PROTEINS CAN BE QUANTITIATED AND LOCALIZED BY HIGHLY SPECIFIC ANTIBODIES

An antibody (immunoglobulin) is a protein synthesized by an animal in response to the presence of a foreign substance, called an antigen

- **Protein, polysaccharides, and nucleic acids are effective antigens**

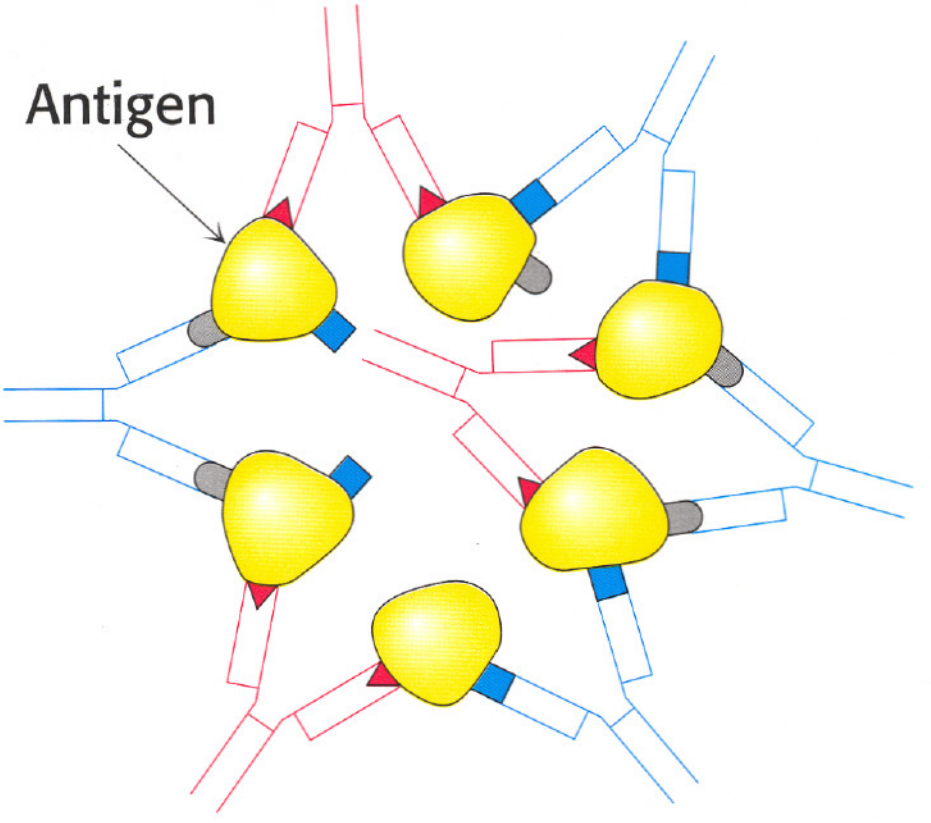
Antibodies that recognize a particular protein can be obtained by injecting the protein into a rabbit; blood is drawn from the immunized rabbit several weeks later and centrifuged. The resulting serum (called an antiserum) usually contains the desired antibody.

- **Monoclonal Antibody**
- **Polyclonal Antibody**
- **Antigenic Determinant (or Epitope)**
- **Immunoglobutin G** ($I_g G$)

Methods to study proteins based on the use of antibodies:

1. **Solid- phase immunoassay**
   - **e.g. enzyme- linked immunosorbent assay (ELISA)**
2. **Western Blotting** (immunoblot)
3. **Fluorescence Microscopy**
4. **Immunoelecton Microscopy**

Polyclonal Antibodies



Monoclonal Antibodies

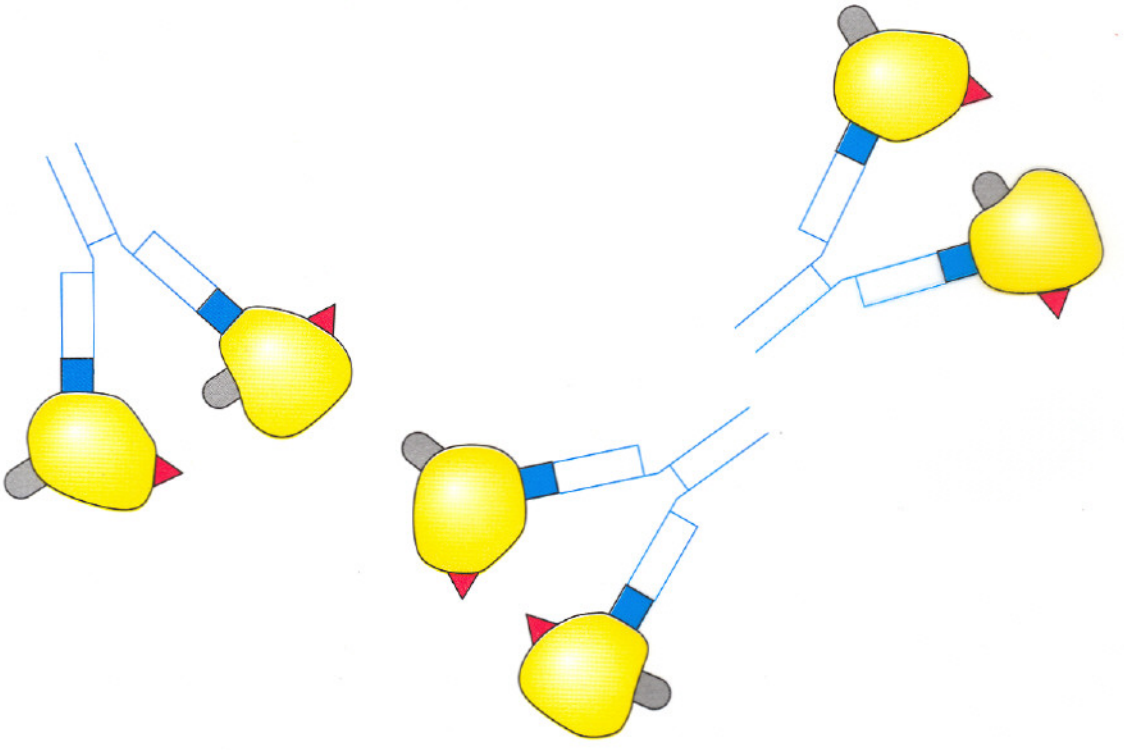


Figure 4-32
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.

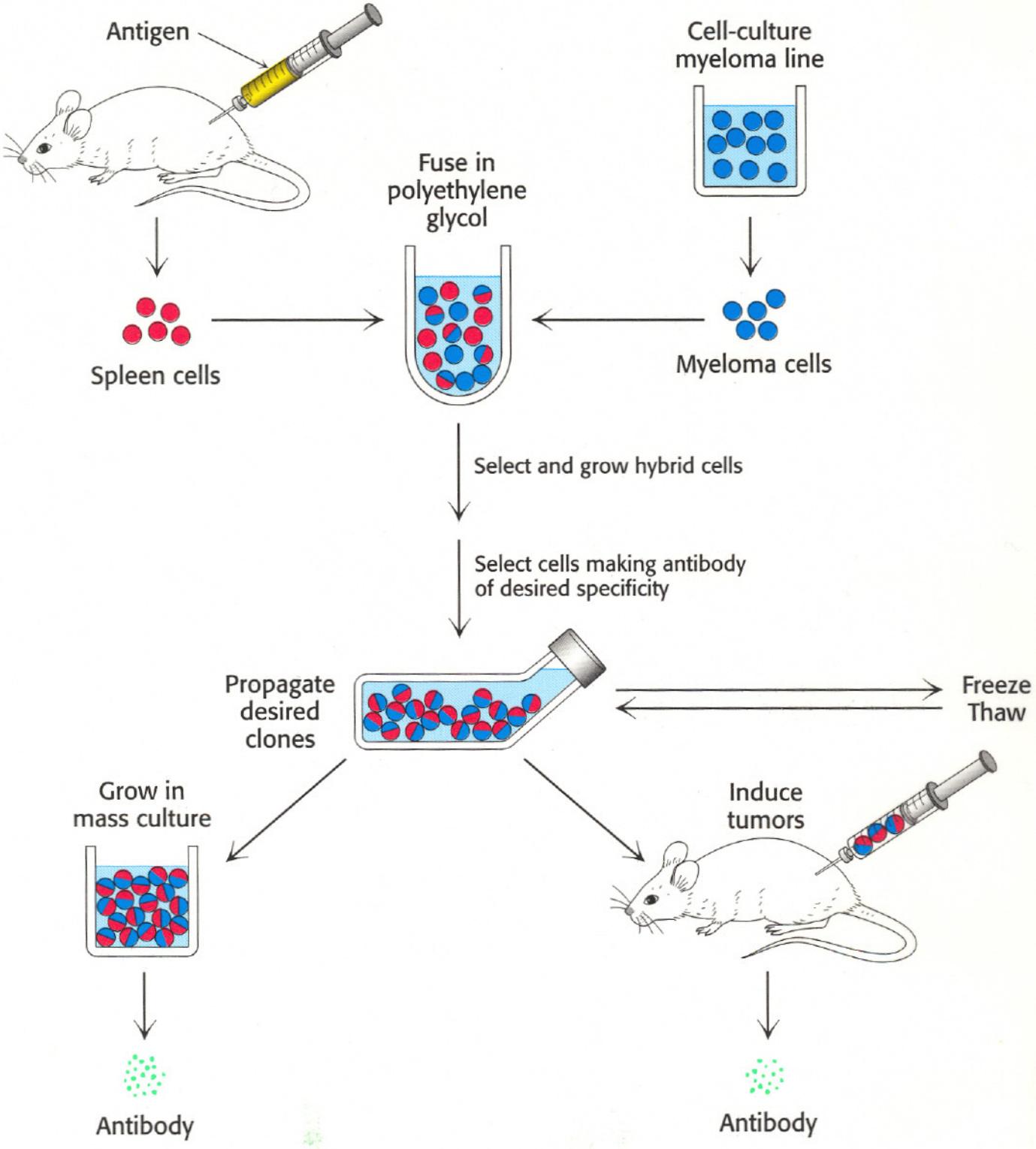
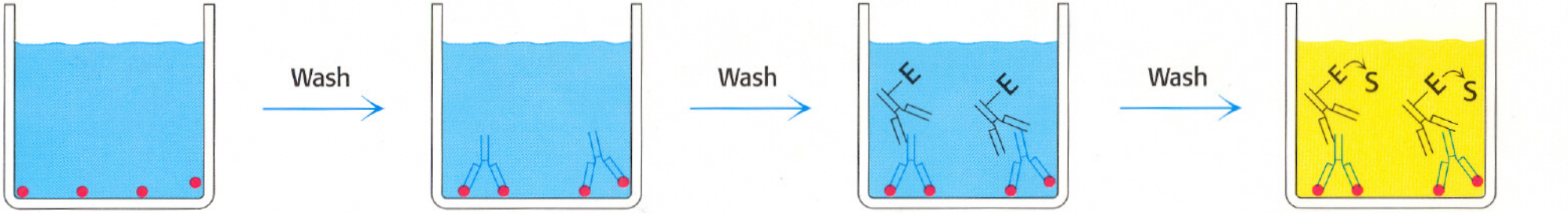


Figure 4-33
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.

(A) Indirect ELISA



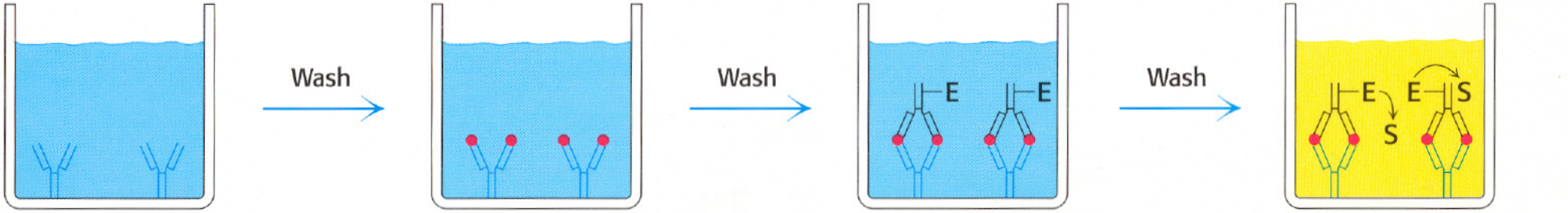
Antigen-coated well

Specific antibody binds to antigen

Enzyme-linked antibody binds to specific antibody

Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of specific antibody

(B) Sandwich ELISA



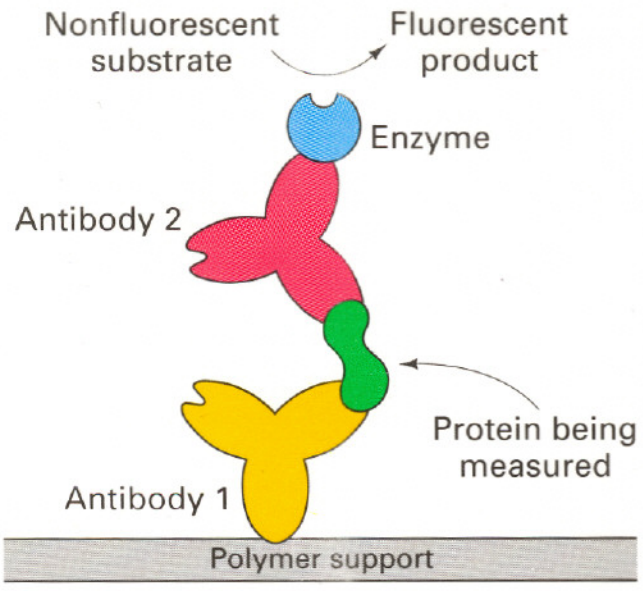
Monoclonal antibody-coated well

Antigen binds to antibody

A second monoclonal antibody, linked to enzyme, binds to immobilized antigen

Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of antigen

Figure 4-35
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.



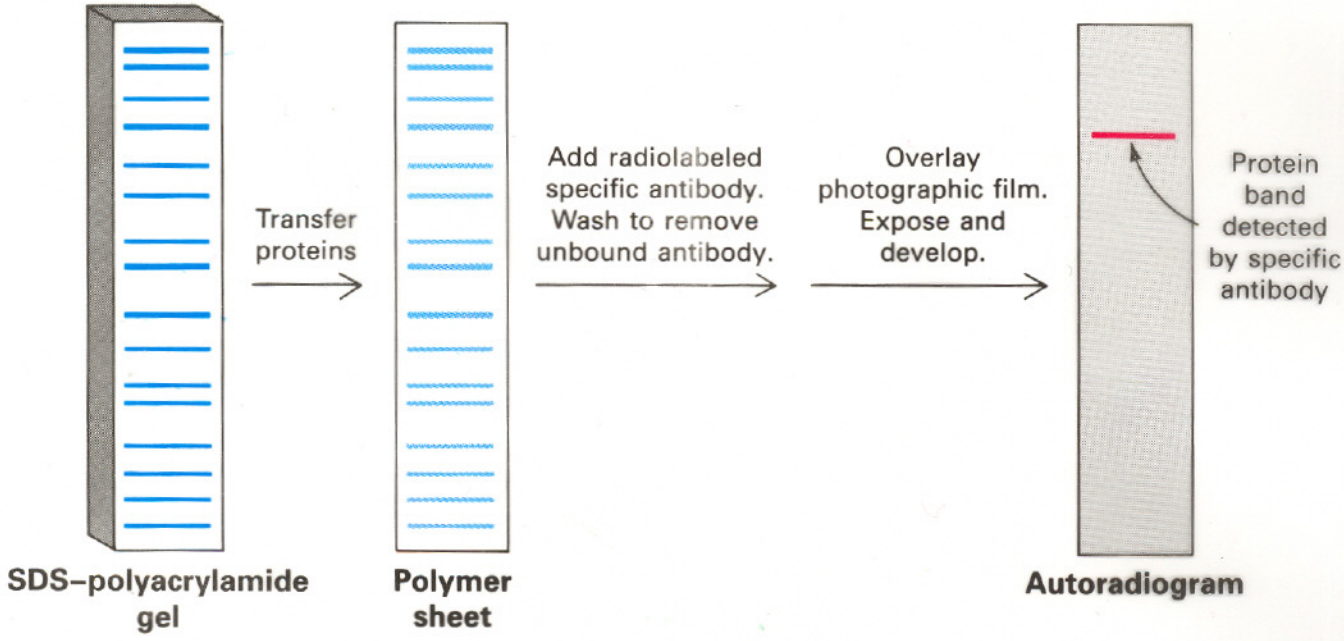


Figure 3-29, page 61; Figure 3-30, page 62

Stryer: *Biochemistry,* Fourth Edition

PEPTIDES CAN BE SYNTHESIZED BY AUTOMATED SOLID-PHASE METHODS

Synthesizing peptides of defined sequence is important for several reasons:

1. **Studying synthetic peptides can help define the rules governing the three- dimensional structure of proteins**

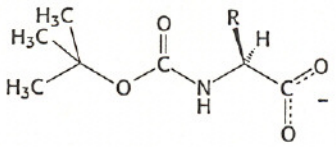
2. **Synthetic peptides can be used to isolate receptors for many hormones and other signal molecules**

3. **Synthetic peptides can serve as drugs**

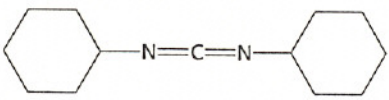
4. **Synthetic peptides can serve as antigens to stimulate the formation of specific antibodies**

Dicyclohexycarbodiimiole (DCC) is used to activate carboxyl groups for the formation of peptide bonds.

Peptides can be readily synthesized by a solid-phase method



t-Butyloxycarbonyl amino acid
(*t*-Boc amino acid)



Dicyclohexylcarbodiimide
(DCC)

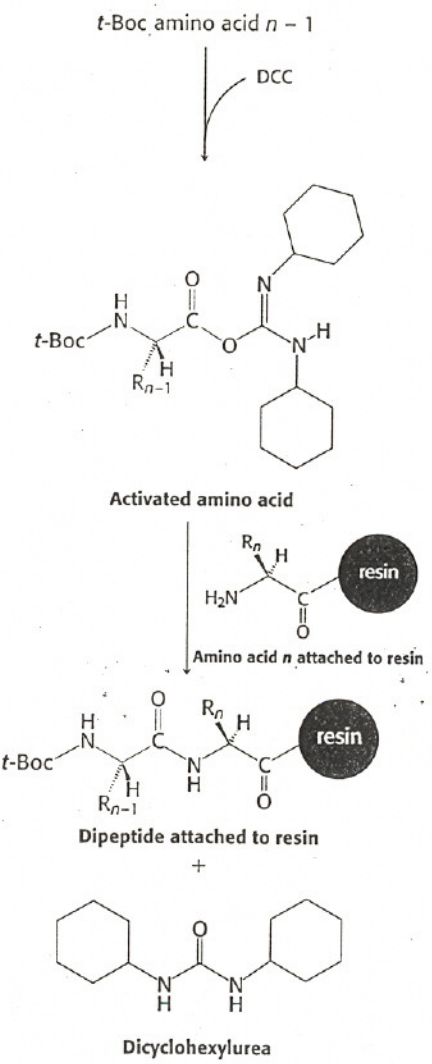


FIGURE 4.41 Amino acid activation. Dicyclohexylcarbodiimide is used to activate carboxyl groups for the formation of peptide bonds.

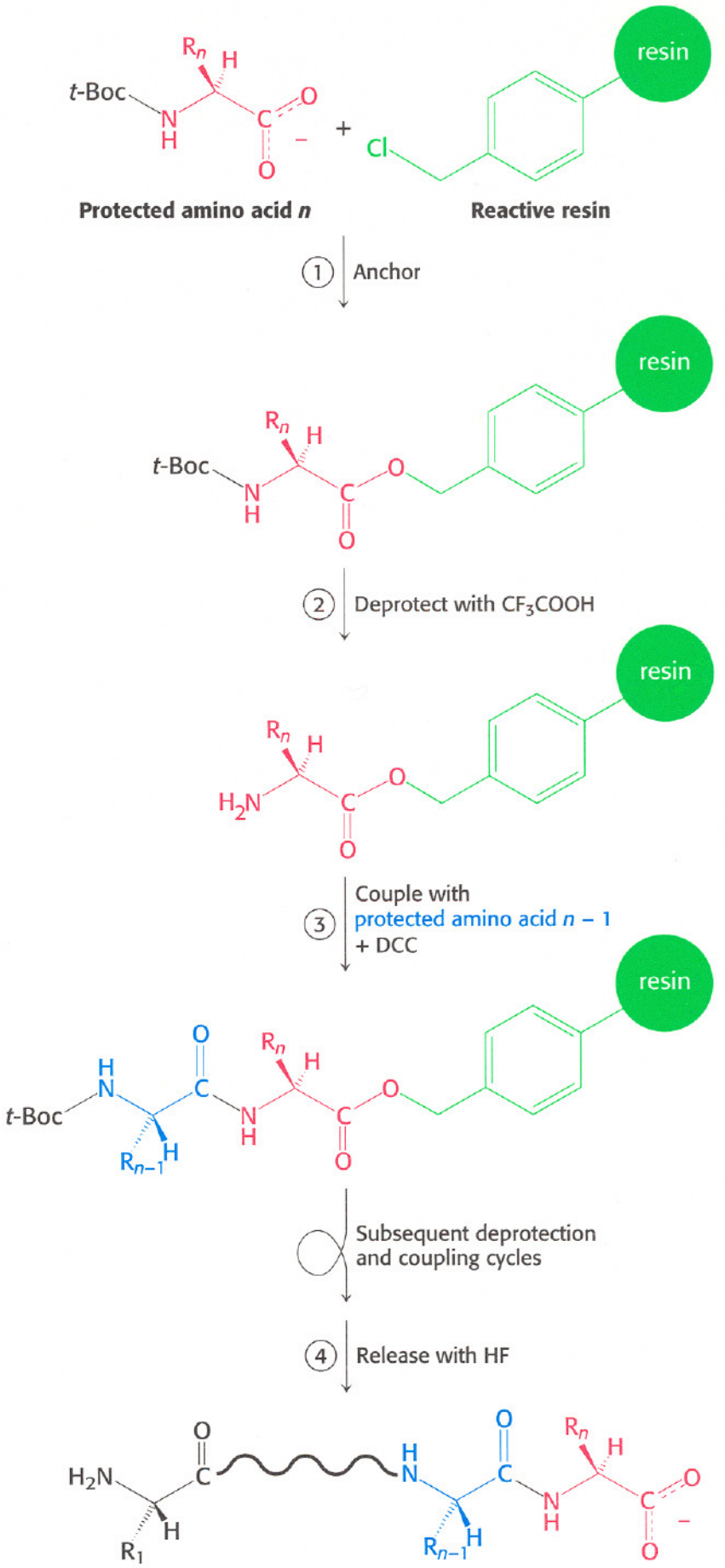


Figure 4-42
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.

X-RAY CRYSTALLOGRAPHY REVEALS THREE-DIMENSIONAL STRUCTURE IN ATOMIC DETAIL

X-Ray Crystallography is a technique that can reveal the precise three- dimensional positions of most of the atoms in a protein molecule

- **Crystals of the protein of interest are needed because the technique requires that all molecules be precisely oriented.**

- **Crystals can be obtained for adding ammonium sulfate or another salt to a concentrated solution of protein to reduce its solubility.**

- **The three components in an x-ray crystallographic analysis are**

    1. **A source of x-rays**
    2. **A protein crystal**
    3. **A detector**

❖ **The structure of more than 200 proteins have been elucidated at atomic resolution**

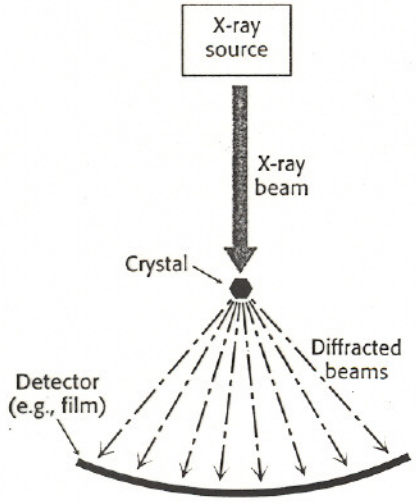


FIGURE 4.49 Essence of an x-ray crystallographic experiment: an x-ray beam, a crystal, and a detector.

FIGURE 4.50 Crystallization of myoglobin.

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY CAN REVEAL THE STRUCTURE OF PROTEINS IN SOLUTION

NMR spectroscopy is able to reveal the atomic structure of macromolecules in solution

NMR spectroscopy and x-ray crystallography are the only two techniques that can reveal the three-dimensional structure of proteins and other biomolecules in atomic detail:

1. **X- ray methods give the highest-resolution images, but crystals are required**

2. **NMR methods are effective with proteins in solution, provided that highly concentrated solutions (~ 1 mm, or 15 mg/ mL for a 15- kd protein) can be obtained. The upper bound on size is about 30 kd**

^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P

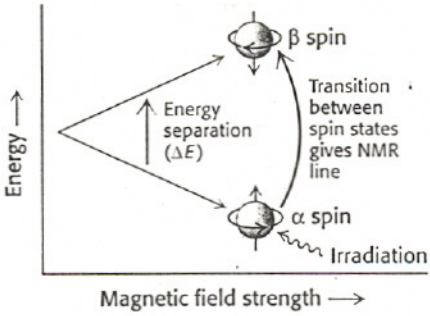


FIGURE 4.43 Basis of NMR spectroscopy. The energies of the two orientations of a nucleus of spin ½ (such as ^{31}P and ^{1}H) depend on the strength of the applied magnetic field. Absorption of electromagnetic radiation of appropriate frequency induces a transition from the lower to the upper level.

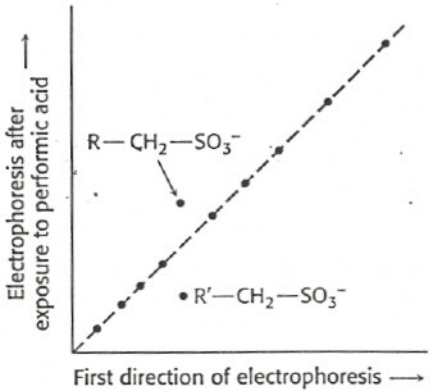


FIGURE 4.27 Diagonal electrophoresis. Peptides joined together by disulfide bonds can be detected by diagonal electrophoresis. The mixture of peptides is subjected to electrophoresis in a single lane in one direction (horizontal) and then treated with performic acid, which cleaves and oxidizes the disulfide bonds. The sample is then subjected to electrophoresis in the perpendicular direction (vertical).