Interview with Robert Tycko: On Amyloids, Alzheimer Disease, and Solid-State NMR

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Dr. Robert Tycko is Senior Investigator and Chief of Solid State NMR and Biomolecular Physics Section in the Laboratory of Chemical Physics in National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK-NIH). In this interview Dr. Tycko speaks about physical and structural basis for polymorphism in amyloid fibrils, the relationship between Aβ polymorphic forms and progression of Alzheimer disease, the conditions to inhibit fibril growth, and the techniques to study and characterize fibrils including prominently solid-state NMR. The video of this interview is available on the YouTube site (URL https://www.youtube.com/watch?v=XBj8TwaJaho).

I. IN Aβ FIBRILS, THE PEPTIDES WITH THE SAME SEQUENCE PRODUCE DIFFERENT STRUCTURES (POLYMORPHS). IS THIS A VIOLATION OF THE ANFINSEN PRINCIPLE, WHICH SUGGESTS THAT THE SEQUENCE DETERMINES MOLECULAR STRUCTURE UNIQUELY?

It does not violate any laws of physics or thermodynamics. It is just the simple fact that there are many different ways in which a peptide can form a fibrillar structure that is sufficiently stable. We have actually studied a 40-residue Aβ peptide and developed detailed structural models for two distinct fibril structures that are formed in vitro. Interestingly, they have different symmetries: one of them has a 2-fold symmetry axis along the direction of fibril growth, while the other has a 3-fold symmetry axis. They are really quite distinct in terms of the data. They look different in electron microscope images. Their mass density (mass per length) is different in EM measurements. When you isotopically label them and record NMR chemical shifts, they are different. Therefore, they really are physically distinct and they propagate independently. If you prepare a pure sample of one, you can use that sample as a seed for growing subsequent generations of fibrils. When fibrils grow from an existing seed, the structure persists along the length of the fibril, that is, the structure is self-propagating.

It is something surprising, but this is what the experiments led us to conclude. Therefore, it was interesting to measure what is the difference in the growth kinetics and differences in thermodynamic stability of the two fibrils. We did that and the article came out about two years ago in the Journal of the American Chemical Society. It turns out that the thermodynamic differences in stability are very small—detectable, but small—meaning on the order of one kT or less. But the stabilities are both very high, so if you take two different fibril structures and separately put them in solution and allow them to come to equilibrium with monomeric or soluble material, the measured solubilities turn out to be very low (submicromolar). This tells you why the polymorphism persists once it develops. It persists because once you

Received 15 June 2015; revised 16 October 2015; accepted 21 October 2015

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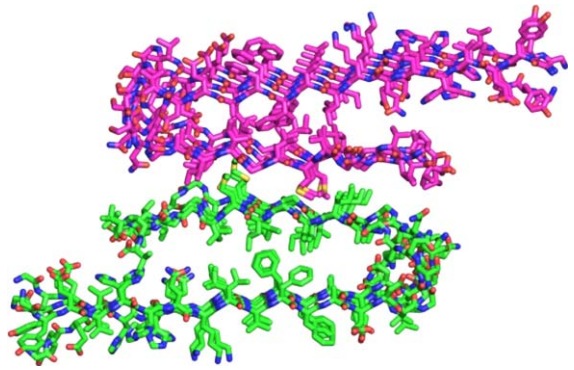
Concepts in Magnetic Resonance Part A, Vol. 44A(4) 182–189 (2015)

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/cmr.a.21353



Structural model of $A\beta_{1\text{-}40}$ fibril [Petkova, Yau & Tycko *Biochemistry* **2006**, *45*, 498].

form a particular fibril structure it essentially uses up all of the monomeric peptide that is left behind. There is very little left, less than a micromolar concentration under typical experimental conditions. And that is not a high enough concentration for new structures to spontaneously nucleate. Therefore, once you have a given structure that is stable it will persist and will not convert into anything else on any reasonable time scale.

However, you can observe interconversion between two different fibril structures if both of them are present. If you mix two different fibril structures, then you can see how gradually those slightly more stable structures will increase in total mass and the less stable structures will decrease. However, this occurs not through an internal structural conversion, it occurs as the less stable fibrils very slowly dissolve and transfer monomers to the more stable fibril structures. And that will be a very gradual conversion on time scale of months or years or decades. You can accelerate that conversion by breaking all the fibrils into short fragments by sonication or by some other procedure. This is because the overall time scale is limited by the number of ends, the density or concentration of ends of the fibrils—that is where all the action happens, that is where the monomeric peptides or soluble peptides come on and off. So the more ends there are, the faster the whole thing will happen. We observed experimentally that there was a partial transfer from one structure to another, so the more stable structures were increasing. However, in order to see it we had to break the fibrils into small fragments—and you do not normally do that in experiments unless you are deliberately trying to do this.

So I think by now we have a reasonable understanding of the biophysical or physical-chemical basis for the polymorphism, and it does not violate any fundamental principles. It is basically a kinetically controlled system, rather than a thermodynamically controlled system.

II. HOW IS THIS DIFFERENT FROM CYTOSOLIC PROTEINS OR MEMBRANE PROTEINS, WHERE THE SEQUENCE UNIQUELY DETERMINES MOLECULAR STRUCTURE?

Internally, the fibril core structure is very rigid. There is no opportunity for it to rearrange internally. That would involve breaking hydrogen bonds and rearranging the packing and sidechain-sidechain interactions—that is not going to happen internally on any appreciable time scales. Unlike in the case of globular proteins, I imagine, where the barriers to structural rearrangements are not so high. For globular proteins in many cases, there is evidence that the proteins are continually undergoing structural fluctuations, transient unfolding and refolding events, which allow them to search for the possible low-energy structures and find the minimum. At least for proteins that have evolved functions in most cases there is essentially one structure that is significantly more stable than others.

Even within that most stable structure, there is always some intrinsic disorder, so it is never perfectly structured. In protein folding simulations that I have seen (done by other groups), as the protein folds it does get temporarily trapped in configurations that are not the native structure. It can get trapped in configurations where it has a lot of the native secondary structure, but does not have the correct tertiary contacts. And those configurations will persist, but they will persist for, say, microseconds—and there is enough thermal energy to rearrange things eventually. In the case of the amyloid structures, they get trapped forever because the kinetic barriers are too high. It is a very extended supramolecular structure—without disrupting the whole thing you cannot rearrange one little part of it.

III. WHY DO THE DIFFERENT POLYMORPHS ARISE IN THE FIRST PLACE?

There we only have a qualitative understanding. For fibrils to start forming, you begin with peptides that are initially in a monomeric state, but then they will tend to aggregate quickly. They form a variety of aggregated structures, some of which may be biologically important: the oligomers that are widely discussed, other kinds of intermediates in the fibril formation process emerging under various conditions on various time scales, depending on exactly how you do the experiments. The oligomers of the peptide are probably somewhat dynamic, and then at some point they randomly adopt a structure that is sufficiently stable—so that it will persist and start to grow. It is probably a structure that resembles the

structure within the fibrils. We do not really know exactly what this critical nucleus structure looks like, but it would make sense if it resembles the structure within mature fibrils, which have grown from that nucleus. And apparently there is more than one kind of nucleation event, more than one kind of critical nucleus structure that is sufficiently stable to grow into a mature fibril. Therefore, there must be this alternative nucleation event that leads to a different fibril structure—that is my understanding. However, we do not know a lot about the actual structural properties of those nuclei.

Apparently, there are many paths. There are two structures formed in vitro that we have studied in detail. However, we know from just looking at many samples that we prepared over the past decade, looking at NMR spectra, looking at electron microscope images of them—we know that there are many more than two. Other laboratories have identified other structures, and there are at least 5 to 10 of them, I would guess, each of which has its own signature in electron microscopy and solid state NMR spectrum. Therefore, there are multiple kinds of nucleation events, apparently. It is surprising that there is such diversity, it is an amazing thing. That was not predicted, I do not think—it was an unexpected result that there would be so many of them.

Experimentally, we found that fibrils that we derived from brain tissue of Alzheimer's patients can have different structures than the structures that we have observed in vitro. They are qualitatively similar, but the structural details are different: the precise conformations, some of the precise intermolecular interactions or interresidue interactions within a peptide molecule—those can be different. So why do we see different structures from brain tissues? Why do we see different structures from one patient to another? Again, it could just be linked to this random nucleation event that determines what the structures are. There is also another possibility. In our experiments, we extract amyloid from brain tissue of Alzheimer's patients obtained from autopsies. Then we use the amyloid fibrils from that tissue as the seed to grow fibrils that we can do our NMR and EM measurements on. However, Alzheimer's disease is generally a slowly progressing disease, so there could be multiple structures that exist at very early stages of the disease. By the time we actually get the tissue, after 10 years, maybe one structure has been selected out because it is particularly resistant to degradation or clearance. Or maybe through the slow interconversion the system reaches the more thermodynamically stable state, so by the time we get the tissue it has actually come to equilibrium. I am not sure if I believe that, but it is possible.

Therefore, we do not really know, but we have observed that the fibrils from brain tissue are typically structurally homogeneous within a given patient. We have seen exceptions to that, and so far the exceptions we have seen actually are from rapidly progressing cases. In rapidly progressing cases, the fibrils presumably have not been present in the brain tissue for as long, so maybe there is a selection and by the time you reach the end of your life, you have selected out a particular structure. Alternatively, within the more heterogeneous mixture of structures that we have seen in a couple of rapidly progressing Alzheimer's cases, some component of that heterogeneous mixture may be particularly neurotoxic in a way that leads to the rapid progression. Therefore, we do not know yet exactly what is accounting for the observations concerning fibrils derived from brain tissue, but this is ongoing work, so maybe we will sort it out in the next couple years.

IV. CAN AMYLOID STRUCTURES OBTAINED IN YOUR LABORATORY BE USED FOR DOCKING/IN-SILICO SCREENING/PHARMACEUTICAL LEAD DEVELOPMENT?

The question is can we use our structural models as the basis for docking calculations or for the development of inhibitors. In addition, I think another important target is imaging agents, so developing compounds that bind selectively—perhaps to particular structures that are most significant in the disease—that could turn out to be important. However, do our structures have sufficient precision for that to be done? I am not sure. When I describe our structures, I refer to them as "detailed molecular structural models" because within the field of structure determination, if you say that you have a structure, people interpret that in a particular way. I want to avoid getting into arguments with people about whether we really have a structure, or we have a model. I think that all structures are models at some level and it is just matter of degree, that is, it is a continuum. I do not think there is a real threshold that separates detailed structural models from structures.

However, from a practical standpoint do we have enough precision in our structural models to use them to actually calculate binding constants of different small molecules to the fibril structures? I would say unlikely because there are certain aspects of the structure that are essentially impossible for us to determine experimentally. In many structure determinations by various experimental methods the reality is such that there are certain aspects of the structure that are known very definitely with high precision, and there are always other aspects that are less certain. That is definitely true of our work. One of the particular aspects

that is very difficult for us to sort out has to do with something that we have called "staggering" in our articles. When you have an amyloid fibril structure, these are primarily β-sheet structures with the cross-β orientation, that is, ribbon-like β-sheets running the length of the fibril. But when you have a situation when there are multiple layers—in particular, when each molecule contributes to more than one layer of β-sheets that are stacked on one another in some way—then there can be a shift of the top layer versus the bottom layer in one direction or the other. Therefore, the β-strand segments that arise from a given molecule do not necessarily sit on top of one another—they can be displaced in one direction or another. When we have looked for that, which we could do by isotopic dilution experiments, we inevitably have found that the contacts between β-sheet layers indicate that they are shifted or staggered in one direction or another. And we do not know in which direction, we have no way of telling. Two ends of the fibrils are structurally inequivalent, so that shifting in one direction is different from shifting in the other direction, but we cannot determine that from our data. Therefore, that is one very specific aspect of the structure that is really indeterminate.

Of course, if you wanted to try to use the structural models to design compounds to inhibit them or dock to them, then you at least have to consider those two possibilities (stagger directions), or maybe it is more than two. And then there are other aspects of the structures – we do not really have sufficient information about the precise aspects of sidechain confirmations and things like that. Therefore, our structures are not at the level of 1.0 Å X-ray crystal structure by any means, and I would not make that claim. However, I think that our structures are what you see. Sometimes when I give talks and show the structural model on the screen, I tell people: "Well, what you can see from the back of the room is certainly correct. When you are sitting in the very front row, some of the details may not be correct." In addition, when we have deposited the coordinates for the structure in the Protein Data Bank, I have tried to do this in a way that, as accurately as possible, reflects the uncertainty of the structure. There are bundles of structures that have disorder, various structural variations, and I have tried to maximize rather than minimize that. Therefore, I have tried to include in those bundles a large range of structures that fit our data equally well or sufficiently well. If you look at what has been deposited in the Protein Data Bank, you will see that there are significant structural variations within the bundle of structures. That gives you a reasonable picture of what we actually know and what we do not know.

Perhaps a more fundamental question is if you want to inhibit the formation of fibril, what do you need to do? The fibrils have a given structure along their length—until you get to the ends. At the ends of the fibrils presumably there are some distortions of the structure. If you want to cap a structure, or prevent its propagation, it seems to me that you probably need to know something about what is happening at the very ends of the fibrils. And we cannot do that. I can imagine futuristic ways of doing that, but at this point we do not really know how much of a distortion of the structure exists at the ends. However, there has to be something because obviously there are dangling hydrogen bonds, dangling interactions at the ends that have to lead to some kind of a distortion.

V. IF YOU HAD TO ESTIMATE THE ACCURACY OF YOUR STRUCTURAL MODELS, WHAT WOULD IT BE?

It is 2.0–2.5 Å range, probably. We can calculate these *rmsd* values from our structural bundles and we get numbers in 2.0–2.5 range. There are certain aspects of it, certain parts of the structure that are 4 Å or something, but then there are other aspects that are much better. Therefore, a single number really cannot capture the whole thing.

There is also a question of how much intrinsic disorder is there. That might vary from one fibril to another. Certainly the quality of the solid-state NMR spectrum and the sharpness of the NMR lines which is some measure of the level of disorder (although it might be difficult to quantify) vary from one fibril type to another and certainly from one fibril-forming protein to another. There are some that give spectacularly sharp solid-state NMR lines, which must mean two things: that they have a very well defined structure, and they have rapid internal dynamics, that is, all motions happen on a very short time scale. Those two things have to be true to get very sharp lines. In other cases, we just never see lines that are quite as sharp, and there is some evidence that there may be intrinsic disorder. Within a single fibril along its length, there can be some variations, which can be a limitation. However, it is hard to quantify these things.

A point that I like to emphasize is this: when we started on this work 15 years ago, very little was known at all about the structures. So one should not set an artificially high bar for the work, which has started from literally zero. We have come very far—not just us, but the field in general has come very far. These are difficult problems, and we have had to figure out a lot of things along the way: which measurements work,

which measurements do not work, what is the right combination of measurements that allow you to get to a full structural model. How do you actually make the samples: this is an issue of polymorphism. We did not expect that to be a problem when we started. There are certain cases where the solid-state NMR spectra are extremely clean and the monomers are not too large and you can isotopically label them the way you need to do for the measurements, you can actually make good samples—then you can get very far and obtain quite precise structures. In addition, there are other cases, where that is just very hard to do. But overall our understanding has improved quite a bit from where it once was.

VI. IS THERE A WAY TO PREPARE AN AMYLOID THAT WOULD DIFFRACT AT HIGH RESOLUTION (ASIDE FROM SHORT PEPTIDES)?

Can you crystallize fibrils? Obviously other groups, principally David Eisenberg's group from UCLA, had spectacular success in determining very high resolution crystal structures for amyloid-forming peptides and in those crystals the structure is a cross-β structure that, if not the same, is very closely related to the structures that one observes in amyloid fibrils. There is the same issue of polymorphism that they observe with various structural variations. An amazing thing about those crystal structures is that the β-sheets are absolutely flat. To obtain a crystal, you need intermolecular β-sheets that are untwisted—otherwise you would not have the periodicity that you need to have, the translational symmetry that you need to have in a crystal structure. And fibrils do tend to twist—indeed, β-sheets in proteins tend to twist. So far, that seems to be limiting the ability to crystallize full-length amyloid-forming peptides or proteins into their amyloid-like state.

So far if you ask what is the real barrier to crystalizing fibrils, determining crystal structures, I think the inherent tendency of β-sheets to twist makes it difficult to crystallize them. To accommodate a fibril with the twist one may need a very large crystal unit cell. Twist is a kind of "soft" degree of freedom. In electron microscope images, some fibrils obviously have a twist, which is somewhat regular in some cases, but not perfectly regular. This also makes it difficult for electron microscopy to get real high-resolution results (that plus the polymorphism again).

Of course, in crystallography you should never say it is impossible because eventually they succeed. Numerous examples have shown that you should never count the crystallographers out because they always figure out a way to do it. The results are spectacular, they can actually get all the details—it is a great technique. So far, it has not worked for large (amyloid-forming) peptides or proteins, but maybe it will. Maybe it already has and we just have not heard about it. But even then there is the issue of polymorphism: which of the structural variants will turn out to be biologically relevant still remains an open question. Our experiments were trying to actually characterize the structures that develop in tissue; it might be hard to apply crystallography to that. Other groups are doing very similar things now for other amyloid-forming proteins: α-synuclein, yeast prion proteins, maybe eventually the mammalian prion protein, using extracts from cells or from tissue and focusing on the biologically relevant polymorphs. That is definitely the trend now in amyloid work.

VII. HOW WIDE-SPREAD IS THE ABILITY TO FORM AMYLOIDS?

Certainly amyloid formation is a very prevalent phenomenon; it seems like something proteins and polypeptides like to do if they do not have something else to do. If there is not a more stable monomeric or oligomeric state, then this is something they tend to revert to. When you look at the structural models of amyloids you can see why they are kind of good, happy structures for polypeptides. There are all those backbone hydrogen bonds that are formed. Most of the residues are in β-strand region of the Ramachandran plane, which is accessible to all amino acids: it is the place where they like to be, avoiding steric clashes among the sidechains. So you can understand why many different sequences would fit into an amyloid structure.

The most common structure that seems to occur is the in-register parallel β-sheet which was first seen by David Lynn, Steve Meredith and Bob Botto in their experiments on the residues 10 to 35 of the Aβ peptide. They found the first evidence for in-register parallel β-sheets in amyloid fibrils. This was very controversial when they reported it in 1998, but that turned out to be correct and the most common amyloid structure. This is the structure that automatically lines up the hydrophobic residues with one another, automatically lines up polar residues, glutamines and asparagines, which can interact with their sidechains in a favorable manner. It also brings together charges, creates rows of charges, which can have a disruptive effect. In a parallel structure if you have many charged sidechains all lined up about 5 Å apart, if they are in a hydrophobic interior of the structure, that is, in a low dielectric environment, then the electrostatic repulsion would easily

disrupt the structure. Therefore, for a polypeptide to form an amyloid structure it has to avoid creating these rows of like charges in a hydrophobic interior. In other words, you can prevent fibril formation if you have a sufficiently highly charged sequence. It is ok if the charges can pair up, but if there is a sequence with a large net charge, that will prevent amyloid from forming. If there are many prolines, that will prevent amyloid from forming. Otherwise, for most sequences it is something they seem to be able to do. That is why it is such a common occurrence.

There are some amyloid-like structures, cross-β type structures in "real" proteins. And then there are various functional amyloids that have been either proven or at least postulated. There are several cases where it is documented that the amyloid state has a biological function. Some of these have sort of an adhesive function, so the curly proteins on the surfaces of bacteria act to allow the bacteria to adhere to surfaces or to one another. HET-s fungal prion is a very remarkable protein that is not polymorphic for which the solid-state NMR spectra were originally obtained in Beat Meier's group at the ETH in Zurich. These are spectacular, fabulous spectra. As a result of their hard work, and also the favorable properties of the protein, they have developed a very detailed structural model for those filaments. Therefore, those are believed to have a biological function in what is called "heterokaryon incompatibility" between two different fungi. Therefore, there is room in nature for amyloids with a useful biological function.

VIII. HOW DO YOU SEE THE SOLID-STATE NMR IN 5 YEARS FROM NOW – IN TERMS OF NEW APPLICATIONS, METHOD DEVELOPMENT, ETC.? WHAT ARE THE AREAS THAT YOU EXPECT TO MOVE TO THE FOREFRONT?

Solid-state NMR is the main experimental technique or set of techniques that we have used to characterize structures of amyloid fibrils. Overall, applications of solid-state NMR for biological system is an area that has grown quite rapidly over the past, let us say, 15 years. There has been a lot of progress because there are now a large enough number of groups, who are just trying things. People have been able to find classes of systems where the solid-state NMR techniques really work and you can get high quality data, you can get sufficiently resolved spectra, and you can actually make the samples and get sufficient sensitivity. The techniques have also come along at the same time, but just exploring different applications has been an important part of it and will remain an important part of it. I think that in the coming 5 years people will find more and more classes of systems where the solid-state NMR methods really work and you can learn things that you cannot learn from other techniques.

Solid-state NMR is great because you can use it to get atomic-level structural constraints, structural information about systems that you really cannot study in detail with other techniques (because these systems are not soluble, or you cannot crystallize them, or they are too large for your technique). In general terms, it is a technique that should have very wide applicability. But then you have to find specific systems where you can actually make the samples and get adequate sensitivity, and where the solid-state NMR spectra are sufficiently simple so that you can actually understand them. We have to identify more of those. Therefore, the amyloid work that I have been involved in is one good example of that but there are other examples that have come out and will continue to come out from a variety of groups who are working on this around the world. And there is still a growing number of groups, so that is a very healthy thing for the field.

When I think about what is the big limitation, a big limitation is really on the biological side. We are limited in terms of the complexity and size of the systems we want to study. We have to isotopically label the proteins (so far it is mostly protein work). We would like to be able to look at proteins that have molecular weights of 100 kDa or more—and in principle you can do that. However, the spectra end up being intractable because there are too many signals if you isotopically label the entire sequence. So molecular biology techniques, protein expression and labeling techniques, segmental labeling and ligation techniques are needed to make proteins that are labeled at a subset of the amino acids in a particular segment of the sequence and to manufacture these proteins in quantities that you actually need for the measurements (typically at least milligrams). My group has worked on that and continues to work on it at some level; many other groups are working on it because it is obviously what needs to be done. And hopefully that will be worked out. If that can be developed on the sample preparation side, the labeling side, if that can be made into a general technique that allows you to get milligram quantities of proteins that are segmentally labeled in a general way, then that would open up all kinds of new applications for the technique.

Then there is always ongoing NMR technique development that is happening all the time. Pulse sequences are getting better. Proton-detected ^{13}C and ^{15}N NMR in solids is something that we worked on a while ago and other groups are doing it a lot now.

Sensitivity is getting better as a result. Magnetic fields are getting higher, etc. Therefore, the sensitivity is there and the methods for getting the structural constraints continue to get better—but they exist already. Making large proteins that are segmentally labeled would very quickly allow you to do all kinds of things that you cannot do now. That is the breakthrough that is needed, I think, more than anything.

On the sensitivity end, there is dynamic nuclear polarization (DNP) technique, which has become very popular, and my laboratory is heavily involved in that also. For solid-state NMR, currently the DNP involves introducing paramagnetic dopants into your sample carrying electron spins, which are intrinsically more highly polarized. This means a bigger population differences between "spin up" and "spin down" states in the magnetic field, so electron spins are more highly polarized than protons by factor of 660. Then polarization is transferred from the electron spins to the nuclei, thereby enhancing the polarization of the nuclei and enhancing the NMR signals proportionally. Those techniques are now becoming more and more widely used.

Currently in solids those experiments are done at low temperatures because the mechanisms that work best for DNP in solids are mechanisms that are effective at low temperatures. Going to low temperatures is a good thing in terms of sensitivity, but it generally costs you a lot in terms of resolution. The NMR lines get broader at low temperatures because the motions that tend to average out structural inhomogeneities become "frozen out"—and then you are stuck with static structural inhomogeneities that lead to broader NMR lines. Therefore, you sacrifice resolution. The amount that you sacrifice seems to vary a lot from sample to sample, but it is always there and it is always significant. It depends on exactly what temperature you are working at, so some groups are trying to do the DNP at higher temperatures, where there is less of a line-broadening effect. That is something that can allow you to look at certain problems that are otherwise difficult to look at.

This kind of breakthrough in sensitivity is important—especially if you are looking at molecules that do not have a large number of isotopically labeled sites. In this case, the spectra are inherently relatively simple and then you can sacrifice resolution and still get meaningful results. Therefore, if you can selectively label or segmentally label relatively short segments of a large protein (or in our case amyloid-forming peptides, which we can chemically synthesize and in this way isotopically label specific residues or segments) then the DNP experiments can be very useful. Hormones that are relatively short peptides or other small molecules bound to receptor proteins—those kinds of systems are perfect targets for DNP experiments if you are interested in what the ligand structure is in the bound state. In the case of hormone-receptor complexes, getting the receptor protein is usually the limitation. Receptor proteins are typically integral membrane proteins that you cannot prepare in large quantities and so you have a sensitivity issue there. With DNP you can overcome that and then you can solve problems that you cannot otherwise solve. This is another thing that is coming along now, and in the next 5 years we will probably see a lot more of that.

IX. WHAT ARE THE MOST PROMISING SYNERGIES INVOLVING SOLID-STATE NMR AND OTHER EXPERIMENTAL TECHNIQUES?

Synergies between different experimental methods are very important in my own laboratory and my own work on amyloids and other systems (for example, we are working on viral capsid proteins and the assemblies that they form). Electron microscopy is invaluable. You cannot live without it, really. Before you do the NMR measurements, you have to be able to see the structures, see the assemblies and make sure it is what you think it is. Otherwise, you can very easily go astray. This polymorphism that we see in fibrils—electron microscopy gives you pretty strong evidence that you have a sample that is morphologically homogenous. That is very important. We use atomic force microscopy to some extent for similar purposes. Some other techniques—calculations, molecular modeling, Molecular Dynamics simulations—are certainly an important component in any of these studies. Electron paramagnetic resonance (EPR) measurements can provide structural information on a longer length scale than what you can readily achieve from solid-state NMR—so that is an obvious combination that NMR people in general are taking advantage of now in their studies. You can also use optical techniques (FRET) as another way of getting long-range distance information. We rely heavily on mass spectrometry. For some of our problems now we are trying to combine solid-state NMR results with hydrogen-deuterium exchange measurements that are detected through mass spectrometry. In a fibril-forming system, that can give you information that is very helpful in identifying the fibril core and the regions that are outside the core. That is a few examples, and there are probably others that I am not thinking of.

During my own training in NMR when I was a student the idea was that you are an NMR expert and so

you should know NMR, but you do not have to know anything else. In fact, the tendency was to avoid learning other techniques. Part of that was a psychological effect. When you become an NMR expert, you really understand NMR in great detail. There is a lot of the mathematics behind it—in some cases, it has very interesting mathematics behind it—that is what attracts many of us to the field in the first place. To do the NMR experiments yourself you have to know a lot of details. It is a field of details. And then you go to some other kind of technique where you are really a novice, you do not know very much about it. Then you feel very uncomfortable because you are used to doing measurements where you know everything about it, you understand exactly how the equipment works, you could build it yourself if you had to, you understand all the physics, all the mathematics behind it, you understand it in great detail. And then you go to do electron microscopy, for example, and you are suddenly not sure how the machine works. So you feel like you are cheating (kind of) by doing that. But that is something that you need to get over, I think. If you do another measurement, then you have to live with the fact that you are not going to be such an expert on it, but still you should do it, and it is still very valuable. And if you do not do it, then your NMR measurements are not going to be as meaningful.

X. WHAT ARE THE PERSPECTIVES OF (BIOMOLECULAR) SOLID-STATE NMR BECOMING MORE USER-FRIENDLY TECHNIQUE, POSSIBLY EVEN A "SERVICE" TYPE OF TECHNIQUE AVAILABLE TO NONEXPERTS?

At least currently, to do any sophisticated solid-state NMR measurements you have to have significant training because there are so many ways in which the measurements can go wrong. You really have to understand how the measurements work and how the instrument works—otherwise you are limited to doing only very simple measurements. As far as the prospects of it becoming a routine technique for general users, I can see how it could be used in a core facility sort of setting, as long as there is at least one person there who has the proper training—meaning someone who has spent at least two years (probably more) doing nothing but solid-state NMR in a laboratory that focuses on those kinds of measurements. If you have somebody like that in a facility who has that expertise and can provide the advice, monitor the progress of the experiments and perhaps help with the interpretation, then it is a technique that could be used by people who are not specifically trained in solid-state NMR. However, you need at least one person who has a great deal of training and expertise.

This model already exists to some extent. There are laboratories that have 900 MHz instruments that are equipped for solid-state NMR—for example, the laboratory in Tallahassee, where they actually do have people with a lot of expertise, both in the instrumentation and the techniques themselves. In a facility like that, with those kinds of people, someone who has prepared an interesting sample could go there and bring the sample and participate in the measurements, but would be able to get plenty of the necessary guidance. At the same time I think there will always be solid-state NMR done in individual solid-state NMR laboratories, so you get mixture of the two models. In a lot of ways this is, of course, dictated by the cost of the equipment. It would be nice if everybody could have a very high field instrument in their own institution, but it is just not economically feasible.

ACKNOWLEDGMENT

This interview has been conducted as part of the educational activities under the NSF grant MCB 1158347 to N.R.S.

BIOGRAPHIES

Robert Tycko received his Ph.D. degree in chemistry under Alex Pines and then trained as a postdoc in Stan Opella's laboratory. After spending eight productive years in AT&T Bell Laboratories, he moved to NIH where he is now a senior investigator in the Laboratory of Chemical Physics in NIDDK. In 2002, Tycko and coworkers published the first detailed structural model of Aβ amyloid. Since then Tycko's group has contributed in many important ways to our understanding of this extraordinary form of protein self-assembly, while at the same time advancing solid-state NMR methodology to study and characterize amyloid fibrils.

Nikolai Skrynnikov obtained his Ph.D. degree in physical chemistry from McGill University. He did his postdoctoral work at ETH Zurich and then University of Toronto, before joining the faculty of Purdue University. His main interests lie in the area of NMR methodology–in particular, combining NMR experiments with MD simulations to study protein dynamics.