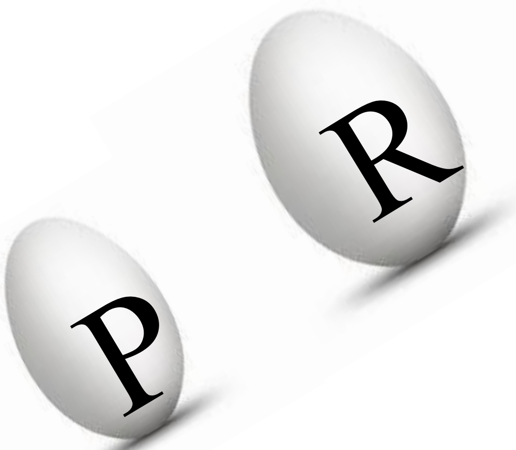
Sources of Plant Proteins



PROTEINS

Polymer of amino acids linked by amide linkage.

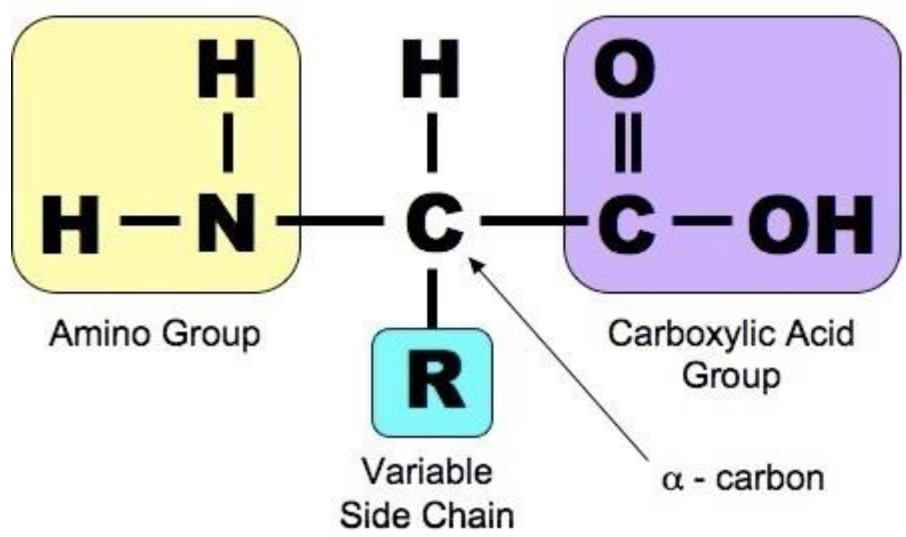
Functions:

1. Nutritional: growth, digestion, metabolism (enzymes).
2. Physical structure: cheese, bread, foaming agent, gel-forming.

AMINO ACIDS

The structure is simple and every amino acid contains an amino group ($-NH_2$) and a carboxyl group ($-COOH$)

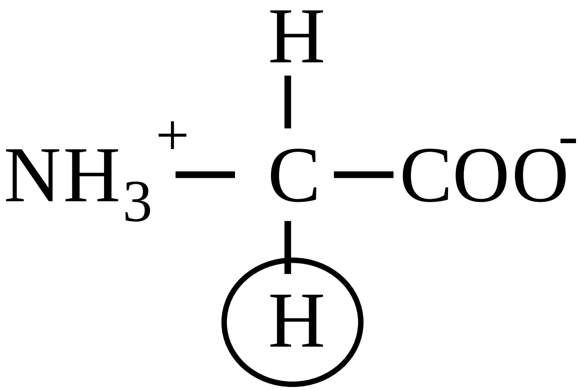
The carbon atom to which the amino group ($-NH_2$) is attached is known as the α–carbon and (except glycine) this carbon atom has four different atoms or groups of atoms attached to it.



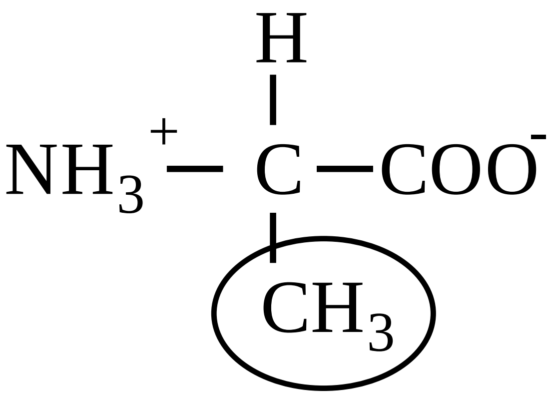
General formula

AMINO ACIDS

Every amino acid also has another atom or group of atoms bonded to the central atom, known as the R group, which determines the identity of the amino acid. For instance, if the R group is a hydrogen atom, then the amino acid is glycine, while if it is a methyl (CH3) group, the amino acid is alanine.



Glycine

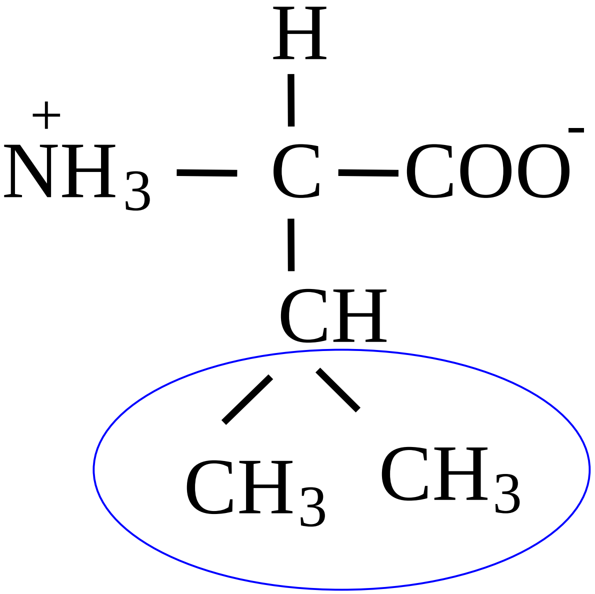


Alanine

AMINO ACIDS

The properties of the side chain determine an amino acid's chemical behavior (that is, whether it is considered **acidic, basic, polar, or nonpolar**).

For example, amino acids such as valine and leucine are **nonpolar** and hydrophobic,



Valine (VAL)

AMINO ACIDS

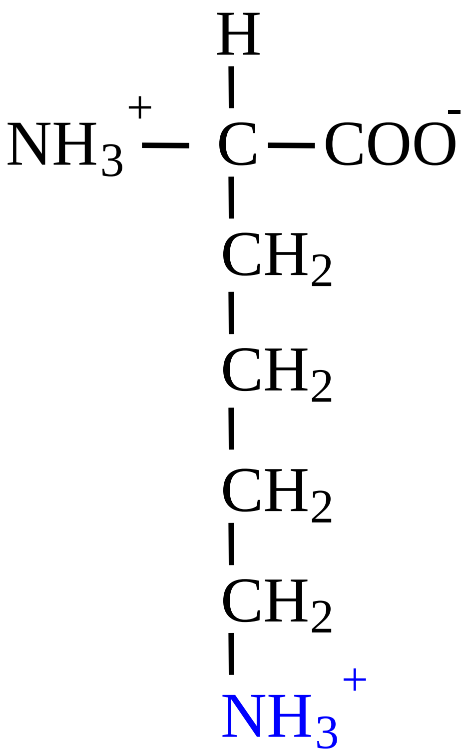
while amino acids like serine and glutamine have hydrophilic side chains and are **polar**.

$$
\begin{array}{ccccc}
 & & H & & \\
 & & | & & \\
NH_3^{+} & - & C & - & COO^{-} \\
 & & | & & \\
 & & CH_2OH & &
\end{array}
$$

Serine (SER)

AMINO ACIDS

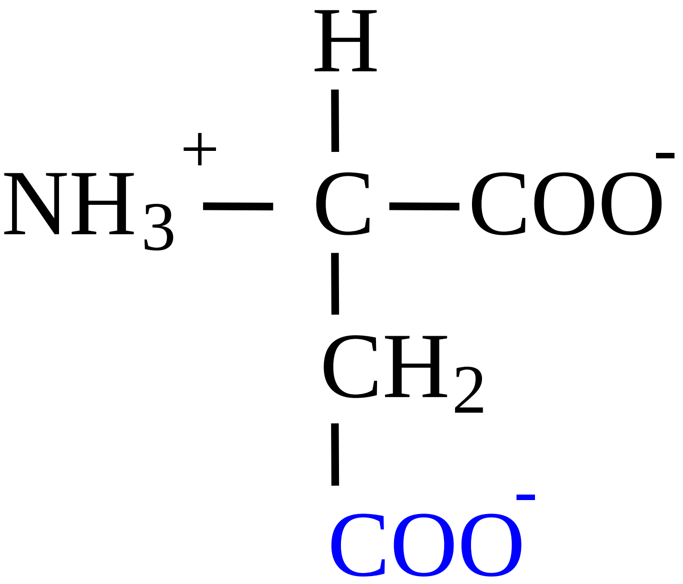
Some amino acids, such as lysine and arginine, have side chains that are positively charged at physiological pH (7.3 to 7.4) and are considered **basic** amino acids.



Lysine (LYS)

AMINO ACIDS

Aspartic acid and glutamic acid are negatively charged at physiological pH and are considered **acidic**.



Aspartic Acid (ASP)

AMINO ACIDS

Essential amino acids

Essential amino acids are the eight amino acids that your body cannot create on its own, and that you must obtain by eating various foods.
Adults need to eat foods that contain the following eight amino acids:

Phenylalanine
Valine
Tryptophan
Threonine
Isoleucine
Methionine
Leucine
Lysine

the ninth amino acid, is only necessary for babies

Histidine

AMINO ACIDS

Physical properties of amino acids

- White crystalline substances
- Soluble in water, acids and alkalies
- Insoluble in organic solvents
- High melting point (more than 200 °C)
- May be sweet (Glycine, Alanine, Valine),
  - tasteless (Leucine) or
  - bitter (Arginine Isoleucine).

AMINO ACIDS

Isoelectric point

The combination of an amino group and a carboxyl group in the same molecule results in it being able to act as an acid or a base; such a substance is said to be AMPHOTERIC.

Amino acids can exist as zwitterions in solution, depending upon pH of the medium.

The pH at which the amino acids exist as zwitterions, with no net charge on them is called **isoelectric pH** or **isoelectric point**.

- In acidic medium, the amino acids exist as cations
- In alkaline medium, they exist as anions.

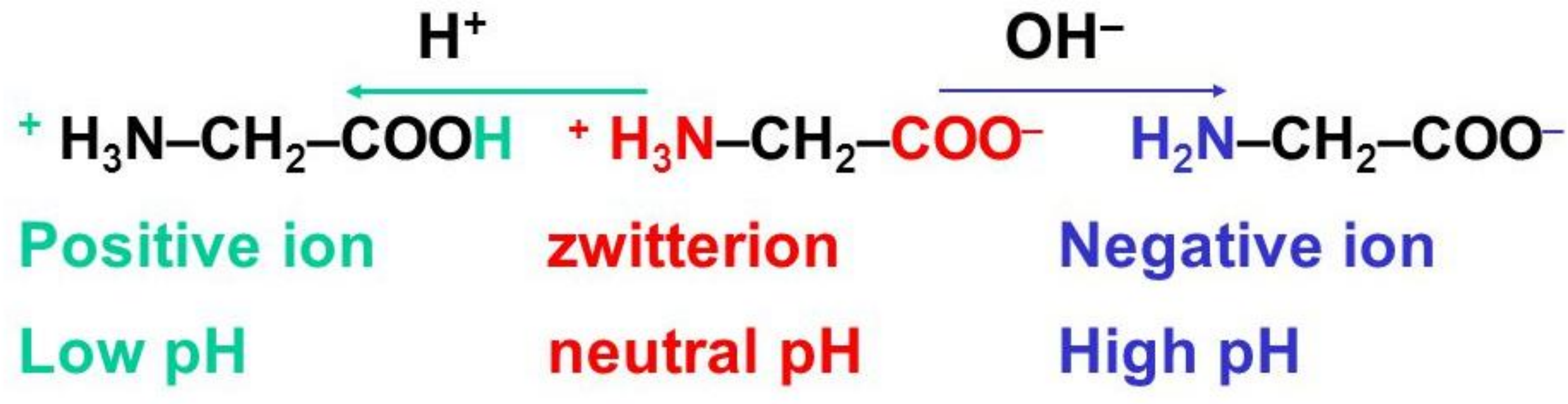
AMINO ACIDS

Isoelectric point

Net charge +1

Net charge 0

Net charge -1



Thus, if acid is added to a neutral solution of an amino acid a positive ion is formed, whereas if alkali is added a negative ion is formed

AMINO ACIDS

Isoelectric point

Therefore an amino acid may be

- Neutral
- Positively charged
- Negatively charges

according to the pH of the system

The isoelectric point is important because at this pH many properties have either a maximum or minimum value.

For example;

- Solubility
- Viscosity of solubilized proteins
- Electrical conductivity

minimum

AMINO ACIDS

Isoelectric point-Milk example

Milk has a pH between 6.5 and 6.7

- In milk, the isoelectric point indicates when the protein, casein, no longer has a positive or negative charge
- The isoelectric point for casein is pH value of 4.6. Once 4.6 is reached, the casein protein will separate from the liquid and clump together.

When acid is added

❖pH can decrease and reach isoelectric point

❖formation of curds

AMINO ACIDS

Isoelectric point

Ionizable groups

$$-\overset{\displaystyle O}{\overset{\|}{C}}-OH \qquad\qquad -NH_2$$

Zwitterion

pKa for $-\overset{\displaystyle O}{\overset{\|}{C}}-OH = 2.2$

pKb for $-NH_2 = 9.5$

What do 2.2 and 9.5 mean?

AMINO ACIDS

Isoelectric point

Effects of pH on the Concentration of Different Groups

	$\text{Log}\,\dfrac{[COO^-]}{[COOH]}$ *	$\text{Log}\,\dfrac{[NH2]}{[NH3^+]}$ **
pH 1	-1.2	-8.5
pH 2.2	0	-7.3
pH 3.5	1.3	-6
pH 5.5	3.3	-3.3
pH 7.5	5.3	-3
pH 9.5	7.3	0
pH 11.5	9.3	2

* pKa = 2.2   ** pKb = 9.5

AMINO ACIDS

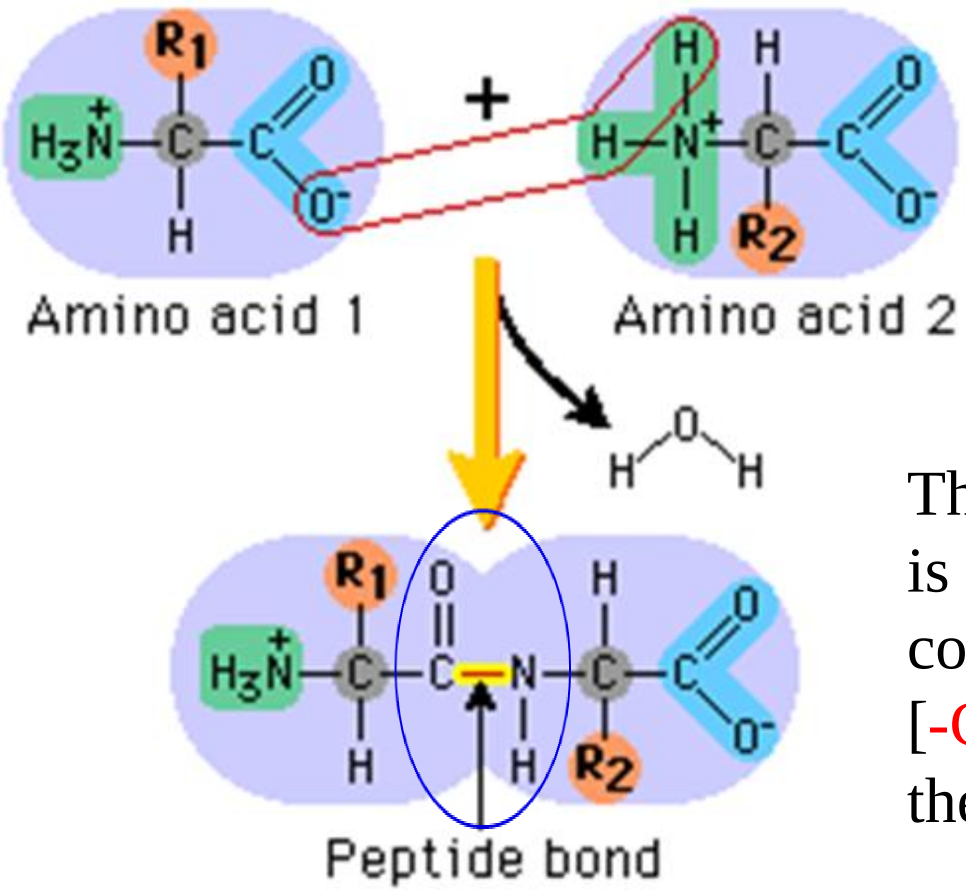
Isoelectric point

pK AND pI OF SOME AMINO ACIDS

	pK_1	pK_2	pK_R	pI
Glycine	2.34	9.78		6.06
Alanine	2.35	9.69		6.02
Isoleucine	2.36	9.69		6.02
Serine	2.21	9.15		5.68
Aspartic Acid	2.09	9.82	3.86	2.97
Asparagine	2.02	8.80		5.41
Glutamic Acid	2.19	9.67	4.25	3.22
Glutamine	2.17	9.13		5.65
Arginine	2.17	9.04	12.48	10.76
Lysine	2.18	8.95	10.53	9.74

PEPTIDES

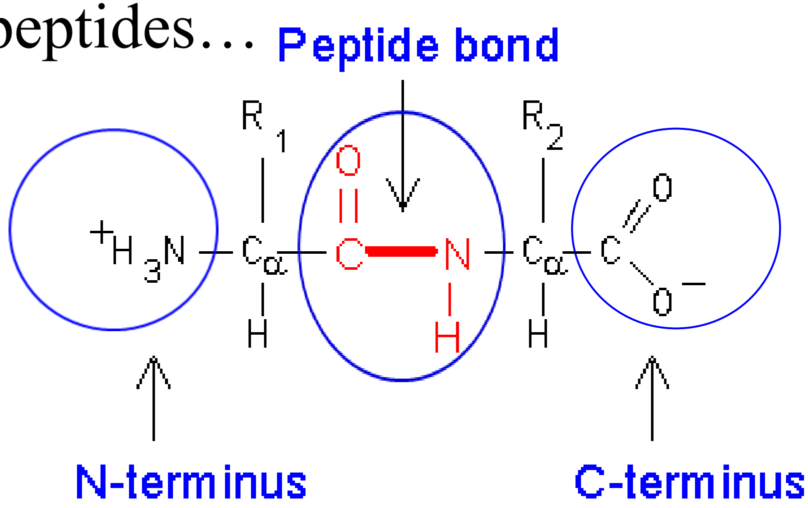
When two amino acid molecules combine, the acid group in one molecule reacts with the basic group in the other with the elimination of water



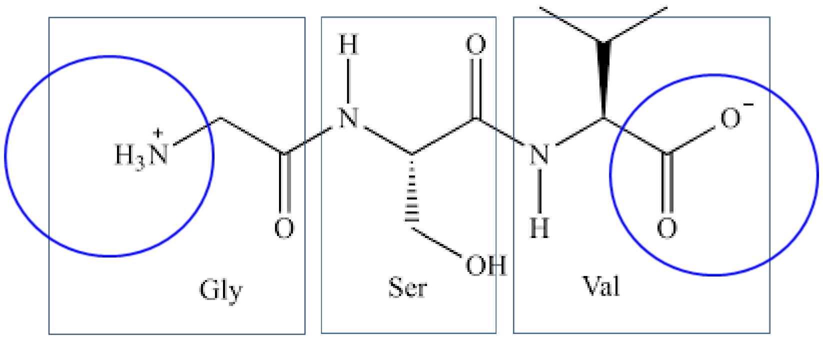
The product formed is a dipeptide and contains the group [-CONH-] which is the peptide linkage

PEPTIDES

A dipeptide still contains an amino and a carboxyl group and may react with another amino acid molecule to form a tripeptide, and so polypeptides…



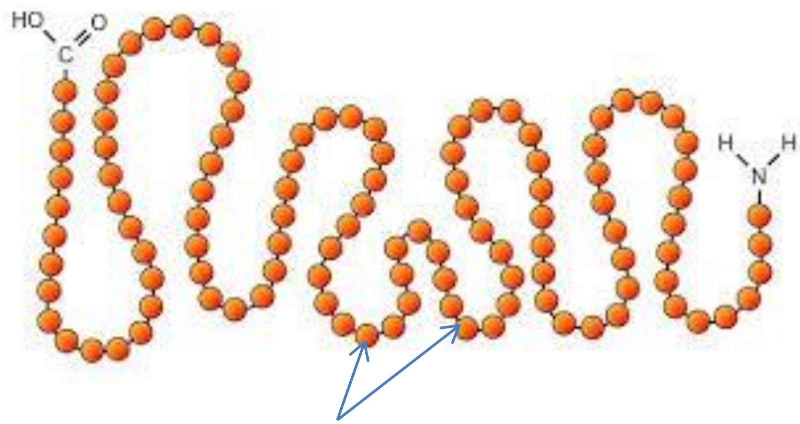
dipeptide



tripeptide

PROTEINS

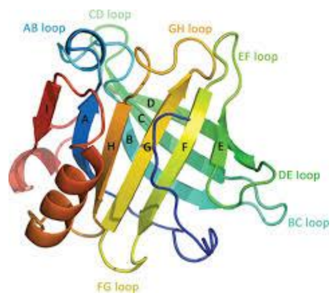
Protein molecules consist of chains of hundreds or thousands of amino units joined together rather like beads on a string.



Amino acid

Protein molecules are composed of large numbers of up to **20 different amino acids** joined together by peptide linkages

Lactoglobulin



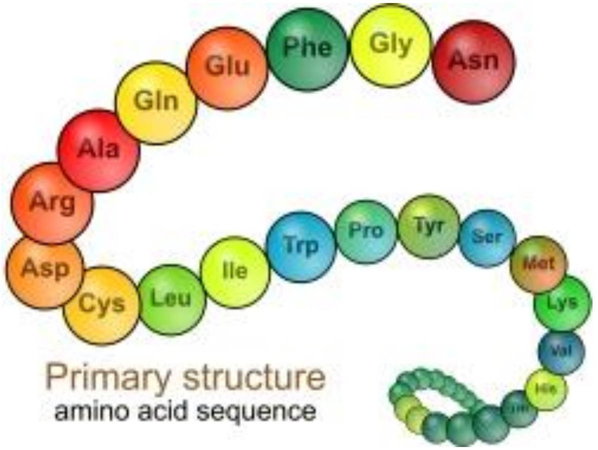
Mwt=42.000

$C_{1864}H_{3012}O_{576}N_{468}S_{21}$

PROTEINS

Protein structure (primary)

The sequence of amino acids linked by peptide bonding into a protein chain is called the **primary structure** of the protein.



The sequence of a protein is determined by the DNA of the gene that encodes the protein. A change in the gene's DNA sequence may lead to a change in the amino acid sequence of the protein. Even changing just one amino acid in a protein’s sequence can affect the protein’s overall structure and function.

PROTEINS

Protein structure (secondary)

The next level of protein structure, **secondary structure**, refers to local folded structures that form within a polypeptide due to interactions between atoms of the backbone. (The backbone just refers to the polypeptide chain apart from the R groups – so all we mean here is that secondary structure does not involve R group atoms.)

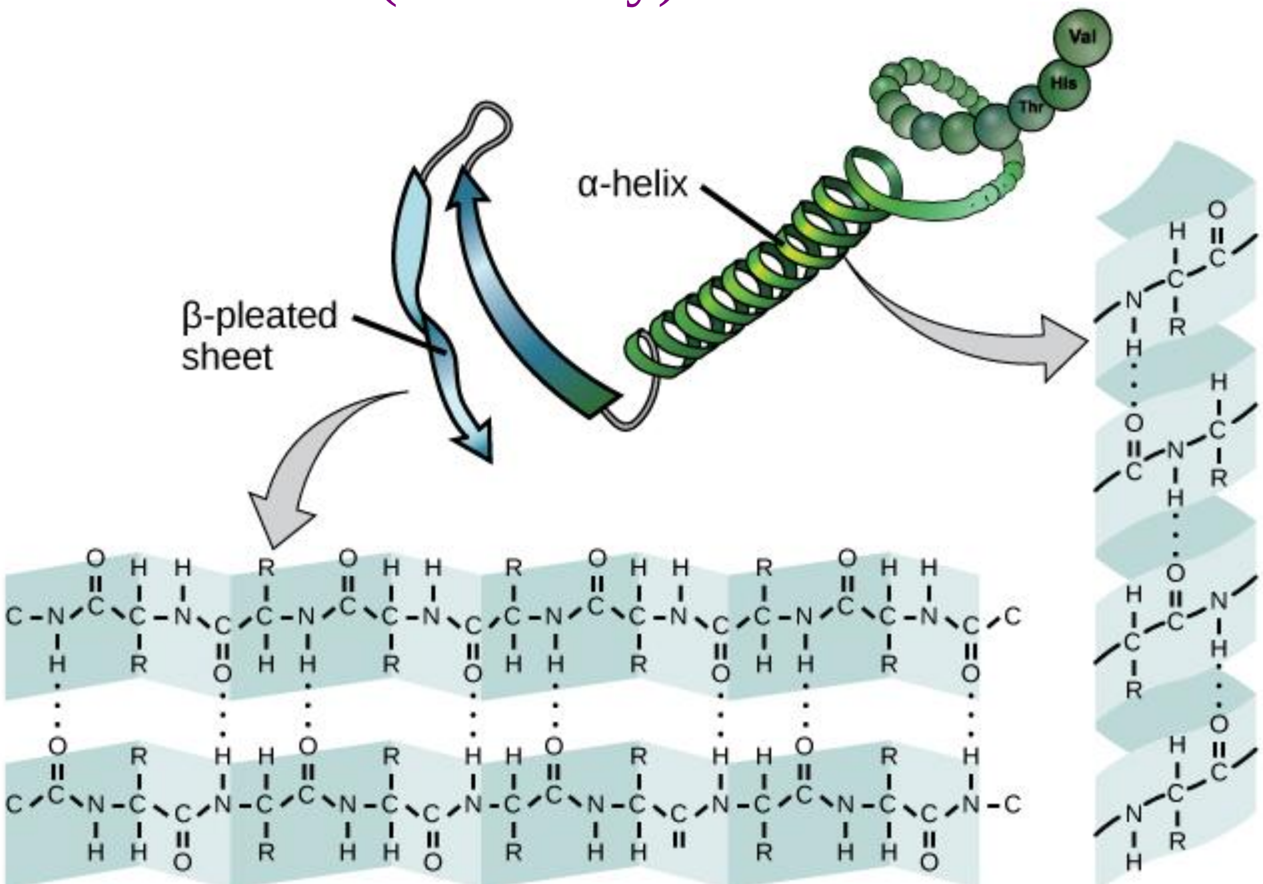
The types of secondary structures are

- α helix
- β pleated sheet

Both structures are held in shape by hydrogen bonds, which form between the carbonyl O of one amino acid and the amino H of another.

PROTEINS

Protein structure (secondary)



In an **α helix**, the carbonyl (C=O) of one amino acid is hydrogen bonded to the amino H (N-H) of an amino (1 to 5). This pattern of bonding pulls the polypeptide chain into a helical structure that resembles a curled ribbon

In a **β pleated sheet**, two or more segments of a polypeptide chain line up next to each other, forming a sheet-like structure held together by hydrogen bonds. The hydrogen bonds form between carbonyl and amino groups of backbone, while the R groups extend above and below the plane of the sheet.

PROTEINS

Protein structure (tertiary)

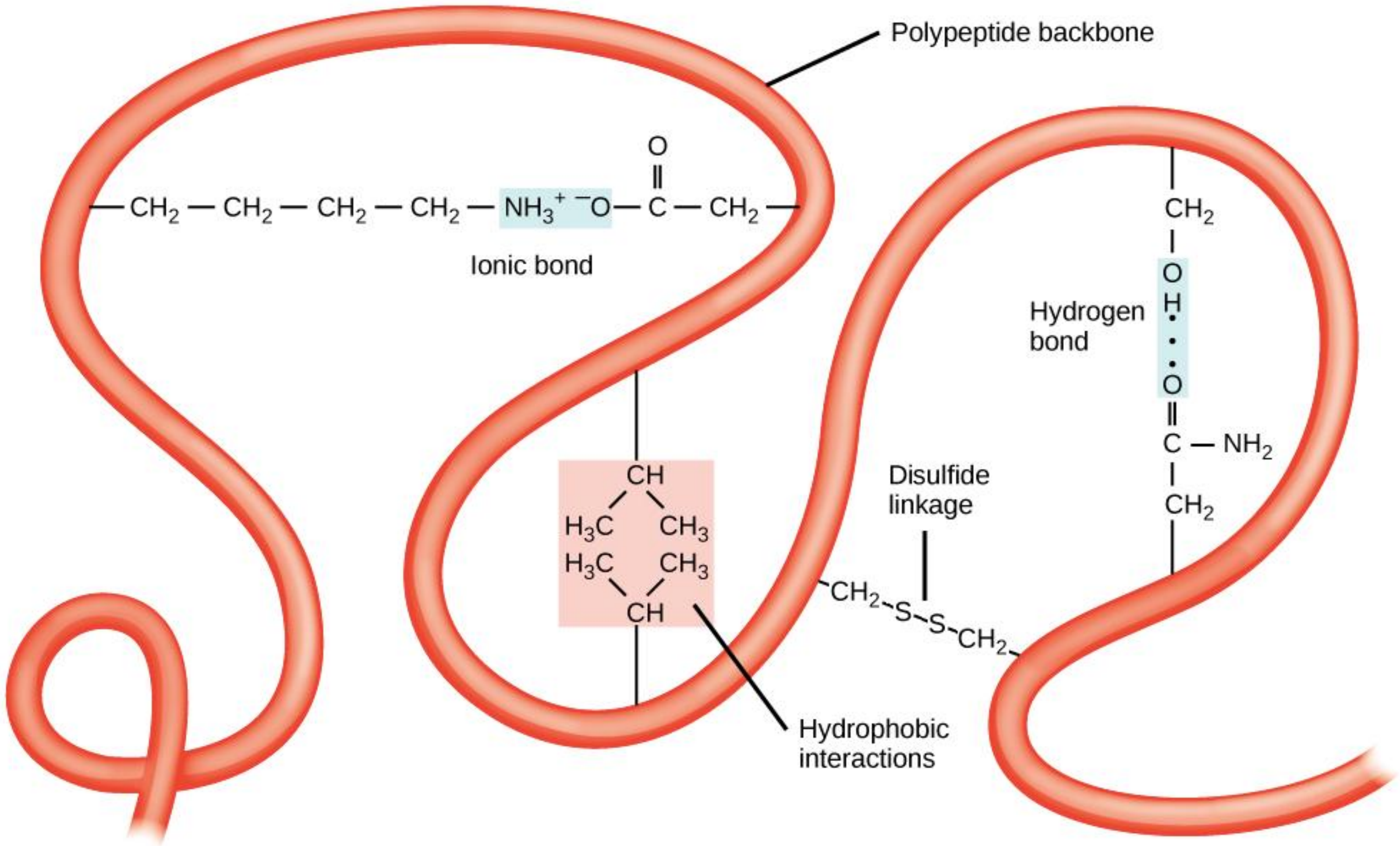
The overall three-dimensional structure of a polypeptide is called its **tertiary structure**. The tertiary structure is primarily due to interactions between the R groups of the amino acids that make up the protein.

R group interactions (cross-linkages) that contribute to tertiary structure include;

1. Hydrophobic attraction: the close association attraction of hydrocarbon side-chains (Nonpolar groups).
2. Ionic bond: between positively charged groups and negatively charged groups (COO^- and NH_3^+).
3. Hydrogen bonds
4. Disulfide bonds: covalent linkages between the sulfur-containing side chains of cysteines.

PROTEINS

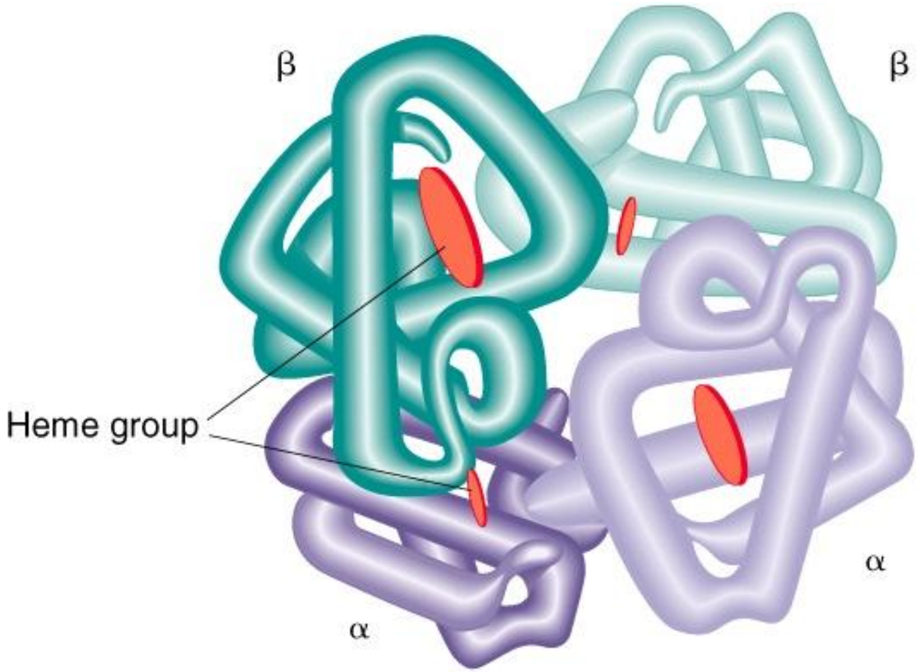
Protein structure (tertiary)



PROTEINS

Protein structure (quaternary)

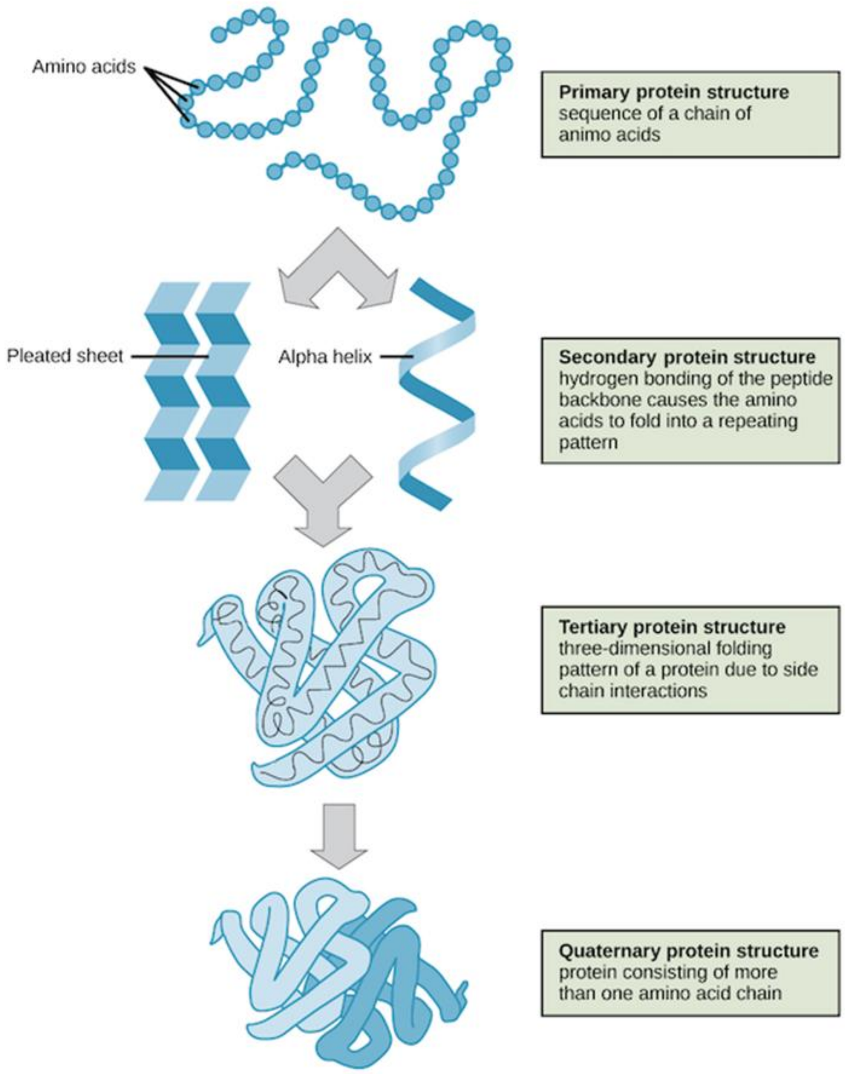
Many proteins are made up of a single polypeptide chain and have only three levels of structure.
However, some proteins are made up of multiple polypeptide chains, also known as subunits. When these subunits come together, they give the protein its **quaternary structure**.



hemoglobin

PROTEINS

Protein structure



PROTEINS

Classification of proteins

Different methods of **protein classification** have been proposed, but currently none of them is universally valid.

Proteins can be classified into groups according to

- their chemical composition
- their shape
- their biological functions

PROTEINS

Classification based on chemical composition

On the basis of their chemical composition, proteins may be divided into two classes: simple and complex (conjugated).

Simple proteins

Also known as homoproteins, they are made up of only amino acids. They are further subdivided as;

- Albumins
- Globulins
- Glutelins
- Prolamines
- Protamines
- Globins
- ..

Examples are plasma albumin, collagen, glutenin (wheat), gliadin (wheat), hordenin (barley), zein (corn), oryzenin (rice) and keratin.

PROTEINS

Classification based on chemical composition

Conjugated proteins

Sometimes also called heteroproteins, they contain in their structure a non-protein portion. These non-protein portions are called as prosthetic groups. Some examples are

glycoproteins (covalently bind one or more carbohydrate units to the polypeptide backbone; e.g., immunoglobulins),
chromoproteins (contain colored prosthetic groups; e.g., Myoglobin, hemoglobin)
phosphoproteins (bind phosphoric acid to serine and threonine residues; e.g., casein).
lipoproteins (in combination with lipids, e.g., LDL, HDL)

PROTEINS

Classification based on shape

On the basis of their shape, proteins may be divided into two classes: fibrous and globular.

Fibrous proteins

Their polypeptide chains form long filaments or sheets.

Collagen (found in connective tissues)

Keratin (found in hair, nail, ..)

Globular proteins

They have a compact and more or less spherical structure, more complex than fibrous proteins.

Globulins (found in blood)

Casein (found in milk)

PROTEINS

Classification based on shape

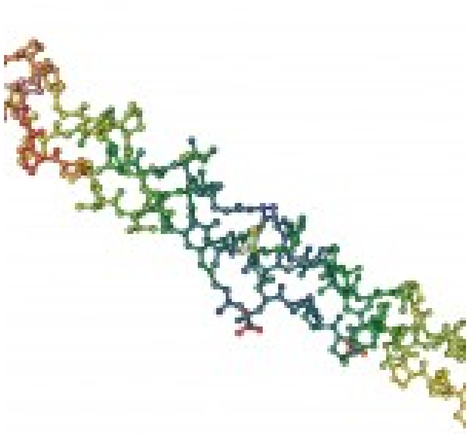
Fibrous Proteins

1. Fibrous Proteins are extended proteins and are insoluble in water.

2. They have long thread like structures.

3. They have helical or sheet like structures.

4. They form structures of body or cells e.g. tendons, ligaments, hairs, etc.



collagen

Globular Proteins

1. Globular Proteins are compact proteins and are soluble in water.

2. They have folded ball like structures.

3. Globular have three dimensional shape.

4. They perform metabolism, transport, immune protection, hormones, etc.

hemoglobin

PROTEINS

Classification based on biological function

Class of Protein	Biological Function	Examples
Enzymatic proteins	Biochemical catalysts	Amylase, catalase, urease
Structural proteins	Provide strength and protect biological structures	Elastin, keratin, collagen
Transport proteins	Transport of ions and molecules in the body	Hemoglobin, myoglobin, lipoproteins
Nutrient and storage proteins	Provide nutrition for growth and repairs	Casein, gluten, ovalbumin
Defense proteins	Defend against other organisms (pathogens)	Antibodies
Regulatory proteins	Regulate metabolism and cellular functions	Insulin, growth hormone
Contractile proteins	Cause contraction and expansion of muscles	Actin, myosin

PROTEINS

Properties of proteins

<u>Molecular weight</u>

Proteins are complex substances, and thus it is extremely difficult to determine their molecular weights.

Their molecular weight is very high- Average 60kDa

Protein	Molecular Weight (kDa)
Myosin	205
Bovine serum albumin	68.9
Ovalbumin	44
Myoglobin	16

Dalton (Da) is an alternate name for the atomic mass unit kilodalton (kDa) is 1000 daltons.

Thus a protein with a mass of 16 kDa has a molecular weight of 16000 grams per mole

PROTEINS

Properties of proteins

Solubility

Proteins vary enormously in their solubilities.

Solubility of proteins depends on the number of polar and nonpolar groups. Some small globular proteins are very soluble while many structural proteins are essentially insoluble.

The solubility of a protein generally increases as the pH moves away from the isoelectric point.

The solubility of proteins is also affected by the presence of salts. Small concentrations of salt increases their solubility.

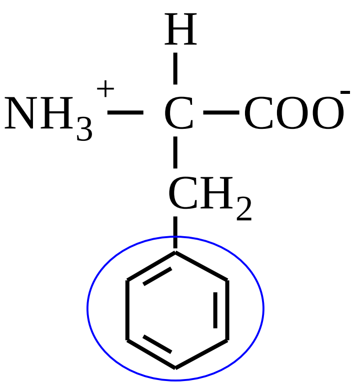
PROTEINS

Properties of proteins

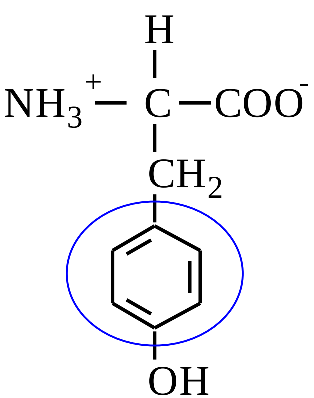
<u>Spectral properties</u>

Proteins contain several chromophores that absorb light in the ultra-violet and infrared regions. Many also display fluorescence.

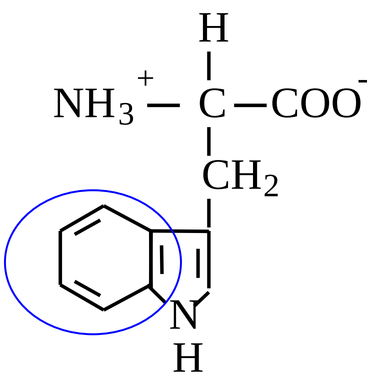
The measurement of light absorption of proteins shows strong bond bands in the UV area at 180-230 nm for the (-CONH-) bonds and at 250-300nm (max at 280 nm) for the aromatic rings of Phenylalanine, Tyrosine and Trytophan.



Phenyalanine



Tyrosine



Trytophan

PROTEINS

Properties of proteins

Optical rotation

In aqueous solution, proteins rotate polarised light to the left.
The magnitude of rotation is dependent on the secondary structureof protein.
All of the twenty natural amino acids except glycine have a chiral center at their alpha-carbon.

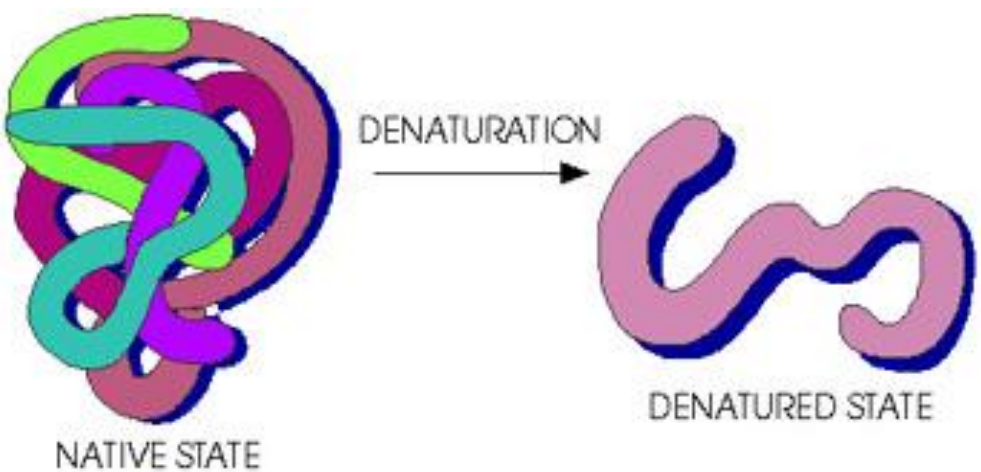
The amino acids of proteins are members of L-series.
D amino acids are very rare in nature (in bacteria as component of cell wall and in certain antibiotics).

PROTEINS

Properties of proteins

<u>Denaturation of proteins</u>

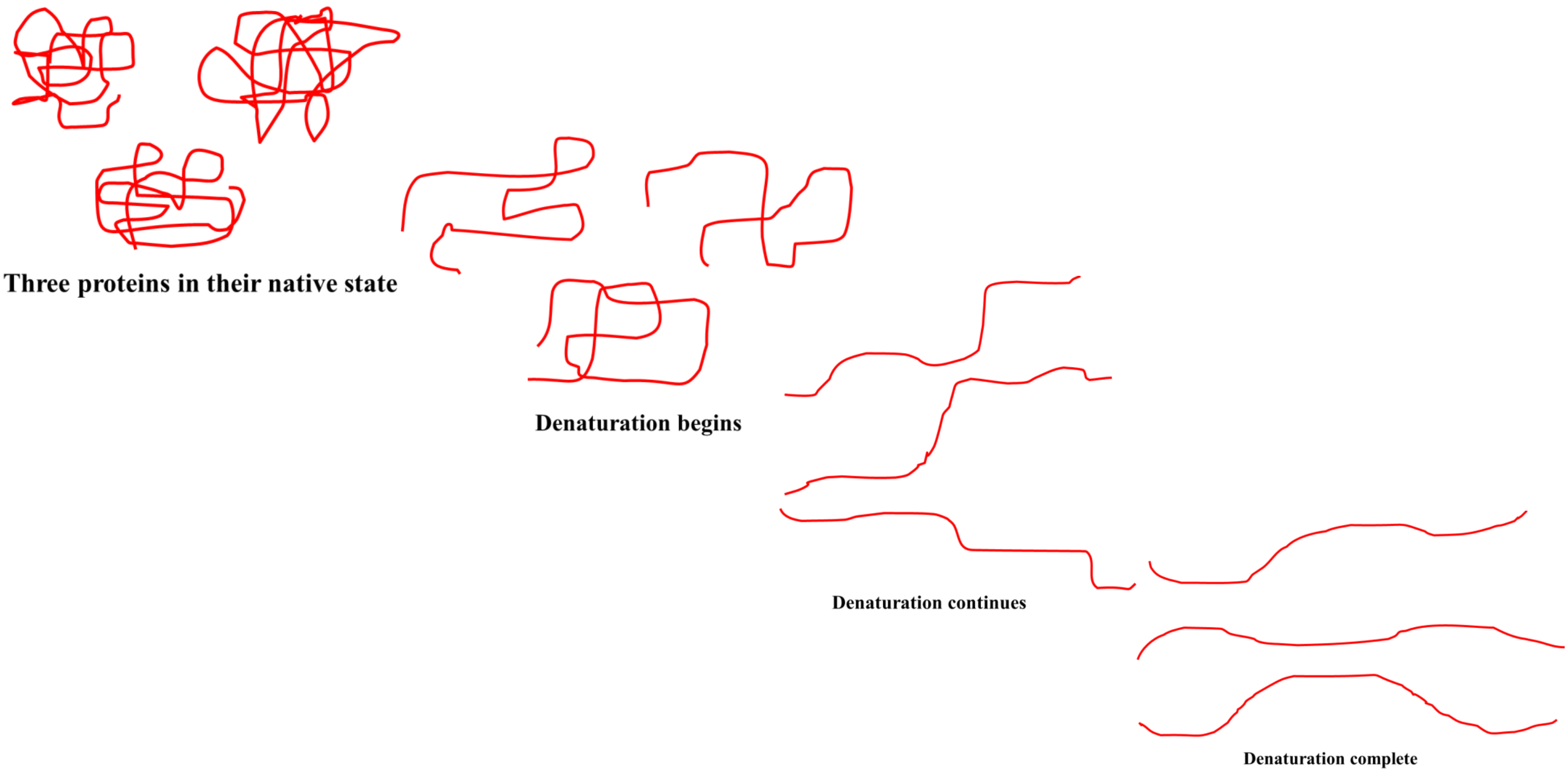
Denaturation is a process in which proteins lose the quaternary structure, tertiary structure and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent, radiation or heat.



PROTEINS

Properties of proteins

<u>Denaturation of proteins:</u> Denaturation occurs because the bonding interactions responsible for the secondary structure and tertiary structure are disrupted.

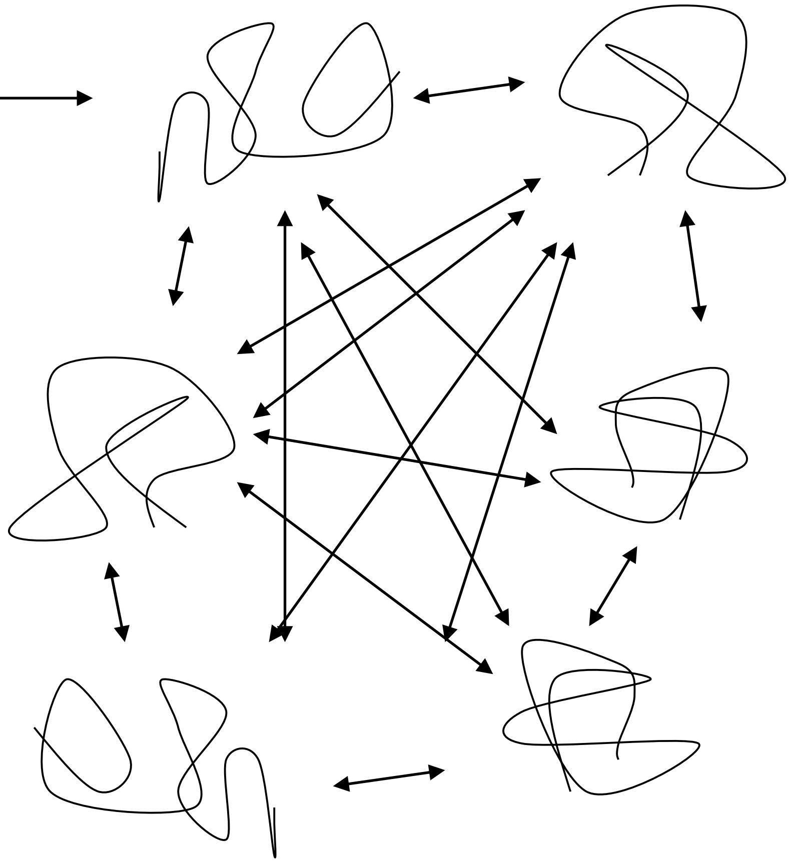


PROTEINS

Properties of proteins

Reversibility of denatured proteins

One native form



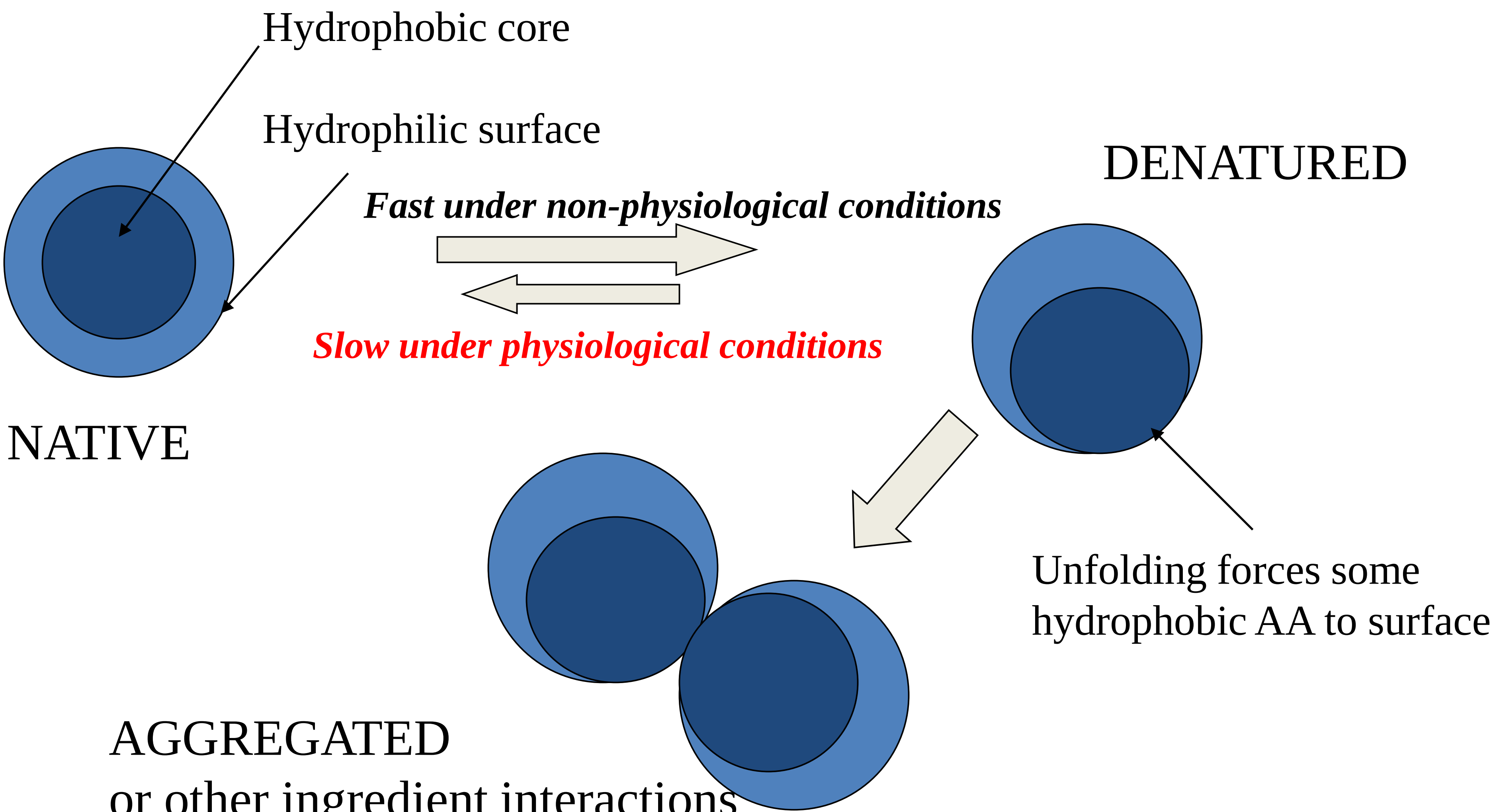
Many denatured forms

Refolding is a complex process – particularly for large proteins or complex proteins

PROTEINS

Properties of proteins

Behaviour of denatured proteins



PROTEINS

Properties of proteins

Denaturation of proteins:

A variety of reagents and conditions can cause denaturation. The most common observation in the denaturation process is the precipitation or coagulation of the protein.

PROTEINS

Properties of proteins

Causes of Protein Denaturation

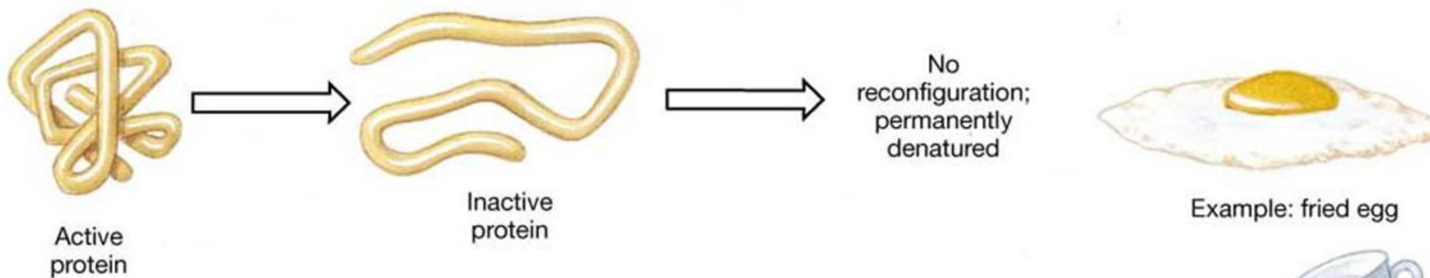
- **Heating** which disrupts the secondary and tertiary structure of the protein.
- **Changes in pH** that interfere with ionic bonding between amino-acid side chains.
- **Detergents**, which make nonpolar amino-acid side chains soluble and thereby destroy the hydrophobic interactions that give rise to the tertiary and quaternary structure of the protein.
- **Oxidizing or reducing agents** that either create or destroy S-S bonds.
- **Reagents such as urea** (H2NCONH2), which disrupt the hydrogen bonds that form the secondary structure of the protein.
- **Ionizing radiation**

PROTEINS

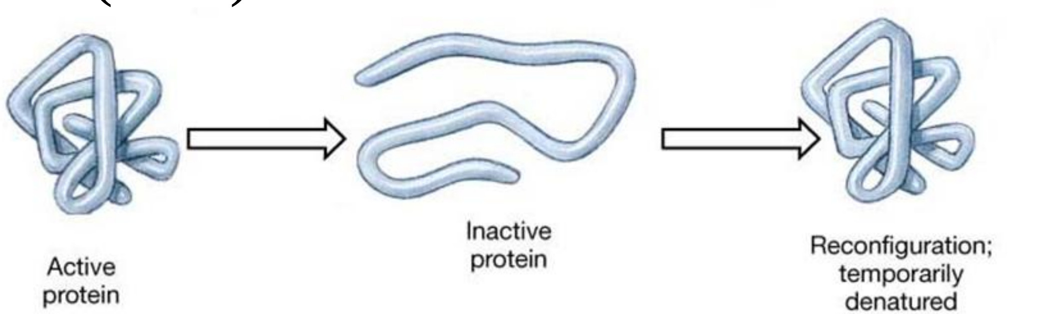
Properties of proteins

Examples for denaturation (heat)

Cooking eggs (extreme)



Warming milk (mild)



PROTEINS

Properties of proteins

Example for denaturation (Change in pH)

Milk forms a solid curd on standing

- bacteria in milk grows
- forms lactic acid
- pH of milk lowers
- reaches to isoelectric point
- milk protein coagulates

PROTEINS

Properties of proteins

Consequences of denaturation

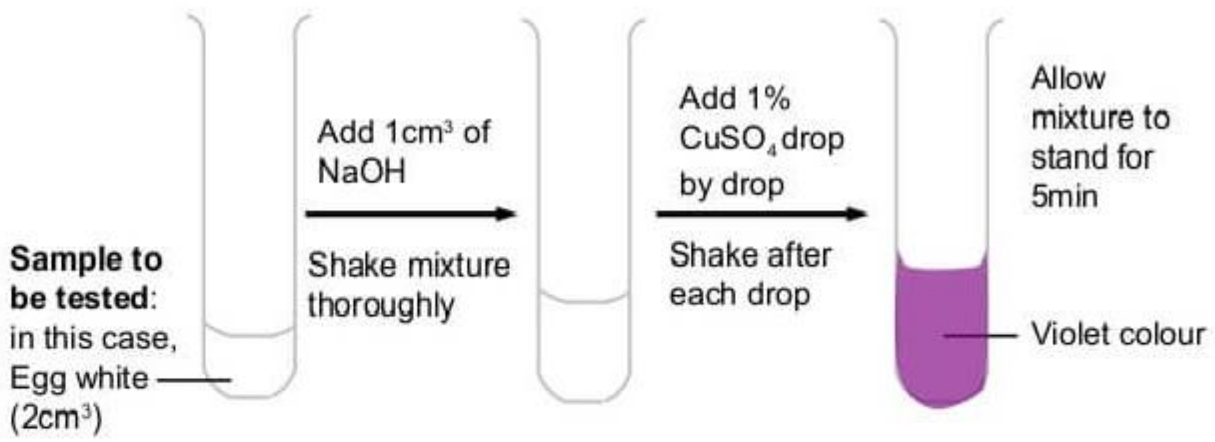
- Loss of enzymatic activity
- Destruction of toxins
- Improved digestibility
- Loss of solubility
- Changes in texture

PROTEINS

Methods to determine proteins

Biuret method

Peptide bonds react with alkaline copper to form a purple compound which absorbs at 540 nm.



PROTEINS

Methods to determine proteins

Ninhydrin method

Ninhydrin (2,2-Dihydroxyindane-1,3-dione) is a chemical used to detect proteins, polypeptides and amino acids. Ninhydrin is the powerful oxidising agent. Amino acids, on heating with ninhydrin, are oxidatively decarboxylated, producing carbon dioxide (CO_2), ammonia (NH_3), and an aldehyde. Reduced ninhydrin then reacts with the liberated ammonia and a blue or purple coloured complex is produced.

A blue or purple colour appears, if proteins, peptides or amino acids are present.

PROTEINS

Methods to determine proteins

Millon's test

Millon's test is used for the detection of amino acid tyrosine.

The proteins, on the addition of Millon's reagent, form a white precipitate first due to denaturation of proteins by mercury salts, which upon heating turn red.

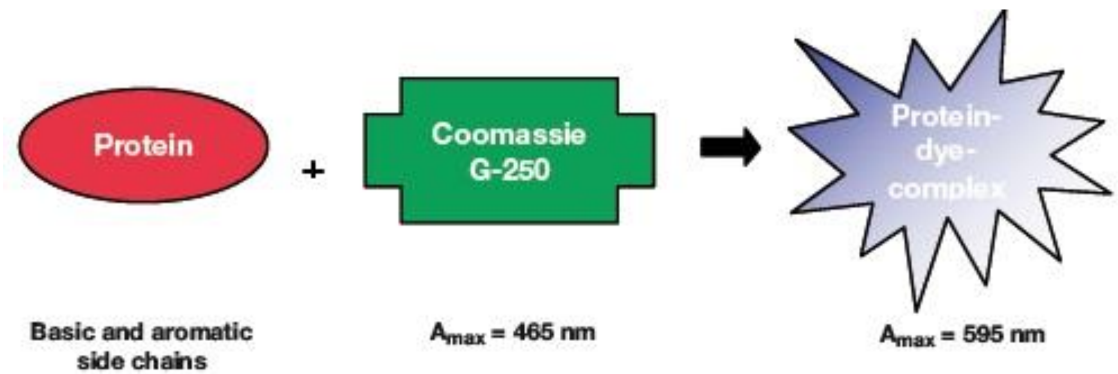
PROTEINS

Methods to determine proteins

Bradford assay

Generally Coomessie brillant blue G-250 bind to protein in acidic solution.

Make a complex which will shift the wavelength of maximum absorbance 465 to 565 nm.



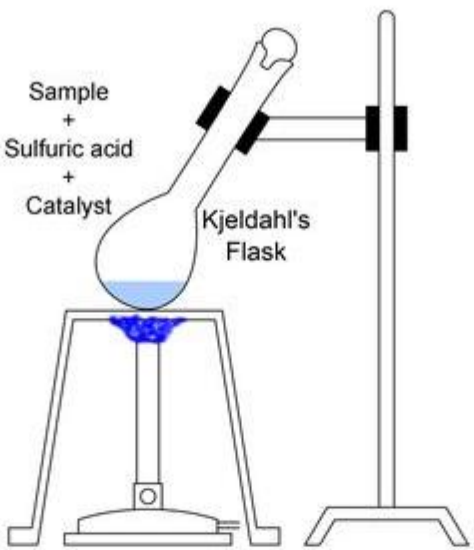
PROTEINS

Methods to determine proteins

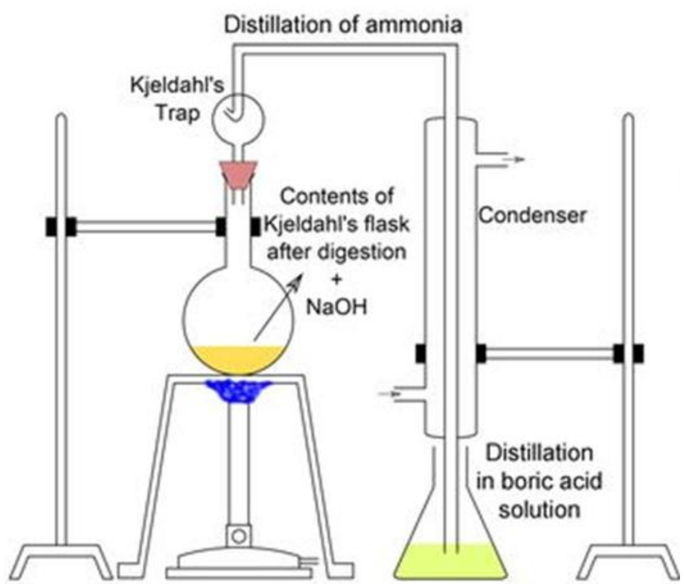
Kjeldahl method

The Kjeldahl procedure measures the nitrogen content of a sample. The protein content then, can be calculated assuming a ratio of protein to nitrogen for the specific food being analyzed. The Kjeldahl procedure can be basically divided into three parts:

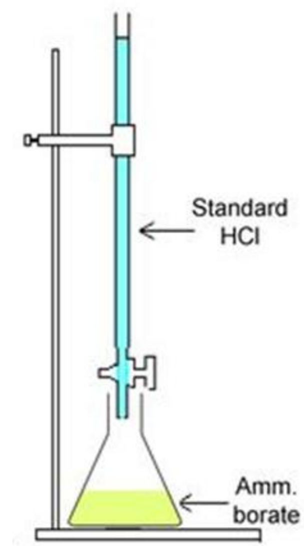
(1) Digestion



(2) Neutralization & Distillation



(3) Titration

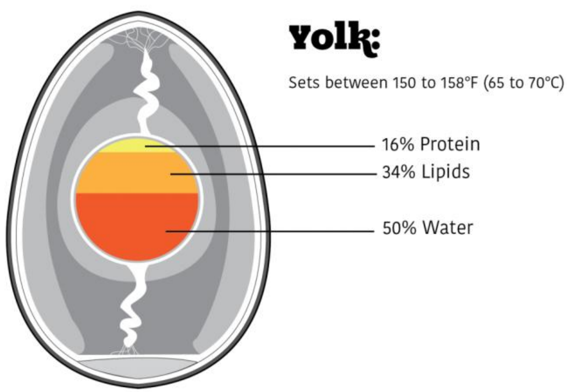


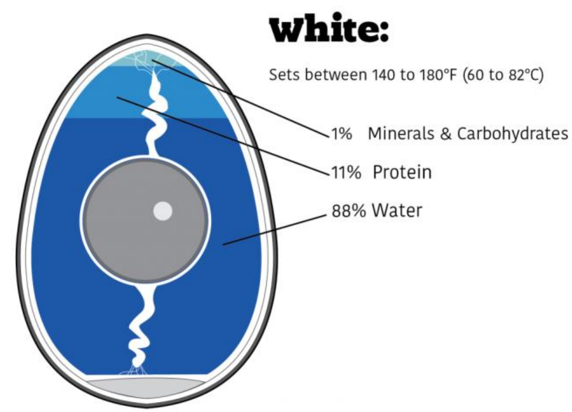
PROTEINS

Egg protein

Yolks and whites are made up of different proteins that have different molecular structures and behave differently when heat is applied.

Egg white proteins are called as **albumens** in overall





Egg white proteins are;
Ovalbumin (54 %)
Conalbumin (12%)
Ovomucin (2%)
Others (32%)

PROTEINS

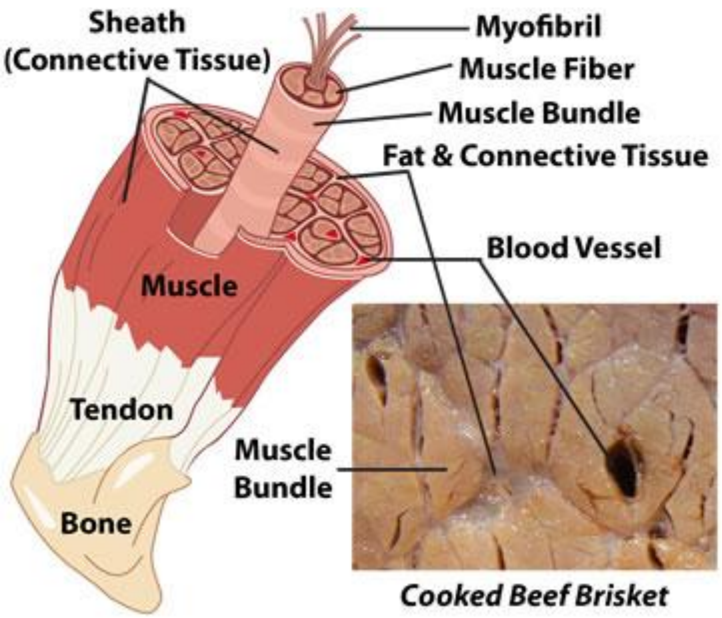
Egg protein functional properties

- ♦ **Foaming** (meringues – egg white and sugar) –
- ♦ **Gelation** (caused by protein denaturation from heat)
- ♦ **Emulsification** – primarily egg yolk proteins
- ♦ Sugar increases denaturation temp
- ♦ Age of an egg increases denaturation temp and increases foam stability

PROTEINS

Meat proteins

1. Myofibrillar (contractile) proteins
2. Stromal (connective tissue) proteins
3. Sarcoplasmic proteins (water soluble, intracellular fluid)



PROTEINS

Meat proteins

1. Myofibrillar (contractile) proteins

myosin	~55%
actin, troponin, tropomyosin	40 - 45%
desmin, synemin, α actinin, nebulin and numerous structural proteins	1 -5%

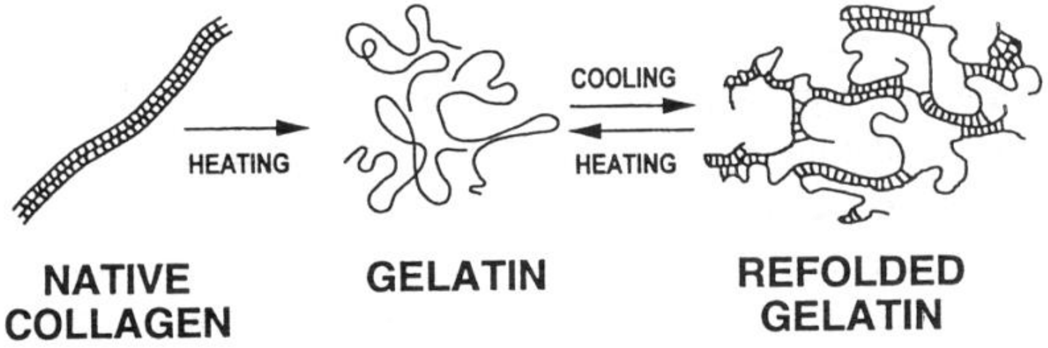
PROTEINS

Meat proteins

2. Stromal (connective tissue) proteins

- Collagen
- Elastin

COLLAGEN CAN BE CONVERTED TO GELATIN



PROTEINS

Meat proteins

3. Sarcoplasmic proteins (water soluble, intracellular fluid)

- Myoglobin (gives color)

Fish Chicken Beef

- Enzyme-Calpain (has a role of tenderization)

PROTEINS

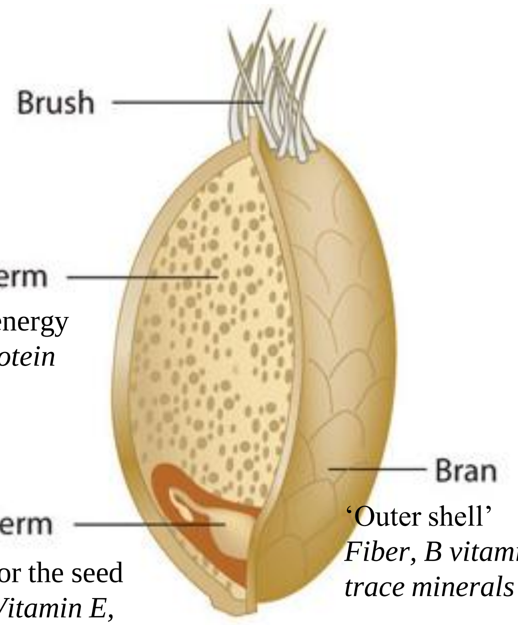
Cereal proteins

PROTEIN CONTENTS OF CEREAL GRAINS
(MOISTURE CONTENTS APPROXIMATELY 12%)

TYPES OF GRAIN	PERCENT PROTEIN
HARD	12-13
SOFT	7.5-10
DURUM	13.5-15
BARLEY	12-15
RYE	11-12
OATS	10-12
CORN	9-10
RICE	7-9

PROTEINS

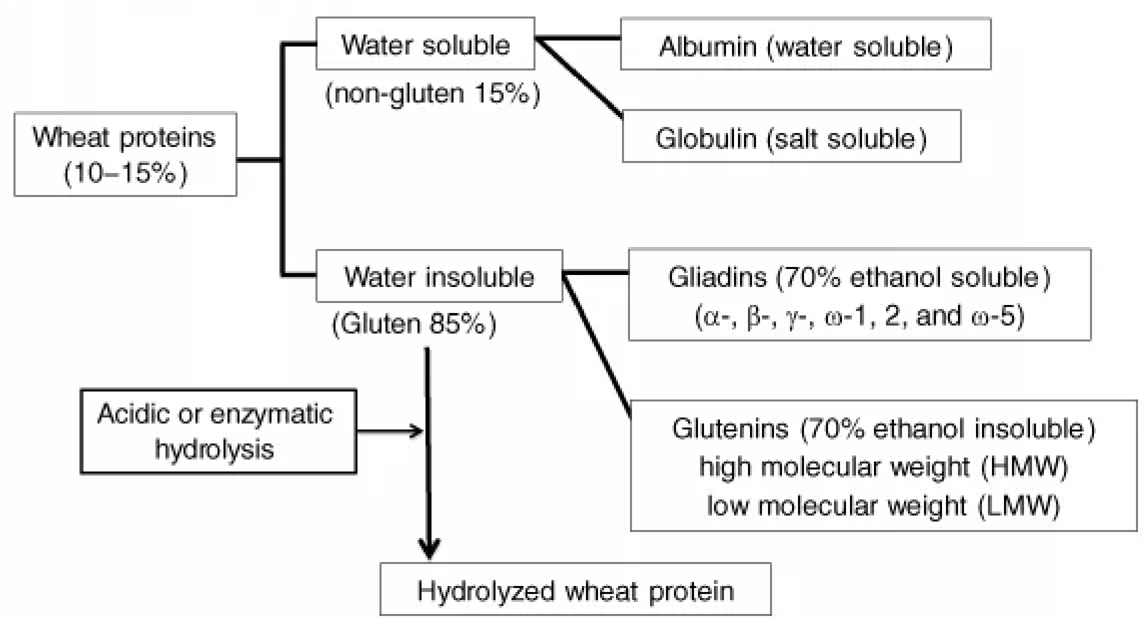
Wheat proteins



Provides energy
Starch, protein

‘Outer shell’
Fiber, B vitamins, trace minerals

Nourishment for the seed
Antioxidants, Vitamin E, B vitamins

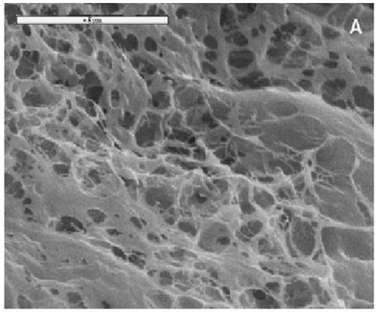


PROTEINS

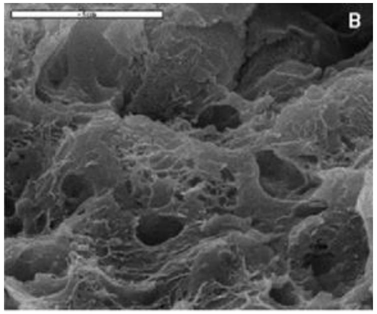
Wheat proteins

GLUTEN

- ❖ Characterized as being elastic, cohesive, and extensive
- ❖ Primarily responsible for structure (intermolecular bonds) associated with breads:
  - Holds leavening gases
  - Expansive - stretches
  - Flexible



(A) standard wheat



(B) gluten-free rice

PROTEINS

Wheat proteins

GLUTEN

- ❖ Characterized as being elastic, cohesive, and extensive
- ❖ Primarily responsible for structure (intermolecular bonds) associated with breads:
  - Holds leavening gases
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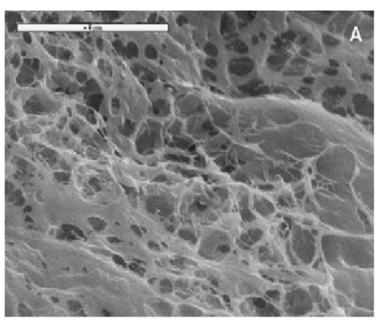
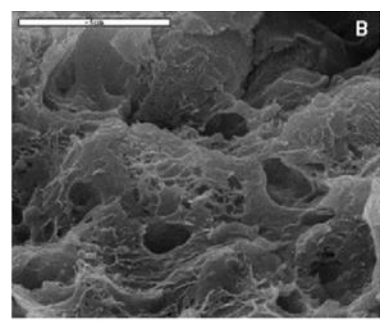
  - Flexible



(A) standard wheat



(B) gluten-free rice

PROTEINS

Protein Quality

The quality of a protein is determined by **its essential amino acid composition and the digestibility and bioavailability of its amino acids**, according to the Food and Agriculture Association and the World Health Organization (FAO/WHO). Several metrics have been developed in an effort to condense these qualitative terms into easy-to-understand scores:

Protein Efficiency Ratio (PER)
Biological Value (BV)
Net Protein Utilization (NPU)
Protein Digestibility Corrected Amino Acid Score (PDCAAS)

As a reference, the list below outlines how the four most popular protein sources fare in each of these metrics:
Whey: PER (3.2), BV (104), NPU (92), PDCAAS (1.00)
Casein: PER (2.5), BV (77), NPU (76), PDCAAS (1.00)
Soy: PER (2.2), BV (74), NPU (61), PDCAAS (1.00)
Egg: PER (3.9), BV (100), NPU (94), PDCAAS (1.00)

PROTEINS

Protein Ranking Scales

Protein Efficiency Ratio determines protein quality by measuring animal growth. In this rating, rats are fed a test protein and are measured for weight gain vs. every gram of consumed protein. The computed value is then compared to a standard value of 2.7, the value corresponding to casein protein's effect on growth. Any value higher than 2.7 indicates an excellent protein source. However, this measure of growth in rats does not strongly correlate to human growth. As other methods (below) were developed, this ratio has become increasingly outdated.

PROTEINS

Protein Ranking Scales

Biological Value determines protein quality by measuring how efficiently the human body uses dietary protein. Specifically, BV measures the nitrogen (largely obtained from dietary protein) that is retained in the body and theoretically used in tissue and muscle formation, and divides it by total amount of nitrogen absorbed from dietary protein. Since BV is a function of how much protein is absorbed and how much ends up being utilized, the theoretical top score is 100.

Net Protein Utilization aims to determine the percentage of amino acids consumed that are eventually converted into proteins and utilized by the body. To maximize NPU values, dietary protein sources must both be easy to digest and provide an effective ratio of essential amino acids. NPU values are usually measured indirectly using protein intake vs. nitrogen excretion.

PROTEINS

Protein Ranking Scales

Protein Digestibility Corrected Amino Acid Score (PDCAAS) measures protein quality based on human essential amino acid requirements and our ability to digest it. The test protein is compared to a standard amino acid profile and is given a score from 0-1, with a score of 1.0 indicating maximum amino acid digestibility.

Whey, casein, and soy receive 1.0 scores
Meat and soybeans 0.9
Vegetables and other legumes 0.7
Whole wheat and peanuts 0.25-0.55

PDCAAS is currently considered the most reliable score of protein quality for human nutrition.