بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

MID | Lecture 14

Amino Acid Ionization & Peptides

Written by : Shahd Thamer
Rand Alkhateeb

Reviewed by : Rand Alkhateeb

Color coding used in the modified:

- *Black: the original slides*
- *Navy Blue: the doctor's explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Effect of pH

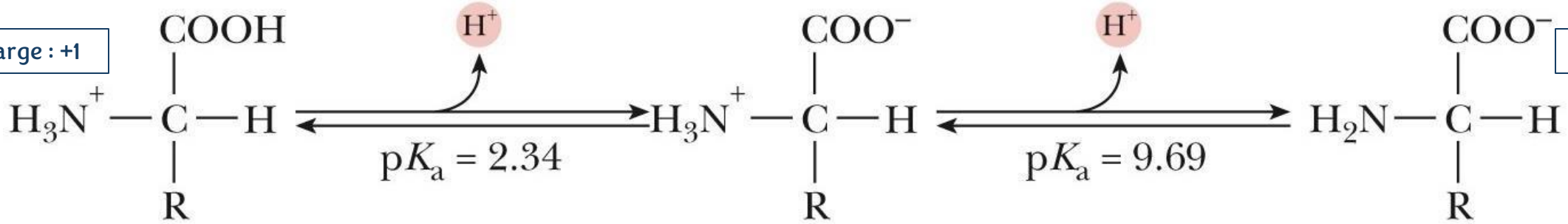
RECALL: PKa is the Ph when the concentration of the acid equals the concentration of the conjugate base.

And when we start raising the ph value in the titration curve we will reach the buffering range in which the amino acid will resist the change in ph (specifically the carboxyl group)

Until we reach the equivalence point at which the isoelectric zwitterion has 100% concentration, we keep raising the ph value until the amino group starts losing its protos (it starts losing them from early on) but until we reach the buffering range at which the resistance happens.

Isoelectric zwitterion

Net charge : +1



Net charge:-1

Because amino acids have ionizable groups that can be ionized:
· Amino group
· Carboxyl group

Such groups can act as buffers.
We can some how agree on an approximate value for the pKa for carboxyl group=2
Amino group=9

Notice that pKa value is variable among amino acids.
When ph is very low each group will be protonated because we have abundance of H+

Note that the isoelectric zwitterion is a charged molecule but the total net charge is zero.

Net charge:0

Q: why the NH3+ group in the isoelectic zwitterionic form is protonated at ph=6?

A: because the ph is lower than the pKa of the amino group so it stays protonated.

· Note that the charge depends on the group itself; what does that mean?

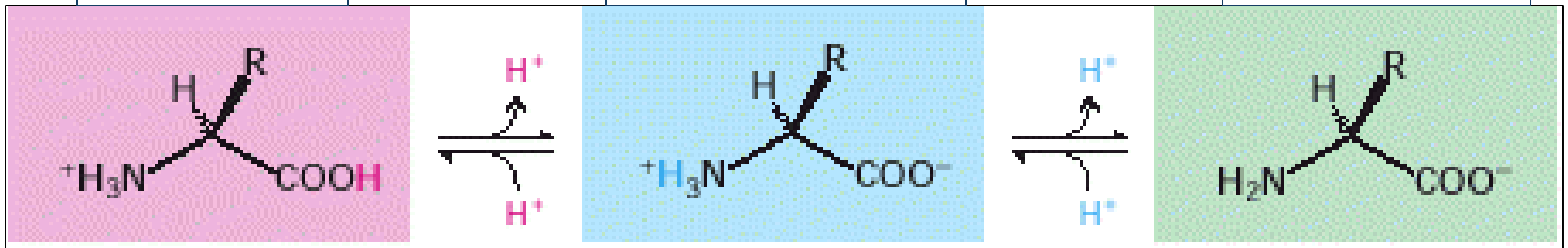
-protonated amino group has a positive charge.

-protonated carboxyl group is neutral.

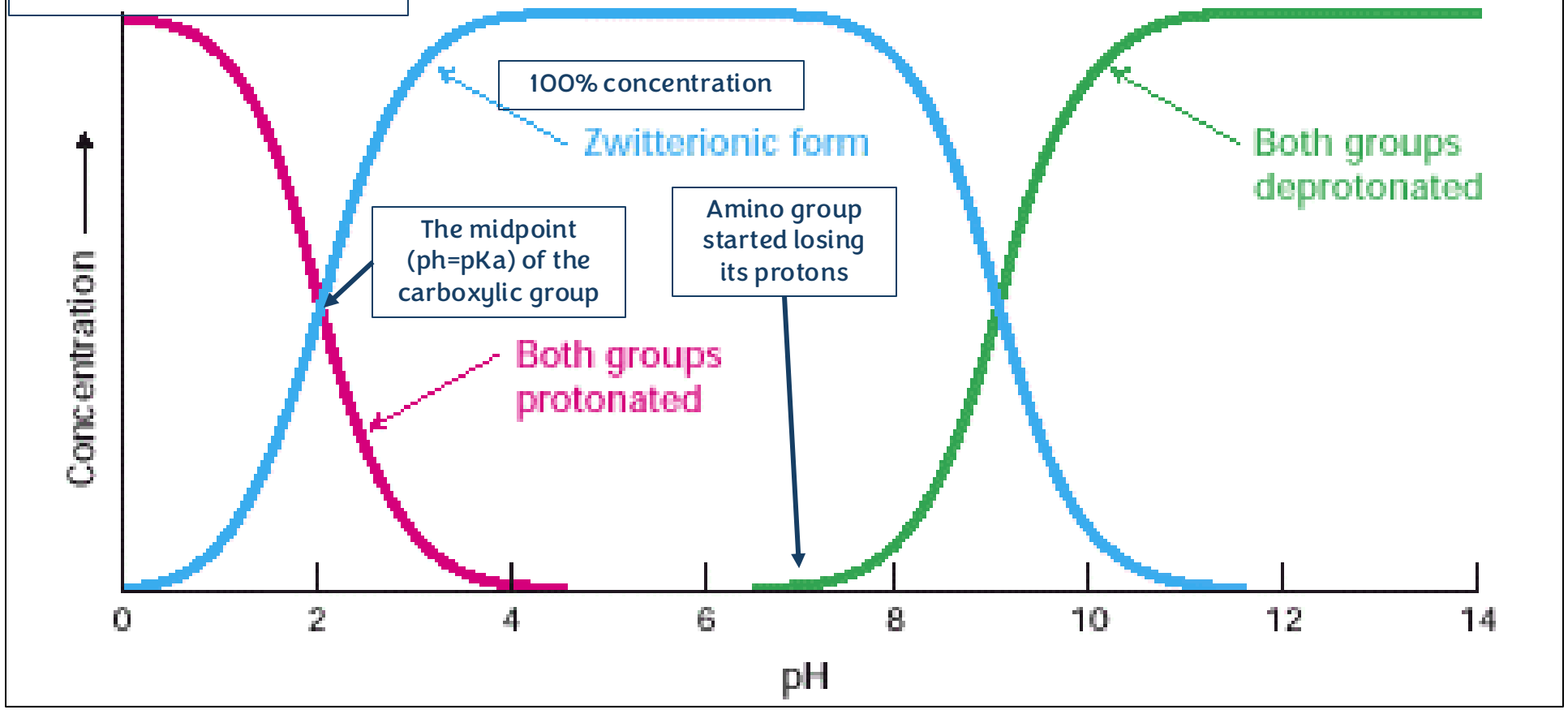
Both groups are protonated

Amino group is the only protonated

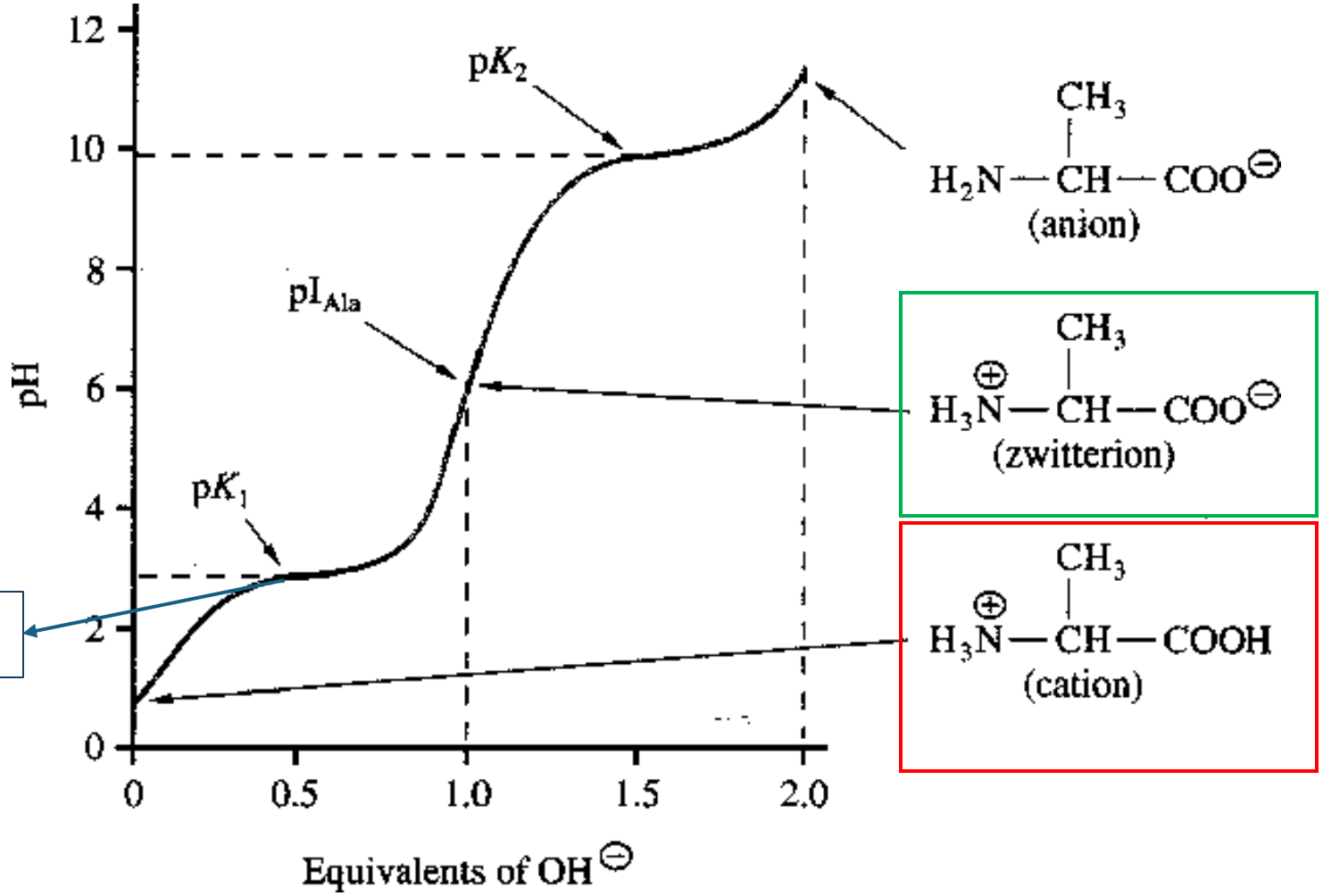
All groups are un protonated



At very low ph the amino acid will be on this form



Example 1 (alanine)



Prevalent resistance for the change of ph.

Isoelectric point pI at 1 eq,
It's the ph when the molecule is charged but neutral.
pI=(pK1+pKa2)/2
PI: the average of pK1 and pK2.
At pI we have the maximal amount of the zwitterionic ion.
· in this case the isoelectric point for alanine is 5.5

Depending on the group (amino group or carboxyl group) we use the equation to determine what is the structure of the acid and the conjugate base.
For example; at ph=2:
Acid: the cation
Conjugate base: zwitterion

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{weak acid}]}$$

Isoelectric Point

- The isoelectric point or pI is the pH where the net charge of a molecules such as an amino acid or protein is zero.
- For the nonpolar and polar amino acids with two pKa's, the isoelectric point is calculated by taking the average of the pKa's of the carboxyl group and the amino group.

$$\mathrm{pI} = \frac{\mathrm{p}K_{\mathrm{a1}} + \mathrm{p}K_{\mathrm{a2}}}{2}$$

Ionization of side chains

- 7 of the 20 amino acids have ionizable side chains near physiological pH.
- These amino acids are tyrosine, cysteine, arginine, lysine, histidine, and aspartic and glutamic acids.
- Each side chain has its own pKa values for ionization of the side chains.

pI of amino acids

In Aspartic acid and Glutamic acid both have an extra carboxyl group that can be negative.

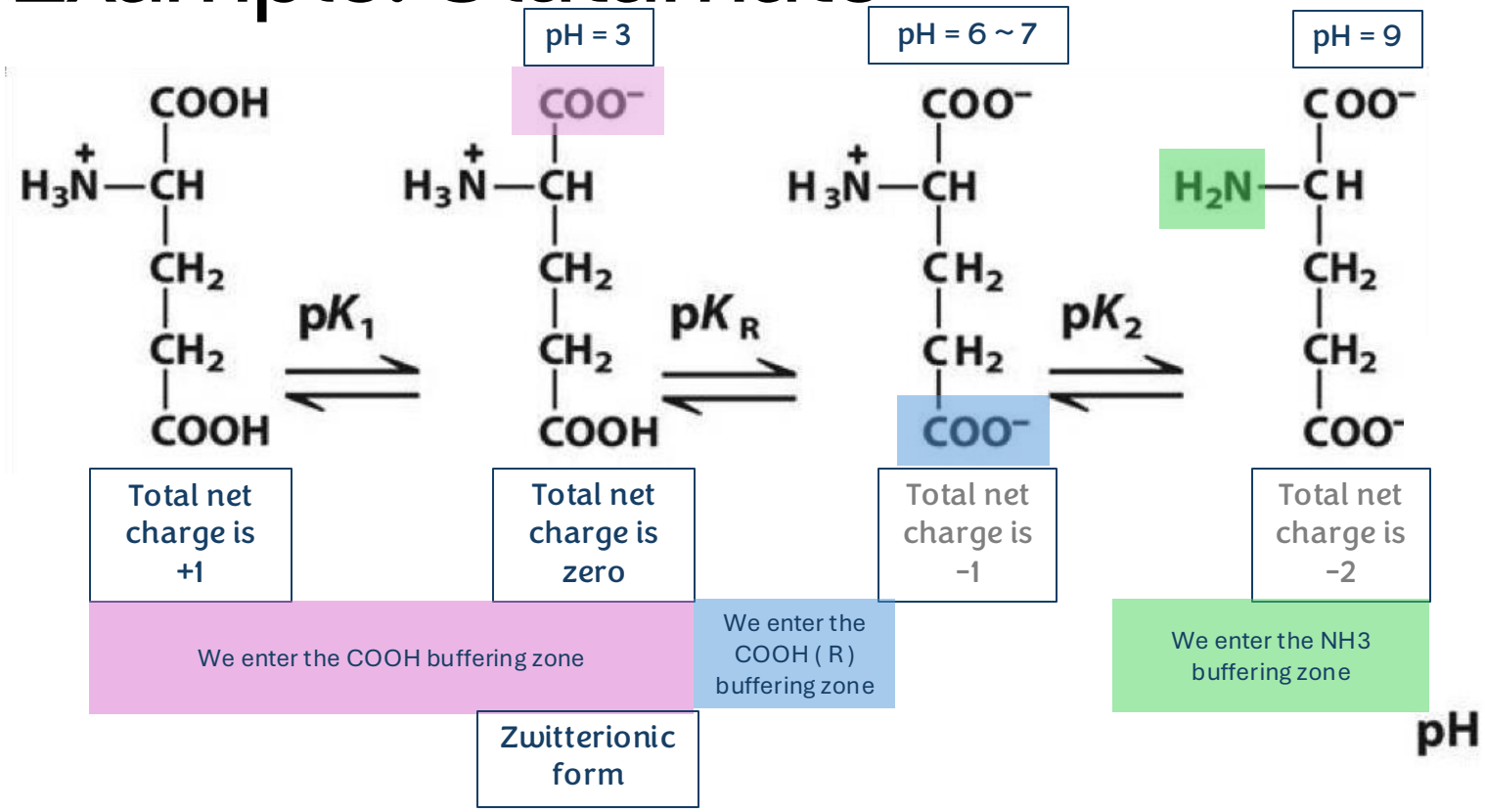
Recall Arginine has R group guanidinium which can be positively charged and has resonance.

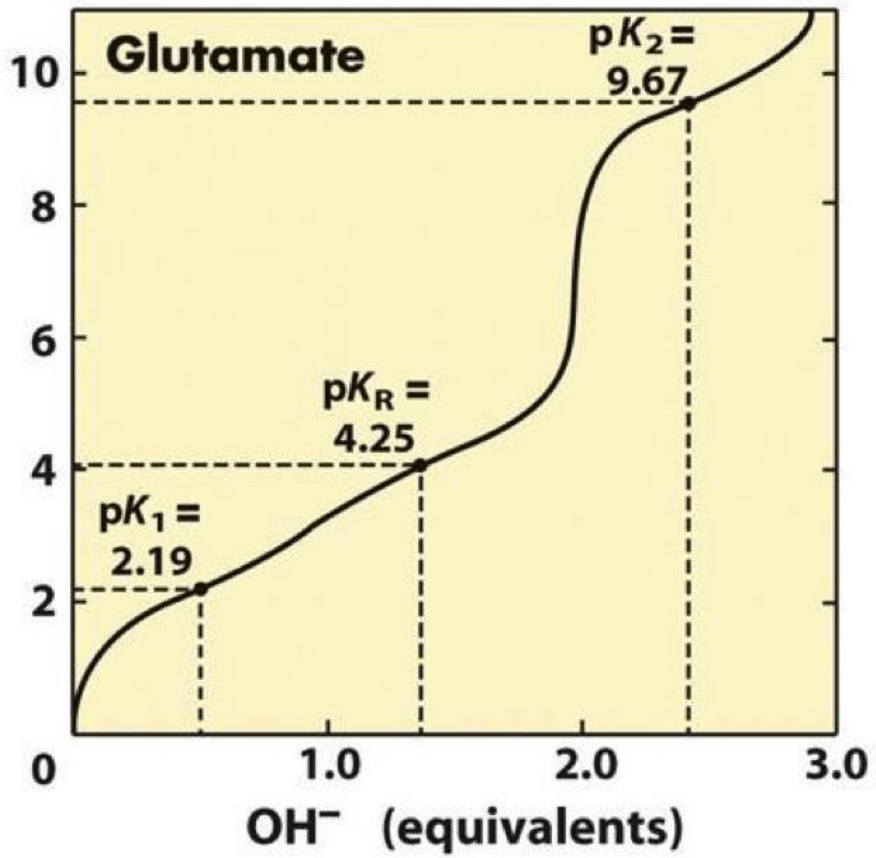
So here in Arginine:
Amino group can be +
Carboxyl group can be –
R group can be +

Amino Acid	Side Chain pK_a (approx.	pI
Arginine	12.5	10.8
Aspartic Acid	4.0	3.0
Cysteine	8.0	5.0
Glutamic Acid	4.1	3.2
Histidine	6.0	7.5
Lysine	11.0	10
Tyrosine	10.0	5.5

Let's consider pKa of -NH_2 = 9 and pKa of –COOH = 2 for all amino acids

Example: Glutamate





- To calculate the isoelectric point of Glu, the pKa's of the two carboxyl groups are averaged.

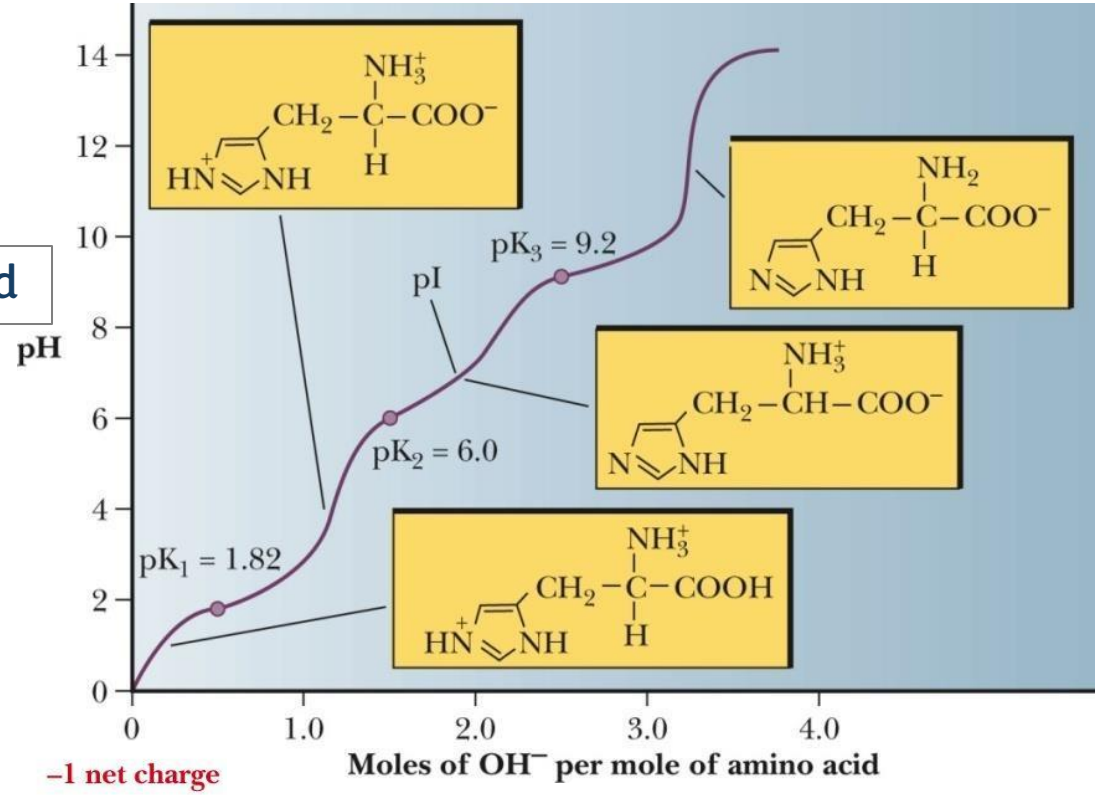
At the point with maximal amount of the zwitterionic form, almost (3)

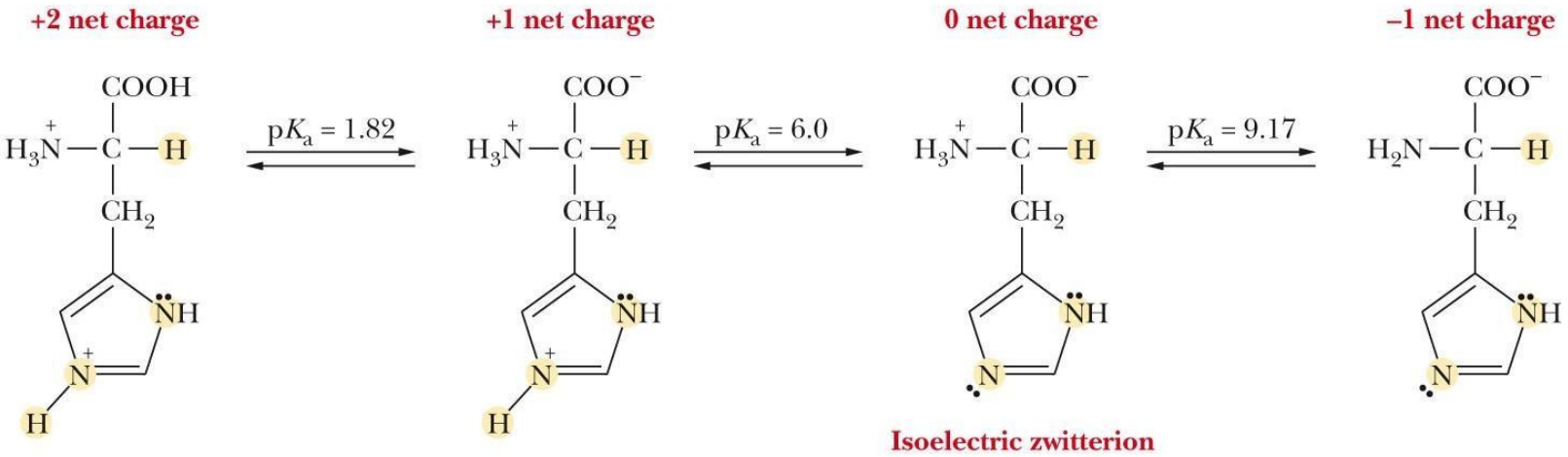
(2.19 + 4.25) / 2 ≈ 3.22

Histidine

- pI = ~7.5 (The imidazole group can be uncharged or positively charged near neutral pH). Protonated (pH< pKa < pH) unprotonated

Note that the pKa's of the side chains are different when amino acids are part of proteins.





Questions

- Draw the titration curve of histidine.
- What is the ratio of conjugate base/acid of glutamate at pH 4.5?
- What is the total charge of lysine at pH 7?

What do you need to know?

- The names of amino acids
- The special structural features of amino acids
- Their abbreviations or designations
- The uncommon amino acids, their precursor and function (if any)
- The pKa of groups
  - not exact numbers, but which ones are acidic, basic, or near neutral

Peptides

Definitions and concepts

- A residue: each amino acid in a (poly)peptide
- Dipeptide, tripeptide, tetrapeptide, etc.
- Oligopeptide (peptide): a short chain of 20-30 amino acids
- Polypeptide: a longer peptide with no particular structure
- Protein: a polypeptide chains with an organized 3D structures
- The average molecular weight of an amino acid residue is about 110

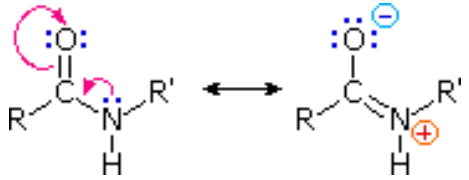
  Residue : a part of a peptide

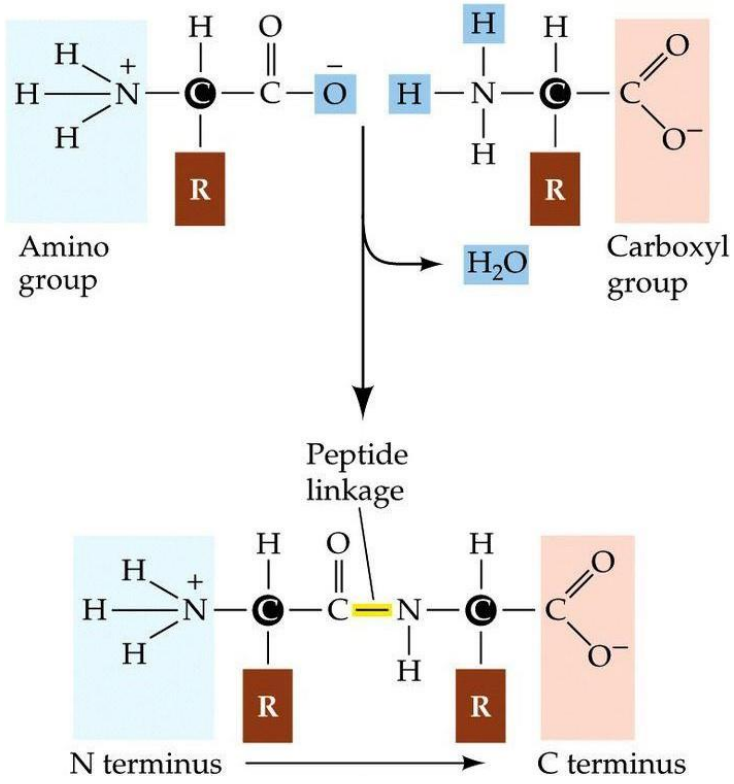
  - The molecular weights of most proteins are between 5500 and 220,000 (calculate how many amino acids)

    The average molecular weight of an amino acid is 110 dalton
- We refer to the mass of a polypeptide in units of Daltons
  - A 10,000-MW protein has a mass of 10,000 Daltons (Da) or 10 kilodaltons (kDa)

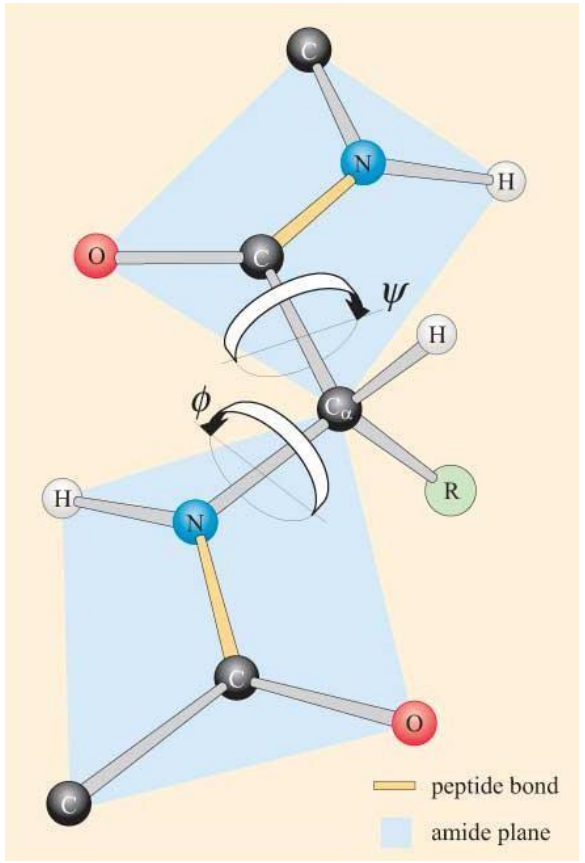
Peptide bond

- It is called an amide bond formed via a condensation reaction.
- Features
  - It has a resonance structure
  - Zigzag structure
  - Double bond
    - Planar, charged, Rigid, Un-rotatable
  - Hydrogen bonding
    - Except proline

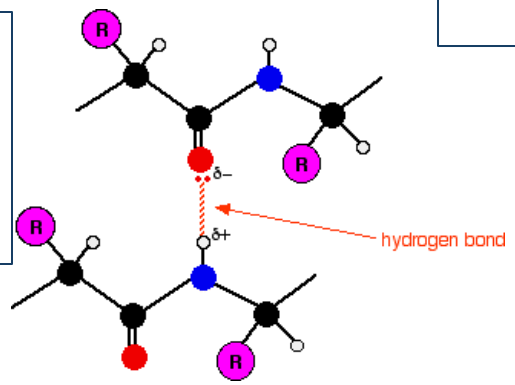


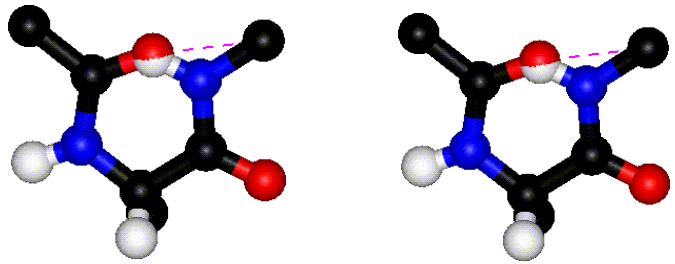


It can't rotate but the surrounding bonds can (bonds between the central carbon & both N & C)



In the hydrogen bonding here; Oxygen is an acceptor and nitrogen is a doner.

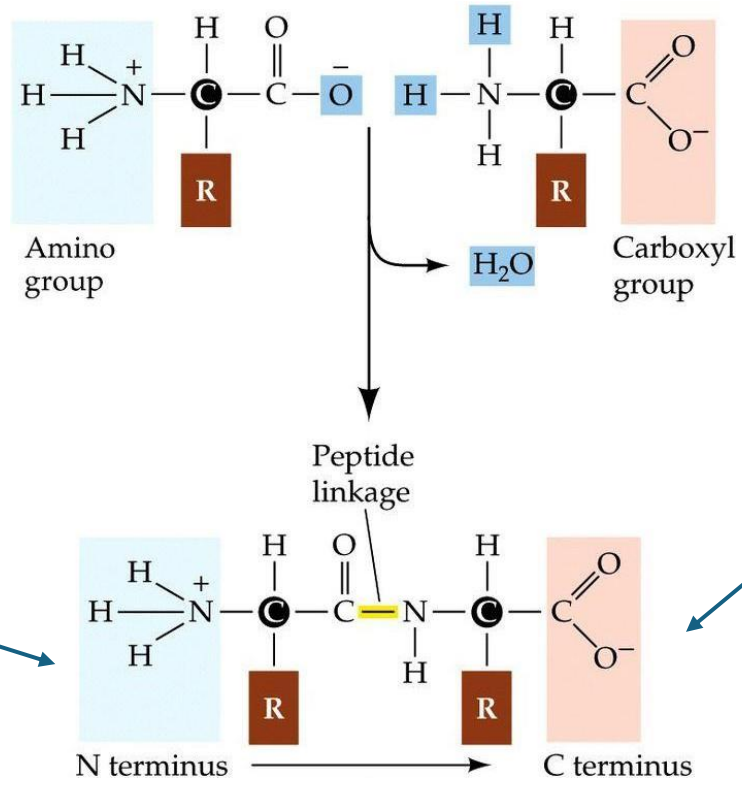




- how amino acids bond between each other? By condensation reaction (just like disaccharides) between carboxyl group from an amino acid and the amino group from a second amino acid, forming a peptide bond with a by product (water).
- In organic chemistry its called amide bond while in biochemistry its called peptide bond in proteins.
- The double bond in the carbonyl can do resonance and the peptide bond becomes a double bond, therefore creating charges on atoms involved in peptide bond
- Crucial question: how can the protein rotate

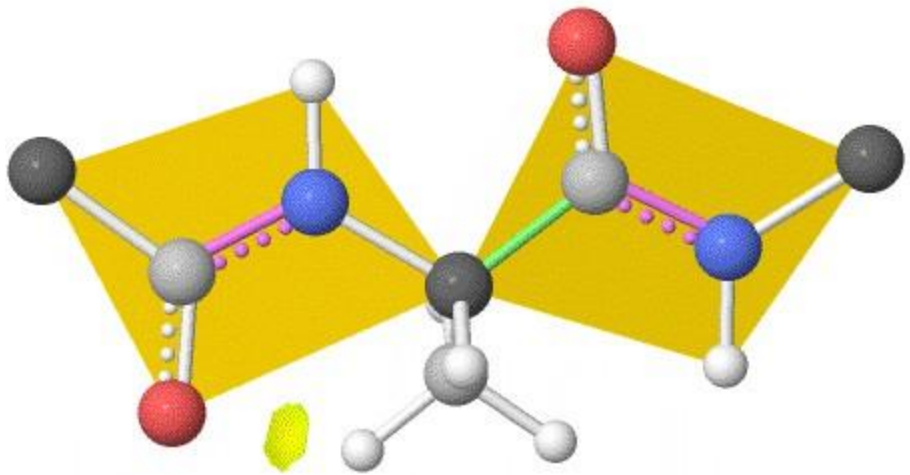
(even though the double bond gives rigidity to the Structure)?

The bonds within the amino acid rotate; central Carbon with the nitrogen; central carbon with the Carboxyl group (which became carbonyl group) This one rotates (to the right and to the left of the Alpha carbon) but the peptide bond itself can not be rotated.



Notice that this end stays untouched

Here we can add another amino acid, therefore elongation happens at the carboxylic end.



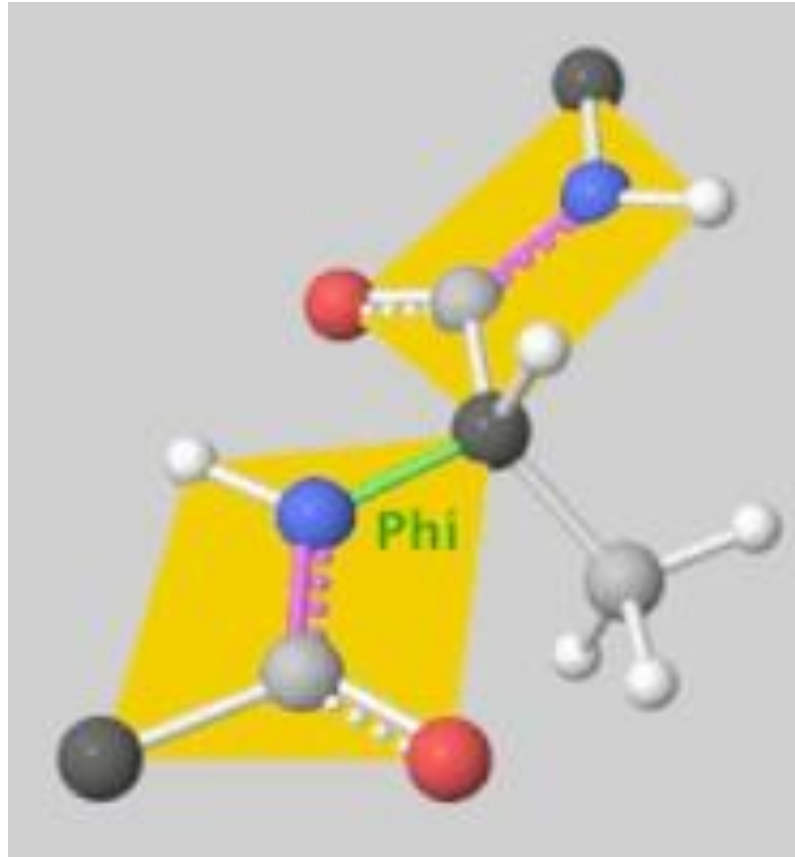
Cα C H N O

Phi φ -175°

Psi ψ -175°

-20° +20°

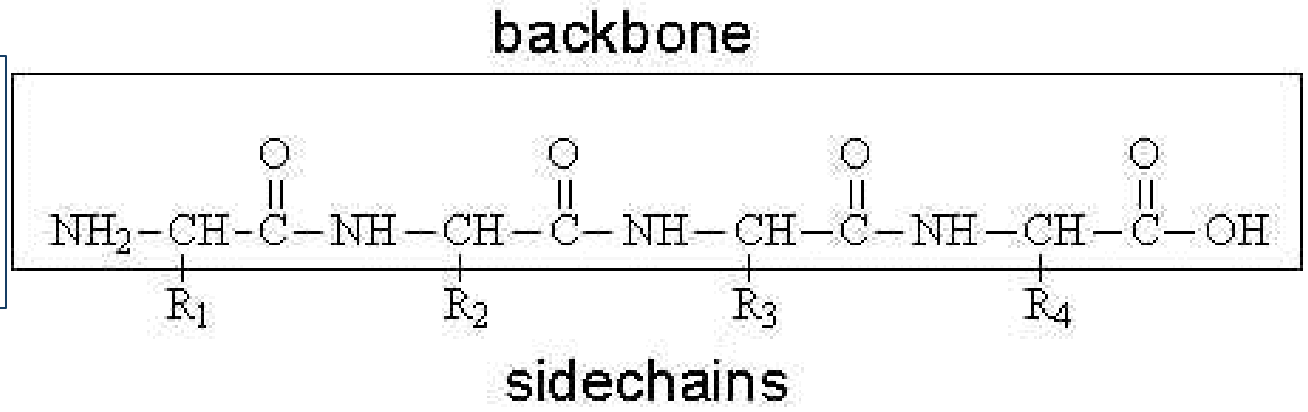
- [ ] Alanine
- [x] Peptide Bonds
- [x] Planes
- [ ] van der Waals[4]
- [x] Show Clashes



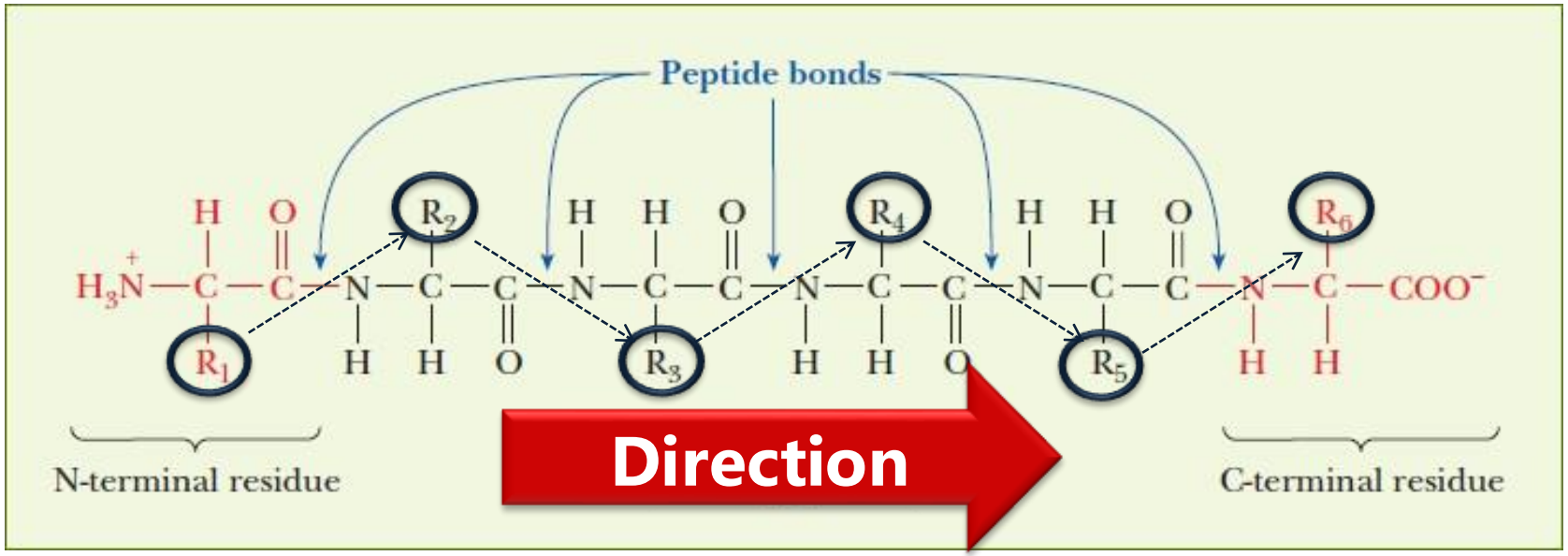
Backbone, orientation and directionality

α-amide N, the α-C, and the α carbonyl C atom

The amino acid starts at the amino terminus and it ends at the carboxy terminus.
So if the doc asks what is the sequence of this amino acid, you start from the amino terminus.



Notice that the order of the peptide bonds is in the trans orientation because if they were on the same side repulsion happens.



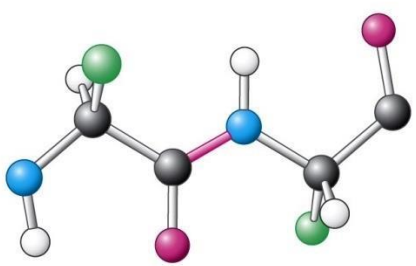
Addition of the amino acid is on the C-terminus

Except for proline

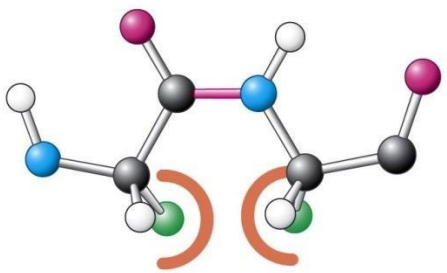
Can't form a hydrogen bond because it's N doesn't have any H to donate since it's forming a bond with the central carbon, a bond with the R group and a peptide bond with C ,(tertiary N)

- Steric hindrance between the functional groups attached to the Cα atoms will be greater in the cis configuration.
- In proline, both cis and trans conformations have about equivalent energies.
- Proline is thus found in the cis configuration more frequently than other amino acid residues.

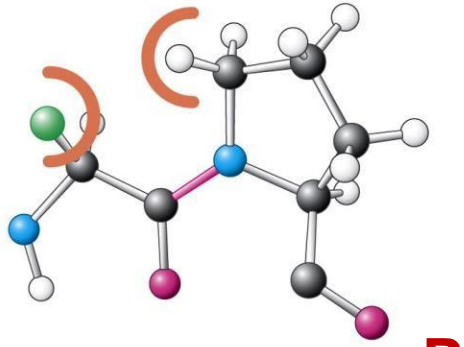
All other amino acids



Trans

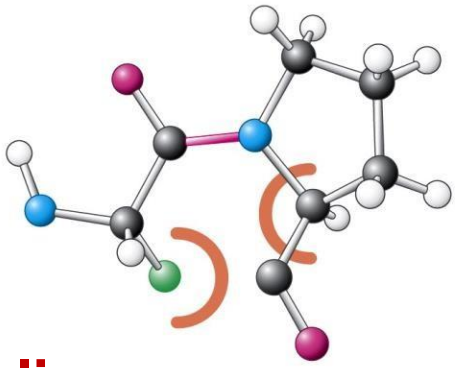


Cis



Trans

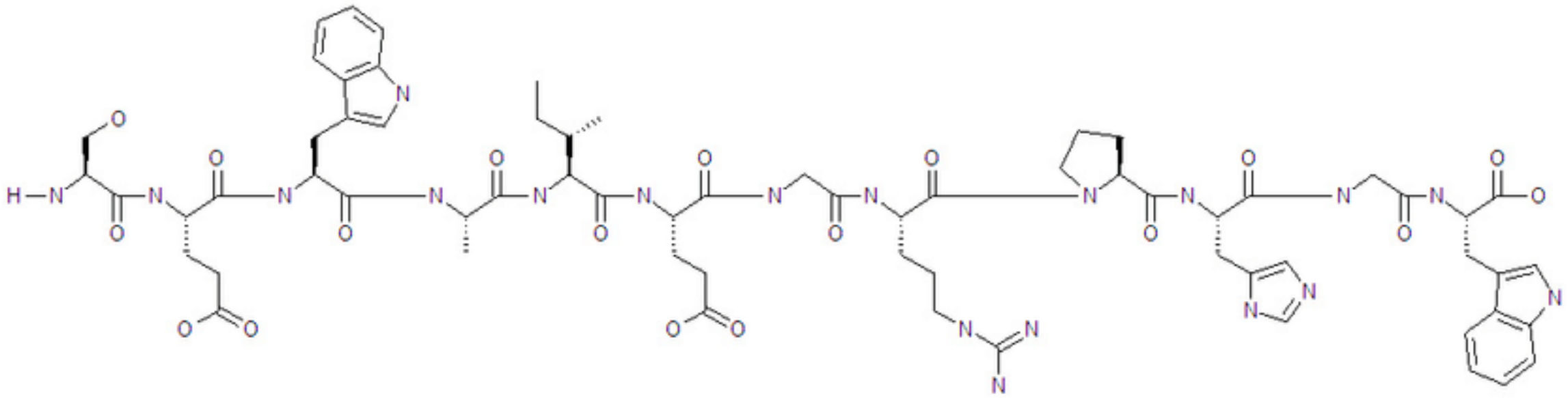
Proline



Cis

The problem with Proline is that the nitrogen group is occupied; so it won't be a proton doner in a peptide bond.
Either in trans or cis orientation, repulsion will happen, so it will form 50% trans and 50% cis.

Practice: *name the amino acid residues*



NH_2—S E W A I E G R P H G W—COOH

The abbreviation is not required

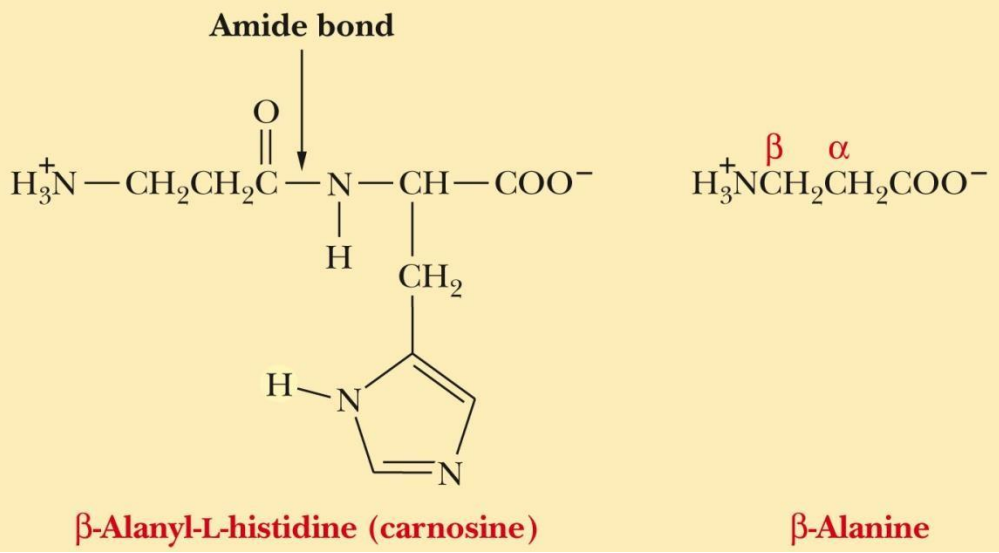
Determine :
- R groups
- peptide bonds
- backbone
- identities of the amino acids (the sequence is provided by the way)

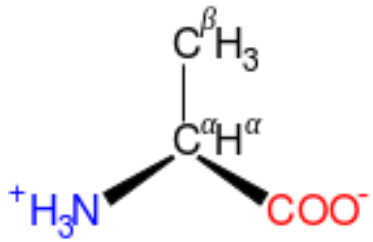
Examples of functional and exceptional peptides

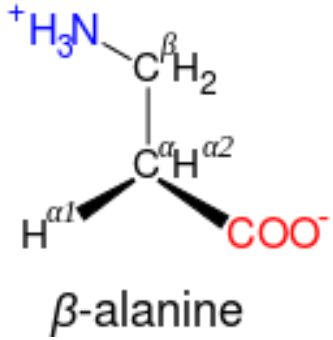
Carnosine (β-alanyl-L-histidine)

You are required to know the name, composition and function.

- A dipeptide of β-alanine and histidine
- The amino group is bonded to the β -carbon of alanine
- It is highly concentrated in muscle and brain tissues
  - Protection of cells from ROS (radical oxygen species) and peroxides
  - Contraction of muscle







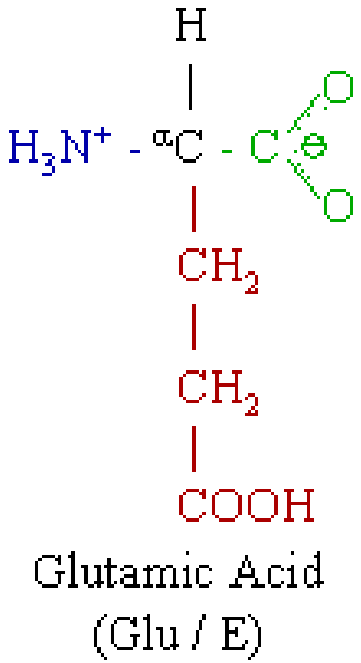
Amino group is on beta carbon not alpha.

Carnosine peptide bond:
Carboxyl group from alanine and amino group from histidine.
(meaning it starts with alanine then histidine)

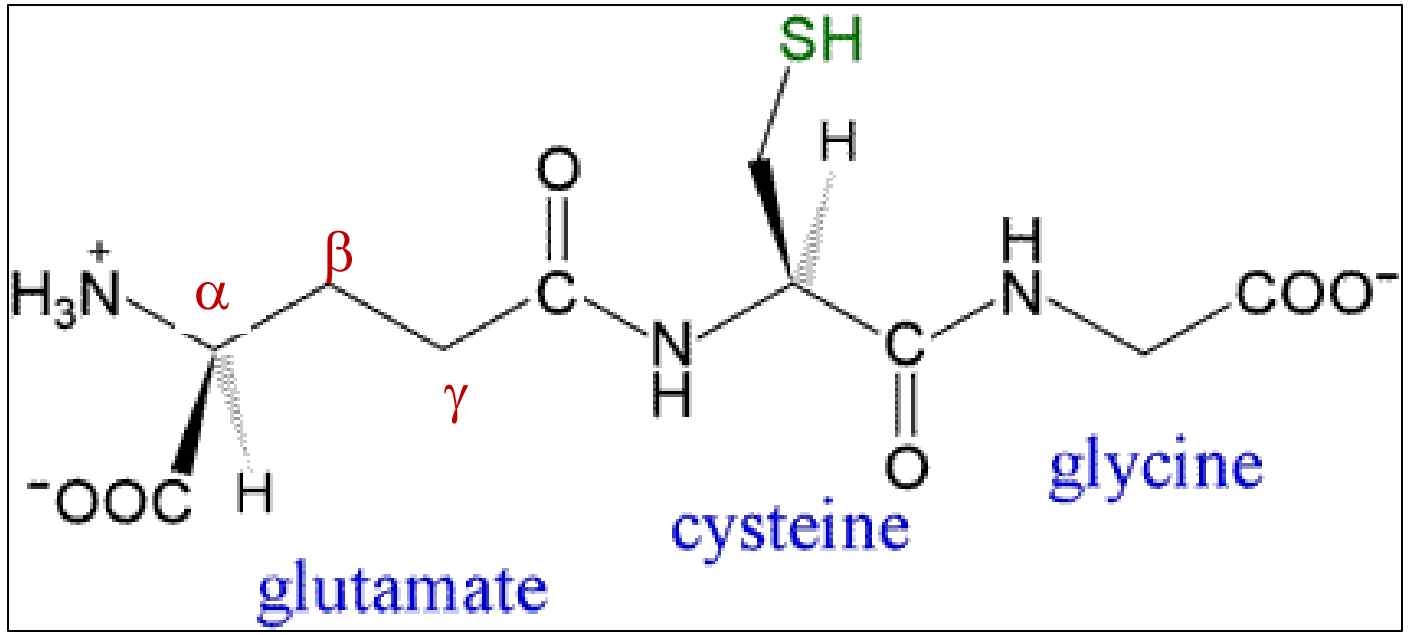
Glutathione

(γ-glutamyl-L-cysteinylglycine)

Tri-peptide
The most dangerous
Consists of:
Glutamate, cysteine, glycine



Glutamic Acid
(Glu / E)



The carboxyl involved in peptide bond is the one in the side chain(connected to γ carbon)

Function of glutathione

Muscle cells require large amounts of **Glutathione** because they perform high rates of aerobic metabolism, which produces significant levels of **reactive oxygen species (ROS)**

- It scavenges oxidizing agents by reacting with them.
- Two molecules of the reduced glutathione molecules form the oxidized form of glutathione by forming a disulfide bond between the —SH groups of the two cysteine residues.

Protects the cells from the reactive oxygen species (free radicals).

Disulfide bond

These free radicals are very dangerous, they ruin any cellular structure, thione group has extra electron and give it to the free radical and form a disulfide bond with another glutathione

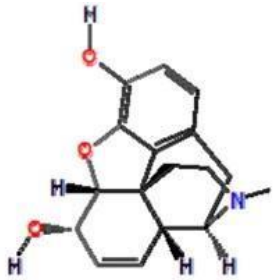
Enkephalins

- Two pentapeptides found in the brain known as enkephalins, and function as analgesics (pain relievers).
  - They differ only in their C-terminal amino acids.
    - Met-enkephalin: Tyr-Gly-Gly-Phe-Met
    - Leu-enkephalin: Tyr-Gly-Gly-Phe-Leu

Sequence is not required.

Both have Phenylalanine which is the active group. One ends with Met, other ends with Leu

  - The aromatic side chains of tyrosine and phenylalanine play a role in their activities.
- There are similarities between the three-dimensional structures of opiates, such as morphine, and enkephalins.



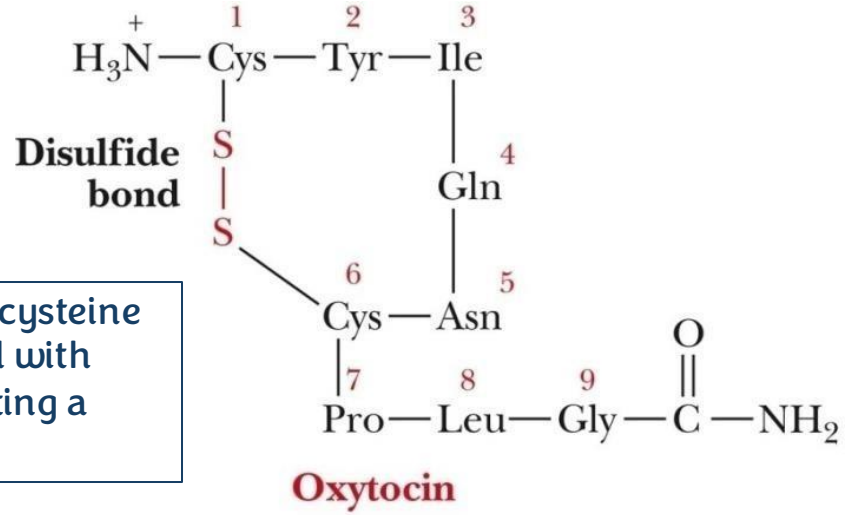
Morphine

Enkephalins

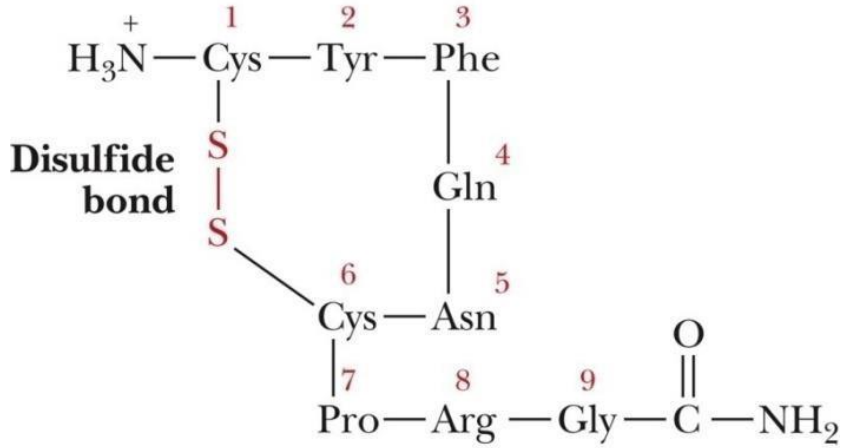
Oxytocin and vasopressin

- Hormones with cyclic structures due to S-S link between Cys.
- Both have amide group at the C-terminus.
- Both contain nine residues, but:
  - Oxytocin has isoleucine and leucine.
  - Vasopressin has phenylalanine and arginine .
- Oxytocin regulates contraction of uterine muscle (labor contraction).
- Vasopressin regulates contraction of smooth muscle, increases water retention, and increases blood pressure.

9 amino acids (first is cysteine forms a disulfide bond with another cysteine creating a ring in the molecule).



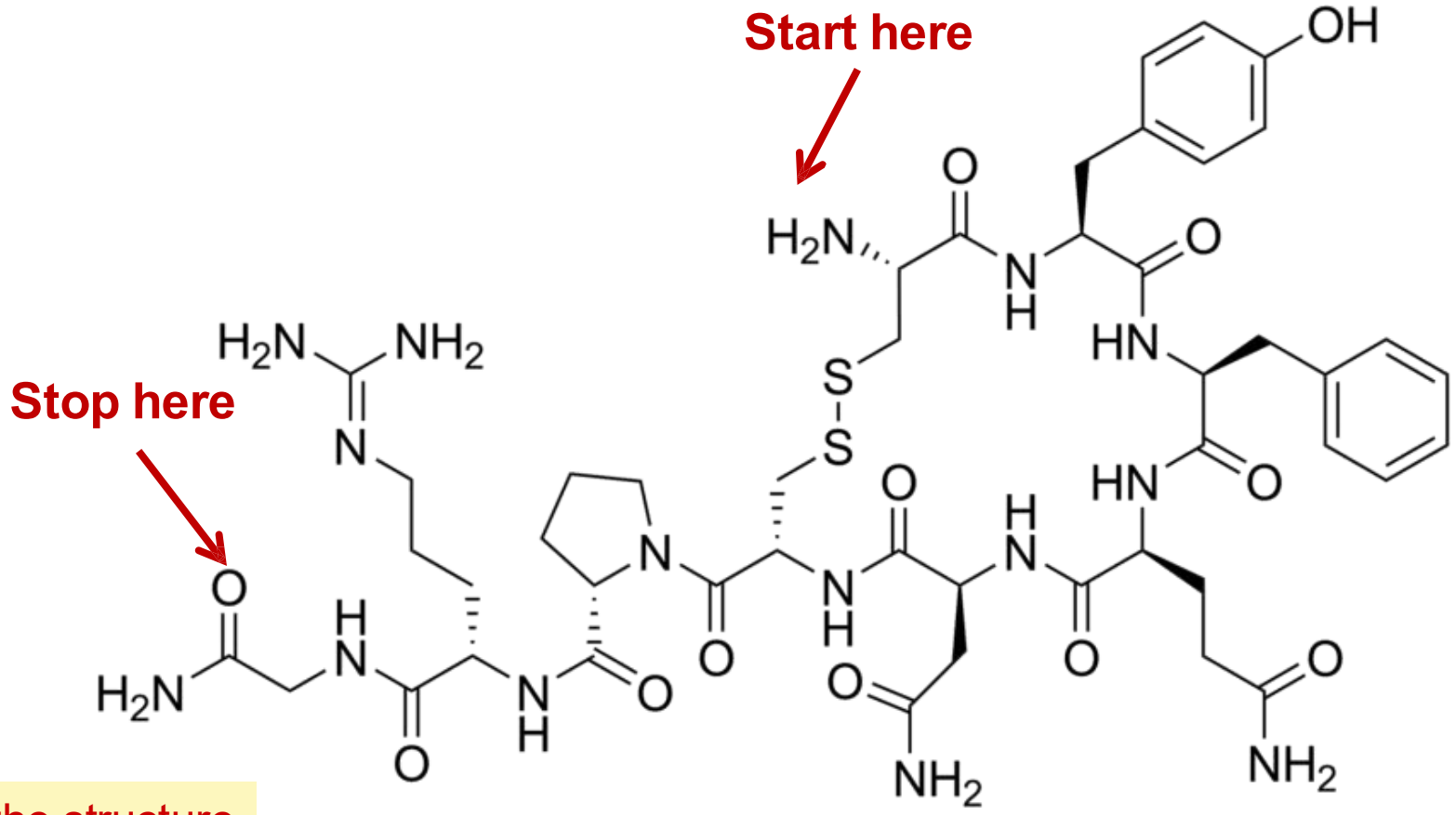
Oxytocin



Vasopressin

Vasopressin

Practice: what is the primary structure?



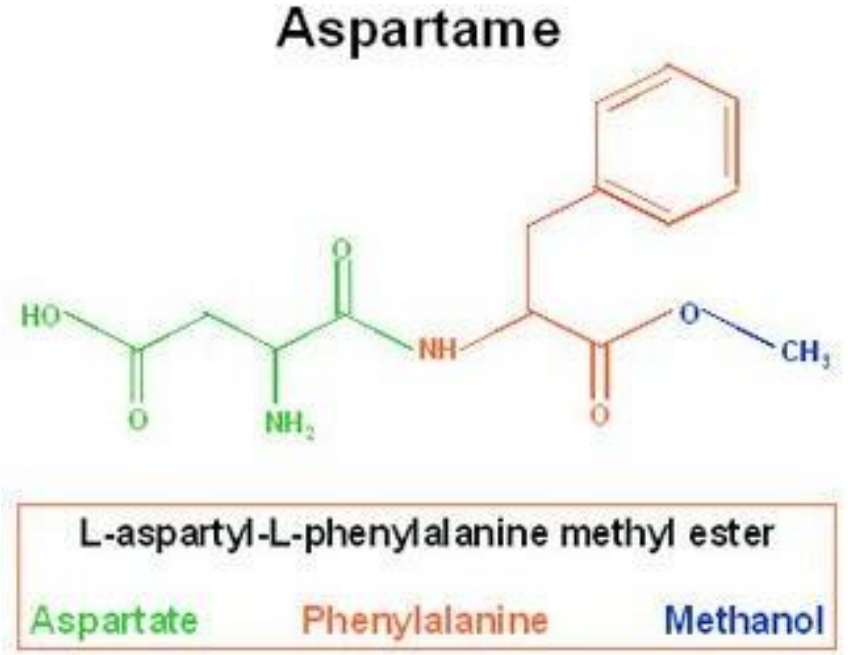
Note: the structure ends with NH2

Aspartame

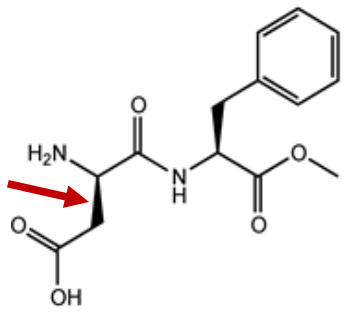
L-Aspartyl-L-phenylalanine (methyl ester)

Sweetener in coffee

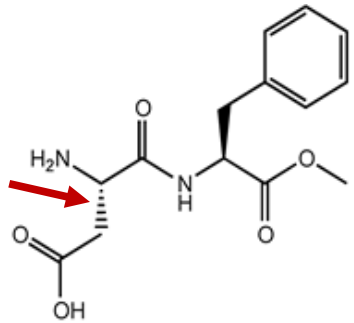
- A dipeptide that is 200 times sweeter than sugar.
- If a D-amino acid is substituted for either amino acid or for both of them, the resulting derivative is bitter rather than sweet.



This methyl group stabilizes the molecule



R,S-Aspartame (bitter)



S,S-Aspartame (sweet)

Aspartame and cancer

Aspartame can cause cancer and is used in diet Cola.

REUTERS® World Business Markets Sustainability Legal Breakingviews Technology Invest

Government Health Policy

Exclusive: WHO's cancer research agency to say aspartame sweetener a possible carcinogen -sources

By **Jennifer Rigby** and **Richa Naidu**

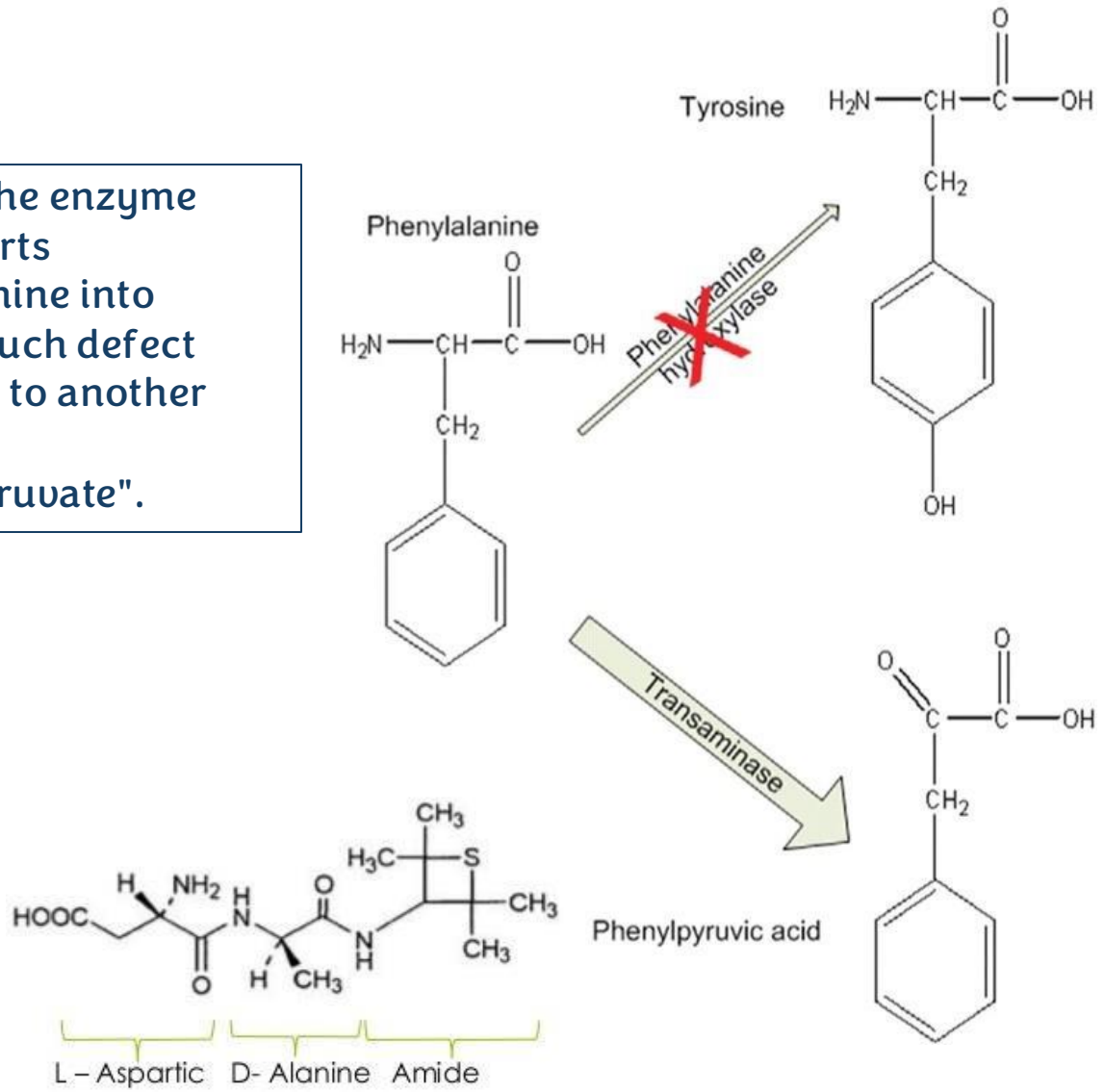
June 29, 2023 10:17 PM GMT+3 · Updated 7 days ago

Phenylketonuria (PKU)

Very common disease in children.

- PKU is a hereditary “inborn error of metabolism” caused by defective enzyme, phenylalanine hydroxylase.
- It causes accumulation of phenylpyruvate, which causes causes mental retardation.
- Sources of phenylalanine such as aspartame must be limited.
- A substitute for aspartame, known as alitame, contains alanine rather than phenylalanine.

Defect in the enzyme that converts phenylalanine into tyrosine; such defect converts it to another molecule "phenylpyruvate".



Test yourself

Quiz (:

ينال المرء مقصدهُ بسعيٍ،
وليس ينال شيئًا إنّ تمنى.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Amino acids Ionaization & peptides

Extra References for the Reader to Use:

Marks' Basic Medical Biochemistry (6th edition)

رسالة من الفريق العلمي:

(نحن مسلمون نُعوَّل على البركة لا الوقت)

وإن كانت القيامةُ غدًا، أُمِرنا بغرسِ الفسيلةِ ولو تأكّدنا يقينًا أنَّا لن نشهدَ أو نجني ثمارها، اغرس الفسيلةَ وذاكِر لآخرِ لحظةٍ،
واتعب لآخر دقيقةٍ،
ولا تظنّ أنّ الأمرَ بالكمِّ أو العددِ،
استعِن باللهِ وتوكَّلْ عليه واعمل بقدرِ وسعك وطاقتك وربّك المعين، وأجرك عليه ((”!

أعاننا اللهُ وإياكُم، ولا تنسونا ووالدينا من صالح دعائكم