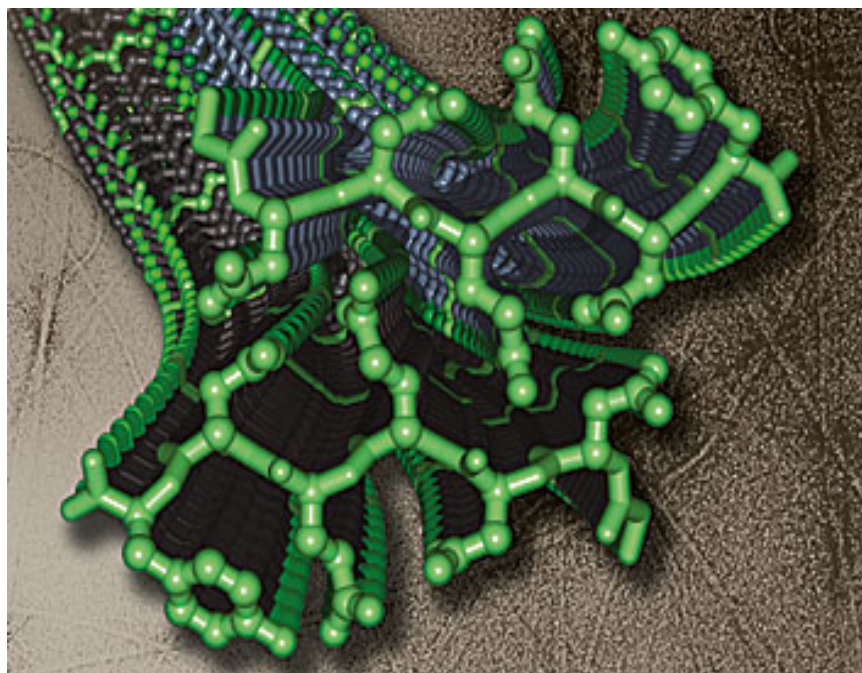


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## Sorting Out What Makes Proteins Clump Together



**Image Title:** Once amyloid fibrils form in tissues and cells, they resemble a towering stack of zippers, each tightly bonded to the one below. - *Nature*, Vol. 435, pp. 773 to 778

Howard Hughes Medical Institute (HHMI) researchers and their colleagues have found the key factors that cause proteins to turn into sticky, fibrous clumps that can grind cellular activity to a halt. The new findings show how some organisms prevent amyloid clumps from forming and point the way toward drugs that might one day do the same for humans.

In many age-related diseases, including Alzheimer's, Parkinson's, and type 2 diabetes, amyloid fibrils accumulate inside cells. Sometimes amyloid deposits build up because cells' ordinary rubbish-disposal mechanisms break down. Some amyloid deposits act as gunk that causes disease by clogging organs, like the kidneys, whereas others kill specific cells, such as neurons.

HHMI investigator David Eisenberg has been searching for a better molecular explanation for how and why amyloid proteins gum up the works. He collaborated on the new study, published online in the *Proceedings of the National Academy of Sciences*, with Lukasz Goldschmidt and Poh Teng, graduate students in his UCLA lab, and Swiss researcher Roland Riek from the Eidgenössische Technische Hochschule in Zurich.

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- David Eisenberg

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Our cells are kept extremely busy linking together amino acids—the building blocks of proteins—in the right order to produce the different proteins we need every day. The order of these amino acids is determined by the genes. According to the genetic code, each triplet of bases in the genes' instructions either calls for a particular amino acid or gives a signal to start or stop making a protein. In previously published work, Eisenberg and his team found that amyloids tend to form in proteins that contain a short amino acid sequence that grabs on to the same sequence in identical protein molecules, forming a particularly tight bond known as a steric zipper. Steric zippers cause proteins that contain them to stack tightly on top of one another, forming a fiber. These fibers can kill cells and clog organs.

Eisenberg's team has been working to develop a computer algorithm capable of predicting whether the structure of any given six-amino-acid protein segment has this peculiar, self-binding stickiness. In the latest research they validated their algorithm using a set of protein segments from the well-studied enzyme ribonuclease A. They showed that when left on their own in a test tube, the segments of protein that they had computationally predicted would form amyloids actually did form long, thin fibrils that could be seen with an electron microscope. In contrast, two test segments predicted *not* to form amyloids retained their normal globular state under the same conditions.

The team also shuffled the amino-acid sequences of the amyloid-forming ribonuclease segments to see whether a different arrangement of the same amino acids would still form amyloids. The rearranged segments did not, which indicated that the specific sequence of amino acids is really what determines a protein segment's propensity to become a self-gluing amyloid.

Having validated the prognostic value of their amyloid-finding algorithm, the team then used it to sift through all the proteins coded by the genomes of the common bacterium *E. coli*, the common yeast *S. cerevisiae*, and human. They applied the same test to 12,000 proteins catalogued in a database for which the three-dimensional structures of the proteins were already known.

“The first surprise to us was just how common these amyloid-prone segments are,” said Eisenberg. His team found that 15 to 20 percent of the tested segments fell into the high-propensity category. But in a living organism, very few of the proteins containing amyloid-prone segments actually switch to the amyloid state. Eisenberg says evolution must have provided powerful prevention mechanisms -- “otherwise,” he says, “we would all fill up with amyloid fibrils.”

When Eisenberg and his team looked for the positions of these high-propensity segments within known protein structures, they quickly found one clue to what keeps their amyloid state at bay. “In folded proteins, these segments tend not to be on the surface, or if they are on the surface, they’re not in the extended form needed to create fibrils,” he said. “So it’s very rare that they are accessible.”

For proteins whose structures don’t always conceal amyloid-prone segments, Eisenberg and his team found that amyloid prevention appears to be accomplished by the protein’s rigidity. “Just one of these high-propensity segments in a loop where it has some flexibility can force a protein to form amyloid fibrils, even if it would never do so otherwise,” he said.

The findings appear to match what is known about proteins that do commonly form amyloids. “Proteins such as amyloid beta, which is associated with Alzheimer’s disease, don’t seem to have a highly structured native state, so they probably offer the exposure and the flexibility that’s necessary for amyloid fibrils to form,” Eisenberg said.

According to Eisenberg, the findings are consistent with previous research showing that amyloid formation can sometimes be triggered by changing the structure of proteins with heat or chemicals, a process known as denaturing. “Those denatured proteins that form amyloids would be ones that unfold and expose previously hidden high-propensity segments,” he said.

Eisenberg’s team now hopes to better define the structural flexibility and other factors that spur these high-propensity segments to start forming amyloid fibrils. With that information, said Eisenberg, “we’ll hopefully be able to discover how to stop this process from happening.”