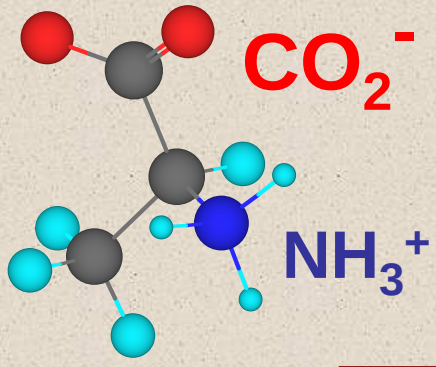
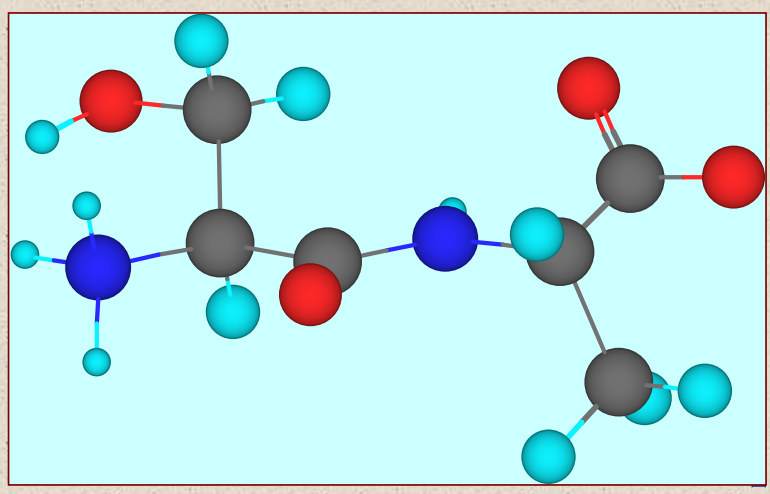
Amino Acids and Proteins

Chapter 27



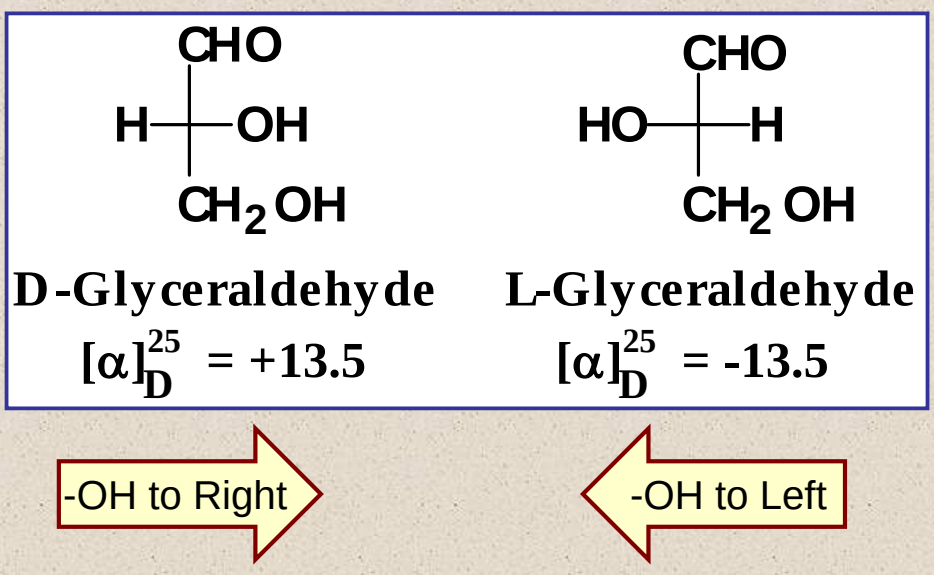


Amino Acids

- Amino acid: a compound that contains both an amino group and a carboxyl group
  - **α-Amino acid:** an amino acid in which the amino group is on the carbon adjacent to the carboxyl group
  - – although α-amino acids are commonly written in the unionized form, they are more properly written in the **zwitterion** (internal salt) form

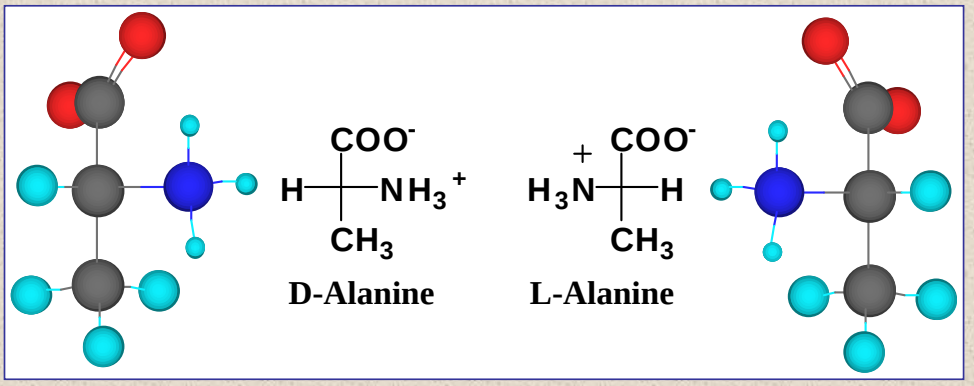
D,L Monosaccharides

In 1891, Emil Fischer made the **arbitrary** assignments of D- and L- to the enantiomers of glyceraldehyde (aldehyde is C1-at the top).

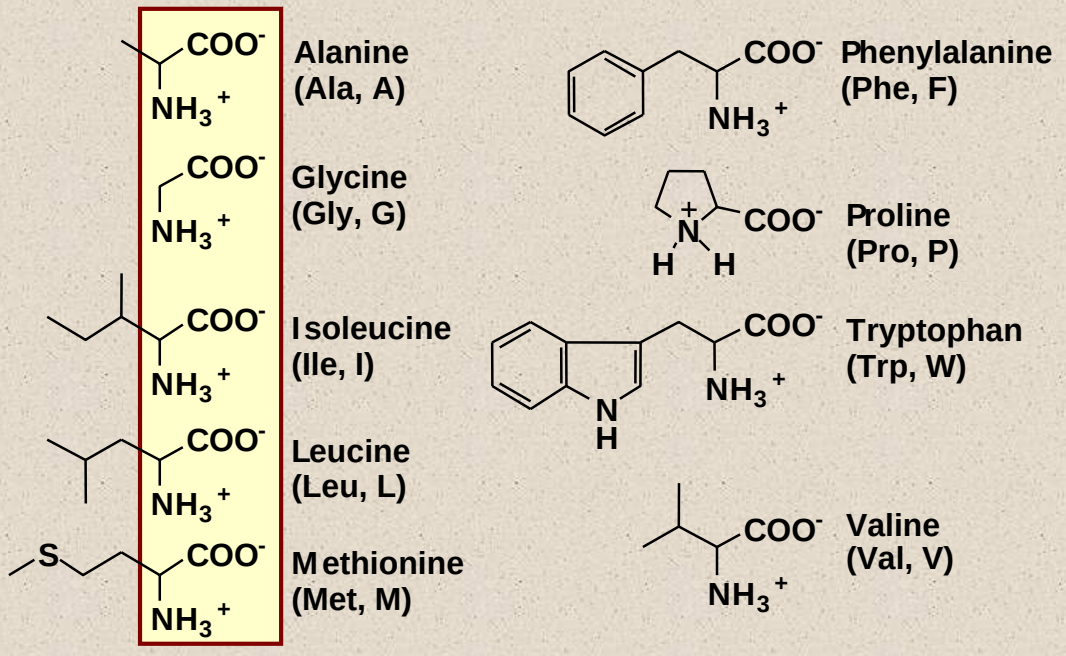


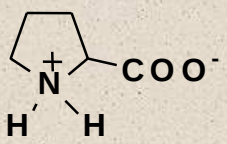
Chirality of Amino Acids

- With the exception of **glycine**, all protein-derived amino acids have at least one stereocenter (**the α-carbon**) and are chiral
  - the vast majority have the **L-configuration** at their α-carbon

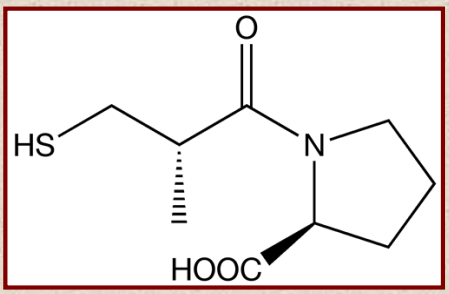


Nonpolar side chains





Proline
(Pro, P)



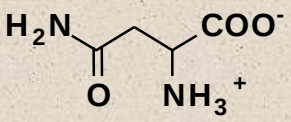
Captopril-
ACE Inhibitor

Angiotensin Converting Enzyme

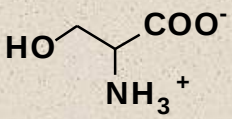
Angiotensin I → **Angiotensin II**

**Angiotensin II is a potent vasoconstrictor so,
ACE inhibitors are used for the treatment of hypertension**

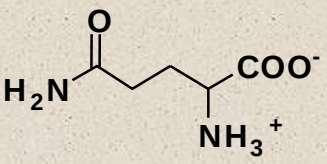
Polar side chains



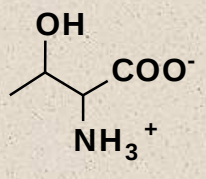
Asparagine
(Asn, N)



Serine
(Ser, S)



Glutamine
(Gln, Q)

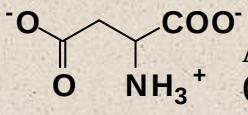


Threonine
(Thr, T)

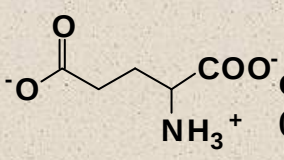
Amide Side Chains

Hydroxyl Side Chains

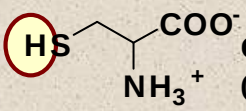
Acidic & Basic Side Chains



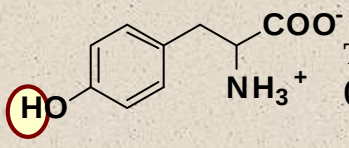
Aspartic acid
(Asp, D)



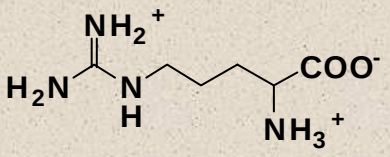
Glutamic acid
(Glu, E)



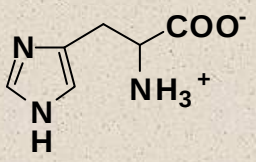
Cysteine
(Cys, C)



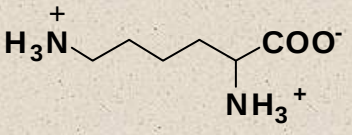
Tyrosine
(Tyr, Y)



Arginine
(Arg, R)



Histidine
(His, H)



Lysine
(Lys, K)

Acid-Base Properties

Nonpolar & polar side chains	pK_a of α–COOH	pK_a of α–NH_3^+
alanine	2.35	9.87
asparagine	2.02	8.80
glutamine	2.17	9.13
glycine	2.35	9.78
isoleucine	2.32	9.76
leucine	2.33	9.74
methionine	2.28	9.21
phenylalanine	2.58	9.24
proline	2.00	10.60
serine	2.21	9.15
threonine	2.09	9.10
tryptophan	2.38	9.39
valine	2.29	9.72

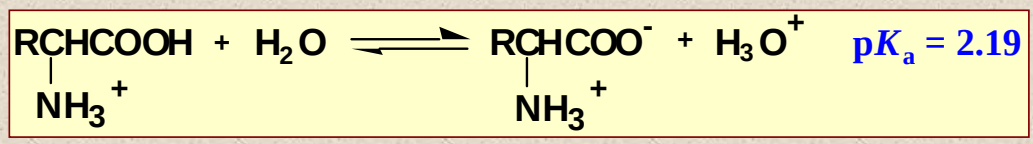
Acid-Base Properties

Acidic Side Chains	pK_a of α–COOH	pK_a of α–NH_3^+	pK_a of Side Chain	Side Chain Group
aspartic acid	2.10	9.82	3.86	carboxyl
glutamic acid	2.10	9.47	4.07	carboxyl
cysteine	2.05	10.25	8.00	sufhydryl
tyrosine	2.20	9.11	10.07	phenolic

Basic Side Chains	pK_a of α–COOH	pK_a of α–NH_3^+	pK_a of Side Chain	Side Chain Group
arginine	2.01	9.04	12.48	guanidino
histidine	1.77	9.18	6.10	imidazole
lysine	2.18	8.95	10.53	1° amino

Acidity: α-COOH Groups

- The average pK_a of an α-carboxyl group is 2.19, which makes them considerably stronger acids than **acetic acid (pK_a 4.76)**
  - the greater acidity is accounted for by the electron-withdrawing inductive effect of the adjacent -NH_3^+ group

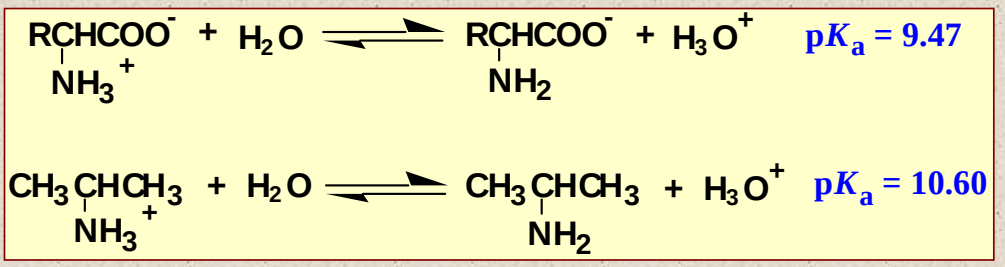


Acidity: side chain -COOH

- Due to the electron-withdrawing inductive effect of the α-NH_3^+ group, side chain -COOH groups are also stronger than acetic acid
  - the inductive effect decreases with distance from the α-NH_3^+ group. Compare:
    - **α-COOH group of alanine (pK_a 2.35)**
    - **β-COOH group of aspartic acid (pK_a 3.86)**
    - **γ-COOH group of glutamic acid (pK_a 4.07)**

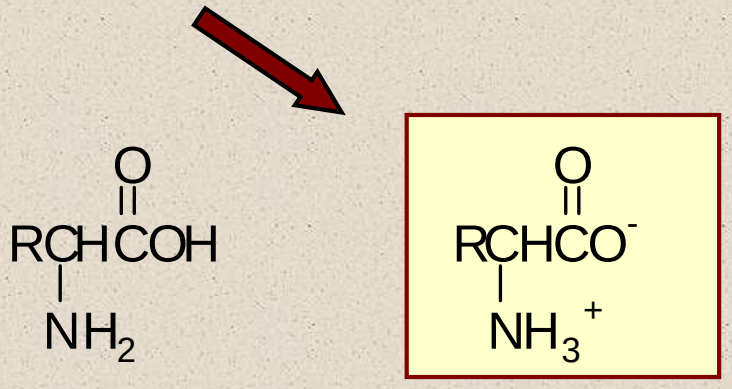
Acidity: α-NH_3^+ groups

- The average value of **pK_a for an α-NH_3^+ group is 9.47**, compared with a value of 10.76 for a 1° alkylammonium ion

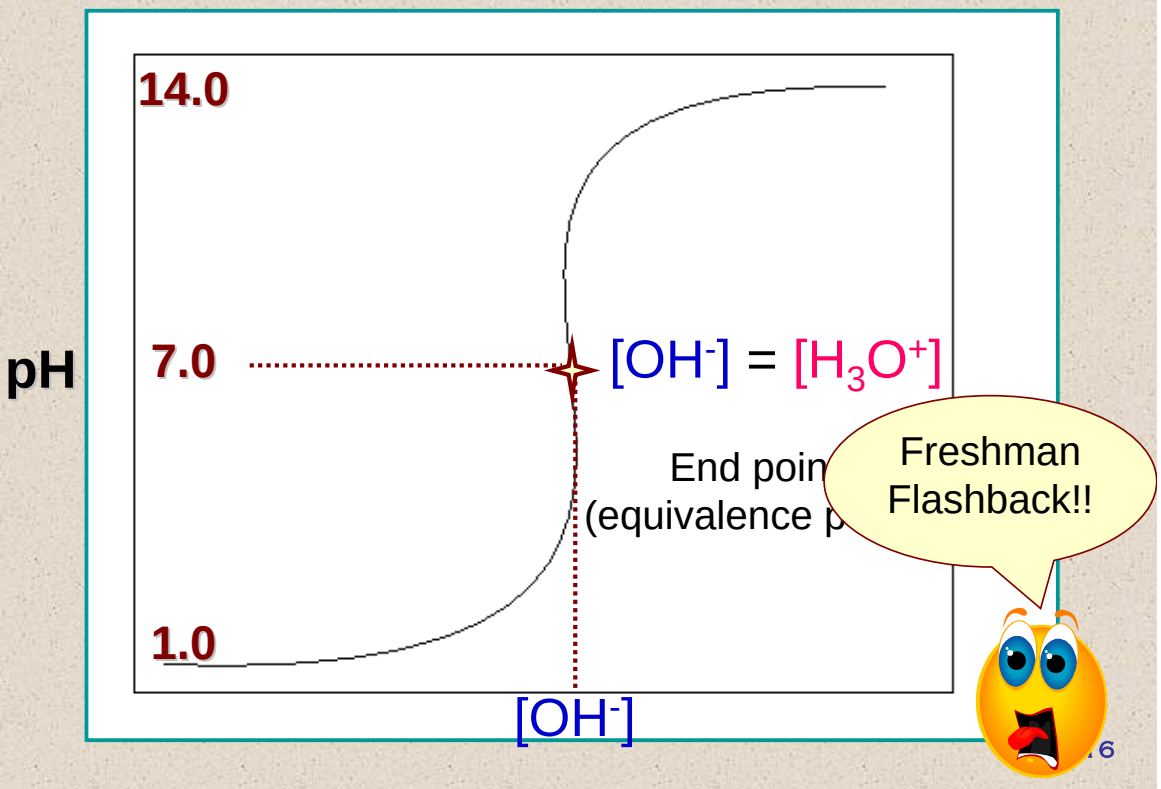


Acid-Base Properties

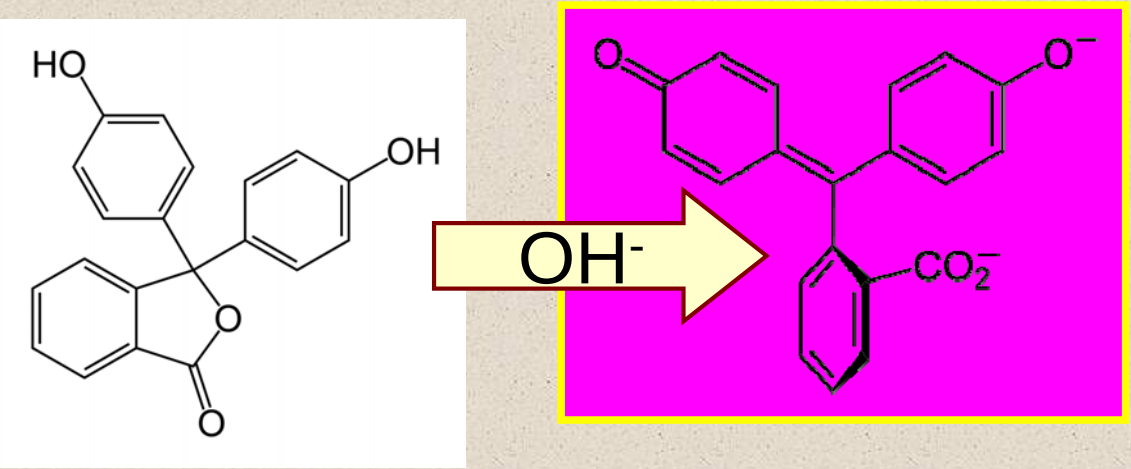
– **Both functional groups display acid-base chemistry**



$NaOH + HCl \rightarrow H_2O + NaCl$



Phenolphthalein



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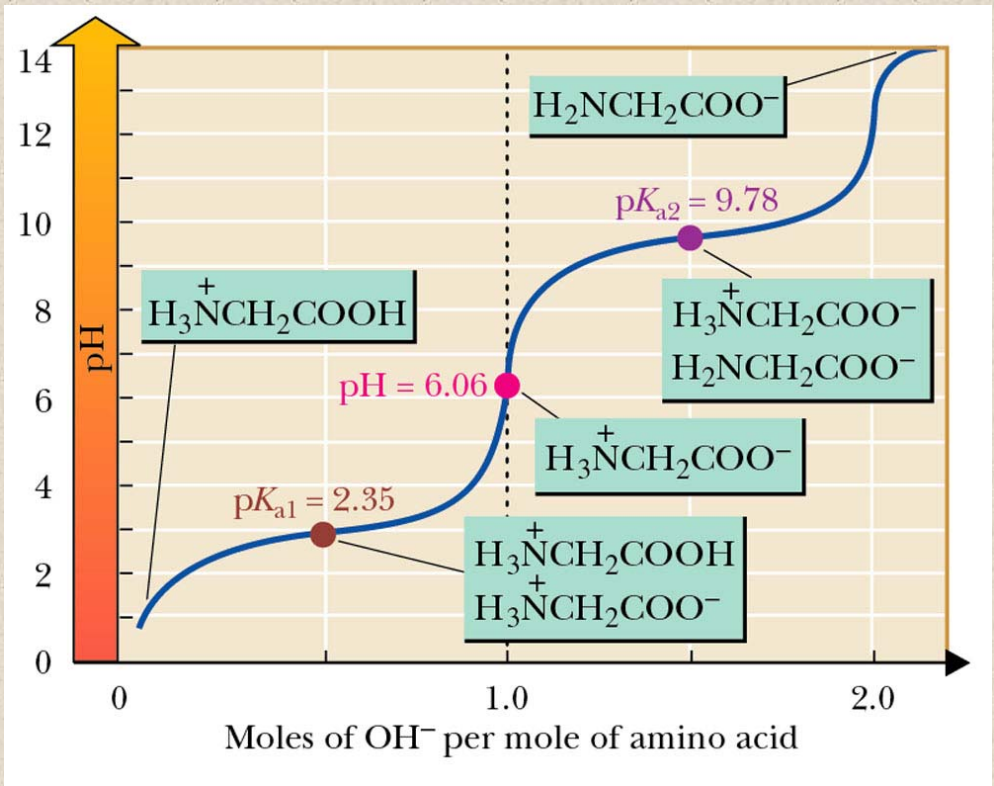
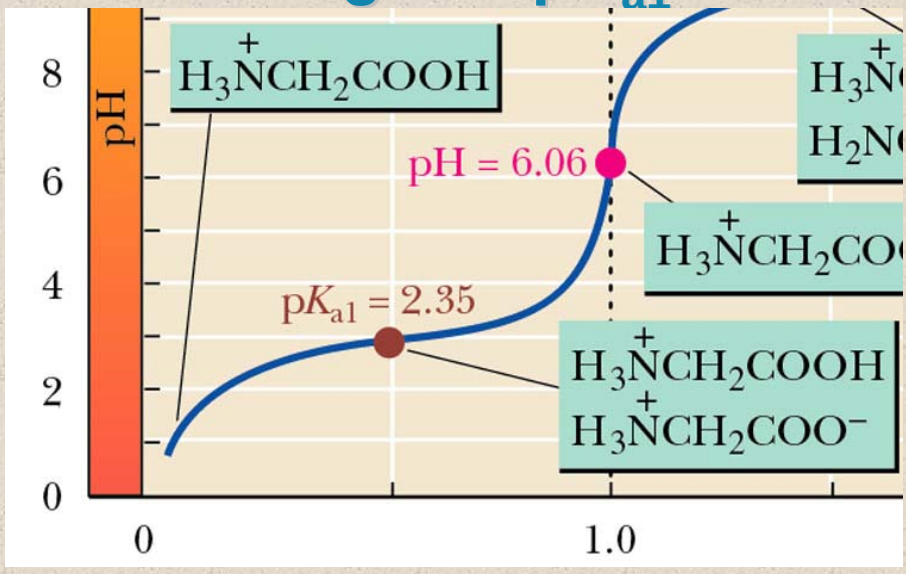
Titration of Amino Acids



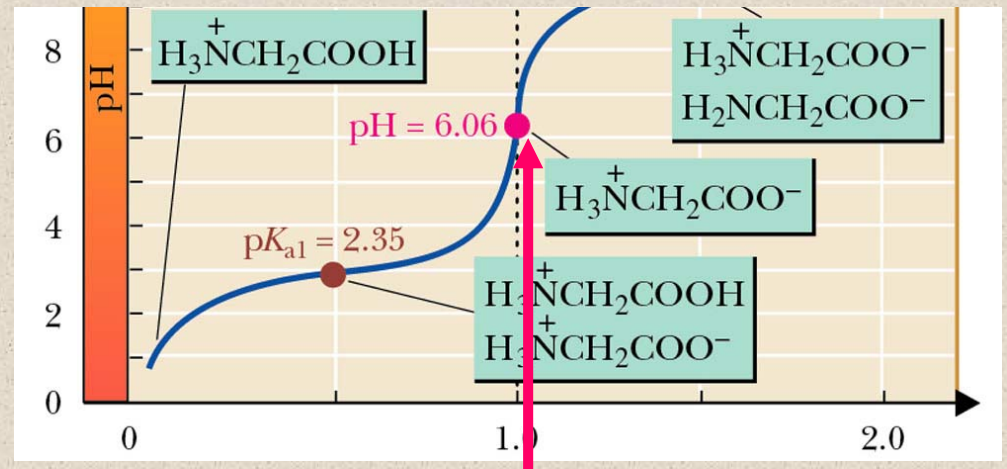
Stage 1– pK_{a1}



$H_3N^+CH_2COOH = H_3N^+CH_2COO^-$

0.5 eq OH^-

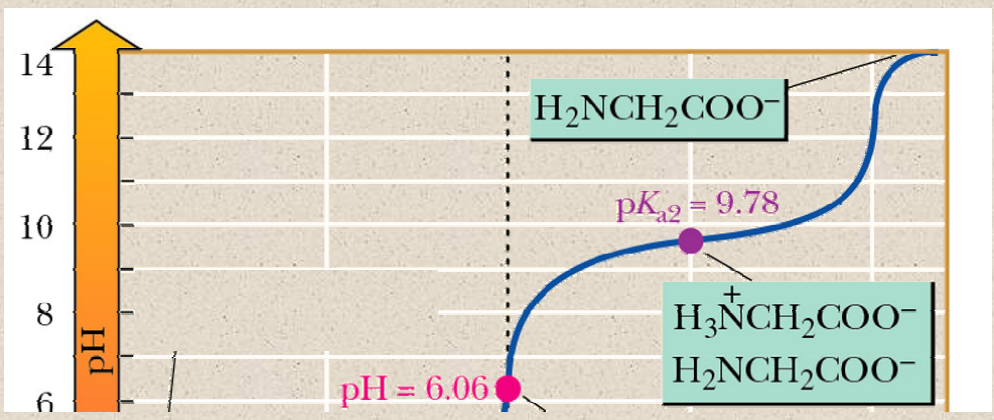
Isoelectric Point



$H_3N^+CH_2COO^-$

1.0 eq OH^-

Stage 2– pK_a2



$H_3N^+CH_2COO^- = H_2NCH_2COO^-$

1.5 eq OH^-

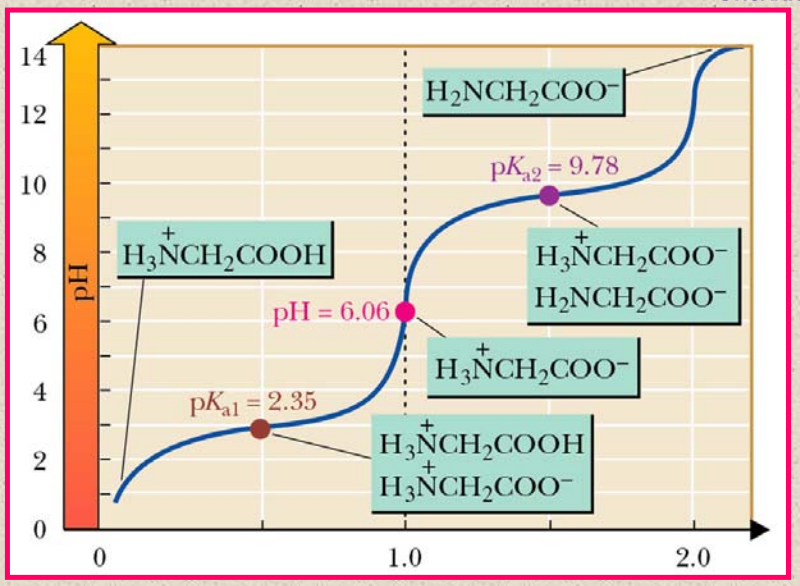
Isoelectric Point

Isoelectric point (pI): the pH at which an amino acid, polypeptide, or protein has no net charge

– the pH for glycine, for example, falls between the pK_a values for the carboxyl and amino groups

$$\mathbf{pI} = \frac{1}{2}\left(\mathbf{p}K_a\ \alpha\text{–COOH} + \mathbf{p}K_a\ \alpha\text{–}NH_3^+\right)$$

$$= \frac{1}{2}(2.35 + 9.78) = 6.06$$



$$pI = \frac{1}{2}\,(pK_a\ \alpha\text{–}COOH\ +\ pK_a\ \alpha\text{–}NH_3^+)$$

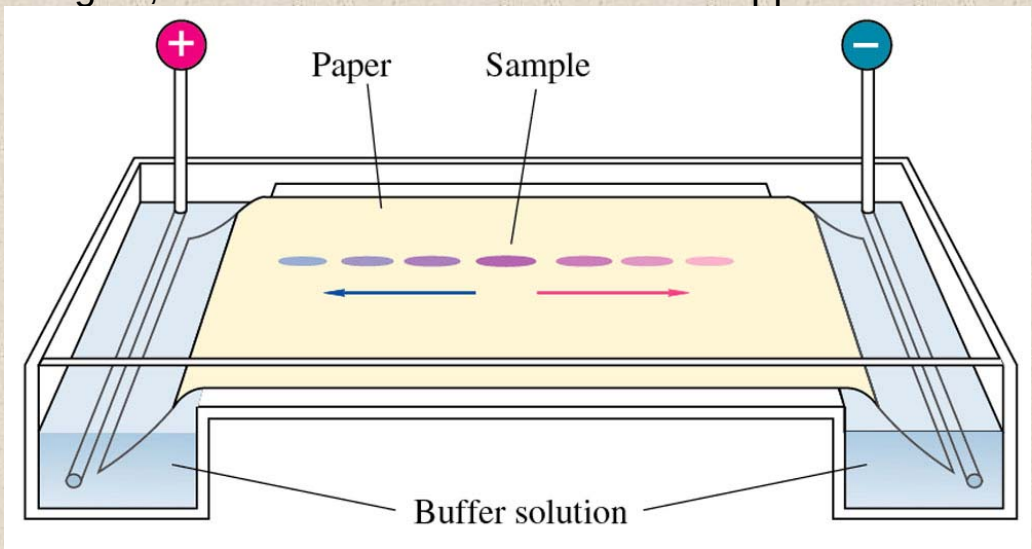
$$= \frac{1}{2}\,(2.35\ +\ 9.78) = \boxed{6.06}$$

Isoelectric Point

Nonpolar & polar side chains	pK_a of α–COOH	pK_a of α–NH_3^+	pK_a of Side Chain	pI
alanine	2.35	9.87	----	6.11
asparagine	2.02	8.80	----	5.41
glutamine	2.17	9.13	----	5.65
glycine	2.35	9.78	----	6.06
isoleucine	2.32	9.76	----	6.04
leucine	2.33	9.74	----	6.04
methionine	2.28	9.21	----	5.74
phenylalanine	2.58	9.24	----	5.91
proline	2.00	10.60	----	6.30
serine	2.21	9.15	----	5.68
threonine	2.09	9.10	----	5.60
tryptophan	2.38	9.39	----	5.88
valine	2.29	9.72	----	6.00

Electrophoresis

- **Electrophoresis:** the process of separating compounds on the basis of their electric charge
  - electrophoresis of amino acids can be carried out using paper, starch, polyacrylamide and agarose gels, and cellulose acetate as solid supports



Electrophoresis

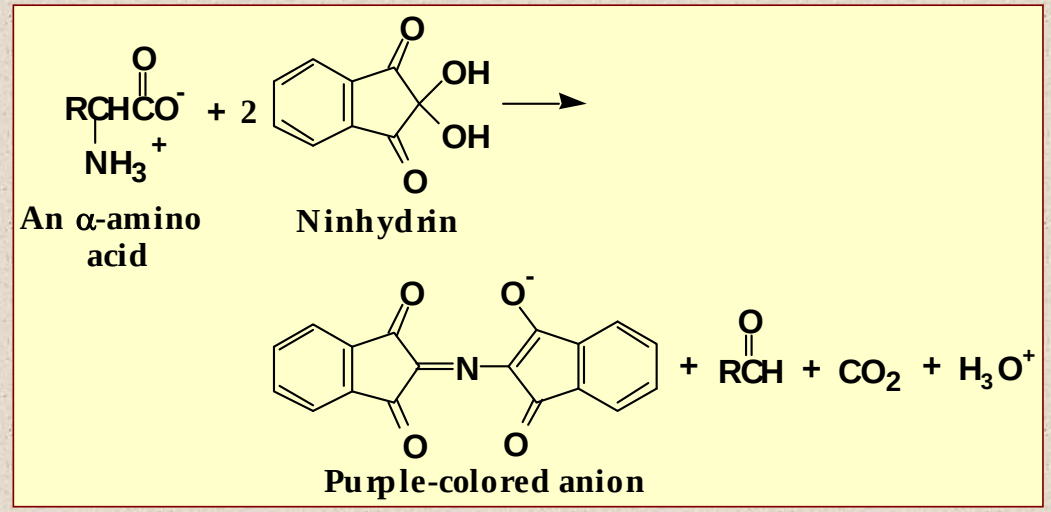
1. a sample of amino acids is applied as a spot on the paper strip
2. an electric potential is applied to the electrode vessels and amino acids migrate toward the electrode with charge opposite their own
3. molecules with a high charge density move faster than those with low charge density

Electrophoresis

- molecules at their isoelectric point remain at the origin
- after separation is complete, the strip is dried and developed to make the separated amino acids visible
- 19 of the 20 amino acids give the same purple-colored anion; proline gives an orange-colored compound

Electrophoresis

a reagent commonly used to detect amino acid is **ninhydrin**

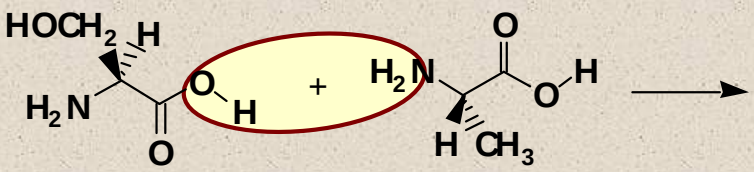


Not Exam Material

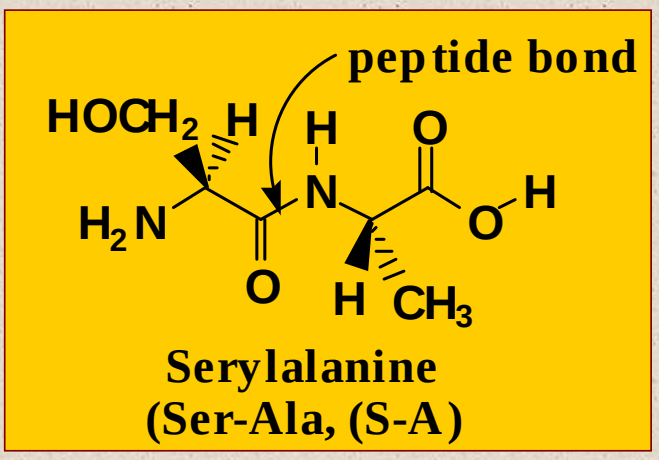
Polypeptides & Proteins

- In 1902, Emil Fischer proposed that proteins are long chains of amino acids joined by amide bonds to which he gave the name peptide bonds
- **Peptide bond:** the special name given to the amide bond between the α-carboxyl group of one amino acid and the α-amino group of another

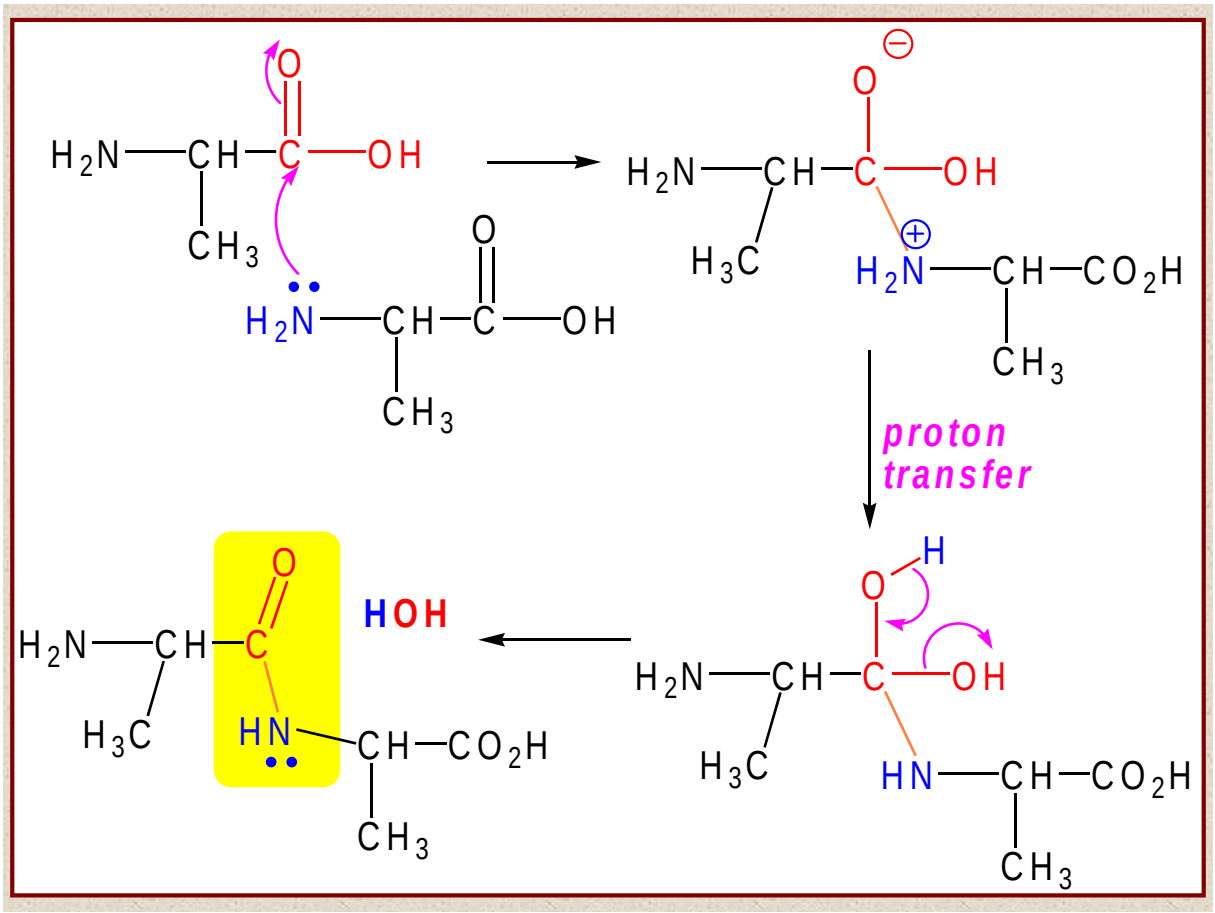
Serylalanine (Ser-Ala)



Serine (Ser, S) **Alanine (Ala, A)**



Serylalanine (Ser-Ala, (S-A)

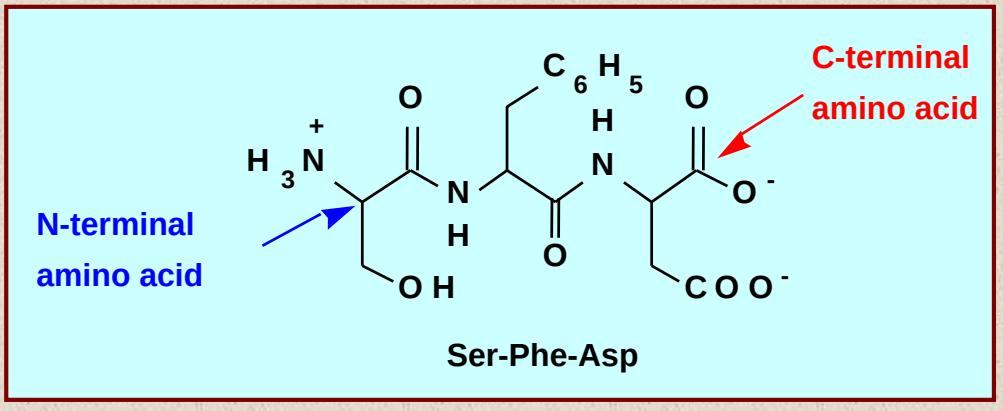


Peptide Terminology

- peptide: the name given to a short polymer of amino acids joined by peptide bonds; they are classified by the number of amino acids in the chain
- dipeptide: a molecule containing two amino acids joined by a peptide bond
- tripeptide: a molecule containing three amino acids joined by peptide bonds
- polypeptide: a macromolecule containing many amino acids joined by peptide bonds
- protein: a biological macromolecule of molecular weight 5000 g/mol of greater, consisting of one or more polypeptide chains

Writing Peptides

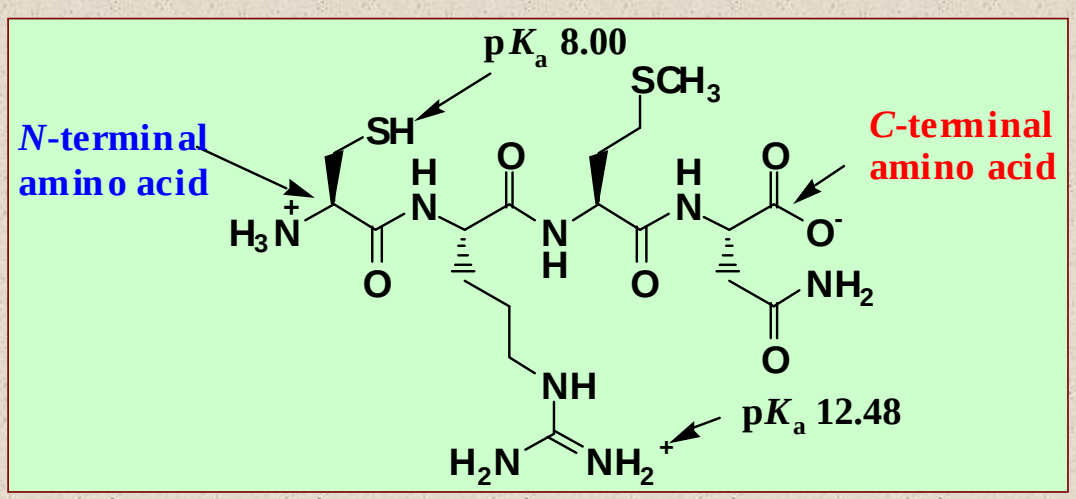
– by convention, peptides are written from the left, beginning with the free -NH_3^+ group and ending with the free -COO^- group on the right



Writing Peptides

– the tetrapeptide Cys-Arg-Met-Asn

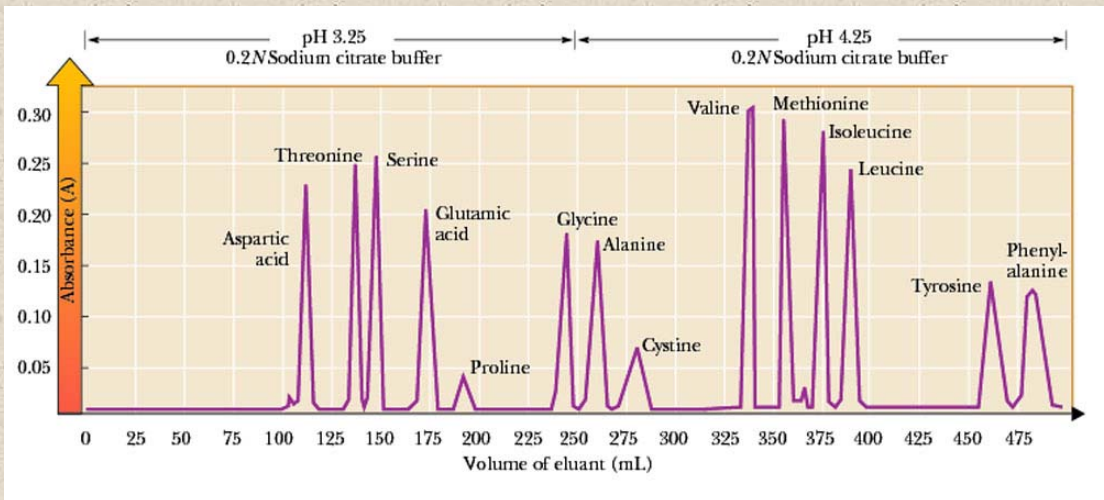
– at pH 6.0, its net charge is +1



Primary Structure

- **Primary structure:** the sequence of amino acids in a polypeptide chain; read from the *N*-terminal amino acid to the *C*-terminal amino acid
- Amino acid analysis:
  - hydrolysis of the polypeptide, most commonly carried out using 6M HCl at elevated temperature
  - quantitative analysis of the hydrolysate (i.e. hydrolyzed solution) by ion-exchange chromatography

Ion Exchange Chromatography



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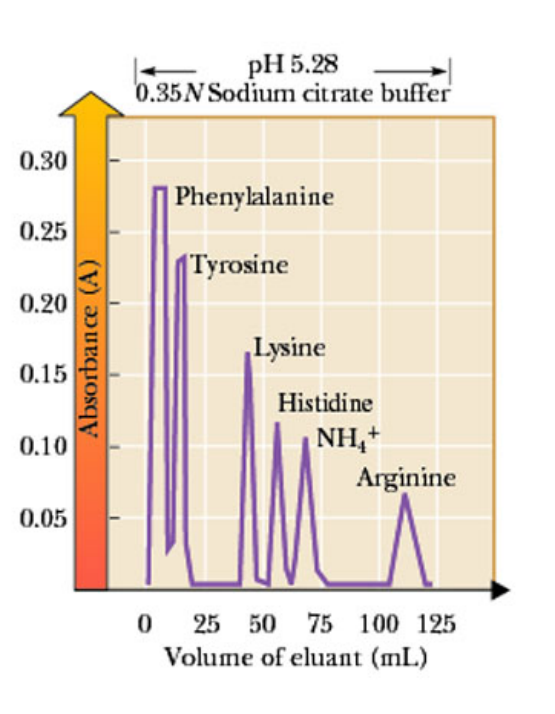
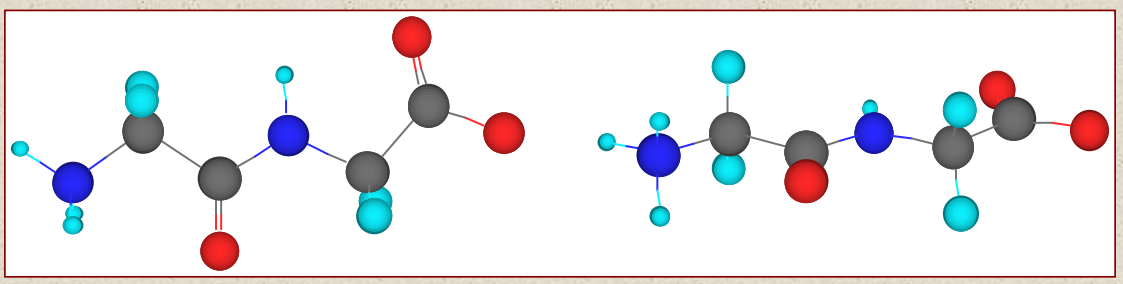


Not Exam Material

Peptide Bond Geometry

– the four atoms of a peptide bond and the two alpha carbons joined to it lie in a plane with bond angles of 120° about C and N

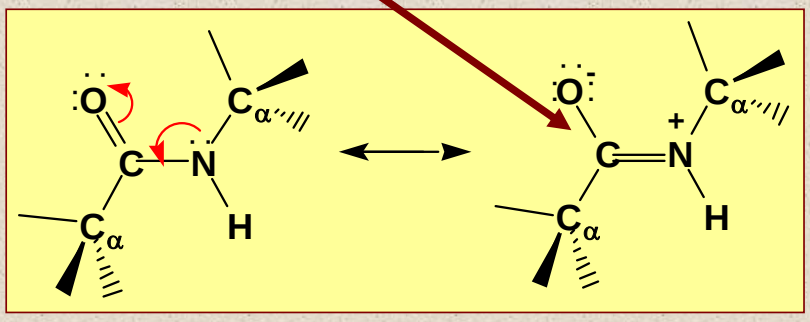
– the model of Gly-Gly is viewed from two perspectives to show the planarity of the six atoms of the peptide bond



Peptide Bond Geometry

– to account for this geometry, Linus Pauling proposed that a peptide bond is most accurately represented as a hybrid of two contributing structures (resonance)

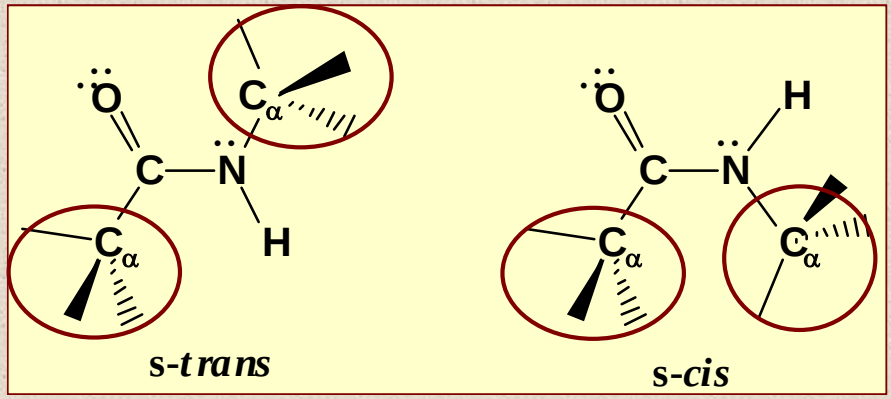
– the hybrid has considerable C-N double bond character and **rotation about the peptide bond is restricted**



Peptide Bond Geometry

– two conformations are possible for a planar peptide bond

– virtually all peptide bonds in naturally occurring proteins studied to date have the *s-trans* conformation

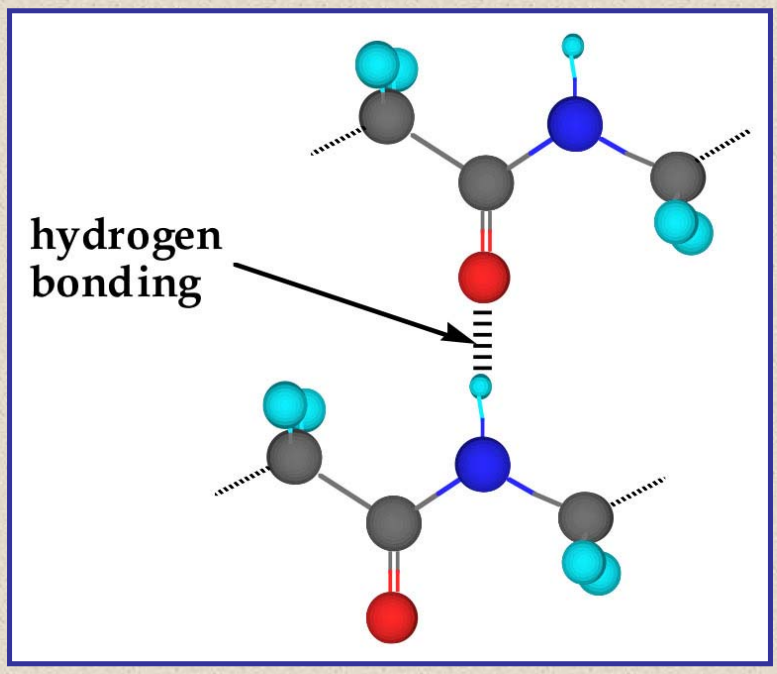


Secondary Structure

- **Secondary structure:** the ordered arrangements (conformations) of amino acids in localized regions of a polypeptide or protein
- To determine from model building which conformations would be of greatest stability, Pauling and Corey assumed that
  1. all six atoms of each peptide bond lie in the same plane and in the s-trans conformation
  2. there is hydrogen bonding between the N-H group of one peptide bond and a C=O group of another peptide bond as shown in the next screen

Secondary Structure

– hydrogen bonding between amide groups



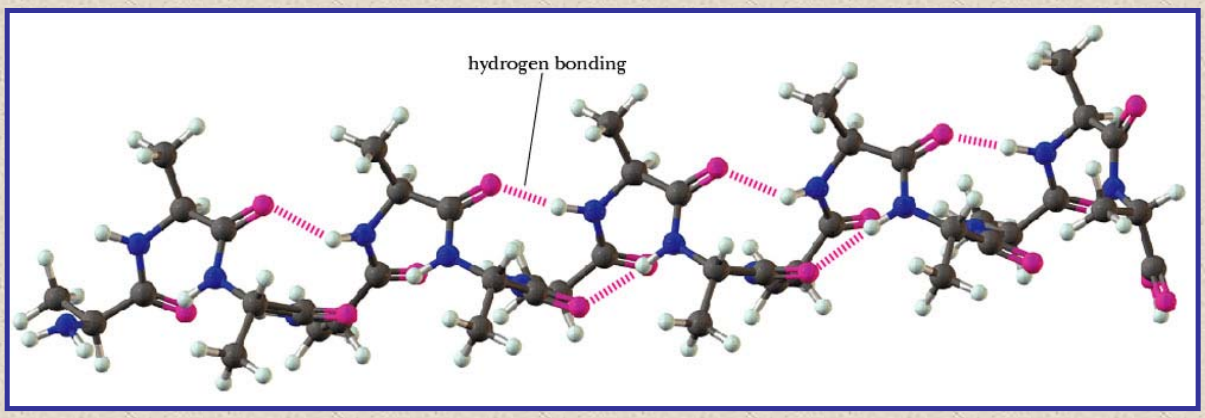
Secondary Structure

- On the basis of model building, Pauling and Corey proposed that two types of secondary structure should be particularly stable
  - ➢α-helix
  - ➢antiparallel β-pleated sheet

α-Helix: a type of secondary structure in which a section of polypeptide chain coils into a spiral, most commonly a right-handed spiral

The α-Helix

– Figure 27.14: the polypeptide chain is repeating units of L-alanine



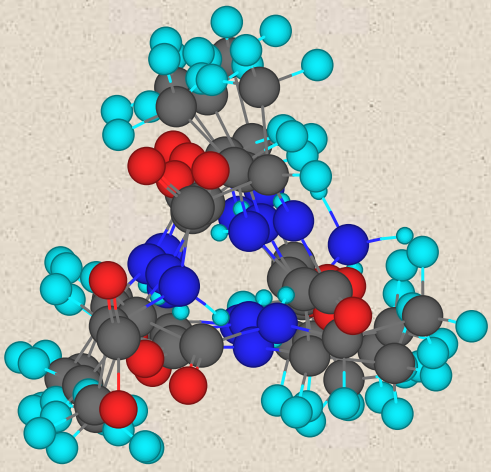
The α-Helix

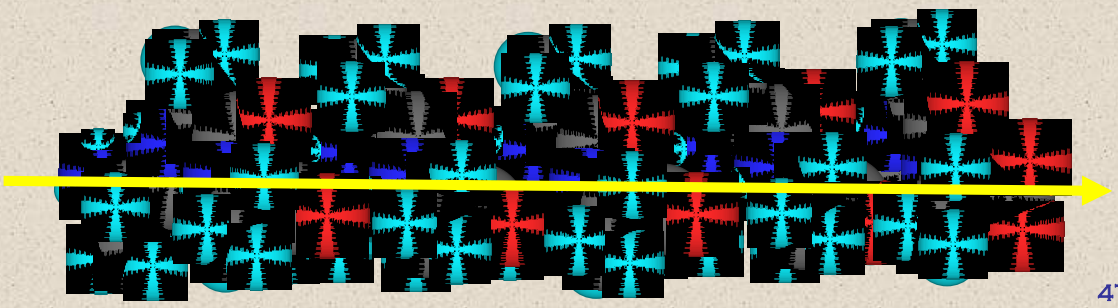
- In a section of α-helix
  - there are 3.6 amino acids per turn of the helix
  - each peptide bond is s-*trans* and planar
  - N-H groups of all peptide bonds point in the same direction, which is roughly parallel to the axis of the helix

The α-Helix

- In a section of α-helix
  - C=O groups of all peptide bonds point in the opposite direction, and also parallel to the axis of the helix
  - the C=O group of each peptide bond is hydrogen bonded to the N-H group of the peptide bond four amino acid units away from it
  - all R- groups point outward from the helix

- An α-helix of repeating units of L-alanine
  - a ball-and-stick model viewed looking down the axis of the helix
  - a space-filling model viewed from the side



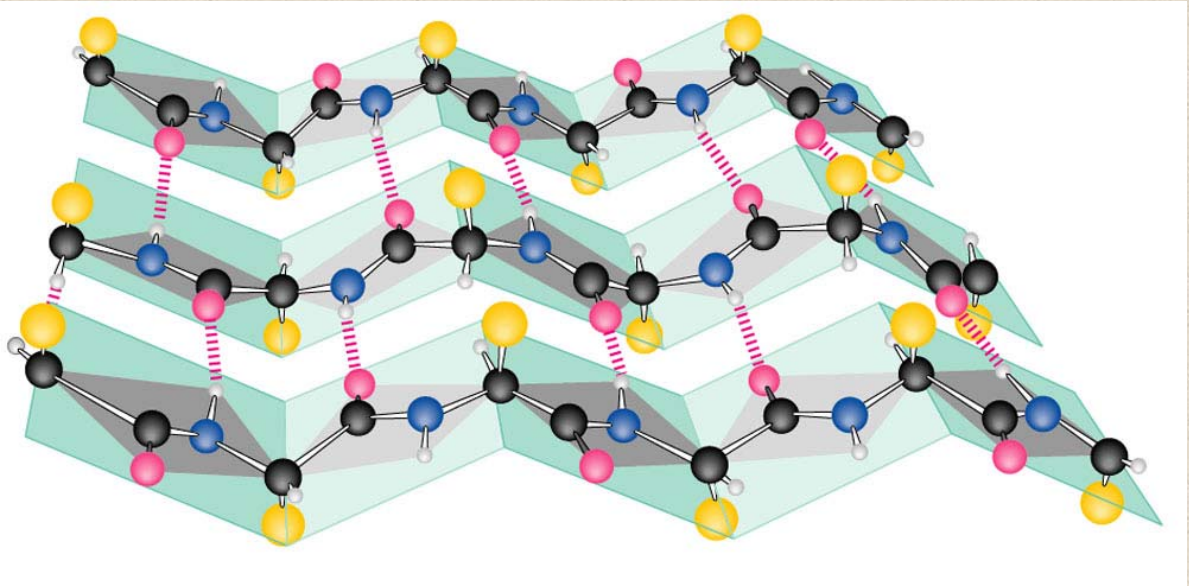


β-Pleated Sheet

- The antiparallel β-pleated sheet consists of adjacent polypeptide chains running in opposite directions
  - each peptide bond is planar and has the s-*trans* conformation
  - the C=O and N-H groups of peptide bonds from adjacent chains point toward each other and are in the same plane so that hydrogen bonding is possible between them
  - all R- groups on any one chain alternate, first above, then below the plane of the sheet, etc.

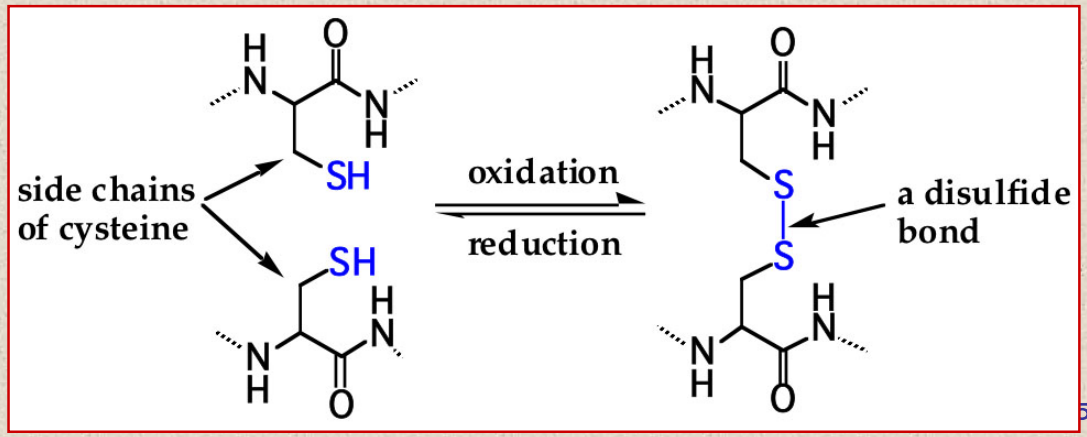
β-Pleated Sheet

β-pleated sheet with three polypeptide chains running in opposite directions

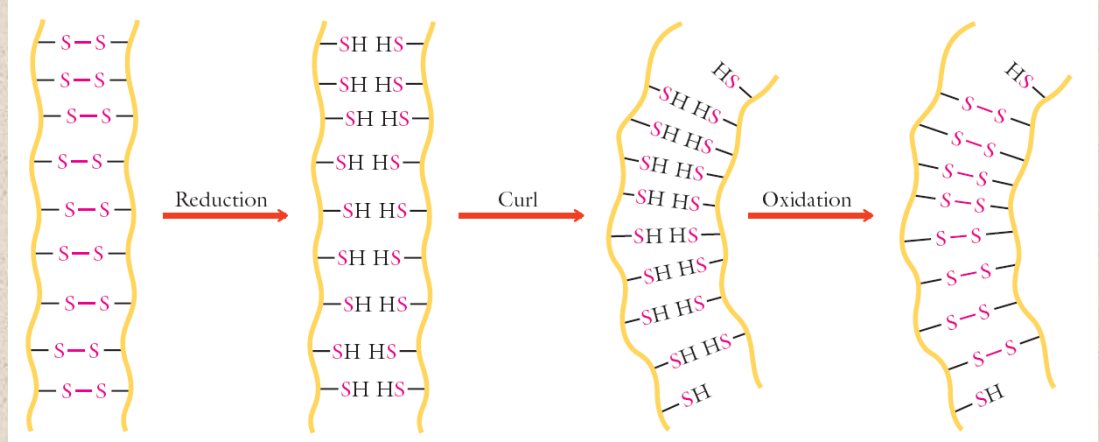


Tertiary Structure

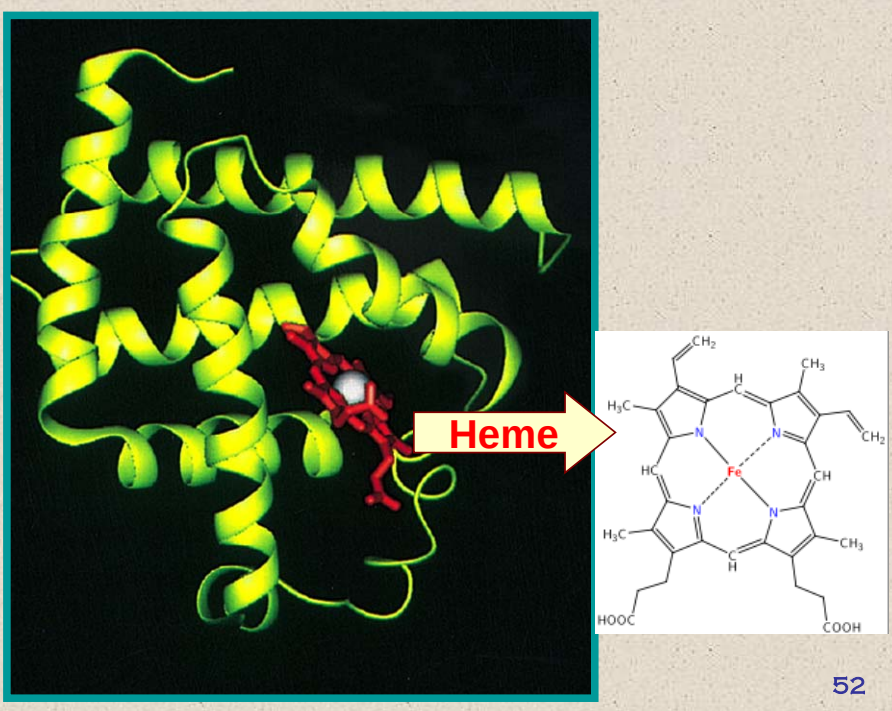
- **Tertiary structure:** the three-dimensional arrangement in space of **all atoms in a single polypeptide chain**
  - disulfide bonds between the side chains of cysteine play an important role in maintaining 3° structure



Permanent waving of hair is accomplished by breaking and reforming cysteine cross-links within the hair fiber:



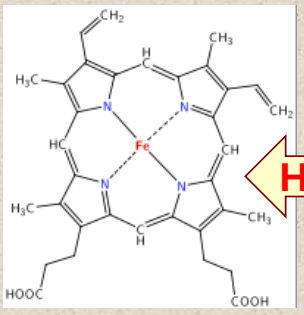
– A ribbon model of myoglobin

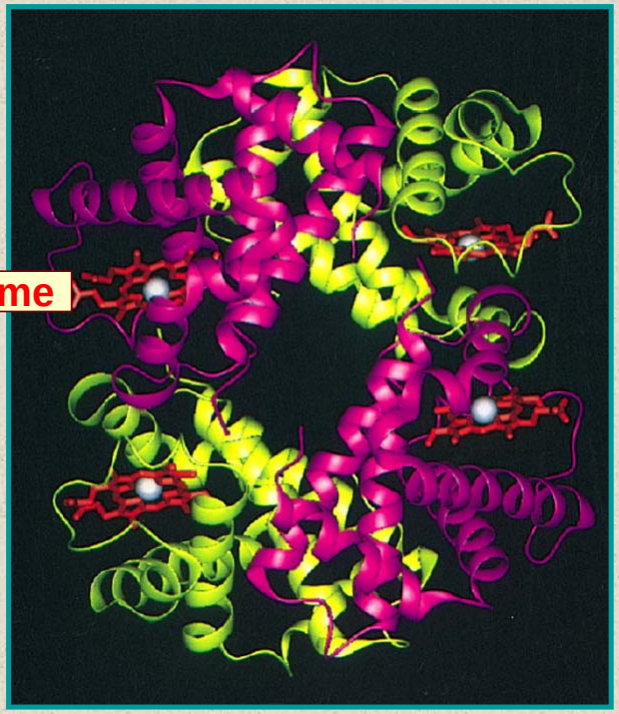


Quaternary structure

- **Quaternary structure:** the arrangement of polypeptide chains into a **noncovalently** bonded aggregation
  - the major factor stabilizing quaternary structure is the hydrophobic effect
- **Hydrophobic effect:** the tendency of nonpolar groups to cluster together in such a way as to be shielded from contact with an aqueous environment

The quaternary structure of hemoglobin



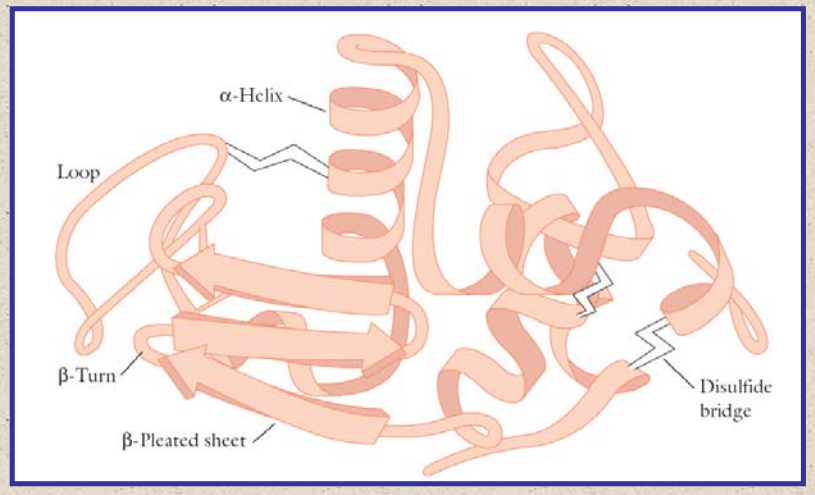


Lysozyme

•Lysozyme is an enzyme found in the cells and secretions of vertebrates.

•Lysozyme hydrolyzes bacterial cell walls which then are susceptible to cell lysis or breaking open.

•Lysozyme from hen egg white contains 129 amino acids which are organized into all four types of secondary structure:



Denaturation

Denaturation is the loss of native conformation due to a change in environmental conditions. The non-functioning protein is called a **denatured protein**.

Denaturation results from the disruptions of the weak secondary forces holding a protein in its native conformation. Disulfide bridges confer considerable resistance to denaturation because they are much stronger than the weak secondary forces.

Denaturation

A variety of denaturing conditions or agents lead to protein denaturation:

- **Increased temperature (or microwave radiation)**
- **Ultraviolet and ionizing radiation**
- **Mechanical energy**
- **Changes in pH**
- **Organic chemicals**
- **Heavy metal salts**
- **Oxidizing and reducing agents**