STRUCTURAL BIOLOGY

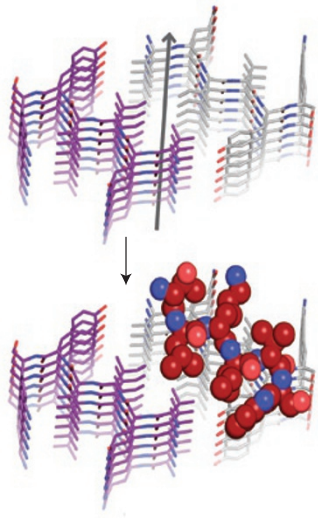
Thwarting amyloid fibers

Two structure-driven studies of the culprits behind diseases associated with amyloid fibers give clues to stopping these agents in their tracks.

Drug design rests firmly on the foundation of basic science research, and one important avenue to a pharmacophore requires a three-dimensional structure. For dozens of diseases associated with proteins that form oligomers or fibers, such as Alzheimer's, this prospect has been hampered by a lack of structural information about the culprit proteins.

Progress toward elucidating amyloid fiber structures accelerated a decade ago with the finding that in one such protein, the yeast prion protein, segments consisting of just 7 amino acids were involved in fiber formation. When expressed alone, these short peptides formed long, needle-like crystals. Solution of the structure revealed that pairs of these β-sheet peptides are held together tightly via the interactions of their protruding side chains. Such pairs layer on top of each other, forming a long fiber (see illustration). "When you look down the fiber, the side chains look like the teeth of a zipper intermeshing, so we called it a steric zipper," says David Eisenberg of his group's first publication of such a structure in 2005. To date, he adds, there are about 90 solved structures of zippers from about 15 disease-related proteins.

With structure information in hand, Eisenberg, at the University of California, Los Angeles, and colleagues set out to inhibit fibril formation by capping fiber ends. They started with a short peptide backbone and designed the side chains to bind tightly to the end of a steric zipper and prevent binding of additional fibril components (Sievers *et al.*, 2011). "Protein computational biology is by no means perfect, but it's getting pretty good. If we make 6–10 different designs, we will get several that are pretty good inhibitors," Eisenberg notes.



Inhibitor design. VQIVYK segments of tau form a steric zipper (purple and gray). A designed inhibitor binds the end of the zipper, preventing addition of other molecules. Reprinted from *Nature*.

Of the inhibitors designed to the VQIVYK segment of the tau protein—which forms amyloid fibers in Alzheimer's disease—the best was specific for the target and, when added in tenfold excess, prevented fibril formation for over 60 hours *in vitro*. Encouraged by this proof of principle, Eisenberg points out that this result reinforces the hypothesis that these short segments are the "adhesive bits" responsible for fibril formation.

Nevertheless, he cautions that to inhibit tau in patients, the engineered peptide would have to cross the blood-brain barrier and have a longer lifetime, "so this peptide is not going to do it." As a start, however, he and his colleagues made this inhibitor of D-amino acids to avert proteolysis *in vivo*. Going a step further, they used non-natural amino acids to engineer an inhibitor for another steric zipper reported in the paper. "Companies now sell some 200 non-natural amino acids, so that gives us a much greater scope in designing inhibitors," says Eisenberg.

To approach structure-based design from another angle, the researchers also analyzed how compounds that others had previously identified as binding to amyloid interact with steric zippers of tau and another protein, amyloid-β (Landau *et al.*, 2011). After a *tour de force* crystallization trial, they solved structures for four mixtures. The analysis revealed a diversity of binding options: some compounds bound between the two β-sheets that form the zipper, whereas others bound between zippers.

Another important observation was that different compounds bound to differently packed polymers of the same peptide or, in other words, different orientations of zippers relative to one another. "What that tells us," explains Eisenberg, "is that fibers are naturally polymorphic: they have different structures, and therefore it will probably take a cocktail of drugs to stop fibrilization." Taken together, these studies offer many promising avenues to follow.

And, of course, these studies were done with steric zippers only—a model for full-length amyloid proteins, albeit one that showed its mettle in these studies.

This work also could have implications for applying fibril-forming proteins in nanotechnology, much as viruses have been harnessed for good in biology research. Eisenberg thinks it will be possible to design amyloid fibers that bind particular compounds. For example, he says: "I see it as a way of designing frameworks that can be binders for various gases."

For now, however, these structure-based studies hint at compounds to focus on in subsequent screens and design approaches with the aim of sorely needed leads to treat devastating diseases.

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RESEARCH PAPERS

Landau, M. *et al.* Towards a pharmacophore for amyloid. *PLoS Biol.* **9**, e1001080 (2011).

Sievers, S.A. *et al.* Structure-based design of non-natural amino-acid inhibitors of amyloid fibril formation. *Nature* **475**, 96–100 (2011).