Biomaterials for Bone Tissue Engineering Applications
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Module 1
Lec 06

So we will continue with the discussion on this cytoskeleton structure little bit more. So this line essentially shows that actin filaments. .

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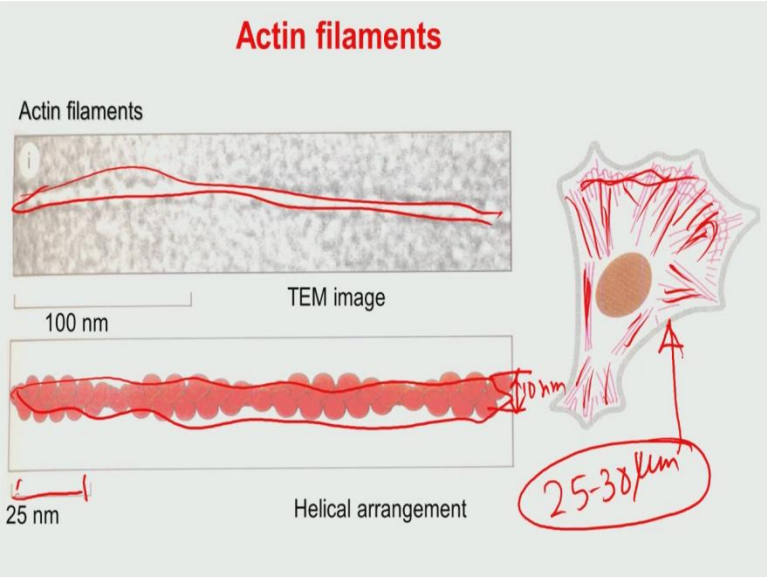

One is the schematic diagram on the right hand side of the slide you can see that how this actin filaments are bunched together inside the Cytoskeleton and it extends throughout the cell. All through the cells. So this is that top panel here in the left hand side you see the actin filaments. This is the transmission from microscope image.

Now based on various structural characterisation both using microscope as well as the competition and analysis so this is that helical arrangement of the actin filaments which are shown here. And now if you look at the dimension of this actin filament. This is typically 25 nanometre so therefore this one can be somewhere between 5 to 10 nanometre. So this is the 10 nanometre around the diameter.

So it is extremely small and if you consider the total cell size is 25 to 30 micron. So there is a difference in terms of 10 to the power 3. So that means you can immediately realise that so many

large number of actin filaments can be accommodated physically within the cytoskeleton structure simply because that cell size is much larger. It is around 25 to 30 micron. Whereas the actin filaments their typical diameter is around 10 nanometre or so.

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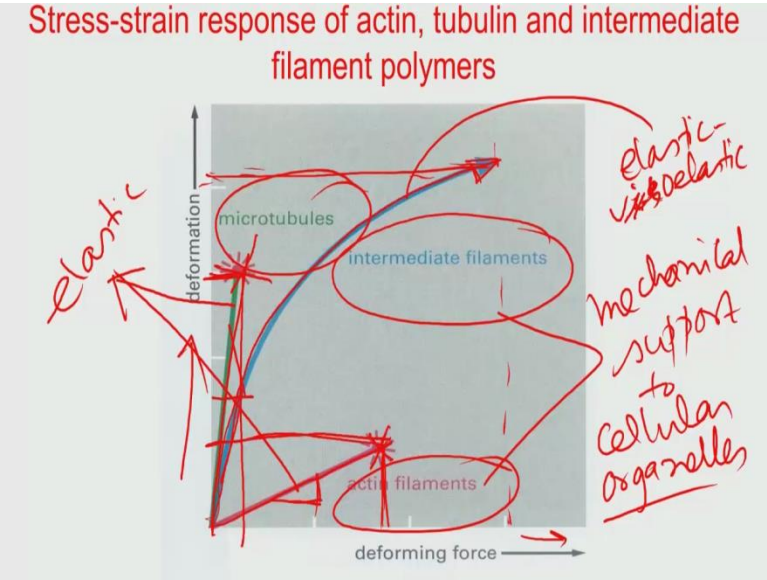

Now in terms of their. Their mechanical properties which is important because you remember that all these intermediate filaments micro tubules and actin filaments together they provide mechanical support to or provide structural mechanical support to various cellular organelles. And therefore some understanding of their mechanical response particularly under let us say under the application of tensile deformation or tensile force that is necessary.

Now to start with if you see that actin filament that mechanical response so it is extremely part linear. A linear elastic response. The same is true for micro tubules as well. However there is a difference in terms of the two response between actin filaments and cytoskeleton because this is here it is the force and this is here is the deformation. So force versus deformation flot. Microtubules has a much larger slope you can see here and actin filaments is not aaa it's a very small slope.

So therefore elastic stiffness wise so there is a difference in terms of the elastic stiffness between the microtubules and the actin filaments. The second points that must attract your attention is the way intermediate filaments they behave during this for during the deformation under force and you see that there is a very small linear region followed by lastly non linear region bleating to

fracture and also in terms of the larger force you can see so both the intermediate filaments can sustain both in terms of large force magnitude as well as larger deformation.

Whereas actin filaments although the force wise it is the second that is the maximum force it can sustain but strain wise or the deformation wise it is much smaller than microtubules. Whereas microtubules they can sustain larger strain but it extremely small force. So this slide essentially captures the characteristic difference in terms of the mechanical response among actin filaments, intermediate filaments and microtubules.

To summarise this particular behaviour these actin filaments and and microtubules they more show very perfect elastic kind of response. Now these microtubules shows elastic and visco elastic type of response. Visco elastic means I will explain to you later Visco elastic means essentially it has a combination of both the viscous fluid and the elastic solid. So this polymers many of the polymeric materials they are essentially exhibit visco elastic response so which is neither fully viscous fluid nor fully viscous solid so it is somewhere intermediate between these two extreme behaviour.

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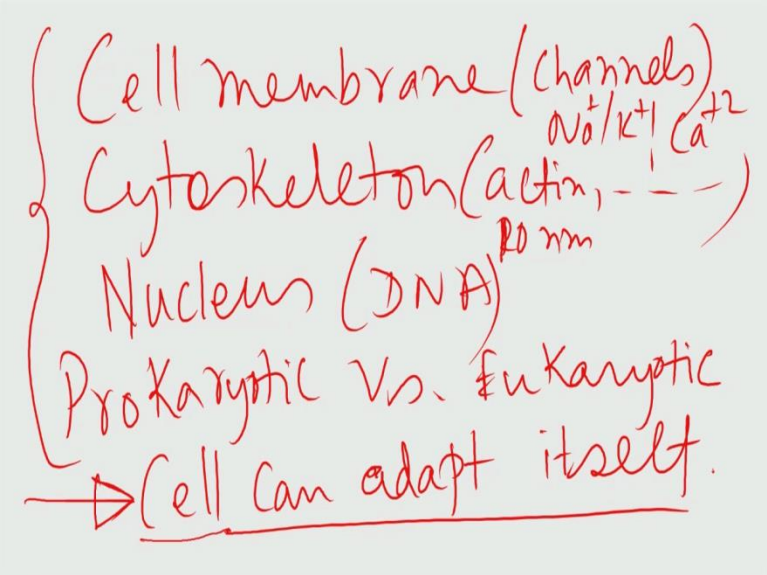

So now enough is about this cell structure description and then we started with the normal cell structure description and then I have explained that how these so to summarise if you can see that couple of things that you need to. So couple of things you need to remember for your own understanding and these things essentially are that what is the typical cell size so that what is the

cell membrane structure in terms of the bilayer phospholiphid bilayer structure double membrane structure.

And in the cell membrane after the cell membrane what is the cytoskeleton structure like actin microtubule and intermediate filament and cell membrane structure also you have to remember that there are there are different channels like sodium potassium or calcium channels are there and this channels some of these channels are voltage sensitive. Then second one is the cytoskeleton structure this actin filament and so on how their structure what is the typical size of this actin filaments and so on.

Third one that you have to see that what is the nuclear structure and inside you have a DNA that is the nucleic acid and then also in the nucleus has a pore complex and pore the difference things and then fourth one is that how this eukaryotic cell structure is different from prokaryotic cell structure. Now this all this 4 points as such I'm trying to summarise here this four points are sufficiently discussed in the last module as well as in the present module.

Now the cell has a unique structural characteristic that is now well established having said that you have to see that how a cell can adapt to an external environment so a cell can adapt itself. Now this cellular adaptation process has manifestations in terms of their way the cells will also function on a material surface as well as how cells would response to external stimulus.

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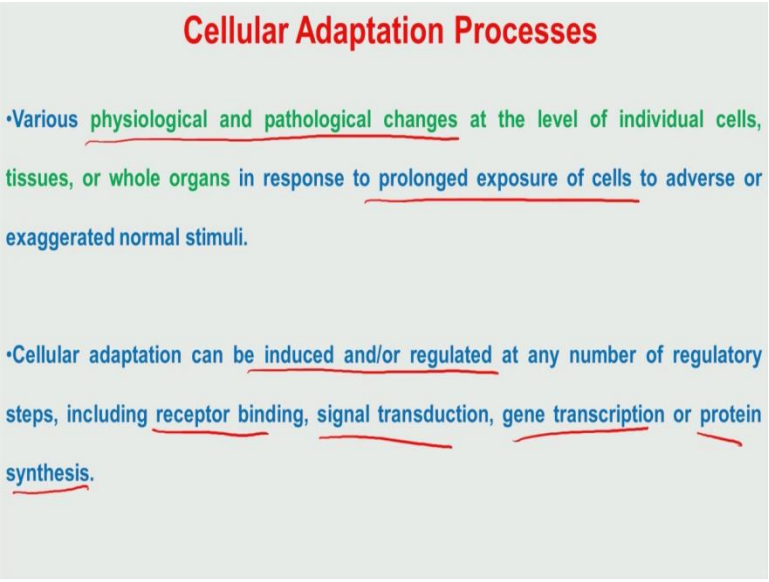

So that is the reason I have made the first statement in this slide that various physiological and pathological changes at the level of individual cells or tissues or whole organs response to prolonged exposure to cells to adverse or (())(7:52) normal stimuli is relevant to the cellular adaptation which can be induced or regulated at any number of regulated steps including receptor binding signal transduction gene transcription and protein synthesis.

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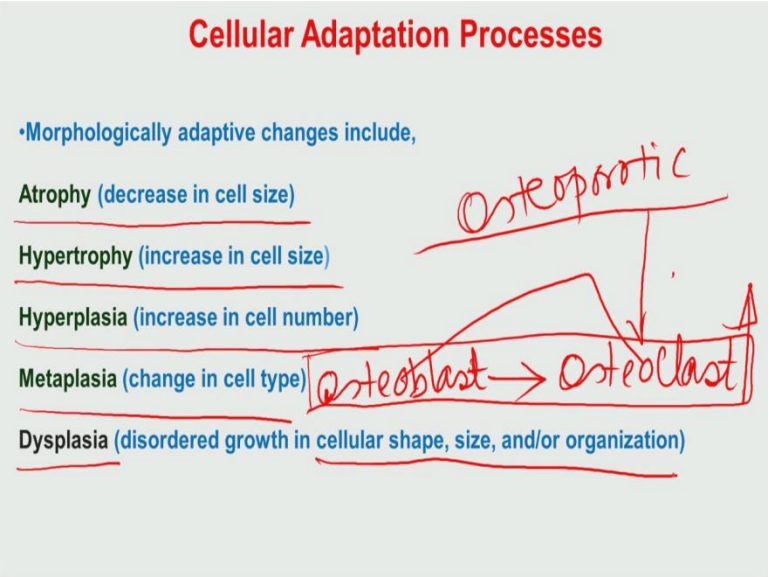

I will try to make an attempt I will make an attempt to explain each of these things in subsequent modules. But to first discuss that what are the different ways a cell can adapt itself. First one is atrophy. Atrophy means decrease in cell size. Hyper mean something larger so hypertrophy means increase in cell size. Third one is hyperplasia. Hyperplacia means increase in cell number so anything is hyper means higher.

Metaplacia means change in cell type. So example of changing cell type for example under certain circumstances osteoblast, I said in the last last to last module. Osteoblast can undergo transition to osteoclast. Osteo means bone osteoblast means cells. Osteoclast means bone resorption cells. Now this kind of transition essentially helps the cell to adapt to certain external stimulation. Now I might have, I might have mentioned in one of the earlier lectures that in a healthy human bone you have to have a appropriate balance between these two cells.

In osteoporotic bone like you know that is this large bone loss and so on and these osteoporotic bone you have a larger concentration of the osteoclast so osteoclast increases and therefore it did

makes a bone osteoporotic. The fifth one is the dysplasia that is a disordered growth in shape size or organisation. So this kind of cell shape or size there is a disordered growth and this leads to the cellular adaptation that is the dysplasia.

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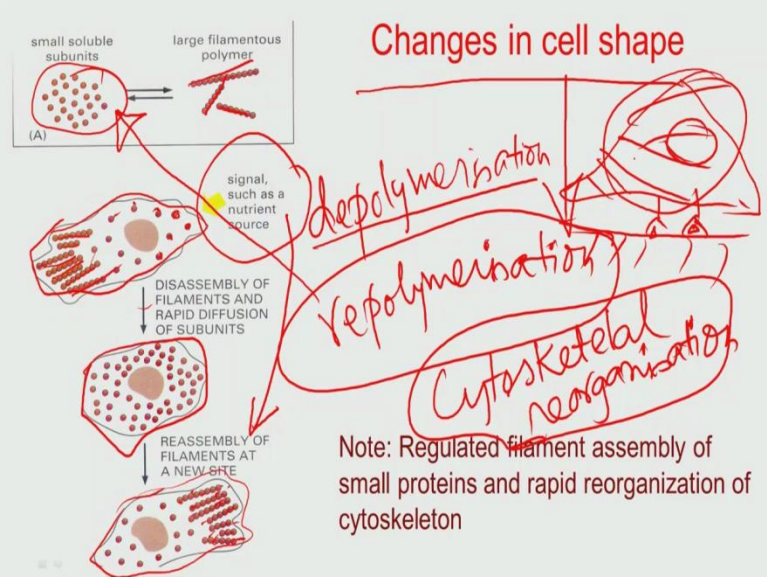

Apart from these the cells also can adapt itself in terms of changes in the cell shape. Now how cell shape is changed. Let me take some time to explain these things in with certain details. Now you have a cell here please follow me how I am how I am making an attempt to explain, so this is your actin filaments in the cytoskeleton which you have seen more details in the last few slides before. Now under certain circumstances as I said this actin filament has a unique characteristics that it can undergo the process called depolymerisation.

Depolymerisation means that actin filaments will break down to smaller monomers and this monomers are now distributed throughout the cell without any preferential space. Either they will prefer their affinity to accumulate at a specific region of a cytoskeleton but under certain signal or nutrient or resource if that continues to happen then this under favourable circumstances this monomers can get again assembled together by a process called repolymerisation and this process enables the enables the formation of actin filaments again but not at the same site where it originally got broken down to small monomers but at a different site and that way the cytoskeleton also can be stretched in that particular direction.

So let me redirect my points once more so the process of depolymerisation helps actin filaments to get broken down to smaller subunits soluble subunits like monomeric units. Now under certain circumstances these are certain signalings environment this repolymerisation of that small soluble subunits can lead to again actin filament formation not at the original place of their the gradation but at other other sites of the cytoplasm. And that way this actin filament depolymerisation and the repolymerisation can get into can lead to the process called cytoskeletal reorganisation.

Now the cytoskeletal reorganisation is one of the precursor to cell material interaction. In fact when a cell would try to adhere on a material substance when a cell would try to adhere on a material substance to protein protein ad adhesion or protein protein interaction then what will happen this depending on some scenario that cell would continuously change its shape and size. So this I will address this point later but at this point of time you might bear in mind that this changes in cell shape due to the depolymerisation repolymerisation is involved in the cell material interaction and therefore this has its own importance in the overall fate processes of a cell.

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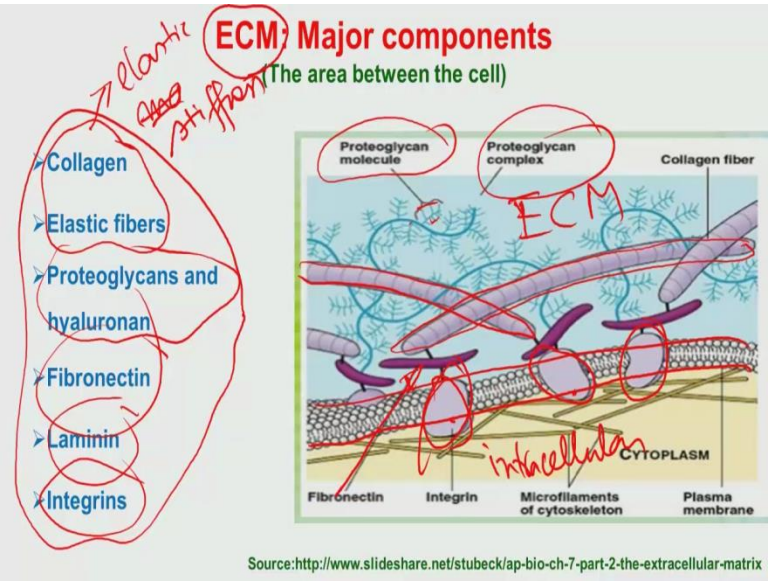

Other things I have mentioned earlier that is extracellular matrix and its importance in the overall cell (())(13:23) processes. Now this slide shows you little bit more details of this extracellular matrix. Now what you see this is your cell membrane right. And in the cell membrane you see

that there are something called transmembrane proteins which I have mentioned to you before and this is that is transmembrane proteins is the integrins and the other plasma membrane here.

Now outside the cell that means towards the other side of the cell membrane opposite to the cytoskeleton or cytoplasm. This entire space where you have the different biological macro molecules and collagenous proteins are there. Now this particular space is very important as far as the functionality of the cells are concerned and this place is known as extracellular matrix. So this is your intercellular region and outside the cell is your extracellular region.

Now what are the various components which has been shown in that ECM. This is a very nice characteristics collagen fibres and later on I will explain you that how this collagen is built up structurally. The other two that has been shown here proteoglycan molecule so these are like proteoglycan molecule or proteoglycan complex is there. And also you have other proteins called fibronectin protein which are also present in the extracellular matrix region.

And these are the sary of all the constituents of the extracellular matrix. You have collagen that is the fibrous protein structure. You have fibronectin, laminin these are also this other structure protein structure. Then you have elastin fibres.

Now these two elements collagen and elastic fibres. They give the elastic extracellular matrix the required elastic stiffness that it has so in other words the presence of collagen and elastic fibres they provide mechanical support to the neighbouring cells that it contain. Proteoglycans hydronium and also this integrins and all they are present. Now integrin proteins is transmembrane proteins has its own role because these transmembrane proteins helps in physic getting physically connected to the proteins which are adsorbed on a material surface. Therefore ECM structure and then transmembrane proteins are important the way a biological cell would interact with that of a synthetic material substance.

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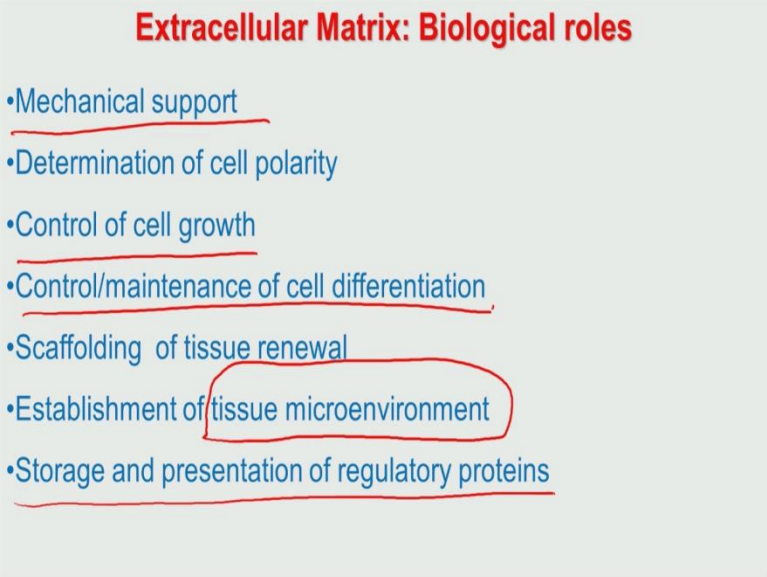

So these are the different roles that extracellular matrix is expected to play improvised mechanical support it also controls the cell growth or control or maintenance of the cell differentiation and it also establish the tissue microenvironment.

Many of this components of the extracellular matrix are synthesised or secreted by the biological cell itself and it form and it goes to outside the cell by a process called exocytosis. So essentially this is in ECM is synthesised by the cell itself and then it then it forms by certain biological processes and into the very nice network of both the different biological molecules macromolecules as well as different fibrous proteins. It also helps extracellular matrix also helps in storing and presenting certain regulatory proteins also.

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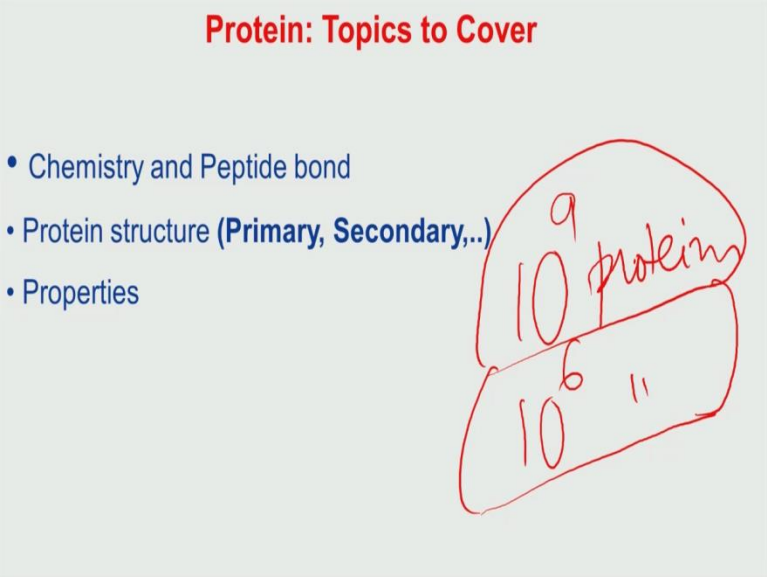

So for quite some time in the last module as well as this module I have mentioned to you that this extracellular matrix contains proteins as well as there are large number of protein molecules which are present if you remember correctly. These are 10 to the power 9 number of proteins which are there in your eukaryotic cells that is truly nucleated cells.

You have around 10 to the power 6 proteins are there in a prokaryotic cells. So these order of magnitude are important and in few of the abundant presence of the protein in a cellular structure as well as extracellular matrix, it is important for somebody who wishes to pursue this research or pursue this particular researcher here or pursue to study this biomaterial science or bone tissue engineering applications that what is the structure of a typical protein.

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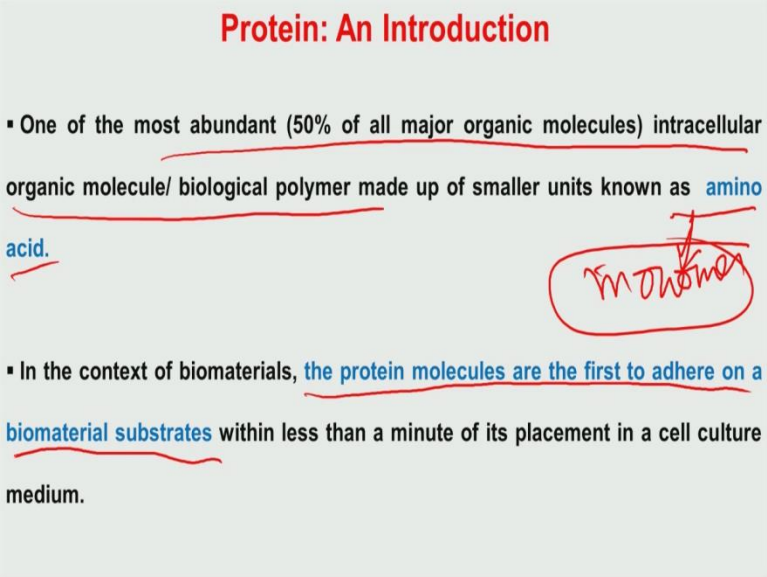

So this slide and few more slides I will explain to you that how this protein structure is built up. So this essentially substantiates what I have mentioned just two minutes ago, so this is one of the most abandoned intracellular organic molecules or biological polymer made up of smaller units known as amino acids. So essentially amino acids is the monomers here in case of the in case of the protein molecule. In the context of the protein biomety there is a protein molecules are the first one to adhere to a biomutual substance before it establishes the interaction with the cell surface receptors.

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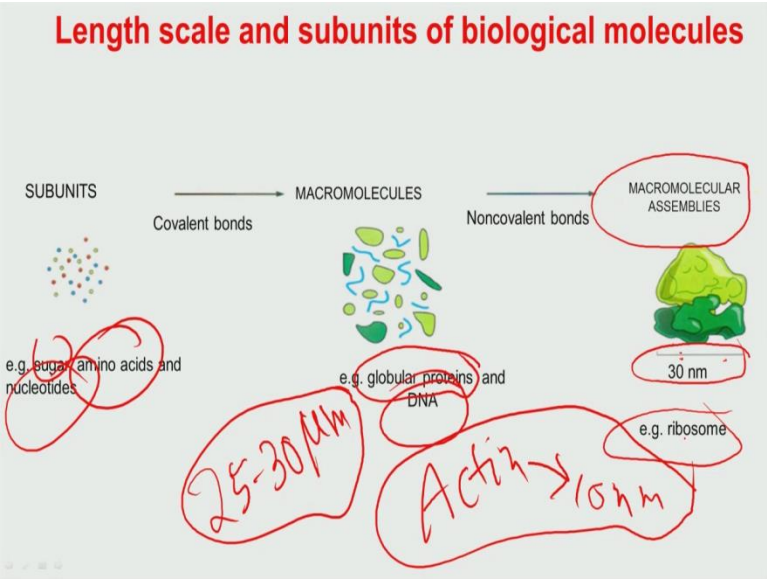

Now these are different biological molecules which are there you have the sugar molecules you have amino acids you have the nucleotides then some of the proteins has a different shape morphology like globular proteins and you have a very characteristics doublenic structure of this deoxyribonucleic acid.

And you also have a macromolecular structure like ribosome. If you look at the length scale this is the 30 nanometre typical ribosome structure and remember your cell size is around 25 to 30 micrometer. So individual organ is 30 nanometre and if you remember that actin filamental structure which is the size somewhere around 10 nanometre. So all the organelles and or all the cytoskeletal elements and different cellular organelles mostly their size with the length scale is in the order of nanometre.

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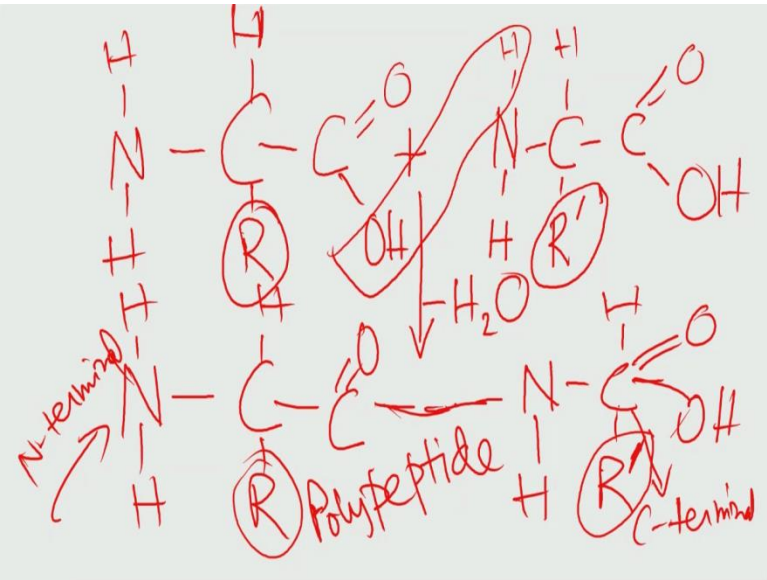

Okay, so this essentially shows you that different level of structure description of a protein but before that let me explain you that how this protein structure is formed or how this amino acid sequence is formed. So you have this one of that amino acids so if I, so this is how amino acid is retained so you have a central carbon atom so. So you have a central carbon atom. You have amide groups so you have nitrogen and you have a 2 hydrogen, so this carbon atom has 1 organic radical this is R. And one is this H. H hydrogen atom and you have a carboxyl group here. So carboxyl group means COOH. And the C essential you can write it COH so this is OH.

So in a physiological medium this amino acids or the protein molecules and other protein molecules particularly is in (())(20:55) conditions, it is not in neutral conditions but just to show you that how this peptide bond forms. So essentially during the reaction of the 2 amino acids. So the way amino acids are retained is always keeping that N terminal in left side and C terminal is in the right side.

So when these 2 amino acids react then this will lead to 1 water molecule and then you can start writing it from left to right. This is N terminal, this is C. This is H, this is R, this is carbon. And you have this oxygen, this is nitrogen and this is H. This is carbon, this is hydrogen, this is (())(21:59). So you have 1 is left. This is H. This is H. So this is your N terminal, so N terminal is always kept at the left and carbon C terminal is always retained at the right or is always kept on the right.

Now other thing that you must have noticed here that 2 amino acids are different simply because if they have RR prime are different and the way these R and R prime the way it is added. That forms is your amino acid sequence in a polypeptide molecules. So essentially protein is nothing but a polypeptide the presence of polypeptide chain. Coming to this polypeptide chain again so in the protein molecules dependant depending on whether you have this R prime R. R prime R double time or different organic radicals are attached. A protein molecule has a different polypeptide, a different amino acid sequence and therefore 2 protein molecules will certainly be different.

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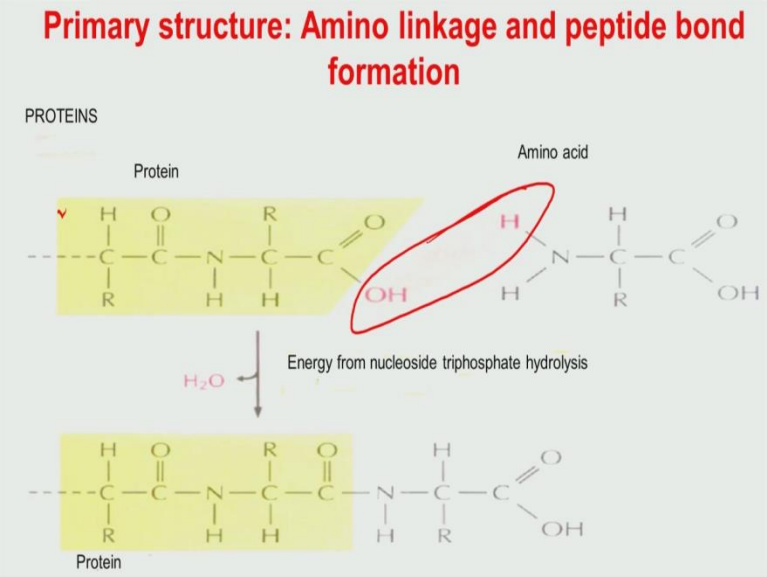

So this has been shown here as I was explaining to you and this amino linkage and peptide bond formation has also been shown in this slide and this is what I was explaining to that O, H and OH and then this will be water molecules that will be released.

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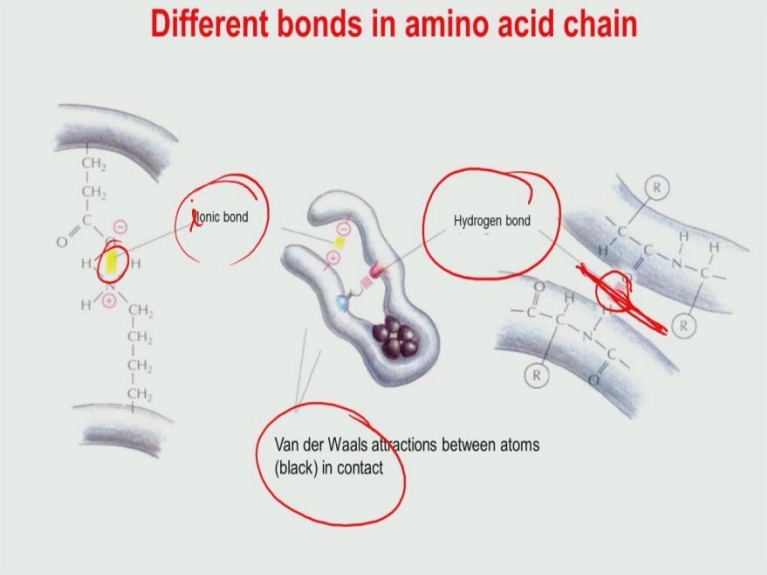

Now different type of bonds in the amino acid chains, it is mostly that we could van der Waals bonds or hydrogen bonds are present and to some extent in some of the cases you have ionic bonds are also there as shown here. So these bonds, one of the advantages of having this hydrogen bonds or Van der Waals bonds in this protein structure is that if required this bonds can

be broken very easily as a result 2 polypeptide chains, they can be separated as and when it is necessary.

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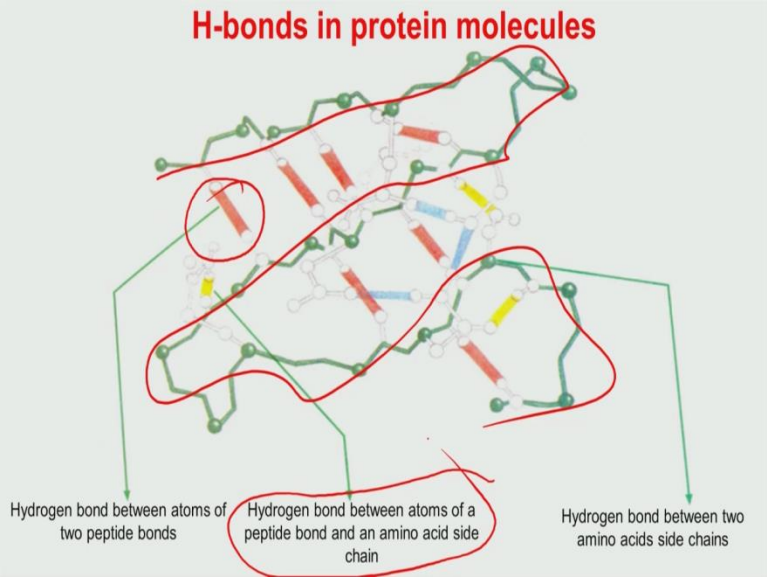

So this is the primary structure of the board that has been shown and the this is typically helical structure and it has a 5 tons in this helical structure and it is typical dimension is 27 Armstrong and each ton is 5 point 4 Armstrong. This slide shows more details about some of this. For a this hydrogen bonds can form, this is a polypeptide chains which is going in the main major backbone chain and these arrivals and bonds that is between atoms of a peptide bond as an amino acid side chain that is also there.

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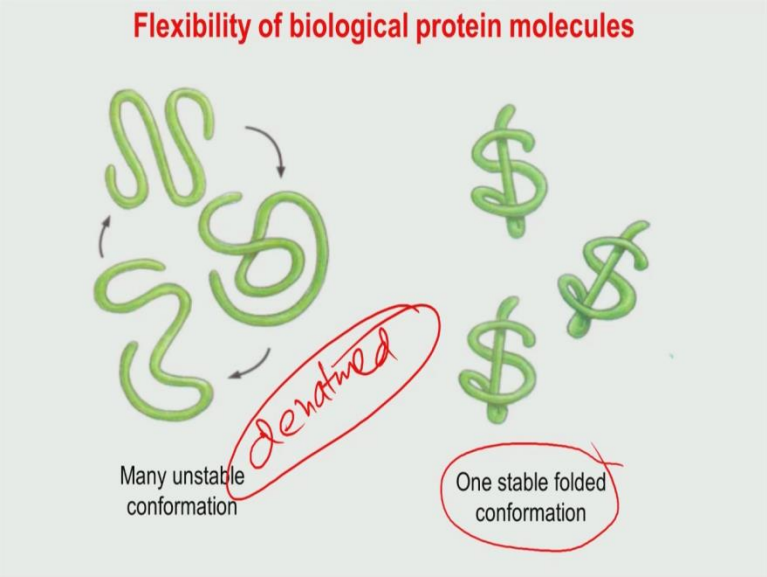

Now as for the stability of the protein molecules in a biological system is concerned the proteins are more stable when it is a folded configuration. The moment it gets straightened then it it becomes very unstable. And one of the ways that so this is called denatured state of a protein. So denaturing takes place when, once when you you use or you add you urea to a protein solution. It immediately gets denatured.

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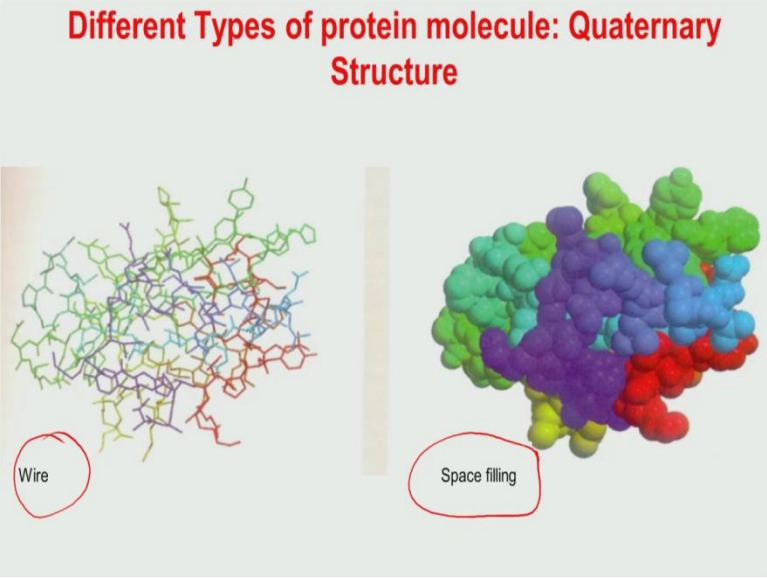

Now protein structure, the understanding of the different protein structure itself is a discipline in itself and then many researchers in convention of biological sciences, they do study the proteonix

as well as the protein engineering and so on. So people do a lot of experimental study as well as they complement that with a computational study. Just to understand the protein structure at different length scale as well as to understand the protein structure how it evolves.

What is the most stable configuration of a protein molecule, which is certainly beyond that scope of this module discussion. However let me also tell you that protein structure at the higher level, it can be described by the wire modeling as well as the space filling module and this has been shown here.

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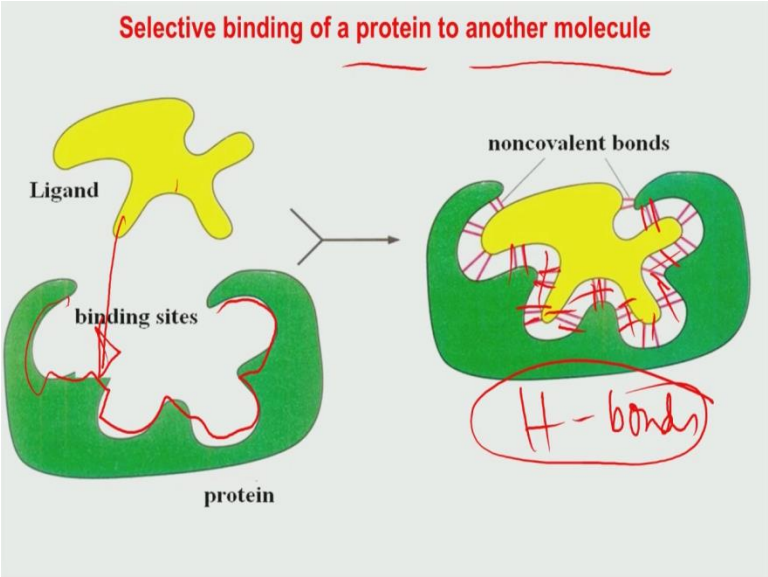

So now that you have some idea about this protein structure. The protein protein interaction is also equally important or protein interaction with another biological macro molecule that has been shown here. So let me first explain this and then I will explain to you what is the relevance of that.

One is the ligands and this is your typical protein structure. It has a particular cleavage and then ups and downs. So these ligands will come and will try to interact or establish the bonding with this protein structure and these bonds are essentially non covalent bonds. Non covalent bonds means it can be either hydrogen bond or it can be van der Waals type of bonds. So these non-covalent bonds are essential extremely weak and therefore they, if these ligands will try to escape from this particular bonding sites which is possible simply by breaking these bonds without much of the difficulty.

So this protein to ligand binding or ligand and to protein binding is a precursor to understand that how a biological cell would interact with a biomaterial substrate which I am going to describe in the next module.