

Amino Acid & Protein Chemistry

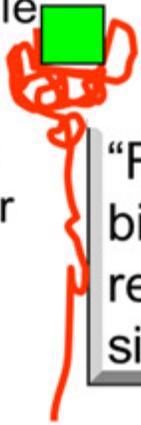
Dr Piyush B. Tailor
Associate Professor
Department of Biochemistry
Govt. Medical College
surat

- More than 300 A.A. in nature
- Commonly found in proteins = 20 only.
- Each amino acids are expressed by DNA-Genetic codon
- 21st = ??????
- 22nd = ??????

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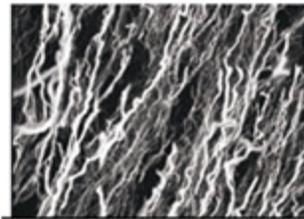
Proteins Shape Dictates Function

Signal molecule

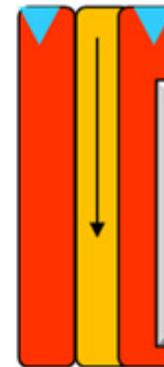


Protein receptor

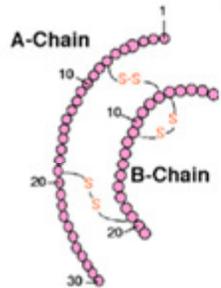
“Receptors” with binding sites that recognize chemical signals



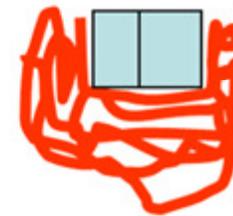
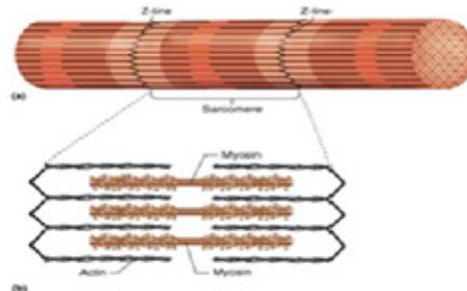
Collagen fibers



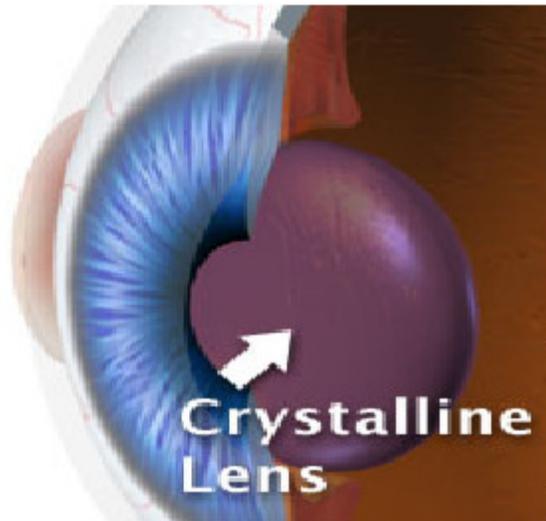
Cylindrical tubes to transport large molecules across the cell membrane



Peptide hormones (insulin)



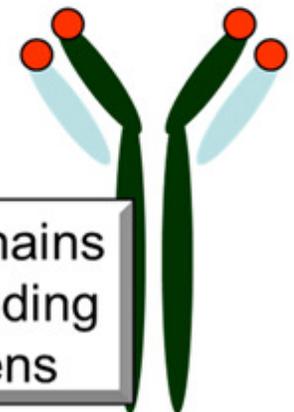
Enzymes that join or split other molecules



Crystalline Lens

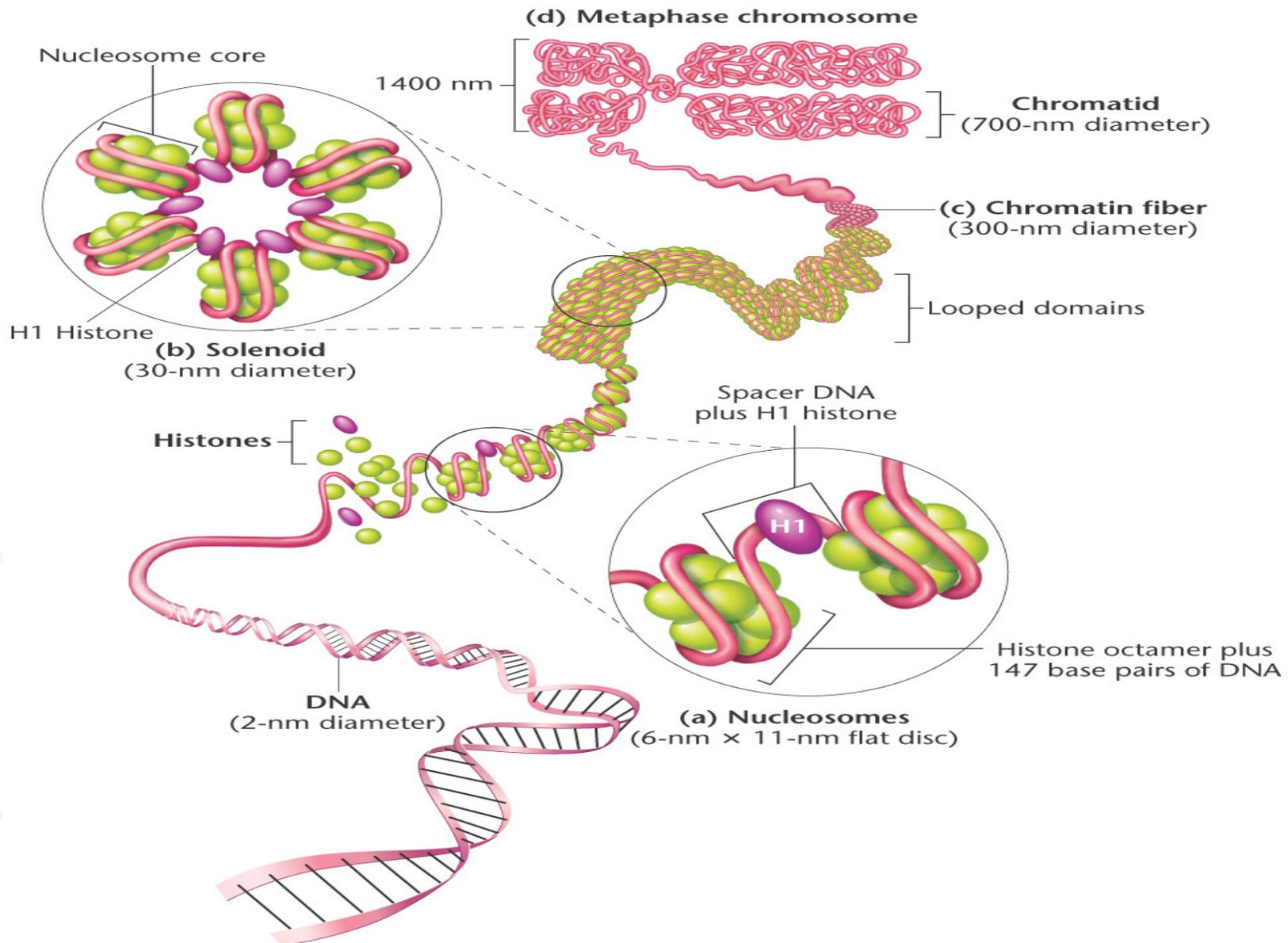


Hemoglobin to transport oxygen

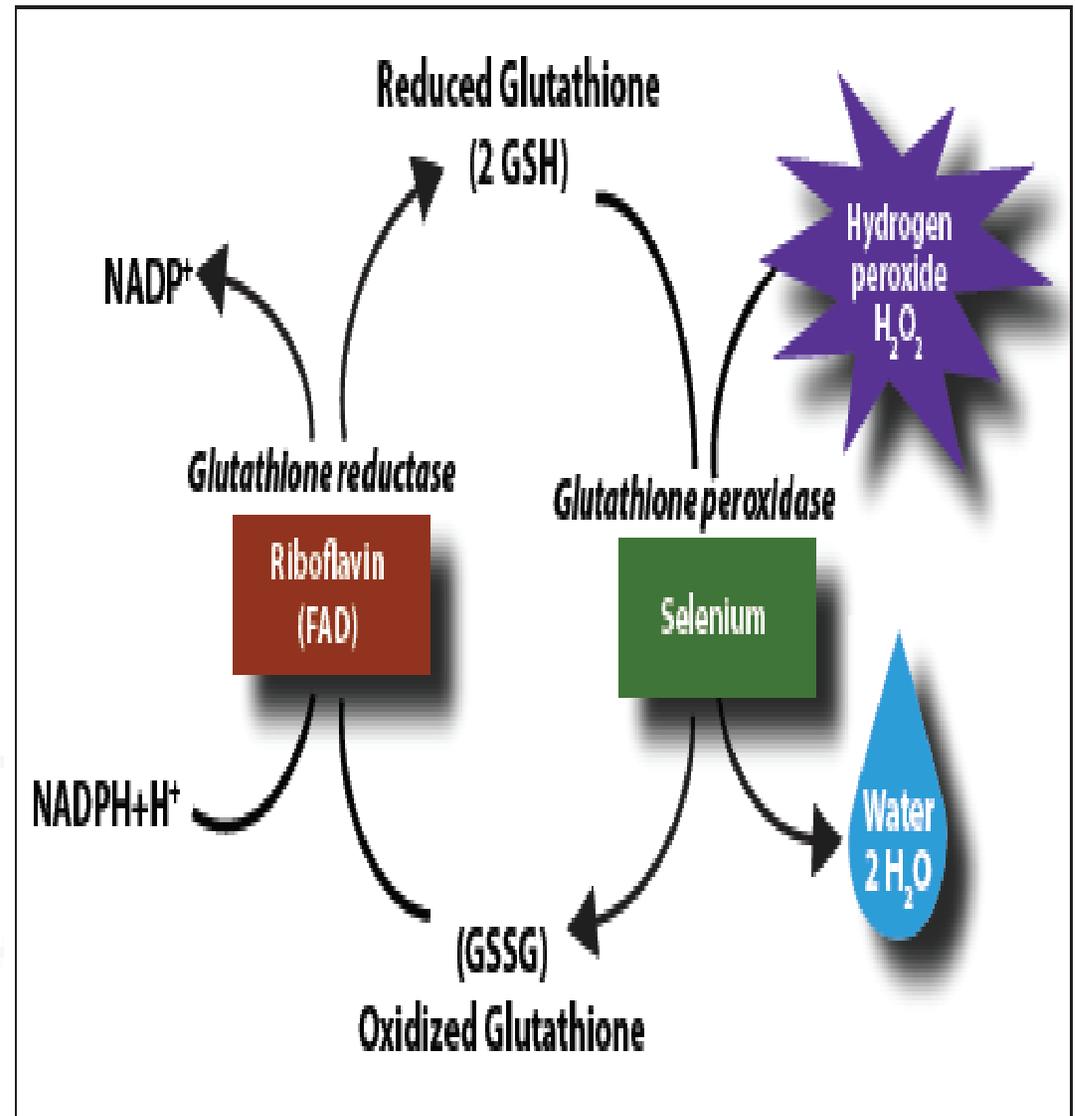
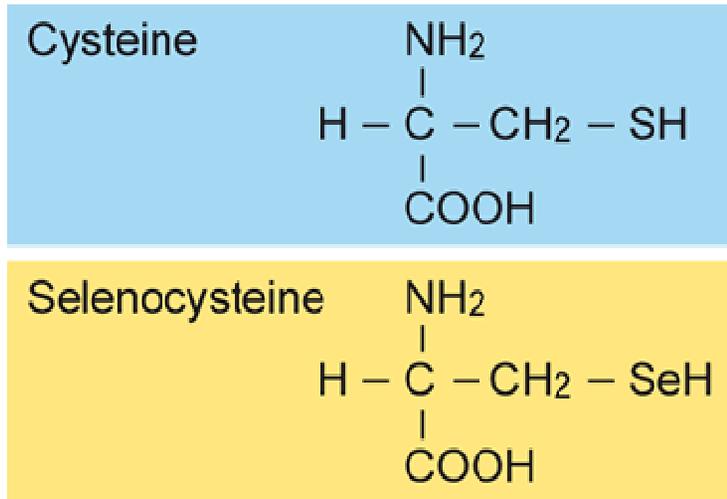


Antibodies - chains bound with binding sites for antigens

Is there any protein in this?

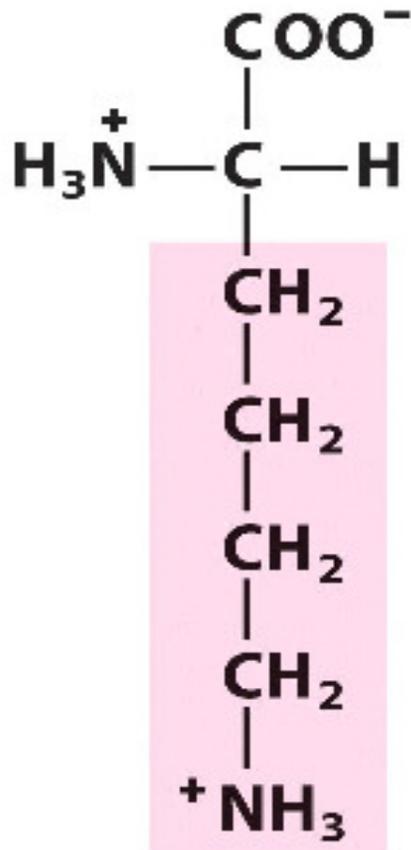


21st Amino Acid = Code By UGA

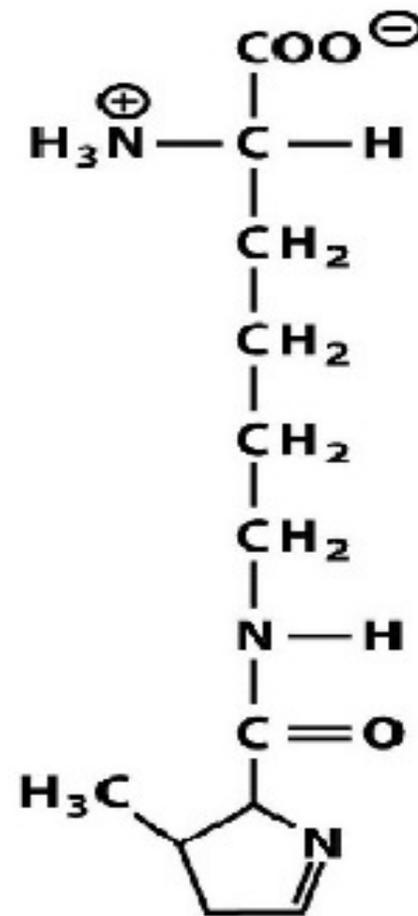


Dr Piyu

22nd Amino acid = Code by UAG



Lysine



Pyrrolysine

Dr

What is the role of Amino acid
other than “Unit of Protein”?

Dr Piyush Tailor

Amino acid Significant

- Neurotransmitters
- Pigment
- Creatinine
- Conjugation
- Detoxification
- Supplement
- Derived Amino acid

Dr. Vishal Tailor

Neurotransmitter

- Dopamine
- Epinephrine
- Nor Epinephrine
- Histamine
- Metatonine
- Seretonine

Dr. Prayush Tailor

FREE
10% Extra

300 Pellets +
30 Pellets Free

**Sugar**TM
Free

~Gold~

Low calorie sugar substitute

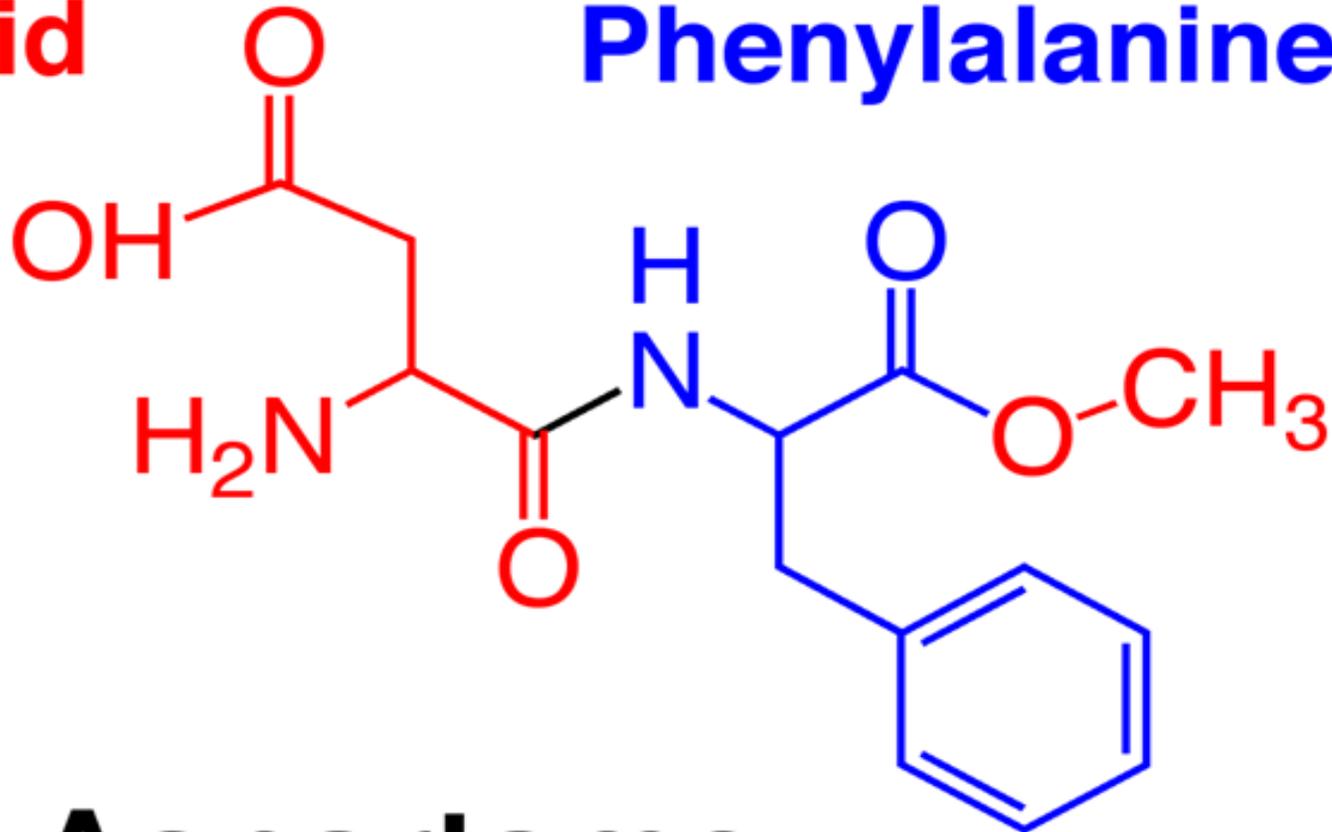
India's
No. 1
sweetener



Aspartame

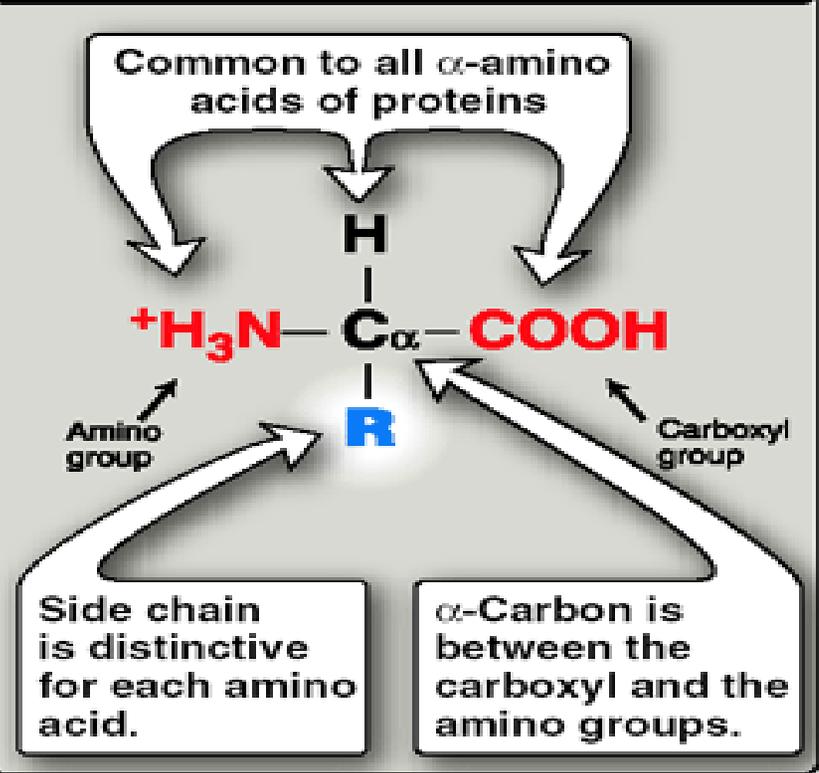
Aspartic acid

Phenylalanine

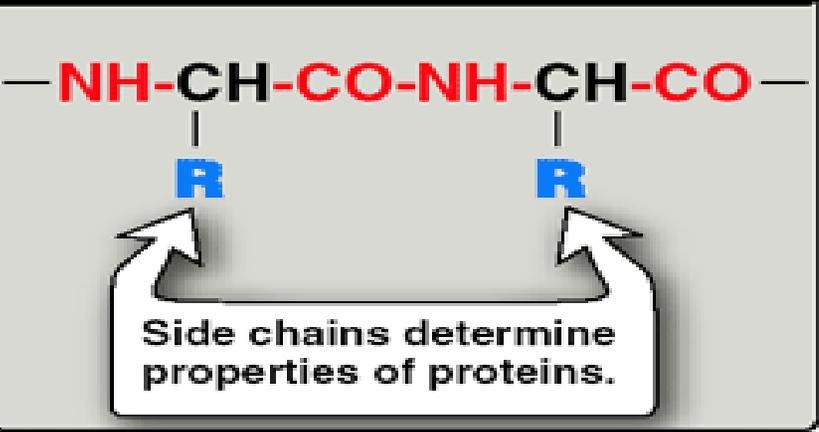


Aspartame

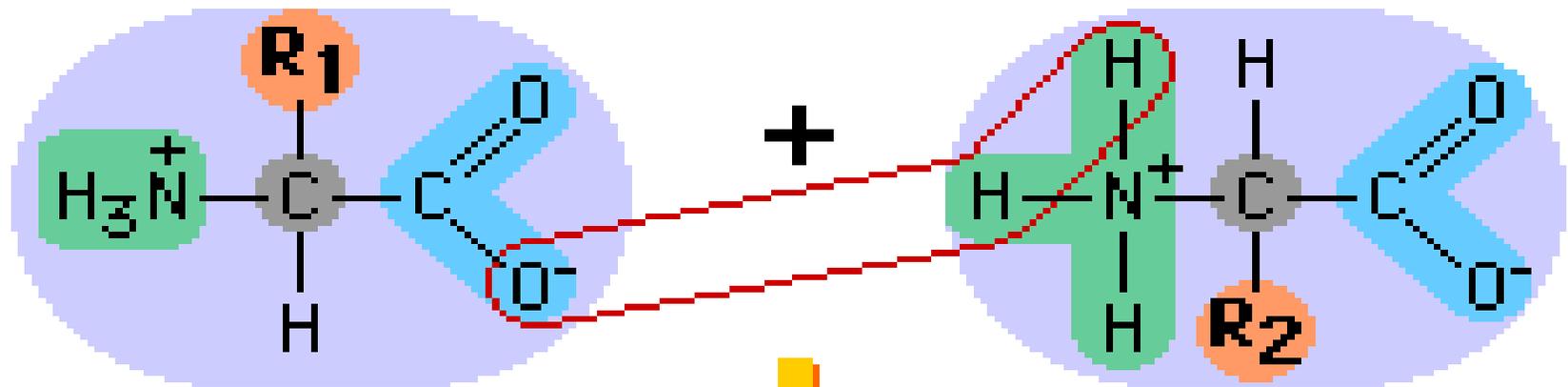
A Free amino acid



B Amino acids combined in peptide linkages

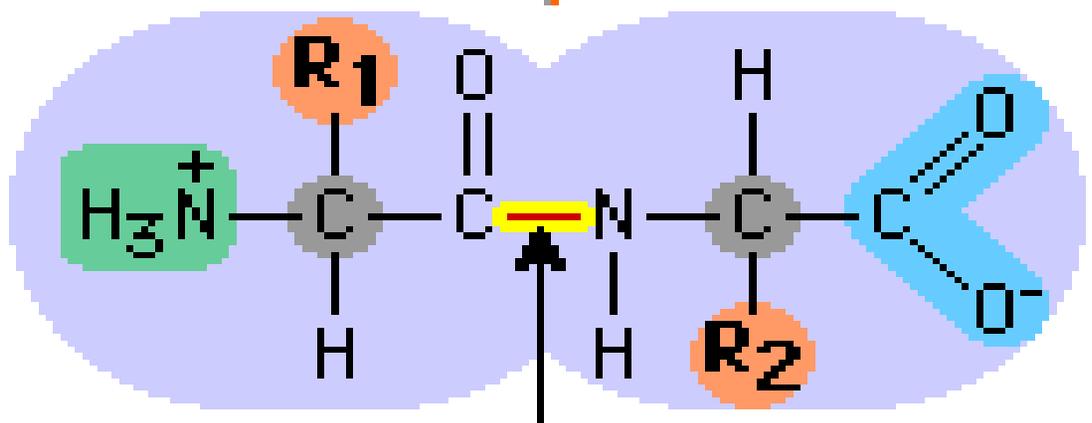
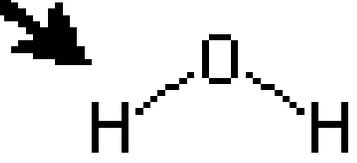


- Each amino acid
 - ✓ **Primary Amino group**
 - ✓ **Carboxyl group**
 - ✓ **Side chain ("R-group)**
- Most of the aminoacids (except proline) are Alpha A.A.
- At physiologic pH,
 - ✓ Carboxyl group = negative = $-\text{COO}^-$
 - ✓ Amino group = positive = $-\text{NH}_3^+$
- **Group are involve in peptide bond**
- **Protein properties depends on A.A. side chains.**



Amino acid 1

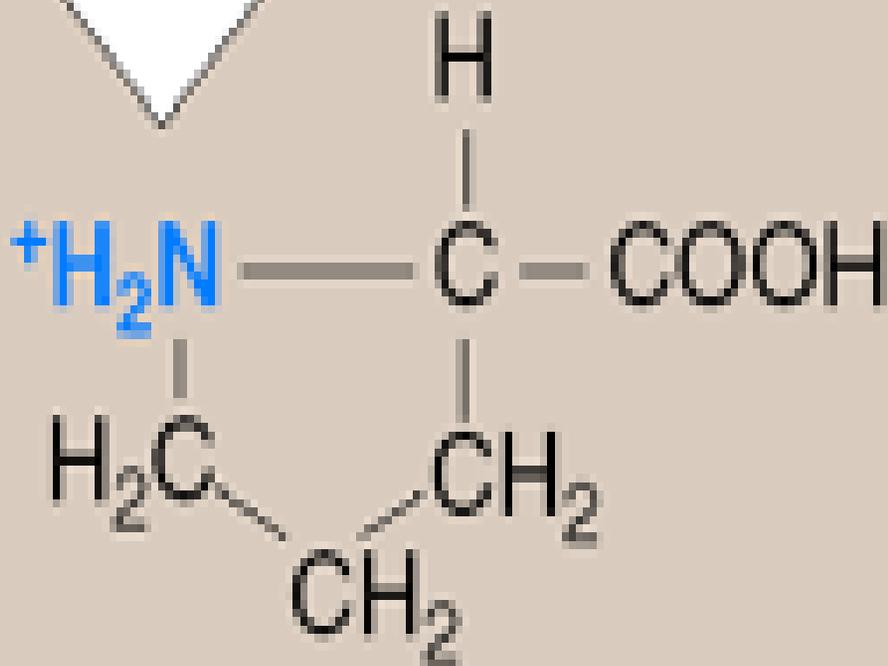
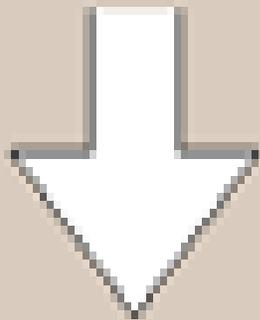
Amino acid 2



Peptide bond

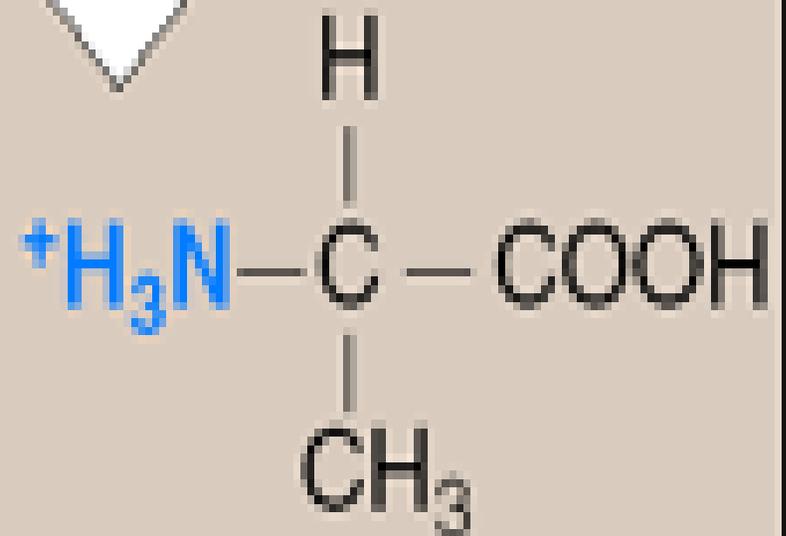
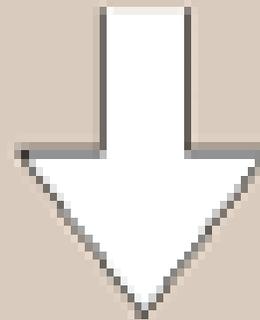
I

Secondary amino group



Proline

Primary amino group



Alanine

Proline

- Secondary amino group
- Unique geometry
- Contributes for formation of fibrous structure of collagen
- Interrupts the α -helices found in globular proteins.

Dr Piyush Tailor

CLASSIFICATION OF AMINOACID

1. Based on structure

2. Based on side chain (polarity)

3. Based on metabolic fates

4. Based on nutritional requirement

Amino acid Classification Based on Structure

A. Aliphatic amino acids:

1. Monoamino monocarboxylic

- Simple a.a.
- Branched a.a.
- Hydroxyl a.a.
- Sulphur containing a.a.
- Amide group containing a.a.

2. Monoamino dicarboxylic

3. Diamino monocarboxylic

B. Aromatic amino acids:

C. Heterocyclic amino acids:

D. Imino acid :

E. Derived amino acids:

A. Aliphatic amino acids

1. Mono amino mono carboxylic acids

➤ **Simple amino acids:**

- Glycine
- Alanine

➤ **Branched chain amino acids:**

- Valine
- Leucine
- Isoleucine

➤ **Hydroxy amino acids:**

- Serine
- Threonine

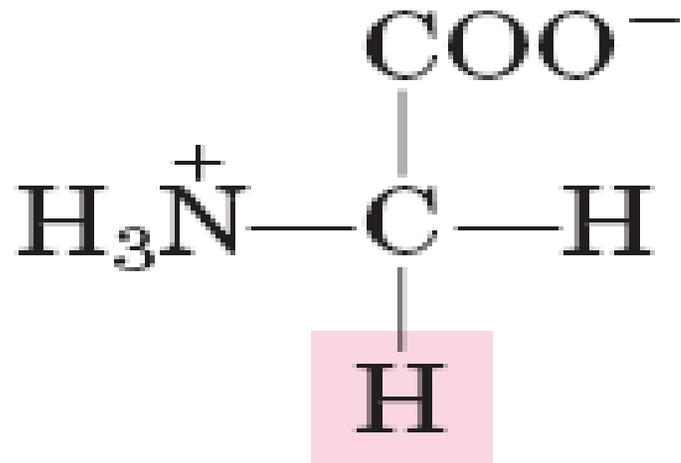
➤ **Sulphur containing amino acids:**

- Cysteine
- Methionine

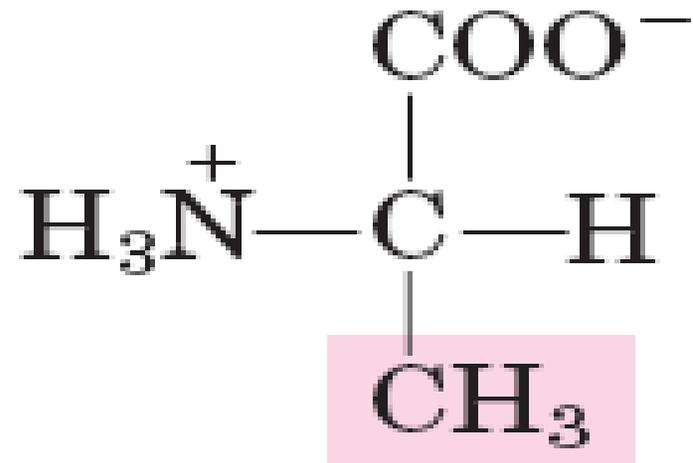
➤ **Amide group containing amino acids:**

- Asparagine
- Glutamine

Simple Amino Acids



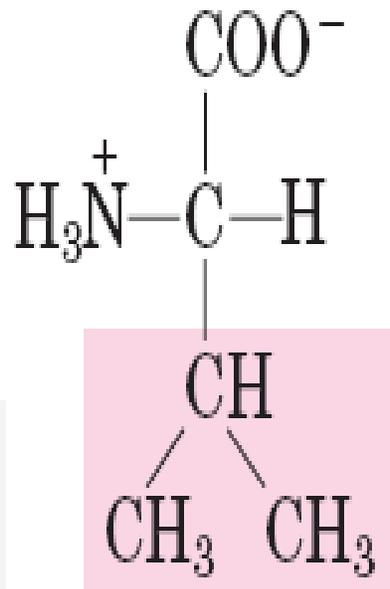
Glycine



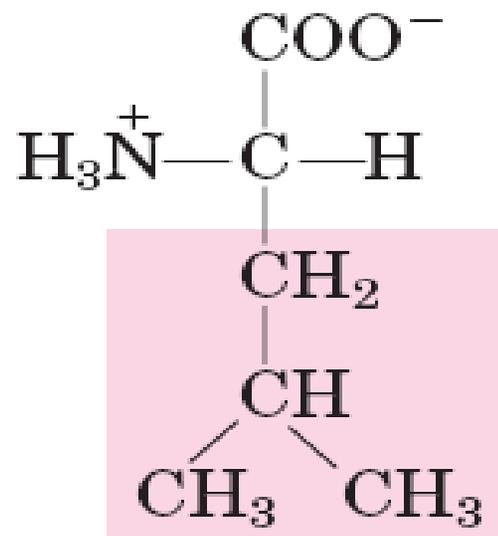
Alanine



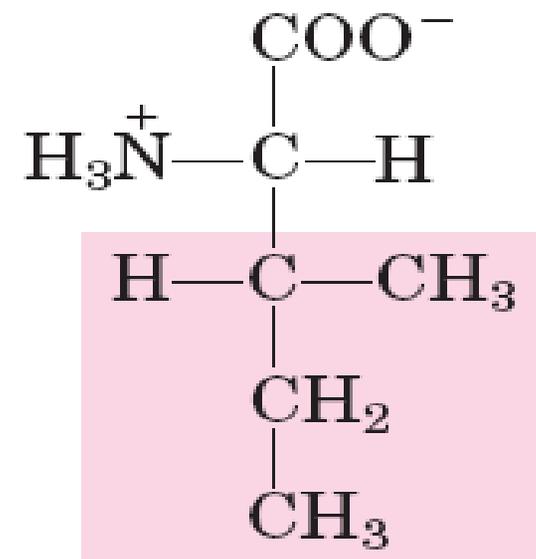
Branched chain Amino Acids



Valine

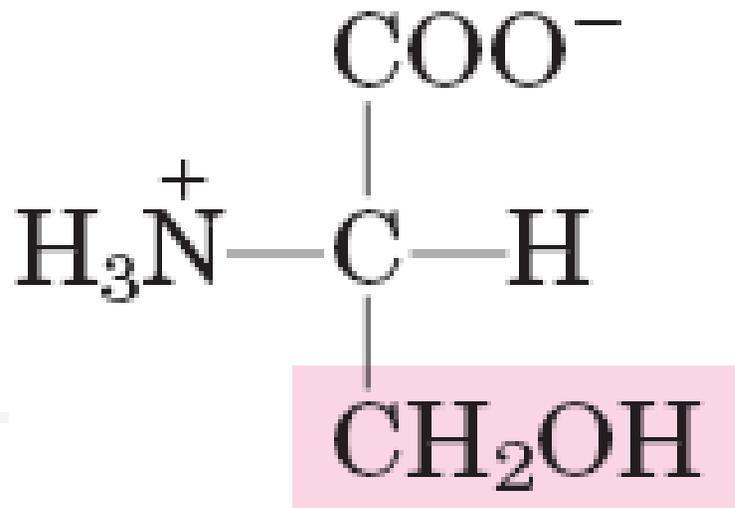


Leucine

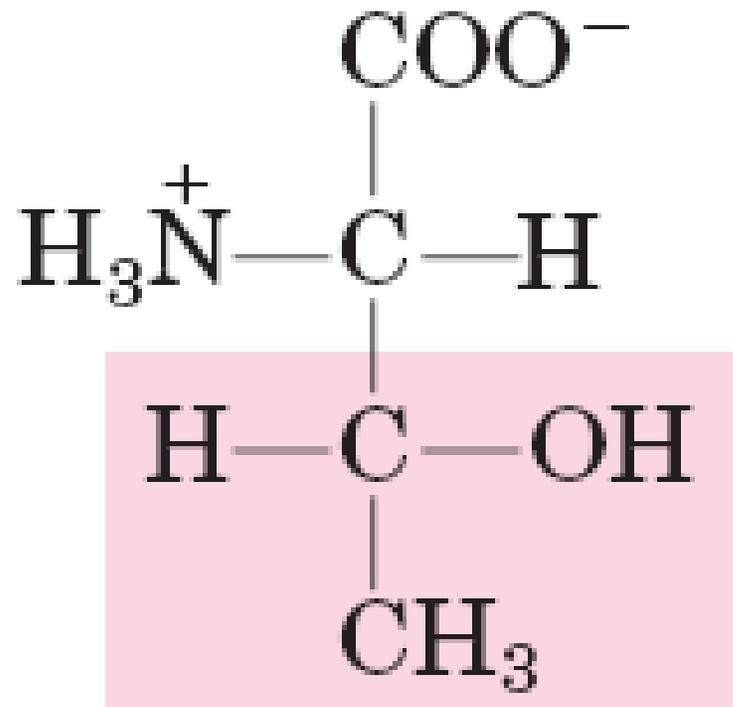


Isoleucine

Hydroxy Amino Acids

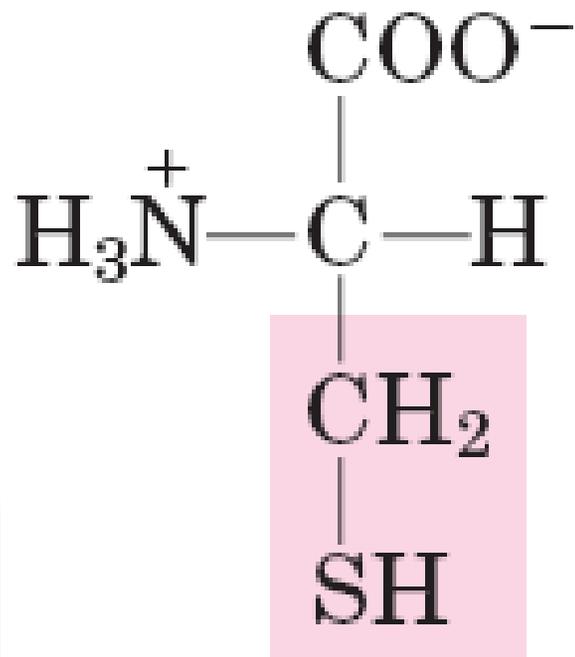


Serine

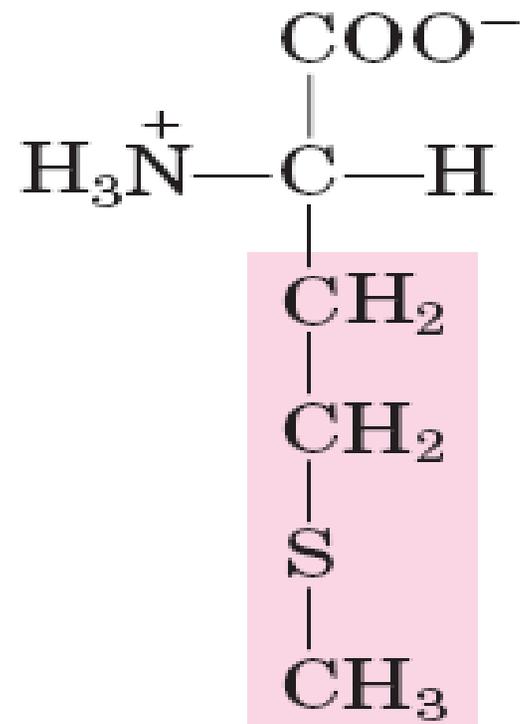


Threonine

Sulphur Containing Amino Acids

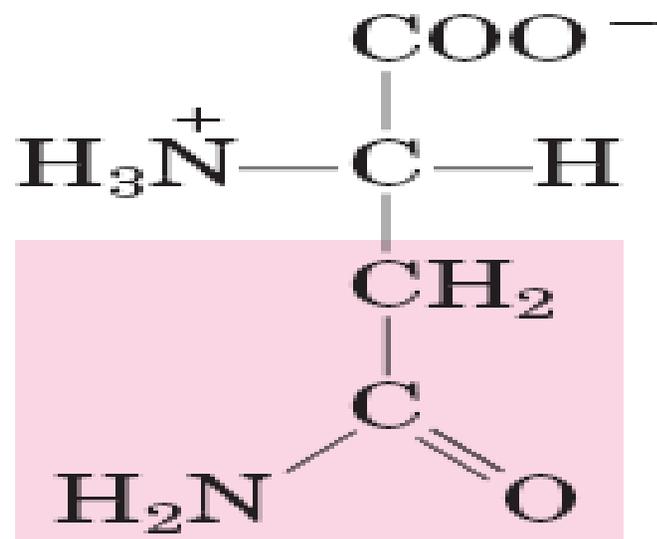


Cysteine

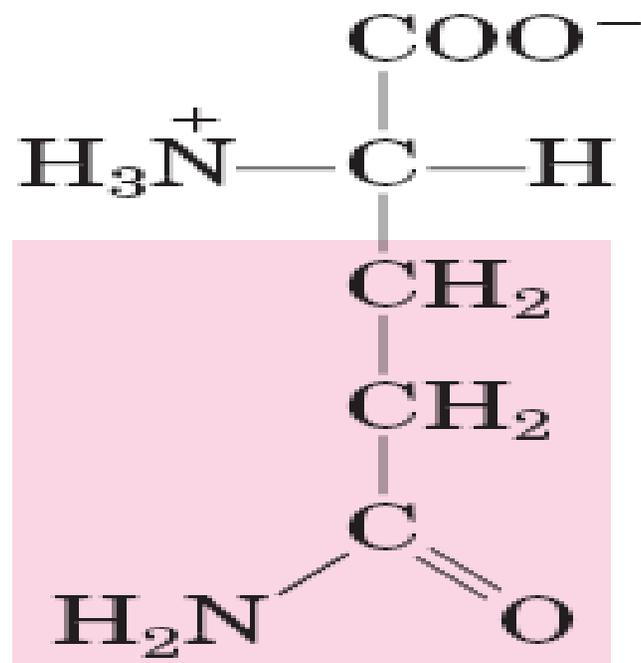


Methionine

Amide Containing Amino Acids



Asparagine



Glutamine

2. Mono amino dicarboxylic acids

Aspartic acid

Glutamic acid

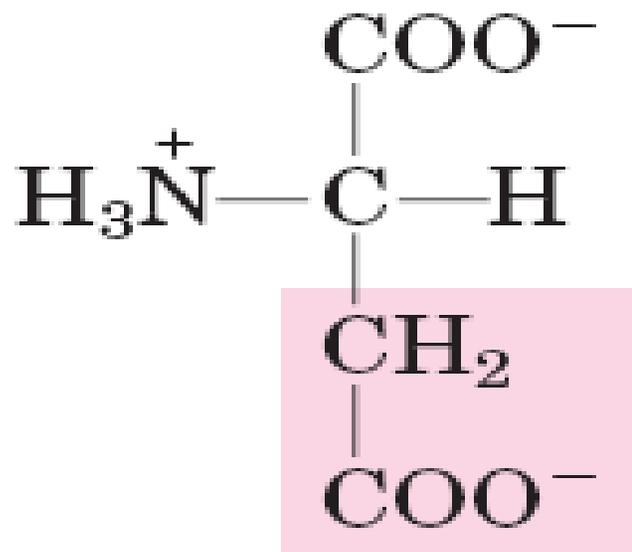
3. Di basic mono carboxylic acids

Lysine

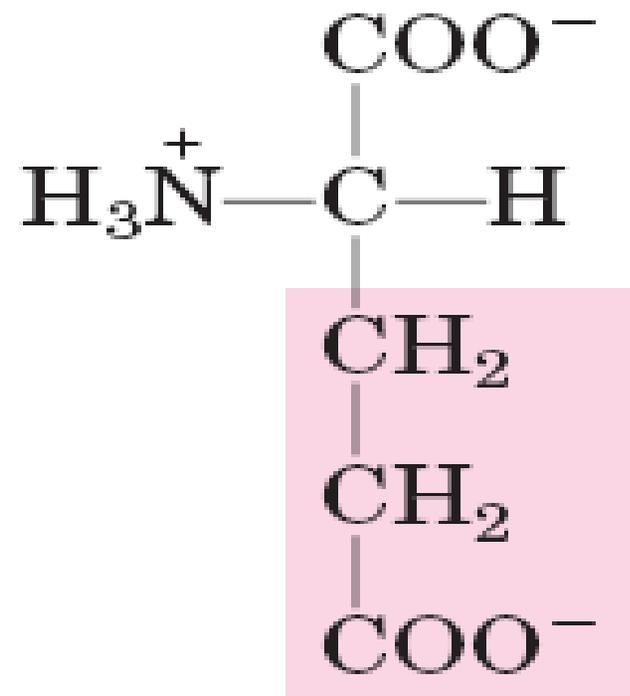
Arginine

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Mono-amino Di-carboxylic Amino Acids

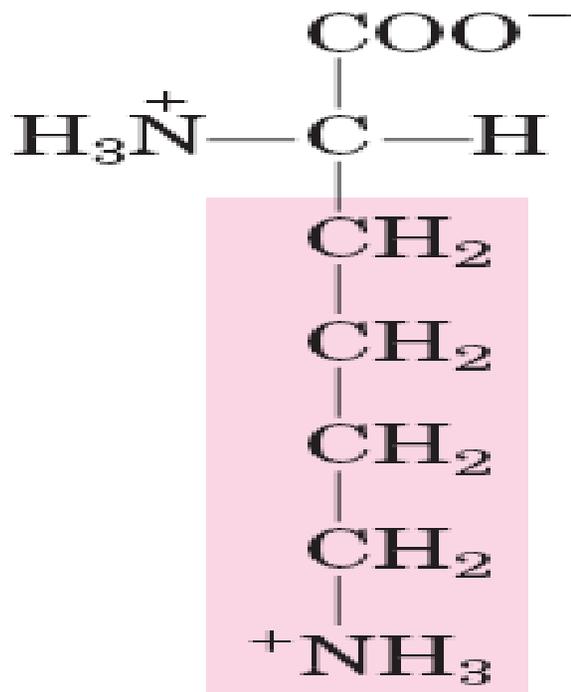


Aspartate

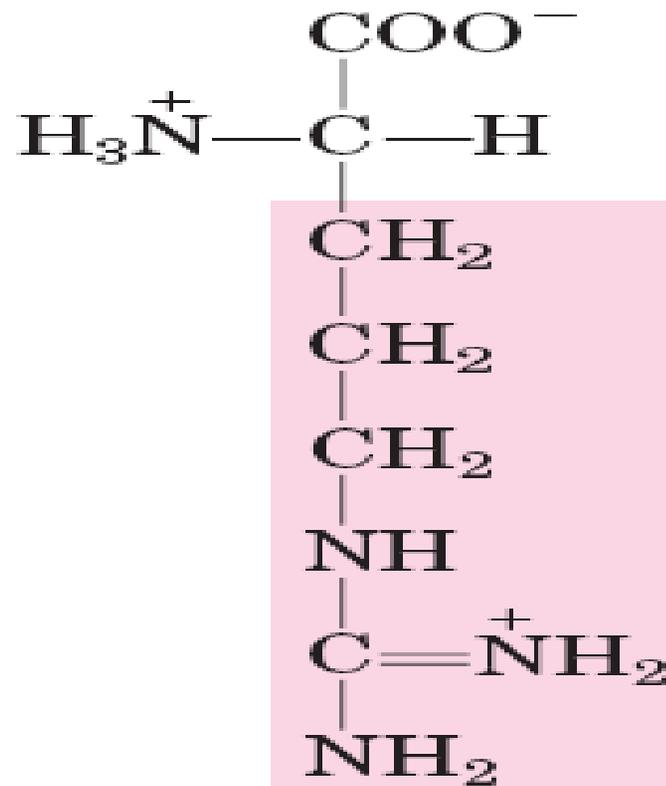


Glutamate

Di-basic Mono-carboxylic Amino Acids



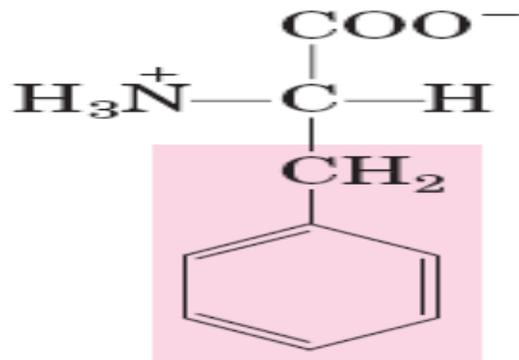
Lysine



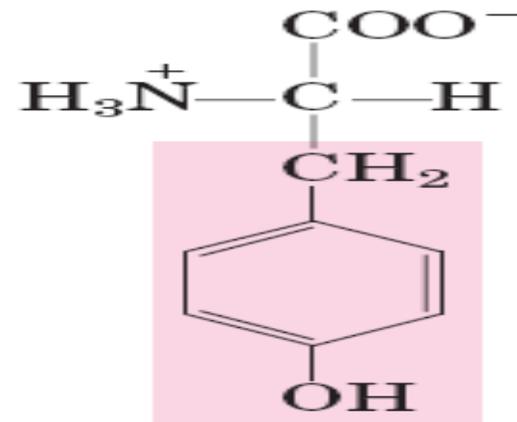
Arginine

B. Aromatic amino acids:

- Phenylalanine
- Tyrosine



Phenylalanine

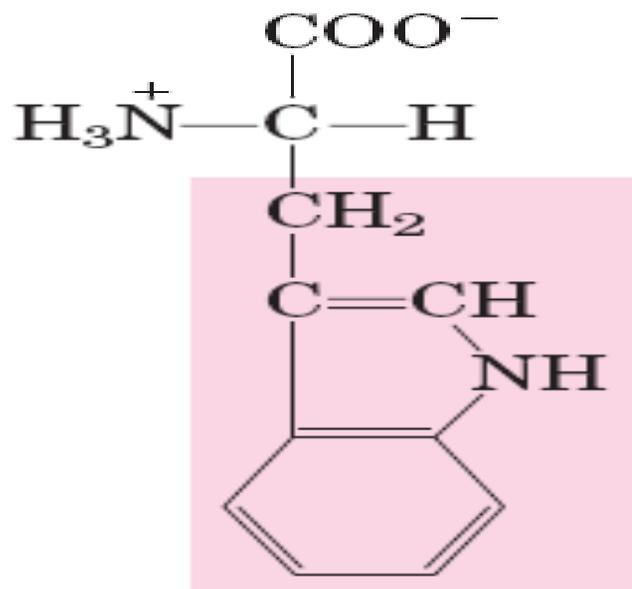


Tyrosine

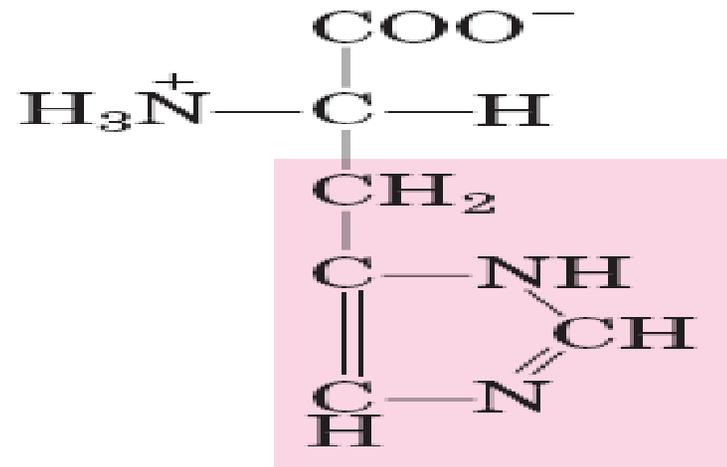
Importance ????

C.Heterocyclic amino acids:

- Tryptophan
- Histidine

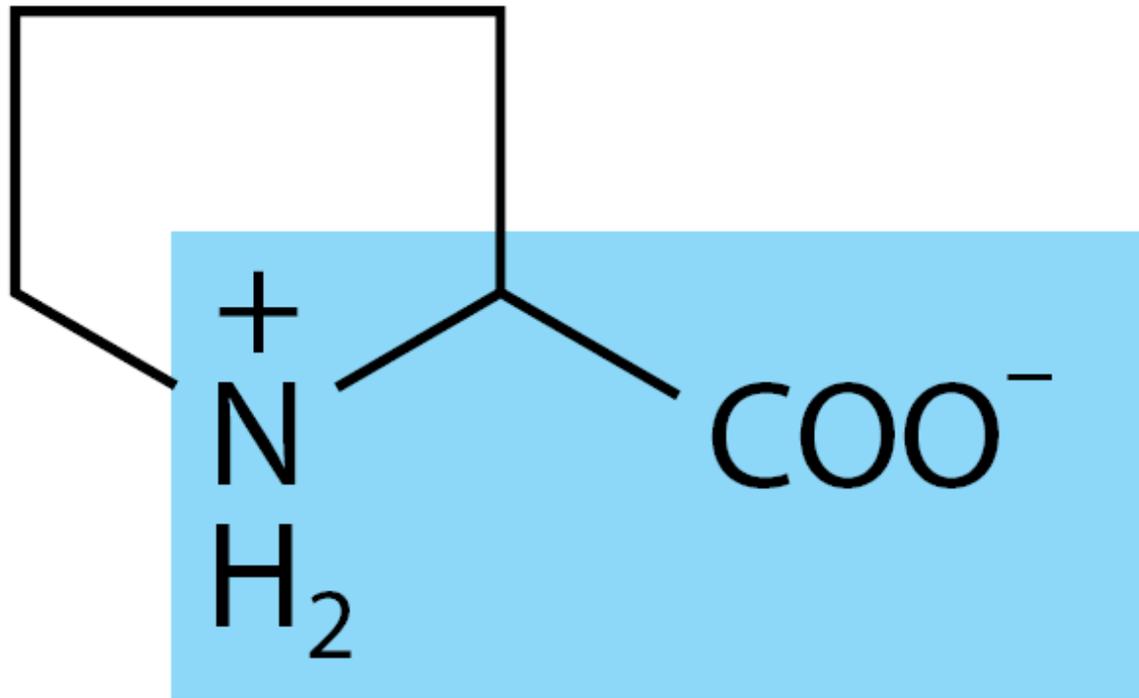


Tryptophan



Histidine

- **D.Imino acid:**
- Proline



D

or

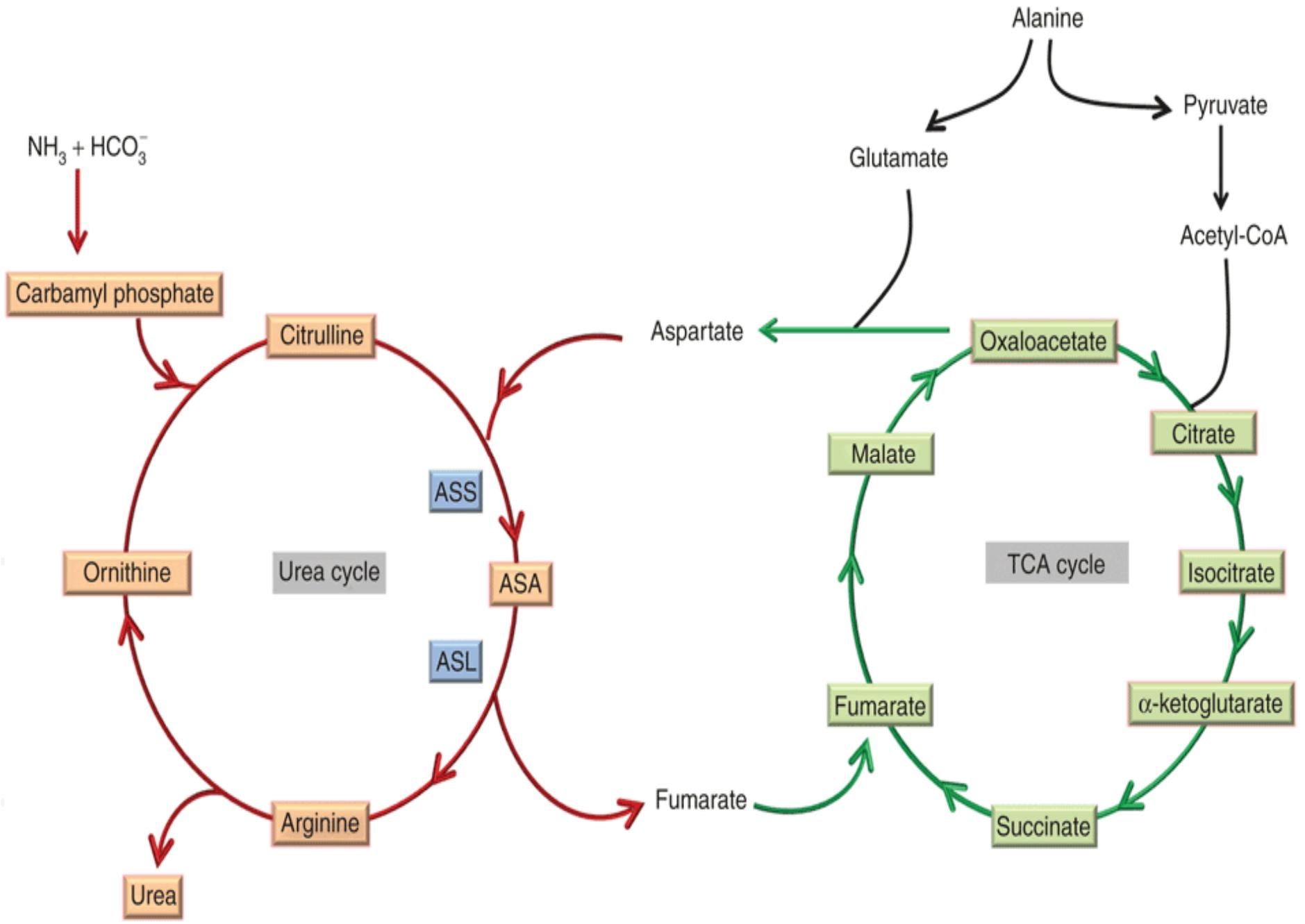
E. Derived amino acids:

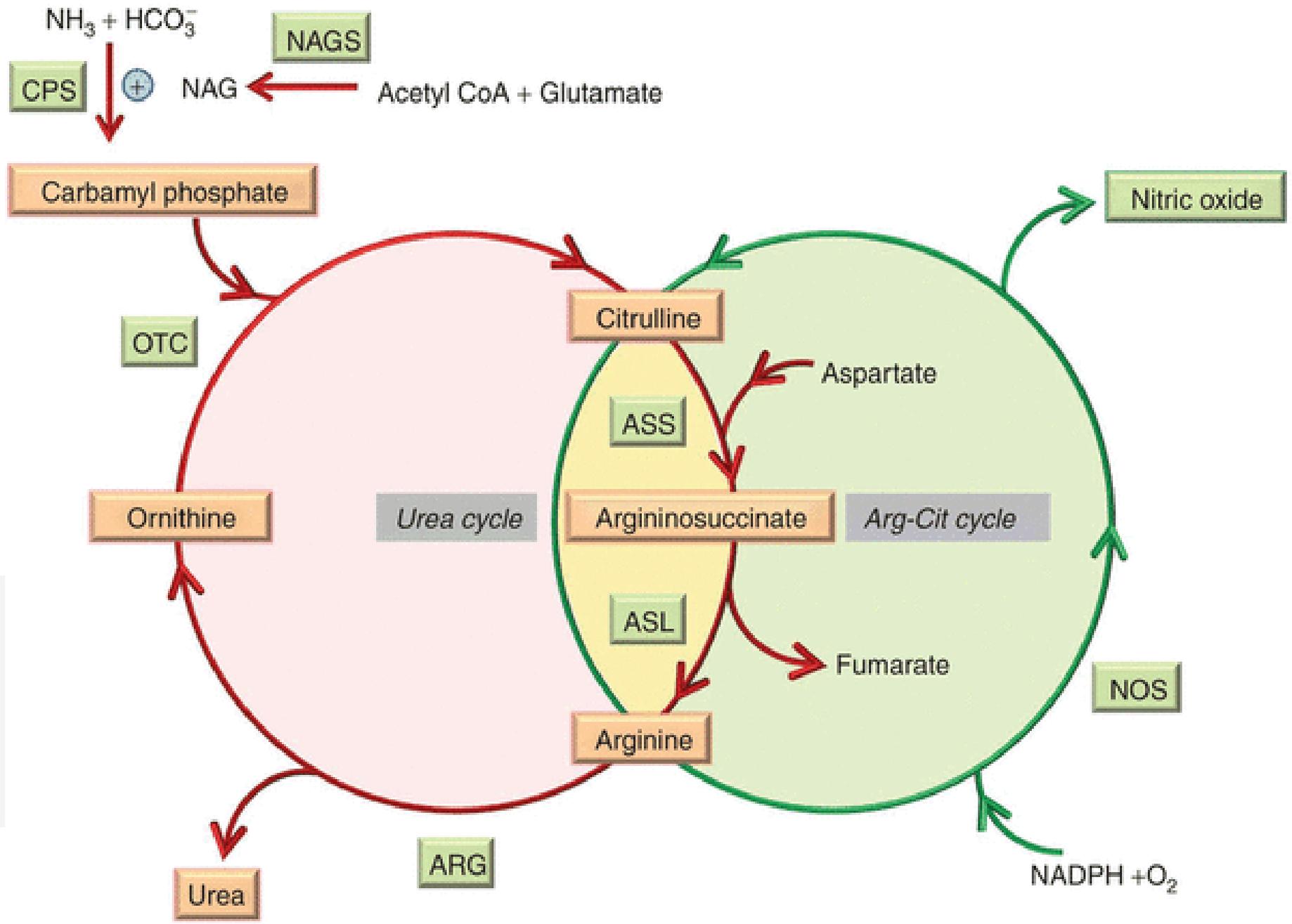
Found in proteins:

- Hydroxy lysine & Hydroxy proline = Collagen.
- Gamma carboxylation of glutamic acid residues of clotting factor.

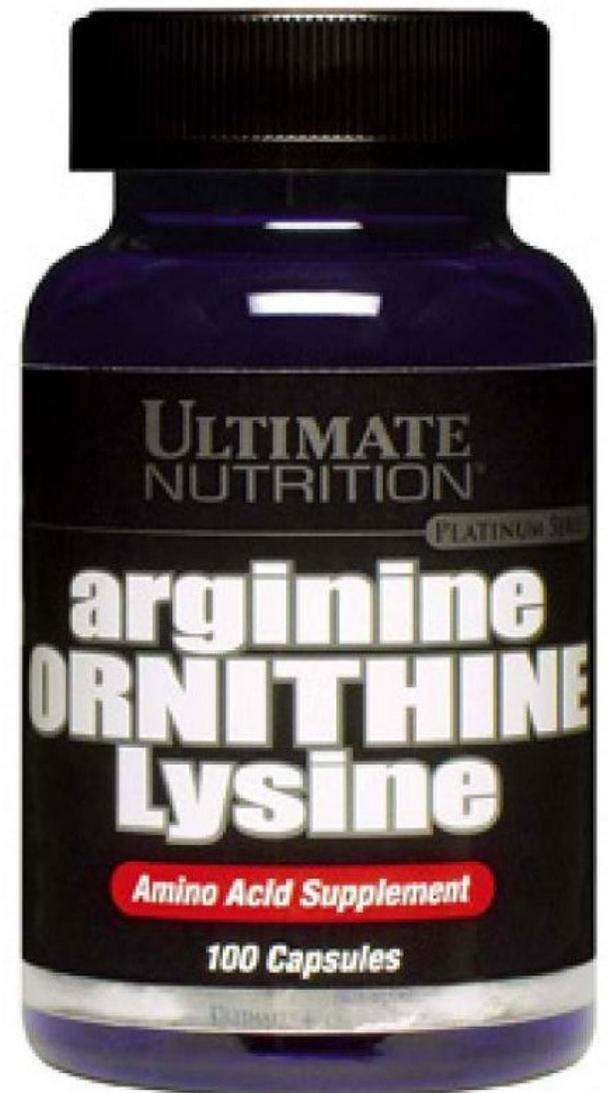
Not found in proteins:(non protein amino acids):

- Ornithine = ?????
- Citruline = ?????
- Homocysteine = Methionine Metabolism

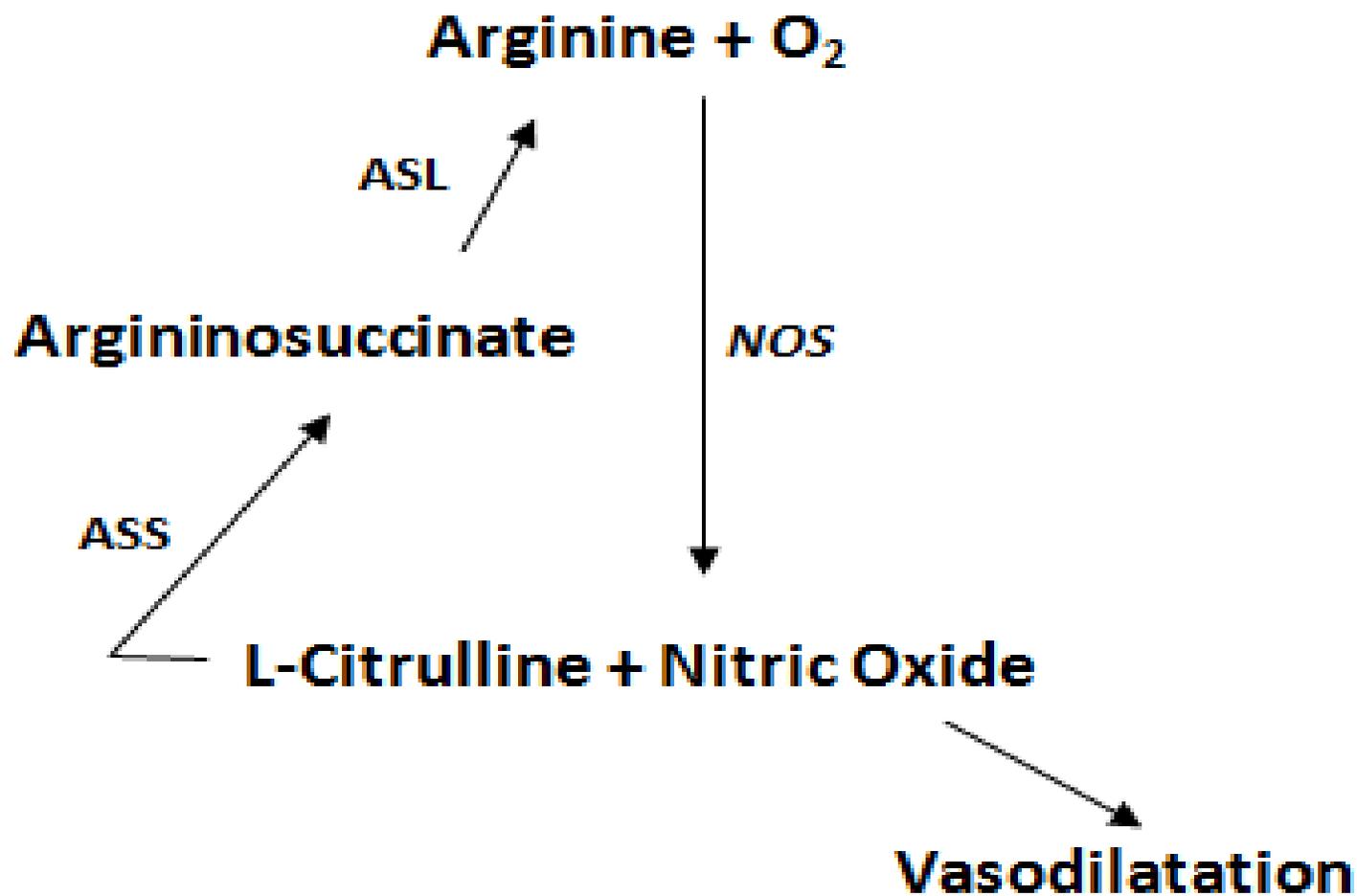




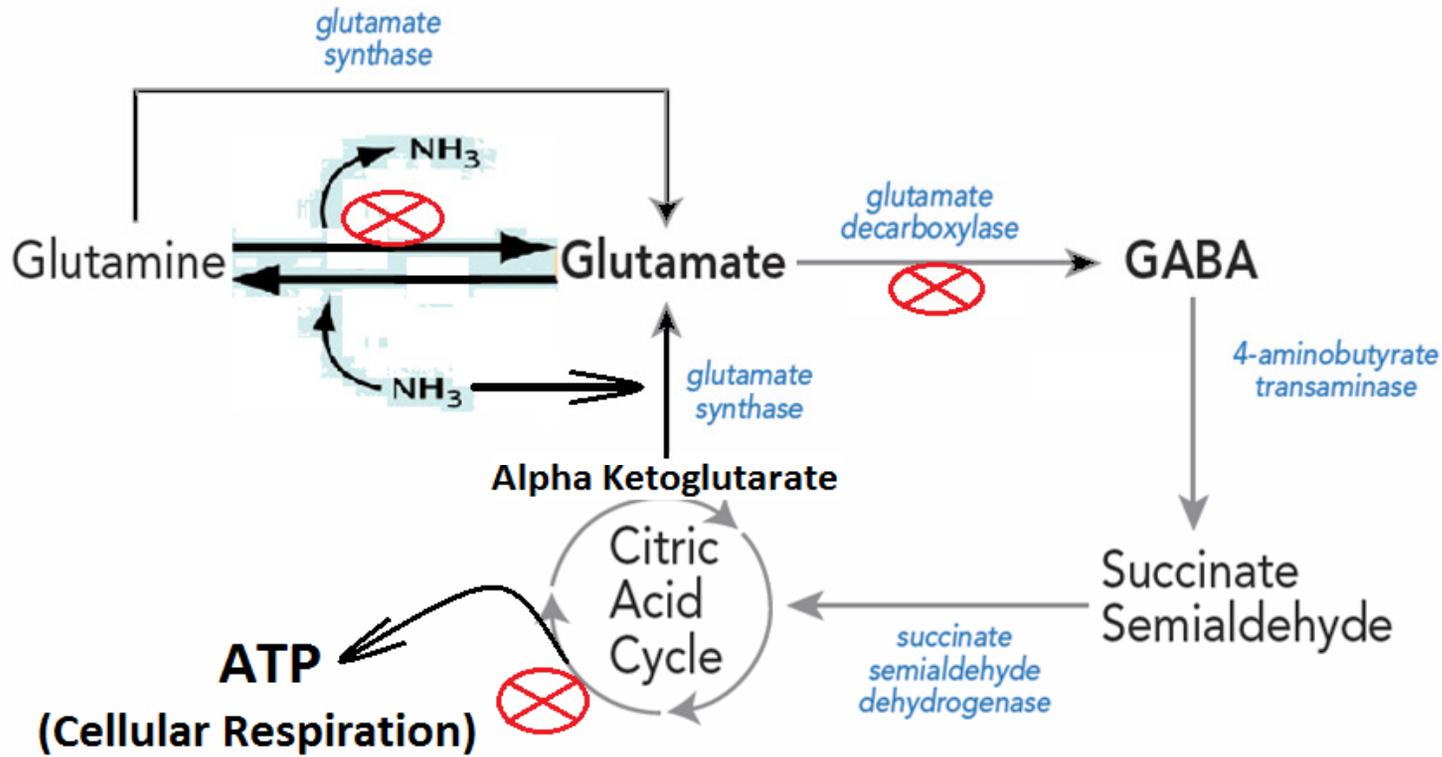
What can be use of this product?



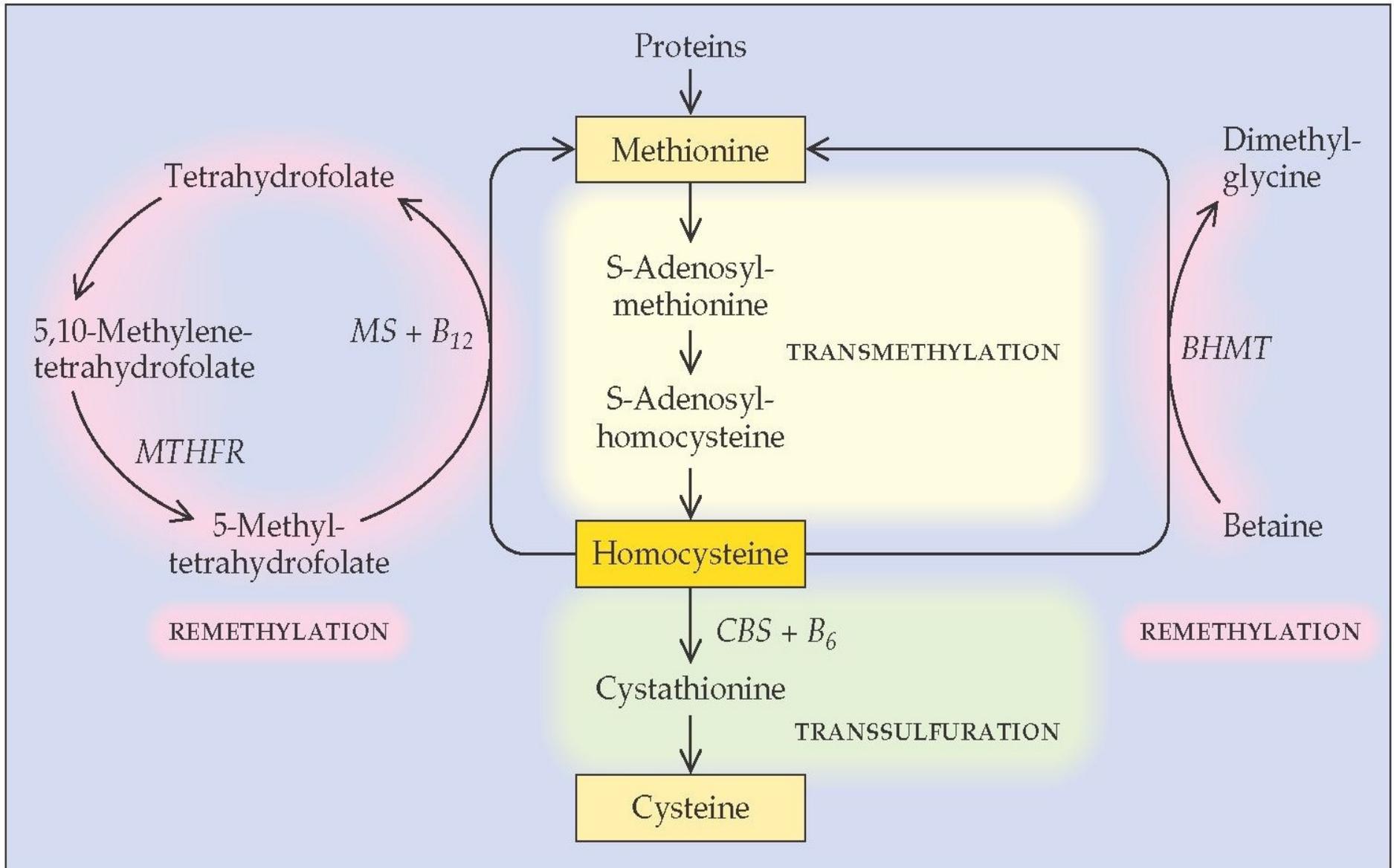
Citrulline – NO Cycle



What can be effect of high NH₃ ?



What can prevent & decrease hyper-homocysteinemia?



E. Derived amino acids:

Non –alpha amino acids:

- Gamma amino butyric acid(GABA)
- Beta alanine
 - Constituent of pantothenic acid & Co-enzyme A.

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Amino acid Classification Based on side chain (Polarity)

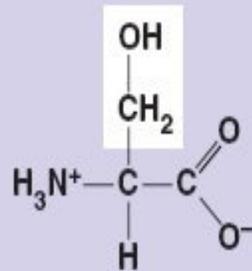
a) Non-polar side chains

b) Polar Un-charged side chain

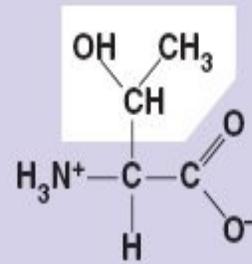
c) Polar charged side chain

- Acidic
- Basic

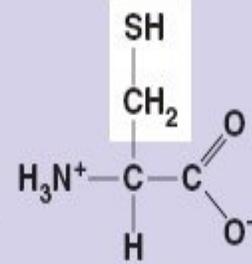
Polar



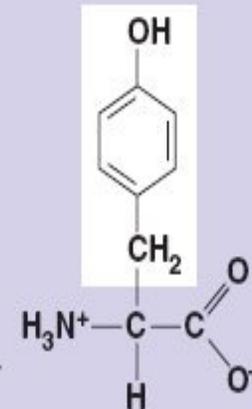
Serine (Ser)



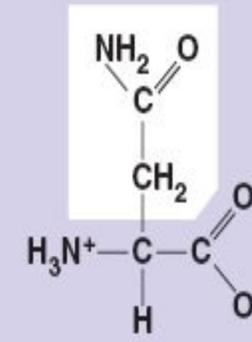
Threonine (Thr)



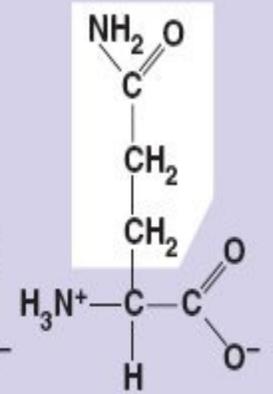
Cysteine (Cys)



Tyrosine (Tyr)



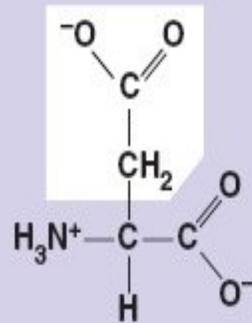
Asparagine (Asn)



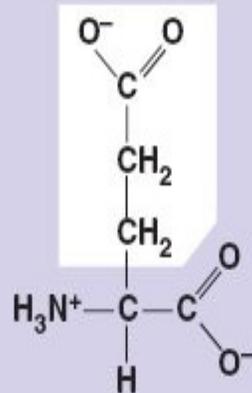
Glutamine (Gln)

Electrically charged

Acidic

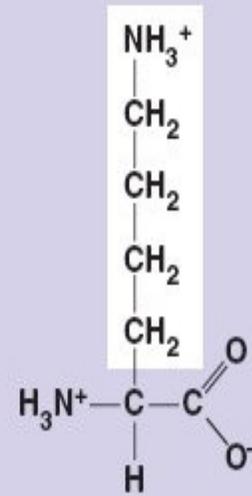


Aspartic acid (Asp)

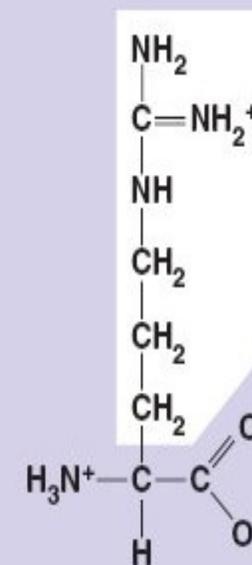


Glutamic acid (Glu)

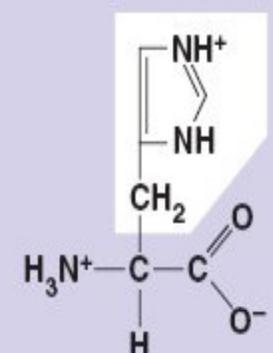
Basic



Lysine (Lys)



Arginine (Arg)



Histidine (His)

Amino acids with nonpolar

- Does not make bond
- Does not participate in hydrogen or ionic bonds
- Does not give off protons. Lipid - like a property
- Promotes hydrophobic interactions
- **In Water & In Polar environment**
 - Cluster together in the interior of the protein
 - Aggragate like droplets of oil in water.
- **In Hydrophobic environment, E.g. membrane**
 - Found on outer side of the protein
 - Interacting with the lipid environment.

Amino acids with Polar - Acidic

- Aspartic and Glutamic acid
- Proton donors
- At physiologic pH
- Fully ionized
- Negatively charged ($-\text{COO}^-$)

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Amino acids with Polar - Basic

- Lysine & Arginine
 - Accept protons
 - At physiologic pH
 - Fully ionized
 - Positively charged. (NH_3^+)
-
- In Histidine, at physiological pH
 - Free amino acid = largely uncharged
 - Into a protein, either positively charged or neutral
 - Plays important in the functioning of Hemoglobin.

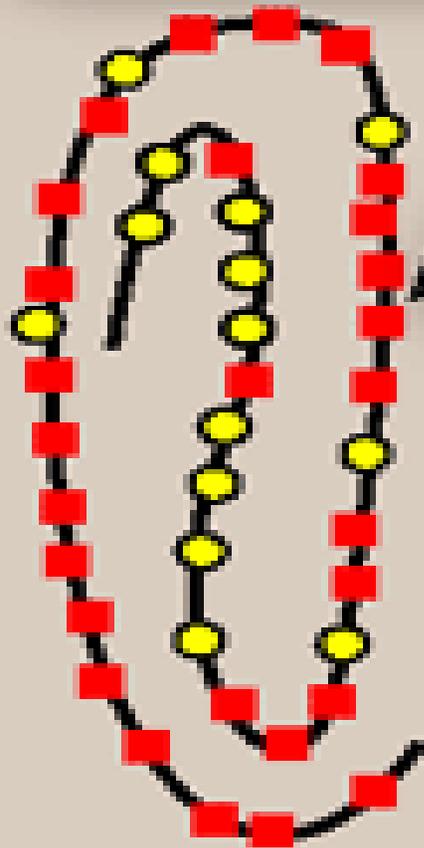
Amino acids with polar uncharged

- Zero net charge at neutral pH

Side chains of Following

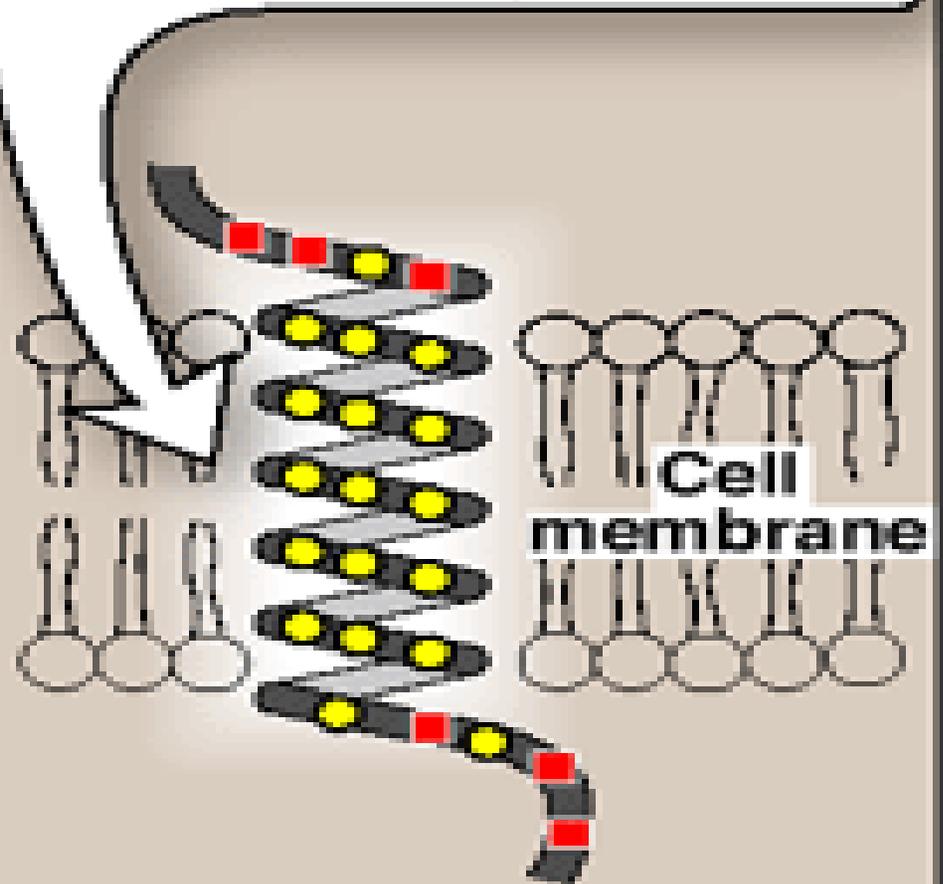
- Cysteine & Tyrosine can lose a proton at an alkaline pH.
- Serine, Threonine & Tyrosine
 - -OH group participate in hydrogen bond
- Asparagine & Glutamine
 - Carbonyl group & Amide group = participate in -OH bonds.

Polar amino acids (■) cluster on the surface of soluble proteins.

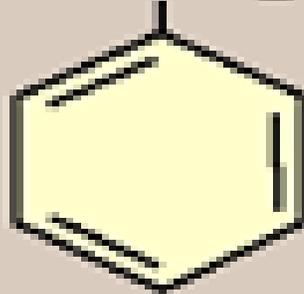
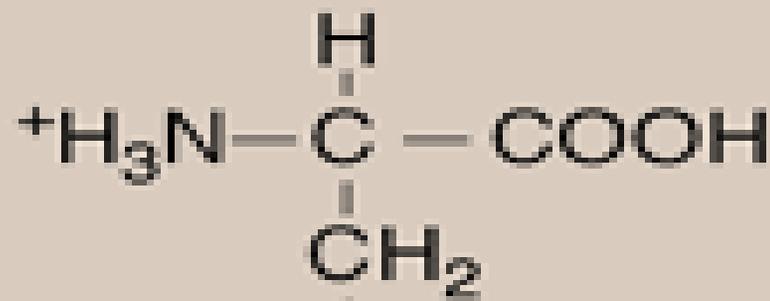


Soluble protein

Nonpolar amino acids (●) cluster on the surface of membrane proteins.



Membrane protein

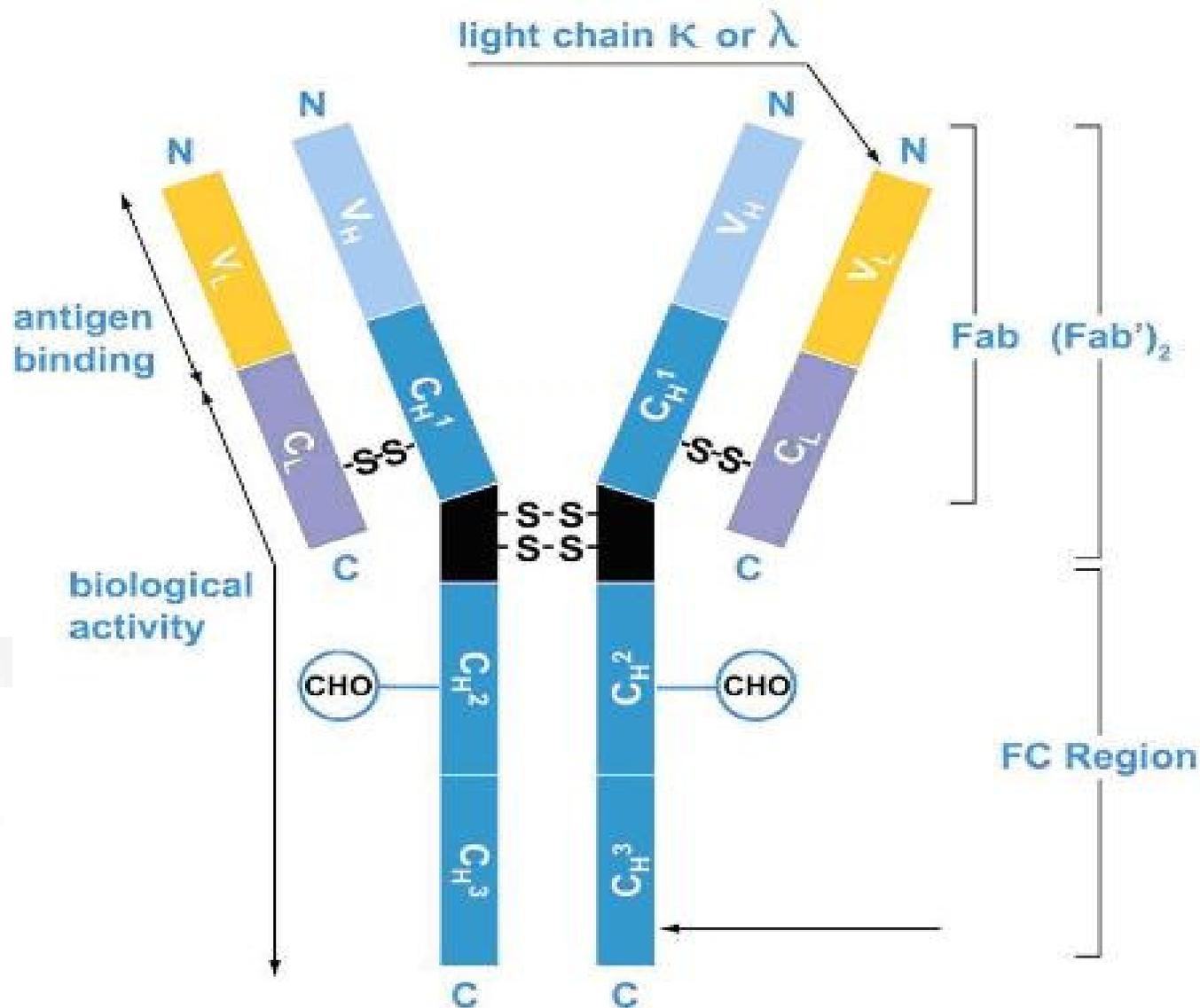


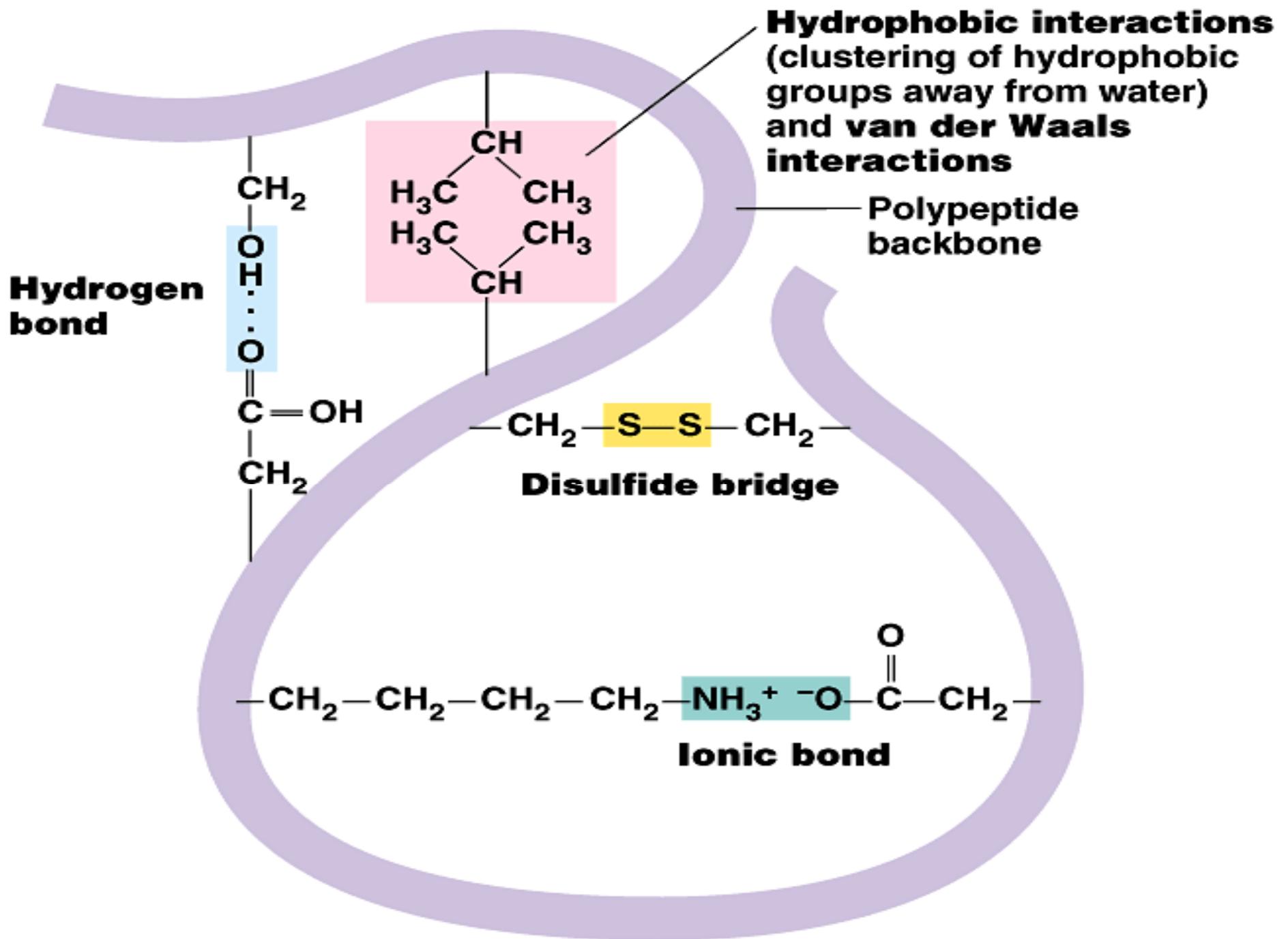
Carbonyl
group

Tyrosine

} Hydrogen
bond

What is there in protein to providing stability and strength?

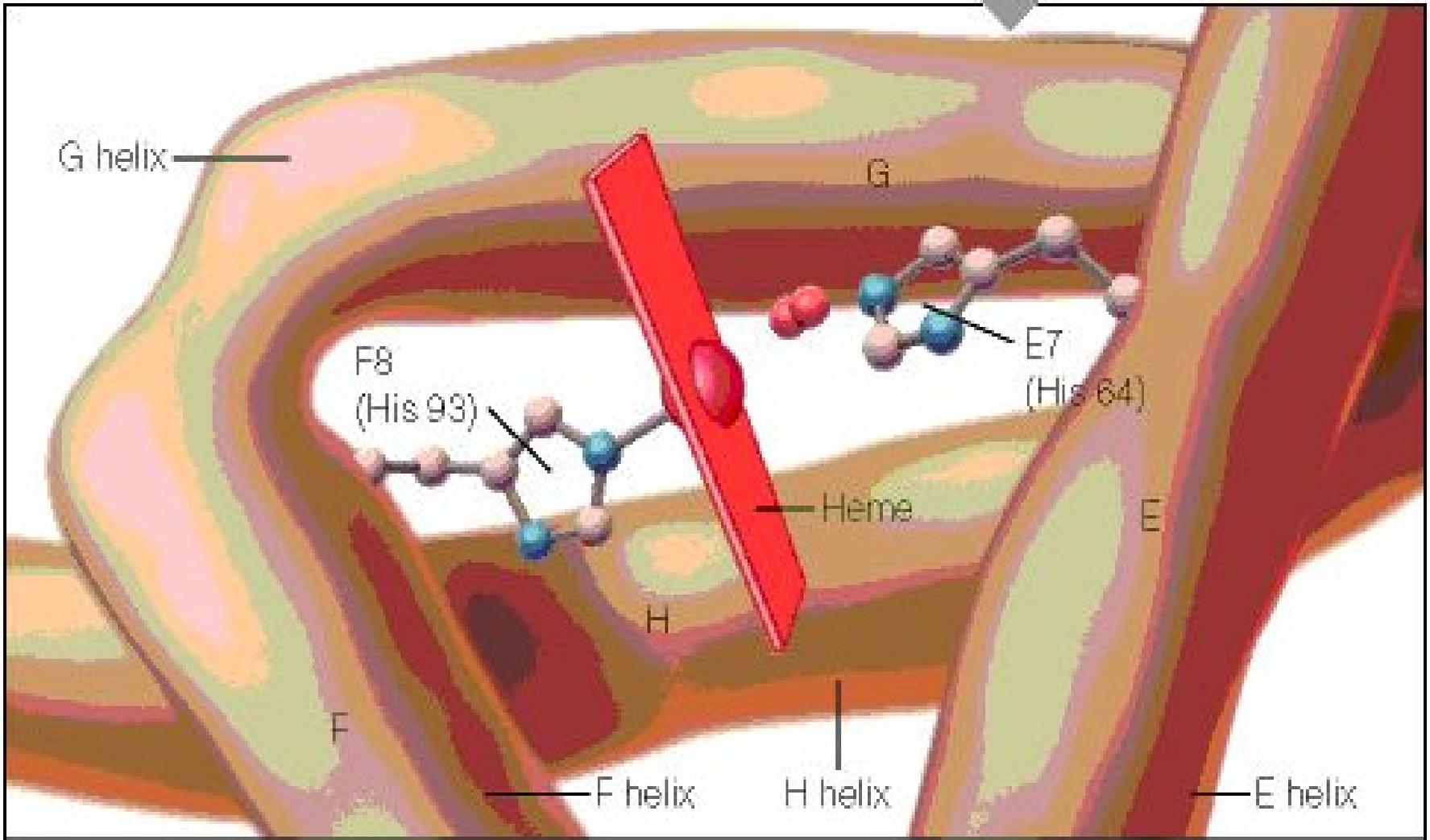


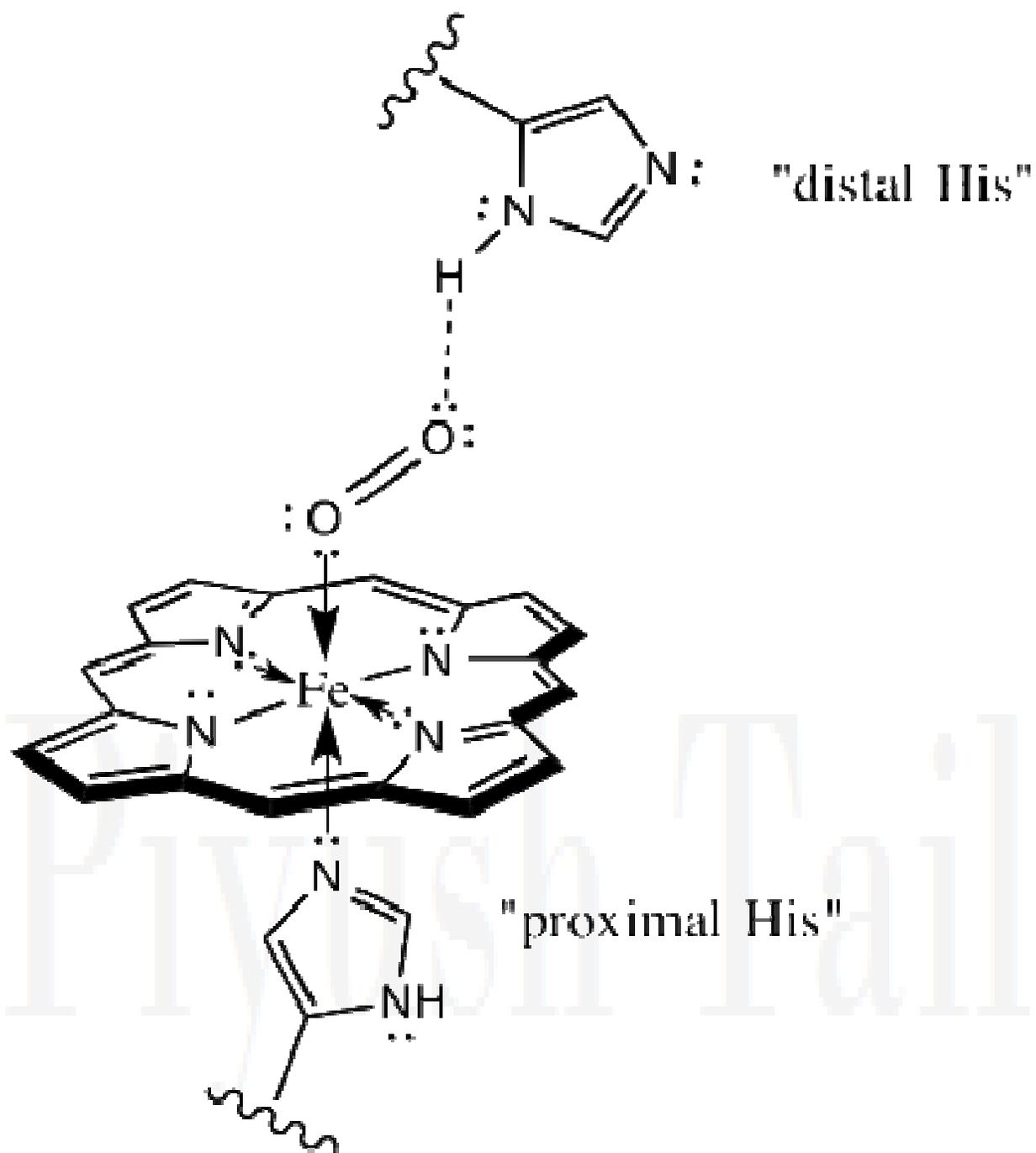


Disulfide bond

- Sulfhydryl group ($-SH$)
- component of the active site of many enzymes.
- $-SH$ groups of two cysteines = oxidized = Dimer = Cystine
- $-OH$ = can attachment with phosphate group.
- $-NH_3$ = can attach with oligosaccharide of glycoproteins.

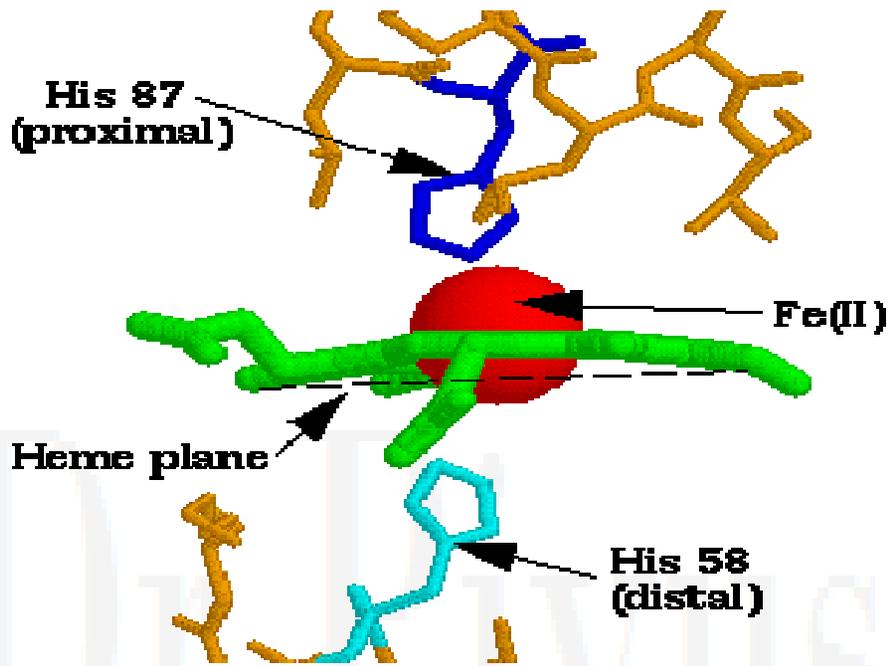
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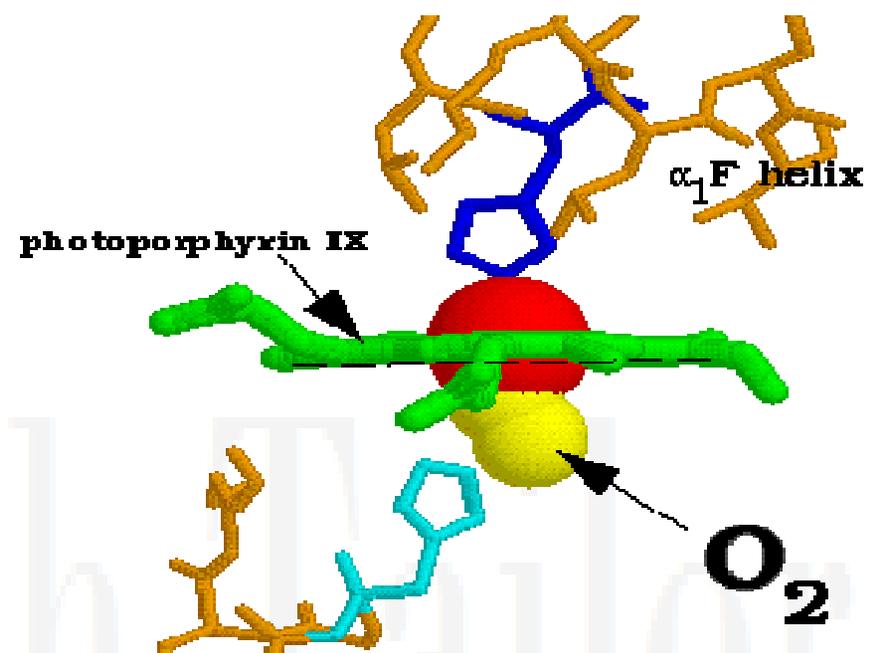


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T-state



R-state



Dr. Prayash Pathor

1 Unique first letter:

C ysteine	=	C ys	=	C
H istidine	=	H is	=	H
I soleucine	=	I le	=	I
M ethionine	=	M et	=	M
S erine	=	S er	=	S
V aline	=	V al	=	V

2 Most commonly occurring amino acids have priority:

A lanine	=	A la	=	A
G lycine	=	G ly	=	G
L eucine	=	L eu	=	L
P roline	=	P ro	=	P
T hreonine	=	T hr	=	T

3 Similar sounding names:

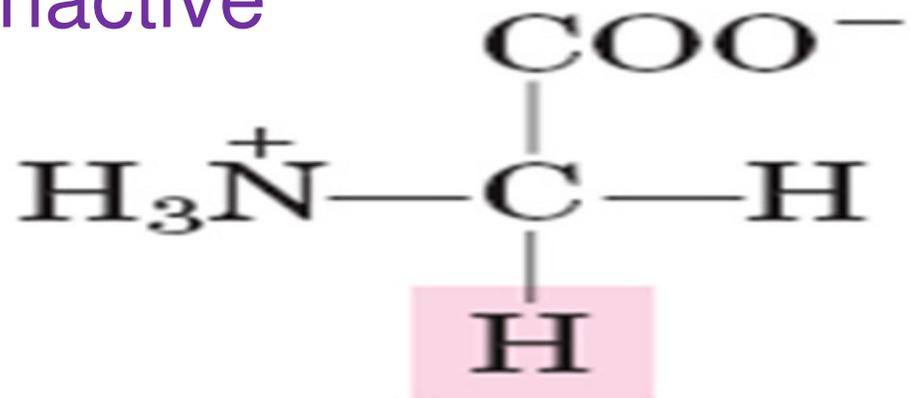
A rginine	=	A rg	=	R	("a R ginine")
A sparagine	=	A sn	=	N	(contains N)
A spartate	=	A sp	=	D	("aspar D ic")
G lutamate	=	G lu	=	E	("glut E mate")
G lutamine	=	G ln	=	Q	("Q-tamine")
P henylalanine	=	P he	=	F	("Fenylalanine")
T yrosine	=	T yr	=	Y	("t Y rosine")
T ryptophan	=	T rp	=	W	(double ring in the molecule)

4 Letter close to initial letter:

Aspartate or asparagine	=	A sx	=	B	(near A)
Glutamate or glutamine	=	G lx	=	Z	
L ysine	=	L ys	=	K	(near L)
Undetermined amino acid	=		=	X	

Optical properties of amino acids

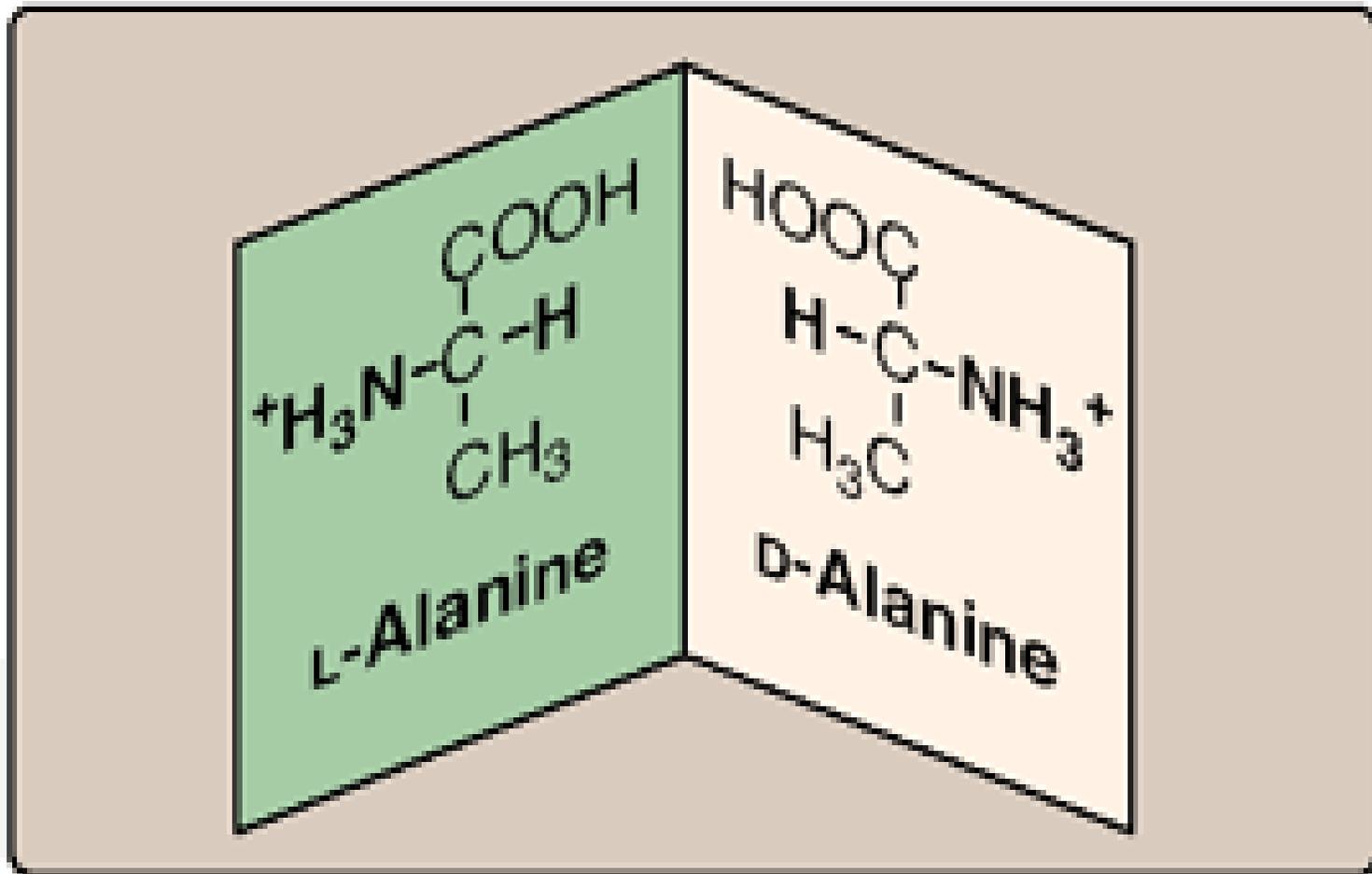
- α -carbon = Four different group
- D and L form
- Stereoisomers,
- Glycine is the exception
- “Glycine is optically inactive”



Glycine

Dr Piyu

Stereoisomer



➤ D-amino acids

- Micro-organisms
- Constituents of certain antibiotics like Actinomycin-D, Polymyxin & Valinomycin
- Bacterial cell wall peptidoglycans.

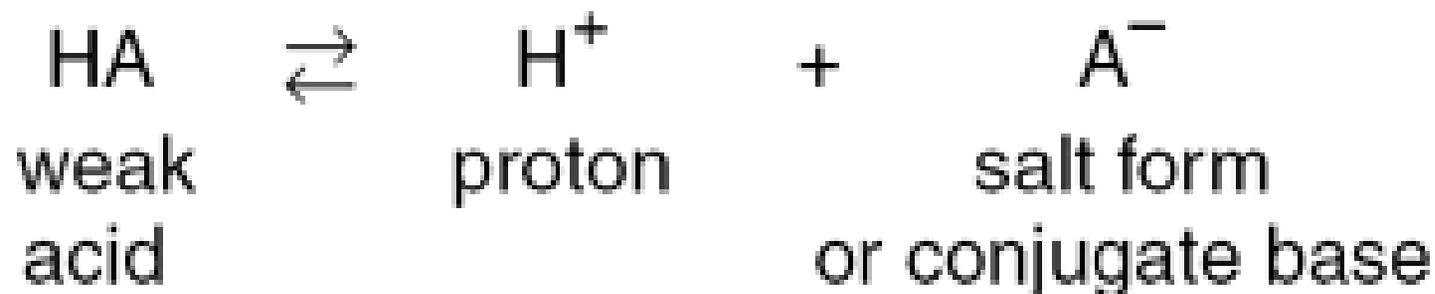
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Acidic and Basic Properties of Amino Acids

- Amino acids in aqueous solution
 - Weakly acidic α -carboxyl groups
 - Weakly basic α -amino groups.
 - Ionizable group in its side chain.
- Can act as buffers.
- $\text{pH} = -\log 1/[\text{H}^+]$
- $\text{pH} = -\log [\text{H}^+]$

Henderson-Hasselbalch equation

- Relationship between
 - pH of the solution
 - Conc. of a weak acid (HA) and its conjugate base (A⁻)



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

Henderson-Hasselbalch equation

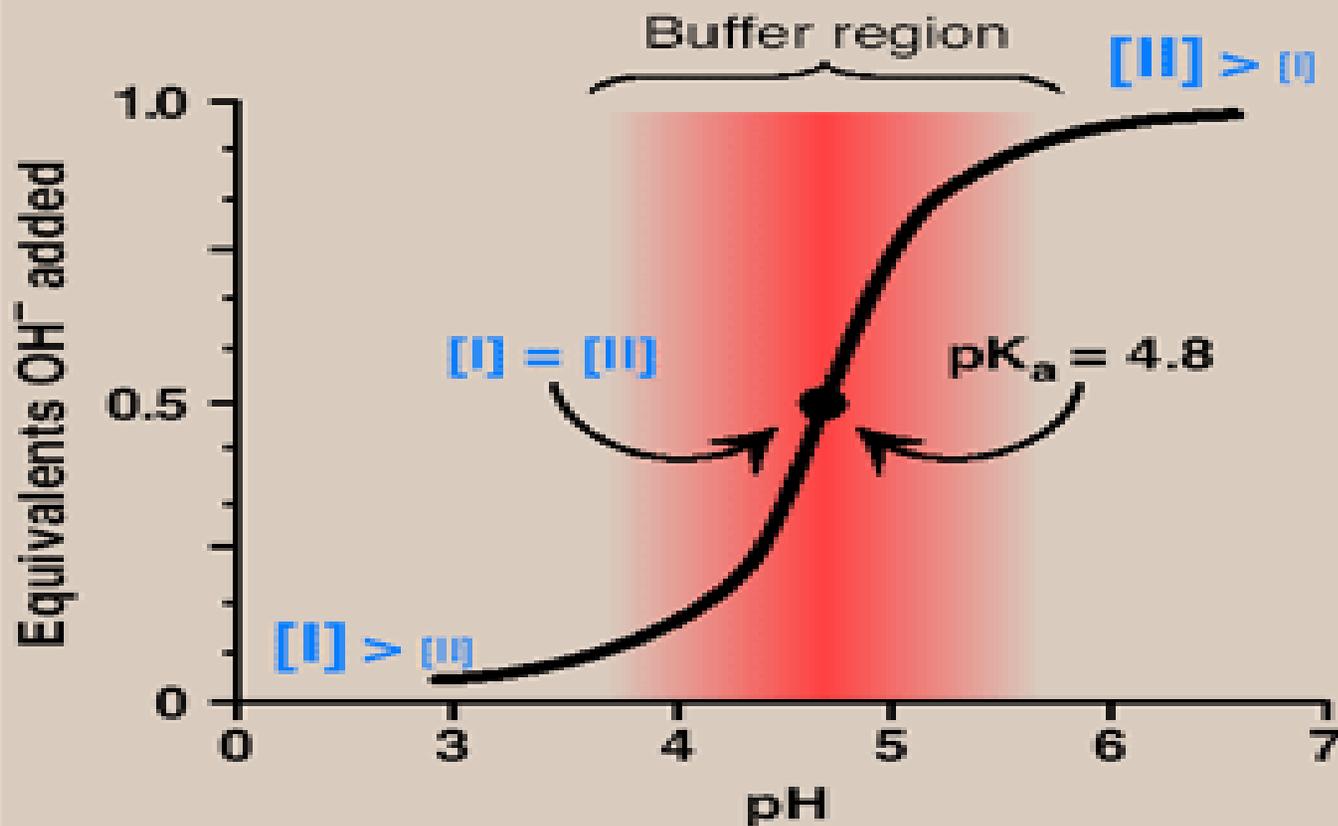
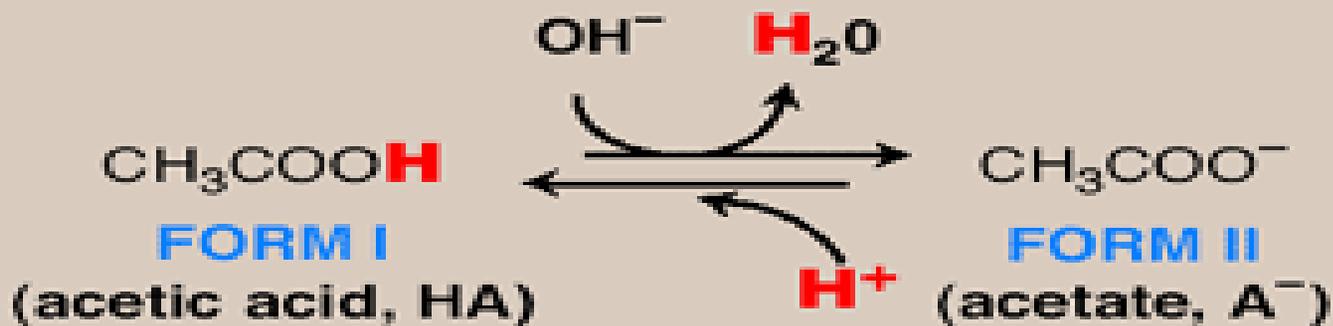
$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

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

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

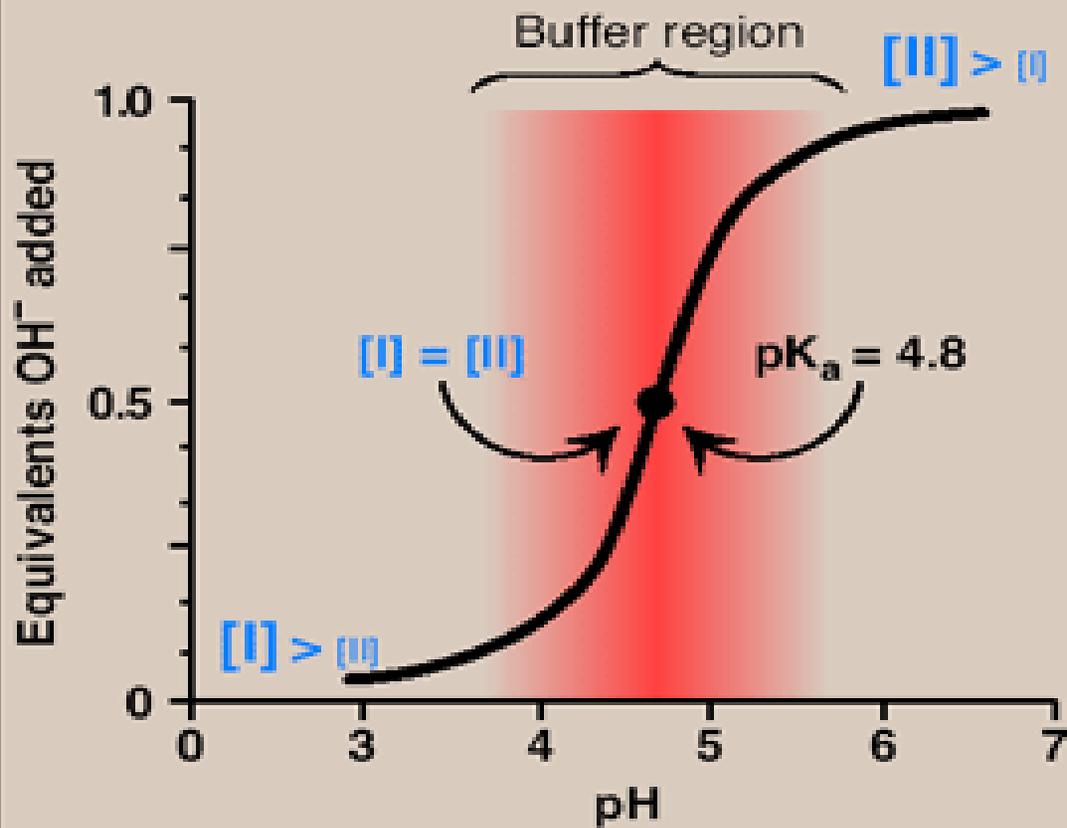
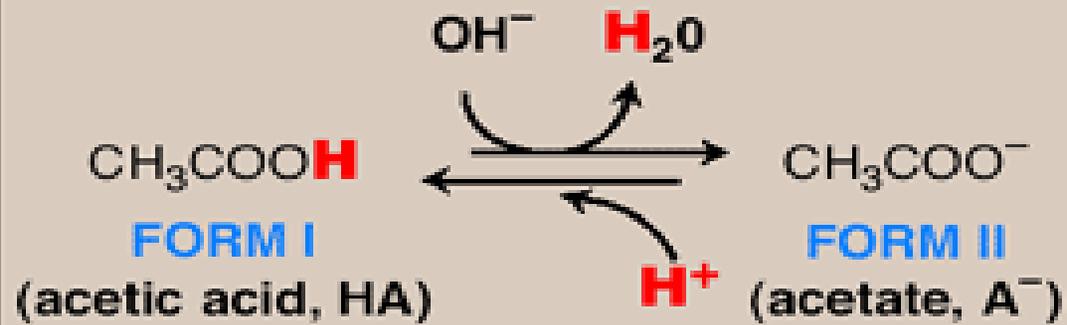
We then use the definitions of pH and pK_a :

$$\text{pH} = pK_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

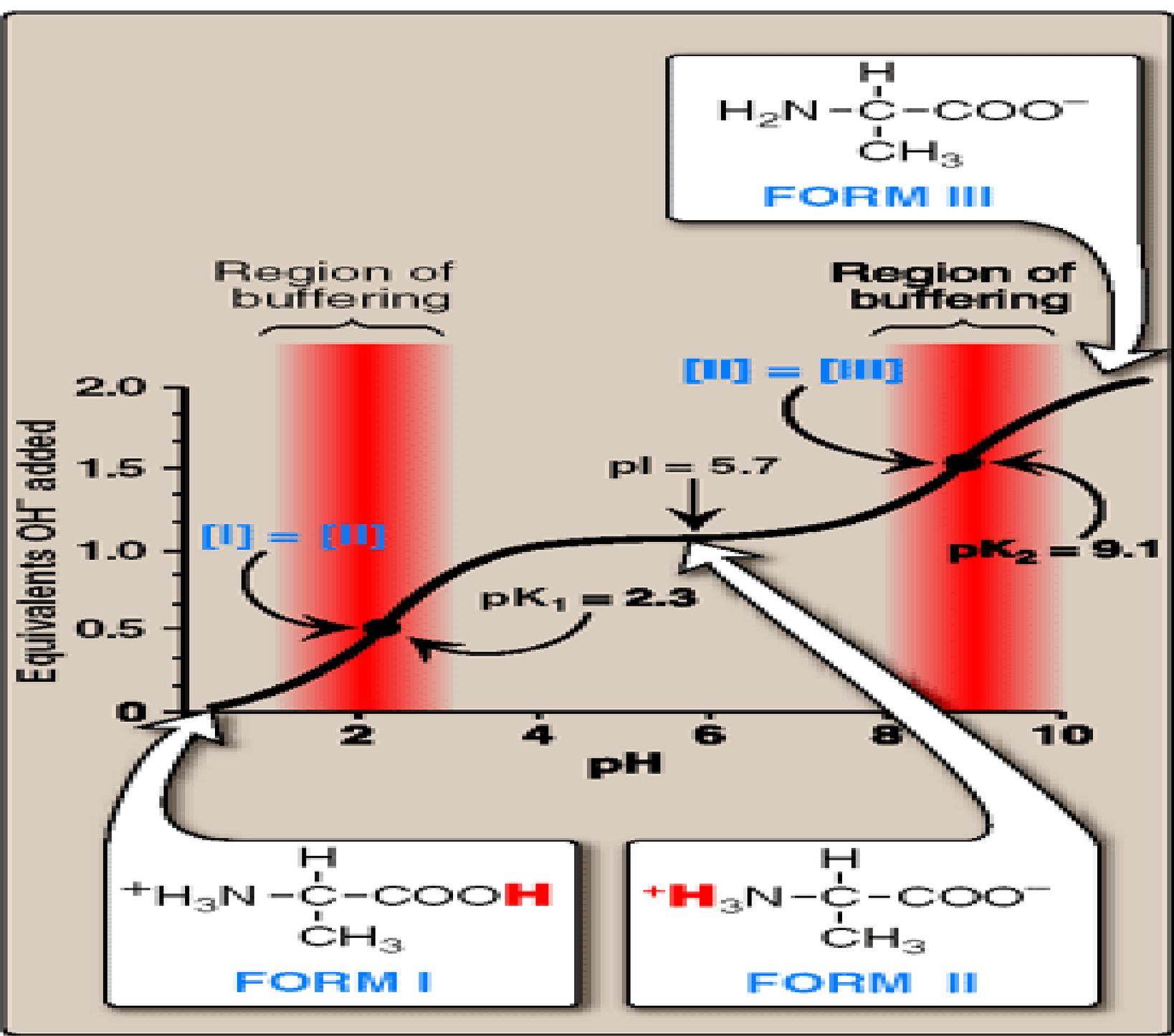


Buffers

- Resists change in pH following the addition of an acid or base.
- Mixing of weak acid (HA) & its conjugate base (A⁻).
- When **HCl** + **A⁻** = **HA**.
- When **HCO₃⁻** + **HA** = H₂CO₃ + **A⁻**.
- **[HA] = [A⁻]**, at that pH = pK_a
- **So, Maximum buffering at a pH = pK_a,**
- But still serve as an effective in ±1 pH of pK_a.
- .



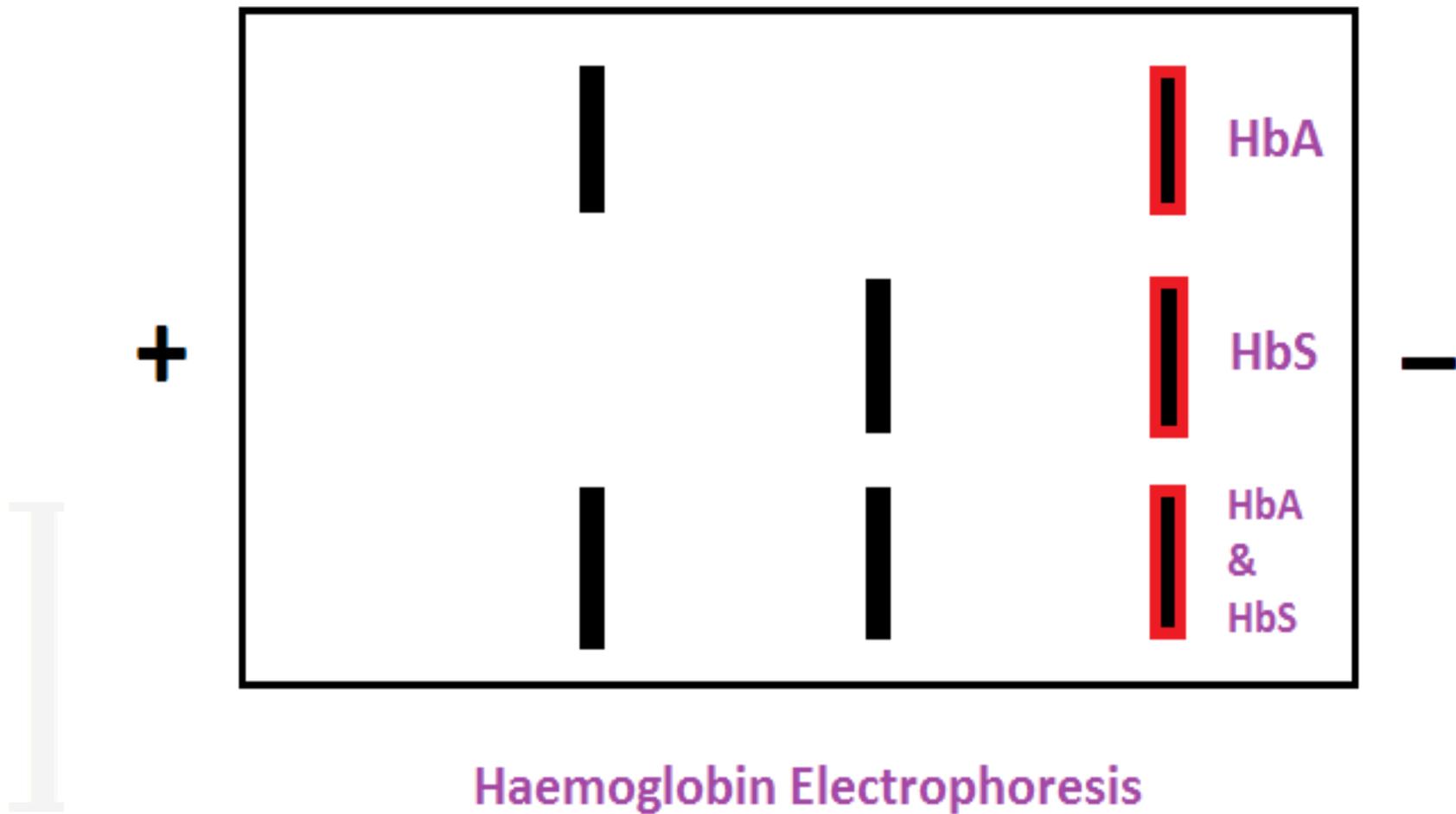
- (HA) = $\text{CH}_3\text{-COOH}$
- (A^-) = $\text{CH}_3\text{-COO}^-$
- pK_a of this 4.8
- Maximum buffering at pH 4.8.
- Buffer capacity between pH 3.8 to 5.8
- At $\text{pH} < \text{pK}_a$
 - Protonated acid form ($\text{CH}_3\text{-COOH}$)
- At $\text{pH} > \text{pK}_a$
 - Deprotonated base form ($\text{CH}_3\text{-COO}^-$)



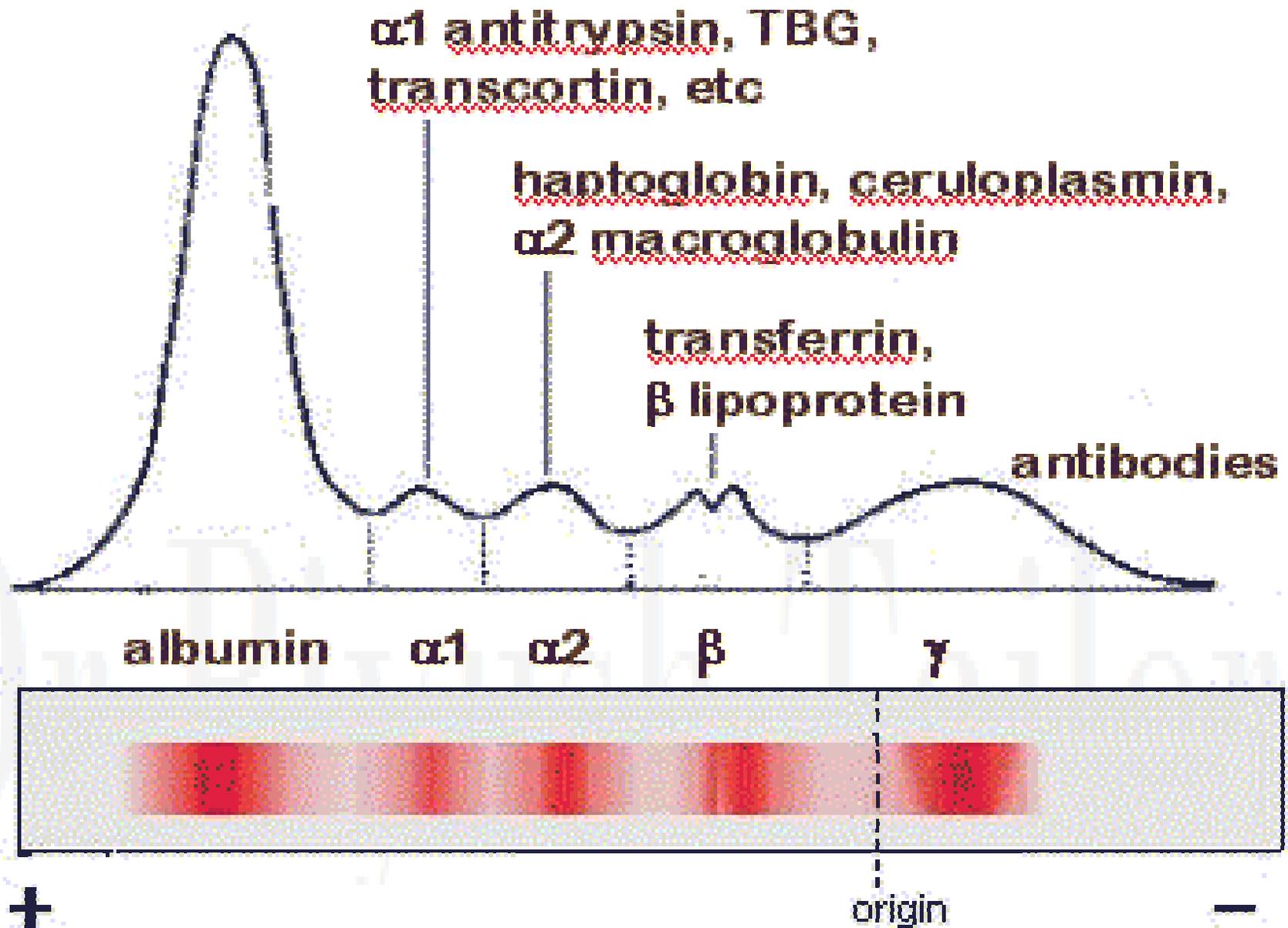
What will be effect of charges, on movement of protein ,in electrical field?

- Separation of plasma proteins is done by it's charges.
- Separation is easier at $\text{pH} > \text{pI}$.
- Thus, the charge on the proteins is negative.

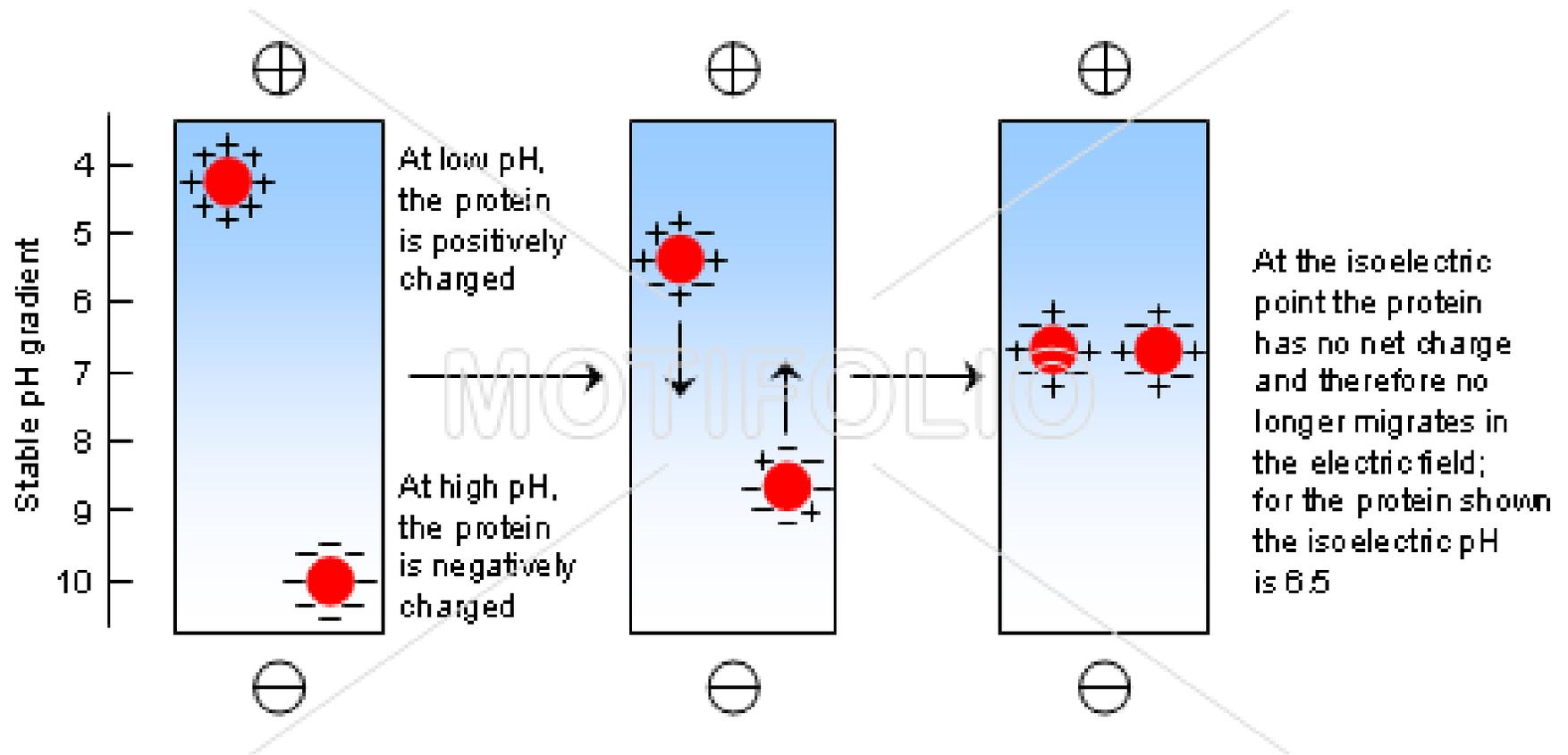
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Plasma Protein

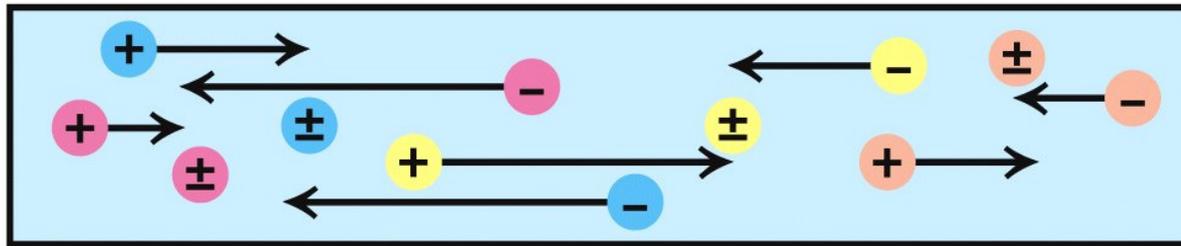


Separation of protein molecules by isoelectric focusing



(A)

Low pH
(+)



High pH
(-)

(B)

Low pH
(+)



High pH
(-)

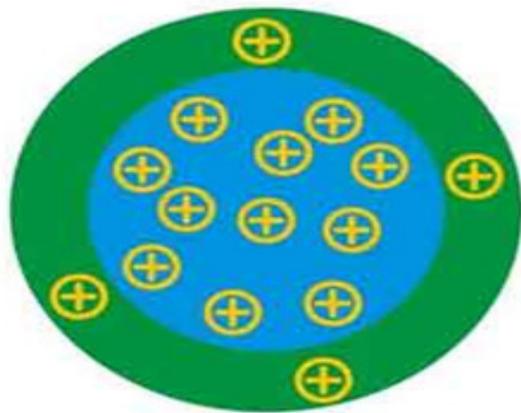
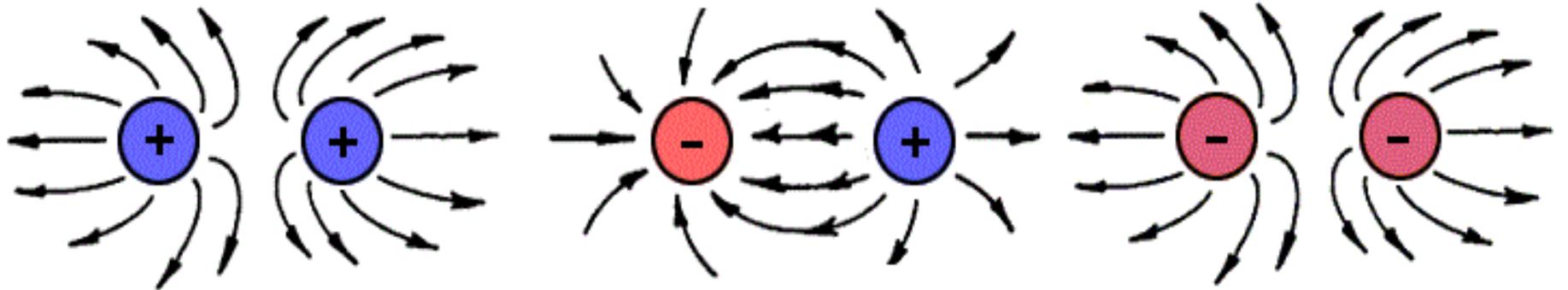
Figure 3.11

Biochemistry, Seventh Edition

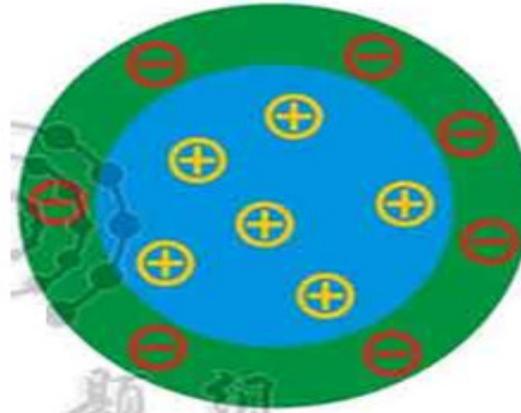
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At What pH

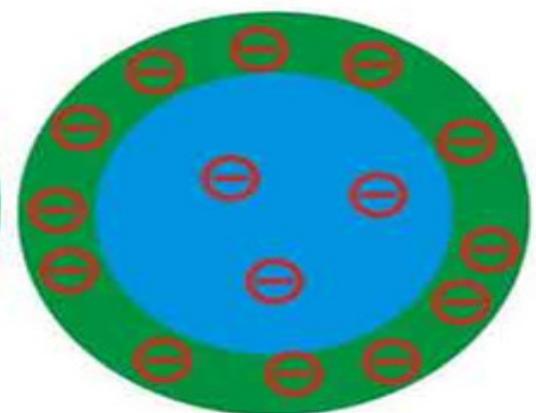
This ion molecule has least solubility?



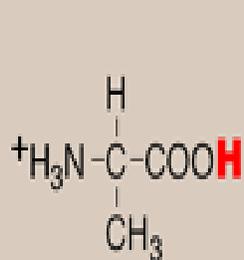
pH < 5



pH = 7



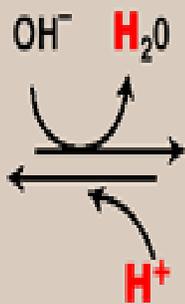
pH > 10



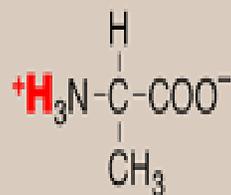
FORM I

Alanine in acid solution
(pH less than 2)

Net charge = +1



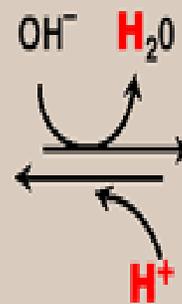
pK₁ = 2.3



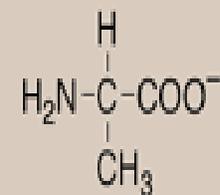
FORM II

Alanine in neutral solution
(pH approximately 6)

Net charge = 0
(isoelectric form)



pK₂ = 9.1



FORM III

Alanine in basic solution
(pH greater than 10)

Net charge = -1

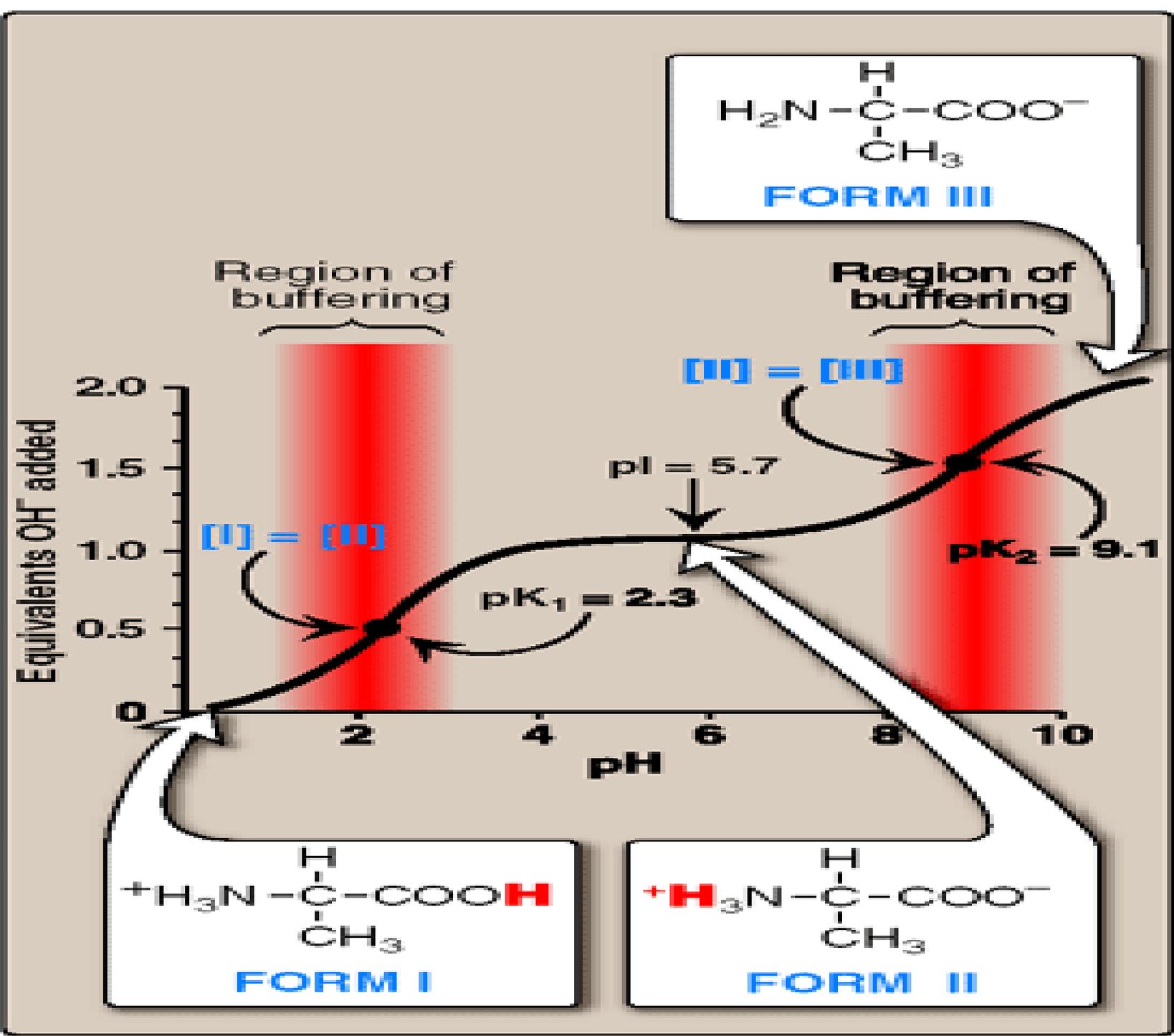
Zwitterion

- E.g. In Alanine,
- At a **low (acidic)** pH,
 - groups are **positive charged** (NH_3^+).
- At a **high (basic)** pH,
 - groups are **negative charged** (COO^-).
- While at one pH = it is dipolar.
- Overall charge is zero.
- pH = pI (Iso-electric pH)

- At physiologic pH, All amino acids have a
 - Negatively charged group ($-\text{COO}^-$)
 - Positively charged group ($-\text{NH}_3^+$)

Ampholyte Amino Acid

- Glutamate, Aspartate, Histidine, Arginine, and Lysine have additional potentially charged groups in their side chains.
- Act either as an **Acid** as well as **Base**



Application of Henderson-Hasselbalch equation

$$K_1 = \frac{[H^+][III]}{[II]}$$

$$pH = pK_1 + \log \frac{[III]}{[II]}$$

Buffer pairs:

- **COO⁻ / COOH** pair can serve as a buffer at around pK₁
- **NH₃⁺ / -NH₂** pair can serve as buffer at around pK₂.

- **Bicarbonate Buffer**

- **HCO₃⁻ / H₂CO₃** = (Strong base / Weak acid)

- **When the pH is equal to pK₁ (2.3),**

- Equal amounts of Forms I and II of alanine.

- **When the pH is equal to pK₂ (9.1),**

- Equal amounts of Forms II and III of alanine.

Henderson & Hesselbatch In Bicarbonate (Blood) Buffer

$$pH = pK_a \text{ H}_2\text{CO}_3 + \log \left(\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \right)$$

$$pH = pK + \log \frac{[\text{HCO}_3^-]}{[\text{PCO}_2 \times 0.03]}$$

$$= 6.1 + \log \frac{24 \text{ mEq/L}}{(40 \times 0.03)}$$

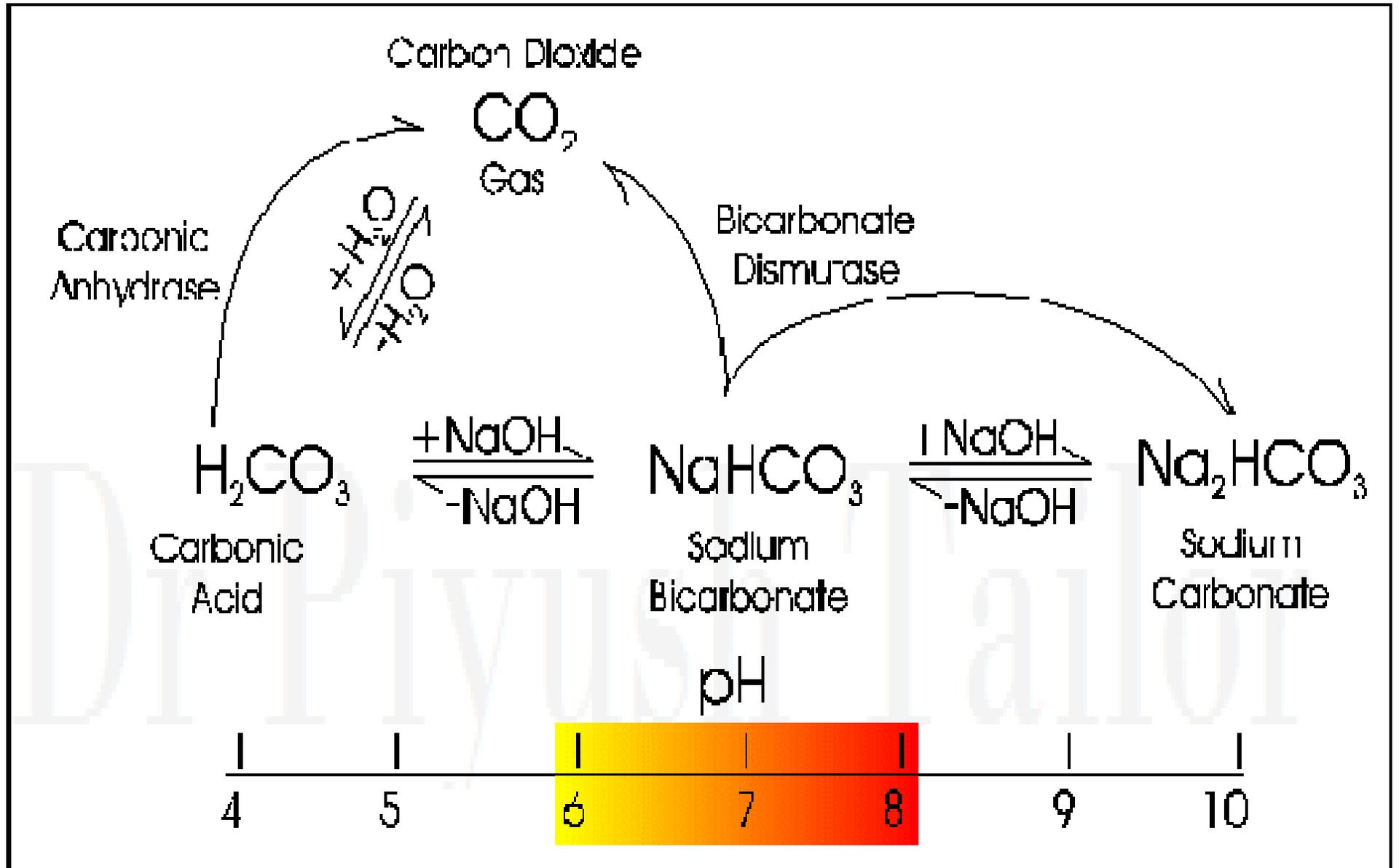
$$= 6.1 + \log \frac{24 \text{ mEq/L}}{(1.2 \text{ mEq/L})}$$

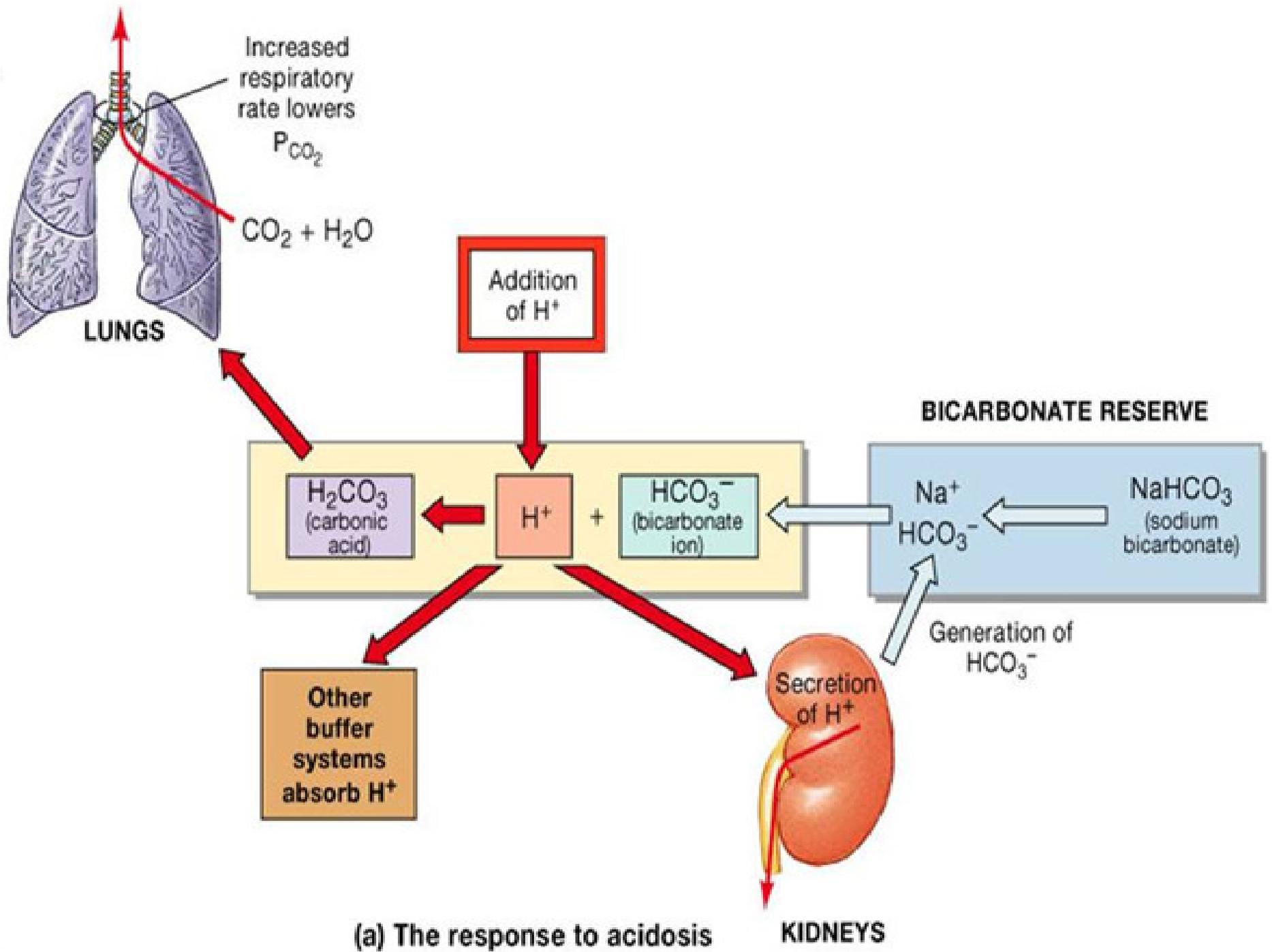
$$= 6.1 + \log \frac{20}{1} \quad (20:1 \text{ ratio})$$

$$= 6.1 + 1.3$$

$$= 7.4$$

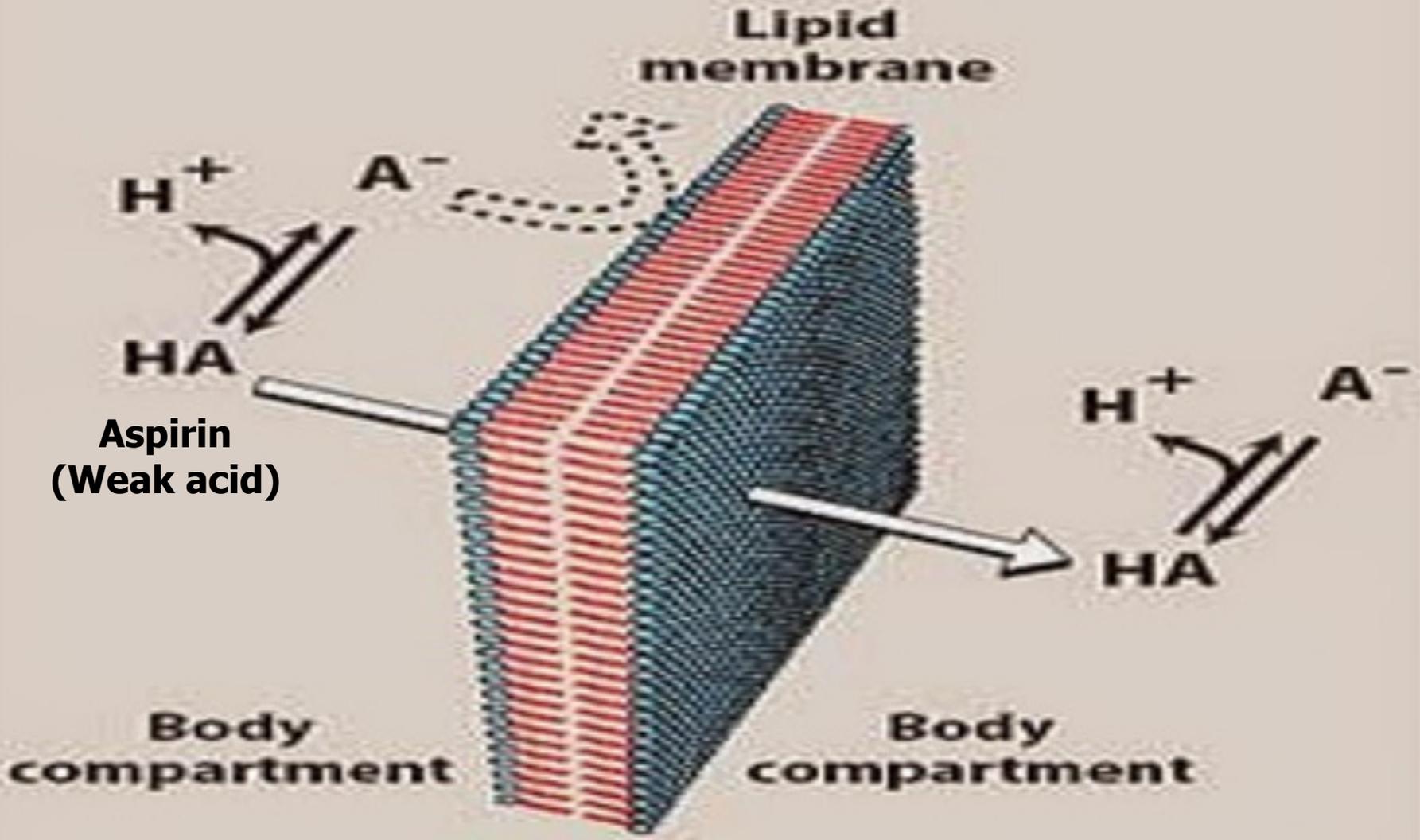
How is it work?



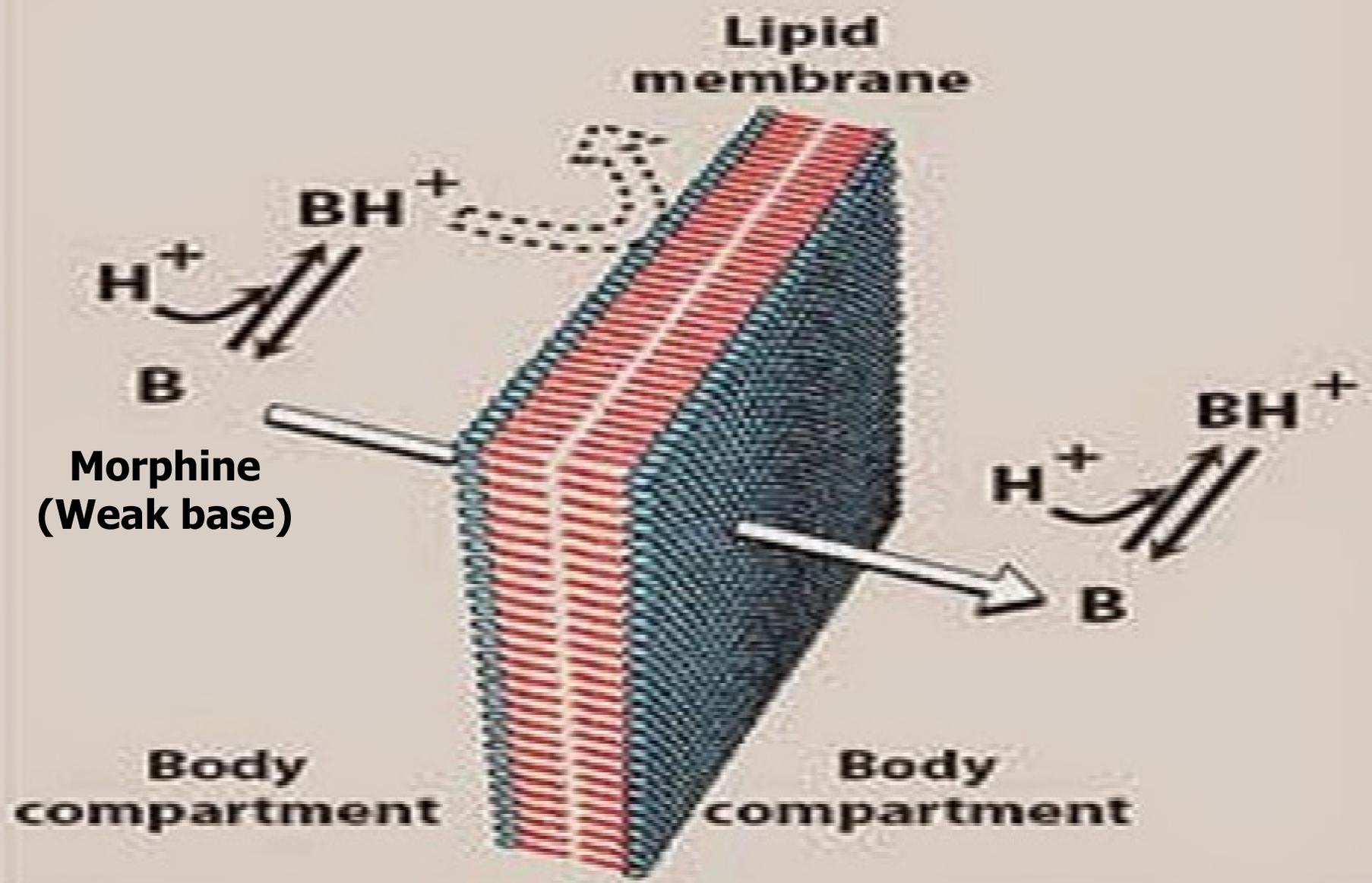


- ✓ **Most drugs are either weak acids or weak bases**
- ✓ **Drug passes through membranes if it is uncharged.**
- ✓ **Absorption of drug depend on**
 - ✓ **Ratio of conc. Of charged and uncharged forms.**
- ✓ **Ratio depend on**
 - ✓ **pH at the site of absorption**
 - ✓ **Strength of the weak acid or base,**
 - ✓ **pK_a of ionizable group of drug.**
- ✓ **Henderson-Hasselbalch equation useful**
 - ✓ **How much will be drug ratio (charge: uncharge drug) at that differ in pH.**
 - ✓ **For example, Stomach (pH 1.0–1.5) & blood (pH 7.4)**

A Weak acid



B Weak base



A

BICARBONATE AS A BUFFER

- $\text{pH} = \text{pK} + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$
- An increase in HCO_3^- causes the pH to rise.
- Pulmonary obstruction causes an increase in carbon dioxide and causes the pH to fall, resulting in respiratory acidosis.



LUNG
ALVEOLI



3. Based on metabolism fate:

Purely ketogenic:

- Leucine is purely ketogenic. it is converted to ketone bodies.

ketogenic and glucogenic:

- Lysine, isoleucine, phenylalanine, tyrosine and tryptophan are partially ketogenic and partially glucogenic.

Purely glucogenic:

- All the remaining 14 amino acids are purely glucogenic as they enter only into the glucogenic pathway.

4. Based on nutritional requirements:

Essential or indispensable:

- Isoleucine
- Leucine
- Lysine
- Tryptophan
- Threonine
- Phenylalanine
- Methionine and
- Valine are essential amino acids.
- Their carbon skeleton cannot be synthesized by human beings and so preformed amino acids are to be taken in food for normal growth.

Semi-essential:

- Histidine and Arginine
- Growing children require them in food. But they are not essential for the adult requirement.

Non-essential or Dispensable:

- The remaining 10 amino acids are non-essential.
- Their carbon skeleton can be synthesized by the body.

Properties of amino acids

➤ Sweet in taste

- Glycine
- Alanine
- Valine
- Serine
- Tryptophan
- Histidine
- Proline

➤ Leucine is tasteless

➤ Bitter

- Isoleucine
- Arginine

➤ Flavouring agent

- Sodium glutamate

➤ Artificial sweetener

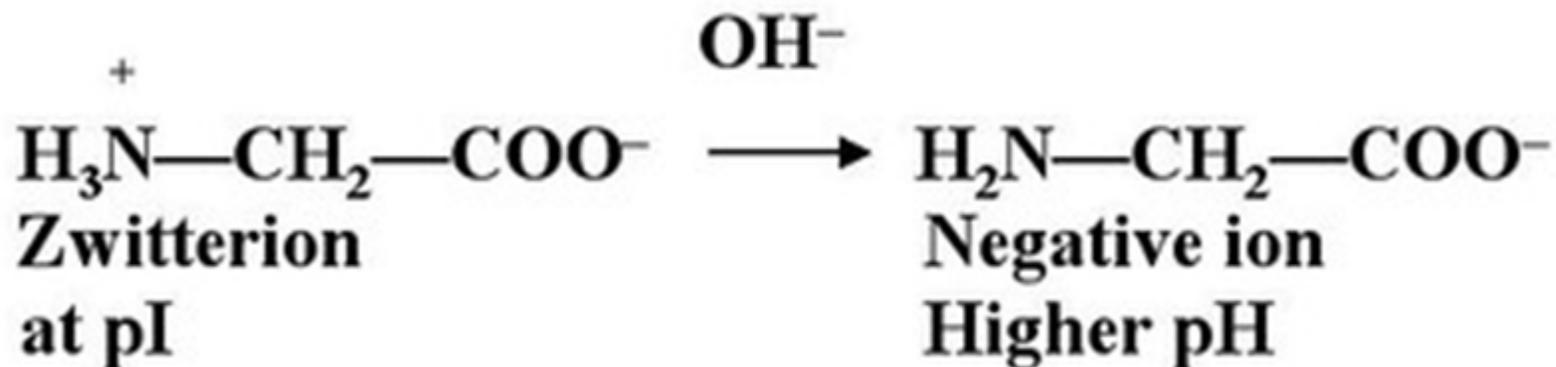
- Aspartame
(aspartic acid & phenylalanine)

Ampholyte and Iso-electric point Zwitterion

- Amino acids can exist as ampholytes or zwitterions in solution, depending on the pH of the medium.
- The pH at which the molecule carries no net charge is known as iso-electric point or iso-electric pH (pI).
- In acidic solution = Cationic in form
- In alkaline solution = Anions in form.

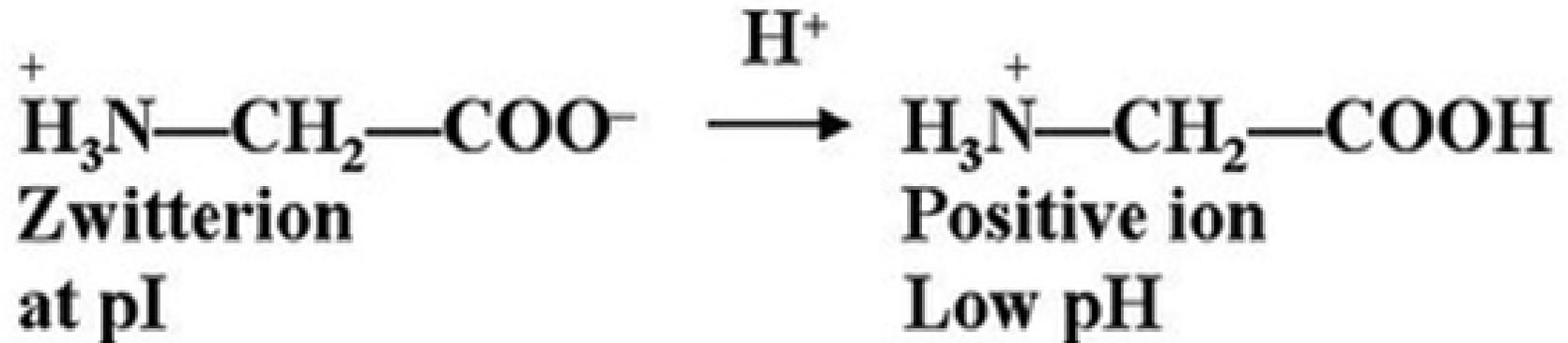
Amino acid in Basic solution

- Amino acid donate H^+ to solution
- So A.A. remains negative charged in basic solution



Amino acid in Acidic solution

Amino acid accept H^+ from solution
So A.A. remains positive charged in basic solution



At iso-electric pH the amino acid

- ✓ **All groups are ionized**
- ✓ **But net charge is “Zero”**

So at iso-electric pH, there is

- ✓ **Least mobility in an electrical field.**
- ✓ **Least Solubility**
- ✓ **Minimum buffering capacity**

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- To such a solution if we add HCl drop by drop, at a particular pH,
 - 50% molecules are in cation form
 - 50% in zwitterion form.
- This pH = pK1 (with regard to COOH).
- If more HCL is added,
 - more molecules become cationic

- If titrate solution with NaOH, molecules acquire the anionic form.
 - 50% of molecules are anions
 - 50% in zwitterion form
- This pH = pK2 (with regard to NH2).

- The iso-electric pH (pI) for mono amino mono carboxylic amino acids can be calculated as :

$$pI = \frac{pK1 + pK2}{2}$$

- Buffering action is maximum in and around pK1 or at pK2
- Buffering minimum at pI.
- In the case of amino acids having more than two ionizable groups, correspondingly there will be more pK values.

- At physiological pH of 7.4, both carboxyl and amino groups of amino acids are completely ionized.
- The pK value of imidazolium group of Histidine is 6.1, and so effective as a buffer at the physiological pH of 7.4.
- The buffering capacity of plasma proteins and hemoglobin is mainly due to histidine residue.

Dr Piyush Tailor

Decarboxylation:

Histidine → Histamine + CO₂

Tyrosine → Tyramine + CO₂

Tryptophan → Tryptamine + CO₂

Amide Formation:

Aspartic acid + NH₃ → Asparagine

Glutamic acid + NH₃ → Glutamine

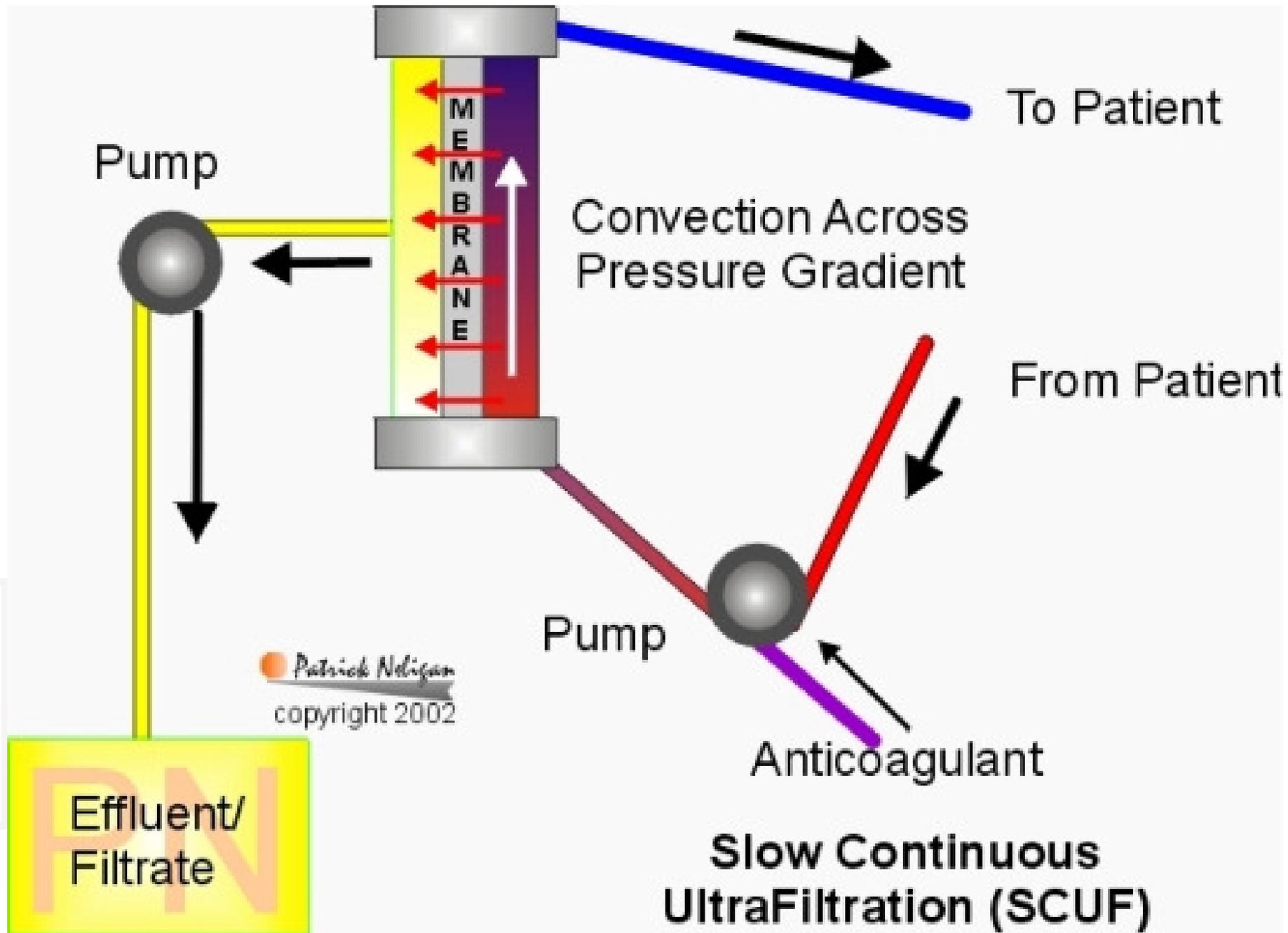
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Story continue.....

Cycloserine = anti-tuberculous drug.

Azaserine = anticancer drug.

Dr Piyush Tailor
.....Never End



- Pellagra can occur in Carcinoid syndrome.
- Vitamin C deficiency causes Scurvy.
- Increase ammonia causes brain toxicity.
- Aspartate & Ornithine is used in hepatotoxicity
- Arginine & Ornithine is useful for athletes.

Dr. Piyush Tailor

1 To find a woman you need Time and Money therefore:

$$\text{Woman} = \text{Time} \times \text{Money}$$

2 "Time is money" so

$$\text{Time} = \text{Money}$$

3 Therefore

$$\text{Woman} = \text{Money} \times \text{Money}$$

$$\text{Woman} = (\text{Money})^2$$

4 "Money is the root of all problems"

$$\text{Money} = \sqrt{\text{Problems}}$$

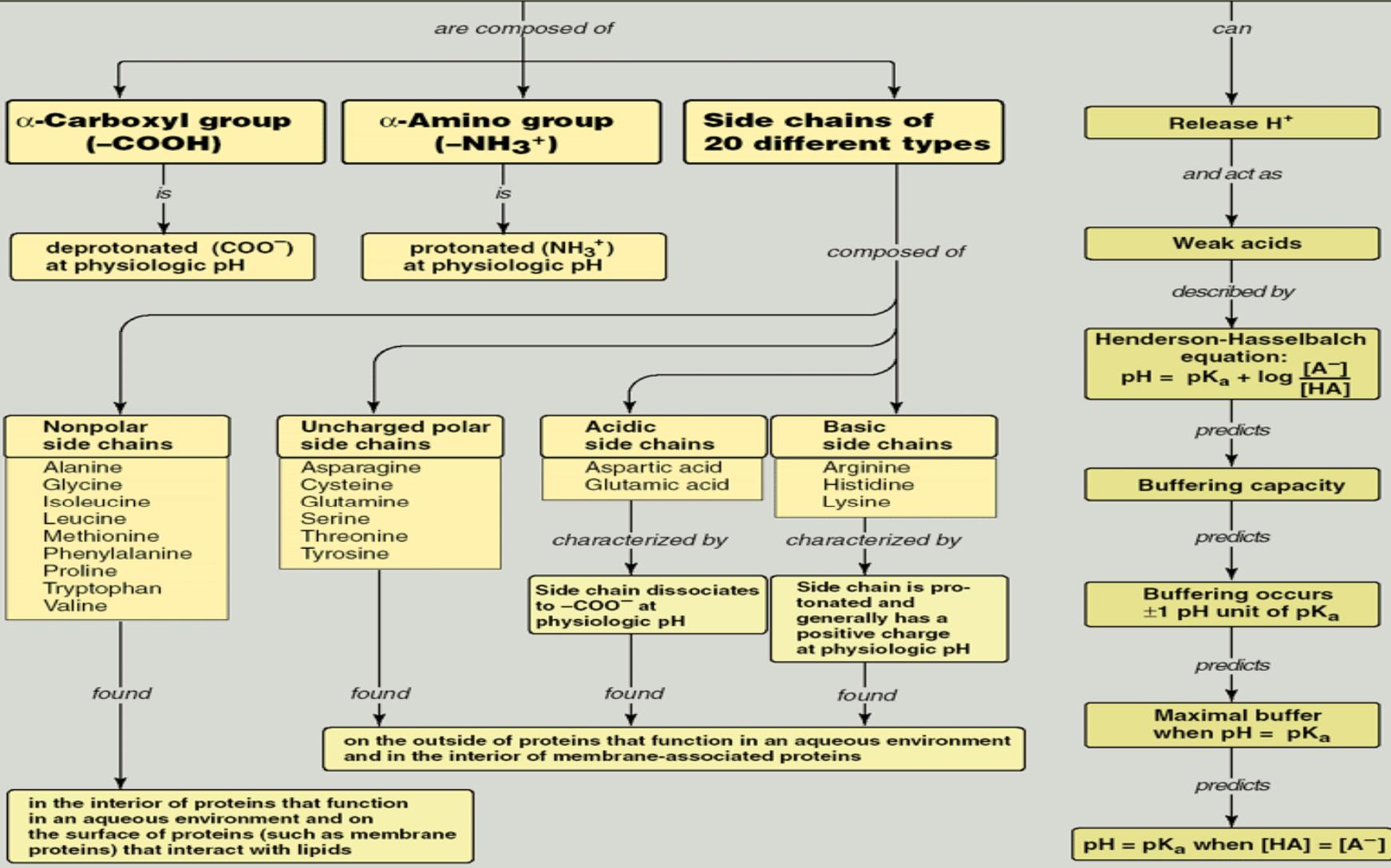
5 Therefore

$$\text{Woman} = (\sqrt{\text{Problems}})^2$$

$$\text{Woman} = \text{Problems}$$

A+

Amino acids (fully protonated)

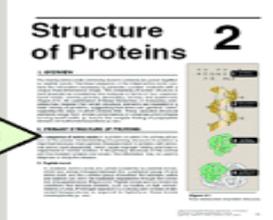


In proteins, most α -COO⁻ and α -NH₃⁺ of amino acids are combined in peptide bonds.

Therefore, these groups are not available for chemical reaction.

Thus, the chemical nature of the side chain determines the role that the amino acid plays in a protein, particularly ...

... how the protein folds into its native conformation.



Thank You

Dr Piyush Tailor