**Principles and Applications of NMR Spectroscopy**
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**Module 06**
**Lecture No 29**

In the previous class we saw the experiment called 2 D HETCOR which was basically starting from hydrogen start from hydrogen magnetization you evolve the hydrogen magnetization and you capture that as Omega H T 1 you transfer the polarization to carbon and detect the carbon polarization or magnetization which is denoted as Omega C T 2 . So, when you do a Fourier transform you get the proton on the Y axis and the carbon on the X axis but we saw that the approach is not really the best approach the reason for this is shown here.

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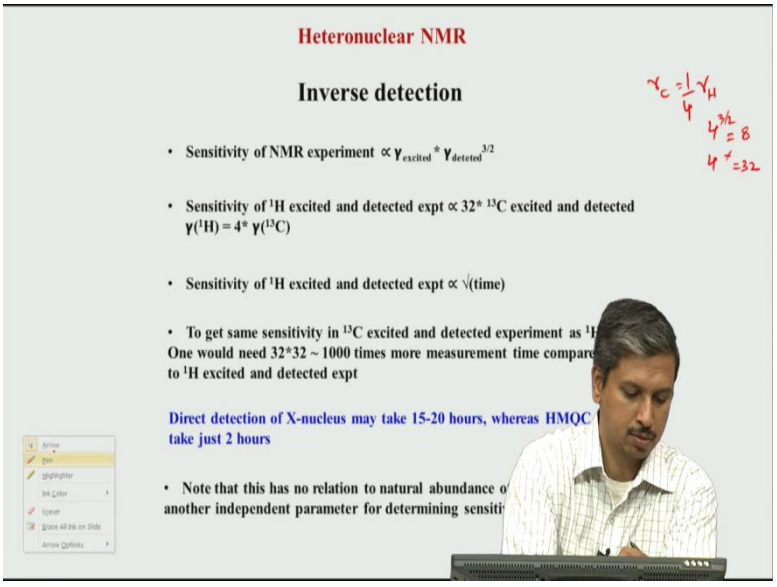


So, we see generally the NMR experiment, the sensitivity of NMR experiment is given like this it is proportional means there are other factors, but one of the factors is this, that it depends on what is the first nucleus that you excite that means in remember we have been seeing all the Y that you have been exciting protons with an RF pulse. So, the Gamma of that nucleus matters and what matters is the what nuclei is detected in the end and in the proton-proton experiment both of these were hydrogens. But in the HETCOR experiment this was hydrogen, but this was carbon okay and carbon is 4 times less compare to proton, so obviously now we can see that if I have both if I want to maximize by sensitivity I should use both hydrogen as excited nucleus and also detect my hydrogen not carbon that would give me

the maximum sensitivity possible. So, let us see for carbon how bad the sensitivity is if you detect carbon.

So, this is what is shown here that if I used proton as excitation but detect carbon there is a 4 times less. So, actually if you look at both carbon and proton excitation, so this is 1 by 4, if I use carbon and this is 1 by 8 if I use carbon. So if I start from carbon excites carbon and I detect carbon, then my sensitivity will be 32 times less compare to if I start from proton and end with proton.

So, let us see again how this comes above, this is coming from this is same equation, so Gamma of carbon is 1 by 4 of gamma of proton so 4 raise to 3 by 2 is basically 8 and in the first one is 4. So if I multiplied these two I will get 32, so what it means is that if I have both carbon as excitation and nucleus and detection also as carbon, my sensitivity is going to be 32 times less compare to hydrogen. Now, if I excite with proton with detect with carbon which is we was (())(3:19) on HETCOR even then there will be a factor of 8 because this will be one that is excitation is one because I am this hydrogen, but if I detect carbon I will get 8 times less sensitive compare to what I would get if I also detect proton. So, therefore as you can see proton excitation and proton detection is always the preferred way to go above okay.

Now the question is why is the sensitivity gain? How does it help us in real practical life, the reason is a following. That suppose I increase that I sensitivity by a factor X, where X is 32 I I have to I save a time by square of that so which is shown here, so we remember this is something which we saw in the earlier in the class first part of the course where we looked at how sensitivity is determine in NMR and we saw one of the factors was a sensitivity is proportional directly to the square root of time. So, if you do now, so let us say that I have a sensitivity let us say I have a two experiment suppose let us say I have two experiments, experiment number one so let us go back to this experiment number one which has a sensitivity let us use a word sensitivity of 10 and I have experiment number 2 or I want the same experiment I called it one prime and I want a sensitivity of 20.

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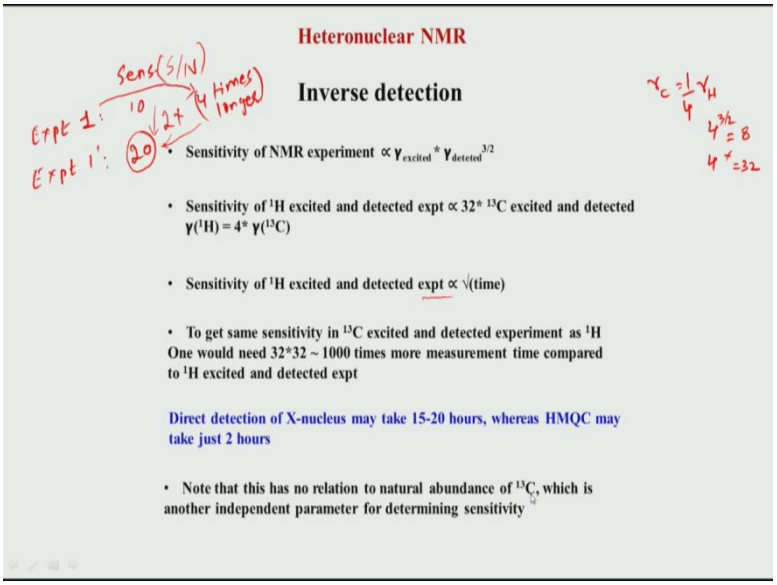


So, what is this written here, so what we are trying to see is here is that I have an experiment number one which has let us say a sensitivity of 10 remember sensitivity is basically signal to noise okay. We will quantify there are different ways to quantify sensitivity, in this course we will always use signal to noise as a measure of sensitivity. So, if let us say my spectrum number one or experiment number one has a sensitivity of 10 that is a signal to noise is 10 and I want to improve the sensitivity and I want to get a signal to noise of 20, so how do I do that? So that means I need a factor of 2 texts, 2 times I have to increase the sensitivity. So according to this rule which is shown here that experiment is the sensitivity is proportional to square root of time it means that I have to run this experiment 4 times longer square okay. So, only if I run this experiment number one, 4 times longer than it will become sensitivity equal to this 20.

So, if I want to increase the sensitivity by X, I have to increase the time by X square, so that is basically the philosophy in all NMR experiment in general that the sensitivity to get an value you have to run experiment suppose you have to go from 2 to 4 that means if you want a factor of 2 gain in sensitivity I need to run it 4 times longer. So, now if you come back to this here if I need to get 32 times more sensitive, remember carbon is 32 times less compare to proton. So, if I want to achieve is the same sensitivity as proton I need to improve my sensitivity by 32 times. That means I have to run the experiment 32 into 32 times longer, if I want to get achieve the same sensitivity for the same sample at the same magnetic field for for a same signal to noise. So, 32 times longer is roughly 1000 times longer 32 square times sorry square of 32 times I have to run longer and that is 1000 times longer.

So, which means that if I want if I use a carbon and proton sorry proton detection and I use a carbon excitation and carbon detection that sensitivity of that experiment is 32 times less which means 1000 times more time is required. So therefore, sensitivity plays a very important role in NMR because of this square factor that means if I want to increase sensitivity by factor of X, I need X square more time to get that, so that is why is very important to minimize the time and where increase the sensitivity or if I increase sensitivity I reduced the time vice a versa. So, this is what is shown here that to get the same sensitivity in an experiment where C 13 is excited and detected and if I want the same sensitivity as a proton 1 D spectrum I would need to record 1000 times longer that is why carbon 1 D NMR experiments are recorded for a longer time generally compare to protons.

So, this is you will see typically in all spectrometers that if you want to get a carbon 13 1 D spectrum we use much longer times because of this reason. Therefore it is important to record everything in a short time, so you see here this is a example that typically suppose I want to take carbon 13 spectrum we usually record about 15-20 hours whereas, 2 D experiment may simply take two hours, so this is what is the trying to get in inverse detection, in inverse detection approach what we do is we directly go for a proton to proton excitation means I start from proton and I end up also in proton. If I do that then may sense time factor I can save a lot of time because now the proton is most sensitive nucleus. So, obviously starting from proton and detecting proton is the best approach.

So, how do we do that? So that approach is known as inverse detection, when we go to 2 D hetronuclear NMR. So, let us see how that can be implemented that let us before going to that you can see here the sensitivity which is we are talking about in terms of time as nothing to do with a natural abundance. So, this is something which uh many times is being confused people think that carbon 13 is less sensitive because of the Gamma or because of natural abundance.

One should keep in mind that both are important, so if I take care of here I am taking care of only the Gamma part. That means I am improving the sensitivity only by looking at the Gamma values. I am not taking care of the sensitivity which comes from the abundance. So, the abundance problem will still remain because carbon 13 is less abundance, but at least I have taken care by using the proton this inverse detection takes care of only the Gamma portion that is improve in sensitivity by exploiting the higher gyromagnetic ratio of proton.

So let us see how now a inverse detection experiment can be implemented or design this is shown here.

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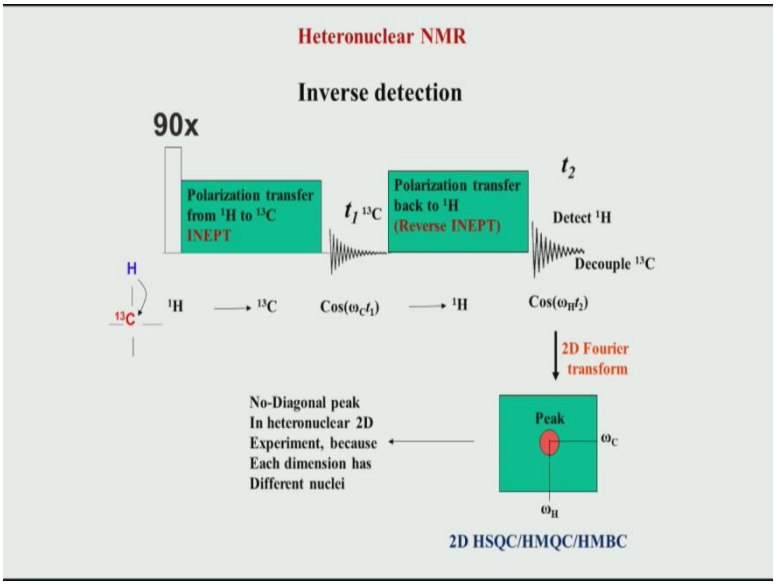


So, we start from a hydrogen RF pulse on the protons which is the maximum sensitivity. So, we start from proton we did apply the pulse on proton, but then instead of directly evolving as we saw here in the previous example we immediately transfer whatever has come to the X-Y plane in this side here by polarization transferred to carbon. So, basically we directly transfer to carbon we using this INEPT approach. So, INEPT approach as I said we will now denote it as a box here we will see the exact sequence of pulses in the next few slides. So, once we transfer the polarization from proton as soon as we apply the pulse to the carbon, the carbon has come to the X-Y plane and of course it has not come we apply a pulse for the carbon which is part of this block.

Then carbon starts evolving because of the chemical shift and that is what is denoted here that during the period T 1 because now the carbon is in the X-Y plane the chemical shift of carbon is present here and that is now captured as T 1. But the sensitivity is higher now, why is it higher because the polarization has come from hydrogen. Once the T 1 evolution period is over after sometime remember we do not as a pictured denotes here it is not that we way till this gets over it is that we stops somewhere in between. So we have to this is the standard principle of 2 D we evolve for some time and then transfer the polarization to the proton.

So, what we are doing now, we are doing it going it back to the proton. So basically you can think of it like this that a person has 1000 rupees and another person has 250 rupees, the

person who has 1000 transfers the money to the person having less money and the person was now less money becomes richer by 1000 rupees, he utilizes it for some purpose and then gives it back to the original person, so this is like that, so we are loaning the magnetic polarization to the carbon, the carbon evolves with it is magnetic chemical shift, then the loan is written back to the proton and polarization is transfer back to the proton again using in the INEPT, but this we called it as reverse INEPT which is just a reverse of this okay. And this is a also INEPT concept but is a reverse and then proton has now got back it is polarization and it will now evolve with it is chemical shift value.

So, you can see here we have starting from proton and ending with proton, so it is a proton to proton detection and excitation but in between we have got carbon okay. So, we will see mathematically have now these two get connected. Now one thing is when we finally detect the hydrogens, protons now we have to decouple the carbon. This is a reverse of what we saw in the previous class, in the previous class we saw that we detect carbon but we decouple protons, here it is the inverse approach that is why we use the word inverse detection.

In inverse approach the proton is detected but the carbon is decoupled again the same idea why need do you need to decouple the proton from carbon that is because protons are couples to carbon and therefore there will be J splitting if I do not decouple and therefore I need to record with decupling the carbon. Now if you go to this let us see mathematically how what happens. So, we have a proton which is attached to carbon, so we excite that proton then we transfer the polarization to carbon 13 and as shown here the carbon 13 evolves with it is chemical shift value, which is now Omega C into T 1 and then we transfer this polarization back to proton like this and what finally evolves is a chemical shift of proton in the direct dimension.

This is called direct dimension this is called indirect dimension, so indirectly the chemical shift of this is captured in finally in the FID. So, now we have basically correlated that 2 chemical shift because remember the final FID is a combination of this component and this term. So, basically we have two terms interconnected, intermodulated each other and if you do a Fourier transform 2-dimensional because I have 2 axis, so now I will get Omega C in the Y axis which is this Omega C is because during T 1 and Omega this is a 2, F 2 dimension is now basically coming from proton okay. So, you see because of this factor here the second dimension is Omega H is because of during T 2 Omega H evolves, but any hydrogen which is connected to carbon only those two hydrogen carbon like this shown here only those carbon

and hydrogen pairs which are connected to each other by J coupling that is by bond only those two proton and carbon pair will show a cross peak.

So, that means this cross peak what we a see in NMR spectrum in H like this 2 D inverse detection will be only those peaks where the protons are connected to carbon. So, if there is no proton attached to this carbon example carbonyls, they will not be coming in this spectrum. So in a 2 D heteronuclear experiment we cannot expect to see a carbonyl directly in a unless, there is a long range coupling means suppose there is a hydrogen somewhere away from here and that is coupled to carbonyl yes they that may show off if there is a coupling.

So, you basical basic requirement is that the hydrogen to carbon what we have transferring the polarization these two should have an interaction between them through J coupling. Remember we are not using the through space interaction here but through bond interaction, so they should be coupled by J coupling. So, this is the basic idea in any inverse detection experiment, so let us see based on this what are the different types of experiment which we are going to see in this course which are known.

So, typically the words we use is HSQC, HMQC or HMVC, so let us see that in this slide in the next slide. So, here before we move on a important point here to notice that you see what has happened here if you look carefully at this terms you see there are one is carbon and other is hydrogen, but if you recall in the homonuclear case we had proton and proton and it was a same proton twice because as I said last time you cannot transfer complete polarization from one hydrogen to another hydrogen.

So, therefore must most of it remains with it is own and that ends up as a diagonal peak. This is what we saw in the previous class but in this here in this type of experiments you see what is happening is we have transfered the magnetization from proton to carbon, so this using this INEPT. So, when we do that INEPT has 100% efficiency, if I know the coupling value, okay, so typically I mean it is not exactly 100 one can never achieve 100% but close to 100 so let us say more than 90%. So, that efficiency can be achieved in an INEPT again as I said we are not going to go into the details of how that works, but that is possible to achieve almost a close to 90% efficiency. So, therefore nothing remains on carbon everything nothing remains on protons everything is transferred to carbon an everything is transferred back to proton.

So, therefore there is no proton-proton evolution here, that means this axis is always carbon and this axis is always proton, so when these two are very different in frequency remember proton frequency let us say is 100 megahertz carbon is something else. So there is no concept of diagonal here, diagonal is only when the two frequencies are same in T 1 and T 2 but there is nothing like this here there is a completely different frequency completely different frequency, so 2 D heteronuclear NMR spectrum does not contain diagonal peak and this is very important point which you should keep. But remember in 3 D we can get again a diagonal, so whenever any two axis are proton-proton, then you can expect to get a diagonal. But whenever there is a carbon proton or proton nitrogen or any proton X nucleus or any X and Y nucleus correlation there is a no concept of diagonal peak because the 2 nuclei are different.

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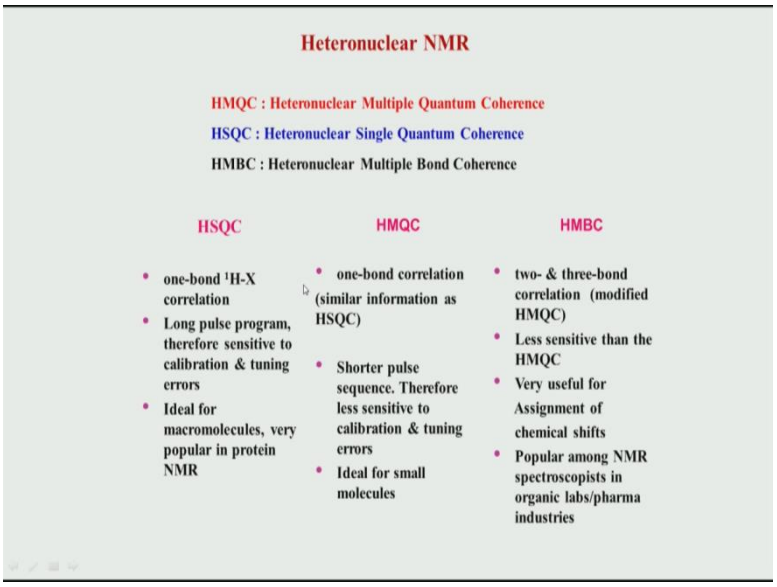


So, now let us go to this list of experiments that we will see in this course, we will look at basically three types of heteronuclear experiment. There are varieties of heteronuclear experiment in the literature in NMR in general, but we cannot go into all the 2 D heteronuclear experiments in this course therefore, we will look at only the most important once which have very routinely used in chemistry and biology for a molecular structure analysis and these are listed here.

We will see the acronym they are acronym for HMQC it stands for Heteronuclear Multiple Quantum Coherence, HSQC Hetero Nuclear Single Quantum Coherence and HMVC is a slight variation it is Multiple Bond Coherence. So, what are the advantages and disadvantages of these experiments, so we can go through this here what is a comparison where do you use

what? So, as a thing written here this is a one bond to proton to carbon correlation, which means that any two proton and carbon pair only if they are directly connected by only a single bond okay will be detected in this experiment in this spectrum okay. So, we will not get any correlation for example from carbonyl because in carbonyl there is no direct attachment of carbon to hydrogen. So, only those carbons which are directly attached to a hydrogen will show a peak or a cross peak in this spectrum.

Same is the case here, so we that is why it is HMQC and HSQC are very similar to each other in terms of the information, in fact they give the same information. We will see that in a experimental spectrum down the line, so this is one major difference. But if you look here HMVC is the slightly different experiment, there you get correlation other way round, you do not get correlation if they are one bond.

So, the idea is the one bond correlation is the suppressed in HMVC, because one bond information any way comes from HSQC, so there is no need to actually do an HSQC, so we suppose I want to get information of a longer correlation suppose I want to get carbonyl information and carbonyl is not directly attached to a hydrogen. So it is little bit away, there would be at least a 2 bond and 3 bond suppression between a proton and a carbonyl and that information that is the protons which chemical shift which is correlated to a carbonyl this captured in this experiment.

So, we basically look at a longer range coupling rather than a shorter range coupling, so we will see this as we go along, so that is the advantage of HMVC, so the idea is one bond any way comes from this experiment, so these experiment HMVC provides a little bit a longer correlation a bigger more information which is useful for structure. Now compare to these if the HSQC and HMQC are similar experiments, then why do we do one over the other the reason is here that HSQC as we will see is a longer experiments in the sense it does not it does not mean in a takes long time to record, what it means is that the whole experiments to execute in spectrometer that is a pulse sequence is longer compare to HMQC. So, therefore HMQC is more sensitive and for in that respect but it is less sensitive for some other purpose, so we will see that later.

So, HSQC is typically used for micro molecules means biomolecules and is very popular in protein NMR or in case of peptides. And HMQC on the other hand is ideal for small molecules that isorganic molecules and that is what typically is done in the case of drug discovery or for solving structures of small organic compounds. Coming to HMVC where is

it is used is useful again as we saw here it is similar in terms of assignment it is useful but it also helps to get the connectivity information which you do not get from these two experiments. So, as we saw here it is more for long range interaction information and other than short ways. So, this is a very popular experiment among spectroscopies and typically it is used in organic chemistry labs and pharmaceutical components.

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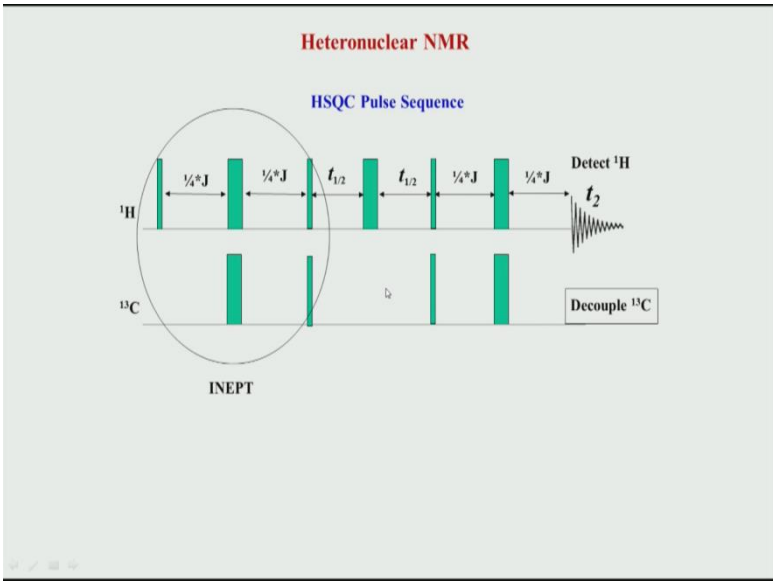


So, let us start from looking at what is an HSQC experiment this is what the pulse program we will look like, so this is a pulse sequence. So what will pulse sequence means, if you recall pulse sequence is the sequence of pulses which are applied in an experiment okay, the sequence of pulses and delays, it is not just the pulses but the delays are also very important. So, these values are very specific values and this whole scheme which is shown here together constitutes one experiment which we will call it as HSQC. So, as we saw here we will see more detail now as we go along, so basically the I you say inverse detection experiment meaning we starts from protons and do some things to protons and comeback to proton in the end and detect the proton FID.

So, let us see how that will look, so this is the pop portion in circle which is denoted as INEPT. So, if you recall in the previous slides we looked at what is an INEPT we shown showed INEPT as a block or a box more like a black box, but now here the actual scheme of INEPT is shown here. So, an INEPT again remember we will not be going to detail mathematical detail or how this works like a magic and achieves a transfer of polarization from proton to carbon, so that part we will not be able to go in detail or what it basically consist of a pulse 90 degree pulse applied on hydrogen, then you give some delay, delay

means a gap which just a ideal delay period and that value of that is given by this formula and then you apply a 180 degree pulse on both of them. So in this picture the fat the broad pulses are called are 180 degrees and short pulses are 90 degrees.

So, remember what is the function of a 180 degree pulse in NMR? It helps to invert the magnetization, so if you go from Z it can take it to - Z or if you go from X it can take it to - X. So, that is called when you take from a - X to + X or - Y to + Y we call it refocusing pulse, when it take it from Z to - Z, we use the word inversion pulse. So you have to, this is just more of a technical jargon which one can that an inversion pulse is just a 180 degree pulse, refocusing pulse is also 180 degree pulse but compare to the 2, inversion is meant for Z magnetization to be taken to - Z, but for refocusing it is going from - X or Y to - X + X or Y.

So, this is how we implement this whole INEPT than you apply one more delay, then there is a magnetization I mean a 90 degree pulse and both carbon and proton. So typically, in a 2 D NMR pictures if you literature and papers you will see like this that we show the top portion as hydrogen and the bottom portion as carbon. And why is it shown like this it is shown because this is a hardware if you go to the hardware details of this when you apply a pulses you apply on a proton channel okay. So, the dedicated dedicated amplifiers, dedicated frequency generators and dedicated preamplifiers etc, are dedicated for protons one set of them and another set will be dedicated to carbon. So, when you apply a pulse on proton channel it is shown like this on the proton line where as when we apply the pulses on carbon channel we will show it like this.

So, this is typically that diagram, so this is like a blue print is a blue print of the experiment which is conducted in a 2 D experiment. So, for an expert this is like all the information of the experiment is contain in this diagram of course this is not a complete diagram on it is own details are omitted here, which we are not going to go into that is the phase cycling and the values of delays here and some more details but overall this picture captures how this 2 D NMR experiment is implemented. So, we will go in the next class into detail of this and see how we can analyze this 2 D spectrum, how this mathematically the 2 D chemical shifts are correlated in HSQC look at some more for examples of HSQC of simple molecules and we will then look at HMQC.