BCH2333 Introduction to Biochemistry

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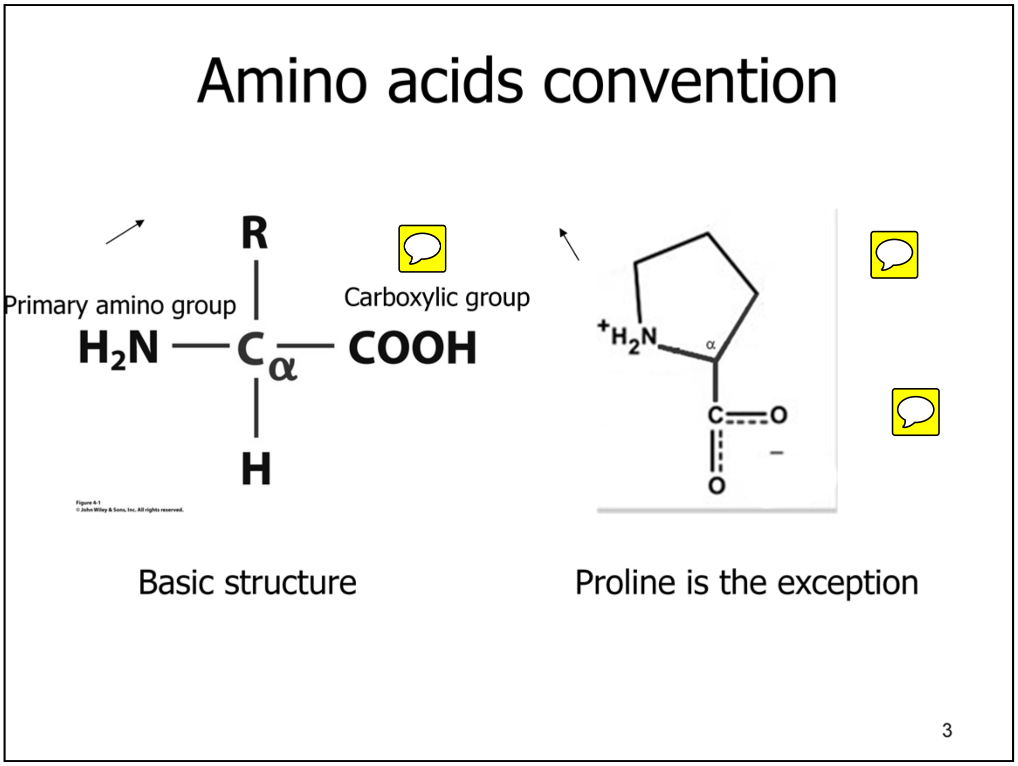
- Lectures 9-12, 13-14: Amino acid, peptides, proteins, and enzyme kinetics

Some information are posted on the course blackboard.

1-jmol structure of all L-amino acids and two D-amino acids.

You can download the jmol software at http://jmol.sourceforge.net/ . You can use jmol with the jmol structure files to familiarize yourself with the structures of amino acids and the differences between L and D amino acids.



Amino acids convention

Primary amino group

Carboxylic group

Figure 4-1
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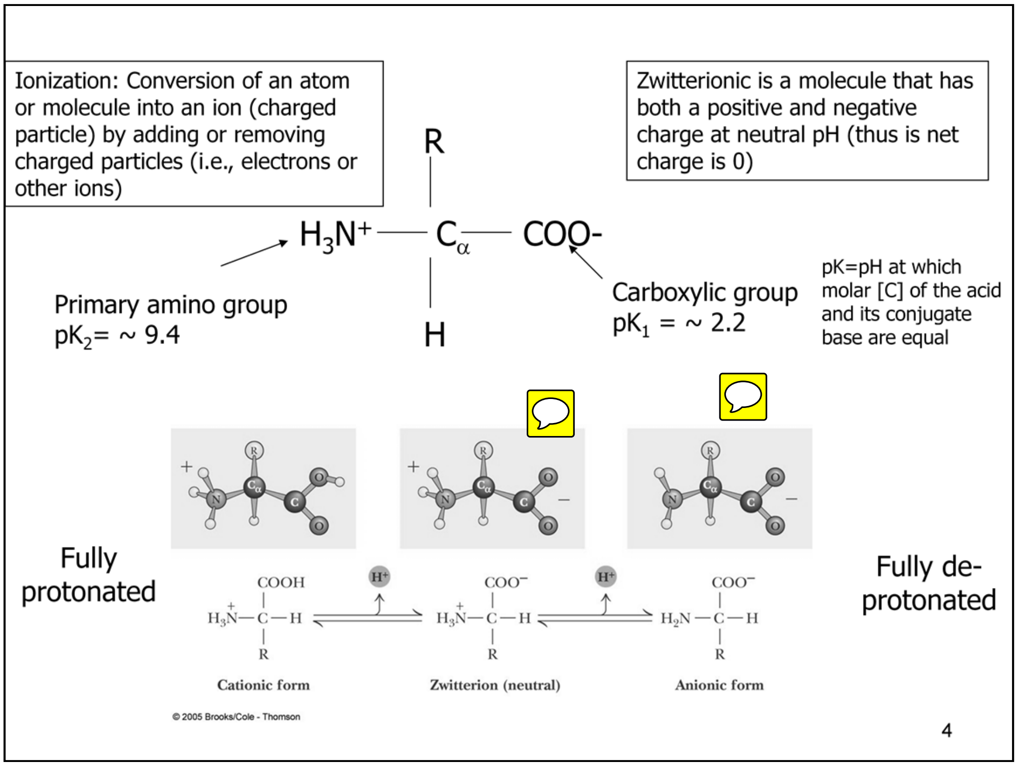
Basic structure

Proline is the exception

An amino acid is a carboxylic acid, a primary amino group, and a variable side chain attached to a carbon. Most amino acids are alpha- amino acids i.e. the amino group is attached to the alpha-carbon. Proline is the exception to this rule. It has a secondary amino group that participates in two bonds with the alpha and the delta carbon. Proline alpha carbon and the secondary amino group are part of a ring structure.



Ionization: Conversion of an atom or molecule into an ion (charged particle) by adding or removing charged particles (i.e., electrons or other ions)

Zwitterionic is a molecule that has both a positive and negative charge at neutral pH (thus is net charge is 0)

$H_3N^+ — C_\alpha — COO^-$ (R above C_α, H below)

Primary amino group $pK_2 = \sim 9.4$

Carboxylic group $pK_1 = \sim 2.2$

pK=pH at which molar [C] of the acid and its conjugate base are equal

Fully protonated

Fully de-protonated

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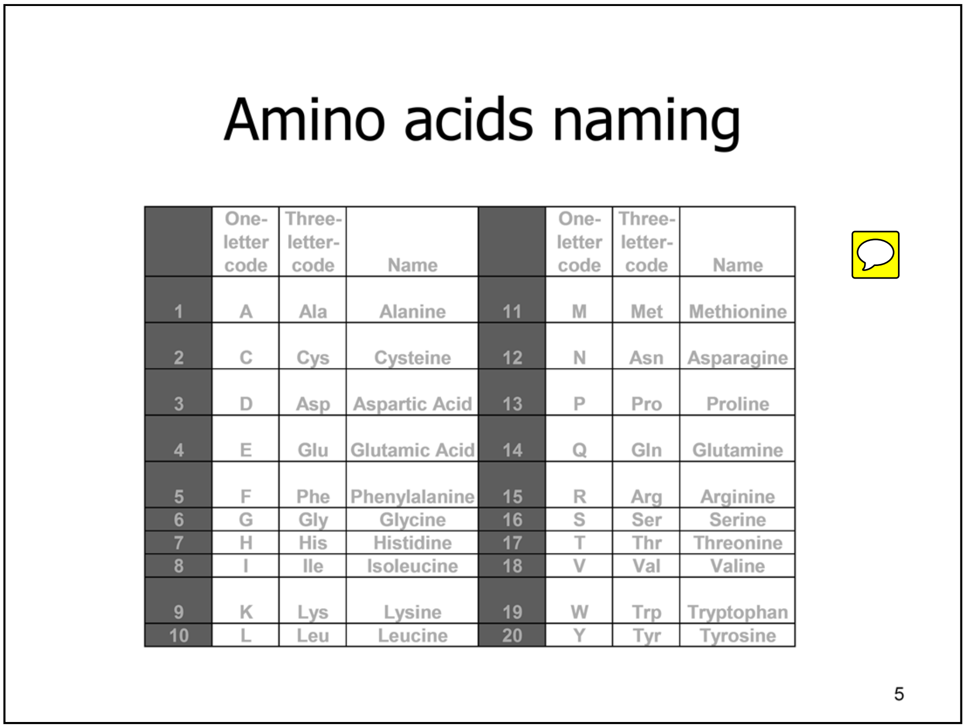
The isoelectric point (pI) is the pH at which the molecule will be neutral. At pH below this point the molecule will be positively charged whereas at pH above this point the molecule will be negatively charged.

You will notice that the pKa of the alpha-carboxylic acid and the protonated alpha-amino groups are relatively similar across the 20 amino acids . The side chain R will affect the pKa. Largest differences for proline and cysteine.

Some of the amino acids have different groups that can be ionized beyond the alpha-carboxylic acid and the alpha-amino group. For example, lysine has one alpha-carboxylic acid, one alpha-amino group and one epsilon amino group. These ionizable side chain will contribute to the pI.



Amino acids naming

	One-letter code	Three-letter-code	Name		One-letter code	Three-letter-code	Name
1	A	Ala	Alanine	11	M	Met	Methionine
2	C	Cys	Cysteine	12	N	Asn	Asparagine
3	D	Asp	Aspartic Acid	13	P	Pro	Proline
4	E	Glu	Glutamic Acid	14	Q	Gln	Glutamine
5	F	Phe	Phenylalanine	15	R	Arg	Arginine
6	G	Gly	Glycine	16	S	Ser	Serine
7	H	His	Histidine	17	T	Thr	Threonine
8	I	Ile	Isoleucine	18	V	Val	Valine
9	K	Lys	Lysine	19	W	Trp	Tryptophan
10	L	Leu	Leucine	20	Y	Tyr	Tyrosine

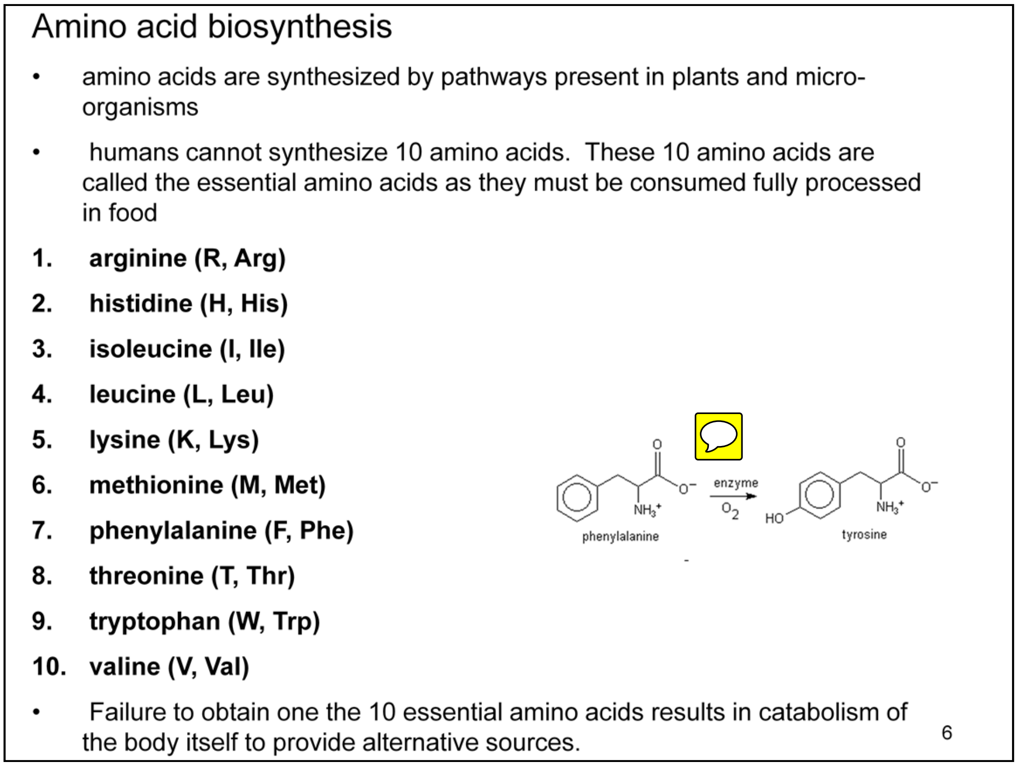
You need to learn the one and the three letter codes. The three letter codes are utilized for amino acids and short peptides. Long peptides and proteins use the one letter codes. As well, many public databases prefer to use the one letter codes because they save storage space (3x less space when using one letter codes instead of three letter codes). Dr. Margaret Oakley Dayhoff developed the one letter codes for the amino acids. For some amino acids the logic is pretty apparent while for others (as she was running out of letters) the codes were pretty far fetched. Here are how the one letter codes were selected.

Most of the one letter codes are easy to remember as the amino acid either start by the same letter or is phonetically the same sound.

Alanine	A	First letter of the name
Glycine	G	First letter of the name
Leucine	L	First letter of the name
Proline	P	First letter of the name
Threonine	T	First letter of the name
Phenylalanine	F	sounds like Fenylalanine
Tryptophan	W	tWiptophan (or, contains Double ring)
Arginine	R	aRginine
Tyrosine	Y	tYrosine
Aspartic Acid	D	asparDic
Asparagine	N	Contains N (or asparagiN)
Glutamic Acid	E	gluE (or glutamEke)
Glutamine	Q	Q-tamine

By the time Dr. Dayhoff got to lysine, there were not too many letters left, so she used the letter K, explaining that K is at least near L in the alphabet.

Lysine	K	K is near L in the alphabet



Amino acid biosynthesis

- amino acids are synthesized by pathways present in plants and micro-organisms
- humans cannot synthesize 10 amino acids. These 10 amino acids are called the essential amino acids as they must be consumed fully processed in food

1. **arginine (R, Arg)**
2. **histidine (H, His)**
3. **isoleucine (I, Ile)**
4. **leucine (L, Leu)**
5. **lysine (K, Lys)**
6. **methionine (M, Met)**
7. **phenylalanine (F, Phe)**
8. **threonine (T, Thr)**
9. **tryptophan (W, Trp)**
10. **valine (V, Val)**

- Failure to obtain one the 10 essential amino acids results in catabolism of the body itself to provide alternative sources.

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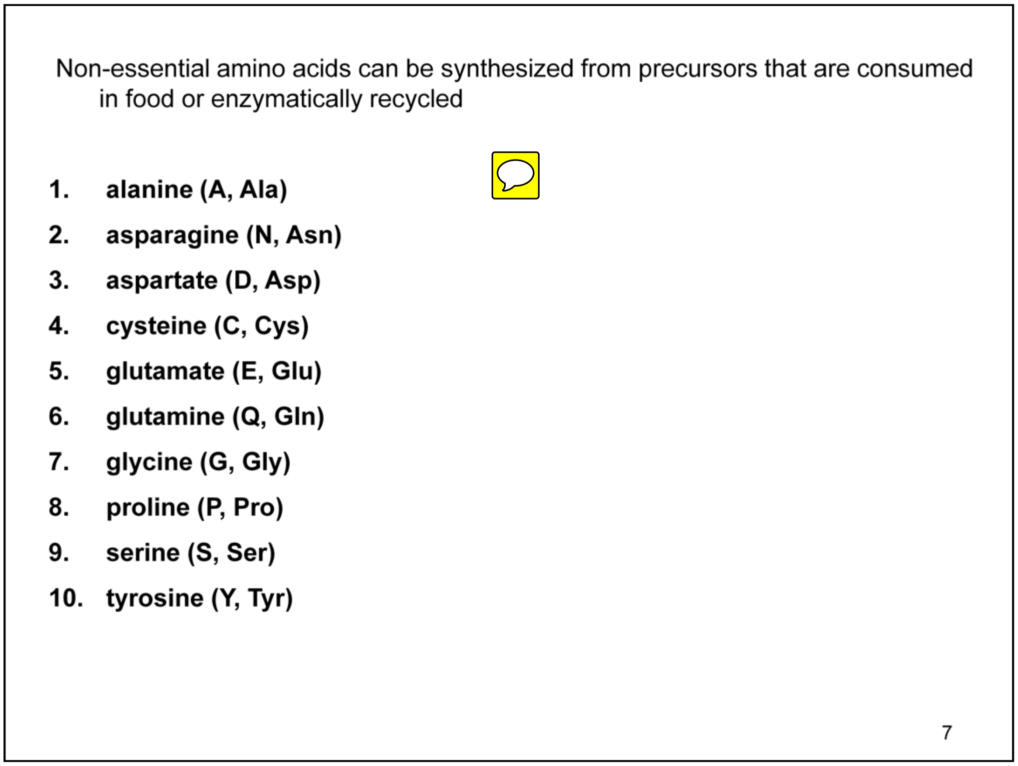
Interestingly, human cannot synthesize many of the amino acids it requires to survive. Instead, it must acquires these amino acids through feeding. Plants can make all of the amino acids. So eating plants can provide the essential amino acids. Another way of obtaining these essential amino acids is to eat other animals (that have obtained these amino acids from plants). Human do not have the enzymes essential for the biosynthesis of all of the amino acids.

If you do not obtain these amino acids from your food, then your body will catabolise itself!!

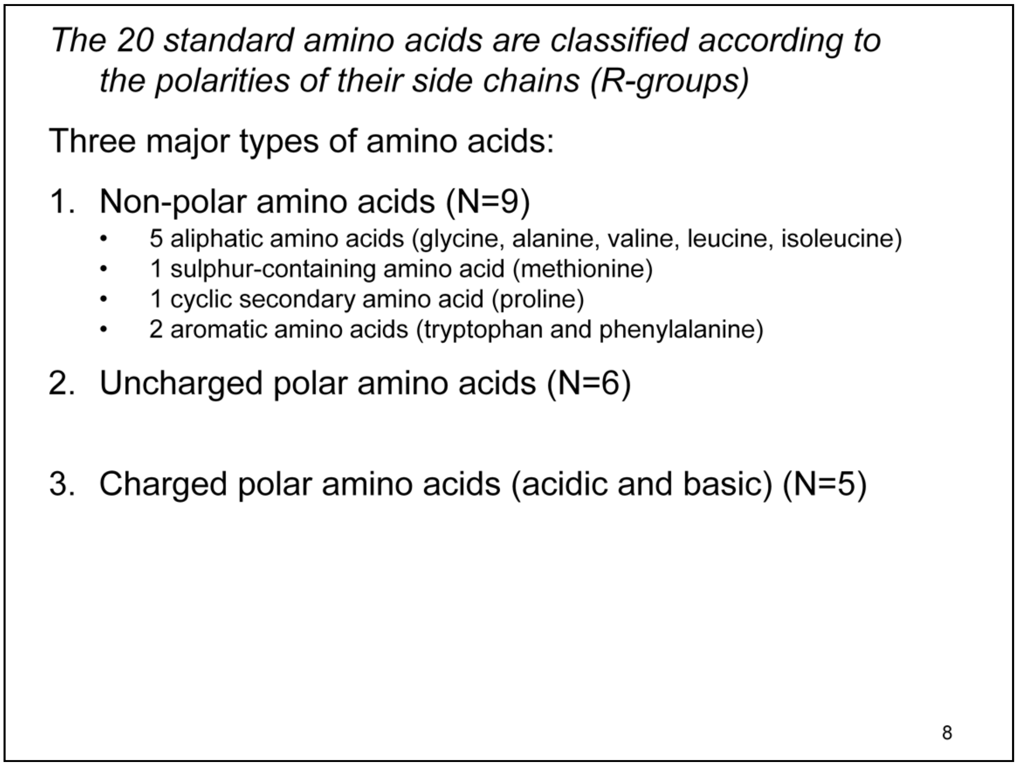
Please note that tyrosine is synthesized from phenylalanine, so if the diet is deficient in phenylalanine, tyrosine will be required as well.



Non-essential amino acids can be synthesized from precursors that are consumed in food or enzymatically recycled

1. **alanine (A, Ala)**
2. **asparagine (N, Asn)**
3. **aspartate (D, Asp)**
4. **cysteine (C, Cys)**
5. **glutamate (E, Glu)**
6. **glutamine (Q, Gln)**
7. **glycine (G, Gly)**
8. **proline (P, Pro)**
9. **serine (S, Ser)**
10. **tyrosine (Y, Tyr)**

For these amino acids, you have the enzymes to either perform a *denovo* synthesis or to do semi-synthesis based on other amino acids.



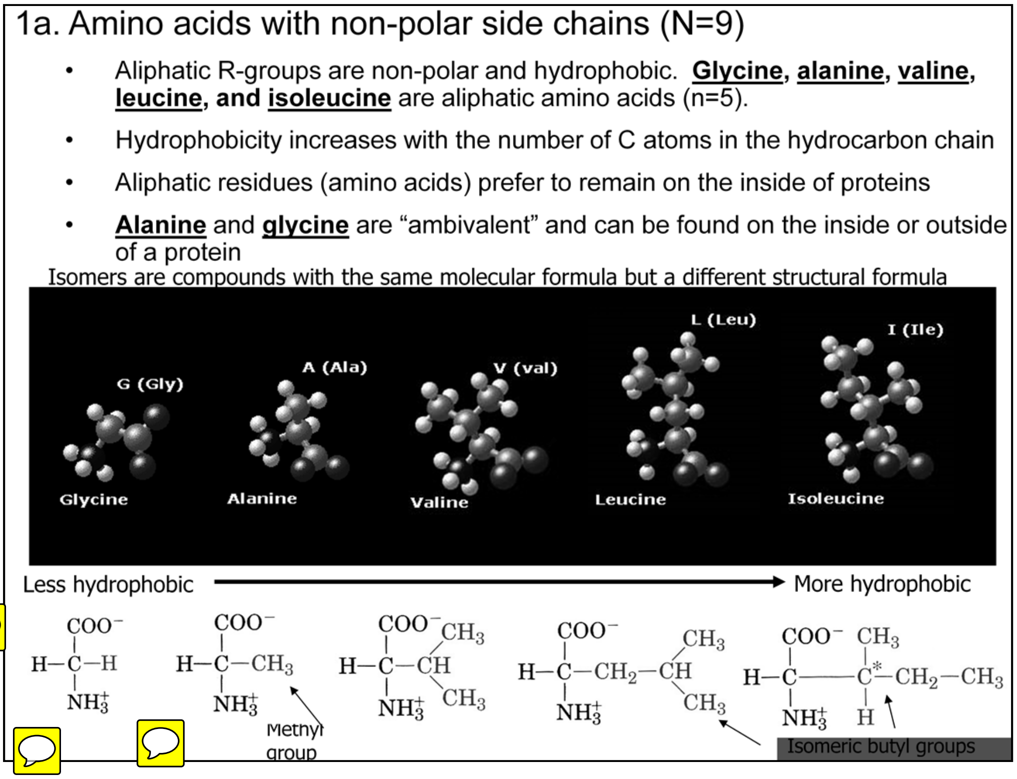
The 20 standard amino acids are classified according to the polarities of their side chains (R-groups)

Three major types of amino acids:

1. Non-polar amino acids (N=9)
   - 5 aliphatic amino acids (glycine, alanine, valine, leucine, isoleucine)
   - 1 sulphur-containing amino acid (methionine)
   - 1 cyclic secondary amino acid (proline)
   - 2 aromatic amino acids (tryptophan and phenylalanine)
2. Uncharged polar amino acids (N=6)
3. Charged polar amino acids (acidic and basic) (N=5)

Amino acids can be classified according to different criteria. Here they are classified according to the properties of their side chains based on basic, acid, uncharged polar side chains, and non polar side chains (next slide).

Three of the common amino acids have side chains with net positive charges at neutral pH: histidine, arginine, and lysine



1a. Amino acids with non-polar side chains (N=9)

- Aliphatic R-groups are non-polar and hydrophobic. **Glycine**, **alanine**, **valine**, **leucine**, and **isoleucine** are aliphatic amino acids (n=5).
- Hydrophobicity increases with the number of C atoms in the hydrocarbon chain
- Aliphatic residues (amino acids) prefer to remain on the inside of proteins
- **Alanine** and **glycine** are "ambivalent" and can be found on the inside or outside of a protein

Isomers are compounds with the same molecular formula but a different structural formula

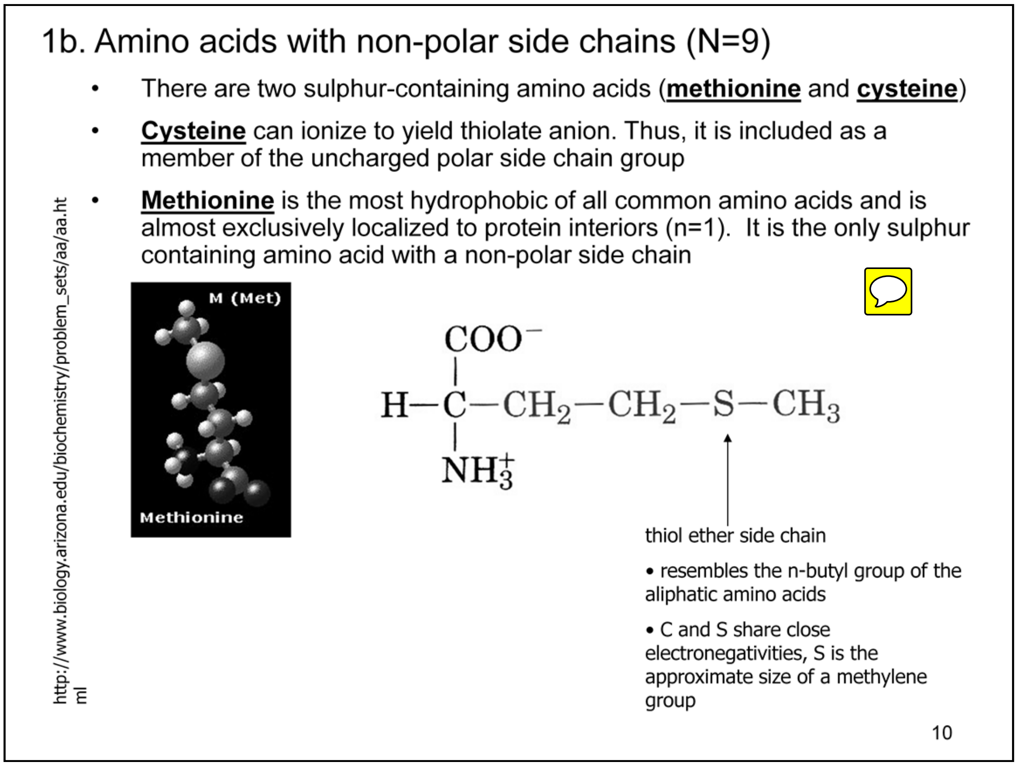
These amino acids do not have a dipole present within their side chains. Basically, as the length of the side chain increases the hydrophobicity increases. In proteins, these amino acids will often be positioned in a way to avoid water molecules and form hydrophobic regions.

When present in proteins alanine and glycine, because of their weak hydrophobicity, can be either facing water molecules or in hydrophobic regions.

Please note that isoleucine has two centers of asymmetry.

As you should remember from organic chemistry, these side chains are not very reactive and therefore are rarely involved in post-translational modification.

The contribution to the pI of these amino acids is primarily determined by the alpha carboxylic acid and alpha primary amine. They have very similar pI ranging from 5.97 to 6.02.



1b. Amino acids with non-polar side chains (N=9)

- There are two sulphur-containing amino acids (**methionine** and **cysteine**)
- **Cysteine** can ionize to yield thiolate anion. Thus, it is included as a member of the uncharged polar side chain group
- **Methionine** is the most hydrophobic of all common amino acids and is almost exclusively localized to protein interiors (n=1). It is the only sulphur containing amino acid with a non-polar side chain

$$\mathrm{H{-}\overset{\displaystyle COO^-}{\underset{\displaystyle NH_3^+}{C}}{-}CH_2{-}CH_2{-}S{-}CH_3}$$

thiol ether side chain

- resembles the n-butyl group of the aliphatic amino acids
- C and S share close electronegativities, S is the approximate size of a methylene group

http://www.biology.arizona.edu/biochemistry/problem_sets/aa/aa.html

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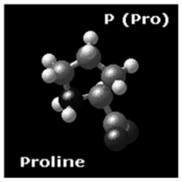
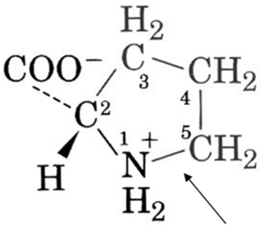
Both Methionine and cysteine contain sulfur in their side chain. However, methionine is classified as having a non-polar side chain whereas cysteine is classified in Voet as having an uncharged polar side group (due to the presence of the thiol/sulfhydryl which can form a thiolate anion). Please note that in some other biochemistry books cysteine is classified as having a non-polar side chain. The side chain is primarily composed of carbon with one sulfur. Sulfur is much more reactive and therefore methionine and cysteine are involved in many post-translational modifications. For example, methionine and cysteine can be oxidized into a sulfoxyde or even as sulfone. As well, cysteine can form a dimer (cystine) and when this occurs in a protein, it forms a bridge within the protein or even between different proteins.

Methionine has a pI of 5.74. In contrast cysteine which has a sulfhydryl group (SH) has a much lower pI of 5.07, because the sulfhydryl side chain has a pKa of 8.37. This means that cysteine SH group is a slightly acid.

1c. Amino acids with non-polar side chains (N=9)

- **Proline** is the only cyclic secondary amino acid (n=1)
- Conformational constraints are imposed by the cyclic pyrrolidine side group
- It is “ambivalent” as it can equally be inside or oriented at the surface of a protein molecule
- Often found in turns or bends of a protein structure





Pyrrolidine side group

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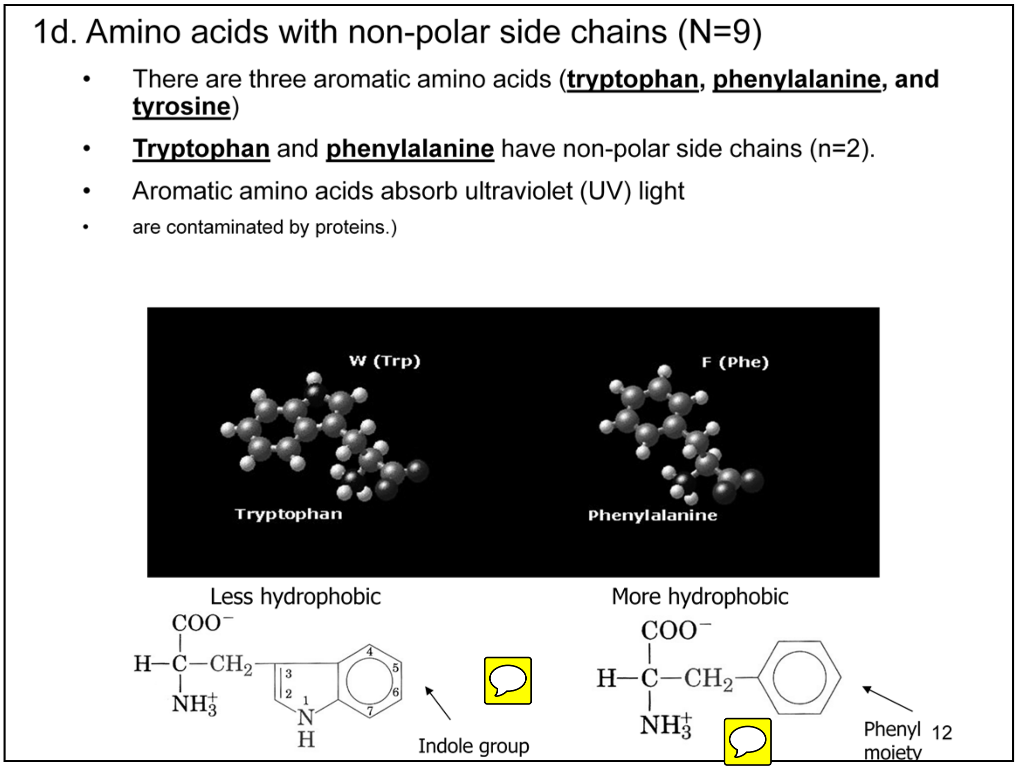

1d. Amino acids with non-polar side chains (N=9)

- There are three aromatic amino acids (**tryptophan**, **phenylalanine, and tyrosine**)
- **Tryptophan** and **phenylalanine** have non-polar side chains (n=2).
- Aromatic amino acids absorb ultraviolet (UV) light
- are contaminated by proteins.)

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Tryptophan and phenylalanine have also non polar side chain. Moreover, they have an aromatic ring within their structure. All of the amino acids that have aromatic rings (regardless of their hydrophobicity) can participate in pi stacking (π-π stacking), hydrogen to pi interaction and cation to pi interaction. For example, although the phenyl moiety in phenyl alanine does not have a dipole, it has a slight negative charge on the aromatic ring compensated by a slight positive charge on the plane of the ring. This slight negative charge permits interactions to occur with positively charged molecules (Na+, NH4+, Li+ and metals). As well phenylalanine and others can participate in pi-pi stacking in which two multiple aromatic rings (from different molecules) form a multilayer sandwich. Finally, they can also form hydrogen bond through weak hydrogen bond to the pi stack of phenylalanine (we will come back to this in the section on protein structure).

The 20 standard amino acids are classified according to the polarities of their side chains (R-groups)

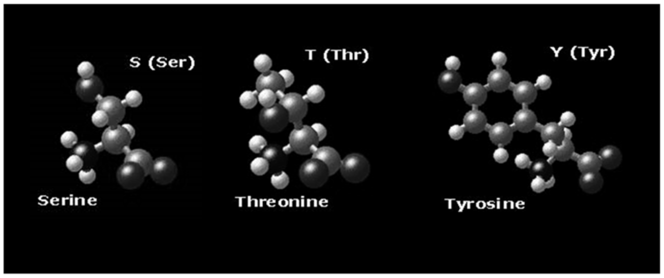
Three major types of amino acids:

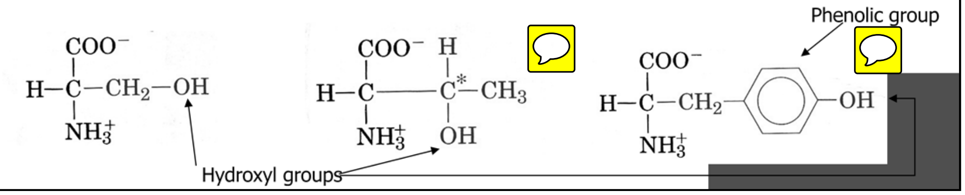
1. Non-polar amino acids (N=9)
2. Uncharged polar amino acids (N=6)
   - 1 sulphur-containing amino acid (cysteine)
   - 3 hydroxyl-containing amino acids (one is an aromatic amino acid)
     (Serine, threonine, tyrosine (aromatic))
   - 2 amide-containing amino acids (asparagine, glutamine)

3. Charged polar amino acids (acidic and basic) (N=5)

2a. Amino acids with uncharged polar side chains (N=6)

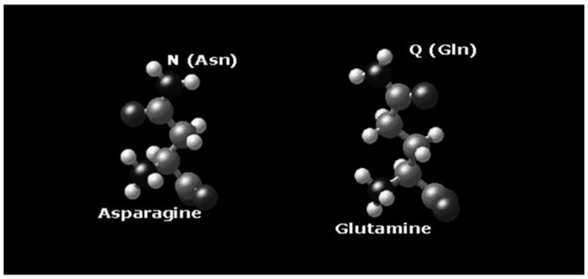
- **Tyrosine** is the third aromatic amino acid and, with **serine** and **threonine**, is also categorized as a hydroxyl amino acid (n=3).
- **Tyrosine** is the only aromatic amino acid with an ionizable side-chain but the pK_r of tyrosine’s phenolic hydroxyl is 10.46 thus is polar and uncharged at physiological pH
- The pK_r of serine and threonine hydroxyl groups are so high that they are considered non-ionizing

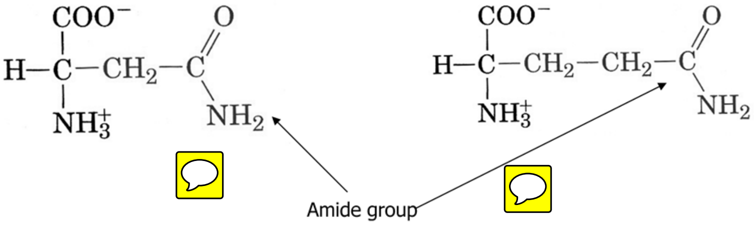




2b. Amino acids with uncharged polar side chains (N=6)

- Amides R-groups are polar and uncharged. **Asparagine** and **glutamine** contain amide side-groups (n=2)
- Amides are not ionizable and very hydrophilic.





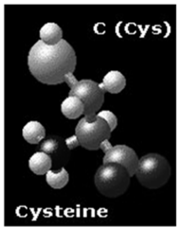
$\mathrm{H{-}C(COO^-)(NH_3^+){-}CH_2{-}C({=}O)NH_2}$

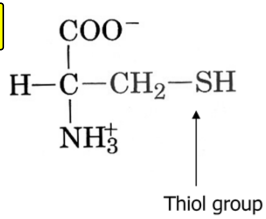
$\mathrm{H{-}C(COO^-)(NH_3^+){-}CH_2{-}CH_2{-}C({=}O)NH_2}$

Amide group

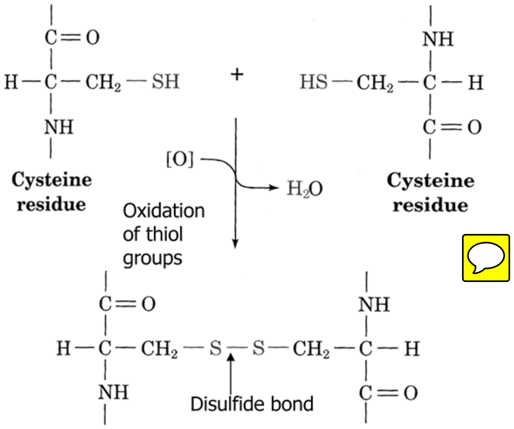
2c. Amino acids with uncharged polar side chains (N=6)

- **Cysteines** can form disulfide bonds with other cysteines by oxidation of their thiol groups (n=1)
- Unlike oxygen, sulphur does not readily hydrogen bond (H_2S is a gas while H_2O is a liquid)





$$\mathrm{H{-}\overset{COO^-}{\underset{NH_3^+}{C}}{-}CH_2{-}SH}$$



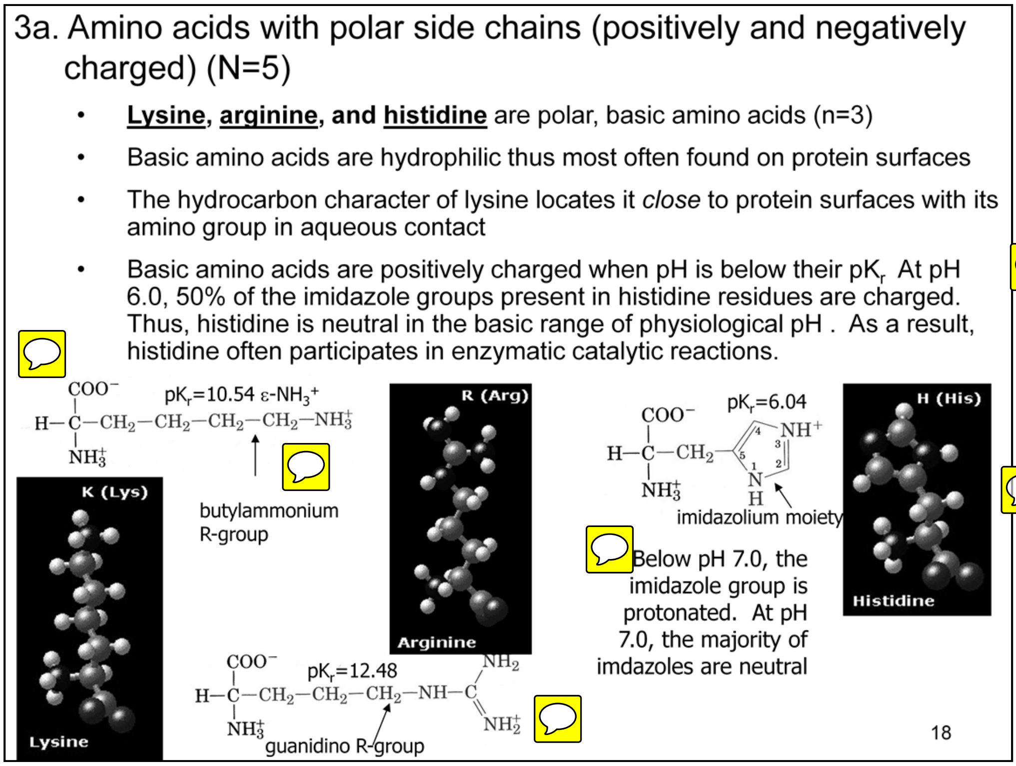
Disulfide bonds can link two different polypeptide chains or cross-link cysteines in the same chain

http://www.biology.arizona.edu/biochemistry/problem_sets/aa/aa.html

The 20 standard amino acids are classified according to the polarities of their side chains (R-groups)

Three major types of amino acids:

1. Non-polar amino acids (N=9)
2. Uncharged polar amino acids (N=6)
3. Charged polar amino acids (acidic and basic) (N=5)



3a. Amino acids with polar side chains (positively and negatively charged) (N=5)

- **Lysine**, **arginine**, **and histidine** are polar, basic amino acids (n=3)
- Basic amino acids are hydrophilic thus most often found on protein surfaces
- The hydrocarbon character of lysine locates it *close* to protein surfaces with its amino group in aqueous contact
- Basic amino acids are positively charged when pH is below their pK_r At pH 6.0, 50% of the imidazole groups present in histidine residues are charged. Thus, histidine is neutral in the basic range of physiological pH . As a result, histidine often participates in enzymatic catalytic reactions.

Below pH 7.0, the imidazole group is protonated. At pH 7.0, the majority of imdazoles are neutral

When arginine is protonated on its epsilon amino group the positive charge is stabilized by resonance between the two amino groups.

Histidine is also classified as basic because the two nitrogens in its ring structure have a weak affinity for protons.

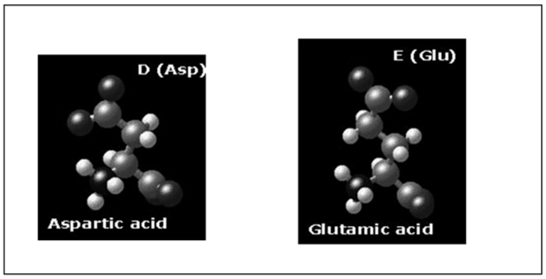
The side chains of lysine and arginine are fully protonated at pH 7, whereas histidine (side chain pK_a of 6.04) is only 10% protonated at pH 7.

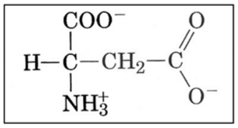
Because histidine side chain has a nearly neutral pK_a, its play important functions in many enzyme catalysis by acting as proton donors and acceptors.

In contrast, arginine and lysine side chains are protonated under physiological conditions and often participate in electrostatic interactions in proteins.

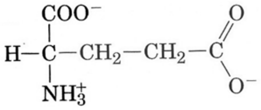
3a. Amino acids with polar side chains (positively and negatively charged) (N=5)

- **Aspartic acid** and **glutamic acid** are polar, acidic amino acids (n=2)
- Both acidic amino acids have two carboxyl groups
- Acidic amino acids are negatively charged above pH 3.0. When ionized, they are also referred to as aspartate and glutamate





$$\mathrm{H{-}\overset{COO^-}{\underset{NH_3^+}{C}}{-}CH_2{-}C(=O)O^-}$$



$$\mathrm{H{-}\overset{COO^-}{\underset{NH_3^+}{C}}{-}CH_2{-}CH_2{-}C(=O)O^-}$$

Industrial use of amino acids

Amino acid	Usage
Glycine	Flavor enhancer for animal feed/pet food i.e. covers the bad taste
Glycine	pH buffering in antiperspirants
Glutamate	MSG in food
Phenylalanine	Needed for production of aspartame
arginine	Dentin hypersensitivity
Tryptophan	sleep

Industrial use of amino acids

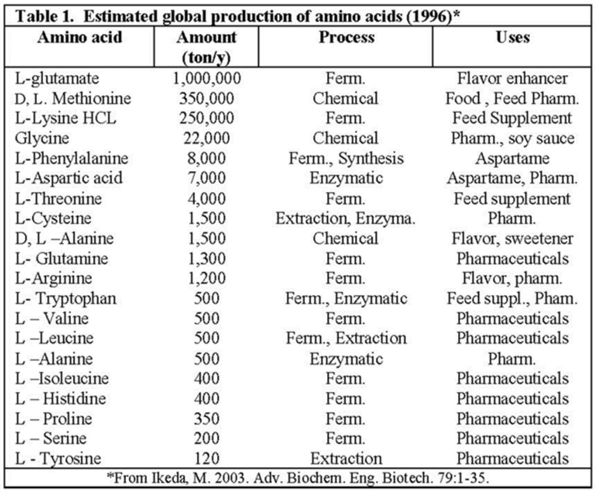
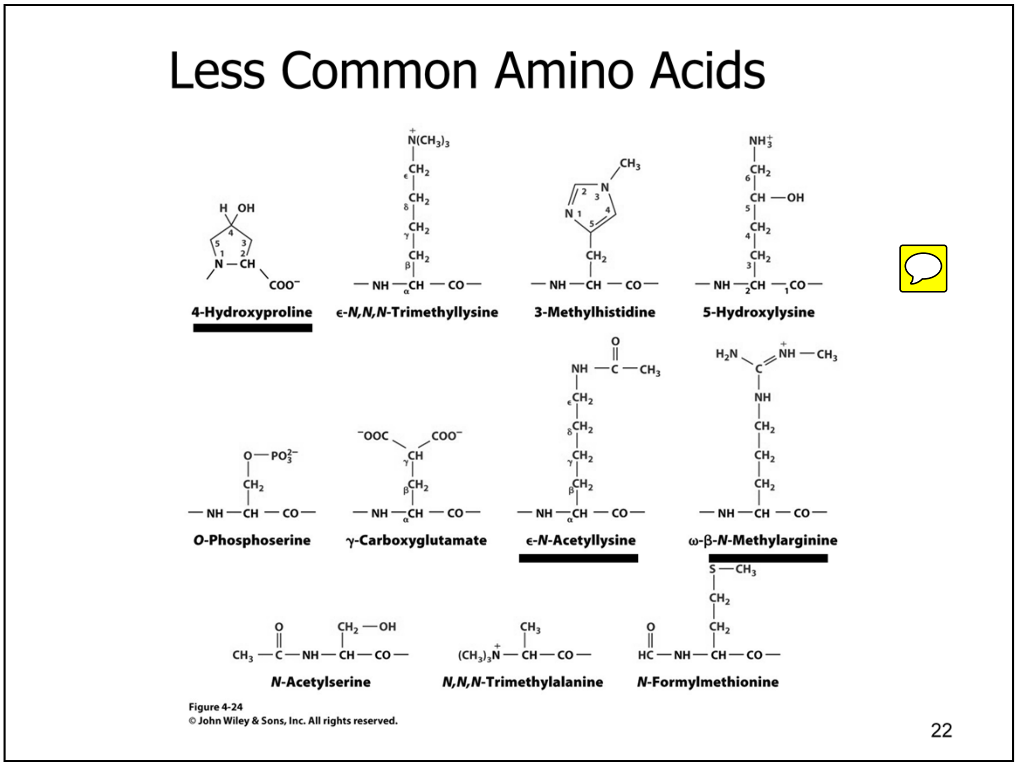


Table 1. Estimated global production of amino acids (1996)*

Amino acid	Amount (ton/y)	Process	Uses
L-glutamate	1,000,000	Ferm.	Flavor enhancer
D, L. Methionine	350,000	Chemical	Food , Feed Pharm.
L-Lysine HCL	250,000	Ferm.	Feed Supplement
Glycine	22,000	Chemical	Pharm., soy sauce
L-Phenylalanine	8,000	Ferm., Synthesis	Aspartame
L-Aspartic acid	7,000	Enzymatic	Aspartame, Pharm.
L-Threonine	4,000	Ferm.	Feed supplement
L-Cysteine	1,500	Extraction, Enzyma.	Pharm.
D, L –Alanine	1,500	Chemical	Flavor, sweetener
L- Glutamine	1,300	Ferm.	Pharmaceuticals
L-Arginine	1,200	Ferm.	Flavor, pharm.
L- Tryptophan	500	Ferm., Enzymatic	Feed suppl., Pham.
L – Valine	500	Ferm.	Pharmaceuticals
L –Leucine	500	Ferm., Extraction	Pharmaceuticals
L –Alanine	500	Enzymatic	Pharm.
L –Isoleucine	400	Ferm.	Pharmaceuticals
L – Histidine	400	Ferm.	Pharmaceuticals
L – Proline	350	Ferm.	Pharmaceuticals
L – Serine	200	Ferm.	Pharmaceuticals
L - Tyrosine	120	Extraction	Pharmaceuticals

*From Ikeda, M. 2003. Adv. Biochem. Eng. Biotech. 79:1-35.



Less Common Amino Acids

4-Hydroxyproline · **ϵ-*N,N,N*-Trimethyllysine** · **3-Methylhistidine** · **5-Hydroxylysine**

***O*-Phosphoserine** · **γ-Carboxyglutamate** · **ϵ-*N*-Acetyllysine** · **ω-β-*N*-Methylarginine**

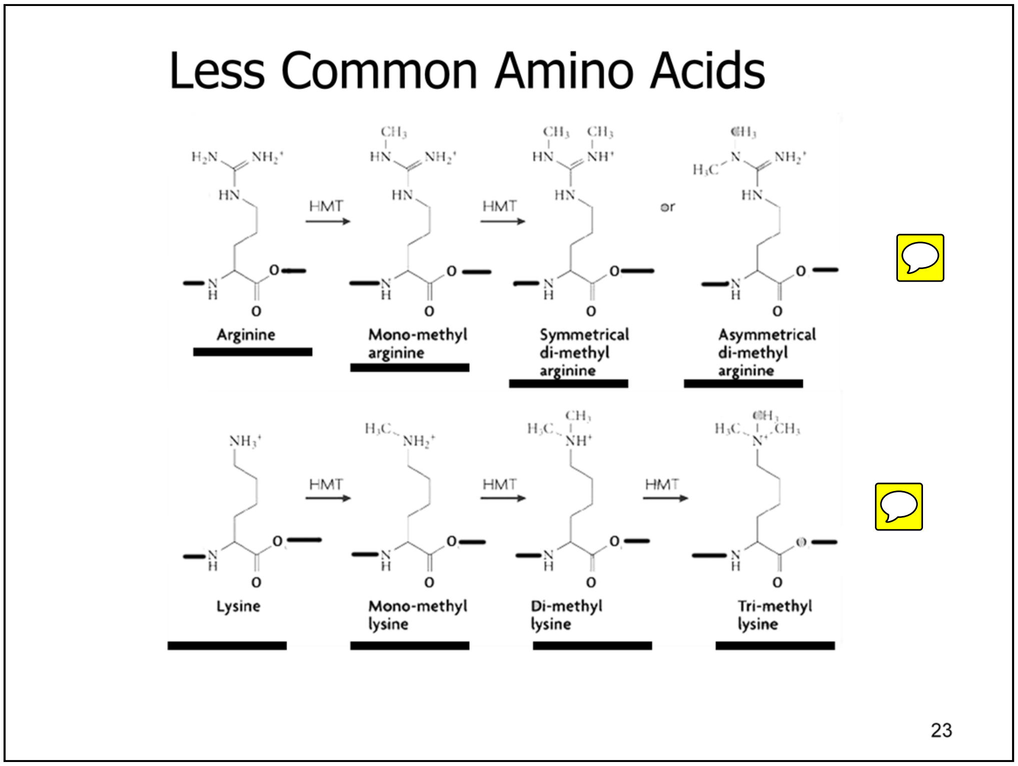
***N*-Acetylserine** · ***N,N,N*-Trimethylalanine** · ***N*-Formylmethionine**

Figure 4-24

Here are some amino acids that are found in proteins, but are relatively rare. Most typically they are formed after the protein is made in cells through a process called post-translational modifications(PTMs) of the protein. PTMs are catalyzed by specific enzymes.

Common PTMs include glycosylation, oxidation, hydroxylation, methylation, acetylation, and phosphorylation.

You need to 1-be able to recognize that they are not one of the common 20 amino acids. 2-know the ones underlined in red.



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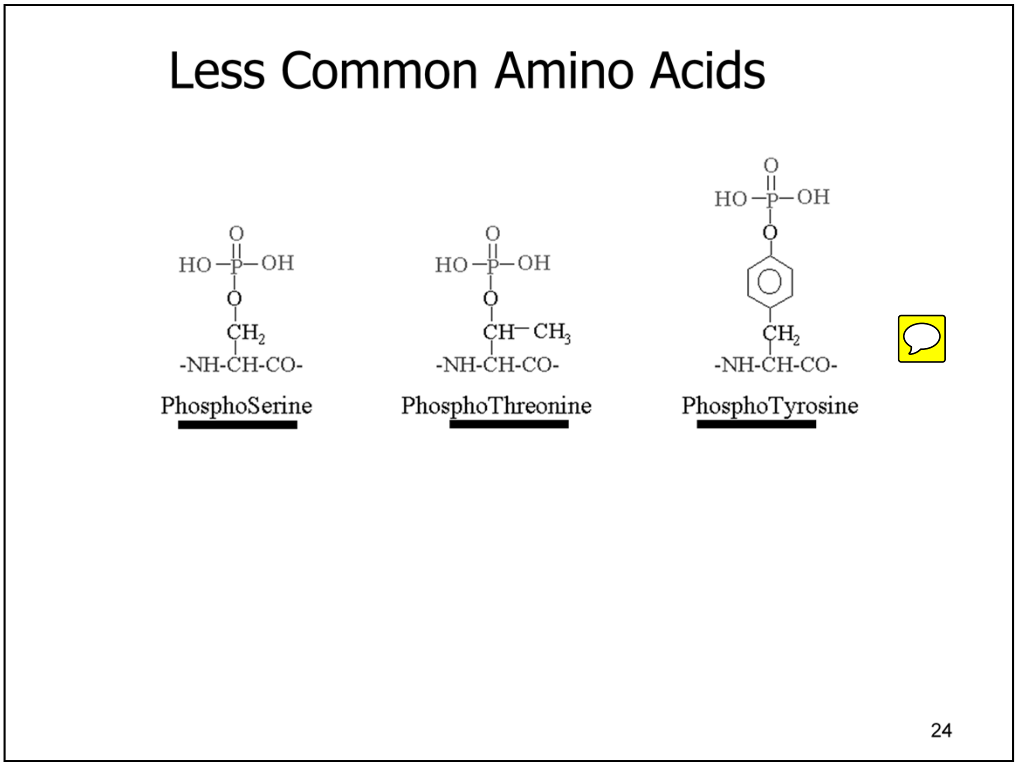


Less Common Amino Acids

PhosphoSerine

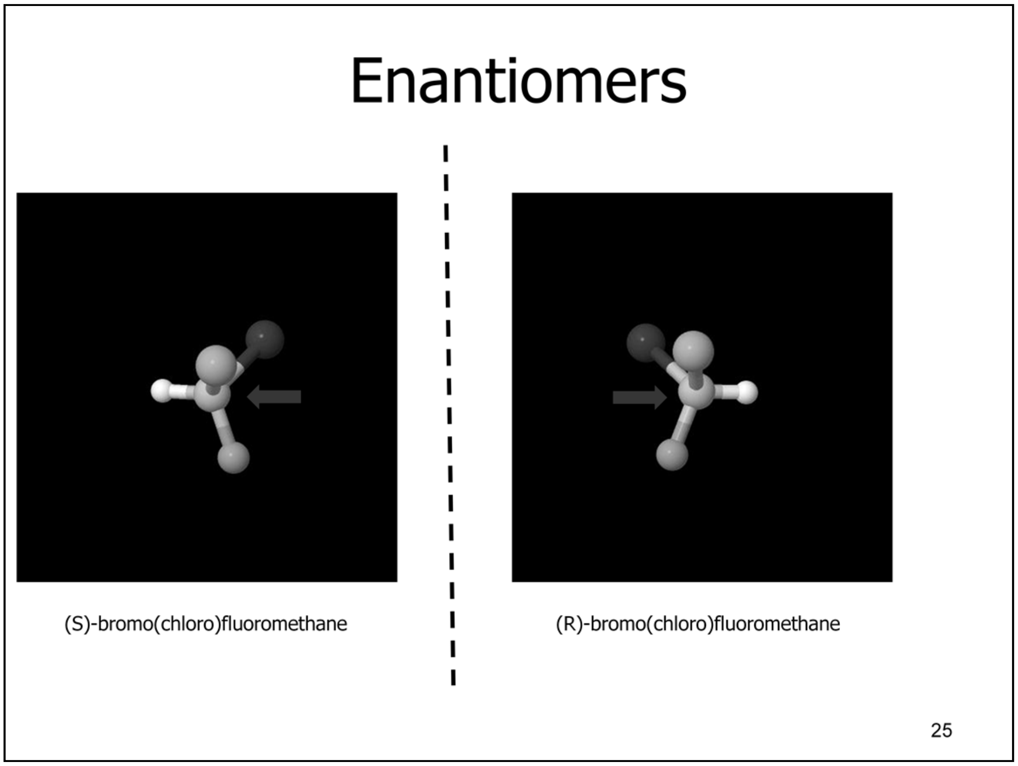
PhosphoThreonine

PhosphoTyrosine

Here are some amino acids that are found in proteins, but are relatively rare. Most typically they are formed after the protein is made in cells through a process called post-translational modifications(PTMs) of the protein. PTMs are catalyzed by specific enzymes.

Common PTMs include glycosylation, oxidation, hydroxylation, methylation, acetylation, and phosphorylation.

You need to 1-be able to recognize that they are not one of the common 20 amino acids. 2-know the ones underlined in red.



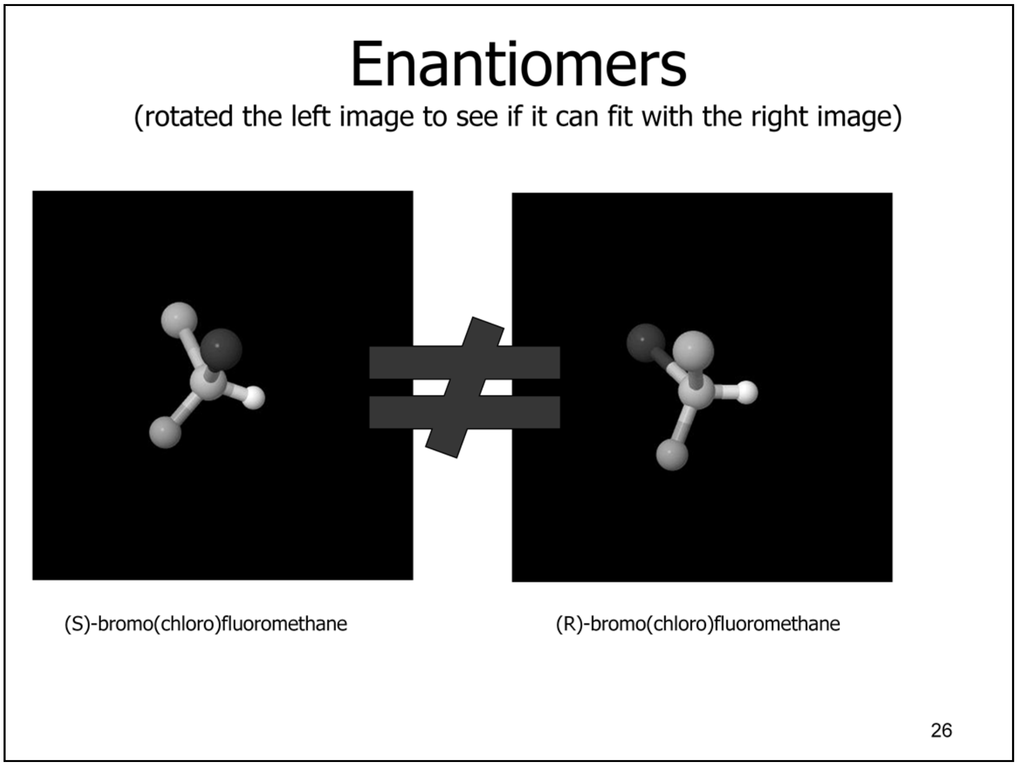
Enantiomers

(S)-bromo(chloro)fluoromethane

(R)-bromo(chloro)fluoromethane

The two enantiomers of fluorochlorobromo methane are presented. Please note that they are mirror images. However, you would not be able to superpose the left image with the right image by rotation. If you try to rotate the left image to superimpose with the right image it will not work.

Enantiomers are types of stereoisomers that are mirror images of each other and that are not superimposable.



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(rotated the left image to see if it can fit with the right image)

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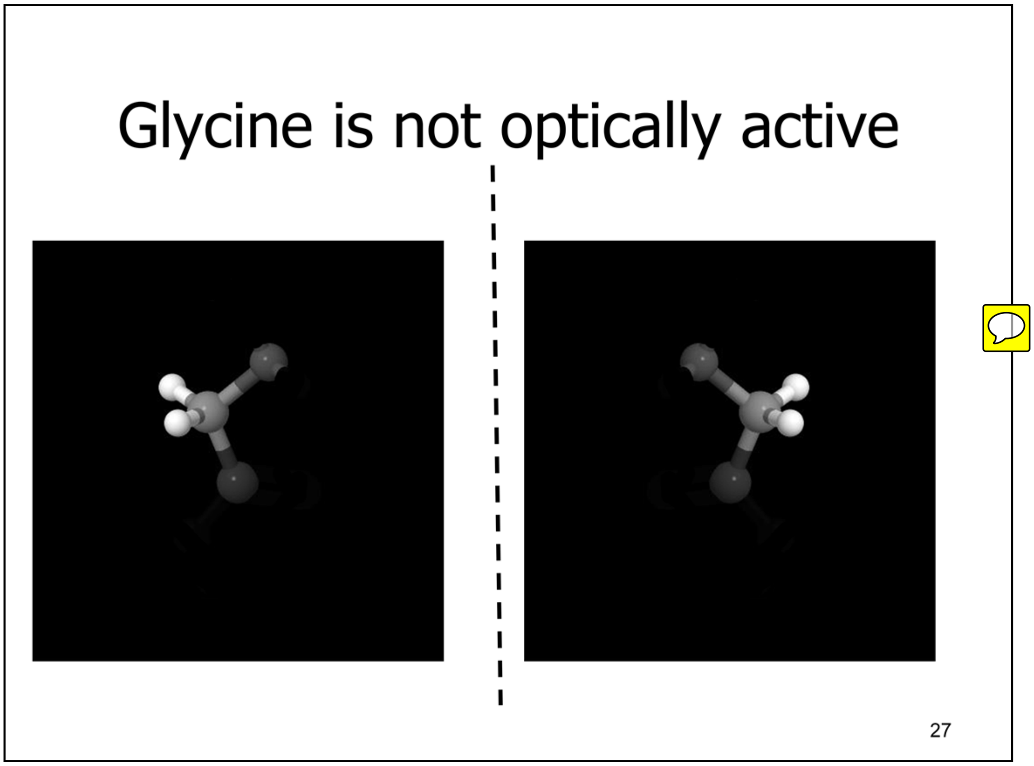
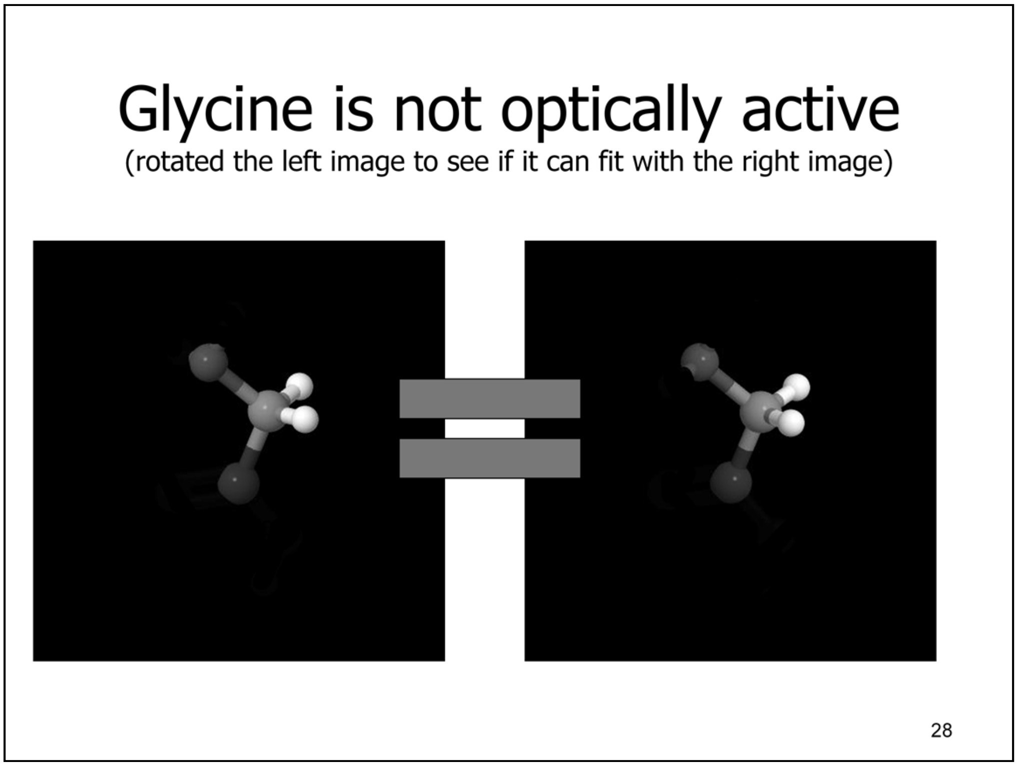


Glycine is not optically active

27

Glycine does not have a stereoisomer. The mirror images of glycine can be superposed. Please note that I have only depicted the side chain of the alpha carbon. The NH2 and CO2H are free to rotate on their bond axis with the alpha-carbon.

Here the blue ball is NH2 whereas the red ball is CO_2H



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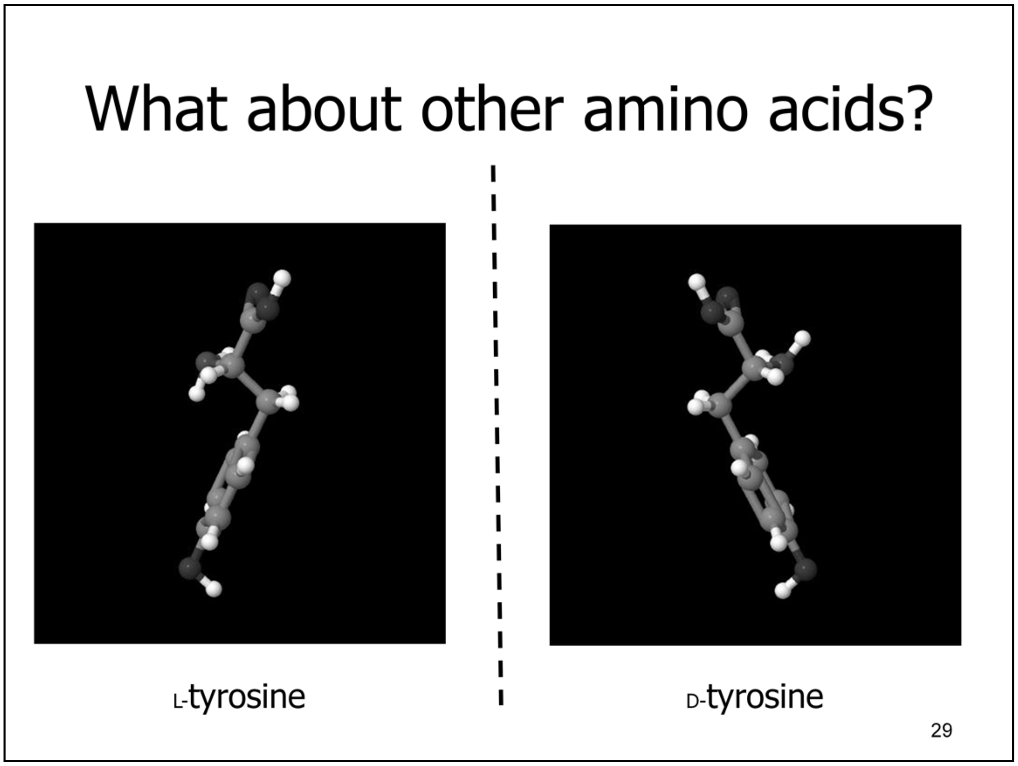


What about other amino acids?

L-tyrosine

D-tyrosine

29

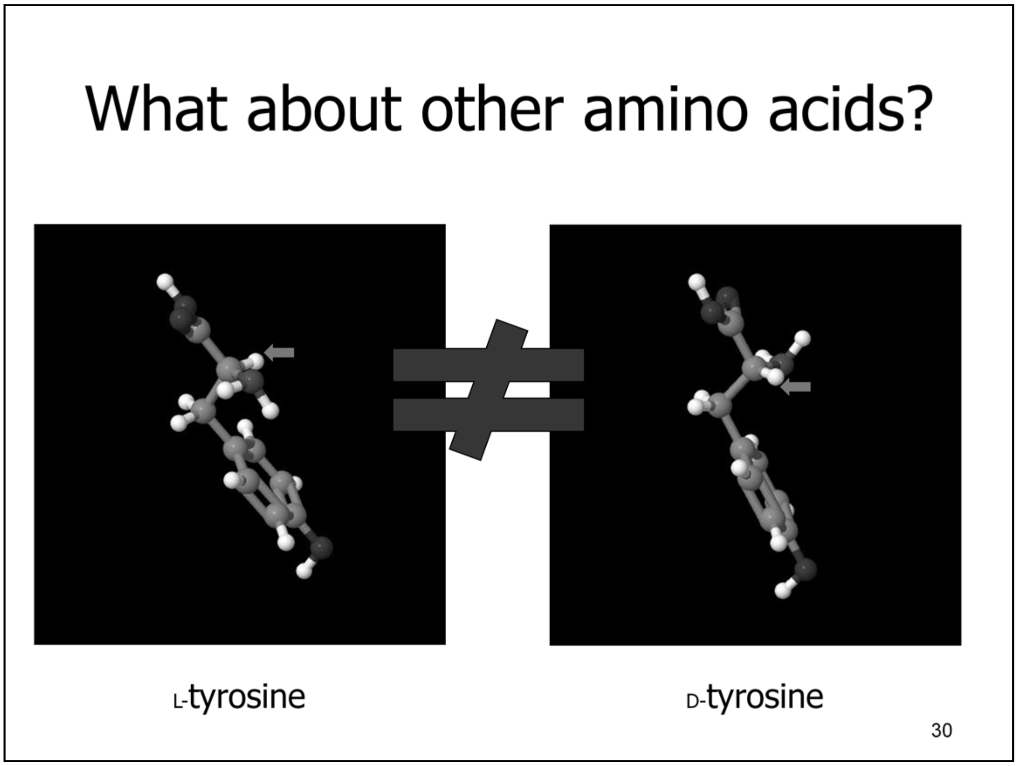
Tyrosine has only one chiral center (the alpha carbon). Therefore it has two stereoisomers and one pair of enantiomers.

Grey=Carbon

White=Hydrogen

Red=Oxygen

Blue=Nitrogen



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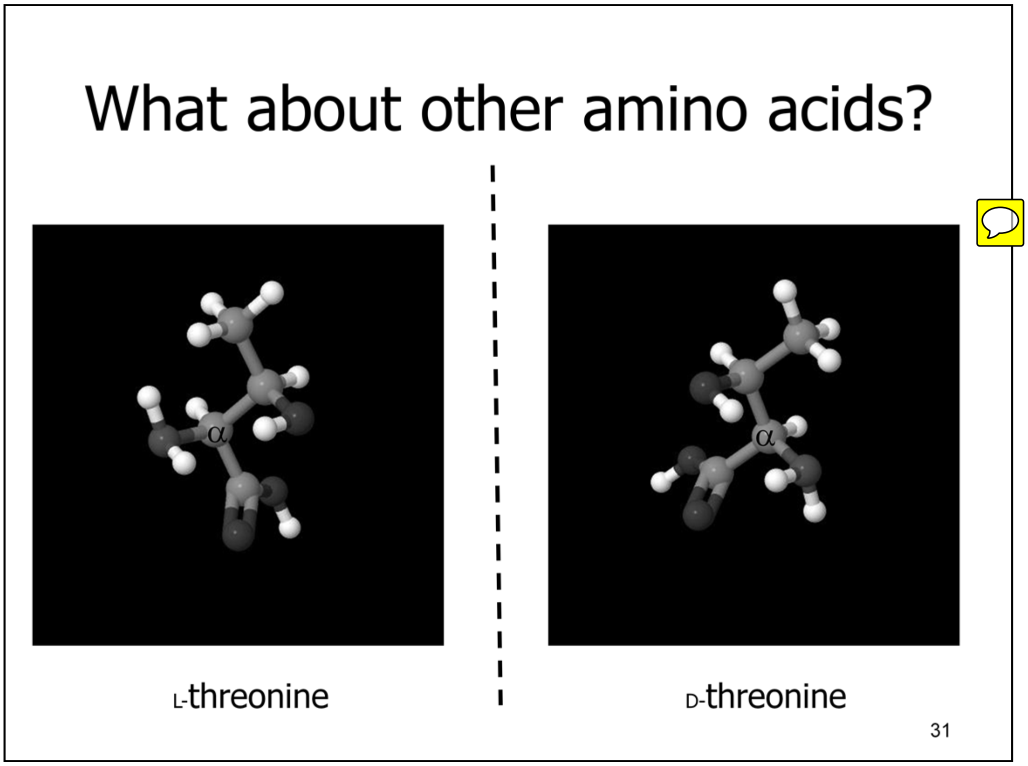


What about other amino acids?

L-threonine

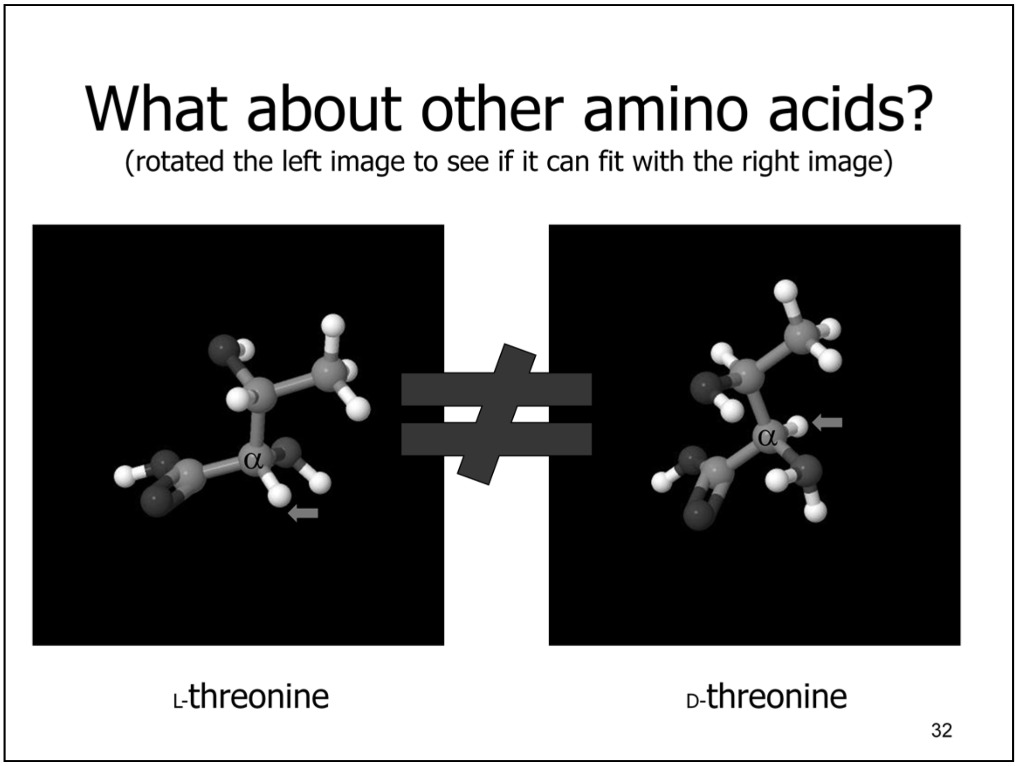
D-threonine

Except for glycine, all of the amino acids have stereoisomers.

Threonine has two different chiral centers and therefore 4 stereoisomers
L-Threonine (2*S*,3*R*) and D-Threonine (2*R*,3*S*)

L-*allo*-Threonine (2*S*,3*S*) and D-*allo*-Threonine (2*R*,3*R*)

Grey=Carbon
White=Hydrogen
Red=Oxygen
Blue=Nitrogen



What about other amino acids?

(rotated the left image to see if it can fit with the right image)

L-threonine

D-threonine

Except for glycine, all of the amino acids have stereoisomers.

Grey=Carbon
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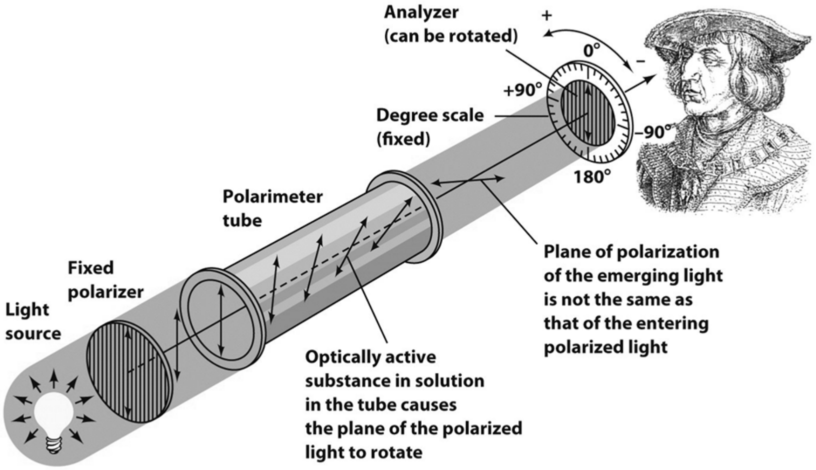
Blue=Nitrogen

Polarimetry

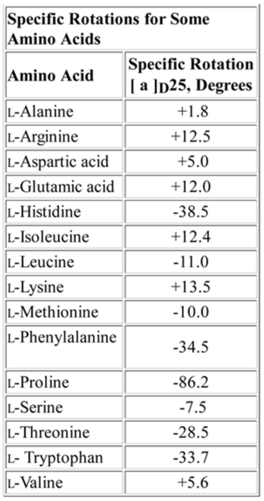


Plane of polarization of the emerging light is not the same as that of the entering polarized light

Optically active substance in solution in the tube causes the plane of the polarized light to rotate

Figure 4-11

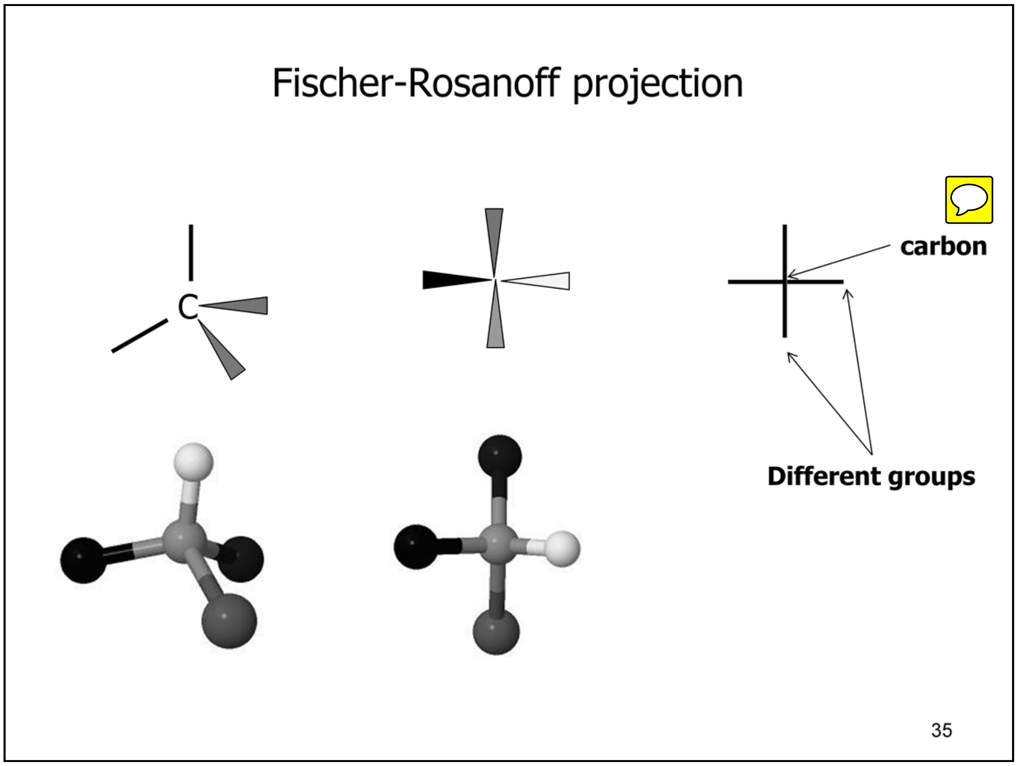
There is no relationship between the Fischer and Cahn-Ingold-Prelog System with the results from polarimetry



Specific Rotations for Some Amino Acids

Amino Acid	**Specific Rotation [a]$_D$25, Degrees**	
L-Alanine	+1.8	**+, d (dextro)**
L-Arginine	+12.5	**+, d (dextro)**
L-Aspartic acid	+5.0	**+, d (dextro)**
L-Glutamic acid	+12.0	**+, d (dextro)**
L-Histidine	-38.5	**-, l (levo)**
L-Isoleucine	+12.4	**+, d (dextro)**
L-Leucine	-11.0	**-, l (levo)**
L-Lysine	+13.5	**+, d (dextro)**
L-Methionine	-10.0	**-, l (levo)**
L-Phenylalanine	-34.5	**-, l (levo)**
L-Proline	-86.2	**-, l (levo)**
L-Serine	-7.5	**-, l (levo)**
L-Threonine	-28.5	**-, l (levo)**
L- Tryptophan	-33.7	**-, l (levo)**
L-Valine	+5.6	**+, d (dextro)**

http://web.virginia.edu/Heidi/home.htm



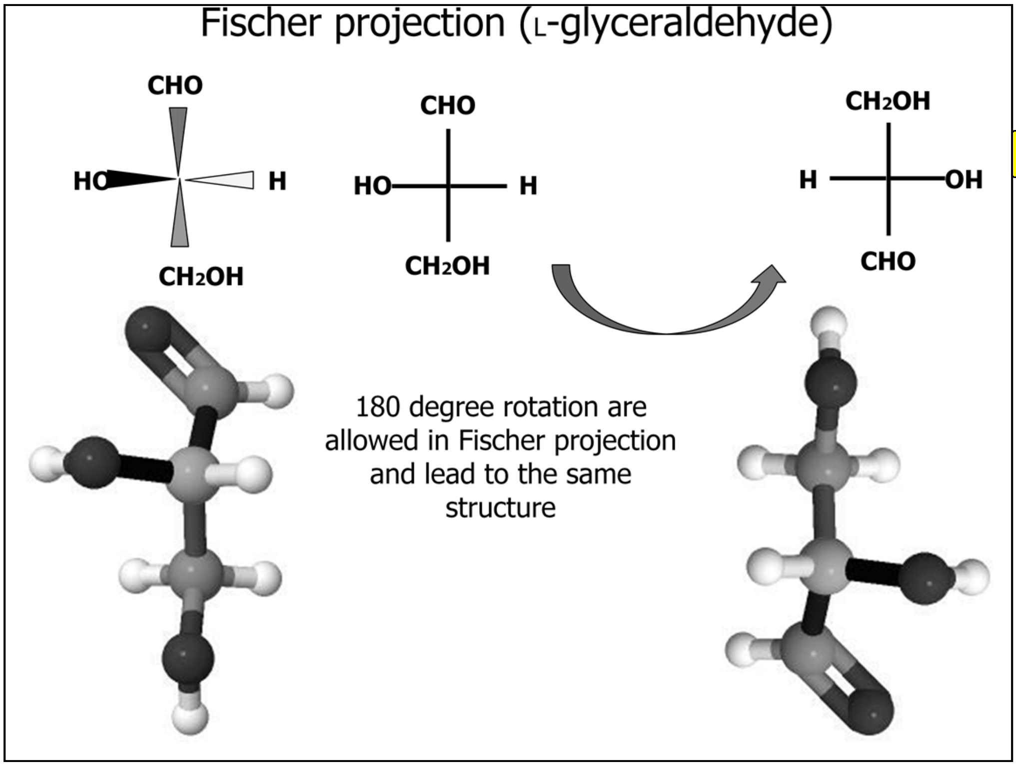
Fischer-Rosanoff projection

Fischer projections are characterized by one or more chiral centers surrounded by four bonds arranged in a plus-shaped pattern. The adjacent chiral centers are aligned vertically. The Fischer projection was originally intended for carbohydrates and its use with other molecules (such as amino acids) can lead to ambiguous structure.

Rules: Chiral carbon is at the center. if multiple carbons connected together they are set on the vertical line.

The vertical line is going behind the plane of the page.

The Horizontal is going out of the plane of the page.



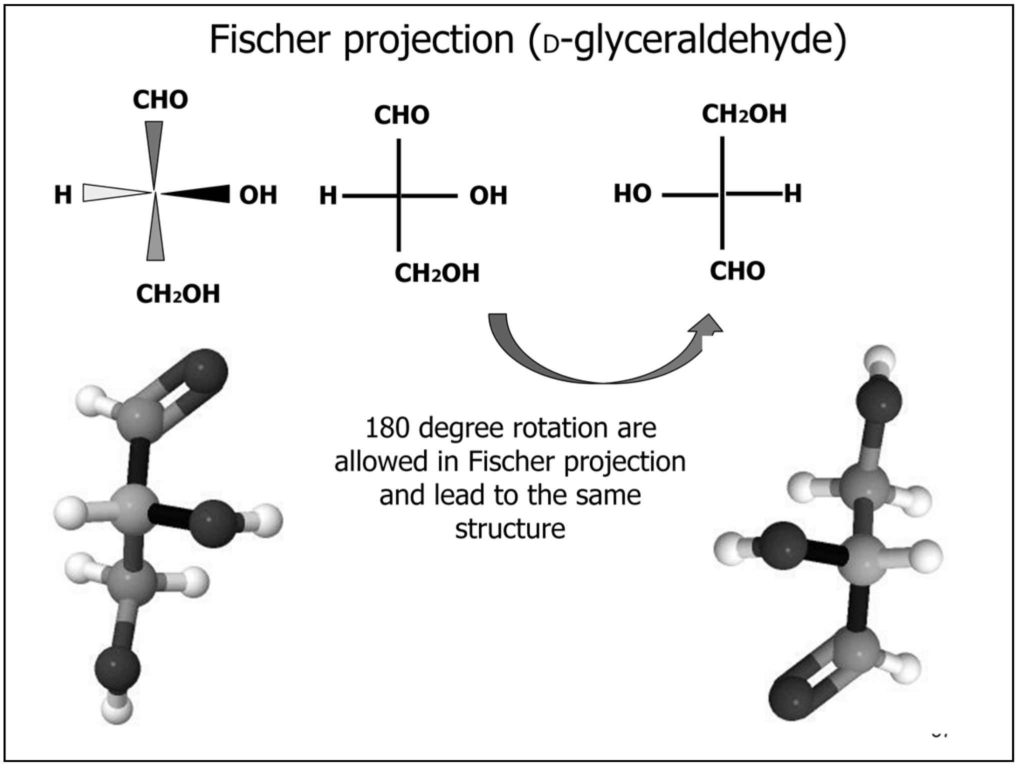
Fischer projection (L-glyceraldehyde)

180 degree rotation are allowed in Fischer projection and lead to the same structure

Fischer projection of L-Glyceraldehyde. I have color coded the chiral carbon orange. The rest of the carbons are grey, oxygens are red, and hydrogens are white.

I also show that 180 degree rotation of the fisher projection does not change the enantiomer

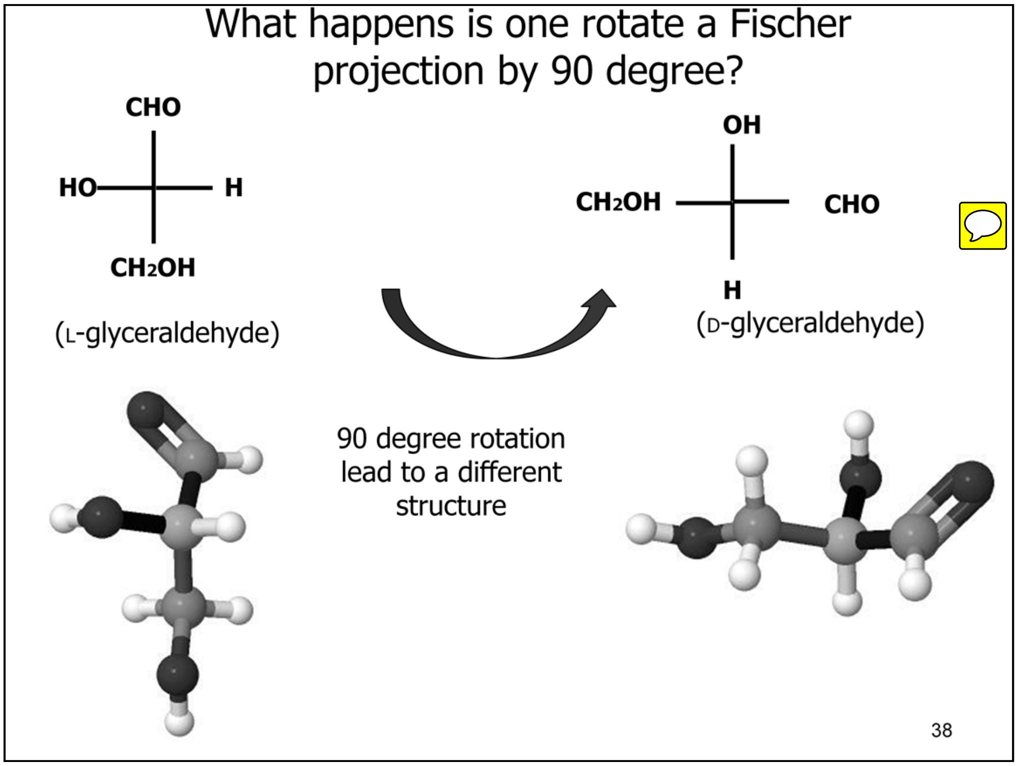
The L-Glyceraldehyde had been previously measured to be (-) by polarimetry whereas the D-glyceraldehyde had been measured to be (+). Fischer selected the representation for L and D glyceraldehydes as illustrated in the two figures. He had a 50% chance of properly selecting and he did. All of the amino acids have an L-configuration. Although the L/D configurations were selected based on glyceraldehyde, it has nothing to do with the levo/dextro system.



Fischer projection (D-glyceraldehyde)

180 degree rotation are allowed in Fischer projection and lead to the same structure

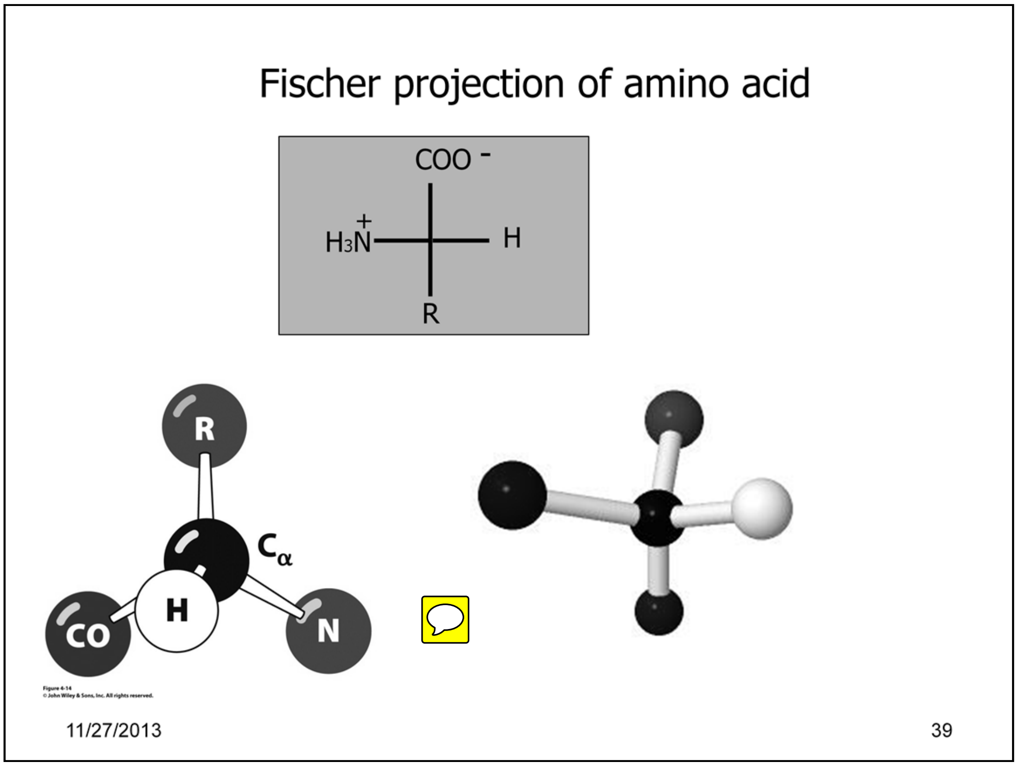
Fischer projection of D-Glyceraldehyde. I have color coded the chiral carbon orange. The rest of the carbons are grey, oxygen is red, and hydrogen is white.



What happens is one rotate a Fischer projection by 90 degree?

90 degree rotation lead to a different structure

I have color coded the chiral carbon orange. The rest of the carbons are grey, oxygen is red, and hydrogen is white. The bounds are color coded from the chiral carbon.



Fischer projection of amino acid

Figure 4-14
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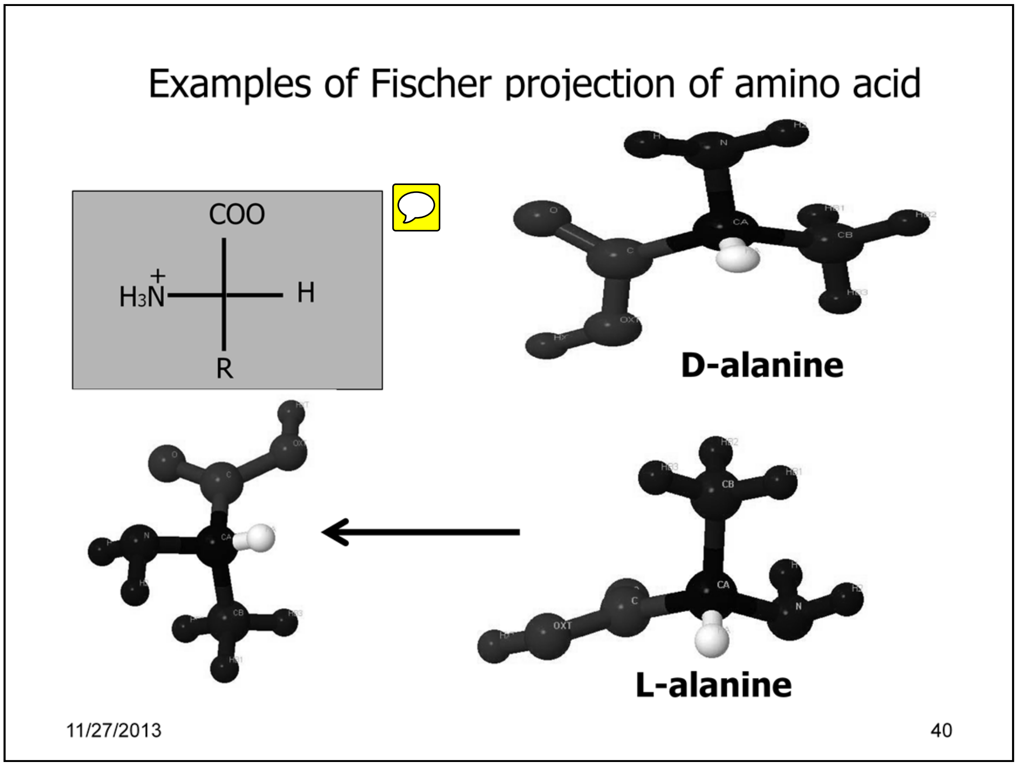
All alpha- amino acids have an L stereochemical configuration (except for glycine which is not stereochemical). The easiest way of remembering the configuration is to use the mnemonic “CORN crib” looking at the alpha carbon through the hydrogen one should read clockwise CO, R, and N where CO stands for the alpha-carboxylic acid, R for side group and N for alpha-amino group. Please remember that in the Fischer projection the carbons are set on the vertical axis. This will be helpful when you try to figure out the configurations of the different amino acids as most R are attached to the alpha-carbon through a carbon.

The Fischer projection is ambiguous when dealing with molecules that have multiple chiral centers.



Examples of Fischer projection of amino acid

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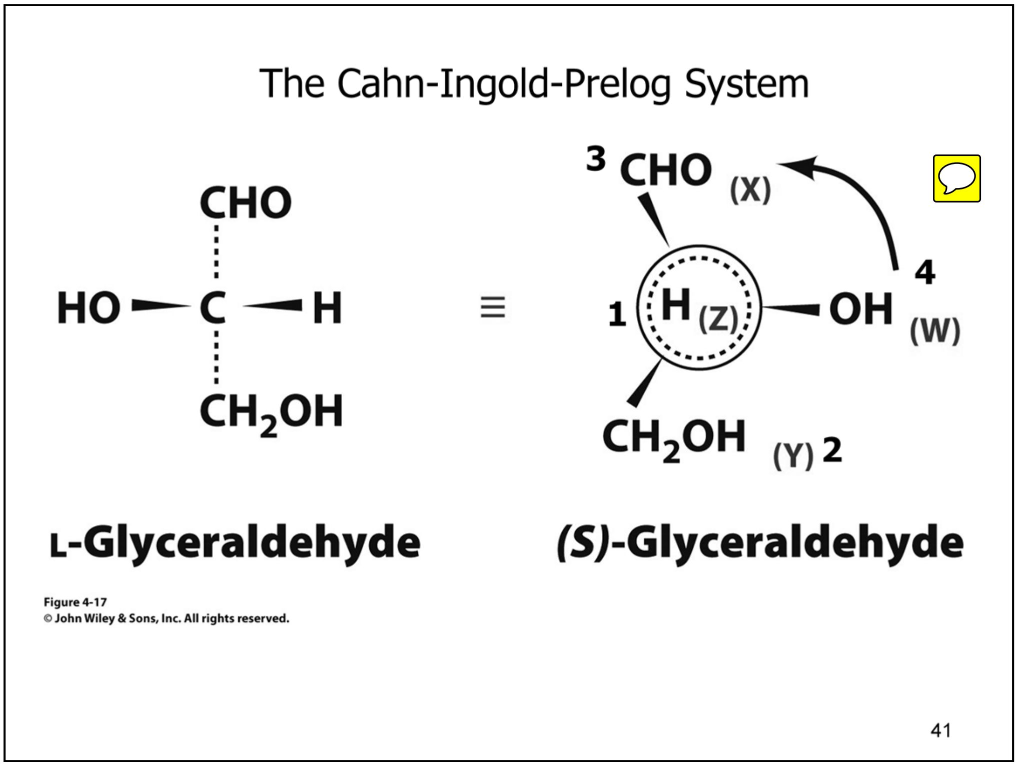
Here is an example on how to verify if a 3D model is the L or D form. At the top right you can see D-alanine and at the bottom right you can see L-Alanine. I have color coded the groups attached to the alpha-carbon according to the color used in the CORN mnemonic. Carboxylic in red, amino group in blue and the CH_3 side chain (R) in purple. You should be able to see that:

1-When you look at the carbon through the hydrogen, the L-alanine forms the clockwise CORN mnemonic.

2- When you look at the carbon through the hydrogen, the D-alanine **<u>does not</u>** form the clockwise CORN mnemonic.

3-You can perform a rotation of the structure of L-Alanine to align it with the L-Amino acid Fischer projection.

4-Rotation of D-alanine will never lead to the Fischer projection for L-amino acid.



The Cahn-Ingold-Prelog System

L-Glyceraldehyde

***(S)*-Glyceraldehyde**

Figure 4-17
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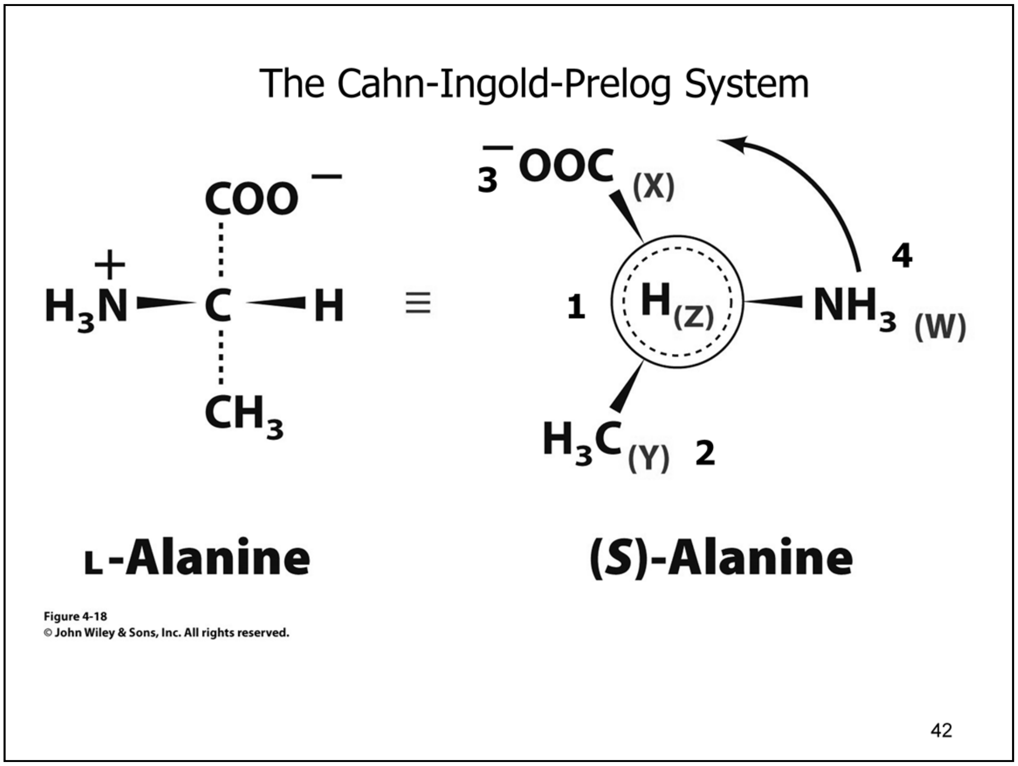
In the Cahn-Ingold system the residues attached to a chiral center are labelled in the following descending order SH > OH > NH2 > COOH > CHO >CH2 OH > CH3>H. You can either used the reversed alphabetic labelling approach (W>X>Y>Z) or numbers (4>3>2>1)

The a-carbon configuration of all the L-amino acids except for cysteine is (S) with the exceptions of L-cysteine and L-cystine , which are ®.

In most amino acids the order of preference in the RS system of the groups around C-alpha are NH', COO-, R, H. However, for cysteine and cystine the group R takes precedence over carboxylate because the atomic number of sulfur attached to C-3 is higher than that of oxygen attached to C-1.

Please note that some amino acids have two chiral centers and the second chiral center is not necessarily the same as the first.



The Cahn-Ingold-Prelog System

L-Alanine

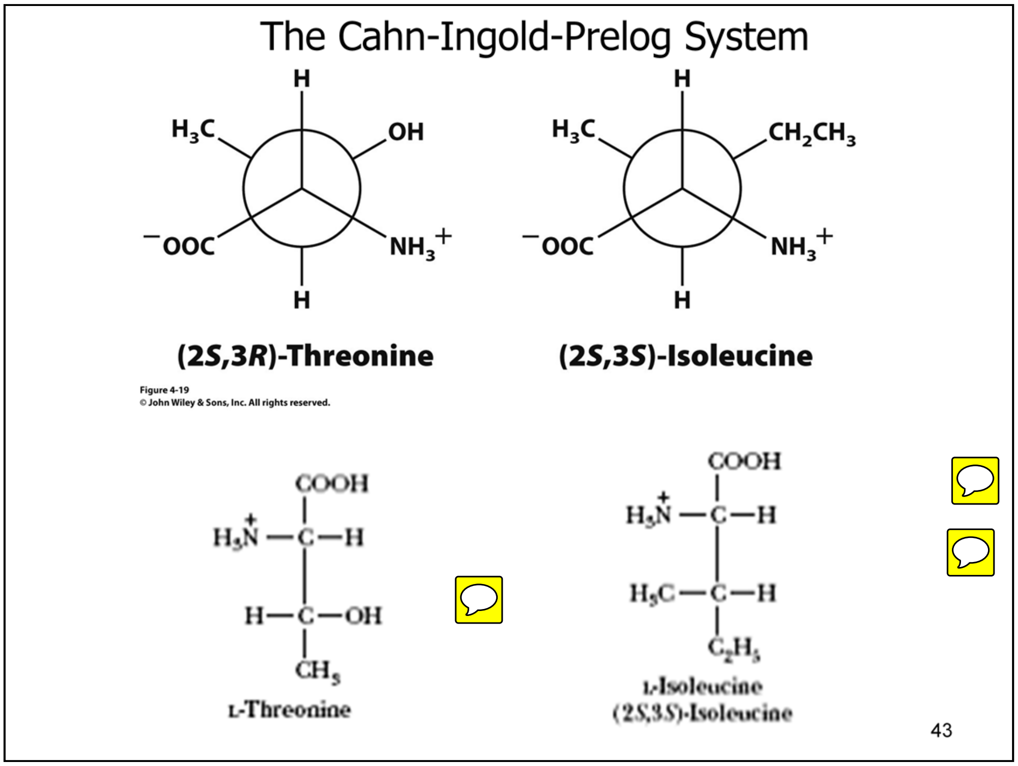
(*S*)-Alanine

Figure 4-18

The a-carbon configuration of all the L-amino acids except for cysteine is (S) with the exceptions of L-cysteine and L-cystine , which are ®.

In most amino acids the order of preference in the RS system of the groups around C-alpha are NH', COO-, R, H. However, for cysteine and cystine the group R takes precedence over carboxylate because the atomic number of sulfur attached to C-3 is higher than that of oxygen attached to C-1).

Please note that some amino acids have two chiral centers and the second chiral center is not necessarily the same as the first. (See next slide)



The Cahn-Ingold-Prelog System

(2*S*,3*R*)-Threonine

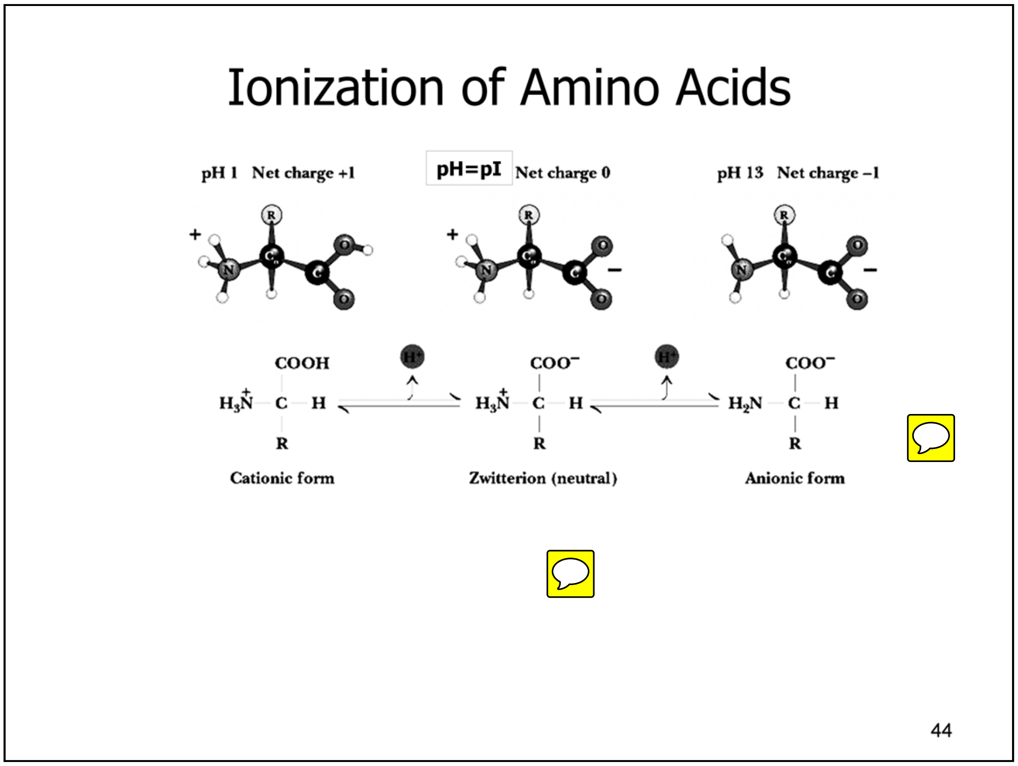
(2*S*,3*S*)-Isoleucine

Figure 4-19

L-Threonine

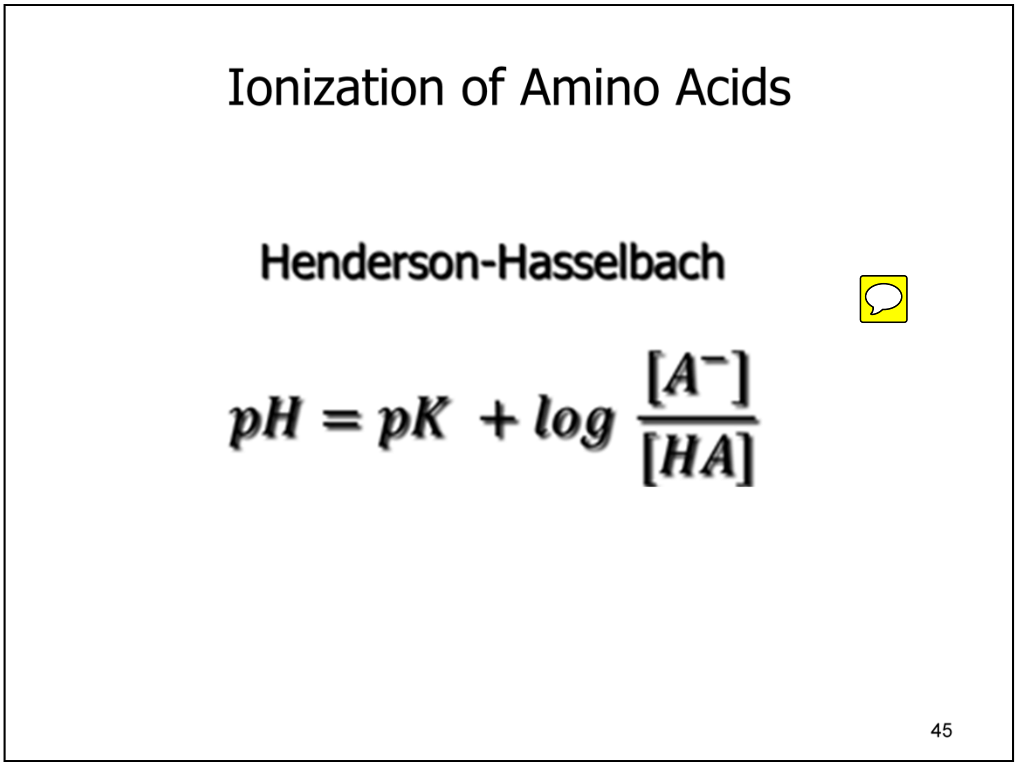
L-Isoleucine
(2*S*,3*S*)-Isoleucine

Here I provide the Newman projections and the Fischer projections of Threonine and isoleucine. Learn those.



Ionization of Amino Acids

The ionization of the alpha amino and alpha carboxyl groups of amino acids will allow three distinct states. At neutral pH, the Zwitterionic state predominates. In this state, the overall charge (disregarding the R group) is 0. At significantly higher pH, loss of a proton from the amino group occurs. This gives the anionic (-1 charged) form. Notice how the pKas for the carboxyl ionization differ from that of acetic acid in solution. *You should be familiar with the pKa values of ionizable side chains.*

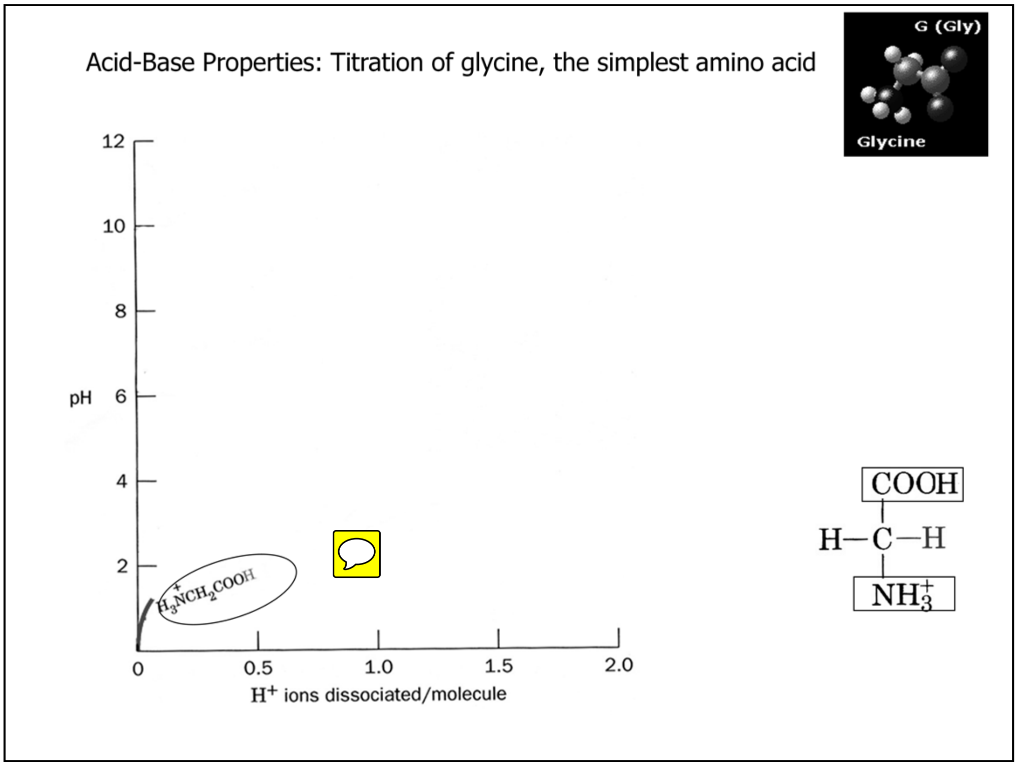


Ionization of Amino Acids

Henderson-Hasselbach

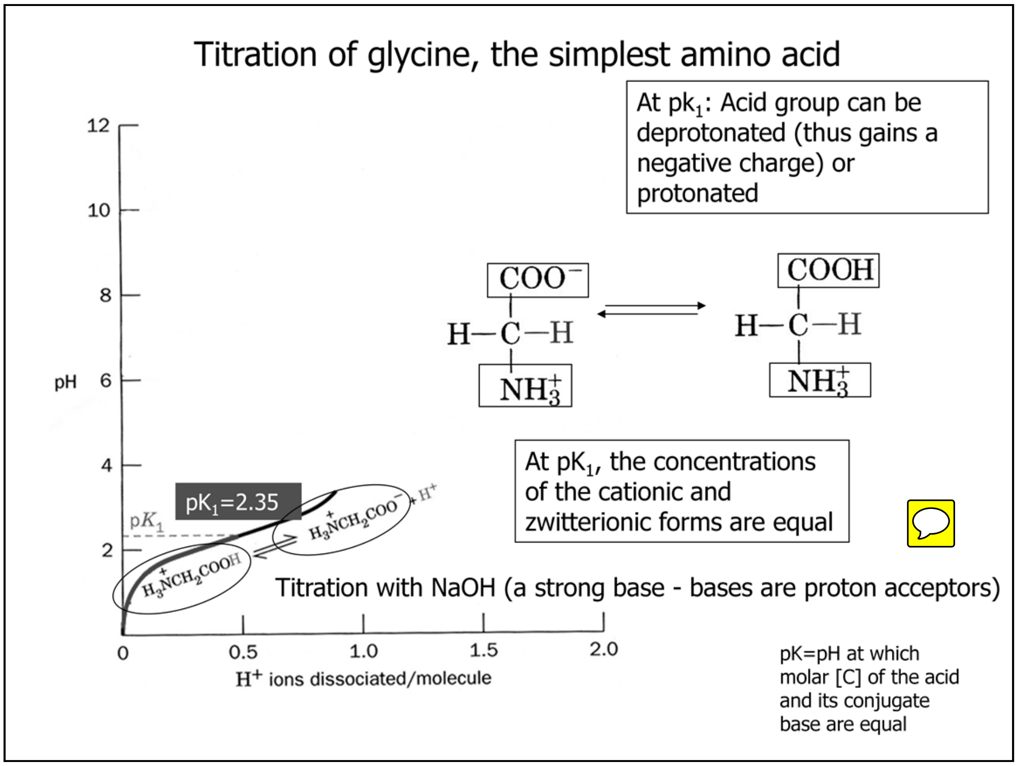
$$pH = pK + \log \frac{[A^-]}{[HA]}$$

The Henderson-Hasselbach predicts that the pH will be equal to the pK when in this case an acid HA is present 50% as HA and is 50% dissociated to A-. This means that the concentration of HA and A- are equal, and their ratio is equal to 1 (log 1=0).



Acid-Base Properties: Titration of glycine, the simplest amino acid

At low pH, both the amino and the carboxyl acid-base groups are fully protonated and the amino acid is in its cationic form .



Titration of glycine, the simplest amino acid

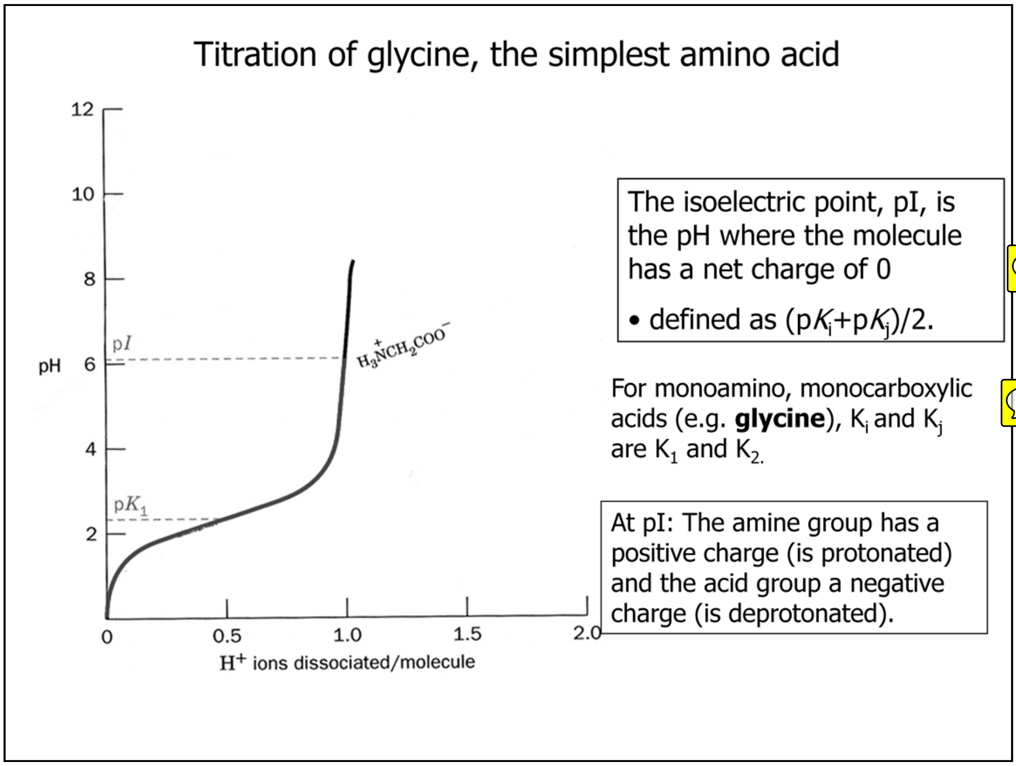
At pk_1: Acid group can be deprotonated (thus gains a negative charge) or protonated

At pK_1, the concentrations of the cationic and zwitterionic forms are equal

Titration with NaOH (a strong base - bases are proton acceptors)

pK=pH at which molar [C] of the acid and its conjugate base are equal

However, by the addition of a base (such as NaOH) the equilibrium can be slowly shifted towards the zwitterion. You will notice that the value of the pH does not change drastically before 1.0 equivalent of NaOH added. In this range the amino acid has buffering capacity (generally +/- 1 pH unit aroud the pK). The pK1 correspond to the pH when 50% is present in its acid and base conjugate.



Titration of glycine, the simplest amino acid

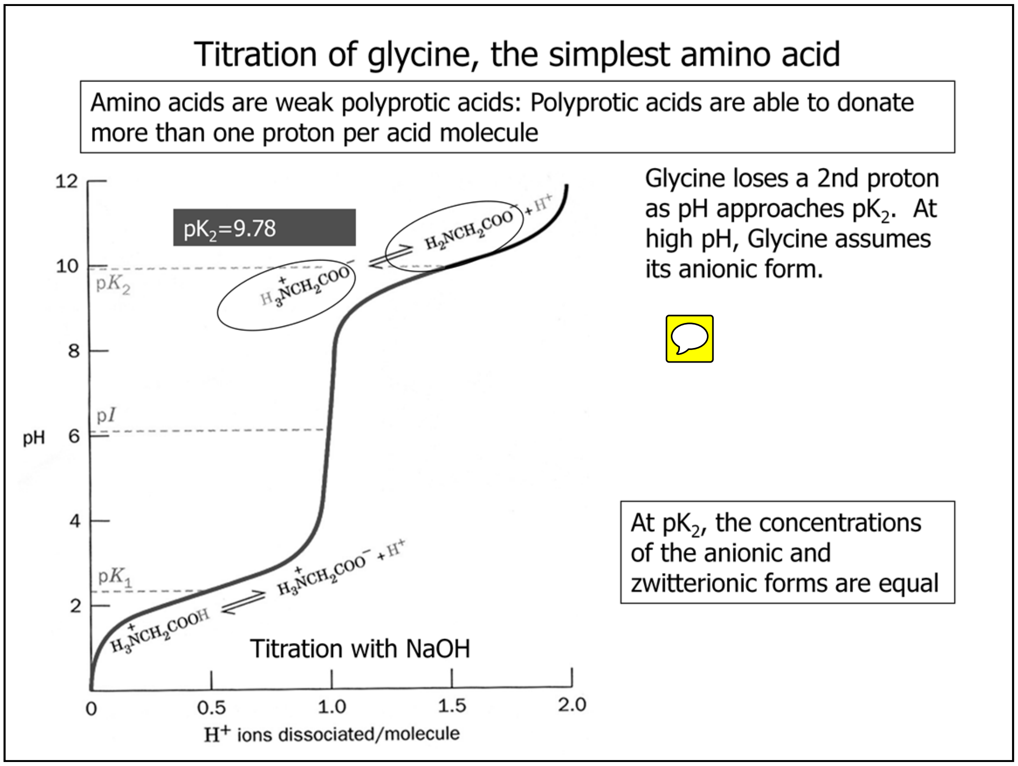
The isoelectric point, pI, is the pH where the molecule has a net charge of 0

- defined as $(pK_i + pK_j)/2$.

For monoamino, monocarboxylic acids (e.g. **glycine**), K_i and K_j are K_1 and K_2.

At pI: The amine group has a positive charge (is protonated) and the acid group a negative charge (is deprotonated).

You will notice that at the equivalent of 1.0 (i.e. 1 equivalent of NaOH added) the pH increases very quickly, at this point the alpha carboxylic acid is fully ionized and cannot provide protons to neutralize OH- and therefore the pH increases.



Titration of glycine, the simplest amino acid

Amino acids are weak polyprotic acids: Polyprotic acids are able to donate more than one proton per acid molecule

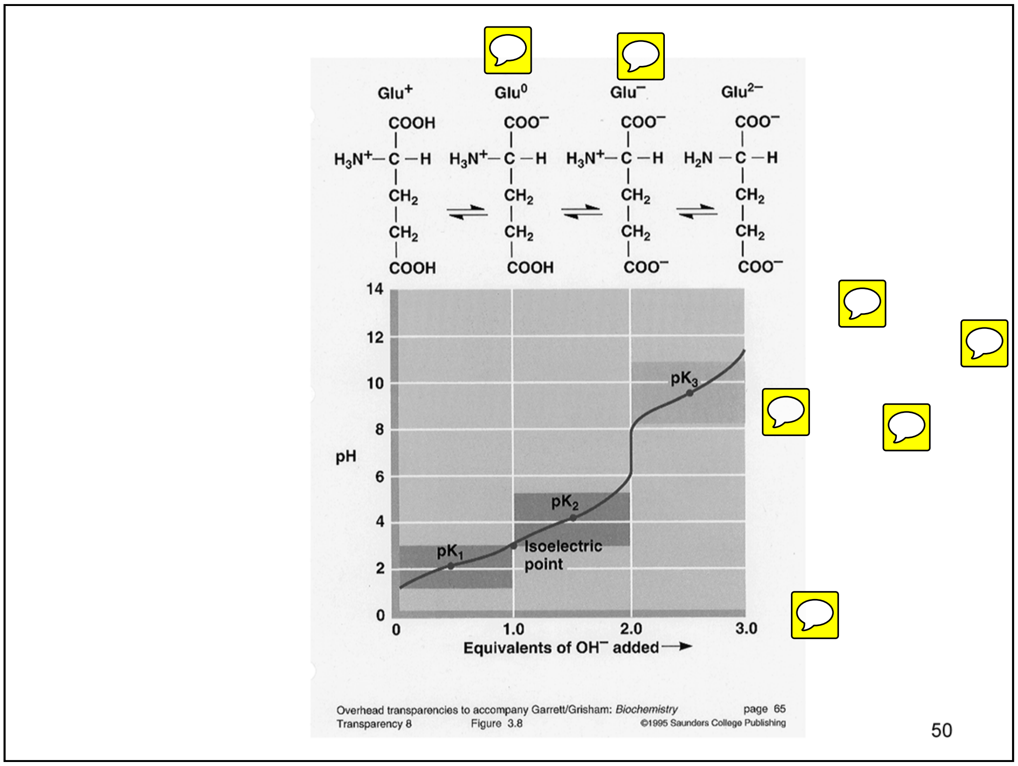
pK_2=9.78

Glycine loses a 2nd proton as pH approaches pK_2. At high pH, Glycine assumes its anionic form.

At pK_2, the concentrations of the anionic and zwitterionic forms are equal

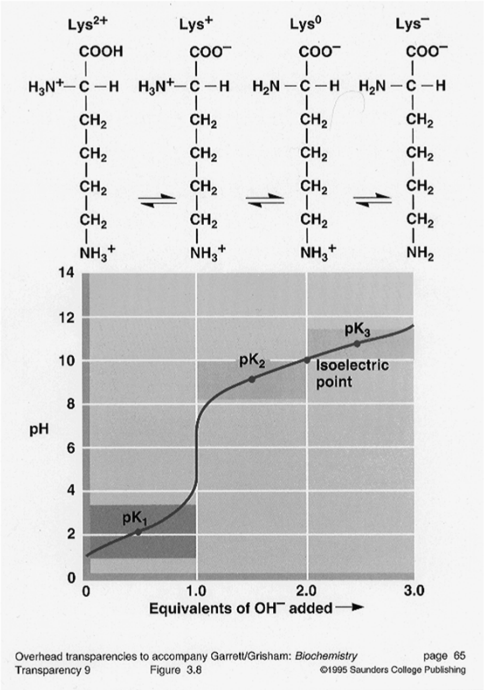
Titration with NaOH

As the pH nears the second pK of glycine, then the buffering capacity will start again and more protons will be liberated that can neutralize the OH- and therefore the pH change decreases. Finally, once all of the protons have been displaced (at 2.0 equivalent) and glycine is in its anionic form then the pH will increase to the pH of the solution of NaOH.



Overhead transparencies to accompany Garrett/Grisham: *Biochemistry* page 65
Transparency 8 Figure 3.8 ©1995 Saunders College Publishing

Please pay attention to the fact that when an amino acid has more than 2 pK, then the pI is found using the pKs that surrounds the zwitterion. In this case pK_1 and pK_2. Therefore the pI is $(pK_1+pK_2)/2$



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Same issue applies here. In this case the zwitterion will occur at pH between pK_2 and pK_3. Therefore the pI is $(pK_2+pK_3)/2$. One easy way to figure this out is to draw the structure of the amino acid in its different charge states along the titration and to find out which structure represent the neutral zwitterion.