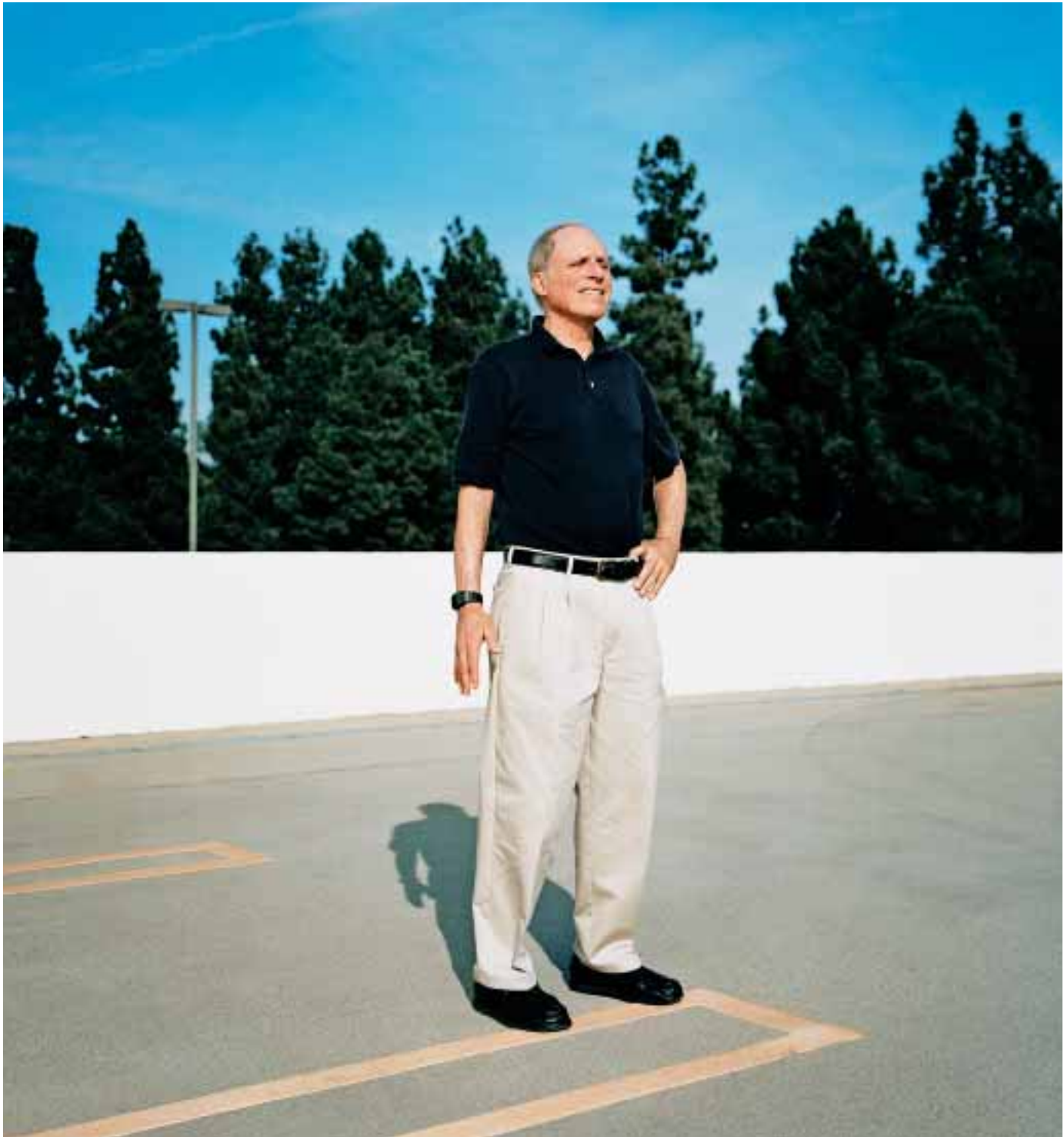


Chasing Amyloid

Though it's taken decades, solving the mystery behind the structure of this errant protein has opened a door to new therapies.



After solving the atomic structure of amyloid, David Eisenberg found the same zipper-like conformation in similar segments of 30 disease-related proteins. Now he's devising new therapies.

João Canziani

SOLVING A PROTEIN'S STRUCTURE—THE THREE-DIMENSIONAL ARRANGEMENT

and spacing of its atoms—is vital for understanding its behavior and is a major step in drug development. But coaxing balky proteins to form crystals for x-ray analysis can consume months or years of frustrating effort. Success requires doggedness and creativity; luck helps, too. ¶ David Eisenberg, an HHMI investigator at the University of California, Los Angeles, lives for such challenges. Using x-ray crystallography and

computational methods, he has specialized in determining the structural details of how proteins bind to other proteins. Now, he is using that knowledge to develop patient therapies.

About 10 years ago, Eisenberg began to attack a particularly stubborn protein—amyloid. A tough, fibrous substance that can accumulate in cells like trash in a landfill, amyloid has been linked to at least two dozen disorders, from Alzheimer's and Parkinson's diseases to type 2 diabetes and “mad cow” disease. These proteins—in fact, almost any protein, apparently—can lose their intricate folded configurations and form fine hair-like amyloid “fibrils.”

Understanding the pathology of amyloid would be vastly aided by learning its atomic structure, but for decades this goal was elusive. Amyloid isn't soluble in water, thereby confounding conventional analysis methods; and it has proved extremely resistant to crystallization, which would illuminate its chemical bonds.

As early as 1935, British scientist William Astbury, who studied the physics of textiles, fired x-rays at strips of poached egg white that he stretched to form amyloid-like fibers. With the x-rays scattering in a pattern that he called “cross-beta,” Astbury inferred that the protein was composed of “beta strands” (short stretches of amino acids) connected by hydrogen bonds to form flat and extended “beta sheets,” packed tightly against each other. It was a good start, but it provided only a rough picture. “Astbury's analysis tells you that the protein is organized into beta sheets with certain spacing, but it doesn't tell you where the atoms are,” explains Eisenberg.

In the 1990s, pathologists began making the connection between amyloid deposits in brain cells and several neurodegenerative disorders—most famously Alzheimer's disease, in which plaques of beta-amyloid are

scattered through the brain. It is still unclear whether the deposits directly kill nerve cells or are the end result of a toxic process during fibril formation.

An important lead came, Eisenberg says, when HHMI investigators Susan Lindquist (Massachusetts Institute of Technology) and Jonathan Weissman (University of California, San Francisco) independently showed that fibril formation could be initiated by a segment made up of about 100 amino acid residues at one end of the protein. Following up on this insight, Eisenberg and graduate student Melinda Balbirnie narrowed the segment down to just seven essential fibril-forming residues, publishing the discovery in 2001. “Melinda and I realized that it took only a tiny bit of the protein to form an amyloid fiber,” says Eisenberg. “To me, that was the paradigm paper.”

In the same paper, they reported that the seven-residue segment could also form crystals—a huge breakthrough. “We were thrilled,” he says, “but there was a big problem: the crystals were 50,000 times smaller than the ones we usually work with. They had beautiful faces and edges, but their width was typically around 1 micrometer.” No existing x-ray beam was narrow enough to probe these “microcrystals.”

Then, luck intervened. At a meeting in Greece in 2003, Eisenberg learned that the European Synchrotron Radiation Facility in Grenoble, France, had just launched a new “microfocus” machine that fired a beam of x-rays only 4 micrometers wide—the size of the largest amyloid crystals. By teaming with European scientists, Eisenberg and graduate student Rebecca Nelson got permission to use the new beam line, and in a matter of weeks they had captured the elusive atomic structure.

Published in 2005, the research confirmed in fine detail that amyloid fibrils contain a cross-beta “spine” made of short amino acid

chains along which beta sheets assemble, forming a lengthening fiber as more units are added. The surprise was that side chains emanating from the sheets interact closely with each other like the teeth of a zipper, locking the sheets tightly in place.

“That was the ‘aha’ moment,” Eisenberg recalls. “That's what causes molecules to form the fibers.” Moreover, this so-called “steric zipper” seals the interface between sheets so that water is excluded, explaining why amyloid is so persistent and insoluble in tissues.

This landmark finding was a jumping-off point for Eisenberg. In a 2007 *Nature* paper, he reported the discovery of similar cross-beta spines in amyloid-forming segments of 30 disease-related proteins, including the amyloid beta and tau proteins that make up the characteristic plaques and tangles in the brains of people with Alzheimer's disease. Toxic fibrils formed by human islet amyloid polypeptide are found in the pancreas of most patients with type 2 diabetes, but their atomic structure wasn't known until Eisenberg reported it in September 2008 in *Protein Science*.

While much remains to be learned about the role of amyloid fibers in disease, the information gained from their atomic structure has already led to work in Eisenberg's lab on potential clinical applications. His group is designing molecular caps, for example, to attach to the ends of amyloid fibers. “It is conceivable that capping the end of a fiber might stop it from lengthening and help to break it down,” he explains.

The pursuit of amyloid and potential human therapies marks a departure for a scientist at age 69 whose body of work has leaned away from medicine (in 1969 he published the definitive book on the properties of ice and water). It's a career move he thinks would have pleased his father.

“He was a doctor and always wanted me to go into medicine,” Eisenberg says. “I thought it was about time I did something that had medical applications.” ■

—RICHARD SALTUS

FOR MORE INFORMATION: To learn about Eisenberg's class on x-ray crystallography, see “Crystal Clear” on page 52.