Proteins crystallize more readily when they are first induced to pair up

Figuring out a protein's role in life is difficult without its three-dimensional structure. And, more often than not, obtaining a structure involves coaxing a protein to form a crystal large enough and ordered enough to diffract x rays sharply.

X-ray crystallography is remarkably successful. So far, about 3000 x-ray-derived structures of human proteins have been deposited in the Worldwide Protein Data Bank. But our bodies make at least as many different kinds of protein as we have genes, about 35 000. Subjecting this sum, the human proteome, to structural analysis is clearly a task for automation.

Ambitious plans exist to crank through the proteome, but they could be frustrated by the reluctance of many proteins to crystallize. Some proteins, notably the transmembrane channels and transporters, defy crystallization because they don't dissolve well in either polar or nonpolar solvents. But for other proteins, it's hard to tell why crystallization is so elusive. Crystallographers are constantly searching for new recipes and ingredients that will yield the crystals they desire.

Now, Todd Yeates, Rey Banatao, and their collaborators from UCLA believe they've found a promising path to crystallization: artificial dimerization.[1] Lacking any symmetry, a protein resembles a messily coiled strand of cooked spaghetti. To form a crystal, each protein molecule must not only find a place in an emergent lattice but also orient itself with respect to the other molecules. If a protein could be induced to form identical dimers, the dimers' rotational symmetry would make orientation easier and promote crystallization.

Some proteins occur naturally as dimers (higher oligomers, too). Before trying to make artificial dimers in the lab, Yeates analyzed structures in the Worldwide Protein Data Bank for evidence that dimerization increases the number of crystalline arrangements a protein can adopt.

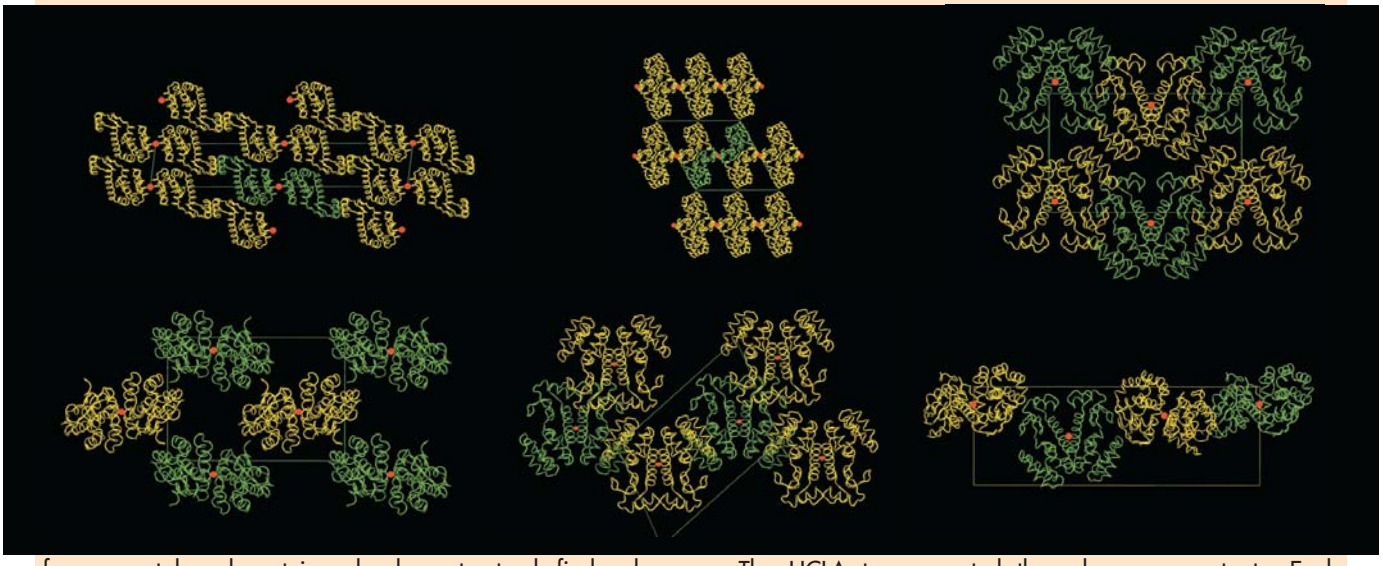
By examining the crystallographic space groups adopted by monomeric and dimeric proteins, Yeates found that dimers do indeed exploit their symmetry to crystallize more readily. According to Yeates's estimate, a protein is 50% more likely to form crystals if it dimerizes.

To induce otherwise monomeric proteins to pair up reliably and identically, Yeates and Banatao took advantage of the disulfide bond that can form between two molecules of a particular amino acid, cysteine. Most proteins contain a cysteine, but the amino acid usually accounts for only a few of the total number of amino acids in a protein.

Through the techniques of genetic engineering, one can substitute any of the several hundred amino acids in a typical protein. Provided the substitutions are few and preserve electrical polarity, a mutant protein will usually keep its original form and function. A monomeric protein's one or two naturally occurring cysteines can be swapped with other amino acids. Likewise, other amino acids can be swapped for cysteine anywhere along the protein's sequence.

But if a mutant protein is to pair up via a disulfide bond, the cysteine substitution must be favorably located: Cysteine's sulfur-bearing thiol group must poke out of the protein's surface rather than into the protein's less accessible interior. Of course, if the point of artificial dimerization is to help determine structure, one doesn't necessarily know in advance whether an amino acid is on the interior or exterior of its protein. Fortunately, proteins are sufficiently predictable that one can make a good guess.

Yeates and Banatao tested their idea on a protein whose structure is already known: the lysozyme from the T4 bacteriophage. In addition to enabling newly replicated viruses to break out of their bacterial hosts, the lysozyme also serves as a model system for crystallization studies.



The UCLA team created three lysozyme mutants. Each mutant had its two naturally occurring cysteines replaced and a cysteine artificially inserted at one of three different, outward-facing sites. As Yeates predicted, the mutants did crystallize readily—into the six new forms shown in the figure. The red dots indicate the engineered disulfide bonds; the white frames and the yellow and green coloring indicate the unit cells.

In another test, the UCLA team added a reducing agent to turn off one of the mutant's ability to dimerize. Of 384 distinct experimental conditions, the disabled mutant crystallized in only 14; the dimerizing mutant crystallized in 98.

Adding cysteine to dimerize a particular protein and promote crystallization is not new. But, says Terese Bergfors of Uppsala University in Sweden, "What the UCLA team has done is take the idea a step further to suggest a general strategy to improve crystallizability."

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Reference

1. D. R. Banatao et al., *Proc. Natl. Acad. Sci. USA* **103**, 16230 (2006).