Chapter 3

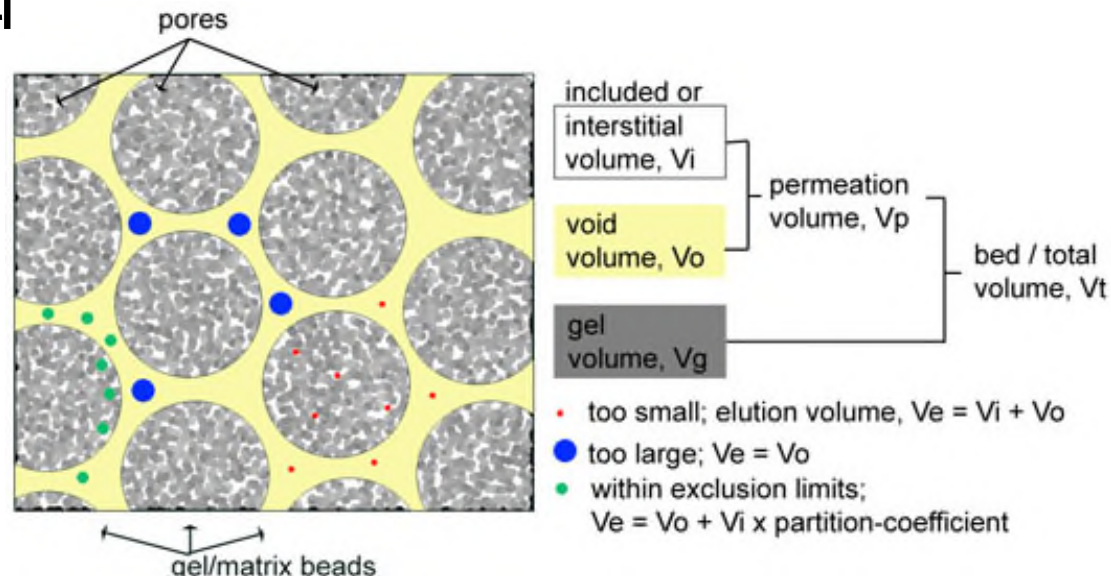
단백질의 탐구 및 단백체학

(3) 겔 거르기 (Gel filtration) chromatography: 크기에 의한 분리

- Resin: 다공성 구슬
  = Sephadex, Sepharose, Biogel
  = Bead size: 100 μm

- 원리: 분배 (partition) chromatograph
  = 분배: 용질이 이동상 (mobile phase)과 고정상 (stationary phase)에 분배되는 정도
  = 이동상: 완충용액
  = 고정상: resin
  = 배제한계 (Exclusion limit): 레진의 구멍의 크기에 의해 결정

- 배제한계 보다 큰 물질: Resin 사이의 공극을 통해 이동
- 배제한계 보다 작은 물질: Resin의 구멍을 통해 이동
- Void volume:
- Gel volume:
- Elution volume:



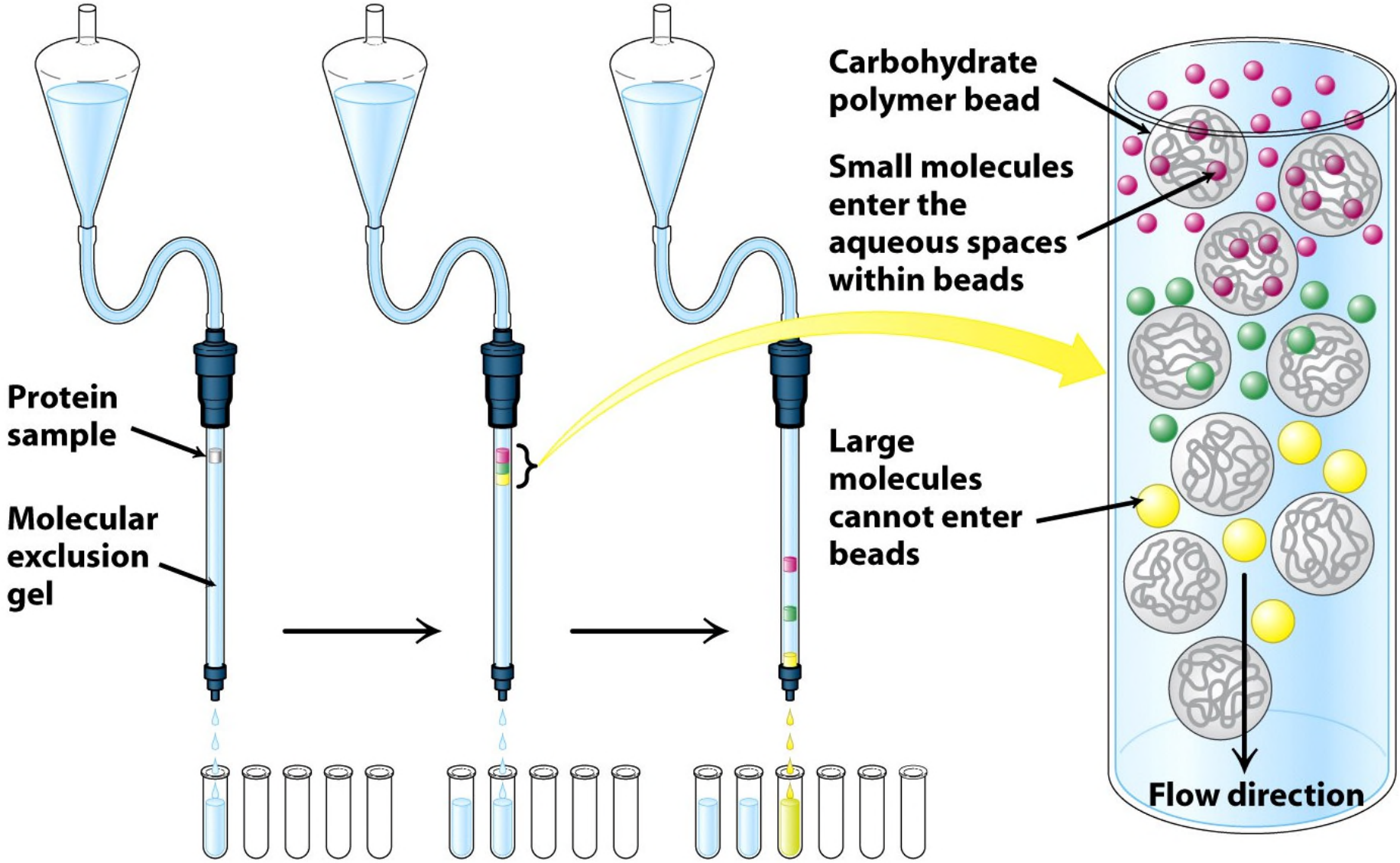


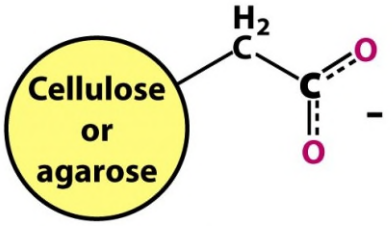
Figure 3-3
Biochemistry, Sixth Edition

(4) 이온 교환 (ion-exchange) chromatography: 전하의 차이를 이용

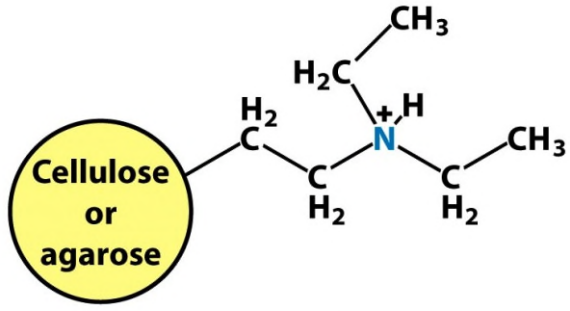
- Resin: 전하를 띈
  = 음이온교환 (Anion): DEAE-cellulose
  = 양이온 (Cation): CM-cellulose

- 분자의 분리는 분자의 전하에 따라 분리된다

- 용출은 buffer의 염 농도를 높인다

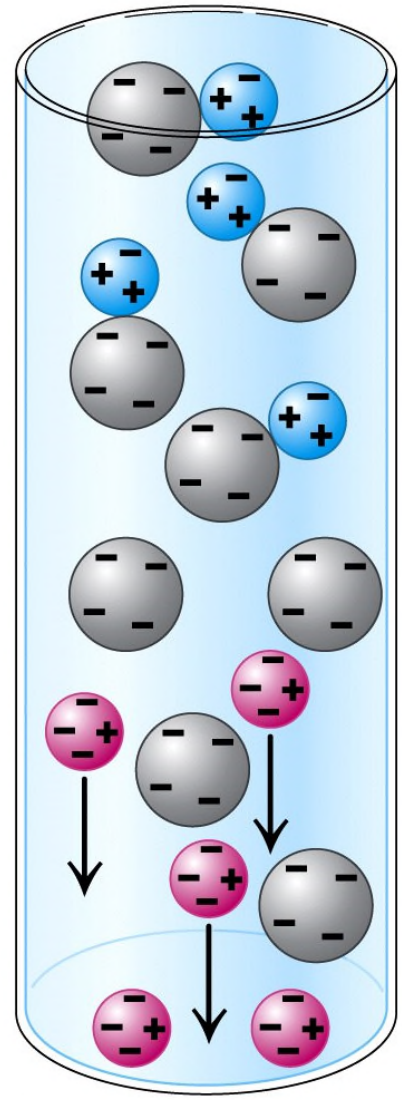


Carboxymethyl (CM) group (ionized form)



Diethylaminoethyl (DEAE) group (protonated form)

Unnumbered figure pg 70
Biochemistry, Sixth Edition



Positively charged protein binds to negatively charged bead

Negatively charged protein flows through

Figure 3-4
Biochemistry, Sixth Edition

(5) 친화 (Affinity) chromatography: 단백질 결합의 특이성을 활용

- Resin: antibody-linked, Substrate-linked
- 장점: 한번에 원하는 단백질의 분리가 가능함
- 용출:
  - = Antibody: low pH (pH 3.0)
  - = Substrate-Enzyme: substrate

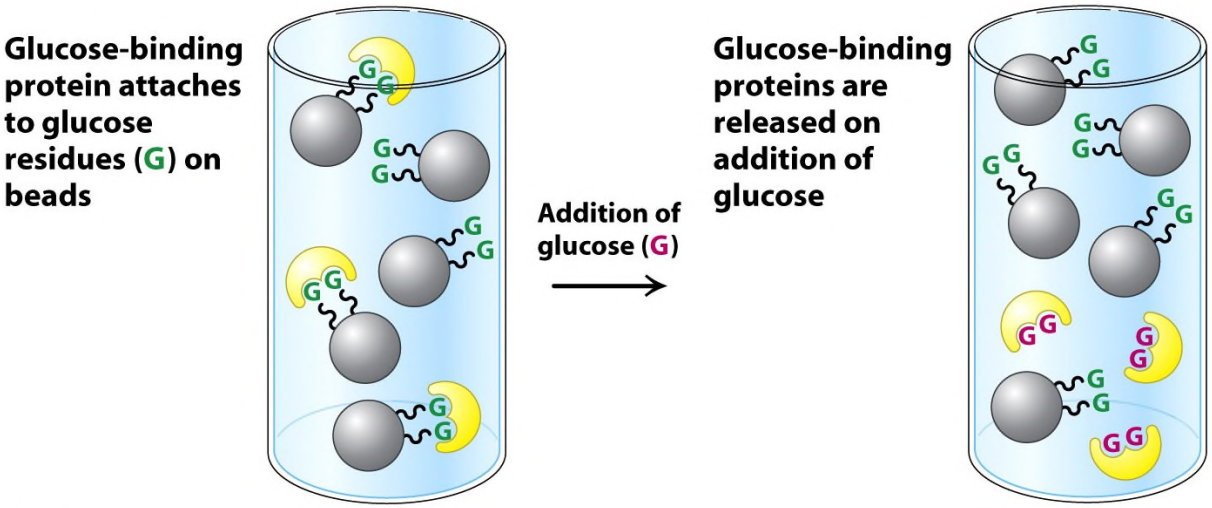
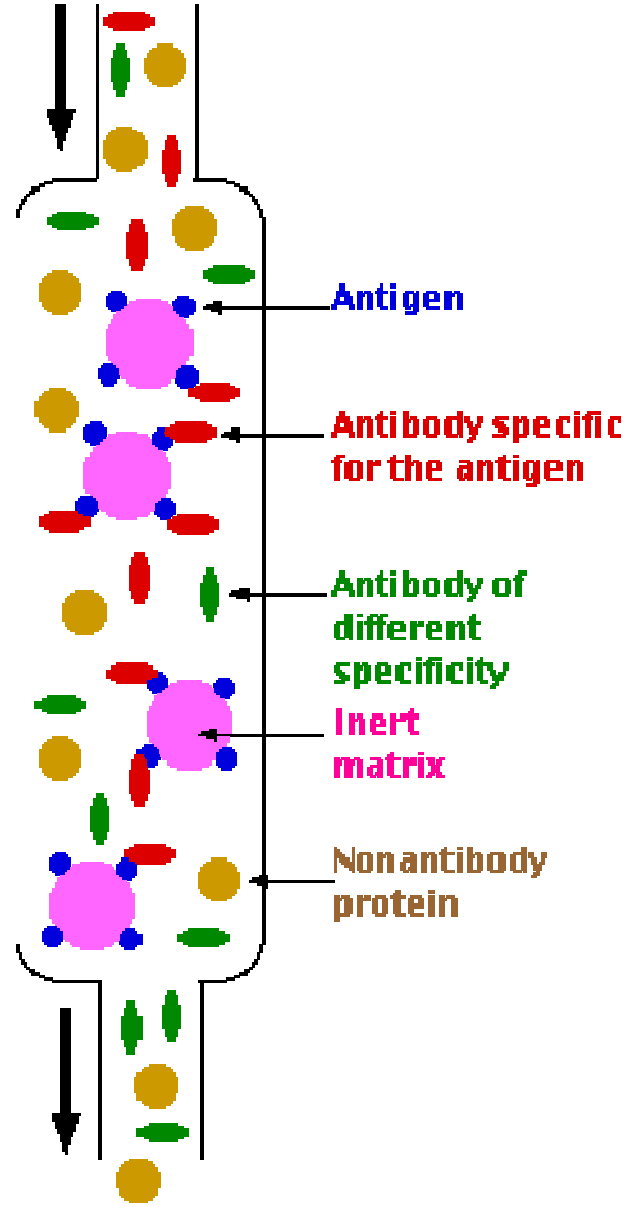


Figure 3-5
Biochemistry, Sixth Edition



(6) 고압액체 크로마토드래피 (High pressure liquid chromatography;HPLC)

- 용매의 압력을 높여 액체크로마토그래피의 성능을 향상시킴
- 시간을 절약할 수 있고, 분해능이 향상됨
- 일반 액체 chromatography의 단점을 보완
  = low resolution:
  = Time consuming:

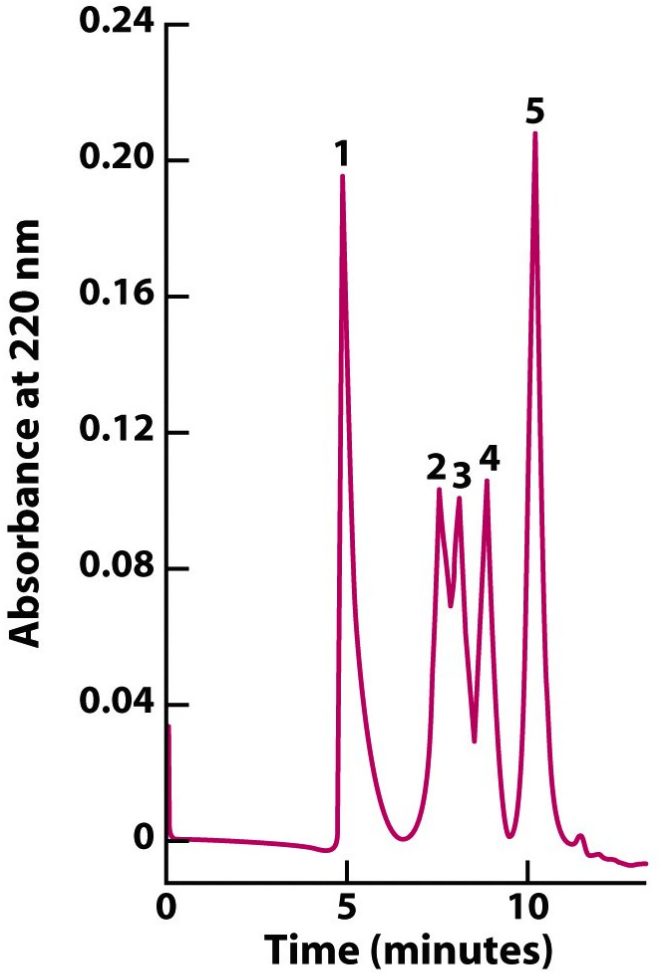


Figure 3-6
Biochemistry, Sixth Edition

4) 전기이동 (영동)법에 의한 단백질의 분리 및 확인

- 단백질 분리효율을 측정하는 방법
  = To check increase of specific activity: assay
  = To check decrease of protein contaminant: electrophoresis

(1) Gel electrophoresis

- 전기영동: 전하를 띈 물질이 전기장 안에서 움직이는 현상

$v = Ez/f$

- v: 이동속도
- E: 전기장의 세기
- z: 분자의 알짜전하
- f: 마찰계수

$f = 6\pi\eta r$

- π: pi
- η: 용매의 점도
- r: 분자의 반지름

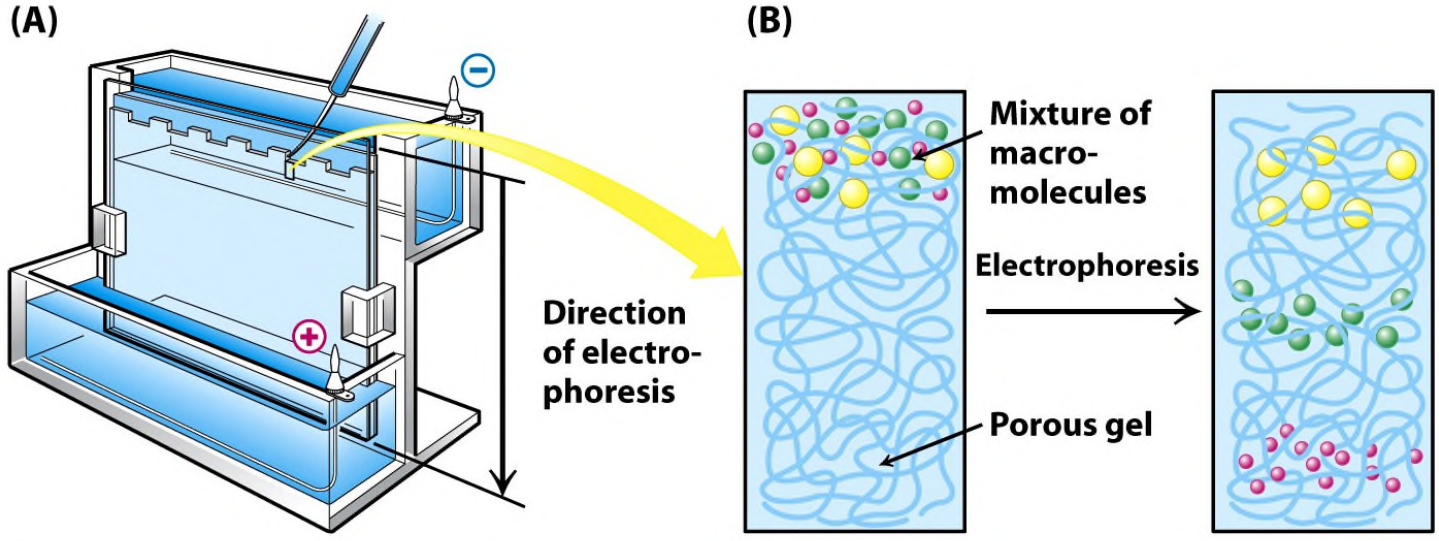


Figure 3-7
Biochemistry, Sixth Edition

- gel electrophoresis의 매질 (matrix)
  = DNA/RNA: Agarose gel
  = Protein : polyacrylamide gel

- 전기영동 시 단백질의 분리 요인:
  = Size
  = Charge
  = Shape

- Polyacrylamide gel
  = Native gel electrophoresis:
    단백질의 알짜전하와 모양에 의해 분리
  = Denatured gel electrophoresis: 크기
  ➔ Soduim dodecyl sulfate (SDS)
  ➔ beta-mercaptoethanol

  = 단백질 검출
  ➔ Autoradiogram: Western
  ➔ Coomassie blue staining

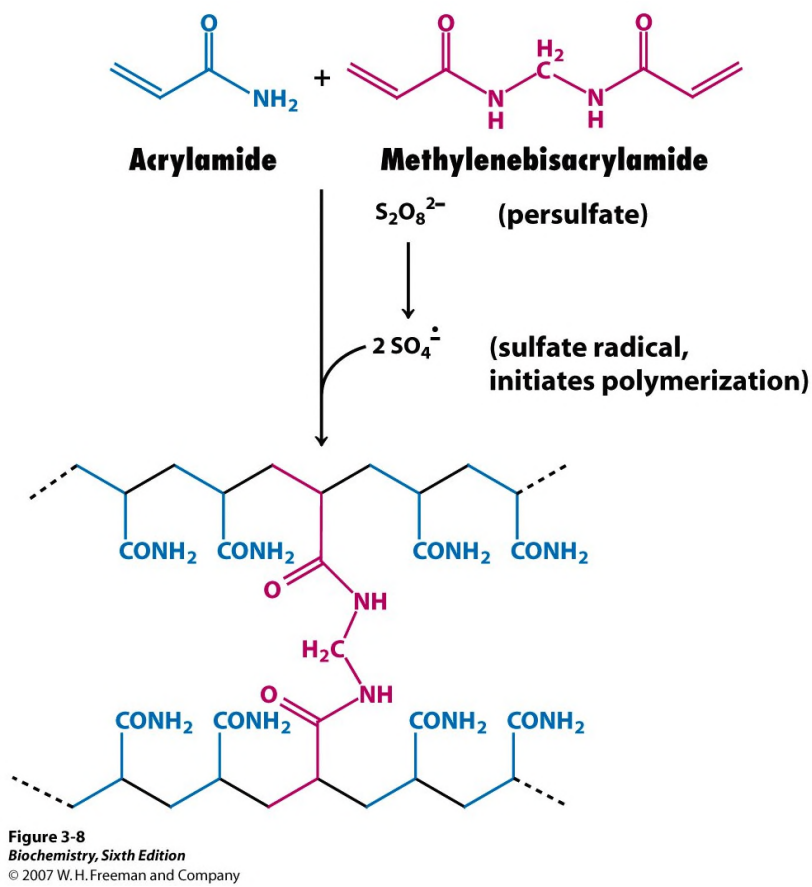
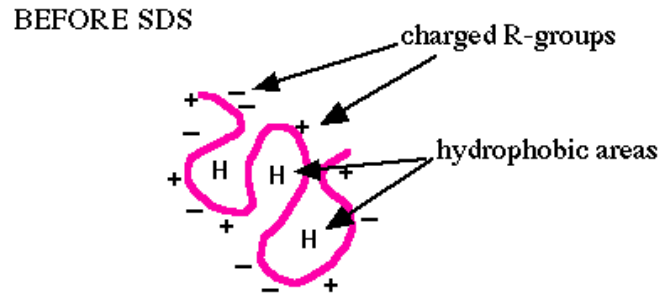
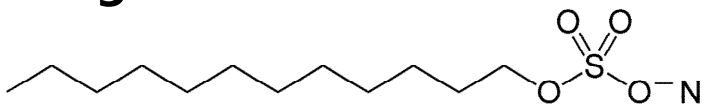
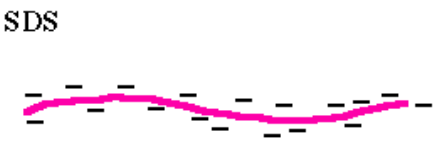


Figure 3-8
Biochemistry, Sixth Edition




SDS



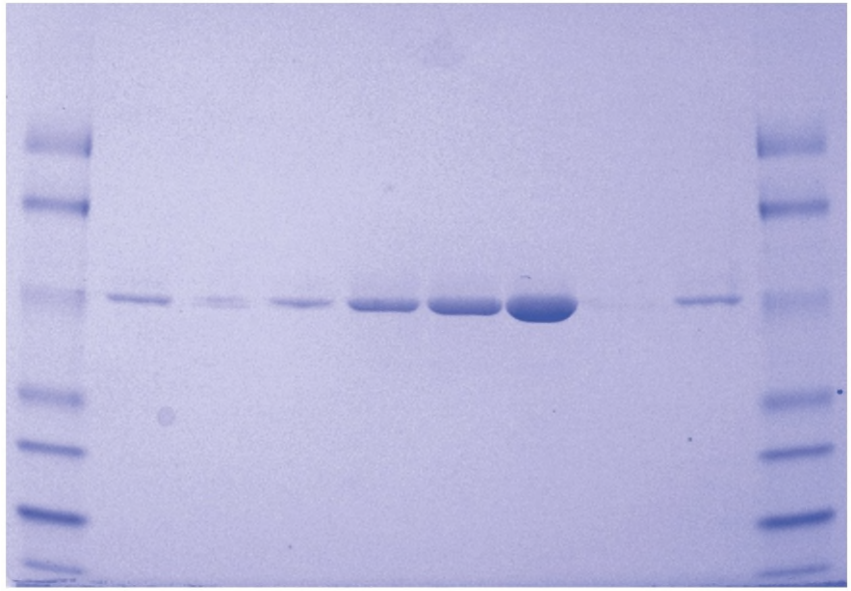
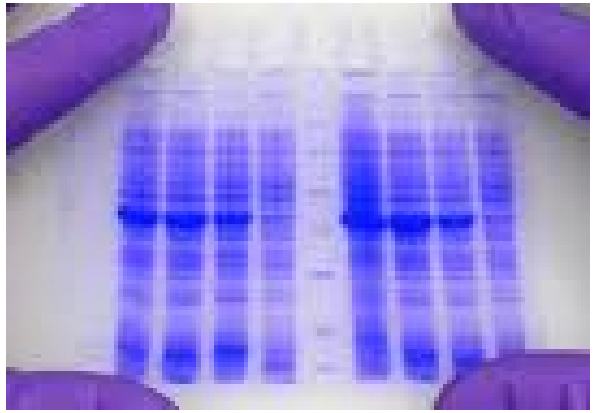


Figure 3-9
Biochemistry, Sixth Edition



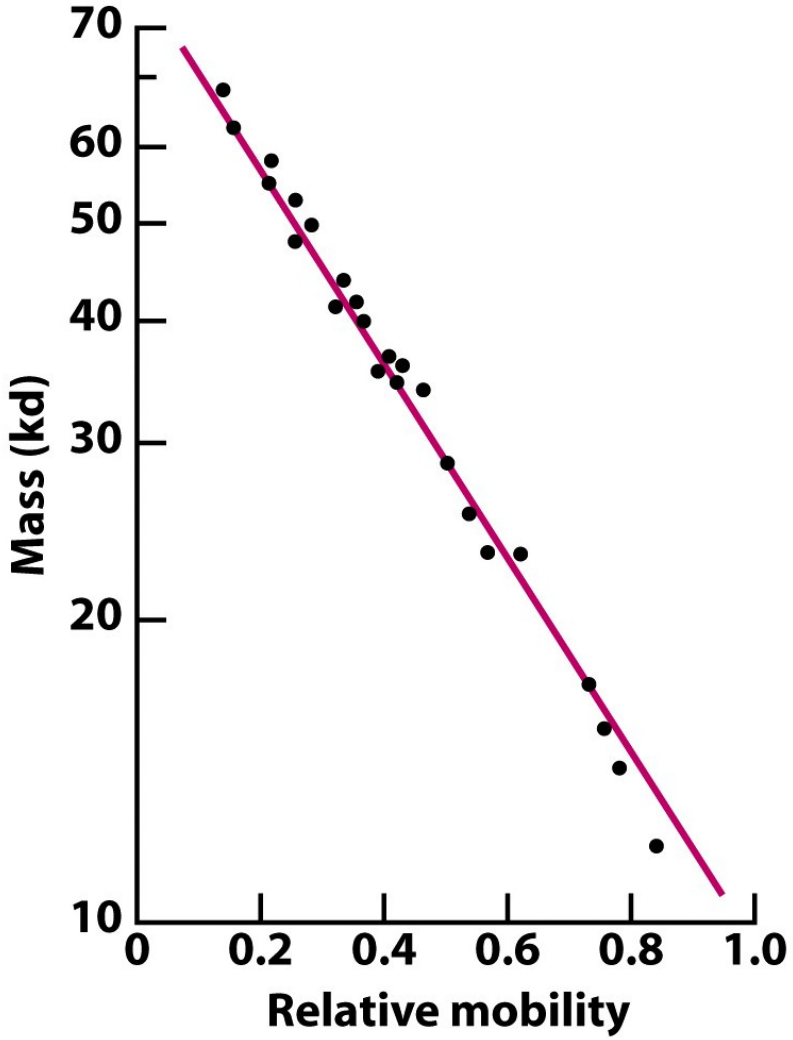


Figure 3-10
Biochemistry, Sixth Edition

(2) 등전점 전기영동 (Isoelectric focusing)

- 등전점 (isoelectric point; pI): t단백질의 알짜전하가 "0"이 되는 pH.
- 단백질의 pI는 이온성 아미노산에 의해 결정된다.
- 등전점에서 단백질은 전기장안에서 움직이지 않는다.
- polyacrylamide gel안에서 pH gradient를 만드는 방법.
  = polyampholytes

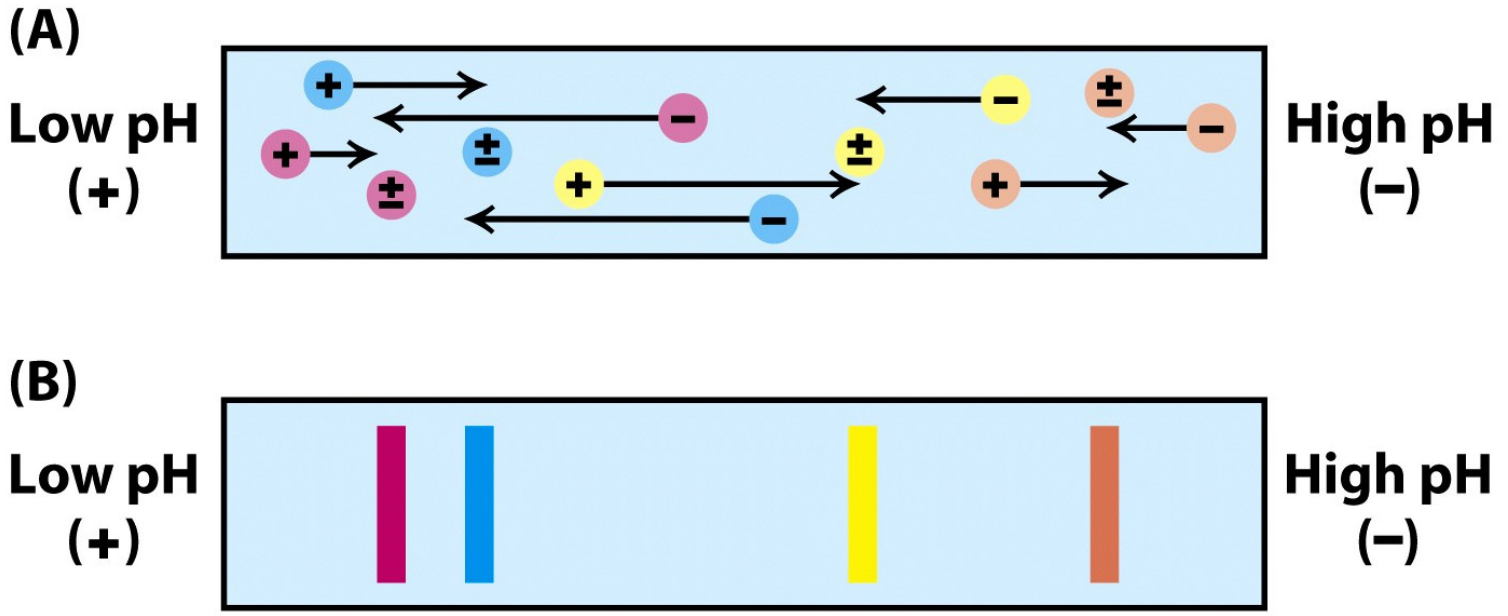


Figure 3-11
Biochemistry, Sixth Edition

(3) 2차원 전기영동 (Two-dimensional electrophoresis)

- 등전점 전기영동법과 SDS-전기영동법을 합쳐 단백질을 분리하는 기술
  = 1차원 전기영동: 등전점 전기영동
  = 2차원 전기영동: SDS-PAGE
- 이 기술은 단백질체 분석에 이용됨.

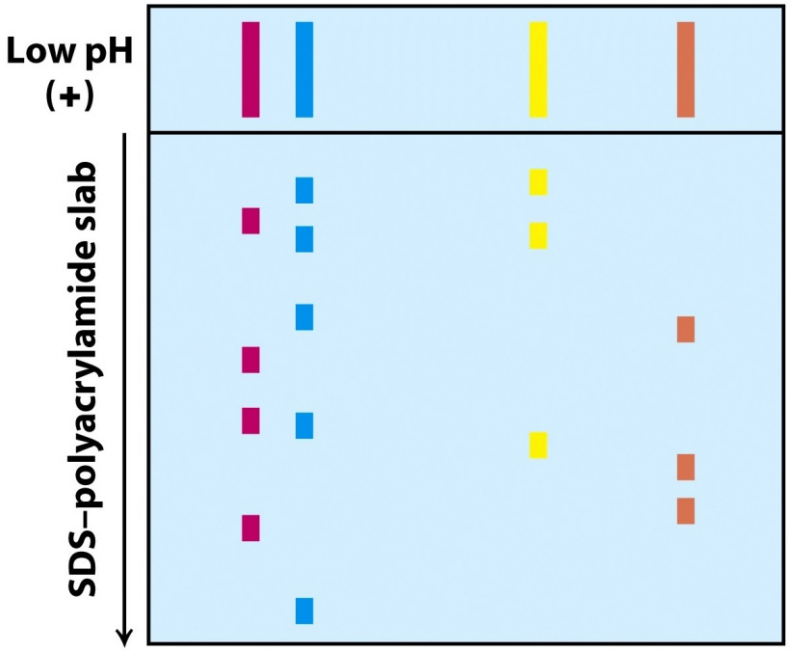


Figure 3-12a
Biochemistry, Sixth Edition

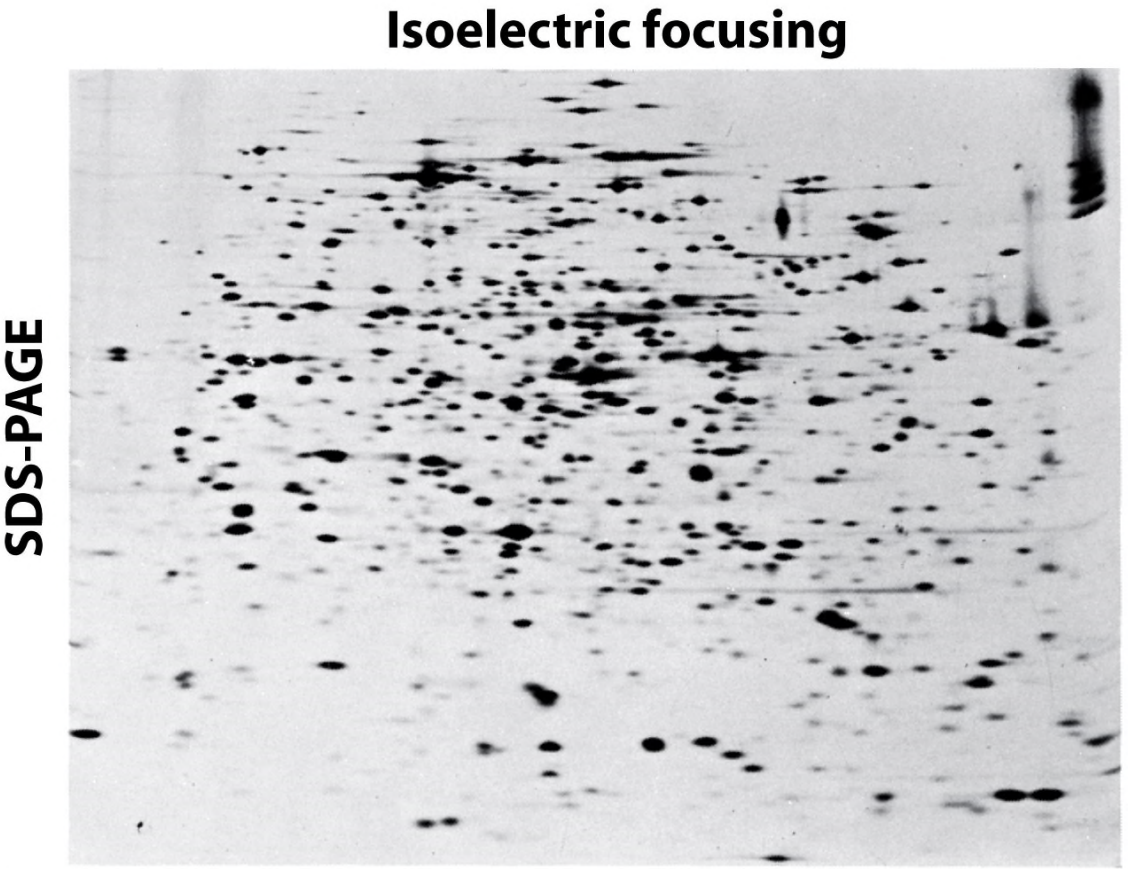
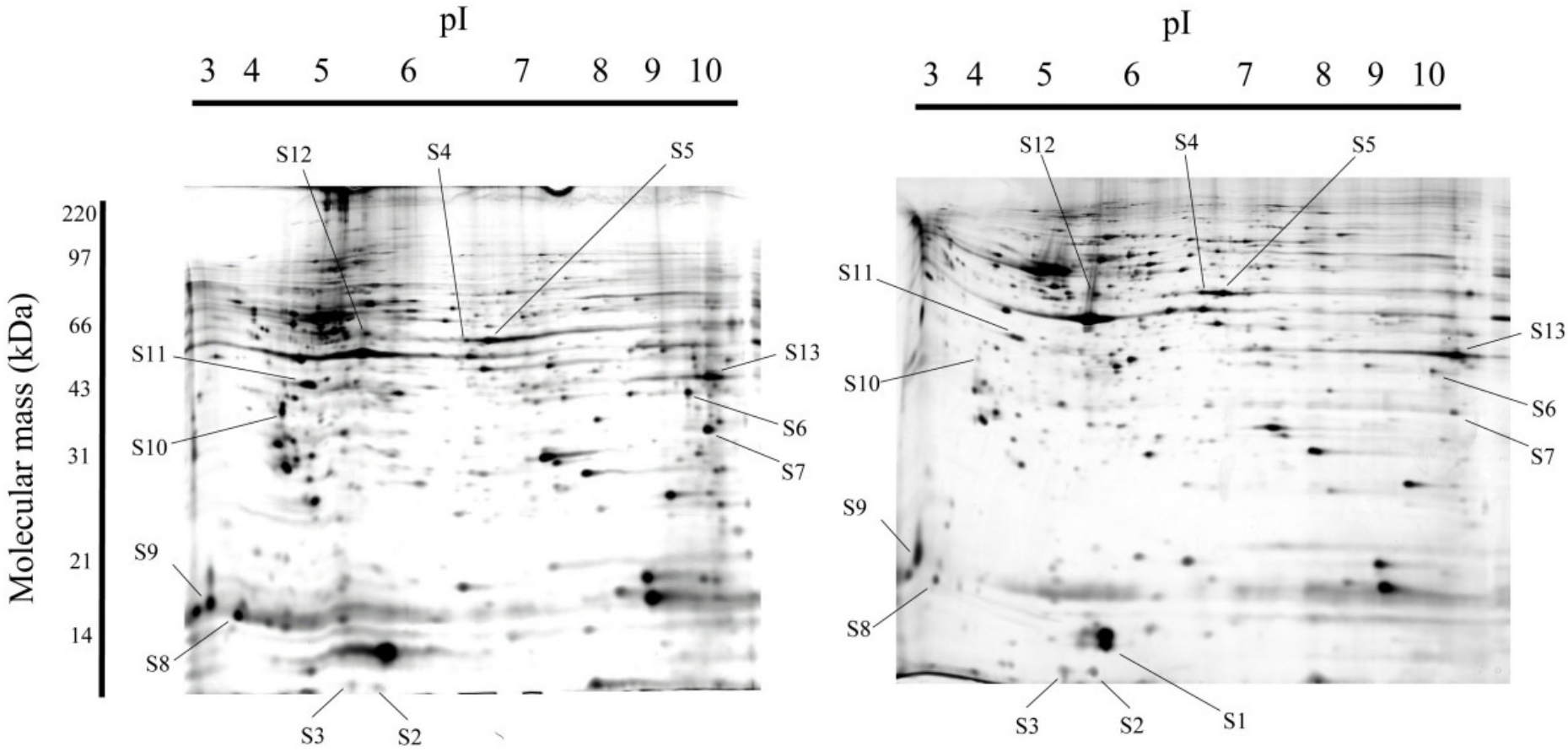


Figure 3-12b
Biochemistry, Sixth Edition

Application of 2D-gel electrophoresis on the proteomics analysis



5) 단백질 정제 계획을 정량적으로 검토할 수 있다.

- 각 정제 단계의 효율을 측정하는 방법
  = 비활성 (specific activity)의 증가를 측정하는 방법: assay
  = 단백질 순도를 측정하는 방법: electrophoresis

(1) To check increase of specific activity

TABLE 3.1 Quantification of a purification protocol for a fictitious protein

Step	Total protein (mg)	Total activity (units)	Specific activity, (units mg^{-1})	Yield (%)	Purification level
Homogenization	15,000	150,000	10	100	1
Salt fractionation	4,600	138,000	30	92	3
Ion-exchange chromatography	1,278	115,500	90	77	9
Gel-filtration chromatography	68.8	75,000	1,100	50	110
Affinity chromatography	1.75	52,500	30,000	35	3,000

Table 3-1
Biochemistry, Sixth Edition

(2) To check protein content

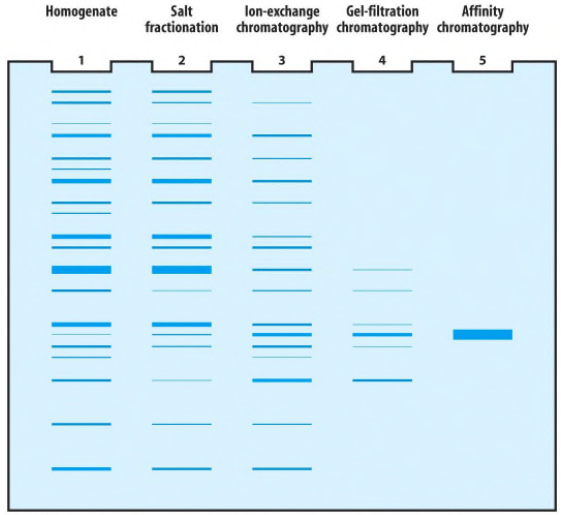


Figure 3-13
Biochemistry, Sixth Edition

6) 초원심분리는 생체분자를 분리하고 그 분자량을 결정할 수 있다.

- 원심분리:

= 생체물질의 침강은 질량과 밀도에 의하여 분리됨mass and density.

= 침강계수: 원심력이 작용하는 장소에서 입자들이 움직이는 속도.

$s = m(1-\upsilon\rho)/f$

m: mass

υ: particle specific volume

ρ: density of medium

f: frictional coefficient

= Svedberg unit (S): ribosome small subunit (40 S), large subunit (60 S)

TABLE 3.2 S values and molecular weights of sample proteins

Protein	S value (Svedberg units)	Molecular weight
Pancreatic trypsin inhibitor	1	6,520
Cytochrome *c*	1.83	12,310
Ribonuclease A	1.78	13,690
Myoglobin	1.97	17,800
Trypsin	2.5	23,200
Carbonic anhydrase	3.23	28,800
Concanavalin A	3.8	51,260
Malate dehydrogenase	5.76	74,900
Lactate dehydrogenase	7.54	146,200

Source: T. Creighton, *Proteins*, 2d ed. (W. H. Freeman and Company, 1993), Table 7.1.

Table 3-2
Biochemistry, Sixth Edition

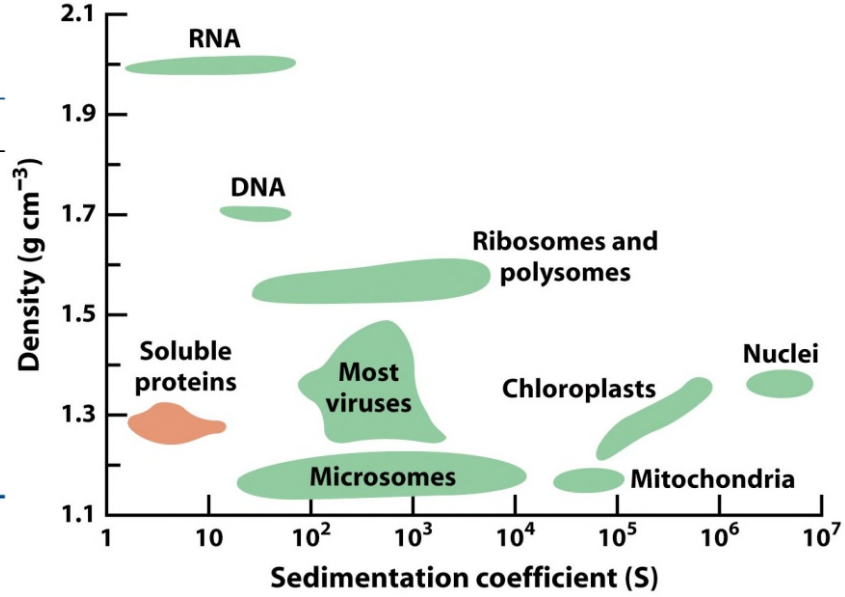


Figure 3-14
Biochemistry, Sixth Edition

- 농도구배 원심분리:
 = 자연상태의 생체분자들의 분자량을 측정할 수 있게함.
  cf) SDS-PAGE 법은 변성된 단백질의 분자량을 측정할 수 있음.

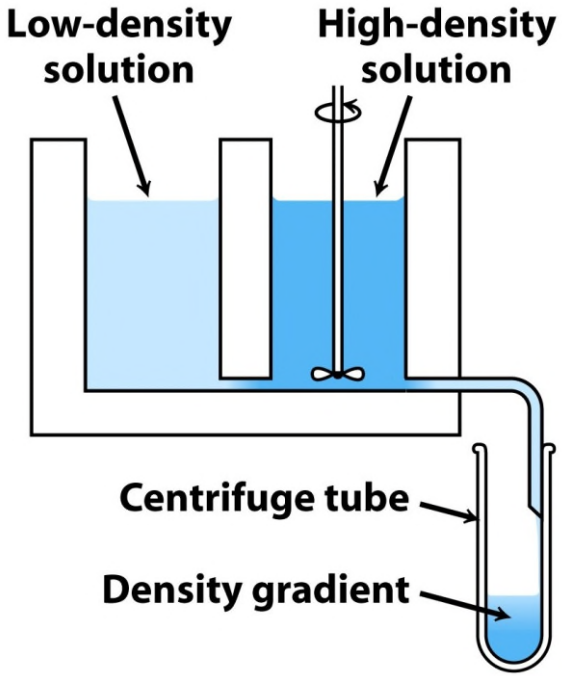


Figure 3-15a
Biochemistry, Sixth Edition

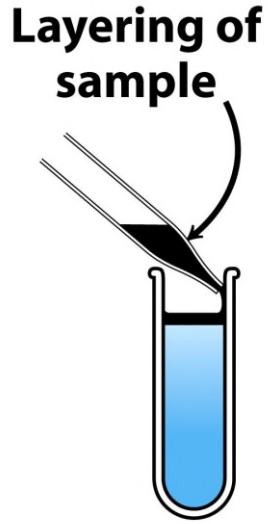


Figure 3-15b
Biochemistry, Sixth Edition

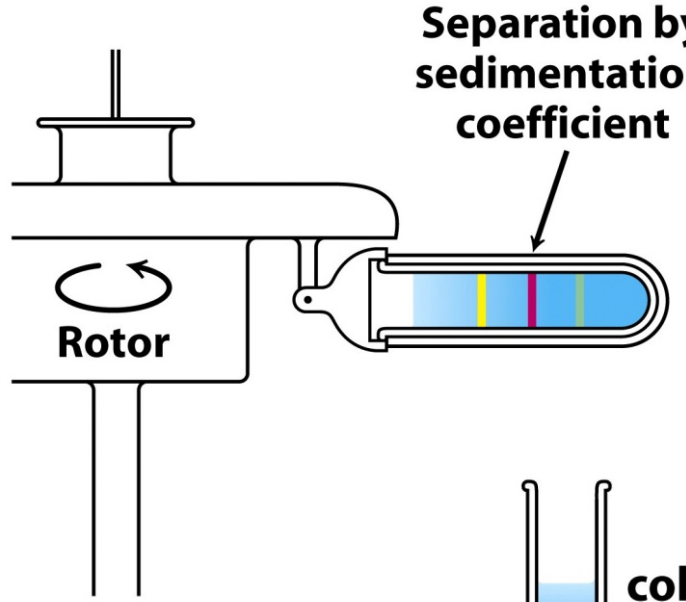


Figure 3-15c
Biochemistry, Sixth Edition

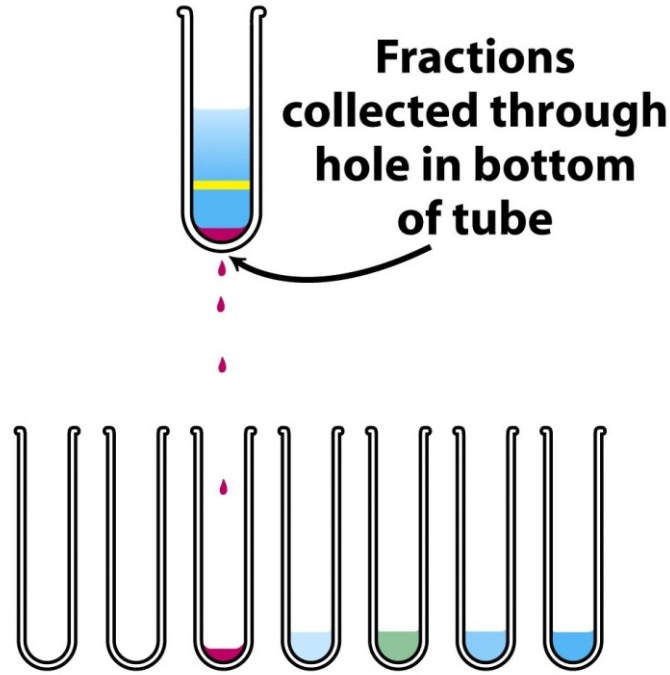


Figure 3-15d
Biochemistry, Sixth Edition

3.3 면역학은 단백질을 연구하는 중요한 기법들을 제공한다.

1. 특정단백질에 대한 항체를 만들 수 있다
   - Antibody: Immunoglobulin G (IgG)
   - Antigen:
     = Epitope (antigenic determinant)
   - 항체의 생산
     = Immunization: inject antigen into rabbit two times (boosting)
     = Antiserum:
     = Polyclonal antibody:

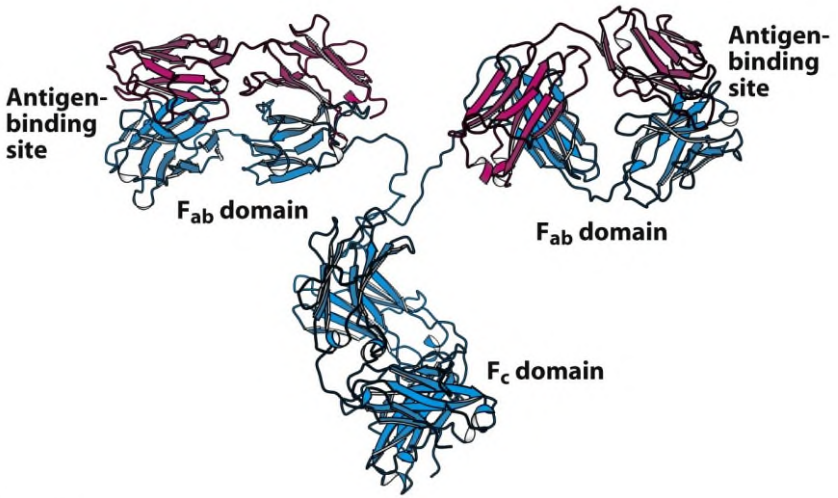


Figure 3-27a
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

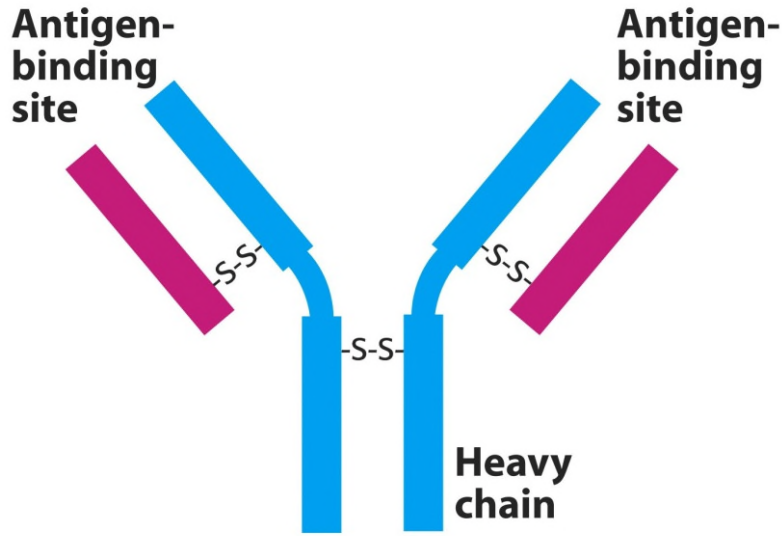


Figure 3-27b
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

Antigen-Antibody reaction

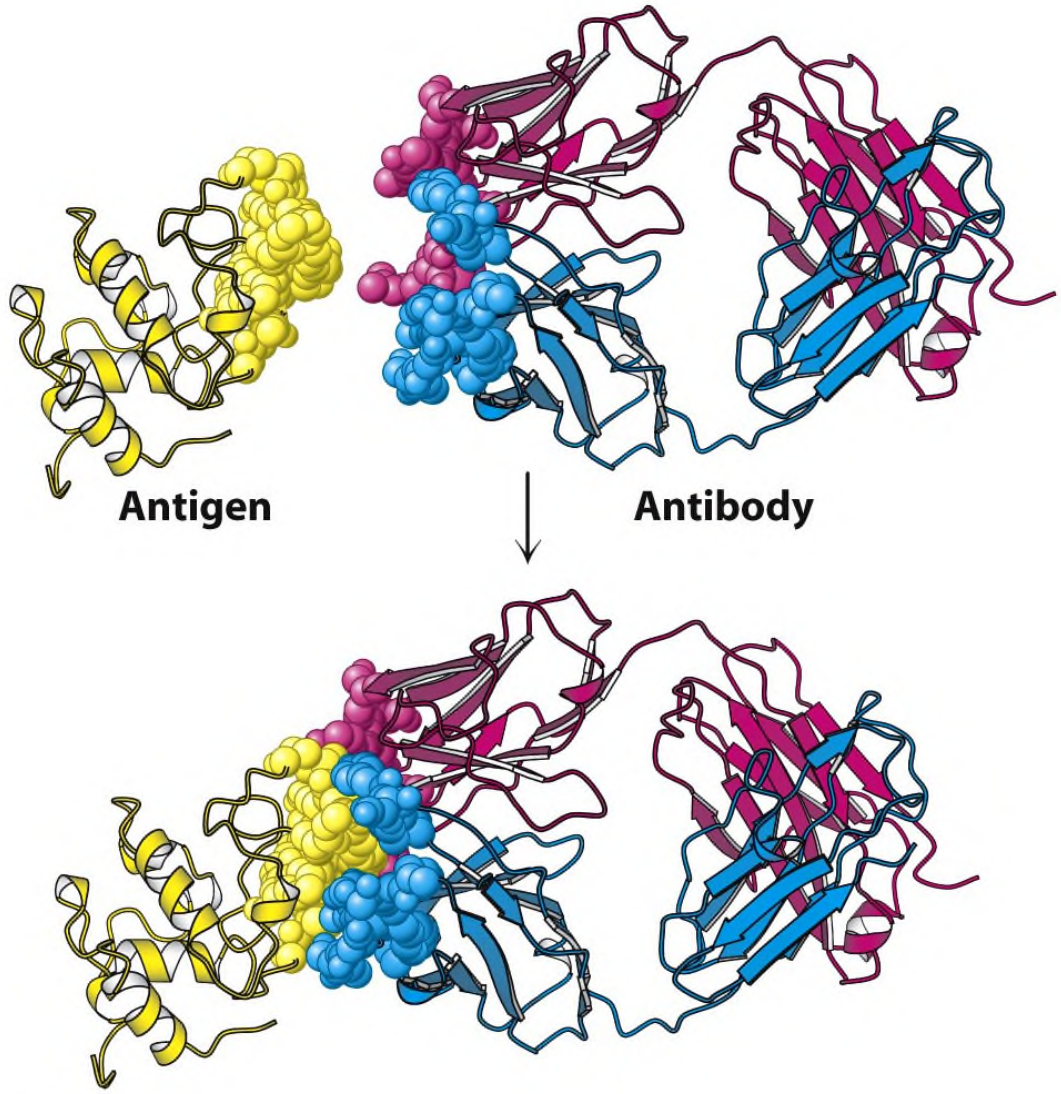


Figure 3-28
Biochemistry, Sixth Edition

2. 단일클론항체 (Monoclonal antibody) 생산
- Single epitope binding antibody

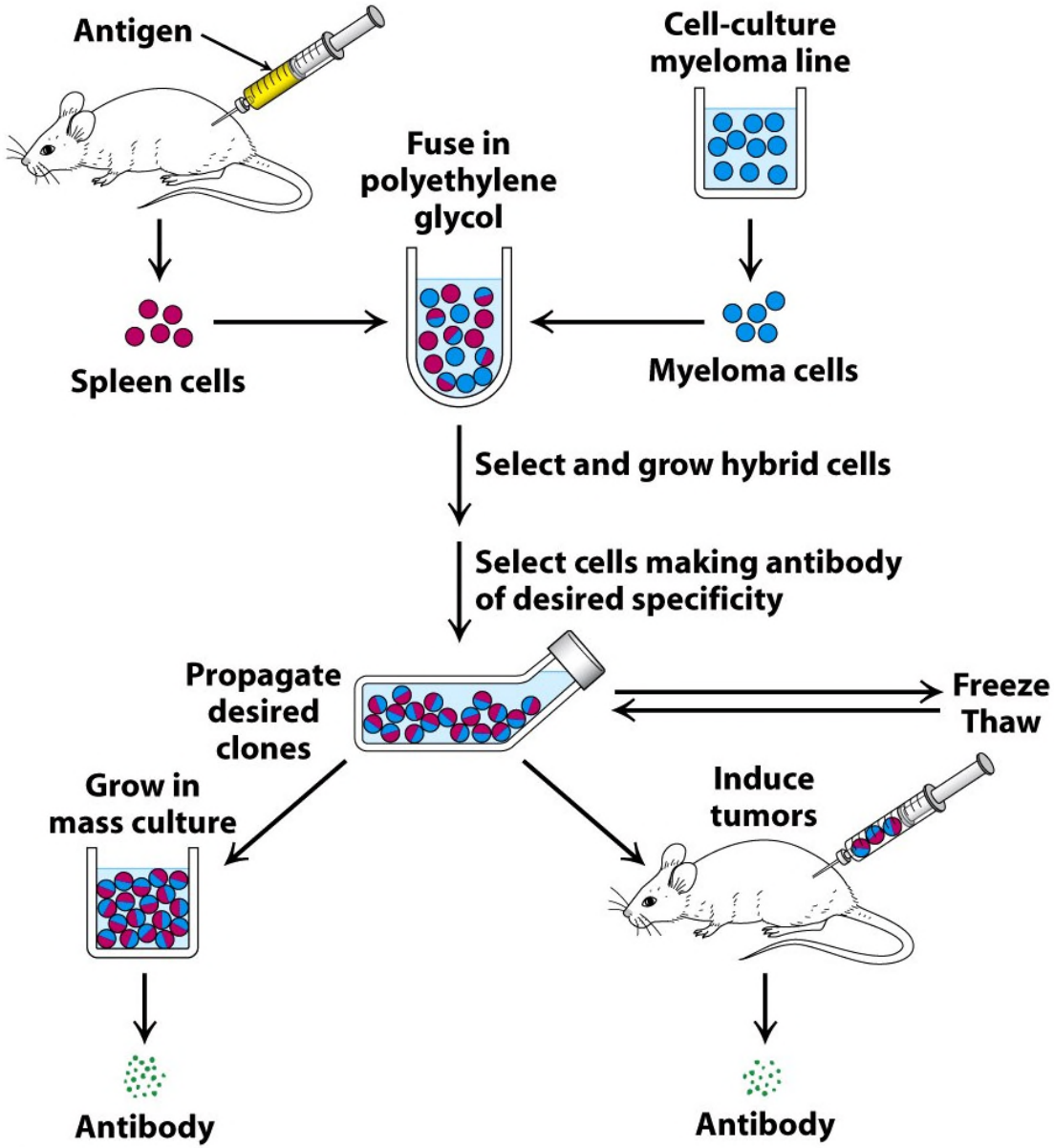


Figure 3-30
Biochemistry, Sixth Edition

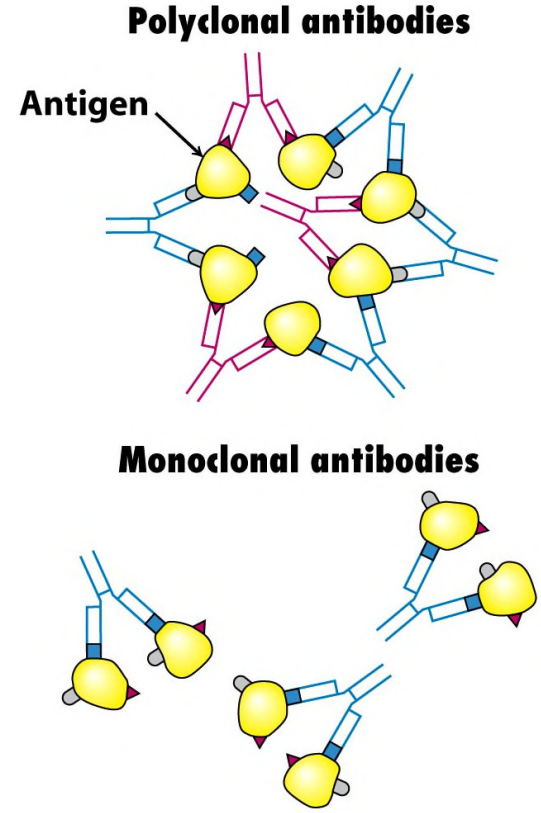


Figure 3-29
Biochemistry, Sixth Edition

Figure 3-31
Biochemistry, Sixth Edition

3. 단백질 검출은 ELISA 기술을 이용하여 가능하다.

- 샘플속에 ng 단백질을 검출할 수 있음
  = AIDS virus 단백질 검출
  = 혈액 중 Insulin 측정
- ELISA (Enzyme Linked Immunosorbent Assay)
  = 간접법: 특정항원에 특이적인 항체의 존재 유무를 측정함
  = Sandwich ELISA: 항원을 직접 측정할 수 있음.

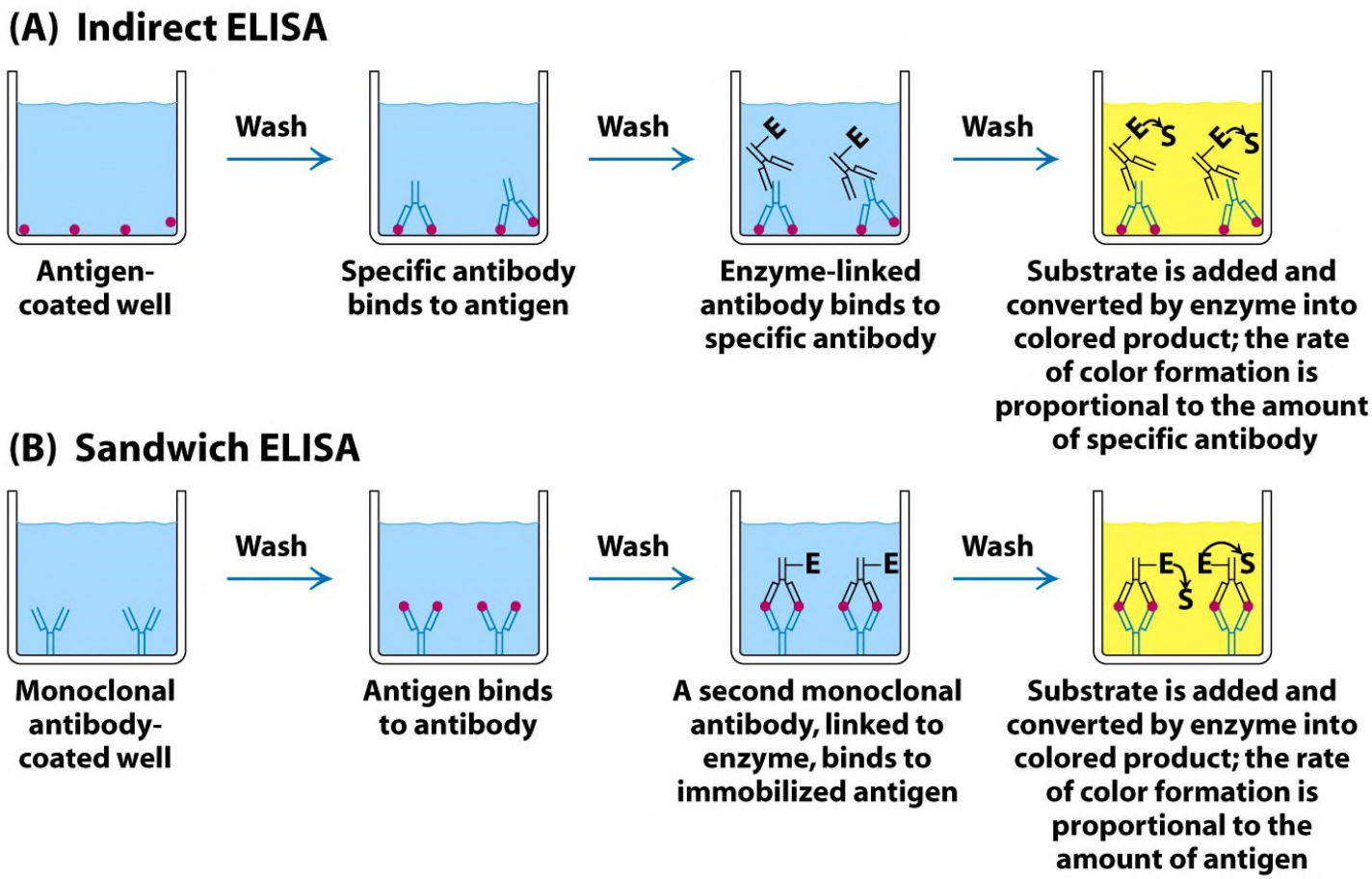


Figure 3-32
Biochemistry, Sixth Edition

4. Western blotting 기술은 gel electrophoresis에 의해 분리된 단백질을 측정할 수 있다.
   - SDS-PAGE: 단백질 크기에 의한 분리
   - 단백질의 검출은 특이항체 (primary Ab)에 의해 결정되고, 그 항체는 anti-IgG 항체 (2nd antibody)에 의해 검출됨

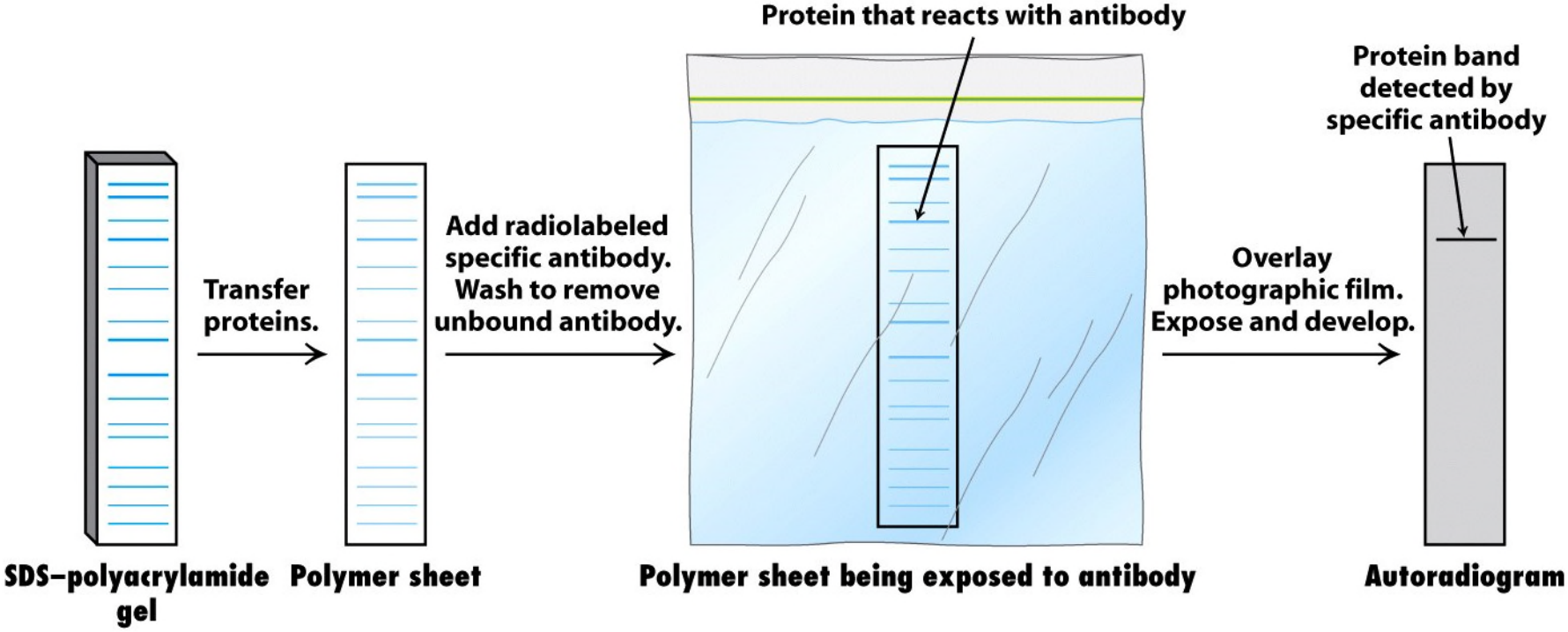


Figure 3-33
Biochemistry, Sixth Edition

5. 형광 표지물은 단백질을 눈으로 확인할 수 있게 해준다.

- 형광물질이 붙어 있는 항체는 세포내 단백질을 검출할 수 있음
  = Fluorescent microscopy
  = Immunohistochemistry
  = Confocal microscopy (공촛점 현미경)
- Green fluorescent protein (GFP): real time protein location

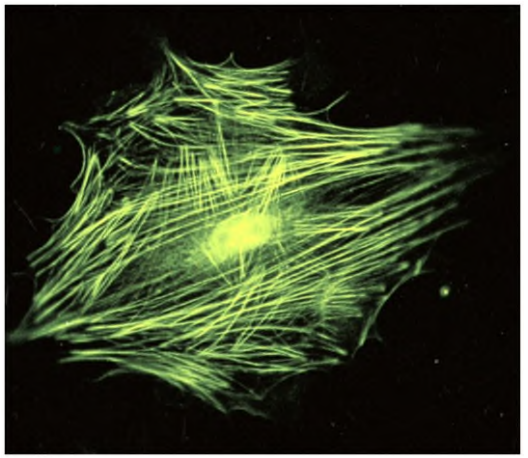


Figure 3-34
Biochemistry, Sixth Edition

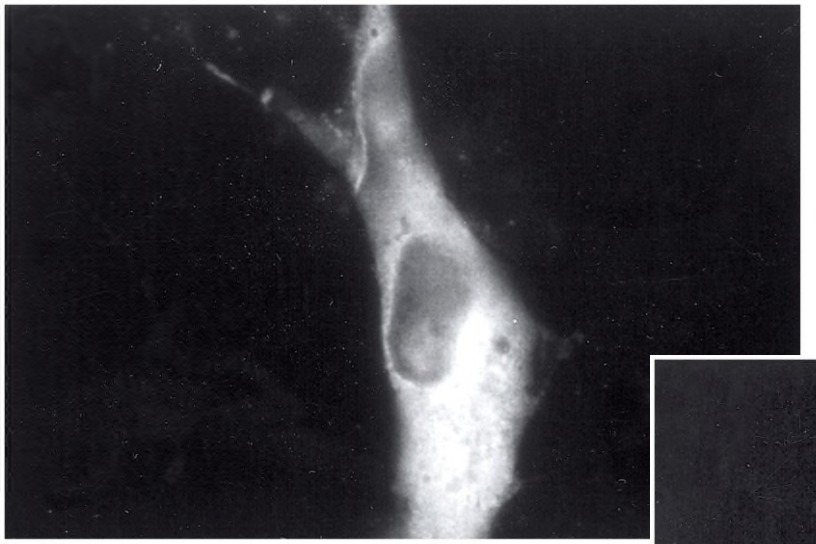


Figure 3-35a
Biochemistry, Sixth Edition

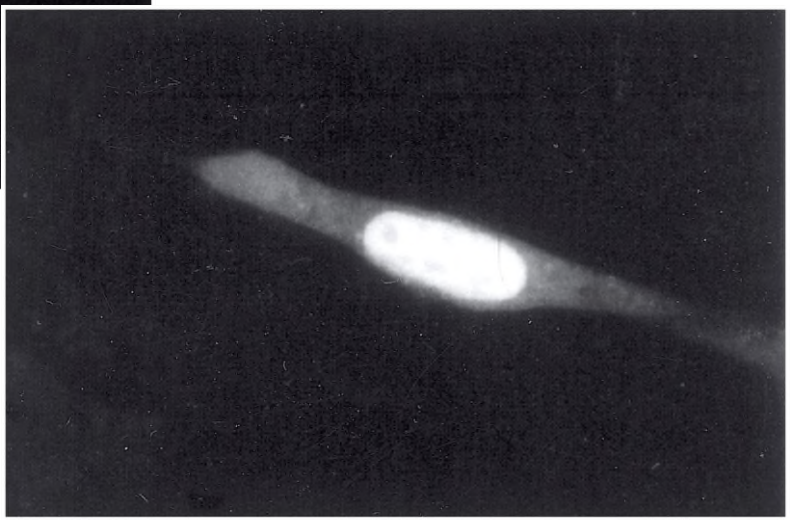


Figure 3-35b
Biochemistry, Sixth Edition

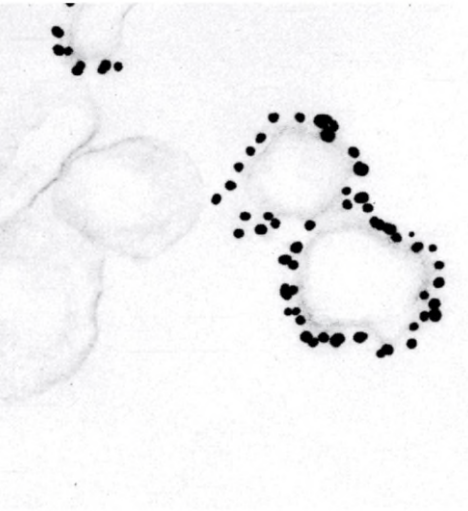


Figure 3-36
Biochemistry, Sixth Edition

3.5 Mass spectrometry

1. The Mass of a protein can be determined by Mass spectrometry.
 - Ionization method
  = Matrix-Assisted Laser Desorption-Ionization (MALDI)
  = Electrospray ionization (ESI):

 - Separation of mass (determine the mass of proteins or peptide):
  = Time of flight (TOF)

 - Mass spectrometry: MALDI-TOF

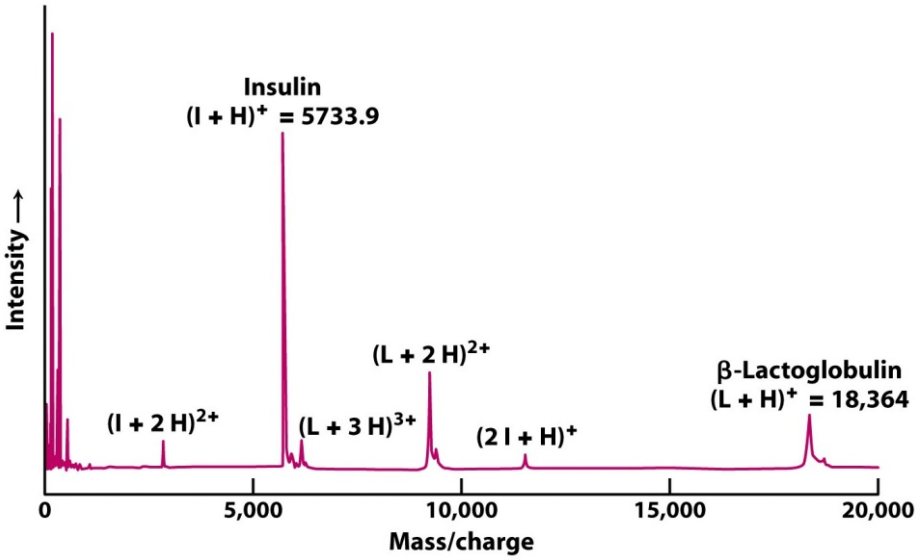


Figure 3-41
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

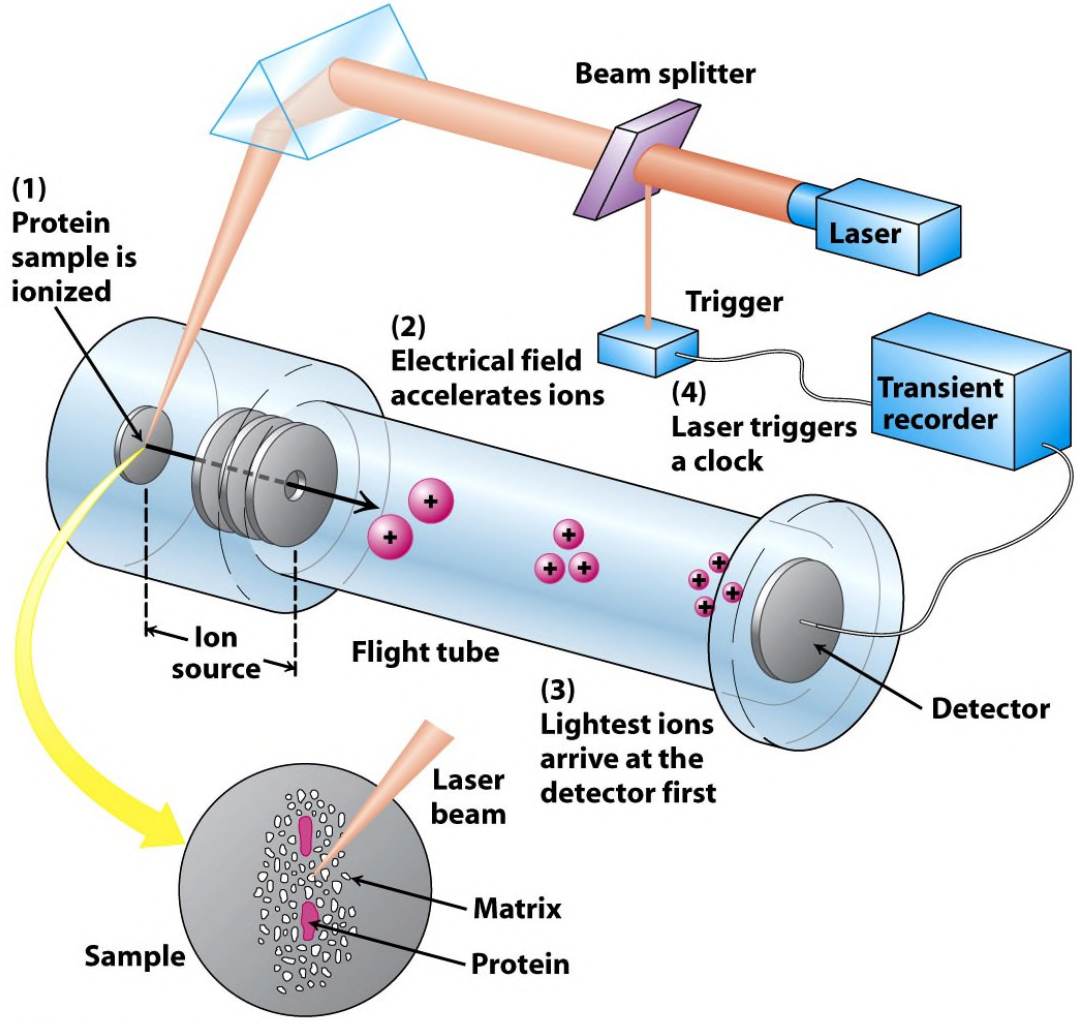


Figure 3-40
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

3.6 3차원 단백질의 구조는 X-ray 결정학과 NMR 분광법으로 결정될 수 있다.

1. X-ray 결정학은 단백질의 3차 구조를 밝힐 수 있음.
   - Protein crystal: depends on the proteins
   - Source of X-ray: x-ray generator or synchrotron radiation
   - Detector: Detect the scattered beam.
   - Electron density determination

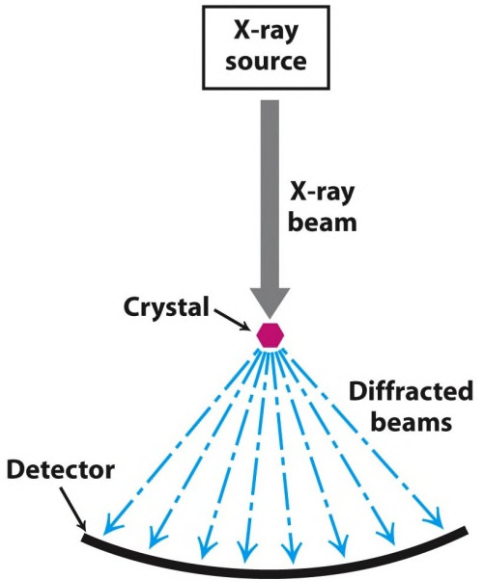


Figure 3-43
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

Figure 3-44
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

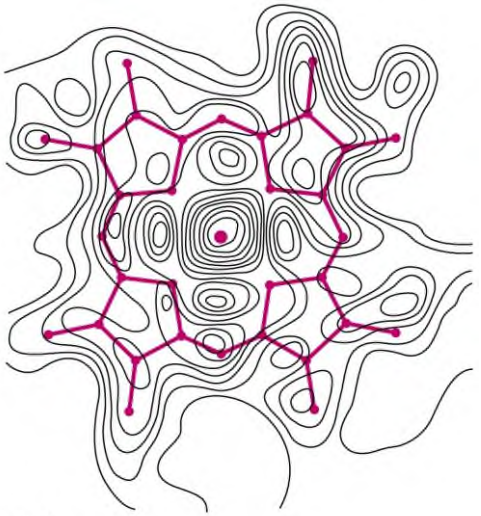
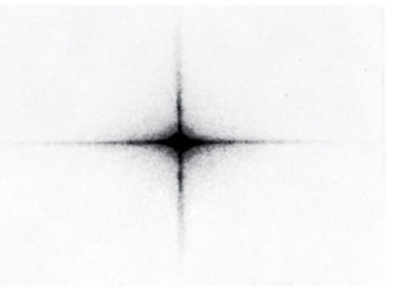


Figure 3-45

Resolution is depended on wave length



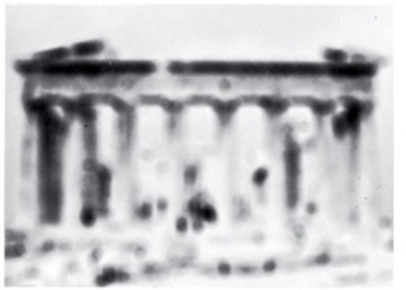


Figure 3-47
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

2. NMR 분광법은 용액속에 존재하는 단백질을 검출할 수 있음.

- Calculate the hydrogen atom distribution in molecules.

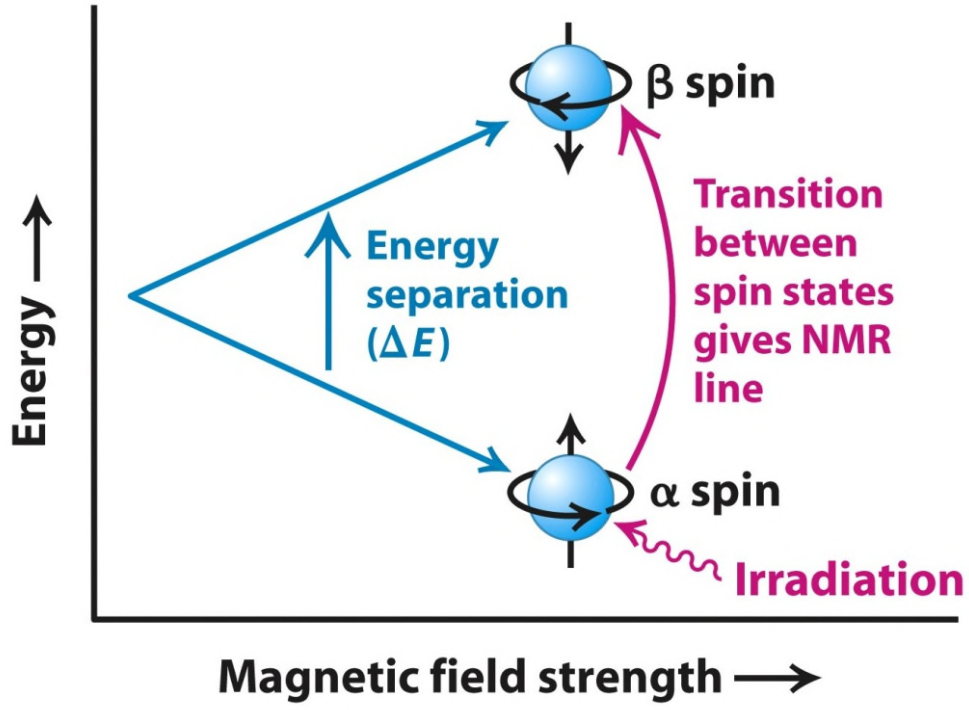


Figure 3-48
Biochemistry, Sixth Edition