One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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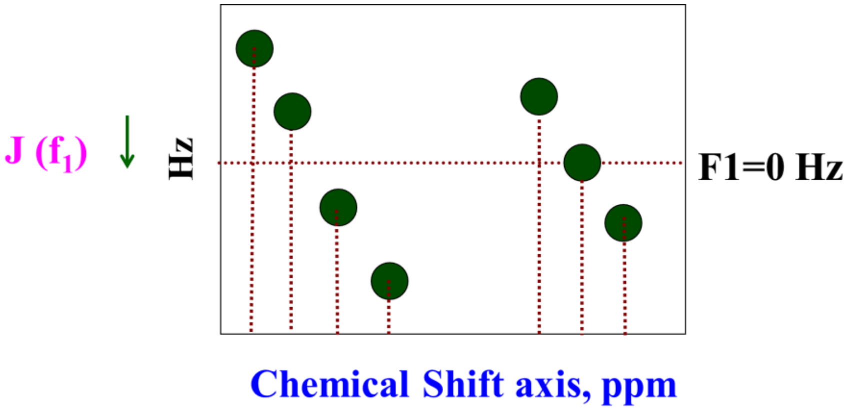
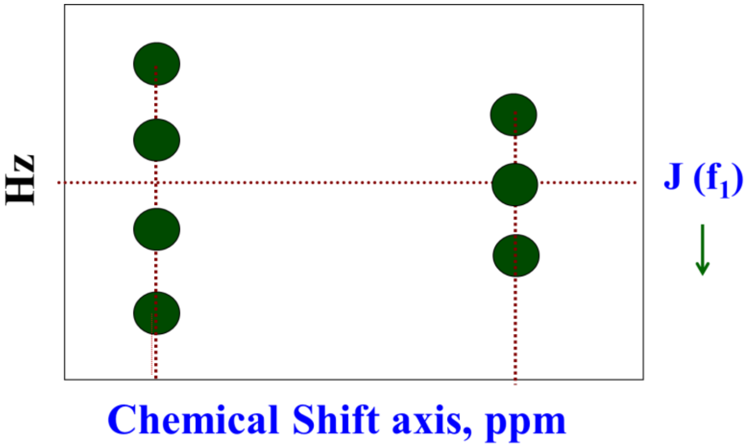
Lecture 50: Homo and Heteronuclear J-resolved

Welcome all of you. In the last class, we started discussing about a new experiment called resolve-type experiment, which is completely different from other experiments we discussed, where we were correlating the information between two abundant spins, or between an abundant spin and the rare spin and also between two dilute spins by using a 2D INADEQUATE experiment. They were all correlation type experiments, but there is another type of experiment, where NMR parameters can be separated out. We have most important two parameters in NMR called J-couplings and chemical shifts. They will be invariably present simultaneously in the one-dimensional spectrum. That will complicate the analysis due to enormous complexity because of N number of interactions for each of the protons or each of the nuclei. Depending upon the interaction strengths the multiplicity pattern will be enormous. So, analysis will be very difficult. One way out is to separate the interactions in two different dimensions. That is what is called J-resolved experiment. We can resolve the two parameters in two dimensions and this is what I discussed about J-resolved. The J-resolved is nothing but a homonuclear sequence spin echo. It is a 90 tau, 180 tau sequence and what is going to happen because of the 180 pulse in between, in the homonuclear case, the chemical shifts are refocused, but J-couplings are not refocused. They continue to evolve. That we discussed long back. So, instead of fixing the t1, vary the t1 and I told you, when I take the example of a doublet, two coupled spins, the intensity of the two peaks keep varying, gets modulated as a function of t1 and also it depends upon the strength of interaction. We saw that. I took another example of CH2 group, an AX2 couple spin system where we monitored the triplet. In the case of the triplet, I said central peaks do not precess. This is what we observed also when we discussed spin echo, only the outer components of the triplet start precessing in the opposite directions. Their intensitities also gets modulated as a function of t1. Their intensity change varies as a function of t1 and also interaction strength. And finally, we observed that when we do the two dimensional Fourier transformation of this, in the F1 dimension, we are going to get the peaks whose separation gives you J-couplings. In the direct dimension, you have both interaction parameters present like chemical shift and J-coupling. And that is why, when we took the example of a triplet and a doublet, we saw peaks were tilted like this. When we saw the peaks, they were tilted by 45 degrees. It is a triplet and of course, in the triplet, because in this dimension, both chemical shift and J-coupling are present, you will see that one peak is moved to the left, other is moved to the right here, from the center of the chemical shift. That is what happens in the 1D spectrum.

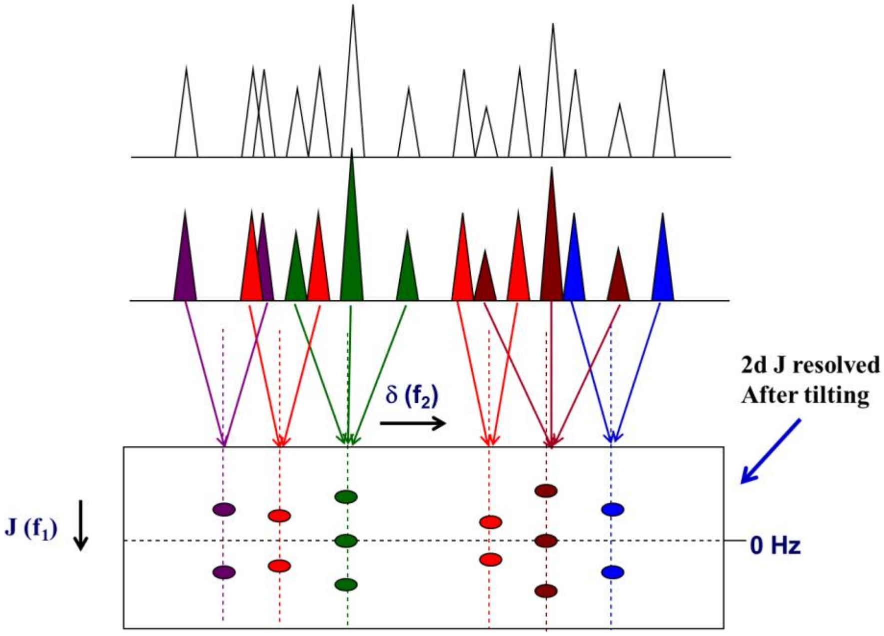


From the center of the chemical shift, one of the coupled peak is moved to the right by half J, and the other is to the right. Of course, in the case of triplet, it is J. This is also J. In this case of this one also, from the center of this, peaks start moving on either side. This is the quartet. It is going to be a quartet. It behaves similar to CH peak doublet, but both the cases, the center of the multiplicity is a chemical shift. Here also, central peak is a chemical shift, but in the indirect dimension, what we can do is, we can measure the J-coupling here between this and this, between this and this, this and this, this and this. That gives a J-coupling. Very easily, you can get J-coupling information.

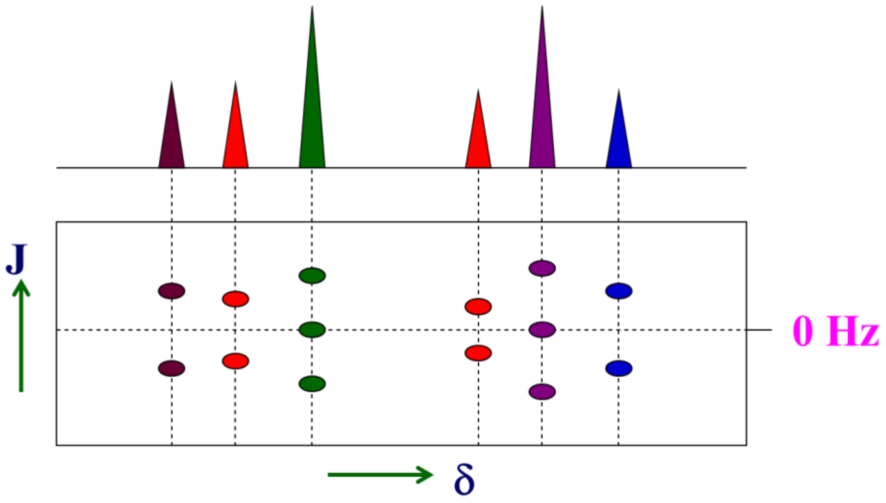
But another thing what we can do is, look here, everything appears tilted by 45 degree. There is a 45degree tilt here. Why cannot I tilt this spectrum by doing what is called processing? I can do a different type of processing. Tilt the entire spectrum by 45 degree, then the peaks which were here started moving these things, peaks which were here started coming like this. For example, in this case, when I tilt it, what is going to happen is, the frequencies will not change. Only this moves here, this moves here and this will move this side, this will move this side, exactly that. Here, this moves this side, this moves this side, this is the center. This is what called a 45 degree tilt.



If you do that, you get a triplet here, you get a quartet here. From the center of the triplet, you measure J-coupling and here, J-coupling is obtained from adjacent separations. Measure the adjacent peak separations, you are going to get the J-coupling. This is called 45 degree tilted J resovled spectrum, not projection, the 45 degree tilting of A3X2 or A2X3 spin system. A2 is going to be a quartet because of X3. X3 is going to be a triplet because of A2 and that is what we see as triplet and a quartet. This is a simple J-resolved experiment. We resolved the J coupling in one dimension and the chemical shifts and J coupling in the other dimension. And by doing the tilting, the advantage is, we removed J-coupling in this dimension also. How it is very useful? Especially in understanding the complex spectrum. You can just see an example like this.

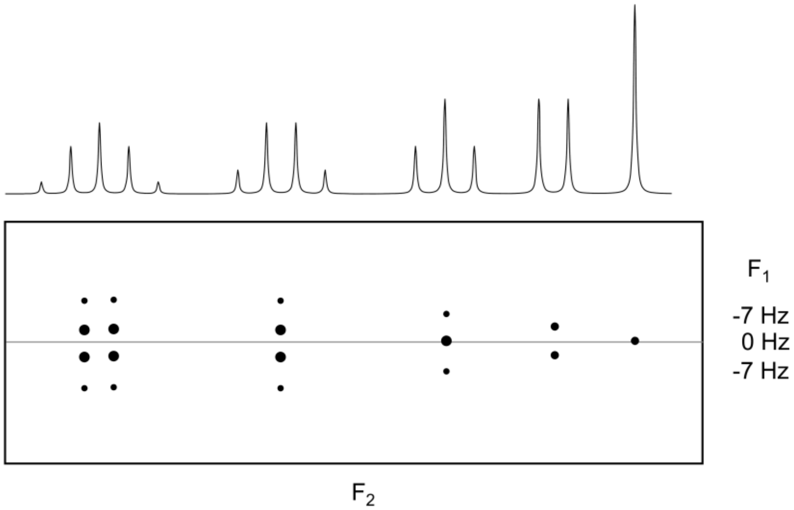


A hypothetical molecule, I am going to put different colors here, the complicated 1D spectrum. This is how it is. That means, I have put deliberately colors, I know this is a doublet, I know this, this and this are triplets, this and this are doublets, this, this and this are triplets and doublet and triplet and deliberately it is numbered. And you can see that there are overlaps here. There is a complexity, spectral complexity is too much to extract the coupling information. This is where J-resolved comes into the picture. What we do is, we take the J-resolved and tilt it by 45 degree. If I do not tilt it by 45 degree, you can still get peaks like this. It can still be okay, but then we are tilting it by 45 degree. Advantage is, you can see that these two peaks which were a doublet is here, this and this peak which were doublets here, the triplet comes here, doublet, triplet and doublet. Such a complex spectrum here, what we did, we simplified it by just removing one of the parameters, resolving it, taking it away, putting in different dimensions. Our analysis becomes simpler. There you will get chemical shifts, and measure here, you get J-couplings. This is the biggest advantage of a J-resolved experiment where the spectral analysis gets drastically simplified. Another biggest advantage is the projection of the 2D spectrum on the F2 axis, in the direct detection axis, gives rise to what is called a broadband proton decoupled proton spectrum. Remember, we discussed broadband proton decoupled carbon 13 spectrum. What did I say at that time? We can irradiate at the second radio frequency, at the center of the proton spectral width with a certain power larger than the coupling strength. Then we can break all the carbons coupled to all the protons, broadband, in a single experiment. That is what we discussed. That is easy. That type of heteronuclear broadband decoupling is very easy to do because the spectrometer frequency is different, detection frequency is carbon 13 and irradiation frequency for decoupling is proton frequency. They are several MHz apart, easy to do. Whereas, if you want to do the broadband decoupling of the proton while detecting the proton, it is not easy. It is very, very challenging because when you are decoupling, you have brought this magnetization into XY plane, you will completely disturb it when you apply RF pulse for decoupling. It is not easy. It is very, very challenging. But if we can decouple somehow, it is possible for us to get the coupling information in one dimension and chemical shift in other dimension in a very simple manner. That is possible, but the simplest experiment you can think of for such broadband proton decoupled proton spectrum is taking the projection of the J-resolved spectrum on the F2 axis. This is what it is. We take the projection. Earlier, we saw doublet, triplet, everything was there. It is a complex spectrum. Now, what we did? We took the projection. See, look at it.



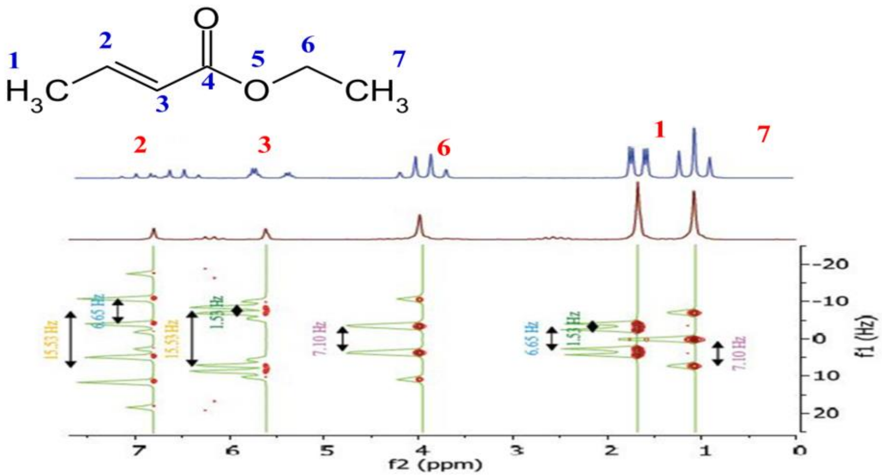
This gives you chemical shift. This gives chemical shift of this proton and this gives you J coupling. What did you do here? There is HH coupling. J_{HH} is completely removed because you are removing all proton-proton couplings here. It is broadband decoupled.

So, directly you can get the chemical shifts. This is the beauty of this experiment. 2D J-resolved, if you take it and take the projection of it on the F2 axis, you are going to get chemical shift of all the protons. Fantastic, broadband decoupled proton spectrum. It is an example, a 45-degree tilted spectrum.



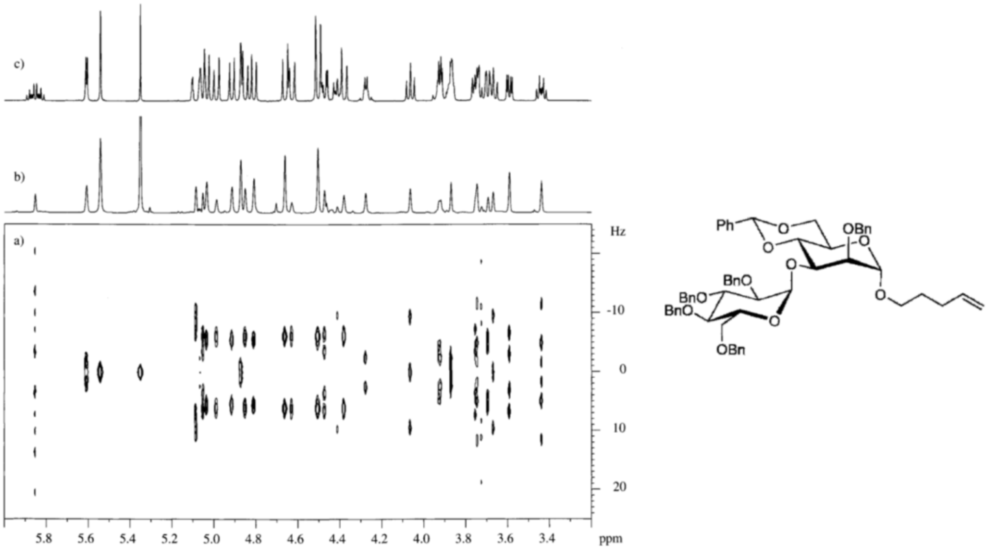
Beauty, what you can see here is, it is a pentet. This pentet comes because of two peaks which are overlapped, but they are two different chemical shifts. Look at it. 1, 3, 3, 1, one quartet, 1, 3, 3, 1, one quartet, but each of them has different chemical shifts here. That you can extract easily. Two quartets, 1, 3, 3, 1, and 1, 3, 3, 1 quartet and this is the chemical shift of one of the quartets here, and the other quartet is here. And this quartet, 1, 3, 3, 1, triplet, doublet and a singlet. Everything is there in a hypothetical spectrum where you know how to resolve it. So, generally what is going to happen is, the homonuclear couplings are between 1 to 20 hertz, not more. HH coupling if you are looking at it, very rarely you get more than 20 Hz, all are within that. So, the type of experiment what you have to do, the experimental aspects I never discussed because it was in the previous courses I extensively discussed about parameters for setting the parameter for experiments. But anyway t1 number of points we required to get the better resolution, especially the 2D-J resolved. If you take 256 points and 512, the spectral width has to be very small only to cover 20 hertz or 50 hertz, maximum 20 or 30 hertz you put it or 50 hertz, the 512 points. The hertz per point becomes very small, you can calculate. For 50 hertz spectral width to be covered with 512 point you have to digitize, your digital resolution become 0.09 Hz per point. That means you can measure the J coupling with very high accuracy in the J resolved, because we do not have to cover a large spectral width.

And this is a simple example ethyl protonate and you see here that a lot of things are crowded here. Of course, looking at the spectrum one can easily assign it. But if you want to do the 2D-J resolved, you can extract it very easily like this. This is a triplet, this is a doublet coming because of various reasons and it is in fact doublet of doublet, you see doublet of doublets are here. This is a quartet, two quartets are here separated out, and easily you can interpret the spectrum. And in a very very complex molecule, this is where the beauty comes into the picture.



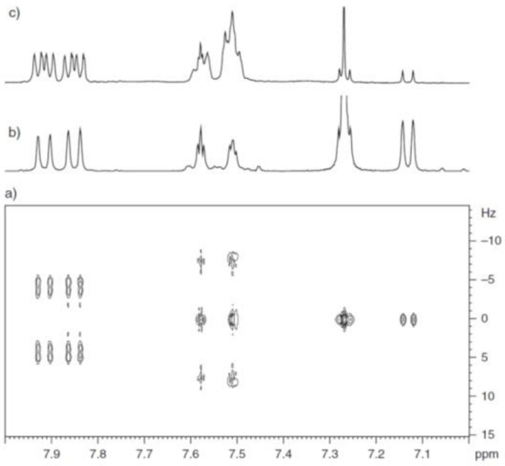
Look at this spectrum of this molecule, very complex molecule. Look at proton spectrum, very complex.

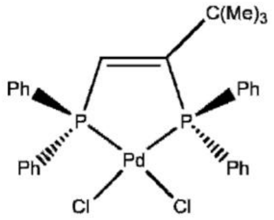
The 500MHz proton homonuclear J-resolved spectrum



You have both J coupling and chemical shifts present here. It is not possible to analyze so easily. Look at this crowded region. See you have to identify which are the coupled multiplicities. If you take two coupled spins which is triplet, which is a quartet, which are the quartet peaks, which are the triplet peaks and their overlap identification is a challenge. But now by doing this 2DJ result, we can assign all the peaks. You see, they are all resolved here. This is the J dimension, this is the chemical shift dimension. Especially when you take the projection of it, you get chemical shifts easily. This is a 45 degree projected J resolved spectrum of this complex molecule. You get chemical shifts here and in this dimension, you measure J couplings. You see how beautifully the complexity of the spectrum could be reduced and analysis becomes fairly simple. This is one more advantage which I wanted to tell.

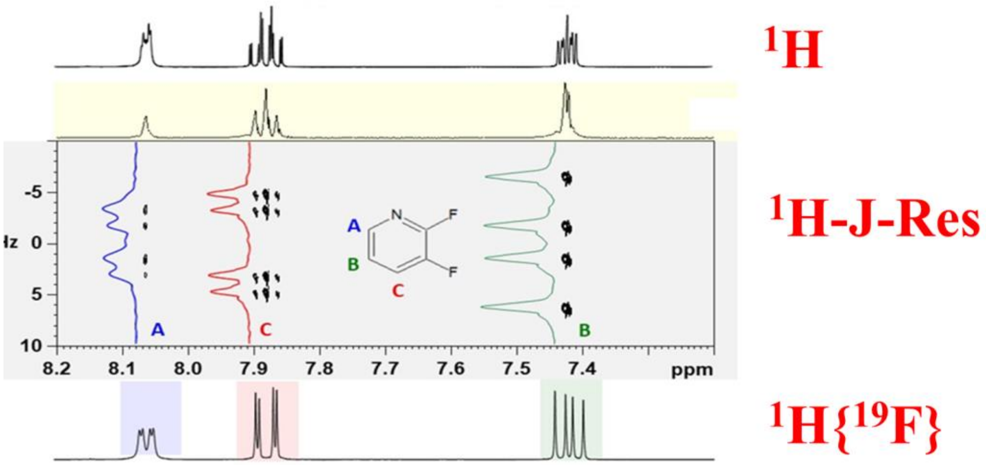
Consider a molecule like this. We have phosphorus, proton, we have proton-proton couplings here and phosphorus is also abundant spin that can also couple to proton. If I take a proton spectrum, we have proton-proton couplings and also proton-posphorus couplings, both are present. So, that means, this is a proton spectrum. You have both homonuclear and heteronuclear couplings are present. What are homonuclear couplings? JHH, and JHP is heteronuclear, both are present. But what we can do is, we can extract only heteronuclear couplings. How is it possible? We have to remove homonuclear couplings. How do you do that? Just now we did, do the proton-proton, J resolved experiment, take a projection.





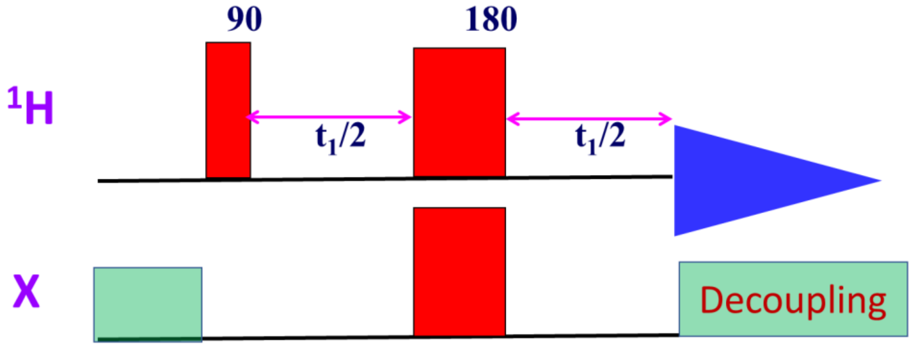
Then in this dimension, you have only chemical shifts and then you have removed the HH coupling, you are getting only proton chemical shifts and you are going to get proton-proton couplings here and here you are going to get HP couplings. See, for example, multiplicity pattern here, center of this correspond to chemical shift of this and this multiplicity pattern correspond to phosphorus proton couplings here also. So, very easily you can measure the heteronuclear couplings by doing homonuclear J ressolved and taking the projection of 45 degree. Tilt by 45 degree and take the projection. This is what you are going to do and this is the simple example of a molecule.

It is a molecule here, 2, 3, difluoro pyridine. There are three groups of protons are here and each proton is coupled to two fluorines.



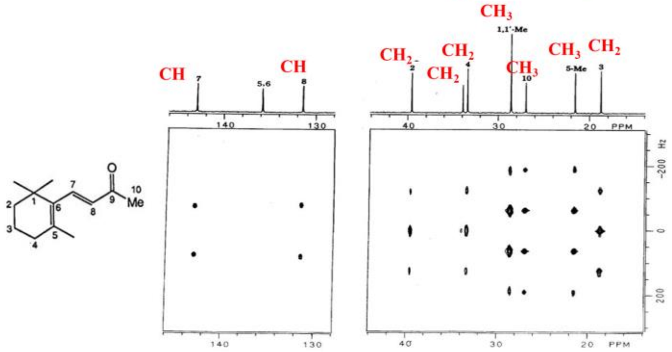
You get HH couplings and HF couplings. This proton can be coupled to this, this and also these two fluorines. Of course, FF coupling you do not see, that is a passive spin, you do not see it. So, if you look at the proton spectrum, three different protons, each of them is a multiplet because of both homonuclear and heteronuclear couplings present. But how do you extract only HF couplings for this molecule? What we do is, we do the proton experiment, J resolved, and take a projection here. When you take projection here, this projection gives me only HF couplings here. Here, this separation gives me HF coupling because proton coupling is removed. Whatever the multiplicity you are getting here as a projection, on this projection, are fluorine-phosphorus coupling. To confirm that, if you do the fluorine decoupling, same four peaks you are going to get here. This is what it is. So, we can get the heteronuclear couplings from the homonuclear J result spectrum and then taking a tilted by 45 degree and take the projection. That helps in getting the heteronuclear couplings. So, these are all homonuclear J resolved experiments and lot of advantages we discussed.

Can we get a heteronuclear J result also? Everything is possible in NMR, not everything, I would say not difficult at all. Why not we get the heteronuclear J resolved spectrum? What we have to do? This is a very simple pulse sequence, we know that. When we have proton and X nuclei, we discussed this also during spin echo modulation and spin echoes. When we apply both these things, 180 pulse simultaneously, both J will evolve. We know that we have discussed a lot about it. And of course, this is a sequence which we did for homonuclear J resolved. Simultaneously here, we are also doing decoupling here. When you are decoupling X nuclei, what is the advantage? You are breaking the coupling between carbon and proton, get individual resonances plus there is an NOE enhancement that will also come into picture.



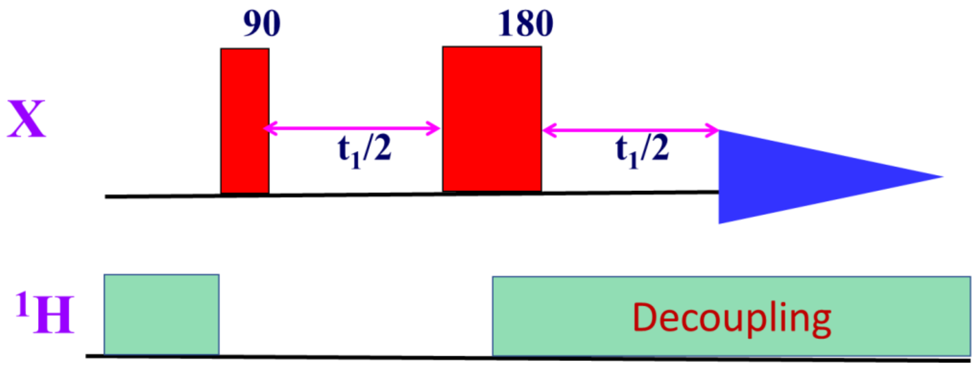
This is a sequence. Simultaneous 180 pulse on 1H and 13C refocuses the carbon chemical shifts, but the heteronuclear J coupling will continue to evolve. That is what I said. In the heteronuclear J resolved, in the spin echo when we discussed, we said simultaneous application of 180 pulses on both the channels, both X nuclei and proton will make sure couplings will evolve, but chemical shifts get refocused. With that idea, that is simple pulse sequence designed, we can continue to do the experiment. And in the t2 dimension, collect the spectrum of heteronuclei like Carbon-13 or nitrogen-15 and do the decoupling of protons. So, this is what happens. What is going to happen? During the spin echo sequence in the t1, chemical shifts are refocused, signal gets modulated only due to heteronuclear J couplings. It is exactly similar to homonuclear J coupling what we discussed. There are several components of the multiplicity, their intensity keeps changing depending upon the delay t1 and the interaction strengths. That continues to happen here also. And there is an experiment for doing this. This experiment is called spin flip or proton flip method. We can do that. We will understand those things later. This experiment is what is called a proton flip experiment or spin flip experiment.

Heteronuclear J-Resolved spectrum, tilted by 45⁰

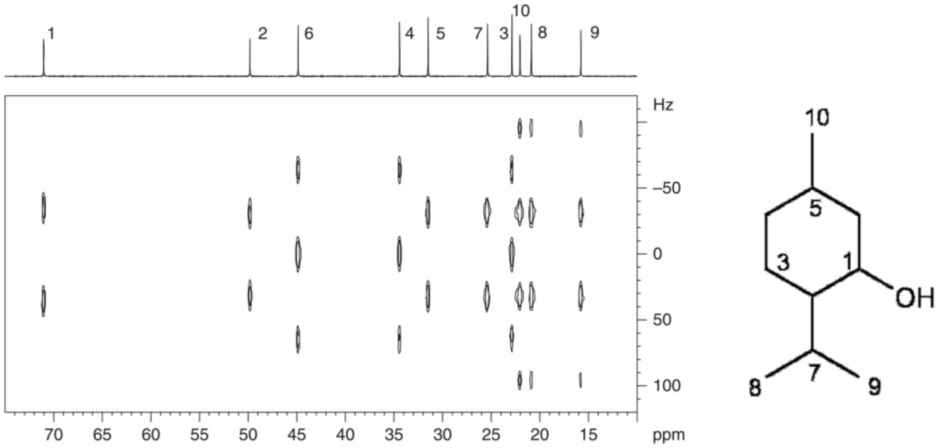


Heteronuclear J result spectrum tilted by 45 degrees like this. This also gives J coupling chemical shift and the F2 dimension. So, when you are tilting it, for example, this is CH2 carbon. CH2 is a triplet because of coupling with carbon with two protons. CH3 is going to be a quartet, beautiful. Again, CH3, quartet, quartet, triplet, triplet and this is CH is a doublet. Very easily, you will measure for this molecule all the carbon-proton one bond couplings easily. Simply do the experiment, tilt by 45 degree, you will measure the J coupling and projection gives you corresponding chemical shifts.

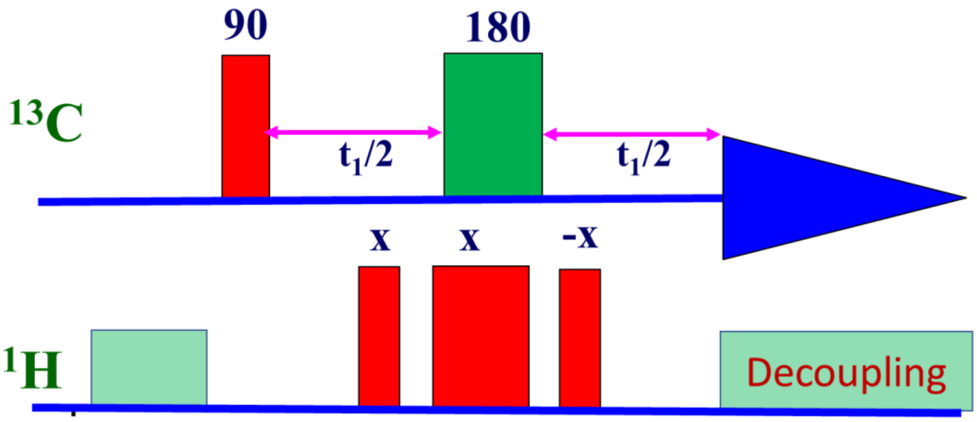
What happens if there is no 180 pulse and only proton decoupling, especially on the proton channel here, there is no 180 pulse. Simply you should understand by now, since we have discussed J modulation at stretch, if there is no 180 pulse, what is going to happen? 180 pulse on X- nucleus refocus chemical shifts and also heteronuclear J couplings. When we discussed J modulation, I said 180 pulse and one of the heteronucleus, will refocus J couplings and also that particular chemical shift. If the 180 pulse is applied on proton, it refocuses the heteronuclear J coupling and but refocuses proton chemical shifts, not carbon. So, but simultaneously application of 180 pulse on both refocuses chemical shifts, but not the J coupling. In which case, if you have to apply only single 180 pulse and not on proton, then it is not a useful experiment, it is a useless experiment that does not give you any information. So, you must apply 180 pulse simultaneously on both proton and carbon channels. So, heteronuclear J resolved can be done by what is called a gated decoupling method.



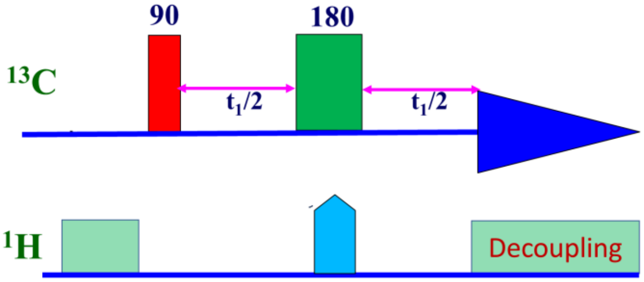
This is what is called a gated decoupling method. What we do here is, in the previous thing, if you see carefully, we started decoupling at the time of acquiring the signal. See here, we start collecting the signal here in the t2 dimension and we apply decoupling power simultaneously and NOE is done here. Whereas here, an experiment is done, where decoupling starts even before during the first half of the t1 period. This is what is called a gated decoupled experiment, where the X-nucleus chemical shifts are refocused due to 180 pulse in the middle of the t1. But heteronuclear J couplings continue to evolve for this period, but not this period. That means, heteronuclear coupling will evolve for half a period, then afterwards decoupling will break it, you will decouple both of them, there is no coupling. So, it evolves only for half a period. What does it mean? The J couplings are reduced by half. It is an experiment, you get enhanced intensity, but J couplings are reduced by half. This is what is going to happen. Look at this heteronuclear J resolved experiment on this menthol molecule, different carbons are identified here.



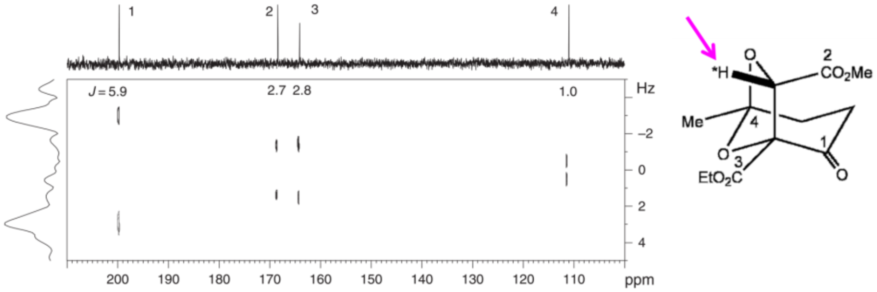
For example, carbon 9 somewhere here, CH3. It is a quartet. But do not directly measure this and say this is a J coupling. It is only half J, because it is a gated decoupled heteronuclear J resolved. I am just giving an example how we can do different types of J resolved experiments. To enhance intensity, we can do this, but only thing is you have to be careful in interpreting. So, the J couplings are not just full valued, but half the value.



And measuring of the long-range J couplings, we can also do. See, we got only one bond J couplings here in all these experiments. Triplet corresponding to this, the quartet corresponding to CH3 coupling. and this is CH and this is CH2, is a triplet like that. But then, what about long-range coupling? That also we discussed when we discussed about carbon 13. For example, this carbon can couple to this carbon, can couple to this proton. Two-bond coupling may be present. Can we get the J coupling, long-range coupling? It is possible to do it. If you want to measure the long-range coupling, certain things have to be done. First, we have to remove one bond coupling, because that will interfere. Similar to your HSQC, HMBC, when you are doing HMBC, you are removing, one bond correlation. Exactly here. One bond JCH has to be removed. We have to remove the evolution of that. That is one of the requirement. Secondly, of course, the advantage of this when you have the long-range coupling is that the spectral width is very small. The digital resolution becomes better. Digital resolution gets enhanced. So, as a consequence, long-range coupling of the order of 10 to 15 hertz, you can measure precisely with a very high accuracy. But only thing is, you have to design an experiment for that. And this is an experiment for that. What it does? Of course, this you know is a homonuclear spin-echo sequence. But on the proton channel, we are applying a BIRD sequence. Yesterday I discussed about BIRD sequence. BIRD is a 90, 180, 90 sequence, like this. What the sequence will do? This sequence when you apply, I discuss this and also explain with the vector diagram, selectively it inverts protons attached to carbon 12. They get selectively inverted. This BIRD has no effect for carbon 13 attached to protons. Only carbons 12 attached to protons are selectively inverted. That is we saw that especially when we were discussing HMBC, I explained this thing. So, this is what is written here. It inverts those protons that share long-range couplings. In this experiment the protons directly bonded remain unaffected, here in this sequence, not in the BIRD I am telling. In the present sequence of J-resolved experiment for long range, this is what happens. It inverts only those protons that share long-range couplings. Protons directly bonded remain unaffected. At the end of t1 period, 1JCH is refocused, only long-range couplings are retained. We designed the experiment such that the one bond couplings are refocused and only long-range couplings are retained. And the F1 dimension gives only long-range couplings here. That is the advantage.

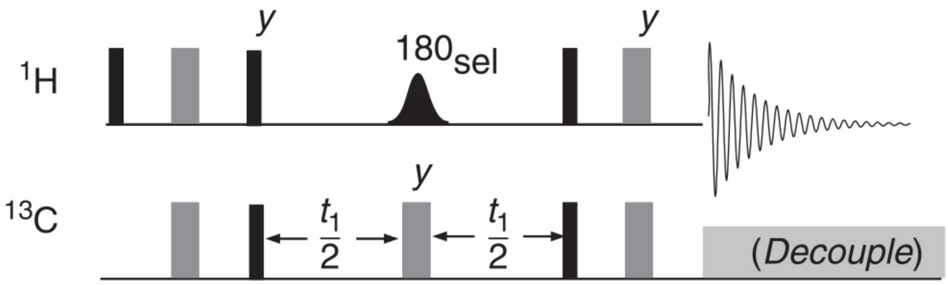


And this is a selective heteronuclear J-result experiment. You can do selective or non-selective both. In the sense, in the previous experiment, here you can do it for all the peaks to get the long-range coupling. They suppress one bond J couplings and all other long-range couplings will be observed. But the same thing, I can ask a question. There are so many protons, so many carbons. Can I get the long-range coupling only for a particular carbon? That is, I have a carbon here and then there are long-range couplings here, here, Can I get only these couplings or only for one particular carbon? It is possible. The same experiment we can make it selective. This is called selective heteronuclear J-resolved experiment, in which case, we apply a selective pulse, not 180 pulse. 180 pulse is selective, not a broadband hard pulse, it is a selective pulse on a particular proton here. When you apply that, then what is going to happen is, selective pulse inverts the selected proton only. All the other JCHs are refocused. Except for that selected proton, all the JCHs are refocused. Only for the selected proton, carbon couplings are retained, long-range couplings are retained. So, indirect dimension always displays simple doublet, triplet, quartets, etcetera, because one carbon experiences couplings with remote proton, long-range couplings. It can be one proton or two protons or three protons. It could be maximum doublet or a triplet or a single quartet like this. This is what happens. This is a selective long-range heteronuclear J-resolved experiment of one molecule is like this.

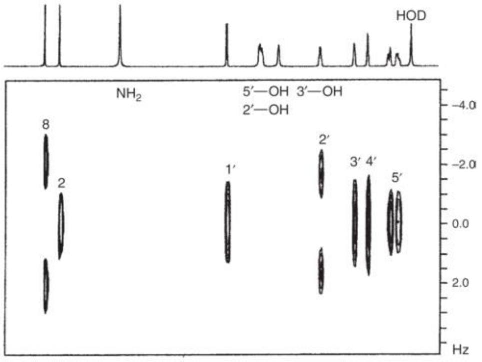


And this is the molecule which is selectively radiated. And there are four different carbons here, 1, 2, 3 and 4. This proton can experience different couplings. What are they? It can experience coupling between 3 bond coupling to 1 here, 2.8 hertz. This separation gives you 2.8. And 2 if you consider here, this proton 1, 2 bond coupling to this proton. For 4, you can consider here, 4 is here, 1, 2, 3 and 4 bond coupling, very small 1 hertz. Measured values, see in the long-range coupling selectively for a particular proton selectively inverts, only for that proton the couplings are retained, for all others are refocused. And we can measure long-range couplings very precisely. And we can use this to distinguish various things like endo form, exo form, etcetera. In fact, in this molecule, it was used to define endo and exo form, because the endo and exoforms of this molecule, the coupling are known for us.

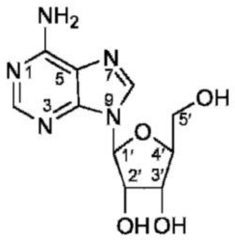
The selective long-range heteronuclear coupling can also be done with a proton detection. But remember here, in this experiment, what we did? Here, we are detecting carbon decoupling proton. Always, I told you, detection of heteronuclei, the dilute spins like carbon 13 is very very time consuming. That is the advantage of doing HSQC. What we did, we detected proton by transfer the magnetization from carbon to proton and back transfer. Why cannot we do that here? If you do that, we can detect the same J coupling with proton detection. Then your experiment becomes faster, sensitivity will go up.



All we have to do is the same. With this, you are already familiar. In an INEPT sequence, heteronuclear J sequence with a selective pulse on the proton, reverse INEPT, trasner the signal back to proton and detect. Exactly what you did for the previous pulse sequence, with a selective proton, you selectively invert and get the couplings to different carbons for a particular carbon. Then what we are going to do is, we can get the couplings to remote or long range couplings, but they are time consuming because we detected carbon 13. Same experiment is done by selective 180 pulse with a polarization transfer and reverse transfer and this is the advantage. In INEPT sequence results in polarization transfer, selective pulse inverts only the selected proton, all the JCH are refocused except for the selected proton. So, the magnetization is back transferred through INEPT and detected. Like in this case, you see here selected this proton, this proton coupling to all the carbons. We are inverting this proton, this proton coupled to remote carbons we see. If you invert other proton, that proton coupled to other carbons you see. This is what it is called selective experiment.



H1' is selectively inverted



And this is an example of a spectrum of a selective long range heteronuclear J resolved with proton detection. This is what it is done. Selectively inverted this one. H prime is selectively inverted. Coupling of H prime to different carbons you can see here because we are detecting proton indirectly.

So, this is what it is, and with this I told you a lot of things about J resolved experiments. Homonuclear J resolved, long range detection, long range J resolved measurement, homonuclear J coupling measurement and homonuclear decoupled broadband spectrum by getting the projection of the proton spectrum after 45 degree tilt. In the heteronuclear case, we also wanted to enhance the sensitivity by selectively inverting a proton and getting the long range coupling of that proton to different carbons. That can be done with the proton detection by doing polarization transfer technique and then back transfer to proton like INEPT and reverse INEPT experiment. Number of things have been done, but remember what I have given is a tip of an iceberg. In principle, this J resolved experiment has been modified enormously. There are number of experiments available, each of them with the improved version to get a particular information. We have two-dimensional version, it can be combined with the HSQC, it can be cooupled with the HMBC also. If you do long range HMBC, if you get a well-resolved spectrum, HMBC I said we always do in a coupled mode. We do not decouple. We can get J coupling also, heteronuclear J couplings. So, we can do with coupled with HMBC, coupled with TOCSY and HSQMBC, varieties of experiments. There are number of papers published, lots and lots of papers have been published, lots of reviews are there. Only for the measurement of J couplings, heteronuclear so many number of pulse sequence have been designed. Many of them have been with improved version and lot of information can be obtained, the excellent experiments have been designed. So, I gave you only the principles and fundamental pulse sequences, but idea is similar. Rest of the things are only slightly improved versions to get better information or to get a better resolution, enhance the sensitivity or speed up the data, like that. That is the way a pulse sequences have been designed. Apart from that, everything remains same. With this, I am coming to the end of this J-resolved experiment. Today, we discussed a lot about J-resolved and hopefully, you understood why it is done. To simplify the spectrum where the information from two parameters can be separated out in two dimensions. It simplifies the analysis. You may ask me a question, what do I do by getting J coupling? J coupling can be utilized for getting structural information. We discussed a lot about Kaplus equation. So, these are all advantages. This is one such 2D experiment. So, with this, I am going to stop. From tomorrow, we will start altogether different type of experiment. Again, it is a correlation experiment, but not through bond, but something else. So, you wait for it till tomorrow. We will discuss something more. Thank you very much.