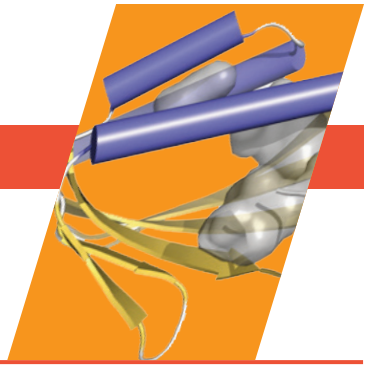
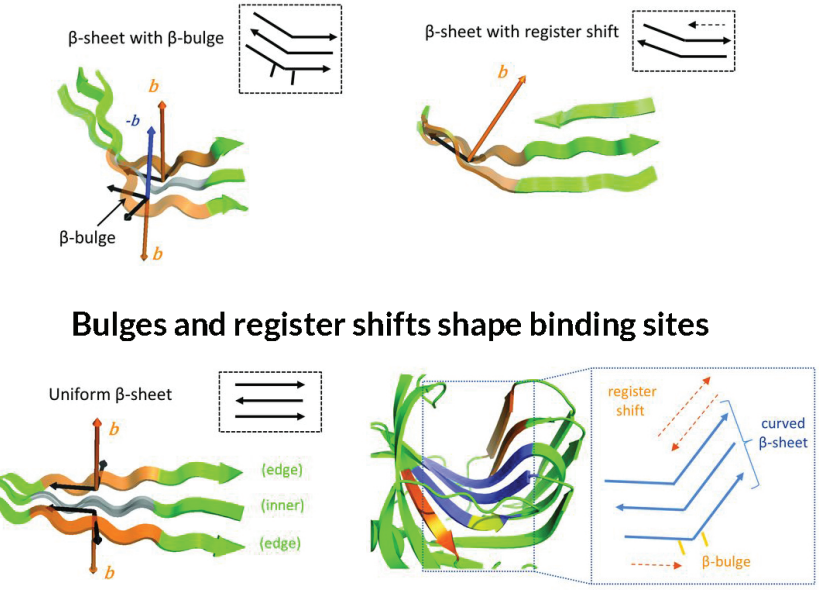
BIOLOGICAL SCIENCES

Bending the (β-Sheet) Curve to Shape Protein Cavities





Rules for β-sheet curvature design. Top left: Classic β bulges are created by placing hydrophobic (or -philic) residues next to each other in neighboring protein strands. Top right: Register shifts are created by terminating the bonding between adjacent strands. Bottom: Coupled with intrinsic β-strand geometry, these features induce curvature in β-sheets.

Curved β sheets are basic building blocks of many protein cavities that, by serving as binding sites for other molecules, are essential to protein function. After analyzing classic protein formations and running folding simulations, researchers designed a series of novel proteins with curved β sheets, inspired by naturally occurring protein superfamilies. They then compared the predicted models to physical examples of these designed proteins using x-ray crystallography. All of the structures closely matched the predicted models, showing that β-sheet curvature can be controlled with atomic-level accuracy. The discovery opens the door to the design of proteins capable of entirely new functions, from improved diagnostic tests and medical treatments to more-efficient catalysis of chemical reactions for industrial processes.

In designing a new ligand-binding protein or enzyme acting on a particular molecule, researchers have traditionally tried to repurpose natural proteins. They look for proteins with cavities having roughly the desired geometry and incorporate mutations to achieve the function of interest. However, this strategy is known to have several limitations: the ideal geometry may not be found and/or the incorporated mutations may change the cavity geometry or be detrimental to the protein's stability. To overcome these limitations, it would be more efficient to design a protein from scratch, with a geometry customized to the target of interest.

Previously, the computational design of proteins from scratch had been limited to structures that were too compact to accommodate cavities. In this work, researchers deciphered the key rules that determine the shape of structures formed by β sheets in natural proteins. In particular, they described the rules that govern how these β sheets bend and

Protein Architecture

Basic (primary) protein structure consists of long sequences of amino-acid molecules linked in a chain. Parts of the chain can be electrostatically attracted to (or repelled by) other parts of the same chain. This causes a protein to fold into compact, characteristic shapes (secondary structures), such as alpha helices and beta (β) sheets. In the latter, parallel protein strands line up so that bonds can form between the strand edges, creating relatively flat, sheet-like structures. These can then be twisted and bent to form pockets of open space in the protein, similar to the way a domed roof can create unobstructed open space underneath. Additionally, bulges, bends, and other protrusions into the space (e.g. side chains that branch off of the main protein chain) can shape the cavity into a unique pocket into which only a complementary shape will fit, like a hand into a glove. These complementary molecules are called "ligands." Ligand binding is the key mechanism of many biological and chemical processes of interest to science, such as drug delivery, molecular signal transmission, and enzyme activity, for example.

wrap to form the cavities of various sizes and shapes that play a key role in protein–protein or protein–molecule interactions. For example, β sheets (normally flat) will bulge if hydrophobic (or -philic) residues are placed next to each other in the protein chain. Also, by terminating the bonding between adjacent strands (a "register shift"), a bend can be created. The researchers then incorporated these rules into existing computational protein design methods and built new types of protein structures having the shapes they were targeting.

To verify that the resulting proteins had the desired properties and adopted the modeled structure, the researchers experimentally characterized them with a variety of techniques. The atomic structures were verified using high-resolution x-ray crystallography at Beamline X4C of the National Synchrotron Light Source and at ALS Beamline 8.2.1. Of the nine solved structures described in this work, six were obtained at the ALS. High-resolution structural analysis is essential for evaluating the accuracy of computational designs and quickly becomes the bottleneck in protein design studies. The ability to quickly screen many crystals at the ALS enhanced the probability of solving the structures.

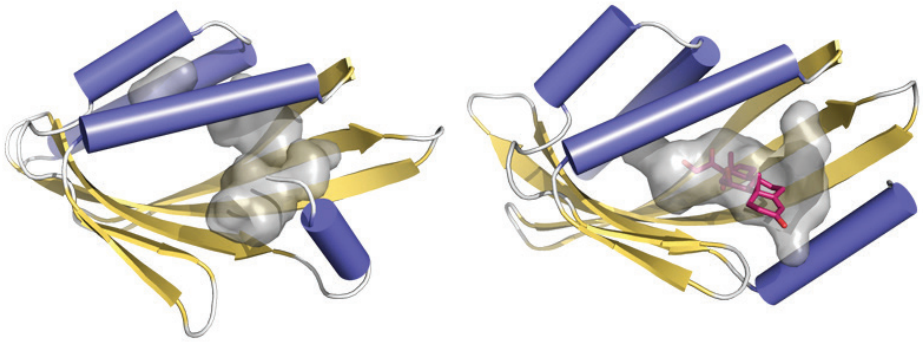


Illustration of how a curved protein surface (yellow-gold) forms a pocket (gray) to potentially fit molecules. The structure at left is based on atomic-resolution data, and the structure at right is based on a computational model. (Credit: Benjamin Basanta)

Interpretation and analysis of the crystallographic data showed that the experimental protein structures were very close to the computational models, validating the new design approach. The researchers were surprised to find that a few small molecules from the crystallization solution had found their way into the cavities of one of the designed proteins—a good indication of the feasibility of follow-up plans for designing an active site there with little change from the initial structure. Now that they have shown that they can design these types of proteins, the researchers plan to use this method to create novel enzymes that could result in reduced chemical waste and more-efficient chemistry in general, as well as sensors for the detection of food and environmental toxins. Overall, the new strategy allows designers to depend less on natural proteins and focus more on custom-designed proteins at the atomic level.

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