Essentials of Biomolecules:
Nucleic Acids, Peptides and Carbohydrates
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Lecture 05
Protein 3D Structures, Folding and Denaturation

Hello everybody and ah welcome back to the biomolecules, so last last lecture we have talked about the structure of the proteins. How the protein assumes its secondary and the tertiary structure.

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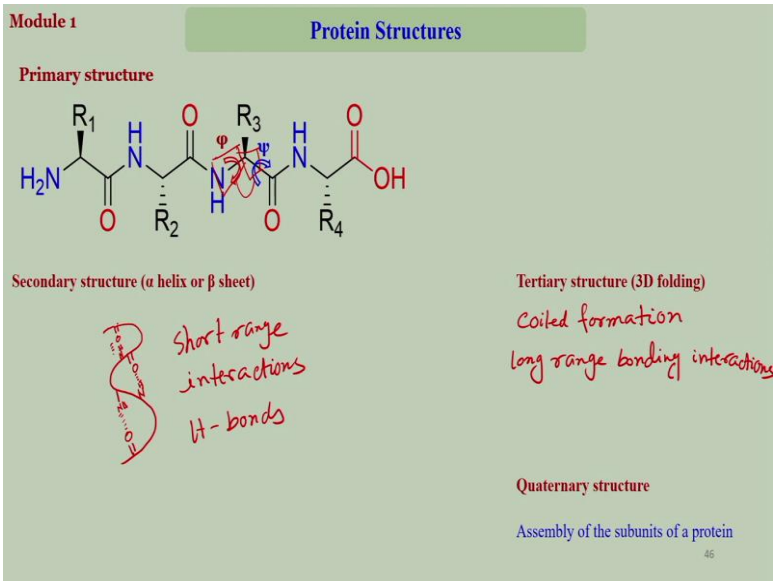

So, ah once again quick recap that you have a primary structure of the protein that is governed by the sequence of the amino acids and we have seen that if you draw a amino acid sequence in this fashion then there are two different kinds of angles that can be forming. One is for the single bonds so one is this bond which is termed as Phi the other one is this bond which is termed as Psi. So, these two bonds are of course in different plane and there is an angle that is created between the planes this is called the dihedral angle.

And so if you rotate one of them fixing the other then you change the torsional angle and therefore the structure of the protein gets changed. So, this is the primary structure of the protein which essentially means the sequence of it the amino acid specific amino acid sequence. Now the secondary structure of the protein which we have seen that it can either assume alpha helix or beta sheet that comes from the rotation around the Phi bond or the Psi bond.

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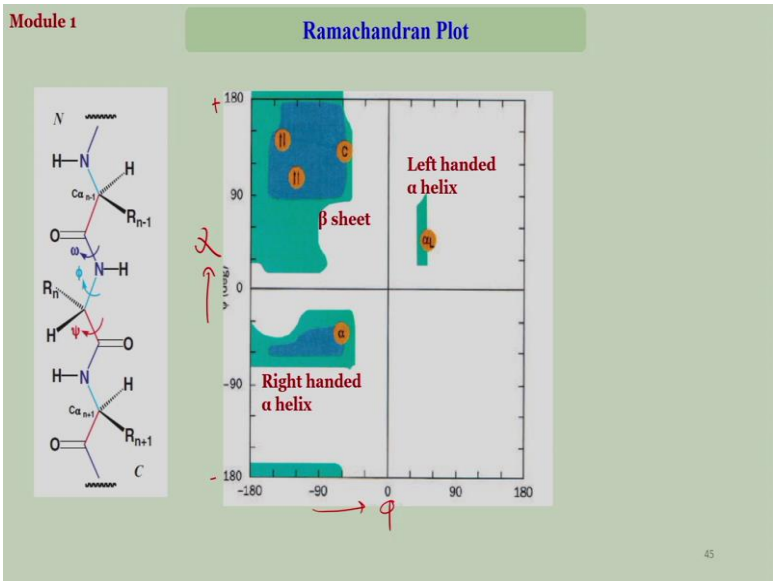

So, as we have seen here and that you can calculate by using the Ramachandran plot, Ramachandran plot essentially tells you that which rotations of the Phi angle and the Psi angle are actually allowed. And there is you can put dots or the points for all the rotations if you make a plot of this is the Phi goes from -180 to +180 which makes it 360 degree rotation similarly the Phi and the Psi goes from -180 to +180 that makes it again a 360 degree rotations.

So, if you try to rotate them simultaneously or keeping one fixed and rotating the other then you will have a plot like this where these coloured points basically tells you that which rotations are energetically allowed and those will of course if your density is the high then that means those kinds of rotations will give you a stable structure of the protein and that is what we call the secondary structure of the protein which is the Alpha either mostly the alpha helix or the beta sheet.

There can be different kinds of alpha helix there can be different kind of beta sheet formation. So, if you assume an alpha helix structures it will look like maybe something like this so where if you rotate one then it will come to such a position that the peptide bond one carbonyl group of the peptide bond and the amine of the other peptide bond may come in close proximity and form the hydrogen bond. Similarly it can be here NH this is C from the backbone and they can come in hydrogen bonding formations that makes it Alpha X4 Alpha helix.

Similarly here can be a carbonyl here can be an H and these are the NH and the CEO of the backbone the peptide this NH this CO. Now this forms the alpha helix structure.

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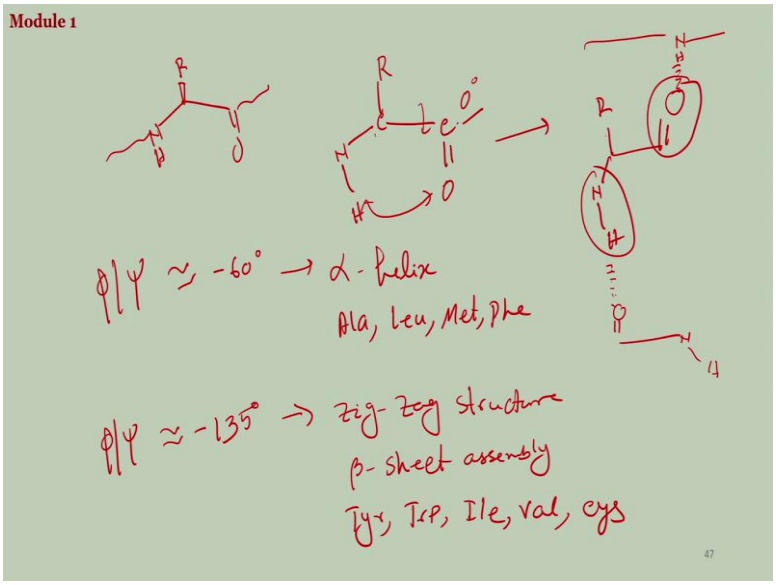

If I consider one of this bond so R if you consider and then what you have is here NH and a CO NH and it goes like this and then you have a CO goes like this so if i make here is your R here is your N and this H see here is your CO it assumes this kind of orientation let us say. So, then if we look at the distance between the NH and the CO so this is around 0 degree angle the distance between the NH and the CO would be like this here and they can come close in proximity and can form maybe hydrogen bonding or may not be I mean they may need to come more closer sometimes.

Now technically if I just make a 180 degree rotation then this CO I am rotating around this bond get flipped off you still have the R and you still have the NH like this. Now if the distance between the CO and the NH gets completely opposite and that creates the change of the secondary structure so here there cannot be a hydrogen bonding between this NH and this CO but it can assume a hydrogen bonding with another CO of a second strand.

So if you consider another molecule of the same protein it cannot have a hydrogen bonding with that. Similarly if you have the other strand they can form hydrogen bonding so that is the difference between the changing of angle by changing the angle you change the geometry of the primary structure that brings you to the secondary structure. So, typically if your Phi and Psi angle as I think I have mentioned it in the last class is around -60 degree then you have a alpha helix formation.

Because it will rotate it and bring it to that level where the NH and the CO can come close in proximity and of course this is governed by the sequence what sequence the peptide has or the

protein has. And very commonly if you have alanine Leusine methionine phenylalanine and there are more they typically tend to form alpha helix kind of structure. And if your Phi versus Psi angle is around -135 degree then that assumes a zigzag structure of the protein and that means they are prone to form beta sheet structure or beta sheet as I will call it assembly.

Because it happens between two molecules or more than two molecules so this is basically a supra molecular architecture or supra molecular arrangement of the different protein molecules on the other hand alpha helix or the hydrogen bonding that is a that exists within the alpha helix is more intra molecular process. And beta sheets is more intermolecular hydrogen bonding and that makes it a supra molecular self-assembly.

So relatively larger amino acid like tyrosine, tryptophan and the crowded wants Isoleucine hydrophobic crowded hydrophobic with branched chains isoleucine, valine, cysteine. These kinds of amino acid if they are present in a sequence or if the protein is rich in such sequences then those segments will be more prone to form beta sheet kind of structure so, within a protein if you consider a protein within a protein a certain segment of the protein can assume alpha helix conformation certain other part of the protein can assume beta sheet confirmations also.

Not necessarily the whole protein will be either alpha helix or beta sheet no. So, that is the secondary structure of the protein alpha helix or the beta sheet and this is formed as I mentioned due to the short-range interaction primarily hydrogen bonds. Now once the secondary structure is formed it undergoes further modifications to assume a tertiary structure of the protein which is basically the functional protein. So, the tertiary structure means is the three-dimensional structures or the folded structure of the protein which comes from coiled formation.

So the protein will further mould into a fold its form or a coiled form that is known as the tertiary structure. And this is because of relatively long-range a bonding interaction that assumes the tertiary structures. I will just show you in a brief period how the interactions will take place? What are the long range introductions that that happens? This is mainly for example that if you have secondary structure which is the alpha helix here.

And then if you have a polar group or let us say an amine functional group here and then a carboxylate functional group sidechain in this case and then they want to come in close in proximity because of the electrostatic interactions. The amine group is positively charged acid

group is negatively charged so they want to come closer together. So, that will force this helix to go further fold so that the negative charge and the positive charge can come closer and form electrostatic bond.

Similarly there are other types of interactions that I will just briefly explain very quick. So, these are the long range arranged bonding interactions that forms a tertiary structure of the protein. Now apart from the starchy structure there is another structure that some proteins have that is known as the quaternary structure. Quaternary structure is basically if a protein sometimes a protein has many subunits more than one unit of a protein there are many other units we call them subunits or they are they can be called all oligomeric proteins.

So, those different subunits of the protein are interconnected of course so that way they are interconnected and form our total structure is known as the quaternary structure. so, it is basically the quaternary structure is basically the assembly of the subunits of a protein.

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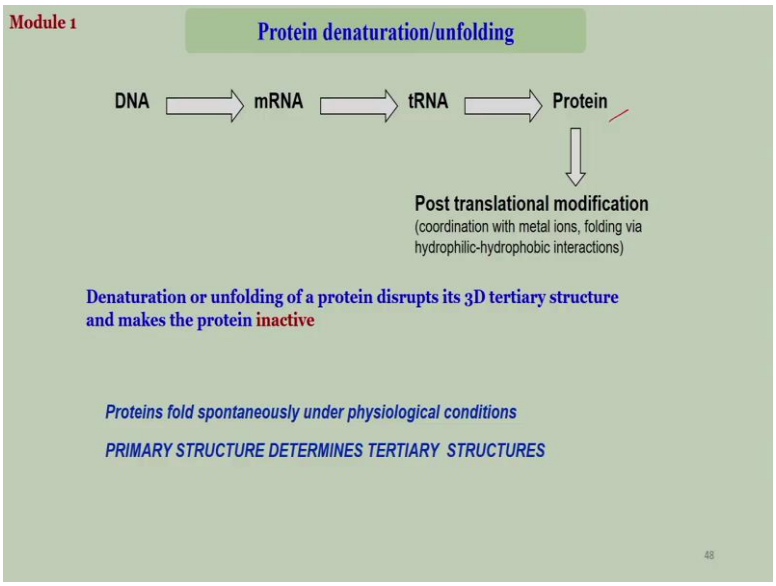

So, as you know that when a protein is formed protein is usually biosynthesized in our cellular systems in the living cells. How is it wire synthesized all the details will have a separate chapter for it that it comes from our genetic code that is DNA and then it goes to formation of a messenger RNA or known as mRNA and from mRNA it is transferred to tRNA and then the tRNA synthesizes the protein.

So, when there is the synthesis of the protein of course it synthesizes the covalently linked primary structures the sequence of the protein. So, once our sequence of the protein is

synthesized then depending upon its sequence it will automatically resume or assume the secondary structure and then the secondary structure will go to the tertiary structures that is the functional protein. So, once a tertiary structure of a protein is formed then only it can do our biological transformation or it will have up certain biological activity.

And of course again and again I am repeating that what kind of tertiary structure a protein will assume is always dictated by the primary structure of the protein that is what sequence it has. Now there are lot of studies that has been done or still been carried out to understand how the protein is folded into that kind of specific structure why not anything else why not a slightly different type of three-dimensional structure possible for the protein. Similarly how you can unfold a protein from the tertiary structure to its primary structure.

And to study the dynamics of those all these processes. So, we call it a typically a denaturation when a protein is unfolded that means you are breaking all the interactions that is present in the tertiary structure and then bringing it back to the kind of primary structure so that is the process is known as as the denaturation of the protein or you can also call it unfolding of a protein. So, the moment you unfold a protein or you force a protein to denature its three-dimensional tertiary structure is disrupted and therefore the protein cannot function anymore this is called the inactive protein.

And we will see just in a brief time that folding of a protein from this primary sequence to the tertiary structure is a spontaneous process under physiological condition and of course the primary structure determines the tertiary structure of the protein. So, now what kind of interactions are present within the tertiary structure of a protein if you understand that then only you can think of breaking those interactions and make and unfold or denature a protein.

You can find a suitable way how to denature a protein or how to unfold a protein and then can find out again methods to study the dynamics of those processes.

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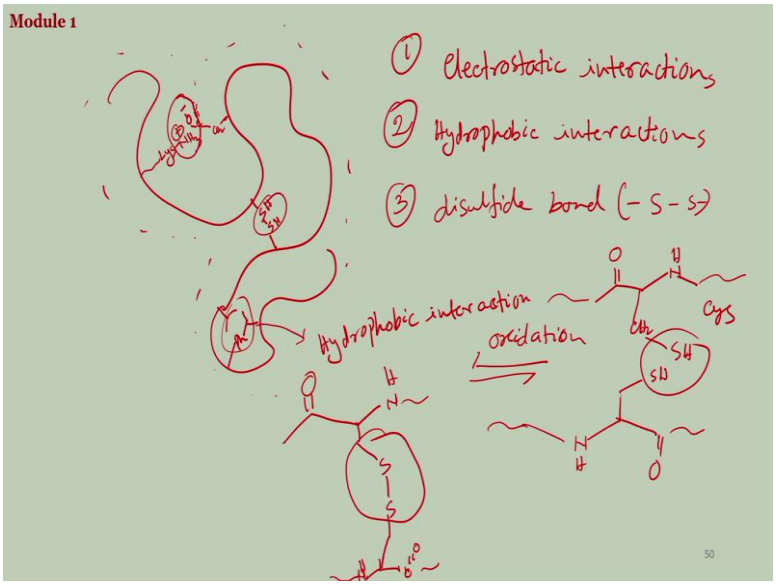

So, if I take an example of a tertiary structures just I am just drawing anything arbitrary like this so what are the forces that makes a tertiary structure or what are the bonding interactions that forces a protein to assume a tertiary structure. One of them is of course the interactions of the sidechains. Sidechains can be of very different types as we have seen we have 20 amino acids with very different functional groups very different properties.

So, depending upon the sidechains the interactions can vary also. So, one is if you have the electrostatic interactions, electrostatic interactions what does that mean? So when you have seen that secondary structure you have seen the hydrogen bonding so were present within the Strand for alpha helix for beta sheet it was it was between the molecules. So here further for the sidechain if I consider for example I consider a lysine.

Lysine as amine sidechain there are 4 CH2 groups I am not drawing them and there is amine here and this amine assumes as you have seen in two it is positive charge form. Now if you have closer in the opposite direction somewhere if you make it a flat structure you will see the distance between this lysine and this residue here is actually very large you have to go all the way here. But because of such interactions like if you have aspartic acid for example aspartic acid is CH2 and then you have a CO then you have OH and this OH of course exists in O minus.

So now you have a positive charge you have a negative charge so if in the secondary structure if there is a chance that this lysine and the aspartic acid somewhere else are to some extent in close proximity then in order to form a better bond in order to form electrostatic interactions

between the positive charge and the negative charge it will force the strand to mould more and bring the positive charge and negative charge within the bonding interactions.

So, that forms the tertiary structure and of course this is a long range interaction as I am saying because if you look the distance between this and this is quite long. But the spatial distribution or the special arrangement forces these two residues to come closer together and that holds the protein. So, one reason for assuming the tertiary structure is electrostatic interaction. Second if there is an electrostatic interaction and then there will be the opposite one the hydrophobic interactions also.

Hydrophobic interactions means so when a protein is in a solution typically in within the biological cells as I have said that it has a pH of 7.4 so more or less and it is an aqueous medium. So, protein is in an aqueous medium in a pH of 7.4 roughly. So, there are water molecules surrounding it and hydrophobic side chains of the amino acids they repel water so they would not want to be exposed towards water their tendency will be to go away from water and height.

So, typically that happens here so if you have multiple number of hydrophobic residues or a hydrophobic sidechains for example the valene in isoleucine and may be fitted phenylalanine and so on so they would want to go or hide away from water and they will form a core. So, if you have for example of valine here I am just making another coil, valine here CH3 CH3 and maybe another valine very close here.

If you have phenylalanine here so they will all try to gather themselves to form a hydrophobic core it says called the Vanderwaals interactions or hydrophobic interactions. So, water molecules are outside all outside here all water molecules surrounding the protein. So, they will Psi away from the protein from the water and want to go inside the interior of the protein. And once they go inside the protein they have the the hydrophobic molecules also have the hydrophobic interactions within them so that is a stability factor.

So second is hydrophobic interactions a third one very common actually is formation of the disulfide bond. So, if you have a cysteine so this is cysteine within one protein molecule and let us say there is another system maybe within the same same protein or can be in a different protein and in a different protein molecule so CH2 and NH. So, these two if you keep if you

take a cysteine and keep it in air so it will immediately undergo oxidation so redox reaction basically that will form CH2 S S CH2 and here is the this CH2.

And here is the rest of it CO and the NH and the rest of it, so it will form a disulfide bond and that is a covalent bond so quite strong bond. So, cysteine-cysteine residues if they are in close proximity that will tend to form a disulfide bond if you keep it in air under aerial condition or anaerobic condition. So, and that will further ah force the helix or the beta sheet to mould it and bring them closer so that they can form the covalent bond.

So, here if you have a self SH residue or the cysteine residue on the on the same strand somewhere else if you have another system let you do and if they are in open chain so they will fold themselves and form the disulfide bond that is the driving force. The formation of the disulfide bond is a driving force for moulding the structure and there are other interactions in the next slide I will show you with proper examples.

So these are primarily 3 types of interactions that dictates the formation of the tertiary structure. So, I will give you an example how the disulfide bond is formed in protein.

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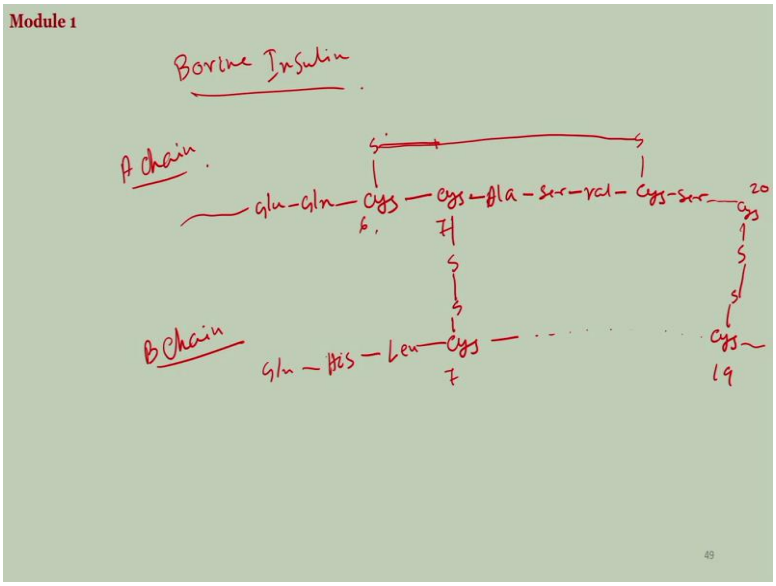

So, very well-studied protein actually is bovine insulin you know insulin is a medicine that is used to treat the diabetic patients right it is basically an enzyme. So, bovine insulin was actually the insulin which used to be used as the medicine to treat such diabetic patients. Now a days it is we can we can manufacture insulin from human sources itself after the discovery of the recombinant DNA techniques.

So, bovine insulin is a very well studied protein and that shows multiple number of disulfide bonds. So, it has two chains it is called a chain and that has a sequence I will just write I am writing the part of the sequence glutamic acid then glutamine then you have a cysteine another cysteine alanine Sherine valine then assistene then I said in and it goes and there is another cysteine at the position 20.

So, this cysteine position is 6 this is 7 position and this is 20th for opposition of the cysteine. Now this cysteine has a SH residue this cysteine has a SH residue and they can form the disulfide bond that locks the protein structure. Similarly the second cysteine I am sorry cysteine alone insulin valine cysteine valine cysteine I'm sorry for here there is SH and this is and this is can form the disulfide bond.

And therefore the protein structure has two mould or has two coil so that these two can come within the bonding distance bonding interactions. Similarly this has a sh and in the next chain of the protein molecule of insulin known as the B chain in this there is another cysteine which comes closer to it opposite. So, before it it has alucian then histidine and then a glutamine and so on. so, this is also the s7th position of it.

And then here there is another cysteine and this position is 19. So, this system has tile this system has a tile and they form disulfide bond very quickly. Similarly the s of this the s of this they form disulfide bond very quickly. So, that overall structure has are many disulfide bonds and that is the driving force for the tertiary structure of the bovine insulin. So, here I have made a schematic presentations of how different interactions are present that makes our tertiary structure of the protein.

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So, this is for example this is the secondary structure of the protein the secondary structure of the protein and it gets down to the tertiary structure of the protein which is the coiled form or the folded form. So, basic laws of chemistry that drives the protein folding are first is the hydrophobic sidechains that has been termed given the colour as black for example this and this they are the hydrophobic side chains as I mentioned valine isoleucine leucine phenylalanine all those they do not have our functional groups or polar sidechains.

So, they are termed as black here they will be buried on the inside of the globular protein where they are hidden from polar water molecules. Water is polar and they are surrounding the protein molecules as I have explained so these things when they form the tertiary structure they want to hide away from water and forms this core kind of structures. So, that is the driving force for the protein to fold in this fashion.

Second is the charged side chains means it can be up to charge on it is positive charge other is the negative charge negative charge is for the acid residues like the aspartic acid and glutamic acid. The positive charges coming from the amine residues for example the lysine and arginine both have a mean sidechains. So, blue and the red are marked for positive and negative sidechains. So, they will be on the surface of the proteins.

Because since they are charged species they would want to interact with water molecules also because the water can reach them is polar or and of course the charged species are also polar so they they primarily would be exposed towards water. And of course they will have the interactions within them bonding interactions or electrostatic interactions within them positive and negative. Polar sidechains other than the charged ones which has polar functional groups will be again on the surface of the protein they would be on the surface.

So that is represented by green dots they would be on the surface of the protein because that is the part where they are exposed to water molecule. So, they can interact with water molecule and get solvated that is a very effective stability factor the solvation energy when a molecule solvated or surrounded by water molecules and that is a highly energetically favourable process. So, the polar sidechains will be on the surface of the protein and they can form the hydrogen bond with the water that is the solvation factor.

Fourth is the cysteine side chains cysteine is tough I have given it as purple colour here for our bond formation SS bond formation that often interact with each other to form covalent disulfide bonds that stabilize the protein structure. So, these are the factors that are responsible for the formation of the tertiary structure of a protein. And obviously now you can see how the primary sequence or how the primary structure of the protein the sequence of the amino acids are dictating the folding of the protein depending upon their sidechains.

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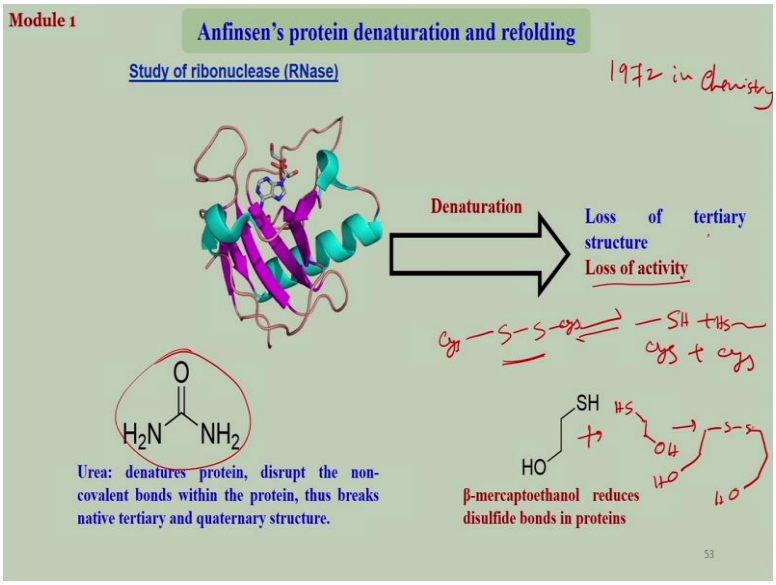

So, a classic study of how the protein folds and unfold was done by Professor Anfinsen in this is called Anfinsen's experiment or Anfinsen's protein denaturation and folding. So, Anfinsen received Nobel Prize in 1972 in Chemistry he has been working for a very long time on the enzyme ribonuclease Rnase. He was one of the key persons who has worked a lot on RNase that is ibonuclease and a part of his research was of course to see how the tertiary structure is formed in the protein.

Why it is such why does it assume such specific structure how you can unfold a protein and can inactivate a protein. So, he has done a complete study and dynamic study for the protein denaturation and refolding. So, it is a sequence of process and that first studies in order to study the folding of the process what he has done he has first tried to denature the protein and how can you denature the protein and then refold the protein again.

So we have seen what are the forces that are involved in the tertiary structure. Now if you want to denature a protein then you have to break all those interactions. And therefore now you can find a way or think of a way how can you denature though or how can you break such kind of interactions then only you can denature the protein. One of them is of course heat you can heat the protein high up and then break all the non covalent interactions but it still has the problem that sometimes the protein degrades by itself or there are other bonds as you will see that especially the disulfide bonds and other which are hard to cleave by heat.

But there are other regions which you can cleverly use to clip those bonds. So, denaturation of a protein means the loss of tertiary structure once. The tertiary structure is lost it will lose its

activity. So, what Anfinsen found out that if you use urea then you can denature a protein. Urea is not very simple reagent of course is a reagent that denatures the protein. It disrupts all the non covalent bonding interactions within present within the protein and therefore you break the native tertiary structure as well as the quaternary structure.

But after denaturation with the urea you still have the disulfide bonds left which you have to cleave and therefore the second reagent is coming which a mercaptoethanol. It is a very simple reagent structure is very simple what it does is that it is anti redox I mean it changes it undo the process of the disulfide bond formation. So, when you have the cysteine here and cysteine there in a protein they formed a disulfide bond.

Now if you treat these with beta mercaptoethanol this will reverse the process it will make plus two individual cysteine by cleaving the disulfide bond. And in the process this molecule itself so this is also and this has also a thymine bond two of these molecules will undergo disulfide bond formation OH S CH2 CH2 OH. So, this will form the disulfide bond and undo this process. so, it is a redox reaction as I have said so this will be oxidized and the protein will be reduced to individual systems. Therefore you can break the cysteine-cysteine disulfide bonds also.

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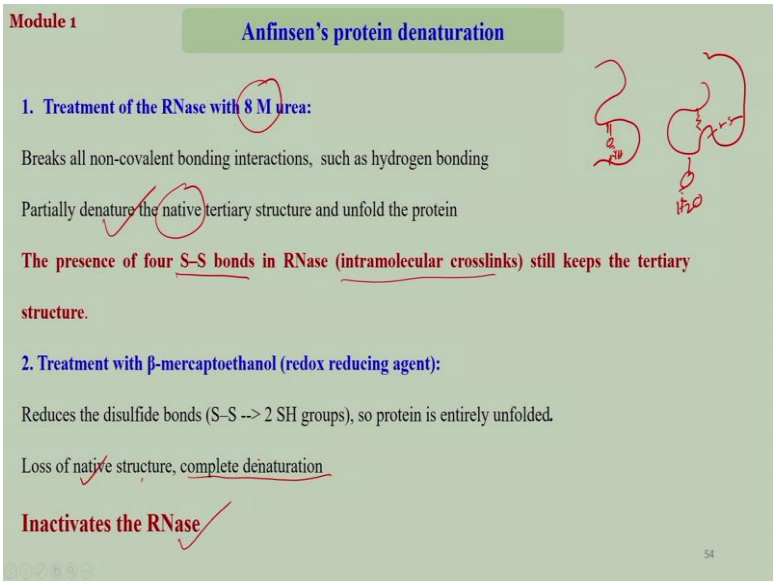

Here is how the process goes on it is done in two steps the first step is treatment of the protein that is in this case RNase with H molar of urea and function has found out that the concentration of urea is optimum if you use it on 6 to 8 molar urea is ideal for denaturation of the protein. So, that is the optimum condition to denature the protein. So, once you use urea 6 to

8 molar then it breaks all the non covalent bonding interactions such as the hydrogen bonding that were present.

Hydrogen bonding so are present within the ah secondary structure also as I mentioned as well as in the tertiary structures with the water molecule within them within the molecule as well as the polar if you have the polar sidechains and they form hydrogen bonding with water also. So, lots of hydrogen bonding will be present within for the tertiary structure of the protein itself as well as the secondary structure of the protein.

So, urea will break all the hydrogen bonding interactions at the same time it will break all the charged interactions all the other Vanderwaals interactions and the electrostatic interactions also like the charge species lysine with the aspartic acid those kinds of interactions will all be broken by using Urea. But still once you destroy all the short-range interactions or the long-range bonding interactions you still have written the disulfide bonds.

So, therefore after treating with urea it partially denatures the native tertiary structure. Second as I said is a treatment with a beta mercaptoethanol it is a redox reducing reagent that will oxidize itself and reduce the disulfide bond back to the cysteine. So, once you treat this with the beta mercaptoethanol then you have completely disrupted the tertiary structure of the protein and that will lose the native structure therefore complete denaturation will happen.

And once you denature the protein that then the protein or in this case the RNase is completely inactivated it cannot do its biological function that is what Anfinsen has found out and have studied. So, after this treatment or of this two consecutive process and Anfinsen has found out that RNase the enzyme RNase does not retain its biological activity. The question is can you refold the protein can you make the protein functional again.

So, that is what is very interesting study actually once you denature the protein can it assume its activity biological function again which means the unfolding structure or unfolded structure and the folded structure of the protein do they exist in equilibrium. So, that is what he has also studied now in order to refold the protein what you have to do so you have urea in it by using urea you have disrupted all the short-range interactions or all the weak interactions weak bonding interactions non covalent interactions I mean.

So, unless and until the urea is present you cannot only grow those bonding interactions. Similarly you have used beta mercaptoethanol to break the disulfide bond. So, you have to remake the disulfide bond again and remove the beta mercaptoethanol.

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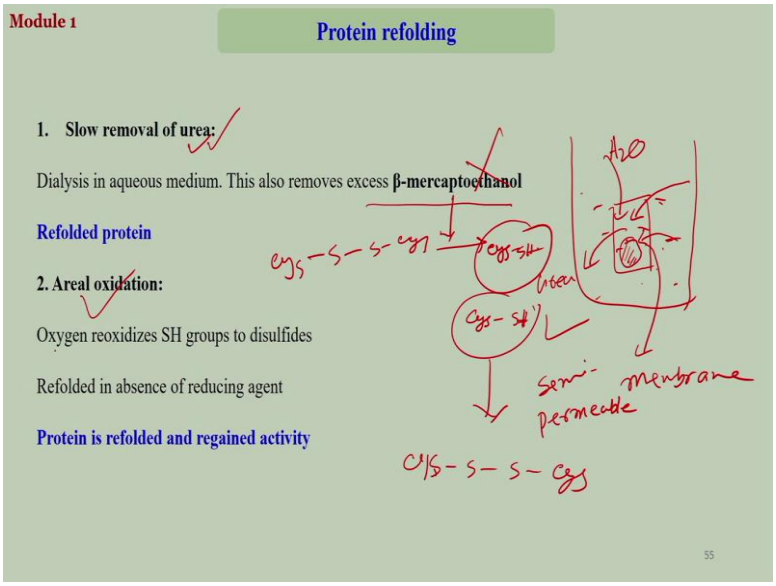

So, two things you have to do one is you have to remove the urea from the solution and co2 also removes the beta mercaptoethanol from the solution and then make the disulfide bond again. So, that is what he has done so slow removal of urea that is the first step for the refolding process to bring back the protein. Slow removal of urea if you if I do not know whether you are aware of this process called dialysis. Dialysis is a process where you have a membrane if you have a vessel full of water or medium then you take a bag the bag is made of or it is a membrane basically.

Semi permeable membrane semi permeable membrane that is what we use as dialysis bag. So, semi permeable membrane if you have then what will it will do and if you have some content inside the bag in a liquid form you know what it will do it will allow only water molecule or the very small molecules to get into it or to come out of it. So, a semi permeable membrane allows the permeation of the small molecules that depends on the molecular weight of it.

Usually the low molecular weight molecules can get into the membrane or can come out of the membrane. So, protein has a very large structure they have their molecular weight in terms of kilo Dalton and on the other hand the urea beta-mercaptoethanol and all those reagents that you have used they have very, very small low molecular weight small molecules. So, if you use a

semipermeable membrane that will allow the larger molecules like protein to stay not to flow out go in or out but allow the small molecules to come in and out.

Then what will happen water molecules will get into the membrane water molecules can get into the membrane and depending upon your chemical potential that will allow the smaller molecules like Urea to come out of it because the concentration of it is a concentration gradient right water is pure water there is no urea in it and inside it you have a high concentration of urea. So, in order to balance the concentration of urea you di will come out over time.

So, if you do a dialysis over time then the smaller molecules because of their concentration gradient will come out of the dialysis bag where your sample was kept. So, if you do a dialysis through this process you can remove the urea the excess of urea that was present. Similarly you can remove the excess of beta-mercaptoethanol that was present in your protein solution. So, now you are after the dialysis you are devoid of urea your protein content does not have urea anymore.

So, once urea is gone now the bonding interactions the electrostatic interactions the polar interactions the hydrogen bonding can again take place. And after the removal of beta mercaptoethanol it cannot do that reduction again if you have the cysteine and cysteine that was reduced by using the beta mercaptoethanol. It was reduced to pure cysteine. So, once you remove the beta mercaptoethanol it cannot do this process again whatever has happened happened furthermore it cannot do the reduction.

So, whatever cysteine free cysteine that was present and the other cysteine that was the prey that was present will remain further reduction will not happen. So, once you remove the beta mercaptoethanol and if you allow it to oxidize again this is done by aerial oxidation. So, after the dialysis if you take your protein content and allow it for aerial oxidation then this process will again resume cysteine SH cysteine SH will again form the disulfide bond.

So you are allowing the protein again to form the disulfide bond the covalent bond and therefore it can regrow its tertiary structure again. So, oxygen oxidizes the SH groups to disulfides refold it in absence of the reducing agent that is the beta mercaptoethanol and once you do both the treatment then protein is again refold it and regained its activity that has been

studied that after you remove all these reagents that you have used at the beginning the protein resumes is the active biological functions and activity.

So that is the study done on is there a lot of studies of course this is one of the famous studies that has been done to understand the protein folding and unfolding kinetics or the dynamics or whether it is a equilibrium process or not.

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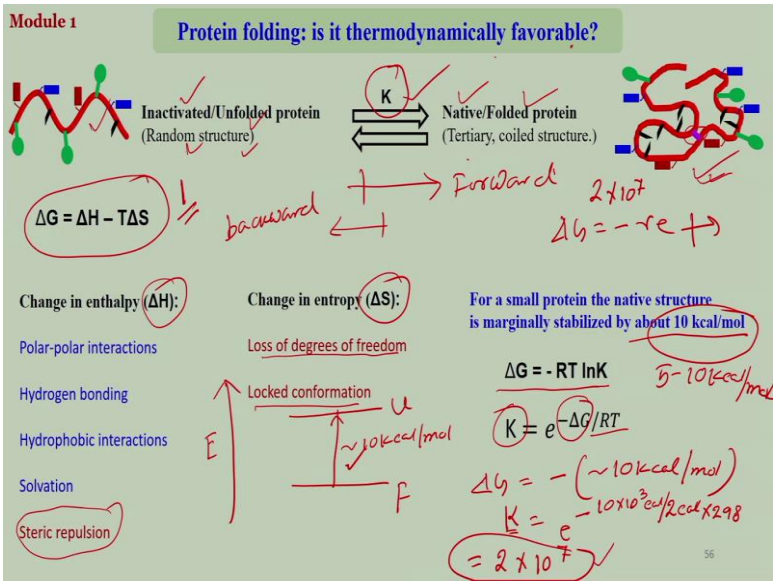

And here comes the thermodynamics of the process so you have that secondary structure of the protein coming to the tertiary structure of the protein. Secondary structure is the inactivated form secondary structure of the protein coming to the tertiary structure of the protein. Secondary structure is the inactivated form or the unfolded form and then when it is the tertiary structure this is called the native form or the folded protein. Tertiary coiled structure secondary structure it has a random structure.

And if they stay in equilibrium then there is equilibrium constant which is defined as K in this case. Now the question is whether the process will move forward direction or whether it will go backward in other words is the folding of a protein is thermodynamically favourable process or not. So, that you can actually understand or that you can explain from studying the Delta G of the system. So, as you know whoever has the basic thermodynamic equation is Delta G equals to Delta H - T Delta S.

So Delta G is a Gibbs free energy for the process Delta H is a change of enthalpy and Delta S is the change of entropy, T is the temperature at which you are doing the experiments in this case

it is the room temperature or the biological cell temperature which is 37 degree Celsius. Now as you know in order to make a process in the forward orientation if you want to go in this forward direction then the Delta G of the process of the overall reaction has to be negative. So, Gibbs free energy has to be negative if you want a forward direction inactivate to protein to activated structure.

For any spontaneous reaction Delta G has to be negative that is called the Gibbs free energy. Now let us see whether this process has a negative Delta G or not. So, in order to calculate Delta G you have to think of Delta H and Delta S. Separately if you see the change of enthalpy Delta H what do you gain or what do you lose why this process unfolded to folded process. So, in the folded tertiary structure you have polar-polar interactions as I have mentioned and that is a favourable introduction. So, Delta H would be negative there. Hydrogen bonding hydrogen bonding against makes a stable structure releases energy that will give you negative Delta H.

Hydrophobic interactions again will stability factor that will give you negative Delta H solvation energy is a huge stability factor and that will release lot of energy. So, Delta H will be negative for this process. And then static repulsion is a unstability or dist ability factor that will require some delta H. So, overall the delta H for the folding process is favourable or you will have negative value of Delta H in this case.

So now think of the Delta S change of entropy. So, entropy means the degrees of freedom whether the Maalik molecular flexible whether the molecules have the freedom to move around the solution whether the atoms within the molecule have higher degrees of freedom or they are free to move. So, in a locked tertiary structure obviously the degrees of freedom is restricted restricted because of different kinds of bonds and the bonding interactions.

As compared to the flexible helical structure or beta sheet confirmations or even the primary structures can assume two random conformations. So, change of entropy is a loss of degrees of freedom for the folded structure. So, it is unfavourable conformation and locked conformation as I have said that the conformation because of disulfide bond here the confirmations is restricted in the protein cannot move.

So, entropically it is not favourable process for enthalpy wise it is a favourable process. Now Delta G equals to – RT lnK if you want to calculate the equilibrium constant K of this process.

So, K is the equilibrium constant K equals to e to the power minus Delta G by RT R is fixed T is fixed because we are doing the reaction at room temperature at a fixed temperature variable is only the minus Delta G. And when you calculate minus Delta G in terms of Delta H and Delta S Delta H is favorable Delta s is not favourable.

And the experiment says that overall you get around change of Delta G of this process unfolded to fold that process is about 5 to 10 kilo calorie per mole a stability factor. So, Delta G is - around I am taking the 10 value 10 kilo calorie per mole for the protein folding process which is actually a very small quantity very small amount of change of energy between the unfolded to the folded structure.

Only 10 kilocalories per mole which means if you have this is the folded form and if you have this is the unfolded form then this difference of energy if this is your energy is only roughly 10 kilo calorie per mole very small change of energy. Now if it is -10 then it comes down to e to the power -10 kilo calorie means 10 into 10 to the power 3 calorie divided by R R in terms of calorie is 2 calorie, so 2 calorie calorie calorie will we cancel each other into temperature room temperature is 298 degree 8 Kelvin.

So, this value will come down to around 2 into 10 to the power 7 that is K. So, this is K, K equals 2 into 10 to the power 7 so you can see a very small change of energy here brings a large change of equilibrium constant 10 to the power 7 is a high rate of conversion. So, this process from the inactivated protein to the folded structure of the protein has a huge equilibrium constant which means it will move forward these directions continuously.

So, in other words if you calculate that from here 2 into 10 to the power 7 equilibrium constant means power if you have one molecule of the unfolded form then you will have around 2 into 10 to the power 7 number of molecule in the folded confirmation which means the most of the protein molecules if you keep it in the solution it will stay as folded confirmation folded structure or the tertiary structure.

In other words the formation of a tertiary conformation or tertiary structure of a protein is hugely thermodynamically favourable process.

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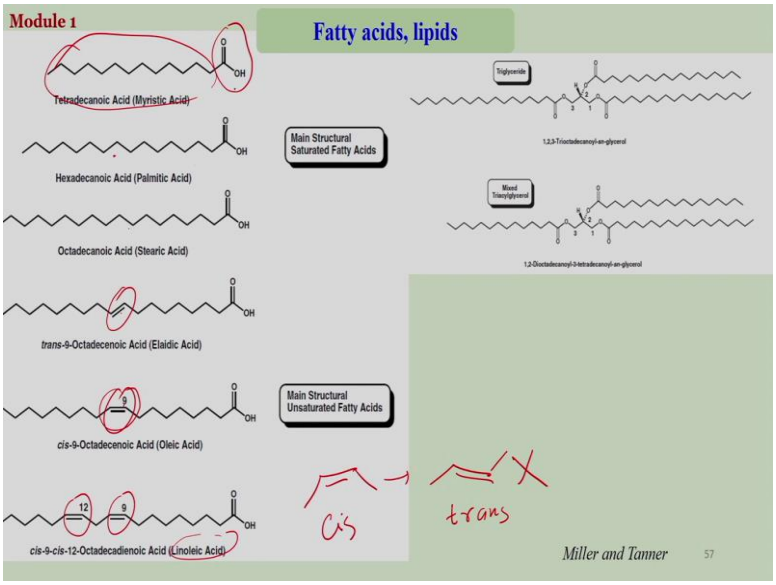

So, now I will move on proteins I am completing the protein part now I will quickly move on to the fatty acid structure and lipids and then complete the module. So, fatty acids I have described in my first lecture of the second lecture. The fatty acids have this kind of structure where you have a carboxylic acid terminal that is polar and then a large hydrophobic side chains and this side chain can vary in number of carbons in terms of number of carbons here you can change the number of carbons are changing number one number two they can have double bonds.

They can be saturated fatty acids or unsaturated fatty acids. So, these are some example of saturated fatty acids for example this is hermitic acid stearic acid these names maybe you have heard and then there are unsaturated fatty acids most naturally unsaturated fatty acids are usually have T's conformation so this you see this is a C’s conformation this is the transform conformations this is C's this is trans trans confirm C's and trans conformation.

Trans conformation is actually bad for health trans fatty acids are bad for health because they are not easily digested C’s fatty acids are easy to digest because they are easy to convert into doing chemical reactions is easy for the C's forms. so C’s fatty acid this is oleic acid and this is with to C’s un-saturations at 9 and 12 positions is lean I guess it is these are also known as omega fatty acids. If you know omega fatty acids are good for our health.

So, these are the some of the fatty acids which has the simple structures with a carboxylic acid terminal. And then you have the triglycerides which are branched chains free hydrocarbon chains and a glycerol moiety here. And then you can have the mixed triglyceride with different carbon chain lengths there can be un-saturation also here.

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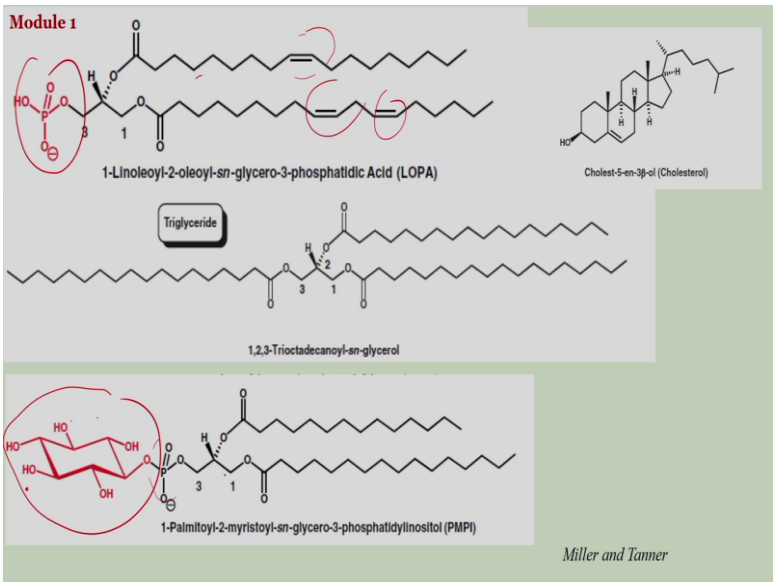

So, these pictures I have taken from the book Miller and Turnner so is this. And there is other example of phospholipids. Phospholipids have a phosphate group here and a lipid moieties. Lipid moieties means basically triglycerides that has sidechains. In this case one of them is a phosphate so the hydrophobic side chains contents on saturations this is one example and then this is other example of the triglycerides this is called glycolipids.

Glycolipids has a glycosidic bond with the sugar attached to his carbohydrate moieties it can have more length chain lengths of carbohydrates also it can have single unit as well. Of course it has a phosphate but not necessarily depends upon the molecule and then it has two hydrocarbon chains. Another is of course the cholesterol, cholesterol has a ring structure and all hydrophobic.

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So, most of these lipids fatty acids are present in our fat of course you know now lipids different kinds of lipids that I have shown they form the membrane of the cells and that is how our cells are very well protected because they are mostly hydrophobic in nature so they do not they make a thick layer and they do not allow the foreign party for foreign bodies to enter into the cells. I will just here you can see how the layer is formed and how they repel the molecules to enter into the to cross the cell walls that is how we are very well protected.

So, lipids they are basically the biomolecules and they are insoluble in water water because of course they have the long hydrocarbon chains. But they are highly soluble in organic solvents

you can make them soluble in various organic solvents. And they are the primarily they constitute the membrane of cells. So, major membrane lipids those are phospholipids that I have shown the contents of fatty acid as well. And a phosphate moiety contents a glycolipid, glycolipids are the lipids which are covalently conjugated to carbohydrates.

One or more chains of sugars can be present this this is the sugar chain one or more sugar chains can be present that is the glycol lipids it is a major constituent of cell membranes. Then the third one is the cholesterol. Cholesterol is a steroid or fat primarily produced in the liver or intestine its present in intestine and liver also. And the membrane lipids they usually form a bilayer, bilayer sheet kind of planers kind of structure in water compared to micelles.

So, if you know how am I so is formed if you have a fatty acid for example and you have the OH or O minus so this is the polar head and this is the non-polar part and we call it a tail. So, if you put the fatty acid in water then what will happen the polar head part would want to interact with the water because it is polar what it is also polar but the tail part the long hydrocarbon chain would want to go away from water because it is a hydrocarbon and therefore it will repel water would not be soluble in water.

So as a result it will form a Groening of things self assembly or aggregations where you can see such kind of structure will be forming. Here all the water is there and therefore the polar head would be exposed towards the water. On the other hand the hydrocarbons would shy away from water and they would want to move away from each other and make a core here. This is how a micelle is formed. And this is our characteristics of most of the fatty acids therefore micelle in water in appropriate concentration.

So, on the other hand in membrane instead of micelle they form a bilayer kind of thing because if you look at the structures they are highly branched structures so micelles will be very hugely crowded and their restraining crowding there that is why they primarily forms the bilayer. This is how a bilayer would look like the polar head here this is the hydrocarbon chain or schematic presentation two have been used hydrocarbon chain naturally the other molecule the hydrocarbon will interact with the hydro hydrocarbon.

So, the opposite direction another layer would be form and since this is polar of course this is exposed to water here this is exposed to water here. So, bilayer in the sense that you have water

then you have a lipid layer which is non water and then a water that is why it is called a bilayer. That is how the same membrane is formed so a cellular membrane basically is this which is a hydrophobic layer outside water outside of water. Now a molecule cannot most of the molecules that are soluble in water they will not allow to cross the cell membranes and that is how the our cells are protected.

So, now I am towards the end of the module one so I will give you a rough synopsis of whatever we have covered in this module.

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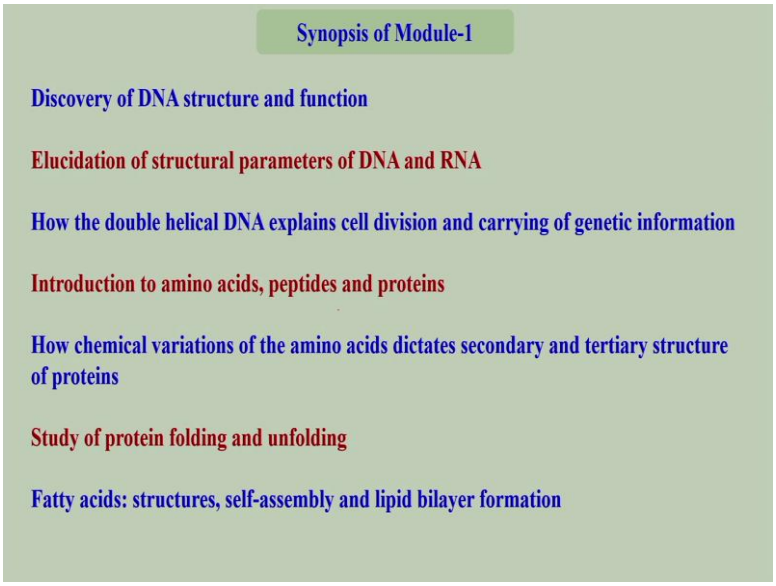

So, we have seen at the beginning that the discovery of DNA structure and how the function of DNA was elucidated from the structure of it. So, elicitation of structural parameters of DNA and RNA both we have seen how the different confirmations different orientations different angles and the functional groups like sugars nuclear bases they are playing role in constituting the final structure of the DNA the tertiary structure of the DNA that is the B DNA structure. How double helical DNA explains cell division and carrying of genetic information how the double strand was able to explain the cell division how the genetic information is transferred to two consecutive cells at the same time.

The same genetic information is passed on to the new cells and then we have talked about the amino acids peptides amino acids peptides and proteins. The basic structures how chemical variations of amino acids they dictate the secondary and the tertiary structure of the proteins. And now today we have seen how is protein folding and unfolding occurs. Finally what are the

structure of the fatty acids the self-assembly and the lipid bilayer formations that forms a major constituent of our cell membrane.

So, with this I will complete the module one and then in the next module would be around I will start some organic synthesis with the next module then again after that module we will come back to biology, thank you.