CHAPTER 4

[redacted] EXPLORING PROTEINS

One of the major goals of biochemistry is to determine how amino acid sequences specify the conformation of proteins

We can want to know how proteins bind specific substrates and other molecules, mediate catalysis, and transduce energy and information

An indispensable step in these studies is the purification of the protein of interest

<u>Key approaches to analyze and purify proteins:</u>

1. **Electrophoresis**
2. **Chromatography**
3. **Ultracentrifugation**
4. Dialysis
5. Salting-out

<u>Other methods used in protein analysis:</u>

1. **Automated peptide sequencing**
2. **X-ray Crystalography**
3. **Nuclear Magnetic Resonance (NMR) Spectroscopy**
4. **Peptide Synthesis**
5. **Antibody Probing**
6. **Recombinant DNA technology**

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PROTEINS CAN BE SEPARATED BY GEL ELECTROPHORESIS AND DISPLAYED

A molecule with a net charge will move in an electric field. This phenomenon is called electrophoresis

Electrophoresis offers a powerful means of separating proteins and other macromolecules, such as DNA and RNA

Electrophoresis is usually carried out in gels, because

1. **Gels suppress convective currents produced by small temperature gradients, a requirement for effective separation**

2. **Gels serve as molecular sieves that enhance separation**

Polyacrylamide gels are choice supporting media for electrophoresis, because

1. **They are chemically inert and are readily formed by the polymerization of acrylamide**

2. **Their pore sizes can be controlled by choosing various concentrations of acrylamide and methylenebisacrylamide (a cross-linking reagent)**

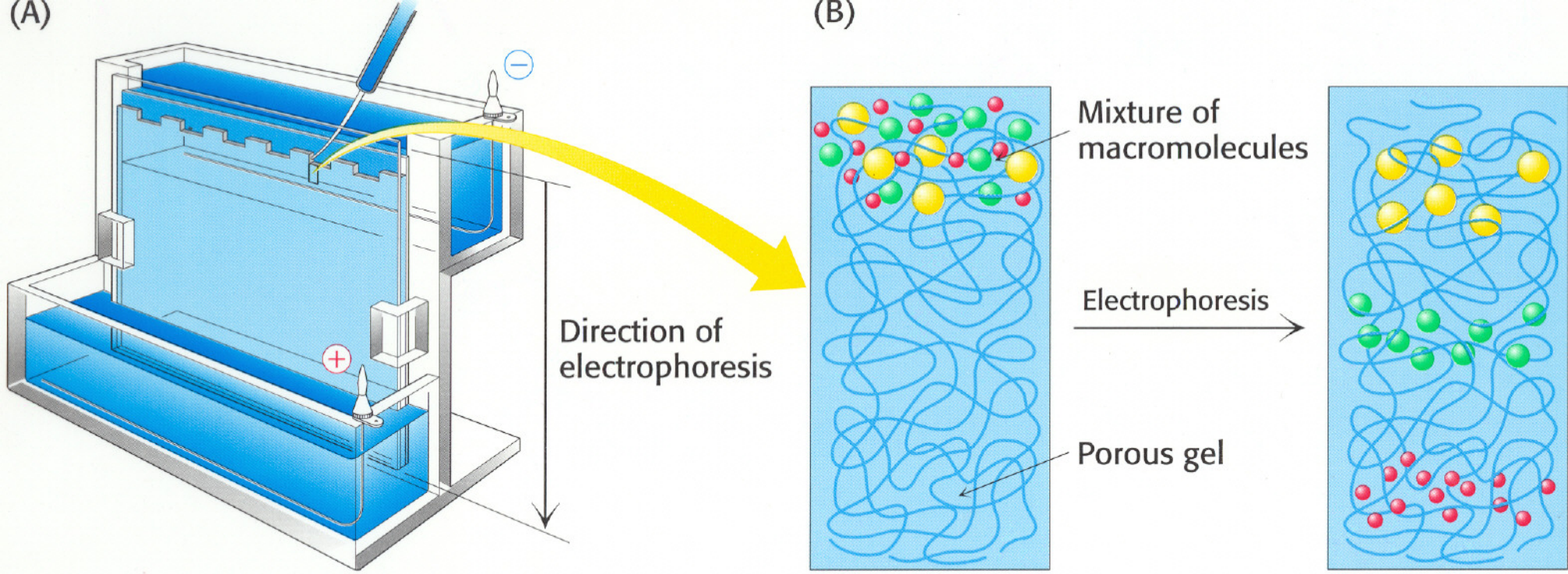
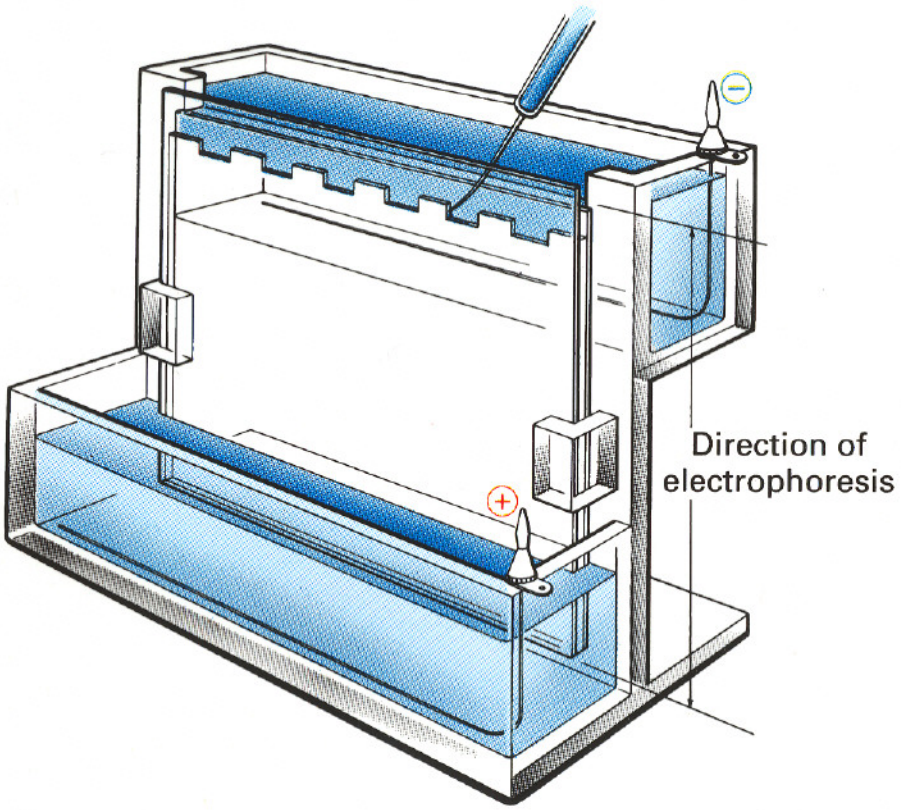
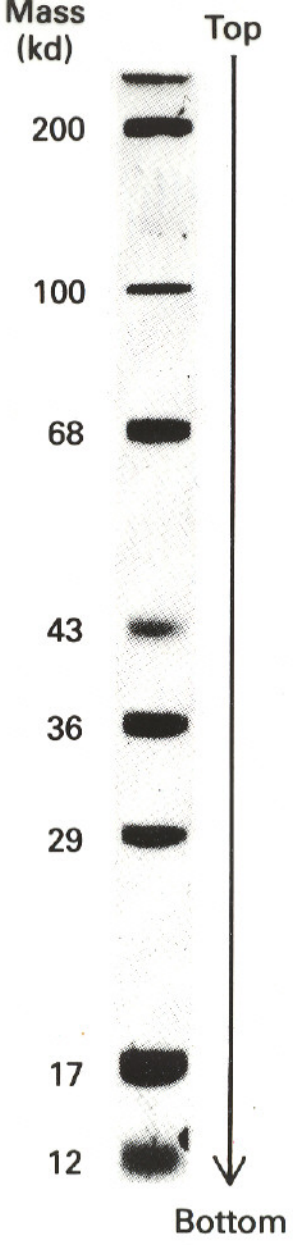


Figure 4-7
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.





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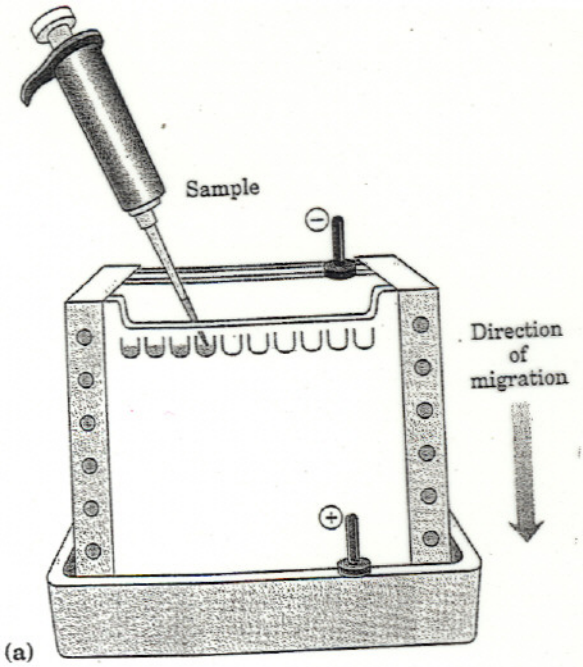


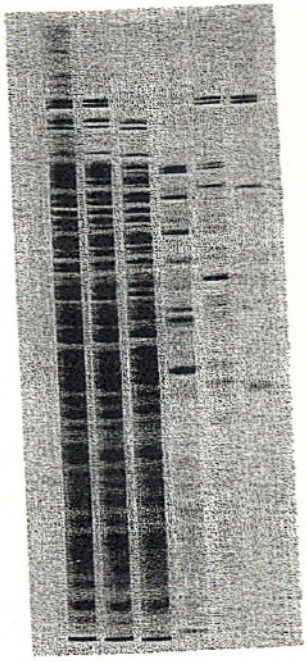
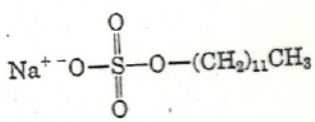


figure 5–19

Electrophoresis. **(a)** Different samples are loaded in wells or depressions at the top of the polyacrylamide gel. The proteins move into the gel when an electric field is applied. The gel minimizes convection currents caused by small temperature gradients, and it minimizes protein movements other than those induced by the electric field. **(b)** Proteins can be visualized after electrophoresis by treating the gel with a stain such as Coomassie blue, which binds to the proteins but not to the gel itself. Each band on the gel represents a different protein (or protein subunit); smaller proteins move through the gel more rapidly than larger proteins and therefore are found nearer the bottom of the gel. This gel illustrates the purification of the enzyme RNA polymerase from the bacterium *E. coli*. The first lane shows the proteins present in the crude cellular extract. Successive lanes (left to right) show the proteins present after each purification step. The purified protein contains four subunits, as seen in the last lane on the right.



Sodium dodecyl sulfate (SDS)

Proteins can be separated largely on the basis of mass by electrophoresis in a polyacrylamide gel under denaturing conditions:

1. Proteins are first dissolved in a solution of sodium dodecyl sulfate (SDS), an anionic detergent that disrupt nearly all nonconvalent interactions in native proteins

2. Mercaptoethanol or dithiothreitol is added to reduce disulfide bonds

3. Finally, the proteins in the gel can be visualized by staining them with silver or a dye such as coomassie blue

Small proteins move rapidly through the gel, whereas large ones stay at the top, near the point of application of the protein mixture

The mobility of most proteins under these conditions is linearly proportional to the logarithm of their mass

Proteins can also be separated electrophoretically according to their isoelectric point (isoelectric focusing)

The isoelectric point (pI) of a protein is the pH at which its net charge is zero

Isoelectric focusing can be combined with SDS-polyacrylamide gel electrophoresis to obtain very high-resolution separations

Proteins are separated in the horizontal direction on the basis of isoelectric point, and in the vertical direction on the basis of mass (2D-gel electrophoresis)

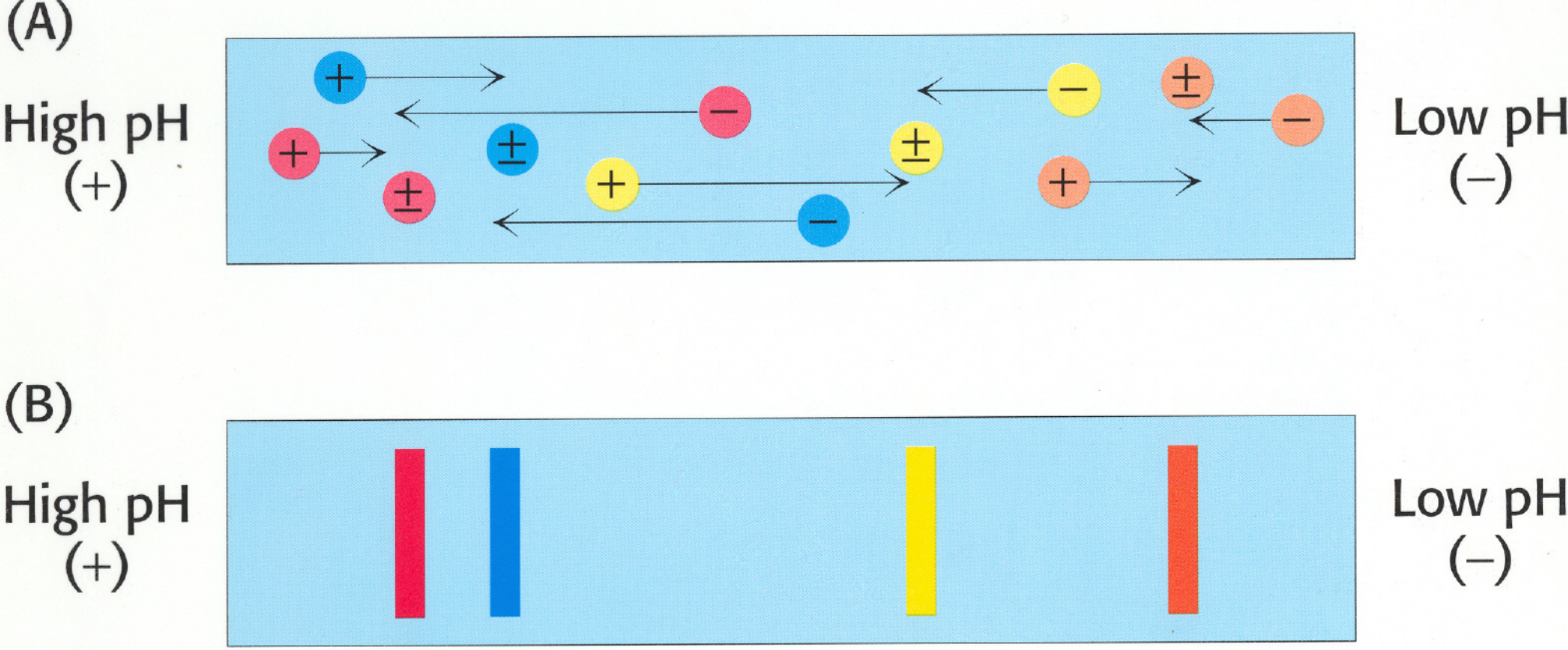


Figure 4-11
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.

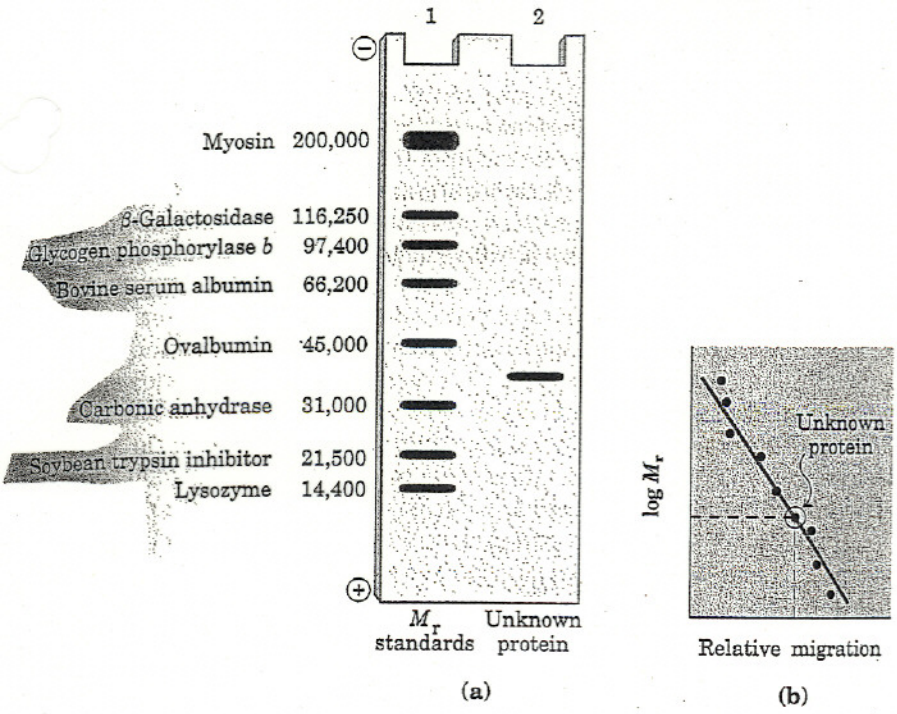


figure 5–20

Estimating the molecular weight of a protein. The electrophoretic mobility of a protein on an SDS polyacrylamide gel is related to its molecular weight, M_r. **(a)** Standard proteins of known molecular weight are subjected to electrophoresis (lane 1). These marker proteins can be used to estimate the molecular weight of an unknown protein (lane 2). **(b)** A plot of log M_r of the marker proteins versus relative migration during electrophoresis is linear, which allows the molecular weight of the unknown protein to be read from the graph.

table 5–6

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

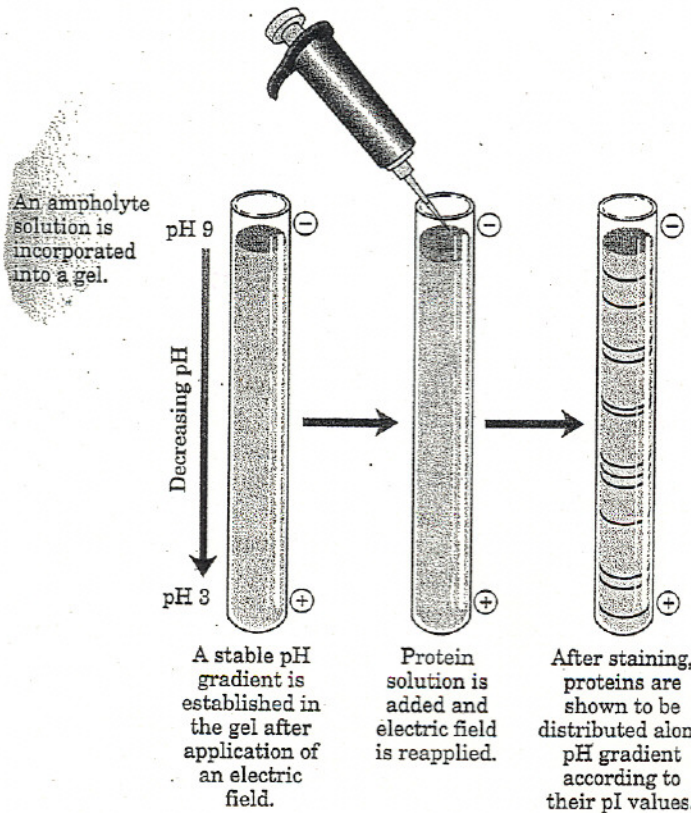
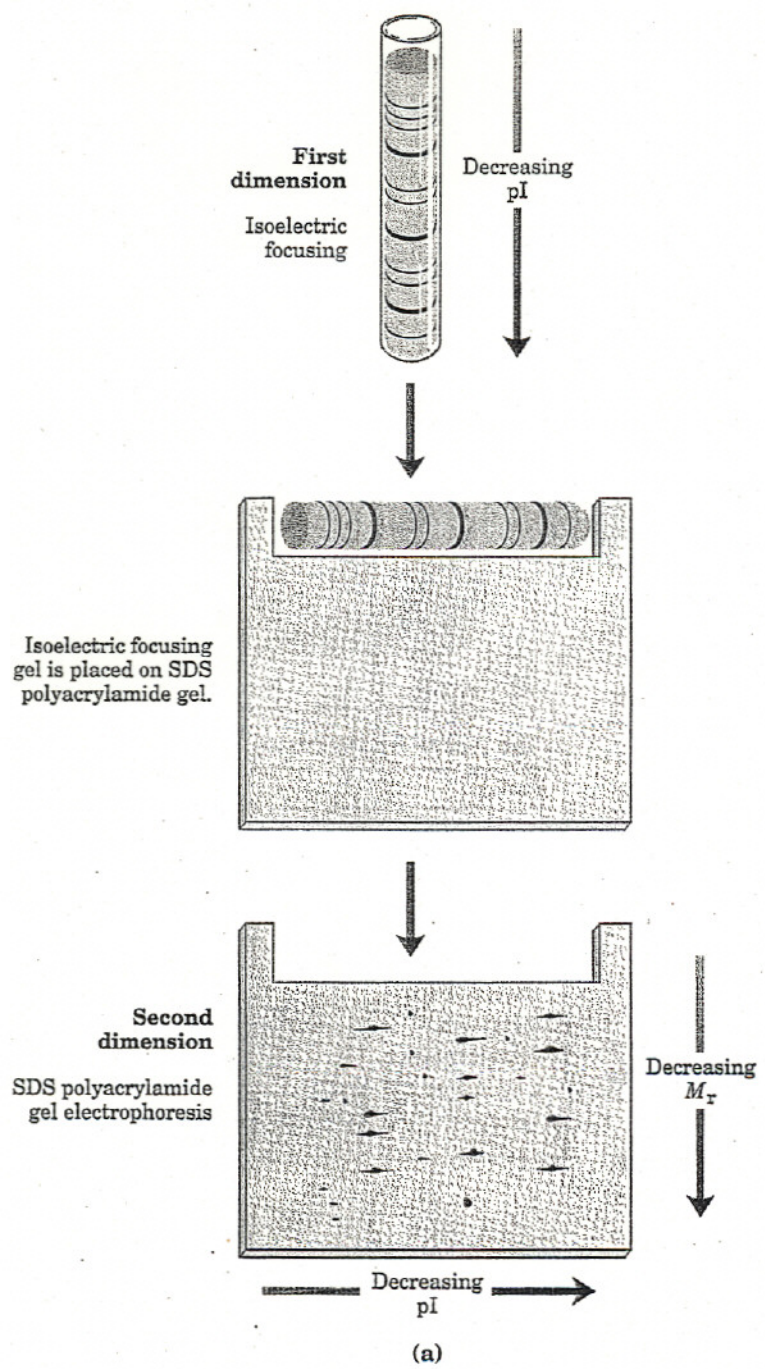
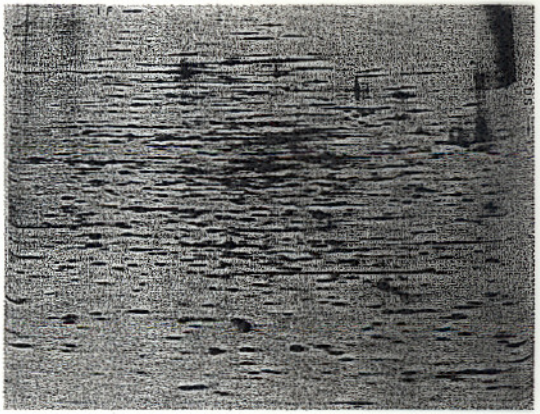


figure 5–21

Isoelectric focusing. This technique separates proteins according to their isoelectric points. A stable pH gradient is established in the gel by the addition of appropriate ampholytes. A protein mixture is placed in a well on the gel. With an applied electric field, proteins enter the gel and migrate until each reaches a pH equivalent to its pI. Remember that when pH = pI, the net charge of a protein is zero.

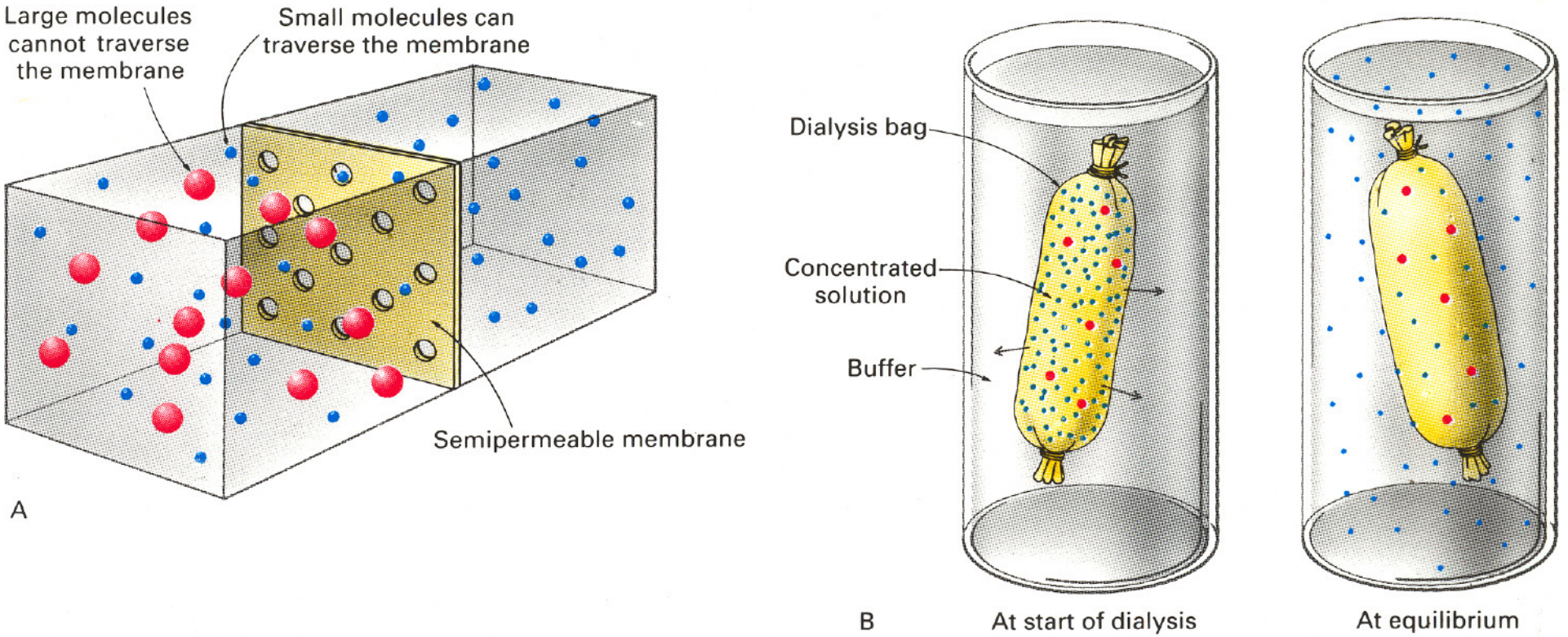




(b)

figure 5–22

Two-dimensional electrophoresis. (a) Proteins are first separated by isoelectric focusing in a cylindrical gel. The gel is then laid horizontally on a second, slab-shaped gel, and the proteins are separated by SDS polyacrylamide gel electrophoresis. Horizontal separation reflects differences in pI; vertical separation reflects differences in molecular weight. **(b)** More than 1,000 different proteins from *E. coli* can be resolved using this technique.



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Set II

PROTEINS CAN BE PURIFIED ACCORDING TO SIZE, SOLUBILITY, CHARGE, AND BINDING AFFINITY

Protein purification is an essential step before a protein can be studied in terms of its three-dimensional structure and mechanism of action

Several thousand proteins have been purified in active form on the basis of such characteristics as size, solubility, charge, and specific binding affinity.

Dialysis: A method used to separate proteins from small molecules.

The dialysis bag is usually made of cellulose semipermeable membrane with pores

Gel-filtration chromatography: A method used to separate proteins on the basis of size. The sample is applied to the top of a column consisting of porous beads made of an insoluble but highly hydrated polymer

- Small molecules can enter these beads, but large ones cannot. Large molecules flow more rapidly through this column emerge first because a small volume is accessible to them

Salting out: A method used to separate or concentrate dilute solutions of proteins based on solubility at high salt concentrations.

- The solubility of most proteins is lowered at high salt concentrations. The dependence of solubility on salt concentrations differs from one protein to another

Ion-

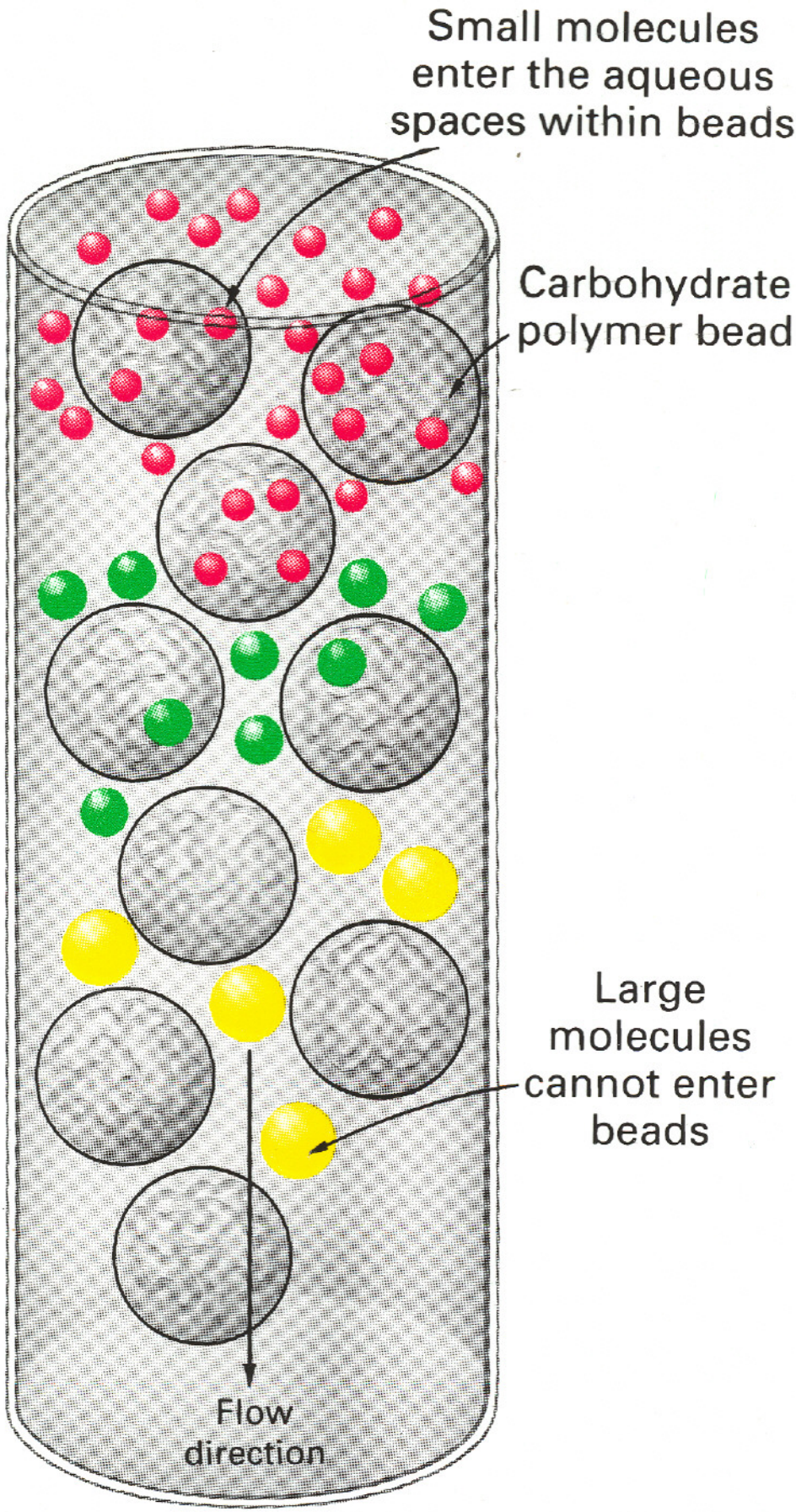
Low-exchange chromatography: It separates proteins on the basis of their net charge.

- If a protein has a net positive charge at pH 7, it will usually bind to a column of beads containing carboxylate groups, whereas a negatively charged protein will not

Negatively charged (anionic) proteins: Use of positively charged diethyl-aminoethyl-cellulose (DEAE-cellulose) columns

Positively charged (cationic) proteins: Use of negatively charged carboxymethyl-cellulose (cm-cellulose) columns

Affinity chromatography: This technique takes advantage of the high affinity of many proteins for specific chemical groups



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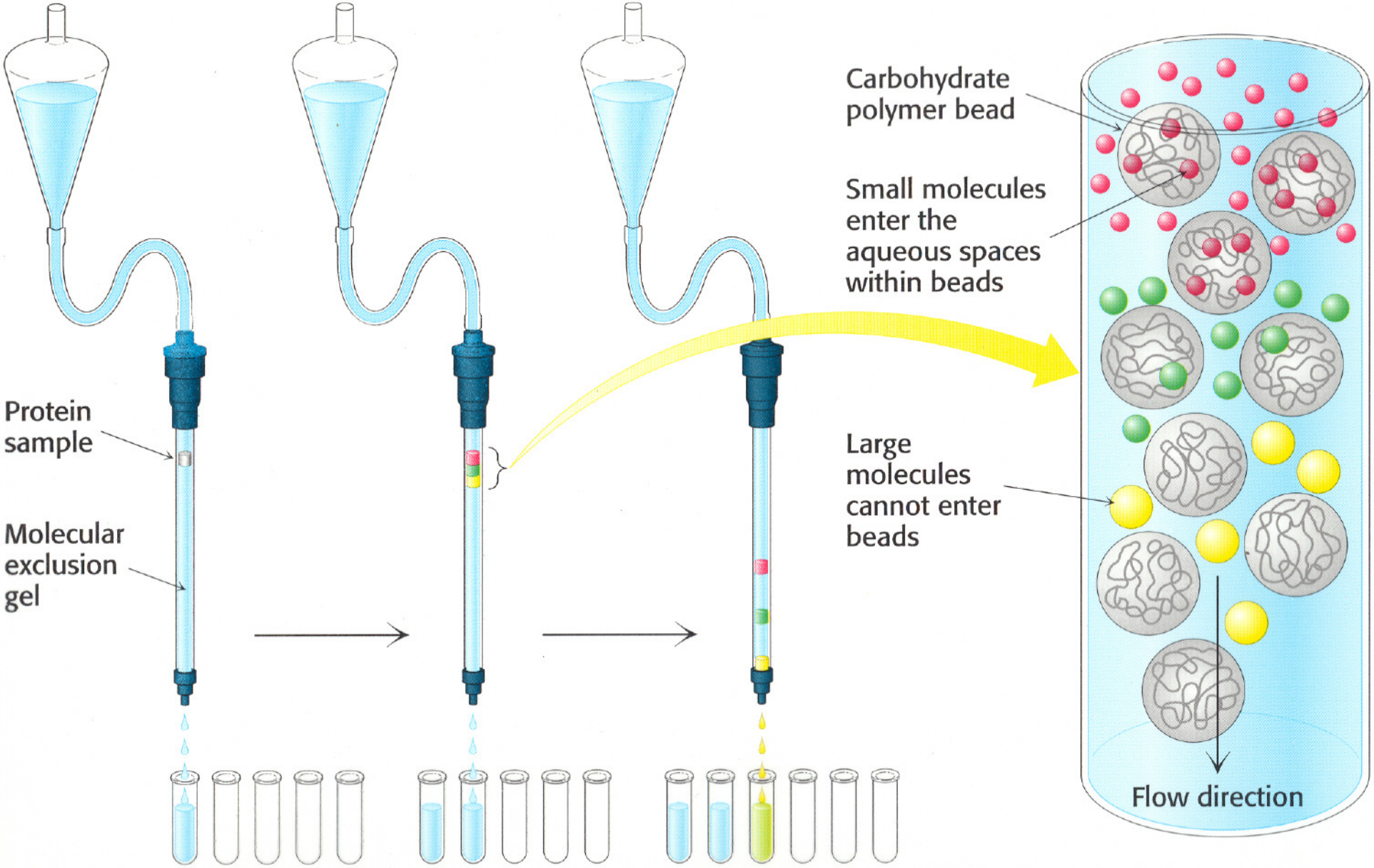
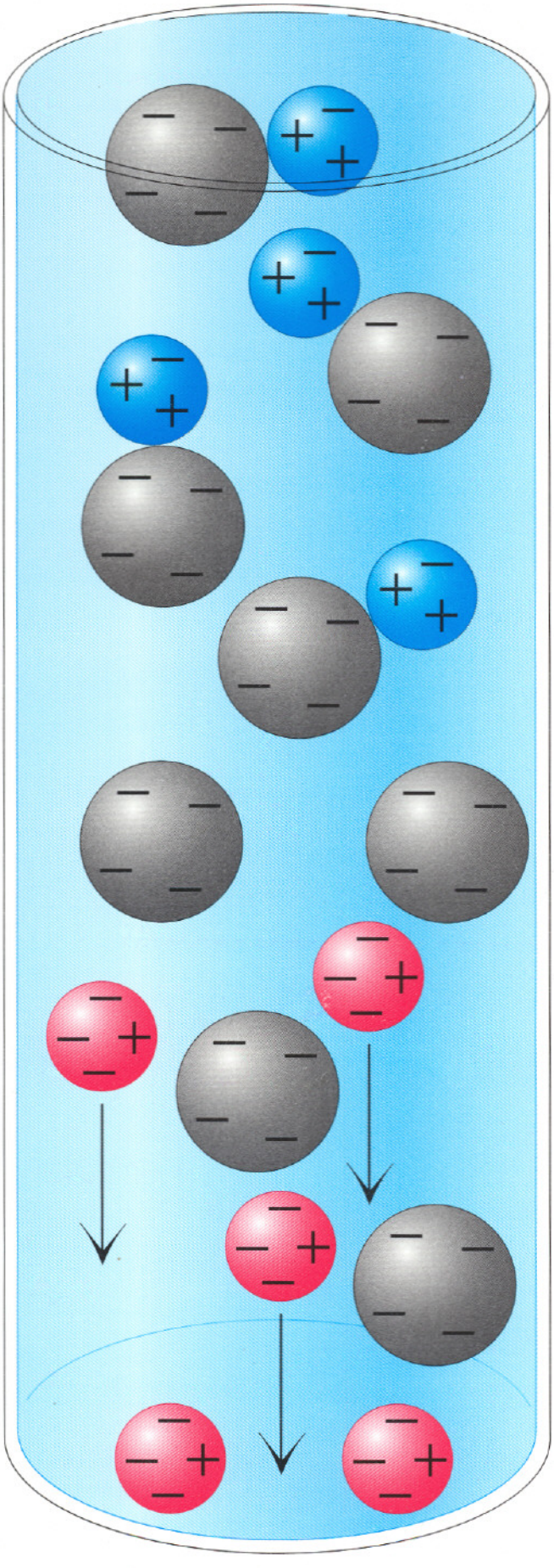


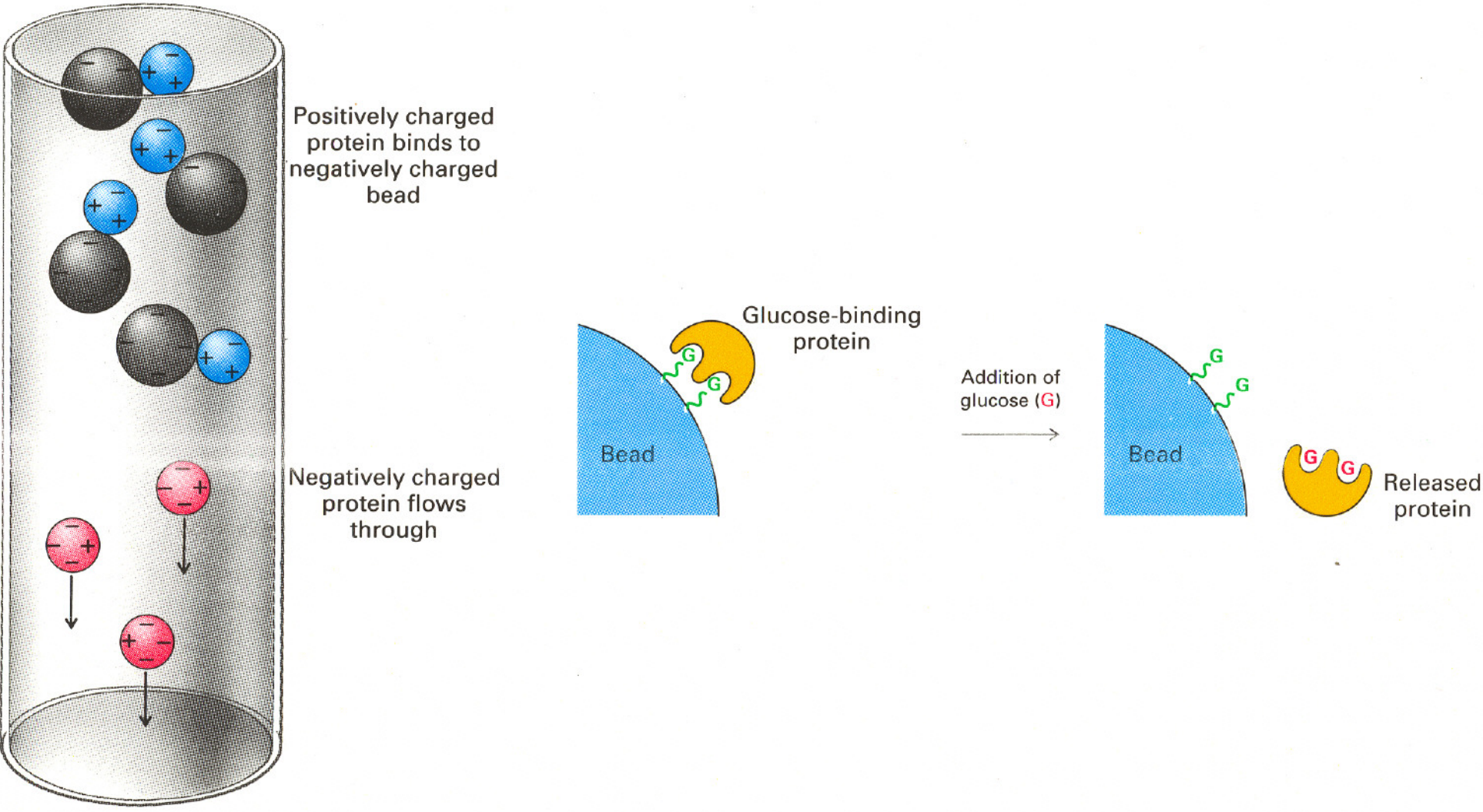
Figure 4-3
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.



Positively charged protein binds to negatively charged bead

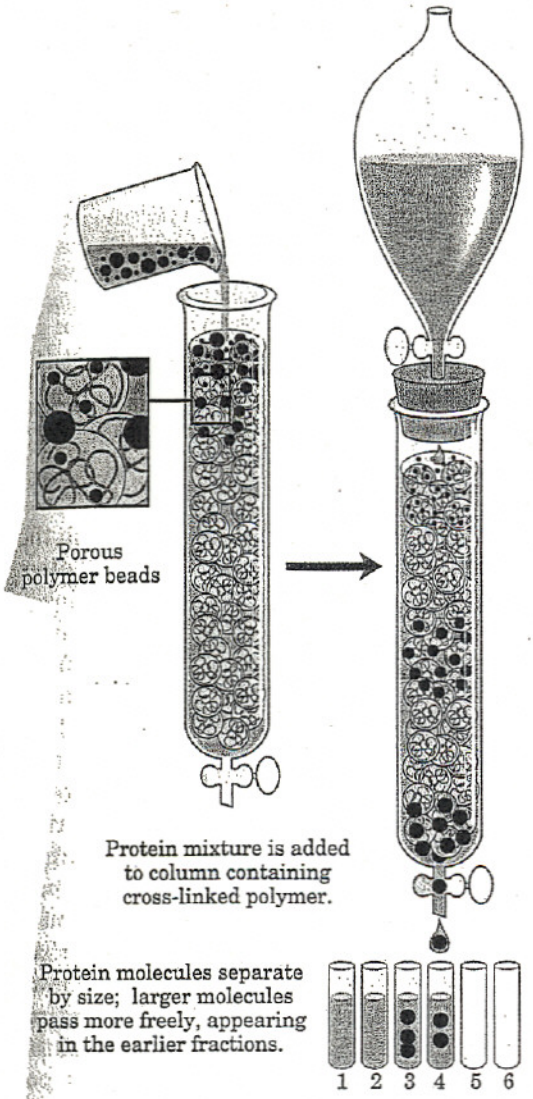
Negatively charged protein flows through

Figure 4-4
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.



Figures 3-9 and 3-10, page 50

Stryer: *Biochemistry,* Fourth Edition



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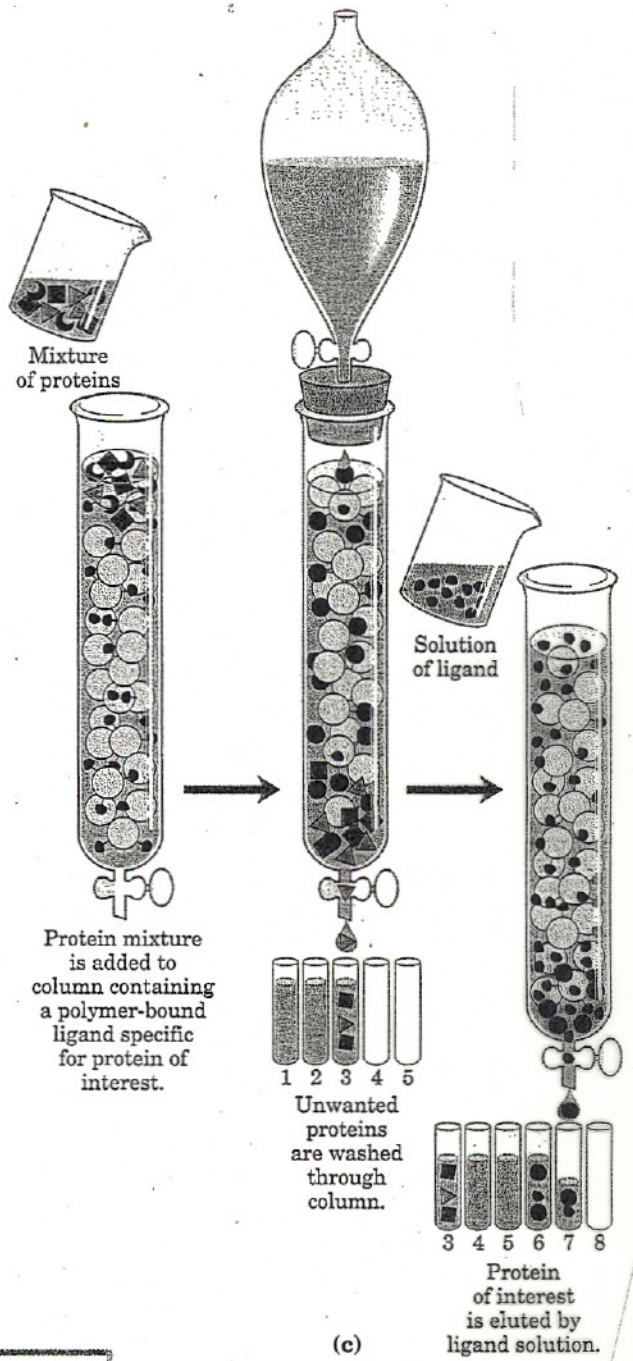

(c)

able 5–5

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

*All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 137.