SENSORS AND PROBES

Fluorescent proteins from scratch

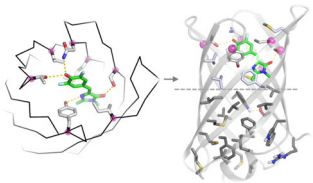
Researchers have carried out the first de novo design of a β-barrel protein and engineered the interior to bind and activate a fluorogenic dye.

The design of protein structures from scratch can yield novel insights into protein structure and function and can also be the ultimate test of whether the rules of protein design are well understood. Although a wide range of α-helical coiled-coil structures have been accurately designed, de novo–designed β-barrel proteins have remained elusive. This is in part because proteins that contain β-strands and sheets tend to aggregate if not perfectly designed.

David Baker, along with his student Jiayi Dou, postdoc Anastassia Vorbieva and team at the University of Washington, decided to tackle this long-standing challenge and design a properly folding β-barrel. Their first design strategy was based on previous work on β-barrel proteins and involved the design of 3D backbones in which each strand was identical, as well as optimization of side chains in silico with a protein modeling software called Rosetta, which can be used to find energy-optimized protein structures. Using this approach, they designed backbones optimized for extensive interstrand hydrogen bonding, which is known to stabilize structures that contain β-sheets. From there, they expressed a set of 41 genes representing optimal designs in *Escherichia coli*; however, all were found to aggregate rather than form monomeric β-barrels.

Despite these discouraging results, the team persevered. Baker recalls, "We had to come up with some new theories about what was wrong with that previous hypothesis and we had to start over again." So, they began from scratch with a strategy using Rosetta that incorporated what they had learned from their previous attempt—namely, that the hydrogen bonding at the top and bottom of their designed barrels was suboptimal, and that the strands had to be allowed to deviate from one another to make good hydrogen bonds without steric clashes.

In examining this issue, they found sources of molecular strain that were preventing the proper hydrogen bonding and folding of their structures. These were largely relieved by the incorporation of glycine residues into the strands to give the strands the flexibility they needed to avoid steric clashes and optimize hydrogen bonding, which Baker calls a surprising result given that glycines are not commonly found in naturally occurring β-strands. These findings led them to the ultimately successful approach of building backbones based on peptide bonds, backbone torsion angle bins, and backbone hydrogen bonds, which enabled them to generate several proteins that were monomeric β-barrels when expressed in bacteria.



Top-down (left) and side (right) views of a designed β-barrel binding the GFP chromophore derivative. Credit: reprinted with permission from Dou et al. (2018), Springer Nature

Once the researchers had unlocked the design of β-barrels, they decided to push the work one giant leap further and develop designer fluorescent proteins. Baker notes, "Fluorescent proteins are very useful in biotechnology and medicine and we would like to design a whole new world of these that are really more suited for their applications than random products of the stochastic evolutionary processes."

For this, they chose to mimic the green fluorescent protein (GFP) by using a small-molecule derivative of the GFP chromophore, which is nonfluorescent in solution but becomes brightly fluorescent when stabilized in a planar conformation. To design an appropriate β-barrel and binding pocket, the team developed a new in silico docking method that enabled them to optimize both the rigid-body degrees of freedom of the chromophore and the amino acids that form the binding site. This work resulted in several proteins that bind and activate the fluorescence of the chromophore, even in the context of living mammalian cells.

This work represents a landmark achievement both for protein design and for fluorescent protein engineering, and is sure to open the door to innovative tool development.

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Research papers
Dou, J. et al. De novo design of a fluorescence-activating β-barrel. *Nature* **561**, 485–491 (2018).