One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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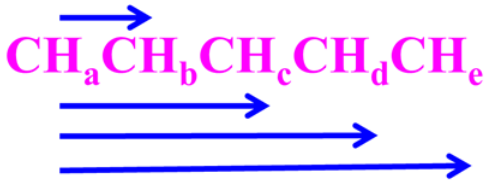
Lecture 59: 1D-TOCSY, PURESHIFT

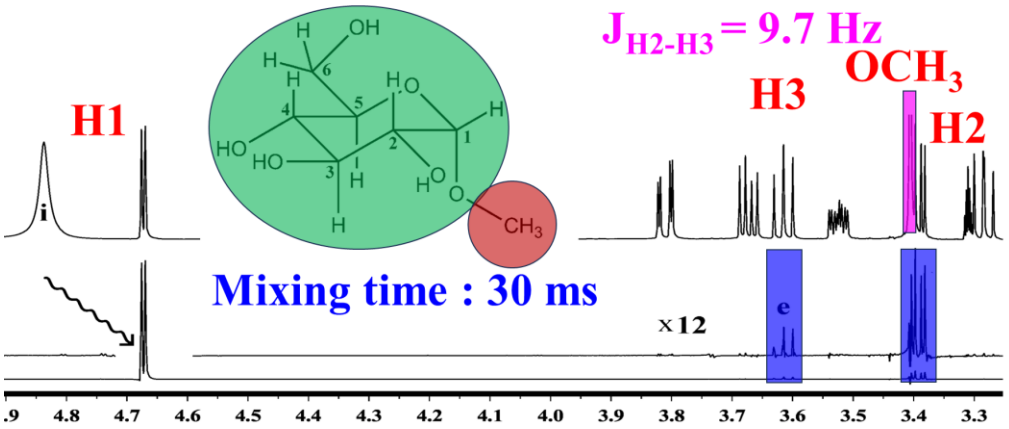
Welcome all of you, we are almost coming to the end of this course with only little bit of materials to be covered. In the last class and in the last couple of classes, I discussed about one dimensional version of several experiments. For example, 1D NOESY which is a steady state experiment, steady state NOE experiment, where we can selectively saturate one of the peaks and then do an identical experiment at off resonance, that is apply RF power far away from the region of interest. So, that any disturbance should be identical for both the experiments. The off resonance saturation, call it as a reference spectrum. And then take the difference between the one which is selectively saturated with the reference spectrum. And if there is any NOE enhancement, if there are peaks which are NOE coupled, you will see the signal enhancement. So, that is what we saw and then we took one or two examples as how NOE can be utilized. And I showed you how in simple cases we do not require to do the two dimensional experiment, which is time consuming. You can do the same 1D version of NOE steady state experiment. In most of the cases for small molecules, if there are isolated peaks, we can do the selective saturation very precisely, that is what is important, rrequency selection for the saturation is very important. If there is a mistake then what will happen the rf spill over to the neighboring peak. As a consequence it is going to be a problem. I showed many examples for E and Z conformation, regiocentric identification of particular substitution in the phenyl ring and a lot of some examples we understood by steady state NOE difference experiment. And we went to TOCSY experiment, in a similar one dimensional way, instead of doing it as 2D. If it is simple molecule and if you have the isolated peak and if you can excite selectively that one and you can see the enhancement of the signal intensity or not. The enhancement will be where you are going to see the signal which are connected to this, because TOCSY is a coherence transfer. The magnetization transfer takes place among the coupled spins. In NOE there is no need of a j coupling. Here j coupling is essential, in the 1D select TOCSY experiment, ofcourse also even 2D TOCSY experiment. We went to one dimensional version and I showed you. I took the simple example of one molecule. I quickly went through in the last 2, 3 minutes. I did not go through very carefully, and I simply rushed through as there was no time. Now we will take that molecule again and see one or two more examples before going to different topic. So, I will start with the analysis of a simple molecule using selective TOCSY on methyl alpha glucoside in a particular solvent.

As I told you when the sugars are there, most of the time they are all generally unbranched carbon chains, the most carbons are CHs and we can think of them as a linear system. that is what I said. So, in the case of linear system I discussed and I said you can do selective excitation. TOCSY is like a diffusion process. What happens I start with, let us say, this one I selectively irradiate proton A. Then what will happen? Start with selective excitation of A it gives magnetization to B depending upon the strength of the coupling and the duration of the mixing time, that is very important. It will give magnetization to that. I keep on increasing the mixing time then slowly it gives the magnetization to C, A will give to C, will give and then to D and then to E. Of course, it goes through A, B, C, D, it is a sequential process. It is propagation of the magnetization in the TOCSY I told you, it goes among all the coupled spins, but it is not unidirectional although I have shown it as unidirectional here.

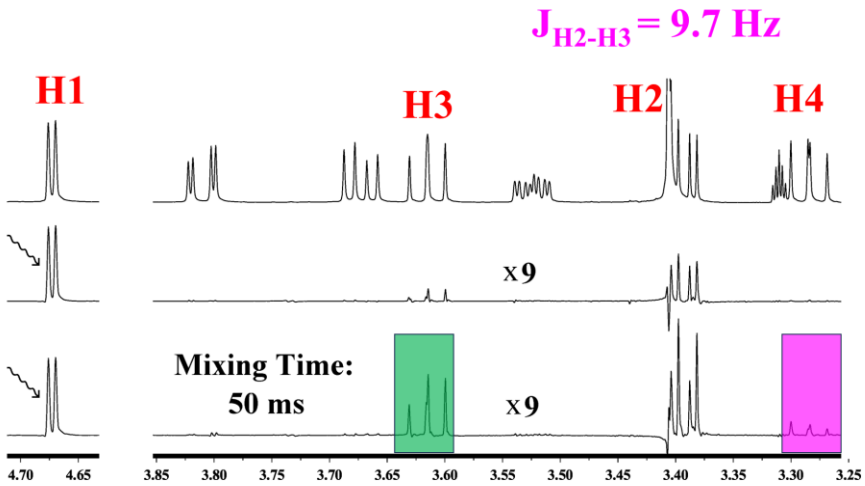


Always the magnetization transfer goes forward and backward, A will give to B and then B also will give to A, all those things we discussed at stretch, when we discussed about TOCSY, especially 2D TOCSY. With this what happens if I selectively excite not the proton which is at the end of the chain, but somewhere in the middle.

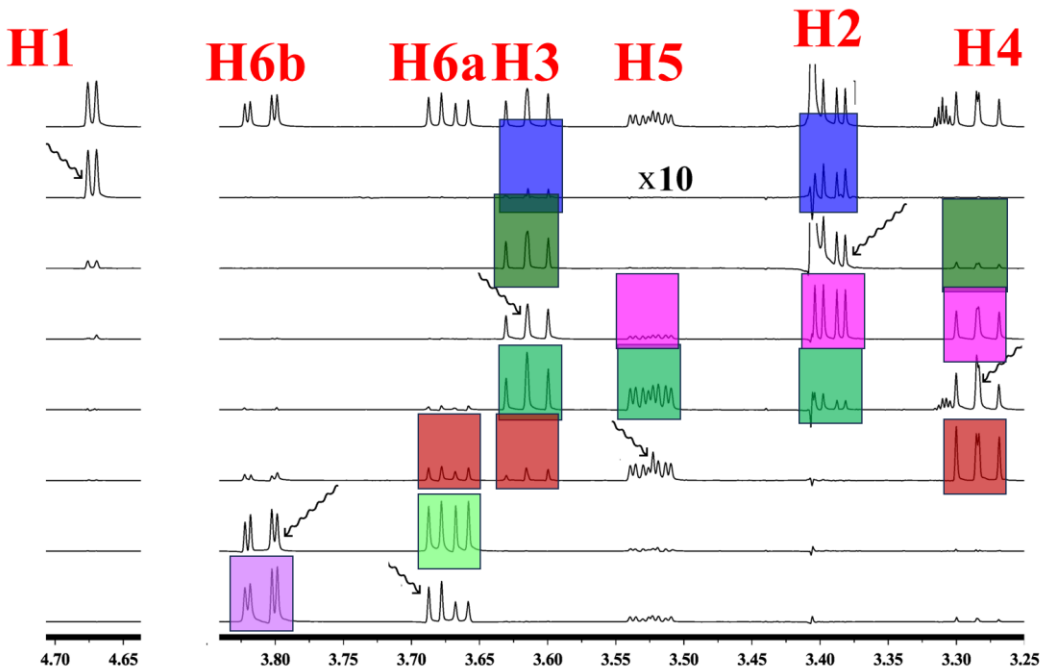
And it can so happen if I start with the selective excitation of, let us say proton C, then the magnetization is given to B and D, because they are next to each other and they are coupled to each other. Depending upon the coupling strength they will be identified, the magnetization transfer takes place for B and D. And then next give the mixing time longer, then it will go to A and E also like this. You know you can start with a particular proton selectively excite one of them and then transfer the magnetization to all the coupled spins of the spin system. So, we can do that and I showed you this in this example how selective excitation is going to be done. If I take for example one of the protons here, this proton, if I selectively excite and enhance the signal intensity you will see there is some enhancement here, and mixing time is very important and the mixing time is given here this is how the selective excitation is done.



And then when you transfer the magnetization it goes to the ones which are immediate coupled partners, and then depending upon the mixing time will go to the next one, next one, like that. You start like this for in this molecule, you start with CH3, you can easily assign that is CH3 and then all other protons we can easily assign. The most important is you should know is H1. Anomeric proton generally in sugars come between 4.5 to 5 around 5.5 ppm depending upon whether is alpha or beta, which type of sugar we have. So, anomeric protons generally easy to detect and easy to assign. And also I told you depending upon whether it is alpha and beta, the coupling strengths are different. I told you earlier axial axial coupling is larger than the axial equatorial and the equatorial equatorial coupling is much smaller than both of them, that is what we discussed. So, depending upon the coupling strength you can even identify alpha and beta isomers. So, we start with this, I am selectively, let us say exciting proton 1, then what is going to happen? you see it is saturating OCH3, which is next to each other, this is proton 1 it is saturating OCH3 next to each other. And then partly it also gives energy to other proton which is coupled to it this, this one. So, that could be H3. It gives to H1, OCH3 and also to H2 and also to H,3 all the 3. For example you see for H3 the magnetization transfer is much less because it is not immediate coupled partner, it goes 2-3 bonds away. So, you require more mixing time. First thing you should understand I have excited proton 1 and I see this enhancement in OCH3 and proton 2, they are very close to each other. And then they are coupled, the strong coupling is there. And there will you are seeing the magnetization transfer. If you carefully see, if you enhance the signal intensity by about 12 times, you will see the polarization transferred to other proton. What is other proton? Obviously, if you go by the chain, it must be proton H3. So, like this we can continue. And then what we will do is we will enhance the mixing time. Remember the previous example, in the previous slide I showed you H3 was less in intensity for 30 milliseconds mixing time. The same H1 is selectively excited and then magnetization transfer is done as you see H4 proton H3 everything is there.



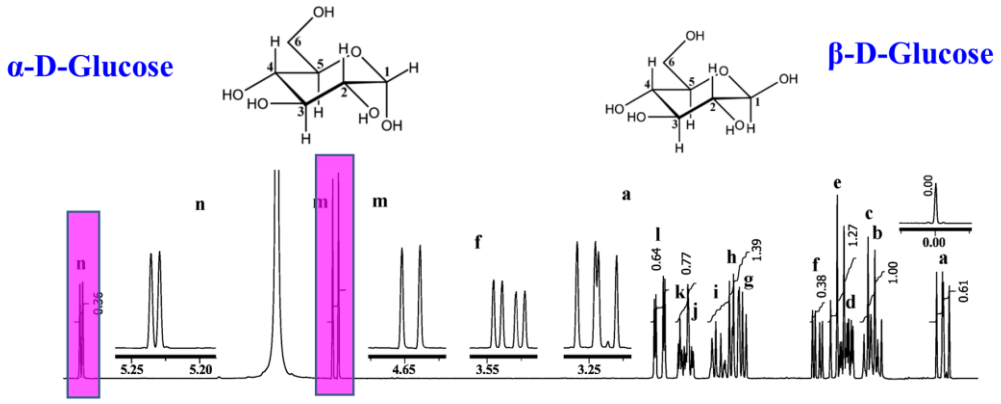
At the same time now because of the mixing time being longer, the magnetization transfer has gone up to H3, proton 3. Alright this is what it is, and then if you see carefully it is going to other proton also not only H3 further, that is H4. What is happening here? nothing we did, simply enhanced the mixing time from 30 millisecond to 50 millisecond. But we excited the same H1, anomeric proton. First is goes to OCH3 which was very close, but then among the J coupled partners of the spin system, it gives to H2 and then to H3, and slowly also it is going to H4. So, like this we can make the assignment easily. Now I know H1 and immediate coupled partner, we saw where the magnetization transfer is H2.



To understand further what we will do is, we will selectively irradiate H2, wherever it is this, the arrow is there, that means that is the place where we are selectively exciting. I selectively excited proton H2, but you see as you observed in the previous example H3 is getting enhanced, of course when H2 is irradiated, there is also small contribution to H1 because H1 and H2 are coupled. And you see H4 is also getting enhanced, you can see that magnetization is transferred to H4 also to some extent. After H2 what we will do is, we will go to H3. We are irradiating proton H3 here. Then what we expect? H3 is far away, we know transfer here negligible, but then major transfer is for H2 and then to H4 because H2 and H4 are immediate coupled partners for that. So, the magnetization transfer is taking place more for H2 and for H4. Alright, we go further. To confirm systematically we can go one after the other, then we start irradiating proton H4. When you irradiate proton H4, of course you can see it is here, when you irradiate H3 small magnetization transfer to H5 is also there. When I excite H4? what do we expect? For immediate coupled partner H3 enhancement is more, and then it further gives magnetization to H5. What next? it will go to H6 also. I am sorry H2 is also there. So, this what is happening and you can see small magnetization transfer to H6 here we are irradiating H3, it is something you know systematically you can go from H3, H4 and then you come to H5 irradiate H5 you will see there is a magnetization transfer to H4, H3 and also here little bit magnetization transfer to other proton. What is the next one in this coupled chain of spin system? coupled chain of spin system, it is H6 obviously. And H6 has two protons A and B. I have identified. So, one is A and you can see partly for the other one also there is a magnetization transfer. To understand this better what we can do is we can selectively excite proton H5, you can see H3, H6 and H4. To confirm more we did one of them which is isolated, which is H6, obviously H6 will give magnetization to other H6A, because they are having a geminal relationship, it gives magnetization to this. So, H6 was selectively irradiated we can see magnetization transfer to other proton of that. On the other hand, if you selectively irradiate this proton, you will also see this. But here when you irradiate this proton, there is a magnetization transfer to H5 also. That shows we can say this is very close to H5. Alright, so, we can understand this thing and complete assignment can be done by selective TOCSY. Selectively I can excite one of the protons and allow the spin system to mix up and then thereby we can transfer the magnetization among the coupled spins.

We will go to another simple example which is a glucose a D glucose. The 2D COSY you have already analyzed that one. And then we also understood D glucose exists as two conformers alpha and beta, with alpha is 64 % and beta is 36%. We can see now and we can almost make the assignment for alpha and beta. We always start with anomeric protons. Anomeric protons I told you which comes down field around 4.5 to 5.5 ppm and each of the anomeric protons will only be a doublet because it can couple to only a single proton, which is proton 2. If you consider anomeric proton is H1, it can only couple to another single proton which is H2. As a consequence it is a doublet. Out of two doublets

you can identify which is beta and which is alpha because I tell you the assignment of alpha and beta is so easy. The beta conformer has a large coupling about 8 Hz because it is axial-axial coupling. Alpha conformer has coupling of only 3.8 Hz which is only axial equatorial coupling. As a consequence, the assignment of anomeric protons can be straightaway done just by looking at the doublet separation. So doublet separation of nearly 8 Hz, I can say it is beta, and other one is alpha. Another one important thing is we have OHs and everything that exchange with D2O, fine.

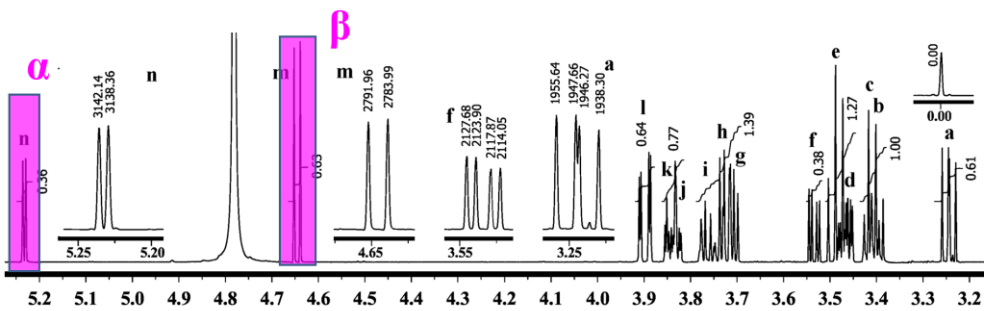


Anomeric Protons

But when you look at the spectrum and also intensity of the alpha and beta anomers, the ratio is 64 : 36 for both the peaks. That is true, because we also know that 63: 36 is the available ratio which is close to the literature value. So 64% and 36% are the two isomers which are supposed to exist in water, that is known. So if you get the intensity ratio 63 to 36, by and large we are close to the literature value and if I identify a peak of intensity 63, relative intensity compared to other one, then we can assign that as a single proton pertaining to beta isomers that is what we do.

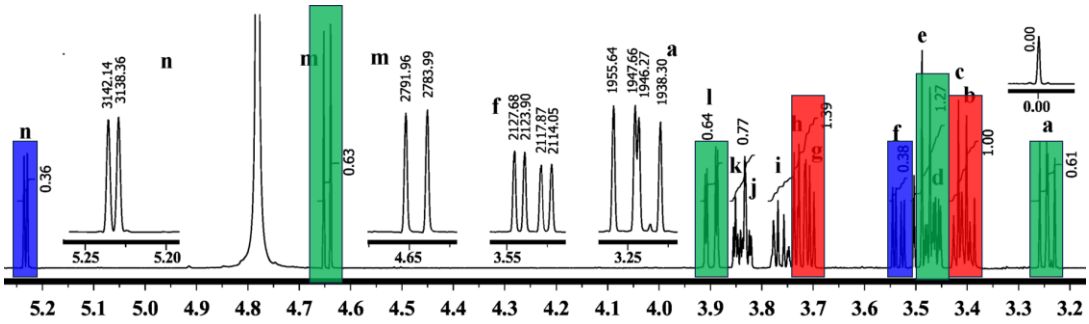
Peak at 4.646 ppm is the H1 proton of β-glucose

Peak at 5.233 ppm is the H1 proton of α-glucose

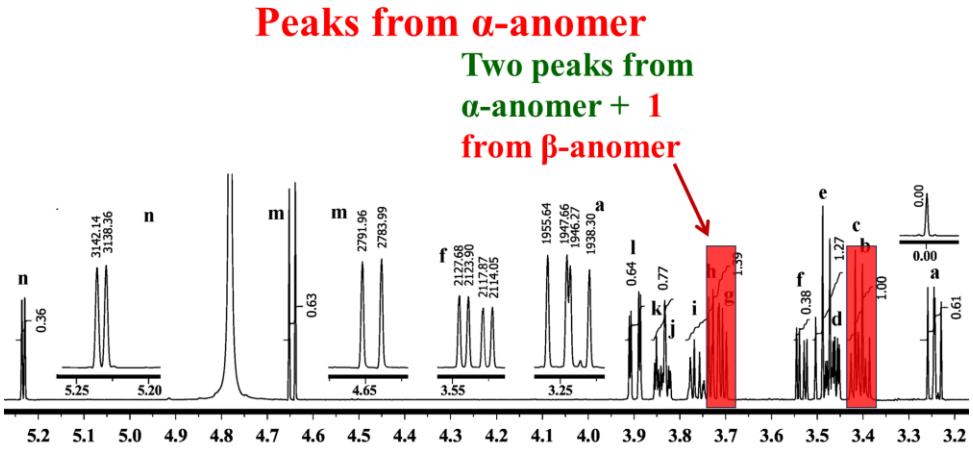


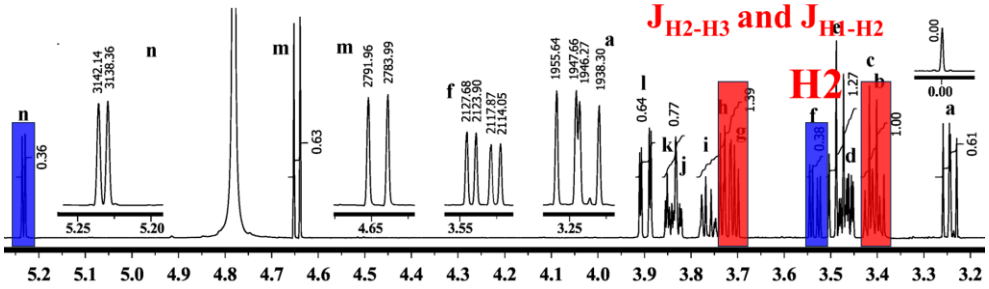
Anomeric Protons

So if we consider two isomers anomeric protons, of course that I already said which is high field and low field, just now we discussed there is no need to go for that. We will see this one now, this is beta this is alpha, fine. What next if you see that all OHs are exchanged the peaks with an integral area of 0.36 correspond to one proton of alpha. I told you, if I measure the integral area a peak of 0.36 intensity, if it is there, it corresponds to alpha anomer. If there is a peak with 0.63 intensity it corresponds to beta anomer. If there is intensity one, what does it mean? It means there is a overlap of alpha and beta anomers together.

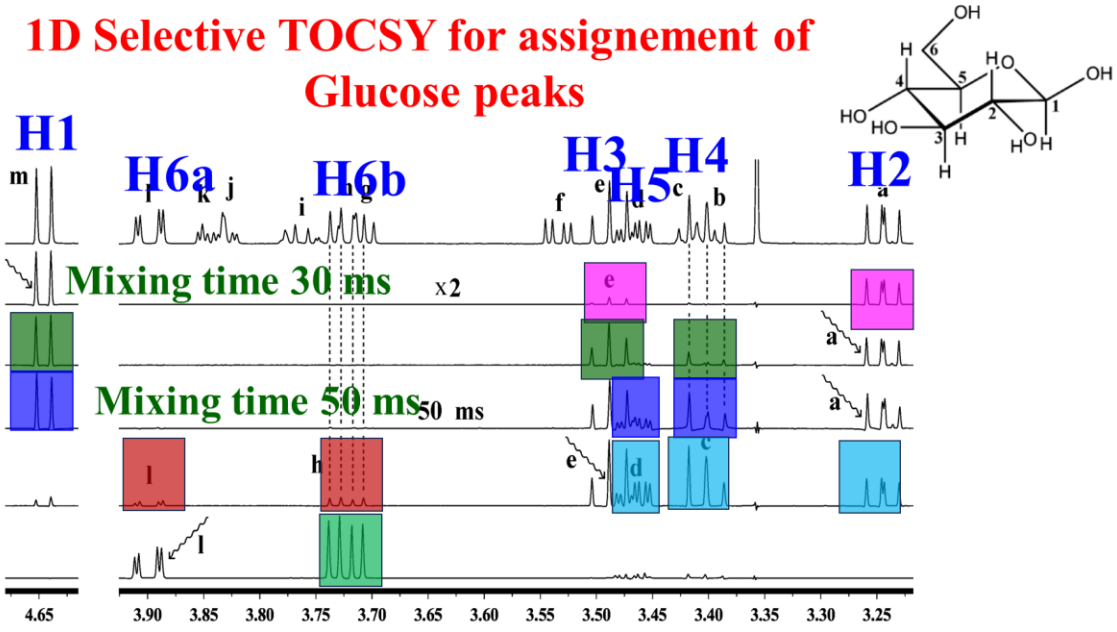


So if there is intensity one, we can identify and say one proton from alpha and one from beta are overlapped. As a consequence, the intensity becomes one. So integral intensities are given here. What we can see is, all the blue things which I have highlighted here all the peaks with intensity 0.36. They are all one proton from alpha. And those which are written in green are intensity 0.63, you can see here 0.64 close to that one, that mean they are all from single proton of beta anomer. But of ourse you can also see some of the peaks with one intensity here. This is one peak from alpha anomer one from beta are overlapped which gives rise to intensity one.

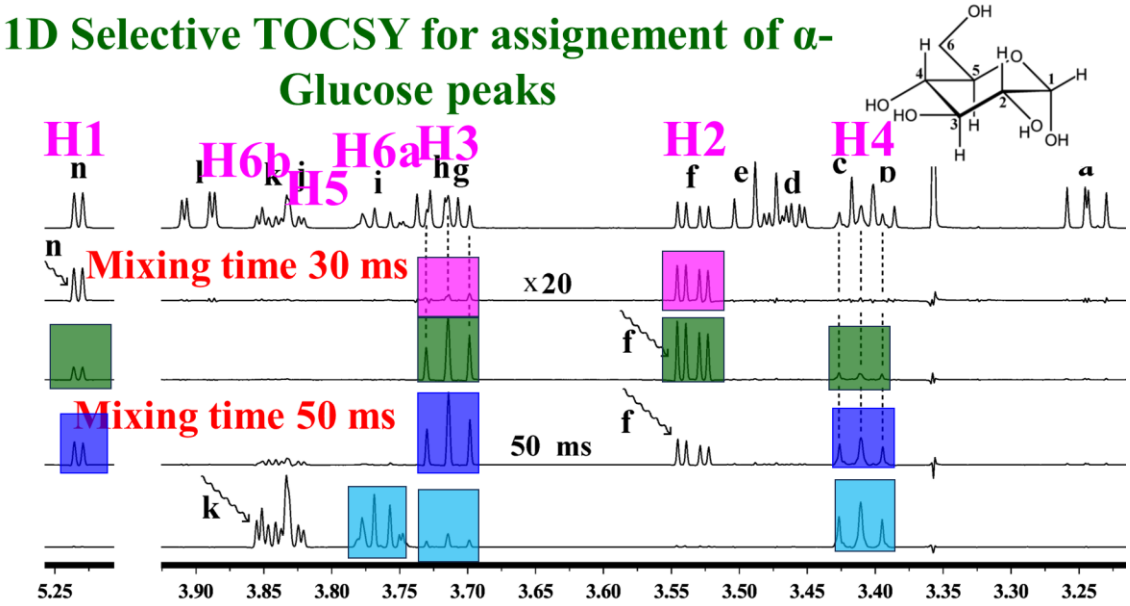




But here there is a interesting thing we have 1.39 intensity what does it tell you it tells you two peaks from alpha and one peak from beta are overlapped here. So by integral intensity area of the integral of the peaks we can start making some assignments. I mean we do not say which proton is which, at least we can say which are the protons coming from alpha and beta how many are overlapped, that information we can say. Here one peak from one alpha and beta are overlapped, two from alpha and one from beta is overlapped; alright. And of course we can also find out from the J coupling and everything identify the peaks very easily. Here it says two peaks from alpha anomer are overlapped. And finally all the assignments we can obtain like this. Now to identify more what we will do is we will do the selective TOCSY. From the previous this thing I showed you we can identify which are the peaks from alpha, which are the peaks from beta isomers by integral intensities and where are the overlapped. Fine. We now specifically assign all the protons based on TOCSY. Of course we can do 2D-TOCSY to identify, but if you do not want to waste much time, then 1D TOCSY is much easier.



As always, I start with the anomeric proton which is down field. I know this is proton H1. I excite selectively that one, give certain mixing time. After exiting that, now give mixing time the polarization transfer takes place. H1, as you know, should give magnetization to H2, to which it is immediately coupled to, and then depending upon the mixing time it can give magnetization to H3 or H4 like that. With 30 millisecond mixing time it is giving magnetization to H2 and partly little bit to H3, a very weak intensity. To confirm more the same plot can be done with enhanced intensity. Of course we can also confirm that by doing selective irradiation of proton H2 instead of H1, If you do irradiation of H2, what will happen? it gives magnetization to H1, to H3 and then also little bit for H4, you can see little bit for H4. To ensure you are okay, our experiment is in the right direction you can give more mixing time, make it 50 milliseconds. You can see intensity enhancement. You can clearly see H1, which is very weak, very clearly you can see. We selectively irradiated proton H3 then where do you expect? the magnetization transfer to H4 and H2, H3 is in between these two. And then depending upon the mixing time we can also see part of magnetization transfer to H5. So that can be H5. And of course H6 also is there and the H5 is DDD and is also coupled to H4 and H6A. See that is another interesting thing. Wee what is happening is if you are irradiating this one, you are going to get the magnetization here. But if you radiate H3, H3 is giving magnetization to H4, H2 and also to H5. To some extent it also gives to H6 one of the H6 protons. That is what is happening here. So what you can do is to confirm further we can selectively excite one of them. Now we will do this one, selectively saturate one of the thing, which could be proton 6, because H5 we have identified H3, H4, H2 everything we have identified, one after the other, and the last one left over is H6. We saturate this one or excite this one and you will see magnetization transfer to H6, because they are in geminal relationship. We can make the assignment. Go further that was our beta isomer.



Now start with the alpha isomer again, where do you start? You start with the anomeric proton, irradiate that if your anomeric proton is always H1, it has to give magnetization to H2. Where is this? The strong signal intensity is here. Then this has to be H2, and also you can see some magnetization is slowly given to that one. That must be H3. So to confirm that irradiate H2, H3 becomes more and then H1 is also getting some signal. Shen you are irradiating H2 some magnetization is given to other protons. Obviously. as I said it is a unbranched chain, look at it, it has to be H4, that is done. And if you enhance the mixing time the intensity become very clear. We can now saturate other protons. For example this one. What else it should be? It has to be H6, one of the H6. When you irradiate that we can see it is giving magnetization to its geminal partner H6a and also to H3, and also to H4. All are getting magnetization transferred. So this way what I suggest is selective saturation or selective excitation of proton allow them to mix up, you can transfer the magnetization to its immediate coupled partner. Enhance the mixing time it can go to the next one, next one, next one, like that. So what you have to do is to irradiate one proton, go to the next one you will see the enhancement there. Go to the next one irradiate that, it gives the magnetization to previous one and also the next one. Then go to the next one, like that systematically you can go one step after step by step and then selectively excite each proton and then allow the spin system to transfer the magnetization among the coupled spins. Depending upon the coupling strength and depending upon the mixing time given, you know which are the coupled protons, because you will see the enhancement in the signal intensity in those protons where the magnetization transfer has taken place. By this way you can make the assignment of all the protons in a simple way by doing one dimensional TOCSY experiment. You do not need to do 2D TOCSY and then of course spend a lot of time. This is okay, if you have to do only one or two experiments. If you have a big very big molecule with hundreds of peaks, and closely spaced, severly overlapped then this type of 1D selective TOCSY is very difficult, because the frequency selection for excitation is not precise. Then it will be very problematic. In such cases usually one can do 2D transient NOE experiment. So this is what I am telling you, 1D type of experiments for faster data acquisition on very simple molecules, where you can see the isolated peaks and when you have a difficulty in identifying which is the proton. Then this type of experiment can be done. So I have covered 1D version of NOESY, 1D version of TOCSY. Of course this is not the end. We have 1D version of NOESY, 1D version of HSQC, 1D version of ROESY, 1D version of COSY, all experiments can be done in a one dimensional way. All this can be applied but I am not showing everything because already we have discussed enough. Only two examples I wanted to show you for one dimensional version of TOCSY and one dimensional version of NOESY where you can utilize selectively for doing this thing. Same thing can be done for ROESY, same thing can be done for HSQC, selectively you can transfer the magnetization to its coupled carbon and then take it back and identify the coupled partner. All these experiments can be in one dimensional way also. You have to

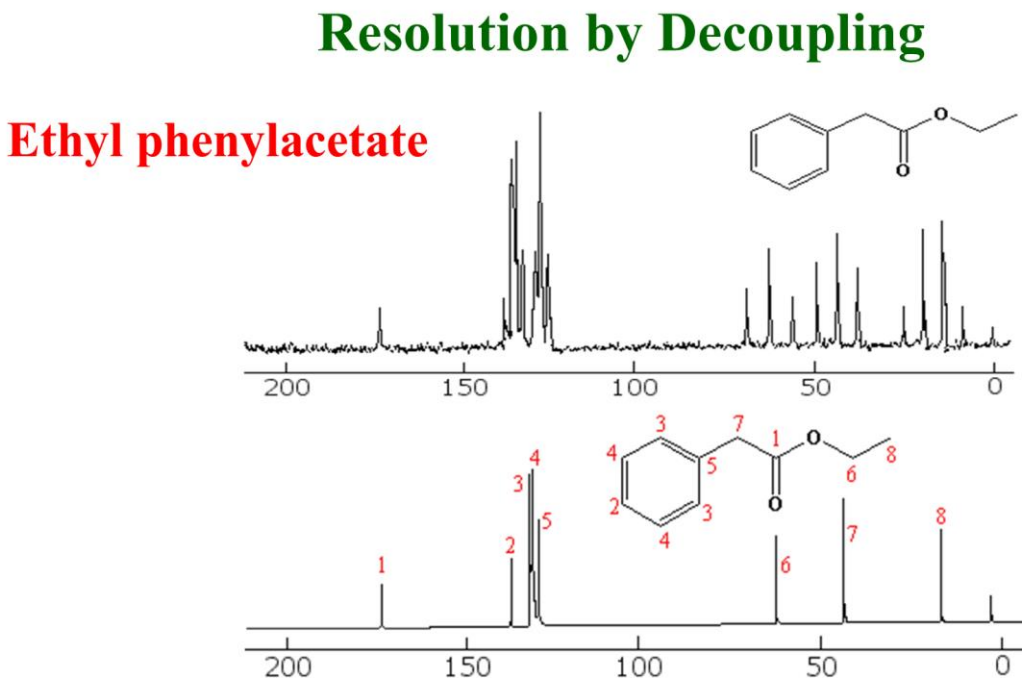
take a judicious decision where to apply this technique, what is the size of your molecule? what information you want to derive? so that there is no overlap, no spillover of the RF by selectively irradiating one peak, and then you should not spill over to the neighboring peak. All this care if you take, and if you have a simple molecule, I suggest you can do simple one dimensional experiment, and get same information as you can get in 2D. But it is a saving of the instrumental time here, that is important thing, you can save a lot of time. With this I think there is no point in going further, what I am going to do is I will go to another topic slightly a different topic and then we will cover that today and see what more we can do later. See I am going to tell something about what is called pure shift NMR. What is a pure shift NMR? We will discuss something different now.

Before going to pure shift two important thing we should discuss in NMR. NMR is as I told you right from the beginning of first class, the sensitivity is the biggest issue. We discussed at stretch about the sensitivity issue. I told you when we were talking about the polarization transfer. One of the ways is to go to high magnetic field, take more amount of the samples, and all those things we discussed. Alternately what I suggested also was we can do polarization transfer experiments, to enhance the sensitivity. So, there are some ways we can address the issues of sensitivity of detection in NMR.

But there is another issue a very key factor for NMR, that is the resolution. Of course, for resolution you have to go to higher and higher magnetic fields. And it also depends upon certain physical parameters in the experiment, for example, the molecules may get aggregated, and if there is no mobility, and if is a viscous solvent like that. Thus you have broadening and resolution issues. But remember the definition of the resolution in NMR is half the sum of the line widths should be greater than the separation. For example, I have two peaks here, this is well resolved I would say, because I take line width of this one, line the width of this one. I will take half of the line width of this, half of the line width of this. Take the sum of these two, half the line width of this peak half the line width of this peak, that should be greater than this. That is half the sum of the line widths should be greater than the separation. In which case we can say these peaks are well resolved. But resolution also is not only because of this, there can be because of severe overlap, that is one thing. And another thing, the severe overlap is also because one particular proton can experience couplings among several other protons or other heteronuclei, with high abundance. It can complicate the spectrum a lot, because of severe overlap, the resolution could be a problem. So, the two sensitivity and resolution of the two key factors in NMR. How do you get the resolution? As I told you resort to very high field, you get the gain in the intensity by B0 to the power of 3 by 2, B0 is the static magnetic field, we discussed this long back in one of the classes. How do you enhance the sensitivity of low abundant spins, broadband heteronuclear decoupling, breaking of the coupling of all the dilute spins like carbon nitrogen with other abundant spins like proton or fluorine. You can do isotopic labeling, enrich the carbon or nitrogen

15 and also to the polarization transfer by developing NMR methodology, which also we discussed. All these things can be done.

So, decoupling is one way to enhance the resolution. If you are interested only in identifying the chemical shifts of the particular carbon or the proton or any heteronuclei. Broadband homonuclear heteronuclear decoupling is very very useful especially when you consider heteronucleus like carbon. We have seen many examples when I was discussing carbon 13 NMR. When there is enormous overlap because each carbon is coupled to different number of protons, it gives rise to a quartet, triplet, doublet and singlet. When there is a severe overlap I showed you we can do broadband decoupling. There are several types of broadband decoupling we discussed, and then when you do the decoupling you break the coupling of all protons and carbons and you get single peak for each chemically inequivalent carbon, that is very easy. Broadband decoupling especially for heteronuclei is very easy to do. And when you do that, see what a simplified spectrum you are going to get. This is a quartet of CH3, the triplet triplet all become singlet.



Look at this one if there is a severe overlap it will be difficult for you to identify quartet, triplet, etc. On the other hand, when you do the broadband decoupling I can identify all the singlets like this. Next question is how do you do the decoupling? How do you achieve decoupling? You can achieve this by manipulating the passive spins. are passive spins? When I am detecting carbon 13, protons are passive spins. So, I can manipulate the passive spins without touching the active spins. I will not perturb the active spins. I only manipulate the passive spins. When I do that I can do heteronuclear decoupling in such a

way I can apply the radio frequency power on the heteronuclei to suppress all the heteronuclear couplings at a time. This we discussed in carbon 13 NMR. Easiest way to do is, apply a radio frequency in an example of carbon couple to a proton. You apply a radio frequency to saturate all the populations of the protons and then you are going to apply another RF at the carbon 13 frequency to detect the signal. The irradiation power should cover the whole range of proton frequencies in which case you break all the protons coupled to all the carbons at a time. This is called heteronuclear broadband decoupling. This is a very easy experiment it can be done. How it works? We discussed long back. If you radiate a proton let us say a set of protons they get saturated and they undergo rapid transitions among all the possible spin states. This rapid transitions break all the spin-spin interaction sbetween protons and the carbon-13. As a consequence all heteronuclear couplings are averaged to 0 and carbon senses only the average spin state of the attached protons, rather than two or more distinct spin states. This is a crude way of discussion, but one can mathematically work out and discuss at stretch. But imagine this is what is going to happen and we can do broad band decoupling. Especially this is the notation also I said. How much should be the decoupling power? It depends upon the strength of the coupling and usually decoupler offset is set at the center of the proton spectrum and the decoupling power should be larger than the largest coupling strength, more than at least twice the larger than that. That is what we do. And what about this 13C-13C scalar coupling at natural abundance they are not usually visible and not intense at all, very very weak intensity and difficult to detect. So, with that heteronuclear decoupling is easy, I tell you not difficult. Now the question comes is what about the complexity of the 1H NMR spectrum. This is where broad band decoupling becomes very important and it is a topic by itself called pure shift NMR. Since the time is getting up we will discuss this in the next class.

Right now I am going to stop here. So, today we discussed a lot about one dimensional TOCSY experiment which we had started from the last week, we showed some examples and then I took another example today alpha and beta isomers, anomers of glucose. I showed you selectively exciting anomeric proton you see the magnetization transfer to proton 2 and then with the mixing time, it will go to proton 3, and then saturated proton 3 it will give to proton 1 and proton 3. Excite the proton 3 it will give to proton 2 and proton 4, also. Like that systematically you can go selectively excite one of the protons and see the saturation and transfer the magnetization to other protons. This is what is done in a one dimensional way. Through this we could identify, we could assign the peaks by doing this 1D TOCSY experiment, all the peaks that pertain into alpha isomer of glucose and all the peaks that pertain into beta isomer of glucose. We could tabulate all the chemical shifts. All those things are not much important, but the concept, the methodology is important, that is what we discussed. Then of course, we went into the pure shift NMR. I have not introduced about the pure shift of homonuclear spins, but heteronuclear pure shift means you will break the heteronuclear couplings get only

chemical shifts that is what we saw in the carbon 13 other heteronuclei. You break the coupling of heteronuclei with abundance spins like protons, and when you do that you are going to get a single peak for each of them. How you do that ? you saturate saturate the protons sitting at the center, applying a radio frequency power. The RF power should be sufficiently larger than the coupling strength. Then you will get single peak for each of them. The resolution and sensitive issues can be also be addressed in NMR by doing one such experiment, not only going to high field, but also by doing decoupling to simplify the complexity. I am going to stop here, we will continue with the other remaining things in the subsequent classes. Thank you very much. Thank you.