Multidimensional NMR Spectroscopy for Structural Studies of Biomolecules
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Lecture – 17
3D NMR Spectroscopy – part II

In the last class we were looking at 2D NMR projections of a 3D spectrum, we will continue with that today and look at another few examples.

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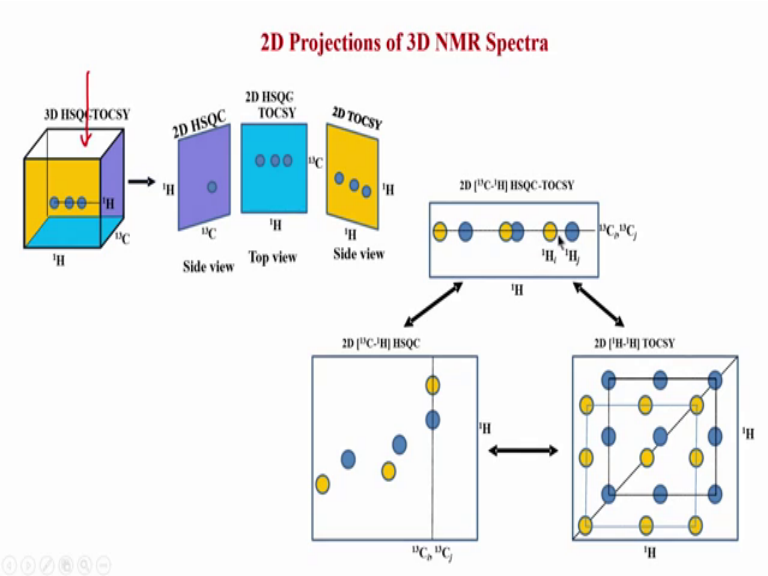

So, let us continue from where we stopped. We looked at this 3D HSQC TOCSY which is shown here and I showed you the different views of this, how it results in different spectra.

So, this is basically the one which I set top views actually side view. So, this I have corrected in the slide here. So, what is this blue colour plane? This is nothing, but an HSQC plane, because it has connection that is correlations of carbon with proton chemical shift and that is this blue colour here on the side what you see. The top view that is if you look from the top is actually this carbon and this proton, the blue colour which you see here now light blue and that is this top view and that is HSQC TOCSY pattern.

So, you have to refer to the previous slides, where we looked at these three spectra separately and with reference to that you will be able to follow how this projections they look. Now the other projection the third projection that is this yellow colour which is the side view I am looking from this side here this side, where my arrow is pointing now. If I look at this side, I will get proton correlations and that is again a spin system. So, this is a spin system, but in a pro carbon they are all collapsed in to one, because there was one diagonal peak and other two were the cross peaks.

So, like this as I mentioned in the previous class that you have different projections which actually were two separate or three separate experiments, but now they are all combined together and they are recorded in a single experiment. So, this is the same projections analysis. So, let us say if you have an overlap. Suppose you have two spin systems here shown like in the blue and another spin system in yellow or orange in HSQC TOCSY in a 2D HSQC TOCSY; that means, these have the same carbon chemical shift, but different proton

So, what happens in a 2D HSQC? You will get two spin systems like this because each molecule each atom will correlate with its own carbon partner and this also will correlate with its own carbon partner. So, total about 3 plus 3 6 peaks you will get in the HSQC, but in a TOCSY now they will form a different family of connections and this is what we saw in the last part of the 2D NMR part where we looked at 2D HSQC TOCSY.

You can see in the 2D TOCSY they will form a connection with each other we know this all the atoms are connected to each other which are coupled to each other. So, this family this is one spin system will separate from the second spin system which will look different. So, 3D is basically able to resolve the peaks which otherwise in a 2D were overlapping. You can see they are overlapping because they come in the same carbon line they are the same carbon chemical shift, but they have different proton chemical shifts. So, because of the virtue of the different proton chemical shift, the 2D TOCSY part was able to resolve. So, with combined 3D will help us to resolve the spin systems.

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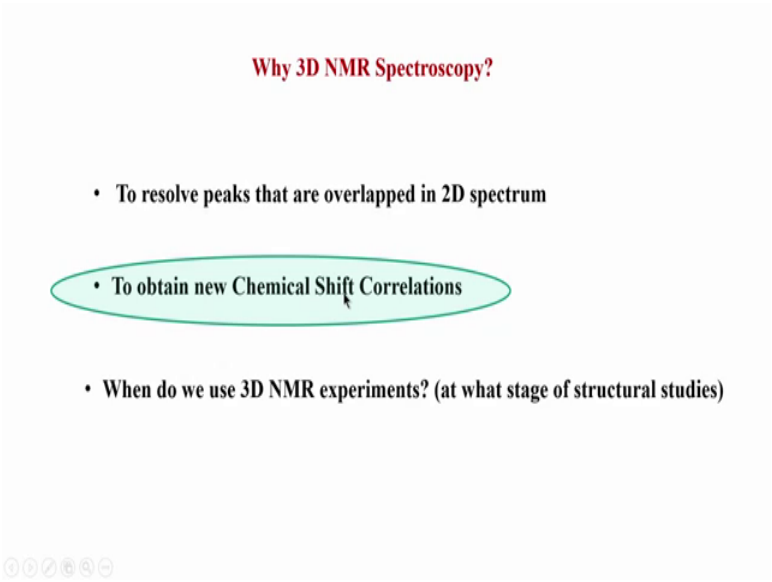

So, 3D basically helps us whenever we have peaks that are overlay overlapped in 2D spectrum.

So, we looked at that now let us go to the next part is how do we use 3D NMR to obtain new chemical shift correlations. So, this is a very important part because now as we saw in the previous one example we added CO. So, we had HSQC which was correlating proton and amide nitrogen h n HSQC, but now we were able to add a CO chemical shift to it and make it as HNCO. So, 3D HNCO is a new experiment in which we correlated the carbonyl now the new chemical shift with the nitrogen and proton.

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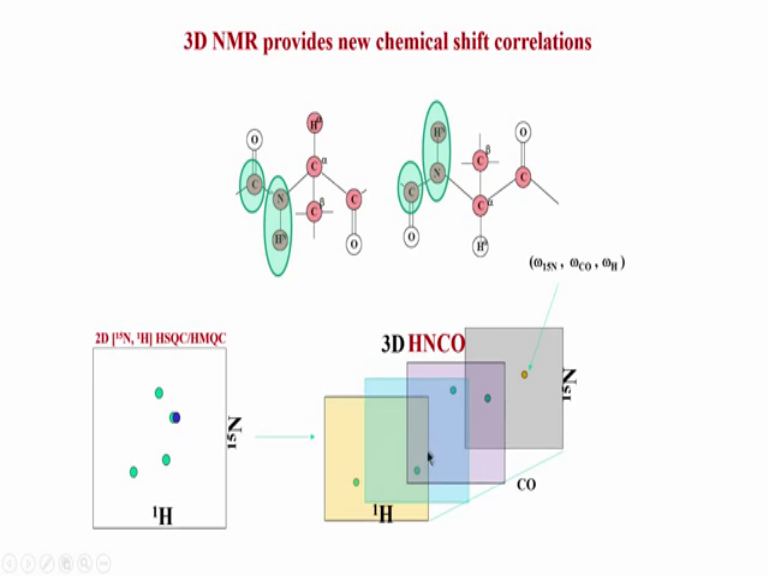

So, that is what we will continue to now look at in this slide in next few slides.

So, this is what I was just mentioning that we had nitrogen proton chemical shift already which is a standard HSQC, but now by introducing a third chemical shift by how do we do that we will come to it slightly in shortly now how they actually we do this connection, but we use some techniques standard techniques. And by connecting these two chemical shifts, I am able to get a 3D spectrum now where I got a new correlation new k information. So, now, for every peak here you see for every peak here I am getting three chemical shifts in the nitrogen, the carbonyl of that previous that is this carbonyl and omega H.

So, remember there is a carbonyl here also, but this nitrogen is not connected to this carbonate, it is actually connected to this carbonate. So, we are looking at this part of this the previous, this is the next amino acid CO rather than the faraway CO here. So, this is an HNCO experiment that is how actually the experiment is in fact, named and this gives us new chemical shift dimension.

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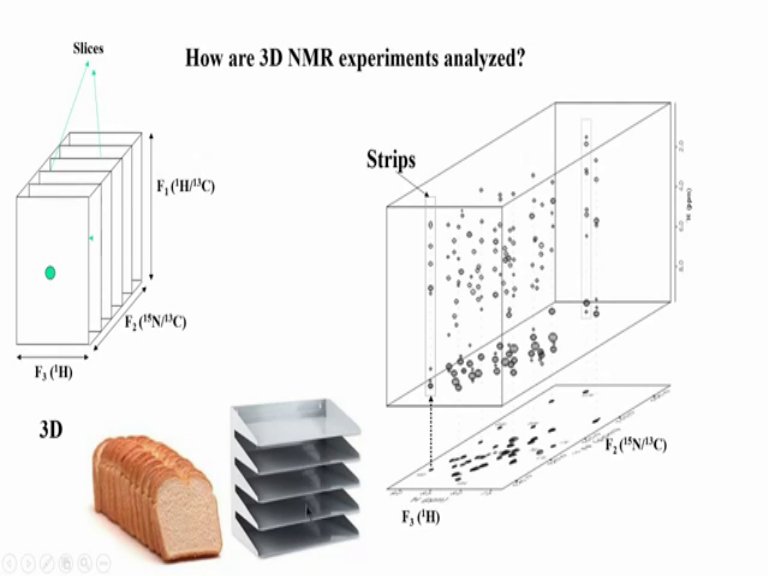

So, how do we actually how are three NMR experiments analysed? So, let us understand that now.

So, 3D NMR experiments as I already showed you some examples they look like this. You have at three dimensions and in one of the dimension you have actually what is called slices you see this slices here and actually if you think of it in a day to day life, they resemble this kind of a bread slices literally they are slices similarly or you can have a slices of files. So, they are 3D is like this. So, essentially all over 3D spectra we analyse slice by slice and then within each slice, you we look at strip you see here now.

So, imagine this is a 3D box I will we will come to the strip analysis more as we go along, but to quickly understand the point is that you have a 3D box here and their consists of peaks. Now if I look from the top view, again remember this concept of projection if I look from the top view then this projection is nothing, but an HSQC ok. So, this is nitrogen or carbon HSQC, but for each peak in this projection in the third dimension, I am getting set of peaks.

So, this is how 3D experiments are in constructed that for a given HSQC peak like this you will get a new set of peaks in the third dimension and all these new peaks are actually new correlations new chemical shift correlations ok. But if I look from the top view, I do not see any of these they all will be compressed and you will end up with a single peak in the 2D projection. So, that is how NMR experiments are analysed, we

always start from a first 2D projection which is usually almost usually always is C 13 or N 15 HSQC and then from there we look in the third dimension which is added here now. So, the third dimension is this here ok.

So, the two dimensions already over these two dimensions which is I am showing now by the third dimension is added and each in third dimension you are getting new sets of correlations chemical shift peaks and then what we do is, for every peak here every point a every spot here we will go and draw a simple strip around that. So, each strip means each of this peaks here it in the projection.

So, if you look at these projections here each of these projection peaks you will have a corresponding strip in this axis here. So, this is how is one can visualize for example, look at this strip here, this strip corresponds to this peak here ok.

So, every peak has a corresponding strip in the third dimension. So, strips and projections these are the two concepts one has to understand very carefully in a 3D NMR experiment and that is how we will be analysing the data.

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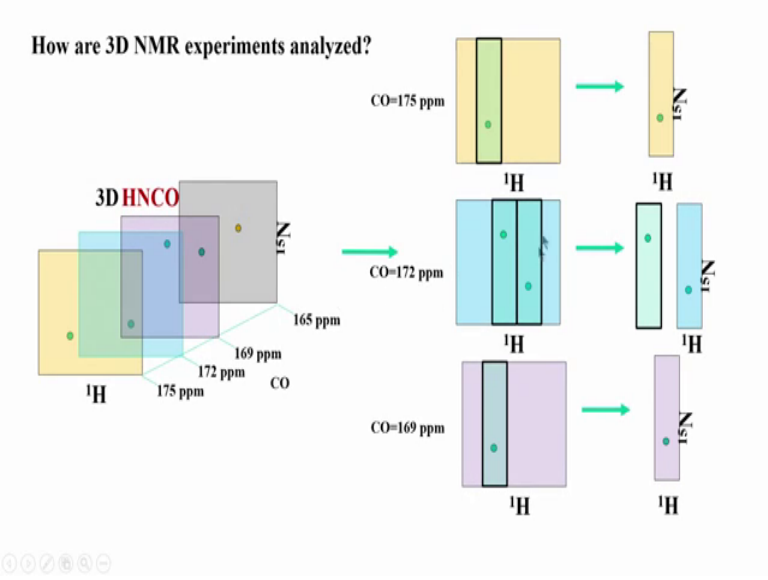

So, now the let us look at this again I will show you how the strip plots we call that as strip plots are made. So, let us say we have a 3D HNCO spectrum this is something we saw and let us say this is CO chemical shifts.

So, each slice here is having a different chemical shift value because we have separated this peaks according to the CO now if you look at this different slices at different chemical shift for example, 175 is this 175 this yellow colour slice that we will have a plane that will look like a plane. And in that plane you see there is a peak here and that peak around that I can cut out a strip and this strip is shown here. So, you can see this strip now in this axis as nitrogen 15 this nitrogen 15 and in this axis is proton.

Now this cutting of strip is not done manually it is done in a computer the computer takes care of that it is not actually physically it a cutting it from spectrum it is only showing us in a screen like this. But it helps us because we can now nicely separate each peaks each of the peak in the 3D into different strips. So, for example, now let us analyse this in 172 ppm plane means slice we had two peaks. So, this green and this blue here ok.

So, these are coming in the same blue plane then if they come like this near protons are different. So, if I draw a strip of some size I can choose the width of the strip whatever I want, I can now represent those two peaks in two different strips ok. Now similarly if you look at 169 ppm plane that has this one single peak here which is shown here, and that appears in at strip plot like this. So, like this I can make strip plots of every peak I can also make for this, but I have not shown that in this particular figure, but you can imagine that it will be same as doing for this.

So, because of the space restrictions I have not shown it, but you can think of it as similar to one of these three ok.

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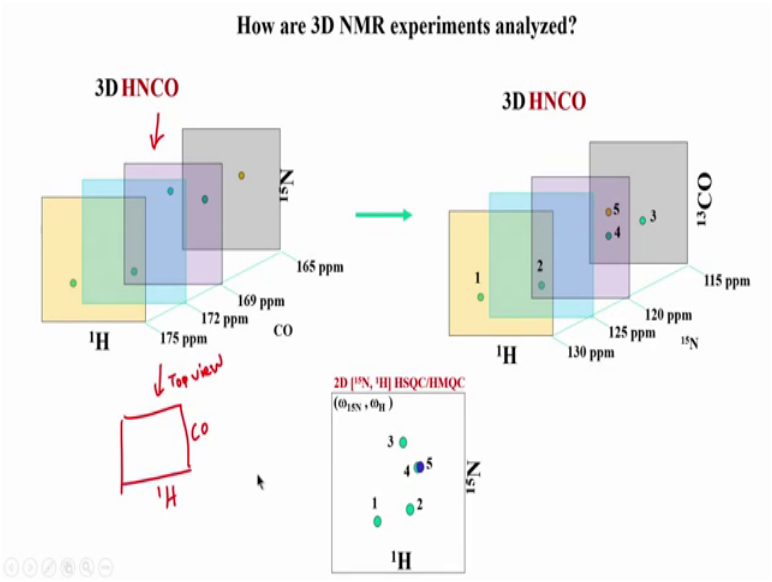

So, this is now what we do is one thing we have to understand is that this is carbonyl ok. So, if I take a top projection like this the projection from here, I will end up with getting if I take a top view I will end up with getting a proton carbonyl correlation. So, there is a top view, but as I said normally if you go back in the slide we saw that actually if you look here in this strip plot here we looked at 2D HSQC as a top view.

So, if you want that as a top view which is normally preferred, then my axis has to be changed I have to rotate this 3D and if I rotate this 3D, I will get now nitrogen here and carbonyl on this side. So, only thing I have done I have simply rotated my 3D nothing else has been done to the spectrum. See if I rotate this 3D. Now nitrogen has come into this part and carbonyl has gone here. So, now, that if I look at the top view of this it will become like HSQC ok.

So, the top view means top view from here as arrow is pointing here that will become the top view. So, that will basically now for each peak in HSQC which will form my base that is basil level basil projection, I can go to the third dimension and get the carbonyl chemical shift. So, hence for all our spectra we will analyse like this we will put ka nitrogen in this axis and we can we will put carbonyl. But remember theoretically there is no reason to do that, you can shift around you can put nitrogen here, carbon here, but the way we look in computers it becomes very easy to have nitrogen here and carbonyl here.

So, it is more of a convenience and convention rather than any other reason; so, nitrogen, proton and carbonyl.

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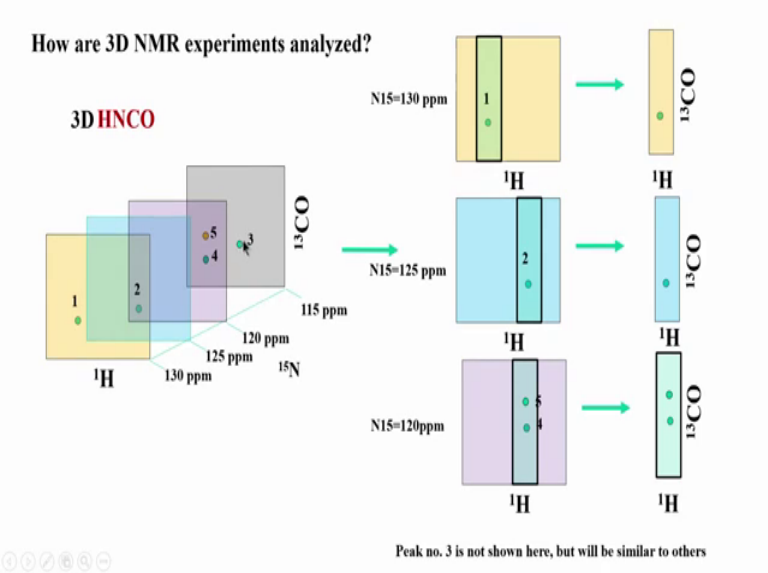

So, this is again a going further how we can analyse this spectra. So, you can see now I have rotated the spectrum I have nitrogen in my this on this axis and this is proton and carbonyl. So, if I take slices now the slices will be along the nitrogen dimension. So, if you recollect few slides ago I showed the slices along carbonyl, here it was co chemical shift, but now you see it has become nitrogen chemical shifts. So, if you have nitrogen chemical shifts then at any given slice value of nitrogen over say 130 ppm slice.

If I take a plane that is proton carbonyl plane now, because this axis is now carbonyl CO. And there I will get a peak which I can perform a strip and then show it in a strip format or look at in a strip format. Similarly nitrogen here for this particular slice is 125 I have a peak and I can look at this. Now if you look at 120 ppm, it turns out that these two are having the same nitrogen chemical shift and why is that? If you recollect in our previous slide here this two are having the same chemical shift in the nitrogen axis and proton axis correct. So, that is what we had the problem that is why we are added one more dimension.

So, nitrogen chemical shift of peak number 4 and 5 are same so; that means, if I go to a picture like this here the 4 and 5 will come in the same slice as in the nitrogen is same. Similarly they will have the same proton chemical shifts. So, look at here this picture, the

proton chemical shift is also same because they are having the same proton and nitrogen chemical shifts that is why they are overlapped. But now because of carbonyl we are able to separate them into two peaks.

So, if I had counted the number of peaks, I would have found in a 2D spectrum I would have found only four peaks, but now because I am able to separate them in a 3D, this is remember still the 3D experiment although I am looking it in 2D manner we are not made it in 2D we are only looking at the slices and strips.

So, we are still in a 3D mode. So, in a 3D I am able to separate them into two peaks. So, this is how basically we analyse 3D NMR experiments by basic looking at the strip plots and looking at the slices along the three dimension third dimension or one of the dimension ok. So, peak three here is not shown, but we will be similar to others. So, this is something I wanted to just stress upon that I have not shown because of space restriction, but you can imagine this also will have a script something looking similar to other peaks.

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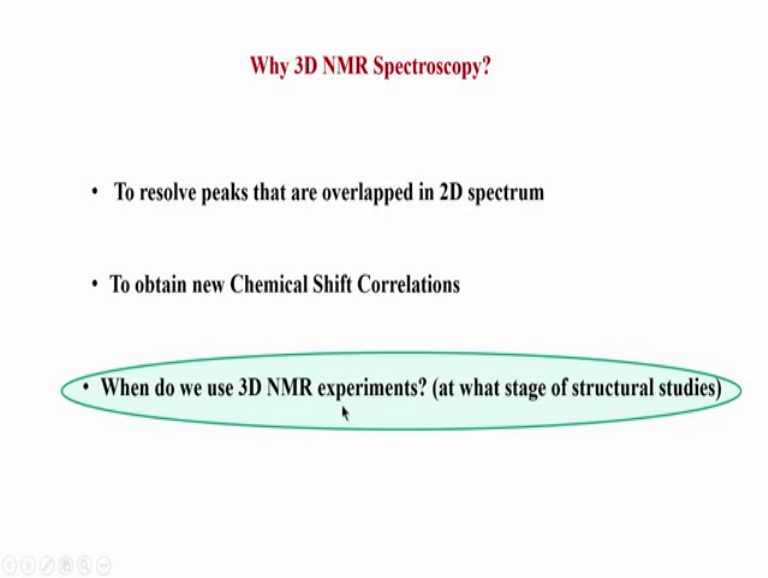

So, we come to the third part why do we need a where do we use NMR 3D NMR expert where do we actually need. So, before we go into actual details of 3D NMR experiments, we need to understand where do we actually use at what stage in our studies.

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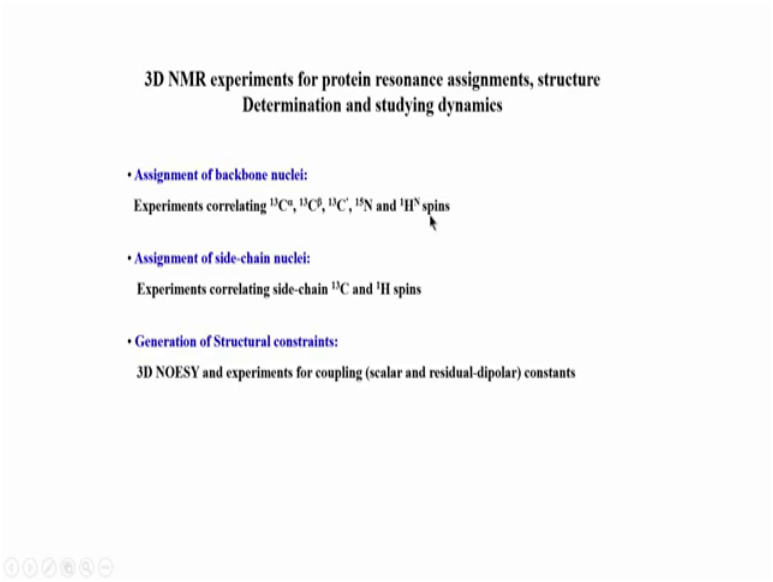

So, this is typically used in three different stages of structure determination. So, for any protein NMR resonance assignment of structure, we need to understand first we have to assign the chemical shift of every carbon and nitrogen in the protein. And this is very important because without assigning or finding out the chemical shift of every amino acid and every carbon of every amino acid we cannot proceed further.

So, that put that part of the work where we actually assign the chemical shift is called backbone assignments, where we look at first the backbone atoms. So, what is listed here are backbone atoms. So, strictly speaking c beta is not backbone, but now is conventionally it is Included in the standard set of experiments, which provide backbone chemical shifts. So, once you have achieved this assignment means identification of each amino acid chemical shift for example, as I said I have 100 amino acid and I have 4 purloins then you.

As I mentioned in the previous class that if have purloins you do not have NH correlations, but otherwise if I have 90 correlations in my HSQC spectrum, every correlation should be assigned to corresponding amino acid. So, that gives this backbone assignment concept. Once you correlate or assign every proton and nitrogen chemical shift to a respective amino acid, then it is very easy to get the carbonyl, C beta C alpha of that amino acid because there are varieties of 3D experiments that we will see which have to be used to get this correlations.

So, one of the use of 3D NMR now is to assign the backbone nuclei. Once the backbone nuclei are assigned the next step when we need another set of 3D experiments which will now help us to correlate or get the chemical shift of the side chain proton and carbon. And this is something different from backbone and the set of experiments required are completely in different principles involving different principles. So, we will see and look at that also and once you have assigned everything all the protons and carbons and nitrogen, then we move on to the final stage of the structure calculation where we look at experiment called 3D NOESY.

So, 3D NOESY basically is 3D NOESY HSQC and 3D NOESY carbon HSQC nitrogen so, on. We will look at that and those experiments are used for and experiments also for 3 dimensional experiments are needed for coupling that is J coupling calculations, and together this information. NOESY remember gives us the distance information and NOESY distance information along with J coupling information we can a combined to get the final structure of the protein.

So, you see that protein 3D NMR is required at every stage of protein structure calculation process. So, let us now simply first understand how we can at least do backbone assignments with 3D then we move on, but we not look at assignment now we will only second part of the course we will be looking at assigning a protein with 3D NMR in the first part which is the up to 3D NMR we will only look at the different basic 3D NMR experiments that are available which can help us in assignment although we will not see how they can be used to assign.

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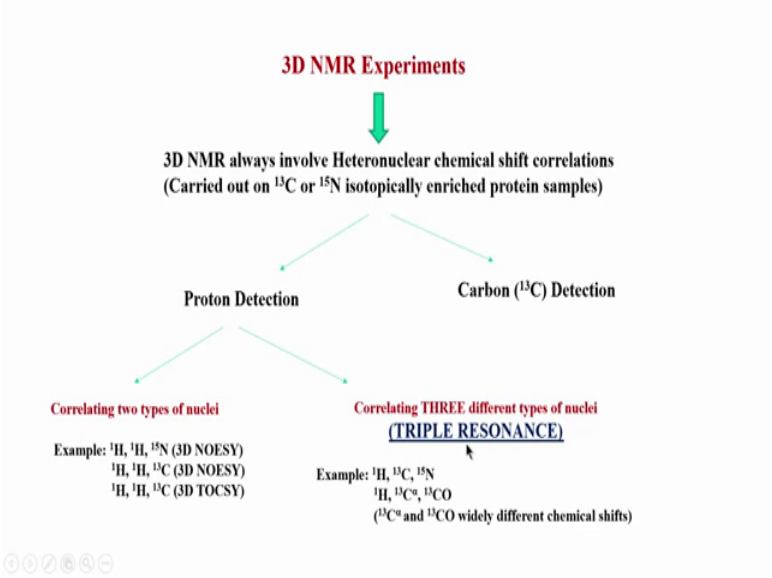

So, this is what I mentioned in the previous classes that we need to whenever we do 3D NMR on protein almost always involve heteronuclear correlation. So, when we talk about 3D it is having at least nitrogen or proton correlated with carbon or nitrogen or carbon correlated with proton, which means that I need to enrich my molecule with these isotopes. The natural abundance it is very difficult to 3D NMR, it will be very insensitive and time consuming. So, we never do 3D NMR on and pro molecule which is an natural abundance we have to somehow enrich the protein with this isotopes for some of them it is possible for many of them it may not be possible.

So, there are in the recent times they have been lot of developments in isotopically enriching proteins or labelling protein. In fact, this is a very rich area of research on its own, because not all proteins can be easily labelled. So, they have been developments technical developments and how to improve the quality of labelling, how to how to label those proteins which are not possible to label in bacteria for example, and so, on. So, that will be part of the second part in this course. So, we will see look at it detail later, but the bottom line is you need to isotopically label the protein and obtain the protein in this form either C 13 or N 15.

Once you do that the 3D NMR experiments can be do in done in two different modes, that is I detect the proton directly id or detect the carbon directly. So, this as I said carbon detection is sensitivity is less, but if I have labelled my protein we C 13 then it is not

such a big deal and now a days there are the special probes in available which can help in improved carbon detection. But we will not look at that in this course our entire course will focus on proton detected experiments 3D NMR when it comes to 3D.

Now and then in 3D NMR you can have two types of proton detection experiments, one is that when we correlate or we have only two nuclei's involved in the chemical shift correlation. So, for example, let us say it may be a 3D NOESY, but we are correlating proton to another proton to nitrogen. So, you see there are only two types of nuclei involved not 3, but it is still a 3D experiment. Similarly I can have proton carbon correlation again it is a 3D experiment, but it has it involves two types of nuclei not three means one is proton other is carbon

Similarly if I do a 3D HSQC TOCSY which I showed you there we saw that two dimensions or proton and proton and the third was carbon. So, we saw in that ex projections in the last lecture in that it has basically only two types of nuclei involved, but nevertheless it was a 3D experiment.

So, we can construct or design 3D NMR experiment where it will have only two nuclei or we can have what is called as triple resonance NMR experiments, where three different types of nuclei are involved. So, this is the popular set of 3D experiments which we used for backbone assignment and side chain assignment as well which we will see, but this experiments are very useful in structure calculations.

You see here we need to have NOESY which has no two nuclei and that is what is done for structure calculation distance information, but for backbone and side chain assignments, we normally rely on triple resonance. Where will where in we will correlate three different nuclei types you see one is nitrogen, one is carbon and other is proton. If you go to if you look at nucleic acids you may have phosphorus here.

So, you may have a phosphorus, carbon and proton. So, that is also triple resonance because it involves three different nuclei that is proton carbon and phosphorus and protein it will be there is no phosphorus. So, we have proton carbon and nitrogen, but remember again we have to label the protein. We have also experiments which correlate proton with calci alpha that is the base the backbone C alpha of a amino acid with CO again backbone that you may think that both are carbon. So, they should actually classify as two nuclei, but then what happens is carbon and carbonyl C alpha and carbonyl have

widely different chemical shifts. So, C alpha come somewhere around 50 ppm and CO comes around 170 ppm to 175 ppm.

So, you see there is a huge difference between the two. So, this type of experiment also sometimes is classified as triple resonance because the chemical shift difference between the two is enormous ok. So, we will look at these 3D types of experiment in the next class and then see how we can start building the basic 3D NMR experiments and from there we will go to look at 3D HNCO 3D HNCA and different types of 3D NMR experiments which are carried out on isotopically labelled proteins and these are the experiments which will be very useful for us to do the assignment of the backbone and side chain. So, we will see that in the subsequent class.