Proteins and Gel-Based Proteomics
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Mod 02 Lecture Number 2

Welcome to the Proteomics course. Before we move on to the proteomics and discuss about what are all different techniques and concepts involved in proteomics, lets first start the basic concepts on proteins.

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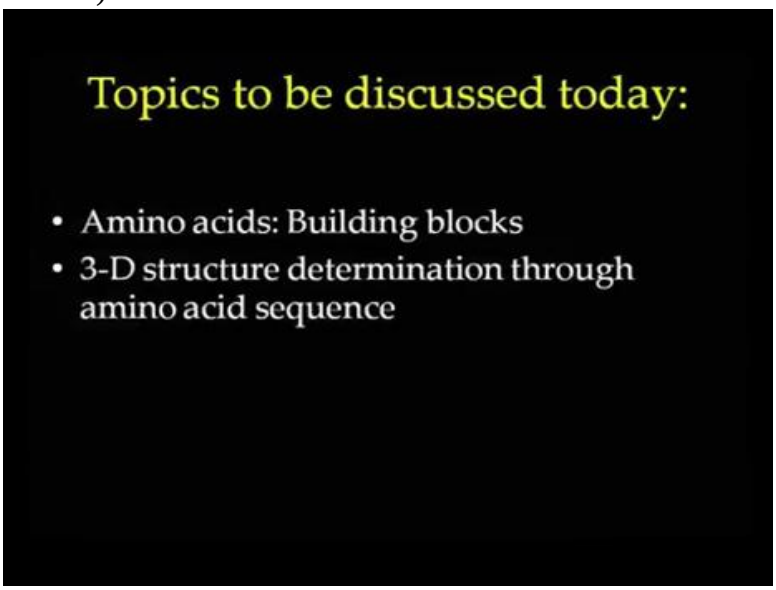

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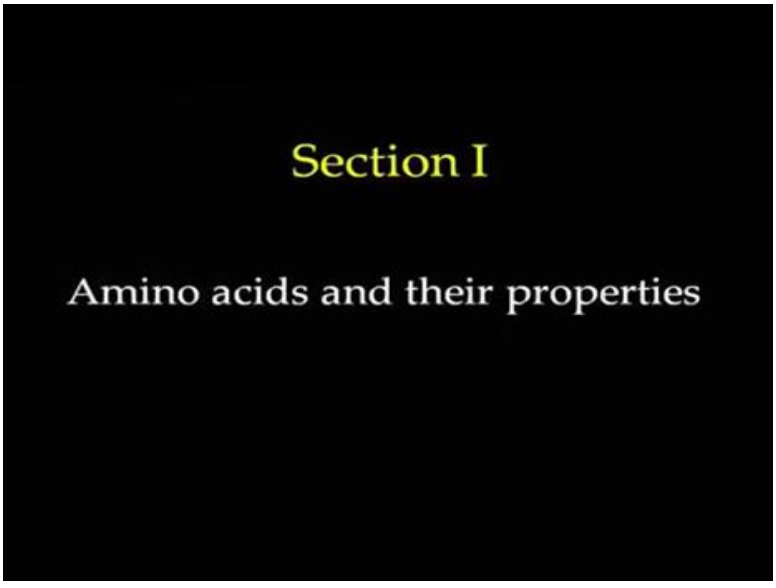

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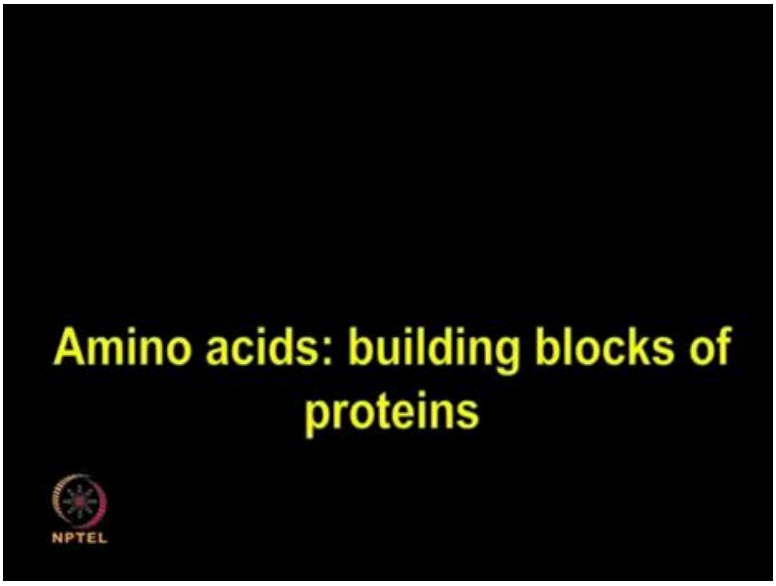

Amino acids, the building block of proteins. Amino acids constitute the basic monomeric unit of proteins which are joined by the peptide bonds. There are 20 standard amino acids which can be arranged in several ways giving rise to numerous proteins having different structural and functional properties.

The diversity and versatility of 20 amino acids enables range of protein functions. Due to the side chain which can vary in size, shape, hydrogen-bonding capacity, hydrophobic characters, charge and chemical reactivity, proteins perform much diverse function as compared to the DNA.

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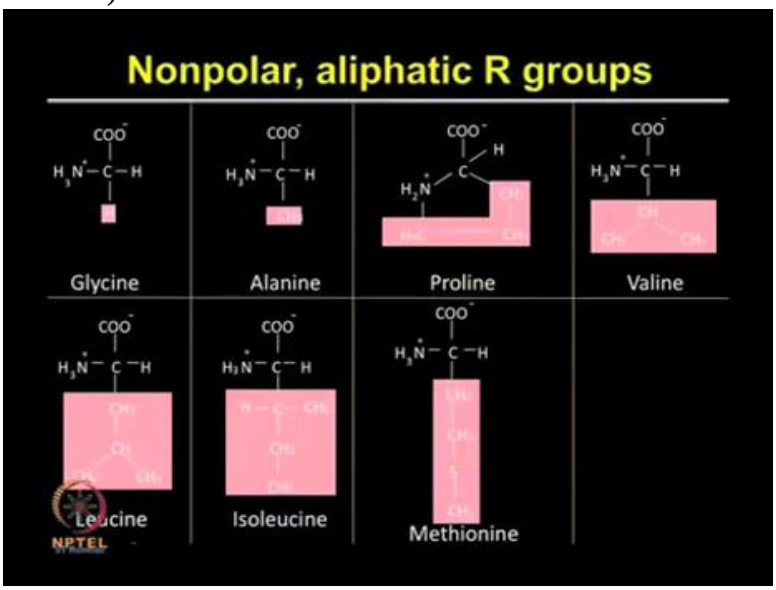

You have already studied about different amino acids in your undergraduate education. I will again try to refresh you on some of those concepts but in more nutshell. Here I have shown various amino acids which non-polar and aliphatic R-Groups starting with the Glycine if you see on the left-hand side, the topmost which is the simplest and a-chiral.

Now next is Alanine which contains a methyl group, Proline which has aliphatic side chains. Proline has very unique feature. It has no free amino group and the side chain is bonded to the N and alphacarbon of the alphacarbon atoms. The ring structure, it provides more conformational restriction and therefore Proline plays very crucial role and unique property in many functions.

Valine, it is branched chain amino acid, Leucine on the left-hand side bottom panel, that is hydrophobic amino acid with isobutyl R group. Isoleucene, it also has hydrophobic amino acid characteristics and it contains chiral side chain. It is one of the essential amino acids.

The last in the group is Metheonine which includes a Thioethane group. Again there are only 2 amino acids which contain Sulphur; and they play some very critical role.

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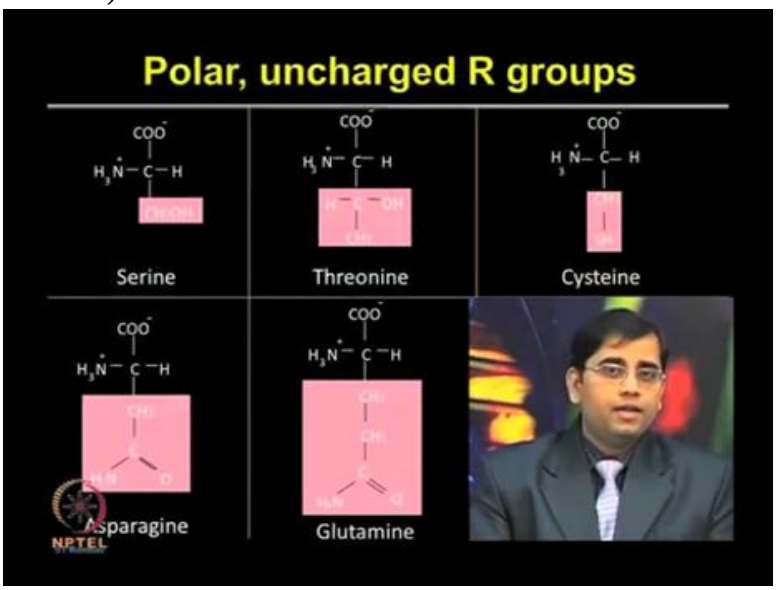

The next category is polar uncharged R groups. Let's start with the Serine which resembles in the structure like Alanine but it contains hydroxyl group. Threonine, it resembles in the structure like Valine and it contains hydroxyl group. It has an additional asymmetric center.

Cystiene, it is similar to Serine but it contains sulfhydryl or thiol group. Two Cystiene molecules form Cystine.

Let's talk about Asparagine which is shown in the left side lower panel. It contains carboxamide side chains as a functional group. Glutamine, the side chain called as amide of Glutamic acid which is formed by replacing the side chain hydroxyl group Glutamic acid with an amino functional group.

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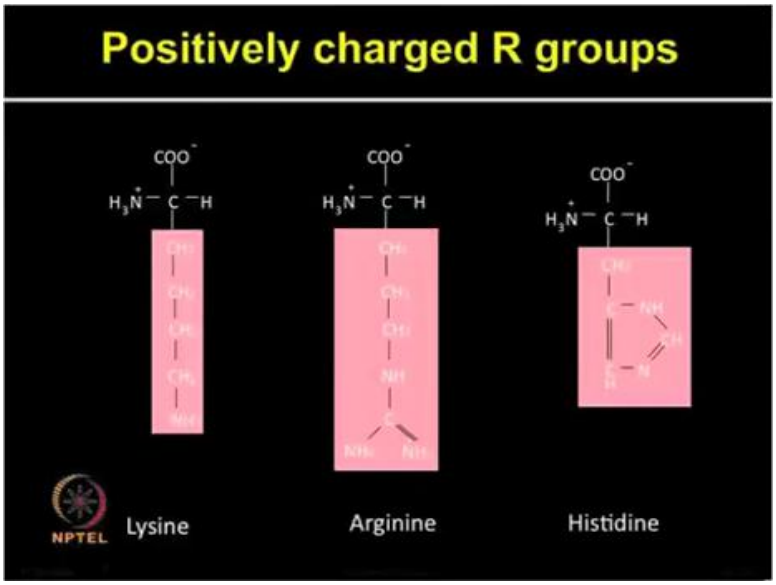

Next category is positively charged R groups. Three amino acids are here; Lysine, Arginine and Histidine. Lysine is a base. It contains capped primary amino group whereas Arginine contains one ADM group.

Histidine, it has a functional METASOL group which is aromatic ring that can be positively charged. Histidine plays very critical role in many enzymatic activities.

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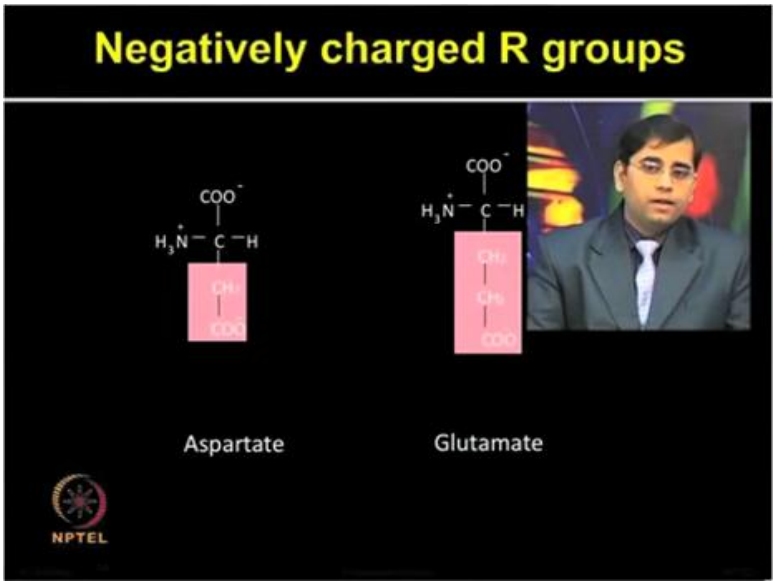

Next group is negatively charged R groups, Aspartate or Glutamate or Aspartic acid or Glutamic acid. The name Aspartate or Glutamate is because at the physiological pH, the side chains of these amino acids lack a proton present in the acid form. Therefore these amino acids are negatively charged.

Aspartate is a carboxylate anion of aspartic acid known as Aspartate, where as the carboxylate anions and salts of Glutamic acids are known as Glutamate.

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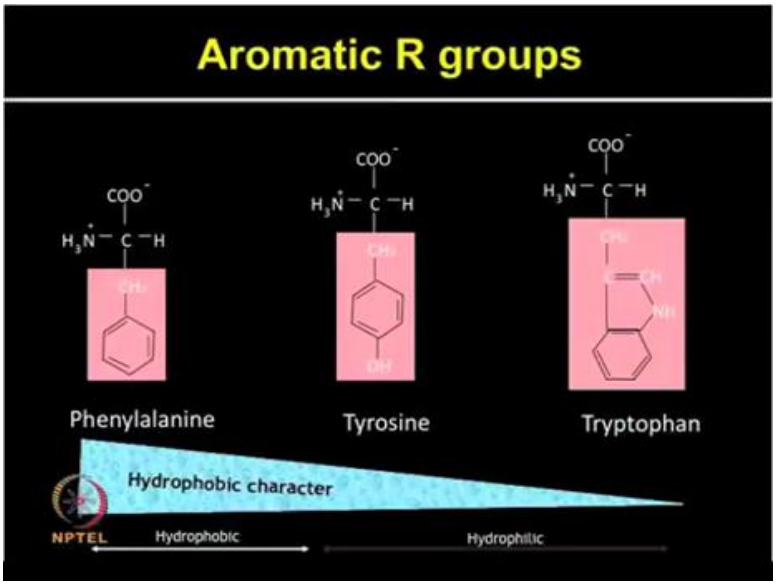

Next category is aromatic R groups. In this one, there are 3 amino acids; Phenylalanine, Tyrosine and Tryptophan. Phenylalanine contains phenyl ring, Tyrosine has one reactive hydroxyl group and Tryptophan contains indole ring, two rings which are fused.

Now if you look at the hydrophobic or hydrophilic characteristics, Phenylalanine is hydrophobic where as Tyrosine and Tryptophan, they are hydrophilic due to the side chain containing hydroxyl and NH reactive groups.

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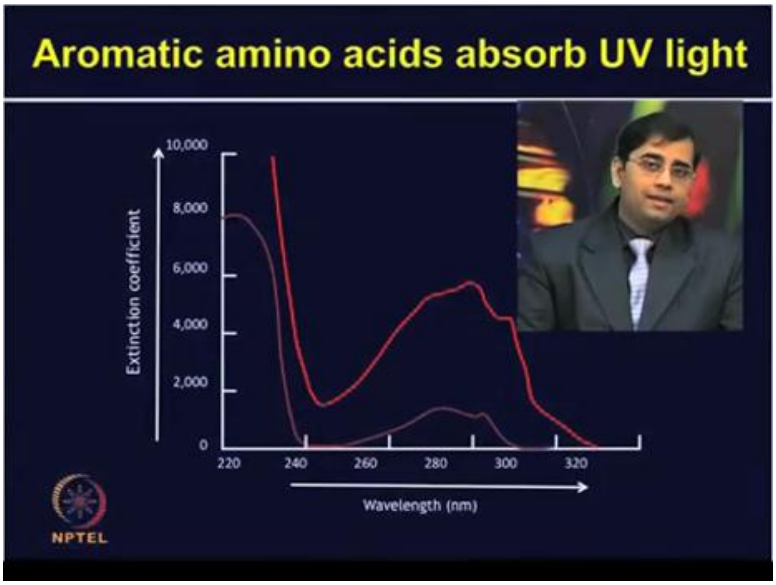

Aromatic amino acids have unique property. They can absorb UV light. All the three amino acids which we just discussed, Tryptophan, Tyrosine and Phenylalanine, they can absorb UV light.

Tryptophan absorption maximizes at 280 nanometers, Tyrosine at 276 nanometers. Phenylalanine, it absorbs light less strongly and at the shorter wavelength. The light absorption at 280 nanometers is used for the protein concentration determination.

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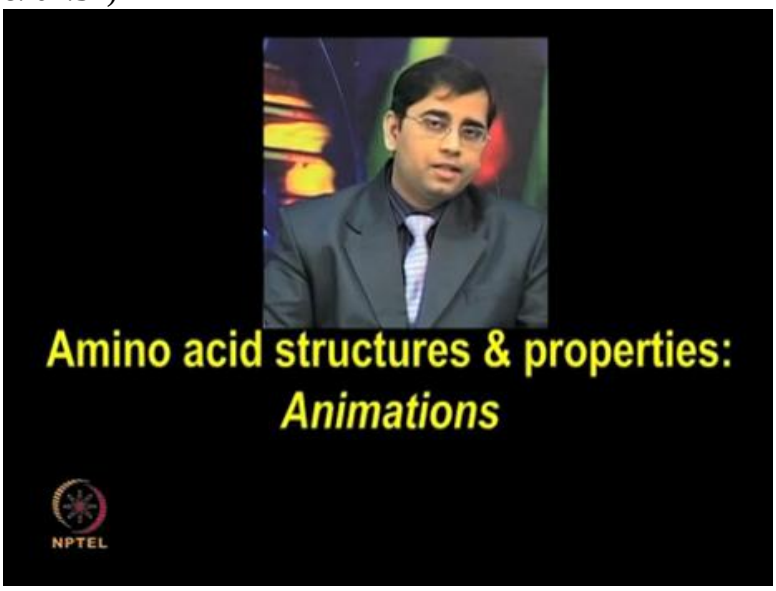

I will refresh some of the concepts discussed in the amino acid structures and properties in following animation.

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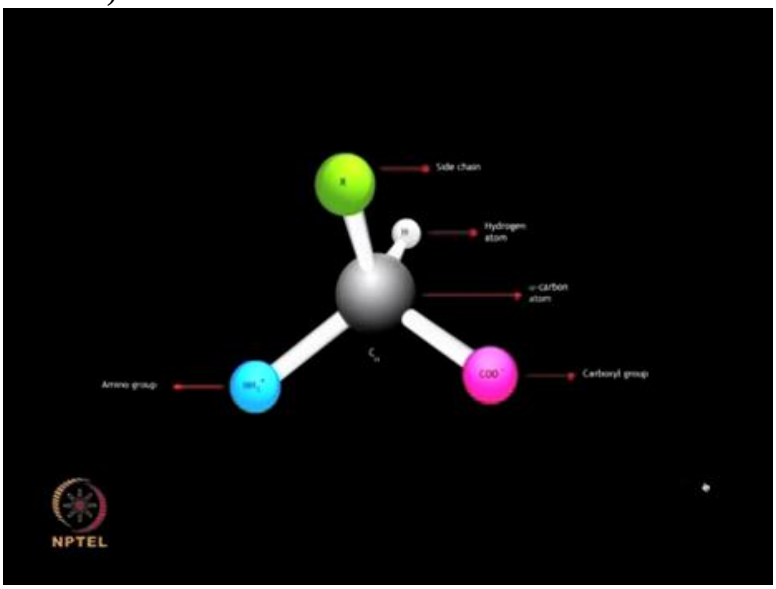

Amino acids are the building blocks or monomers that make up proteins. They consist of a central alphacarbon atom bonded covalently to an amino group, a carboxyl group, a hydrogen atom and a variable side chain called as R group. Amino acids are the basic monomeric constituents of proteins found in varying amounts depending upon the type of protein.

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They are classified based on the properties of their side chains or R groups which vary in size, structure and charge.

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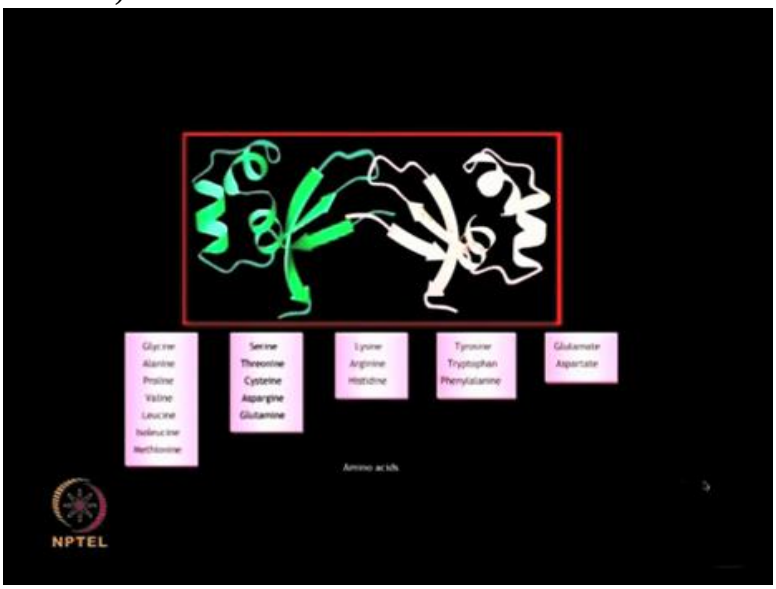

Polarity of side chain is one of the main basis for the classification.

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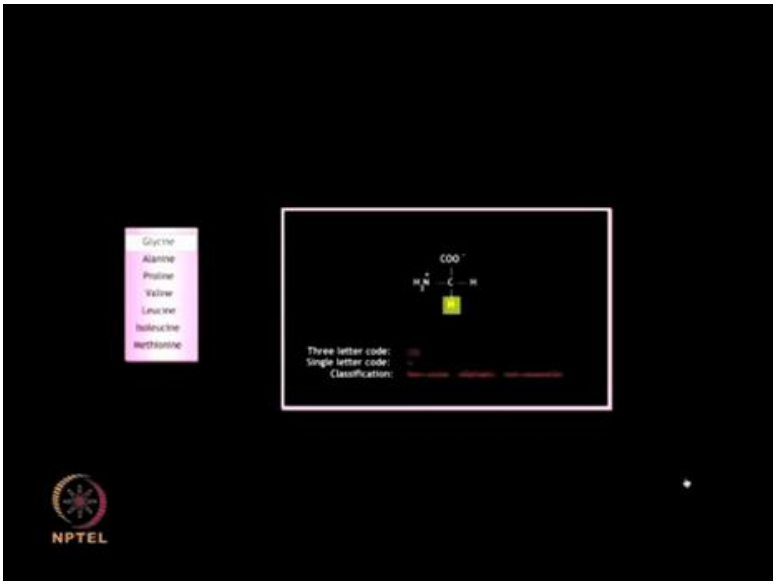

Amino acids having non-polar aliphatic side chains include Glycine,

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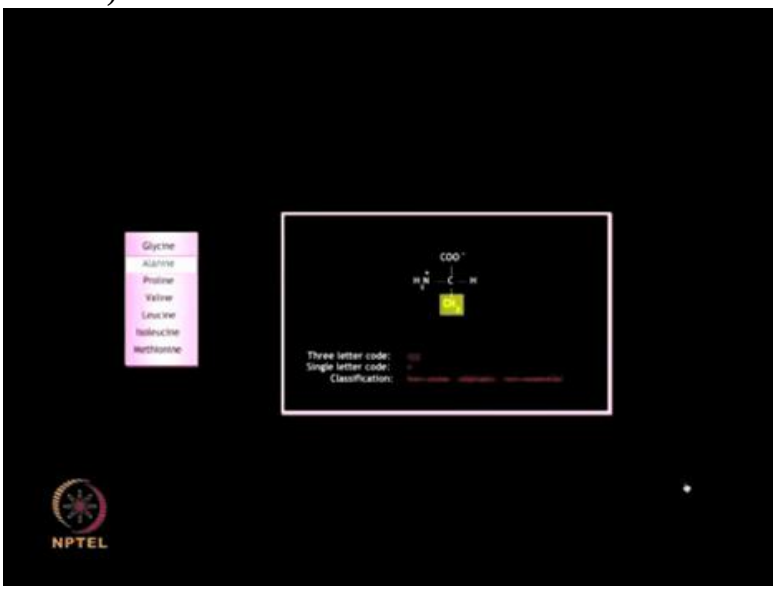

Alanine,

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Proline,

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Valine,

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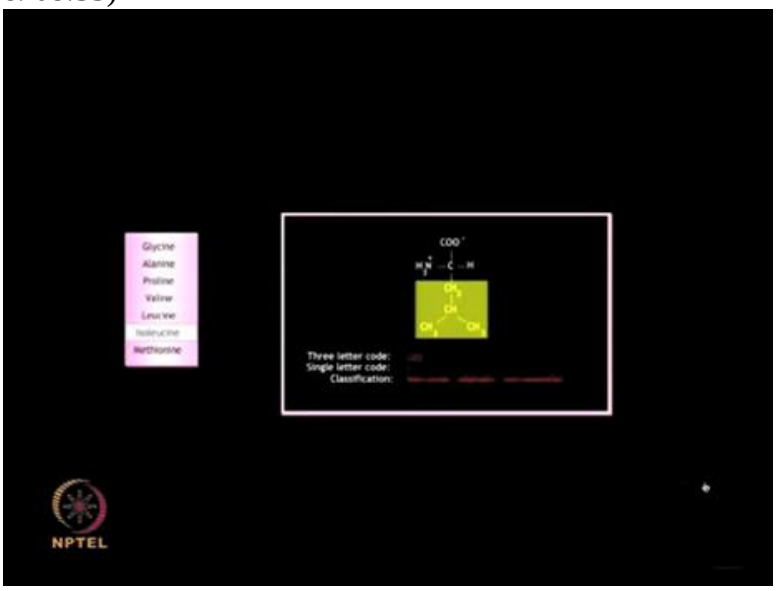

Leucene,

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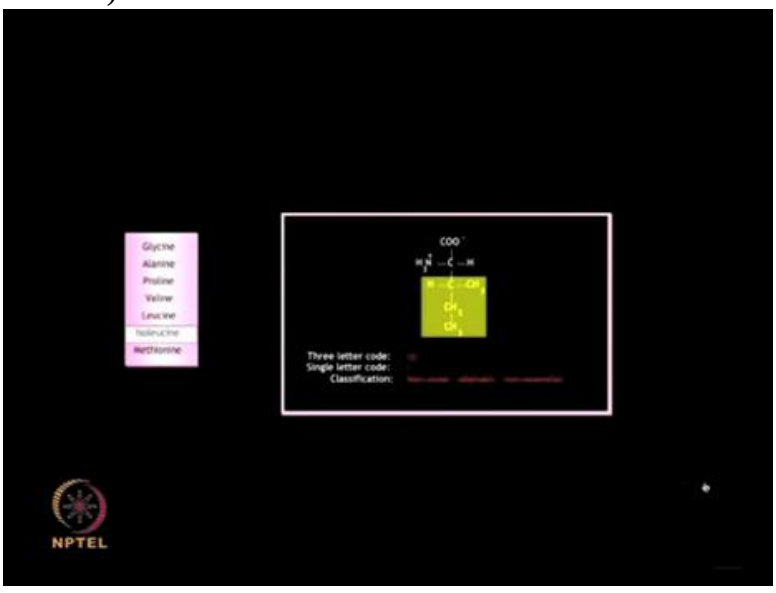

Isoleucene

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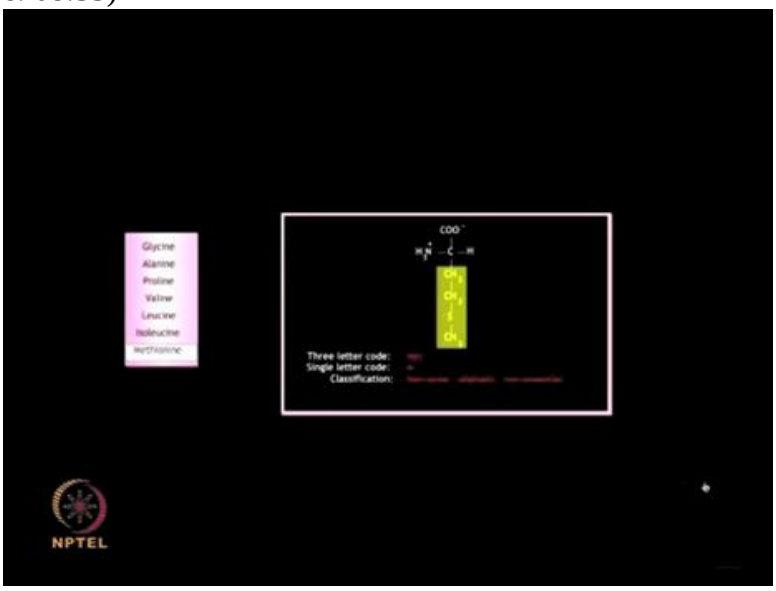

and Metheonine.

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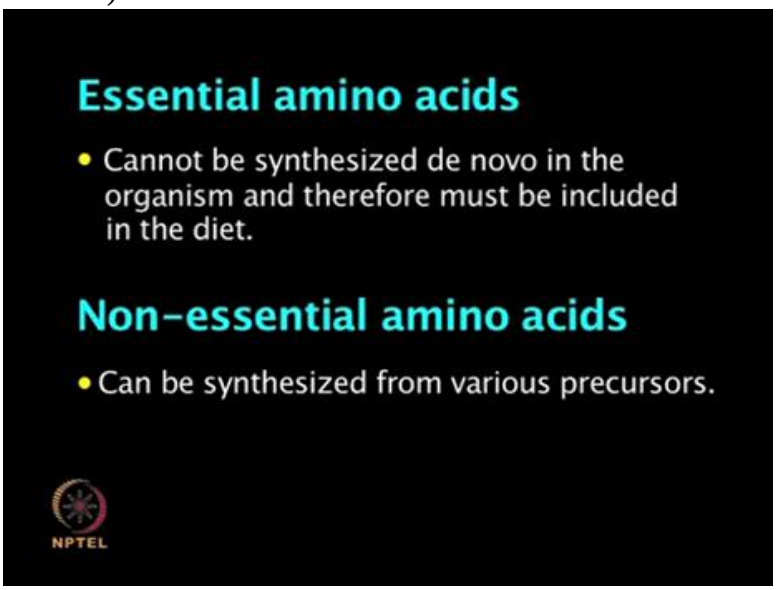

Essential amino acids are those that cannot be synthesized de-novo in the organism and therefore must be included in the diet. Non-essential amino acids on the other hand, can be synthesized from various precursors.

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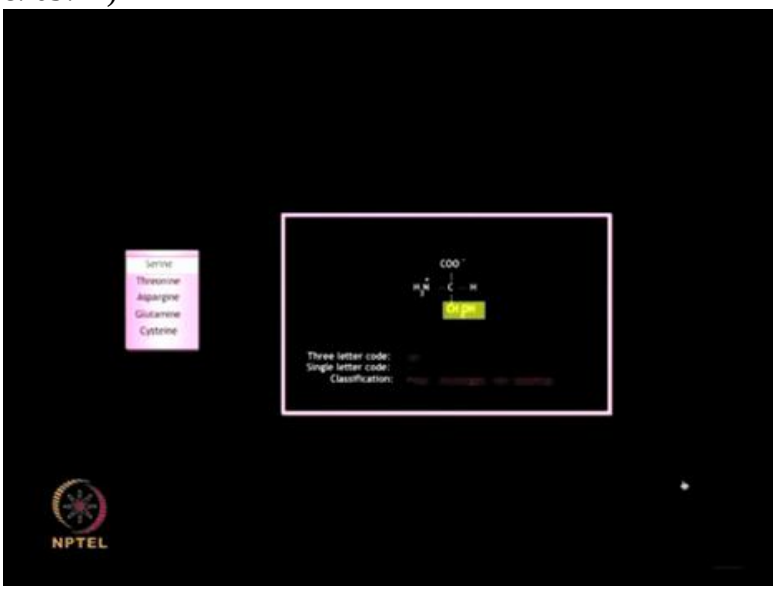

Serene,

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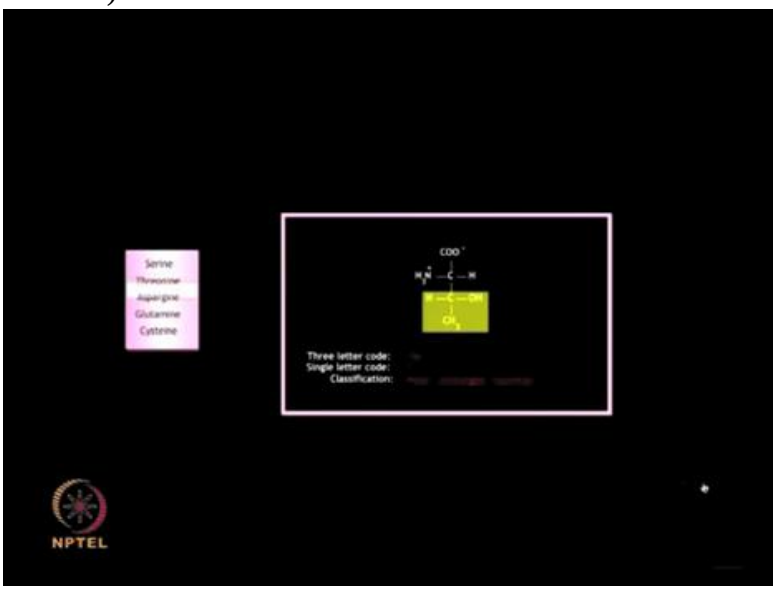

Theonine,

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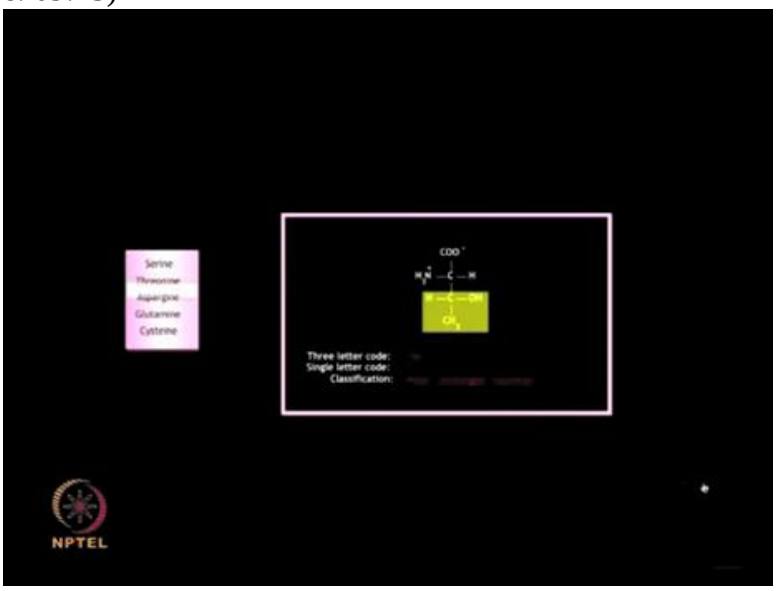

Aspargene,

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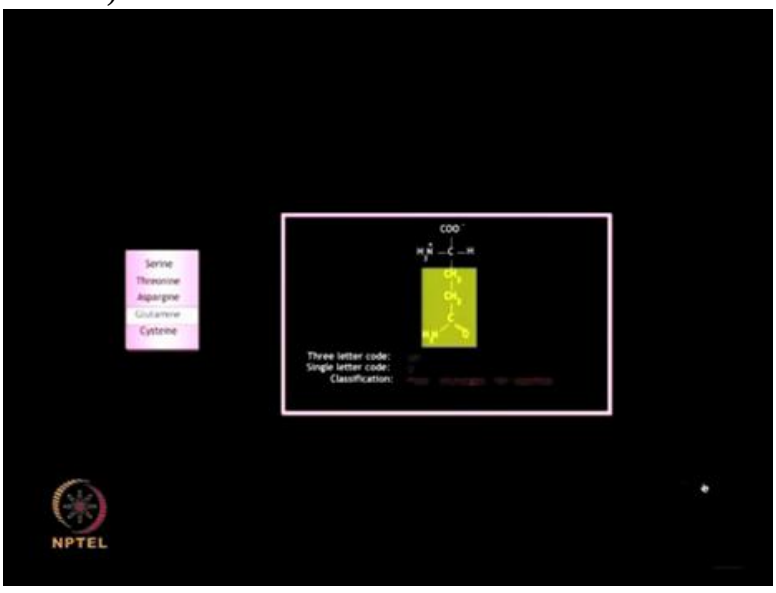

Glutamine

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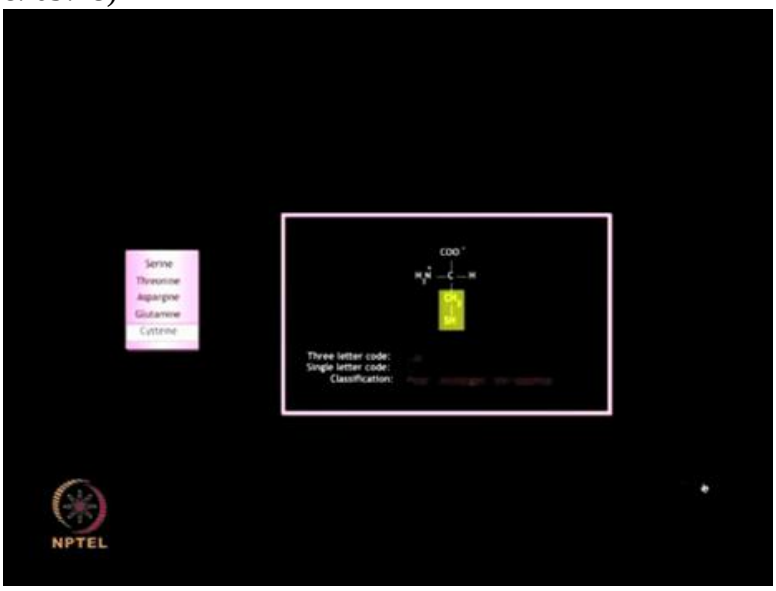

and Cysteine consist of polar but uncharged side chains.

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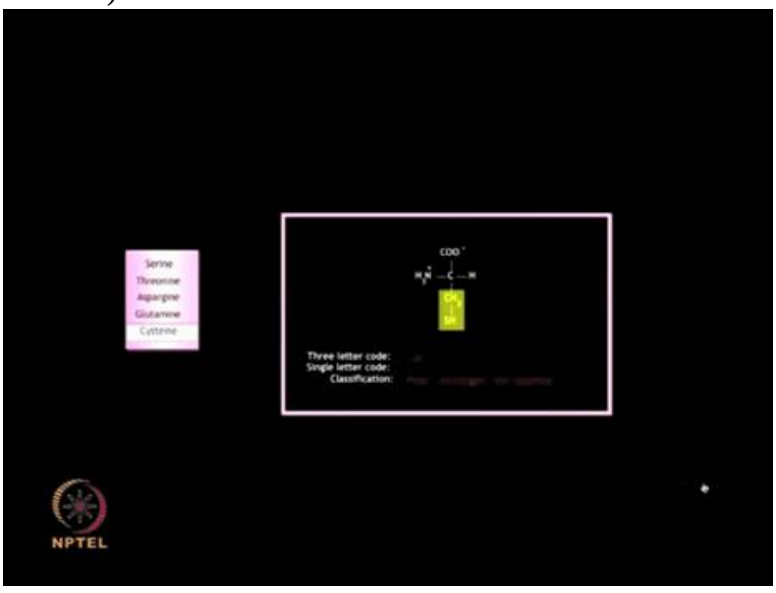

Lysine,

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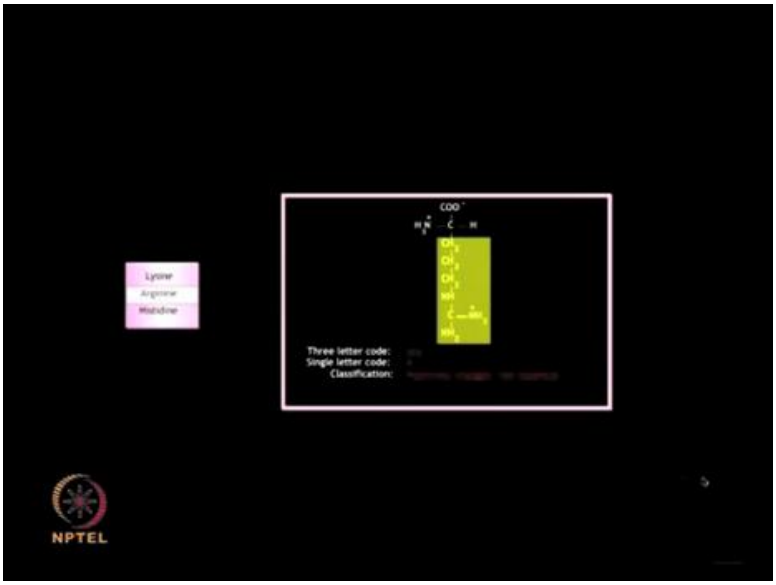

Arginine and

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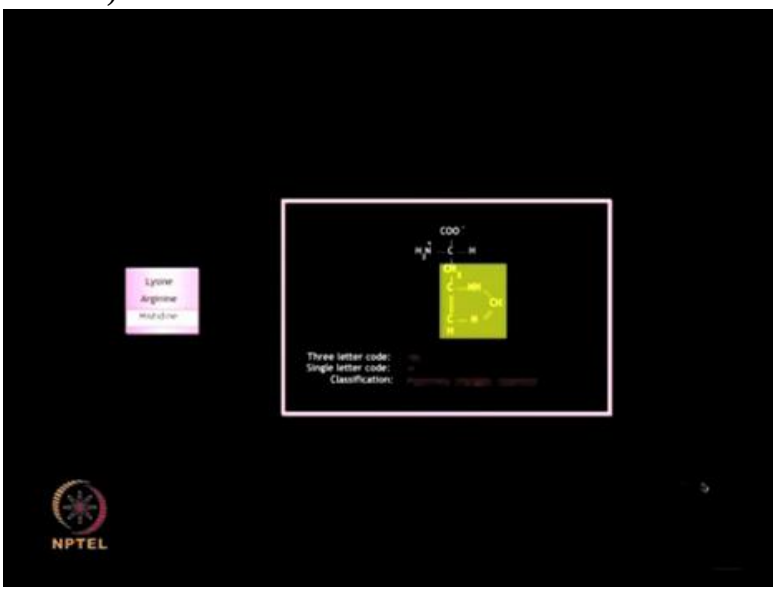

Histidine, these have positively charged side chains.

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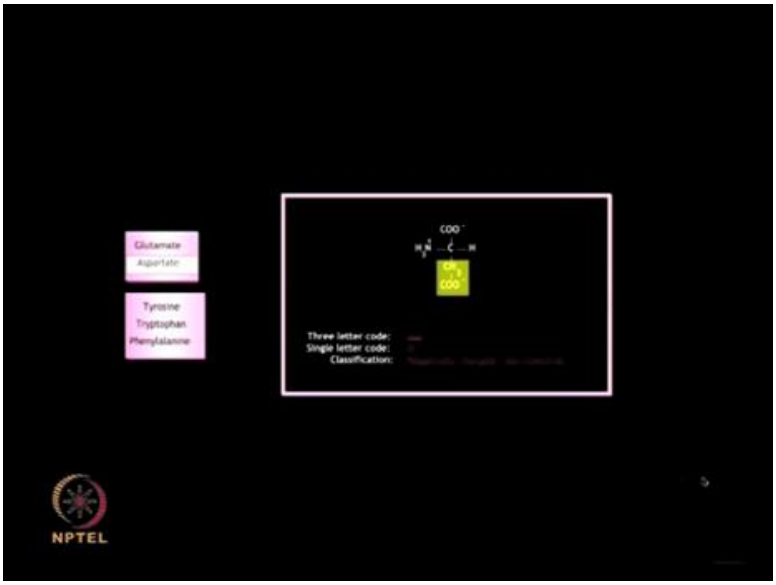

Aspartic acid and

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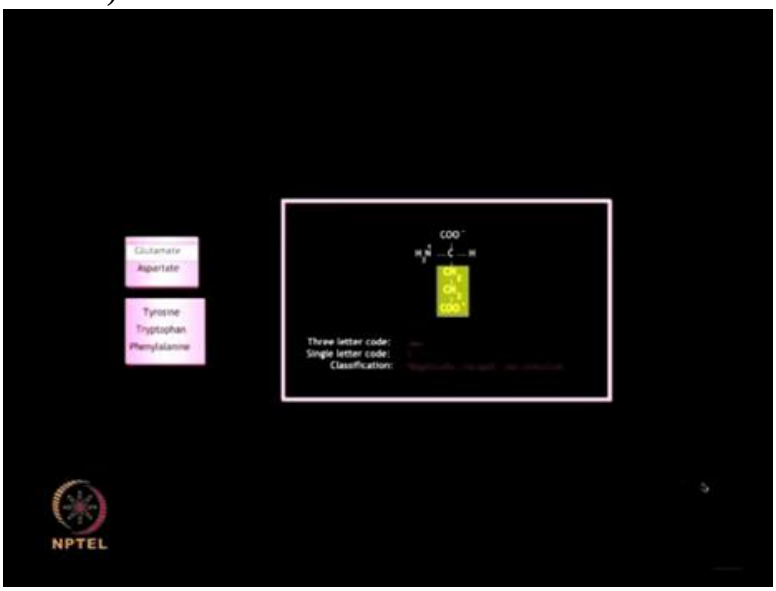

Glutamic acid are polar and negatively-charged amino acids.

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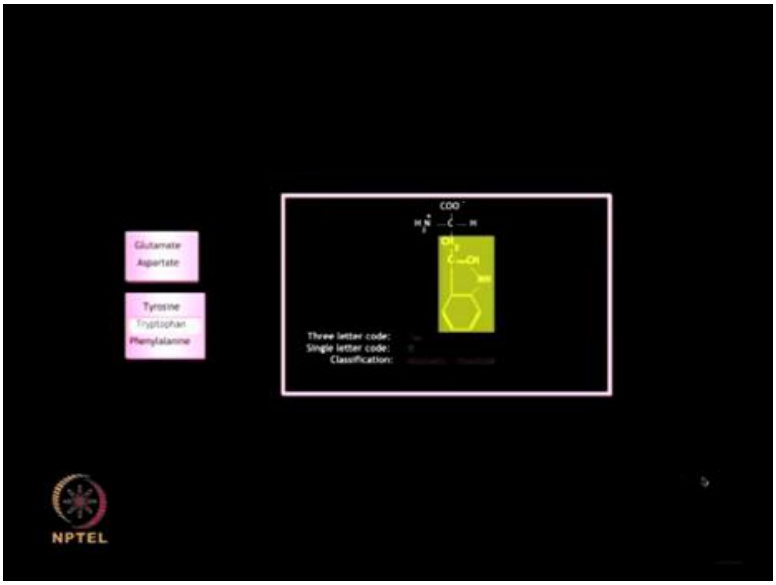

Tryptophan,

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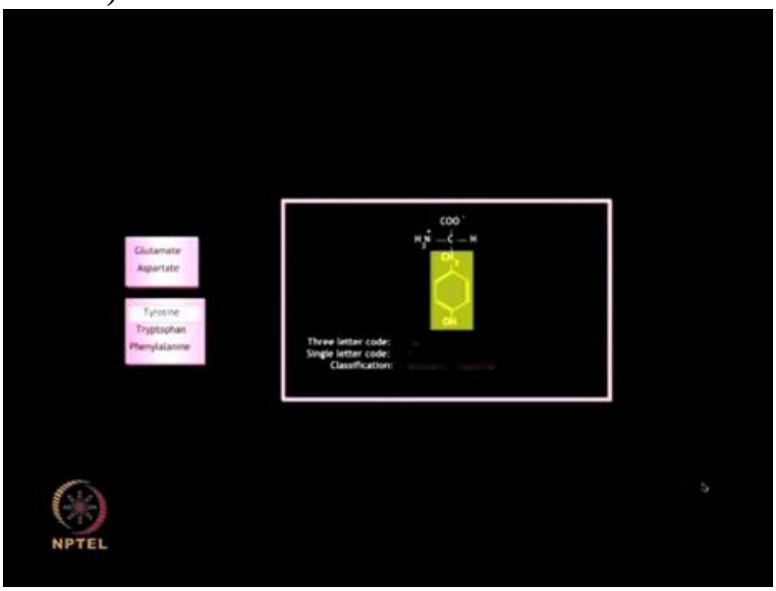

Tyrosine and

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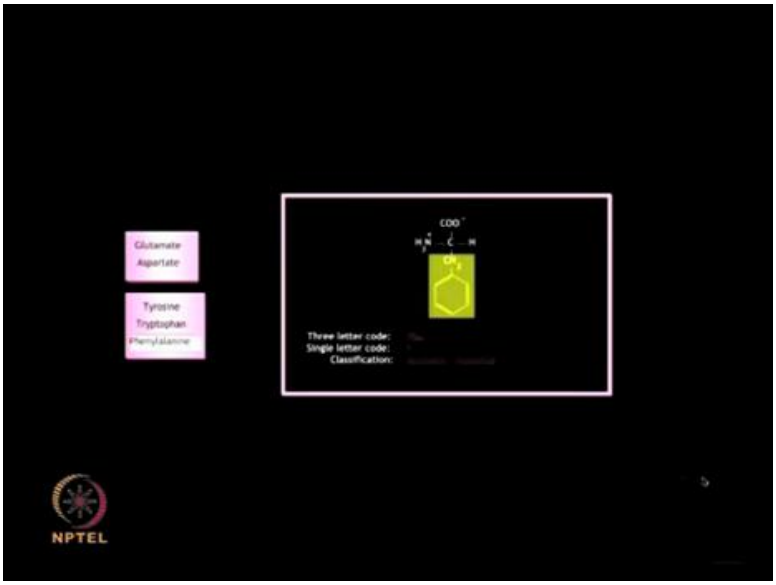

Phenylaniline are all essential amino acids having aromatic side chains.

After having discussed different type of group of amino acids, let's look at the basic constituents of amino acids and different isoforms which it can form; amino acids having 4 different groups which are connected to the alphacarbon atom.

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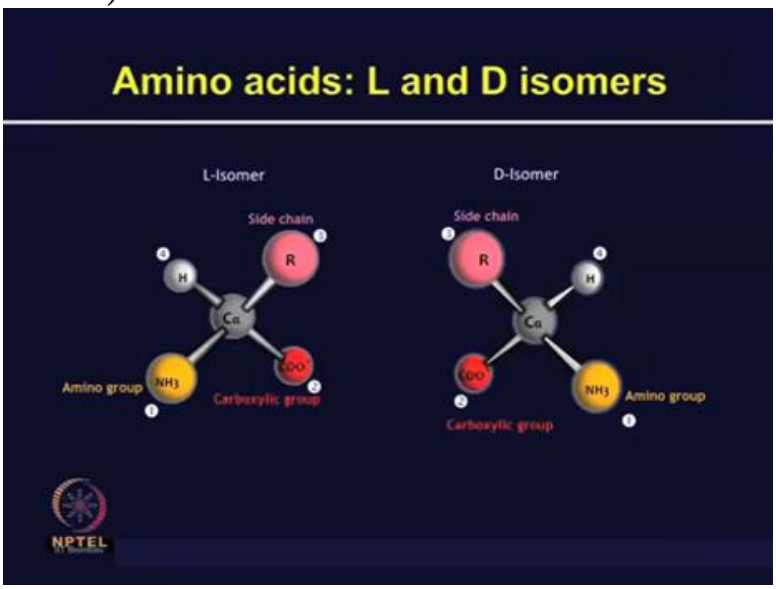

It can form 2 mirror images which can exist in the L or the D isomers which are shown in the slide here. The alpha amino acids are chiral.

There could be R or S configurations in the amino acids depending upon the priority groups but only L amino acids are present in the proteins.

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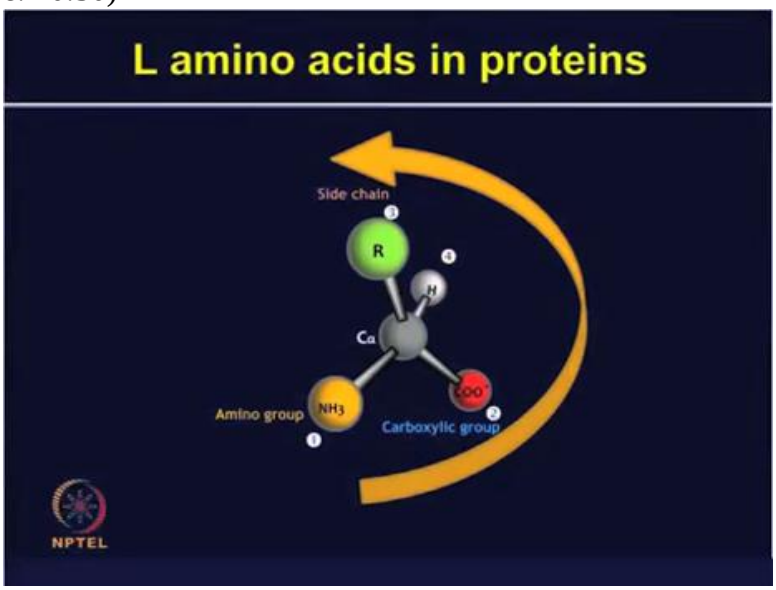

All the L amino acids have S configuration which describes the counter-clockwise direction as shown here from the highest to the lowest priority groups which is an indicative of chiral center with the S configurations.

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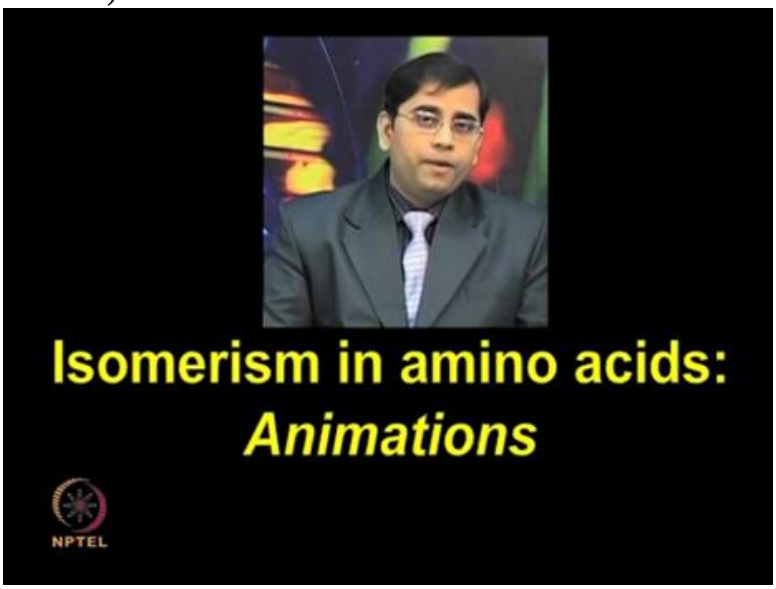

Some of the isomerism properties of amino acids will be discussed in following animation. Before learning about the isomerism, let's first know what chirality is. The term chirality arises from the Greek term chire meaning handedness.

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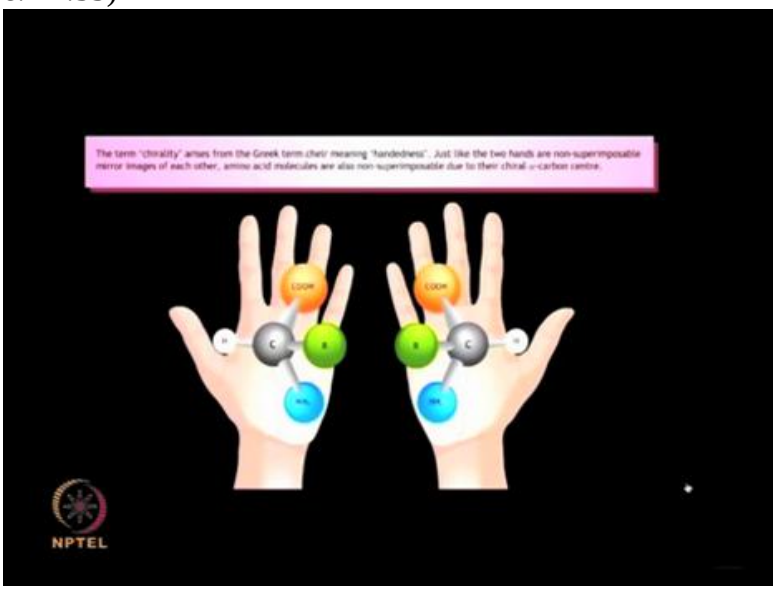

Just like the two hands are non-superimposable mirror images of each other, amino acid molecules are also non-superimposable due to their chiral alphacarbon center.

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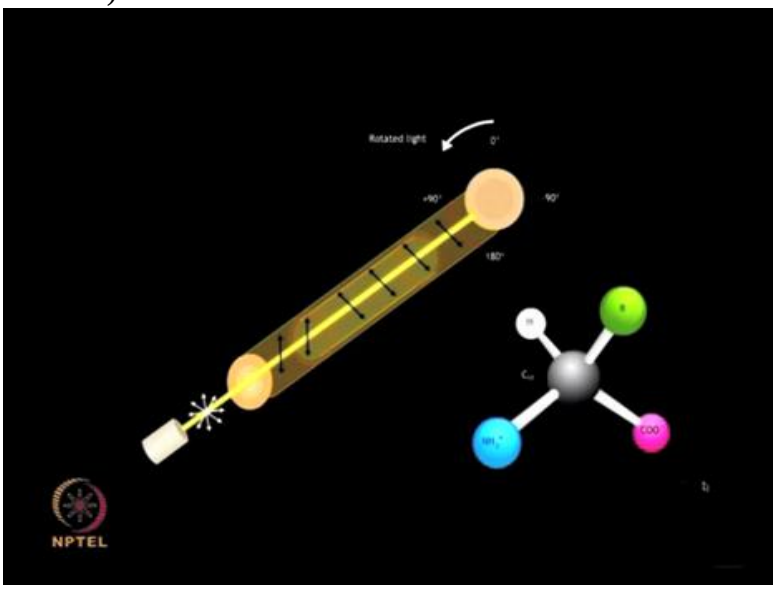

All amino acids except Lysine contain asymmetric center that makes them chiral in nature due to which they can rotate the plane of polarized light.

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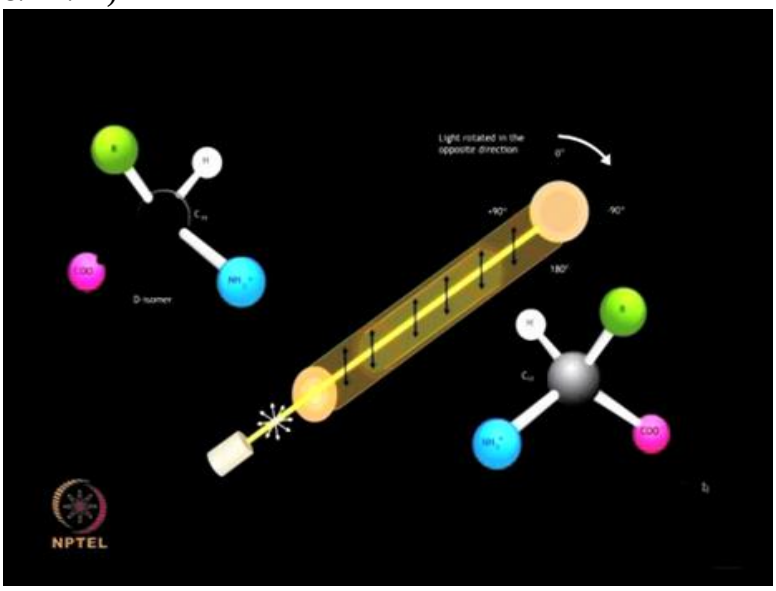

The two enantiomers designated as D and L rotate the plane of polarization in opposite directions.

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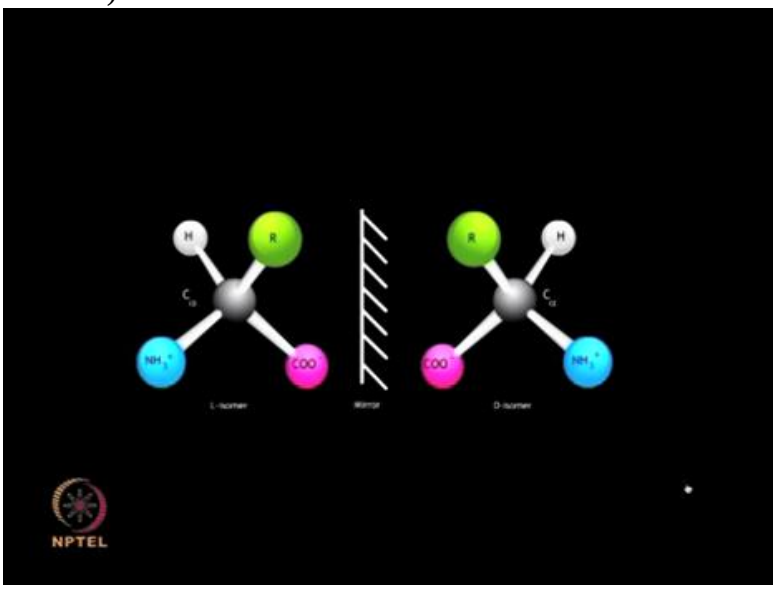

The two enantiomers of amino acids are non-superimposable mirror images due to the special arrangements of 4 different groups about the chiral carbon atom.

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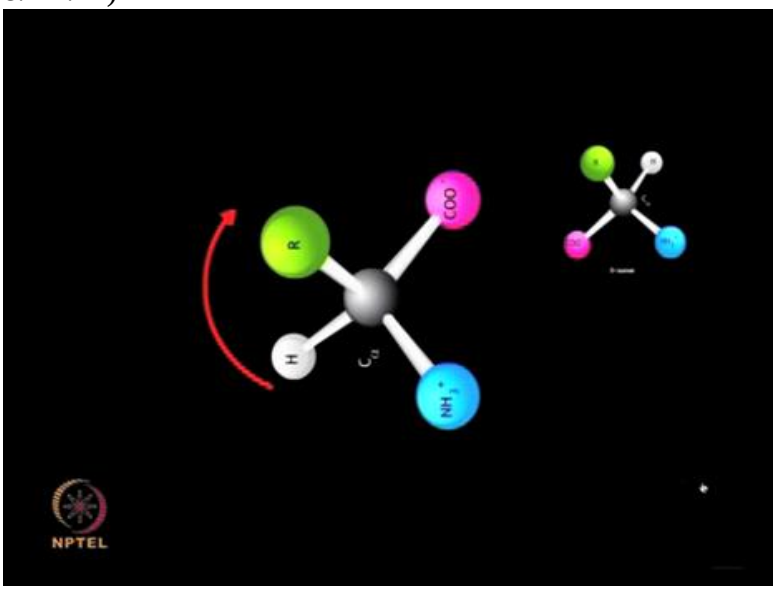

Rotation of either isomer about its central axis will never give rise to the other isomeric structure.

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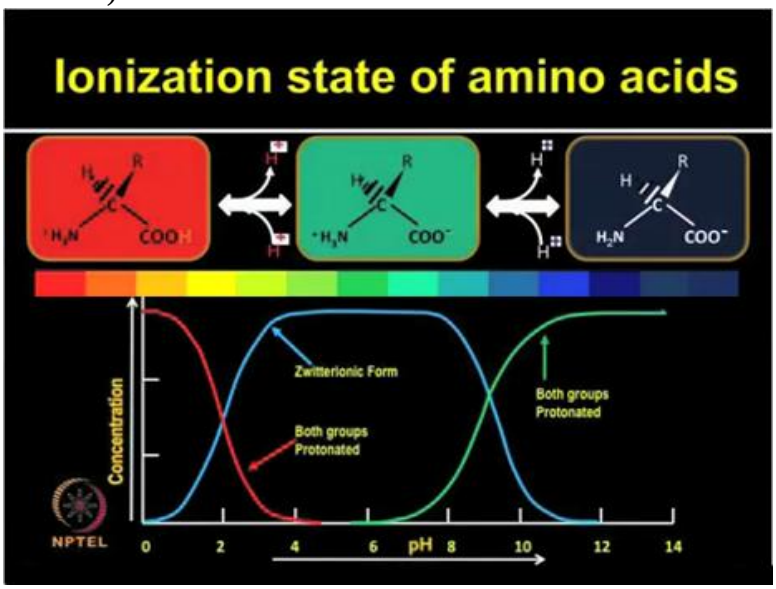

Let's now talk about ionization state of amino acids. The ionization state of amino acid varies with its pH. In the acidic solution, if you follow the slide from the left to right, the amino group is protonated NH3+.

Carboxylic group is un-dissociated COOH. At the neutral pH amino acid exists as dipolar ions or zwitterions. Amino group is protonated NH3+ and carboxylic group is deprotonated COO-. Now this dipolar form can exist till pH9. Now when you move to the basic pH, the protonated amino group loses its proton and forms NH2.

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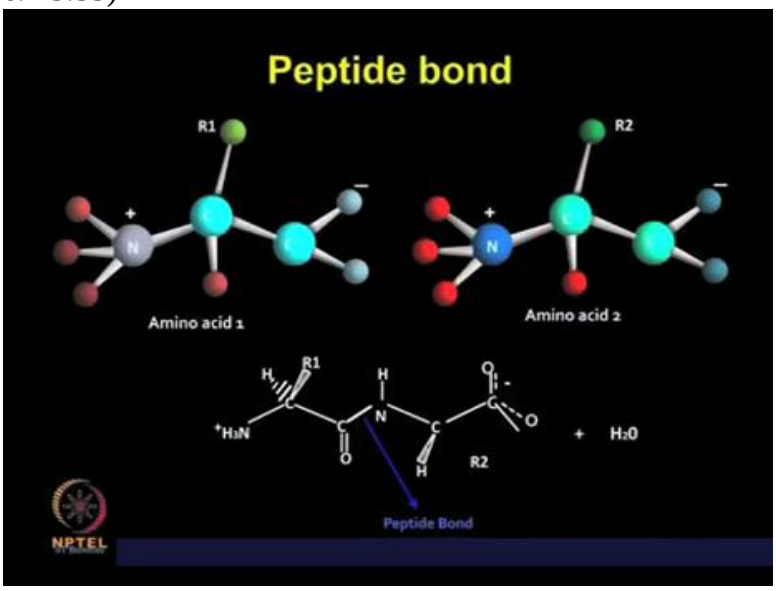

Let's now talk about peptide bonds. Peptide bond can link amino acids to form the polypeptide proteins. The alpha-carboxylic group of 1 amino acid linked with the alpha amino group of another amino acid as you can see here. These two amino acids are forming a bond and peptide bond is formed with accompanying loss of water molecule.

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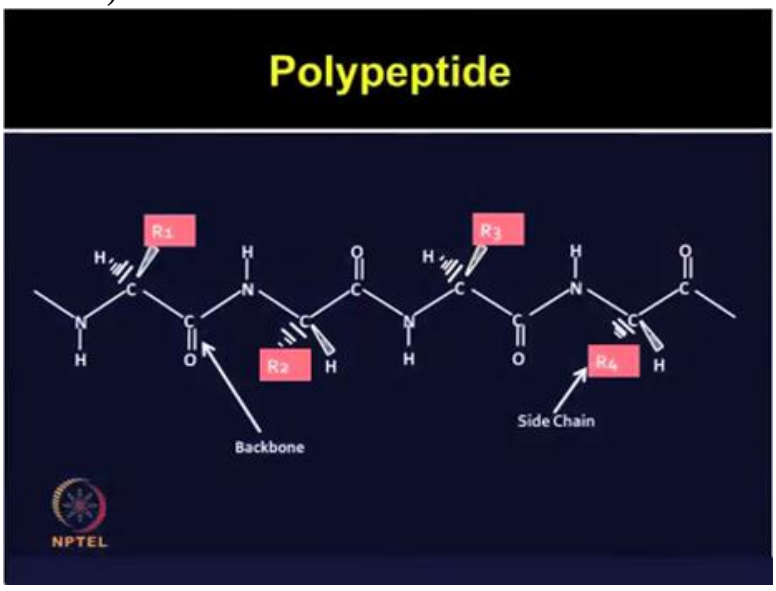

Many amino acids are linked together. They form a polypeptide as you can see in this slide. Multiple peptide bonds are present.

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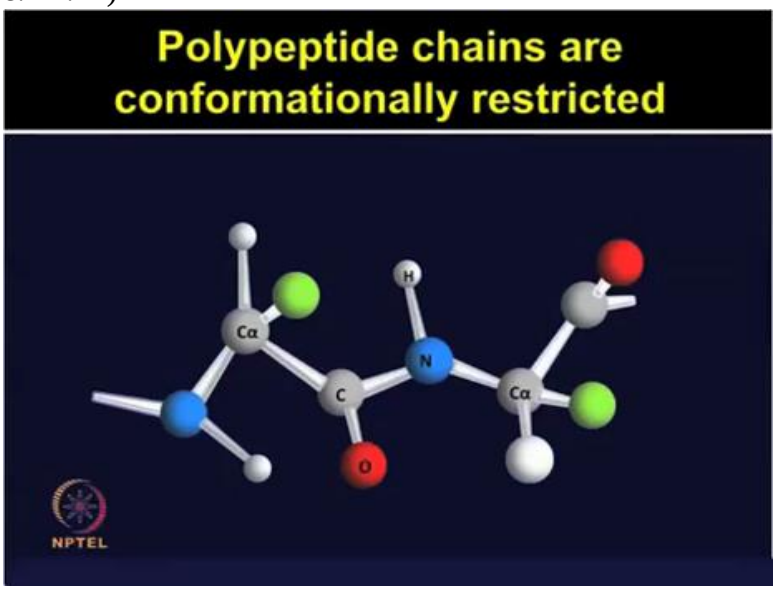

The polypeptide chains are conformationally restricted. Therefore peptide bond is planar. Amino acid pairs, they are linked by the peptide bonds and all the six atoms lie in the same plane as you can see here; alphacarbon, carbon, oxygen, nitrogen, hydrogen and another alphacarbon.

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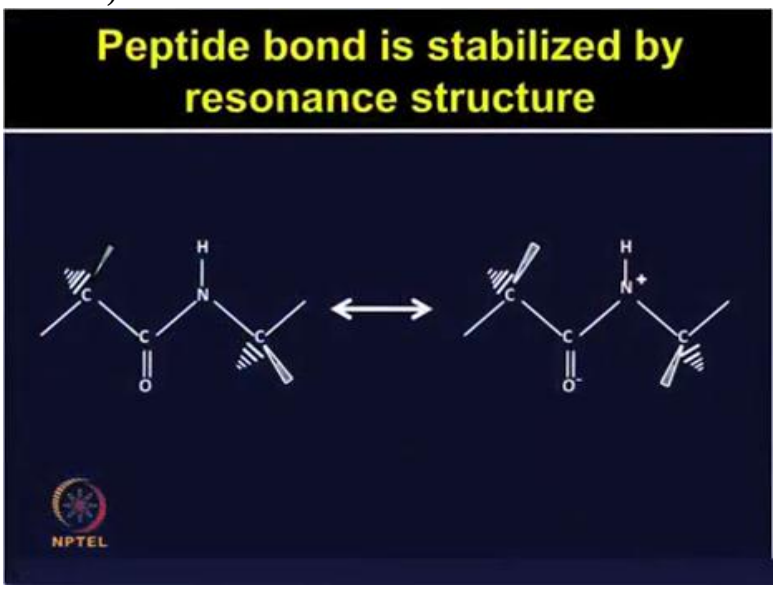

Peptide bonds can be stabilized by the resonance structure. Peptide bond is rigid because of its partial double bond characters (tics) which arise due to the resonance structures present in peptide bond. There could be two forms, Cis-form and the trans-form.

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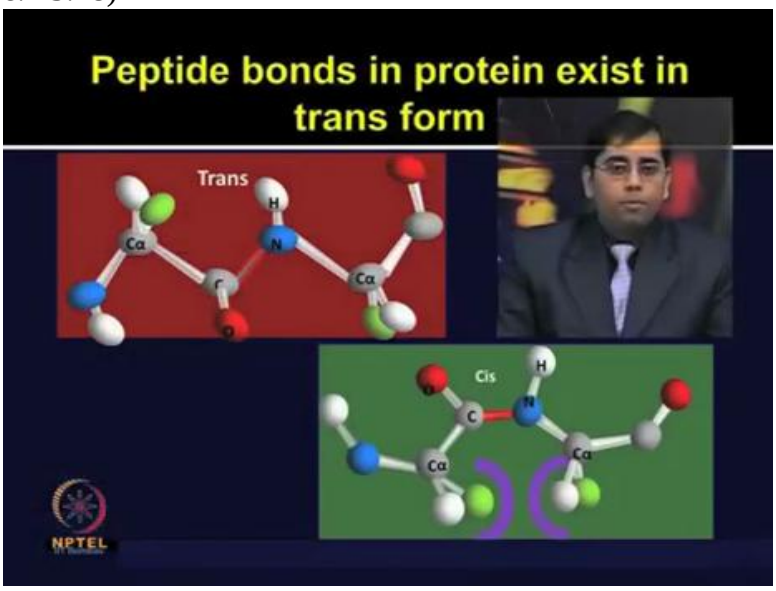

Peptide bonds in protein exist in the Trans form. If you see the top panel, the Trans configuration there are two c alpha on the opposite sides of the peptide bonds.

This configuration is allowing less steric clashes where as if you look at the bottom panel, the Cis configuration there are two alphacarbons on the same side of peptide bond, so there is more probability of having steric clashes. Therefore peptide bond in proteins exists in the Trans form.

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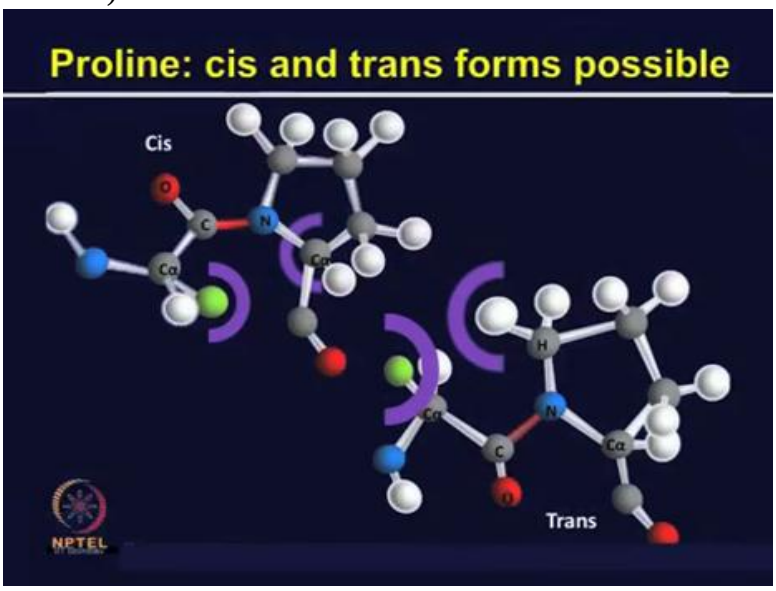

Now Proline is a unique amino acid as we discussed earlier. Proline with peptide bonds, it can form both Cis and the Trans forms. So, as you can see here, it can avoid the steric clashes

and both Cis and Trans configurations are possible. Some of the concepts of peptide bonds will be described in the following animation.

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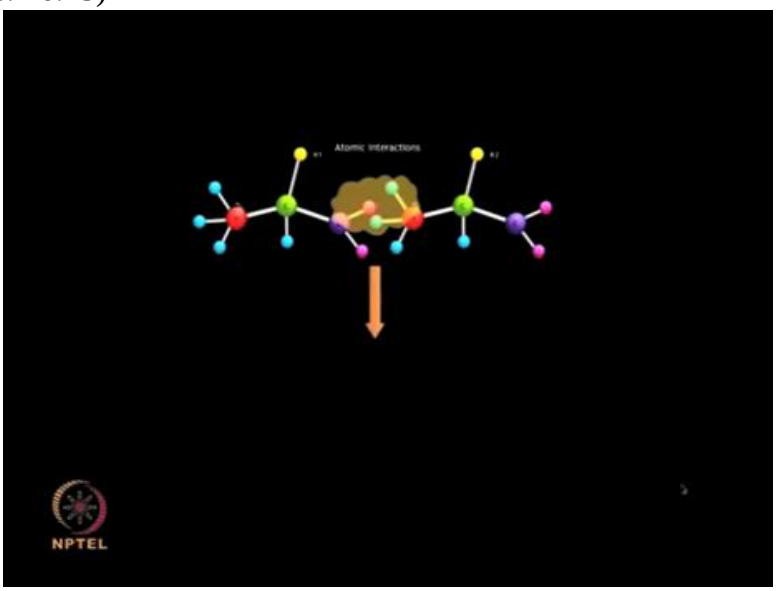

Amino acids are the building blocks or monomers that make the proteins. Amino acids are oriented in a head-to-tail fashion and linked together such that they carboxyl group of one amino acid combines with the amino group of another amino acid.

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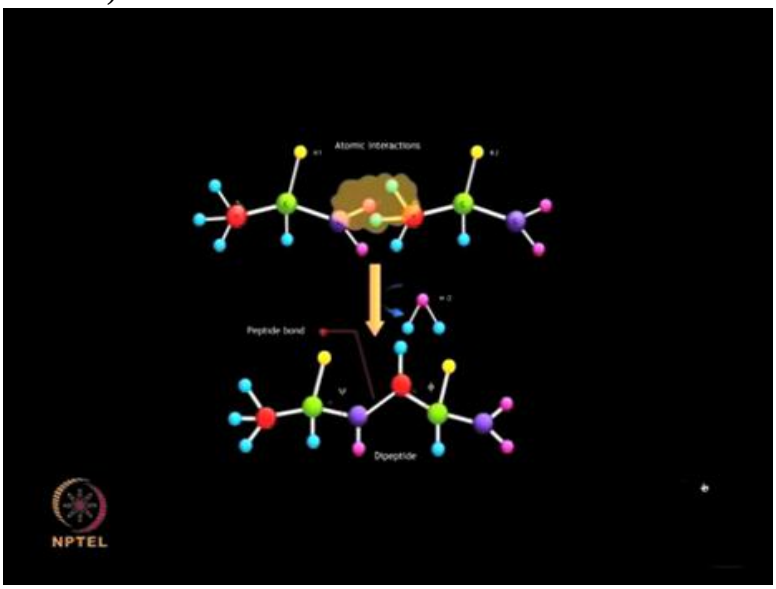

Two amino acids join together by means of such a condensation reaction with the loss of water molecule forms a di-peptide. Many such amino acids link together and form polypeptide.

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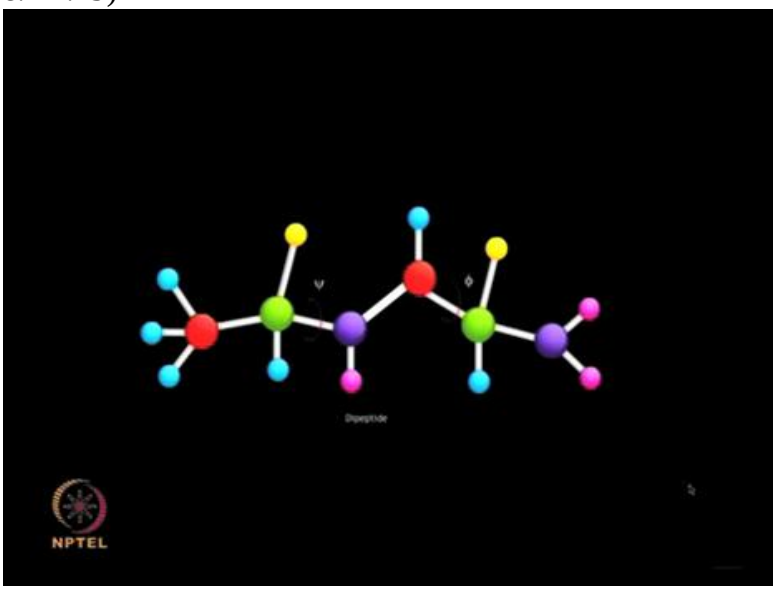

The peptide bond is rigid due to its partial double bond character which arises from the resonance structure.

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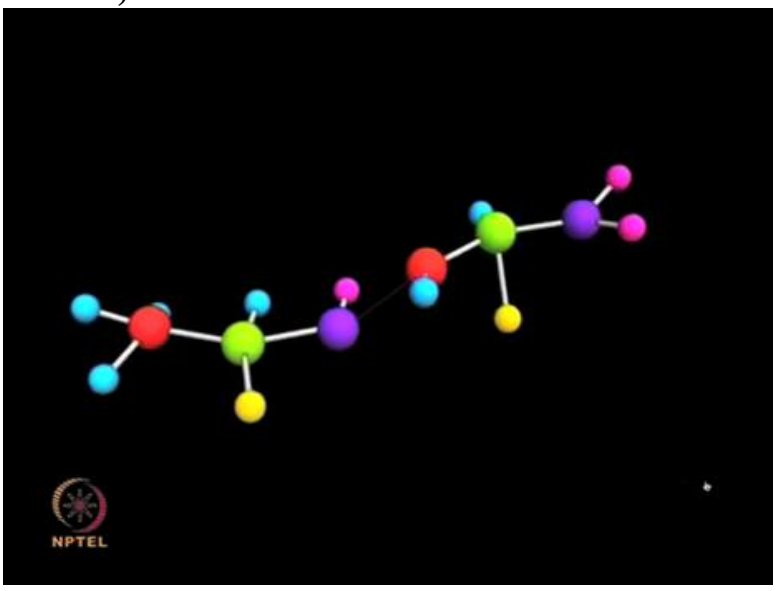

However, the bonds between the alphacarbon and amino and carboxyl groups are pure single bonds that are free to rotate.

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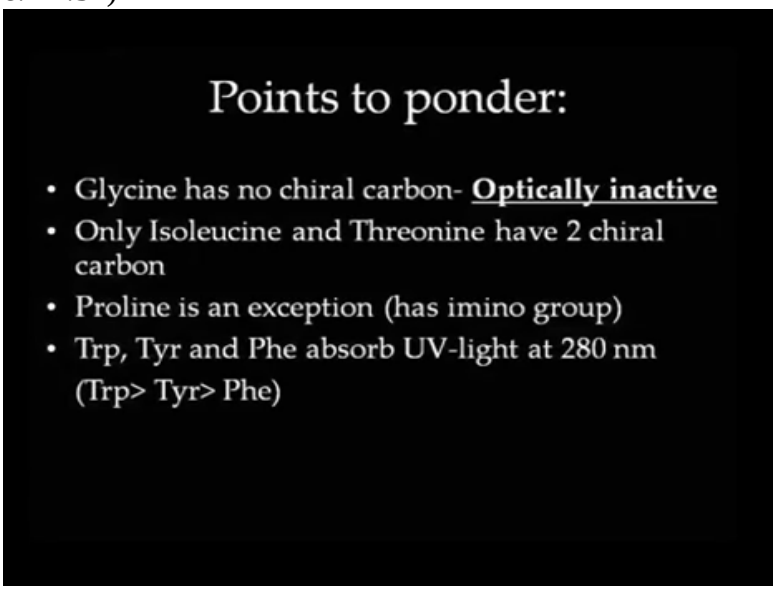

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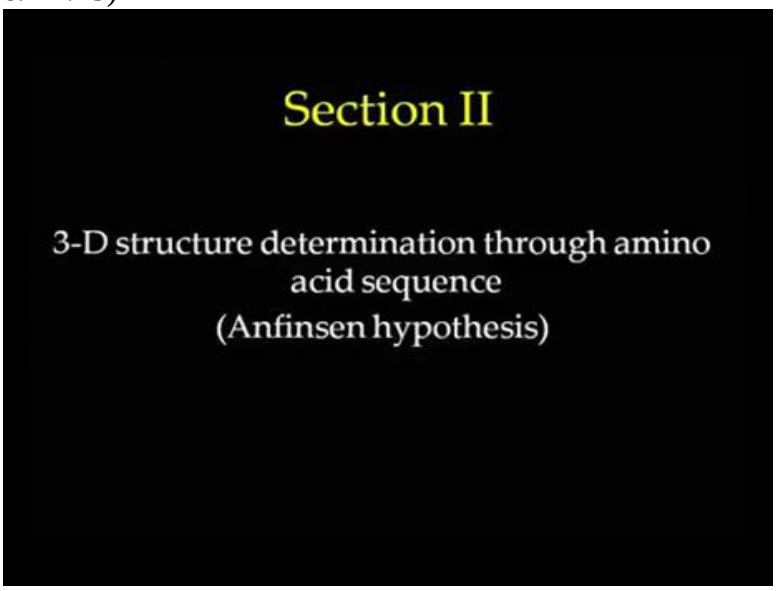

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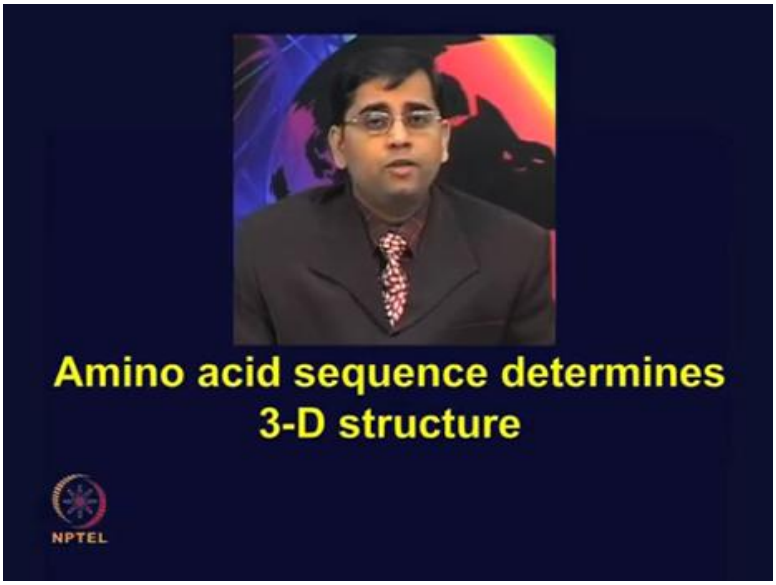

Amino acid sequences determine three dimensional structures of proteins. So there is very intricate sequence-structure relationship. The amino acid sequence dictates the conformations which are adopted by the polypeptide chains at secondary and tertiary levels.

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Scientist Anfinsen, he did a classical experiment where he tested the ability of reduced and unfolded proteins to spontaneously fold into native state by using protein ribonuclease A. The experiment established that the primary amino acid sequence of a protein contains all the information which is required for the proper protein folding into its native form.

The fundamental discovery of Anfinsen led him to receive the Nobel Prize in Chemistry in 1972.

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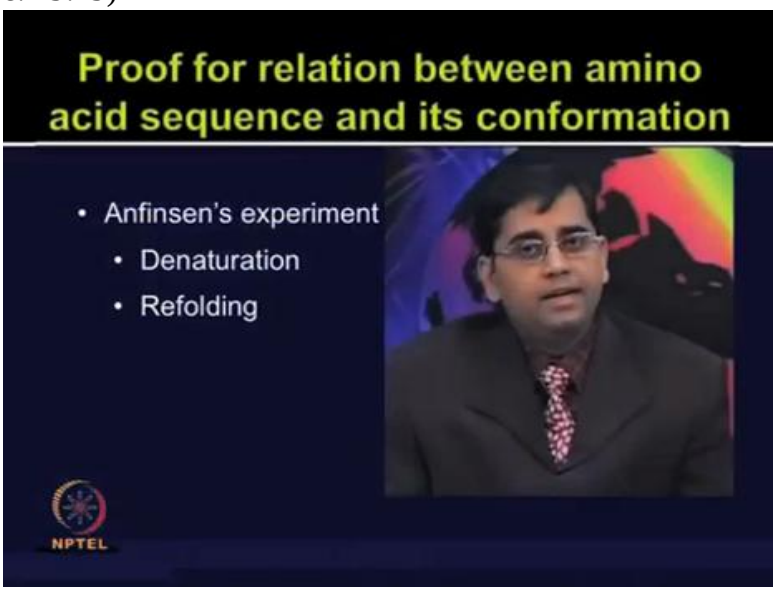

So let's explain you how this experiment worked. To establish the proof for relationship between amino acid sequence and its confirmation, Christian Anfinsen in 1950s performed an experiment where he performed two steps, denaturing and refolding. So how denaturation and refolding works?

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So in this classical experiment, Anfinsen used protein ribonuclease A (Rnase A). He used few denaturants such as urea or guanidine hydrochloride and beta Mercaptoethanol which breaks the disulfide bonds. So let's look at these components a little bit more detail.

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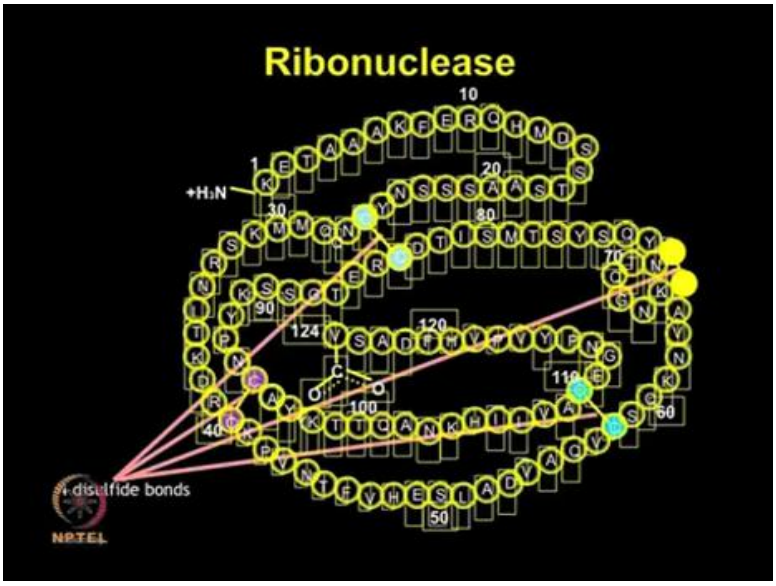

Lets first about ribonuclease Rnase A protein. This protein has contributed greatly to our understanding of protein folding in vitro from the landmark experiment of Anfinsen.

As you can see in the structure, ribonuclease has 124 amino acid residues and it forms 4 disulfide bridges which are located between Cysteine residues of 26 and 84, 40 and 95, 58 and 110, and 65 and 72. This protein catalyses the hydrolysis of RNA.

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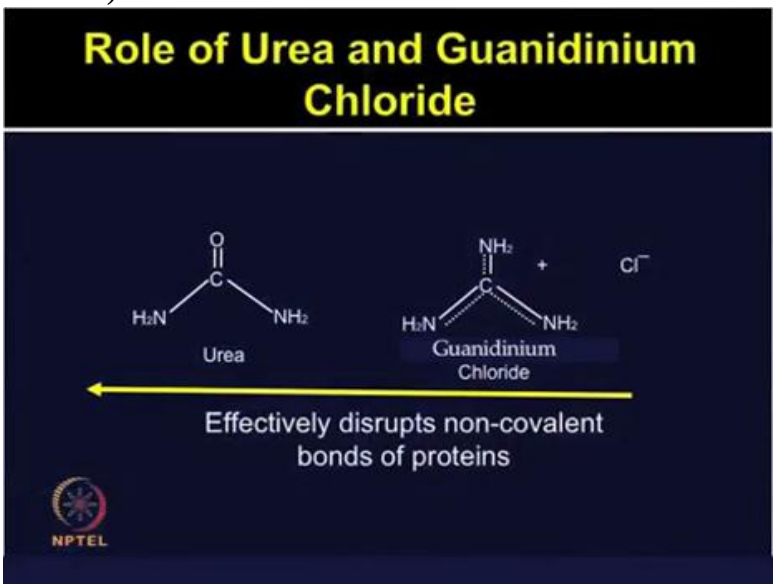

So what is the role of urea and guanidinium chloride? Urea is an organic compound which has two amino groups joined by a carbonyl group and used at a concentration of around 6 molar for denaturing the proteins by breaking the non-covalent interactions. Both urea and guanidinium chloride can effectively disrupt the protein's non-covalent interactions.

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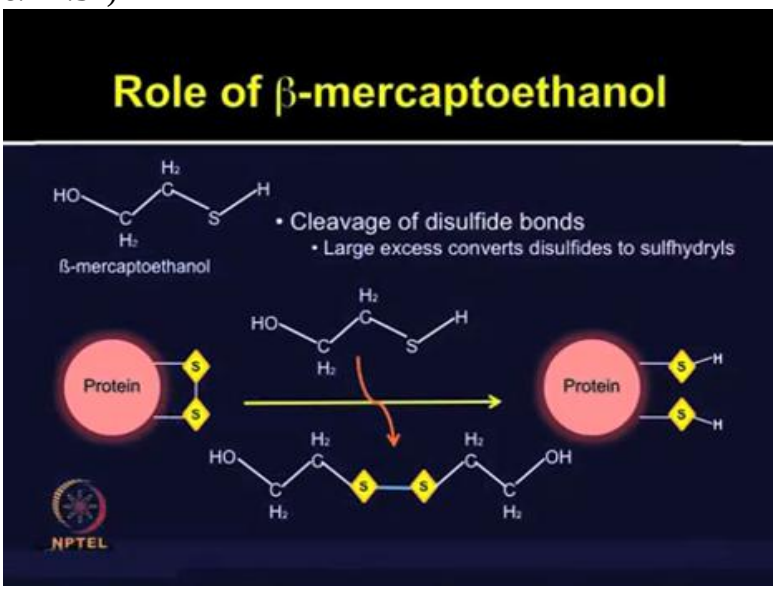

What is the role of beta Mercaptoethanol? Beta Mercaptoethanol is commonly used to reduce the di-sulphide linkages in proteins and thereby it disrupts tertiary and quaternary structures. As you can see in the structure here in the slide, in the presence of excess of beta Mercaptoethanol the disulfide or Cysteines can be fully converted into sulfhydryls or Cysteines.

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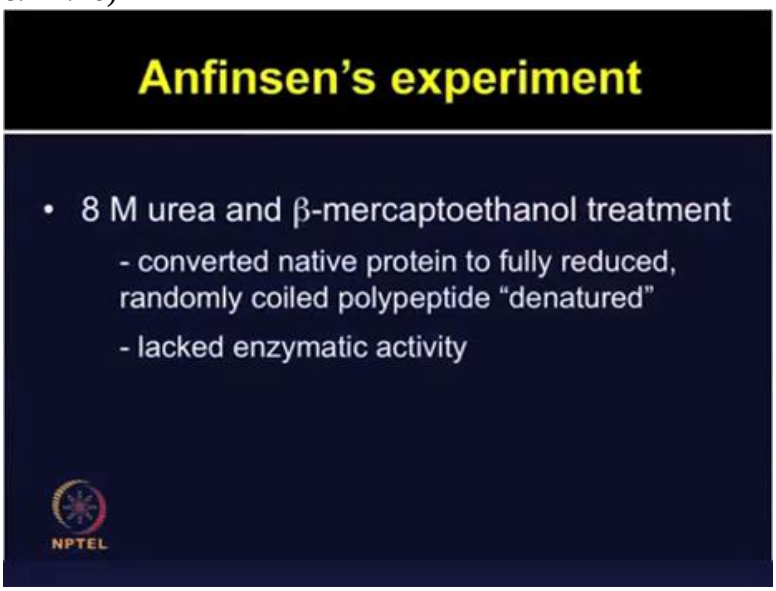

In Anfinsen's experiment, he used 8 molar of urea and beta Mercaptoethanol treatment which converted the native proteins to fully reduced state into the randomly coiled polypeptides known as the denatured structure. The denatured polypeptide lacked enzymatic activity.

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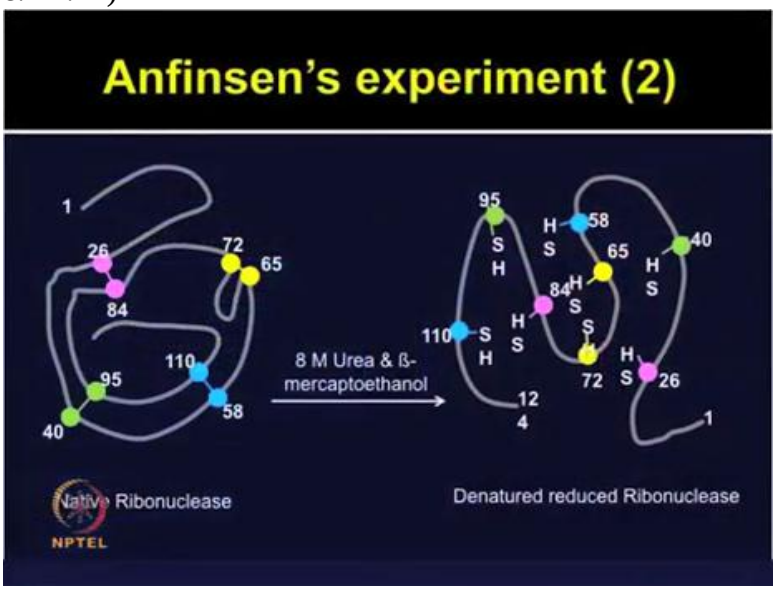

Such a disbursed ribonuclease protein, it contains 124 amino acid residues and forms 4 disulfide linkages. These linkages are formed between the Cysteines as shown here of 26 and 84, 40 and 95, 58 and 110, and 65 and 72. The ribonuclease native conformation is lost when it was treated with 8 molar urea and beta Mercaptoethanol.

As you can see here, the native ribonuclease has formed denatured, reduced ribonuclease due to the breaking of disulfide and non-covalent interactions. Anfinsen noticed that when ribonuclease was oxidized in air and urea was removed by the process of dialysis, the enzyme activity slowly recovered and as a result of the protein folding.

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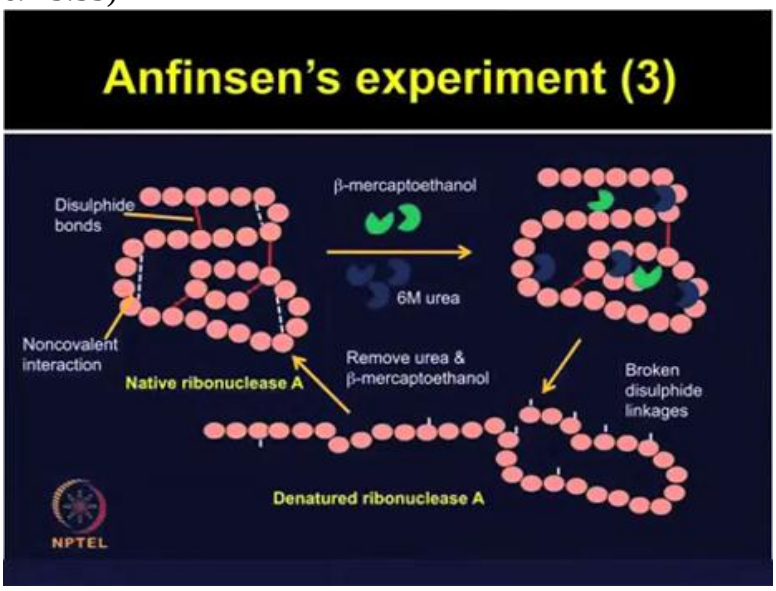

As you can see here, if you have used beta Mercaptoethanol and 6 molar urea, all the disulfide and covalent bonds are breaking. Once urea is removed, then slowly protein folding occurs. It results into the reformation of tertiary structure and active site.

When Anfinsen repeated this experiment in presence of denaturant urea; that led to regeneration of less than 1% of enzyme activity. So what could be the reason?

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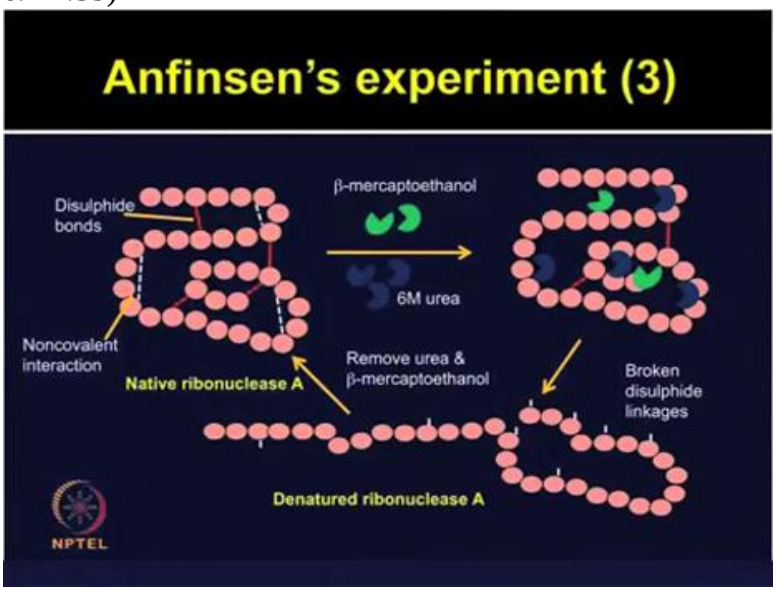

In fact urea prevented the correct disulfide pairing which resulted into the scrambled form, scrambled ribonuclease.

Now if you mathematically calculate due to the presence of 4 disulfide bonds here and presence of 8 Cysteine residues, it can actually give rise to 105 different forms in which these 4 disulfide bonds can be formed. In the absence of urea, the correct disulfide bridge formation occurred and it allowed folded and thermodynamically stable state to be reached in ribonuclease protein.

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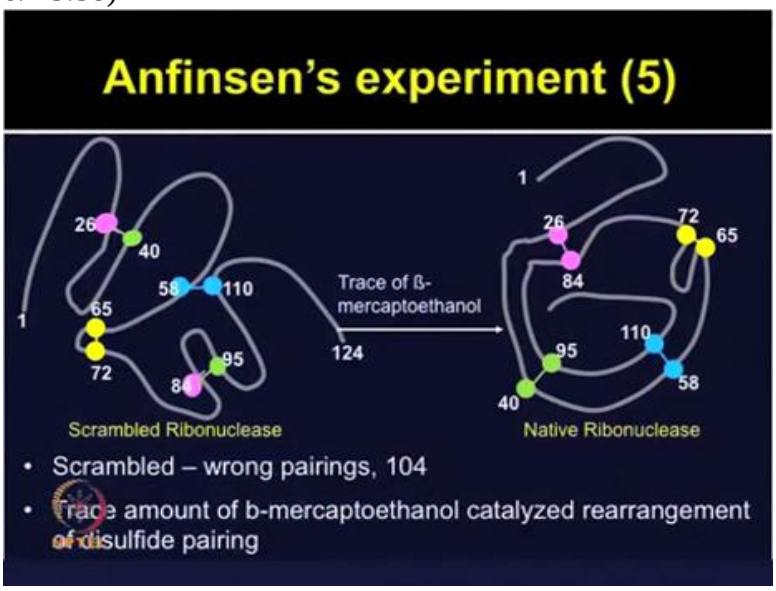

Now this figure shows here that in presence of trace amount of beta Mercaptoethanol and complete removal of denaturant urea, the refolding of ribonuclease was accurate and 4 intra-chain disulfide bonds were reformed in the same positions where they were expected in the native ribonuclease.

The random distribution of disulfide bonds was obtained when denaturants were used as you can see in this scrambled state which indicates that weak bonding interactions were required for the correct positioning of disulfide bonds and achieve the native conformation.

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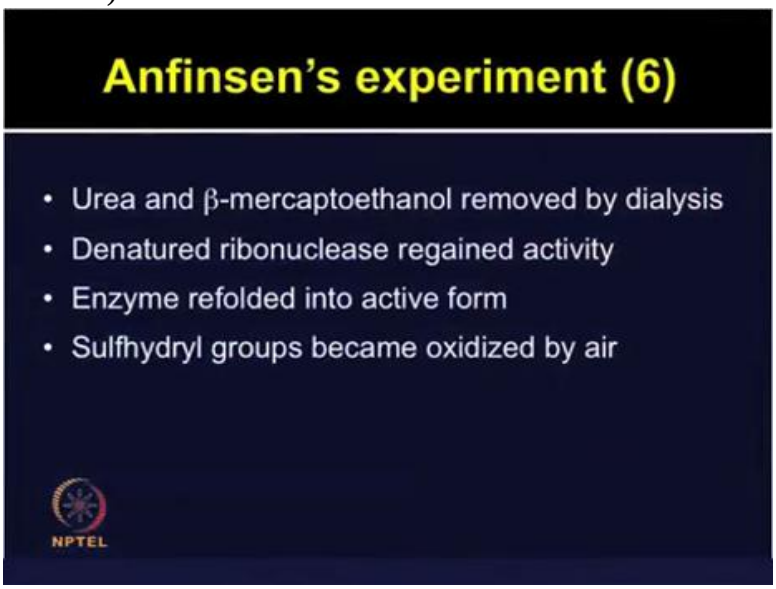

So in Anfinsen's experiment, he removed urea and beta Mercaptoethanol by dialysis process. The denatured ribonuclease regained its enzyme activity. The enzyme was refolded into the active form and the sulfhydryl groups became oxidized in presence of air.

The experiment proved that information required for specific catalytic active structure of ribonuclease is contained in its amino acid sequence. The classical study of Anfinsen proved that all information which is crucial for protein folding resides in its primary sequence.Let me explain you this experiment in following animation.

In Anfinsen's experiment, ribonuclease A in its native state has 4 disulfide bonds between its Cystiene residues.

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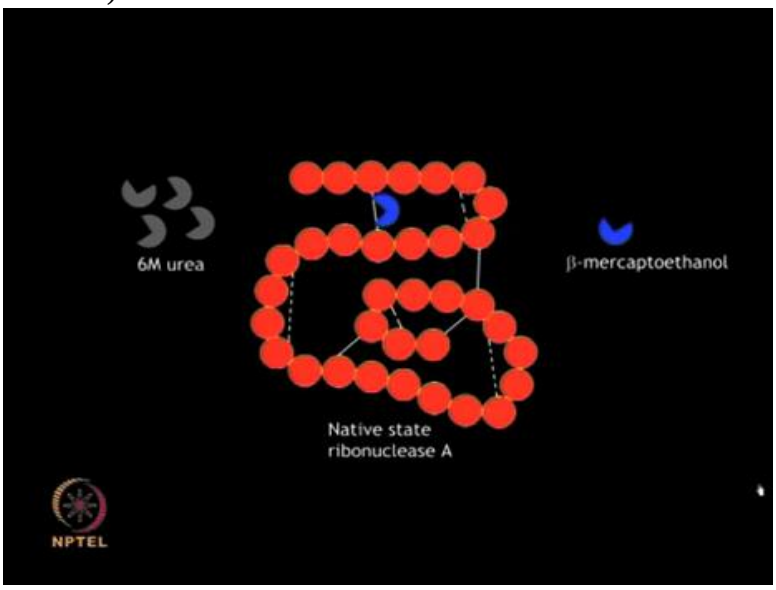

When treated with beta Mercaptoethanol and 6 molar urea, the protein undergoes denaturation and the disulfide linkages are broken.

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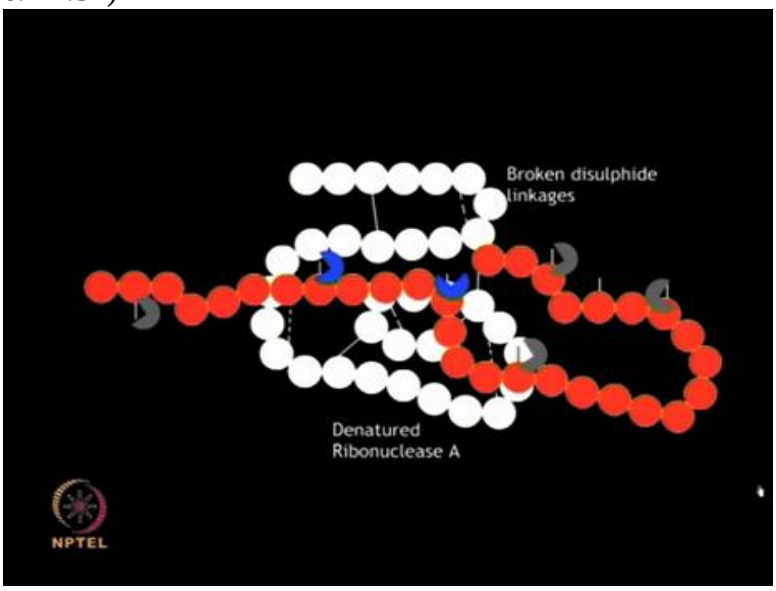

The enzyme activity is lost in its denatured state.

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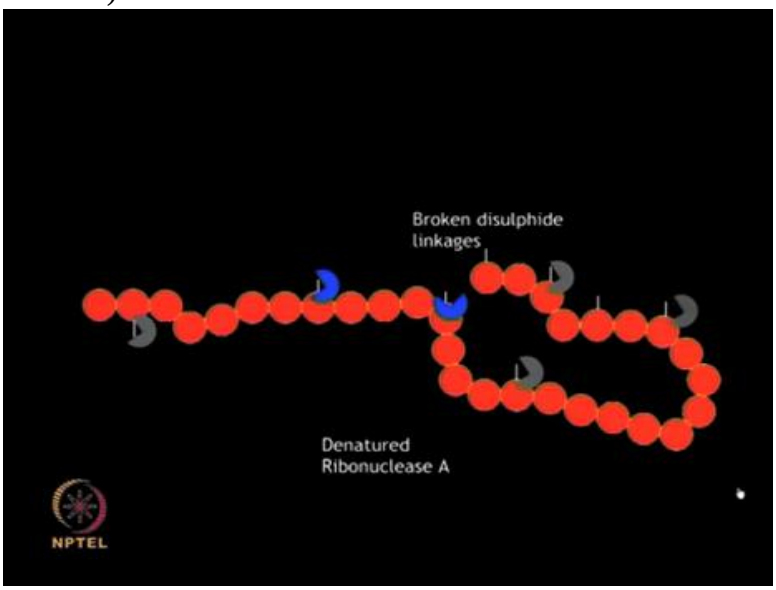

It was observed by Anfinsen that…

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… removal of urea and beta Mercaptoethanol led to the refolding of enzymes to assume its native state…

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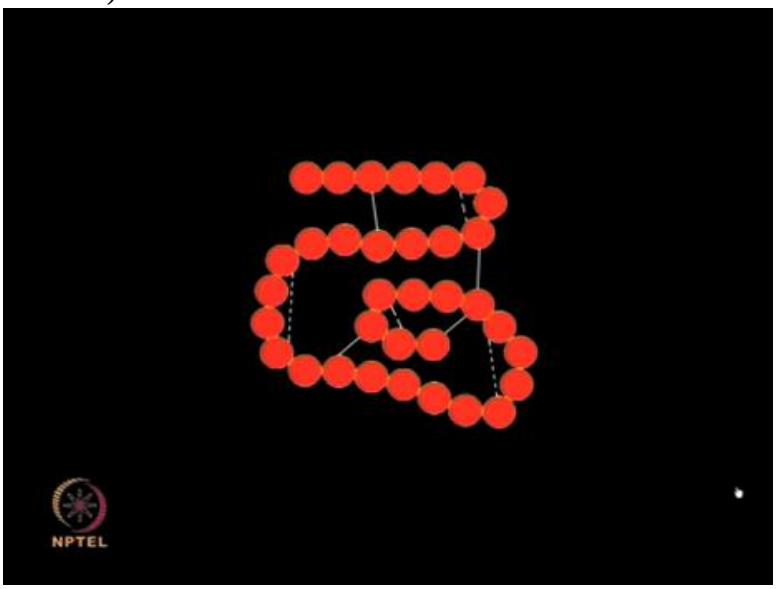

… with more than 90% enzyme activity being intact.

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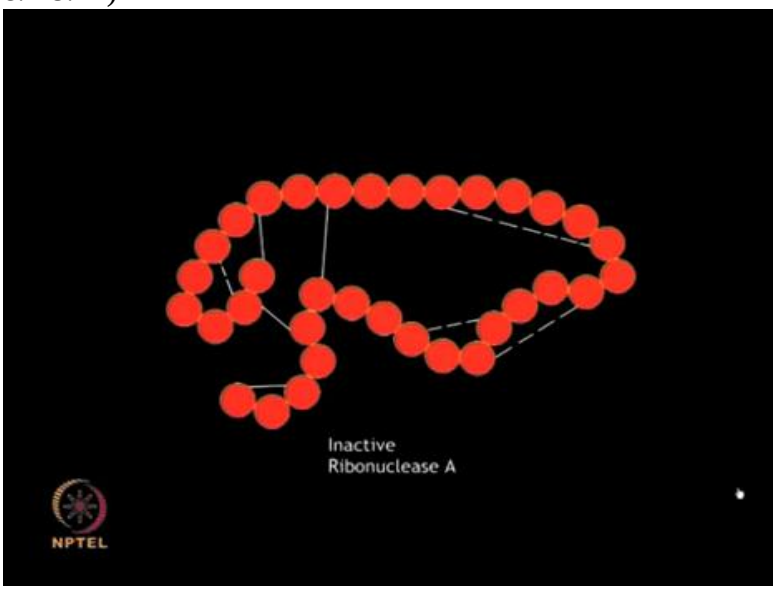

However if only beta Mercaptoethanol was removed in presence of urea, the formation of disulfide bonds was random which led to enzyme with only around 1% activity.

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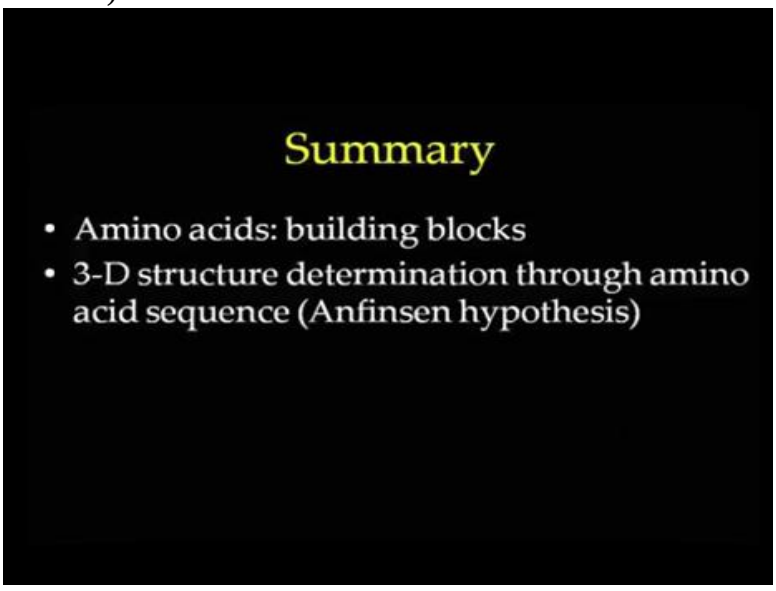

In summary we refresh our concepts on amino acids which are the building blocks. We then talked about a classical experiment of Anfinsen which has proved that all the information which is crucial for protein folding resides within the primary amino acid sequence.

We will continue our discussion on some basic concepts of proteins in the next class. Thank you.