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Researchers Get First Peek at Amyloid's Spine

Howard Hughes Medical Institute researchers have provided the first detailed look at the core structure of the abnormal protein filaments found in at least 20 devastating diseases, ranging from Alzheimer's to Creutzfeldt-Jakob disease, the human version of "mad cow" disease.

The images reveal that the filaments form a short zipper that is closed and stuck. To get a more realistic picture of what the fibrils look like, however, one should picture a towering stack of zippers, each of which is tightly bonded to the one below.

The first atomic details of the interconnected protein segments were reported in the June 9, 2005, issue of *Nature*.

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In each disease, a different protein transforms into the misfolded threads known as amyloid fibrils. Scientists believe that the various proteins share a common underlying feature that explains how they assemble into the persistent fibrils that can accumulate in the brain and other tissues.

"To do something about these diseases, you have to be able to see the parts at the atomic level," said senior author David Eisenberg, a Howard Hughes Medical Institute (HHMI) investigator at the University of California, Los Angeles. "Only then can you design an intervention."

The common trait of these different proteins was discovered more than thirty years ago. But even the most advanced technologies have been unable to capture anything more than a fuzzy image.

"We call it the spine of the amyloid," said Eisenberg, who is also director of the UCLA-Department of Energy Institute of Genomics and Proteomics. "A little bit of each protein forms the spine, and the rest of the protein is hanging out in globular domains that decorate the spine and give the fibril its thickness and bumpiness. Once these amyloid fibrils form in tissues or cells, they are very hard to get rid of."

Now, he and his colleagues report the first detailed look at one protein's version of the shared core feature. In this case, it was a yeast prion, a misfolded protein that has the additional knack of being able to infect other cells or organisms, said first author Rebecca Nelson, a graduate student. Unlike in people, the yeast prion causes a condition which may be beneficial. In people, scientists do not know the role of the fibrils in the disease process in most associated diseases, but the formation of fibrils is associated with diseases.

According to yeast prion expert Jonathan Weissman, an HHMI investigator at the University of California, San Francisco, determining this structure "is a monumental achievement that will open up a new era in the structural analysis of amyloids."

The path to the discovery began several years ago, when co-author Melinda Balbirnie had narrowed down the stretch of prion necessary for fibrils to only seven amino acids, which were located at one end of the entire protein. Filling a test tube with just those snippets was enough to form short thin threads with the same essential characteristics of the common amyloid spine, a structure known as a cross-beta sheet.

Once it begins, the structure of a growing amyloid fiber is irresistible to other identical proteins or, as in this study, the crucial peptide subcomponents. The fibril spine elongates as pairs of the short beta-sheet segments stack up like teeth in a zipper.

When Balbirnie added more peptides to the test tube, she found that microcrystals formed and dropped to the bottom of the test tube. The crystals were about 50,000 times smaller than those normally used to determine atomic details of protein architecture. The researchers tried one mathematically intensive technique to analyze the microcrystals. It showed a fuzzy picture similar to other fibrils, telling them they were on the right track but not giving them the details they were seeking.

Nelson picked up the project four years ago. "Because the crystals were so small, we ended up trying lots of techniques," she said. "We formed collaborations with people who were experts in those areas. Every time we'd come up with a new idea, it was exciting. Then, when we were able to determine the structure, it was twice as exciting."

The breakthrough came when the researchers teamed up with European crystallographer Christian Riek, who had designed and built a special x-ray beam as narrow as the crystals were tiny, and Anders Madsen, a Danish student working at the synchrotron in France who was skilled in special methods for mounting and manipulating the samples to collect good x-ray diffraction data.

"With the first calculation, we were able to see the structure and how we would be able to model atoms into that map," Nelson said. "That was really the ah-ha moment."

The final detailed structure is broadly consistent with other lower-resolution models, such as the two stacked beta sheets composed of the main chain of amino acids. The surprise came with the molecular side chains that give each amino acid its unique identity and hold the pairs of beta sheets in formation.

Nelson expected to see only the ends of the side chains reaching out and touching each other, the way they do in the DNA double helix. Instead, she found interdigitated connections akin to zippers and Velcro.

"This gives a structural explanation about why the fibers grow almost infinitely, and why prions are infectious," said Roland Riek of The Salk Institute. "Like a zipper, you have one end that never ends; you always have a free binding site for a growing fiber." In a related paper in *Nature*, Riek reported that the infectious ability of a fungal prion depends on its beta sheet structure, which he proposed would look similar to the detailed structure from Eisenberg's lab.

In another interesting finding, the zipped up fibril core is dry. "Proteins love water," said Eisenberg. "When they are soluble, there is water all around them. When the zipper is formed, water is forced out of the interface between the two beta sheets. Once you have this dry interface, it's hard to open up. Imagine trying to pry open two long pieces of Velcro."

In preliminary follow-up experiments, Eisenberg and his colleagues have found 10 short segments from other amyloid and prion proteins from hamsters, mice, and people that exhibit the same behavior in the test tube.

"We think virtually any protein can be converted into this type of structure," said Christopher Dobson of Cambridge University, United Kingdom, who wrote an accompanying commentary in *Nature*. "This is the first model that gives an atomic-level image of how the molecules might be stacked together in such a fibril."