One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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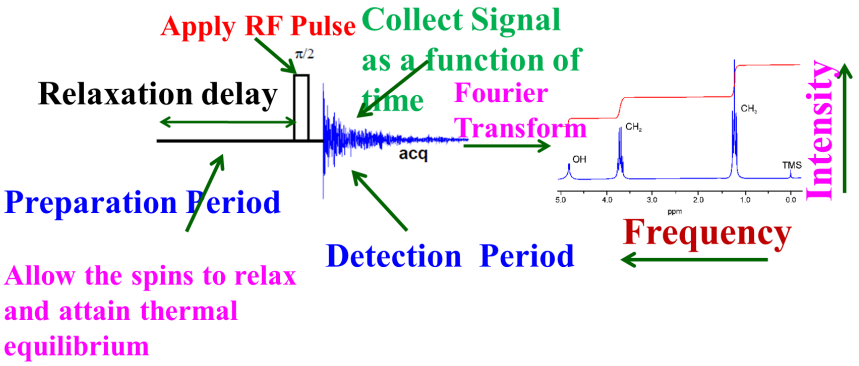
Lecture 37: 2D NMR

Welcome all of you. Almost several weeks we discussed in depth about one-dimensional NMR, right from the rudimentary concepts to interaction parameters, multiplicity pattern, chemical shifts, the factors affecting chemical shifts and how do we get multiplicity pattern when one proton is coupled to groups of other protons, etc. We analyzed lots of one-dimensional spectra, Proton, carbon and various other heteronuclei. Based on the multiplicity pattern we could get lot of information. How do we make the assignment everything and also we could extract the coupling information between two abundant spins, coupled spins, abundant and rare spin, that is dilute spin. From the analysis of the satellite spectra, not only we get the heteronuclear couplings, we also got homonuclear couplings and then we extended this analysis further. We understood afterwards about spin echoes, what is the spin echo? how does it happen? I said a 90 degree pulse followed by a delay with another 180 plus a delay was a conventional spin echo sequence and the Hahn echo was 90 tau 90 tau, which was first given by Erwin Hahn. A spin echo is a time reversal experiment and we understood how the spin echo works and we also understood what is a J modulation? what will happen to the spin echo sequence? especially when we have homonuclear spins, what happens when you have heteronuclear spins, when the chemical shift gets refocused, whether the J coupling gets refocussed or not; all those things we understood. We extended this further for the polarization transfer experiment where we saw that we can transfer the polarization from abundant spin to rare spin, like dilute spin proton to carbon, phosphorus to rhodium, phosphorus to nitrogen like that. And then we can use all these methods not only for polarization transfer but also for the identification of different carbons attached to different number of protons based on the whether the carbon is attached to 1 proton, 2 protons or 3 protons or quaternary carbon. By using INEPT experiment we were able to identify them. We can use it for spectral editing especially for carbon that polarization transfer is used in DEPT experiment. I said by using DEPT experiment we can identify different carbons also. So, with the J modulation we understood APT, DEPT, INEPT all experiments we understood. Of course DEPT and INEPT are polarization transfer experiments, but APT is not. It is only a J modulation experiment. But all these things can be utilized for identification of different carbons plus polarization transfer. So, lot of things we understood about 1D NMR and we spent several hours on that and now it is time to go for advanced subject called 2 dimensional NMR, where we can use this method for further simplifying the experiment or further simplifying the spectra or for aiding the analysis of the spectra,

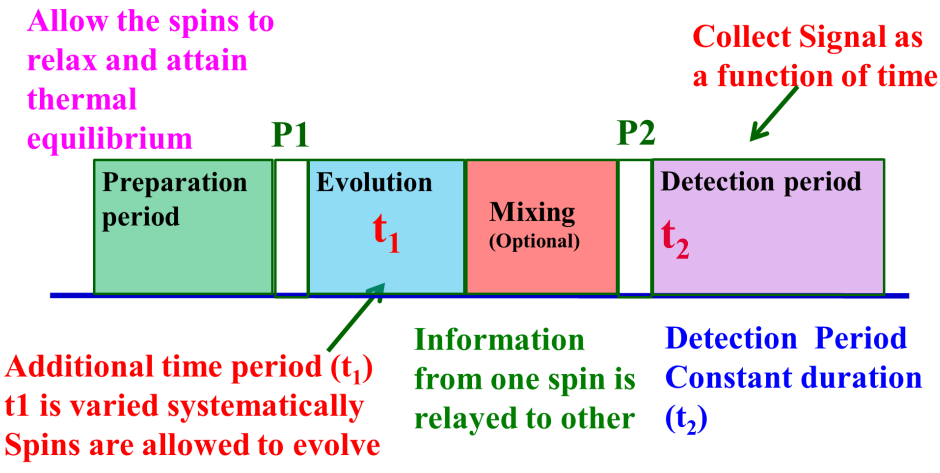
getting more information which you cannot get from the one dimensional spectrum. Lot of things one can do. So we will start from today 2 dimensional NMR. Of course in 2 dimensional NMR, there are several experiments, there is also one experiment called 2D NOESY, I will introduce before that a concept of NOESY. So, I have not introduced yet but I am going to introduce it later.

But right now we will start with 2D experiment. With this let us see how it goes. We will ask a question what is 2D NMR and why it is needed? The first question we have to ask what is a 2D? why do you need 2D NMR. First, we have to understand the limitations of 1D NMR. What are the limitations of 1D NMR. It depends upon the size of the molecule. What happens so far we have analyzed only small molecules, small organic molecules spectra, small molecules heteronuclear spectra etcetera, that is fine. But what happens as you go to bigger and bigger molecules? Take for example a big protein consisting of let us say 10 or 20 amino acids each amino acid running from CH3 to NH protons. So many protons will be there, each of them form a spin system. So many peaks will be there and they will all be overlapped and the protons spectra will be within only 0 to 10 ppm range. Then what will happen? so many peaks overlap, so much of complexity, it is very difficult to analyze the spectrum. So what it means? as the size of the molecule keeps increasing, the number of transitions and the complexity of the spectrum keeps increasing. Of course assuming the symmetry is not there, if the symmetry is there, with higher symmetry then what will happen is number of transitions get reduced. The spectrum becomes less complex, that is well known. But basically remember for the 1D NMR there is a limitation for the size of the molecules. As the size of the molecule keep becoming bigger and bigger, the spectra become very complex. That is one limitation. The second is the assignment problem due to spectral complexity. S far we were analyzing the spectra very easily. I used to say that CH3 proton CH2 proton, CH3 is a triplet because of CH2, CH2 is a quartet because of this, et. All those things we were able to analyze which is quartet, which is a triplet. This is quartert because of three protons, equivalent protons and this is triplet because of the two equivalent protons. All those things we knew and we are able to assign in a straightforward way. The analysis is a straightforward analysis, simple analysis. This is possible only when the spectrum is not complex, and well dispersed. If you go to a simply bigger molecule slightly bigger molecule with enormous overlap and spectral complexity you cannot make the assignment in a easy way, because there each proton can have many couplings. I showed you one example of a case where one phosphorus was having four different couplings and give 90 peaks. Like that there could be enormous complexity. In which case assignment of the peaks for a partiular chemically inequivalent spin is a very very challenging task. And next is, it is not possible to separate the interaction J and delta. Remember the chemical shift and J coupling will be present both simultaneously in a 1D NMR. I took 1D NMR spectrum and when we analyze we said chemical shift but that is also having multiplicity pattern because of J couplings. Wo both are present you cannot

separate them in 1D. So it is very difficult job, because when you separate these two interactions your spectrum analysis becomes easy. There is a lot of simplicity is there in the spectrum. So this is a big issue and this is one limitation. Also you cannot correlate the interactions. For example, if I want to say CH3 proton is coupled to CH2, how did I understand that? based on the multiplicity. I know this CH3 is a triplet because of CH2 CH2 is a quartet because of CH3. I know multiplicity I can correlate. If there is enormous complexity in the spectrum how do you even identify the multiplicity pattern? how do you do the correlation? not easy. Let us say one proton is coupled to several other protons in different groups with different chemically inequivalent protons, and there is a overlap, how do you identify that? So which is coupled to which is very difficult to correlate. These are some of the practical limitations of 1D NMR. Continuing further there are forbidden transitions which you cannot detect in 1D NMR. I told you selection rule is delta m is equal to plus or minus 1. The change in the magnetic quantum number between two spin states either should be plus 1 or minus 1. Anything above that or below are called forbidden transitions in NMR. And you cannot detect. It is not possible to detect. So that is one limitation. Another thing is simultaneous detection of different nuclei is not possible. That is another very interesting thing. For example if I want to take a 1D NMR spectrum of proton I have to tune the probe, and you do the experiment and let us say on a 400 megahertz spectrometer at 400 megahertz. I will put the sample and I get only proton signal. If I have to do carbon 13 NMR in the same spectrometer, I have to do some changes. I have to change the frequency of the probe and tune the probe everything. It is not easy, you cannot get everything so easily. You have to do some changes and then see the carbon signal. That means both simultaneously cannot be detected at the same time. I do one experiment where I get carbon signal and detect carbon and also proton. Not possible to do. These are all limitations of 1D NMR. It puts you some constraints. So how do you overcome this? And first of all what is 1D? Aafter understanding the limitations of 1D NMR, let us see what is a 1D NMR means. We have been analyzing all along, since several weeks. They are all 1D NMR one dimensional NMR. What do you mean by 1 dimensional NMR?

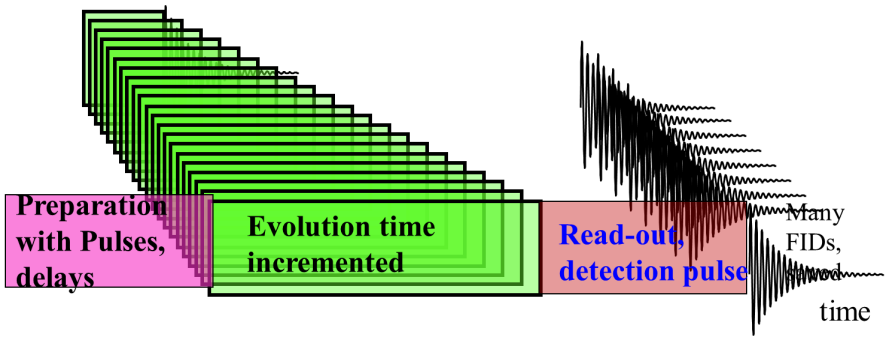


This is simple experiment which I told you. Apply radio frequency pulse and start collecting the signal. This is called a dead time delay. You do not worry these are all not discussed in this course. But in the previous courses I explained this. So I apply radio frequency pulse and start collecting the signal as a function of time, and then after collecting the signal what we do is we do the Fourier transformation. When you do the Fourier transformation what you are going to get is the spectrum like this. Very simple. This is a conventional one dimension NMR. Apply radio frequency pulse, collect the signal and do the Fourier transformation. And here you have the intensity and what you have here the chemical shift frequency. Okay. Or in other words, in short I can put it like this. There is a delay here; this is called a relaxation delay, before you apply radio frequency pulse. After putting the sample in a magnetic field you have to wait for some time.Or after you apply a radio frequency pulse before you apply another pulse you have to wait for some time because a spins have to come back to thermal equilibrium. They have to attain equilibrium. Only then you can apply another pulse to get maximum signal. And this is called a relaxation delay. The spins take some time to relax and come back to thermal equilibrium. And that I call also as a preparation period. I am preparing the spins to get the signal. This is called a preparation period, Okay, where spins are allowed to relax and attain thermal equilibrium. And this is where I am collecting the signal. This is called detection period. It is called detection period. And now you understand one thing in this 1D NMR experiment. The signal is detected in the one time period and you do the Fourier transformation and you get different frequencies. That is all. So what does it mean? one-dimensional NMR means you collect the signal in one time domain, do the Fourier transformation and get the frequency spectrum. This is a conventional simple one-dimensional NMR. Then what is it 2D NMR?



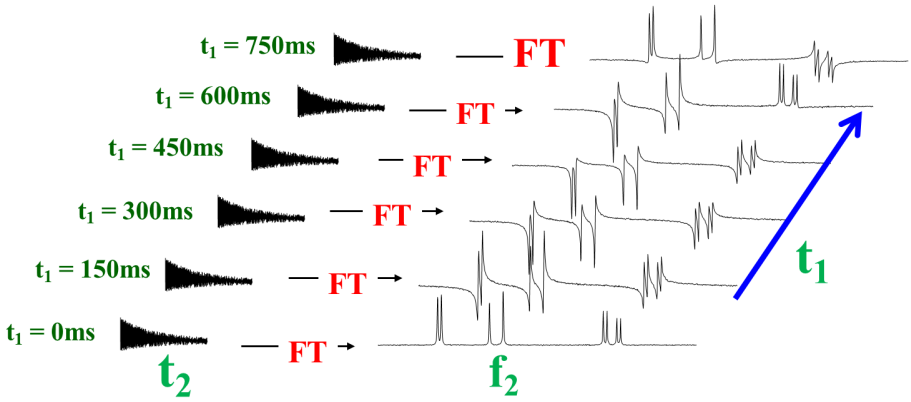
A general pulse sequence for the 2D NMR is given like this. What did we say here? One thing I wanted to tell you, here you must understand we have a preparation period and a detection period there are two things, which are present in 1D NMR. Go to here as

always there is a preparation period that is a period where you allow the spins to come to thermal equilibrium, fantastic. Then we are going to have another time period called t1, the evolution period, another time period called detection period, it is called t2. We have a mixing period in between these two. Optionally we can use it whenever you want for some specific experiments, okay. And after the preparation period I am going to apply a pulse p1 a pulse some 90 degree or whatever the angle, I apply a RF pulse then I'll allow the spins to evolve with time spins are evolving after 90 degree pulse. What do you mean by evolution? spins start undergoing evolution because of chemical shifts, J couplings, etc. The precession can be there, since there could be free precision after the pulse because of chemical shifts and J couplings, okay. And then what I am going to do is, this time I don't keep it constant. I can systematically vary it in every experiment. I keep this one constant and then collect a signal. I will go further and here we have a mixing period I will come to this later, then I will explain. I have mixing period where information is transferred from one spin to another spin, The information is relayed, taken from one spin given to another nuclear spin, and apply another radio frequency pulse. This is called a detection pulse. We apply to detect the signal. Then you start collecting the signal like we did in 1d. And do the Fourier transformation. Now this is a constant period, no doubt. But this one is not a single period, In this, in one experiment I keep this t1 constant, do one experiment. Then what I will do, I vary this t1 for some value, small increment. Every time I keep incrementing. Then I collect the signal. Increment, collect the signal apply pulse increment, apply pulse collect signal increment this by some other value. Do this like that n number of experiments I do every time I increment this and collect the signal and I am also collecting the signal at this place. Whenever I increment every time when I collect the signal. What is going to happen? I can create a time domain. signal analogues to this one. This is what happens. You see it is like this. Diagrammatically this is what is happening.

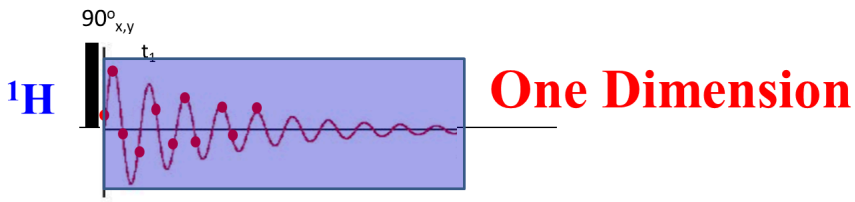


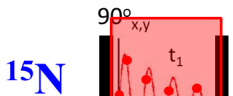
Look at it very carefully this is a preparation period, this is an evolution period, and mixing period and detection period. The mixing period can be absent. I don't need to use it. That is optional. I am preparing, I am allowing this to be prepared then I will put some delay t1, I will collect the signal. Initially delay is 0, there is no t1. I collect the signal I

increase t1 by some value, collect the signal. Increase t1 by some value. See here it is moving and then collect the signal. I keep on doing this I increase it and again collect signal. Look what is happening? every time I am increasing the evolution time, what is happening for this one? that is constant detection period that t2 is constant, I am not changing that. Only t1 period I am changing systematically, and then I collected the signal. So t1 is incremented systematically and the signal is collected for each t1 period. Now how you look at it pictorially, you can see like this. This is the preparation period, is constant here. I keep on increasing the time and I apply radio frequency pulse and every time I collect the signal. This is always constant. This period I am not incrementing. Look at this one here there is increment in the time evolution period, this is how we collect the signal. But what will happen? how do you get the 1D signal in two to dimensions? After each t1 increment this t2 period I am keeping it constant I told you, I am collecting the signal. This is called t2 period; this is called t1 period. There are two time periods. This is evolution period is called t1 and detection period is called t2. Remember t2 is constant only t1 is getting varied here. I keep this constant collect the signal. Now increment t1 collect the signal in the t1 do the Fourier transformation, there are some phase distortions, increment do the Fourier transformation. Like this for different t1 periods I collect the signal. This is always kept constant, but here you see phase is getting changed. The different t1 periods started with the 0 millisecond kept on increasing delay and every time see the phases are changing. What I am going to do is I will do a trick I will take any one of the signals here, for example this proton and then measure the intensity and phases of that particular peak for all the t1 values, and plot it.



For example take this one, this signal is positive, negative, negative, negative, negative, again a positive. So it starts like this intensity also is changing. Start with negative goes positive changes in the

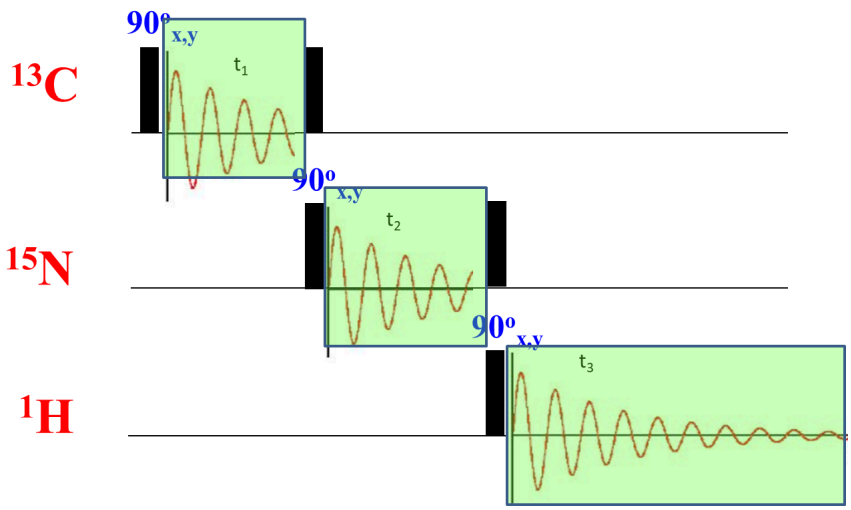




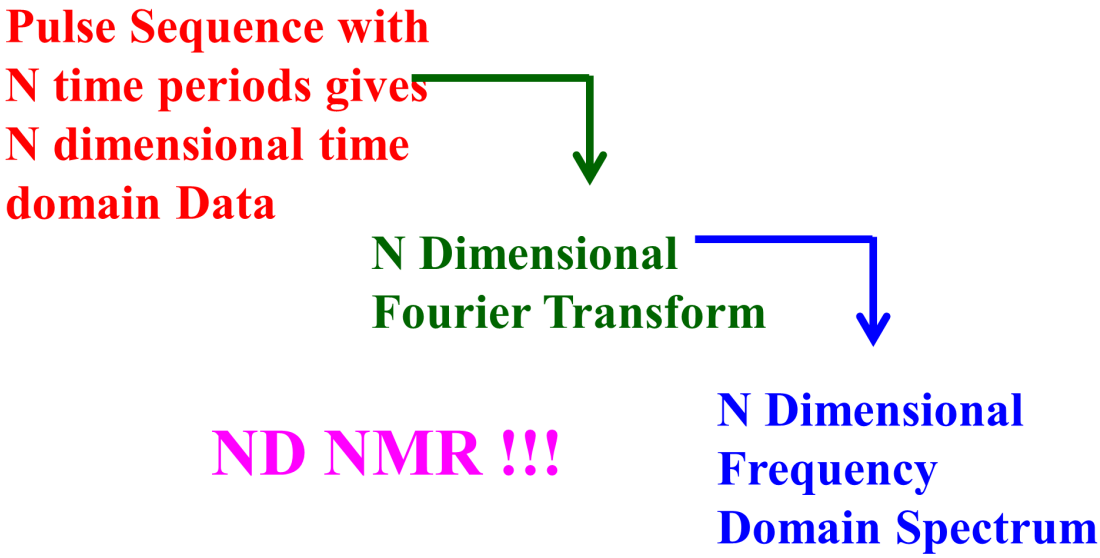
intensity. So there is oscillation going on. What is this oscillation? It is similar to this one, decaying function. Oscillatory and decaying function, time domain function which is decaying. And typically if you collect not one or two points about hundred to 128 to 512 t1 points will be collected into a single data file. And then what is going to happen is we create an FID in the t1 dimension. It is artificially we create an FID. For each t1 period if you collect the signal and do Fourier transformation we get a series of 1d spectra like this, a series of 1d spectra for each t1 point. But look at it now, the intensity is not oscillating, it is going like an oscillatory function, okay. And this period is always constant. Here it is another time domain as if you are collecting the signal and do the Fourier transformation for series of 1d, you see it is an oscillatory function and this what is the phase of the signal is systematically changing with t1. That is what is happening here. All right, we will go further we will see the plot of the signal intensity for a peak as a function of t1, how it is going to happen. And this is an interferogram. This signal is oscillating here like this is like an interferogram, the time domain signal is similar to what we saw in the t2 period, okay. It is what we did, we created a pseudo FID, a pseudo FID is created, similar to FID which we collect in the t2 domain. So what we do in the 1d NMR experiment is a one time domain data, do single Fourier transformation, get single frequency spectrum, it is a one dimension NMR. In the other case we have two time domains, then what we have to do we have to do Fourier transformation for both the time domains to get the frequency domain spectrum that is what I explained to you when I explained 1d NMR since we have two time domains we do two Fourier transformation you get a two frequency domain spectra. Remember one time domain one Fourier transformation gives one domain spectrum. That is the single domain NMR spectrum. Two time domain, double Fourier transformation you get two frequency domain spectrum. That is what is going to happen. Mow look at this one, how it happens. We collected the signal like this, that is what I showed you. This is a t1 point now I will do the Fourier transformation along this axis. I get frequency here here. I will go for go further I will do the Fourier transformation both in this dimension and in this dimension. This is a t1 dimension, this is a t2 dimension, also called f1 dimension and f2 dimension frequency 1 and frequency 2, also called omega 1 or omega 2, they are also called direct dimension and indirect dimension. This is what the nomenclature which is used. Okay. But now what I did is I collected the signal, did the Fourier transformation, first in this dimension and then afterwards I did the Fourier transformation in the other dimension also. Then we are going to get frequency spectrum in both the dimensions. That is interesting. You got the frequency spectrum in both the dimensions. This is a two-dimensional frequency domain spectrum, you got the point. The two-dimensional frequency domain spectrum we got it, then how many dimensions we can have in NMR so far I told you about 2d NMR, two dimensions. How many dimensions you can have like that? we can have any number of dimensions but there is a limit for it. For example consider this one, I apply one pulse collect signal in one time domain and get the

frequency in one domain, you get one dimension NMR. This is 1d NMR. One time domain, one dimensional Fourier transform, one frequency domain transform Fourier transformation, you get one domain frequency domain spectra. This is one dimension. So one time domain is one dimension, we go to this one, it could be homonuclear or heteronuclear doesn't matter. Here I have one time domain t1, here I have another time domain t2 there are two time domains, this is called two dimensions dimensional NMR.

With different time periods, different time domains you can have. One time domain here one dimension. This is two dimensions. Go further you have t1 here, t2 here and t3 here this is three dimensions, this is 3d NMR, three dimension gives rise to 3d NMR. So, what defines the dimensionality in NMR? Now you already understood with this whatever I discussed, meaning a pulse sequence in an experiment which I am going to explain soon, a pulse sequence with n time periods if you consider, time periods is t1 t2 t3 etcetera, they are all given like that t1 t2 t3 they are all different time periods. This is 3D NMR.

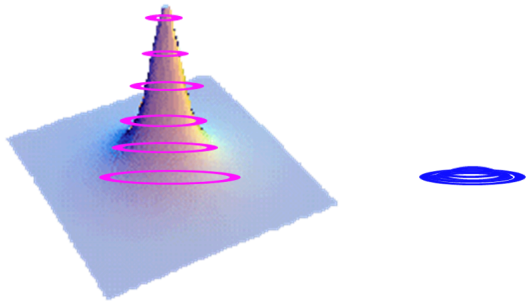


If I have n time periods with n minus 1 incremented, n minus 1 systematically we increment to create a pseudo FID and then only one of them is kept constant. So of the n time domains n minus 1 is incremented, only one time domain is kept constant. This gives rise to n dimensional spectra. So, dimensionality is defined by the number of time domains, time periods you have so t1, t2, t3 etcetera. You can have n number of time periods and you can as well have two dimension two dimension means two time periods, three dimension means three time periods, 4d means four time periods. Of course you can extend like this beyond 5d and etcetera is very difficult because signal will decay, theoretically is different practically you cannot have more than 5d or 60 etcetera 5d you can have but beyond that it is very difficult. So you can have different dimensionality in NMR. you understood what is the dimensionality. The dimensionality is defined by the time periods. So what defines the dimensionality? if you go further I am sorry the pulse sequence with the n time periods gives n dimensional data time domain data, the pulse sequence with n time periods gives n time domain data and you do n dimensional Fourier transformation you are going to get n dimensional frequency spectrum, understand the logic now. You can have a pulse sequence with the different pulse sequences. With n time periods you get n dimensional time domain data you do n dimensional Fourier transformation you get n dimensional frequency spectrum and this is called nd NMR, n dimensional NMR. you understood what is the n dimensional number now.



So n can be anything n can be 2 3 4 5 if n is 2 you have two dimensions, if n is 3 by 3 dimensions. How do you get the two dimensional spectra? just for illustration purpose we can have different type of experiment called resolved or correlated experiment like that.

As we go ahead I will discuss all those things. In the resolved experiment I will resolve two parameters in two different dimensions, in the correlated experiment I will correlate two information in two dimensions. What is the type of spectrum you see, you are plotting the spectrum like this. It is called stack plots, you are plotting this like one dimensional spectrum like pattern in different stacks. These different stacks are stacked one above the other. This is called a stack plot. This is the way people used to record the NMR spectrum when the 2d NMR was discovered in late 70s or early 80s. It has been replaced now. This type of representation of the 2d data is not done anymore. just I am trying to tell you, this was the way started with is called a stack plot. But now what is the way it is done is called contour plot. The stack plot is a contour plot, the same thing is represented as a contour here. Can you see any difference here? Look at it, this peak is put as a contour with different circles one within the other, severa concentric circles, several circles are within that. Look at this peak here, the strong peak is here and these four peaks which are the stack plots. See several number of spectra are there. Here they are plotted like this, so many one-dimensional spectra like that are plotted and the same thing is put as a contour here. This each contour correspond to particular peak in a stack plot. And this is f1 dimension, omega 1 dimension, indirect dimension, f2, omega 2, direct dimension. Nowadays you don't represent the signal or spectrum in stack plot everything is a contour plot. So what the 2d NMR you are going to record, the spectrum of 2d NMR is nothing but a contour plot. It is a 2d spectrum it is nothing but this one transformed into this. Then you may ask me a question how can I transform peak to contour.



I will give you an analogy, try to understand this how do we translate stack plots into contour plots, okay. Look at this one, I have a stack like this, go to the bottom, it is like a mountain, steep mountain. Go to the bottom. go one round measure the area and put a circle. This is a contour. Go slightly above in the mountain, go slightly above, go one more round measure the area. And this bottom circumference is much more compared to the next one. Put another circle it is within this. Go slightly above, measure the area. Put this within this. Go slightly above, put it like this. Go above like this, like this, and what we are doing we measure the area of thes contours at different places and converted into a contours like this. So the contour is nothing but the area of this peak. If you take the area of this contour it is nothing but the area of this peak, that is all. Just imagine a steep

mountain at the bottom you go one round put one circle, go slightly above come back one circumference measure the area put one, like that you can keep on going to different heights every time we measure the circumference, measure the area, and put a circle like this. This is how we translate the stack plot into contour plot. Alright so the area of the contour provides the integral area of the peak, that is all. This is how we converted stack plot into contour plot. Now the question is what is the time constraint in higher dimension NMR? you may ask me a question I want to do a 2D NMR, 3D NM,R 4D NMR, but you should understand before doing any experiment what is the time constraint involved, how much time it will take to do an experiment. Based on that you have to decide your experiment. Say for example 1D NMR nowadays in the present day spectrometers takes less than few minutes, 5 minutes for proton NMR, 10-15 minutes for carbon 13 NMR like that. Forget about exotic nuclei like nitrogen 15 etc. It should take more time, if it is not labeled. But by and large 1D NMR for nuclei like fluorine, phosphorus and proton are very fast. If you go to 2D NMR it may take several minutes that is, 15 20 30 minutes like that. Go to 3D NMR, it will take several hours. Go to 4D NMR, it may take days. If you go to 5D NMR it will take several weeks. That means if you have to do one experiment you have to spend several weeks. And what is the cost of the NMR instrument? the cost of the NMR instrument runs into several crores, 800 mega it may be about 15 crores. When so much of money has been invested nobody will give you one week or three weeks to do an experiment. So, it is the judiciously you have to decide depending upon the information you want to derive, whether you want to do higher dimension experiment or not. So, there is a time constraint. So, the measurement time increases exponentially with increasing in the dimensionality. All right and also what will happen as the dimensionality increases, the measurement time increases, the sensitivity also decreases. It goes by square root of the measurement time. The sensitivity goes by square root of the measurement time, and the dimensionality also depends upon the size of the molecule. You cannot take a small molecule and do 5D NMR what is the use of that? If you want to do a n-dimensional NMR, multi-dimensional NMR it depends upon the molecular size. For example a molecule size of let us say few kilo Dalton 20 to 30 or so just 2D NMR will do. Slightly more than that 30, 40, 50, KDa, the 3D NMR will do, slightly above 40 or 50, 4D NMR will do. If you go to 50, 60, 80 and 90 like that you not only you need 4D NMR you need additional experiment like deuteration etcetera. So, requirement of the dimensionality of NMR depends upon molecular size also. So, increased information will be there when you have increased dimension, because of increased resolution that I am going to explain. So, how do you choose a required dimensionality for any given molecule etcetera we will discuss in the next class. Right now since the time is getting over, I do not want to continue further. But in this class we started with understanding what is the 2D NMR? what defines dimensionality. I said dimensionality is defined with the time. An experiment with a pulse sequence with n time periods with n-1 incremented only one time domain is

constant is going to give n-dimensional NMR. We have n time periods, n-dimensional Fourier transformation you do and you get n-dimensional frequency spectrum. And we have two different ways of representation of the 2D data, it was stack plot and contour plot stack. The plot is outdated, I showed you how we can use contour plot. The area of the contour is nothing but the integral area of the peak in the stack plot. So, we can use that. And of course the dimensionality of NMR depends upon how much time constraint is there I explained to you. 2D NMR may take few minutes, 3D NMR may take few minutes or let us say 1 or 2 hours, 3 hours, 2 hours like that, 4D takes days, 5D takes weeks. And also resolution enhancement will be there and the sensitivity goes by the square root of the measurement time. So, there are certain restrictions also involved in this thing. So, it and also depends upon the molecular size that also I explained to you. So, what is the size of the molecule you have to use for getting the different dimensionality. But the question is how do you choose a required dimensionality for a given molecule? That we will discuss in the next class. Thank you very much.