PROTEIN STRUCTURE

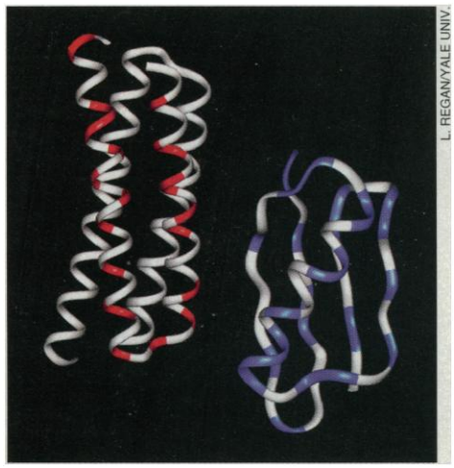
Amino Acid Alchemy Transmutes Sheets to Coils

BALTIMORE—In January 1994, George Rose and Trevor Creamer threw down the gauntlet. They challenged their fellow protein researchers to try their hands at a bit of alchemy: radically transforming the basic three-dimensional (3D) structure of a protein while altering no more than 50% of its amino acid building blocks. As a reward, they offered $1000 of their own money. While that may seem like a rash bet—50%, after all, is a substantial change—Rose and Creamer knew that pairs of natural proteins differing in up to 70% of their amino acid sequences virtually always fold up into the same general 3D structure. "Realistically, we didn't expect to have to pay this for a long time," says Rose, a biophysicist at the Johns Hopkins University School of Medicine in Baltimore. "It turned out we were not safe at all."

Late last month, Yale University biochemist Lynne Regan gave a symposium here, presenting her team's work to Rose and a roomful of Hopkins faculty, postdocs, and graduate students. She walked away with a cool grand. Regan and her Yale colleagues Seema Dalal and Suganthi Balasubramanian earned the prize by converting the 3D structure of a small protein from one that resembles a sheet with four flat strips lying side by side to another that resembles a collection of four helical telephone cords bunched together.

The result, published in this month's issue of *Nature Structural Biology*, "is very impressive," says Peter Kim, a protein designer at the Massachusetts Institute of Technology's Whitehead Institute for Biomedical Research, who saw the work presented at a recent Gordon Conference. The unexpectedly small number of amino acid differences between the two structures, he says, could have implications for efforts to infer the shapes of new proteins by comparing their sequences to those of known ones. The result is also a step toward the long-sought goal of being able to predict a protein's 3D structure from its sequence, which would be a boon to genome researchers and drug designers.

Rose and Creamer launched their competition—dubbed the "Paracelsus Challenge" after the 16th century Swiss physician and alchemist—in hopes of sparking greater understanding of why proteins fold into particular patterns. At least two other groups beside Regan's had sought the prize. One team—Johns Hopkins structural biologists Neil Clarke and Shao-Min Yuan—even worked with the same two basic protein structures, a sheet and a set of coils. Clarke and Yuan started with the coiled structure and tried to transform it into a sheet. That change had the best chance of success, they thought, because "we knew the β sheet was a very stable structure," says Clarke. As a result, he and Yuan hoped that a small complement of altered amino acids would hold the sheet together. "But we clearly didn't succeed," says Clarke.



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Reforming a protein. Splicing pieces of a coil-forming sequence (red) into a sheetlike protein *(right)* transforms its structure to coils. Blue indicates sequence retained in the hybrid protein.

Clarke and Yuan's work was what inspired Regan and her colleagues to try their hand. After hearing Clarke give a talk at a protein-design meeting in the spring of last year, says Regan, the trio got to talking about how the prize could be won. They decided to try going the other way: altering a sheetlike section of a molecule called protein G, which is found on the surface of *Streptococcus* bacteria, into a set of coils resembling those found in an RNA-binding protein called Rop. "We knew the properties of both these proteins very well," says Regan. "But we knew a lot more about what stabilizes helixes than sheets."

In their studies of Rop, for example, they had found that substituting the amino acids alanine and leucine for other amino acids in spots where the four coils all face a central core made the protein even more stable than the standard version. Likewise, they found that they could destabilize the sheetlike region of protein G—a section known as the B1 domain—by removing key amino acids such as threonine.

By plucking out such amino acids from the B1 structure and replacing them with α-helix formers such as alanine and leucine, the Yale team came up with a preliminary design for their transformed protein. They then plugged the substitutions into a computer program used to predict how amino acid sequences will fold and weeded out amino acid placements that clearly wouldn't allow the protein to fold into a bundle of helixes. In the end, they settled on a sequence that retained 50% of the amino acids in B1 but was identical to Rop in another 41%. They called their hybrid protein Janus, after the Roman god of new beginnings, who bears two faces.

With Janus's blueprint complete, the researchers synthesized a stretch of DNA encoding the new protein's amino acid sequence and inserted it in an *Escherichia coli* bacterium, which expressed the DNA sequence and built the protein. Finally, the researchers purified the protein and characterized it via a variety of methods, including one called circular dichroism, which relies on the scattering of polarized light to differentiate protein structures such as helixes and sheets. "We were very encouraged," says Regan. "Right from the beginning, it looked really helical."

That was enough to win the team the Paracelsus laurels, but they didn't stop tinkering with Janus. They have begun working backward, restoring more and more of B1's sheet-forming sequence to Janus in an attempt to see just how much of the original sequence they can add back and still get a helical fold. Already, the group has been able to design Janus analogs that share as much as 60% of the sequence of the β sheet–forming B1, but still fold into a cluster of helixes.

"This is something that should give people at least some pause," says Kim. He explains that genomics researchers commonly compare newly discovered gene sequences to those of genes that code for proteins with a known structure. If the new gene's protein product resembles the known one at 30% of its amino acid positions or more, it is considered very likely to have a similar shape and function. But the new Janus analogs share twice that number of amino acids with B1, yet form a very different structure. For genomics researchers working with the 30% standard, says Kim, "there are times we will be fooled."

Kim and others say the study also has important implications for researchers trying to learn the rules of protein folding. Most important, it underscores the growing understanding that not all amino acid residues have equal influence over the structure of a protein. Learning all the rules that govern how those structures are formed will take more protein alchemy studies, and perhaps a few more competitions. But as for the next competition's prize, Rose says he's tapped out. "Virtue," he says, "will have to be its own reward."

–Robert F. Service