Essential of Biomolecules: Nucleic Acid, Peptides and Carbohydrates

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Lecture -31
Molecular Probes: PNA and LNA

Hello, everybody and welcome back to the lectures. So, today we will start a new module which is module 9 that we termed as molecular probes. And specifically, I will be talking about 2 of them in detail that is PNA which is known as peptide nucleic acids and then LNA locked nucleic acid. So, we have in the last previous lecture, we have completed module 8, and where we have talked about some of the techniques that we use in modern chemical biology or in organic chemistry.

We use organic chemistry to understand or to study biomolecules or to study biological processes. So, this is the new module, and we are moving from the modern techniques to a little bit in the molecular level. So, those were the instrumentations. Now will be talking about the molecules which are used to study the details of the biomolecules. So, probes I have talked about already, I think, time to time, so, probes are basically.

When you talk about the probe, probe is something that you use to track your target. For example, I have given this analogy before also, like, if you are and if the GPS in your mobile is on, then you can be tracked. You can know where you are your own location, as well as the other people if they want, they can also know where is your location or in other words, they can spot you where you are. So the GPS in this case is a probe.

So tracker is something that will tell your exact location or your exact stand. So when you do titration, for example, in the school days, we used to titrations where we use indicator and that indicator is usually the colorless or it changes color from one color to the other. So basically that will give you. So, if it is colorless and then you reach neutralization point and then it changes it gives you a distinct color it appears in a distinct color such as pink and all these things.

There are many different types of indicators that we have used. So, by seeing the color, you can know that you have reached your neutralization point. So, that is your indicator. So, the change of color or the appearance of a new color tells you that something is happening that is a probe. So, it is a kind of a reporting, the molecules are reporting to you that I have reached the point where you want. So, that is termed as probe which you can use to track something.

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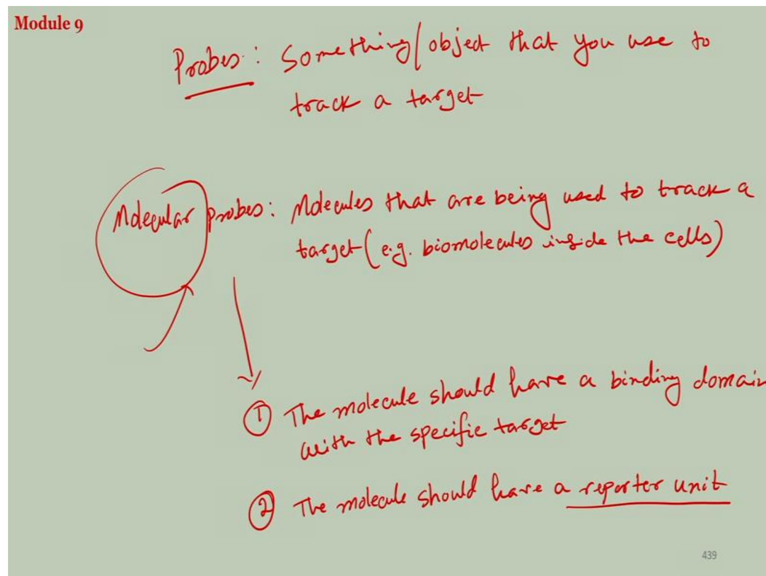

Probe is something or an object that you use to track our target when we talk about molecular probes or molecules that are used to track a target. So certain molecules that you can use that are being used to track our target. So, in this case, target means all biomolecules or inside the cells example, bio molecules inside the cells. So if you want to study something inside the cell. You need a molecule. You need to send a molecule that will go into the cell that will be bound to the target that you want to study and then report you back.

It should give you a signal also that I have reached the target and this is what is happening inside. So, those are called the molecular probes. So, it has usually 2 parts. One is of course, you need a molecule that can bind to a target inside the cell, may be outside the cell also in vitro in we the both. So, you need a certain molecule that will go and bind be bound to that target that is 1 part, 2nd part is there has to be a tracker here.

So, it should contain 2 components. One is the binding domain the molecules should have a binding domain with the target should have a binding domain with the specific target number 1, number 2. The molecule should have a reporter unit. Reporter unit means something that will tell you that I have reached there. And this is what is happening, whatever is happening, you should be able to see it so that is called the reporter unit.

For example, we use fluorescence molecule and other things. Radioactive leveling that you use in organic chemistry that is also a reporter unit, because you can track it, you can see where it is going. So, these are the 2 basic components that are essential for a molecule to act as a good molecular probe. It should have a binding domain, something that will specifically bind to your target and it should have a reporter unit. Well, so, now, what kind of molecules that can be used as molecular probe.

Obviously, you can think of many different ways, such as if you want to target a specific protein. Sometimes if you know something about the protein if you know about the active side of the protein and if you know the substrate that the protein binds to then you can use the substrate. You can synthesize the substrate in your laboratory, attach it with the fluorophore and then send it to the cell. And you can expect that since protein substrate binding is highly specific, you can highly expect that your substrate will go and will only bind to the target protein.

And of course, the substrate has fluorescence. So, you can see where it is if you do a fluorescence imaging, fluorescence imaging if you study then you can see where inside the cell your molecule is. And you can pretty much say that since my molecule is there, the protein must be there also.

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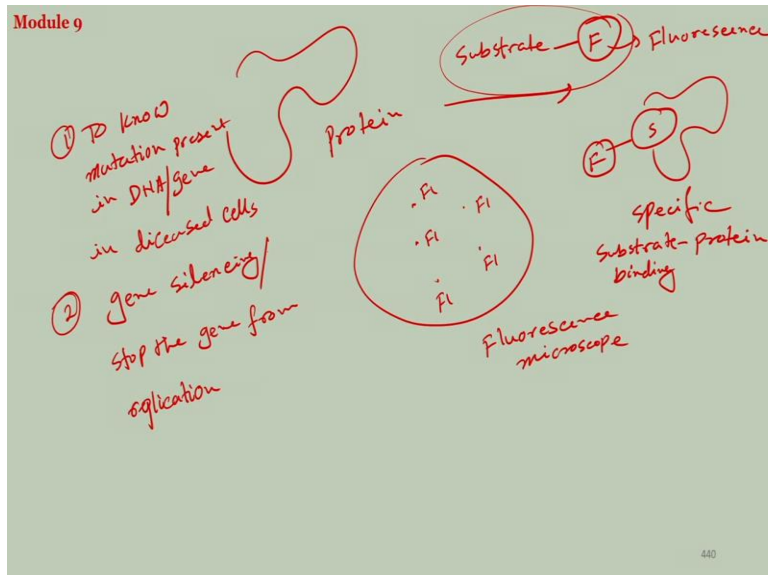

So, if you have a protein active site if you use a substrate usually an organic molecule and then you attach it with a fluorescence molecule, this is a fluorescent molecule. Fluorescence attachment is there. Then what you can expect is that your substrate would be here is for substrate attached with fluorescence and these substrates should be specific substrate protein binding.

And you should see the fluorescence, by if you do the image, the image will tell you where if you if this is your cell, you can see the dots, fluorescence dots basically, these are all fluorescence FL I am writing FL, FL, FL, FL, if you see fluorescence marker inside the cell under a fluorescent microscope. So, this is your fluorescence microscope. Then you can see, and then you can actually tell that my protein is more likely to be in these positions.

Obviously, you have to do the reference study also, this can only be your substrate fluorescence as well, but there are ways to eliminate them. With a reference you can do a base line corrections and if you have without the protein and all these things, then you can make a matching that will tell you if your molecule only is giving you with the fluorescence, so that can be eliminated. So, ideally if you see the tracks, if you see the sports then you can say that your proteins are also present in these positions.

So, that is how you can track the activity of our protein and this can be called a molecular probe. Now, similarly, if you want to track a DNA, if you want to track a gene, especially the diseased genes, something is mutated, for example, you are expecting a mutation that has happened in the genome. And in the diseased cells, you want to know whether the mutations are present there or even where that gene is present what the gene is functioning.

That is one to know mutations present in DNA or gene. In normal cells or diseased cells, that is one. So, you basically want to know whether your gene is perfect or not. Second is also very important. So, if there is a mutation, or if your gene is not functioning as you want, for example. What happens in tumor cells or cancer cells is the cells undergo rapid cell divisions and rapid cell division means your DNA is also getting replicated.

So more number of DNA is getting replicated that is why you are getting more cell divisions. Now, if you want one way to stop this, one way to cure tumor or cancer is to kill these cells. So, indirectly if you can stop the DNA replication, then the cells will automatically be cleaved because if you can stop the replication of the DNA, then there will not be any cell divisions possible.

And then the growth of the tumor cells will be stopped eventually the cells will die because there will be no DNA inside it. So they cannot function. So, which we call it so, if you stop DNA replication means silencing the gene. If you can stop the activity of the gene, or silence the gene this is known as gene silencing or stop the gene from replication. So, if you can use something, a molecular probe, a certain molecule that will go and that will stop the replication of the DNA, then your job is done.

So, we can use molecular probes to do these 2 things; one is of course to see if there is any mutation in the gene, if there is any mismatch, number 1, because that is very, very essential to understand the early growth of tumor cells or early presence of tumor cells. Second is if there is a problem in the gene, whether we can silence the gene. And these are 2 things which can be done using artificial nucleic acids which we call the molecular probes.

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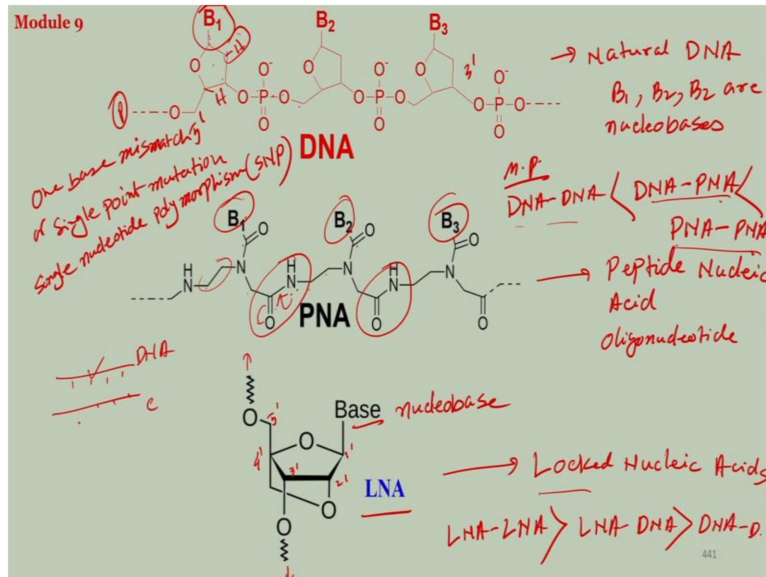

So, 2 of these have become very popular and widely used in research. To some extent even they are in clinical trial 1 or 2 of them. But mostly in vitro we use them a lot in order to know if there is a mutation that is present in your target gene. And if you want to even stop the replication of the genes, one is known as PNA. This is peptide nucleic acids other is LNA locked nucleic acid or acids.

Both of these are artificially synthesized all uniquely tides. They are not natural. They are not obtained in our body. None of them we have synthesized them and we use them as molecular probes. The reason is they show PNA both PNA and LNA show activities that is sometimes better than our DNA. So first let go to the structural features. And then also, we will discuss about that importance what they do, actually.

This is a skeleton of the natural DNA. This is a naturally occurring DNA. I have done it other way around actually, this is 5 prime, it should have been the phosphate here. So it starts with the phosphate here. This is the 5 prime end, this is 3 prime end, it will move on. So 5 prime O, here you have the nucleo base. So B1, B2, B3 are nucleo bases they can vary ATGC. So, this is the deoxyribose sugar with the hydrogen here.

And this is the ways, it will move on at the 3 prime direction. And at 1 prime sugar you have the nucleo base, nucleo base 3 prime sugar 3 prime sugar. That is how it is going on. Now, you can

synthesize DNA oligonucleotides. Now that we have learned how to synthesize the DNA oligonucleotides you can even purchase them nowadays, there are a lot of companies that sell oligonucleotides. So it is basically the synthesized once.

When the question is why you cannot use DNA itself synthesized DNA oligonucleotides itself as molecular probes. So you have a gene inside the cell that you want to, for example, that you want to see whether there is a mutation or not, what is the way to know to understand a mutation? The simple way is if you can calculate the melting temperature. So, your gene has a melting temperature because that is perfectly complimentary.

If there is one base mismatch, the melting temperature will be lesser. In other words, the binding if you have a DNA this is the complimentary then there will be strong binding, if there is a mutation, the binding would be weaker. So, this weaker binding factor you have to consider that is your point of target basically, if you can use something that will nullify or that will make use of that weak binding part.

Now, one way is that you inject or you incubate your gene where there is a mutation, which means a mismatch with a foreign DNA that you have synthesized, and this is perfectly complimentary to this, then it should go and bind stronger. The problem is DNA - DNA binding, you already have a double stranded DNA. If you use another DNA from outside, then what does this DNA has to do?

It has to first unwind this double helix and then make a new double strand with your probe. That is little bit hard to do because both our DNA - DNA double helix. So, thermodynamically the process is not that much physical it is not perfect. The difference that you will see in your experimental data will not give you 100% certainty. That my DNA has gone there and have been bound to there because the difference of the melting temperature would not be match.

There is always the chance of experimental error also. So, I have talked about before, I think that if you have a single bass mismatch in your DNA, it changes the melting temperature by roughly around 5 to 10 degrees Celsius. So, one base mismatch, or single point mutation. Single point

mutation also is known as single nucleotide this is very important to remember because this is the term you will come across in many different places if you are studying Chemical Biology.

Single nucleotide polymorphism. in short, this is called as SNP single nucleotide polymorphism that basically means a single base mismatch that is present in the DNA. And many different types of cancers are actually because of the presence of a point mutation, single point mutations. Even some certain kinds of diabetes are responsible for single point mutations, epileptic behaviors, epilepsy because of the some of the other single point mutations or single nucleotide polymorphisms in the gene.

So, it is very important to study them or it is very important to find out what kind of mutations are present there and where they are present in the gene. So, molecular probes is one of the way which will determine in the laboratory or even in the cells, what where is the mutation that is happening or what kind of mutation they are is or how much is the mutation amount, all of them. So, using our DNA as the molecular probe is not the best idea.

Because the change or the difference that you will get is, is not that much high or not very distinctive. So, you will need to develop something that will have a stronger interaction with your target if the interaction is stronger than a mismatch will be equally weaker. So the difference that you get between a strong binding and a mismatched binding would be large enough to experimentally judge that will see in the subsequent slides.

So this is the natural DNA peptide nucleic acid is a synthesized artificial oligonucleotide. And as the name suggests, it is a combination of peptide and nucleic acids. So, if you see this is how the structure of a PNA looks like. In DNA you have started with the phosphate and the 5 prime end from the sugar. In peptide nucleic acid, there is no sugar. The backbone is a peptide backbone. In DNA your backbone is for sugar and phosphate PNA, there is no sugar there is no phosphate, there is peptide as the backbone.

So it starts with here CH2 CH2 little bit flexible in CH2 and here this is your peptide bond. So, it is not even a real amino acid. There is no real amino acid present here because this is a CH2 CH2

that is not a part of N amino acid. This part is to some extent is present you can said glycine but it is not really a glycine. There is end in between here. So this is artificial peptide. Backbone has peptide bonds. It has peptide bonds but they are not part of any amino acids. And here, what is the nucleo base nucleo base is present here with it again.

This is an amide bond or a peptide bond you can say this amide bond and then our nucleic base is attached here. If you go by the structural similarities between DNA and PNA, there is 2 things that is common. One is of course; all of them have nucleo bases. Here also you have the nucleo bases. Other thing is, if you go by the carbon chain, or if you go by the atomic chain length, then the distance between B 1 and B 2 is almost the same in PNA, as was in DNA. Let us see in DNA how many atoms are present? How many intervening atoms are present?

So, 1234567891010 atoms I think are present between B1 and B2, here 1234567899 so very close to this. So, the intervene, so the distance if you go by the chain between B1 and B2 are almost the same as in DNA. But of course, the flexibility is very different. Here, this is DNA confirmation is locked. Here also you have as we have talked about, you have the resonating structures, you have a double bond character here, but the other parts are pretty much flexible. So, this is the only similarity between DNA and PNA, apart from that there is no other similarity.

And the PNA because the nucleo bases are present, it can hybridize, the nucleo bases can form hydrogen bonding as well as the 5 stacking interactions that were present in the original DNA also. So, that is about peptide nucleic acids. And then comes other one that is called the locked nucleic acid or LNA locked nucleic acid. This is same as DNA. You also use a sugar here but it is not a deoxyribose sugar. You see, this is a nucleo base.

Base is attached at the C1 prime position which is here this is your 5 prime end, this is your 3 prime. So 1 prime, 2 prime, 3 prime, 4 prime and 5 prime. As usual 5 prime is attached to the phosphate and you can move on 4 prime, 3 prime has OH which you can move on. So in this case, the DNA is proceeding oligonucleotidel aligal not the DNA. So you can make the oligomers in this direction and in this direction this is 2 prime, this 1 prime.

So usually, in DNA what you see 2 prime has a free hydrogen here, then there is no hydroxyl group. Here you see, there is basically arrival not a deoxyribose. But ribose pattern here with the oxygen there, but this is not a free hydroxyl. There is one more here there was one more CH2 at the 4 prime position which usually is free here there is nothing at 4 prime is just H in this case 4 prime also has CH 2 OH which that has been fused with the 2 prime hydroxyl group.

And you have made a bridge sequence and that is where the differences. So, the confirmation of this sugar is usually locked you cannot rotate it because that is what you have locked it here you cannot move then the bonds will be broken to allowed and these are pretty strong bonds. So, your rotation around the flexibility in the sugar is totally gone. Here you had some flexibility here that flexibility is fully gone. That is why this is known as locked nucleic acids. So let us start with the PNA first.

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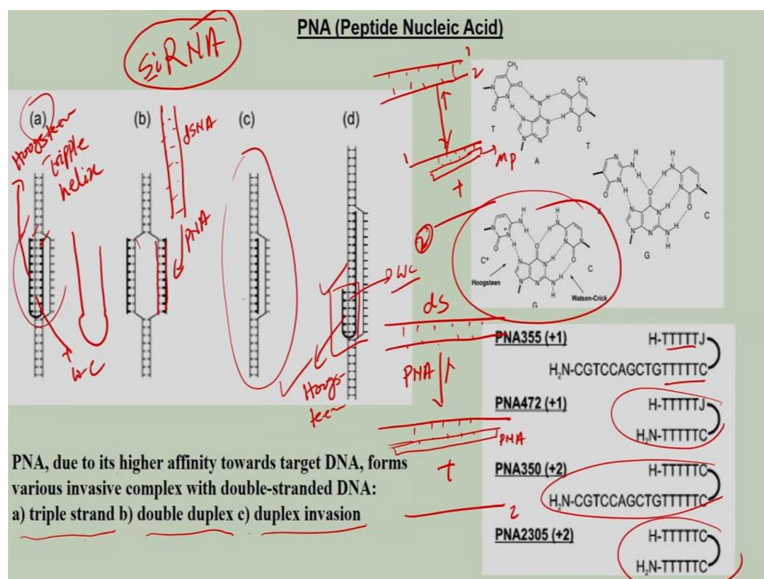

Peptide nucleic acid. You can see in the literature, I have not written the details of it around 1995 or 96. Not very long ago. The PNA was first developed by Nielsen, Peter Nielsen. So he first developed the peptide nucleic acids as artificial base pairs. There are many, many different kinds of artificial base pairs that are available in the literature. So PNA and LNA and there is one more that I should talk about is the gene silence are SiRNA.

I am mentioning it otherwise I will forget. At least you can you should know the name SiRNA is basically an RNA sequence which is used for gene silencing. So Si mean silencer, SiRNA is RNA that is used for silencing a gene and that is given clinically used to treat certain tumor cells. Okay, so peptide nucleic acids were used to study its binding or its hybridization with the target DNA.

And these are the patterns or these are the ways that a peptide nucleic acid can act. So, if you have there are many different types, let us start with this is the easiest thing. So, as I was saying the property or the characteristics of molecular probes should be if you have a target DNA. This is a double stranded DNA if you want to study the property, or if you want to study if there is a mutation.

What you need to do? is your molecule or probe which you will be using from the external shows should get into this is your molecular probe MP I am writing should be able to get inside the double helix disrupt the double helix and make a new one plus the complimentary. So, this is DNA 1, this is DNA 2, 1 it can bind 2 can be made free because this binding is stronger compared to this.

So, the equilibrium this way, equilibrium will be mostly towards this because this will be energetically favorable process PNA is one such thing PNA has a stronger binding affinity with the target DNA. It is a double stranded DNA, ds DNA. Now, you treat these with a PNA then what will happen PNA, DNA binding is very strong and this will force. So, this is PNA, this is single stranded DNA plus this will force.

The double helix to unwind this is DNA 2 will present as it is and then PNA 1 DNA. If this is your complimentary sequence, then they will be binding strongly and these processes highly favorable thermodynamically. So that is one mode of binding. So that is what it is happening here. And in this case, PNA does not have to be very long; it does not have to be as long as your target DNA, the short sequence of PNA is, is strong enough to make a displacement of the double helix.

So here in this region, your double helix gets displaced it is unhybridized. Now, with the complementary strand coming out of the helix, and the PNA is getting in, there is a new double helix that has been formed because it is thermodynamically favorable. So this is one way of binding. Second is this, this is called double displacement. Originally, you had this is the double stranded DNA ds DNA which is the original sequence.

Now, we have treated this with the PNA as a sequence of the PNA is such that it is complimentary to part of your target DNA. So, your target DNA has been unhybridized in this part separated out one of this target DNA is hybridized to the PNA, the sequence of the PNA such that there is the other one is also complimentary to another PNA, it can be the another PNA or it can be the even the same PNA if the sequence matches.

So, it also forms 2 PNA forms 2 double strands there and that is a huge binding factor or huge advantage thermodynamically highly favorable process if you have 2 different PNA both are complimentary to the target, then it will fully open the target DNA. So, that is a second kind of binding property or binding pattern I would say third is this, this is called triple helix. In this case your PNA has gone invaded, it is called the innovation.

This is called the innovation into the double helix. So, your PNA, because this is complimentary to the target DNA, it has invaded into the double helix and displaced it. Now, in this case, you have made a PNA which has a U shape this and then there is something flexible hairpin kind of this is complementary to this. This has one sequence, this has other sequence, and both are complimentary to the single strand.

It is the same sequence basically, this and this have the same sequence. So, this we will have a double helix hybridization. And then one other hand, you will have a non Watson Crick Hoogsteen base pairing also here that forms a DNA triple helix here Watson Crick and Hoogsteen both can be possible that is called the triple helix, triple helix are not usual for DNA. Nowadays we use the triple helix as a method to unwind target DNA.

Because the energetically this is so much favorable that you get fully denatured target DNA. So that is called the triple helix formations; one helix here, one helix here 2 double helix. 3 components are involved 1, 2, 3 components are involved. That is why it is called the triple helix. Same thing you can see here is also triple helix, this is a longer part, this is a shorter part, but in this region, basically it is forming a triple helix, this is the Watson Crick.

The other one is Hoogsteen, this is Watson Crick base pairing that part is the Hoogsteen for in the triple helix, this is called 2 double helix diy double helix or something and this is the single invasive. So, these are the hybridizations that you can see PNA due to it is higher affinity towards target DNA forms various innovative complexes with double stranded DNA what is called the triple strand that is this, this is called the double duplex.

You have one duplex here; you have a second duplex here. So this is called a double duplex. And C is the duplex invasion. So it is a invasion into the single duplex and this is occurs, that triple stand again. So, these are the ways that a PNA can be bound, these are the sequences you can see this, this has the same sequence. So it will be hybridized here it will be hybridized there, this is the longer DNA. So the other part this is also the bent, this is also the bent all of them are bent structures. So they are forming in the triple helix basically.

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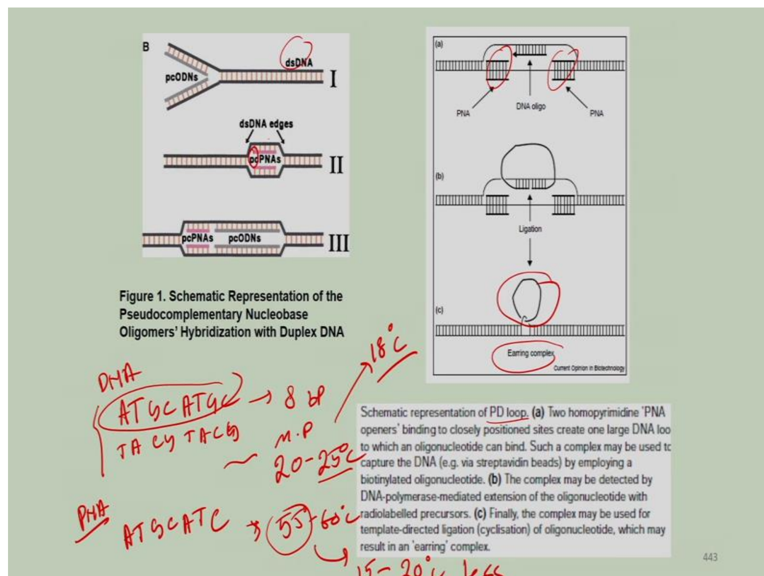

Here also you can see schematic representations. This is even more variation or called the Pseudocomplimentary Nucleobase. So you can forget that pc term you can forget just consider the PNA If you have a DNA, is a double stranded DNA here, you add the PNA into it, you can see there is 2 double helix that can be formed. That is how you can make a lot of designs to prevent the target DNA from the dehybridizations here.

This is the PNA bound here, bound here. This is the DNA oligon. And this is for the ligation. You are forming a different kinds of complex here to study different things. Many variations that I just I want to show you 2 homopyrimidine PNA openers, you can go through it. So this is a little bit away from the main design a little bit more modification from the main design. So the question is what makes PNA to have so much strong binding with the target DNA?

So in other words, I should mention here very important, as I said that is binding is strong means higher melting temperature. So, basically if you calculate the melting temperature or melting point, if you have a DNA - DNA means DNA double helix and if you have DNA - PNA double helix, DNA - PNA double helix has much higher melting temperature compared to DNA - DNA. Similarly, this again has much higher melting temperature compared to our PNA - PNA double helix, it both are PNA strands, very strong interactions, very strong hybridization.

So, melting point of a PNA - DNA is higher than our DNA - PNA, then the DNA – DNA. The question is why is that? Both cases you have nucleobases, normal nucleobases ATGC. The difference is in the backbone. So what makes the backbone? So much advantage compared to a DNA - DNA double helix. First thing that you can notice here is in DNA, if you have phosphate groups, negative charged.

So when you want to make hybridization, DNA - DNA hybridization, there is always the repulsion, repulsive force between the phosphate groups. That is a good repulsive force. Actually, it destabilizes the double helix. And that is why I have mentioned when you want to get DNA hybridization; you always need to use a salt to overcome the repulsive repulsion between the phosphates.

So never the less 2 DNA, if you bring closer together, even if they are complimentary there will be a repulsion between the phosphate groups that is a destabilizing factor for the DNA double helix formation. PNA on the other hand, if you look at the backbone is neutral does not have a charge. Therefore, if you make a hybridization of DNA with a PNA, then this repulsion will not be there.

So that destabilation factor in DNA - DNA is not present in DNA - PNA or in PNA - PNA. That is why is one of the reason why the melting temperature is higher. Or in other words, the hybridization is stronger. Second is the flexibility. The confirmation if you see of the PNA and in the DNA, PNA has more favorable confirmation. It allows better hybridization, better stalking between the nucleobases.

So, that is another advantage or that is another stabilizing factor for PNA - PNA double helix or DNA - PNA double helix. So, these 2 are the prime factors that are responsible for better binding property of the PNA, though why you would use it as a molecular probe. Another thing that is of high concern, when we want to use something to move into the cell to inject into the cell is that the cells as I have said repeatedly.

The cells are very highly protective usually; they do not allow foreign bodies to enter the cells. So, if you use DNA as a probe if you want to inject synthesize DNA oligonucleotide into the cell that most of the times the cell will not allow the DNA to enter because DNA is highly dangerous virus, bacteria those contents DNA. And so, the cells know that DNA are highly notorious and they will have a lot of impact on its own gene.

So, they do not usually allow the DNA to get into it. And our cells have enzymes called the nucleus is your heard the name nucleuses before, nucleuses is the enzyme that will cleave the DNA into pieces. Even DNA and RNA both would be cleaved by the nucleuses. So the moment you will use an artificial DNA. The moment it gets into the cell, it will be broken into pieces and you cannot do any you cannot study anything with that.

PNA one the other hand because it has a more peptide backbone, cell walls are friendly to peptides. Though they allow the PNA to enter into the cell and it does not have phosphate bonds. So, it is not a substrate for the nucleus. Nucleus cannot break PNA, because nucleus usually breaks the phosphate and the sugar bonds. So, nucleus cannot break PNA, nucleus cannot destroy PNA. And since it has a more like a peptide backbone cells sometimes allow the molecules to get into it.

So, it is a better molecular proof in terms of when you want to do the living cell studies. And of course, it has the advantage that it will have a stronger binding more or less is the same for locked nucleic acid. This is too some extent stable with nucleuses because of these structural variations new places do not take this as substrate most of the times it is too some extent stable with nucleuses and of course, it has better binding property.

So, also LNA when it comes to LNA - LNA double helix has higher melting temperature compared to LNA - DNA and compared to DNA - DNA. So, this is melting point. I will give you one example. If you have let us say ATGCATGC 12345678. This has the 8 base pair sequence. DNA TACGCTACG is the double helix. So if this is a DNA usually this will have a melting point which is close to maybe 20, 25 degrees Celsius. 20 to 25 degrees at most close room temperature.

Now same, I am just writing this if you have a PNA let us say this is your DNA, this is a PNA either of them ATGCATGC, if this is PNA strand, that will have melting point close to 55 degree to 60 degree Celsius. So much difference this is close to let us say 55 degree Celsius for the PNA- DNA sequence, PNA - DNA sequence will have a 55 degree Celsius melting temperature. And the DNA - DNA sequence having the same sequence is only 25 degrees Celsius.

So the binding strength is that high for PNA. And now, if you talk about a mismatch, if there is a single base mismatch somewhere, it will reduce the melting temperature by around 7 degrees. So, in this case, if it is 25 - 7, so, around 18 degree Celsius, if there is a mismatch, if there is a

mismatch in PNA I have seen, I have also worked to some extent with the PNA, that it changes the melting temperature by at least 15 to 20 degree.

So, here it was 5 to 7 degree change. In this case, it would be around 15 to 20 degree less, if you have a single base mismatch there. So, if the difference is high, then you can see if you by simply studying the melting point, you can see that there is some mismatch over here, because this cannot be an experimental error 15 to 20 degrees Celsius cannot be an experimental error, 5 degree can be here in the there instrument problem, instrumental error, experimental error is so much close to the normal one. This is a huge difference.

So, you can pretty well understand and study or find out even what kind of mutations are present when you use the PNA as a probe. Here I will give you an example a real example that we had done.

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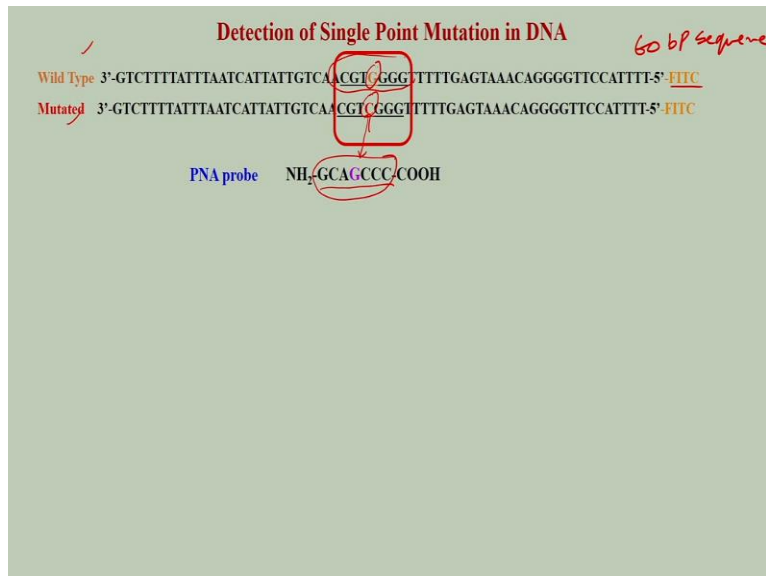

So, this is a DNA wild type means the original DNA, 60 molar long DNA this has a 60 base pairs sequence. He has a fluorescence level that you can forget. And this is a mutated DNA where there is only a single mutation single change, the original one was G and the mutated one was C same 16 base pairs sequence. This is actually a sequence that is present in rice blast pathogen that destroys crops, especially their eyes.

This is called the sub mildew and that pathogen has this DNA sequence here. The problem is when you use pesticide to kill those pathogens; eventually those pathogens have mutated themselves so that the pesticide does not work on them. This is the known mutation that the pathogens have done within themselves. So that they will survive, even if you use the pesticide they will survive.

Because the pesticide does not bind with this sequence, when there is C it does put bind, so you cannot kill that. So, this is the part, small sequence which is changed into this, of course, which is important where the pesticide used to bind only this much sequence this much is the important part of it. And this is the important part where you have this mutation. So, we wanted to find out how much mutations is present or if in general.

This is a 60 molar long sequence with only a single change in the genome. If you are able to find that out. It is actually hard to find that out. So, we have seen that if you use a DNA sequence instead of PNA. And you hybridize both wild type and the mutated they almost give you the same results, because it is too long. And finding out about the small difference here is in the DNA was too hard. DNA probe does not tell you the difference between the wild type and the mutations.

Now, if we have used a PNA probe with this sequence, G means it will hybridize with this with the mutated version, then it shows a huge difference. Now, if you hybridize this separately with wild type and mutation, then you can see the high change of melting temperature. So that when through that you can understand that this is your mutation, this is your not mutated or wild type, only a very short sequence 1234567 nucleobase sequence, very short sequence.

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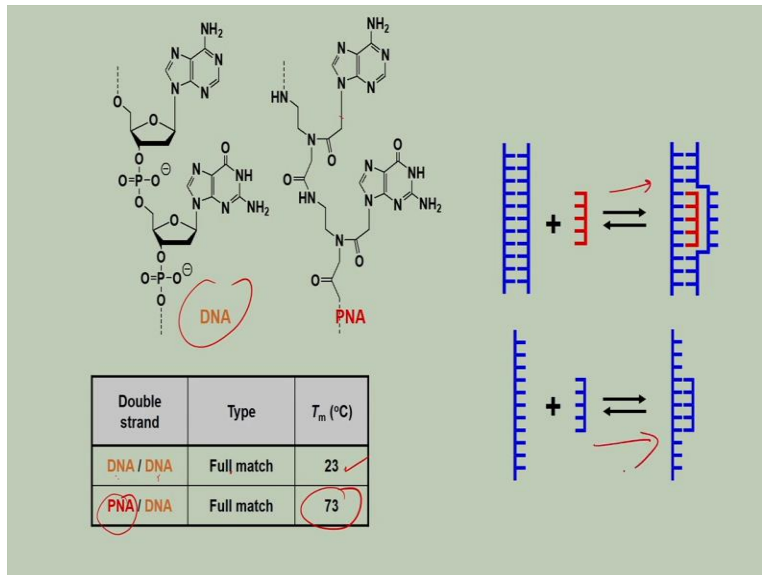

Double strand	Type	T_m (°C)
DNA / DNA	Full match	23
PNA / DNA	Full match	73

This is here it is there in proper geometry. This is the DNA sugar phosphate backbone, this is the PNA within the same nucleobases that are here, but the backbone is no peptide backbone. See here, double strand if you have our DNA - DNA double strand full match, then the temperature is 23 degrees Celsius, if you consider that this DNA - DNA has it a 23 degree Celsius melting temperature.

The same length using only a PNA instead of this DNA full match has a melting temperature around 73. So, high huge difference and these are the patterns. So, thermodynamically pattern this one is thermodynamically favorable, this one is of course, thermodynamically favorable.

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Peptide Nucleic Acids (PNA)

PNA

DNA

Advantages of PNA

PNA has neutral backbone and therefore is an uncharged species

PNA hybridization is independent of ionic strength

PNA probes are better target specific and has higher melting temperature than its DNA analogue

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This we can discuss in the next class, so, how PNA can be used as a molecular probe to make different designs and to find out the mutations in the cells. This week I can discuss very briefly. The advantages and disadvantages of PNA that I was talking about. So, this is a PNA this is a DNA advantages of PNA is PNA has neutral backbone, as I have talked about, and therefore, is an uncharged species.

PNA hybridization is independent of ionic strength; you do not need to use salt because there is no charge. PNA probes are better target specific and has higher melting temperature than its DNA analog that we have seen. So, details of those including the synthesis, we will see that next lecture. Thank you.