NMR Spectroscopy for Chemists and Biologists
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Lecture 54
2D Heteronuclear Experiments 2

So, we have been discussing heteronuclear correlations experiments two dimensional experiments. We discussed last time various options and various ways of recording heteronuclear correlations. So, we will continue with that now some more options which are present here.

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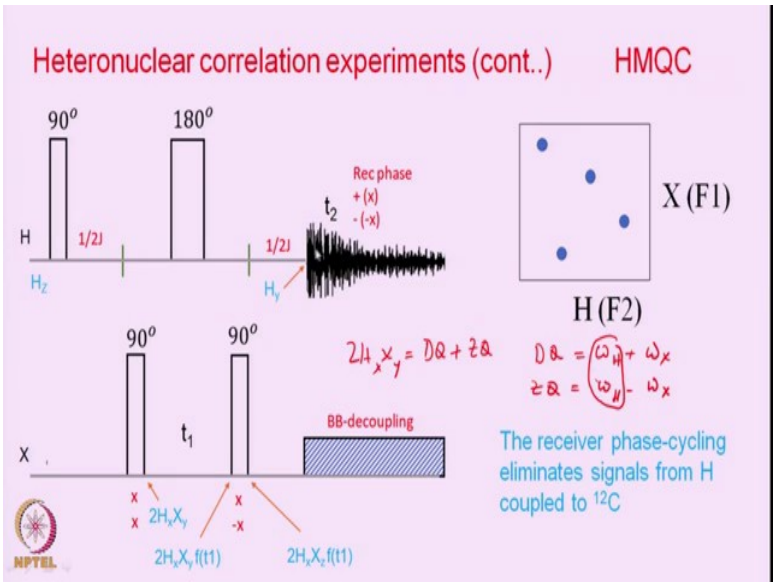

And so, one of them which I will now discuss is so called heteronuclear multiple quantum coherence spectroscopy and that is abbreviated as HMQC. The pulse sequence for that is given here. So you start with a 90 degree pulse on the proton channel. So, the magnetization comes down to the transverse plane and then you evolve it for the period $\frac{1}{2J}$ under the influence of the chemical shift as well as the coupling.

But it is the coupling evolution which is important for us and then after the $\frac{1}{2J}$ evolution under the coupling you apply 90˚pulse on the *X* channel. So at this point this magnetization is converted into multiple quantum coherence at this point, this will now be hold during the

period t_1. In the middle of the t1 period we apply 180˚ pulse on to the proton channel and then at the end of the t_1 period we apply a 90˚ pulse again on the *X* channel.

So, that the magnetization now is actually transfer back to the proton and then at this point the magnetization is anti-phase with respect to the *X* nucleus and anti-phase magnetization as we know is not observable. So, we evolve further $\frac{1}{2J}$period once more. So, it is converted into in-phase magnetization and after that you collect the data on the proton channel and you decouple the *X* nucleus by broadband decoupling. So, the product operator calculations can be done as we have discussed this quite extensively.

So I am only going to tell you about the salient features of these calculations which are relevant for this. Now, let us look at this $2 H_x X_y$ and ofcourse you notice here what I given here is the phase cycling *X*-*X* for this and *X* -*X* for this and when you record the data the first scan you add it and the second scan you subtract it. So, it is plus and minus. So the reason for that we will see soon.

Now, first let us look at what happens here. We have got here relevant product operator turn which is *2 Hx Xy* what is this? You recall the discussion of the product operator this we know $2H_xH_y = DQ + ZQ$. As, double quantum goes $DQ = \omega_H + \omega_X$ and the zero quantum should go as $ZQ = \omega_H - \omega_X$.

Now, we notice in the middle of the t_1 period we apply 180 pulse on the proton channel therefore from each one of those this frequency is refocused. So till the time here and we come up to this point at the end of the t_1 period the ω_H frequency in the double quantum and in the zero quantum is refocused. So what remains is only ω_X therefore effectively during the t_1 period I only have the *X* frequencies and *X* frequency the operator term and of course they will evolve with the characteristics frequencies.

The second thing is both this double quantum and zero quantum frequencies do not evolve under the coupling between *H* and *X* if you are considering it 2 spin system heteronuclear 2 spin system. For example, the CH group or NH group where we have created the double quantum and zero quantum using the one bond coupling the *J* is a one bond coupling here it does not evolve under the coupling constant.

Therefore, at the end of this t_1 period there is no coupling evolution contribution there will only be chemical shift evolution okay at this point for the 2 spin system. Therefore, this operator term does not change process there is no coupling evolution this we will remain as $2H_x X_y$ or you may add $H_x X_x$ if you are considering chemical shift evolution it can also cause another term which is X_{y+x}.

So, but we consider one of this here it does not matter which one you consider that is you can do that by phase cycling and you get here $2 H_x X_y$ and then you will have a function which is ft 1 which will depend on the chemical shift evolution during this period t_1, okay. When you apply a 90˚pulse on to the X channel now once more this will get converted into $H_x X_z$ because this is Xy here when you apply 90x pulse that will get converted into Xz.

So now you see it is proton magnetization, here it was double quantum and zero quantum coherence. Now, it is proton magnetization, single quantum proton. Therefore, I have here now I come back here. So it is proton magnetization, anti-phase proton magnetization. Now when you evolve it further now under the influence of the coupling between the two this will evolve under the coupling proton x coupling and it will evolve into Hy to Hx Hz will evolve into $H_y f(t_1)$ will remain there.

Now, you know this H_y is now in-phase magnetization of proton. Therefore, we can actually collect the data and decouple the X during this acquisition time, okay. So now therefore what we have achieved? We have only chemical shift information in the t_1 period. Therefore if when you do two dimensional Fourier transformation I have along the F_1 axis X chemical shift and then here I have the proton magnetization here it evolve with the chemical shift of the proton and there is no coupling.

Therefore, I only have along the F_2 axis the proton magnetization. Therefore I have peaks here very similar to those we observed in the HSQC spectrum. So, one peak and no fine structure here for each HX pair, okay. So this therefore spectrum will appear very similar to the HSQC spectrum. There will be some other factors which we will consider and that will happen when you have more complex spin systems.

Like if the proton is coupled to some other proton that proton will evolve the coupling between the two protons will evolve here during this period. Although the chemical shift of the proton is refocused but the proton -proton coupling evolution will happen in case of more complicated spin systems. That is you have one proton and another proton things like that in

the proton-proton coupling as all. All that will enter into this $f(t_1)$ and that can lead to some other complications in the spectra.

Now the second thing will happen is if you are doing it experimented natural abundance. So, you see the proton is attached to every carbon all those carbon or the nitrogen which is evolved. But the carbon natural abundance is 1.1 percent and 15 abundance is 0.37 percent but you are going to excite all the protons which are attach to carbon 12 as well.

So, what happens to those one? Those will not get transferred here because there is no coupling evolution for those ones and therefore they will continue to evolve like this and now you see this is the entire period this 180 pulse is in the middle of the period here from here to here. So therefore, this will get refocused at this point.

Now, if you add and subtract as you do in receivers phase here, one scan you add, other scan you subtract and that magnetization which is coming from directly through without passing through this channel we will get cancelled out. So therefore, that is the reason for this phase cycling here and this does not affect the magnetization which comes from this pathway.

And therefore, you have to do this sort of phase cycling to eliminate magnetization which is coming from the ^{12}C. If you have molecules which are carbon13 label then it does not matter anyway everything will pass though this and it is not affected by this phase cycling you will get a clean signal, okay.

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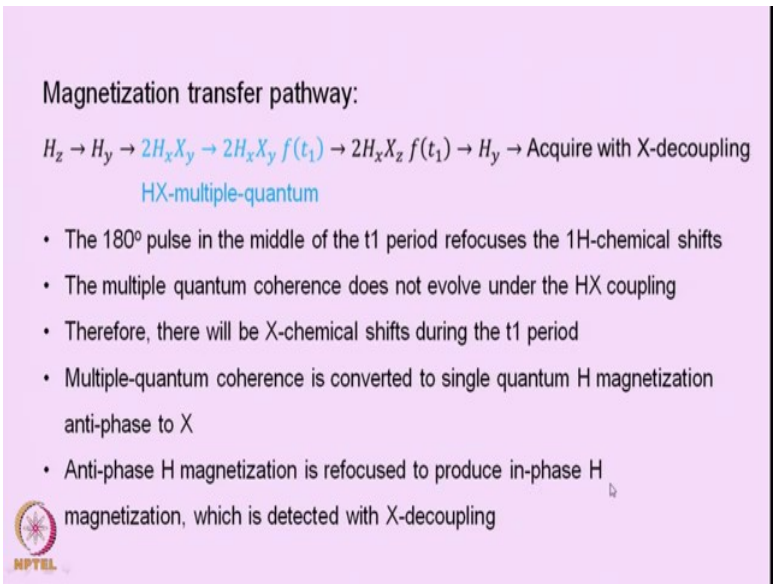

Now so therefore, let us look at the magnetization transfer pathway once again in more explicit term, you start with the proton magnetization H_z converted into H_y and evolve it during the $\frac{1}{2J}$ period and the relevant part of the product operator which is important for us and other things do not contribute and that will be $2H_x X_y$ which are said is multiple quantum which is a summation of zero quantum and double quantum they will both eventually be retain in this manner during the t1 period because it do not evolve under the proton X coupling, this are one bond they do not evolve under.

So, this operated term remains as it is and then when you apply a 90 pulse on the X channel you convert it into proton magnetization which is anti-phase to X and then during the next $\frac{1}{2J}$ period you refocus this into in-phase proton magnetization and then after that you can acquire with X decoupling.

So, here are the salient features repeated once more the 180 pulse in the middle of the t_1 period refocuses the proton chemical shifts, multiple quantum coherence does not evolve under the HX coupling. Therefore, there will be X chemical shifts only during t_1 period. The multiple quantum coherence is converted into single quantum magnetization anti-phase to X then anti-phase proton magnetization is refocused to produce in-phase proton magnetization which is detected with X decoupling.

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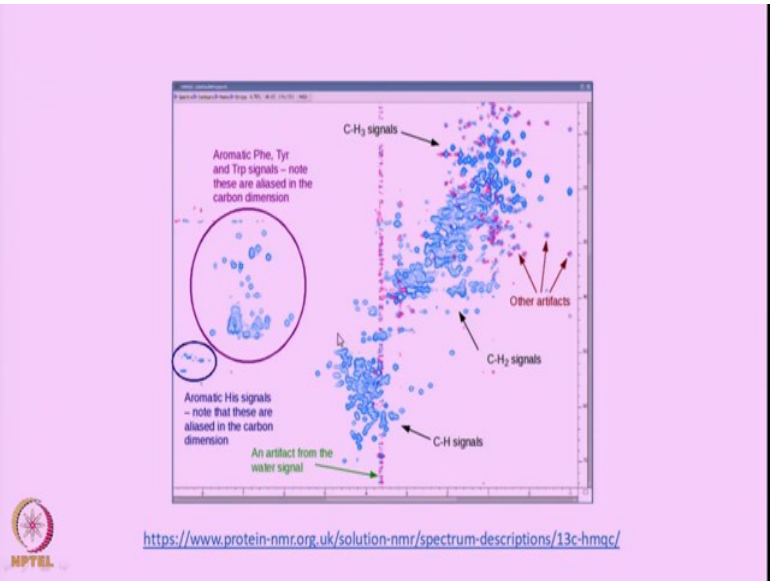

So, here is an experimental spectrum of a particular large molecule which is a protein. So, here on this axis you have the proton this axis you have the carbon here and see you can see lot of crowding of the peaks there look all the peaks are seen here this are the CH_3 signals here and this are the protein. So these belong to the methyl because the methyl protons appear at this chemical shift and the carbons of the methyl appear at this chemical shift. And therefore, this is the CH_3 signals.

And here you have the Υ protons and the β protons of the side chains in the proteins of the amino acids those one appear here in the protons chemical shift and the carbon chemical shift occurs here. So this is up to 20 ppm here, this is between 20 to 40 ppm you have this β and the Υ chemical shifts and ofcourse you also have the δ chemical shifts which are coming here, the Υ and the δ and the side chains of the amino acids they will come here and the glycine α also appear at this point.

Which is around the forty to 40 to 45 ppm and then in this place you have the α protons and the betas of the serine's they will appear in this area, the carbon chemical shifts will be in this between 50 to 65 ppm you will have the CH signals of the protein alphas and the β of the serine's and they will also appear in this area and this are coming from the aromatic signals.

Notice however that the aromatic carbon chemical shifts appear between 100 to 120 ppm but they are appearing in this area and this is because you allowed them to fold into this area, you are not excited the carbon chemical shift all the way from 0 ppm to 200 ppm, you have restricted the carbon excitation here up to 70 ppm only. Therefore, the ones which are outside of that one will get folded into this area.

And therefore they are coming in this region. You identify them on the basis of the proton chemical shift. The proton chemical shifts are between 6 to 7.5 ppm and therefore you know that these are coming from the aromatic carbons and not from the aliphatic carbons, okay. So but this is fine we can still analyse this. So the reason why you choose only a small chemical shift range here is because you improve the resolution, your spectral width is reduced, so the acquisition time can be increased along the F_1 dimension .

So they have better resolution along there F_1 dimension and this are particularly important in case of carbons because the carbon chemical shift range is quite large and generally it is not possible to excite the wide chemical shift range with simple hard pulses. So the one special

tricks that be used, we need high power and all of those complications. So therefore, you allow them to fold. So, you can see analyse even the folded peaks here as well.

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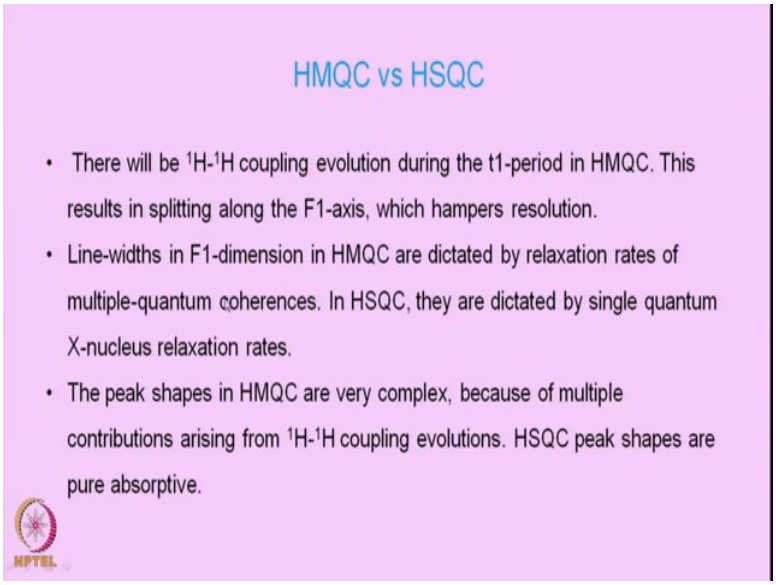

Okay, so now let us do a comparison of the spectra from HMQC and HSQC.

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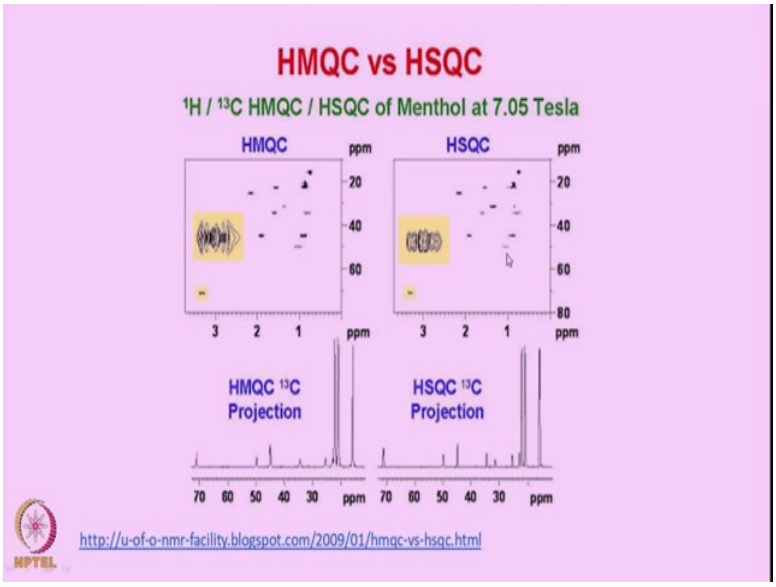

They look similar but there are some differences between the two. This is from a particular molecule this is HMQC spectrum and this is the HSQC spectrum by enlarge look very similar but look except for this area this one does look very different, okay. So these are blow ups here and what it shows is that the resolution in the HMQC appears to be smaller than the resolution in the HSQC spectrum.

And this happens because of the proton-proton coupling evolution during the t_1 period which I mentioned to you. There when the complicated spin systems are there it is not just a HC

group or suppose it is a CH_2 group or CH_2-CH_3 kind of a group where there is a proton-proton coupling evolved. So every proton which is present here which is at the carbon chemical shift here will be coupled to some other proton and that proton-proton chemical shift leads to additional operator terms in your $f(t_1)$ function and that leads to sort of complications in the F_1 dimensions.

So, this leads to loss of resolution in this area and this ones are the projections along the carbon axis. So you take projection like this and this is the projection of the spectrum from the HMQC and this is from the HSQC. By enlarge the resolution here is better although you cannot see it is projection in the 1D. When you blow them up and see as it is done here you can see there will be a improvement in the resolution in the HSQC and no complications from the proton-proton coupling. So typically therefore, when you have complex spin systems one wants to record an HSQC spectrum.

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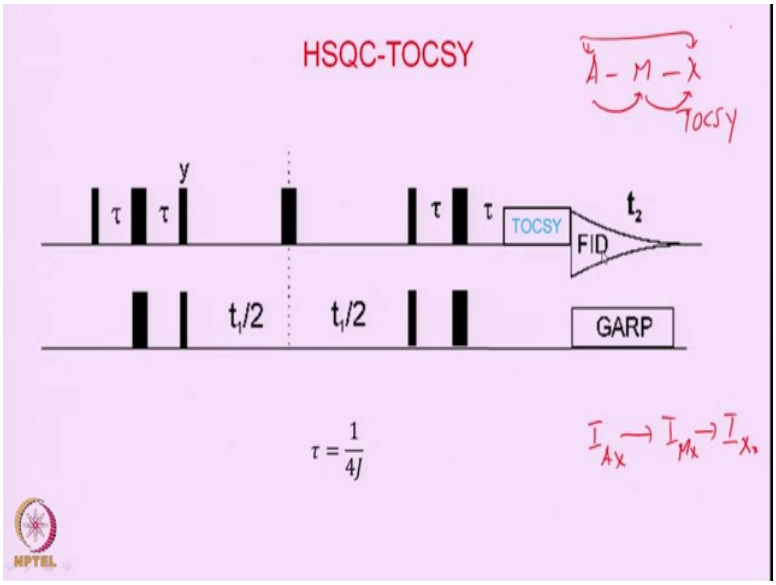

Okay, now we consider different ways of correlating protons and the *X* nucleus but now we can combine further these experiments with other proton-proton experiment to get more information on the molecule which you are having. Say for example, here HSQC is combine with the TOCSY spectrum. We discussed about the TOCSY and the TOCSY is kind of a relay experiments in the homonuclear case.

So, proton magnetization is transferred through the coupling network in the TOCSY just to remind you what we discuss in the case of TOCSY. So if you have an *AMX* spin system magnetization is transferred from the *A* to the *M* in the TOCSY. So, if you have a proton step

of *AMX* and each one of the maze ofcourse attach to a carbon. Now in the TOCSY you remember that we had the transfer of coherence from here to here and then from here to here.

So this resulted in a cross peak between this two. So that is the relay. So the relay that happens is used now to correlate the *X* nucleus chemical shift to the whole set of protons which are attach in the coupling network. And how is this experiment done? It is very simple, okay let write this is the TOCSY. So this is just to remind you because HSQC is a fresh in your mind TOCSY may not be fresh therefore I just put that here there.

Now, this is the HSQC part all the way from here to here is the HSQC part, right. So this is the INEPT transfer to begin with from proton to the *X* nucleus $\tau = \frac{1}{4J}$. So $2\tau = \frac{1}{2J}$, so with that adjustment magnetization is completely transfer to the *X* nucleus and this is anti-phase here and this magnetization evolves during the t1 period and there is 180° pulse supplied in the middle.

So that there is no coupling evolution between the *X* nucleus and the proton that is decoupled there and then from here the magnetization transferred back to the protons this is anti-phase protons magnetization with respect of the carbon or the *X* whatever is the *X*. And then during the next two τ period the anti-phase magnetization is refocused into the in-phase magnetization. Now you recall that the TOCSY transfer happens from in-phase to in-phase.

So, here if I have the magnetization of *A* spin *X* here and this will be transferred to magnetization of *M* spin into the x magnetization only and this will also go to the okay I said *X* there and this is also *X* here *X*, *X*. Okay if it where *Z* then ofcourse I_{AZ} will go to I_{MZ} between I_{XZ}. So therefore, in the TOCSY there isotropic mixing and that leads to in-phase magnetization gets transferred to the connected nuclei

So here therefore, if you started from one particular proton let us say a *A* proton is couple to a particular carbon and you will come here all the way up to that particular proton magnetization once more and then during this period you relay it from *A* to *M* and *M* to *X* and then after that you collect the FID.

So, therefore you have created transverse magnetization of all the 3 spins here which coupled to each other. And during that one all since all of them are in-phase magnetization we do a broadband decoupling of the carbon or the *X* nucleus then you will have the relay in the along

F_2 axis and that is an important information you can identify spin systems on the basis of such a kind of a magnetization transfer.

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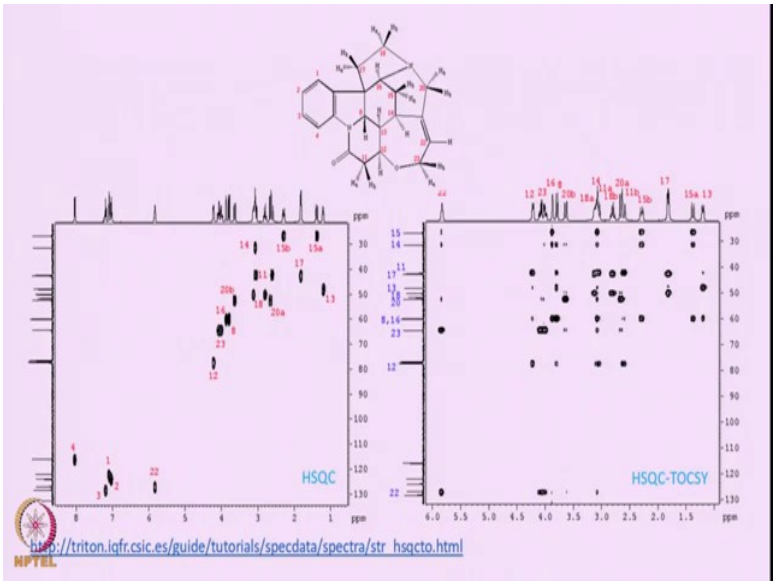

Okay, so here is an example. So you see here this is the normal HSQC spectrum for a particular molecule. The molecule is shown here and you see here this is a carbon proton correlation spectrum so you have here 2 peaks and this is 2 carbons there are 2 carbons here and they are connected to the protons at the respective chemical shifts or this particular carbon is connected to 2 protons and this are one bond correlations.

So you are seeing from here to here this is both the 2 protons on a particular one and then you will have here similarly the particular carbon connected to 2 protons which are non-equivalent you will see two peaks here and this is another carbon coupled here and one another carbon. So therefore these are all simple one bond correlations between the carbons and the connected protons, then what happened here? Now this is the corresponding TOCSY spectrum, HSQC TOCSY.

Therefore, magnetization is transferred from this carbon to this proton or to this proton and then from this proton it is relate further to other protons which are located on another carbon not on the same carbon this two proton are located on the same carbon. And therefore, you got them at the same carbon chemical shift this. Now at the same carbon chemical shift because of the proton- proton relay that happens through the TOCSY there is a relay to proton which is on another carbon.

Therefore, you will see this one will go to these protons here which are connected a different carbon and that will show up here as well and where they are present for example this one here you can see there is a carbon here and this another one is appearing at this point. So this one for example this carbon is appearing at this point and that shows relay to these 3 protons okay here there is only 1 carbon 1 proton but this proton is coupled to other proton, 3 other protons there that is this one here, right.

This one is, this one here and it is connected to other protons which have this chemical shift. So which are this proton chemical shifts here. So, this is how you established a network of coupled spin in a molecule it will allow you to identify the resonances in unambiguous manner.

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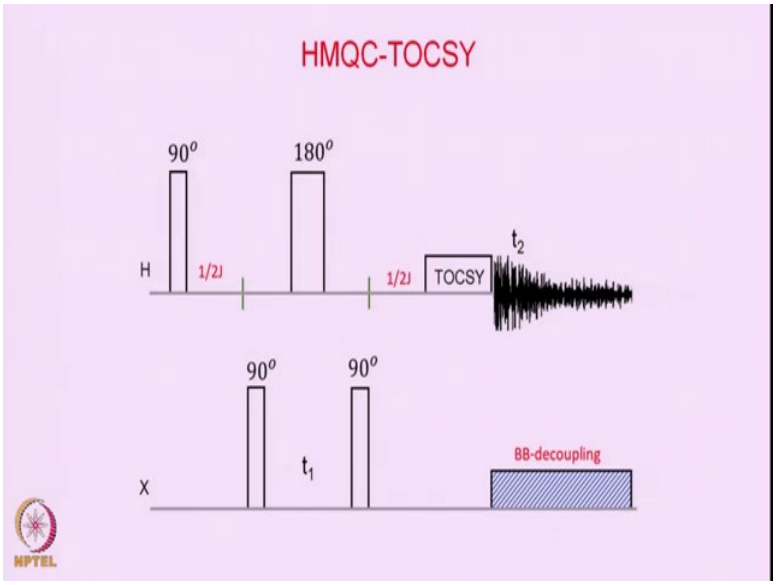

Okay, we can do the same thing with HMQC, HMQC also allows the same thing to be done. So, you go through the enter QC spectrum until this point and introduce the TOCSY block. If the TOCSY block here allows you to relay the magnetization through the proton coupling network and to collect the data here proton magnetization and do the decoupling. So, the spectrum will look very similar except ofcourse for the proton-proton coupling complications which happened as I indicated in the HMQC spectrum.

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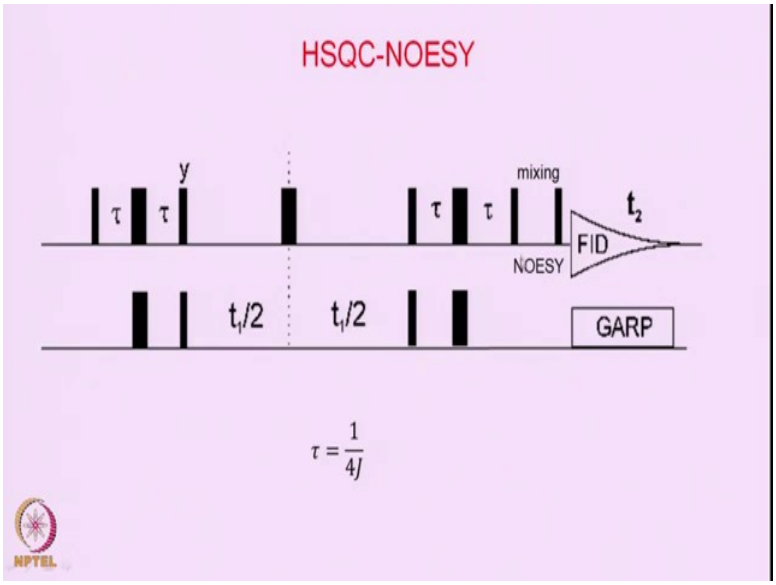

Now so, we you can also introduced the NOESY, okay. So now, what will you do is up till here the pulse sequence is the same as HSQC, right. So, this is HSQC from here you come up to this and which is the NOESY. So at this point therefore I have the magnetization of the protons but now this is along the *Z* axis. When I apply the 90˚pulse here I put the magnetization along the *Z* axis.

So now, during this time period this is a mixing time during this mixing time then I have relay of magnetization from a particular proton to another proton which is close by in space and this happened through the dipolar coupling. Therefore, this is a NOESY mixing. So earlier it was *J* coupling mixing in the TOCSY now here it is a dipolar coupling mixing.

So, there is a relay of magnetization from this *z* of one particular proton to another proton and then after that ofcourse you have at this point both the *z* magnetizations of if I take 2 protons *A* and *M* then I will have here relay I have the mixture of I_{AZ} and I_{MZ} which the relay has happened through the dipolar interaction and through the mixing period and then ofcourse you collect the data since it is *z* magnetization this last 90˚pulse converts *z* magnetization into transverse magnetization.

And so, that you can generate in-phase transverse magnetizations and you collect the data here as an FID and this will contain frequencies of both of the spins. And you decouple here since it is all in-phase magnetization you can decouple. So, and then all other parameters remain as they are in the HSQC mixing time you cannot optimize to get what information

you want in your spectrum. How much relay you want to do and you can optimize the mixing time and intensities it will get affected accordingly.

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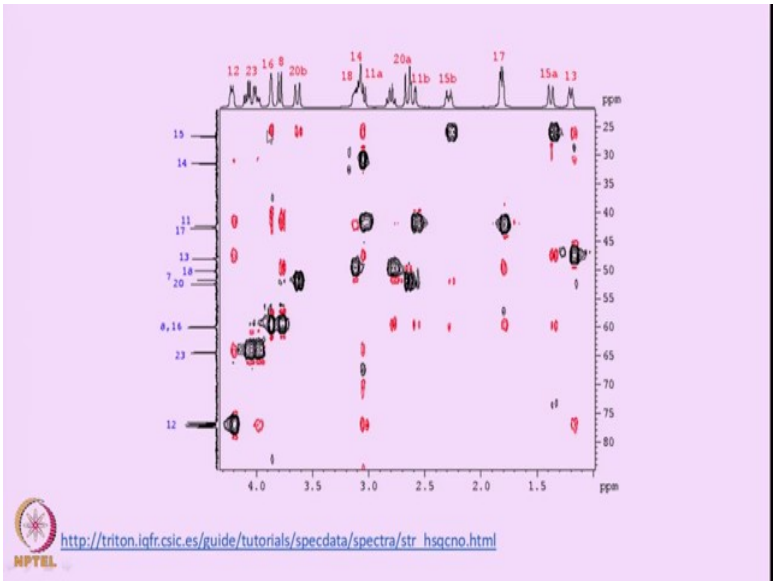

Okay, so here is an experimental spectrum incidentally if when you are working with the small molecule the correlation peaks and NOESY peaks will have opposite signs and it is very helpful because you can then identify which are peaks which are coming from NOESY and which once are coming from the normal HSQC correlation.

So, all this black things which are present in this experiment they all are coming from the normal HSQC correlations proton-carbon correlations and then this red once which are present here these ones are coming because of NOE transfer this is proton-proton NOE transfer and they appear with the different sign. And therefore, it becomes very easy to figure out which protons are close by in space and that is an important information for structure determination of small molecules.

If you have a large molecule ofcourse this situation will change in large molecules the NOE you will have an opposite sign compare to that for the small molecules. Therefore in that case this one also appear with the same sign as the direct correlation peaks.

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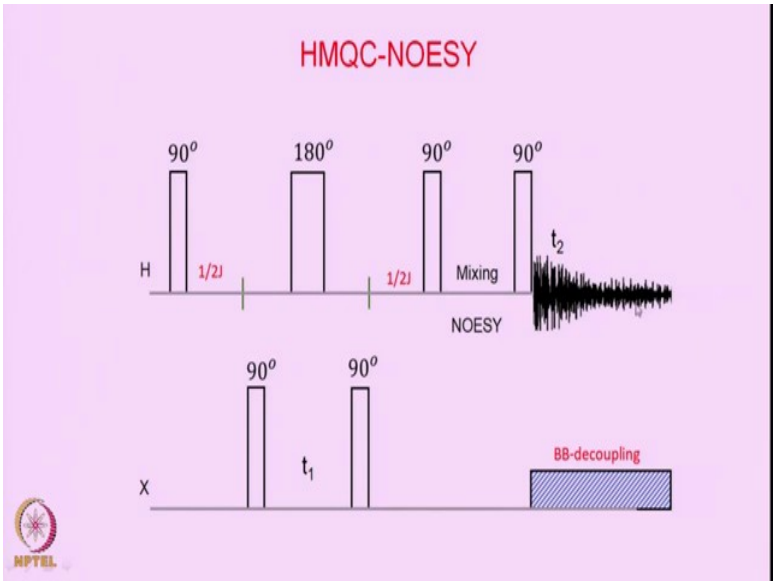

Okay, same experiment can do with the HMQC NOESY as well and the same block is introduced here until this point it is HMQC and you apply 90˚pulse here to convert that magnetization into the *Z* axis and once you put in the *Z* axis then you can do a NOESY mixing at this point. Then you will have the last 90˚pulse to convert the *z* magnetization back in to the transverse magnetization you collect the data and then you decouple the *X* nucleus.

Okay, so I think we have covered here important heteronuclear experiments and also we have shown to combine this heteronuclear correlation with proton-proton correlations to extent the information content of the two dimensional spectra. You can relay the magnetization within the proton network either using the TOCSY scheme or using the NOESY scheme and you can combine it with the heteronuclear correlations either through HSQC experiment or through the HMQC experiment. So this will be extremely useful in obtaining resonance assignments in small molecules, big molecules and likewise. So, I think we will stop here.