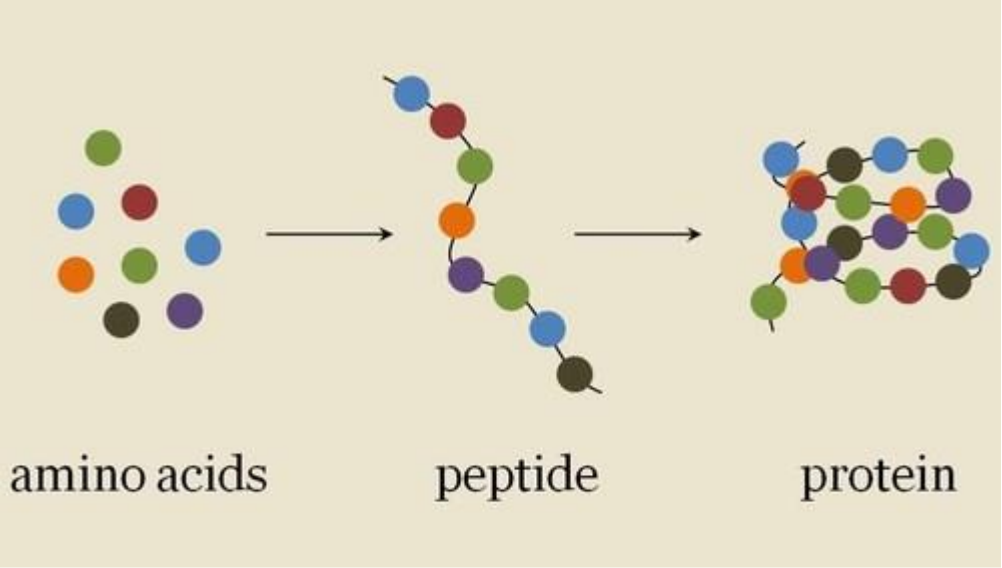
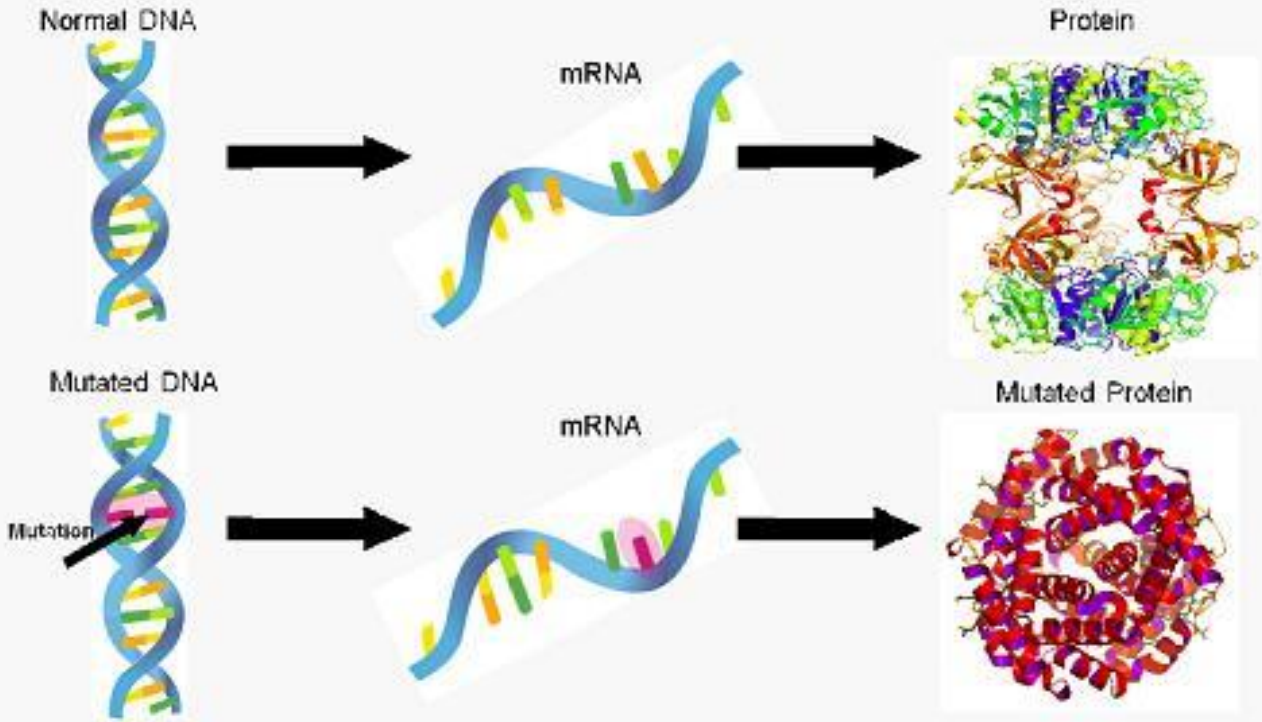
Amino Acids, Peptides, and Proteins



Why Are Mutations Important?

Many Serious Diseases Are Driven by a Change in Protein Structure



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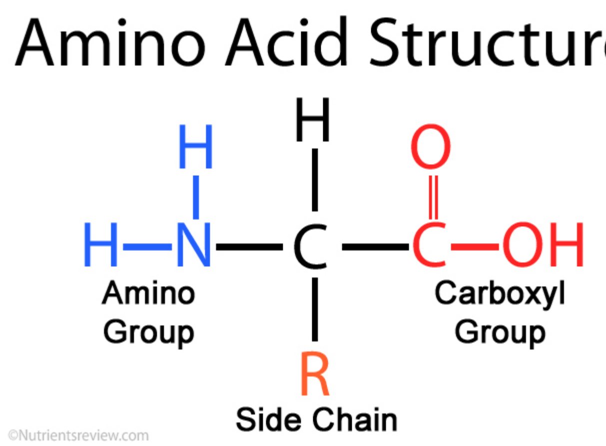
Amino Acid Structure

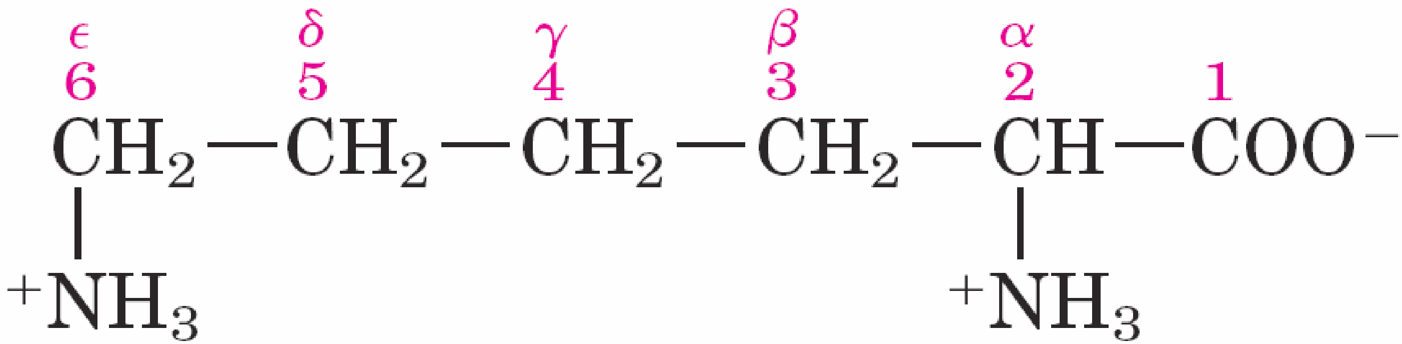
- Proteins: polymers of amino acids, with each **amino acid residue** joined
- **Amino Acids share common structure**

  All 20 of the common amino acids are α-amino acids

  A **carboxyl group** and an **amino group** bonded to the same carbon atom (the α carbon).
- Different α –amino acids are distinguished by their different side chains (R group).The α-carbon atom is thus a **chiral center**.



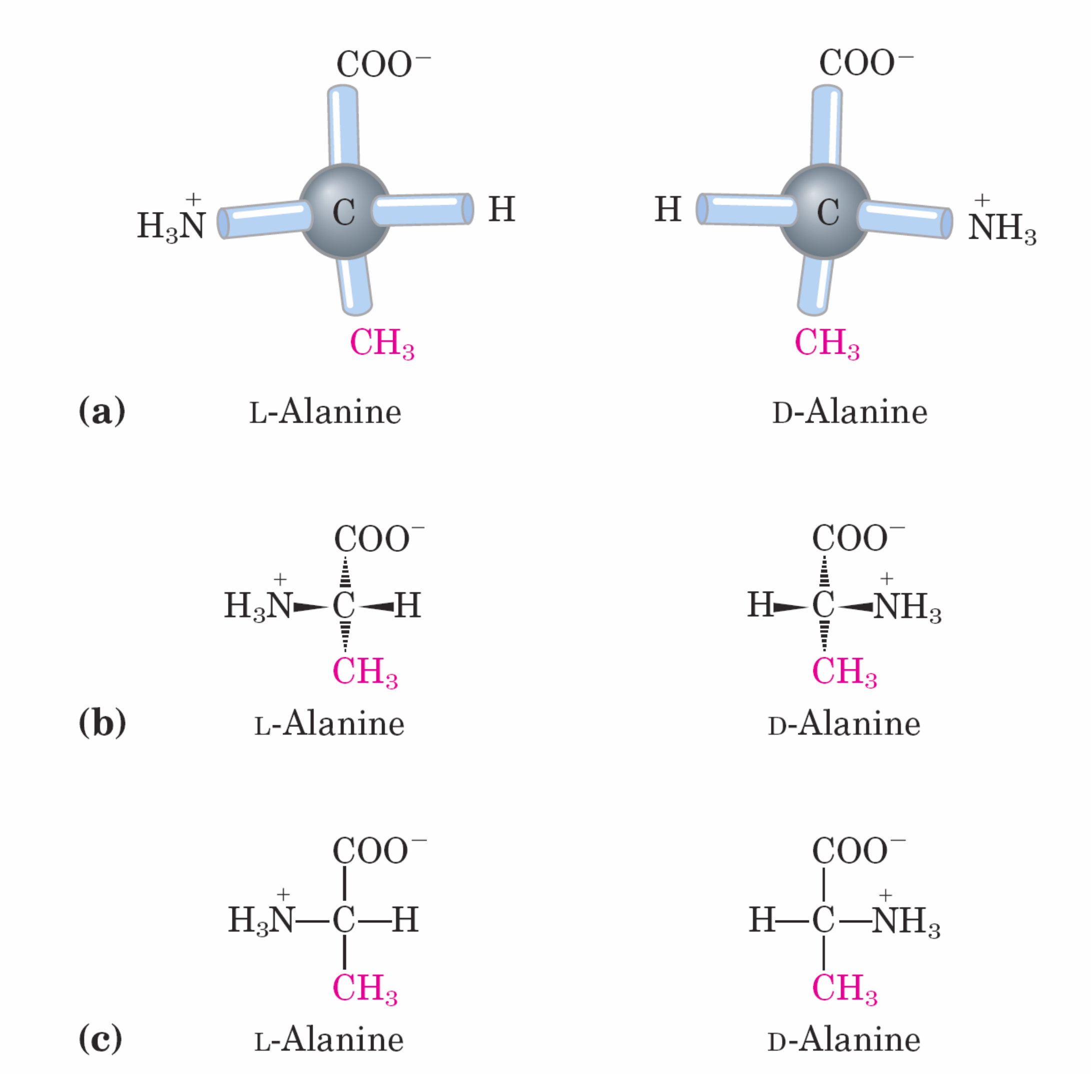
Nomenclature: Carbons



Lysine

The additional carbons in an R group are commonly designated β, γ, δ, ε.

Amino acids have two stereoisomers



L: Levo

D: Dextro

（透視）

Perspective formulas

（投射）

Projection formulas

Amino acid can be classified by R group

- The 20 common amino acids can be grouped into five main classes based on the properties of their R groups

- Polarity: or tendency to interact with water at biological pH. The polarity of the R groups varies widely, from nonpolar and hydrophobic (water-insoluble) to highly polar and hydrophilic (water-soluble).

- The 20 common amino acids can also be classified by their side chains:
    - Aliphatic
    - Aromatic
    - Hydroxyl or sulfur-containing
    - Basic
    - Acidic and their amides

TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

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

pKa – The acid strength of weak acids
(代表一種 酸 解 離 氫 離子 的能力)

$$R{-}COOH \rightleftharpoons R{-}COO^- + H^+$$
$$R{-}NH_3^+ \rightleftharpoons R{-}NH_2 + H^+$$

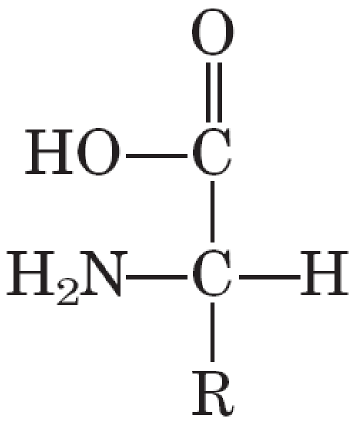
pI(isoelectric point)- the pH value at which number of positive charges equals the number of negative charges

isoelectric pH (pI) of alanine

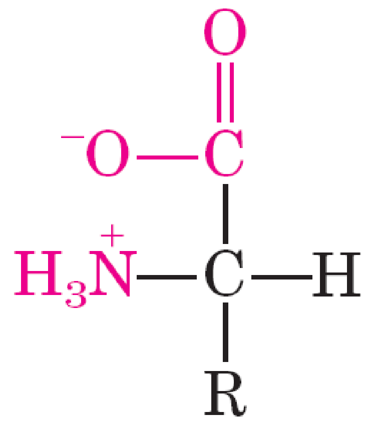
$$pI = \frac{pK_1 + pK_2}{2} = \frac{2.35 + 9.69}{2} = 6.02$$

hydropathy index - a measure of polarity of an amino acid residue.

Amino acids can act as acids and bases



Nonionic form



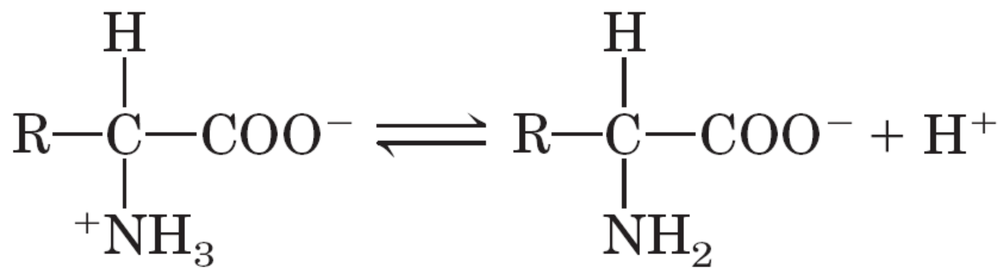
Zwitterionic form

The nonionic form does not occur in significant amounts in aqueous solutions.

The zwitterion predominates at neutral pH.

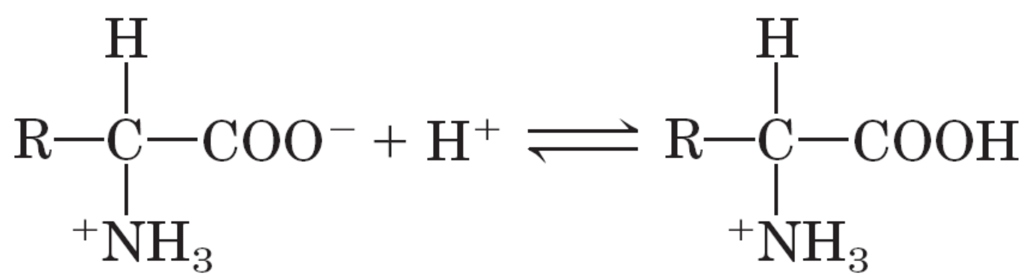
Amino acids can act as acids and bases

as an acid (proton donor)



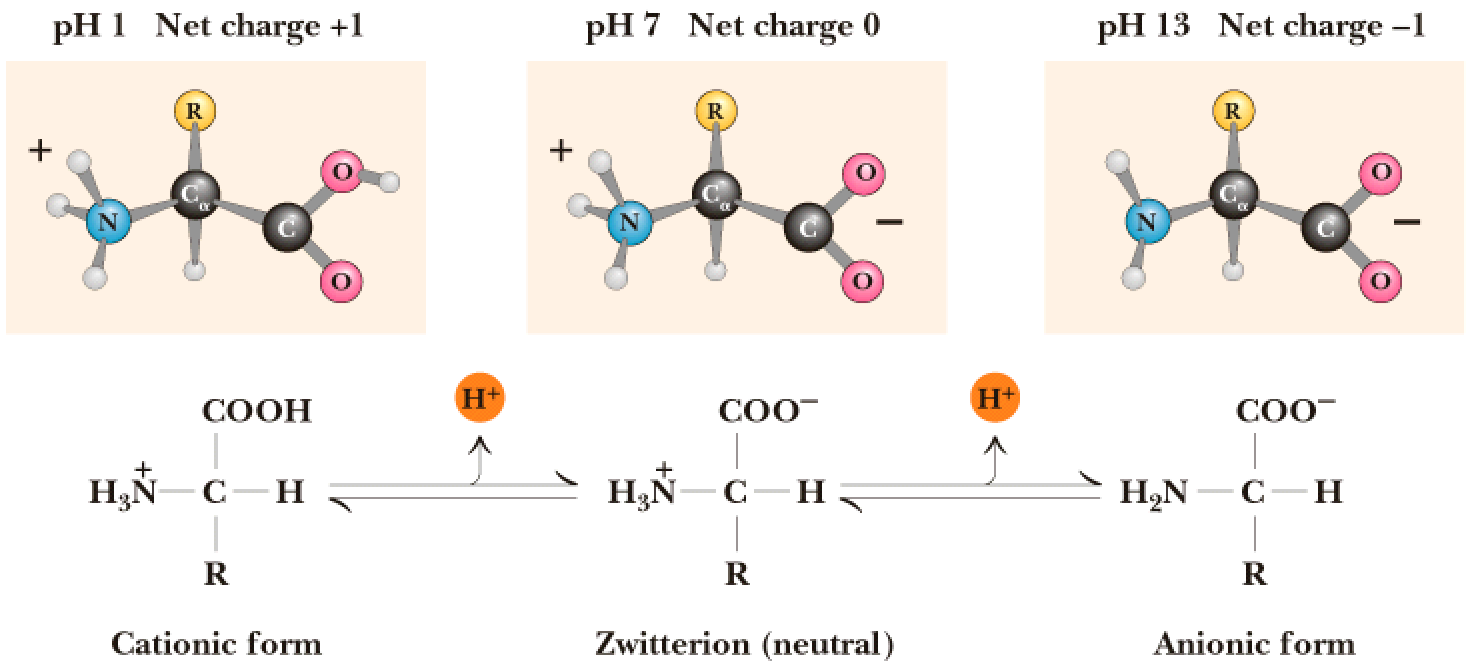
Zwitterion

or a base (proton acceptor)



Zwitterion

Isoelectric point
(pI)



Saunders College Publishing

Amino acids have characteristic titration curves

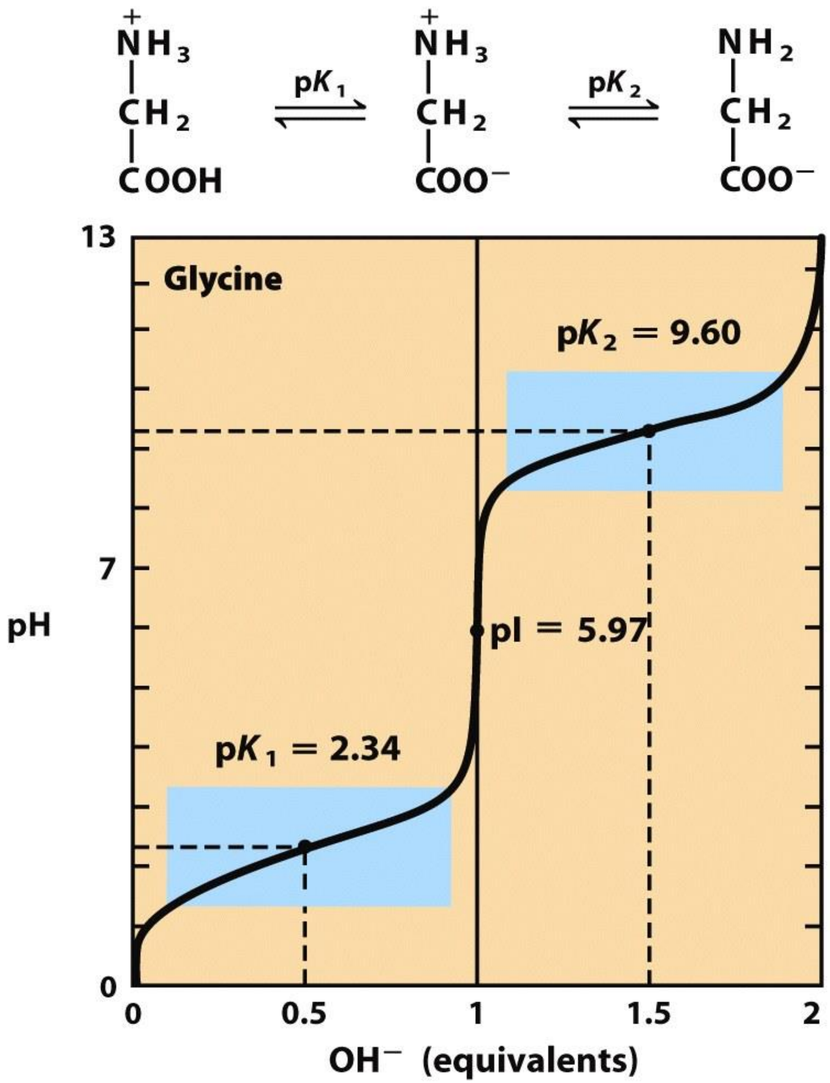


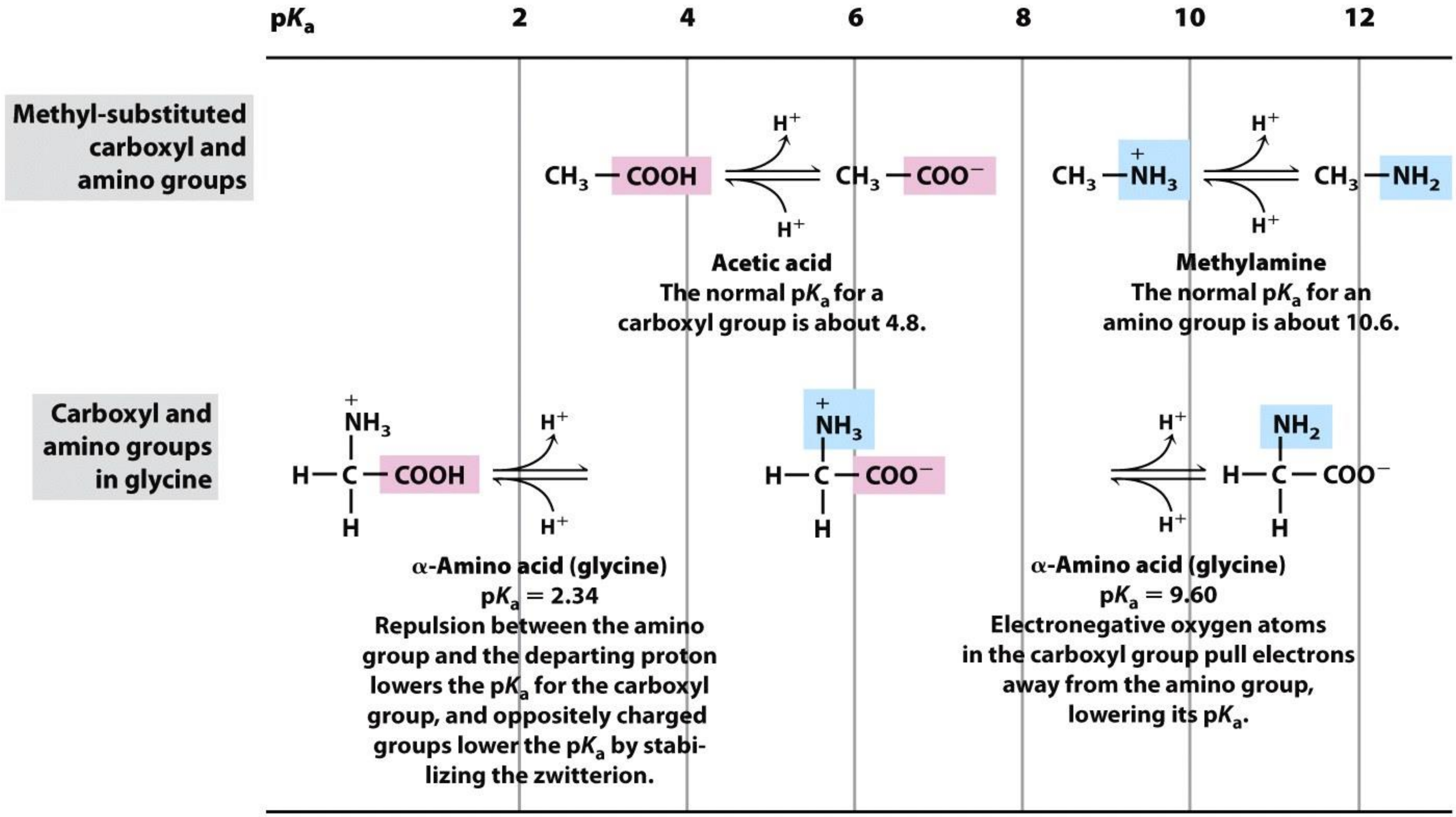
Figure 3-10
Lehninger Principles of Biochemistry, Fifth Edition

$$pI = \frac{1}{2}(pK_1 + pK_2)$$

$$= \frac{1}{2}(2.34 + 9.6)$$

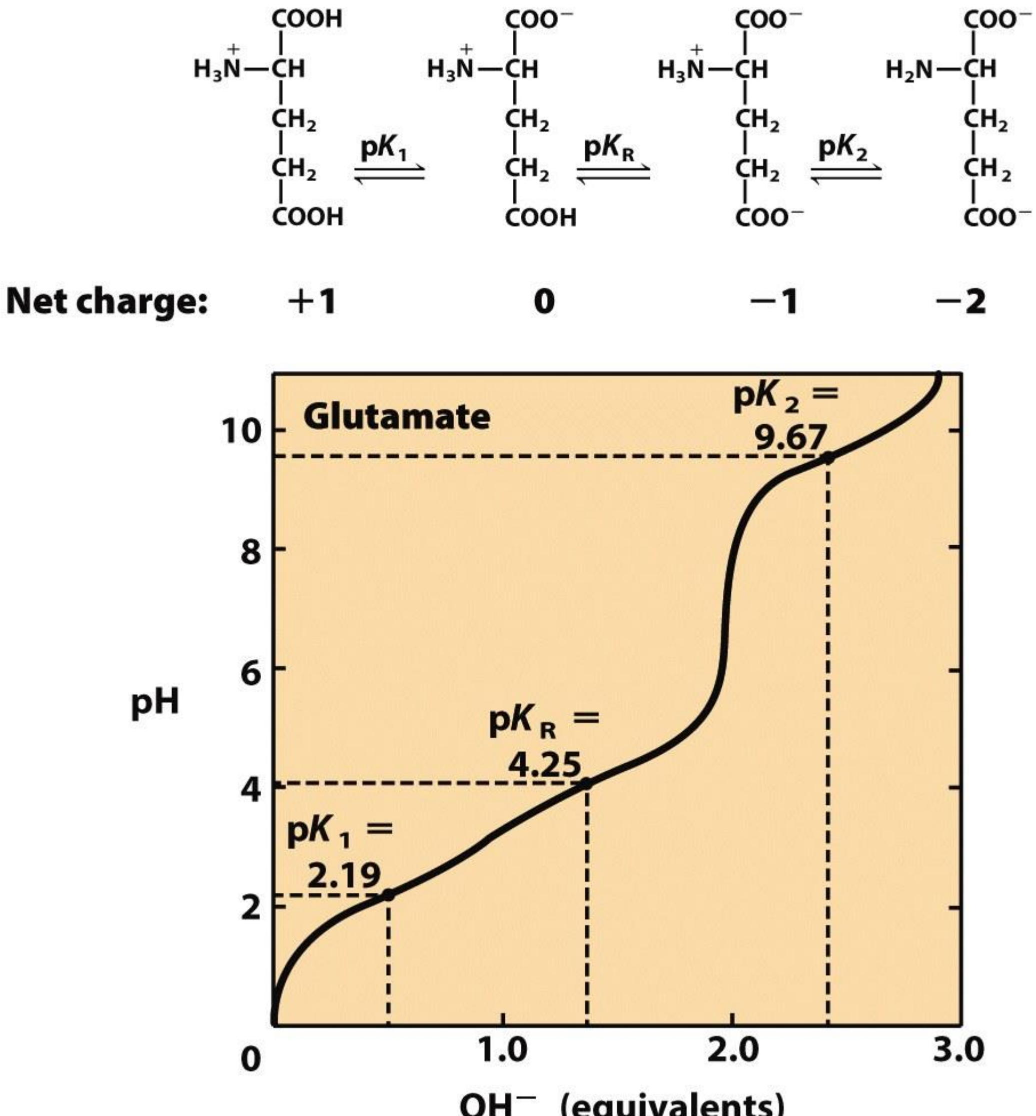
$$= 5.97$$

Effect of the chemical environment on pK_a



The pK_a values for the ionizable groups in glycine are lower than those for simple, methyl-substituted amino and carboxyl groups. These downward perturbations of pK_a are due to intramolecular interactions. They can be caused by chemical groups that happen to be positioned nearby.

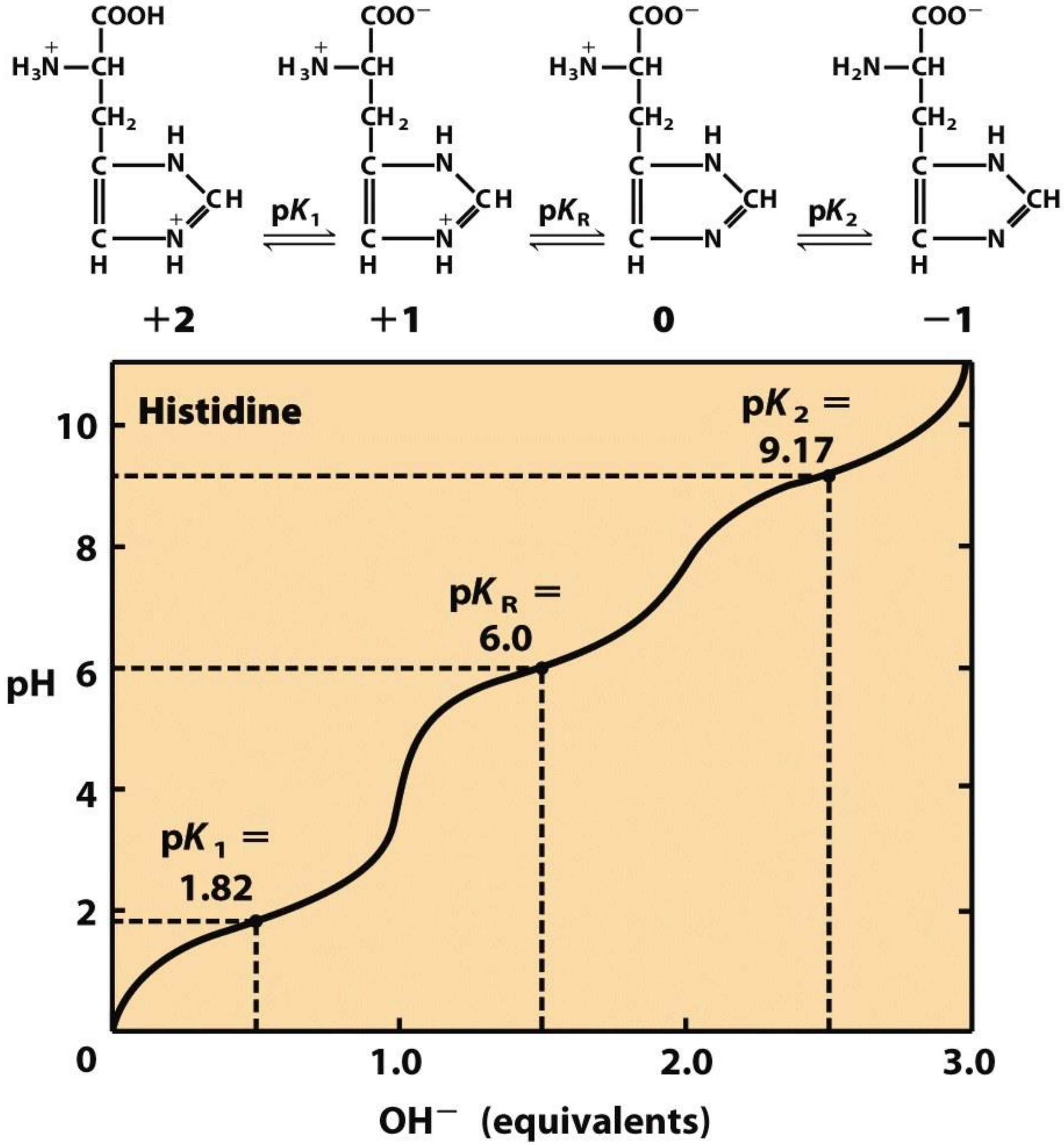
Titration curves for glutamate



$$pI = \frac{2.19 + 4.25}{2}$$

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

Titration curves for histidine



$$pI = \frac{6.0+9.17}{2}$$

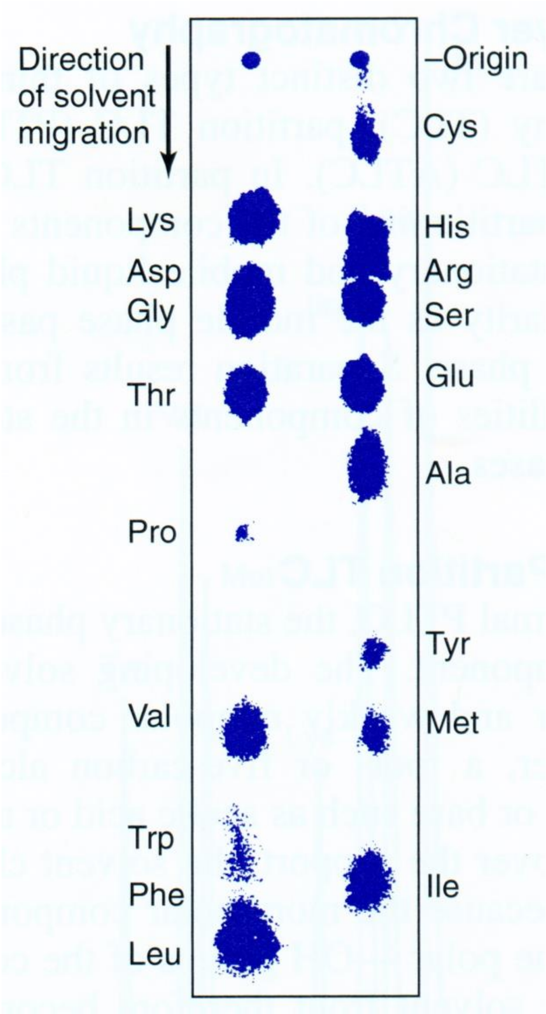
Figure 3-12b
Lehninger Principles of Biochemistry, Fifth Edition

Typical range of pK values for ionizable groups in proteins

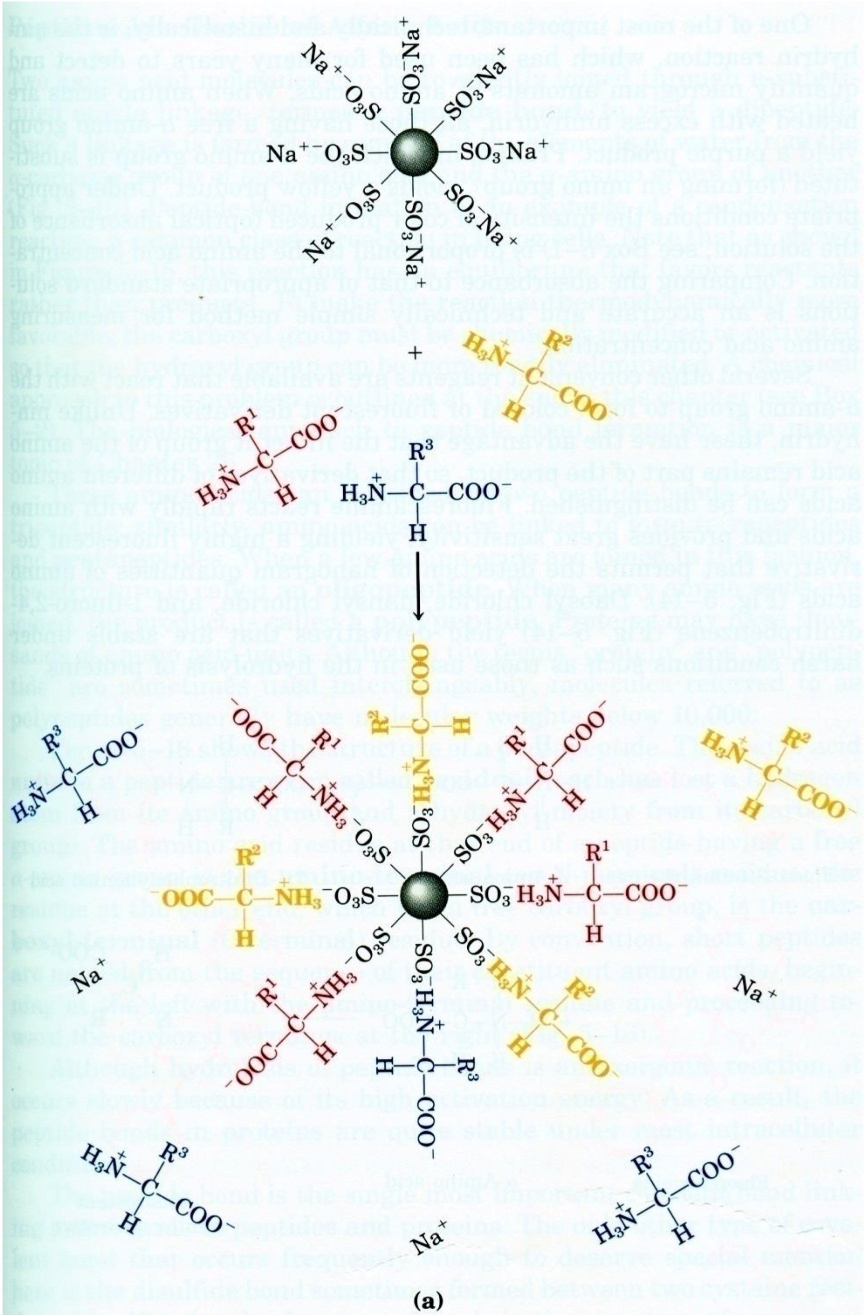
Dissociating Group	p*K* Range
α-Carboxyl	3.5–4.0
Non-α COOH of Asp or Glu	4.0–4.8
Imidazole of His	6.5–7.4
SH of Cys	8.5–9.0
OH of Tyr	9.5–10.5
α-Amino	8.0–9.0
ε-Amino of Lys	9.8–10.4
Guanidinium of Arg	~12.0

Various techniques separate amino acids

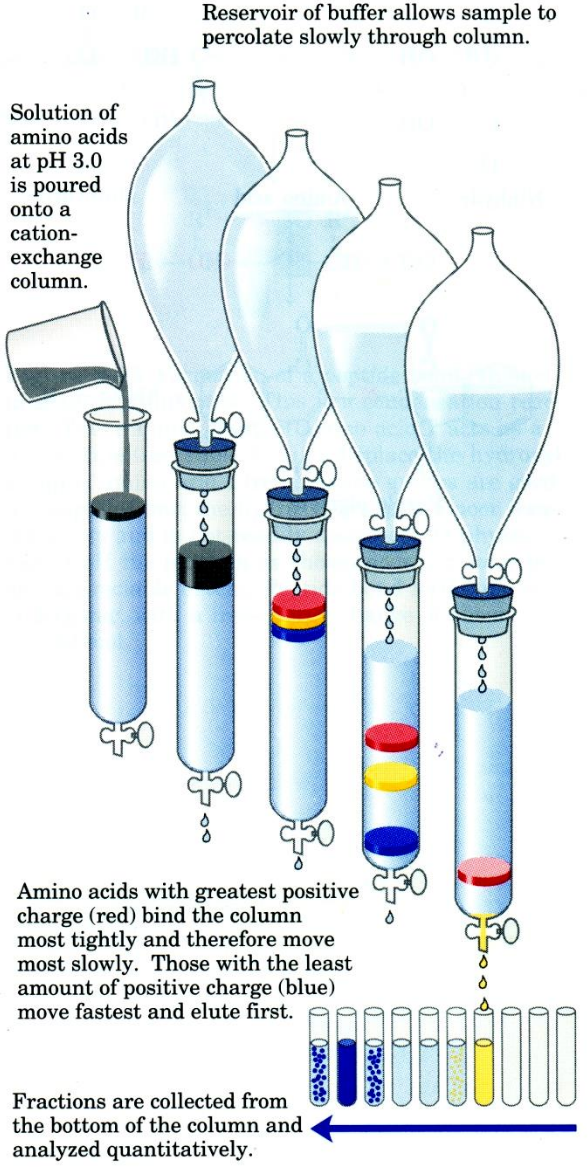
Paper chromatography



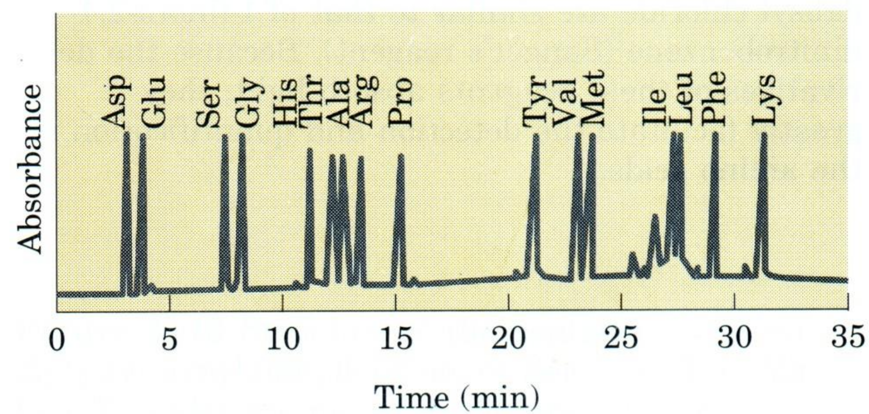
Ion exchange chromatography
separates amino acids
by electric charge



(a)


Reservoir of buffer allows sample to percolate slowly through column.
Solution of amino acids at pH 3.0 is poured onto a cation-exchange column.
Amino acids with greatest positive charge (red) bind the column most tightly and therefore move most slowly. Those with the least amount of positive charge (blue) move fastest and elute first.
Fractions are collected from the bottom of the column and analyzed quantitatively.

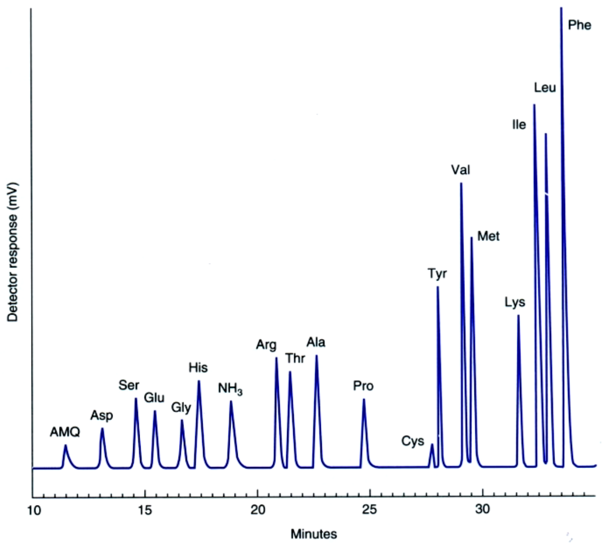
(b)


Absorbance
Asp
Glu
Ser
Gly
His
Thr
Ala
Arg
Pro
Tyr
Val
Met
Ile
Leu
Phe
Lys
0
5
10
15
20
25
30
35
Time (min)

Analysis of amino acids

High Performance liquid chromatography (HPLC)

Amino Acid Analyzer



The 20 standard amino acids of proteins

Amino acids with aliphatic side chains

G: R=-H

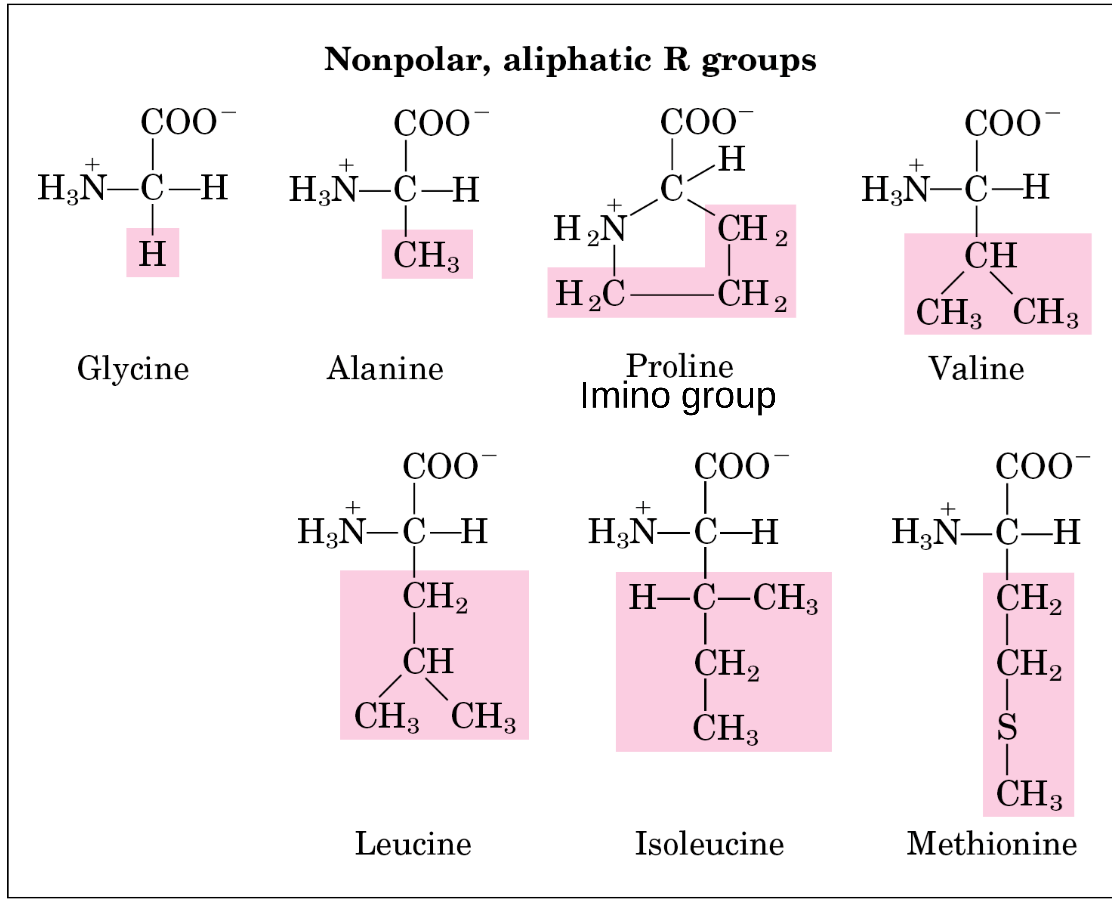
A: R=$-CH_3$

V: R=$-C<\begin{array}{l}CH_3\\CH_3\end{array}$

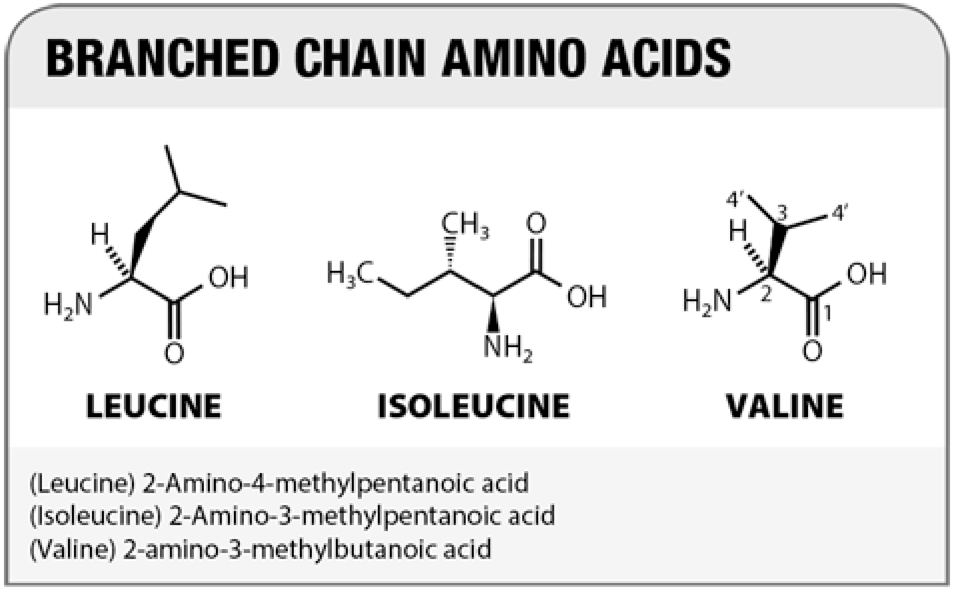
L: R=$-CH_2-CH<\begin{array}{l}CH_3\\CH_3\end{array}$

M: R=$-CH_2-CH_2-S-CH_3$

I: R=$-CH<\begin{array}{l}CH_2-CH_3\\CH_3\end{array}$



Small amino acids- glycine and alanine
Branched chain amino acids-valine, leucine and isoleucine



Improve exercise performance and reduce muscle breakdown

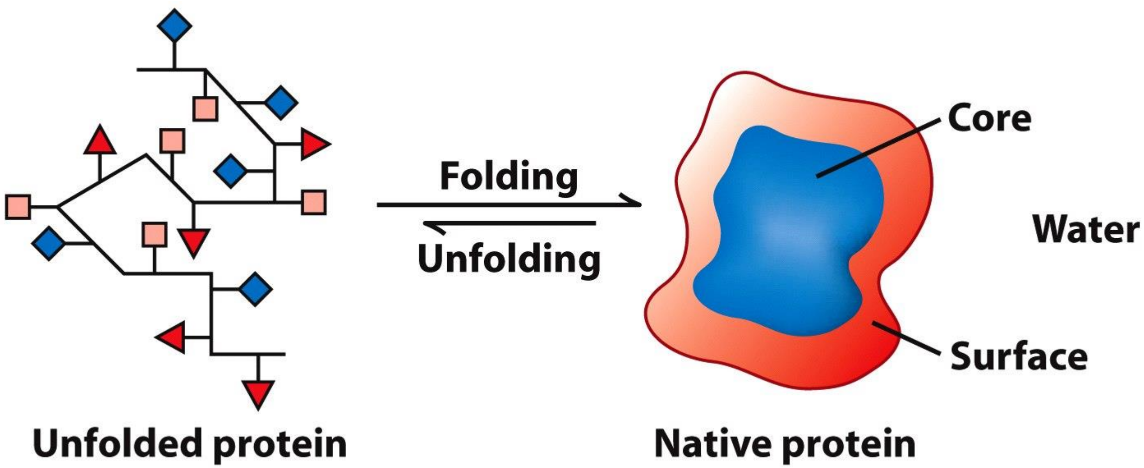
Of the essential amino acids, three account for as much as 33% of muscle tissue – leucine, isoleucine, and valine.

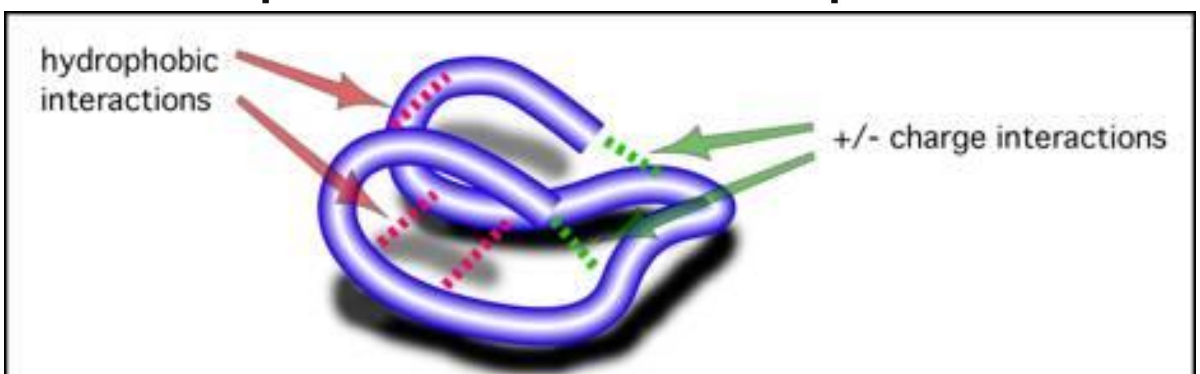
Classification of the L-α-amino acids of proteins based on their relative hydrophilicity or hydrophobicity

Hydrophobic	Hydrophilic	
Alanine	Arginine	Histidine
Isoleucine	Asparagine	Lysine
Leucine	Aspartic acid	Serine
Methionine	Cysteine	Threonine
Phenylalanine	Glutamic acid	
Proline	Glutamine	
Tryptophan	Glycine	
Tyrosine		
Valine		

Properties of the Amino Acid Side Chains

- The more hydrophobic amino acids such as **isoleucine** are usually found *within the core of a protein molecule, where they are shielded from water.*

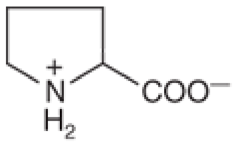




The protein folding pattern is primarily determined by hydrophobic amino acids burying themselves in the center, away from water; additional interactions between charged amino acids help the protein adopt its final shape.

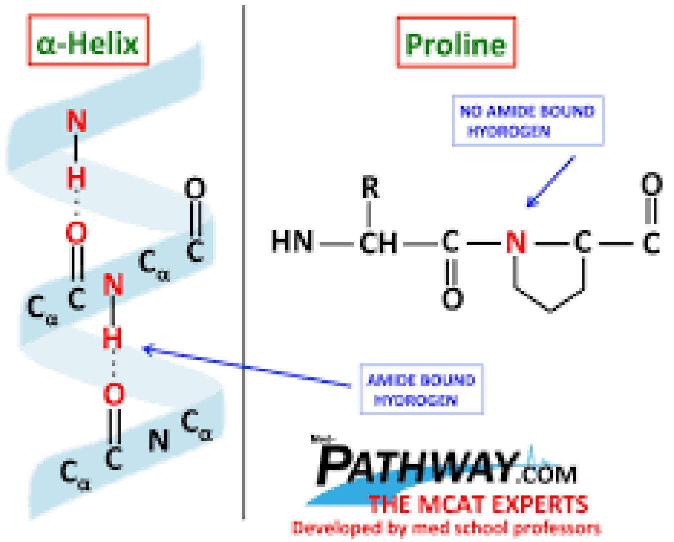
Properties of the Amino Acid Side Chains

- ***Proline***: only amino acid which the side chain forms a covalent bond with the α–amino group.

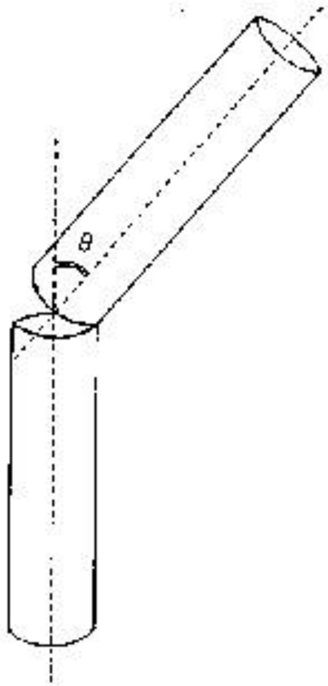


- The rigid ring of proline: protein structure where the protein must fold back on itself (so-called "turns").

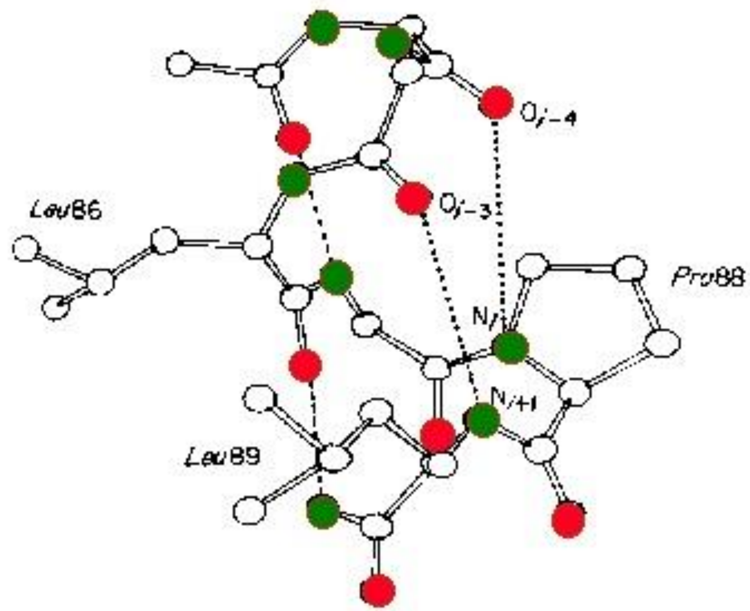
- Proline acts as a structural disruptor in the middle of regular secondary structure elements such as alpha helices and beta sheets.



Proline is a helix-breaker but participates in formation of other motifs

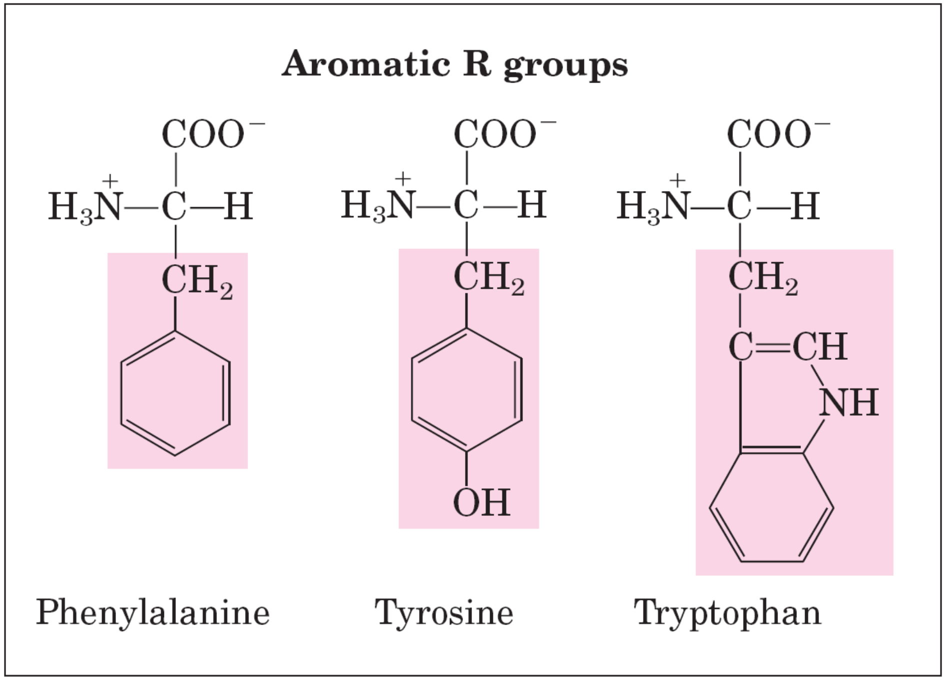


In helices

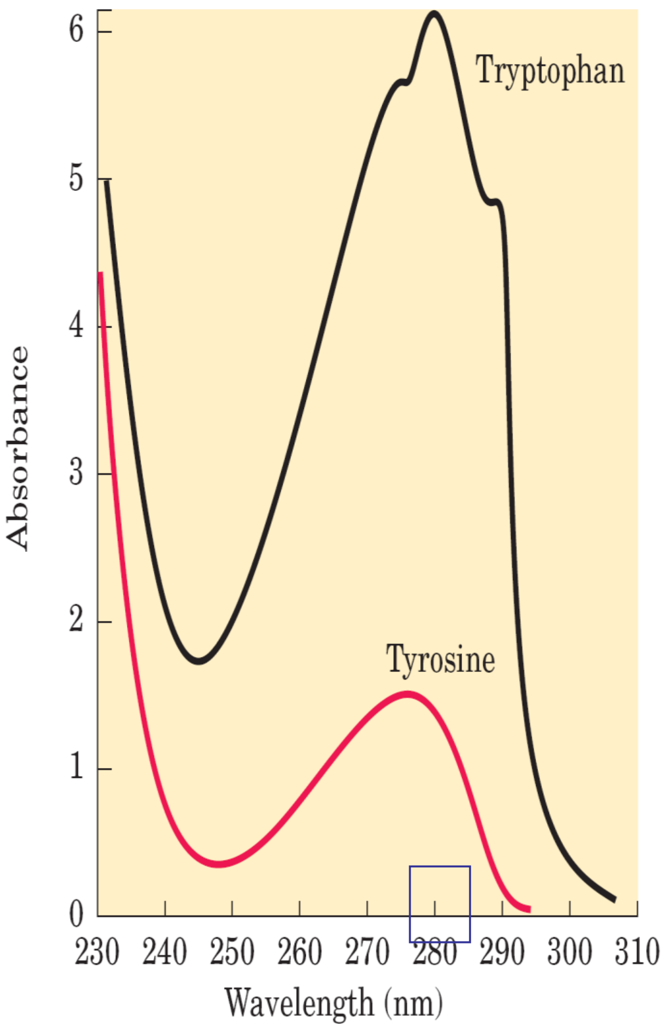


In turns

Aromatic amino acids

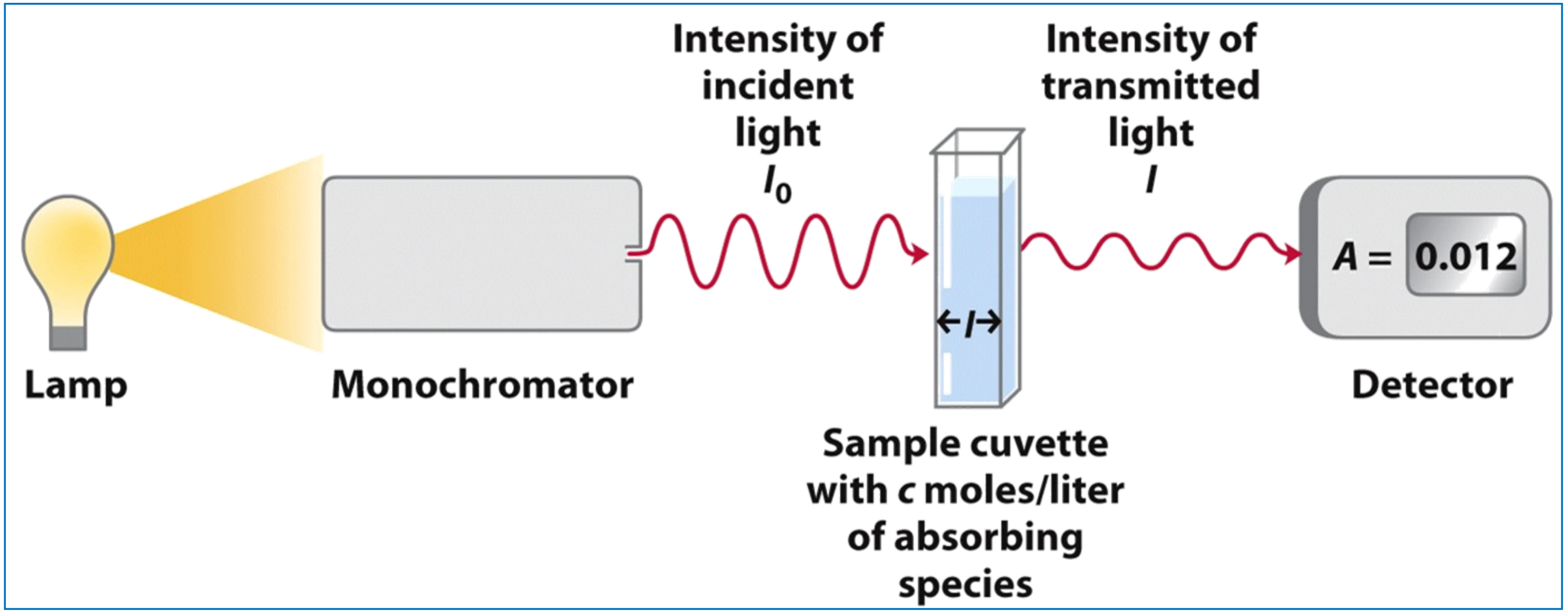


Phenyl group Phenolic group Indolyl group



Phenylalanine: one of the most hydrophobic amino acids.

The principle components of a spectrophotometer



Lambert-Beer Law

$$\log \frac{I_0}{I} = \varepsilon\, c\, l = \text{Absorbance (A)} = \text{Optical density (OD)}$$

I_0= Intensity of the incident light

I= Intensity of the transmitted light

ε = *molecular absorption coefficient*

c = concentration

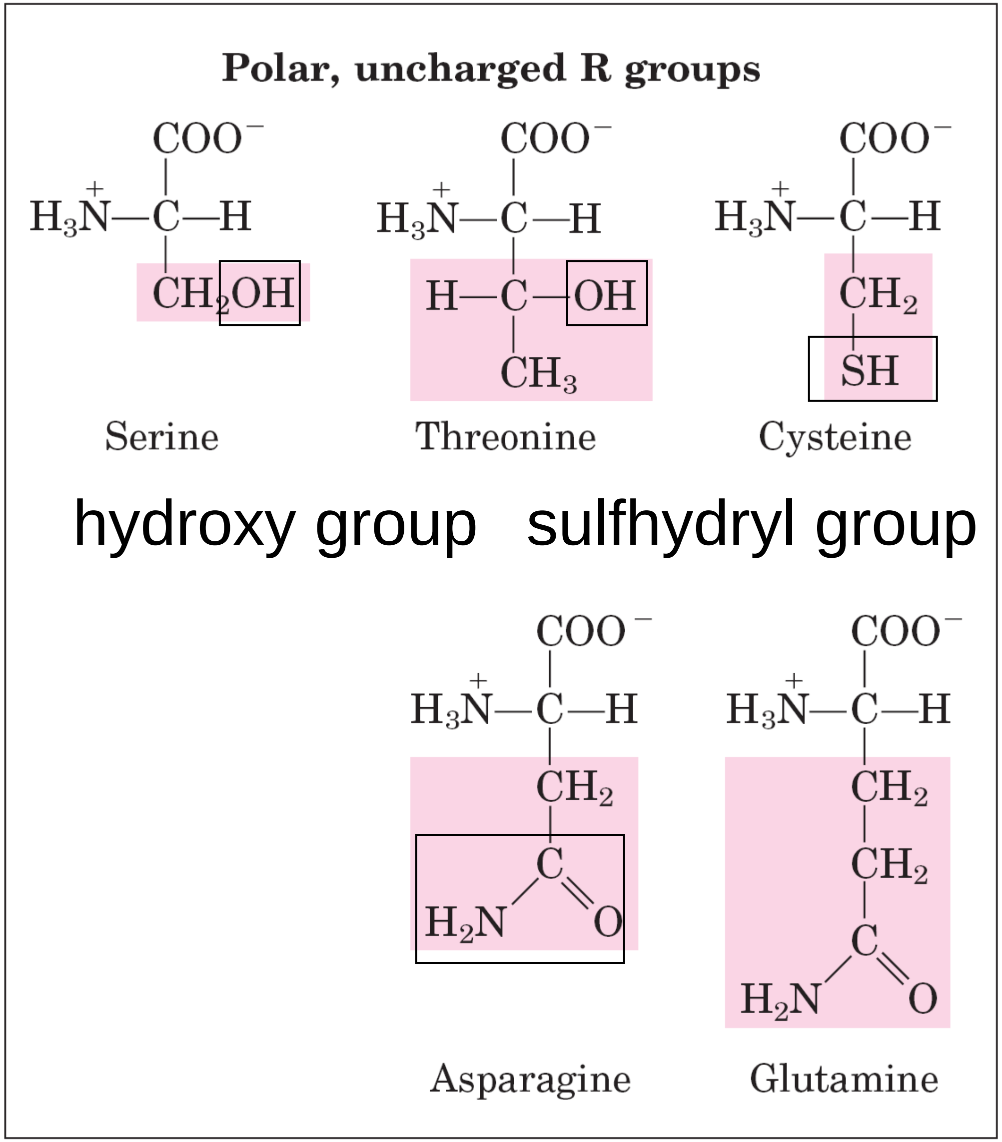
l= path length

OD_{280} = 1.2→ 1 mg/ml

Amino acids with hydroxy or sulfur containing side chains

Hydroxy amino acids

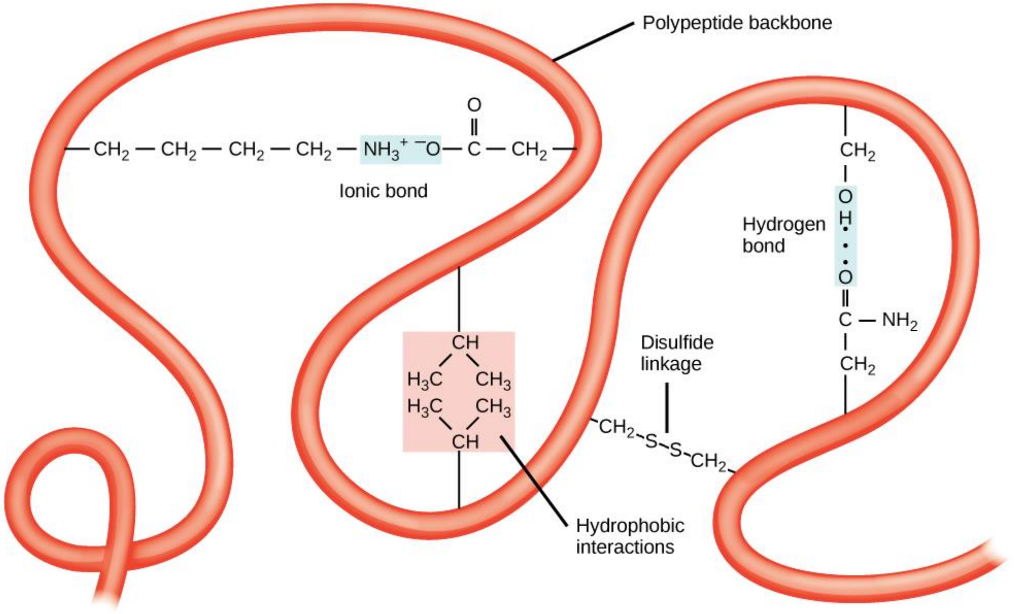
Sulfur amino acids



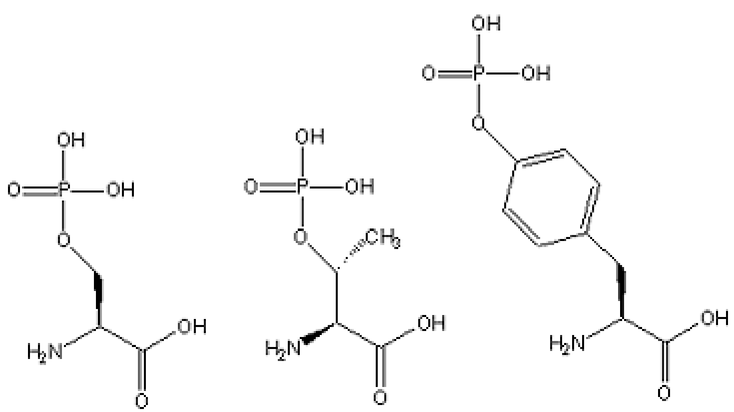
Amide group

Properties of the Amino Acid Side Chains

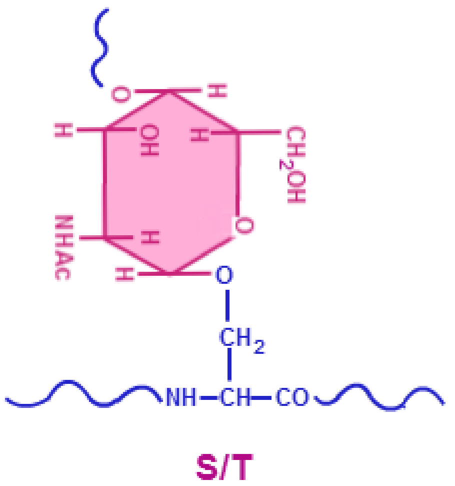
- The –OH group of serine and the –SH group of cysteine are good nucleophiles and often play key roles in enzyme activity.
- Asn and Gln are hydrophilic and tend to be on the surface of a protein molecule, in contact with the surrounding water.



Post transaltional modification



Phospho Serine, Threonine and Tyrosine amino acids



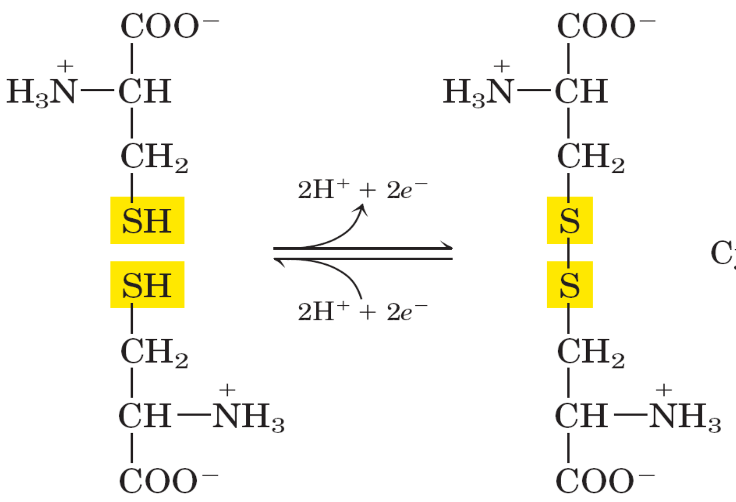
phosphorylation

glycosylation

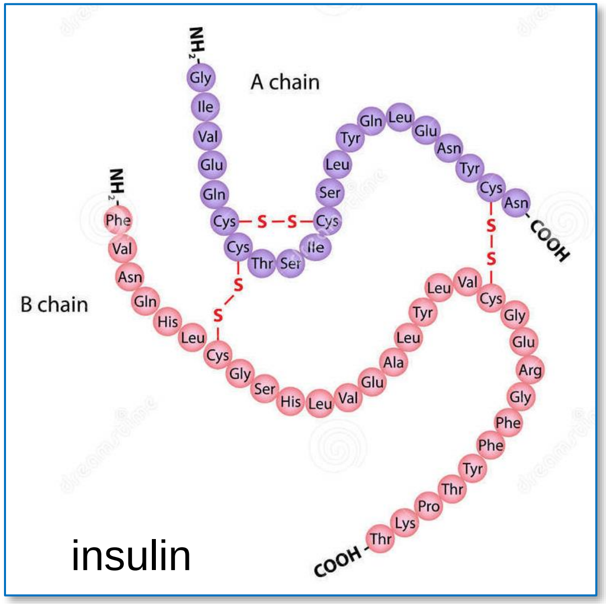
Formation of a disulfide bond by the oxidation of two molecules of cysteine

Cysteine

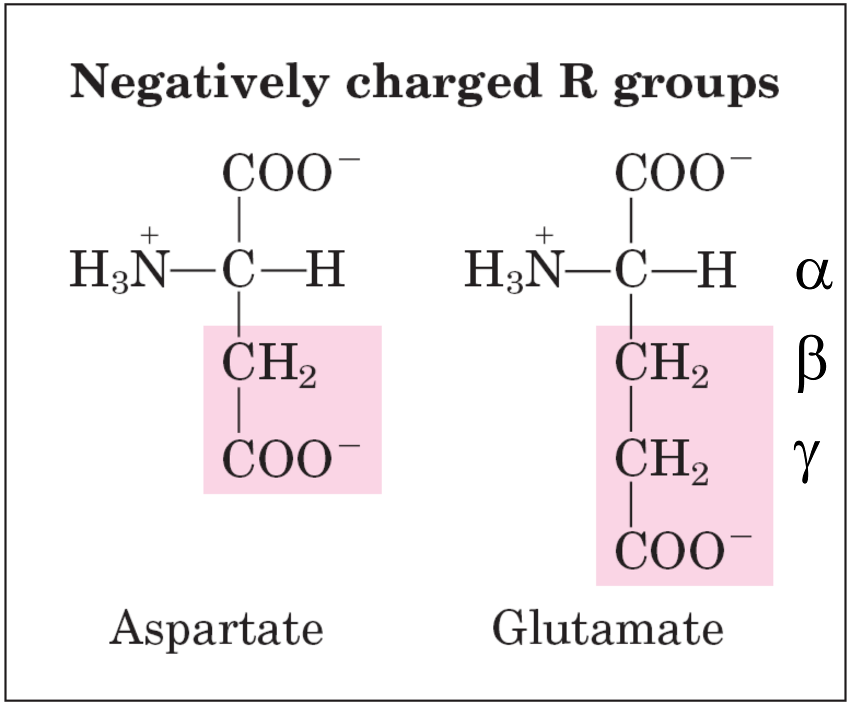
Cysteine



Cystine



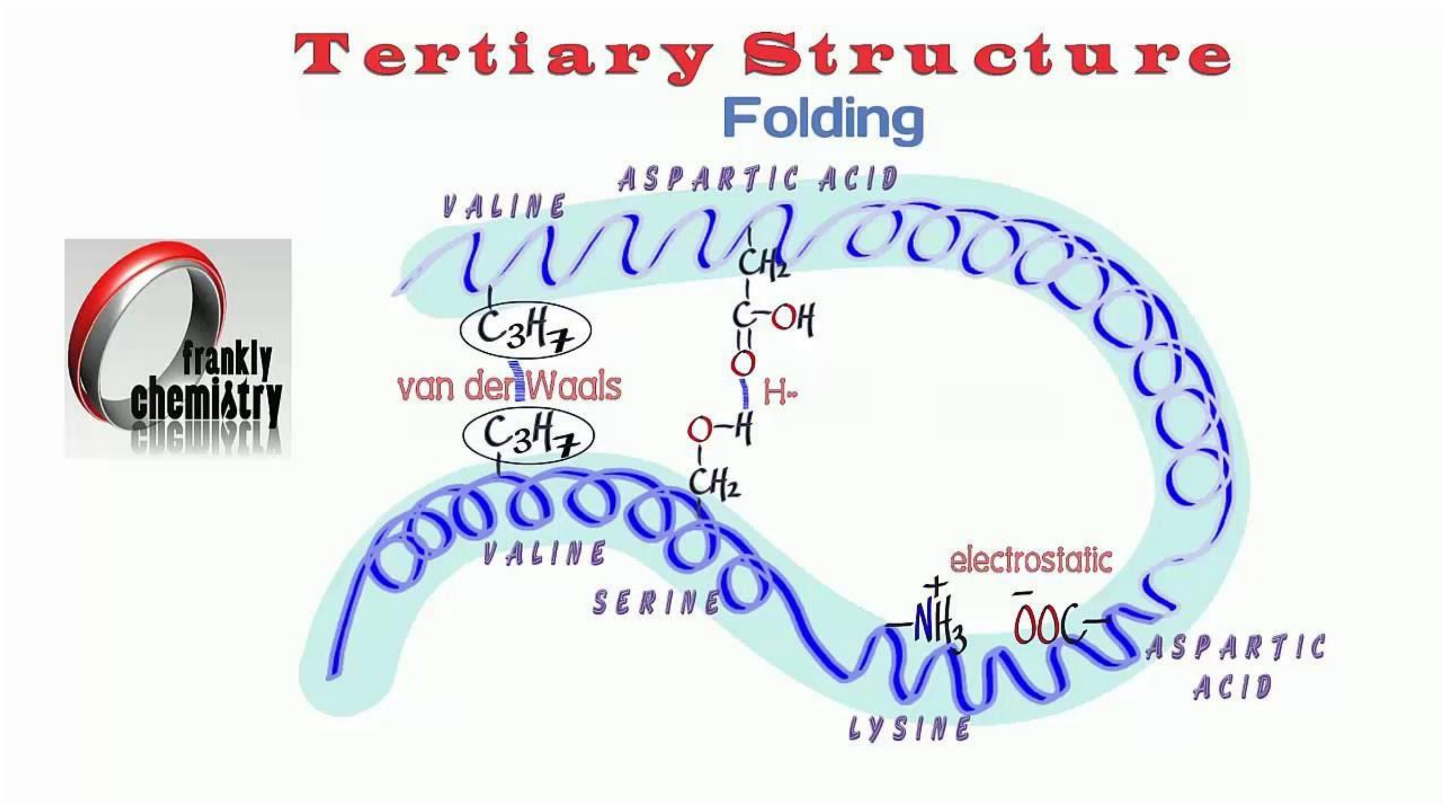
Acidic amino acids



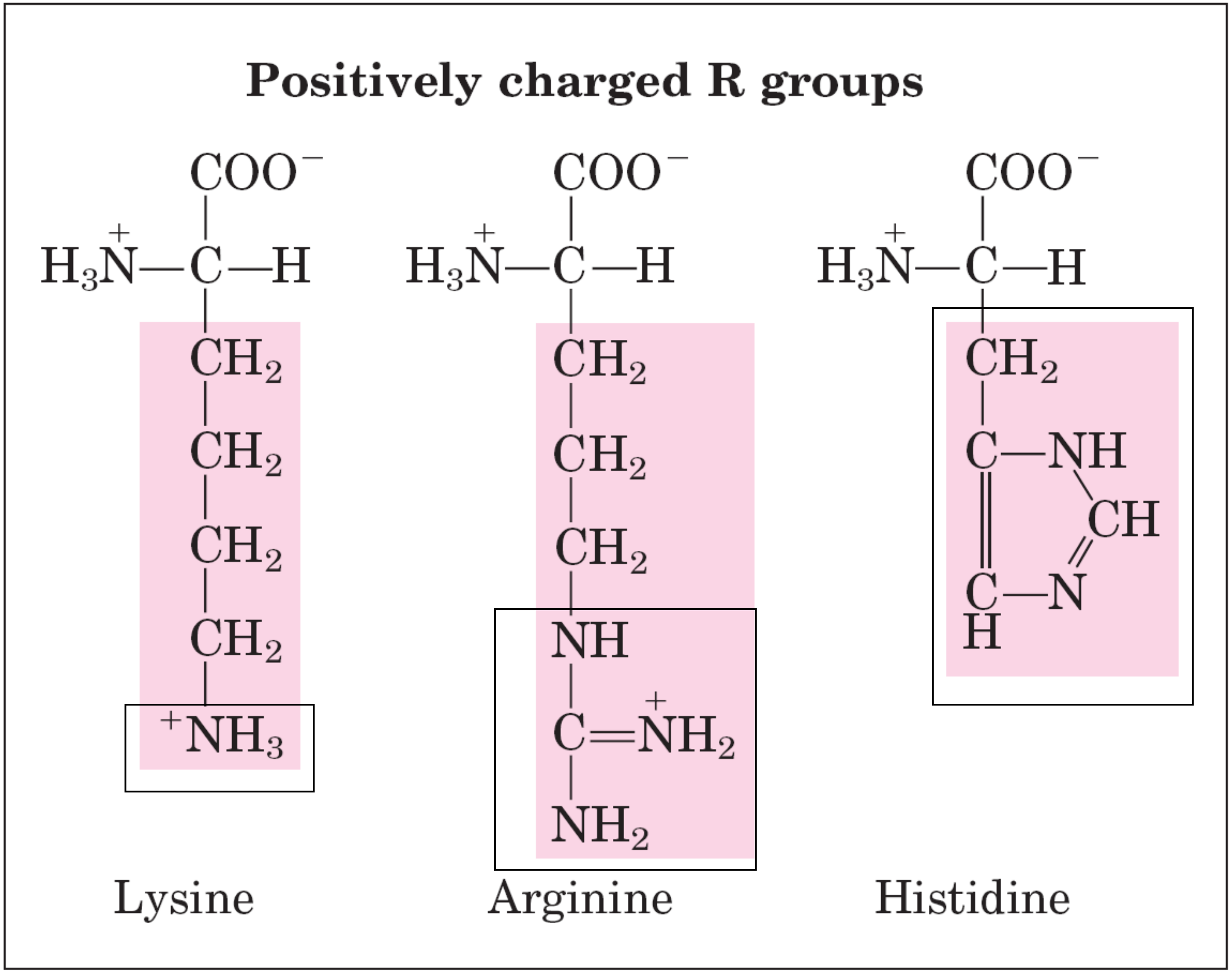
COOH= carboxy group

Properties of the Amino Acid Side Chains

- Aspartic acid (pK_a = 3.9) and glutamic acid (pK_a = 4.2) typically carry <u>negative charges </u>at pH 7.



Basic amino acids



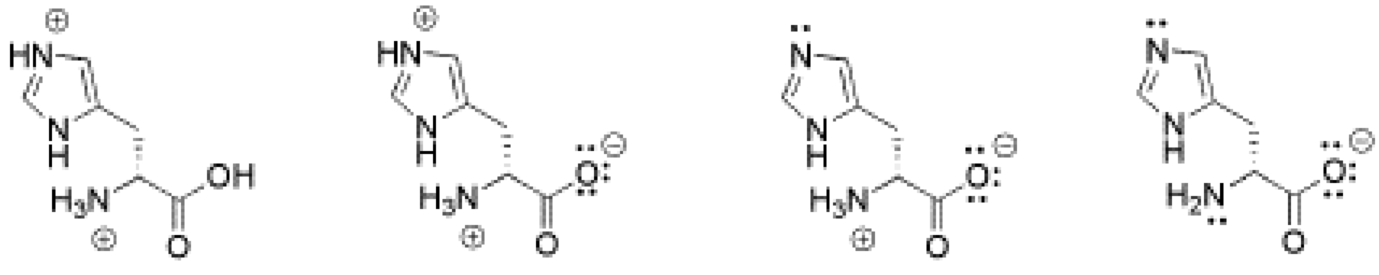
amino group   guanidino group   Imidazole group

Properties of the Amino Acid Side Chains

- Lysine (pK_a = 10.0) and arginine (pK_a = 12.5) are the more basic amino acids.
- Their side chains are almost always positively charged under physiological conditions.

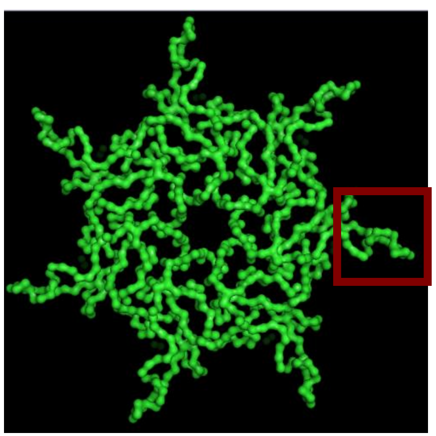
Histidine

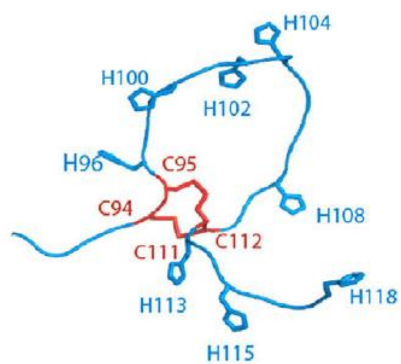
- Histidine contains a side chain imidazole. At the physiological pH (pH ~7.4), the side chains of histidine is only partially charged



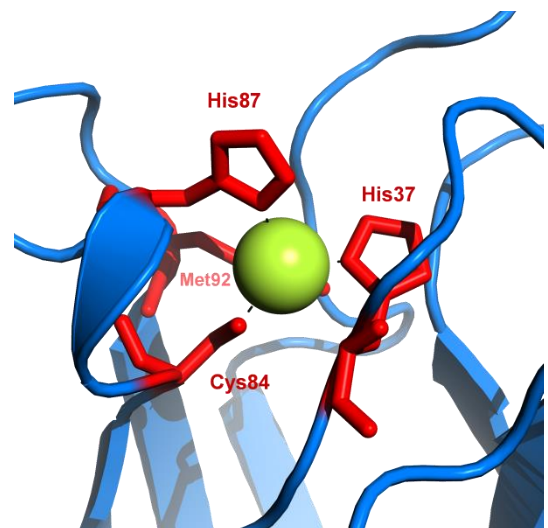
at pH = 1 at pH = 4 at pH = 8 at pH = 11

- The imidazole side chain of histidine is a common coordinating ligand in metalloproteins and is a part of catalytic sites in certain enzymes.

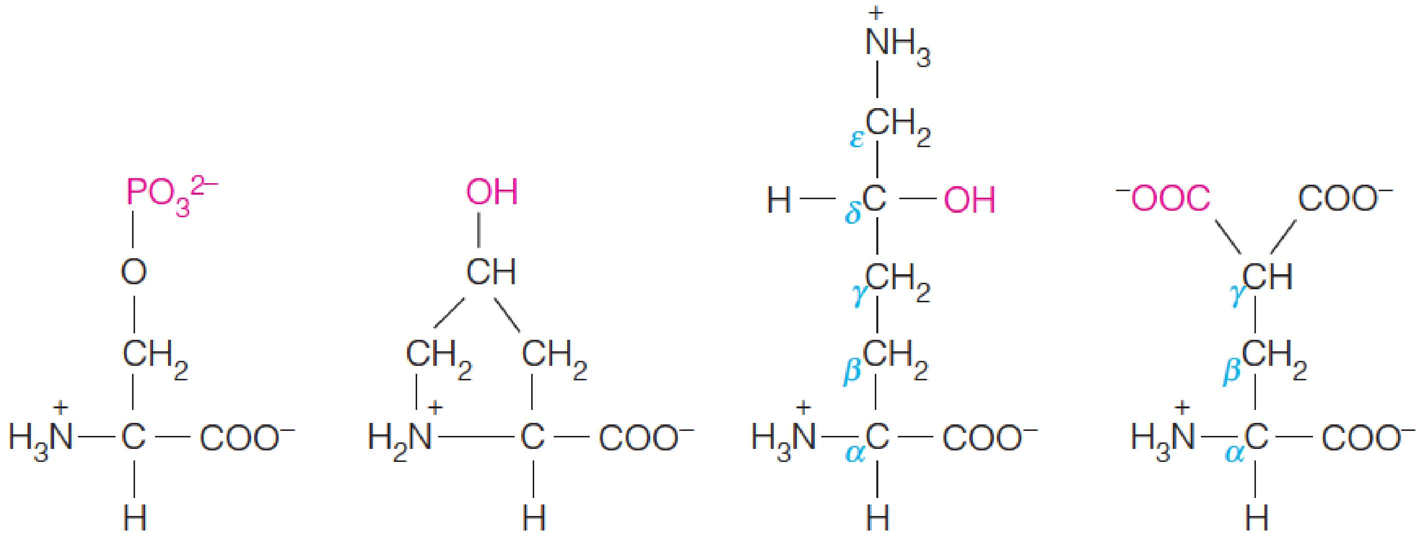




GroES of *H. pylori*

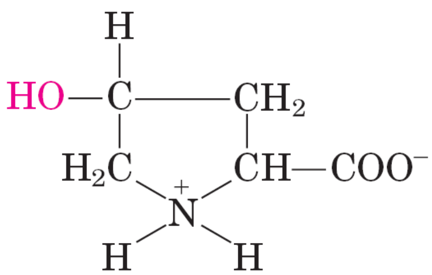


Amino acids can undergo ***post-translational modification*** resulting in modified amino acids with unique properties



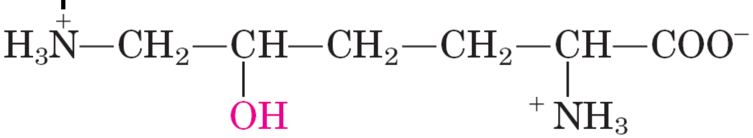
Phosphoserine **4-Hydroxyproline** **δ-Hydroxylysine** **γ-Carboxyglutamic acid**

Nonstandard amino acids



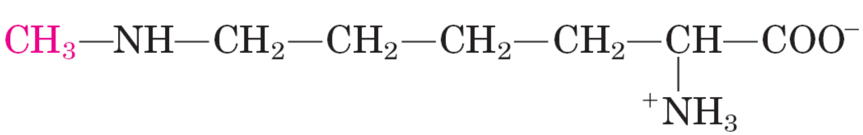
4-Hydroxyproline

plant cell wall connective tissue



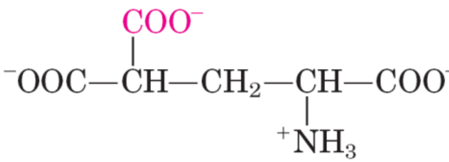
5-Hydroxylysine

collagen



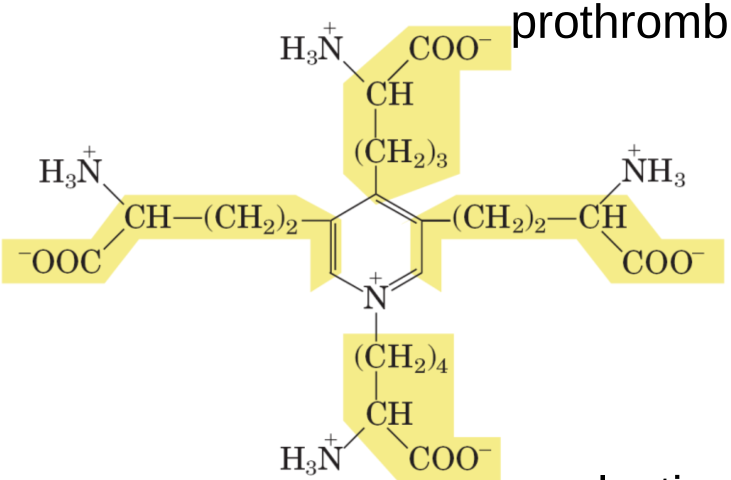
6-*N*-Methyllysine

myosin



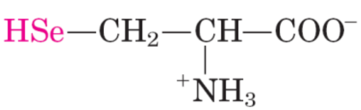
γ-Carboxyglutamate

blood-clotting protein prothrombin



Desmosine

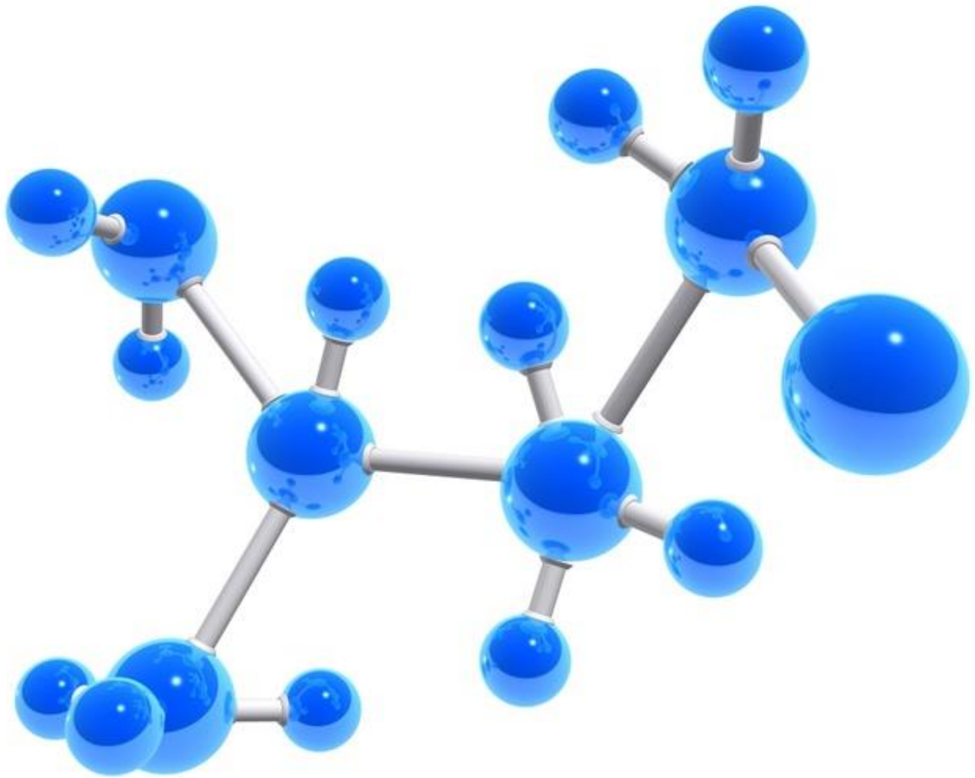
elastin

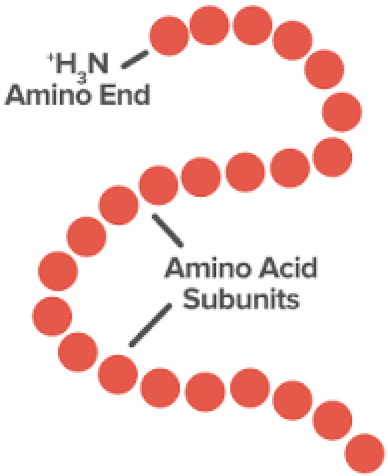


Selenocysteine

glutathione peroxidase

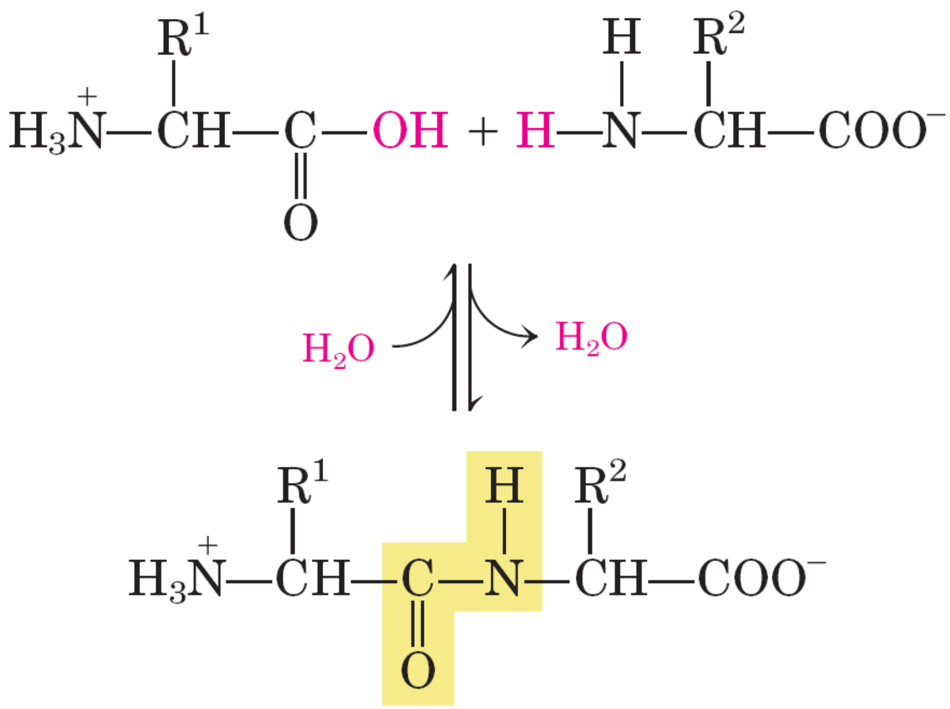
Peptides





Peptides: polymers of amino acids

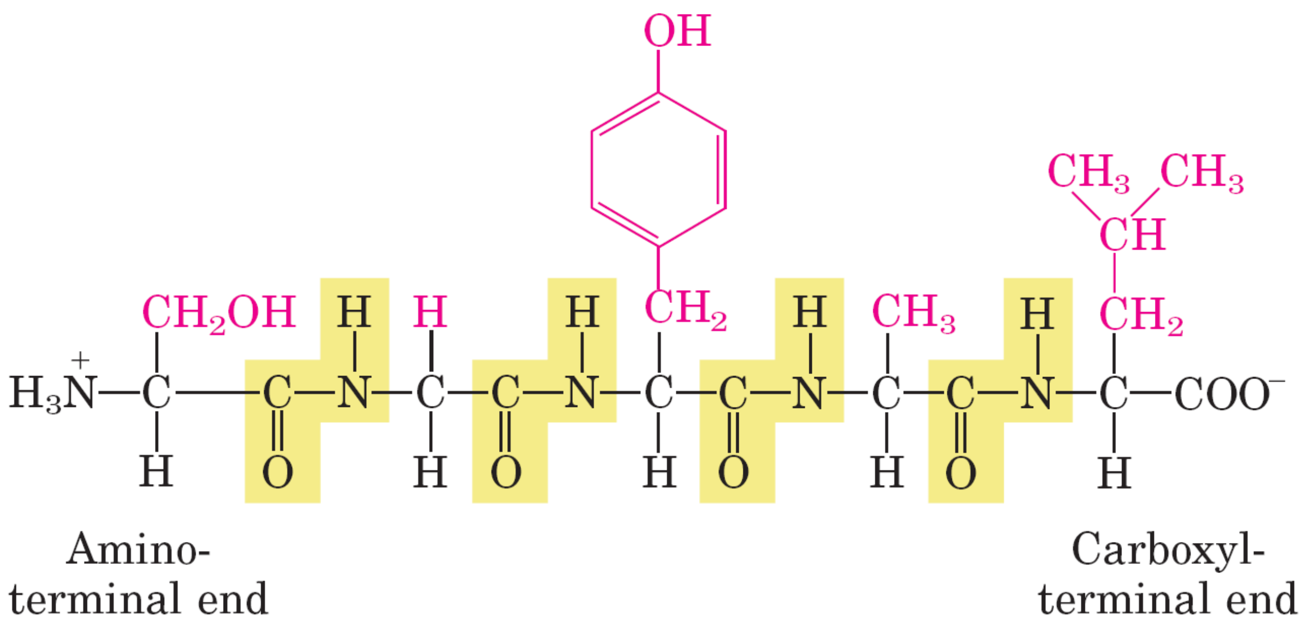
Peptide bond: two amino acid molecules can be covalently joined through a substituted amide linkage



Formation of a peptide bond by condensation

Oligopeptide: a few amino acids are joined, the structure is called an oligopeptide

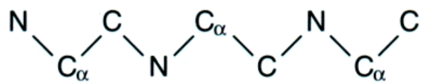
Polypeptide: many amino acids are joined, the product is called polypeptide



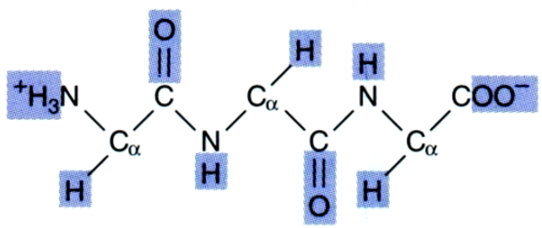
The pentapeptide serylglycyltyrosylalanylleucine, or Ser-Gly-Tyr-Ala-Leu

How to draw the peptide structure

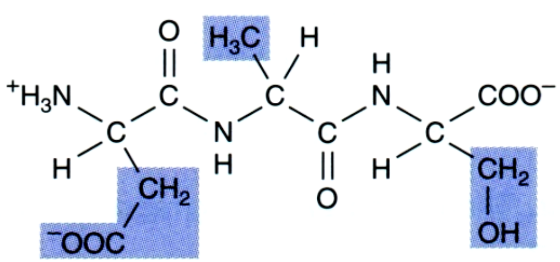
Write a zigzag formed from the repeating sequence of main chain, or "backbone," atoms: α-nitrogen, α-carbon, carbonyl carbon.



Complete the amino and carboxyl terminals, add a hydrogen to each α-carbon and to each peptide nitrogen, and add oxygen to the carbonyl carbon.



Add appropriate R groups (shaded) to each α-carbon atom.



Abbreviations

Glu-Ala-Lys-Gly-Tyr-Ala

E A K G Y A

Amino acid	pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)
	pK_a values		
Nonpolar, aliphatic R groups			
Glycine	$\boxed{2.34}$	9.60	
Alanine	2.34	9.69	
Proline	1.99	10.96	
Valine	2.32	9.62	
Leucine	2.36	9.60	
Isoleucine	2.36	9.68	
Methionine	2.28	9.21	
Aromatic R groups			
Phenylalanine	1.83	9.13	
Tyrosine	2.20	9.11	10.07
Tryptophan	2.38	9.39	
Polar, uncharged R groups			
Serine	2.21	9.15	
Threonine	2.11	9.62	
Cysteine	1.96	10.28	8.18
Asparagine	2.02	8.80	
Glutamine	2.17	9.13	
Positively charged R groups			
Lysine	2.18	8.95	10.53
Histidine	1.82	9.17	$\boxed{6.00}$
Arginine	2.17	9.04	$\boxed{12.48}$
Negatively charged R groups			
Aspartate	1.88	9.60	3.65
Glutamate	2.19	$\boxed{9.67}$	$\boxed{4.25}$

Net Electric Charge of Peptides

NH_3^+-Glu-His-Trp-Ser-Gly-Leu-Arg-Pro-Gly-COOH

9.67 4.25 6.0 12.48 2.34

+3		+2		+1		0		-1		-2
	2.34 pK_1		4.25 pK_2		6.0 pK_3		9.67 pK_4		12.48 pK_5	

pH=3 +2

pH=8 0

pH=11 -1

$$pI = \frac{6.0+9.67}{2} = 7.835$$

Many peptides have physiologic activity

A ***neuropeptide*** is a peptide that is active in association with neural tissue.

A ***lipopeptide*** is a peptide that has a lipid connected to it, and ***pepducins*** are new modulators of G protein–coupled receptors (GPCRs)

A ***peptide hormone*** is a peptide that acts as a hormone.

A ***proteose*** is a mixture of peptides produced by the hydrolysis of proteins.

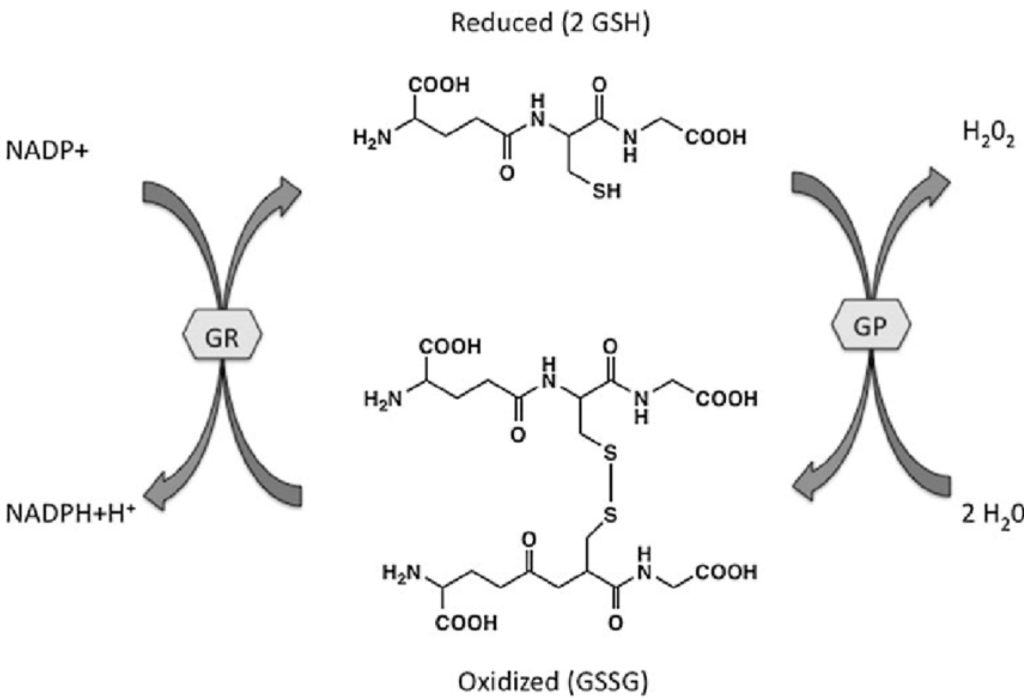
Many peptides have physiologic activity

a. Glutathione (GSH)

γ-Glu-Cys-Gly (tripeptide)

$$2GSH + X_{oxidized} \longrightarrow GSSG + X_{reduced}$$

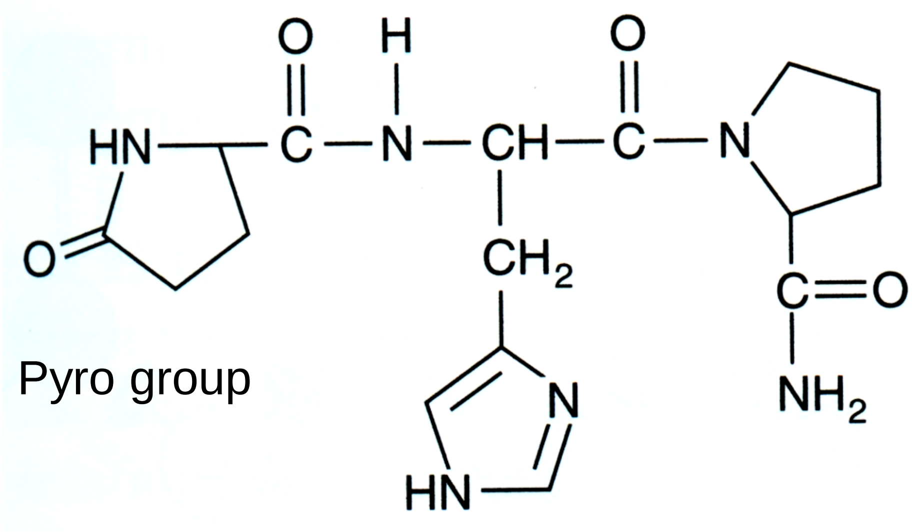
(Glutathione disulfide)



Glutathione (GSH) is an important antioxdant agent.

b. TRH (Thyrotropin-releasing hormone)

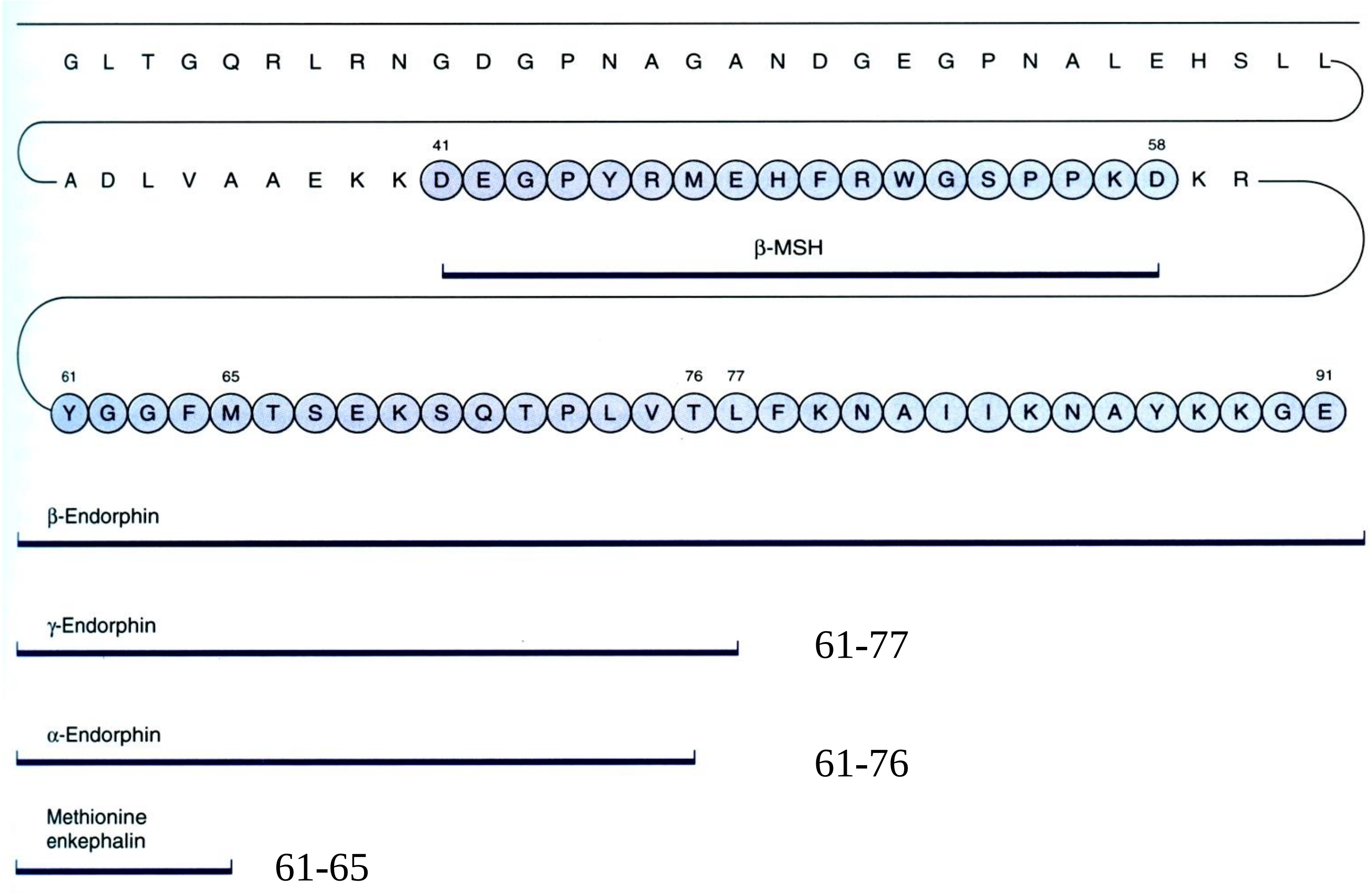
<Glu-His-Pro-NH_2



由下視丘（Hypothalamus）所製造的肽類激素（Peptide Hormone）

，能夠調節腦下垂體前葉分泌促甲狀腺激素（甲促素，thyroid stimulating hormone、TSH）與催乳素

c. β-lipotropin



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61-76

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腦垂體分泌的一種激素，可分解脂肪

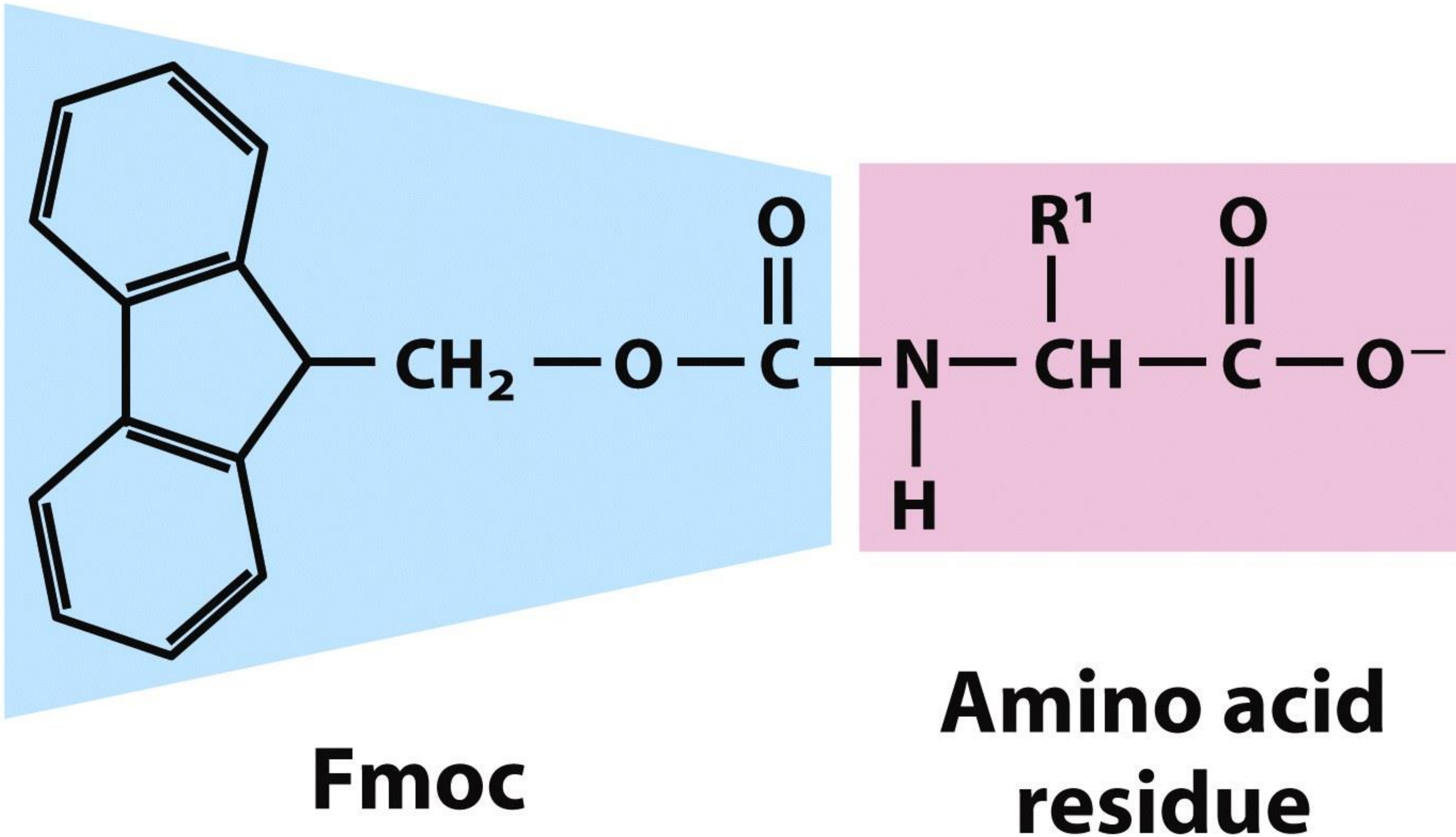
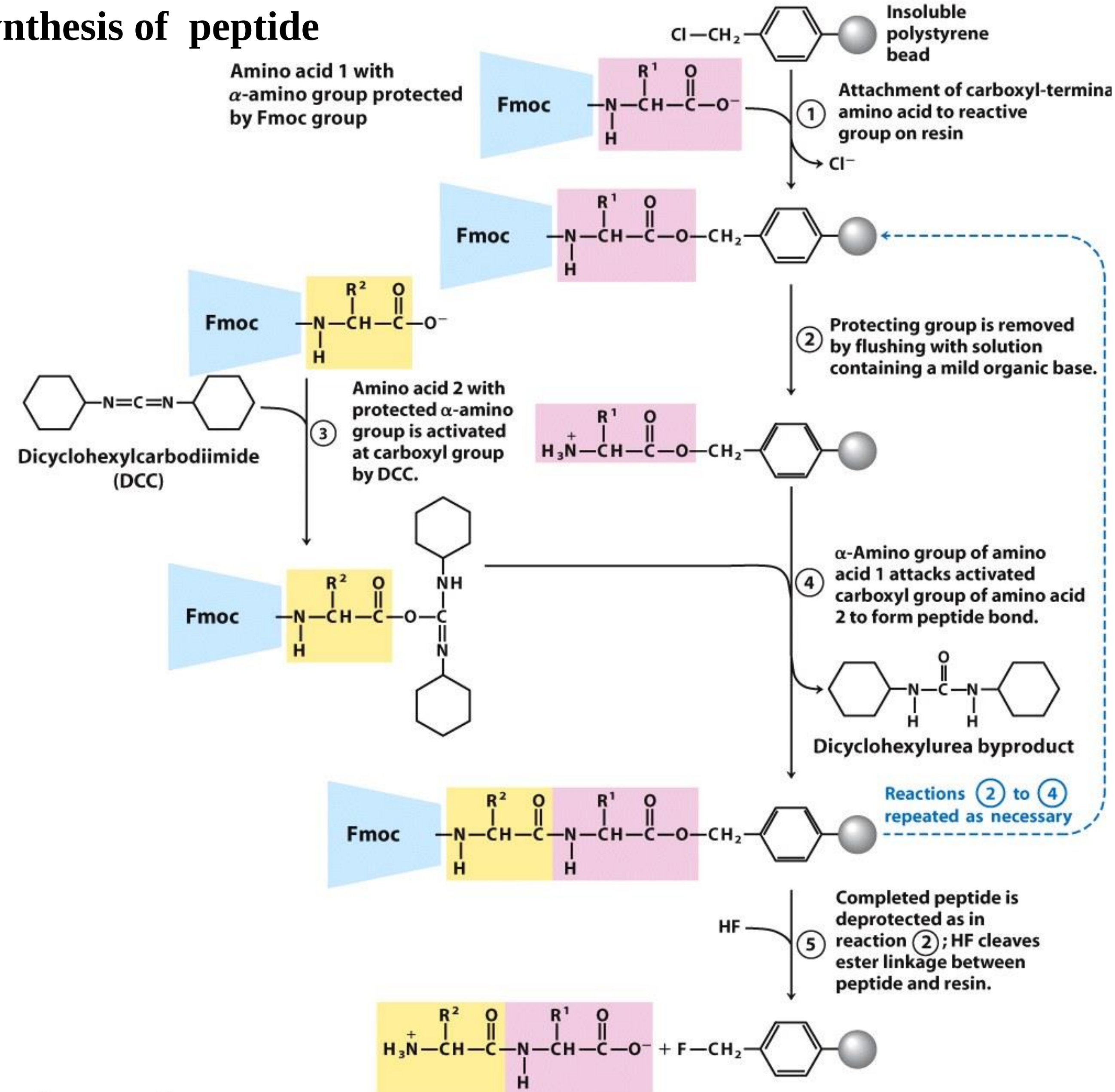


Figure 3-29a
Lehninger Principles of Biochemistry, Fifth Edition

Solid phase synthesis of peptide

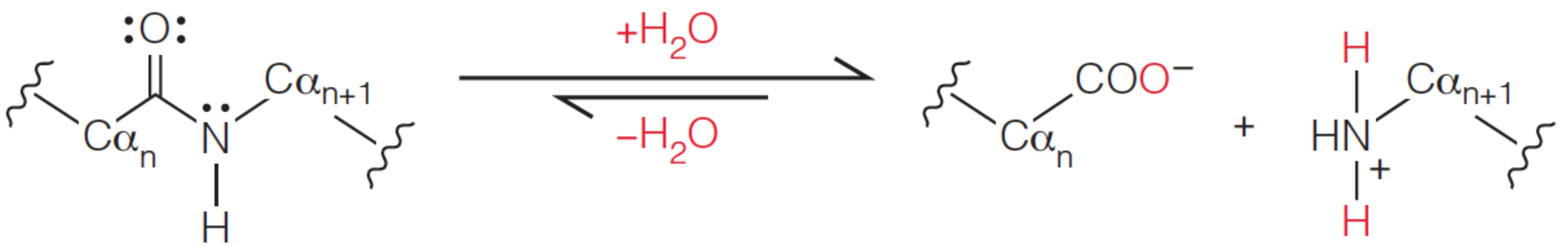


R. Bruce Merrifield
1921–2006

Unnumbered 3 p101
Lehninger Principles of Biochemistry, Fifth Edition

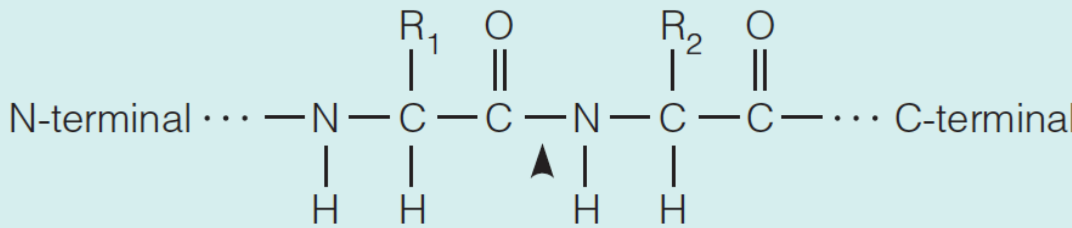
Figure 3-29b
Lehninger Principles of Biochemistry, Fifth Edition

Peptides and the peptide bond



- While hydrolysis of peptide bonds is thermodynamically favored in aqueous solutions, the reaction is exceedingly slow at physiological pH and temperature
- Peptide bond hydrolysis can be achieved by:
  - Strong mineral acid (*e.g.*, 6 *M* HCl) cleaves all peptide bonds (including the Asn and Gln amide bonds)
  - Chemicals that cleave at specific sites (*e.g.*, CNBr cleaves at Met)
  - Proteolytic enzymes (proteases) that cleave at specific sites

Sequence specificities of some proteolytic enzymes



Enzyme	Preferred Site[a]	Source
Trypsin	R_1 = Lys, Arg	From digestive systems of animals, many other sources
Chymotrypsin	R_1 = Tyr, Trp, Phe, Leu	Same as trypsin
Thrombin	R_1 = Arg	From blood; involved in coagulation
V-8 protease	R_1 = Asp, Glu	From *Staphylococcus aureus*
Prolyl endopeptidase	R_1 = Pro	Lamb kidney, other tissues
Subtilisin	Very little specificity	From various bacilli
Carboxypeptidase A	R_2 = C-terminal amino acid	From digestive systems of animals
Thermolysin	R_2 = Leu, Val, Ile, Met	From *Bacillus thermoproteolyticus*

Separation of peptides