Principles and Applications of NMR Spectroscopy
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Module 7
Lecture No 31

In the last class we looked at the experiment HSQC and saw some examples of how the peak pattern appears in this spectrum. So, we will continue now with another experiment which is a 2 D Heteronuclear multiple quantum coherence experiment and that is called as HMQC. So, information wise 2 D HSQC and HMQC are exactly same, you do not get any extra information in HMQC which you do not get in HSQC as far as practical aspects of using these experiments are concerned. But let us how we look at how HMQC is different from HSQC and where it does have better advantage as compare to HSQC.

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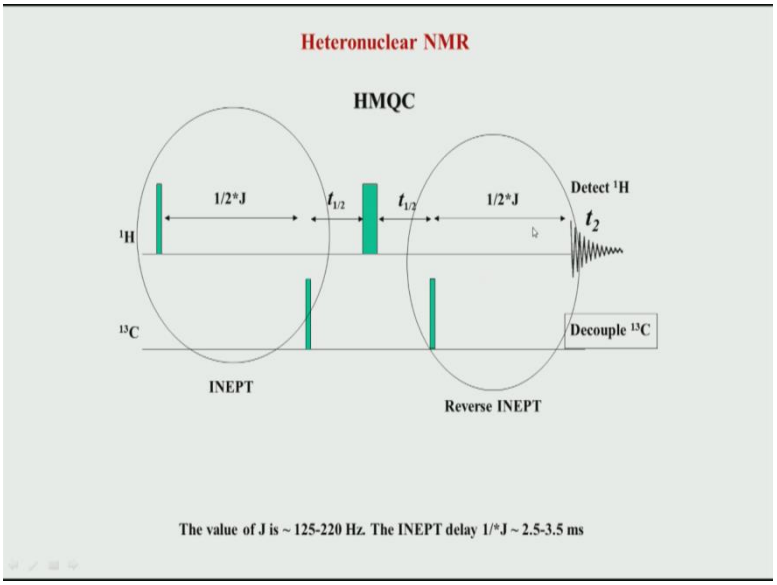

So, the pulse program is shown here you can see this is similar to a little bit similar to what we saw in HSQC and this is the called the proton channel, this horizontal line and the pulses applied in the proton channel are shown here and pulses applied on the carbon channel as is shown here. So, one thing if you notice is that the number of pulses here as much less than what you saw in the HSQC and that is already you can see the first advantage of HMQC there it has much less number of pulses then HSQC. So, what it means is that you can get the same information what you got in HSQC with much simpler pulse program and why is this number of less number of pulses useful, in general each pulse is not again 100% efficient due to several reasons and that inefficiency gets multiply as we apply more and more pulses.

Typically in NMR, the 180 pulses are not very good efficient as compare to the 90 degree pulse, so 180 degree pulse are very bad in terms of the efficiency and therefore one of the areas of research in NMR is how to designed a very good 180 degree pulse, there are several papers published over the years or this looking at a inversion this is like an inversion pulse or a good refocusing pulse. The reason this is bad I mean there is because it does not excite the full chemical shift range.

Remember in carbon in the case of carbon, the chemical shifts go from 0 to 200ppm. So, now if you are let us say if you are working at a 800 megahertz NMR spectrometer, that is 800 megahertz is for hydrogen, so for carbon it will be 200 megahertz 1 over 4. So now for carbon 200megahertz and if you look at 200ppm, if you calculate this 200ppm scale in hertz term in terms of hertz, it is 40 kilo hertz, 40,000 hertz. So, that means you have pulse which you are applying in carbon if at all you apply 180 degree like we saw in the HSQC, your range of coverage has to be 40,000 hertz and that is not typically possible with a simple 180 like this, therefore you need a broadband means a broad coverage 180 degree pulse. And therefore there are been series of different ways methods proposed in literature how to achieve this.

So, let us now look at HMQC, it is a much simpler experiment in terms of the number of sequence pulses. So, is similar to what we saw in HSQC you need to transfer polarization from proton to carbon and that is typically done with using a INEPT block, but this INEPT is not same as the INEPT you saw in the HSQC experiment because remember if you we saw 2 180 degrees and so on, but without this 180 is also we can achieve this transfer and that is what is shown here but it is slightly different. So, we will still called it as a regular INEPT, then you have a reverse transfer back like we saw in HSQC again it is not looking at all similar to what we saw in HSQC but it is a polarization transfer back to proton by using J coupling, so let us called this as reverse INEPT and in between is the evolution or chemical shift of carbon takes place and that is what is captured as at FID in the indirect dimension.

So, this is very similar to in terms of the way we look at in HSQC, we got a transfer of polarization from hydrogen to carbon, we evolve the carbon, then go back to proton and detect the proton, so similar considerations apply if you want a very good transfer efficiency then you are delays here, have to be tuned to the right values. So, the delay here is 1 over 2 J this is should be 1 over 2 J. So, let me correct this, it should be 1 over 2 J and this value of J is 125 to 220 hertz depending on the carbon which you are looking at and therefore the value

if you calculate this roughly around in this range. So, this is the again practical point of view if you want to achieve a 100% transfer one has to then keep those values in this range.

And if now let us look again analyze it mathematically, how this how the FID will be captured, Similar to what we saw in HSQC, we are looking at a hydrogen carbon pair where there attached directly by one bond okay. So, therefore this hydrogen to this carbon is coupled by J coupling and that is achieved the transfer of polarization from proton to carbon is achieved by using a INEPT, so the carbon polarization now comes here, during this period it evolves by the chemical shift of carbon and that is what we call it as captured as (())(6:49) omega C. Of course in all these experiments till date whatever we have seen, we are only looking at cosine part, but this is something which is have done to simplify this whole exercise and actually in reality we do not do like this.

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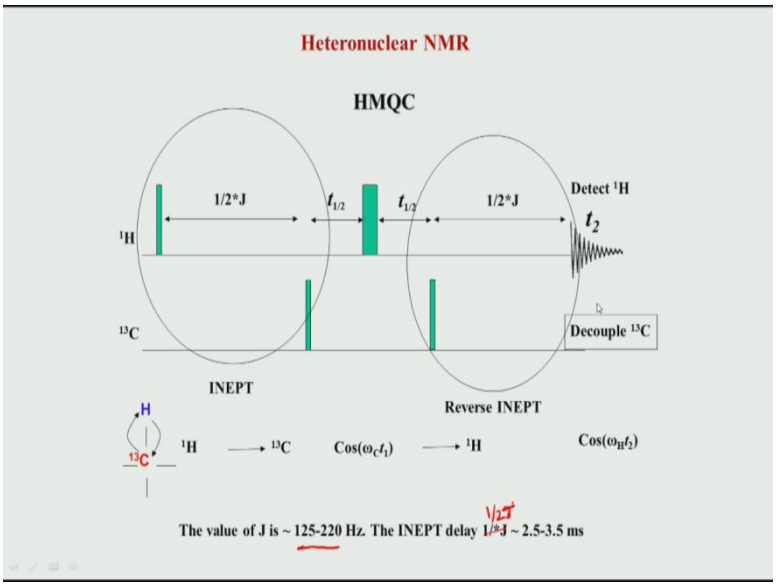

In reality we also have a sign component with use the word complex signal, but in this entire course where the idea is to get a qualitative picture of how NMR different experiments work, so we are not going into details of how the actually the experiments are conducted, but we will see in the advance topic section, we can cover we cover what is called Quadrature detection; in a Quadrature detection you have to get both the cosine and sine component of the signal, because that is what a gives you finally the signal with sine discrimination.

So, let us time being not considered those aspects, we will ignore those we will just say that idea is to capture the chemical shift as a sinusoidal modulation and that is what is done here during this period, the carbon evolves because of the chemical shift. Or again similar to what

we saw in HSQC, there is a 180 degree pulse on the proton applied on the proton channel right in the center of the T 1 part, so half T 1 is this side, half T 1 is this side and in the center is the one. This is done similar to what we saw earlier, this is done for decoupling protons form carbon. So, that is achieved by doing applying is 180 in the center and then we come back to the proton here by reverse INEPT and then whatever has come back we detect the proton chemical shift during the T 2 detection period. And here again we decouple protons from carbon, carbons from protons, so there is no J coupling interaction between these 2.

So, this is very important so we now basically got an FID which combines this chemical shift of carbon with the chemical shift of protons, but again remember we are looking at this pair here. So, every pair which has a proton has transfer to it is neighboring carbon, come back to proton. So, we are captured this chemical shift correlation or connection between these 2 in these 2 pair in this pair okay, so everyone bond pair proton carbon attached will show of in your spectrum.

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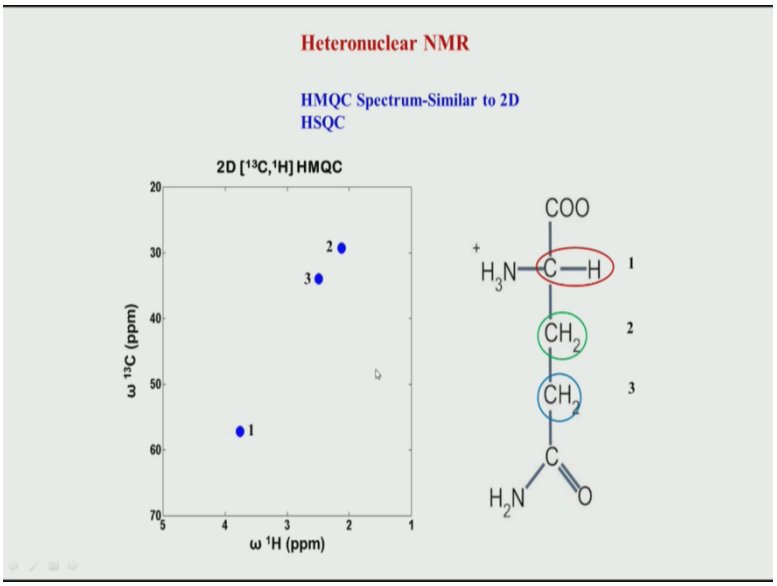

So, let us look at example spectrum again looking at the molecule Glutamine again this going to be exactly similar to HSQC, HMQC spectrum similar to 2 D HSQC. So, this entire chemical shift everything are same, so here we will not go through the exercise of finding out which is which because it has already being assigned here. So, one is this carbon proton 2 is this and 3, so very similar to HSQC this is not a real spectrum, this is a schematic but in reality where we hardly different the any difference and if you look at a if the bigger picture means at the overall. But smalls fines details definitely will be not same, so let us see what

are the similarities and differences, now between the 2 experiments that we have seen HSQC and HMQC.

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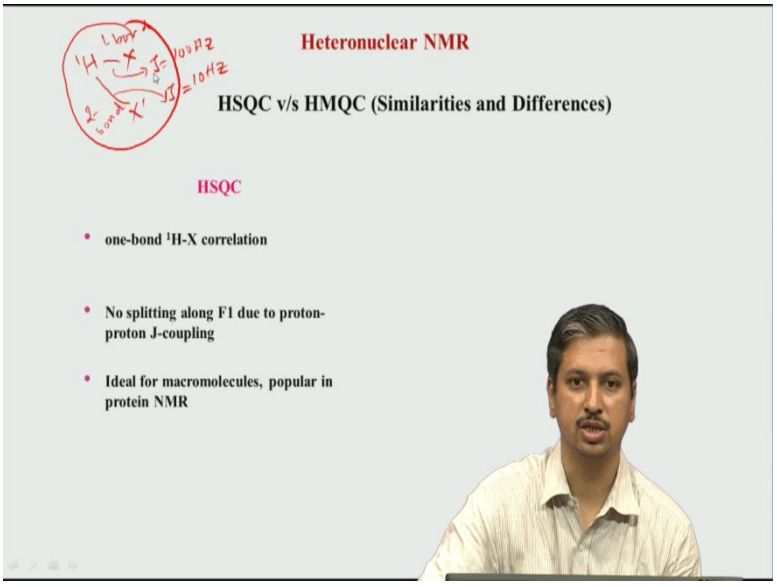

So, HSQC gives one bond proton X correlations, we have only looked at carbon if you look at fluorine or if you look at any other nucleus. You get the same information you get between proton and the directly coupled X atom. Now the question is how do we know it is directly coupled the reason is that we have tuned the J value or the INEPT deal experiments remember we looked at the INEPT pulse sequence block and there we discuss that you have to calculate the delays exactly for the coupling value.

If you do not set the delay in INEPT according to the coupling value the efficiency of transfer is less. So, therefore it to get maximum transfers, we have to tune it to the coupling value, so if you tune it to a different coupling value suppose there is one more X and you tune it to that coupling, so let me give make it more clear, when drawing a schematic here. So, let say I have a hydrogen, it is coupled to X and down the line there is one more X prime in the molecule and there is a connection by let us say 2 bond and this is 1 bond.

So, if at if J is J value will be some value let us say 100 hertz, but this J value will not be 100 it will be very less because it is far away 2 bonds, so let us say it is 10 hertz, so now what I can do is in my HSQC or HMQC, I can tuned the delay according to the value of this J value. So, if I tune it means I arrange it or keep it at the corresponding to 1 by 2 J of 10 hertz, then I will get a maximum transfer from here to here.

But this to this transfer will be not very good because I did not choose the delay period to be tuned to this value. But if I tuned my delay period to this value, then this coupling will be not observed or means not will take place okay so, this is how we can selectively transfer the polarization from proton to a neighboring X nucleus, which is depending on the coupling value. So, I can choose to transfer either this direction to go to here or I can decide to go this way just based on the simple idea of 1 by 2 J in the INEPT part based on the J value, so I should know roughly the J value of my system.

So, this whole process is exactly what we as going to do we do in between HSQC and HMQC and as well as HMBC. So, we will come to HMBC in a short way, so the idea in HMBC is called heteronuclear multiple bond correlations, so that means we go to more than one bond and that is in that experiment we suppress the direct one bond correlation

So, direct one bond correlation comes from HSQC or HMQC, which is what we are discussing here, but in HMBC we go to higher a longer bond and we suppress because we how do we suppress we simply suppress by tuning our delay in INEPT sequence, which we saw INEPT portion by either for this coupling 1 by 2 J or using this coupling. And again this is rough example but we should more roughly or us to some fair extend the coupling value. So, now let us look at the other important features of HSQC is that, there is no splitting along the omega in the T 1 axis due to proton-proton J coupling. Now what is this thing? let us go back and visit the sequence, so you can see here this is for HMQC.

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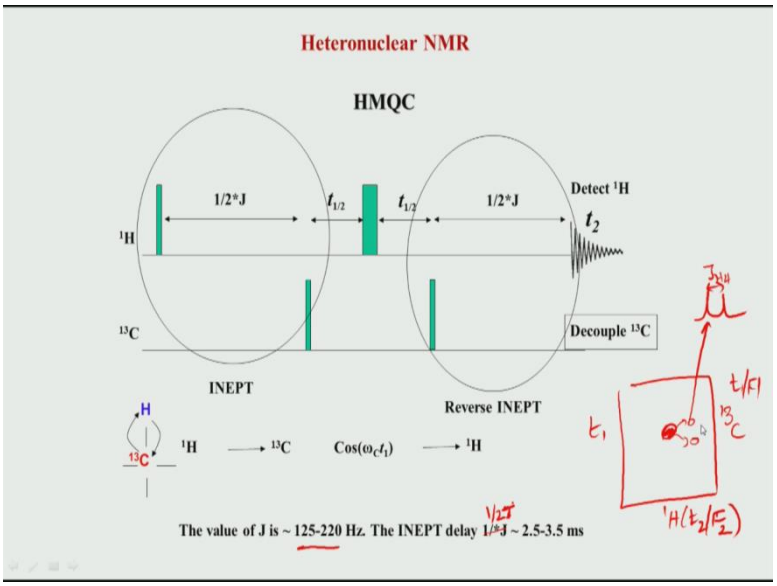

Here what happens is once you excite the proton here the first pulse, then there is no 90 degree pulse anywhere, okay. So, the magnetization of proton remains in the X and Y plane there is no other 90 degrees in like in the HSQC to take it to the Z direction, so when it is in X Y plane the proton during all this period it is, then coupled to each other okay. The protons are coupled to each other, whenever proton is in X-Y plane it will be coupled to all others protons, so that proton-proton coupling is active is away is there throughout the sequence. That means it is also present during this sequence this part and what is this part; this part is basically the indirect vertical axis dimension it is in a 2 D.

So, in the 2 D, so again let me draw it as an example or a schematic. So, 2 D is like this, you have carbon 13 here this is called T 1 or we use the word F 1and this is proton this is T 2 or we can called it as F 2, F 2 means frequency. So, now this peak let us say we get a peak like in this correlation, for during this period that is T 1 which is shown here, here then in the T 1 part there will be a slight splitting because of proton to proton coupling. So, this is basically like the J coupling between 2 protons, so I am write it as JHH okay. So, we did not see that in the spectrum which I showed you because that was more like a schematic drawing but in reality if you give a very long value here this portion, then you will fine couplings between proton to proton will start appearing in the along the vertical axis, so this peak will not appear as a single peak it will appear as 2 peaks.

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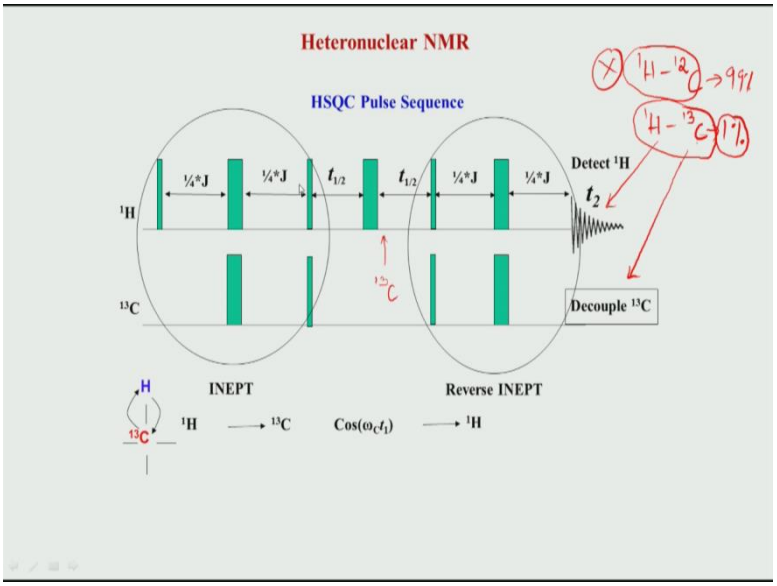

So, this is called F 1 splitting and that does not happened in the HSQC, and why it does not happened in the HSQC. Let us go back to HSQC, in HSQC what happens is you can see there is no splitting the reason is that you have here during this portion of the time. Because of this

180 one because of this 90 degree pulse the proton goes to Z axis okay, so we are not going in mathematical details.

So, I am just qualitatively explaining why there is F 1 splitting in HSQC is because the hydrogen during this portion, when the carbon 13 is having a chemical shift evolution during that period the proton is along Z axis. And when the proton is along Z axis there it cannot be coupled to any other protons, so that is a theorem in NMR not theorem but there is rule in NMR which we will not go in detail but if you keep a proton along Z axis, you can now keep it uncoupled to any other nucleus in any other protons and during that whole period as long as it is in the Z direction it will not be coupled to any carbon any proton sorry.

And this is just a 180, it is not a 90 degree pulse, so that will not affect that coupling. So, coupling will keep in 0, I mean it will not be coupled between 2 protons. That is not there in HMQC in HMQC it is along X-Y plane and therefore here it is coupled. So, this is a big difference between the 2 experiment that in HSQC there is no splitting or in HMQC there is splitting, okay.

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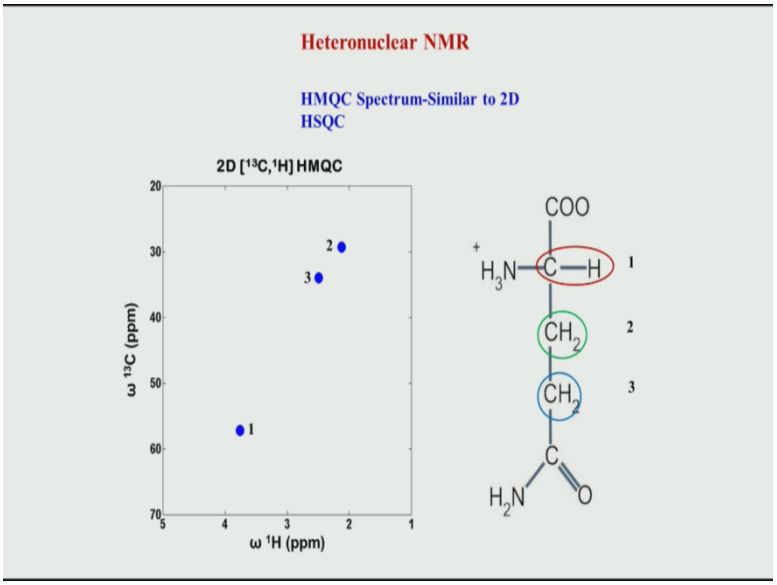

So this is a set one major difference between the 2 and because of this reason normally we like it for a bigger molecule because sensitivity is a major issue and there is another reason why sensitivity is bad for HMQC. So, as for as the HMQC overall sensitivity if you give a rank it, comes second rank or X to HSQC because of this number one, this splitting problem, number 2, there is what is called relaxation problem the multiple quantum coherence you relaxes much faster compare to single quantum coherence okay.

So, that is why multiple quantum is a less sensitive compare to single quantum. But another but that is what we prefer this overall sensitivity being better in HSQC we prefer this for biomolecules where in biomolecules the molecules are very large in size, so therefore the signal to noise in biomolecules is slightly all always lower compare to small molecules. So, when we say biomolecules we are talking about peptides bigger peptides we are talking about proteins and these are the their correlation time, the rotational correlation time is very high, so therefore the sensitivity or T 2 relaxation is very short. So, whenever T 2 is very short in NMR the signal to noise will be very poor in that for that molecule. So, therefore whichever experiment has the better sensitivity is always preferred.

But having said that HSQC is always not that good in the sense there are many number of pulses you apply, we saw that in the case were you further INEPT we needed a 180 degree pulse which has to be broad band because 180 has to cover a big range from 0 to 200 ppm in carbon or in HMQC if you recollect or let we go back we do not have any 180 on the carbon channel. So 180 on carbons is not so critical in HMQC and you do not need to have a special 180 degree pulse which is broad band. So, that is why HMQC is is easy to apply and second reason HMQC is preferred to small molecules is that it is a very simple experiment in terms of number of pulses and now a days or in the recent times there have been many experiments, which have exploited this to accelerate the time required to do NMR experiment.

So, there are some accelerated NMR approaches and which we cannot go in detail because some more research oriented topic in that was developed around this because that exploits some of the features of HMQC, which you cannot do in HSQC. So, so on so force so therefore HMQC is of is a preferred for small molecules and for ideally for large molecules, where you want to get maximum sensitivity where speed may not be the factor, you want to maximize and we can do many more sensitivity enhanced approaches in HSQC. So, these are basically the broad differences between these 2 and we will now move to the third experiment called HMBC HMBC.

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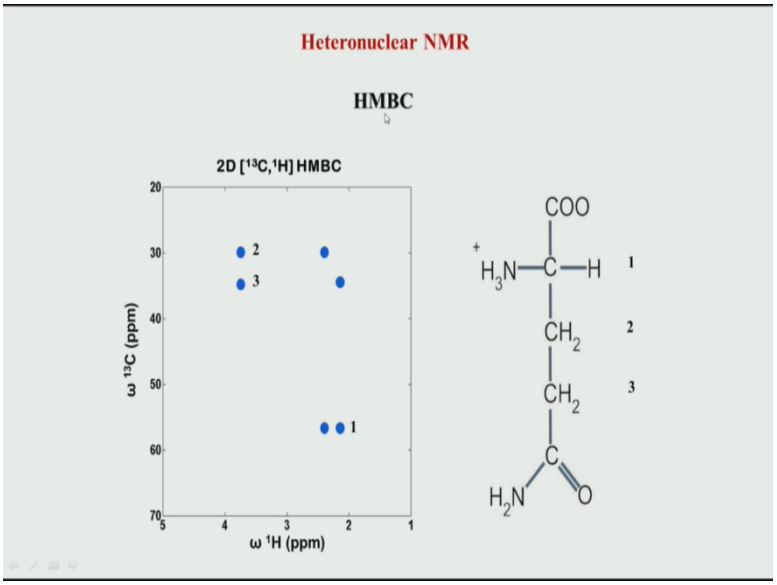

So, this is as the name suggests it is called heteronuclear Multiple Bond Coherence Spectroscopy. So, now what is the information extra here we get which we do not see in a last 2 experiments, we will have a look here? So, here if you look at this, this is again a schematic and this is the molecule Glutamine which we have seen in HSQC analysis. So, what we will see that this proton is coupled to this carbon by one bond but as I said they can also have a further remote coupling to another carbon which is 2 bond okay, which will be smaller comparatively.

This is typically let us say 140, this will be much less above 10 or even less and you can also have a further coupling this side even more. So, this is 3 bonds which is even less which is 2 to 5 hertz, sometimes even less than 2 hertz. So, it varies but there is a finite small coupling possible up to 3 bonds, we can go further but that is much-much weaker and we can ignore it, so we will focus only on 2 bonds or 3 bonds carbon proton long range coupling.

So, what we can do as we saw in the previous slides there we can tuned means we can adjust other word delay value period in the INEPT part of the HSQC such that I get only coupling to the longer range coupling values but I can suppress the intermediate the direct strong coupling. So, because the strong coupling means this pair, we have already got it from HSQC, so we do not need to again have another experiment to give the same information.

So this HMBC was designed to get information of a longer range coupling we will see shortly how that helps to resolve some kind of ambiguities where HMQC, HMBC works by going to 2 bond and 3 bond. I do not have the pulse sequence shown here, but it is similar to very-very

similar to HSQC or HMQC sorry not HSQC and what is done is that delay value the J INEPT value is simply tuned to long range coupling and so that the short range that is this is suppressed by appropriately choosing the INEPT delay values.

So, that is all it changes we make in the experiment and HMQC now gets converted into HMBC. So we will not go into the pulse sequence details, but let us now look at information what is information wise we get. So let us come to this spectrum, so here in this line okay this is the line this vertical dotted line corresponds to the proton number 1, which we saw in the HSQC it was around 3.5 and the carbon this carbon was around 60ppm. So, somewhere here but now we have suppressed that correlation and tuned our correlation to longer range. So, now I will start seeing this proton to this carbon correlation and that is what is 1 to 1 proton number 1, which is on this horizontal line and 2 in the vertical here through the carbon value, so that is 1-2 combination which is here and similarly I will get 1-3 combination which is not shown by an arrow but it is what I will get here.

So, I will get 1-2 and 1-3 connectivity because my I have tuned this experiment to a longer range and suppress the short range interaction or in terms of seeing in the peaks. Now similarly, let us go to the second carbon here this carbon proton is now has 2 bond coupling to here at 2 bond coupling to here, of course it has also to carbonyl this carbon and also to this carbonyl Amide. But that is not shown in this because it is only going from 20 to 70, we will see in the next few slides examples where we can actually exploit this carbonyl connection to this proton.

So, that you can see let us time being only focused on this, so similar to what we saw in the case of first proton. Now in this line which corresponds to the middle carbon proton if you recollect, we saw there was a peak around 2.2 and we got a peak somewhere here. Now, that is gone because it is suppress, but you are seeing a long range information and that is coming here. So, on the hydrogen which is corresponding to 2, I am getting a peak at for the carbon number 1 which is this carbon and carbon number 3 which is this carbon. Now let us look at the last case last proton in this molecule again similar, we saw that we have a proton here which is number 3, so we had a peak around 2.5ppm in the proton and we are not seeing a peak at this position because that is the direct 1 bond which is suppressed and all we are going to see here is the connection from 3 to a longer range.

So, 3 to 2 that is hydrogen number 3 to carbon number 2 and hydrogen number 3 to carbon number one so that is what we see here okay. But what is suppressed is the direct connection,

we will also able to see connection or correlation between the proton number 3 and this carbonyl or proton number 2, proton number 3 and this carbonyl, but this is very far away, this is much closer but as a spectrum is only plotted from 20 to 70 that peak comes which comes at 170 is not shown, okay.

But this interaction now you can see is starts giving us longer range information. It is helping us to tell us that this proton is not only attached to this carbon which we get from HSQC, it is also telling us that what are other carbons which are close that is 2 or 3 bonds away from this proton. So, in a way it is helping us to get a connectivity information, so we will see this in the next class, we will see how this information a long range connectivity correlation information will help us 2 resolve ambiguities, which we will get in sometimes in you see that COSY a 2 D homo nuclear COSY and 2 D HSQC together is not sufficient to get the complete in structure information connectivity information and that can come from HMBC and we will also look at one more final experiment in next class this is the 2 D hetero nuclear experiment before we start moving to the last part of the course on structural analysis, so second last part.