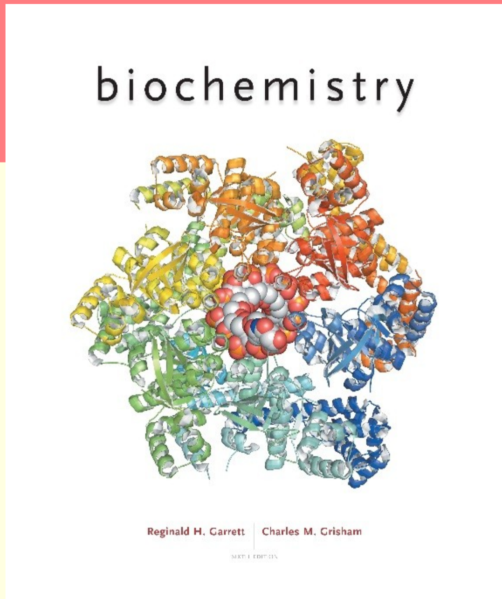

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Charles M. Grisham

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Chapter 4
Amino Acids

with a look at hydrophobic interactions from Chapter 2

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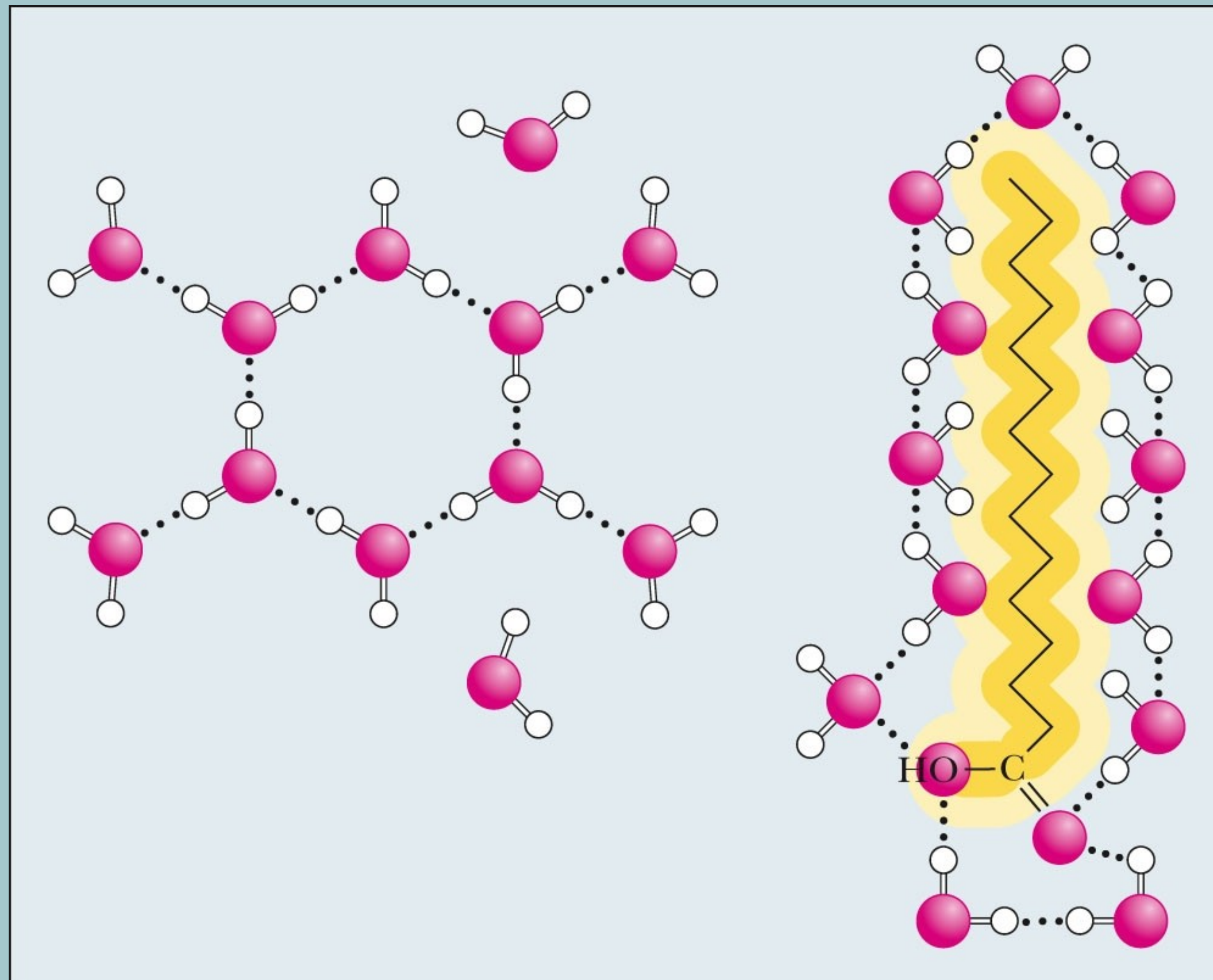
Outline

- What are hydrophobic interactions?
- What are the structures and properties of amino acids?
- What are the stereochemical properties of amino acids?
- What are the acid-base properties of amino acids?
- What are the spectroscopic properties of amino acids?
- How are amino acid mixtures separated and analyzed?
- What reactions do amino acids undergo?

Hydrophobic Interactions

- A nonpolar solute "organizes" water at its surface into an H-bonded clathrate-like structure
- This is an increase in "order" of water
- This is an unfavorable decrease in ENTROPY
- Nonpolar molecules associate to reduce the nonpolar surface area and thus minimize the decrease in ENTROPY

Figure 2.5
(left) A network of transiently H-bonded water molecules.
(right) A clathrate cage of more ordered, H-bonded water molecules around a nonpolar solute molecule.



Hydrophobic Interactions

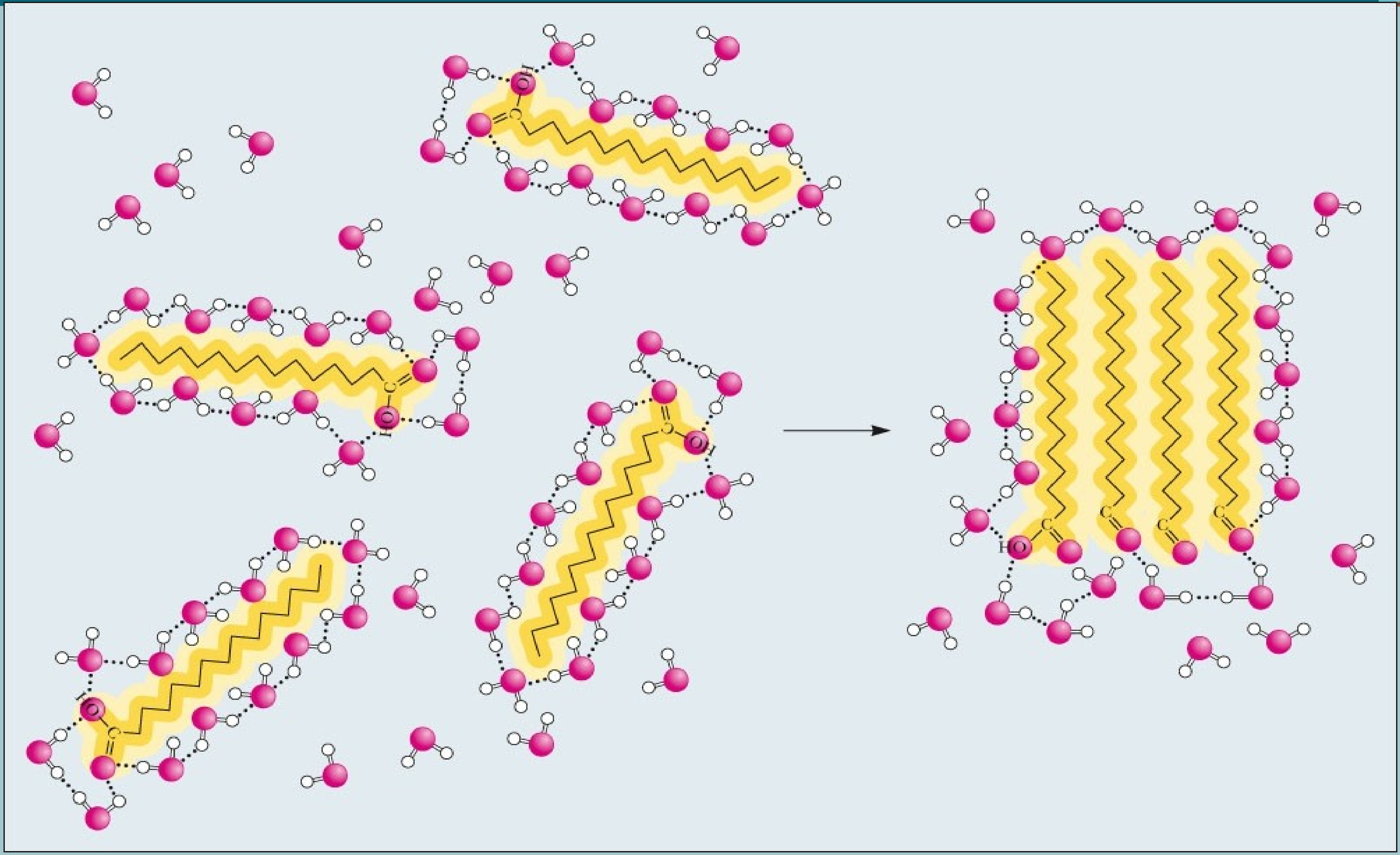


Fig. 2.6 Association of nonpolar molecules releases water from the clathrate structures, increasing the entropy of solvent water. This entropy-driven association of nonpolar solutes is called hydrophobic interaction.

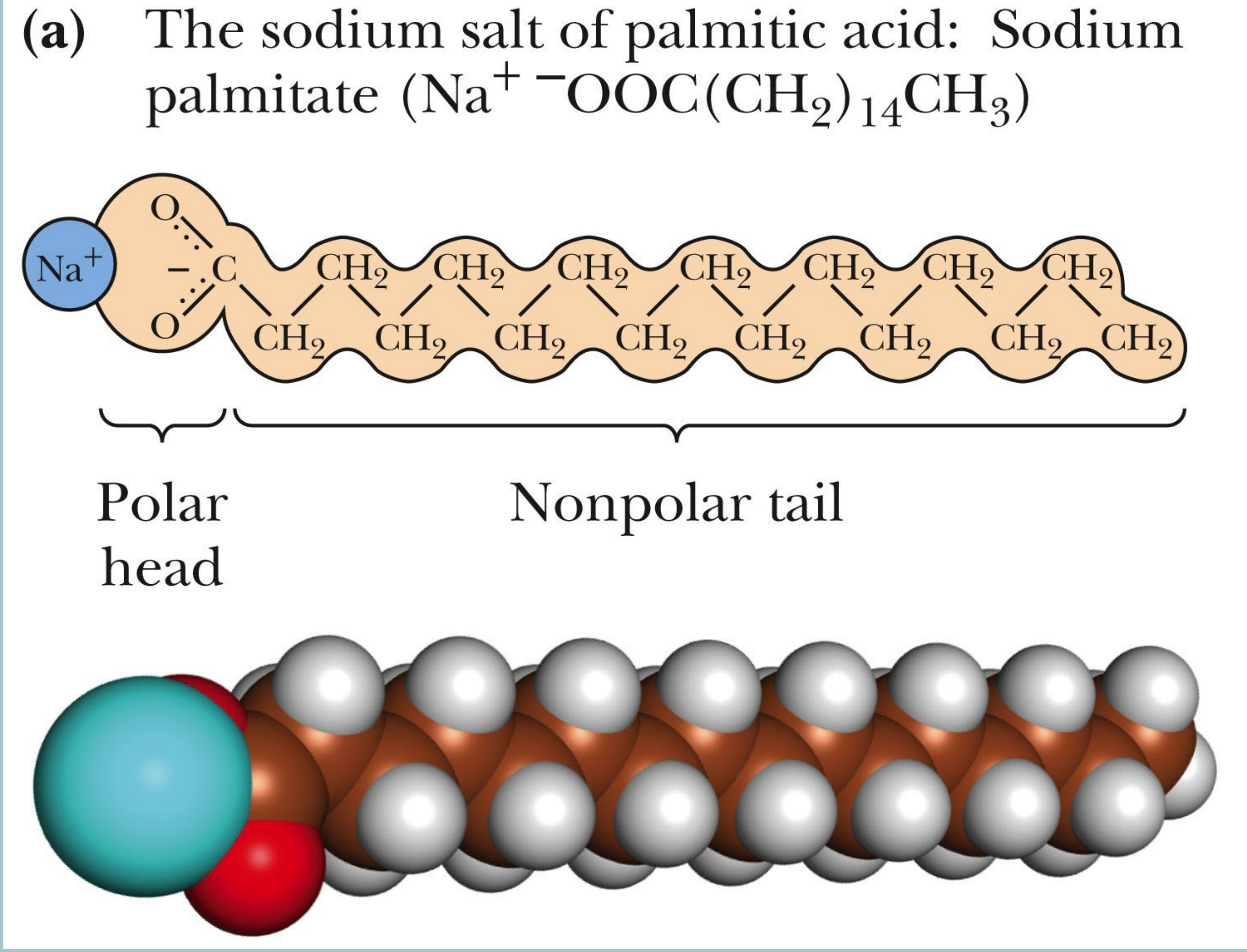
Amphiphilic Molecules

Also called "amphipathic“

- Refers to molecules that contain both polar and nonpolar groups
- These molecules are attracted to both polar and nonpolar environments
- Good examples - fatty acids and detergents, such as SDS (sodium dodecyl sulfate), and most common amino acids

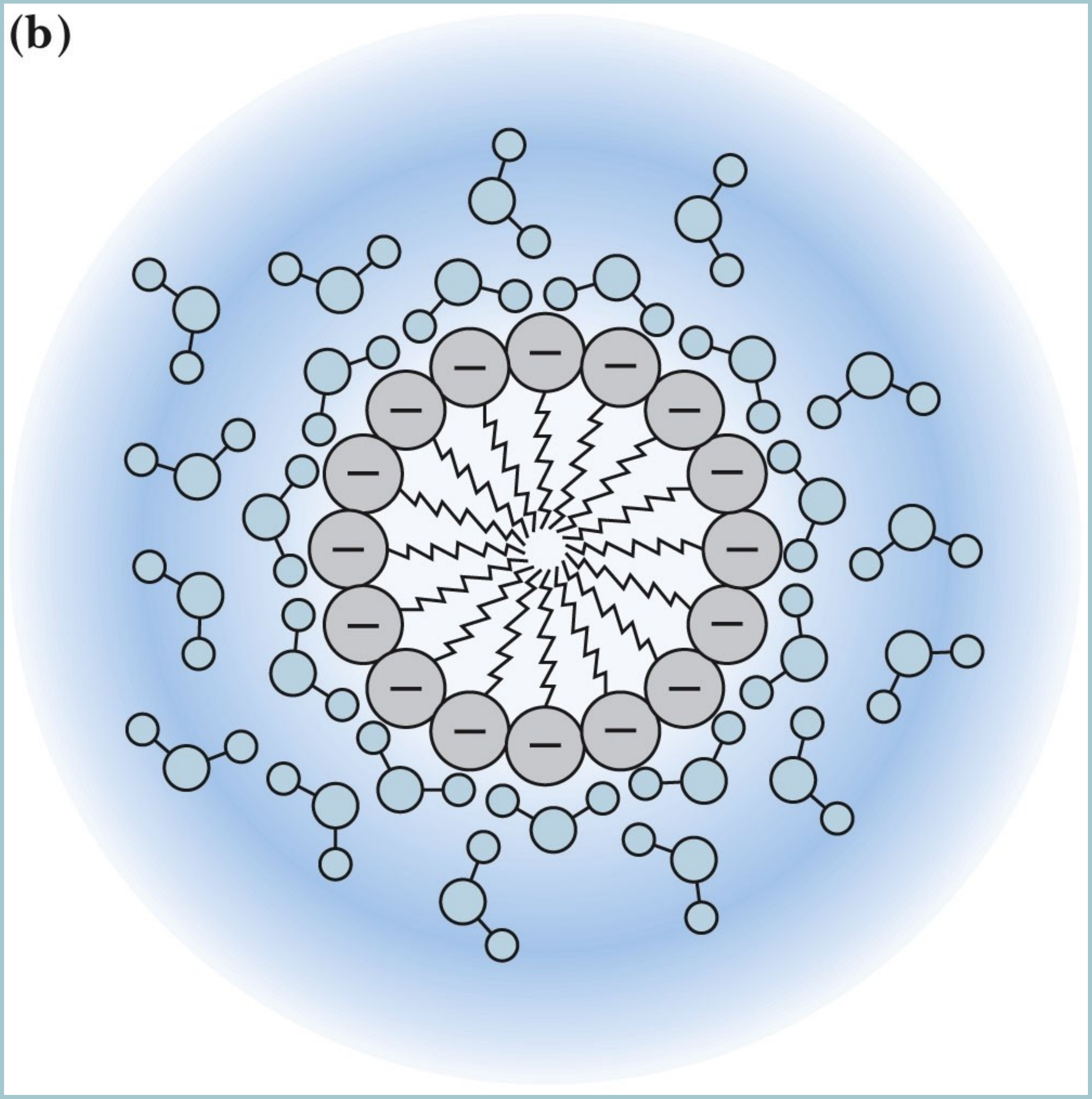
Amphiphilic Molecules

Figure 2.7 An amphiphilic molecule: sodium palmitate. Amphiphilic molecules are frequently symbolized by a ball and zig-zag line structure  ,where the ball represents the hydrophilic polar head and the zig-zag represents the nonpolar hydrophobic hydrocarbon tail.



Section through a Micelle

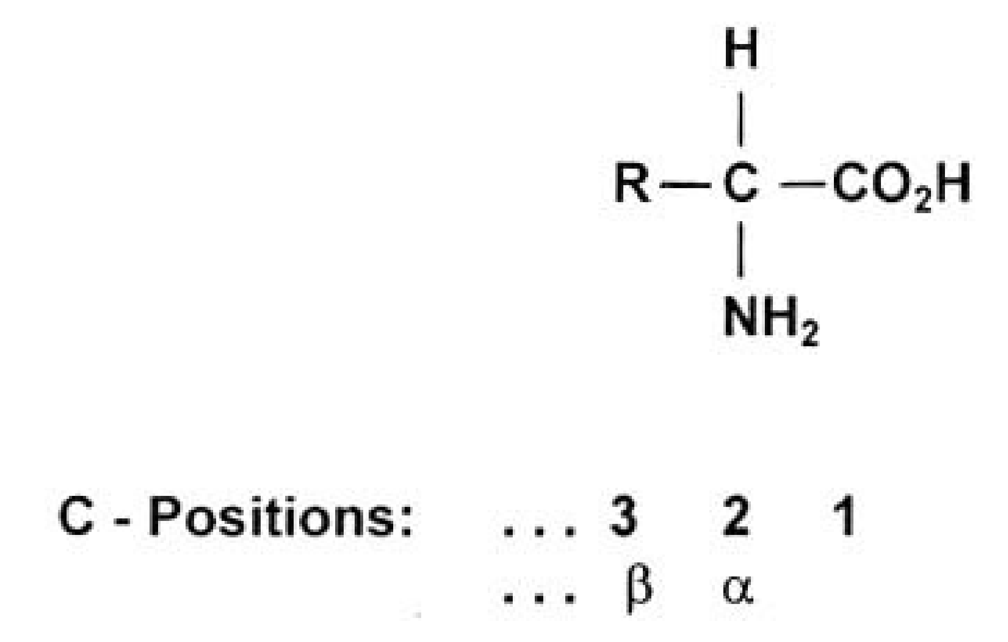
Figure 2.7b Micelle formation by amphiphilic molecules in aqueous solution. Negatively charged carboxylate head groups orient to the micelle surface and interact with the polar H2O molecules via H bonding. The nonpolar hydrocarbon tails cluster in the interior of the spherical micelle, driven by hydrophobic exclusion from the solvent and the formation of favorable van der Waals interactions. Because of their negatively charged surfaces, neighboring micelles repel one another and thereby maintain a relative stability in solution.



What Are the Structures and Properties of Amino Acids?

- Amino acids contain a central tetrahedral carbon atom, the α-carbon
- Amino acids can polymerize via peptide bonds between the α-amino and α-carboxyl groups
- There are 20 common (aka standard) amino acids found in proteins
- Several amino acids occur only rarely in proteins
- Some amino acids can be modified after incorporation in proteins (i. e., post-translational modification)
- Some amino acids are not found in proteins

Amino Acid Structure



Molecular weight range for standard amino acids

75 - 204

Gly - Trp

Average molecular weight per residue in protein is ~110

(this excludes H_20 lost in polymerization)

Amino acids are chiral molecules

“To hold, as ‘twere, the mirror up to nature.”
William Shakespeare, Hamlet

All objects have mirror images, and amino acids exist in mirror-image forms. Only the L-isomers of amino acids occur commonly in nature.

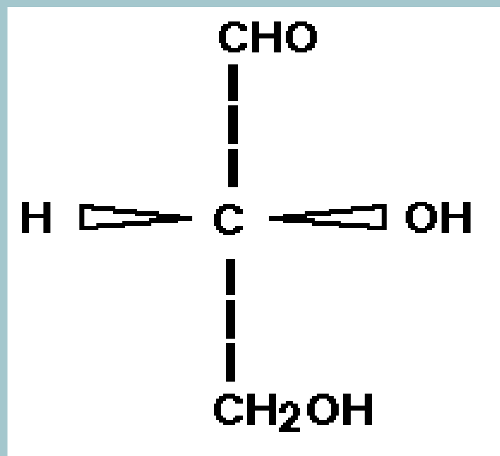
Three Sisters Wilderness, Oregon

Stereochemistry of Amino Acids

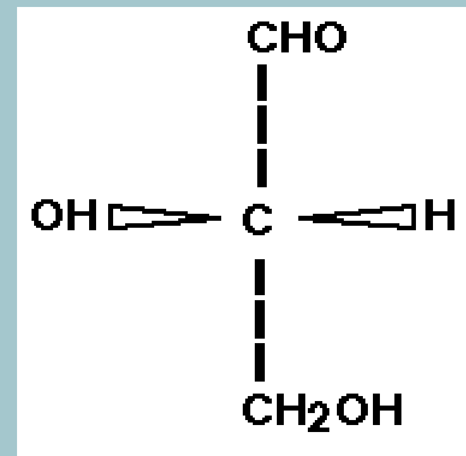
- All but glycine are chiral due to an asymmetric α-carbon
- L-amino acids predominate in nature and are the form incorporated by ribosomes into proteins
- D,L-nomenclature is based on D- and L-glyceraldehyde
- R,S-nomenclature system is superior, since amino acids like isoleucine and threonine (with two chiral centers) can be named unambiguously

Distinguishing D from L amino acids

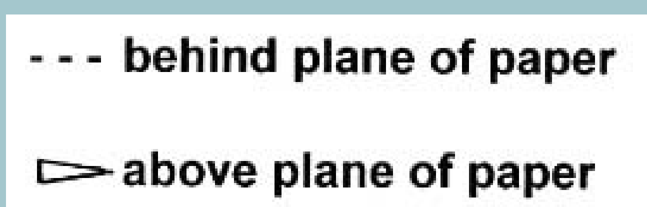
Note: D, L is not d, l ; D, L doesn't indicate rotation of plane polarized light.
L designation is assigned by structural analogy with L-glyceraldehyde

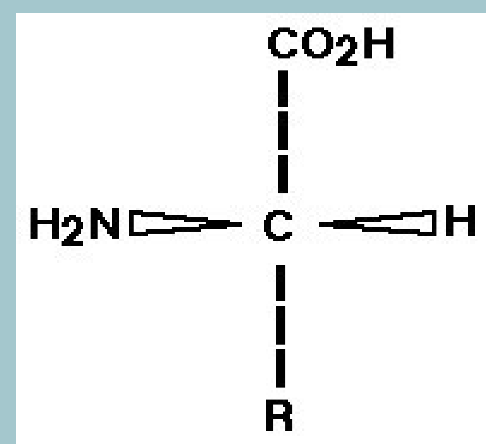


D - glyceraldehyde



L - glyceraldehyde

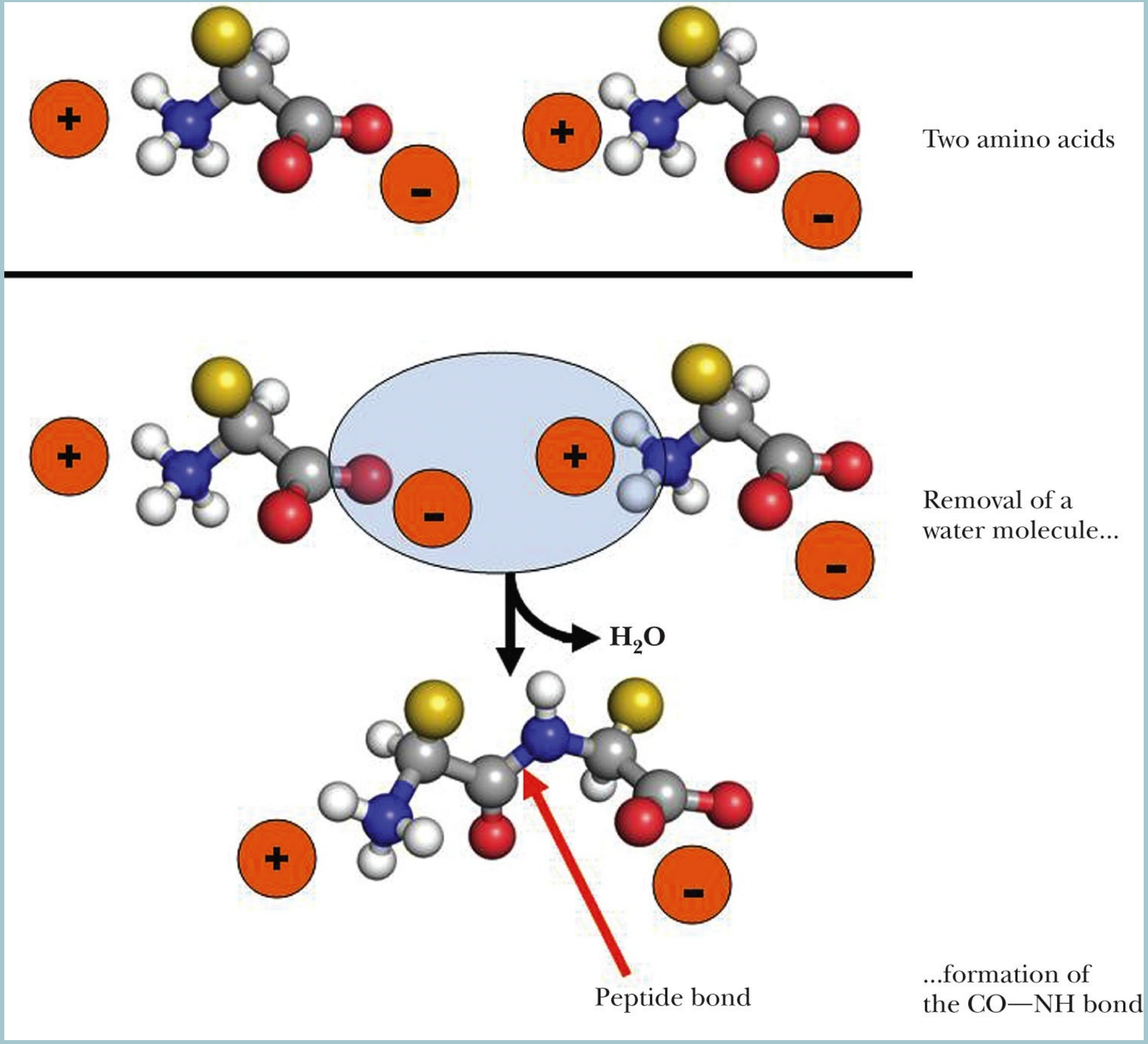




L - amino acid

Amino Acids Can Join Via Peptide Bonds

Figure 4.2 Two amino acids can react with loss of a water molecule to form a covalent bond. The bond joining the two amino acids is called a peptide bond.

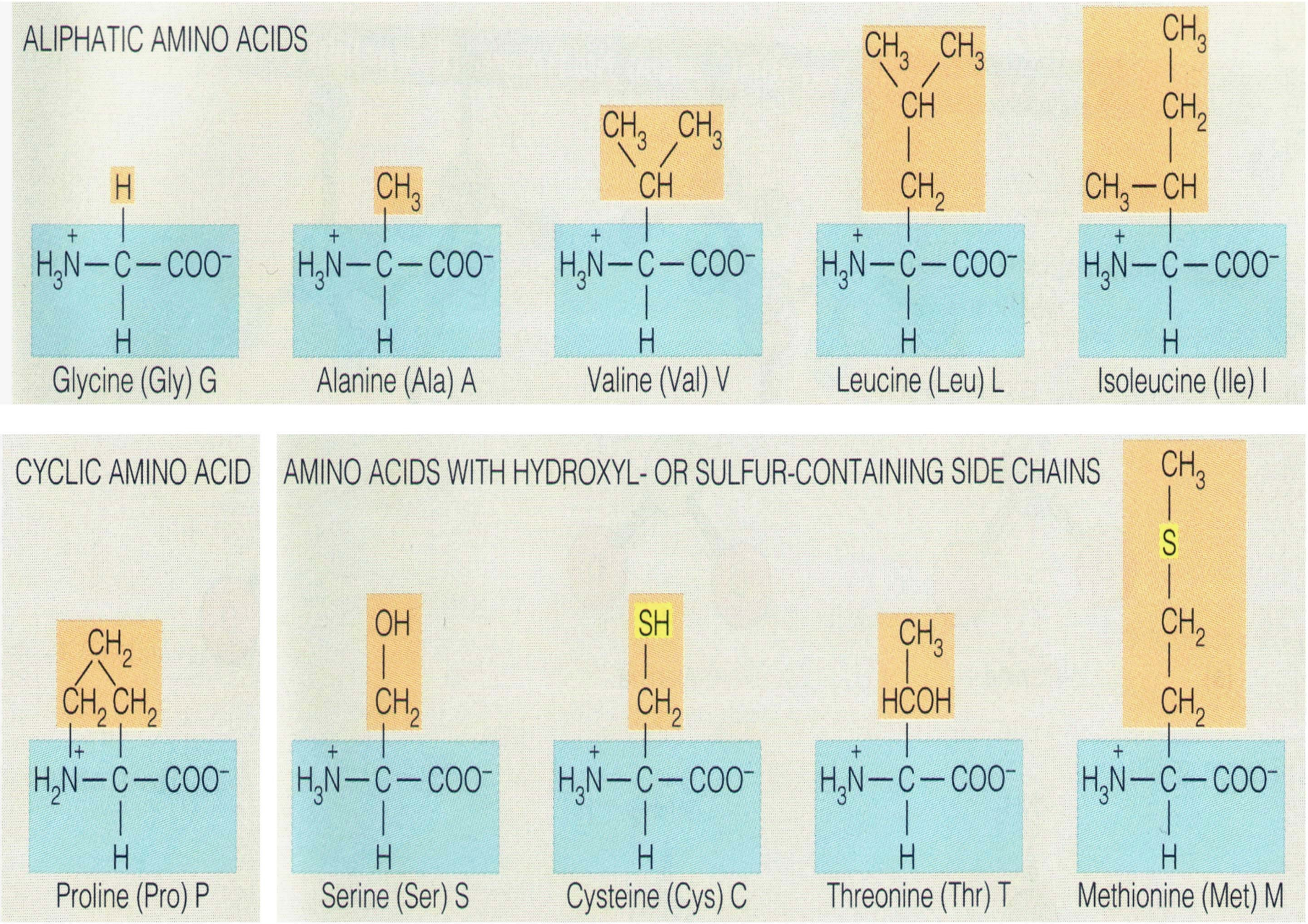


The 20 common amino acids

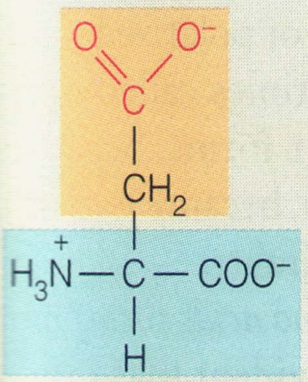
You should know:

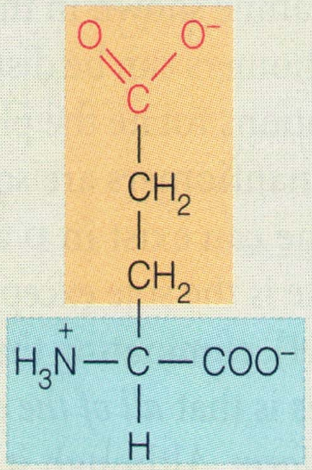
- Names
- Structures (Be able to draw.)
- Approximate pK_a values
- 3-letter and 1-letter abbreviations
- Classifications based on physical properties, e.g., non-polar aliphatic amino acids, basic, etc. (Be careful in accepting G&G designation. They can be misleading.)

20 Standard Amino Acids

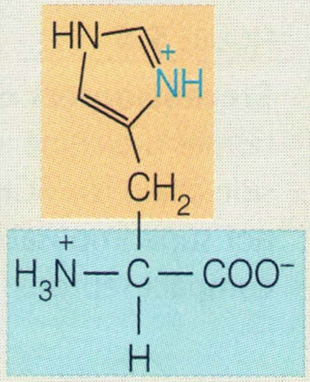


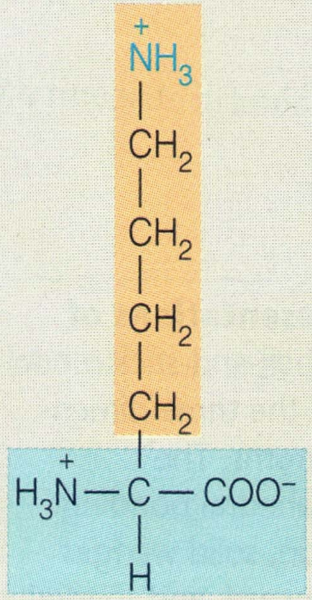
ACIDIC AMINO ACIDS AND THEIR AMIDES

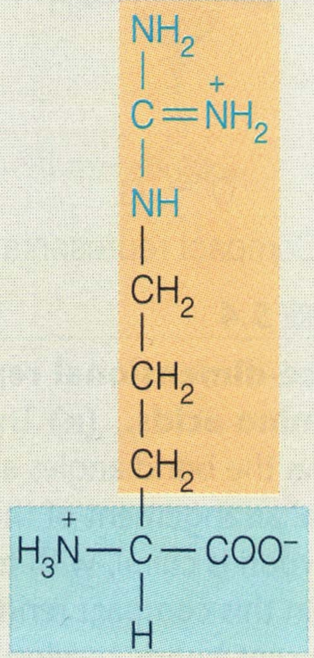

Aspartic acid (Asp) D

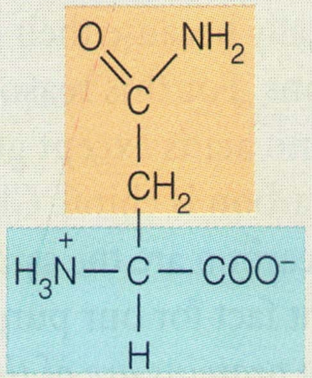

Glutamic acid (Glu) E

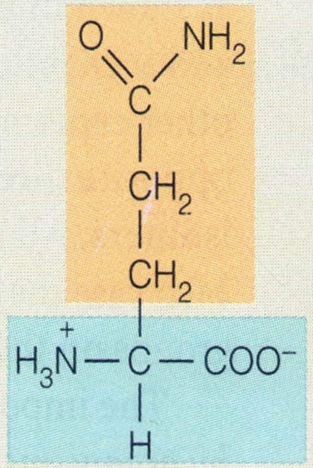
BASIC AMINO ACIDS


Histidine (His) H

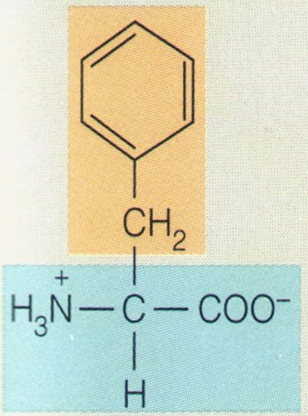

Lysine (Lys) K

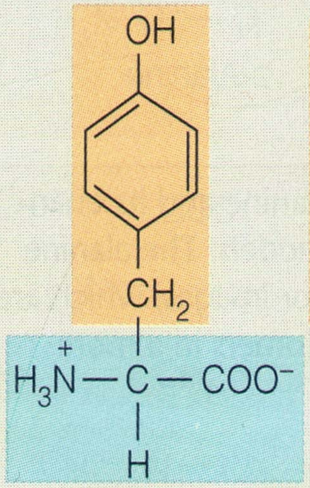

Arginine (Arg) R

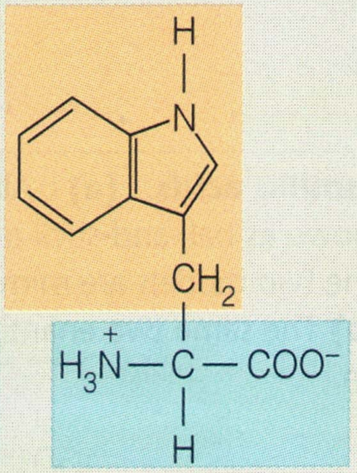

Asparagine (Asn) N


Glutamine (Gln) Q

AROMATIC AMINO ACIDS


Phenylalanine (Phe) F


Tyrosine (Tyr) Y


Tryptophan (Trp) W

Frequency of Amino Acid Residues in Proteins

Nonessential Amino Acids Require Fewer Reactions for Synthesis

	Amino Acid	Reaction Steps	Mole % in Proteins†
1	Alanine	1	7.9
2	Aspartic acid	1	5.3
3	Glutamic acid	1	6.7
4	Asparagine	2	4.1
5	Glutamine	2	4.0
6	Serine	5	6.9
7	Glycine	6	6.9
8	Proline	6	4.8
9	Cysteine	7	1.5
10	Threonine	6	5.4
11	Valine	9	6.7
12	Isoleucine	13	5.9
13	Leucine	14	10.0
14	Lysine	14	5.9
15	Methionine	17	2.4
16	Arginine	24	5.4
17	Histidine	27	2.3
18	Phenylalanine	29	4.0
19	Tyrosine‡	30	3.0
20	Tryptophan	33	1.1

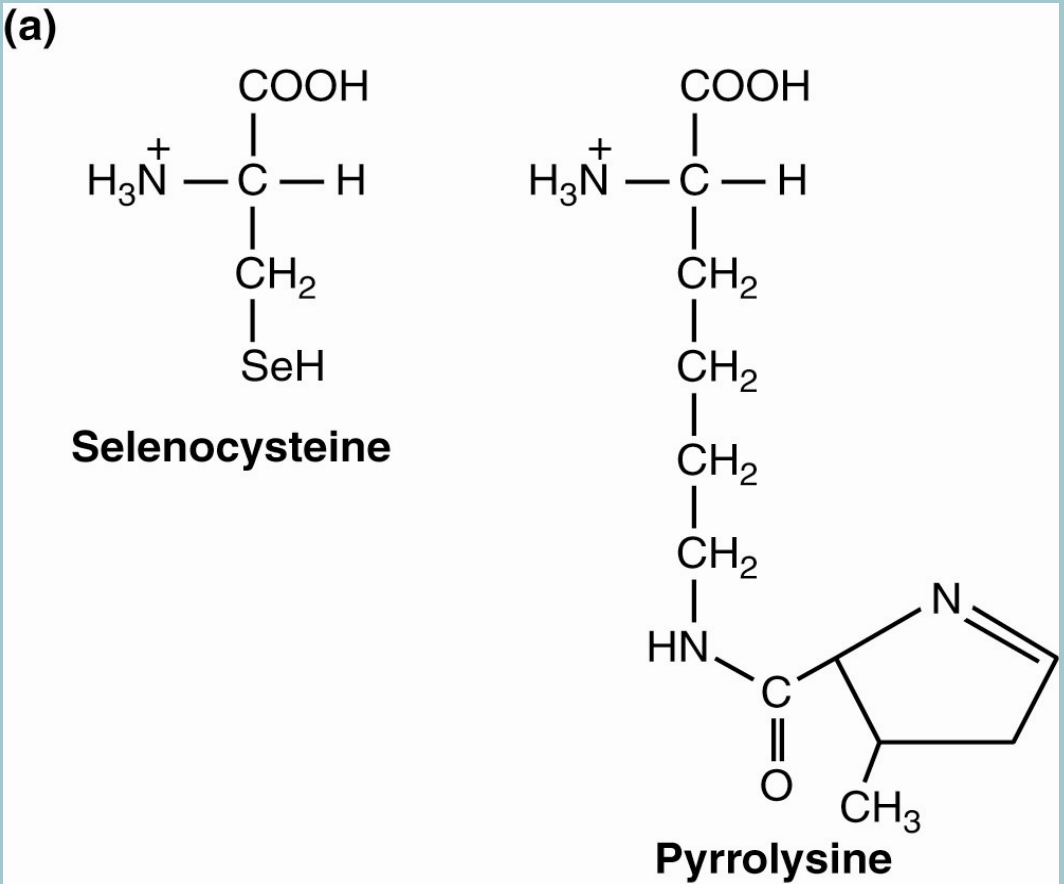
†Mole percentages are taken from amino acid representations among proteins in the Swiss-Prot protein knowledgebase: ca.expasy.org/sprot.
‡Note that "nonessential" tyrosine can be made only from "essential" phenylanine.

From Chap. 25, p891 of G&G

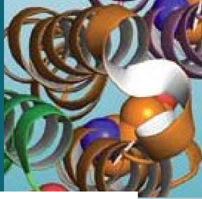
Two Additional Standard Amino Acids Occur Rarely in Proteins

- **Selenocysteine** (Sec, U) in a few proteins in many, but not all, organisms; humans have 25 selenoproteins, plants none.
- **Pyrrolysine** (Pyl, O) in several methanogenic archaeal species & one bacterium

Fig. 4.4



Several Post-translationally Modified Amino Acids Occur Rarely in Proteins



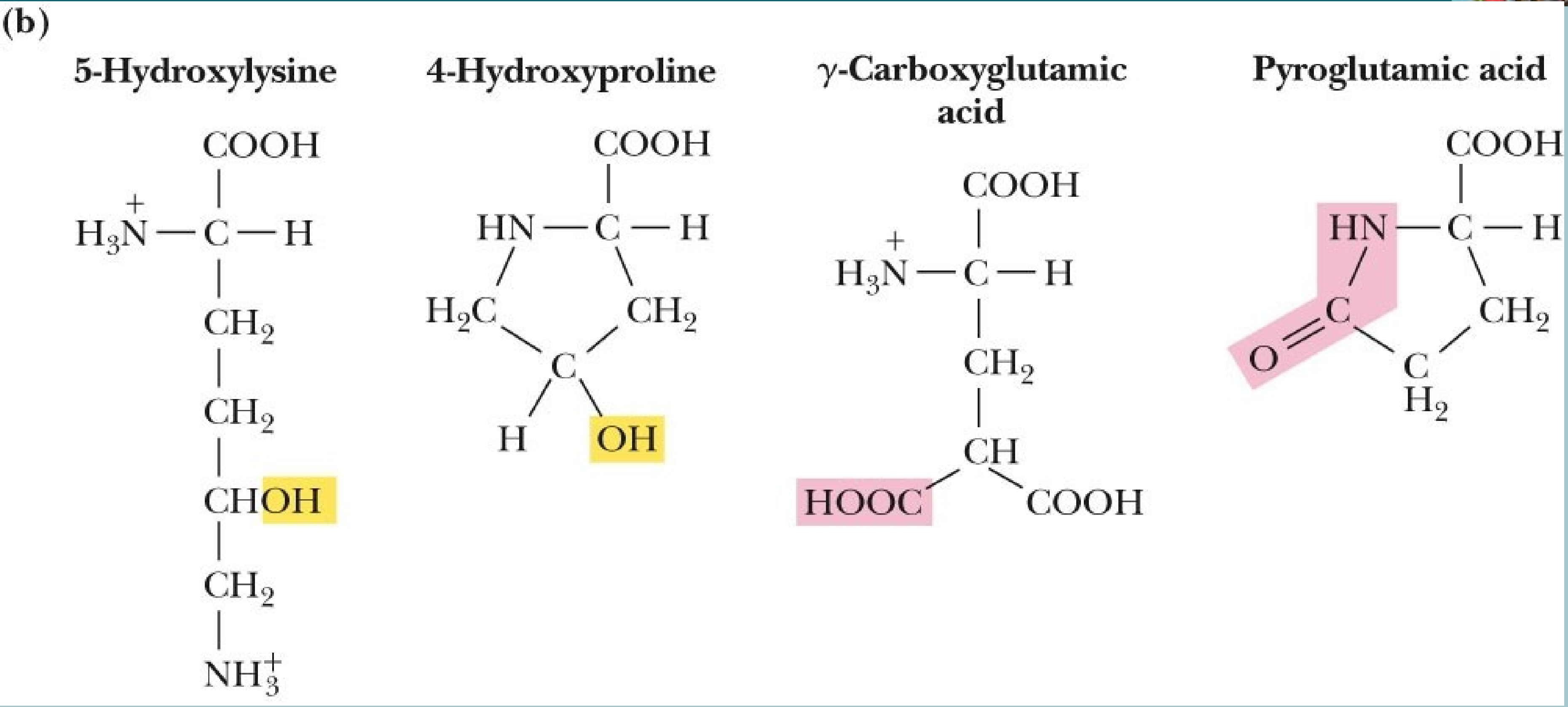
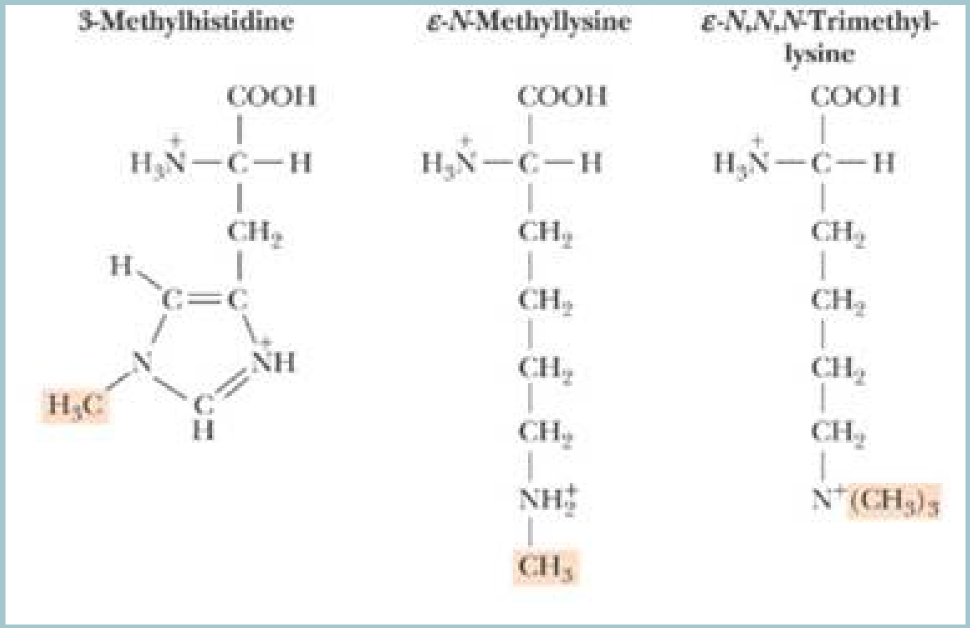
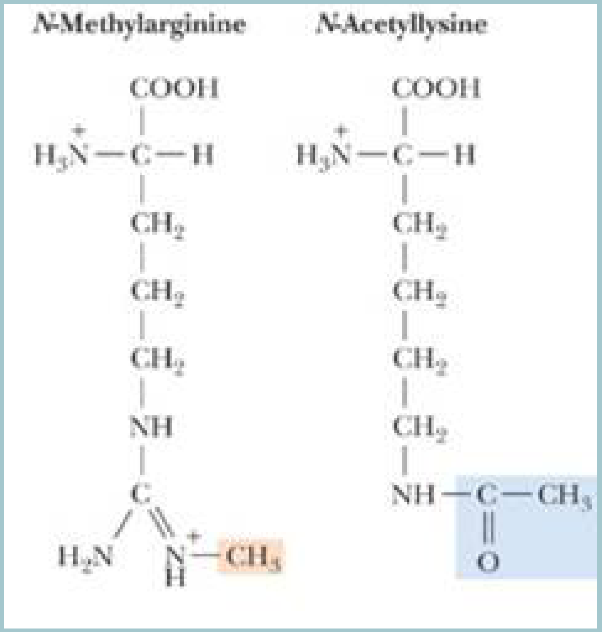
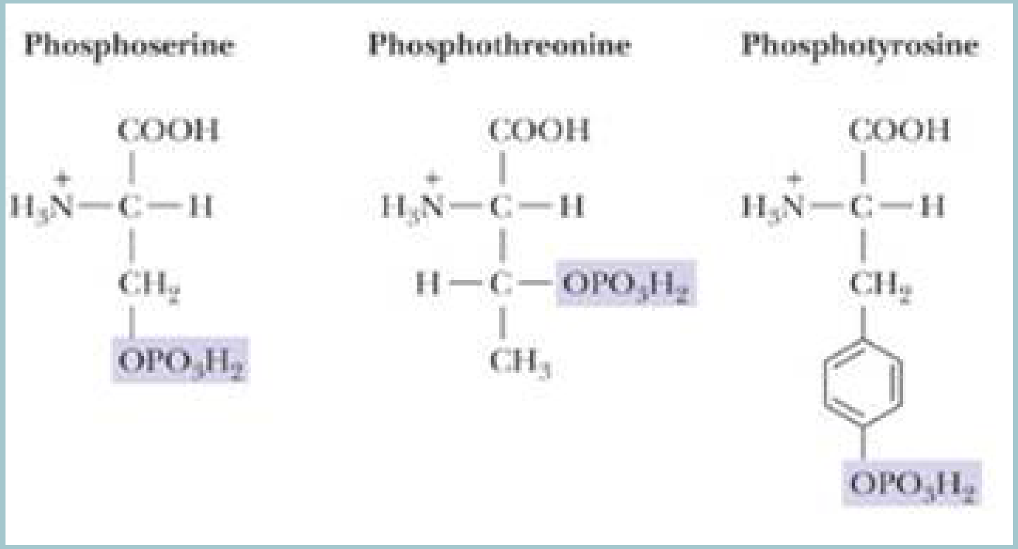


Figure 4.4 (b) Some amino acids are less common, but nevertheless found in certain proteins. Hydroxylysine and hydroxyproline are found in connective-tissue proteins; carboxyglutamate is found in blood-clotting proteins; pyroglutamate is found in bacteriorhodopsin, a light-driven proton pump (see Chap. 9).

Several Post-translationally Modified Amino Acids Occur More Often in Proteins and are Usually Involved in Signaling/Regulation





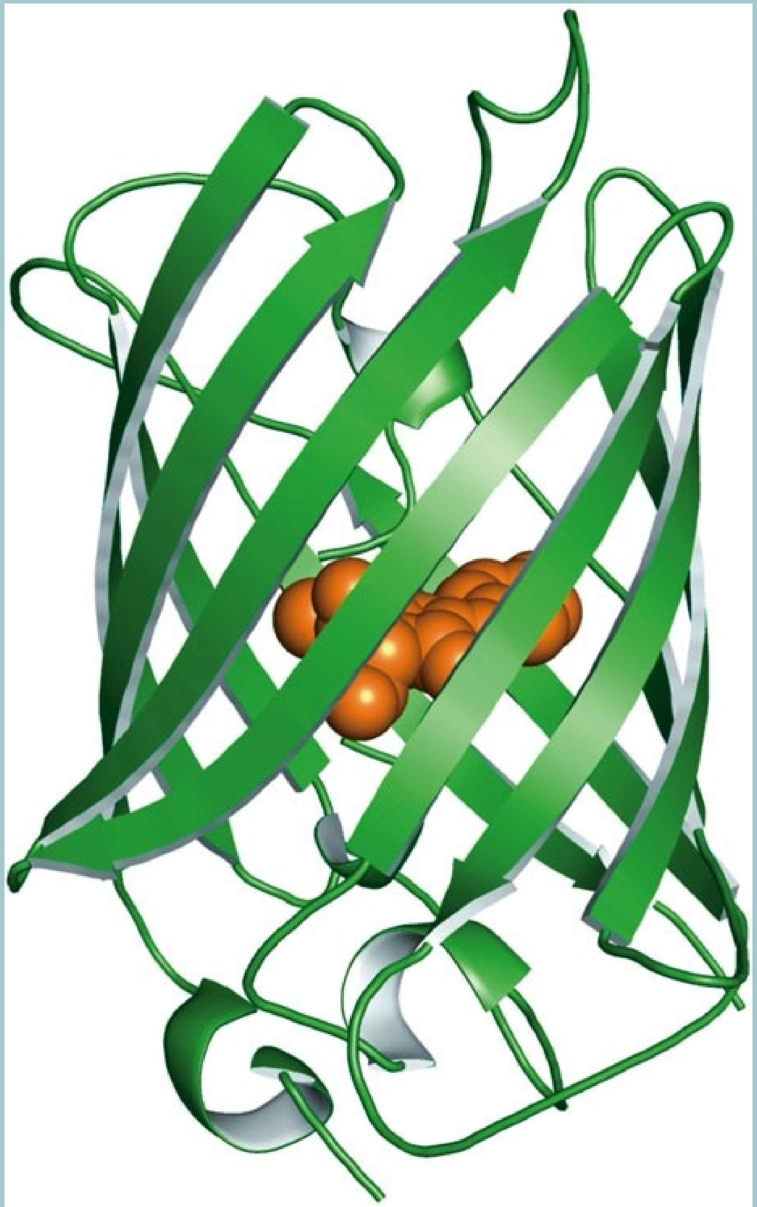


You can see additional modifications in Chapter 5, Table 5.5 on page 135.

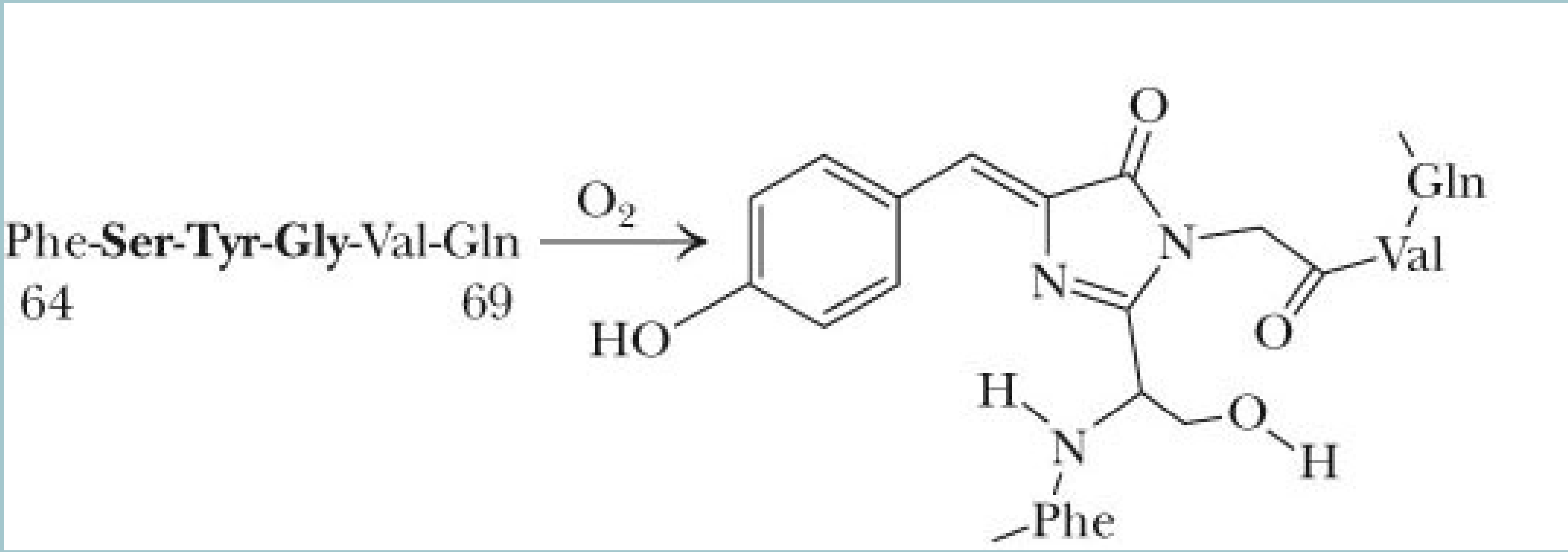
GloFish: Available only in the USA (except California)
GFP-recombinant Zebrafish

Green Fluorescent Protein: An Unusual Post-translational Auto-Modification

A jellyfish (Aequorea victoria) native to the northwest Pacific Ocean contains a **green fluorescent protein**. GFP is a naturally fluorescent protein. Genetic engineering techniques can be used to "tag" virtually any protein, structure, or organelle in a cell. The GFP chromophore (orange) lies in the center of a β-barrel protein structure.



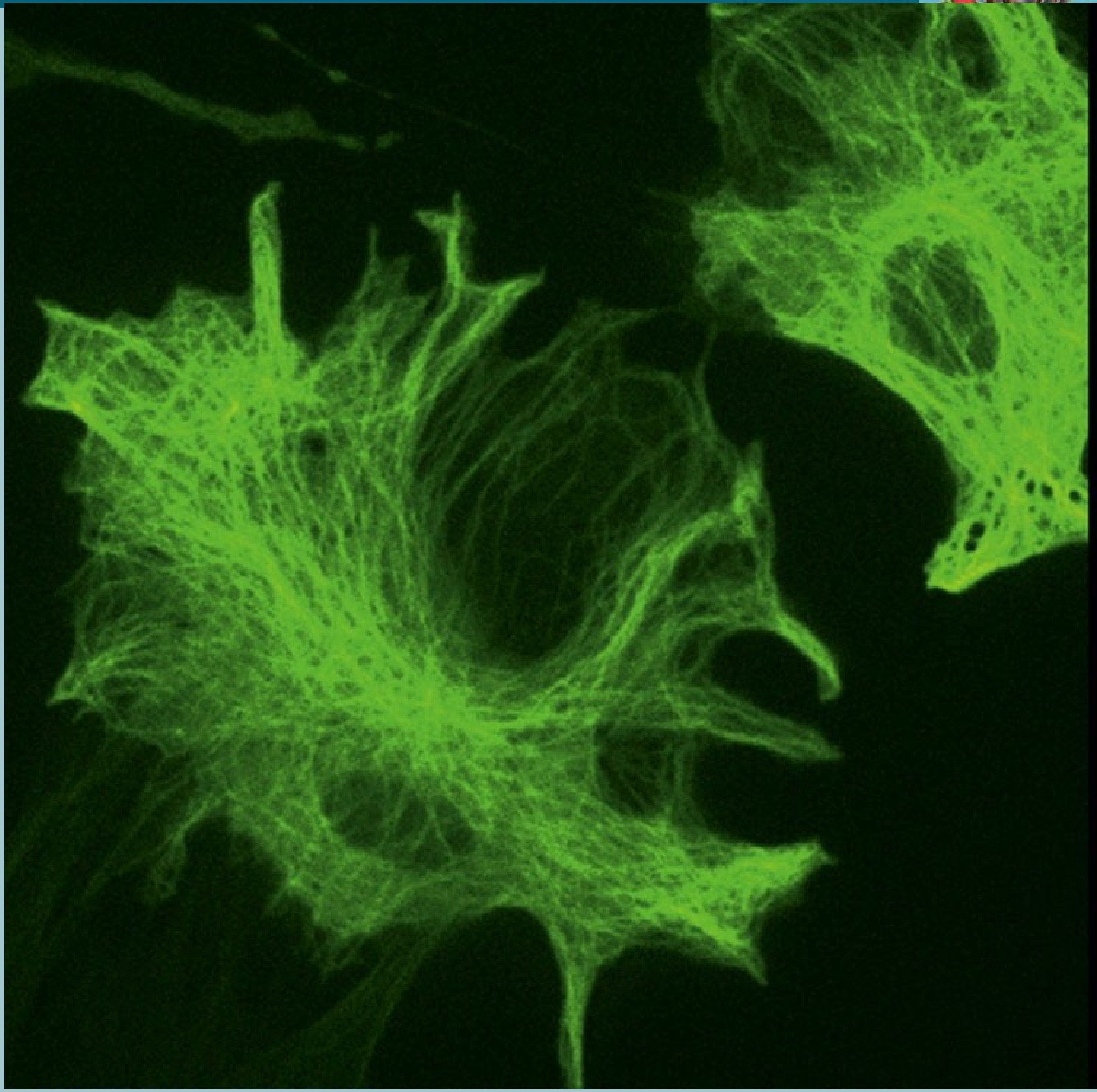
Green Fluorescent Protein Chromophore

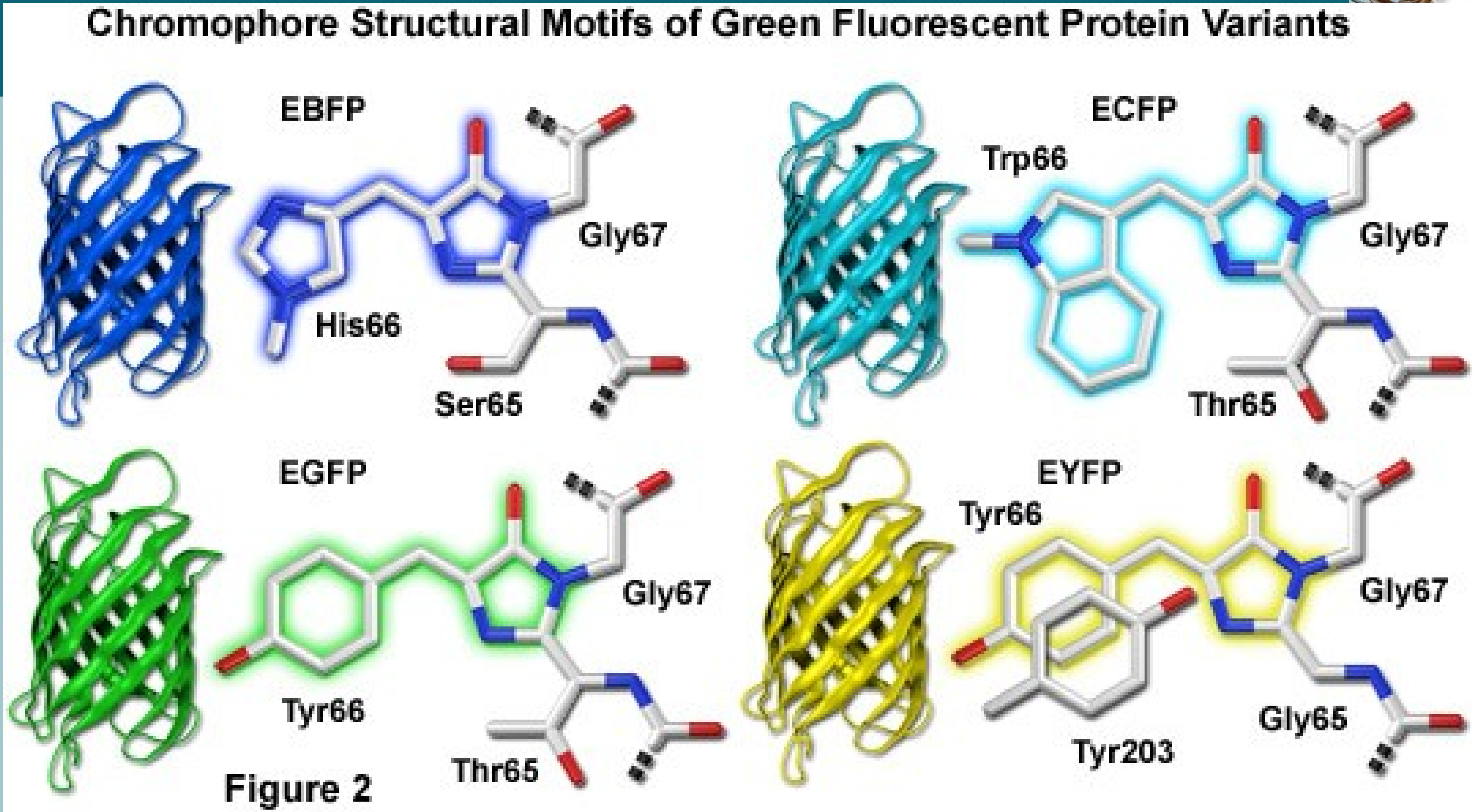


The chromophore of GFP is an oxidative product of the sequence –FSYGVQ-.

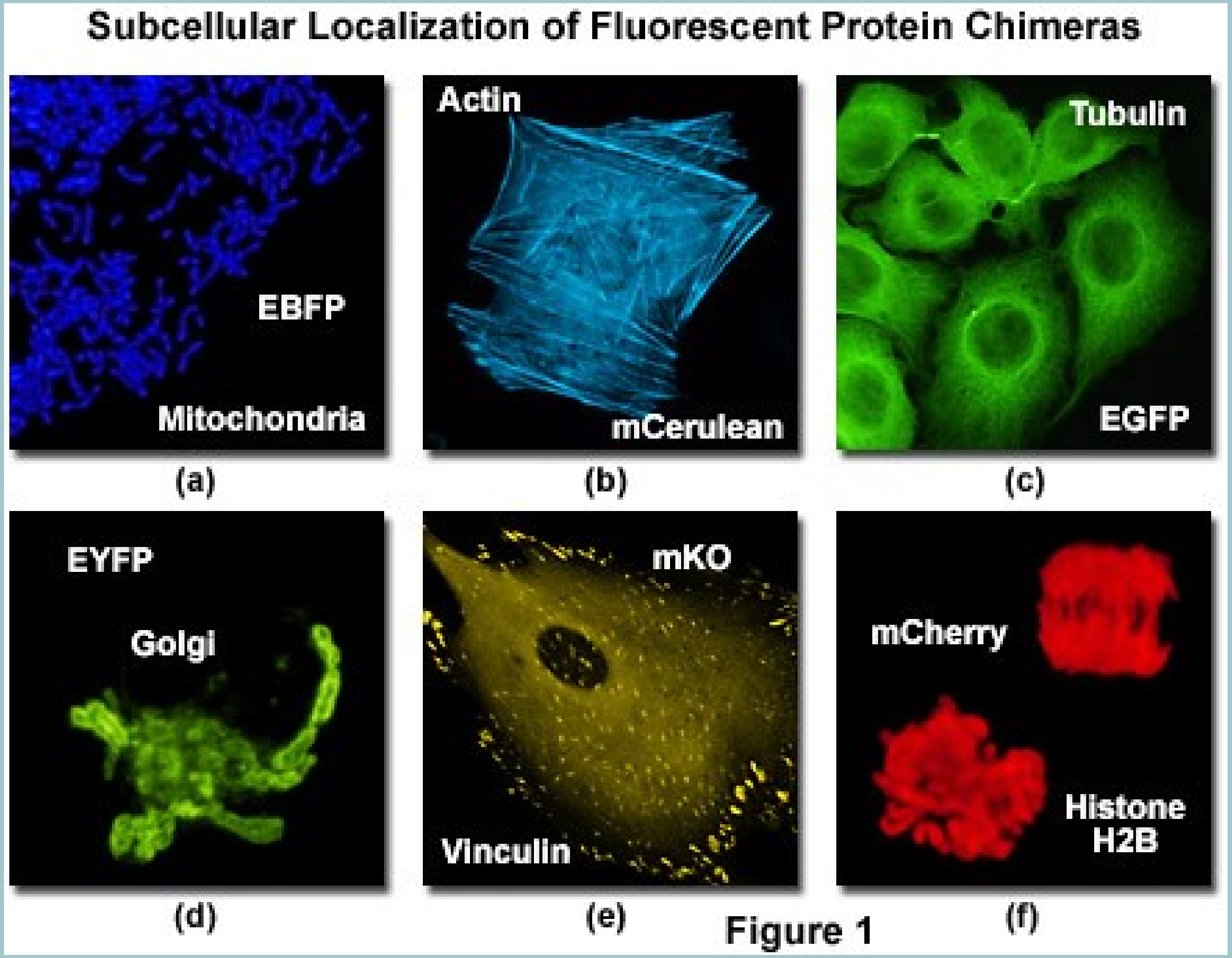
Yellow fluorescent protein

Amino acid substitutions in GFP can tune the color of emitted light. Shown here is an image of African green monkey kidney cells expressing yellow fluorescent protein (YFP) fused to α-tubulin, a cytoskeletal protein.




Chromophore Structural Motifs of Green Fluorescent Protein Variants
EBFP
His66
Gly67
Ser65
ECFP
Trp66
Gly67
Thr65
EGFP
Tyr66
Gly67
Thr65
EYFP
Tyr66
Gly67
Gly65
Tyr203
Figure 2

The Fluorescent Protein Color Palette



From http://www.olympusconfocal.com/applications/fpcolorpalette.html

Fluorescent protein-tagging of brain neurons

Expression of combinations of three different fluorescent proteins in a mouse brain produces ten different colorations of neurons. Individual neurons in a mouse brain appear in different colors in a fluorescence microscope. This “Brainbow” method enables many distinct cells within a brain circuit to be viewed at one time. This technique may facilitate analysis of neuronal circuits on a large scale.

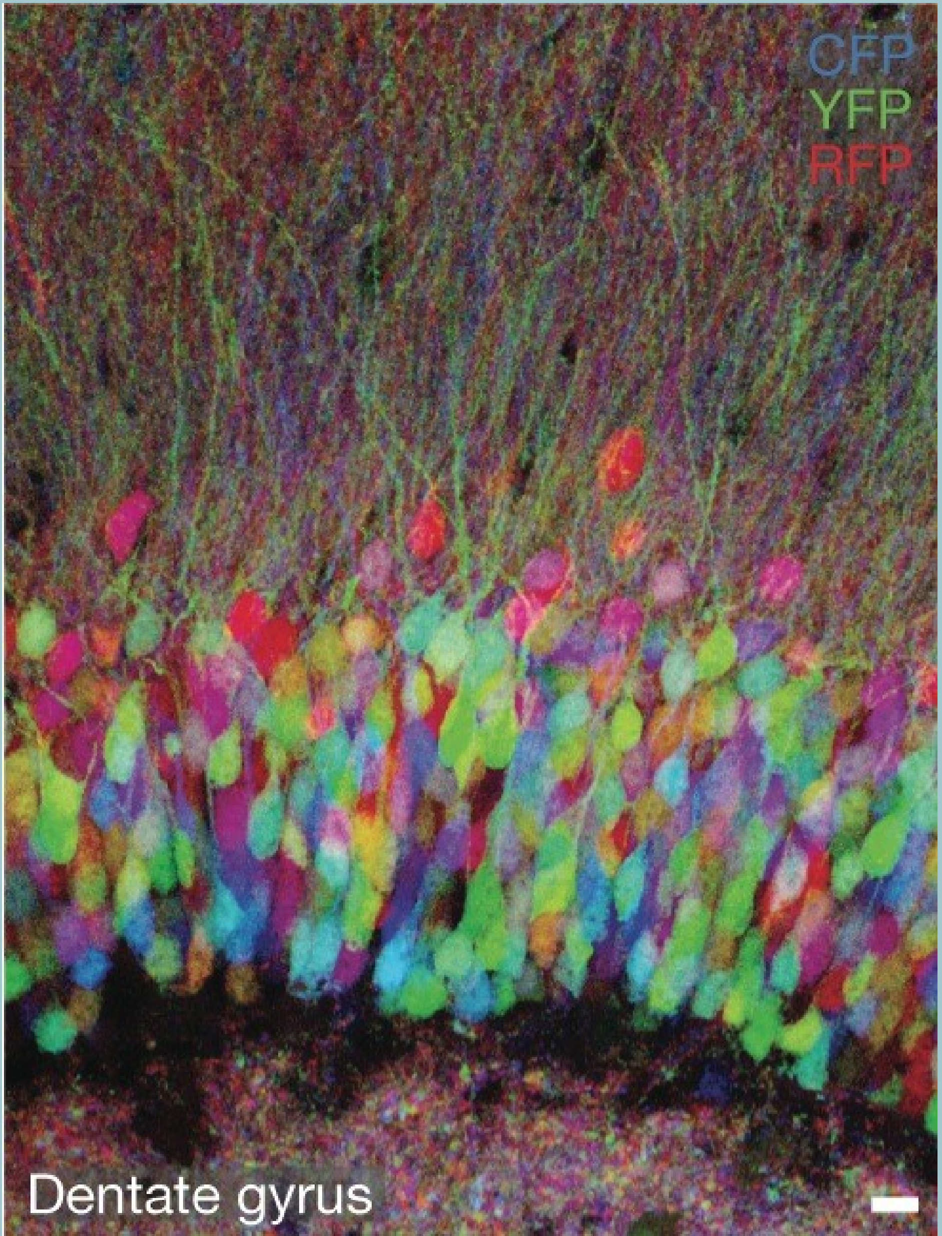
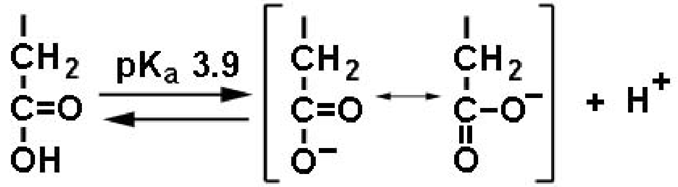


TABLE 4.1 pK_a Values of Common Amino Acids

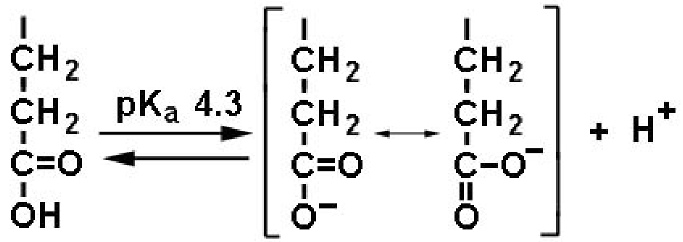
Amino Acid	α-COOH pK_a	α-NH_3^+ pK_a	R group pK_a
Alanine	2.4	9.7	
Arginine	2.2	9.0	12.5
Asparagine	2.0	8.8	
Aspartic acid	2.1	9.8	3.9
Cysteine	1.7	10.8	8.3
Glutamic acid	2.2	9.7	4.3
Glutamine	2.2	9.1	
Glycine	2.3	9.6	
Histidine	1.8	9.2	6.0
Isoleucine	2.4	9.7	
Leucine	2.4	9.6	
Lysine	2.2	9.0	10.5
Methionine	2.3	9.2	
Phenylalanine	1.8	9.1	
Proline	2.1	10.6	
Serine	2.2	9.2	~13
Threonine	2.6	10.4	~13
Tryptophan	2.4	9.4	
Tyrosine	2.2	9.1	10.1
Valine	2.3	9.6	

Side Chain pKa Values

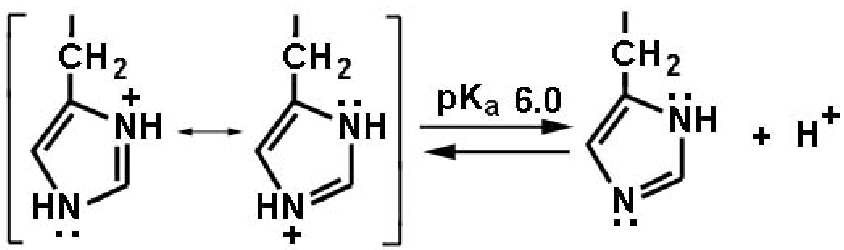
Aspartic Acid



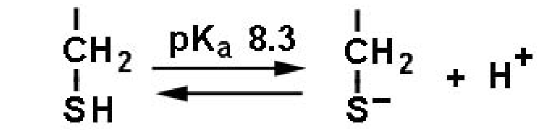
Glutamic Acid



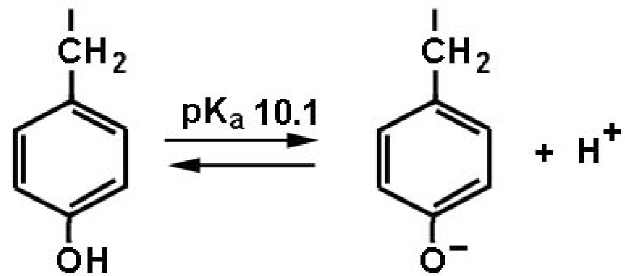
Histidine



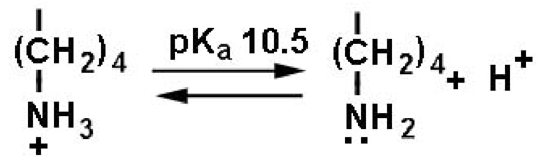
Cysteine



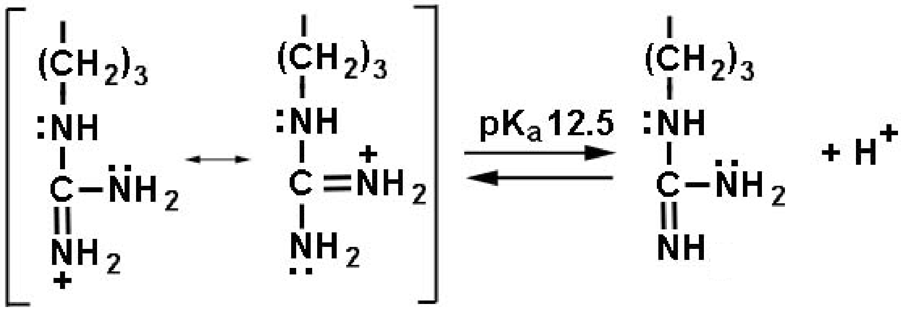
Tyrosine



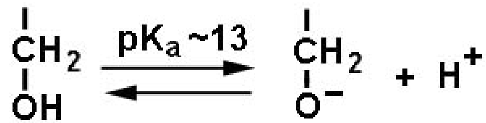
Lysine



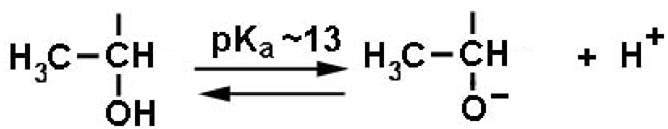
Arginine



Serine



Theronine



pKa values can be changed by the environment

pKa vs. dielectric constant of the medium

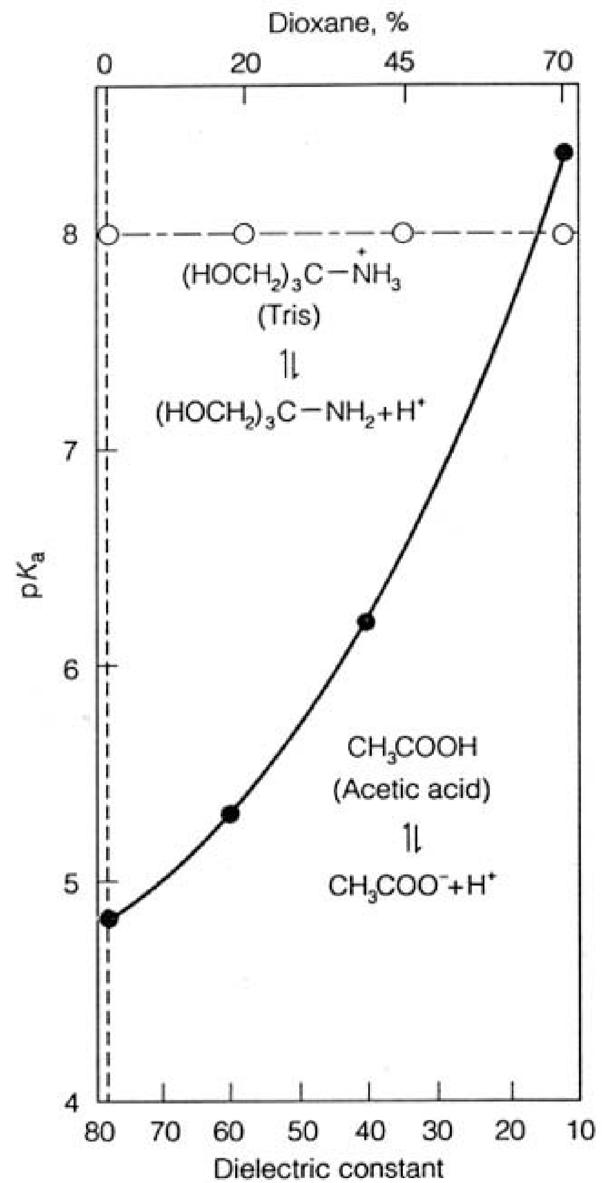
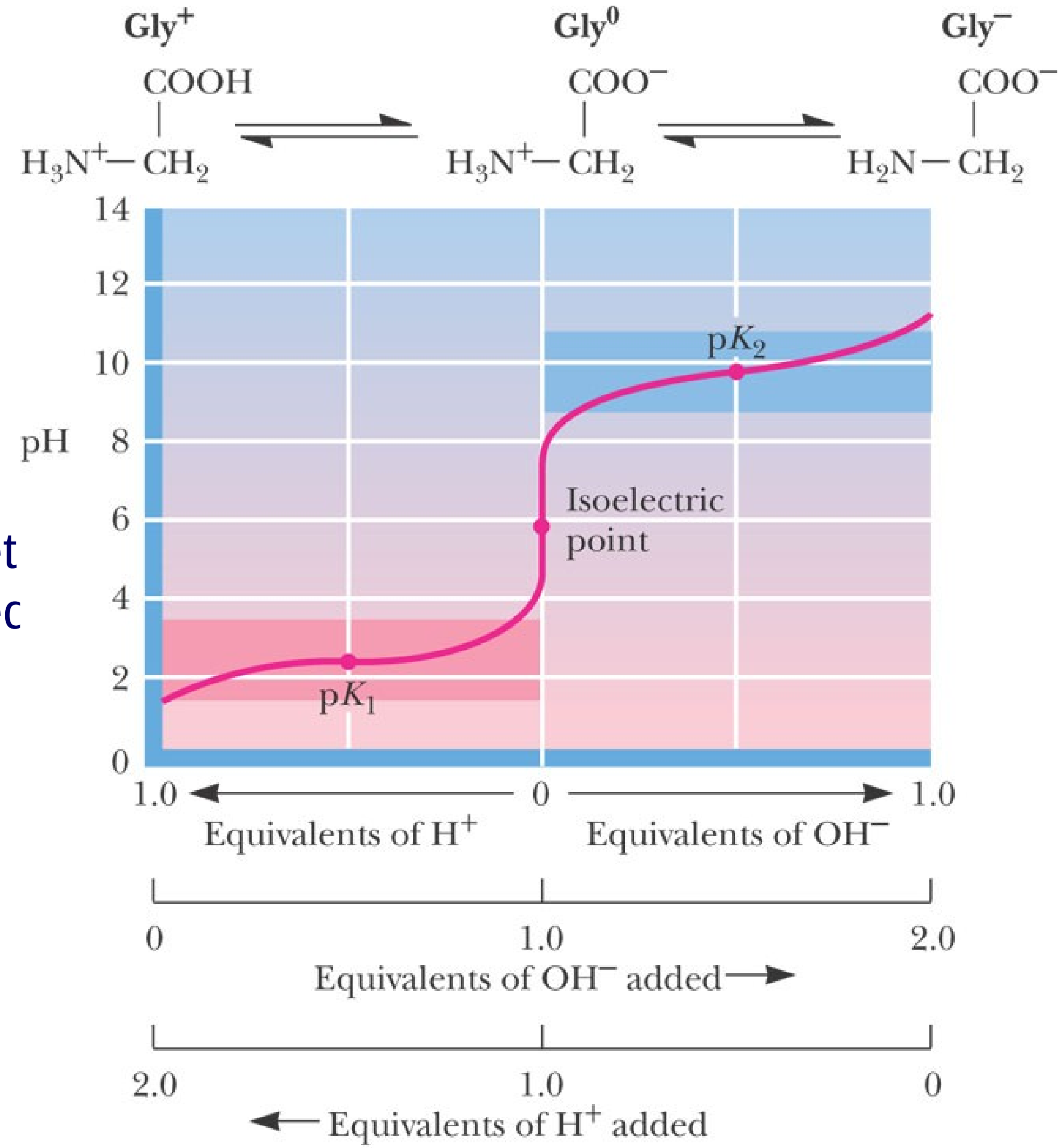


Figure 4.6 Titration of glycine, a simple amino acid. The isoelectric point, pI, the pH where the molecule has a net charge of 0, can be calculated as (pK_1+ pK_2)/2.



© 2005 Brooks/Cole - Thomson

Titrations of polyprotic amino acids

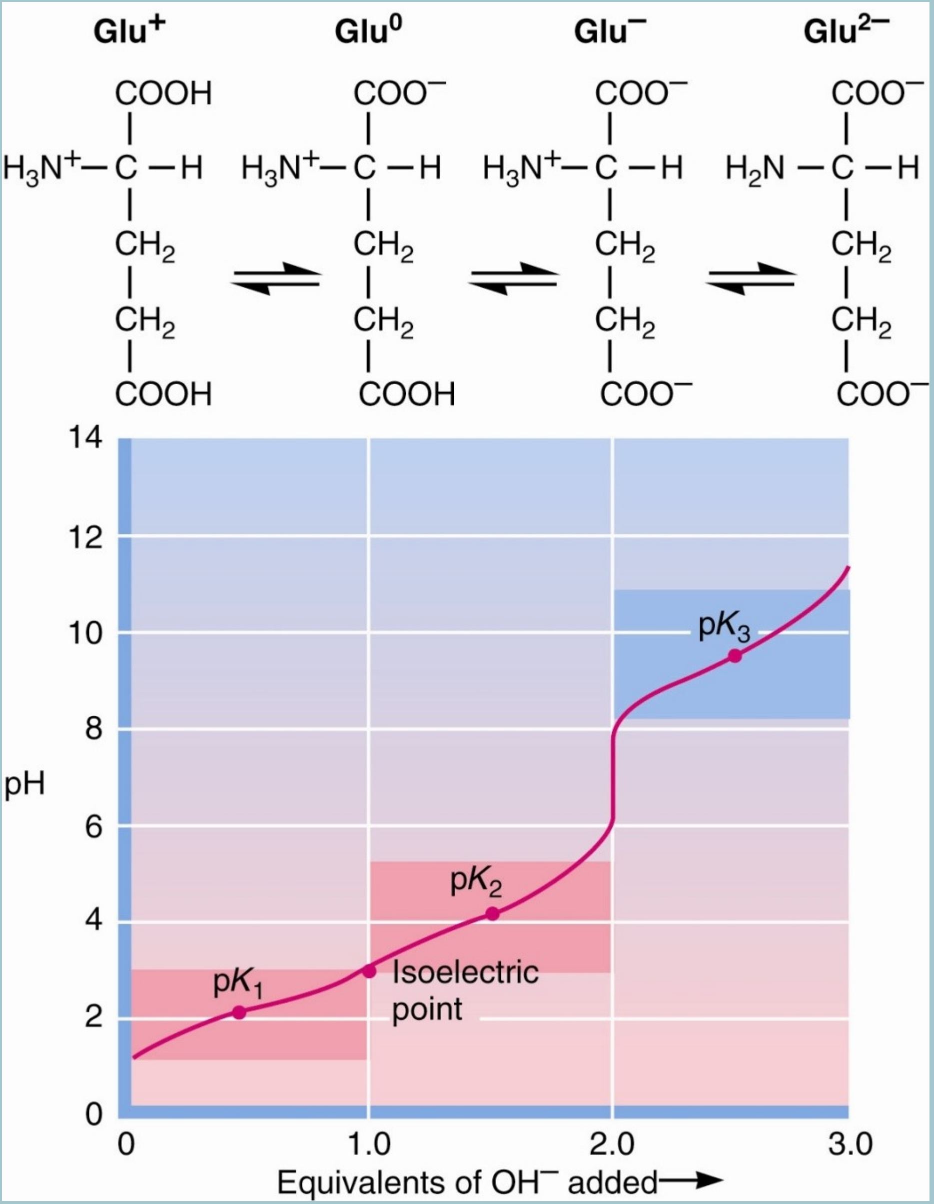
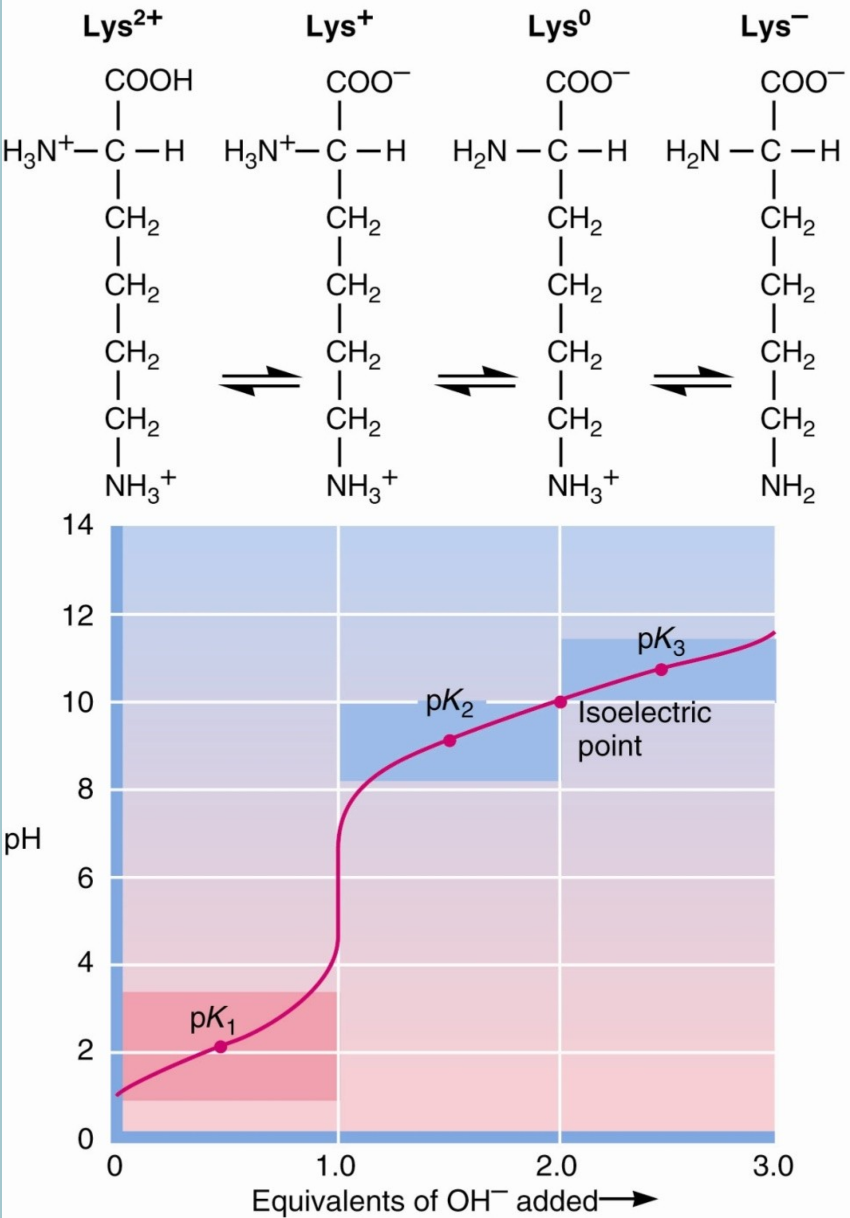


Figure 4.7 Titration of glutamic acid

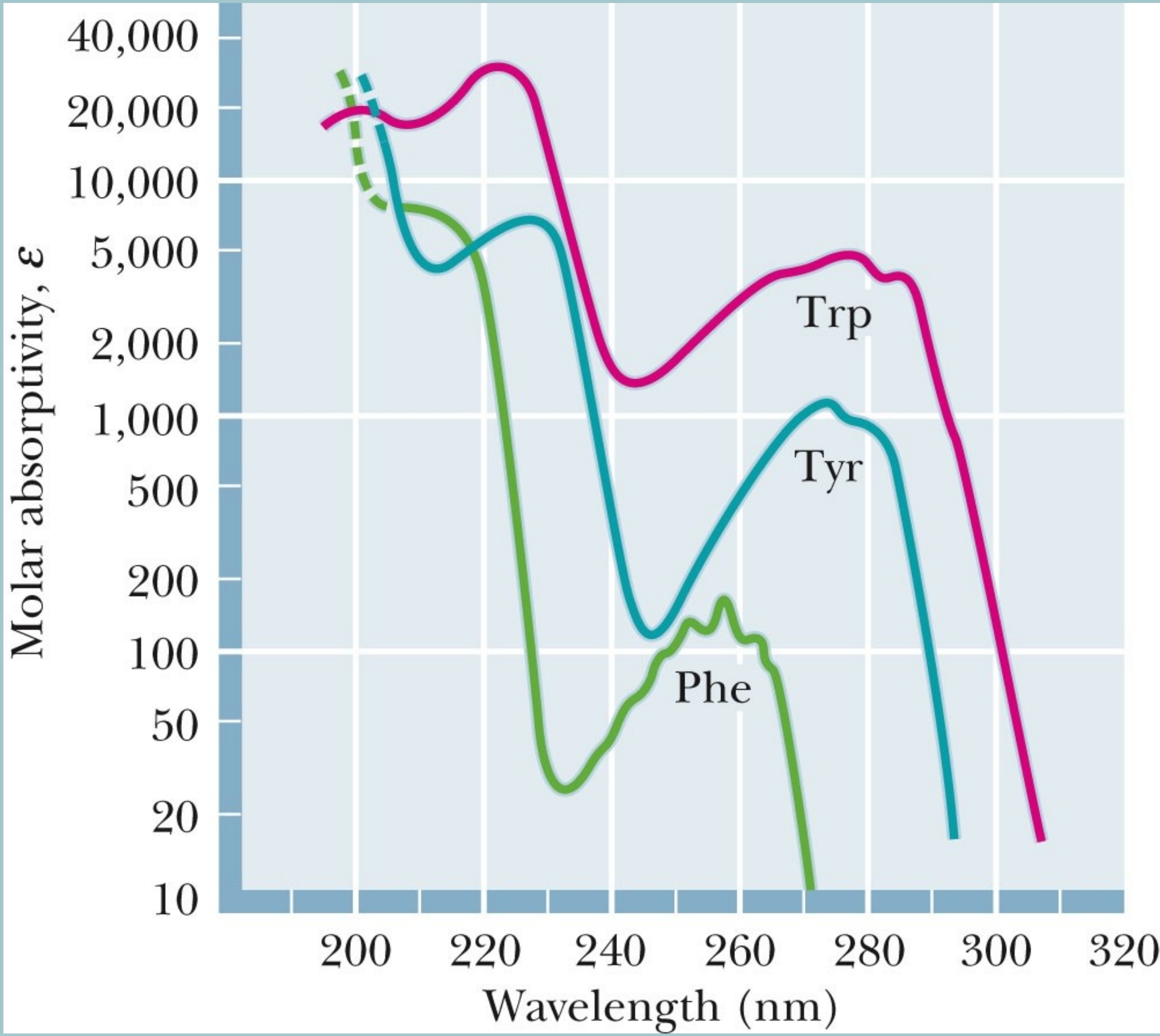
Titrations of polyprotic amino acids

Figure 4.7 Titration of lysine.



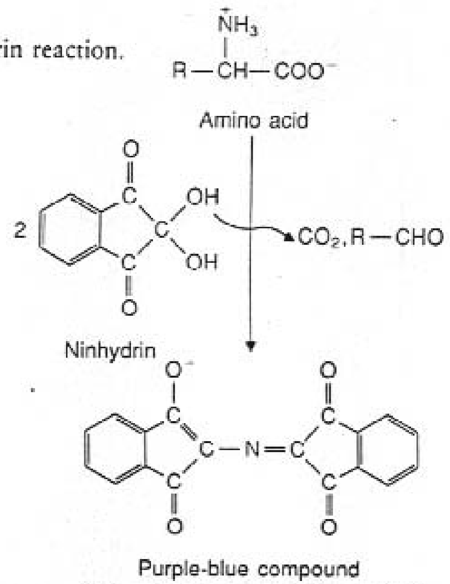
Spectroscopic Properties

Figure 4.10 The UV spectra of the aromatic amino acids at pH 6.

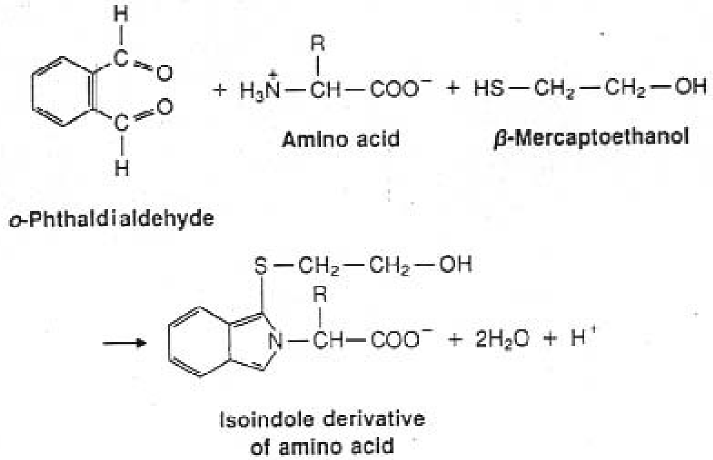


Reactions for Amino Acid Quantitation

The ninhydrin reaction.



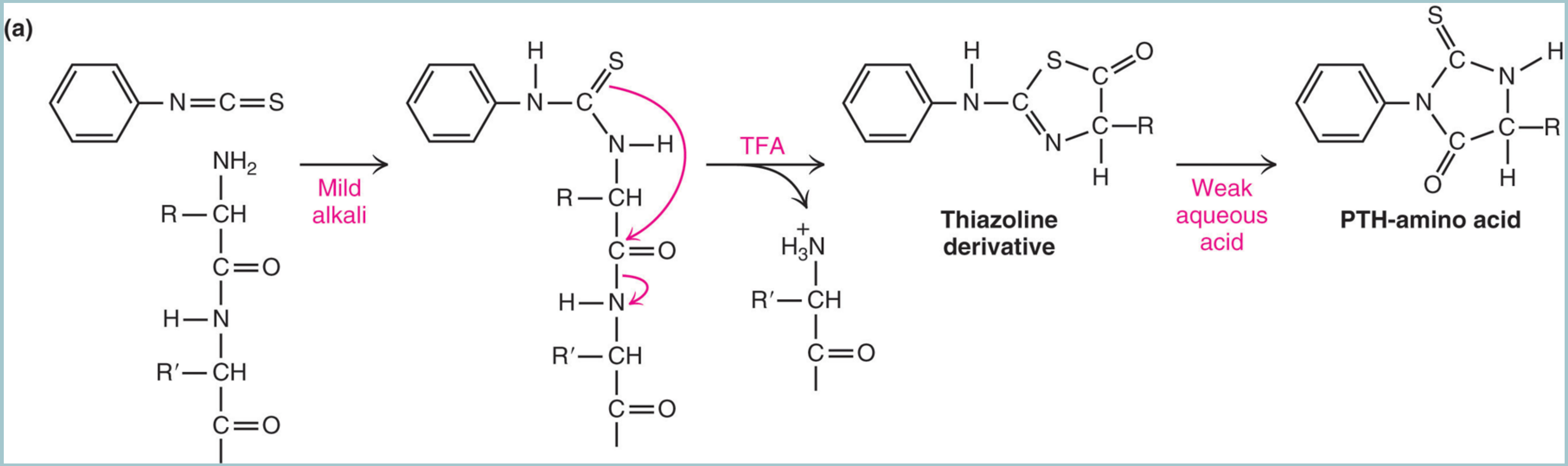
(Ruhemann's Purple)



(fluorescent)

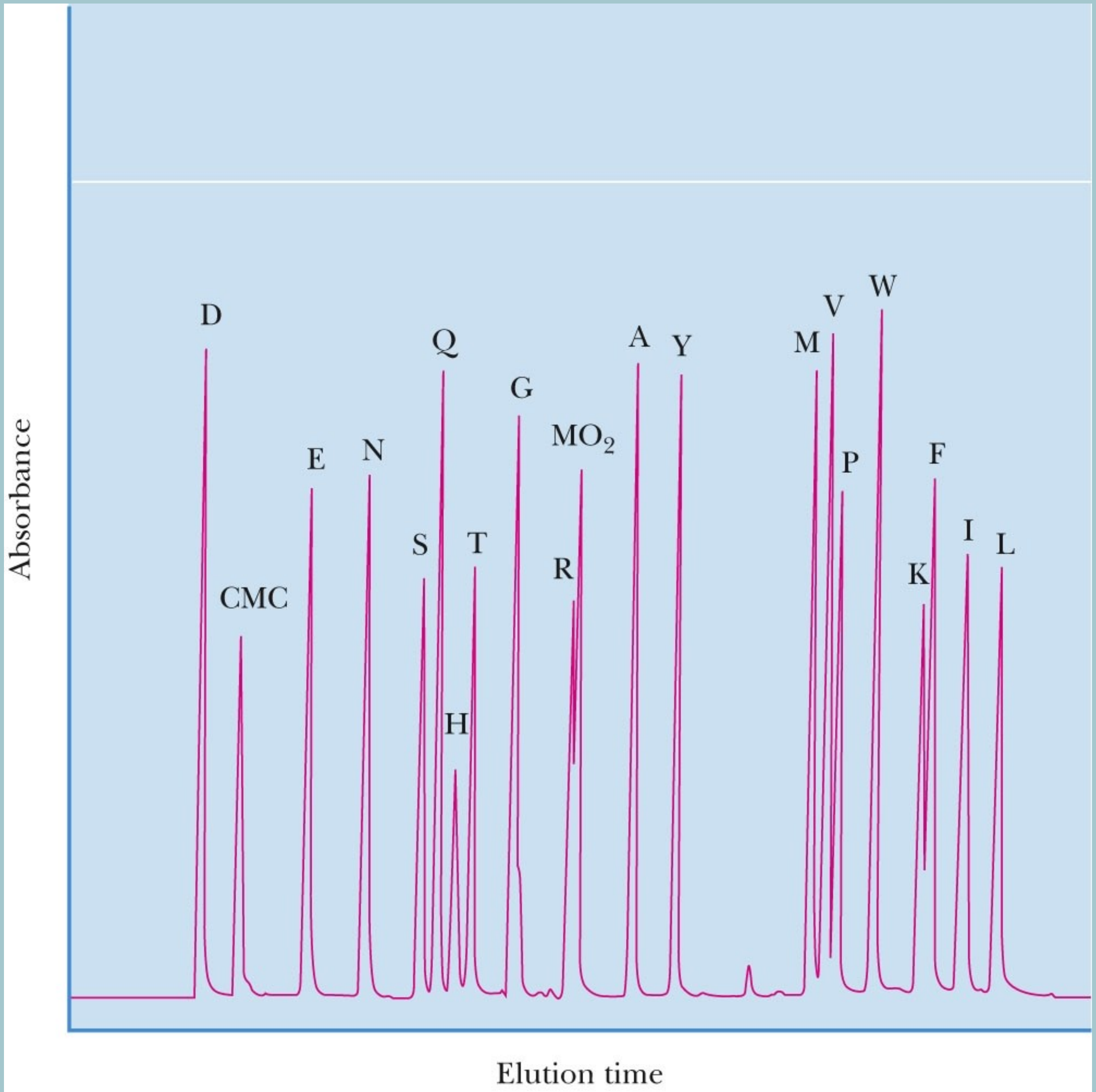
Reactions for Amino Acid Quantitation

Fig. 4.8 (a) Edman reagent, phenylisothiocyanate, reacts with the α-amino group of an amino acid or peptide to produce a phenylthiohydantoin (PTH) derivative, that can be detected by uv asorbance at 254 nm.



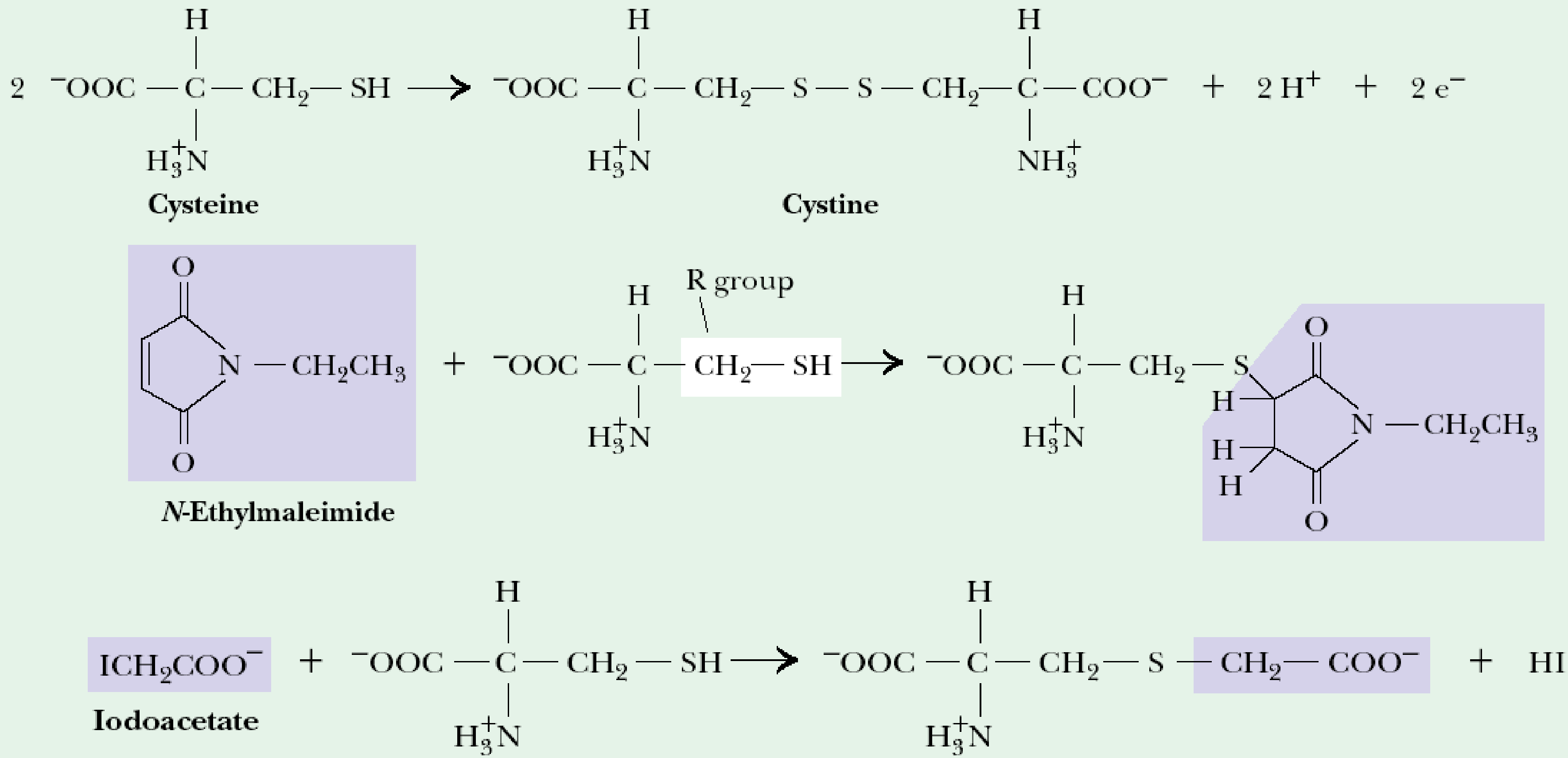
Separation of Amino Acids for Quantitation

Figure 4.13 Gradient separation of common PTH-amino acids by HPLC (High Performance/ Pressure Liquid Chromatography). Areas under peaks are proportional to the moles of each amino acid.



Some Reactions of Amino Acid Side Chains

CYSTEINE



LYSINE