Lehninger

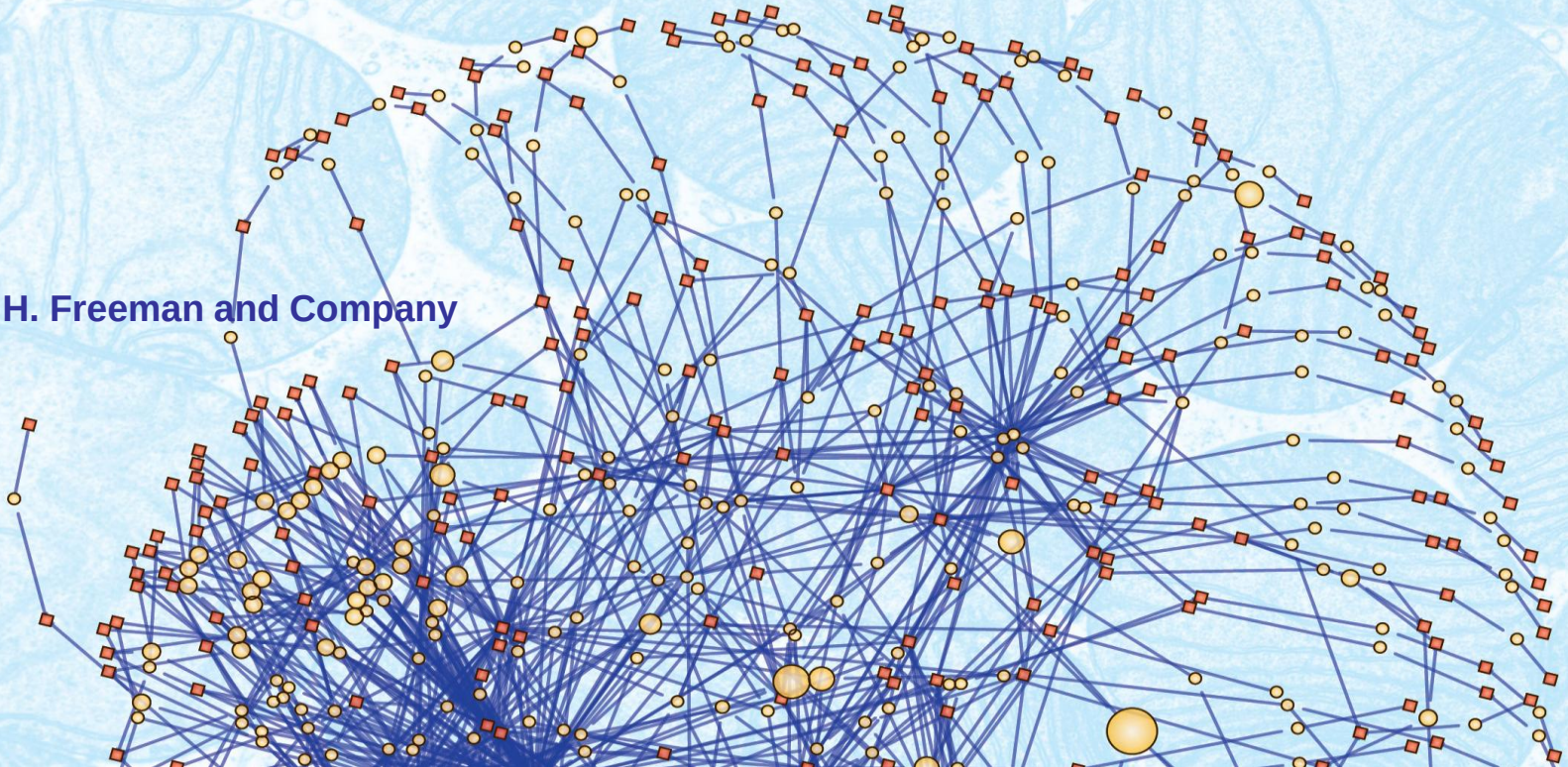
SIXTH EDITION

# Principles of Biochemistry

David L. Nelson | Michael M. Cox

## 3 | Amino Acids, Peptides, Proteins





# Proteins: Main Agents of Biological Function

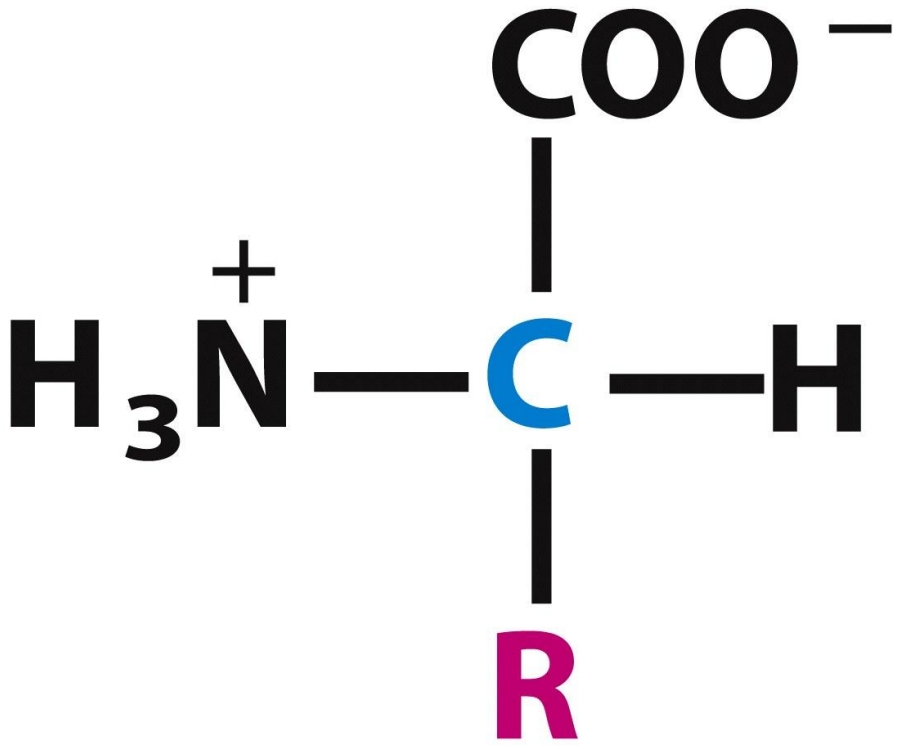
- Catalysis:
  - –enolase (in the glycolytic pathway)
  - –DNA polymerase (in DNA replication)
- Transport:
  - –hemoglobin (transports $O_2$ in the blood)
  - –lactose permease (transports lactose across the cell membrane)
- Structure:
  - –collagen (connective tissue)
  - –keratin (hair, nails, feathers,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

**Figure 3-1c**
***Lehninger Principles of Biochemistry, Fifth Edition***

# Amino Acids: Building Blocks of Protein

- Proteins are heteropolymers of $\alpha$-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



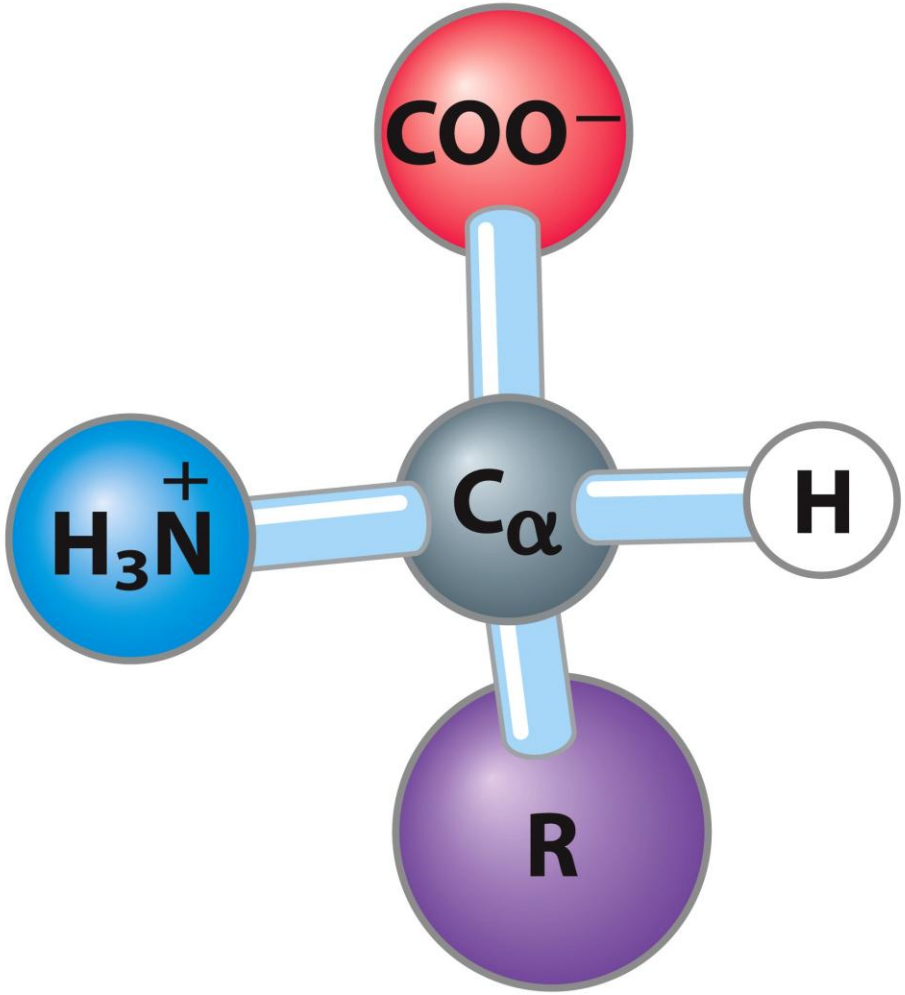




**Figure 3-2**
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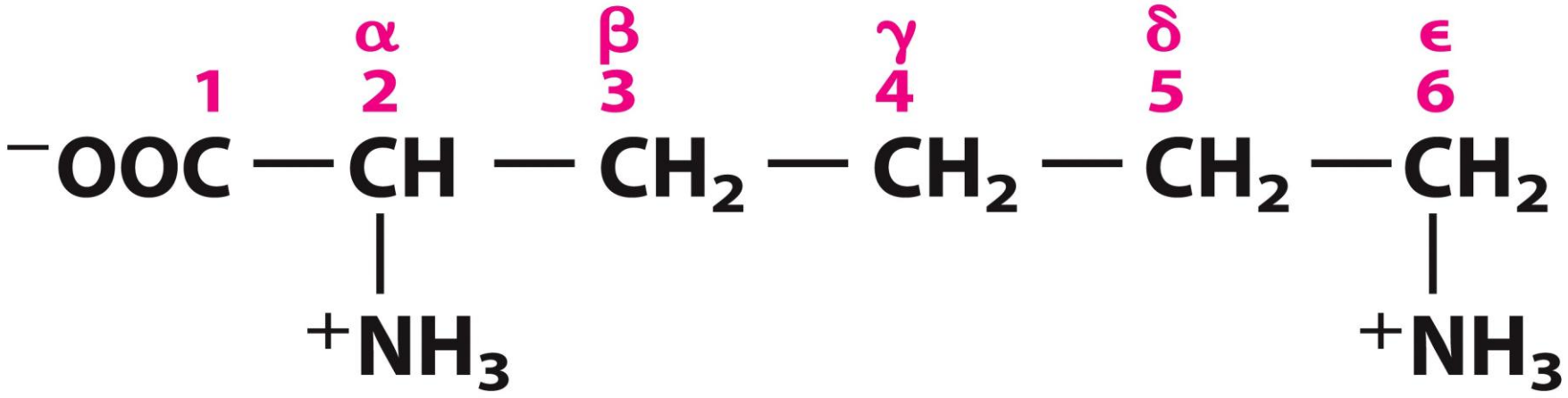
# Amino Acids: Atom Naming

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




**Lysine**

**Unnumbered 3 p78**

*Lehninger Principles of Biochemistry*, Sixth Edition

# 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

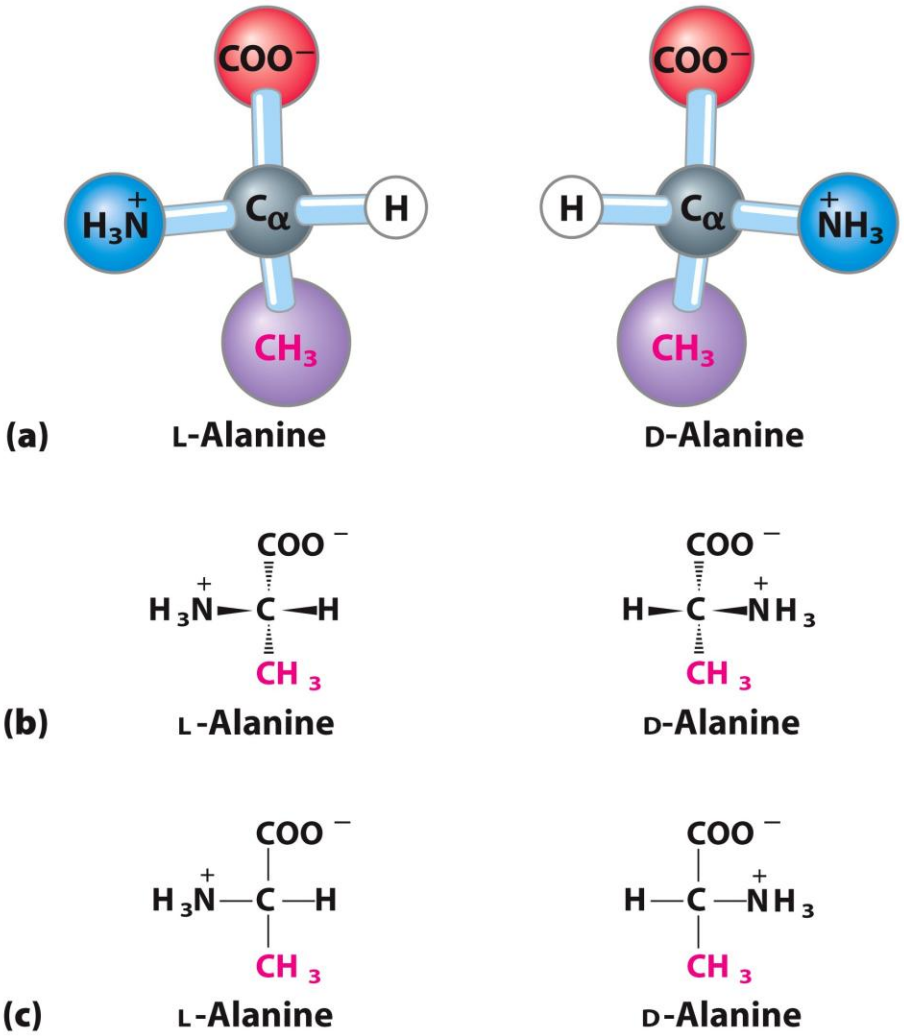



**Figure 3-3**
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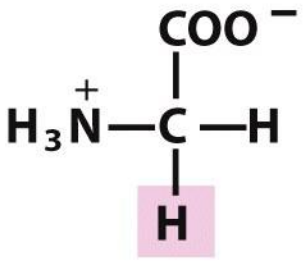
# Amino Acids: Classification

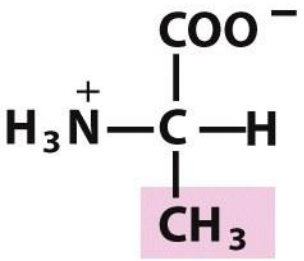
Common amino acids can be placed in five basic groups depending on their R substituents:

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

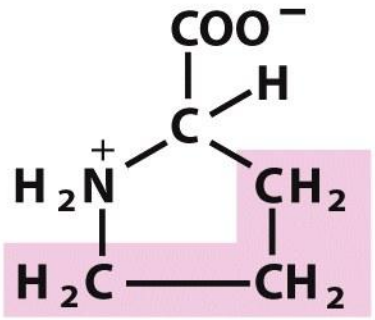
# Nonpolar, aliphatic R groups



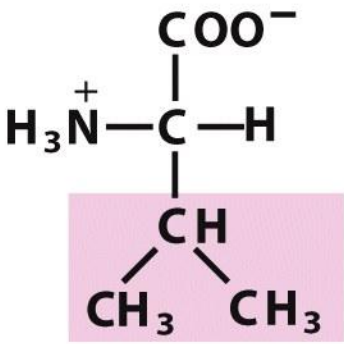
Glycine



Alanine

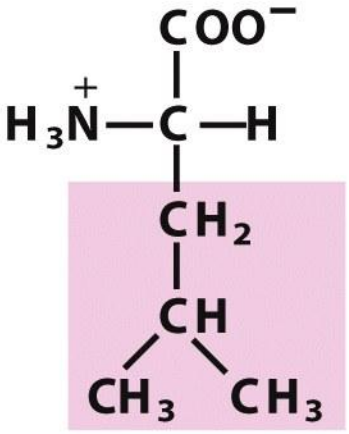


Proline

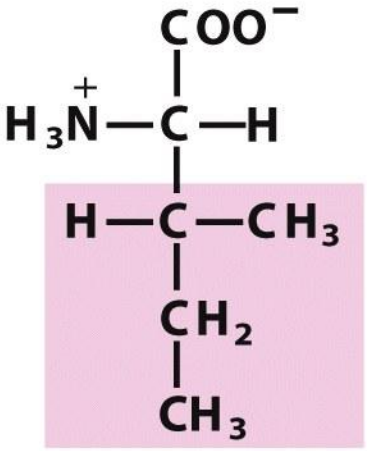


Valine

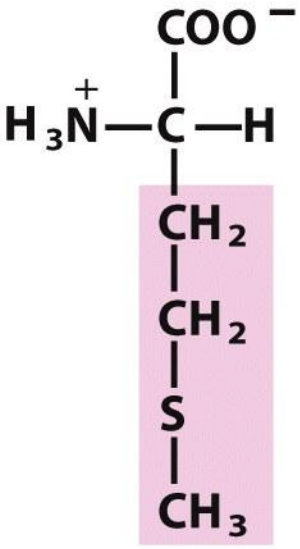
These amino acid side chains are ***hydrophobic***



Leucine



Isoleucine



Methionine

**Figure 3-5 part 1**
***Lehninger Principles of Biochemistry, Fifth Edition***

# Aromatic R groups

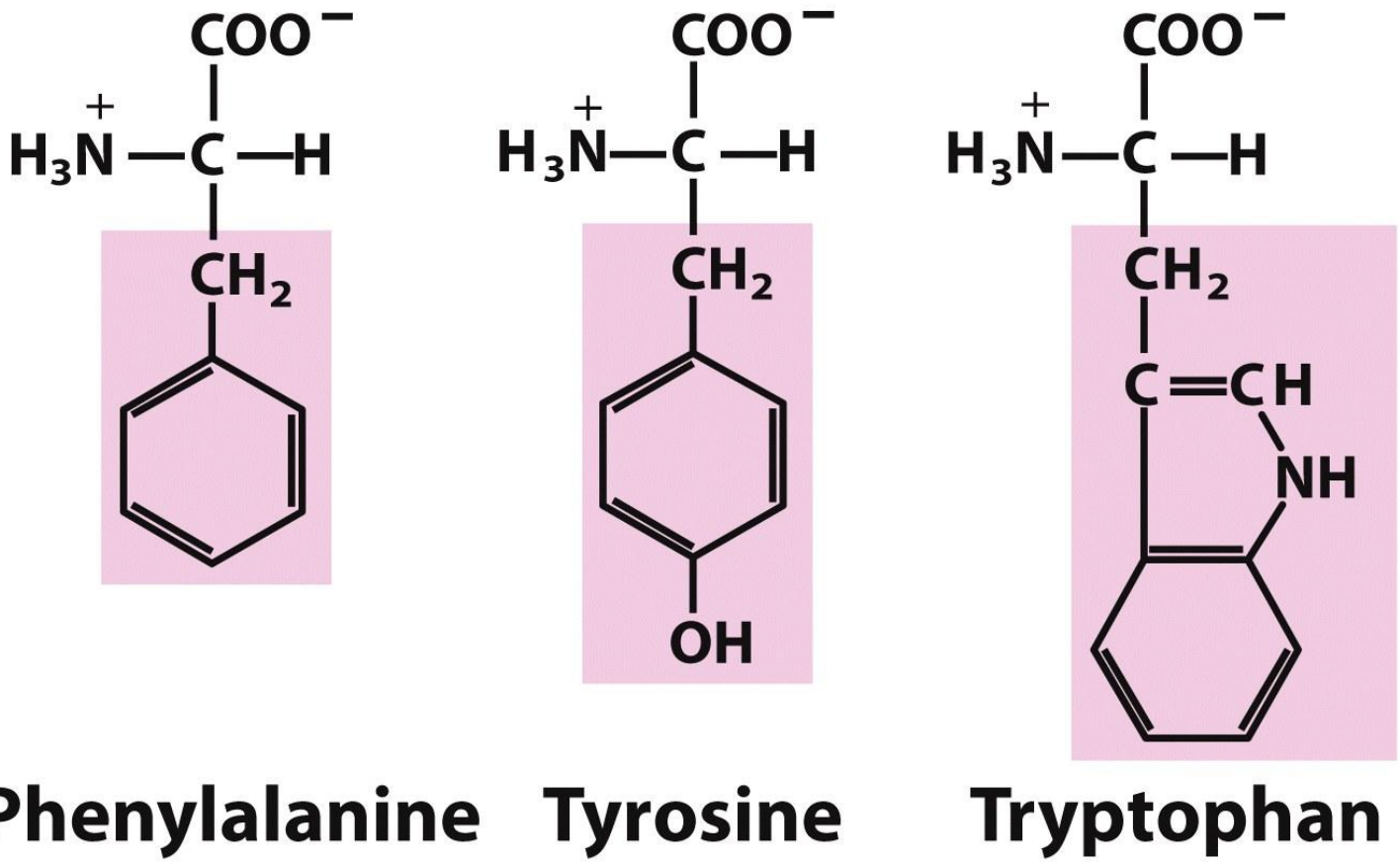



**Figure 3-5 part 2**
***Lehninger Principles of Biochemistry, Fifth Edition***


These amino acid side chains ***absorb UV light at 270-280 nm***

# Positively charged R groups

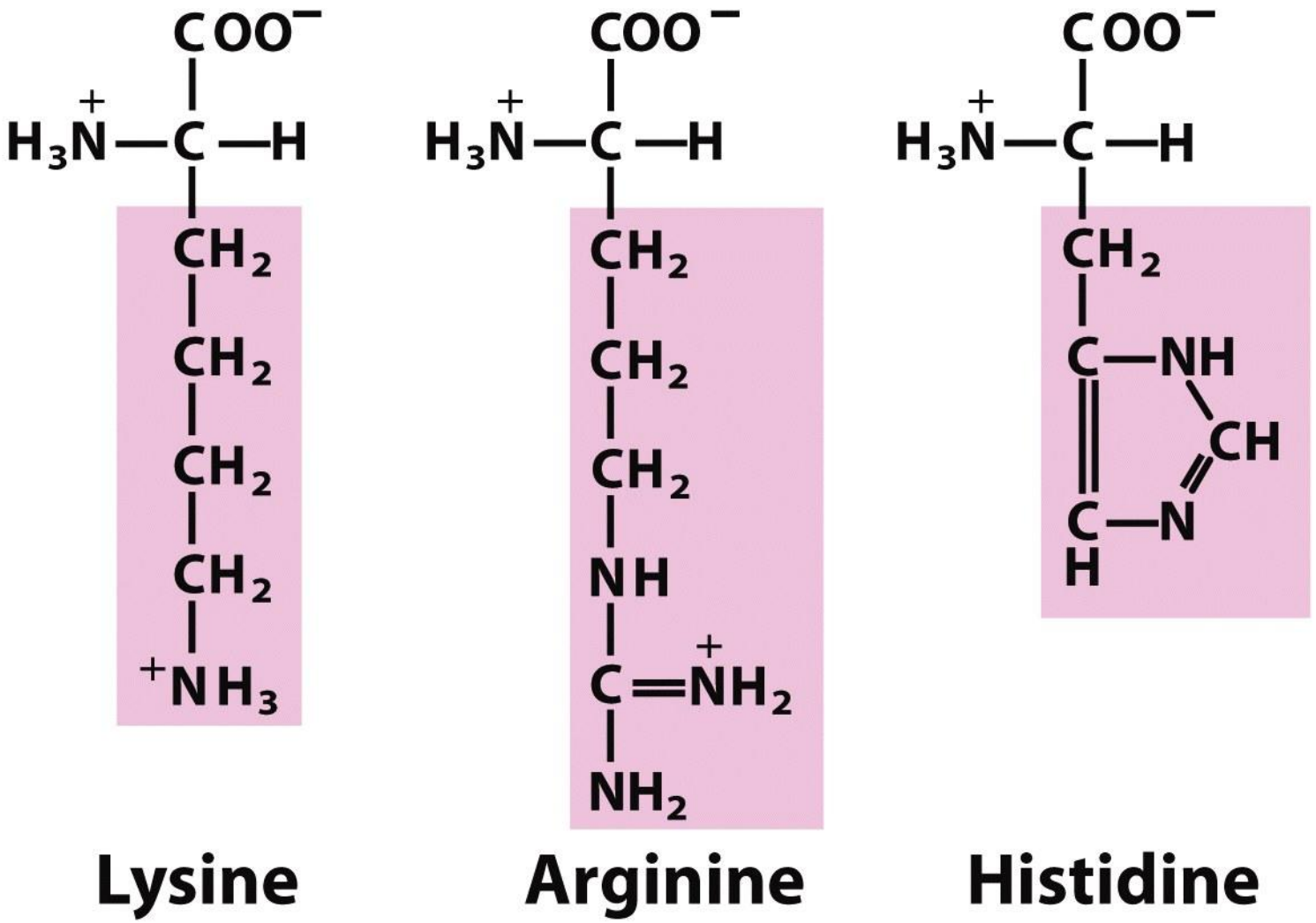



Figure 3-5 part 4
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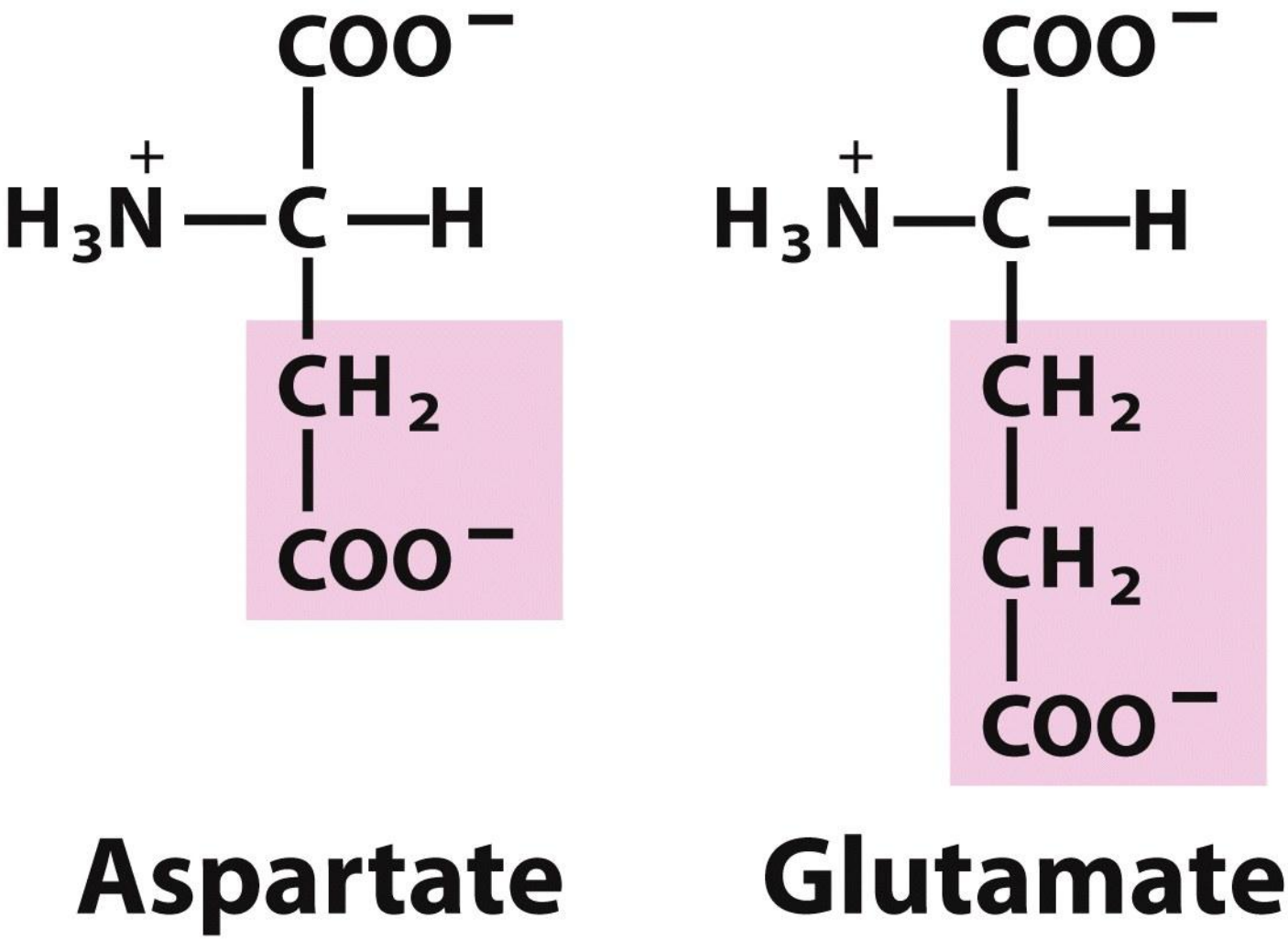
These are ***basic amino acids***

# Negatively charged R groups

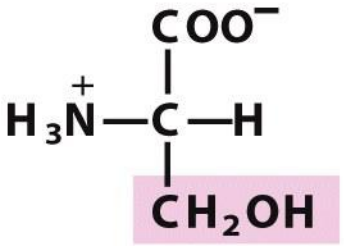



**Aspartate** **Glutamate**

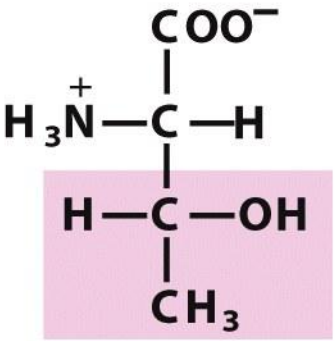
**Figure 3-5 part 5**
***Lehninger Principles of Biochemistry, Fifth Edition***


These are ***acidic amino acids***

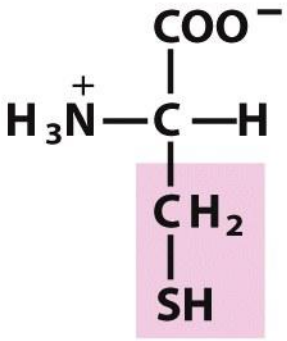
# Polar, uncharged R groups



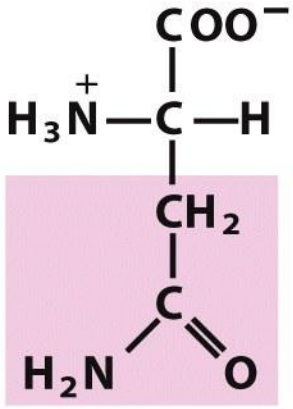
Serine



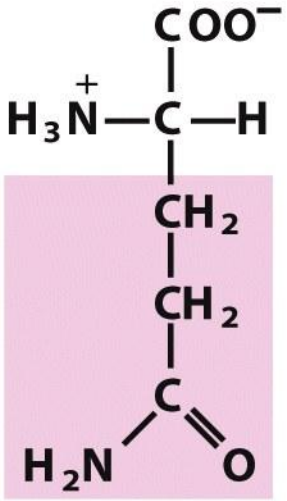
Threonine



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.

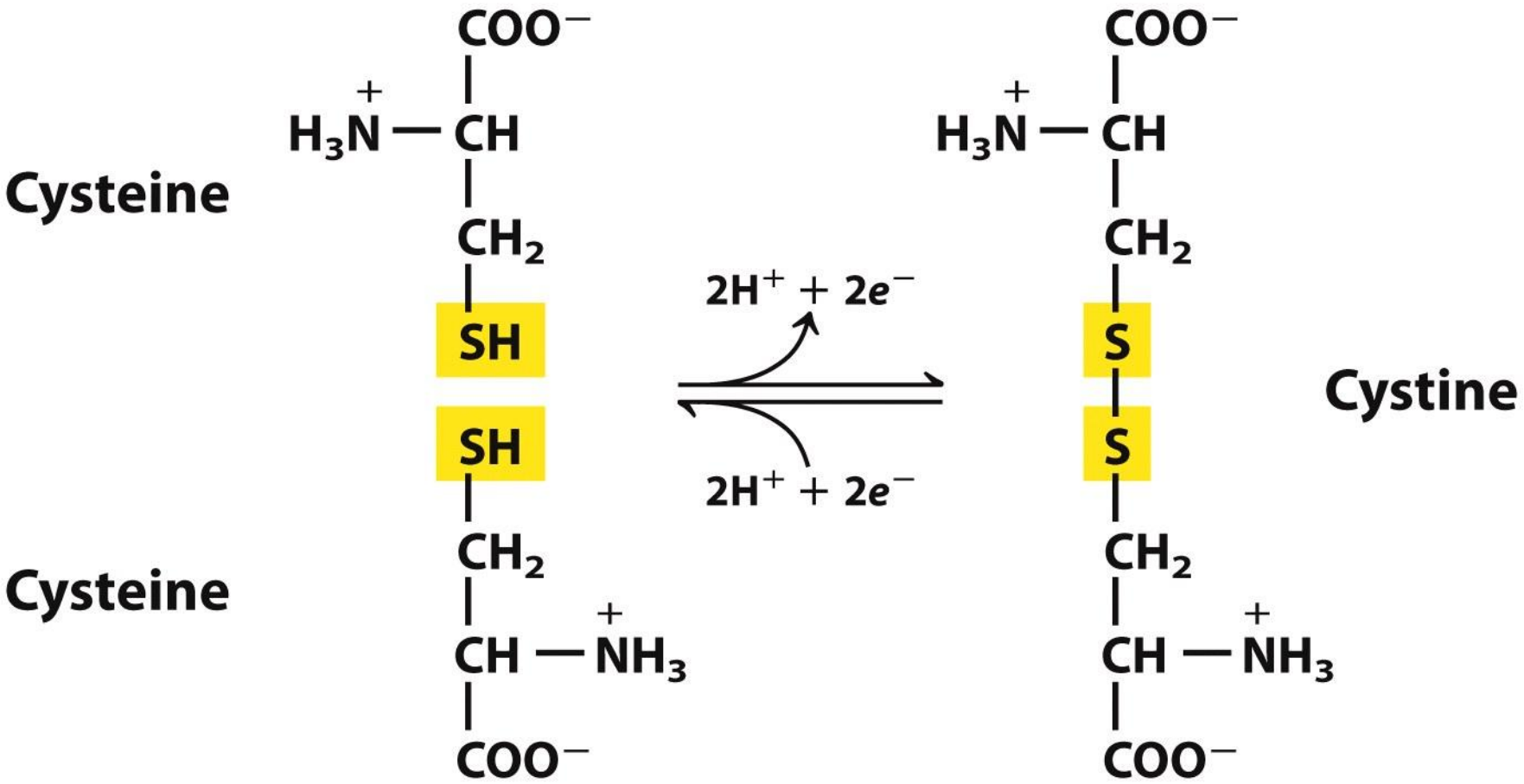



**Figure 3-7**
***Lehninger Principles of Biochemistry, Fifth Edition***

# 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

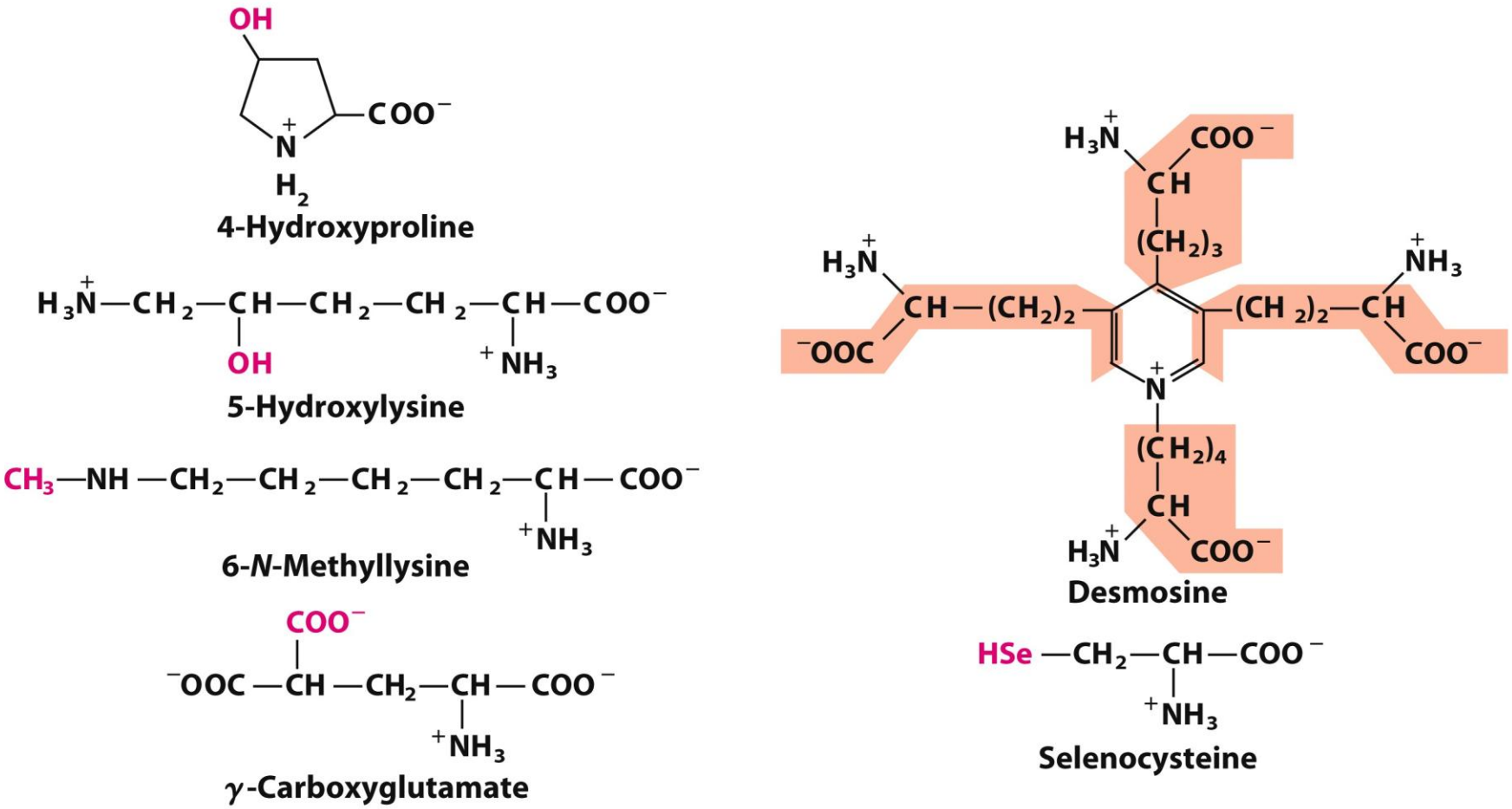
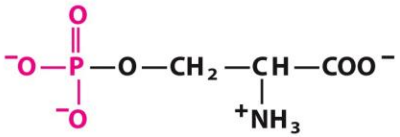
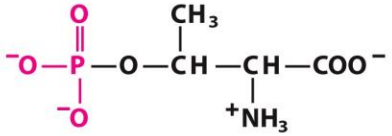



**Figure 3-8a**
*Lehninger Principles of Biochemistry*, Sixth Edition

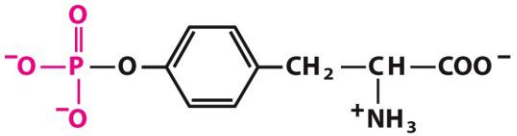
# Reversible Modifications of Amino Acids



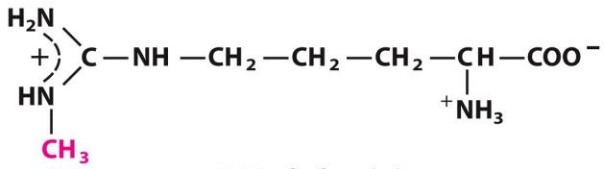
Phosphoserine



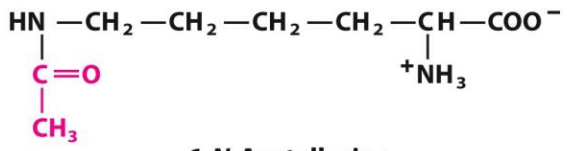
Phosphothreonine



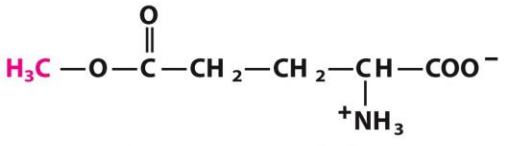
Phosphotyrosine



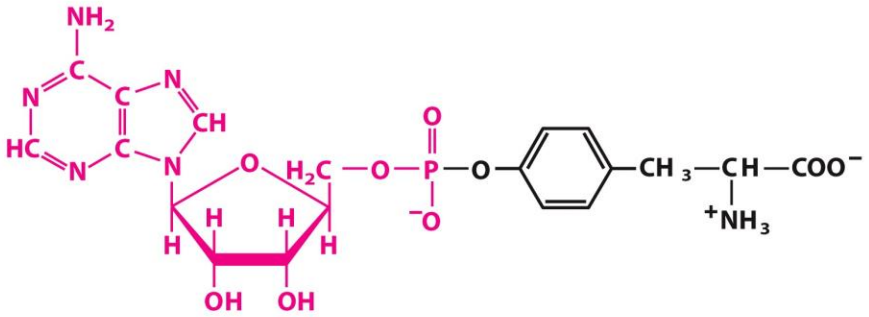
σ-*N*-Methylarginine



6-*N*-Acetyllysine



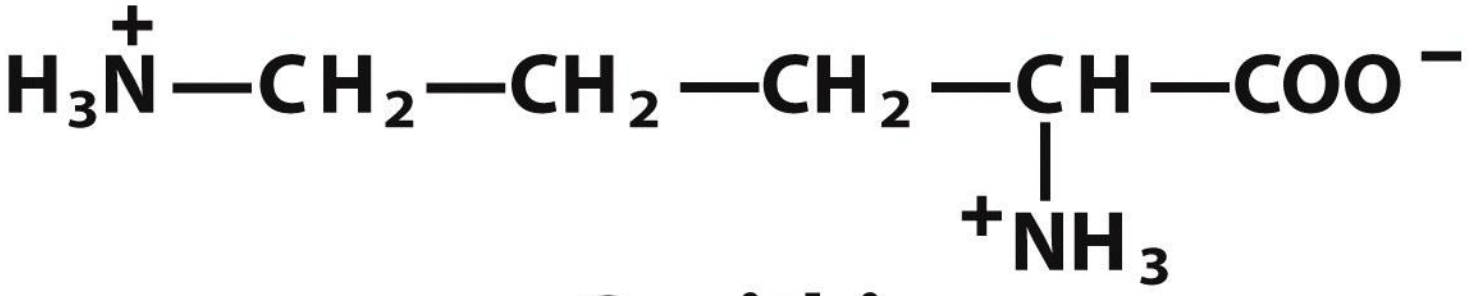
Glutamate γ-methyl ester



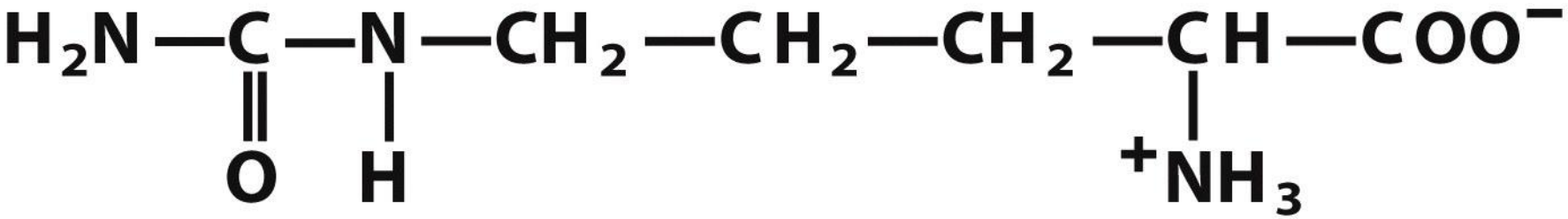
Adenylyltyrosine

**Figure 3-8b**
*Lehninger Principles of Biochemistry*, Sixth Edition

# Important Amino Acids in Urea Metabolism



**Ornithine**

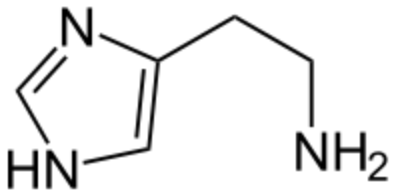


**Citrulline**

**Figure 3-8c**
***Lehninger Principles of Biochemistry, Fifth Edition***

# A Derivative of amino acids

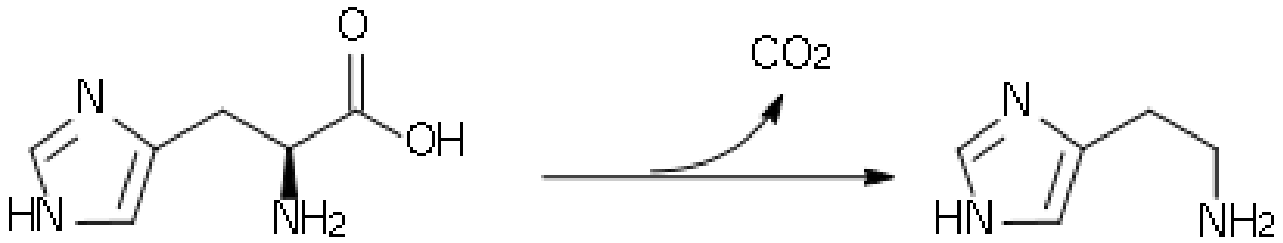
• **Histamine** is an organic nitrogen compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter.




• 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.

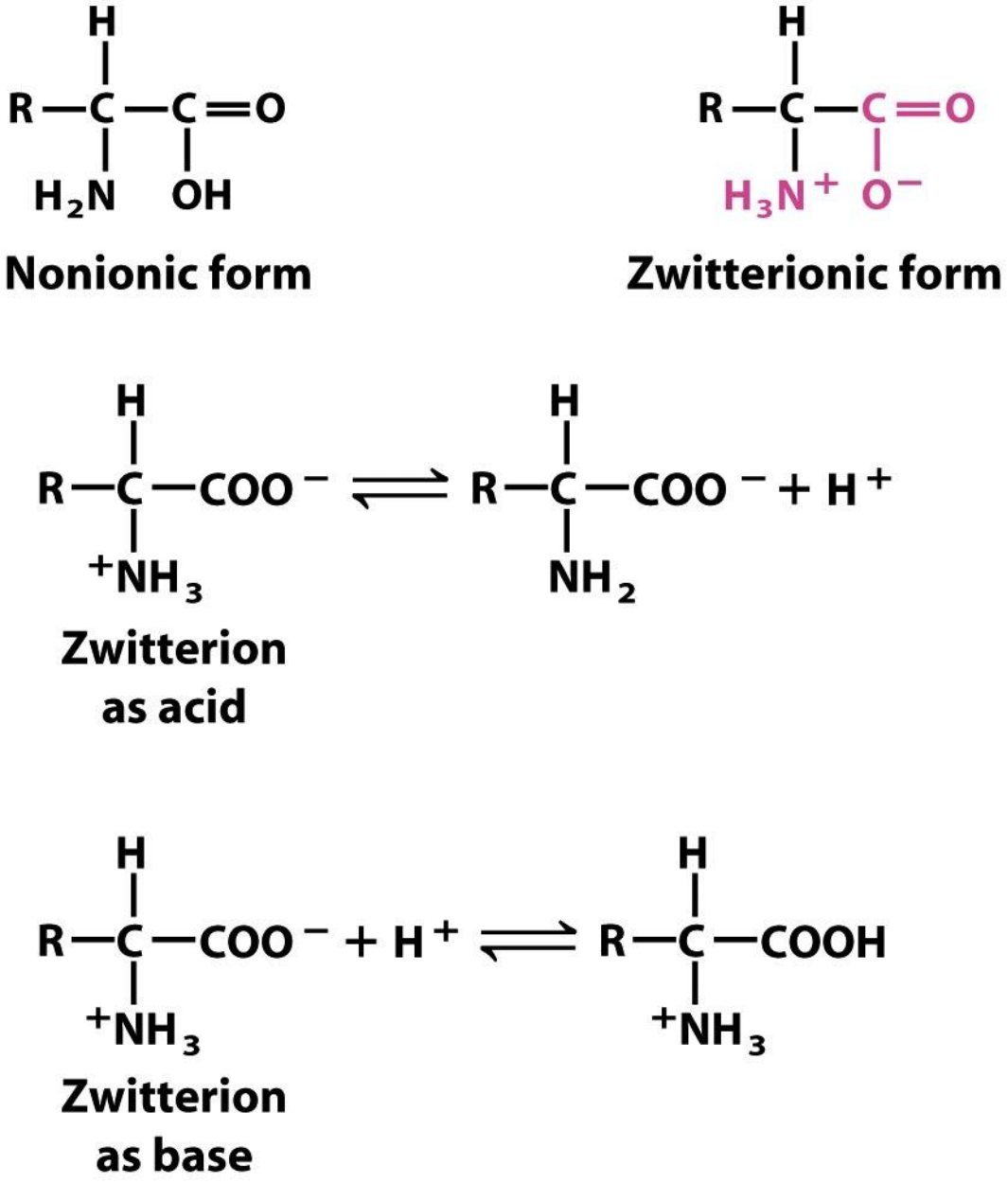



**Figure 3-9**
***Lehninger Principles of Biochemistry, Fifth Edition***

# Cation → Zwitterion → Anion

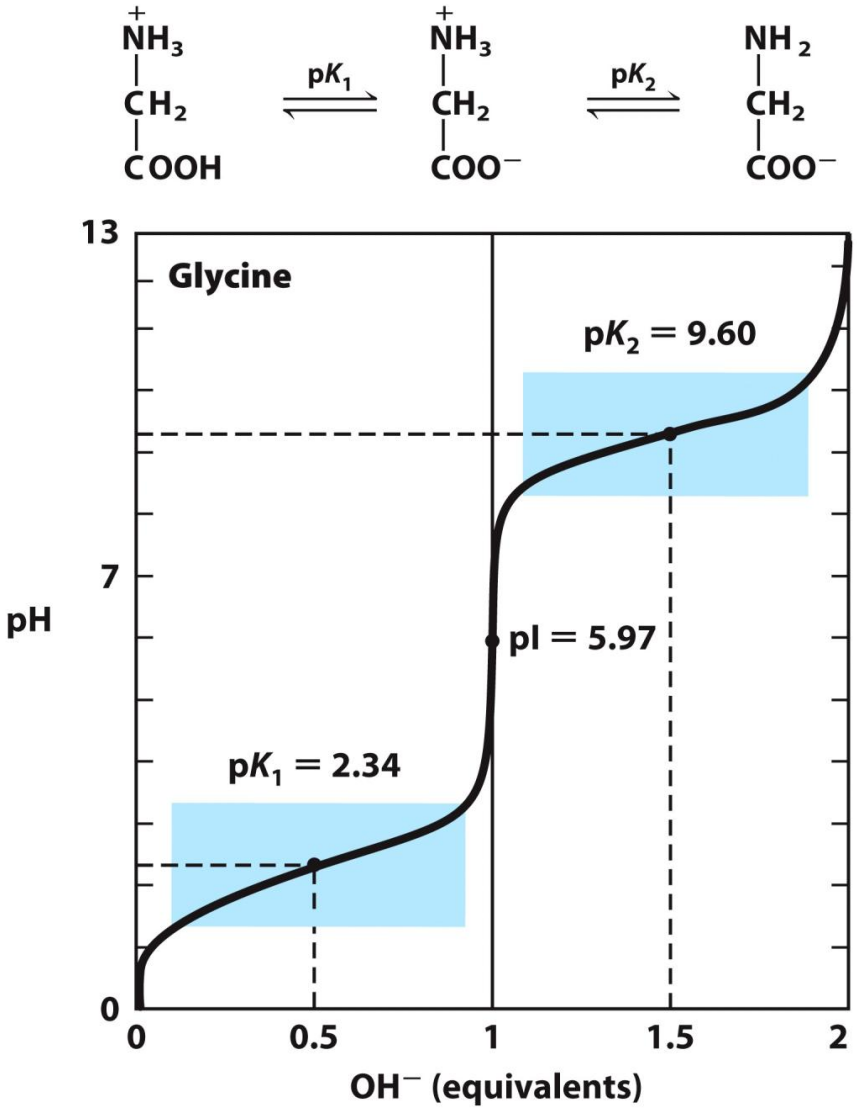



**Figure 3-10**
*Lehninger Principles of Biochemistry*, Sixth Edition

# Chemical Environment Affects p$K_a$ Values

$\alpha$-carboxy group is much more acidic than in carboxylic acids

$\alpha$-amino group is slightly less basic than in amines

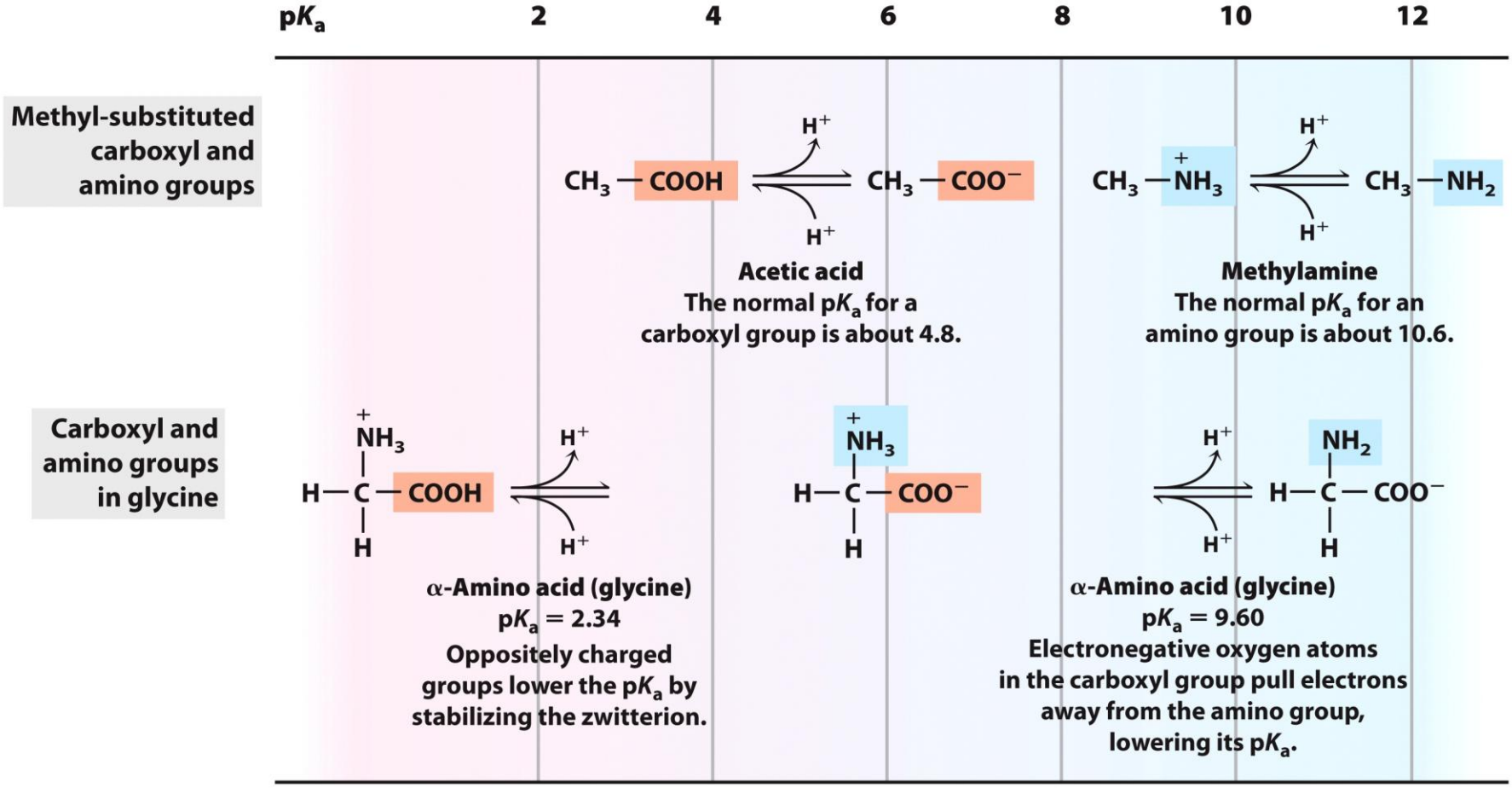



**Figure 3-11**

*Lehninger Principles of Biochemistry*, Sixth Edition

# Amino acids can act as buffers

Amino acids with uncharged side chains, such as glycine, have two p$K_a$ values:

The p$K_a$ of the $\alpha$-carboxyl group is 2.34

The p$K_a$ of the $\alpha$-amino group is 9.6

It can act as a buffer in two pH regimes.

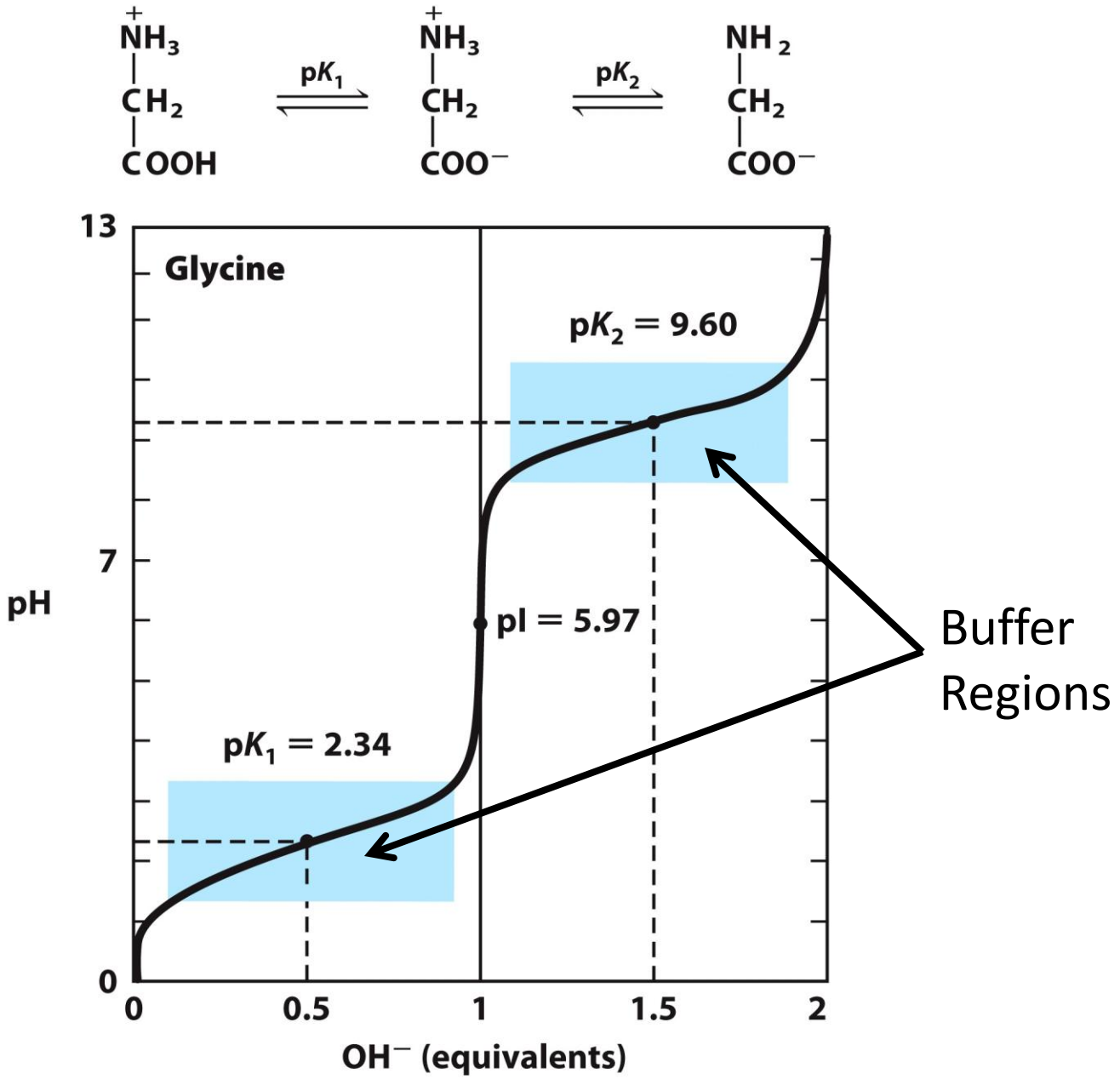



**Figure 3-10**
*Lehninger Principles of Biochemistry*, Sixth Edition

# Amino acids carry a net charge of zero at a specific pH (the pI)

- Zwitterions predominate at pH values between the p$K_a$ 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
- p$K_a$ values are discernable if two p$K_a$ values are more than two pH units apart

*Why is the side-chain p$K_a$ so much higher?*

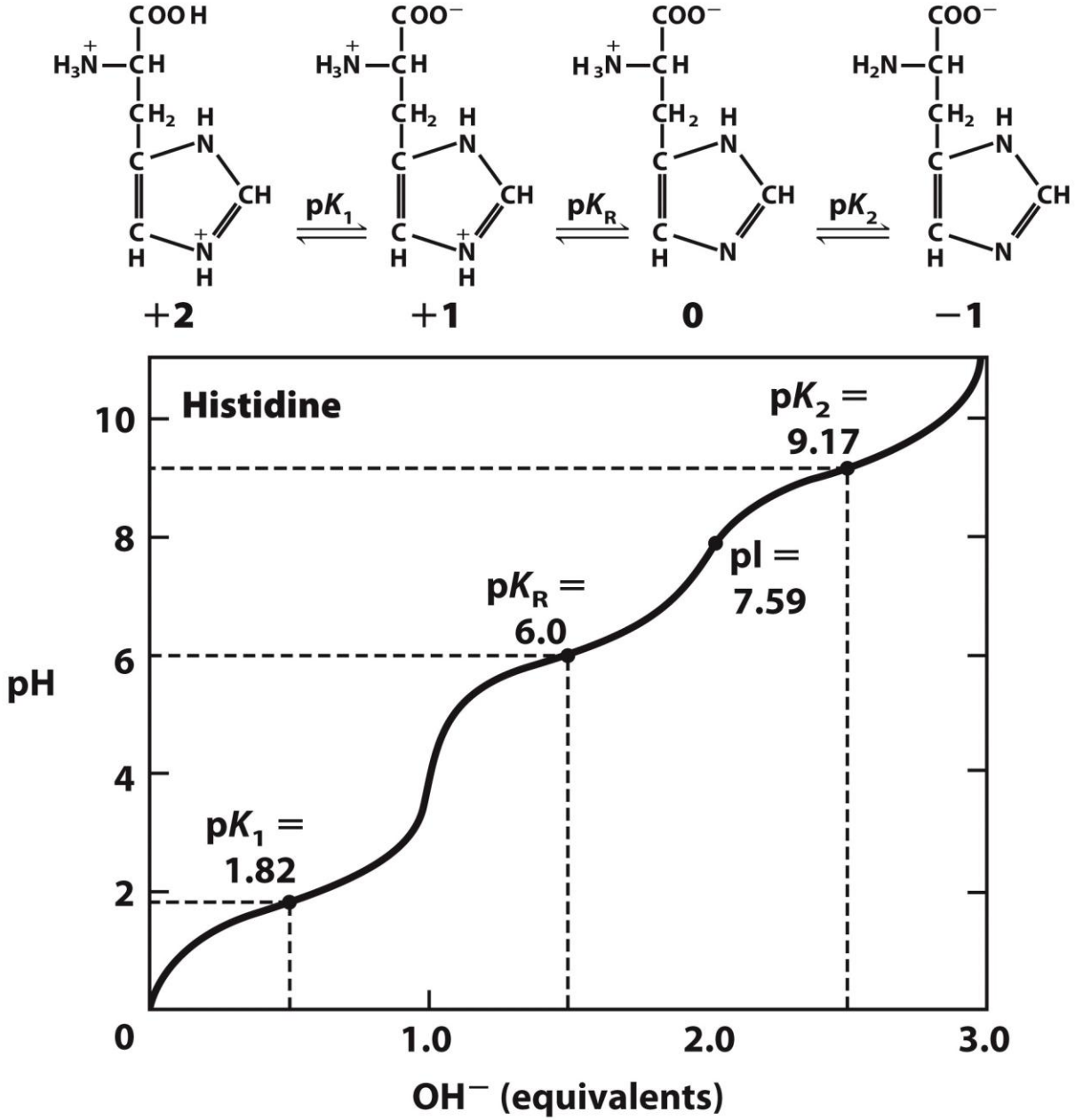


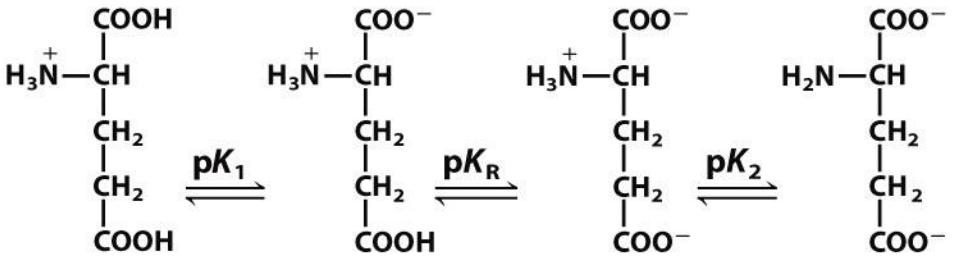

**Figure 3-12b**
*Lehninger Principles of Biochemistry*, Sixth Edition

# 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?*

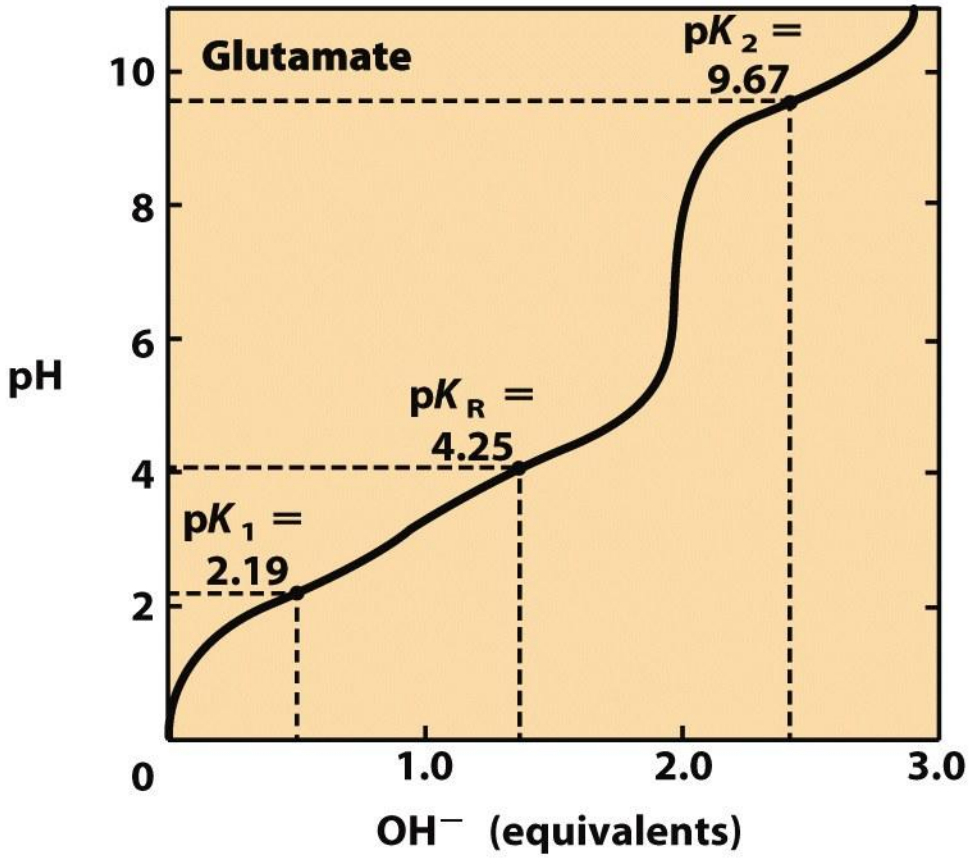



**Figure 3-12a**
*Lehninger Principles of Biochemistry, Fifth Edition*

# Formation of Peptides

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

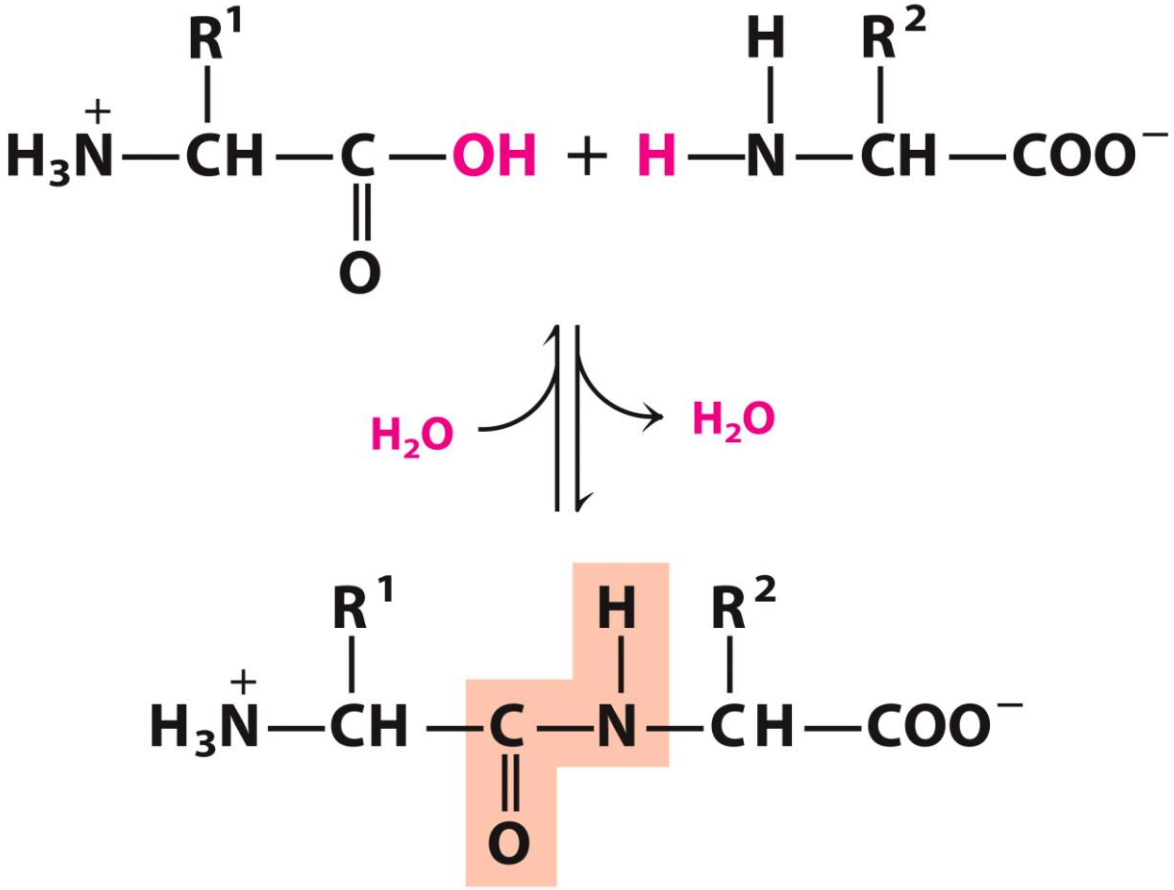



**Figure 3-13**
*Lehninger Principles of Biochemistry*, Sixth Edition

# Peptide ends are not the same

Numbering (and naming) starts from the amino terminus

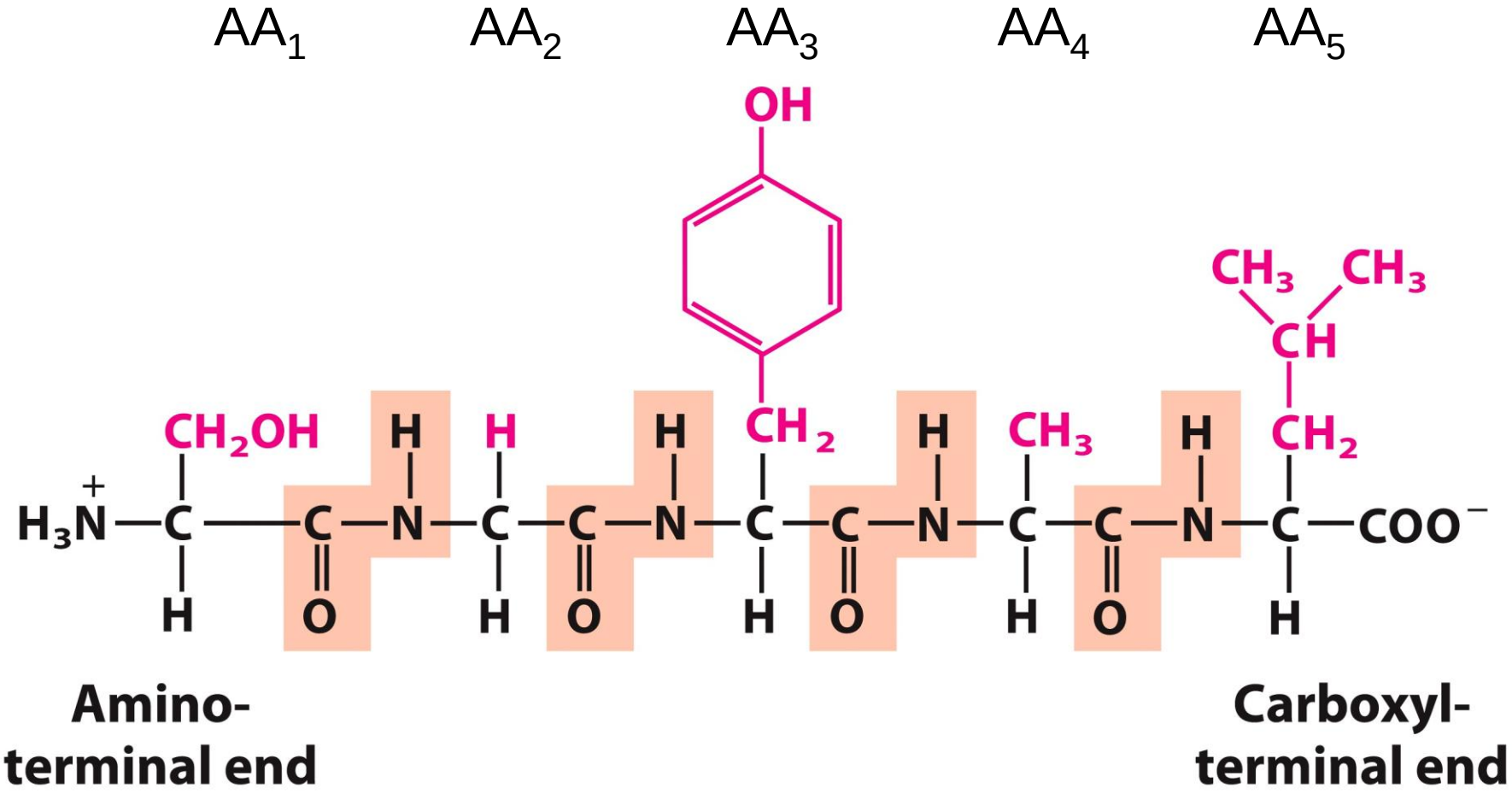
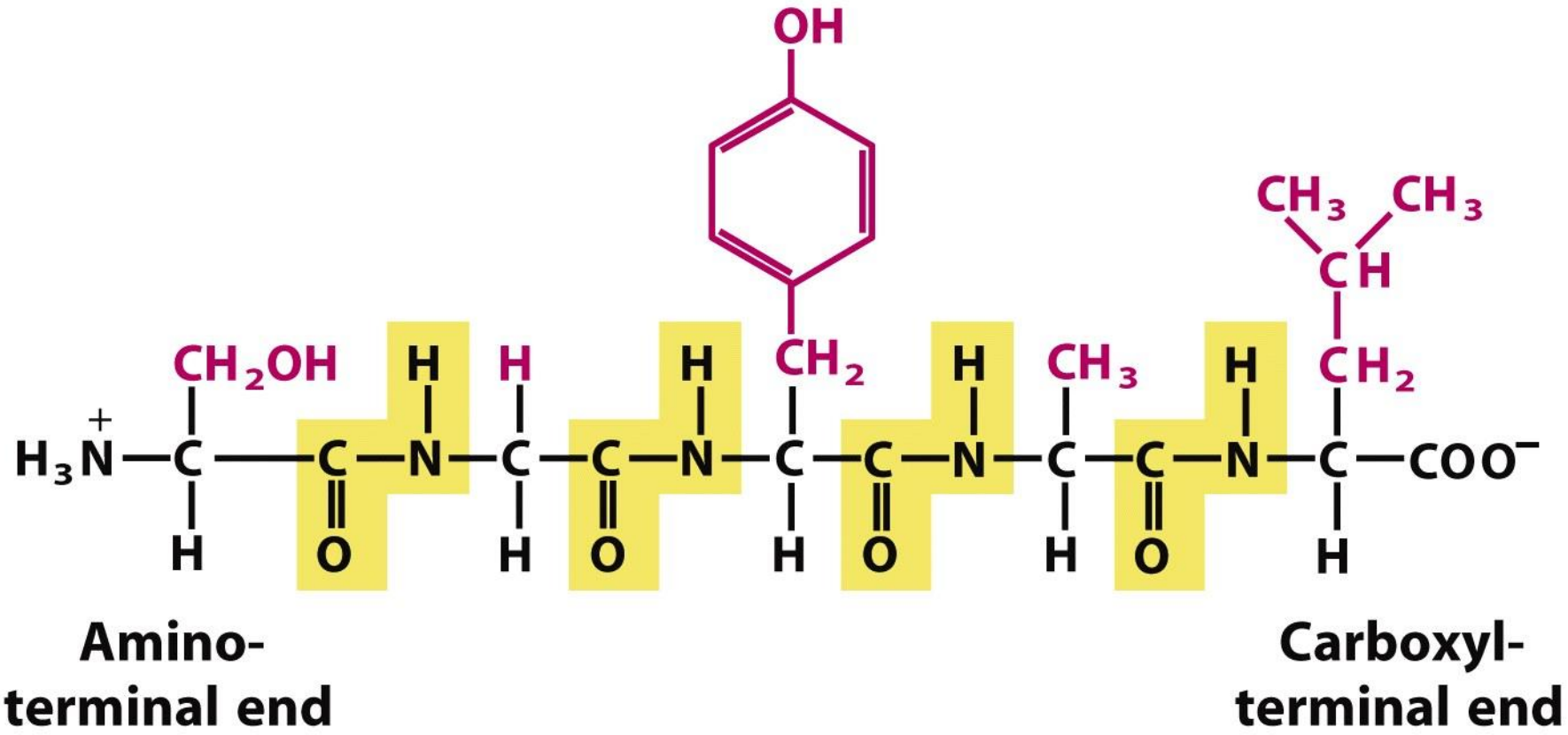



**Figure 3-14**
*Lehninger Principles of Biochemistry*, Sixth Edition

# Naming peptides: start at the N-terminus

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



**Alanylglutamylglycyllysine**

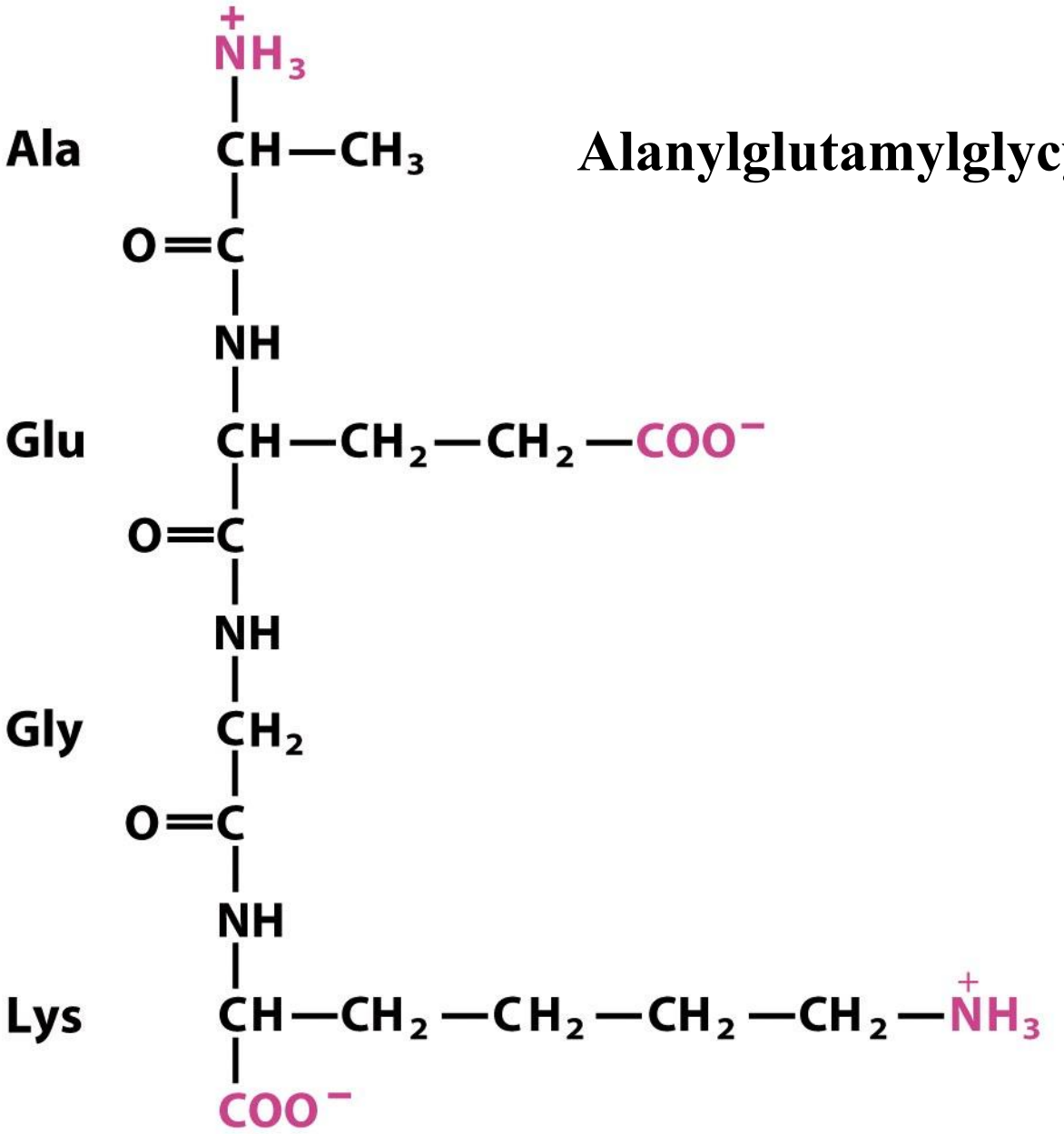
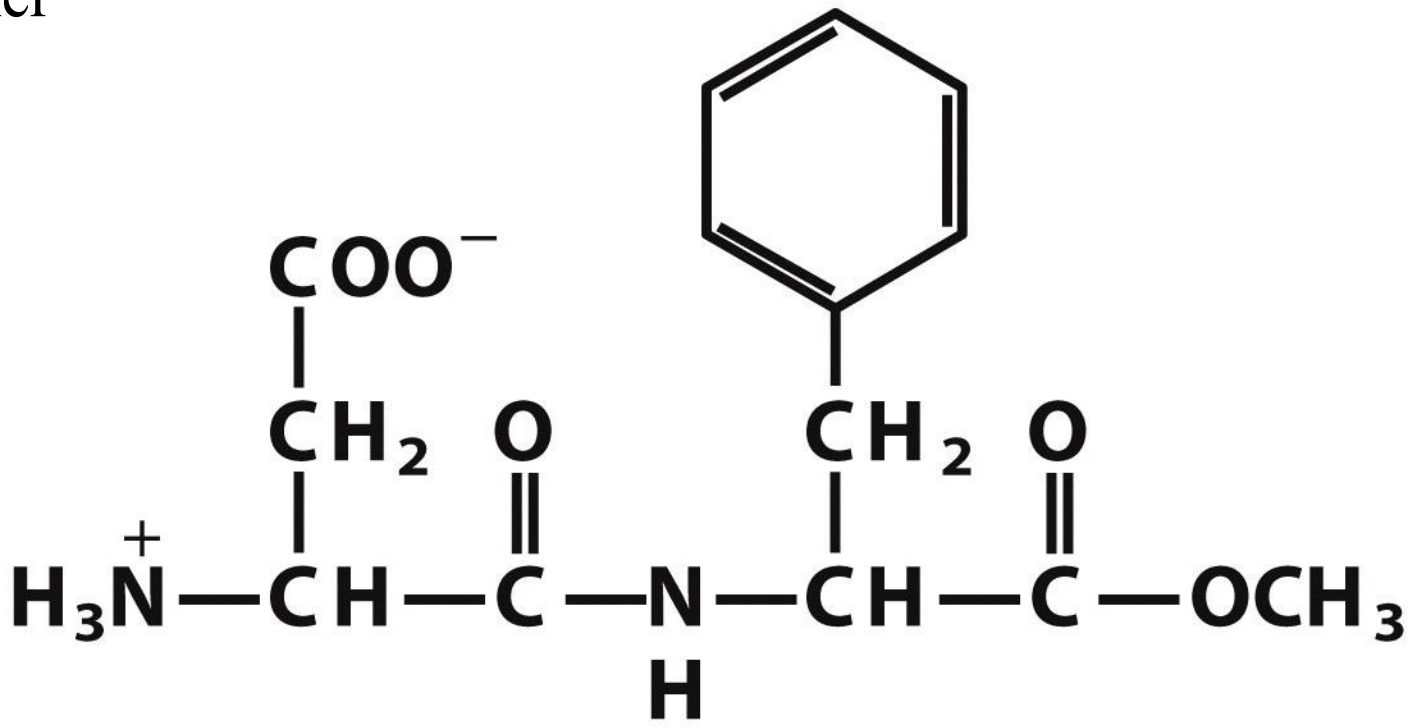



**Figure 3-15**
*Lehninger Principles of Biochemistry, Fifth Edition*

# 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 $\alpha$-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 | $\beta_1$-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

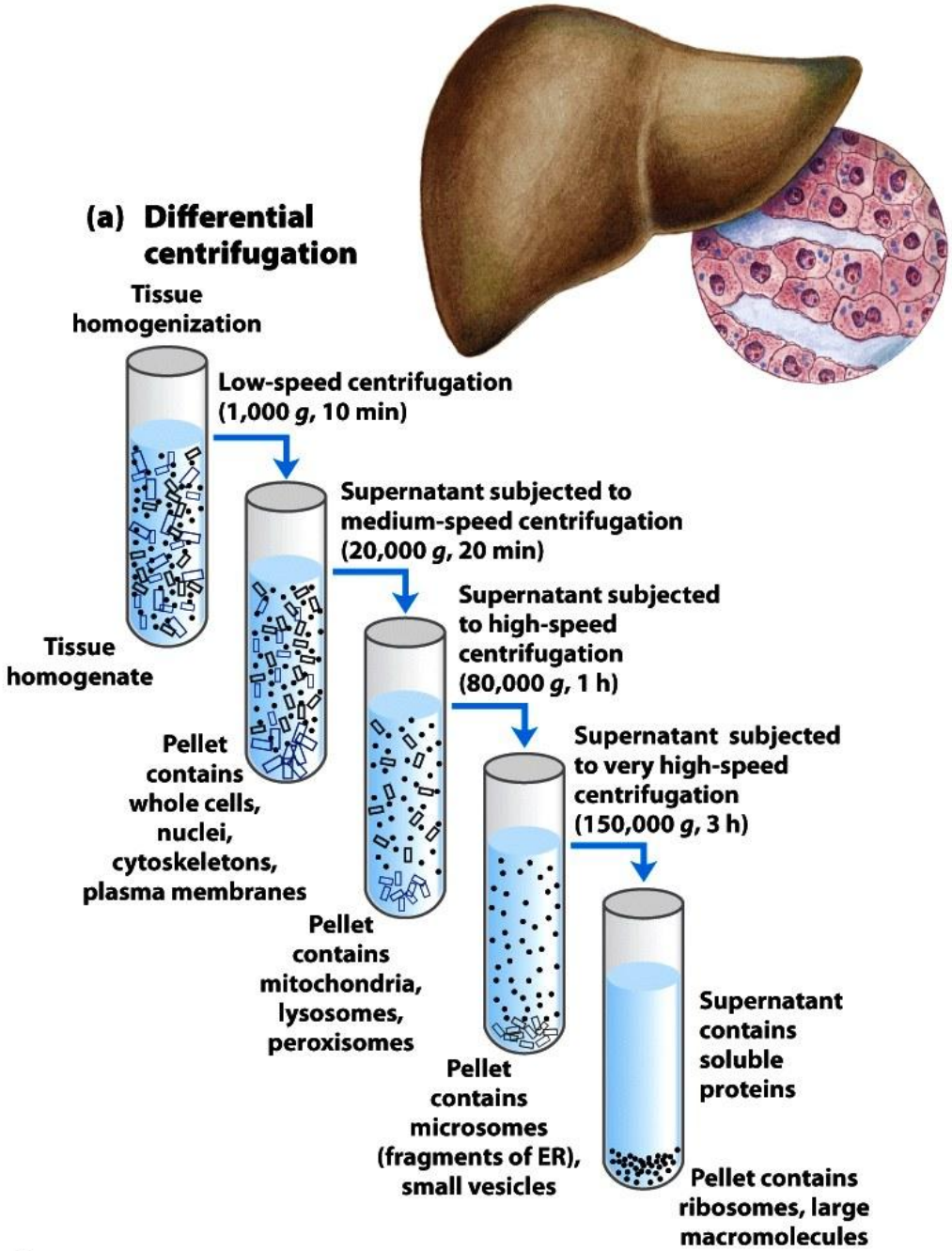


**Figure 1-8**

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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

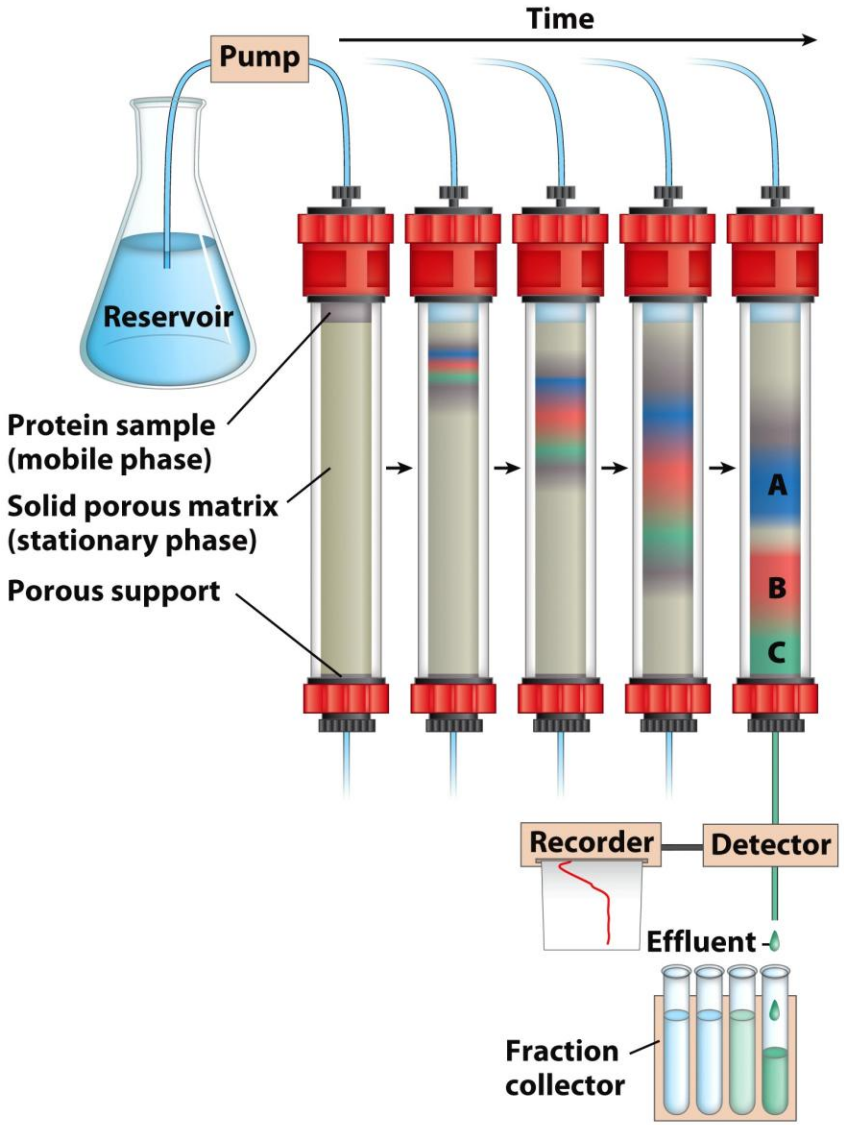


**Figure 3-16**
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# Separation by Charge

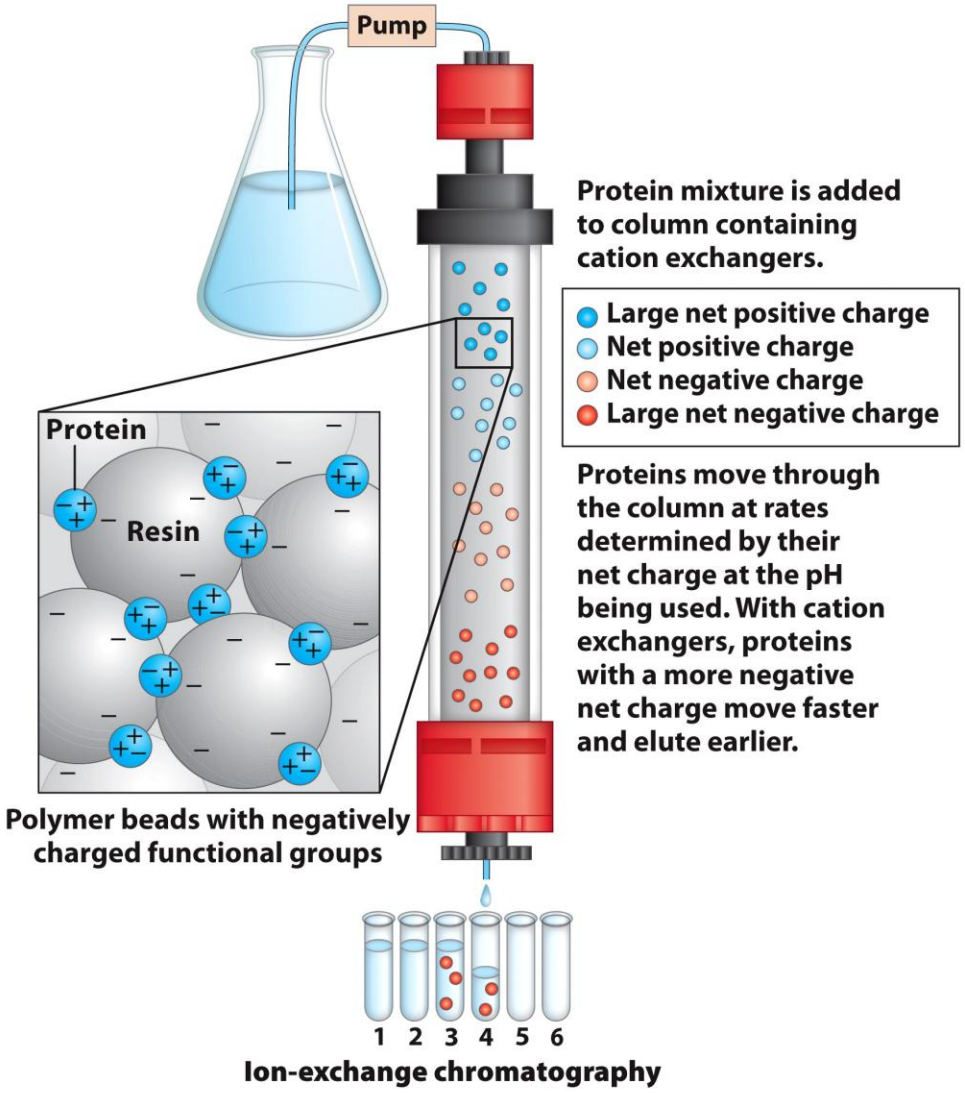


**Figure 3-17a**
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# Separation by Size

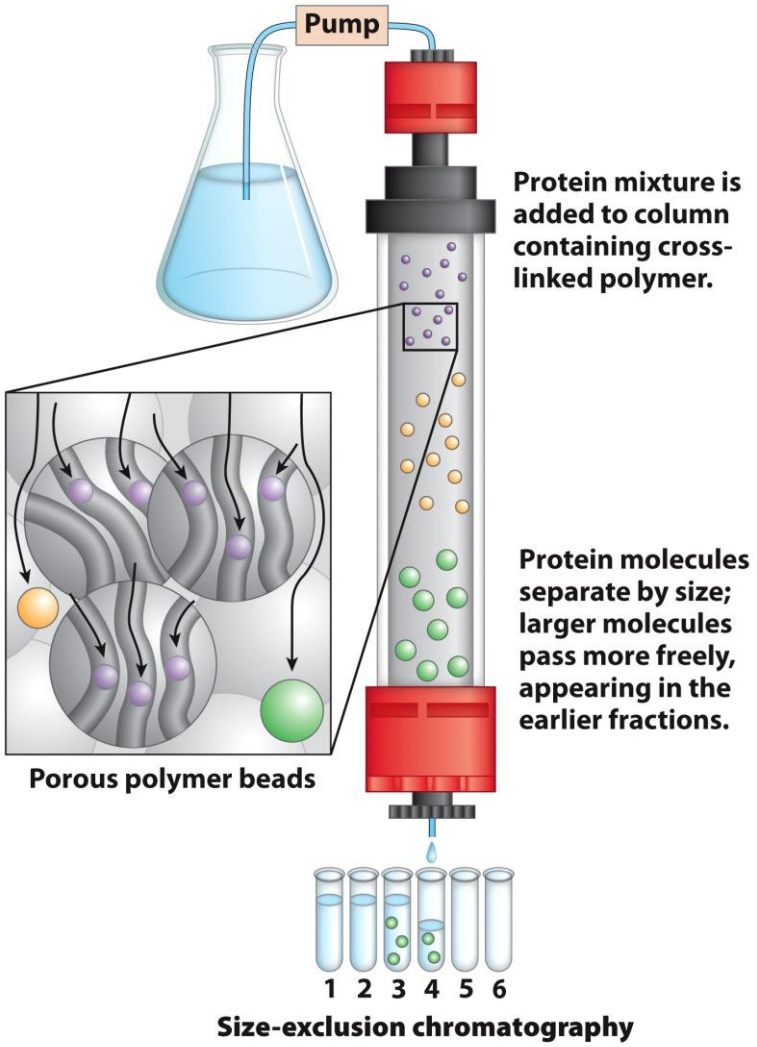


**Figure 3-17b**
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# Separation by Affinity

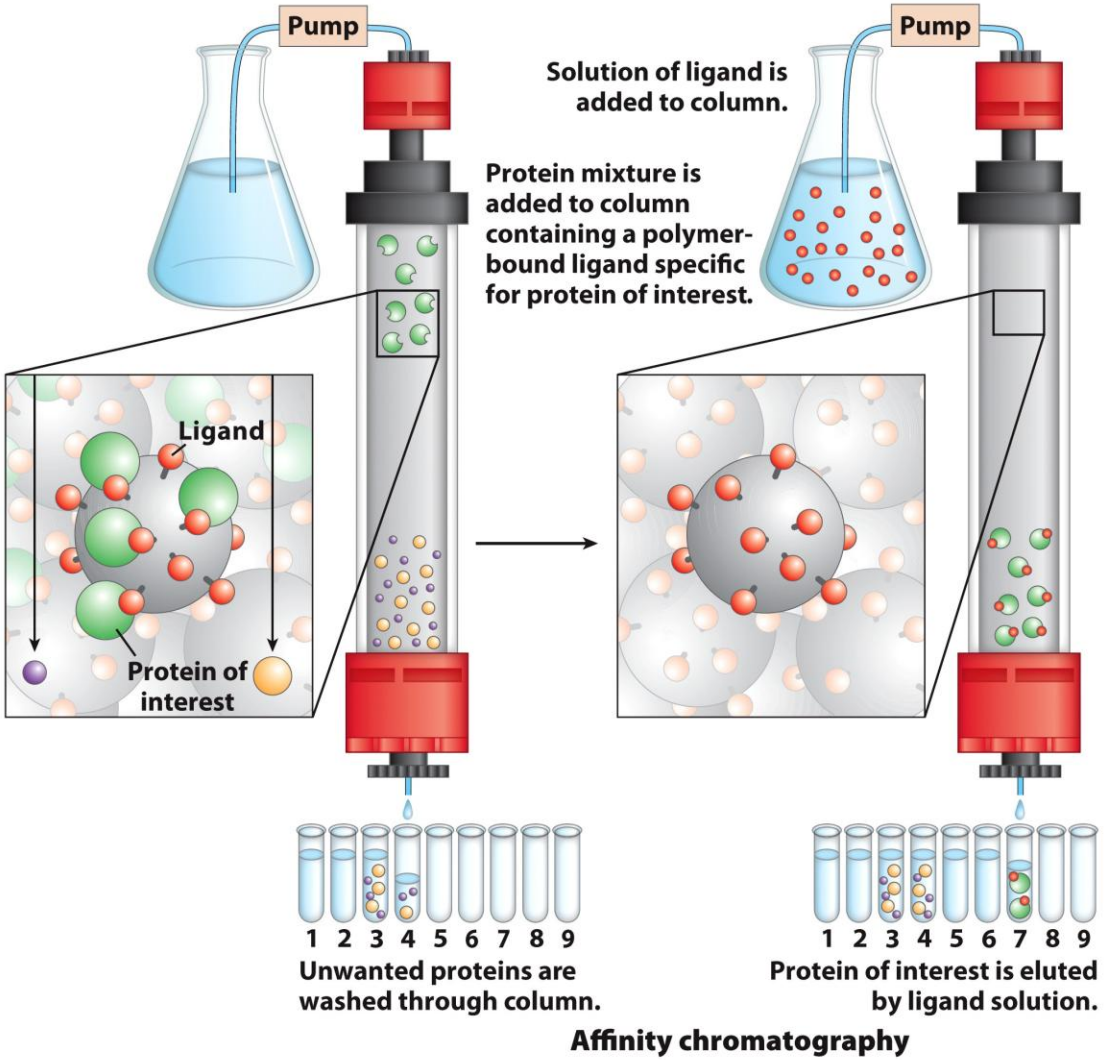


**Figure 3-17c**
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# Electrophoresis for Protein Analysis

- 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

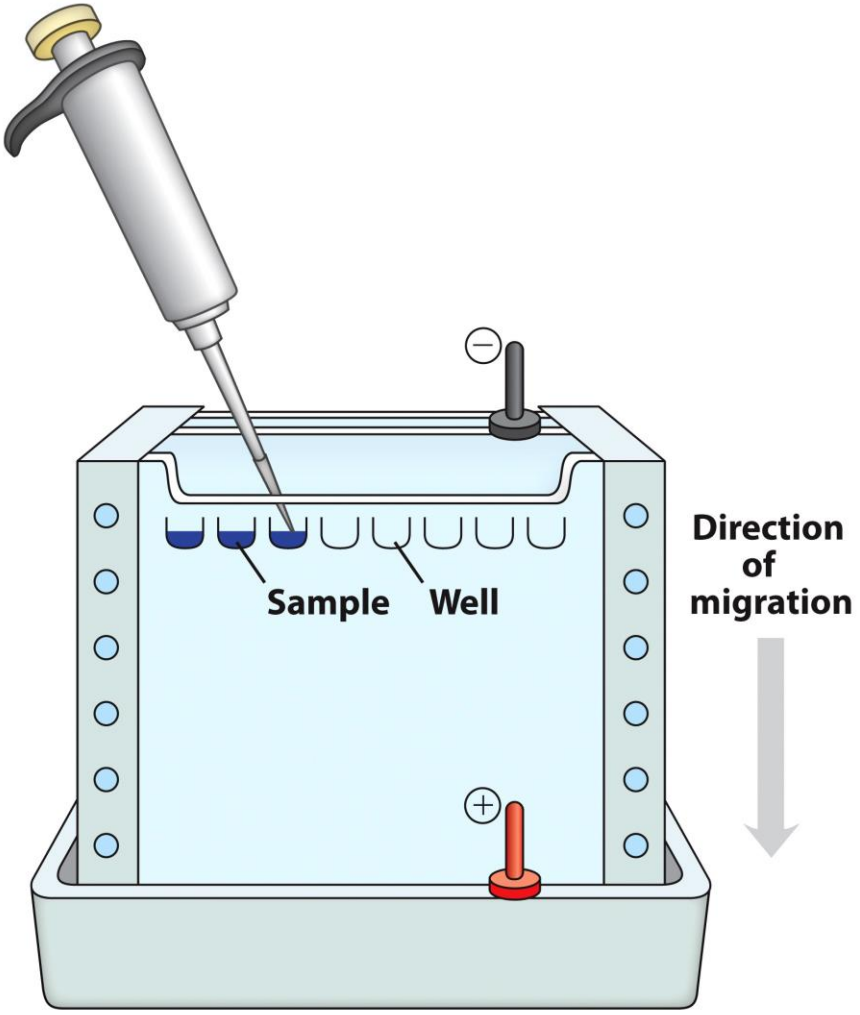


**Figure 3-18a**
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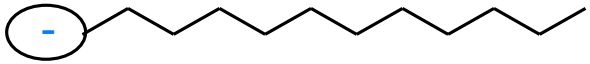
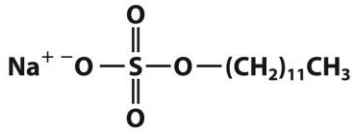
# SDS PAGE: Molecular Weight

- SDS – sodium dodecyl sulfate – a detergent




- 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

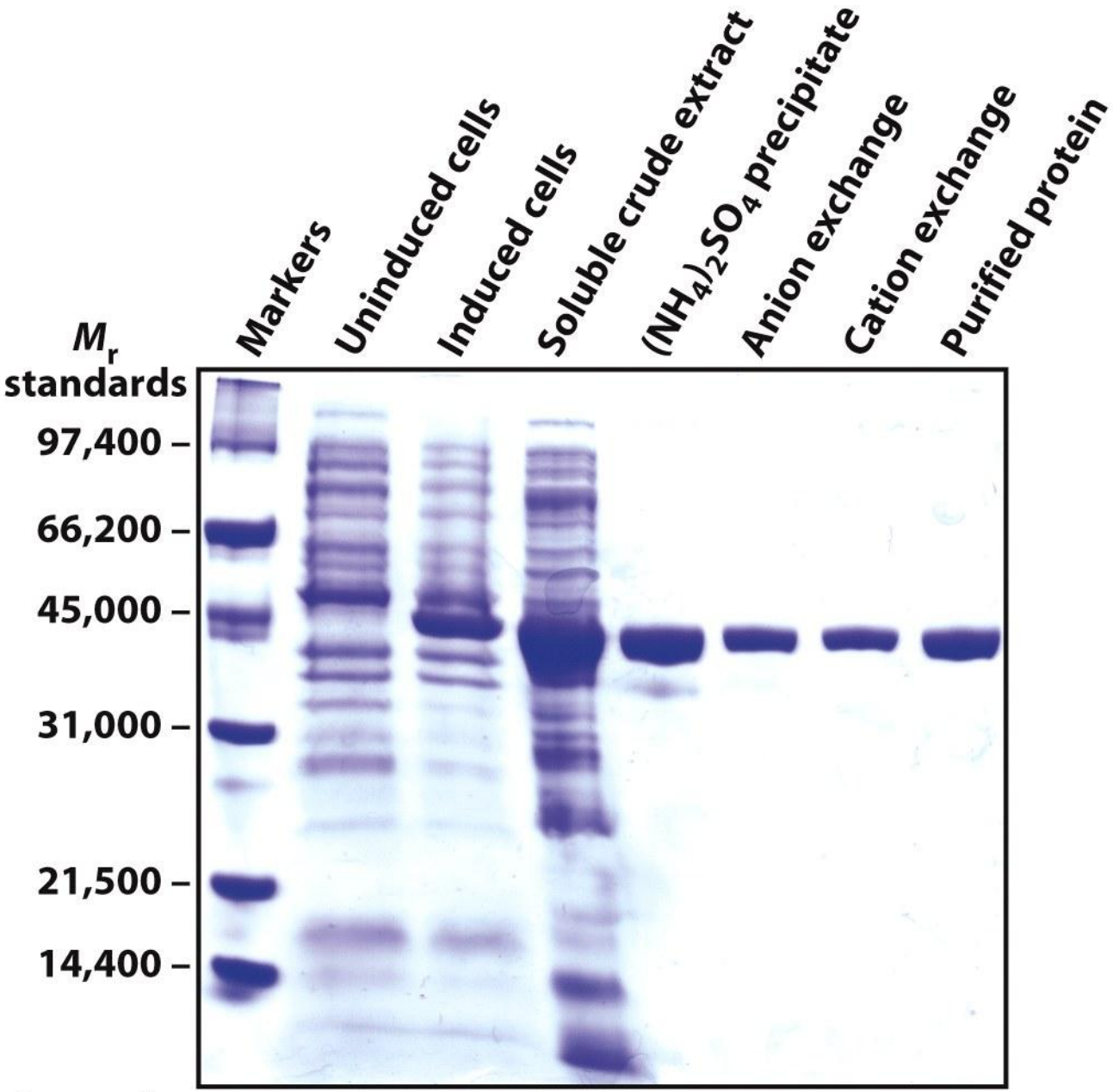



**Figure 3-18b**

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# SDS-PAGE can be used to calculate the molecular weight of a protein

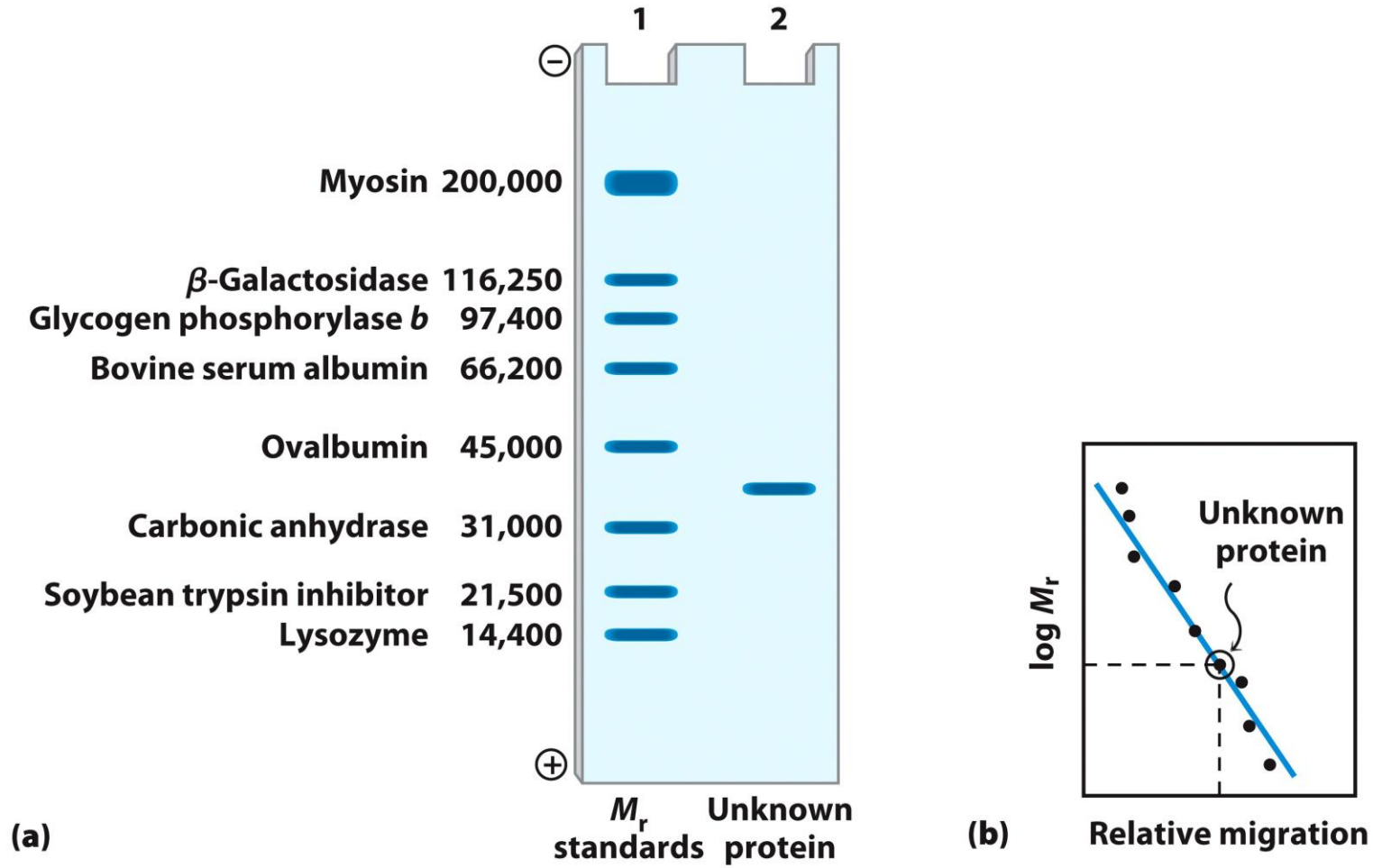


**Figure 3-19**
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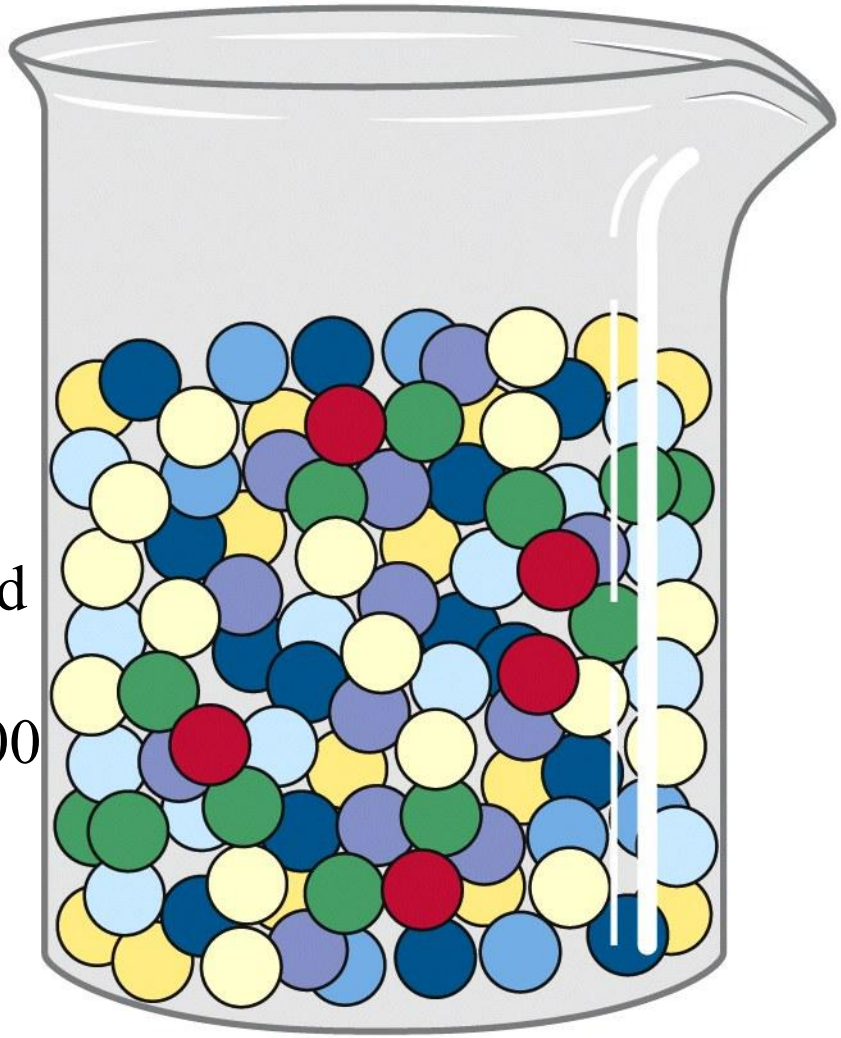
# SDS Gel Electrophoresis

# Specific Activity

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

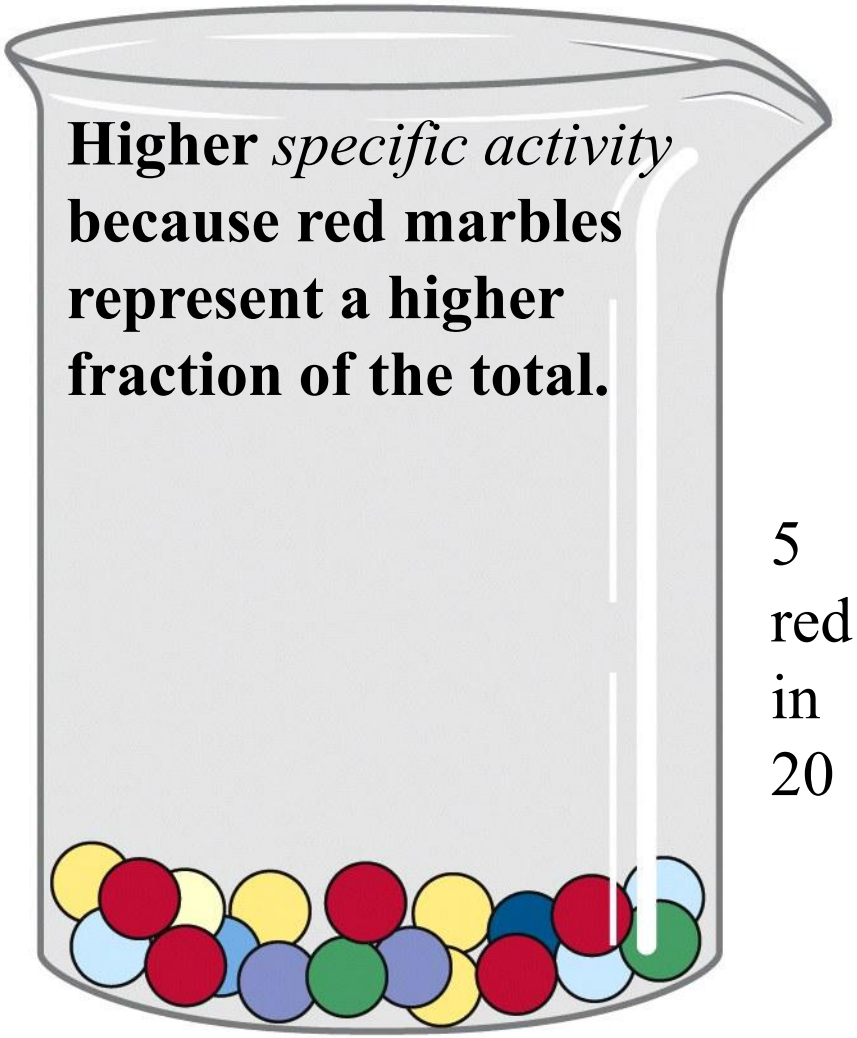
- ***Activity:*** Total units of enzyme in a solution
- ***Specific Activity:*** number of enzyme units / mg of total protein
- In a purification, many steps are used
- After each step, total protein ↓ (sometimes activity ↓) but specific activity ↑

**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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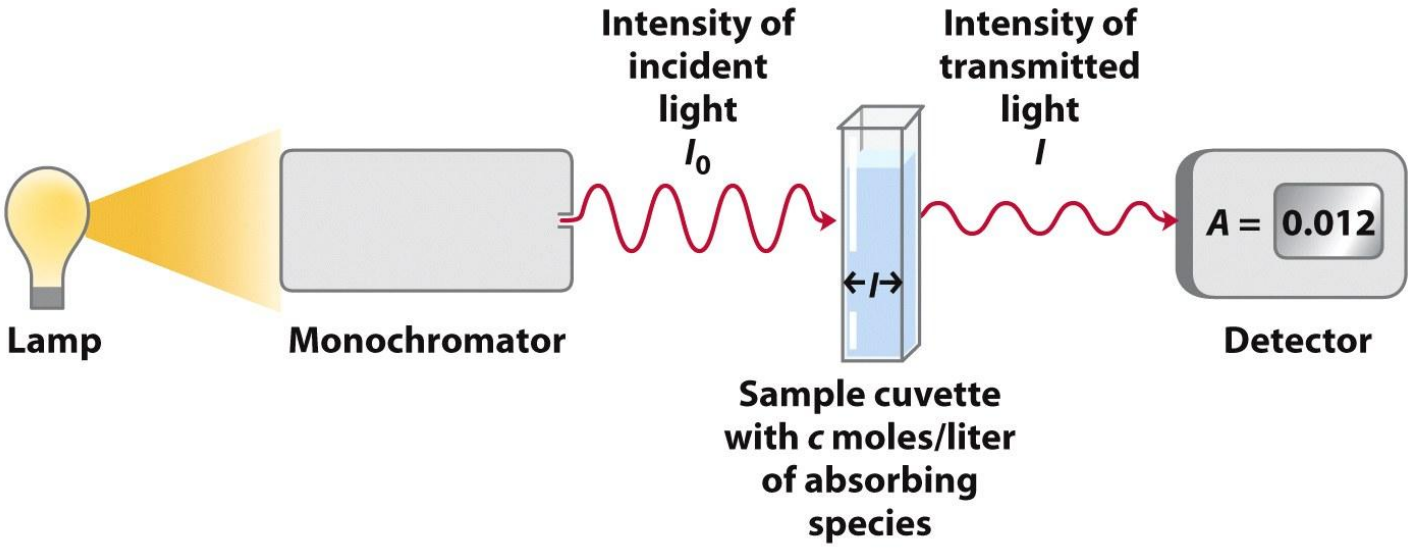
# Spectroscopic Detection of Aromatic Amino Acids

- 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: $A = \varepsilon \cdot c \cdot l$



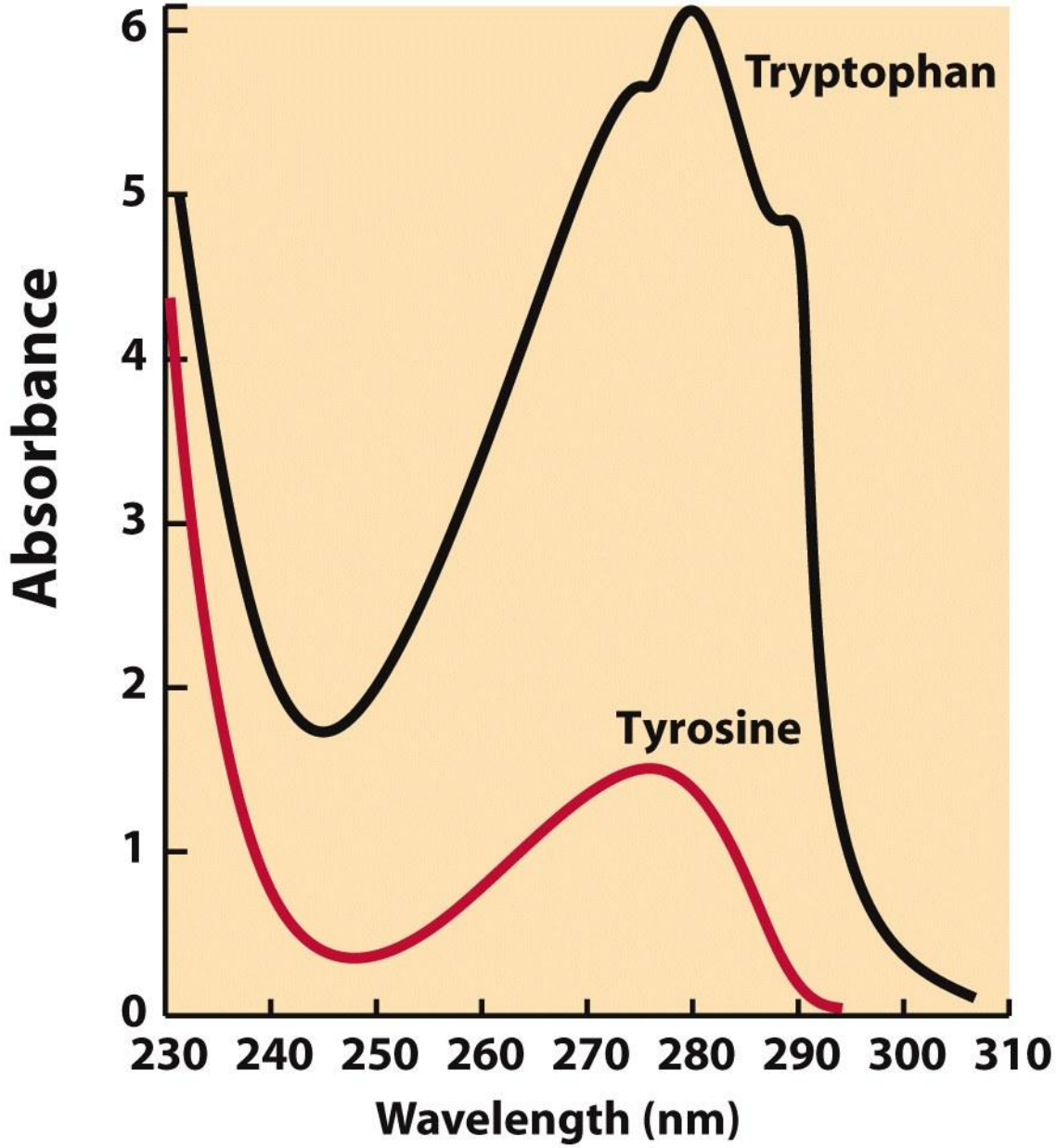



**Figure 3-6**
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# Proteases Can Be Used to Cleave Proteins

- 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