IIT KHARAGPUR | NPTEL ONLINE CERTIFICATION COURSES

NPTEL

Dairy and Food Process and Products Technology

PROF. TRIDIB KUMAR GOSWAMI
AGRICULTURAL AND FOOD ENGINEERING DEPARTMENT
IIT KHARAGPUR

Lecture 26

Folding Protein – What is it?

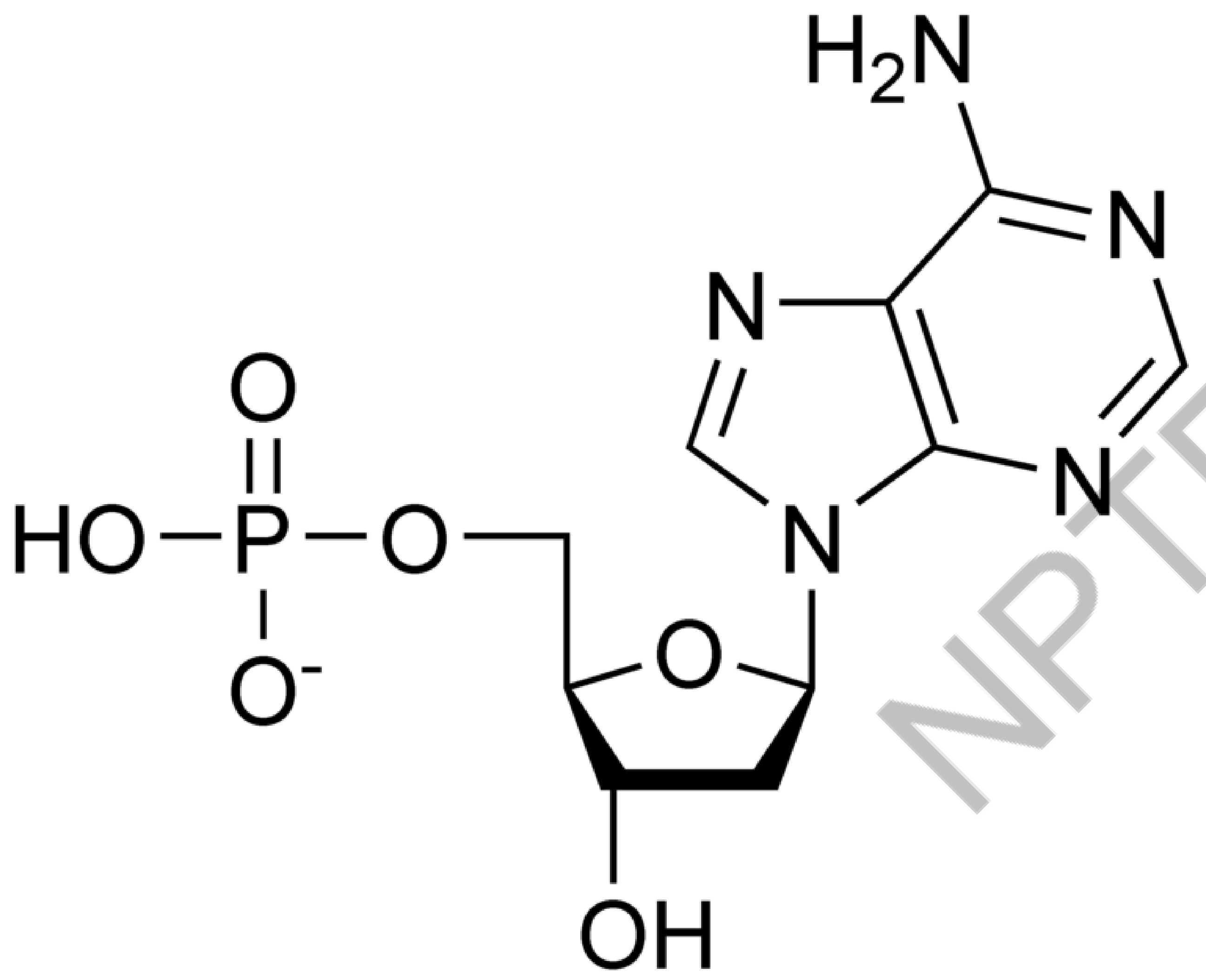
Protein folding is the **physical process** by which a **protein** chain acquires its **native 3-dimensional** structure, a **conformation** that is usually **biologically functional**, in an **expeditious** and **reproducible** manner.

Physical process: **Physical changes** are changes affecting the form of a **chemical substance**, but not its **chemical composition**. **Physical changes** are used to separate **mixures** into their component **compounds**, but can not usually be used to separate compounds into **chemical elements** or simpler compounds

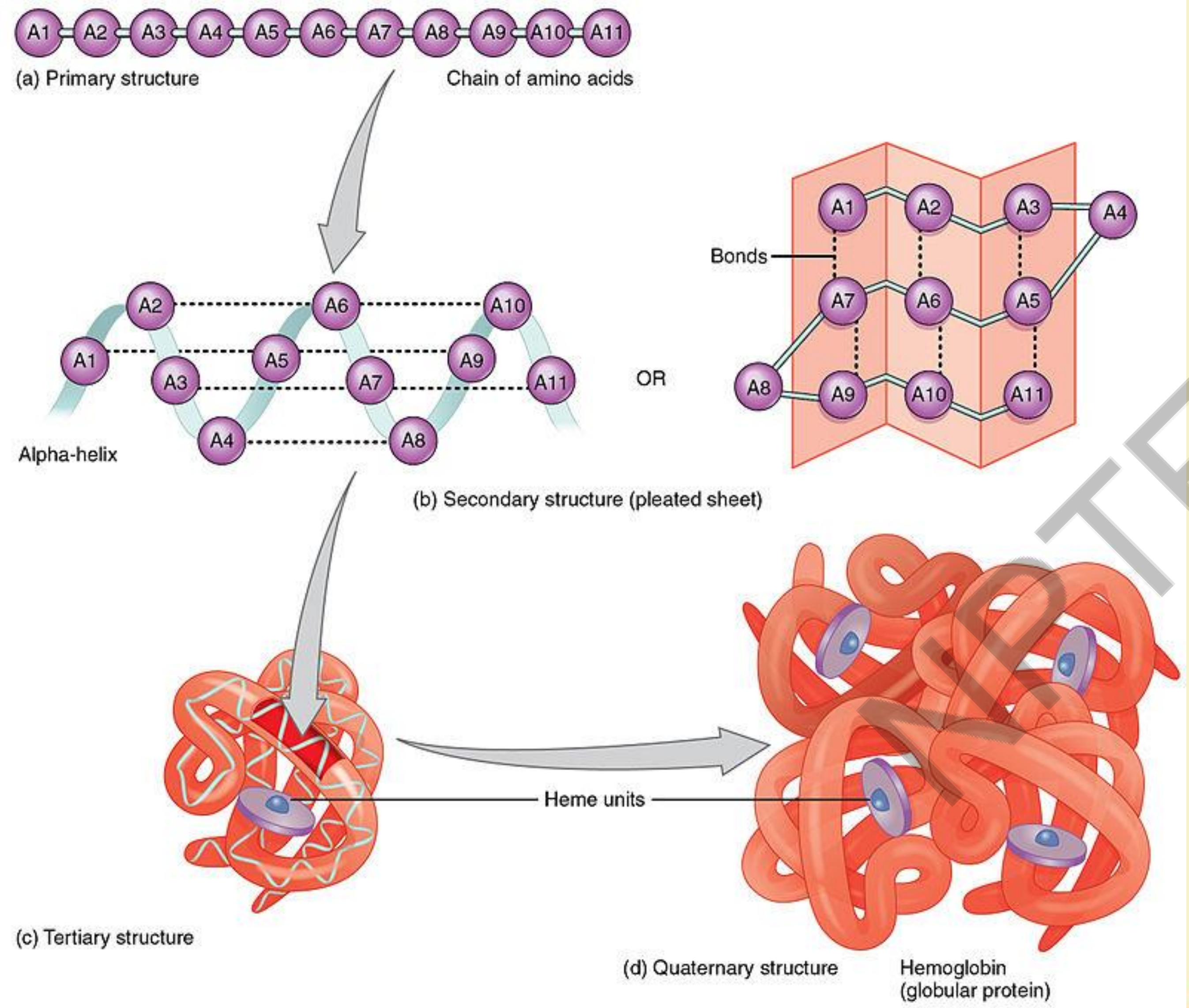
Native state of a **protein** or **nucleic acid** is its properly folded and / or assembled form, which is operative and functional. The native state of a **biomolecule** may possess all four levels of **biomolecular structure**, with the secondary through quaternary structure being formed from weak interactions along the covalently-bonded backbone. This is in contrast to the **denatured** state, in which these **weak interactions** are **disrupted**, leading to the **loss** of these forms of structure and retaining only the biomolecule's primary structure.

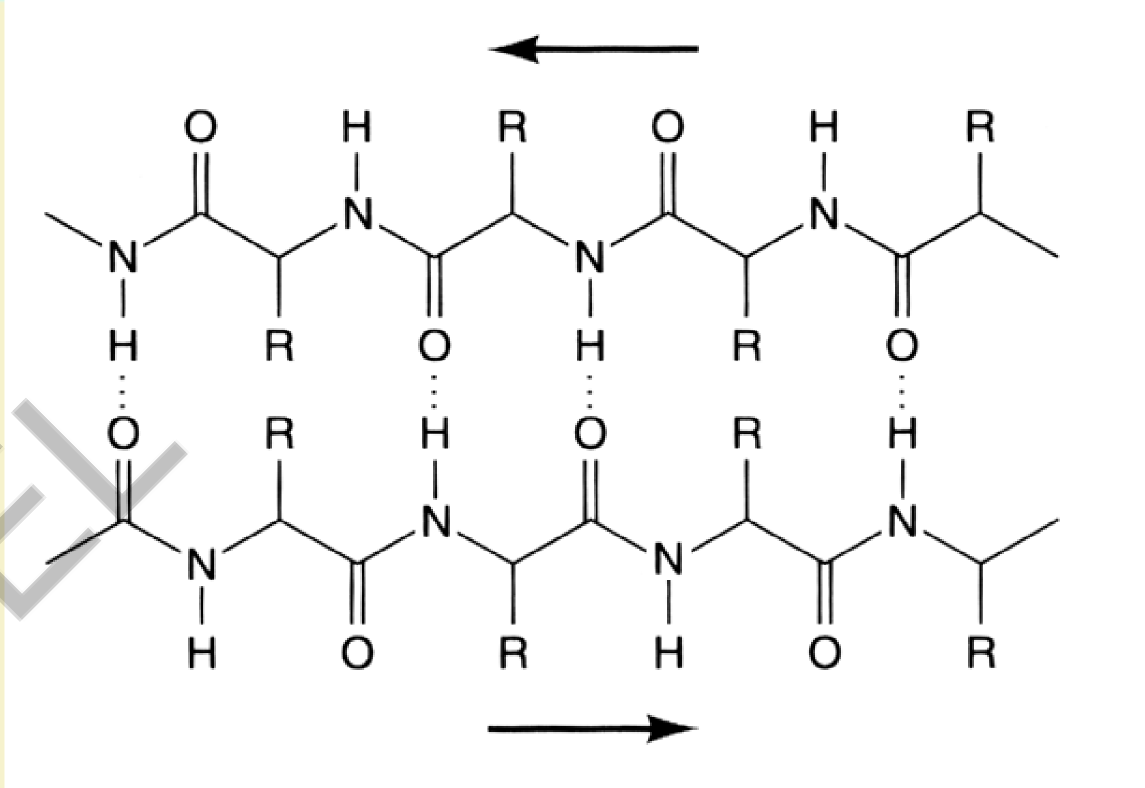
Nucleic acids are **biopolymers**, or small **biomolecules**, essential to all known forms of **life**. They are composed of **nucleotides**, which are **monomers** made of three components: a 5-carbon sugar, a **phosphate** group and a **nitrogenous base**. If the **sugar** is a compound **ribose**, the **polymer** is **RNA** (**ribonucleic acid**); if the sugar is derived from ribose as **deoxyribose**, the **polymer** is **DNA** (**deoxyribonucleic acid**)

Nucleotides are **organic molecules** that serve as the **monomer** units for forming the **nucleic acid polymers** as **deoxyribonucleic asid (DNA)** and **ribonucleic acid (RNA)**, both of which are essential **biomolecules** in all **forms of life** on **Earth**. Nucleotides are the **building blocks** of nucleic acids; they are composed of **three subunit** molecules: a **nitrogenous base**, a **5-carbon sugar** (**ribose or deoxyribose**), and **at least one phosphate group**. They are also known as ***phosphate* nucleotides**.



The **Nucleotide** contains the five-carbon sugar **deoxyribose** (at center), a **nitrogenous base** called adenine (upper right), and one **phosphate group** (left). The deoxyribose sugar joined only to the nitrogenous base forms a **Deoxyribonucleoside** called **deoxyadenosine**, whereas the whole structure along with the phosphate group is a **nucleotide,** a constituent of DNA with the name **deoxyadenosine monophosphate**.





An anti-parallel beta pleated sheet displaying hydrogen bonding within the backbone

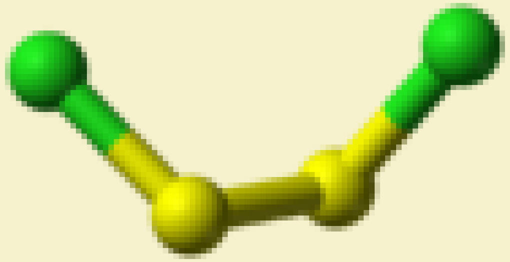
Source: Wikipedia

Structure:-

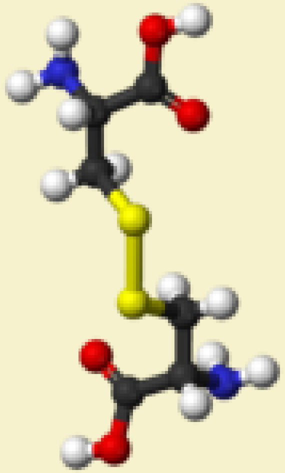
Primary Structure: Amino Acid Sequence, which is a polyamide.

Secondary Structure: Regularly repeating local structures stabilized by hydrogen bonds. The most common examples are the α-helix, β-sheet (**β-pleated sheet**) and turns. Because secondary structures are local, many regions of different secondary structure can be present in the same protein molecule.

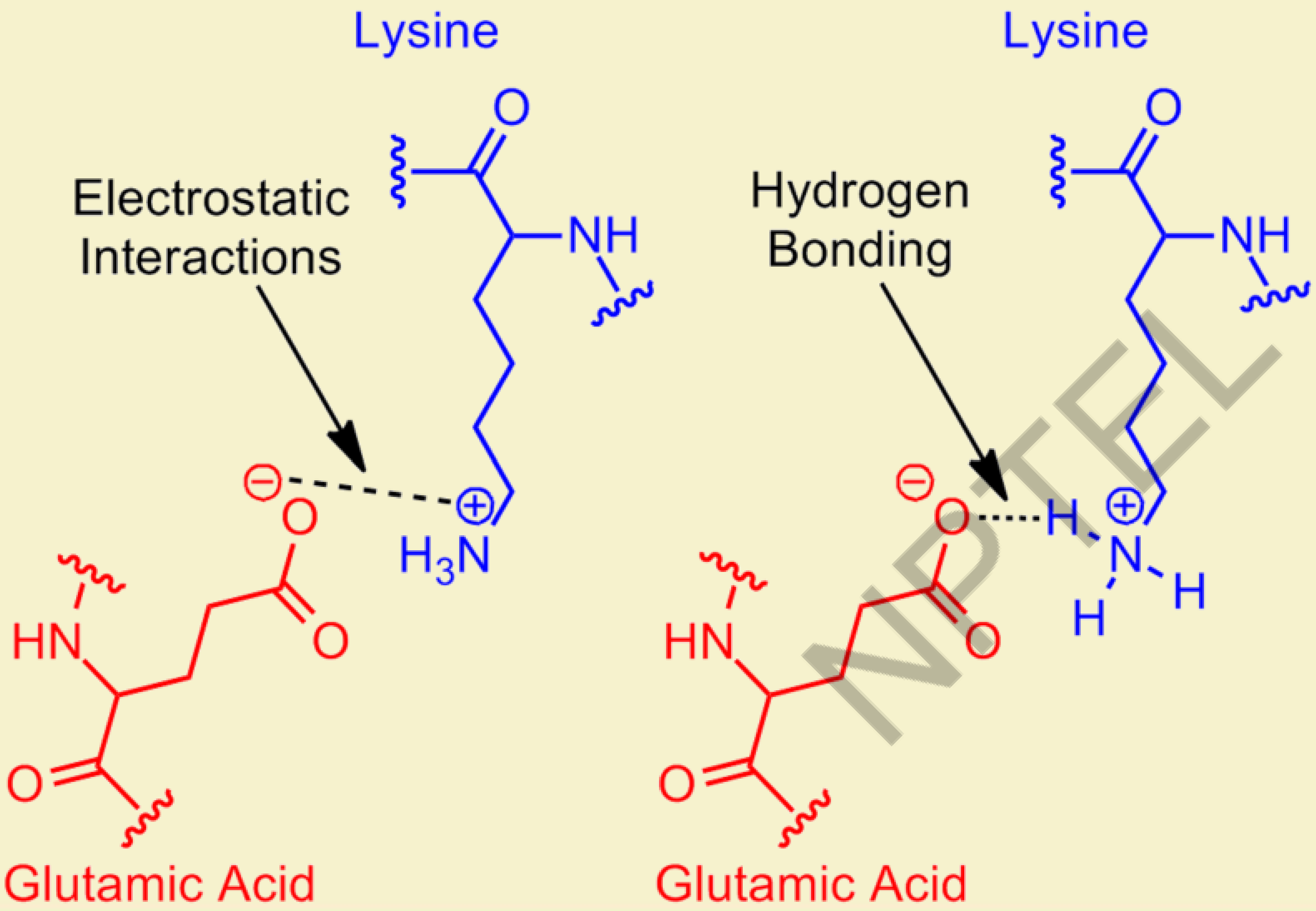
Tertiary structure:- the overall shape of a single protein molecule; the spatial relationship of the secondary structures to one another. Tertiary structure is generally stabilized by nonlocal interactions, most commonly the formation of a hydrophobic core, but also through salt bridges, hydrogen bonds, disulfide bonds, and even post translational modifications. The term "tertiary structure" is often used as synonymous with the term *fold*. The tertiary structure is what controls the basic function of the protein.



Disulfide bonds



Post-translational modification (PTM) refers to the covalent and generally enzymatic modification of proteins following protein biosynthesis.



Example of salt bridge between amino acids: glutamic acid and lysine demonstrating electrostatic interaction and hydrogen bonding

Quaternary structure: Quaternary structure:- the structure formed by several protein molecules (polypeptide chains), usually called protein subunits in this context, which function as a single protein complex.

In structural biology, a **protein subunit** is a single protein molecule that assembles (or "*coassembles*") with other protein molecules to form a protein complex.

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Peptides are short chains of **amino acid** monomers linked by **peptide** (amide) bonds. The covalent chemical bonds are formed when the carboxyl group of one **amino acid** reacts with the **amino** group of another.

The first **difference** is that proteins are made of only 20 different **amino acids**, while **peptides** can be made of this 20 "protein" **amino acids** and of other naturally available **amino acids**. Another basic **difference** is the size of the chain, that is, the number of **amino acids** that a protein or a **peptide** contains.

Peptide just refers to two or more amino acids liking together. A **dipeptide** consists of two amino acids linking together. A **polypeptide** is more that two amino acids linking together.

Long strings, or 'chains' of thousands of the 20 **amino acids** that form proteins linking together form the proteins that exist in the body. These chains generally consist of repeated sequences of specific **amino acids**. ... It is the SIDE GROUPS which make **each amino acid different** from the others.

A **protein** is a chain of **amino acids** connected together. You can think of this like a beaded necklace. The beads (**amino acids**) are connected together by a string (bond), which forms a long chain (**protein**). Therefore, a **protein** is "intact" or "whole."

Amino acids are the basic building blocks of enzymes, hormones, proteins, and body tissues. A **peptide** is a compound consisting of 2 or more **amino acids**. Polypeptides and proteins are chains of 10 or more **amino acids**, but **peptides** consisting of more than 50 **amino acids** are classified as proteins.

Nucleic Acids (RNA and DNA) are made up of a series of nucleotides. The center of an **amino acid** is the carbon bonded to four different groups. The fourth group, R, is different for each **amino acid**. A nucleotide is composed of a five-carbon sugar, a nitrogenous base and a phosphate group.

All **amino acids** have the alpha carbon bonded to a hydrogen atom, carboxyl group, and **amino** group. The "R" group varies among **amino** **acids** and determines the differences **between** these protein monomers. ... The genetic code is the sequence of nucleotide bases in nucleic **acids** (DNA and RNA) that code for **amino acids**

20 amino acids are there in our body's proteins, nine are essential to your diet because our cells cannot manufacture them: **histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine**.

Twenty-**two amino acids** are naturally incorporated into polypeptides and are called proteinogenic or natural amino acids. Of these, **20** are encoded by the universal genetic code. The remaining 2, selenocysteine and pyrrolysine, are incorporated into proteins by unique synthetic mechanisms.

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Lecture 28

20 amino acids are there in our body's proteins, nine are essential to your diet because our cells cannot manufacture them: **histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine**.

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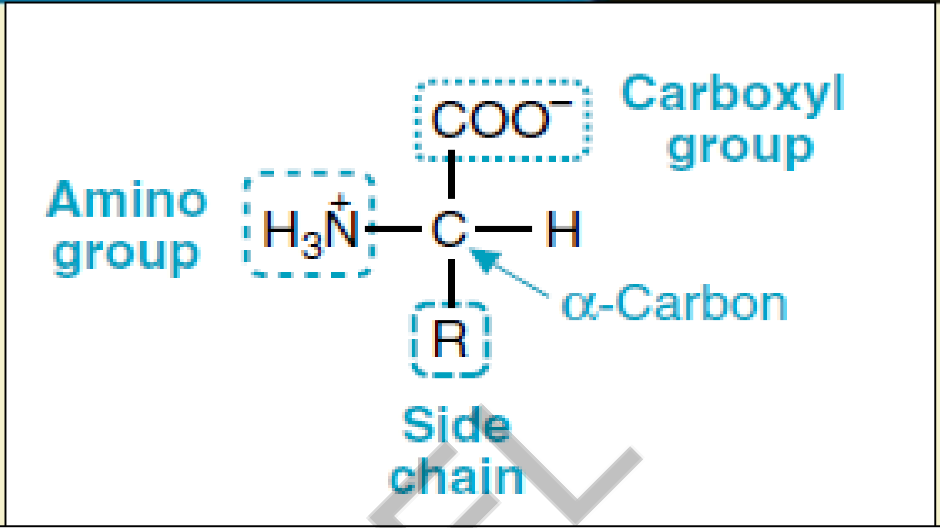
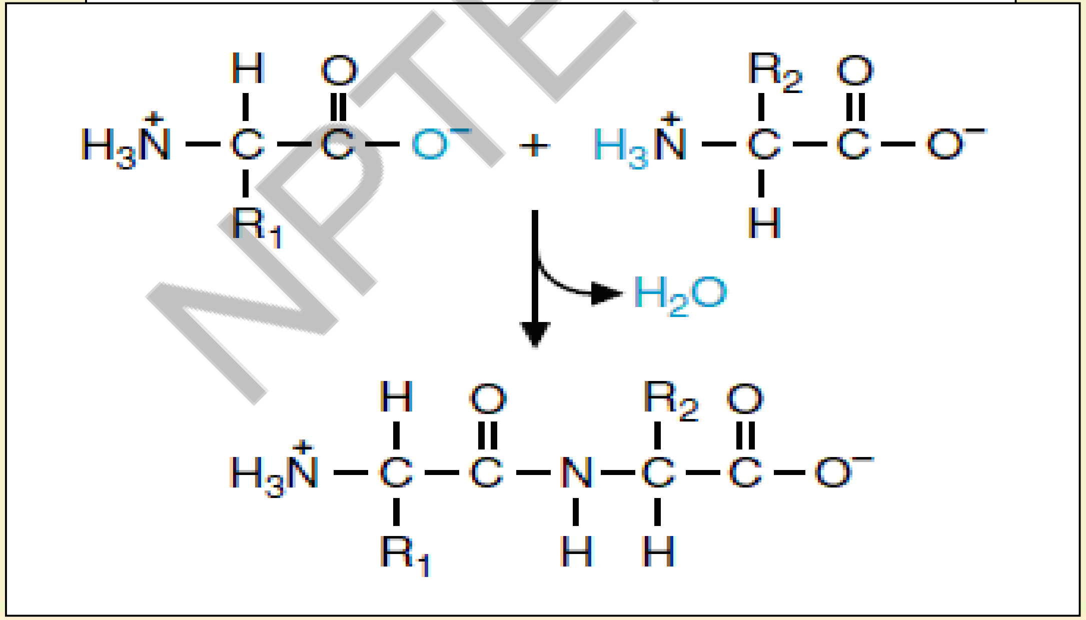
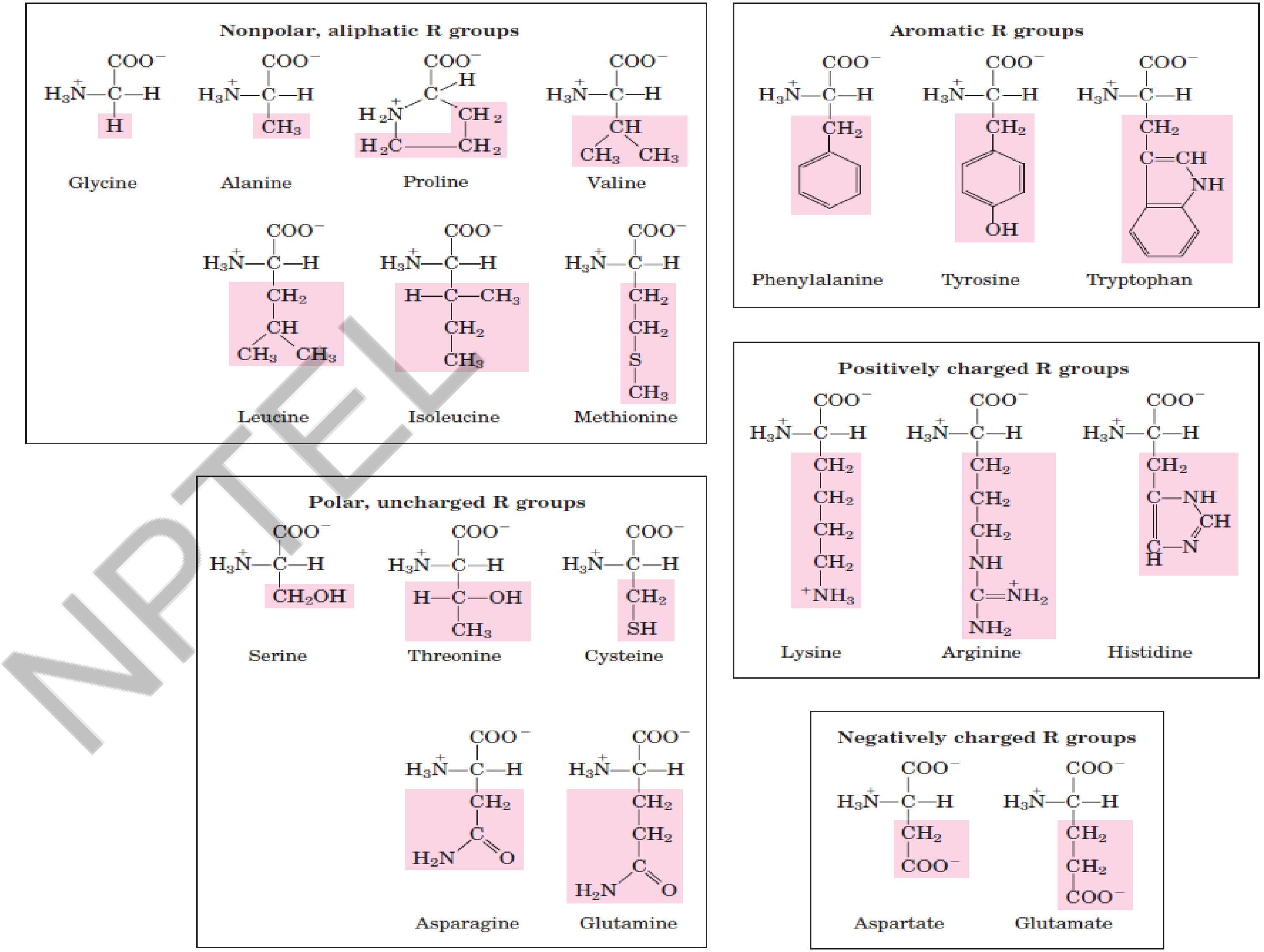

Carboxyl group
Amino group
α-Carbon
Side chain


H_2O

- Amino acids with nonpolar side chains.
- Aromatic R Groups.
- Amino acids with uncharged polar side chains.
- Positively Charged (Basic) R Groups.
- Amino acids with acidic side chains.



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Proteins of milk:-

– C, H_2, O_2, N_2, S, P (some times) – characterized chiefly by N_2.

– ***Proteins are made up of amino acids with specific properties that are determined by the side chains of amino acids in the polypeptide chain.***

– ***Conformation of the protein depends on the hydrogen bonds, hydrophobic interactions, and salt bridges formed between peptide chains.***

– ***Regular arrangements include β sheets and α helices.***

–***Temperature, ionic strength, and pH affect protein conformation.***

–*Normal milk – 2.8 to 4% (30 to 35 g / kg) – remains constant – influenced by lactation stage of the animal.*

– **Two types – Casein (80%) and Lactalbumin or whey or serum protein (18%) – derived genetically.**

- **Lactoglobulin – 0.05 to 0.07%.**

-- Casein --

- **four main types of casein have genetic variation**
- **α_{s1} casein, α_{s2} casein, β casein, and κ casein.**
- **These are phosphorylated and hydrophobic and associate**

With themselves and each other.

- They represent 38%, 10%, 36%, and 13% of whole casein.
- Overall 21 variants of casein have been identified that occur by genetic mutation.
- Caseins have distinct disordered molecular structures that lack disulfide bridges.
- Caseins are very heat stable and withstand temperature about 150 ^{o}C before getting dissociated.
- Hydrophobicity is due to

- high ratios of apolar amino acids
- apolarity is even in valine, leucine, isoleucine, phenylalanine and proline.
- Apolarity ranges between 35 to 45%.

Hydrophobicity is counteracted by

- ✓ High phosphate content
- ✓ Low concentration of sulphur containing amino acids such as methionine and cysteine that allow the caseins to be reasonably water soluble.

Caseins susceptibility to proteolysis is due to the

- lack of secondary and tertiary structures of (α_{s1} casein, casein, β casein).

α-Caseins

α_{S1}-casein	α_{S2} - casein
□ 199 amino acids and is app. 23.6 kDa. □ highest charge of all the casein molecules. □ Consists of at least eight phosphoserine units. □ It has 17 proline residues that ultimately disrupt the formation of secondary structures, such as α - helices and β - sheets.	□ 207 amino acids and is app 25.4 kDa. □ least hydrophobic casein molecule. □ Several genetic variants, contain between 10 and 13 phosphoserine units. □ Contains two cysteine residues. □ It exists as a dimer in milk.

β-Casein

- β-casein consists of 209 amino acids and is approximately 24 kDa.
- It is the most hydrophobic casein molecule.
- There are six known genetic variants that contain between zero and five phosphoserine units.
- Similar to α_{S1}-casein, β-casein has few secondary structures due to the presence of 35 proline residues.

γ-Casein

- γ-casein is derived by hydrolysis of β-casein by the enzyme plasmin.
- Three variants have been identified near the C - terminal end of the β-casein molecule.

K - Casein

- κ - casein consists of 169 amino acids and is approximately 19 kDa, and it contains both glycosylated and phosphorylated residues.
- It can exist as a dimer up to a decamer with the subunits held together by disulfide linkages.
- Unlike the other caseins, it is not sensitive to calcium and surrounds the micelles, keeping them intact.
- It usually contains one phosphoserine unit;
- however, genetic variants containing two or three phosphoserine units have been identified.
- Further, nine variants have been identified that demonstrate different degrees of glycosylation.

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Lecture 30

Casein Micelle

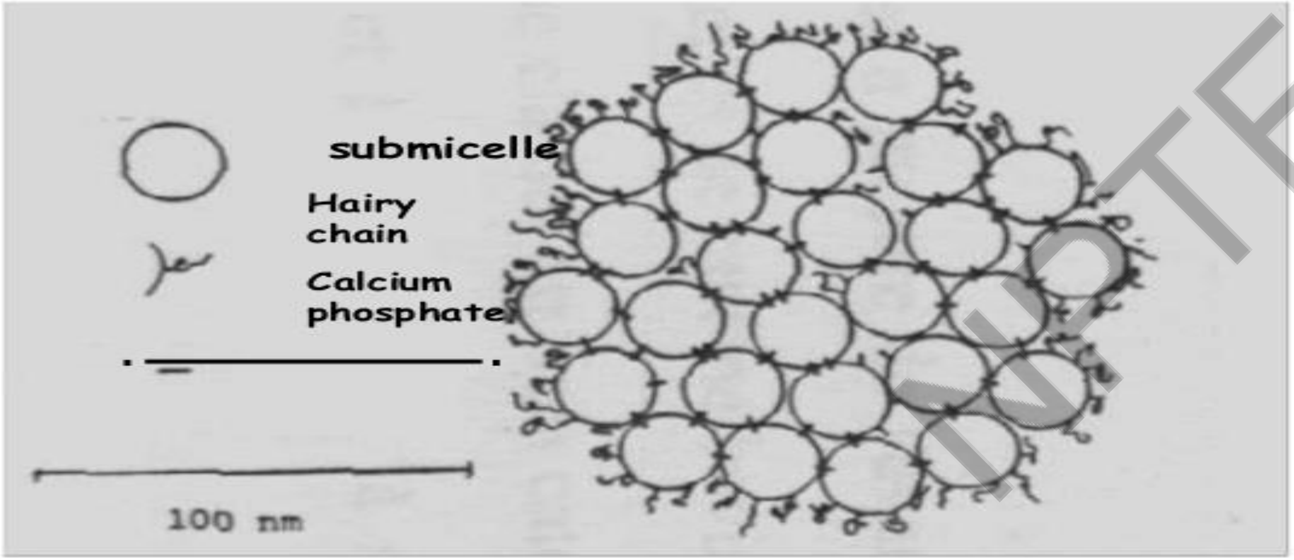
Molecules of Casein + Minerals (Calcium phosphate)

Composition:

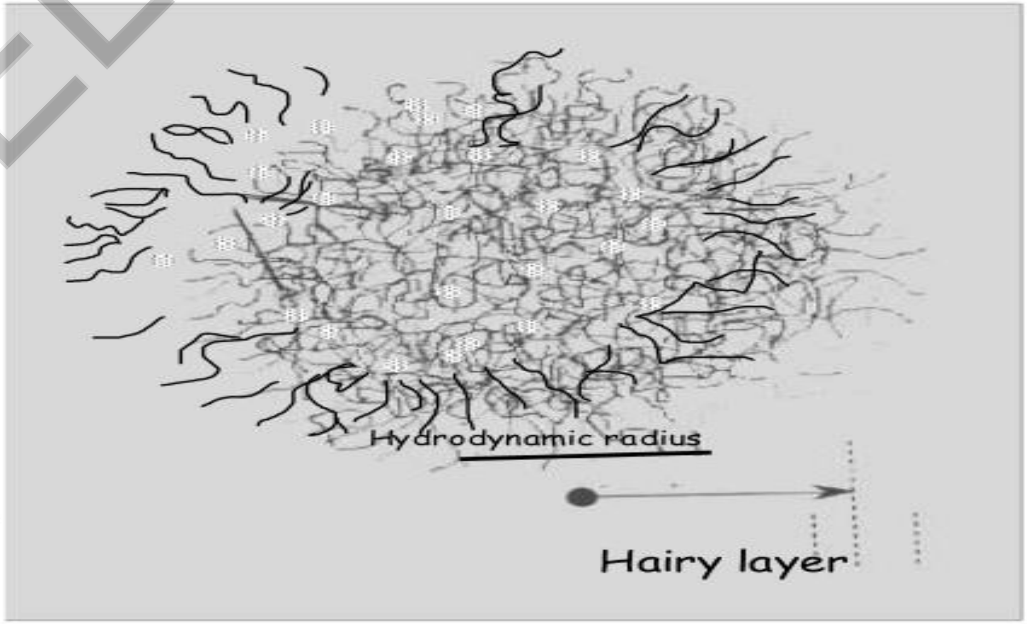
- **93% caseins : 4 phosphoproteins**
  - α_{s1} - CN : 36%
  - α_{s2} - CN : 10%
  - β – CN : 34%
  - κ - CN : 12%
- **7% : colloidal mineral complex containing phosphate, calcium, magnasium and citrate**

Main Models of Casein Micelles

- Structure with sub-units
- Open structure



Walstra (1990)



Casein Micelles

- The polymers are built up of hundreds and thousands of individual casein molecules and form a colloidal solution
- These molecular complexes are known as casein micelles
- Micelles may be as large as 0.4 µm., can only be seen under electron microscope.
- A medium sized micelle consists of about 400 to 500 submicelles which are bound together.
- α and β caseins are mainly concentrated in the middle of the sub-micelles, while κ casein predominates in the surface.

- The hydrophilic protruding chain of the κ casein protrudes from the surface of the sub-micelles forming a hairy layer (5 – 10 nm).
- κ casein deficient sub-micelles are mainly located in the centre of the micelle, whereas κ casein rich sub micelles predominate on the surface giving the whole micelle a hairy surface layer.
- Hairy layer of κ casein's protruding chain is partially responsible for the micelle's stability through a major contribution to the negative charge of the micelles.

- **Calcium phosphate and hydrophobic interactions between sub-micelles are responsible for the integrity of the casein micelles.**

- **Adding an excess calcium and phosphate results in aggregation of sub-micelles into larger units of micelles.**

- **Reason for this aggregation is presumably due to the deposition of Ca-phosphate in the sub-micelle which lowers their electric charge and makes them more compact**

Caseins : Phospoproteins

- β-casein: $-Glu-\overset{P}{\underset{15}{Ser}}-Leu-\overset{P}{\underset{17}{Ser}}-\overset{P}{\underset{18}{Ser}}-\overset{P}{\underset{19}{Ser}}-Glu- \ldots -Gln-\overset{P}{\underset{35}{Ser}}-Glu-$
- α_{s1}-casein: $-Gly-\overset{P}{\underset{46}{Ser}}-Glu-\overset{P}{\underset{48}{Ser}}-Thr- \ldots -Glu-\overset{P}{\underset{64}{Ser}}-Ile-\overset{P}{\underset{66}{Ser}}-\overset{P}{\underset{67}{Ser}}-\overset{P}{\underset{68}{Ser}}-Glu- \ldots -Asn-\overset{P}{\underset{114}{Ser}}-Ala-$
- α_{s2}-casein:
  - $-Val-\overset{P}{\underset{8}{Ser}}-\overset{P}{\underset{9}{Ser}}-\overset{P}{\underset{10}{Ser}}-Glu-Glu- \ldots -Gly-\overset{P}{\underset{56}{Ser}}-\overset{P}{\underset{57}{Ser}}-\overset{P}{\underset{58}{Ser}}-Glu-Glu-\overset{P}{\underset{61}{Ser}}-Ala-$
  - $-Ile-\overset{P}{\underset{16}{Ser}}-Gln-Glu- \ldots -Leu-\overset{P}{\underset{129}{Ser}}-Thr-\overset{P}{\underset{131}{Ser}}- \ldots -Glu-\overset{P}{\underset{143}{Ser}}-Thr$
- κ-casein: $Glu-Ala-\overset{P}{\underset{149}{Ser}}-Pro-Glu-$

Casein Precipitation:-

One characteristic property of casein is its ability to precipitate at pH 4.6

Due to the complex nature of the casein molecules and that of the micelles formed from them, precipitation can be caused by a number of factors or agents.

Casein

- It is a snow-white odourless and tasteless granular substance, contributes to the whiteness of milk.

- **Found in combination with calcium as**
  - **“Calcium Caseinate” dispersed state as colloidal.**
- **On boiling fresh milk**

– a thin layer of finely precipitated casein, together with other milk constituents, including fat, forms a thin layer over the surface of the milk. With added acid – curdles.

- **Used in**

– manufacture of hard, water resistant, non inflamable plastics,

– substitute for celluloid, horn, tortoise shell, a wide variety of toilet articles such as, combs, buttons, and rims for eyeglass,

- **fountain-pen barrels, electrical insulations, high-grade paper (80% of casein), glue, cold water paint**

– textile and leather industries, as spreader over foliage (horticultural work) to spread and adhere better, as casein fiber (Aralac) as in hat industry in the manufacture of fur-felt hats.

– filler or binder in certain foods, serves as the base material for a meat sauce and as a binder in sausage.

Thank You!!