Equal length (3). Manners, Winnik, and co-workers have shown that this approach can be extended to the generation of very well-defined micellar BCPs (see the figure) (4). Labeling by fluorescent dyes verifies the high definition of these objects. Thus, CDSA provides the access to 1D objects with fully defined length and sequence.

Qiu et al. performed the next step and achieved full 2D control. In earlier work (8), they extended CDSA to 2D epitaxial growth of micelles with lenticular shape (2). The latest approach uses epitaxial crystallization on a cylindrical seed micelle. To this core, a mixture of a BCP from PFs and poly(2-vinylpyridine) (P2VP) and the homopolymer PF is added in a solvent chosen to selectively solubilize the different blocks. Self-assembly leads to rectangular, fully defined platelets. The ratio of the BCP to the homopolymer controls the aspect ratio of these objects that can grow up to a size of 60 µm by 10 µm. Hence, a living 2D CDSA has been achieved, resulting in platelets with internal structure and nearly without defects. Qiu et al. explain the high definition of the platelets in terms of a simple geometric model.

In additional studies, one of the BCPs (P2VP) was cross-linked by addition of platinum nanoparticles. Dissolution of the non-cross-linked cores led to stable hollow nanoframes that could be manipulated by optical tweezers and laid down on a substrate. Thus, the platelets formed by controlled 2D self-assembly add a new building block to nanotechnology. These systems may open the way to a large number of applications in various different fields, ranging from sensors to microelectronics (9). At the same time, the 2D self-assembly having a precision near to crystallization presents a fundamental observation to be studied in further detail.

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PROTEIN DESIGN

Inspired by nature

Designed proteins have structural features resembling those of natural active sites

By Ravit Netzer and Sarel J. Fleishman

Over the past decade, scientists have made exciting progress in designing protein folds entirely on the computer and then successfully synthesizing them in the laboratory (1–5). These designer proteins had the same structure in experiment as in the model and were very stable; however, they lacked important structural features seen in protein interfaces and enzyme active sites. In two reports on pages 680 and 687 of this issue, Boyken et al. (6) and Jacobs et al. (7) use the Rosetta biomolecular modeling software to design proteins that include some of these features. Experiments show that these new designs retain high structural precision and stability.

Although understanding and control of biomolecular structure are far from complete, the high precision and stability observed in earlier design experiments (1–5) are clearly essential ingredients for designing enzymes and binding partners from scratch. But natural proteins have other features that are crucial for fulfilling their molecular function. Computer algorithms optimize surface polarity, core hydrophobicity, and backbone regularity; in a nutshell, they optimize stability. By contrast, evolution selects proteins for their ability to perform a vital molecular function, often at the expense of stability. Numerous molecular structures of natural proteins show that the active site is the protein’s most energetically perturbed region, with multiple same-charge chemical groups, buried polar atoms, hydrophobic surfaces exposed to water, or broken and twisted α helices and β sheets. Can design algorithms be extended to encode such features, and if so, do the resulting proteins still show high stability and precision?

To address this question, Boyken et al. aimed to design buried hydrogen bonds in protein-protein interfaces starting from designed helical coiled coils. In nature, polar interactions encode high-precision binding specificity; these interactions typically require supporting networks of polar groups on both binding partners for accurate positioning (see the first figure). Because of this complexity, designed binding partners in previous work have not contained elaborate polar networks (8).

To overcome this limitation, Boyken et al. designed a large repertoire of coiled coils, including novel topologies of dimers, trimers, and tetramers. They developed an algorithm (called HBNet) to find side-chain constellations in which all polar atoms are connected through stabilizing hydrogen-bond networks (see the first figure). The authors then synthesized 114 designed oligomers and characterized the structures of various topologies. Most of them showed high precision rela-
tive to the models, formed only the intended oligomers, and were stable at temperatures as high as 95°C.

Furthermore, the hydrogen-bond networks in these oligomers were reminiscent of the simplicity and elegance of the DNA double helix, where every base on one strand is paired to a complementary base on the other through hydrogen bonds. Inspired by the double helix, Boyken et al. designed long coiled coils built from modular parts, each with its own constellation of polar side chains. These modular coiled coils may provide the basis for a new generation of protein-based molecular structures of programmable shape, similar to DNA origami. Unlike DNA, however, these assemblies could be easily interfaced with proteins of desired function.

Jacobs et al. address a complementary question: how to construct new proteins with features seen in protein active sites, diverse geometries, including some with cavities that could allow small-molecule binding (see the second figure). Three designs were hyperstable; moreover, the molecular structure of one of them precisely recapitulated the computational model, and another required a further round of computations to fix a design flaw.

Jacobs et al.’s modular design approach has natural and protein-engineering parallels; indeed, gene recombination is the main means of diversification in natural protein families and is regularly used by protein engineers (9). The new work extends the reach of modular design to combinations of fragments from nonhomologous proteins, for which genetic recombination is unlikely.

The remarkable selectivities and efficiencies seen in natural protein binders and enzymes require a balance between stabilizing features that specify molecular structure...