Computational design of symmetric eight and nine-bladed β-propellers

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Pseudo symmetric, repeat proteins are favoured targets for computational protein design as they allow for the creation of larger domains with limited amino acids by exploiting their symmetric and repeating nature. One of the most common pseudo symmetric, repeat domains is the β-propellers fold. In addition, they fulfil many functions from sugar binding to enzymatic and protein-protein interaction mediation, thus increasing the potential applications of the designed proteins. Each propeller is built from 4-stranded anti-parallel β-sheets also known as a blade, repeated around a central axis. The number of blades differs from four to ten with seven and eight being the most common. The first successful computational protein design of a β-propeller was the 6-bladed Pizza protein[1]. The RE3volutionary design method[2] makes use of ancestral sequence reconstruction and symmetry based template construction methods incorporated in Rosetta. Each blade of the pizza protein possess the same amino acid sequence. When two or three repeats of this sequence are expressed, they self-assemble into the 6-bladed domain.

The same design method was employed to design the eight or nine bladed Cake protein[3]. The protein consists repeating units of 42 amino acids, when eight repeats are expressed, the protein adopts the nine bladed fold. However, when nine repeats are expressed, the protein will adopt a nine bladed fold. This structural plasticity was unseen among β-propellers monomers. Its existence might explain the wide diversity of repeat numbers observed in β-propellers by allowing the change from even to odd numbers. Identical to the Pizza protein, smaller repeat fragments of Cake will self-assemble into either the eight-bladed protein or the nine-bladed protein. The structures of most these assemblies as well as the monomeric eight-and nine-bladed propellers were confirmed with X-ray diffraction experiments.

While the structural plasticity of the Cake protein is novel, we also wanted to create a protein that could only adopt the rare nine-bladed propeller fold. In order to achieve this a three-blade repeat (124 amino acids) was designed with a similar design strategy, with the idea the three-fold symmetry would prevent formation of eight bladed propellers. Two variants, Scone-E and Scone-R were created[4]. Crystallography revealed however that both designs adopted an eight-fold conformation. This failed design showcases that more research is needed to create a specific sequence for large β-propellers. In addition to this the Scone-E protein could only be crystallized upon addition of the polyoxometalate STA. This charged molecule interacts with multiple positively charged regions on the protein surface, neutralizing them. It can also bind multiple chains thus facilitating protein contacts, resulting in higher symmetric space groups.

Some of the designed proteins in this research behaved unexpectedly, thus illustrating the importance of accurate structure determination by X-ray diffraction to validate the designed proteins. In addition the design lessons on larger β-propellers could prove instrumental in the design of new functional proteins based on this common natural protein fold.

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