

JUNE 29, 2001

## Researchers Use Computers to Redesign Protein Folding

Researchers have used an advanced computer program to redesign how a small protein folds into its final three-dimensional form. The re-engineered protein folds 100-times faster than the natural protein and has a folding pathway that is completely reversed.

Understanding protein folding is an important scientific challenge, said David Baker, a Howard Hughes Medical Institute (HHMI) investigator at the University of Washington School of Medicine and senior author of the study, which was published in the July 2001 issue of *Nature Structural Biology*. Folding determines how proteins first synthesized as long chains of amino acids assume the three-dimensional architecture that enables them to function as enzymes and other key cellular components.

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— **David Baker**

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Baker and University of Washington colleagues Sehat Nauli and Brian Kuhlman redesigned the folding pathway of protein G, a small protein produced by streptococcal bacteria. Their work concentrated on altering the order in which two key structural elements of the protein are formed during folding.

"This experiment represented a test of our fundamental understanding of protein folding," said Baker. "Over the last decade or so, as the folding of naturally occurring proteins has been explored, researchers have begun to understand the general principles behind folding. As a test of that understanding, we tried to redesign the way a protein folds — the basic idea being that if you understand something, you should be able to rationally redesign it with predictable results." Baker said that Stephen Mayo, an HHMI investigator at California Institute of Technology, and others have made impressive gains in computational protein design that have been used to redesign and stabilize naturally occurring proteins.

Baker and his colleagues chose protein G for their studies because it possesses considerable symmetry, with two hairpin-shaped backbone regions that flank a helical region of the protein. The researchers found that during natural folding, the second hairpin is formed first. This is the rate-limiting step in the folding process, said Baker. But Baker and his colleagues knew that another protein, called protein L, has the same basic backbone shape as protein G, yet because of differences in its amino acid sequence, the first hairpin folds before the second hairpin.

The researchers began their attempts to switch the folding pathway by examining in detail the structure of the hairpins of protein G. “Our initial hypothesis was that the amino acid structure of the second hairpin underwent many more favorable interactions than those of the first hairpin, and that’s why it folds up first in the naturally occurring protein,” said Baker. “In fact, when we examined the first hairpin, we realized that it had a number of sub-optimal features. So, our next objective was to replace the backbone conformation of the first hairpin with a much more favorable structure for folding.” Using computer software they developed to calculate the energy of folding of all combinations of amino acid sequences, the scientists arrived at an optimal sequence for a backbone conformation of the first hairpin that was likely to fold more readily. They next produced a gene for this new protein, which they expressed in bacteria to produce the re-engineered protein.

“When we began to characterize this redesigned protein, the first surprise was that it was almost twice as stable as the wild-type protein,” said Baker. “The second equally amazing surprise came when we found that it folded a hundred times faster than the naturally occurring protein.” Such a higher folding rate is extremely unusual, explained Baker, because mutations in proteins rarely increase folding rate.

Next, the researchers tested the re-engineered protein G to determine whether it had switched its folding pathway to that of protein L. “We found to our delight that the folding mechanism WAS completely switched,” said Baker. “The first hairpin in this designed protein folds up first, and the second hairpin folds up last,” he said. Since the initial study of the redesigned protein, x-ray crystallography studies by Baker and his colleagues have confirmed that the redesigned protein matches the structure predicted by their computer model.

“The significance of these results is that they show we’re making progress in understanding the fundamental principles underlying folding,” said Baker. Also, he said, the ability to engineer proteins with increased stability could prove useful. “One might imagine re-engineering a protein to be a therapeutic, or correcting a protein that causes a disease because it tends to aggregate or misfold,” Baker said.

One of the next steps will be to extend the computer-aided protein design technique to develop new backbone conformations not seen before in nature. “A fundamental question and a Holy Grail of protein design — is whether we can discover viable protein structures that evolution simply missed,” Baker

said.