Lehninger

SIXTH EDITION

Principles of Biochemistry

David L. Nelson | Michael M. Cox

3 | Amino Acids, Peptides, Proteins

Proteins: Main Agents of Biological Function

- Catalysis:
  - –enolase (in the glycolytic pathway)
  - –DNA polymerase (in DNA replication)
- Transport:
  - –hemoglobin (transports O_2 in the blood)
  - –lactose permease (transports lactose across the cell membrane)
- Structure:
  - –collagen (connective tissue)
  - –keratin (hair, nails, feathers, horns)
- Motion:
  - –myosin (muscle tissue)
  - –actin (muscle tissue, cell motility)

Figure 3-1c
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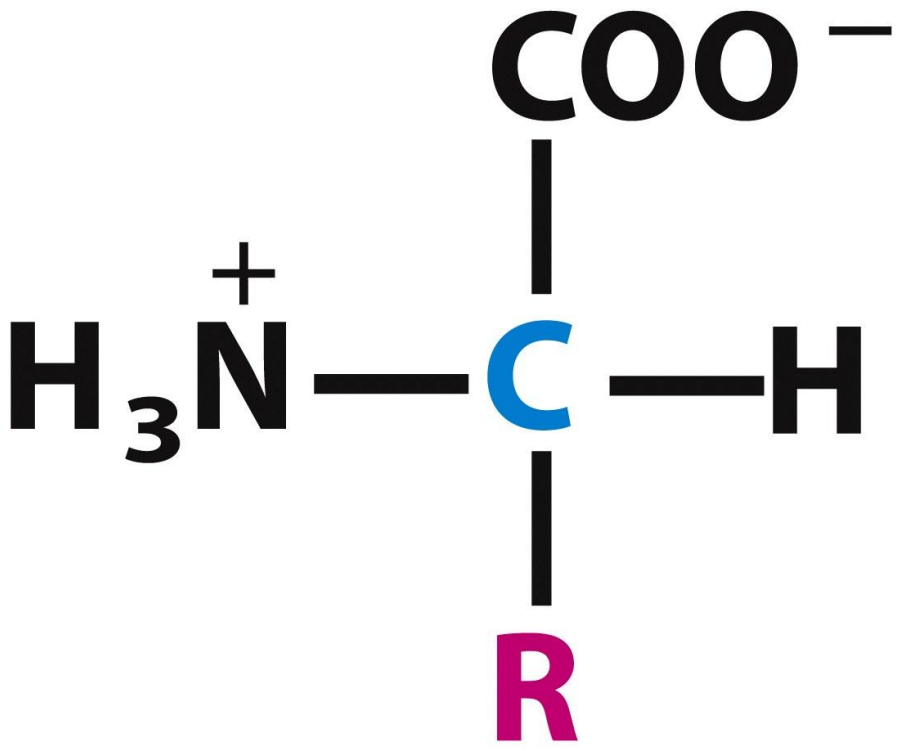
Amino Acids: Building Blocks of Protein

- Proteins are heteropolymers of α-amino acids
- Amino acids have properties that are well suited to carry out a variety of biological functions:
  - Capacity to polymerize
  - Useful acid-base properties
  - Varied physical properties
  - Varied chemical functionality

Amino acids share many features, differing only at the R substituent



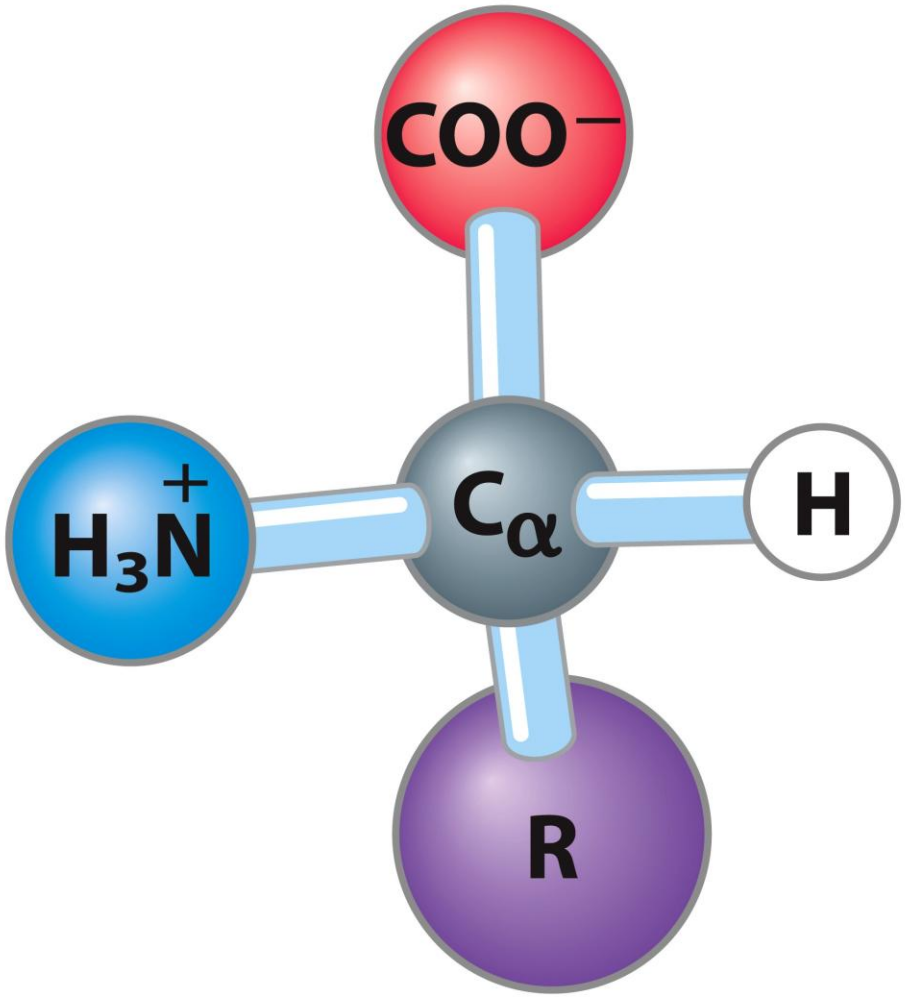


Figure 3-2
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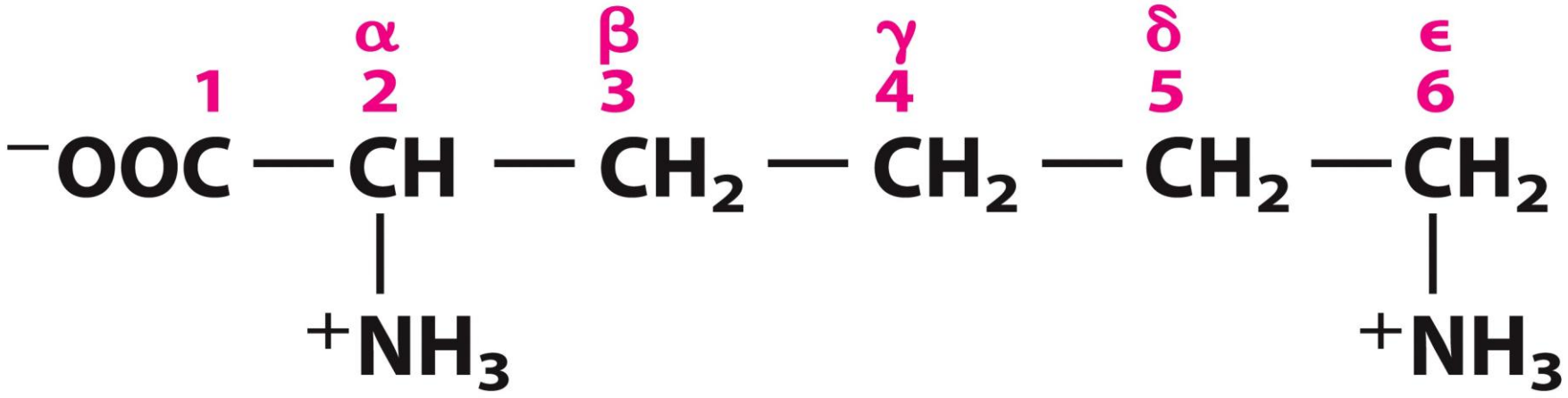
Amino Acids: Atom Naming

- Organic nomenclature: start from one end
- Biochemical designation:
  - start from α-carbon and go down the R-group



Lysine

Unnumbered 3 p78
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Most α-Amino Acids are Chiral

- The α-carbon has always four substituents and is tetrahedral
- All (except proline) have an acidic carboxyl group, a basic amino group, and an alpha hydrogen connected to the α-carbon
- Each amino acid has an unique fourth substituent R
- In glycine, the fourth substituent is also hydrogen

Proteins only contain L amino acids

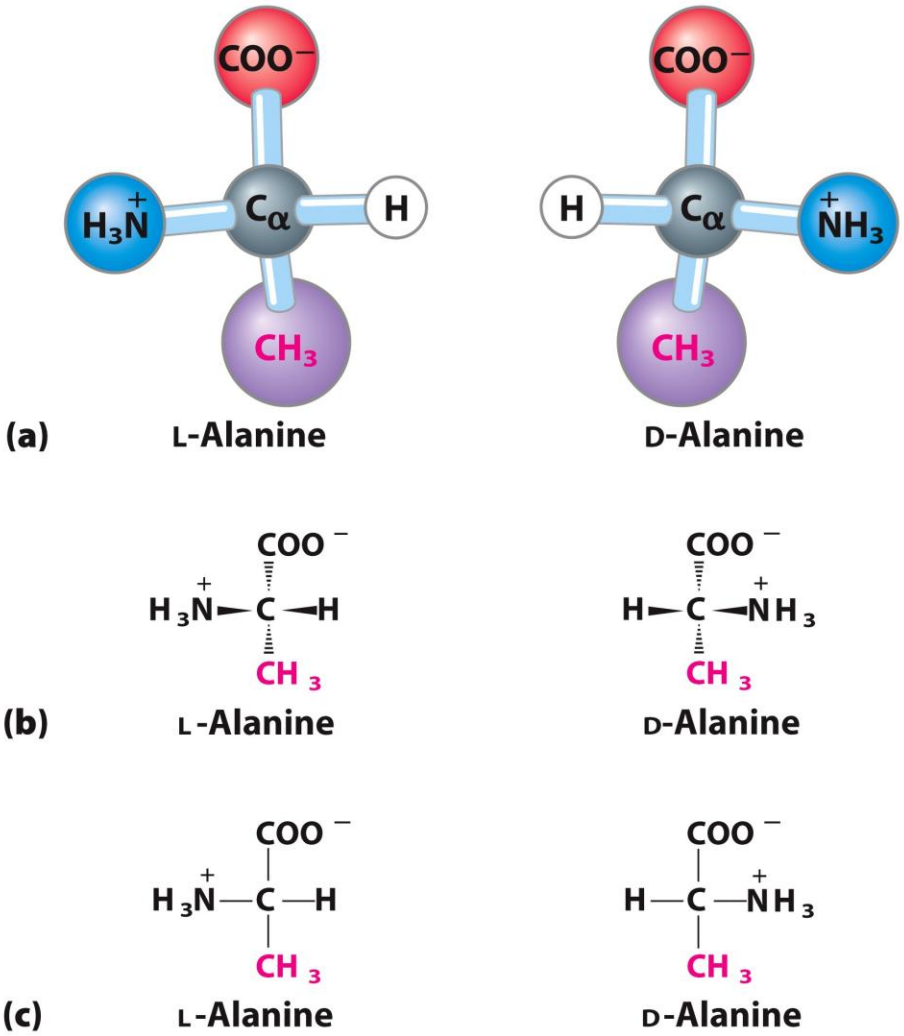

Figure 3-3
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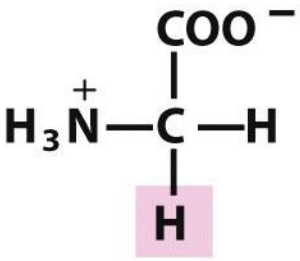
Amino Acids: Classification

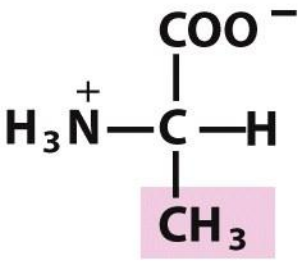
Common amino acids can be placed in five basic groups depending on their R substituents:

- Nonpolar, aliphatic (7)
- Aromatic (3)
- Positively charged (3)
- Negatively charged (2)
- Polar, uncharged (5)

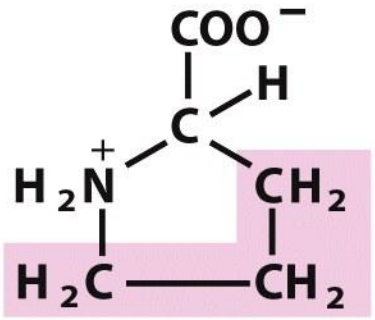
Nonpolar, aliphatic R groups



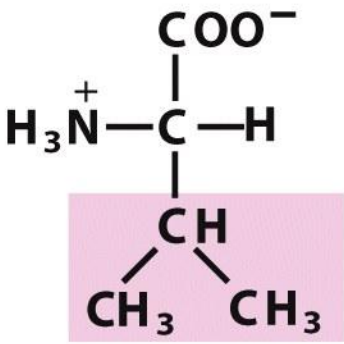
Glycine



Alanine

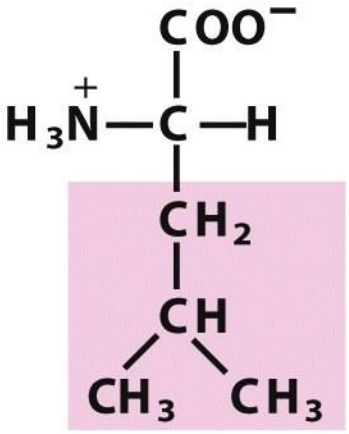


Proline

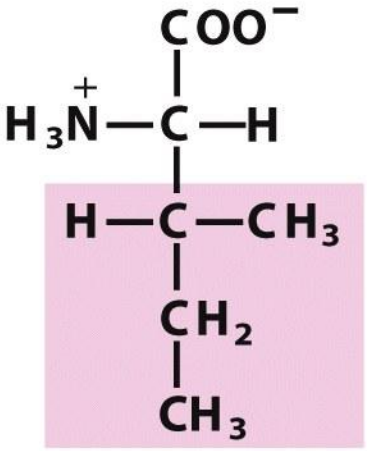


Valine

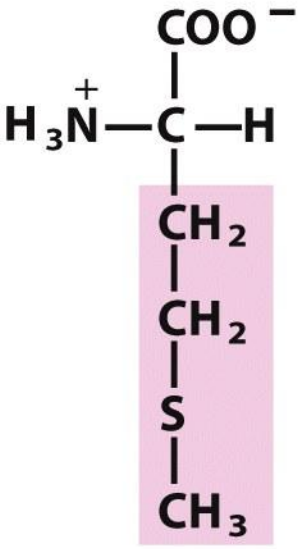
These amino acid side chains are ***hydrophobic***



Leucine



Isoleucine



Methionine

Figure 3-5 part 1
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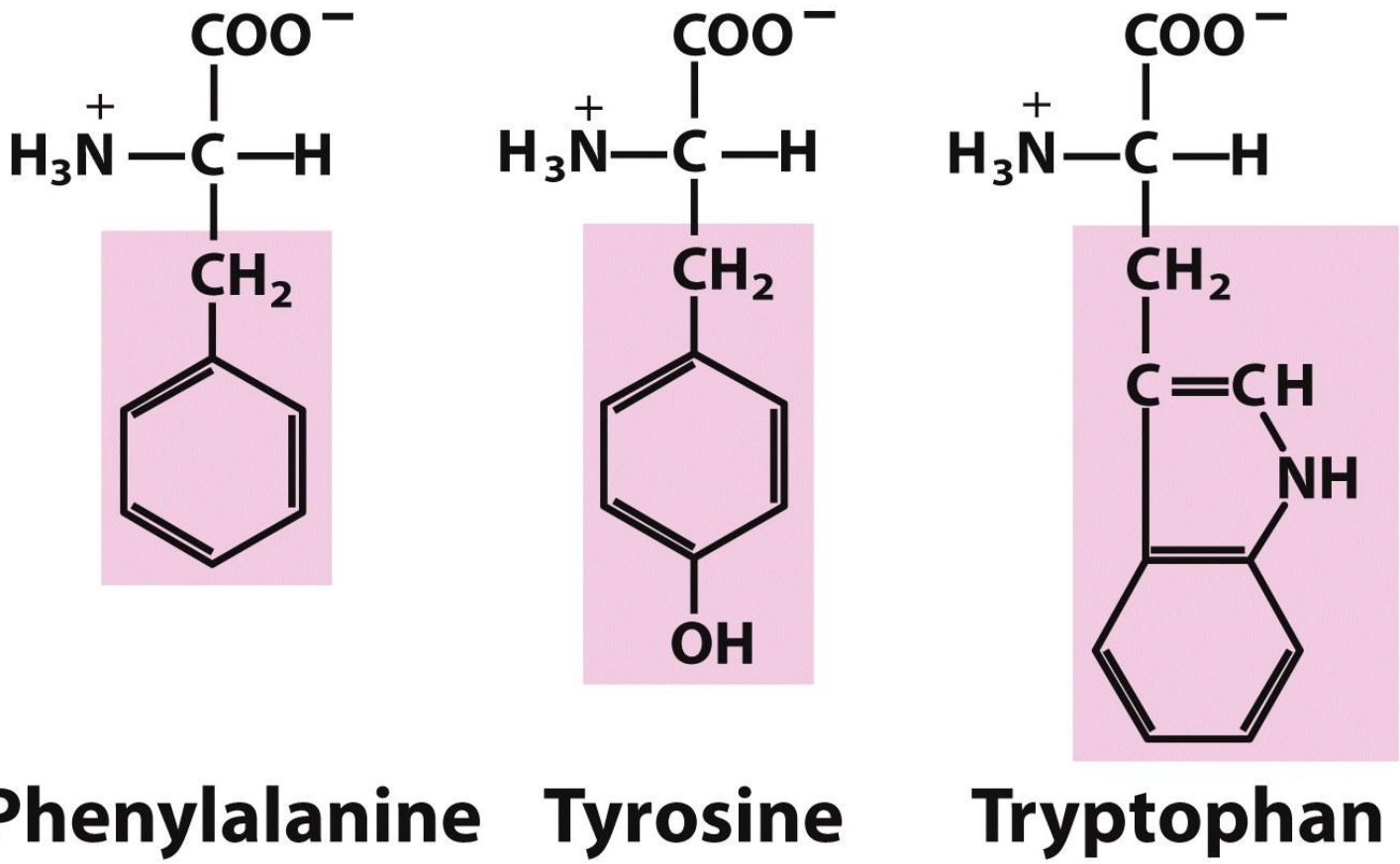
Aromatic R groups



Phenylalanine **Tyrosine** **Tryptophan**

Figure 3-5 part 2
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These amino acid side chains ***absorb UV light at 270-280 nm***

Positively charged R groups

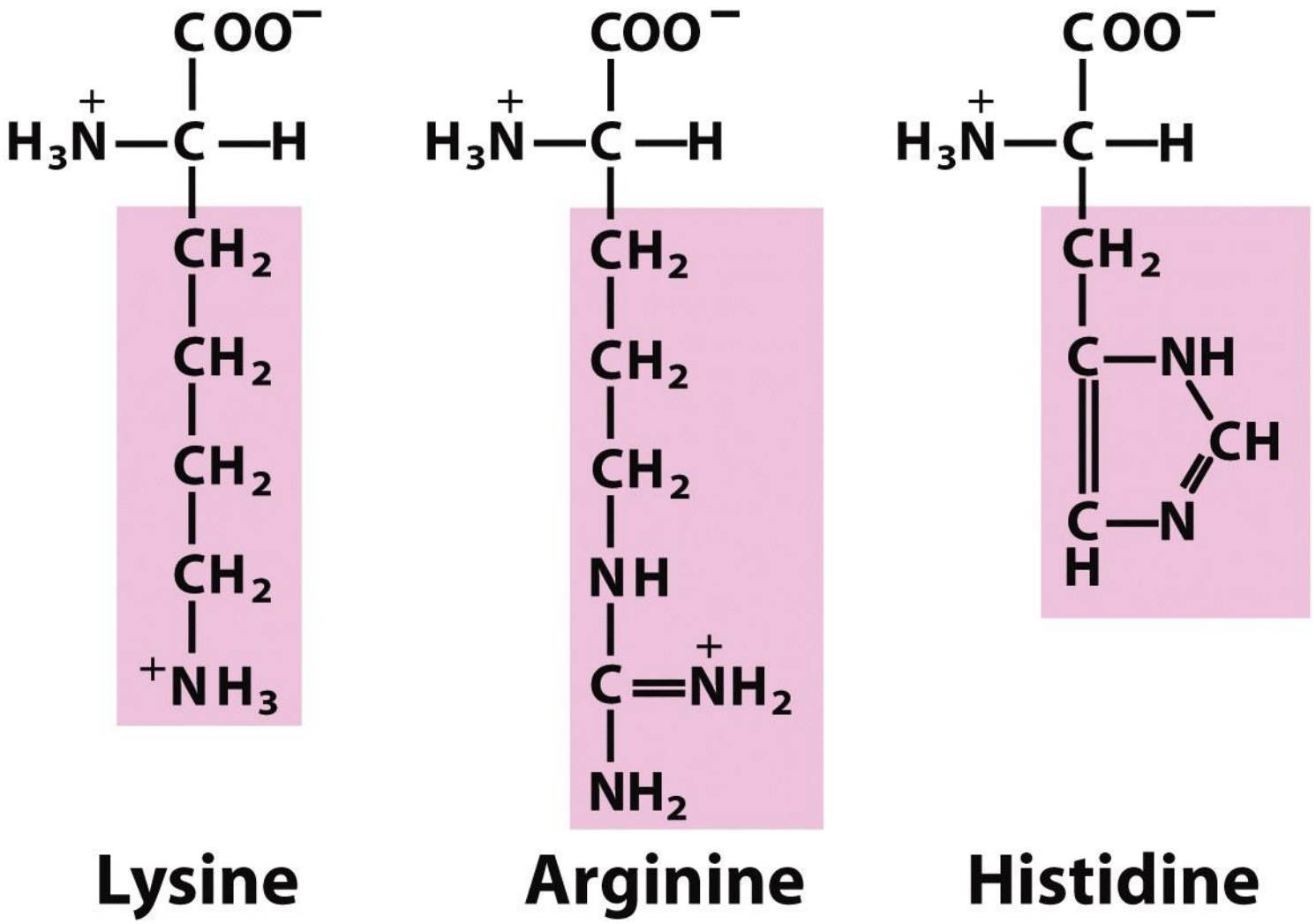

Figure 3-5 part 4
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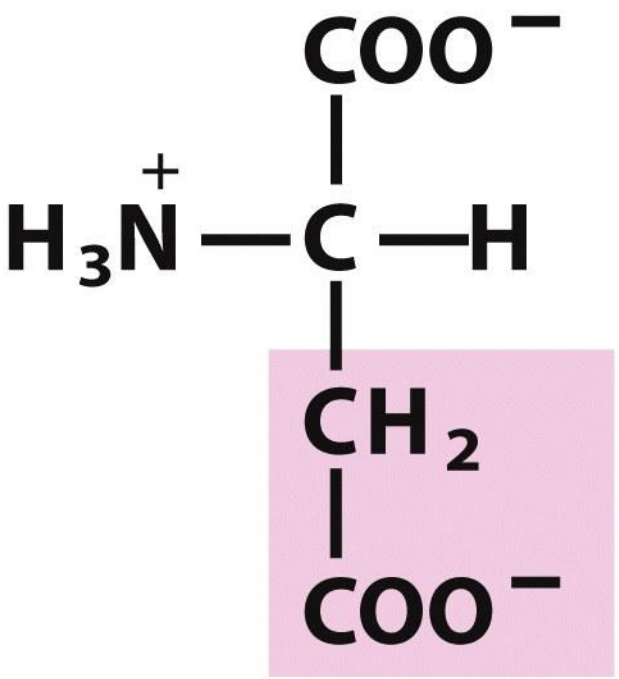
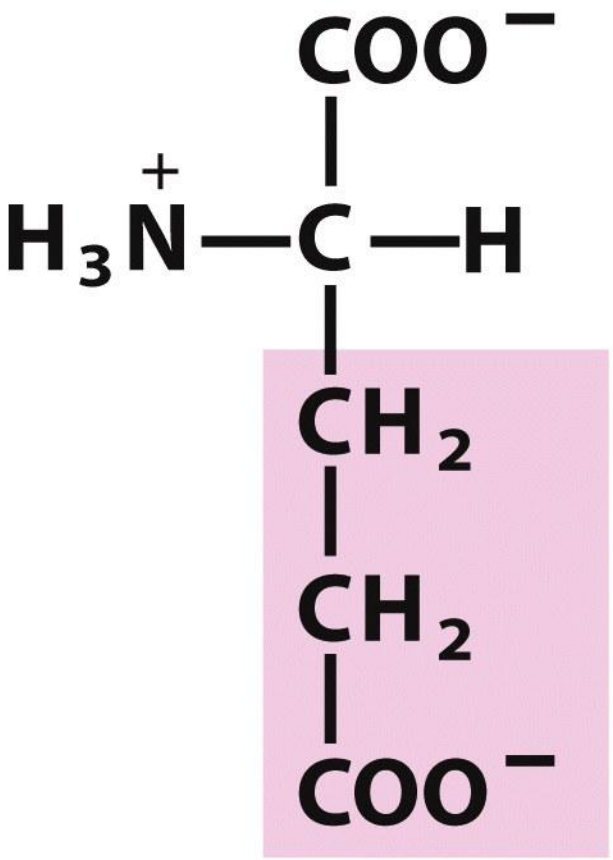
These are ***basic amino acids***

Negatively charged R groups



Aspartate



Glutamate

Figure 3-5 part 5
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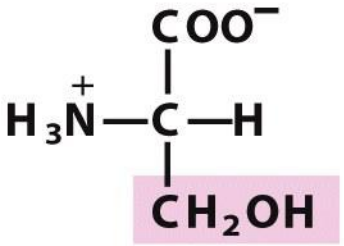
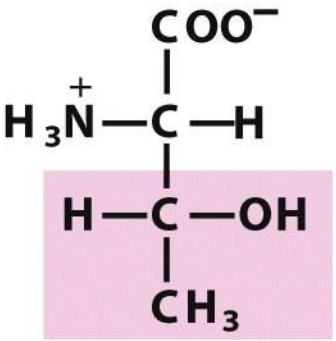
These are ***acidic amino acids***

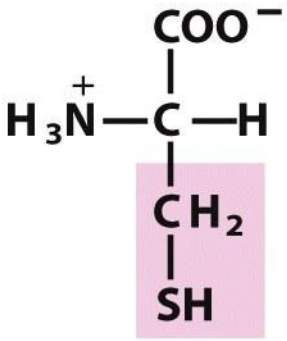
Polar, uncharged R groups



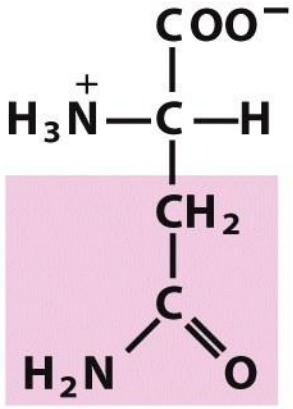
Serine



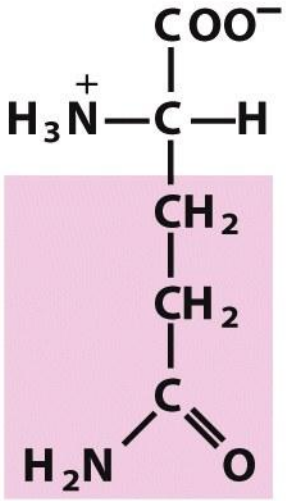
Threonine



Cysteine



Asparagine



Glutamine

These amino acids side chains can form ***hydrogen bonds***

Cysteine can form ***disulfide bonds***

Reversible formation of a disulfide bond by the oxidation of two molecules of cysteine. Disulfide bonds between Cys residues stabilize the structures of many proteins.

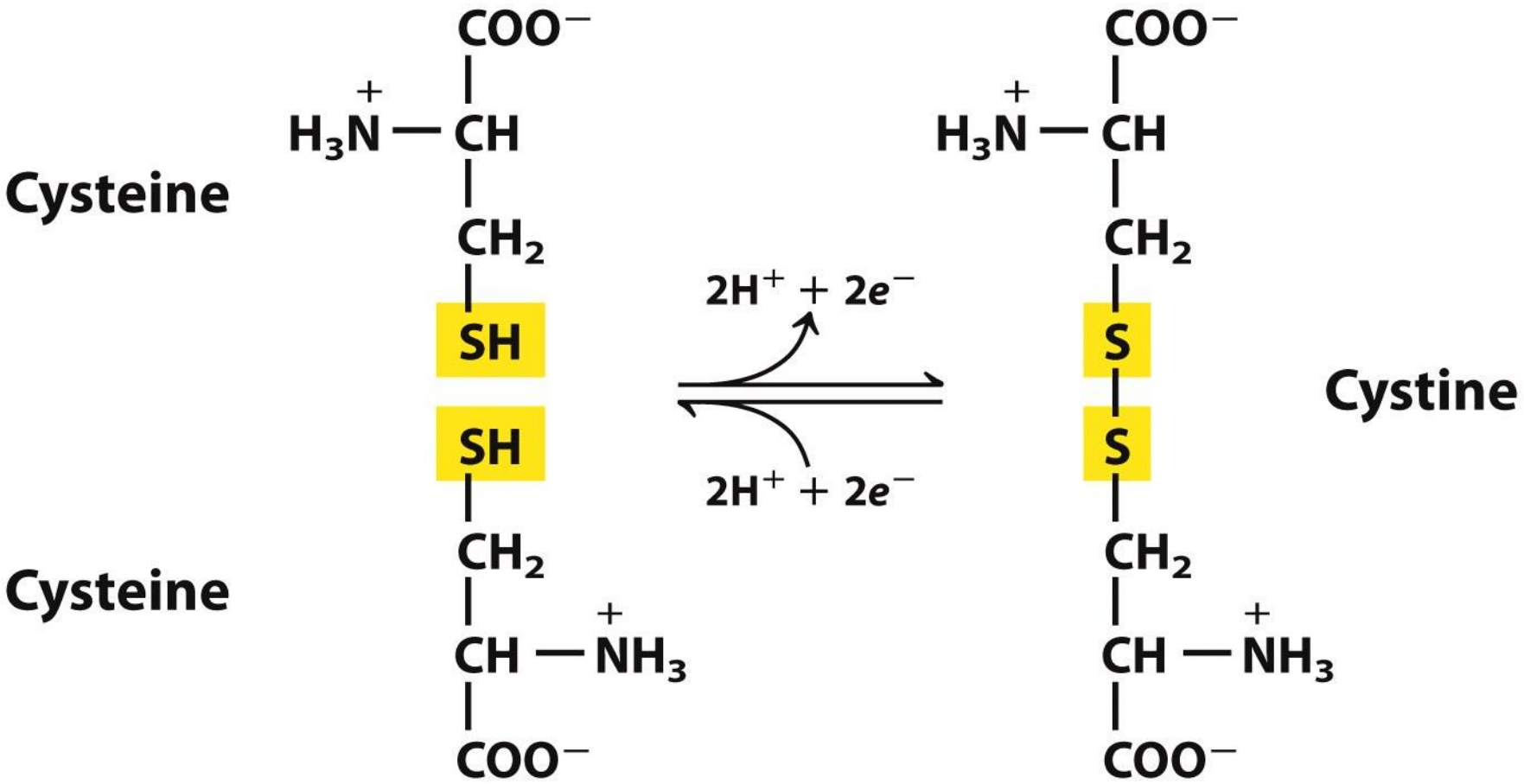


Figure 3-7
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Uncommon Amino Acids in Proteins

Not incorporated by ribosomes

Arise by post-translational modifications of proteins

Reversible modifications, esp. phosphorylation is important in regulation and signaling

Modified Amino Acids Found in Proteins

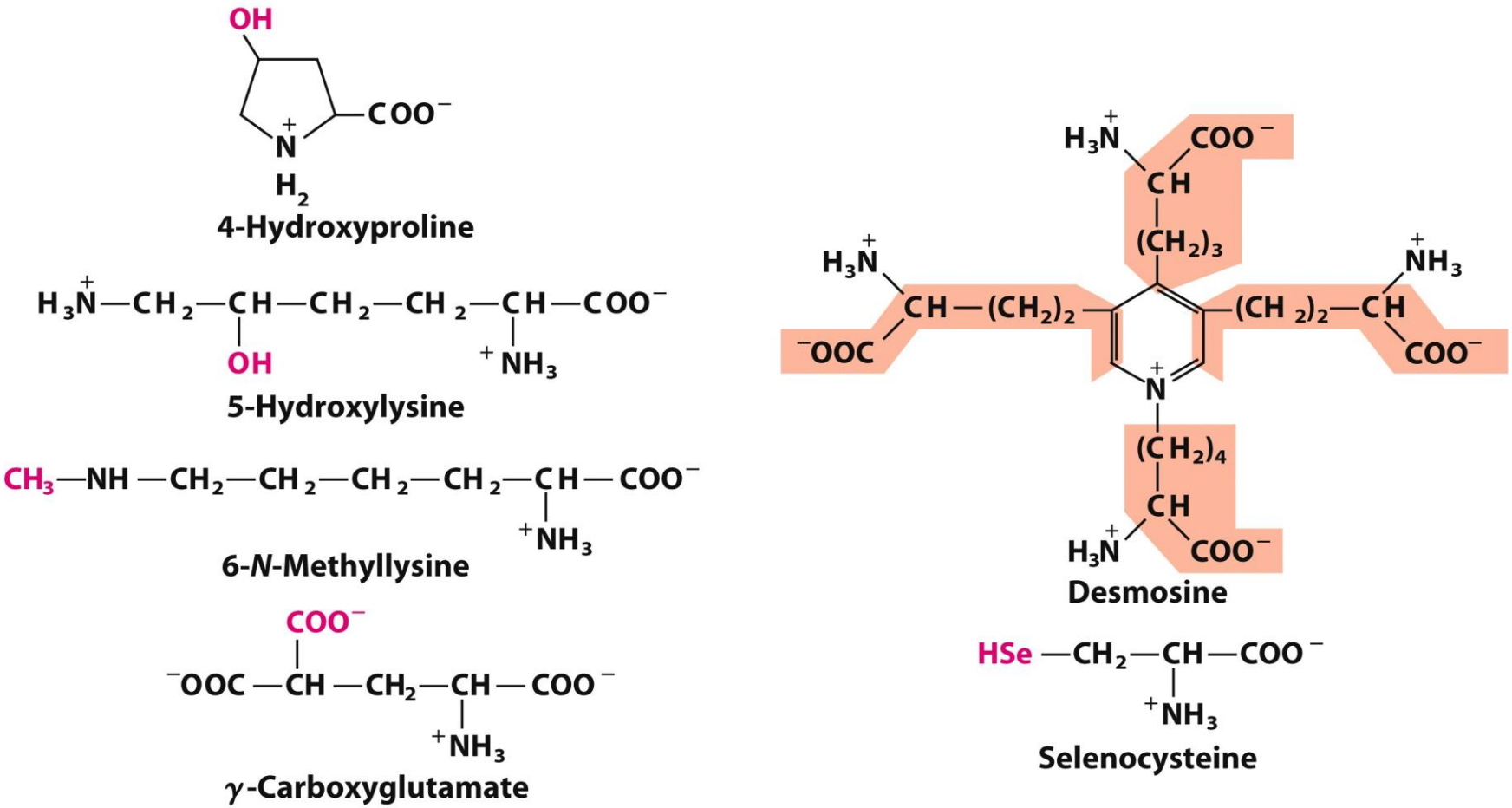

Figure 3-8a
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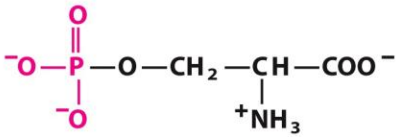
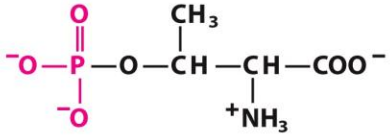
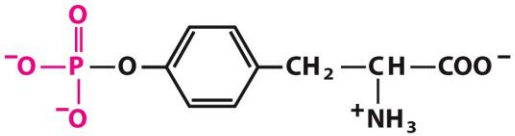
Reversible Modifications of Amino Acids



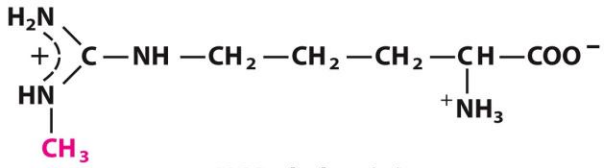
Phosphoserine



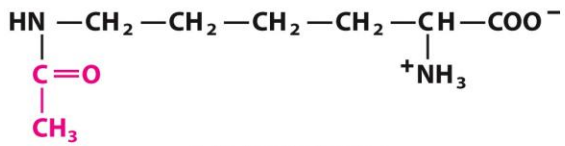
Phosphothreonine



Phosphotyrosine



σ-*N*-Methylarginine



6-*N*-Acetyllysine

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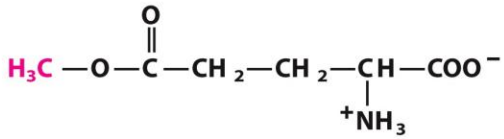
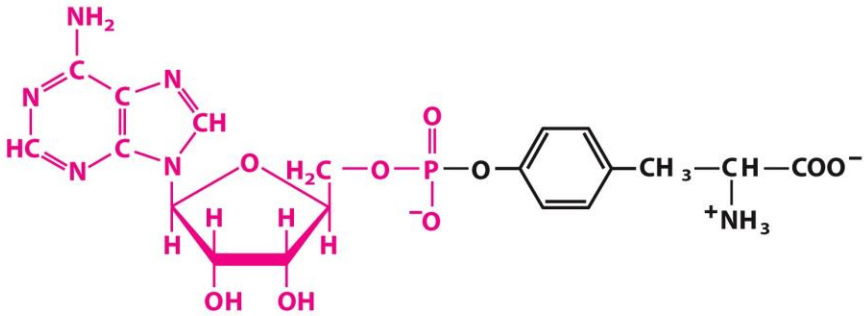
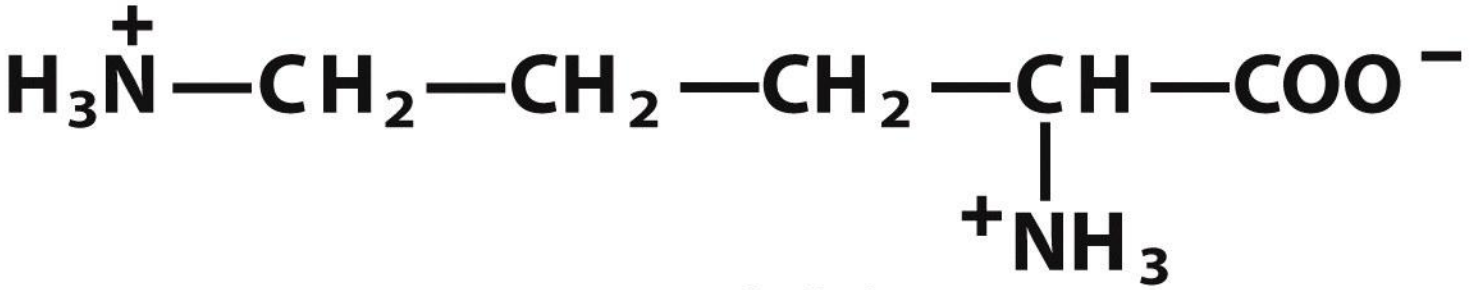


Glutamate γ-methyl ester

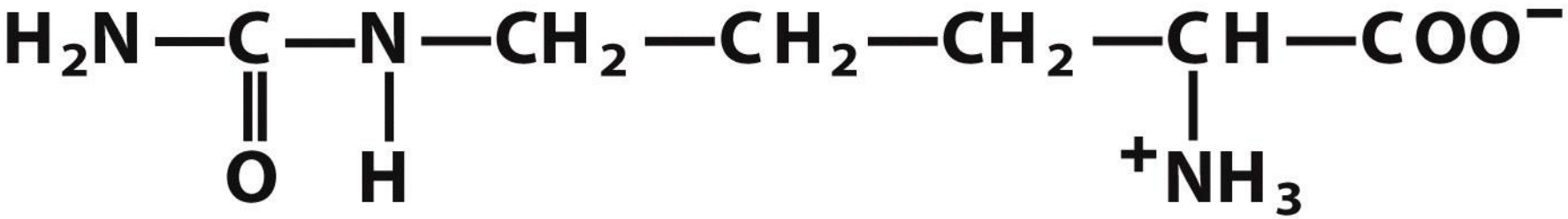


Adenylyltyrosine

Important Amino Acids in Urea Metabolism



Ornithine



Citrulline

Figure 3-8c
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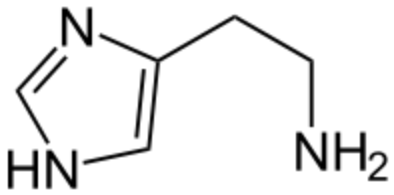
A Derivative of amino acids

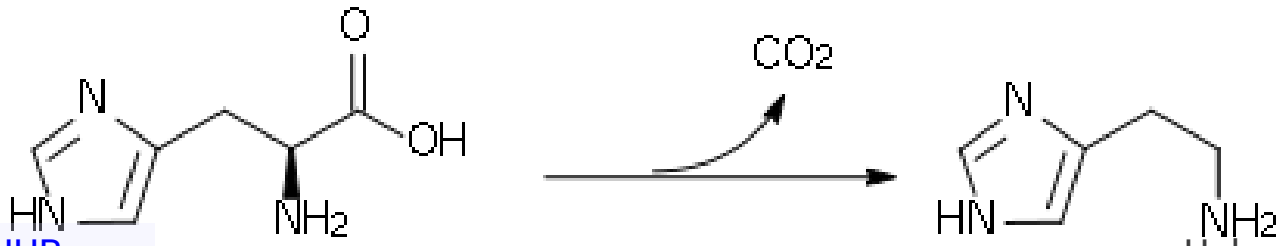
• **Histamine** is an organic nitrogen compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter.



• Histamine triggers the inflammatory response

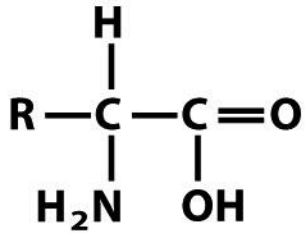
• Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues

• Histamine is derived from the decarboxylation of the amino acid histidine, a reaction catalyzed by the enzyme *L-histidine decarboxylase*

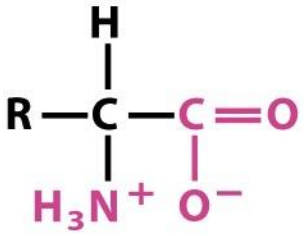


Ionization of Amino Acids

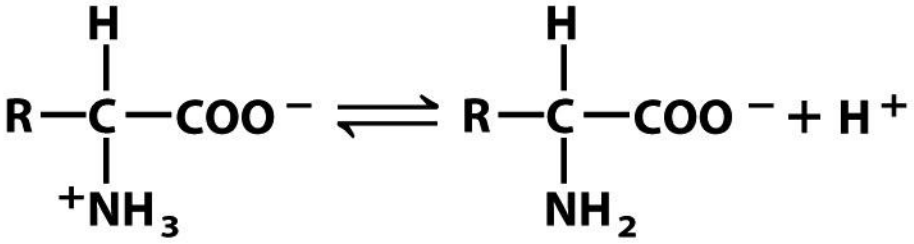
- At acidic pH, the carboxyl group is protonated and the amino acid is in the cationic form
- At neutral pH, the carboxyl group is deprotonated but the amino group is protonated. The net charge is zero; such ions are called Zwitterions
- At alkaline pH, the amino group is neutral $–NH_2$ and the amino acid is in the anionic form.



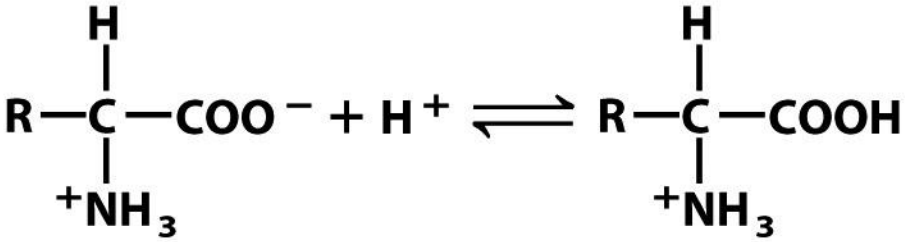
Nonionic form



Zwitterionic form



Zwitterion as acid



Zwitterion as base

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Cation → Zwitterion → Anion

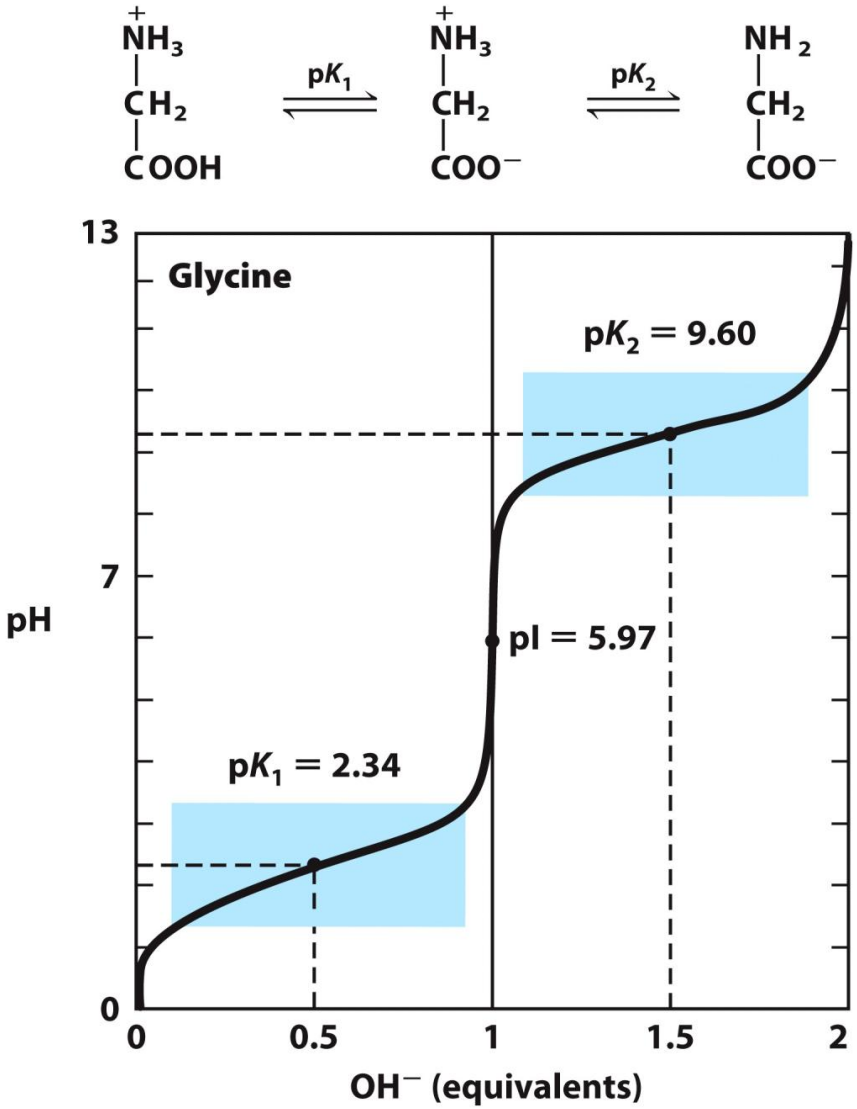

Figure 3-10
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Chemical Environment Affects pK_a Values

α-carboxy group is much more acidic than in carboxylic acids

α-amino group is slightly less basic than in amines

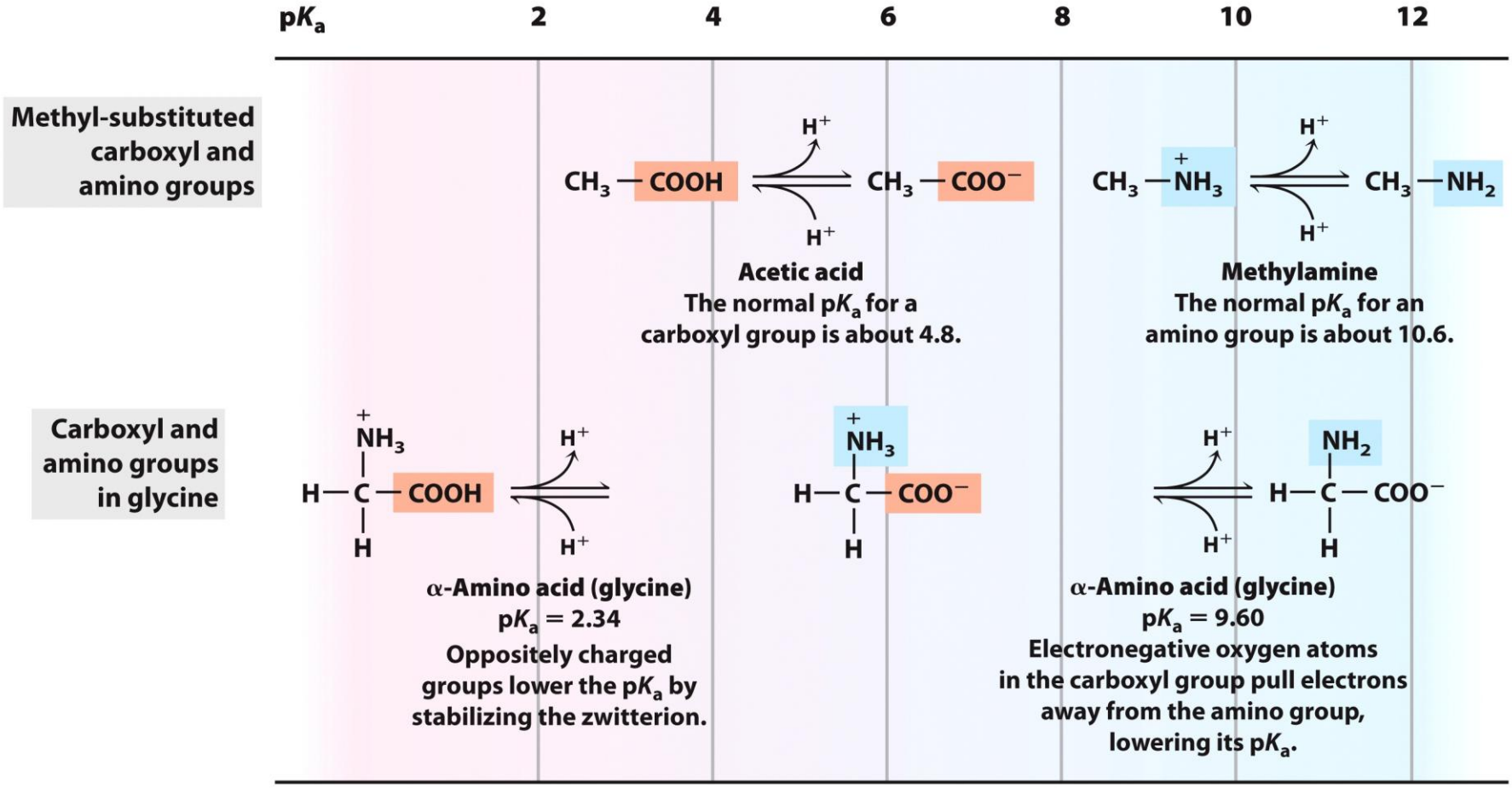

Figure 3-11

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Amino acids can act as buffers

Amino acids with uncharged side chains, such as glycine, have two pK_a values:

The pK_a of the α-carboxyl group is 2.34

The pK_a of the α-amino group is 9.6

It can act as a buffer in two pH regimes.

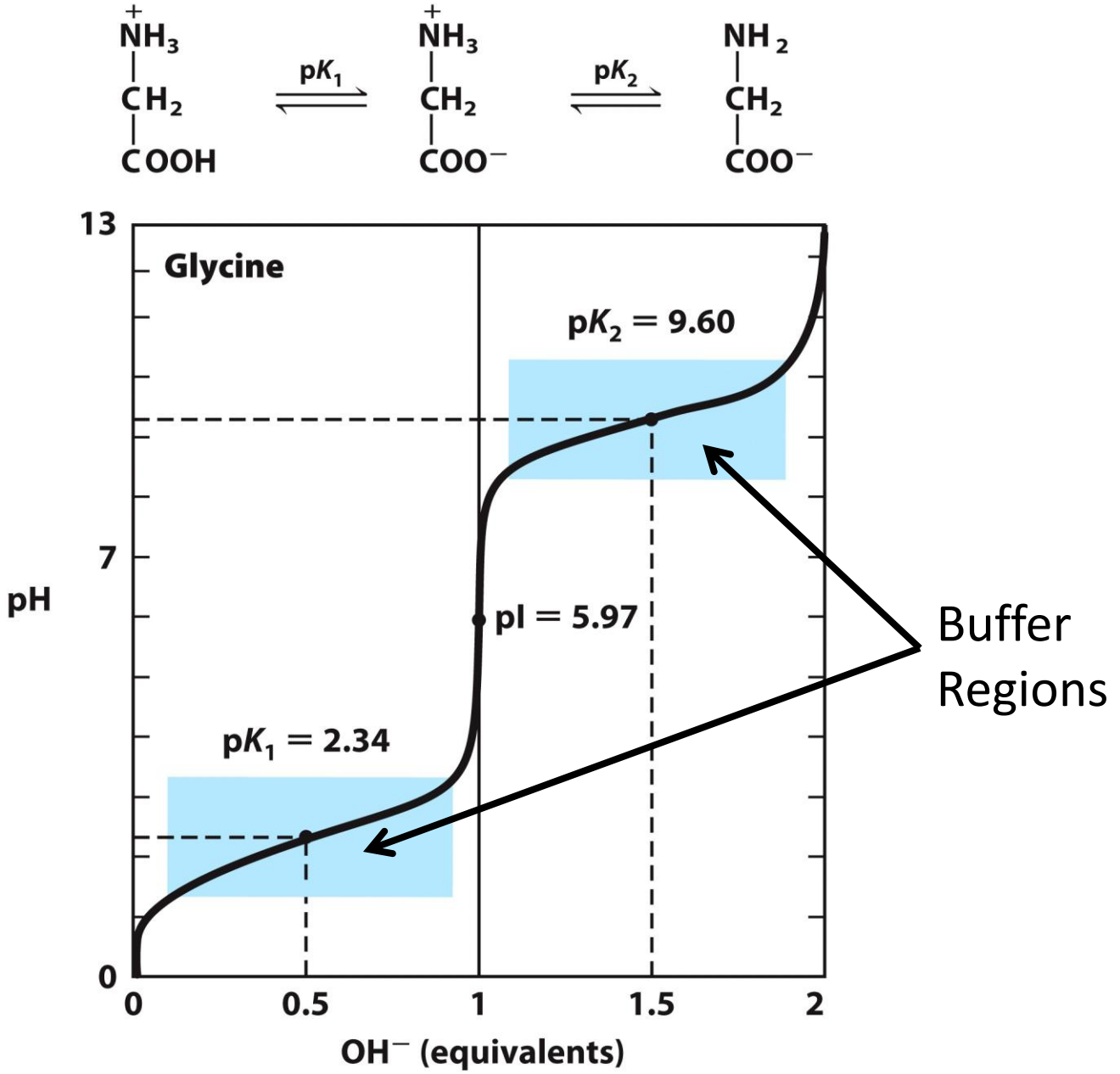

Figure 3-10
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Amino acids carry a net charge of zero at a specific pH (the pI)

- Zwitterions predominate at pH values between the pK_a values of the amino and carboxyl groups
- For amino acids without ionizable side chains, the Isoelectric Point (equivalence point, pI) is

$$pI = \frac{pK_1 + pK_2}{2}$$

- At this point, the net charge is zero
  - AA is least soluble in water
  - AA does not migrate in electric field

Ionizable Side Chains Can Show Up in Titration Curves

- Ionizable side chains can be also titrated
- Titration curves are now more complex
- pK_a values are discernable if two pK_a values are more than two pH units apart

Why is the side-chain pK_a so much higher?

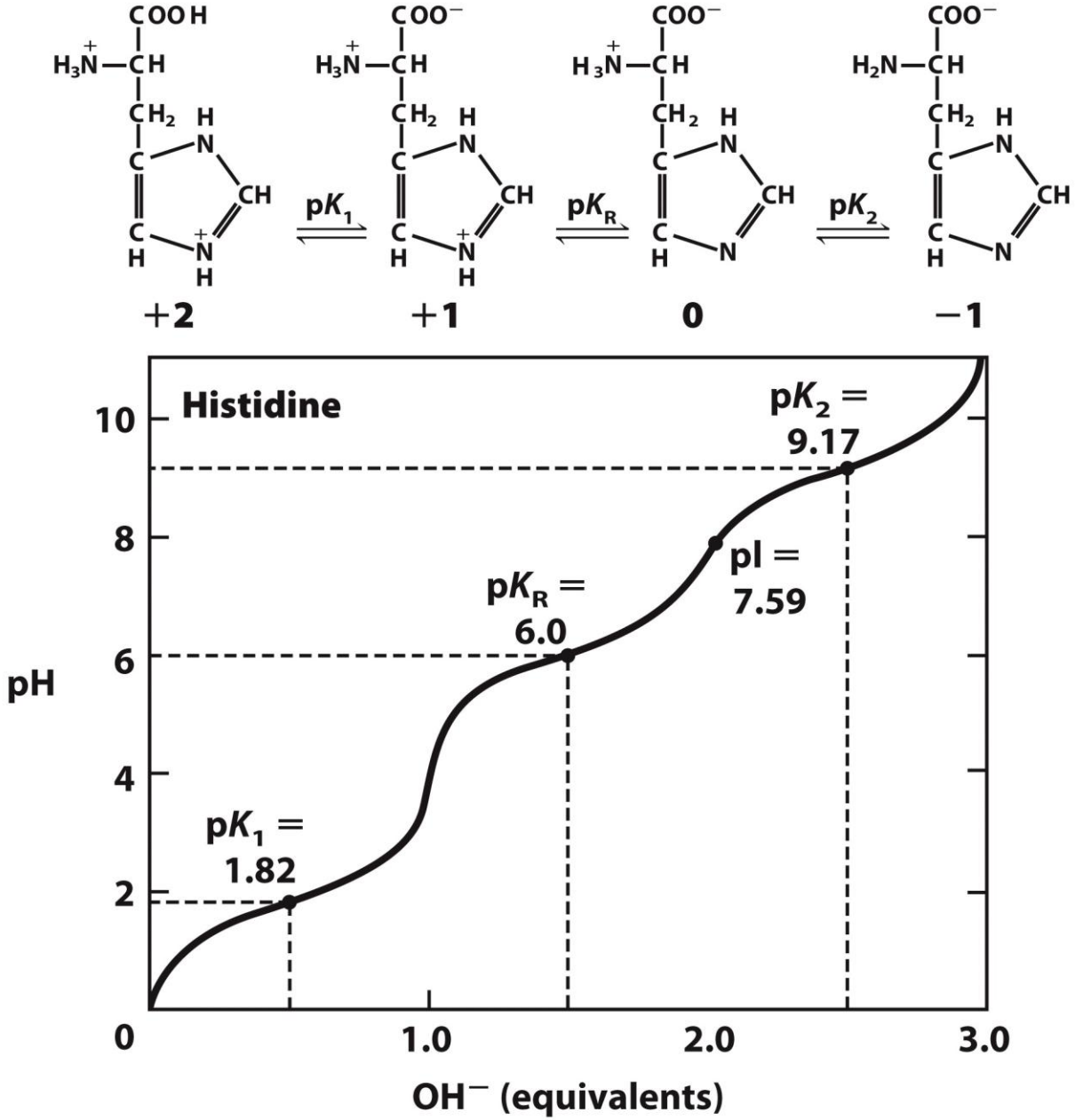

Figure 3-12b
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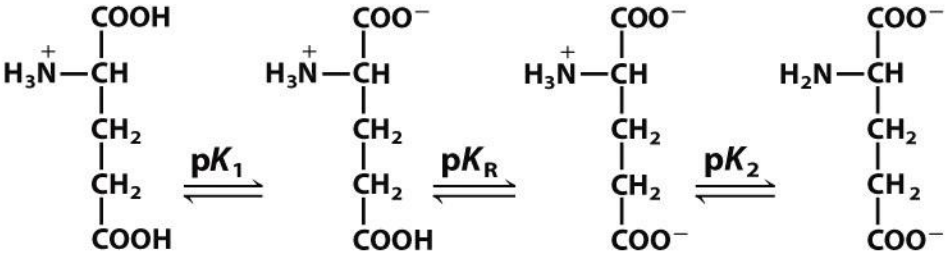
How to Calculate the pI When the Side Chain is Ionizable

- At the pI, *the net charge of the molecule is zero*
- Identify species that carries a net zero charge
- Identify the species on either side of the neutral form (0 charge)
- Take average the two pK_a values

What is the pI of histidine?

- $(pK_R + pK_2)/2 = pI$
- $(6 + 9.17)/2 = 7.58$



Net charge: +1 0 −1 −2

What is the pI of glutamate?

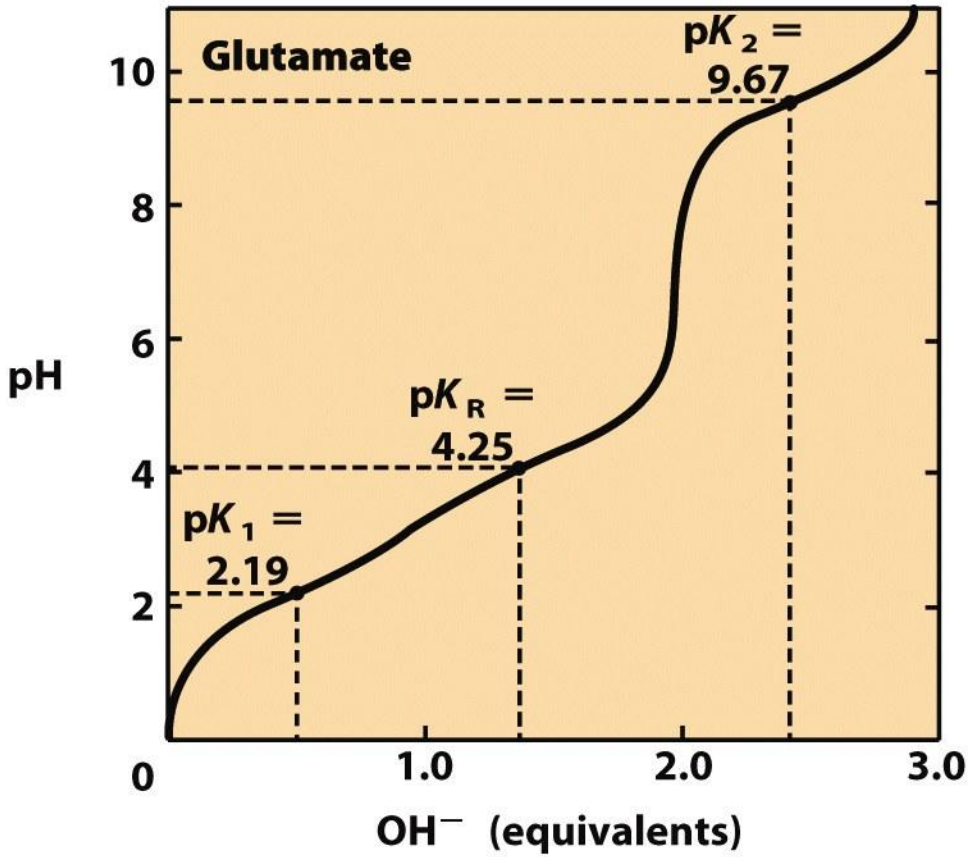


Figure 3-12a
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Formation of Peptides

- Peptides are small condensation products of amino acids
- They are "small" compared to proteins (M_w < 10 kDa)

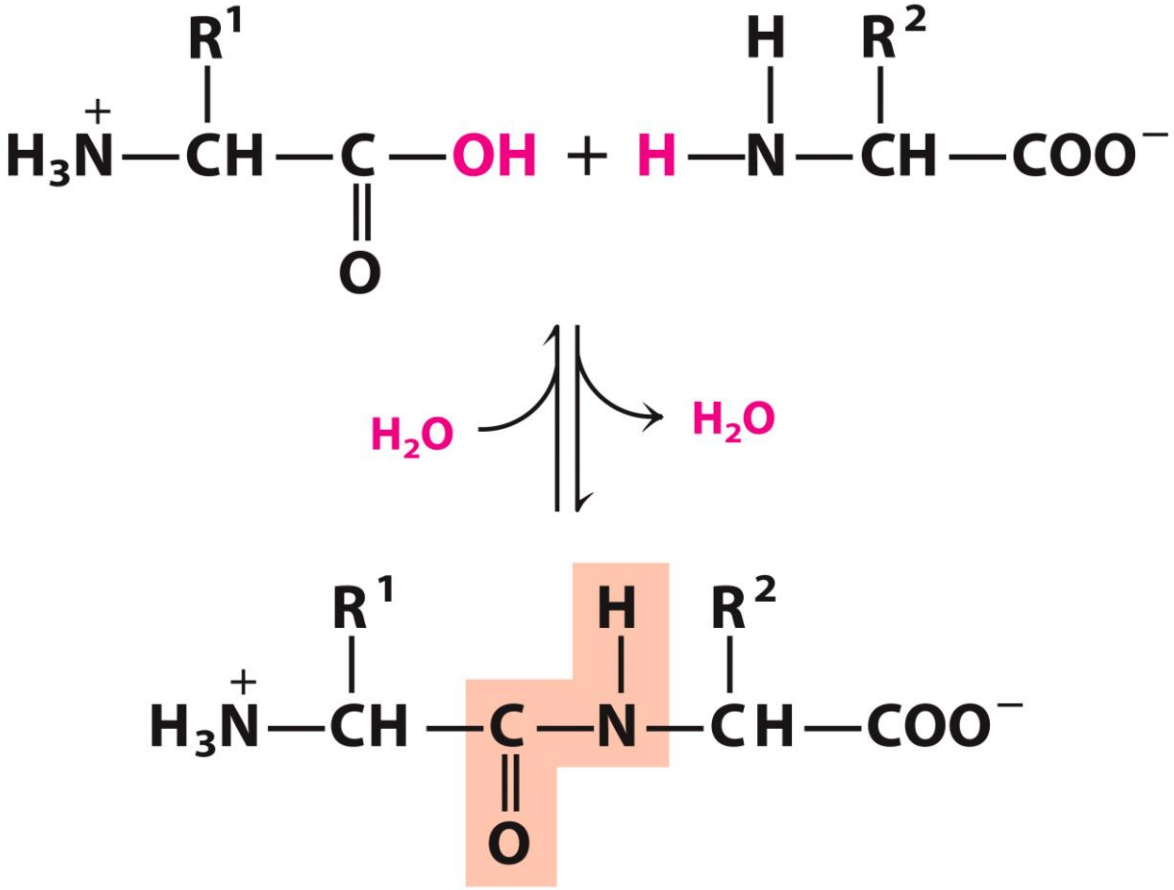

Figure 3-13
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Peptide ends are not the same

Numbering (and naming) starts from the amino terminus

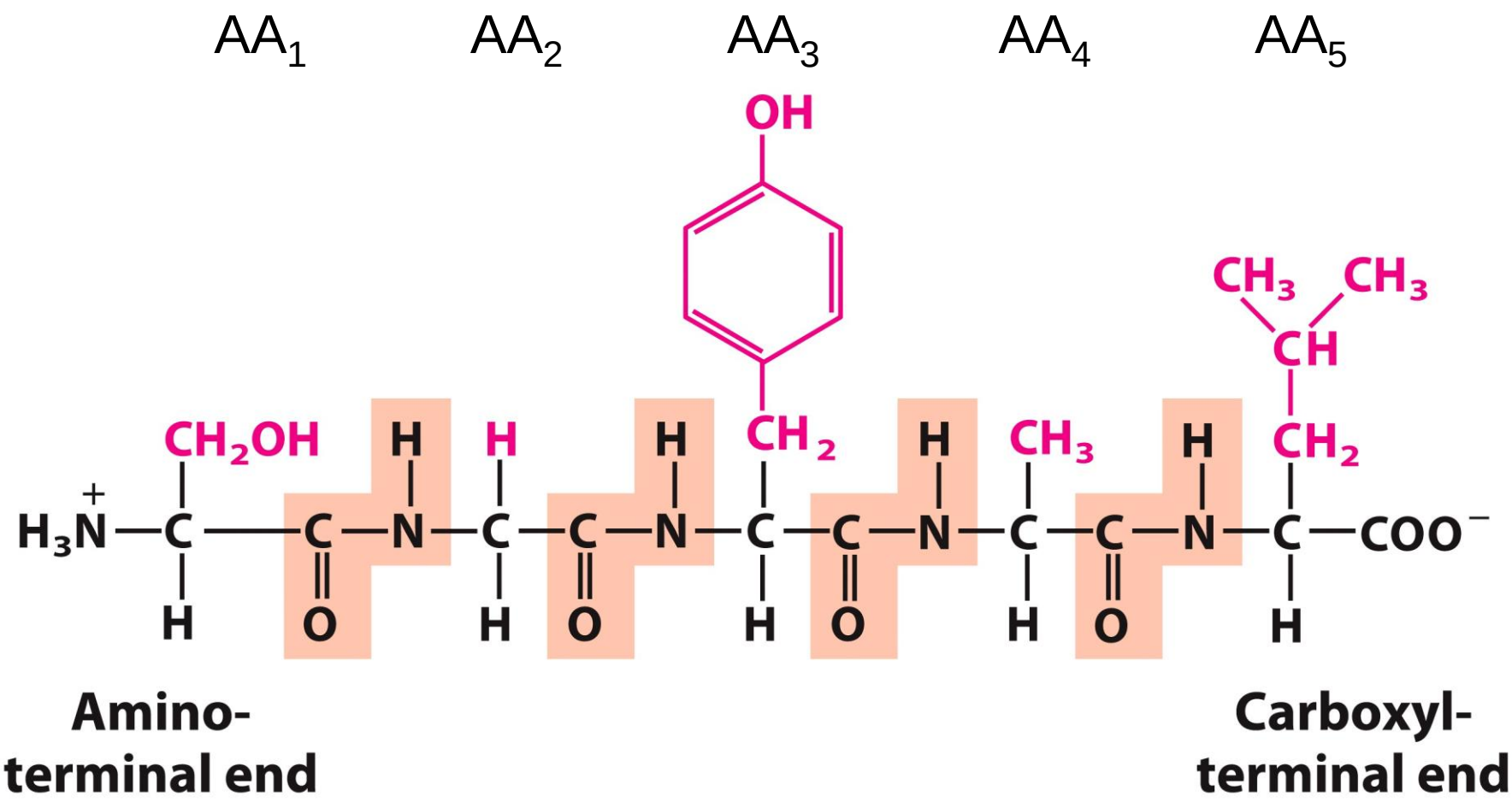

Figure 3-14
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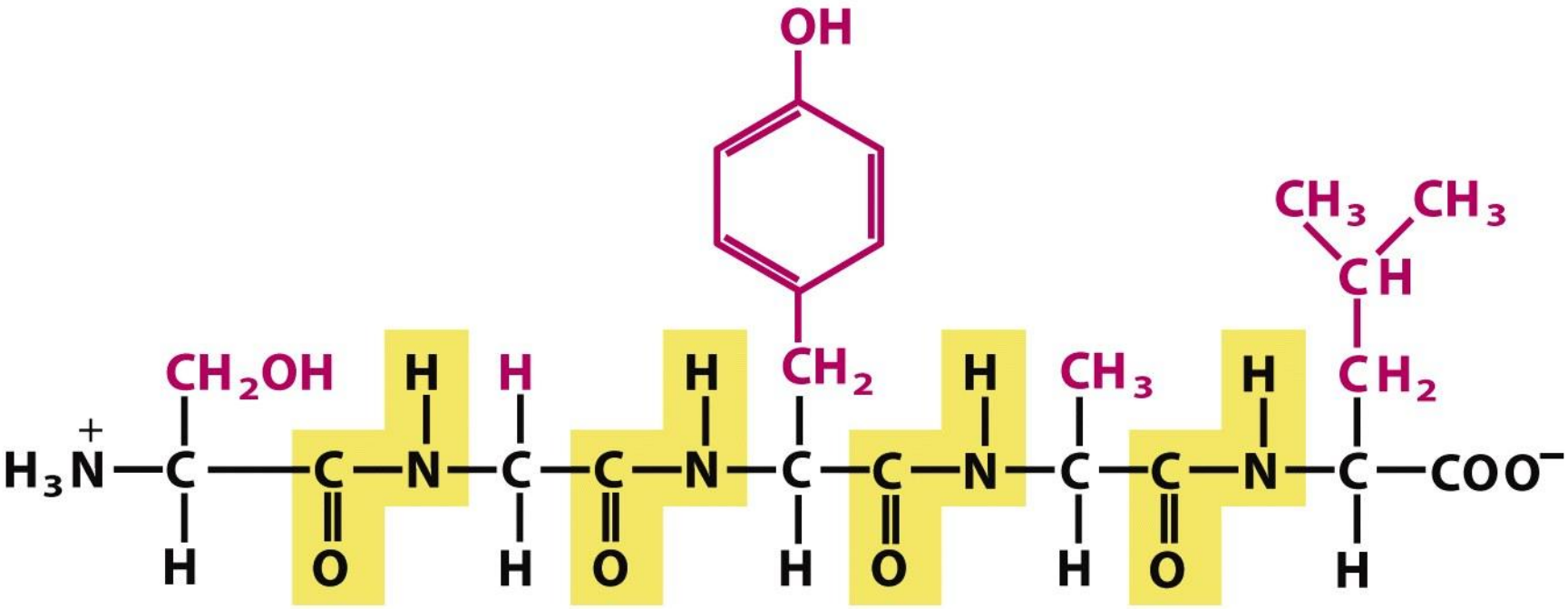
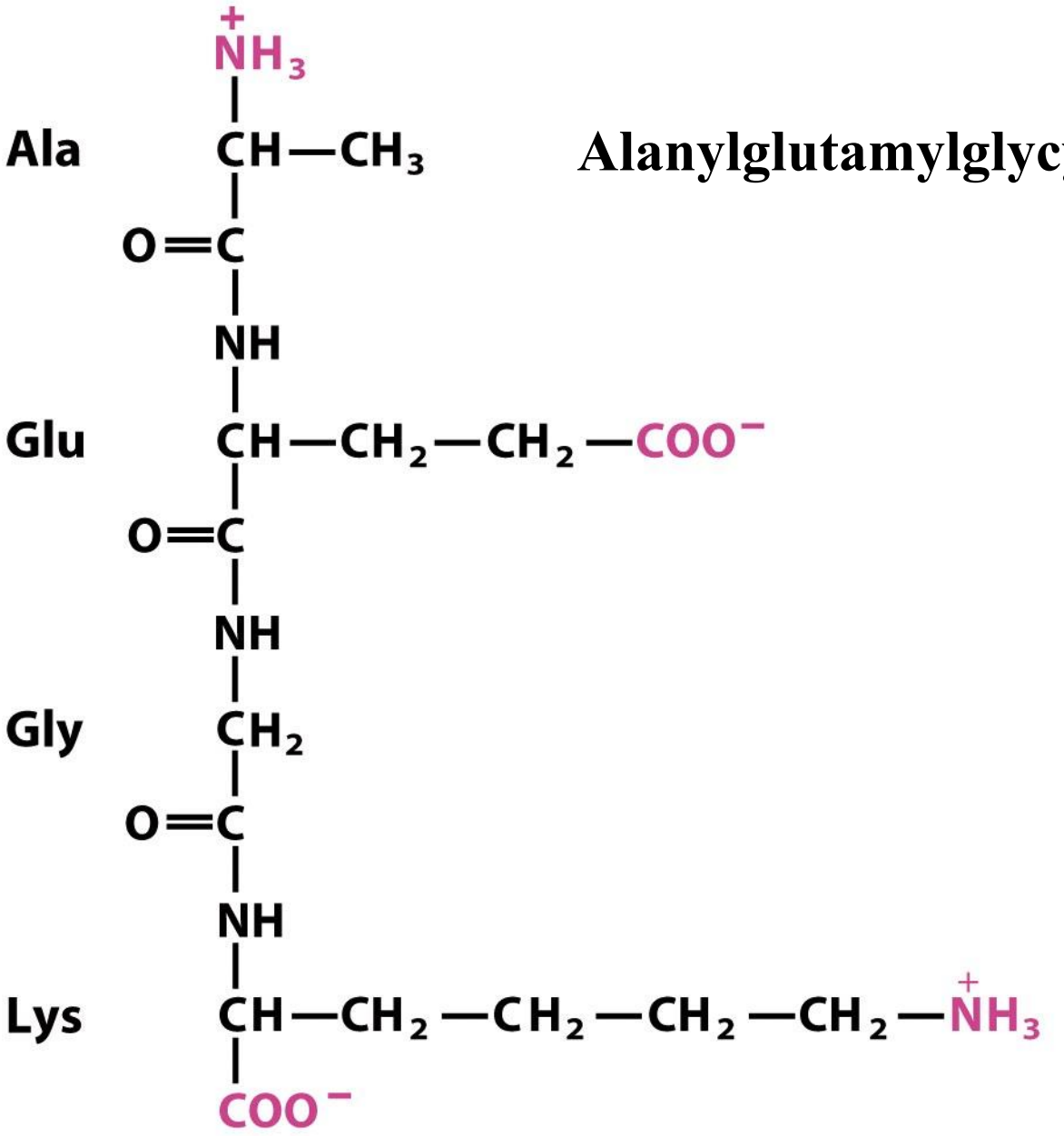
Naming peptides: start at the N-terminus

The pentapeptide serylglycyltyrosylalanylleucine,
Ser–Gly–Tyr–Ala–Leu, or SGYAL



Amino-terminal end

Carboxyl-terminal end



Alanylglutamylglycyllysine

Figure 3-15
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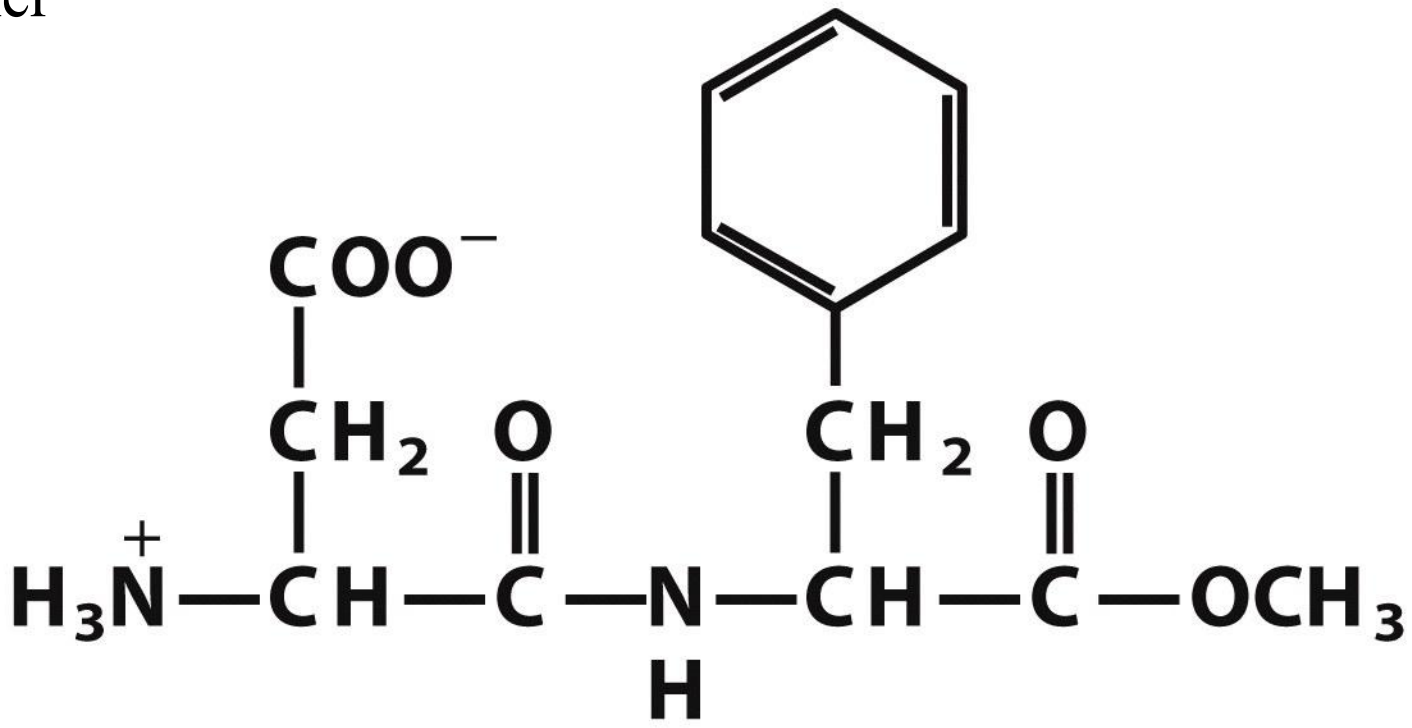
Peptides: A Variety of Functions

- Hormones and pheromones:
  - insulin (sugar uptake)
  - oxytocin (childbirth)
  - sex-peptide (fruit fly mating)
- Neuropeptides
  - substance P (pain mediator)
- Antibiotics:
  - polymyxin B (for Gram - bacteria)
  - bacitracin (for Gram + bacteria)
- Protection, e.g. toxins
  - amanitin (mushrooms)
  - conotoxin (cone snails)
  - chlorotoxin (scorpions)

Artificial sweetener



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

Unnumbered 3 p83
Lehninger Principles of Biochemistry, Fifth Edition

Proteins are:

- Polypeptides (covalently linked α-amino acids) + possibly –
  - cofactors,
  - coenzymes,
  - prosthetic groups,
  - other modifications

- **Cofactor** is a general term for functional non-amino acid component
  - – Metal ions or organic molecules
- **Coenzyme** is used to designate an organic cofactors
  - – NAD^+ in lactate dehydrogenase
- **Prosthetic groups** are covalently attached cofactors
  - – Heme in myoglobin

Polypeptide size and number varies greatly in proteins

TABLE 3–2 Molecular Data on Some Proteins

	Molecular weight	Number of residues	Number of polypeptide chains
Cytochrome *c* (human)	12,400	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (chicken egg white)	14,300	129	1
Myoglobin (equine heart)	16,700	153	1
Chymotrypsin (bovine pancreas)	25,200	241	3
Chymotrypsinogen (bovine)	25,700	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	66,000	609	1
Hexokinase (yeast)	107,900	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 3-2
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Classes of Conjugated Proteins

TABLE 3–4 Conjugated Proteins

Class	Prosthetic group	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

Table 3-4
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Proteins Can Be Separated and Purified

- Protein source is normally a tissue or cells

1. Open these cells releasing their proteins into solution (crude extract)
2. Differential centrifugation
3. Once extract or organelle fraction is ready, many techniques can be used

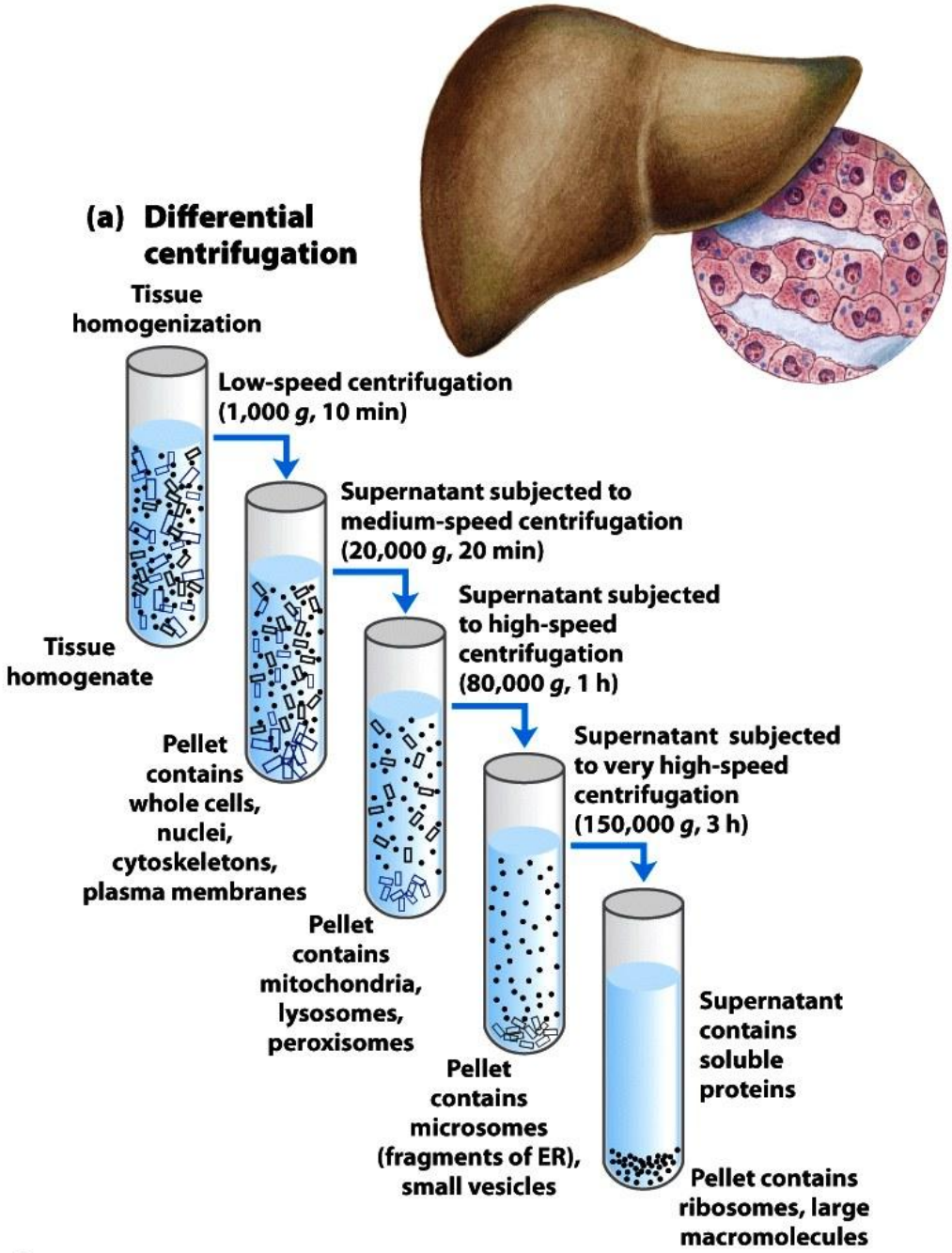

Figure 1-8
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A mixture of proteins can be separated

- Separation relies on differences in physical and chemical properties
  - Charge
  - Size
  - Affinity for a ligand
  - Solubility
  - Hydrophobicity
  - Thermal stability
- Chromatography is commonly used for preparative separation

Protein separation

- *“Salting out”:* some proteins come out of solution (precipitate) at high salt concentration (while others stay in solution). $(NH_4)_2SO_4$ is normally used
- *Dialysis:* separation of proteins from solvent because proteins are large. Proteins are put in a semi-permeable bag which is soaked in a larger volume of the correct buffer and salt concentration. Ions and buffer will equilibrate (going in) while proteins cannot go out. Can be used to remove $(NH_4)_2SO_4$

Column Chromatography

<u>Chromatography</u> is commonly used for preparative separation

- Stationary phase
- Mobile phase
- Effluent

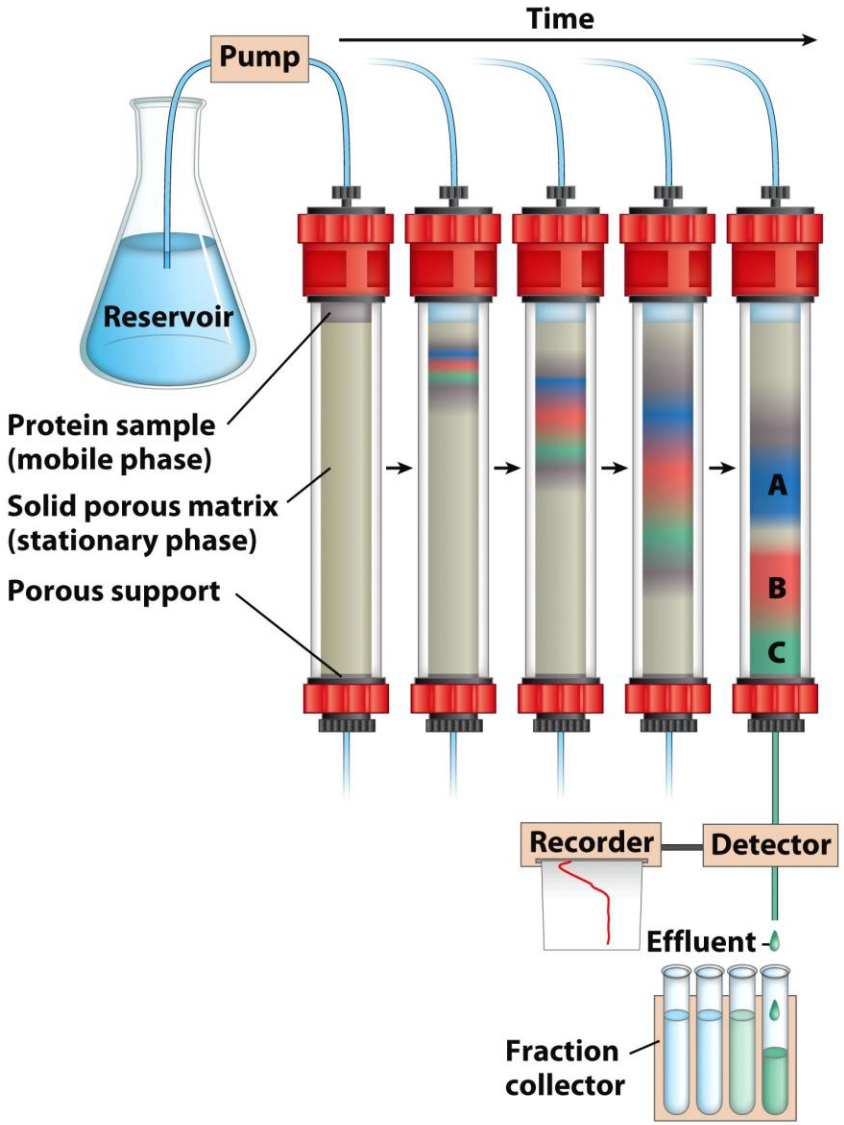

Figure 3-16
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Separation by Charge

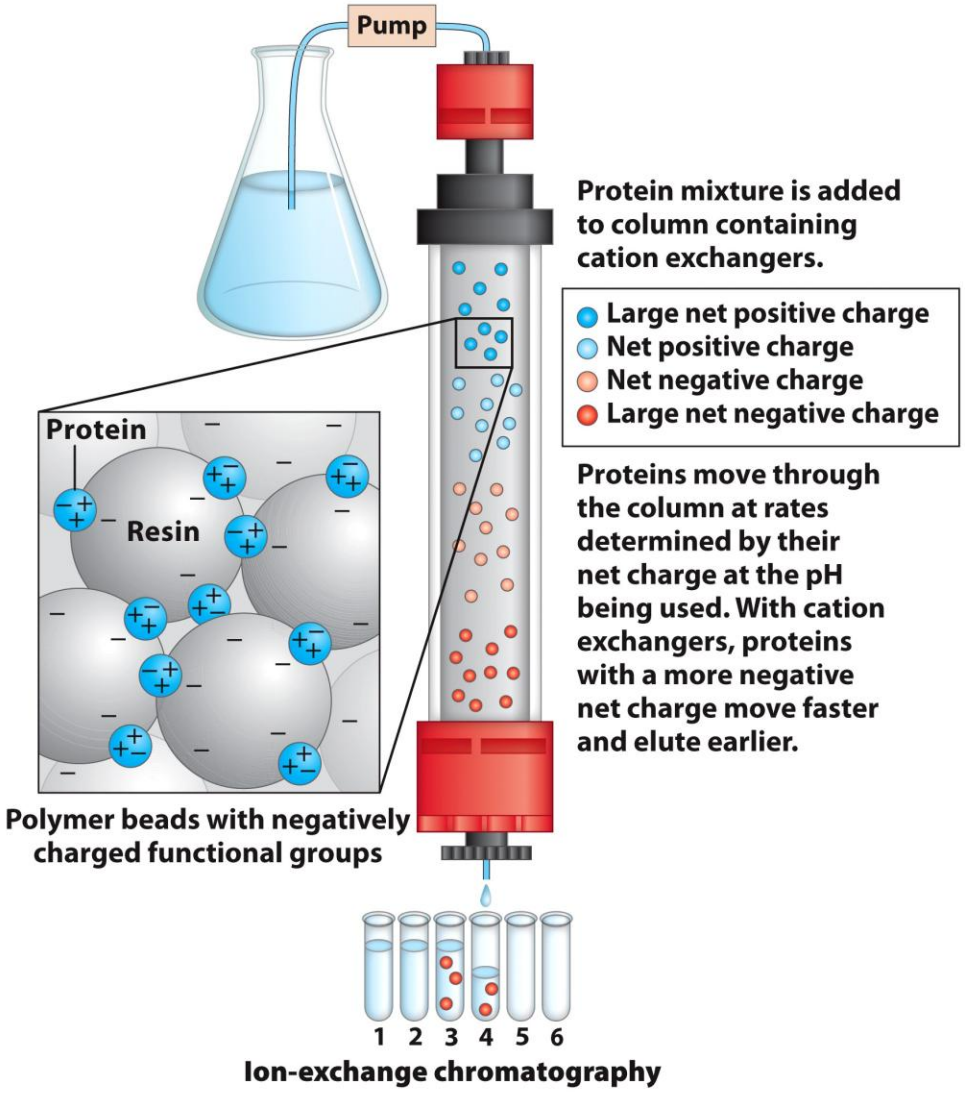

Figure 3-17a
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Separation by Size

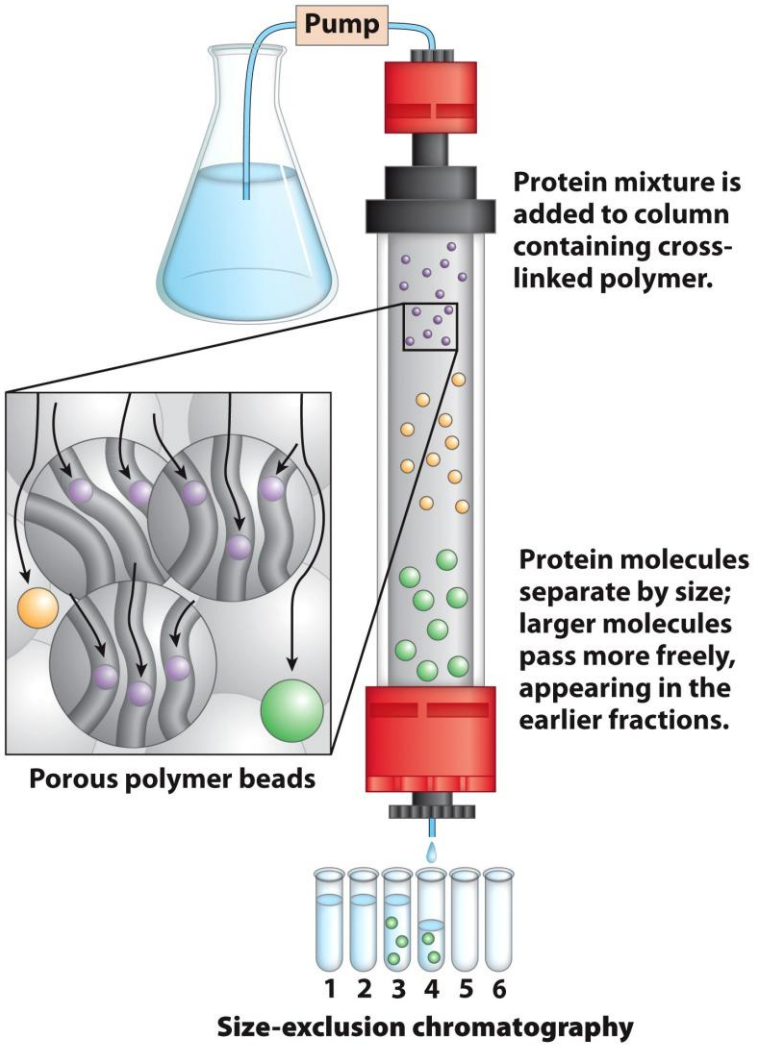

Figure 3-17b
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Separation by Affinity

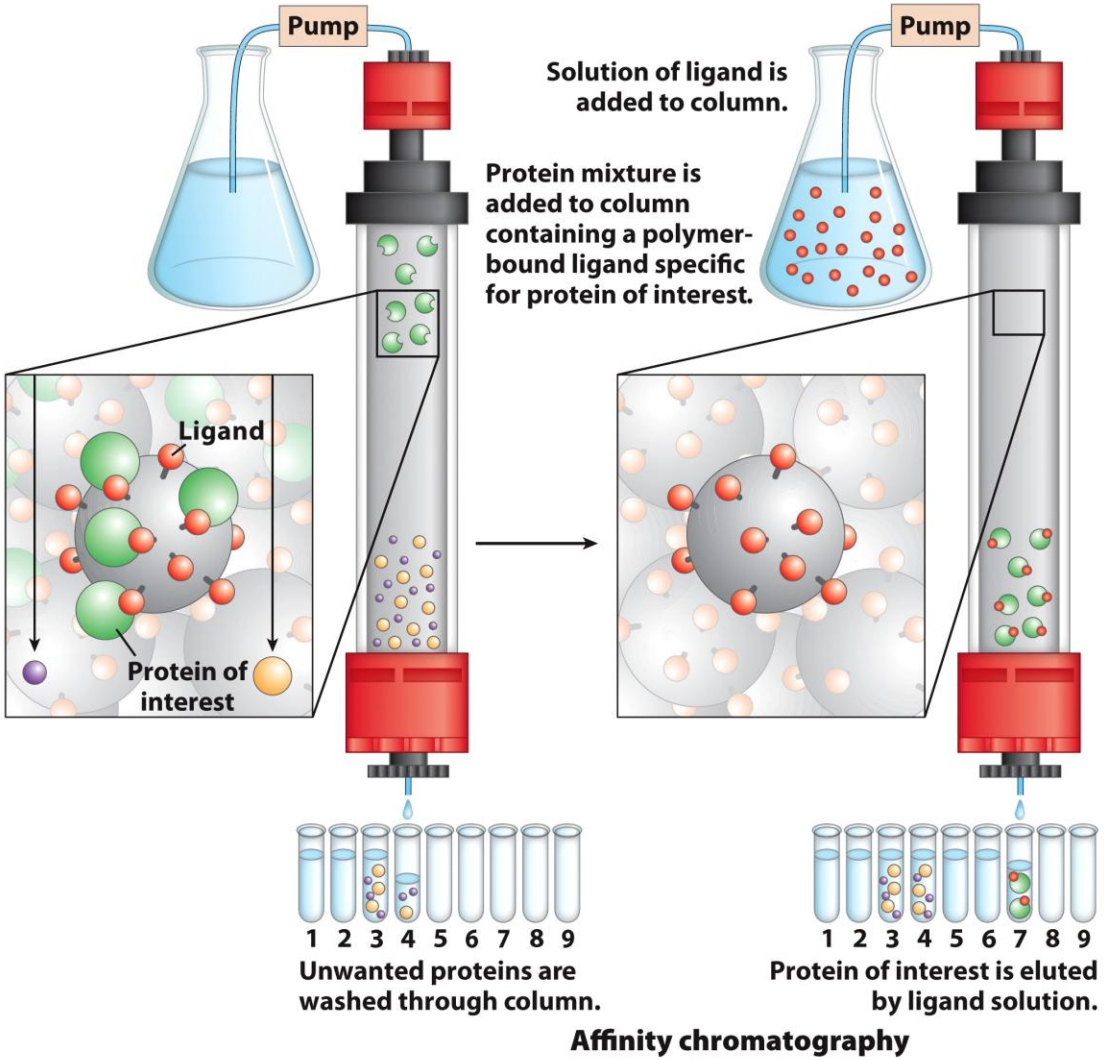

Figure 3-17c
Lehninger Principles of Biochemistry, Sixth Edition

Electrophoresis for Protein Analysis

- Separation in analytical scale is commonly done by electrophoresis
- Electric field pulls proteins according to their charge
- Gel matrix hinders mobility of proteins according to their size and shape

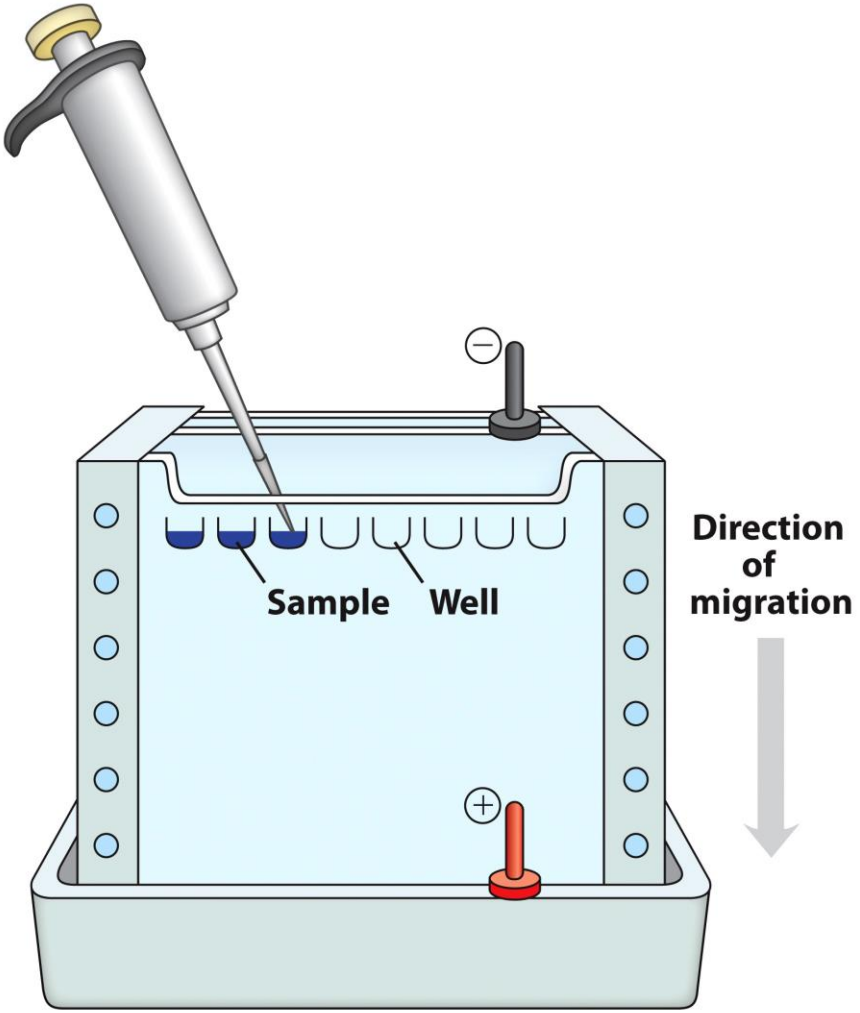

Figure 3-18a
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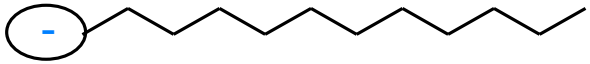
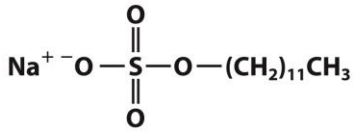
SDS PAGE: Molecular Weight

- SDS – sodium dodecyl sulfate – a detergent





Sodium dodecyl sulfate
(SDS)

- SDS micelles binds to, and unfold all the proteins
  - SDS gives all proteins an uniformly negative charge
  - The native shape of proteins does not matter
  - Rate of movement will only depend on size: small proteins will move faster

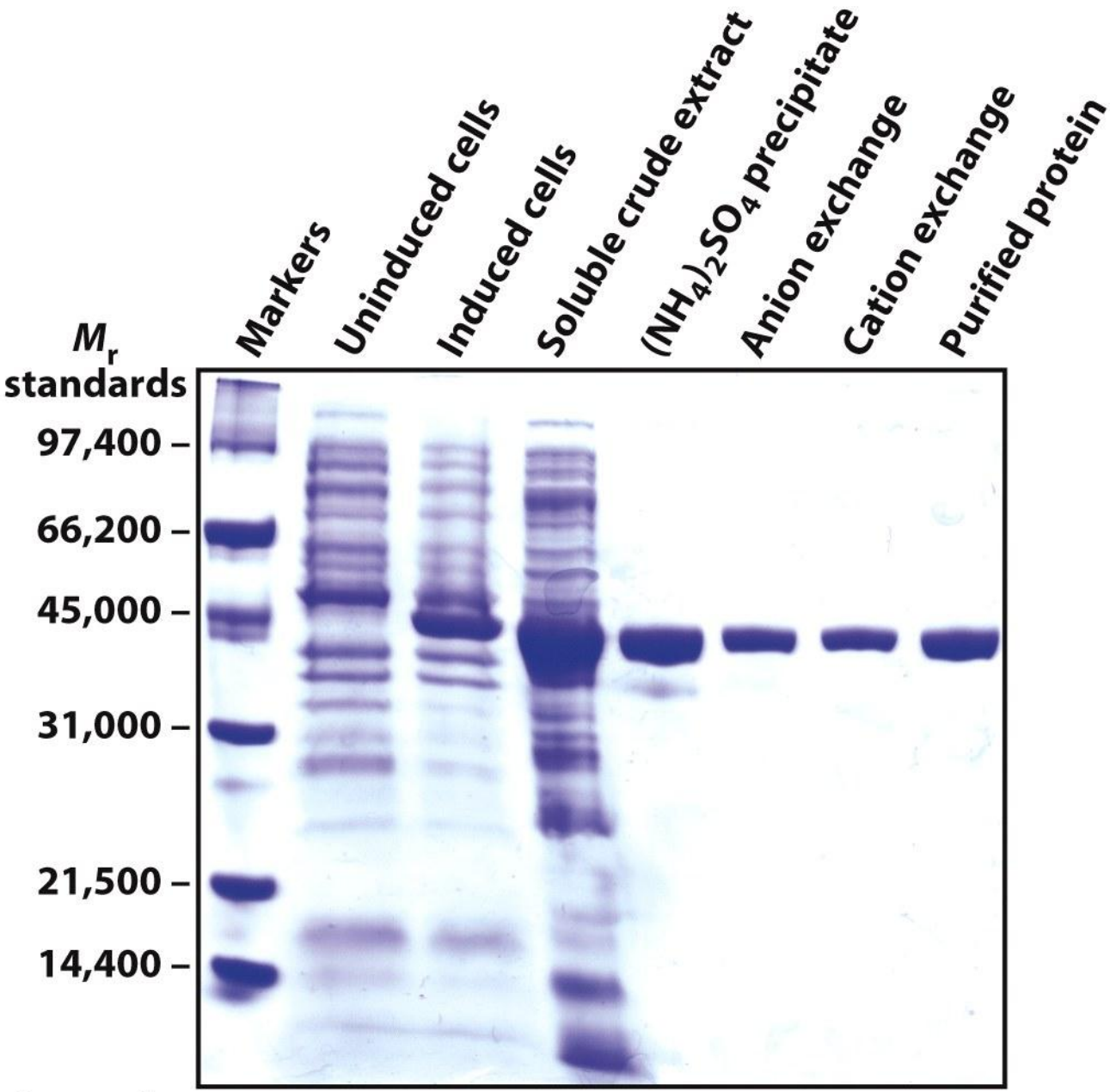


Figure 3-18b
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SDS-PAGE can be used to calculate the molecular weight of a protein

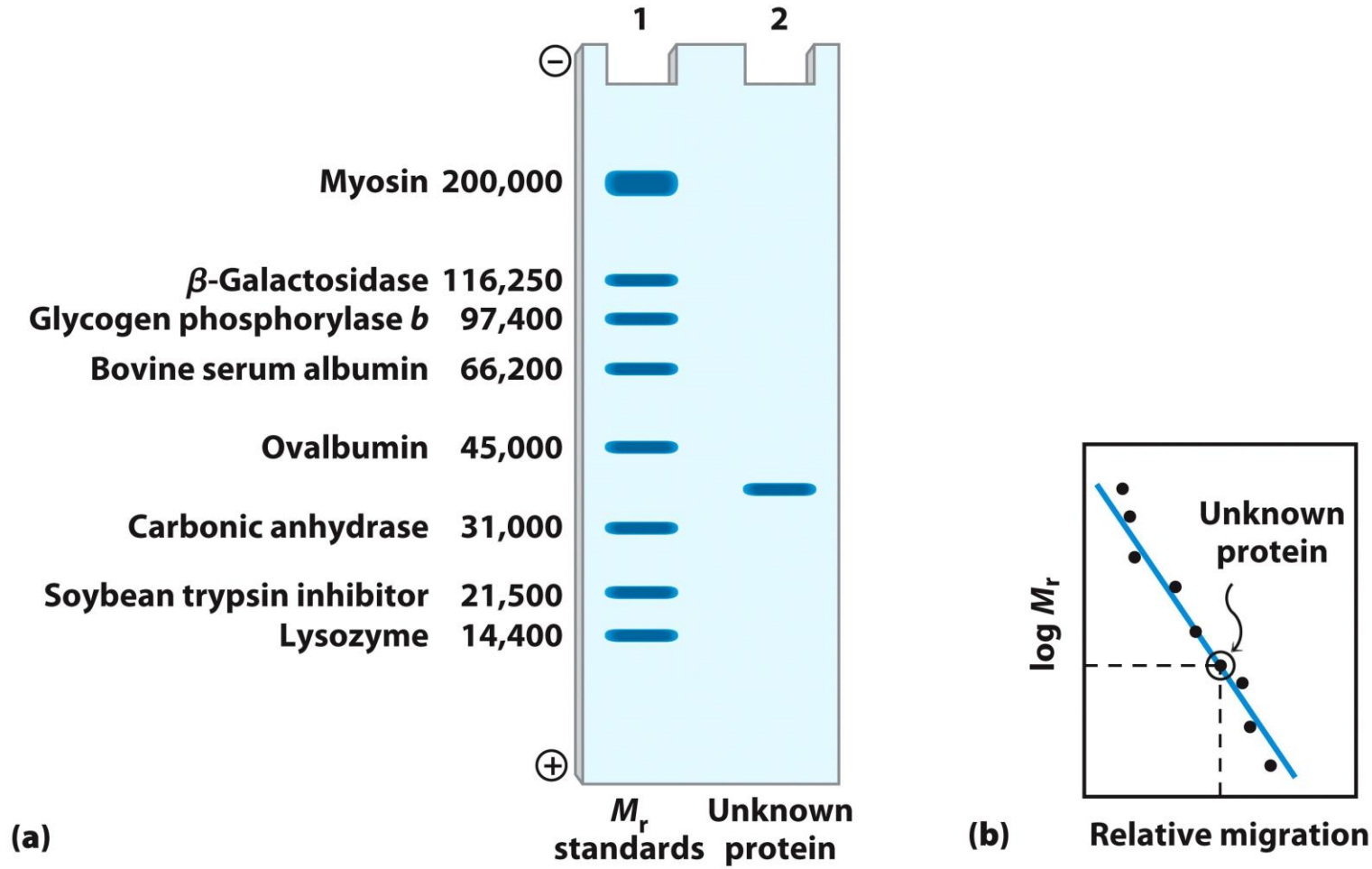

Figure 3-19
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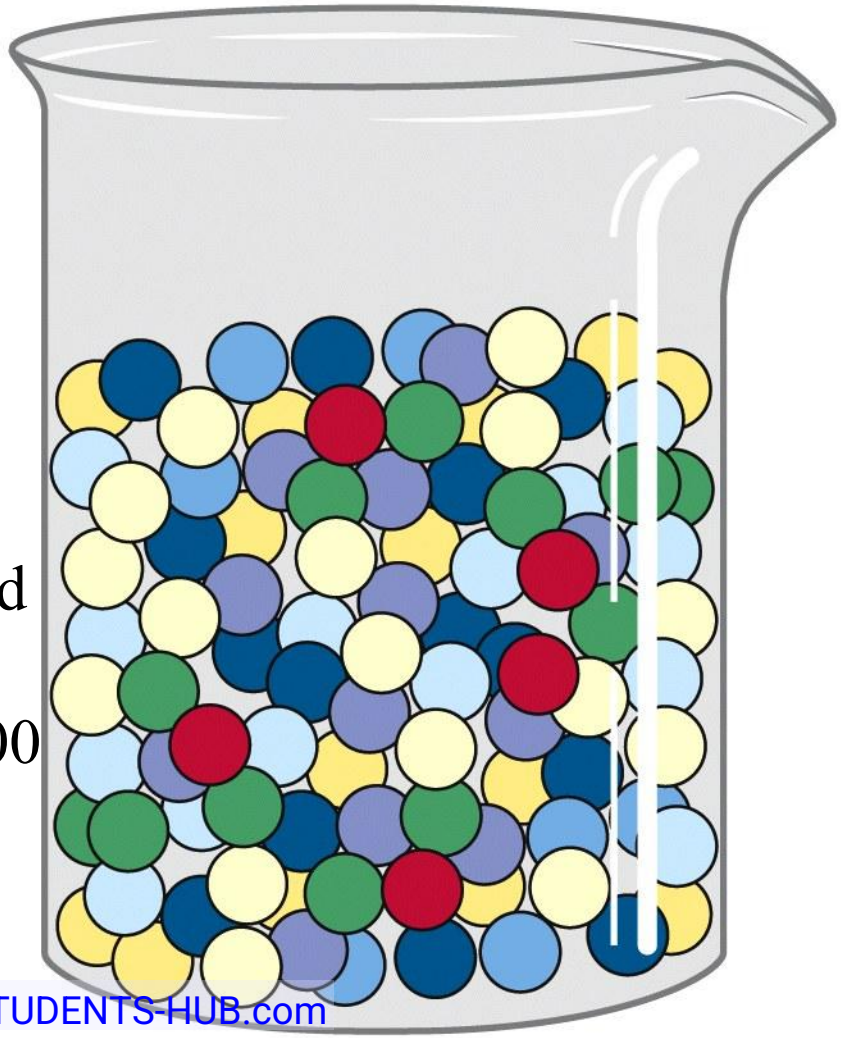
SDS Gel Electrophoresis

Specific Activity

- 1 unit of enzyme activity: amount of enzyme causing transformation of 1 μmol of substrate / min at 25 ^{o}C

- ***Activity:*** Total units of enzyme in a solution
- ***Specific Activity:*** number of enzyme units / mg of total protein
- In a purification, many steps are used
- After each step, total protein ↓ (sometimes activity ↓) but specific activity ↑

If the marbles represent proteins, both beakers contain the same *activity* of the protein represented by the red marbles.



5 red in 100

Higher *specific activity* **because red marbles represent a higher fraction of the total.**

5 red in 20

TABLE 3–5 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

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 91.

Table 3-5
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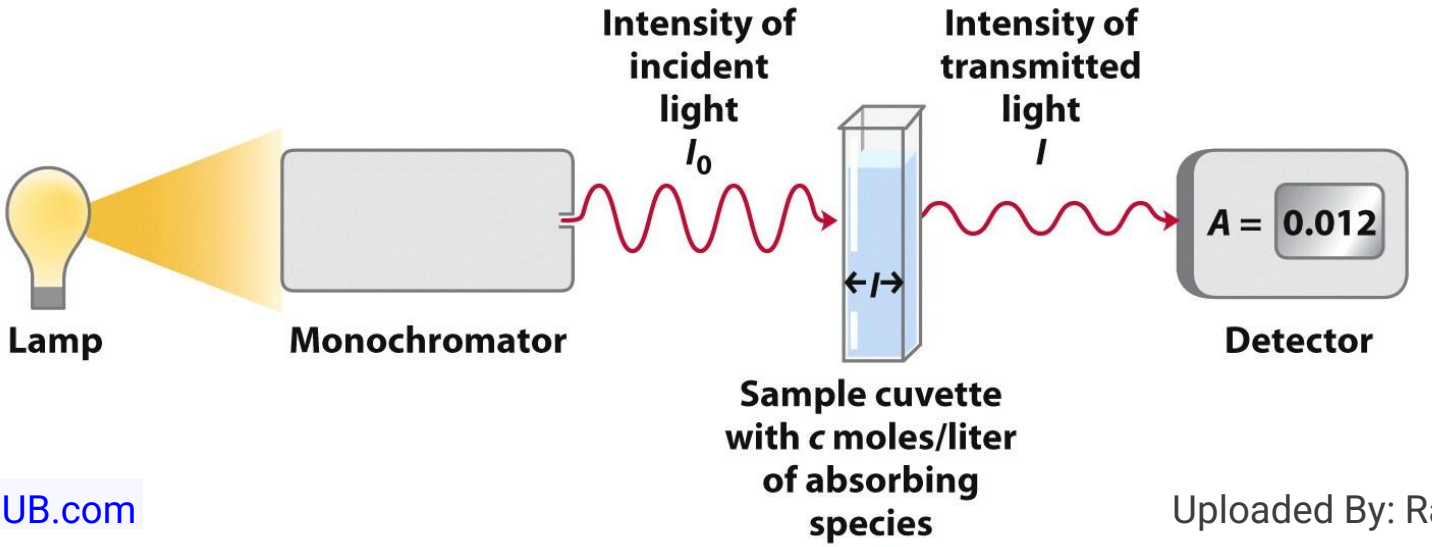
Spectroscopic Detection of Aromatic Amino Acids

- The aromatic amino acids absorb light in the UV region
- Proteins typically have UV absorbance maxima around 275-280 nm
- Tryptophan and tyrosine are the strongest chromophores
- Concentration can be determined by UV-visible spectrophotometry using Beers law: $A = \varepsilon \cdot c \cdot l$



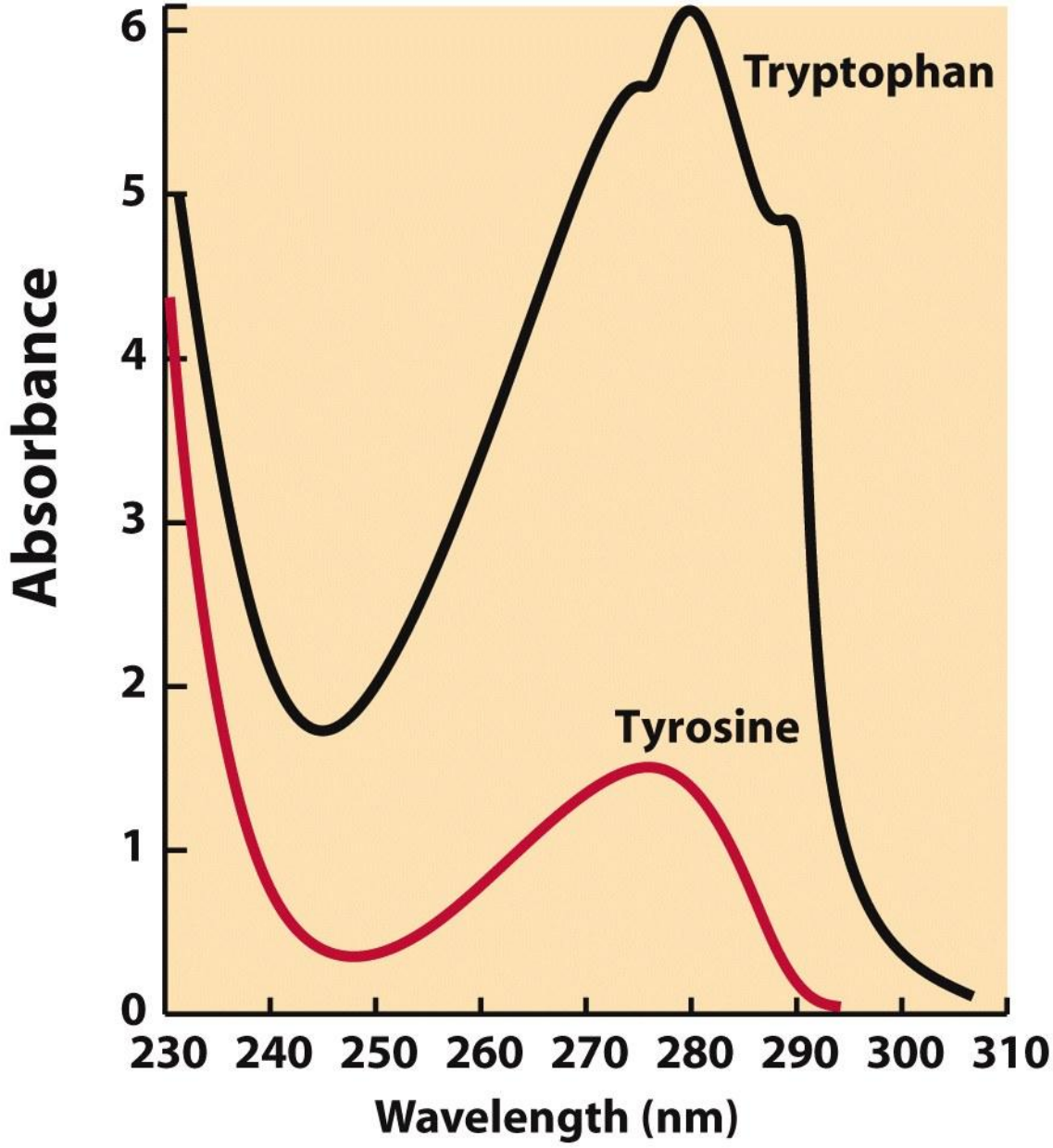


Figure 3-6
Lehninger Principles of Biochemistry, Fifth Edition

Proteases Can Be Used to Cleave Proteins

- Enzymes that catalyze the hydrolysis of peptide bonds are **proteases**
- Different kinds of proteases
- Trypsin, cleavage points: K,R (C)
  peptide: WTRCTTSRLPLKSSWSSRWSET

  will be cleaved by trypsin into:
  WTR + CTTSR + LPLK + SSWSSR + WSET

TABLE 3–7 The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

Reagent (biological source)*	Cleavage points†
Trypsin (bovine pancreas)	Lys, Arg (C)
Submaxillarus protease (mouse submaxillary gland)	Arg (C)
Chymotrypsin (bovine pancreas)	Phe, Trp, Tyr (C)
Staphylococcus aureus V8 protease (bacterium *S. aureus*)	Asp, Glu (C)
Asp-*N*-protease (bacterium *Pseudomonas fragi*)	Asp, Glu (N)
Pepsin (porcine stomach)	Leu, Phe, Trp, Tyr (N)
Endoproteinase Lys C (bacterium *Lysobacter enzymogenes*)	Lys (C)
Cyanogen bromide	Met (C)

*All reagents except cyanogen bromide are proteases. All are available from commercial sources.

†Residues furnishing the primary recognition point for the protease or reagent; peptide bond cleavage occurs on either the carbonyl (C) or the amino (N) side of the indicated amino acid residues.

Table 3-7
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Protein Sequences as Clues to Evolutionary Relationships

- Sequences of homologous proteins from a wide range of species can be aligned and analyzed for differences
- Differences indicate evolutionary divergences
- Analysis of multiple protein families can indicate evolutionary relationships between organisms, ultimately the history of life on Earth