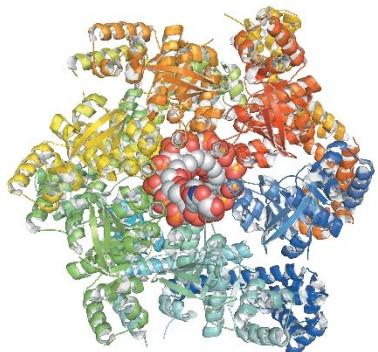


biochemistry



Reginald H. Garrett | Charles M. Grisham  
SIXTH EDITION



Reginald H. Garrett  
Charles M. Grisham

[www.cengage.com/chemistry/garrett](http://www.cengage.com/chemistry/garrett)

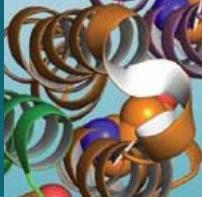
# Chapter 4.7, 5.1, 5.2, 5.7, 5.8

# Protein Structure/Function Overview

# Techniques for Protein Purification and

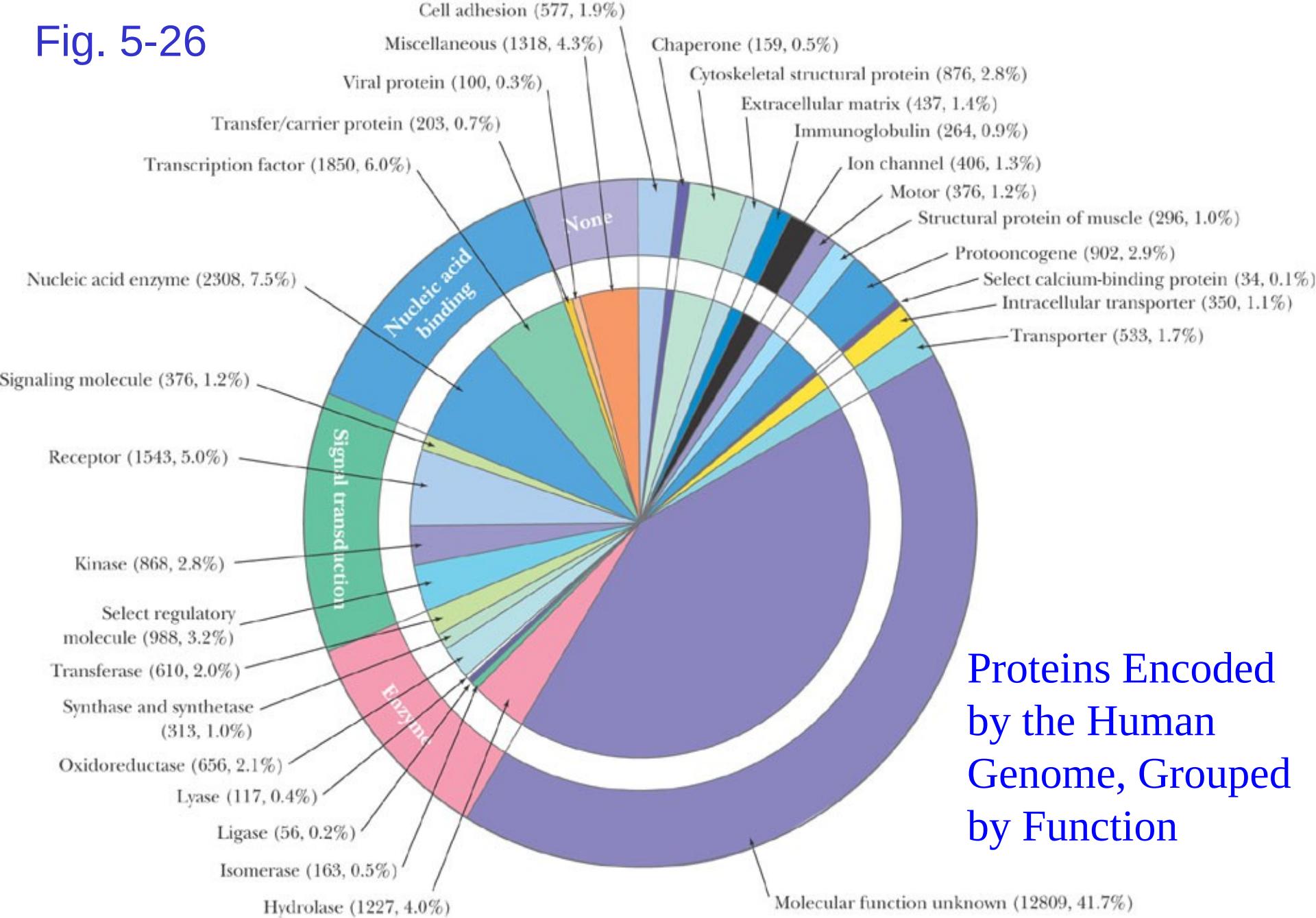
# Characterization

# Outline



- Protein functions
- Protein terminology and types
- Levels of protein structure: primary, secondary, tertiary, quaternary

Fig. 5-26



Proteins Encoded  
by the Human  
Genome, Grouped  
by Function

# 5.8 What Are the Many Biological Functions of Proteins?



The functionality of proteins can be enhanced by post-translational binding of co-factors (usually small, noncovalent, needed for enzyme activity) and prosthetic groups (usually covalent).

**TABLE 5.6** Some Common Conjugated Proteins

| Name   | Nonprotein Part   | Association             | Examples   |
|--|---|-------------------------|--|
| Lipoproteins                                 | Lipids  | Noncovalent             | Blood lipoprotein complexes (HDL, LDL)                 |
| Nucleoproteins                               | RNA, DNA  | Noncovalent             | Ribosomes, chromosomes                                 |
| Glycoproteins                                | Carbohydrate groups   | Covalent                | Immunoglobulins, LDL receptor                          |
| Metalloproteins and metal-activated proteins | $\text{Ca}^{2+}$ , $\text{K}^+$ , $\text{Fe}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Co}^{2+}$ , others | Covalent to noncovalent | Metabolic enzymes, kinases, phosphatases, among others |
| Hemoproteins                                 | Heme group  | Covalent or noncovalent | Hemoglobin, cytochromes                                |
| Flavoproteins                                | FMN, FAD  | Covalent or noncovalent | Electron transfer enzymes                              |

## Sec. 4.7 What is the Fundamental Structural Pattern in Proteins?

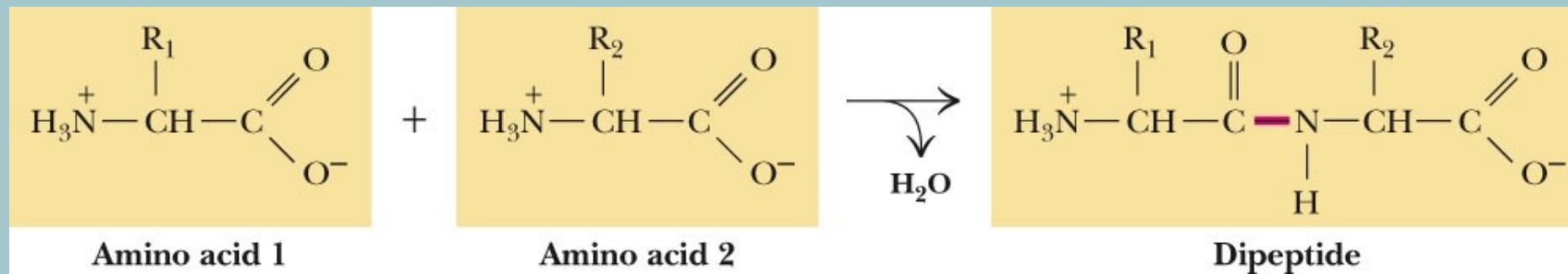
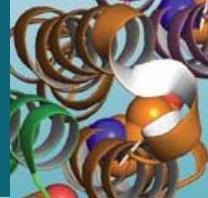


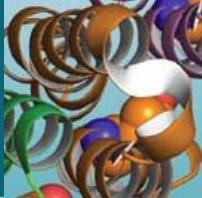
Figure 4.14 Peptide formation is the creation of an amide bond between the carboxyl group of one amino acid and the amino group of another amino acid.

## 4.7 What is the Fundamental Structural Pattern in Proteins?



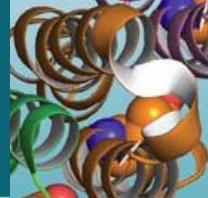
- Proteins are unbranched polymers of amino acids
- Proteins may be composed of one or more polymer chains
- The peptide backbone of a protein consists of the repeated sequence  $\text{--N-C}_\alpha\text{-C}_\text{o}\text{--}$ 
  - “N” is the amide nitrogen of the amino acid
  - “ $\text{C}_\alpha$ ” is the alpha-C of the amino acid
  - “ $\text{C}_\text{o}$ ” is the carbonyl carbon of the amino acid

# “Peptides”



- Short polymers of amino acids
- Each amino acid unit is called a residue
- 2 residues – dipeptide
- 3 residues – tripeptide
- 12-20 residues – oligopeptide
- >20 residues – polypeptide
- Polypeptides above 50 residues – protein

# “Protein”



- One polypeptide chain - a monomeric protein
  - More than one - multimeric protein
  - Homomultimer - one kind of chain
  - Heteromultimer - two or more different chains
- Hemoglobin, for example, is a heterotetramer. It has two alpha chains and two beta chains held together by weak interactions.
- .

# “Protein”



## Classified By Shape

- Fibrous – Collagen  
Elastin  
Fibroin (silk)  
Keratin (wool, hair, skin, horn)
- Globular – Most proteins and enzymes

## Classified By Solubility in Aqueous Solutions

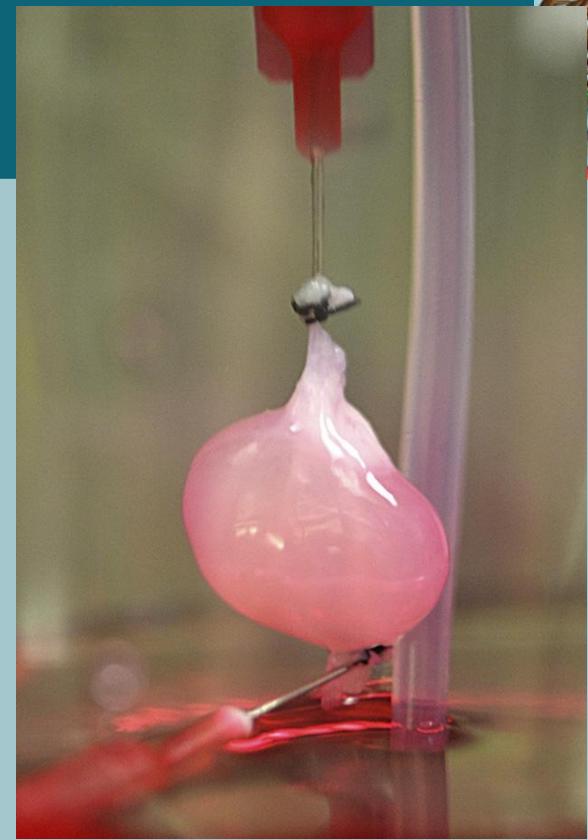
- Soluble – e.g. Cytosolic
- Insoluble – e.g. Membrane-bound or structural

# Bioengineered organs



The insoluble collagen scaffolding of a rat kidney, left behind after removing all cells with mild detergent.

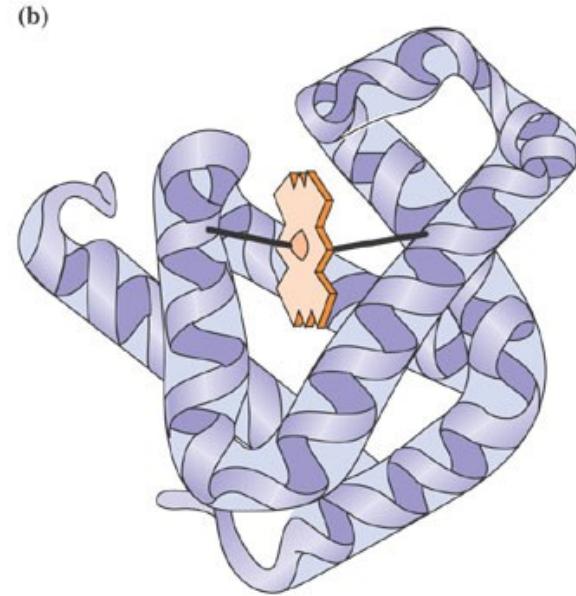
Ott Laboratory, Massachusetts General Hospital Center for Regenerative Medicine,  
April 2013



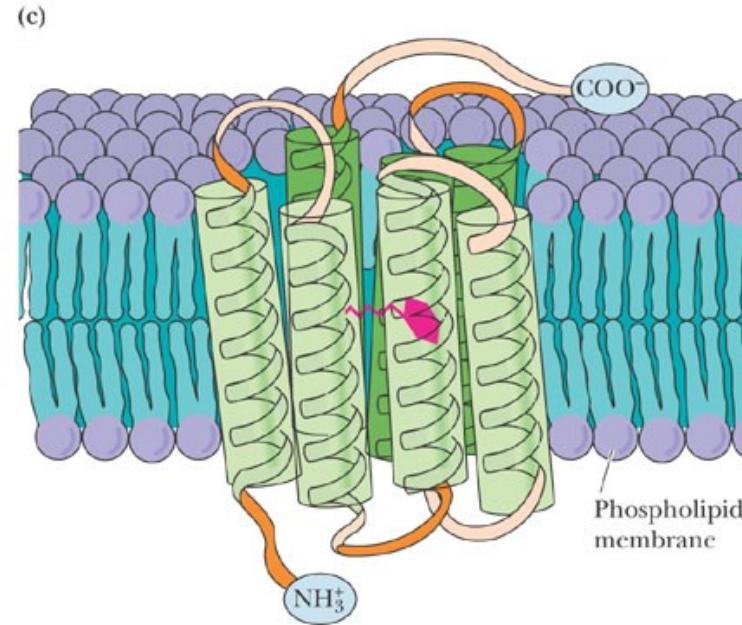
To create a functional lab-made kidney, the scaffolding was reseeded with rat neonatal kidney cells and human endothelial cells.



Collagen, a fibrous protein



Myoglobin, a globular protein



Bacteriorhodopsin, a membrane protein

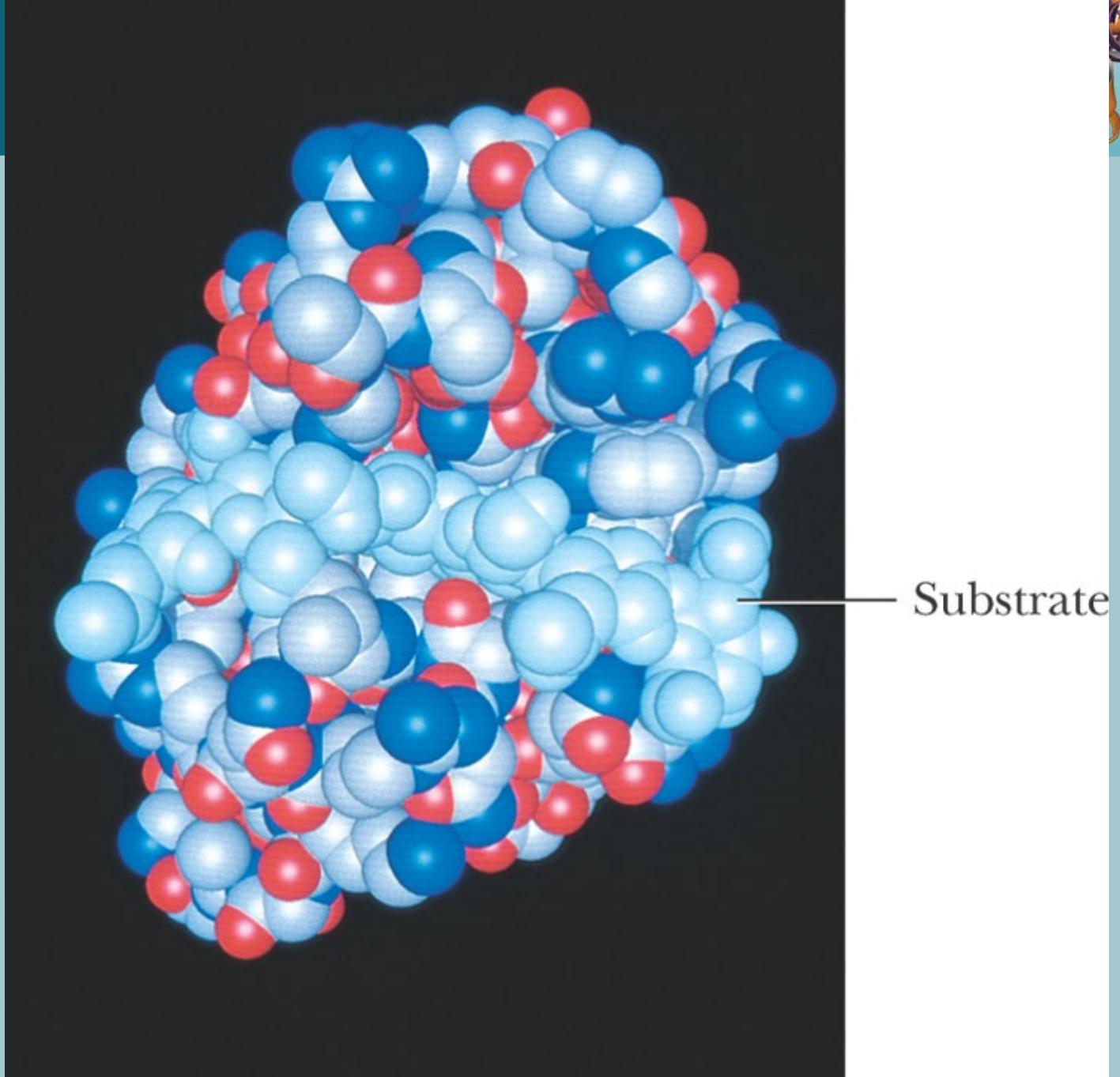
© 2005 Brooks/Cole - Thomson

**(a)** Proteins having structural roles in cells are typically fibrous and often water insoluble. Collagen is a good example. Collagen is composed of three polypeptide chains that intertwine.

**(b)** Soluble proteins serving metabolic functions can be characterized as compactly folded globular molecules, such as myoglobin. The folding pattern puts hydrophilic amino acid side chains on the outside and buries hydrophobic side chains in the interior, making the protein highly water soluble. **(c)** Membrane proteins fold so that hydrophobic amino acid side chains are exposed in their membrane-associated regions. The portions of membrane proteins extending into or exposed at the aqueous environments are hydrophilic in character, like soluble proteins. Bacteriorhodopsin is a typical membrane protein; it binds the light-absorbing pigment, *cis*-retinal, shown here in red.

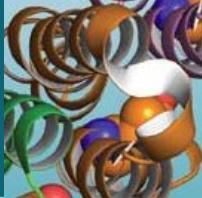
(*a, b, Illustration: Irving Geis. Rights owned by Howard Hughes Medical Institute. Not to be reproduced without permission.*)

Space-filling model of the soluble, globular enzyme lysozyme with bound oligosaccharide substrate.



## Protein Structure

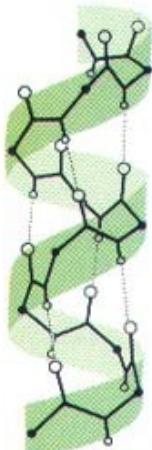
1. Primary: sequence of amino acids and their linkage by peptide bonds to form polymer, including post-translational modifications and disulfide bonds
2. Secondary: regular recurring arrangements of polymer in space, i.e., the helices formed by the polymer backbone
  - a. Super secondary structure (motif): short-range associations of secondary structural elements, often through sidechain interactions
  - b. Domains: associations of lower order structure to form a 3-D unit
3. Tertiary: folding of all helical units to produce the complete 3-D polypeptide structure
4. Quaternary: interaction between protein monomers(i.e., subunits) to form oligomeric proteins



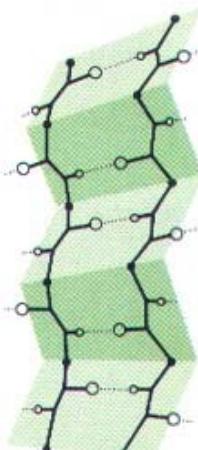
# The four levels of protein structure organization



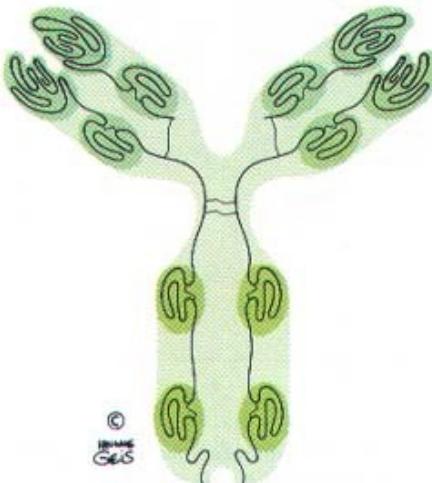
(a) Primary structure (amino acid sequence in the protein chain)



$\alpha$  helix

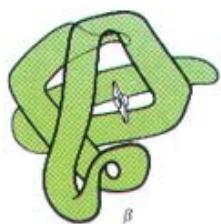


$\beta$  sheet



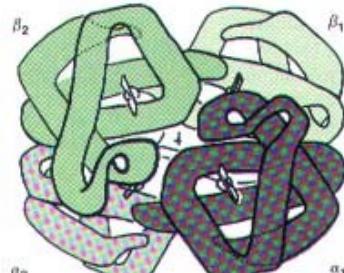
Domains (dark color) in an antibody molecule

(c) Local folding



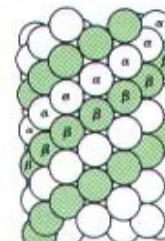
One complete protein chain ( $\beta$  chain of hemoglobin)

(d) Tertiary structure



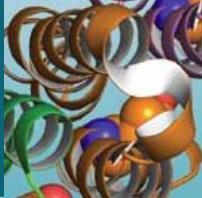
The four separate chains of hemoglobin assembled into an oligomeric protein

(e) Quaternary structure

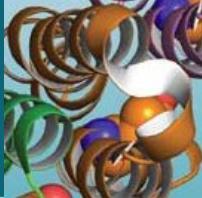


$\alpha$  (white) and  $\beta$  (color) tubulin molecules in a microtubule

(f) Quaternary structure



# 5.1 What Architectural Arrangements Characterize Protein Structure?

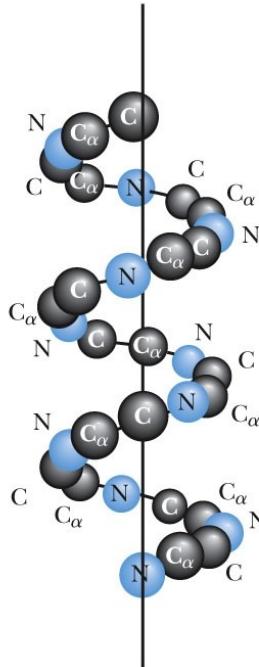


## Secondary structures in proteins

Figure 5.3 The  $\alpha$ -helix and the  $\beta$ -pleated strand are the two principal secondary structures found in proteins.

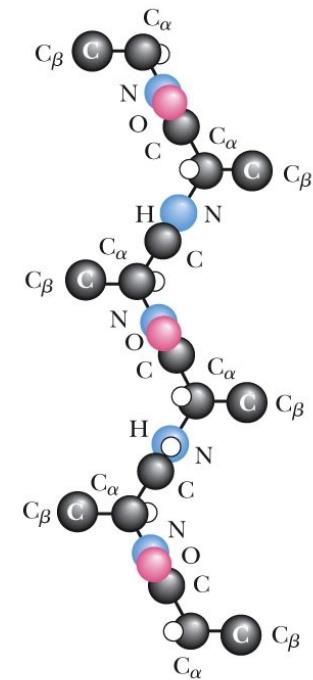
$\alpha$ -Helix

Only the N — C <sub>$\alpha$</sub>  — C backbone is represented. The vertical line is the helix axis.

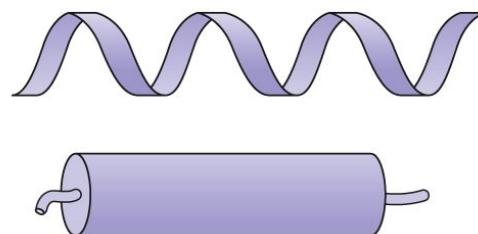


$\beta$ -Strand

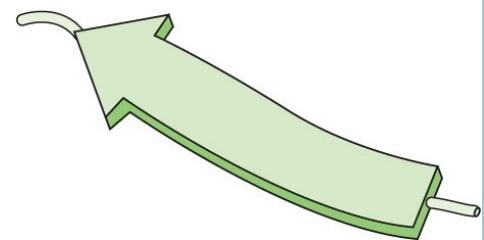
The N — C <sub>$\alpha$</sub>  — C<sub>O</sub> backbone as well as the C <sub>$\beta$</sub>  of R groups are represented here. Note that the amide planes are perpendicular to the page.



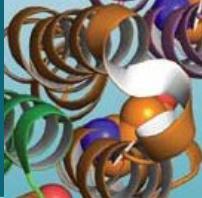
“Shorthand”  $\alpha$ -helix



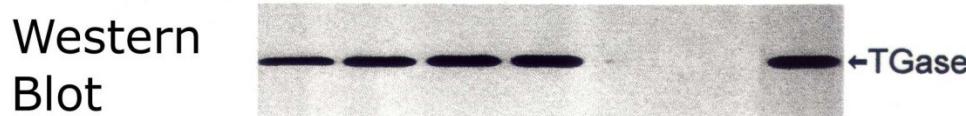
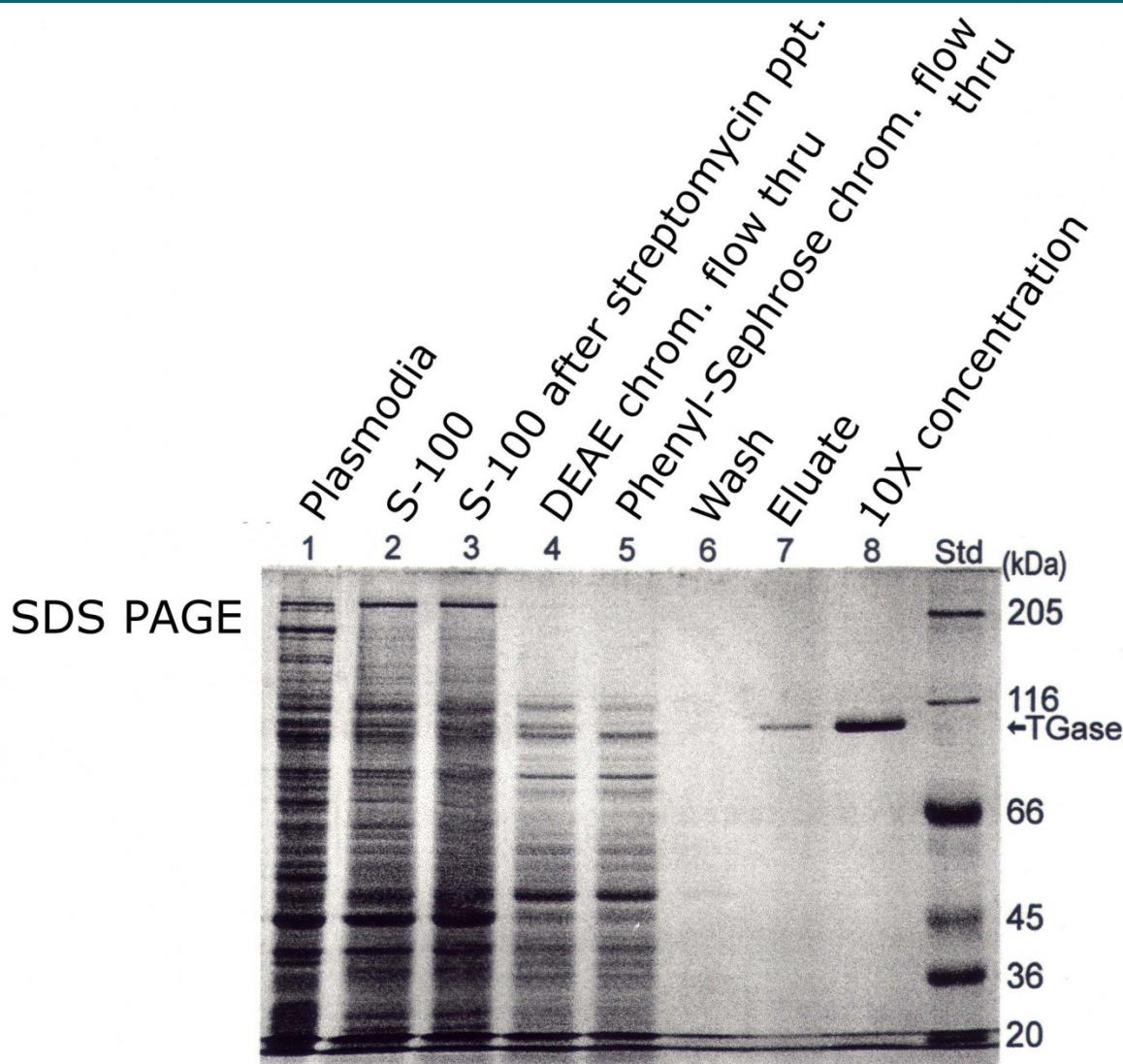
“Shorthand”  $\beta$ -strand



# Outline: Techniques for Protein Purification and Characterization



- Electrophoresis
  - Paper/PAGE/SDS-PAGE/Isoelectric Focusing
- Centrifugation
  - Velocity/Differential/Density Gradient
- Chromatography
  - Ion/Reverse Phase/Affinity/Gel Filtration
- Dialysis/Ultrafiltration
- Salting In/Salting Out



Transglutaminase Purification

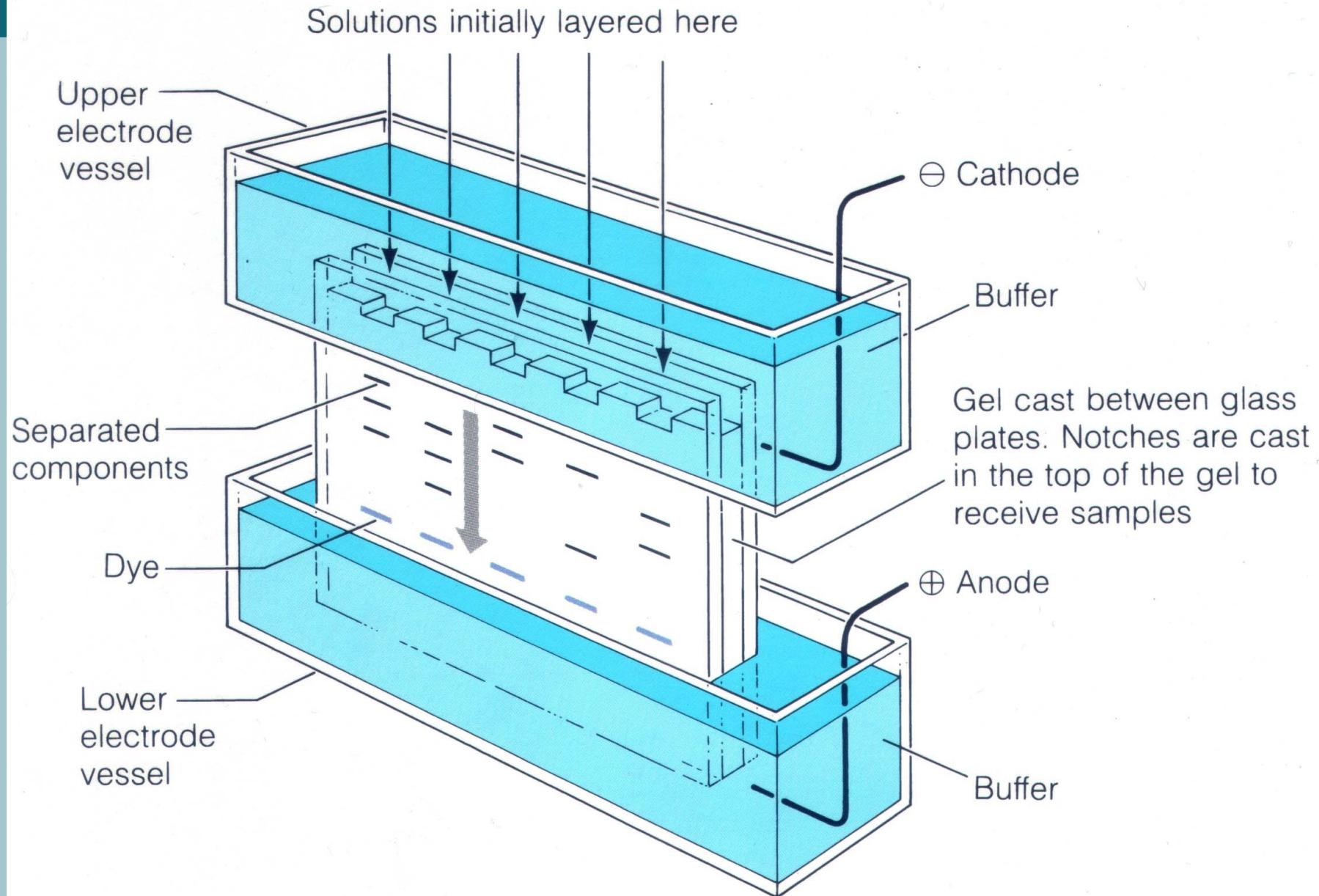
# Electrophoresis



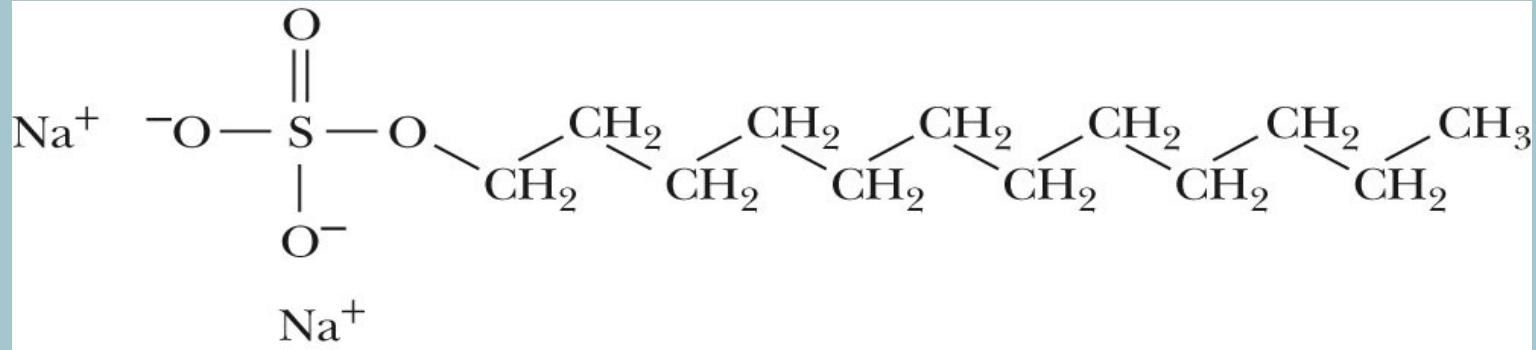
- Paper
- Gel (usually polyacrylamide, i.e. PAGE)
  - Native
  - Denaturing (usually SDS-PAGE)

Plot  $\log M_r$  vs. distance migrated of standards to determine apparent molecular weight of unknown protein
- Isoelectric Focusing
  - Proteins band at their pIs in pH gradient stabilized by multiple buffers
  - + anode in low pH tank, - cathode in high pH tank
  - Proteins that differ in pI by  $\leq 0.1$  can be separated

## Gel electrophoresis

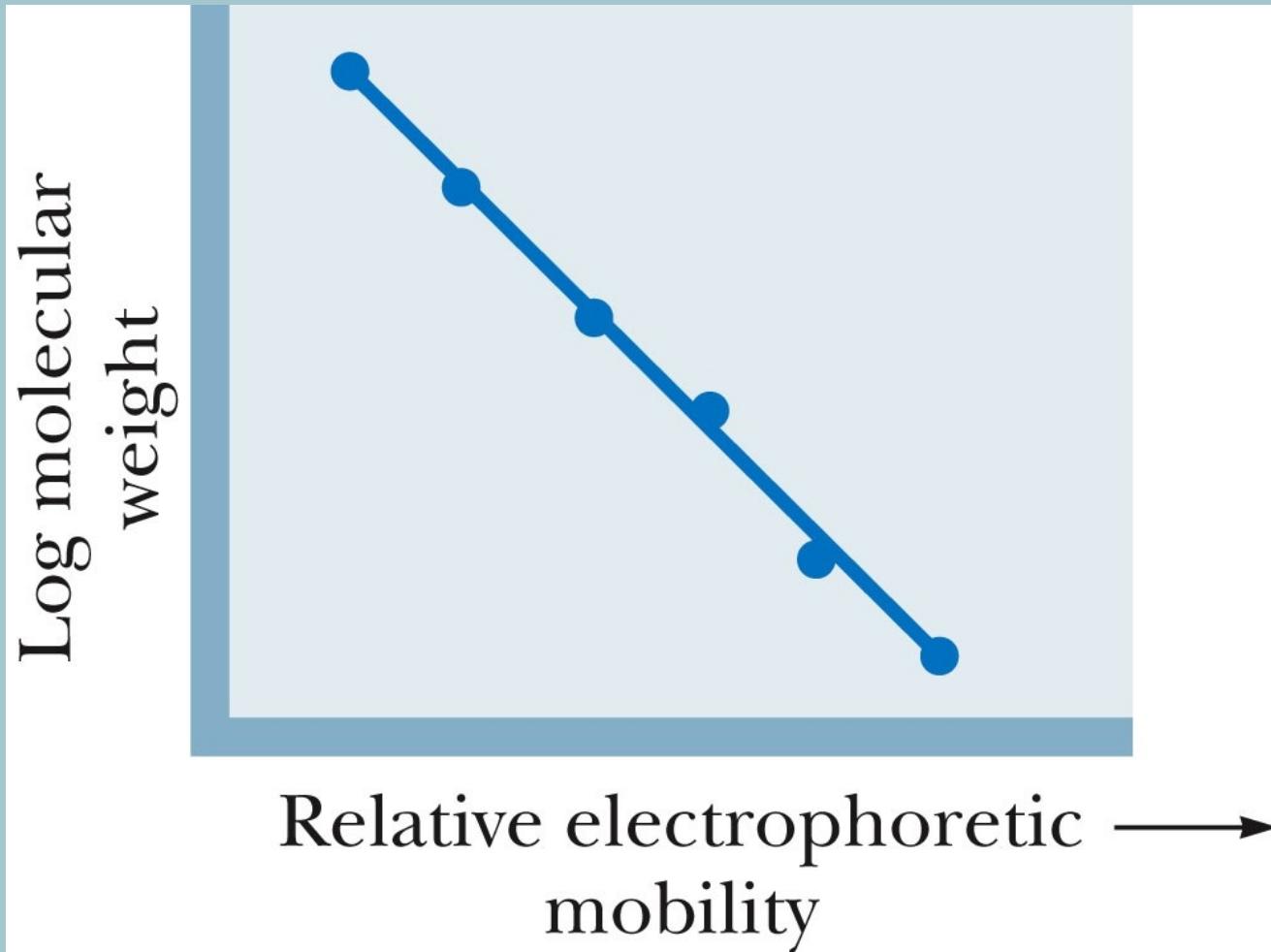
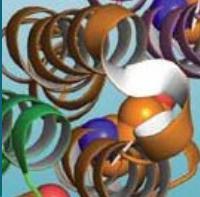


# SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)



Techniques, Figure 5, p. 113  
The structure of sodium dodecyl sulfate (SDS).

# SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)



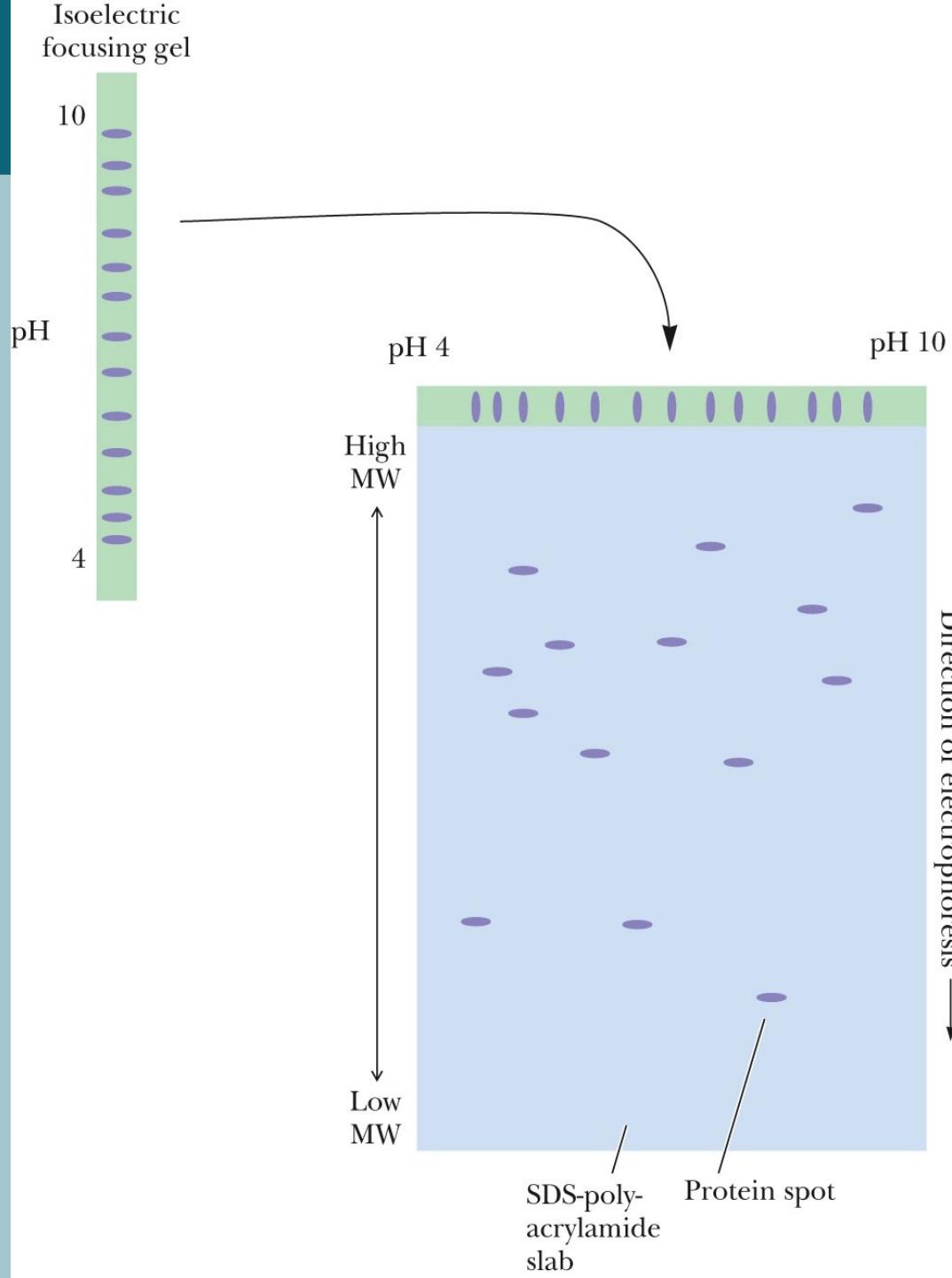
Techniques, Figure 6, p. 113 A plot of protein mobility versus log of molecular weight of individual proteins.

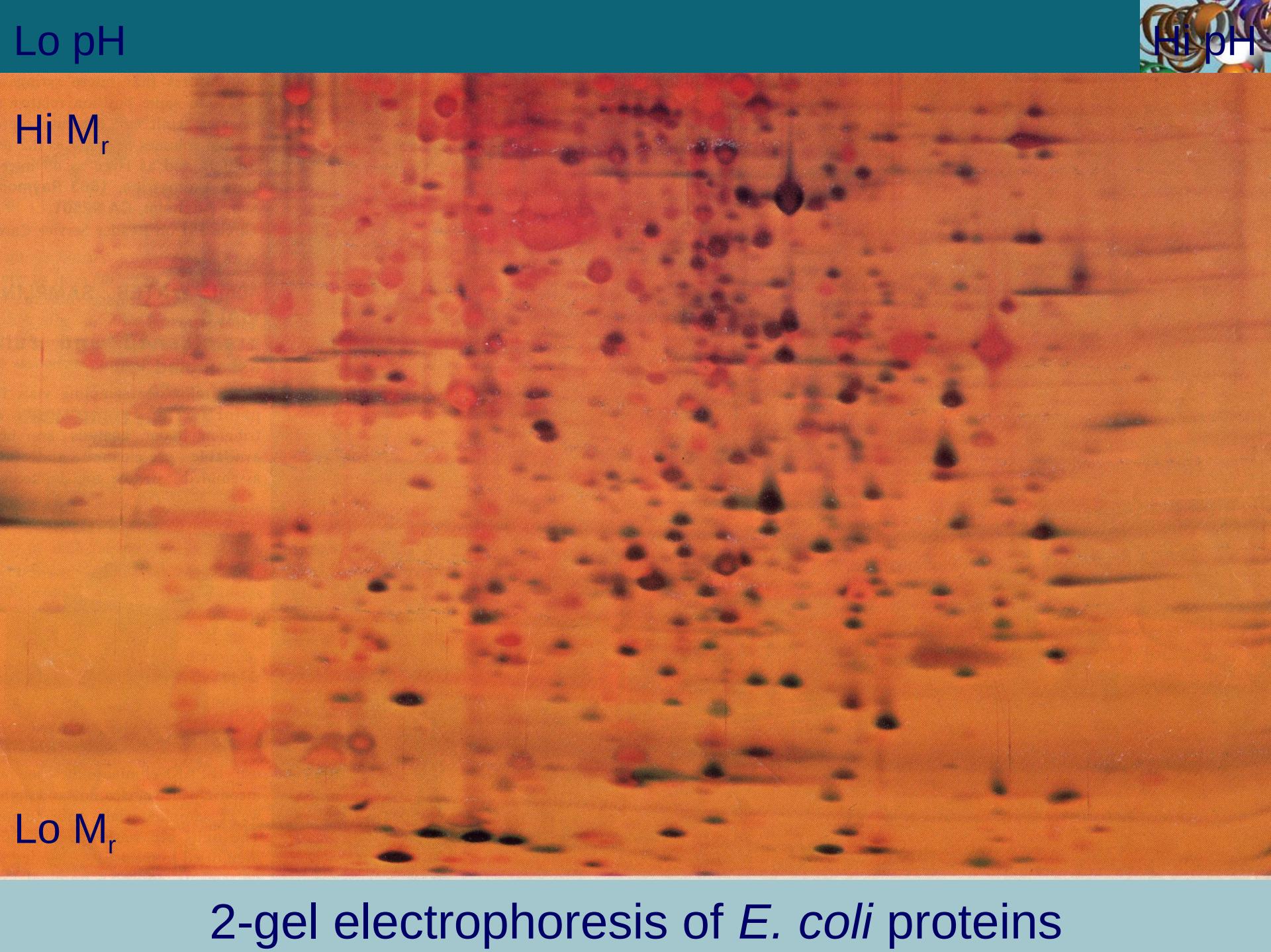
# Two-Dimensional Gel Electrophoresis

Techniques, Figure 7, p. 114

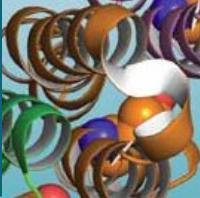
A two-dimensional electrophoresis separation.

Macromolecules are first separated according to charge by isoelectric focusing in a tube gel. The gel containing separated molecules is then placed on top of an SDS-PAGE slab, and the molecules are electrophoresed into the SDS-PAGE gel, where they are separated according to size.



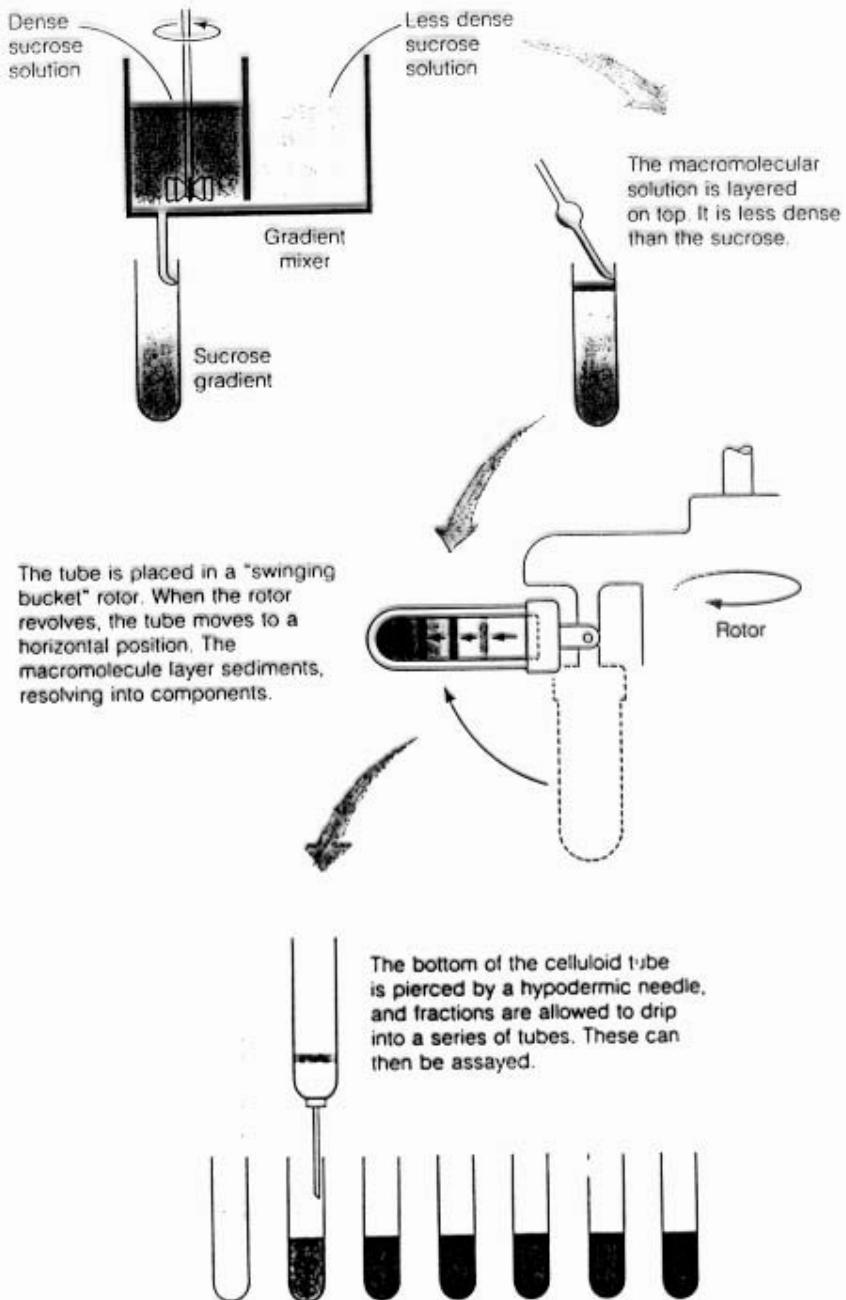


# Centrifugation

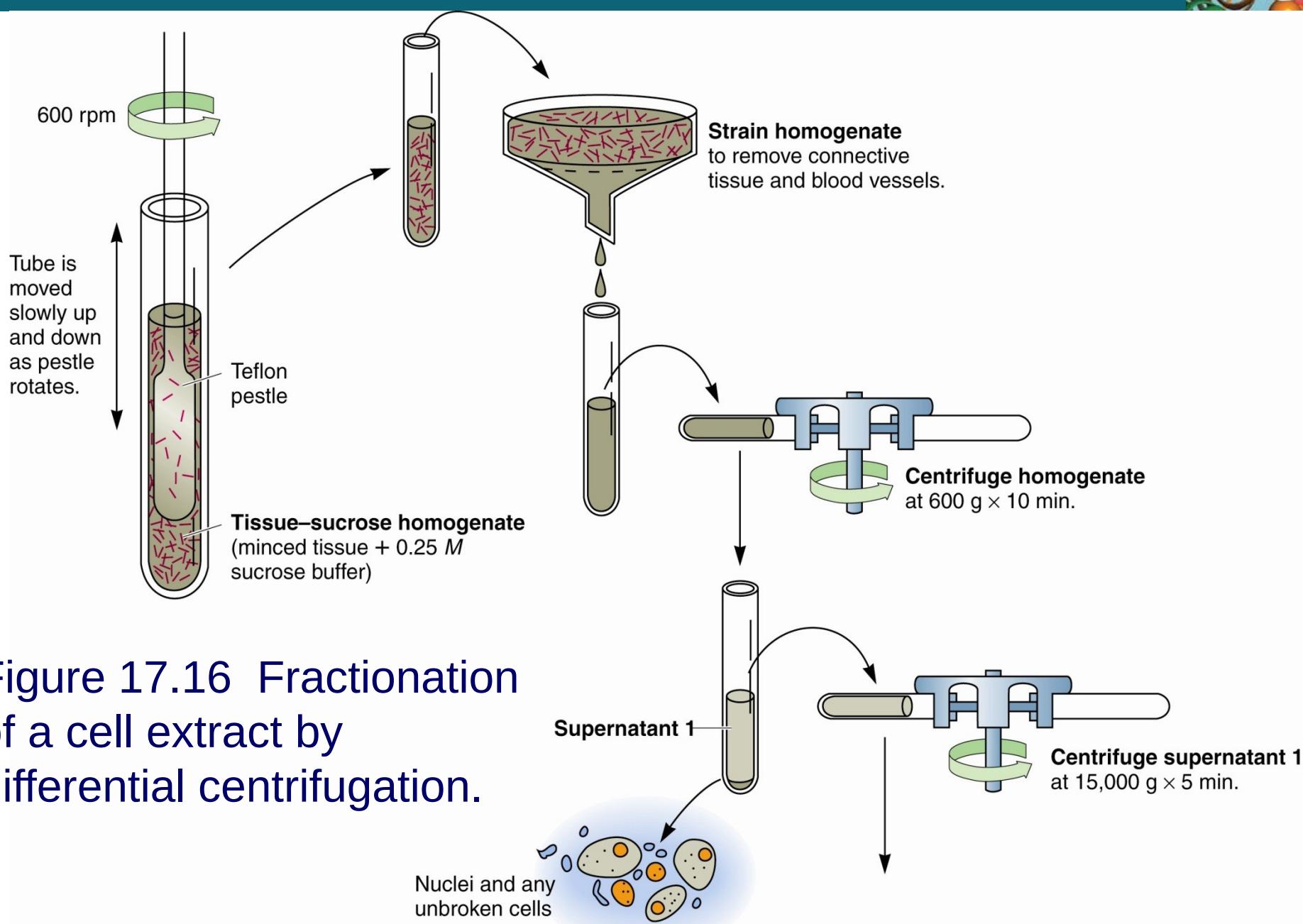


- Non-equilibrium
  - Zonal, or Velocity (can be isokinetic)  
material layered on top of buffered solution of higher density, or on a preformed density gradient made with sucrose or glycerol
  - Differential  
material pelleted from solution spun at different speeds
- Equilibrium (i.e., isopycnic)
  - Molecules or organelles banded at their density in a density gradient, usually formed during centrifugation using CsCl (for DNA) NaCl (for lipoprotein), sucrose (for organelles), Percol (for organelles), etc.

## Zonal Centrifugation



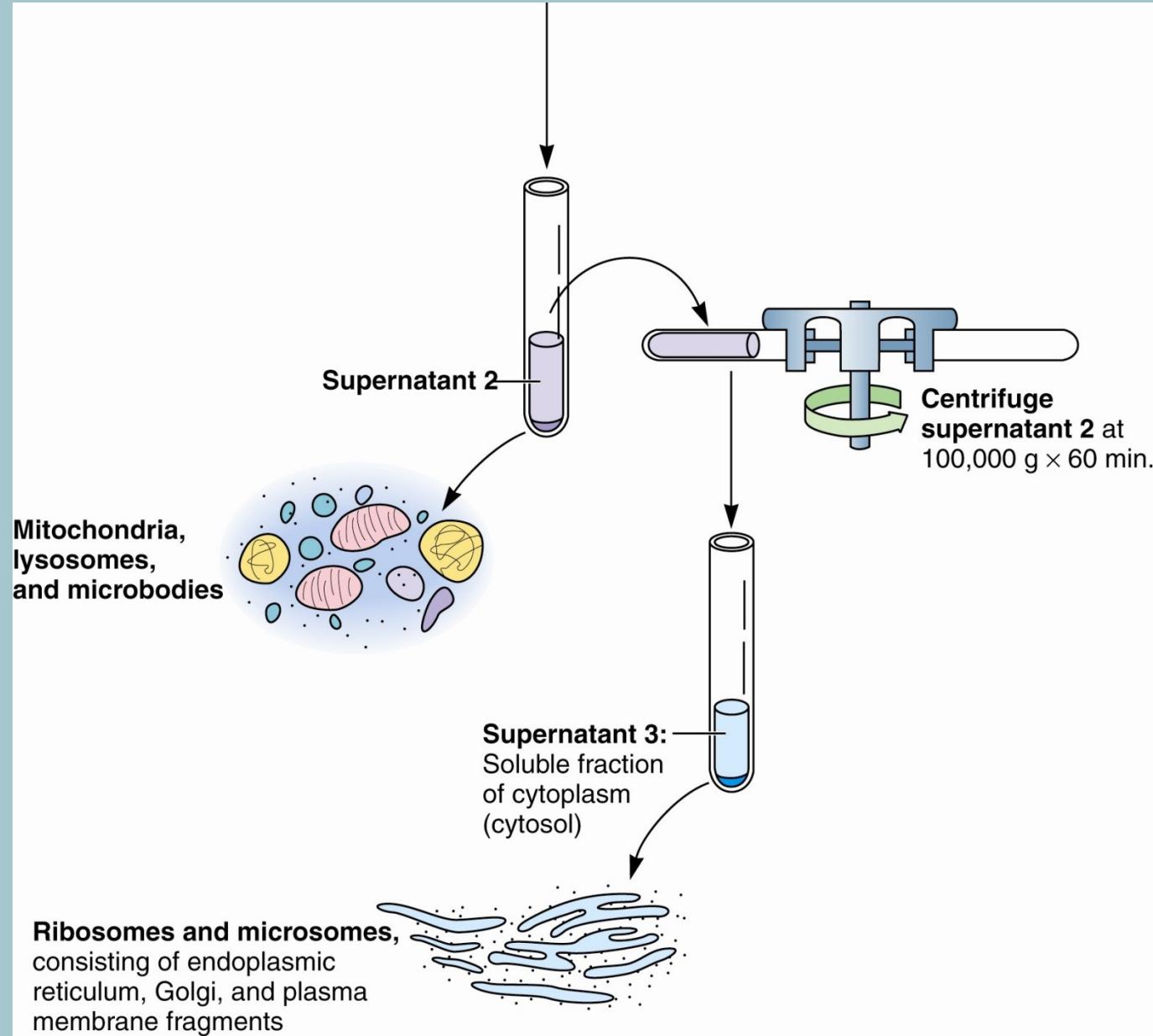
# Metabolic Pathways are Compartmentalized Within Cells

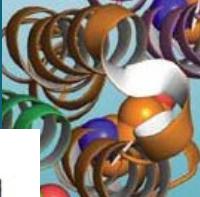


# Metabolic Pathways are Compartmentalized Within Cells



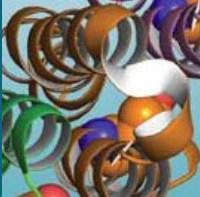
Figure 17.16  
Fractionation of  
a cell extract by  
differential  
centrifugation.





## Sedimentation Conditions for Different Cellular Fractions

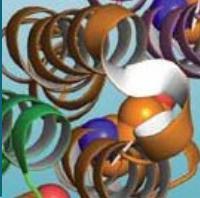
| Fraction Sedimented                        | Centrifugal Force ( $\times g$ ) | Time (min) |
|--|----------------------------------|------------|
| Cells (eukaryotic)                         | 1,000                            | 5          |
| Chloroplasts;<br>cell membranes;<br>nuclei | 4,000                            | 10         |
| Mitochondria;<br>bacteria cells            | 15,000                           | 20         |
| Lysosomes;<br>bacterial membranes          | 30,000                           | 30         |
| Ribosomes                                  | 100,000                          | 180        |



# Chromatography

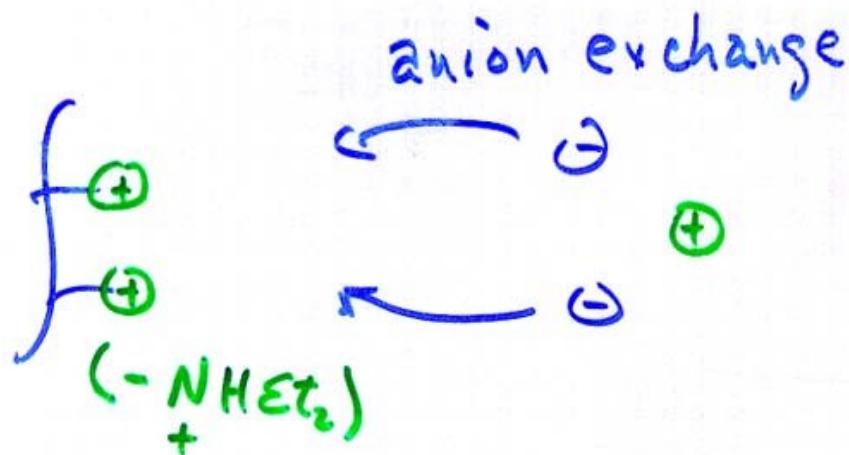
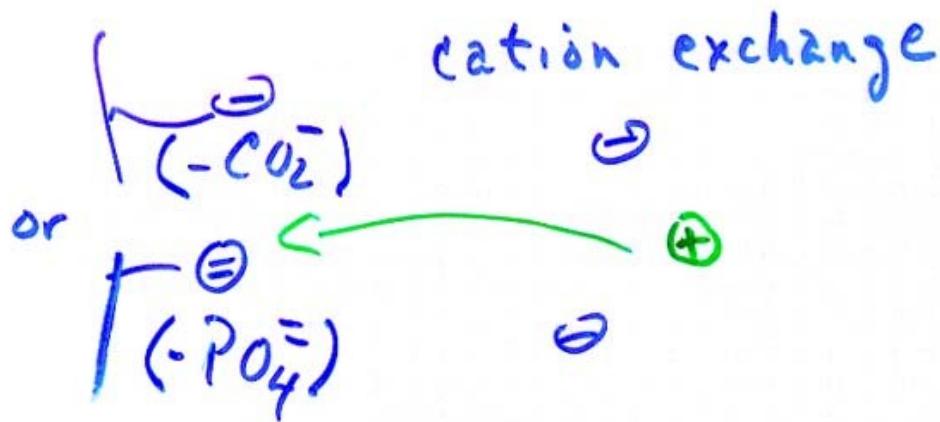
- Ion exchange
  - Anion exchanger has + charged groups
  - Cation exchanger has – charged groups  
load in low [salt]; use gradient of increasing [salt] to elute
- Hydrophobic (i.e., Reverse phase)
  - Resin is hydrophobic  
load in polar solution; elute with nonpolar solution
  - Hydrophobic groups covalently-linked to polar resin  
load in high [salt]; use gradient of decreasing [salt] to elute
- Affinity
  - Resin carries ligand that binds target protein  
elute with solution that disrupts ligand-protein interaction

# Chromatography



- Gel filtration
  - Resin is insoluble porous beads  
layer protein solution on top of resin column  
elute by flowing buffer through the column
  - Plot  $\log M_r$  v. elution volume to determine apparent molecular weight

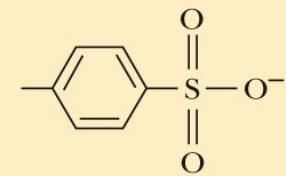
## Ion Exchange Chromatography



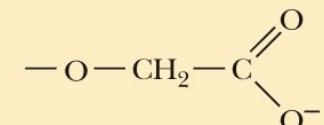
**Techniques, Figure 2,  
p. 112**  
**Cation (a) and anion  
(b) exchange resins  
used commonly for  
biochemical  
separations.**

**(a) Cation Exchange Media**

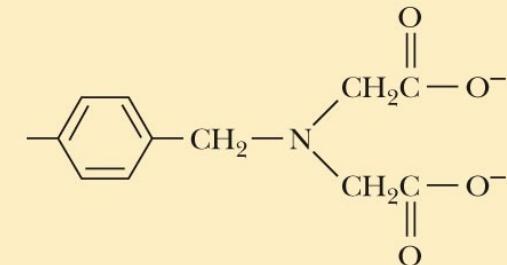
Strongly acidic, polystyrene resin (Dowex-50)



Weakly acidic, carboxymethyl (CM) cellulose



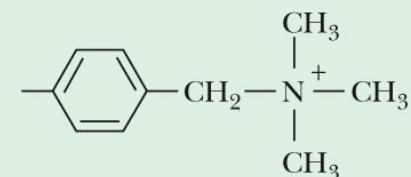
Weakly acidic, chelating, polystyrene resin  
(Chelex-100)



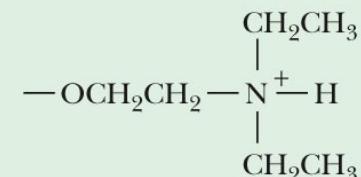
**(b) Anion Exchange Media**

**Structure**

Strongly basic, polystyrene resin (Dowex-1)

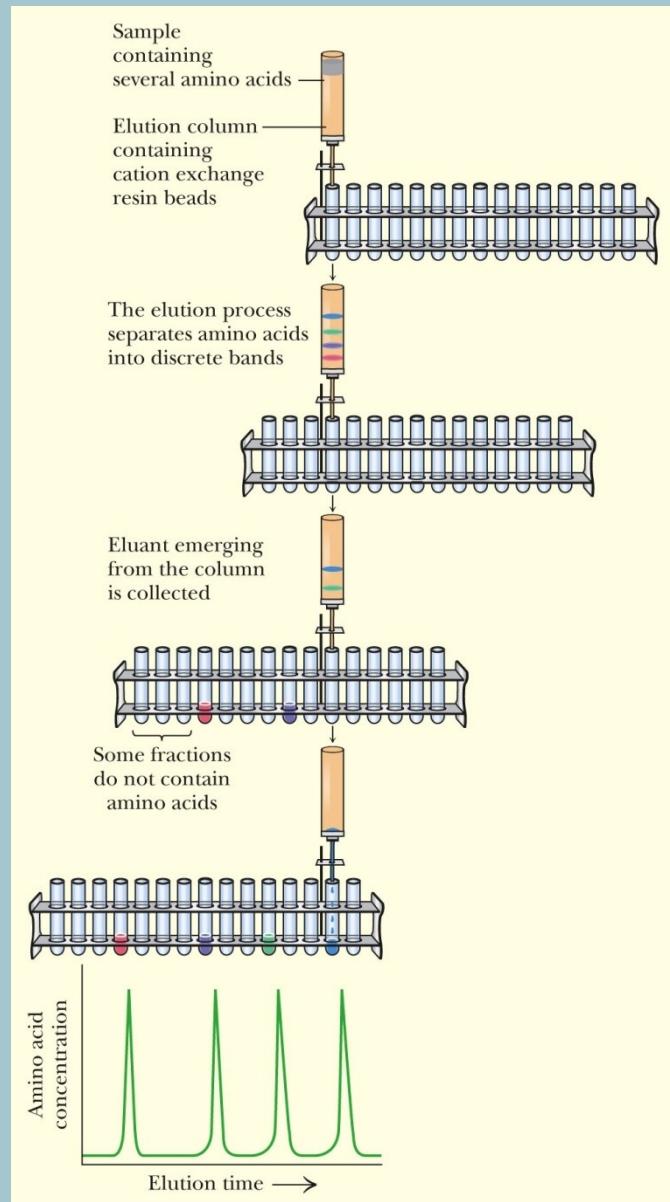


Weakly basic, diethylaminoethyl (DEAE)  
cellulose

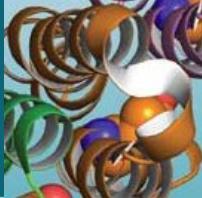


# Ion Exchange Chromatography Can Be Used to Separate Molecules on the Basis of Charge

Techniques, Figure 3, p.113  
The separation of amino acids (shown as colored bands) on a cation exchange column.

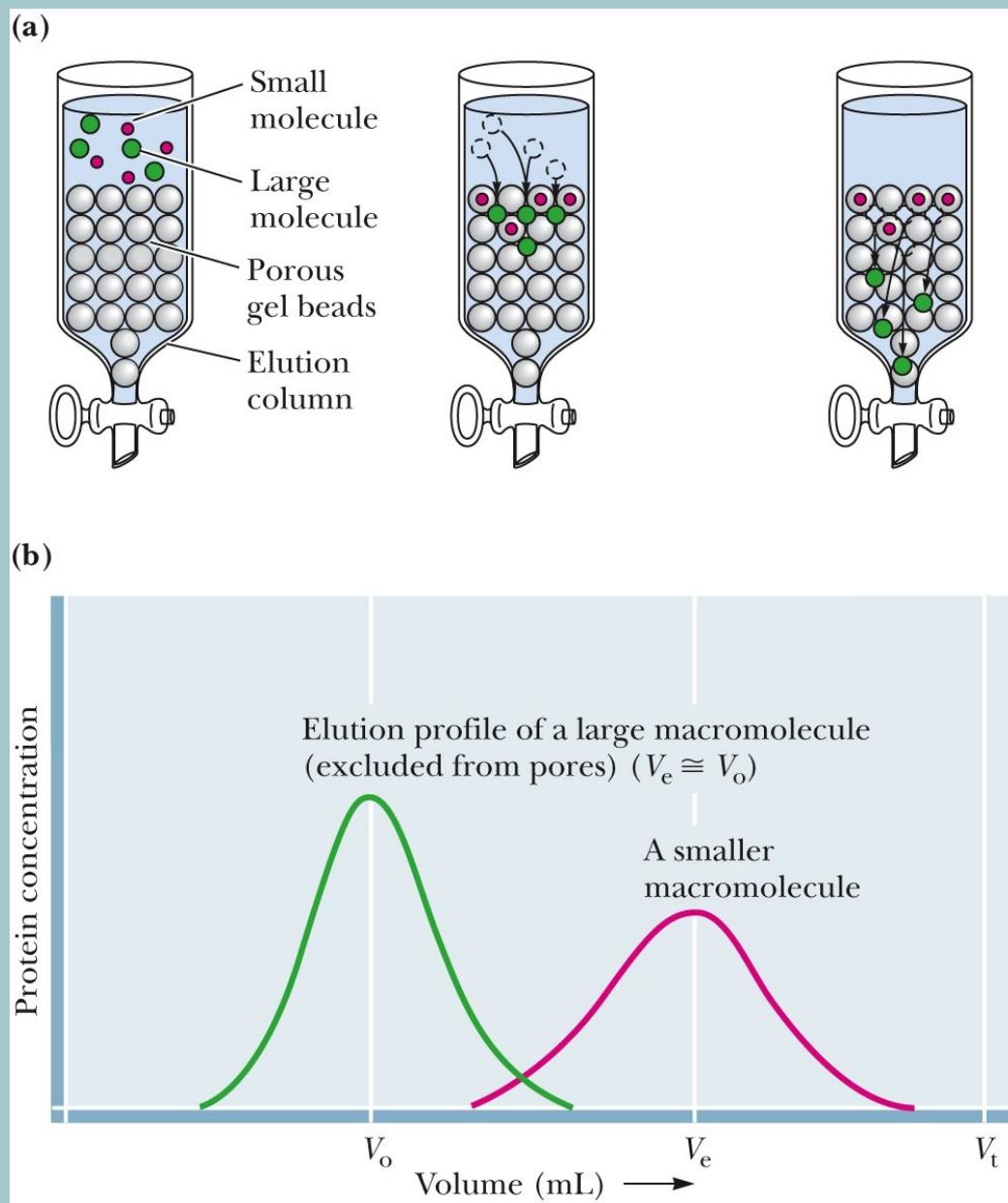


# Gel Filtration Chromatography Can Be Used to Separate Molecules on the Basis of Size



Techniques, Figure 4 (a),  
p. 113

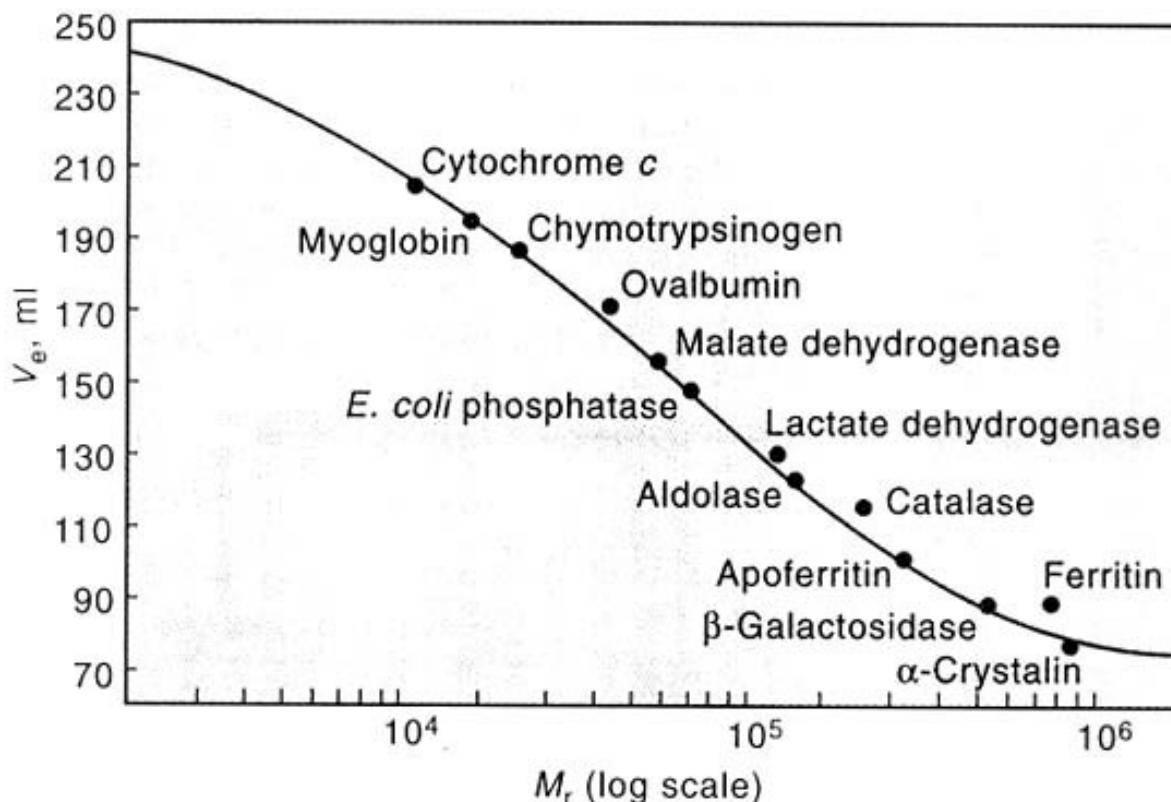
A gel filtration chromatography column. Larger molecules are excluded from the gel beads and emerge from the column sooner than smaller molecules, whose migration is retarded because they can enter the beads.  
(b) An elution profile.



## Determination of $M_r$ by Gel Filtration



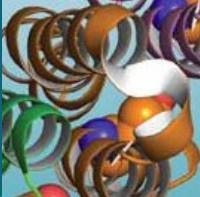
Plot of elution volume ( $V_e$ ) versus the logarithm of the protein molecular weight. A cross-linked dextran (Sephadex G-2000 at pH 7.5) was used. The farther apart two proteins are on this plot, the easier it is to separate them by gel-exclusion chromatography. (Source: Adapted from P. Andrews, *Biochemical J.* 96:595, 1965.)



# Other Techniques for Macromolecules



- Dialysis and Ultrafiltration
  - Use semipermeable membrane or filter with defined pore size
- Fractionation by selective precipitation or solubilization
  - Salting in  
solubilization of protein at mM [salt]
  - Salting out  
ppt. of proteins at M [salt], usually ammonium sulfate
- Selective precipitation by heat denaturation
  - Remove denatured proteins by centrifugation, leaving active proteins in solution



### Dialysis

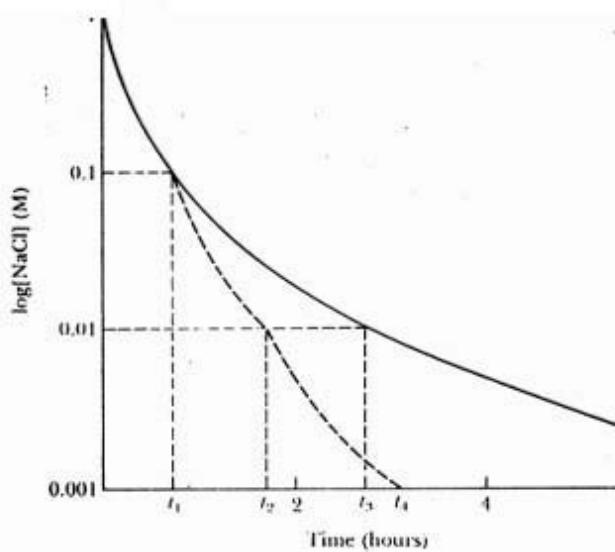
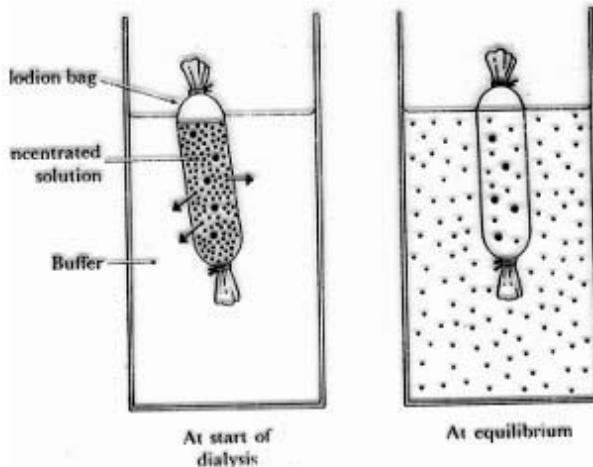
in cellulose (or collodion) bag

Exclusion limits vary

from 6000 to 50,000 Daltons

with 12,000 Daltons used

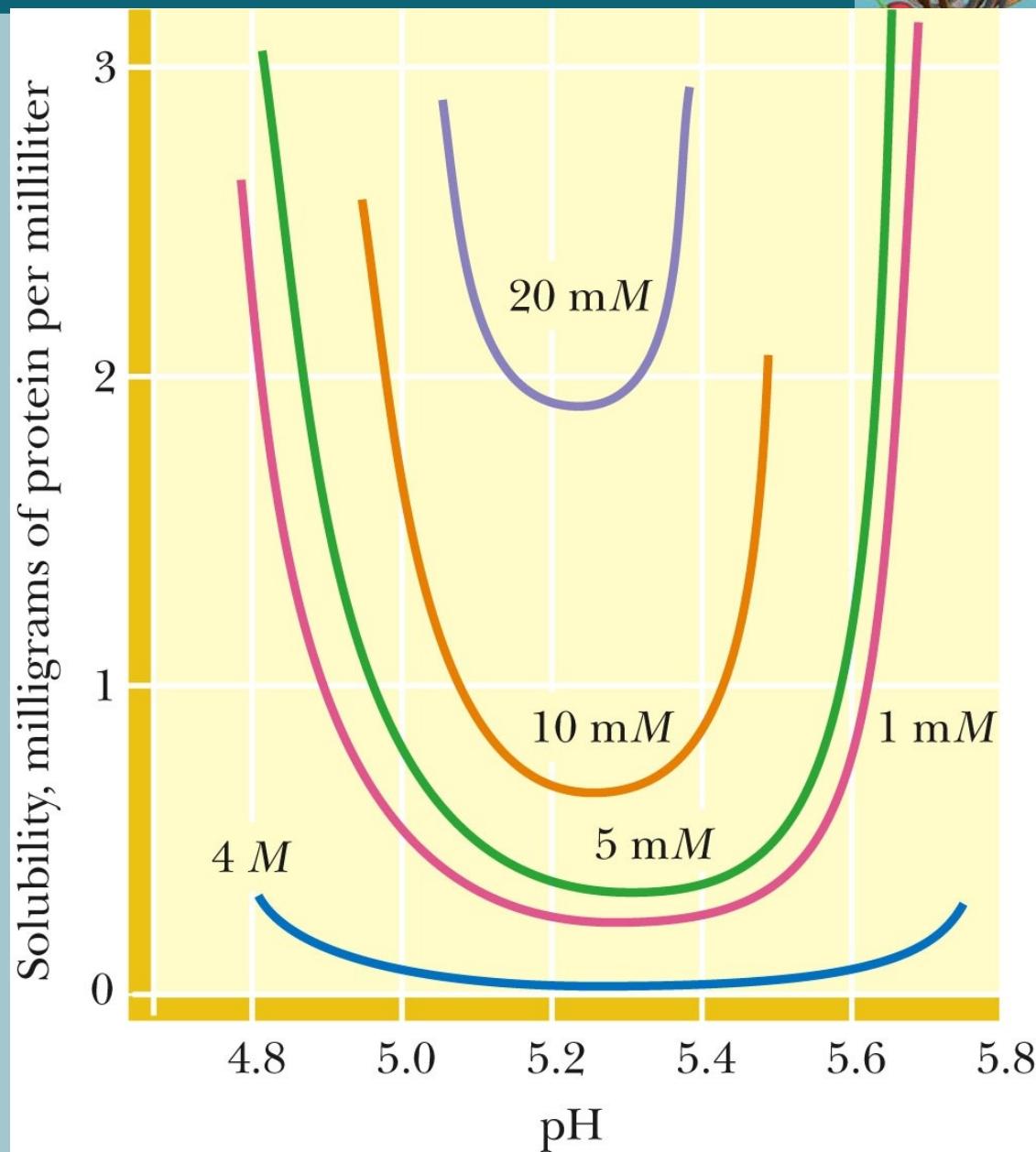
most frequently



## 5.2 How Are Proteins Isolated and Purified from Cells?

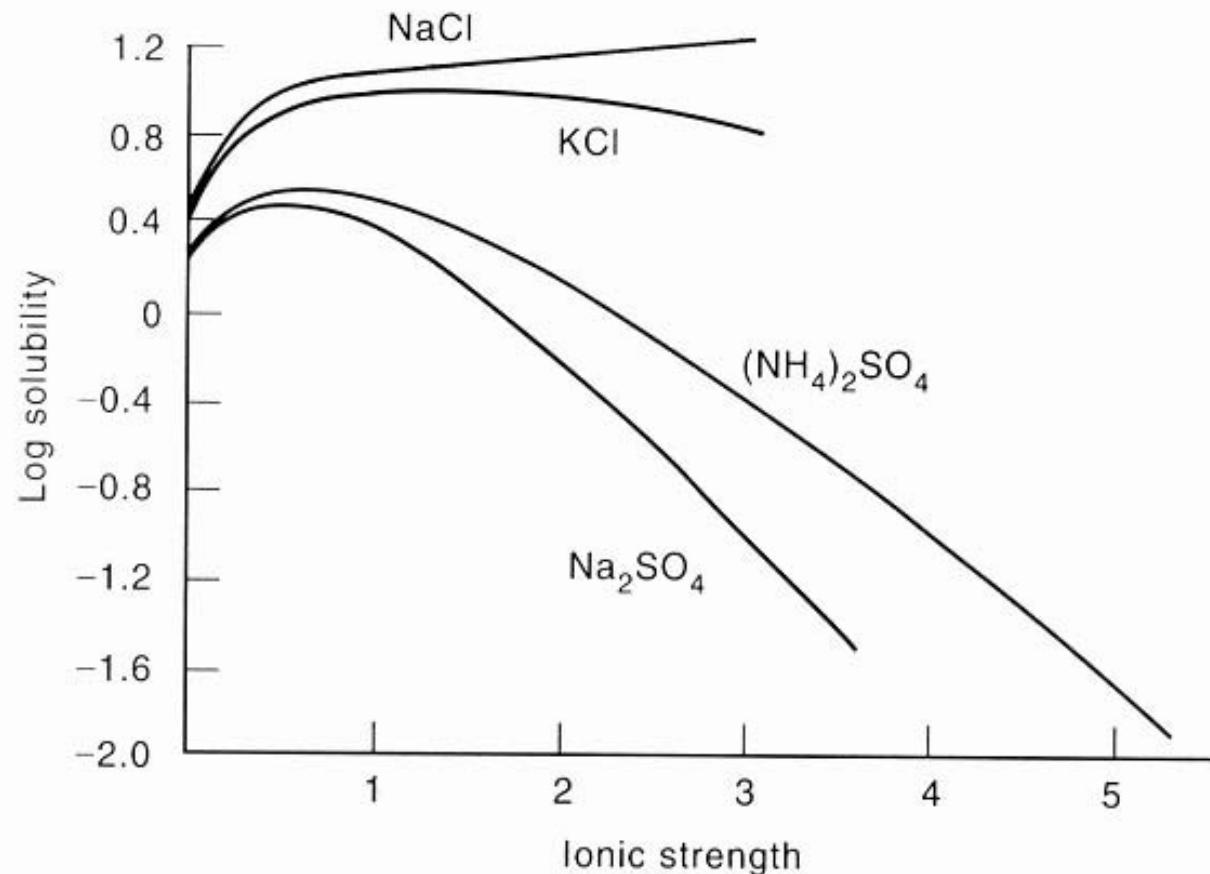


Figure 5.7 The solubility of most globular proteins is markedly influenced by pH and ionic strength. This figure shows the solubility of a typical protein as a function of pH and various salt concentrations.



## Salting-in & out

Solubility of horse carbon monoxide hemoglobin in different salt solutions. The addition of a moderate amount of salt (salting in) is required to solubilize this protein. At high concentrations, certain salts compete more favorably for solvent, decreasing the solubility of the protein and thus leading to its precipitation (salting out). (Source: E. J. Cohn and J. T. Edsall, *Proteins, Amino Acids, and Peptides as Ions and Dipolar Ions*. Copyright ©1942, Reinhold, New York, N.Y.)



## Sec. 5.2 How Are Proteins Isolated and Purified from Cells?



**TABLE 5.1** Example of a Protein Purification Scheme: Purification of an Enzyme from a Cell Extract

| Fraction                          | Volume (mL) | Total Protein (mg) | Total Activity* | Specific Activity† | Percent Recovery‡ |
|-----------------------------------|-------------|--------------------|-----------------|--------------------|-------------------|
| 1. Crude extract                  | 3,800       | 22,800             | 2,460           | 0.108              | 100               |
| 2. Salt precipitate               | 165         | 2,800              | 1,190           | 0.425              | 48                |
| 3. Ion exchange chromatography    | 65          | 100                | 720             | 7.2                | 29                |
| 4. Molecular sieve chromatography | 40          | 14.5               | 555             | 38.3               | 23                |
| 5. Immunoaffinity chromatography§ | 6           | 1.8                | 275             | 152                | 11                |

A typical protein purification scheme uses a series of separation methods. Note the dramatic increase in specific activity\* of the enzyme through a series of five different purification procedures.

\*The term “specific activity” refers to the activity of the enzyme per mg of protein.

# Purification of Lactose Carrier Protein

