QnAs with Robert Tycko

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The complexity of diseases, such as Alzheimer's disease (AD), has hampered treatments despite decades of study. Recent technological advances and interdisciplinary investigations, however, are helping researchers better understand such diseases. Robert Tycko, a Senior Investigator in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) at the National Institutes of Health (NIH) in Bethesda, Maryland, leverages his background in physical chemistry and condensed matter physics to characterize the molecular structure of biomedically relevant proteins and polypeptides, including the amyloid-β peptide that causes the telltale plaques seen in the brains of patients with AD. Tycko draws on his experience developing structure determination methods, including solid-state NMR (ssNMR) and cryogenic-electron microscopy (cryo-EM), to uncover what amyloid-β fibrils look like at the molecular level, potentially leading to improved diagnostic and treatment options. PNAS recently spoke to Tycko, who was elected to the National Academy of Sciences in April 2020, about his current research.

PNAS: Your Inaugural Article (1) details the molecular structure of amyloid-β fibrils seen in patients with AD. How were you able to determine the structure?

Tycko: Amyloid-β fibrils are polymorphic, meaning they can have a variety of molecular structures, depending on their growth conditions. In an earlier paper, using ssNMR, we found that there is a single predominant polymorph for 40-residue amyloid-β fibrils in the brain tissue of typical Alzheimer's disease patients, but we did not determine its molecular structure (2). In the new paper (1), we show that this predominant polymorph has surprising structural properties. The most puzzling aspect was that our initial mass-per-length measurements indicated that the fibrils were comprised of three β-sheet subunits. But when we took cryo-EM images, they revealed that the structure had twofold symmetry, meaning that there were two—not three—subunits that were symmetrically related by rotation around the axis of fibril growth. When we were able to further analyze the cryo-EM images, we discovered that the basic subunit is effectively one-and-a-half β-sheets. Two such subunits then provide the three β-sheets that we had seen with the mass-per-length measurements.

This structural organization hasn't been seen before, but we have reason to believe that it is very common in the brain tissue of Alzheimer's patients. These results may provide a basis for designing small molecules that could be used as imaging agents to target this specific structure or perhaps inhibit the growth of this structure and slow the progression of the disease.

PNAS: How does this work fit into the broader context of your research, and has the technology improved to better aid your investigations?

Tycko: Prior to joining the NIH in 1994, I worked at Bell Labs in New Jersey, where I used ssNMR to study problems in materials science and solid-state physics. In the physical sciences, ssNMR is an essential technique for studying various properties of materials, including their structure, electronic and magnetic properties, internal motions, and phase transitions. When I first

Robert Tycko. Image credit: Sasha K. Tycko (photographer).

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moved to the NIH, my main goal was to find biological problems where ssNMR measurements could have a similar impact. I was fortunate to join the Intramural Research Program of NIDDK, where we have a lot of freedom and support to choose our own research directions.

I had never heard of amyloid fibrils before moving to the NIH. But when I did, I realized that there weren't effective techniques to study amyloid fibril structures because these fibrils are inherently insoluble and noncrystalline. It turned out that sophisticated ssNMR methods work very well on amyloid fibrils and, eventually, we built up enough information to put together complete molecular structural models based on the experimental data. Amyloid formation and amyloid structures are very widely studied by biochemists, biologists, and medical researchers, so this work has had an impact outside the NMR community. That's what I've always hoped, that our work will have a broad impact.

Of course, electron microscopy has also been around for a long time. However, only recently—within the last 3 or 4 years—have both the equipment and the image-analysis software improved to the point where high-resolution amyloid fibril structures can be determined from cryo-EM images. In this new paper (1), we use a combination of cryo-EM and ssNMR to characterize the unusual subunit structure. For example, we propose that the "half β-sheets" in each subunit are formed by amyloid-β molecules with β-hairpin conformations, and we use ssNMR measurements to support this proposal. One message from this paper is that it's important to combine methods to get a full picture of an amyloid fibril or of other types of protein assemblies.

PNAS: In addition to your studies on amyloid-β fibrils, you are working to improve existing imaging methods, including ssNMR and magnetic resonance imaging. How are you improving those techniques?

Tycko: My group is now working on two other, unrelated magnetic resonance projects. One project, which we call time-resolved ssNMR, seeks to solve the molecular structures of intermediate states in time-dependent processes, such as the early stages of amyloid fibril formation or protein self-assembly. We are developing rapid mixing and rapid freezing methods to trap these structures on a millisecond timescale. These are processes that are traditionally examined by optical spectroscopy or fluorescence measurements, but those methods don't give you the detailed molecular structural information that's available from ssNMR.

Our MRI project seeks to push the spatial resolution of MRI to the single micrometer or submicron level. MRI is obviously an important clinical technique that is very widely used. But the spatial resolution is typically on the order of a millimeter or maybe a tenth of a millimeter, so not very high. We're trying to get to a resolution that will allow you to see organelles within cells. The experiments depend on new technology that operates at very low temperatures, and we use magnetic resonance tricks like dynamic nuclear polarization to enhance the NMR signals. It is definitely possible to reach the single micrometer level, and we're very close. Once we've gotten it to work, the next question is, of course, what will we use it for? Contrast mechanisms in MRI are different from in optical microscopy, so hopefully we will see things that can't be seen with established methods.

1 U. Ghosh, K. R. Thurber, W.-M. Yau, R. Tycko, Molecular structure of a prevalent amyloid-β fibril polymorph from Alzheimer's disease brain tissue. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2023089118 (2021).

2 W. Qiang, W. M. Yau, J. X. Lu, J. Collinge, R. Tycko, Structural variation in amyloid-β fibrils from Alzheimer's disease clinical subtypes. *Nature* **541**, 217–221 (2017).