Amyloid Structures from Alzheimer's Disease Patients

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Lu and colleagues report the structures of β-amyloid fibrils seeded from the brain extracts of two Alzheimer's disease patients, a game-changing study that could open new avenues for a structure-based design of diagnostic imaging agents and aggregation inhibiting drugs.

In the September 12 issue of *Cell* (Lu et al., 2013), a research team led by Robert Tycko at the National Institutes of Health in collaboration with Stephen C. Meredith at the University of Chicago reported a landmark study of β-amyloid(1–40) (Aβ40) fibril structures derived from post-mortem human brain tissue. This work is a major breakthrough for Alzheimer's disease (AD) research and culminates a decades-long effort to develop technologies to address this previously intractable structural problem.

The only established method for solving atomic-resolution structures of non-crystalline peptide or protein fibrils is solid-state nuclear magnetic resonance (SSNMR) spectroscopy. However, performing SSNMR on specimens extracted directly from brain tissue was not previously possible, because such fibrils were neither isotopically labeled nor in sufficient quantity to conduct a full structural study. The investigators solved this technical problem by (1) extracting microgram quantities of amyloid plaques from 1 g of tissue from each patient, (2) characterizing the fibril fragments by transmission electron microscopy (TEM), and then (3) seeding the growth of synthetic ^{13}C,^{15}N-labeled Aβ40. Although fibrils of such peptides prepared in vitro can have variations in morphology (Paravastu et al., 2009), the Lu et al. (2013) study shows that in vivo morphologies can be faithfully reproduced by the seeding protocol in sufficient quantity to carry out SSNMR measurements, as previously demonstrated for in vitro samples (Petkova et al., 2005). This approach enables structural measurements with an unprecedented level of detail for the fibrils underlying AD.

On a technical level, the outcome is breathtaking, and the publication is beautifully illustrated with light microscope and TEM images and corresponding two-dimensional and three-dimensional SSNMR data sets and structures. The authors use the full armament of spectroscopic tools to perform unambiguous backbone assignments, determine backbone dihedral angles, measure internuclear distances, and determine relative orientations of molecular segments. The data sets give full sequence coverage and, when combined with symmetry restraints from dark-field TEM, simulated annealing calculations converge to unique structures. In the past, obtaining such results would have required years of spectrometer measurement time and dozens of samples with site-specific isotopic labels, but major progress in the data collection and interpretation from the SSNMR community has alleviated this bottleneck. In addition to studies from the Tycko group, other research teams have solved structures of alternative polymorphs (Bertini et al., 2011) and examined small molecule interactions with Aβ40 (Lopez del Amo et al., 2012), and SSNMR technologies have been utilized to solve high-resolution structures of the HET-s(218–289) prion (Wasmer et al., 2008) among others (reviewed in Comellas and Rienstra, 2013). Collectively, a powerful set of SSNMR and TEM methods has emerged and gives high confidence in the new structures.

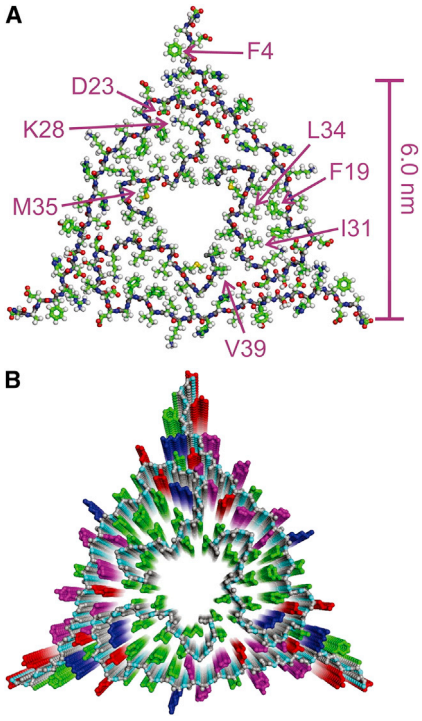

Figure 1. High-Resolution Structure of Aβ40 Fibrils Seeded from Alzheimer's Patient Tissue
(A) Three-fold symmetry repeat unit illustrating backbone (gray) and side chain (green) atoms.
(B) View of the idealized structure along the fibril axis. Adapted from Figure 5 of Lu et al. (2013) Cell *154*, 1257–1268, with permission from Elsevier.

The patient I form yields a 3-fold symmetric structure (Figure 1) with a number of significant implications. First, the authors note that, due to the burial of C-terminal residues in the fibril core, quantitation using monoclonal antibodies specific to C-terminal epitopes may be unreliable. Conversely, production of antibodies with complementary

specificities may enable more accurate assays of pathogenic and non-pathogenic forms of Aβ40 in vivo.

Second, the distinctive conformational features of brain-derived fibril structures may provide a basis for the rational design of small molecule inhibitors or imaging agents. For example, thioflavin T analogs such as Pittsburgh compound B have proved clinically useful as positron emission tomography imaging agents. The diagnostic specificity of such compounds may be enhanced by targeting differences in conformation between in vivo and in vitro forms of the fibrils. Such strategies could have broad implications also for differential diagnosis of Alzheimer's, Parkinson's, and other neurodegenerative disorders (Bagchi et al., 2013).

Finally, the structural results contribute profoundly to the longstanding debate regarding the etiology and progression of AD. The results argue strongly in favor of Aβ fibrils as a causative or at least contributing agent in AD, yet they also raise a number of significant questions. Given the inherent polymorphism of such small peptides, why are the fibrils from individual patients not polymorphic? Is the initial fibril formation stochastic, and then the same form predominantly replicated and transported throughout the brain? Or are several fibril forms nucleated in the brain, but some clearance mechanisms are defective, selecting for a population of one form? Do patients with common clinical histories have the same fibril form? If so, can AD diagnosis and treatment be personalized at the molecular level?

Answers to these questions are likely only to be clarified by further studies of larger patient populations using the fabulous SSNMR technologies now available and applied in this pioneering study.

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