3 Amino Acids, Peptides, and Proteins

Text

3.1 Amino Acids

P1 Principle 1

In every living organism, proteins are constructed from a common set of 20 amino acids. Each amino acid has a side chain with distinctive chemical properties. Amino acids may be regarded as the alphabet in which the language of protein structure is written.

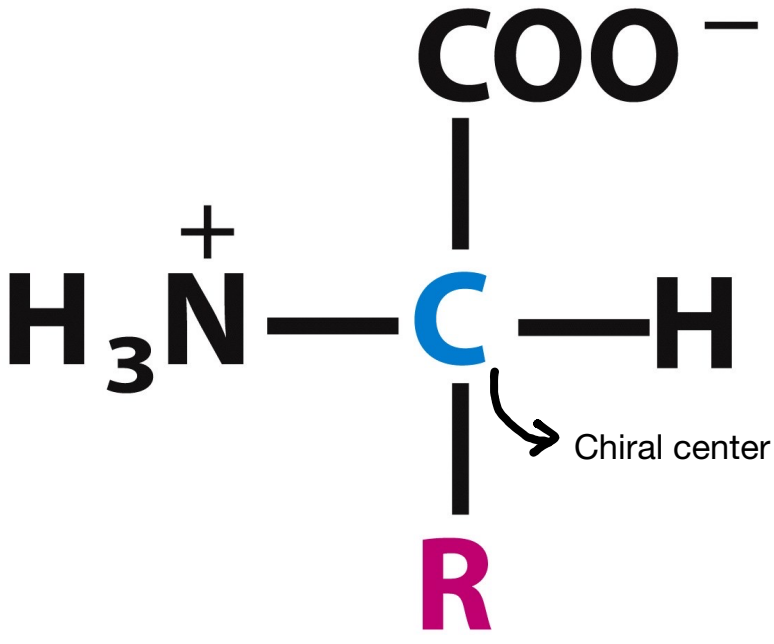
Protein functional group

— Proteinogenic amino acids: amino acids that are joined biosynthetically into proteins during translation

* Non-proteinogenic amino acids: amino acids that don't use to produce proteins, they have another different function.

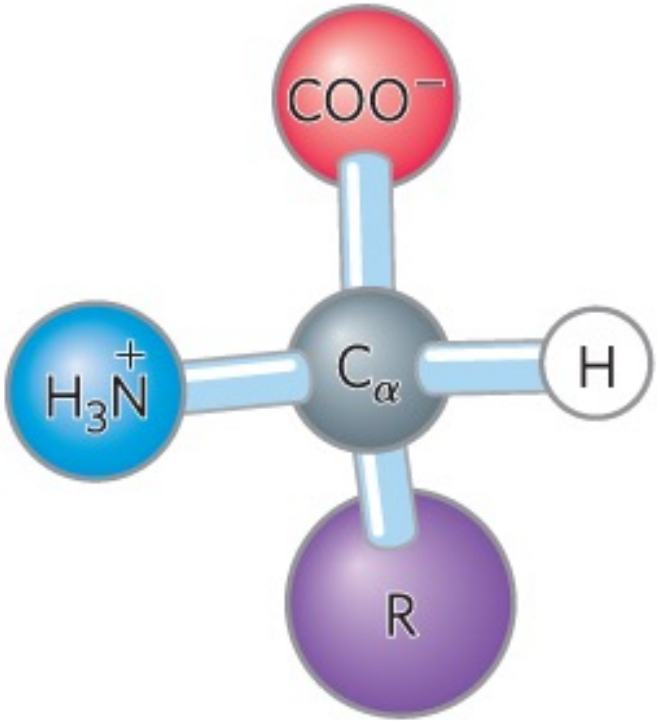
Amino Acids Share Common Structural Features

- *α* carbon and four substituents
- *α* carbon is the **chiral center**
- tetrahedral (Angle =109.5)



Amino Acid Substituents

- four substituents:
  - a carboxyl group
  - an amino group
  - a hydrogen atom
  - an **R group** (a side chain unique to each amino acid)
    - glycine has a second hydrogen atom instead of an R group



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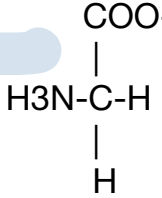
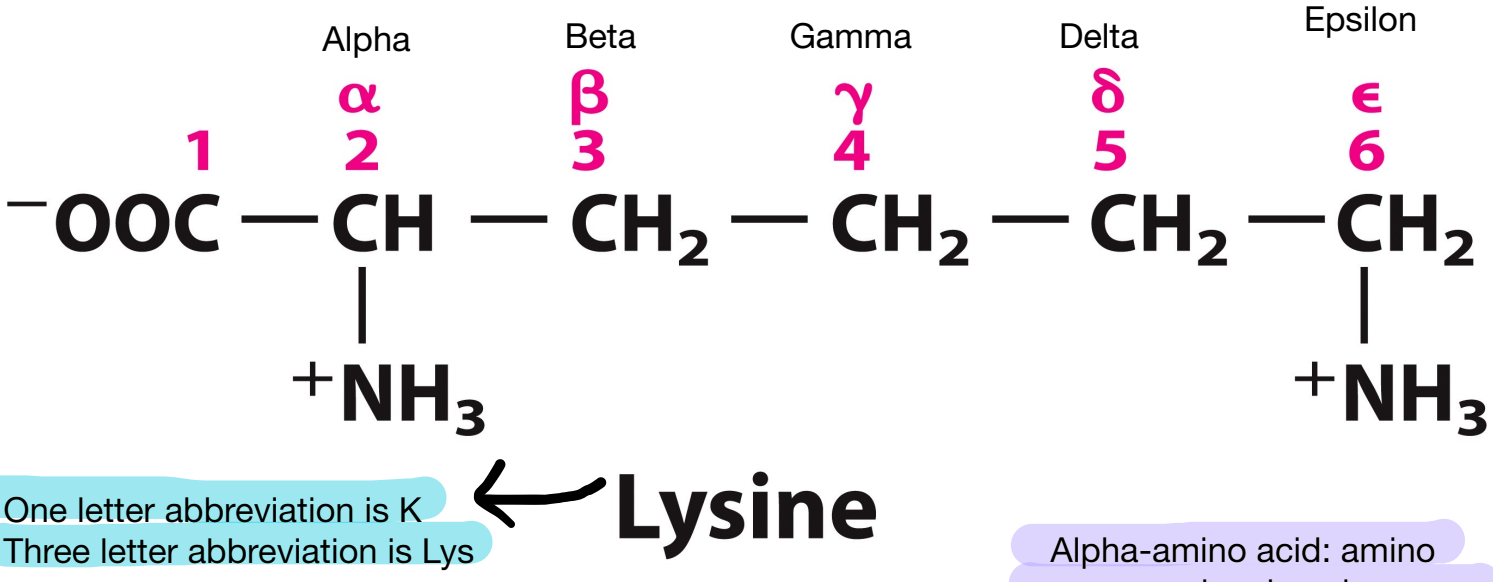


Amino Acids: Atom Naming

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



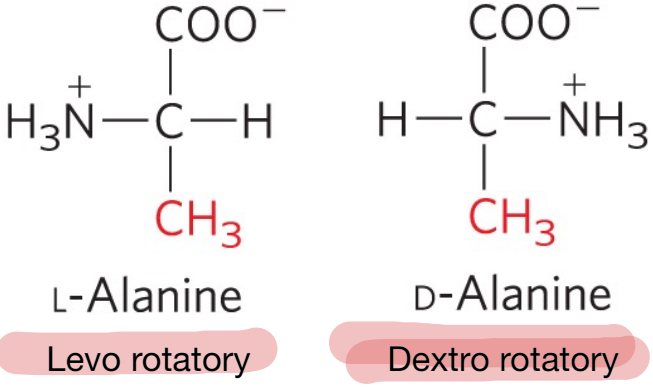
One letter abbreviation is K
Three letter abbreviation is Lys

Alpha-amino acid

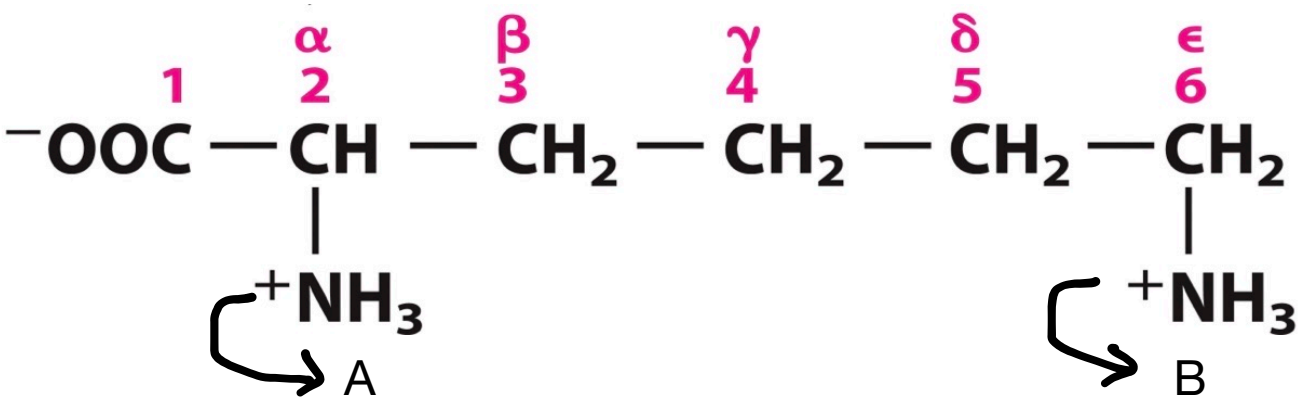
Alpha-amino acid: amino group and carboxyl group found at same carbon, this carbon is alpha carbon

The Amino Acid Residues in Proteins are L Stereoisomers

- two possible stereoisomers = **enantiomers**
- **optically active**
- D, L system specifies **absolute configuration**



- Amino acid must be in the same configuration at the protein.
- All Amino acid in the proteins in our body is L configuration.



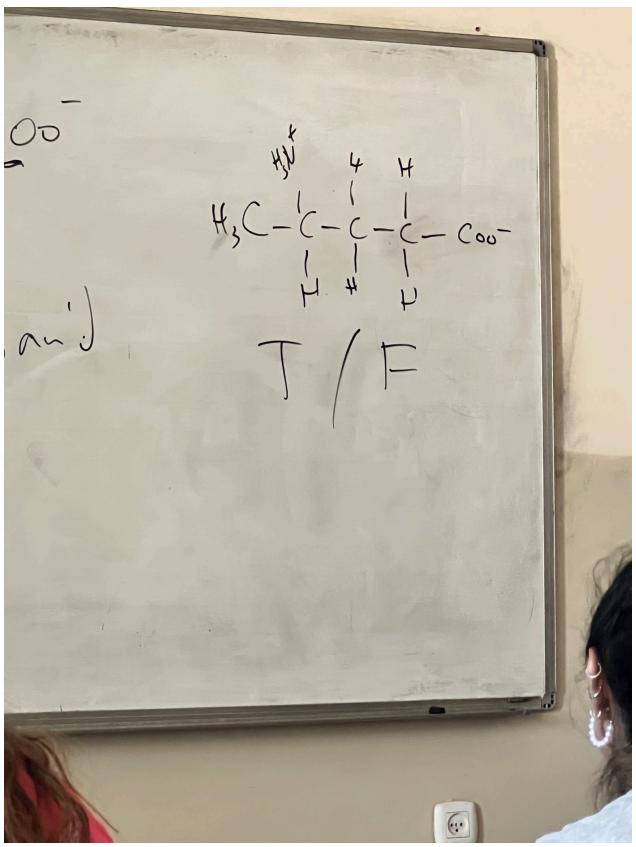
What the type of amino acid if:

1- A delete

Not alpha amino acid

2- B delete

Alpha amino



This is amino acid but not alpha amino acid

How do we determine if the amino acid is L configuration or D configuration?

To determine if an amino acid is L or D, look at the α carbon, so that the hydrogen atom is directly behind it. This should place the three other functional groups in a circle. Follow from COOH to R to NH2, or CORN. If this is in a clockwise direction, the the amino acid is in the L-isomer.

Amino Acids Can Be Classified by R Group

- Common amino acids can be placed in ***five main classes*** depending on their R substituents:
  - nonpolar, aliphatic (7)
  - aromatic (3)
  - polar, uncharged (5)
  - positively charged (3)
  - negatively charged (2)

YouTube Video:

1) Memorize the 20 Amino Acids in 9 Minutes

Table 3-1

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 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index[b]	Occurrence in proteins (%)[c]		
Nonpolar, aliphatic R groups										
Glycine	Gly G	75	2.34	9.60		5.97	–0.4	7.2	7.3	7.3
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8	9.4	7.2
Proline	Pro P	115	1.99	10.96		6.48	–1.6[d]	5.2	4.4	4.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6	7.1	8.2
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1	10.6	9.9
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3	6.0	7.6
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3	2.2	2.2
Aromatic R groups										
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9	4.0	4.5
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	–1.3	3.2	3.0	3.9
Tryptophan	Trp W	204	2.38	9.39		5.89	–0.9	1.4	1.3	1.1
Polar, uncharged R groups										
Serine	Ser S	105	2.21	9.15		5.68	–0.8	6.8	6.1	5.7
Threonine	Thr T	119	2.11	9.62		5.87	–0.7	5.9	5.4	4.5
Cysteine[e]	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9	1.2	0.8
Asparagine	Asn N	132	2.02	8.80		5.41	–3.5	4.3	3.7	3.4
Glutamine	Gln Q	146	2.17	9.13		5.65	–3.5	4.2	4.5	2.0
Positively charged R groups										
Lysine	Lys K	146	2.18	8.95	10.53	9.74	–3.9	5.9	4.7	6.8
Histidine	His H	155	1.82	9.17	6.00	7.59	–3.2	2.3	2.4	1.6
Arginine	Arg R	174	2.17	9.04	12.48	10.76	–4.5	5.1	5.6	5.9
Negatively charged R groups										
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	–3.5	5.3	5.1	5.0
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	–3.5	6.3	6.0	8.2

[a] M_r values reflect the structures as shown in Figure 3-5. The elements of water (M_r 18) are deleted when the amino acid is incorporated into a polypeptide.

[b] A scale combining hydrophobicity and hydrophilicity of R groups. The values reflect the free energy (G) of transfer of the amino acid side chain from a hydrophobic environment to water. This transfer is favorable ($G<0$; negative value in the index) for charged or polar amino acid side chains, and it is unfavorable ($G>0$; positive value in the index) for amino acids with nonpolar or more hydrophobic side chains. See Chapter 11. Source: Data from J. Kyte and R. F. Doolittle, *J. Mol. Biol.* 157:105, 1982.

[c] The first value in each row is the average occurrence in more than 1,150 proteins. Source: Data from R. F. Doolittle, in *Prediction of Protein Structure and the Principles of Protein Conformation* (G. D. Fasman, ed.), p. 599, Plenum Press, 1989. The second and third values are, respectively, from the complete proteomes of nine mesophilic bacterial species and seven thermophilic bacterial species. Mesophiles grow at commonly encountered temperatures, whereas thermophiles grow at elevated temperatures up to and beyond the boiling point of water. The decline in glutamine occurrence in thermophiles may reflect a tendency of this amino acid to deaminate at high temperatures. Source: Data from A. C. Singer and D. A. Hickey, *Gene* 317:39, 2003.

[d] As originally composed, the hydropathy index takes into account the frequency with which an amino acid residue appears on the surface of a protein. As proline often appears on the surface in β turns, it has a lower score than its chain of methylene groups would suggest.

[e] Cysteine is generally classified as polar, despite having a positive hydropathy index. This reflects the ability of the sulfhydryl group to act as a weak acid and to form a weak hydrogen bond with oxygen or nitrogen.

P1 Clicker Question 1

Which amino acid has three pK_a values?

A. leucine

B. proline

C. glycine

D. threonine

E. cysteine

Clicker Question 1, Response

Which amino acid has three pK_a values?

E. cysteine

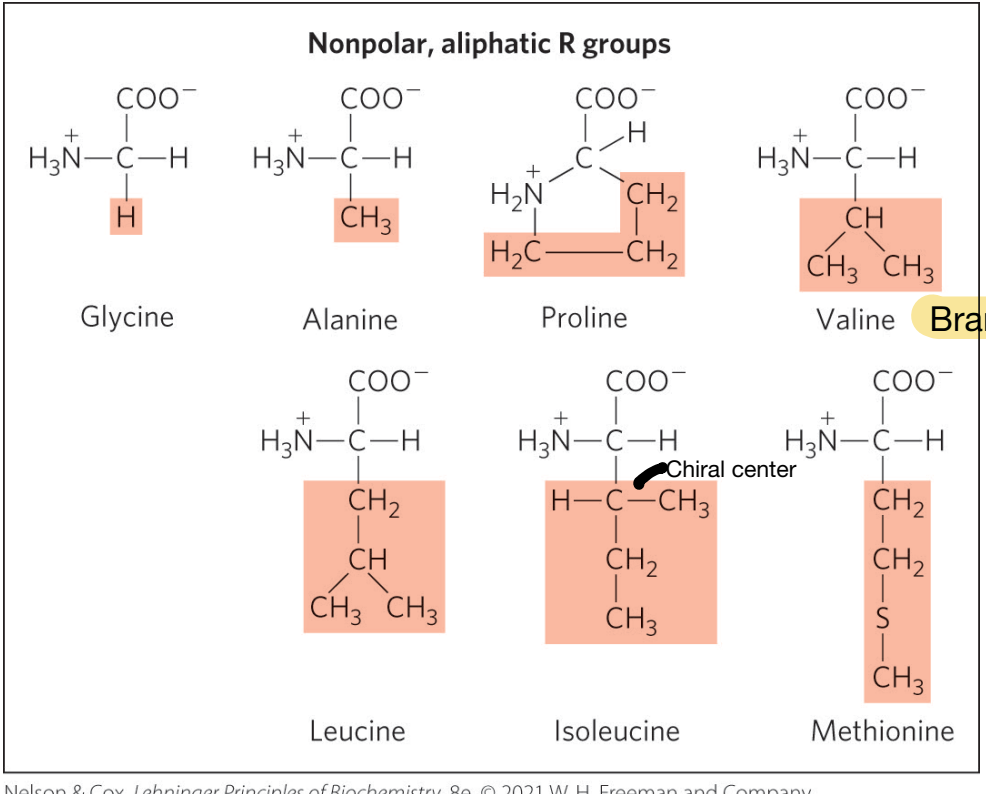
Cysteine has three pK_a values:

pK_1 (—COOH): 1.96

pK_2 (—NH_3^+): 10.28

pK_3 (R group): 8.18

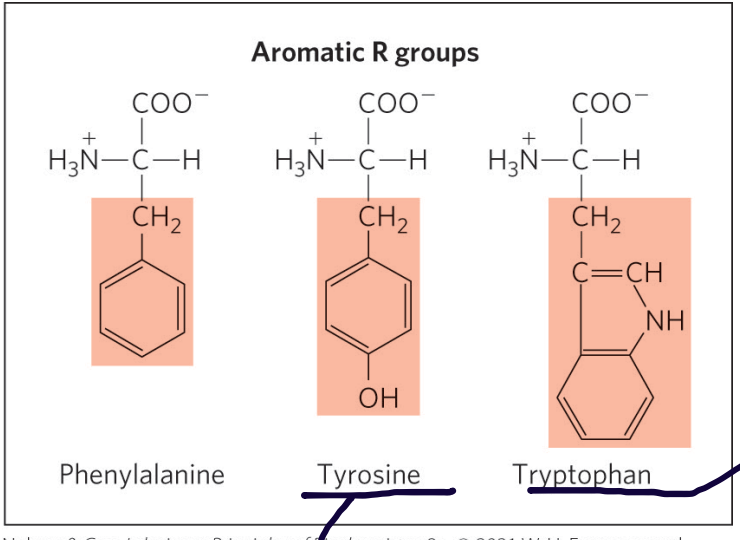
Nonpolar, aliphatic R groups



- **hydrophobic** effects stabilizes protein structure

Branched amino acid

Aromatic R Groups



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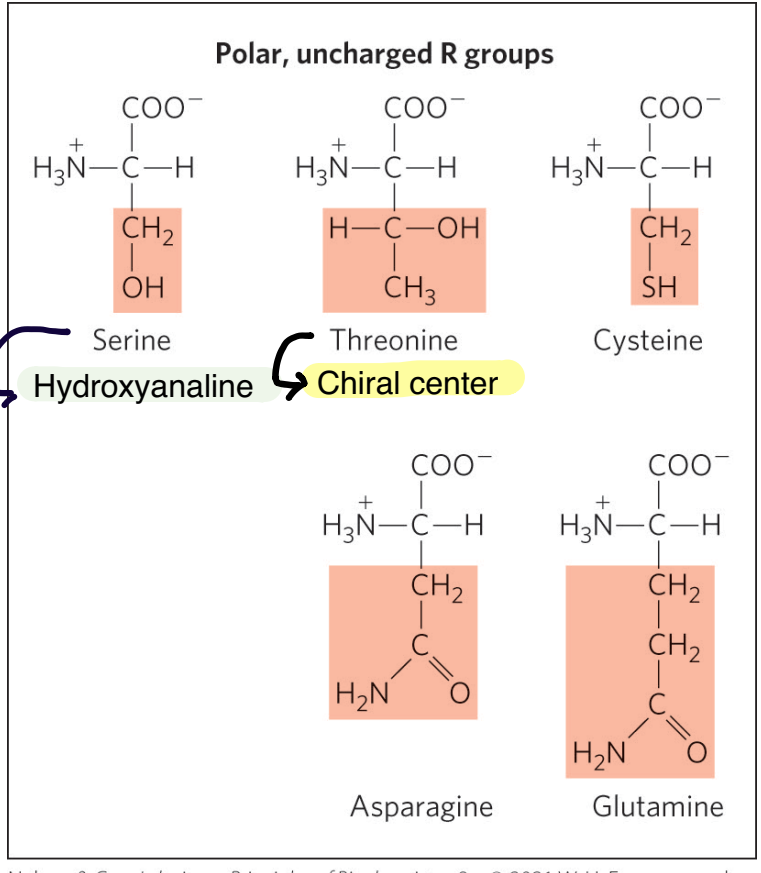
- R groups absorb UV light at 270–280 nm
- can contribute to the hydrophobic effect

Complex and is the amino acid that have the biggest R group

The first amino acid discovered

Polar, Uncharged R Groups



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- R groups can form ***hydrogen bonds***
- cysteine can form ***disulfide bonds***

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

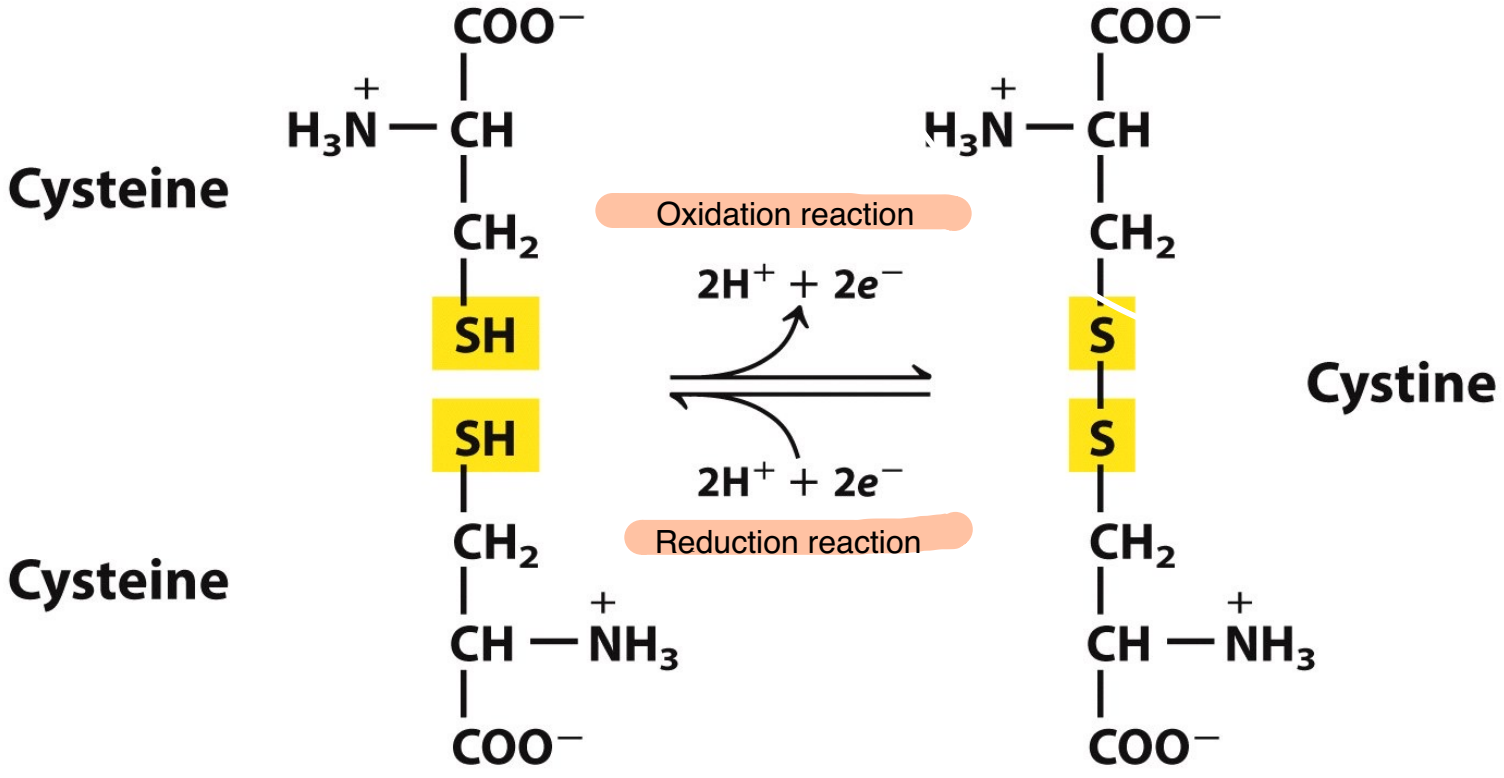

Figure 3-7
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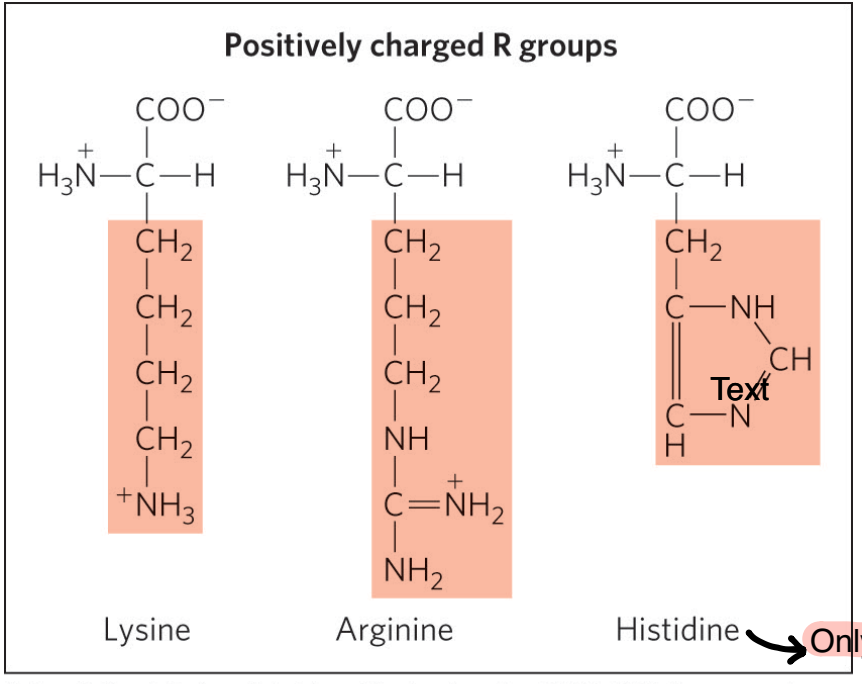
Positively Charged R Groups



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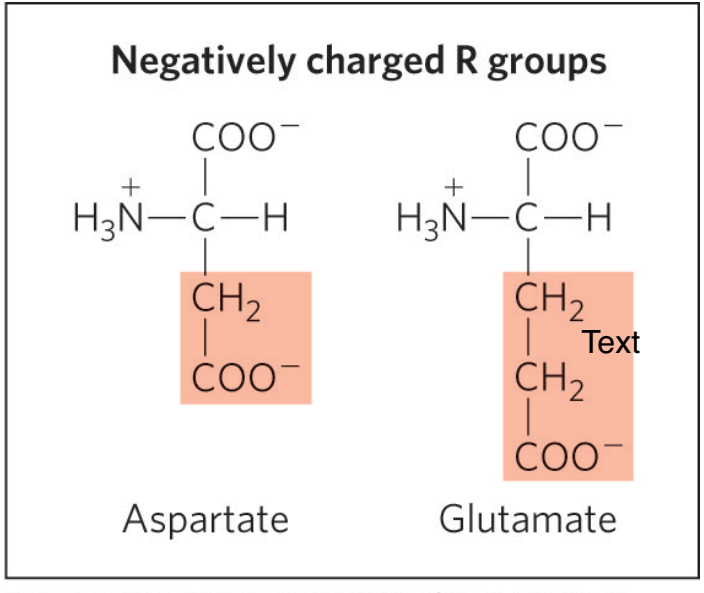
- have significant positive charge at pH 7.0
- ***Basic amino acids***

Only amino acid that can use as a buffer at neutral pH (pH=7)

Negatively Charged R Groups



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- have a net negative charge at pH 7.0
- ***Acidic amino acids***

Text

Produce by oxidation of asparagine and glutamine

P1 Clicker Question 2

Which amino acid would MOST likely be found in the interior of a globular protein?

A. Ala

B. D

C. glutamate

D. cysteine

E. Lys

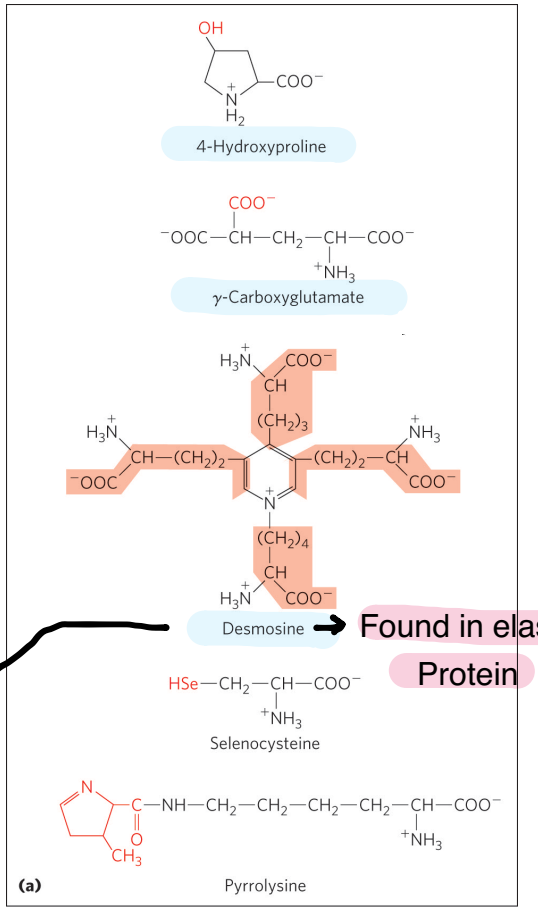
Clicker Question 2, Response

Which amino acid would MOST likely be found in the interior of a globular protein?

A. Alanine (Ala)

Alanine has a nonpolar, aliphatic R group, —CH_3. Nonpolar, hydrophobic side chains tend to cluster together within proteins, stabilizing protein structure through the hydrophobic effect.

Uncommon Amino Acids Also Have Important Functions



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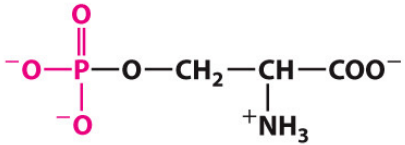
Elastin found in blood vessels

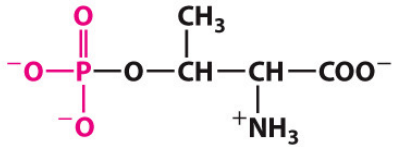
- modifications of common amino acids:
  - modified after protein synthesis (e.g., 4-hydroxyproline, found in collagen)
  - modified during protein synthesis (e.g., pyrrolysine and N-formylmethionine)
  - modified transiently to change protein's function (e.g., phosphorylation)
  - Not incorporated by ribosomes (except for selenocysteine)
  - free metabolites (e.g., ornithine, intermediate in arginine biosynthesis/urea cycle)

Text

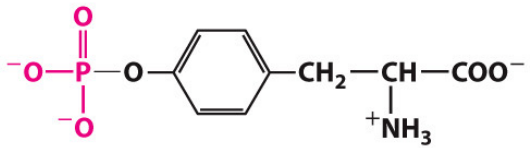
Reversible Modifications of Amino Acids



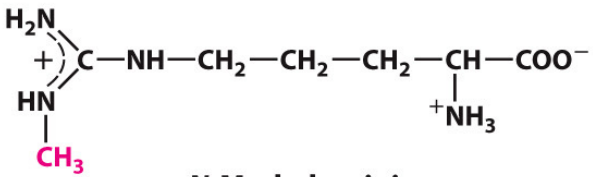
Phosphoserine



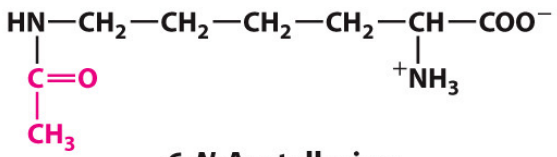
Phosphothreonine



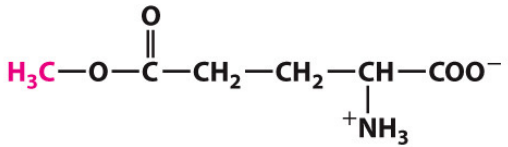
Phosphotyrosine



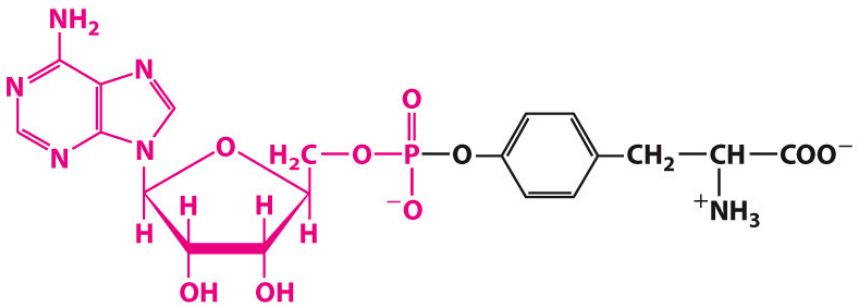
ω-*N*-Methylarginine



6-*N*-Acetyllysine



Glutamate γ-methyl ester



Adenylyltyrosine

Figure 3-8b
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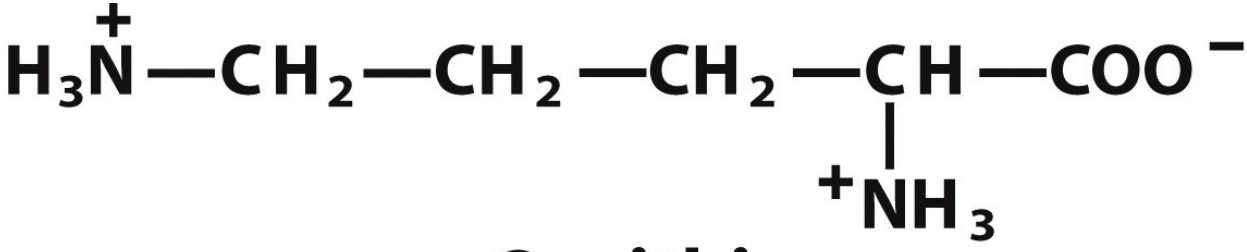
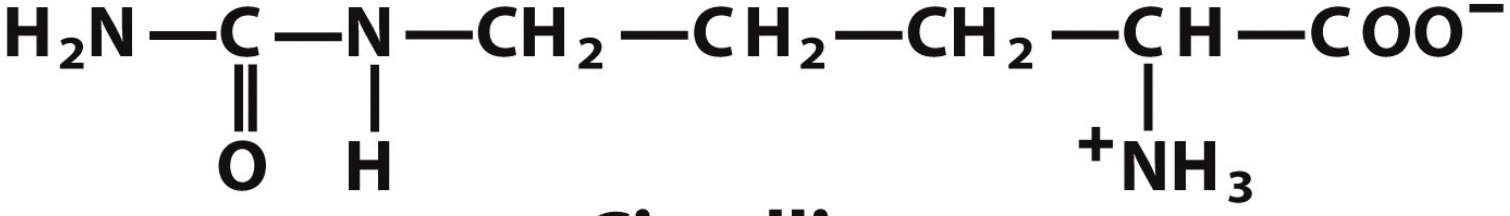
Important Amino Acids in Urea Metabolism



Ornithine



Citrulline

Figure 3-8c
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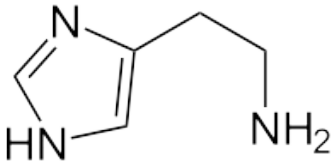
A Derivative of amino acids

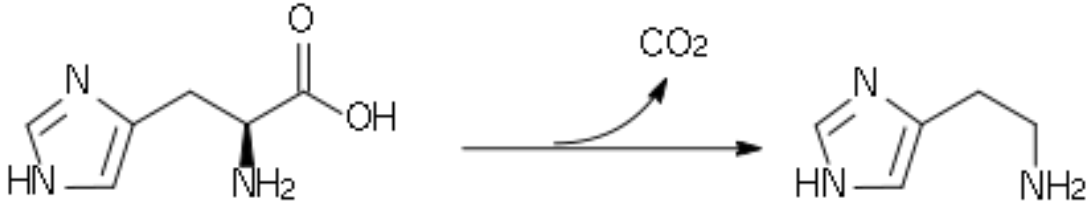
• **Histamine** is an organic nitrogen compound involved in local immune responses as well as regulating physiological function in the gut and acting as a neurotransmitter.



• Histamine triggers the inflammatory response

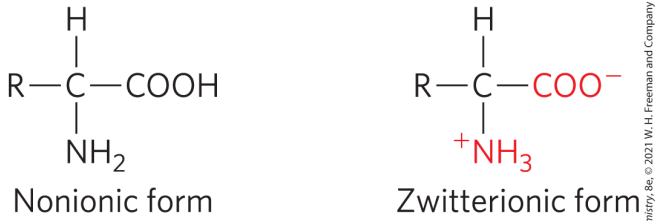
• Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues

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

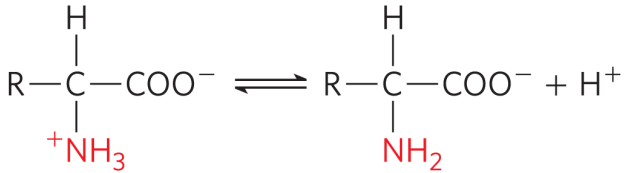


Amino Acids Can Act as Acids or Bases

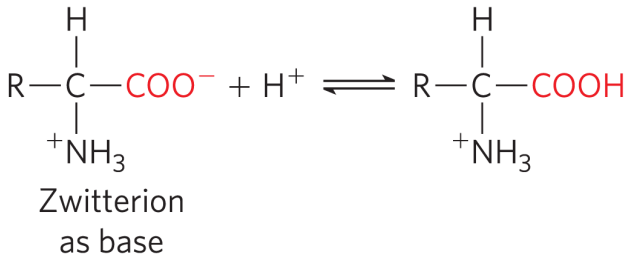
Not found in nature



Acidic medium



Basic medium



- amino groups, carboxyl groups, and ionizable R groups = weak acids and bases
- **zwitterion** occurs at neutral pH

The most soluble amino acid in water is charged amino acid (positive or negative)

Example

+H3N—CH2–COOH —>+H3V—CH2–COO- —> N2H—CH2–COO-

Net charge=+1 Net charge=0 Net charge=-1

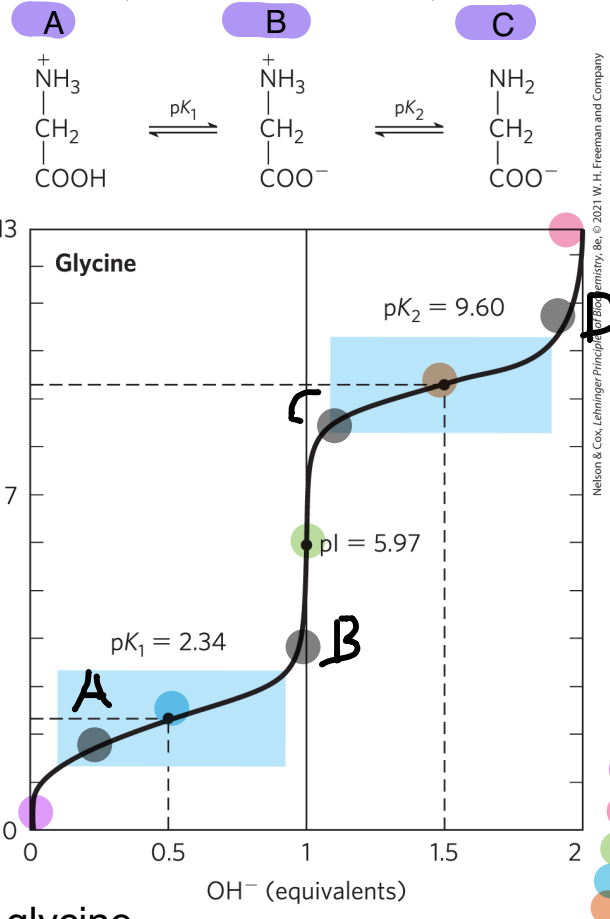
- The charge of amino acid change from +1 to 0 to -1
- This change in charge occur in process called simple titration

Example : Found the concentration of A,B orC at black point ● in titration curve

A- 75%A and 25%B
B- 25%A and 75%B
C- 75%B and 25%C
D- 25%B and 75%C

Titration of Amino Acids

cation ⇌ zwitterion ⇌ anion



- —COOH has an acidic pK_a (pK_1)
- —NH_3^+ has a basic pK_a (pK_2)
- the pH at which the net electric charge is zero is the **isoelectric point (pI)**

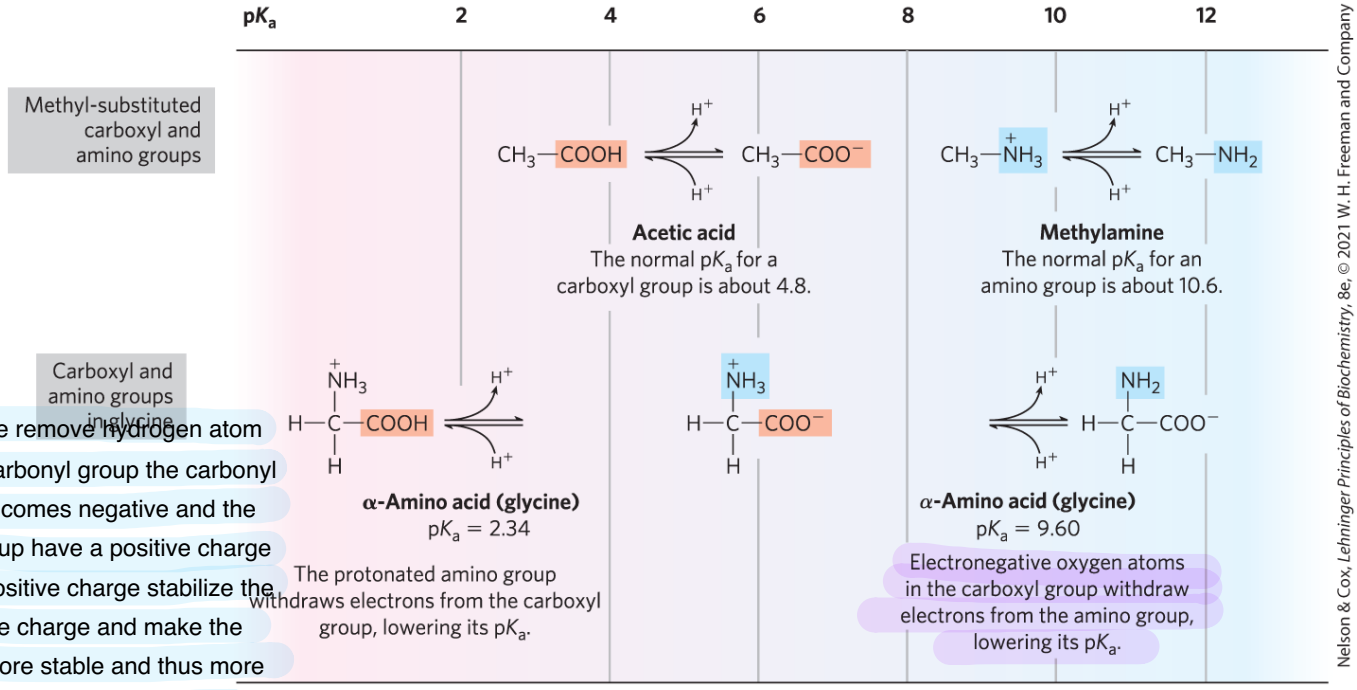
100% A
100% C
100% B
50%A and 50%B
50%B and 50%C

YouTube Video:

1) Titration curve of glycine

Effect of the Chemical Environment on pK_a

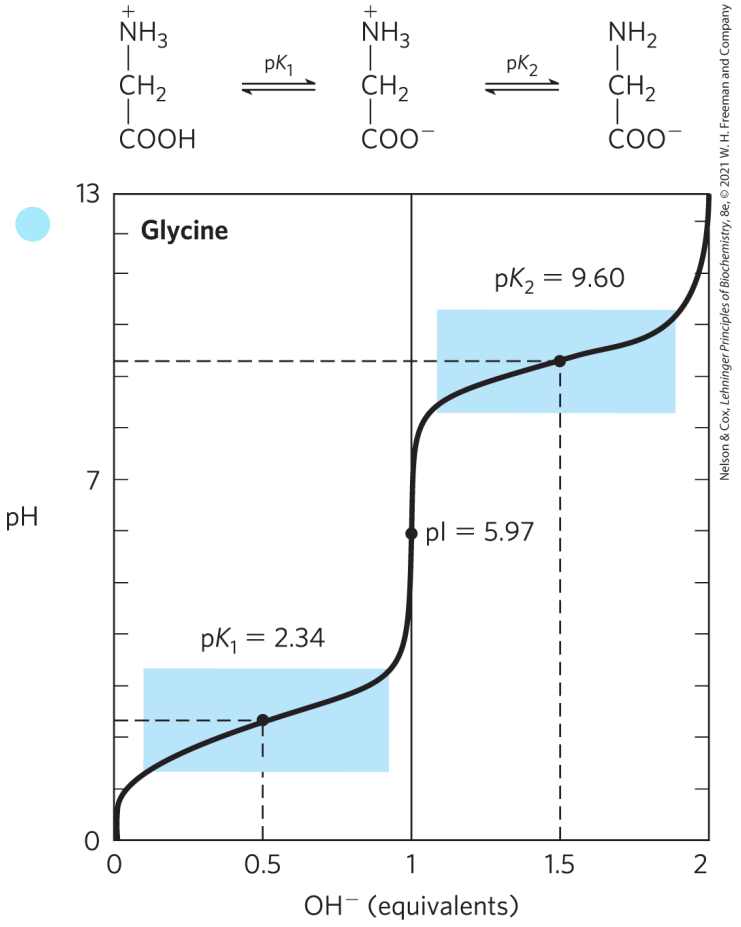
- α-carboxyl group is more acidic than in carboxylic acids
- α-amino group is less basic than in amines



When we remove hydrogen atom from the carbonyl group the carbonyl group becomes negative and the amino group have a positive charge and this positive charge stabilize the negative charge and make the glycine more stable and thus more acidic than acetic acid

Information from a Titration Curve

- quantitative measure of the pK_a of each ionizing group
- regions of buffering power
- relationship between its net charge and the pH of the solution
  - **isoelectric point, pI**

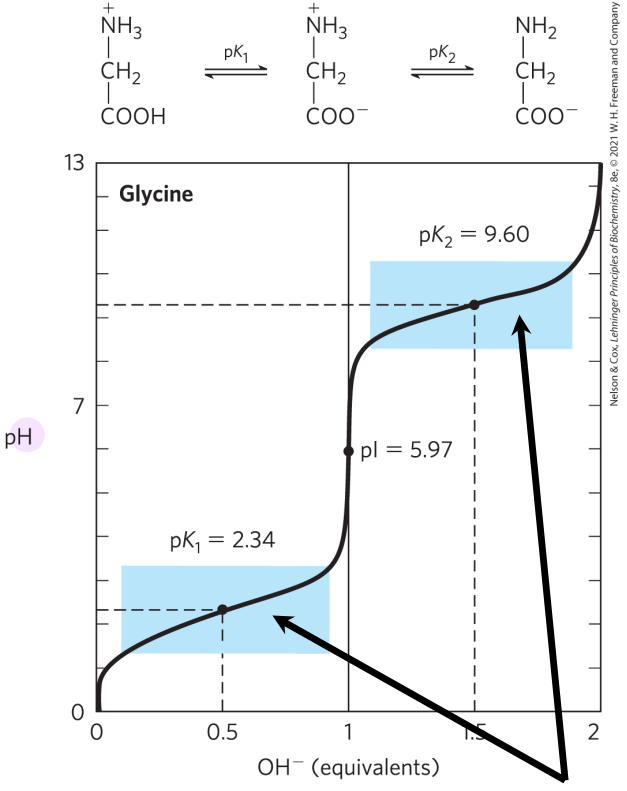


Amino Acids as Buffers

A buffer consists of a weak acid and its conjugate base or a weak base and its conjugate acid. The best buffer consists of 50%acid and 50% conjugate base or 50% base and 50% conjugate acid

- buffers prevent changes in pH close to the pK_a
  - Buffer region =-+pka
  - Example: pka=3.45

  Buffer region from 2.45 to 4.45
- glycine has two buffer regions:
  - centered around the pK_a of the α-carboxyl group (pK_1 = 2.34)
  - centered around the pK_a of the α-amino group (pK_2 = 9.6)



buffer regions

Isoelectric Point, pI

- for amino acids without ionizable side chains, the **isoelectric point** (**pI**) is:

Lowest solubility in water

$$\mathrm{pI} = \frac{\mathrm{p}K_1 + \mathrm{p}K_2}{2}$$

- pH = pI = net charge is zero (amino acid least soluble in water, does not migrate in electric field)
- pH > pI = net negative change
- pH < pI = net positive charge

P1 Clicker Question 3

Given that all 20 common amino acids have at least two oppositely charged groups, which statement is NOT correct?

A. Solutions of amino acids can act as buffers.
B. All amino acids are zwitterions.
C. All amino acids carry at least one charge across the full range of their titration curves.
D. Amino acid isoelectric points should be near neutrality.
E. It is not possible to fully deprotonate amino acids.

Clicker Question 3, Response

Given that all 20 common amino acids have at least two oppositely charged groups, which statement is NOT correct?

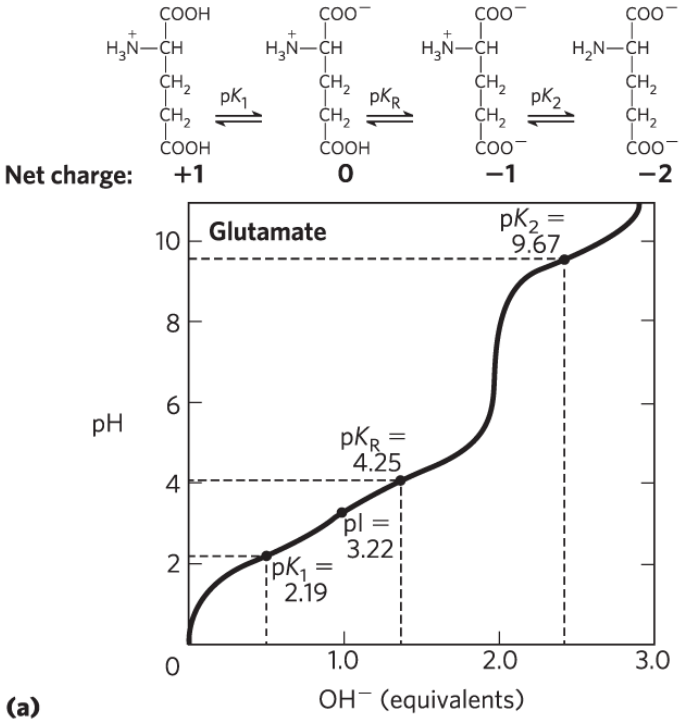
E. It is not possible to fully deprotonate amino acids.

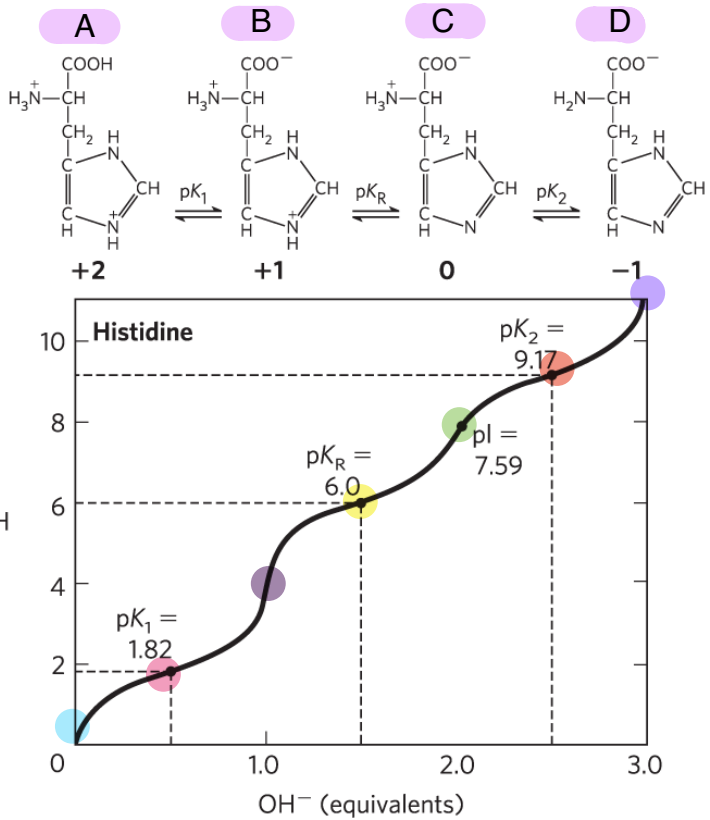
At sufficiently high pH values, the carboxyl group, amine group, and any ionizable R groups are fully deprotonated and the amino acid carries a negative charge.

Amino Acids Differ in Their Acid-Base Properties

- ionizable side chains:
  - have a pK_a value
  - act as buffers
  - influence the pI of the amino acid
  - can be titrated (titration curve has 3 ionization steps)

Titration of Amino Acids with an Ionizable R Group





Text

100% A
50%A and 50% B
100%D
50%C and 50%D
50%B and 50%C
100%C
100%B

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 glutamate?

- $(pK_R + pK_1)/2 = pI$
- $(2.19 + 4.25)/2 = 3.22$

P1 Clicker Question 4

At what pH values will histidine have a net neutral charge?

A. below 1.82
B. between 1.82 and 6.0
C. between 6.0 and 9.17
D. above 9.17
E. There are no pH values where histidine will have a net neutral charge.

Clicker Question 4, Response

At what pH values will histidine have a net neutral charge?

C. between 6.0 and 9.17

Histidine has three pK_a values: pK_1 (—COOH): 1.82; pK_2 (—NH_3^+): 9.17; pK_3 (R group): 6.0. Below pH 1.82, histidine has a net +2 charge. Between pH 1.82 and 6.0, the —COOH group loses its proton and histidine has a net +1 charge. Between 6.0 and 9.17, the R group loses its proton and histidine has a net neutral charge.

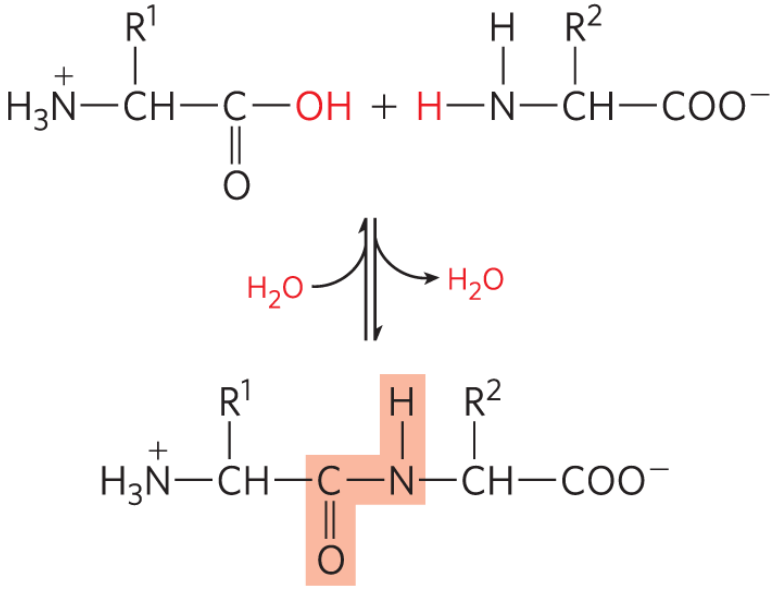
3.2 Peptides and Proteins

P2 Principle 2

In proteins, amino acids are joined in characteristic linear sequences through a common amide linkage, the peptide bond. The amino acid sequence of a protein constitutes its primary structure, a first level we will introduce within the broader complexities of protein structure.

Peptides Are Chains of Amino Acids

- **peptide bond:**
  - covalent
  - formed through **condensation**
  - broken through **hydrolysis**



All dehydration process is condensation process but not all condensation process is dehydration process

YouTube Video:

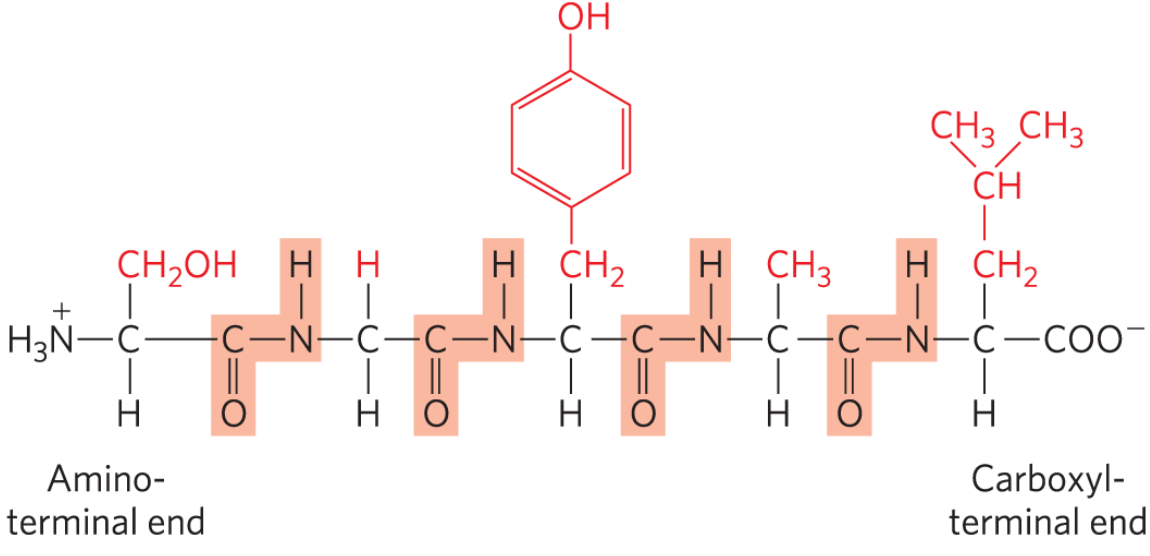
1) Proteins (Osmosis)

Peptide Types by the Number

- dipeptide = 2 amino acids, 1 peptide bond
- tripeptide = 3 amino acids, 2 peptide bonds
- **oligopeptide** = a few amino acids
- **polypeptide** = many amino acids, molecular weight < 10 kDa
- **protein** = hundreds - thousands of amino acids, molecular weight > 10 kDa

Peptide Terminals

numbering (and naming) starts from the **amino-terminal residue** (*N*-terminal)



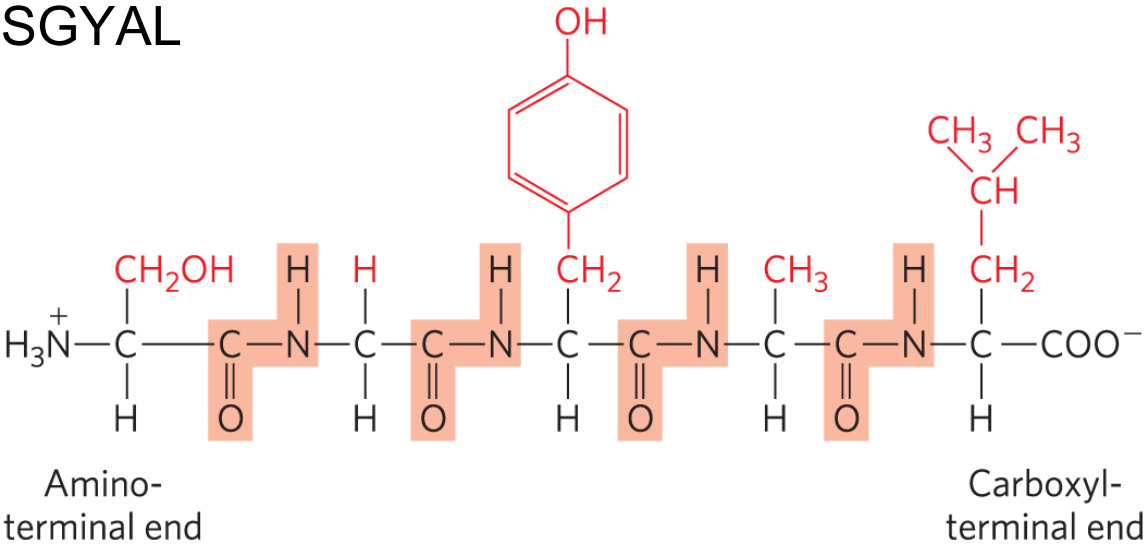
N-terminal

C-terminal

All amino acid in peptide lose water molecule so it called amino acid

Naming Peptides

- full amino acid names:
  serylglycyltyrosylalanylleucine
- three-letter code abbreviations:
  Ser–Gly–Tyr–Ala–Leu
- one-letter code abbreviation:
  SGYAL



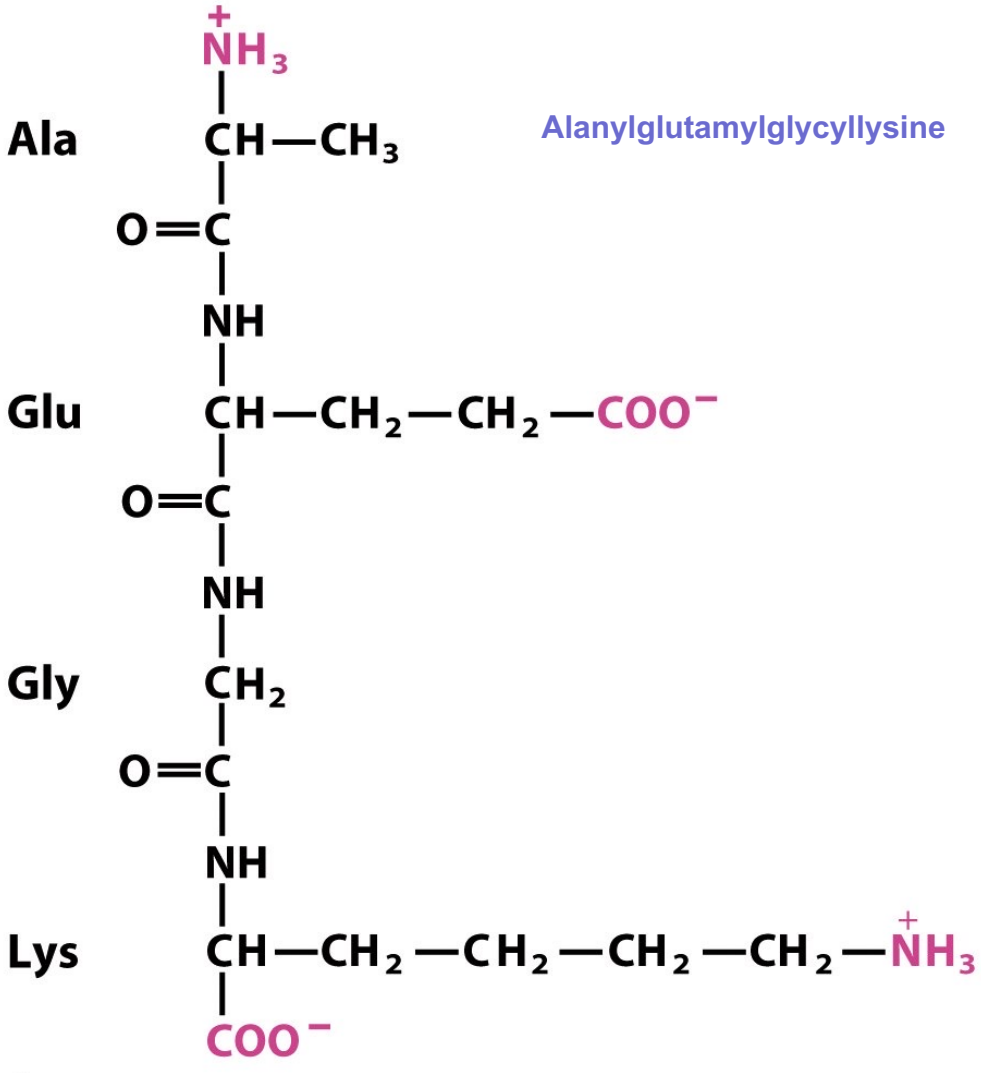


Figure 3-15
Lehninger Principles of Biochemistry, Fifth Edition

P2 Clicker Question 5

Which statement is correct about peptides?

A. Peptides have no *α*-carboxyl groups.
B. Peptides have their amino acid sequences written from the *N*-terminus.
C. Peptides do not have isoelectric points.
D. Peptides are not biologically active.
E. Peptide bonds are broken through condensation reactions.

Clicker Question 5, Response

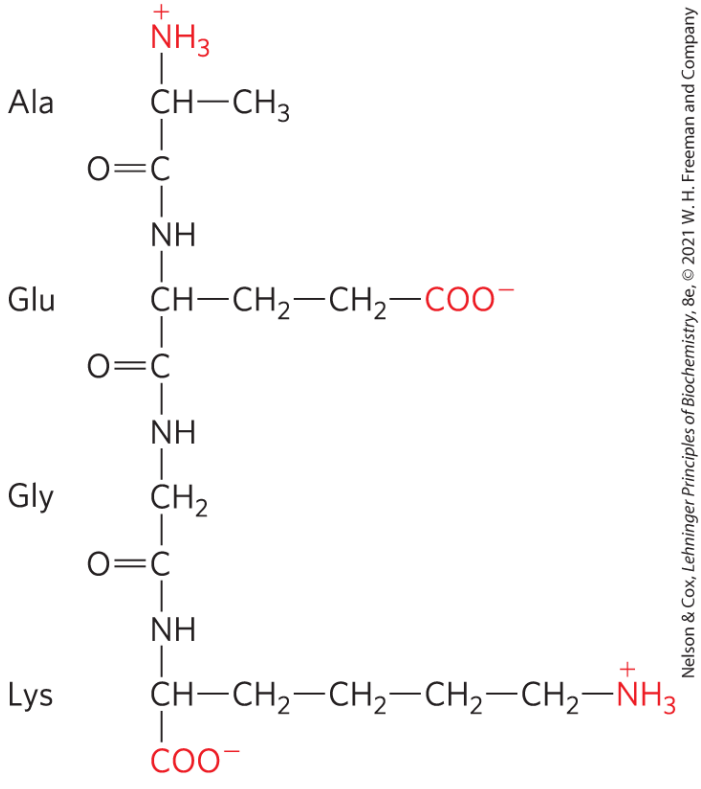
Which statement is correct about peptides?

B. Peptides have their amino acid sequences written from the *N*-terminus.

Peptides are named beginning with the amino-terminal (*N*-terminal) residue.

Peptides Can Be Distinguished by Their Ionization Behavior

- ionizable groups in peptides:
  - one free α-amino group
  - one free α-carboxyl group
  - some R groups



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

- length of naturally occurring peptides = 2 to many thousands of amino acid residues

Table 3-2 Molecular Data on Some Proteins

Protein	Molecular weight	Number of residues	Number of polypeptide chains
Cytochrome *c* (human)	12,400	104	1
Myoglobin (equine heart)	16,700	153	1
Chymotrypsin (bovine pancreas)	25,200	241	3
Hemoglobin (human)	64,500	574	4
Hexokinase (yeast)	107,900	972	2
RNS polymerase (*E. coli*)	450,00	4,158	5
Glutamine synthetase (*E. coli*)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

Peptide Subunits

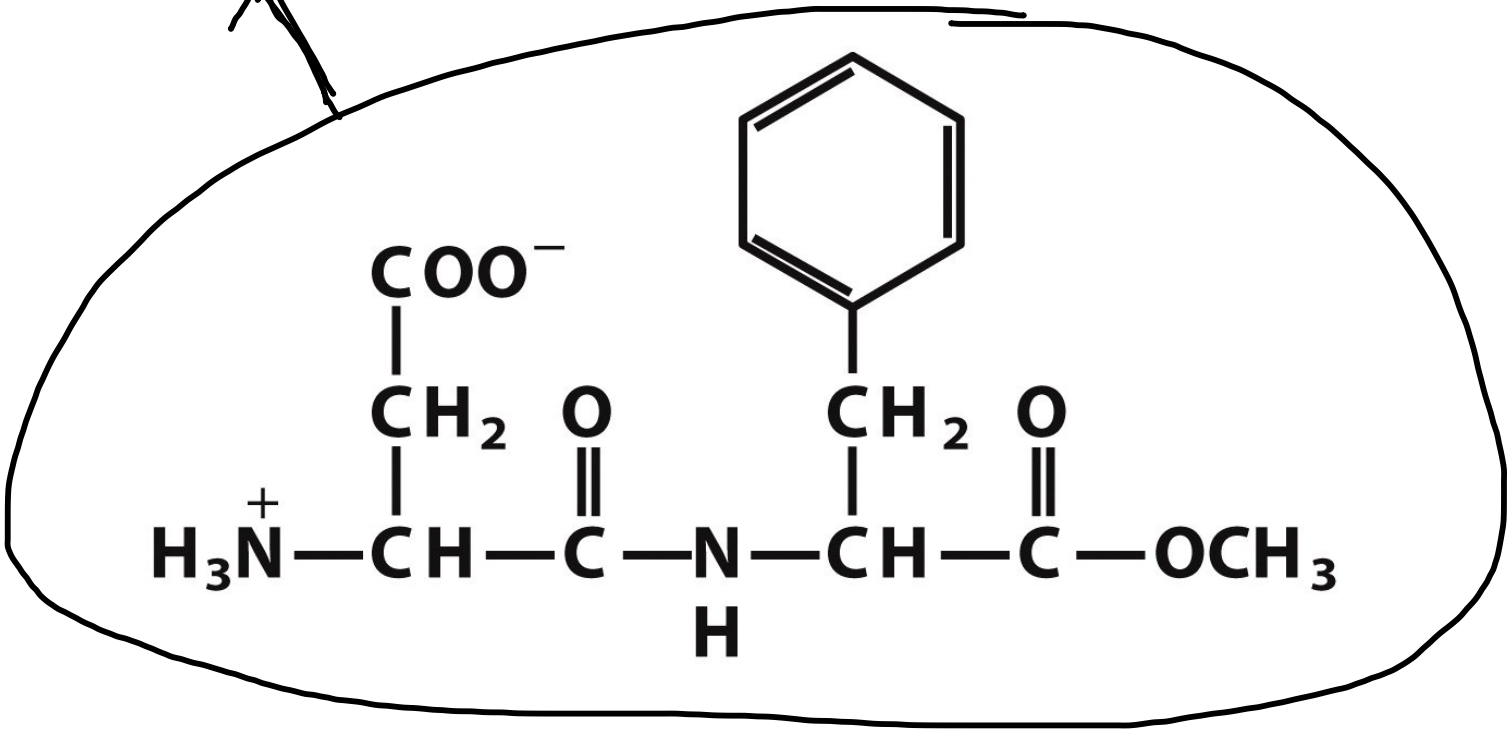
- **multisubunit** protein = 2+ polypeptides associated noncovalently

- **oligomeric** protein = at least 2 identical subunits
  - identical units = **protomers**

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

P2 Clicker Question 6

The cytoskeletal component filamentous actin is made of many subunits, all of which are globular actin. Which term refers to globular actin in this context?

A. prosthetic group
B. protomer
C. ligand
D. cofactor
E. polymorphism

Clicker Question 6, Response

The cytoskeletal component filamentous actin is made of many subunits, all of which are globular actin. Which term refers to globular actin in this context?

B. protomer

Identical units in a multisubunit protein are referred to as protomers.

Amino Acid Composition of Proteins

- amino acid composition is highly variable

Table 3-3 Amino Acid Composition of Two Proteins

Amino Acid	Bovine cytochrome *c*: Number of residues per molecule	Bovine cytochrome *c*: percentage of total	Bovine chymotrypsinogen: Number of residues per molecule	Bovine chymotrypsinogen: Percentage of total
Ala	6	6	22	9
Arg	2	2	4	1.6
Asn	5	5	14	5.7
Asp	3	3	9	3.7
Cys	2	2	10	4
Gln	3	3	10	4
Glu	9	9	5	2
Gly	14	13	23	9.4
His	3	3	2	0.8
Ile	6	6	10	4
Leu	6	6	19	7.8
Lys	18	17	14	5.7
Met	2	2	2	0.8
Phe	4	4	6	2.4
Pro	4	4	9	3.7
Ser	1	1	28	11.4
Thr	8	8	23	9.4
Trp	1	1	8	3.3
Tyr	4	4	4	1.6
Val	3	3	23	9.4
Total	104	102	245	99.7

Estimating the Number of Amino Acid Residues

- number of residues = molecular weight/110
- average molecular weight of amino acid = ~128
- molecule of water removed to form peptide bond = 18

Text

128 – 18 = 110 ⇒ so we use 110 to foun MW.

Some Proteins Contain Chemical Groups Other Than Amino Acids

- **conjugated** proteins = contain permanently associated chemical components
  - non–amino acid part = **prosthetic group**

specific non-polypeptide unit required for the biological function of some proteins

- **lipoproteins** contain lipids
- **glycoproteins** contain sugars
- **metalloproteins** contain specific metals

Table 3-4 Conjugated Proteins

Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1-Lipoprotein of blood (Fig. 17-2)
Glycoproteins	Carbohydrates	Immunoglobulin G (Fig. 5-20)
Phosphoproteins	Phosphate groups	Glycogen phosphorylase (Fig. 6-39)
Hemoproteins	Heme (iron porphyrin)	Hemoglobin (Figs 5-8 to 5-11)
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase (Fig. 19-9)
Metallproteins	Iron Zinc Calcium Molybdenum Copper	Ferritin (Box 16-1) Alcohol dehrogenase (Fig. 14-12) Calmodulin (Fig. 12-17) Dinitrogenase (Fig. 22-3) Complex IV (Fig. 19-12)

P2 Clicker Question 7

Which statement is correct about proteins?

A. Proteins can consist of more than one polypeptide chain.
B. Proteins consist solely of polymerized amino acids.
C. Proteins all have similar amino acid compositions.
D. Proteins can, by definition, consist of no more than 2,000 amino acid residues.
E. Proteins do not have ionizable groups.

Clicker Question 7, Response

Which statement is correct about proteins?

A. Proteins can consist of more than one polypeptide chain.

Multisubunit proteins have two or more polypeptide chains that are noncovalently associated.

3.3 Working with Proteins

P3 Principle 3

For study, individual proteins can be separated from the thousands of other proteins present in a cell, based on differences in their chemical and functional properties arising from their distinct amino acid sequences. As proteins are central to biochemistry, the purification of individual proteins for study is a quintessential biochemical endeavor.

جوهري،محوري

مسعى

Proteins Can Be Separated and Purified

- separated based on:
  - size
  - charge
  - binding properties
  - protein solubility → (Polar or nonpolar

Methods for Purifying Proteins

تكسير و طحن

Main source of protein

- first step = break open tissue or microbial cells
  - **crude extract** = releases proteins in solution

تجزئة

By use centrifugation

- second step = **fractionation** = separate proteins into **fractions** based on size or charge
  - “salting out” = lower solubility of proteins in salt to selectively precipitate proteins

When I add salt to test tube comtain protein dissolved in water, the water will connected to salt and thus water not connected to protein so protein molecules will gather on each other And precipitate

- third step = **dialysis** = use semipermeable membrane to separate proteins from small solutes

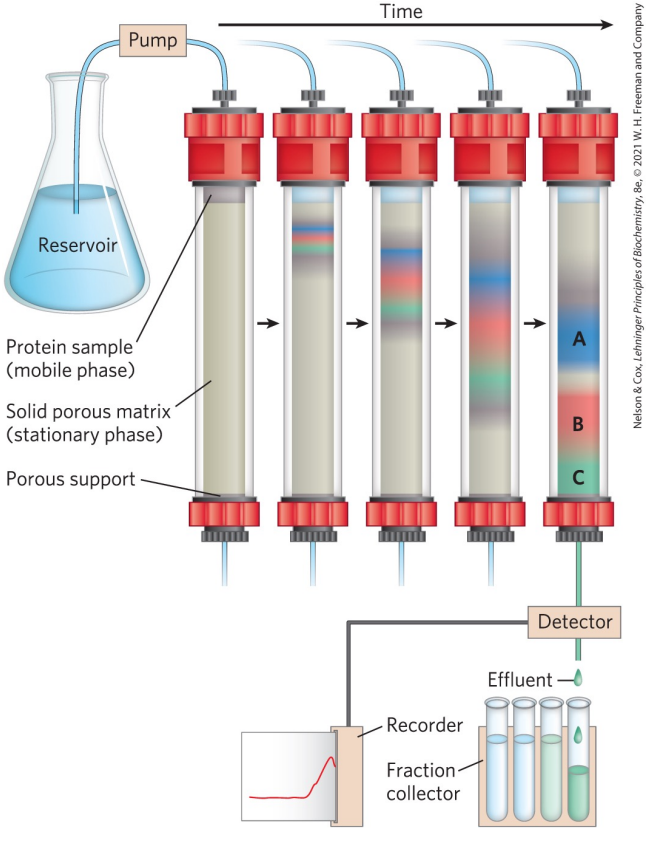
Important in the renaturation process

the reconstruction of a protein or nucleic acid (such as DNA) to their original form especially after denaturation.

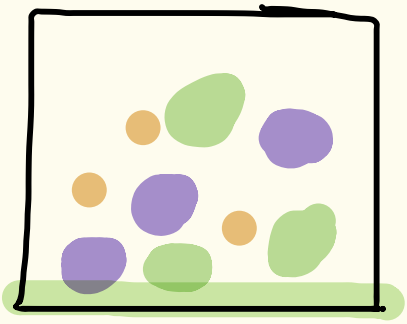
Chromatography is commonly used for preparative separation in which the protein is often able to remain fully folded → Remain functional

Column Chromatography

- first step = buffered solution (mobile phase) migrates through porous solid material (solid phase)
- second step = buffered solution containing protein migrates through solid phase
- protein properties affect migration rates



Salting out



Beaker

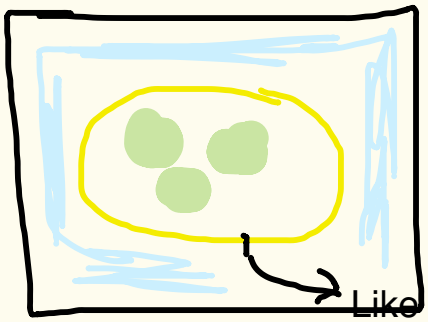
Protein 1

Protein 2

Protein 3

We add ammonium salt so let's say protein 3 will precipitate then this precipitate undergo dialysis

Dialysis



Beaker

Dialysis bag

Correct buffer

Like filter, it allow passage of solution and small solute and not all protein to go out

—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 (غارقة) 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 $(NH_4)_2SO_4$

Also called buffer exchange

P3

Clicker Question 8

Which component is absolutely necessary for the purification of a protein?

A. column chromatography
B. the gene sequence of the protein
C. a means of detecting the protein
D. a centrifuge

Clicker Question 8, Response

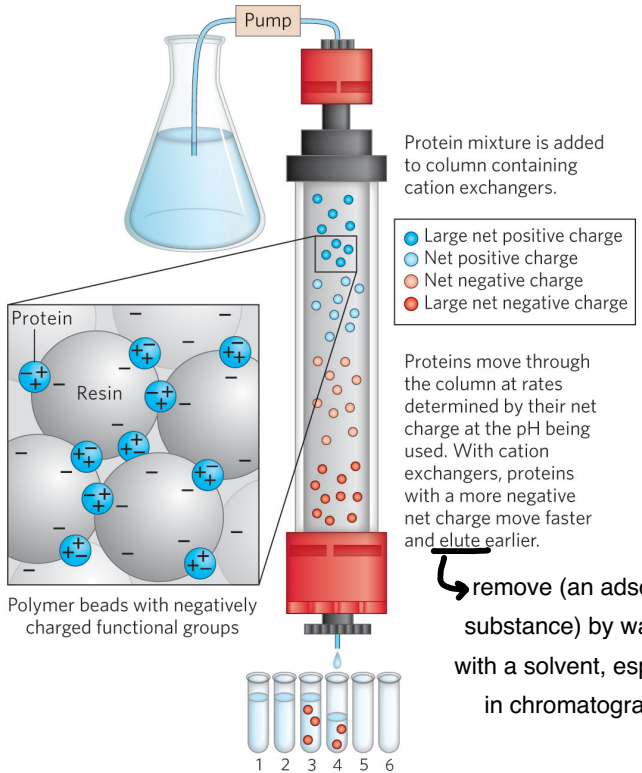
Which component is absolutely necessary for the purification of a protein?

C. a means of detecting the protein

To study a protein in detail, researchers must be able to separate it from other proteins in pure form and must have the techniques to determine its properties.

Ion-Exchange Chromatography

- separates based on sign and magnitude of the net electric charge
- pH and concentration of free salt ions affect protein affinity
- uses bound charged groups:
  - **cation exchangers**
  - **anion exchangers**



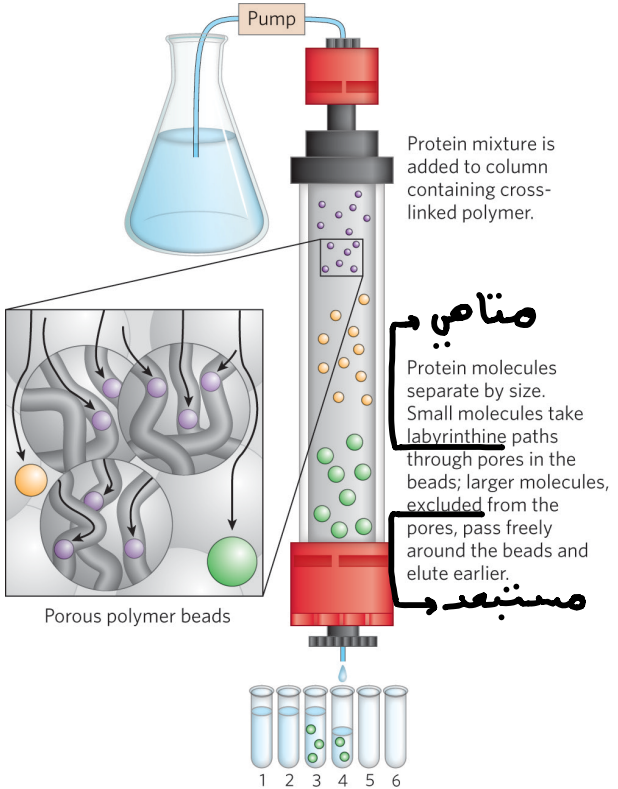
(a) Ion-exchange chromatography

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

remove (an adsorbed substance) by washing with a solvent, especially in chromatography.

Size-Exclusion Chromatography

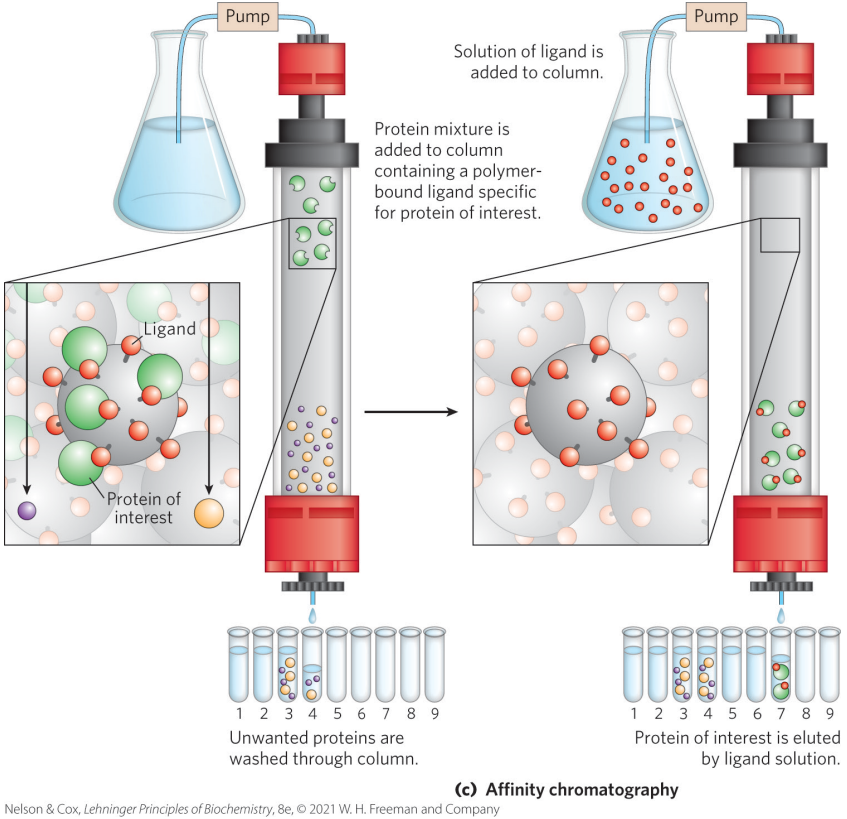
- also called gel filtration chromatography
- separates based on size
- large proteins emerge from the column before small proteins do



Affinity Chromatography

- separates based on binding affinity
- eluted by high concentration of salt or ligand

There are certain ligand in the stationary phase that certain protein bound to it and other proteins not bound and move down.



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P3

Clicker Question 9

Which protein would elute first from a gel filtration column?

A. protein A, with M_r = 27,000
B. protein B, with M_r = 58,400
C. protein C, a homodimer with protomer M_r = 11,300
D. protein D, with M_r = 15,600

Clicker Question 9, Response

Which protein would elute first from a gel filtration column?

B. protein B, with M_r = 58,400

Size-exclusion chromatography, also called gel filtration, separates proteins according to size. In this method, large proteins emerge from the column sooner than small ones do.

High-Performance Liquid Chromatography

- uses high-pressure pumps to move proteins down the column
- greatly improves resolution

P3 Clicker Question 10

A new protein resembling myosin was reported. Unlike myosin, it binds calcium. Its isoelectric point and molecular weight are *very similar* to those of myosin. Which method would BEST separate the new protein from myosin if those two proteins were in the same buffer solution?

A. ion-exchange chromatography
B. size-exclusion chromatography
C. affinity chromatography
D. dialysis
E. fractionation

Clicker Question 10, Response

Which method would BEST separate the new protein from myosin if those two proteins were in the same buffer solution?

C. affinity chromatography

Attaching calcium to the beads in the column would create an affinity matrix that could help purify the protein. Proteins that do not bind to calcium would flow more rapidly through the column than the new protein, which does bind calcium.

P3 Clicker Question 11

The specific activity of a protein in crude cellular extract is 15 units/mg. Following a purification process, involving precipitation with ammonium sulfate and multiple chromatography steps, the specific activity of the protein is 12,000 units/mg. What is the purification factor?

A. 180,000
B. 800
C. 0.00125
D. 15
E. 12,000

Clicker Question 11, Response

The specific activity of a protein in crude cellular extract is 15 units/mg. Following a purification process, involving precipitation with ammonium sulfate and multiple chromatography steps, the specific activity of the protein is 12,000 units/mg. What is the purification factor?

B. 800

The ratio of the final specific activity (12,000 units/mg) to the starting specific activity (15 units/mg) gives the purification factor (800).

Proteins Can Be Separated and Characterized by Electrophoresis

- **electrophoresis** = visualize and characterize purified proteins

- can be used to estimate:
  - number of different proteins in a mixture
  - degree of purity
  - isoelectric point
  - approximate molecular weight

YouTube Video:

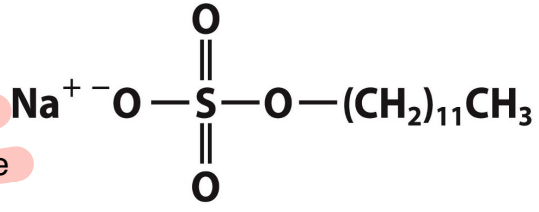
1) SDS-PAGE, Sodium Dodecyl Sulfate–PolyAcrylamide Gel Electrophoresis–Animation

Sodium Dodecyl Sulfate (SDS)

- **sodium dodecyl sulfate (SDS)** = a detergent
  - binds and partially unfolds proteins
  - gives all proteins a similar charge-to-mass ratio
  - electrophoresis in the presence of SDS separates proteins by molecular weight
    - smaller proteins migrate more rapidly

Heavy protein—> more negative change

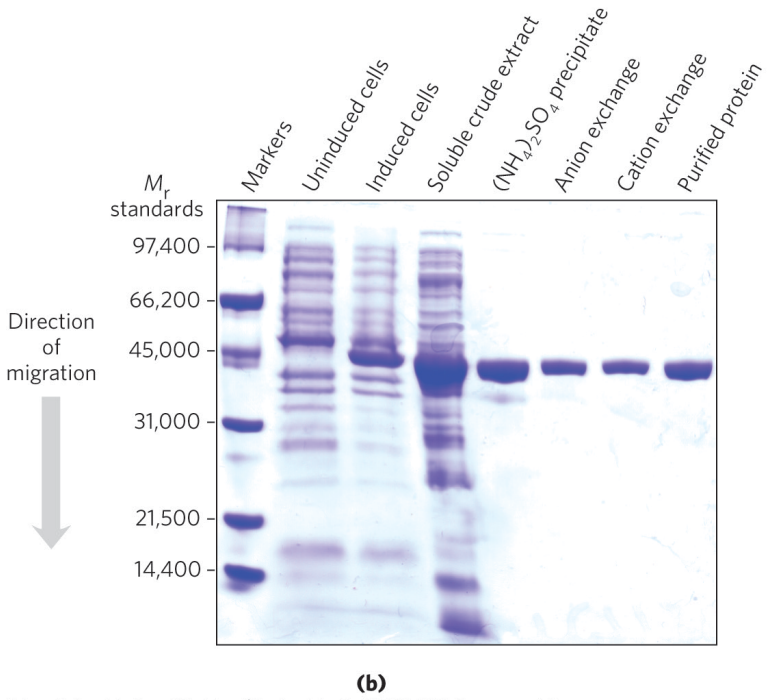
light (خفيف) protein—>less negative change



Sodium dodecyl sulfate (SDS)

Electrophoresis for Protein Analysis

- uses cross-linked polymer polyacrylamide gels
- proteins migrate based on charge-to-mass ratio
- visualization = Coomassie blue dye binds to proteins



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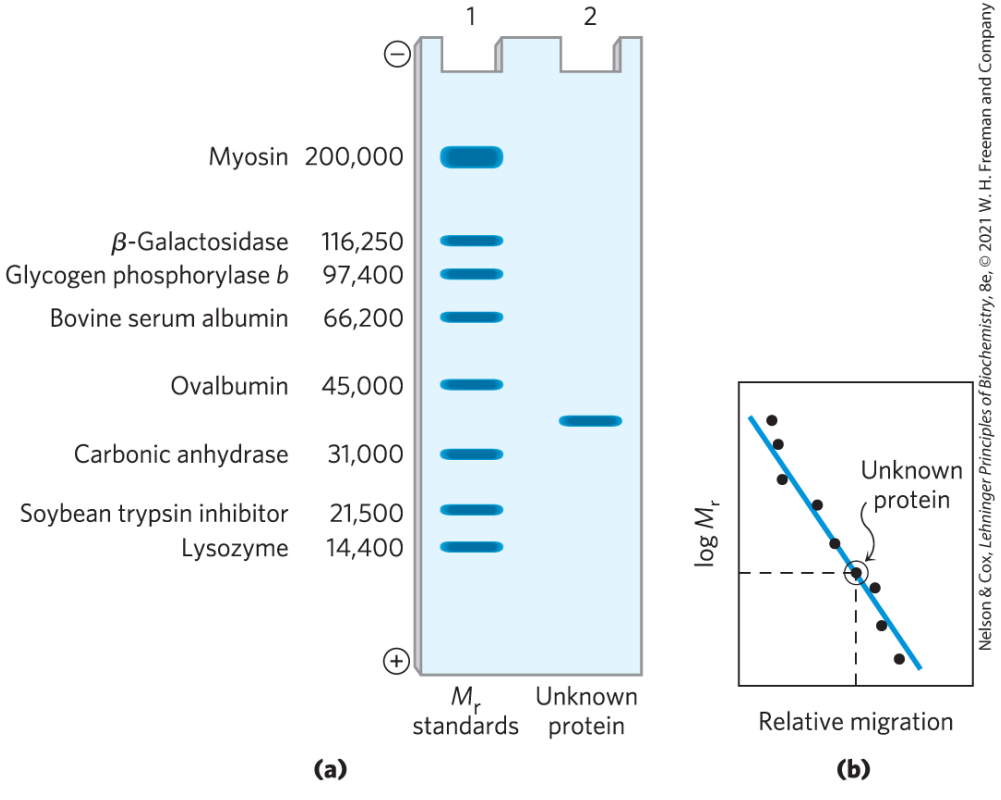
(a) Gustoimages/Science Source; (b) Dr. Julia Cox.

After adding SDS, all proteins become have the same charge, shape, and charge to mass ratio, then I put this all protein in polyacrylamide gel inside a machine thin I make electric field, all protein will move to the positive side of electric field depending on size, small protein will move faster than big protein.

Estimating the Molecular Weight of a Protein

- plot of log M_r of marker proteins vs. relative migration during electrophoresis = linear



Migration of Proteins during Electrophoresis

$$\mu = \frac{V}{E} = \frac{Z}{f}$$

μ = electrophoretic mobility
V = velocity
E = electrical potential
Z = net charge
f = frictional coefficient

migration of a protein in a gel during electrophoresis = function of size and shape

P3 Clicker Question 12

Denaturing gel electrophoresis separates proteins based on differences in:

A. size and shape.
B. molecular mass.
C. charge.
D. amino acid content.

Clicker Question 12, Response

Denaturing gel electrophoresis separates proteins based on differences in:

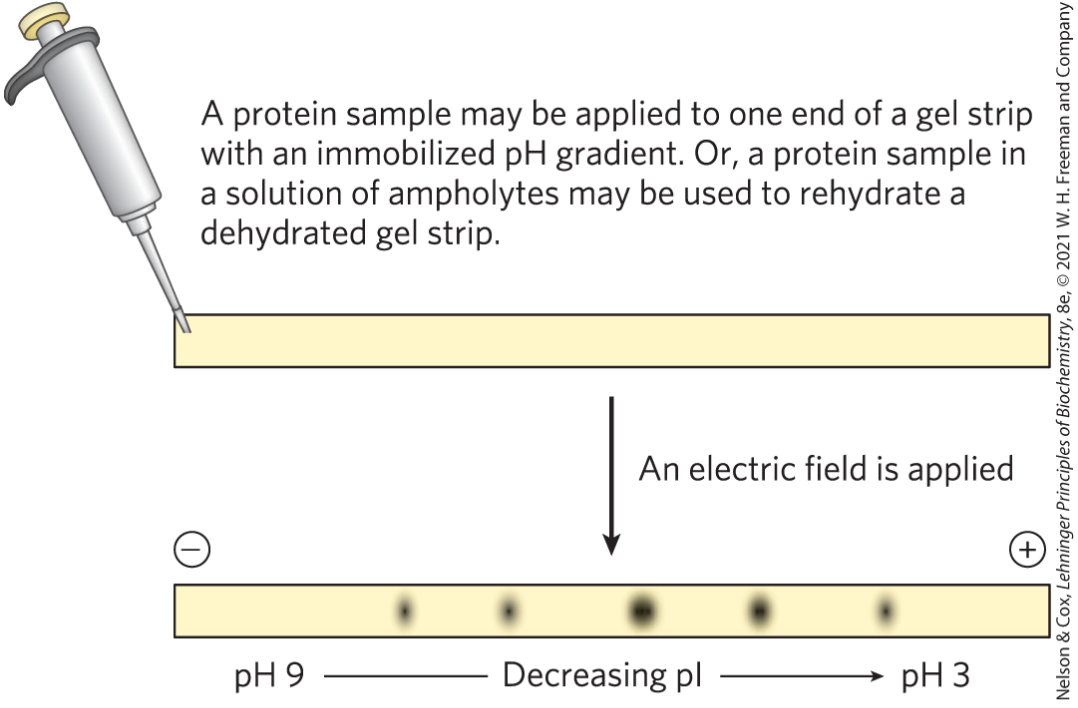
A. size and shape.

$$\mu = \frac{V}{E} = \frac{Z}{f}$$

μ = electrophoretic mobility
V = velocity
E = electrical potential
Z = net charge
f = frictional coefficient

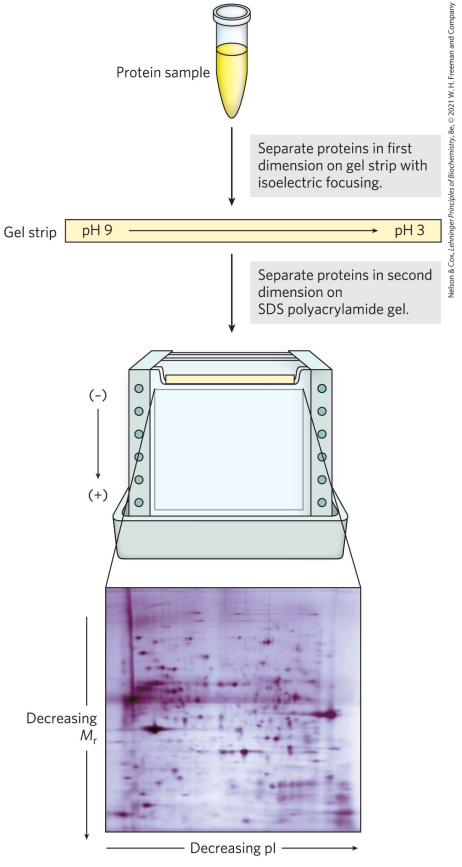
Thus, the migration of a protein in a gel during electrophoresis is a function of its size and its shape.

Using Isoelectric Focusing to Determine the pI of a Protein



Two-Dimensional Electrophoresis

- permits resolution of complex protein mixtures of proteins
- more sensitive than individual methods

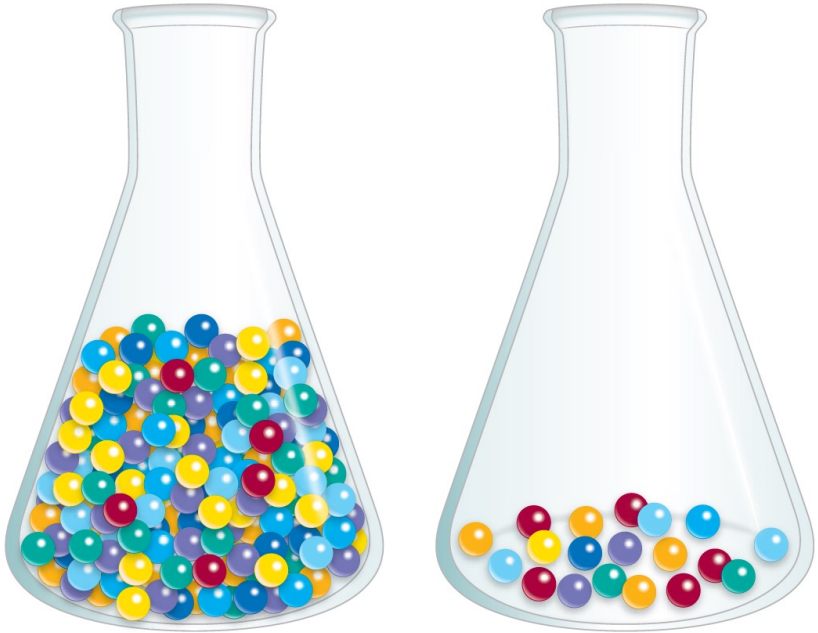


Specific Activity Describes the Purity of the Protein of Interest

- Proteins in a complex mixture often require more than one purification to completely isolate the protein of interest.
- During purification, determination of the location of the protein of interest can be performed by tracking its behavior.
- If a protein has a specific function (e.g., binding insulin), the fraction that binds insulin best after each purification step will contain the most of the protein of interest.
- The function of the protein is called the "**activity**."
- The ratio of activity to total protein concentration is called the "**specific activity**."

Unseparated Proteins Are Detected and Quantified Based on Their Functions

- can monitor enzyme purification by assaying specific activity:
  - **activity** = total enzyme units in a solution
  - **specific activity** = number of enzyme units per mg of total protein



5 red in 100

5 red in 20

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 ↑

Sequential Purification Steps Decrease Sample Size

Table 3-5 A Hypothetical Purification Table for an 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 chromatography80	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 90.

final specific activity: starting specific activity ratio = purification factor

percentage of the final activity/percentage starting activity = percent yield

YouTube Video:

1) Protein Purification

P3 Clicker Question 13

What measurement increases during purification of an enzyme?

A. activity
B. total protein
C. specific activity
D. fraction volume

Clicker Question 13, Response

What measurement increases during purification of an enzyme?

C. specific activity

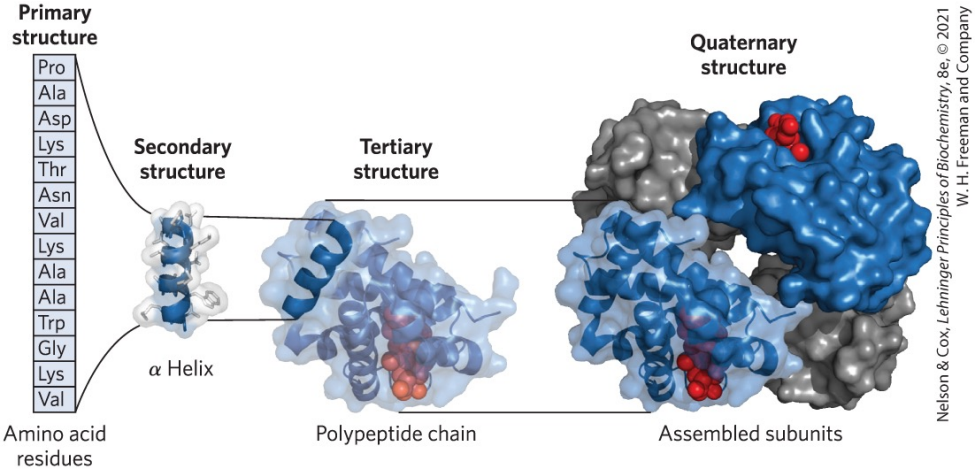
The specific activity is the number of enzyme units per milligram of total protein. The specific activity is a measure of enzyme purity: it increases during purification of an enzyme and becomes maximal and constant when the enzyme is pure.

3.4 The Structure of Proteins: Primary Structure

Levels of Structure in Proteins

- four levels:
  - **primary structure** = covalent bonds linking amino acid residues in a polypeptide chain
  - **secondary structure** = recurring (تواتري، تتابعي) structural patterns
  - **tertiary structure** = 3D folding of polypeptide
  - **quaternary structure** = 2+ polypeptide subunits

All proteins have three level of structure. Primary,secondary, and tertiary. But some proteins have another level of structure is quaternary



YouTube Video:

1) Protein Structure and Folding

P2 Clicker Question 14

What is the highest level of protein structure in human insulin, which has two polypeptides of different mass linked by several disulfide bonds?

A. primary
B. secondary
C. tertiary
D. quaternary

Clicker Question 14, Response

What is the highest level of protein structure in human insulin, which has two polypeptides of different mass linked by several disulfide bonds?

D. quaternary

Insulin has two polypeptide subunits. When a protein has two or more polypeptide subunits, their arrangement in space is referred to as quaternary structure.

Principle 4

Shaped by evolution, amino acid sequences are a key resource for understanding the function of individual proteins and for tracing broader functional and evolutionary relationships.

The Function of a Protein Depends on Its Amino Acid Sequence

- amino acid sequence confers 3D structure
- 3D structure confers function
- most human proteins = **polymorphic** = have amino acid sequence variants
- **Edman degradation** = classic method of sequencing amino acids

Studying Protein Structure Using Proteases

- **proteases** = catalyze hydrolytic cleavage of peptide bonds

Table 3-6 The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

Reagent (biological source)	Cleavage points
Trypsin (bovine pancreas)	Lys, 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)

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

P4 Clicker Question 15

Trypsin catalyzes the hydrolysis of peptide bonds in which a Lys or Arg residue contributes a carbonyl group LL-37, an antimicrobial peptide with 37 residues has 6 Lys residues and 5 Arg residues. How many smaller peptides will LL-37 yield upon trypsin cleavage?

A. 6
B. 5
C. 12
D. 37
E. 11

Clicker Question 15, Response

LL-37, an antimicrobial peptide with 37 residues has 6 Lys residues and 5 Arg residues. How many smaller peptides will LL-37 yield upon trypsin cleavage?

C. 12

A polypeptide with 11 Lys and Arg residues will usually yield 12 smaller peptides upon cleavage with trypsin. Moreover, all except one of these will have a carboxyl-terminal Lys or Arg.

Mass Spectrometry Provides Information on Molecular Mass, Amino Acid Sequence, and Entire Proteomes

- **mass spectrometry** = measure molecular mass with high accuracy يرتب بشكل خاص
  - can sequence short amino acid sequences (20 to 30 amino acid residues)
  - can document the entire cellular **proteome**

is the entire set of proteins that is, or can be, expressed by a genome, cell, tissue, or organism at a certain time. It is the set of expressed proteins in a given type of cell or organism, at a given time, under defined conditions.

General Steps Involved in Mass Spectrometry

- first step: ionize **analytes** in a vacuum

Substance that we want to study it

- second step: introduce charged molecules to electric and/or magnetic field
- third step: charged molecules move through field as a function of the mass-to-charge ratio, *m/z*
- fourth step: deduce mass (*m*) of analyte

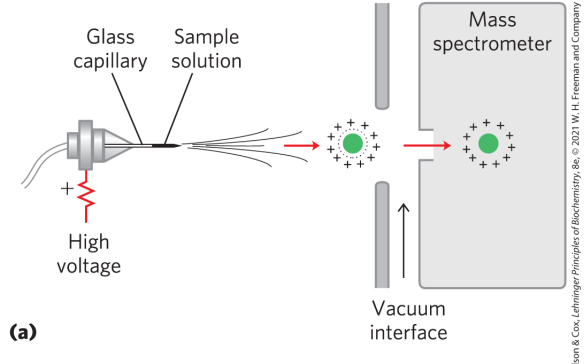
نستنتج

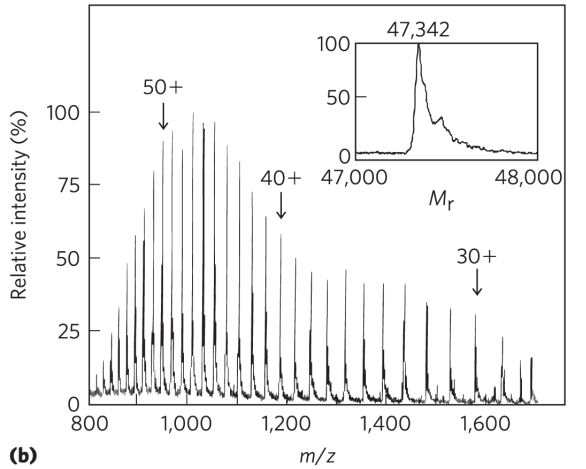
Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI MS)

- proteins placed in light-absorbing matrix
- pulse of laser light ionizes and desorbs proteins from matrix

Electrospray Ionization Mass Spectrometry (ESI MS)

- macromolecules forced directly from the liquid to gas phase





Methods for Analyzing the Mass-to-Charge Ratio, *m*/*z*

- **time of flight (TOF)** = ion acceleration in an electric field depends on *m*/*z*
- **Orbitrap** = traps ions in orbit, electron trajectory converted to *m*/*z*

P4 Clicker Question 16

Which statement is true about mass spectrometry?

A. Mass spectrometry can be performed on analytes in the liquid phase.
B. Mass spectrometry can obtain the sequences of multiple polypeptide segments of 100 residues each.
C. The mass (*m*) of an analyte is used to deduce the mass-to-charge ratio, *m/z*, with high precision.
D. MALDI MS requires treatment of proteins with a protease before injection into a mass spectrometer.
E. Mass spectrometry can monitor changes in the cellular proteome as a function of metabolic state.

Clicker Question 16, Response

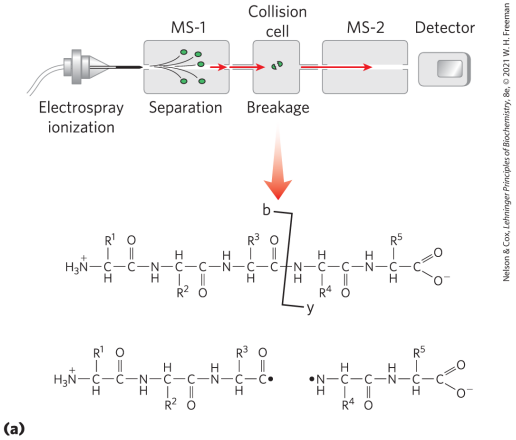
Which statement is true about mass spectrometry?

E. Mass spectrometry can monitor changes in the cellular proteome as a function of metabolic state.

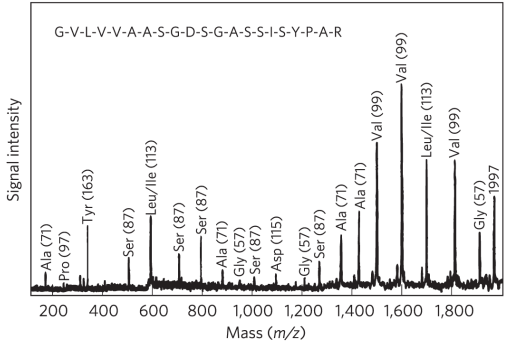
When coupled to peptide separation protocols, mass spectrometry can document a complete cellular proteome. Changes in the cellular proteome can be monitored as a function of metabolic state or environmental conditions.

Extracting Amino Acid Sequence Information Using Tandem MS

- **tandem MS (MS/MS)** = two mass filters in tandem
  - first = sorts peptides produced by cleavage
  - second = measures *m*/*z* ratios of charged fragments



(a)



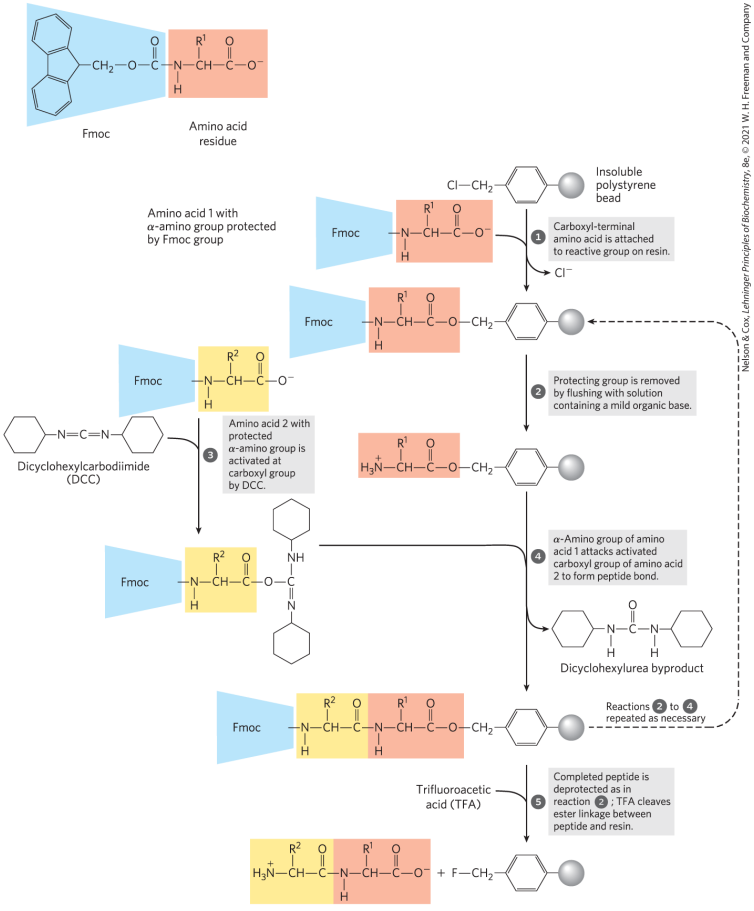
(b)

Analyzing Complex Protein Mixtures Using Liquid Chromatography (LC)

- **LC-MS/MS** = liquid chromatography-tandem MS
  - chromatography on complex mixture of peptides
  - resolved peptides introduced to MS successively
  - identifies proteins and protein abundance

Small Peptides and Proteins Can Be Chemically Synthesized

- the Merrifield method
- one end of peptide = attached to resin in column
- protective chemical groups block unwanted reactions



P4 Clicker Question 17

Chemical synthesis of peptides by the Merrifield method:

A. involves synthesizing the peptide and then attaching it to a solid support.
B. is nearly as efficient as peptide bond synthesis in biochemical systems.
C. has a practical upper limit of a polymer of ~500 amino acid residues.
D. proceeds from the carboxyl terminus to the amino terminus.

Clicker Question 17, Response

Chemical synthesis of peptides by the Merrifield method:

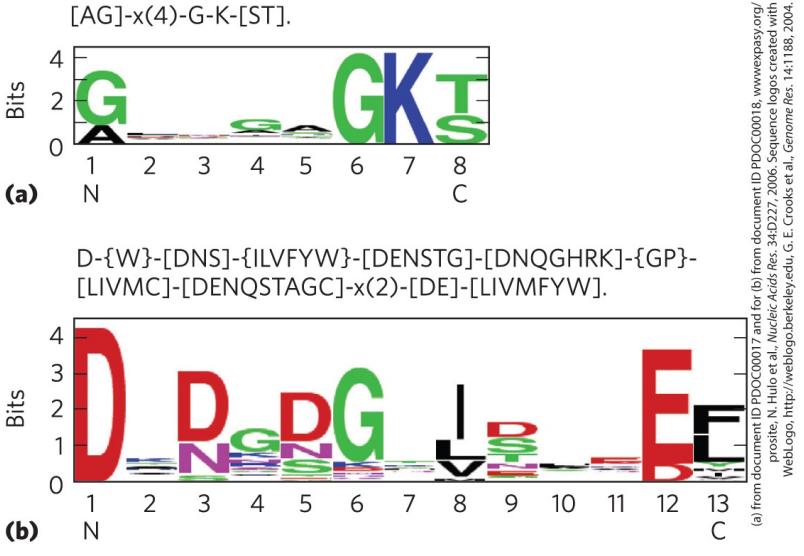
D. proceeds from the carboxyl terminus to the amino terminus.

Chemical synthesis proceeds from the carboxyl terminus to the amino terminus, the reverse of the direction of protein synthesis in vivo.

Amino Acid Sequences Provide Important Biochemical Information

- amino acid sequence can inform:
  - 3D structure
  - function
  - cellular location
  - evolution

- **consensus sequence** = reflects most common amino acid at each position



Protein Sequences Help Elucidate the History of Life on Earth

- **bioinformatics:**
  - identifies functional segments in new proteins
  - establishes sequence and structural relationships to known proteins

- essential amino acid residues = conserved over evolutionary time

- less important amino acid residues = vary over evolutionary time

Defining Members of Protein Families

- **homologs** = **homologous proteins** = members of protein families
  - **paralogs** = homologs in same species
  - **orthologs** = homologs in different species
  - identified by comparing protein sequences to a database of protein sequences

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

P4 Clicker Question 18

Which statement is false?

A. protein structure is commonly defined at four levels.
between organisms.
B. The function of a protein is a result of its amino acid sequence.
C. Orthologs are homologs found in the same species.
D. It is possible to change the amino acid sequence of a protein and have no effect on its function.

Clicker Question 18, Response

Which statement is false?

C. Orthologs are homologs found in the same species.

Homologs from different species are called orthologs.

P4 Clicker Question 19

The amino acid sequences of proteins:

A. can be used to establish evolutionary relationships between organisms.
B. are an example of tertiary structure.
C. are similar within a given organism.
D. are also referred to as “consensus sequences.”

Clicker Question 19, Response

The amino acid sequences of proteins:

A. can be used to establish evolutionary relationships between organisms.

Protein sequences are a rich source of information about protein structure and function. Bioinformatics can analyze changes in the amino acid sequences of homologous proteins over time to trace the evolution of life on Earth and establish evolutionary relationships between organisms.