NMR spectroscopy for Structural Biology
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Lecture: 29
Application of NMR in the Area of Structural Biology: Structure of DNA and RNA 6

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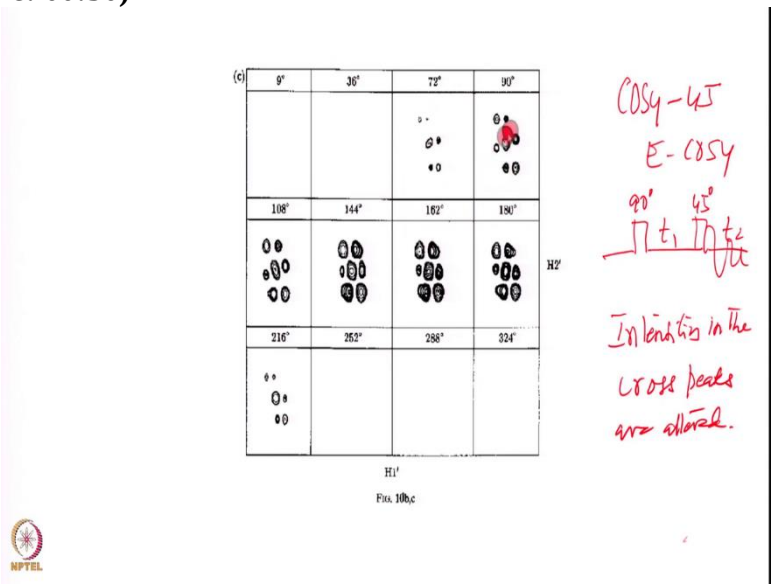

So,. Now we are going to invoke a different kind of an experiment to simplify the cross peak fine structure and this is called as COSY 45 or one can also use what is called as the ECOSY. And COSY 45 is simply a modification from the COSY experiment the COSY experiment has normally this sort of sequence here T_1 T_2 and the COSY 45 means this angle is 45° this flip angle is 45°.

This is 90° and this angle is 45° you can also use a smaller one you can also use 30° and 40° or whatever but a smaller angle not 90°. What is the result of this? The result of this is the intensities in the multiplet intensities in the cross peak are altered. This as a result what happens is you may not see all the components you will see some components and therefore you will see a better fine structure.

But the some intensities will go to zero therefore the cancellations will be less and you will see a better fine structure in this cross peaks. Now you can see the number of components is almost reduced to half when we do this, this sort of an experiment there. So, in this of course this will not be present there and this one this is the 1' 2' peak here these peaks are not present and you see this structure is reduced this structure reduced number of components is reduced.
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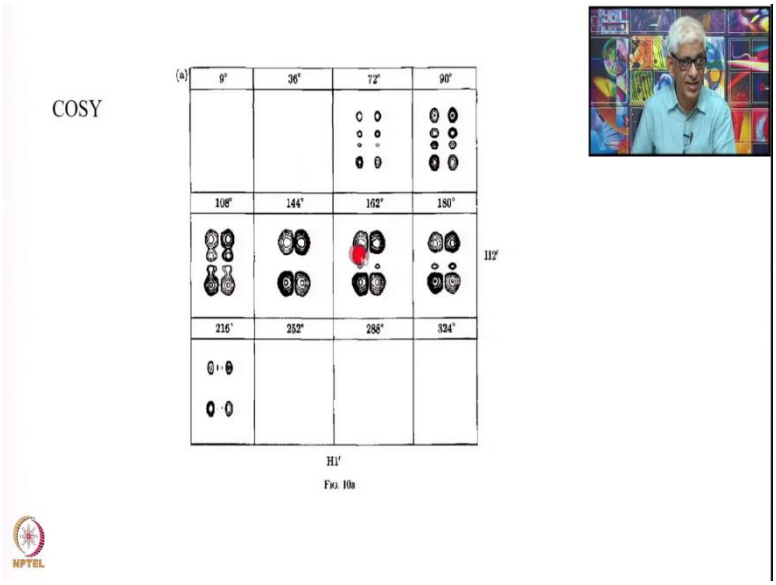

Fig. 10a

You can compare this with the peak here see the kind of a structure what we had in this here see these ones are more components here but the many components are cancelled and when the components are cancelled you are not able to measure the coupling constants but you need to get those coupling constants. So, therefore you need to do extract those informations. Now we see you can see more components here in this because the cancellations are reduced.

Because the intensities of the components are altered by this use of this 45° flip angle for this pulse. Now you can see many more components here therefore you can do a simulation which will be which will allow you to extract these coupling constants. So, once again you can go here and then of course you do not see the cross peak here as as expected. So, similarly for the 1 prime to double prime peak this is the 1', 2" peak.

Now we see the cone this of course does not matter because the 1', 2' coupling is not there it is zero and this structure remains the same.

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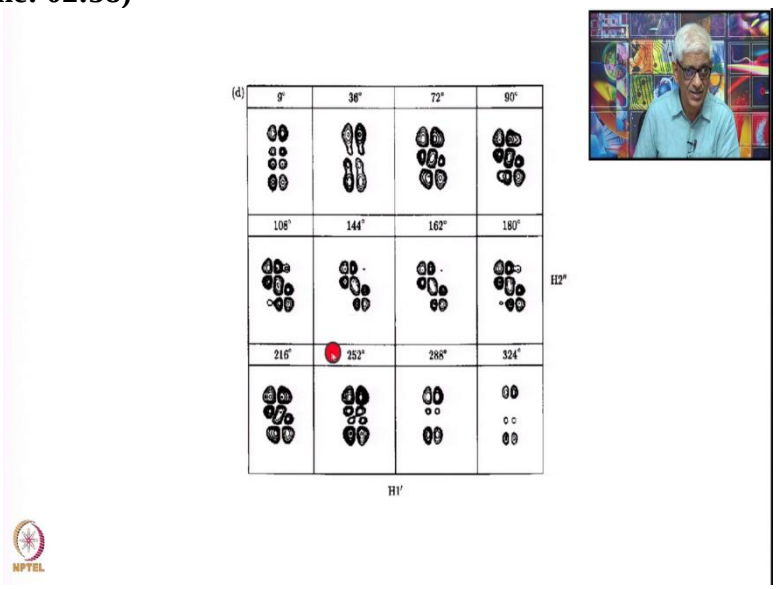

But most importantly you can see here the way these peaks which were earlier present in this area this portion they have been they have been reduced to zero intensity therefore you do not see these peaks there and you will see better resolution within the fine structure you will see 8

components here instead of the 16 components you are seeing only 8 components and that is what is very clear. So, once you have this 8 components.

So, you can actually measure these individual coupling constants very clearly in this area. So, in all the C2' and all this corresponds to the C2 parameter geometry area this is the C3' endo geometry area. This one is little bit more complex but here it becomes much more clearer with regard to the components which are present there. So, and this of course becomes similar to this here and the C2' endo geometry is very well characterized by this fine structure here.

You can clearly see 4 here and 4 there and these will be the plus minus plus minus and then you have the plus minus plus minus here and these ones are extremely useful for calculating the coupling constants.

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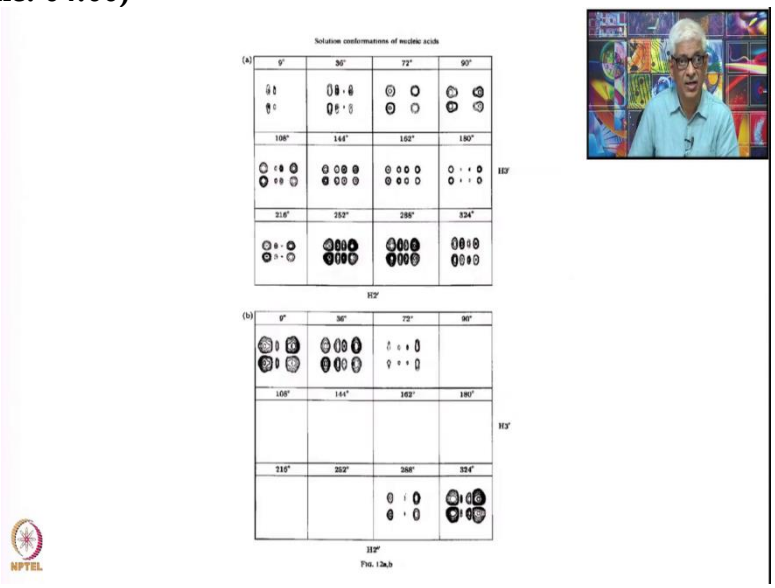

So, this I will demonstrate to you in certain other things and similarly this one is now for the 2', 3' and 2", 3' peaks these are for the 2", 3' peak and these are for the 2", 3' cross peaks. Now these are for the COSY 90 these are not for the COSY 45 and in this case all the components present will not calculate this fine structure right.

This it can be calculated in the same way as I indicated to you for the 1', 2' and the 1', 2" cross peaks taking the splitting patterns of the individual nuclear individual protons and combining them with the splittings of the other ones you will calculate the structures here. So, you see there are many cancellations depending upon the sugar geometry you will have different kinds of fine structures here.

So, one has to simulate these and all the peak patterns have to match at the same time. So, you want to choose a certain set of coupling constants you not only must match the 1 prime 2 prime 1 prime 2 double prime must also match the 2 prime 3 prime and 2 double prime 3 prime fine structures. Then only you can be confident that your coupling constant calculations are correct.

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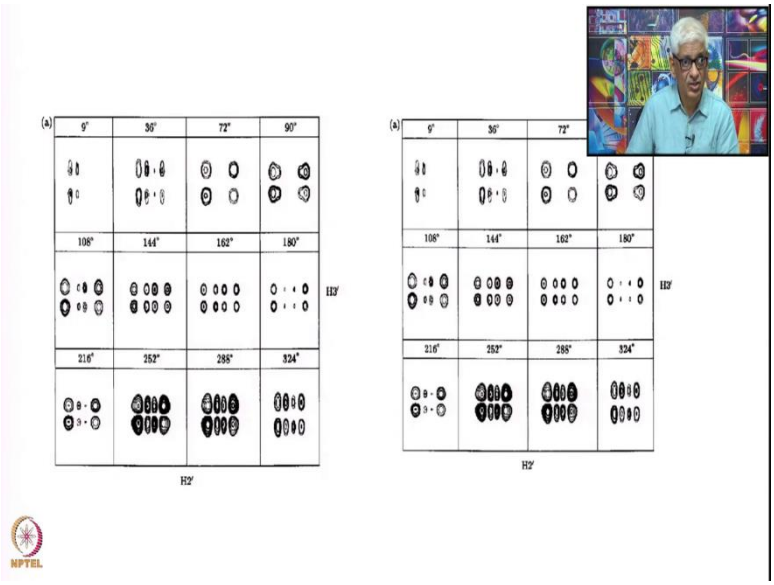

This is the same thing 2', 3' peaks how these fine structures are present in the individual as a function of the pseudo rotation angle there. So, this one cannot remember these ones but whenever there is one faced with a particular situation then you must this forms a database. Using this database one can compare your experimental spectra with this and simulate this using this of course this software was written for simulation purposes.

And so, all this data is already published in progress in NMR and those you can make use this is the database you calculate this pattern and experimentally the external spectra you can compare and you can estimate the sugar geometries.

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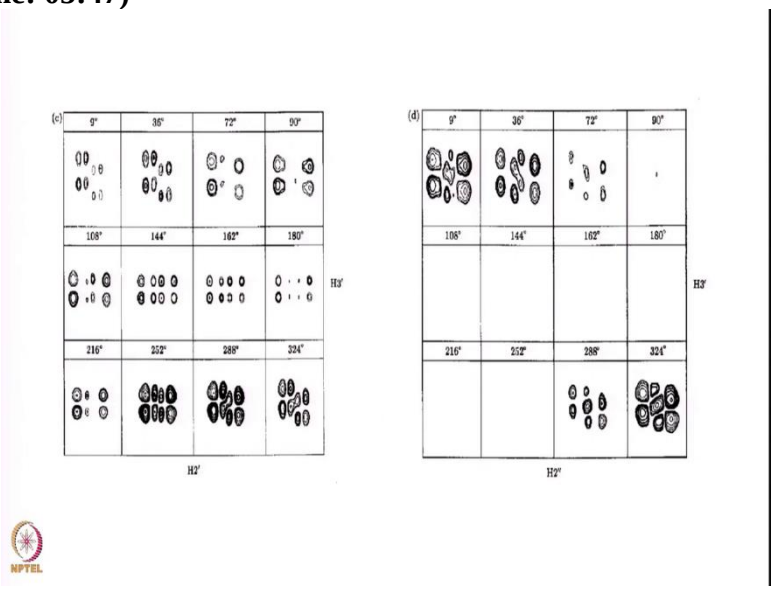

Now this is for the 2", 3' . Now this is for the COSY 45 in the COSY 45 you can see how these ones will change. So, here you see for the COSY 45 to 2', 3' peak this actually looks much more simpler in the 2', 3' for the 2', 3' coupling constant of course will be there because that is about 6 to 7 hertz. So, you will see in the C3' and domain also you will see that all these cross peaks 2', 3' cross specs will be present 2", 3' prime peaks will not be present.

But the 2', 3' peaks will be present for the north region of course this will be present all of these will be present and you can see this calculation that how the pattern happens this is in the COSY 45. And once you have that you can actually see how the pattern number of components

is reduced this resolution will allow you to estimate the coupling constant. And this is for the 2", 3' peak the 2", 3' peak will not be present in the C2' endo geometry here.

Because this is 2", 3' coupling is zero at this in this domain in this whole area from here to here that coupling is zero therefore you will not see this you will only see for these and this is for the COSY 45. You can combine this with the COSY 40 experiment by and large it is better to simulate these ones than the COSY 90 because the number of components is relatively less.

Therefore you can obtain better dispersion of the multiple components and calculate the structure.

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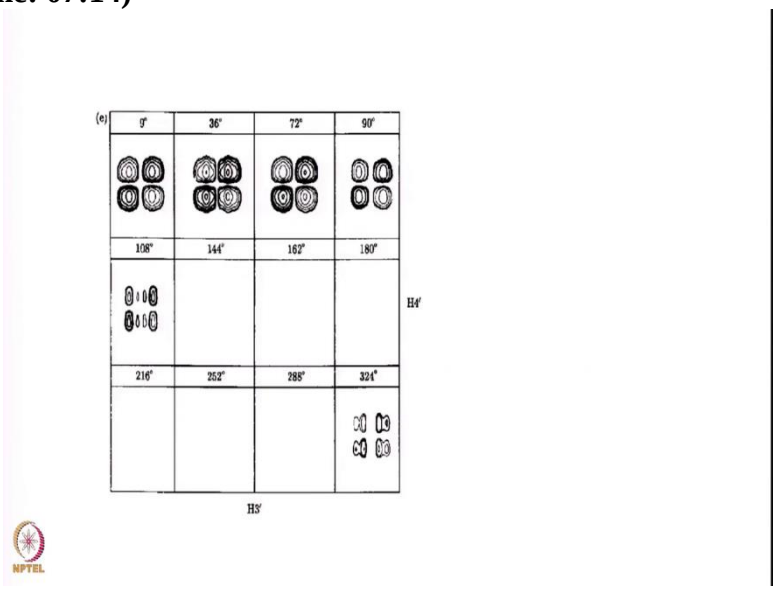
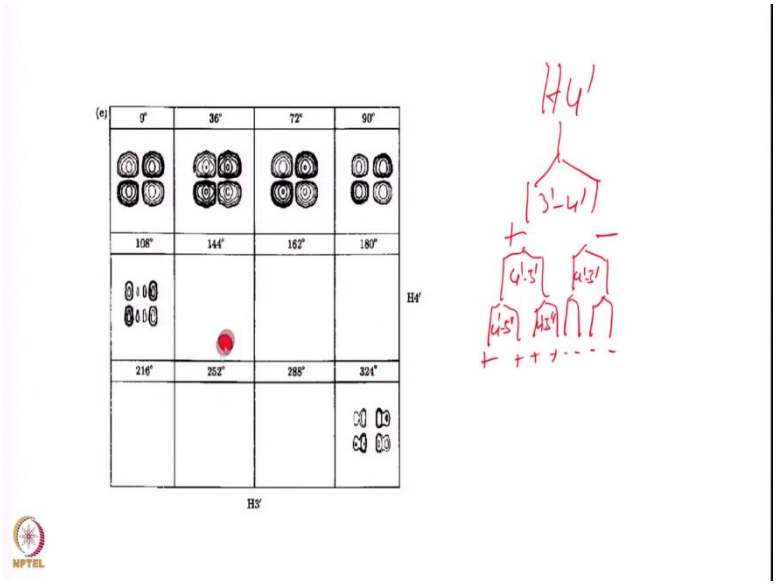


Now this is for the 3', 4' peak. Now the 3 prime 4 prime peak. So, you see in the C2' endo geometry region 3 point 4' coupling is zero in the C2' geometry it is very strong in the C3' endo geometry that is in the northern region 3', 4' peak is very large therefore you will see very strong cross peak this is for the COSY 90 this is not for COSY 45 and you will see only 4 these ones you will see a very strong peak.

And the fine structures with here will depend upon 3', 4' coupling 3', 2' coupling and that is all the 2 couplings which are present you can calculate the fine structures based on the coupling constant values. And of course when you are calculating the 4's one has to take care of the fine structure of the 4' proton as well. The 4' proton has a fine structure because of the coupling with 4', 3'.

But it also has the fine structure with 4', 5', 5" one such remember that here. So, 4' will have a fine structure due to this is a 3', 4' but it will also have couplings to 4', 5', 4' ,5', 4', 5' then you have 4', 5". So, plus plus, plus plus, minus minus, minus minus. So, this will be 4', 5" double prime.

So, therefore this we this has 8 components here therefore this structure will be very complex.



So, this structure will be very complex along this axis all of that is not resolved and many of those ones will overlap finally you will see only this much the total width is sort of the sum of the various coupling constants there. Therefore within that you have these various fine structures plus minuses and any way only thing you can see is that the 3 prime 4 prime peaks are not present in the in this domain which is the C2 prime endo geometry.

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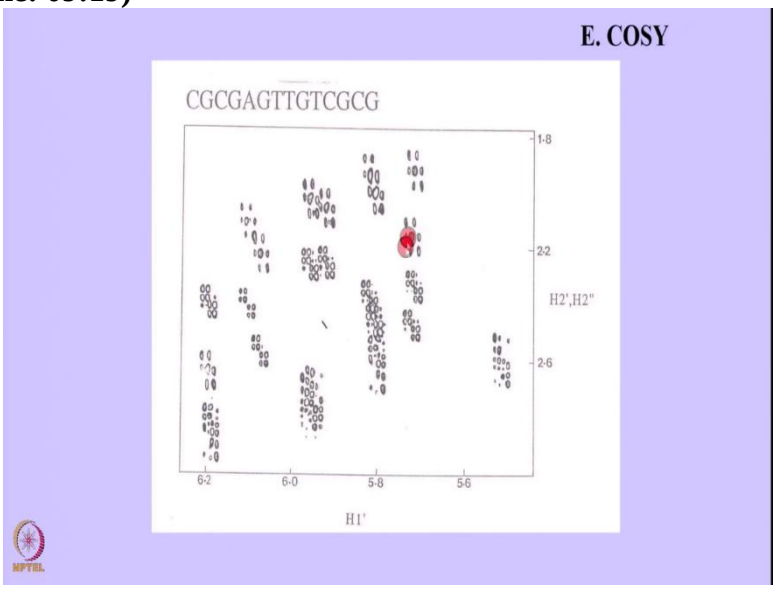

Now this is the COSY 45 experiment for this oligomer for the experimental spectrum for this oligomer. Now you can see here the fine structures of the 1' these are the top ones are the 1', 2' peaks the corresponding bottom ones are the 1', 2". Now there are two 1', 2' there are 2 nucleotides here and these ones are the corresponding 1', 2" peaks this is the 1', 2' peak of one nucleotide this is the 1', 2' of another nucleotide and the corresponding 2" are here you match the centers.

So, this and this form a pair this and this form a pair and similarly in this area there is an overlap of 2 nucleotides. So, there are two 1', 2' peaks here and correspondingly the two 1', 2" peaks are present here but there is one more nucleotide here which is embedded into this area this is overlapping quite a substantial overlap of this here. Here again there is a quite a substantial overlap of the 1', 2', 1', 2" peaks.

This one is very clear there is a 1', 2', 1', 2" 1', 2', 1', 2". Now you can see the better resolution because of the COSY 45 if you had taken this COSY 40 you would not have been able to just separate out these ones components very clearly. So, now you can use this to calculate. So, once again here also you can see 2 here one here and one there this is one this is 2 and here there are 2.

Now notice here this fellow the 1', 2" peak is on the top 1', 2', prime is down and this is because of the terminal 1 something is this is identified and this pattern is very characteristic of the 1', 2" peak you can see that all in all of these places but there is an extensive overlap of peaks here. Now we will see we will have to use different tricks to separate this out we will see what to do there but I just indicate you to the experimental example.

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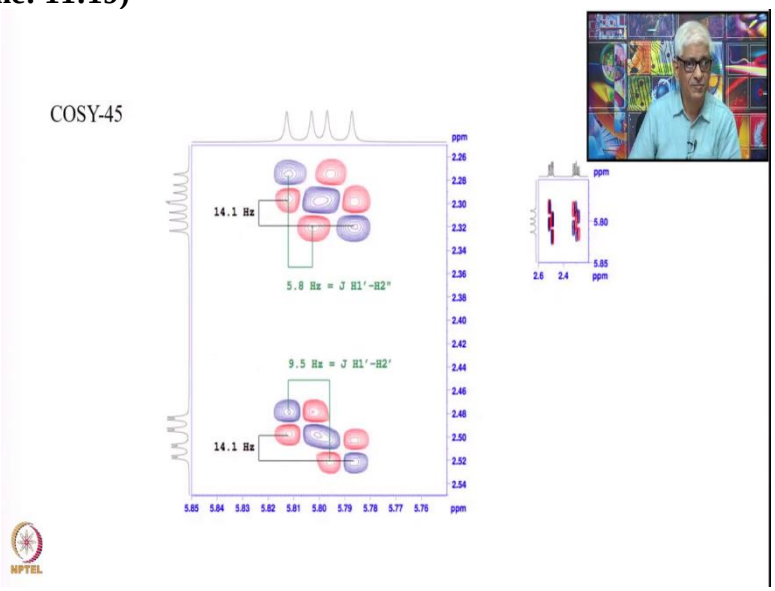

Now this is an example which clearly shows using COSY 45, how one can identify the coupling constants. So, you see the fine structures here what is present along this axis is the 1 prime multiplet this is 1' the 4 components you have the 1', 2' coupling and 1', 2" coupling and what is this peak this peak is my 1', 2'peak this is my 1', 2" peak.

So, you can see here the fine structure there are 8 components there are 8 components here also 8 components here depending on the coupling constant what we have there and you the various coupling constants are indicated here you have the 2', 2" coupling indicated here and then the 1', 2', coupling indicated here. You measure the 1', 2', 2" coupling along this axis.

And measure the 1', 2'coupling along this axis or 1', 2" coupling along this axis also in this one you can measure the 1', 2'coupling which is 9.5 hertz this is the larger and then this is the same 14.1 hertz is the 2', 2" coupling is the same. So, therefore you can determine 1', 2" coupling 1', 2" coupling and the 2', 2" couplings all the 3 couplings one can measure from this 2 cross peaks.

So, this is illustrated in this experimental spectrum this is a very beautiful spectrum and you can actually see the fine structures in these ones.

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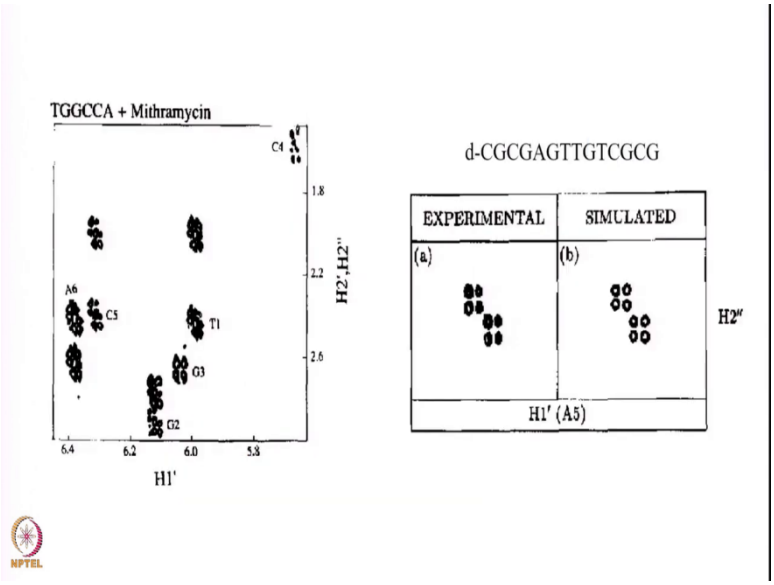

Now this is another experimental spectrum and then the simulated spectrum shown here for this one particular cross peak the same spectra which I showed you earlier the big one and one particular cross peak of that one is this is the A5 nucleotide. A5 nucleotide that is this one here this is the 5 and the 1', 2" coupling of that you see here this is the experimental spectrum. And this is the simulator spectrum and the overlay on this perfectly overlapping and when you do that of course you can get the coupling constants very clearly.

Now this is a complex here with some drug and you one can study what happens when the drug is binding whether there is a change in the sugar geometry as a result of the binding of a particular drug and so on. So, this is a particular application to demonstrate that there is a change in the coupling constant the sugar geometry actually changes from C2' endo to C3' endo when this happens there.

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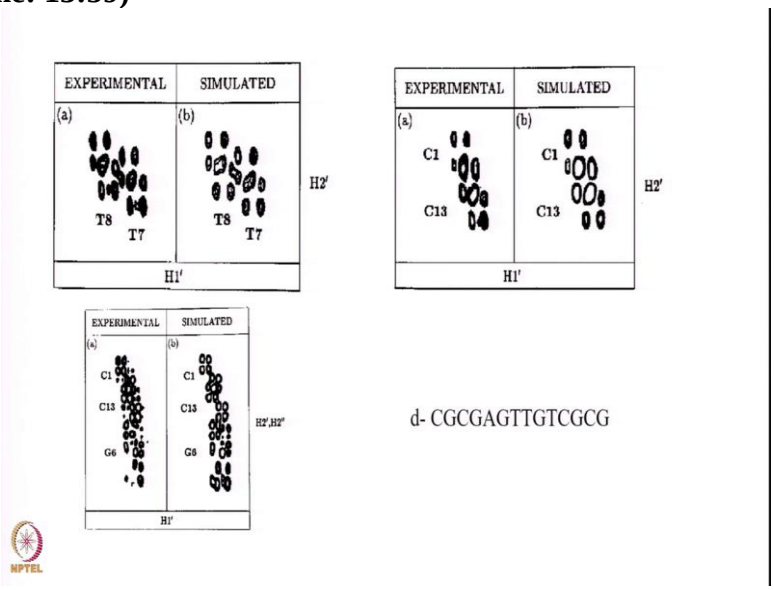

Now here are the simulations of the same spectrum which I showed earlier. So, you remember this overlapping areas here you remember the overlapping this was the way which was so, many nucleotides are getting overlapped there in the COSY 45 experimental spectrum there are 3 nucleotides here 3 nucleotides there C1 C13 and this contains both of the H2' is 2" cross peaks all of them are present in this area.

Now, one could simulate this, this could only by simulation you can extract this coupling counts use vary these different parameters there how many coupling constants are present you can actually vary those coupling constants and simulate this to match this perfectly. So, this is the wonderful demonstration of how to extract the coupling constants by simulations. Now and these are the little simpler ones you have the T8 and T7 there are 2 nucleotides here these are the 1', 2'peaks.

And then this is the T7 experimental spectrum and the simulated spectrum and these are identical and you can confidently determine the coupling constants. Now here there is an overlap of C1 and C13 here to 1', 2' peaks are overlapping in this area and at see you look at that the way the chemical shifts are and the coupling constants are the patterns are looking different. So, this one; of course now you simulate it perfectly to get these coupling constants from there.

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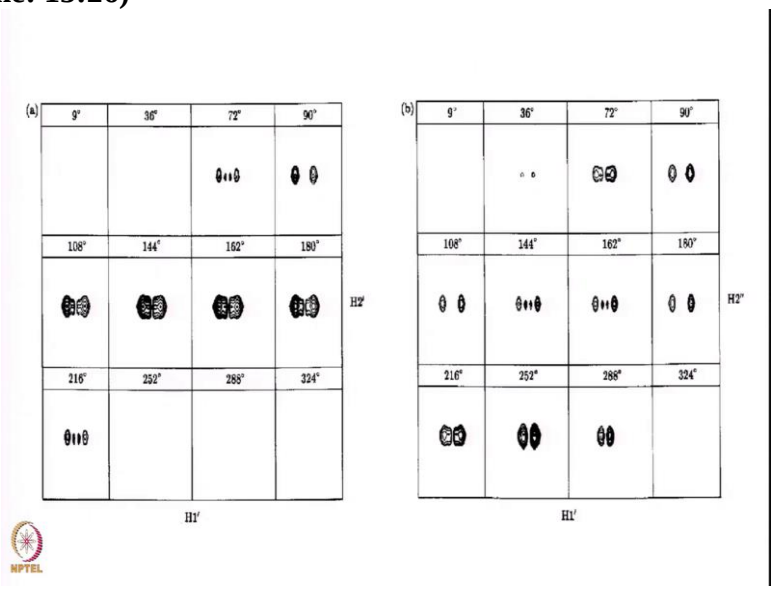

Now that is so, much for using the COSY and the COSY 45 but now you get into even more difficulties of course that the simulation was done fine but can we do something more well. So, we said that we have this coupling information a redundant coupling information along both the frequency axis. We have the coupling information along the F_2 axis as well as the H1 axis for example the H1 prime coupling is present along H1', H2' coupling is present along with 2 axis and the chemical shifts may overlap. Therefore what we do is here we use a decoupling technique decoupling technique this is constant time constant time COSY we had discussed this during the methods.

So, what happens is this will result in decoupling along F_1 axis therefore in this. Now you do not see the splitting along the F_1 axis at all you only have couplings along the F_2 axis H1 prime is the F_2 axis this is my F_1 axis if I am looking at that therefore you do not see the splitting along this axis. Therefore this simplifies the cross pick fine structures much more.

As a result of which you can measure this coupling also relatively better and if there are overlaps of the peaks that also will get resolved these are the simulations for the different sugar geometries how it happens. And once again for the 1', 2' there will be no peak here because the C3' endo geometry for the 9th these are C3' endo and 1' 2 from coupling is zero.

There you will not see as you start increasing it starts picking up numbers and you will see the highest intensities in this area for this particular peak 1', 2' and once again it will be 0 here and this is for the 1', 2" once again this one is smaller here because this coupling constant is not that large as compared to the 1 prime 2 prime coupling. But you can see the 4 components little bit better here because the relative intensities are different and that will allow you to measure the individual coupling constants in this.

Because what you see here is the fine structure of the H1' alone all on this axis you do not have the H1' has is what it is a doublet of a doublet it has 2 couplings 1' to 2' and 1' to 2". So, you can see the 4 components here although there is some cancellation in the middle in this area that is why the central peaks have lower intensity but nonetheless you can actually calculate measure the separations you get an idea as to what they will be they may not be precise but you will get the precise values after the simulations.

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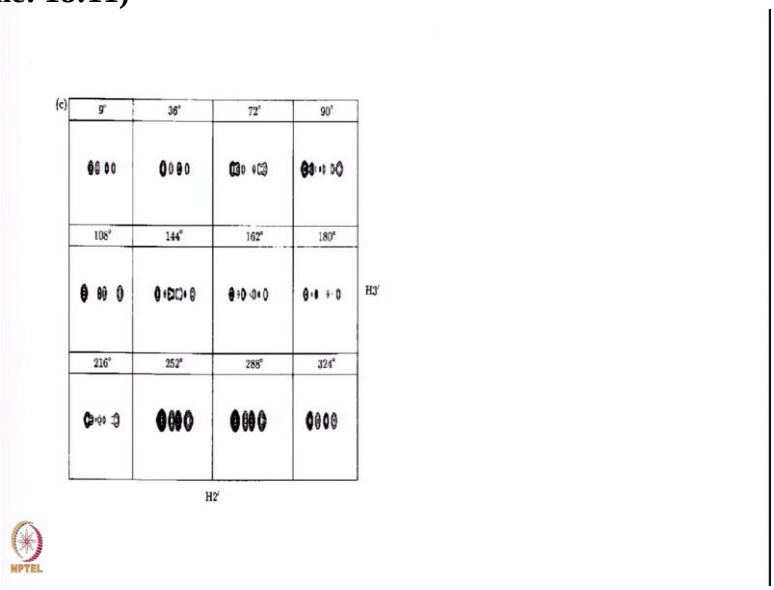

Now this is for the 2', 3' in the same area for the same for entire region of the sugar geometries. So, this forming database all of these simulations for my database for this is again F_1 decoupled COSY this is constant time COSY constant and COSY or this is also called as F_1 decoupled COSY. So, you will only see the fine structure of the put what is present along this axis.

You will not see the cross fine structure here. So, this will show the fine structure of the 2' proton. The 2' proton has what couplings it has a 2', 3', 1', 2' and 2', 2" prime. So, it has those 3 coupling constants there and this will be 8 components there but of course all of them are not sufficiently resolved. So, but you can see 4 components there these ones.

And so, you can see better representation of this more components here. Here actually you see more components there see same as 2 here it should be 8 components you see actually see 8 of them there because the relative intensities they you can see this separate them better here. But the intensities become more then of course they tend to merge and then you will start seeing this sort of a pattern.

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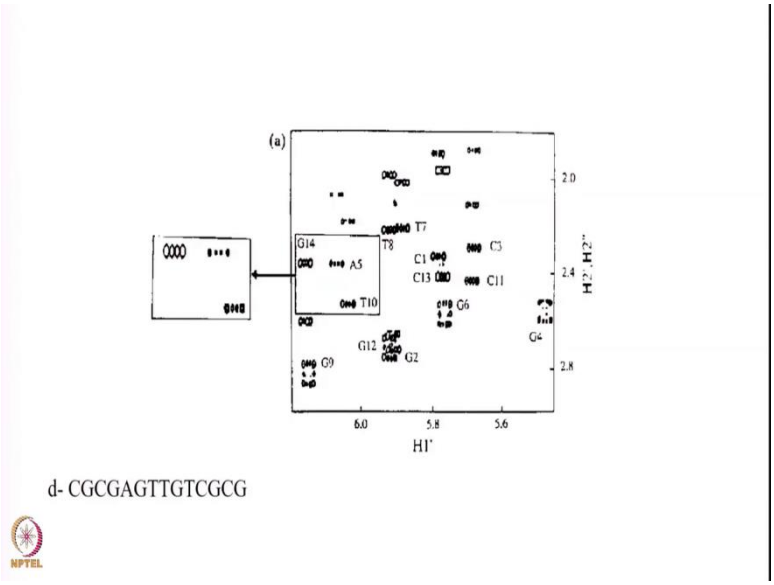

Now this is the same spectrum which I showed you earlier of the same molecule which was let me show you a corresponding one I will go back and show you that this is constant time COSY. This spectrum is of the same one as this one this is the same region it is the same region of the same molecule with the constant time COSY see. Now you can see all of them are. So, well resolved the ones which are present here every one of them is one one one one line.

So, 2', 2" correspond you can also figure out which one which 2 are in the line. So, this corresponds to this, this corresponds to this. So, you can see you can draw the lines. Now you see here that there are 2 overlapping which they are very complex. Now you see here there are 2 nucleotides here G2 and G12 and they are both the 2', 2" they are both present here they are all overlapping and the G9 2' and 2" peaks are also overlapping there.

And we since we remove the coupling constant along this axis they have become. Now one line here and one line there. So, similarly here for the G12 one line there one line down another G2 one line here one line there. So, each one of them is giving you one, one line it is right. So, this is one line here one line there and the correspondingly one line here one line there and similarly this one, one line here one line here one line here and one line here.

So, that will allow you to figure out how many peaks are overlapping. So, even in such a complex situation by doing this constant time COSY you are able to separate out these various components then you can actually simulate this to calculate the coupling constants.

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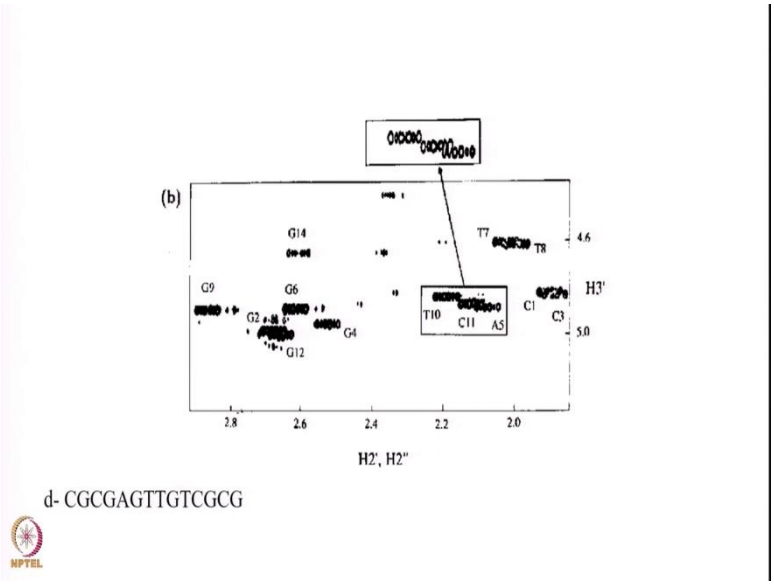

Now this is the 2', 2", 3' cross peak region this is the extremely complex area extremely complex look at the overlaps here. Therefore this you could not have analyzed without the F_1 decoupling this is again a constant time COSY. This is again a constant time COSY or also called as F_1 decouples COSY and you see this area. So, how complex it is if you had the coupling constant information here this would have been impossible to analyze.

If you had the coupling information along this axis as well you would not have able to separate out all of these components. So, now we can see clearly 3 lines 1 2 3 and there is a blow up here. So, similarly there are 2 lines here the T7 T8 they are. So, close and similarly C1 C3 these ones are again G2 G12 which you saw earlier also in the case of 1', 2' 2" as well.

So, therefore while. Now by doing this you are able to figure out the individual patterns chemical shifts and the individual coupling constants. You have to be able to measure all of these coupling constants at the same time then only you will have the confidence in those ones.

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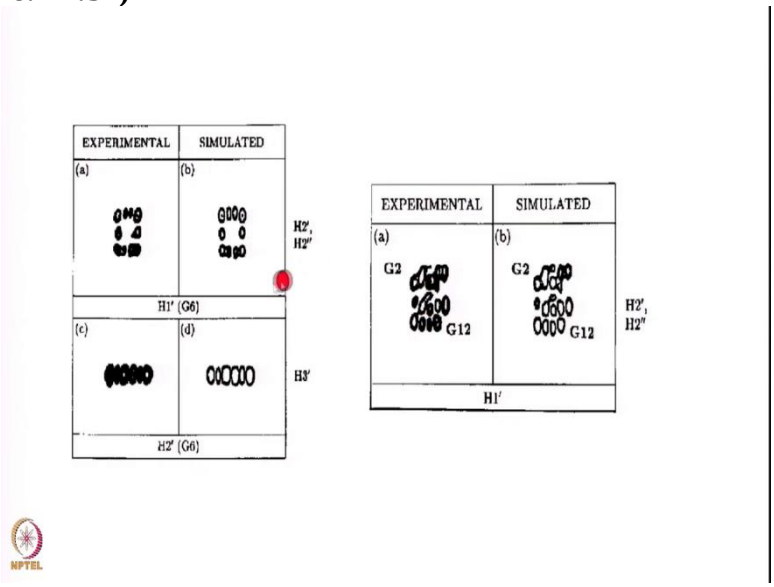

Now you see these are the simulations having obtained those experimental spectra. Now to generate where there are overlaps you will have to resort to the simulations. So, you have this experimental spectrum here the 1', 2", 2', 2" are overlapping here in this and that one is

distinctly seen this was of the G6 residue which was there below and 1', 2', 2" and this is the 2', 3' of the same residue.

So, using the same coupling constants you must be able to fit all the cross peak fine structures the 1', 2', 2". So, you have the 2' here the 2" here 2' there 2" there and then you measure this fit the all the coupling constants and get the fine structure this is the 2' and 3'. Now in this case experimental. Now experimental and simulated once again you perfectly generate the simulations to get this individual.

Two nucleotides overlapping even so, you could get the coupling because you have one line each along both along the vertical along this axis and you have the fine structures along this axis. Therefore you can measure these coupling constants quite clearly you can yeah. So, you can see here this one and this one and this one and this one these 2 belong to G2 top one belongs to G2 and this one belongs to G12. So, therefore you can actually measure this and separate the coupling constants.

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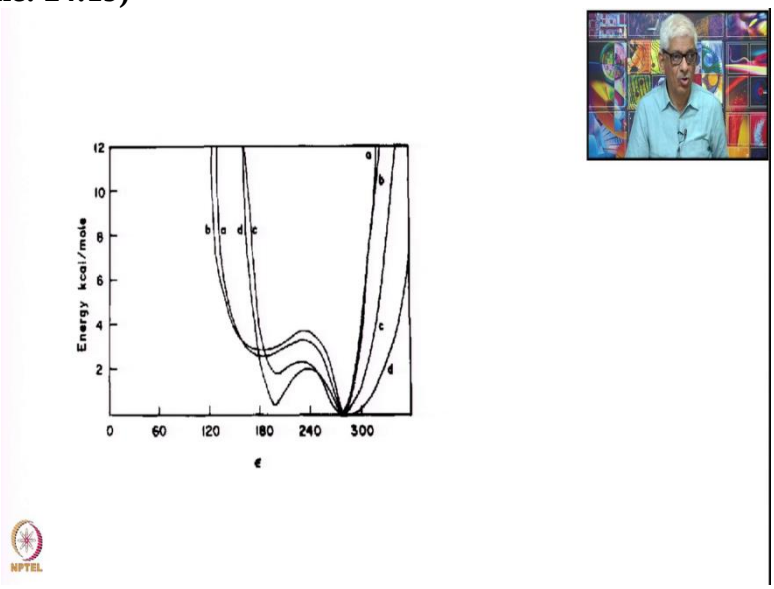

That is so, much for the sugar geometry. Now we turn to the one of the torsion angles along the backbone and that is ε torsion angle the C3', O3' torsion angle in the along the backbone and this actually is quite restricted. How do we know this is restricted you do determine this by there is no measurable parameter here to find out this coupling constant NMR parameter.

Although you can sometimes you can use the P31 spectra and here you also determine this by energy calculations you calculate the energy of the system for a particular dinucleotide and what are the favourable energies what is the most stable state? It turns out that when you do this that you have the lowest energy for this epsilon torsion angle is around 270 degree and here you have a very nice minimum here with very small energy.

And as you go anywhere you go away from there the energy goes up very rapidly if the energy is like this is very unlikely that these will get populated in any normal situations. Therefore by and large when you are actually calculating you generally consider only this value or this value for the ε torsion angle. When you build the models for the structure calculations then you will have to use this sort of constraints you therefore what constraints you use first of all you have the assignments.

And then you have energy values here for the epsilon torsion angle and the sugar geometry determined from the coupling constants that is that forms the input for the structure calculations. And we have not yet come to the distances we will use the distances that will form a particular part from the NOECY is the ultimate finally for to calculate the structure because you have the various interproton distances which we measure and that is the one which you are going to use for structure calculations.

This will form I guess we will go that into the next to next class. So, here so, let me summarize once more. We had the first of all we obtained the assignments using the NOESY and base to 1' connectivity is base to 2', 2" connectivities and then from 1' to 2', 2" connectivities. And then we analyze in the COSY spectrum the fine structures in the cross peaks of one of the sugar ring 1',2', 2", 2', 3', 3', 4'.

And we saw how there is a distinct pattern of cross peaks depending upon the sugar geometry with that we get a well idea about the sugar geometry constraints. This we have to use for the structured calculation which will take up in the next class.