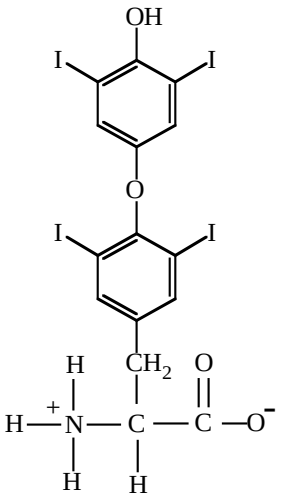
Chapter 3 - Amino Acids

α-Amino Acids are the main constituents of proteins. But they also can function as neurotransmitters (glutamate, γ-aminobutyric acid), hormones (thyroxine; see right), as bacterial cell wall components (*D*-alanine) and are intermediates in many metabolic pathways.

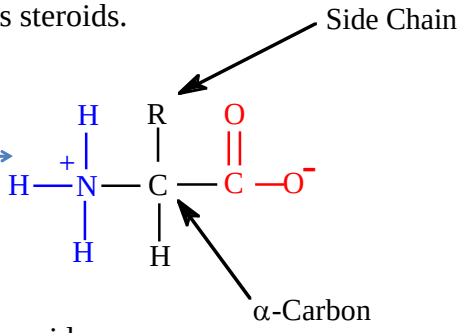


e.g. Glycine is a precursor to heme.

Hormones are chemical messengers secreted into the bloodstream by one cell that affect the metabolism of another cell.

They may be **hydrophilic** such as amino acids and peptides or they may be **hydrophobic** such as steroids.

Formula for α-AA at pH 7 →



α-NH_2 - base high pH form

α-COOH - acid low pH form

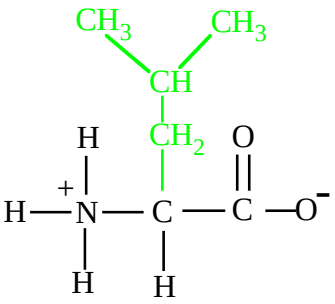
A **Protein** is a **Biopolymer** composed of a linear chain of α-AA. Only 20 α-amino acids are encoded in genetic material - DNA.

A **Peptide** is a short protein, up to 20 AA.

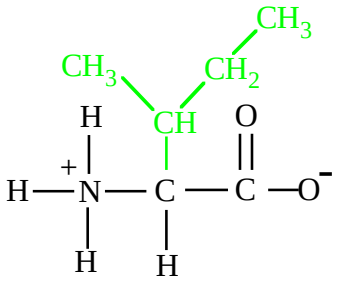
AA Side-Chain Classification:

A. Aliphatic, non-polar, hydrophobic

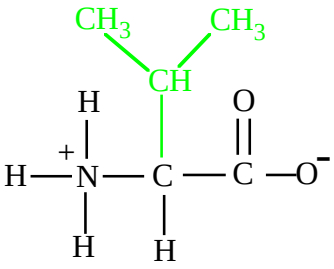
Leucine Leu



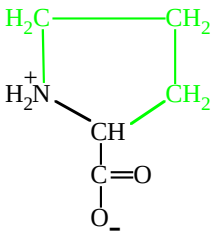
Isoleucine Ile



Valine Val

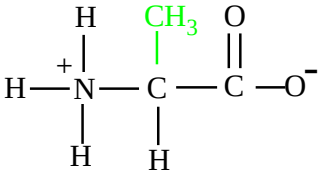


Proline Pro

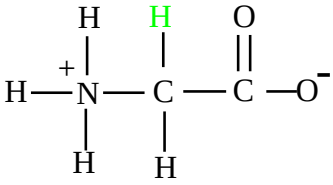


A cyclic α-imino acid.

Alanine Ala

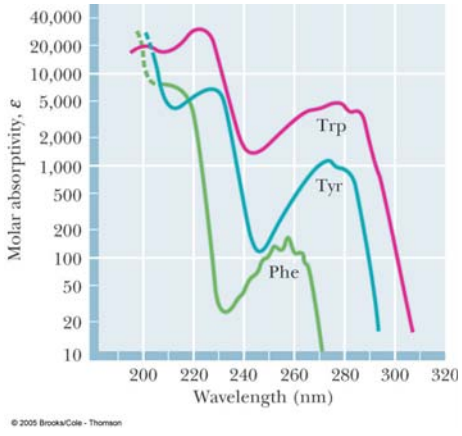


Glycine Gly

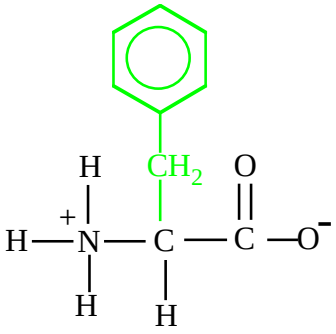


B. Aromatic Amino Acids - in order of hydrophobicity

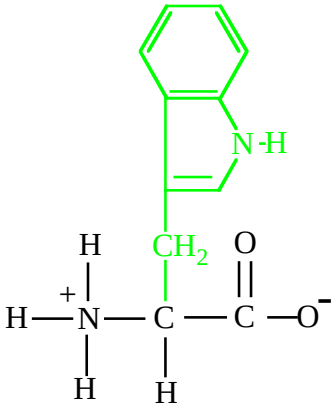
They absorb **ultra-violet light** because of conjugated double bonds.



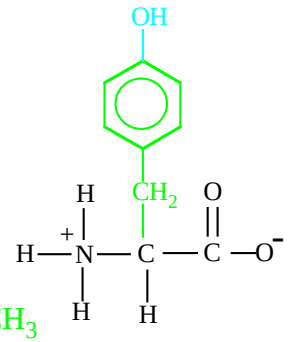
Phenylalanine Phe



Tryptophan Trp Indole



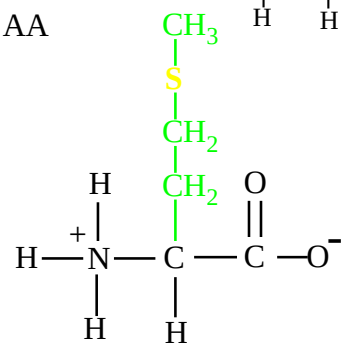
Tyrosine Tyr



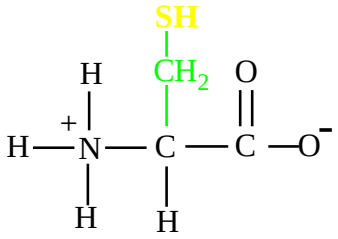
Phenolic hydroxyl pK_a = 10.1

C. Sulphur-containing AA

Methionine Met

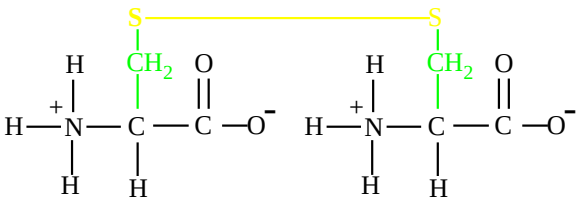


Cysteine Cys



Thiol $pK_a \sim 8.3$

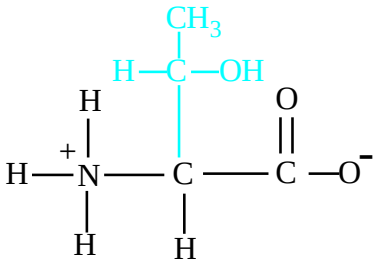
Two nearby Cys can form a disulphide bond in an oxidizing environment.



D. Hydroxyl Amino Acids - polar

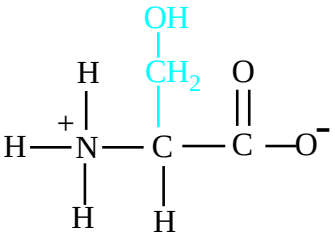
Threonine Thr

Alcohol $pK_a = 13.6$



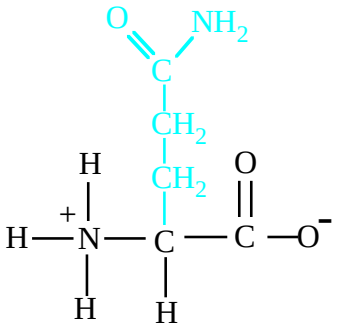
Serine Ser

Alcohol $pK_a = 13.6$



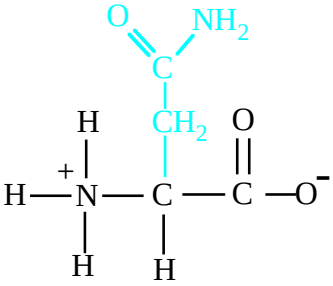
E. Amide-containing side-chains

Glutamine Gln



Amide side-chains do not ionize!

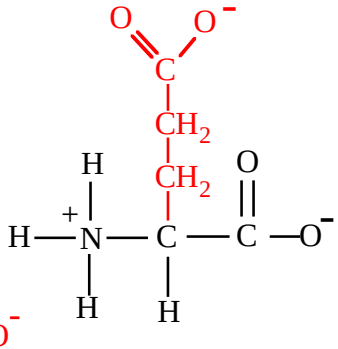
Asparagine Asn



F. Acidic AA (negative charge in the side-chain at pH 7)

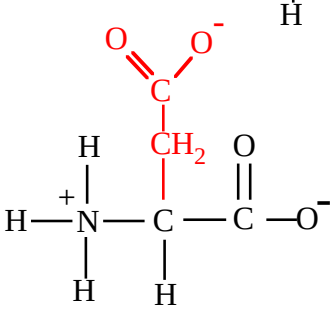
Glutamic Acid Glu

Carboxyl pK_a = 4.2



Aspartic Acid Asp

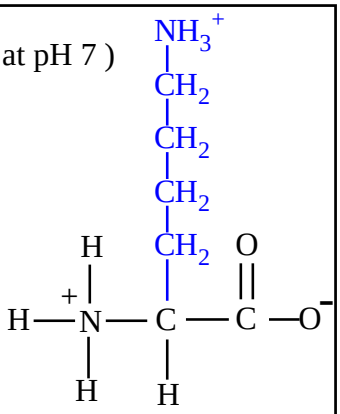
Carboxyl pK_a = 3.9



D. Basic (Positive charge in the side-chain at pH 7)

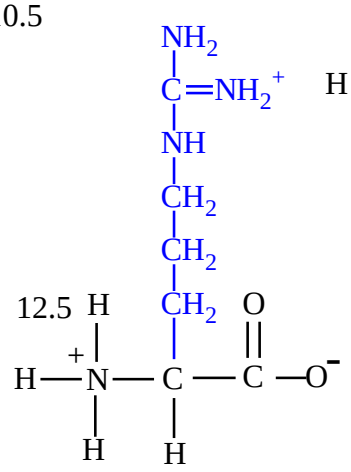
Lysine Lys

ε-Amino pK_a = 10.5



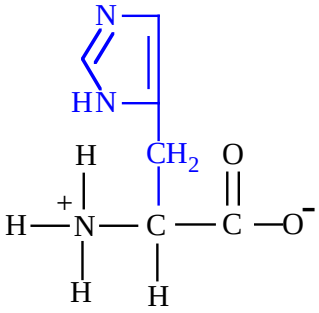
Arginine Arg

Guanidino pK_a = 12.5

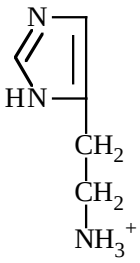


Histidine His

Imidazole pK_a = 6.0

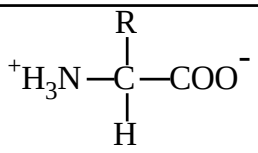


Histamine



Side chains in Groups A and B carry no net electric charge at pH 7 and remain stationary in an electric field.

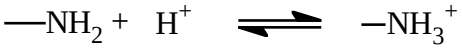
Zwitterion "hybrid ion".



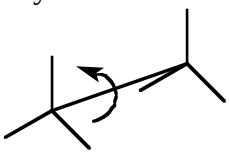
Average values for AA carboxyl and amino pK_a's are 2.2 and 9.5.

At pH 7, acidic amino acids have lost a proton from their side chains and are **anionic**. They move toward a positive electrode.

At pH 7, basic amino acids bind a H^+ and are **cationic**. They move toward a negative electrode.



Molecular **Conformation** refers to differences in the spatial arrangement of groups joined by covalent bonds due to bond **rotation**.

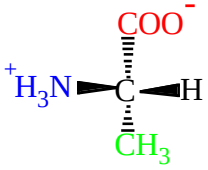


Isomers have the same molecular formula but a different arrangement of the groups. *e.g.* Leu, Ile.

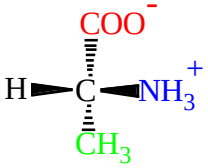
All AA except Glycine, have 4 different groups attached to the tetrahedral αC. The α-C is **asymmetric** / **chiral** (handed).

There are 2 possible arrangements of the 4 groups called **stereoisomers**.

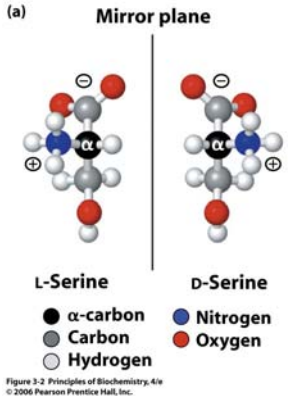
They are non-superimposable mirror images / **enantiomers**.

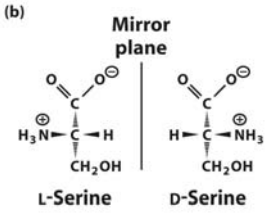


L-Ala

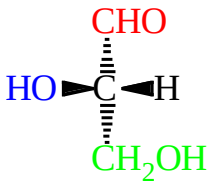


D-Ala

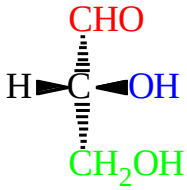




D- and *L*-glyceraldehyde are reference molecules for assignment of stereochemistry (absolute configuration).



L-Glyceraldehyde



D-Glyceraldehyde

The aldehyde and acid are aligned and the hydroxyl and amino groups are aligned.

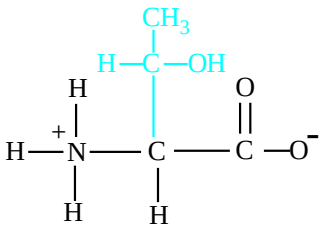
These molecules are optically active: they rotate the plane of monochromatic plane-polarized light in opposite directions.

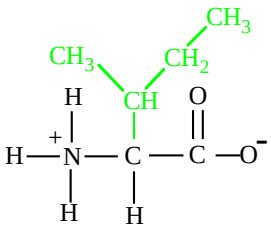
Dextrorotatory (+) or Levorotatory (-),

or RS (Rectus, Sinister).

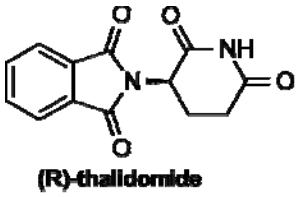
All AA in proteins are *L*-. Some *D*- are found in antibiotic peptides.

Thr and Ile contain two chiral C's and 4 stereoisomers.

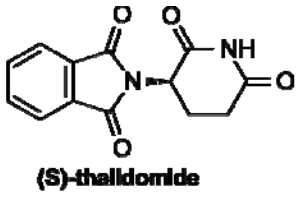




Thalidomide is a sedative. The + form is therapeutic, the mirror image is teratogenic and causes embryo malformation.

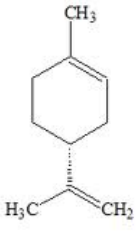


(R)-thalidomide

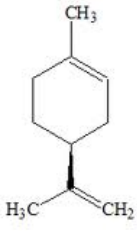


(S)-thalidomide

R-limonene: fresh citrus, orange-like



(R)



(S)

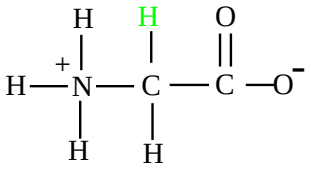
S-limonene: harsh, turpentine-like, lemon

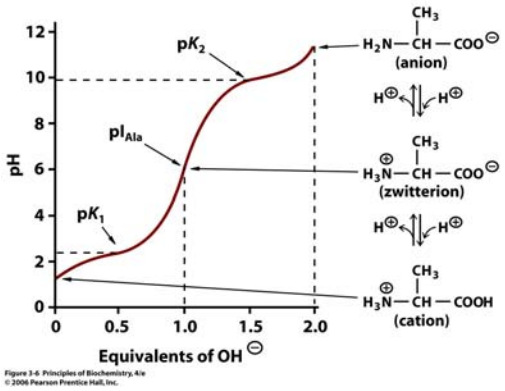
Titration of Gly with OH^-:

The titration curve will have two buffering regions, one for each group.

Amino $pK_a = 9.6$ Carboxyl $pK_a = 2.3$

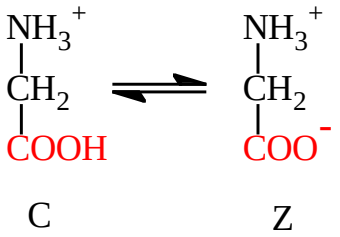
Average values for AA carboxyl and amino pK_a's are 2.2 and 9.5.





Draw Gly at low pH (C).

Add OH^- and raise the pH to 2.3:
The strongest acid (lowest pK_a) will ionize first.



C Z

After adding 0.5 equivalents of OH^-,
$pH = pK_{a1} = 2.3$ and [C] = [Z].

After adding 1.0 equivalent of OH^- (first end point), Gly is Zwitterionic, and the pH is midway between 2.3 and 9.6.

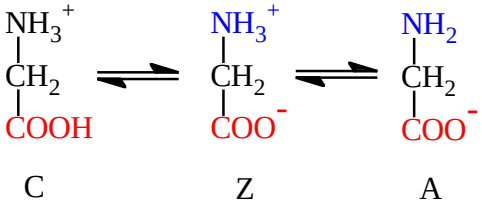
$$pH = \frac{2.3 + 9.6}{2} = 6.0 = pI$$

The **Isoelectric Point** is the pH at which the concentration of the Z-form of an AA is maximum. On average there is no net charge on the AA.

At the pI, the AA is stationary in an electric field.

After addition of 1.5 equivalents of OH^-, $pH = pK_{a2} = 9.6$ and A = Z.

After addition of 2.0 equivalents of OH^-, the pH ~ 12 and Gly is 100% A.

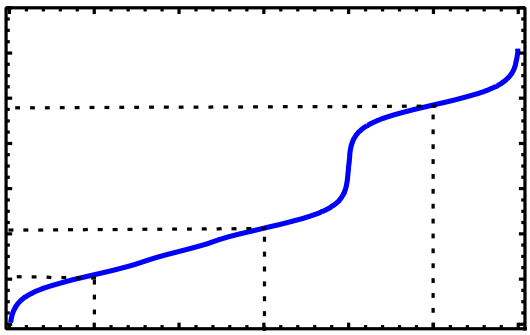


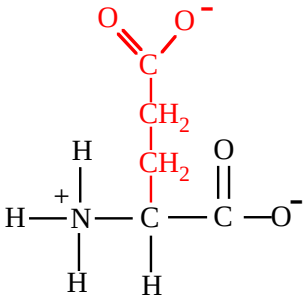
C Z A

12 other AA with non-ionizable side chains have titration curves just like Gly and Ala.

Titration of Glutamic Acid

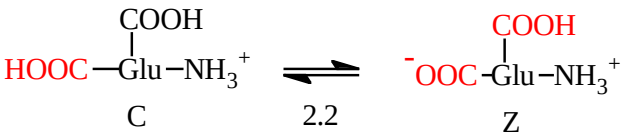
There are 3 buffering regions centred on pH 2.2, 4.25, and 9.7.





At 4.25 < pH < 9.7

What is the pI?

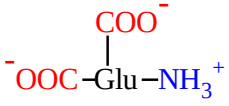


At pH 2.2 there is 50% C, 50% Z.
At pH 4.3 there is 50% Z, 50% A1.

4.3

At pH 9.7 there is 50% A1, 50% A2

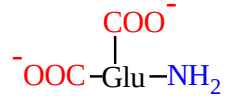
A1



So 1/2 way between 4.3 and 2.2 there will be 100% Z.

9.7

A2



So $pI = \frac{2.2 + 4.3}{2} = 3.25$

Titration of His:

There are 3 buffering regions centred on pH 1.8, 6.0, and 9.2.

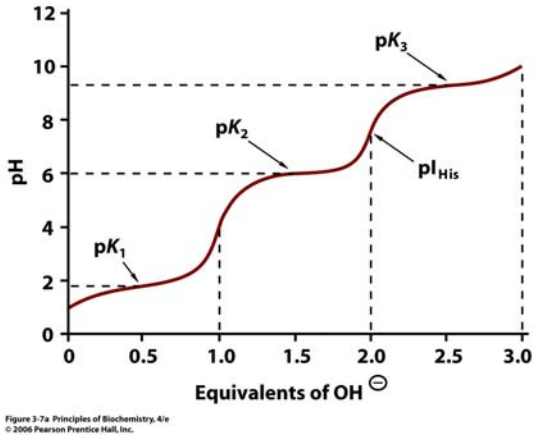


Figure 3-7a Principles of Biochemistry, 4/e
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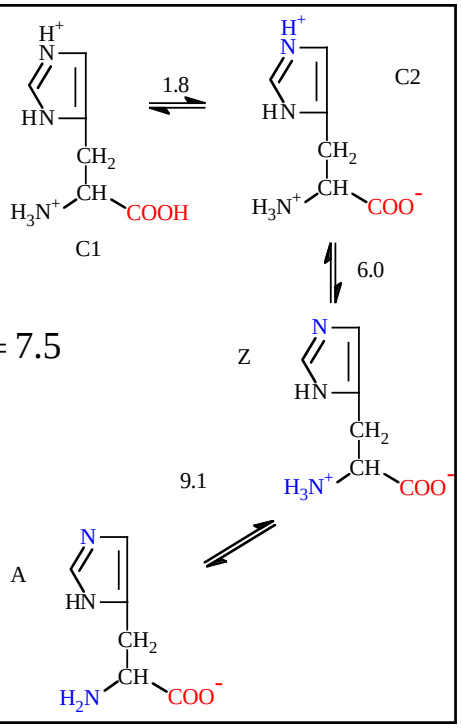
At pH 1.8 [C1] = [C2]

At pH 6.0 [C2] = [Z]

At pH 9.1 [Z] = [A]

What is the pI?

$$pI = \frac{9.1 + 6.0}{2} = 7.5$$



Sample Buffer Question:

To 50 mL of 0.1 M acetate buffer pH 4.75 , is added 1 mL of 0.2 M HCl.

What is the new pH? The pK_a of acetic acid is 4.75.

Sketch and label the titration curve:

What do I know?

pH = 4.75 = pK_a, so $[A^-] = [HA]$

$[A^-] + [HA] = 0.1$ M

$[A^-] = [HA] = 0.05$ M

Moles:

A^-: 0.05 M x 0.050 L = 0.0025 mole

HA: 0.05 M x 0.050 L = 0.0025 mole

HCl: 0.2 M x 0.001 L = 0.0002 mole

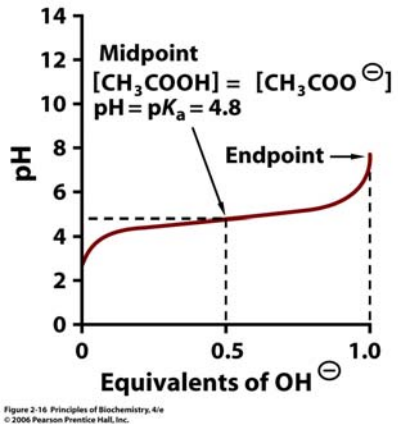


Figure 2-16 Principles of Biochemistry, 4/e
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$$HA \rightleftharpoons A^- + H^+$$

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

HCl: 0.002 mole of HCl will neutralize 0.002 mole of buffer (A^-).

i.e. 0.0002 H^+ react with 0.002 A^- to form 0.0002 new HA.

So HA_{new} = 0.0025 + 0.0002 = 0.0027 moles.

A^-_{new} = 0.0025 – 0.0002 = 0.0023 moles.

Plug into HH: $pH = 4.75 + \log\{\frac{[0.0023 mole / 0.051 L]}{[0.0027 mmoles / 0.051 L]}\}$

= 4.75 + log{0.85} = 4.75 – 0.07 = 4.68

Near the pK_a, a small addition of H^+ has caused only a small change in pH.

A second question:

To 50 mL of 0.1 M acetate buffer, pH 2.75, is added 1 mL of 0.2M NaOH. What is the new pH?

Here we cannot assume that we have equal amounts of HA and A^-.

Plugging into HH: $2.75 = 4.75 + \log\{\frac{[A^-]}{[HA]}\}$ and $-2.0 = \log\{\frac{[A^-]}{[HA]}\}$

Take the antilog of both sides gives:

$$10^{-2} = \frac{[A^-]}{[HA]} \qquad [HA] \bullet 10^{-2} = [A^-]$$

We also know that A^- + HA = 0.1 M

So 0.01 HA + HA = 0.1 M

We find HA = 0.099 M and A^- = 0.001 M

This is what we started with. Now, what happens when OH^- is added?

NaOH: 0.001 L x 0.2 M = 0.0002 mole

i.e. 0.0002 OH^- + 0.0002 HA → 0.0002 A^- + 0.0002 H_2O

So moles HA_{new} = (0.099 M x 0.050 L) – 0.0002 = 0.00475 mole

and A^-_{new} = (0.001 M x 0.050 L) + 0.0002 = 0.00025 mole

Plug into HH:

$$pH = 4.75 + \log\{\frac{[0.00025 mole / 0.051 L]}{[0.00475 mole / 0.051 L]}\}$$

= 4.75 + log {0.053} = 4.75 – 1.28 = 3.47

Far from the pK_a, a small addition of OH^- has caused a **large** change in pH.

Amino Acid Purification:

Amino acids, proteins, and nucleic acids are often purified using methods that separate the molecules based on differences in net charge.

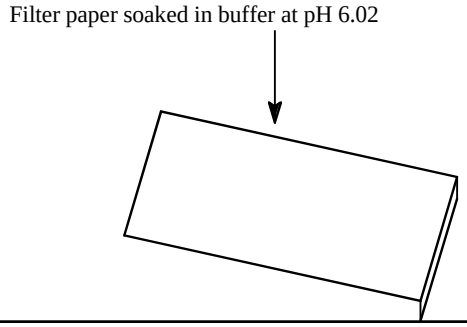
Electrophoresis separates molecules by applying an electric field. Ions move in the field according to their net charge.

In **paper electrophoresis** the ions move through a buffer solution that permeates the paper.

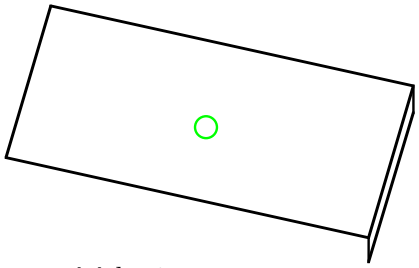
e.g. a mixture of Ala, Lys, and Asp at pH 6 will have different charges.

AA	Net Charge
Ala	0
Lys	+
Asp	-

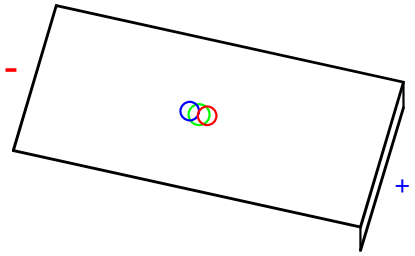
Ala will be stationary, **Lys** will move toward the cathode (-) and **Asp** will move toward the anode (+).



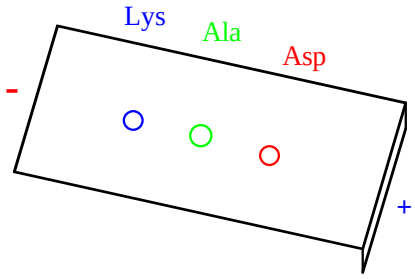
Spot a mixture of AA



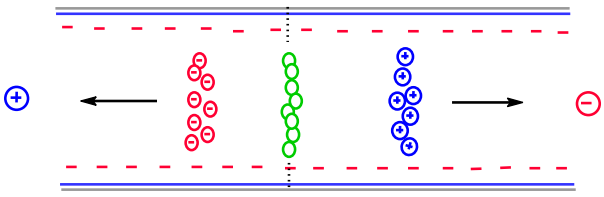
AA begin to separate.



After electrophoresis

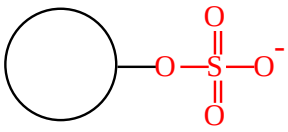


Amino acids can also be separated in thin silica capillaries - **Capillary Electrophoresis**.

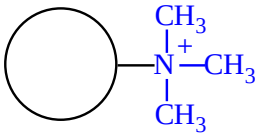


Ion-Exchange Chromatography

Polystyrene or silica-based beads have anionic or cationic functional groups attached.



Cation Exchanger



Anion Exchanger

Sulfonic acid is a strong acid and is **anionic** at pH 2 whereas all amino acids are **cationic**.

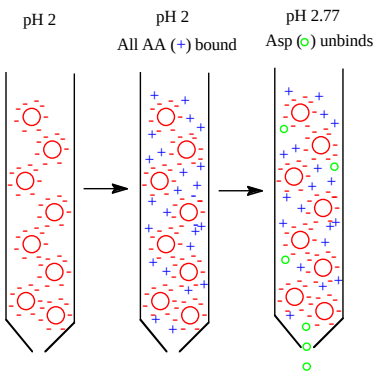
Beads with sulphonic acid are packed into a cylindrical column.

A mixture of AA is applied in a buffer at pH 2 and bind to the sulphonic acid.

The AA are removed by washing the beads with buffers at higher pH.

When the pH reaches the pI of an AA it unbinds from the beads and washes out of the column.

Differences in the pI of each AA cause them to be dissociated at different pHs.



Here is a typical elution profile:

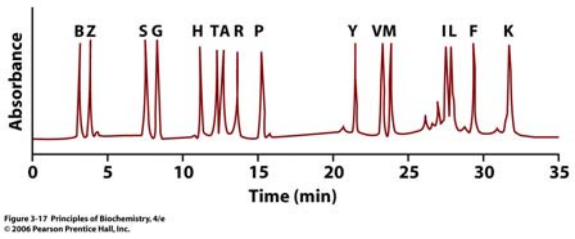


Figure 3-17 Principles of Biochemistry, 4/e
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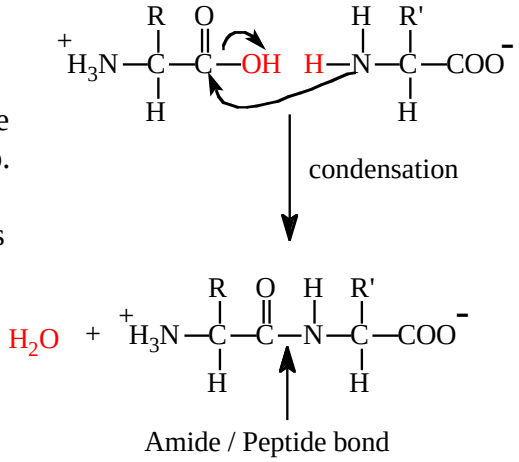
Peptides

The **condensation** of AA does not occur in neutral water as the OH is not a good leaving group.

You will learn how cells do this in CHEM/MBIO 2780.

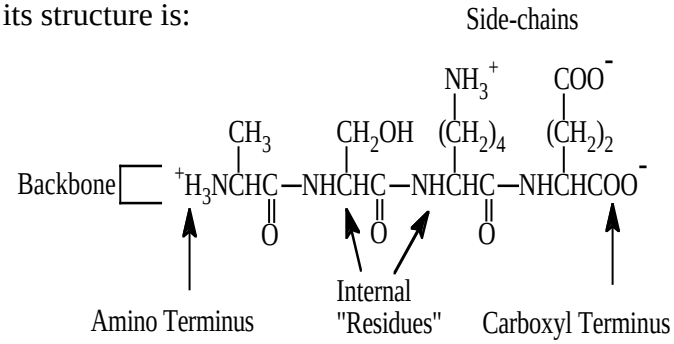


Polypeptide: more than 20 AA.

Protein: 1 or more polypeptides.

Alanyl-Seryl-Lysyl-Glutamate is a tetrapeptide.

At pH 7 its structure is:



Notes:

1. Repeating backbone is non-ionizable, except the ends.

2. Side-chains vary.

3. pK_a, pI of side chains and termini are similar to the AA.

4. Amino terminus at left, carboxyl terminus at right.

Biologically-Active Peptides

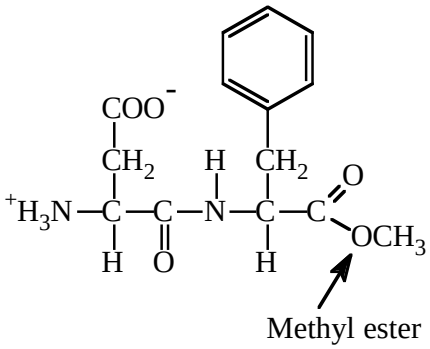
Enkephalins are natural analgesics. They bind to specific receptors in the brain and diminish the perception of pain. *e.g.*

Tyr-Gly-Gly-Phe-Met

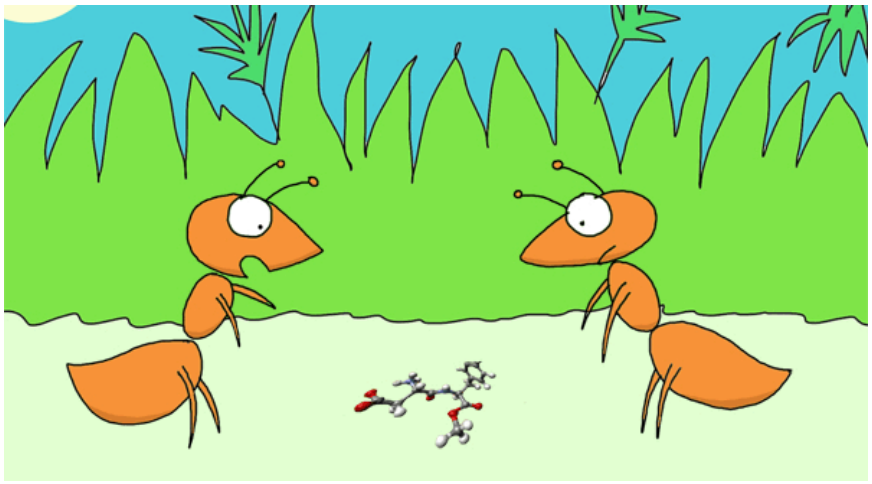
Peptides can adopt many **conformations** because of rotation about single covalent bonds.

Binding to a target receptor may force them to adopt a single conformation.

Aspartame: a synthetic dipeptide 150 X sweeter than sugar.



Nuh-huh. Aspartame



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