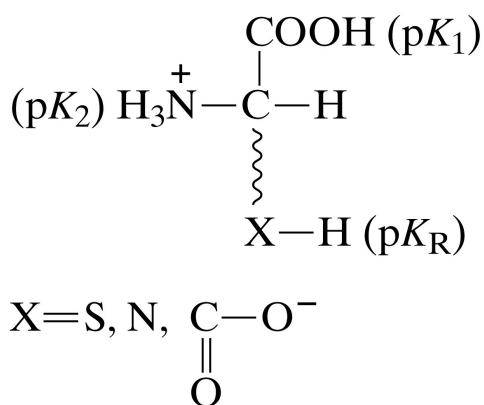


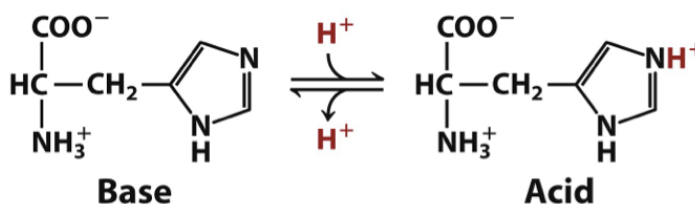
**Amino Acids as Acids, Bases and Buffers:**

- Amino acids are weak acids
- All have at least **2 titratable protons** (shown below as fully protonated species) and therefore have 2 pKa's
  - o  $\alpha$ -carboxyl (-COOH)
  - o  $\alpha$ -amino (-NH<sub>3</sub><sup>+</sup>)
- Some amino acids have a **third** titratable proton in the R group and therefore a third pKa
  - o Showing all protonated:



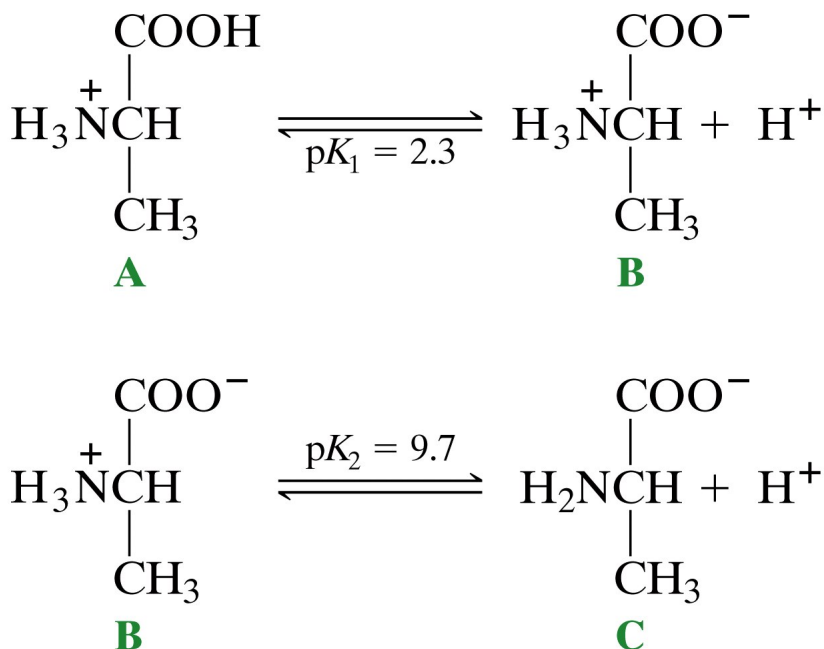
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- Cysteine (Cys, C) has -SH
- Tyrosine (Y, Tyr) has -OH
- Lysine (Lys, K) has -NH<sub>3</sub><sup>+</sup>
- Arginine (Arg, R) has =NH<sub>2</sub><sup>+</sup>
- Histidine (His, H) has  $\text{NH}^+$
- Aspartate (Asp, D) has -COOH
- Glutamate (Glu, E) has -COOH



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Histidine side chain ionization  
Imidazolium ion (an acid):



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**pKa Table for amino acids:**\* First column ( $pK_{a1}$ ) = COOH\* Second column ( $pK_{a2}$ ) =  $NH_3^+$ \* Third column ( $pK_{aR}$ ) = R group  $H^+$ **Table 3.2**

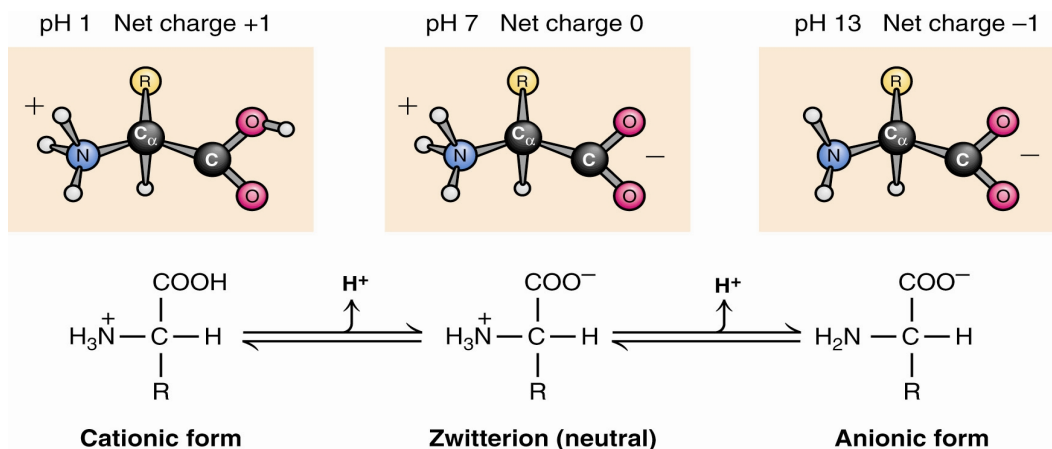
Table of  $pK$  values for the 20 amino acids found in proteins.  $pK_1$  values are assigned to the  $\alpha$ -carboxyl group,  $pK_2$  values to the  $\alpha$ -amino group, and  $pK_R$  to ionizable groups in the R side chain (see structure).

Name	$pK_1$	$pK_2$	$pK_R$
Glycine	2.4	9.8	
Alanine	2.3	9.7	
Valine	2.3	9.6	
Leucine	2.4	9.6	
Isoleucine	2.4	9.7	
Methionine	2.3	9.2	
Phenylalanine	1.8	9.1	
Proline	2.0	10.6	
Serine	2.1	9.2	
Threonine	2.6	10.4	
Cysteine	1.8	10.8	8.3
Asparagine	2.0	8.8	
Glutamine	2.2	9.1	
Tyrosine	2.2	9.1	10.9
Tryptophan	2.4	9.4	
Aspartate	2.0	10.0	3.9
Glutamate	2.2	9.7	4.3
Histidine	1.8	9.2	6.0
Lysine	2.2	9.2	10.8
Arginine	1.8	9.0	12.5

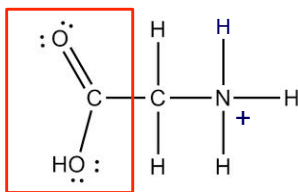
Table 3-2 Concepts in Biochemistry, 3/e  
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**AMINO ACIDS AS WEAK ACIDS:**

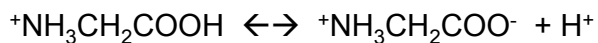
- Properties of amino acids in proteins and peptides are determined by the R group but also by the charges of the titratable group. Will ultimately affect protein structure.
- Important to know which groups on peptides and proteins will be protonated at a certain pH.



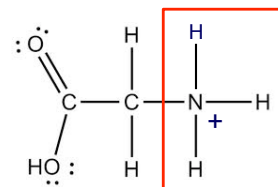
*The degree of dissociation depends on the pH of the solution.*

**Glycine**

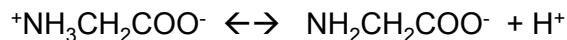
The first dissociation is the carboxylic acid group (using glycine as an example):



$$K_{a1} = \frac{[^+\text{NH}_3\text{CH}_2\text{COO}^-][\text{H}^+]}{[^+\text{NH}_3\text{CH}_2\text{COOH}]}$$



The second dissociation is the amino group in the case of glycine:



$$K_{a2} = \frac{[\text{NH}_2\text{CH}_2\text{COO}^-][\text{H}^+]}{[^+\text{NH}_3\text{CH}_2\text{COO}^-]}$$

How do we do this??

Example – Alanine

1. Draw the fully protonated structure

Q: Which protons come off when?

A: Look at pKa table for amino acids

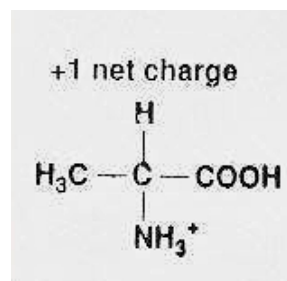
**Alanine has 2 pKas:**

$\alpha$ -COOH (pKa = 2.3) comes off first (has lower pKa)

$\alpha$ -NH<sub>3</sub><sup>+</sup> (pKa = 9.9)

Others come off SEQUENTIALLY in ascending order of pKa.

2. Write out structures for sequential deprotonation and place pKa values over the equilibrium arrows.



Alanine

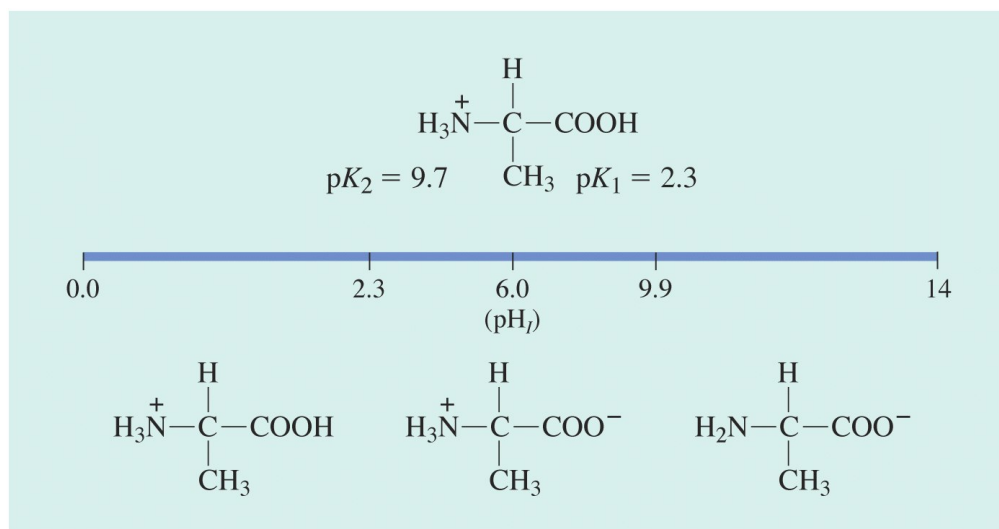


Figure 3-4b Concepts in Biochemistry, 3/e  
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Fully protonated  
Net charge = +1

1<sup>st</sup> proton removed  
Net charge = 0

2<sup>nd</sup> proton removed  
Net charge = -1

So, from looking at the net charges, at different pH's, amino acids can have different charges!  
Very important for protein structure!!

Remember that the  $pK_a = pH$  when  $\frac{1}{2}$  of an available amount of an ionizable group is ionized.

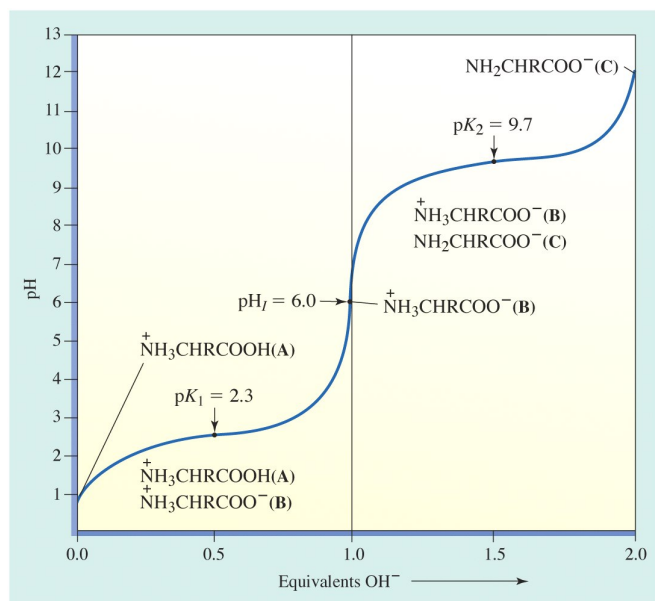


Figure 3-4a Concepts in Biochemistry, 3/e  
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- Let's take a look at the titration curve for Alanine

- Looks very much like what we saw for acetic acid last time except that it has 2 midpoints ( $pK_a$ 's) – one for each proton  $\alpha$ -COOH and  $\alpha$ -NH<sub>3</sub><sup>+</sup>

- At beginning, all protonated
- Need one equivalent of base for each proton
- At each HALF equivalent =  $pK_a$ 
  - 50% protonated/50% deprotonated
- At end all deprotonated

- Flat parts of curve are **BUFFERING REGIONS: Acts as buffer in TWO pH ranges.**
  - +/- 1 pH unit from  $pK_a$
- For our purposes, to determine whether the proton is ON or OFF at a certain pH use the following RULES
- - $pH = pK_a$  Equal amounts of protonated and deprotonated species exist

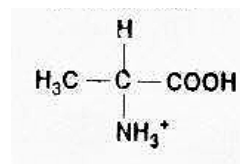
$pH < pK_a$
H <sup>+</sup> on, substance protonated
$pH > pK_a$
H <sup>+</sup> off, substance deprotonated

if pH is LESS than the  $pK_a$  of a particular group, that group will be predominantly protonated

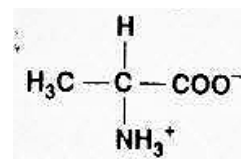
if pH is GREATER than the  $pK_a$  of a particular ionizable group, that group will be predominantly deprotonated

For example: Alanine at different pH's (see  $pK_a$  table)

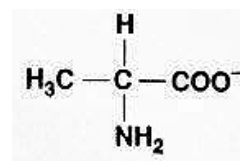
At pH 1.5: pH is less than the  $pK_a$  of both the  $\alpha$ -COOH and the  $\alpha$ -NH<sub>3</sub><sup>+</sup>, therefore, both protons are ON



At pH 7: pH is greater than the  $pK_a$  of the  $\alpha$ -COOH  $\rightarrow$  H<sup>+</sup> OFF  
pH is less than the  $pK_a$  of the  $\alpha$ -NH<sub>3</sub><sup>+</sup>  $\rightarrow$  H<sup>+</sup> ON



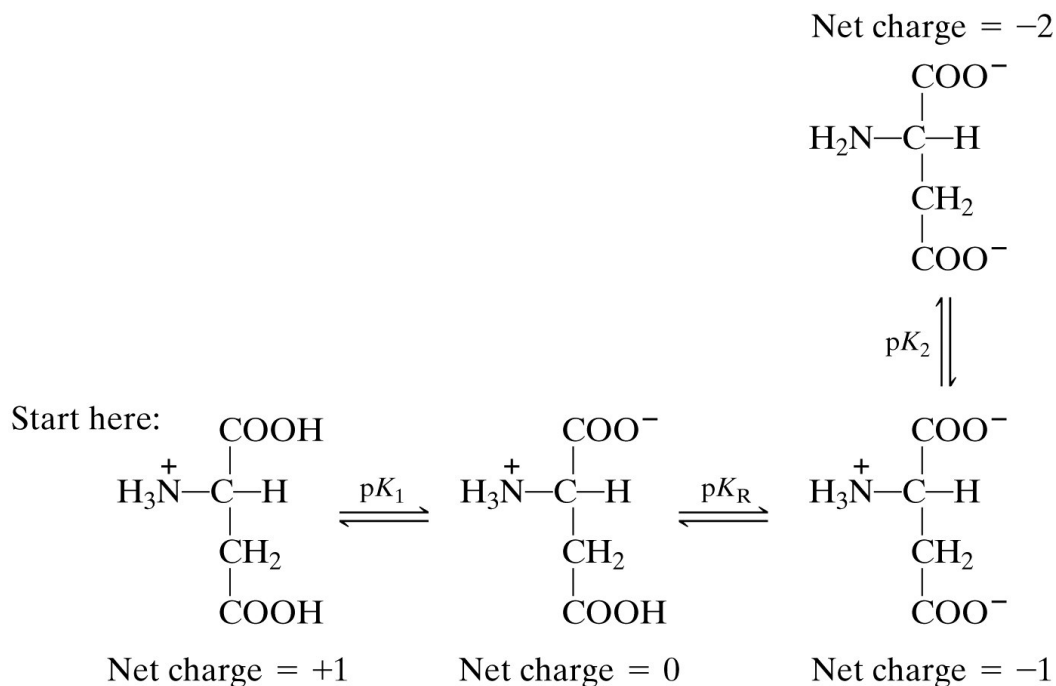
At pH 10.5    pH is greater than the pKa of the  $\alpha$ -COOH  $\rightarrow$  H<sup>+</sup> OFF  
 pH is greater than the pKa of the  $\alpha$ -COOH  $\rightarrow$  H<sup>+</sup> OFF



Apply same rules if there are 3 titratable protons:

1. Determine what the pKa's of the titratable protons are by looking at the pKa table
2. Draw the structures and the equilibria representing the complete deprotonation of the amino acid
  - a. Start with fully protonated and then remove in order of pKa values
  - b. Put pKa values above equilibrium
3. Determine at the pH of interest whether the proton is ON or OFF using the above rules

For example, Aspartate (D, Asp): Asp has 3 titratable protons



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1. pKa's for the three groups (look at Table 3.2)
2. Draw the structures from fully protonated to fully deprotonated

Note that all amino acids are at one point, electrically neutral at some pH value.

This **pH = isoelectric point (pI)**

### How do you calculate pI?

1. Draw out the complete ionization of amino acid
2. Determine net charge on each ionized form
3. Find the structure that has no net charge
4. Take the average of the pKa's that are around the structure

$$pI = \frac{pK_{a1} + pK_{a2}}{2}$$

5. Note do **NOT** just take the average of all pKa's.

pI

Ala:  $\frac{2.3 + 9.9}{2} = 6.1$

### What about Asp?? pKas: 2, 3.9, 10 (from Table 3.2)

$$pI = (2 + 3.9) / 2 = 2.95$$

Amino acids can be separated on the basis of their charges at a certain pH

### HOW ARE AMINO ACIDS MADE?

- Many organisms can make all 20 of the amino acids
  - o Bacteria, yeast, and plants
- Some amino acids are made from common metabolic intermediates directly
  - o For example, alanine is made from pyruvate (transamination of pyruvate with glutamate as the amino donor)
- Some amino acids are made as products from long and complex pathways
  - o For example, aromatic amino acids are made from the **shikimic acid pathway**
- Humans and other animals CANNOT make some of the 20 amino acids
  - o These are **ESSENTIAL AMINO ACIDS**

**Arginine** and **Histidine** are essential only in babies or in people with extreme metabolic stress disease – Conditional Essential Amino Acids

- Foods vary in “protein quality”
  - o Content of essential amino acids

**Table 19.2**

### Essential and nonessential amino acids in humans

Essential	Nonessential
Arginine <sup>a</sup>	Alanine
Histidine <sup>a</sup>	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine <sup>b</sup>

<sup>a</sup>Arg and His are essential in juveniles but not adults.

<sup>b</sup>Tyr is synthesized from Phe.

Table 19-2 Concepts in Biochemistry, 3/e  
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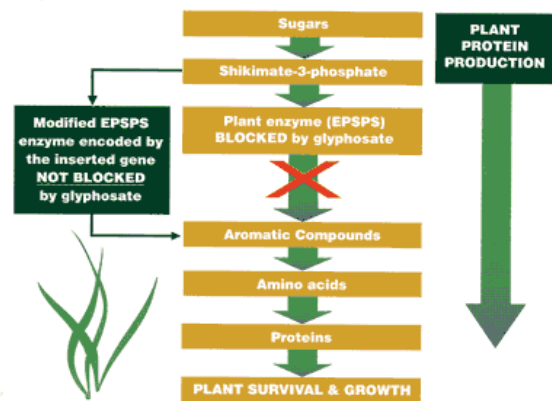


- Animal proteins are often of a “higher quality” than vegetable proteins
  - Cereals are deficient in **Lys**
  - Legumes are low in **Met** and **Cys**
  - So vegetarians need variety
- Since human cannot make all 20 aa's – we are susceptible to **protein malnutrition** especially in children and elderly adults
- Disorder called **Kwashiorkor**
  - Protein disorder in children
  - Prevalent in overpopulated areas, particularly sections of Africa, Central & South America, and South Asia
  - Areas of famine, limited food supply, diet mainly consisting of starchy vegetables
  - Diet is adequate in calories (energy) but deficient in amino acids
- Symptoms include: lethargy, irritability, protruding belly, changes in skin pigment, hair changes, increased infections due to damaged immune system, enlarged liver, renal problems.
- Treatment: Depends on severity
- Increase calories from proteins usually in form of dry milk
- If not treated soon enough, permanent physical and intellectual disabilities can result

## HOW CAN WE EXPLOIT THE FACT THAT WE HAVE ESSENTIAL AMINO ACIDS?

EXAMPLE: Use in agriculture:

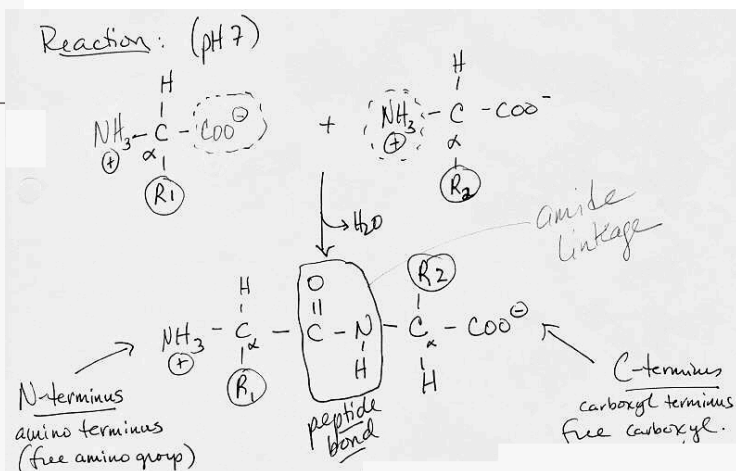
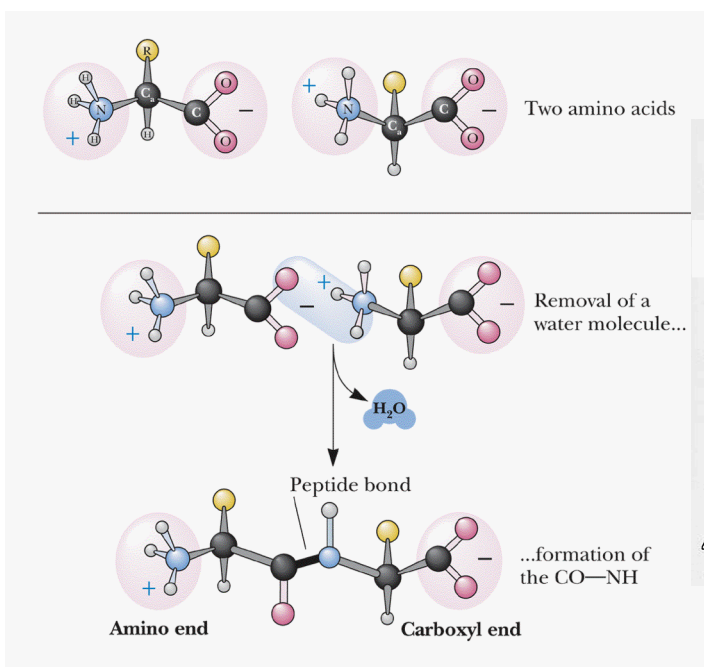
- Pathways leading to essential amino acids are good targets for herbicides
- **Glyphosate (RoundUp) (N-phosphonomethyl-glycine)**
  - Inhibitor of **EPSP synthase**, an enzyme in the shikimic acid pathway
  - Blocks the production of aromatic amino acids
  - Non-selective killer of anything green!
- Glyphosate is relatively non-toxic to humans because we do NOT make our own aromatic amino acids.
- RoundUp Ready soybeans and cotton carry an altered gene for EPSP synthase – introduced into the plants using biotechnology
- Altered gene encodes a protein that resists the RoundUp inhibitor



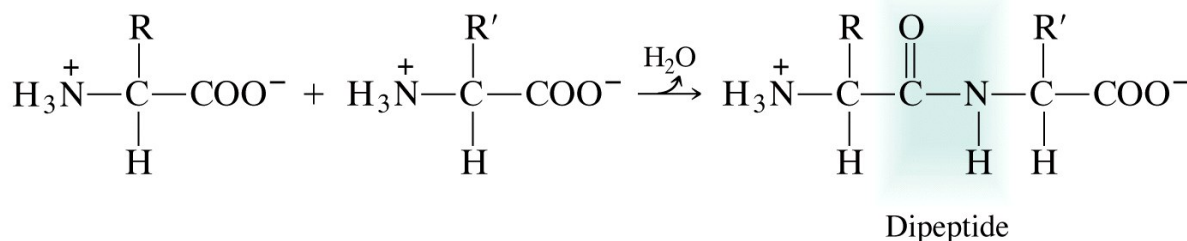
## PEPTIDES and PROTEINS

- Learned basic chemistry of amino acids – structure and charges
- Chemical nature/charges of amino acids is **CRUCIAL** to the structure and function of proteins
- Amino acids can assemble into chains (peptides, polypeptides, proteins)
  - o Can be very short to very long
    - Dipeptide = two amino acids linked
    - Tripeptide = three amino acids linked
- Amino acids sometimes called **RESIDUES**
- Identity and function of a protein or peptide is determined by
  - o Amino acid **composition**
  - o **Order** of amino acids in the chain
  - o Enormous variety of possible sequences
    - e.g., if you have a protein with 100 aa, there are  $1.27 \times 10^{130}$  possible sequences!
- Amino acids are linked by **COVALENT BONDS = PEPTIDE BONDS**
- Peptide bond is an **amide linkage** formed by a **condensation reaction** (loss of water)
- Brings together the **alpha-carboxyl** of one amino acid with the **alpha-amino** of another
- Portion of the AA left in the peptide is termed the amino acid **RESIDUE**
  - o Amino acids sometimes called **RESIDUES**
- R groups remain **UNCHANGED** – remain active
- N-terminal amino and C-terminal carboxyl are also available for further reaction
- Reaction is **NOT** thermodynamically favorable (not spontaneous)
  - o Need energy and other components and instructions to correctly assemble
    - This is the process of **protein translation**

### FORMATION OF THE PEPTIDE BOND







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### Animation of peptide bond formation:

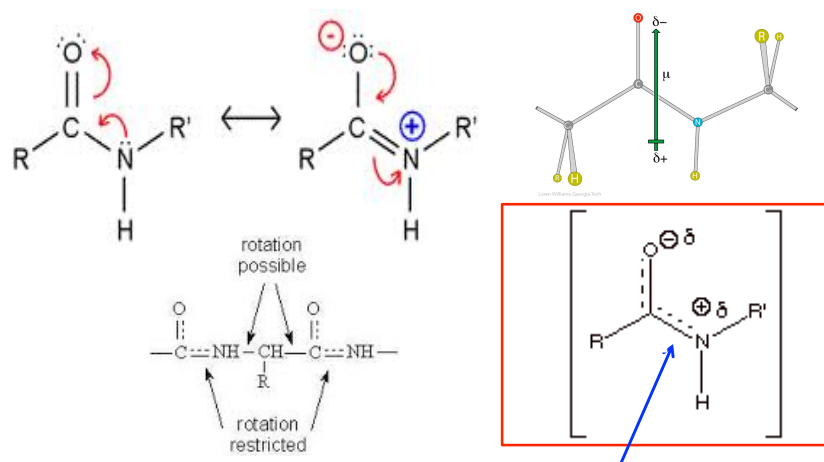
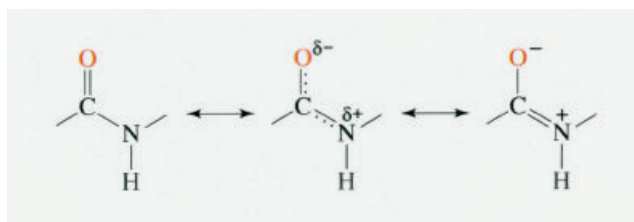
<http://intro.bio.umb.edu/111-112/111F98Lect/PeptideBond.html>

<http://www.wisc-online.com/objects/ViewObject.aspx?ID=BIC007>

- Peptides are always written in the N → C direction
- Each peptide has ONLY ONE free amino group and ONE free carboxyl group; others are neutralized by formation of the peptide bond

### IMPORTANT FEATURES OF THE PEPTIDE BOND:

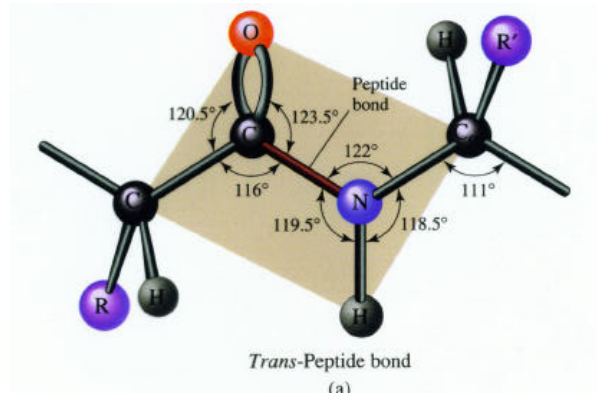
1. Peptide bonds have **double bond character** resulting from resonance stabilization (C-N bond has 40% double bond character) C-N and C-O have partial double bond character



**Resonance Results in Partial Double Bond Character of the Peptide Bond (Amide Bond):**

**Rotation Restricted**

- Results in the peptide bond being **PLANAR**
  - o C, N, H, O are all in the same plane (C $\alpha$ 's are also in plane)
  - o *p* orbitals can overlap to form partial double bonds between the nitrogen and carbon and the carbon and oxygen

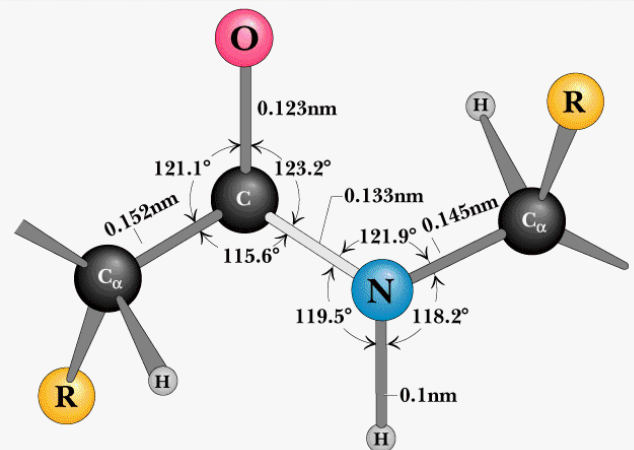


- Stronger than a normal bond because of the double bond character
- No tendency to protonate
- No significant rotation occurs around the peptide bond itself.

- **PEPTIDE BACKBONE** is made up of these rigid planar units with limited rotation around the C $\alpha$  carbons – N-C $\alpha$ 1 (phi) and the C $\alpha$ 2-C (psi) bonds. These bonds link the peptide groups in the peptide chain

- o Crucial for protein structure
- o Rotation can occur at the  $\alpha$ -carbons
- o However, rotation is limited due to steric hindrance (R groups)
- o These limitations limit the shapes that can be assumed by protein chains

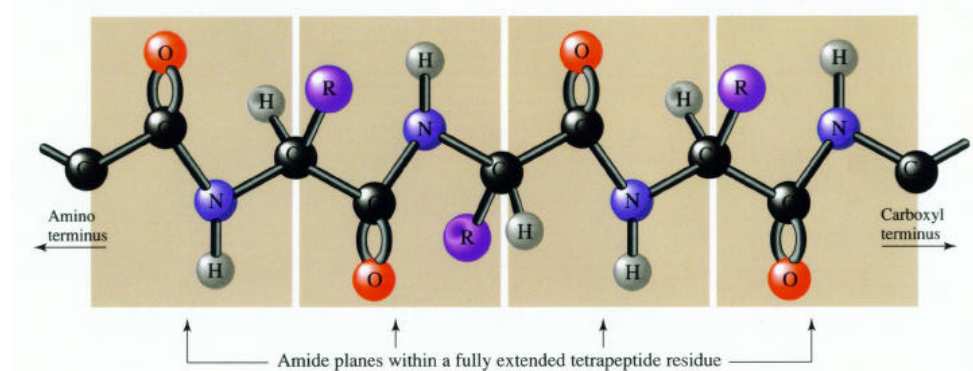
#### PEPTIDE BOND----RIGID AND PLANAR



**H AND O TRANS, NO ROTATION ABOUT C-N BOND(PARTIAL DOUBLE BOND CHARACTER) HOWEVER ROTATION ALLOWED ABOUT**

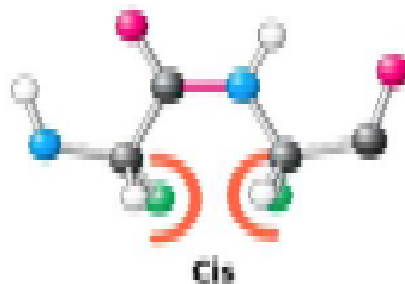
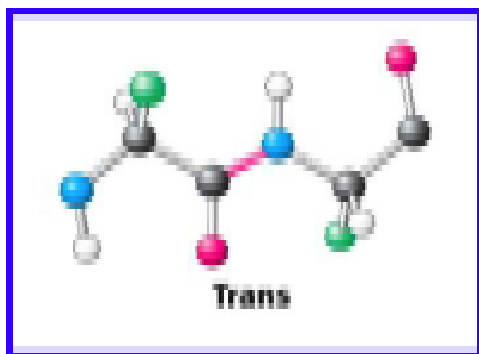
**C ( $\alpha$ ) -N**

**C ( $\alpha$ ) -CARBONYL CARBON**



**These limitations force C $\alpha$  carbons on either side of the peptide bond to be, with few exceptions, *trans* to each other (opposite sides of the peptide bond)**

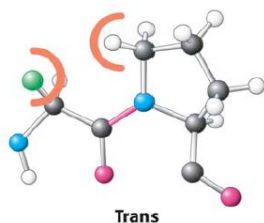
- a. Cis configuration is rare; steric hindrance is greater
- b. Trans is much more stable (sterically favored)
- c. R-groups (on the C $\alpha$  carbons) also end up on opposite sides of the peptide bond
  - i. (R-groups away from each other – favored)



**ALMOST EXCLUSIVELY TRANS CONFIGURATION**

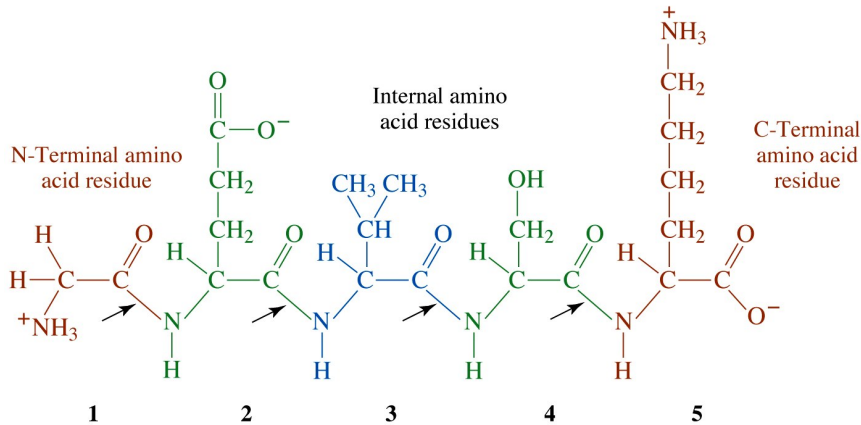
## Proline

- Proline is the exception with ~ 10% of the proline residues in proteins following a *cis* peptide bond



### SUMMARY:

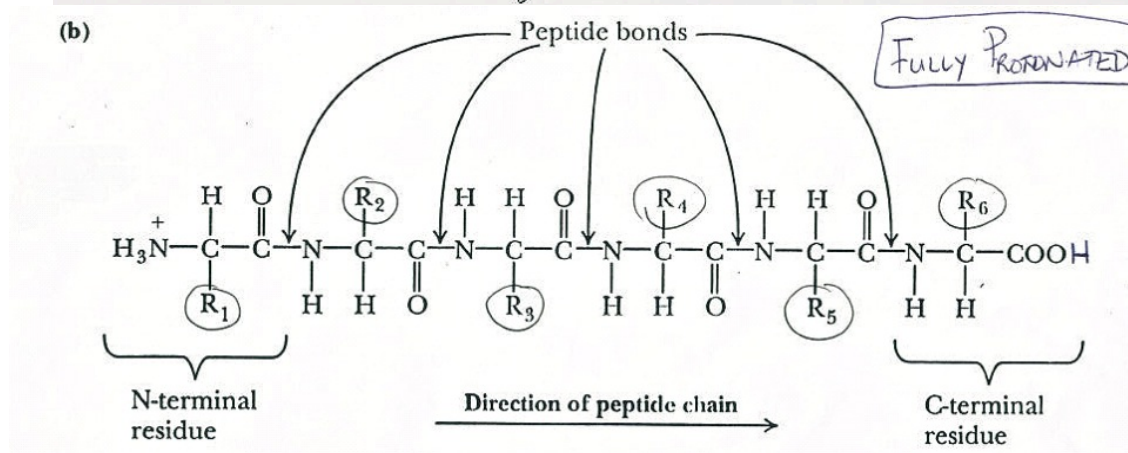
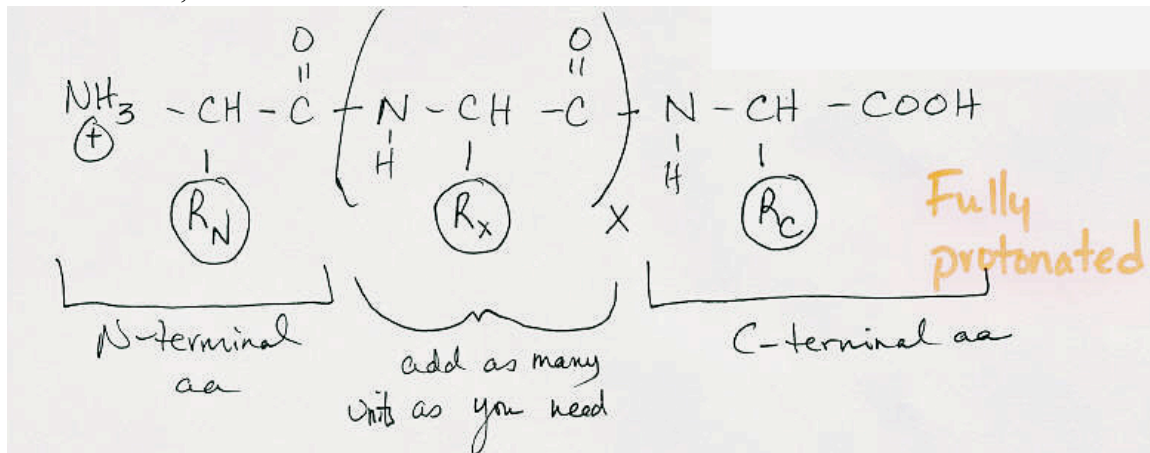
1. C $\alpha$ , O, C, N and H atoms are planar
2. No rotation around the peptide bond
3. C $\alpha$  (with R groups) groups are in *trans* configuration (i.e. H on the amide Nitrogen is opposite to the Oxygen of the carbonyl)
4. Peptide backbone is rigid

**DRAWING PEPTIDES GIVEN THEIR AMINO ACID SEQUENCE:**

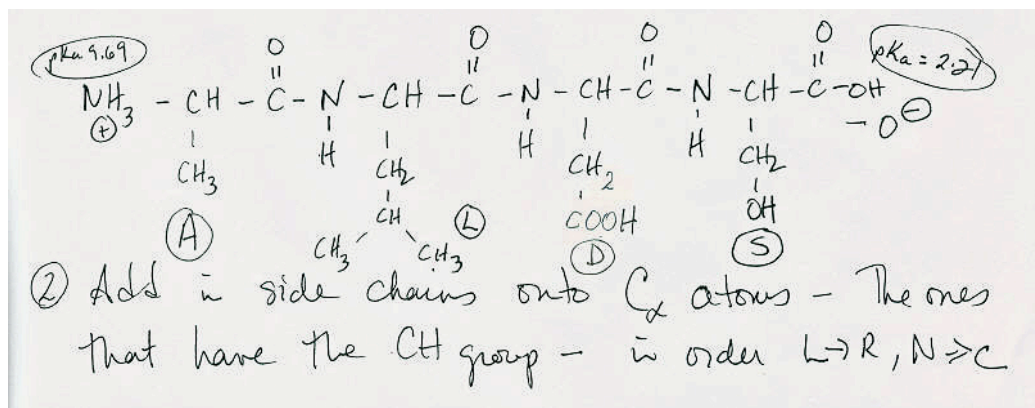
- Peptide = ALDS
- Left → Right = N → C-terminus (amino to carboxyl termini)
- Want to draw it at pH 5

- 1) Draw the PEPTIDE BACKBONE fully protonated for as many amino acids as you have. For convenience, convention and ease, put the R groups down and the carbonyls up.

In our case, we have FOUR amino acids:



2. Add in the side chains onto the C $\alpha$  carbons – the ones that have the CH group – in order left to right. Draw side chains fully protonated as well.



3. Determine if groups are ionized at the pH that you are interested in.

Remember if pH > pKa, the H<sup>+</sup> is OFF (deprotonated)

Let's say pH 5:

- Terminal carboxyl group is **DEPROTONATED** (5 > 2.21)
- Terminal amino group is **NOT DEPROTONATED** (5 < 9.69)
- Carboxyl group on Aspartate is **DEPROTONATED** (5 > 3.9)

Go back to fully protonated figure above and remove 2 protons: One from the terminal carboxyl, and one from the side chain of Asp.

**Note:** In di, tri, or polypeptides, there is only **ONE** free amino group (N-terminus) and **ONE** free carboxyl group **UNLESS** present in R groups.

You should be able to write out the full structures of peptides at different pH values.

Calculate an approximate pI for the peptide Val-Cys-Arg-Phe-His-Asp-Gln.

$\alpha$ -carboxyl COOH (pKa 2.2)

Asp R-COOH (pKa 3.9)

His imidazole + (pKa 6.0)

$\alpha$ -amino NH<sub>3</sub><sup>+</sup> (pKa 9.6)

Cys-SH (pKa 8.3)

Arg guan. + (pKa 12.5)

Sketch out starting with fully protonated and remove protons in order until you get the neutral species.

Take the average of the pKa's surrounding the neutral species.

What can you do with the charge information?

- 1) Calculate net charge of a peptide at a specific pH
- 2) Determine pH range of a peptide based on given structure and charge
- 3) Calculate pI of simple peptide

Net charge at low pH = +3, so have to add 3 equivalents of base to get to net charge zero. 3 equiv. base would completely titrate the  $\alpha$ -carboxyl, R-carboxyl, and His imidazole, so pI would be halfway between pK of imidazole and pK of  $\alpha$ -amino group, i.e. (6.0 + 8.3)/2 so pI = 7.15.