

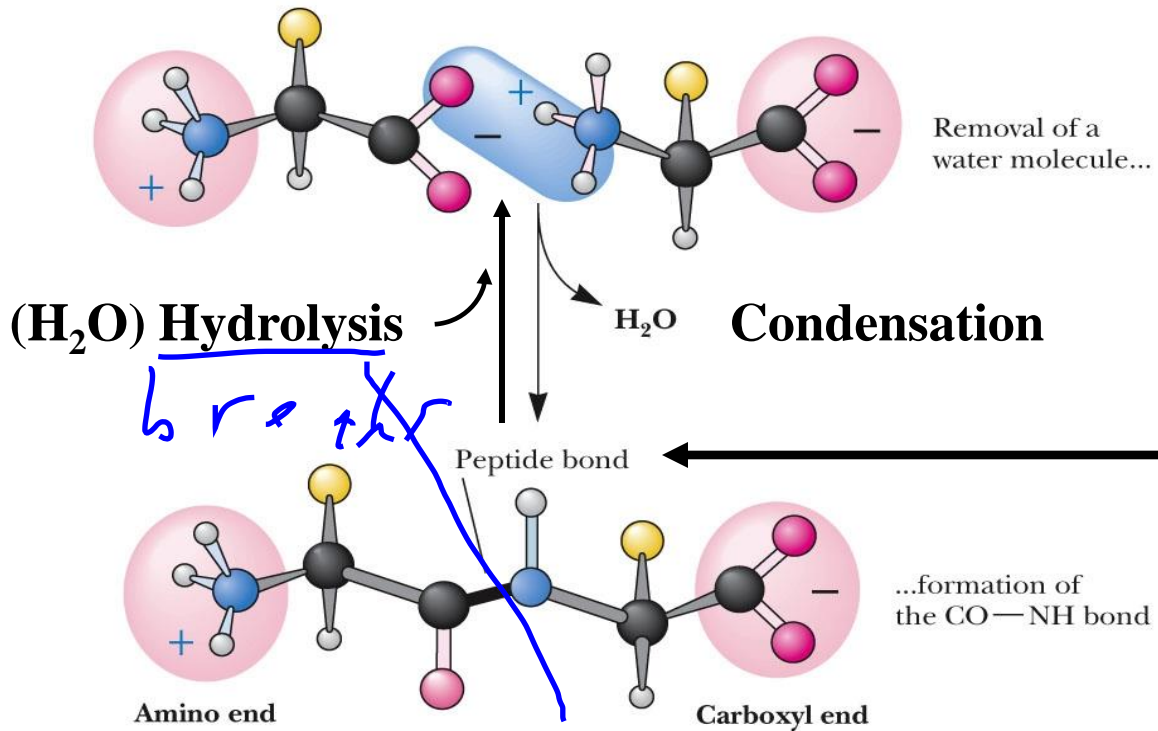
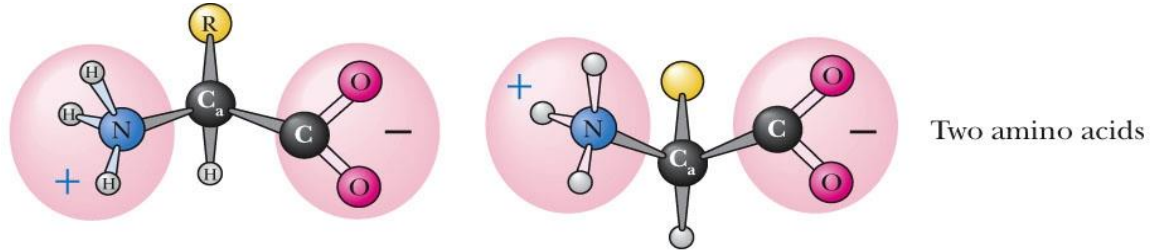
LECTURE TWO

Ionization, pH, Titration Curves Acid-Base Chemistry Amino Acids

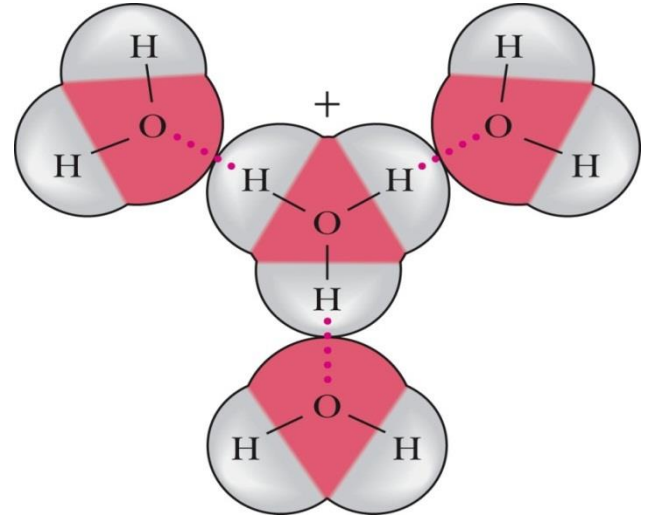
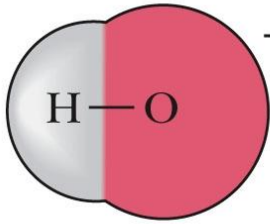
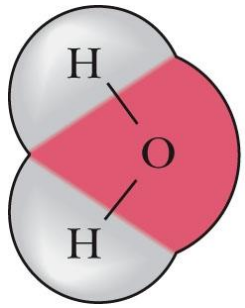


WATER IS A NUCLEOPHILE

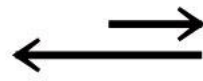
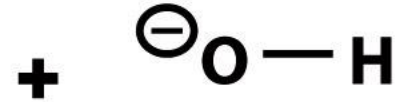
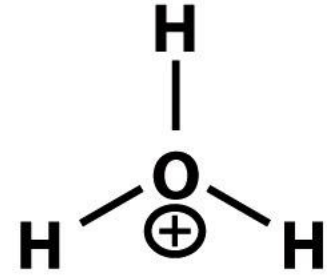
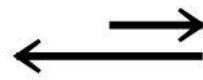
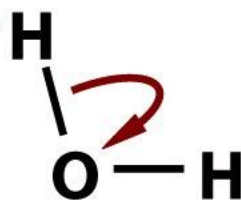
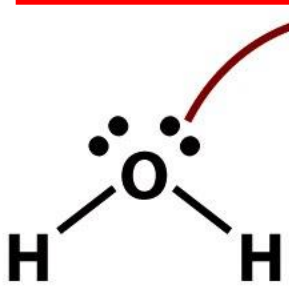
W E A K w



WATER CAN IONIZE



nucleophilic attack



[H⁺]

PROPERTIES OF WATER



- Electrical Polarity (net dipole)
- Highly cohesive *Attract each other*
- Solvates polar molecules and weakens ionic ones
- Ionizable *- weak nucleophile*

WHAT IS pH?

- Søren Sørensen of Denmark devised the pH scale
- pH is the negative logarithm of the hydrogen ion concentration
- If $[H^+] = 1 \times 10^{-7} M$
- Then $pH = 7$

$$pH = -\log_{10}[H^+]$$

$$pK_w = pH + pOH = 14$$

pH SCALE

TABLE 2.2 pH Scale

The hydrogen ion and hydroxyl ion concentrations are given in moles per liter at 25°C.

pH	[H ⁺]		[OH ⁻]	
0	(10 ⁰)	1.0	0.000000000000001	(10 ⁻¹⁴)
1	(10 ⁻¹)	0.1	0.00000000000001	(10 ⁻¹³)
2	(10 ⁻²)	0.01	0.0000000000001	(10 ⁻¹²)
3	(10 ⁻³)	0.001	0.000000000001	(10 ⁻¹¹)
4	(10 ⁻⁴)	0.0001	0.0000000001	(10 ⁻¹⁰)
5	(10 ⁻⁵)	0.00001	0.000000001	(10 ⁻⁹)
6	(10 ⁻⁶)	0.000001	0.00000001	(10 ⁻⁸)
7	(10⁻⁷)	0.0000001	0.0000001	(10⁻⁷)
8	(10 ⁻⁸)	0.00000001	0.000001	(10 ⁻⁶)
9	(10 ⁻⁹)	0.000000001	0.00001	(10 ⁻⁵)
10	(10 ⁻¹⁰)	0.0000000001	0.0001	(10 ⁻⁴)
11	(10 ⁻¹¹)	0.00000000001	0.001	(10 ⁻³)
12	(10 ⁻¹²)	0.000000000001	0.01	(10 ⁻²)
13	(10 ⁻¹³)	0.0000000000001	0.1	(10 ⁻¹)
14	(10 ⁻¹⁴)	0.00000000000001	1.0	(10 ⁰)

QUANTIFY THE IONIZATION OF WATER

pH SCALE

pH of various fluids at 25°C

acidosis - diabetes
alkaa

Fluid	pH
Household lye	13.6
Bleach	12.6
Household ammonia	11.4
Milk of magnesia	10.3
Baking soda	8.4
Seawater	8.0
Pancreatic fluid	7.8–8.0
Blood plasma	7.4
Intracellular fluids	
Liver	6.9
Muscle	6.1
Saliva	6.6
Urine	5–8
Boric acid	5.0
Beer	4.5
Orange juice	4.3
Grapefruit juice	3.2
Vinegar	2.9
Soft drinks	2.8
Lemon juice	2.3
Gastric juice	1.2–3.0
Battery acid	0.35

DISSOCIATION OF WEAK ELECTROLYTES

Consider a weak acid, HA

- The acid dissociation constant is given by:



$$K_a = \frac{[H^+][A^-]}{[HA]}$$

DERIVE THE HENDERSON- HASSELBALCH EQUATION

THE HENDERSON-HASSELBALCH EQUATION

Know this! You'll use it constantly.

- For any acid HA, the relationship between the pK_a , the concentrations existing at equilibrium and the solution pH is given by:



$$pH = pK_a + \log_{10} \frac{[A^-]}{[HA]}$$

pK_a VALUES

TABLE 2.4 Acid Dissociation Constants and pK _a Values for Some Weak Electrolytes (at 25°C)		
Acid	K _a (M)	pK _a
HCOOH (formic acid)	1.78×10^{-4}	3.75
CH ₃ COOH (acetic acid)	1.74×10^{-5}	4.76
CH ₃ CH ₂ COOH (propionic acid)	1.35×10^{-5}	4.87
CH ₃ CHOHCOOH (lactic acid)	1.38×10^{-4}	3.86
HOOCCH ₂ CH ₂ COOH (succinic acid) pK ₁ [*]	6.16×10^{-5}	4.21
HOOCCH ₂ CH ₂ COO ⁻ (succinic acid) pK ₂	2.34×10^{-6}	5.63
H ₃ PO ₄ (phosphoric acid) pK ₁	7.08×10^{-3}	2.15
H ₂ PO ₄ ⁻ (phosphoric acid) pK ₂	6.31×10^{-8}	7.20
HPO ₄ ²⁻ (phosphoric acid) pK ₃	3.98×10^{-13}	12.40
C ₃ N ₂ H ₅ ⁺ (imidazole)	1.02×10^{-7}	6.99
C ₆ O ₂ N ₃ H ₁₁ ⁻ (histidine–imidazole group) pK _R [†]	9.12×10^{-7}	6.04
H ₂ CO ₃ (carbonic acid) pK ₁	1.70×10^{-4}	3.77
HCO ₃ ⁻ (bicarbonate) pK ₂	5.75×10^{-11}	10.24
(HOCH ₂) ₃ CNH ₃ ⁺ (<i>tris</i> -hydroxymethyl aminomethane)	8.32×10^{-9}	8.07
NH ₄ ⁺ (ammonium)	5.62×10^{-10}	9.25
CH ₃ NH ₃ ⁺ (methylammonium)	2.46×10^{-11}	10.62

*The pK values listed as pK₁, pK₂, or pK₃ are in actuality pK_a values for the respective dissociations. This simplification in notation is used throughout this book.

†pK_R refers to the imidazole ionization of histidine.

Data from CRC Handbook of Biochemistry, The Chemical Rubber Co., 1968.

pKa VALUES

$$\begin{aligned}\text{Eg: } K_a &= \frac{[\text{H}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]} \\ &= 1.76 \times 10^{-5} \text{ M}\end{aligned}$$

$$\text{p}K_a = -\log K_a = 4.8$$

****Remember****

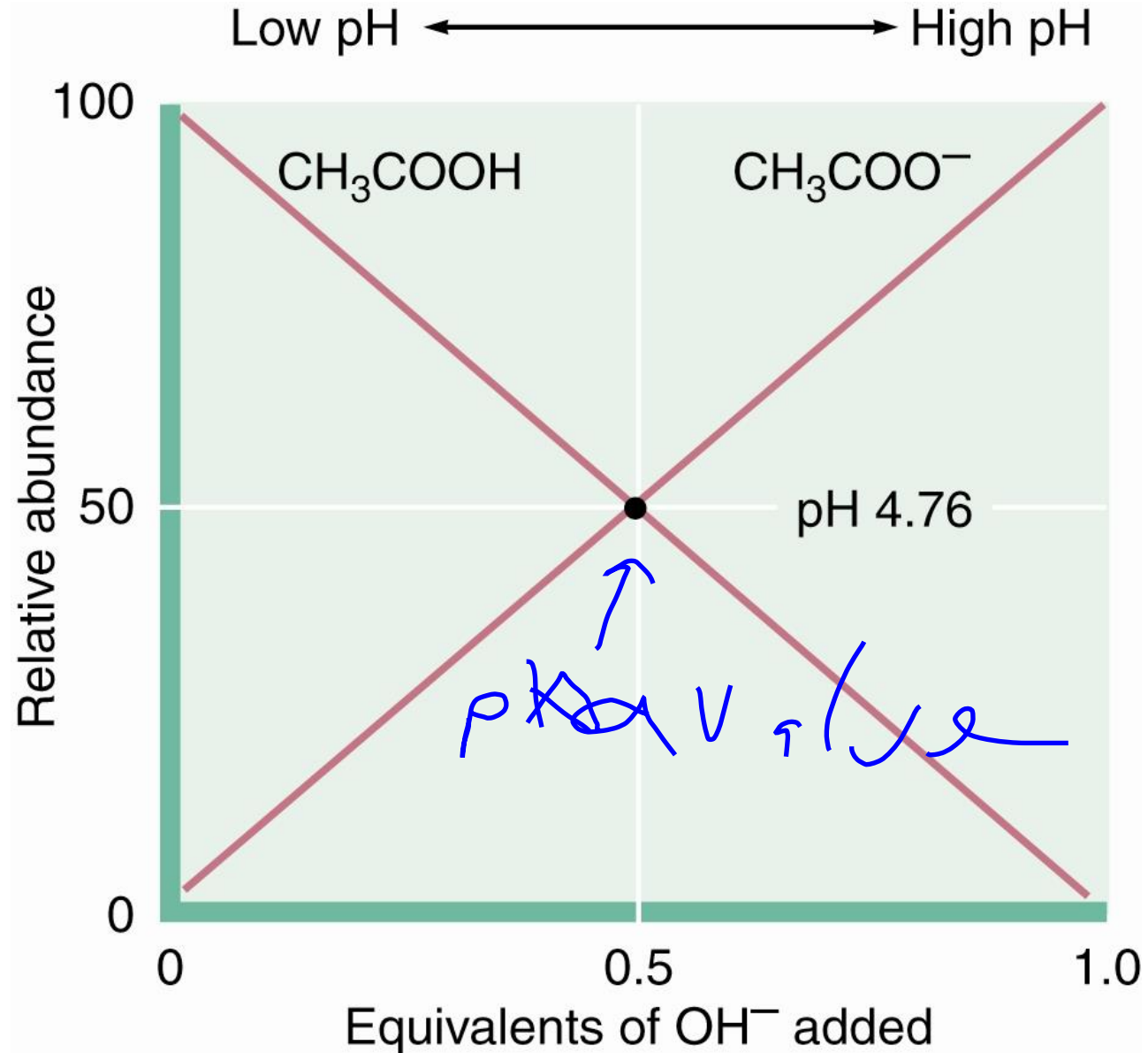
pH = measure of acidity

pKa = measure of acid strength

Weak acid \rightarrow low K_a , high pKa

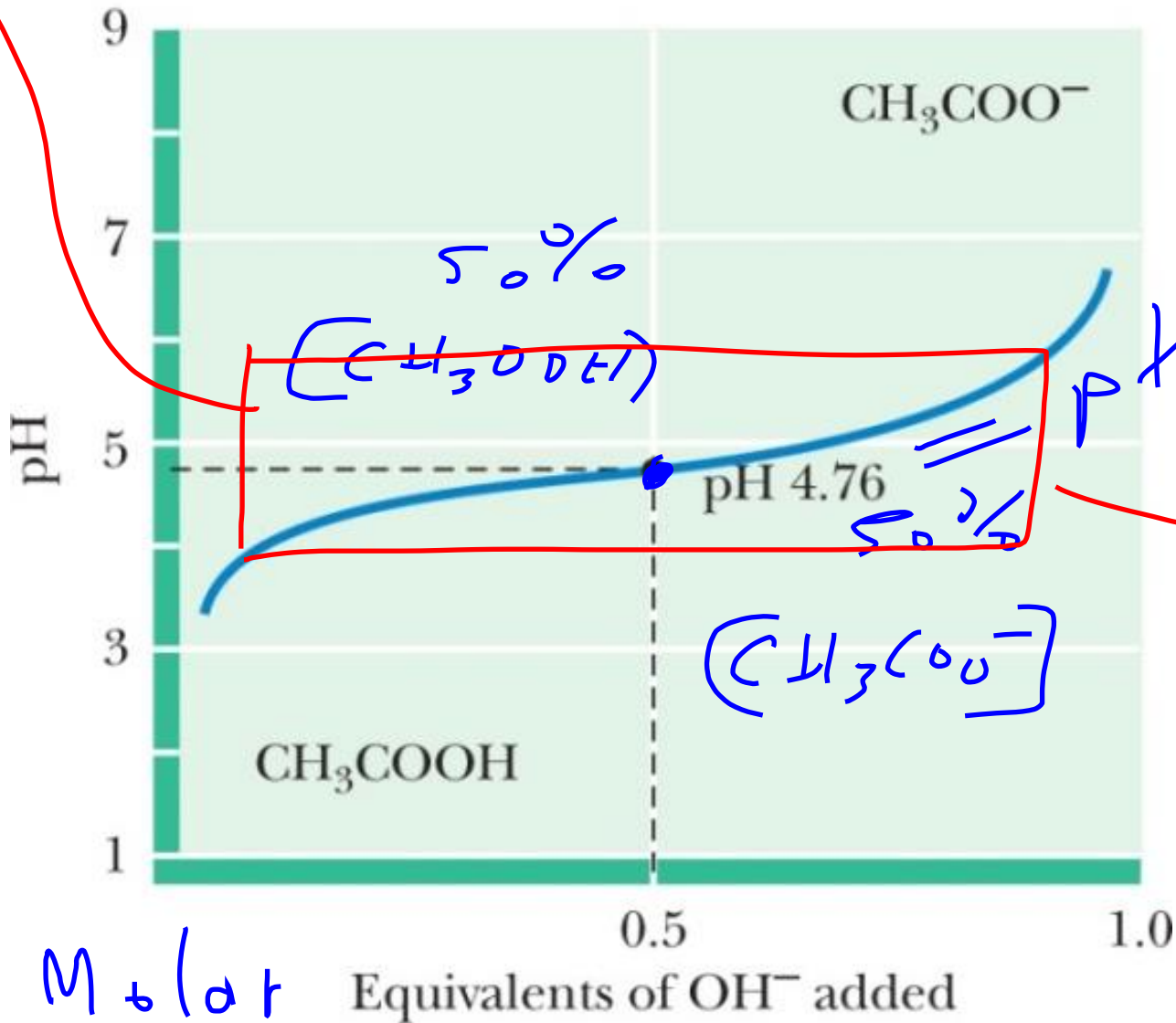
TITRATION CURVES

Titration curves illustrate the progressive dissociation of a weak acid



TITRATION CURVE OF ACETIC ACID

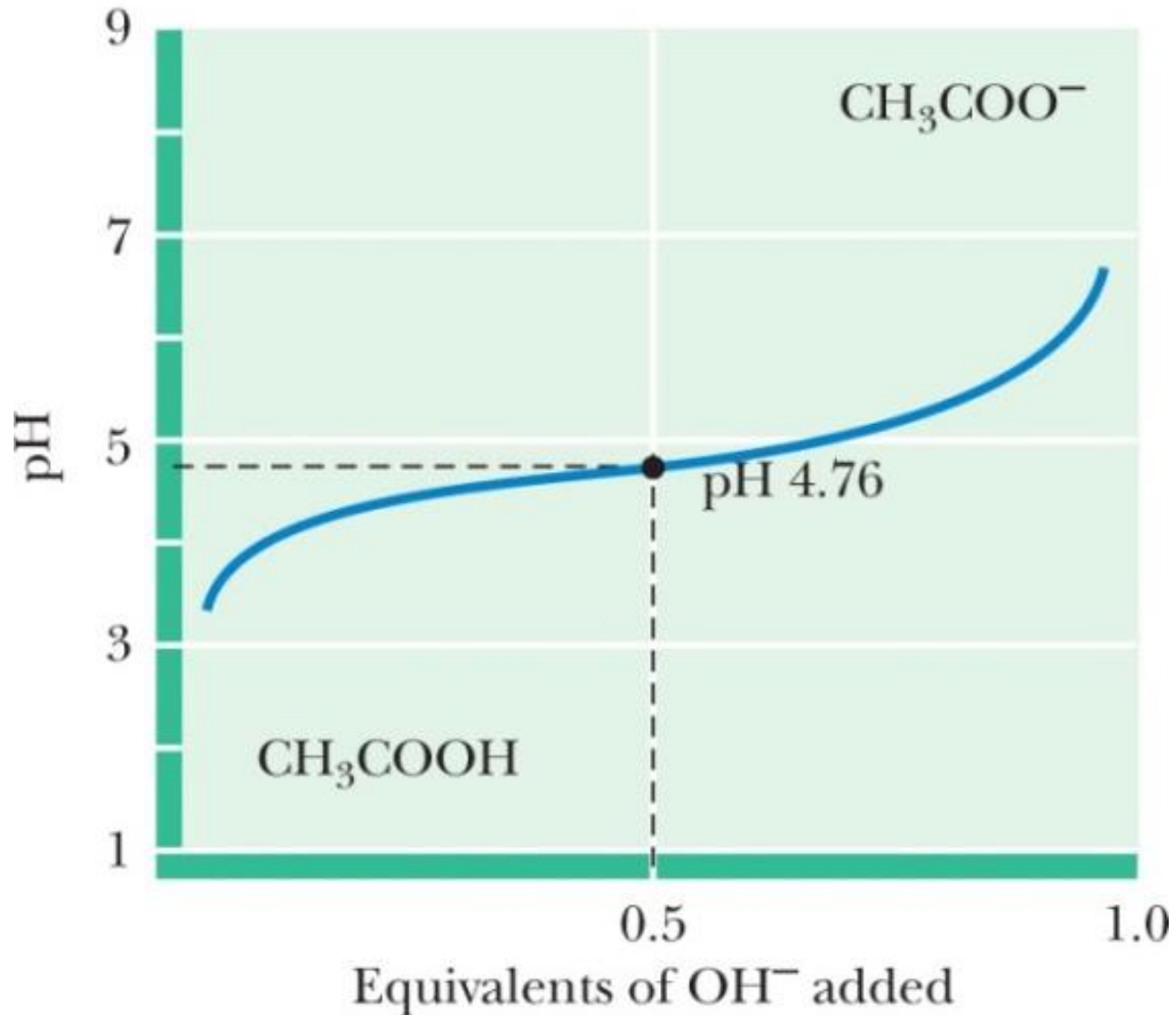
buffering capacity



pKa
buff
ratio
+ 1:1 pt

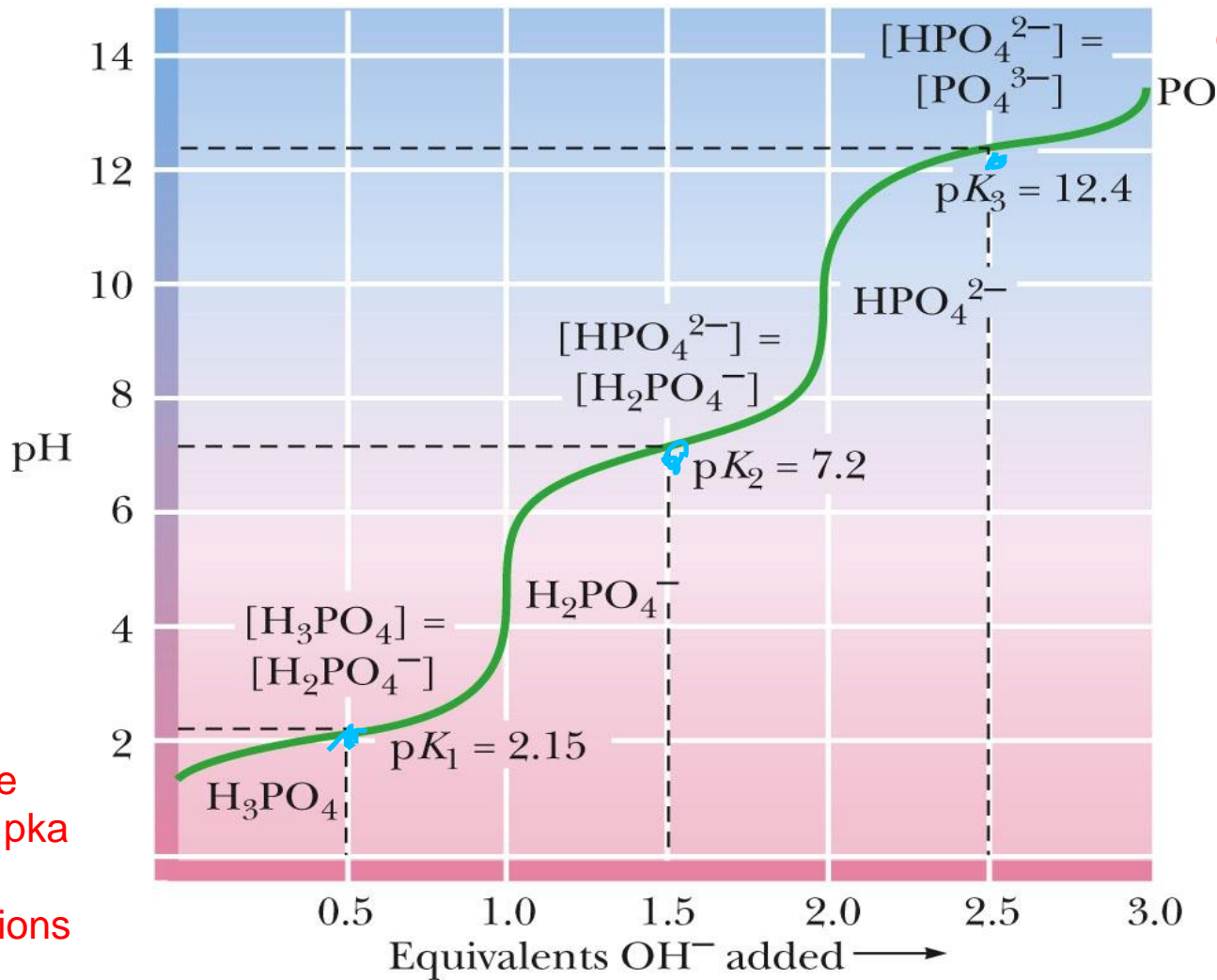
Molar

TITRATION CURVE OF ACETIC ACID



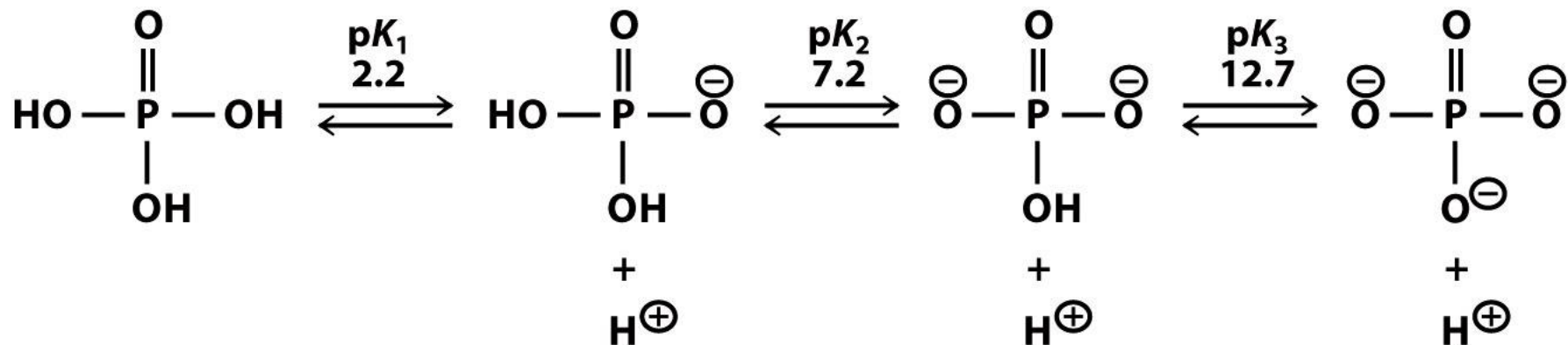
TITRATION CURVE OF PHOSPHORIC ACID

Need 3 molar equivalents to titration completely



3 ionizable groups, 3 pka values, 3 buffer regions

TITRATION CURVE OF PHOSPHORIC ACID



WHAT ARE BUFFERS AND WHAT DO THEY DO?

- Buffers are solutions that resist changes in pH as acid and base are added
- Most buffers consist of a weak acid and its conjugate base
- The plot of pH versus base added is flat near the pK_a
- Buffers can only be used reliably within a pH unit of their pK_a

~~***~~ BUFFER SYSTEM OF BLOOD PLASMA

bicarbonate <->

carbonic acid <->

CO₂ system

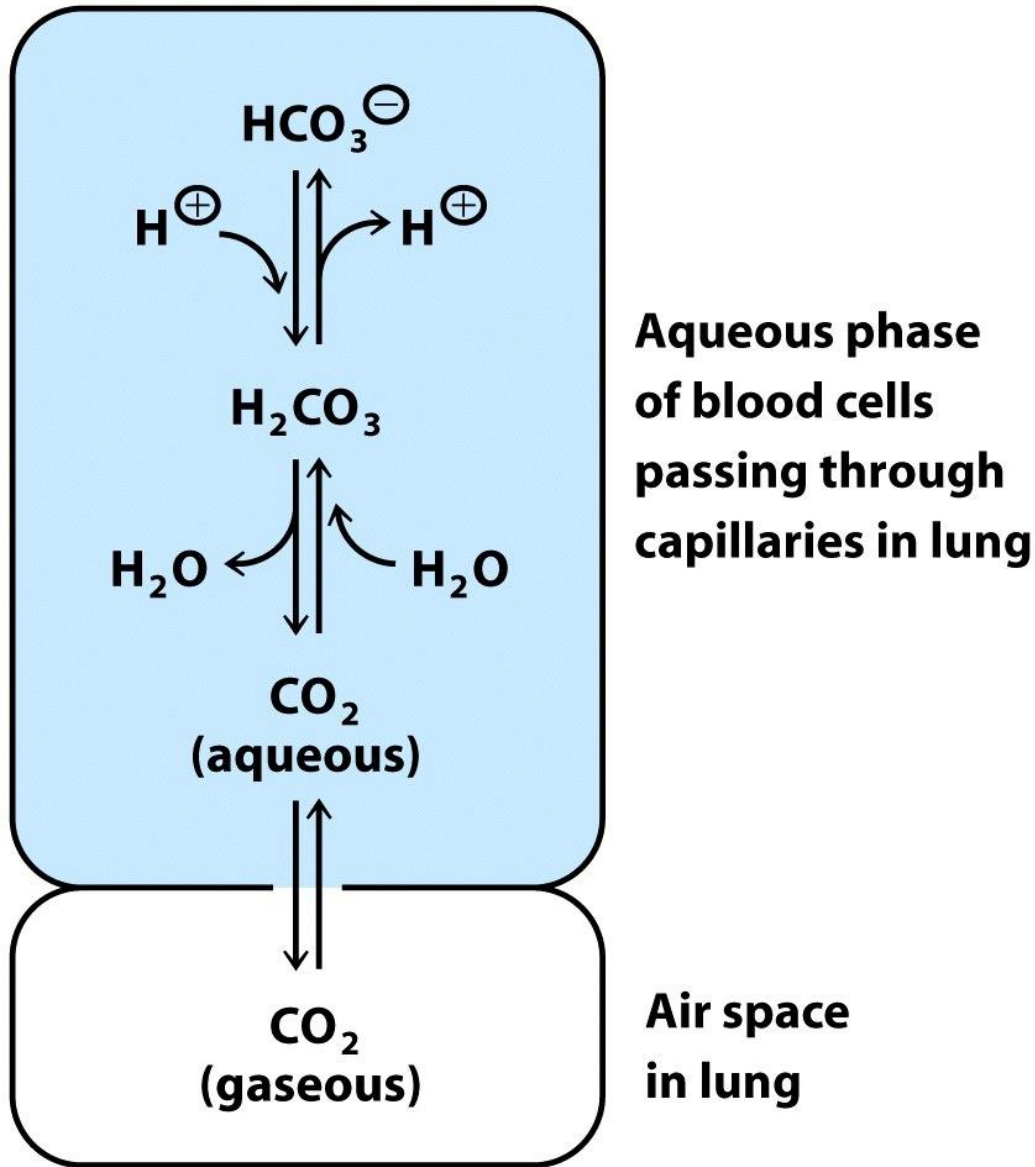


Figure 2-20 Principles of Biochemistry, 4/e
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SUMMARY

1) Properties of H₂O – Ionization, nucleophile

2) pH scale – pH calculations, $\text{pH} = -\log [\text{H}^+]$

3) Henderson-Hasselbach Equation:

$$\text{pH} = \text{pKa} + \log [\text{A}^-]/[\text{HA}]$$

$$\text{pKa} = -\log \text{Ka} \text{ (acid dissociation constant)}$$

Weaker acid, small Ka, large pKa

4) pKa – Titration Curves

-# of pKa values = # ionizable groups

-eg, 2 ionizable groups → 2 pKa values, 2 buffering regions

2 mol. Eq. to completely titrate

-buffering capacity → ± 1 pH unit above/below pKa value

5) Blood → CO₂ / H₂CO₃ / HCO₃⁻ buffering system

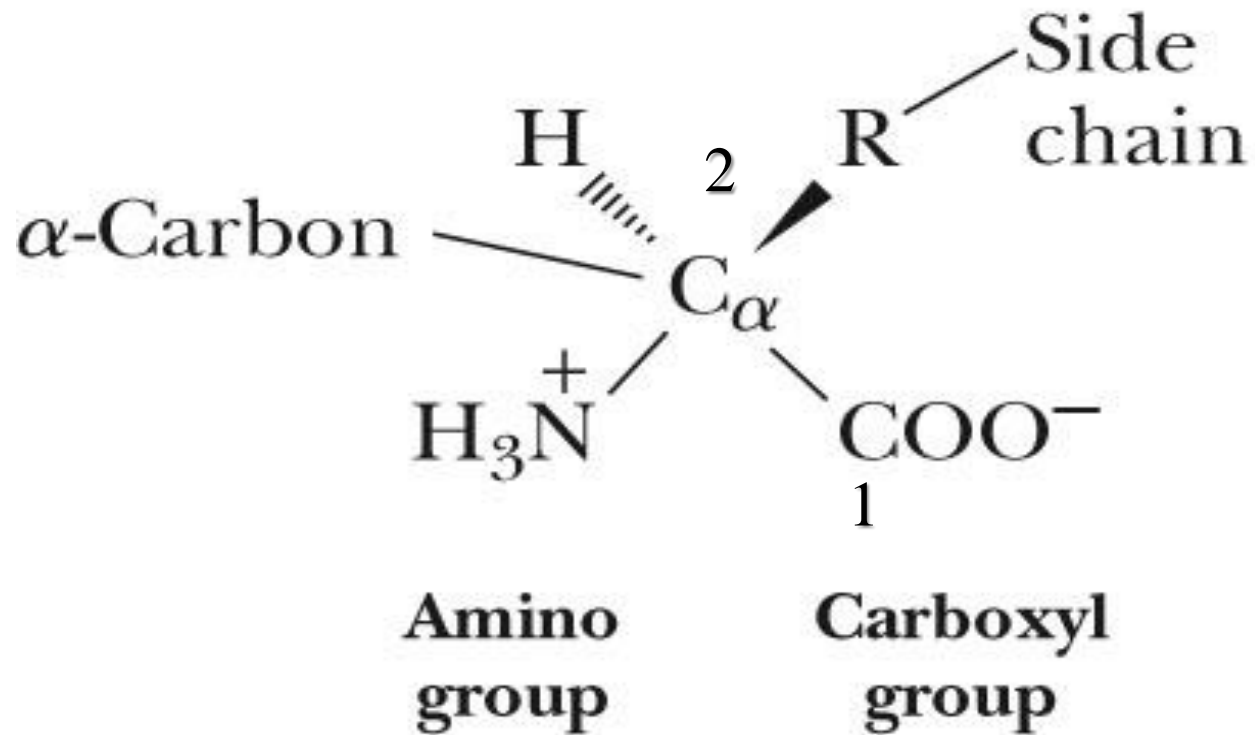
AMINO ACIDS: ROLE OF PROTEINS

- Enzymes eg. Serine proteases
- Bind molecules for storage and support
- Shape Cells - Cytoskeleton
- Assemblies of proteins can do mechanical work
- Hormones, receptors for hormones
- Specialized functions eg. Antibodies
- Transport proteins eg. Hemoglobin

OUTLINE

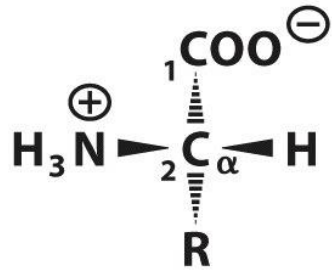
- What are the structures and properties of amino acids?
- What are the acid-base properties of amino acids?
- What reactions do amino acids undergo?
- What are the optical and stereochemical properties of amino acids?
- What are the spectroscopic properties of amino acids?
- How are amino acid mixtures separated and analyzed?
- What is the fundamental structural pattern in proteins?

AMINO ACIDS

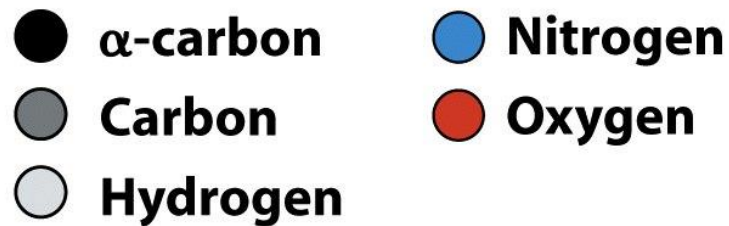
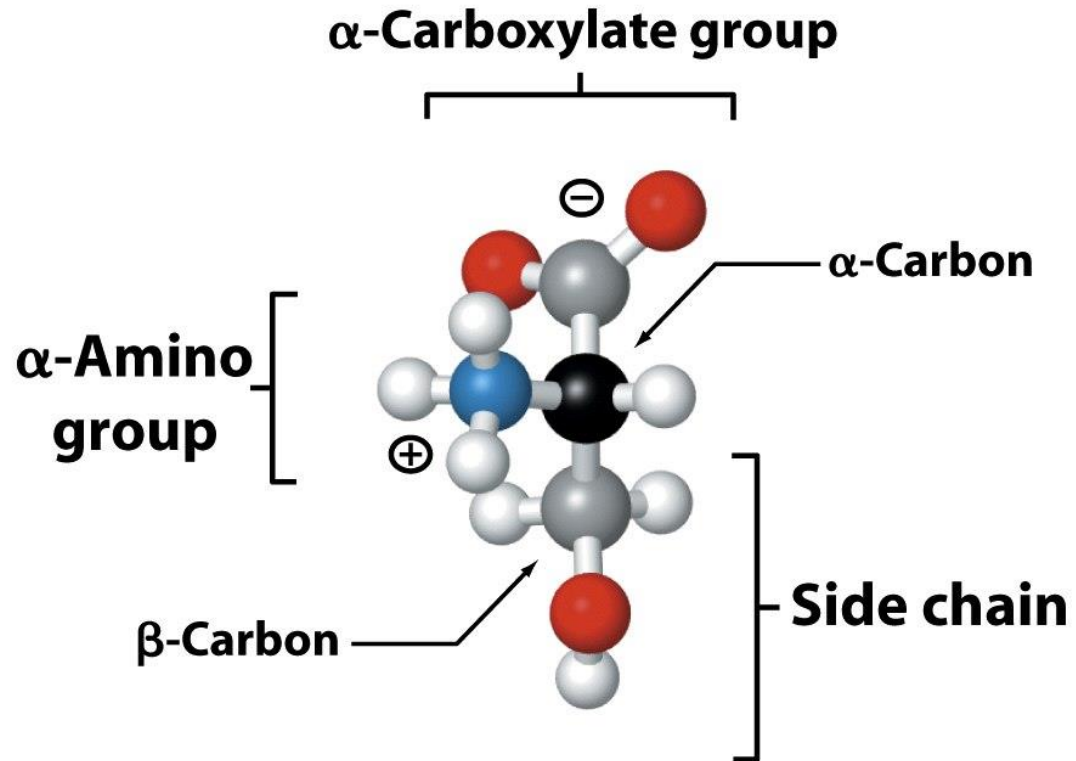


AMINO ACIDS

(a)



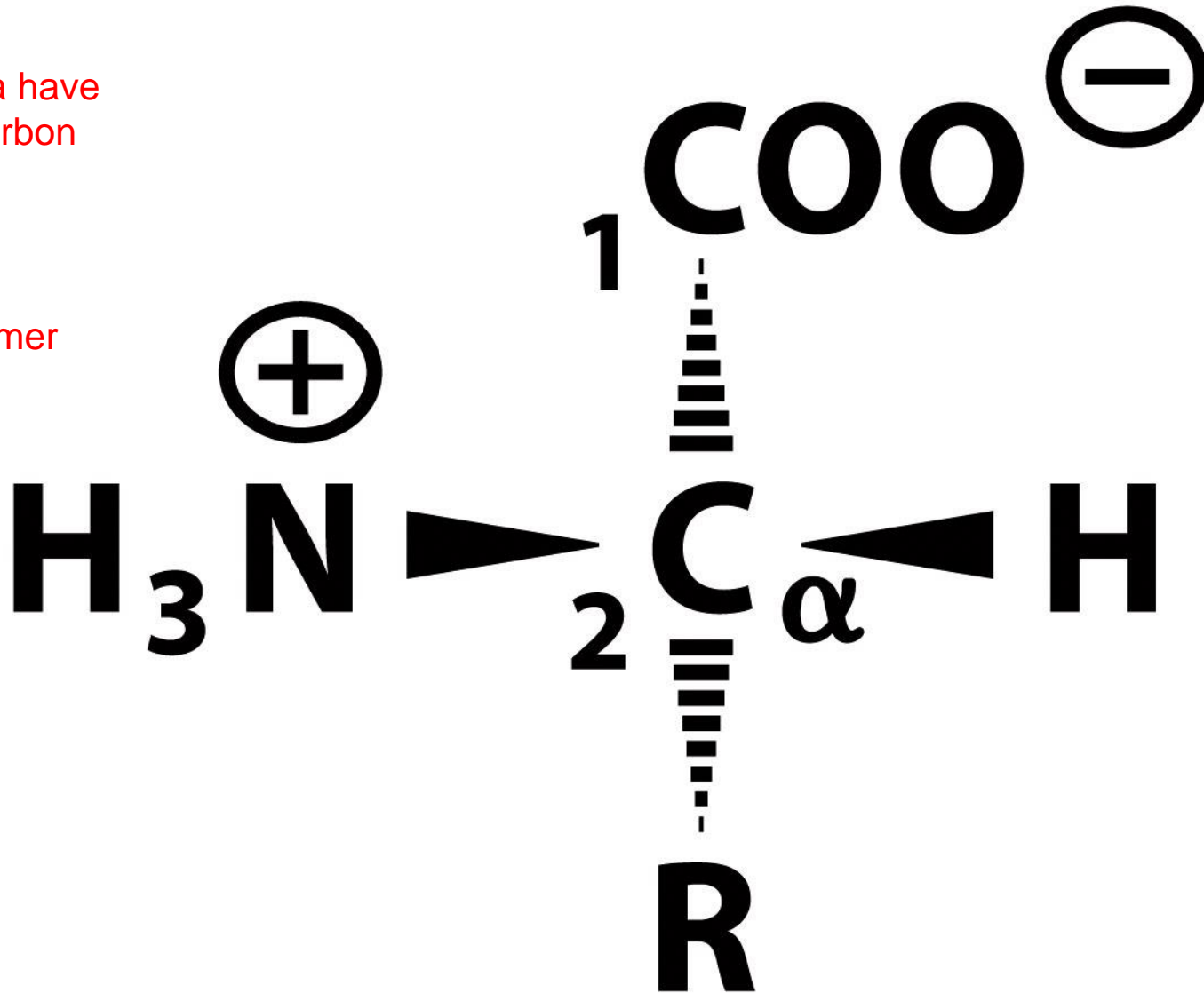
(b)



AMINO ACIDS

19/20 aa have
chiral carbon

stereoisomer



AMINO ACIDS

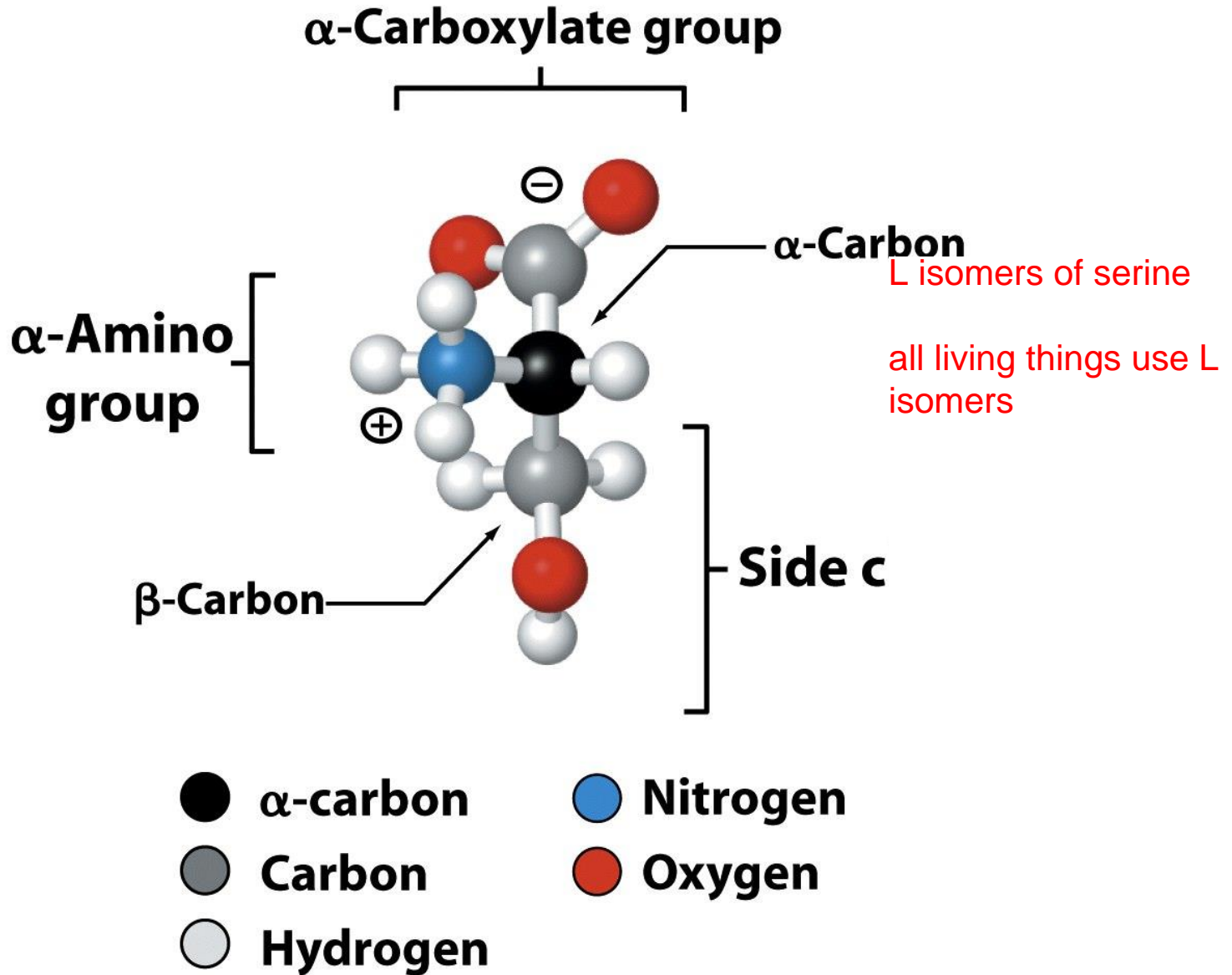
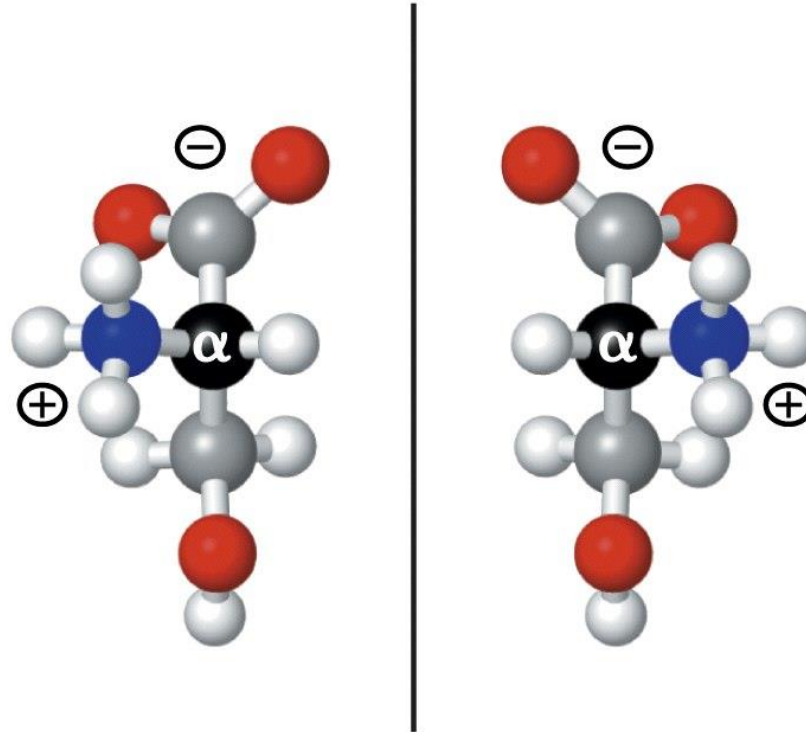


Figure 3-1b Principles of Biochemistry, 4/e
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AMINO ACIDS

Mirror plane



L-Serine

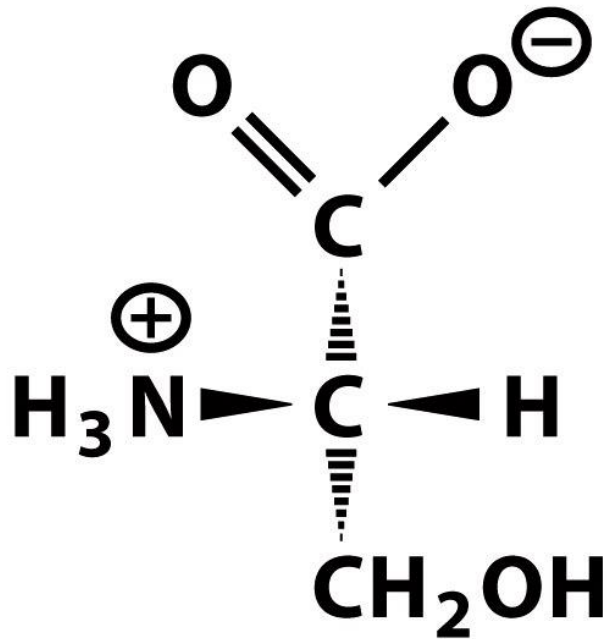
D-Serine

● **α -carbon**
● **Carbon**
○ **Hydrogen**

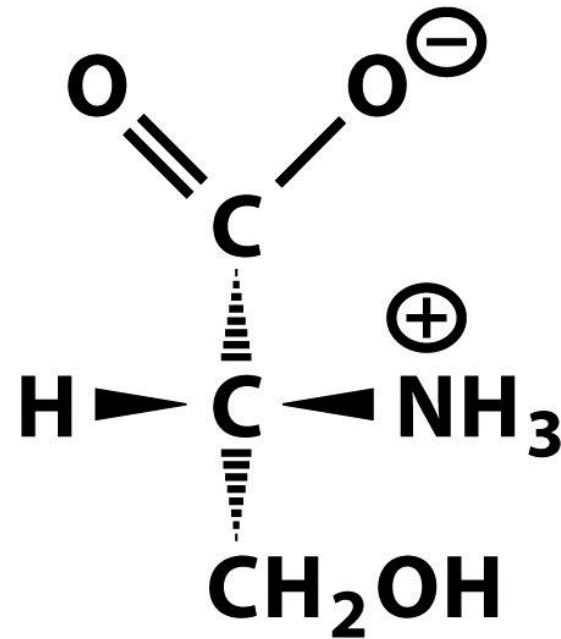
● **Nitrogen**
● **Oxygen**

AMINO ACIDS

**Mirror
plane**



L-Serine



D-Serine



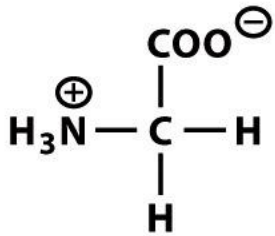
THE 20 COMMON AMINO ACIDS

You should know names, structures, pK_a values, 3-letter and 1-letter codes

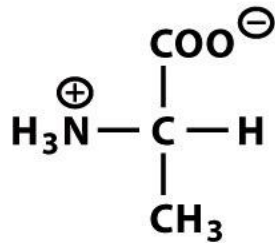
- Non-polar amino acids
- Polar, uncharged amino acids
- Acidic amino acids
- Basic amino acids

ALIPHATIC R GROUPS

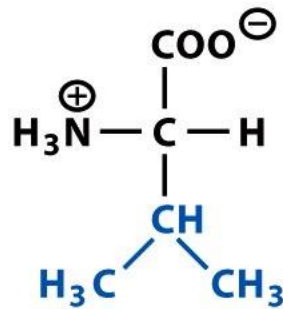
hydrophobic



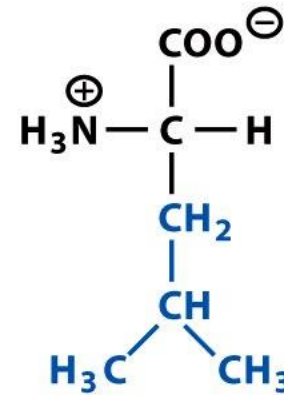
Glycine [G]
(Gly)



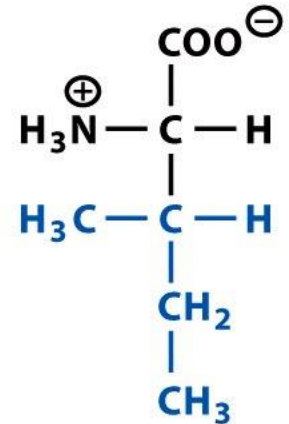
Alanine [A]
(Ala)



Valine [V]
(Val)



Leucine [L]
(Leu)

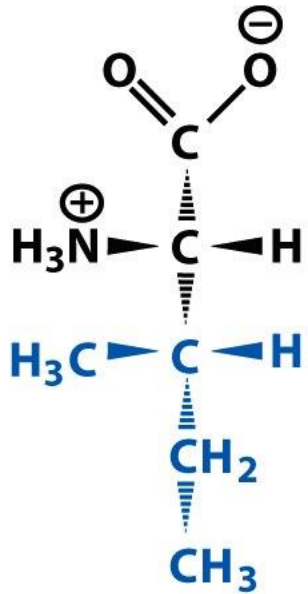


Isoleucine [I]
(Iso)

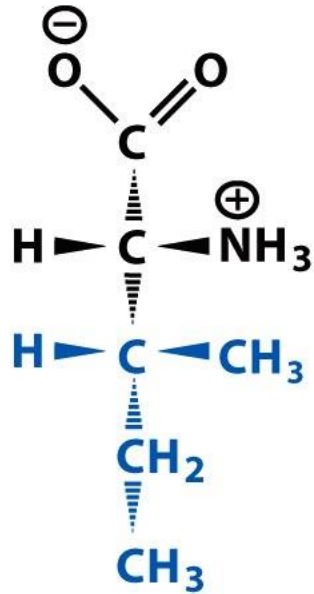
2 chiral carbons,
4 stereoisomers

ALIPHATIC R GROUPS

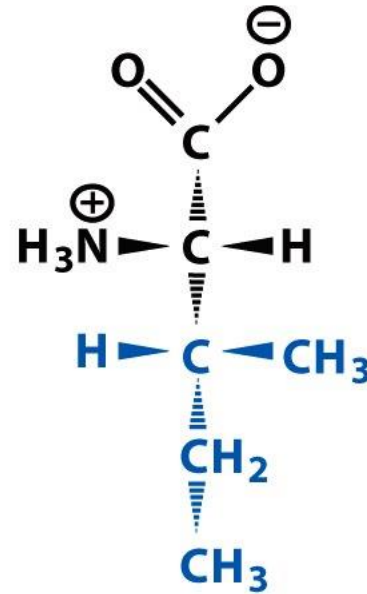
*2 Chiral Carbons



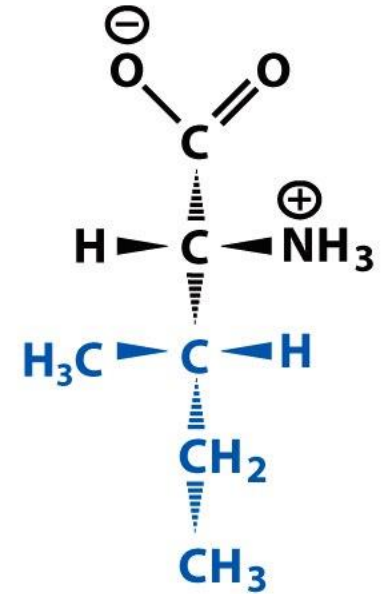
L-Isoleucine



D-Isoleucine

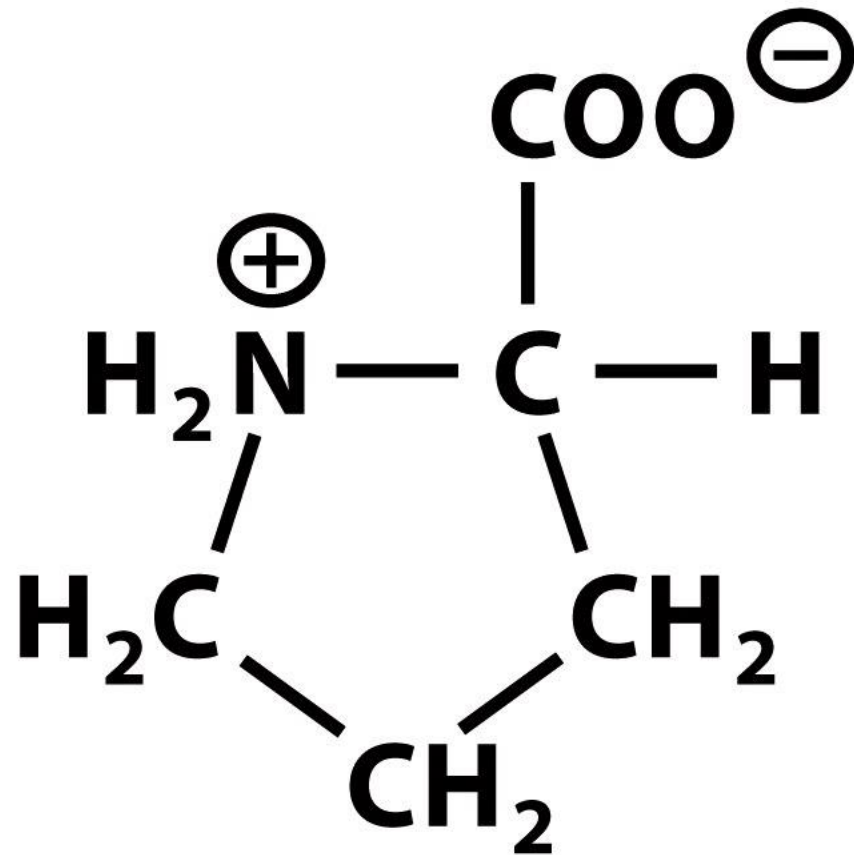


L-Alloisoleucine



D-Alloisoleucine

ALIPHATIC R GROUPS

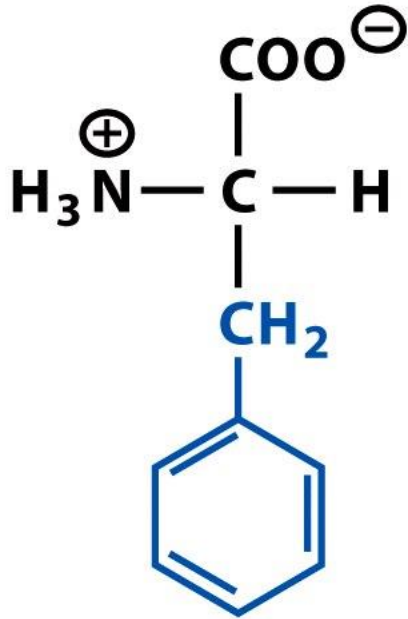


Proline [P]

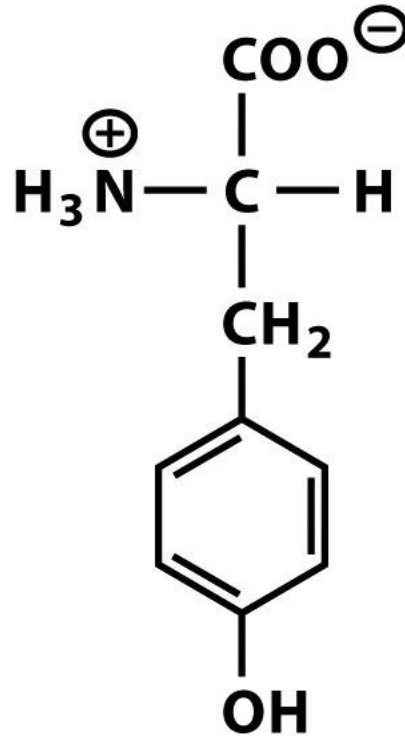
(Pro)

AROMATIC R GROUPS

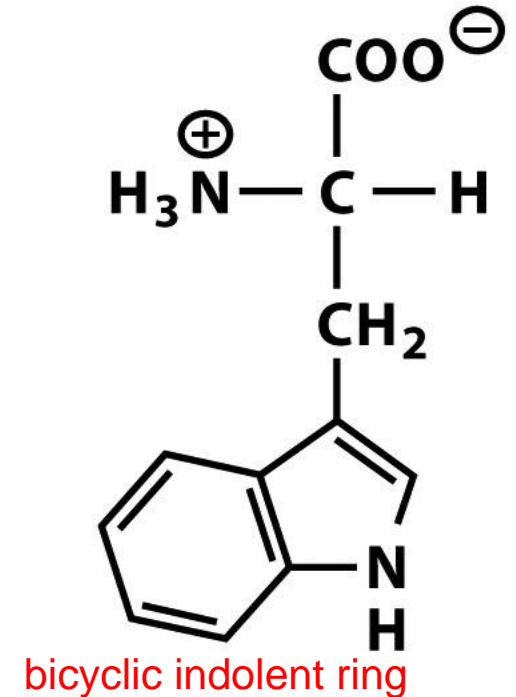
absorbs UV light and can use assays to determine protein concentrations



**Phenylalanine [F]
(Phe)**



**Tyrosine [Y]
(Tyr)**

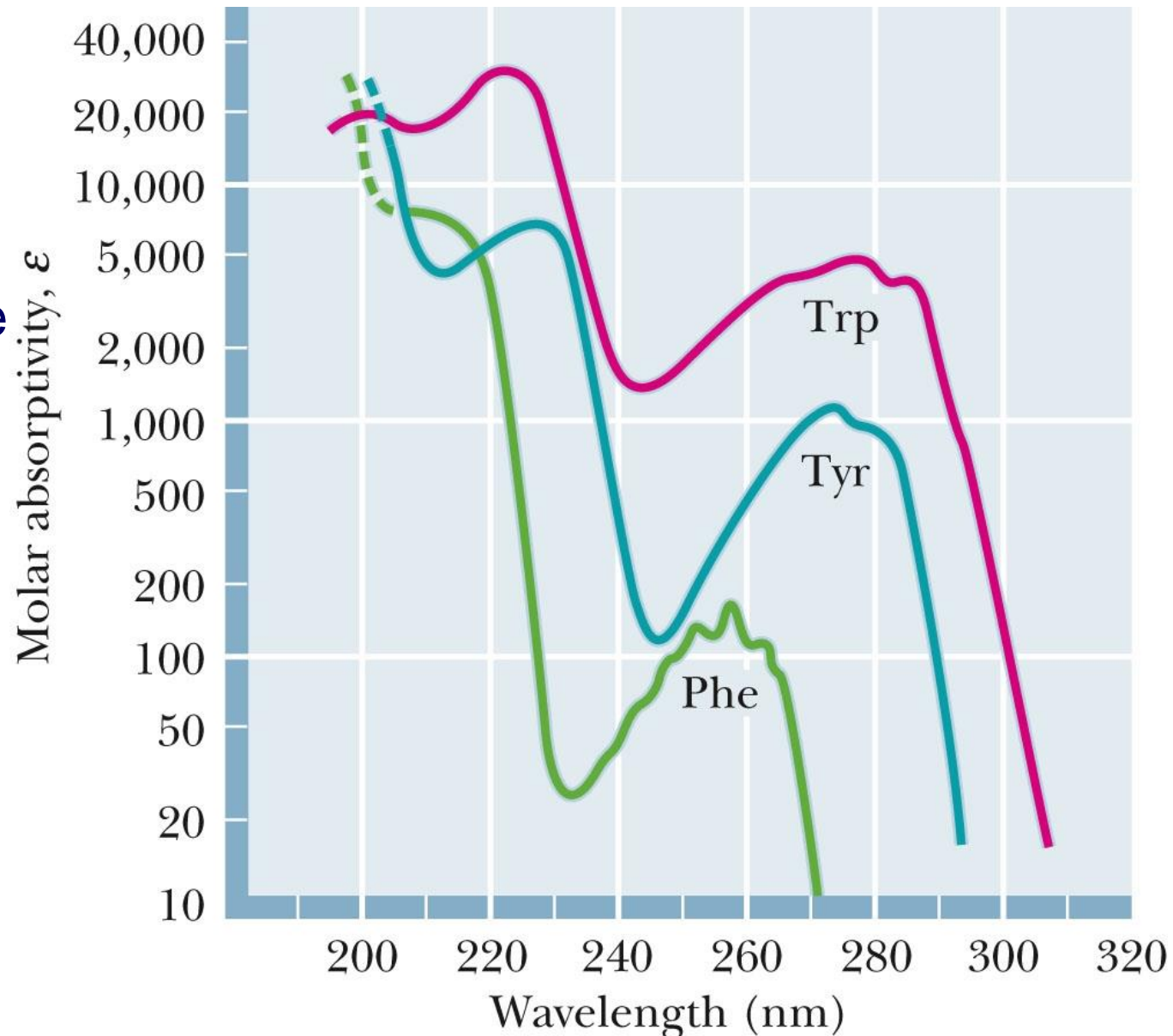


**Tryptophan [W]
(Trp)**

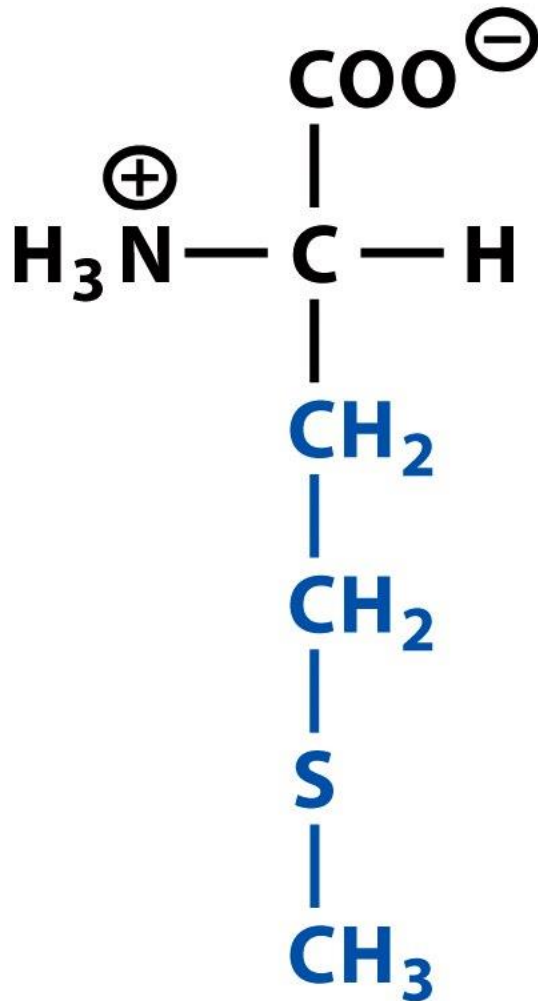
SPECTROSCOPIC PROPERTIES

The UV spectra of the aromatic amino acids at pH 6. A good diagnostic device for amino acids

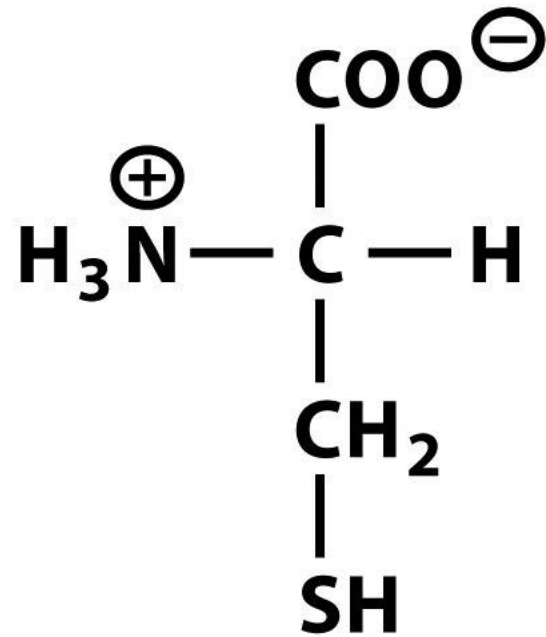
A = something c l, a is absorbing and you can get concentration of sample.



SULFUR R GROUPS



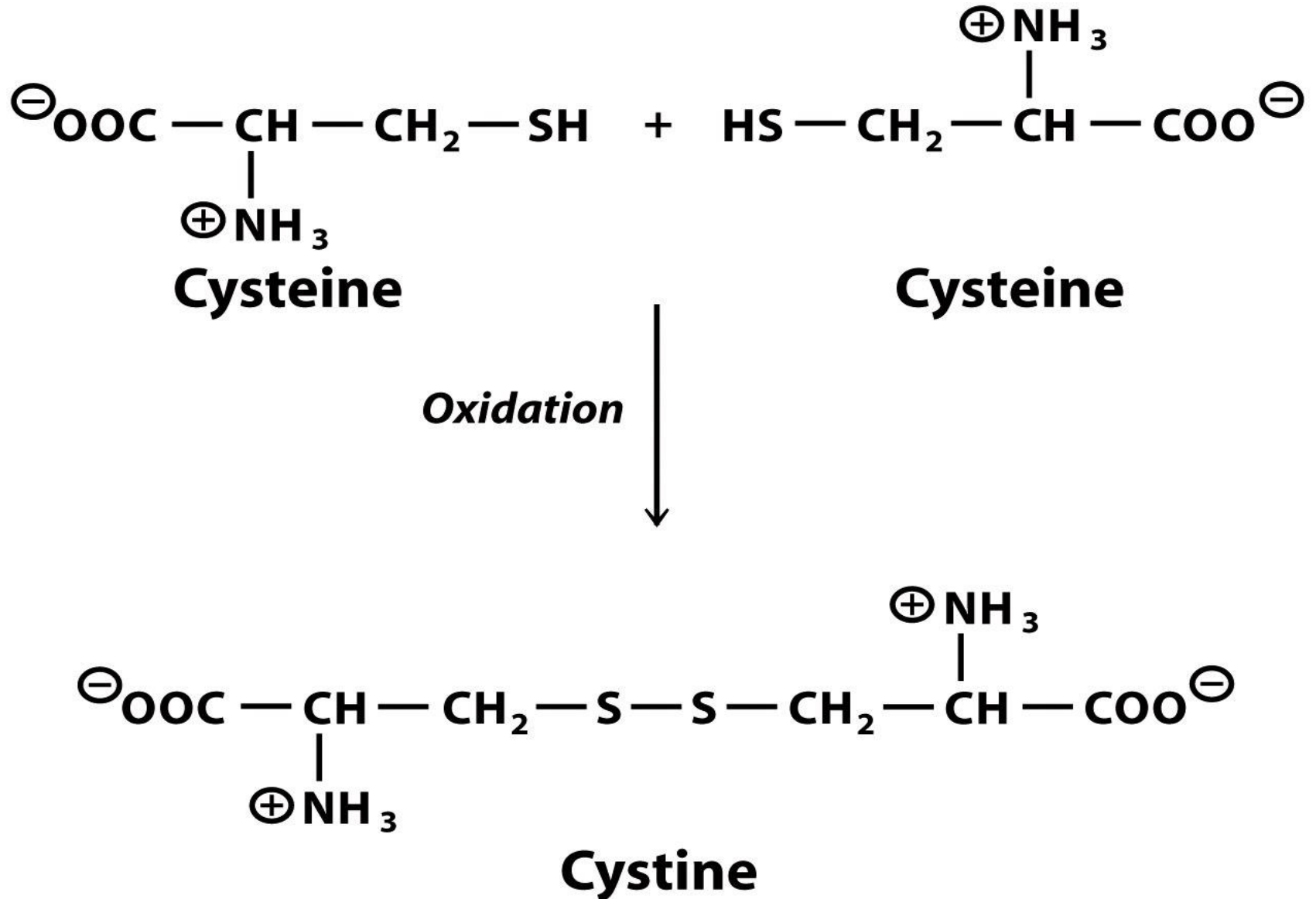
Methionine [M]
(Met)



cystine - formed from two cysteines,
disulfur linkage

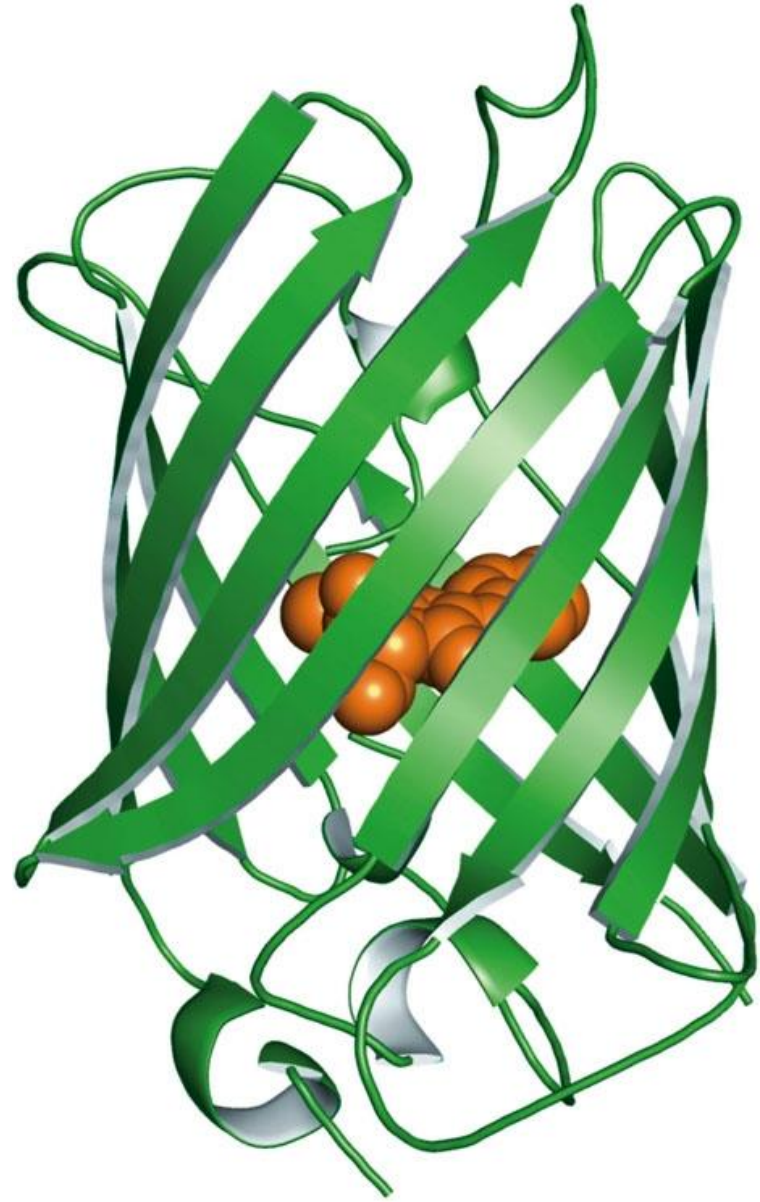
Cysteine [C]
(Cys)

REACTIONS OF AMINO ACIDS

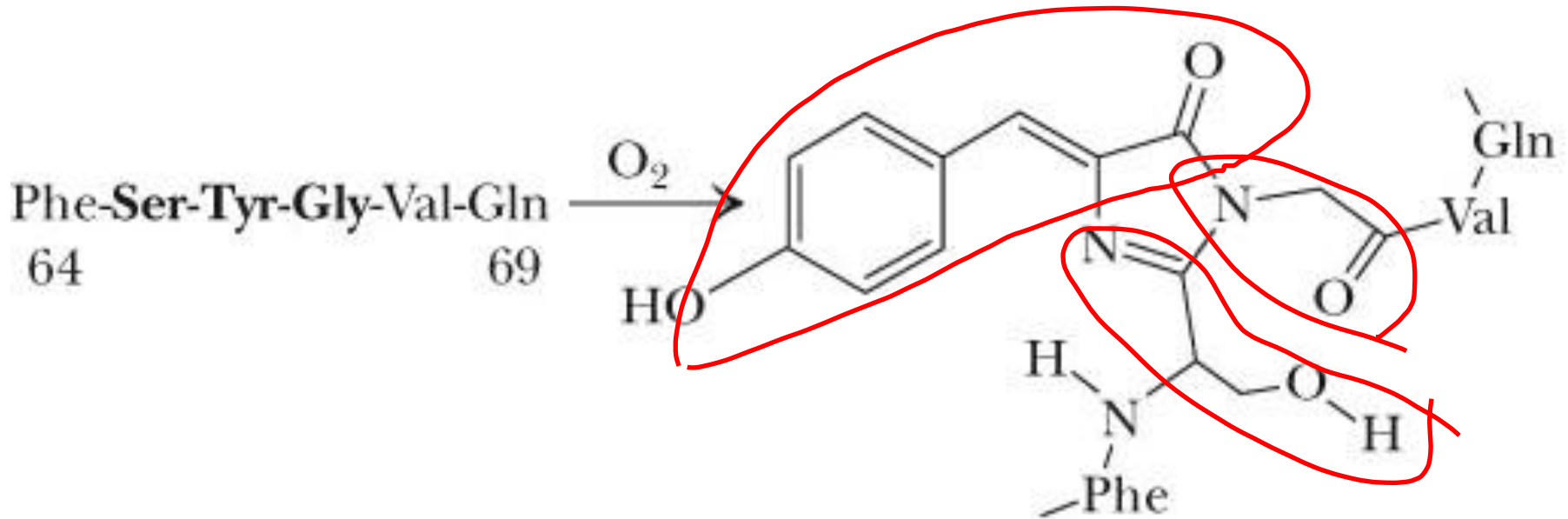


GREEN FLUORESCENT PROTEIN

A jellyfish (*Aequorea victoria*) native to the northwest Pacific Ocean contains a **green fluorescent protein**. GFP is a naturally fluorescent protein. Genetic engineering techniques can be used to “tag” virtually any protein, structure, or organelle in a cell. The GFP chromophore lies in the center of a β -barrel protein structure.



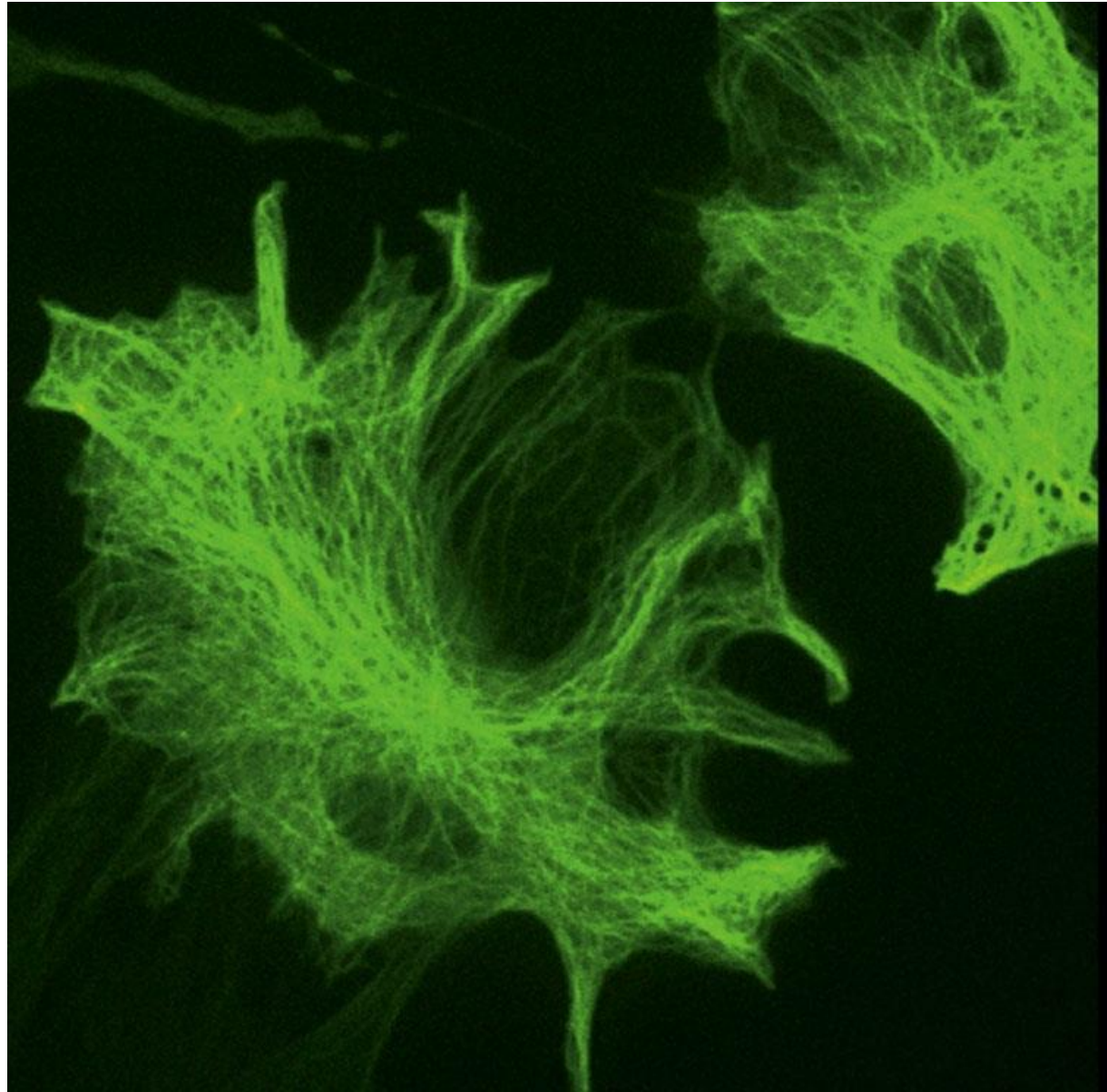
GREEN FLUORESCENT PROTEIN



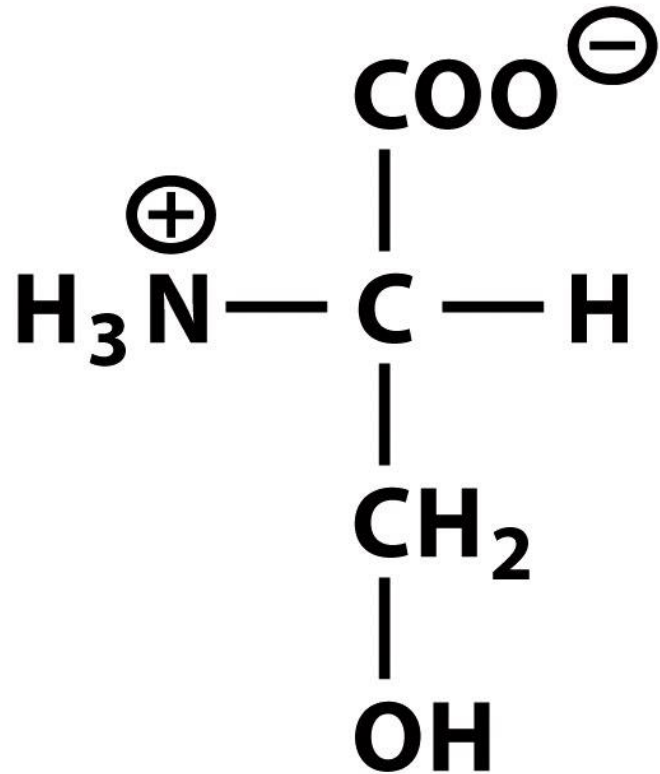
The prosthetic group of GFP is an oxidative product of the sequence –FSYGVQ-.

YELLOW FLUORESCENT PROTEIN

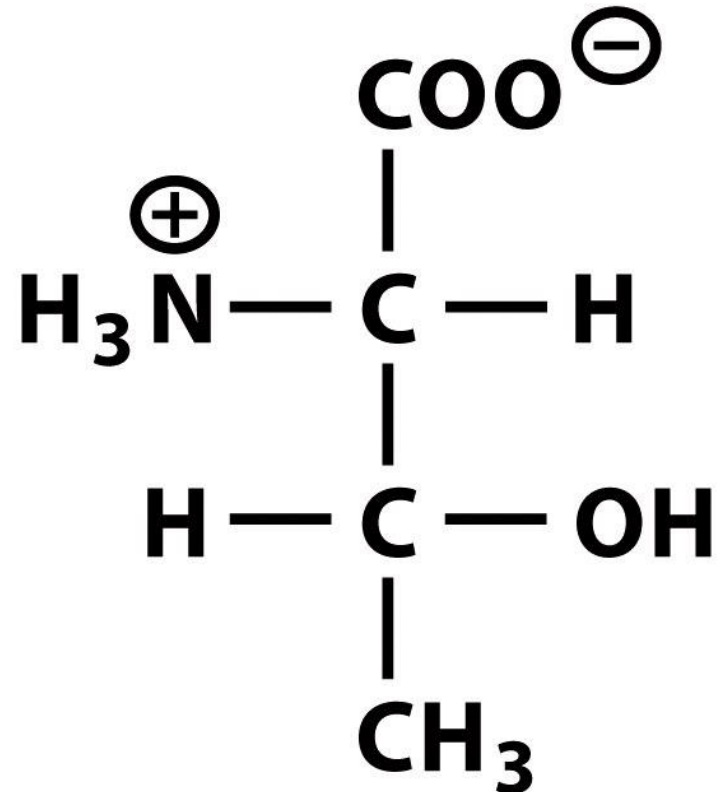
Amino acid substitutions in GFP can tune the color of emitted light. Shown here is an image of African green monkey kidney cells expressing yellow fluorescent protein (YFP) fused to α -tubulin, a cytoskeletal protein.



R SIDE CHAINS WITH ALCOHOL GROUPS



Serine [S]
(Ser)



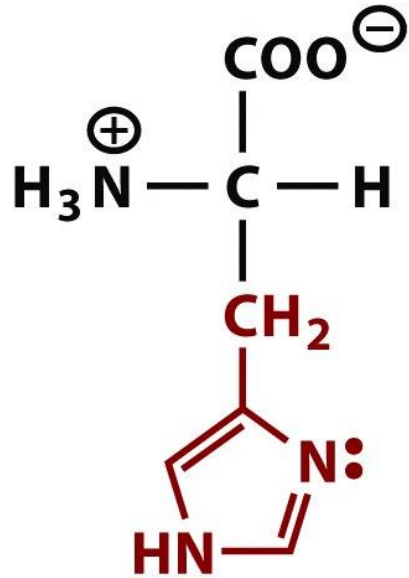
Threonine [T]
(Thr)

HOMework



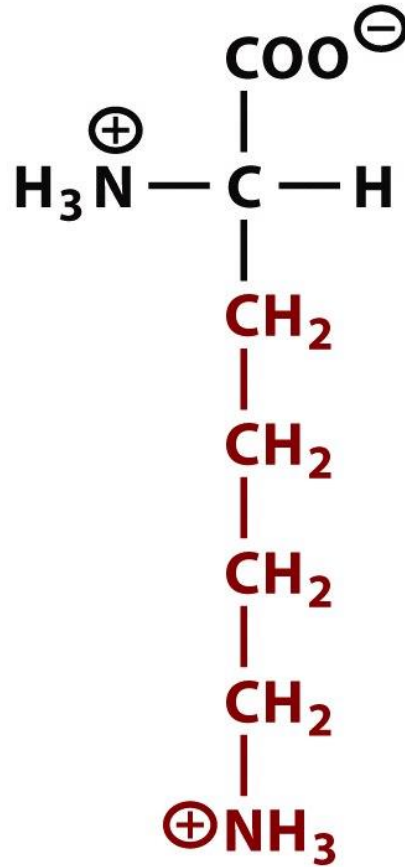
Draw the four stereoisomers for Threonine

BASIC R GROUPS



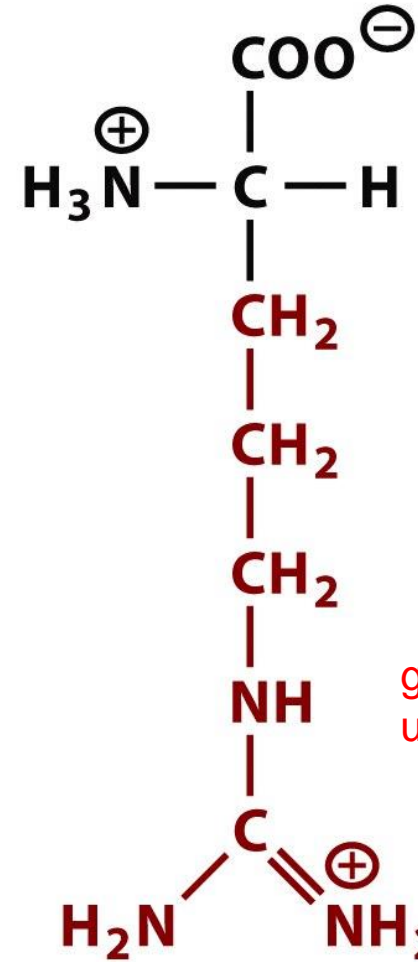
imidazole ring, found in acid base catalysis

Histidine [H]
(His)



Epsilon amino group

Lysine [K]
(Lys)



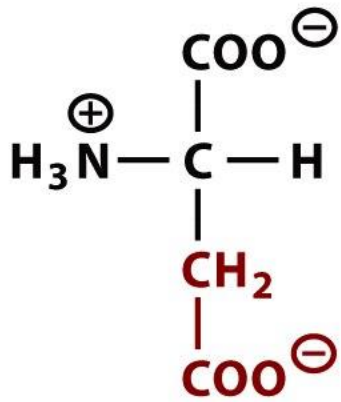
guanidinium

Arginine [R]
(Arg)

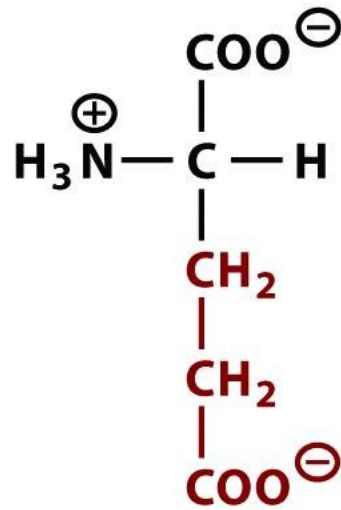
ACIDIC R GROUPS AND THEIR AMIDE DERIVATIVES

negative charge at physiological

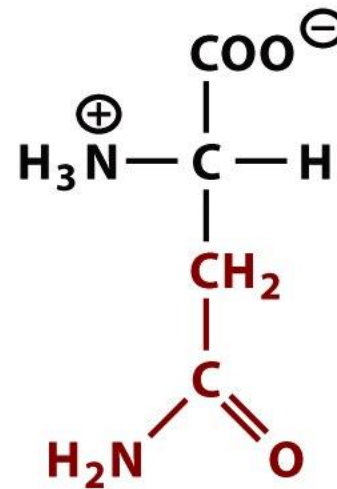
no charge



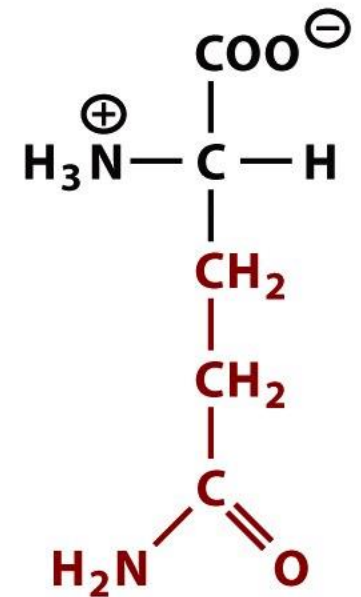
**Aspartate [D]
(Asp)**



**Glutamate [E]
(Glu)**



**Asparagine [N]
(Asn)**



**Glutamine [Q]
(Gln)**

important
slide, see
picture
taken on
tablet

SUMMARY TABLE OF AMINO ACIDS

most exist as zwitterions, no net charge

Amino acid	Three-letter abbreviation	One-letter abbreviation	Amino acid	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A	Methionine	Met	M
Arginine	Arg	R	Phenylalanine	Phe	F
Asparagine	Asn	N	Proline	Pro	P
Aspartic acid	Asp	D	Serine	Ser	S
Cysteine	Cys	C	Threonine	Thr	T
Glutamine	Gln	Q	Tryptophan	Trp	W
Glutamic acid	Glu	E	Tyrosine	Tyr	Y
Glycine	Gly	G	Valine	Val	V
Histidine	His	H	Asparagine or aspartic acid	Asx	B
Isoleucine	Ile	I	Glutamine or glutamic acid	Glx	Z
Leucine	Leu	L			
Lysine	Lys	K			

HYDROPATHY TABLE OF AMINO ACIDS

Hydropathy: The Relative hydrophobicity/philicity of each AA

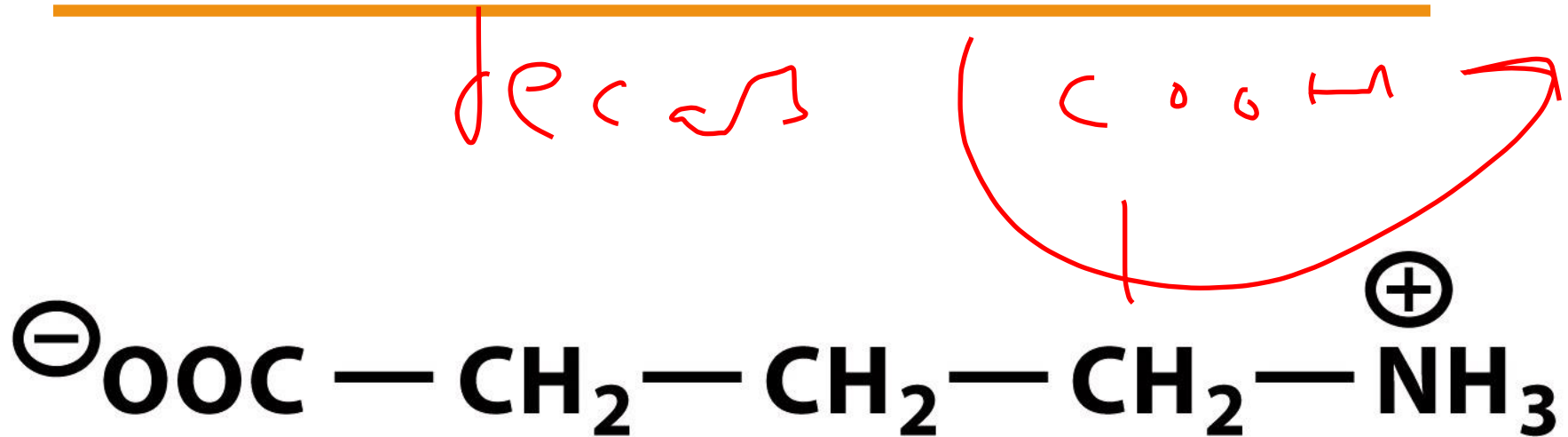
Side Chain	Hydropathy Index
Isoleucine	4.5
Valine	4.2
Leucine	3.8
Phenylalanine	2.8
Cysteine	2.5
Methionine	1.9
Alanine	1.8
Glycine	-0.4
Threonine	-0.7
Serine	-0.8
Tryptophan	-0.9
Tyrosine	-1.3
Proline	-1.6
Histidine	-3.2
Glutamic acid	-3.5
Glutamine	-3.5
Aspartic acid	-3.5
Asparagine	-3.5
Lysine	-3.9
Arginine	-4.5

more hydrophobic, and more energy needed to move from lipid bilateral

negative and less energy needed to move from lipid bilayer, hydrophilic

*From Kyte, J., and Doolittle, R., 1982. A simple method for displaying the hydrophobic character of a protein. *Journal of Molecular Biology* 157:105-132.

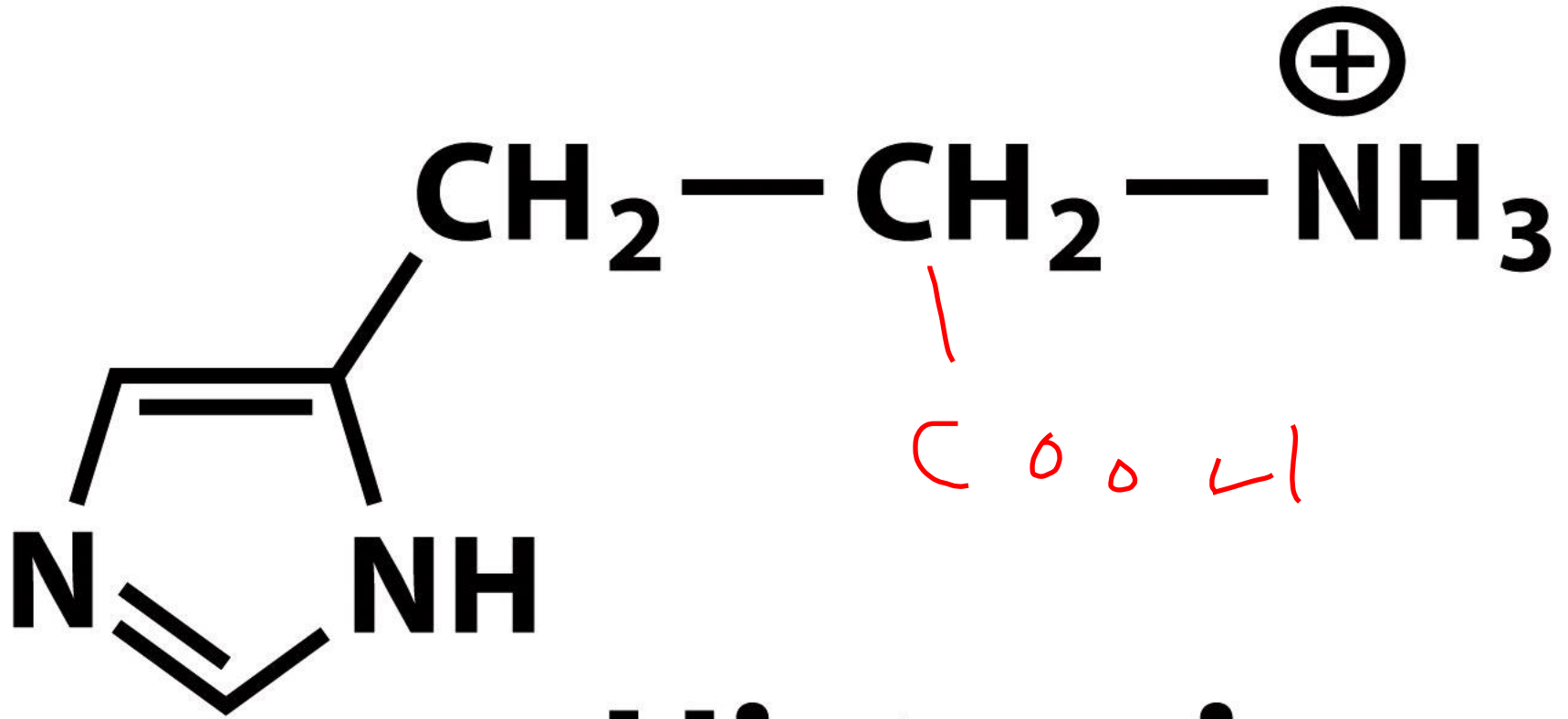
AMINO ACID DERIVATIVES



γ -Aminobutyrate

neurotransmitter in mammalian brain, derived from glutamic acid

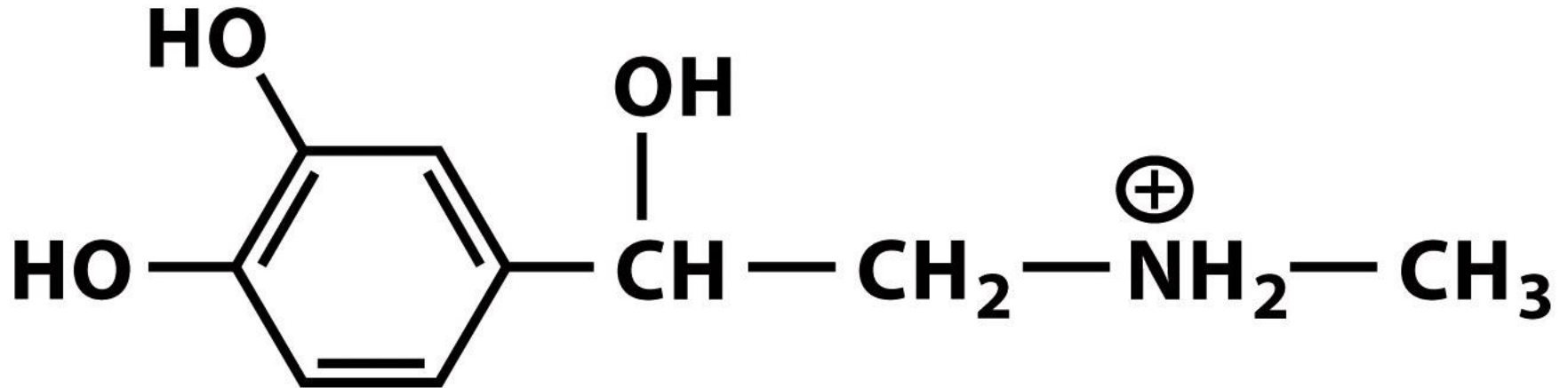
AMINO ACID DERIVATIVES



Histamine

derived from histidine, helps in hcl and vaso constriction

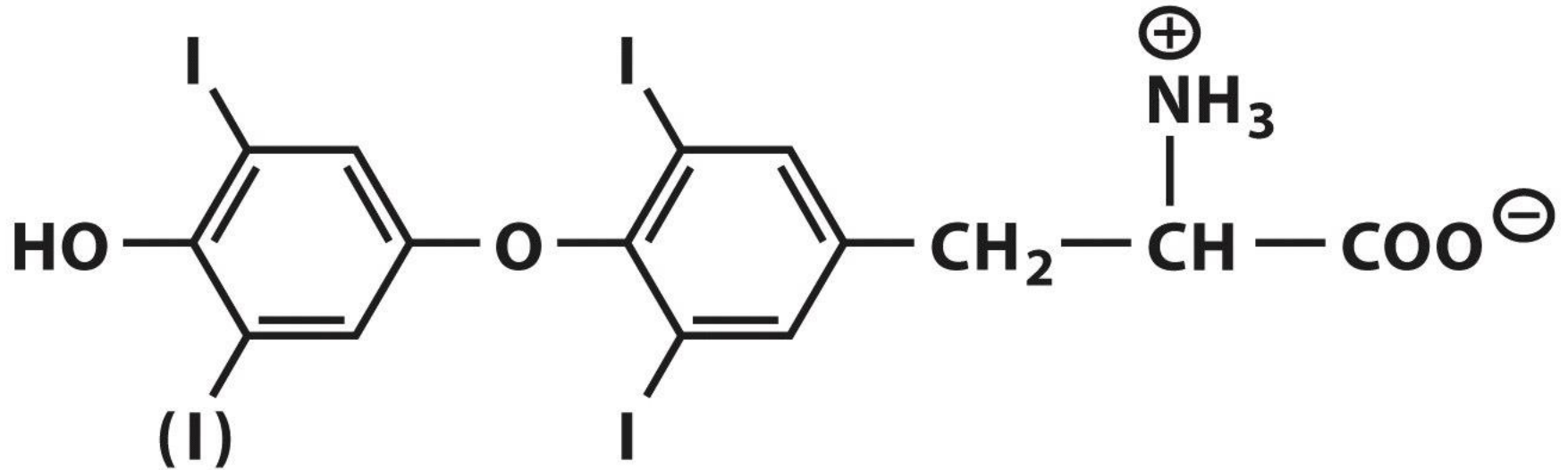
AMINO ACID DERIVATIVES



**Epinephrine
(Adrenaline)**

tyrosine derivative.

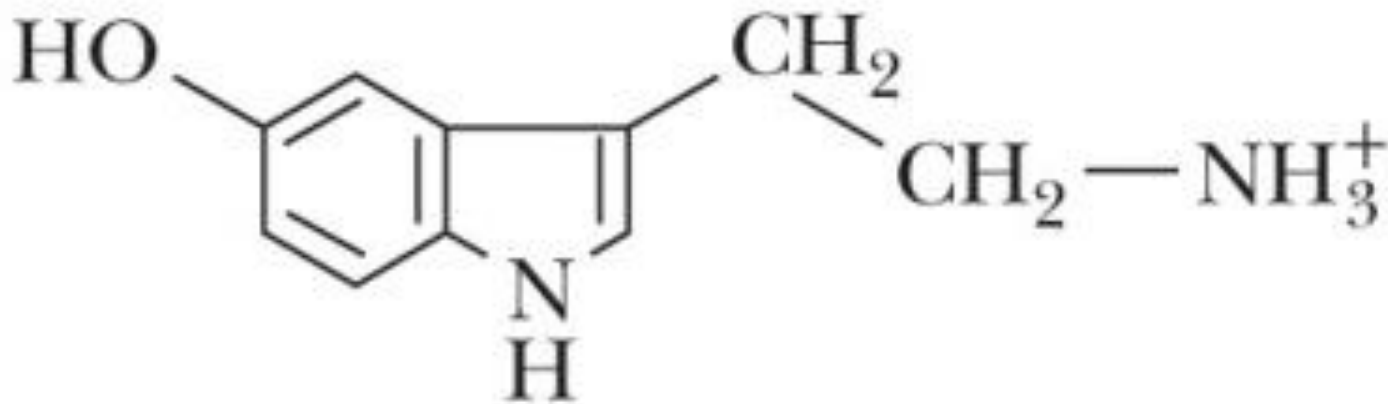
AMINO ACID DERIVATIVES



Thyroxine / Triiodothyronine

also tyrosine derivative. thyroid hormone

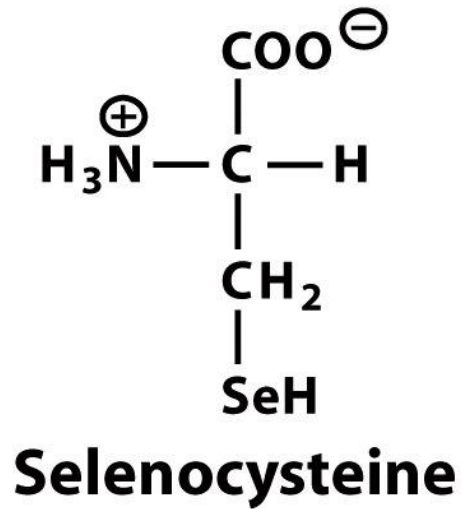
AMINO ACID DERIVATIVES



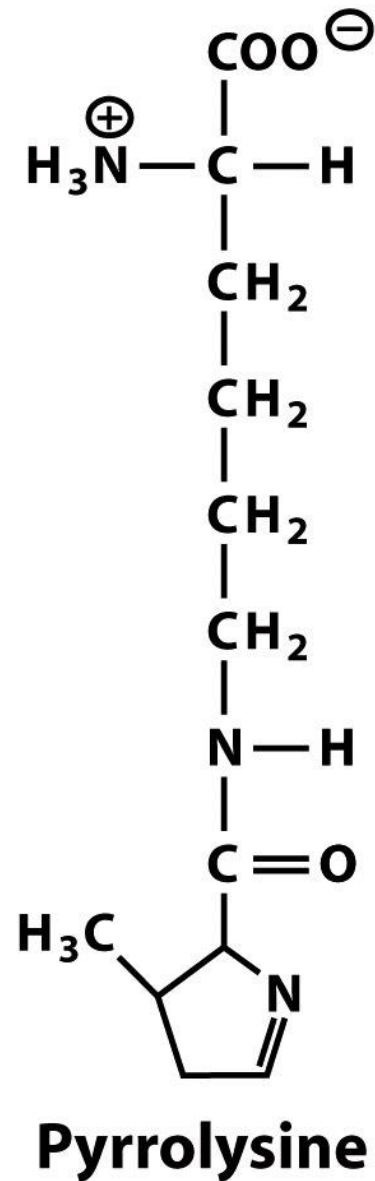
Serotonin

tryptophan

AMINO ACID 21 AND 22?



bacteria incorporate these two amino acids in their proteins. similar to cysteine and lysine respectively.



SUMMARY

Properties of AAs – R group defines AA, 20 AAs, most exist as Zwitterions (no net charge), 19 have α -C that is chiral/asymmetrical, exist as stereoisomers (mirror images, not superimposable), L/D Designation

a) Aliphatic : hydrophobic

Proline: pyrrolidine ring (causes changes in direction)

b) Aromatic : phenol groups, absorb UV light, hydrophobic

c) Sulfur: Cys \rightarrow cystine

d) Alcohol: less hydrophobic

e) Basic: His \rightarrow imidazole ring

Lys \rightarrow (α , ϵ amino groups), Arg \rightarrow (guanidinium group)

f) Acidic + Amide derivatives: Asp, Glu negatively charged

Both e) and f) hydrophilic

g) Iso/Thr (4 stereoisomers)

h) AA derivatives