One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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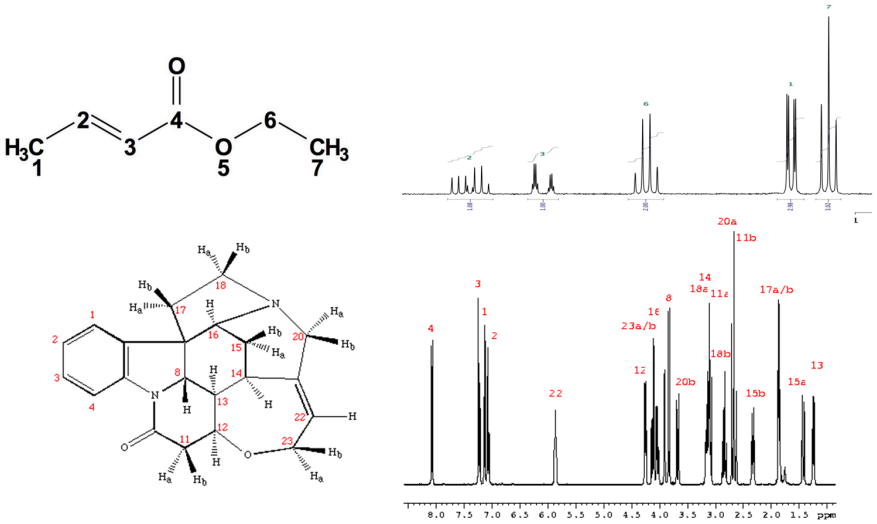
Lecture 38: 2D NMR-II

Welcome all of you. Since the last class, we started discussing about 2 dimensional NMR. In the last class, I even discussed about what is 2D NMR, what are different time periods. Any 2D experiment has a basic pulse sequence, which consists of a preparation period, evolution period, mixing period and the detection period, I said. Whereas the conventional simple 1D NMR, apply a pulse, collect the signal. One time domain you will collect the signal, do the Fourier transformation, you get a one dimensional spectrum. You design a pulse sequence, which has two time periods, one is constant, other isa incremented, and create a pseudo time domain data. There also you will have a oscillatory function, which is time domain signal. Do the double Fourier transformation of it, you get a two dimensional spectrum. You have a n time periods where n minus 1 periods are varied, one is kept constant, you get the n dimensional spectrum. That is what I explained to you about what is n dimensional NMR. And of course, there is a time constraint. As you go to higher and higher dimensionality, time requirement for doing the experiment is more and more. 1D NMR may be taking few minutes, 2D NMR, few hours, half an hour to one hour, 3D experiment few hours, 4D experiment few days, 5D may take weeks. And also another thing is, it depends upon the molecular size. There is a constraint of the time and depending upon the molecular size, you should decide what experiment you want. For example, small organic molecules which we have been discussing, 1D will do most of the time. You do not require 2D, but slightly complex molecule, you can go to 2D. A bigger protein or you know, 30, 40 kilo Dalton molecular weight, you can go to 3D. Even more you can go to 4D. Slightly higher 80, 90 kilo Dalton. Not only you have to use 4D, plus you have to use some other experimental techniques like labeling and varieties of other experiments. That is what I said. But now we will start today with a discussion on how do you choose the dimensionality for your given molecule. Let us start with that.

For example, I consider a molecule like this, which we analyzed the spectra, in the 1D NMR, very easily you can analyze. Simply look at this CH3 a triplet, and this is a quartet, this is a triplet, this is a quartet and this is a quartet, you know, quartet of triplet, this is triplet. Very easily wecan look at the 1D NMR spectrum, I can analyze it. The triplet, quartet, everything. Not much of a difficulty is there in analyzing this. So, you do not require more than 1D for this. And 2D and higher experiments are not needed at all. You

do not need both either 2D or any higher dimensions. 1D will do, that will give you the required information.

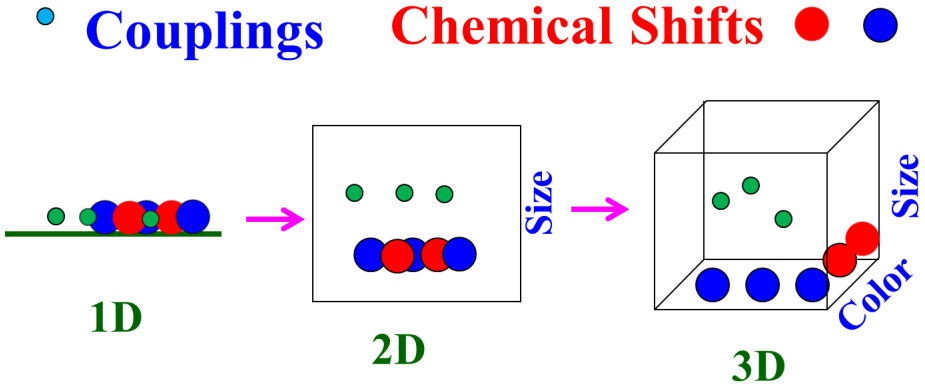


On the other hand, go to a molecule like this. This molecule is called strychnine. This is 1D NMR spectrum. Compared to this, it is slightly difficult, slightly complex, not very complex. If you are already working in NMR, spend let us say couple of weeks or months already working in that, if you have worked for a couple of months, this is fairly simple. You can interpret it and analyze in less than half an hour to one hour, maybe if not half a day, if you are inexperienced, maybe within a day you can analyze it. It would not take much time. So, 1D NMR will fairly do your job. But in case if you want to make it faster and if you think it is still difficult to analyze because of complex multiplicity, overlap, etc, 2D will do. You can do a 2D experiment for this and definitely 2D is enough to get the required information for assignment of the peaks, etc. And you do not need 3D and higher dimensions for this molecule. Definitely you do not require.

Go to a molecule like this. Is it 13C and 15N labeled ubiquitin, a big protein, a typical HNCO experiment, a three dimensional spectrum is given here. So many peaks are there in this. For such type of molecule and there too, if carbon and nitrogen are 13C and 15N labeled 3D is definitely required. You may not be able to do only with 2D. And then if you want to go to even bigger molecule as I said we need 4D etc. So, this is how we can choose the dimensionality.

Then the question is what are the benefits of higher dimensions? What do you get by going to higher dimensions? Look at this molecule. I have taken as an example. These are small spheres, I call them as couplings. I have two different nuclei. One is red, other

is blue, bigger spheres. They are different chemical shifts. Of course, they are two different nuclei. They will come at different resonating frequencies.



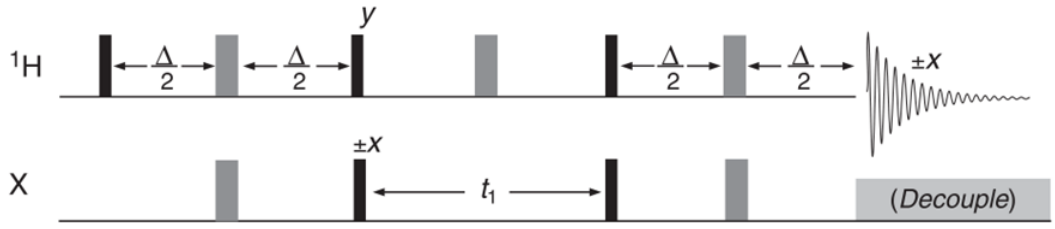
For understanding purpose, I will say they are all overlapped here. It could be same protons or assume that different chemical shift from different molecules are there. They are overlapped, different chemical shifts and couplings are here. They are all overlapped here. This is the complexity the 1D spectrum. How do you resolve it? How do you analyze this one? It is a challenge. What we do is you go to 2D NMR. What I do in the 2D, I am going to let us say make all smaller spheres on one dimension. I will bring it here and bigger I will keep it here. I will change in different dimensions. In this dimension, based on the size of different spheres, I will change it. I will make the difference. So, now small spheres are here, bigger spheres are here. That is okay. That is one way. I can go further. I can make three dimensions where I can have different small sized balls are here, different colors here, bigger spheres, one is here, one blue color is there, red color is here. Based on the color I changed it. Here based on the size I changed it. What did I use? I used three different parameters, size, blue color and red color or otherwise two parameters, size along this axis and color along this axis and this axis. I resolved it. See, this was the spectrum, 1D spectrum crowded. Now, you see well resolved, very easy to analyze. So, what is the advantage of this? Higher dimension significantly simplify the spectral complexity and gives spectral dispersion, higher dispersion, better dispersion. Meaning instead of crowded spectrum you will resolve it. Instead of bringing it very close, you can pull them apart exactly what it did by dispersion means you are pulling them apart so that you can easily analyze the spectrum. This is the benefit of higher dimension.

I would say if I go to it, most of the time we are dealing with 2D experiment in this course, we do not go to 3D and other things because that is going to be enormous, time-consuming and we do not have time to discuss all those things. Most of the time 2D will be sufficient, unless you are working biochemist, working biophysicist, working on

a very big protein, etc. where you require 3D and special experiments. We will stick to only 2D experiments in this course. Broad classification of 2D experiments, I have made, it is my own interpretation, we have two classifications, two way of classifying. One I call them correlation experiments, other is resolved type experiments. There are two types of experiments, correlation and resolved. In the correlation experiment, what we are going to do, the information in two dimensions are correlated, related to two, two information are related, their example, COSY, TOCSY, etc. How they are related, I am going to explain to you. For example, the chemical shift here, chemical shift of another nuclei here may be related, we can correlate this with this like that. These are all correlation experiments. And in other case, we have we have resolved information. I can resolve in two dimensions. For example, in the previous example I showed you, coupling in one dimension, chemical shift in the other dimension. Coupling and chemicals are two different parameters, they come together in the 1D NMR spectrum. Can I resolve in two dimensions? Can I take coupling in this dimension and chemical shift in other dimension? That is called resolving, they are called resolved type experiments and they could be J-resolved. And again, both these experiment could be homonuclear type and heteronuclear type. For example, you can have a homonuclear correlation experiment and a homonuclear resolved experiment. You can have homonuclear, heteronuclear resolved experiment and heteronuclear correlation, both are possible. And homonuclear experiments, each 2D experiment mind you, when you design a new experiment, it is given an acronym. For example, COSY, TOCSY, J-resolved, etc. Similarly, heteronuclear experiment called HETCOR, HSQC, HMBC, etc. Some of these things we will discuss in the next few classes. So, what we can understand? One thing is simplifying the spectrum, and the better resolution, easy for analysis, that is okay. Apart from this, what else we get from the 2D spectrum? What else we can get? One is, we can get the coupling pathways, you do not get in 1D. Of course, coupling constant we get in 1D, but in a better way we can get it. Long range couplings we can get. Reduced dipolar couplings, we can get nuclear Overhauser effect, diffusion constant, all those things we can get it here. And all these information we can obtain, both for heteronuclear spin system or homonuclear spin system. All this we can get from the 2D spectrum. And there is a plethora of information, but mind you all these things we can do in solution state and also in the solid state. Solution state NMR and solid state NMR are different. Of course, nuclear spin do not know whether they are in solution state or solid state. The information content what you get from solution state, type of spectra are different. We get sharp signals, well-resolved, much better than solid state, because in solid state. There is additional information. The additional parameters which you have to remove to get the sharp peaks. I discussed this in one of the advanced courses, in the previous course about solid state NMR. So, I do not want to go into the details, but remember in solids also the 2D experiment can be carried out where we can get chemical shift anisotropy information, homonuclear dipolar couplings, heteronuclear dipolar couplings,

quadrupolar couplings, etc. All this information we can derive in solids. Again in solids also you can get plethora of information both in liquid state and solid state. You can do 2D NMR, you can also do higherdimensional NMR and then we get lot of information which you cannot get from the 1D NMR in easy way. This is the advantage of 2D NMR. What are the typical 2D experiments commonly employed? We will deal with the solution state in this course only. We are not discussing solid state. We have COSY experiment called correlation spectroscopy. SECSY means spino-echo correlation spectroscopy, NOESY, the nuclear over-hazard effect spectroscopy, ROESY rotating frame Overhauser effect spectroscopy. DQF-COSY, the double quantum filtered correlated spectroscopy. HSQC heteronuclear single quantum correlation spectroscopy. Each letter is highlighted with a different color that is a abbreviation here. And HMBC heteronuclear multiple bond correlation spectroscopy, DOSY diffusion ordered spectroscopy. GgHSQC gradient based version of HSQC. The gradient can be used with other experiments also, but I have given you only this. All these experiments are there, but these are all the acronyms. I have given you only couple of them, but NMR is a huge ocean. If you go to the literature and see in the books more than several hundreds of such sequences have been designed, hundreds of such experiments. In NMR spectroscopy when you design a new experiment to get a new information, we will give an acronym for this. This acronym tells you what is that experiment, what it does in a short form, what it gives. So, many such experiments are possible. We will discuss couple of them as we go ahead in this course.

Then one question may come. There are so many experiments here, say COSY, NOESY, ROESY, etc. How did these experiments were designed? How do we know which is COSY, which is NOESY, which is ROESY, etc.? How are they designed, first of all?



Remember, each experiment has its own pulse sequence. What is a pulse sequence? It is a combination of pulses and delays. See, we have been discussing the pulse sequence, especially when we discuss spin echo, INEPT, etc, where we saw this is an INEPT type sequence. We discussed this which is spin echo,. We earlier discussed that. So, each experiment has its own pulse sequence and this is a pulse sequence. Pulse sequence is given like this and each of them has different pulses, 90 pulse, 180 pulse, different delays. I told you different delays plays a big role. When we discussed spin echo, INEPT and APT, I said 1/2J, 1/4J, 1/J, etc. How the spin vectors become in-phase, anti-phase, etc. we discussed. So, all those delays matters a lot. Basically, each experiment is a

combination of different pulses and different delays like this. And how do you choose this? What is a delay? What is a pulse sequence? There is a way, you have to understand spin dynamics and then do it. That is the job of a real hardcore NMR spectroscopist. They will do it. And how do you choose the delay everything? That all depends upon experiment, they are tailored for a particular information. Already we discussed this. When we have 1/2J, we found CH vectors become anti-phase in character. The CH vector we saw in the spin echo. Like that we can tune it. In the INEPT experiment, we saw that 11/4J, 1/2J, 3/4J, where we can identify CH, CH2, CH3.

All those things were designed only based on the delays. So, delay and everything is tuned or tailored to a specific coupling constant. Pulse duration, what it does then? What these durations will do? Different durations here. These pulse durations creates coherences. What is a coherence? It brings the magnetization from Z axis to X axis or Y axis. It is magnetization in the XY plane, transverse plane is a coherence. It creates coherences between coupled spins and allows the modulation to encode from one spin to another spin. All those things will be done in the pulse duration. And finally, after doing everything in any pulse sequence like this, finally, we are going to collect the signal. And we apply a pulse here and then start collecting the signal. Final step is to turn on the receiver and start recording the signal as a function of time. This is how we design the pulse sequence. Pulse sequences are nothing but the combination of pulses and delays. Pulses are 180 pulses, 90 pulses, different angles, flip angle pulses. They can be tuned. Delays are tuned for a particular information to be derived. And what happens in any 2D experiment? We have tuned delay. We have applied 90 pulses. There are delays in between. What happens? This is where the information is transferred from one nuclear spin to another nuclear spin. This transfer of magnetization establishes relation between the spins within the molecule. How does it establish relation? The magnetization transfer mechanism is different for different experiments.

Transient NOE (z magnetization transfer via NOE)

INEPT (antiphase to antiphase coherence transfer via J-coupling)

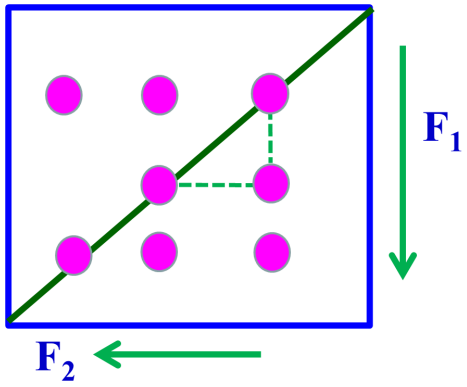
TOCSY (multiple in-phase to in-phase coherence transfers via J coupling)

ROESY (NOE transfer in the x–y plane during spin lock)

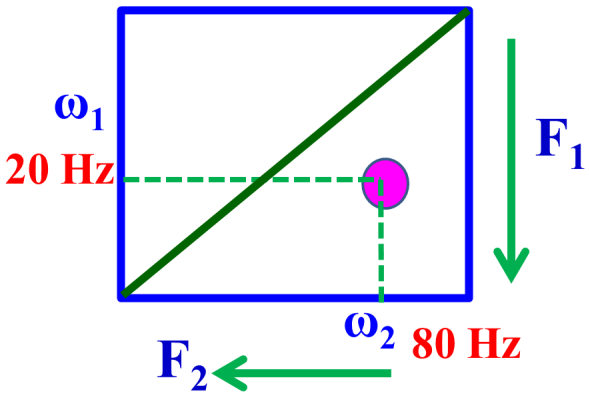
So, we will understand how the transfer of magnetization takes place within the molecule and what are different types of magnetization transfer mechanisms. We will discuss some of the things when we go ahead further. But just to give you an idea, what are the magnetization transfer mechanisms, transient NOE? Here, the Z magnetization is transferred via NOE. NOE along the Z axis, the Z magnetization is transferred because of NOE, that is through space interaction. No need of J coupling, no need of covalent bonds. That is a transient NOE. That is a transfer mechanism. Mechanism of magnetization transfer between two interacting spins is through Z magnetization. INEPT, we discussed this in the last two or three classes. It is anti-phase to anti-phase coherence transfer. I told you, when we apply a 90 pulse after 1/2J, the spin vectors become anti-phase. Simultaneously, you apply two 90 pulses on proton and carbon. The anti-phase coherence of proton jumps to carbon 13. That is what we saw. It is the INEPT experiment where anti-phase to anti-phase coherence transfer takes place, where we require J coupling for mechanism of this transfer, J coupling is needed. TOCSY is another experiment which we discussed. It is a multiple in-phase to in-phase coherence transfer. This also requires J coupling. Using J coupling, we can do multiple in-phase to in-phase coherence transfer. ROESY is a NOE transfer in the XY plane during spin lock. What is a spin lock? We will discuss later. Using the particular magnetization transfer mechanism, a particular specific 2D experiment is designed. How? We have different pulse sequences and different delays, and each of these experiment has some mechanism where the magnetization is transferred to derive particular information of a molecule. This particular magnetization transfer mechanism is specific to particular 2D experiment. For example, it is a COSY, HETCOR, HSQC, HMBC, NOESY. These are all the experiments which have been designed with a particular magnetization transfer mechanism in mind. That is how most of the pulse sequences are designed. You may ask me question, what are the common experiments, there are 500, 600, or 1000 different experiments in NMR. Everything you cannot learn, that is the ocean. But what are the common experiment which we require for our day-to-day utility? That is they are COSY, phase sensitive COSY, DQF-COSY, soft-COSY, relay COSY, TOCSY, INADEQUATE, J-resolved, ROESY. These are all common today experiment. We will discuss couple of them. It may not be possible to discuss everything in this one course, but we will discuss some important experiment and we analyze the spectrum. If you go to HETCOR experiment, COLOC correlation of long range correlation couplings, HETCOR relay, it is called HEHAHA, homonuclear correlation, HETCOR, heteornuclear J-Resolve. These are all certain 2D experiments. We also have what are called inverse experiments. For example, all these experiments are called direct detection experiment, direct detection experiments. In this sense, in these experiments, for example, heteronuclear carbon-13, nitrogen-15, etcetera are detected directly. Whereas, there are other experiment called inverse experiments, where you can detect the information about the dilute spin indirectly

through abundant spins. I will explain to you when we go to HSQC, HMQC, etcetera. They are, for example, HSQC experiment, heteronuclear single quantum coherence, HMQC, heteronuclear multiple quantum coherence, HMBC, heteronuclear multiple bond coherence like that.

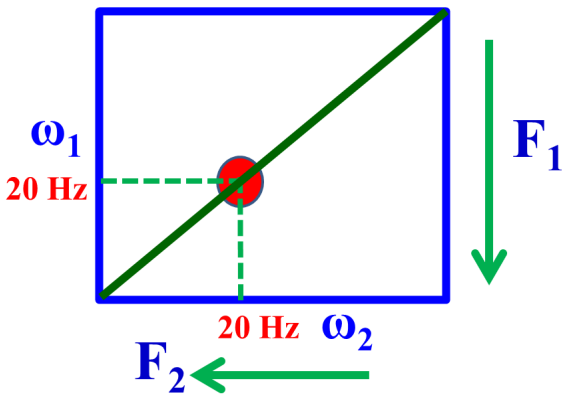
Varieties of experiments are possible. Assume that you know how to do an experiment. You have done an experiment, simple experiment. But how do you interpret the data? Of course, assignment of the peaks in a COSY experiment, they are all different things that you know how to do that. But generally, if a COSY spectrum or any 2D spectrum is given, how do you interpret the spectrum? There is a way to interpret. I am going to show you that. Any experiment, 2D experiment, especially homonuclear, I am talking about homonuclear 2D experiments.



You can have diagonal peaks and also cross peaks. For example, peaks which are sitting on this diagonal, the peaks which are sitting exactly on the diagonal, they are called diagonal peaks. And the diagonal means, it should be from the bottom left corner to the right top corner. You cannot have this as a diagonal, it is an anti-diagonal. Diagonal refers to that you start from the bottom left most corner, you draw a line to the right top most corner, that is diagonal. And there are peaks situated outside the diagonal here. They are called cross peaks. So, any homonuclear experiment, especially correlated experiments like COSY, TOCSY, etc., you will get cross peaks like this and also diagonal peaks. We will now do a 2D experiment, homonuclear or whatever it is. Let us say I am going to get a eak here. This is a diagonal. There is no peak on the diagonal. And I am going to put the value, this is 20 hertz, this is 80 hertz. This is my, some value I have written. This is a peak here from the center of the peak, come vertically down. And of course, this is f2 dimension, this is f1 dimension. If you measure the frequency in this dimension like this, they are all in f1 dimension. If you measure in this dimension, they are all f2 dimension. This is f1 dimension, and this is f2 dimension. That is how you can measure the frequencies.

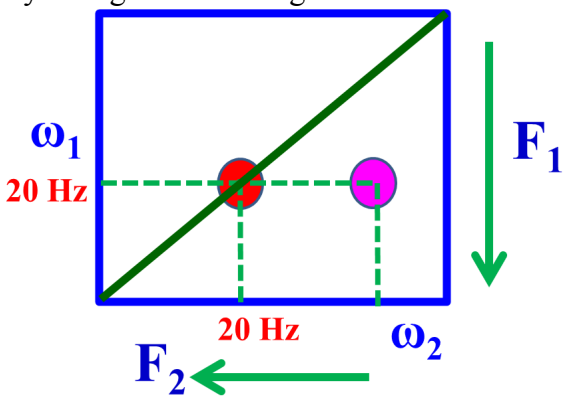


Now, look at it. I have a peak here. From this peak, draw a line vertically down or draw a line horizontally. You see here, if you draw a line here, you hit here. I will say this is omega 2, this correspond to 80 hertz. Go along this axis, this correspond to omega 1, here it pertains to 20 hertz. That means, I have a peak which in the f1 dimension is at 20 hertz, in the f2 dimension is 80 hertz. What does it mean? It means the signal evolved during t1 period, in the t1 period is f1 period, at a frequency of 20 hertz. During the process of 2D mixing, it so happens, the same signal is transferred somewhere to another signal which evolved at 80 hertz in t2 dimension. Somehow, something happened in the molecule during the experiment, the signal which was coming at 20 hertz in the t1 period transferred some of its magnetization in some way. How we will do? We will worry about it later. Somehow, in some way, it transfers part of its magnetization to another spin. It evolved at 80 hertz. See, in the t1 period, it was at 20 hertz. Somehow, in the t2 period, it evolved in some way to another signal, it gives another spin and evolved at 80 Hz. You understand? This is how you have to interpret the signal. If I have a peak here, go along this axis, this is the frequency in omega 1 dimension, this is the frequency in the omega 2 dimension.



I will give you another example. Here, in this example, a peak is appearing at 20 Hz in the f1 dimension. I have peak on the diagonal, go horizontally, I get 20 Hz, go vertically down, I get 20 Hz. So, a peak on the diagonal, if you go horizontally and vertically, it comes at the same frequency.

Something interesting, what does it mean? There is a signal from a particular spin, which evolves at a particular frequency in t1, which is 20 Hz. It continued and evolved at 20 Hz in the t2 also without any change. It did not get affected at all. This signal is not affected during the mixing period. It simply continued at the same frequency at 20 Hz. That is how you have to interpret. The peak on the diagonal is at 20 hertz. That means that is remaining unaffected in the t2 dimension. Whatever was its frequency in the t1 dimension continues to remain the same in the t2 dimension.



We will give another example. I have a peak like this. If we go along f1 axis, it correspond to 20 hertz. There are two peaks exactly at 20 hertz. This also at 20, this also at 20 because if you draw a horizontal line, it correspond to 20 Hz. If you draw a vertical line, this correspond to 20 Hz and this correspond to 80 Hz. What does it mean? There was a signal, in the experiment in the t1 dimension during evolution period, which was at 20 Hz But what happened during the experiment, it remained same also in the t2 dimension. It came at 20 Hz. At the same time, it gives part of its signal to another spin, which comes at 80 Hz in the t2 dimension. This is the point. You have to understand the signal which was at 20 hertz in the t1 dimension continue to remain same in the t2 dimension. Also, it gives part of its magnetization to another spin, which evolves at 80 hertz. This is 80. This is how you have to interpret the 2D spectrum. You understood how we can interpret a 2D spectrum. So, if you look at this one, these are typical varieties of 2D experiments. This I already showed you 1D NMR spectrum. This is a 2D NMR spectrum of a molecule. Look at it. This is a 3D NMR. I showed you this also earlier. This is how when you see a spectrum, you should imagine, and say hey, this is 1D

spectrum, this is a 2D spectrum. Whether it is COSY, ROESY, TOCSY, is a different question, but this is a 3D experiment. There are 3 dimensions here. 3 dimensions here, here and here, 3 dimensions here. There are 2 dimensions. Here only 1 dimension here. This is multi-dimensional experiment, typically multi-dimensional.

And for this, approximately molecular weight is about 500. This is about 10,000. This is about 30,000 for 3D. 30 kilo Dalton and 10 like that. We have already discussed. So, basically what I wanted to tell you in this class is, we discussed a lot about the 2D and we discussed about the dimensionality. And I told you how you have to choose a dimensionality based on the molecular size, complexity of the spectrum. For a simple molecule, let us say water which gives you one single peak in ^{1}H NMR. You do not require even high frequency spectrometer, it is not necessary. Whether it is 50 MHz or 100 MHz, you will get one single peak. A bigger molecule, a slightly bigger molecule 1D will do. Even bigger molecule with large spectral complexity, you can go to 2D to simplify the spectrum. Go to 3D for even bigger. I also told you, as you go to higher and higher dimension, it takes enormous amount of time. The benefit of higher dimension is you can spread the information in different dimensions. It aids, helps you in simplifying the spectrum and aids you in getting the required information. And I showed you so many experiments are there. They are called correlated experiments and resolved type experiments. We can broadly classify into two types of 2D experiments. Correlated experiment again can be homonuclear and heteronuclear. Similarly, resolved experiment can be homonuclear and heteronuclear. Examples of correlated experiments are COSY, NOESY, ROESY, TOCSY etc. Resolved experiments are heteronuclear, J-Resolved, Homonuclear J-resolved, etc. Especially in the case of heteronuclear experiment, we have HETCOR, COLOC, HMBC, you can do inverse experiment like HSQC, HMBC, HMQC etc. Varieties of experiments. All these acronyms are there, different experiments have been given different acronyms or designed pulse sequences to extract some information, particular information in a molecule. You can get particular type of information. The question is how do you design these pulse sequences? These pulse sequences are nothing but different RF pulses and the delays. The delays are tailored for a particular value of J coupling and the pulses could be 90 pulses, 180 pulses, etc. different types of pulses, applied along different axis, x-axis, y-axis etc. And there are different ways, they do different things. During the delays, there could be transfer of magnetization. The transfer of magnetization can be a different mechanism. It can be Z-magnetization transfer for NOE, in phase to in phase for TOCSY, multiple in phase to in phase for TOCSY. It is the anti phase to anti phase for INEPT, like that there are several mechanism of transfer of magnetization. In the ROESY, there is a spin lock. During the spin lock there is a transfer of magnetization. So, there are several ways we can do it. And there are commonly used experiment, I said COSY, TOCSY, ROESY etc. How do you interpret, there is an easy way. I took the simple example, any homonuclear experiment, for example, correlation experiment, we have a diagonal peak and also

several cross peaks. For diagonal peaks, the diagonal is drawn from the bottom left corner to the right top corner. And if the peak is sitting on that, they are diagonal peaks, any other peaks outside of that are called cross peaks. I explained to you how do you interpret a 2D spectrum. Take a particular peak, draw a line horizontally along F1 dimension, draw vertically for F2 dimension. Take a peak, if you go horizontally and let us say I get some 20 Hz, come down vertically, I get 20 hertz. How do you interpret that? It means there was a signal at 20 hertz for a particular spin, which was giving signal at 20 Hz in the t1 dimension, which remains unaltered, evolved with the same frequency in the t2, that is why in t2 also it remains at 80. Alternately, there is one peak let us say at 20 hertz in t1 become 80 in the t2. What happened? It remained at 80, 20 became 80 during the process, in the experiment. In the t2 dimension, it became 80, t1 it was 20. It can so happen it can remain 20 also give it to 80. In another experiment I showed you, there are number of ways you can get the information. How do you interpret the 2D data I have explained to you. This is the way you have to explain, interpret the 2D spectrum. And typical 2D data I showed you how we can look at the theory and find out. And with this basic introduction to 2 dimensional NMR, next, we will jump into analysis of a COSY spectrum, what a COSY does, what a ROESY does, what a TOCSY does, some few examples of experiments we take and start interpreting those spectra. So, from next class, we will start with a 2D COSY experiment. I am going to stop now for today. Thank you very much.