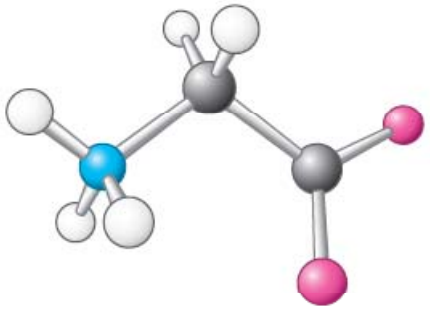
Chapter 3

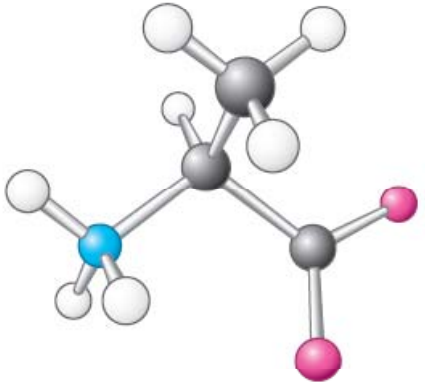
Amino acids, peptides, and proteins

Ball-and-stick models show the arrangement of atoms and bonds in space.

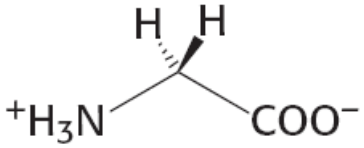
Glycine (Gly, G)

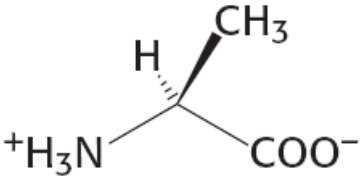


Alanine (Ala, A)

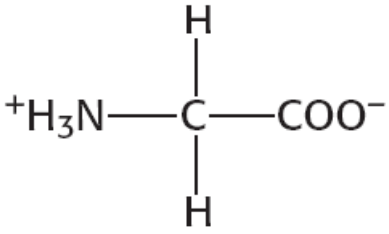


Stereochemically realistic formulas show the geometrical arrangement of bonds around atoms

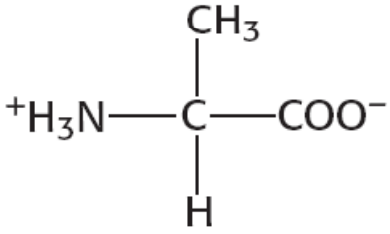




Fischer projections



Glycine (Gly, G)



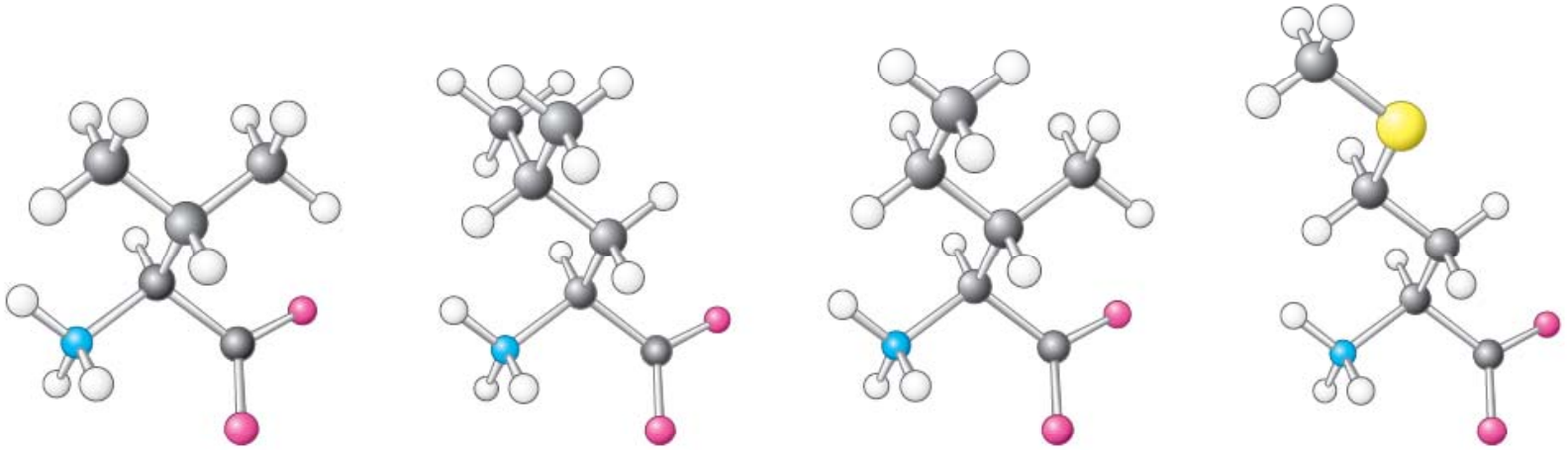
Alanine (Ala, A)

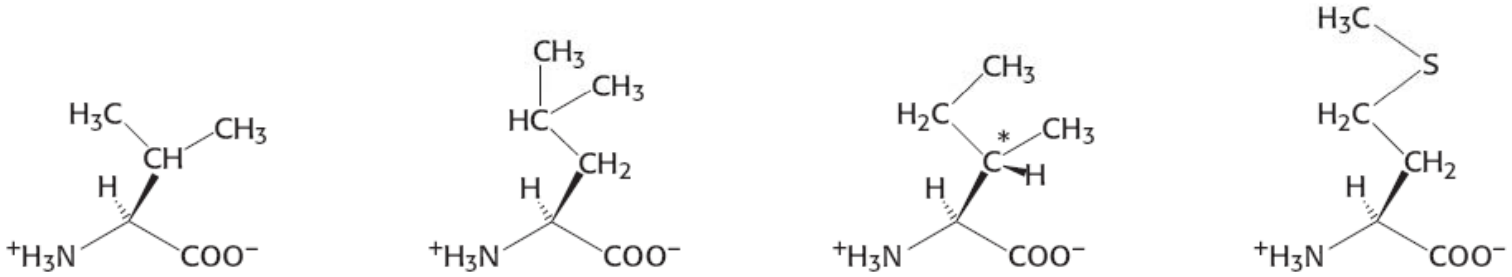
Valine
(Val, V)

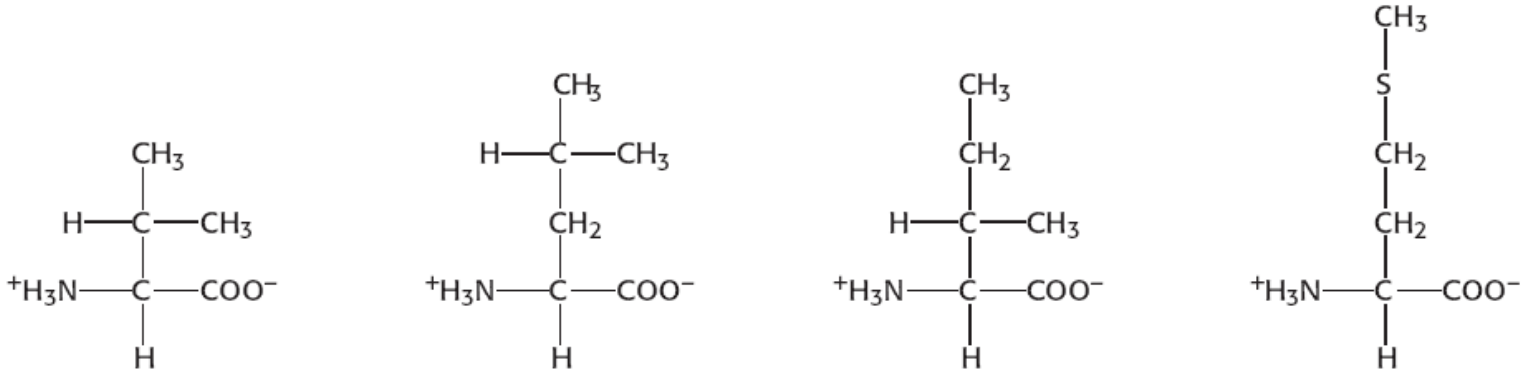
Leucine
(Leu, L)

Isoleucine
(Ile, I)

Methionine
(Met, M)





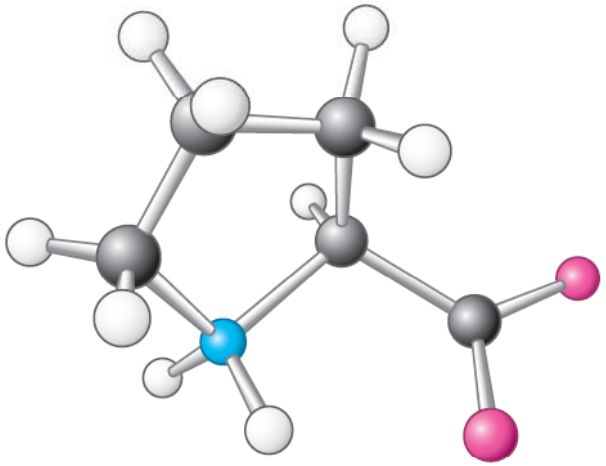


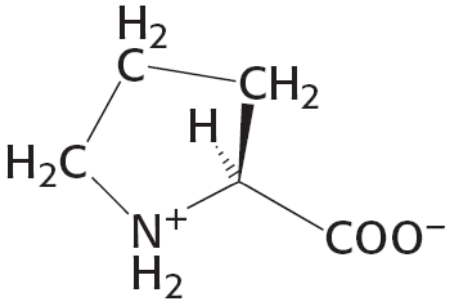
Valine
(Val, V)

Leucine
(Leu, L)

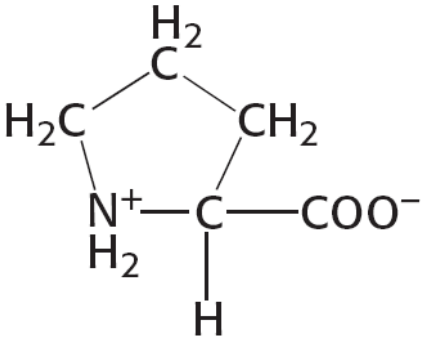
Isoleucine
(Ile, I)

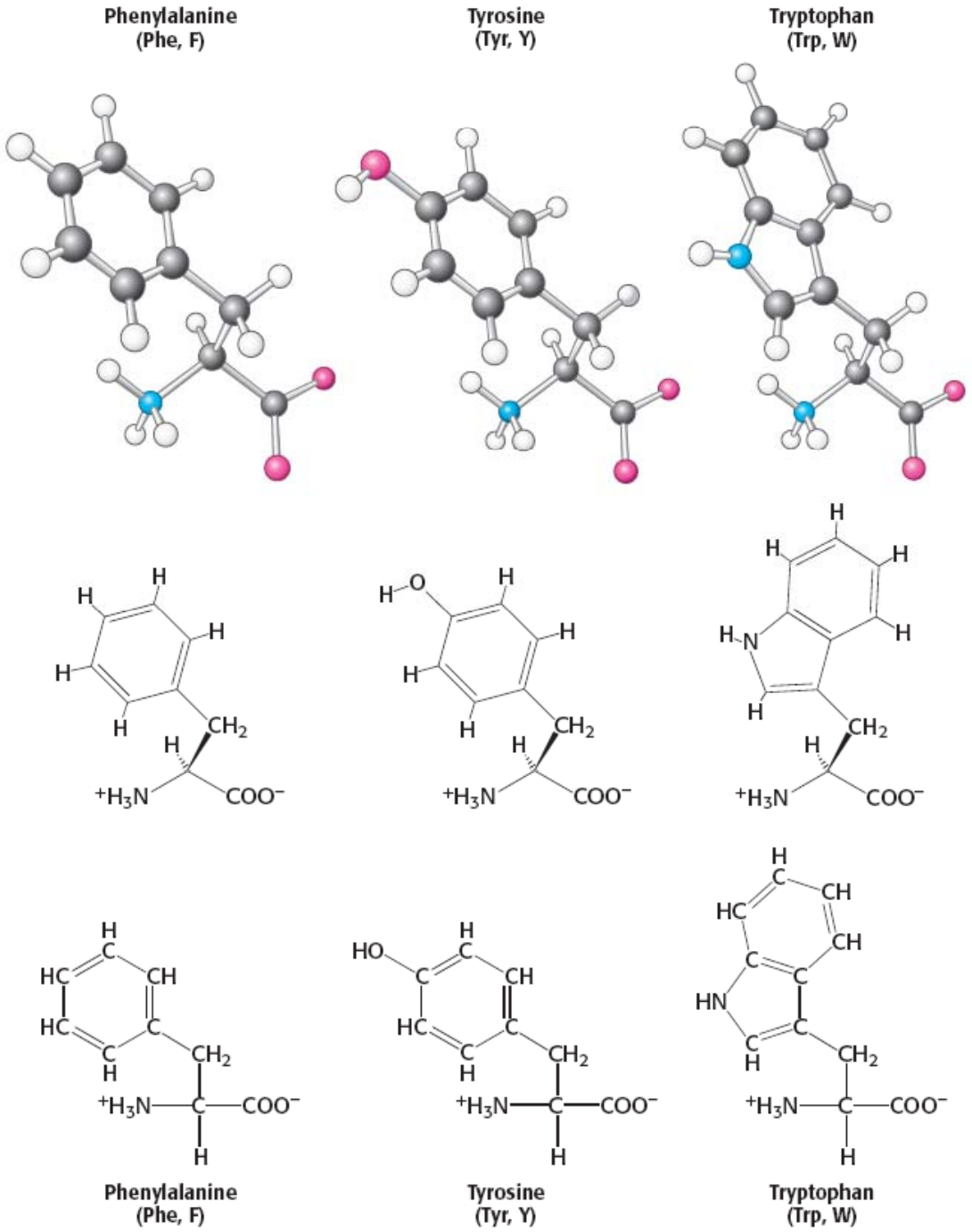
Methionine
(Met, M)





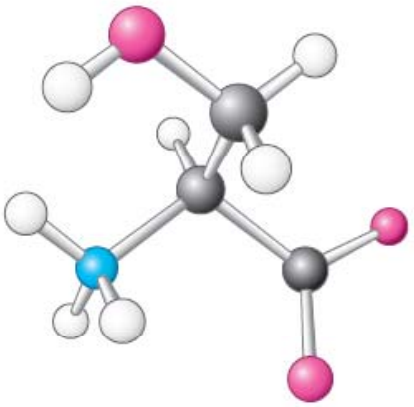
Proline
(Pro, P)

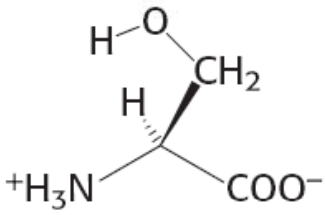


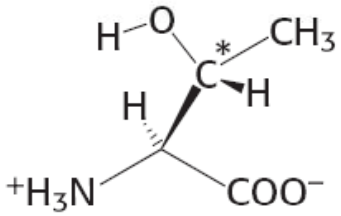

Phenylalanine
(Phe, F)
Tyrosine
(Tyr, Y)
Tryptophan
(Trp, W)
Phenylalanine
(Phe, F)
Tyrosine
(Tyr, Y)
Tryptophan
(Trp, W)

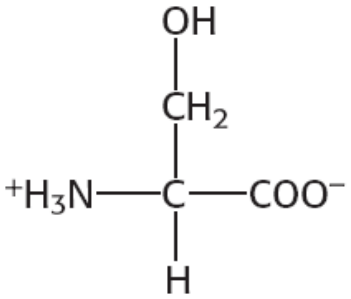
Serine
(Ser, S)

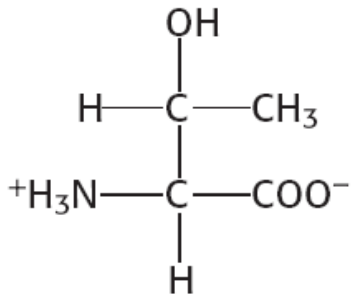
Threonine
(Thr, T)





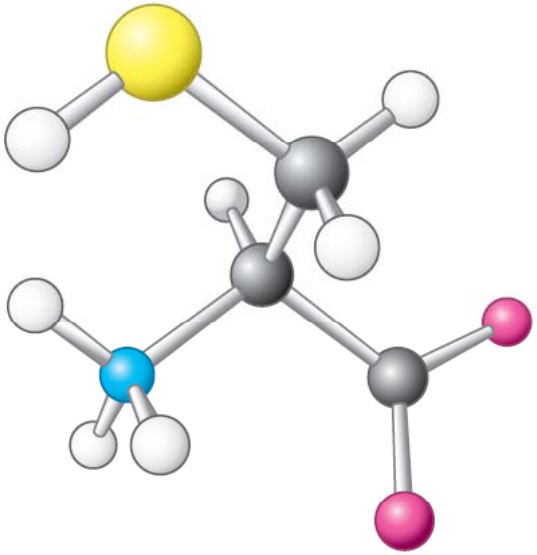


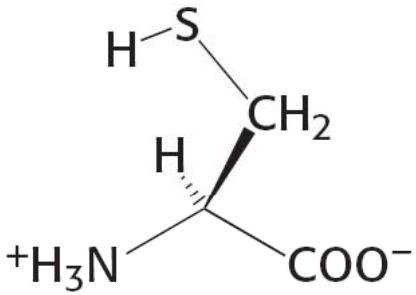




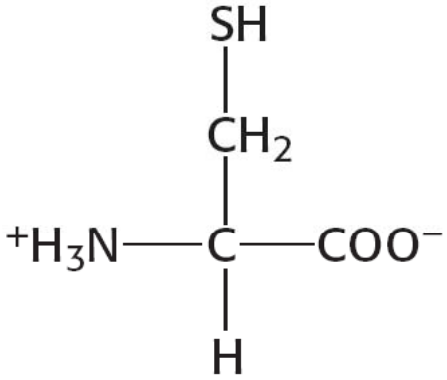
Serine
(Ser, S)

Threonine
(Thr, T)

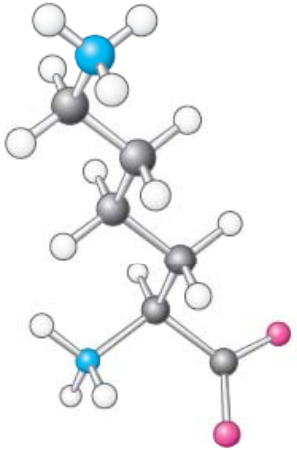




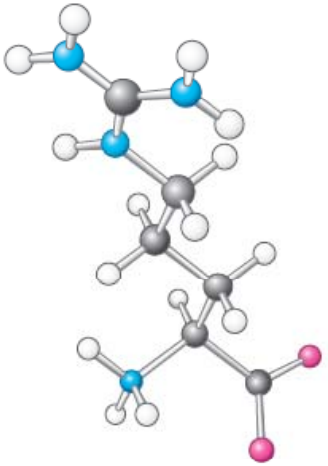
Cysteine
(Cys, C)



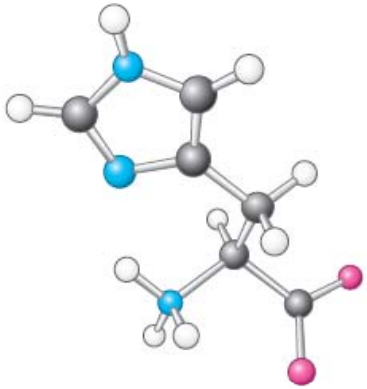
Lysine
(Lys, K)

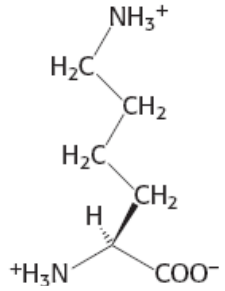


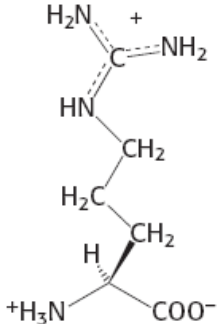
Arginine
(Arg, R)

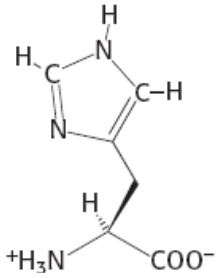


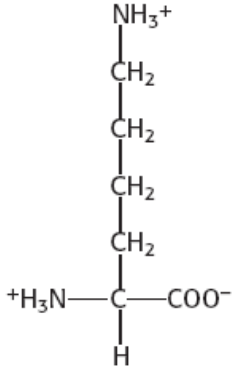
Histidine
(His, H)



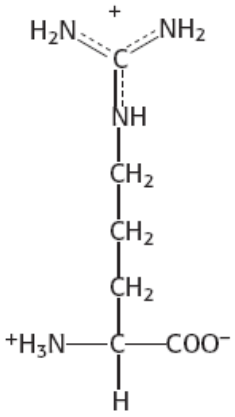




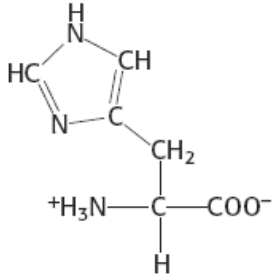




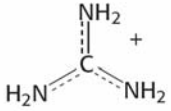
Lysine
(Lys, K)



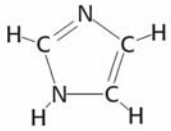
Arginine
(Arg, R)



Histidine
(His, H)



Guanidinium



Imidazole

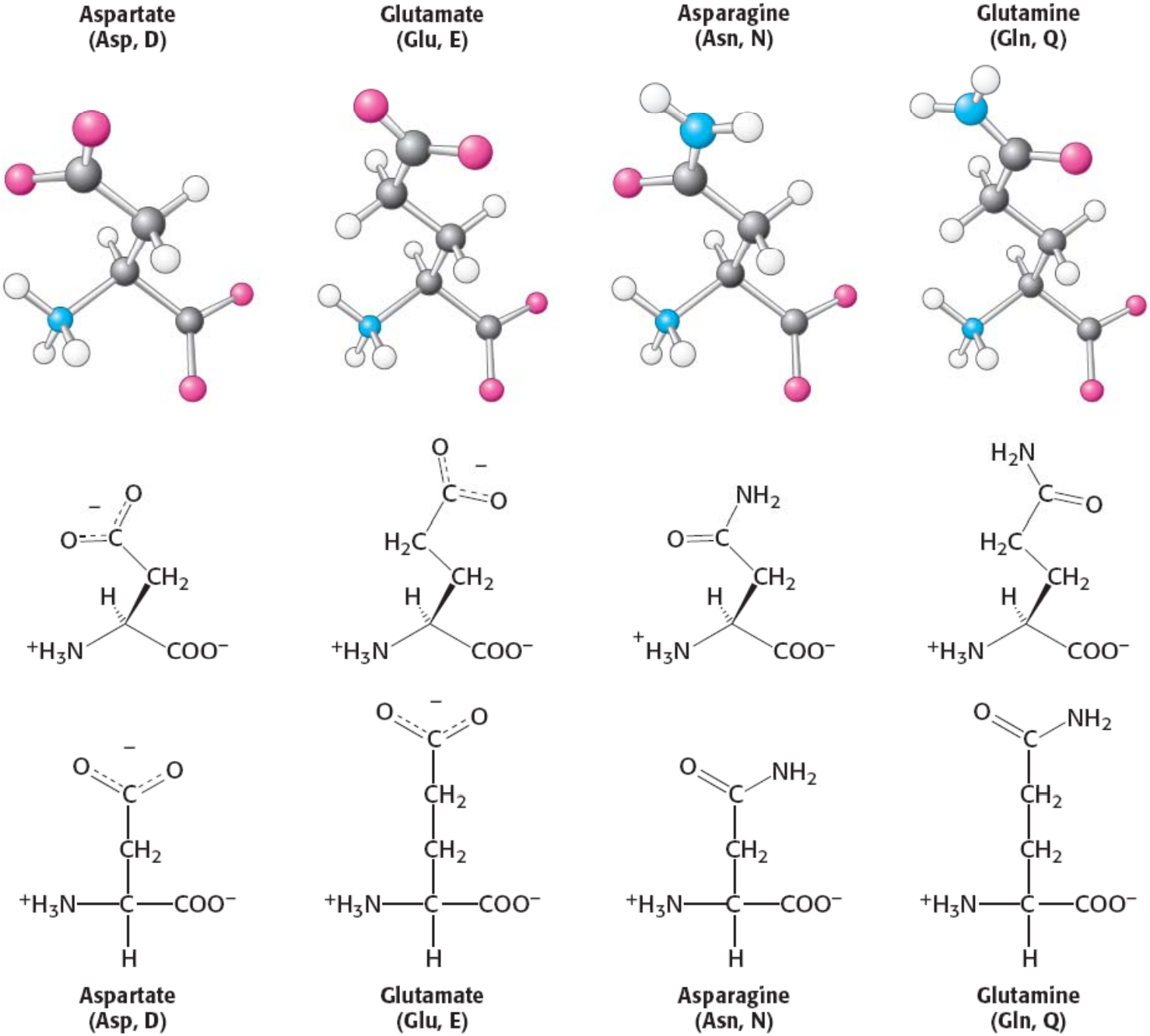
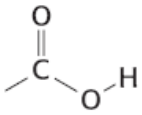
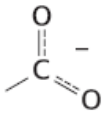
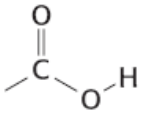
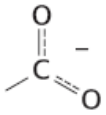
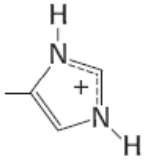
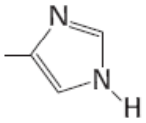
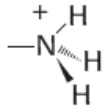
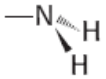
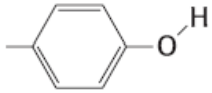
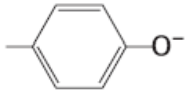
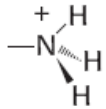
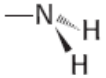
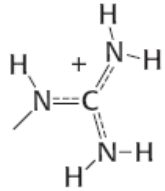
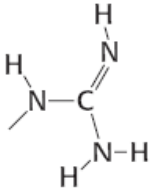

Aspartate
(Asp, D)
Glutamate
(Glu, E)
Asparagine
(Asn, N)
Glutamine
(Gln, Q)
Aspartate
(Asp, D)
Glutamate
(Glu, E)
Asparagine
(Asn, N)
Glutamine
(Gln, Q)

TABLE 3.1 Typical pK_a values of ionizable groups in proteins

Group	Acid		Base	Typical pK_a*
Terminal α-carboxyl group		⇌		3.1
Aspartic acid Glutamic acid		⇌		4.1
Histidine		⇌		6.0
Terminal α-amino group		⇌		8.0
Cysteine		⇌		8.3
Tyrosine		⇌		10.9
Lysine		⇌		10.8
Arginine		⇌		12.5

*pK_a values depend on temperature, ionic strength, and the microenvironment of the ionizable group.

TABLE 3.2 **Abbreviations for amino acids**

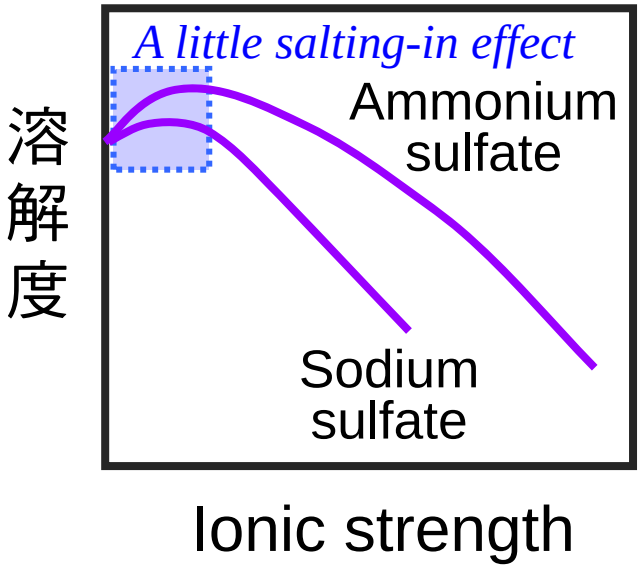
Amino acid	Three-letter abbreviation	One-letter abbreviation	Amino acid	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A	Methionine	Met	M
Arginine	Arg	R	Phenylalanine	Phe	F
Asparagine	Asn	N	Proline	Pro	P
Aspartic Acid	Asp	D	Serine	Ser	S
Cysteine	Cys	C	Threonine	Thr	T
Glutamine	Gln	Q	Tryptophan	Trp	W
Glutamic Acid	Glu	E	Tyrosine	Tyr	Y
Glycine	Gly	G	Valine	Val	V
Histidine	His	H	Asparagine or aspartic acid	Asx	B
Isoleucine	Ile	I			
Leucine	Leu	L	Glutamine or glutamic acid	Glx	Z
Lysine	Lys	K			

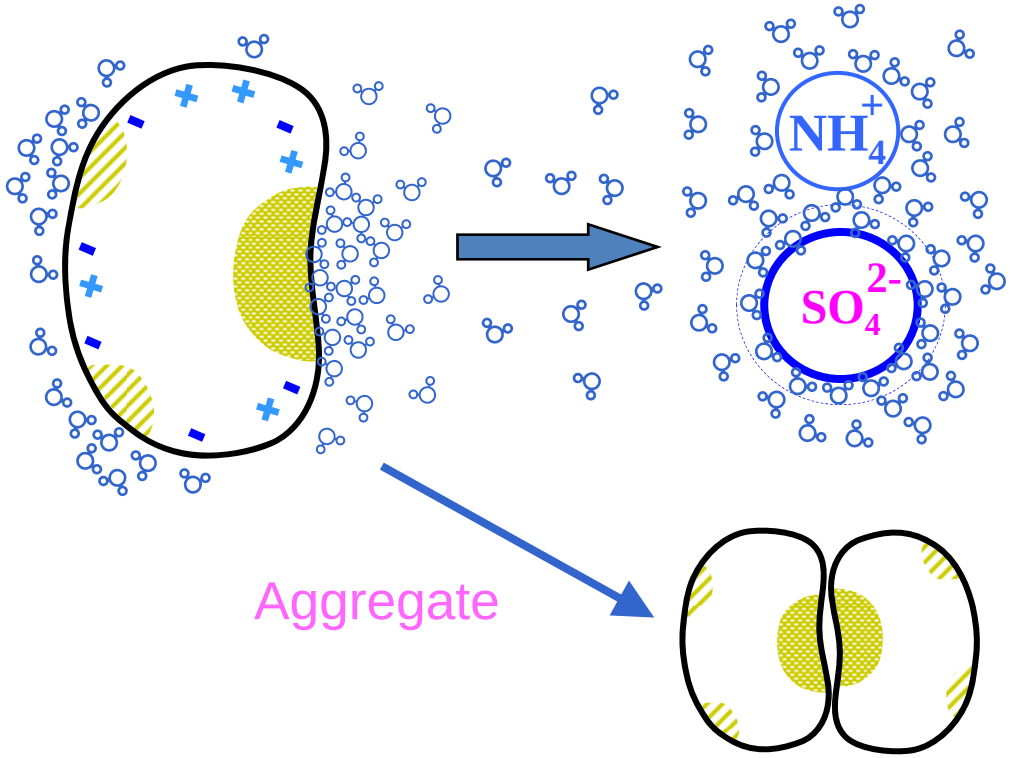
Chapter 3

3.3 Working with proteins

鹽析 Salting-out ：硫酸銨沈澱

Ammonium sulfate precipitation

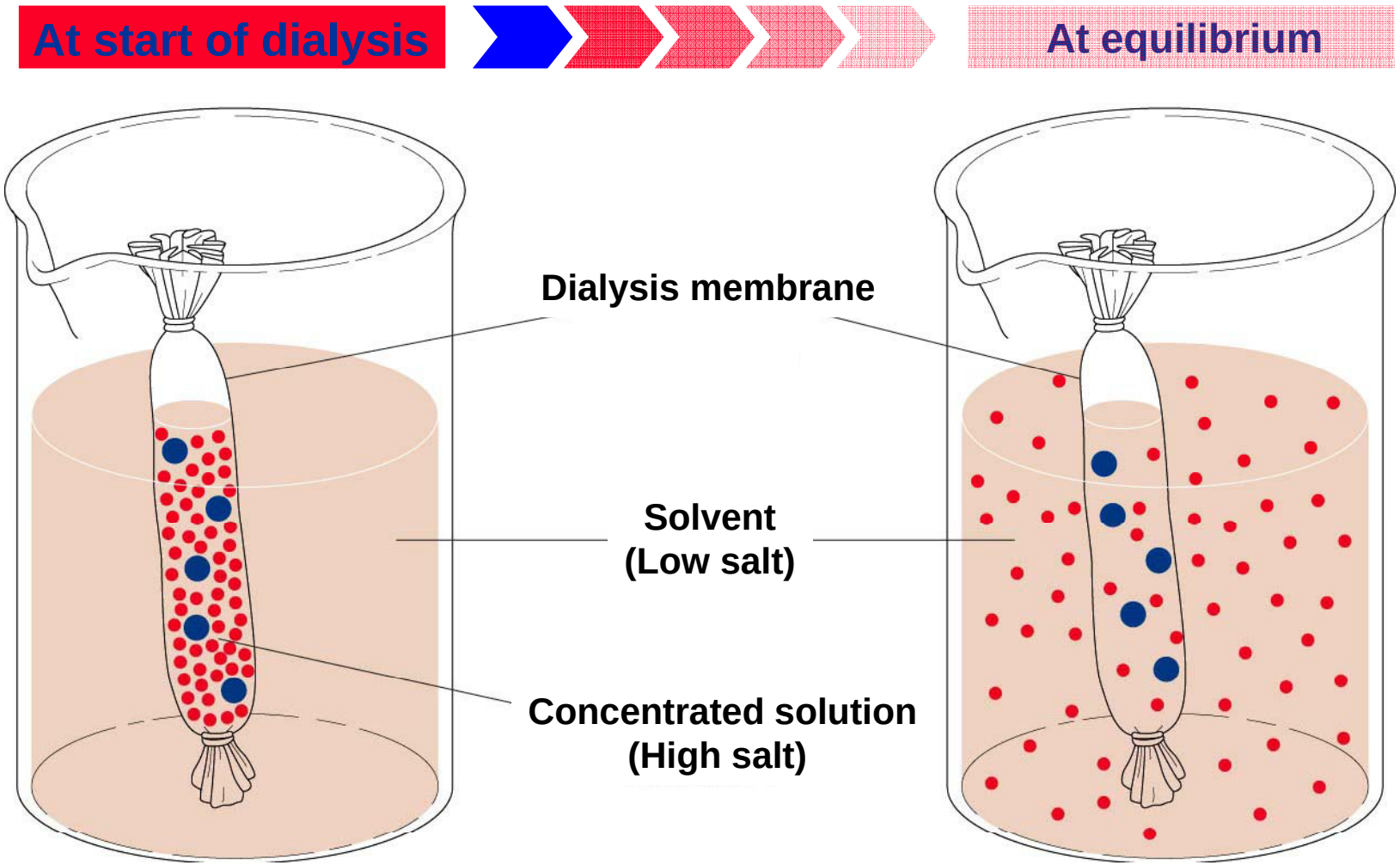




蛋白質分子表面的疏水性區域，都聚集許多水分子，當鹽類加入時，這些水分子被抽出，以便與鹽離子進行水合，暴露出來的疏水性區域互相結合，形成沈澱。

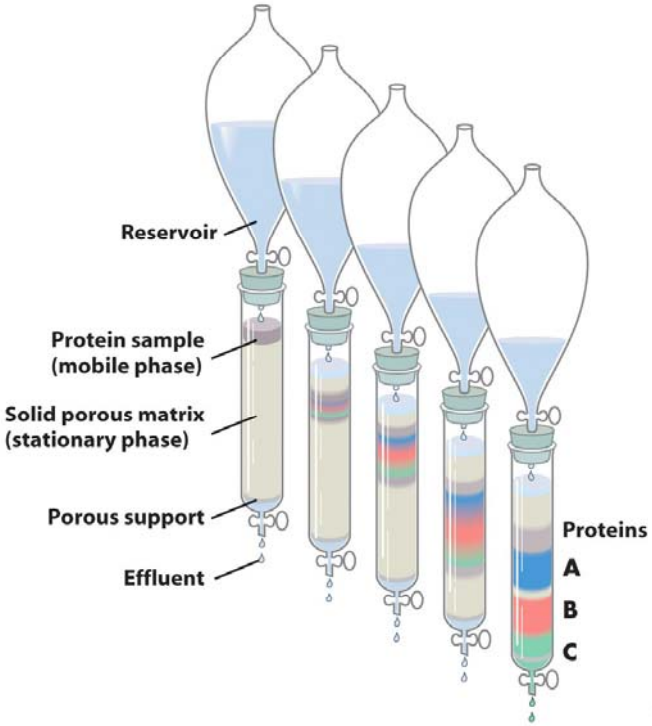
= hydrophobic

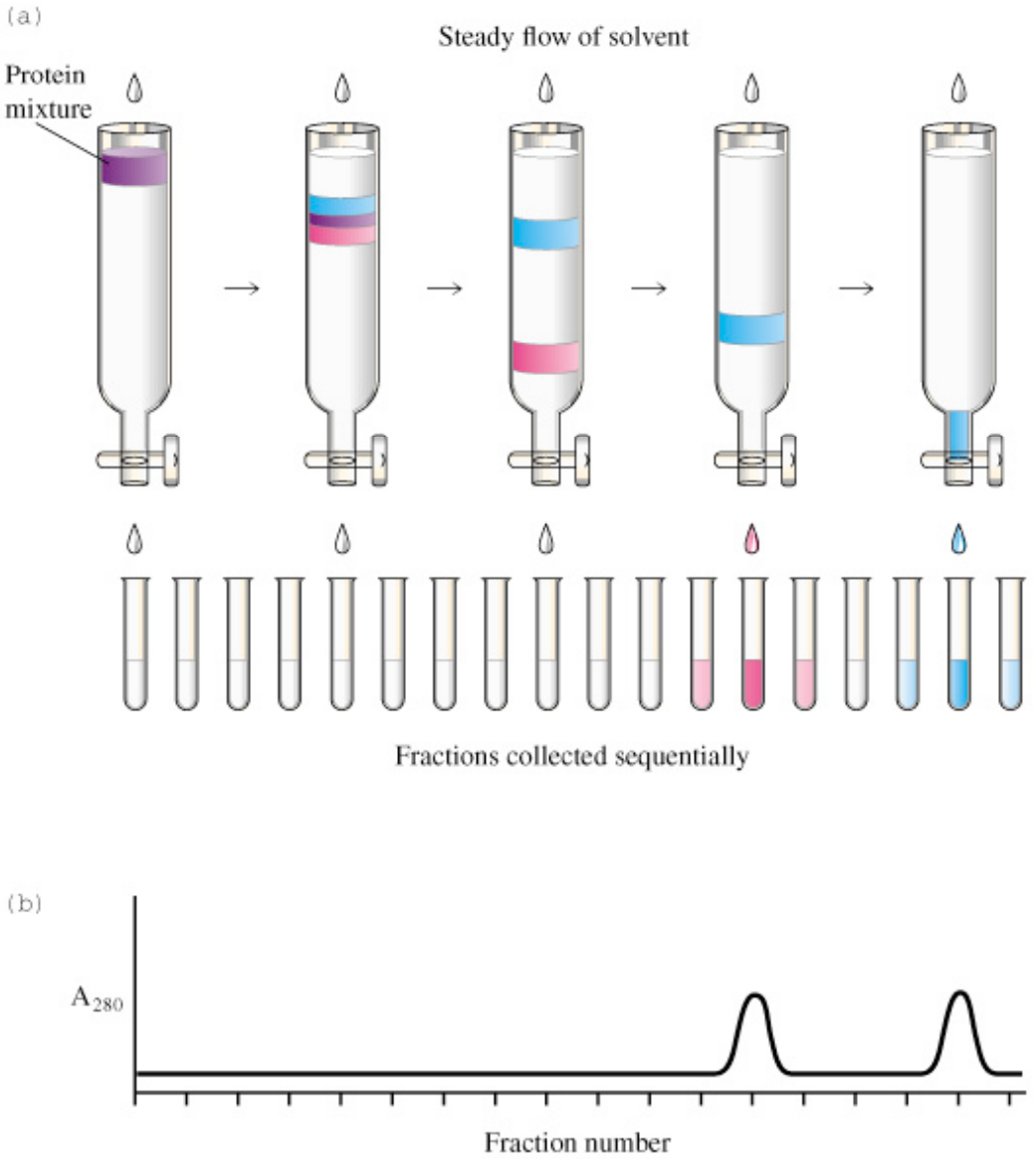
透析(dialysis)：desalting and exchange buffer



MWCO: molecular weight cut-off

Column chromatography

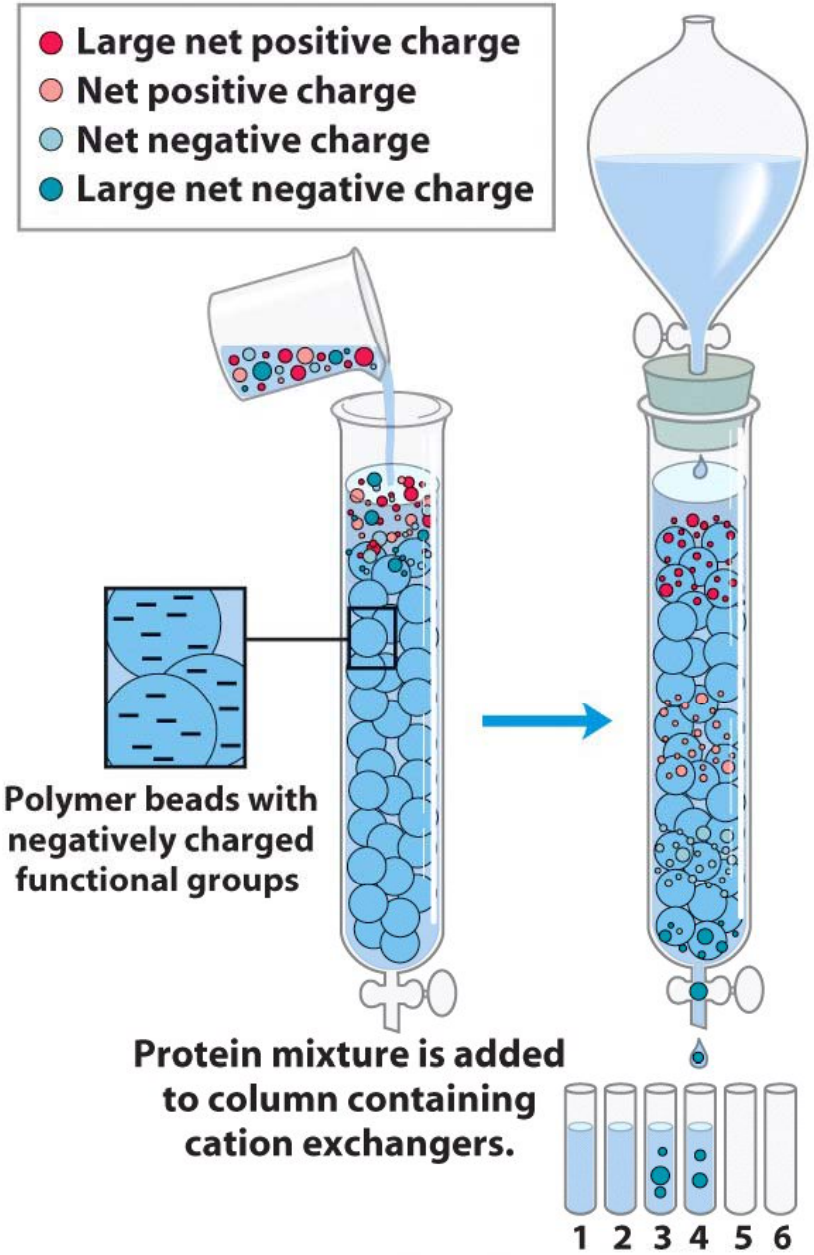




P. 86

Principles of biochemistry, 3rd edition, Horton, Moran, Ochs , Rawn, Scrimgeour

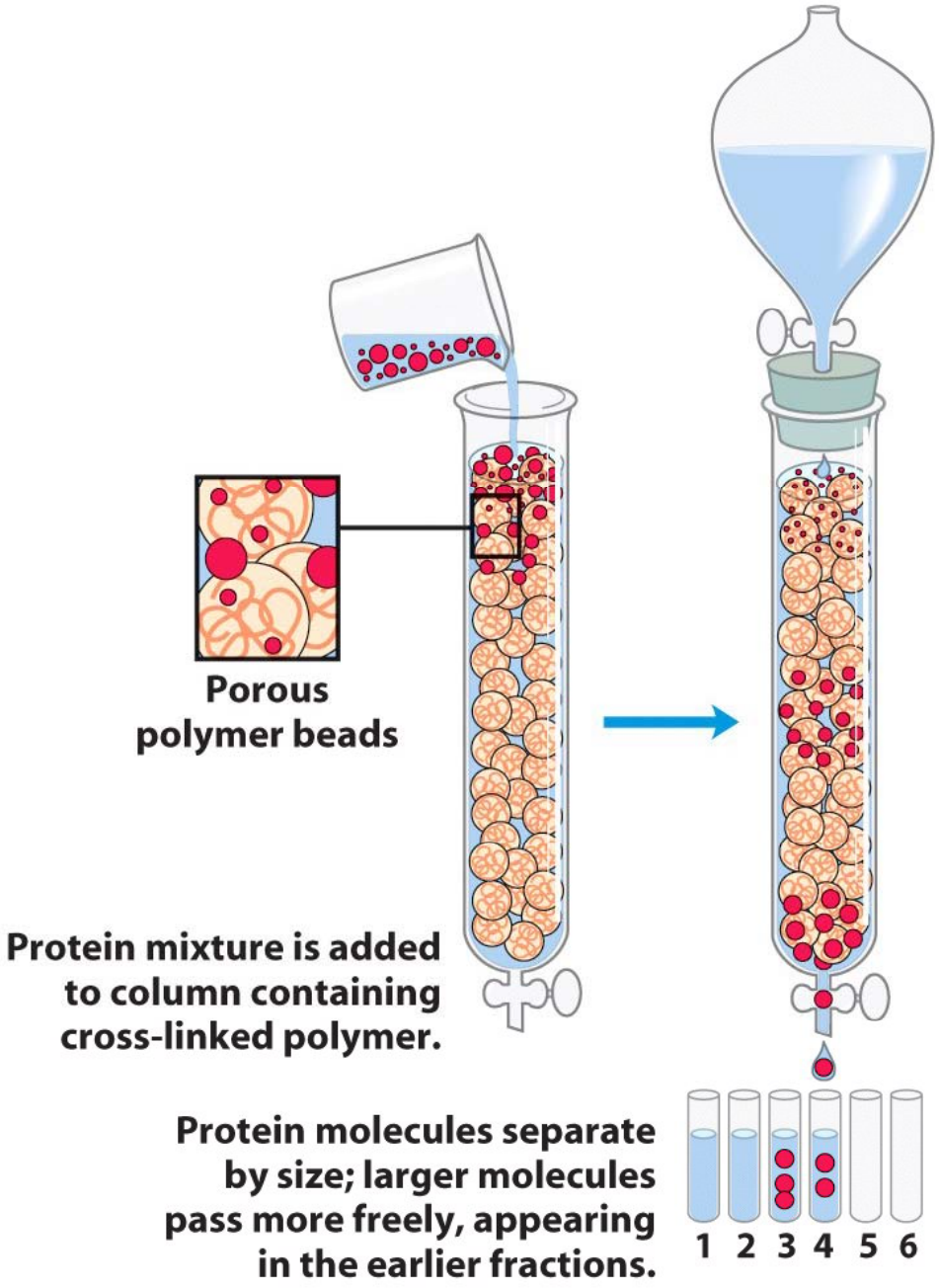
Ion exchange chromatography



- based upon the overall charge of molecules

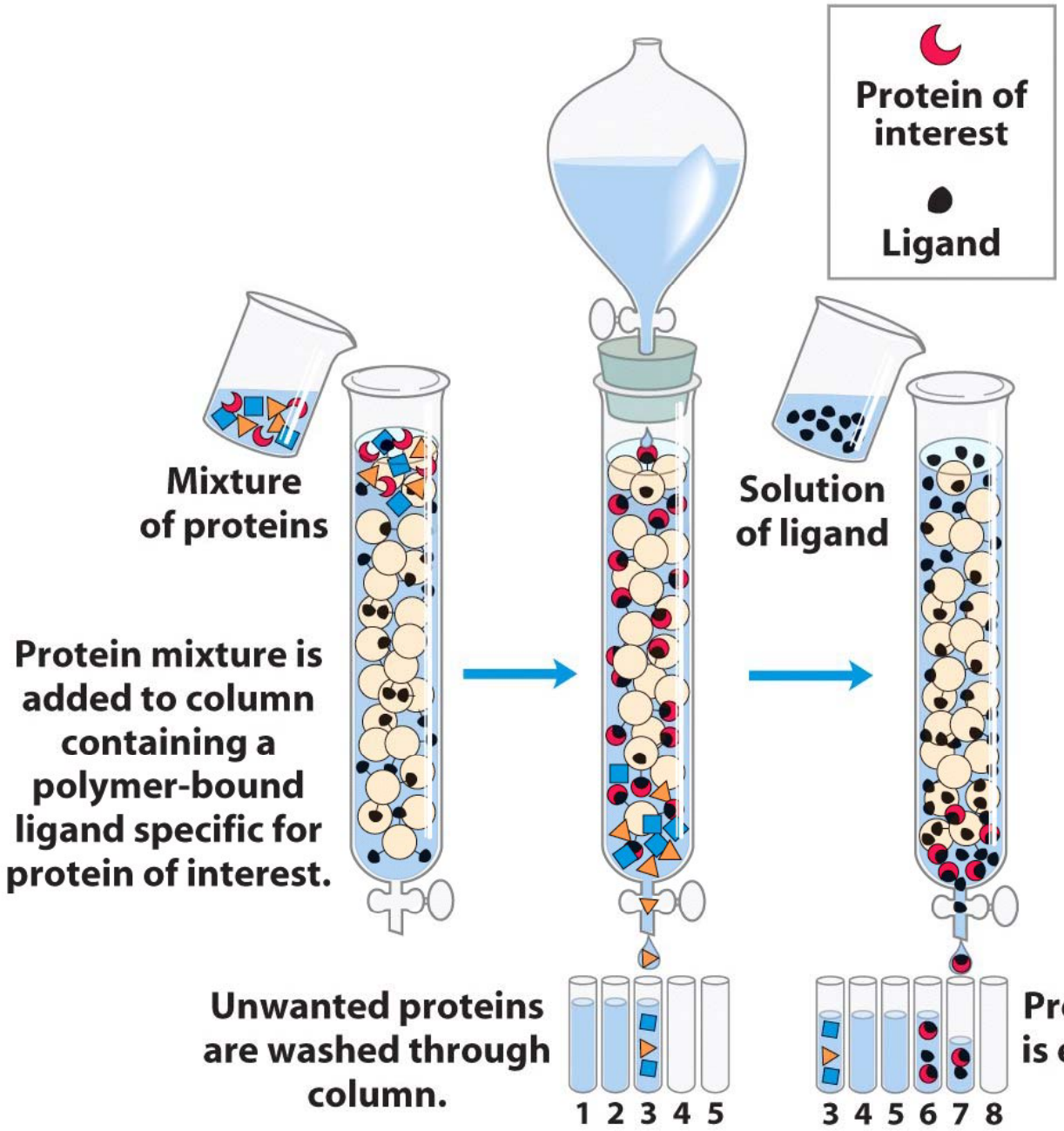
P. 87

Size-exclusion chromatography



- based upon molecular size

Affinity chromatography



- **specific binding interactions between column matrix and target proteins**

Purification table 純化表

純化流程 *Procedure or step*	總體積 *Fraction volume (ml)*	總蛋白質量 *Total protein (mg)*	總活性 *Activity (units)*	比活性 *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

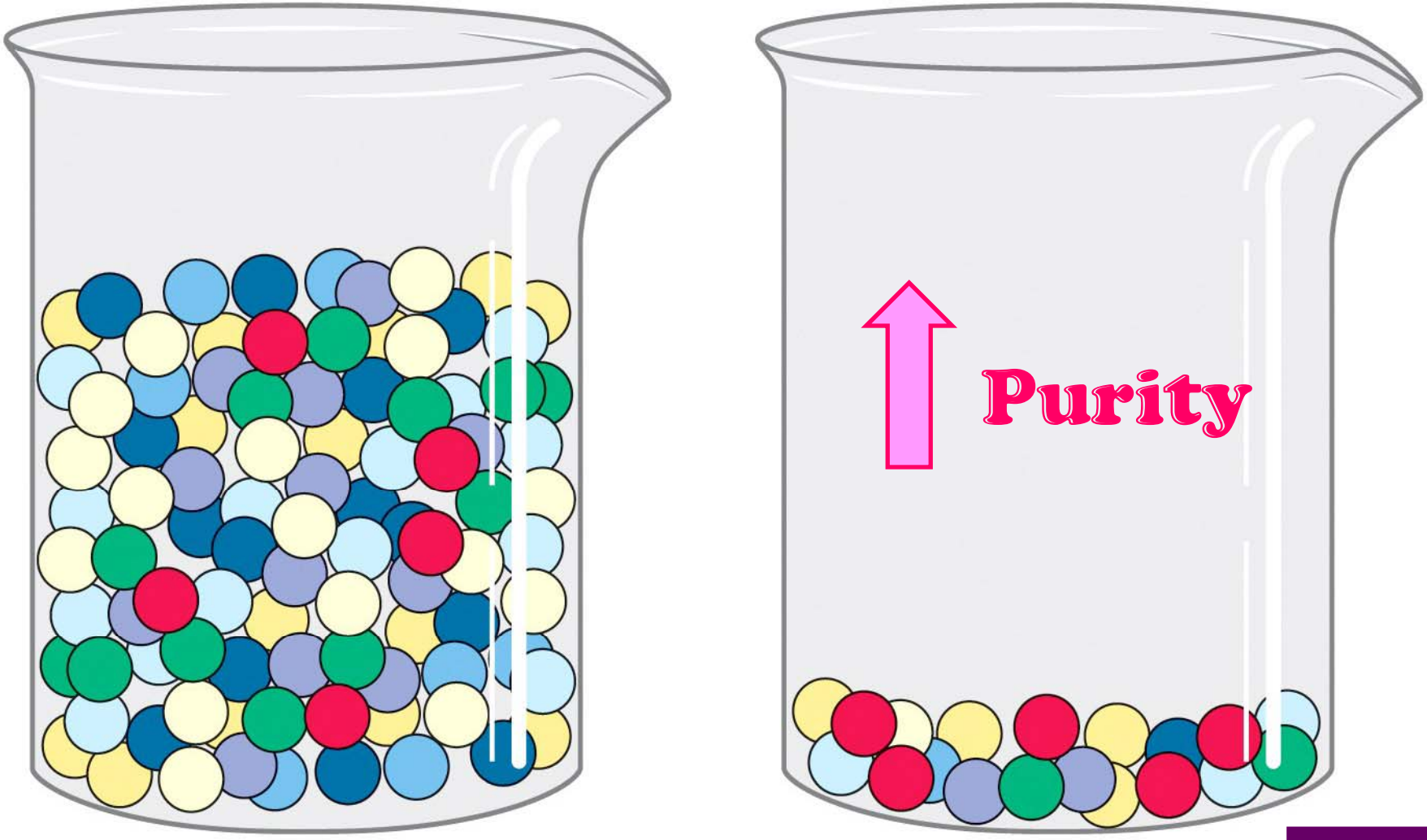
Note: All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 94.

1.0 unit of enzyme activity : transformation of 1.0 μmol of substrate per min at designated assay conditions.

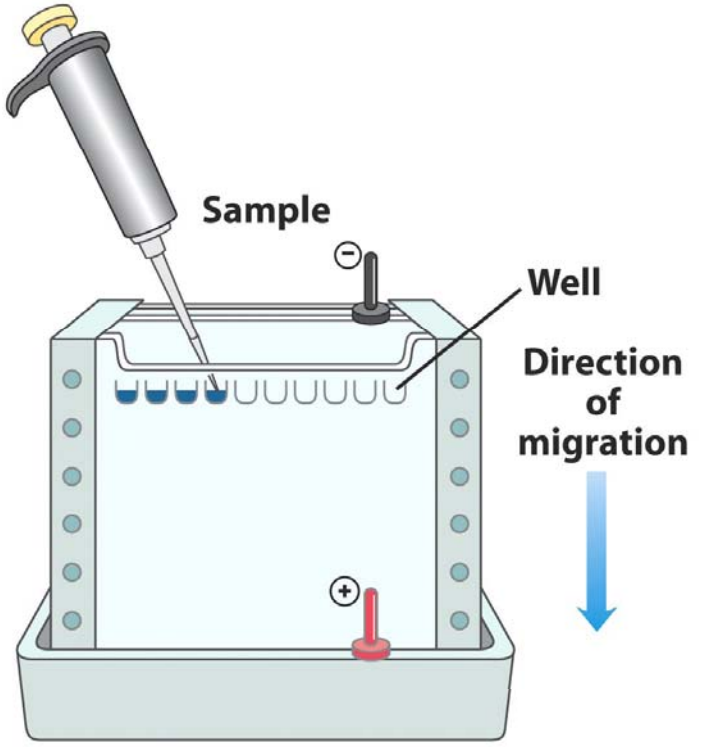
Specific activity: μmol/min/mg

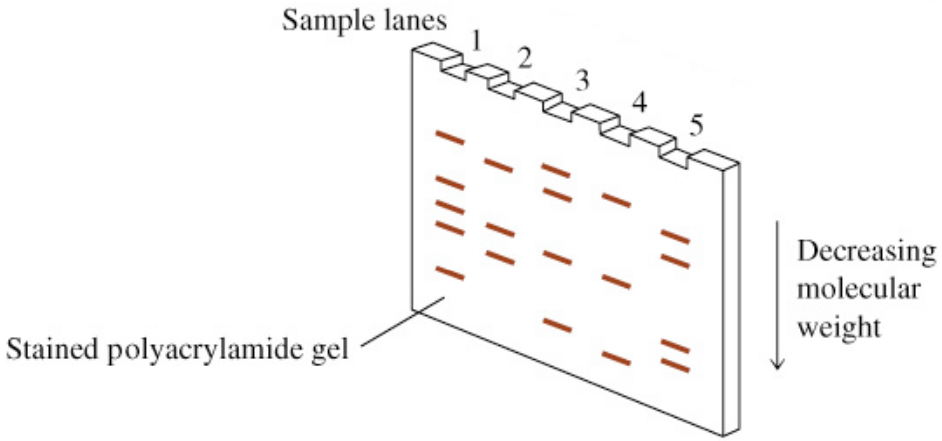
P. 88

The specific activity is a measure of enzyme purity



Electrophoresis 電泳

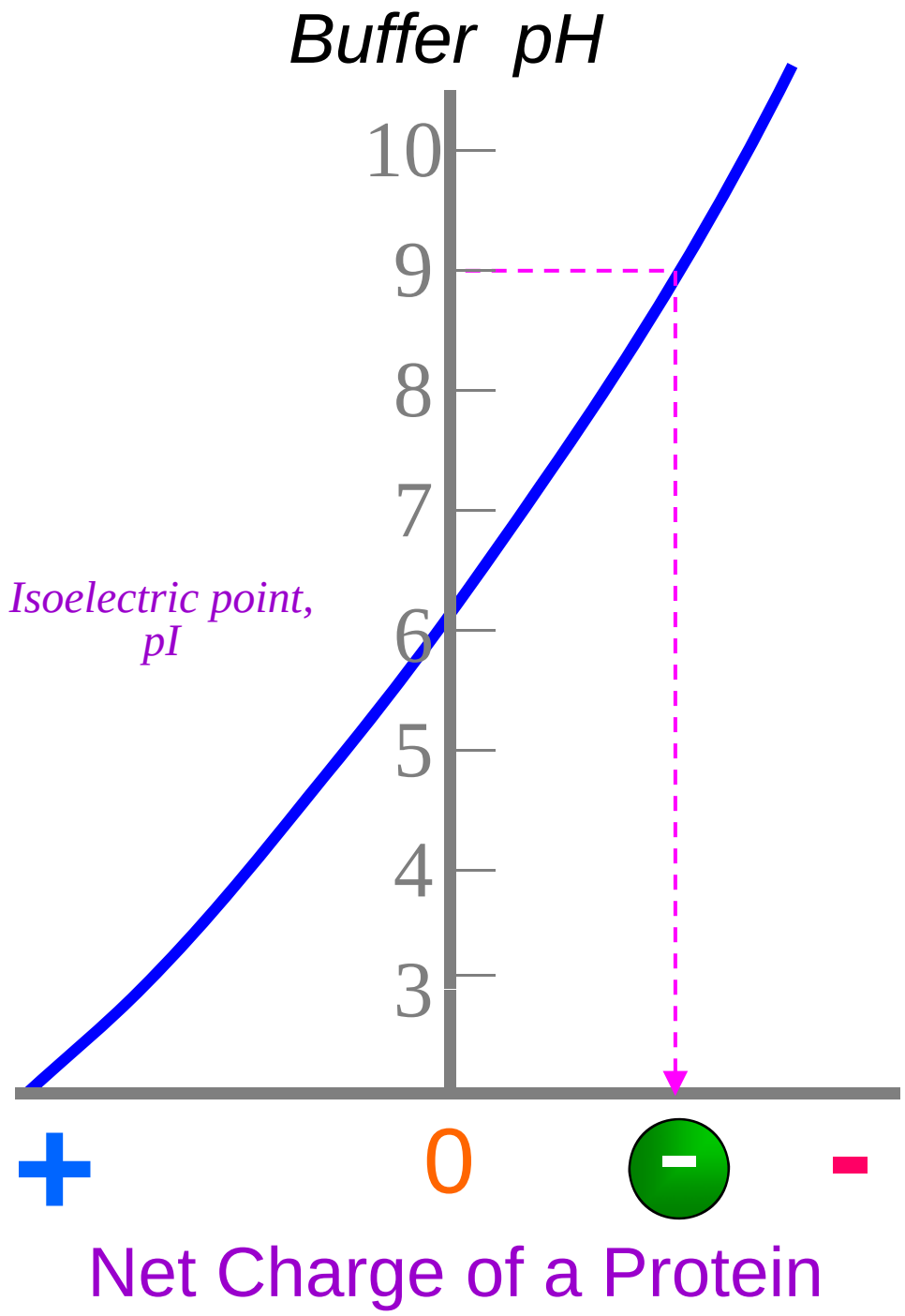




- **Polyacrylamide gel electrophoresis (PAGE)** Separates molecules on a polyacrylamide gel matrix when an electric field is applied
- **SDS-PAGE.** Sodium dodecyl sulfate (SDS) coats proteins with **negative charges**. Coated polypeptide chains then separate by **molecular mass**.

P. 89

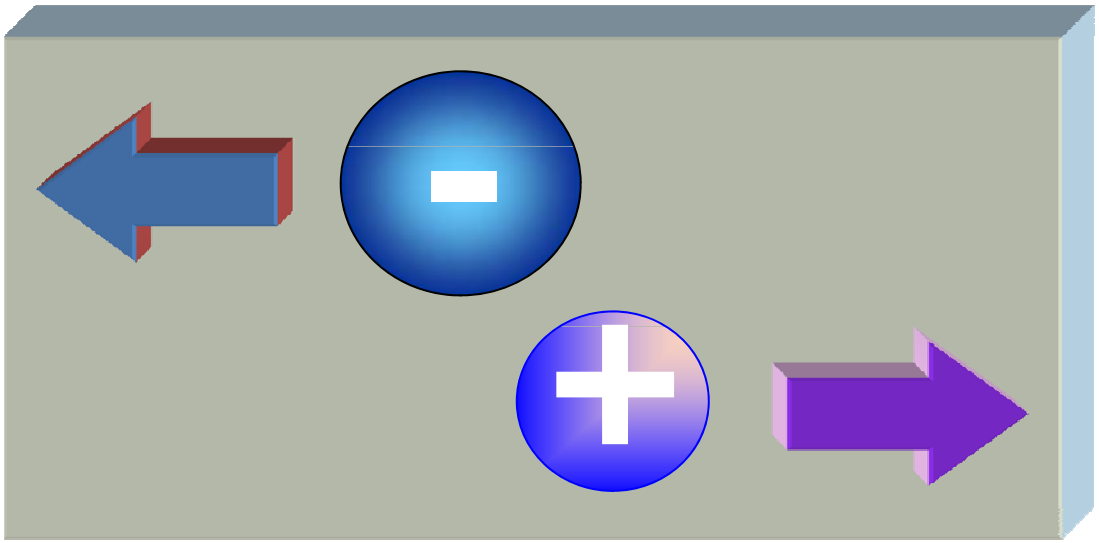
環境影響分子的帶電性質



影響泳動率的因素

$$\text{Mobility } (\mu) = \frac{Z}{f}$$

外加電流電壓 (*current, voltage*)



\+ -

Net Charge (Z)

分子的等電點

Isoelectric point

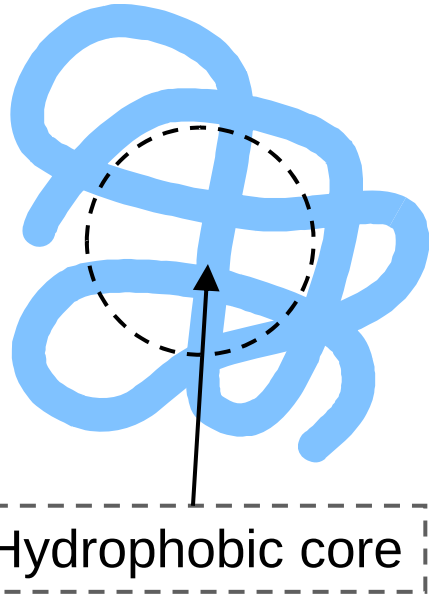
Friction (f)

分子量 分子形狀

Molecular weight, shape

SDS 在蛋白質表面均勻敷上一層負電

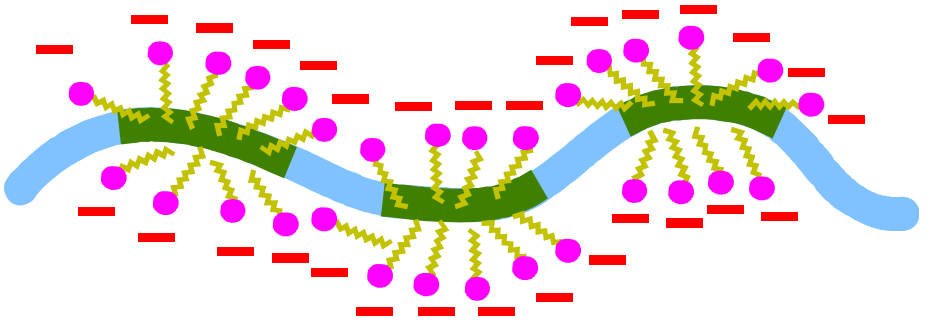
Native protein



SDS

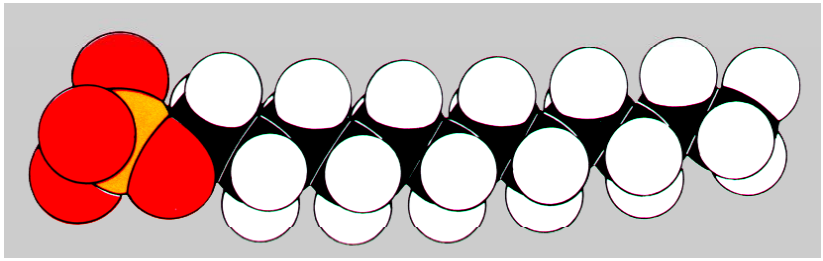
boiling

Protein is denatured to linear form



Its surface covered with negatively charged SDS uniformly

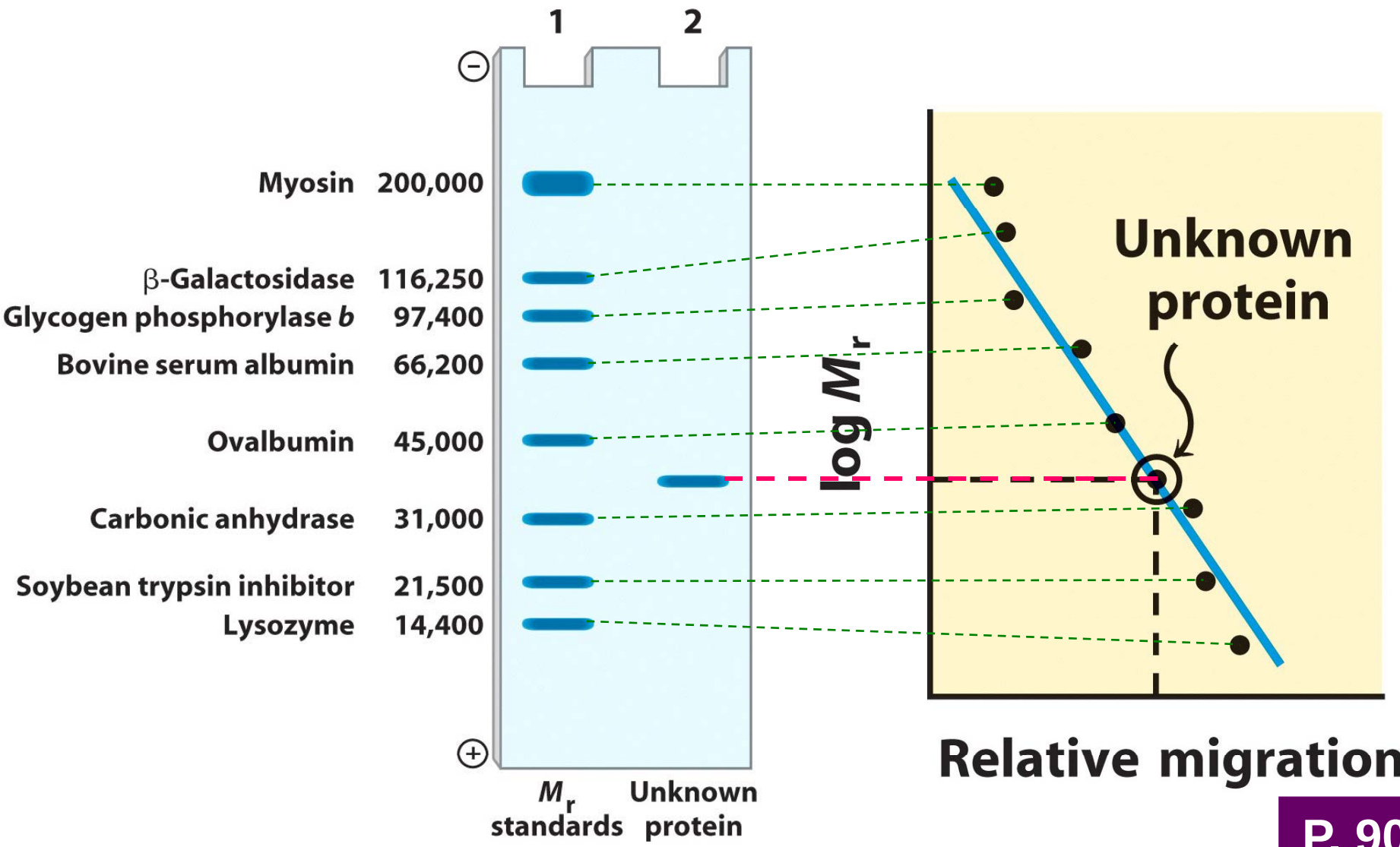
Polar head



Non-polar tail

Sodium dodecyl sulfate (SDS)

Estimating the molecular weight of a protein



P. 90

Isoelectric focusing

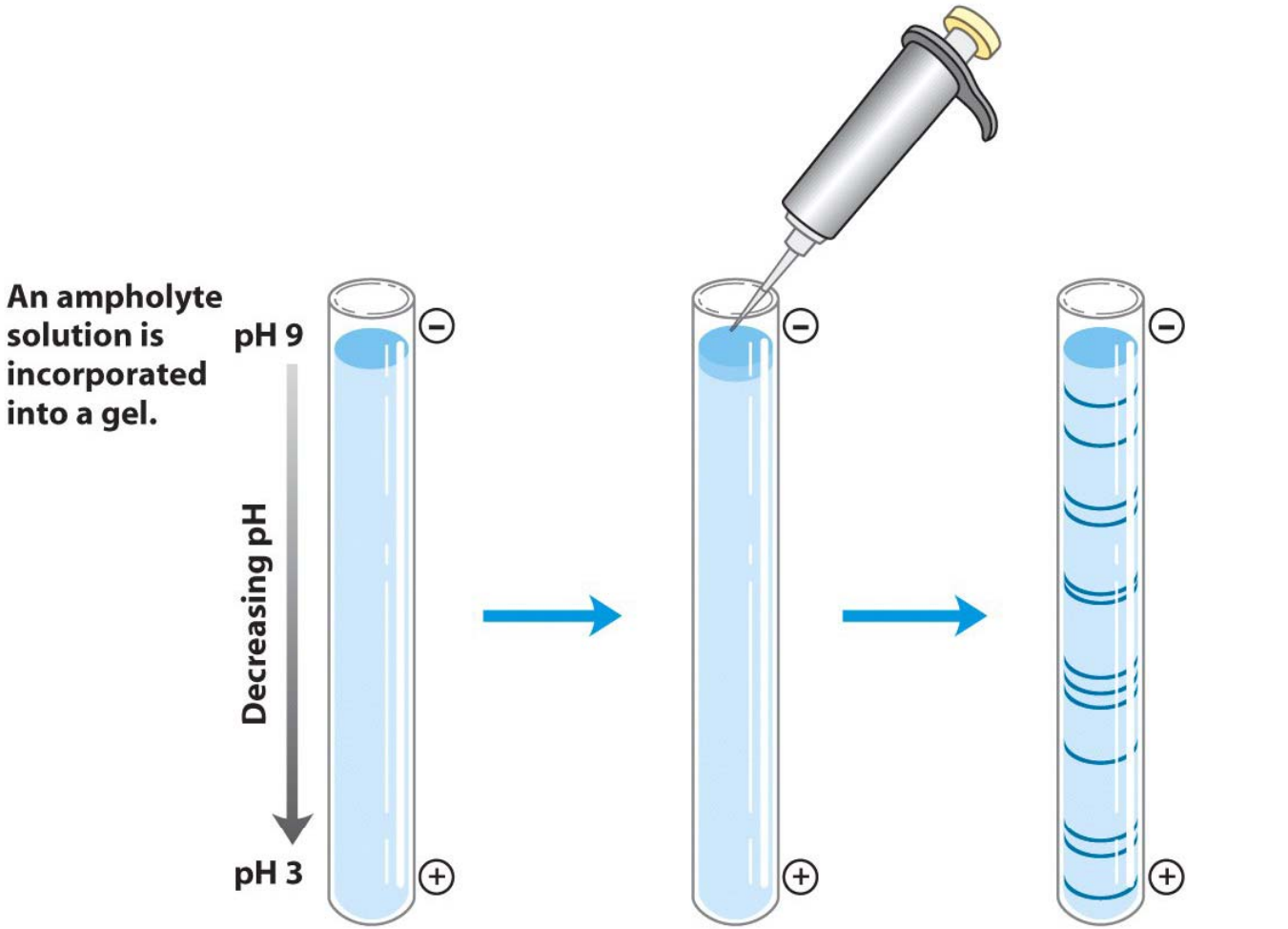
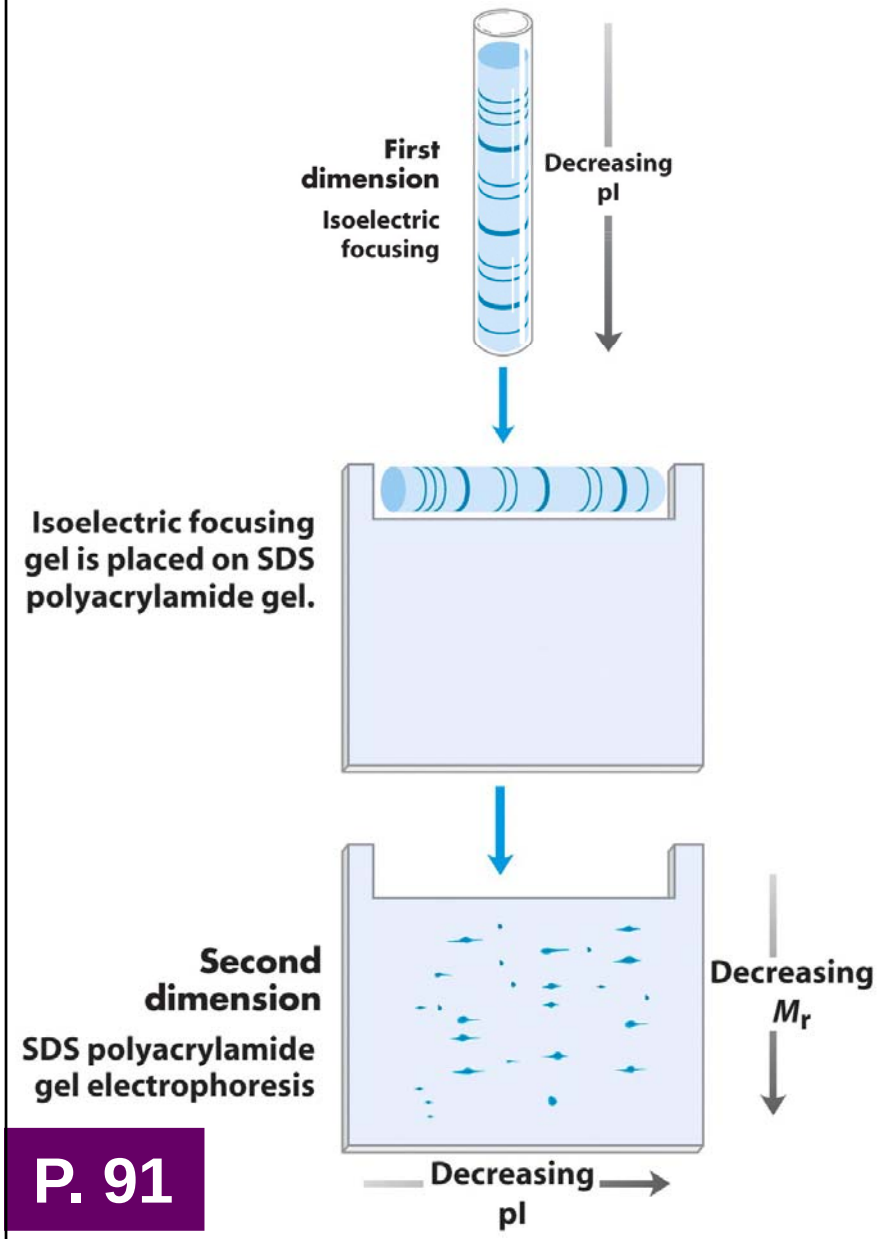
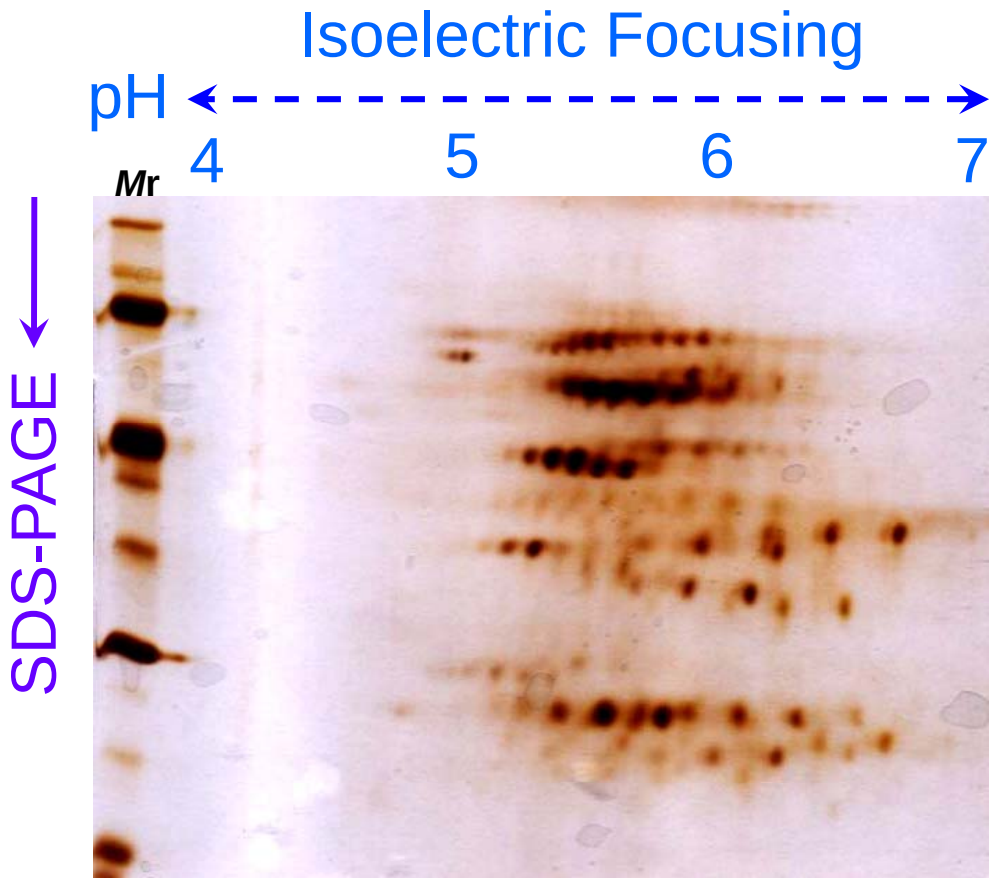


TABLE 3–6 The Isoelectric Points of Some Proteins

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

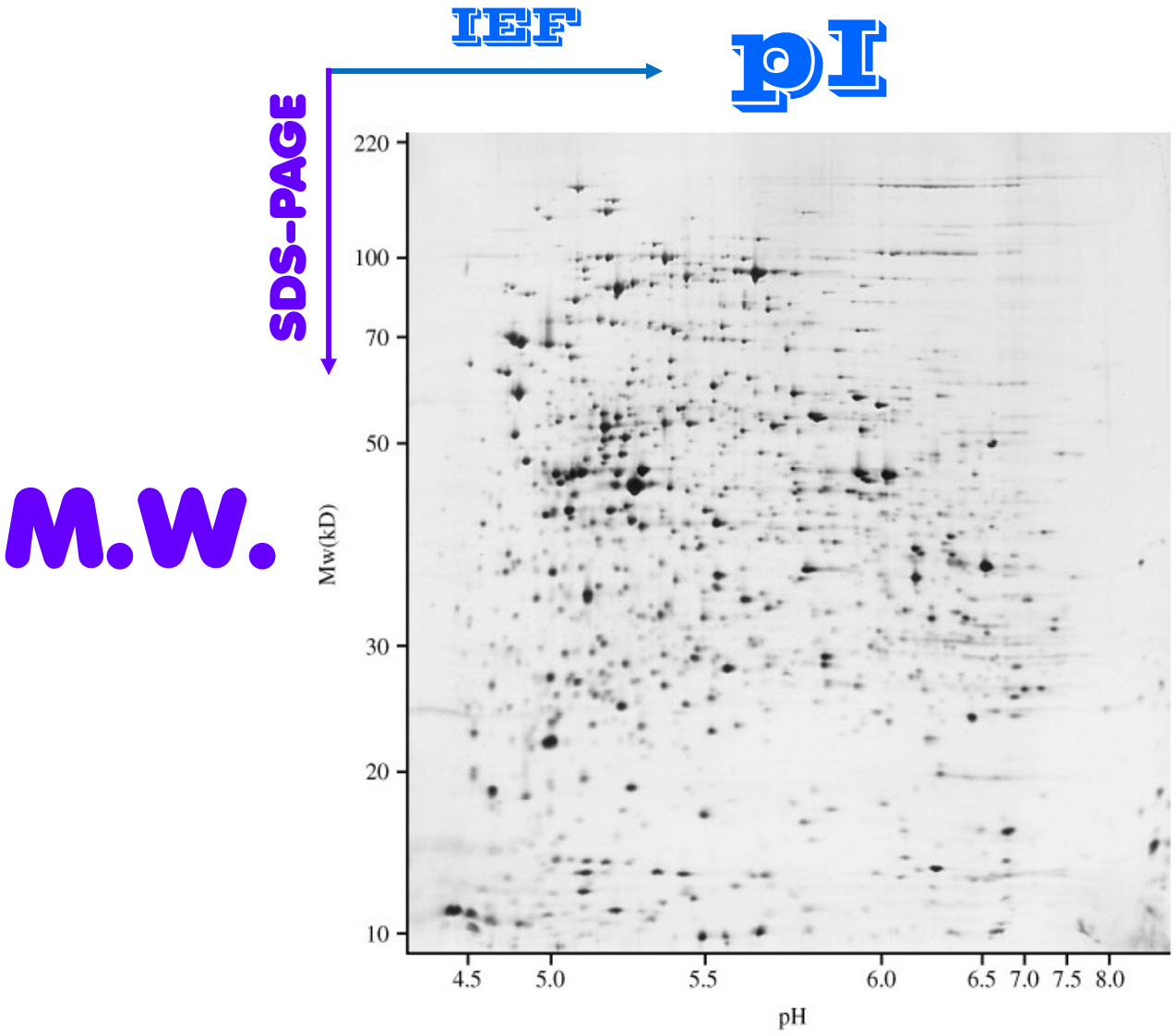
Two-dimensional gel electrophoresis





Silver staining

Two-dimensional gel electrophoresis



Principles of biochemistry, 3rd edition, Horton, Moran, Ochs , Rawn, Scrimgeour