Amino acid

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- Protein are the most abundant biological macromolecules, occurring in all cells and all parts of cells.
- Proteins also occur in great variety; thousands of different kinds may be found in a single cell.
- The proteins of every organism, from the simplest of bacteria to human beings, are constructed from the same set of 20 amino acids
- These 20 amino acids in many different combinations and Sequences
- enzymes, hormones, antibodies, transporters, muscle fibers, the lens protein of the eye, feathers, spider webs, rhinoceros horn, milk proteins, antibiotics, mushroom poisons, and myriad other substances having distinct biological activities

Amino acids

- Proteins are polymers of amino acids, with each **amino acid residue** joined to its neighbor by a specific type of covalent bond.
- Asparagine was first found in asparagus, and
- glutamate in wheat gluten
- tyrosine was first isolated from cheese
- glycine was so named because of its sweet taste.

Amino Acids Share Common Structural Features

- All 20 of the common amino acids are α-amino acids. They have a carboxyl group and an amino group bonded to the same carbon atom
- They differ from each other in their side chains, or **R groups**, which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water

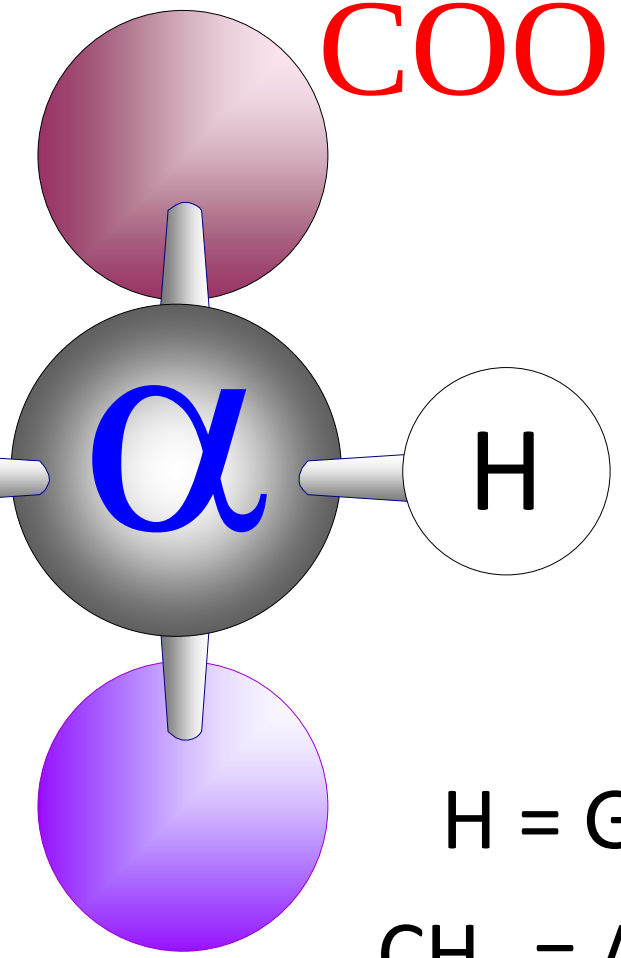
Amino Acid Structure

Carboxylic group

COO^-

Amino group

$+H_3N$



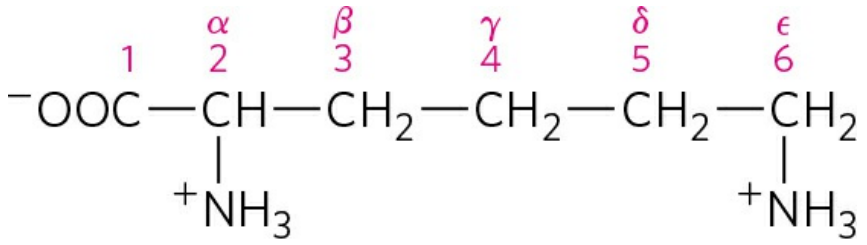
R group

H = Glycine

CH_3 = Alanine

- For all the common amino acids except glycine, the α carbon is bonded to four different groups: a carboxyl group, an amino group, an R group, and a hydrogen atom
- The α-carbon atom is thus a **chiral cente**
- tetrahedral arrangement of the bonding orbitals around the α-carbon atom, the four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible stereoisomers.
- Since they are nonsuperposable mirror images of each other, the two forms represent a class of stereoisomers called **enantiomers**

- The additional carbons in an R group are commonly designated β, γ, δ, ε, and so, proceeding out from the α carbon



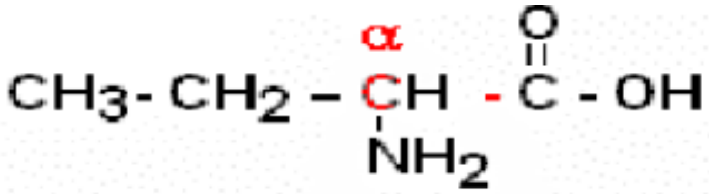
Lysine

Amino acids may be characterized as α, β , or γ amino acids depending on the location of the amino group in the carbon chain.

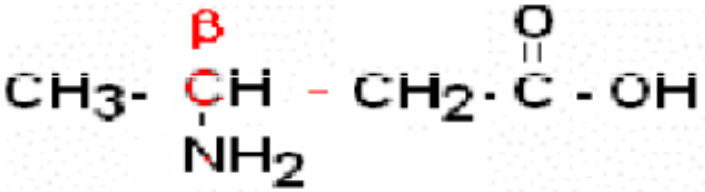
α are on the carbon adjacent to the carboxyl group.

β are on the 2nd carbon

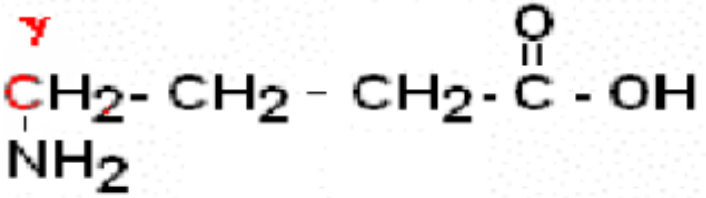
γ on the 3rd carbon from the carboxyl group



α– aminobutanoic acid



β – aminobutanoic acid



γ – aminobutanoic acid

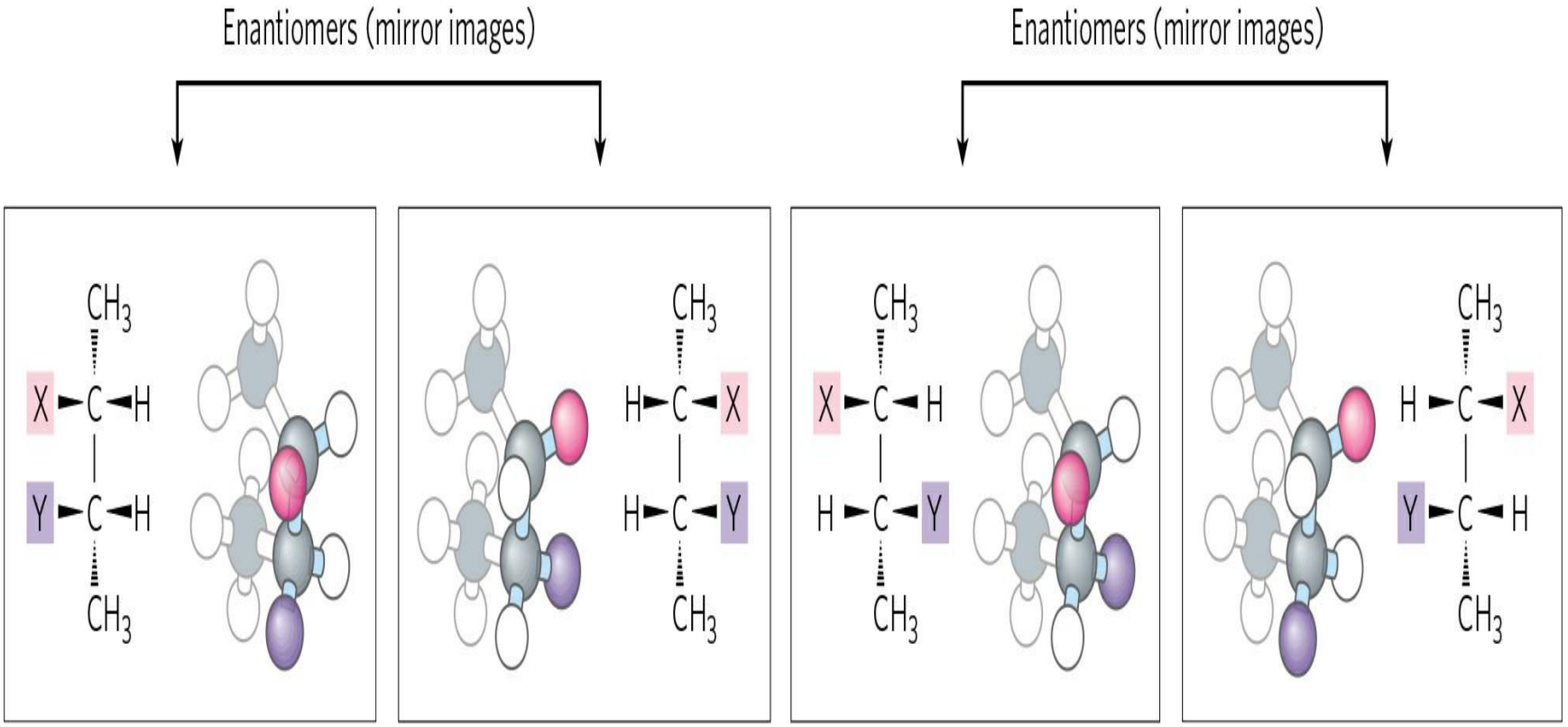
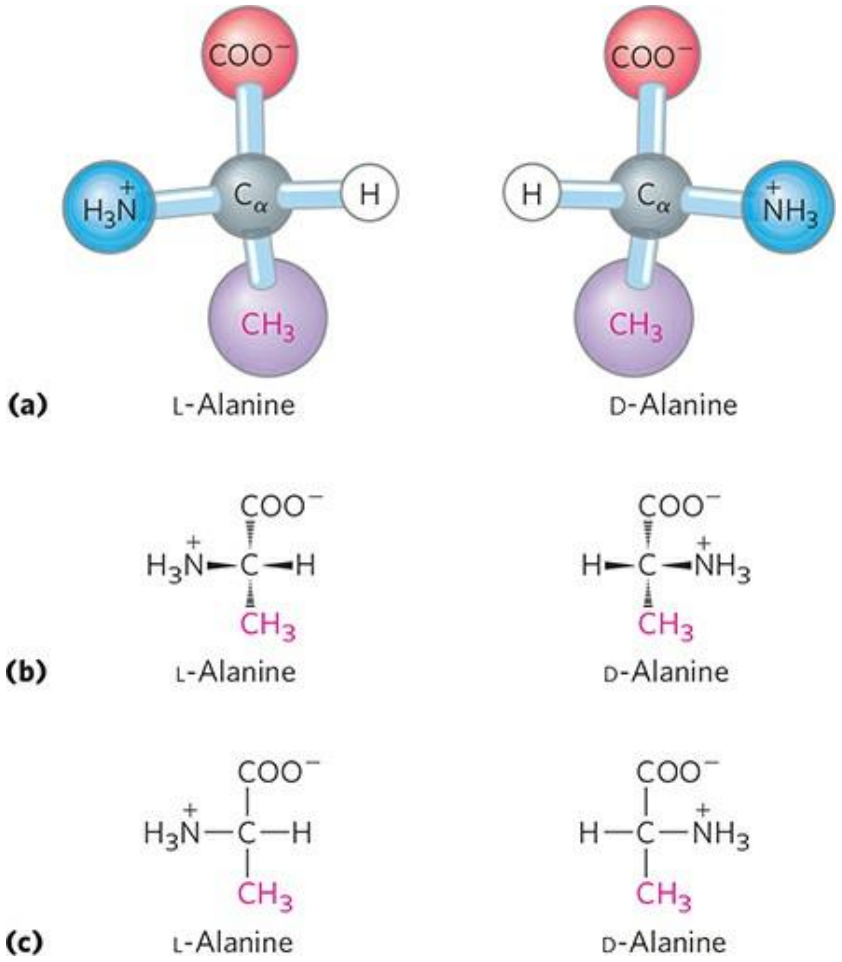
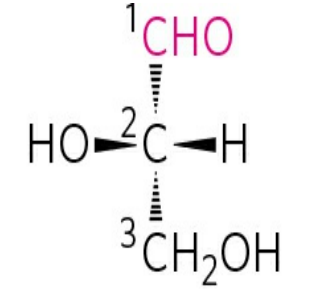

Enantiomers (mirror images)
Enantiomers (mirror images)
CH3
X
C
H
Y
C
H
CH3
CH3
H
C
X
H
C
Y
CH3
CH3
X
C
H
H
C
Y
CH3
CH3
H
C
X
Y
C
H
CH3

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

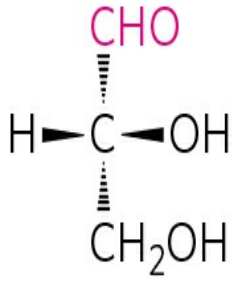
Amino acid	Abbreviation/symbol	M_r[a]	pK_a values: pK_1 (—OCOOH)	pK_2 (–NH$_3^+$)	pK_R (R group)	pI	Hydropathy index[b]
Nonpolar, aliphatic R groups							
Glycine	Gly G	75	2.34	9.60		5.97	−0.4
Alanine	Ala A	89	2.34	9.69		6.01	1.8
Proline	Pro P	115	1.99	10.96		6.48	−1.6[d]
Valine	Val V	117	2.32	9.62		5.97	4.2
Leucine	Leu L	131	2.36	9.60		5.98	3.8
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5
Methionine	Met M	149	2.28	9.21		5.74	1.9
Aromatic R groups							
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9
Polar, uncharged R groups							
Serine	Ser S	105	2.21	9.15		5.68	−0.8
Threonine	Thr T	119	2.11	9.62		5.87	−0.7
Cysteine[e]	Cys C	121	1.96	10.28	8.18	5.07	2.5
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5
Positively charged R groups							
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5
Negatively charged R groups							
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5



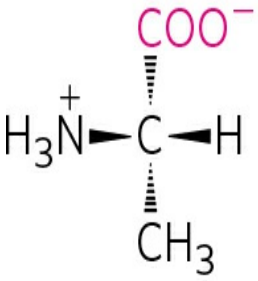
- **(a)** The two stereoisomers of alanine, L- and D-alanine, are nonsuperposable mirror images of each other (enantiomers).
- **(b, c)** Two different conventions for showing the configurations in space of stereoisomers. In perspective formulas (b), the solid wedge-shaped bonds project out of the plane of the paper, the dashed bonds behind it.
- In projection formulas (c), the horizontal bonds are assumed to project out of the plane of the paper, the vertical bonds behind. However, projection formulas are often used casually and are not always
- intended to portray a specific stereochemical configuration.



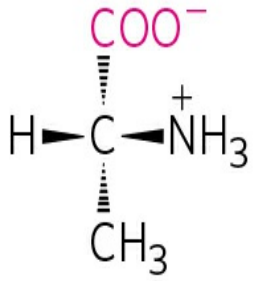
L-Glyceraldehyde



D-Glyceraldehyde



L-Alanine



D-Alanine

- The absolute configurations of simple sugars and amino acids are specified by the **D, L system**
- For all chiral compounds, stereoisomers having a configuration related to that of L-glyceraldehyde are designated L,

  and stereoisomers related to D-glyceraldehyde are designated D.
- The functional groups of L-alanine are matched with those of L-glyceraldehyde by aligning those that can be interconverted by simple, one-step chemical reactions
- the carboxyl group of L-alanine occupies the same position about the chiral carbon as does the aldehyde group of L-glyceraldehyde, because an aldehyde is readily converted to a carboxyl group via a one-step oxidation

The Amino Acid Residues in Proteins Are L Stereoisomers

- In living system, D and L isomers are as different as the right hand and the left. The formation of stable, repeating substructures in proteins generally requires that their constituent amino acids be of one stereochemical series.
- Cells are able to specifically synthesize the L isomers of amino acids because the active sites of enzymes are asymmetric, causing the reactions they catalyze to be stereospecific.

Amino Acids Can Be Classified by R Group

- The amino acids into five main classes based on the properties of their R groups, particularly their **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)

CLASSIFICATION OF AMINOACIDS

1.Based on structure :

A. Aliphatic amino acids:

a. Mono amino mono carboxylic acids

b. Mono amino dicarboxylic acids

c. Dibasic monocarboxylic acids

B. Aromatic amino acids

C. Heterocyclic amino acids

D. Imino acid

E. Derived Amino acids

1.Based on structure:-

A. Aliphatic amino acids:

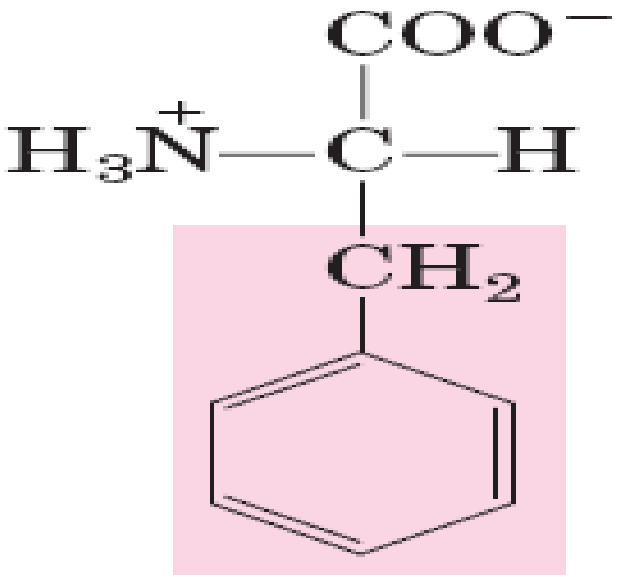
a. Mono amino mono carboxylic acids:

1. Simple amino acids: glycine, alanine
2. Branched chain amino acids: Valine, Leucine, Isoleucine
3. Hydroxy amino acids: Serine, Threonine
4. Sulphur-containing amino acids: Cysteine, Methionine
5. Amino acids with amide group: Asparagine, Glutamine

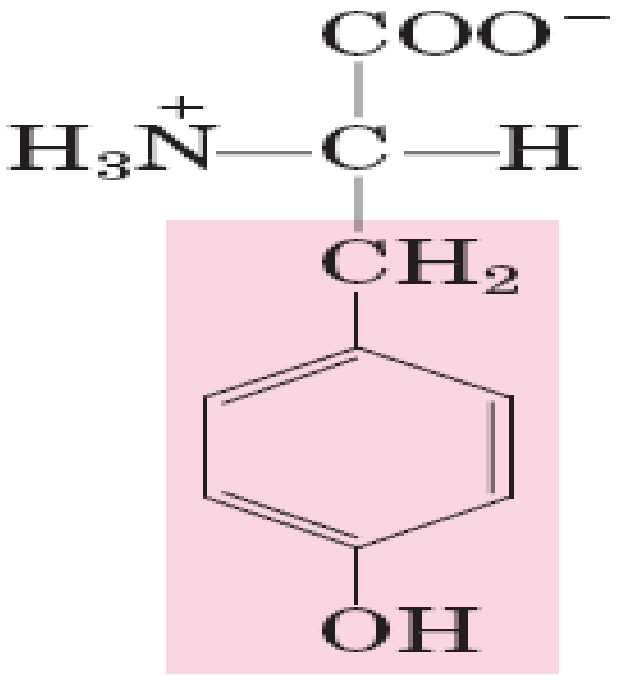
b.Mono amino dicarboxylic acids: Aspartic acid, Glutamic acid

c. Di basic mono carboxylic acids: Lysine, Arginine

B.Aromatic amino acids:



Phenylalanine

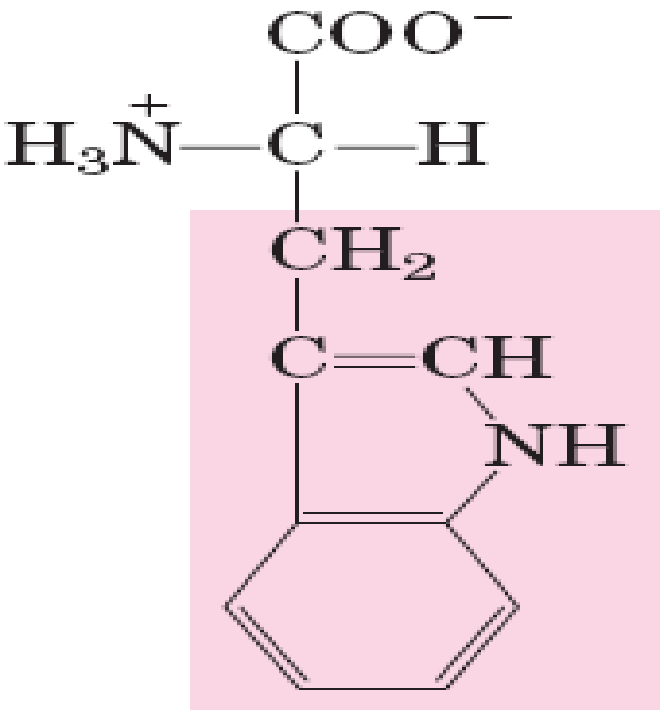


Tyrosine

Phe;F; Benzene gp

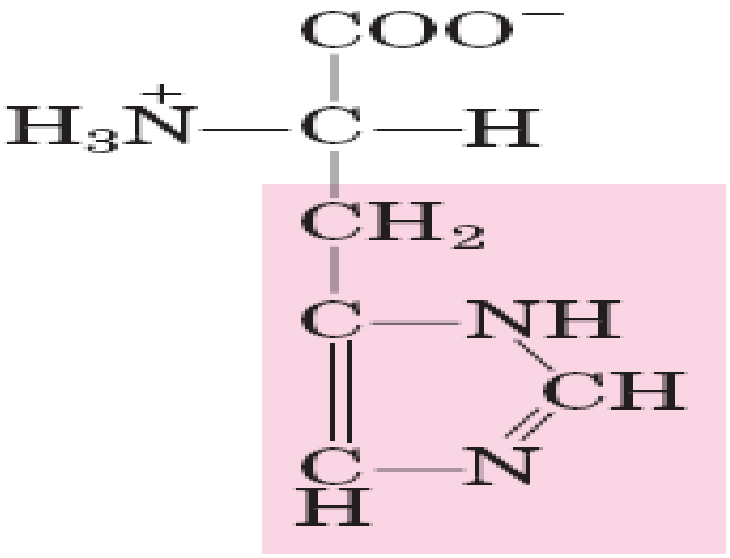
Tyr;Y; Phenol gp

C.Heterocyclic amino acids:



Tryptophan

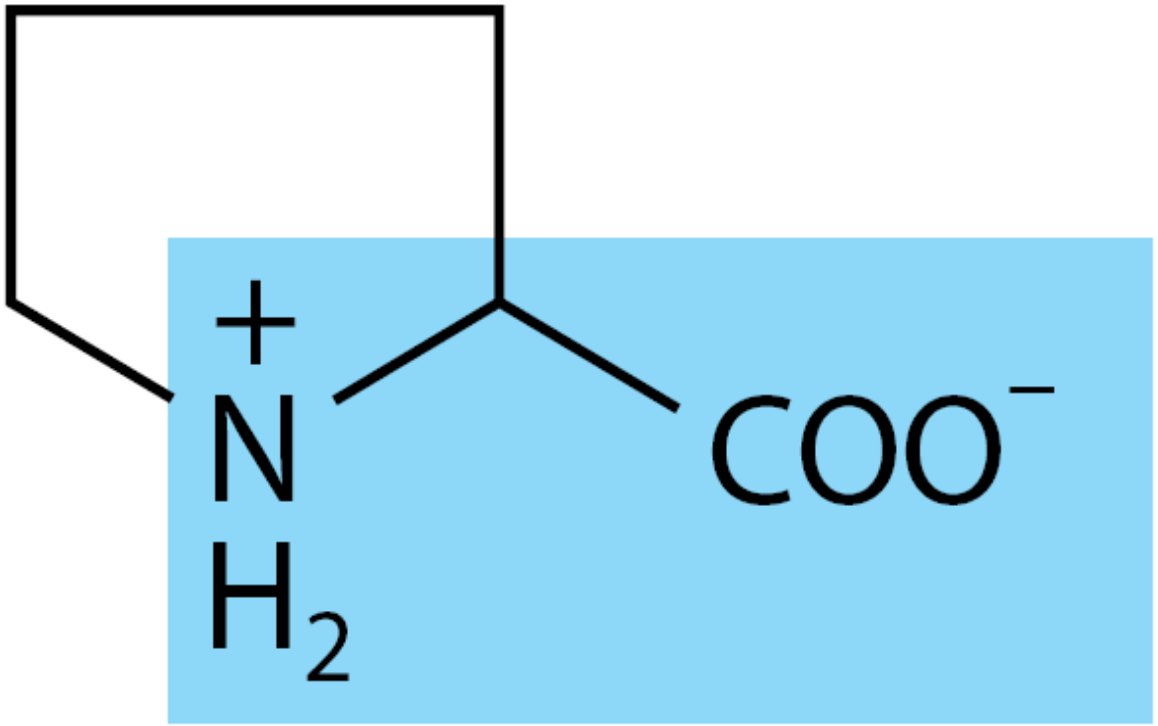
Trp;W; Indole gp



Histidine

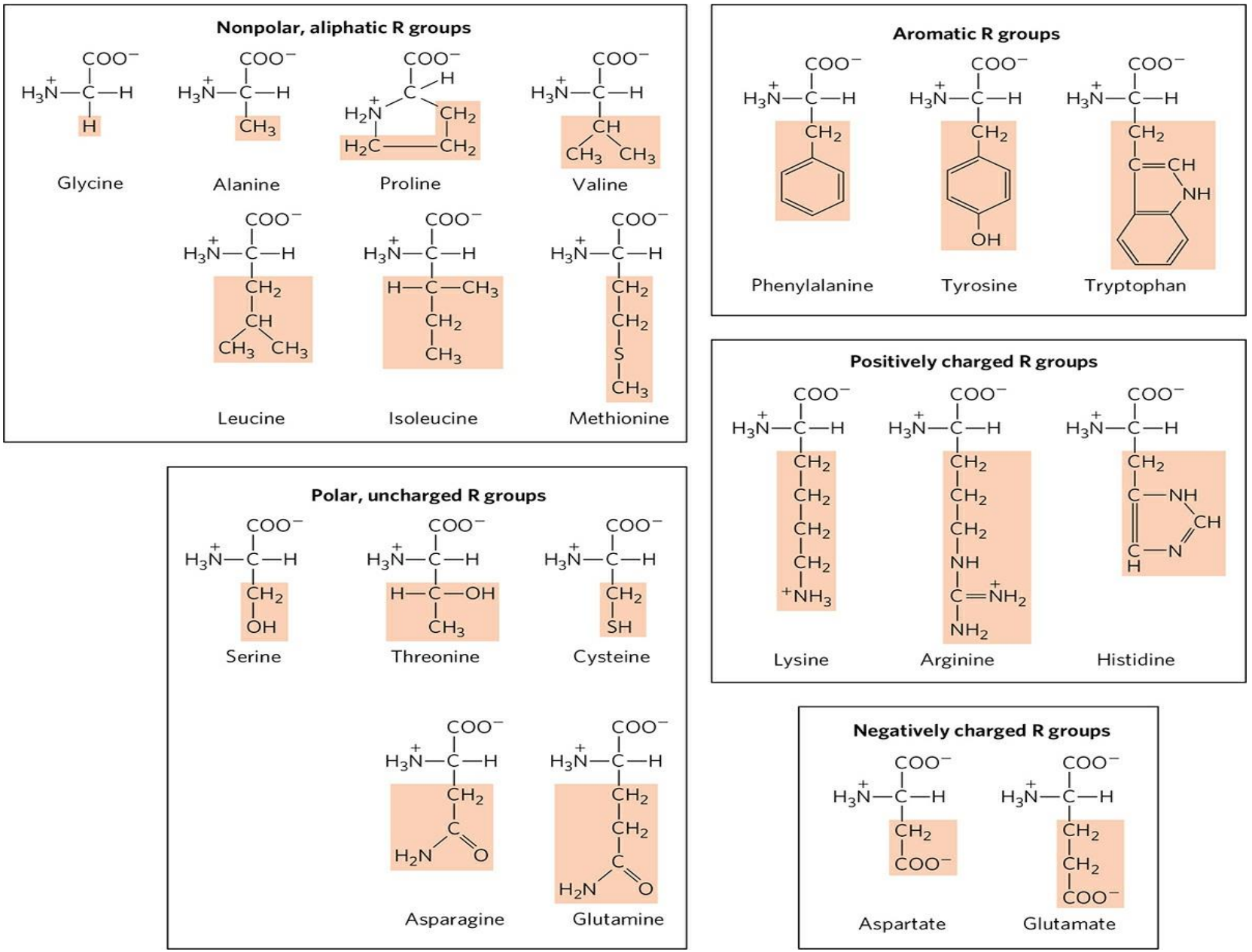
His;H;Imidazole gp

D.Imino acid:



Pro; P (Pyrrolidine gp)

Proline


Nonpolar, aliphatic R groups
Glycine
Alanine
Proline
Valine
Leucine
Isoleucine
Methionine
Aromatic R groups
Phenylalanine
Tyrosine
Tryptophan
Positively charged R groups
Lysine
Arginine
Histidine
Polar, uncharged R groups
Serine
Threonine
Cysteine
Asparagine
Glutamine
Negatively charged R groups
Aspartate
Glutamate

Nonpolar, Aliphatic R Groups

- The R groups in this class of amino acids are nonpolar and hydrophobic.
- The side chains of **alanine**, **valine**, **leucine**, and **isoleucine** tend to cluster together within proteins, stabilizing protein structure through the hydrophobic effect.
- **Glycine** has the simplest structure. Although it is most easily grouped with the nonpolar amino acids, its very small side chain makes no real contribution to interactions driven by the hydrophobic effect.
- **Methionine**, one of the two sulfur-containing amino acids, has a slightly nonpolar thioether group in its side chain.
- **Proline** has an aliphatic side chain with a distinctive cyclic structure. The secondary amino (imino) group of proline residues is held in a rigid conformation that reduces the structural flexibility of polypeptide regions containing proline.

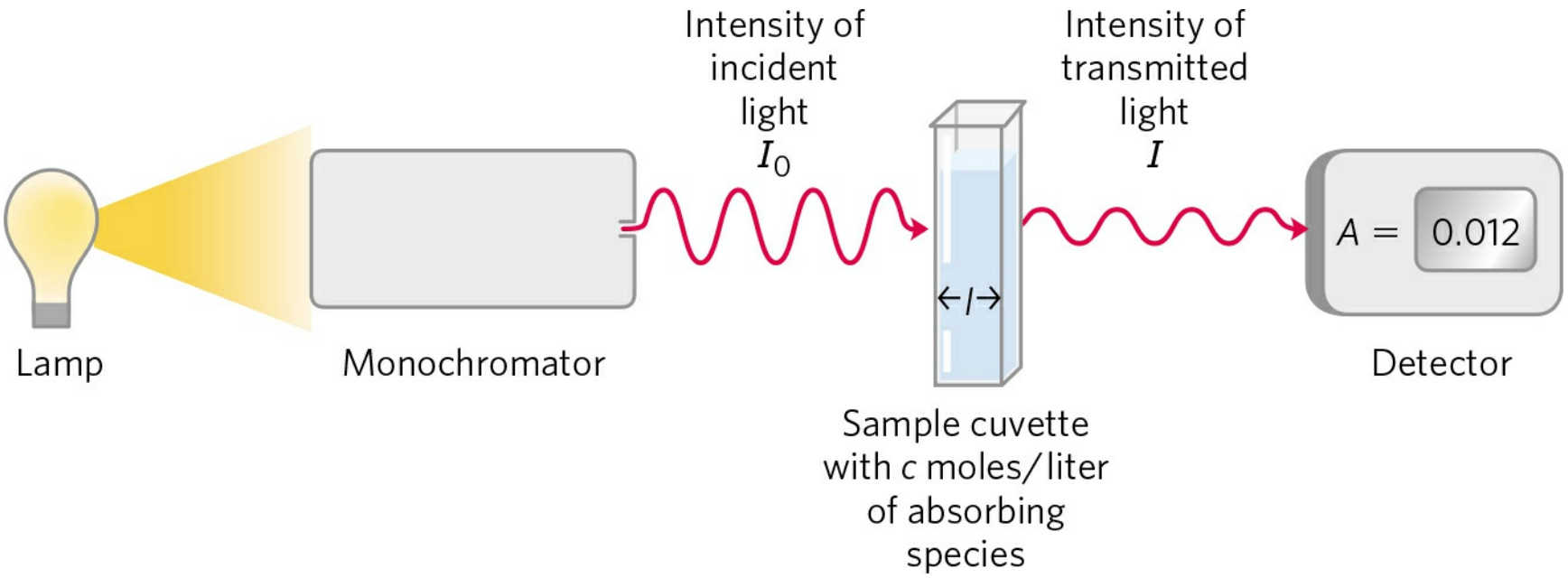
Aromatic R Groups

- **Phenylalanine**, **tyrosine**, and **tryptophan**, with their aromatic side chains, are relatively nonpolar (hydrophobic). All can contribute to the hydrophobic effect.
- The hydroxyl group of tyrosine can form hydrogen bonds, and it is an important functional group in some enzymes.
- Tyrosine and tryptophan are significantly more polar than phenylalanine because of the tyrosine hydroxyl group and the nitrogen of the tryptophan indole ring.

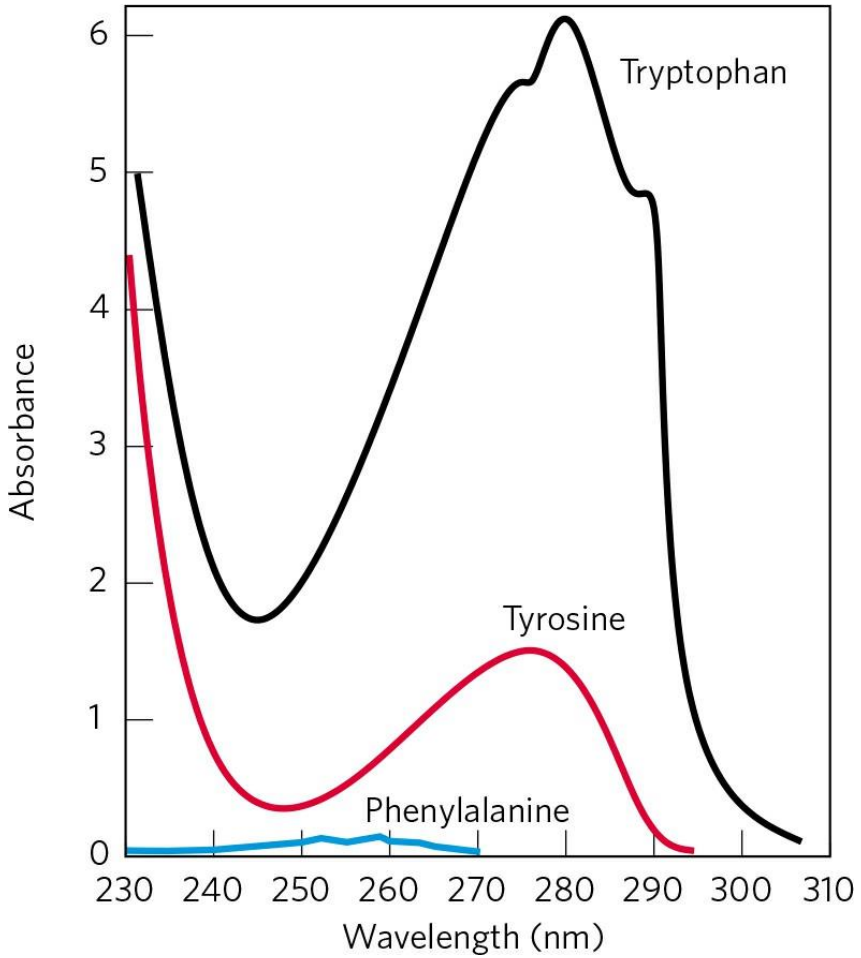
LAMBERT BEER LAW

- A wide range of bio molecules absorb light at characteristic wavelengths, just as tryptophan absorbs light at 280 nm. Measurement of light absorption by a spectrophotometer is used to detect and identify molecules and to measure their concentration in solution. The fraction of the incident light absorbed by a solution at a given wavelength is related to the thickness of the absorbing layer (path length) and the concentration of the absorbing species .
- These two relationships are combined into the Lambert-Beer law,

$$A = \log_{10}\left(\frac{I_o}{I}\right) = \epsilon c l$$


Intensity of incident light I_0
Intensity of transmitted light I
$A = 0.012$
$\leftarrow l \rightarrow$
Lamp
Monochromator
Sample cuvette with c moles/liter of absorbing species
Detector

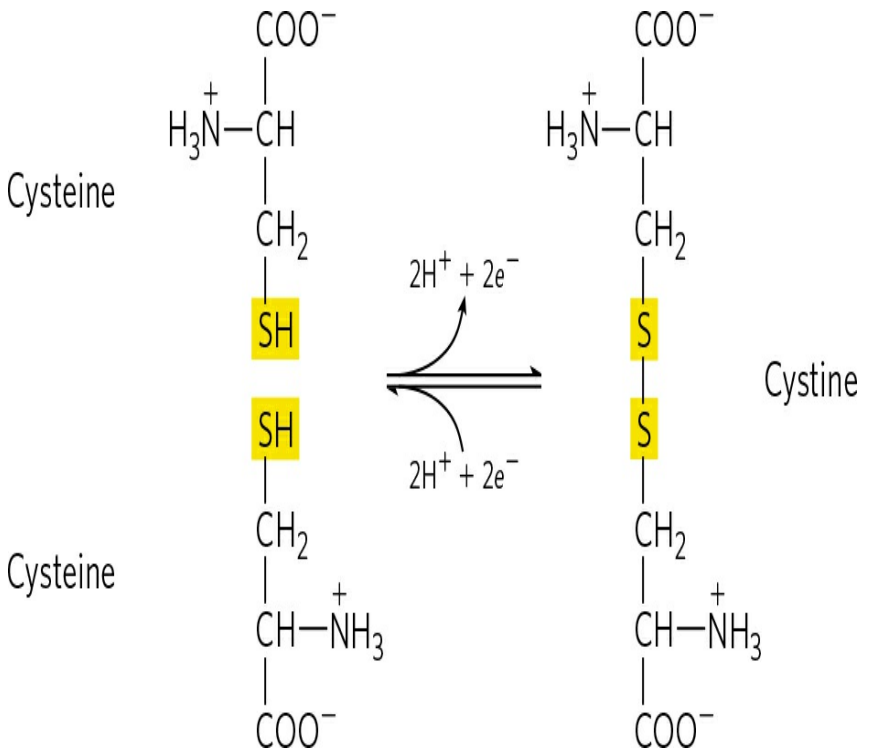
- Tryptophan and tyrosine, and to a much lesser extent phenylalanine, absorb ultraviolet light. This accounts for the characteristic strong absorbance of light by most proteins at a wavelength of 280 nm, a property exploited by researchers in the characterization of proteins.



Polar, Uncharged R Groups

- **Serine**
- **Threonine**
- **cysteine**,
- **Asparagine**
- **glutamine**.

- Cysteine is readily oxidized to form a covalently linked dimeric amino acid called **cystine**,



Positively Charged (Basic) R Groups The most hydrophilic R groups are those that are either positively or negatively charged.

The amino acids in which the R groups have significant positive charge at pH 7.0 are **lysine**, which has a second primary amino group at the ε position on its aliphatic chain;

- **arginine**, which has a positively charged guanidinium group; and **histidine**, which has an aromatic imidazole group.
- As the only common amino acid having an ionizable side chain with p*K*a near neutrality, histidine may be positively charged (protonated form) or uncharged at pH 7.0.
- His residues facilitate many enzyme-catalyzed reactions by serving as proton donors/acceptors.

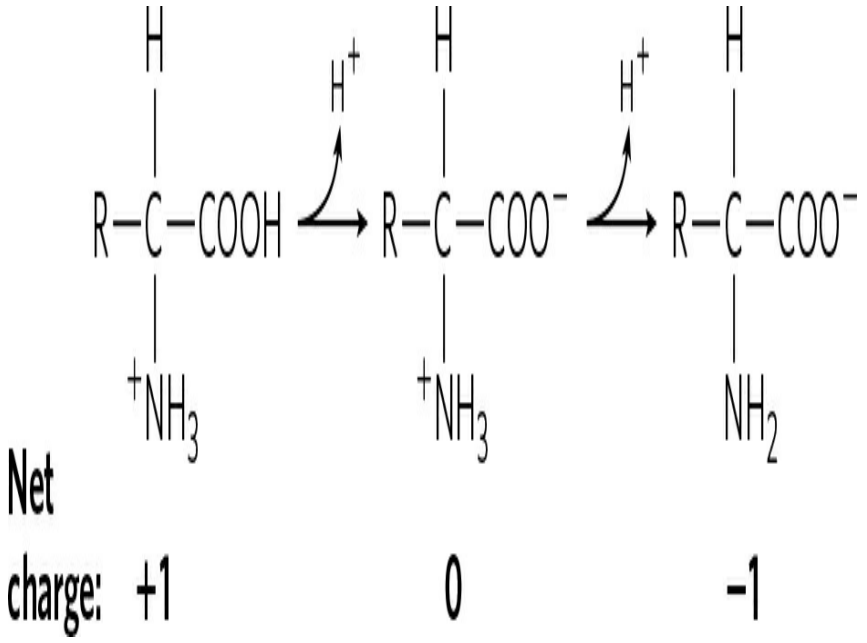
Negatively Charged (Acidic) R Groups The two amino acids having R groups with a net negative charge at pH 7.0 are **aspartate** and **glutamate**, each of which has a second carboxyl group.

Uncommon Amino Acids Also Have Important Functions

- **4-hydroxyproline**, a derivative of proline,
- **5-hydroxylysine**, derived from lysine. The former is found in plant cell wall proteins, and both are found in collagen, a fibrous protein of connective tissues.
- **6-*N*-Methyllysine** is a constituent of myosin, a contractile protein of muscle.
- ***γ*- carboxyglutamate**, found in the blood-clotting protein prothrombin and in certain other proteins that bind Ca2+ as part of their biological function.
- Complex is **desmosine**, a derivative of four Lys residues, which is found in the fibrous protein elastin.
- Selenocysteine contains selenium rather than the sulfur of cysteine. Actually derived from serine, selenocysteine is a constituent of just a few known proteins.
- Pyrrolysine is found in a few proteins in several methanogenic (methane-producing) archaea and in one known bacterium; it plays a role in methane biosynthesis.
- Some 300 additional amino acids have been found in cells.
- **Ornithine** and **citrulline** deserve special note because they are key intermediates (metabolites) in the biosynthesis of arginine and in the urea cycle

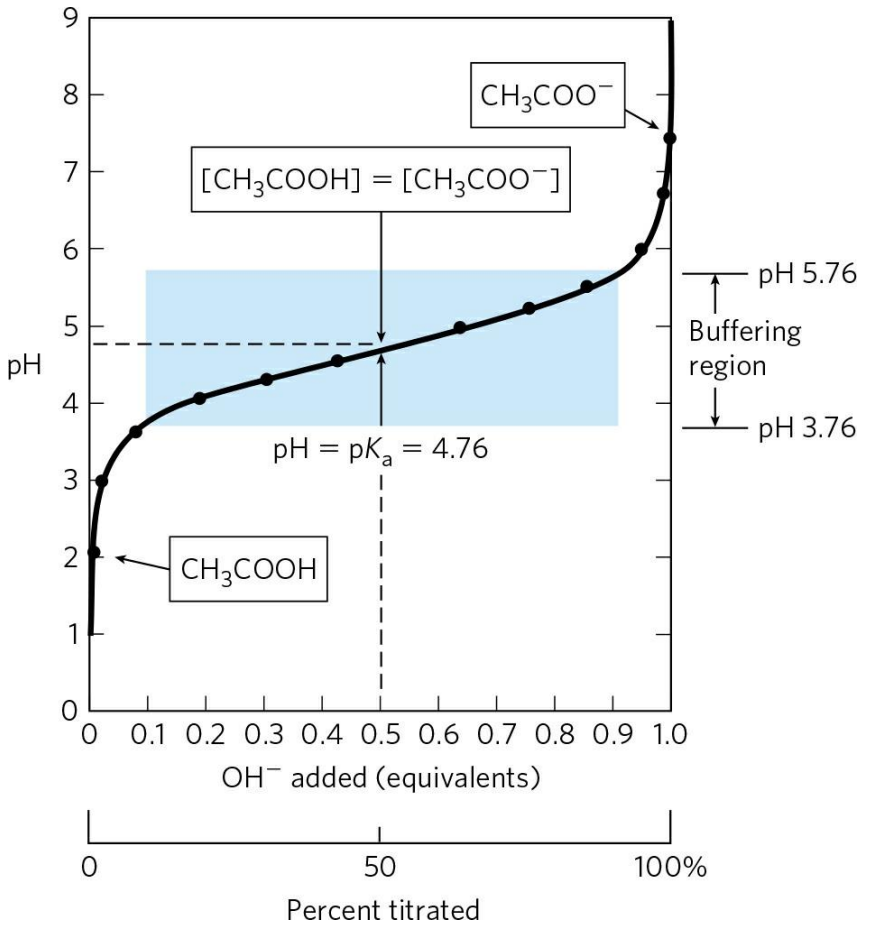
Amino Acids Can Act as Acids and Bases

- When an amino acid lacking an ionizable R group is dissolved in water at neutral pH, it exists in solution as the dipolar ion, or **zwitterion** , which can act as either an acid or a base.
- Substances having this dual nature are **amphoteric** and are often called **ampholytes .**
- A simple monoamino monocarboxylic α-amino acid, such as alanine, is a diprotic acid when fully protonated; it has two groups, the —COOH group and the NH3 group, that can yield protons:



Amino Acids Have Characteristic Titration Curves

- Acid-base titration involves the gradual addition or removal of protons The two ionizable groups of glycine, the carboxyl group and the amino group, are titrated with a strong base such as NaOH.
- The plot has two distinct stages, corresponding to deprotonation of two different groups on glycine. Each of the two stages resembles in shape the titration curve of a monoprotic acid, such as acetic acid and can be analyzed in the same way



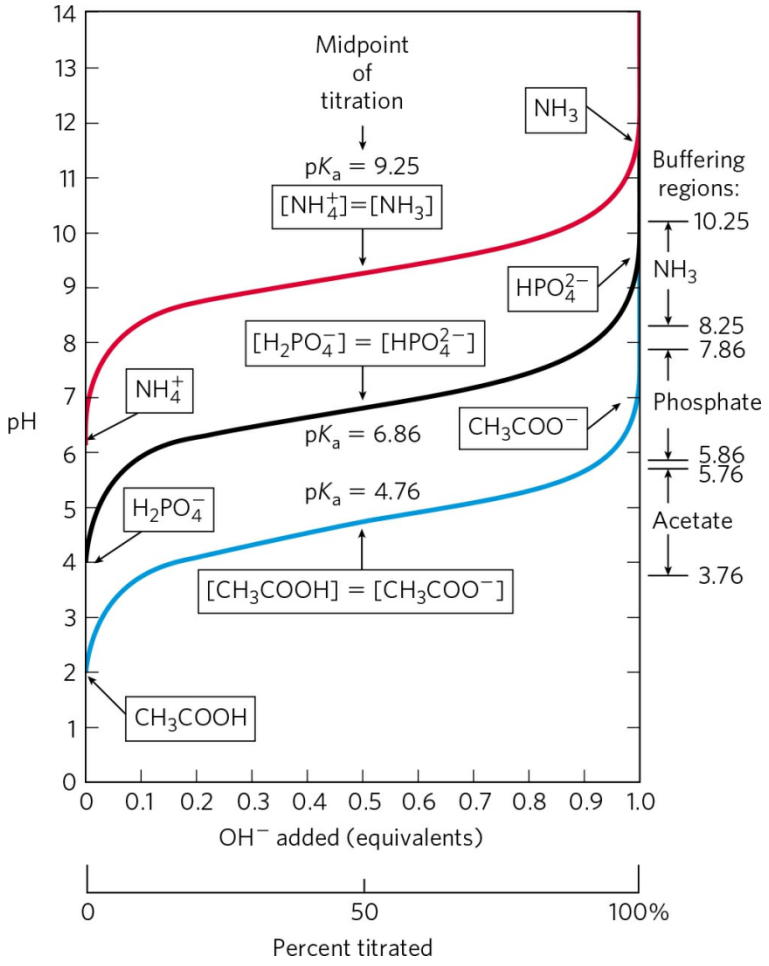
Titration Curves

- At very low pH, the predominant ionic species of glycine is the fully protonated form,

  +H3N—CH2—COOH. In the first stage of the titration, the —COOH group of glycine loses its proton
- At the midpoint of this stage, equimolar concentrations of the proton-donor (+H3N—CH2—COOH) and proton-acceptor (+H3N—CH2—COO−) species are present.

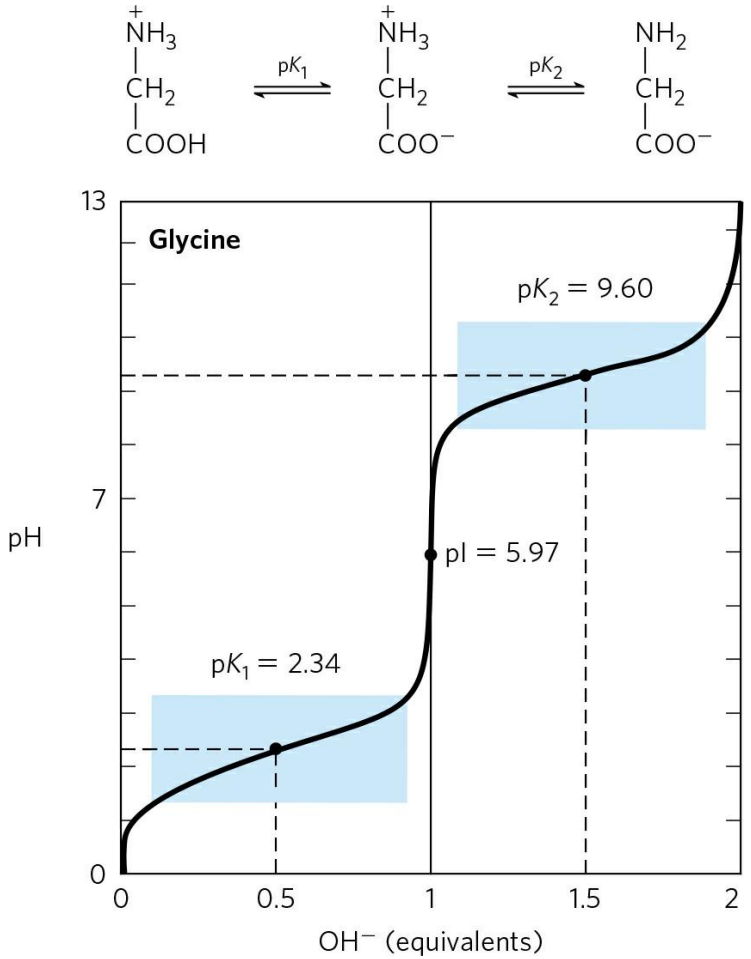
Titration Curves

- Although the titration curves of these acids have the same shape, they are displaced the pH axis because the three acids have different strengths.
- Acetic acid, with the highest *K*a (lowest p*K*a) of the three, is the strongest of the three weak acids (loses its proton most readily); it is already half dissociated at pH 4.76.
- Dihydrogen phosphate loses a proton less readily, being half dissociated at pH 6.86.
- Ammonium ion is the weakest acid of the three and does not become half dissociated until pH 9.25.



Titration Curves

- For glycine, the pH at the midpoint is 2.34, thus its —COOH group has a p*K*a (labeled p*K*1) of 2.34.
- pH and p*K*a are simply convenient notations for proton concentration and the equilibrium constant for ionization
- As the titration of glycine proceeds, another important point is reached at pH 5.97. Here there is another point of inflection, at which removal of the first proton is essentially complete and removal of the second has just begun
- At this pH glycine is present largely as the dipolar ion (zwitterion) +H3N—CH2—COO
- The second stage of the titration corresponds to the removal of a proton from the NH4 group of glycine. The pH at the midpoint of this stage is 9.60, equal to the p*K*a (labeled p*K*2) for the NH4 group.
- The titration is essentially complete at a pH of about 12, at which point the predominant form of glycine is H2N—CH2—COO−.



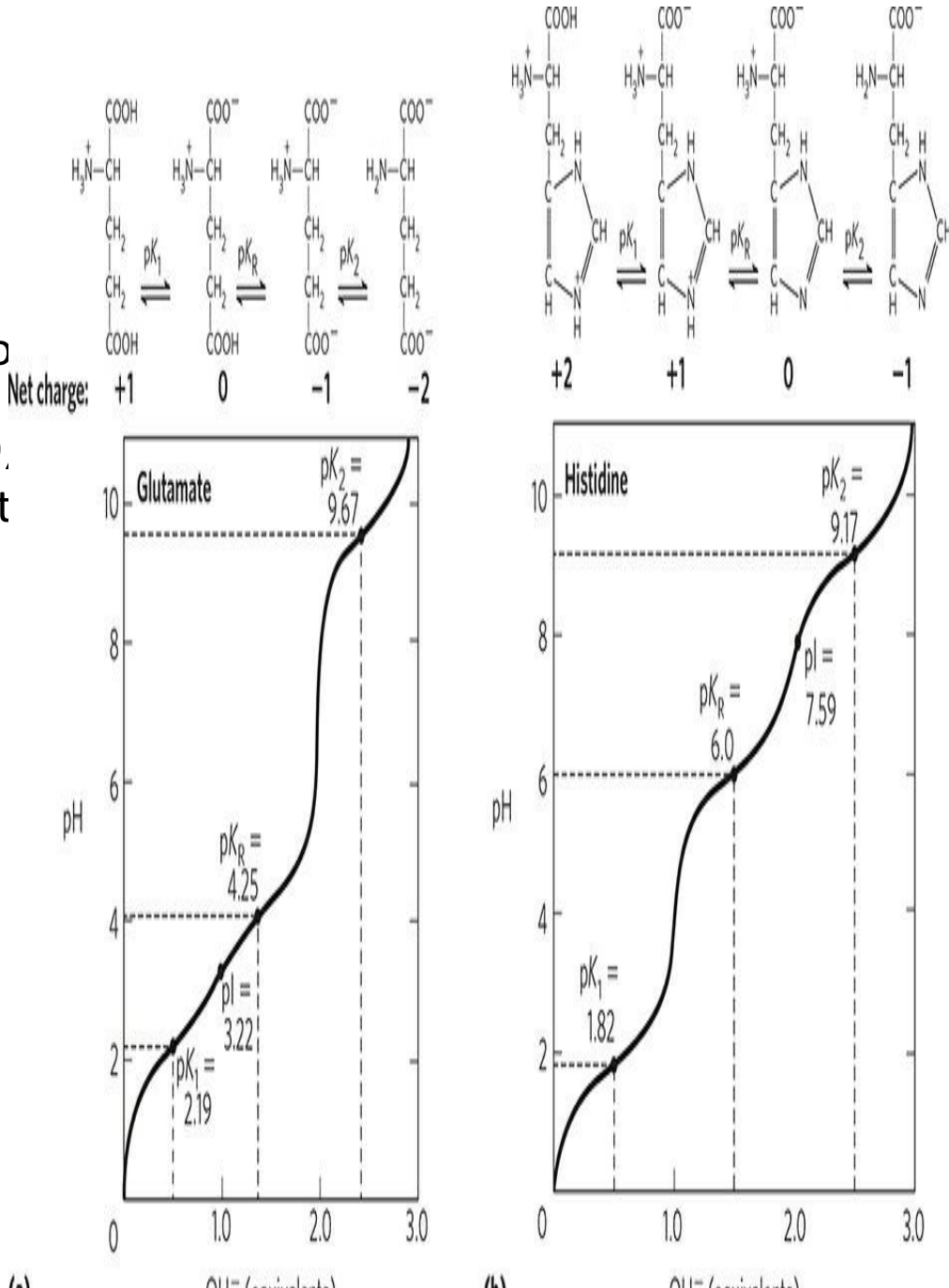
Titration Curves Predict the Electric Charge of Amino Acids

- At pH 5.97, the point of inflection between the two stages in its titration curve, glycine is present predominantly as its dipolar form,
- fully ionized but with no *net* electric charge . The characteristic pH at which the *net* electric charge is zero is called the **isoelectric point** or **isoelectric pH**, designated **pI**

Amino Acids Differ in Their Acid-Base Properties

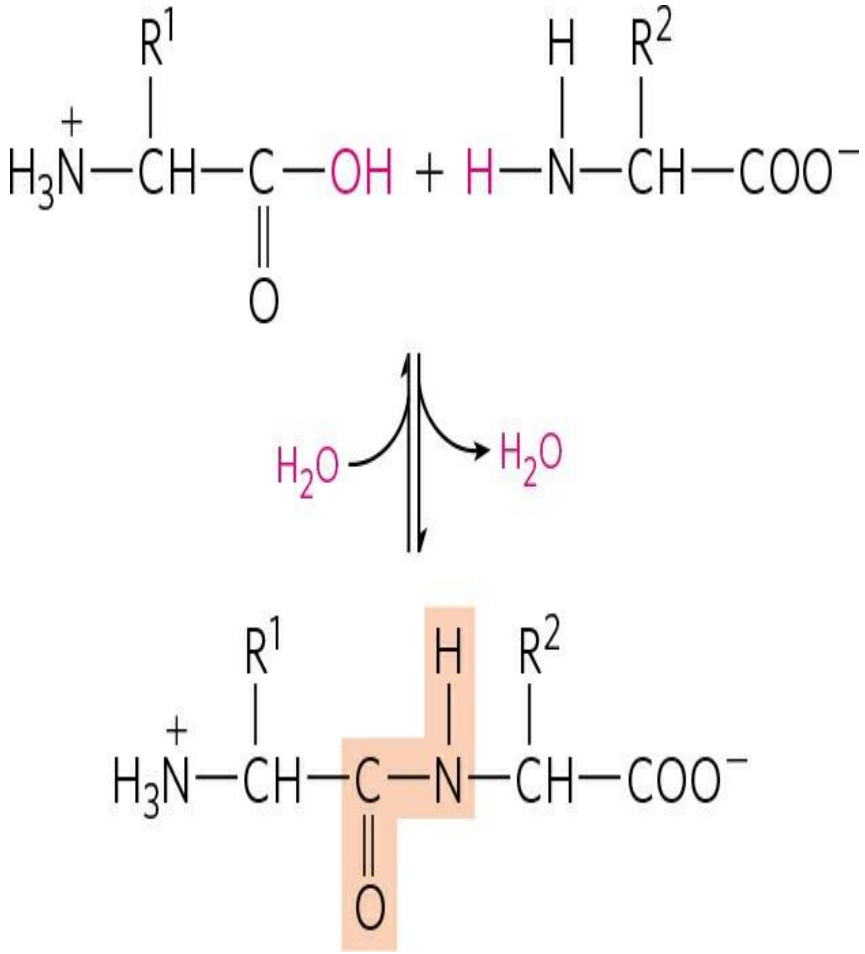
- p*K*a values: p*K*a of the —COOH group in the range of 1.8 to 2.4, and p*K*a of the NH4 group in the range of 8.8 to 11.0. The differences in these p*K*a values reflect the chemical environments imposed by their R groups.
- an ionizable R group have more complex titration curves, with *three* stages corresponding to the three possible ionization steps; thus they have three p*K*a values

- The titration curves for two amino acids of this type, glutamate and histidine.
- Glutamate has a pI of 3.22, considerably lower than that of glycine.
- This is due to the presence of two carboxyl groups, which, at the average of their p*K*a values (3.22),
- contribute a net charge of −1 that balances the +1 contributed by the amino group
- histidine, with two groups that are positively charged when protonated, is 7.59 (the average of the p*K*a values of the amino and imidazole groups), much higher than that of glycine



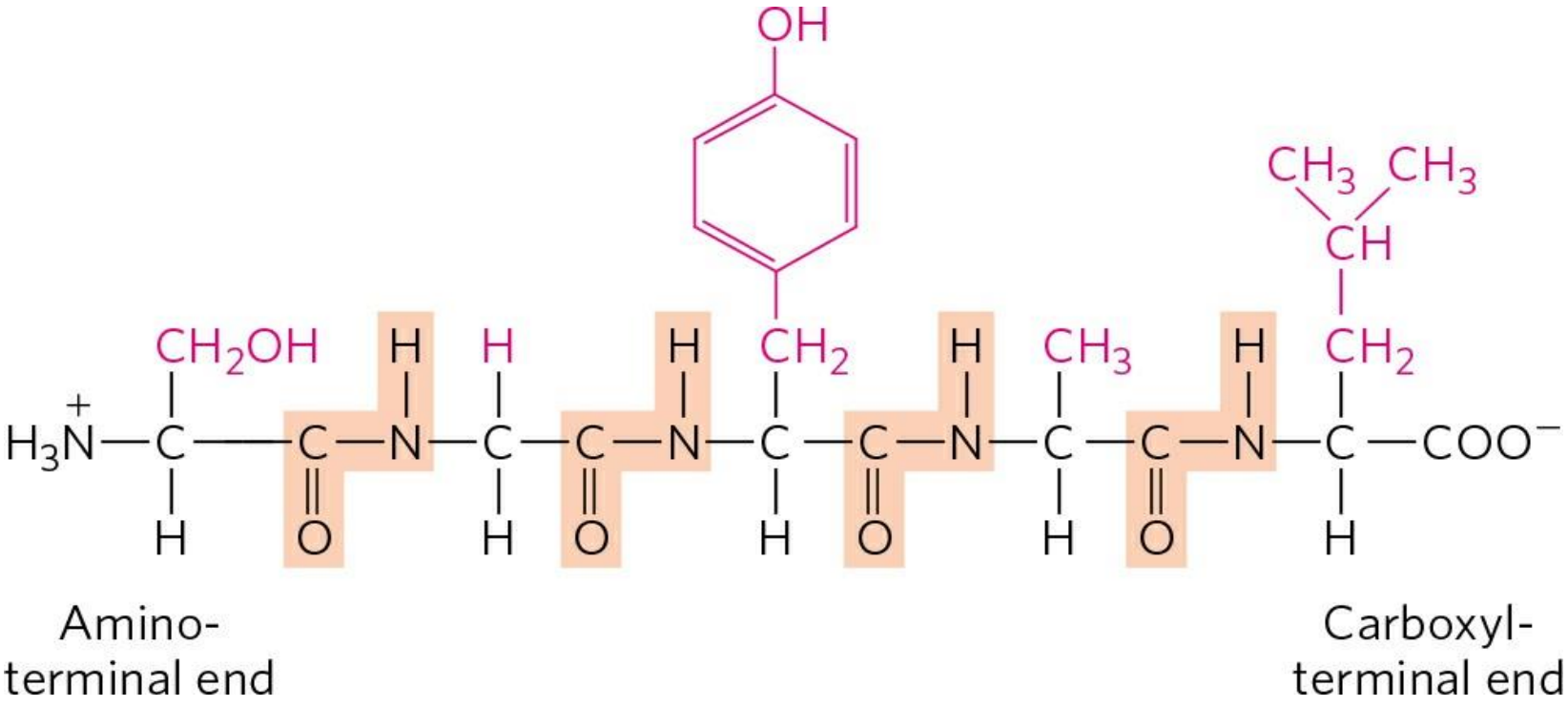
Peptides and Proteins

- **Peptides Are Chains of Amino Acids**
- Two amino acid molecules can be covalently joined through a substituted amide linkage, termed a **peptide bond**, to yield a dipeptide.
- Such a linkage is formed by removal of the elements of water (dehydration) from the α-carboxyl group of one amino acid and the α-amino group of another'



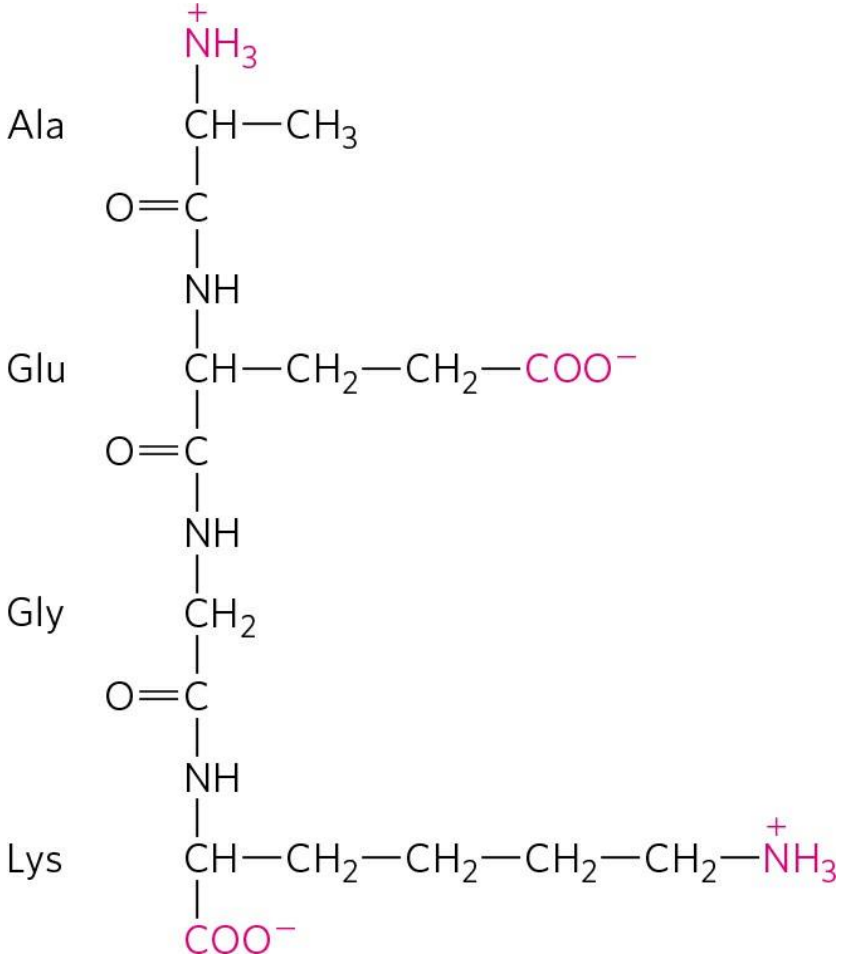
- Three amino acids can be joined by two peptide bonds to form a tripeptide
- Four amino acids can be linked to form a tetrapeptide,
- Five to form a pentapeptide, and so forth.
- When a few amino acids are joined in this fashion, the structure is called an **oligopeptide**.
- When many amino acids are joined, the product is called a **polypeptide**

- an amino acid unit in a peptide is often called a residue. In a peptide, the amino acid residue at the end with a free *α*-amino group is the **amino-terminal** (or *N*-terminal) residue; the other end, which has a free carboxyl group, is the **carboxyl-terminal**



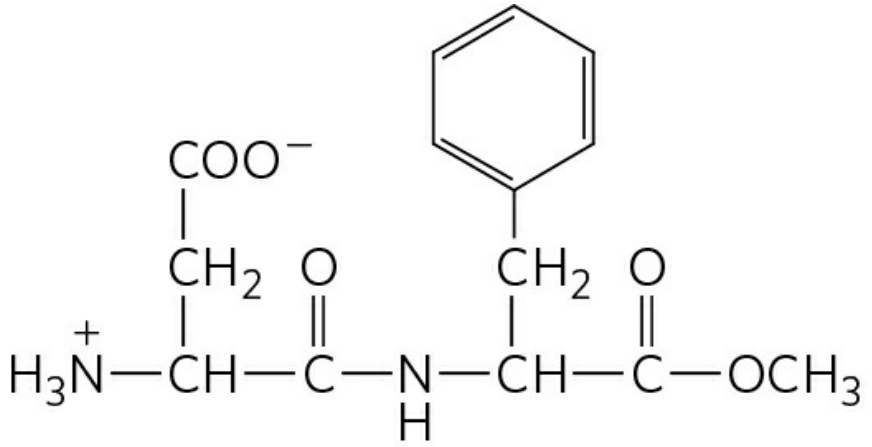
Peptides Can Be Distinguished by Their Ionization Behavior

- Peptides contain only one free α-amino group and one free α-carboxyl group, at opposite ends of the chain . These groups ionize as they do in free amino acids.
- The α-amino and α-carboxyl groups of all nonterminal amino acids are covalently joined in the peptide bonds, which do not ionize and thus do not contribute to the total acid-base behavior of peptides.
- the R groups of some amino acids can ionize and in a peptide these contribute to the overall acid-base properties of the molecule .
- Thus the acid-base behavior of a peptide can be predicted from its free α-amino and α-carboxyl groups combined with the nature and number of its ionizable R group.



Biologically Active Peptides and Polypeptides Occur in a Vast Range of Sizes and Compositions

- No generalizations can be made about the molecular weights of biologically active peptides and proteins in relation to their functions.
- Naturally occurring peptides range in length from two to many thousands of amino acid residues. Even the smallest peptides can have biologically important effects.
- Consider the commercially synthesized dipeptide L-aspartyl-L-phenylalanine methyl ester, the artificial sweetener better known as aspartame or NutraSweet.



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

- The amino acid composition of proteins is also highly variable.
- The 20 common amino acids almost never occur in equal amounts in a protein. Some amino acids may occur only once or not at all in a given type of protein;
- others may occur in large numbers ;
- composition of bovine cytochrome *c* and chymotrypsinogen , the inactive precursor of the digestive enzyme chymotrypsin.
- These two proteins, with very different functions, also differ significantly in the relative numbers of each kind of amino acid residue.

- We can calculate the approximate number of amino acid residues in a simple protein containing no other chemical constituents by dividing its molecular weight by 110.
- Although the average molecular weight of the 20 common amino acids is about 138,
- The smaller amino acids predominate in most proteins. If we take into account the proportions in which the various amino acids occur in an average protein the average molecular weight of protein amino acids is nearer to 128.
- Because a molecule of water (*M*r 18) is removed to create each peptide bond, the average molecular weight of an amino acid residue in a protein is about 128 − 18 = 110.

Some Proteins Contain Chemical Groups Other Than Amino Acids

- for example the enzymes ribonuclease A and chymotrypsin, contain only amino acid residues and no other chemical constituents; these are considered simple proteins.
- some proteins contain permanently associated chemical components in addition to amino acids; these are called **conjugated proteins**.
- The non–amino acid part of a conjugated protein is usually called its **prosthetic group**.
- Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups ,for example,
- **lipoproteins** contain lipids, **glycoproteins** contain sugar groups, and **metalloproteins** contain a specific metal.
- Some proteins contain more than one prosthetic group. Usually the prosthetic group plays an important role in the protein's biological function.

TABLE 3-4 Conjugated Proteins

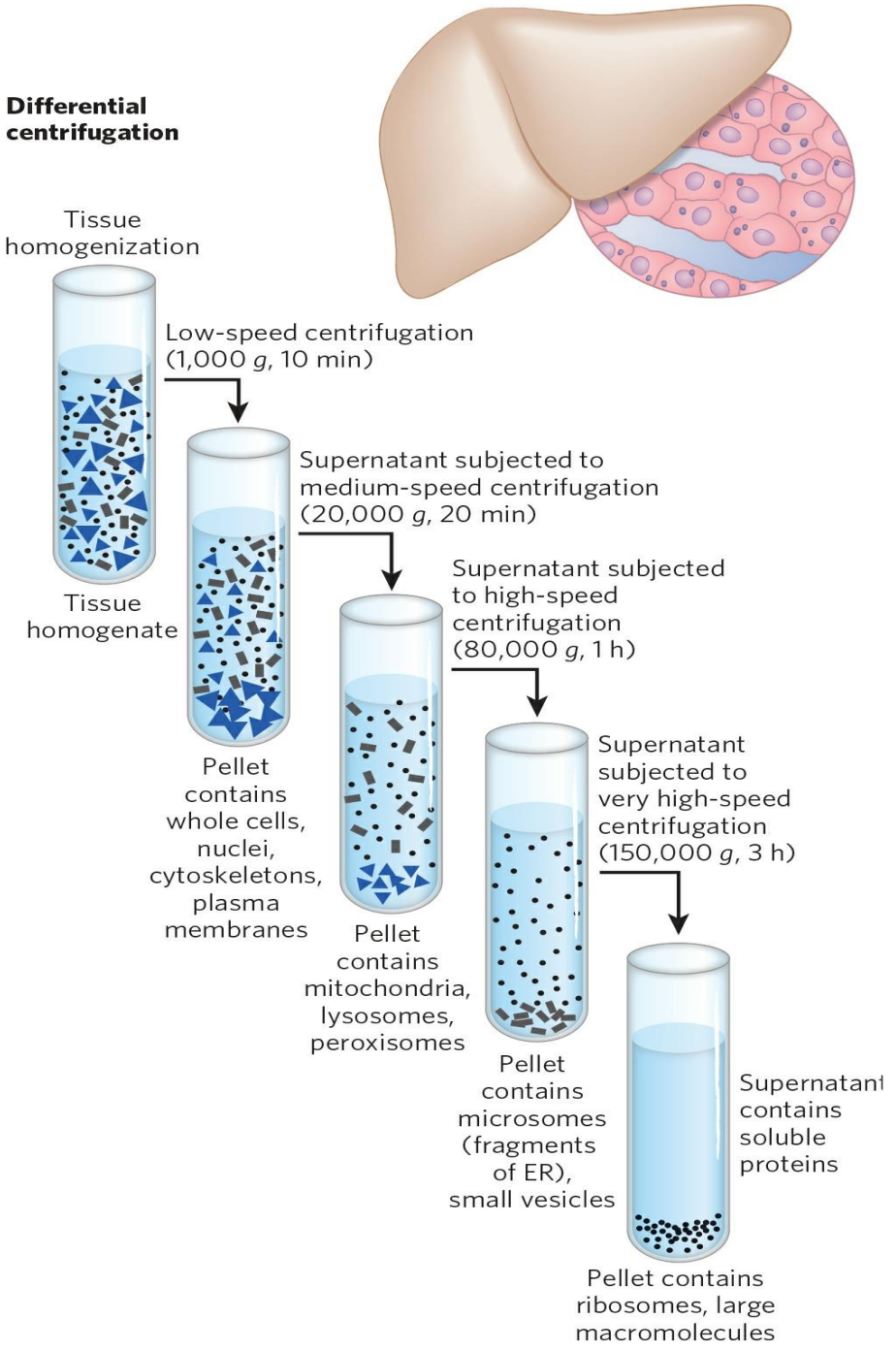
Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

Proteins

- Proteins Can Be Separated and Purified
- methods involving DNA cloning and genome sequencing that can simplify the process of protein purification
- The newer method, n artificially modify the protein being purified, adding a few or many amino acid residues to one or both ends.
- Convenience thus comes at the price of potentially altering the activity of the purified protein.
- The purification of proteins in their native state (the form in which they function in the cell) usually relies on methods.

- The source of a protein is generally tissue or microbial cells.
- The first step in any protein purification procedure is to break open these cells, releasing their proteins into a solution called a **crude extract**.
- If necessary, differential centrifugation can be used to prepare subcellular fractions or to isolate specific organelles
- Once the extract or organelle preparation is ready, various methods are available for purifying one or more of the proteins it contains.

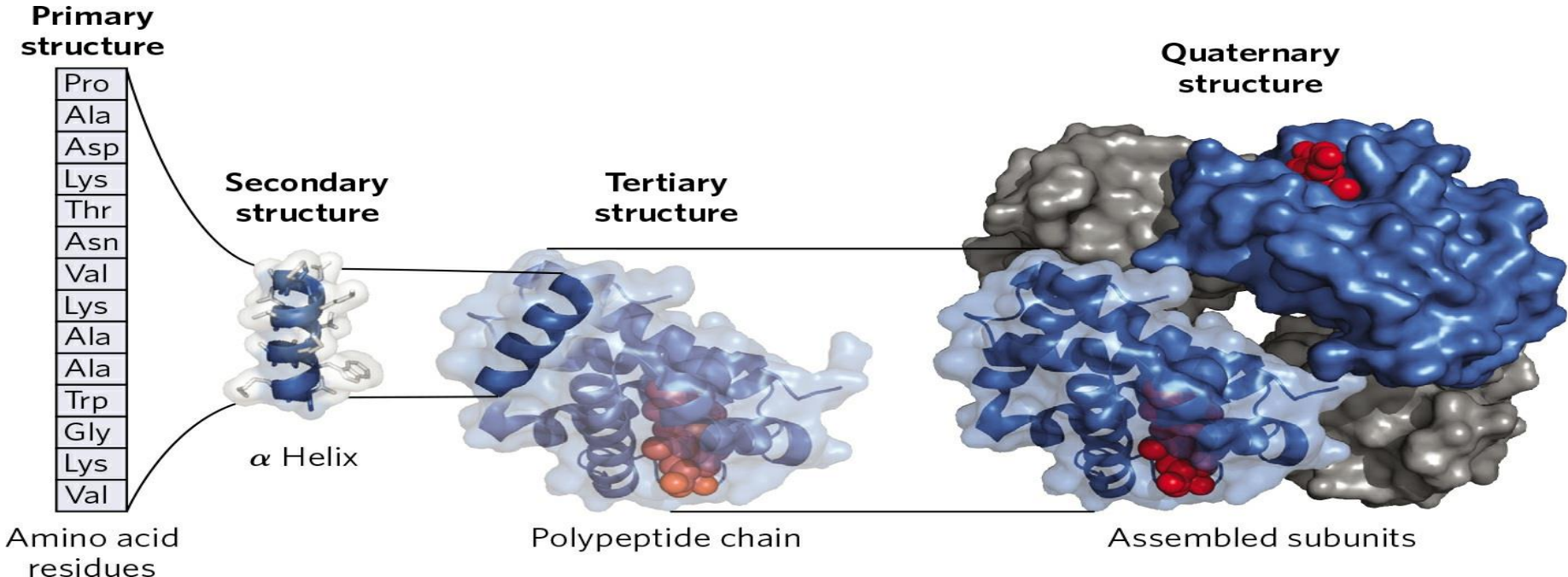
- Commonly, the extract is subjected to treatments that separate the proteins into different **fractions** based on a property such as size or charge, a process referred to as **fractionation**
- Early fractionation steps in a purification utilize differences in protein solubility, which is a complex function of pH, temperature, salt concentration, and other factors.
- The solubility of proteins is lowered in the presence of some salts, an effect called “salting out.”
- The addition of certain salts in the right amount can selectively precipitate some proteins, while others remain in solution.
- Ammonium sulfate ((NH4)2SO4) is particularly effective and is often used to salt out proteins.
- The proteins thus precipitated are removed from those remaining in solution by low-speed centrifugation.



The Structure of Proteins: Primary Structure

Primary structure

- all covalent bonds (mainly peptide bonds and disulfide bonds) linking amino acid residues in a polypeptide chain
- The most important element of primary structure is the *sequence* of amino acid residues



Secondary structure refers to particularly stable arrangements of amino acid residues giving rise to recurring structural patterns

Tertiary structure describes all aspects of the three-dimensional folding of a polypeptide.

Quaternary structure

When a protein has two or more polypeptide subunits, their arrangement in space

The Function of a Protein Depends on Its Amino Acid Sequence

First proteins with different functions always have different amino acid sequences.

Second thousands of human genetic diseases have been traced to the production of defective proteins.

The defect can range from a single change in the amino acid sequence (as in sickle cell disease) to deletion of a larger portion of the polypeptide chain (as in most cases of Duchenne muscular dystrophy: a large deletion in the gene encoding the protein dystrophin leads to production of a shortened, inactive protein).

Finally, on

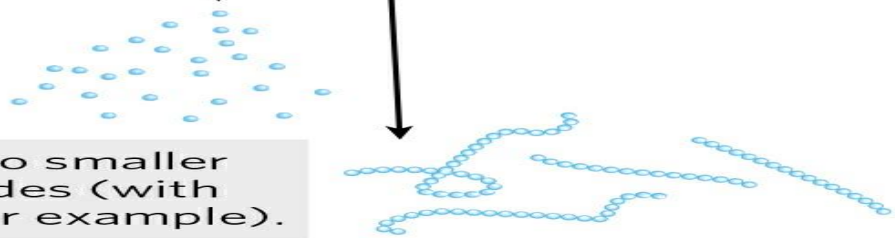
- comparing functionally similar proteins from different species, we find that these proteins often have similar amino acid sequences. Thus, a close link between protein primary structure and function is evident.

Protein Chemistry Is Enriched by Methods Derived from Classical Polypeptide Sequencing

- The methods used in the 1950s by Fred Sanger to determine the sequence of the protein insulin .
- The sequence of a protein can usually be predicted from the sequence of the gene encoding it, information now readily available in evergrowing genomic databases.
- However, the traditional sequencing protocols have provided a rich array of tools for biochemists, and almost every step makes use of methods that are widely used in biochemistry labs, sometimes in quite different contexts.

Determine amino terminus (react with FDNB). Gly is at amino terminus.

Determine amino acid content (by acid hydrolysis). Select cleavage reagents based on presence of target amino acids in protein.



Cleave into smaller polypeptides (with trypsin, for example).

Sequence each polypeptide.

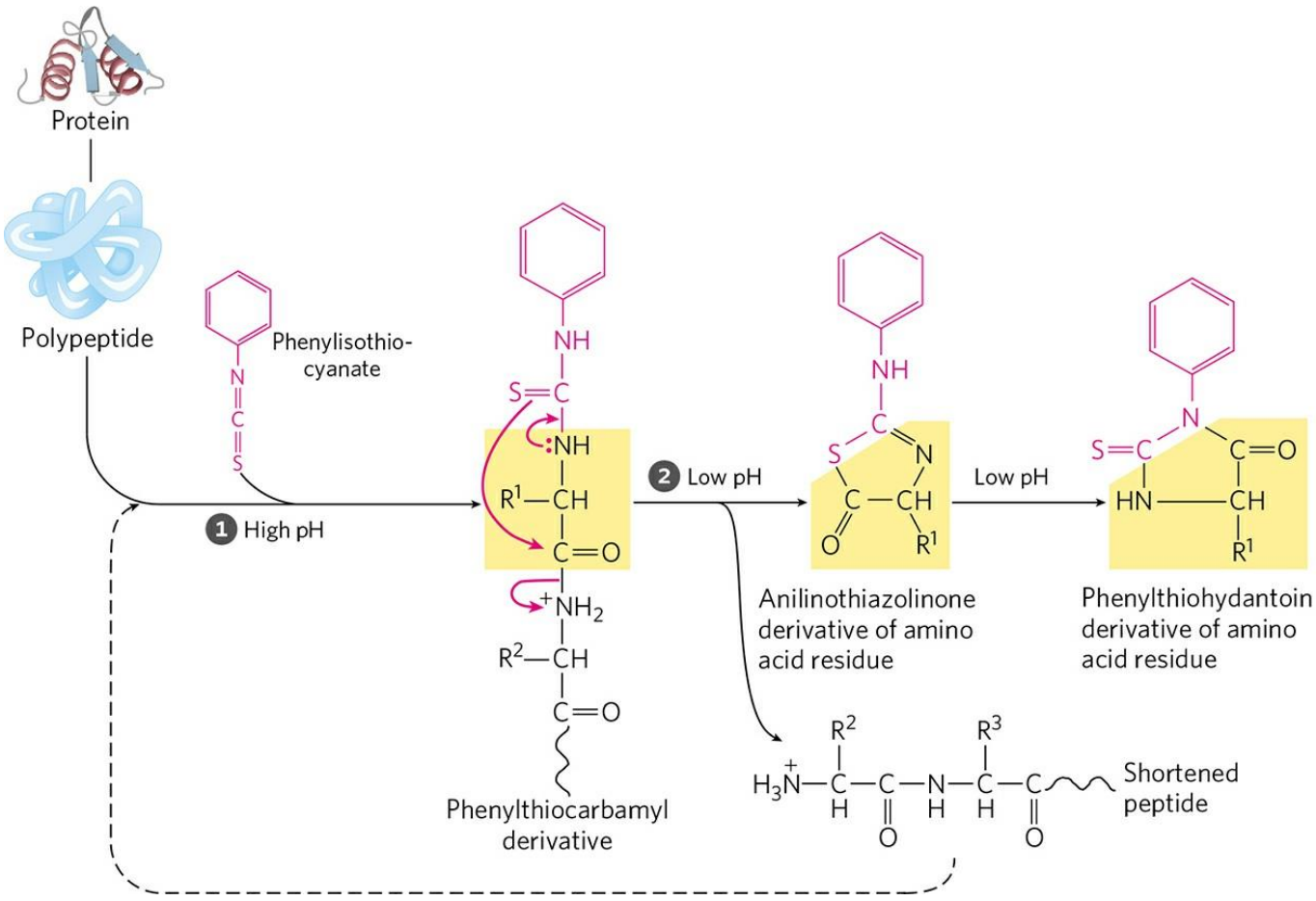
1. DCGGAHYLVLLAGPTIRSGTMR
2. AQGAFNPSCGVIQHAWIKMWILAAGTE
3. GGPVIATYEQDGGTSRYAPK
4. QGYASULAIEFTR

Determine order of polypeptides in protein. Peptide 3 is at the amino terminus. Peptide 2 is at the carboxyl terminus (it does not end in an amino acid residue that defines a trypsin cleavage site).

Order others by overlaps with sequences of peptides obtained by cleaving the protein with a different reagent, such as cyanogen bromide or chymotrypsin.

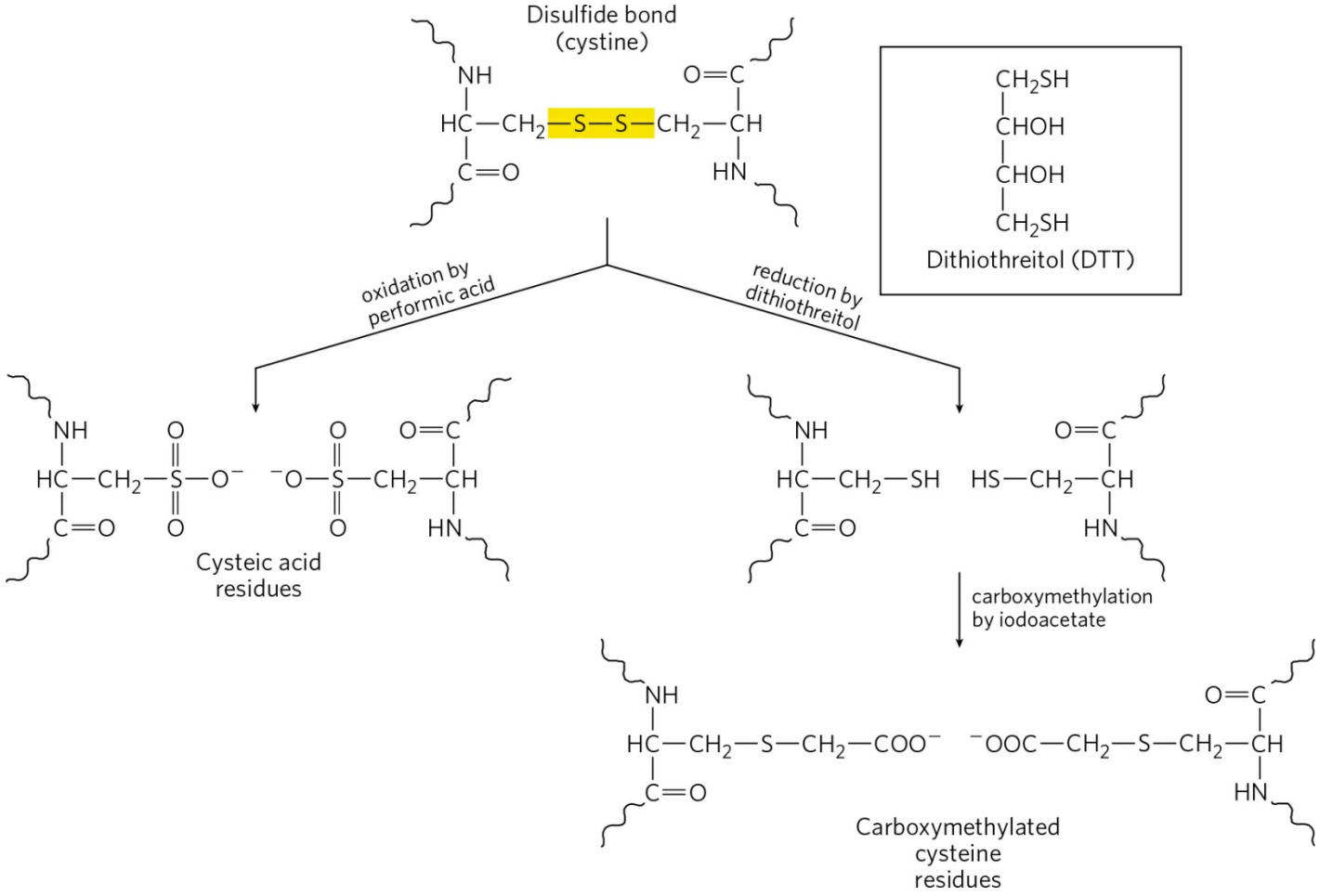
- The chemical sequencing process itself is based on a two-step process developed by Pehr Edman .
- The **Edman degradation** procedure labels and removes only the amino-terminal residue from a peptide, leaving all other peptide bonds intact.
- The peptide is reacted with phenylisothiocyanate under mildly ALKALINE conditions, which converts the amino-terminal amino acid to a phenylthiocarbamoyl (PTC) adduct.
- The peptide bond next to the PTC adduct is then cleaved in a step carried out in anhydrous trifluoroacetic ACID, with removal of the amino-terminal amino acid as an anilinothiazolinone derivative.

- The derivatized amino acid is extracted with organic solvents, converted to the more stable phenylthiohydantoin derivative by treatment with aqueous ACID, and then identified.
- The use of sequential reactions carried out under first basic and then acidic conditions provides a means of controlling the entire process.
- Each reaction with the amino-terminal amino acid can go essentially to completion without affecting any of the other peptide bonds in the peptide.
- The process is repeated until, typically, as many as 40 sequential amino acid residues are identified. The reactions of the Edman degradation have been automated



Identify amino-terminal residue of polypeptide.

Purify and recycle remaining peptide fragment though Edman process.


Disulfide bond
(cystine)
Dithiothreitol (DTT)
oxidation by
performic acid
reduction by
dithiothreitol
Cysteic acid
residues
carboxymethylation
by iodoacetate
Carboxymethylated
cysteine
residues

Thank you