Essentials of Biomolecules: Nucleic Acids, Peptides and Carbohydrates

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Lecture - 32
Molecular Probes: PNA and LNA- II

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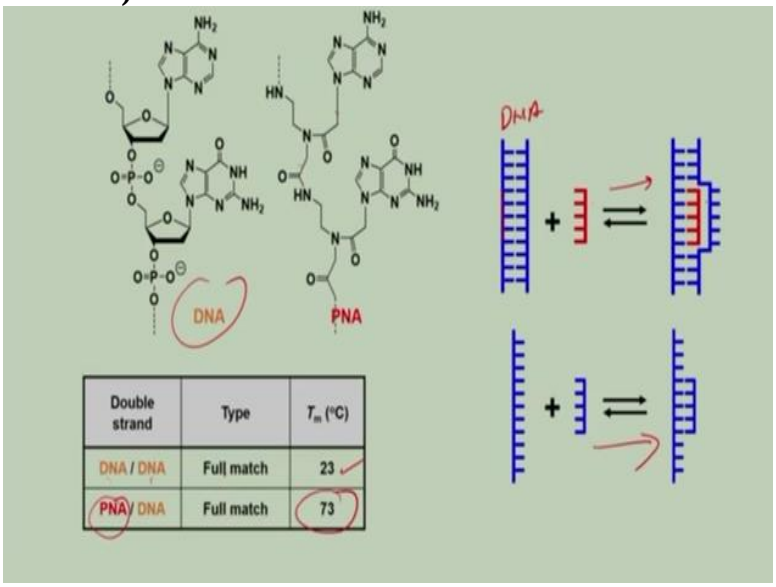

Hello everybody and welcome back to the lectures; So, in the previous lecture in this module we are basically talking about modified oligonucleotides how we can develop new chemically synthesized oligonucleotides that are different than DNA that are different than RNA. There are many numbers of modified oligonucleotides that have been synthesized and that are available in literatures.

Out of those, I am talking about mostly 2 of the modified oligonucleotides which are of major use in biochemistry or in chemical organic chemistry as well as bio organic chemistry. So one of them that I have talked about is PNA, peptide nucleic acids that is different than DNA in the sense that it has a peptide backbone instead of phosphates and sugar backbone. So normal DNA has a sugar, sugar phosphate backbone. PNA has a peptide backbone, peptide bone, peptide bone, peptide backbone, both of them has nucleic acids.

So PNA also has the nuclear bases attached so that is the structure of the DNA and its advantage is that we have talked first advantage and the most important advantage is that it has stronger binding affinity towards its complimentary DNA or it is complimentary

oligonucleotide it can be DNA, it can be RNA, it can be even another PNA and we have also shown that if you if you have a single base mismatch in a large target DNA.

Then PNA DNA double helix double strand melting temperature would be changed or there will be a difference of melting temperature by around at least 20 degree due to presence of a single base mismatch. So PNA DNA binding is pretty strong melting temperature is very high 73 degrees Celsius, the same double strand, the naturally occurring DNA DNA has melting temperature only 23 degrees Celsius and competitive PNA DNA will have a very high 73 degrees Celsius.

So binding or the hybridization degree of hybridization or the strength of the hybridization is pretty high for PNA DNA double helix and that is the use of it that you can use this binding property or this hybridization property to find out presence of single point mutations and we have talked about that these are some of the patterns and the binding patterns that are generated because of PNA, this is called DNA double helix this is DNA double helix.

And if you have a synthesized PNA, which is complementary to part of the DNA, maybe this then this PNA has the ability to disrupt the local hybridization of the DNA and mix a new DNA, PNA hybridized complex or double strand because this has higher energy. So, this is called the displacement of the DNA double helix. Another one is simply if you have a single stranded DNA and if you have a PNA that they will hybridized.

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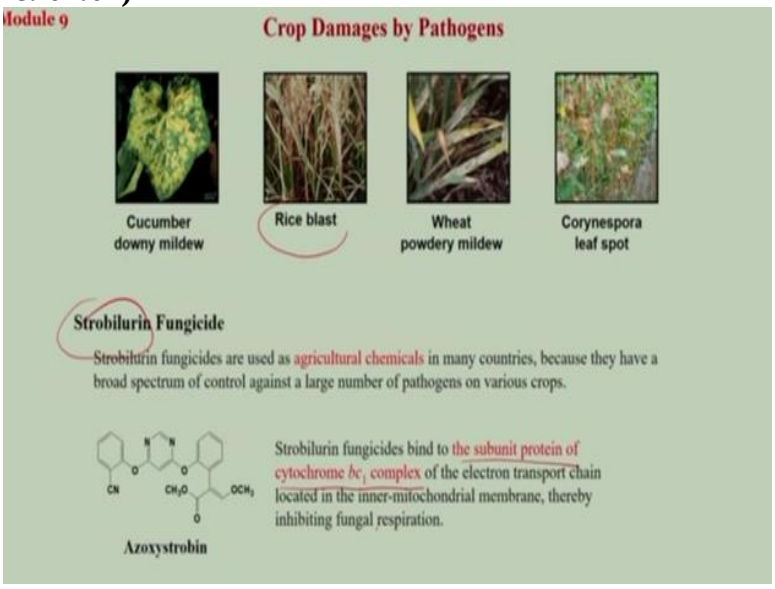

Today also that how we have used or other people also have used in a different manner. PNA use a PNA, how can you detect presence of a single bass mutation? So, in this case I will talk about first the relevance of finding out the mutations why do you need to detect the presence of mutations? Of course, all of the tumour cells have one or more than mutations present in their diseased genes.

Apart from that, this from the agricultural stuffs that the pathogens that attacked the agricultural product crops cucumber downy mildew is a kind of pathogen that will damage the cucumber leaf you can see this this is how it looks like when the leaves have attacked by the pathogens. Rice blast is another kind of pathogen that kills rice wheat powdery mildew also that you can see the white spots on the leaves that are affected by the pathogens.

Corynespora leaf spot the same thing the leaves are damaged and the crops are killed basically by the pathogens. So, mostly when the crops are attacked by pathogens or other insecticides, then we use pesticides to kill those insecticides. So strobilurin is fungicide so, these pathogens are basically the fungus. So this cucumber downy mildew is a fungus rice blast is a fungus, wheat powdery mildew is a fungus and this also is a fungus.

So it will use fungicide to kill those fungus strobilurin is fungicide that is usually used on these kinds of crops. This is the structure of a variation of strobilurin, this is also strobilurin, and this is the structure. How does it work is a fungicide that are used as agricultural chemicals in many countries, because they have a broad spectrum of control against a large number of pathogens on various crops. So, this is a widely used fungicide, that can kill a lot of pathogens and a lot of fungus fungal attack on the crops, how does it work?

It bind to the subunit protein of cytochrome bc 1 complex so, cytochrome bc 1 is a part of the protein and that part that is basically the active site of the protein and that active site binds, the pocket of the active site binds with this product or with this organic molecule and as a result of the electron transport chain located in the inner mitochondrial membrane, thereby inhibiting the fungal respiration.

So once this molecule binds or docs into the protein, then the protein cannot do its function and that protein is associated with the fungal respiration. So, if the protein cannot function,

fungal growth will be stopped, and the fungus should be killed eventually so that is how this molecule works.

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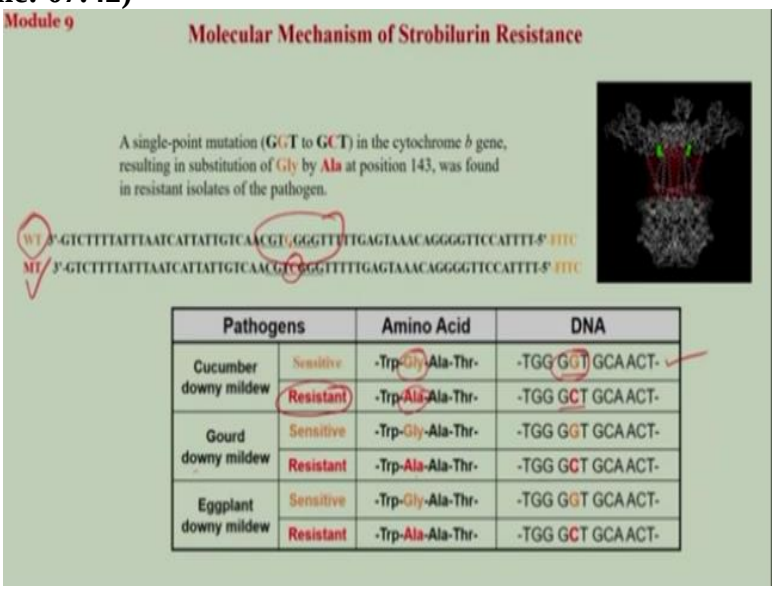

So over the time, the pathogens are clever, and it happens not only for the crops pathogens but also for our different types of antibodies we develop different types of antibiotics we take we consume and the virus or fungus, they actually muted themselves, they become immune to those antibiotics. So, if you remember penicillin was one of the most widely used antibiotic and now, the microorganisms they have developed.

Immune system against the penicillin so we have to use most of the times different kinds of antibiotics apart from penicillin, penicillin group antibiotics, but not exactly penicillin. So, different molecules basically having the same property as penicillin. So, because the microorganisms they can, be immuned or they can develop, they can change their genomic systems very fast once they see that they are under threat.

So, the same thing happens for these kinds of fungus or this type of pathogens also for the crops, these crops, they muted themselves in their genome. So, this is the sequence of the wild type DNA that is present in the pathogen or in the fungus, this is the most important part of it. So G, so this is the parent DNA. So GGT GGT and this actually codes for minoacid glycine so, that is, what is the function of this wild type DNA?

That is part of this code on of the wild type DNA, it codon for the amino acid glycine and because of this so, it is the active site is tryptophan, glycine, alanine, theanine and that has the

structure of the protein that makes constitutes a structure of the protein 3 dimensional structure. It makes the cavity or the active site of the protein where that strawberry molecules comes and docks into it perfectly and that is helped the pathogen gets killed.

Now, because of excessive use of the fungicides or when those organic molecules strobilurin the microorganisms or the fungus have mutated itself how this G they have mutated into C cytosine so, this has now become GCT. Now, what does that do? The single change of this mutation actually changes the amino acid, it now codes for alanine instead of glycine. The moment it becomes alanine, rest are all as it is the active site of the protein gets changed.

So, the organic molecule this which was bound initially, which used to be bound to the active site of the protein can no longer bind and therefore it cannot kill the pathogen or become resistant so that microorganisms become resistant to the fungicide. So this mutation, this single point mutation actually saves the microorganism for from getting killed. So they will persist and they will destroy more crops that is how they have become resistant.

Similarly, downy mildew have the same mutation here same change of amino acid, eggplants downy mildew have the same. Even rice blast has the same, exactly the same mutation and exactly the same change of amino acid and that is how the microorganisms are now resistant. So we have developed actually, so this is a long DNA, I do not 60 more DNA. So question is, how do you find out pathogens whether the pathogens are mutated or not?

This is a single point mutation out of 60 long base DNA base pairs, how to find a presence of a single mutation single change. So similar same exactly same thing happens for the disease when tumour cells or cancer cells is mutated, how would you find out the presence of single point mutation and that we have done it using PNA as a molecular probe.

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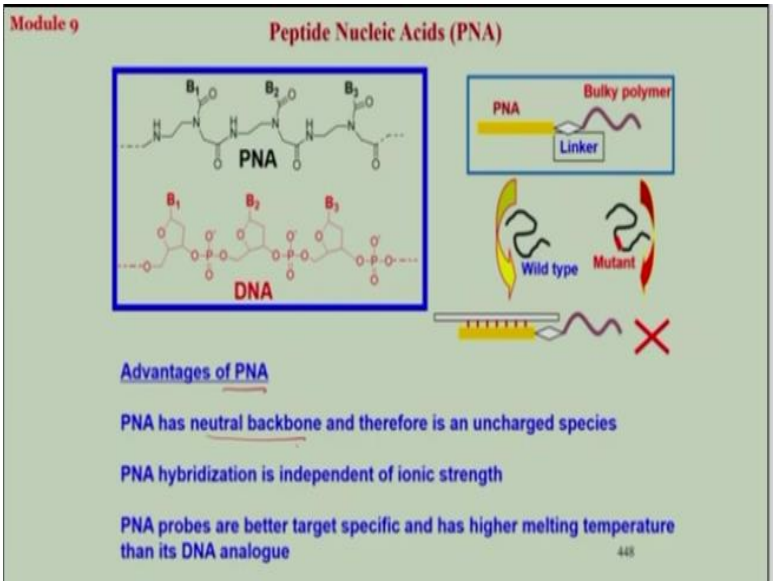

So, this is the structure of PNA, this is the structure of DNA, I have talked about already. Advantages of using the PNA I think I have mentioned already that PNA is neutral backbone, there is no search in the backbone and therefore it is an uncharged species. PNA hybridization is independent of ionic strength, this is very important actually. Since this is neutral no matter how much salt you use or you do not need to use a salt.

When you have PNA PNA hybrid digestion, you do not need to counteries, the charge usually you have to use sodium or magnesium chloride when you have DNA hybridization to neutralize the negative charge on the phosphates that is not required in PNA period probes are better targets specific and has higher melting temperature than its DNA analogues that already have been discussed.

So, now what we have done is also a change or modifications that we have a PNA and that we have attached with a polymer which is a heavier weight. So, it will make the whole thing bulky. Now, if you have the wild type DNA and the single mutated DNA, just we have taken this DNA. This is the wild type with the parent genomic sequence and then the mutated with a single point mutation sequence then what happens the wild type DNA is perfectly complimentary to the PNA that we have used.

So, they will have a full hybridization, proper hybridization and the mutant will have weak hybridization or almost no hybridization here. So, that will make this whole complex bulky and this here, your mutant DNA will move.

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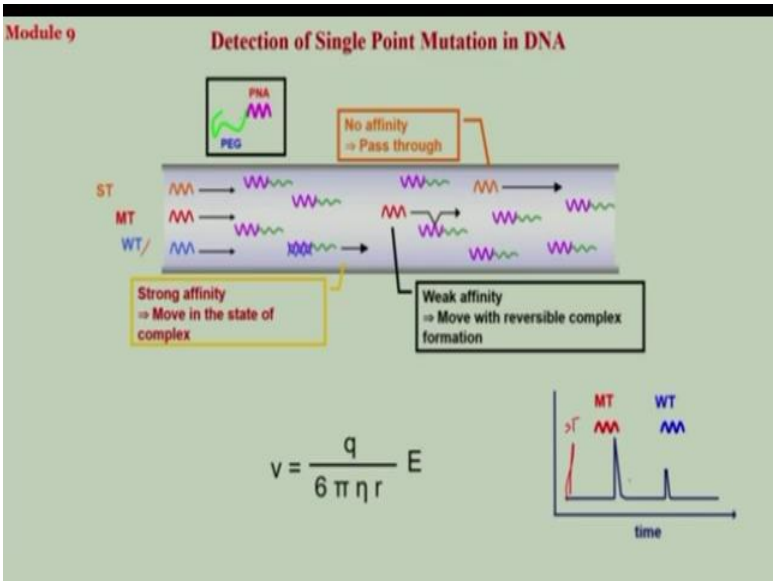

So, this kind of video or this is animation I had shown you before also, we have used this for actually for the PNA and the polygon analogue. We have done capillary electrophoresis, affinity capillary electrophoresis that I had talked about before. This is our PNA and this is the polymer bulky polymer which is polyethylene glycol that was attached to the PNA and this PNA is complimentary to the wild type.

Which means it has one bass mismatch with the mutant and this is a scramble, Scrambled sequence basically that means, that has no similarity that does not form any hybridization with the PNA and this is injected into the capillary and now, this is your sample, we have injected a mixture of the wild type, mutant and a scramble DNA which does not hybridize at all with the PNA. Now, what it does to go so, once these moves through the capillary, the wild type is to hybridize here actually have done.

So, wild type and the PNA they forms a strong complex mutant because of its single point mutation, it changes the melting temperature by around 20, 25 degree. So under the experimental condition, if you do this experiment with a little higher temperature, lets 50 degree then this hybridization would not be very weak they will hybridize and open up hybridize and open up, because it will be in the melting temperature range.

So, therefore, because of this strong hybridization, it will move as a complex and you have bulky polymer, so it will move very slowly. Mutant will have very weak hybridization, so, it will move faster than this and scramble has no hybridization. So, no interaction with PNA it

will move straight and that is what it will look like, scramble will be something here. I will show you the actual data, then will come the mutant then will come the wild type over time.

So, by this method, you can actually separate out the presence of a mutation or whether you have a mutation present in it. Number 1, number 2, how much amount and this quantitative detection of mutations is very, very rare actually, there are a limited number of methods that are available, which can actually detect the number of mutations, the qualitative mutations can be detected quantitatively it is actually hard to do.

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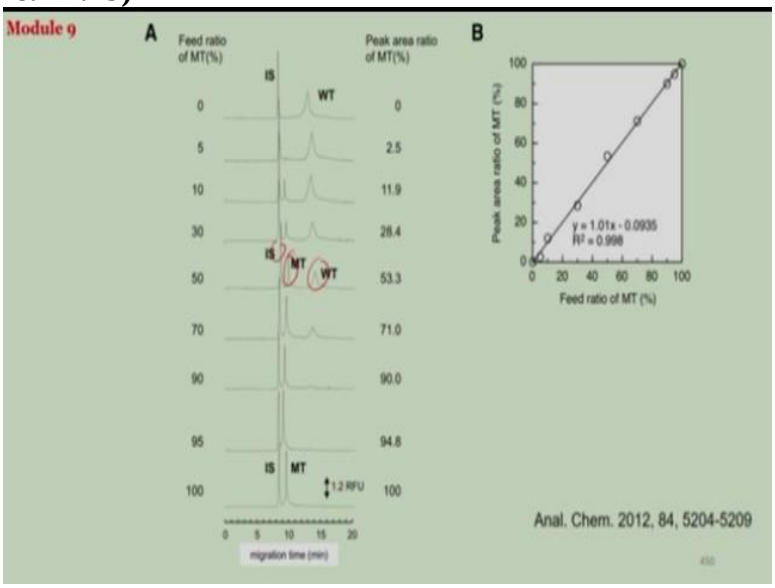

So, this is the actual result, this is the wild type, this is the scrambled sequence as you move on different parameters, this is the wild type this is the mutant, this is the scramble. So, mutant and scramble are appearing very close together wild type is far apart, so well separated here.

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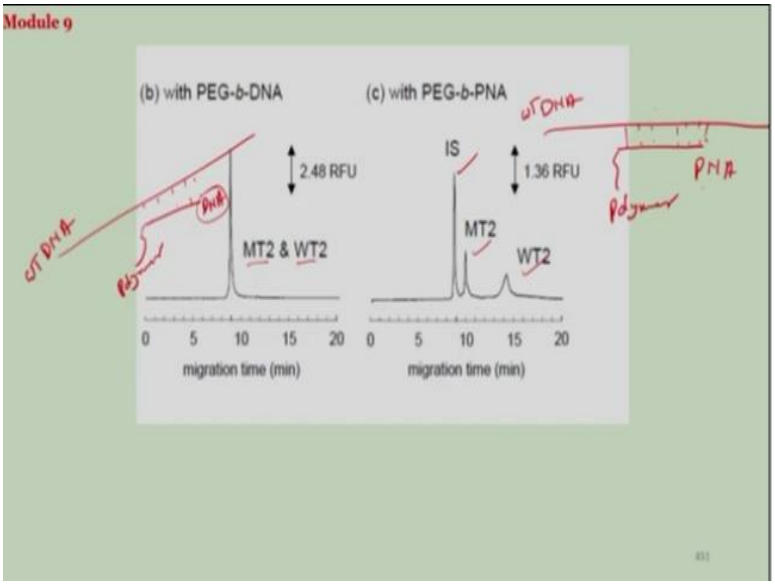

So in this case, what we have done is this was our PNA that had the polymer and this one was that target DNA, wild type DNA and that was forming perfect complimentary here complex formation. Now instead of this PNA polymer if you use the DNA same thing DNA polymerase they exactly same sequence, but in the DNA and if you have the same wild type target DNA this is where I am trying to show you the important in the advantage of DNA.

Then this will have a double helix, PNA DNA double helix DNA DNA double helix. If you use these and do the electrophoresis see mutant and wild type they are not separated, they are merged together in this peak, which means the presence of a single bass mismatch in the target DNA is not been able to be detected by using the DNA as the probe. Because the hybridization between the corporate complimentary and mutated, both are almost similar.

Melting temperature is not very different, so thermodynamic parameters are almost the same? That is why they are more both of them are moving as a complex almost similarly, that is why you are not able to detect them. PNA on the other hand, is able to detect all 3 of them scramble sequence, mutated sequence, wild type sequence very nice well separation is a beautiful separation actually very clean separation that you can see. So because the binding affinity of the PNA with the target DNA is high and with the mutant is very low, so that is the distinction.

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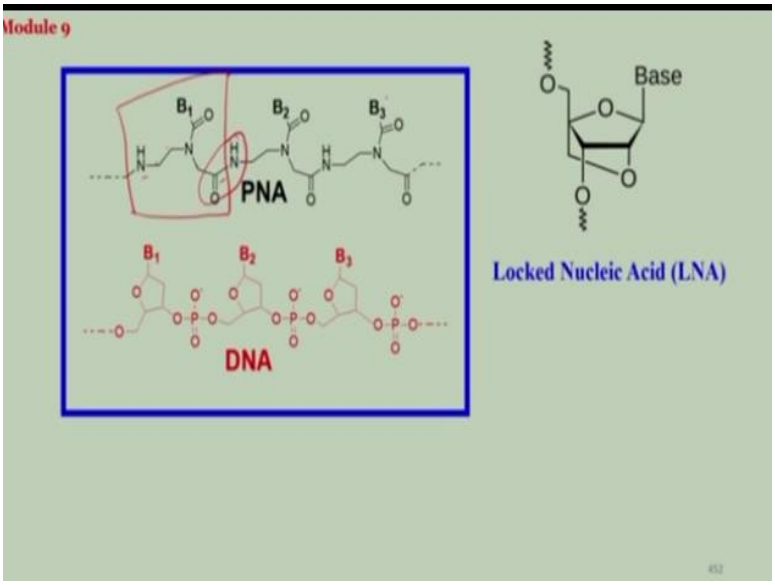

So, one of the example, there are plenty of examples using the PNA and using LNA using the other oligonucleotides to show that they are target affinity are pretty high and pretty selective actually that is what you mostly want pretty selective. So, this is the PNA structure, this is the LNA structures. So, I have so far explained the importance or the advantage, advantages of using the wider oligonucleotides.

Wider modified oligonucleotides are synthesized in the laboratory and a lot of people are doing a lot of research to develop new kinds of structures, which will have importance in detecting chemical processes or biological processes in the living cells and I have talked only 2 of them. So now I will be talking about the synthesis of it, how can you synthesize these kinds of oligonucleotides so I will start first with the PNA.

So, again we have to look at the structure of the PNA this has the backbone, which has ethylenide CH2, CH2, NH2 and N this N is attached with basically amide bond and then you have the nuclear is attached to it covalently then here you have CH2 intervening. So this is basically kind of a glycine. So CH2 and then CO and then that is fused with the first amine bond, first peptide bond is synthesized here and then it is going on like this.

So basically here to here is your monomer this is your monomer and then they are fused together different with the different nucleo bases and you can do the peptide coupling. So, all the peptide couplings are happened so, basically all the monomers are attached together and this peptide you are synthesizing the peptide bond. So, you will be using the same chemistry as peptide coupling that we have done before and that is the difference with the DNA.

So, in DNA, you had a phosphate, now you have a peptide, you had a sugar, now we had this and then later we will see once the PNA is finished, then I will talk about the LNA which has the structure.

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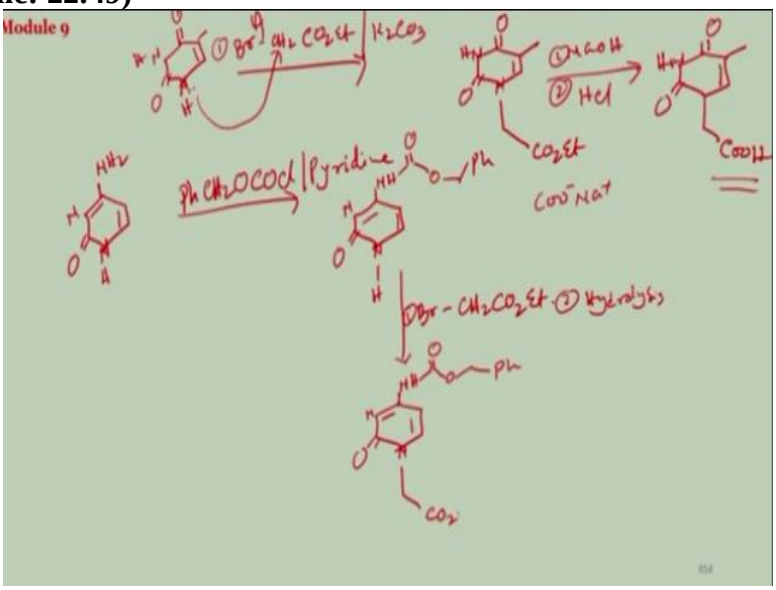

So, if I write the monomer CH2 CH2 N Co2 R basically here you can couple you can attach the nuclear base CH2 CH2 NH BoC, this would be your skeleton backbone skeleton and on top you will attach the nuclear base here and then you have to make this into an acid then you can go ahead with the coupling. So, how do you synthesize this? First is CH2 CH2 N NH2, NH2 we start with this, this molecule is ethylene diamine.

So, what you have to do is you have to make one of these NH2 react with this and the other amine, you have to protect by the BoC. So, first is the production by the BoC, BoC nitride if you use and this is little bit tricky reaction, you have to use one equivalent of it and in a controlled way then one of these amine would be protected by the BoC. So a little bit tricky reactions you have to be very careful.

So that the both are not easily if you use the BoC hydrate or any other electrophile both of these amine will be protected or will react. So you have to be very careful so that you can get only one side protection. Next reaction is this with your to make a CH2 and a start. so this is done CO let us say ET we, we take here CH2 Br. Now it is easy I am not writing the executive, the other agent, I am writing the main component substitution.

They will have NH Co2 ET and you have NH BoC, then you are 1 skeleton is ready. Now you have to attach this with that nuclear base, if you take thiamine this is your thiamine here treat this with Br-CH2 Co2 Et, in presence of mild ways potassium carbonate that is one then what will happen? You will have the same chemistry that we have seen nucleophilic displacement. So, you will form is amine this and now if you hydrolyse with sodium hydroxide.

Sodium hydroxide would hydrolyse this into COO-, COO- sodium salt of the acid of the acid basically and then further you have to neutralize it into an acid. So, you have to use HCl because after hydrolysis what you will have you will have No COO- Na + and that you acidify using the HCl. So, it will be COOH that will give you this, of course, there is still a chance that when this is reacting, reacting this NH can also react that can also happen.

So, under suitable experimental conditions, you have to optimize the reaction condition show that the reaction of the side products is low. So this is done, if you have cytosine NH then you have to if you just treat this with this reagent then nucleophilic reaction will happen here at the same time even a better nucleophilic reaction would happen through the amine this aside residium and that you do not want so, you have to first block this protect this.

PhCH2OCOCl in presence of pyridine you need a Lewis base then what you will have you will have in NH CO O CH2 Ph this is a production of the amine and now you can react this way CH2Co2Et and get your selected H and then this is 1, number 2 is hydrolysis, so this will become your acid you will have this for the cytosine, these are the pyridine in basis.

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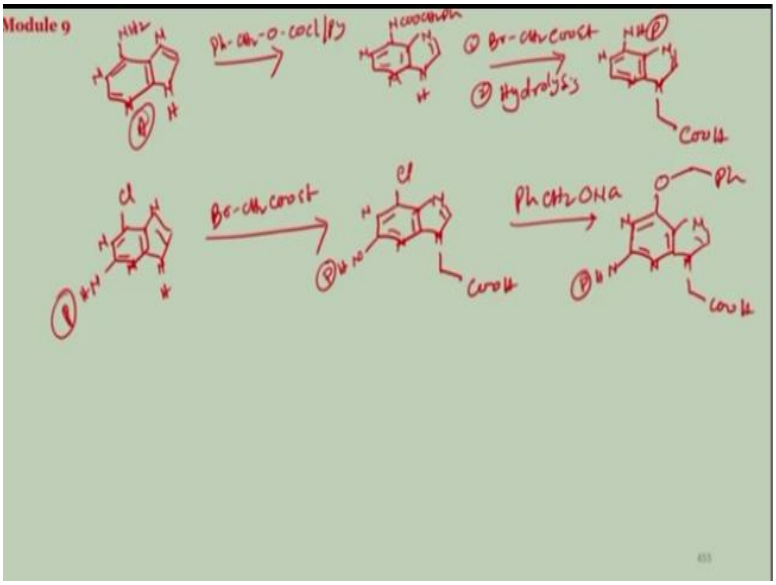

If you go for the purine base this of course, we have to link it at the 9 position here. So, first thing again you can use the Ph CH2 O COCl pyridine to protect thiamine, NCOOCH2Ph. Here is in and then similarly Br CH2COOEt number 1, number 2 hydrolysis, that will give you the monomer for the nuclear base N9 you want, NH I am writing P for the protection, this is for adenine.

Now for guanine it can be a little bit different than a straight forward you can still do it straight forward, straightforward production and then using this step or you can start with a modified one, insist of go and in itself. We can start with that chloric version of it, because it will hydrolyse and produce a double bond O here. For the first step is BrCH2COOEt you can do this protection before also and after also so it is better to do this production before.

NH NH start with this production and then this it will give you CHCOOH followed by hydrolysis have Cl, Nitrogen, NH P production and you have N here and then PhCH2ONA sodium salt of the alcohol. PhCH2OH- basically cyanide and if you do a straightforward hydrolysis, you will have a double bond O here. So this would be protected alcohol even better instead of double bond O, you can have the protections and that will facilitate you are for the reaction steps actually.

So, these are the nuclear waste skeletons that you can synthesize, so I will take one of these examples, for example the thiamine, this is the one you have synthesized.

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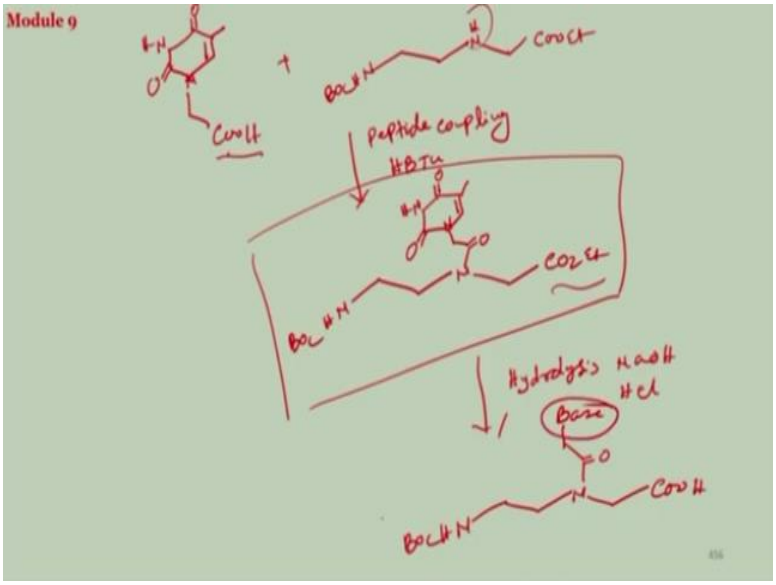

COOH synthesize this and plus you have before synthesised CH2, CH2, this one was NH BoC. So on NH and CH2 COOEt that had synthesized now these 2 can be coupled so, you have an acid here, you have amine here, secondary amine, then if you couple together using the peptide coupling agent same as peptide coupling that you use, you can use HBTu for anything else or any other kinds of peptide coupling agent.

Then you will have you will form a new peptide bond between this acid and this amine. So, this amine Co2Et NHBoC N CO and here you will have the thiamine N NH this so monomer is ready. Only one step is left over here you have to hydrolyse the study into the acid hydrolysis as you have done by sodium hydroxide and then followed by addition of HCl to acidify, so this is your end that will make you here acid this will keep your BoC here.

There is a CH2, I am sorry, there is a CH2 here, and here there is a CH2 so base this should be your monomer. Now take multiple of monomers one by one and go on coupling them according to your sequence and you can you can synthesize your PNA. Likewise, synthesis of PNA is little bit tricky L is not very high and many steps actually takes little bit time and if you want to purchase it, you can, you can also purchase it from some of the pharmaceutical companies, it is very expensive.

So you can try to synthesize it in your own lab, if you want to use them so that is the organic synthesis part of the DNA and now I will show you how you can synthesize the LNA.

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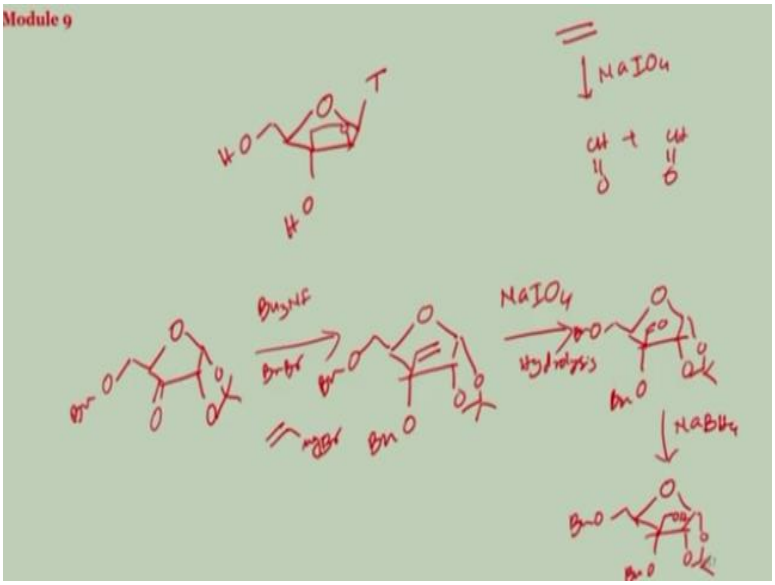

So draw the structure of LNA let us take the example of thiamine so, here there are many varieties. One is of course, the one I had shown you here this starting with the 4 prime end or the one I have here is a little bit different that is this OH and then here is the lock, this is the lock. So this is another variation of the lock to nucleic acids instead of here, it can be here as well of course, the original one was this.

And that was developed by if I can remember correctly just for Vengal is the person who first reported or had shown the importance of the locked nucleic acids. So very quickly I will show you the synthesis of it you can start it from here, this you have done before production of these 2 with acidel group here you start with the ketone and O you can start with the production for example O TMS or TV TV TMS anything, maybe that different one.

Let us start with O DmT so you can synthesize this of course because DmT production can be done. This is simple acetyl production that we have seen in the sugar chemistry when you have done. Here to this O benzyl and then you can have the double bond here. This can be O DmT or here they have done O Bn, O Bn production for both. So this is done by of course it is a nucleophilic substitution with a venile and amine.

So benzyl bromide done, and then aesthetician, I mean so first step is we will see NF floridian agent and then you have BnBr benzyl bromide will make it O Bn and then you can have the venile magnesium bromide ,venile magnesium bromide basically. It is basically a grignard reagent however grignard reagent reacts on a carbonyl carbon, it will have this this is the positive part, this is the negative part.

So it this will go and attack here it will open up the O-, in order to stabilize the O- you can use the Bn Br, so it can take O Bn here that is how it will be forming. Now what do you have to do? If this is your target, you need 1 CH2 here you have 2 CH2, so you need 1CH2 basically here and 1CH2 OH. That is what you need in this position so you have to hydrolyse it into or you have to reduce it into CH2OH what is the way to do it?

If you do the iodine reactions here sodium hydroxide NaIO4 sodium fluoride, NaIO4 treatment will give you followed by hydrolysis of course. If you remember if you have ethylene group CH2 CH2, if you treat it with NaIO4, then what you get you basically get an aldehyde CH O, CH O that is what you will have here. You have O Bn here you can have O Bn and then you will have an aldehyde here and then this aldehyde is been reduced to the alcohol in NaBH4.

So, the borohydride reduction you can do or you can use the other suitable maybe diabol can also be used to reduce one electron reduction, these O Bn in and that will give you OH here O Bn and the last step is basically a coupling here.

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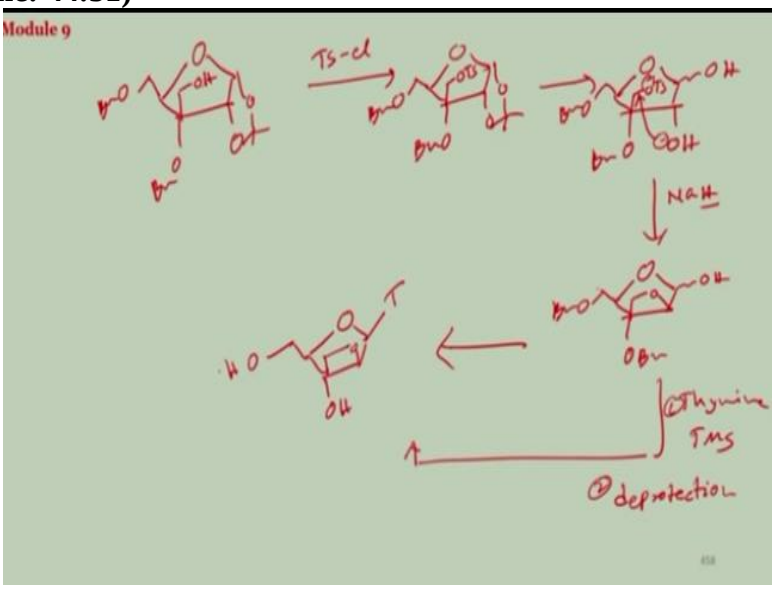

So, we have this OH protected here O Bn protected first I will unlock this before that if I unlock this and there would not be one reaction here, either this has to react here or this OH has to react here and open up the eliminate 1 hydroxyl elimination of 1 hydroxyl is required here and if we want to eliminate that this is a not a good living roof. So toxcile chloride I will make it a good living roof first and then we will eliminate this becomes O toxcilide.

O benlyide and then I will open this off de protection OTS that will give you a OH here it will give you OH there O Bn, O Bn and now if you use sodium hydride is H- basically that will take off this it will make a O- here, this O- will be very reactive, it can do this reaction elimination OH. So, basically now you have O and you have OH bond O Bn, O Bn and then this can be hydrolysed de protected to give you OH.

CH2 OH, here you will have the OH and here you will have OH and you can do the normal way that we have done the TMS the thiamine coupling, you can use thiamine or before the production here you can use the thiamine actually. I will go here straight thiamine T, here you can have the thiamine and then TMS that will make the thiamine active and you can do the coupling here that will give you this followed by the hydrolysis, de production.

Number 2 is the de production of the all the protecting groups. It will roughly give you the monomer and then you can carry on you can make this DMT as usual and this makes phosphor amide bond making it suitable for the DNA coupling. So, this monomer is basically DNA framework, the monomer would be the same as we have done the phosphor every day to chemistry for DNA, so, that you can further do.

So, this is one of the examples of the locked nucleic acids which through which you can restrict the conformational flexibility of the oligonucleotide that you will be synthesized synthesizing would be restricted here and all these have the advantages that this is not a new, not a natural oligonucleotide smaller, more of unnatural oligonucleotide and many times they are stable inside the living cells because the cells are the proteins, enzymes that are there.

Which actually degrades foreign DNA and the virus particular virus DNA molecule, viral DNA and all these. So, those cannot recognize this as a substrate so, this kinds of molecules if you take inside the cells, they will survive longer and therefore, will allow you to do your biological studies so, with this basically I will complete the module.

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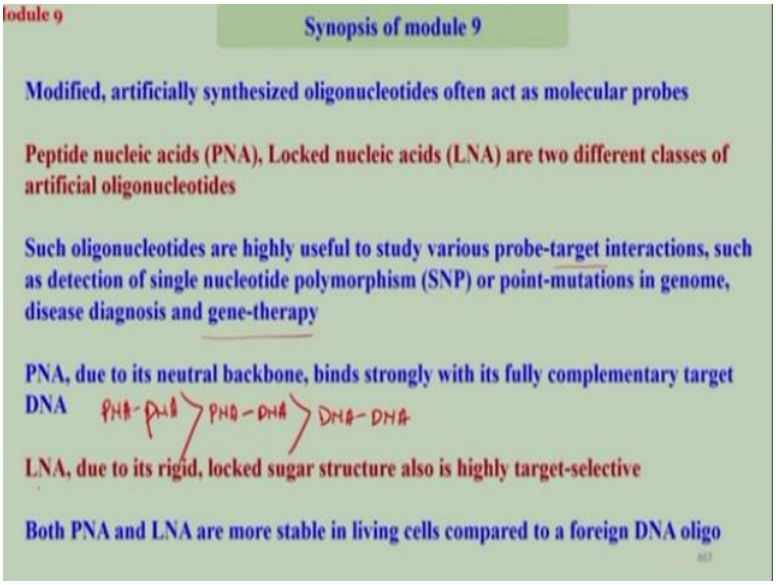

So, in this module what we have talked about is, we have talked about the synthesis of artificial oligonucleotides which we call as molecular probes. So, in general molecular probes, basically probes in terms of molecules, so molecular probes are the molecules or the oligonucleotides or the polymeric materials that are used to detect something in a molecular level and especially to detect something inside biological cells.

The challenges are, if you want to do that, most of the molecules that you inject into the cells would be destroyed immediately. So, therefore, studies that you want to do inside the living cells cannot be done, because all the probe molecules that you are injecting will be immediately destroyed. Cells do not allow most of the foreign particles to enter into the cells, that is where especially PNA, the peptides, nucleic acids and the locked nucleic acids I have talked here.

Because these are the kinds of modifications that can survive in the living cells much longer. So, these are 2 different classes of artificial oligonucleotides that can be synthesized. This oligonucleotides are highly useful to study various probe target interactions, this is the probe target can be anything target can be protein target can be DNA as I had shown that detection of single point mutations in DNA that is one of the aspect protein DNA interactions.

Whether your protein is manipulated or not inside the living cells that can be studied or analysed. So, in general probe target interactions, most of the applications of the PNA and locked nucleic acids you will find that they are to study the RNA, the mRNA, especially the

messenger RNA in the presence of the messenger RNA or where the messenger RNA is moving, leaving detection or time dependent detections of the movement of the messenger.

RNAs in the cells are studied a lot in these kinds of artificial oligonucleotides. So, probe target interactions, such as detection of single nucleotide polymorphism or insort SNP, which basically means the presence of single mutations in the genome. This is diagnosis, many times that diseases which are associated with gene mutations can be studied using this and gene-therapy, that is another very important aspect of it if you want to silence the gene.

If you want to neutralize the function of a gene, then PNA is one of the good molecule to be used. So your gene will have higher binding affinity much higher about binding affinity to each PNA and when the form are stabilized hybridized complex your gene cannot go for further DNA replication, because for DNA replications they have to unwind and once PNA once there same goes for gene silencing RNA also is similar Si RNA we call them.

They actually go and bind very strongly or very formally with your target gene and therefore, the activity of the gene is further lost it cannot undergo cell divisions. So, gene therapy this is called or gene even in the diagnostics, the sequence of a gene can also be detected or also be diagnosed using the molecular probes why PNA I have already shown that PNA duty it is neutral backbone, bind strongly with this fully complimentary target DNA.

So in the in here, one second I will mention the PNA PNA duplex double strand has much higher melting temperature or much higher binding energy compared to PNA-DNA, which is of course, much higher compared to the natural DNA DNA double helix. So, LNA due to its rigid locked sugar structure also is highly target selective. So, whenever you are using a probe to detect some your target, so of course, the probe has to be highly selective towards the target only it should not bind to all molecules.

It should only bind to the molecule of your interest so, selectivity is very important and that is where these 2 small liquids are highly advantages of locked nucleic acid also, it is very target selective. Both PNA and LNA are more stable, as I was saying are more stable in living cells compared to our foreign DNA oligo. So if you send our DNA, foreign DNA synthesized DNA into the cell it will immediately be cleaved by different enzymes present in the cell.

They are called the nucleus, nucleus are the enzymes that destroys DNAs or even RNAs. The reason is being virus and bacteria they will enter into the cells. So nucleus if they can figure out that those species have DNA they can immediately destroy. That is how that is what our immune system is also, it is a part of our immune system. So if you use DNA oligonucleotide synthesized as to probe they are not very stable into the cell.

They are not going to persist in the cells would be chopped off. So, you cannot do your studies using these oligo DNA oligo. PNA and LNA, PNA is highly nucleus stable, it is not chopped off by the nucleus because PNA has the peptide backbone and nucleus cleaves only the phosphodiester bonds. So, PNA is very stable LNA because of its locked structures and much unknown structures, it is also quite stable by the nucleus.

So, they will persist longer in the living cells so, to allow you to do your studies. So, these are some of the advantages associated with synthetic molecular probes and I have shown you they are experimental utilities also. There are many more things that are there in the literature in the text book also that you can go through. So with this, I will conclude this module. Thank you.