Working with Proteins

Dialysis

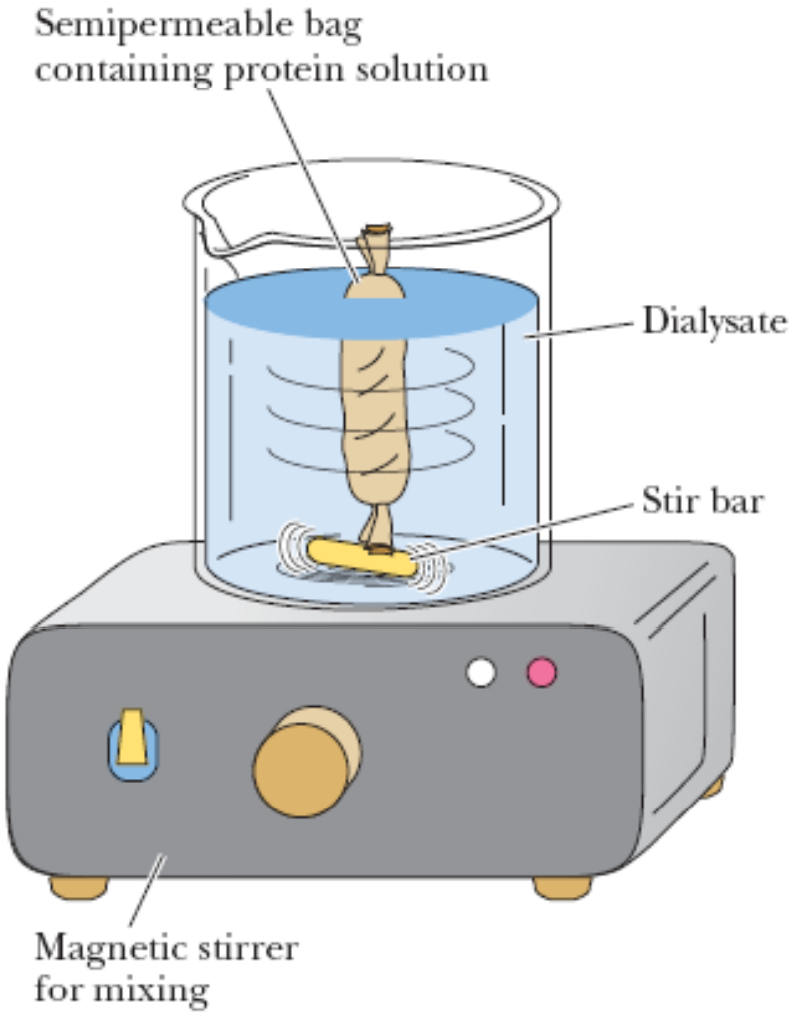
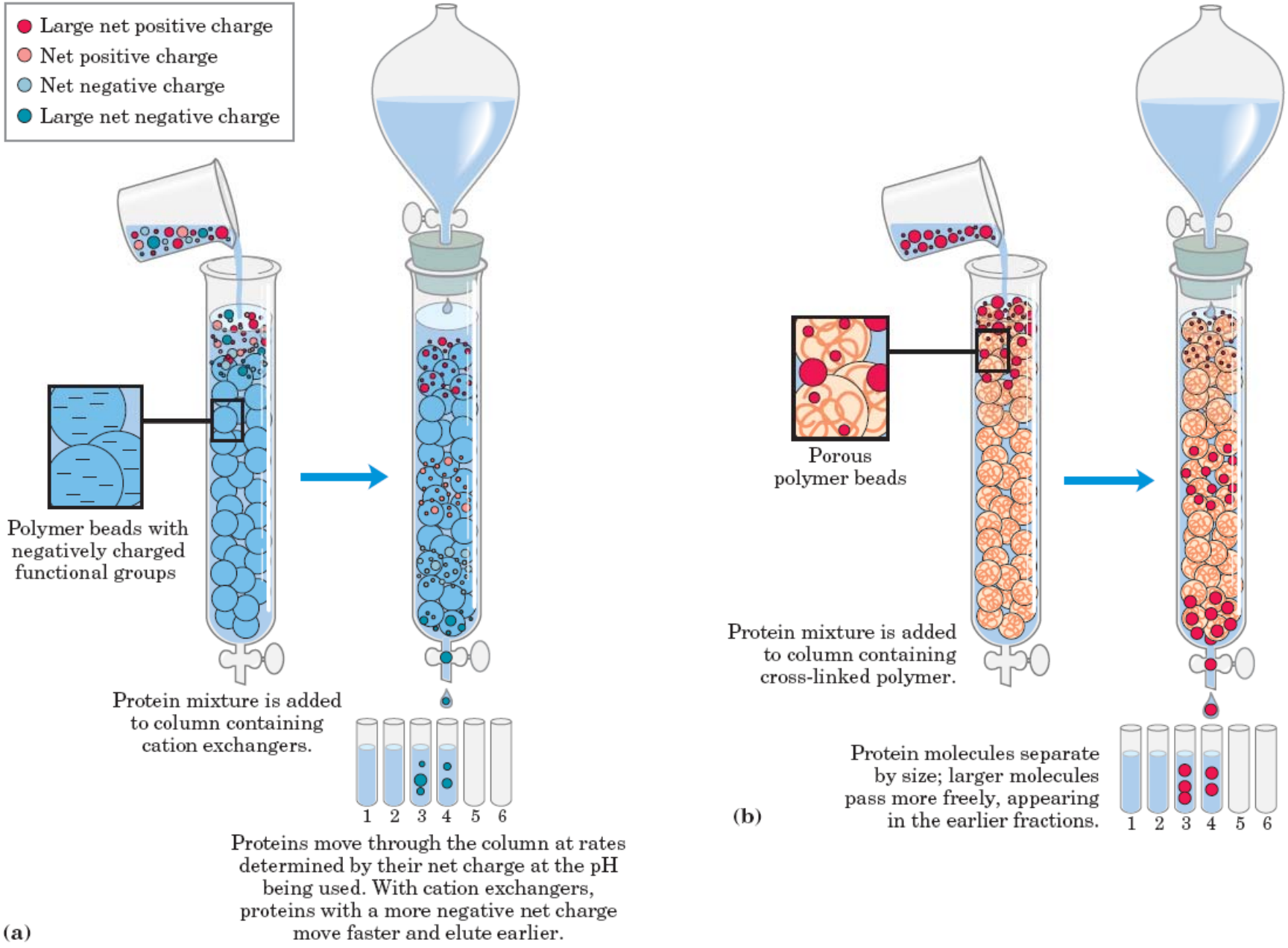
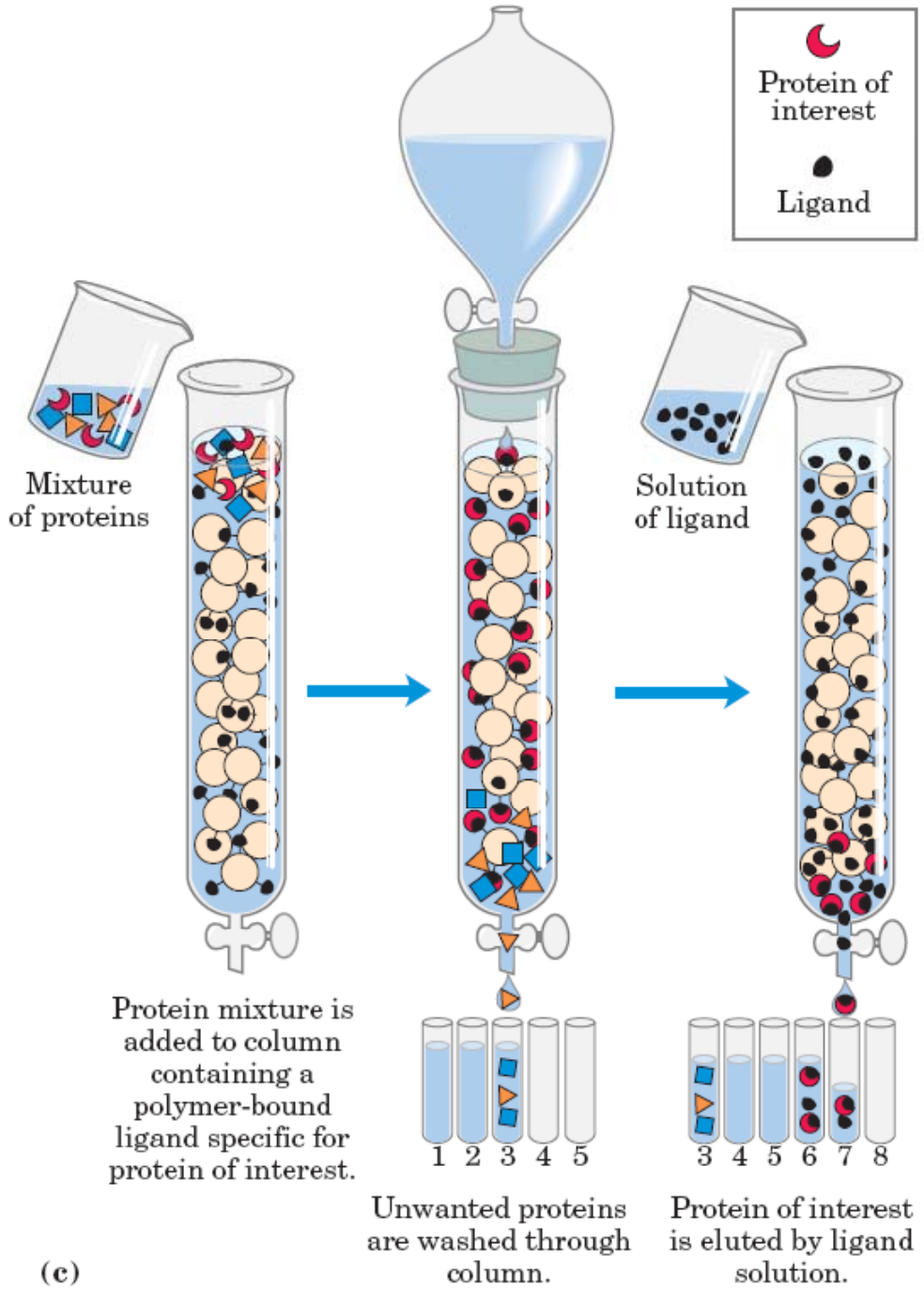


Figure 1 A dialysis experiment. The solution of macromolecules to be dialyzed is placed in a semipermeable membrane bag, and the bag is immersed in a bathing solution. A magnetic stirrer gently mixes the solution to facilitate equilibrium of diffusible solutes between the dialysate and the solution contained in the bag.

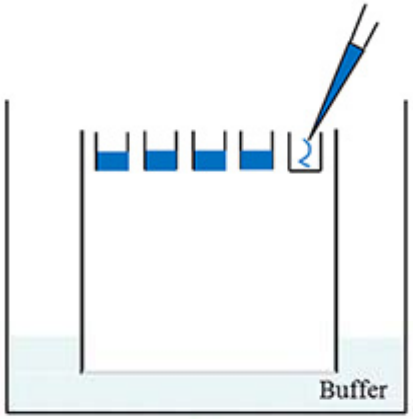
Chromatography



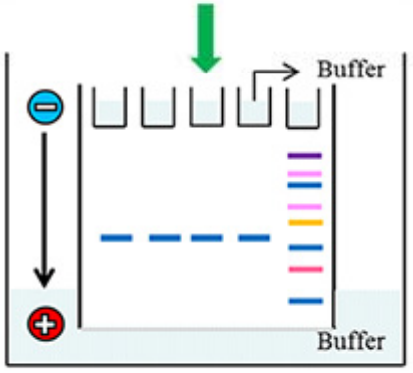

Protein of interest
Ligand
Mixture of proteins
Solution of ligand
Protein mixture is added to column containing a polymer-bound ligand specific for protein of interest.
1 2 3 4 5
Unwanted proteins are washed through column.
3 4 5 6 7 8
Protein of interest is eluted by ligand solution.

(c)

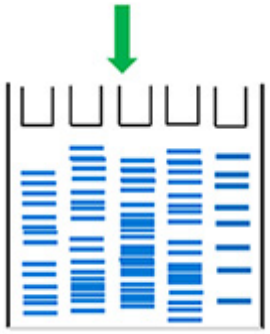
Electrophoresis



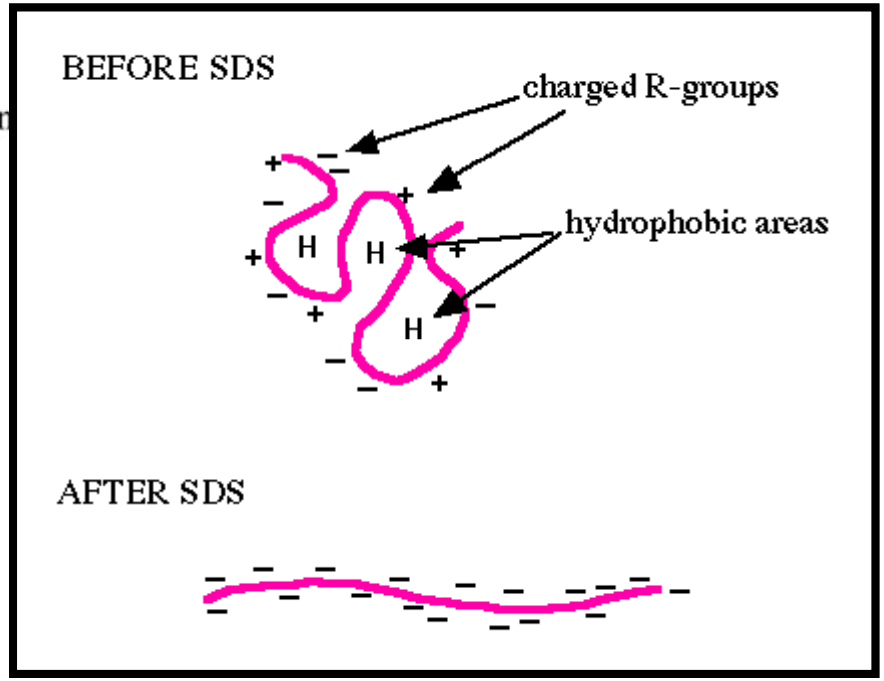
Protein samples and marker loaded in vertical SDS-PAGE syste



Direction of migration of samples in vertical SDS-PAGE system



SDS-PAGE gel after Coomassie blue staining



Electrophoresis

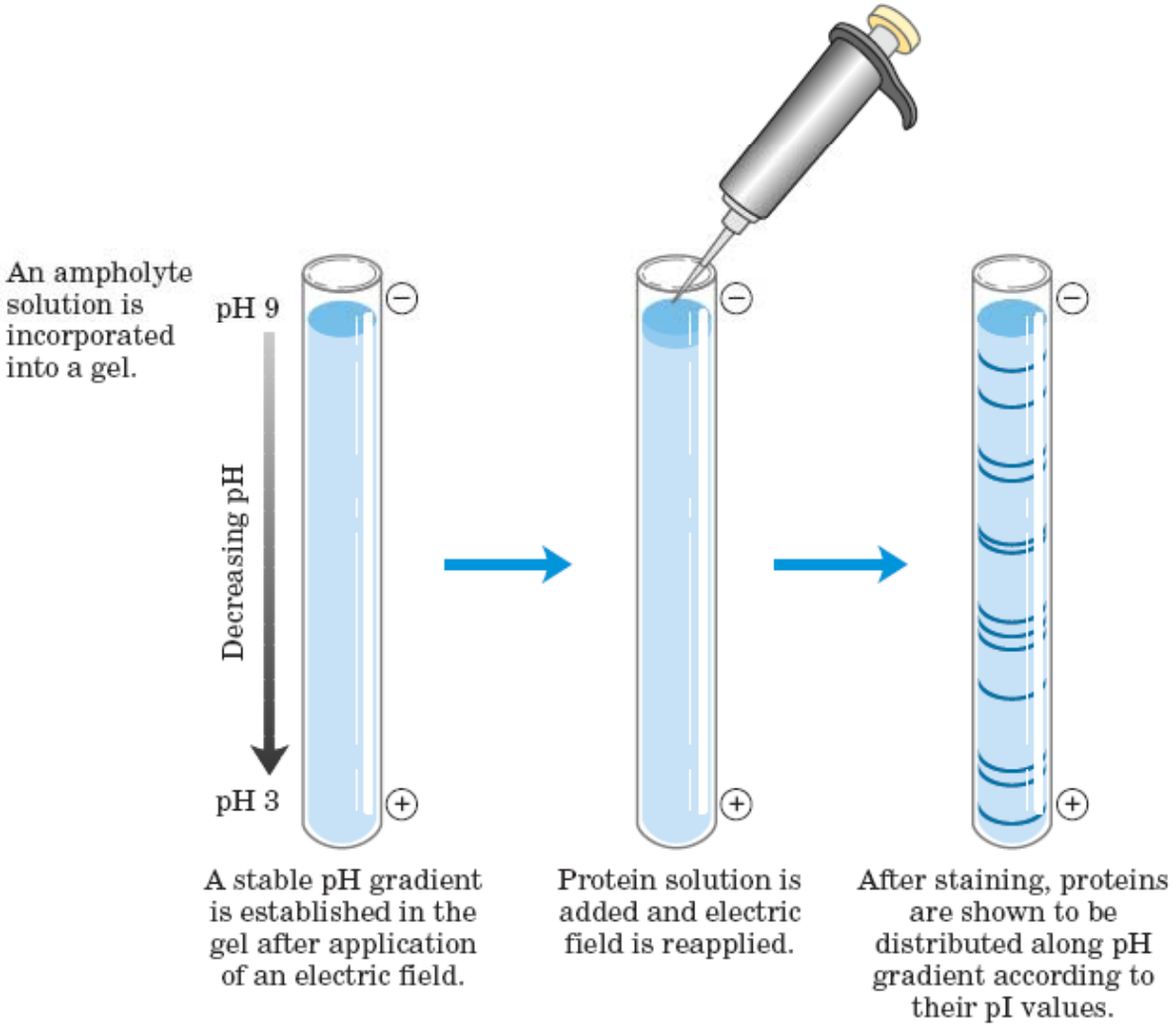
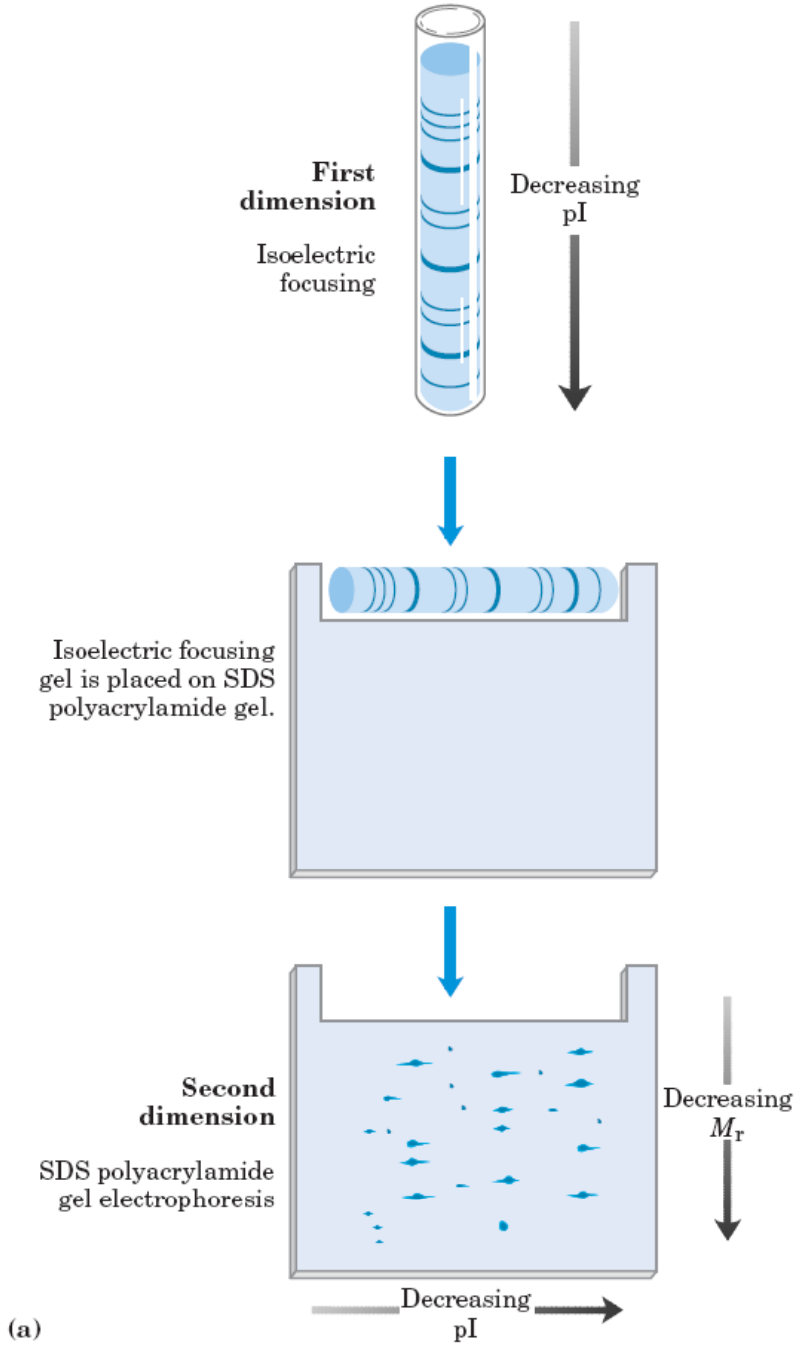


FIGURE 3–21 Isoelectric focusing. This technique separates proteins according to their isoelectric points. A stable pH gradient is established in the gel by the addition of appropriate ampholytes. A protein mixture is placed in a well on the gel. With an applied electric field, proteins enter the gel 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 3–22 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.

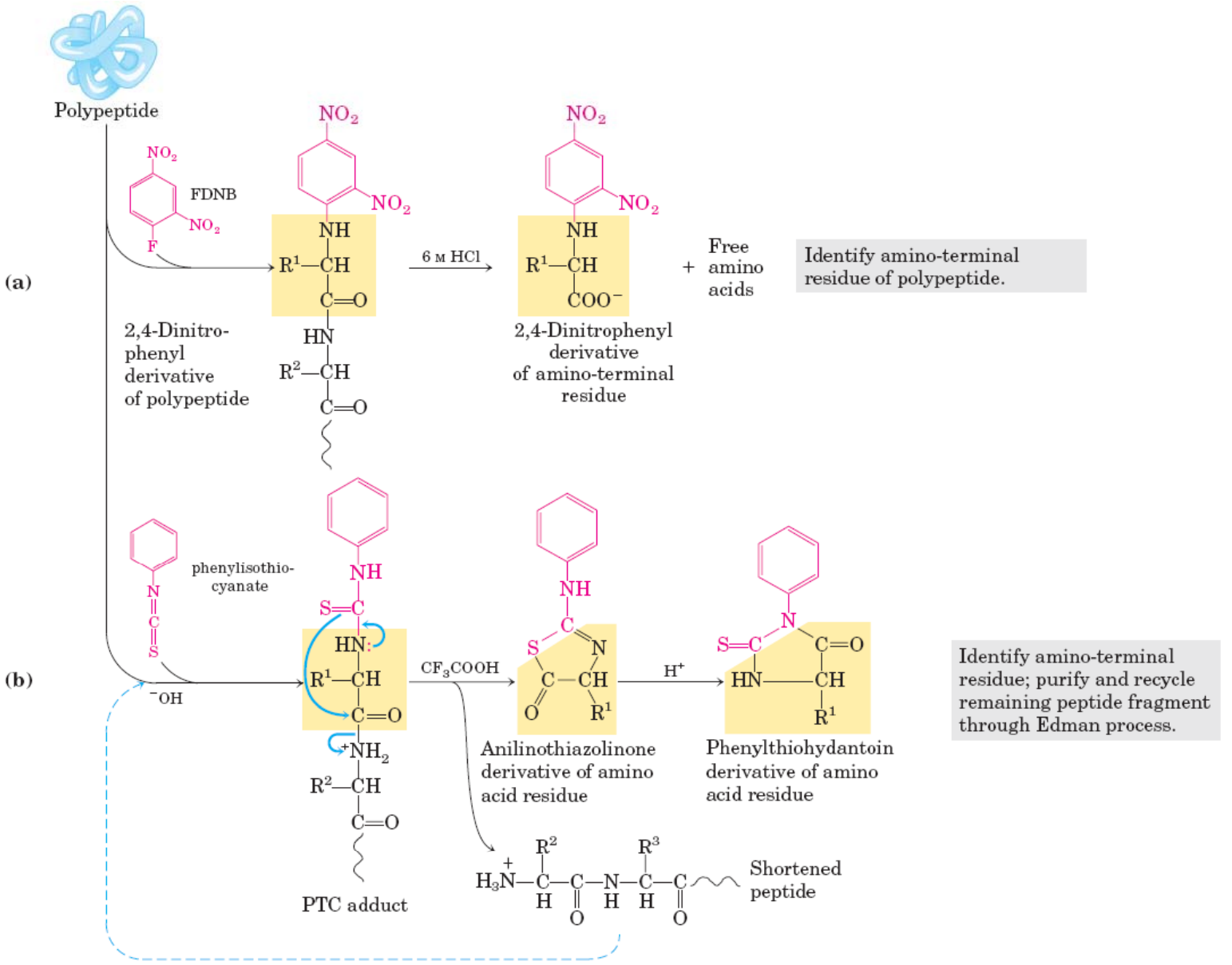


FIGURE 3–25 **Steps in sequencing a polypeptide.** (**a**) Identification of the amino-terminal residue can be the first step in sequencing a polypeptide. Sanger's method for identifying the amino-terminal residue is shown here. (**b**) The Edman degradation procedure reveals the entire sequence of a peptide. For shorter peptides, this method alone readily yields the entire sequence, and step (**a**) is often omitted. Step (**a**) is useful in the case of larger polypeptides, which are often fragmented into smaller peptides for sequencing (see Fig. 3–27).

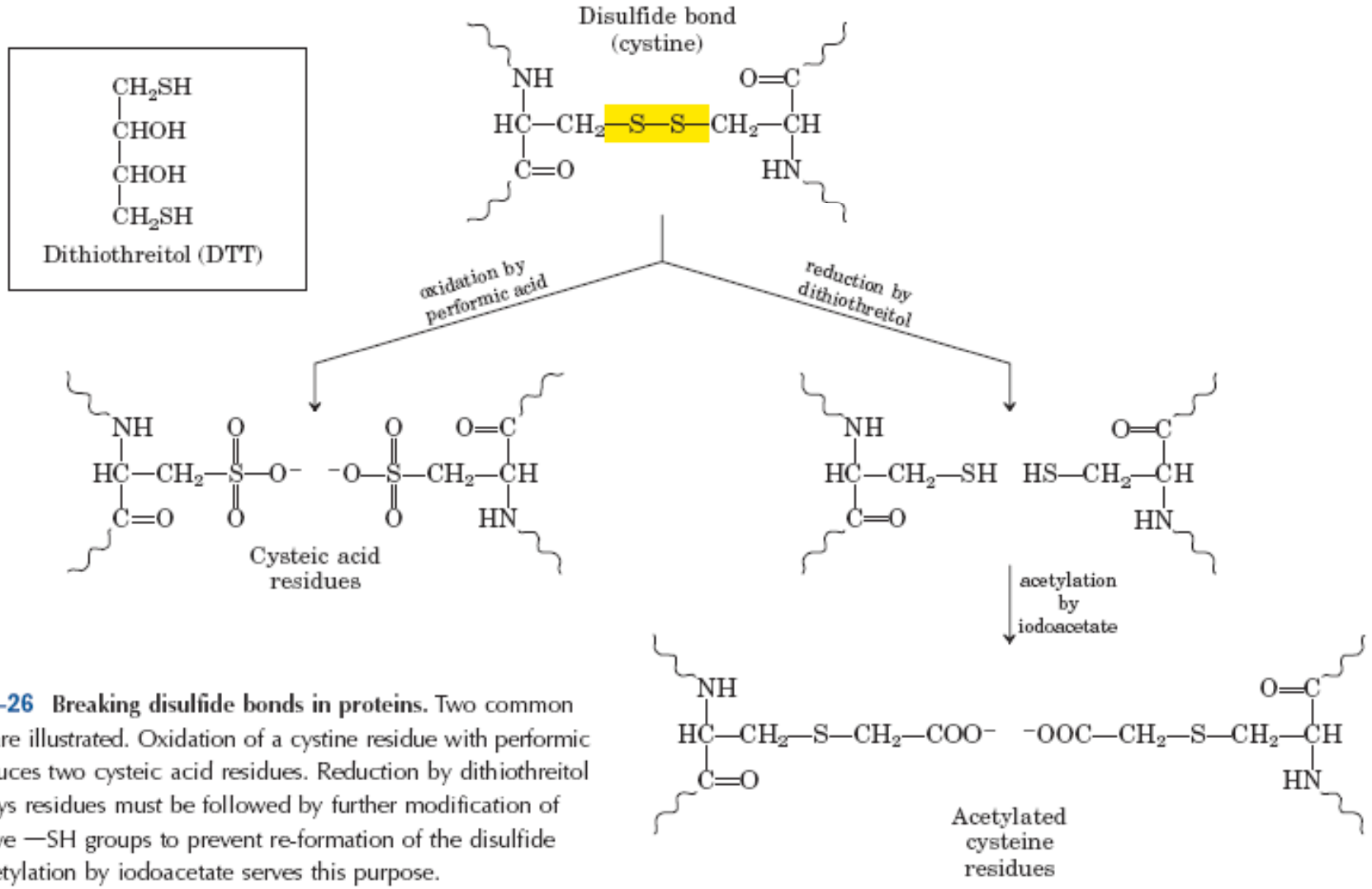


FIGURE 3–26 **Breaking disulfide bonds in proteins.** Two common methods are illustrated. Oxidation of a cystine residue with performic acid produces two cysteic acid residues. Reduction by dithiothreitol to form Cys residues must be followed by further modification of the reactive —SH groups to prevent re-formation of the disulfide bond. Acetylation by iodoacetate serves this purpose.

TABLE 3–7 The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

*Reagent (biological source)**	*Cleavage points*[†]
Trypsin (bovine pancreas)	Lys, Arg (C)
Submaxillarus protease (mouse submaxillary gland)	Arg (C)
Chymotrypsin (bovine pancreas)	Phe, Trp, Tyr (C)
Staphylococcus aureus V8 protease (bacterium *S. aureus*)	Asp, Glu (C)
Asp-*N*-protease (bacterium *Pseudomonas fragi*)	Asp, Glu (N)
Pepsin (porcine stomach)	Phe, Trp, Tyr (N)
Endoproteinase Lys C (bacterium *Lysobacter enzymogenes*)	Lys (C)
Cyanogen bromide	Met (C)

*All reagents except cyanogen bromide are proteases. All are available from commercial sources.

[†]Residues furnishing the primary recognition point for the protease or reagent; peptide bond cleavage occurs on either the carbonyl (C) or the amino (N) side of the indicated amino acid residues.

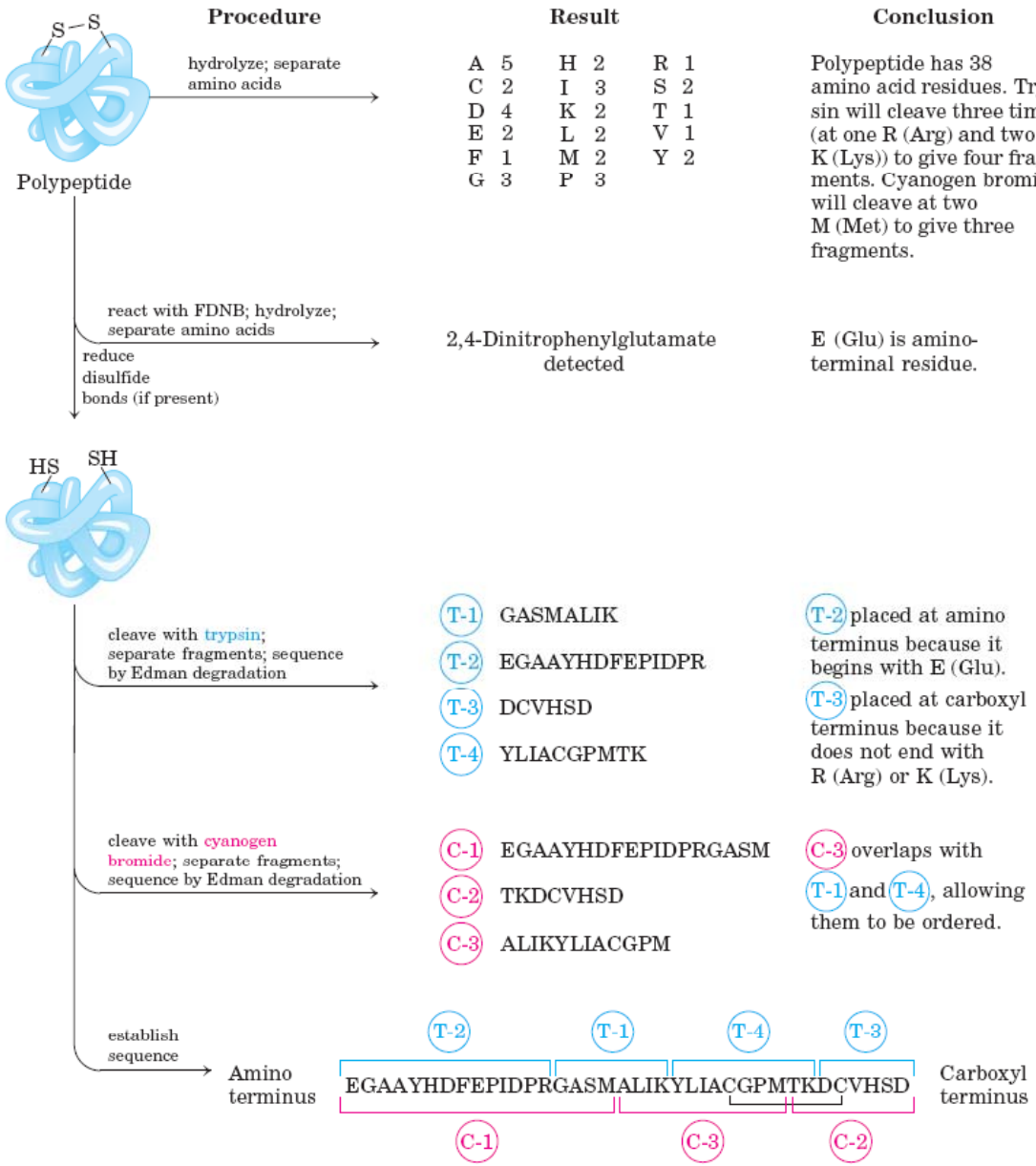


FIGURE 3–27 Cleaving proteins and sequencing and ordering the peptide fragments. First, the amino acid composition and amino-terminal residue of an intact sample are determined. Then any disulfide bonds are broken before fragmenting so that sequencing can proceed efficiently. In this example, there are only two Cys (C) residues and thus only one possibility for location of the disulfide bond. In polypeptides with three or more Cys residues, the position of disulfide bonds can be determined as described in the text. (The one-letter symbols for amino acids are given in Table 3–1.)