citing because, before their studies, it was not expected that sequences as short as 25 residues in length could fold into stable tertiary structures.

Now, Dahiyat and Mayo take these studies one step further through the design of a sequence composed of only natural amino acids that adopts the zinc finger motif. As input to their program, they introduced the coordinates of the backbone atoms from the crystal structure of the second domain of the zinc finger protein Zif268. The program then evaluated a total of 10^{62} possible side chain–rotamer combinations to find a sequence capable of stabilizing this fold without a bound metal ion. The resulting protein sequence shares a small hydrophobic core with its predecessor from Zif268. However, in the newly designed protein FSD-1 the core is enlarged through the addition of hydrophobic residues that fill the space vacated by the removal of the metal-binding site (see the figure). This increase in the size of the hydrophobic core together with the enhancements in the propensity for forming the appropriate secondary structure provide an adequate driving force for folding. The designed miniprotein actually folds into the desired structure as assessed by nuclear magnetic resonance spectroscopy, and the observed structure closely resembles the three-dimensional structure of Zif268.

Because of its small size, the protein is marginally stable. A Van't Hoff analysis of the thermal unfolding curve gives a change in the enthalpy (ΔH_{vH}) of approximately –10 kcal/mol, and indicates that the protein is about 90 to 95% folded at low temperatures (*13*). The small value ΔH_{vH} and the lack of strong cooperativity in the unfolding transition are expected for a native-like protein of this very small size (*14*). Thus, FSD-1 is the smallest protein known to be capable of folding into a unique structure without the thermodynamic assistance of disulfides, metal ions, or other subunits. This important accomplishment illustrates the impressive ability of Dahiyat and Mayo's program to design highly optimized sequences.

This new achievement caps a banner year for de novo protein design. Earlier, Regan (*15*) answered the challenge of changing a protein's tertiary structure by altering no more than 50% of its sequence. And although Dahiyat and Mayo have demonstrated that the stabilizing metal-binding site is not necessary in their system, Caradonna, Hellinga, and co-workers (*16*) have made impressive progress in automating the introduction of functional metal-binding sites into the three-dimensional structures of natural proteins. Further, other workers (*17*) have used less automated approaches to successfully introduce functionally and spectroscopically interesting metal-binding sites into de novo designed proteins.

To date, the most computationally intensive protein design problems have been the redesign of natural proteins of known three-dimensional structure. But the new automated approaches open the door to the de novo design of structures with entirely novel backbone conformations. It will be interesting to see if Dahiyat and Mayo's approach of designing an optimal sequence for a given fold is sufficient, or if it will be necessary also to destabilize alternate possible folds. Indeed, when using an earlier version of their algorithm to repack the interior of the coiled coil from GCN4, they had to retain the identity of a buried Asn residue from the wild-type protein. Although the inclusion of this Asn actually destabilized the desired fold, it was nevertheless essential to avoid the formation of alternate, unwanted conformers (*18*). The ability to ask such focused questions will reveal much about how natural proteins adopt their folded conformations while simultaneously allowing the design of entirely new polymers for applications ranging from catalysis to pharmaceuticals.

References and Notes

1. B. I. Dahiyat and S. L. Mayo, *Science* **278**, 82.
2. K. E. Drexler, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5275 (1981); C. Pabo, *Nature* **301**, 200 (1983).
3. W. F. DeGrado, Z. R. Wasserman, J. D. Lear, *Science* **243**, 622 (1989); J. W. Bryson *et al.*, *ibid.* **270**, 935 (1995); M. H. J. Cordes, A. R. Davidson, R. T. Sauer, *Curr. Opin. Struct. Biol.* **6**, 3 (1996).
4. R. Munoz and L. Serrano, *Proteins* **20**, 301 (1994); C. A. Kim and J. M. Berg, *Nature* **362**, 267 (1993); D. L. Minor and P. S. Kim, *ibid.* **367**, 660 (1994); C. K. Smith, J. M. Withka, L. Regan, *Biochemistry* **33**, 5510 (1994).
5. J. Janin, S. Wodak, M. Levitt, B. Maigret, *J. Mol. Biol.* **125**, 37 (1978).
6. J. W. Ponder and F. M. Richards, *ibid.* **193**, 775 (1987); J. R. Desjarlais and T. M. Handel, *Protein Sci.* **4**, 2006 (1995); X. Jing, E. J. Bishop, R. S. Farid, *J. Am. Chem. Soc.* **119**, 838 (1997).
7. J. U. Bowie, J. F. Reidhaar-Olson, W. A. Lim, R. T. Sauer, *Science* **247**, 1306 (1990).
8. S. Kamtekar, J. M. Schiffer, H. Xiong, J. M. Babik, M. H. Hecht, *ibid.* **262**, 1680 (1993).
9. K. J. Lumb and P. S. Kim, *ibid.* **271**, 1137 (1996); Y. Yu, O. D. Monera, R. S. Hodges, P. L. Privalov, *J. Mol. Biol.* **255**, 367, (1996).
10. A. C. Braisted and J. A. Wells, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5688 (1996).
11. J. M. Berg, *ibid.* **85**, 99 (1988).
12. M. D. Struthers, R. P. Cheng, B. Imperiali, *Science* **271**, 342 (1996).
13. This Van't Hoff analysis of the protein is approximate because of the lack of definition of the pre- and posttransition baselines.
14. P. Alexander, S. Fahnestock, T. Lee, J. Orban, P. Bryn, *Biochemistry* **31**, 3597 (1992).
15. S. Dalal, S. Balasubramanian, L. Regan, *Nat. Struct. Biol.* **4**, 548 (1997).
16. A Pinto, H. W. Hellinga, J. P. Caradonna, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5562 (1997); C. Coldren, H. W. Hellinga, J. P. Caradonna, *ibid.*, p. 6635.
17. B. R. Gibney, S. E. Mulholland, F. Rabanal, P. L. Dutton, *ibid.* **93**, 15041 (1996); M. P. Scott, J. Biggins, *Protein Sci.* **6**, 340 (1997); P. A. Arnold, W. R. Shelton, D. R. Benson, *J. Am. Chem. Soc.* **119**, 3181 (1997); G. R. Dieckman *et al.*, *ibid.*, p. 6195.
18. P. B. Harbury, T. Zhang, P. S. Kim, T. Alber, *Science* **262**, 1401 (1993); K. J. Lumb and P. S. Kim, *Biochemistry* **34**, 8642 (1995).
19. L. G. Presta and G. D. Rose, *Science* **240**, 1632 (1988); J. S. Richardson and D. C. Richardson, *ibid.*, p. 1648.

WEBWATCH

Mathematical broth

http://math.wisc.edu/~griffeat/welcome.html

The Primordial Soup Kitchen is a site devoted to research on cellular automata (CA), collections of mathematical objects that individually are governed by simple rules but are highly complex in the aggregate. Depending on the rules, automata can evolve into ordered states from disordered beginnings. The Primordial Soup Kitchen relies on a culinary metaphor: the menu includes CAffeine (Java applications for cellular automata), Lagniappe (bite-sized descriptions of recent automata research by various researchers), and the Kitchen Sink (Web links to other resources). The chef, D. Griffeat of the University of Wisconsin, has prepared a thoroughly nutritious Web page for CA studies.

An educational voyage

http://www.reef.edu.au

The Reef Education Network (REN) Web page, developed by the University of Sydney, is a basic guide to one of the most complex ecosystems on Earth. The REN is aimed at an audience of laypersons and educators and contains a great deal of sophisticated information about reef communities. "Ask a Brain Coral" offers answers to questions about reef biology, and the Research Hut describes the people and laboratories involved in reef research. Users of the page can create their own online notebooks with links to items in the Web site.

One-stop site shop

http://www.scicentral.com

SciCentral is structured as a meta-directory of science and engineering on the Web. The site provides many links to resources in all fields of science, including daily necessities such as e-mail and phone directories and lists of college and university Web pages. Other tools are included, too, including travel guides for the frequent-flying researcher, links to media coverage of science, and grant information on the Web.

Edited by David Voss

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