Fundamentals of Protein Chemistry

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Module - 12
Special Topics in Protein Chemistry
Lecture - 60
Overview of Course

In our last lecture of this course on fundamentals of protein chemistry, we will be looking at an overview of the course as to what has been covered in the specific modules and understanding of protein chemistry which has been conveyed to you, the importance of an understanding of their structure and their function.

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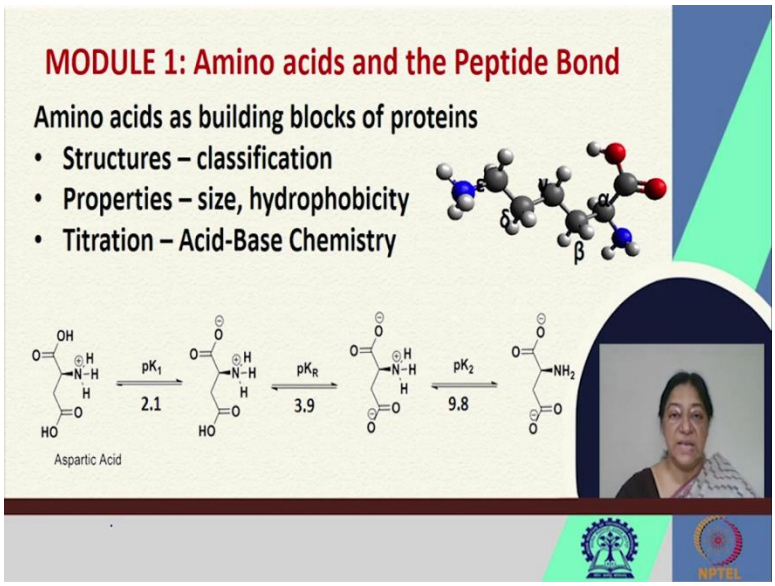

We start off with the first module that dealt with amino acids and the peptide bond. We looked at the amino acids as building blocks of proteins and understood their classification, their properties in terms of their size, their hydrophobicity and how we can do specific titrations in the acid-base chemistry that they are involved in.

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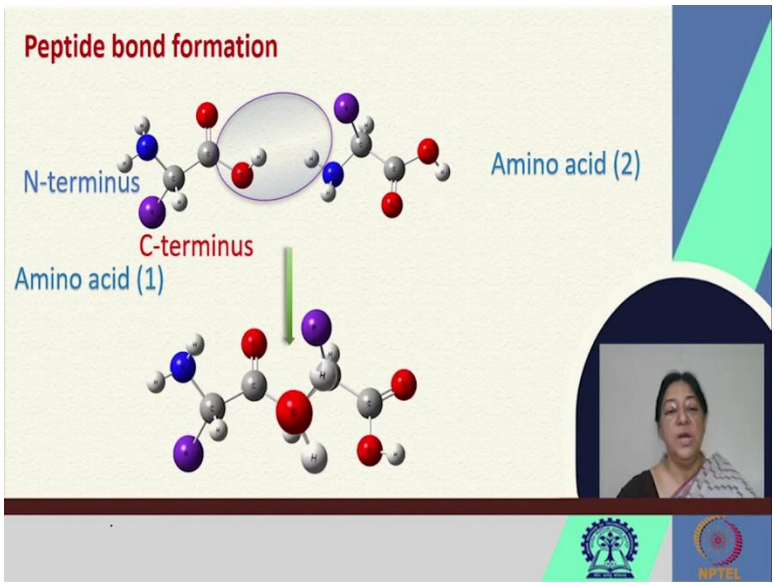

In the formation of the peptide bond we realize the presence of two amino acids in a specific condensation reaction, that would occur for the elimination of water and the formation of the peptide bond.

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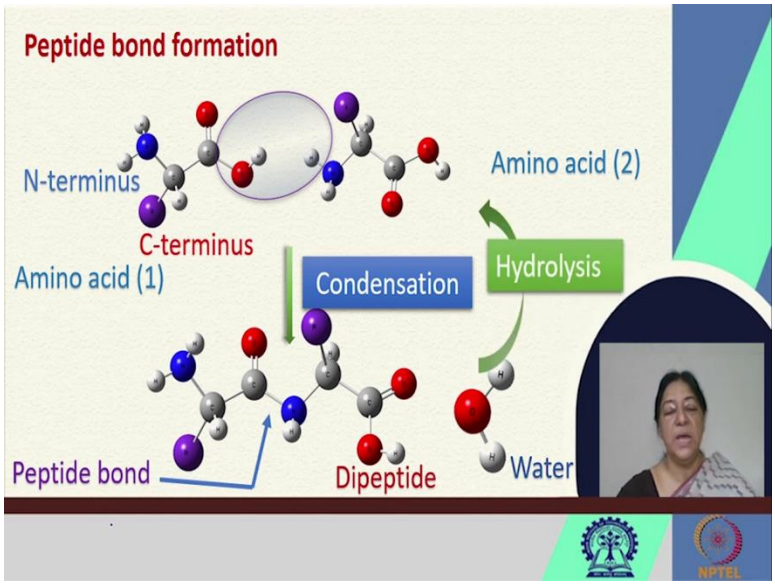

This [refer to slide] peptide bond, the covalent bond that linked the two amino acids together, could go on to form the larger protein structure that we went to learn on.

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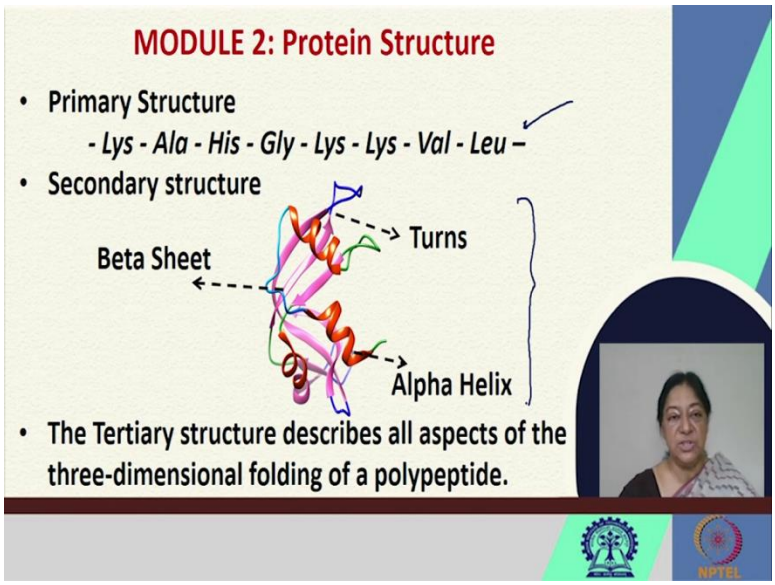

In the process of learning the protein structure, we got an idea of the specific types of amino acids that were linked together to form the primary structure. We found out the formation of secondary structures in terms of the α-helix and the β-sheet and the hydrogen bonding that formed these [refer to slide] specific secondary structures. The tertiary structure describes all the aspects of the three-dimensional folding of a polypeptide, in the specific interactions that were observed.

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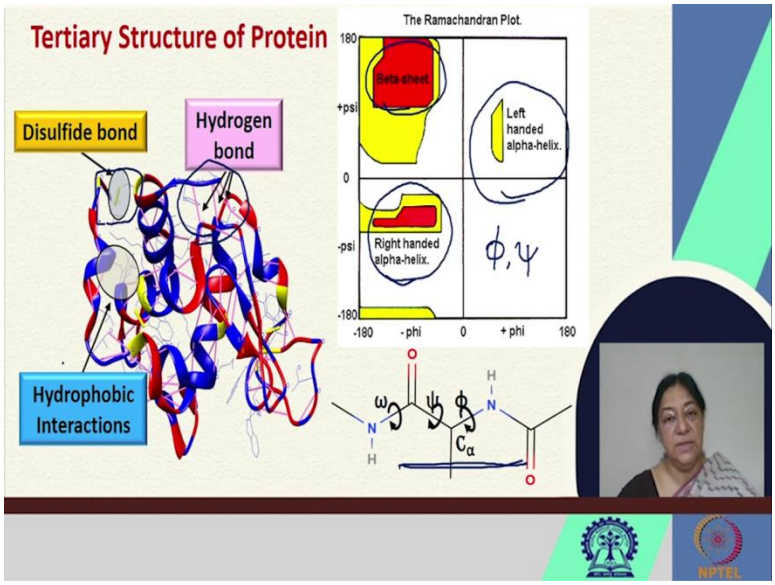

The specific interactions involved the geometry in terms of the rotations about the single bonds, giving us the Ramachandran plot; that gave us an idea of the location of the specific φ, ψ angles, where we could identify regions of the α-helix, the β-sheet and the left-handed α-helix.

The tertiary structure was formed from non-covalent interactions, where we had interactions such as the hydrogen bond, disulphide linkages that were the only other covalent linkages possible and hydrophobic interactions, in addition to Van der Waals forces. These held the protein structure together in a unique three-dimensional manner, that gives us our biochemical processes in the body.

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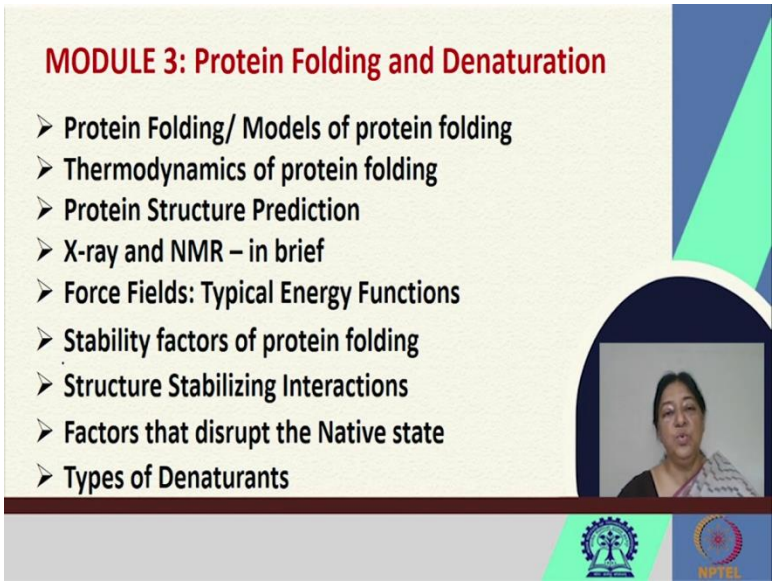

In protein folding and denaturation we looked at several models, the thermodynamics, a brief introduction as to what we meant by protein structure prediction in terms of X-ray and NMR. Energy functions that were a cause of the stability factors that resulted in protein folding and then we looked at factors that could disrupt the native state, in an understanding of denaturants that would lead to unfolding of the protein.

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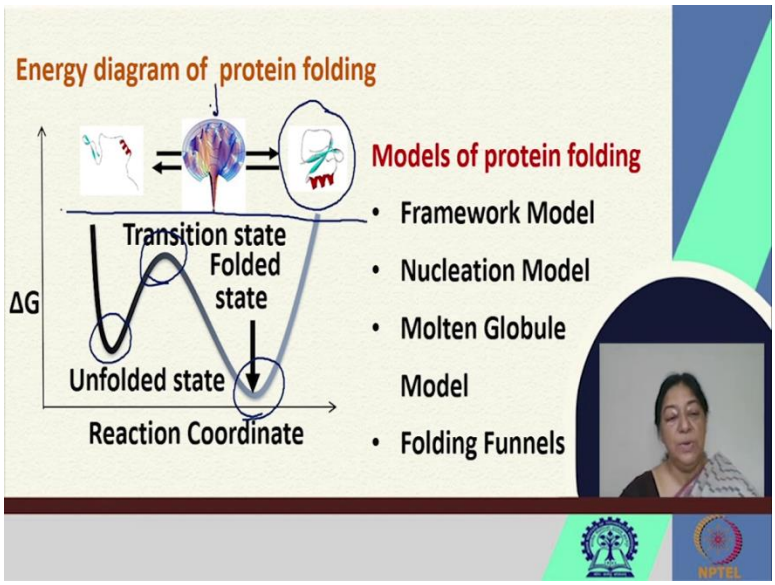

In this [refer to slide] case we looked at an unfolded state, we had a transition state and a stable folded state and if we looked at it from a theoretical standpoint, we understood that there were very many possibilities for an unfolded conformation that finally led to the folded three-dimensional structure, that was unique for the protein.

The models of protein folding that we looked at, were the specific types in terms of the framework, nucleation, molten globule and the specific folding funnels, that gave us an indication of how we can get to the native form of the protein.

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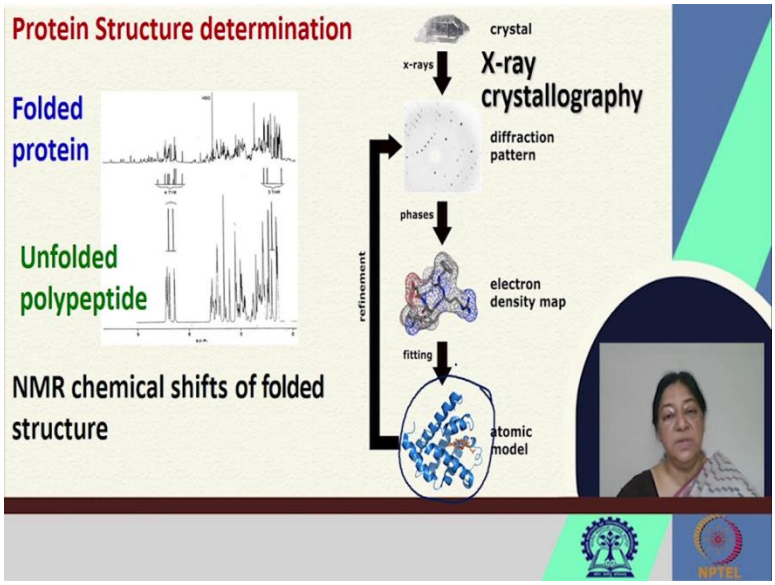

The protein structure determination methods were just looked at in brief and understood from an NMR chemical shift point of view for the folded structure and the X-ray crystallography that gave us the specific diffraction pattern, followed by the electron density map that would finally

give us an atomic model. The xyz coordinates that was so important to understand, the three-dimensional structure for which we could understand the function of the protein, followed by methods to inhibit enzymes if the need arises.

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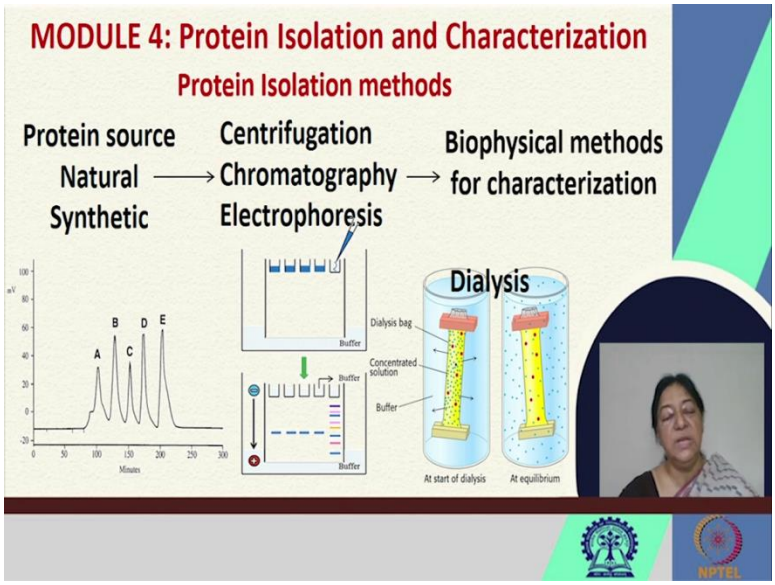

In the protein isolation methods that we looked at, the protein sources could be natural or synthetic. They could be expression of protein followed by centrifugation methods, chromatographic methods, electrophoresis methods, followed by specific biophysical methods for characterization.

In the gel filtration methods, we learned about how we can get the specific chromatogram based on the sizes of the protein. We looked at ion exchange chromatography, where we could exploit the charge on the protein for the separation and affinity chromatography, where we looked at the specific affinity for a specific ligand or a small molecule.

This would be followed by electrophoresis to get the molecule away, followed by dialysis to get the purified protein.

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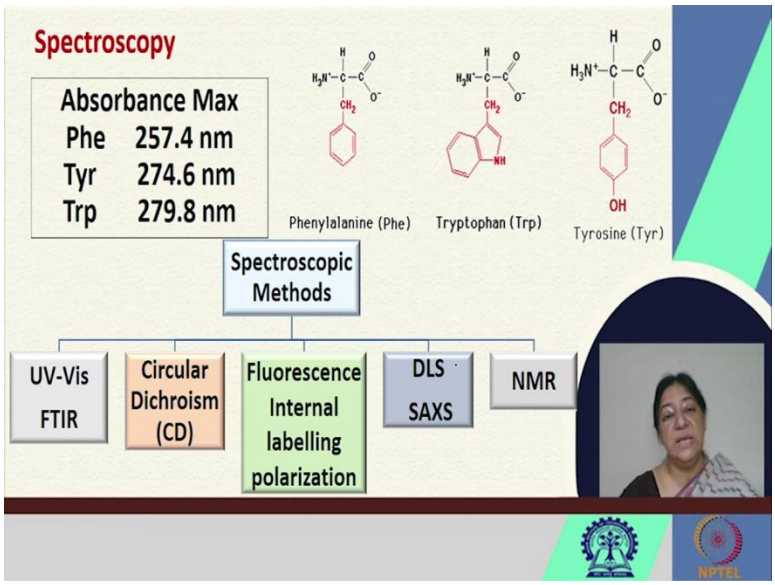

In the spectroscopic characteristics of the protein, we realize that the aromatic amino acids present in the protein would give us some signal that could be monitored for the specific studies, in an idea not only to understand the presence of the protein, but to understand the function of the protein and to see whether there were structural disruptions in the protein due to the change in the signals.

The spectroscopic methods that were looked at briefly were UV visible, FTIR, circular dichroism, fluorescence internal labeling polarization and DLS; we did not go into SAXS or NMR in that much detail. But the idea is that there are methodologies, biophysical techniques that can give us indications about our integrity of structure in this fashion, give us ideas about protein ligand binding; a specific subset of which is enzyme substrate binding.

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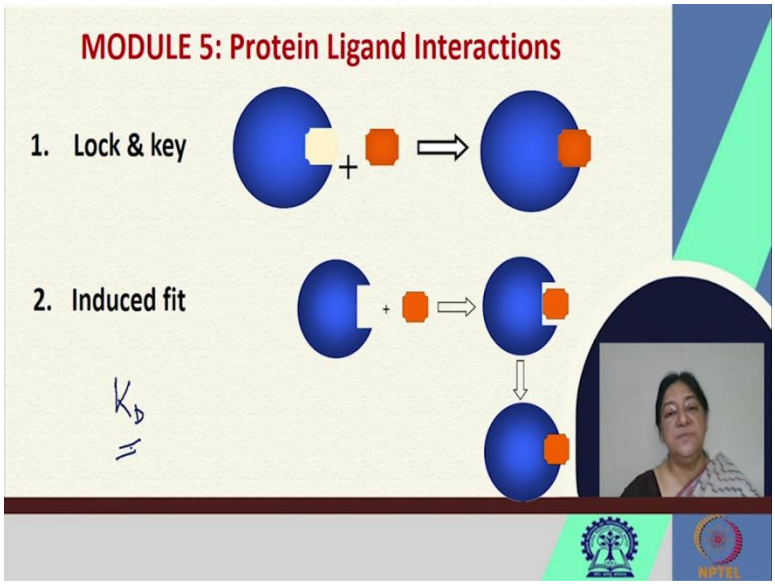

When we look therefore at protein ligand interactions, the idea was to look at a specific methodology as to how they interacted, what methods could be used to identify or to get an idea of the specific dissociation constants that we were involved in or interested in, to understand how tight the binding was. So we looked at a specific affinity, a specific binding characteristic.

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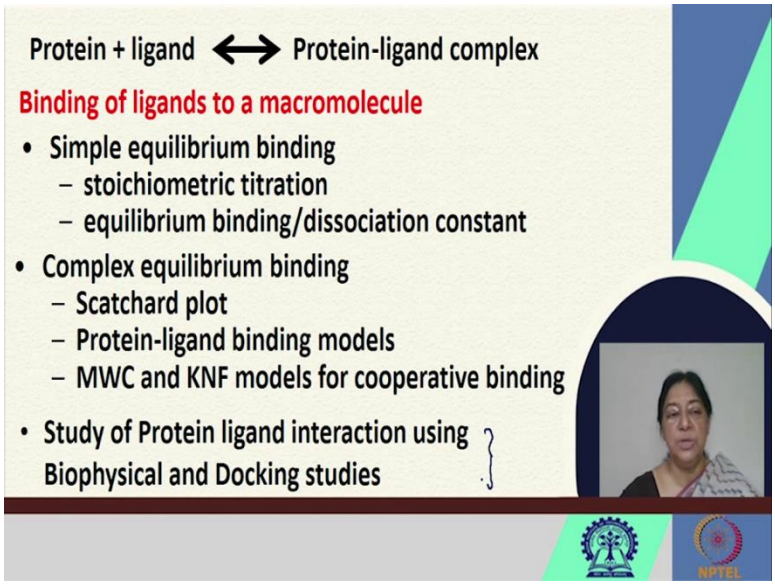

In this case we looked at simple equilibrium binding where there was stoichiometry involved, a dissociation constant that came as an important factor and then complex equilibrium binding, where there could be multiple sites, cooperativity, that would give us an idea of how our protein is interacting with the ligand.

This could also be done experimentally through many biophysical studies and through docking studies, giving us an indication as to where the small molecule is binding to the three-dimensional protein.

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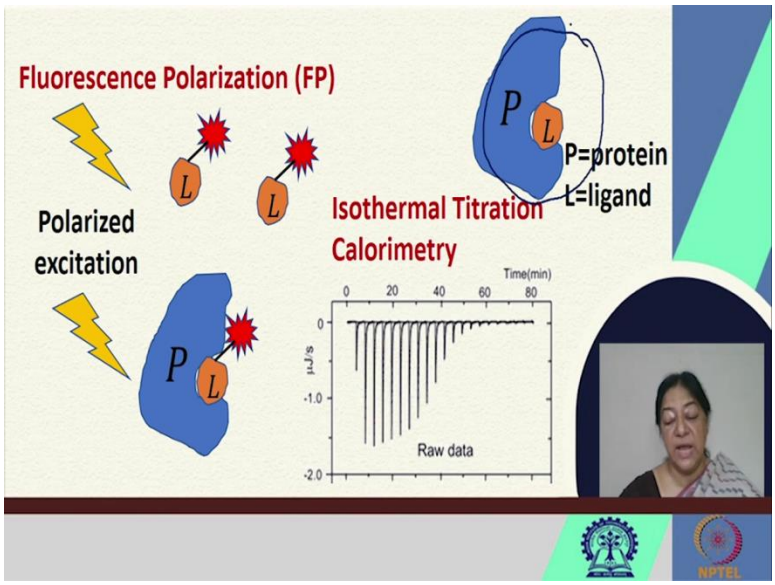

The fluorescence polarization methods were also discussed as was discussed an isothermal titration calorimetric method, in the understanding of these important protein ligand interactions.

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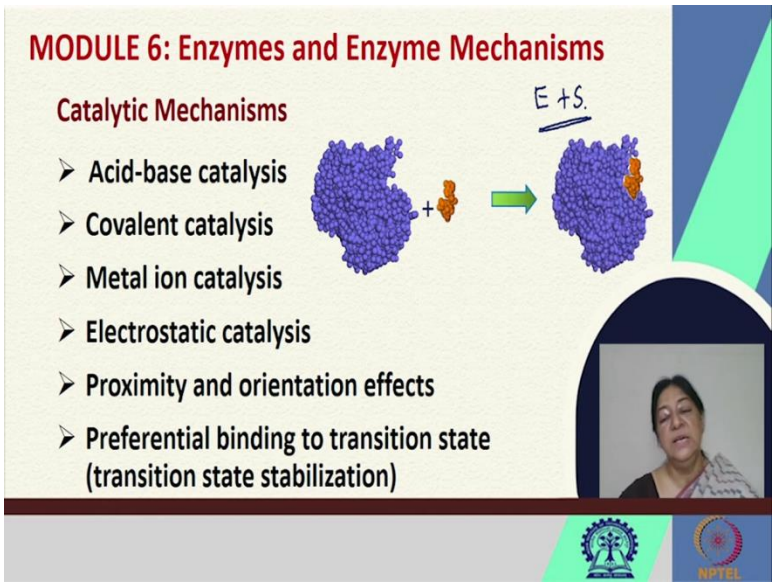

The subset or a special example of protein ligand interaction, are enzyme substrate interactions. These enzyme substrate interactions that give us our enzyme substrate complex, forms a product leaving the enzyme intact. We looked at specific enzyme classes, specific enzyme mechanisms and we realized that we do not want this enzyme substrate complex to bind too tightly because then the release would be difficult.

Nevertheless the molecular recognition that is so important, is very specific to the nature of the active site of the protein and the nature of the substrate and we realize that the catalytic mechanisms could be of different types.

We could have acid-base type, covalent type, metal ion catalysis, electrostatic catalysis, proximity and orientation effects that were important in the recognition of the ligand, in this case the substrate with the protein, the enzyme and we have the preferential binding to the transition state as we looked at the specific types of formations of the enzyme substrate complexes.

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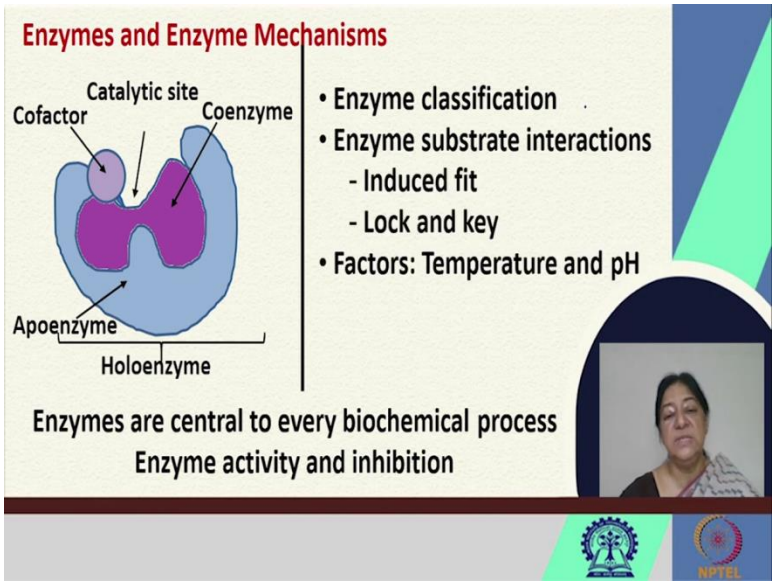

So when we look [refer to slide] at enzymes, we know they are central to every biochemical process and enzyme activity inhibition and an understanding of this is extremely important. Whether we have cofactors or coenzymes involved, the location of the catalytic site, the specific residues that line the active site that are involved in the reaction, are extremely important.

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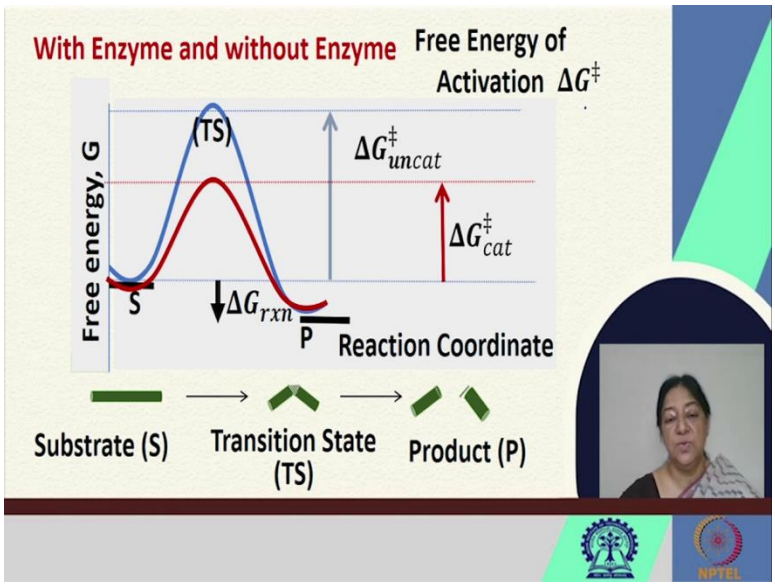

We looked [refer to slide] at all these specific processes and the factors that are important for the specific enzyme catalytic reaction, where we have the specific energetics associated with the catalysed reaction and an uncatalyzed reaction.

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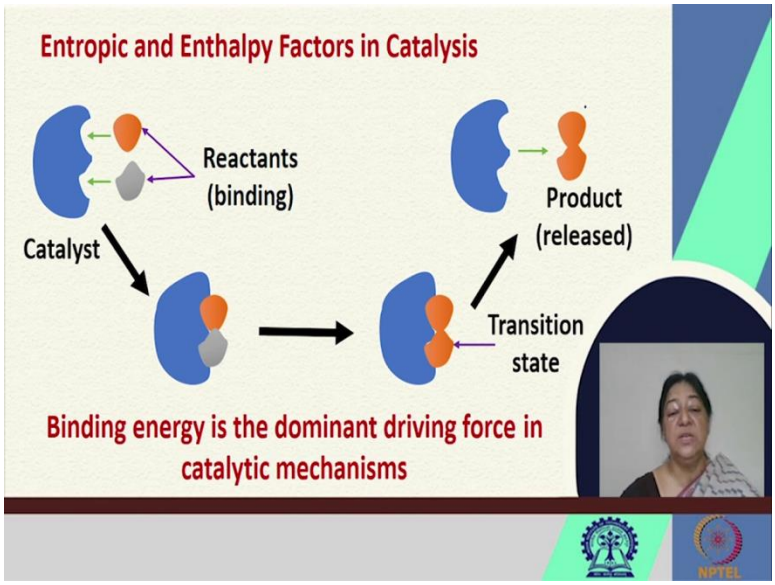

Another important factor was the entropic and enthalpy factors in catalysis, where we had the reactants binding, the catalyst being the enzyme, the formation of the transition state and the release of the product. So the binding energy associated with the recognition process, is the dominant driving force in these catalytic mechanisms.

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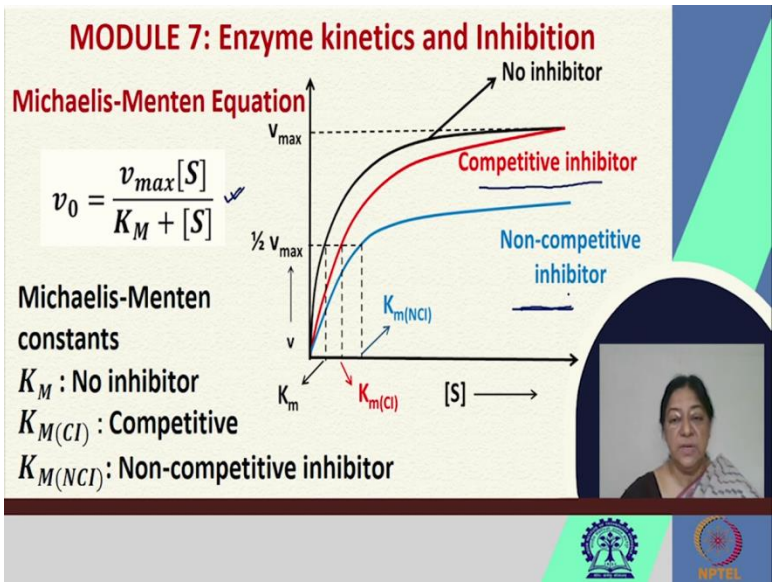

Our next module looked at enzyme kinetic procedures and inhibition of enzymes, where we looked at Michaelis-Menten kinetics, the specific graphs and the specific expressions that were found and we realized that we could have non-competitive; competitive and non-competitive inhibitors and an understanding of how they could be regulated to inhibit the action of the enzyme.

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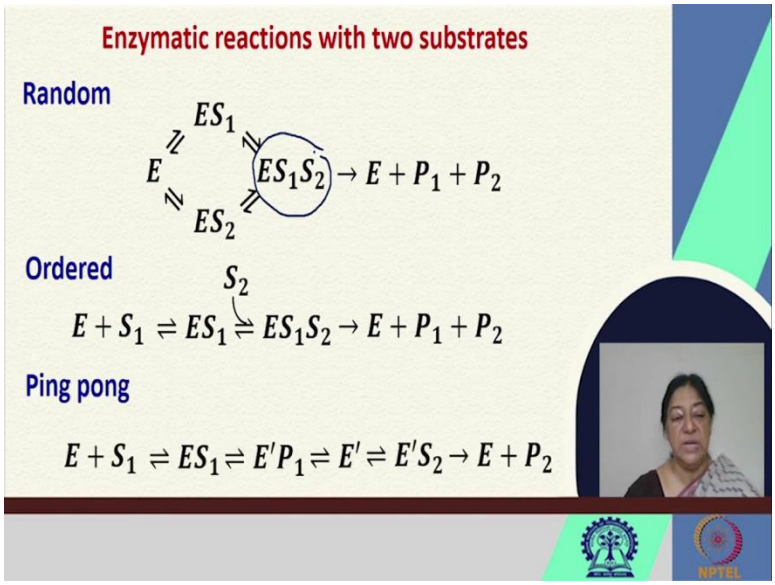

When we looked at these [refer to slide] specific Michaelis-Menten kinetics, we also had the possibility of two substrates being present and in this case there could be random binding, where we form a ternary complex with the formation of products.

There could be specific ordered binding in the formation of one substrate, then allowing an enzymatic conformational change to allow the binding of the second substrate and the ping-pong mechanism.

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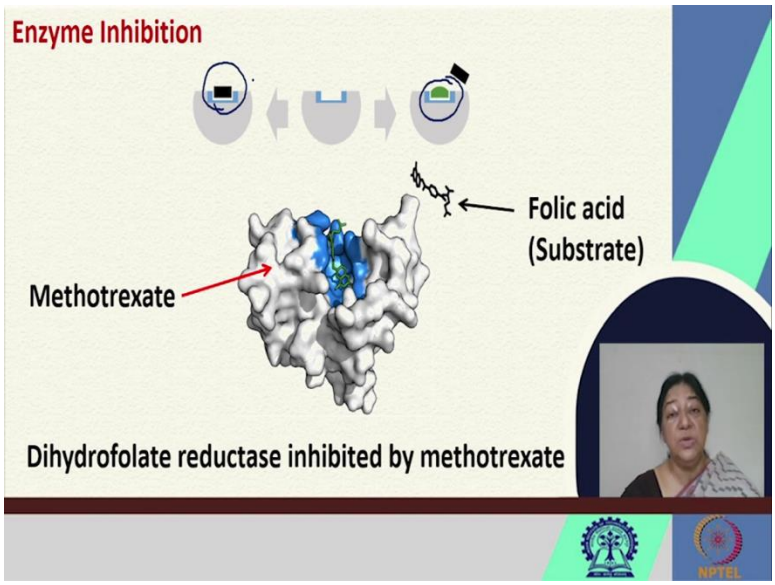

In enzyme inhibition, we looked at a specific example where we have dihydrofolate reductase, inhibited by methotrexate. The idea of competitive inhibition is that, the binding is to the same active site and the residues we looked at were specific mechanisms of enzymes.

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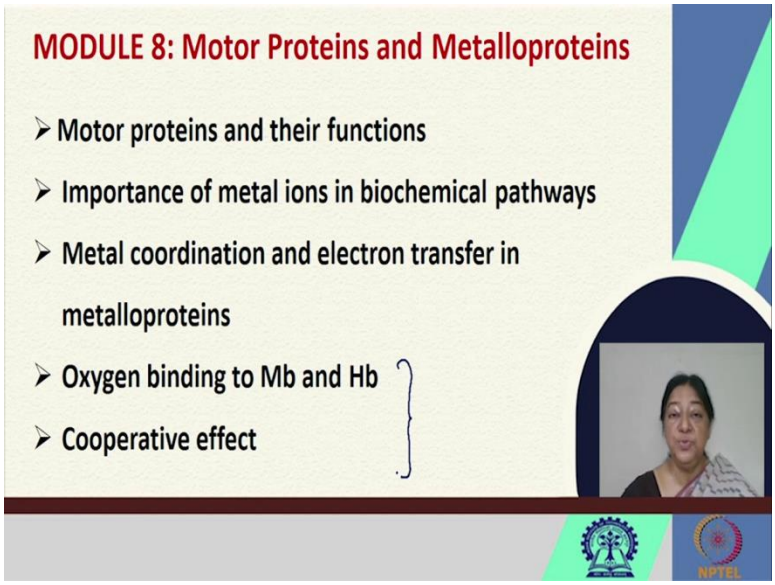

Then after this module we went on to look at very specific classes of proteins. In module 8 we looked at motor proteins and metalloproteins. The motor proteins and their functions, the importance of metal ions in biochemical pathways and how we have specific metal coordination and electron transfer in metalloproteins.

After that at the end of that module, we looked at a special case of haemoglobin and myoglobin in their oxygen binding and the overall cooperative effect that was observed in haemoglobin and

how it was important in oxygen binding. How we had the storage protein and the transport protein characteristics in this case.

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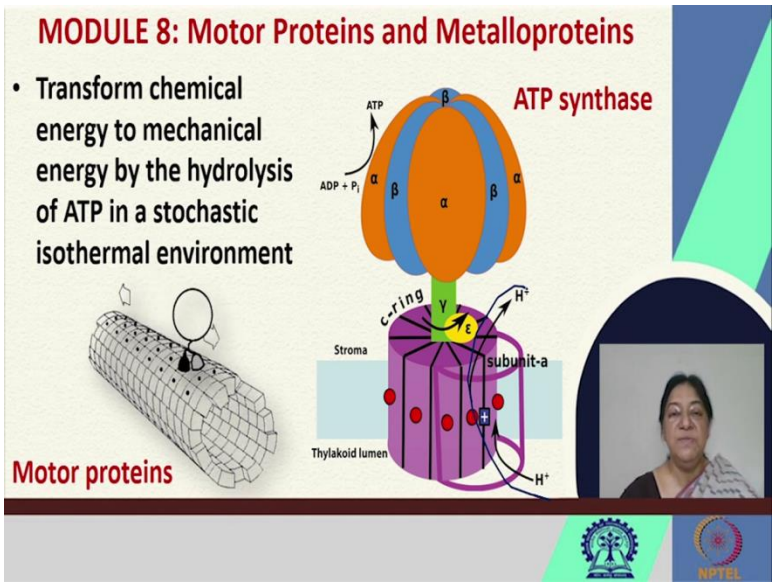

So, in case of the motor proteins we had the transformation of chemical energy to mechanical energy and these were associated by the hydrolysis of ATP in a isothermal environment.

The ATP synthase that was a proton regulated pump. As we saw in our understanding of how this motor protein could generate ATP, the energetic factor that is required for many reactions. The hydrolysis of which produces a large amount of energy, that is used as the driving force of many reactions and processes.

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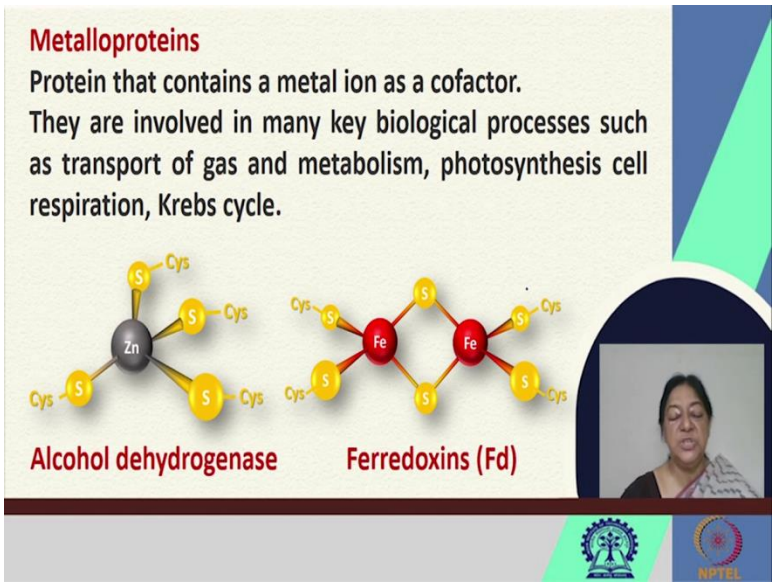

In the case of metalloproteins we understood that the metal ion is a cofactor and they are involved in many key biological processes such as the transport of gas, metabolism, photosynthesis, cell respiration and Krebs cycle. We looked at the structure of the ferredoxins and alcohol dehydrogenase, in terms of specific metal binding to these proteins.

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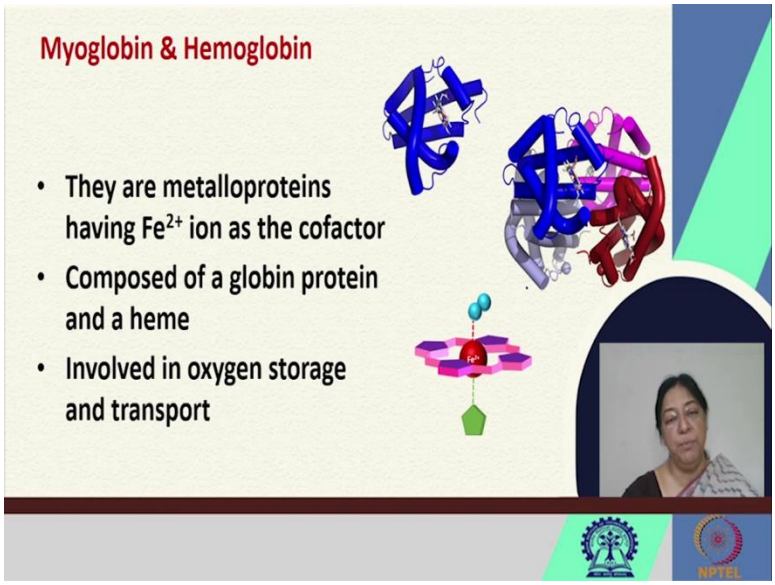

In the case of myoglobin and haemoglobin; the storage and transport proteins, the importance of the iron metalloprotein that has iron as the cofactor, that has the presence of the heme and the involvement in specific important processes in the body.

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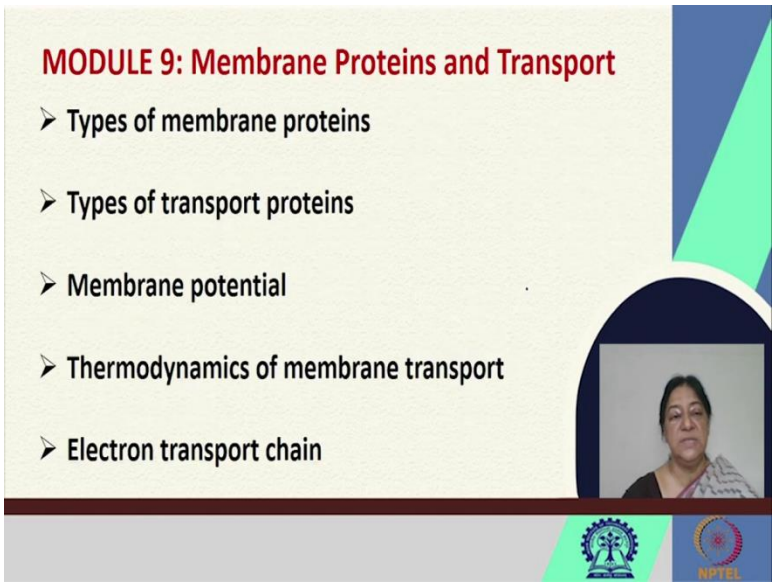

In module 9, we looked at membrane proteins and their transport. The different types of membrane proteins possible, the transport occurrences, membrane potential and the thermodynamics of membrane transport and how we have an electron transport chain, a complex of several proteins that result in very important reactions for biochemical processes.

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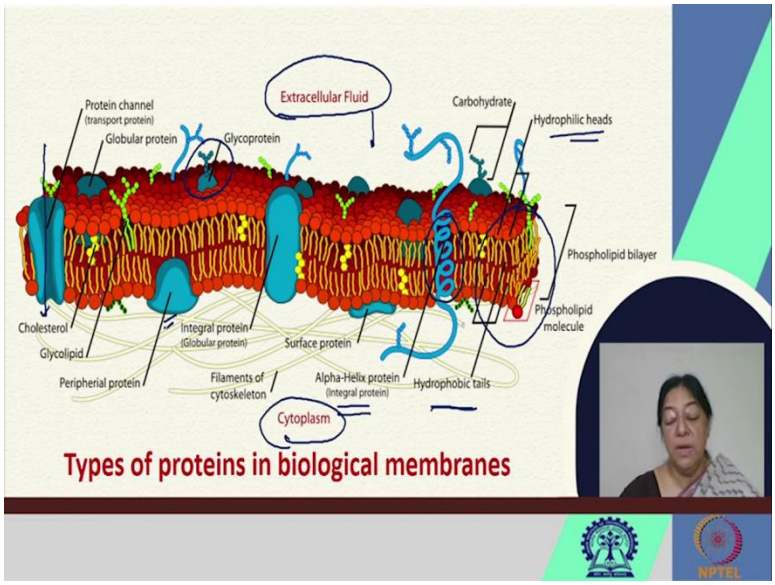

In the types of proteins and biological membranes, we understood that this is a very complex structure in terms of the presence of the lipid bilayer. The lipid bilayer where we have the hydrophilic heads, followed by the hydrophobic tails. The hydrophobic tails would interact with specific amino acid residues on the surface of what were called integral proteins; an α-helix integral protein given as an example here [refer to slide].

The hydrophilic heads on both sides, whether it was the extracellular fluid or the cytoplasm, formed this cell membrane in what is called a fluid mosaic model, where we have the recognition through glycoproteins, we have protein channels, cholesterol that gives the fluidity to the membrane and peripheral proteins that were involved in the important aspect of transfer across the membrane.

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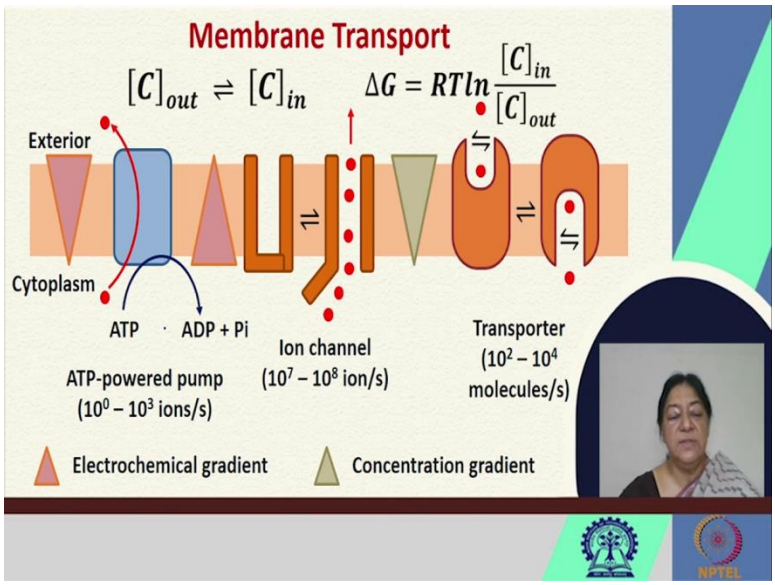

The membrane transport occurred through an electrochemical gradient, as well as a concentration gradient and we could have ATP powered pumps, ion channels and transporter proteins, that would be involved in this very important factor of membrane transport.

The different types of membrane transport in terms of the ports that we had, the antiport, the symport and so on and so forth. We looked at the concentration variations and the free energy of the transport.

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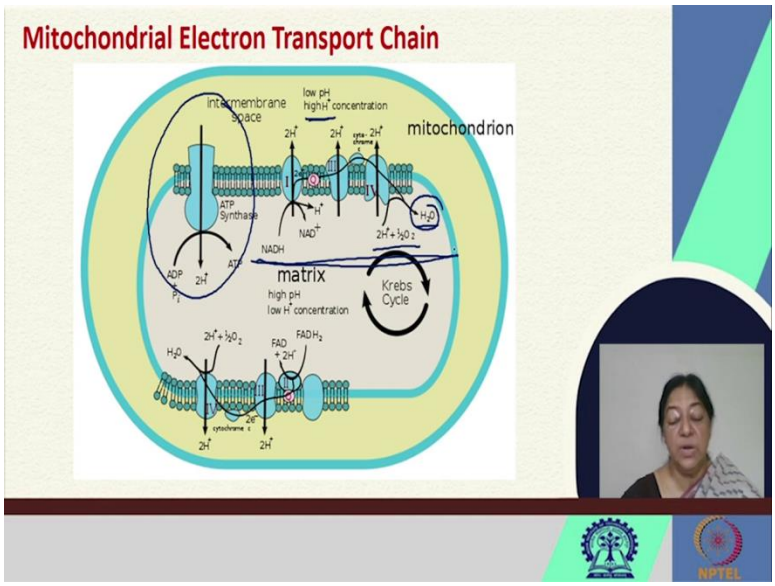

The mitochondrial electron transport chain, gave us an idea [refer to slide] about the reaction where we had the oxygen go to water and how the variations were present with the pH, in terms of the proton concentration across the membrane and this proton concentration is a variation in terms of how our ATP is working and how we have our whole electron transport chain working.

The function that occurred with specific cofactors, specific proteins that were involved in this very important process.

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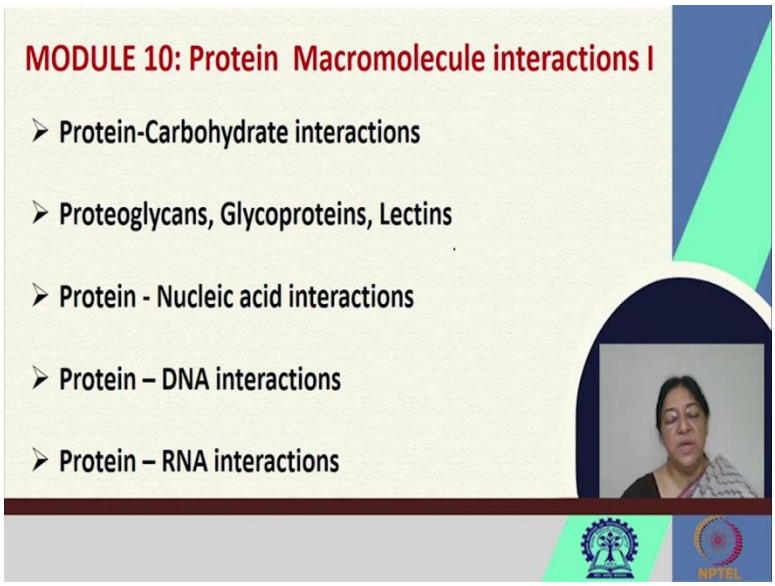

In module 10 and 11, we looked at protein macromolecule interactions. Considering that protein-protein interactions are extremely important in many biological processes, we understand that the specific interactions with carbohydrates, with nucleic acids, are important in many aspects of the processes that occur in the body.

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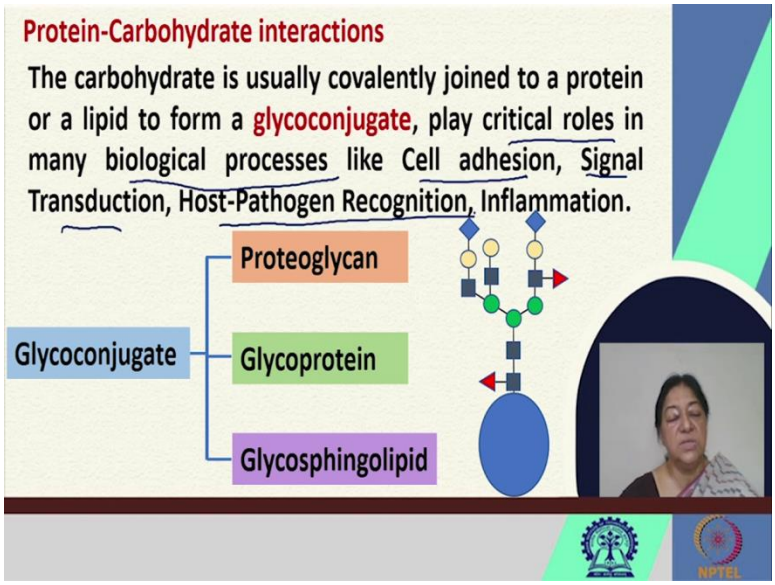

If we look at protein carbohydrate interactions, the carbohydrate we found out is usually covalently joined to a protein or a lipid, that forms a glycoconjugate and this plays critical roles

in many biological process like cell addition, signal transduction, host-pathogen recognition and inflammation.

So, the specific protein-protein recognition that occurs in terms of the glycoconjugate, where we have the proteoglycan, the glycoprotein or the glycosphingolipid in terms of the lipid interactions, is extremely important in many processes.

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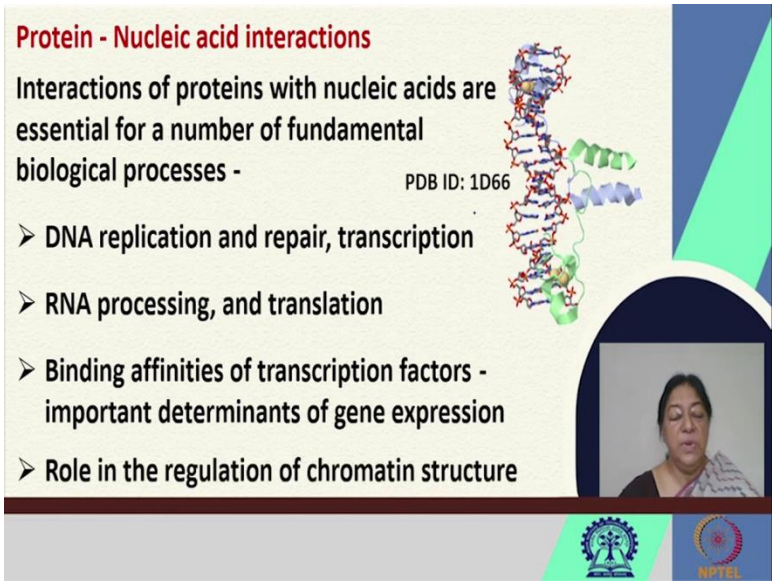

The protein nucleic acid interactions are fundamental for biological processes of DNA replication and repair, transcription processes and RNA processing and translation. We have the binding affinities of the transcription factors that are very important determinants of gene expression and we have the role in the regulation of the chromatin structures.

We realize the importance of each of these interactions, in the specific roles that they have been designed to play. We have therefore, all these processes that are important in an understanding of the protein.

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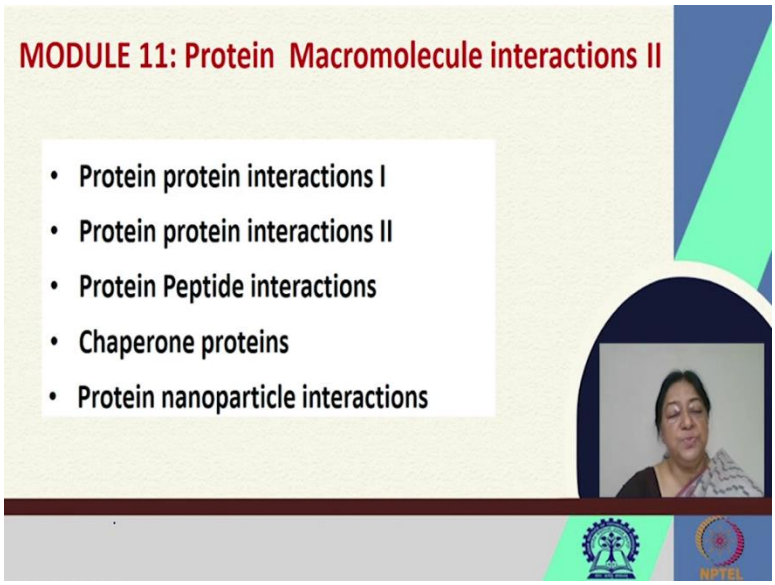

In module 11 we revisited protein macromolecule interactions, in looking at protein peptide interactions, protein-protein interactions, chaperone proteins and protein nanoparticle interactions.

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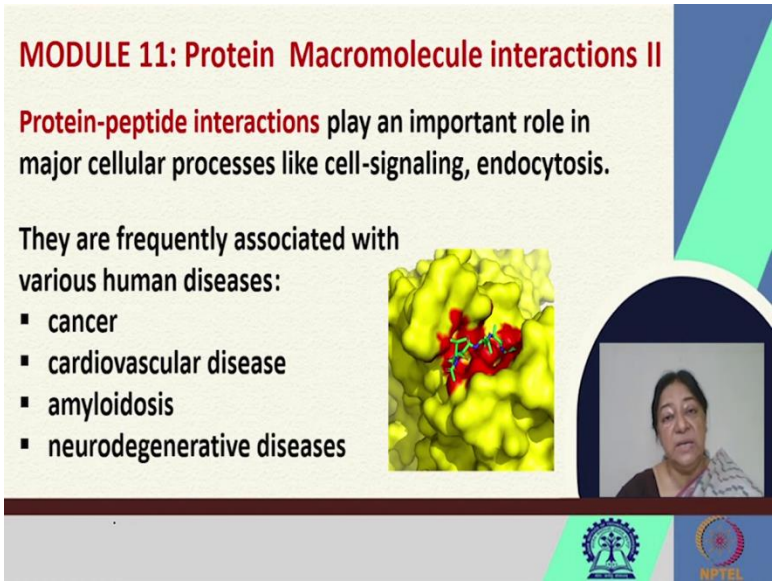

In the case of protein peptide interactions, we realize their important role in major cellular processes like cell signalling, endocytosis and how they are associative with various human diseases. The specific recognition sites that are present in an understanding of how inhibitors may be designed, with an understanding of the active site involvement in the molecular recognition process.

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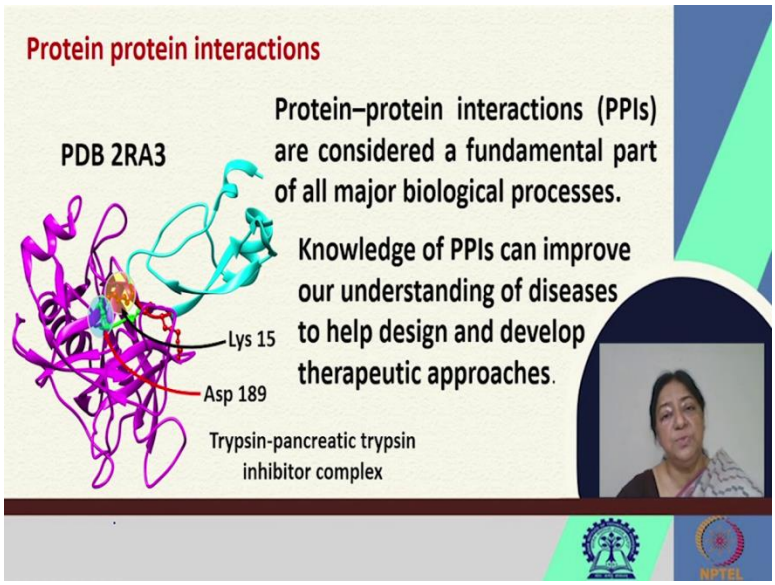

In protein-protein interactions therefore, we understood their fundamental part in all biological major processes. The knowledge of how an understanding of their interactions can improve and help design specific therapeutic approaches.

So, whether we are looking at the specific types of residues involved or the types of interactions involved, in this [refer to slide] case for example there is a specific ionic interaction. Whether we are looking at a hydrophobic type or a hydrogen bonding type, all of these interactions involved in the molecular recognition process, are extremely important in an understanding of these interaction processes that occur.

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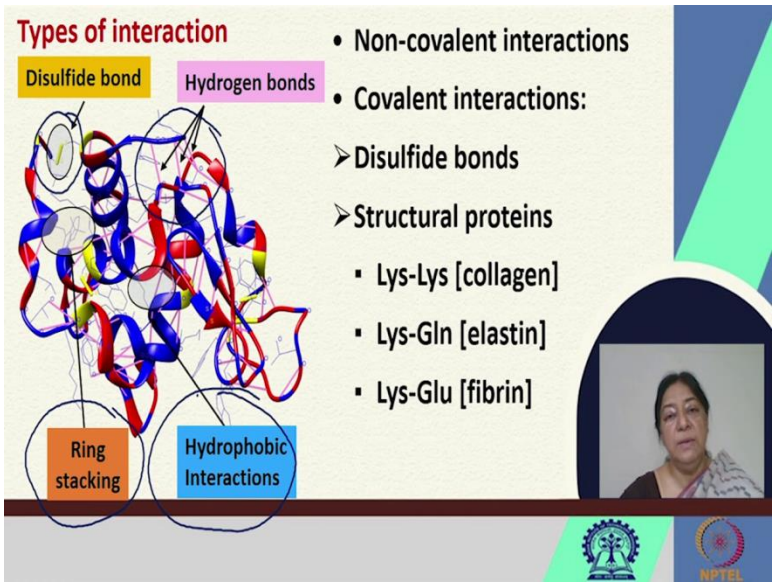

So, whether they are the disulfide bonds that are formed, the hydrogen bonding, the ring stacking or hydrophobic interactions, all of these play a very important role in not only the structural integrity, but also in the molecular recognition processes.

All the non-covalent interactions, the covalent interactions, disulfide bonds and specific interactions associated with structural proteins, that give us an indication of how they may be studied and understood.

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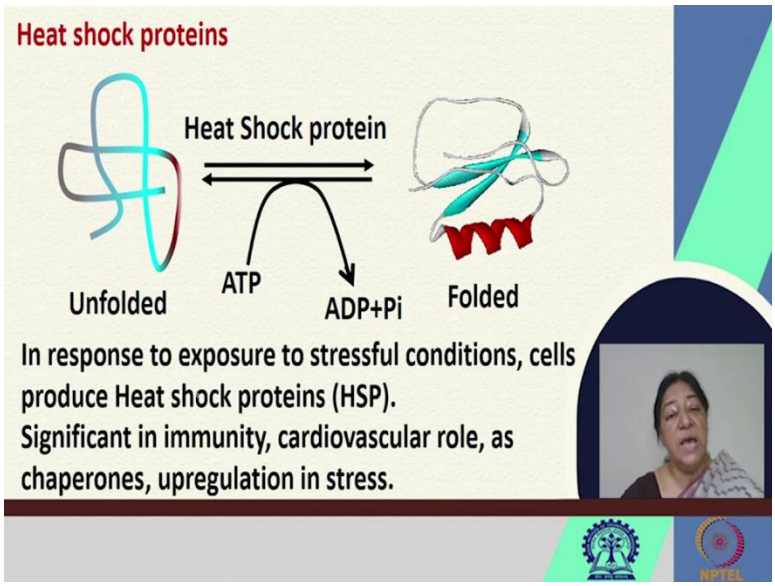

For heat shock proteins, the chaperone proteins, they occur in a manner or at the process where we have ATP hydrolysis taking an unfolded protein to a folded protein. So in response to exposure to stressful conditions, cells produce these heat shock proteins. The realization that there is an unfolded protein that could form a folded protein.

These play a significant role in immunity, cardiovascular roles and diseases, as chaperones and they have an upregulation under stress condition.

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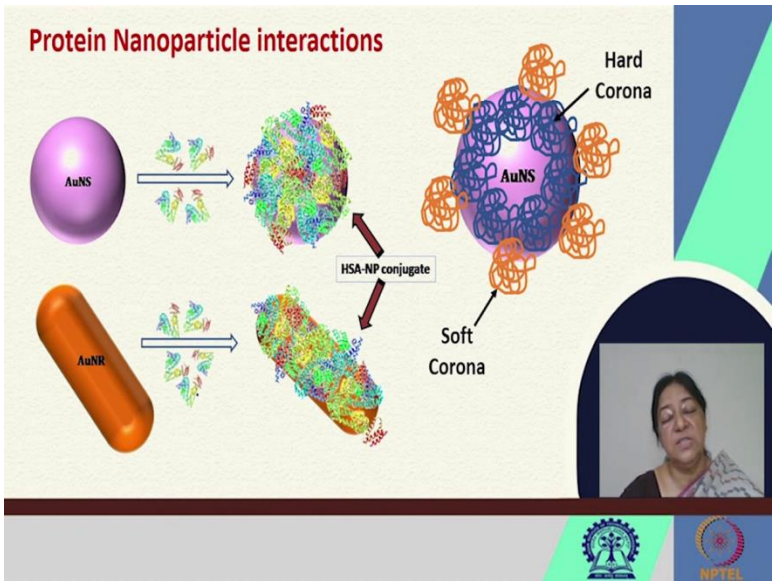

In protein nanoparticle interactions, we looked at how the nanoparticles could have what is called a corona around them and how the shapes and sizes could affect this corona formation. How they could be used for drug delivery and an understanding of protein nanoparticle interactions in terms of the metals that are used, in terms of the shapes and what spectral characteristics could change accordingly.

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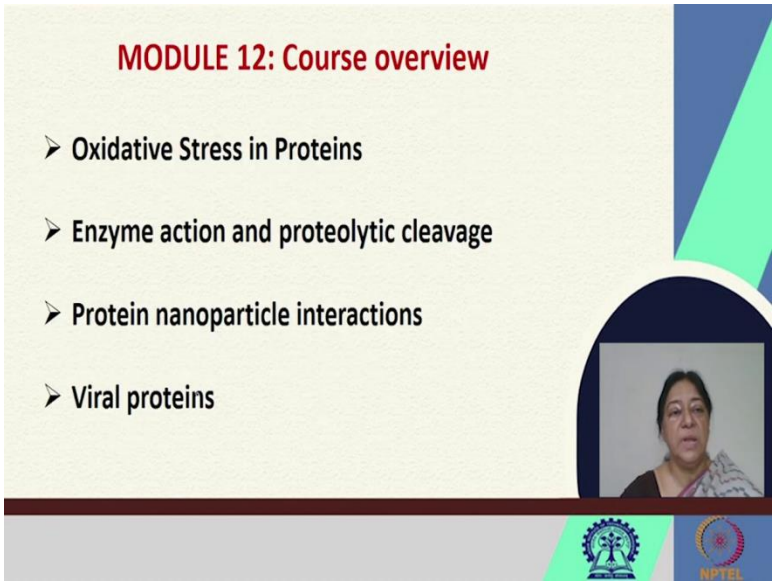

In module 12 where we are looking at the course overview, we came across special topics like oxidative stress in proteins, enzyme action and proteolytic cleavage, protein nanoparticle interactions and viral proteins.