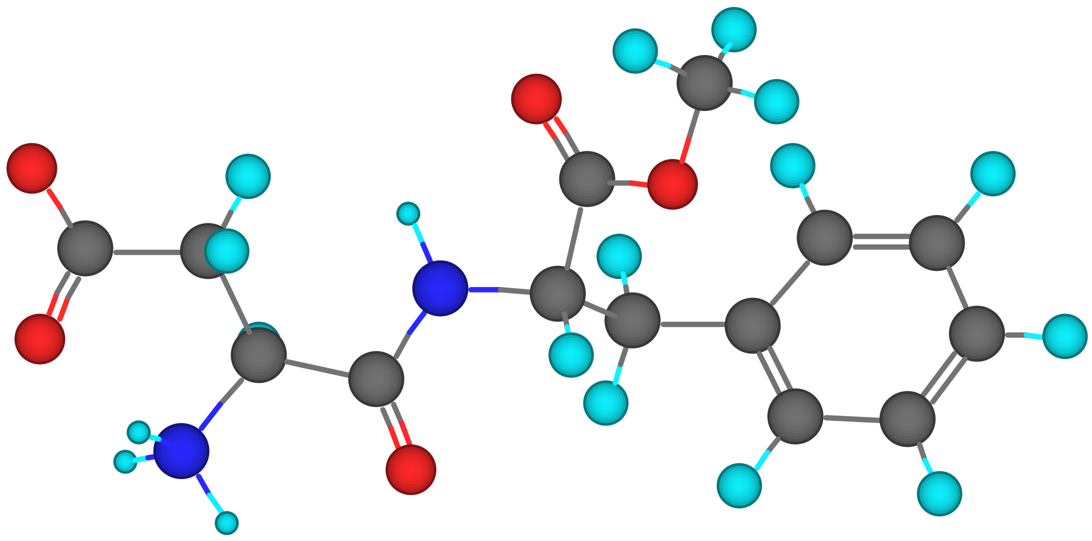
Amino Acids & Proteins



Patcharee Boonsiri

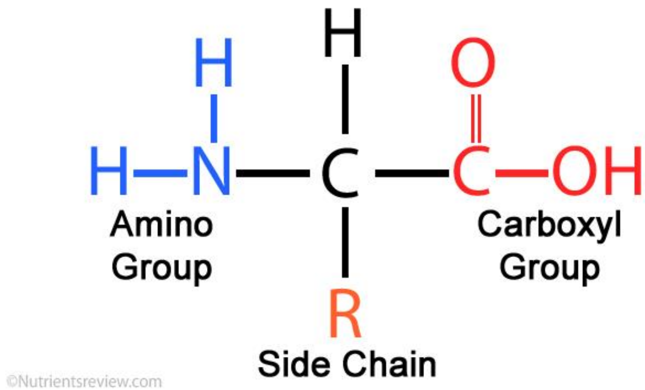
Outline

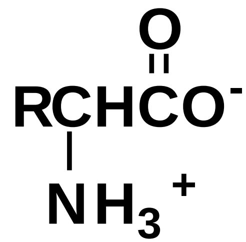
1. Amino acid and classification
    - Properties of amino acids
    - Test for amino acids
    - Role of amino acids
2. Peptide bond and its properties
3. Protein
    - Level of protein structure
    - Protein folding
    - Protein denaturation
    - Test for proteins
    - Roles of proteins in living organisms

Amino acid and classification

Amino acid

contains an **amino group** and a **carboxyl group**.

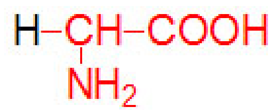
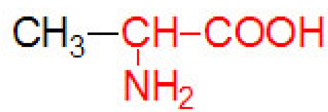
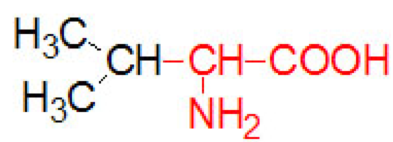
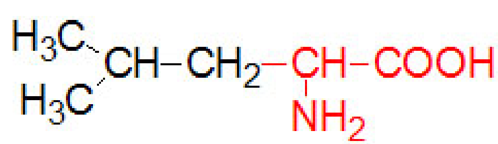
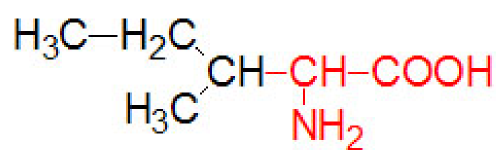




Zwitterion form

α-Amino acid: the amino group is on the carbon adjacent to the carboxyl group.

Amino acids with aliphatic R-groups

Amino Acid	Symbol	Structure*	pK_1 (COOH)	pK_2 (NH_2)	pK R Group
Amino Acids with Aliphatic R-Groups					
Glycine	Gly – G		2.4	9.8	
Alanine	Ala – A		2.4	9.9	
Valine	Val – V		2.2	9.7	
Leucine	Leu – L		2.3	9.7	
Isoleucine	Ile – I		2.3	9.8	

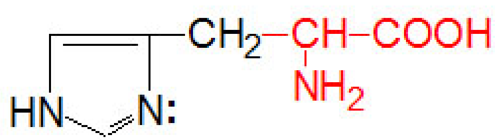
https://themedicalbiochemistrypage.org/amino-acids.php

Amino acids with –OH groups and sulfur-containing R-groups

Amino Acid	Symbol	Structure*	pK_1 (COOH)	pK_2 (NH_2)	pK R Group
Non-Aromatic Amino Acids with Hydroxyl R-Groups					
Serine	Ser – S	$HO-CH_2-CH(NH_2)-COOH$	2.2	9.2	≈13
Threonine	Thr – T	$H_3C(HO)CH-CH(NH_2)-COOH$	2.1	9.1	≈13
Amino Acids with Sulfur-Containing R-Groups					
Cysteine	Cys – C	$HS-CH_2-CH(NH_2)-COOH$	1.9	10.8	8.3
Methionine	Met – M	$H_3C-S-(CH_2)_2-CH(NH_2)-COOH$	2.1	9.3	

https://themedicalbiochemistrypage.org/amino-acids.php

Basic amino acids

Amino Acid	Symbol	Structure*	pK_1 (COOH)	pK_2 (NH_2)	pK R Group
Basic Amino Acids					
Arginine	Arg – R	$HN(C(=NH)NH_2)-CH_2-CH_2-CH_2-CH(NH_2)-COOH$	1.8	9.0	12.5
Lysine	Lys – K	$H_2N-(CH_2)_4-CH(NH_2)-COOH$	2.2	9.2	10.8
Histidine	His – H		1.8	9.2	6.0

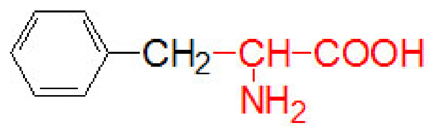
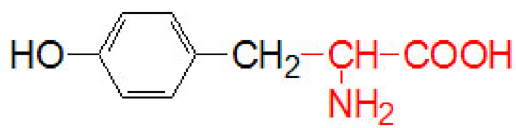
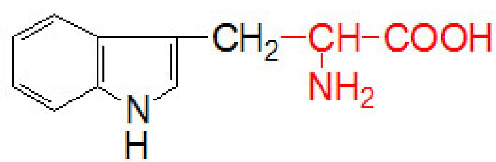
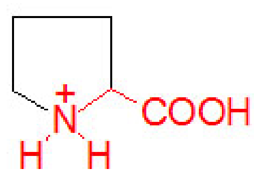
https://themedicalbiochemistrypage.org/amino-acids.php

Acidic acids and their amides

Amino Acid	Symbol	Structure*	pK_1 (COOH)	pK_2 (NH_2)	pK R Group
Acidic Amino Acids and their Amides					
Aspartic Acid	Asp – D	$HOOC-CH_2-CH(NH_2)-COOH$	2.0	9.9	3.9
Asparagine	Asn – N	$H_2N-C(=O)-CH_2-CH(NH_2)-COOH$	2.1	8.8	
Glutamic Acid	Glu – E	$HOOC-CH_2-CH_2-CH(NH_2)-COOH$	2.1	9.5	4.1
Glutamine	Gln – Q	$H_2N-C(=O)-CH_2-CH_2-CH(NH_2)-COOH$	2.2	9.1	

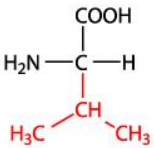
https://themedicalbiochemistrypage.org/amino-acids.php

Amino acids with aromatic rings and imino acid

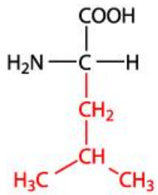
Amino Acid	Symbol	Structure*	pK_1 (COOH)	pK_2 (NH_2)	pK R Group
Amino Acids with Aromatic Rings					
Phenylalanine	Phe – F		2.2	9.2	
Tyrosine	Tyr – Y		2.2	9.1	10.1
Tryptophan	Trp – W		2.4	9.4	
Imino Acids					
Proline	Pro – P		2.0	10.6	

*Backbone of the amino acids is red, R-groups are black

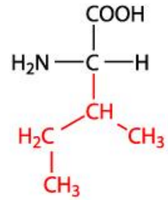
Essential amino acids



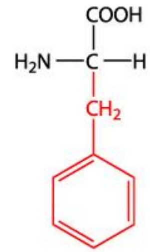
Valine



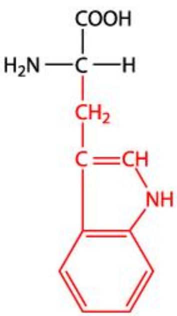
Leucine



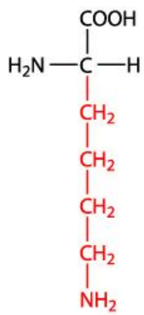
Isoleucine



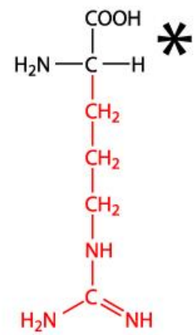
Phenylalanine



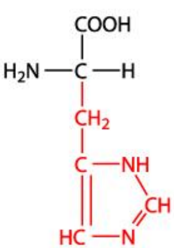
Tryptophan



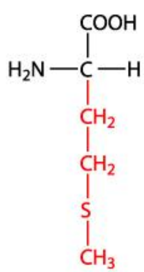
Lysine



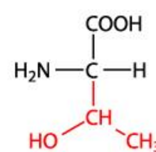
Arginine



Histidine



Methionine



Threonine

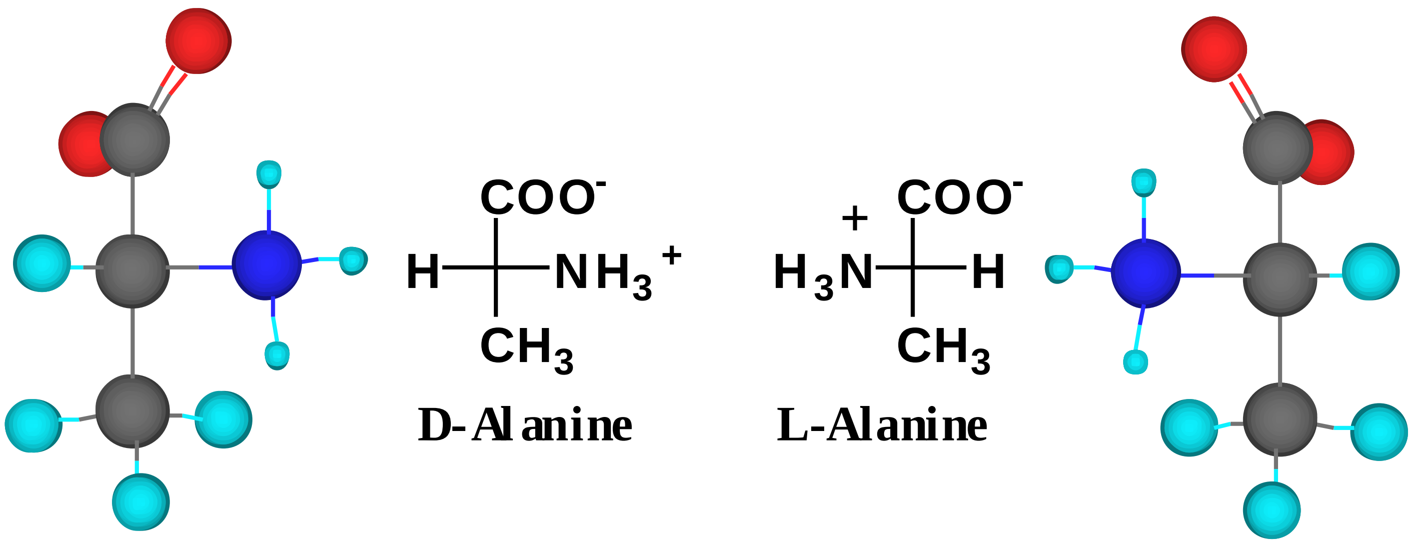
They cannot be synthesized by the body

They must come from food

Properties of amino acids

Chirality of amino acids

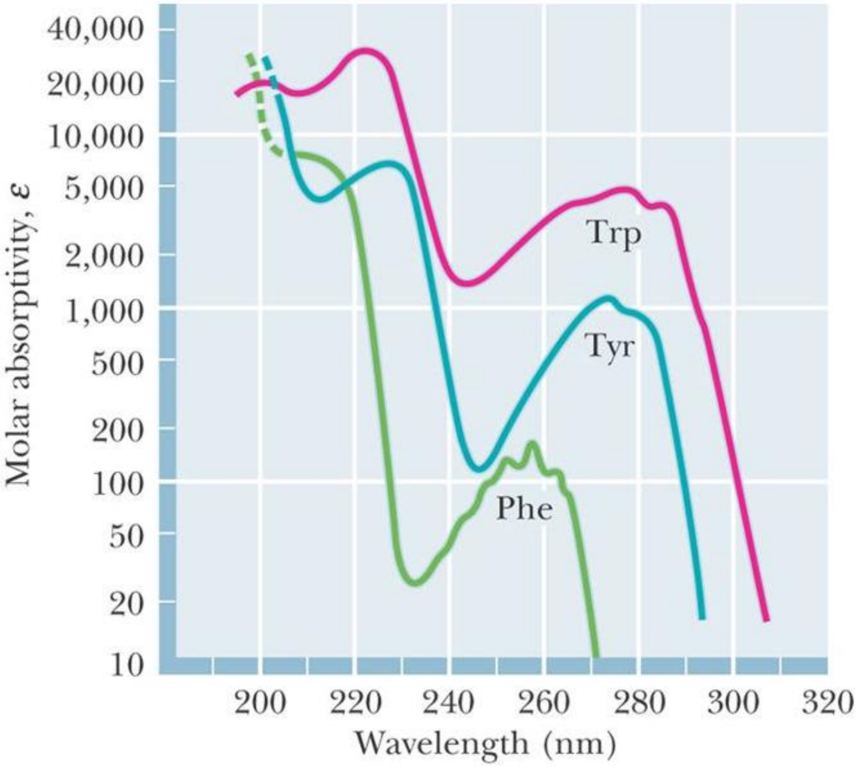
Amino acids have at least one stereocenter (the α-carbon) and are chiral except glycine

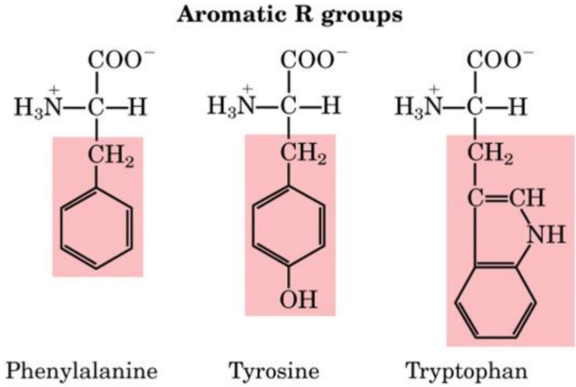


L-configuration at their α-carbon.

Optical property of amino acids

The ultraviolet absorption spectra of the aromatic amino acids at pH 6. (*From Wetlaufer, D.B., 1962. Ultraviolet spectra of proteins and amino acids.* Advances in Protein Chemistry **17**:*303–390.*)

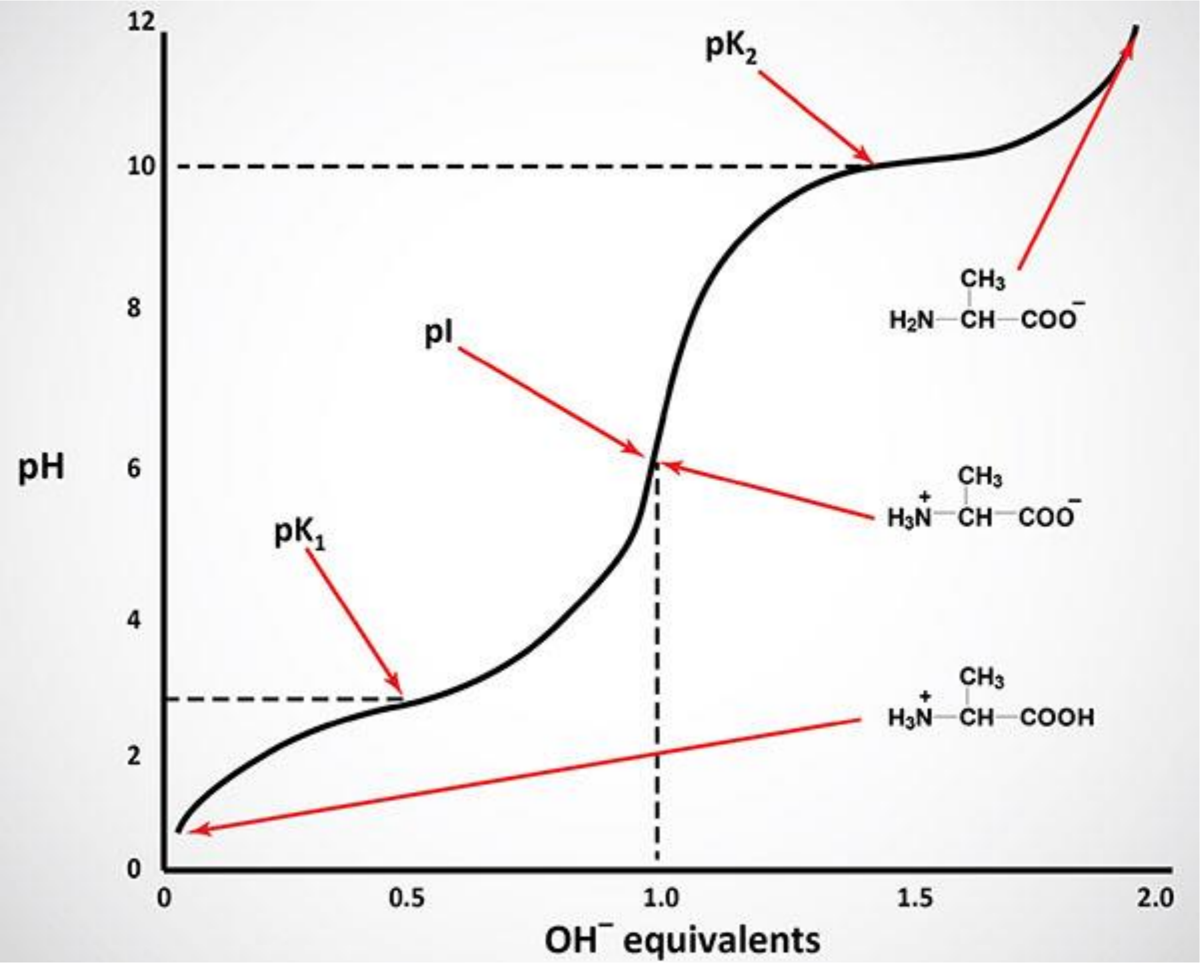




The aromatic R-groups in amino acids absorb UV light with an absorbance maximum in the range of 280nm

https://slideplayer.com/slide/4617706/15/images/37/The+ultraviolet+absorption+spectra+of+the+aromatic+amino+acids+at+pH+6.jpg

Acid-base properties



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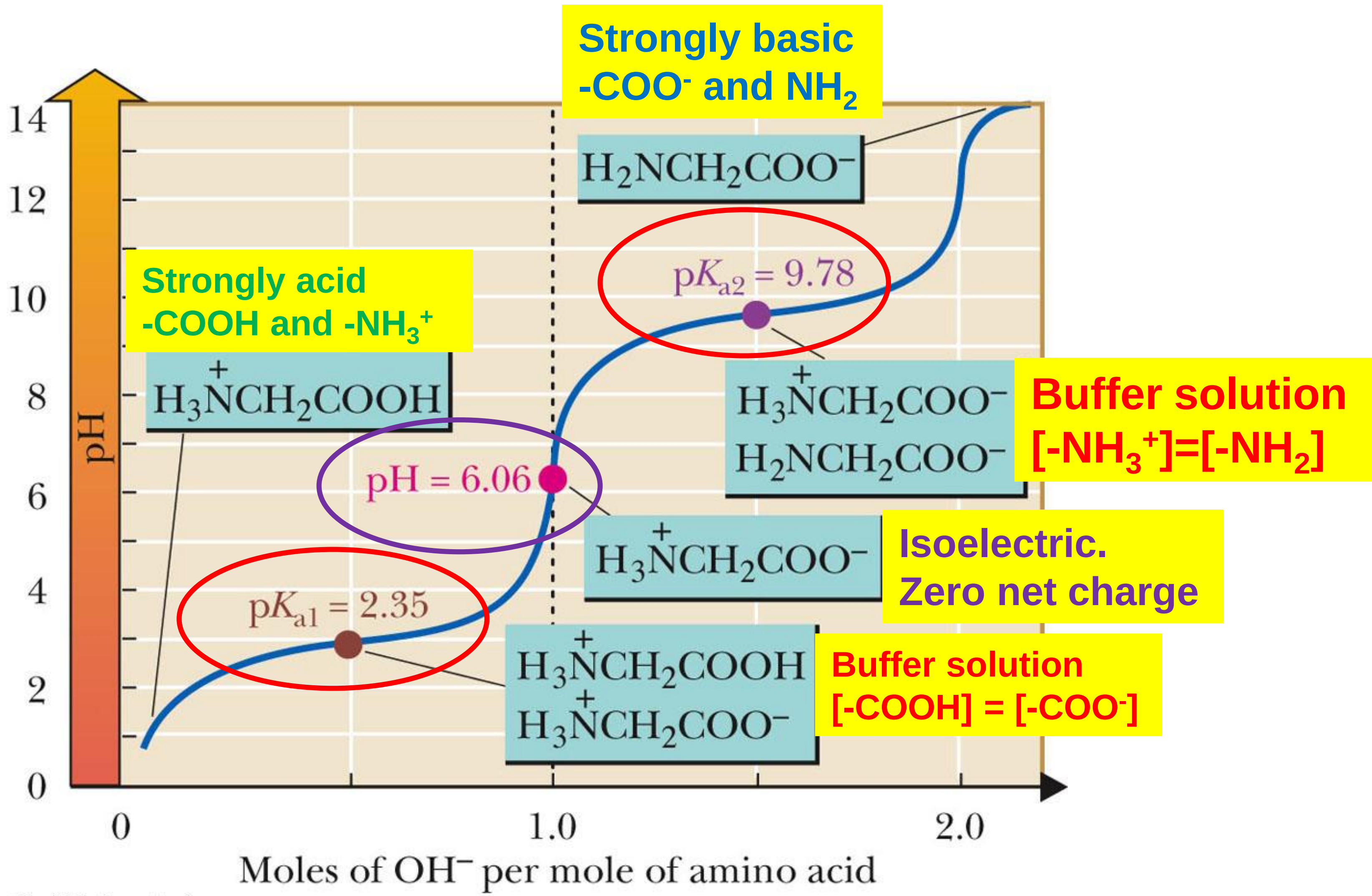
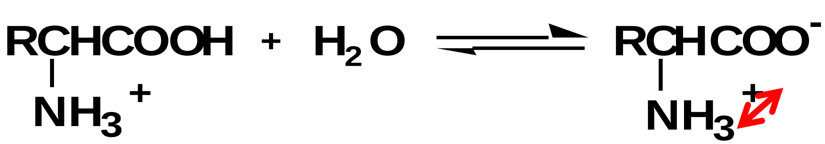
Titration of glycine with NaOH



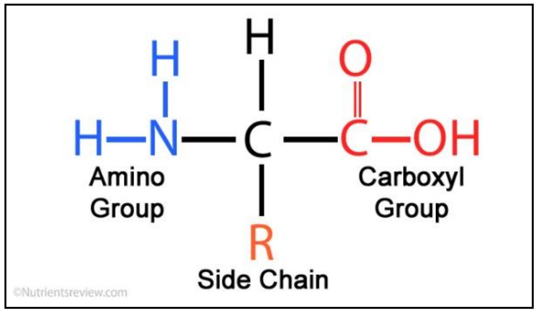
Acidity: α-COOH Groups

The average pK_a of an α-COOH is 2.19
[stronger acids than acetic acid (pK_a 4.76)].



pK_a = 2.19

Note that the NH_2 will be protonated at these low pH



Acidity: side chain -COOH

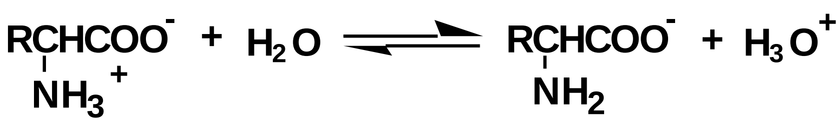
Due to the electron-withdrawing inductive effect of the α-NH_3^+ group, side chain -COOH groups are also stronger than acetic acid.

The effect decreases with distance from the α-NH_3^+ group. Compare:

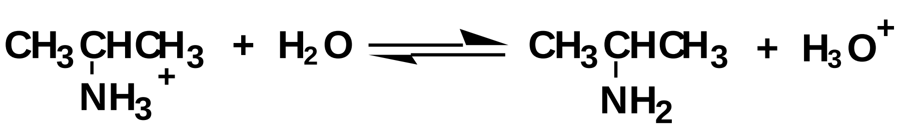
- **α-COOH group of alanine (pK_a 2.35)**
- **β-COOH group of aspartic acid (pK_a 3.86)**
- **γ-COOH group of glutamic acid (pK_a 4.07)**

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.



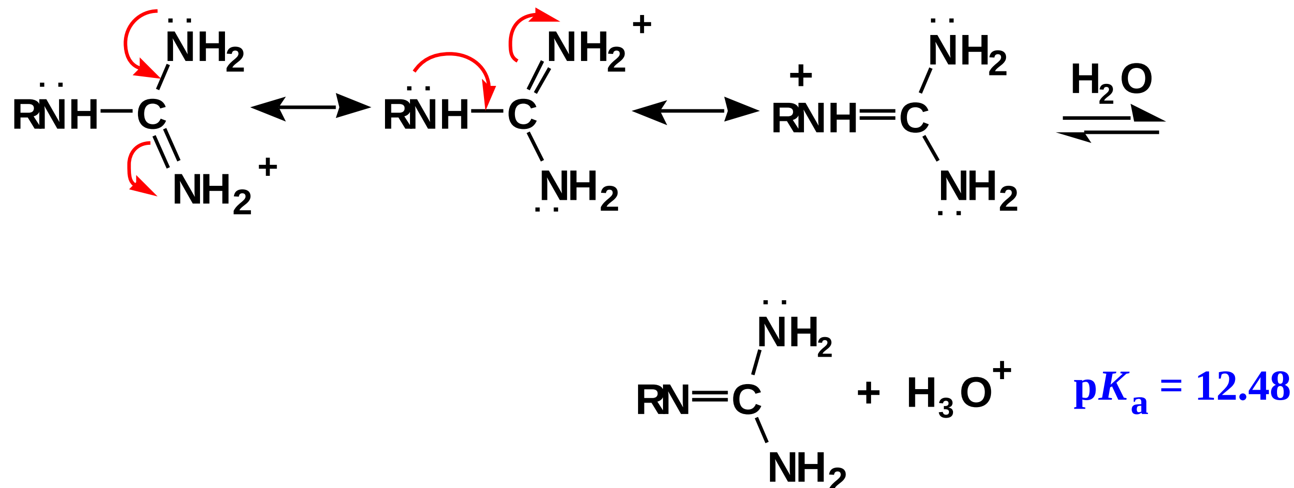
pK_a = 9.47



pK_a = 10.60

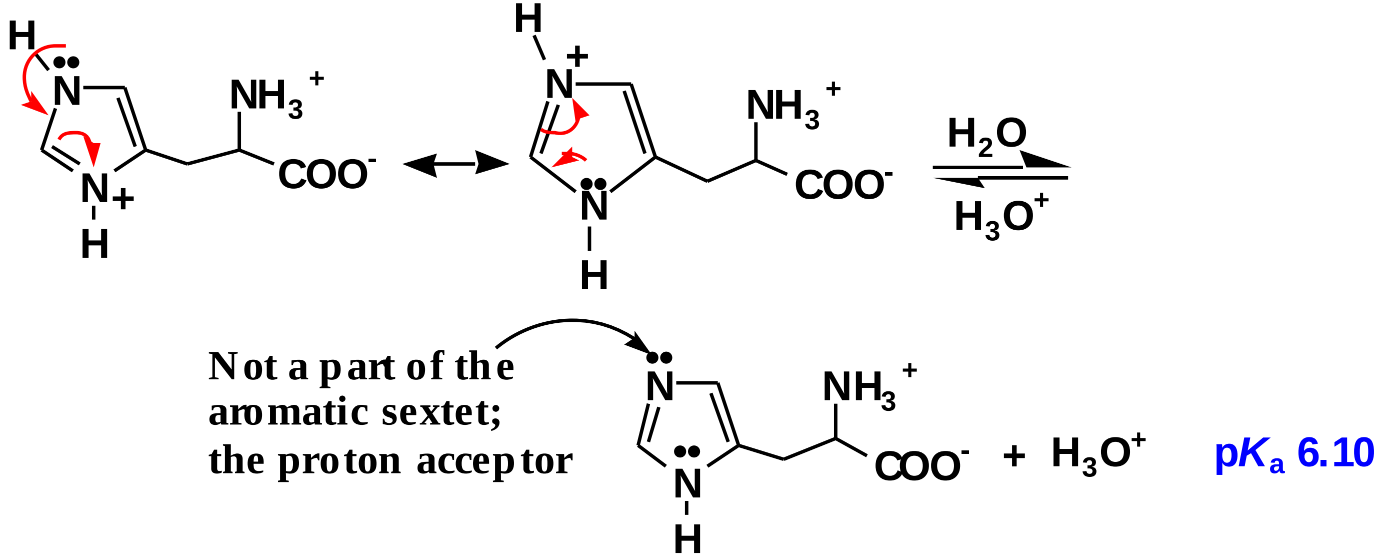
Details: The Guanidine Group of Arg

The basicity of the guanidine group is attributed to the large resonance stabilization of the protonated form relative to the neutral form.



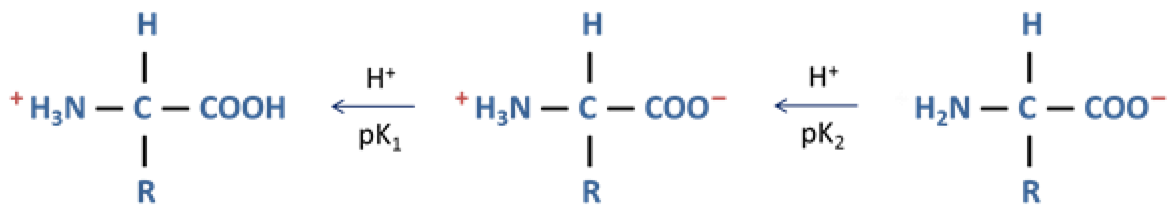
Details: Imidazole Group

The imidazole group is a heterocyclic aromatic amine



Isoelectric point (pI)

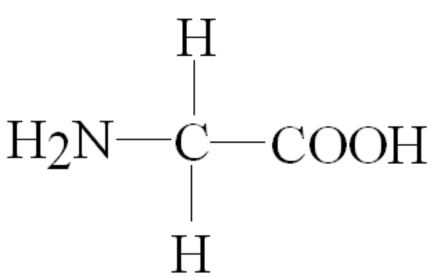
pH at which an amino acid, polypeptide, or protein has a net charge = 0



cation (at low pH) zwitterion anion (at high pH)

Example: pI of glycine

falls between the pK_a values for the carboxyl and amino groups



glycine

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

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

Isoelectric Point (pI) net charge = 0

If pH is lower than pI then more protonated molecules
If higher than pI then more negative charge

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

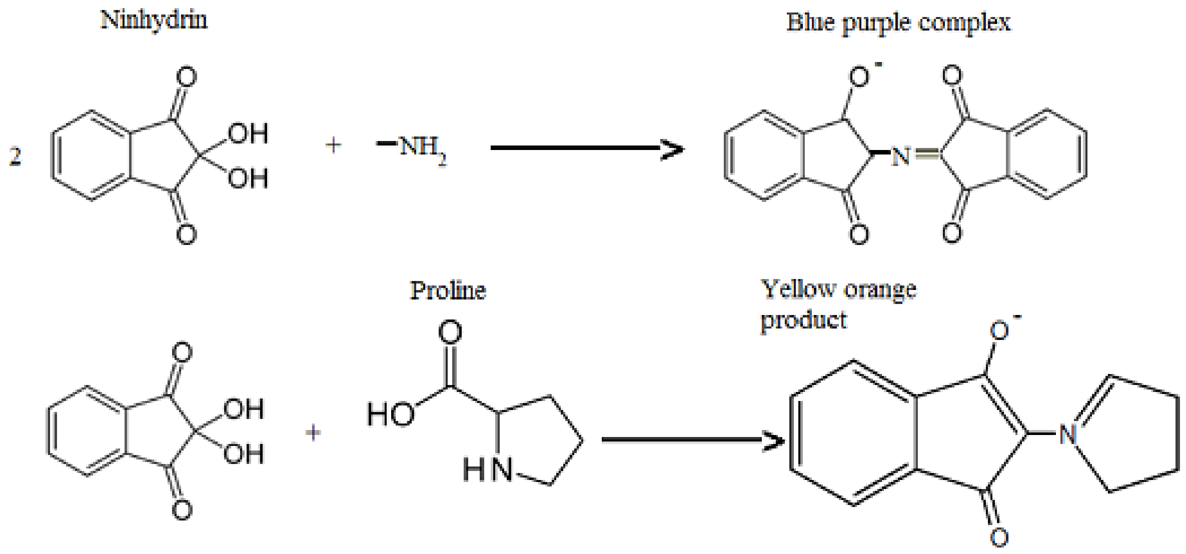
Isoelectric Point (pI)

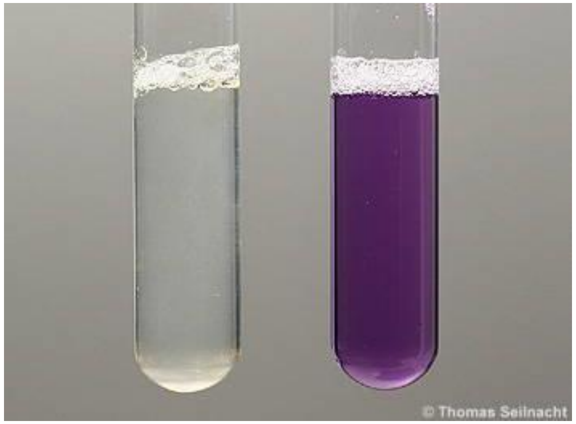
Acidic Side Chains	pK_a of α–COOH	pK_a of α–NH_3^+	pK_a of Side Chain	pI
aspartic acid	2.10	9.82	3.86	2.98
glutamic acid	2.10	9.47	4.07	3.08
cysteine	2.05	10.25	8.00	5.02
tyrosine	2.20	9.11	10.07	5.63

Basic Side Chains	pK_a of α–COOH	pK_a of α–NH_3^+	pK_a of Side Chain	pI
arginine	2.01	9.04	12.48	10.76
histidine	1.77	9.18	6.10	7.64
lysine	2.18	8.95	10.53	9.74

Ninhydrin test for amino group (-NH_2)

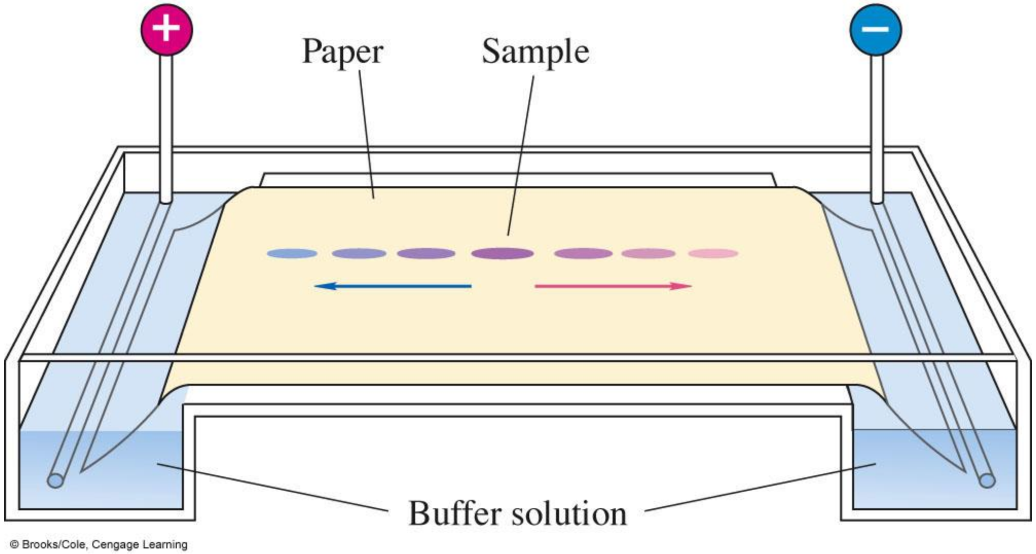
Purple product for all amino acids
except proline(imino acid) which gives yellow product





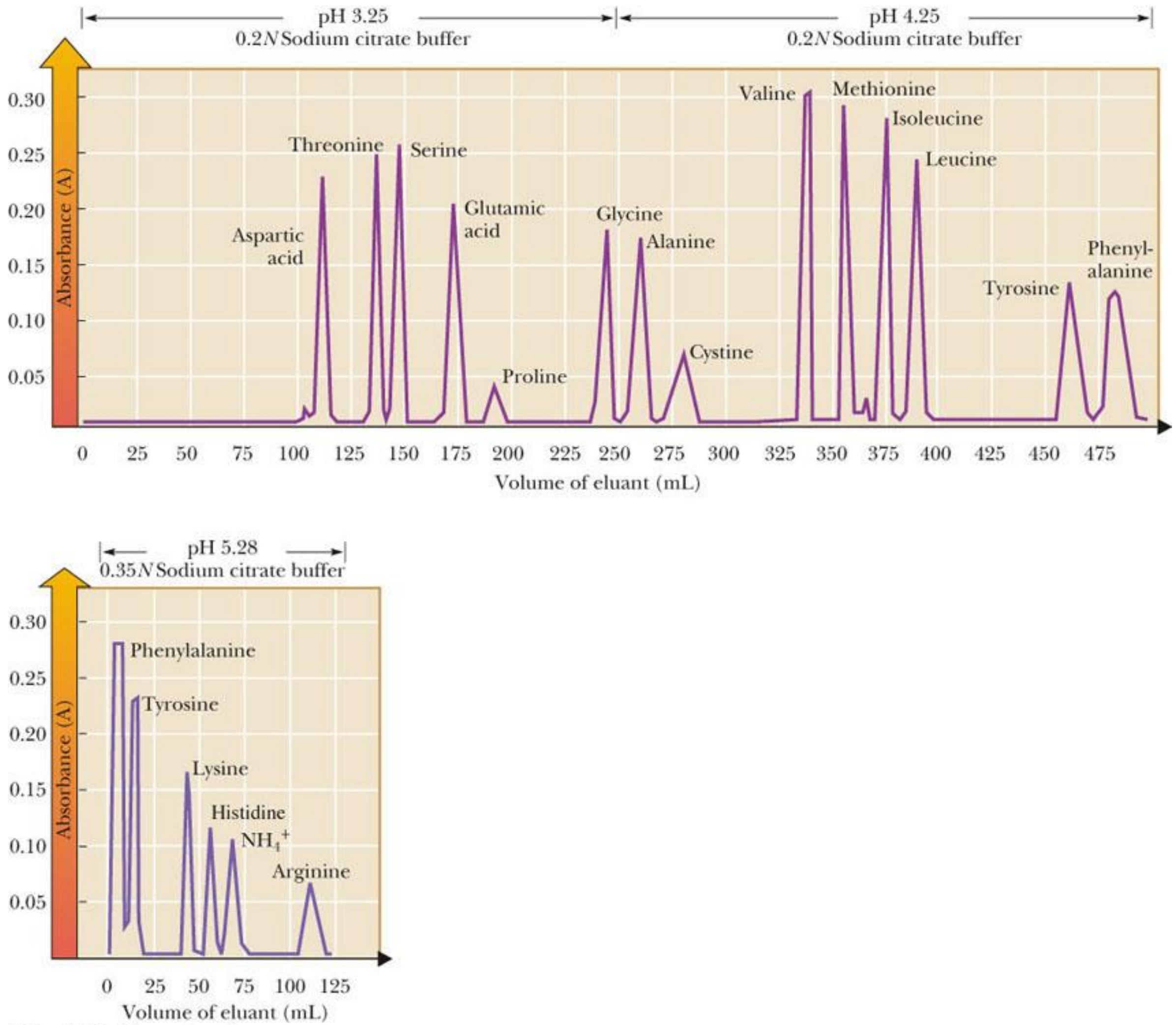
https://biochemistryisagoodthing.files.wordpress.com/2013/02/ninhydrin-1.png

Electrophoresis can separate amino acids based on their electric charge



- An electric potential is applied to the electrode vessels and amino acids migrate toward the electrode with charge opposite their own.
- Molecules with a high charge density move faster than those with low charge density.
- Molecules at isoelectric point remain at the origin.
- After derivitization with ninhydrin, 19 of the 20 amino acids give the same purple-colored anion; proline gives an orange-colored compound.

Ion exchange chromatography for analysis of a mixture of amino acids based on their electric charge



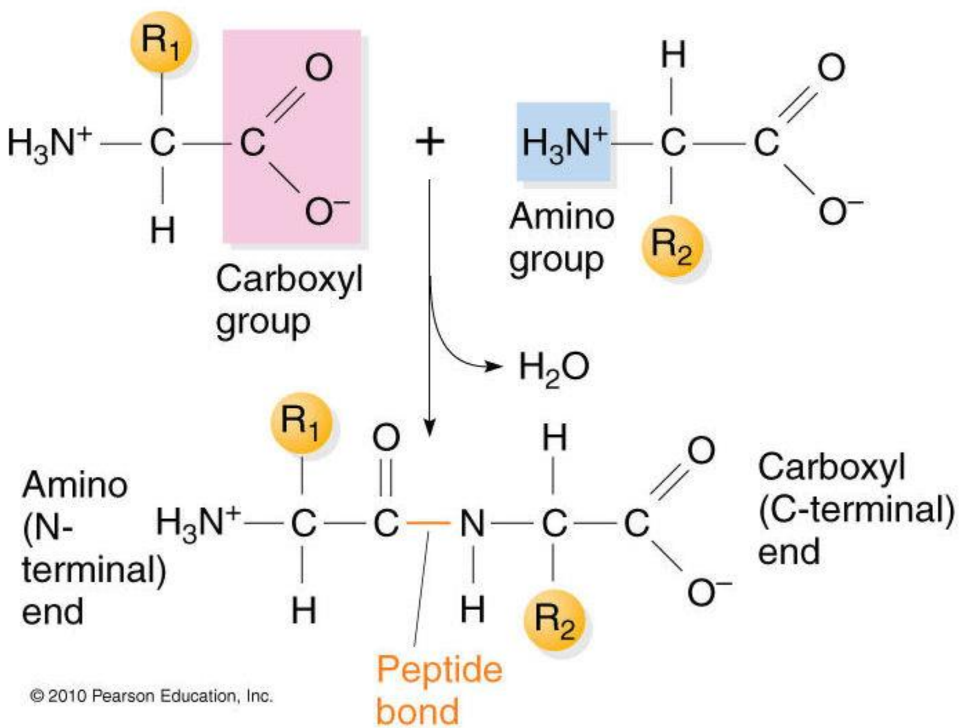
Role of amino acids

1. Monomer for proteins
2. Use for biosynthesis e.g. porphyrin, urea, purine, pyrimidine
3. Amino acids in some polypeptides: hormones and neurotransmitters
4. Tryptophan use for synthesis of serotonin, melatonin, niacin
5. Tyrosine use for synthesis of melanin, catecholamines
6. Glutamic acid use for synthesis of GABA (gamma-aminobutyric acid
7. Arginine use for synthesis of nitric oxide

Polypeptides & Proteins

Proteins are long chains of amino acids joined by peptide bonds

Peptide bond: amide bond between the α -carboxyl group of one amino acid and the α-amino group of another



Peptides

Peptide: a short polymer of amino acids joined by peptide bonds

Polypeptide: many amino acids joined by peptide bonds

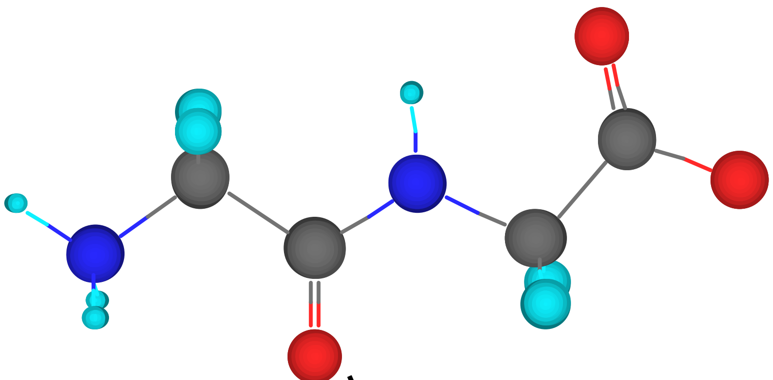
Protein: one or more polypeptide chains, molecular weight 5000 g/mol or greater

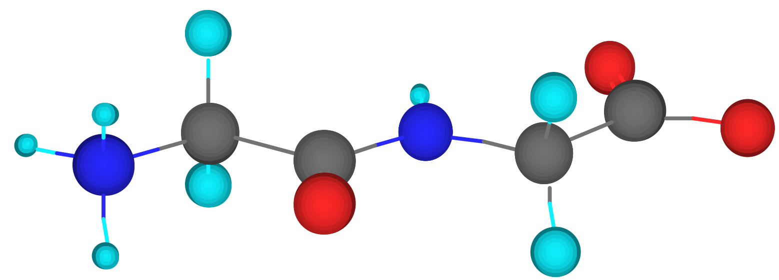
Peptide Bond Geometry

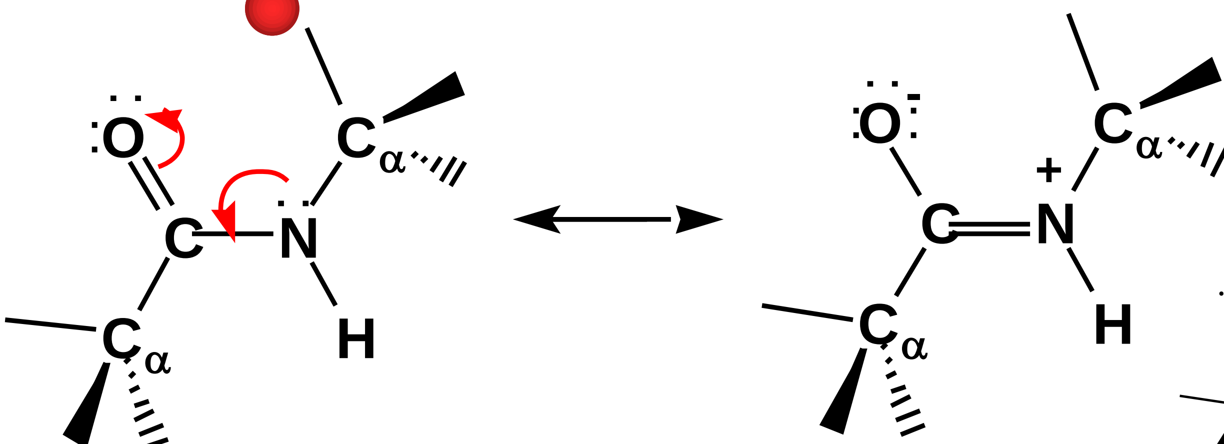
The four atoms of a peptide bond and the two alpha carbons joined to it lie in a plane with bond angles of 120° about C and N

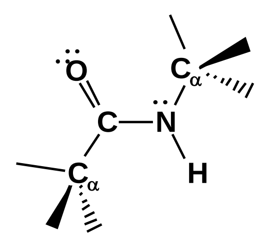
Model of the zwitterion form of Gly-Gly

peptide bond is restricted and the preferred *s*-trans geometry.

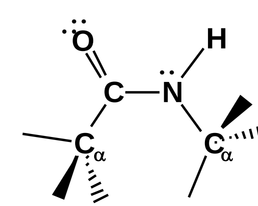






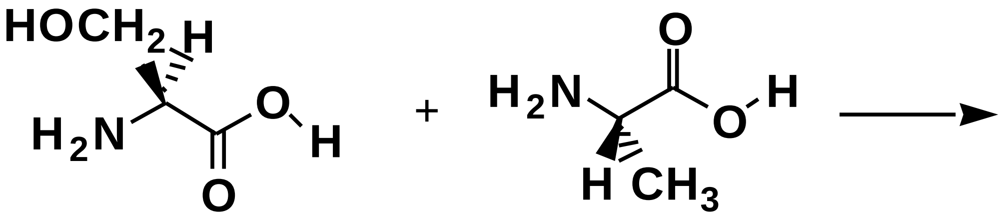


s-*trans*



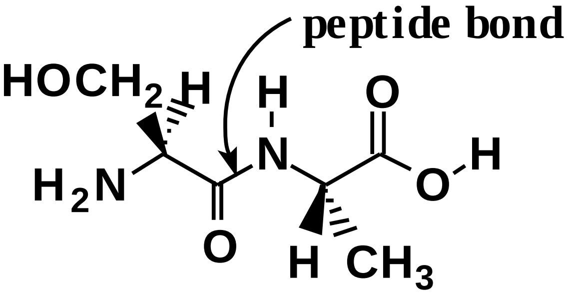
s-*cis*

Dipeptide e.g. serinylalanine (Ser-Ala)

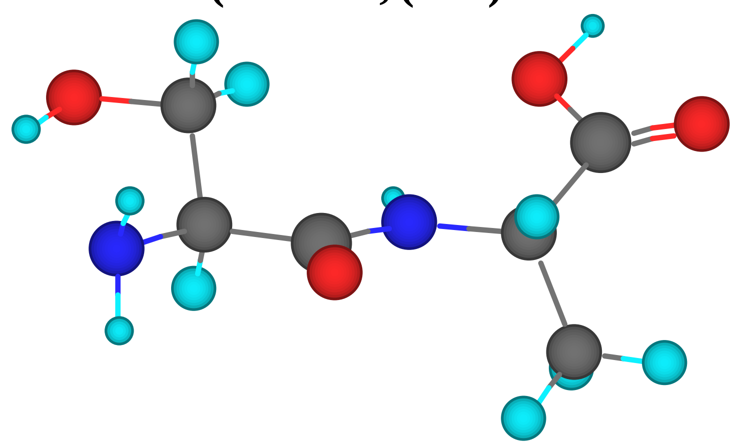


Serine
(Ser, S)

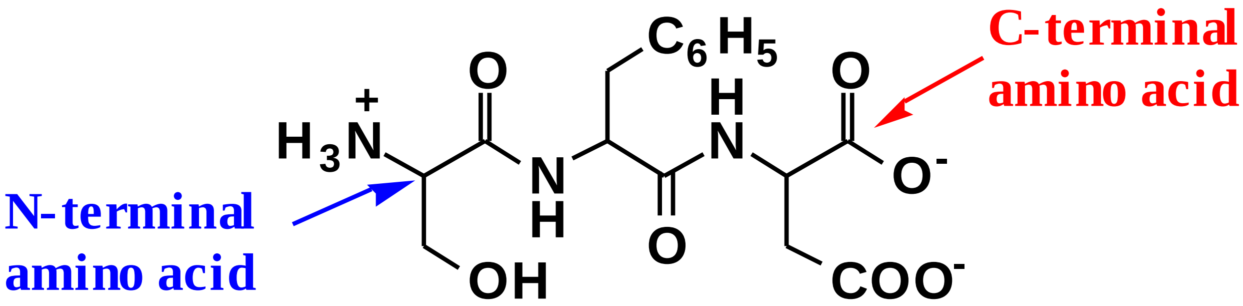
Alanine
(Ala, A)



Serinylalanine
(Ser-Ala, (S-A)



Tripeptide



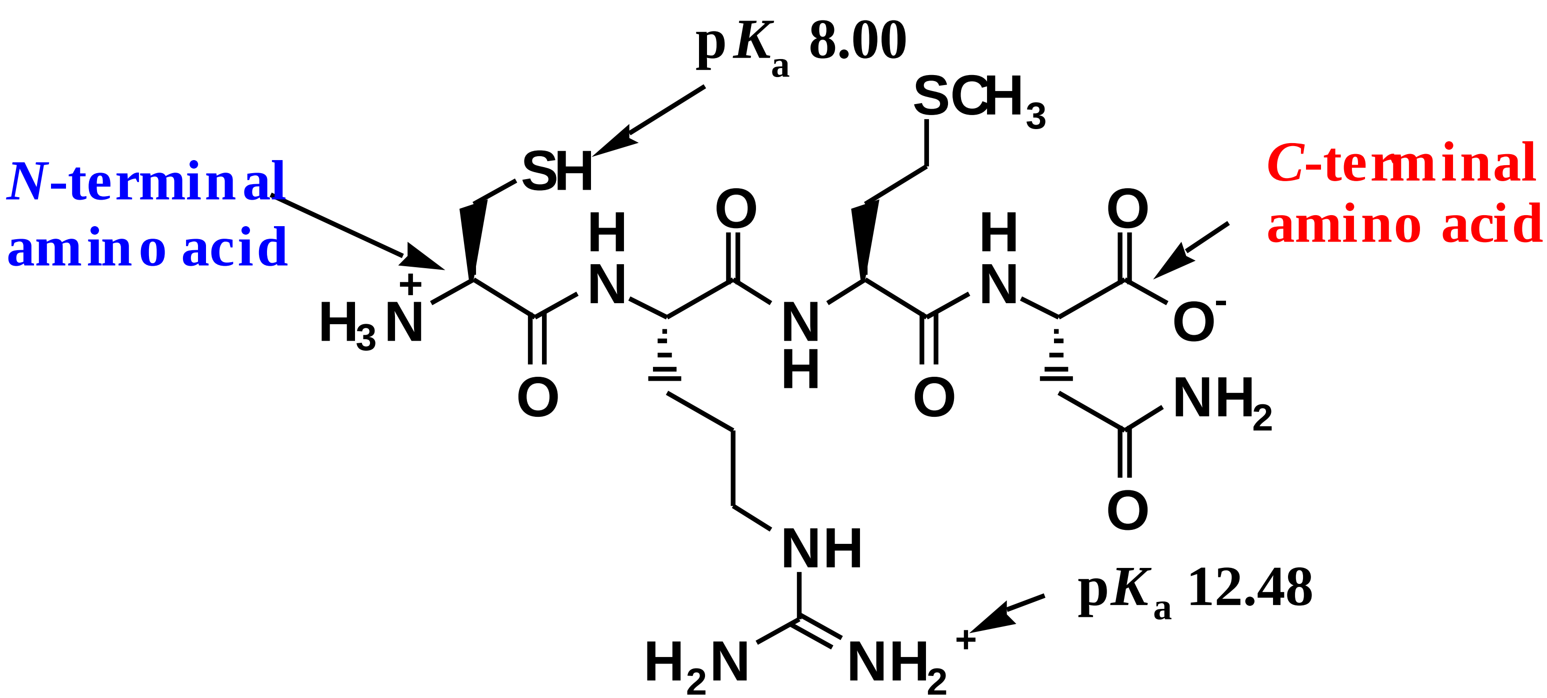
Ser-Phe-Asn

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

Tetrapeptide

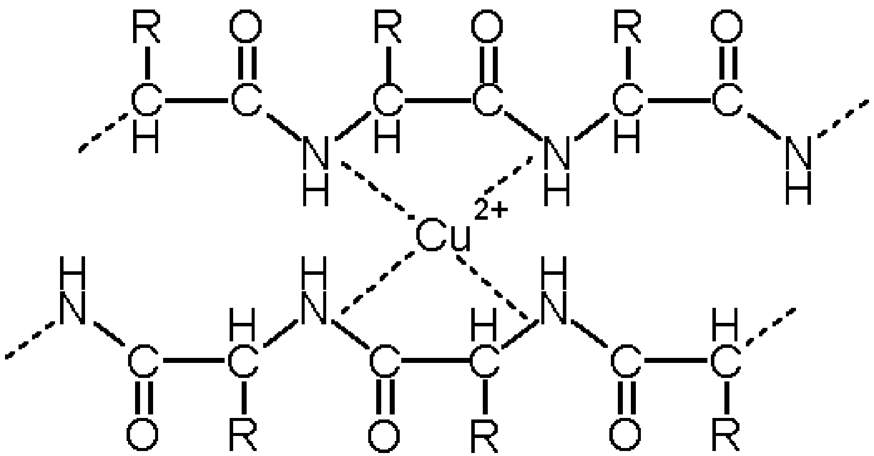
Cys-Arg-Met-Asp
At pH 6.0, its net charge is +1.

At pH 8 it would be half ionized



Biuret test

Peptide bonds of protein react with Cu $^{2+}$ in alkaline condition



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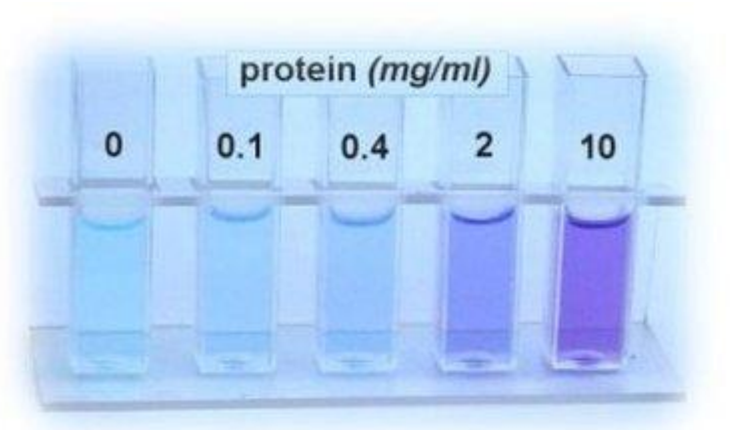

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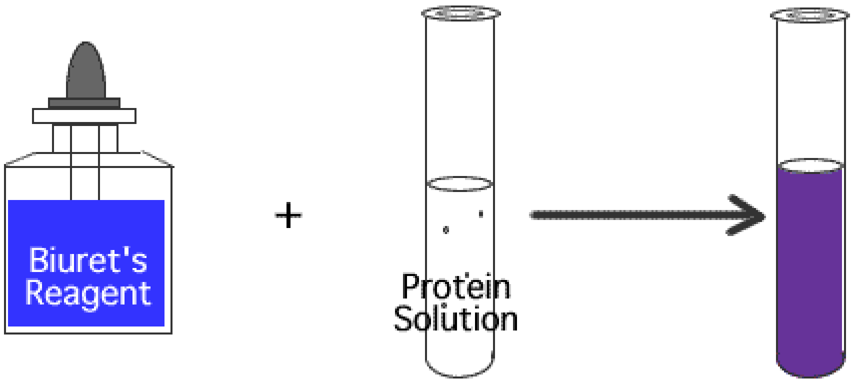


Proteases can be used to catalyze the hydrolysis of specific peptide bonds.

Enzyme	Catalyzes Hydrolysis of Peptide Bond Formed by Carboxyl Group of
Trypsin	Arginine, lysine
Chymotrypsin	Phenylalanine, tyrosine, tryptophan

Carboxypeptidase cleavage of C terminal AA

Treatment of peptide with carboxypeptidase cleaves the peptide linkage adjacent to the free alpha carboxyl group. It may then be identified.

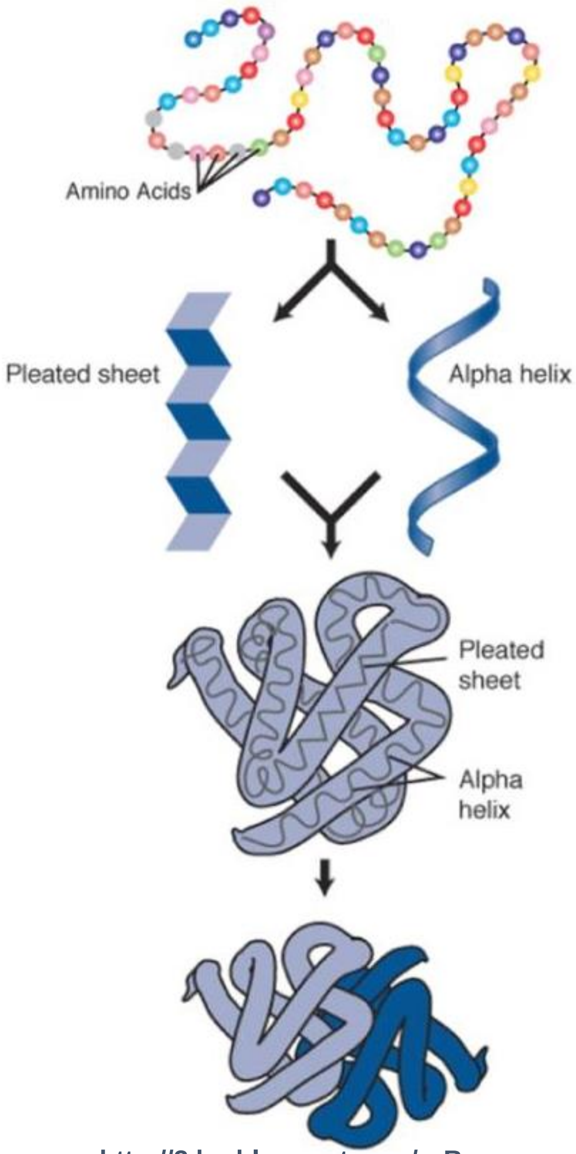
Level of protein structure

Primary structure

Secondary structure

Tertiary structure

Quaternary structure



Levels of protein organization

Primary protein structure
is sequence of a chain of amino acids

Secondary protein structure
occurs when the sequence of amino acids are linked by hydrogen bonds

Tertiary protein structure
occurs when certain attractions are present between alpha helices and pleated sheets.

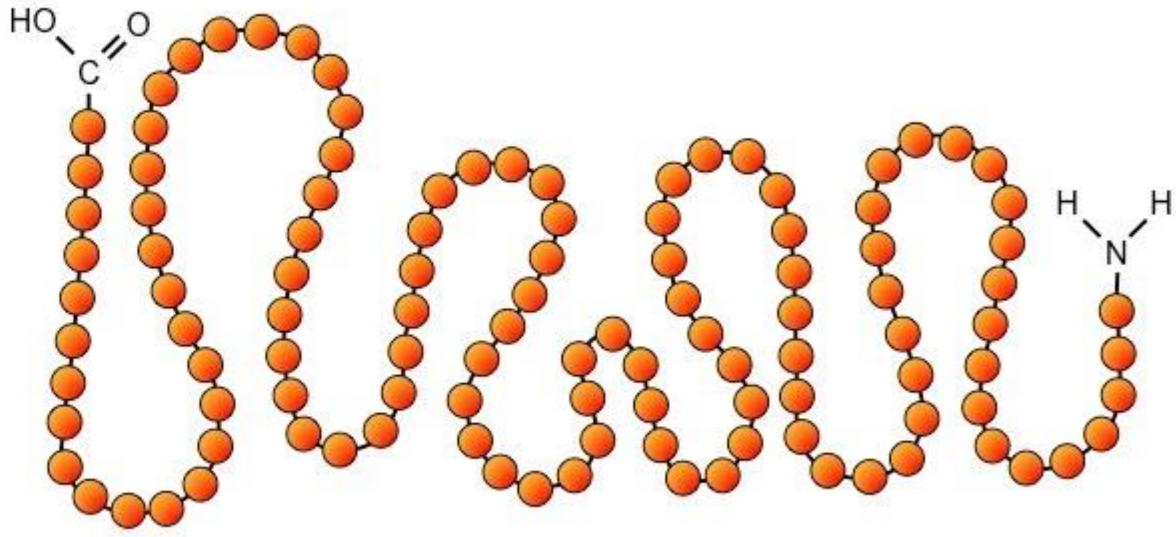
Quaternary protein structure
is a protein consisting of more than one amino acid chain.

http://3.bp.blogspot.com/-nB-UqGed7XY/Tq7dSxXA2EI/AAAAAAAAABE/50uNbPAq8ds/s1600/protein+strure.jpg

Primary Structure

The sequence of amino acids in a polypeptide chain

read from the *N*-terminal amino acid to the *C*-terminal amino acid



https://ib.bioninja.com.au/_Media/primary-structure_med.jpeg

Protein sequencing: the way to know amino acid composition of protein

Edman degradation method: Cleaves the ***N*-terminal** amino acid of a polypeptide chain

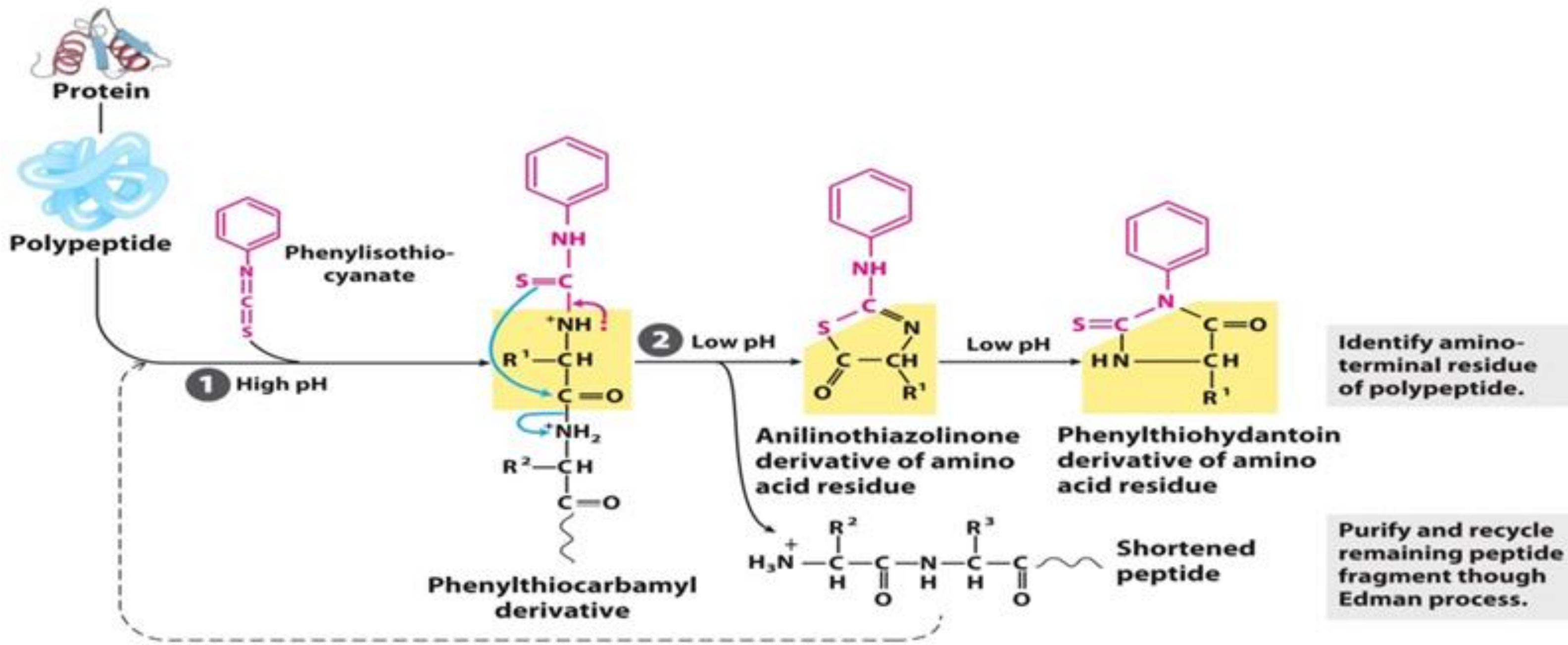
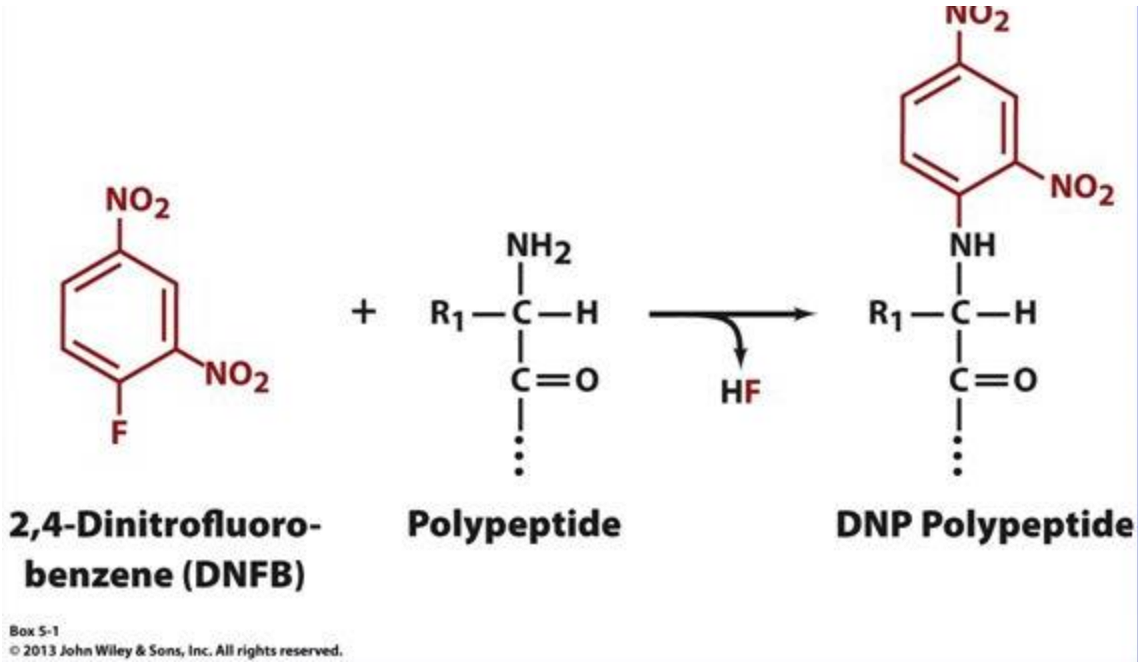


Figure 3-27
Lehninger Principles of Biochemistry, Sixth Edition

Sanger's method: DNFB bind to terminal amino groups of a protein



Bind to terminal amino groups to form a yellow dinitrophenyl derivative

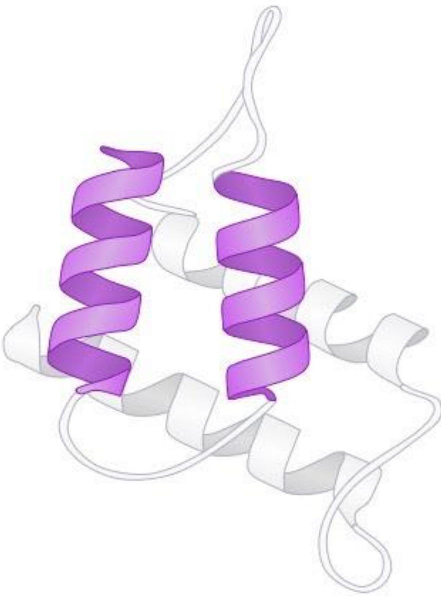
Hydrolyze protein

Identify terminal amino acid chromatographically

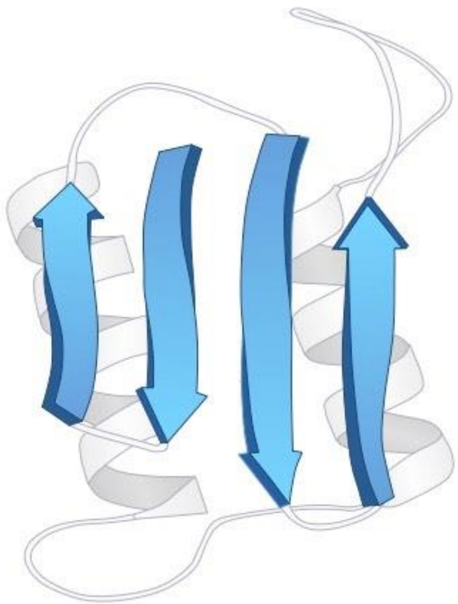
Secondary structure

The ordered arrangements (conformations) of amino acids in localized regions of a polypeptide or protein.

- α-Helix
- β-pleated sheet



α - helices

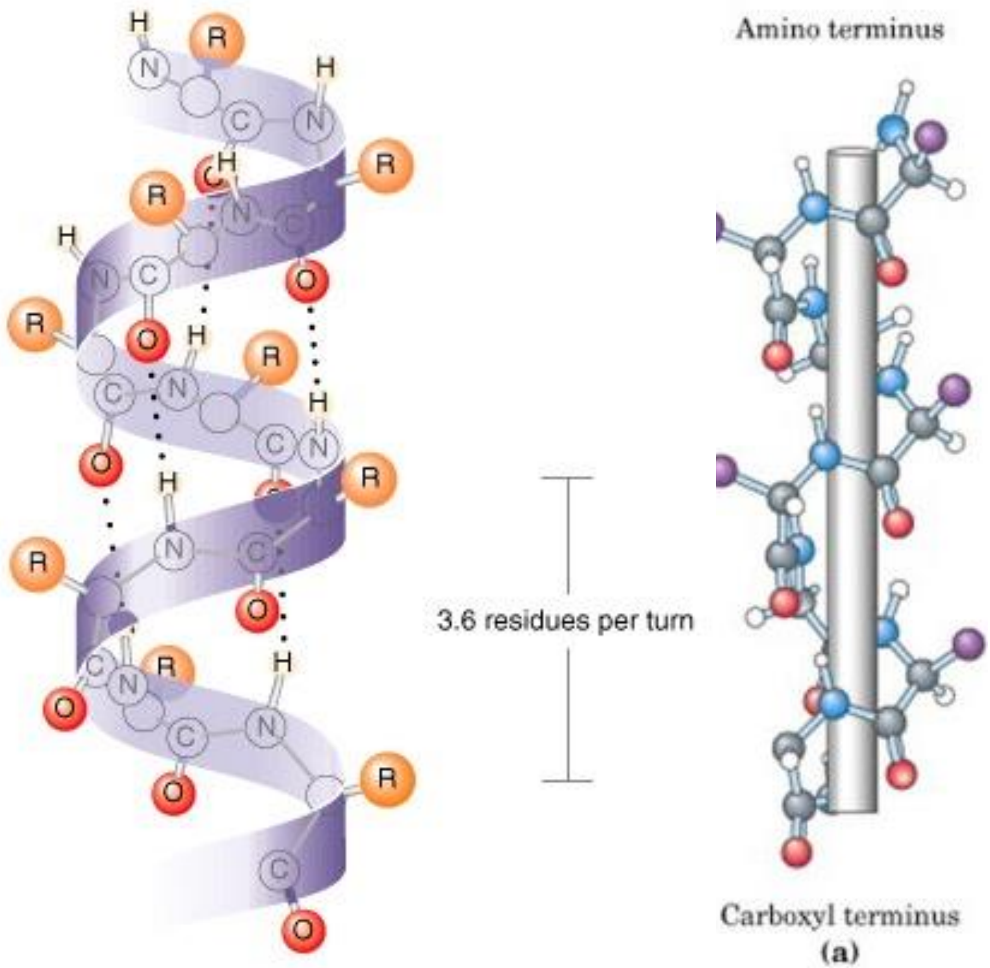


β - pleated sheets

https://ib.bioninja.com.au/_Media/secondary-structure_med.jpeg

α-helix

a section of polypeptide chain coils into a right-handed spiral



- All R- groups point outward from the helix
- 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
- 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 H-bond to the N-H group of the peptide bond four amino acid units away from it

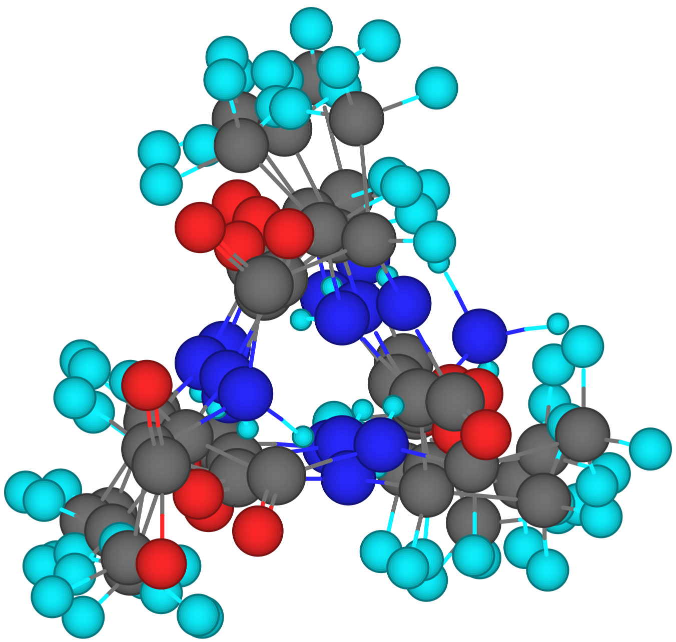
https://www.mun.ca/biology/scarr/F09-05.jpg

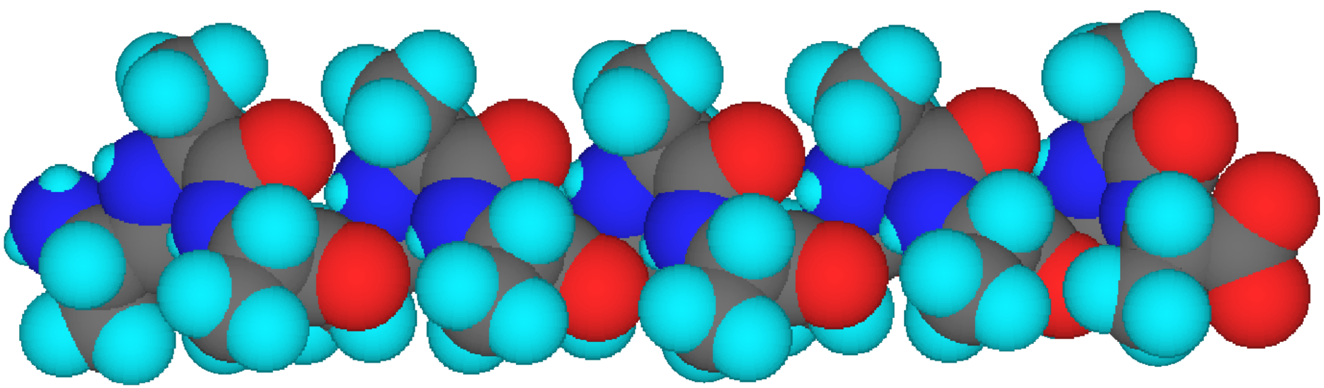
https://d2jmvrsizmvf4x.cloudfront.net/TUwBGO5gR6WxxRoOiVMN_a-helix.jpg

Example

An a-helix of repeating units of L-alanine

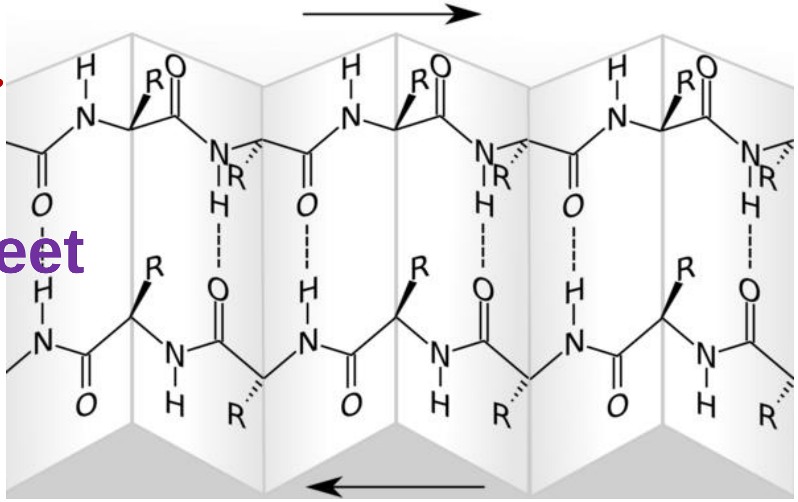
- A ball-and-stick model viewed looking down the axis of the helix.
- A space-filling model viewed from the side.

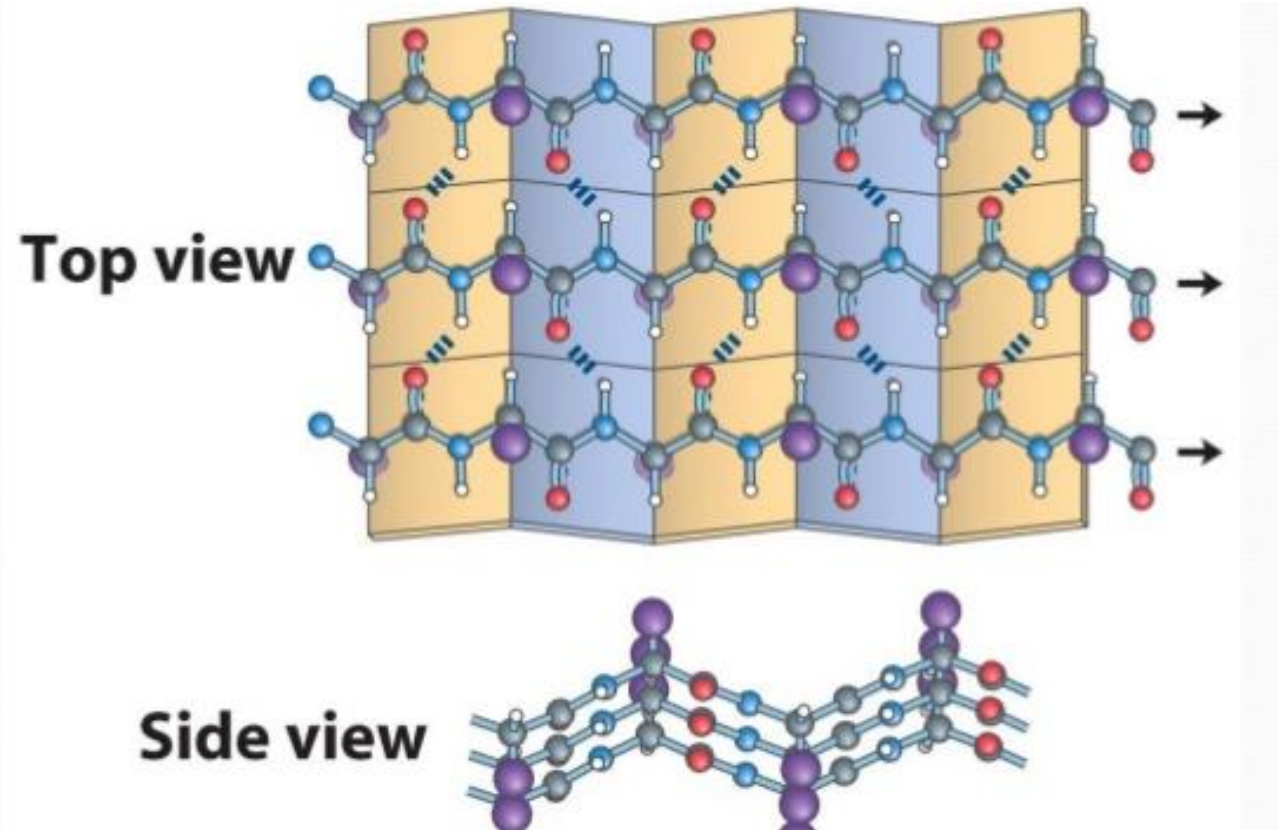




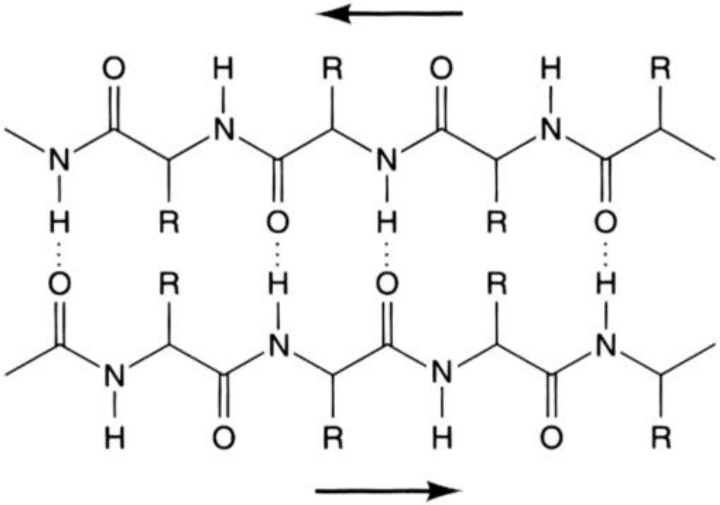
β-Pleated Sheet

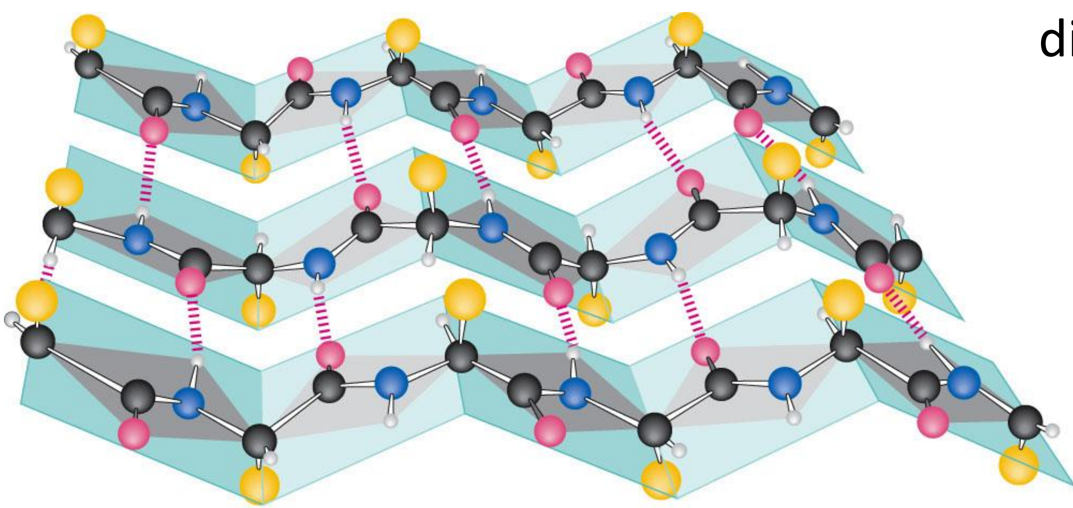
parallel β-pleated sheet





antiparallel β-pleated sheet





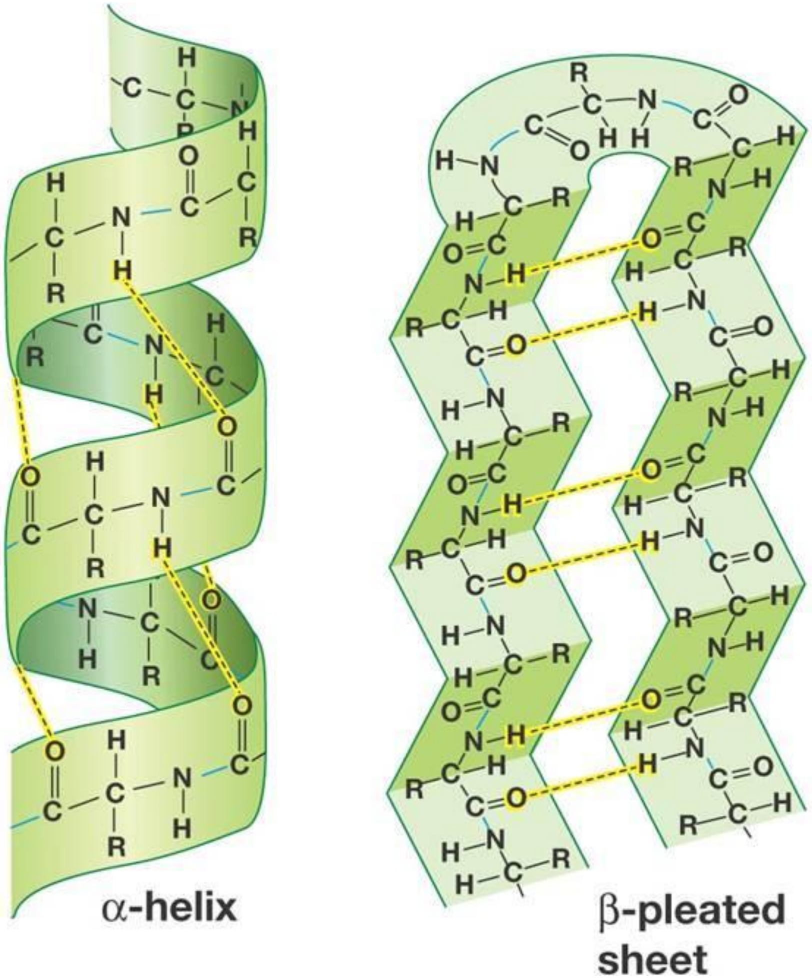
http://www.smh.com.au/content/dam/images/4/f/7/w/b/image.imgtype.videoThumbnail.729x410.png/1463630273256.jpg

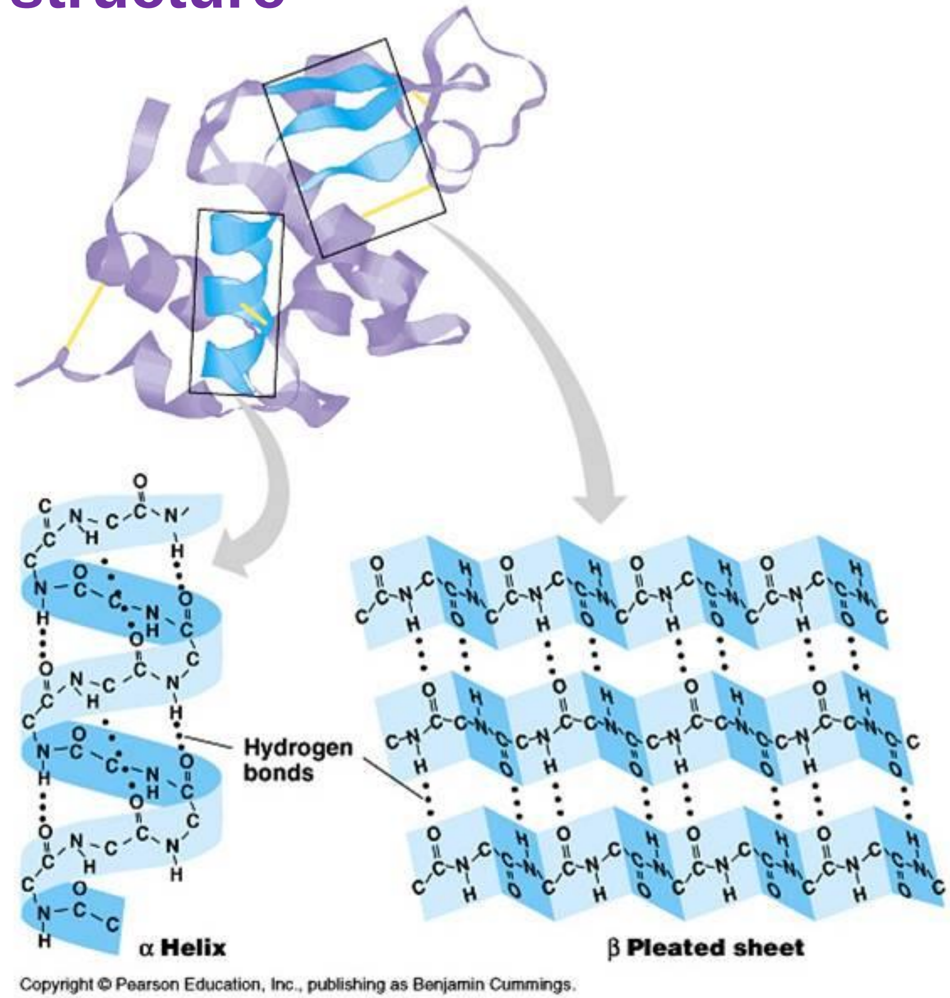
- The antiparallel β-pleated sheet consists of adjacent polypeptide chains running in opposite directions:
  - Each peptide bond is planar and has the s-*trans* conformation
  - The C=O and N-H groups of peptide bonds from adjacent chains point toward each other and are in the same plane → H-bond is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

Amino Acid	Sericin	Fibroin	Wool Keratin	Spider Silk
Glycine	13.9	43.7	8.4	37.1
Alanine	5.9	28.8	5.5	21.1
Valine	2.7	2.2	5.6	1.8
Leucine	1.1	0.5	7.8	3.8
Isoleucine	0.7	0.7	3.3	0.9
Serine	33.4	11.9	11.6	4.5
Theronine	9.7	0.9	6.9	1.7
Aspartic Acid	16.7	1.3	5.9	2.5
Glutamic Acid	4.4	1.0	11.3	9.2
Phenylanine	0.5	0.6	2.8	0.7
Tyrosine	2.6	5.1	3.5	---
Lysine	3.3	0.3	2.6	0.5
Histidine	1.3	0.2	0.9	0.5
Arginine	3.1	0.5	6.4	7.6
Proline	0.6	0.5	6.8	4.3
Tryptophan	0.2	0.3	0.5	2.9
Cystine	0.1	0.2	9.8	0.3
Methionine	0.04	0.1	0.4	0.4

Table 2 Amino Acid Composition (mole %) of Spider dragline silk and Other Protein Fibers

Combination of secondary structure can form tertiary structure

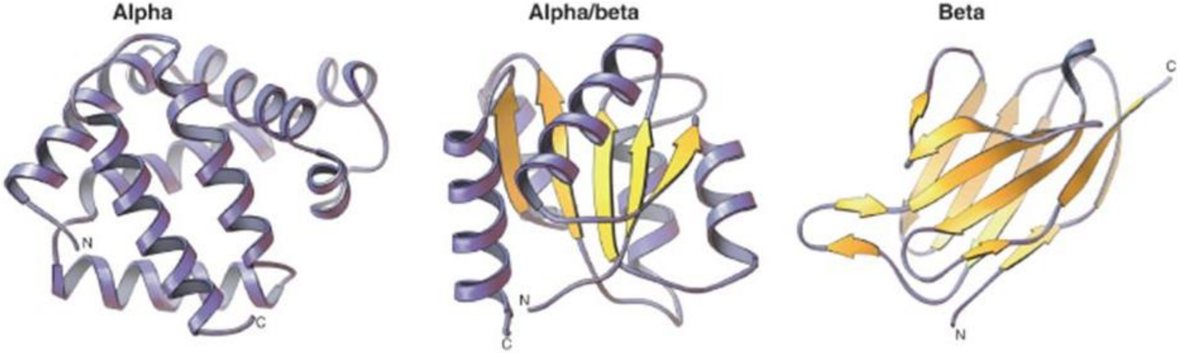




https://d2jmvrsizmvf4x.cloudfront.net/d5G5CpdjToNgGKgwJg6Q_picture21327254818885.jpg

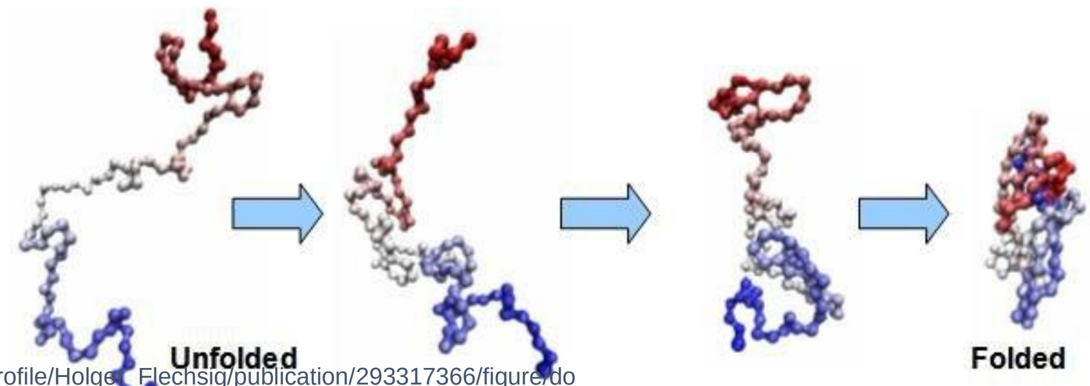
Tertiary structure

The three-dimensional arrangement in space of all atoms in a single polypeptide chain (combination of secondary structure)

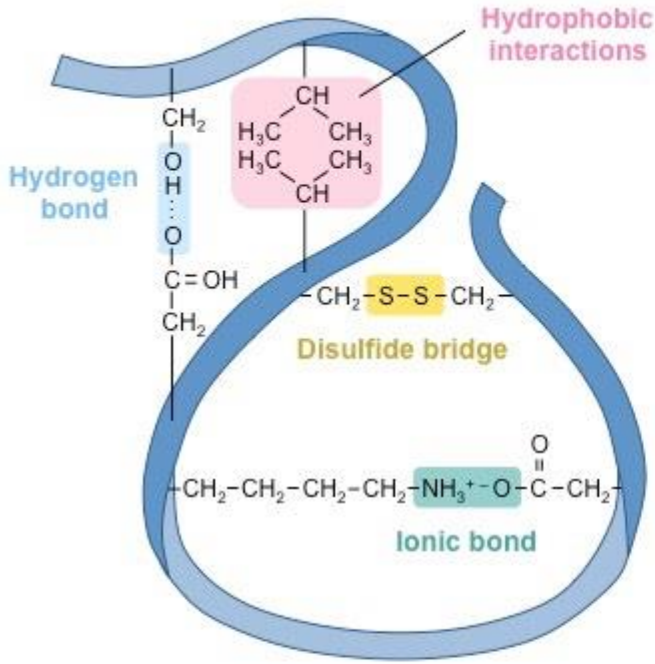


Protein folding

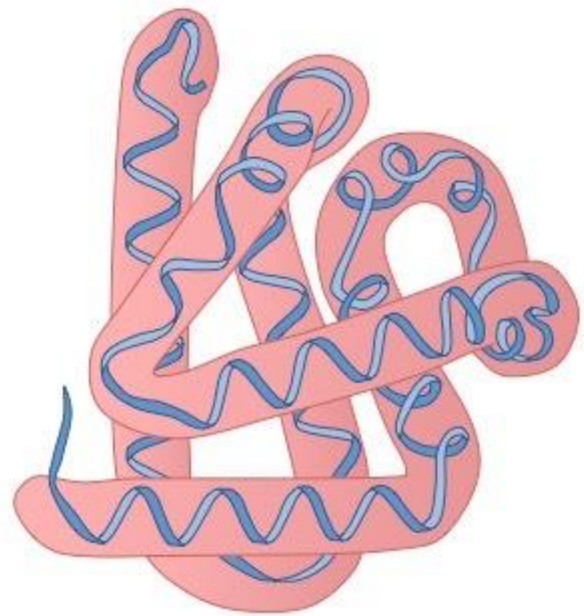
A physical process that a protein chain acquires its native 3-dimensional structure, a conformation with biological function



https://www.researchgate.net/profile/Holger_Flechsig/publication/293317366/figure/download/fig1/AS:650031413604361@1531990974211/Protein-folding-schematic-representation-Under-the-folding-process-the.png



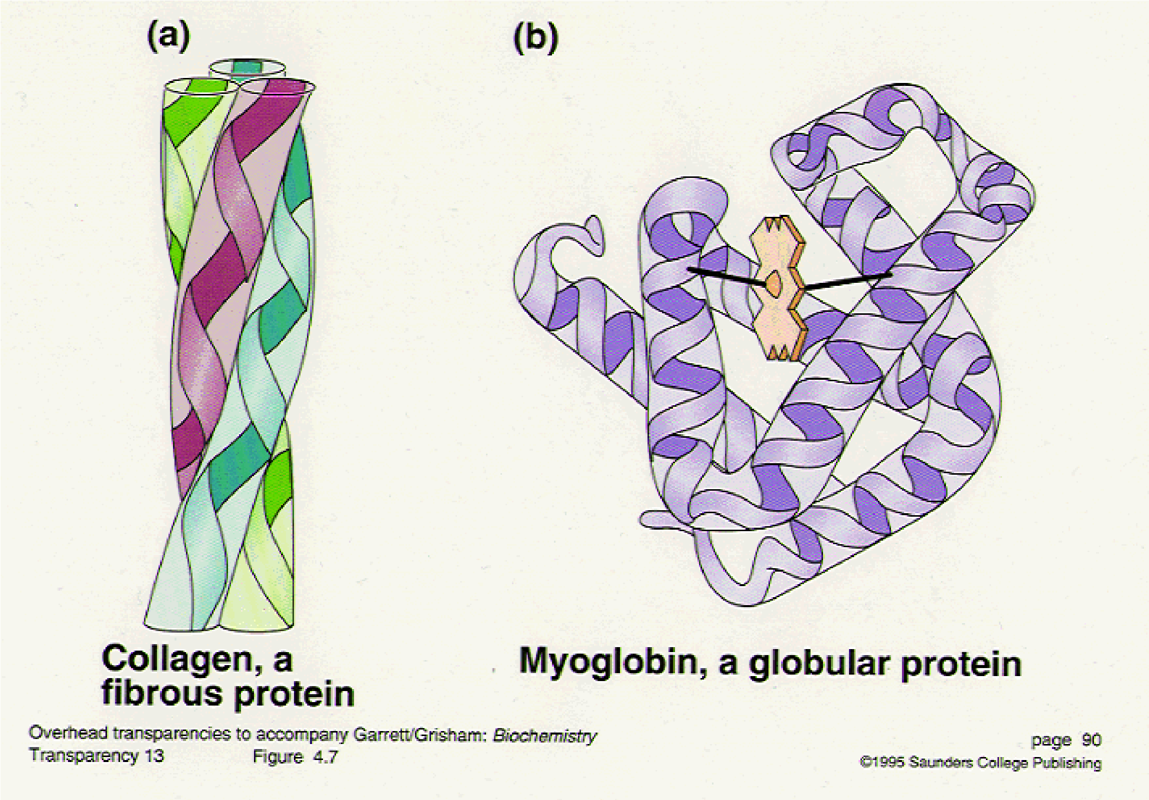
Types of side chain interactions



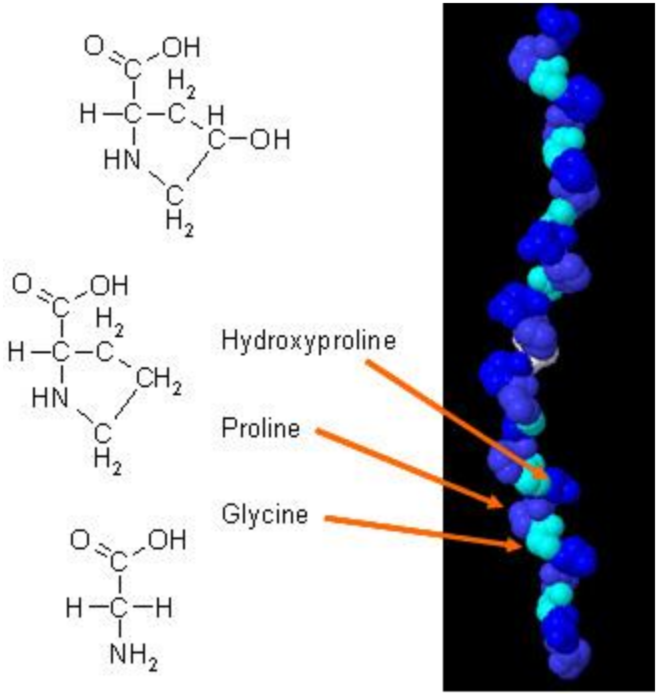
Overall 3D shape (3º Structure)

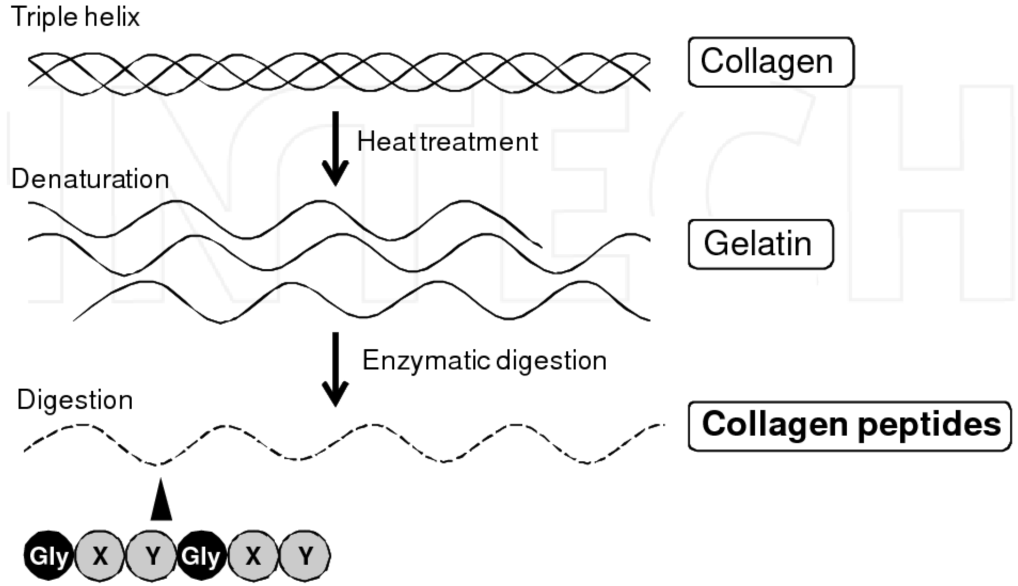
https://ib.bioninja.com.au/_Media/tertiary-structure_med.jpeg

- **Fibrous protein**
- **Globular protein**



Fibrous protein
Example: Triple helix e.g. collagen

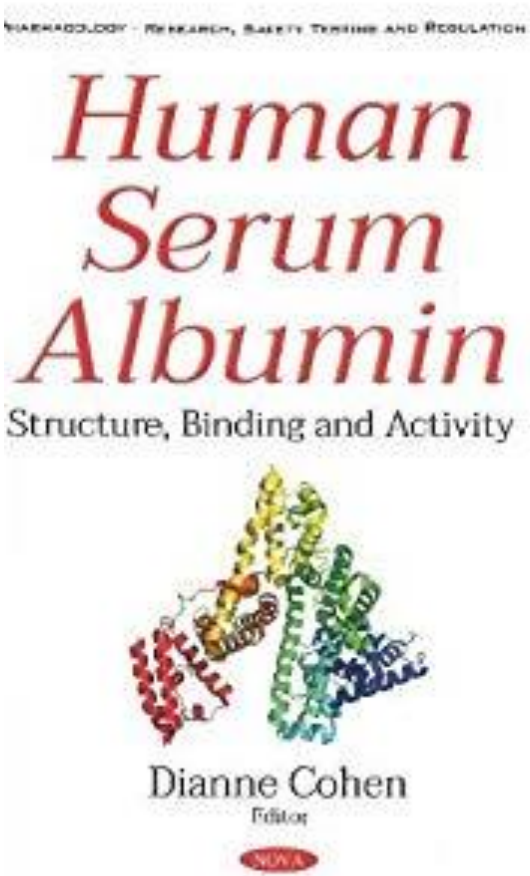




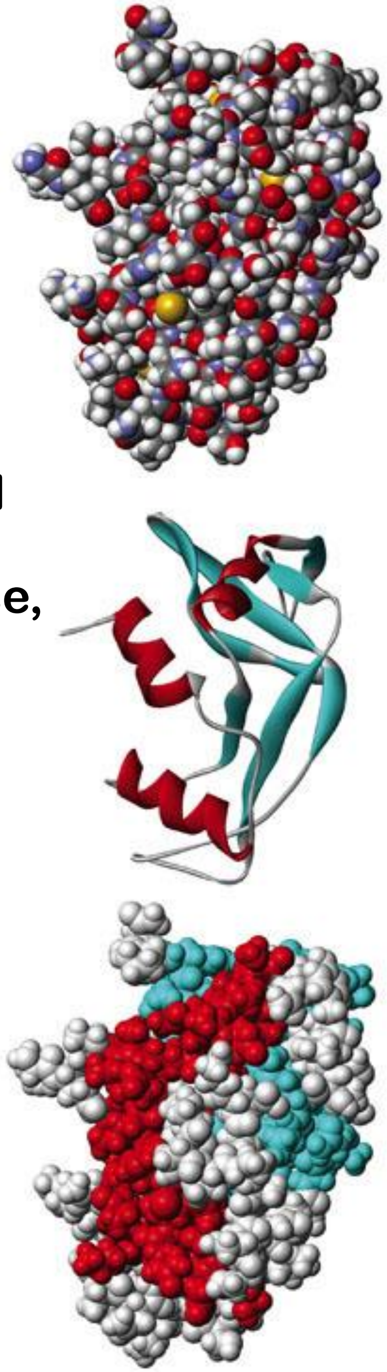
https://d3i71xaburhd42.cloudfront.net/27a3d34beaa8c6776b2babe8affd78e74bd1fa77/3-Figure1-1.png

https://upload.orthobullets.com/topic/9013/images/collagen-alpha-chain.jpg

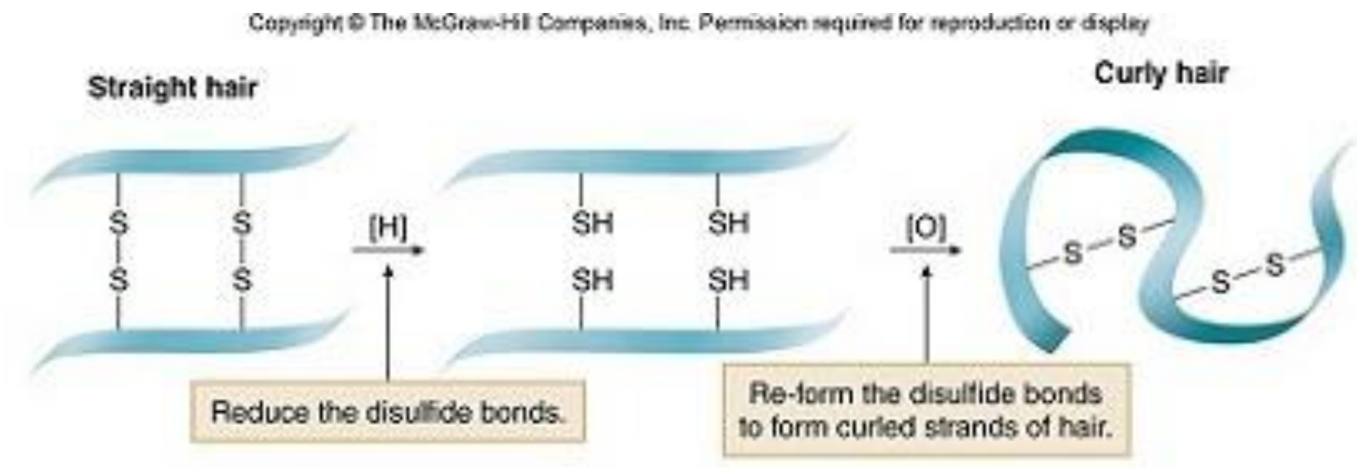
Globular protein
Example:

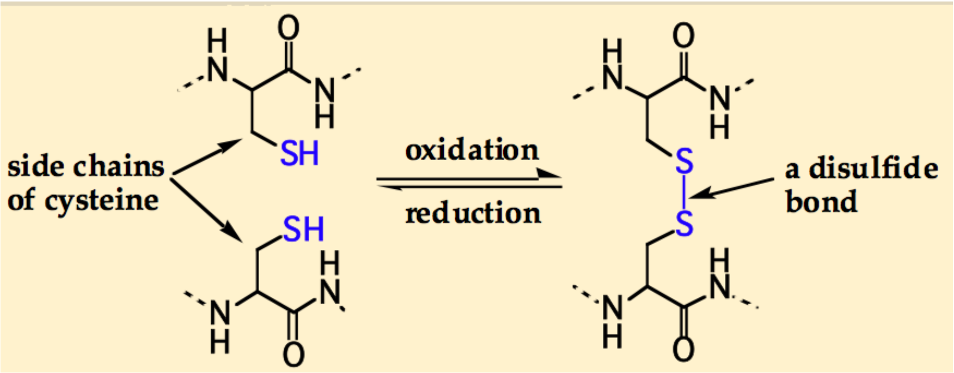


Merrifield synthesized the enzyme ribonuclease, a protein containing 124 amino acids



Disulfide bond and hair curling





Disulfide bonds between the side chains of cysteine

Quaternary structure

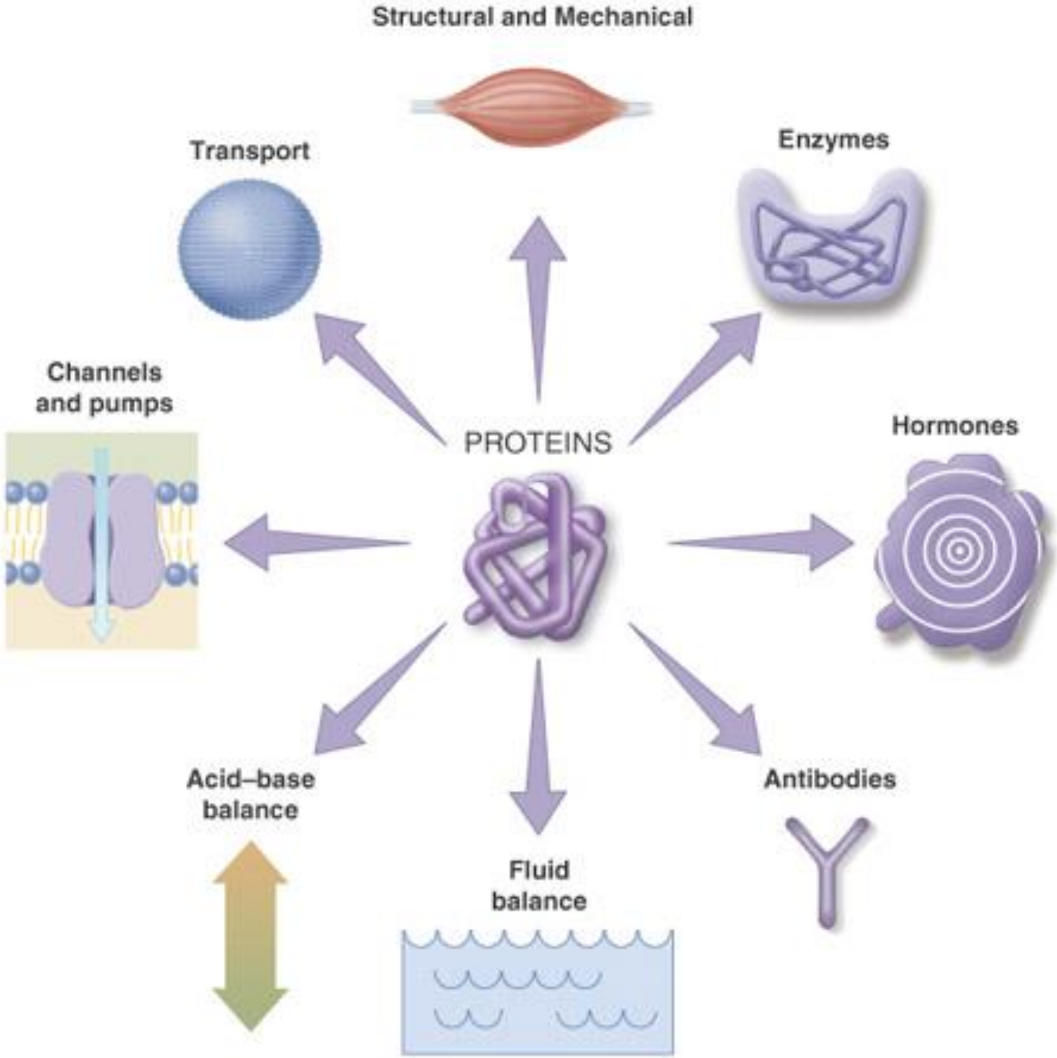
The arrangement of polypeptide chains into a noncovalently bonded aggregation

hydrophobic effect is the major factor stabilizing quaternary structure

Quaternary structure of hemoglobin
The β-chains in yellow, the heme ligands in red, and the Fe atoms as white spheres.

Protein	Number of Subunits
Alcohol dehydrogenase	2
Aldolase	4
Hemoglobin	4
Lactate dehydrogenase	4
Insulin	6
Glutamine synthetase	12
Tobacco mosaic virus protein disc	17

Role of proteins



https://plantbasedscientist.files.wordpress.com/2013/10/proteinfunctions.jpg

Role of proteins

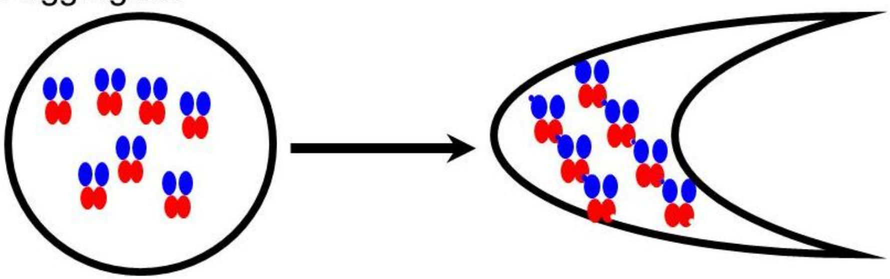
Class of Protein	Function in the Body	Examples
Structural	Provide structural components	*Collagen* is in tendons and cartilage. *Keratin* is in hair, skin, wool, and nails.
Contractile	Movement of muscles	*Myosin* and *actin* contract muscle fibers.
Transport	Carry essential substances throughout the body	*Hemoglobin* transports oxygen. *Lipoproteins* transport lipids.
Storage	Store nutrients	*Casein* stores protein in milk. *Ferritin* stores iron in the spleen and liver.
Hormone	Regulate body metabolism and nervous system	*Insulin* regulates blood glucose level. *Growth hormone* regulates body growth.
Enzyme	Catalyze biochemical reactions in the cells	*Sucrase* catalyzes the hydrolysis of sucrose. *Trypsin* catalyzes the hydrolysis of proteins.
Protection	Recognize and destroy foreign substances	*Immunoglobulins* stimulate immune responses.

Sickle cell hemoglobin

Small change in Hb

The change in cell structure arises from a change in the structure of hemoglobin.

A single change in an amino acid causes hemoglobin to aggregate.



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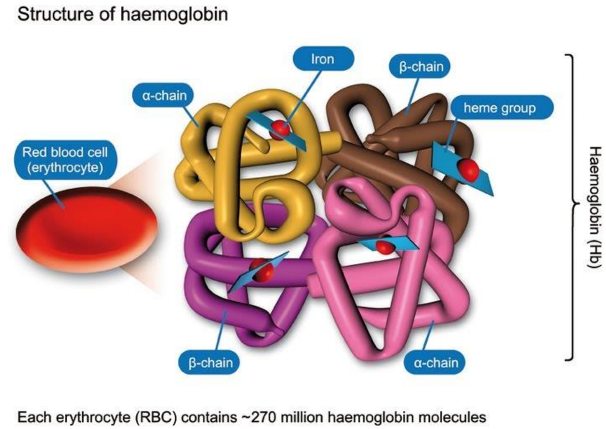
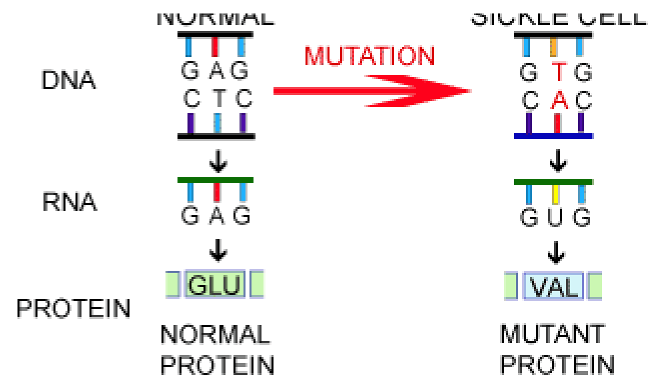
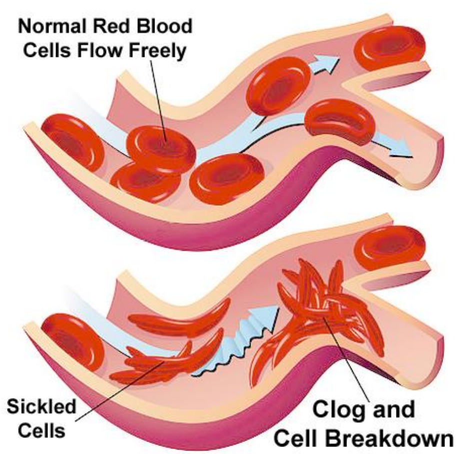
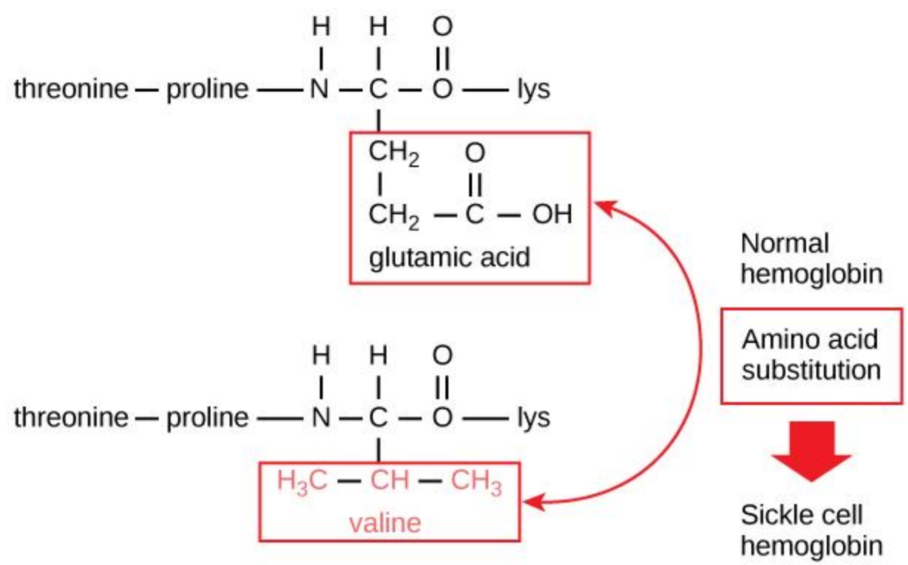




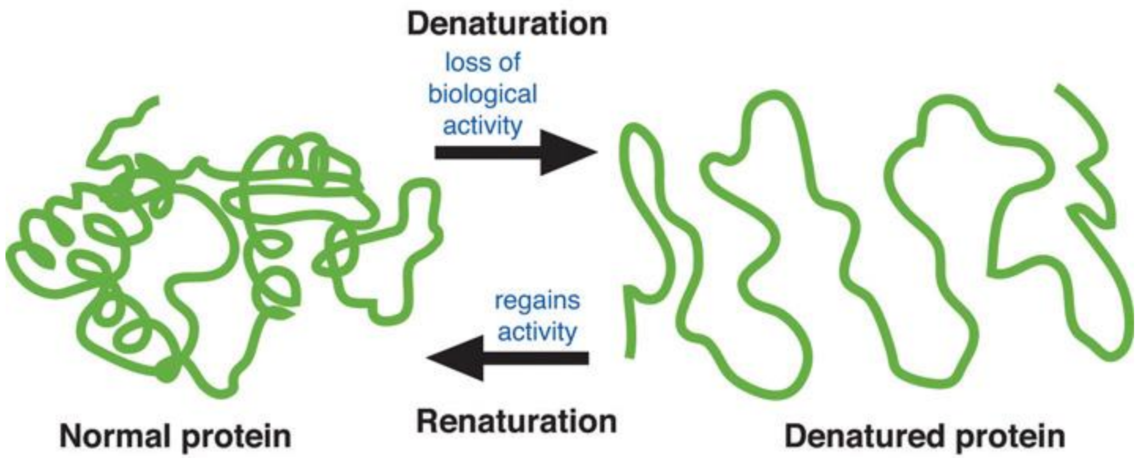




http://bioinformatics.org/jmol-tutorials/jtat/hemoglobin/images4all/sickle4.png

Protein denaturation

agents: pH, temp, ionic strength, solubility



https://2012books.lardbucket.org/books/an-introduction-to-nutrition/section_10/ca6893e3fff0790df3f609815c619145.jpg

Example albumin (white egg)

https://image.slidesharecdn.com/denaturationofprotein2-150330074227-conversion-gate01/95/denaturation-of-protein-8-638.jpg?cb=1427719453

Summary

Amino acids (AA) are monomer of proteins. AA can be classified according to their side chains.

AA joined by peptide bond can form polypeptides/proteins.

AA joined by peptide bond can form polypeptides/proteins.

There are 4 level of protein structure: primary, secondary, tertiary and quaternary structure.

AA, polypeptides and proteins play role in the body.