NMR spectroscopy for Structural Biology
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Lecture: 56
Basics of solid state NMR spectroscopy - I

Hello, good morning. So welcome to today's lecture, the application of NMR spectroscopy in structural biology. Till now, we have seen how NMR spectroscopy can be used to elucidate the structures of biomolecules say protein, nucleic acid and all those. Now, slowly I want to transition into application of solid state NMR spectroscopy, which is one of the advanced technique in NMR spectroscopy, little more complex than the liquid state and we are going to learn the basics of solid state NMR spectroscopy; how it is different than the liquid state NMR spectroscopy, what extra tricks and methods that you need to imply in solid state NMR spectroscopy and those how we can use to elucidate the structures of proteins and nucleic acid and what kind of structure we are talking about here. So let us slowly start into this.

So if you just look at the matters, what kind of matters we can define? The matters can be defined into say gas, or it can be liquid and solid. These are the different states of matter. Now gas is randomly moving, randomly oriented. Now in this case of gas, as you know, spins will be randomly oriented.

So if they are just randomly tumbling, then the net spin quantum number will be 0 and it will be difficult to do any NMR spectroscopy on this. Now liquid state, you can have two kind of liquid, one will be isotropic, again in solution state we have seen this isotropic behavior, the molecules are tumbling here and there and therefore that is what we were investigating in liquid state NMR. Isotropic behavior was exploited in liquid state NMR spectroscopy to understand the structural biology aspects of protein and nucleic acid. In some cases, this liquid can be anisotropic, we can orient these liquids and that probably we have seen in the RDC experiment, how residual dipolar coupling, the partial orientation of these molecules gives us the signal or the parameter that is used for structural biology. Another third one is solid state. In solid you can have anisotropic molecules, solid can be ordered or disordered.

Now first let us talk about anisotropic molecule. So anisotropic where the orientation is fixed, it is not tumbling so much. Now if you take the case of membrane protein, they are embedded in the membrane, they are oriented at a certain angle. They are not freely tumbling, therefore there is some anisotropic condition introduced in this membrane protein. Now talking about the ordered solid, it can be either crystal, right.

So if there is a long-range order then that is crystal or it can be even fibrils, amyloid fibrils that we had talked previously little bit and now we are going to talk more. Amyloid fibers are arranged in a particular fashion. These are the protein assembly. So these are ordered solid. The disordered solids are glasses.

So if you look at all these molecules like a membrane or the crystal or fibrils or glasses, all these can be classified as a solid or these are amenable for solid state NMR. Now isotropic proteins, the membranes and the fibrils, these are biological material. So in this section of this course, we are going to focus more on membrane protein and the fibrils, how we can deduce their structural and dynamics information using solid state NMR. Now isotropic already we have seen in liquid state NMR. So why solid state NMR? Why it is important? If we can do everything by liquid state NMR that there is no need but there are many biological materials that are not amenable for liquid state NMR and therefore we need to invoke the power of solid state NMR to deduce their structural and dynamic property.

In solid acid NMR, you do not need solubility of these materials, the biological materials or even you do not need long range order that is required for X-ray crystallography. So because it does not require solubility of the material, it does not require long range order of the material, solid state NMR is suitable for following samples. Suppose your protein is not crystallizing very well, it is forming only small crystals like a microcrystal or nano-crystal. Even if you are going to synchrotron and you are not getting enough diffraction data, do not worry that is a sample for solid state NMR. So we can use solid state NMR of this nanocrystal and microcrystal.

Another samples if your protein is not soluble, they are precipitated, they are perfectly fine for solid state NMR. Because these are non-soluble proteins and there is no long range order, you cannot do crystallography, but these are perfectly amenable for solid-state NMR. The third sample is aggregated protein. In next slide, I am going to talk about aggregated

protein. So you can see these are protein fibrils. Many proteins aggregate under cellular condition and one can investigate these aggregated proteins using solid-state NMR.

The fourth one is membrane protein. As we discussed the anisotropic is introduced, anisotropic order is introduced in membrane protein. Here is in green I am showing you the membrane, lipid bilayer where a protein is embedded. Now this protein is not freely tumbling in solution, there is a restriction. Because of this restriction of the motion, these proteins cannot be completely amenable to liquid state NMR.

Some parts, which are flexible can still be seen in liquid state NMR but not whole protein. Now such proteins, membrane embedded protein is perfectly fine for solid state NMR. The last one, one can classify in this whole like a protein inside a whole cell. Now if you really want to mimic what happens in vivo, a solid state NMR is a great tool to look at what happens in vivo. So you can investigate the protein inside the whole cell, just you have to do probably label this protein, isotopically label these proteins.

And the rest environment will be un-labeled. So the selectively labeled protein of interest can be still very well investigated in natural cellular setting. So if solid state NMR is so nice, what are the challenges? Before we go to challenge, let us look at some of these two proteins, which are very popular for solid state NMR. These are amyloid fibers and membrane protein. Why amyloid fibers? Christopher Robson, once upon he said, under certain condition all proteins and peptides has tendency to form amyloid fibers.

And this protein misfolding is one of the common like a common phenomenon that happens in many disease. Some or other protein like if they are not properly folded or upon certain stimuli, they can misfold and aggregates and they forms this fiber like structure. These fibers like structure has been seen in many disease like Alzheimer's disease, one of the horrendous disease where memory loss happens, this is neurodegeneration. In this disease, one of the protein called β-amyloids get aggregated and deposited in the neurofibrillar plaque or tangle. Another second common neurodegenerative disease which is called Parkinson's disease, this is classified by tremor, rigidity, loss of posture, instability and all those one protein called α-synuclein aggregates and forms this amyloid like fiber.

The third one is Huntingtin, this is again a neurodegenerative disease or you have heard about mad cow disease, a prion disease like CJD, Creutzfeldt-Jakob disease or Kuru disease. Many of such neurodegenerative disease in the neurons of a human brain some or other protein gets aggregated. These proteins are not soluble. So if you want to do any structural study, you want to design a drug against them, the solid state NMR offers a great tool because you can look at the structural details of these molecules. We will be discussing those how we can do that.

Not only neurodegeneration, even the type 2 diabetes, one protein called amylin or IAPP, human islet amyloid polypeptide, the small peptide of 37 amino acid, which is co-secreted with insulin. You see this in normal case, this is co-secreted with insulin, this helps in gastric emptying but as type 2 diabetes triggers, this protein starts aggregating and aggregation of this protein starts rapturing the insulin producing β cell. Now that is the problem and then your insulin producing β cell starts depleting and that is actually aggregate the type 2 diabetes. So these are the protein aggregates that are amenable for solid state NMR based structural biology. Another popular class for solid state NMR is membrane protein.

There are many proteins, at least one-third of the open reading frame codes for membrane protein. Some of the example you can see G-protein coupled receptor, GPCRs or ion channels or even transporters, these all can be classified as a membrane protein and they are amenable for solid state NMR. GPCR in particular very, very interesting protein molecule because they are the target for many drugs. About 60% of drugs are targeted to GPCRs. Similarly, ion channel, they control many important function like potassium channel, calcium channel, proton channel, all of these either in human or in other organism, they plays very, very important role in physiology.

So they are again amenable for solid state NMR. You cannot only look at the structure, you can look at the dynamics, which is very important for their functioning and signaling and solid state NMR offers a great tool for looking at the structure as well as the dynamics and even thermodynamics. The transporters like ATP, ADP transporters, so there are many examples of membrane protein that can be looked at with solid state NMR. But if this technique is so great, what are the challenges? So let us look at the challenges now.

Problem is inherent line broadening. So, for giving you a perspective, I am taking a small molecule here. You look at, there are not too many protons here and I just do not want to give you the signal for proton.

Let us look at the carbon. We have 1, 2, 3, 4, 5, 6, 7, 8 carbons in aliphatic region and there are some 6 carbons in aromatic region. So all these carbons, if you record a solid state 13C, you get really really broad signal. If you record proton, you get a really like a broad signal and you do not get any resolution. You do not know which signal is coming from where. However, on the other hand if you record a solution state spectrum, you get a beautiful well-resolved spectrum that we are used to see it.

Now here again you 13C spectrum for this in solution you get a really well resolved spectrum. Here we can identify each of these peaks, the carbons where they are coming from. So we can do lots of interesting stuff that we have seen in the previous section. But, now with solid state NMR with this broad line what to do? We have no choice. So we have to think something else.

Why these are coming? Why these are broad signal? Because these molecules are not tumbling. Spin dynamics induces the line broadening. These spins are fixed. So, there is anisotropic interactions happening between these spins and that essentially makes this line extremely, extremely broad.

So a static solid state NMR spectrum which is called powder spectrum are very broad compared to solid state NMR and this is the result of anisotropic interactions that happens between the spins. So this is one of the major problem when we initiate recording solid state NMR spectrum. So why actually signals in solid are broad? Just to recapitulate what we learned in the previous section, if you just look at the molecules in solution, all of them are randomly oriented, tumbling fast. Now because of the tumbling you get an average chemical shift, say one of these, okay. But if you start restricting their motion, so like suppose one spin set is like this, that will give a signal here.

If your spin sets are slightly tilted, you get a signal here. If it is more tilted, you get a signal here. Since in solid because of the motions are almost frozen you get a various peaks like all of these and what we see a resultant signal which I showed you in the previous slide.

So because of differently oriented fixed motion of these spins you get a resultant signal of those spins and that is really, really broad for proton even more broader. Now that is the reason why anisotropic interactions broadens the orientation.

So to explain it even more I have chosen various sets of spins here, if you look at they are color coded in different case. These are spin pockets oriented in different orientation and because of their different orientation they are giving a different peaks at their chemical shift and now since we are seeing and resultant of that which is called powder spectrum you can see a really broad spectrum. This is the reason why in solid state, we get really broad signal here powder pattern. Because of this free trembling, that is happening in solution, you get sharp peaks here. So liquid, you have rapid random tumbling actually it is averaging the chemical shift, anisotropic chemical shifts and couplings are more or less average.

In solid interactions are there, the anisotropic interactions are there that introduces various other terms that I am coming in next slide. These orientation interactions leads to broad line and that limits the resolution in the NMR spectrum of biological macromolecule. So what are those interactions? So if you decouple the Hamiltonian that are there, this is the total Hamiltonian, then out of that this is external and this is internal Hamiltonian. External Hamiltonian as we know there are two, one is static field where we are doing the NMR spectrum, NMR experiments like if you are doing on say 600 megahertz, it is a 14 tesla or 800 megahertz or 1 gigahertz.

So depending upon what magnetic field we are choosing that is a one of the external Hamiltonian that introduce static field. Then another external perturbation that we use is RF field, B1 field that we give the pulse. So these two are source for external Hamiltonian. Now internal Hamiltonian as we know there is a chemical shift term we have learned earlier.

Then because of these spins are not tumbling, so in anisotropic interactions, the spin-spin coupling is introduced and that is dipole-dipole coupling, that is a short-range interactions. You know this is r^3, so that is a short-range interaction that is introduced because of this anisotropic interactions. Then we have also dipole-dipole or dipole-quadrupole interactions that are long-range interaction, then we have J-coupling that we have seen previously. So these are internal Hamiltonian that contributes to the NMR phenomena, chemical shift, dipolar coupling, J coupling and quadrapolar coupling.

If you look at the order of these perturbation, the static field is really big compared to the RF field that we have. Quadrapolar coupling is quite big. So this is in megahertz if you talk in terms of frequency. This is typically in kilohertz. This is again kilohertz to megahertz. Now, these again are in kilohertz order and J coupling are in terms of hertz.

Now what happens, so these actually in liquid state if we compare because of tumbling we do not have this dipolar coupling, we do not have this quadrupolar coupling. So only interactions that we have other than external we have a chemical shift and we have a little bit of dipolar coupling, long range dipolar coupling that we have seen in NOESY spectrum that is exploited for getting the distance constraints in the NOESY spectrum and we have a J coupling. So this is case for liquid state or isotropic liquid and that is how we have a well resolved spectrum. However, in anisotropic liquid just now we talked about the membrane protein. So we have again this anisotropic interaction introduced the dipolar coupling.

It has a long-range dipolar coupling that is still small. We have a J coupling and we can have a quadrapolar coupling. These are basically extra interactions we have, these two additional interactions that we are having, the CSA, chemical shift anisotropy and dipolar coupling. So chemical shift anisotropy and dipolar coupling, these two extra interactions are responsible for this line broadening that we have seen in solid state NMR. So essentially, what we want for structural biology, if you look at the tumbling of molecules, generally in solution, molecule tumbles all the way from picosecond-nanosecond to the second time scale.

What kind of molecular tumbling we have? Like I say unfolded protein - folded protein, all those tumbles in nanosecond time scale that we have seen earlier. If we put this protein in a micelle, there is restriction in the motion that tumbles in microsecond. If this embedded in bilayer, then further the tumbling slows down and interactions are introduced, dipolar coupling and chemical shift anisotropy or even in the fibril that we have seen or aggregated protein all these basically introduce the additional interactions. The tumbling slows down and these are amenable for solid state NMR. However, these freely tumbling proteins are amenable for liquid state NMR.

So because of this tumbling in solution the lines here were sharp and because of restricted tumbling or almost no tumbling you have a line really broad here. Here in liquid state we have a sharp line, in solid state we have a broad line. The chemical shift anisotropy is introduced and that is causing the line broadening. So spectral resolution is sharp here, it is a broad here. Now what we want? So we want, because the orientation gives really important structural parameter like chemical shift anisotropy that we talked, quadrapolar coupling, dipolar coupling, J coupling, all of these were available in liquid state. However, in liquid state, what we have only available, little bit of chemical shift anisotropy, quadrapolar coupling, dipolar coupling were averaged out and J coupling we had.

So this J coupling and little bit of chemical shift anisotropy and long range dipolar coupling that we have in NOESY spectrum that were giving us structural information. However, in solid all of these are at our disposal. So now, can we exploit these important structural information for doing the structural biology aspects? But you know lines are broader, so we have to do some tricks. What tricks we have to do to get a sharp line and retaining this structural information? So we have to mimic the inherent averaging process in solid that we have because of free tumbling to obtain the high resolution and also isotropic information. So what we have to do? We have to mimic the liquid state condition by doing something so that lines are sharp and we can mimic this isotropic information.

Now goal one for us is to increase the resolution and sensitivity. We have to increase the resolution, lines become sharp and it should be also sensitive. So, we have to remove the anisotropic part and retain only the isotropic part. We have to remove the anisotropic part, which is introduced by the dipolar coupling, chemical shift anisotropic and we have to retain only isotropic part. So, that can be done by decoupling or averaging of the interaction, we have to decouple or average the interaction.

The second thing that we have to do goal 2 is selectively, precisely get back the anisotropic interaction for elucidation of geometric parameter. So, some of these like interactions that we had. These are important structural parameter, chemical shift anisotropy, dipolar coupling. So can we also selectively introduce those and retain those. So that is done by something called recoupling or reintroduction of the full interaction.

So first, we decouple to get sharp line and selectively introduce some of these anisotropic interactions for structural parameters. So, these are two prime goals of doing experiment

in solid. So essentially what we want best out of the both world, sharp line and retaining the important structural parameter. We need a sharp line structural resolution and we want using a reintroduction of this structural parameter, this chemical shift anisotropy, dipolar coupling, J coupling and quadrapolar coupling.

So this is obtained by something called decoupling and this is obtained by something called recoupling. If you do both of these, then we can achieve sharp line and also retain all of the important structural parameters. So this is something basics of solid state NMR I gave you, the nut and bolts of like a basic toolkit of solid state NMR. Now in next class we are going to talk how we can do decoupling and how we can do recoupling. Decoupling is for sharper line, recoupling for introduction of structural information.

With this, I am closing today's lecture and hope to see you in next class with lots of interesting questions. Thank you very much and see you in the next class.