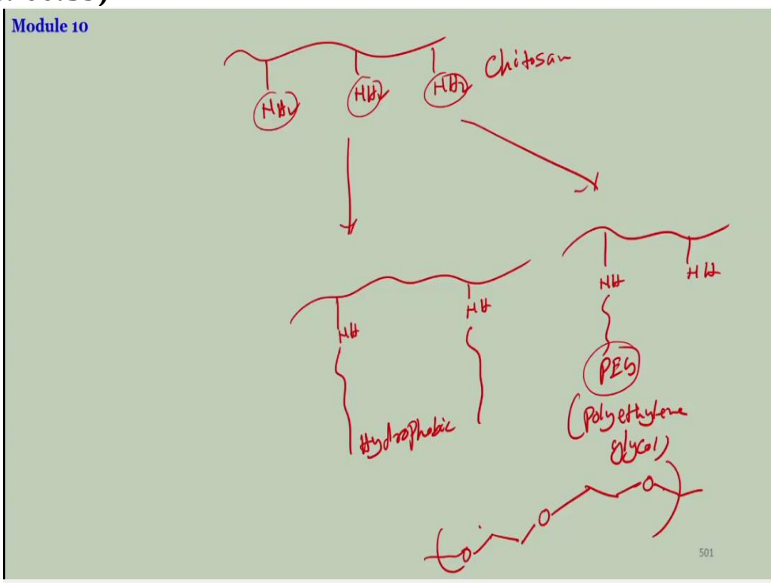
Essentials of Biomolecules: Nucleic Acids, Peptides and Carbohydrates

Prof. Dr. Lal Mohan Kundu
Department of Chemistry
Indian Institute of Technology – Guwahati

Lecture- 35
Carbohydrate chemistry - 3: Synthesis of Nanoparticles: Recap of All Modules

Hello everybody and welcome back. So, we are discussing about the carbohydrates and carbohydrate based molecules that can have various applications. And on that course of discussions we have come to the synthesis of or development of nanomaterials or nanoparticles.

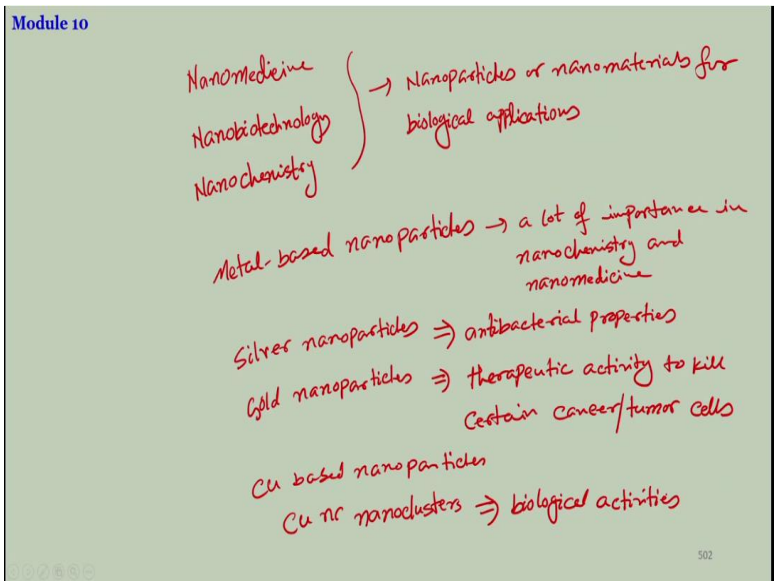
(Refer Slide Time: 00:59)



That I have talked about in the last lecture that the macro molecular carbohydrates that contain a long polymorphic chains, sometimes the modifications, this one was our constitution, which has amine groups as branched chains in its polymorphic chain structure. And in the last lecture I have talked about that how you can use these kinds of functional groups to do various chemical modifications for your biological applications.

And I have also talked about a little bit that you can synthesize the nanoparticles or the nanomaterials out of these molecules by themselves. So, basically I will start today with the importance of the nanomaterials or now, which we can call nanomedicine or nanobiotechnology you can say even nanochemistry.

(Refer Slide Time: 01:54)



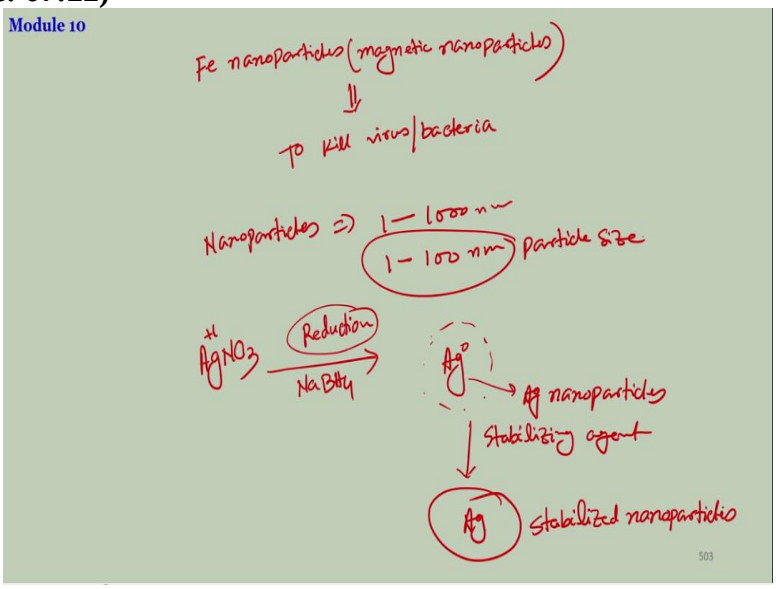
So, that basically covers the area of using or developing new nanomaterials or new nanoparticles or nanomaterials for biological applications such as drug delivery, such as using the molecules themselves as the medicines, such as in order to study several binding property activities inside the cells, taking probe molecules into the cells in a nanoparticle format. And since we are talking about this, the most abundant amount of nanomaterials or a nanoparticle that has gained enormous importance are the metal based nanoparticles.

So, I will just talk very briefly without this metal based nanoparticles, I think nanobiology or nanobiotechnology or nanomedicine is incomplete so I will very briefly talk about this and then will conclude metal based nanoparticles have gained a lot of importance nanochemistry in general and particularly nanomedicine. For example, silver nanoparticles. Silver nanoparticles show very good amount of antibacterial property. Similarly, gold nanoparticles, they have actually, for certain types of cancers, they show pretty good amount of anti-cancer activity.

So they are used as therapeutics. So they have therapeutic activity to kill certain cancer or tumor cells. So, gold nanoparticles in literature, you will find there are many different types of gold nanoparticles that have been synthesized that have been developed and people are doing research on them many of those varieties, so pretty good activity against the tumor cell lines. Similarly, nowadays, copper mediated nanoparticles.

Especially copper based nanoparticles, especially, the copper nanoclusters, this will very good biological activity. Anti-bacterial anti-tumor or both In general biological activities lot of cellular studies have been performed using the copper nanoclusters.

(Refer Slide Time: 07:22)



And today a lot of people are trying to develop even iron nanoparticles. Iron nanoparticles have one particular property that they are magnetic in nature. So they are usually called being called magnetic nanoparticle. Magnetic nanoparticles and they are being used to treat certain types of virus to kill virus. Viruses and bacteria because of this, they are magnetic properties they are being used to destroy certain types of viruses that have themselves certain magnetic activity or the bacteria.

So, these are some of the examples of the nanoparticles being used in medicinal chemistry or nanobiotechnology. So, now, the question is how these nanoparticles are usually synthesized. So, as I have mentioned nanoparticles usually have the size range 1 nanometer to you can say up to 1000 nanometer for biological good biological activity, it usually should be between 1 to 100 nanometere particle size and nanoclusters have larger size than 100 nanometers, but they fall within 1000 nanometer maybe 300, 400 nanometers sometimes.

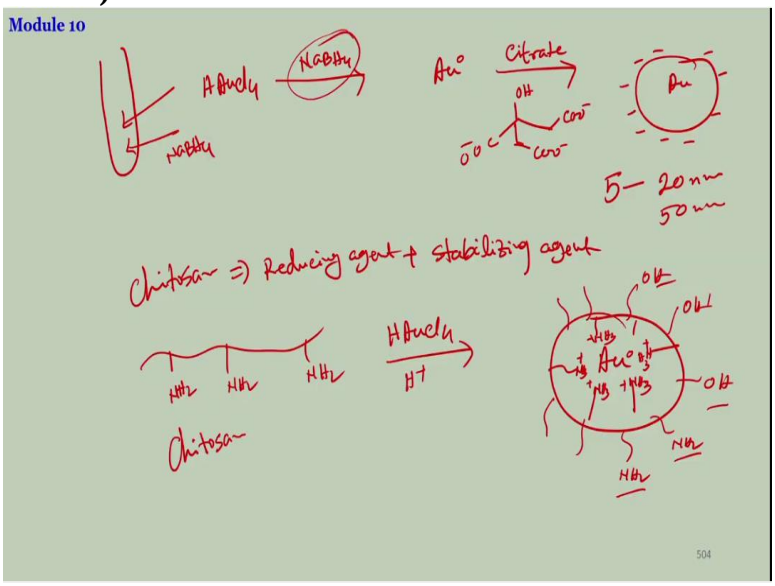
So, what you are actually doing here is you are using certain components you are using a chemical for example, this metal things metal based compounds and you are reducing the size of the metal that is done usually. So, for example, if you take silver if you consider synthesis of

silver nanoparticles, then initiate, you take salt of silver, maybe a silver nitrate salt. So this is a salt, where silver exists in +1 oxidation state. Now if you can reduce these under controlled manner reduction, then you form silver 0.

And if you have a particular type of environment here environment there that actually forces these particles to reduce their size or to aggregate or disperse, but it will reduce their size and that is when it forms a nanoparticles. So, silver 0 oxidation state will exist as silver nanoparticles Ag nanoparticles which will be surrounded by it is environment and most often the environment is the reducing agent that you have used that is the first environment actually surrounding environment.

So, you can use chemicals typically if you want to reduce silver nitrate to silver 0, you can use reducing agents such as sodium borohydride, then it will be surrounded by counter enhance and that will be the first reason for the formation of silver 0 into nanoparticle form or quite often, once these nanoparticles are forming, they are not quite stable. So, you need a certain stabilizing agent and here we usually use large macromolecules so, that will further stabilized the nanoparticles I will show one example. So, this is your stabilized nanoparticles.

(Refer Slide Time: 12:30)

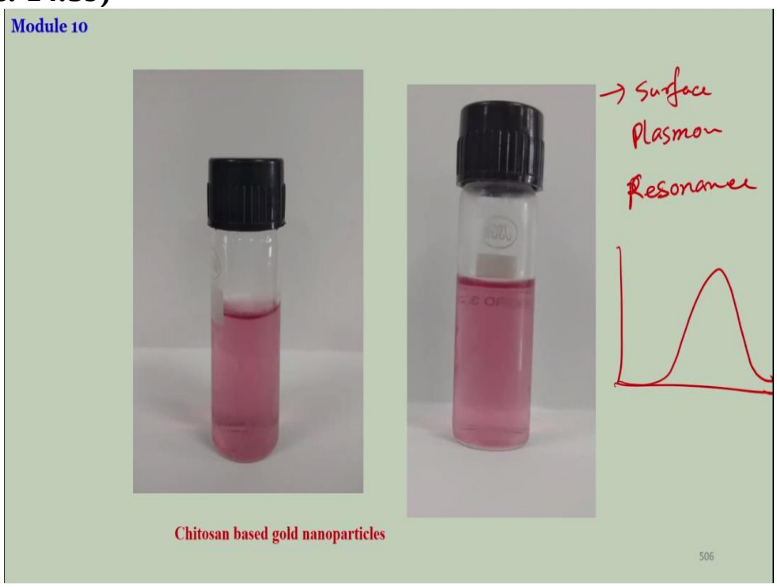


For example, if you want to synthesize gold nanoparticles typically people start with gold oric chloride. HAUCL 4 is a liquid make a solution of gold oric chloride and then we use a reducing agent here. Sodium borohydrate so you take a test tube add gold oric chloride add sodium

borohydrate in a formal medium keep on staring for some time, it depends upon your reaction conditions, it can take from minutes to hours, but it finishes usually typically either half an hour to 1 hour it will be done most of the times then you will have gold 0.

And then if you use certain stabilizing agent, the most common stabilizing agent people use is citrate. Citrate base basically cyclic acid structure, this is OH COO COO - I think, COO minus. So that way you can surround the gold with the charge density and here you will have all the negative charge on the surface because of the carboxylate enhance and that is the reason why gold nanoparticles are formed and gold nanoparticles have usually very small size. You can even purchase nowadays golden nanoparticles from 5 nanometer of to 20 nanometer or maybe 50 nanometer. So, it can be as small as 5 nanometer it can go large also.

(Refer Slide Time: 14:39)



So, I will show you this is how it looks like, but this is not citrate stabilized this is another gold nanoparticles we have synthesized in our laboratory. This is basically we have done using the polymer like chitosan we have used. So, this is how it looks like when the nanoparticles are synthesized this pink color actually demonstrates that you nanoparticles have been formed. And in this case the nanoparticles are dispersed well dispersed in the solution is not precipitated out because this is typical for gold.

Because gold nanoparticles are so small that you cannot usually precipitate it out they are dispersed in solutions and they are so, color. The reason for this color is you can read a little bit

that is surface plasmon resonance because of particle, particle interactions basically, surface plasmon resonance is the reason why the nanoparticles so colors and you can pretty much see them in if you take a if you visible spectroscopy. You can see where before gold oric chloride does not show any we went.

Once you started synthesizing then the nanoparticles are new nice bend will come up that is the responsible for this color and this bend is because of the surface plasmon resonance bend. So, the problem here is if you want to use these nanoparticles for biological purpose, then these nanoparticles have to be non toxic. That is the first criteria and when as I have said when you using the reducing agent, the reducing agent stays on the nanoparticles it provides a environment it provides an interaction factor.

So, it stays with the nanoparticle that is synthesized and this reducing agents are toxic. These are pure chemicals. So, ideally, the nanoparticles that are being developed using the chemicals as the reducing agent are not good for application into biology. So, nowadays that is why people use natural products such as chitosan such as Aloe Vera such as silk such as citrate. Cyclic acid is also a natural product often and does not source much toxicity. So use of those materials is as reducing agent and they also act as a stabilizing agent also.

So I will show you just for example, if you take the example of chitosan reducing agent plus stabilizing agent both. So, if this is your chitosan you have amine you have amine roof here, amine group there and here you have the hydroxyl groups as well. So, those hydroxyl groups are also responsible for reducing your nanoparticles. If you use; gold oric chloride and without the reducing agent you can develop a method without using other chemical as reducing agents.

It will you have to do such certain kinds of optimization to use this then you will have gold nanoparticles surrounded by chitosan here would be amine he would be amine so, positives. Basically, when you are using this acid, the chloride is basically have a H + also. So, that will protonate amine into a NH3 +. And those captions are responsible for stabilizing the nanoparticles for stabilizing that forces the particles to be in the nanoparticle form.

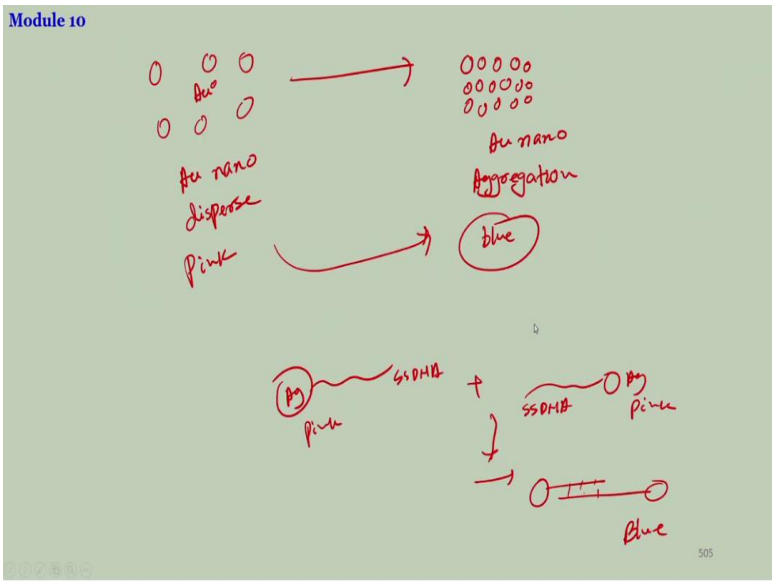
And on the surface you can have the other ones on the unreacted amines can be there hydroxyls that are present they can be there. So, depending upon what agent what polymeric component you are using, it can be chitosan. It can be PEG which is polyethylene glycol. There are many it can be peptides you can synthesize nanoparticles with a stabilizing agent and they are supposed to be nontoxic in nature because you whatever you have used throughout is kind of natural units and such kind of nanoparticles have advantages.

Number 1 is of course, I have talked about the nanoparticle itself, for example, gold has anti-tumor activity. So, you can use this itself to kill the tumor cells. Number 2, since you have stabilized or since you have recovered your nanoparticles or nanoparticle are merged with the macro molecular structures. You can do lot of chemistries on the surface of the nanoparticles. So, in here you have hydroxyl if you use something else, you can have carboxylic acid and here you have already amines it is here.

So you can attach the targeting moieties or other kinds of components that you want, you can add as peptide here, you can change its viscosity, you can change its solubility property by adding either the hydrophobic group if you want it to be more insoluble, you can add more hydrophilic group in order to make it more dispersed or more soluble you can add DNA to it lot of studies have been done lot of nanoparticles have been synthesized.

Where DNA are attached to study different activities, which is including the single point mutations. Gold nanoparticles have been enormously used and very nicely used actually, to find out presence of single base mutation in DNA.

(Refer Slide Time: 21:54)



And gold has another kind of property that when they are in dispersed mode hold 0 nanoparticles not silver gold. So, gold nanoparticles in disperse they are so pink color as you have seen this color. So, that is the dispersed nanoparticles pretty transparent you can see now if you vary the concentration and make it more dense this is also gold nano but aggregation because of high concentrations the atoms aggregate the nanoparticles aggregate.

So, this is aggregation the reason for aggregation are various it can be automatic interactions or it can be the interaction between the surfaces the functional groups present in the surface can interact between each other also. So, these are the functional groups present in the surface. So, depending upon your choice you can make your surface in such a way that it will facilitate aggregation.

So, for example, those who are biologics maybe knowing that biotin avidin binding is very strong and people in biotechnology in bio in chemical biology, this is one of the oldest techniques if you want to study binding between 2 components, one component is attached with the biotin other component is attached with it avidin so, that you will know that they will come instantly closer together.

So, here also such kinds of tricks have been used to make the aggregation so, if you have a mixture of biotin stabilized gold and another one is avidin stabilized gold then if you mix them

together they will immediately form aggregation. So, you will have gold aggregation and this aggregated gold has a color of intense blue, intense blue color you will get. So, this change of color you can measure of course using the spectroscopic techniques using the UV. This change of color is also a way to study different biological aspects.

And this is what has been used very nicely to find out presence of single base mismatch in DNA. For example, if one gold nanoparticle you attach with a single stranded DNA and another golden nanoparticle you attach with another SS DNA single stranded DNA. If these 2 are complimentary then you will have hybridization perfect hybridization and that will give you aggregation. So, it will show you blue color this is pink, this is pink gold mixed together that will give you blue color.

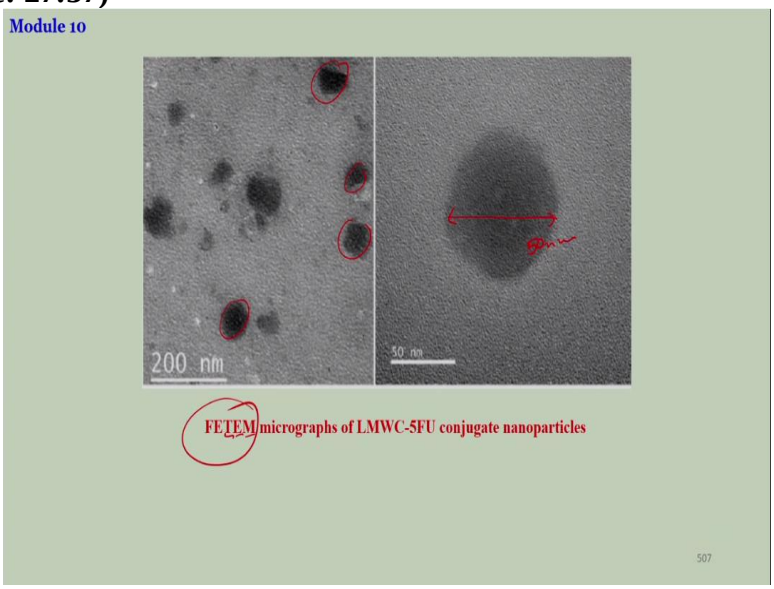
If these are not matched properly, if there is single presence of mutation here, then they will not aggregate. So, and you will not see the blue color. So, like that this is the plant I have explained, there are a lot of tricks in it. So, these kinds of phenomena have been used very nicely to study lot of biological activities lot of chemical activities as well. So, like that you can develop many nanoparticle materials.

And one important thing I forgot to mention I should mention is that you have stabilized these with the polymorphic metrical another reason of using such bulky large structured material is that because of their hydrophobic filling property or sometimes they have hydrophobic property, you can make use of them to encapsulate or to trap small molecules, such as drugs. I have talked about using only the nutrition or only the macro molecule and the drug.

Now I am talking about using the metal nanoparticles stabilized by this macromolecules and encapsulated drugs. This is where the most of the drug delivery applications came. So, you can have if you have your drug hydrophilic, then it can easily be entrapped on the surface because these are hydrophilic in nature now, to some extent, so your drugs can be encapsulated here, and then whole thing as a nanoparticle form will get into the cell.

If your drug is hydrophobic, then you can make these things hydrophobic by attaching the long carbon chain hydrophobic carbon chain and then your drugs will be inside will go inside if these are hydrophobic in nature hydrophobic in nature, drugs can be bound here bound here. So and the whole thing stays as a nanoparticle kind of form quite stable. And then you can take that into the cell. So drug delivery applications are huge when it comes to metal based nanoparticles. So, with this, I will conclude this module.

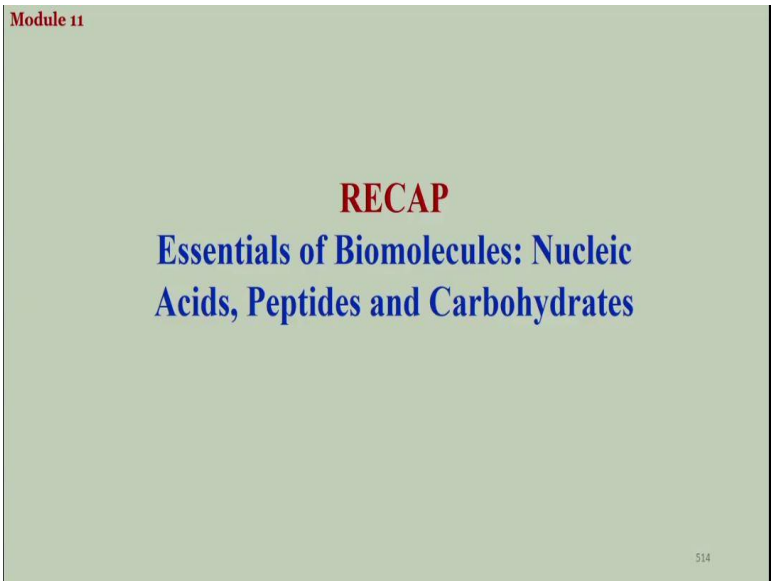
(Refer Slide Time: 27:37)



This is how the size of the nanoparticles look like. This is the 200 scale. This is called FETEM fuller electron transmitted electron microscopy. Transmitted electron microscopy is TEM and TEM is the Fourier Transform factor. So this is the size of the nanoparticles. This is the size of the nanoparticles size of the nanoparticles, this is some of our picture actually, if you look at a single nanoparticle. This is the size which means this basically is around 50 nanometer.

So, this is well within 100 nanometer range and we have synthesized by modification of the chitosan with the drug there. So, like that it is so, if you read it in a high hand machine, it will show you the image like this. So, with this, I will conclude this module. And today, I guess is going to be my last lecture of this course. So, I will give you a recap of whatever we have covered in this course, so far.

(Refer Slide Time: 28:49)



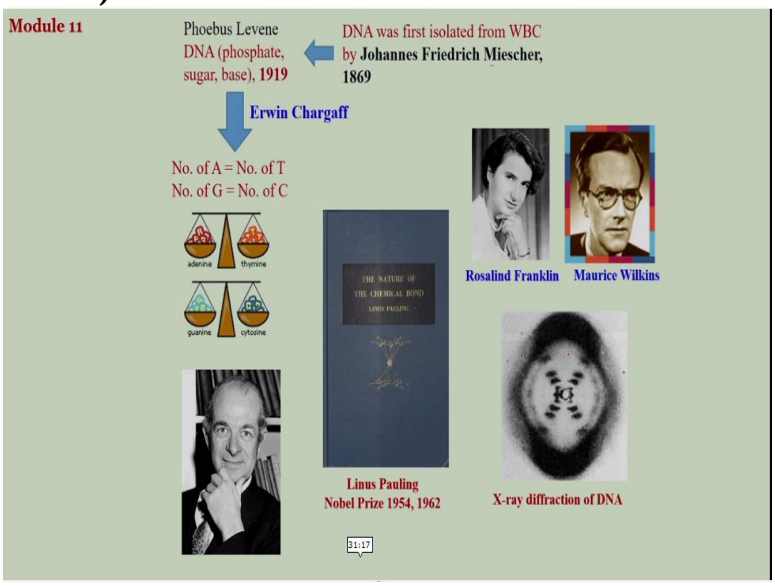
So, the title of this course, obviously, is essential so biomolecules nucleic acids peptides and we have later on added carbohydrates I have divided into 10 modules this the last one being the module 11 which is the recap basically. And over time the first course content that I had shown you, we have done kind of nominal modifications in 1 or 2 modules, change the names for examples had. So, this is the total overall module features that we have covered. First one being nucleic acids and proteins.

Second module was on synthesis of artificial nucleobases or as well as natural nucleobases and nucleotides. Third module is DNA replication polymerases DNA sequencing, and PCR. Module 4 was on DNA damage mutations and cancer. Module fifth we have talked about DNA to proteins, the process of transcription, translation and the genetic code. Module 6 is about protein sequencing methods.

So, I had divided the module into 2, it was a single module, these 2, 6 and 7 was single module. So module 6 is on protein sequencing methods. Module 7, we have talked about peptide synthesis, the organic synthesis of peptides and its application into therapeutics. Module 8, we have talked about certain modern techniques that we use in Chemical Biology for diagnosis of disease. Module 9 was on molecular probes PNA and LNA.

Module 10 was this module that we have just finished is the chemistry of carbohydrates, sugars and carbohydrates and how we can use the carbohydrates as biomolecular probes. So I will give you a brief overview of whatever we have learned so far, the key points and the key aspects of those modules. So let us start with module 1 nucleic acids and proteins.

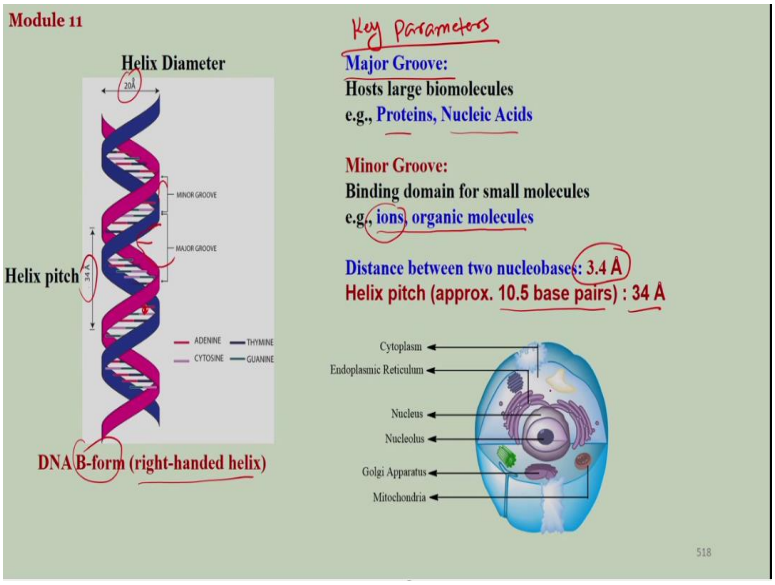
(Refer Slide Time: 31:29)



So, in this module, we have actually seen how the double helix structure of DNA was discovered or evolved. Taking all the information's taking all the right kind of information that were available that time. For example, it was started with the Miescher who has first isolated the DNA contents the unit's phosphate, sugar and bass. And then Chargaff found out that the equivalency of A and T and the equivalency of G and C number of adenine in would be equal to number of thymine.

That number of guanine was equal to number of cytosine and this equal factor exists in all DNA is that he has isolated. So, and using these 2 key information's and using the book that gives the bond distances that gives the angle that gives that talks about the electronegativity of the atoms. Watson and Crick has finally established the double helix structure of DNA taking the experimental digital X-ray diffraction data that was given by Rosalind Franklin and Maurice Wilkins.

(Refer Slide Time: 32:48)

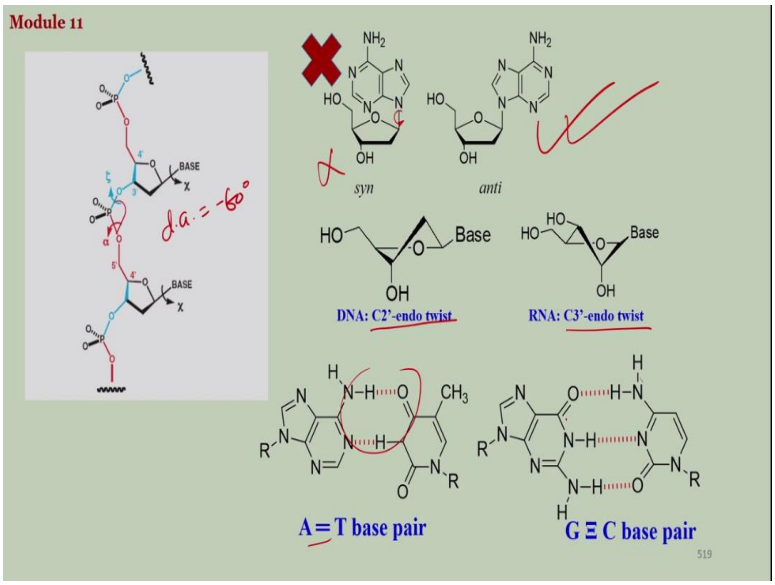


And so, this is the double helix structure, basically, which is actually a right handed helix. The key parameters or features of a double helix DNA or we call it B form of the DNA is that the distance or the diameter of the helix is 20 angstrom the distance between 2 nuclear bases distance between 2 nuclear bases. So, nuclear bases are perpendicular to each other. So, here is one nucleobase here is another nucleobase as it is given here.

This is the distance between the neighboring nuclear bases, they are in this form is around 3.4 angstrom. And in a helix pitch, that distance is 34 angstrom. That means, one complete turn is 34 angstrom which means each helix pitch or each complete turn contains about 10.5 base pairs on average. And because of the double helix structure or helical pattern, it forms 2 kinds of groups one is called the major group, which is explained here, that is a large cavity.

And the major group because it is large it is for or it was binding with large other biomolecules, such as proteins, such as other nucleic acids like RNA. It also has a minor group with a small cavity here. And that is being small, it only allows binding with the very small molecules like the ions in like the drugs like other small organic molecules. So, these are the key features or the key parameters of the double helix. This is where a DNA stays inside the chromosome that we have talked about stays inside the nucleus.

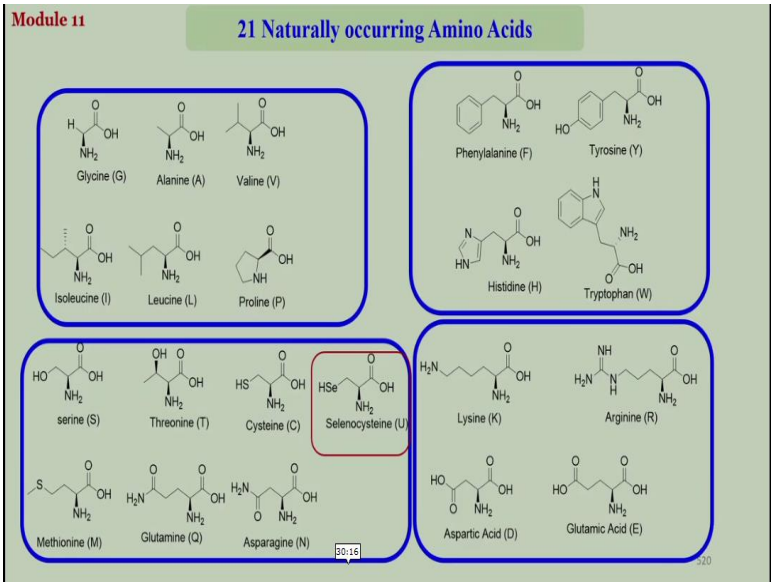
(Refer Slide Time: 35:05)



When it comes to the structures, this is the how the structural orientation happens this angle it has a dihedral angle minus 60 degree. So, that is very important for a DNA that makes the DNA helix actually that is the backbone structure sugar is oriented a little bit tilted from the backbone or sometimes you can just think of like this, if this is the backbone, this is your sugar, this is your sugar and then the basis is like this nucleobase is like this.

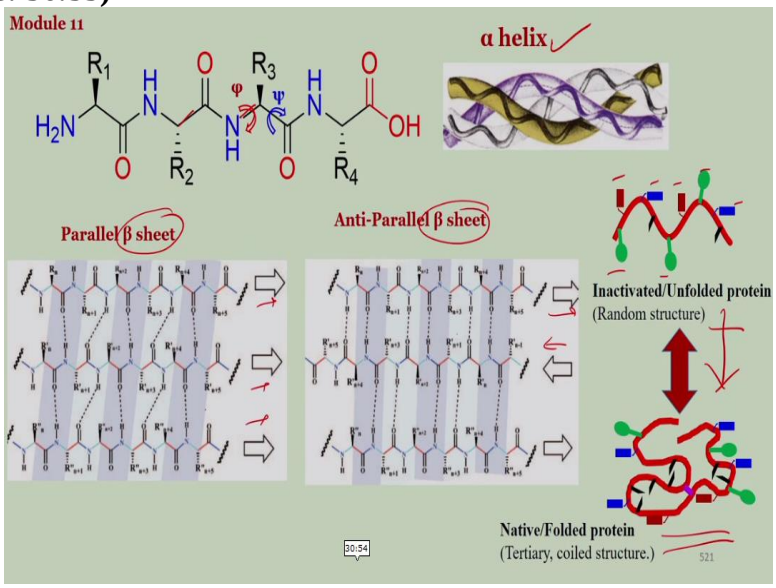
When it comes to the confirmation of the sugar nuclear base sin geometry where this nuclear waste will fall on top of the sugar is not allowed as it is, this is the actual geometry anti geometry that exists in DNA. Similarly, the sugar Parker conformation exist as C 2 prime endo twist for DNA and C 3 prime endo twist in RNA and because of this it can acting the A T base pairs and G C base pairs with 2 hydrogen bonds and 3 hydrogen bonds respectively.

(Refer Slide Time: 36:37)



Then after the DNA we have talked about the amino acids, we have seen all the natural occurring amino acids they are structures, they are very essence as we have talked many times I think.

(Refer Slide Time: 36:55)



And we have seen how want to have the sequence of protein or sequence of a peptide, you call it the primary structure, then it does not stay in that sequence forever, because of its single bond rotation, here is also a single bond, because of the presence of the single bonds the bonds can rotate. And it can assume certain orientations. That is called for storing is of course; we call them the secondary structure that is because of the local interactions.

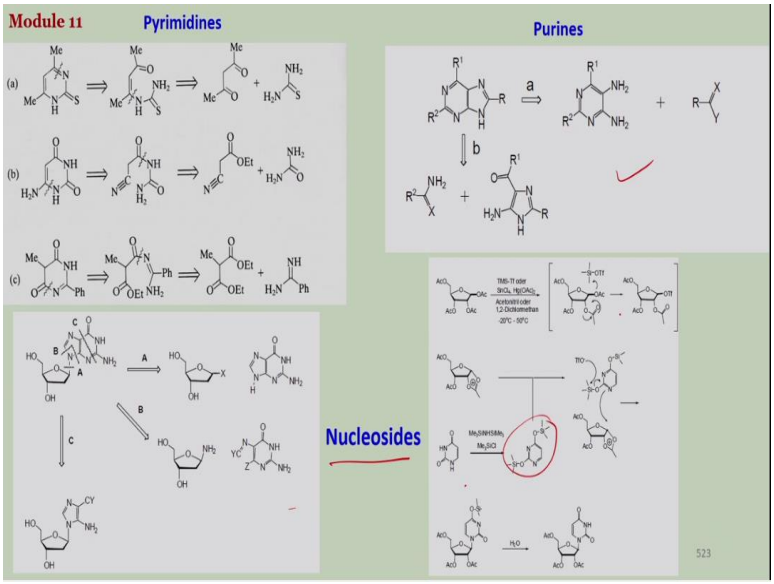
And we have seen that it can assume 2 kinds of confirmations, one is the helix structure, which is like this. This is a picture of the collagen it can either be a helix or it can assume beta sheet confirmation. So, that is a parallel confirmation. So, this plane can be of 2 types. One is the parallel and other is anti-parallel as it is mentioned and the beta confirmations are basically because of the inter molecular hydrogen bondings and by stacking alpha helix was because of the intra molecular hydrogen bonding interactions and electrostatic interactions.

So, this this this are the secondary structure of amino acids or secondary structure of the proteins and then large scale interactions little bit distant interactions gives a folded structure or a 3 dimensional structure of the protein that is because of the variation of the side chains that are present in the protein. So, hydrophilic side chains are there hydrophobic chains are there basic side chains acidic side chains.

All these factors are there and because of their long range interactions they kind of fold into each other that gives you the 3 dimensional structure or the tertiary structure of the protein. And as a result it forms hydrophobic core, it forms the hydrophilic surface. Usually we have seen the interior of the protein is hydrophobic in nature that does not allow water to come in the surface of the protein contains the all the polar side chains or the charged side chains and that interact with the polar solvents such as water or other electrostatic molecules.

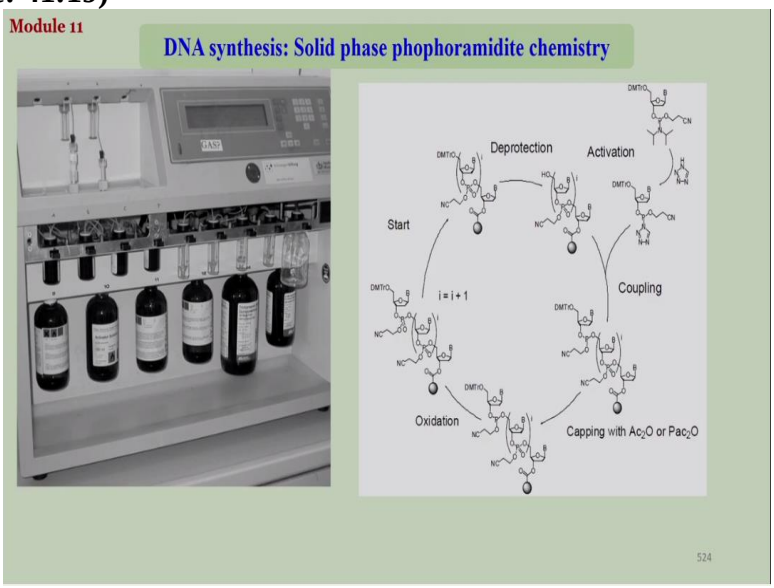
And we have seen also that from unfolded secondary structure of the protein to the folded tertiary structure of the protein. This is a very spontaneous process. So, this is the ultimate geometry or ultimate confirmation that a protein exists. So, those who are about module one. Now, coming back coming to module 2 second module, we have seen how we can develop how the nucleobases that are present in nature or you can make a lot of different modifications for our own purpose. And how can you also synthesize the real nucleotides such as DNA in your laboratory.

(Refer Slide Time: 40:30)



So, these are some of the methodologies that we have talked about, this is the synthesis of pyrimidine components in general that basically means the synthesis of the pyramid in nucleobases also such as thymine uracil and cytosine, this is how we can synthesize the purines, adenine and guanine and there are various derivatives. These are the techniques how you can synthesize the nuclear sides which means attaching the basis on to the sugar that has various 2 or 3 approaches we have talked about. This is one of the example how thymine this is uracil uracil was attached with the sugar moiety in a multi step reactions.

(Refer Slide Time: 41:19)



And then we went on with the synthesis of the DNA oligonucleotides that we call polymeric structure of the nucleotides or polymeric structure of the nucleotides. So, this is mostly you have

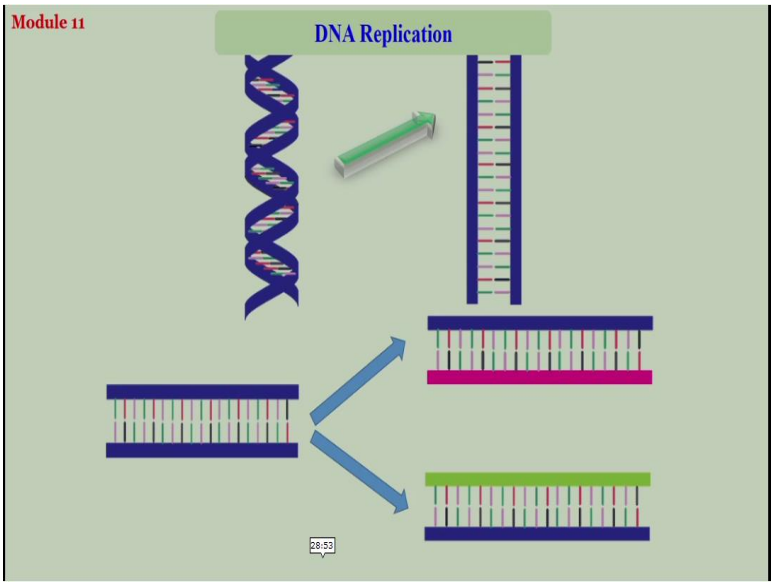
talked about the solid phase phosphoramidite chemistry phosphoramidite was the monomer this that you have synthesized in using the organic synthesis methods. So, which basically has a sugar moiety attached with your desired nuclear base and in the 3 prime end you have phosphorus with it protected way.

And your 5 prime in also has a DMT production. So it is basically about the reaction that will allow you to continue this way to make bonds and go further. So, it goes on a series of reaction cycles series of reaction steps also. And everything we have talked about that everything can be done in a machine, all the DNA synthesizer or you can call it also oligo synthesizer and this is the place where the synthesis actually happens.

It goes through a lot of steps through activation then coupling then capping to make sure that that the unreacted ones. That are remained in the solid phase does not react in the next step, then oxidation, then the cycle continues. And using this technique, you can synthesize short oligonucleotides short DNA sequences that can have a sequence up to maybe 3040 5050 is little bit too large, but at least 30 base pairs long DNA.

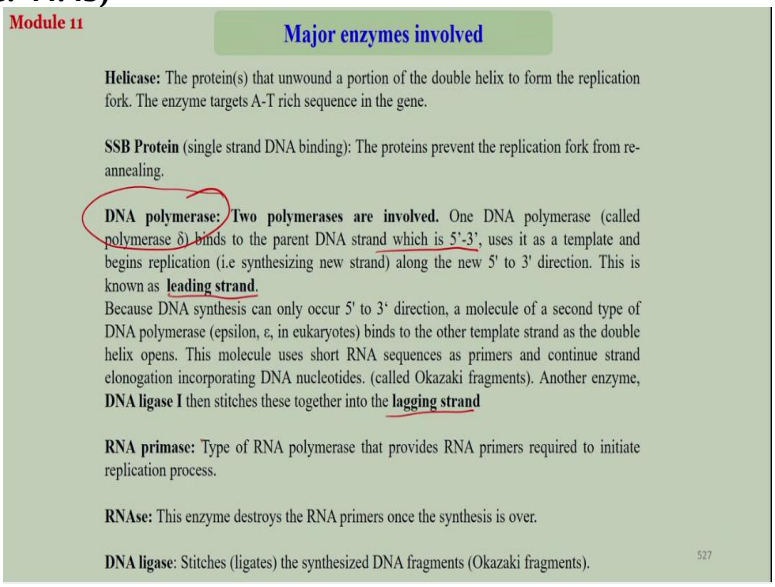
And then came a very large module that was module 3, this is where we have talked about the biological process that goes on inside the cells, that is DNA replication. And then, how we can how we have developed technologies to use the kind of the process of replication to synthesize more number of more copies of DNA. So, basically from the cell to a machine. We have also talked about DNA sequencing techniques and all of this actually it was the same kind of this idea of DNA replication.

(Refer Slide Time: 44:07)



So in the end replication is basically how you can multiply how the multiplication of DNA or gene happens inside our cells. From one DNA, how you can get 2 copies of double stranded DNA. That is what happens during cell division process. One cell divides into 2 cells, and each cell has a nucleus. So each cell contents all the genetic molecules that is DNA. So, one DNA from the parent cell has to be divided into 2 DNA which is actually the same as the mother one. So that is what is the process of replication.

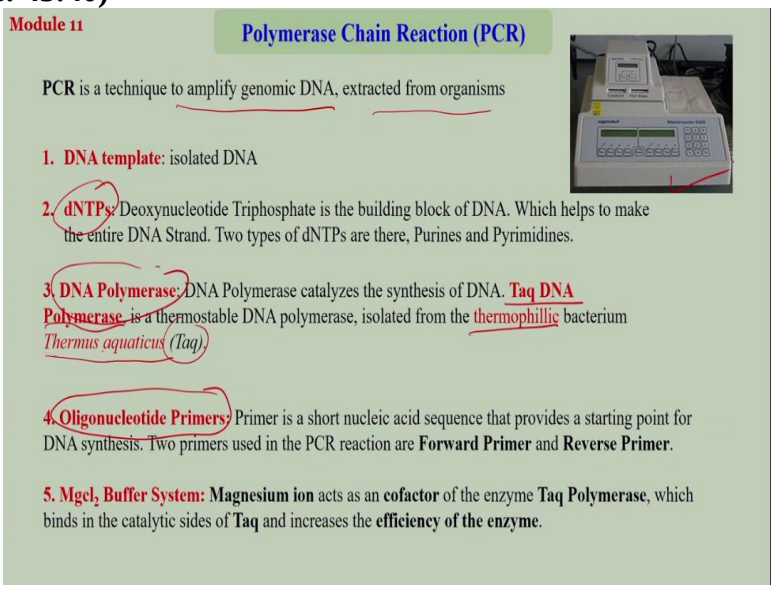
(Refer Slide Time: 44:45)



And we have seen that it goes through a series of steps, involving a lot of enzymes involving lots of other small molecules involving oligonucleotides, such as RNA. So helicase is the key enzyme present SSP protein we have talked about the most important enzyme we have talked

about was DNA polymerase. And that you need 2 kinds of polymerase for DNA replication, one for the 5 prime to 3 prime strand, other is for the leading strand, other for the lagging strand that is the 3 prime to 5 prime strand. So, 2 types of DNA polymerase are essential for cellular replication of DNA. And then other enzymes of course, RNA prime is RNAs or DNA ligase.

(Refer Slide Time: 45:40)



And all sorts and then how to use the cellular process into a laboratory. So if you need to copy if you need to make more number of DNA from our given isolated DNA, how can you do that in your laboratory? And that was called the polymerase chain reaction or PCR is the technique where you can amplify the genomic DNA that you have isolated from certain organisms. And we have developed certain steps to make this process possible.

You need a DNA template, which is basically the DNA that are isolated and then you need the individual components that we call dNTPs triphosphates nucleotide deoxybase nucleotide triphosphates that are basically the building blocks for the synthesis of the counter strand. And then, the key important was the use of the polymerase which you have used to for the cellular process. Cells used to, we cannot in this case, we have seen that we do not need to use 2 polymerases you can use single polymerase.

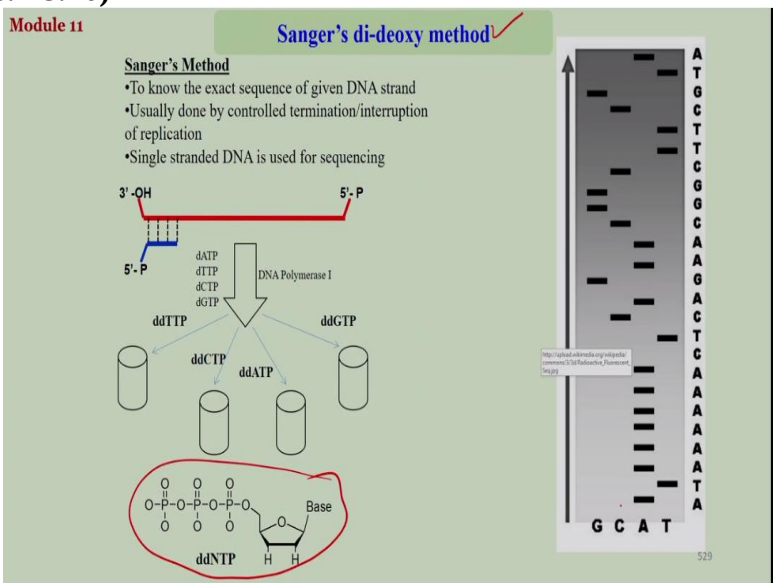
But the problem was that in the process you need to break your double stranded DNA into single strand and that requires high heat. So, the key question was, will the polymerase be stable in that high temperature. So, you cannot do your reactions at high temperature because polymerases the

normal polymerases they are used to function at the physiological temperature which is 37 degrees Celsius in our body.

And there came the major breakthrough that people have found out the discovery or isolation of Taq DNA polymerase. That is basically a thermophilic bacteria, which functions at a very high temperature close to 100 degrees Celsius. And this is the thermos aquaticus this organism microorganism or the bacteria from the bacteria or the Taq polymerase was isolated. And the name also came from there, the Taq because of the thermos aqueous Taq is aqueous that makes it Taq.

So use of Taq polymerase it solved your problem of high temperature during the course of the reaction. And then things become easier. Of course, we have learned already how to synthesize oligonucleotide. You used that as primers here. And then you could do the cycle of process that will every time multiply your DNA and that can be done in this machine call the PCR machine.

(Refer Slide Time: 48:40)

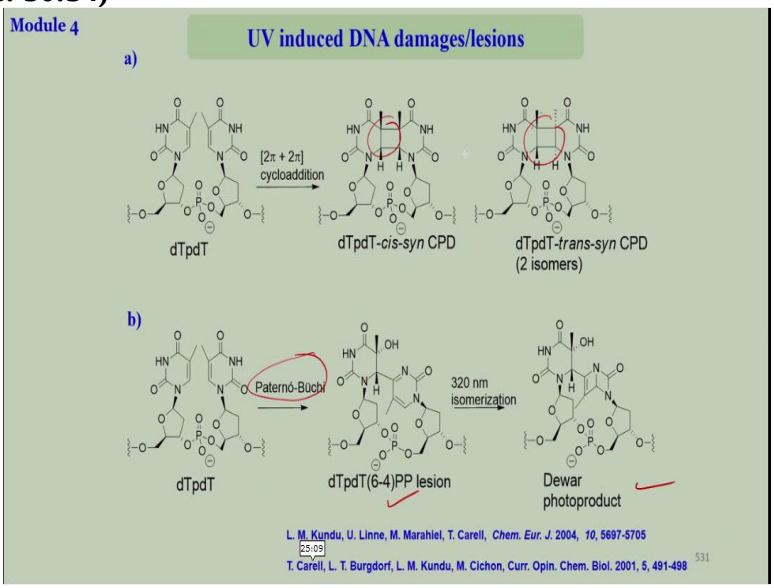


And then one of the best one of my very favorite topic was the sequencing of DNA. How can you find out the actual sequence of the DNA and the most I have talked about was the Sanger's di-deoxy method which is one of the best method actually for sequencing our DNA. And here we have seen how a synthesized molecule which is the di-deoxy was used di-deoxy NTP was used as a stopper during the replication of the counter strand.

So every time in each of these vials you have added shorten amount of the ddNTP. And that was actually stopping at every base when you have been elongating the synthesis of the counter strand using the enzyme polymerase. And by studying those fragmented patterns, you can see the in the gel electrophoresis diagram very nice. Every nuclear base will give you a band break off every nuclear base, or the stock of every nuclear base will give you a band.

And it is one of the simplest analysis techniques that you can imagine, through which you can actually write down the sequence of the whole gene or the whole long DNA very easily. The module 4 we have seen here the chemistry that actually goes on inside our cells or that are the main key reasons for the development of mutations, which in turn, are the reasons for aging and cancers. So how the DNA gets damaged, how those damaged damages are responsible for mutations and how those mutations are adapted to cancer.

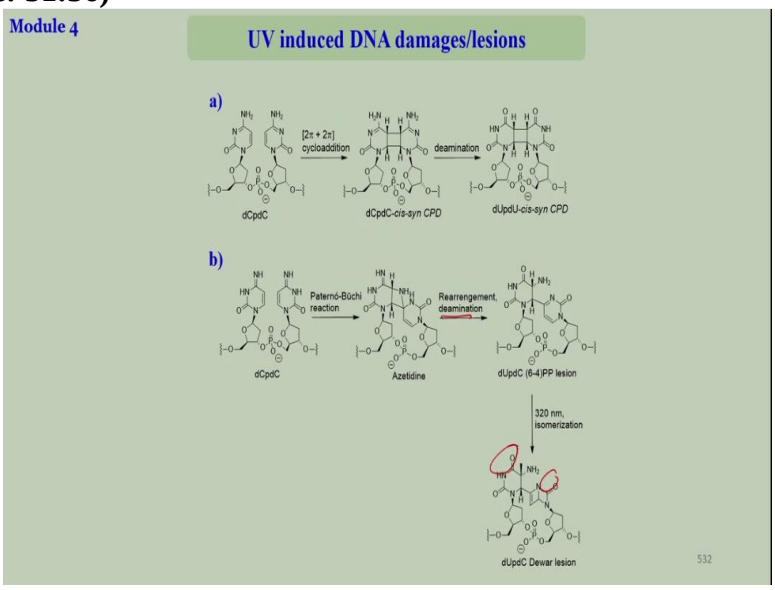
(Refer Slide Time: 50:34)



And here we have of course, talked about first with the UV induced DNA lesions, that we have seen that if you have a dinucleotide that can form the cyclobutane pyrimidine dimers. By doing a photochemistry, it can be 2 different types one is 2 plus 2 cycloaddition reaction standard other is also 2 plus 2 cycloaddition but a different one Paterno-Buchi that gives you formation of the damages like this. And these damages because once they are the chemical nature of the individual nucleobases are now changed.

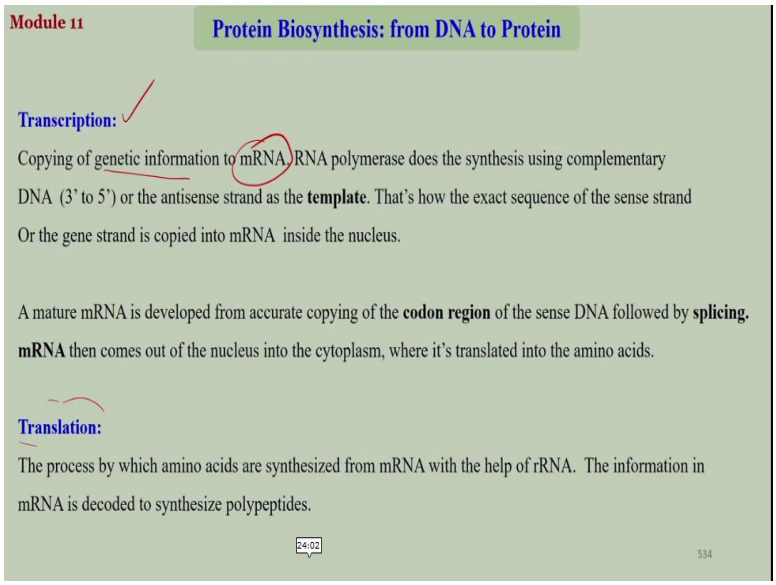
So if you have started with T this is all T this is no longer T this is no longer T. This is no longer T this is no longer T here you have a matched one. So, nucleobases has changed their chemical structures, which obviously means that they have changed their chemical properties also. And those change of chemical properties are the reasons this is for cytosine.

(Refer Slide Time: 51:36)



Cytosine, we have seen the 8 undergoes deamination also. That gives you conversion from cytosine to uracil is the deamination. And those are the structural changes that are responsible for developing mutations. And those mutations are the key reasons for the growth of the tumor cells or the cancers. Module 5 in module 5, we have seen how the proteins are bio synthesized in cells and they take the information's that are present in DNA that are presenting the gene. Those information's are used to synthesize or as directions to synthesize the proteins of a particular sequence and that has 2 keys steps that is one is transcription or the other one is translation.

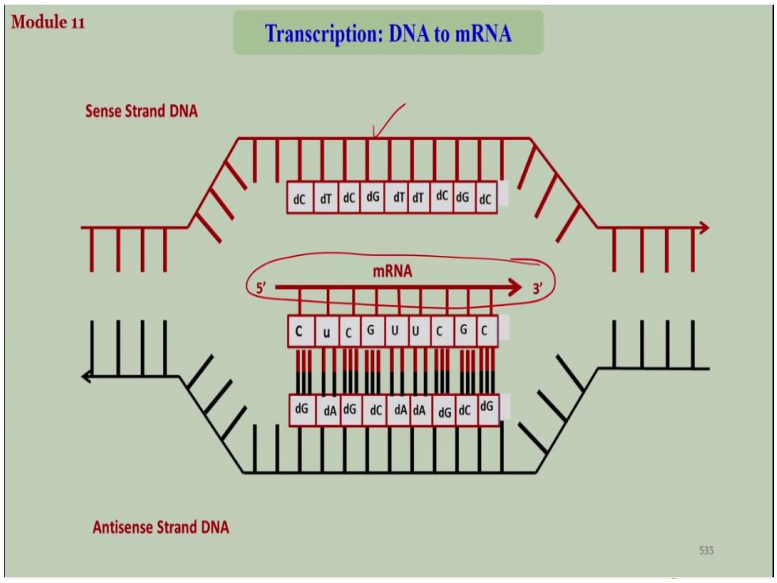
(Refer Slide Time: 52:40)



The process of transcription was how you can synthesize or how you can take the information from the nucleus to the cytoplasm. Because protein is synthesized in the cytoplasm, DNA stays in nucleus. So, how is this information transfer of information are happens from the nucleus to the cytoplasm that is basically your transcription. And here we have seen how mRNA was synthesized taking the genetic code as the template.

So, that is the first thing. Second is how mRNA with its sequence with this information is governing the synthesis of the protein or in other words, how the language present in the nucleic acid language, the codeon present in mRNA is translated into an amino acid language, which is protein.

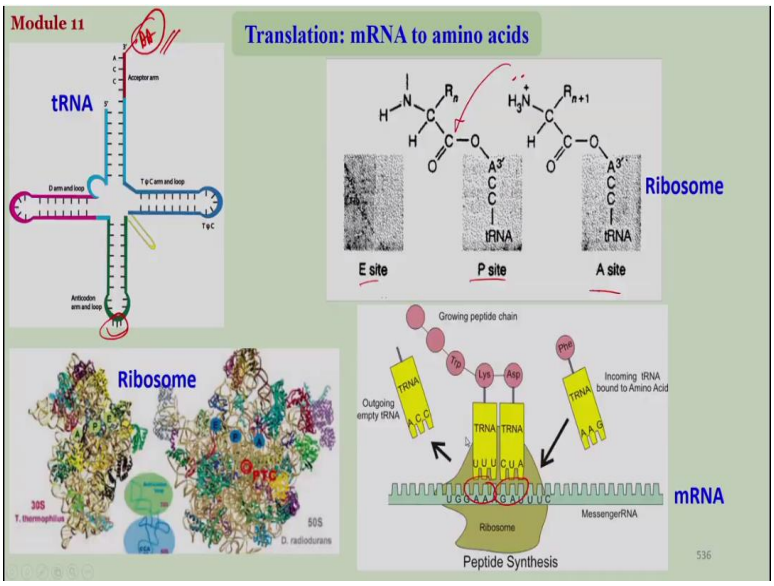
(Refer Slide Time: 53:46)



So, here was the process of transcription that we have talked about that in the genome you have double stranded DNA, 5 prime to 3 prime 3 prime to 5 prime which we have called sense strand and antisense strand. So, idea is that you have to this sense strand contents all the information. So, you have to synthesize a copy of the strand. So, best way is that you use the antisense in the cell you had used antisense strand as the template and synthesize the complimentary mRNA sequence from DNA to RNA.

And this mRNA sequence contains the exact sequence cell copy of the parent DNA and this comes out of the nucleus. So, that is what was the process of transcription or this is a means the messenger. So, RNA is behaving as a messenger of the gene.

(Refer Slide Time: 54:45)

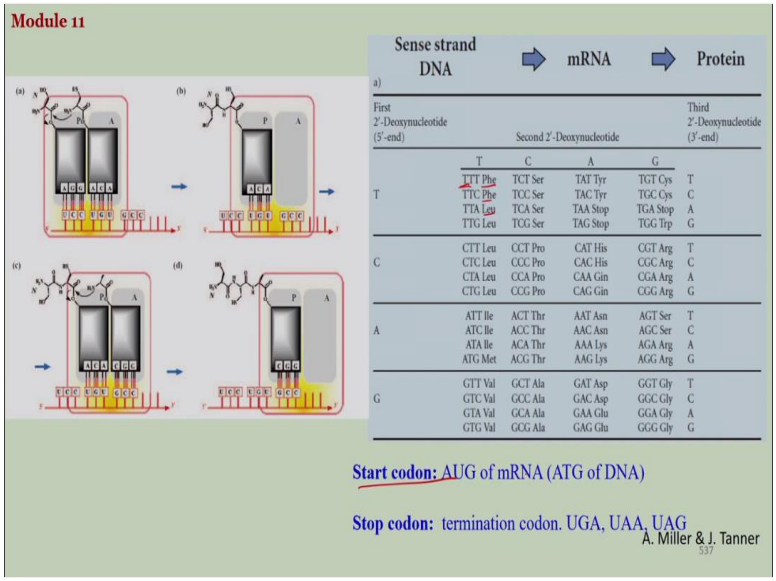


Next came the translation, how is the nucleic acid sequence in mRNA is translated into amino acids and there we have seen the activity of this is why the amino acid is attached activity of tRNA transfer RNA, which has of course, a complicated structure and which contents which carries amino acid here and each of the tRNA carries a 3 letter code RNA code here and this is the complimentary to the mRNA sequence part of the mRNA sequence.

And we have seen that the whole thing happens in a resolver which is called ribosome acts as a platform where all the synthesis happens. And the details of the process we have talked about that ribosome has 3 sides, A side B side and A side where the mRNA comes and binds. So, each tRNA comes and binds to its opposite mRNA strand and each tRNA content amino acids this tRNA contents also an amino acid they come close in proximity and then the reaction happens this attacks now, this may be the peptide coupling happens there.

This is where the peptide coupling happens B side A side this and it goes on goes on goes on and here comes the codon we have talked about because this is the place the tRNA this specific 3 letter alphabet it binds with the mRNA. So, this is very important which we call anticodon and this is responsible for which amino acid will be coming here.

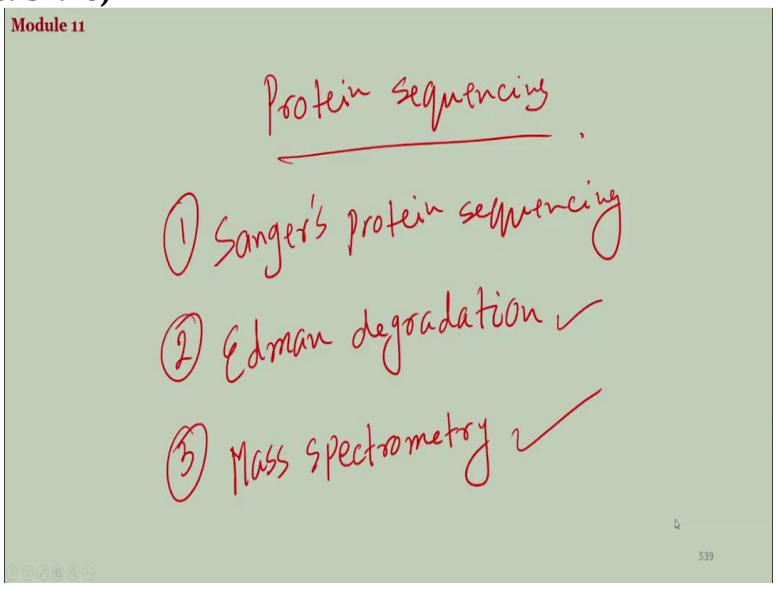
(Refer Slide Time: 56:47)



And then that we call it a codon so, codon came into picture here for which Professor Quran is very famous. So, here we had given a table charge of which codon is responsible or which codon determines which amino acid sequence you can have all these codon this also have a start codon without that start codon the protein synthesis does not start this is the start codon and you also have a stop codon you have to stop somewhere once it reaches the stop codon.

The synthesis stops there because the stop codons does not carry the amino acids. Module 6 was on protein sequencing. And here we have discussed how to at least 2 different methods we have talked about.

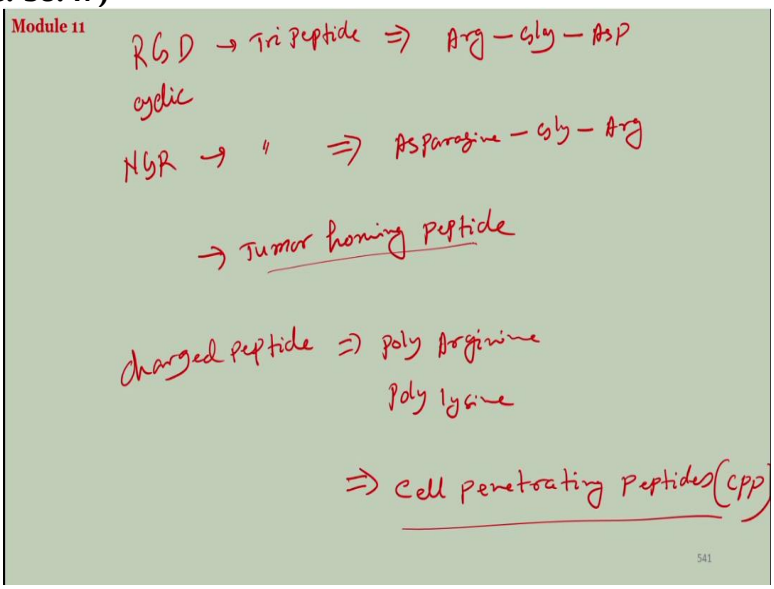
(Refer Slide Time: 57:48)



One is Sanger's method again and another one was what was it Edman's degradation method and third method is of course, using the mass spectrometry. So, Sanger's protein sequencing method, Edman's degradation method and mass spectrometry method all of these methods are associated with a lot of other side reagents to because you have to hydrolyze we have that sort we have seen how to hydrolyze the peptide sequences large protein units into the smaller peptide sequences. The other ways these methods cannot handle such a large length of molecule.

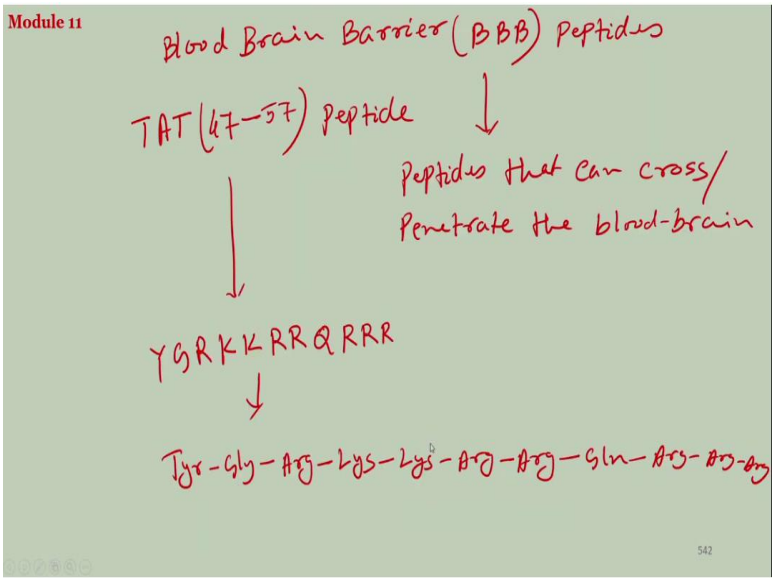
So, Sanger's method was primarily used for in Terminal amino acid determination and then full sequence determination we have seen that was done by Edman's degradation method, module 7 was on an organic synthesis How can you develop short sequences of peptides in your laboratory and they are applications into therapeutics.

(Refer Slide Time: 58:47)



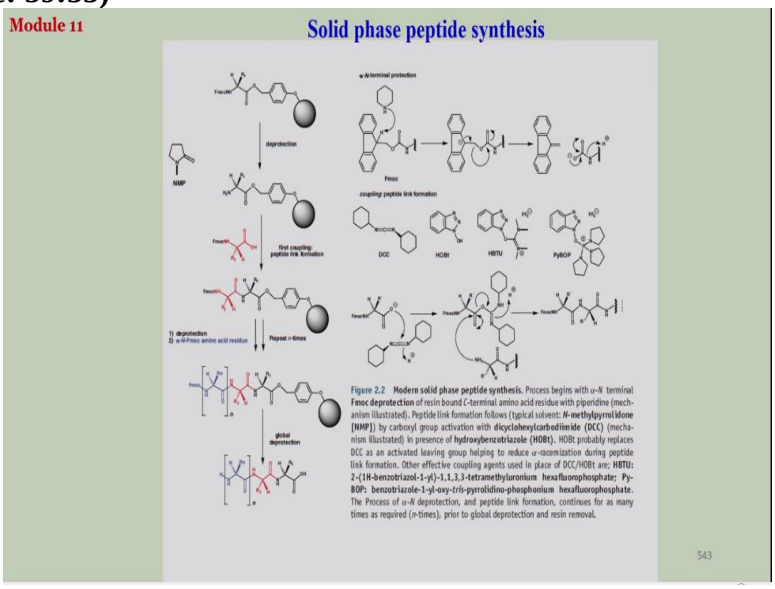
Here we have seen that certain peptides so, very good therapeutic activities like RGD peptide NGR peptide then you have these are basically the tumor homing peptides. So, that can get into the tumor cells specifically. And then you have cell penetrating peptides, which are polymeric peptide with a high positive charge density such as arginine such as lysine, they can penetrate into the cells.

(Refer Slide Time: 59:19)



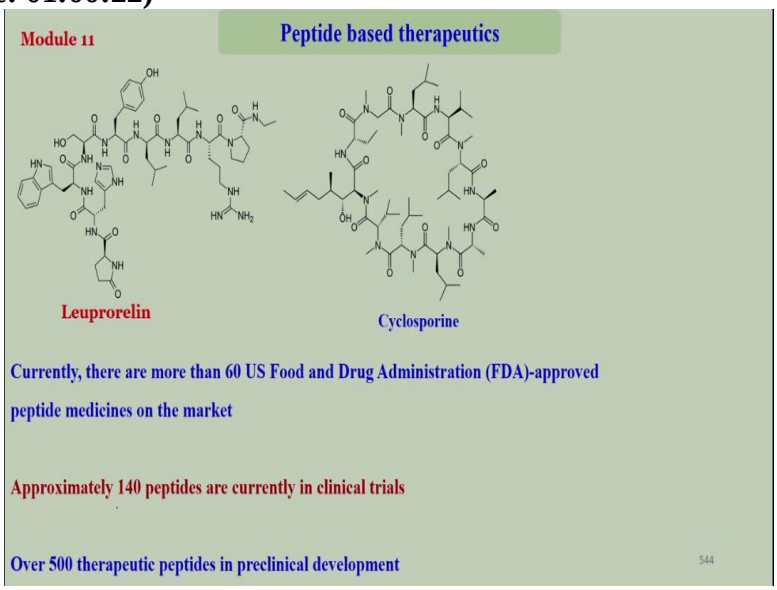
So, similarly, if you are very important is the blood brain barrier peptides, because it is actually very difficult to take any mutation into the brain cells, because brains brain cells are over protective. So, certain kinds of peptides such as this sequence, they are actually they can invade or they can cross the brain blood brain barrier. So, such kinds of peptides can be used as therapeutics to carry other drugs along with them into the brain cells particularly.

(Refer Slide Time: 59:55)



Then we have talked about how the solution phase chemistry you can adapt to synthesize small peptides, but the best one is of course, by using the solid phase peptide synthesis protocol, which goes in a multi-step organic chemistry reactions all happens in the solid phase using a reason and you can synthesize fairly are length good length of the peptide from there.

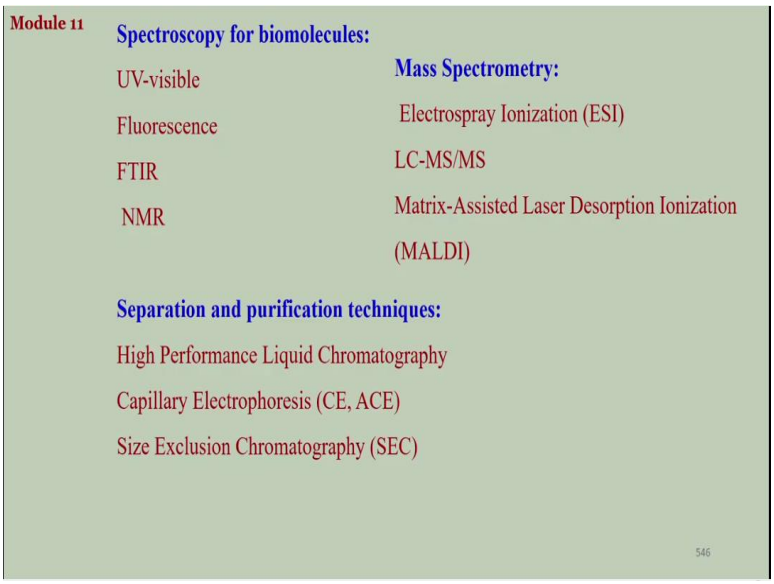
(Refer Slide Time: 01:00:22)



Some of the peptides that has been used as drugs that are currently in the market. This is a little bit complicated geometry, this is a cyclic peptide. This is open chain peptide, and there are many peptides still in clinical trials. There are some peptides already in the market. A lot of peptides that we currently do research on for various aspects to study various aspects everyday new sequence of peptides are synthesized and they are activities that have been proven, but they are not yet in the market of course.

Module 8 we have given some descriptions of some of the technologies, molecular technologies as well as instrumental techniques that we nowadays adapt to for biomolecular research into Chemical Biology into biotechnology into bio organic chemistry, whatever you call it.

(Refer Slide Time: 01:01:22)

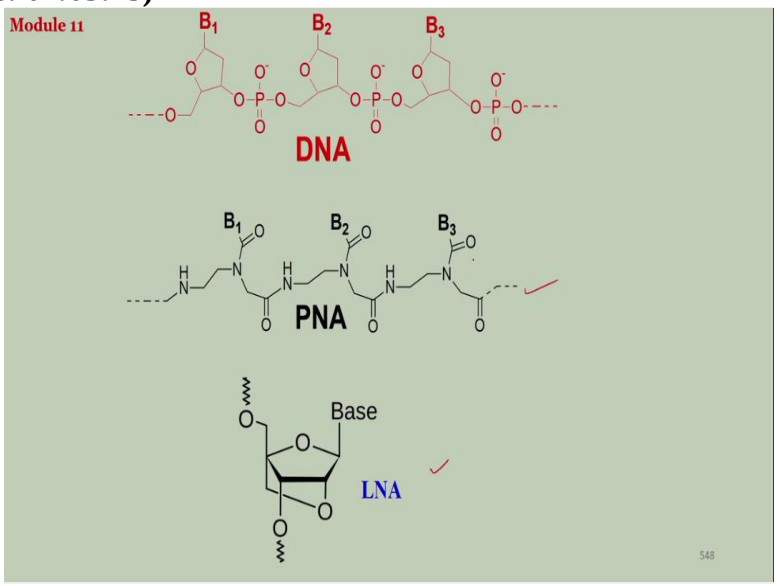


For example, and I have given an overview of the spectroscopic techniques, how UV visible fluorescence FTR NMR I have not talked about, these are the some of the spectroscopic techniques that can be used on the biomolecules they can be mass spectrometry, we have talked about that electrospray ionization, we have talked about high and mass spectroscopy, that is mass mass MS MS. We have talked about for very large polymeric biomolecules macromolecules that has very high molecular weight.

You can use the multi matrix assisted laser desorption ionization technique to find their purity or to do their characterization. So, actually, you can also do protein sequencing using this mass mass and as well as MALDI technique. And then we have talked about the separation techniques for the biomolecules since they are very large molecules, it is hard to separate them, we are dropped out HPLC we have talked about capillary electrophoresis, affinity, capillary electrophoresis. And then size exclusion chromatography also among a few others.

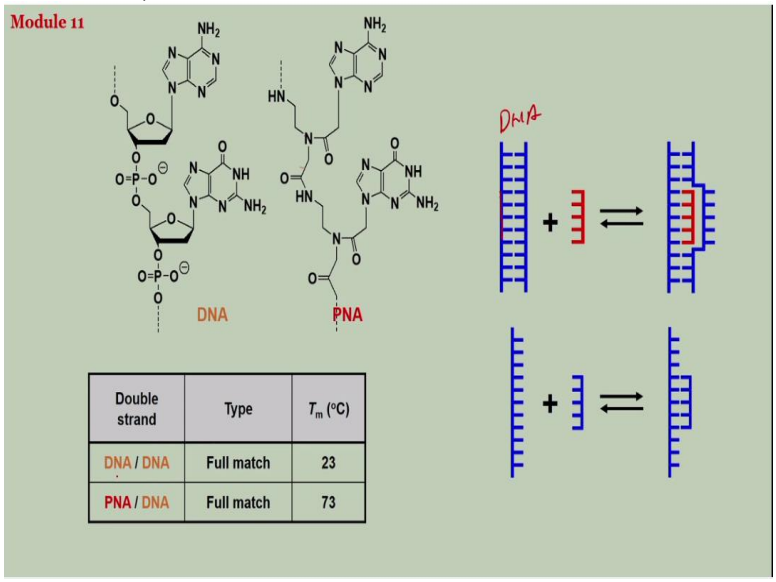
Module 9 was on molecular probes. How we have synthesized as organic chemists. Our aim is to develop a new molecules that can have that have utilities in biology. So, peptides nucleic acids, PNA and locked nucleic acids LNA are the modified versions of the oligonucleotides. They are different than DNA and they can be used for to study the biological activities or biological interactions as well as to find out the presence of mutations in DNA.

(Refer Slide Time: 01:03:15)



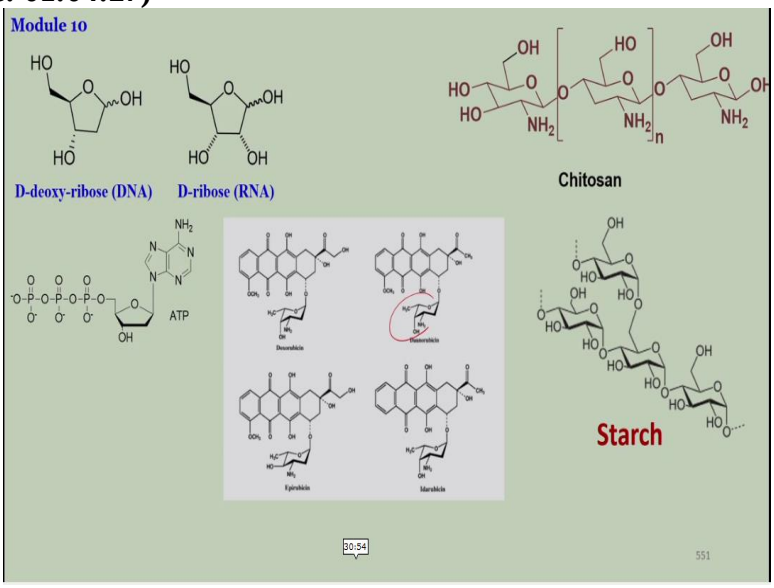
So, we have described the structure of DNA, how the PNA and how they LNA are different, what are their structural features? What are their advantages? What are their disadvantages? For example, both PNA and LNA especially the PNA, because it has a peptide backbone, it can inward into the cells very easily, it is not destroyed by the metabolic systems mostly the nucleus, this is stable nuclear stable this is also to some extent nuclear stable DNA is very unstable to nucleus. Any foreign DNA would be cleaved by the nucleus. So this if you want to carry a foreign molecules, these 2 are the better choices.

(Refer Slide Time: 01:03:56)



So we have seen why that use of PNA if you use that make a very strong hybridization with the target. So, that enables it are very good candidate or molecular proof to find out the mutations present in the gene. In the last module on carbohydrates, where we have seen the structures of the sugars, we have seen the structure of the ribose and D-deoxy-ribose.

(Refer Slide Time: 01:04:27)



We have talked long back, we have seen the structures of the large carbohydrate macromolecules that are present in nature such as chitosan such as starch such as cellulose and many others and some of the drugs that content carbohydrates also and we have seen some of the reactions, how you can play around with the chemistry of sugars. And finally, we have talked about how the large macromolecules you can use for medicinal purposes such as nanoparticles such as drug delivery.

So, this was about the layout and the more or less whole about the course that I have covered, and with this I will be done to for this course. And I hope that I was able to describe certain methods, I was able to give a overview of the biomolecules, how they are structured look like, how their activities occur, how they act inside the cells. And I hope that I was also able to describe certain methodologies, certain developments, the techniques, modern ways modern techniques.

That we adopt nowadays in our laboratory to study the biological molecules. So, with this I wish all of you to have good health and thank you for attending the lecture and all the best for your future. Thank you.