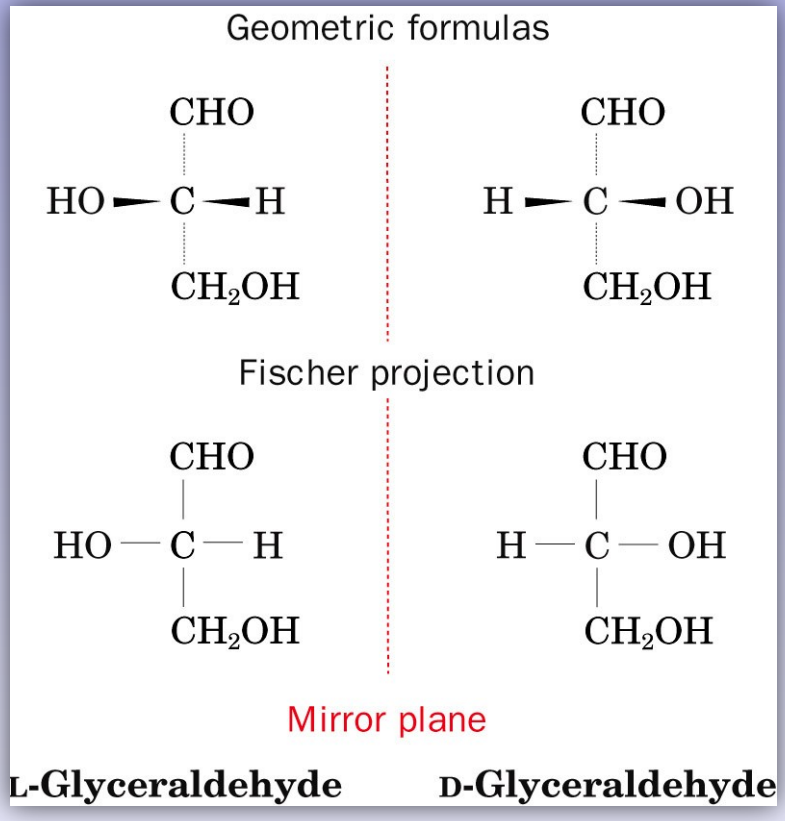
α-Amino Acids and Oligopeptides: Structure, Properties & Purification

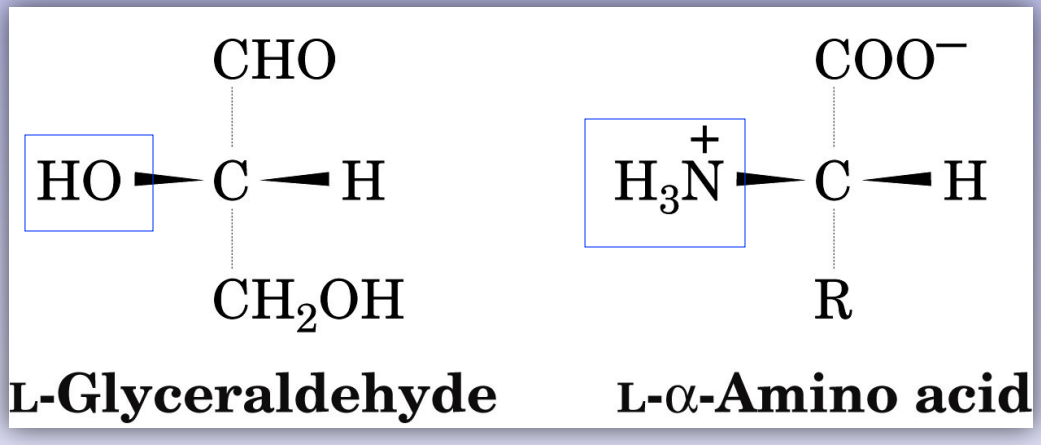
CHEM 420 – Principles of Biochemistry
Instructor – Anthony S. Serianni

Chapter 4: Voet/Voet, *Biochemistry*, 2011
Fall 2015

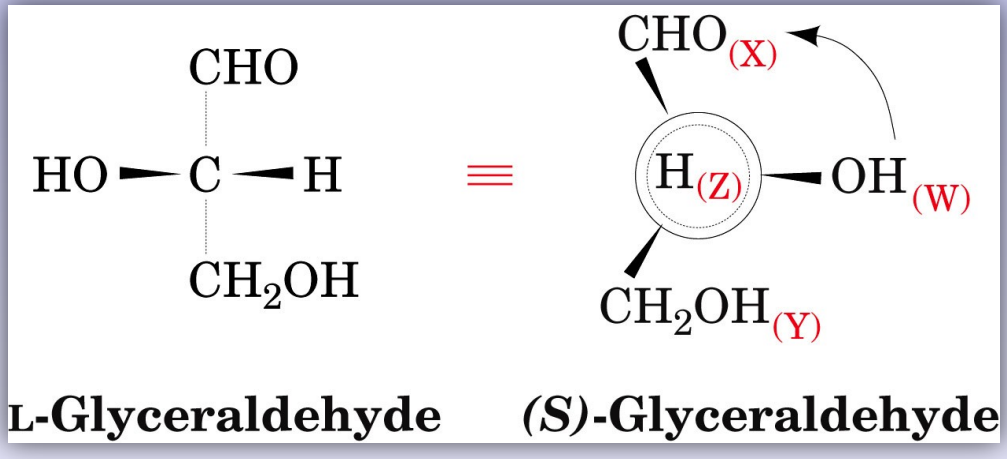
September 7 & 9



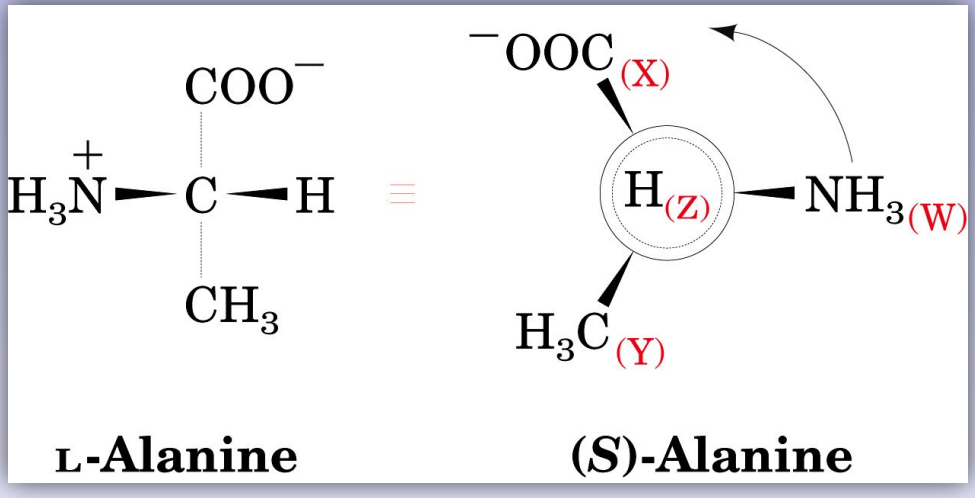
Fischer convention for assigning the absolute configuration of glyceraldehyde enantiomers. Glyceraldehyde is the reference compound for assigning D/L configuration of α-amino acids.



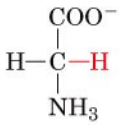
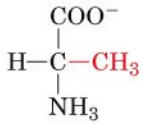
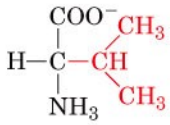
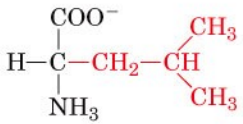
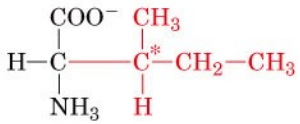
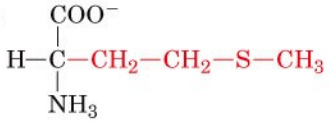
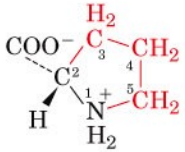
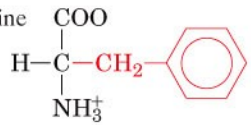
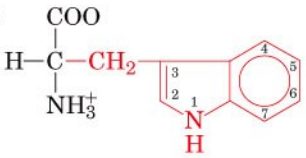
Correlation between glyceraldehyde configuration and α-amino acid configuration: L-series



Correlation between D/L convention and R/S convention for assignment of the absolute configuration of glyceraldehyde

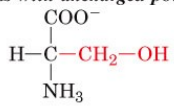
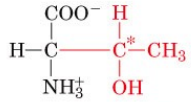
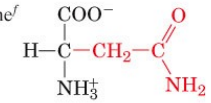
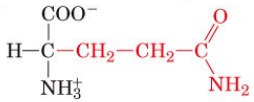
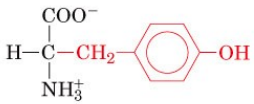
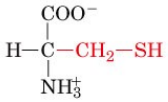
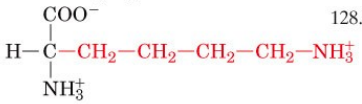
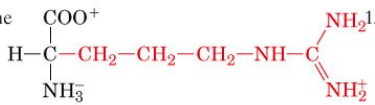
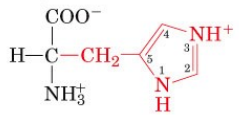
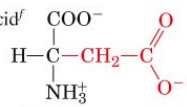
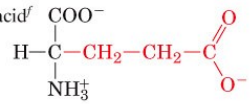


The structural formula of L-alanine

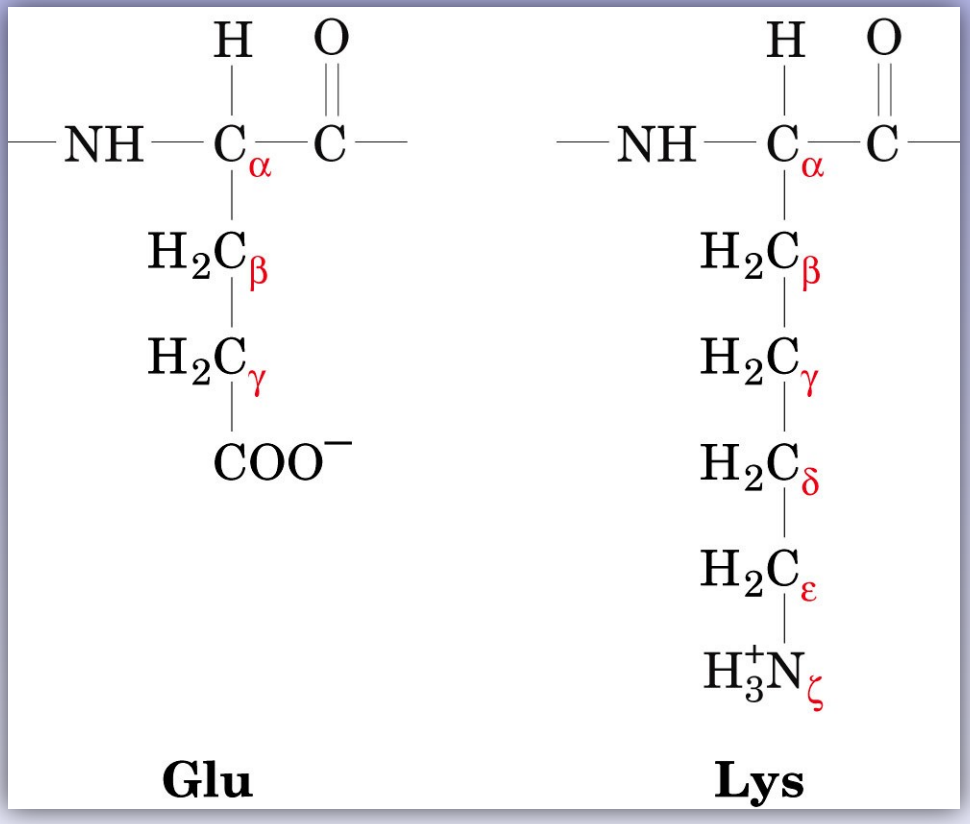
Name, Three-letter Symbol, and One-letter Symbol	Structural Formula[a]	Residue Mass (D)[b]	Average Occurrence in Proteins (%)[c]	pK_1 α-COOH[d]	pK_2 α-NH_3^+[d]	pK_R Side Chain[d]
Amino acids with nonpolar side chains						
Glycine Gly G		57.0	6.8	2.35	9.78	
Alanine Ala A		71.1	7.6	2.35	9.87	
Valine Val V		99.1	6.6	2.29	9.74	
Leucine Leu L		113.2	9.5	2.33	9.74	
Isoleucine Ile I		113.2	5.8	2.32	9.76	
Methionine Met M		131.2	2.4	2.13	9.28	
Proline Pro P		97.1	5.0	1.95	10.64	
Phenylalanine Phe F		147.2	4.1	2.20	9.31	
Tryptophan Trp W		186.2	1.2	2.46	9.41	

(*continued*)

Classification of α-amino acids according to side-chain structure

Name Three-letter Symbol, and One-letter Symbol	Structural Formula[a]	Residue Mass (D)[b]	Average Occurrence in Proteins (%)[c]	pK_1 -COOH[d]	pK_2 $-NH_3^+$[d]	pK_R Side Chain[d]
Amino acids with uncharged polar side chains						
Serine Ser S		87.1	7.1	2.19	9.21	
Threonine Thr T		101.1	5.6	2.09	9.10	
Asparagine[f] Asn N		114.1	4.3	2.14	8.72	
Glutamine[f] Gln Q		128.1	3.9	2.17	9.13	
Tyrosine Tyr Y		163.2	3.2	2.20	9.21	10.46 (phenol)
Cysteine Cys C		103.1	1.6	1.92	10.70	8.37 (sulfhydryl)
Amino acids with charged polar side chains						
Lysine Lys K		128.2	6.0	2.16	9.06	10.54 ($-NH_3$)
Arginine Arg R		156.2	5.2	1.82	8.99	12.48 (guanidino)
Histidine[e] His H		137.1	2.2	1.80	9.33	6.04 (imidazole)
Aspartic acid[f] Asp D		115.1	5.2	1.99	9.90	3.90 (-COOH)
Glutamic acid[f] Glu E		129.1	6.5	2.10	9.47	4.07 (-COOH)

Classification of α-amino acids according to side chain structure



Greek letter convention used to identify the side-chain atoms of L-glutamic acid and L-lysine.

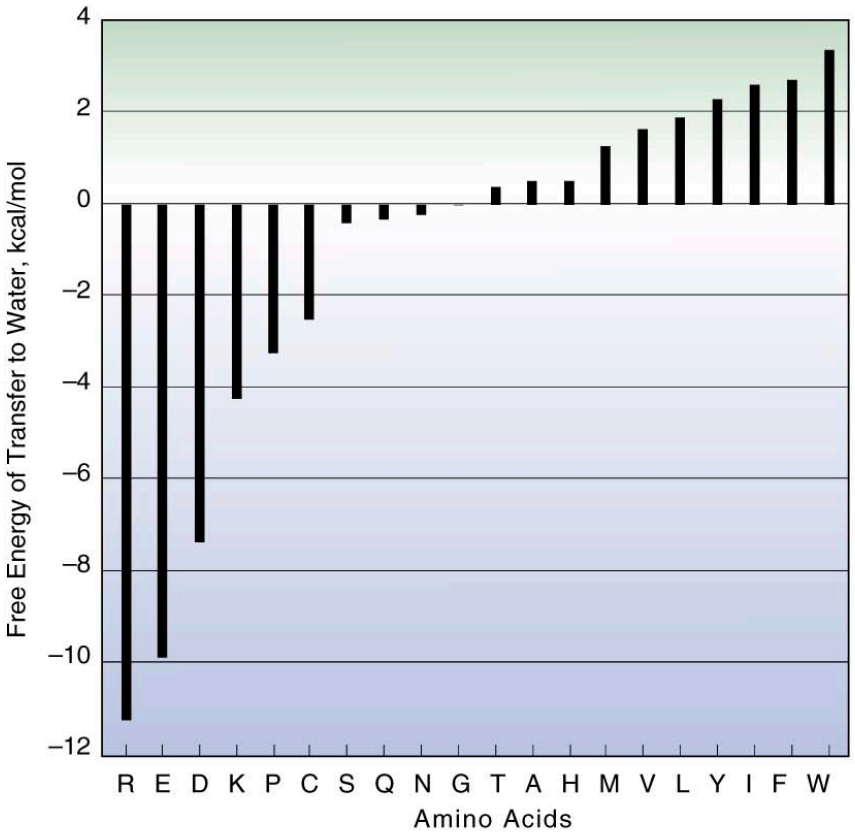
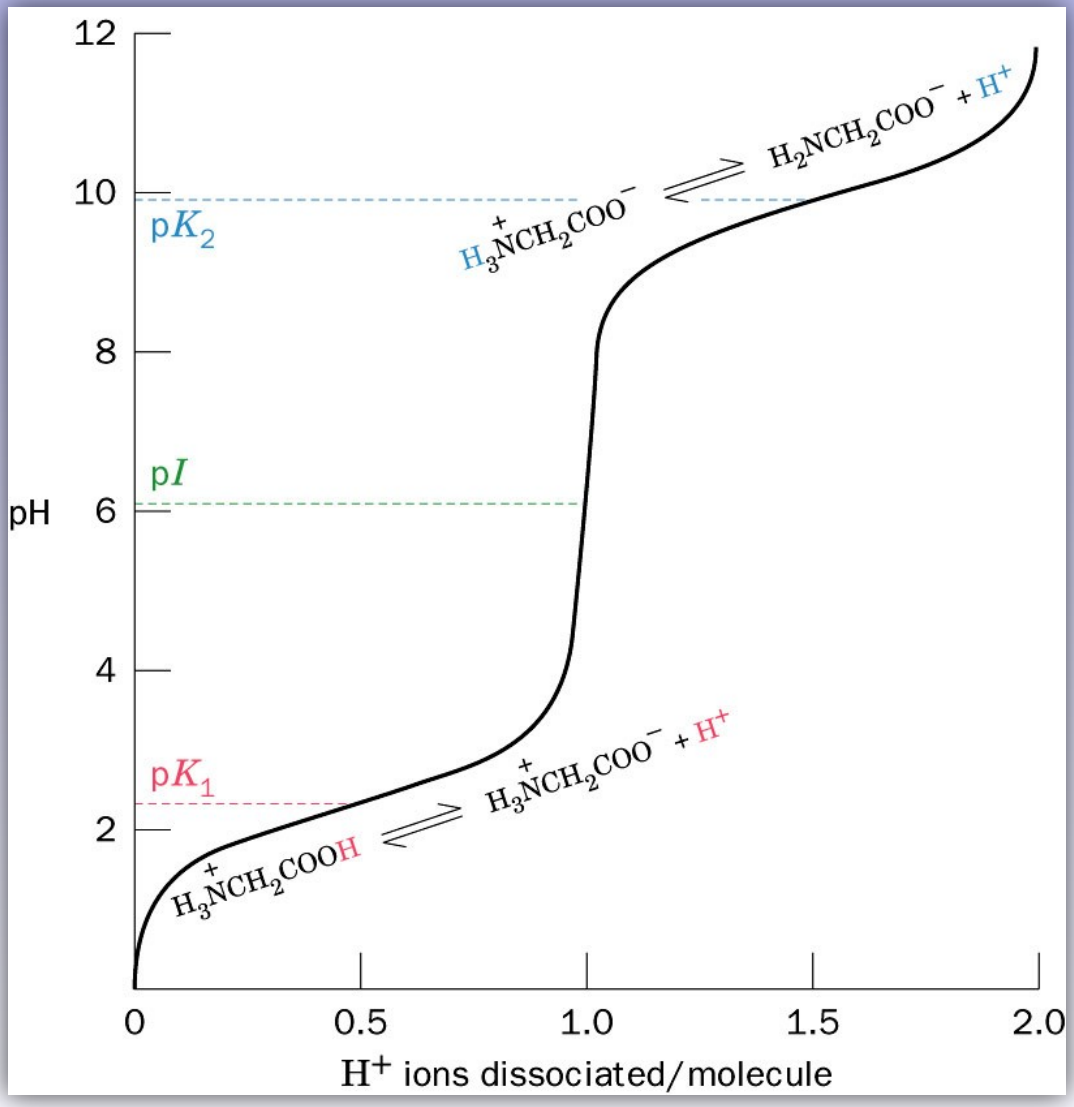
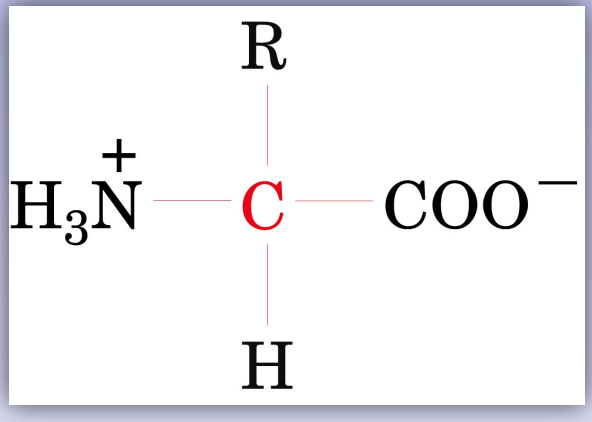


Figure 3.22. Relative hydrophobicity of amino acid side chains. Based on data from Von Heijne, G. and Blomberg, C. *Eur. J. Biochem.* 97:175, 1979; and from Nozaki, Y. and Tanford, C. *J. Biol. Chem.* 246:2211, 1971.

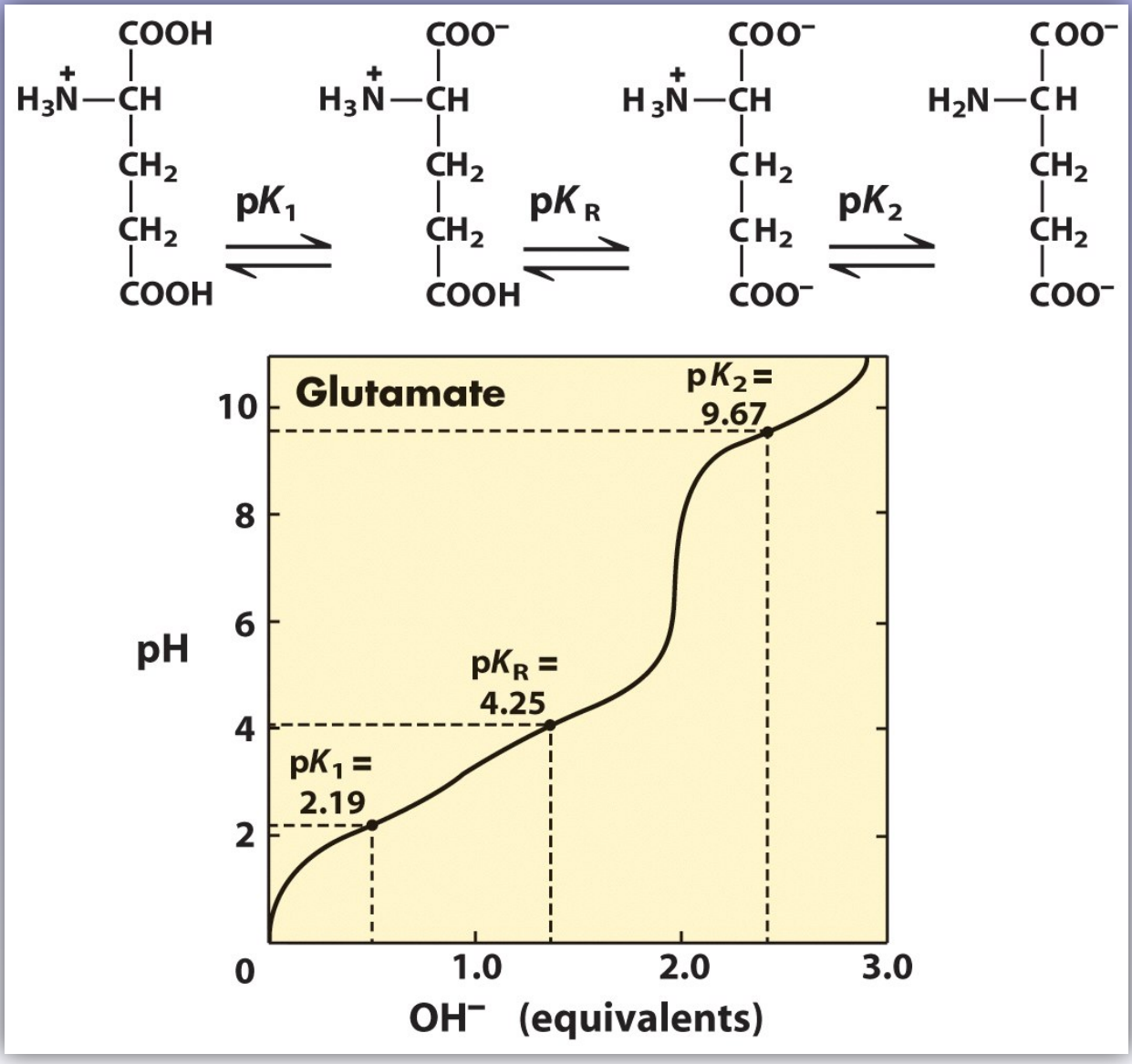
Based on partition of the amino acid between organic solvent and water. Negative values indicate a preference for water and positive values indicate a preference for organic solvent (dioxane) relative to glycine.



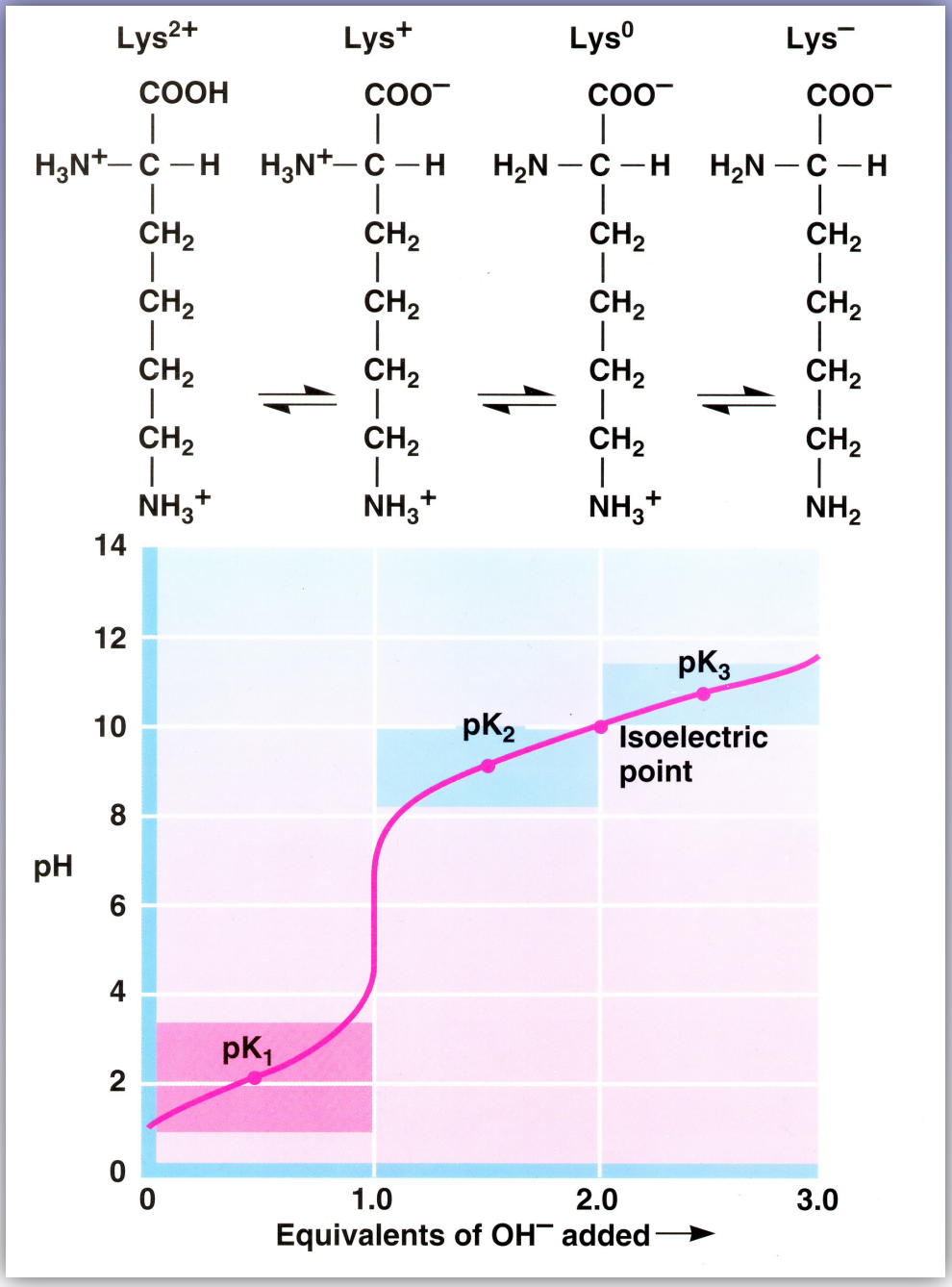
Titration curve for L-glycine (non-ionizable side chain)



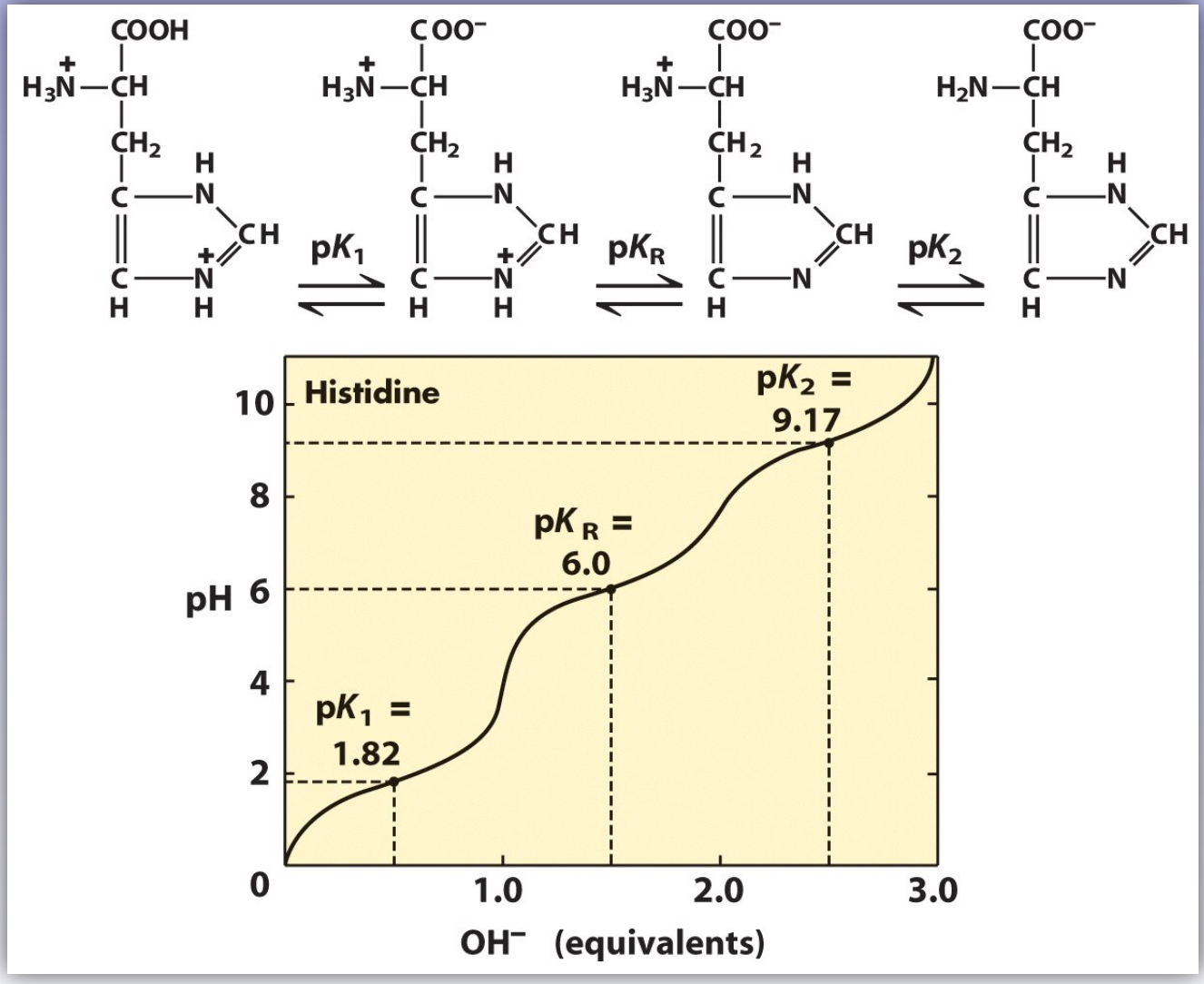
Zwitterionic form of an α-amino acid that occurs at physiological pH (R = non-ionizable); this ionic form is amphoteric.



Titration curve for L-glutamic acid



Titration curve for L-lysine



Titration curve for L-histidine

TABLE 5.3 Typical ranges observed for pK_a values of groups in proteins

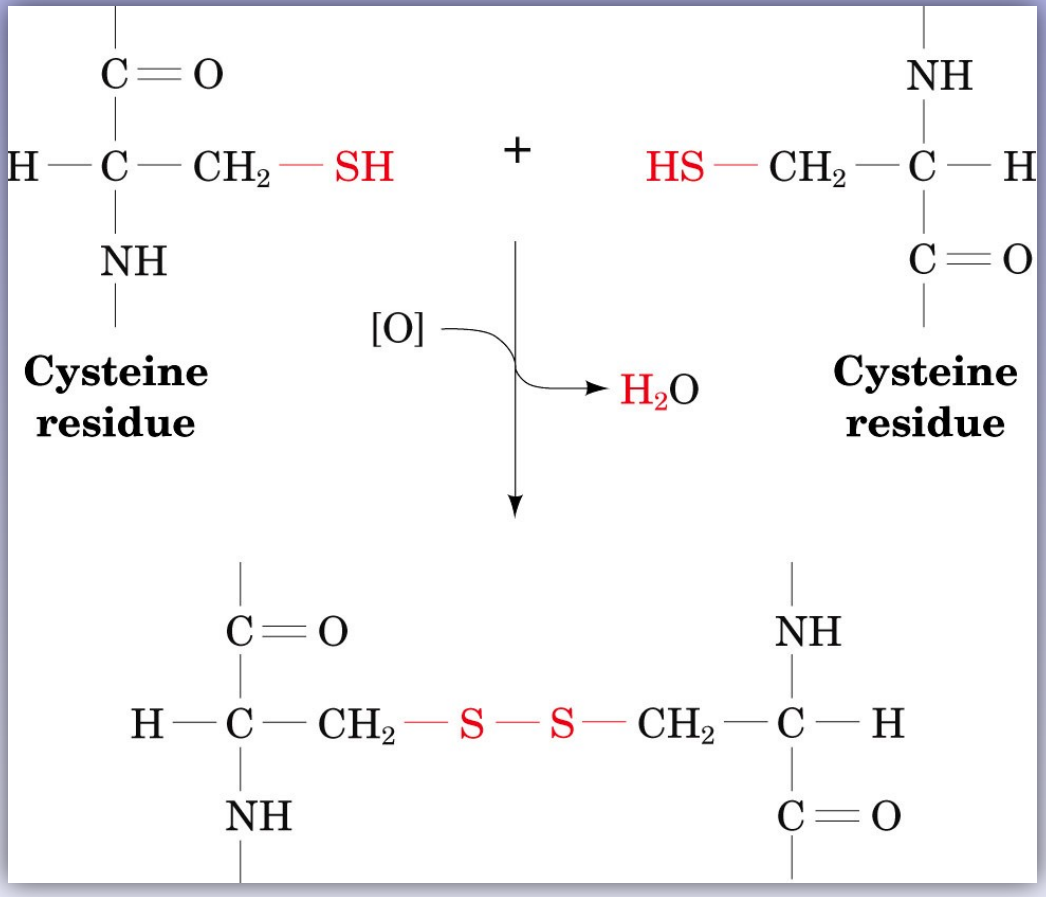
Group Type	Typical pK_a Range[a]
α-Carboxyl	3.5–4.0
Side chain carboxyls of aspartic and glutamic acids	4.0–4.8
Imidazole (histidine)	6.5–7.4
Cysteine (—SH)	8.5–9.0
Phenolic (tyrosine)	9.5–10.5
α-Amino	8.0–9.0
Side chain amino (lysine)	9.8–10.4
Guanidinyl (arginine)	~12

[a]Values outside these ranges are observed. For example, side chain carboxyls have been reported with pK_a values as high as 7.3.

Table 2-3. Perturbed pK_a Values of Amino Acid Residues in Enzymes

Enzyme	Residue	pK_a	ΔpK_a	Study
Papain	Cys25	3.3	–5.4	Pinitglang, 1997
Papain	His	9.5	+3.0	
Acetoacetate decarboxylase	Lys115	6.0	–4.5	Kokesh, 1971; Frey, 1971
β-Galactosidase	Asp	7.5	+3.5	
UDP-galactose 4-epimerase	Tyr149	6.1	–4.1	Liu, 1997; Berger, 2001

[a]The pK_a of the amino acid side chain in solution minus that of the same residue in the enzyme active site. The normal values of pK_a in solution are given in table 1-2 in chapter 1.



Conversion of two L-cysteine molecules to the L-cystine dimer involves redox chemistry. The reaction is enzyme-catalyzed *in vivo*.

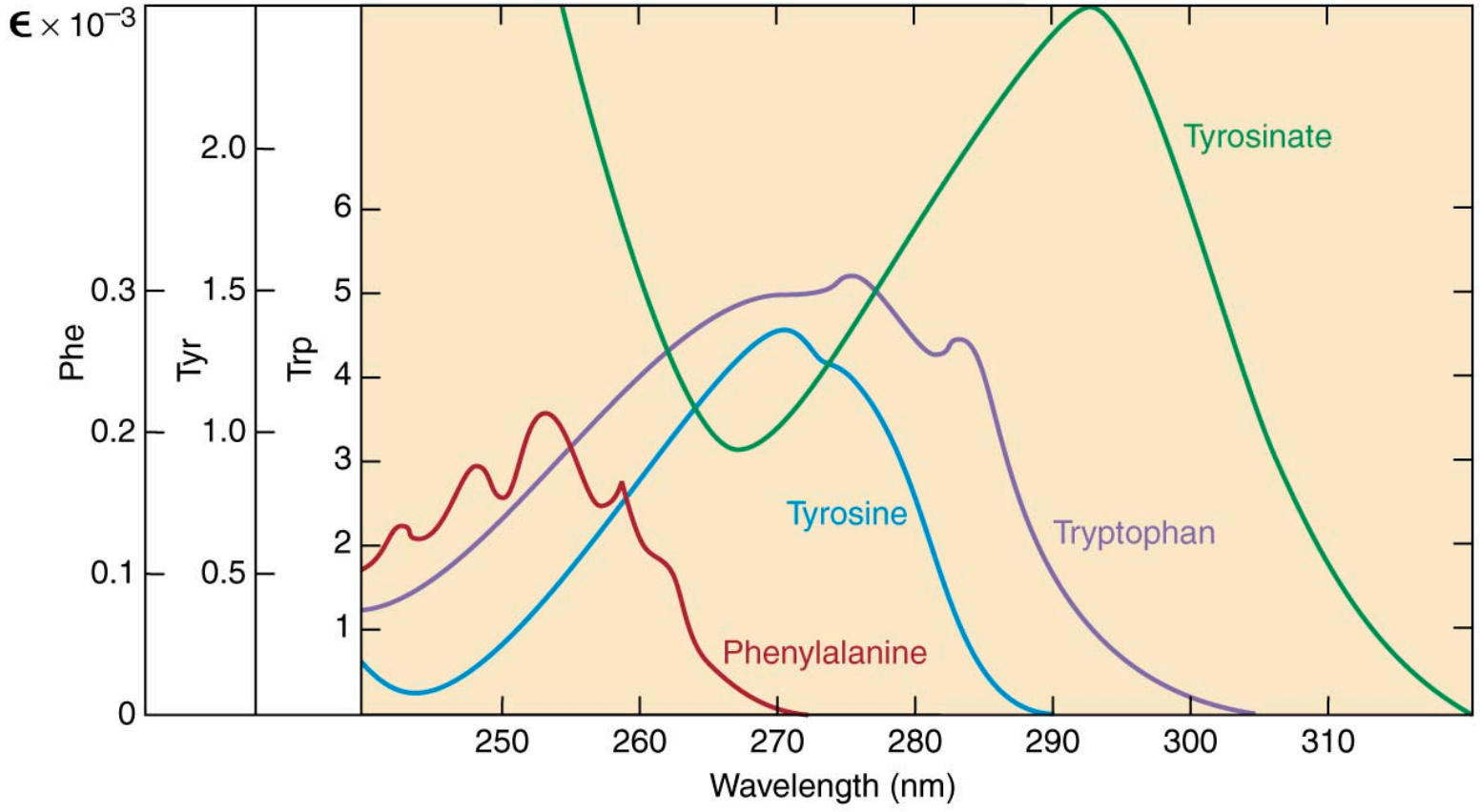


Figure 3.71. Ultraviolet absorption for chromophores of Phe, Tyr, Trp, and tyrosinate. Redrawn from d'Albis, A. and Gratzer, W. B. In: A. T. Bull, J. R. Lagnado, J. O. Thomas, and K. F. Tipton (Eds.), *Companion to Biochemistry*. London: Longmans, 1974, p. 170.

Note different extinction coefficients (left axes) for the different chromophores

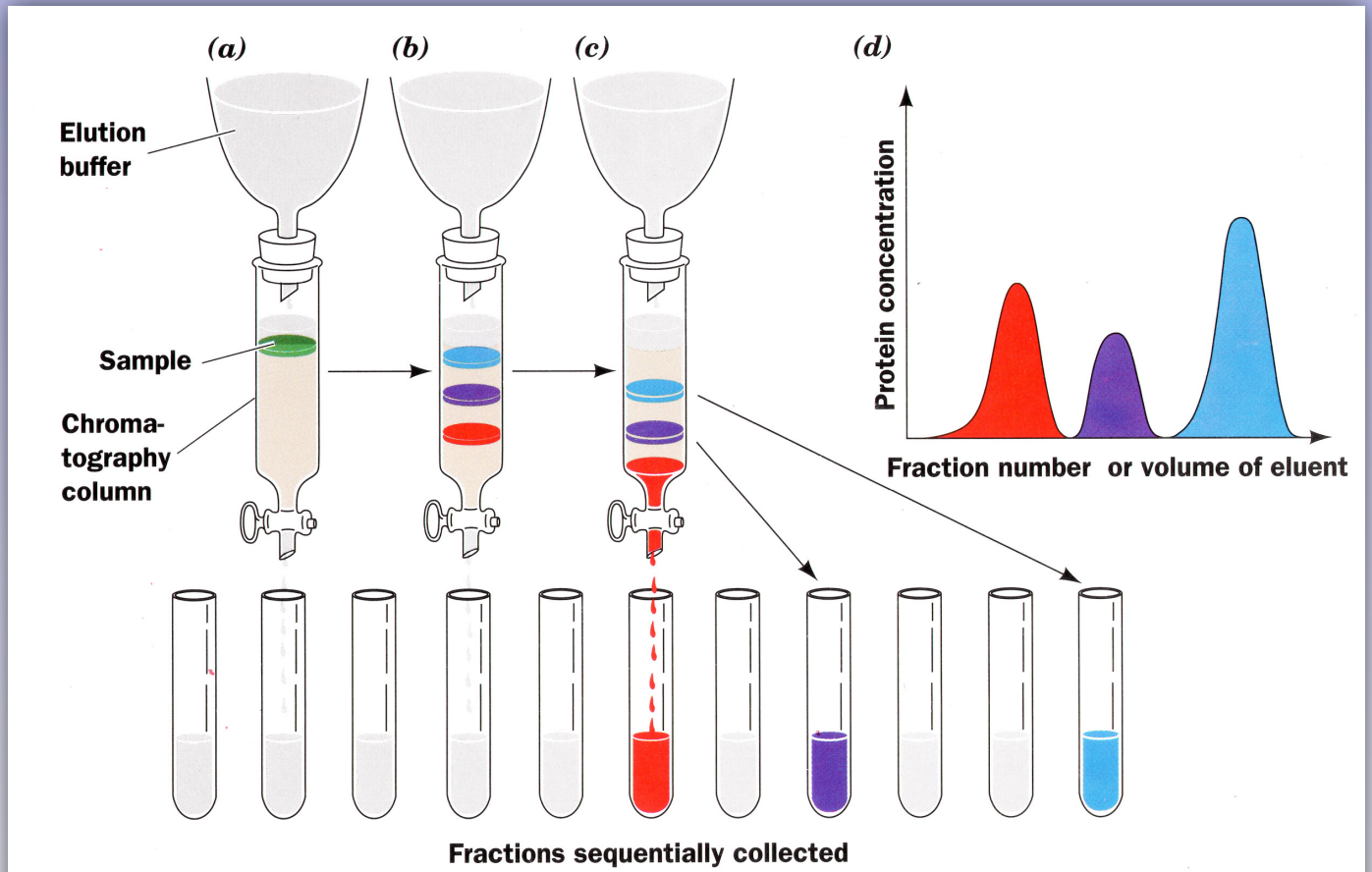
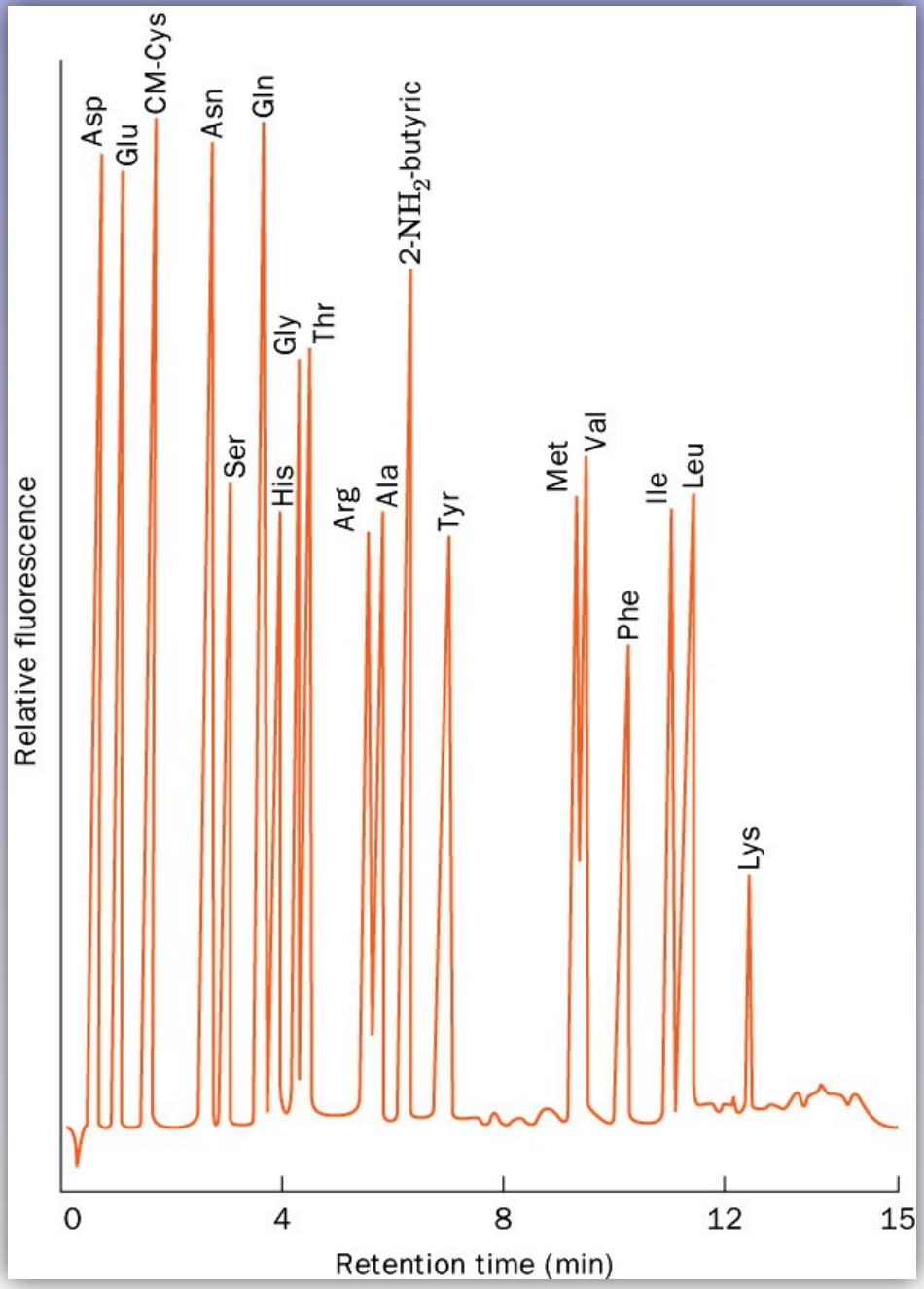


Figure 5-6. Ion exchange chromatography.



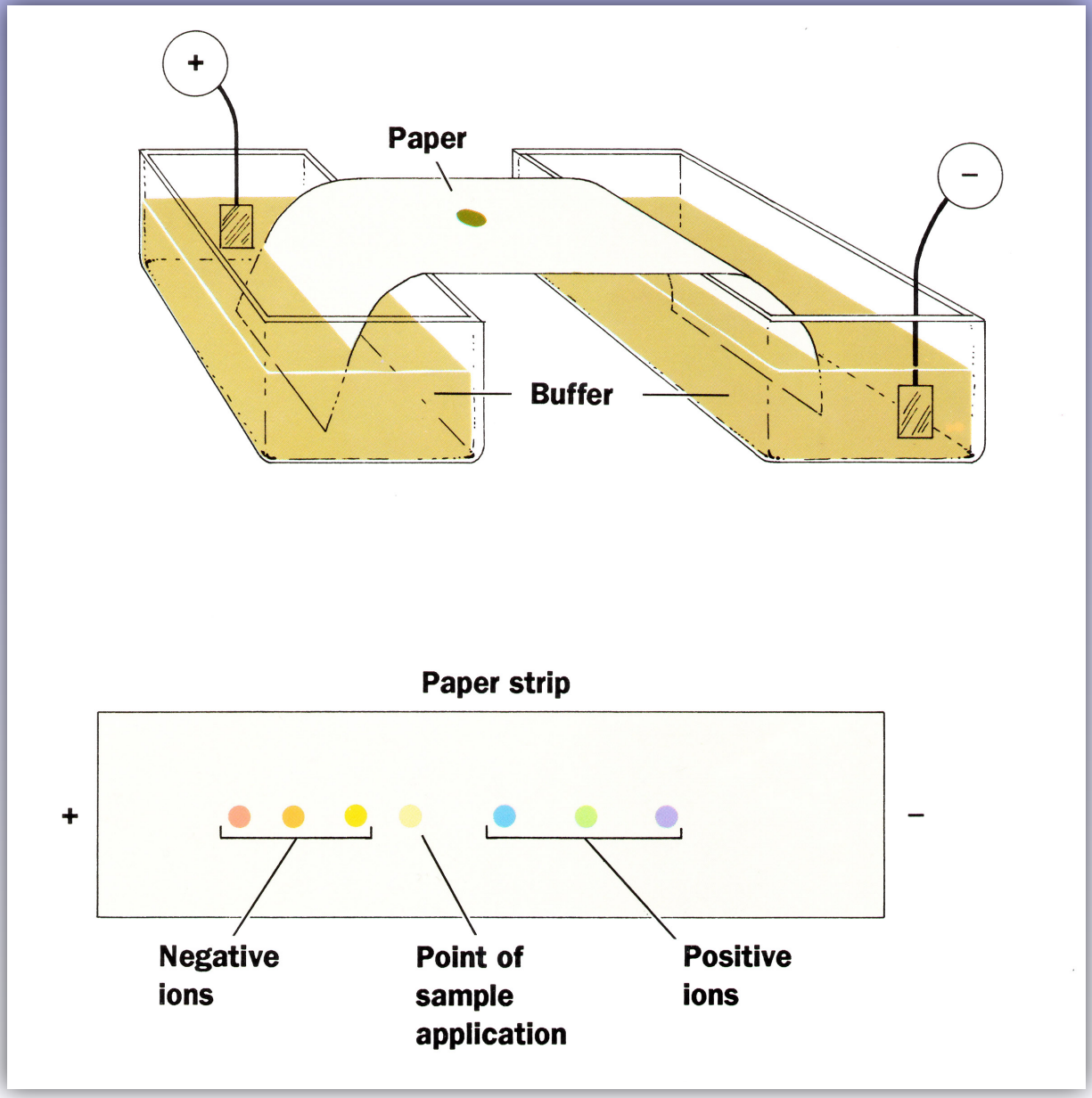
Amino acid analysis (separation) via HPLC. This type of chromatographic separation is used to determine the amino acid composition of a purified oligopeptide or protein.

The chromatographic HPLC columns are commonly packed with ion-exchange resins.

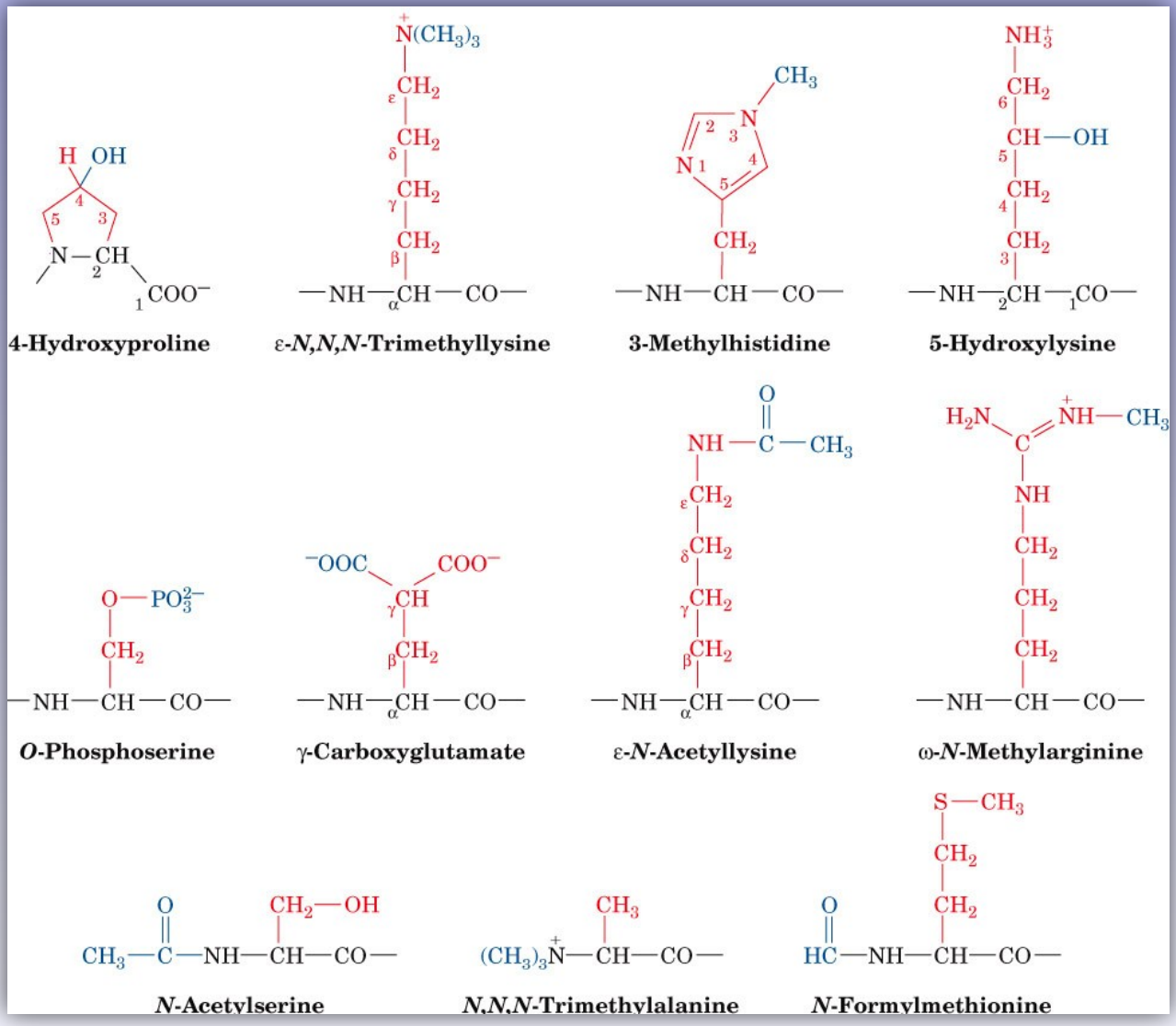
TABLE 3–3 Amino Acid Composition of Two Proteins

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

*In some common analyses, such as acid hydrolysis, Asp and Asn are not readily distinguished from each other and are together designated Asx (or B). Similarly, when Glu and Gln cannot be distinguished, they are together designated Glx (or Z). In addition, Trp is destroyed. Additional procedures must be employed to obtain an accurate assessment of complete amino acid content.



Paper electrophoresis: Separation of small charged molecules

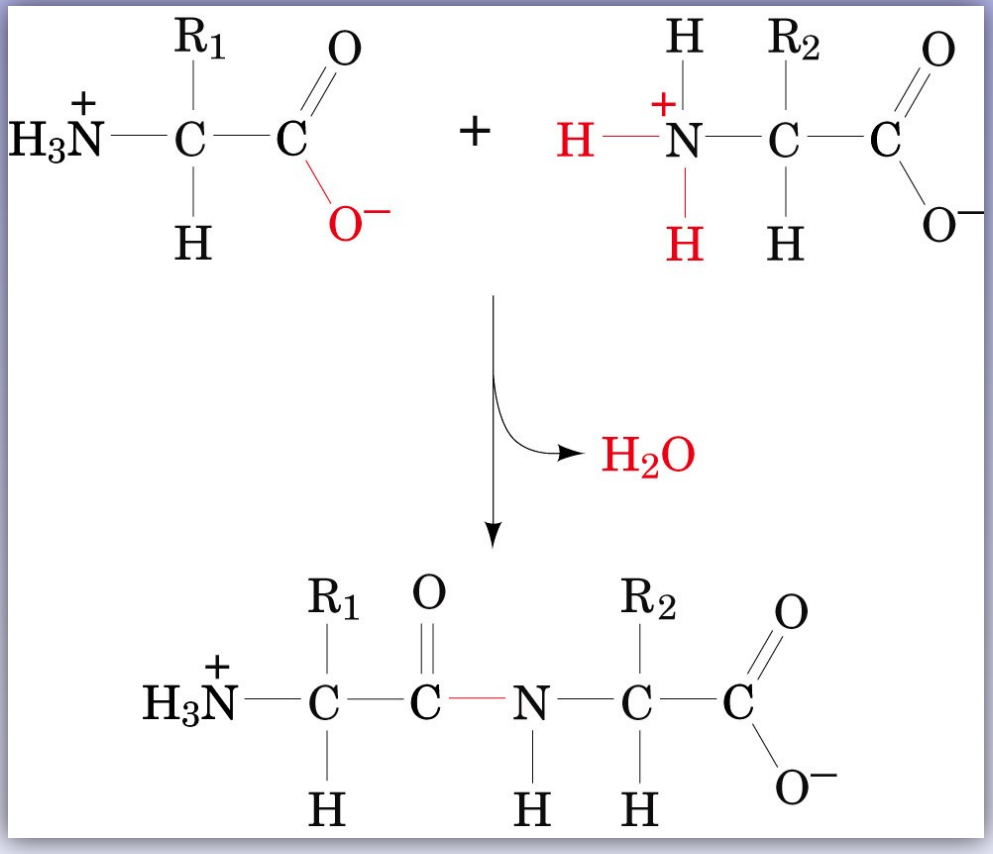


Uncommon amino acids that are components of some proteins; introduced by post-translational modification.

Biosynthesis of α-amino acids *in vivo*

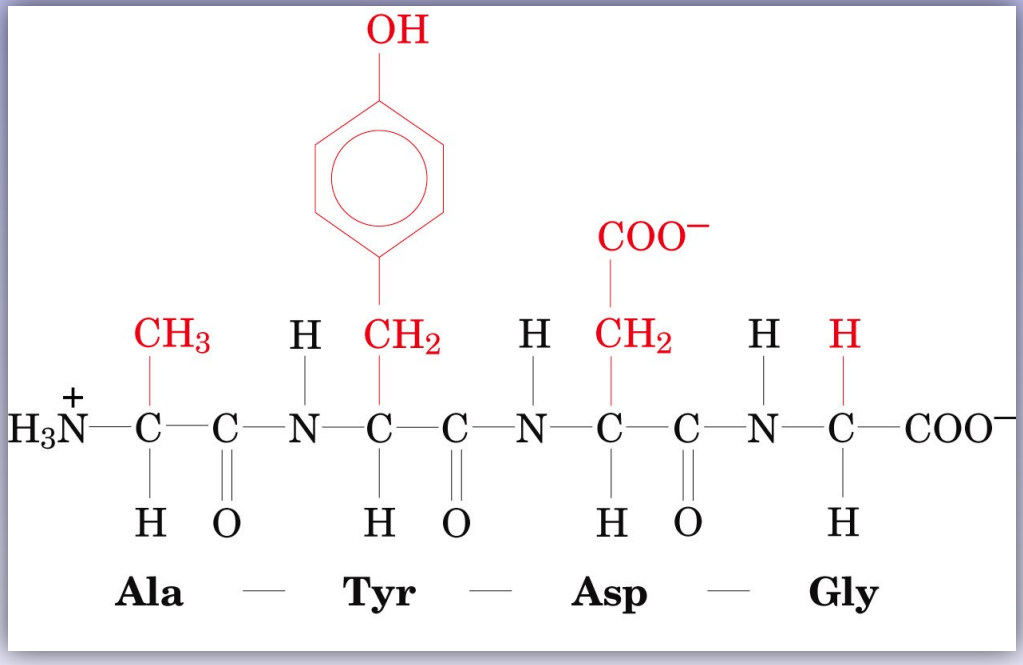
Essential α-amino acids (humans)

L-histidine
L-isoleucine
L-leucine
L-lysine
L-methionine
L-phenylalanine
L-threonine
L-tryptophan
L-valine



Condensation of two α-amino acids with the loss of water to produce a dipeptide. Peptide (amide) bond formation is enzyme-catalyzed *in vivo*.

Nomenclature: Oligopeptides



Structure and ionic form of the tetrapeptide, Ala-Tyr-Asp-Gly, at neutral pH. α-Amino acids are identified by three-letter or one-letter symbolisms (AYDG), and oligopeptide sequences are always written from the *N*-terminus (left) to the *C*-terminus (right).

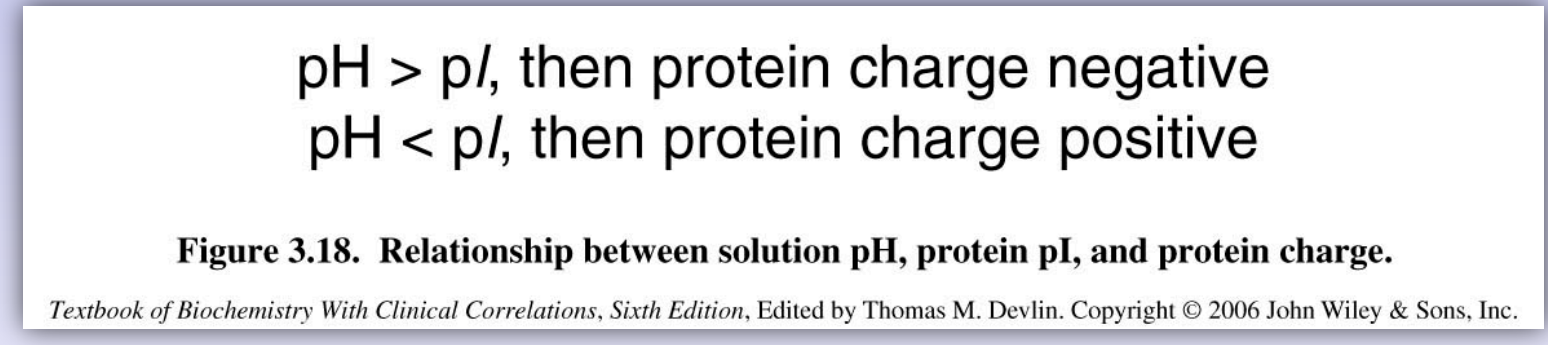


Figure 3.18. Relationship between solution pH, protein pI, and protein charge.

Figure 3.19. Titration curve of human serum albumin at 25°C and an ionic strength of 0.150. Redrawn from Tanford, C. *J. Am. Chem. Soc.* 72:441, 1950.

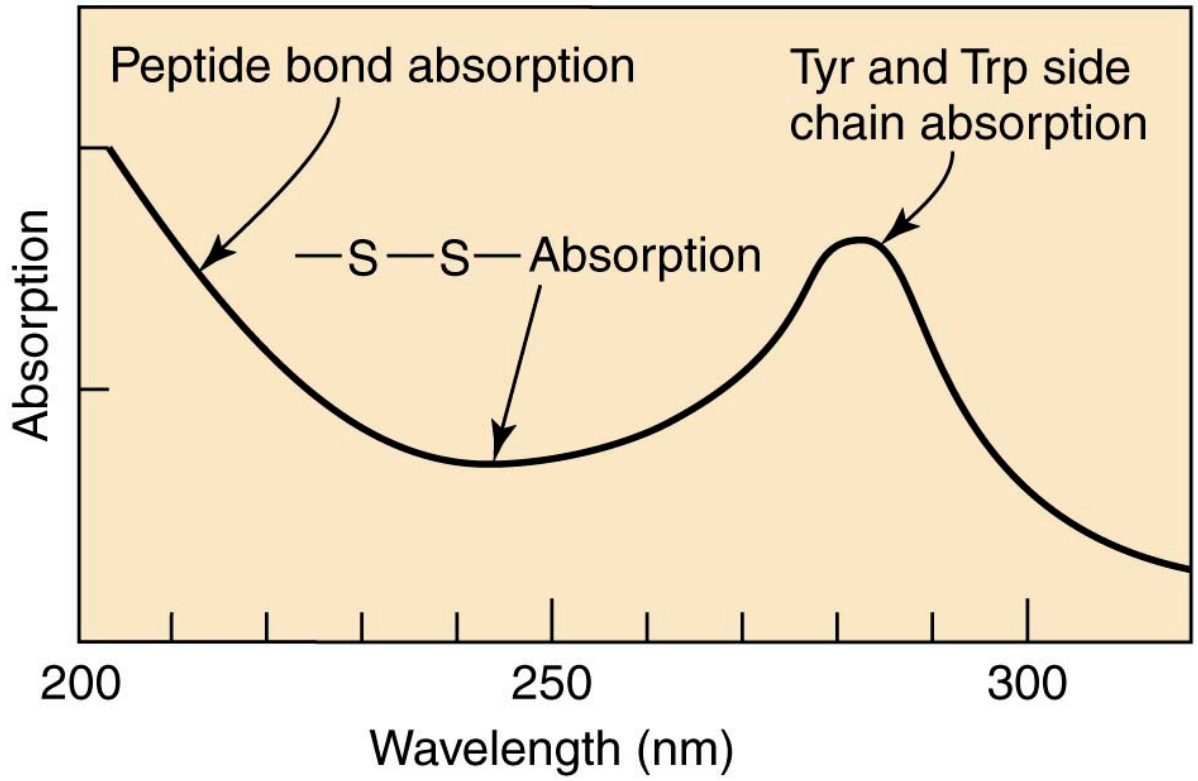


Figure 3.70. Ultraviolet absorption spectrum of the globular protein α-chymotrypsin.

Typical UV spectrum of a protein