NMR spectroscopy for Structural Biology
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Lecture: 57
Basics of solid state NMR spectroscopy - II

So welcome to today's class. In the last class, we discussed the basics of solid state NMR spectroscopy and how we are going to use that in structural biology. So I introduce you the difference between solid state NMR and liquid state NMR spectroscopy and what are the protein molecules that are applicable for solid-state NMR, where we can apply solid-state NMR for looking at the structural and dynamic aspects of biological macromolecules. I also discussed about inherent problems with solid-state NMR, and what can be toolkit for overcoming some of those problems. Let us repeat little bit and then we move forward.

So in the previous class I discussed that we want to mimic the inherent averaging process that is in solution state NMR to obtain high resolution isotropic information. So inherent averaging process that is present in solution state NMR. If you do that, if we mimic that solution state like condition, then we can get the isotropic information. And to do that what we have to do? We have to enhance the resolution and sensitivity.

So how we are going to enhance the resolution and sensitivity? By removing the anisotropic part that were present in solid and retain only isotropic part. If you remove anisotropic part, retain only isotropic part by something called decoupling or averaging of interaction, and then we can get a sharper line. But by doing this we are like getting rid of important structural parameters. So we have to introduce those, get back those anisotropic part for elucidation of geometric parameter like a dipolar coupling or chemical shift anisotropy. So that is called recoupling or reintroducing this information.

So first, we decouple to get sharper lines, then we recouple to get important structural geometric parameter. With doing this we can achieve best out of both world, the sharper line, which is generally seen in the solution state NMR, and all the structural parameter that are inherently present in the solids. So if you do that we can achieve the structural aspects or dynamics motional aspects of protein molecules in solid state animal. So how to do it? Actually, it all started when John Kendrew in 1958 proposed this idea of a spinning

sample at an angle which is called magic angle. So you know this is our main magnetic field B_0 and in solution state we know that our sample tube was something like this.

So all the spins were aligned like this. So our spins were placed along the magnetic field. Now John Kendrew proposed since dipolar coupling is culprit, so can we do something? Can we orient our sample at an angle, which is called a magic angle, this θ_m in the magnetic field and then we spin them faster and faster and faster and faster. Because of this faster spinning, the interactions that we talked will be averaged out.

This θ_m is a magic angle, why this is magic angle? Because you know $(3\cos^2 \theta - 1)$ is dipolar coupling term and if you want to make it average, so we have to put it 0. So now you can calculate what will be our θ, that will be 54.7 degree. So if we orient our sample at that angle which is called magic angle, we are putting our sample in this rotor, this is the fins that are in the rotor and we have to spin with air faster, faster and faster and because of that actually this anisotropic interactions that is there will be averaged out. So, what are the samples where we are putting samples? All sorts of samples whether it is membrane protein or fibril protein or aggregated protein any of these we pack in this rotor.

That rotor is placed at an angle called magic angle in the magnet and then we are spinning and you see what happens because of this spinning here. So if we spin it, because of this spinning at magic angle, so let us say in a static case we have seen the peaks were really broad, and I am taking the simplest sample, glycine where there are only two carbons.

One with CH_2 that is α-carbon and one carbonyl carbon CO. So for α-carbon we are getting one peak here really broad peak here and then for CO we are getting a really broad peak here. That is for a static case, if you are not spinning. It is glycine, the simplest biological molecule that you can think of. When we start spinning, so we are spinning speed is here say 0.85 kHz that is a kilohertz means like this many rotation per second.

Then lines starts becoming really like a little sharper and you can see now these whatever we have discussed orientation dependence, chemical shift is start appearing here. If we start little faster like a 3 kHz, we see now line are slowly getting sharper. If we go to 5.5 kHz, two lines are now really have become sharp, and if you go to 12 kHz, we see only two peaks, beautiful two peaks, one coming here at the Cα, one coming here at the CO.

So glycines, spinning at 12 kHz is giving us really sharp peak. So we have to spin it. You remember this 12 kHz means 12,000 rotation per second. So this is quite fast spinning if you compare with your ultracentrifuge that is, that is 1 lakh rpm that is rotation per minute. Here we are talking 12,000 rotation per second.

So this is damn fast. You have to spin that damn fast. So this material that is there that is made has to be really robust and the mechanical aspects also has to be very robust. So these are spin with air and that is given from outside and here are the fins and it has to spin very stably in a narrow passage. So this is made up of zirconium oxide.

It is a really robust material because we are doing experiment at various temperatures. So it should be temperature insensitive and it can spin really, really fast. So this is one of the moderate spin that we can think of 12 kHz. it can nowadays can go up to 110 kHz and that is a special arrangement that you need for spinning. But because of this spinning now we are going to get really sharp lines.

As we discussed we have to spin it, spin it very faster and this spinning is called magic angle spinning. We are spinning our rotors at an angle, which is called magic angle and this actually averaging out our anisotropic interactions. Now we have a different kind of rotor because for depending upon samples we have to spin it at different speed and we have all the way like this is say 7 mm, then 4 mm rotor. This is 7 mm means 7 millimeter is the outer diameter of this rotor, then 4 mm, 3.2 mm, 2.5 mm, 1.3 mm or even like you can see now nowadays we have a 0.8 mm rotor, that is really fast spinning rotor.

So here is a 3.2 mm and you need a nanogram of sample for doing solid state NMR, just with a coin this are compared. If you reduce your outer diameter that means we can spin faster and faster.

So outer diameter of magic angle spinning rotors determine the maximum rotation frequency. Like if we are having 6 mm rotor that means we can spin maximum to 8 kHz. If we have 4 millimeter rotor we can spin up to 15 kHz. If you have 2.5 mm rotor, you can spin up to 30 kHz.

If you are going say 1.9 millimeter of rotor, you can spin up to 40 kHz, 42 kHz. If you are using 1.3 mm rotor, we can go all the way up to 65 to 70 kHz. Now the fastest rotor, the fastest available rotor is 0.8 mm that can go 100 or 110 kilohertz.

So now, this different size of rotors are essential for spinning faster and faster. Now since we are miniaturizing this rotor size, so that means the sample that we can pack in this is also going to be very, very small. Sample volume depends upon what is the inner diameter of this rotor. What I talk to you is outer diameter of the rotor.

If inner diameter if we talk that is going to be very small. Because this material has to be very stable, that is why it is made up of zirconium oxide. Now this is the top cap which has a fins that drives the spinning. So essential volume that like the effective volume that is there is very small for these smaller rotors, right. So the MAS frequency also depends upon what is kind of the size. So smaller size faster rotation, bigger size slower rotation.

That also determines what kind of experiment that we are going to implement. Here since we are spinning fast, so we can average out really lots of the stronger interaction. Therefore, we can do something called even proton detection that probably I am going to talk to you briefly. Here mostly we have to do carbon detection.

3.2 rotor can spin up to 42 kHz and that has a speed of 240 m/sec when rolling along the ground and that needs only 46 hours to roll around the whole earth. That is the fastest I am talking. So, we have to spin these rotors really, really fast to average out the isotropic interactions that we have talked about. Because of this, we are going to get basically the sharper lines that we had talked.

So the powdered pattern gives us broad line and the sideband, but we are spinning faster then we can get a sharper line. This MAS frequency is chosen so that we get out of this sideband and then once we get a sharper line then we can introduce some of the recoupling experiment that I had talked to you earlier. So how we do? So you remember we are doing two things. We are first decoupling the unwanted interaction and then selectively introducing the wanted interactions. So decoupling, so suppose here is one spin coupled

with four spins, so here is a rare spins in red, which we can call it carbon 13 or N15 and that is surrounded by abundant spin protons.

So we have homonuclear dipolar coupling between proton-proton and we have a heteronuclear dipolar coupling between carbon and proton. This is relatively weaker coupling and this is quite strong coupling. So if we spin faster and faster we can like weaken this coupling. So if you look at the order of the dipolar coupling, proton-proton, since the proton-proton distance is shorter about 1.8 angstrom, the coupling in terms of kilohertz is about 21 kHz.

The carbon-proton coupling, the distance is 1.1, the here coupling is about 23 kHz, and another if the distance is like increasing up to 2, you have a big coupling like 3.8 kHz. So depending upon what is the distance between these two dipoles, your coupling also varies and because of this, with the MAS, we can weaken this coupling and then we can do something called decoupling.

We selectively irradiate these couplings by a series of pulses which is called decoupling pulses, then we can get rid of this heteronuclear coupling. So because we are getting rid of this heteronuclear coupling, we are going to get sharper line. So that is what we do in a typical experiment, in heteronuclear decoupling experiment. The first thing just to take you on track, first thing we did magic angle spinning.

We put our sample in this rotor. We put that in the magnet at an angle, which is called magic angle and then we are spinning faster and faster and faster depending upon what is our requirement all the way from 10 kHz to 100 kHz. That average out some of those heteronuclear couplings. Then next thing we are doing is dipolar decoupling. What we do in this dipolar decoupling? So we always start polarization from the proton which is I spin and then we transfer that on carbon. Typically that I am going to talk to you in the next slide. But in decoupling what we do? In decoupling, we selectively irradiate the proton and we detect on carbon. Because of this constant irradiation on proton, we are getting rid of these heteronuclear dipolar coupling.

So in this simple experiment what we are doing? We are having two spin, one proton, one carbon, proton is I spin, carbon is S spin. We are applying a 90° pulse detecting carbon

while decoupling protons. So S spin is detected and I spin is decoupled, now this is what we get a sharper line. So because of magic angle we are getting this weakened coupling, because of irradiation we are removing these couplings. So now because of this RF application we have removed this coupling and therefore this is kind of isolated spin, which probably gives us, which is going to give us better resolution.

The next important parameter that is in solid state is called cross polarization. This is something like Hartman-Hahn condition that we have discussed in liquid state. So cross polarization is what? You know the proton, the gyromagnetic ratio is high, carbon it is one-fourth. Proton is more sensitive, carbon is less sensitive. So can we exploit that more sensitivity of proton for our benefit and for doing that what we do is called cross polarization.

We are polarizing proton and carbon simultaneously and transferring that polarization from proton to carbon to enhance the sensitivity. So how we do that? Let us start again with two spins, I spin and S spin. First, we are exciting protons using 90° here. So now, proton is excited.

So we apply X pulse so it is in Y. Now then we are simultaneously applying the RF on proton and carbon and this is ramped actually, ramped because of it gives the stable performance at high MAS. So we are ramping here and then we are matching that Hartmann-Hahn condition. If you match that condition, now the polarization from proton is transferred to carbon. So we have enhanced the sensitivity.

Now we will decouple the proton and detect on carbon. So we did two things, we started with proton, transfer our magnetization to carbon using this cross polarization, we decouple proton and then detected on carbon. So because of this we are enhancing the sensitivity of carbon. So resolution was enhanced using magic angle spinning and decoupling and cross polarization is increasing the sensitivity. So these two are basic building block for any solid state NMR experiment. The cross polarization CP and magic angle spinning that is MAS.

So what is the cross polarization condition? So you have to have these,

$\Delta\omega^{nut}{}_s = \omega_r 2\omega_f$ and $\Delta\omega_H - \Delta\omega_C = \omega_r$

in spinning condition to have this Hartmann-Hahn magical condition. So suppose we are spinning at say 10 kHz. So difference between the frequency that is applied on proton and frequency applied on carbon, so here is 10 kHz, that should be either 10 or 20 or something like this. And then we can establish this Hartman-Hahn condition and we can increase the sensitivity. Therefore, to conclude, magic angle spinning is essential for getting the sharper line. Then it is supplemented by heteronuclear dipolar coupling.

So these two leads to resolution whereas cross polarization leads to sensitivity of the signal. So CP and MAS these two are basic building block of any solid state NMR experiment. So as we saw this we have to do X nuclei detection that is carbon or nitrogen, while decoupling the proton. This proton decoupling is removing the heteronuclear dipolar coupling. So we have to apply a high power decoupling on proton that is going to be order of 100 kHz.

Now if we do that we are going to get a really sharp line. So magic angle spinning remove anisotropic interactions and remaining was removed by this high power decoupling and then we are doing cross polarization to enhance the sensitivity. Now we are almost achieving the liquid state light spectrum. So for this L-valine and L-phenylalanine if you look at here, the liquid state spectrum shown in blue and solid state spectrum shown in red. You are getting really, really beautiful sharp lines almost comparable to liquid state.

Now we achieved it, we wanted to always have a sharper line. So now we had a sharp line. So what are the disadvantage of the solid state? Why we cannot do solid state for everything? This disadvantage is now because of lower sensitivity and because we are detecting on carbon, so that is a lower sensitivity. To compensate that sensitivity, we really need larger samples. So, we are putting about 30 to 40 milligram in 3 or 4 millimeter or 3.2 millimeter rotor to get such signals and we have to probably record little longer to achieve the same signal to noise ratio for in the solid-state NMR.

So these are some of the disadvantage, but still we can detect these samples in their states what it should be and this is very much useful like a say for pharma sample. So many of the medicine that we consume comes in a tablet, right and if you dissolve them then there is a possibility that their property will change. Now you do not want to perturb anything, just take that pack in the rotor, record the spectrum and you see what kind of the molecular configuration and the orientation of these moieties are there in their formulated state. So

solid state NMR is a great boon if we are doing that in the formulated state and still we are achieving very sharp line, so we can tell everything that is needed.

Advantage we are getting by doing this CP and MAS is really high resolution. Now we can see it almost compared to and we are getting all the peaks that are there in the liquid state NMR. So we detected on carbon, can we detect on proton? Because sensitivity was less, if we replace that carbon with proton we are going to get it better sensitivity. So can we think of detecting now proton as well? See proton the inherent property that because of high dipolar coupling we have a really broad signal here, you can see the broad signal.

Now if you are no MAS, no decoupling we are really getting broad signal you can see how much it is dispersed. Now we start spinning and still no decoupling, then we start to see some feature, some resolution and depending upon how fast we are spinning it. If we are spinning it say 20 kHz still there is a line broad, but if you go about 100 kHz we will start getting this sharp features and we apply MAS magic angle spinning and then do proton-proton decoupling, then we are really going to get sharp lines. So for proton detection you need damn fast magic angle spinning and high power decoupling for proton-proton decoupling and still we can detect it.

Still we can detect proton, so we are not losing sensitivity. But you remember, I just showed you the rotors that is used for fast spinning.

It is a 1.3 millimeter or 0.9 or 0.8 millimeter. Now if rotor size is decreasing, the OD, I am talking about 0.8, 0.9 or 1.3. The rotor size is decreasing, the effective volume that is available for putting your sample is also reducing. So you really need to put it small amount of the sample inside the rotor.

If we can are happy with that, if your problem is getting solved with that small nanogram of sample, you can still spin very faster, detect a well resolved spectrum using protons and you can get all the structural parameters. So, that gives you little bit of perspective how we initiate our experiment using solid state NMR. Here onwards I will take little bit of transition because 1D is not good enough for structural biology you know, right. So we need to transition into 2D. So how we are going to use two dimensional NMR spectroscopy

for structural elucidation of biological macromolecules in the next class and I hope you are getting along with me.

So I would request you to go back and little bit read about the basics tools of solid state NMR and the next class we start with the two dimensional aspects of solid state NMR and how we can utilize those two dimensional spectrum for getting the structural information for biological macromolecules. Thank you very much.