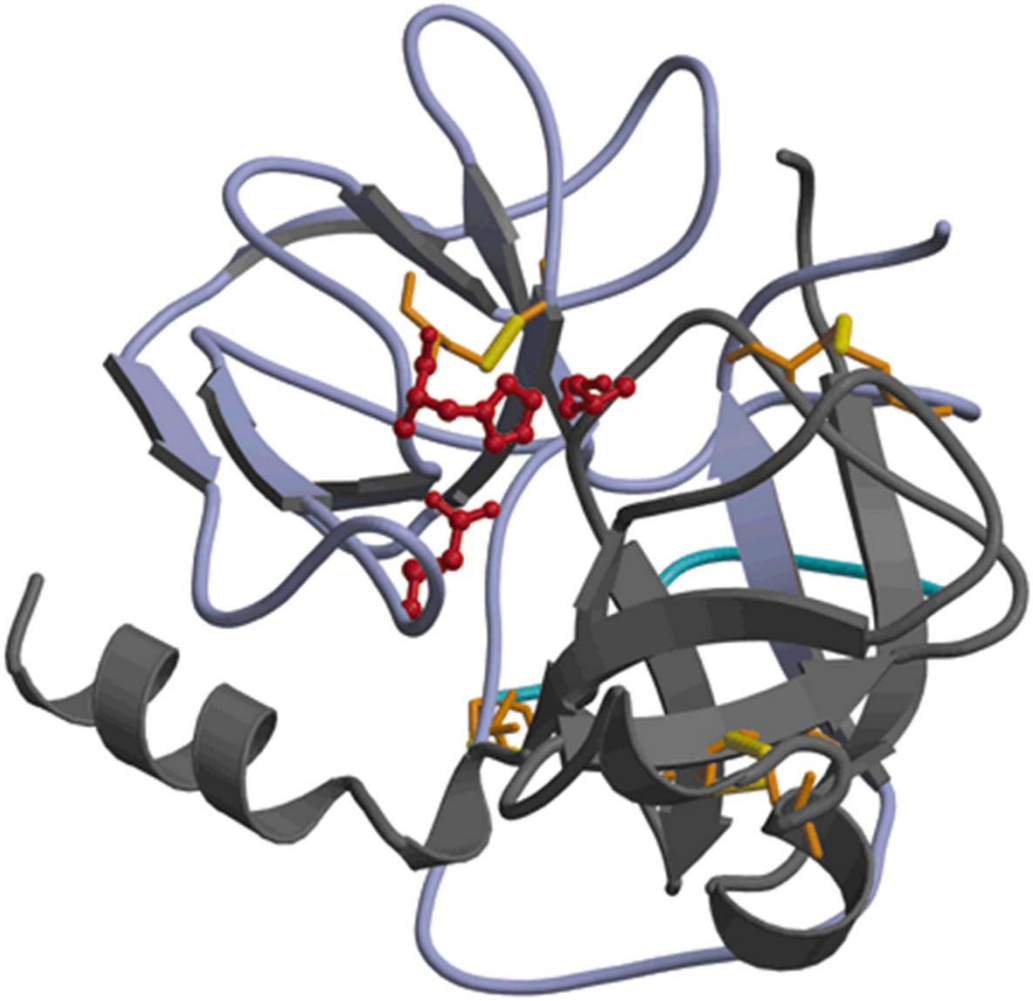
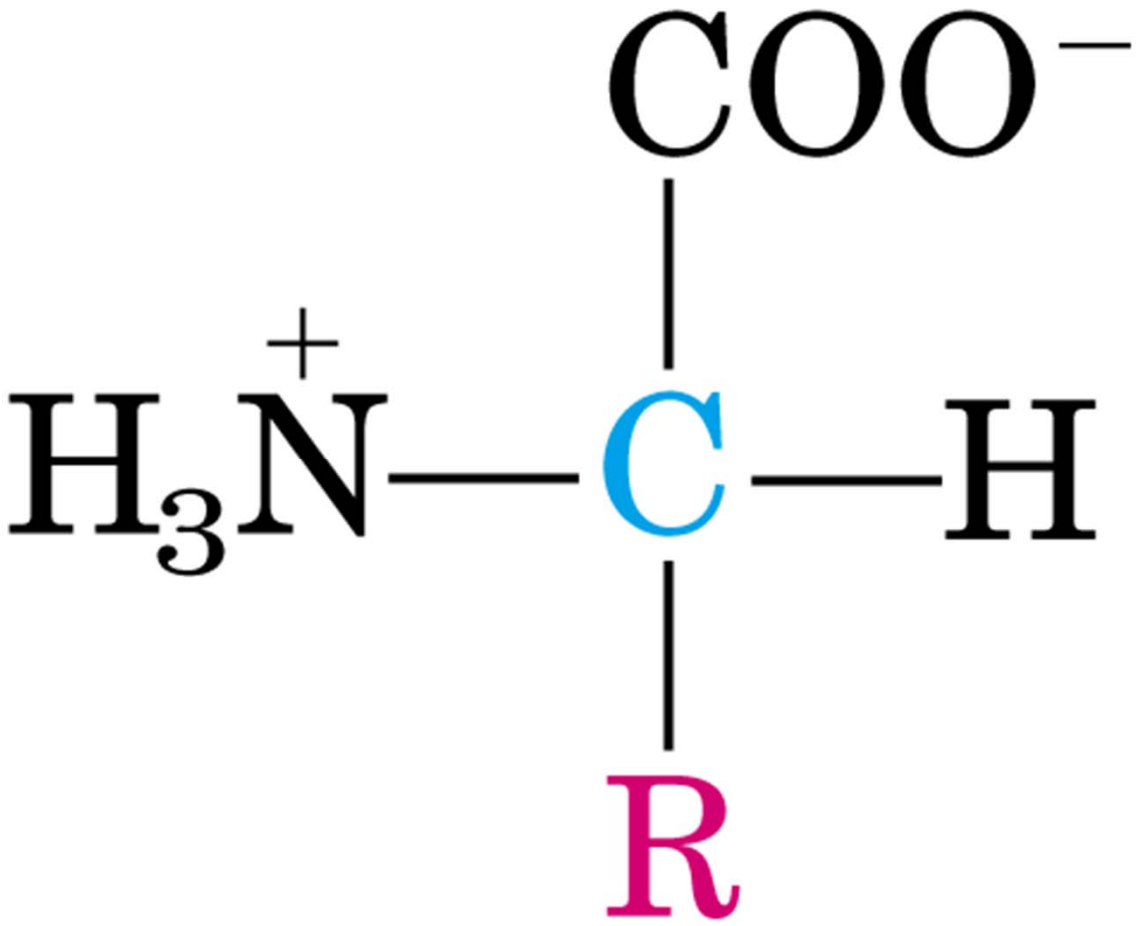
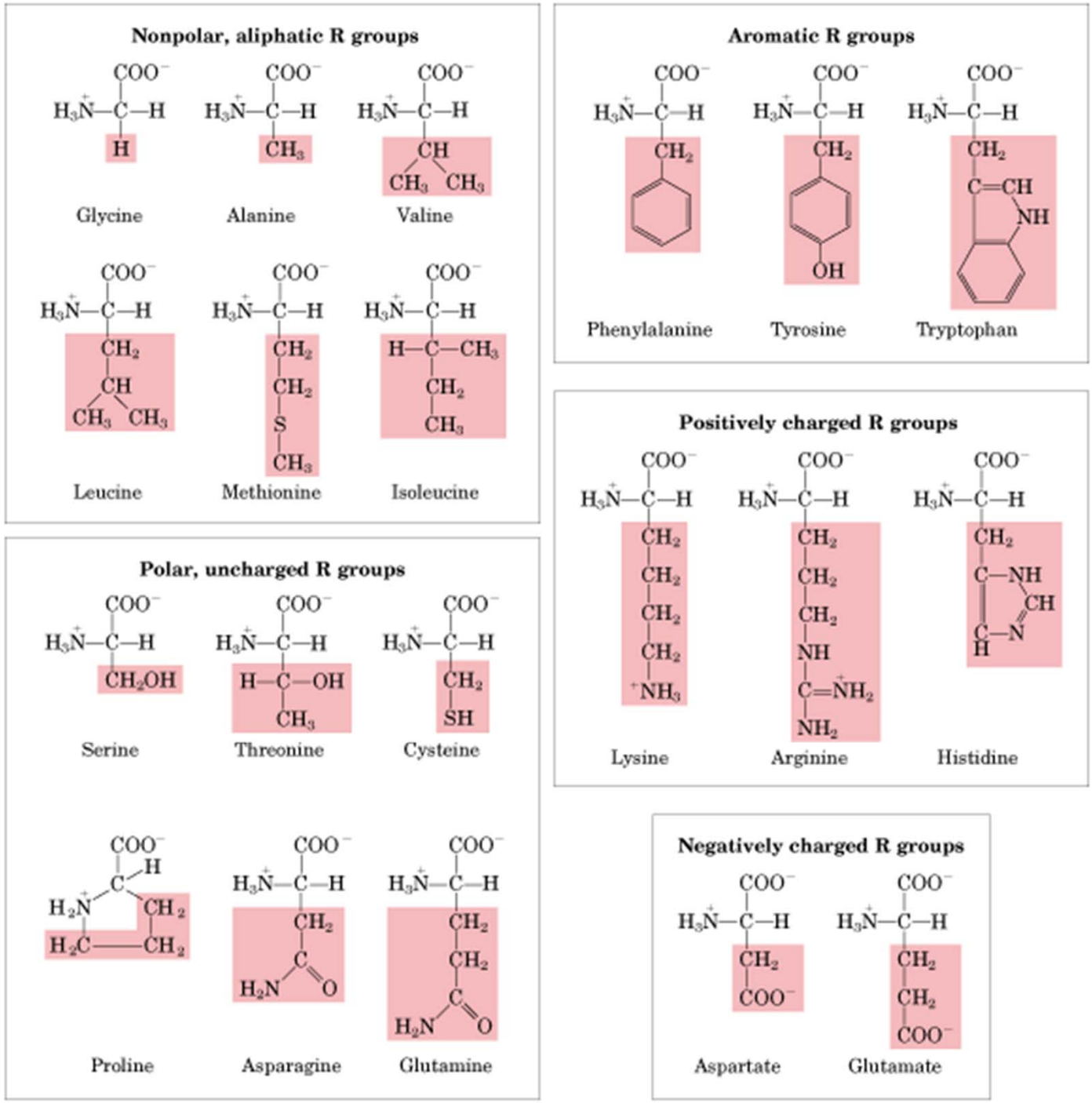
Biochemistry for Bioseparation Engineering

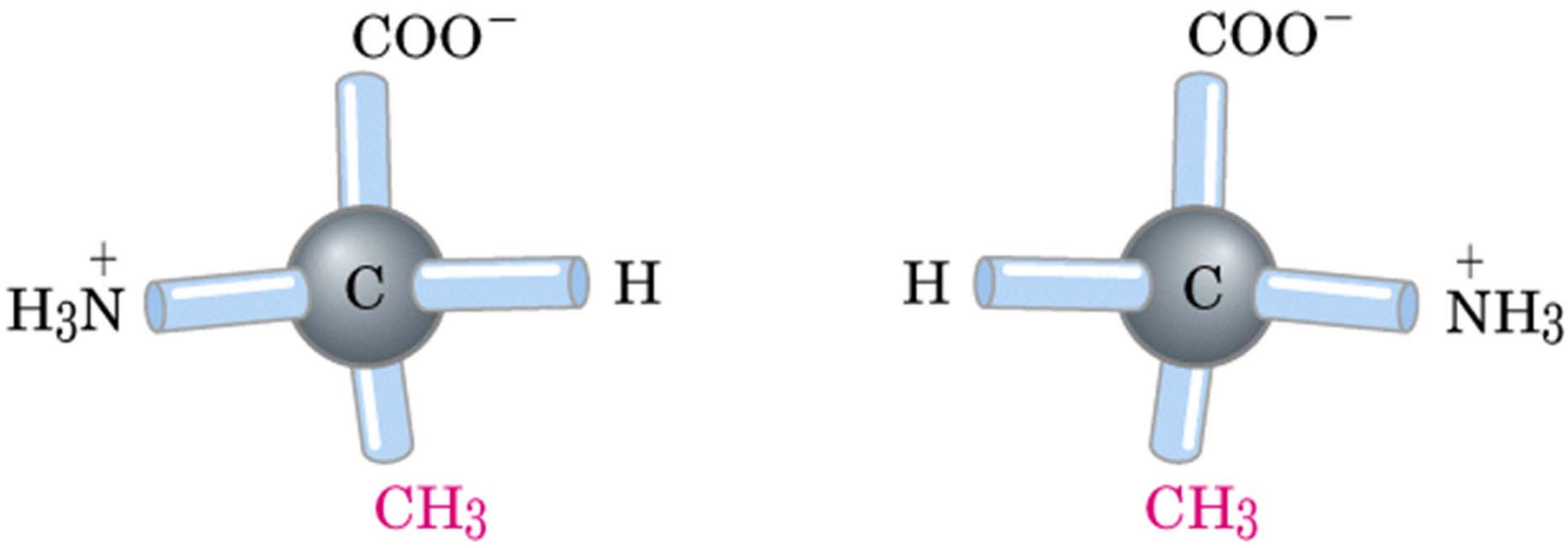
3-Dimensional Structure of Protein



Basic Structure of Amino Acid

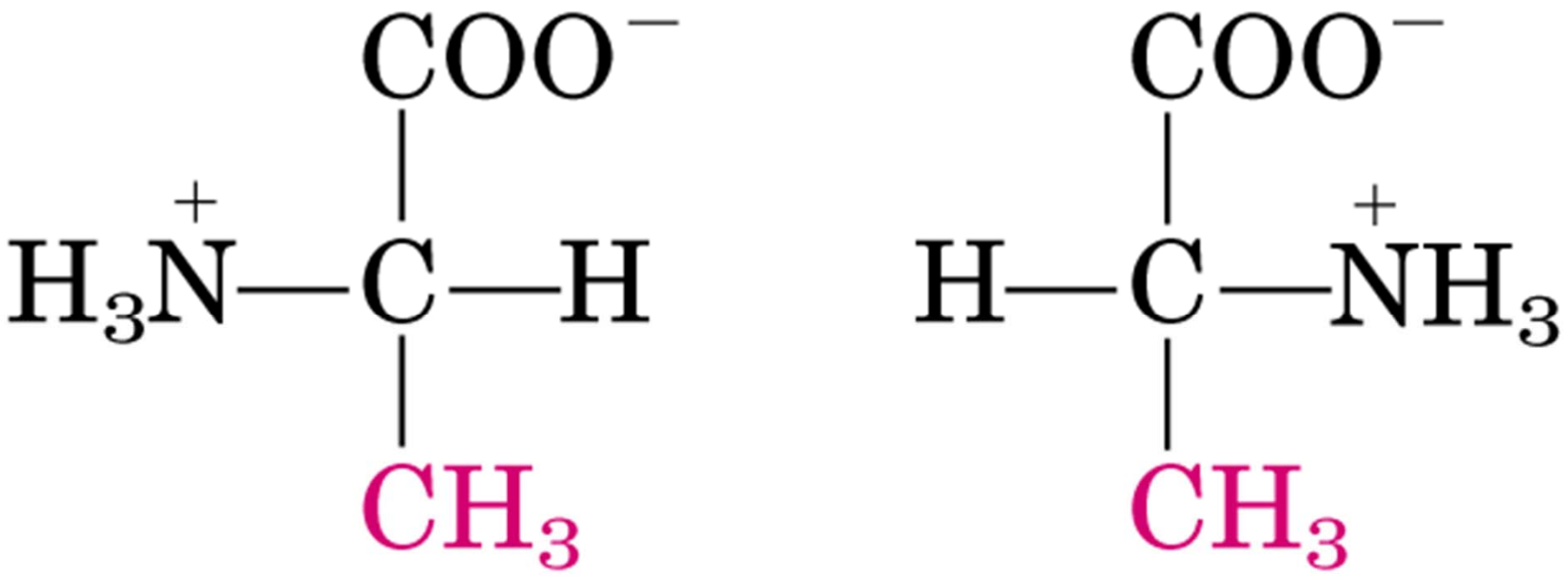



Nonpolar, aliphatic R groups
Glycine
Alanine
Valine
Leucine
Methionine
Isoleucine
Aromatic R groups
Phenylalanine
Tyrosine
Tryptophan
Positively charged R groups
Lysine
Arginine
Histidine
Polar, uncharged R groups
Serine
Threonine
Cysteine
Proline
Asparagine
Glutamine
Negatively charged R groups
Aspartate
Glutamate



L-Alanine D-Alanine

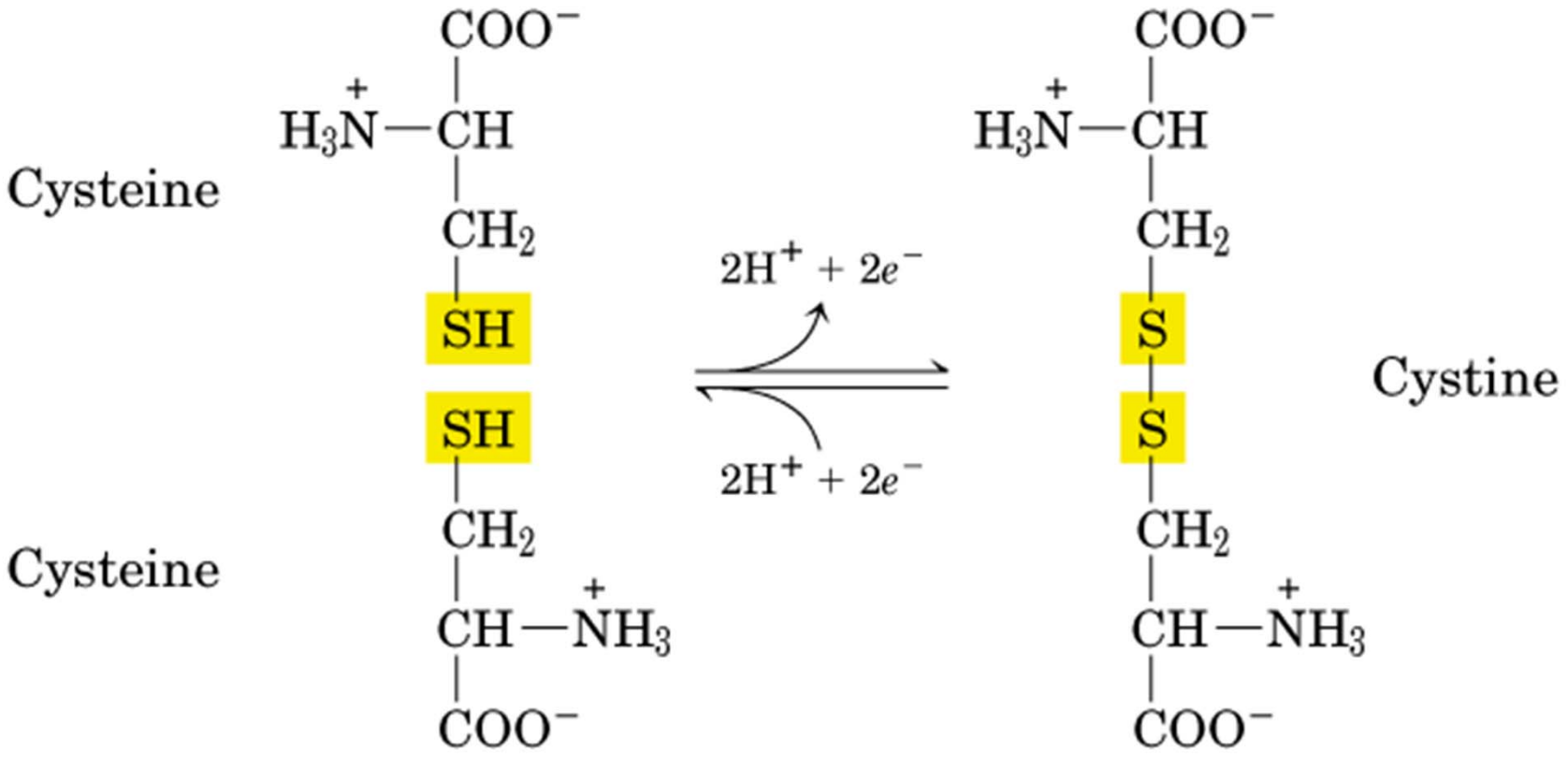
(a)

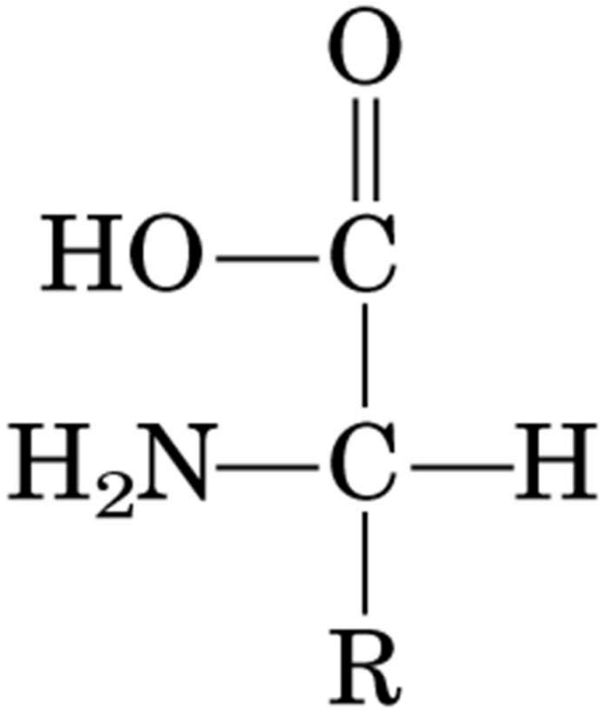


L-Alanine D-Alanine

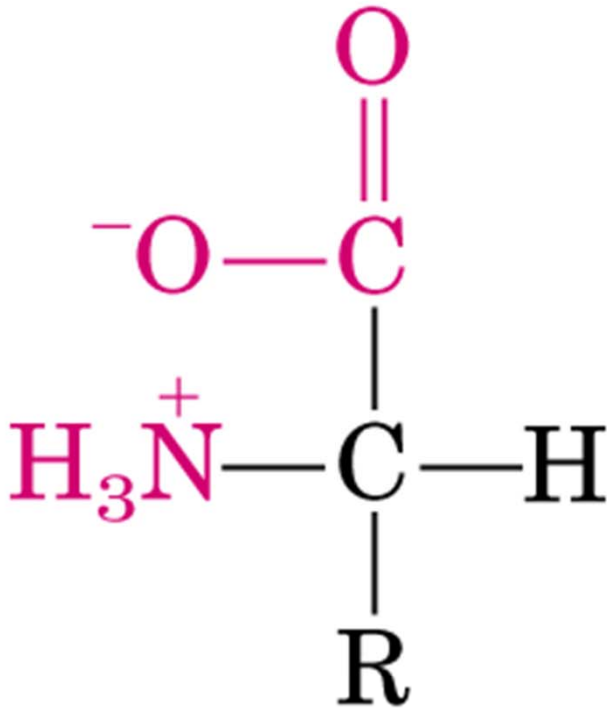
(c)

Disulfide Bond





Nonionic form

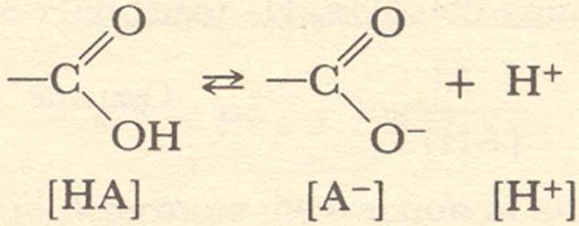


Zwitterionic form

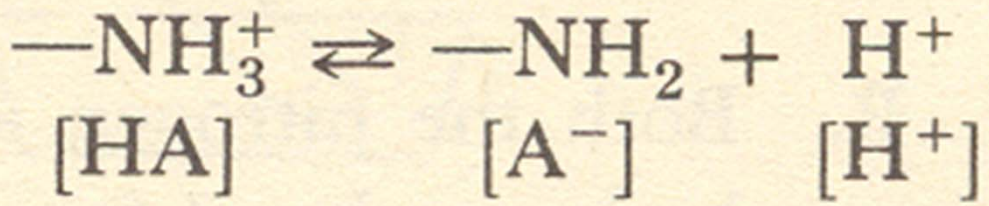
Similarly, the pK_a of a general acid, HA, is defined as $-\log K_a$, in which K_a is the equilibrium constant for the dissociation $HA \rightleftarrows H^+ + A^-$:

$$K_a = \frac{[H^+][A^-]}{[HA]} \tag{2.1}$$

All amino acids have at least two dissociation constants, one for the carboxyl group (K_{a_1}),



the amino group (K_{a_2}),



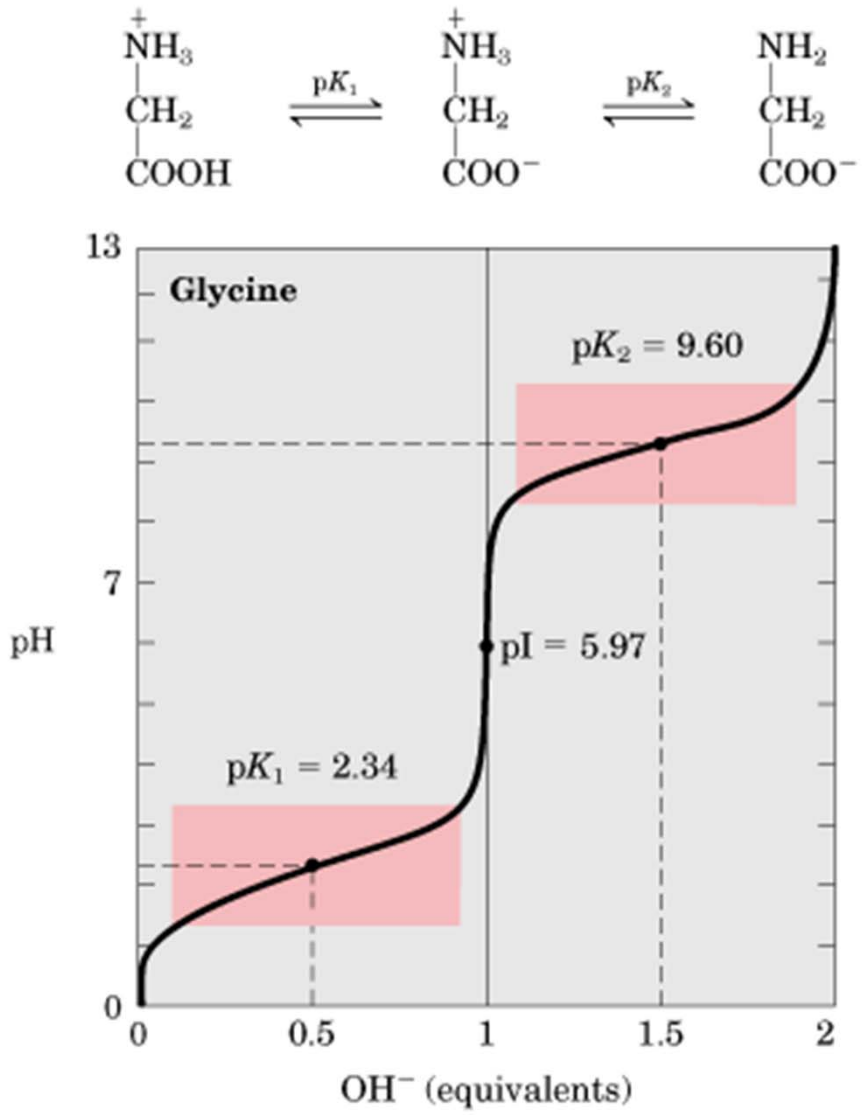
The pH of a solution and the dissociation constant, K_a, of an ionizable group in the solution are related by the Henderson–Hasselbalch equation,

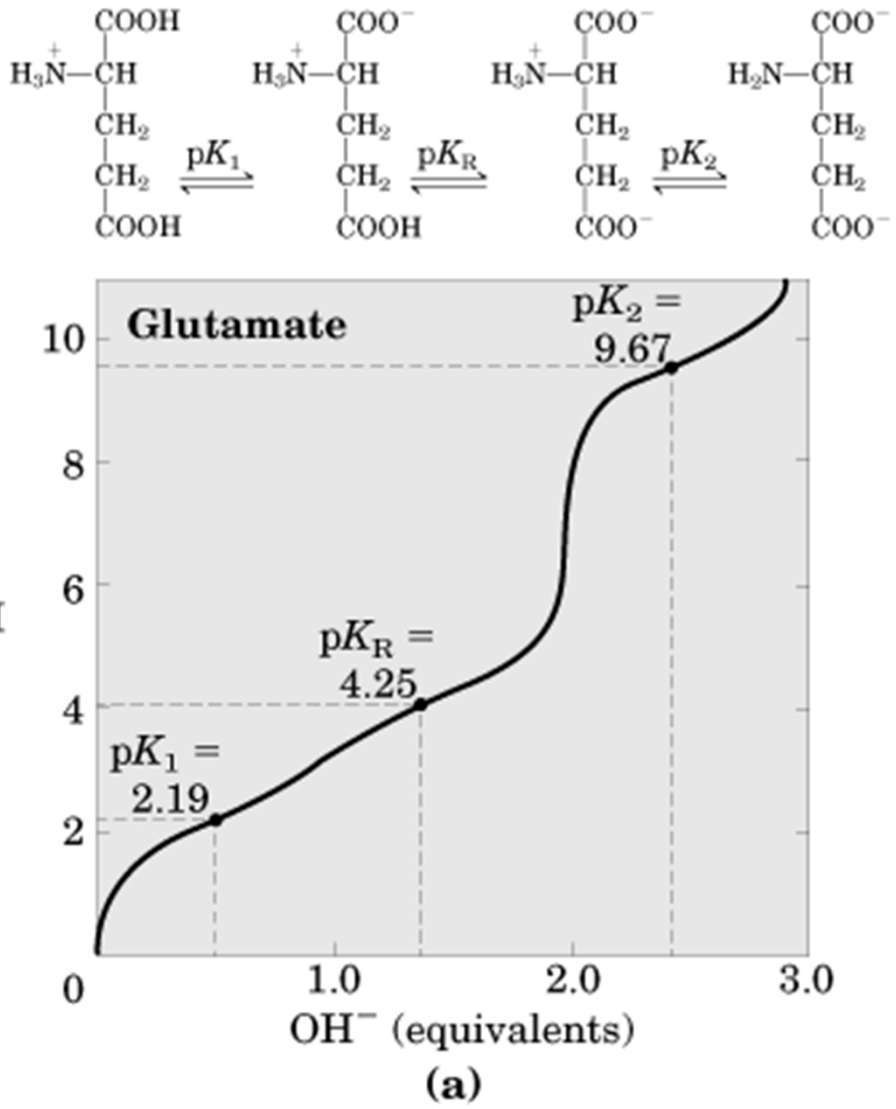
$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} \tag{2.2}$$

This equation can be used to determine the fraction of ionizable groups found in each of the possible ionization states in solution at a known pH. Note that at $\mathrm{pH} = \mathrm{p}K_a$, half of the ionizable groups are dissociated.

The p*I*, or isoelectric point, of an amino acid is the pH at which it carries no *net* charge. For monoamino, monocarboxylic acids, p*I* is defined by the simple relationship

$$\mathrm{p}I = \tfrac{1}{2}(\mathrm{p}K_{a_1} + \mathrm{p}K_{a_2}) \tag{2.3}$$


NH3+
CH2
COOH
pK1
NH3+
CH2
COO−
pK2
NH2
CH2
COO−
Glycine
pK2 = 9.60
pI = 5.97
pK1 = 2.34
pH
13
7
0
0
0.5
1
1.5
2
OH− (equivalents)


Glutamate
pK2 = 9.67
pKR = 4.25
pK1 = 2.19
pH
10
8
6
4
2
0
1.0
2.0
3.0
OH⁻ (equivalents)
(a)

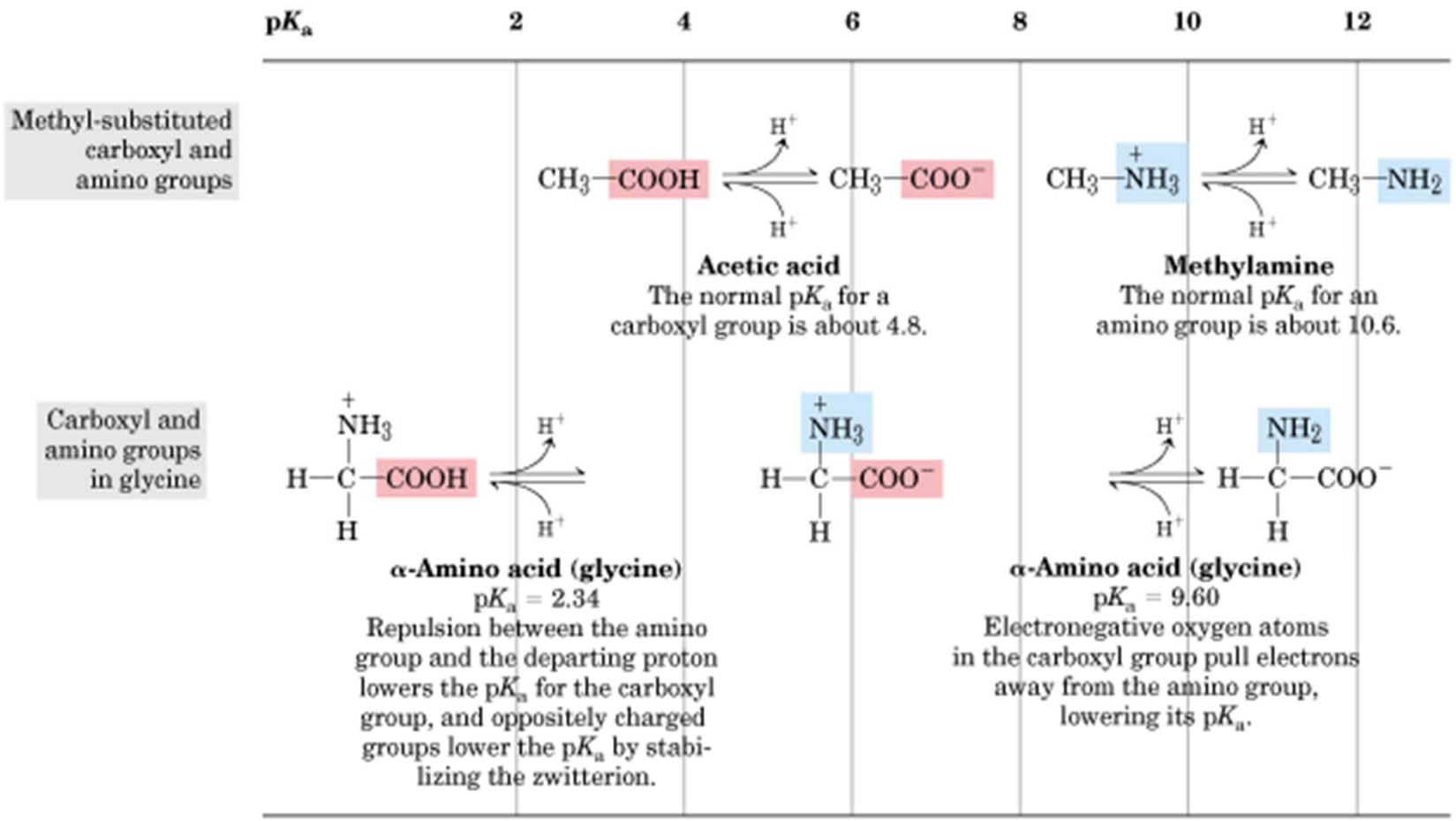

pKa
2
4
6
8
10
12
Methyl-substituted carboxyl and amino groups
CH3—COOH
CH3—COO−
H+
H+
Acetic acid
The normal pKa for a carboxyl group is about 4.8.
CH3—NH3+
CH3—NH2
H+
H+
Methylamine
The normal pKa for an amino group is about 10.6.
Carboxyl and amino groups in glycine
NH3+
H—C—COOH
H
H+
H+
α-Amino acid (glycine)
pKa = 2.34
Repulsion between the amino group and the departing proton lowers the pKa for the carboxyl group, and oppositely charged groups lower the pKa by stabilizing the zwitterion.
NH3+
H—C—COO−
H
NH2
H—C—COO−
H
H+
H+
α-Amino acid (glycine)
pKa = 9.60
Electronegative oxygen atoms in the carboxyl group pull electrons away from the amino group, lowering its pKa.

table 5–1

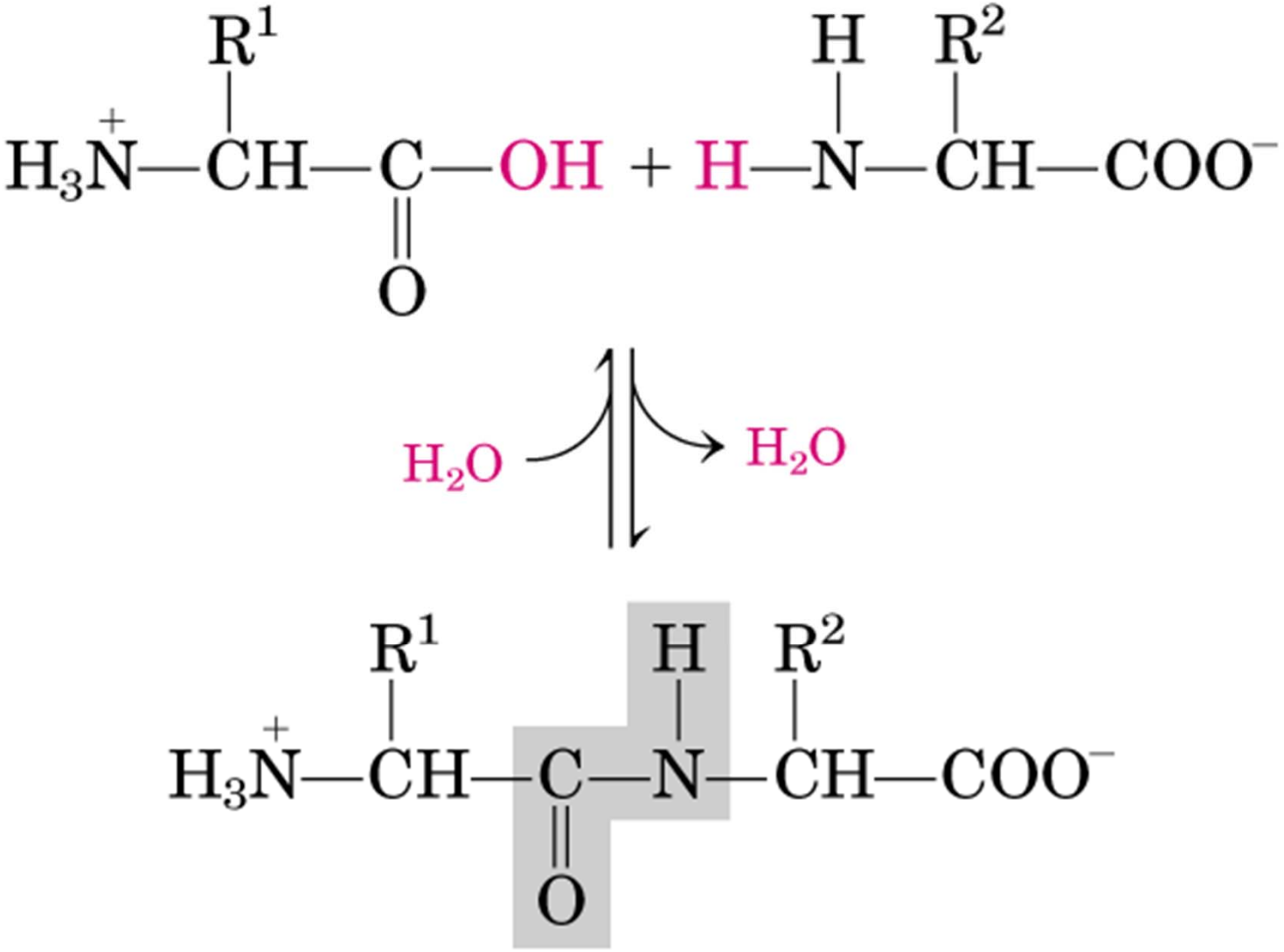
Properties and Conventions Associated with the Standard Amino Acids

Amino acid	Abbreviated names		M_r	pK_a values: pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups									
Glycine	Gly	G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala	A	89	2.34	9.69		6.01	1.8	7.8
Valine	Val	V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu	L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile	I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met	M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups									
Phenylalanine	Phe	F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr	Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp	W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups									
Serine	Ser	S	105	2.21	9.15		5.68	−0.8	6.8
Proline	Pro	P	115	1.99	10.96		6.48	1.6	5.2
Threonine	Thr	T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys	C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn	N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln	Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups									
Lysine	Lys	K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His	H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg	R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups									
Aspartate	Asp	D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu	E	147	2.19	9.67	4.25	3.22	−3.5	6.3

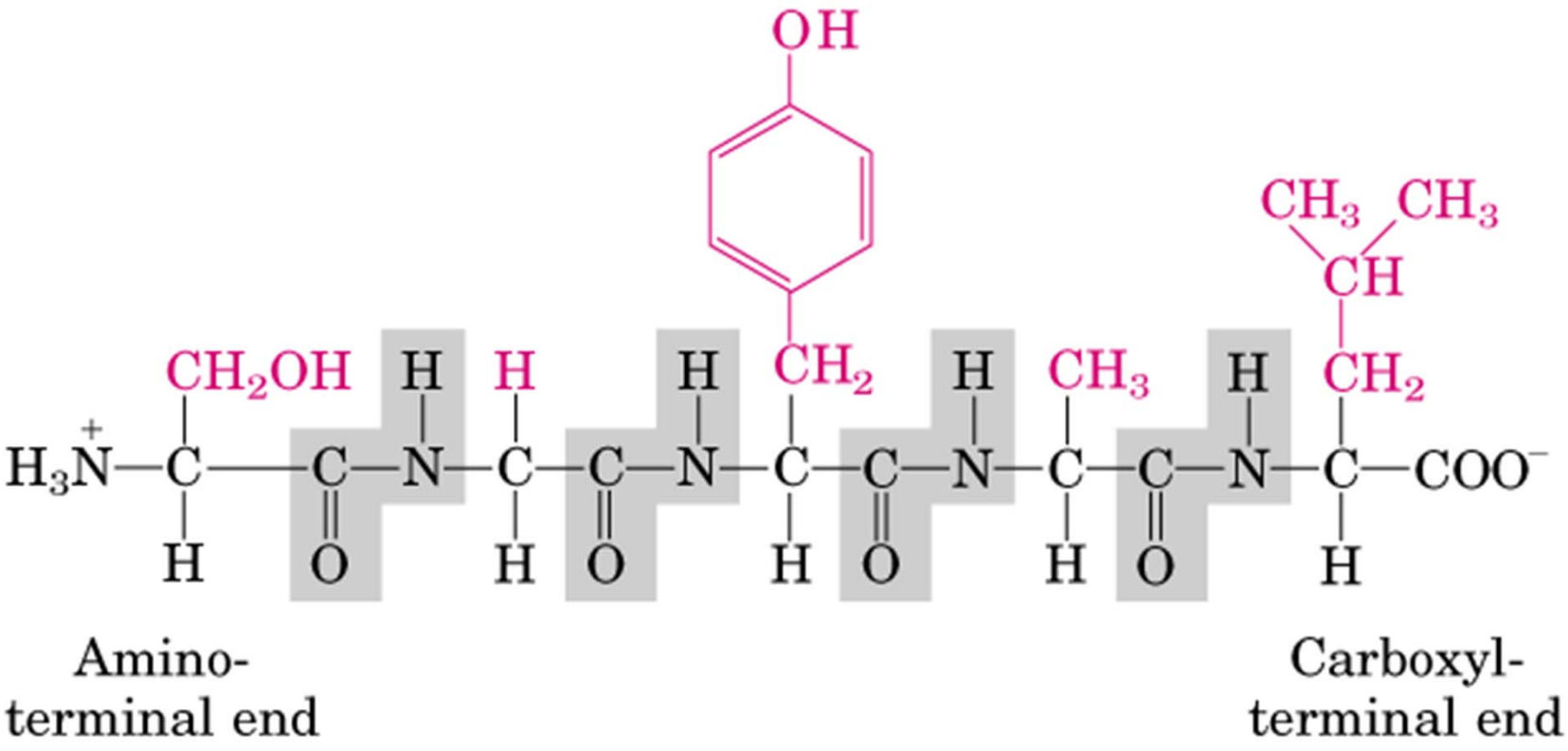
*A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (− values) or a hydrophobic environment (+ values). See Chapter 12. From Kyte, J. & Doolittle, R.F. (1982) *J. Mol. Biol.* **157**, 105 – 132.

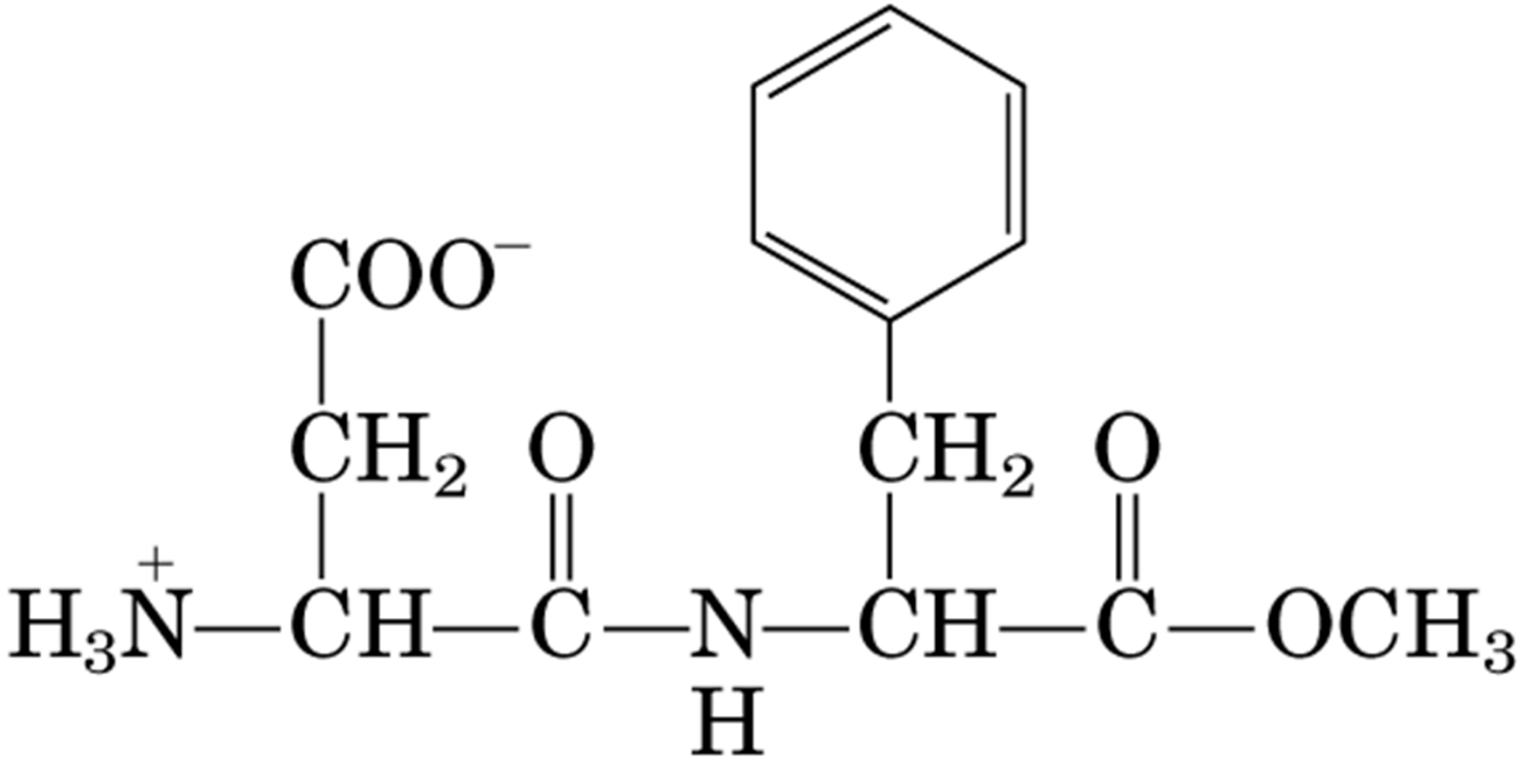
†Average occurrence in over 1150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed) Plenum Press, NY, pp. 599–623.

Peptide Bond



N- and C-terminals of Peptide





L-Aspartyl-L-phenylalanine methyl ester
(aspartame)

table 5–2

Molecular Data on Some Proteins

	Molecular weight	Number of residues	Number of polypeptide chains
Cytochrome *c* (human)	13,000	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (egg white)	13,930	129	1
Myoglobin (equine heart)	16,890	153	1
Chymotrypsin (bovine pancreas)	21,600	241	3
Chymotrypsinogen (bovine)	22,000	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	68,500	609	1
Hexokinase (yeast)	102,000	972	2
RNA polymerase (*E. coli*)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (*E. coli*)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

table 5–3

Amino Acid Composition of Two Proteins*

Amino acid	Number of residues per molecule of protein	
	Bovine cytochrome *c*	Bovine chymotrypsinogen
Ala	6	22
Arg	2	4
Asn	5	15
Asp	3	8
Cys	2	10
Gln	3	10
Glu	9	5
Gly	14	23
His	3	2
Ile	6	10
Leu	6	19
Lys	18	14
Met	2	2
Phe	4	6
Pro	4	9
Ser	1	28
Thr	8	23
Trp	1	8
Tyr	4	4
Val	3	23
Total	104	245

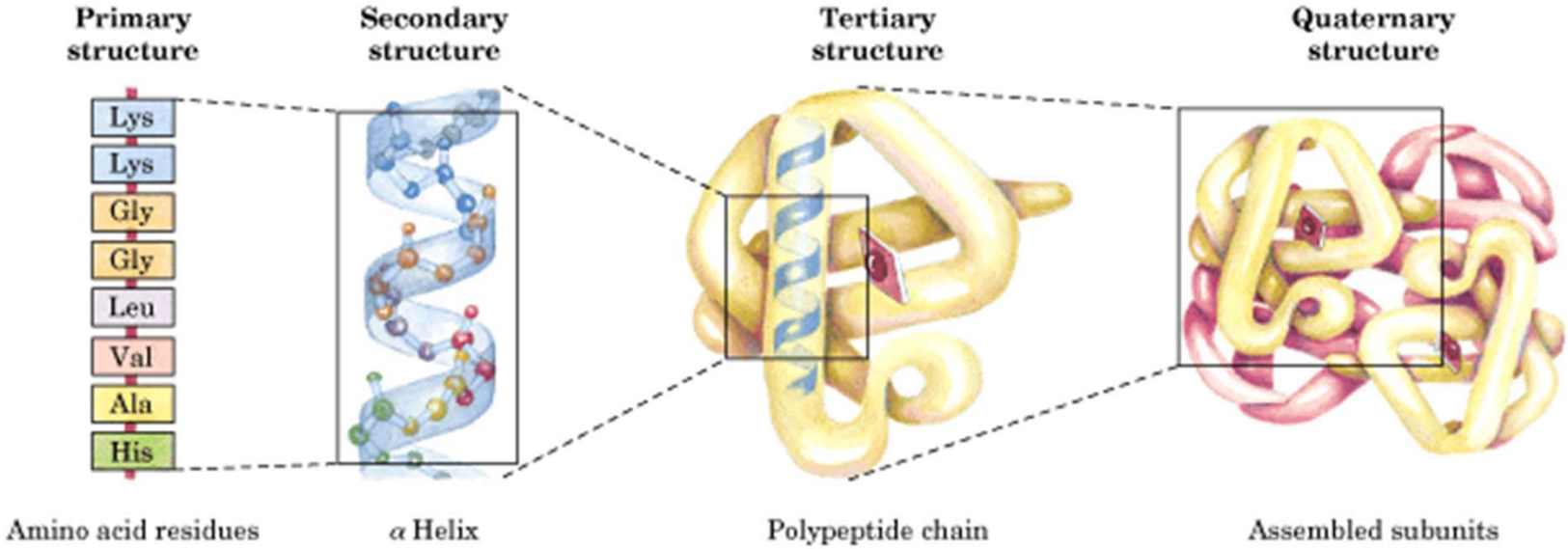
*Note that standard procedures for the acid hydrolysis of proteins convert Asn and Gln to Asp and Glu, respectively. In addition, Trp is destroyed. Special procedures must be employed to determine the amounts of these amino acids.

table 5–4

Conjugated Proteins

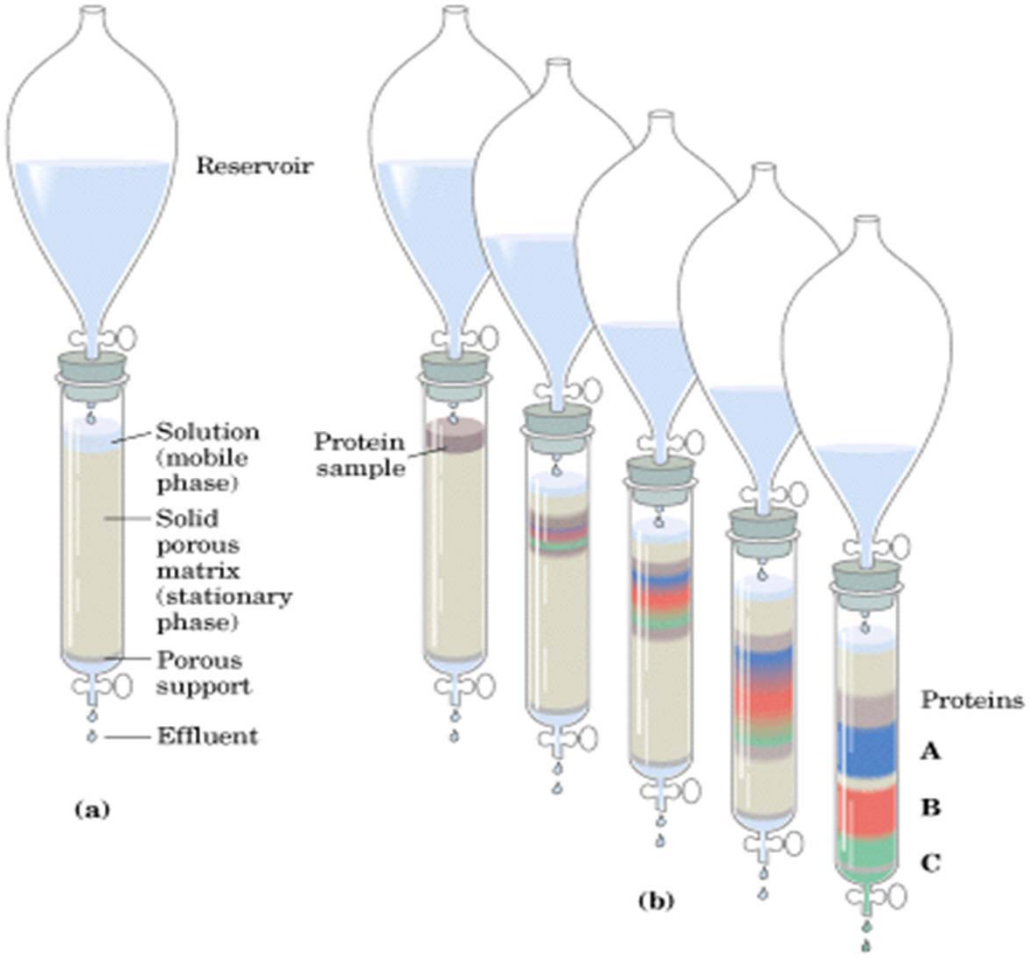
Class	Prosthetic group(s)	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

단백질의 구조

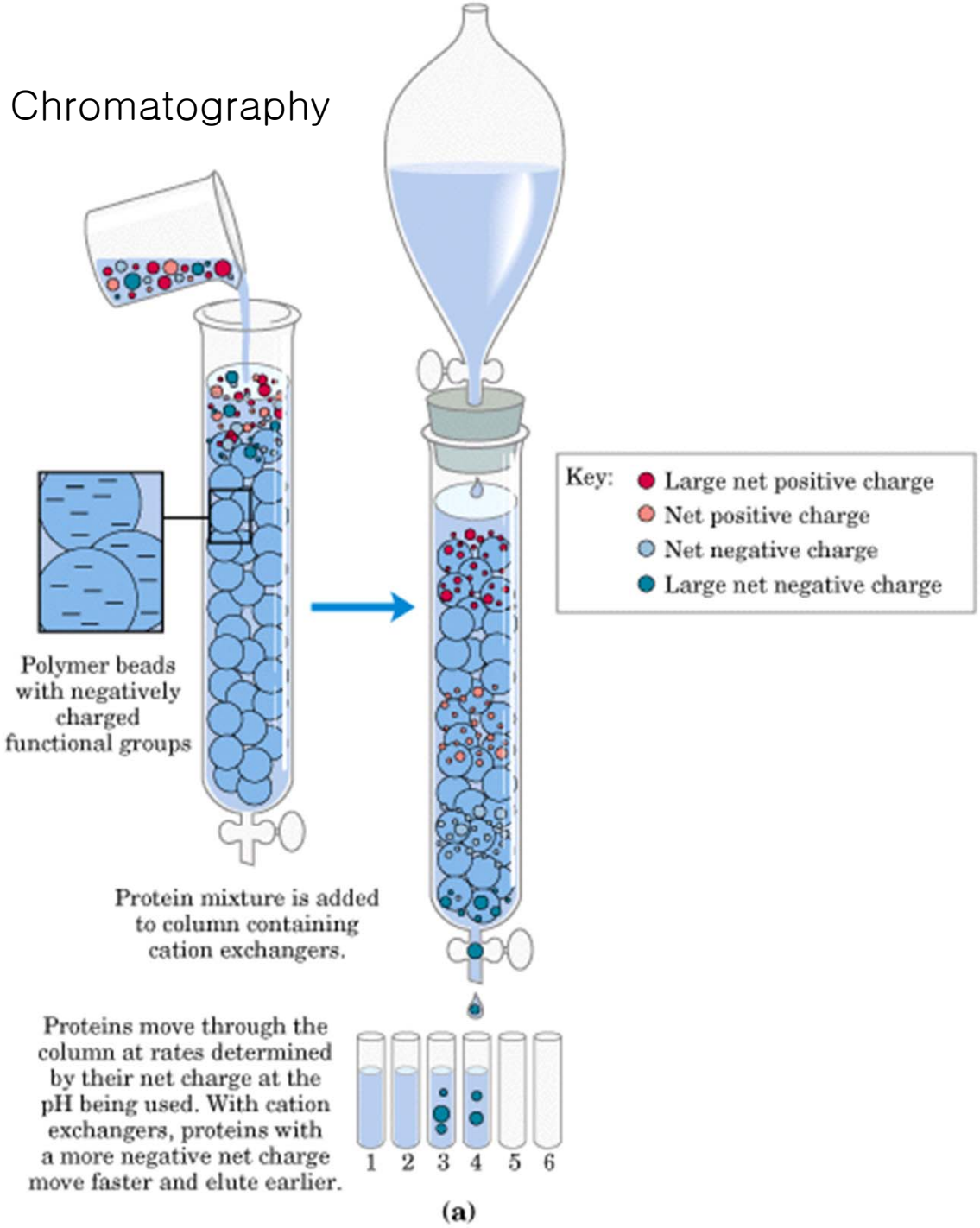

Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Lys
Lys
Gly
Gly
Leu
Val
Ala
His
Amino acid residues
α Helix
Polypeptide chain
Assembled subunits

단백질의 분리 정제

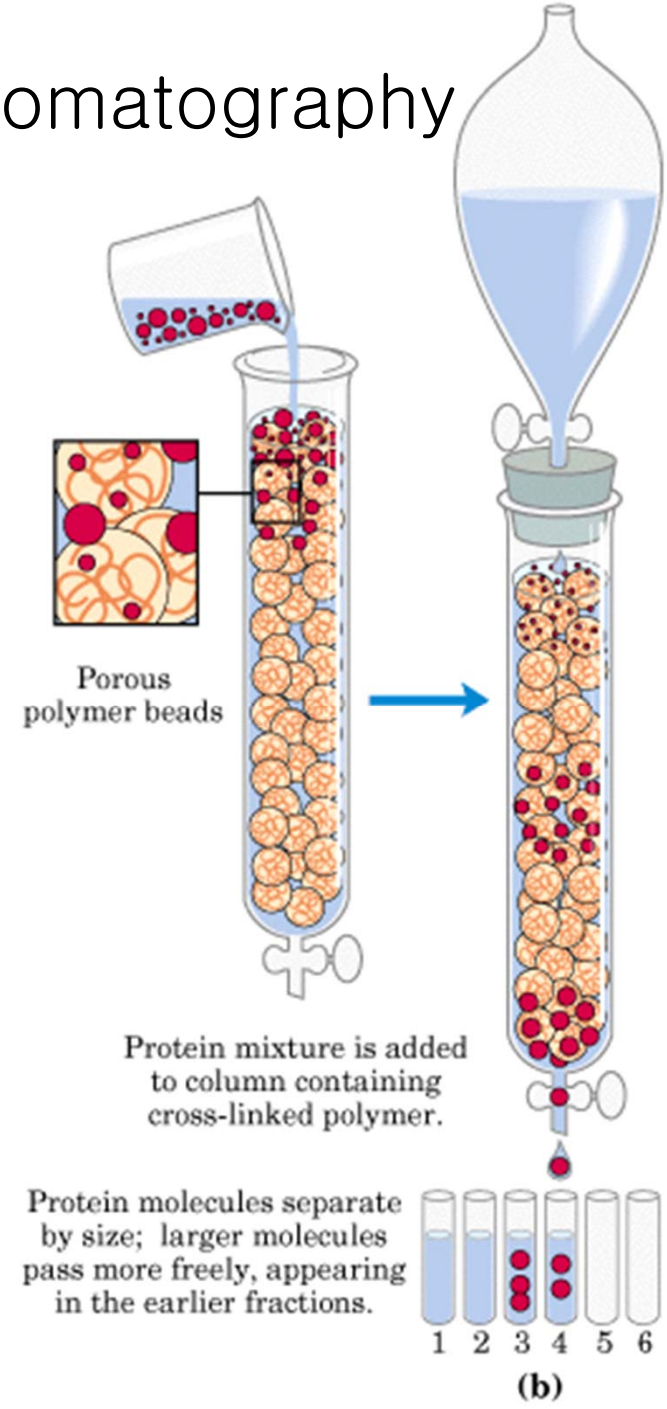
Principles of Chromatography



Ion Exchange Chromatography



Gel Filtration Chromatography



Affinity Chromatography

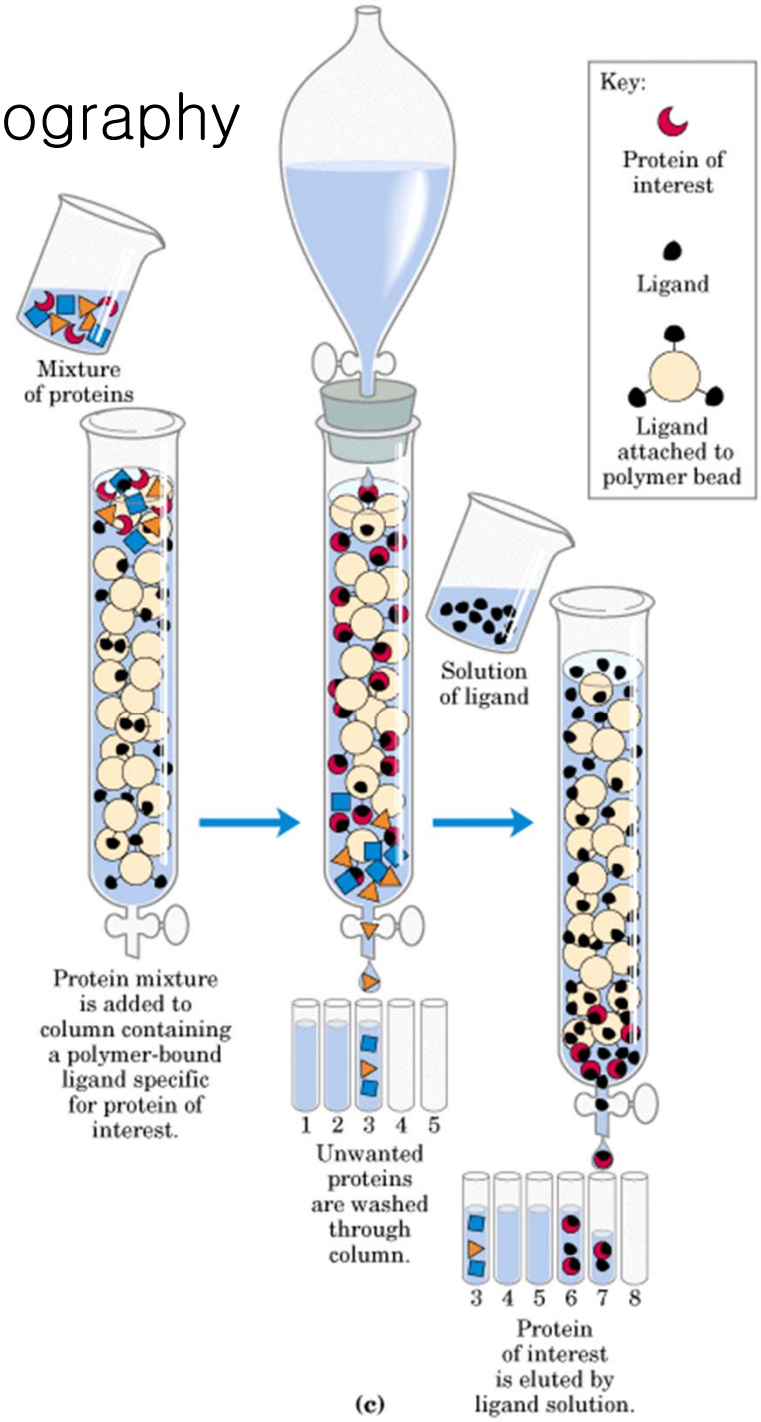


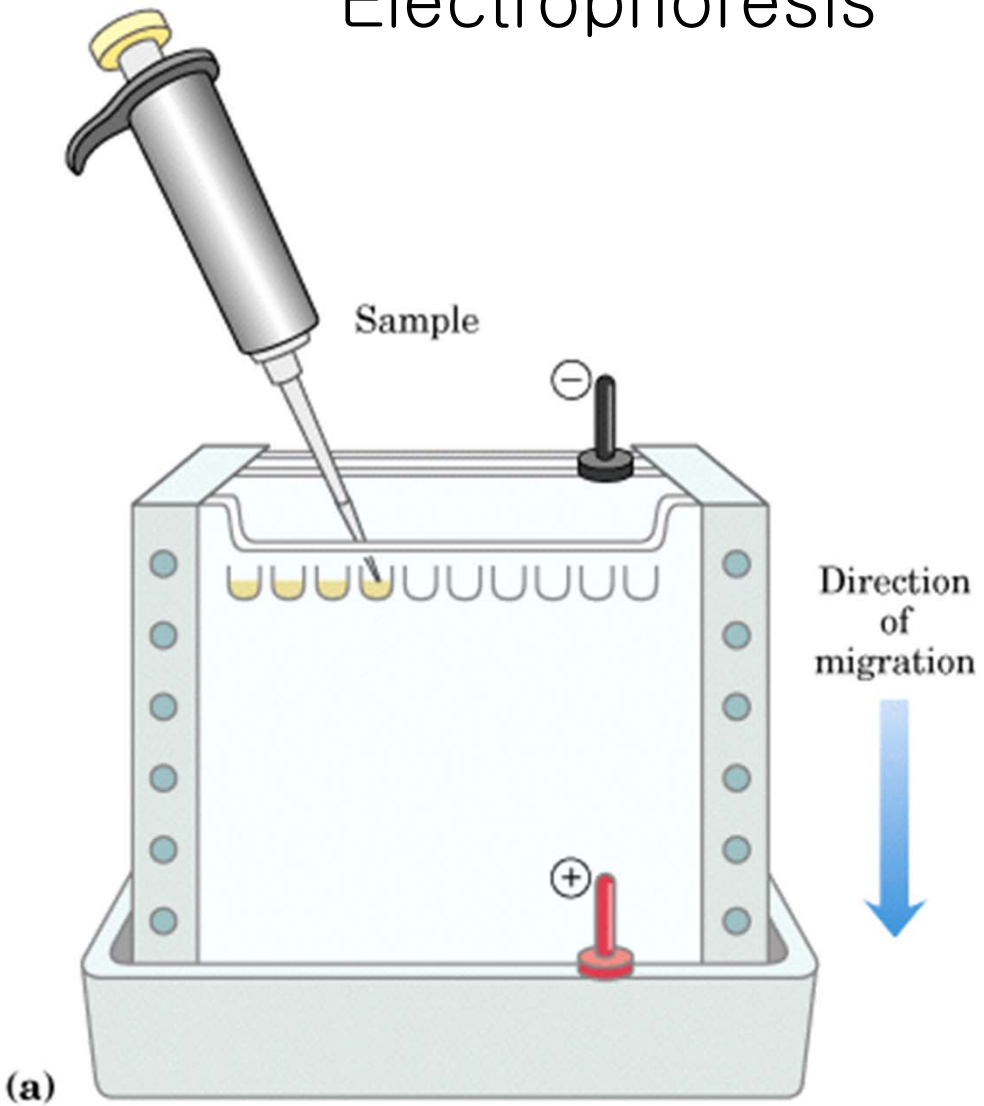
table 5–5

A Purification Table for a Hypothetical Enzyme*

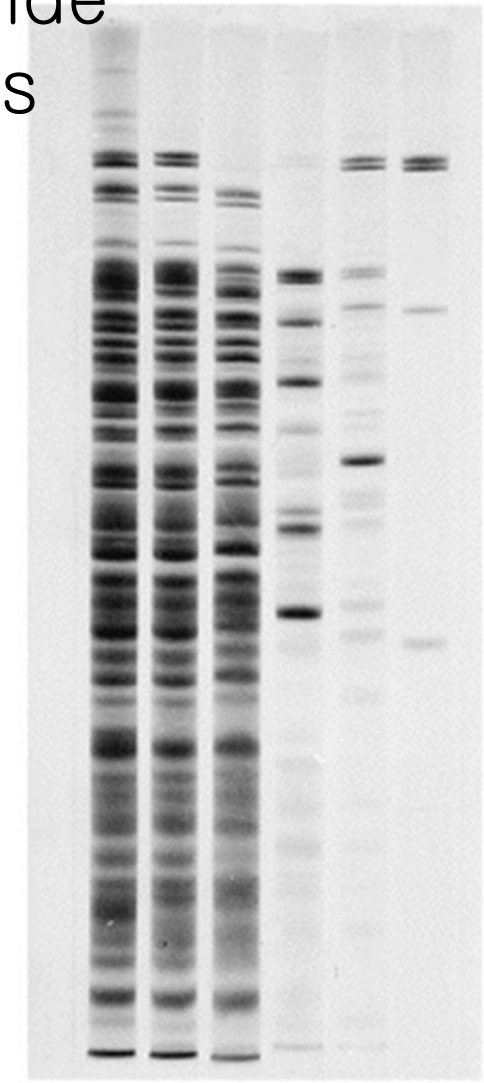
Procedure or step	Fraction volume (ml)	Total protein (mg)	Activity (units)	Specific activity (units/mg)
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	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.

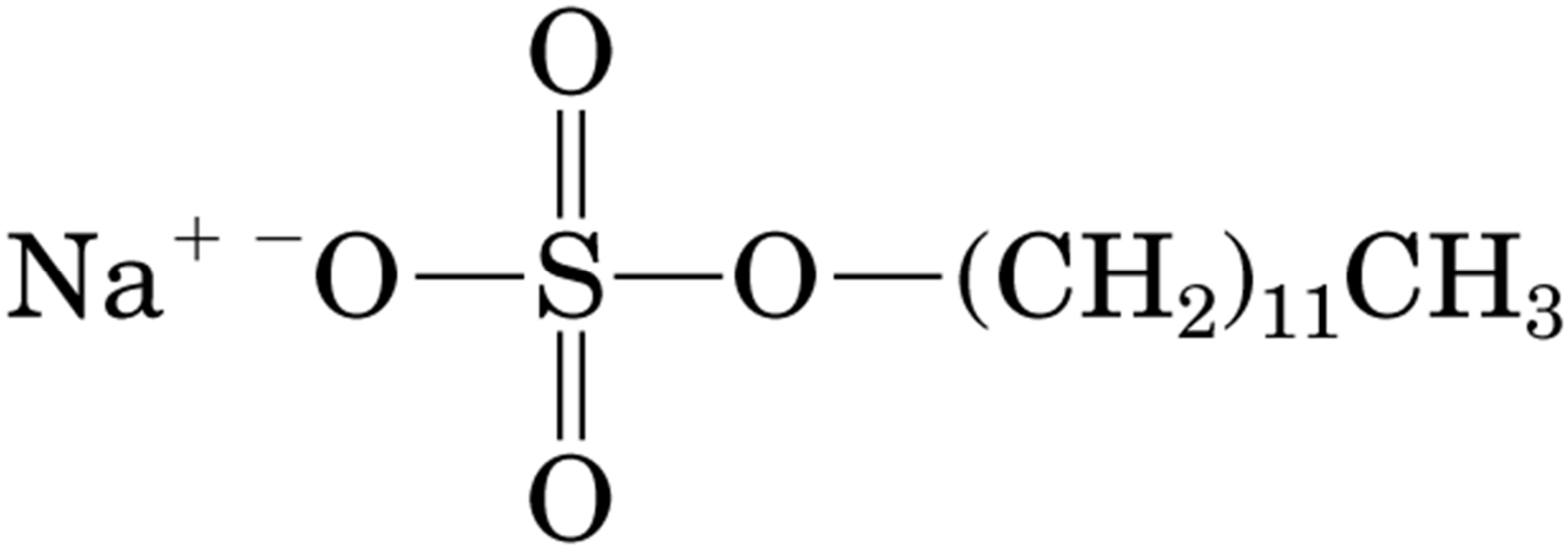
Electrophoresis



SDS-Polyacrileamide Gel Electrophoresis



(b)



Sodium dodecyl sulfate
(SDS)

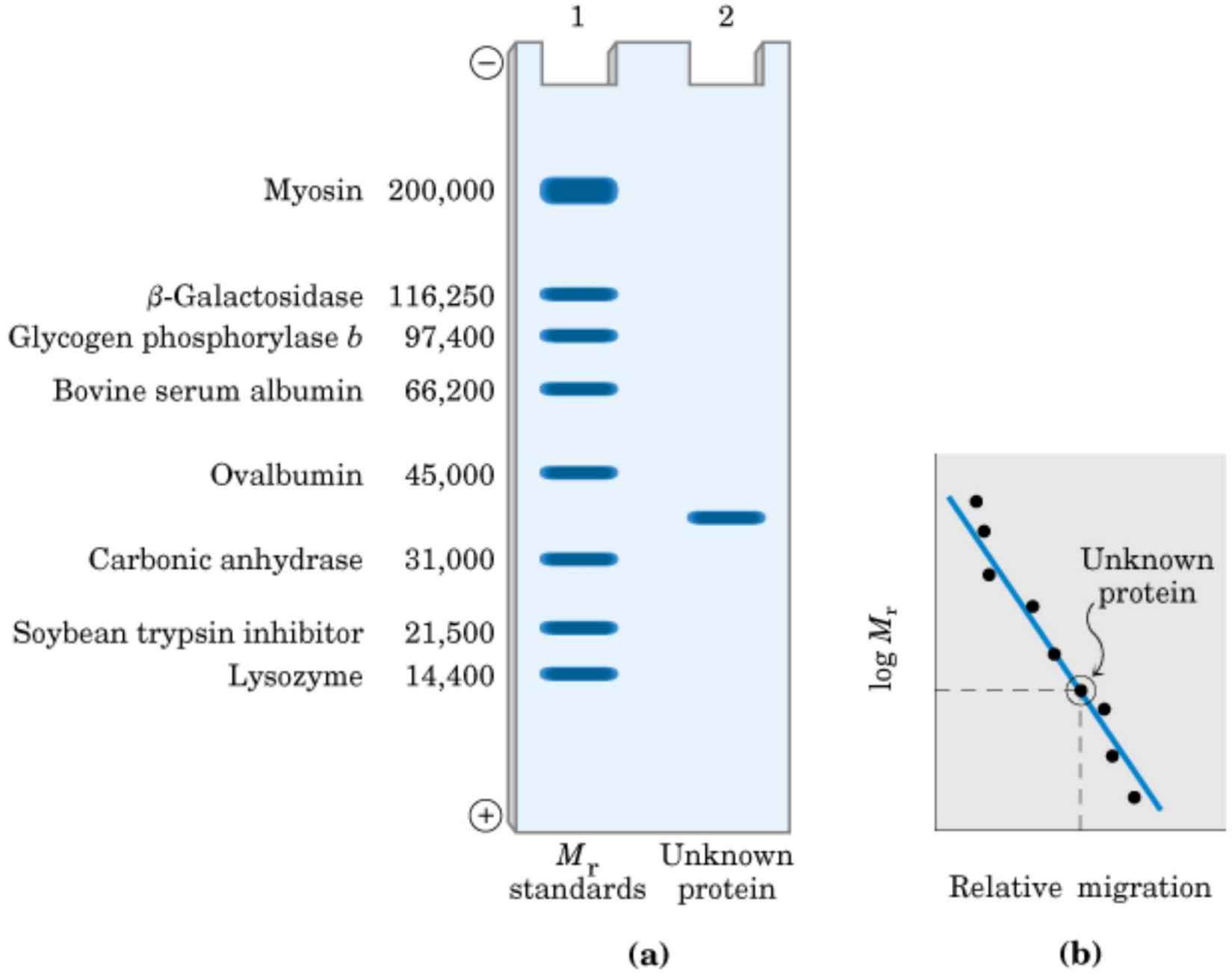
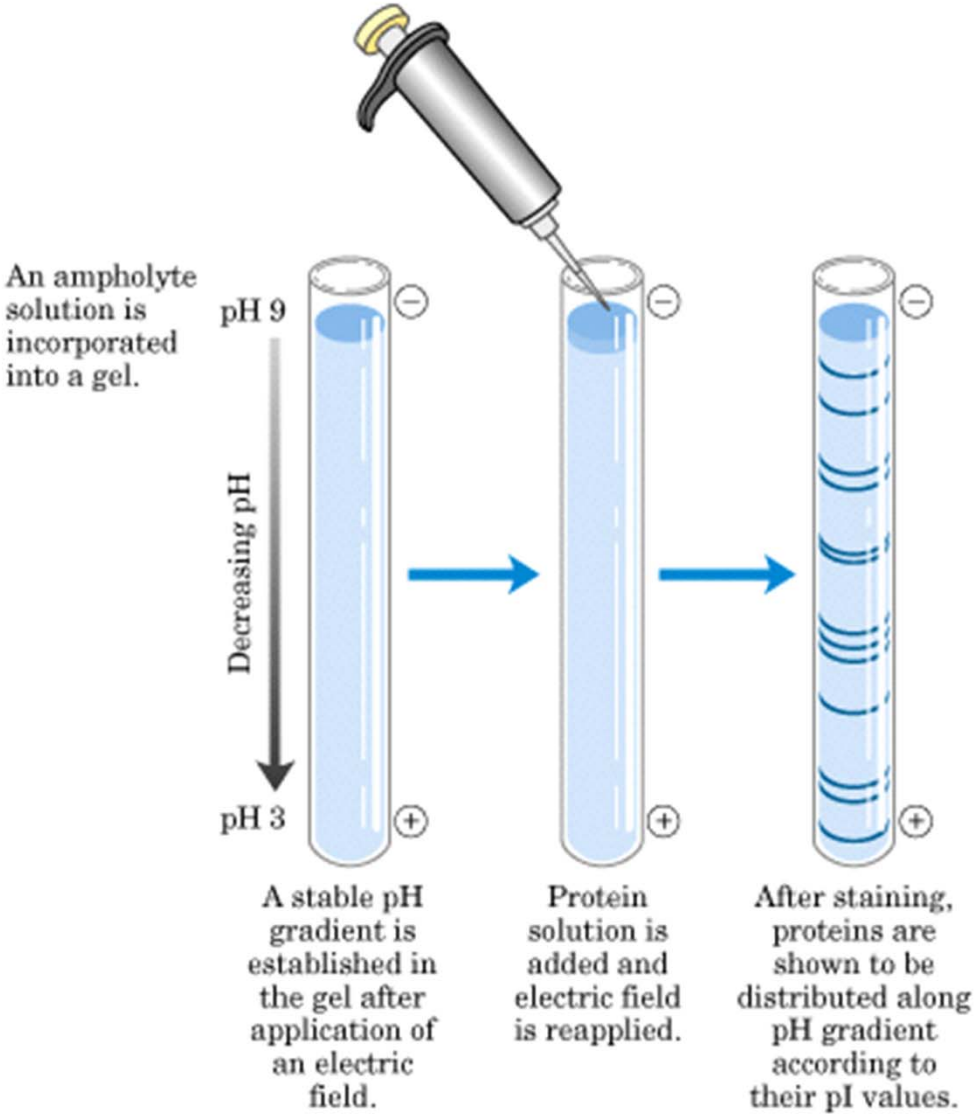

1
2
Myosin 200,000
β-Galactosidase 116,250
Glycogen phosphorylase b 97,400
Bovine serum albumin 66,200
Ovalbumin 45,000
Carbonic anhydrase 31,000
Soybean trypsin inhibitor 21,500
Lysozyme 14,400
M r standards
Unknown protein
(a)
log M r
Unknown protein
Relative migration
(b)

table 5–6

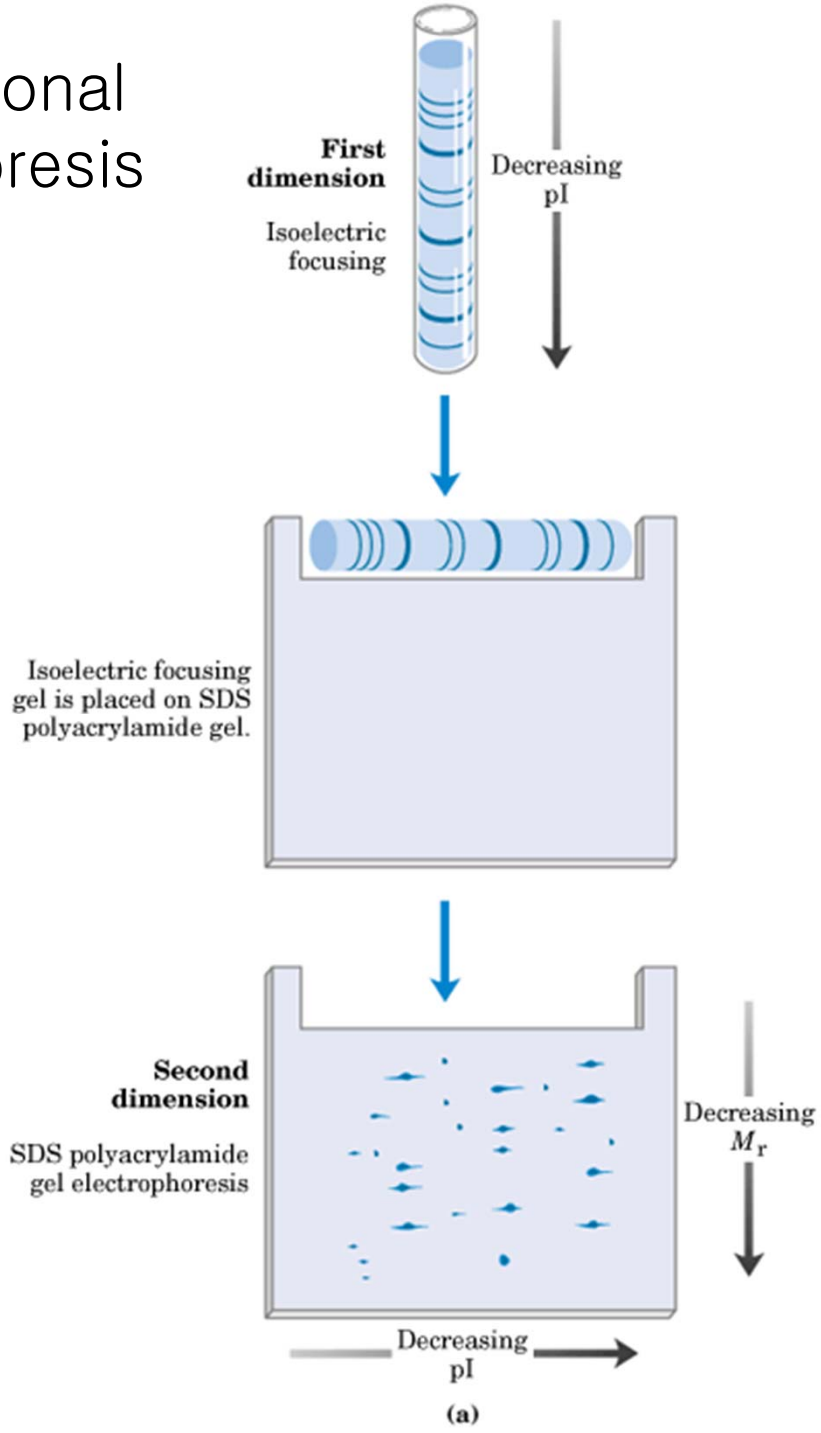
The Isoelectric Points of Some Proteins

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

Isoelectric Focusing



2–Dimensional Electrophoresis



2-Dimentional Electrophoresis

(b)