtration chromatography;
Molecular sieve chromatography



Porous gel beads:

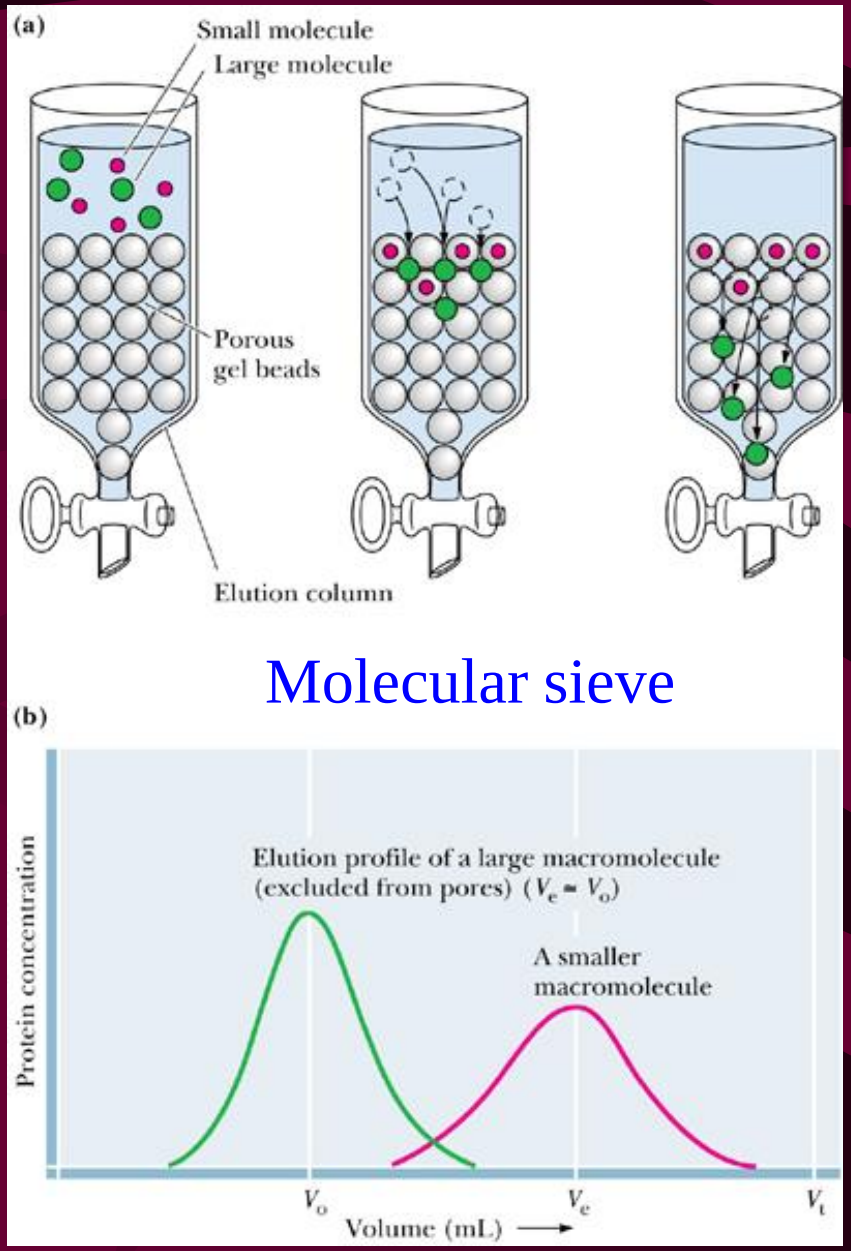
Dextran polymers (*Sephadex*)

Agarose (*Sepharose*)

Polyacrylamide (*Sephacryl* or *BioGel P*)

Pore size of beads

Protein molecular dimension



Molecular sieve

Electrophoresis

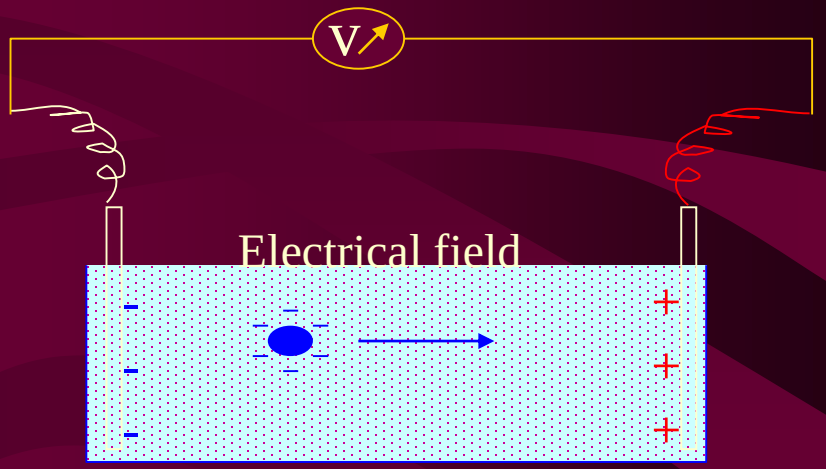
Electrophoresis are based on the movement of ions in an electrical field

$$F = \frac{Eq}{d} \qquad F_f = 6\pi r\eta v$$

$F >> F_f$ v↑ F_f↑ $F = F_f$

$$Eq / d = 6\pi r\eta v ;$$

$$v = Eq / d\, 6\pi r\eta$$



E: the voltage; q: the charge of molecule; d: the distance between tow electrodes; r: the radius of the molecule; η: the viscosity of the medium; v: moving velocity

Medium: solution and porous support matrix (polyacrylamide, agarose)