One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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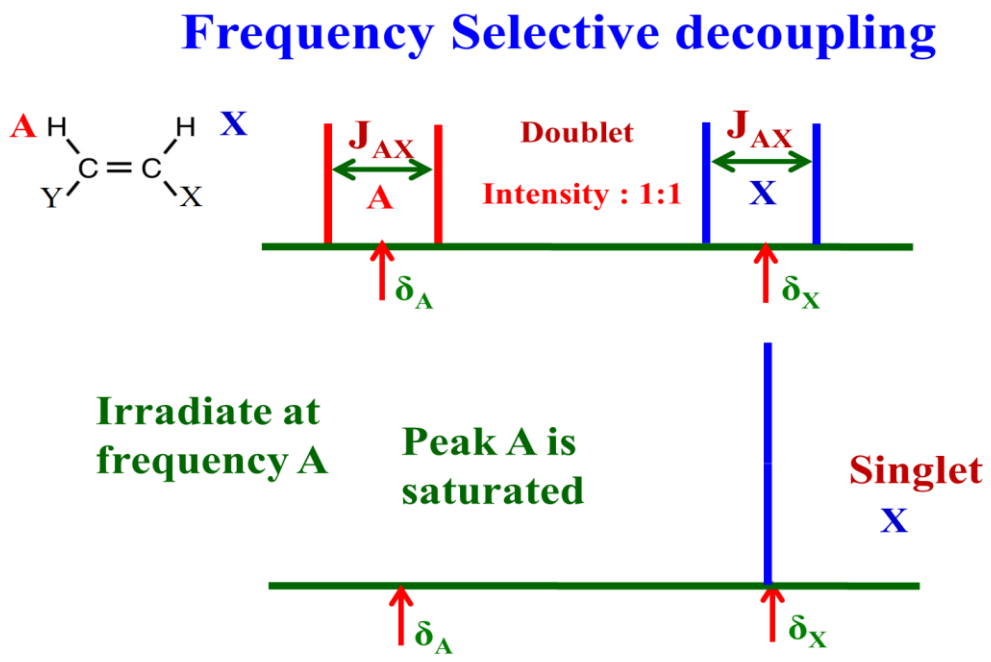
Lecture 60: PURSHIFT NMR

We have almost come to the end of the course, and probably this is the last class I guess unless if there is something which I would like to summarize later, but probably this is the last class. And since last class we have been discussing about pure shift NMR another interesting topic. What is a pure shift NMR? For that I just introduced you what are the key factors of NMR. One is sensitivity, other is the resolution. Of course, there are ways to address the sensitivity by going to higher magnetic field, increasing the sample concentration, and also adapting polarization transfer experimental methodologies, all that we discussed. What about the resolution? One thing, for the resolution also we can go to higher magnetic field. Higher the magnetic field larger the chemical shift dispersion, and you get better resolution, that is one way. But what happens in some complex molecules? The complexity comes because of large number of couplings. What are the large number of couplings? It can be heteronuclear, homonuclear. In the heteronuclear case, I said, you can reduce the spectral complexity just by doing what is called the broadband decoupling. When you do the broadband decoupling you will break the coupling of all heteronuclear dilute spins with the abundant spins, like carbon 13 decoupled with protons. Then you get individual peaks for each of the carbons, that is one way. But what is the loss there? You get better resolution, but you got rid of the coupling information. That is important thing, coupling is very useful information. By doing decoupling you simplify the spectrum, reduced the complexity, but you have lost the spectral information, does not matter. But in many examples when there is spectral complexity simplifying this to identify chemical shifts is an important criteria to assign the peaks. And then of course you can worry about getting the J coupling information from the multiplicity later. If you want to get the chemical shift information decoupling is one of the options, I mean the broadband decoupling. In the case of heteronuclear broadband decoupling is easy. I told you. We have seen that in carbon 13, several examples and you can do this for the any other nuclei also. Broadband decoupling is easy, especially when there are two nuclei completely different, because you can irradiate at the frequency of one nuclei while observing the different nuclei. There is no interference between these two, because they are all MHz away. The frequency is separated by several MHz. That is OK. But what happens in the case of the protons? if I have to do the proton NMR. Then we will understand the proton NMR complexity, and how we can overcome that, in this class. We will start with by looking at the complexity

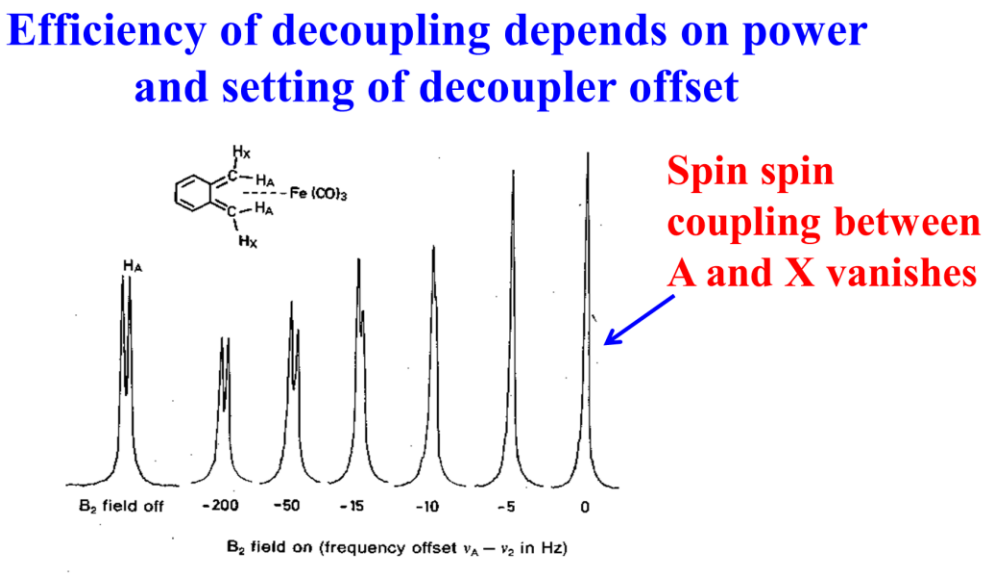
of the ^{1}H NMR spectrum. Why does complexity comes? First thing the chemical shift dispersion is very small, about 10 ppm maximum, in a 10 ppm range. Let us consider 500 MHz spectrometer, 10 ppm is 5000 Hz. In a 5000 Hz range, if you take a reasonably big molecule with at least 10 to 20 or 30 different protons. Each of them interacting with other protons. Then there will be a lot of complexity, because of multiple couplings experienced by each proton. Multiplicity becomes more and more with more and more couplings. And also because of the poor chemical dispersion there is extensive signal overlap. Signal overlap will be so much that it will be very difficult for you to identify the chemical shifts. So, in the many of the examples we took in analysis of the proton spectra and other heteronuclei, they were all fairly simple. If you go to a slightly bigger molecule it is not easy, the multiplicity becomes more. So, the complexity of the spectrum comes because of poor chemical shift dispersion and each spin will be experiencing pair wise interactions among lot of other spins, which gives rise to extensive signal overlap complicating the spectrum. Alright. What can we do to overcome that? like I said in the heteronuclear case we can do broadband decoupling. Of course, we can lose the coupling information, but to simplify the spectral complexity, can we do broadband homonuclear decoupling of protons, like we did for heteronuclei? This is the question. Of course, there are challenges, if you can do that you can reduce the spectral complexity. You can get a better resolution, the interpretation of the chemical shfits become very easy. You can identify which is the proton? what is the chemical shift of each of the individual protons, because when you remove the complexity each peak corresponds to the particular chemical shift of chemically inequivalent protons. There is an advantage. But what is the disadvantage? you have lost coupling information. The coupling information is very useful in getting the conformation of the molecules. Remember I told you about the Karplus equation, where 3J coupling can be used to get the conformation. So there is a disadvantage, we lose that information. So, it does not matter. But how we can do that, in case we are going to compromise only for the chemical shift information? and we are not worried about loss of coupling information? There is a challenge in broadband decoupling of 1H protons. What is the challenge? When you are doing the decoupling you remember I apply a decoupling power. In the heteronuclear case I will be detecting carbon 13, while applying a very high RF power CW power or with different pulses at the proton frequency, such that we can break the couplings. But here what we are doing? It is a broadband decoupling of proton while we observe the proton. That means remember what was the sequence of carbon 13 we apply the carbon 13 pulse and start collecting the signal. But in the case of proton, simultaneous you are applying decoupling power while detecting protons. Remember you have to apply the decoupling power at the time of detecting, that is a challenge here. So, broadband decoupling power during acquisition, when you apply RF power on proton while detecting proton will destroy all the transverse magnetization, it will complicate the issue. So, broadband decoupling of the proton spins while simultaneously detecting the

protons is a challenge. It is not easy, although not impossible. Of course, you can do by different ways, indirect ways, but decoupling proton by applying RF power, CW power and detecting the protons, is not easy, it is a challenge. So, broadband decoupling is not possible especially for the protons. I am talking about protons, the broadband decoupling of proton while detecting proton is a challenge. What people do at times in the good old days, we were doing what is called frequency selective decoupling. What do you mean by frequency selective decoupling? I can irradiate at a single frequency and see the change, that is happening at the other part of the spectrum. We have to do many such individual decoupling experiments. I can irradiate with several frequencies at a time also and see the change in the spectrum, you can do that. Then that type of decoupling helps you to identify which proton is coupled to which proton, based on where the spectrum gets simplified. That was the method adapted to identify the coupled partner, in good old days. How we do the homonuclear selective decoupling, exactly what we do for the broadband decoupling. The only thing is we saturate the particular proton signal, selectively saturate one of them. Apply RF power on one of the protons and saturate that proton. Then apply RF for detection. Or a conventional way saturate a proton and simply detect the signal after applying RF on a selected proton, and monitor the change, that is what was being done. What happens if you do the frequency selective decoupling?

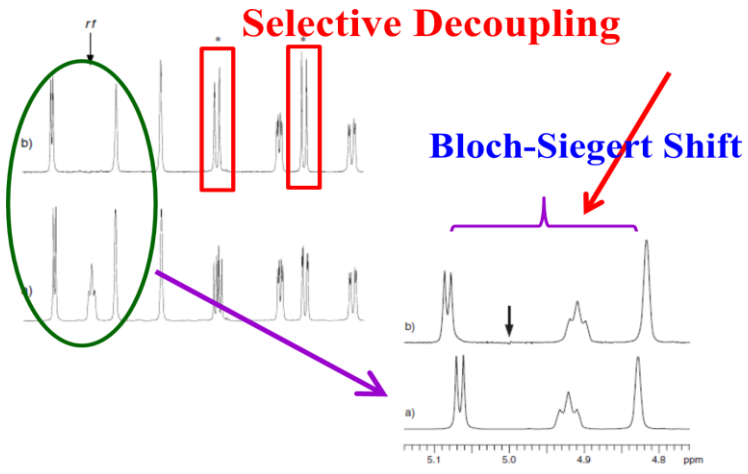


Look at it, I have a two coupled spin system, AX. This center gives a chemical shift, this center gives a chemical shift and this separation gives you J coupling. Alright, what you are going to do, you selectively saturate this peak you get a singlet, because I am breaking the coupling between this and this. I know then this is coupled to this. That is

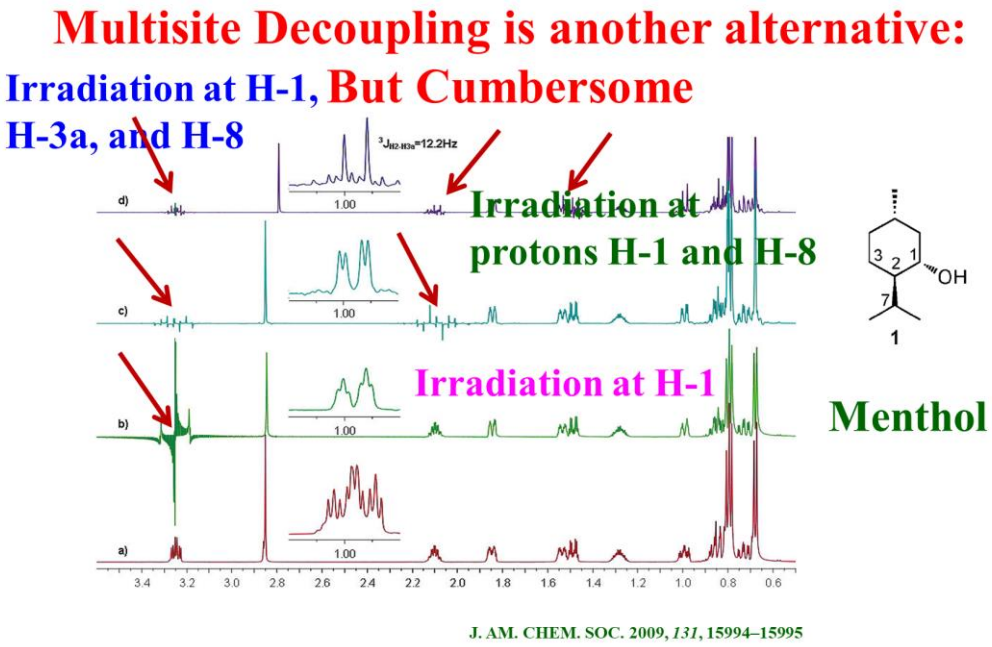
okay. I lost the coupling information, but you see I simplified the spectrum. The spectrum which was a doublet became a singlet. This is a frequency selective decoupling. For a small molecule, it is fine, you can do it. What happens if your molecule is very big, with N number of protons? With large number of protons the frequency selective decoupling is a challenge. First of all you have to have a perfect frequency selection, and you have to apply RF so that neighboring peaks are not disturbed. And also it is strenuous experiment. You can do several such experiments. Hundreds of such peaks you have to irradiate. that is okay, but it is a difficult job. How we do is like this, select the two protons which are coupled , let us say I am irradiating X proton, see at the doublet of A, like what I showed in the previous slide. When we do that depending upon the RF power where we are hitting and what is the RF power, the decoupler offset I have to set perfectly on the other coupled partner.



For example this is a doublet, I have to hit perfectly the center of this one. Then you will see the change here. But if the decoupler offset is far away, then the efficiency will not be very good, so decoupler won't be efficient.

You see  here, as the

decoupler offset becomes larger and larger, the coupling will not be removed. The only thing is there is reduction in the coupling bit, bit. Only when it is exactly on resonance, at a particular chemical shift of the coupled partner, then you will hit that with second RF. Then you are going to get a singlet, then you have broken the coupling. So it is a selective decoupling. See when you do selective decoupling like this, there are many complications like this. This type of thing is called Bloch-Siegert shift. You don't worry about it, so this is what we discussed in one of the previous courses, I will not go into that. Also we can do one more experiment, called multi-site decoupling.



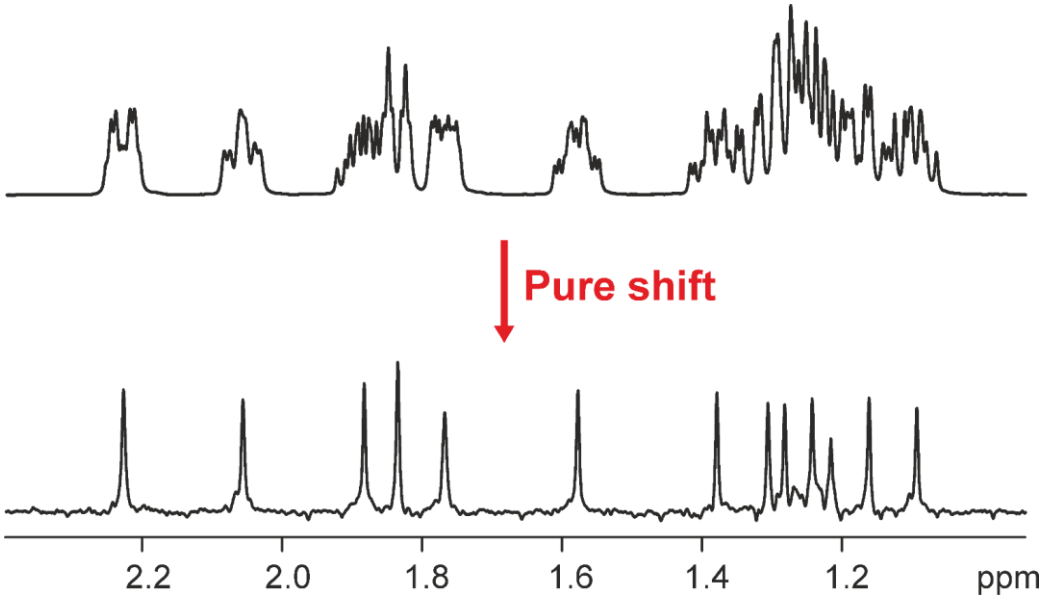
It is another experiment but little cumbersome experiment. Why it is cumbersome? take the example of menthol. Here you see we we can look at the proton here, one of the protons, look at this region here, in this region, which is expanded here. The peak looks like quartet of a triplet or something like that, whatever we are getting, it looks like a pentet of a triplet, some funny pattern is there. This is coming from which region? we do not know, which proton is this? it is experiencing coupling with several other protons. Now I want to identify and simplify this complexity. we selectively irradiate the proton H1, here. And you see changes here. This became two triplets, doublet of triplets. Further selectively irradiate at proton H1 and H8, which is H1? This is H1, and this is H8. You see now the complexity became much better, instead of doublet of triplet it became doublet of a doublet. Go further, you can saturate this. You irradiate at, H1, H3a and H8, all the three. Then you are going to get the coupling between two protons. That is now a doublet, you can simply measure the separation and get the coupling, the three bond coupling. See this is very easy, but what we did is multi-site decoupling. Wd have

irradiated with RF power simultaneously at three protons, and every time we saw the change in the complexity pattern. And finally we are going to get a doublet like this. Then measure the coupling in a straight forward manner. This is on one way. And of course we can also do the broadband decoupling of protons, that is possible by adopting certain experimental methodologies. Not straight away by hitting with RF power like this, as I told you decoupling of proton while detecting proton is not a easy thing, it is a challenge. Nevertheless, one of the easiest ways you can think of is to do the broadband decoupling. What is the advantage ? why we have to do? The advantage is the resolution, I will tell you what is the resolution you are going to get. Consider a molecule like this.

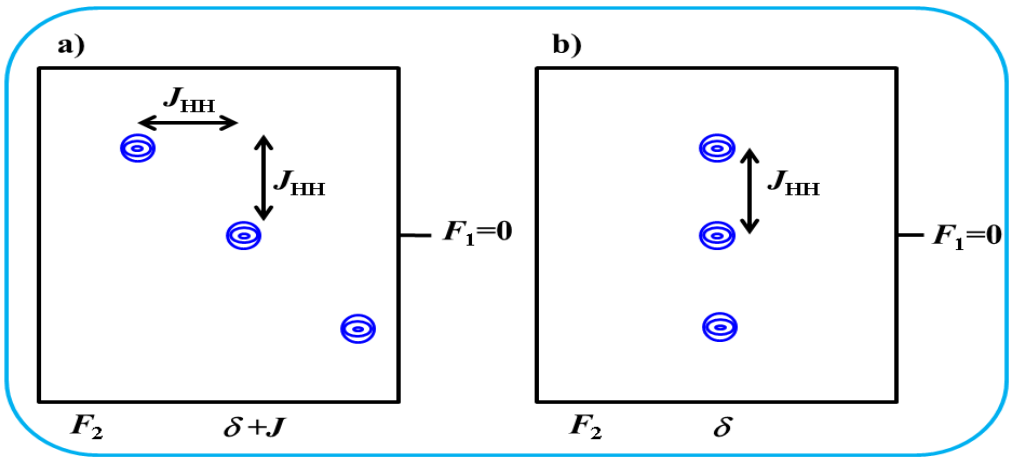
Resolution improvement due to decoupling

$H_3C\text{-}CH\text{-}CH_3$ $\quad J_{CH\text{-}CH3} = 10$ Hz

I look at proton don't worry about symmetry and everything, assume that symmetry is not there, X or Y are there or something like that, if you break the symmetry, assuming that no symmetry this CH proton is coupled to two CH3 protons of equal strength. I will say 10 Hz, then it is going to be a septet. The septet is a seven line pattern. Each peak is separated by 10 Hz. So what is the width from one one end of the peak to other end of the multiplicity peak. If you consider, it is going to be 7 lines, it is 70 Hz. Assume that I am going to do the decoupling. And after decoupling the natural line width is 2 Hz. What does it mean? from this end to this end there are seven peaks, you measure the separation each is, 10 Hz, and 10 hertz like this this let us say about 60 hertz or whatever you get and then if you do the decoupling, you get 2 Hz line width. That means this multiplicity pattern has been reduced to a single peak of line width 2 Hz. What does it mean? it amounts to telling that the line width has come down by 30 times, the reduction in the line width is 30 times. Alternately I can say I have got the high resolution spectrum of the same thing without decoupling at 9 GHz, analogous to that. If you get a high resolution spectra at 9 GHz, then you know this multiplicity pattern will be highly dispersed with only 2 Hz line width, then it is easy to see that. So analogous of this decoupling, the broadband decoupling can be compared to the recording the spectrum in a very high field magnetic field, high field spectrometer. So the resolution improvement is almost an order of magnitude. And this what it is in a pure shift NMR. If you do that broadband decoupling this is the spectrum, complex spectrum look at this complexity in this region, when we do this broadband decoupling each peak correspond to single peak. There are so many protons are there each of them gives rise to a particular chemical shift of each of them. So all this multiplicity patterns disappears, and we are going to get this. It is as good as telling I have got this is a spectrum, let us say instead of at 500 megahertz, I would say I have obtained this spectrum at 5 GHz. If you obtain this spectrum at 5 GHz you get this type of resolution, you can imagine like that. It is just a comparison.



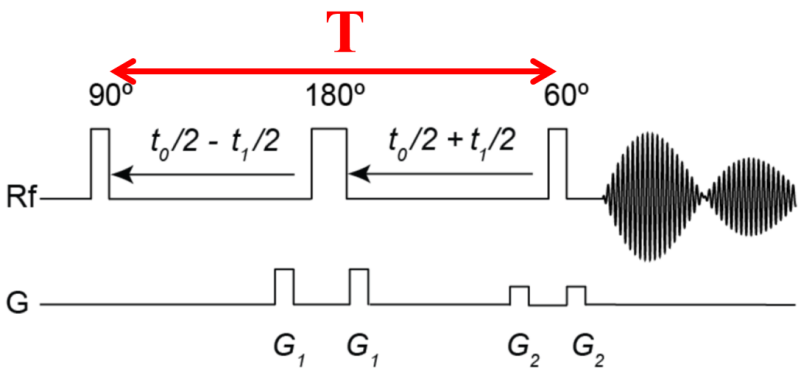
All right, let us say I want to do the broadband decoupling, what are the approaches? and how do I do that? When I say broadband decoupling is a challenge, how do I do that? There are two ways of doing. In the 2d experimental way, where I can do the broadband homonuclear decoupling in the indirect dimension, or I can also do the broadband decoupling during acquisition, which is even more challenging. Just now I said during acquisition you cannot do the broadband decoupling, it is a challenge. But of course we can do somehow. There are two ways of doing broadband decoupling, homonuclear decoupling, both in the indirect dimension and also in the direct dimension. All right the way you are going to get broadband decoupled homo decoupled proton spectra are called pure shift spectra. That means couplings are completely removed, what you are going to get is only chemical shifts for each chemically inequivalent proton. A single peak we get for each chemically inequivlaent protons. And that type of spectra we saw. Just now I showed you, they are called pure shift spectra. So this is a way you can get get rid of coupling information and get sharp lines with highly resolved spectrum. This is called pure shift spectrum. Okay. And what is the simplest way of doing this broadband decoupling?



Remember when I talked about j-resolved experiment I said in the homonuclear j-resolved experiment, we saw that there is delta plus j information in the direct dimension and J information in the indirect dimension. And the spectrum we saw is tilted by 45 degre. If you do the tilting by 45 degree and take the projection, we get a single peak. For example, the triplet appears like a single peak. So F2 projection of the 45 degree tilted J-resolved spectrum is nothing but the broadband decoupled proton spectrum. It is proton broadband decoupled proton spectrum. That is one way. So I can call it as a pure shift spectrum because there is no j coupling information. So you can have N number of peaks, N number of couplings, do the J-resolved, tilt the spectrum by 45 degrees and take the projection. This is what is called a pure shift spectrum. Though it is simplest possible way, but there are certain issues in that when you have strong coupling effects, etc. We will not go into more details, but what is done in the pure shift experiment is something important spins are manipulated in such a way the average evolution of the magnetization is only under chemical shifts. We have to manipulate spin dynamics such that the magnetization evolves only under the chemical shift and the J couplings are removed or refocused. Or it should not evolve. Thus something we can do, we will ensure j couplings do not evolve or refocus even if it evolves, somehow gets decoupled. That is the way we can do. But to do that or to prevent the evolution of J couplings, there are two possible ways. One is refocusing of j evolution, other is what is called a constant time experiment. When you do constant time experiment J couplings do not evolve. When the J couplings do not evolve, it is as good as only chemical shift evolution. That is one way you can refocus J couplings, prevent their evolution. Otherwise you do constant time experiment where J couplings do not evolve at all. These are two possible ways. There are many ways so you can these two things i am telling you. If you do the j refocusing what we do, first the spins are inverted. Select one of the spins, which is an active spin, all the other passive spins phases are inverted. What will happen? all at the passive spins are inverted active spin phase remains unaltered, remains same unchanged. Then what will happen active and passive spin coupling is broken, that means it refocuses the coupling between active and passive spins. What it means is, in J refocusing experiment, simply understand spins are inverted, the passive spins are inverted the phase of the active spin remains unaltered. This refocuses the coupling between active and passive spins. That is what it is. Then we say active spins are decoupled and are detected. All right, how do you acquire the data with J refocusing, is the next question. We can do J refocusing, let us say. How do you acquire data, while doing J refocusing? What we do is we acquire chunks of FID. Okay, and that is acquired independently for a particular time, which is 2/J with J refocusing. If J is refocused at midpoint of each, we have a different chunks of FID chosen and series of chunks are acquired, concatenate each of them and construct a composite FID and do the Fourier transformation. That is one way. It is like collecting the chunks of FID, and each time at the midpoint of it we have to ensure that Js are refocused. Take several such things, artificially you concatenate, bring them together

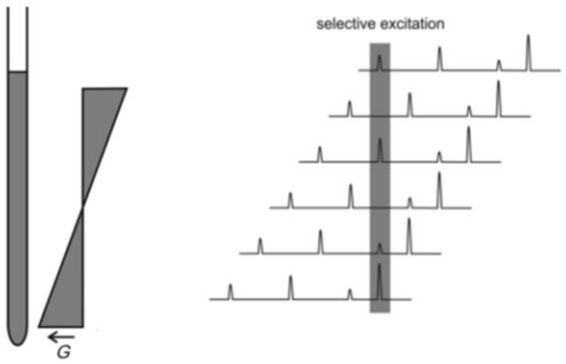
construct a composite FID, composite all these things as in FID, and do the Fourier transformation. That is what we do, then J couplings in such a situation will not evolve, and the fid has only chemical shift evolution. The J is refocused at the center of each of the chunks of FIDs, small small FID chunks you take and at the center of each of them J couplings are refocused, only chemical shifts will evolve. That is okay. So concatenated FID is Fourier transformed and we get the pure shift, and large number of such chunks you take, of course large does not mean a big number, about 20, 30 or 40 chunks is more than sufficient beyond that FID would have died down. Even 30 or 32 chunks is very difficult to get. But if you take that many number of chunks, you get narrow peak. if you have a smaller number of chunks, you get broad peaks. So narrow lines will come if you have large number of chunks. And of course the chunk size also matter. The shorter chunk size will generate cleaner spectrum as the maximum deviation from j coupling, exact j coupling is reduced. There won't be much deviation.

And there is also what is called a direct detection approach, that is very important very interesting thing. In this case while acquiring the signal we can apply J refocusing elements in between the FID. I am collecting FID like this, in between I can put J refocusing at several places and collect the FID. At these places what will happen, if there are N number of such things applied in between while collecting the signal. In each of these elements, the J gets refocused, and whole FID is acquired in a single shot. But then while acquiring the data J is refocused. So there are several J refocusing elements. What are the refocusing elements? like BIRD frequency, anti-Z-COSY, time reversal methods, low flip angles, etc., so many things are there. Each of these J refocusing element reverses the effect of J and then suppressed at a given evolution time. That is important thing. Of course, constant time experiment is what is normally done. Of course very often in the constant time experiment what is done, the evolution time is kept constant in the 2D experiment. In the 2D experiment, I told you there is evolution time and detection time.

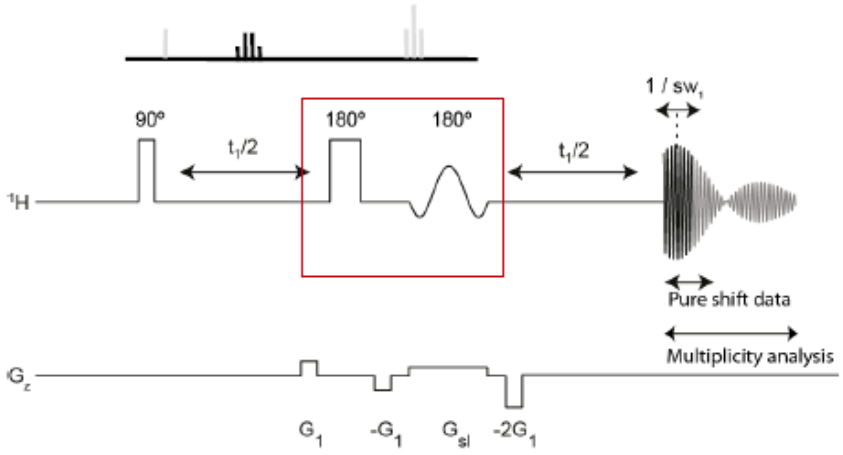


In this case, the evolution time is always kept constant, with a particular delay. Let us say T. In between we apply a 180 pulse, you know it like a spin echo. Whenever I have a 180 pulse in between, the homonuclear, you know the chemical shifts get refocused. J will continue to evolve. But what you should do is, don't allow the J to evolve. You apply 180 pulse at the center. Instead of incrementing the t1, you change the position of the 180 degree pulse. Don't increment t1, you change the position of the 180 pulse, keeping this total period constant. In which case what happens, the 180 pulse is positioned after, let us say particular time, followed by this delay. This ensures then there is no J modulation. So chemical shift only evolves during t1. It is called a constant time experiment. The homonuclear scalar couplings are not in influenced by 180 pulses and evolves during total delay, but then coupling is not getting modulated as a function of t1. So evolution of J coupling in the indirect dimension is completely prevented. This is called a constant time experiment. Then you may ask me a question what about carbon-carbon homonuclear couplings. 13C abundance is very small, the carbon 13- carbon 13 coupling is one in 10,000. Generally we will not worry, so they are not popular. They are very popular only in the constant time HSQC experiment but not for homonuclear experiments. If you collect the FID of such type of experiment, and do the Fourier transformation, you get a 2d spectrum. This is a pure shift spectrum. In the constant time experiment t1 dimension is kept constant and then acquire the 2D data, keeping T constant, moving the 180 degree pulse in such a way you will have the incremented t1 but total period remains constant, and do the double Fourier transformation. In the indirect dimension you are going to get 2D spectrum which is pure shift and you get conventional spectrum in the direct dimension. And also another way is BIRD decoupling. We discussed this when we discussed the carbon 13 NMR. What does BIRD do? I told you BIRD decouples carbon 13 bound protons from carbon 12 bound protons. That also I told you. It selectively inverts carbon 12, pictorially we understood, through the vector diagram how it works. The carbon 12 bound protons are selectively inverted while carbon 13 bound protons are unaffected, we saw that when we discussed BIRD, especially BIRD decoupling, when we try to do HSQC, HMQC, etc. Following that INEPT filtering is done to suppress the signals arising from carbon 12 protons, which are not decoupled. Finally frequency and spatially selected decoupling you can do. Another experiment, you apply a linear gradient along the sample. That is very interesting. When you apply a linear gradient what happens? the different regions of the sample experience different magnetic fields. For example this is your NMR tube, apply a linear gradient along this axis, then each part of the sample has a different magnetic field, because there is a gradient. When you have different magnetic fields, the resonating frequencies are different. So you get location dependent frequency shift. Then you know each part of the sample experiences different magnetic field. So this results in location dependent frequency shift. And the frequency and spatially selected decoupling one can do, by

concept called Zanger Sterk method. This is a very famous experiment, given by great scientists Zanger and Sterk.

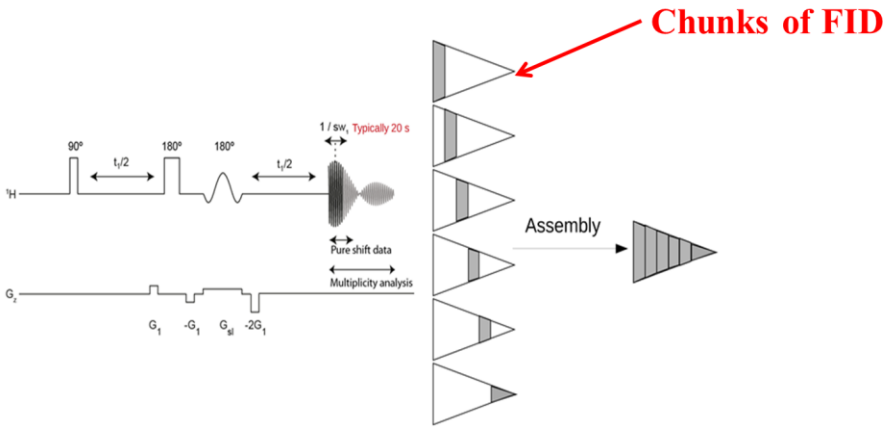


And what they do is like this, sample is there in the NMR tube, apply a gradient along this axis, and then a selective pulse during the gradient. Then what happens different parts of the sample in different slices, different spins are excited. Essentially the whole spectrum is excited by a selective pulse during gradient. This is what you can do. You can apply a selective pulse during gradient and excite the whole spectrum at a time. And the selectively excited spins are decoupled by inverting all other spins. How do you achieve this? For this the proposed pulse sequence of Zanger and Sterk. This is achieved by the combination of pulses hard and soft 180 pulses of the same strength, during the gradient field. And this is what the pulse sequence of Zanger and Sterk, the combination of hard and 180 soft pulse, both are applied.



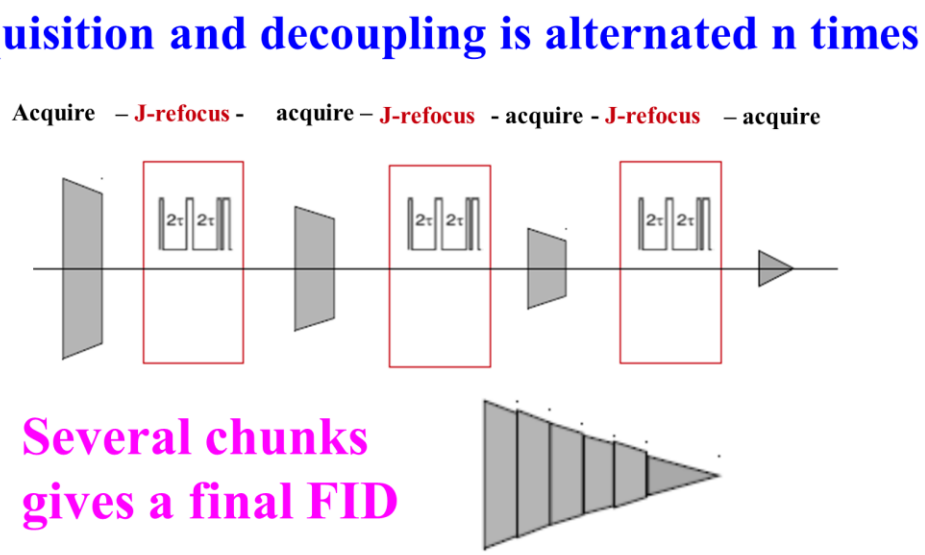
And then this period is kept as t1/2. When you select apply this 180 pulse hard and soft pulse we can do selective decoupling here. As I said you can do the selectively invert all other protons you can do the decoupling. The homonuclear decoupling can be achieved.

And you may ask me a question, fine, we can do the decoupling. What is the strength of the gradient? how much you have to put? These are all more of the details, you don't worry about it. Finally I tell you how to create a 1D FID. The scalar couplings, you should know evolve slowly compared to chemical shifts. Why? because chemical shift interaction strength is larger than J coupling, which is of the order of 5 to 10 hertz or 15 hertz for protons, homonuclear. Whereas, the chemical shifts of the order of several KHz, so they evolve faster. Scalar couplings evolve very slowly. As a consequence, we collect the free induction decay, the initial portion of the FID for a short time, before the J coupling starts coming into the picture. If you collect FID, then only you have chemical shift information in the initial portion of the FID, the J couplings take some time to evolve. Here in the beginning portion of the FID, the chemical shifts would have evolved, collect only this much then there won't be any interference of the J coupling. So this is the important idea. To do that how do you create the FID. The initial points of each FID is taken arranged as a new FID consecutively, and then you do the Fourier transformation of the new FID. This is how it is done.



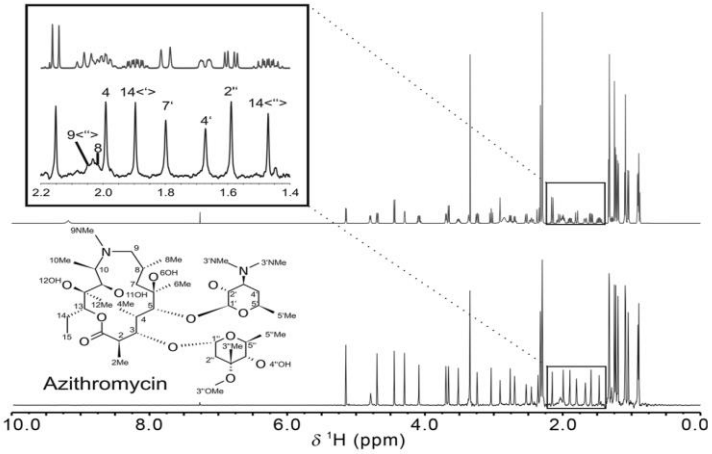
The different FID chunks are taken, assembled here like this, and then do the Fourier transformation in the indirect way. Several times you will have this for t1, and each time you collect the FID, the different chunks like this, and then after assembling this, you will get a real assembled chunks of FID like this. And what should be the size of the chunks? Remember this is 1/2SW1. What is SW1? it is nothing but the width of the J split multiplets, the widest j split multiplets in the spectrum. For example I told you the multiplicity pattern is this much, and this spread is 25 Hz, so this SW1, the size of the chunk should be 1/2x25. That is 1/50 Hz. That should be the size of the chunk. Okay. The number of t1 increments also depends upon how many chunks you have. Usually as I told you it cannot be more than thirty two or sixty four. Sixty four also is too much, around thirty two chunks or so we collect, concatenate the FID. This is how it is done indirectly. That is one type of experiment, otherwise I told you the real time spin manipulation as

one of the two two ways of doing. I can do in the indirect way or in the direct dimension spin manipulation. That is what I told you. How do you do the real time spin manipulation, it is very easy way. I can do the homonuclear decoupling by using BIRD or some or something, some J refocusing and acquisition is interrupted after every regular intervals. And we can perform either selective or BIRD decoupling while acquiring the signal like this. This is acquisition and decoupling alternated N times.



See you acquire the signal in between using a J refocusing. Again acquire signal J refocusing in between. Like this keep on repeating this N times, the whole experiment N times. All these several chunks of FID together gives an FID. The final FID is like this. In between FIDs chunk is there. This what is done. If you look at it the real time manipulation is like this. It is a fid in principle if you don't do any J refocusing in between. This is the type of spectrum you are going to get. But we can refocus with the window several times in between, this is a refocus window. And then if you collect this FID you are going to get an FID devoid of all J couplings. You get only chemical shifts. That is nothing but a pure shift spectrum, in which case you don't need a special processing technique. I forgot to tell you, in all other previous experiments where you concatenate the data, assemble it and do the Fourier transformation, there are special pulse programs required to concatenate and do the processing. But in this direct detection, there is no such thing. There is no special data processing technique required. Directly acquire the FID, while in between at different intervals you do the J refocusing, you are going to collect the signal and do the Fourier transformation, get a simple spectrum without j couplings. And this is an experiment where we can do this type of decoupling and at different intervals J would be refocused. This is a famous Klaus and Zanger experiment. The decoupling is achieved by a combination of hard and band

selective 180 pulse, and the gradients of same strength is used during excitation. Of course there are certain troubles in this type of experiment, when you do this pure shift experiment, it is not easy especially when you do this type of direct detection experiment. What will happen is while acquiring and interrupting the FID decoupling due to J refocusing, at the same time magnetization is also relaxing. That gives rise to artifacts and pulses should be very very short. Otherwise especially for large molecules there are shorter t2 relaxation times, the signal would have relaxed before that. So all these things will have to be taken into account. It will give rise to some artifacts in the spectrum, many a times unavoidable artifacts will be there, some can be avoided. So with this I show you a simple a pure shift spectrum of a molecule called azithromycin.



This is taken from the one of the papers of Gareth Morris group. You can see this region is expanded here, this is conventional spectrum this is pure shift. Okay, this is instant homo decoupled spectrum, that means decoupling while doing acquisition and conventional way of Zanger Sterk decoupling. The direct decoupling would have taken more than 10 hours. This is direct detection the same thing what I said like a earlier way we can create a 2D way by indirectly either by using ct constant time experiment or j refocusing elements, and do a 2D experiment. That would taken lot of time. But this convention direct detection experiment while you are are interrupting the fid simultaneously at different intervals for J refocusing. This would take only a small time. And this is a typical experiment, you see this is a TOCSYspectrum.

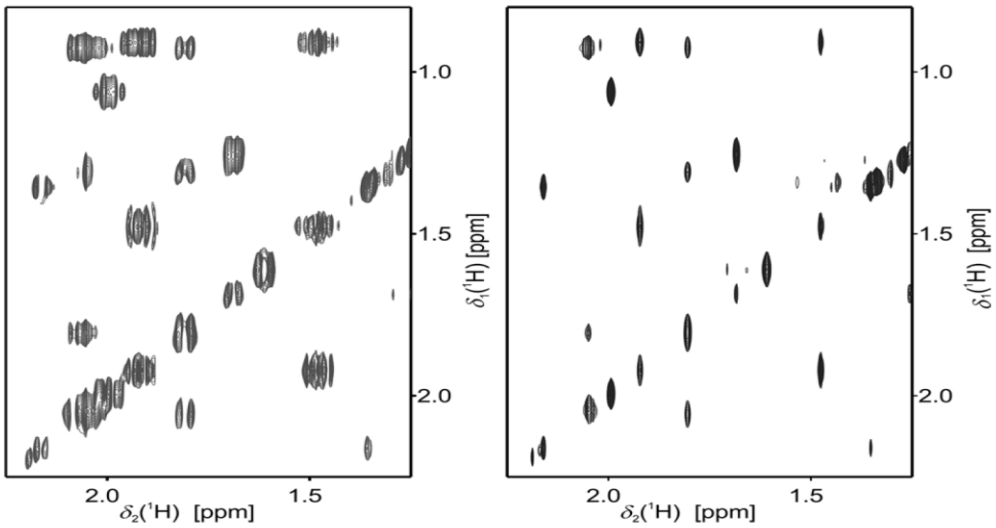
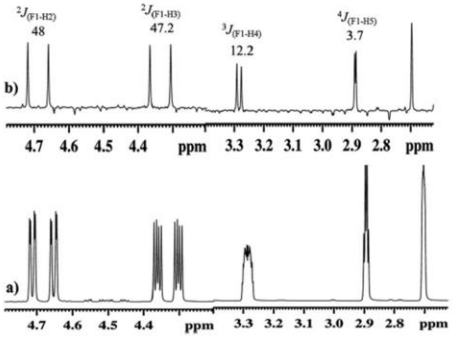
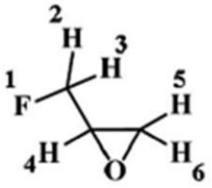


Figure 4. Expanded view of a regular TOCSY of 40 mM azithromycin in $CDCl_3$ (left) and an ω_2-decoupled one (right). Spectra were acquired with a mixing time of 60 ms and 256 increments in the indirect dimension. Total acquisition times were approximately 2 h for the regular TOCSY (8 scans per increment) and approximately 20 h for the homonuclear decoupled TOCSY (64 scans per increment).

I tell you where do you find the utility of it. In a simple example in our own study in our own molecules, this is a molecule we are looking at, if i take the proton spectrum here we have proton proton and proton fluorine couplings, both present in this case. Which are the FH couplings here? in this. You cannot directly say which is FH coupling unless there is only one fluorine coupled to a single proton, but each of them have a proton proton coupling, and also proton fluorine couplings. Look at this spectrum. This spectrum is pure shift spectrum, where homonuclear HH couplings are removed. Then what are you going to get here? you get only FH couplings, Now if you measure the separation you will get the FH couplings, the direct extraction of FH coupling is possible in the pure shift experiment, okay.

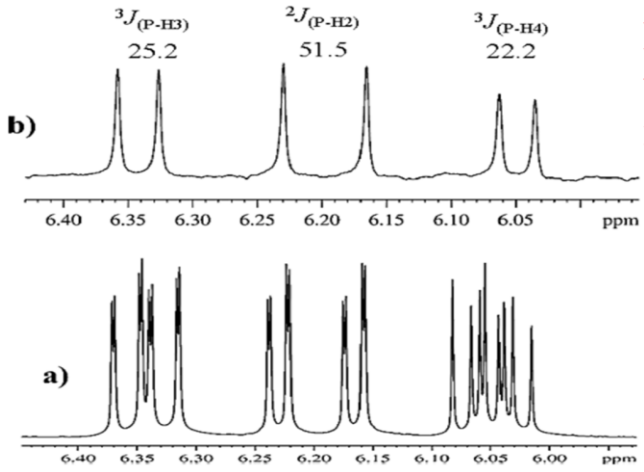
Pure shift spectrum when heteronuclei are present



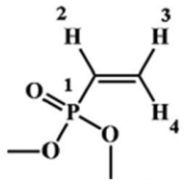


Epichlorohydrin in $CDCl_3$

So this is another advantage and look at this molecule we have phosphorus here and proton proton couplings.

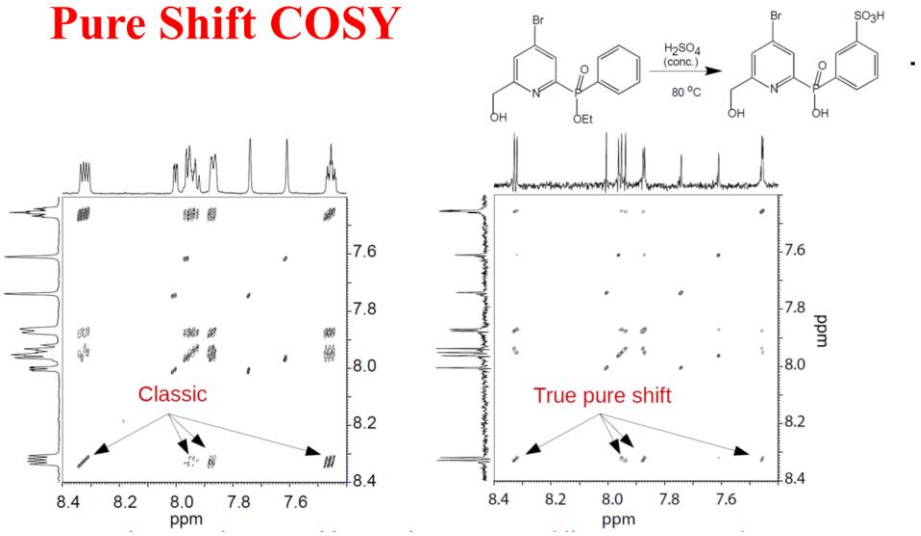


Dimethyl vinylphosphonate in $CDCl_3$

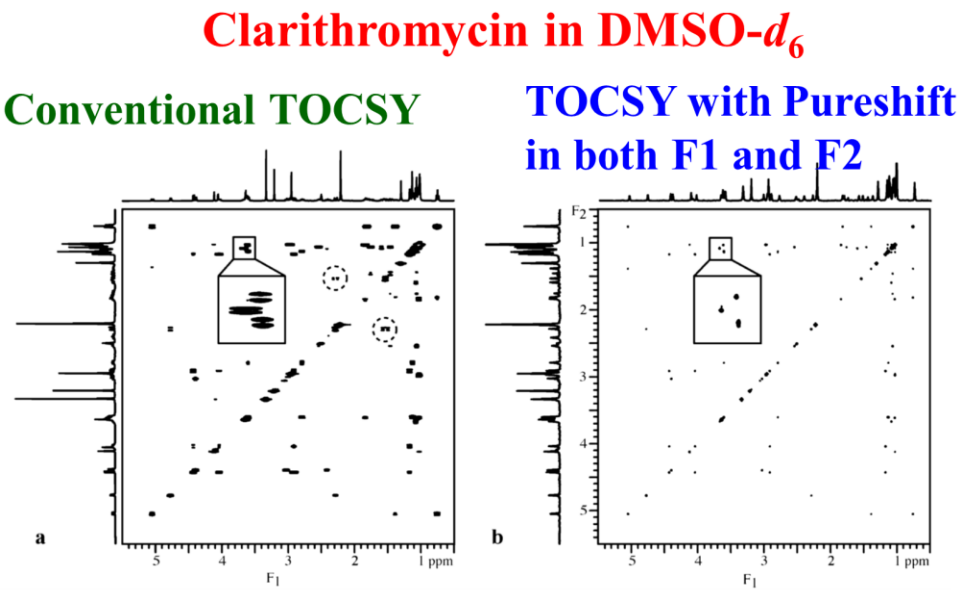


This spectrum has both FP and HH couplings, both phosphorus proton and proton proton coupling complicate this spectrum. Do the pure shift, here HH couplings are removed, each of this if you measure the doublet separation gives you a phosphorus proton couplings. That is one advantage. So all these pure shift methods have their own benefits and limitations and there are many many improvements available and PSYCHE is the best experiment of pure shift, available. I have not discussed PSYCHE. There is no time. An interested student can see that. But all these 2D experiments also come with the blend of all these pure shift experiments, the blend of 2D experiments. For example, I can have pure shift COSY, pure shift TOCSY, pure shift HSQC, pure shift ROESY, pure shift NOESY, pure shift DOSY, all these experiments are possible. Okay This is a pure shift HSQC experiment. This is HSQC. See the line broadening coming because of coupling. This is pure shift, see the sharpness of the lines.

And look at this one, it is a pure shift COSY. Look at the multiplicity pattern here and here. A simple combination of 2 experiments, so combining together is a blend of 2 pulse sequences, you get a pure shift COSY experiment.



Similarly this is a conventional TOCSY, look at the broadening of the peaks because of multiplicity pattern. You look at this, these are all TOCSY with pure shift in both the dimensions. Double pure shift TOCSY.



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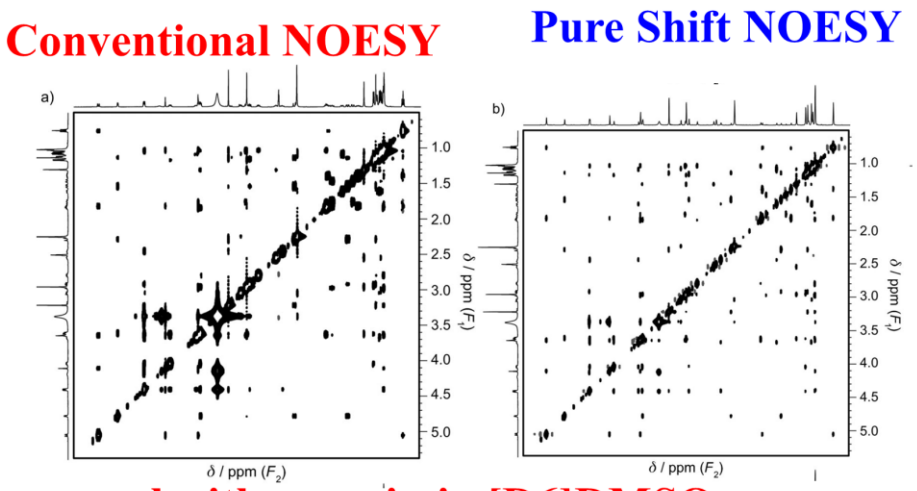
And this is pure shift NOESY. This is the conventional NOESY, this is a pure shift NOESY, look at the sharpness of this signal.



These are all the works of very famous people like Gareth Morrois groups, and other groups. They have been doing lot of these type of experiments, and pure shift. So with this I can show you another thing from the same group. This is is the DQFCOSY. This is a constant time DQFCOSY. Due to constant time the J evolution is prevented. Look at the sharpness of the peaks here, so many such experiments are possible. So I will stop here. What I wanted to tell in this class is, another type of experiment called pure shift experiments. There are several things we discussed, how do you do the pure shift, basically remember pure shift means you break all the homonuclear couplings, it is a broadband homonuclear decoupling, which is a fairly a challenging task. I told you you cannot break the homonuclear coupling in a broadband way, and then detect the proton, because it is not possible, it will disturb the transverse magnetization. But there are several ways of doing it indirectly, in a two-dimensional way. What we can do is, we can have J refocusing elements in the t1 period, there could be constant time experiment, BIRD decoupling, varieties of the refocusing elements are there. Any of them can be utilized, then we can have pure shift in one dimension and the conventional spectrum in other dimension. You can also have direct detection experiment where you can interrupt the FID at regular intervals and each interval you can have a J refocusing, and get the FID together. It is a real-time FID. Do the Fourier transformation, no special processing is required. Whereas in the other type of pure shift experiments, you have to collect the chunk for a different time periods. There are several experiments I discussed you have to take collect the chunks of FID assemble it and then do the Fourier transformation. In the direct detection experiment no need of special processing and you can do the J refocusing while acquiring the signal, in the direct detection. And all these pure shift

experiments can be combined with many existing well-known 2D experiments. For example 2D COSY, 2D TOCSY, 2D DOSY, 2D NOESY, etc. In all these things there is an advantage. We saw a lot of advantages in the COSY, HSQC, the signals were very broad because of couplings present, various couplings. But they can be removed with pure shift. And we saw in the pure shift the peaks were very very sharp. These are all advantages. I want to tell you people depending upon what you want to do, if you want to lose the coupling information and get a better spectrum for analysis, sharp spectrum you can do one-dimensional way or in also two-dimensional way. Of course pure shift when you do in a two-dimensional way take the projection and you can find out that each peak corresponds to particular chemical shift of the chemically inequivalent protons. But you are losing coupling information. But there are several experiments we can do the pure shift, also we can reintroduce coupling. This way you can measure coupling while using pure shift also. There is a very big article written by us in the progress in NMR spectroscopy that if any of the student is interested can see. Very recently in 2023 we have published that paper in progress in NMR spectroscopy. So what i wanted to tell you in this class is to give just an idea about new experiment called pure shift, not new experiment which has been in practice for several years, but only few groups are doing, but its utility is enormous. Those who want to do can use these methods. So with this i am going to stop here. I think this will be the last class of this course, I wanted to talk about DOSY, again 2D DOSY experiment where we can utilize diffusion parameter, see molecules in a given system.

You may take a sample in a NMR tube, the molecules can be undergoing diffusion, because of thermal agitation, and the diffusion depends upon various parameters. It depends upon molecular weight hydrodynamic radius of the molecules. And then we can measure the diffusion by an experiment called 2D DOSY, the two-dimensional DOSY experiment called diffusion ordered spectroscopy. Different molecules based on the diffusion coefficients gets separated out. The multiple components can be separated out by using DOSY. That has lot of applications also. If I start now, that will be a big topic, you know although i was thinking of discussin this, because of lack of time i could not discuss DOSY. But any of you who are interested to learn DOSY you can send me an email, one special class only for half an hour I can take to talk about DOSY experiment, which is very useful, especially for multiple component analysis if you are working on metabolomics you want to find out the different components present in let us say body fluids like serum or urine. you can find out by doing this type of experiment. You can separate out the components based on the diffusion coefficient, provided there there is change in the diffusion coefficients. And DOSY is done in a very very different way by applying field gradients. How? I have to explain to you how the defocusing take place how the the refocusing takes place like that. That is a very big topic, we discussed this field gradients, defocusing and refocusing in one of the previous courses. But we can adapt the strategy. If the molecules are undergoing translational motion, and if they are

diffusing, we can measure the diffusion coefficients. There are several applications of that, since lack of time I am not discussing. I tried my best to give you what are best possible concepts and many varieties of applications of NMR. Of course, all these things cannot be covered just in one hour for each of the topics, like DOSY, pure shift. Each of these pulse sequences we need to discuss. Just the summary is you can do this type of experiment simplify your spectral complexity which can aid your analysis, both in 1D way or combine them. Take a blend of pure shift experiment, you can also do it 2D way. With this i am going to stop here. Thank you very much for all of you for attending this course.